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(54) Title: HYDROPHILIC LINKERS FOR CONJUGATION

(57) Abstract: Cell binding agent-drug conjugates comprising hydrophilic linkers, and methods of using such linkers and conjugates are provided.

HYDROPHILIC LINKERS FOR CONJUGATION

FIELD OF THE INVENTION

The present invention relates to the preparation of hydrophilic linkers used for the conjugation of a drug, in particular, a cytotoxic agent or a chromophore molecule to a cell binding molecule. The present invention also relates to methods of making cell-binding agent-drug (e.g. cytotoxic agent) conjugates comprising either modification of drugs with these hydrophilic linkers first, followed by reaction with cell-binding agents; or modification of cell-binding agents with these hydrophilic linkers first, followed by reaction with drugs.

BACKGROUND OF THE INVENTION

Targeted therapies have been the much focused in the pharmaceutical research and development for many years. They are a cornerstone of “precision medicine” (President Barack Obama, State of the Union Address, Jan. 20, 2015, www.whitehouse.gov/precisionmedicine) that uses specific information about a person’s tumor to help diagnose, plan treatment, find out how well treatment is working, or make a prognosis (F. S. Collins, *New Engl. J. Med.* 2015; 372:793-795). So far many different targeted therapies have been approved for use in cancer treatment by US FDA, European EMA and Chinese CFDA. These therapies include hormone therapies, signal transduction inhibitors, gene expression modulator, apoptosis inducer, angiogenesis inhibitor, immunotherapies, and toxin delivery molecules. The hormone therapies act by preventing the body from producing the hormones or by interfering with the action of the hormones, which in turn can slow or stop the growth of hormone-sensitive tumors. Signal transduction inhibitors block a cell responds to signals from its environment, in particular, prevent the ability of cancer cells to multiply quickly and invade other tissues. Gene expression modulator can modify the function of proteins that play a role in controlling gene expression. Apoptosis inducers cause cancer cells to undergo a process of controlled cell death. Angiogenesis inhibitors block the growth of new blood vessels to tumors (a process called tumor angiogenesis). Immunotherapies trigger the immune system to destroy cancer cells. Most immunotherapies are monoclonal antibodies that recognize specific molecules on the surface of cancer cells. Toxin delivery molecules use a transport vehicle/method to deliver a toxin drug specifically to the cancer cells.

The much promising approach that has been studied extensively during the past three decades is the toxin delivery molecules. There are several systemic deliveries of chemotherapeutical drugs for targeted treatment of tumor: Heat-activated targeted drug delivery; Tissue-selective drug delivery for cancer using carrier-mediated transport systems; Tumor-activated prodrug therapy for targeted delivery of chemotherapy; Pressure-induced filtration of drug across vessels to tumor; Promoting selective permeation of the anticancer agent into the tumor; Two-step targeting using a bispecific antibody; Site-specific delivery by an antibody conjugate; And light-activation of macromolecules. Many carriers have been studied in different forms or in special formulations for the target delivery of anticancer drugs, such as Albumin-based drug carriers; Carbohydrate-enhanced chemotherapy; Proteins and peptides based drug carriers; Fatty acids as targeting vectors linked to active drugs; Microsphere carriers; Monoclonal antibodies as carriers; Vitamins, e.g. folates as carriers; Nanoparticle carriers,; Liposome carriers, e. g. pegylated liposomes (enclosed in a polyethylene glycol bilayer); Polyethylene glycol (PEG) carriers; Single-chain antigen-binding molecule carriers; Polymeric micelle carriers; Lipoprotein-based drug carriers; Dendrimers; etc. Ideally the delivery vehicle has to be site-specific, non-toxic, biocompatible, non-immunogenic, and biodegradable (Scott, R; et al (2008) Expert Opin. Drug Deliv. 5, 459) and avoid recognition by the host's defense mechanisms (Saltzman, W.; (2008). "Drug delivery systems" Access Science. McGraw-Hill Co.). For this criterion, using a monoclonal antibody (mAb) as a delivery vehicle for chemotherapeutical drugs has been much successful. By combining the unique targeting capabilities of monoclonal antibodies with the cancer-killing ability of cytotoxic drugs, the antibody-drug conjugates (ADCs) allow sensitive discrimination between healthy and diseased tissue (ADC Review, J. Antibody-drug Conjugates, - Jun 1, 2013). Beside the successful market approval of adotrastuzumab emtansine (T-DM) in 2013 and Brentuximab vedotin in 2011 by US FDA, there are over 40 different ADC drugs currently in clinical trials in USA (www.clinicaltrials.gov). But there are still many challenges among ADC development, such as the linker selection for improvement of the therapeutic index, mAbs linked to a careful selection of the targets, a better understanding of the mechanism of action, and the management and understanding of ADC off-target toxicities. It has been known that the linker between the delivery vehicles, in particular, an antibodies and the cell-killing toxins plays a critical role in the development of targeted drug delivery systems, as the nature of the linker significantly affects the potency, selectivity and the pharmacokinetics of the

resulting conjugates (Zhao, R. Y. et al (2011) J. Med. Chem. 54, 3606; Acchionea, M. et al (2012) mAbs, 4, 362; Doronina, S. et al, (2006) Bioconjug Chem, 17, 114; Hamann, P. et al. (2005) Bioconjug Chem. 16, 346). So far, four types of linkers had often been used for preparation of cell binding agent-drug conjugates that have entered the clinic: (a) acid-labile linkers, exploiting the acidic endosomal and lysosomal intracellular microenvironment; (b) linkers cleavable by lysosomal proteases; (c) chemically stable thioether linkers that release a lysyl adduct after proteolytic degradation of the antibody inside the cell; and (d) disulfide-containing linkers, which are cleaved upon exposure to an intracellular thiol (Zhao, R. Y. et al, 2011 J. Med. Chem. 36, 5404).

Conjugates of cell-binding agents with drugs or modified chemical compounds via different types of linkers have been described (U.S. Patent Nos. 4,680,338, 5,122,368, 5,141,648, 5,208,020, 5,416,064; 5,475,092, 5,543,390, 5,563,250 5,585,499, 5,880,270, 6,214,345, 6,436,931, 6,372,738, 6,340,701, 6,989,452, 7,129,261, 7,375,078, 7,498,302, 7,507,420, 7,691,962, 7,910,594, 7,968,586, 7,989,434, 7,994,135, 7,999,083, 8,153,768, 8,236,319, WO2014080251, Zhao, R.; et al, (2011) J. Med. Chem. 36, 5404; Doronina, S.; et al, (2006) Bioconjug Chem, 17, 114; Hamann, P.; et al. (2005) Bioconjug Chem. 16, 346). Typically, in these conjugates, the cell-binding agents are first modified with a bifunctional agent such as SPDP (*N*-succinimidyl 3-(2-pyridyldithio) propionate), SMPDP (*N*-succinimidyl 4-methyl-4-(2-pyridyldithio) pentanoate), SPDB (*N*-succinimidyl 4-(2-pyridyldithio) butanoate), or SMCC (succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate), to introduce an active disulfide or a maleimido moiety. Reaction with a thiol-containing cytotoxic drug provides a conjugate in which the cell-binding agent, such as a monoclonal antibody, and drug are linked via disulfide bonds or thioether bonds.

However, the use of the cell binding molecule-drug conjugates, such as antibody-drug conjugates (ADCs), in developing therapies for a wide variety of cancers has been limited both by the availability of specific targeting agents (carriers) as well as the conjugation methodologies which result in the formation of protein aggregates when the amount of the drugs that are conjugated to the carrier (i.e., the drug loading) is increased. Normally the tendency for cytotoxic drug conjugates to aggregate is especially problematic when the conjugation reactions are performed with the hydrophobic linkers. Since higher drug loading increases the inherent potency of the conjugate, it is desirable to have as much drug loaded on the carrier as is consistent with retaining the affinity of the carrier protein. The presence of aggregated protein, which may be nonspecifically toxic and immunogenic, and

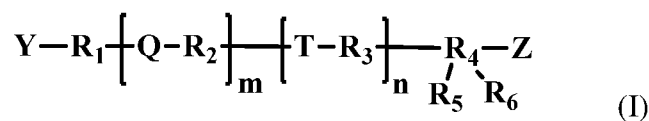
therefore must be removed for therapeutic applications, makes the scale-up process for the production of these conjugates more difficult and decreases the yield of the products.

We have invented a series of hydrophilic linkers containing phosphinate, sulfonyl, and/or sulfoxide groups which can improve methods for conjugating cytotoxic drugs to a carrier (cell binding molecules) in high a drug loading without aggregation (PCT/IB2012/056700 and PCT/CN2014/072769). Here we describe the extending innovation of the hydrophilic linkers for better conjugation, much conditional drug release, loading with two different kinds of drugs or molecules per a linker, and/or linking with a pair of sites of a cell binding molecule per a linker for specific or better conjugation.

10 SUMMARY OF THE INVENTION

The present invention provides hydrophilic linkers containing phosphamide, phosphinate, sulfonamide, sulfonyl, sulfonimide, and/or sulfoxide groups to link drugs to a cell-binding agent (e.g., an antibody). The preferred formula of the cell binding molecule – hydrophilic linker- drug conjugates can be represented as: Cb-(-L-Drug)_n, wherein Cb is a cell-binding agent, L is a hydrophilic linker, Drug is a drug molecule, and n is an integer from 1 to 20. The advantages in applying the hydrophilic linker in the cell molecule-drug conjugate are: a). Reducing the aggregation of the conjugates in water based media; b). enabling higher drug-per-cell binding molecule-ratio conjugate, resulting in higher potency; c). Being retained inside the target cell after the drug-linker released from the conjugates, which can combat permeability-glycoprotein (Pgp)-expressing multidrug resistant (MDR) cells; d). Enabling loading two different kinds of drugs per a linker; f). Conjugating a pair of sites of a cell binding molecule per a linker.

In one aspect of the present invention, the hydrophilic linker is represented by Formula (I) wherein Y can react with a cell-binding agent and Z can react with a cytotoxic drug:



Wherein:

Y represents a functional group that enables reaction with a cell-binding agent;

Q and T are either $-X_1-P(=O)(OM)-$, or $X_1-S(O_2)-$, or $-X_1-S(O)-$; or $-X_1-$

30 $P(=O)(OM)-X_2-$, or $-X_1-P(=O)[X_2-R_4-Z]-X_3-$, or $-X_1-P(=O)[X_2-R_1-Y]-X_3-$, or $-X_1-S(O_2)-X_2-$, or $-X_1-S(O)-X_2-$;

X_1 , X_2 and X_3 are independently selected from N(R_7), O, or S; In addition, when X_1 is either N(R_7), or O, or S, then either X_2 or X_3 , or another X_1 connects to $-P(=O)$, $-S(O)$, or $-S(O_2)$ can be CH_2 .

m and n are integer from 0 to 5, but not 0 at the same time;

5 Z represents a functional group that enables linkage of a cytotoxic drug via a disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, amine (secondary, tertiary, or quaternary), imine, cycloheteroalkane, heteroaromatic, alkoxime or amide bond;

10 R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and R_7 are the same or different and are H, linear alkyl having from 1-6 carbon atoms, branched or cyclic alkyl having from 3 to 6 carbon atoms, linear, branched or cyclic alkenyl or alkynyl, or 1-6 carbon atoms of esters, ether, amide, or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$, wherein p is an integer from 0 to about 1000, or combination thereof.

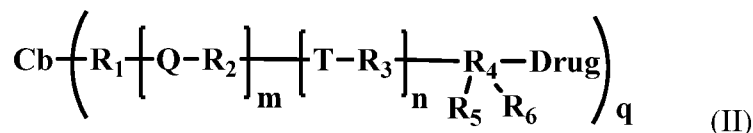
15 Additionally R_1 , R_2 , R_3 and R_4 are respectively a chain of atoms selected from C, N, O, S, Si, and P that covalently connects the cell-surface binding ligand, the phosphinate or sulfonyl group, the conjugated drug and among themselves (R_1 , R_2 , R_3 and R_4). The atoms used in forming the hydrophilic linker may be combined in all chemically relevant ways, such as forming alkylane, alkylene, alkenylene, and alkynylene, ethers, polyoxyalkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amides, ureas, semicarbazides, 20 carbazides, alkoxyamines, alkoxyamines, urethanes, amino acids, peptides, acyloxyamines, hydroxamic acids, or combination thereof.

M is H, or Na, or K, or $N^+R_1R_2R_3$ or a pharmaceutical salt. R_1 , R_2 and R_3 are described above.

25 In another embodiment, when the hydrophilic linkers of the Formula (I) has two or more Y groups, in particular the two same Y groups, at such case, either Q , or/and T is $-X_1-P(=O)[X_2-R_1-Y]-X_3-$, then the hydrophilic linkers of the Formula (I) can be linked to two or more sites, particularly to a pair of sites of cell binding molecules.

30 In yet another embodiment, when the hydrophilic linkers of the Formula (I) has two Z or more groups, at such case, either Q , or/and T is $-X_1-P(=O)[X_2-R_4-Z]-X_3-$, then the hydrophilic linkers of the Formula (I) can be linked to two or more drugs, in particular, two different drugs.

In another aspect, this invention provides a cell-binding agent-drug conjugate of Formula (II), in which the cell-binding agent, Cb, and the drug, Drug, have reacted at the two ends of the hydrophilic linker:



5 wherein:

Cb represents a cell-binding agent;

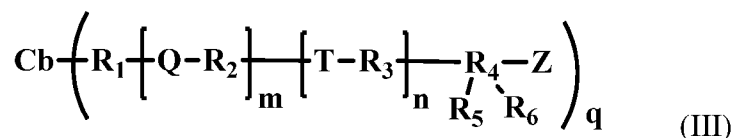
Drug represents the drug linked to the cell-binding agent via the hydrophilic linker by a disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, cycloheteroalkane, heteroaromatic, alkoxime or amide bond;

10 q is 1 ~ 20; m, n, R₁, R₂, R₃, R₄, R₅, R₆ and M are described the same previously in Formula (I).

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Drug]-X₃-, or -X₁-P(=O)[X₂-R₁-Cb]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-;

15 X₁, X₂ and X₃ are independently selected from N(R₇), O, or S; In addition, when X₁ is either N(R₇), or O, or S, then either X₂, or X₃, or another X₁ that connects to -P(=O), -S(O), or -S(O₂) can be CH₂.

In a further aspect, the present invention provides a modified cell-binding agent of Formula (III), in which the cell-binding agent, Cb, has reacted with the hydrophilic linker, which still has Z, a group capable of reacting with a drug:

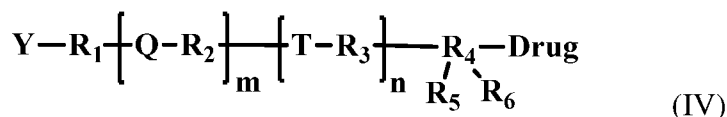


20 Wherein Cb, Z, m, n, q, R₁, R₂, R₃, R₄, R₅ and R₆ are defined the same as in Formula (I) and (II).

25 Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Z]-X₃-, or -X₁-P(=O)[X₂-R₁-Cb]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-;

X₁, X₂ and X₃ are independently selected from N(R₇), O, or S; In addition, when X₁ is either N(R₇), or O, or S, then either X₂, or X₃, or another X₁ that connects to -P(=O), -S(O), or -S(O₂) can be CH₂.

In an even further aspect, the present invention provides a modified drug of Formula (IV), in which the drug, Drug, has reacted with the hydrophilic linker, which still has Y, a group capable of reacting with the cell-binding agent:



5 Wherein Y, Drug, m, n, q, R₁, R₂, R₃, R₄, R₅ and R₆ are defined the same as in Formula (I) and (II).

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Drug]-X₃-, or -X₁-P(=O)[X₂-R₁-Y]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-;

10 X₁, X₂ and X₃ are independently selected from N(R₇), O, or S; In addition, when X₁ is either N(R₇), or O, or S, then either X₂, or X₃, or another X₁ that connects to -P(=O), -S(O), or -S(O₂) can be CH₂.

15 The present invention further relates to a method of making a cell-binding molecule-drug conjugate of Formula (II), wherein the drug is linked to a cell-binding agent via the hydrophilic linker.

The present invention also relates to a method of making a modified cell-binding molecule of Formula (III), wherein the cell-binding molecule is reacted with the hydrophilic linker.

20 The present invention also relates to a method of making a modified drug of Formula (IV), wherein the drug is reacted with the hydrophilic linker.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows synthesis of phosphamide linkers containing maleimide groups and the application of these linkers in the conjugation of an antibody with drugs.

25 Figure 2 shows the synthesis of phosphamide linkers containing disulfide bonds and the application of these linkers in the conjugation of an antibody with drugs.

Figure 3 shows the synthesis of phosphamide linkers containing maleimide groups and the application of these linkers in the conjugation of an antibody with drugs.

30 Figure 4 shows the synthesis of phosphamide linkers containing maleimide, hydrazone, or thioether groups and the application of these linkers in the conjugation of an antibody with drugs.

Figure 5 shows the synthesis of hinder phosphamide linkers containing maleimide groups and the application of these linkers in the conjugation of an antibody with drugs.

Figure 6 shows the synthesis of phosphamide linkers containing disulfide or oxime groups and the application of these linkers in the conjugation of an antibody with drugs.

5 Figure 7 shows the synthesis of phosphamide linkers containing maleimide and polyethylene glycol groups and the application of these linkers in the conjugation of an antibody with drugs.

Figure 8 shows the synthesis of phosphamide linkers containing maleimide, polyethylene glycol and azido groups and the application of these linkers in the conjugation of an antibody with two different compounds. Drug₁ and Drug₂ here can be a cytotoxic agent for therapeutic application or a chromophore compound for monitoring the interaction of the conjugates with targeted cells as well.

Figure 9 shows the synthesis of phosphamide linkers containing disulfide, polyethylene glycol or ketone groups, and these linkers are used for conjugation of a protein with two different compounds. Drug₁ and Drug₂ here can be a cytotoxic agent for therapeutic application or a chromophore compound for monitoring the interaction of the conjugates with targeted cells.

Figure 10 shows the synthesis of the sulfonamide and the sulfinamide linkers containing a maleimide group and the application of these linkers in the conjugation of an antibody with a cytotoxic drug.

Figure 11 shows the synthesis of a sulfonamide linker containing a disulfide, polyethylene glycol or ketone group, and the linker is used for conjugation of a protein with two different compounds. Drug₁ and Drug₂ here can be a cytotoxic agent for therapeutic application or a chromophore compound for monitoring the interaction of the conjugate with targeted cells.

Figure 12 shows the synthesis of phosphamide linkers containing thioether or disulfide groups, and these linkers are used to link two drug/compounds per linker. Drug here can be a cytotoxic agent for therapeutic application or a chromophore compound for monitoring the interaction of the conjugates with targeted cells.

Figure 13 shows the synthesis of an antibody conjugate via the phosphamide linkers containing two function groups, wherein one group is linked to a cytotoxic agent, MMAF for targeted killing, and the other one is linked to a fluorochrome group for monitoring the interaction of the conjugate with a targeted cell.

Figure 14 shows the synthesis of the phosphamide linkers containing two function groups.

Figure 15 shows the synthesis of an antibody conjugate via a phosphamide linker containing both a tubulysin analog and a MMAF analog on the linker.

5 Figure 16 shows the synthesis of the phosphamide linkers containing two different cytotoxic drugs (PBD dimer & MMAF analogs).

Figure 17 shows the synthesis of antibody conjugates via the phosphamide linkers of the present patent. The linkers can be conjugated two different drugs (e.g. a PBD analog and a MMAF analog) per linker, or can be linked to a pair of cysteine sites of an antibody.

10 Figure 18 shows the synthesis of a sulfonamide linker containing a disulfide, polyethylene glycol, azido, or a triazole group, and the linker is for conjugation with two different compounds.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

15 “Alkyl” refers to an aliphatic hydrocarbon group which may be straight or branched having 1 to 8 carbon atoms in the chain. “Branched” means that one or more lower C numbers of 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-
20 dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methyl-hexyl, 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 C₁-C₈ alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R', -SR', -
25 -S(O)₂R', -S(O)R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; where each R' is independently selected from -C₁-C₈ alkyl and aryl. “Halogen” refers to fluorine, chlorine, bromine or iodine atom; preferably fluorine and chlorine atom.

30 “Heteroalkyl” refers to C₂-C₈ alkyl in which one to four carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N.

“Carbocycle” refers to a saturated or unsaturated ring having 3 to 8 carbon atoms as a

monocycle or 7 to 13 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, arranged as a bicycle [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicycle [5,6] or [6,6] system. Representative C₃-C₈ 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 "C₃-C₈ carbocycle" refers to a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated nonaromatic carbocyclic ring. A C₃-C₈ carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R', -SR', -S(O)R', -S(O)₂R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; where each R' is independently selected from -C₁-C₈ alkyl and aryl.

"Alkenyl" refers to an aliphatic hydrocarbon group containing a carbon-carbon double bond 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" refers to an aliphatic hydrocarbon group containing a carbon-carbon triple bond 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, 5-pentynyl, *n*-pentynyl, hexylnyl, heptynyl, and octynyl.

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH₂-), 1,2-ethyl (-CH₂CH₂-), 1,3-propyl (-CH₂CH₂CH₂-), 1,4-butyl (-CH₂CH₂CH₂CH₂-), and the like.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by

the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene, propargyl and 4-pentynyl.

5 “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 an aromatic group, wherein one or several H atoms are replaced independently by -R', -halogen, -OR', or -
 10 SR', -NR'R'', -N=NR', -N=R', -NR'R'', -NO₂, -S(O)R', -S(O)₂R', -S(O)₂OR', -OS(O)₂OR', -PR'R'', -P(O)R'R'', -P(OR')(OR''), -P(O)(OR')(OR'') or -OP(O)(OR')(OR'') wherein R', R'' are independently H, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, arylalkyl, carbonyl, or pharmaceutical salts.

15 “Heterocycle” refers to a ring system in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group of O, N, S, Se, B, Si and P. Preferable heteroatoms are O, N and S. Heterocycles are also described in The Handbook of Chemistry and Physics, 78th Edition, CRC Press, Inc., 1997-1998, p. 225 to 226, the disclosure of which is hereby incorporated by reference. Preferred nonaromatic heterocyclic include, but are not limited to epoxy, aziridinyl, thiranyl, pyrrolidinyl, pyrazolidinyl,
 20 imidazolidinyl, oxiranyl, tetrahydrofuranyl, dioxolanyl, tetrahydropyranyl, dioxanyl, dioxolanyl, piperidyl, piperazinyl, morpholinyl, pyranyl, imidazolinyl, pyrrolinyl, pyrazolinyl, thiazolidinyl, tetrahydrothiopyranyl, dithianyl, thiomorpholinyl, dihydropyranyl, tetrahydropyranyl, dihydropyranyl, tetrahydropyridyl, dihydropyridyl, tetrahydropyridinyl, dihydrothiopyranyl, azepanyl, as well as the fused systems resulting
 25 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,
 30 isothiazolyl, triazolyl, 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”, “heterocyclic” 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.

“Arylalkyl” refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like.

“Heteroarylalkyl” refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolymethyl, 2-furylethyl and the like.

Examples of a “hydroxyl protecting group” include, but are not limited to, methoxymethyl ether, 2-methoxyethoxymethyl ether, tetrahydropyranyl ether, benzyl ether, *p*-methoxybenzyl ether, trimethylsilyl ether, triethylsilyl ether, triisopropylsilyl ether, *t*-butyldimethylsilyl ether, triphenylmethylsilyl ether, acetate ester, substituted acetate esters, pivaloate, benzoate, methanesulfonate and *p*-toluenesulfonate.

“Leaving group” refers to a functional group that can be substituted by another functional group. Such leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, and iodide), methanesulfonyl (mesyl), *p*-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

The following abbreviations may be used herein and have the indicated definitions: Boc, *tert*-butoxy carbonyl; BroP, bromotrispyrrolidinophosphonium hexafluorophosphate; CDI, 1,1'-carbonyldiimidazole; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIAD, diisopropylazodicarboxylate; DIBAL-H, diisobutylaluminium hydride; DIPEA, diisopropylethylamine; DEPC, diethyl phosphorocyanidate; DMA, *N,N*-dimethyl acetamide; DMAP, 4-(*N,N*-dimethylamino)pyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; DTT, dithiothreitol; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; ESI-MS, electrospray mass spectrometry; HATU, O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HPLC, high pressure liquid chromatography; NHS, *N*-Hydroxysuccinimide; MMP, 4-methylmorpholine; PAB, *p*-aminobenzyl; PBS, phosphate-buffered saline (pH 7.0~7.5); PEG, polyethylene glycol; SEC, size-exclusion chromatog-

raphy; TCEP, tris(2-carboxyethyl)phosphine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Val, valine.

“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 solvate” or “solvate” refer to an association of one or more solvent molecules and a disclosed compound. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid and ethanolamine.

“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 via reaction the free acidic or basic forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an

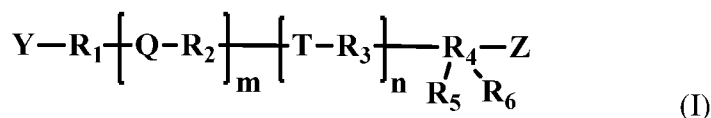
organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

5 The novel conjugates disclosed herein use the hydrophilic linkers. Examples of some suitable linkers and their synthesis are shown in Figures 1 to 17.

THE HYDROPHILIC LINKERS

The synthetic routes to produce hydrophilic linkers as well as the preparation of the conjugates of drugs to a cell binding molecules of the present invention are shown in
10 Figures 1-18. The hydrophilic linkers possess three elements: a) Substituents that are either phosphamide, or phosphinate, or sulfonamide, or sulfonyl, or sulfonamide, and/or sulfoxide, or mixed of these groups, b) A group, such as but not limited to, a *N*-hydroxysuccinimide ester group, maleimido group, disulfide group, haloacetyl group, alkoxyamino group, and/or hydrazide group, capable of reaction with a cell-binding agent,
15 and c) A group, such as but not limited to, a disulfide, maleimide, haloacetyl, aldehyde, ketone, azide, amine, alkoxyamine and hydrazide, capable of reaction with a drug. The hydrophilic substituents can be introduced by methods described herein. For example of the phosphamide substituents, they can be formed through directly condensation of phosphorus (V) oxychloride with amino molecules which are described in the figures 1, 2, 3, 4
20 and 5. For the mixed the phosphinate/ phosphamide substituents, they can be introduced by first treating a commercially available ammonium phosphinate with an acrylate via Michael addition, then substitution of excess amount of dibromo alkane to a phosphinate group, and followed by condensation with an amino compound, which are exemplified in the figures 6, 7, 8 and 9. For example of the sulfonamide and the sulfinamide substituents, they
25 can be through directly condensation of sulfuryl chloride and thionyl chloride with amino compounds, which are exemplified in the figure 10. The sulfonyl/ sulfonamide substituent can be formed through condensation of chlorosulfonic acid with an amine which is exemplified in the figures 11 and 18. The detail synthesis of the hydrophilic linkers and their uses for the preparation of cell binding ligand-drug conjugates of this invention are disclosed in
30 the figures 1~18.

Preferably, the hydrophilic linkers are compounds of the Formula (I) below:



wherein:

Y represents a functional group that enables reaction with a cell-binding agent;

Q and T are either $-X_1-P(=O)(OM)-$, or $-X_1-S(O_2)-$, or $-X_1-S(O)-$; or $-X_1-$

5 $P(=O)(OM)-X_2-$, or $-X_1-P(=O)[X_2-R_4-Z]-X_3-$, or $-X_1-P(=O)[X_2-R_1-Y]-X_3-$, or $-X_1-S(O_2)-X_2-$, or $-X_1-S(O)-X_2-$;

X_1 , X_2 and X_3 are independently selected from $N(R_7)$, O, or S; In addition, when X_1 is either $N(R_7)$, or O, or S, then either X_2 or X_3 , or another X_1 connects to $-P(=O)$, $-S(O)$, or $-S(O_2)$ can be CH_2 .

10 m and n are integer from 0 to 5, but not 0 at the same time;

Z represents a functional group that enables linkage of a cytotoxic drug via an alkyl, alkenylene, alkenylene, alkynylene, aromatic, heteroalkyl, disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, amine (secondary, tertiary, or quaternary), imine, cycloheteroalkylene, heteroaromatic, alkoxime or amide bond;

15 R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are the same or different and are H, linear alkyl having from 1-6 carbon atoms, branched or cyclic alkyl having from 3 to 6 carbon atoms, linear, branched or cyclic alkenyl or alkynyl, or 1-6 carbon atoms of esters, ether, amide, or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$, wherein p is an integer from 0 to about 1000, or combination thereof.

20 M is H, or Na, or K, or $N^+R_1R_2R_3$ or a pharmaceutical salt. R_1 , R_2 and R_3 are described above.

In another embodiment, R_1 , R_2 , R_3 , and R_4 can be respectively a chain of atoms selected from C, N, O, S, Si, and P that covalently connects the cell-surface binding ligand, the phosphinate or sulfonyl or sulfoxide group, the conjugated drug and themselves (R_1 , R_2 , R_3 and R_4). The atoms used in forming the hydrophilic 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, alkoxyamines, urethanes, amino acids, acyloxyamines, hydroxamic acids, and many others. In addition, it is to be understood that the atoms forming the linker (L) may be either saturated or unsaturated, or may be radicals, or may be cyclized upon each other to form divalent cyclic structures, includ-

25
30

ing cyclo alkanes, cyclic ethers, cyclic amines, arylenes, heteroarylenes, and the like in the linker.

Examples of the functional group, Y, that enables reaction with a cell-binding agent include amine reacting agents such as but not limited to *N*-hydroxysuccinimide esters, p-nitrophenyl esters, dinitrophenyl esters, pentafluorophenyl esters; thiol reactive agents such as but not limited to pyridyldisulfides, nitropyridyldisulfides, maleimides, haloacetates and carboxylic acid chlorides.

Examples of the functional group, Z, which enables linkage of a cytotoxic drug, include groups that enable linkage via a disulfide, thioether, thioester, peptide, hydrazone, ester, carbamate, carbonate, alkoxime or an amide bond. Such functional groups include, but are not limited to, thiol, disulfide, amino, carboxy, aldehydes, maleimido, haloacetyl, hydrazines, alkoxyamino, and/or hydroxy.

In preferred embodiments, R₁, R₂, R₃, and R₄, are linear alkyl having from 1-6 carbon atoms, or polyethyleneoxy unit of formula (OCH₂CH₂)_p, p = 1~100.

In another embodiments, when the hydrophilic linkers of the Formula (I) has two or more Y groups, in particular the two same Y groups, at such case, either Q, or/and T is – X₁–P(=O)[X₂–R₁–Y]–X₃–, then the hydrophilic linkers of the Formula (I) can be for linking to two or more sites, particularly to a pair of sites of cell binding molecules. Wherein X₁, X₂ and X₃ are independently selected from N(R₇), O, CH₂ or S; R₁ is defined above.

In yet another embodiments, when the hydrophilic linkers of the Formula (I) has two Z or more groups, at such case, either Q, or/and T is – X₁–P(=O)[X₂–R₄–Z]–X₃–, then the hydrophilic linkers of the Formula (I) can be for linking for two or more drugs, in particular, two different drugs. Wherein X₁, X₂ and X₃ are independently selected from N(R₇), O, CH₂ or S; R₄ is defined above.

The synthesis of 2-dithio-pyridyl containing cross-linkers of Formulae (I) is shown, for example, in figures 2, 6, 9, 11 and 12. The synthesis of maleimido-containing cross linkers of the Formula (I) is shown, for example, in figures 1, 3, 4, 5, 7, 8, 10, 13, and 17. The synthesis of thioether-containing cross linkers of the Formula (I) is shown, for example, in figures 1, 3, 4, 5, 7, 8, 10, 12 and 17. The synthesis of polyethylene glycol-containing hydrophilic cross linkers of Formula (I) is shown, for example, in figures 7, 8, 9, 11, 12, 13, 14, 15, 16, 17 and 18. The synthesis of azide-containing hydrophilic cross linkers of Formula (I) for Huisgen 1,3-dipolar cycloaddition of azides to alkynes (also called click chemistry) is shown, for example, in figures 8, 9, 13 and 18. The synthesis of

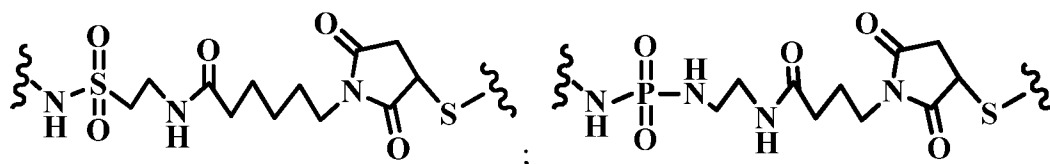
hydrophilic cross linkers of Formula (I) bearing hydrazide, or ketone, or alkoxyimino moieties enabling linkage via acid-labile bonds is shown, for example, in figures 6, 9, 11, 14, 15 and 16. The synthesis of hydrophilic linkers of Formula (I) which can be linked two drugs per a linker, or linked two sites per a linker is shown, for example, in figure 1, 2, 3, 4, 5, 6, 7, 17 and 18. The synthesis of hydrophilic linkers of Formula (I), which can be linked two different compounds /drugs per a linker, is shown, for example, in figures 8, 9, 11, 14, 15, 16 and 17. The synthesis of hydrophilic linkers of Formula (I), which can be linked one cytotoxic compound and one chromophore molecule per a linker, is shown, for example, in figure 13.

10 CELL-BINDING AGENT-DRUG CONJUGATES

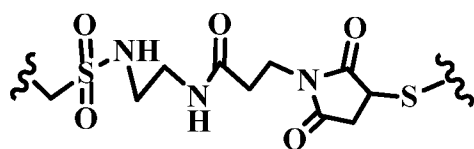
The conjugates of the present invention can be represented by the following formula, Cb-(-L-Drug)_n, wherein Cb is a cell-binding agent, L is a hydrophilic linker, Drug is a drug molecule, and n is an integer from 1 to 20.

The hydrophilic linker L may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe" or "af"), p-aminobenzyloxycarbonyl ("PAB"), 4-thiopentanoate ("SPP"), 4-(N-maleimidomethyl)cyclohexane-1 carboxylate ("MCC"), (4-acetyl)aminobenzoate ("SIAB"), 4-thio-butyrates (SPDB), 4-thio-2-hydroxysulfonyl-butyrates (2-Sulfo-SPDB), ethyleneoxy -CH₂CH₂O- as one or more repeating units ("EO" or "PEO"). Additional linker components are known in the art and some are described herein.

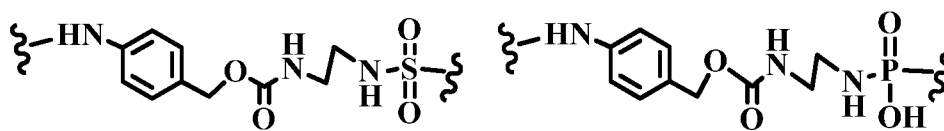
Example structures of these components containing linkers are:



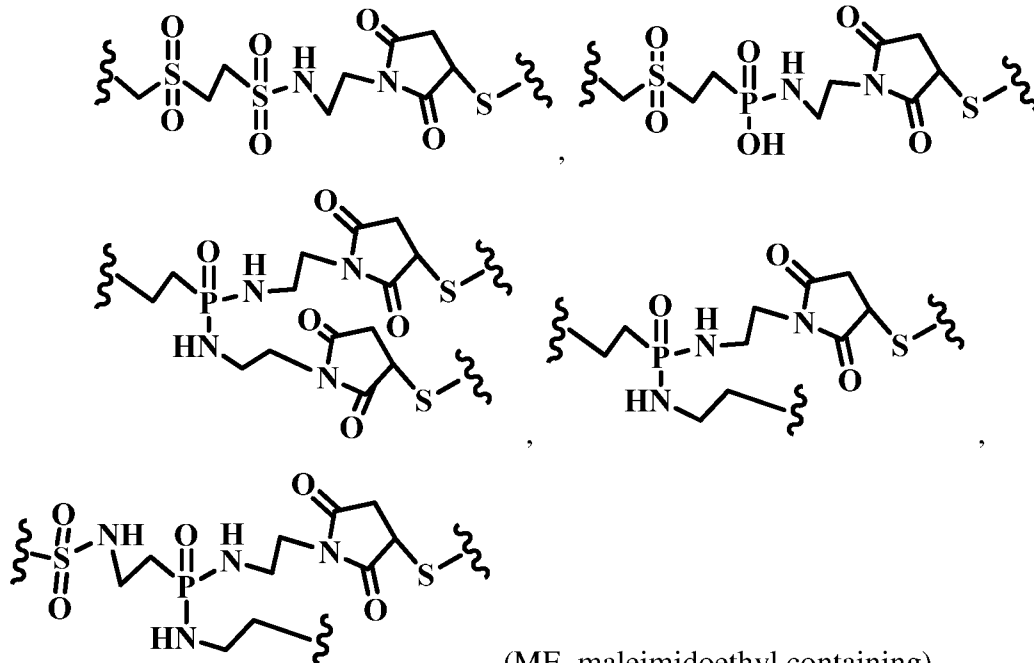
(MC, 6-maleimidocaproyl containing)



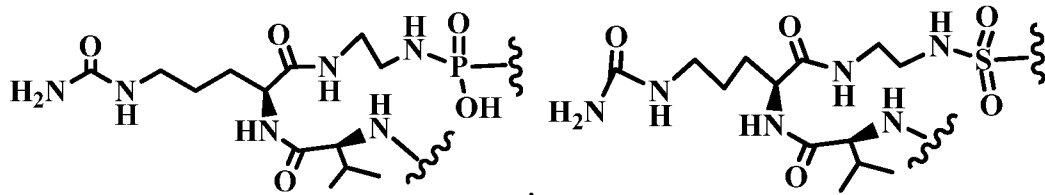
(MP, maleimidopropanoyl containing)



(PAB, p-aminobenzyloxycarbonyl containing)

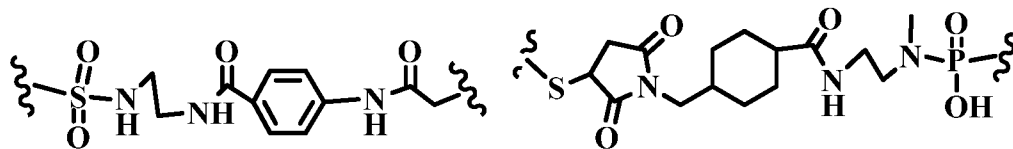


(ME, maleimidoethyl containing).

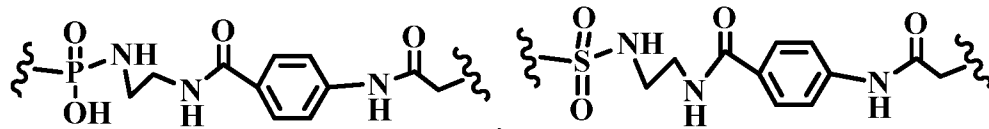


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(valine-citrulline containing)

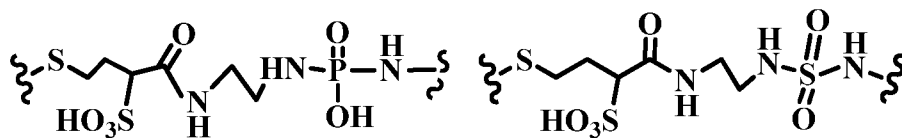


(MCC, 4-(N-maleimidomethyl)cyclohexane-1 carboxylate)



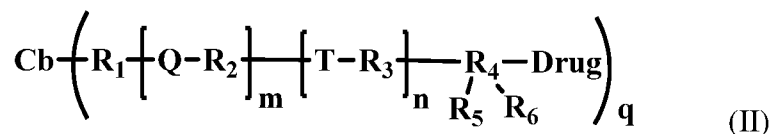
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((4-acetyl)aminobenzoate containing)



(4-thio-2-hydroxysulfonyl-butyrates, 2-sulfo-SPDB)

Preferably, the conjugates have the following Formula (II):



wherein:

Cb represents a cell-binding agent;

Drug represents the drug linked to the cell-binding agent via the hydrophilic linkers of this invention by an alkyl, alkylene, alkenylene, alkynylene, ether, polyoxyalkylene, ester, amine, imine, polyamine, hydrazine, hydrazone, amide, urea, semicarbazide, carbazide, alkoxyamine, urethanes, amino acid, peptide, acyloxylamine, hydroxamic acid, disulfide, thioether, thioester, carbamate, carbonate, heterocyclic ring, heteroalkyl, heteroaromatic, or alkoxime bond, or combination thereof.

q is 1 ~ 30; m, n, R₁, R₂, R₃, R₄, R₅, R₆ and M are described the same previously in Formula (I).

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Drug]-X₃-, or -X₁-P(=O)[X₂-R₁-Cb]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-; Wherein X₁, X₂ and X₃ are the same described in Formula (I).

In another embodiment, when the conjugate of the Formula (II) has two or more Cb groups, in particular the two same Cb groups, at such case, either Q, or/and T is -X₁-P(=O)[X₂-R₁-Cb]-X₃-, then the conjugate of the Formula (II) is linked to two or more sites, particularly a pair of sites of cell binding molecules.

In yet another embodiments, when the conjugate of the Formula (II) has two or more Drug groups, at such case, either Q, or/and T is -X₁-P(=O)[X₂-R₄-Drug]-X₃-, then the conjugate of the Formula (II) is linked to two or more drugs, in particular, two or more different drugs.

As described in more detail below, the drug can be any of many small molecule drugs, including, but not limited to, tubulysins, calicheamicins, auristatins, maytansinoids, CC-1065 analogs, morpholinos doxorubicins, taxanes, cryptophycins, epothilones, and benzodiazepine dimers (e.g., dimmers of pyrrolobenzodiazepine (PBD) or tomaymycin), indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines).

To synthesize the conjugate, the cell-binding agent can be first modified with the hydrophilic linkers of the present invention to introduce reactive groups of disulfide, maleimido, haloacetyl, azide, 1-yne, ketone, aldehyde, alkoxyamino, or hydrazide. Synthesis of the cell-binding agent-drug conjugates linked via disulfide bonds is achieved by a

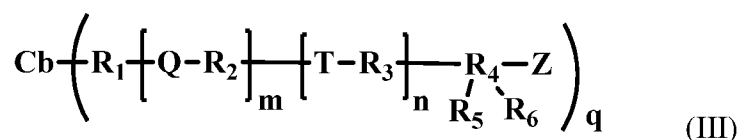
disulfide exchange between the disulfide bond in the modified cell-binding agent and a drug containing a free thiol group. Synthesis of the cell-binding agent-drug conjugates linked via thioether is achieved by reaction of the maleimido or haloacetyl or ethylsulfonyl modified cell-binding agent and a drug containing a free thiol group. Synthesis of conjugates bearing an acid labile hydrazone link can be achieved by reaction of a carbonyl group with the hydrazide moiety in the linker, by methods known in the art (see, for example, P. Hamann et al., Hinman, L. M., et al, *Cancer Res.* 53, 3336-334, 1993; B. Laguzza et al., *J. Med. Chem.*, 32; 548-555, 1959; P. Trail et al., *Cancer Res.*, 57; 100-105, 1997).

Alternatively, the drug can be modified with the hydrophilic linkers of the present invention to give a modified drug of Formula (IV) bearing functionality capable of reacting with a cell binding agent. For example a thiol-containing drug can be reacted with the hydrophilic linker of Formula (I) bearing a maleimido, or a haloacetyl, or an ethylsulfonyl substituent at neutral pH in aqueous buffer to give a drug connected to the hydrophilic linker via a thioether link. A thiol-containing drug can undergo disulfide exchange with a hydrophilic linker bearing a pyridyldithio moiety to give a modified drug attached via a disulfide bond to the hydrophilic cross linker. A drug bearing a hydroxyl group or a thiol group can be reacted with a hydrophilic linker bearing a halogen of this invention, in the presence of a mild base, to give a modified drug bearing an ether or thiol ether link. A hydroxyl group containing drug can be condensed with a hydrophilic cross linker of Formula (I) bearing a carboxyl group, in the presence of a dehydrating agent, such as EDC or DCC, to give an ester link. An amino group containing drug can similarly undergo condensation with a carboxyl group on the hydrophilic linker of Formula (I) to give an amide bond.

The conjugate may be purified by standard biochemical means, such as gel filtration on a Sephadex G25 or Sephacryl S300 column, adsorption chromatography, and ion exchange or by dialysis. In some cases, a small molecule as a cell-binding agent (e.g. folic acid, melanocyte stimulating hormone, EGF etc) conjugated with a small molecular drugs can be purified by chromatography such as by HPLC, medium pressure column chromatography or ion exchange chromatography.

MODIFIED CELL-BINDING AGENTS

The cell-binding agent modified by reaction with linkers of the present invention are preferably represented by the Formula (III)



Wherein Cb, q, m, n, R₁, R₂, R₃, R₄, R₅, R₆ and Z, as well as the substituents inside are described the same previously in Formula (I) and (II).

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Z]-X₃-, or -X₁-P(=O)[X₂-R₁-Cb]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-; Wherein X₁, X₂ and X₃ are the same described in Formula (I).

In another embodiment, when the compound of the Formula (III) has two or more Cb groups, in particular the two same Cb groups, at such case, either Q, or/and T is -X₁-P(=O)[X₂-R₁-Cb]-X₃-, then the compound of the Formula (III) is linked to two or more sites, particularly a pair of sites of cell binding molecules.

In yet another embodiment, when the compound of the Formula (III) has two or more Z groups, at such case, either Q, or/and T is -X₁-P(=O)[X₂-R₄-Z]-X₃-, then the compound of the Formula (III) can be used to link to two or more drugs, in particular, two different drugs.

In preferred embodiments, Z is a disulfide substituent, maleimido, haloacetyl, alkoxyamine, hydrazine group, or an *N*-hydroxysuccinimide ester, and Cb linked with R₁ is through thioether, hydrazone, amide, alkoxime, carbamate, or disulfide bond. The modified cell-binding agent can be prepared via a reaction of the cell-binding agent with the hydrophilic linkers by methods known in the art for other cross-linkers (U.S. Patent Nos. 5,846,545, 5,585,499, 5,475,092, 5,414,064, 5,208,020, and 4,563,304; Carlsson, J. et al. *Biochem. J.* (1978) 173, 723-737(1978); Goff, D. A., *Bioconj. Chem.* (1990), 1, 381-386; L. Delprino et al. *J. Pharm. Sci.* (1993), 82, 506-512; S. Arpicco et al., *Bioconjugate Chem*(1997), 8, 327-337).

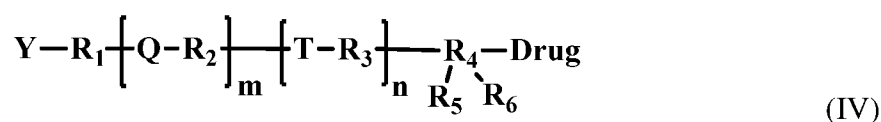
Advantageously, because the phosphamide, phosphinate, sulfonamide, sulfonyl, sulfonimide, and/or sulfoxide groups on the hydrophilic linkers are soluble in water or require only a small percentage of organic solvent to maintain solubility in aqueous solution, the reaction between the cell-binding agent and the cross-linker can be conducted in aqueous solution. The cross-linking reagent is dissolved in aqueous buffer, optionally containing a small amount (typically <10% by volume) of a polar organic solvent that is miscible with water, for example different alcohols, such as methanol, ethanol, and propanol, acetone, acetonitrile, tetrahydrofuran (THF), 1,4-dioxane, dimethyl formamide (DMF),

dimethyl acetamide (DMA), or dimethylsulfoxide (DMSO) at a high concentration, for example 1-100 mM, and then an appropriate aliquot is added to the buffered aqueous solution of the cell-binding agent. An appropriate aliquot is an amount of solution that introduces 1-10 cross-linking groups per cell-binding agent, preferably 1-6 groups, and the volume to be added should not exceed 10 %, preferably 5 %, and most preferably 0-3 % of the volume of the cell-binding agent solution. The aqueous solutions for the cell-binding agents are buffered between pH 6 and 9, preferably between 6.5 and 7.5 and can contain any non-nucleophilic buffer salts useful for these pH ranges. Typical buffers include phosphate, triethanolamine HCl, HEPES, and MOPS buffers, which can contain additional components, such as cyclodextrins, sucrose and salts, for examples, NaCl and KCl. After the addition the reaction is incubated at a temperature of from 4 °C to 45 °C, preferably at ambient temperature. The progress of the reaction can be monitored by measuring the increase in the absorption at 280, or 320 nm, or another appropriate wavelength. After the reaction is complete, isolation of the modified cell-binding agent can be performed in a routine way, using for example gel filtration chromatography, or adsorptive chromatography.

The extent of modification can be assessed by measuring the absorbance of the nitropyridine thione, dinitropyridine dithione, pyridine thione, carboxamidopyridine dithione and dicarboxamidopyridine dithione group released. The hydrophilic cross-linkers described herein have diverse functional groups that can react with any cell-binding agent that possesses a suitable substituent. For example cell-binding agents bearing an amino or hydroxyl substituent can react with cross linkers bearing an N-hydroxysuccinimide (NHS) ester, cell-binding agents bearing a thiol substituent can react with cross linkers bearing a maleimido or haloacetyl group. Additionally, cell-binding agents bearing a carbonyl substituent can react with the linkers bearing a hydrazide or an alkoxyamine. One skilled in the art can readily determine which linker to use based on the known reactivity of the available functional group on the cell-binding agent.

MODIFIED CYTOTOXIC DRUGS

The cytotoxic drugs modified by reaction with cross-linkers of the present invention are preferably represented by the Formula (IV):



wherein Y, m, n, R₁, R₂, R₃, R₄, R₅, R₆ and Drug, as well as the substituents inside the Formula (IV) are described the same previously in Formula (I) and (II).

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Drug]-X₃-, or -X₁-P(=O)[X₂-R₁-Y]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-; Wherein X₁, X₂ and X₃ are the same described in Formula (I).

In another embodiment, when the compound of the Formula (IV) has two or more Y groups, in particular the two same Y groups, at such case, either Q, or/and T is -X₁-P(=O)[X₂-R₁-Y]-X₃-, then compound of the Formula (IV) can be linked to two or more sites, particularly a pair of sites of cell binding molecules.

In yet another embodiment, when the compound of the Formula (IV) has two or more Drug groups, at such case, either Q, or/and T is -X₁-P(=O)[X₂-R₄-Drug]-X₃-, then the compound of the Formula (II) is linked to two or more drugs, in particular, two or more different drugs.

In preferred embodiments, Y is a disulfide substituent, a maleimido, a haloacetyl, an alkoxyamino group, carboxylic acid, or an *N*-hydroxysuccinimide ester.

The modified drugs can be prepared by reacting the drug with the linkers of the present invention to give a modified drug of Formula (IV) bearing functionality capable of reacting with a cell binding agent. For example a thiol-containing drug can be reacted with the linker of Formula (I) bearing a maleimido substituent at neutral pH in aqueous buffer to give a drug connected to the hydrophilic linker via thioether linkage. A thiol-containing drug can undergo disulfide exchange with a hydrophilic linker bearing a pyridyldithio moiety to give a modified drug attached via a disulfide bond to the hydrophilic linker. A drug bearing a hydroxyl group can be reacted with a linker bearing a halogen, in the presence of a mild base, to give a modified drug bearing ether linkage. A hydroxyl group containing drug can be condensed with a linker of Formula (I) bearing a carboxyl group, in the presence of a dehydrating agent, such as EDC or dicyclohexylcarbodiimide (DCC), to give ester linkage. A drug bearing a thiol group can be reacted with a linker bearing a maleimido or a vinylsulfonyl, or a haloacetyl group, to give a modified drug bearing thioether linkage. An amino group containing drug can similarly undergo condensation with a carboxyl group on the hydrophilic linker of Formula (I) to give an amide bond. The modified drug can be purified by standard methods such as column chromatography over silica gel or alumina, crystallization, preparatory thin layer chromatography, ion exchange chromatography, or HPLC.

CELL-BINDING AGENTS

The cell-binding molecule that comprises the conjugates and the modified cell-binding agents of the present invention 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 cell binding agents include, but are not limited to, large molecular weight proteins such as, for example, full-length antibodies (polyclonal antibodies, monoclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific 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, microbial antigens or a protein generated by the immune system that is capable of recognizing, binding to a specific antigen or exhibiting the desired biological activity (Miller et al (2003) J. of Immunology 170:4854-4861); interferons (such as type I, II, III); peptides; lymphokines such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, 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 TGF α , TGF β , 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); bioactive 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, 5 327, 71-93).

In general, a monoclonal antibody is preferred as a cell-surface binding agent if an appropriate one is available. And the antibody may be murine, human, humanized, chimeric, or derived from other species.

Production of antibodies used in the present invention involves *in vivo* or *in vitro* 10 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 15 Laboratory Manual", Harlow and Lane, eds., Cold Spring Harbor Laboratory Press, New York (1988), which is incorporated herein by reference. 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 20 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 25 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 30 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., *Virology* 8, 396 (1959)) supplemented with 4.5 gm/l glucose, 0~20 mM glutamine, 0~20% fetal calf serum, several ppm amount of heavy metals, such as Cu, Mn, Fe, or Zn, etc, or/and the heavy metals added in their salt forms, 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. Nos. 5,859,205 and 6,797,492; Liu et al, *Immunol Rev.* 222:9-27 (2008); Almagro et al, *Front Biosci.* 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) each incorporated herein by reference. 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.

5 Examples of such mice are: the Xenomouse. (Abgenix/Amgen), the HuMAb-Mouse (Medarex/BMS), the VelociMouse (Regeneron), see also U.S. Pat. No. 6,596,541 , 6,207,418, No. 6,150,584, No. 6,111,166, No. 6,075,181, No. 5,922,545, Nos. 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 consid-
10 erably less immunogenic in man than murine mAbs (Kipriyanov et al, Mol Biotechnol. 26:39-60 (2004); Houdebine, Curr Opin Biotechnol. 13:625-9 (2002) each incorporated herein by reference). 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
15 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
20 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
25 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
30 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, a dendrimer, as long as such attachment permits the peptide or protein to retain its antigen binding specificity.

Examples of antibodies used for conjugation of drugs via the hydrophilic linkers of this prevention for treating cancer, autoimmune disease, and/or 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), Anrukizumab (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), Atorolimomab (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, Ecomeximab (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- β 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), Nerelimumab (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), Pentumomab (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-
 5 IL-6), Siplizumab (anti-CD2), (Smart) MI95 (anti-CD33), Solanezumab (anti-beta amy-
 loid), Sonepcizumab (anti-sphingosine-1-phosphate), Sontuzumab (anti-episialin),
 Stamulumab (anti-myostatin), Sulesomab (LeukoScan, (anti-NCA-90 (granulocyte anti-
 gen), Tacatuzumab (anti-alpha-fetoprotein), Tadocizumab (anti-integrin $\alpha_{IIb}\beta_3$), Talizumab
 (anti-IgE), Tanezumab (anti-NGF), Taplitumomab (anti-CD19), Tefibazumab (Aurexis,
 10 (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),
 15 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 $\alpha_v\beta_3$), Volociximab (anti-integrin $\alpha_5\beta_1$), Votumumab
 20 (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-
 25 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),
 30 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) .

10 Other antibodies as cell binding molecules/ligands include, but are not limited to, are antibodies against the following antigens: Aminopeptidase N (CD13), Annexin A1, B7-H3 (CD276, various cancers), CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA242 (colorectal), 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 (Hodgkin's lymphoma), CD33 (leukemia, autoimmune diseases), CD38 (multiple myeloma), CD40 (lymphoma, multiple myeloma, leukemia (CLL)), CD51 (Metastatic melanoma, sarcoma), CD52 (leukemia), 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 (delta-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 (interleu-
 5 kin 2 receptor, T-cell leukemia and lymphomas), IL-6R (interleukin 6 receptor, multiple myeloma, RA, Castleman's disease, IL6 dependent tumors), Integrins ($\alpha\beta3$, $\alpha5\beta1$, $\alpha6\beta4$, $\alpha11\beta3$, $\alpha5\beta5$, $\alpha\nu\beta5$, 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-
 10 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,
 15 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,
 20 multiple myeloma, NHL, other cancers, RA and SLE), TPBG (trophoblast glycoprotein, Renal cell carcinoma), TRAIL-R1 (Tumor necrosis apoptosis 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);
 25 Novellino et al, Cancer Immunol Immunother. 54(3), 187-207 (2005). Franke, et al, Cancer Biother Radiopharm. 2000, 15, 459-76).

The cell binding agents, more preferred antibodies, can be any agents that are able to against tumor cells, virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells, activated cells, myeloid cells, activated T-cells, B cells, or melano-
 30 cytes. More specifically the cell binding agents can be any agent/molecule that is able to against any one of the following antigens or receptors: CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD12w, CD14, CD15, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30,

CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42,
 CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD51, CD52, CD53, CD54,
 CD55, CD56, CD58, CD59, CD61, CD62E, CD62L, CD62P, CD63, CD66, CD68, CD69,
 CD70, CD72, CD74, CD79, CD79a, CD79b, CD80, CD81, CD82, CD83, CD86, CD87,
 5 CD88, CD89, CD90, CD91, CD95, CD96, CD98, CD100, CD103, CD105, CD106,
 CD109, CD117, CD120, CD125, CD126, CD127, CD133, CD134, CD135, CD138,
 CD141, CD142, CD143, CD144, CD147, CD151, CD147, CD152, CD154, CD156,
 CD158, CD163, CD166, .CD168, CD174, CD180, CD184, CDw186, CD194, CD195,
 CD200, CD200a, CD200b, CD209, CD221, CD227, CD235a, CD240, CD262, CD271,
 10 CD274, CD276 (B7-H3), CD303, CD304, CD309, CD326, 4-1BB, 5AC, 5T4 (Trophoblast
 glycoprotein, TPBG, 5T4, Wnt-Activated Inhibitory Factor 1 or WAIF1), Adenocarcinoma
 antigen, AGS-5, AGS-22M6, Activin receptor-like kinase 1, AFP, AKAP-4, ALK, Alpha
 intergrin, Alpha v beta6, Amino-peptidase N, Amyloid beta, Androgen receptor,
 Angiopoietin 2, Angiopoietin 3, Annexin A1, Anthrax toxin protective antigen, Anti-
 15 transferrin receptor, AOC3 (VAP-1), B7-H3, Bacillus anthracis anthrax, BAFF (B-cell
 activating factor), B-lymphoma cell, bcr-abl, Bombesin, BORIS, C5, C242 antigen, CA125
 (carbohydrate antigen 125, MUC16), CA-IX (or CAIX, carbonic anhydrase 9), CALLA,
 CanAg, Canis lupus familiaris IL31, Carbonic anhydrase IX, Cardiac myosin, CCL11(C-C
 motif chemokine 11), CCR4 (C-C chemokine receptor type 4, CD194), CCR5, CD3E
 20 (epsilon), CEA (Carcinoembryonic antigen), CEACAM3, CEACAM5 (carcinoembryonic
 antigen), CFD (Factor D), Ch4D5, Cholecystokinin 2 (CCK2R), CLDN18 (Claudin-18),
 Clumping factor A, CRIPTO, FCSF1R (Colony stimulating factor 1 receptor, CD115),
 CSF2 (colony stimulating factor 2, Granulocyte-macrophage colony-stimulating factor
 (GM-CSF)), CTLA4 (cytotoxic T-lymphocyte-associated protein 4), CTAA16.88 tumor
 25 antigen, CXCR4 (CD184), C-X-C chemokine receptor type 4, cyclic ADP ribose hydrolase,
 Cyclin B1, CYP1B1, Cytomegalovirus, Cytomegalovirus glycoprotein B, Dabigatran,
 DLL4 (delta-like-ligand 4), DPP4 (Dipeptidyl-peptidase 4), DR5 (Death receptor 5), E.
 coli shiga toxin type-1, E. coli shiga toxin type-2, ED-B, EGFL7 (EGF-like domain-
 containing protein 7), EGFR, EGFRII, EGFRvIII, Endoglin (CD105), Endothelin B
 30 receptor, Endotoxin, EpCAM (epithelial cell adhesion molecule), EphA2, Episialin,
 ERBB2 (Epidermal Growth Factor Receptor 2), ERBB3, ERG (TMPRSS2 ETS fusion
 gene), Escherichia coli, ETV6-AML, FAP (Fibroblast activation protein alpha), FCGR1,
 alpha-Fetoprotein, Fibrin II, beta chain, Fibronectin extra domain-B, FOLR (folate recep-

tor), Folate receptor alpha, Folate hydrolase, Fos-related antigen 1.F protein of respiratory syncytial virus, Frizzled receptor, Fucosyl GM1, GD2 ganglioside, G-28 (a cell surface antigen glyvolipid), GD3 idio type, GloboH, Glypican 3, N-glycolylneuraminic acid, GM3, GMCSF receptor α -chain, Growth differentiation factor 8, GP100, GPNMB

5 (Transmembrane glycoprotein NMB), GUCY2C (Guanylate cyclase 2C, guanylyl cyclase C(GC-C), intestinal Guanylate cyclase, Guanylate cyclase-C receptor, Heat-stable enterotoxin receptor (hSTAR)), Heat shock proteins, Hemagglutinin, Hepatitis B surface antigen, Hepatitis B virus, HER1 (human epidermal growth factor receptor 1), HER2, HER2/neu, HER3 (ERBB-3), IgG4, HGF/SF (Hepatocyte growth factor/scatter factor), HHGFR, HIV-

10 1, Histone complex, HLA-DR (human leukocyte antigen), HLA-DR10, HLA-DRB , HMWMAA, Human chorionic gonadotropin, HNGF, Human scatter factor receptor kinase, HPV E6/E7, Hsp90, hTERT, ICAM-1 (Intercellular Adhesion Molecule 1), Idiotype, IGF1R (IGF-1, insulin-like growth factor 1 receptor), IGHE, IFN- γ , Influenza hemagglutinin, IgE, IgE Fc region, IGHE, IL-1, IL-2 receptor (interleukin 2 receptor),

15 IL-4, IL-5, IL-6, IL-6R (interleukin 6 receptor), IL-9, IL-10, IL-12, IL-13, IL-17, IL-17A, IL-20, IL-22, IL-23, IL31RA, ILGF2 (Insulin-like growth factor 2), Integrins (α 4, α IIb β 3, α v β 3, α 4 β 7, α 5 β 1, α 6 β 4, α 7 β 7, α II β 3, α 5 β 5, α v β 5), Interferon gamma-induced protein, ITGA2, ITGB2, KIR2D, LCK, Le, Legumain, Lewis-Y antigen, LFA-1(Lymphocyte function-associated antigen 1, CD11a), LHRH, LINGO-1, Lipoteichoic acid, LIV1A,

20 LMP2, LTA, MAD-CT-1, MAD-CT-2, MAGE-1, MAGE-2, MAGE-3, MAGE A1, MAGE A3, MAGE 4, MART1, MCP-1, MIF (Macrophage migration inhibitory factor, or glycosylation-inhibiting factor (GIF)), MS4A1 (membrane-spanning 4-domains subfamily A member 1), MSLN (mesothelin), MUC1(Mucin 1, cell surface associated (MUC1) or polymorphic epithelial mucin (PEM)), MUC1-KLH, MUC16 (CA125), MCP1(monocyte chemotactic protein 1), MelanA/MART1, ML-IAP, MPG, MS4A1 (membrane-spanning 4-

25 domains subfamily A), MYCN, Myelin-associated glycoprotein, Myostatin, NA17, NARP-1, NCA-90 (granulocyte antigen), Nectin-4 (ASG-22ME), NGF, Neural apoptosis-regulated proteinase 1, NOGO-A, Notch receptor, Nucleolin, Neu oncogene product, NY-BR-1, NY-ESO-1, OX-40, OxLDL (Oxidized low-density lipoprotein), OY-TES1, P21,

30 p53 nonmutant, P97, Page4, PAP, Paratope of anti-(N-glycolylneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1,CD279), PDGF-R α (Alpha-type platelet-derived growth factor receptor), PDGFR- β , PDL-1, PLAC1, PLAP-like testicular alkaline phosphatase, Platelet-derived growth factor receptor beta, Phos-

phate-sodium co-transporter, PMEL 17, Polysialic acid, Proteinase3 (PR1), Prostatic carcinoma, PS (Phosphatidylserine), Prostatic carcinoma cells, Pseudomonas aeruginosa, PSMA, PSA, PSCA, Rabies virus glycoprotein, RHD (Rh polypeptide 1 (RhPI), CD240), Rhesus factor, RANKL, RhoC, Ras mutant, RGS5, ROBO4, Respiratory syncytial virus, RON, Sarcoma translocation breakpoints, SART3, Sclerostin, SLAMF7 (SLAM family member 7), Selectin P, SDC1 (Syndecan 1), sLe(a), Somatomedin C, SIP (Sphingosine-1-phosphate), Somatostatin, Sperm protein 17, SSX2, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), STEAP2, STn, TAG-72 (tumor associated glycoprotein 72), Survivin, T-cell receptor, T cell transmembrane protein, TEM1 (Tumor endothelial marker 1), TENB2, Tenascin C (TN-C), TGF- α , TGF- β (Transforming growth factor beta), TGF- β 1, TGF- β 2 (Transforming growth factor-beta 2), Tie (CD202b), Tie2, TIM-1 (CDX-014), Tn, TNF, TNF- α , TNFRSF8, TNFRSF10B (tumor necrosis factor receptor superfamily member 10B), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B), TPBG (trophoblast glycoprotein), TRAIL-R1 (Tumor necrosis apoptosis Inducing ligand Receptor 1), TRAILR2 (Death receptor 5 (DR5)), tumor-associated calcium signal transducer 2, tumor specific glycosylation of MUC1, TWEAK receptor, TYRP1(glycoprotein 75), TRP-2, Tyrosinase, VCAM-1 (CD106), VEGF, VEGF-A, VEGF-2 (CD309), VEGFR-1, VEGFR2, or vimentin, WT1, XAGE 1, or cells expressing any insulin growth factor receptors, or any epidermal growth factor receptors.

In another specific embodiment, the cell-binding ligand -drug conjugates via the hydrophilic linkers of this invention are used for the targeted treatment of cancers. The targeted 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, Metastatic 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 cell-binding-drug conjugates via the hydrophilic linkers of this invention are used in accordance with the compositions and methods 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, Behets 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 osteomyelitis, 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
5 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
10 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
15 disease), Inclusion body myositis, Inflammatory demyelinating polyneuropathy, 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,
20 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, Ocular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord thyroiditis, Palindromic rheumatism,
25 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,
30 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, Schizophre-

nia, 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),
 5 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 via the hydrophilic linkers of this invention for the treatment or prevention of an autoimmune disease can be, 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
 15 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)
 20 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 both a receptor or a receptor complex expressed on an activated lymphocyte which is associated with an autoimmune disease. The receptor or
 25 receptor complex can comprise an immunoglobulin gene superfamily member (e.g. CD2, CD3, CD4, CD8, CD19, CD20, CD22, CD28, CD30, CD33, CD37, CD38, CD56, CD70, CD79, CD79b, CD90, CD125, 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-
 30 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 cell 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 neuramimidase, 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 bacterial, fungi, pathogenic protozoa, or yeast polypeptides including, e.g., LPS and capsular polysaccharide 5/8) that is capable of eliciting an immune response. Examples of antibodies available 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 cell binding molecules–drug conjugates via the hydrophilic linkers 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, Arcanobacterium 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, Burkholderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), Campylobacteriosis, Candidiasis (Moniliasis; Thrush), Cat-scratch disease, Cellulitis, Chagas Disease (American trypanosomiasis), Chancroid, Chickenpox, Chlamydia, Chlamydomydia 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, Diphylobothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), Enterococcus infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum (Fifth disease), Exanthem subitum, Fasciolopsiasis, Fasciolosis,

5 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

10 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,

15 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,

20 Microsporidiosis, *Molluscum contagiosum*, Mumps, Murine typhus (Endemic typhus), *Mycoplasma pneumoniae*, Mycetoma, Myiasis, Neonatal conjunctivitis (*Ophthalmia neonatorum*), (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), Nocardiosis,

25 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 pneumoniae*,

30 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),
 5 Tinea cruris (Jock itch), Tinea manuum (Ringworm of the Hand), Tinea nigra, Tinea pedis (Athlete's foot), Tinea unguium (Onychomycosis), 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
 10 hemorrhagic fever, Viral pneumonia, West Nile Fever, White piedra (Tinea blanca), Yersinia pseudotuberculosis infection, Yersiniosis, Yellow fever, Zygomycosis.

The cell binding molecule, which is more preferred to be an antibody described in this patent that are against pathogenic strains include, but are not limit, Acinetobacter baumannii, Actinomyces israelii, Actinomyces gerencseriae and Propionibacterium
 15 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,
 20 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,
 25 Chlamydia 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
 30 parasites, Cyclospora cayentanensis, Taenia solium, Cytomegalovirus, Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4) – Flaviviruses, Dientamoeba fragilis, Corynebacterium diphtheriae, Diphyllbothrium, 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,

5 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,

10 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

15 ozaenas, Klebsiella rhinoscleromatis, 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

20 pseudomallei, Neisseria meningitidis, 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,

25 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

30 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, Staph-

ylococcus 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*,
 5 *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 encephali-
 titis virus, *Vibrio colerae*, Guanarito virus, West Nile virus, *Trichosporon beigelii*, *Yersinia*
pseudotuberculosis, *Yersinia enterocolitica*, Yellow fever virus, *Mucorales* order
 10 (*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 pertenuis*, *Treponema caratenum*, *Borrelia vincentii*, *Borrelia*
burgdorferi, *Leptospira icterohemorrhagiae*, *Pneumocystis carinii*, *Brucella abortus*,
 15 *Brucella suis*, *Brucella melitensis*, *Mycoplasma* spp., *Rickettsia prowazekii*, *Rickettsia*
tsutsugumushi, *Chlamydia* spp.; pathogenic fungi (*Aspergillus fumigatus*, *Candida albicans*,
Histoplasma capsulatum); protozoa (*Entamoeba histolytica*, *Trichomonas tenax*,
Trichomonas hominis, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Leishmania*
donovani, *Leishmania tropica*, *Leishmania braziliensis*, *Pneumocystis pneumonia*, *Plasmo-*
dium vivax, *Plasmodium falciparum*, *Plasmodium malariae*); or Helminths (*Schistosoma*
japonicum, *Schistosoma mansoni*, *Schistosoma haematobium*, and hookworms).

Other antibodies as cell binding ligands used 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: *Poxviridae*, *Herpesviridae*, *Adenoviridae*,
 25 *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*
 30 (*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 via the hydrophilic linkers of the invention together with a pharmaceutically acceptable carrier, diluent, or excipient for treatment of cancers, infections or autoimmune disorders. The method for treatment of cancers, infections 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
5 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 via the linkers of the invention will be supplied as solutions or as a lyophilized solid that can be redissolved in sterile water for injection. Examples of suitable protocols of conjugate administration are as follows. Conjugates are
10 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 μ g to 20 mg/kg of body weight per week, i.v. (range of 10 μ g to 200 mg/kg per injection). 8 weeks after treatment, the patient may receive a second course of treatment. Specific clinical
15 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).

20 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.

25 In general terms, the conjugates via the linkers 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 μ g/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
30 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 via the linkers 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/weekly/biweekly/monthly 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, or per week, per two week or per month. 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 intranasal, 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.

DRUGS/CYTOTOXIC AGENTS

Drugs that can be conjugated to a cell-binding molecule in the present invention are small molecule drugs including cytotoxic agents, which can be linked to or after they are modified for linkage to the cell-binding agent. A "small molecule drug" is broadly used herein to refer to an organic, inorganic, or organometallic compound that may have a molecular weight of for example 100 to 1800, more suitably from 120 to 1400. Small molecule drugs are well characterized in the art, such as in WO05058367A2, and in U.S. Patent No. 4,956,303, among others and are incorporated in their entirety by reference. The drugs include known drugs and those that may become known drugs.

Drugs that are known include, but not limited to,

1). Chemotherapeutic agents: a). Alkylating agents: such as Nitrogen mustards: chlorambucil, chlornaphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, mannomustine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiotepa, trofosfamide, uracil mustard; CC-1065 (including its adozelesin, carzelesin and bizelesin

synthetic analogues); Duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); Benzodiazepine dimers (e.g., dimmers of pyrrolbenzodiazepine (PBD) or tomaymycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines); Nitrosoureas: (carmustine, lomustine, chlorozotocin, fotemustine, 5 nimustine, ranimustine); Alkylsulphonates: (busulfan, treosulfan, improsulfan and pipsulfan); Triazines: (dacarbazine); Platinum containing compounds: (carboplatin, cisplatin, oxaliplatin); aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate and 10 trimethylolomelamine]; b). Plant Alkaloids: such as Vinca alkaloids: (vincristine, vinblastine, vindesine, vinorelbine, navelbin); Taxoids: (paclitaxel, docetaxol) and their analogs, Maytansinoids (DM1, DM2, DM3, DM4, maytansine and ansamitocins) and their analogs, cryptophycins (particularly cryptophycin 1 and cryptophycin 8); epothilones, eleutherobin, discodermolide, bryostatins, dolostatins, auristatins, tubulysins, cephalostatins; 15 pancratistatin; a sarcodictyin; spongistatin; c). DNA Topoisomerase Inhibitors: such as [Epipodophyllins: (9-aminocamptothecin, camptothecin, crisnatol, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantrone, retinoic acids (retinols), teniposide, topotecan, 9-nitrocamptothecin (RFS 2000)); mitomycins: (mitomycin C)]; d). Anti-metabolites: such as {[Anti-folate: DHFR inhibitors: (methotrexate, trimetrexate, 20 denopterin, pteropterin, aminopterin (4-aminopteroic acid) or the other folic acid analogues); IMP dehydrogenase Inhibitors: (mycophenolic acid, tiazofurin, ribavirin, EICAR); Ribonucleotide reductase Inhibitors: (hydroxyurea, deferoxamine)]; [Pyrimidine analogs: Uracil analogs: (ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-Fluorouracil, floxuridine, 25 ratitrexed (Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine)]; folic acid replenisher, such as frolinic acid}; e). Hormonal therapies: such as {Receptor antagonists: [Anti-estrogen: (megestrol, raloxifene, tamoxifen); LHRH agonists: (goserelin, leuprolide acetate); Anti-androgens: (bicalutamide, flutamide, calusterone, dromostanolone propionate, epitiostanol, goserelin, leuprolide, mepitiostane, nilutamide, testolactone, 30 trilostane and other androgens inhibitors)]; 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),
5 bafetinib (INNO-406), bosutinib (SKI-606), cabozantinib, vismodegib, iniparib, ruxolitinib, CYT387, axitinib, tivozanib, sorafenib, bevacizumab, cetuximab, Trastuzumab, Ranibizumab, Panitumumab, ispinesib; g). antibiotics, such as the enediyne antibiotics (e.g. calicheamicins, especially calicheamicin γ 1, δ 1, α 1 and β 1, see, e.g., J. Med. Chem., 39 (11), 2103–2117 (1996), Angew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin,
10 including dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin; chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine,
15 doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; f). Others: such as Polyketides (acetogenins), especially bullatacin
20 and bullatacinone; 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),
25 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,
30 Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A) ; Thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, Disulfiram, Salinosporamide A.; Anti-adrenals, such as aminoglutethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestrabucil; bisantrene; edatraxate;

defofamine; demecolcine; diaziquone; eflornithine (DFMO), elfomithine; elliptinium acetate, etoglucid; gallium nitrate; gacytosine, hydroxyurea; ibandronate, lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®]; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziuone; 2, 2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verrucarin A, roridin A and anguidine); urethane, siRNA, antisense drugs, and a nucleolytic enzyme.

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, ceftazidime, cefepime, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, cefteteram, ceftibuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cefuzonam, *cephamycin* (cefoxitin, cefotetan, cefmetazole), oxacephem (flomoxef, latamoxef); f). Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin; 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, 5 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 phenoxymethyl-penicillin, clometocillin, procaine 10 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, 15 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, 20 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, glycylicyclines (e.g. tigeicycline); u). Other 25 types of antibiotics: annonacin, arspenamine, 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. fosfomicin), nitrofurantoin, paclitaxel, platensimycin, pyrazinamide, 30 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). Neuramini-

dase inhibitors: oseltamivir, zanamivir, peramivir; e). Nucleosides & nucleotides: abacavir, aciclovir, adefovir, amdoxovir, apricitabine, brivudine, cidofovir, clevidine, 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) The drugs used for conjugates via a hydrophilic linker of the present invention also include radioisotopes. Examples of radioisotopes (radionuclides) are ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{64}Cu , ^{68}Ga , ^{86}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{133}Xe , ^{177}Lu , ^{211}At , or ^{213}Bi . Radioisotope labeled antibodies are useful in receptor targeted imaging experiments or can be for targeted treatment such as with the antibody-drug conjugates of the invention (Wu et al (2005) Nature Biotechnology 23(9): 1137-1146). The cell binding molecules, e.g. an antibody can be labeled with ligand reagents through the hydrophilic linkers of the present patent that bind, chelate or otherwise complex a radioisotope metal, using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al, Ed. Wiley-Interscience, New York, N.Y., Pubs. (1991). Chelating ligands which may complex a metal ion include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocyclics, Dallas, Tex.).

6). The pharmaceutically acceptable salts, acids or derivatives of any of the above drugs.

In another embodiment, the drug in the Formula (II) and (IV) can be a chromophore molecule, for which the conjugate can be used for detection, monitoring, or study the interaction of the cell binding molecule with a target cell. Chromophore molecules are a compound that have the ability to absorb a kind of light, such as UV light, fluorescent light, IR light, near IR light, visual light; A chromophore molecule includes a class or subclass of xanthophores, erythrophores, iridophores, leucophores, melanophores, and cyanophores; a class or subclass of fluorophore molecules which are fluorescent chemical compounds re-emitting light upon light; a class or subclass of visual phototransduction molecules; a class or subclass of photophore molecules; a class or subclass of luminescence molecules; and a class or subclass of luciferin compounds.

The chromophore molecule can be selected from, but not limited, Non-protein organic fluorophores, such as: Xanthene derivatives (fluorescein, rhodamine, Oregon green, eosin, and Texas red); Cyanine derivatives: (cyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine, and merocyanine); Squaraine derivatives and ring-substituted squaraines, including Seta, SeTau, and Square dyes; Naphthalene derivatives (dansyl and prodan derivatives); Coumarin derivatives; Oxadiazole derivatives (pyridyloxazole, nitrobenzoxadiazole and benzoxadiazole); Anthracene derivatives (anthraquinones, including DRAQ5, DRAQ7 and CyTRAK Orange); Pyrene derivatives (cascade blue, etc); Oxazine derivatives (Nile red, Nile blue, cresyl violet, oxazine 170 etc). Acridine derivatives (proflavin, acridine orange, acridine yellow etc). Arylmethine derivatives (auramine, crystal violet, malachite green). Tetrapyrrole derivatives (porphin, phthalocyanine, bilirubin).

Or a chromophore molecule can be selected from any analogs and derivatives of the following fluorophore compounds: CF dye (Biotium), DRAQ and CyTRAK probes (BioStatus), BODIPY (Invitrogen), Alexa Fluor (Invitrogen), DyLight Fluor (Thermo Scientific, Pierce), Atto and Tracy (Sigma Aldrich), FluoProbes (Interchim), Abberior Dyes (Abberior), DY and MegaStokes Dyes (Dyomics), Sulfo Cy dyes (Cyandye), HiLyte Fluor (AnaSpec), Seta, SeTau and Square Dyes (SETA BioMedicals), Quasar and Cal Fluor dyes (Biosearch Technologies), SureLight Dyes (APC, RPEPerCP, Phycobilisomes)(Columbia Biosciences), APC, APCXL, RPE, BPE (Phyco-Biotech);

Examples of the widely used fluorophore compounds which are reactive or conjugatable with the linkers of the invention are: Allophycocyanin (APC), Aminocoumarin, APC-Cy7 conjugates, BODIPY-FL, Cascade Blue, Cy2, Cy3, Cy3.5,

Cy3B, Cy5, Cy5.5, Cy7, Fluorescein, FluorX, Hydroxycoumarin, Lissamine Rhodamine B, Lucifer yellow, Methoxycoumarin, NBD, Pacific Blue, Pacific Orange, PE-Cy5 conjugates, PE-Cy7 conjugates, PerCP, R-Phycoerythrin(PE), Red 613, Seta-555-Azide, Seta-555-DBCO, Seta-555-NHS, Seta-580-NHS, Seta-680-NHS, Seta-780-NHS, Seta-APC-780, Seta-PerCP-680, Seta-R-PE-670, SeTau-380-NHS, SeTau-405-Maleimide, SeTau-405-NHS, SeTau-425-NHS, SeTau-647-NHS, Texas Red, TRITC, TruRed, X-Rhodamine.

The fluorophore compounds that can be linked to the linkers of the invention for study of nucleic acids or proteins are selected from the following compounds or their derivatives: 7-AAD (7-aminoactinomycin D, CG-selective), Acridine Orange, Chromomycin A3, CyTRAK Orange (Biostatus, red excitation dark), DAPI, DRAQ5, DRAQ7, Ethidium Bromide, Hoechst33258, Hoechst33342, LDS 751, Mithramycin, PropidiumIodide (PI), SYTOX Blue, SYTOX Green, SYTOX Orange, Thiazole Orange, TO-PRO: Cyanine Monomer, TOTO-1, TO-PRO-1, TOTO-3, TO-PRO-3, YOSeta-1, YOYO-1. The fluorophore compounds that can be linked to the linkers of the invention for study cells are selected from the following compounds or their derivatives: DCFH (2',7'-Dichlorodihydro-fluorescein, oxidized form), DHR (Dihydrorhodamine 123, oxidized form, light catalyzes oxidation), Fluo-3 (AM ester, pH > 6), Fluo-4 (AM ester, pH 7.2), Indo-1 (AM ester, low/high calcium (Ca²⁺)), SNARF(pH 6/9). The preferred fluorophore compounds that can be linked to the linkers of the invention for study proteins/antibodies are selected from the following compounds or their derivatives: Allophycocyanin(APC), AmCyan1 (tetramer, Clontech), AsRed2 (tetramer, Clontech), Azami Green (monomer, MBL), Azurite, B-phycoerythrin(BPE), Cerulean, CyPet, DsRed monomer (Clontech), DsRed2 ("RFP", Clontech), EBFP, EBFP2, ECFP, EGFP (weak dimer, Clontech), Emerald (weak dimer, Invitrogen), EYFP (weak dimer, Clontech), GFP (S65A mutation), GFP (S65C mutation), GFP (S65L mutation), GFP (S65T mutation), GFP (Y66F mutation), GFP (Y66H mutation), GFP (Y66W mutation), GFPuv, HcRed1, J-Red, Katusha, Kusabira Orange (monomer, MBL), mCFP, mCherry, mCitrine, Midoriishi Cyan (dimer, MBL), mKate (TagFP635, monomer, Evrogen), mKeima-Red (monomer, MBL), mKO, mOrange, mPlum, mRaspberry, mRFP1 (monomer, Tsien lab), mStrawberry, mTFP1, mTurquoise2, P3 (phycobilisome complex), Peridinin Chlorophyll (PerCP), R-phycoerythrin(RPE), T-Sapphire, TagCFP (dimer, Evrogen), TagGFP (dimer, Evrogen), TagRFP (dimer, Evrogen), TagYFP (dimer, Evrogen), tdTomato (tandem dimer), Topaz, TurboFP602 (dimer, Evrogen), TurboFP635 (dimer, Evrogen), TurboGFP (dimer, Evrogen), TurboRFP (dimer,

Evrogen), TurboYFP (dimer, Evrogen), Venus, Wild Type GFP, YPet, ZsGreen1 (tetramer, Clontech), ZsYellow1 (tetramer, Clontech).

In yet another embodiment, the preferred cytotoxic agents that conjugated to a cell-binding molecule via a hydrophilic linker of this patent are tubulysins, maytansinoids, taxanoids (taxanes), CC-1065 analogs, daunorubicin and doxorubicin compounds, benzodiazepine dimers (e.g., dimers of pyrrolbenzodiazepine (PBD), tomaymycin, anthramycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidino-benzodiazepines), calicheamicins and the enediyne antibiotics, actinomycin, azaserines, bleomycins, epirubicin, tamoxifen, idarubicin, dolastatins, auristatins (e.g. monomethyl auristatin E, MMAE, MMAF, auristatin PYE, auristatin TP, Auristatins 2-AQ, 6-AQ, EB (AEB), and EFP (AEFP)), duocarmycins, thiotepa, vincristines, hemiasterlins, nazumamides, microginins, radiosumins, alterobactins, microsclerodermins, theonellamides, esperamicins, PNU-159682, and their analogues and derivatives above thereof.

Tubulysins that are preferred for conjugation in the present invention are well known in the art and can be isolated from natural sources according to known methods or prepared synthetically according to known methods (e. g. Balasubramanian, R.; et al. *J. Med. Chem.*, 2009, 52, 238–240. Wipf, P.; et al. *Org. Lett.*, 2004, 6, 4057–4060. Pando, O.; et al. *J. Am. Chem. Soc.*, 2011, 133, 7692–7695. Reddy, J. A.; et al. *Mol. Pharmaceutics*, 2009, 6, 1518–1525. Raghavan, B.; et al. *J. Med. Chem.*, 2008, 51, 1530–1533. Patterson, A. W.; et al. *J. Org. Chem.*, 2008, 73, 4362–4369. Pando, O.; et al. *Org. Lett.*, 2009, 11 (24), pp 5567–5569. Wipf, P.; et al. *Org. Lett.*, 2007, 9 (8), 1605–1607. Friestad, G. K.; *Org. Lett.*, 2004, 6, pp 3249–3252. Hillary M. Peltier, H. M.; et al. *J. Am. Chem. Soc.*, 2006, 128, 16018–16019. Chandrasekhar, S.; et al. *J. Org. Chem.*, 2009, 74, 9531–9534. Liu, Y.; et al. *Mol. Pharmaceutics*, 2012, 9, 168–175. Friestad, G. K.; et al. *Org. Lett.*, 2009, 11, 1095–1098. Kubicek, K.; et al., *Angew Chem Int Ed Engl*, 2010, 49: p. 4809-12. Chai, Y.; et al., *Chem Biol*, 2010, 17: 296-309. Ullrich, A.; et al., *Angew Chem Int Ed Engl*, 2009, 48, 4422-5. Sani, M.; et al. *Angew Chem Int Ed Engl*, 2007, 46, 3526-9. Domling, A.; et al., *Angew Chem Int Ed Engl*, 2006, 45, 7235-9. Patent applications: Zanda, M. ; et al, *Can. Pat. Appl. CA 2710693* (2011). Chai, Y.; et al. *Eur. Pat. Appl. 2174947* (2010), *PCT WO 2010034724*. Leamon, C.; et al, *PCT WO 2010033733*, *WO 2009002993*. Ellman, J.; et al, *PCT WO 2009134279*; *PCT WO 2009012958*, *US appl. 20110263650*, *20110021568*, Matschiner, G.; et al, *PCT WO 2009095447*. Vlahov, I.; et al, *PCT WO 2009055562*, *WO 2008112873*. Low, P.; et al, *PCT WO 2009026177*. Richter, W., *PCT WO 2008138561*.

Kjems, J.; et al, PCT WO 2008125116. Davis, M.; et al, PCT WO 2008076333. Diener, J.; et al, U.S. Pat. Appl. 20070041901, WO 2006096754. Matschiner, G.; et al, PCT WO 2006056464. Vaghefi, F.; et al, 5 PCT WO 2006033913. Doemling, A., Ger. Offen. DE 102004030227; PCT WO 2004005327; WO 2004005326; WO2004005269. Stanton, M.; et
5 al, U.S. Pat. Appl. Publ. 20040249130. Hoefle, G.; et al, Ger. Offen. DE 10254439 ; DE 10241152; DE 10008089. Leung, D.; et al, WO 2002077036. Reichenbach, H.; et al, Ger. Offen. DE 19638870; Wolfgang, R.; US 20120129779, Chen, H.,US appl. 20110027274. The preferred structure of tubulysins for conjugation of cell binding molecules are described in the patent application of PCT/IB2012/053554

10 Calicheamicins and their related enediyne antibiotics that are preferred for cell-binding molecule-drug conjugates of this patent are described in: Nicolaou, K. C. et al, Science 1992, 256, 1172-1178; Proc. Natl. Acad. Sci USA. 1993, 90, 5881-5888), U.S. Patent Nos. 4,970,198; 5,053,394; 5,108,912; 5,264,586; 5,384,412; 5,606,040; 5,712,374; 5,714,586; 5,739,116; 5,770,701; 5,770,710; 5,773,001; 5,877,296; 6,015,562; 6,124,310;
15 8,153,768.

Maytansinoids that are preferred to be used in the present invention including maytansinol and maytansinol analogues are described in U.S. Patent Nos. 4,256,746, 4,361,650, 4,307,016, 4,294,757, 4,294,757, 4,371,533, 4,424,219, 4,331,598, 4,450,254, 4,364,866, 4,313,946, 4,315,929 4,362,663, 4,322,348, 4,371,533, 4,424,219, 5,208,020,
20 5,416,064, 5,208,020; 5,416,064; 6,333,410; 6,441,163; 6,716,821, 7,276,497, 7,301,019, 7,303,749, 7,368,565, 7,411,063, 7,851,432, and 8,163,888 .

Taxanes, which includes Paclitaxel (Taxol), a cytotoxic natural product, and docetaxel (Taxotere), a semi-synthetic derivative, and their analogs which are preferred for conjugation via the hydrophilic linkers of the present patent are exemplified in: K C.
25 Nicolaou et al., J. Am. Chem. Soc. 117, 2409-2420, (1995); Ojima et al, J. Med. Chem. 39:3889-3896 (1996); 40:267-278 (1997); 45, 5620-5623 (2002); Ojima et al., Proc. Natl. Acad. Sci., 96:4256-4261 (1999); Kim et al., Bull. Korean Chem. Soc., 20, 1389-1390 (1999); Miller, et al. J. Med. Chem., 47, 4802-4805(2004); U.S. Patent No. 5,475,011 5,728,849, 5,811,452; 6,340,701; 6,372,738; 6,391,913, 6,436,931; 6,589,979; 6,596,757;
30 6,706,708; 7,008,942; 7,186,851; 7,217,819; 7,276,499; 7,598,290; and 7,667,054.

CC-1065 analogues and doucarmycin analogs are also preferred to be used for a conjugate with the hydrophilic linkers of the present patent. The examples of the CC-1065 analogues and doucarmycin analogs as well as their synthesis are described in:

e.g. Warpehoski et al, J. Med. Chem. 31:590-603 (1988), D. Boger et al., J. Org. Chem; 66; 6654-6661, 2001; U. S. Patent Nos: 4169888, 4391904, 4671958, 4816567, 4912227, 4923990, 4952394, 4975278, 4978757, 4994578, 5037993, 5070092, 5084468, 5101038, 5117006, 5137877, 5138059, 5147786, 5187186, 5223409, 5225539, 5288514, 5324483, 5332740, 5332837, 5334528, 5403484, 5427908, 5475092, 5495009, 5530101, 5545806, 5547667, 5569825, 5571698, 5573922, 5580717, 5585089, 5585499, 5587161, 5595499, 5606017, 5622929, 5625126, 5629430, 5633425, 5641780, 5660829, 5661016, 5686237, 5693762, 5703080, 5712374, 5714586, 5739116, 5739350, 5770429, 5773001, 5773435, 5786377, 5786486, 5789650, 5814318, 5846545, 5874299, 5877296, 5877397, 5885793, 5939598, 5962216, 5969108, 5985908, 6060608, 6066742, 6075181, 6103236, 6114598, 6130237, 6132722, 6143901, 6150584, 6162963, 6172197, 6180370, 6194612, 6214345, 6262271, 6281354, 6310209, 6329497, 6342480, 6486326, 6512101, 6521404, 6534660, 6544731, 6548530, 6555313, 6555693, 6566336, 6,586,618, 6593081, 6630579, 6,756,397, 6759509, 6762179, 6884869, 6897034, 6946455, 7,049,316, 7087600, 7091186, 7115573, 7129261, 7214663, 7223837, 7304032, 7329507, 7,329,760, 7,388,026, 7,655,660, 7,655,661, 7,906,545, and 8,012,978.

Daunorubicin/Doxorubicin Analogues are also preferred for conjugation via the hydrophilic linkers of the present patent. The preferred structures and their synthesis are exemplified in: Hurwitz, E., et al., Cancer Res. 35, 1175-1181 (1975). Yang, H. M., and Reisfeld, R. A., Proc. Natl. Acad. Sci. 85, 1189-1193 (1988); Pietersz, C. A., E., et al., E., et al., " Cancer Res. 48, 926-9311 (1988); Trouet, et al., 79, 626-629 (1982); Z. Brich et al., J. Controlled Release, 19, 245-258 (1992); Chen et al., Syn. Comm., 33, 2377-2390, 2003; King et al., Bioconj. Chem., 10, 279-288, 1999; King et al., J. Med. Chem., 45, 4336-4343, 2002; Kratz et al., J Med Chem. 45, 5523-33. 2002; Kratz et al., Biol Pharm Bull. Jan. 21, 56-61, 1998; Lau et al., Bioorg. Med. Chem. 3, 1305-1312, 1995; Scott et al., Bioorg. Med. Chem. Lett. 6, 1491-1496; 1996; Watanabe et al., Tokai J. Experimental Clin. Med. 15, 327-334, 1990; Zhou et al., J. Am. Chem. Soc. 126, 15656-7, 2004; WO 01/38318; U.S. Patent No.). 5,106,951; 5,122,368; 5,146,064; 5,177,016; 5,208,323; 5,824,805; 6,146,658; 6,214,345; 7569358; 7,803,903; 8,084,586; 8,053,205.

Auristatins and dolastatins are preferred in conjugation via the hydrophilic linkers of this patent. The auristatins (e. g. auristatin E (AE) auristatin EB (AEB), auristatin EFP (AEFP), monomethyl auristatin E (MMAE), Monomethylauristatin (MMAF), Auristatin F phenylene diamine (AFP) and a phenylalanine variant of MMAE) which are synthetic

analogs of dolastatins, are described in Int. J. Oncol. 15:367-72 (1999); Molecular Cancer Therapeutics, vol. 3, No. 8, pp. 921-932 (2004); U.S. Application Nos. 11134826, 20060074008, 2006022925. U.S. Patent Nos. 4414205, 4753894, 4764368, 4816444, 4879278, 4943628, 4978744, 5122368, 5165923, 5169774, 5286637, 5410024, 5521284, 5530097, 5554725, 5585089, 5599902, 5629197, 5635483, 5654399, 5663149, 5665860, 5708146, 5714586, 5741892, 5767236, 5767237, 5780588, 5821337, 5840699, 5965537, 6004934, 6033876, 6034065, 6048720, 6054297, 6054561, 6124431, 6143721, 6162930, 6214345, 6239104, 6323315, 6342219, 6342221, 6407213, 6569834, 6620911, 6639055, 6884869, 6913748, 7090843, 7091186, 7097840, 7098305, 7098308, 7498298, 7375078, 7462352, 7553816, 7659241, 7662387, 7745394, 7754681, 7829531, 7837980, 7837995, 7902338, 7964566, 7964567, 7851437, 7994135.

The benzodiazepine dimers (e. g. dimmers of pyrrolbenzodiazepine (PBD) or (tomaymycin), indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolodinobenzodiazepines) which are preferred cytotoxic agents according to the present invention are exemplified in the art: US Patent Nos . 8,163,736; 8,153,627; 8,034,808; 7,834,005; 7,741,319; 7,704,924; 7,691,848; 7,678,787; 7,612,062; 7,608,615; 7,557,099; 7,528,128; 7,528,126; 7,511,032; 7,429,658; 7,407,951; 7,326,700; 7,312,210; 7,265,105; 7,202,239; 7,189,710; 7,173,026; 7,109,193; 7,067,511; 7,064,120; 7,056,913; 7,049,311; 7,022,699; 7,015,215; 6,979,684; 6,951,853; 6,884,799; 6,800,622; 6,747,144; 6,660,856; 6,608,192; 6,562,806; 6,977,254; 6,951,853; 6,909,006; 6,344,451; 5,880,122; 4,935,362; 4,764,616; 4,761,412; 4,723,007; 4,723,003; 4,683,230; 4,663,453; 4,508,647; 4,464,467; 4,427,587; 4,000,304; US patent appl. 20100203007, 20100316656, 20030195196.

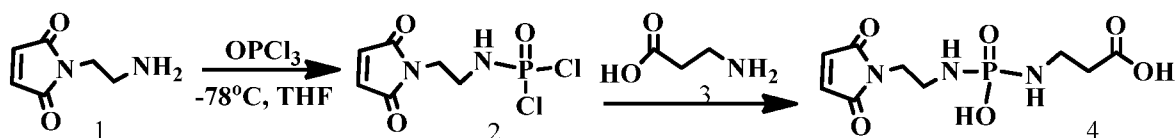
The drugs/ cytotoxic agents used for conjugation via a hydrophilic linker of the present patent can be any analogues and/or derivatives of drugs/molecules described in the present patent. One skilled in the art of drugs/cytotoxic agents will readily understand that each of the drugs/cytotoxic agents described herein can be modified in such a manner that the resulting compound still retains the specificity and/or activity of the starting compound. The skilled artisan will also understand that many of these compounds can be used in place of the drugs/cytotoxic agents described herein. Thus, the drugs/cytotoxic agents of the present invention include analogues and derivatives of the compounds described herein.

All references cited herein and in the examples that follow are expressly incorporated by reference in their entireties.

EXAMPLES

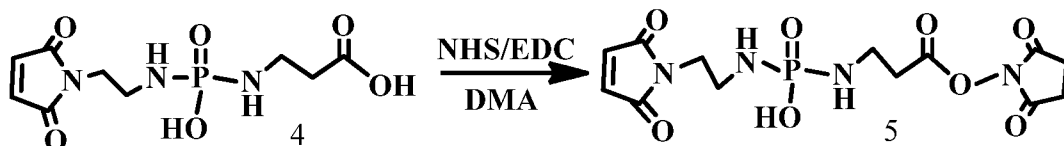
The invention is further described in the following examples, which are not intended to limit the scope of the invention. Cell lines described in the following examples were maintained in culture according to the conditions specified by the American Type Culture Collection (ATCC) or Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DMSZ), or The Shanghai Cell Culture Institute of Chinese Academy of Science, unless otherwise specified. Cell culture reagents were obtained from Invitrogen Corp., unless otherwise specified. All anhydrous solvents were commercially obtained and stored in Sure-seal bottles under nitrogen. All other reagents and solvents were purchased as the highest grade available and used without further purification. The preparative HPLC separations were performed with Varain PreStar HPLC. NMR spectra were recorded on Varian Mercury 400 MHz Instrument. Chemical shifts (δ) are reported in parts per million (ppm) referenced to tetramethylsilane at 0.00 and coupling constants (J) are reported in Hz. The mass spectral data were acquired on a Waters Xevo Qtof mass spec with Waters Acquity UPLC separations module and Acquity TUV detector.

Example 1: 3-(((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)(hydroxy)-phosphoryl)amino)propanoic acid (**4**)



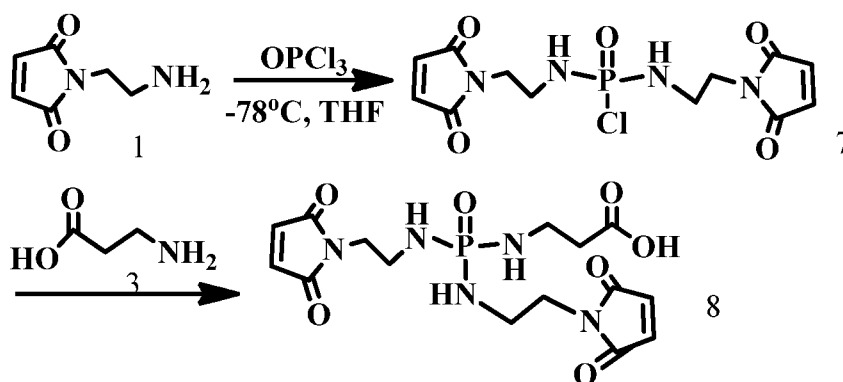
N-2-ethyl-malimide hydrochloride salt (1.0 g, 5.66 mmol) in THF (50 ml) cooled at -78°C was added phosphoryl trichloride (0.86 g, 5.66 mmol). After stirred at -78°C for 2 h to form (2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)phosphoramidic dichloride (**2**), 3-aminopropanoic acid (0.51g, 5.70 mmol) in the mixture of THF/ H_2O (2:1, 30 ml) and triethylamine (1.0 g, 9.90 mmol) was added to the solution. The resulting mixture was stirred at RT for 3 h, concentrated under vacuum and purified on the SiO_2 column eluted with $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:20 ~1:10) to afford the title compound **4** (1.28 g, 78% yield). ESI MS m/z - $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_6\text{P}$ (M-H), caclcd. 290.06, found 290.10.

Example 2. N-Hydroxysuccinimidyl 3-(((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)(hydroxy)phosphoryl)amino)propanoate (**5**).



3-(((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)(hydroxy)-phosphoryl)amino)propanoic acid (**4**) (0.50 g, 1.72 mmol) in DMA (30 ml) was added NHS (0.20 g, 1.74 mmol) and EDC (0.81 g, 4.22 mmol). The mixture was stirred under Ar overnight, evaporated and purified on SiO₂ chromatography eluted with acetone/CH₂Cl₂ (1:10 ~1:3). The fractions containing the product were pooled, evaporated, solidified in C₂H₅OH/Dioxane/Hexane to afford the title compound (392 mg, 58% yield). ESI MS m/z- C₁₃H₁₆N₄O₈P (M-H), cacl. 387.08, found 387.20.

Example 3. 3-((Bis((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-phosphoryl)-amino)propanoic acid (**8**).



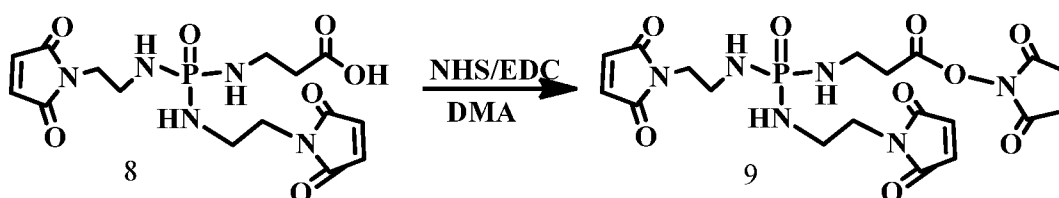
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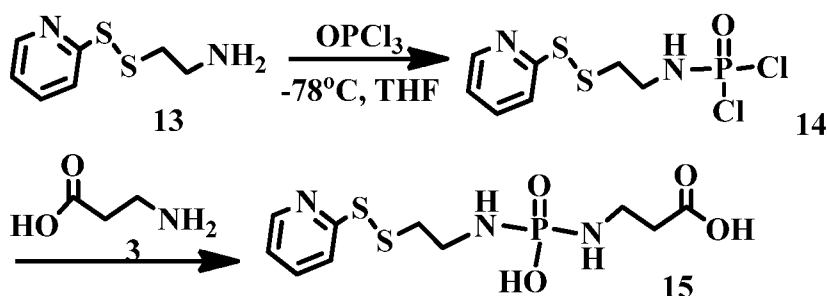
N-2-ethyl-malimide hydrochloride salt (2.0 g, 11.32 mmol) in THF (100 ml) cooled at -78°C was added phosphoryl trichloride (0.86 g, 5.66 mmol). After stirred at -78°C for 1 h, the mixture was added triethylamine (1.0 g, 9.90 mmol) and the resulting solution was stirred at RT for 3 h to generate bis(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-phosphoramidic chloride (**7**). Then 3-aminopropanoic acid (0.51g, 5.70 mmol) in the mixture of THF/H₂O (2:1, 30 ml) and triethylamine (1.51 g, 14.90 mmol) was added to the solution. The resulting mixture was stirred at 35°C for 3 h, concentrated under vacuum and purified on the SiO₂ column eluted with H₂O/CH₃CN (1:20 ~1:10) to afford the title compound **8** (1.47 g, 63% yield). ESI MS m/z- C₁₅H₁₉N₅O₇P (M-H), cacl. 412.11, found 412.20.

Example 4. N-Hydroxysuccinimidyl 3-((Bis((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)phosphoryl)-amino)propanoate (**9**).



3-((Bis((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)phosphoryl)amino)propanoic acid (**8**) (0.55 g, 1.33 mmol) in DMA (30 ml) was added NHS (0.20 g, 1.74 mmol) and EDC (0.78 g, 4.06 mmol). The mixture was stirred under Ar overnight, evaporated and purified on short SiO₂ chromatography eluted with EtOAc/CH₂Cl₂ (1:3 ~1:1).
 5 The fractions containing the product were pooled, evaporated in vacuum to afford the title compound (536 mg, 79% yield). ESI MS m/z+ C₁₉H₂₃N₆N_aO₉P (M+Na), caclcd. 533.13, found 533.20.

Example 5. 3-((hydroxy((2-(pyridin-2-yl)disulfanyl)ethyl)amino)phosphoryl)amino)propanoic acid (**15**).



10

2-(pyridin-2-yl)disulfanyl)ethanamine hydrochloride salt (1.40 g, 7.52 mmol) in THF (60 ml) cooled at -78°C was added phosphoryl trichloride (1.15 g, 7.56 mmol). After stirred at -78°C for 2 h to form (2-(pyridin-2-yl)disulfanyl)ethylphosphoramidic dichloride (**14**), 3-aminopropanoic acid (0.67g, 7.52 mmol) in the mixture of THF/H₂O (2:1, 30 ml) and triethylamine (20 g, 19.80 mmol) was added to the solution. The resulting mixture was stirred at RT for 3 h, concentrated under vacuum and purified on the SiO₂ column eluted with H₂O/CH₃CN (1:20 ~1:10) to afford the title compound **15** (1.69 g, 66% yield). ESI MS m/z- C₁₀H₁₅N₃O₄PS (M-H), caclcd. 336.03, found 336.20.

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Example 6. N-Hydroxysuccinimidyl 3-(((2-(pyridin-2-yl)disulfanyl)ethyl)amino)-(hydroxy)phosphoryl)amino)propanoate (**16**).

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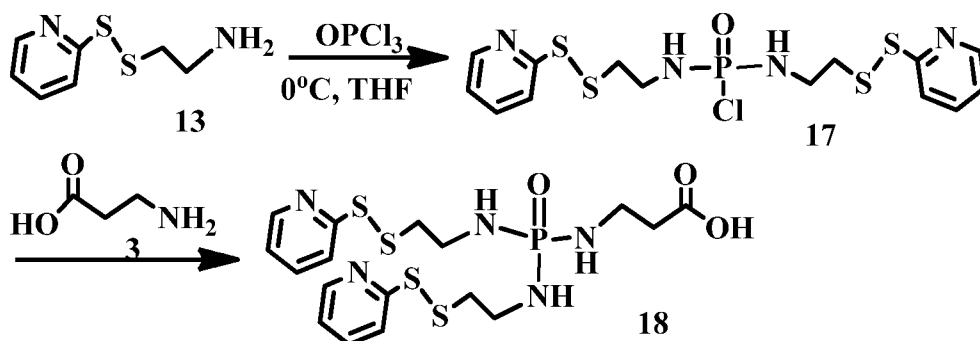


3-(((2-(pyridin-2-yl)disulfanyl)ethyl)amino)-(hydroxy)-phosphoryl)amino)propanoic acid (**15**) (0.60 g, 1.78 mmol) in DMA (30 ml) was added NHS (0.22 g, 1.91 mmol) and EDC (0.81 g, 4.22 mmol). The mixture was stirred under Ar overnight, evaporated and purified on short C-18 chromatography eluted with water/dioxane at 4°C. The fractions containing the product were pooled, frozen at -78°C, lyophilized to afford the title com-

25

pound **16** (477 mg, 61% yield). ESI MS m/z - $C_{14}H_{19}N_4O_6PS_2$ (M-H), caclcd. 433.05, found 433.20.

Example 7. 3-((Bis((2-(pyridin-2-yl)disulfanyl)ethyl)amino)phosphoryl)amino)propanoic acid (**18**).

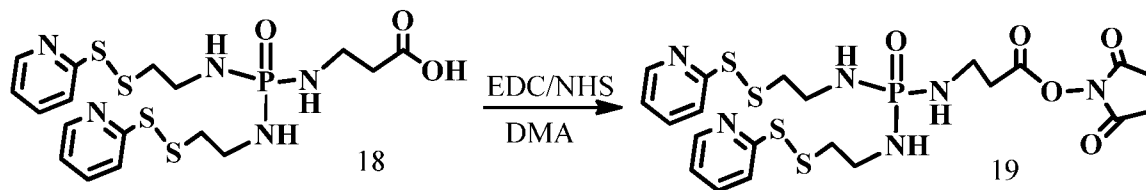


5

2-(pyridin-2-yl)ethanamine hydrochloride salt (2.10 g, 11.29 mmol) in THF (100 ml) cooled at $-78^{\circ}C$ was added phosphoryl trichloride (0.85 g, 5.59 mmol). After stirred at $-78^{\circ}C$ for 1 h, the mixture was added triethylamine (1.0 g, 9.90 mmol) and the resulting solution was stirred at RT for 3 h to generate bis(2-(pyridin-2-yl)disulfanyl)ethylphosphoramidic chloride (**17**). Then 3-aminopropanoic acid (0.61g, 6.85 mmol) in the mixture of THF/H₂O (2:1, 30 ml) and triethylamine (1.80 g, 17.82 mmol) was added to the solution. The resulting mixture was stirred at $35^{\circ}C$ for 3 h, concentrated under vacuum and purified on the SiO₂ column eluted with H₂O/CH₃CN (1:20 ~1:10) to afford the title compound **18** (1.47 g, 63% yield). ESI MS m/z - $C_{17}H_{23}N_5O_3PS$ (M-H), caclcd. 504.05, found 504.20.

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Example 8. 2,5-dioxopyrrolidin-1-yl 3-((bis(2-(pyridin-2-yl)disulfanyl)ethyl)amino)phosphoryl)amino)propanoate (**19**).

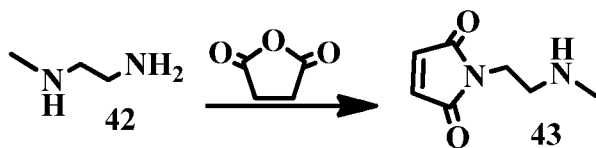


3-((Bis((2-(pyridin-2-yl)disulfanyl)ethyl)amino)phosphoryl)amino)propanoic acid (**18**) (0.52 g, 1.03 mmol) in DMA (30 ml) was added NHS (0.20 g, 1.74 mmol) and EDC (0.80 g, 4.16 mmol). The mixture was stirred under Ar overnight, evaporated and purified on short SiO₂ chromatography eluted with EtOAc/CH₂Cl₂ (1:4 ~1:1). The fractions containing the product were pooled, evaporated in vacuum to afford the title compound **19**

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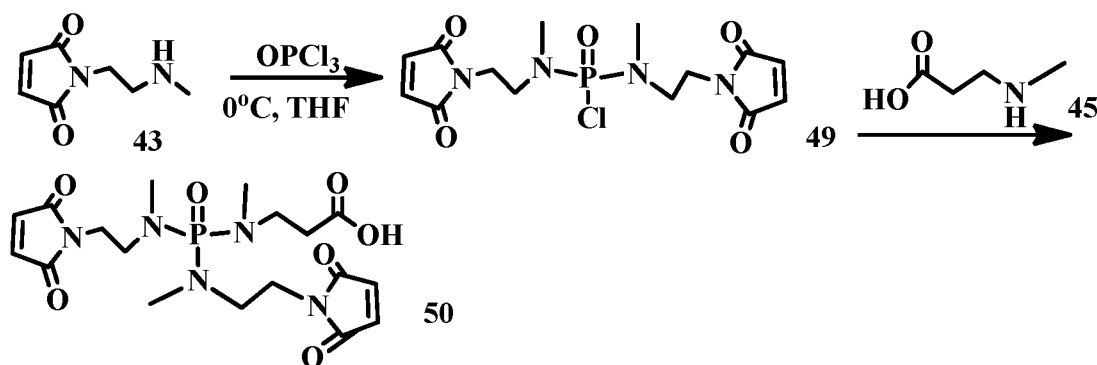
(536 mg, 79% yield). ESI MS m/z + $C_{21}H_{27}N_6NaO_5PS_4$ (M+Na), cacl'd. 625.06, found 625.20.

Example 9. 1-(2-(Methylamino)ethyl)-1H-pyrrole-2,5-dione (**43**)



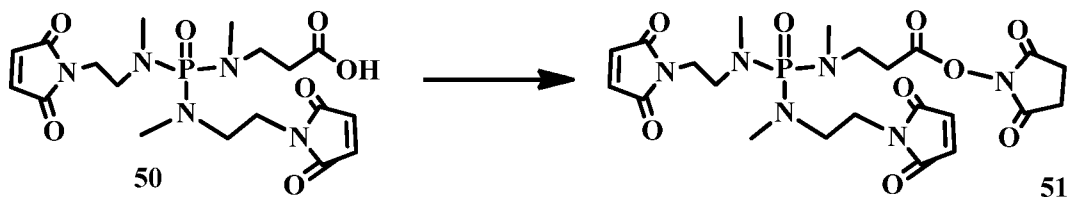
5 N¹-methylethane-1,2-diamine (10.23 g, 138.08 mmol) in DMA (150 ml) was added succinic anhydride (13.82 g, 138.08 mmol) at 0°C. After stirring under Ar at 0°C for 1 hr then RT for 4 h, the mixture was evaporated, redissolved in acetic acid (100 ml, 98%) and Ac₂O (0.5 mL), then heated at 80 °C for 8 h. The mixture was concentrated, purified on C-18 flush chromatography eluted with water/CH₃OH (100% water to 60% water containing
10 0.3% HCl) to afford the title compound as a hydrochloric acid salt. (13.68 g, 52% yield). ESI MS m/z + 155.10 (M + H).

Example 10. 3-((bis((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)phosphoryl)(methyl)amino)propanoic acid (**50**)



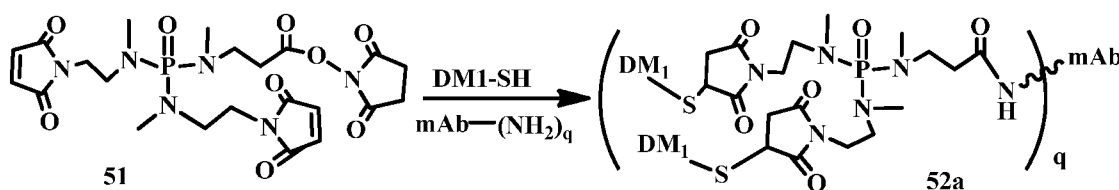
15 1-(2-(Methylamino)ethyl)-1H-pyrrole-2,5-dione, HCl salt (**43**) (4.05 g, 21.00 mmol) in THF (100 ml) cooled at -78°C was added phosphoryl trichloride (1.59 g, 10.50 mmol). After stirred at -78°C for 1 h, the mixture was added triethylamine (1.2 g, 11.88 mmol) and the resulting solution was stirred at RT for 3 h to generate bis(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl(methyl))-phosphoramidic chloride (**49**). Then 3-
20 (methylamino)propanoic acid (1.21 g, 11.73 mmol) in the mixture of THF (30 ml) and triethylamine (1.20 g, 11.88 mmol) was added to the solution. The resulting mixture was stirred at 45°C for 3 h, concentrated under vacuum and purified on the SiO₂ column eluted with H₂O/CH₃CN (1:20 ~1:10) to afford the title compound **50** (2.44 g, 51% yield). ESI MS m/z - $C_{18}H_{25}N_5O_7P$ (M-H), cacl'd. 454.16, found 454.20.

Example 11. 2,5-dioxopyrrolidin-1-yl 3-((bis((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)phosphoryl)(methyl)amino)propanoate (**51**)



Compound **50** (1.10 g, 2.41 mmol) in DMA (40 ml) was added NHS (0.40 g, 3.48 mmol) and EDC (1.80 g, 9.37 mmol). The mixture was stirred under Ar overnight, evaporated and purified on short SiO₂ chromatography eluted with acetone/CH₂Cl₂ (1:6 ~1:2). The fractions containing the product were pooled, evaporated in vacuum to afford the title compound **51** (971 mg, 73% yield). ESI MS *m/z*+ C₂₂H₂₉N₆N_aO₉P (M+Na), cacl'd. 575.17, found 575.20.

Example 12. Conjugation of two DM1 per linker with an antibody for (**52a**).

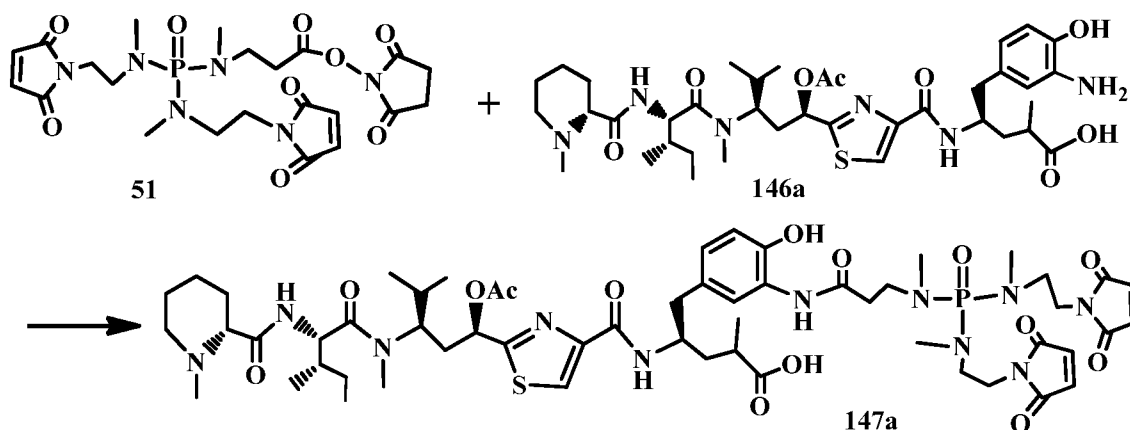


The compound **51** (35 μ L, 20 mM in DMA), buffers (60 μ L, 100 mM NaH₂PO₄, pH 5.0~7.0) and DM1 (85 μ L, 20 mM in DMA) were incubated at 15~30 $^{\circ}$ C for 20 min ~2.5 h. Then the mixture was added to a mixture of 2.0 mL of 10 mg/ml antiHer2 antibody in pH 6.5~8.0 PBS buffer, 1.0~2.0 mL of 100 mM NaH₂PO₄, pH 7.5 buffer. The subject mixture solution was incubated at RT for 2~24 h, purified on G-25 column eluted with 100 mM NaH₂PO₄, 50 mM NaCl pH 5.5~7.5 buffers to afford 16.5~18.3 mg of the compound **52a** (~86% yield) in 11.6~14.2 ml buffers. The DM1/antibody ratio was 6.8~7.8, which was calculated according to the reference (Zhao, R. Y. et al, J. Med. Chem. 2011, 54, 3606). It was 95~99% monomer analyzed by SEC HPLC (Tosoh Bioscience, Tskgel G3000SW, 7.8 mm ID x 30 cm, 0.5 ml/min, 100 min)

Alternatively, the compound **51** (35 μ L, 20 mM in DMA), was added to a mixture of 2.0 mL of 10 mg/ml antiHer2 antibody in pH 6.5~8.0 PBS buffers, 0.5~1.7 mL of 100 mM NaH₂PO₄, pH 6.5~8.0 buffer. After incubated at RT for 2 h, the mixture was purified on G-

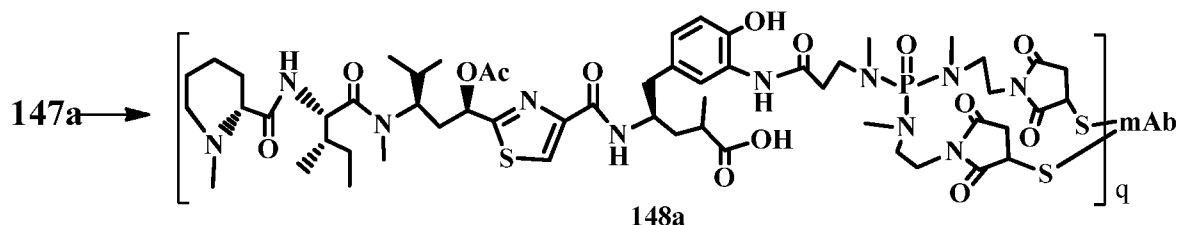
25 column eluted with 100 mM NaH₂PO₄, 50 mM NaCl pH 5.5~7.5 buffers. Then to the collected subject solution (4.5~6.5 ml) were added DM1 (70 μL, 20 mM in DMA) and DMA (0.1~0.5 ml). The mixture was then incubated at RT for 2~16 h, purified on G-25 column eluted with 100 mM NaH₂PO₄, 50 mM NaCl pH 5.5~7.5 buffers to afford 5 15.8~17.3 mg of the compound **52a** (~81% yield) in 15.5~17.4 ml buffers. The DM1/antibody ratio was 7.1~7.7, which was calculated according to the reference (Zhao, R. Y. et al, J. Med. Chem. 2011, 54, 3606). It was 95~99% monomer analyzed by SEC HPLC (Tosoh Bioscience, Tskgel G3000SW, 7.8 mm ID x 30 cm, 0.5 ml/min, 100 min).

Example 13. Compound **147a** bearing a hydrophilic linker of the present patent con-
jugated with a tubulysin analog.



The compound **51** (120 mg, 0.217 mmol) in THF (3.0 ml) was added compound **146a** (Huang Y. et al, Med Chem. #44, 249th ACS National Meeting, Dever, CO, Mar. 22~26, 2015; WO2014009774) (151 mg, 0.199 mmol) in THF (3.0 ml) and buffer (5 ml, 15 100 mM Na₂HPO₄, pH 7.2). After stirred at RT for 4 h, the mixture was concentrated and purified with C-18 preparative HPLC (250 mm x ID 30 mm), eluted with water/ethanol (90% water to 50% water in 35 min, v = 65 ml/min). The fractions containing the product were pooled, concentrated and crystallized with EtOH/Hexane to afford the title compound (159 mg, 67% yield). ESI MS m/z+ C₅₆H₈₂N₁₁N_aO₁₄PS (M+Na), caclcd. 1218.35, 20 found 1218.40.

Example 14. Conjugated compound **147a** to an antibody for **148a**.



To a mixture of 2.0 mL of 10 mg/ml antiHer2 antibody in pH 6.0~8.0, were added of 0.70 ~ 2.0 mL PBS buffer of 100 mM NaH₂PO₄, pH 6.5~7.5 buffers, TCEP (28 μL, 20 mM in water) and the compound **147a** (35 μL, 20 mM in DMA). The mixture was incubated at RT for 2~16 h, then DHAA (135 μL, 50 mM) was added in. After continuous incubation at RT overnight, the mixture was purified on G-25 column eluted with 100 mM NaH₂PO₄, 50 mM NaCl pH 6.0~7.5 buffer to afford 16.8~17.9 mg of the conjugate compound **148a** (~87% yield) in 13.1~14.9 ml buffer. The drug/antibody ratio (DAR) was 2.8~3.7, which was determined UPLC-Qtof mass spectrum. It was 96~99% monomer analyzed by SEC HPLC (Tosoh Bioscience, Tskgel G3000SW, 7.8 mm ID x 30 cm, 0.5 ml/min, 100 min) and a single band measured by SDS-PAGE gel.

Alternatively, a mixture of 2.0 mL of 10 mg/ml antiHer2 antibody in pH 6.0~8.0 PBS buffer, 0.50~1.5 mL of 100 mM NaH₂PO₄, pH 6.0~8.0 buffers, and DTT (30 μL, 20 mM in water) was incubated at 15~37°C for 1~5 h and then purified on G-25 column eluted with 100 mM NaH₂PO₄, 50 mM NaCl pH 6.0~8.0 buffer. The pooled fractions (3.8~5.8 ml) was added 0.2~0.6 mL DMA and compound **147a** (35 μL, 20 mM in DMA), and incubated at RT for 4~12 h. After addition of DHAA (135 μL, 50 mM) and continuous incubation at RT overnight, the mixture was purified on G-25 column eluted with 100 mM NaH₂PO₄, 50 mM NaCl pH 7.5 buffer to afford 15.5~16.8 mg of the conjugate compound **148a** (~80% yield) in 13.8~15.9 ml buffers. The drug/antibody ratio (DAR) was 2.6~3.8, which was determined UPLC-QTOF mass spectrum. It was 96~98% monomer analyzed by SEC HPLC (Tosoh Bioscience, Tskgel G3000SW, 7.8 mm ID x 30 cm, 0.5 ml/min, 100 min) and a single band measured by SDS-PAGE gel.

Example 15. *In vitro* cytotoxicity evaluation of conjugates **52a** and **148a**:

The targeted cells (e.g. N-87, SKOV3 and HL60 cells, 6,000 cells) were cultured in the presence of various concentrations the antiHer2 antibody conjugate **52a** and **148a** for 96 hours after which cell viability was measured by propidium iodide exclusion and analyzed by flow cytometry using a Becton Dickinson FACSort (Becton Dickinson, Franklin Lakes, NJ). Red fluorescent intensity (emission at 617 nm in the FL2 channel) of the cells excited at 488 nm was measured. The regions for viable cells were also set using both the forward light scatter and right-angle light scatter properties of the cells. The loss of viability was determined by the loss of cells from within the gated region defining viable cells. The average number of viable cells per 6 replicate cultures was calculated. The

survival fraction was plotted versus conjugate concentration to determine the IC₅₀ value (50% cell killing concentration) of the conjugates **52a** and **148a**.

The cytotoxicity results:

IC ₅₀ nM	N87 cell (Ag+)	SK-OV-3 cell (Ag+)	HL60 cell (Ag-)
Conjugate 52a	0.027 nM	0.021 nM	>50 nM
Conjugate 148a	0.038 nM	0.032 nM	>50 nM

5 Specificity of conjugate **52a** for N87 cell was over 1850 (IC₅₀> 50/ IC₅₀= 0.027), and for SK-OV-3 cell was over 2380.

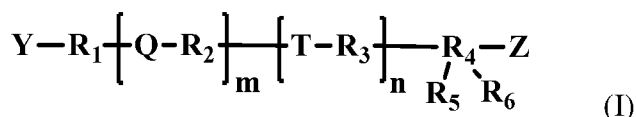
 Specificity of conjugate **148a** for N87 cell was over 1315 (IC₅₀> 50/ IC₅₀= 0.038), and for SK-OV-3 cell was over 1560.

10 Both conjugate **52a** and conjugate **148a** were extremely potent and much specifically targeting the antigen positive tumor cells.

CLAIMS

What is claimed is:

1. A hydrophilic linker compound of the Formula (I)



Wherein:

Y represents a functional group that enables reaction with a cell-binding agent;

Q and T are either $-X_1-P(=O)(OM)-$, or $-X_1-S(O_2)-$, or $-X_1-S(O)-$; or $-X_1-P(=O)(OM)-X_2-$, or $-X_1-P(=O)[X_2-R_4-Z]-X_3-$, or $-X_1-P(=O)[X_2-R_1-Y]-X_3-$, or $-X_1-S(O_2)-X_2-$, or $-X_1-S(O)-X_2-$;

X_1 , X_2 and X_3 are independently selected from $N(R_7)$, O, or S; In addition, when X_1 is either $N(R_7)$, or O, or S, then either X_2 , or X_3 , or another X_1 that connects to $-P(=O)$, $-S(O)$, or $-S(O_2)$ can be CH_2 .

m and n are integer from 0 to 5, but not 0 at the same time;

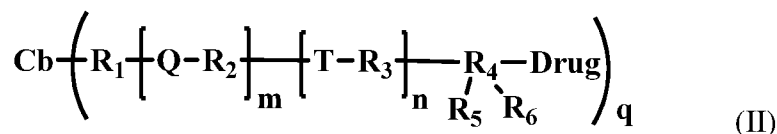
Z represents a functional group that enables linkage of a cytotoxic drug via an alkyl, alkenyl, alkynyl, aromatic, heteroalkyl, disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, amine (secondary, tertiary, or quaternary), imine, cycloheteroalkane, heteroaromatic, alkoxy or amide bond;

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and R_7 are the same or different and are H, linear alkyl having from 1-6 carbon atoms, branched or cyclic alkyl having from 3 to 6 carbon atoms, linear, branched or cyclic alkenyl or alkynyl, or 1-6 carbon atoms of esters, ether, amide, or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$, wherein p is an integer from 0 to about 1000, or combination thereof.

Additionally R_1 , R_2 , R_3 and R_4 are respectively a chain of atoms selected from C, N, O, S, Si, and P that covalently connects the cell-surface binding ligand, the phosphinate or sulfonyl group, the conjugated drug and among themselves (R_1 , R_2 , R_3 and R_4). The atoms used in forming the hydrophilic 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, urethanes, amino acids, peptides, acyloxyamines, heterocyclics, hydroxamic acids, or combination thereof.

M is H, or Na, or K, or $N^+R_1R_2R_3$ or a pharmaceutical salt. R_1 , R_2 and R_3 are described above.

2. A cell-binding agent-drug conjugate compound of Formula (II)



wherein:

Cb represents a cell-binding agent/molecule;

Drug represents a drug linked to the cell-binding agent via the hydrophilic linker by an alkyl, alkylene, alkenylene, alkynylene, ether, polyoxyalkylene, ester, amine, imine, polyamine, hydrazine, hydrazone, amide, urea, semicarbazide, carbazide, alkoxyamine, urethanes, amino acid, peptide, acyloxylamine, hydroxamic acid, disulfide, thioether, thioester, carbamate, carbonate, heterocyclic ring, heteroalkyl, heteroaromatic, or alkoxime bond, or combination thereof;

Q and T are either $-\text{X}_1-\text{P}(=\text{O})(\text{OM})-$, or $-\text{X}_1-\text{S}(\text{O}_2)-$, or $-\text{X}_1-\text{S}(\text{O})-$; or $-\text{X}_1-\text{P}(=\text{O})(\text{OM})-\text{X}_2-$, or $-\text{X}_1-\text{P}(=\text{O})[\text{X}_2-\text{R}_4-\text{Drug}]-\text{X}_3-$, or $-\text{X}_1-\text{P}(=\text{O})[\text{X}_2-\text{R}_1-\text{Cb}]-\text{X}_3-$, or $-\text{X}_1-\text{S}(\text{O}_2)-\text{X}_2-$, or $-\text{X}_1-\text{S}(\text{O})-\text{X}_2-$;

X_1 , X_2 and X_3 are independently selected from $\text{N}(\text{R}_7)$, O, or S; In addition, when X_1 is either $\text{N}(\text{R}_7)$, or O, or S, then either X_2 , or X_3 , or another X_1 that connects to $-\text{P}(=\text{O})$, $-\text{S}(\text{O})$, or $-\text{S}(\text{O}_2)$ can be CH_2 .

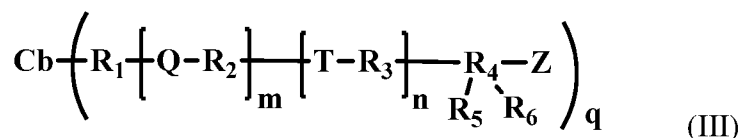
m and n are integer from 0 to 5, but not 0 at the same time; q is 1 ~ 30.

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and R_7 are the same or different and are H, linear alkyl having from 1-6 carbon atoms, branched or cyclic alkyl having from 3 to 6 carbon atoms, linear, branched or cyclic alkenyl or alkynyl, or 1~6 carbon atoms of esters, ether, amide, or polyethyleneoxy unit of formula $(\text{OCH}_2\text{CH}_2)_p$, wherein p is an integer from 0 to about 1000, or combination thereof.

Additionally R_1 , R_2 , R_3 and R_4 are respectively a chain of atoms selected from C, N, O, S, Si, and P that covalently connects the cell-surface binding ligand, the phosphinate or sulfonyl group, the conjugated drug and among themselves (R_1 , R_2 , R_3 and R_4). The atoms used in forming the hydrophilic linker may be combined in all chemically relevant ways, such as forming alkyl, alkylene, alkenylene, and alkynylene, ethers, polyoxyalkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amides, ureas, semicarbazides, carbazides, alkoxyamines, alkoxyamines, urethanes, amino acids, peptides, acyloxylamines, heterocyclics, hydroxamic acids, or combination thereof.

M is H, or Na, or K, or $\text{N}^+\text{R}_1\text{R}_2\text{R}_3$ or a pharmaceutical salt. R_1 , R_2 and R_3 are described above.

3. A compound of Formula (III):



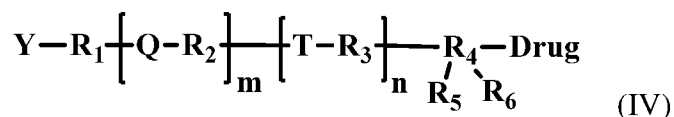
Wherein:

Cb, Z, m, n, q, R₁, R₂, R₃, R₄, R₅ and R₆ are defined the same as in Claims 1 and 2.

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Z]-X₃-, or -X₁-P(=O)[X₂-R₁-Cb]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-;

X₁, X₂ and X₃ are independently selected from N(R₇), O, or S; In addition, when X₁ is either N(R₇), or O, or S, then either X₂, or X₃, or another X₁ that connects to -P(=O), -S(O), or -S(O₂) can be CH₂.

4. A compound of Formula (IV):



Wherein:

Y, Drug, m, n, q, R₁, R₂, R₃, R₄, R₅ and R₆ are defined the same as in Claim 1 and 2.

Q and T are either -X₁-P(=O)(OM)-, or -X₁-S(O₂)-, or -X₁-S(O)-; or -X₁-P(=O)(OM)-X₂-, or -X₁-P(=O)[X₂-R₄-Drug]-X₃-, or -X₁-P(=O)[X₂-R₁-Y]-X₃-, or -X₁-S(O₂)-X₂-, or -X₁-S(O)-X₂-;

X₁, X₂ and X₃ are independently selected from N(R₇), O, or S; In addition, when X₁ is either N(R₇), or O, or S, then either X₂, or X₃, or another X₁ that connects to -P(=O), -S(O), or -S(O₂) can be CH₂.

5. The compound of Formula (I) of claims 1, wherein Q, or/and T is -X₁-P(=O)[X₁-R₁-Y]-X₃-, then the compound of the Formula (I) can be used to link to two or more sites, particularly to a pair of sites of cell binding molecules. Wherein X₁, X₂ and X₃ are independently selected from N(R₇), O, CH₂ or S; and R₁ and R₇ are defined in Claim 1.

6. The compound of Formula (I) of claims 1, wherein Q, or/and T is -X₁-P(=O)[X₂-R₄-Z]-X₃-, then the compound of the Formula (I) can be used for linking two or more drugs, in particular, two different drugs. Wherein X₁, X₂ and X₃ are independently selected from N(R₇), O, CH₂, or S; and R₄ and R₇ are defined in Claim 1.

7. The conjugate compound of Formula (II) of claims 2, wherein Q, or/and T is -X₁-P(=O)[X₁-R₁-Cb]-X₃-, then the compound of the Formula (II) is linked to two or more sites, particularly a pair of sites of cell binding molecules. Wherein X₁, X₂ and X₃ are independently selected from N(R₇), O, CH₂, or S; and R₁ and R₇ are defined in Claim 1.

- 8.** The compound of Formula (II) of claims **2**, wherein Q, or/and T is $-X_1-P(=O)[X_2-R_4-$ Drug]- X_3- , then the compound of the Formula (II) is linked to two or more drugs, in particular, two different drugs. Wherein X_1 , X_2 and X_3 are independently selected from N(R_7), O, CH_2 , or S; and R_4 and R_7 are defined in Claim **1**.
- 9.** The compound of Formula (III) of claims **3**, wherein Q, or/and T is $-X_1-P(=O)[X_2-R_1-$ Cb]- X_3- , then the compound of the Formula (III) is linked to two or more sites, particularly a pair of sites of cell binding molecules. Wherein X_1 , X_2 and X_3 are independently selected from N(R_7), O, CH_2 , or S; and R_1 and R_7 are defined in Claim **1**.
- 10.** The compound of Formula (III) of claims **3**, wherein Q, or/and T is $-X_1-P(=O)[X_2-R_4-Z]-$ X_3- , then the compound of the Formula (III) can be used for linking to two or more drugs, in particular, two different drugs. Wherein X_1 , X_2 and X_3 are independently selected from N(R_7), O, CH_2 , or S; and R_4 and R_7 are defined in Claim **1**.
- 11.** The compound of Formula (IV) of claims **4**, wherein Q, or/and T is $-X_1-P(=O)[X_2-R_1-Y]-$ X_3- , then the compound of the Formula (IV) can be used for linking to two or more sites, particularly to a pair of sites of cell binding molecules. Wherein X_1 , X_2 and X_3 are independently selected from N(R_7), O, CH_2 , or S; and R_1 and R_7 are defined in Claim **1**.
- 12.** The compound of Formula (IV) of claims **4**, wherein Q, or/and T is $-X_1-P(=O)[X_2-R_4-$ Drug]- X_3- , then the compound of the Formula (IV) is linked to two or more drugs, in particular, two different drugs. Wherein X_1 , X_2 and X_3 are independently selected from N(R_7), O, CH_2 , or S; and R_4 and R_7 are defined in Claim **1**.
- 13.** The Formula (II) and (IV) of claims **2**, **4**, **8** and **12** wherein the Drug is selected from:
- 1). Chemotherapeutic agents: a). Alkylating agents: such as Nitrogen mustards: chlorambucil, chlornaphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, mannomustine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiotepa, trofosfamide, uracil mustard; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); benzodiazepine dimers (e.g., dimmers of pyrrolobenzodiazepine (PBD) or tomaymycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines); Nitrosoureas: (carmustine, lomustine, chlorozotocin, fotemustine, nimustine, ranimustine); Alkylsulphonates: (busulfan, treosulfan, improsulfan and piposulfan); Triazenes: (dacarbazine); Platinum containing compounds: (carboplatin, cisplatin, oxaliplatin); aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramidate,

triethylenethiophosphoramidate and trimethylolomelamine]; b). Plant Alkaloids: such as Vinca alkaloids: (vincristine, vinblastine, vindesine, vinorelbine, navelbin); Taxoids: (paclitaxel, docetaxol) and their analogs, Maytansinoids (DM1, DM2, DM3, DM4, DM5, DM6, DM7, maytansine and ansamitocins) and their analogs, cryptophycins (particularly cryptophycin 1 and cryptophycin 8); epothilones, eleutherobin, discodermolide, bryostatins, dolostatins, auristatins, tubulysins, cephalostatins; pancratistatin; a sarcodictyin; spongistatin; c). DNA Topoisomerase Inhibitors: such as [Epipodophyllins: (9-aminocamptothecin, camptothecin, crisnatol, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantrone, retinoic acids (retinols), teniposide, topotecan, 9-nitrocamptothecin (RFS 2000)); mitomycins: (mitomycin C)]; d). Anti-metabolites: such as {[Anti-folate: DHFR inhibitors: (methotrexate, trimetrexate, denopterin, pteropterin, aminopterin (4-aminopteroic acid) or the other folic acid analogues); IMP dehydrogenase Inhibitors: (mycophenolic acid, tiazofurin, ribavirin, EICAR); Ribonucleotide reductase Inhibitors: (hydroxyurea, deferoxamine)]; [Pyrimidine analogs: Uracil analogs: (ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-Fluorouracil, floxuridine, ratitrexed (Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine)]; folic acid replenisher, such as frolic acid]; e). Hormonal therapies: such as {Receptor antagonists: [Anti-estrogen: (megestrol, raloxifene, tamoxifen); LHRH agonists: (goserelin, leuprolide acetate); Anti-androgens: (bicalutamide, flutamide, calusterone, dromostanolone propionate, epitiostanol, goserelin, leuprolide, mepitiostane, nilutamide, testolactone, trilostane and other androgens inhibitors)]; 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). antibiotics, such as the enediyne antibiotics (e.g. calicheamicins, especially calicheamicin γ 1, δ 1, α 1 and β 1; dynemicin, including dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins,

cactinomycin, carabycin, carminomycin, carzinophilin; chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; f). Others: such as Polyketides (acetogenins), especially bullatacin and bullatacinone; 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), Ca²⁺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.; Anti-adrenals, such as aminoglutethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; eflornithine (DFMO), elfomithine; elliptinium acetate, etoglucid; gallium nitrate; gacytosine, hydroxyurea; ibandronate, lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®]; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verrucaric acid, roridin A and anguidine); urethane, siRNA, antisense drugs;

2). Anti-autoimmune disease agents: 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, mycophenolate, prednisone, sirolimus, tacrolimus.

3). Anti-infectious disease agents, a). Aminoglycosides: amikacin, astromycin, gentamicin (netilmicin, sisomicin, isepamicin), hygromycin B, kanamycin (amikacin, arbekacin,

beknamycin, 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, ceftazidime, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, ceftazidime, ceftriaxone, cefuroxime, cefuzonam, *cephamycin* (cefoxitin, cefotetan, cefmetazole), oxacephem (flomoxef, latamoxef); f). Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin; 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, glycylicyclines (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). A radioisotope that can be selected from (radionuclides) ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{64}Cu , ^{68}Ga , ^{86}Y , ^{99}Tc , ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{133}Xe , ^{177}Lu , ^{211}At , or ^{213}Bi .

6). A chromophore molecule, which can be one that has the ability to absorb a kind of light, such as UV light, florescent light, IR light, near IR light, visual light; A class or subclass

of xanthophores, erythrophores, iridophores, leucophores, melanophores, cyanophores, fluorophore molecules which are fluorescent chemical compounds re-emitting light upon light, visual phototransduction molecules, photophore molecules, luminescence molecules, luciferin compounds; Non-protein organic fluorophores, such as: Xanthene derivatives (fluorescein, rhodamine, Oregon green, eosin, and Texas red); Cyanine derivatives: (cyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine, and merocyanine); Squaraine derivatives and ring-substituted squaraines, including Seta, SeTau, and Square dyes; Naphthalene derivatives (dansyl and prodan derivatives); Coumarin derivatives; Oxadiazole derivatives (pyridyloxazole, nitrobenzoxadiazole and benzoxadiazole); Anthracene derivatives (anthraquinones, including DRAQ5, DRAQ7 and CyTRAK Orange); Pyrene derivatives (cascade blue, etc); Oxazine derivatives (Nile red, Nile blue, cresyl violet, oxazine 170 etc). Acridine derivatives (proflavin, acridine orange, acridine yellow etc). Arylmethine derivatives (auramine, crystal violet, malachite green). Tetrapyrrole derivatives (porphin, phthalocyanine, bilirubin); Any analogs and derivatives of the following fluorophore compounds: CF dye (Biotium), DRAQ and CyTRAK probes (BioStatus), BODIPY (Invitrogen), Alexa Fluor (Invitrogen), DyLight Fluor (Thermo Scientific, Pierce), Atto and Tracy (Sigma Aldrich), FluoProbes (Interchim), Abberior Dyes (Abberior), DY and MegaStokes Dyes (Dyomics), Sulfo Cy dyes (Cyandye), HiLyte Fluor (AnaSpec), Seta, SeTau and Square Dyes (SETA BioMedicals), Quasar and Cal Fluor dyes (Biosearch Technologies), SureLight Dyes (APC, RPEPerCP, Phycobilisomes)(Columbia Biosciences), APC, APCXL, RPE, BPE (Phycobiotech), Allophycocyanin (APC), Aminocoumarin, APC-Cy7 conjugates, BODIPY-FL, Cascade Blue, Cy2, Cy3, Cy3.5, Cy3B, Cy5, Cy5.5, Cy7, Fluorescein, FluorX, Hydroxycoumarin, Lissamine Rhodamine B, Lucifer yellow, Methoxycoumarin, NBD, Pacific Blue, Pacific Orange, PE-Cy5 conjugates, PE-Cy7 conjugates, PerCP, R-Phycoerythrin(PE), Red 613, Seta-555-Azide, Seta-555-DBCO, Seta-555-NHS, Seta-580-NHS, Seta-680-NHS, Seta-780-NHS, Seta-APC-780, Seta-PerCP-680, Seta-R-PE-670, SeTau-380-NHS, SeTau-405-Maleimide, SeTau-405-NHS, SeTau-425-NHS, SeTau-647-NHS, Texas Red, TRITC, TruRed, X-Rhodamine, 7-AAD (7-aminoactinomycin D, CG-selective), Acridine Orange, Chromomycin A3, CyTRAK Orange (Biostatus, red excitation dark), DAPI, DRAQ5, DRAQ7, Ethidium Bromide, Hoechst33258, Hoechst33342, LDS 751, Mithramycin, PropidiumIodide (PI), SYTOX Blue, SYTOX Green, SYTOX Orange, Thiazole Orange, TO-PRO: Cyanine Monomer, TOTO-1, TO-PRO-1, TOTO-3, TO-PRO-3, YOSeta-1, YOYO-1. The fluorophore compounds that can be linked to the linkers of the invention for study cells are selected from the following compounds or their derivatives: DCFH (2'7'Dichlorodihydro-fluorescein, oxidized form), DHR

(Dihydrorhodamine 123, oxidized form, light catalyzes oxidation), Fluo-3 (AM ester. pH > 6), Fluo-4 (AM ester. pH 7.2), Indo-1 (AM ester, low/high calcium (Ca²⁺)), SNARF(pH 6/9), Allophycocyanin(APC), AmCyan1 (tetramer, Clontech), AsRed2 (tetramer, Clontech), Azami Green (monomer, MBL), Azurite, B-phycoerythrin(BPE), Cerulean, CyPet, DsRed monomer (Clontech), DsRed2 ("RFP", Clontech), EBFP, EBFP2, ECFP, EGFP (weak dimer, Clontech), Emerald (weak dimer, Invitrogen), EYFP (weak dimer, Clontech), GFP (S65A mutation), GFP (S65C mutation), GFP (S65L mutation), GFP (S65T mutation), GFP (Y66F mutation), GFP (Y66H mutation), GFP (Y66W mutation), GFPuv, HcRed1, J-Red, Katusha, Kusabira Orange (monomer, MBL), mCFP, mCherry, mCitrine, Midoriishi Cyan (dimer, MBL), mKate (TagFP635, monomer, Evrogen), mKeima-Red (monomer, MBL), mKO, mOrange, mPlum, mRaspberry, mRFP1 (monomer, Tsien lab), mStrawberry, mTFP1, mTurquoise2, P3 (phycobilisome complex), Peridinin Chlorophyll (PerCP), R-phycoerythrin(RPE), T-Sapphire, TagCFP (dimer, Evrogen), TagGFP (dimer, Evrogen), TagRFP (dimer, Evrogen), TagYFP (dimer, Evrogen), tdTomato (tandem dimer), Topaz, TurboFP602 (dimer, Evrogen), TurboFP635 (dimer, Evrogen), TurboGFP (dimer, Evrogen), TurboRFP (dimer, Evrogen), TurboYFP (dimer, Evrogen), Venus, Wild Type GFP, YPet, ZsGreen1 (tetramer, Clontech), ZsYellow1 (tetramer, Clontech).

- 7). The pharmaceutically acceptable salts, acids or derivatives of any of the above drugs.
- 14.** The compound of Formula (II) and (IV) of claims **2, 4, 8** and **12**, wherein a "Drug" is a chromophore molecule, the conjugate compounds of Formula (II) and (IV) of claims **2, 4, 8** and **12** can be used for detection, monitoring, or study the interactions and/or functions of the cell binding molecule, and/or the conjugate with a target, particularly, a targeted cell.
- 15.** The conjugate compound of claims **2, 4, 8** and **12**, wherein "Drug" is preferred from tubulysins, calicheamicins, auristatins, maytansinoids, CC-1065 analogs, morpholino doxorubicins, taxanes, cryptophycins, epothilones, and benzodiazepine dimers (e.g., dimmers of pyrrolbenzodiazepine (PBD) or tomaymycin), indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines), siRNA or a combination thereof, and/or pharmaceutically acceptable salts, acids or derivatives of any of the above molecules.
- 16.** The conjugate compounds of claims **2, 3, 7,** and **9** wherein the cell binding agent/molecule is selected from an antibody, a protein, a vitamin (e.g. folate), peptides, a polymeric micelle, a liposome, a lipoprotein-based drug carrier, a nano-particle drug carrier, a dendrimer, and a combination of above thereof.
- 17.** The cell-binding molecule/agent according to claims **2, 3, 7, 9** and **16** is preferred an antibody, a single chain antibody, an antibody fragment that binds to the target cell, a monoclonal

antibody, a single chain monoclonal antibody, or a monoclonal antibody fragment that binds 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, a resurfaced antibody, a resurfaced single chain antibody, or a resurfaced antibody fragment that binds to the target cell, a humanized antibody or a resurfaced antibody, a humanized single chain antibody, or a humanized antibody fragment that binds to the target cell, a lymphokine, a hormone, a vitamin, a growth factor, a colony stimulating factor, or a nutrient-transport molecule.

18. The cell-binding molecule/agent according to claims **2, 3, 7, 9** and **16** can be any agent that is able to target against a tumor cell, a virus infected cell, a microorganism infected cell, a parasite infected cell, an autoimmune disease cell, an activated tumor cells, a myeloid cell, an activated T-cell, an affecting B cell, or a melanocyte.

19. The cell-binding molecule/agent according to claims **2, 3, 7, 9** and **16** can be any agent/molecule that is able to against any one of the following antigens or receptors: CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD12w, CD14, CD15, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD51, CD52, CD53, CD54, CD55, CD56, CD58, CD59, CD61, CD62E, CD62L, CD62P, CD63, CD66, CD68, CD69, CD70, CD72, CD74, CD79, CD79a, CD79b, CD80, CD81, CD82, CD83, CD86, CD87, CD88, CD89, CD90, CD91, CD95, CD96, CD98, CD100, CD103, CD105, CD106, CD109, CD117, CD120, CD125, CD126, CD127, CD133, CD134, CD135, CD138, CD141, CD142, CD143, CD144, CD147, CD151, CD147, CD152, CD154, CD156, CD158, CD163, CD166, .CD168, CD174, CD180, CD184, CDw186, CD194, CD195, CD200, CD200a, CD200b, CD209, CD221, CD227, CD235a, CD240, CD262, CD271, CD274, CD276 (B7-H3), CD303, CD304, CD309, CD326, 4-1BB, 5AC, 5T4 (Trophoblast glycoprotein, TPBG, 5T4, Wnt-Activated Inhibitory Factor 1 or WAIF1), Adenocarcinoma antigen, AGS-5, AGS-22M6, Activin receptor-like kinase 1, AFP, AKAP-4, ALK, Alpha intergrin, Alpha v beta6, Amino-peptidase N, Amyloid beta, Androgen receptor, Angiopoietin 2, Angiopoietin 3, Annexin A1, Anthrax toxin protective antigen, Anti-transferrin receptor, AOC3 (VAP-1), B7-H3, Bacillus anthracis anthrax, BAFF (B-cell activating factor), B-lymphoma cell, bcr-abl, Bombesin, BORIS, C5, C242 antigen, CA125 (carbohydrate antigen 125, MUC16), CA-IX (or CAIX, carbonic anhydrase 9), CALLA, CanAg, Canis lupus familiaris IL31, Carbonic anhydrase IX, Cardiac myosin, CCL11(C-C motif chemokine 11), CCR4 (C-C chemokine receptor type 4, CD194), CCR5, CD3E (epsilon), CEA (Carcinoembryonic antigen), CEACAM3, CEACAM5 (carcinoembryonic antigen), CFD (Fac-

tor D), Ch4D5, Cholecystokinin 2 (CCK2R), CLDN18 (Claudin-18), Clumping factor A, CRIPTO, FCSF1R (Colony stimulating factor 1 receptor, CD115), CSF2 (colony stimulating factor 2, Granulocyte-macrophage colony-stimulating factor (GM-CSF)), CTLA4 (cytotoxic T-lymphocyte-associated protein 4), CTAA16.88 tumor antigen, CXCR4 (CD184), C-X-C chemokine receptor type 4, cyclic ADP ribose hydrolase, Cyclin B1, CYP1B1, Cytomegalovirus, Cytomegalovirus glycoprotein B, Dabigatran, DLL4 (delta-like-ligand 4), DPP4 (Dipeptidyl-peptidase 4), DR5 (Death receptor 5), E. coli shiga toxin type-1, E. coli shiga toxin type-2, ED-B, EGFL7 (EGF-like domain-containing protein 7), EGFR, EGFRII, EGFRvIII, Endoglin (CD105), Endothelin B receptor, Endotoxin, EpCAM (epithelial cell adhesion molecule), EphA2, Episialin, ERBB2 (Epidermal Growth Factor Receptor 2), ERBB3, ERG (TMPRSS2 ETS fusion gene), Escherichia coli, ETV6-AML, FAP (Fibroblast activation protein alpha), FCGR1, alpha-Fetoprotein, Fibrin II, beta chain, Fibronectin extra domain-B, FOLR (folate receptor), Folate receptor alpha, Folate hydrolase, Fos-related antigen 1.F protein of respiratory syncytial virus, Frizzled receptor, Fucosyl GM1, GD2 ganglioside, G-28 (a cell surface antigen glycolipid), GD3 idiootype, GloboH, Glypican 3, N-glycolylneuraminic acid, GM3, GMCSF receptor α -chain, Growth differentiation factor 8, GP100, GPNMB (Transmembrane glycoprotein NMB), GUCY2C (Guanylate cyclase 2C, guanylyl cyclase C(GC-C), intestinal Guanylate cyclase, Guanylate cyclase-C receptor, Heat-stable enterotoxin receptor (hSTAR)), Heat shock proteins, Hemagglutinin, Hepatitis B surface antigen, Hepatitis B virus, HER1 (human epidermal growth factor receptor 1), HER2, HER2/neu, HER3 (ERBB-3), IgG4, HGF/SF (Hepatocyte growth factor/scatter factor), HHGFR, HIV-1, Histone complex, HLA-DR (human leukocyte antigen), HLA-DR10, HLA-DRB, HMWMAA, Human chorionic gonadotropin, HNGF, Human scatter factor receptor kinase, HPV E6/E7, Hsp90, hTERT, ICAM-1 (Intercellular Adhesion Molecule 1), Idiotype, IGF1R (IGF-1, insulin-like growth factor 1 receptor), IGHE, IFN- γ , Influenza hemagglutinin, IgE, IgE Fc region, IGHE, IL-1, IL-2 receptor (interleukin 2 receptor), IL-4, IL-5, IL-6, IL-6R (interleukin 6 receptor), IL-9, IL-10, IL-12, IL-13, IL-17, IL-17A, IL-20, IL-22, IL-23, IL31RA, ILGF2 (Insulin-like growth factor 2), Integrins (α 4, α _{IIb} β 3, α v β 3, α 4 β 7, α 5 β 1, α 6 β 4, α 7 β 7, α 11 β 3, α 5 β 5, α v β 5), Interferon gamma-induced protein, ITGA2, ITGB2, KIR2D, LCK, Le, Legumain, Lewis-Y antigen, LFA-1(Lymphocyte function-associated antigen 1, CD11a), LHRH, LINGO-1, Lipoteichoic acid, LIV1A, LMP2, LTA, MAD-CT-1, MAD-CT-2, MAGE-1, MAGE-2, MAGE-3, MAGE A1, MAGE A3, MAGE 4, MART1, MCP-1, MIF (Macrophage migration inhibitory factor, or glycosylation-inhibiting factor (GIF)), MS4A1 (membrane-spanning 4-domains subfamily A member 1), MSLN (mesothelin), MUC1(Mucin 1, cell surface associated (MUC1) or polymorphic epithelial mucin (PEM)), MUC1-KLH, MUC16

(CA125), MCP1(monocyte chemotactic protein 1), MelanA/MART1, ML-IAP, MPG, MS4A1 (membrane-spanning 4-domains subfamily A), MYCN, Myelin-associated glycoprotein, Myostatin, NA17, NARP-1, NCA-90 (granulocyte antigen), Nectin-4 (ASG-22ME), NGF, Neural apoptosis-regulated proteinase 1, NOGO-A, Notch receptor, Nucleolin, Neu oncogene product, NY-BR-1, NY-ESO-1, OX-40, OxLDL (Oxidized low-density lipoprotein), OY-TES1, P21, p53 nonmutant, P97, Page4, PAP, Paratope of anti-(N-glycolylneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1,CD279), PDGF-R α (Alpha-type platelet-derived growth factor receptor), PDGFR- β , PDL-1, PLAC1, PLAP-like testicular alkaline phosphatase, Platelet-derived growth factor receptor beta, Phosphate-sodium co-transporter, PMEL 17, Polysialic acid, Proteinase3 (PR1), Prostatic carcinoma, PS (Phosphatidylserine), Prostatic carcinoma cells, Pseudomonas aeruginosa, PSMA, PSA, PSCA, Rabies virus glycoprotein, RHD (Rh polypeptide 1 (RhPI), CD240), Rhesus factor, RANKL, RhoC, Ras mutant, RGS5, ROBO4, Respiratory syncytial virus, RON, Sarcoma translocation breakpoints, SART3, Sclerostin, SLAMF7 (SLAM family member 7), Selectin P, SDC1 (Syndecan 1), sLe(a), Somatomedin C, SIP (Sphingosine-1-phosphate), Somatostatin, Sperm protein 17, SSX2, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), STEAP2, STn, TAG-72 (tumor associated glycoprotein 72), Survivin, T-cell receptor, T cell transmembrane protein, TEM1 (Tumor endothelial marker 1), TENB2, Tenascin C (TN-C), TGF- α , TGF- β (Transforming growth factor beta), TGF- β 1, TGF- β 2 (Transforming growth factor-beta 2), Tie (CD202b), Tie2, TIM-1 (CDX-014), Tn, TNF, TNF- α , TNFRSF8, TNFRSF10B (tumor necrosis factor receptor superfamily member 10B), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B), TPBG (trophoblast glycoprotein), TRAIL-R1 (Tumor necrosis apoptosis Inducing ligand Receptor 1), TRAILR2 (Death receptor 5 (DR5)), tumor-associated calcium signal transducer 2, tumor specific glycosylation of MUC1, TWEAK receptor, TYRP1(glycoprotein 75), TRP-2, Tyrosinase, VCAM-1 (CD106), VEGF, VEGF-A, VEGF-2 (CD309), VEGFR-1, VEGFR2, or vimentin, WT1, XAGE 1, or cells expressing any insulin growth factor receptors, or any epidermal growth factor receptors.

20. The tumor cell according to claim **18** is selected from lymphoma cells, myeloma cells, renal cells, breast cancer cells, prostate cancer cells, ovarian cancer cells, colorectal cancer cells, gastric cancer cells, squamous cancer cells, small-cell lung cancer cells, none small-cell lung cancer cells, testicular cancer cells, or any cells that grow and divide at an unregulated, quickened pace to cause cancers.

21. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate compounds of claim **2** and/or **8**, and a pharmaceutically acceptable salt, carrier, diluent,

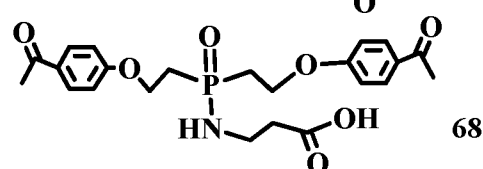
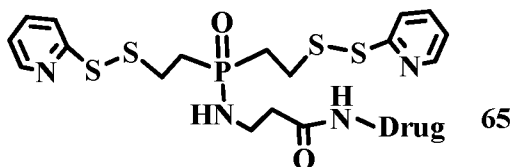
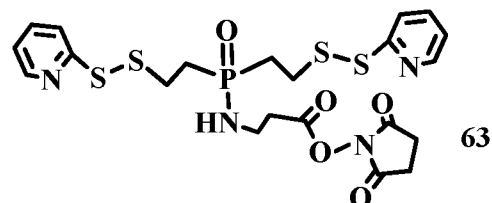
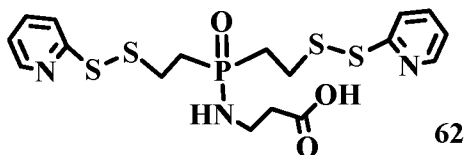
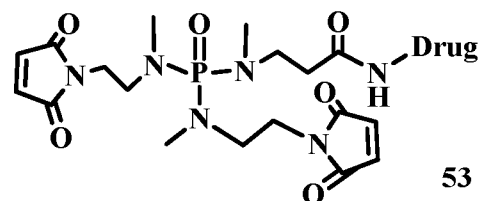
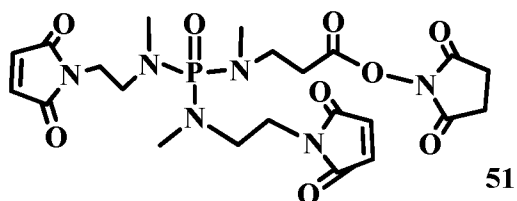
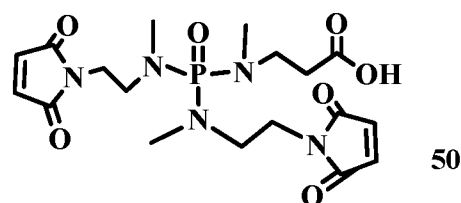
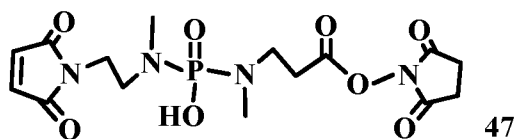
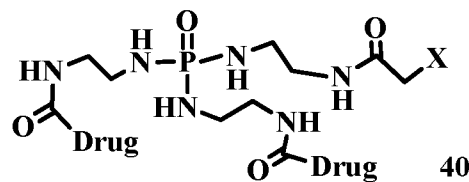
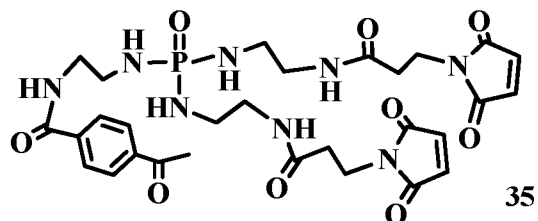
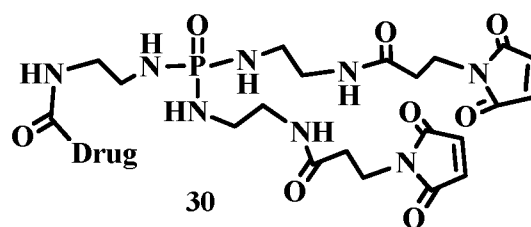
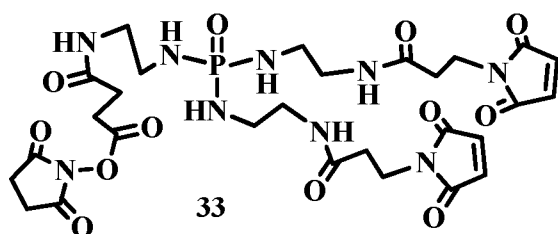
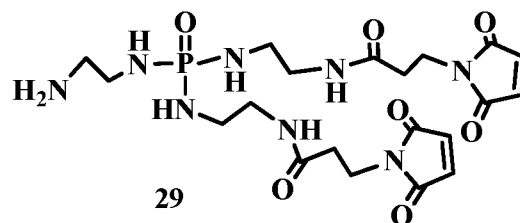
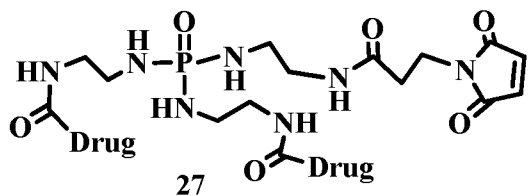
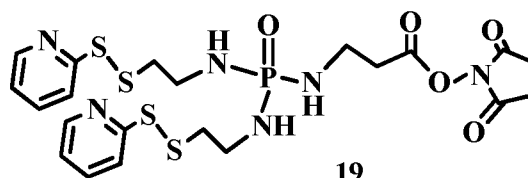
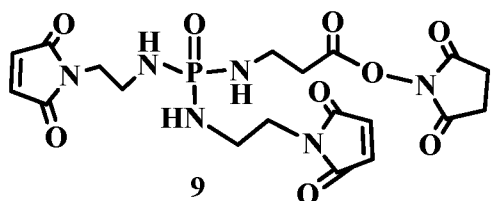
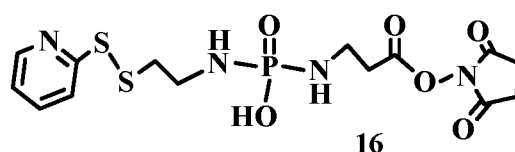
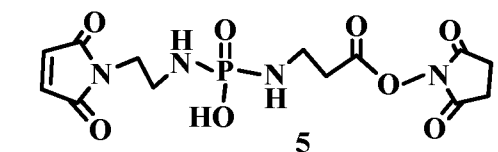
or excipient therefore, or a combination therefore, for the treatment or prevention of a cancer, or an autoimmune disease, or an infectious disease.

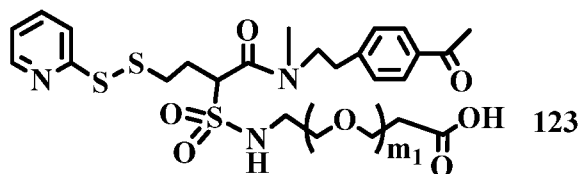
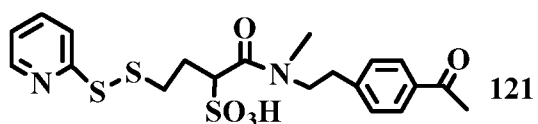
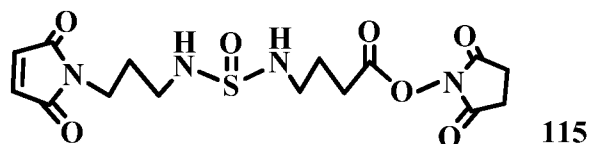
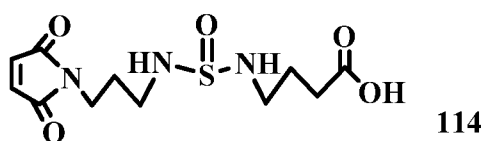
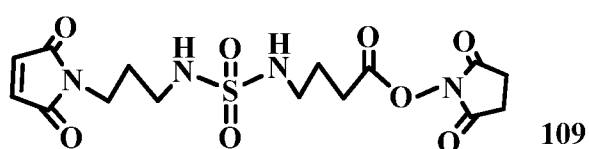
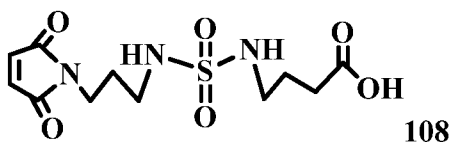
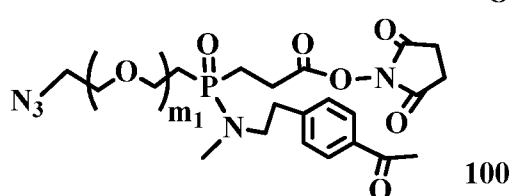
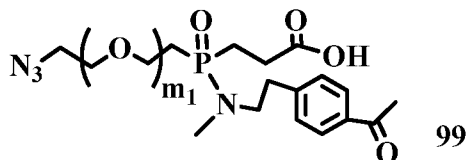
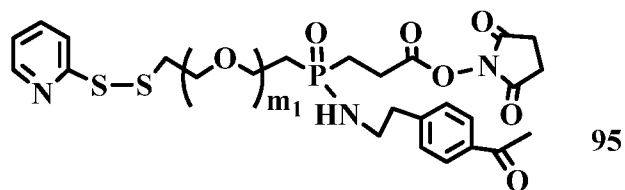
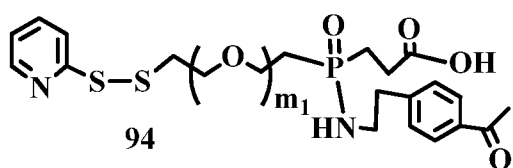
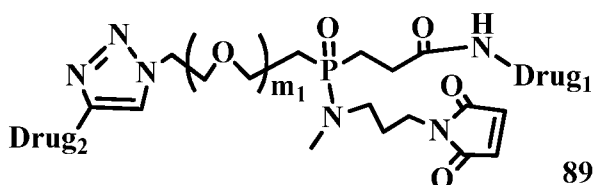
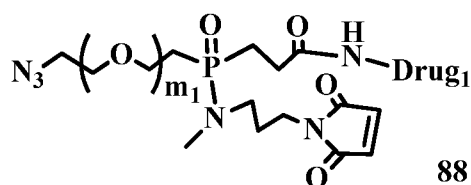
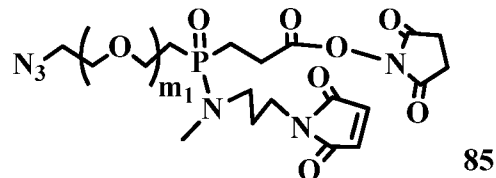
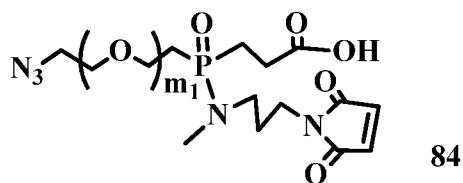
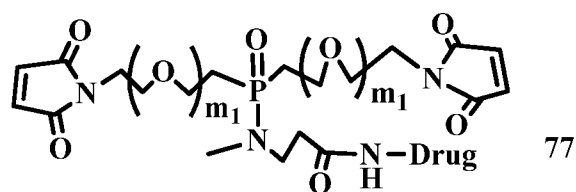
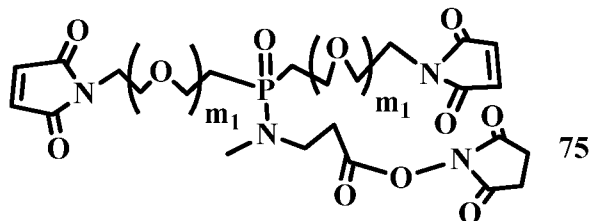
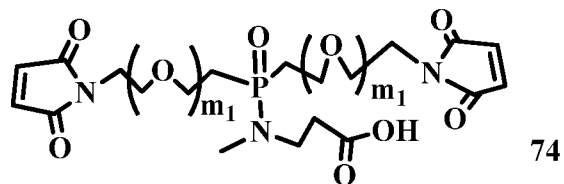
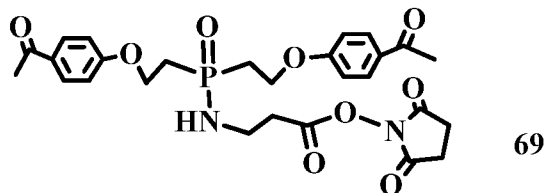
22. The conjugate compounds of claims **2**, **4**, **8** and/or **12**, wherein the drug/cytotoxic agent is selected from a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, a nucleolytic enzyme and a chromophore molecule.

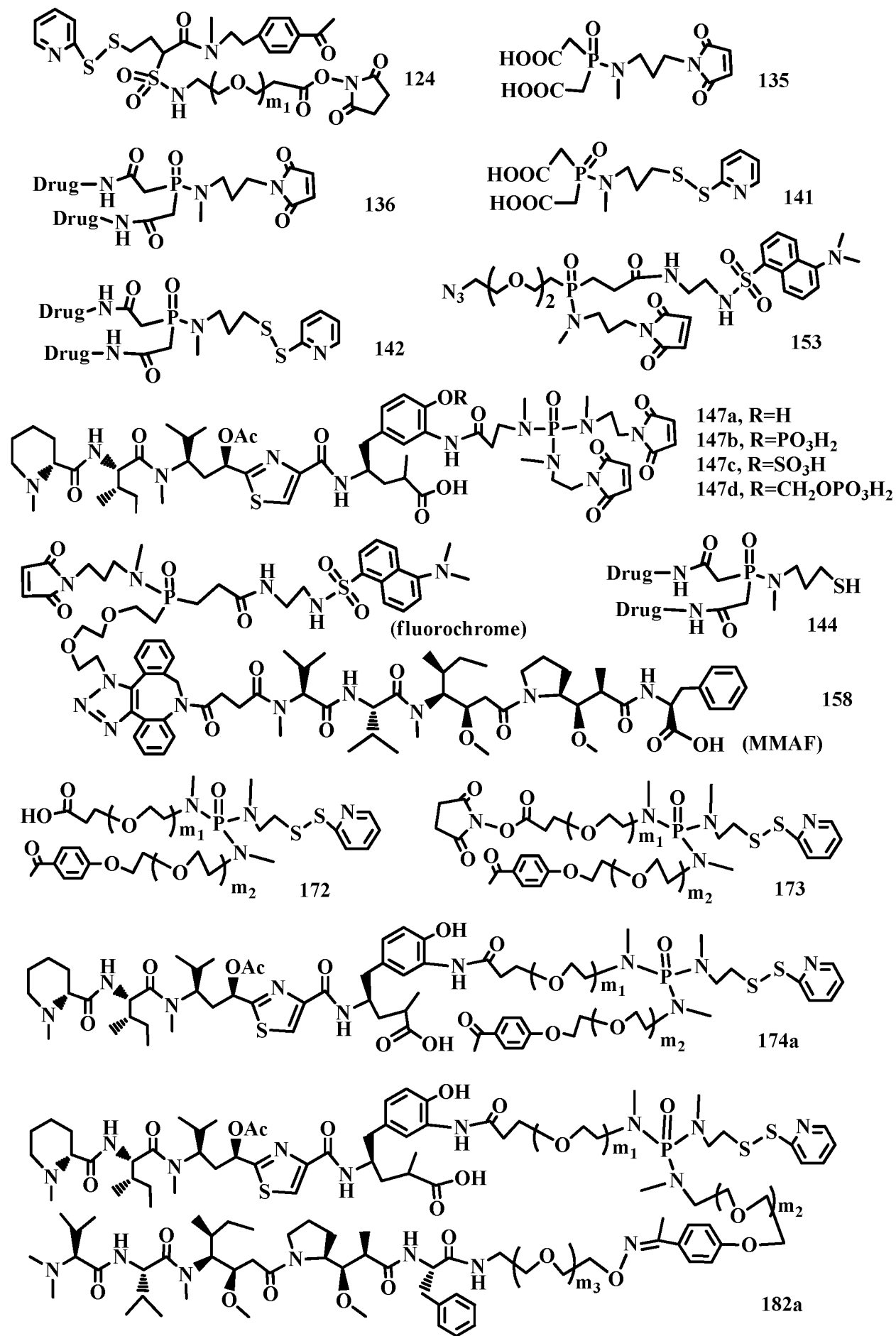
23. The compounds of Formula (I) of claims **1**, **5** and **6**, wherein the linkage components of the compounds of Formula (I) can be composed of one or more linker components of: 6-maleimidocaproyl (MC), maleimido propanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), lysine-phenylalanine (lys-phe), p-aminobenzyloxycarbonyl (PAB), 4-thio-pentanoate (SPP), 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (MCC), 4-thio-butyrates (SPDB), maleimidoethyl (ME), 4-thio-2-hydroxysulfonyl-butyrates (2-Sulfo-SPDB), pyridinyl-dithiol (PySS), alkoxy amino (AOA), ethyleneoxy (EO), 4-methyl-4-dithio-pentanoic (MPDP), azido (N₃), alkynyl, dithio, peptide, and/or (4-acetyl)aminobenzoate (SIAB).

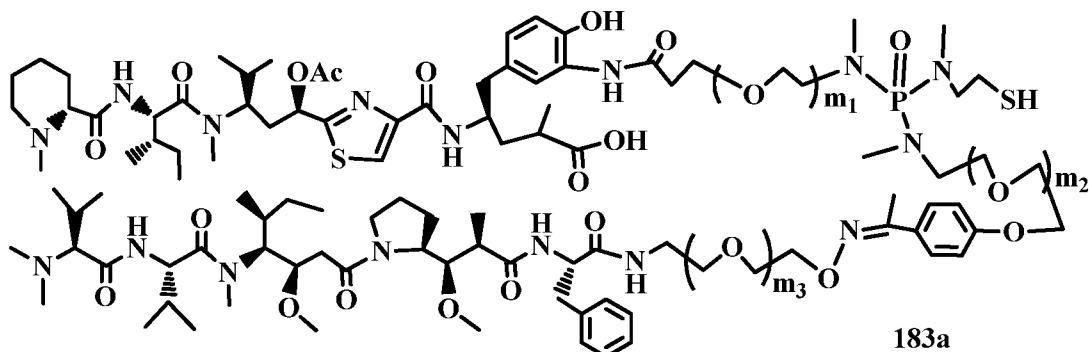
24. The conjugate compounds of claims **2**, **8** and/or **22**, wherein "Drug" is preferably selected from tubulysins, maytansinoids, taxanoids (taxanes), CC-1065 analogs, daunorubicin and doxorubicin compounds, benzodiazepine dimers (e.g., dimers of pyrrolbenzodiazepine (PBD), tomaymycin, anthramycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines), calicheamicins and the enediyne antibiotics, actinomycin, azaserines, bleomycins, epirubicin, tamoxifen, idarubicin, dolastatins/auristatins (e.g. monomethyl auristatin E, MMAE, MMAF, auristatin PYE, auristatin TP, Auristatins 2-AQ, 6-AQ, EB (AEB), and EFP (AEFP)), duocarmycins, thiotepa, vincristine, hemiasterlins, nazumamides, microginins, radiosumins, alterobactins, microsclerodermins, theonellamides, esperamicins, and their analogues and derivatives thereof.

25. A compound according to claims **1** and **4** containing a functional group that enables reaction with a cell-binding agent comprises the formula compounds 5, 9, 16, 19, 27, 29, 30, 35, 40, 47, 50, 51, 53, 62, 63, 65, 68, 69, 74, 75, 77, 84, 85, 88, 89, 94, 95, 99, 100, 108, 109, 114, 115, 121, 123, 124, 135, 136, 141, 142, 144, 147a, 147b, 147c, 147d, 153, 158, 172, 173, 174, 182, 183, 193, 194, 195, 202, 203, 204, 207, 208, and 209, of which are illustrated below:

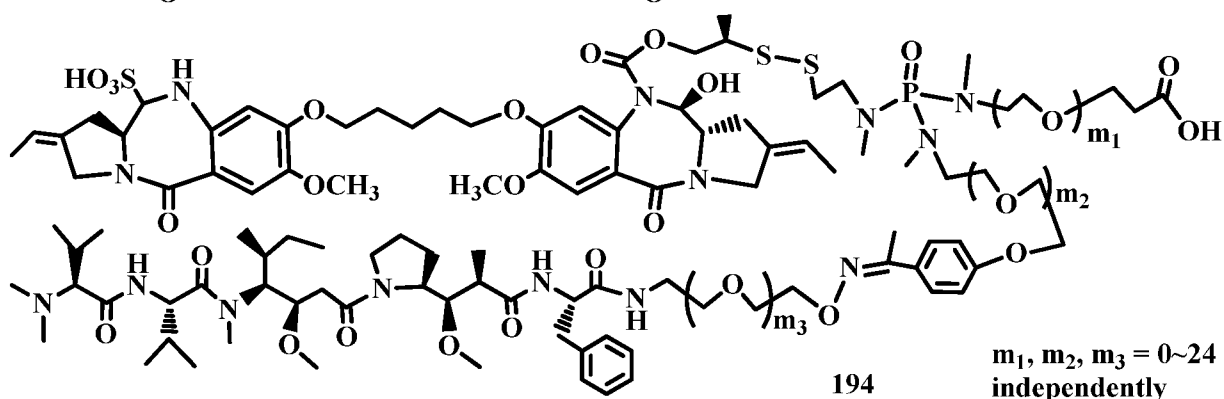
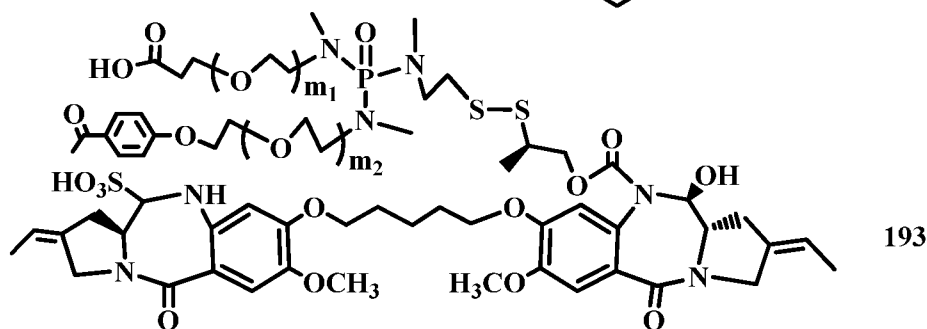




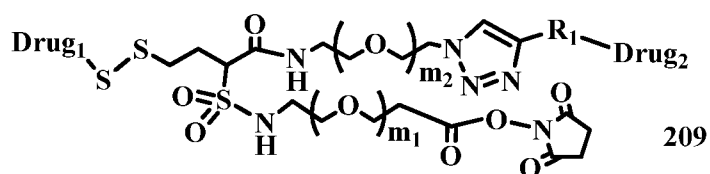
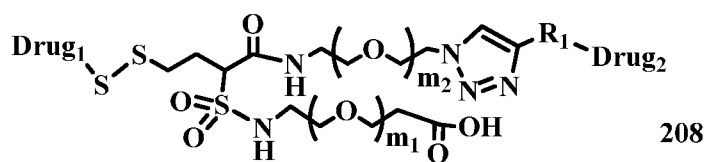
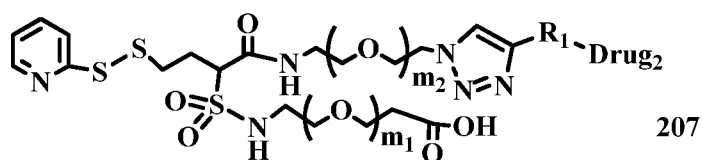
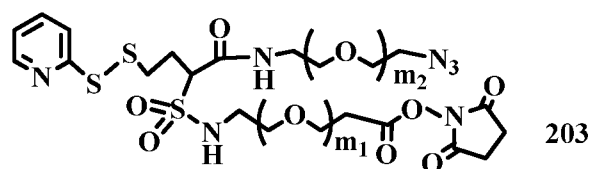
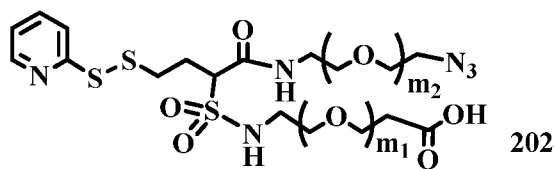




$m_1, m_2, m_3 = 0\sim 24$
independently

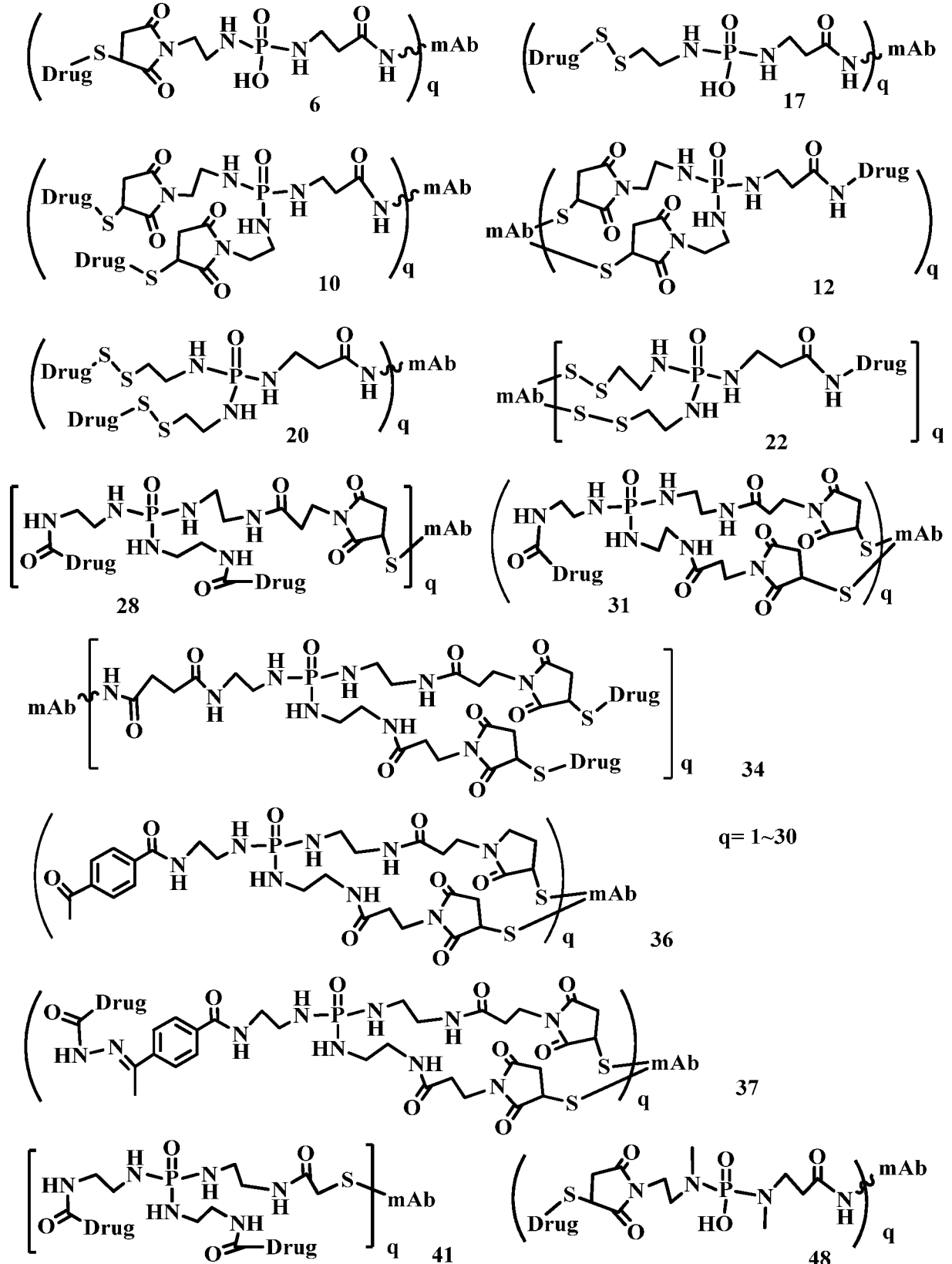


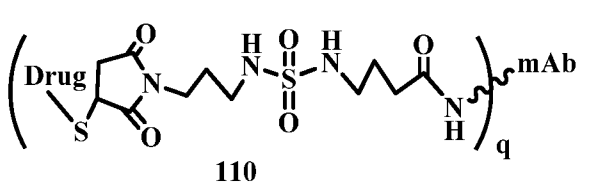
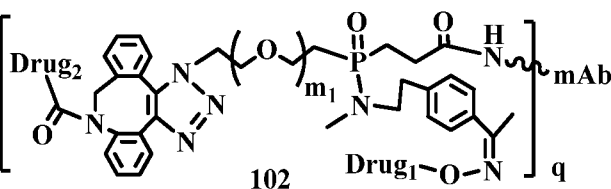
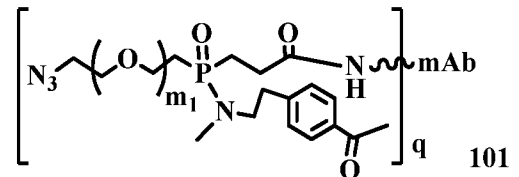
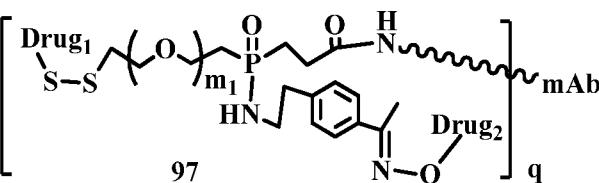
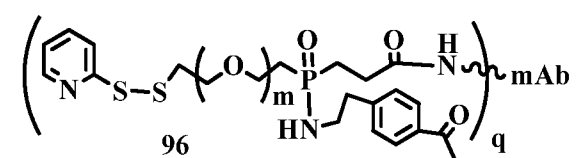
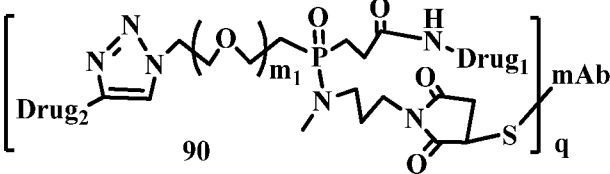
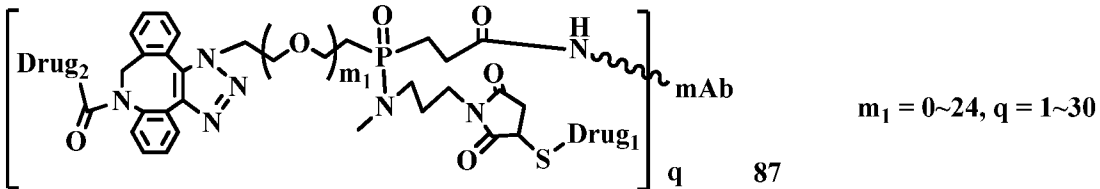
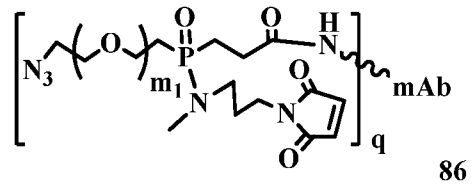
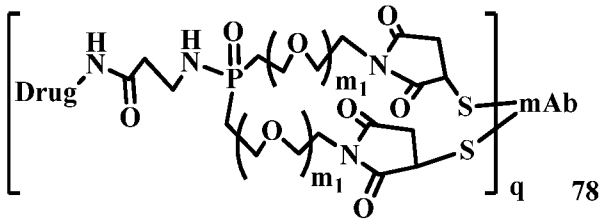
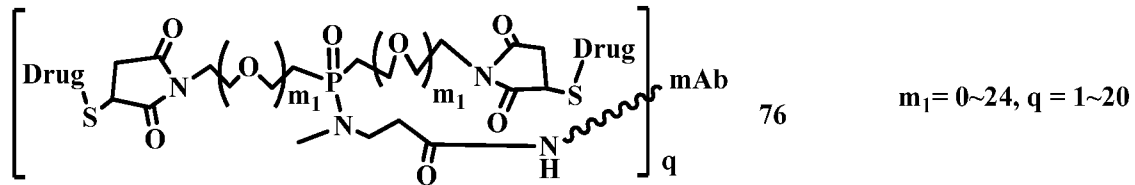
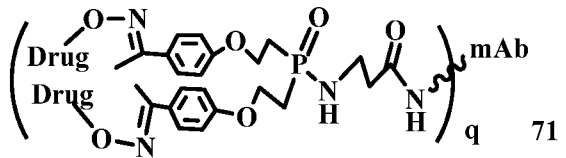
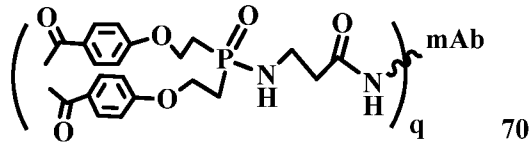
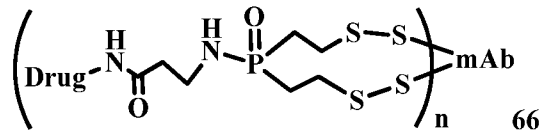
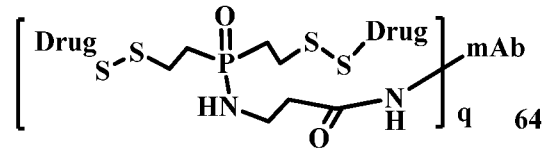
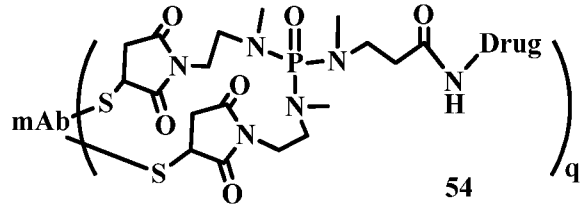
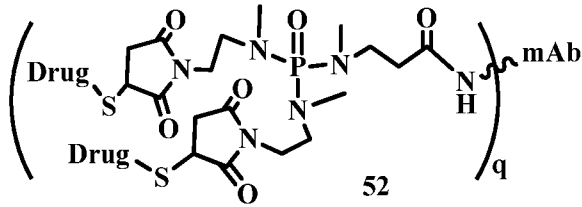
$m_1, m_2, m_3 = 0\sim 24$
independently

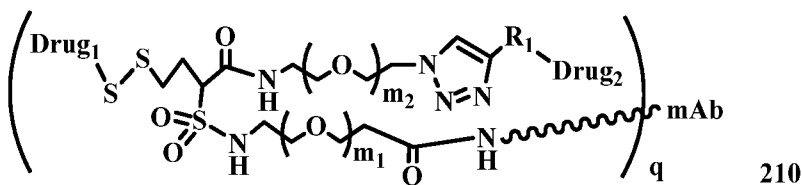
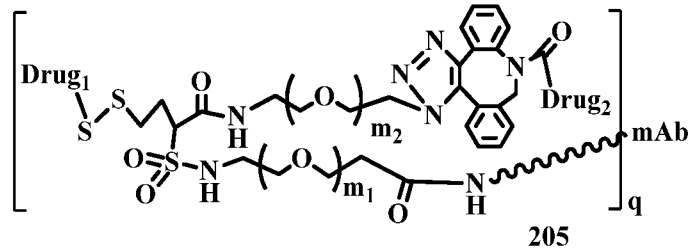
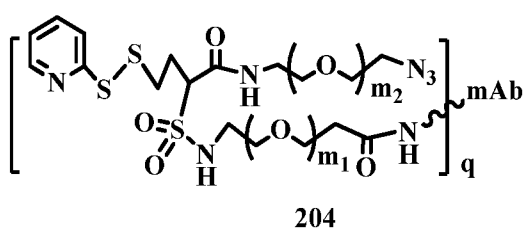
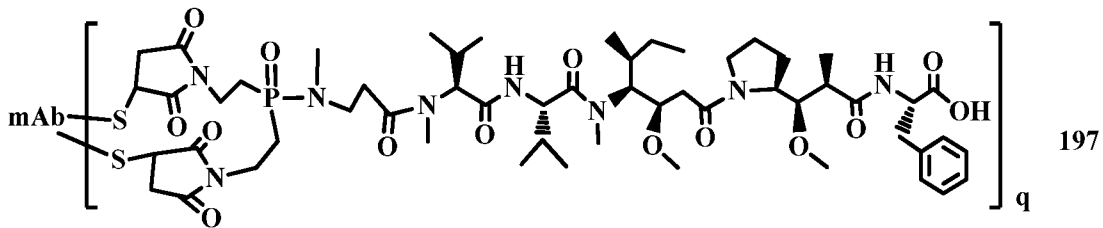
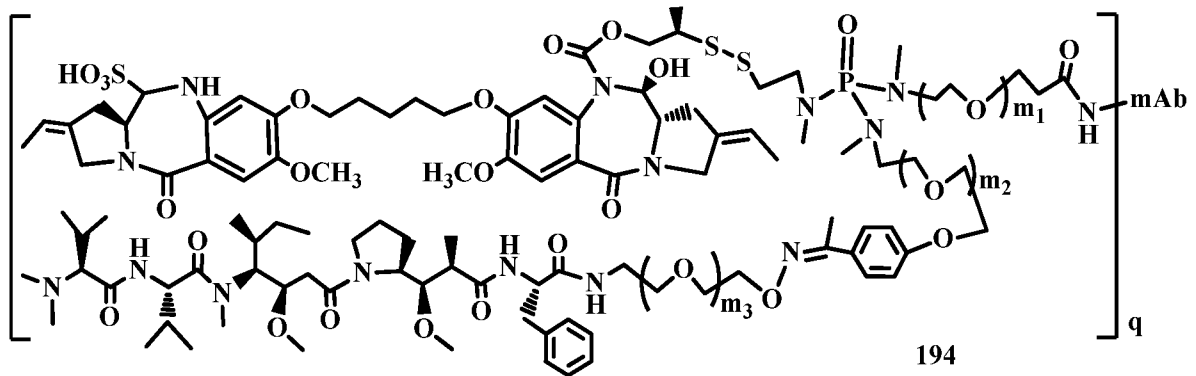
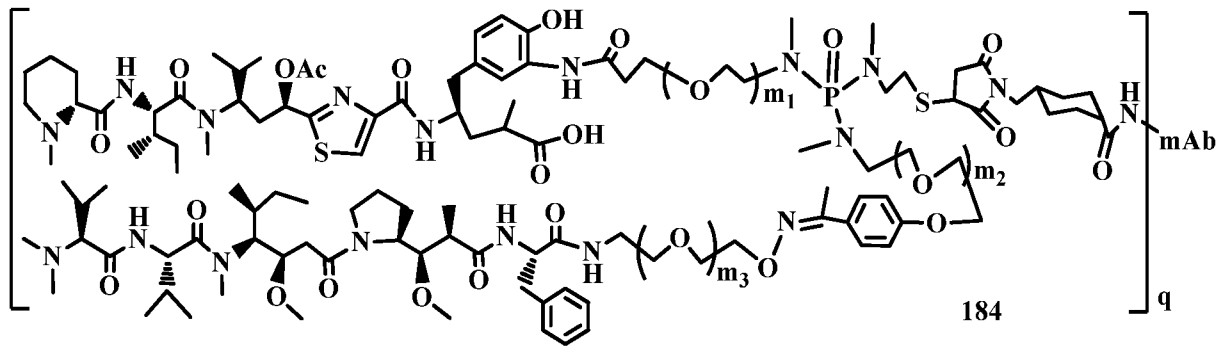
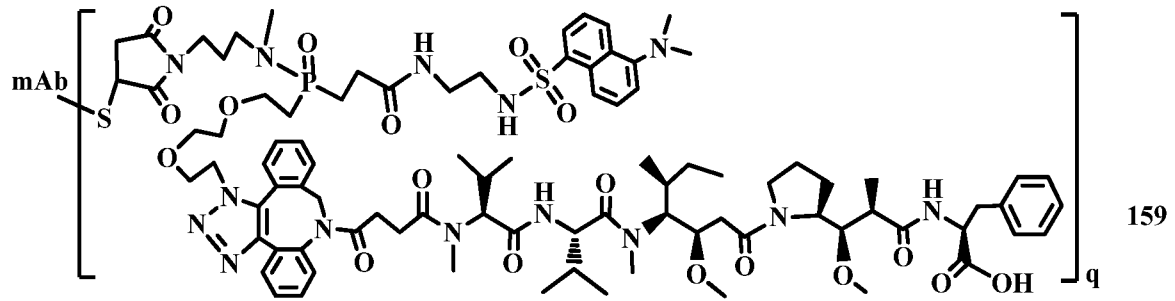


Wherein m_1, m_2 and m_3 are 0~24 independently, R_1 and Drug are defined in claims 1, 13, 14.

26. The compound according to claims 2 and 3 comprises the formula of 6, 17, 10, 12, 20, 22, 28, 31, 34, 36, 37, 41, 48, 52, 54, 64, 66, 70, 71, 76, 78, 86, 87, 90, 96, 97, 101, 102, 110, 116, 125, 126, 127, 137, 143, 145, 148a, 148b, 148c, 148d, 159, 184, 185, 194, 197, 205, 210, which are illustrated below:







Wherein m_1 , m_2 and m_3 are 0~24 independently, R_1 , q and Drug are defined in claims 1, 13, 14.

- 27.** The conjugate of claim **2**, **7**, or **8**, having in vitro, in vivo or ex vivo cell killing activity.
- 28.** The conjugate compound of claim **2**, **7**, and/or **8**, can comprise either a peptides of 1~20 units of natural or unnatural amino acids, or a p-aminobenzyl unit, or a 6-maleimidocaproyl unit, or a disulfide unit, or a thioether unit, or a hydrozone unit, a triazole unit, or an alkoxime unit.
- 29.** The conjugate of claim **2**, **7** and/or **8**, wherein the linkage components of this invention can be cleavable by a protease.
- 30.** A pharmaceutical composition comprising a therapeutically effective amount of the conjugate of claim **2**, **7** or/and **8**, administered concurrently with the other therapeutic agents such as the chemotherapeutic agent, the radiation therapy, immunotherapy agents, autoimmune disorder agents, anti-infectious agents or the other conjugates for synergistically effective treatment or prevention of a cancer, or an autoimmune disease, or an infectious disease.
- 31.** The synergistic agents according to claim **30** are preferably selected from one or several of the following drugs: Abatacept (Orencia), Abiraterone acetate (Zytiga®), Acetaminophen /hydrocodone, Adalimumab, afatinib dimaleate (Gilotrif®), alemtuzumab (Campath®), Alitretinoin (Panretin®), ado-trastuzumab emtansine (Kadcyla™), Amphetamine mixed salts (Amphetamine/dextroamphetamine, or Adderall XR), anastrozole (Arimidex®), Aripiprazole, Atazanavir, Atezolizumab (MPDL3280A), Atorvastatin, axitinib (Inlyta®), belinostat (Beleodaq™), Bevacizumab (Avastin®), Cabazitaxel (Jevtana®), Cabozantinib (Cometriq™), bexarotene (Targretin®), blinatumomab (Blincyto™), Bortezomib (Velcade®), bosutinib (Bosulif®), brentuximab vedotin (Adcetris®), Budesonide, Budesonide/formoterol, Buprenorphine, Capecitabine, carfilzomib (Kyprolis®), Celecoxib, ceritinib (LDK378/Zykadia), Cetuximab (Erbix®), Ciclosporin, Cinacalcet, crizotinib (Xalkori®), Dabigatran, dabrafenib (Tafinlar®), Darbepoetin alfa, Darunavir, imatinib mesylate (Gleevec®), dasatinib (Sprycel®), denileukin diftitox (Ontak®), Denosumab (Xgeva®), Depakote, Dexlansoprazole, Dexmethylphenidate, Dinutuximab (Unituxin™), Doxycycline, Duloxetine, Emtricitabine/Rilpivirine/Tenofovir disoproxil fumarate, Emtricitabine/tenofovir/efavirenz, Enoxaparin, Enzalutamide (Xtandi®), Epoetin alfa, erlotinib (Tarceva®), Esomeprazole, Eszopiclone, Etanercept, Everolimus (Afinitor®), exemestane (Aromasin®), everolimus (Afinitor®), Ezetimibe, Ezetimibe/simvastatin, Fenofibrate, Filgrastim, fingolimod, Fluticasone propionate, Fluticasone/salmeterol, fulvestrant (Faslodex®), gefitinib (Iressa®), Glatiramer, Goserelin acetate (Zoladex), Imatinib (Gleevec), Ibritumomab tiuxetan (Zevalin®), ibrutinib (Imbruvica™), idelalisib (Zydelig®), Infliximab, Insulin aspart, Insulin detemir, Insulin glargine, Insulin lispro, Interferon beta 1a, Interferon beta 1b, lapatinib

(Tykerb®), Ipilimumab (Yervoy®), Ipratropium bromide/salbutamol, Lanreotide acetate (Somatuline® Depot), lenaliomide (Revlimid®), lenvatinib mesylate (Lenvima™), letrozole (Femara®), Levothyroxine, Levodopa, Lidocaine, Linezolid, Liraglutide, Lisdexamphetamine, MEDI4736 (AstraZeneca, Celgene), Memantine, Methylphenidate, Metoprolol, Modafinil, Mometasone, Nilotinib (Tasigna®), Nivolumab (Opdivo®), ofatumumab (Arzerra®), obinutuzumab (Gazyva™), olaparib (Lynparza™), Olmesartan, Olmesartan/hydrochlorothiazide, Omalizumab, Omega-3 fatty acid ethyl esters, Oseltamivir, Oxycodone, palbociclib (Ibrance®), Palivizumab, panitumumab (Vectibix®), panobinostat (Farydak®), pazopanib (Votrient®), pembrolizumab (Keytruda®), Pemetrexed (Alimta), pertuzumab (Perjeta™), Pneumococcal conjugate vaccine, pomalidomide (Pomalyst®), Pregabalin, Quetiapine, Rabeprazole, radium 223 chloride (Xofigo®), Raloxifene, Raltegravir, ramucirumab (Cyramza®), Ranibizumab, regorafenib (Stivarga®), Rituximab (Rituxan®), Rivaroxaban, romidepsin (Istodax®), Rosuvastatin, ruxolitinib phosphate (Jakafi™), Salbutamol, Sevelamer, Sildenafil, siltuximab (Sylvant™), Sitagliptin, Sitagliptin/metformin, Solifenacin, Sorafenib (Nexavar®), Sunitinib (Sutent®), Tadalafil, tamoxifen, Telaprevir, temsirolimus (Torisel®), Tenofovir/emtricitabine, Testosterone gel, Thalidomide (Immunoprin, Talidex), Tiotropium bromide, toremifene (Fareston®), trametinib (Mekinist®), Trastuzumab, Tretinoin (Vesanoid®), Ustekinumab, Valsartan, vandetanib (Caprelsa®), vemurafenib (Zelboraf®), vorinostat (Zolinza®), ziv-aflibercept (Zaltrap®), Zostavax., and their analogs, derivatives, pharmaceutically acceptable salts, carriers, diluents, or excipients therefore, or a combination above therefore,

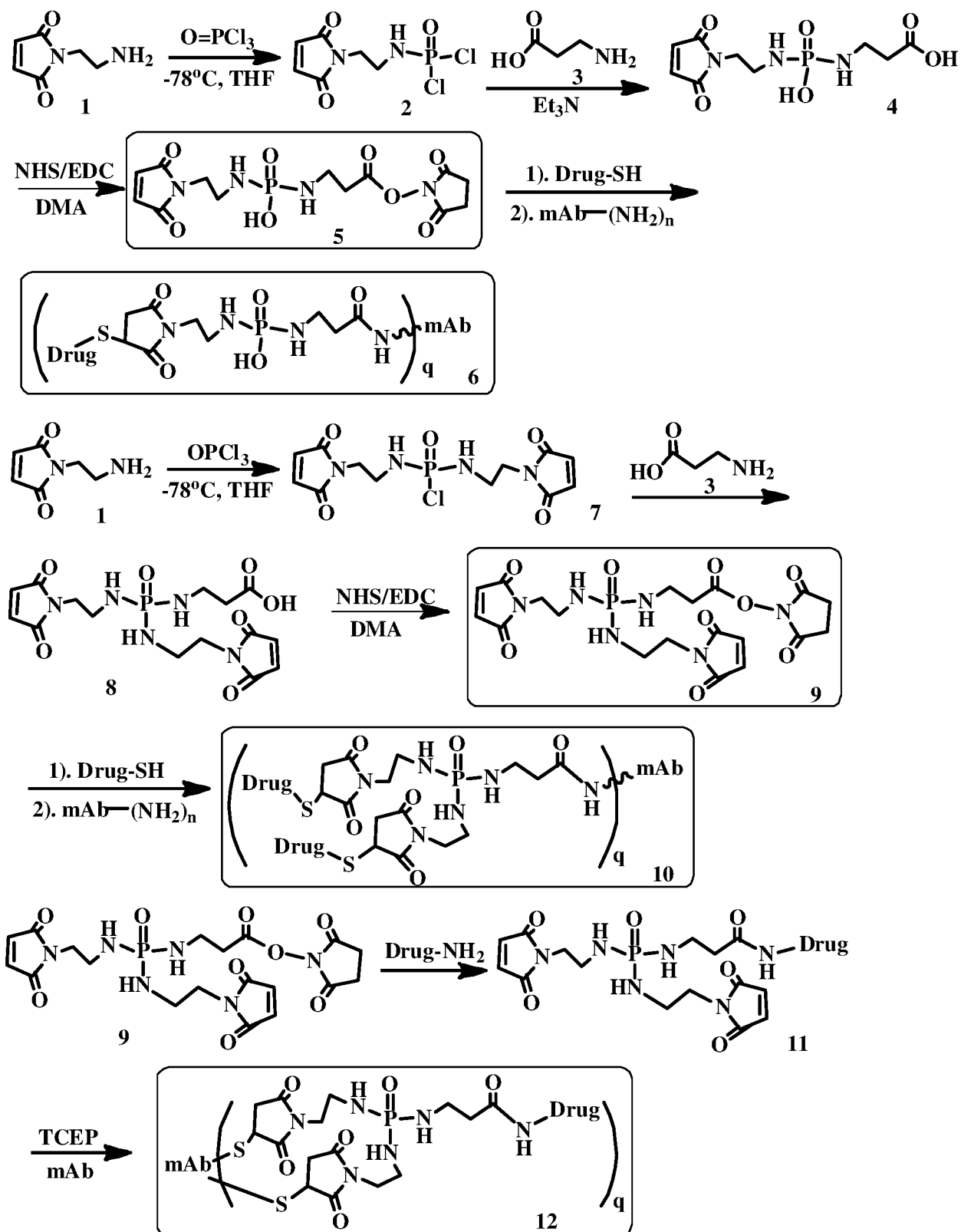


Figure 1

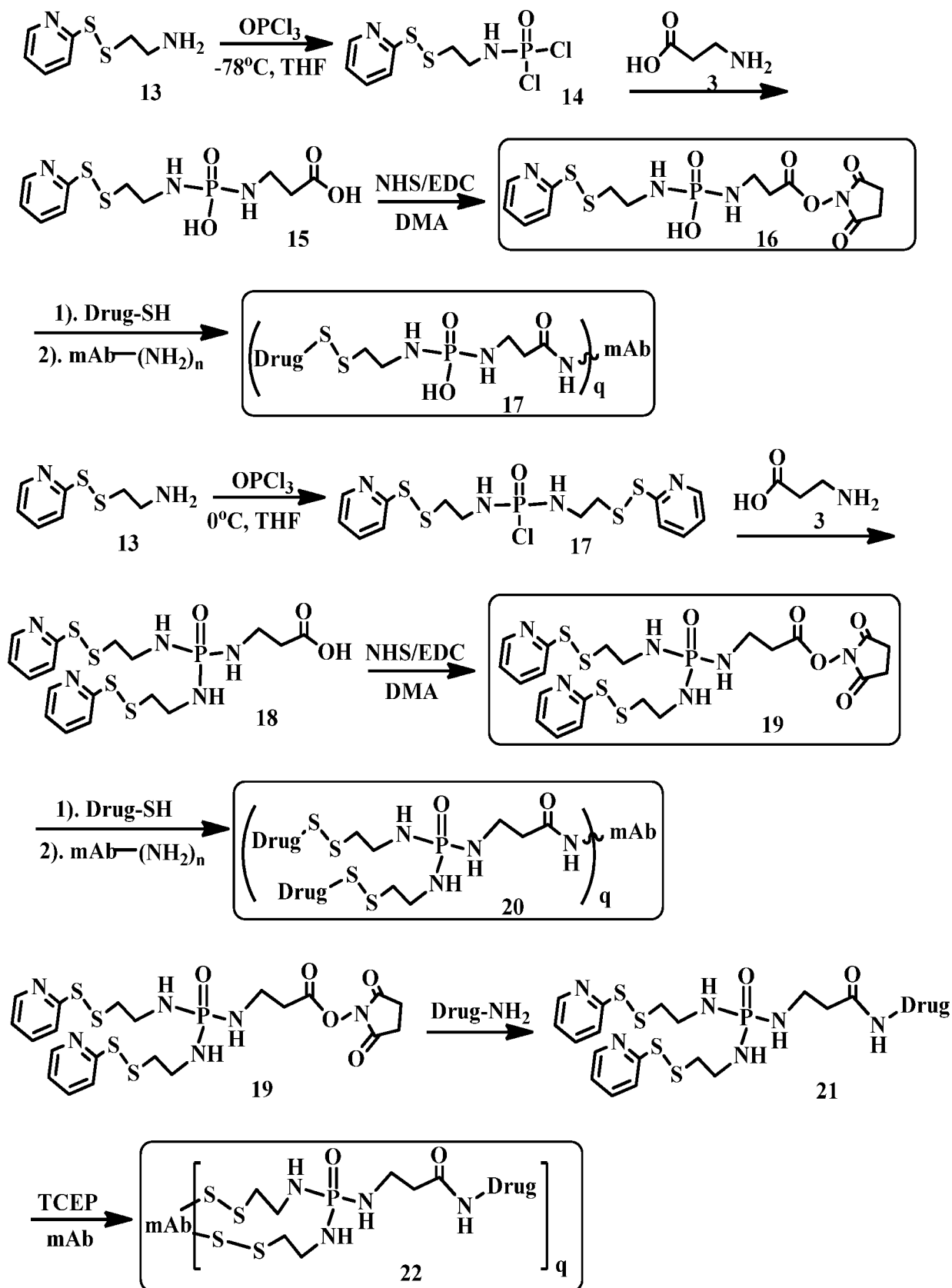


Figure 2

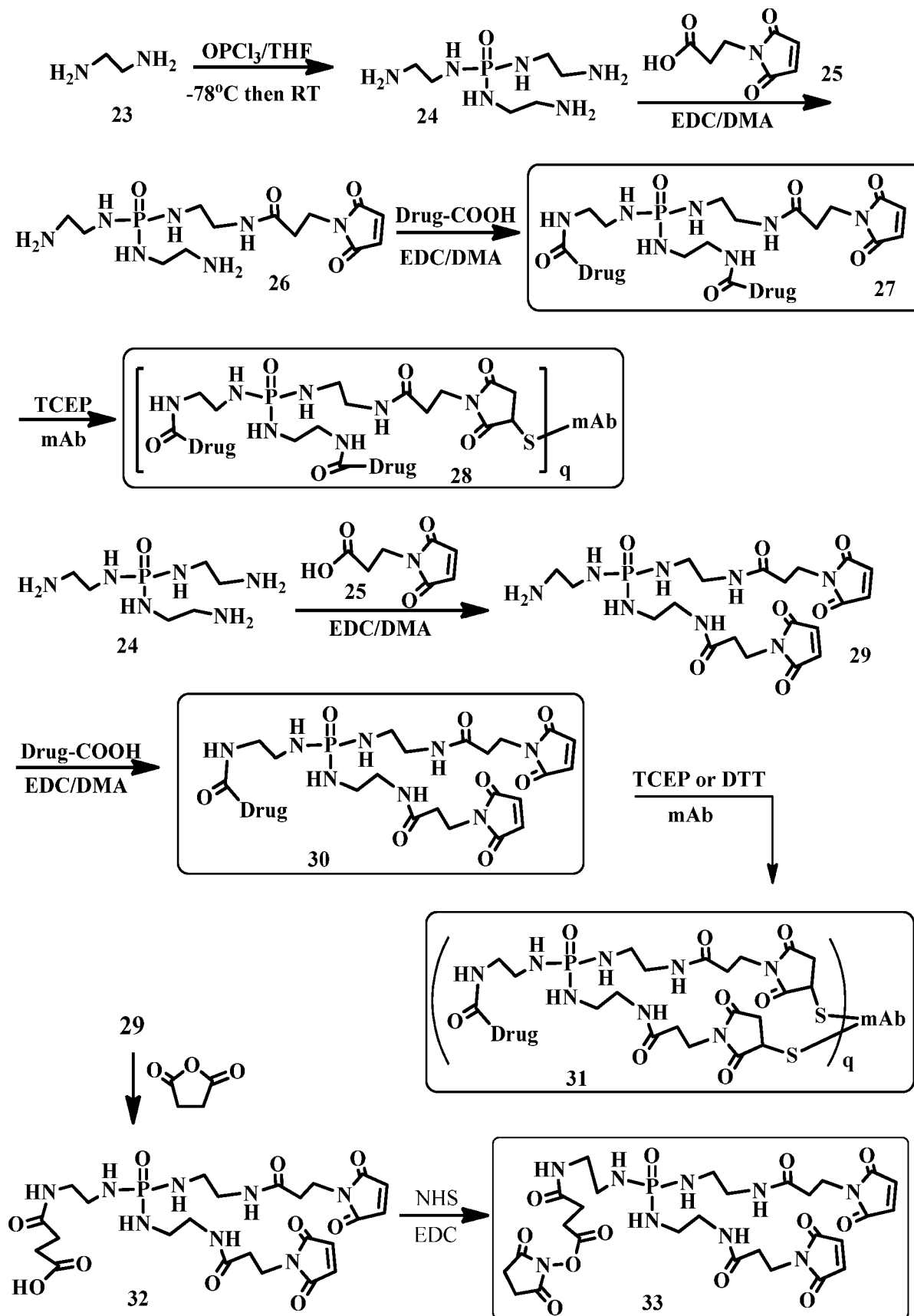


Figure 3

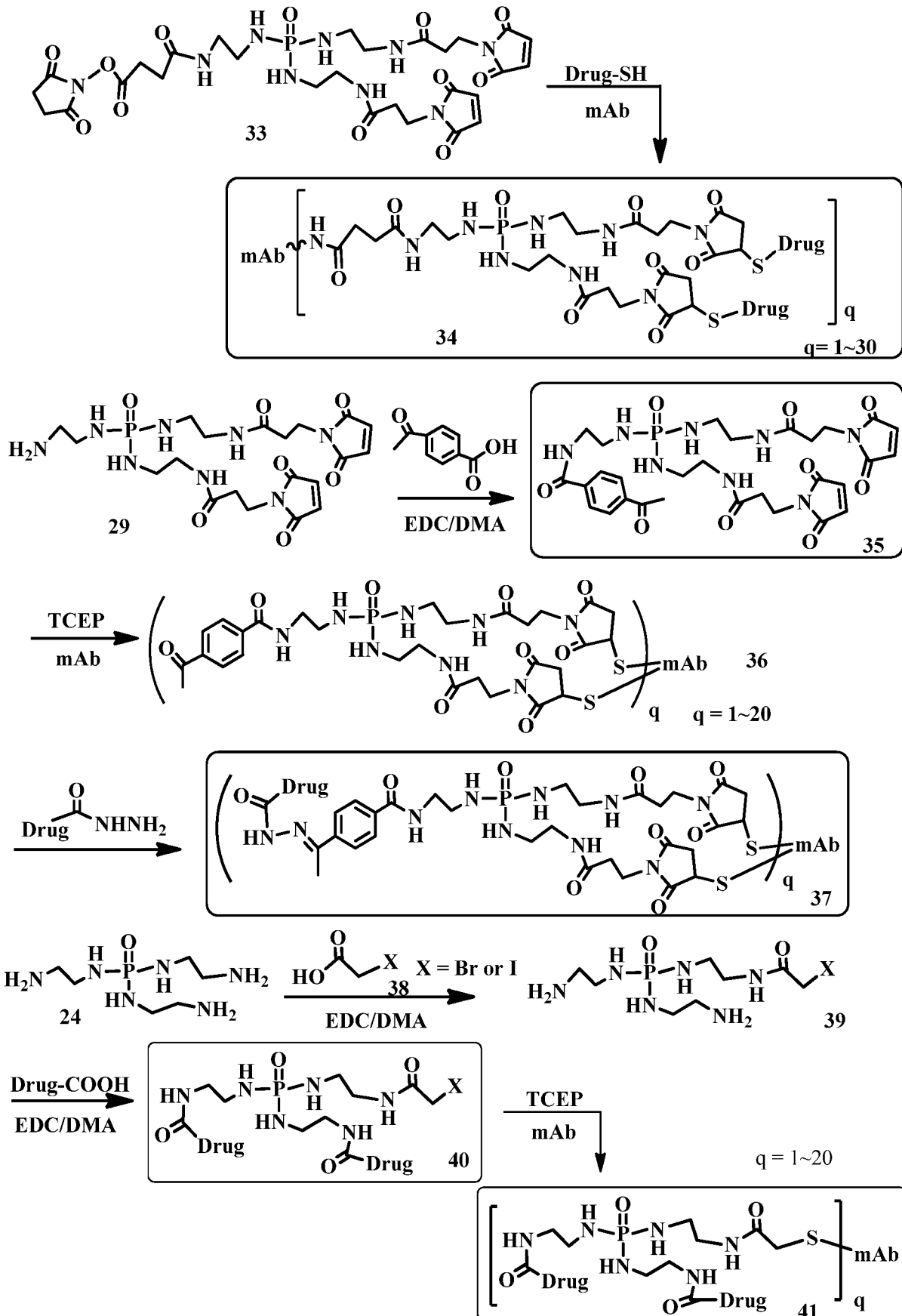


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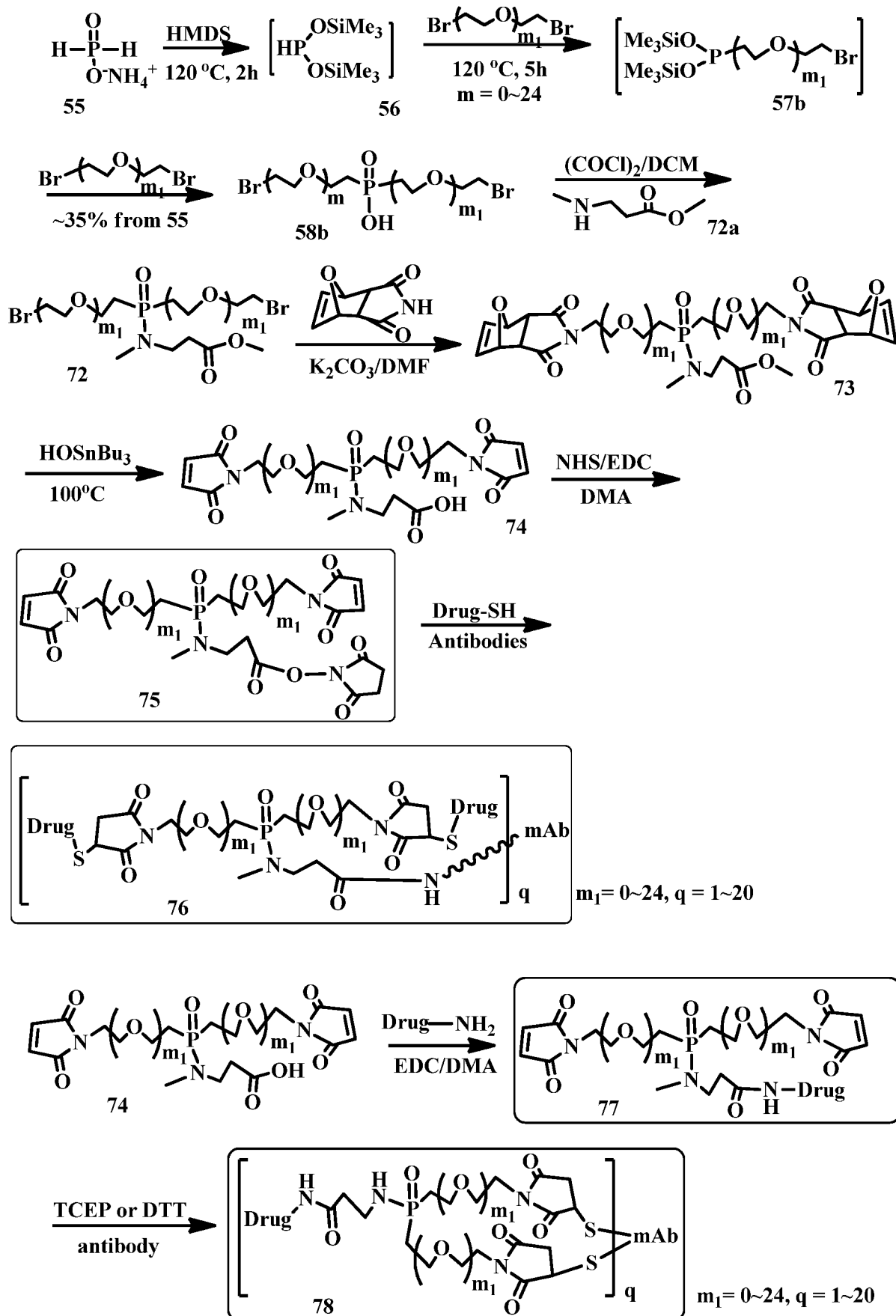


Figure 7

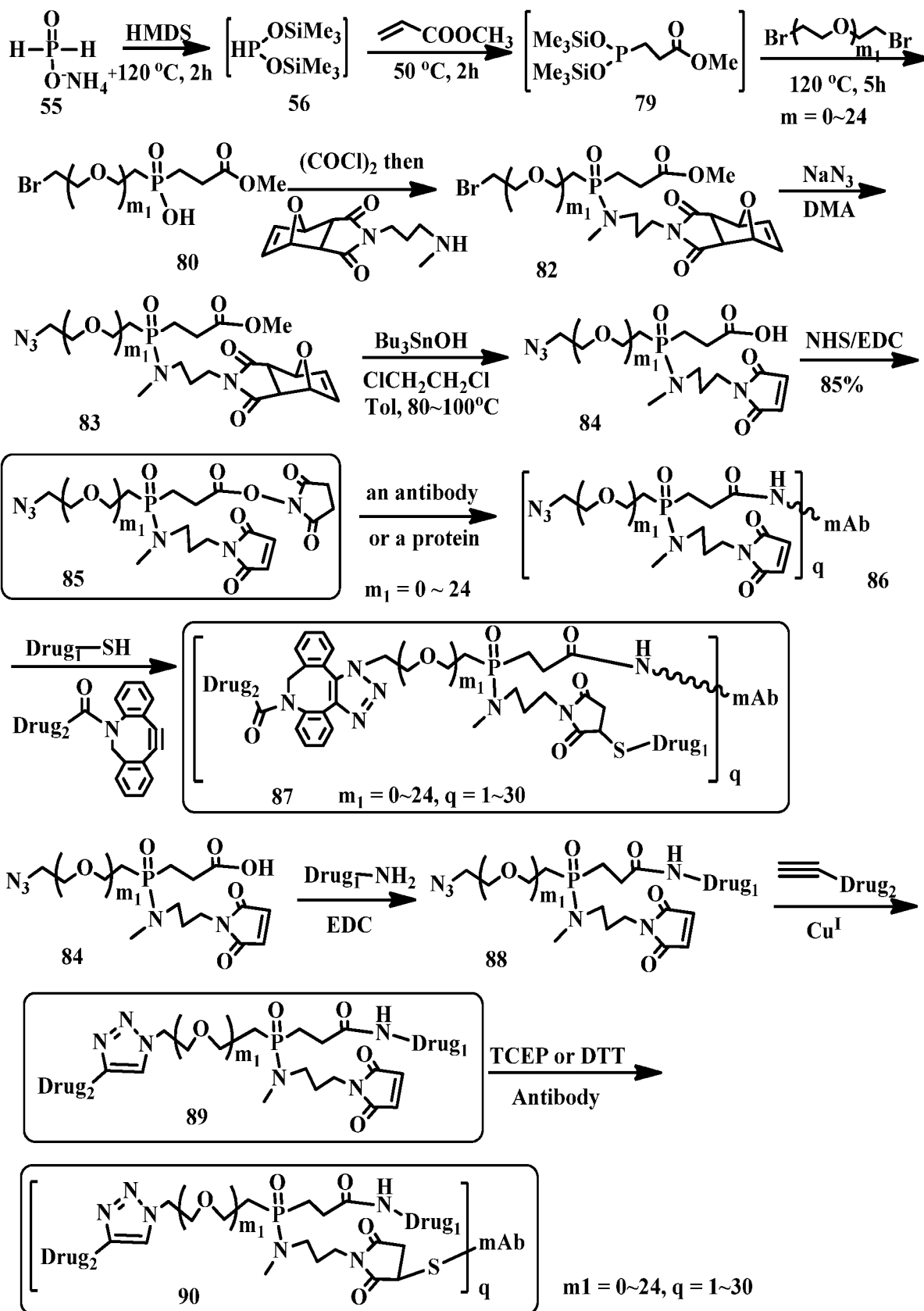


Figure 8

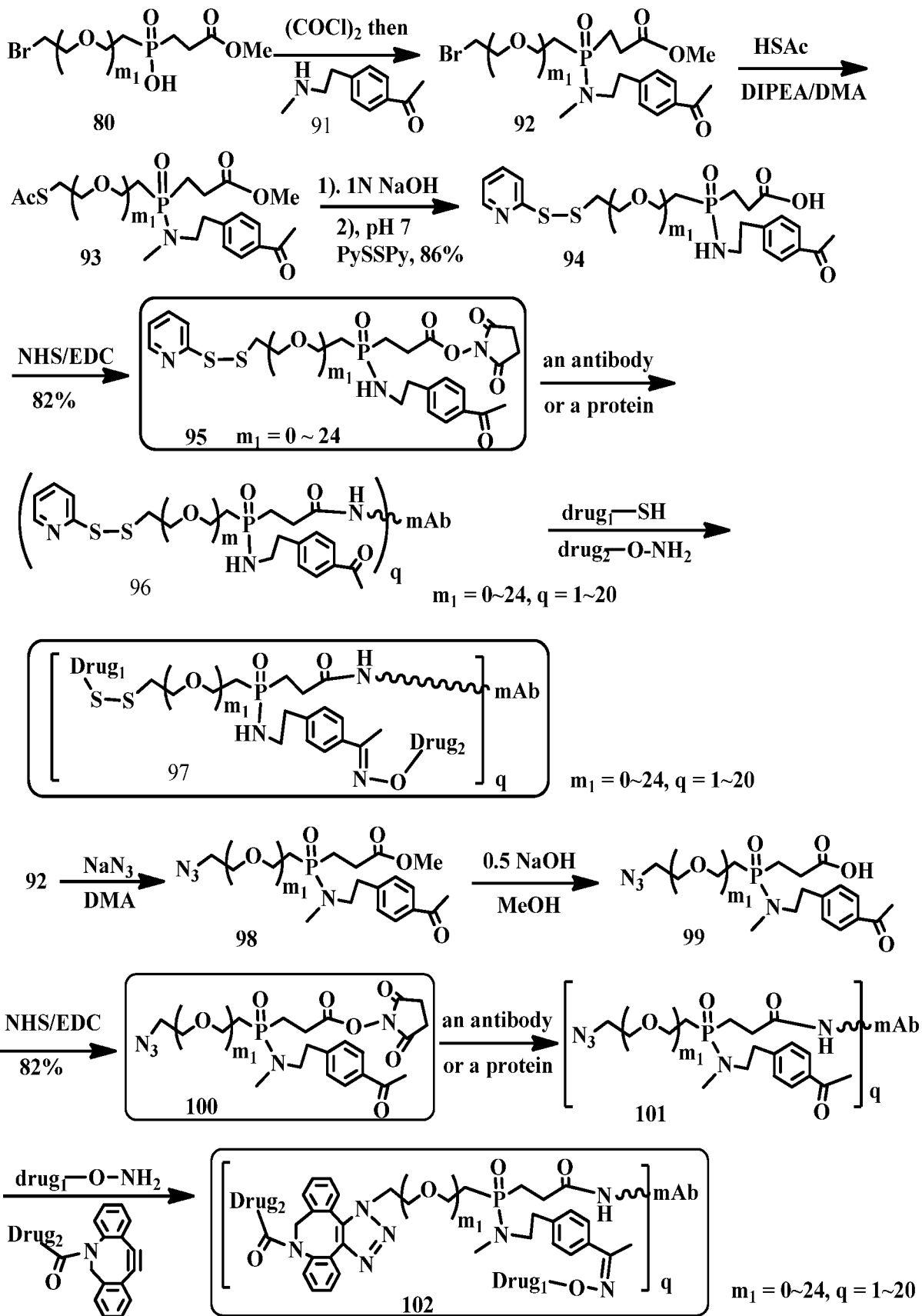


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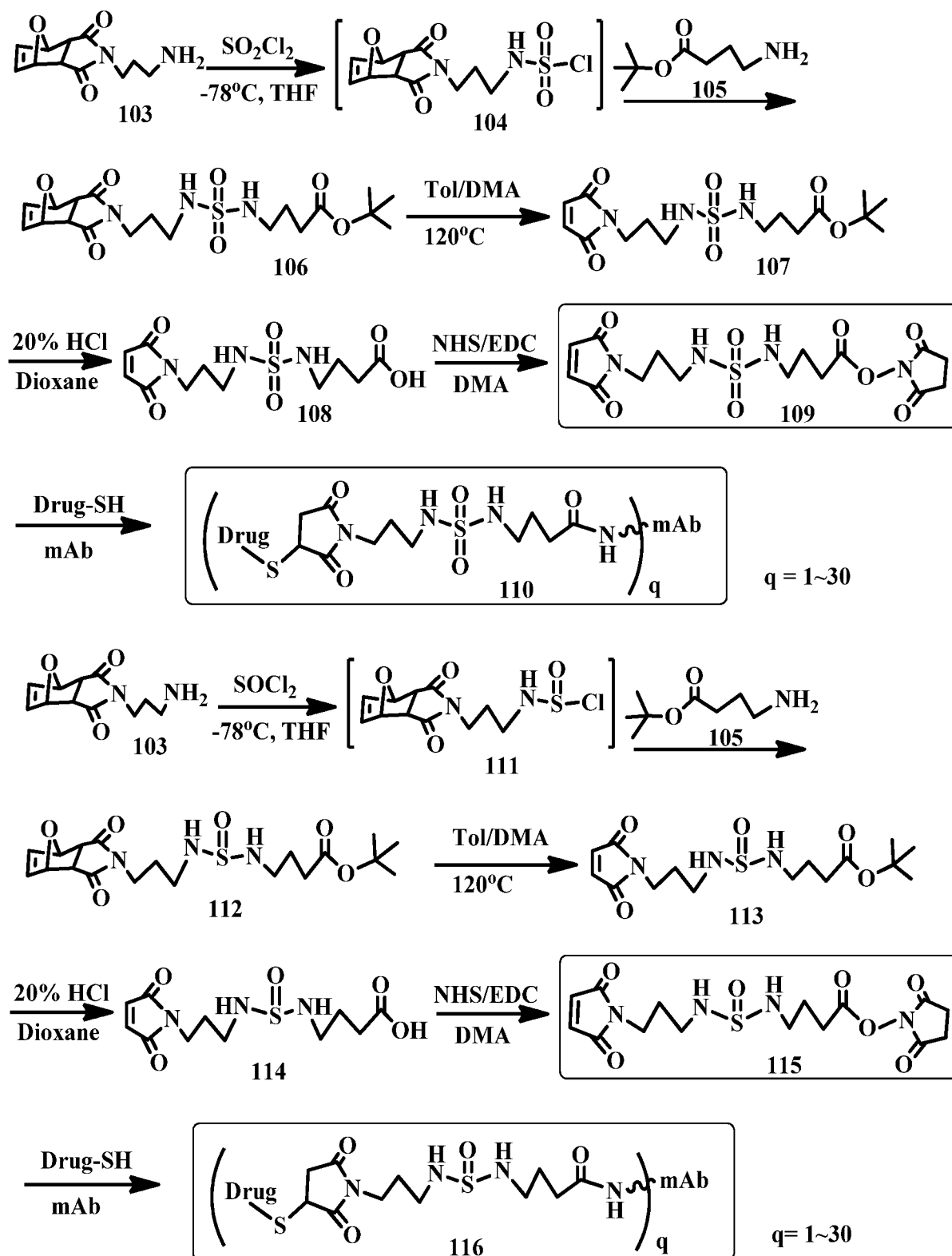


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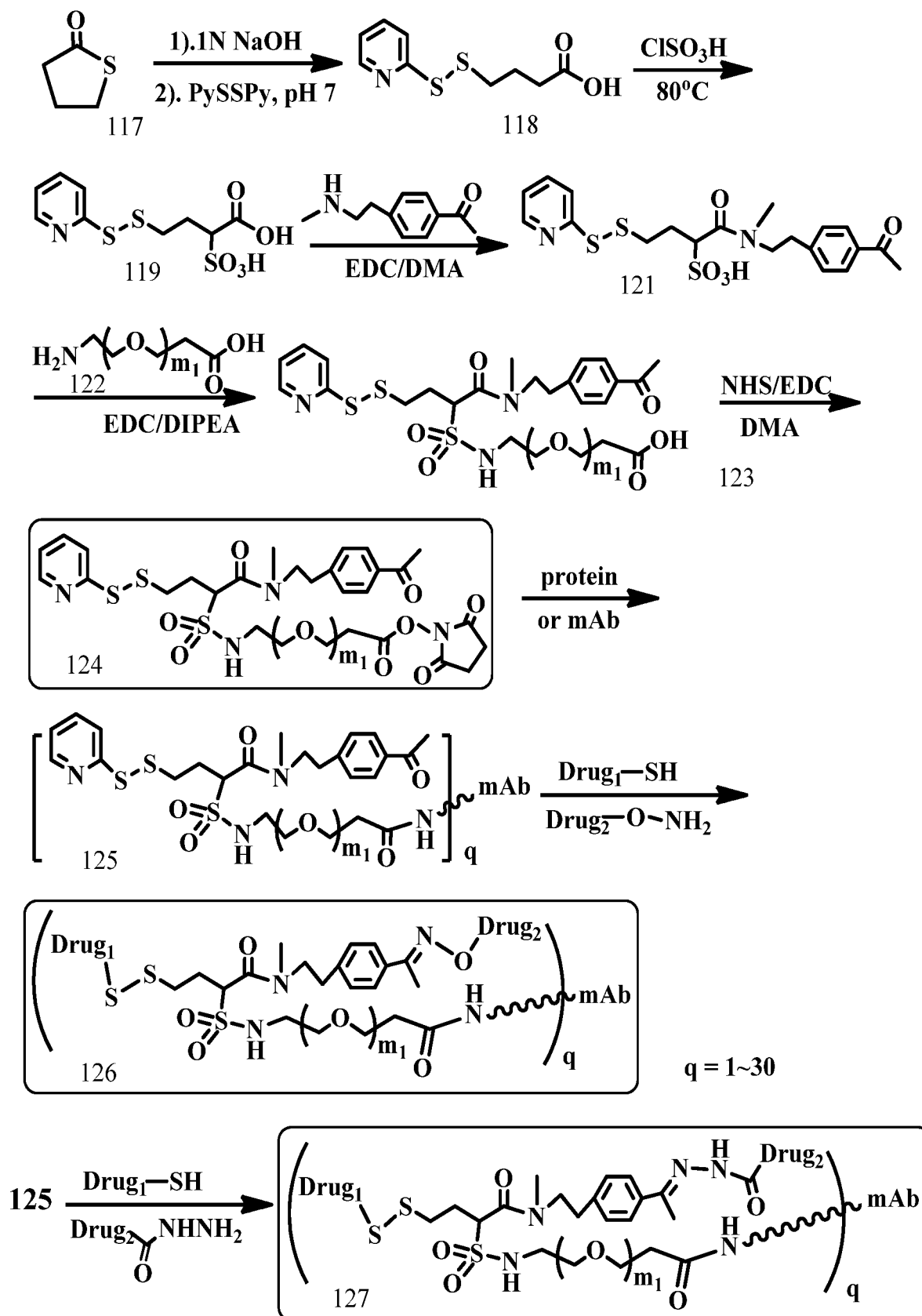


Figure 11

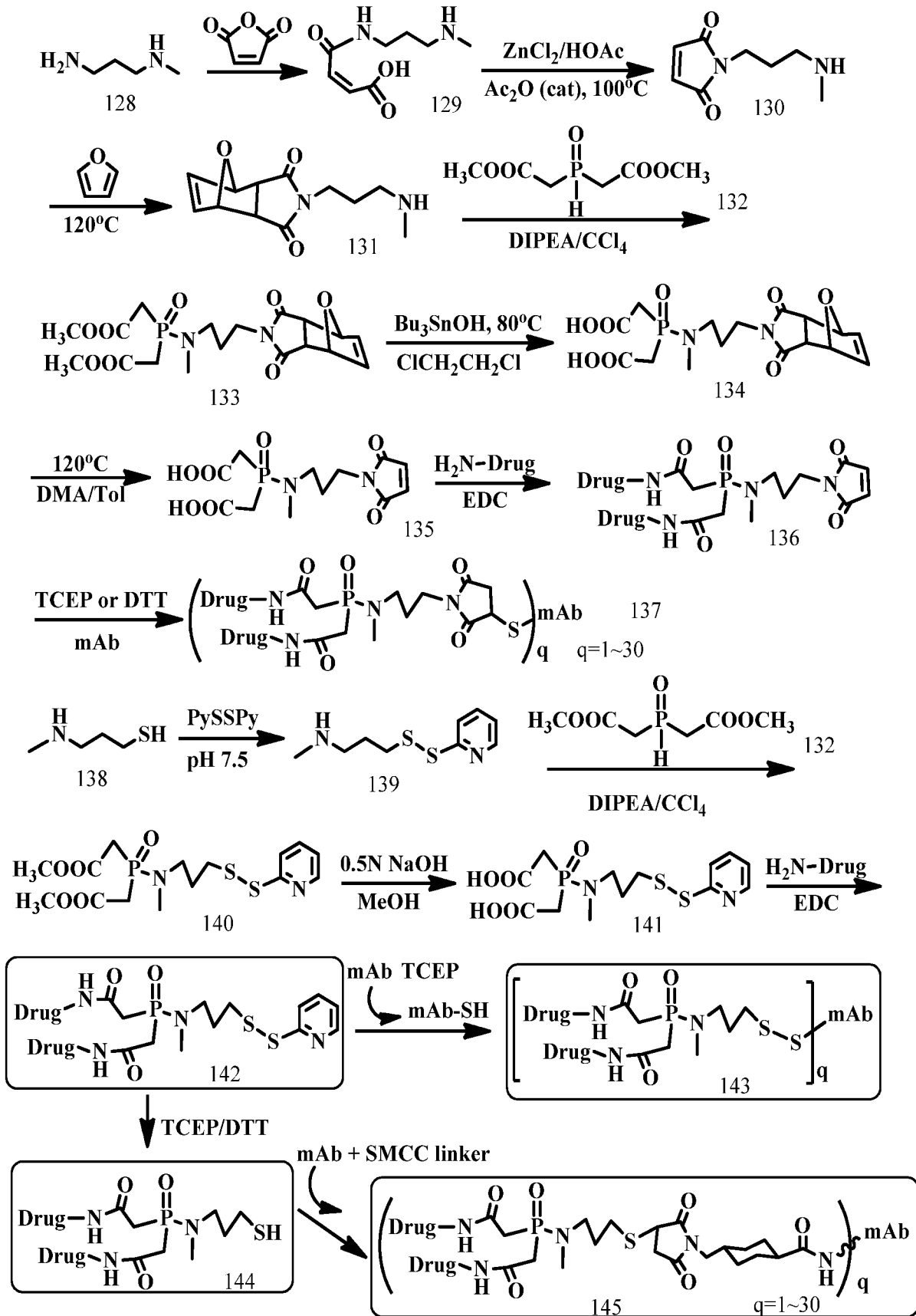


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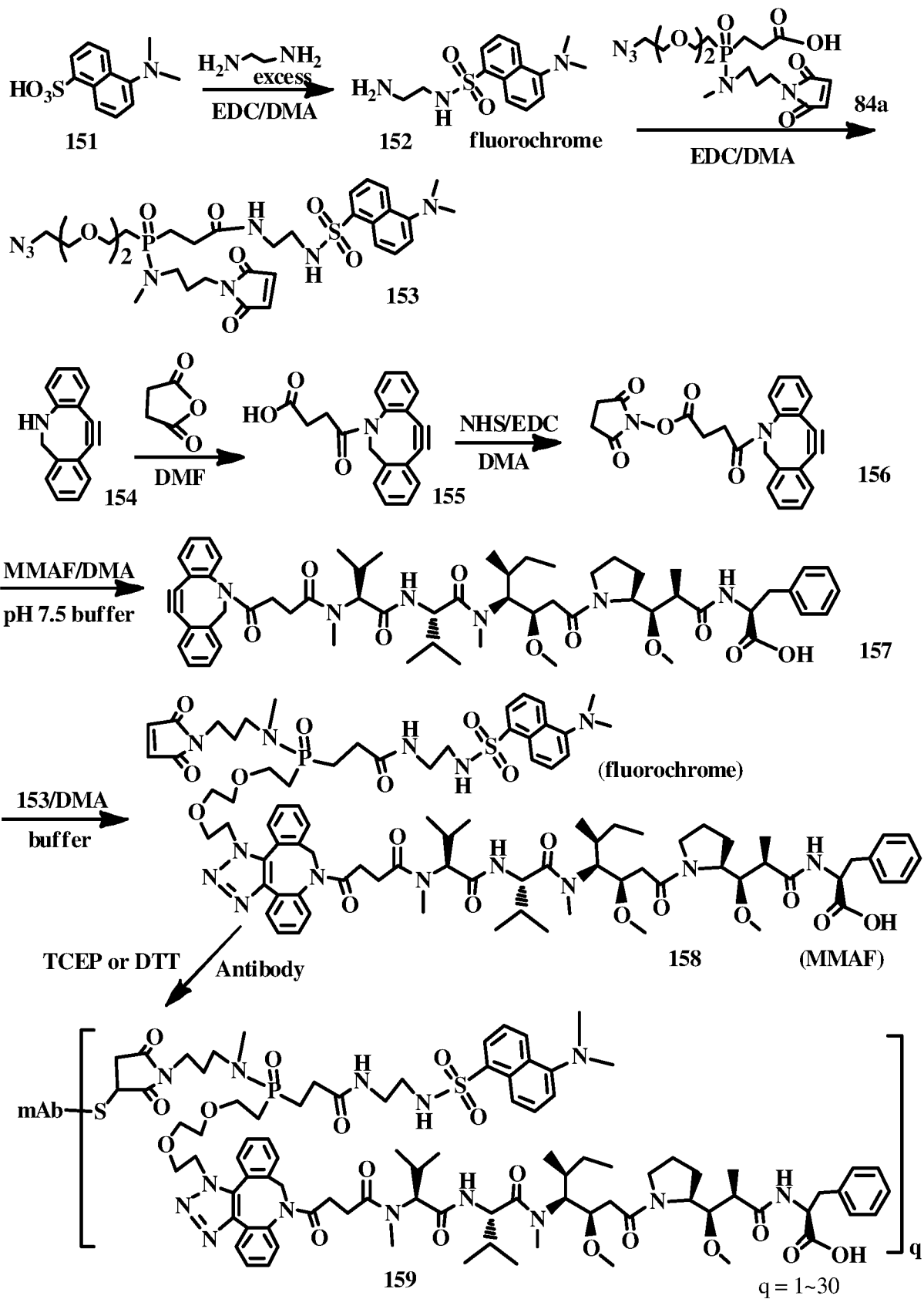


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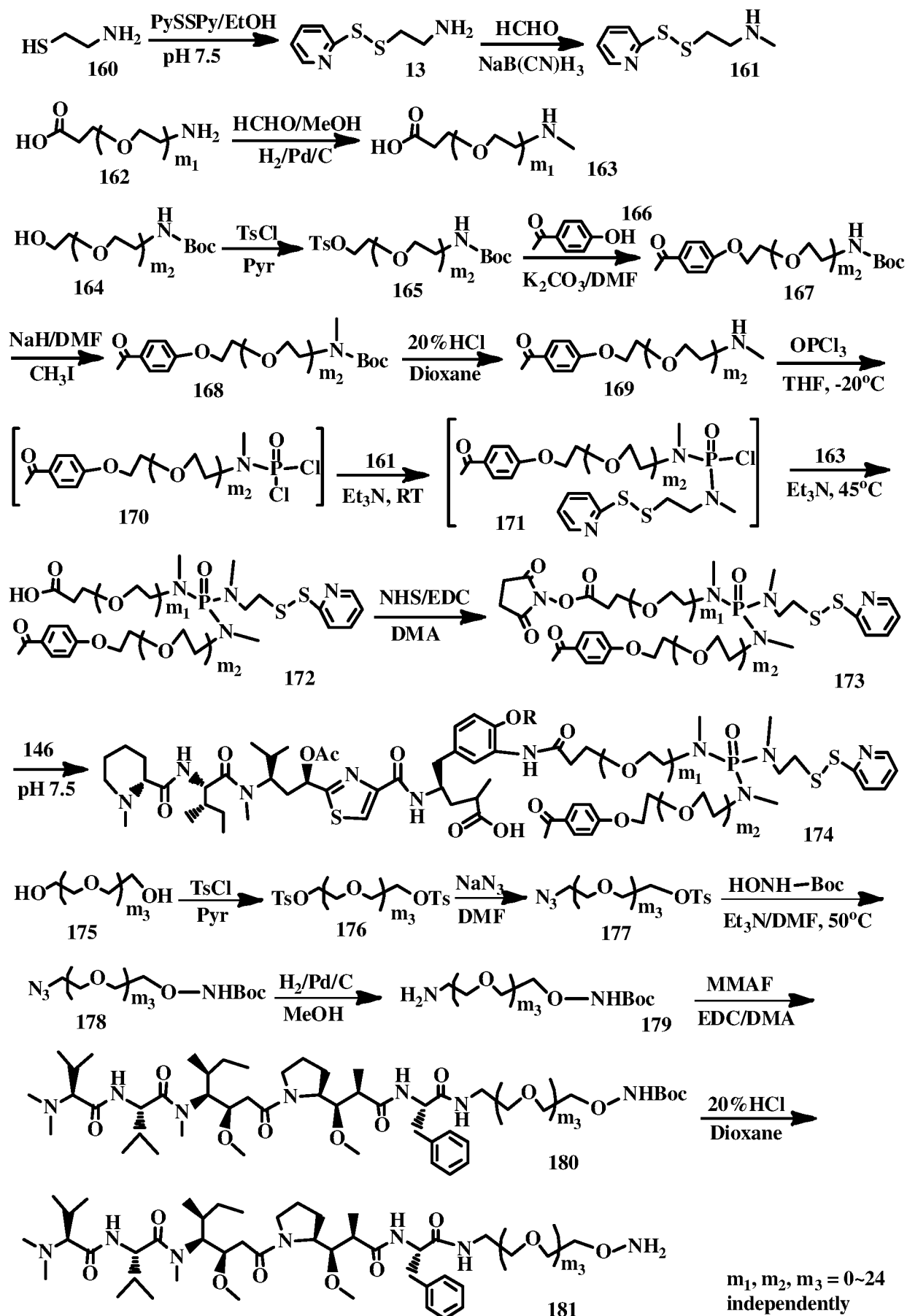


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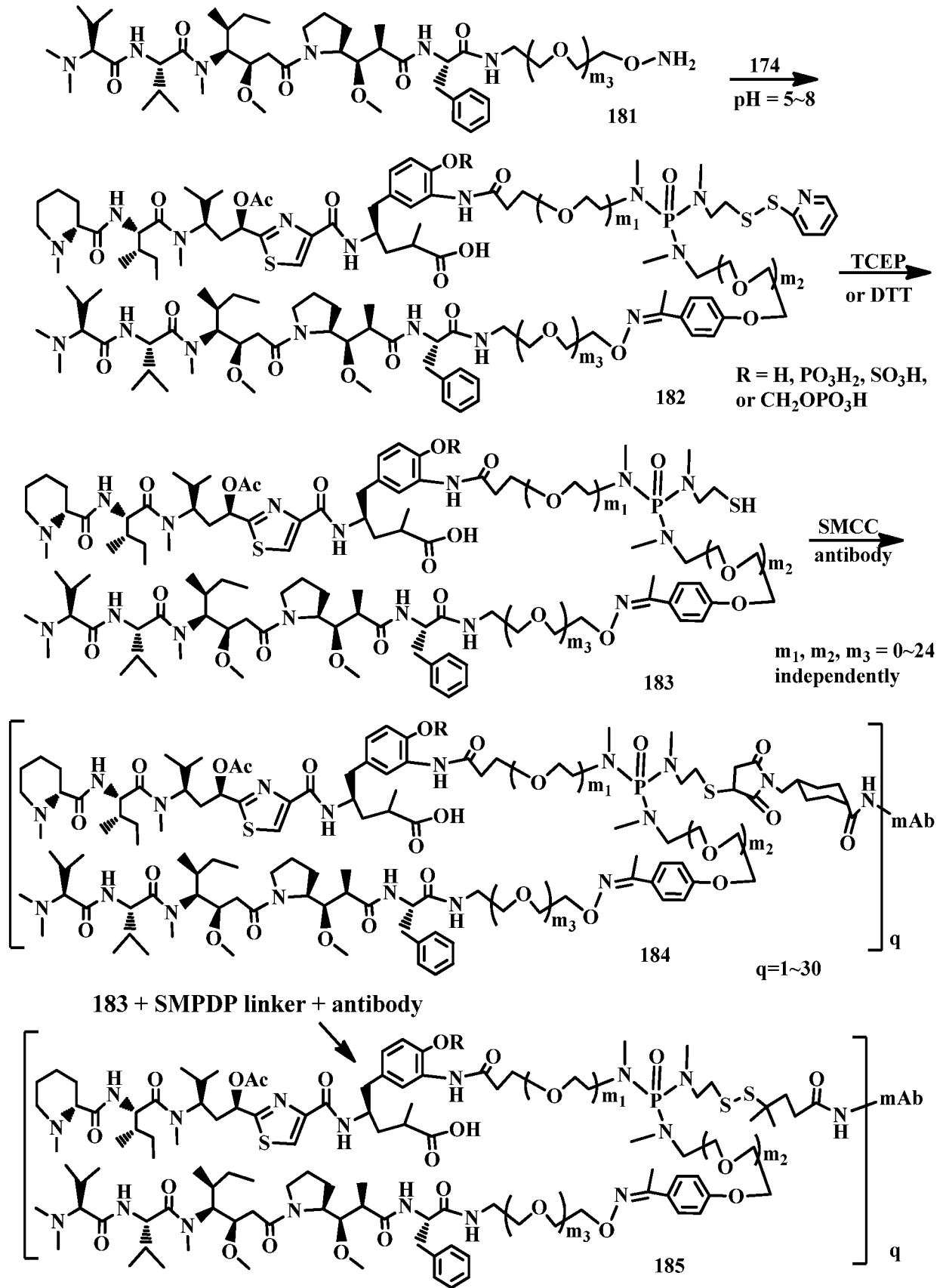


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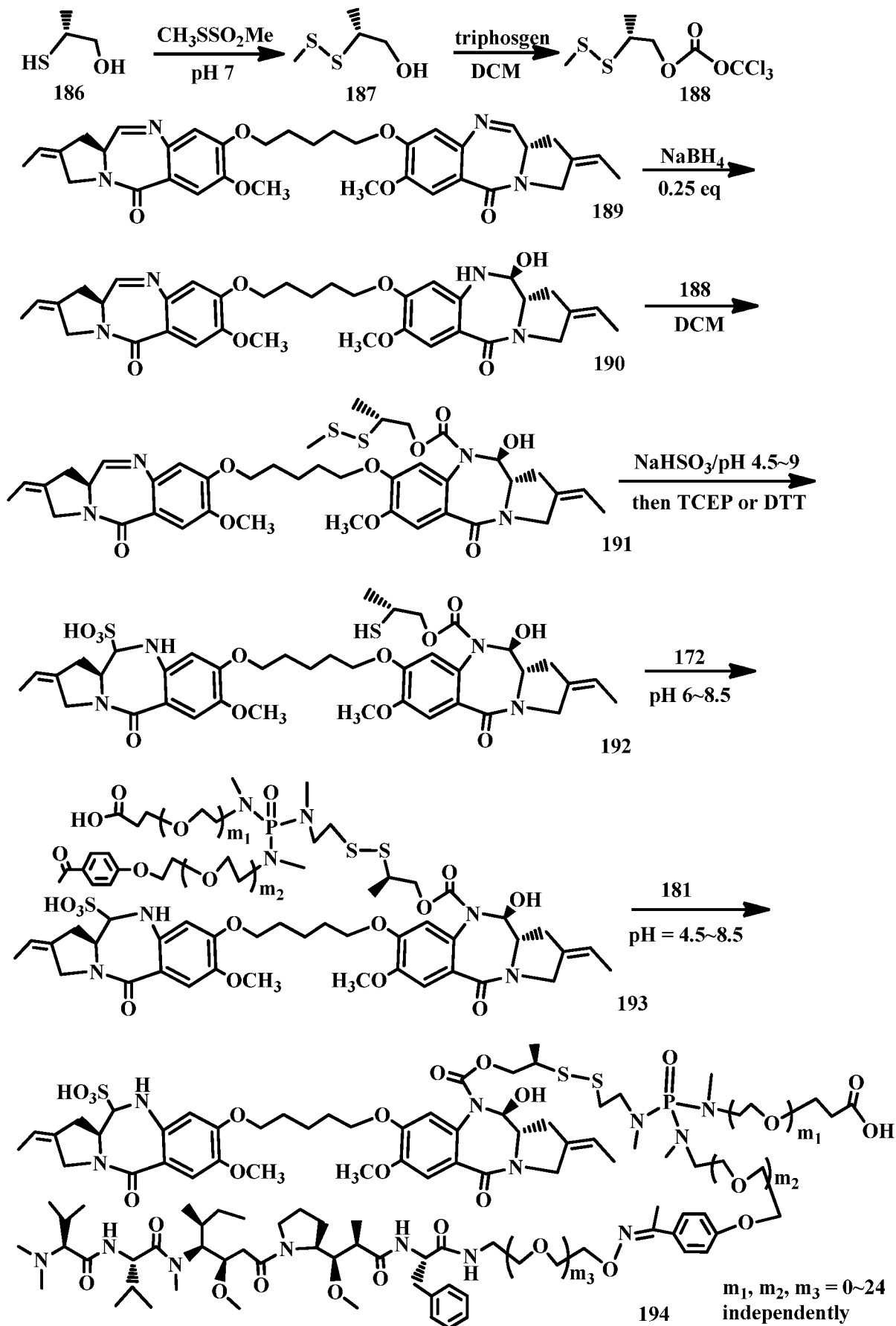


Figure 16

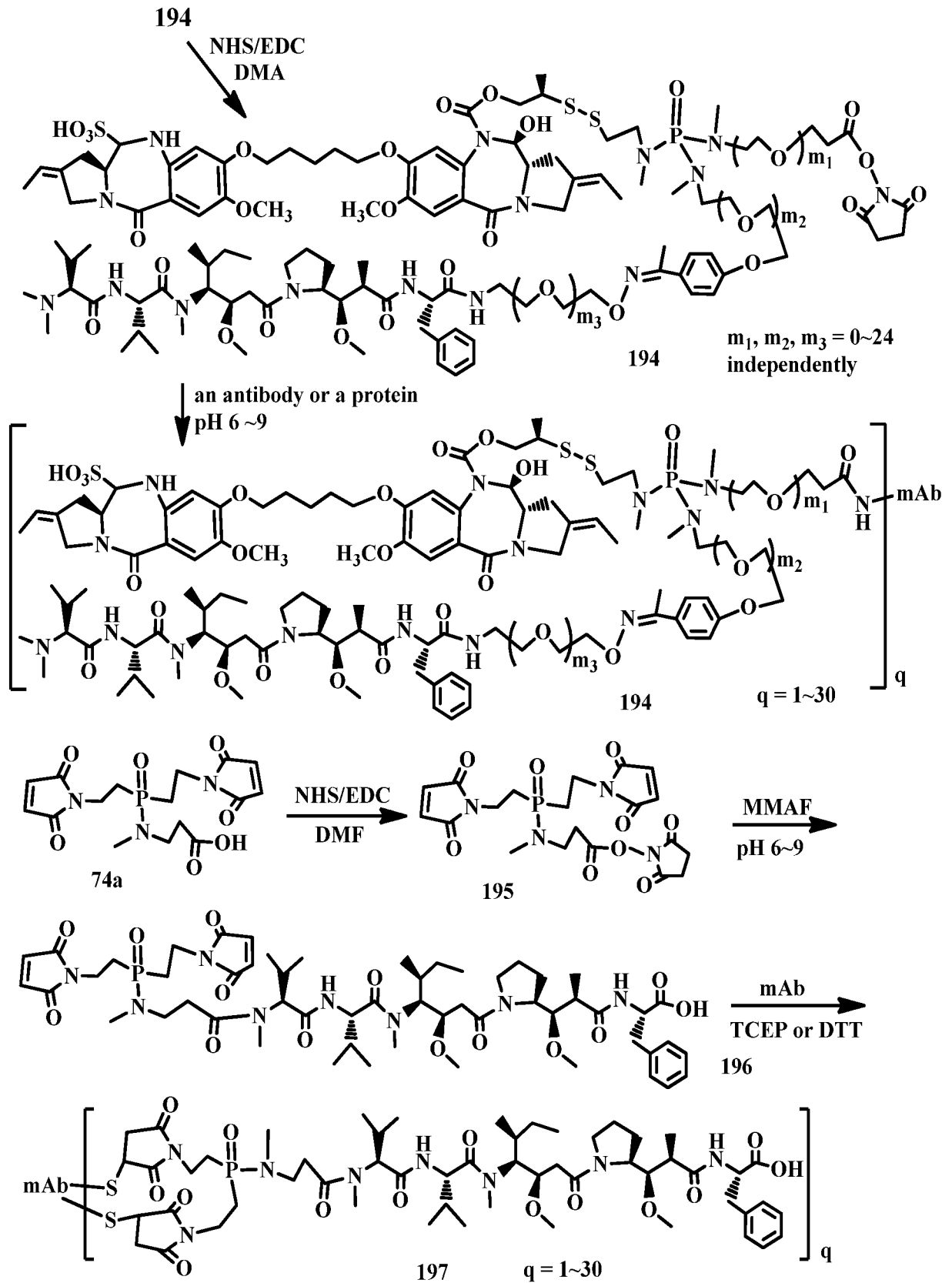


Figure 17

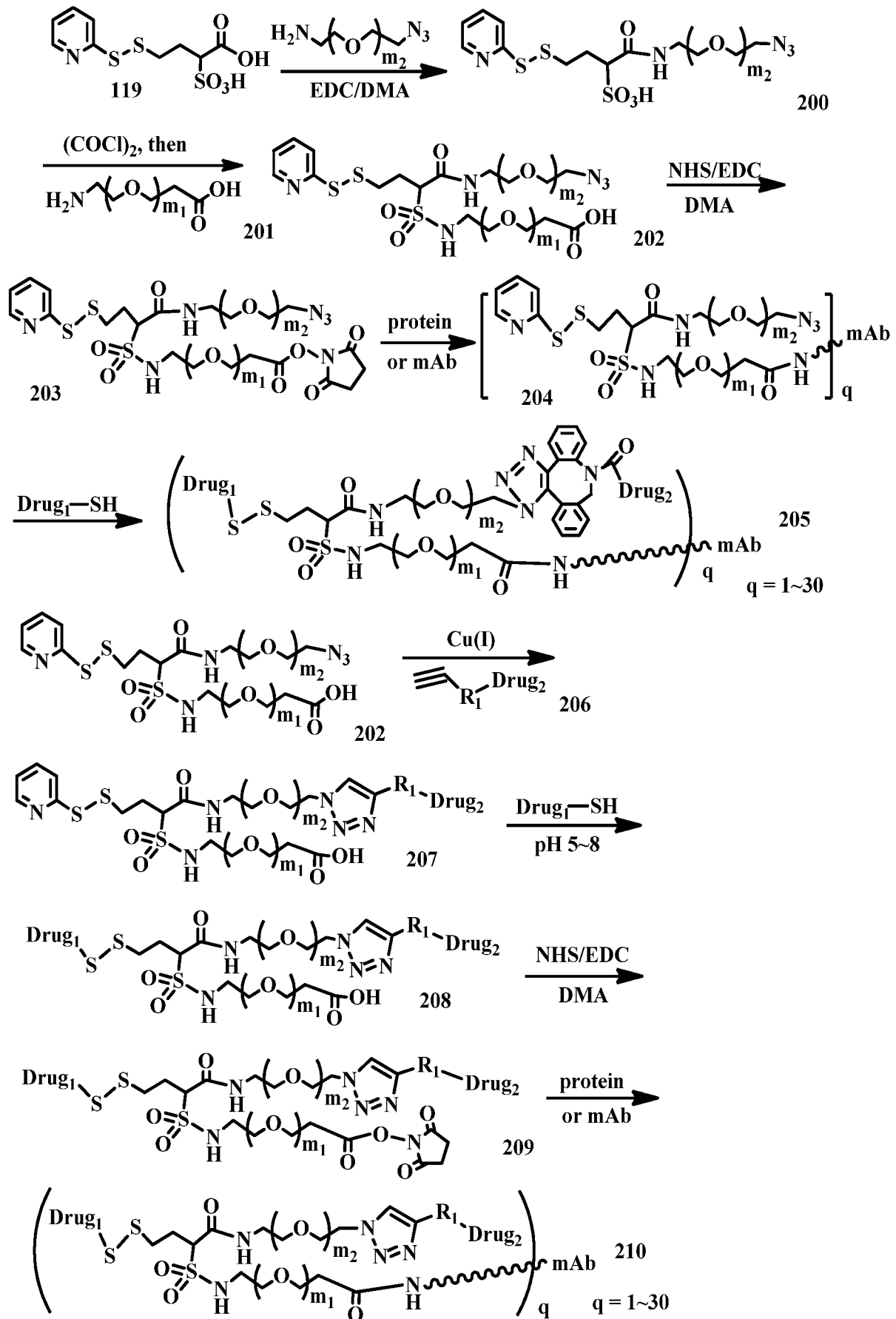


Figure 18