(54) Title: NOVEL LINKERS AND THEIR USES IN SPECIFIC CONJUGATION OF DRUGS TO A BIOLOGICAL MOLECULE

(57) Abstract:
Disclosed are linkers containing a 2,3-disubstituted succinic group, or 2-monosubstituted, or 2,3-disubstituted fumaric or maleic group for conjugation of two or more compounds/cytotoxic agents per linker to a cell-binding molecule through bridge linking pairs of thiols on the cell-binding molecule, as well as methods of making such linkers, of using such linkers in making homogeneous conjugates, and of application of the conjugates in treatment of cancers, infections and autoimmune disorders.
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NOVEL LINKERS AND THEIR USES IN SPECIFIC CONJUGATION OF DRUGS TO A BIOLOGICAL MOLECULE

FIELD OF THE INVENTION

The present invention relates to linkers used for the specific conjugation of compounds, in particular, cytotoxic agents to pairs of sulfur atoms of a biological molecule at two drugs per linker. The present invention also relates to methods of making cell-binding agent-drug (cytotoxic agent) conjugates in a specific manner comprising either modification of drugs with these linkers first, followed by reaction with prepared cell-binding agents; or modification of cell-binding agents with these linkers first, followed by reaction with drugs.

BACKGROUND OF THE INVENTION

The big challenge of chemotherapeutic drugs is their narrow therapeutic windows due to they normally cannot discriminate between normal and malignant cells, thus causes side effects which limit the tolerated doses below the clinically effective ones. In contrast, immunotherapy, normally in the form of monoclonal antibodies (mAb) can specifically bind to certain proteins or molecules of malignant cells, leaving normal cells unharmed, and thus has less side effects and bigger therapeutic windows than chemotherapy. Monoclonal antibodies (mAb) can target against malignant cells by several mechanisms, such as, 1). Making the cancer cell more visible to the immune system (Villaruz, L. C. et al, 2014, Transl Lung Cancer Res, 3, 2-14; Camacho, L. H. 2015 Cancer Med 4, 661-72); 2). Blocking growth signals (Dillman, R. O. 2011, Cancer Biother Radiopharm, 26, 1-64; Ferris, R. L. et al 2010, J Clin Oncol, 28, 4390-9); 3). Stopping new blood vessels from forming (Arrilaga-Romany, I., et al, 2014, Expert Opin Investig Drugs, 23, 199-210); 4). Delivering radiation to cancer cells (Chapuy, B. et al, 2007, Biotechnol J. 2, 1435-43); 5). Delivering chemotherapy drug to cancer cells (Chari R. J. 2008 Acc Chem Res. 41, 98-107; Mullard A. 2013, Nature Reviews Drug Discovery 12, 329–332; Zhao, R. J. 2012, J. Med. Chem., 55, 766-782 ); 6). Delivering enzyme to cancer cells (Francis R. J. et al, 2002, Br. J. Cancer 87, 600-7). One of these above strategies, delivering chemotherapy to cancer cells called antibody–drug conjugates (ADCs), which enables to target and deliver drugs to cancer cells leaving normal cells largely unaffected by the exquisite targeting ability of antibodies, has been intensely exploitation in the last two decades. In particular, since US FDA approvals of Adcetris (brentuximab vedotin) in 2011 and Kadcyla (ado-trastuzumab
emtansine) in 2013, the applications of antibody-drug conjugate (ADC) as a promise
targeted treatment of cancers have been exploded and almost every major pharmaceutical
and biotech company has adopted this approach (Chari, R. et al, Angew. Chem., Int. Ed.
Future Oncol, 2015, 11, 549). Currently there are more than 50 ADC drugs in the clinic
trials according to www.clinictrails.gov.

The first-generation ADCs, including Kadcyla and Adcetris, are produced through
nonselective conjugation of native lysine amines or interchain cysteine thiols on an anti-
body respectively to a cytotoxic drug. Since there are over 50 surface-exposed lysines and
8 hinge cysteine residues in IgG1 antibodies, this nonselective conjugation results in
randomly cross-linkage of cytotoxic drugs to practically all areas of the antibody molecule,
particularly having a diverse population of ADCs with a wide distribution of drugs per
Clin. Cancer Res. 10, 7063). Thus some of the undesired ADC subpopulation could lead
to shorter circulation half-life, lower efficacy, potentially increased off-target toxicity and
a wide range of in vivo pharmacokinetic (PK) properties (Hamblett, K. J. et al, Clin.
Chem. Biol. 20, 161–167). In addition, with this classical conjugation, the batch-to-batch
consistency in ADC production can be challenging and may require diligent manufactur-
ing capabilities (Wakankar, A. mAbs, 2011, 3, 161–172).

Therefore, biotechnology companies and academic institutions are highly focusing on
establishing novel reliable methods for site-specific ADC conjugation. So far, there are
several approaches developed in recent years for site selective ADC preparation
(Panowski, S. 2014, mAbs 6, 34). They include incorporation of unpaired cysteines, e.g.
engineered reactive cysteine residues, called THIOMAB from Genentech (Junutula, J. R.,
32; US Patents 8,309,300; 7,855,275; 7,521,541; 7,723,485, WO2008/141044), genetical-
ly introduced glutamine tag with Streptocvertillum mobaraense transglutaminase (mTG)
161–167; US Patent 8,871,908 for Rinat-Pfizer) or with Microbial transglutaminase
20130189287 for Innate Pharma; US Pat 7,893,019 for Bio-Ker S.r.l. (IT)), incorporation
of thiolfucose (Okeley, N. M., et al 2013 Bioconjugate Chem. 24, 1650), incorporation of

IgG₁ and IgG₄, 4 for IgG₂ and 11 for IgG₃. The light chain of the IgG₁ is connected to the heavy chain by a disulfide bond between the last cysteine residue of the light chain and the fifth cysteine residue of the heavy chain. But, for IgG₂, IgG₃ and IgG₄, the light chain is linked to the heavy chain by a disulfide bond between the last cysteine residue of the light chain and the third cysteine residue of the heavy chain (Liu, H. and May, K., 2012, mAbs 4, 17-23). On the ranks of the susceptibility of disulfide bonds in human IgG₁ antibodies by experimental reduction, differential alkylation, and LC-MS analysis (Liu, H, et al Anal. Chem., 2010, 82, 5219–5226), inter chain disulfide bonds are more susceptible to reduction than intra chain disulfide bonds, and the disulfide bonds between the light chain and heavy chain were more susceptible than disulfide bonds between the two heavy chains. The upper disulfide bond of the two inter heavy chain disulfide bonds was more susceptible than the lower one. Furthermore, disulfide bonds in the CH2 domain were the most susceptible to reduction. Disulfide bonds in VL, CL, VH, and CH1 domains had similar and moderate susceptibility, while disulfide bonds in the CH3 domain were the least susceptible to reduction (Liu, H, et al Anal. Chem., 2010, 82, 5219–5226).

Based on the more susceptibility of inter chain disulfide bonds in human IgG₁ antibodies, several institutions and companies adopted the chemically specific conjugation strategy through rebridging reduced interchain disulfide bonds of a native antibody, such as, using bromo or dibromo-maleimides, called next generation maleimides (NGMs) (Schumacher, F.F., et al 2014, Org. Biomol. Chem. 12, 7261–7269; UCL Cancer Institute), applying bis-alkylating reagents via a three-carbon bridge (Badescu, G., et al., 2014, Bioconjug. Chem. 25, 1124–1136., WO2013/190272, WO2014/064424 for PolyTherics Ltd), with di-substituted heteroaryl bridge (US Pat Appl. 2015/0105539 for Concortis Biosystem), or through di-maleimide as a bridge (WO2014/114207). We have also used bromo maleimide and dibromomaleimide linkers to conjugate both drugs and antibodies for a quite while (WO2014/009774, PCT/IB2012/053554). However, these above bridge linkers were designed in the way to conjugate only one cytotoxic agents to a pair of disulfide bonds, and therefore at most of time they only produced ADCs at DAR less than 2 (drugs per antibody), due to limited numbers (about two pairs) of reduced disulfide bonds are more accessible for conjugation.

As one of the major issues for ADCs is the limited numbers or amount of cytotoxic compound that ultimately reaches the tumor, and thus the favorable DAR over 3 is much important factor for improvement of ADC therapeutical index (Epenetos, A. A. et al, Cancer Res., 1986, 46, 3183–3191; Chari, R. V. Acc. Chem. Res., 2008, 41, 98-107, Zhao,
R. Y. et al, 2011, J. Med. Chem. 54, 3606-3623), we therefore disclose novel disulfur bridge linkers of this invention that not only are able to conjugate two or more drugs per linker for achieving higher DARs (\( \geq 4 \)), but also can selectively rebridge pairs of reduced inter chain disulfide bonds on surface of antibody, which are generated by overloaded TCEP or DTT reduction agents. And the over reduced pairs of thiol groups that are inaccessible reached by the bridge linkers can be recoupled (regenerated) by an oxide, e.g. dehydroascorbic acid (DHAA) or Cu(II), to form back disulfide bonds at the end of conjugation. In principal, this rebridging back of reduced disulfide bonds results in more stable or longer half-life of ADCs in comparison with traditional thiol linked ADCs. Furthermore, as the “ring-opened” succinimide ring linker bearing mono-thioether bond has improved in vitro stability, improved PK exposure, and improved efficacy as compared to the mono-thiol-maleimide-conjugated ADCs (Tumey, L. N, et al, 2014, Bioconjug. Chem. 25, 1871–80; Lyon, R. P, et al. 2014, Nat. Biotechnol. 32, 1059–62), due to the latter is prone to payload loss via a retro-Michael type reaction of the maleimide conjugation (Shen, B. Q, et al, 2012, Nat Biotechnol. 30, 184–9; Tumey, L. N, et al, 2014 Bioconjug Chem. 25, 1871–80), the bridge linkers of this invention containing a 2,3-disubstituted succinic group, or 2-monosubstituted, or 2,3-disubstituted fumaric or maleic (\( \text{trans (E)} \)- or cis (\( \text{Z} \))-butenedioic) group have less payload loss as compared to their nonhydrolyzed bromo or dibromo-maleimide linkers which were tested in our lab. In other words, the methods of the instant invention can be used to for the immunoconjugates that carry a combination of drugs, in particular different drugs, which can be delivered simultaneously and specifically to a particular target site, where the therapeutic molecules in the medicament are highly homogeneous, with lot-to-lot consistency. The major advantages of such immunoconjugates include: simultaneous targeted delivery of multiple drugs that act synergistically in targeting malignant cells; combining drugs that act in different phases of the cell cycle to increase the number of target cells exposed to a particular pharmaceutical drug or effect; minimized exposure to non-target cells, tissues or organs; precise control over drug payloads and drug ratios leading to homogeneous final products. In short, the bridge linkers of the invention can make homogeneous production of specific ADCs in a simple manner.

**SUMMARY OF THE INVENTION**

The present invention provides linkers containing a 2,3-disubstituted succinic group, or 2-monosubstituted, or 2,3-disubstituted fumaric or maleic (\( \text{trans (E)} \)- or cis (\( \text{Z} \))-butenedioic) group to link two drugs to a cell-binding agent (e.g., an antibody). The
preferred formula of the cell-binding molecule-linker-drug conjugates can be represented as:

\[ \text{Cb} \left\langle \text{S} \right\rangle \left\langle \text{L} \right\rangle \left\langle \text{Drug}_1 \right\rangle \left\langle \text{Drug}_2 \right\rangle_{n} \]

wherein Cb is a cell-binding agent, L Cb is a cell-binding agent, L is a linker containing succinic, fumaric or maleic group; \( \text{Drug}_1 \) and \( \text{Drug}_2 \) are a drug molecule; \( n \) is an integer from 1 to 30; and two S (sulfur) elements from Cb bridgely link to L, which covalently connects two or more drugs. The advantages in applying the linker in the cell molecule-drug conjugate are: a). Retaining the stability of the conjugates by covalently cross-linking (re-bridging) the pairs of reduced disulfur atoms of the cell-binding agents, particularly of antibodies; b). Enabling conjugation of the cytotoxic agents/drugs to specific sites of a cell-binding molecule, e.g. the inter chain disulfide bond sites of IgG antibodies, resulting in homogeneous production of ADC.

In one aspect of the present invention, the linker is represented by Formula (I)

\[
\begin{align*}
\text{Z}_1 & \rightarrow \text{R}_1 \rightarrow \text{X}_1 \quad \text{O} \\
\text{U} \quad \text{U}' \quad \text{X}_2 & \rightarrow \text{R}_2 \rightarrow \text{Z}_2
\end{align*}
\]

(1)

Wherein

- represents an optional single bond;

- represents either a single bond or a double bond;

\( \text{U} \) and \( \text{U}' \) represent the same or different leaving group that can be substituted by a thiol. Such leaving groups are, but are not limited to, a halide (e.g., fluoride, chloride, bromide, and iodide), methanesulfonyl (mesyl), \( p \)-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), trifluoromethylsulfonate, nitrophenol, \( N \)-hydroxysuccinimide (NHS), phenol; dinitrophenol; pentafluorophenol, tetrafluorophenol, difluorophenol, monofluorophenol, pentachlorophenol, imidazole, dichlorophenol, tetrachlorophenol, 1-hydroxybenzotriazole, 2-ethyl-5-phenylisoxazolium-3'-sulfonate, or an intermediate molecule generated with a condensation reagent for Mitsunobu reactions.

It provided that when \( \text{U} \) represents a single bond, both \( \text{U} \) and \( \text{U}' \) are not H; when \( \text{U} \) represents a double bond, either \( \text{U} \) or \( \text{U}' \) can be H, but are not H at the same time.

\( \text{Z}_1 \) and \( \text{Z}_2 \) are the same or different a function group that enables to react with a cytotoxic drug, to form a disulfide, ether, ester, thioether, thioester, peptide, hydrazone, carbamate, carbonate, amine (secondary, tertiary, or quarter), imine, cycloheteroalklyne, heteroaromatic, alkylxime or amide bond;

\( \text{R}_1 \) and \( \text{R}_2 \) are the same or different, and are absent, 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 \(R_1\) and \(R_2\) are respectively a chain of atoms selected from C, N, O, S, Si, and P, preferably having 0–500 atoms, which covalently connects to \(X_1\) or \(X_2\) and \(Z_1\) or \(Z_2\). The atoms used in forming the \(R_1\) and \(R_2\) may be combined in all chemically relevant ways, such as forming alkylene, alkenylene, and alkynylene, ethers, polyoxyalkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amides, ureas, semicarbazides, carbazides, alkoxyamines, alkoxylamines, urethanes, amino acids, peptides, acyloxyamines, hydroxamic acids, or combination thereof.

\(X_1\) and \(X_2\) are independently selected from NH, N(R₃), O, S or CH₂; \(R₃\) is 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.

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, \(\text{Drug}₁\) and \(\text{Drug}₂\), have reacted at the ends of the bridge linker:

![Diagram](image)

Wherein:

\(Cb\) represents a cell-binding agent, preferred an antibody;

Inside the bracket (parentheses) are the linker-drug components that are conjugated to pairs of sulfur atoms of the cell-binding molecule. The sulfur atoms are preferred pairs of thiols reduced from the interchain disulfide bonds of the cell-binding agent by a reduction agent, such as DTT and/or TCEP;

\(\text{Drug}₁\) and \(\text{Drug}₂\) represent the same or different cytotoxic agents, which linked to the cell-binding agent via the bridge linker by a disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, cycloheteroalkyne, heteroaromatic, alkoxime or amide bond;
n is 1 ~ 30; “—”, “—”, , R₁, R₂, X₁ and X₂ are described the same previously in Formula (I).

In a further aspect, the present invention provides a modified cell-binding agent of Formula (III), in which the cell-binding agent, Cb, through its pair of thiols generated with reduction of disulfide bonds, has reacted with the bridge linker, which has Z₁ and Z₂, the function groups capable of reacting with a drug:

Wherein “—”, “—”, Cb, Z₁, Z₂, n, R₁, R₂, X₁, and X₂ are defined the same as in Formula (I) and (II).

In an even further aspect, the present invention provides a modified drug of Formula (IV), in which the drug, Drug₁ and Drug₂, have reacted with the linker of Formula (I), which still has the 2,3-disubstituted succinic group, or 2-monosubstituted, or 2,3-disubstituted fumaric or maleic (trans (E)- or cis (Z)-butenedioic) group capable of reacting with a pair of sulfur atoms of the cell-binding agent:

Wherein “—”, “—”, Drug₁, Drug₂, U, U’, R₁, R₂, X₁, and X₂ are defined the same as in Formula (I) and (II).

The present invention further relates to a method of making a cell-binding molecule-drug conjugate of Formula (II), wherein the drugs, Drug₁ and Drug₂ are linked to a cell-binding agent via the bridge 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 bridge linker of Formula (I).

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

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the synthesis of a bridge linker containing polyethylene glycols and the application of this linker in the conjugation of an antibody with drugs via amide bonds.
Figure 2 shows the synthesis of a bridge linker containing polyethylene glycols and the application of this linker in the conjugation of drugs to an antibody via amide bonds.

Figure 3 shows the synthesis of a bridge linker containing polyethylene glycols and the application of this linker in the conjugation of drugs to an antibody via oxime linkage.

Figure 4 shows the synthesis of a bridge linker containing polyethylene glycols and the application of this linker in the conjugation of two drugs to an antibody via hydrazone linkage.

Figure 5 shows the synthesis of bridge linkers containing polyethylene glycols and the application in the conjugation of two different drugs per linker to an antibody via amide linkage.

Figure 6 shows the synthesis of bridge linkers and the application in the conjugation of two different drugs per linker to an antibody via hinder amide linkage.

Figure 7 shows the synthesis of bridge linkers containing peptides or polyethylene glycols and the application of these linkers in the conjugation of two (different) drugs to an antibody via hydrazone linkage.

Figure 8 shows the synthesis of the conjugatable analogs of MMAE, Tubulysin and PBD cytotoxic drugs.

Figure 9 shows the synthesis of the conjugatable analogs of PBD, MMAF, and Tubulysin D cytotoxic drugs.

Figure 10 shows the synthesis of the conjugates of cell-binding molecule-tubulysin analogs via the bridge-linker.

Figure 11 shows the synthesis of the conjugates of both PBD dimer analog and Tubulysin B analog per linker, or both MMAE and Tubulysin D analog per linker, to an antibody.

Figure 12 shows the synthesis of the conjugates of both PBD dimer analog and MMAF analog per linker, or both PBD dimer and Tubulysin B analog per linker, to an antibody.

Figure 13 shows the synthesis of the conjugates of both Maytansinoid analog and Tubulysin B analog per linker, to an antibody.

Figure 14 shows the synthesis of the conjugates of both Maytansinoid analog and PBD dimer analog per linker, or two Tubulysin B analogs per linker, to an antibody.

Figure 15 shows the synthesis of the conjugates of both two MMAF analogs per linker, or two Tubulysin B analogs per linker, containing polyethylene glycols, to an antibody.
Figure 16 shows the comparison of the anti-tumor effect of conjugate compounds 127, 129 and 142 with T-DM1 using human gastric tumor N87 cell model at dosing, 3 mg/kg, i.v., one injection. All the four conjugates did not cause the animal body weight loss (top figure). The animals at control group were sacrificed at day 37 due to the tumor volume larger than 1500 mm$^3$ and they were too sick. The three compounds 127, 129 and 142 were better than T-DM1: All 6/6 animals at the groups of compound 127 and 129 had completely no tumor measurable at day 13 till day 60 (the end of experiment). All 6/6 animals at the group of Compound 142 group had no tumor measurable at day 21 and 2/6 animals had tumor growth (measurable) back at days 48, which still inhibited the tumor growth for over 55 days. In contrast T-DM1 at dose of 3 mg/Kg was not able to eradicate the tumors completely although it had inhibited the tumor growth for about 28 days.

DETAILED DESCRIPTION OF THE INVENTION
DEFINITIONS

"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, 2,3dimethylbutyl, 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 C$_1$-C$_8$ alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C$_1$-C$_8$ alkyl,-O-(C$_1$-C$_8$ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH$_2$, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N$_3$, -NH$_2$, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from -C$_1$-C$_8$ alkyl and aryl.

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

"Heteroalkyl" refers to C$_2$-C$_8$ alkyl in which one to four carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N.

"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, hexenyl, 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, hexynyl, heptynol, 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 (-CH₃CH₂CH₂-), 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=CH₂-).

“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.
“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 -SR’, -NR’R”, -N=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, alkylnyl, heteroalkyl, aryl, arylalkyl, carbonyl, or pharmaceutical salts.

“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, thiiranyl, pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxiranyl, tetrahydrofuranyl, dioxolanyl, tetrahydropyranyl, dioxanyl, dioxolanyl, piperidyl, piperezinyl, morpholinyl, pyrran, imidazolinyl, pyrrolinyl, pyrazolinyl, thiazolidinyl, tetrahydrothiopyranyl, dithianyl, thiomorpholinyl, dihydropyranyl, tetrahydropyranyl, dihydropyranyl, tetrahydropyridyl, dihydropyridyl, tetrahydropyrimidinyl, dihydrothiopyrananyl, azepanyl, as well as the fused systems resulting from the condensation with a phenyl group.

The term “heteroaryl” or aromatic heterocycles refers to a 5 to 14, preferably 5 to 10 membered aromatic hetero, mono-, bi- or multicyclic ring. Examples include pyrrolyl, pyridyl, pyrazolyl, thienyl, pyrimidinyl, pyrazinyl, tetrazolyl, indolyl, quinolynyl, purinyl, imidazolyl, thienyl, thiazolyl, benzothiazolyl, furanyl, benzofuranyl, 1,2,4-thiadiazolyl, isothiazolyl, triazolyl, tetrazolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, carazolyl, 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 aryalkyl 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-benzimidazolylmethyl, 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 trifluoromethanesulfonate.

The following abbreviations may be used herein and have the indicated definitions:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boc</td>
<td>tert-butoxy carbonyl; BroP, bromotrispyrrolidinophosphonium hexafluorophosphate;</td>
</tr>
<tr>
<td>CDI</td>
<td>1,1′-carbonyldiimidazole; DCC, dicyclohexylcarbodiimide; DCE, dichloroethane;</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane; DIAD, diisopropylazodicarboxylate; Dibal-H, diisobutylaluminium hydride;</td>
</tr>
<tr>
<td>DPEA</td>
<td>DIPEA, diisopropylethylamine; DEPC, diethyl phosphorocyanidate;</td>
</tr>
<tr>
<td>DMA</td>
<td>DMA, N,N-dimethyl acetamide; DMAP, 4-((N), (N)-dimethylamino)pyridine; DMF, N,N-dimethylformamide;</td>
</tr>
<tr>
<td>DMSO</td>
<td>DMSO, dimethylsulfoxide; DTT, dithiothreitol; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride;</td>
</tr>
<tr>
<td>HATU</td>
<td>ESI-MS, electrospray mass spectrometry; HATU, O-(7-azabenzoazol-1-yl)-(N), (N), (N)-tetramethyluronium hexafluorophosphate; HOBT, 1-hydroxybenzotriazole; HPLC, high pressure liquid chromatography;</td>
</tr>
<tr>
<td>MMP</td>
<td>NHS, N-Hydroxysuccinimide; MMP, 4-methylmorpholine; PAB, (p)-aminobenzyl;</td>
</tr>
<tr>
<td>PBS</td>
<td>PBS, phosphate-buffered saline (pH 7.0–7.5); PEG, polyethylene glycol;</td>
</tr>
<tr>
<td>SEC</td>
<td>SEC, size-exclusion chromatography; TCEP, tris(2-carboxyethyl)phosphine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Val, valine.</td>
</tr>
</tbody>
</table>

“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 ethanalamine.

“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.

"Administering" or “administration” refers to any mode of transferring, delivering, introducing or transporting a pharmaceutical drug or other agent to a subject. Such modes
include oral administration, topical contact, intravenous, intraperitoneal, intramuscular, intraleisional, intranasal, subcutaneous or intrathecal administration. Also contemplated by the present invention is utilization of a device or instrument in administering an agent. Such device may utilize active or passive transport and may be slow-release or fast-release delivery device.

The novel conjugates disclosed herein use the bridge linkers. Examples of some suitable linkers and their synthesis are shown in Figures 1 to 15.

**THE BRIDGE LINKERS**

The synthetic routes to produce bridge linkers as well as the preparation of the conjugates of drugs to a cell binding molecules of the present invention are shown in Figures 1-15. The bridge linkers possess two elements: a) A Substituent that is a 2,3-disubstituted succinic group; or 2-monosubstituted, or 2,3-disubstituted fumaric group; or 2-monosubstituted, or 2,3-disubstituted maleic group; which can react to a pair of thiols to form covalent thioether bonds, and b) A group, such as but not limited to, a disulfide, maleimide, haloacetyl, aldehyde, ketone, azide, amine, alkoxyamine, hydrazide, ethenesulfonfyl, acyl halide (acid halide), acryl (acyloyl), and/or acid anhydride group, capable of reaction with a drug. The bridge substituents of 2,3-disubstituted succinic group; or 2-monosubstituted, or 2,3-disubstituted fumaric group; or 2-monosubstituted, or 2,3-disubstituted maleic group; can be introduced by direct condensation of these 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric or maleic with an amine, an alcohol, or a thiol group to form amide, ester or thioester bonds. The synthesis of these bridge linkers is exemplified in the Figures 1, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14 and 15.

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

![Chemical Structure](image)

(1)

Wherein

--- represents an optional single bond;

\[==\] represents either a single bond or a double bond;

It provided that when \[==\] represents a single bond, both U and U’ are not H; when

\[==\] represents a double bond, either U or U’ can be H, but are not H at the same time.
Wherein the component:

\[
\begin{array}{c}
\text{O} \\
\text{U} \\
\text{O} \\
\text{U}'
\end{array}
\]

, which can be 2,3-disubstituted succinic group, or 2-monosubstituted or 2,3-disubstituted fumaric group, or 2-monosubstituted or 2,3-disubstituted maleic group, is capable of reacting with a pair of sulfur atoms of the cell-binding agent. The sulfur atoms are preferred pairs of thiols reduced from the interchain disulfide bonds of the cell-binding agent by a reducing agent, such as dithiothreitol (DTT), dithioerythritol (DTE), L-glutathione (GSH) and tris (2-carboxyethyl) phosphine (TCEP), or/and beta mercaptoethanol (β-ME, 2-ME).

U and U' represent the same or different leaving group that can be substituted by a thiol. Such leaving groups are, but are not limited to, a halide (e.g., fluoride, chloride, bromide, and iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), trifluoromethylsulfonate, nitrophenol, N-hydroxysuccinimide (NHS), phenol; dinitrophenol; pentafluorophenol, tetrafluorophenol, difluorophenol, monofluorophenol, pentachlorophenol, imidazole, dichlorophenol, tetrachlorophenol, 1-hydroxybenzotriazole, 2-ethyl-5-phenylisoxazolium-3'-sulfonate, or an intermediate molecule generated with a condensation reagent for Mitsunobu reactions.

Z₁ and Z₂ are the same or different a function group that enables to react with a cytotoxic drug, to form a disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, amine (secondary, tertiary, or quarter), imine, cyclohexeroalkyene, heteroaromatic, alkoxime or amide bond;

R₁ and R₂ are the same or different, and are absent, 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 (OCH₂(CH₂)₈, or polypropyleneoxy unit of formula (OCH₂(CH₃)CH₂)p wherein p is an integer from 0 to about 1000, or combination thereof.

Additionally R₁ and R₂ are respectively a chain of atoms selected from C, N, O, S, Si, and P, preferably having 0~500 atoms, which covalently connects to X₁ or X₂ and Z₁ or Z₂. The atoms used in forming the R₁ and R₂ may be combined in all chemically relevant ways, such as forming alkylene, alkenylene, and alkynylene, ethers, polyoxyalkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amidines, ureas, semicarbazides, carbazides, alkoxyamines, alkoxylamines, urethanes, amino acids, peptides, acyloxyamines, hydroxamic acids, or combination thereof.
X₁ and X₂ are independently selected from NH, N(R₃), O, S or CH₂; Wherein R₃ is 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 polyethyleneglycol unit of formula (OCH₂CH₂)ₚ, wherein p is an integer from 0 to about 1000, or combination thereof.

In another embodiment, R₁, R₂, and R₃, can be respectively a chain of atoms selected from C, N, O, S, Si, and P which covalently connects the cell-surface binding molecule and/or the conjugated drug. The atoms used in forming the bridge linker may be combined in all chemically relevant ways, such as forming alkylene, alkenylene, and alkyndiene, ethers, polyaqualkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amides, ureas, semicarbazides, carbazides, alkoxyamines, alkoxyamines, urethanes, amino acids, acylxoyamines, 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, including cyclo alkanes, cyclic ethers, cyclic amines, arenes, heteroarenes, and the like in the linker.

Examples of the functional groups, Z₁ and Z₂, which enable linkage of a cytotoxic drug, include groups that enable linkage via a disulfide, thioether, thioester, peptide, hydrazone, ester, carbamate, carbonate, alkoxy or an amide bond. Such functional groups include, but are not limited to, thiol, disulfide, amino, carboxy, aldehydes, ketone, maleimido, haloacetyl, hydrazines, alkoxyamino, and/or hydroxy.

Examples of the functional groups, Z₁ and Z₂, that enable reaction with the terminal of amine of a drug/cytotoxic agent can be, but not limited to, N-hydroxysuccinimide esters, p-nitrophenyl esters, dinitrophenyl esters, pentafluorophenyl esters, carboxylic acid chlorides or carboxylic acid anhydride; With the terminal of thiol can be, as but not limited to, pyridyldisulfides, nitropyridyldisulfides, maleimides, haloacetates, methylsulfone phenyloxadiazole (ODA), carboxylic acid chlorides and carboxylic acid anhydride; With the terminal of ketone or aldehyde can be, as but not limited to, amines, alkoxyamines, hydrazines, acylxoyamine, or hydrazide; With the terminal of azide can be, as but not limited to, alkyn. Examples of these function groups are displayed below:

\[
\begin{align*}
\text{N-hydroxysuccinimide ester;} & \\
\text{maleimide;}
\end{align*}
\]
\[
R_S-S_{\delta} \quad \text{disulfide;} \quad X_1-\text{acyl halide (acid)}
\]

\[
\begin{align*}
&\text{SO}_2 X_2 \quad \text{haloacetyl;} \\
&\text{O} \quad \text{ethenesulfonyl;} \\
&\text{O} \quad \text{acryl (acyroyl);} \\
\end{align*}
\]

\[
\begin{align*}
&Ts-O \quad 2-\text{(tosyloxy)acetyl;} \\
&Ms-O \quad 2-\text{(mesyloxy)acetyl;} \\
\end{align*}
\]

\[
\begin{align*}
&O_2N \quad 2-\text{(nitrophenoxy)acetyl;} \\
&O_2N \quad 2-\text{(fluorophenoxo)acetyl;} \\
&O_2N \quad 2-\text{(difluorophenoxo)acetyl;} \\
&O_2N \quad 2-\text{(pentfluorophenoxo)acetyl;} \\
&O_2N \quad \text{methylsulfone phenyloxadiazole (ODA);} \\
&O_2N \quad \text{anhydride;} \\
&O_2N \quad \text{alkoxy amino;}
\end{align*}
\]

\[
\text{H}_2\text{NN} \quad \text{azido;}
\]

\[
\text{H}_2\text{NN} \quad \text{alkynyl, or}
\]

\[
\text{H}_2\text{NN} \quad \text{hydrazide.}
\]

Wherein \(X_1\) is F, Cl, Br, I or \(Lv_3\); \(X_2\) is O, NH, N(R\(_1\)), or CH\(_2\); \(R_5\) and \(R_3\) are H, R\(_1\), aromatic, heteroaromatic, or aromatic group wherein one or several H atoms are replaced independently by -R\(_1\), -halogen, -OR\(_1\), -SR\(_1\), -NR\(_1\)R\(_2\), -NO\(_2\), -S(O)R\(_1\), -S(O)\(_2\)R\(_1\), or -COOR\(_1\); \(Lv_3\) is a leaving group selected from nitrophenol; N-hydroxysuccinimide (NHS); phenol; dinitrophenol; pentafluorophenol; tetrafluorophenol; difluorophenol; monofluorophenol; pentachlorophenol; triflate; imidazole; dichlorophenol; tetrachlorophenol; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3'-sulfonate, anhydrides formed its self, or formed with the other
anhydride, e.g. acetyl anhydride, formyl anhydride; or an intermediate molecule generated with a condensation reagent for peptide coupling reactions, or for Mitsunobu reactions.

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

The key step of synthesis of the bridge linker containing 2,3-disubstituted succinic group, or 2-monosubstituted or 2,3-disubstituted fumaric group, or 2-monosubstituted or 2,3-disubstituted maleic group is the condensation of the 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric acid, or 2-monosubstituted or 2,3-disubstituted maleic acid, or its acid derivatives, with the other components containing an amine (1° or 2° amines), alcohol, or thiol on their terminal, as shown in the following scheme (Ia):

\[
\begin{array}{c}
\text{LV₁} \quad \text{LV₂} \\
\text{U} \quad \text{U'} \\
\end{array}
\quad + \quad \begin{array}{c}
\text{X-R} \\
\text{U} \quad \text{U'} \\
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{X-R} \\
\text{U} \quad \text{U'} \\
\end{array}
\]

Wherein X₁ or X₂ described in Formula (I) as NH, N(R₃), O, or S; R is R₁ and/or R₂ that described in Formula (I); R₁ is the same defined in Formula (I).

LV₁ and LV₂ are the same or independently OH; F; Cl; Br; I; nitrophenol; N-hydroxy-succinimide (NHS); phenol; dinitrophenol; pentafluorophenol; tetrafluorophenol; difluorophenol; monofluorophenol; pentachlorophenol; triflate; imidazole; dichlorophenol; tetrachlorophenol; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3'-sulfonate, anhydrides formed its self, or formed with the other anhydride, e.g. acetyl anhydride, formyl anhydride; or an intermediate molecule generated with a condensation reagent for peptide coupling reactions, or for Mitsunobu reactions, e.g. condensation reagents are: EDC (N-(3-Dimethylaminopropyl)-N'-'ethylcarbodiimide), DCC (Dicyclohexyl-carbodiimide), N,N'-Diisopropylcarbodiimide (DIC), N-Cyclohexyl-N'-2-morpholino-ethyl)carbodiimide metho-p-toluenesulfonate (CMC,or CME-CDI), 1,1'-Carbonyldiimi-dazole (CDI), TBTU (O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate), N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), (Benzotriazol-1-yl oxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), Diethyl cyanophosphonate (DEPC), Chloro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 1-[(Dimethylamino)(morpholino)methylene]-1H-[1,2,3]triazolo[4,5-b]pyridine-1-ium 3-oxide
hexafluorophosphate (HDMA), 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP), Chlorotripyrrolidinophosphonium hexafluorophosphate (PyCloP), Fluoro-N,N,N′,N′-bis(tetramethylene)formamidinium hexafluorophosphate (BTFFFH), N,N,N′,N′-Tetramethyl-S-(1-oxido-2-pyridyl)thiuronium hexafluorophosphate, O-(2-Oxo-1(2H)pyridyl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TPTU), S-(1-Oxido-2-pyridyl)-N,N,N′,N′-tetrathiomethylthiuronium tetrafluoroborate, O-[(Ethoxycarbonyl)cyano-methylenamino]-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HOTU), (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate(COMU), O-(Benzotriazol-1-yl)-N,N,N′,N′-bis(tetramethylene)uronium hexafluorophosphate (HBPYU), N-Benzyl-N′-cyclohexylcarbodiimide (with, or without polymer-bound), Dipyrrolidino(N-succinimidyl-oxycarbene)hexafluorophosphate (HSPyU), Chlorodipyrrolidinocarbenium hexafluorophosphate (PyClU), 2-Chloro-1,3-dimethylimidazolidinium tetrafluoroborate(CIB), (Benzotriazol-1-yloxy) dipiperidinocarbenium hexafluorophosphate (HBPipU), O-(6-Chlorobenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (CTCU), Bromotris(dimethylamino)phosphonium hexafluorophosphate (BroP), Propylphosphonic anhydride (PPACA, T3P®), 2-Morpholinoethyl isocyanide (MEI), N,N,N′,N′-Tetramethyl-O-(N-succinimidyl)uronium hexafluorophosphate (HSTU), 2-Bromo-1-ethyl-pyridinium tetrafluoroborate (BEP), O-[(Ethoxycarbonyl)cyanomethylenamino]-N,N,N′,N′-tetracyclohexyluronium hexafluorophosphate (TOTU), 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (MMTM, DMTMM), N,N,N′,N′-Tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU), O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TDBTU), 1,1′-(Azodicarbonyl)dipiperidine (ADD), Di-(4-chlorobenzyl) azodicarboxylate (DCAD), Di-tert-butyl azodicarboxylate (DBAD), Diisopropyl azodicarboxylate (DIAD), Diethyl azodicarboxylate (DEAD).

The detail examples of the synthesis of the bridge linkers are shown in the figures 1–10. Normally the bridge substituents of 2,3-disubstituted succinic group, or 2-monosubstituted or 2,3-disubstituted fumaric group, or 2-monosubstituted or 2,3-disubstituted maleic group can be condensed with linker components containing function groups capable to react to drugs of desired conjugation.

**CELL-BINDING AGENT-DRUG CONJUGATES**
The conjugates of the present invention can be represented by the following formula,

$$\text{Cb} \overset{L}{\rightarrow} \overset{\text{Drug}_1}{\rightarrow} \overset{\text{Drug}_2}{\rightarrow} S\overset{n}{\rightarrow} S,$$

wherein Cb is a cell-binding agent, L is linker containing succinic, fumaric or maleic group, Drug$_1$ and Drug$_2$ are a drug molecule, n is an integer from 1 to 30, and two S (sulfur) elements from Cb bridgely link to L, which covalently connects two or more drugs (per bridge linker L).

The bridge 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-aminobenzylxycarbonyl ("PAB"), 4-thiopentanoate ("SPP"), 4-(N-maleimidomethyl)-cyclohexane-1 carboxylate ("MCC"), (4-acetyl)aminobenzoate ("SIAB"), 4-thio-butyrate (SPDB), 4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB), ethyleneoxy –CH$_2$CH$_2$O– 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-aminobenzylxycarbonyl containing)
(ME, maleimidoethyl containing).

(valine-citrulline containing)

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

((4-acetyl)aminobenzoate containing)

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

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

\[ \text{Drug}_1 - R_1 - \text{X}_1 - \text{S} - \text{X}_2 - R_2 - \text{Drug}_2 \]

wherein:

Cb represents a cell-binding agent, preferably an antibody, which conjugates to \text{Drug}_1 and \text{Drug}_2 via a pair of sulfur atoms (thiols). The conjugatable thiol groups can generally be generated from dithiothreitol (DTT), dithioerythritol (DTE), L-glutathione (GSH) and tris (2-carboxyethyl) phosphine (TCEP), or/and beta mercaptoethanol (\(\beta\)-ME, 2-ME) reduction of pairs of disulfide bonds on the surface of cell-binding molecule.

\text{Drug}_1 and \text{Drug}_2 represent the same or different cytotoxic agents, linked to the cell-binding agent via the bridge linker through an alkyl, alkenylene, alkynylene, ether, polyoxyalkylene, ester, amine, imine, polyamine, hydrazine, hydrazone, amide, urea, semicarbazide, carbazide, alkoxysamine, urethanes, amino acid, peptide, acyloxyamine,
hydroxamic acid, disulfide, thioether, thioester, carbamate, carbonate, heterocyclic ring, heteroalkyl, heteroaromatic, or alkoxyime bond, or combination thereof.

n is 1 ~ 30; "—","—", R₁, R₂, X₁ and X₂ are described the same previously in Formula (I).

As described in more detail below, Drug₁ and Drug₂ can be any of many small molecule drugs, including, but not limited to, tubulysins, calicheamincins, auristatins, maytansinoids, CC-1065 analogs, morpholinones doxorubicins, taxanes, cryptophycins, epothilones, and benzodiazepine dimers (e.g., dimers of pyrrolobenzodiazepine (PBD) or tomaymycin), indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines).

To synthesize the conjugate, the cell-binding agent can be first modified with the bridge linkers of the present invention through reduction of disulfide bonds of the cell-binding molecule. The yielded a pair of free thiols can react to the bridge linker of Formula (I) at pH 5~9 aqueous media with or without addition of 0~30% of water mixable (miscible) organic solvents, such as DMA, DMF, ethanol, methanol, acetone, acetonitrile, THF, isopropanol, dioxane, propylene glycol, or ethylene diol, to introduce the reactive groups of Z₁ and Z₂ containing disulfide, maleimido, haloacetyl, azide, 1-yne, ketone, aldehyde, alkoxymino, or hydrazide groups. Then a reactive group of a cytotoxic agent reacts to the modified cell-binding molecule accordingly. For example, 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 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., 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). Synthesis of conjugates bearing triazole linkage can be achieved by reaction of a 1-yne group of the drug with the azido moiety in the linker, through the click chemistry (Huisgen cycloaddition) (Lutz, J-F. et al, 2008, Adv. Drug Del. Rev. 60, 958–970; Sletten, E. M. et al 2011, Acc Chem. Research 44, 666–676).

Alternatively, the drug can react with the bridge linkers of the present invention that have conjugated to a cell-binding molecule to give a modified cell-binding molecule linker of Formula (III) bearing functionalities. For example, a thiol-containing drug can be
reached with the modified cell-binding molecule bridge linker of Formula (III) bearing a maleimido, or a haloacetyl, or an ethylsulfonyl substituent at pH 5.5–9.0 in aqueous buffer to give a cell-binding molecule-drug conjugate via a thioether linkage. A thiol-containing drug can undergo disulfide exchange with a modified bridge linker of Formula (III) bearing a pyridyldithio moiety to give a conjugate a disulfide bond linkage. A drug bearing a hydroxyl group or a thiol group can be reacted with a modified bridge linker of Formula (III) bearing a halogen, particularly the alpha halide of carboxylates, in the presence of a mild base, e.g. pH 8.0–9.5, to give a modified drug bearing an ether or thiol ether link. A hydroxyl group containing drug can be condensed with a bridge cross linker of Formula (I) bearing a carboxyl group, in the presence of a dehydrating agent, such as EDC or DCC, to give ester linkage, then the subject drug modified bridge linker undergoes the conjugation with a cell-binding molecule. A drug containing an amino group can condensate with a carboxyl ester of NHS, imidazole, nitrophenol; N-hydroxysuccinimide (NHS); phenol; dinitrophenol; pentafluorophenol; tetrafluorophenol; difluorophenol; monofluorophenol; pentachlorophenol; triflate; imidazole; dichlorophenol; tetrachlorophenol; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenyloxazolium-3′-sulfonate on the cell-binding molecule-bridge linker of Formula (III) to give a conjugate via amide bond linkage.

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/MOLECULES**

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

![Chemical Structure](image)

Wherein “—”, “——”, Cb, Z₁, Z₂, n, R₁, R₂, X₁, and X₂ are defined the same as in Formula (I) and (II).
In preferred embodiments, Z₁ and Z₂ are a disulfide substituent, maleimido, haloacetyl, alkoxyamine, azido, ketone, aldehyde, hydrazine, alkyne, an N-hydroxysuccinimide ester, or a carboxyl ester formed with phenol; dinitrophenol; pentafluorophenol; tetrafluorophenol; difluorophenol; monofluorophenol; pentachlorophenol; triflate; imidazole; dichlorophenol; tetrachlorophenol; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxa-zolium-3'-sulfonate. Z₁ and Z₂ can then react with a cytotoxic agent through disulfide, thioether, hydrazine, amide, alkoxime, carbamate, ester, ether bond or heteroaromatic ring. The modified cell-binding agent can be prepared via a reaction of the cell-binding agent with the bridge linkers of Formula (I) as described in Formula (II) above.

In order to achieve a higher conjugation yield of the alkyne group on the bridge linkers with a pair of free thiols on the cell-binding molecule, preferably on an antibody, a small percentage of organic co-solvent may be required to add to the reaction mixture, as well in the solution after the reaction to maintain solubility of the Formula (III) in aqueous solution. To modify the cell-binding agents, the cross-linking reagent (bridge linker) of Formula (I) can be first dissolved in 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 1-500 mM. Meanwhile, the cell-binding molecule, such as antibody dissolved in an aqueous buffer pH 5-9.5, preferably pH 6-8.5, at 1-35 mg/ml concentration was treated with 1-20 equivalent of TCEP or DTT for 20 min to 12 hour. After the reduction, DTT can be removed by SEC chromatographic purification. TCEP can be optionally removed by SEC chromatography too, or staying in the reaction mixture for the next step reaction without purification. Furthermore, the reduction of antibodies or the other cell-binding agents with TCEP can be performed with a bridge linker of Formula (I), for which the cross-linking conjugation for the cell-binding molecules can be achieved simultaneously along with the TCEP reduction.

The aqueous solutions for the modification of 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 of the bridge linker of Formula (I) into the solution containing the reduced cell-binding molecules, the reaction mixture 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
decrease in the absorption at 254 nm, or increase in the absorption at 280 nm, or the other 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 via UV spectra. For the conjugation without a chromophore group, the modification or conjugation reaction can be monitored by LC-MS, preferably by UPLC-QTOF mass spectrometry, or Capillary electrophoresis–mass spectrometry (CE-MS). The bridge cross-linkers described herein have diverse functional groups that can react with any drugs, preferably cytotoxic agents that possess a suitable substituent. For examples, the modified cell-binding molecules bearing an amino or hydroxyl substituent can react with drugs bearing an N-hydroxysuccinimide (NHS) ester, the modified cell-binding molecules bearing a thiol substituent can react with drugs bearing a maleimido or haloacetyl group. Additionally, the modified cell-binding molecules bearing a carbonyl (ketone or aldehyde) substituent can react with drugs 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 linkers.

**MODIFIED CYTOTOXIC DRUGS**

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

\[
\text{Drug}_1 - R_1 - X_1 - O_\text{U} \quad \text{O} \quad X_2 - R_2 - \text{Drug}_2
\]

(IV)

Wherein “\(\text{O}\)”, “\(-\)”, U, U’, Drug\(_1\), Drug\(_2\), R\(_1\), R\(_2\), X\(_1\), and X\(_2\) are defined the same as in Formula (I) and (II).

The modified drugs can be prepared via reaction of the drug with the linkers of the Formula (I) to give a modified drug of Formula (IV) bearing functionality of an 2,3-disubstituted succinic group, or 2-monosubstituted or 2,3-disubstituted fumaric group, or 2-monosubstituted or 2,3-disubstituted maleic group. But for drugs containing a thiol, or the drugs undergoing to link a cell-binding molecule via the bridge linkers through thioether, thioester or disulfide bond, it is therefore preferred that the Drug\(_1\) or Drug\(_2\) may be synthesized to connect to R\(_1\), or R\(_2\) in a piece of components via the linkage of thioether,
thioester or disulfide bond first. Then the synthesized R₁-Drug₁ or R₂-Drug₂ component is assembled to 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric acid, or 2-monosubstituted or 2,3-disubstituted maleic acid to form the bridge linker modified drugs of Formula (IV).

For examples of the synthesis, a thiol-containing drug can be reacted with the linker of components R₁ or R₂ bearing a maleimido substituent at neutral pH in aqueous buffer to give a R₁-Drug₁ or R₂-Drug₂ compartment bearing thioether linkage, and following by condensation with either 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric acid, or 2-monosubstituted or 2,3-disubstituted maleic acid to give a modified drug of Formula (IV) bearing thioether linkage. A drug bearing a hydroxyl group can be reacted with a linker component R₁ or R₂ bearing a halogen, or a tosylate, or a mesylate, in the presence of a mild base, to give a R₁-Drug₁ or R₂-Drug₂ compartment bearing ether linkage, and following by condensation with 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric acid, or 2-monosubstituted or 2,3-disubstituted maleic acid to give a modified drug of Formula (IV) bearing thioether 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 a modified drug of Formula (IV) via ester linkage. A drug bearing a thiol group can also react the linker of components R₁ or R₂ bearing a maleimido or a vinylsulfonyl, or a haloacetyl group, give a R₁-Drug₁ or R₂-Drug₂ compartment bearing thioether linkage, and following by condensation with a compartment of 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric acid, or 2-monosubstituted or 2,3-disubstituted maleic acid to give a modified drug of Formula (IV) bearing thioether linkage. An amino group containing drug can similarly undergo condensation with a carboxyl group on the bridge linker of Formula (I) to give a modified drug of Formula (IV) bearing amide bonds. 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, Fv [Parham, J. Immunol. 131, 2895-2902 (1983)], fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR's, diabody, triabody, tetraabody, miniantibody, small immune proteins (SIP), 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 TGFα, TGFβ, insulin and insulin like growth factors (IGF-I, IGF-II) G-CSF, M-CSF and GM-CSF [Burgess, Immunology Today, 5, 155-158 (1984)]; vaccinia growth factors (VGF); fibroblast growth factors (FGFs); smaller molecular weight proteins, poly-peptide, peptides and peptide hormones, such as bombesin, gastrin, gastrin-releasing peptide; platelet-derived growth factors; interleukin and cytokines, such as interleukin-2 (IL-2), interleukin-6 (IL-6), leukemia inhibitory factors, granulocyte-macrophage colony-stimulating factor (GM-CSF); vitamins, such as folate; apoproteins and glycoproteins, such as transferrin [O'Keefe et al, 260 J. Biol. Chem. 932-937 (1985)]; sugar-binding proteins or lipoproteins, such as lectins; cell nutrient-transport molecules; and small molecular inhibitors, such as prostate-specific membrane antigen (PSMA) inhibitors and small molecular tyrosine kinase inhibitors (TKI), non-peptides or any other cell binding molecule or substance, such as bioactive polymers (Dhar, et al, Proc. Natl. Acad. Sci. 2008, 105, 17356-61); 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, 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 procedures or combinations thereof. Methods for producing polyclonal anti-receptor peptide antibodies are well-known in the art, such as in U.S. Pat. No. 4,493,795 (to Nestor et al). A monoclonal antibody is typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen (Köhler, G.; Milstein, C. (1975). *Nature* 256: 495-497). The detailed procedures are described in “Antibodies--A Laboratory Manual”, Harlow and Lane, eds., Cold Spring Harbor Laboratory Press, New York (1988), 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 cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT (hypoxanthine-aminopterin-thymine). Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact specified receptors or inhibit receptor activity on target cells.

A monoclonal antibody used in the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques, such as using protein-A affinity chromatography; anion, cation, hydrophobic, or size exclusive chromatographies (particularly by affinity for the specific antigen after protein A, and sizing column chromatography); centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., *Virol. 8, 396 (1959)*) supplemented with 4.5 gm/l glucose, 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. 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 considerably less immunogenic in man than murine mAbs (Kipriyanov et al, Mol Biotechnol. 26:39-60 (2004); Houdebine, Curr Opin Biotechnol. 13:625-9 (2002) 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 Methods. 231:249-60 (1999)) and exchanging constant regions of a mAb can improve its ability to mediate effector functions of binding and cytotoxicity.

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

Apart from an antibody, a peptide or protein that bind/block/target or in some other way interact with the epitopes or corresponding receptors on a targeted cell can be used as a binding molecule. These peptides or proteins could be any random peptide or proteins that have an affinity for the epitopes or corresponding receptors and they don't necessarily have to be of the immunoglobulin family. These peptides can be isolated by similar techniques as for phage display antibodies (Szardening, J Recept Signal Transduct Res. 2003; 23(4):307-49). The use of peptides from such random peptide libraries can be similar to antibodies and antibody fragments. The binding molecules of peptides or proteins may be conjugated on or linked to a large molecules or materials, such as, but is not limited, an albumin, a polymer, a liposome, a nano particle, 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 bridge 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-Ilb), Adalimumab (anti-TNF-α), Adecatumumab (anti-EpCAM, CD326), Afelimomab (anti-TNF-α); Afutuzumab (anti-CD20), Alacizumab pegol (anti-VEGFR2), ALD518 (anti-IL-6), Alemtuzumab (Campath, MabCampath, anti-CD52), Altumomab (anti-CEA), Anatumomab (anti-TAG-72), Anrukinzumab (IMA-638, anti-IL-13),
Apolizumab (anti-HLA-DR), Arcitumomab (anti-CEA), Aselizumab (anti-L-selectin (CD62L), Atlizumab (tocilizumab, Actemra, RoActemra, anti-IL-6 receptor), Atorolimumab (anti-Rhesus factor), Bapineuzumab (anti-beta amyloid), Basiliximab (Simulect, antiCD25 (a 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 (Scintum, 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 (a chain of IL-2 receptor), Daratumumab (anti-CD38 (cyclic ADP ribose hydrolase), Denosumab (Prolia, anti-RANKL), Detumomab (anti-B-lymphoma cell), Dorlimomab, Dorlixizumab, Ecromeximab (anti-GD3 ganglioside), Eculizumab (Soliris, anti-C5), Edobacomab (anti-endotoxin), Edrecolomab (Panorex, MAb17-1A, anti-EpCAM), Efalizumab (Raptiva, anti-LFA-1 (CD11a), Efungumab (Mycograb, anti-Hsp90), Elotuzumab (anti-SLAMF7), Elsilimomab (anti-IL-6), Enlimomab pegol (anti-ICAM-1 (CD54)), Epitumomab (anti-episialin), Epratuzumab (anti-CD22), Erlizumab (anti-ITGB2 (CD18)), Ertumaxomab (Rexomun, anti-HER2/neu, CD3), Etaracizumab (Abegrin, anti-integrin α,β3), Exbivirumab (anti-hepatitis B surface antigen), Fanolesomab (NeutoSpec, anti-CD15), Faralimomab (anti-interferon receptor), Farletuzumab (anti-folate receptor 1), Felizumab (anti-respiratory syncytial virus), Fezakinumab (anti-IL-22), Figitumumab (anti-IGF-1 receptor), Fontolizumab (anti-IFN-γ), Foravirumab (anti-rabies virus glycoprotein), Fresolimumab (anti-TGF-β), Galiximab (anti-CD80), Gantenerumab (anti-beta amyloid), Gavilimomab (anti-CD147 (basigin)), Gemtuzumab (anti-CD33), Girentuximab (anti-carbonic anhydrase 9), Glembatumumab (CR011, anti-GPNMB), Golimumab (Simponi, anti-TNF-α), Gomiliximab (anti-CD23 (IgE receptor), Ibalizumab (anti-CD4), Ibritumomab (anti-CD20), Ivogomab (Indimacis-125, anti-CA-125), Imcromab (Myoscint, anti-cardiac myosin), Infliximab (Remicade, anti-TNF-α), Intetumumab (anti-CD51), Inolimomab (anti-CD25 (a chain of IL-2 recep-
tor), 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), Luatumumab (anti-CD40), Lumiliximab (anti-CD23 (IgE receptor), Mapatumumab (anti-TRAIL-R1), Maslimob (anti-T-cell receptor), Matuzumab (anti-EGFR), Mepolizumab (Bosatria, anti-IL-5), Metelimumab (anti-TGF beta 1), Milatuzumab (anti-CD74), Minretumomab (anti-TAG-72), Mitumumab (BEC-2, anti-GD3 ganglioside), Morolimumab (anti-Rhesus factor), Motavizumab (Numax, anti-respiratory syncytial virus), Muromonab-CD3 (Orthoclone OKT3, anti-CD3), Nacoloma (anti-C242), Naptumomab (anti-5T4), Natalizumab (Tysabri, anti-integrin α4), Nebacumab (anti-endotoxin), Necitumumab (anti-EGFR), Nerelimomab (anti-TNF-α), Nimotuzumab (Theracim, Theraloc, anti-EGFR), Nofetumumab, Ocrelizumab (anti-CD20), Odulimomab (Afolimomab, anti-LFA-1 (CD11a)), Ofatumumab (Arzerra), anti-CD20), Olaratumab (anti-PDGF-R α), Omalizumab (Xolair, anti-IgE Fc region), Oportunumab (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- \textit{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), Ritolumumab (anti-HGF), Rituximab (MabThera, Rituxanmb, anti-CD20), Robatumumab (anti-IGF-1 receptor), Rontalizumab (anti-IFN-α), Rovelizumab (LeukArrest, anti-CD11, CD18), Ruplizumab (Antova, anti-CD154 (CD40L)), Satumomab (anti-TAG-72), Sevirumab (anti-cytomegalovirus), Sibrotuzumab (anti-FAP), Sifalimumab (anti-IFN-α), Siltuximab (anti-IL-6), Siplizumab (anti-CD2), (Smart) MJ95 (anti-CD33), Solanezumab (anti-beta amyloid), Sonepcizumab (anti-sphingosine-1-phosphate), Sontuzumab (anti-episialin), Stamulumb (anti-myostatin), Sulesomab (LeukoScan, anti-NCA-90 (granulocyte antigen), Tacatumumab (anti-alpha-fetoprotein), Tadocizumab (anti-integrin α1β3), Talizumab (anti-IgE), Tanezumab (anti-NGF), Taplitumomab (anti-CD19),
Tefibazumab (Aurexis, (anti-clumping factor A), Telimomab (anti-tenascin C), Teneliximab (anti-CD40), Teplizumab (anti-CD3), TGN1412 (anti-CD28), Ticilimumab (Tremelimumab, (anti-CTLA-4), Tigatuzumab (anti-TRAIL-R2), TNX-650 (anti-IL-13), Tocilizumab (Atlizumab, Actemra, RoActemra, (anti-IL-6 receptor), Toralizumab (anti-CD154 (CD40L)), Tositumomab (anti-CD20), Trastuzumab (Herceptin, (anti-HER2/neu), Tremelimumab (anti-CTLA-4), Tucotuzumab celmoleukin (anti-EpCAM), Tuvirumab (anti-hepatitis B virus), Urtoxazumab (anti- *Escherichia coli*), Ustekinumab (Stelara, anti-IL-12, IL-23), Vapaliximab (anti-AOC3 (VAP-1)), Vedolizumab, (anti-integrin α₄β₇), Veltuzumab (anti-CD20), Vepalimomab (anti-AOC3 (VAP-1), Visilizumab (Nuvion, anti-CD3), Vitaxin (anti-vascular integrin avb3), Volociximab (anti-integrin α₃β₁), Votumumab (HumaSPECT, anti-tumor antigen CTAA16.88), Zalutumumab (HuMax-EGFr, (anti-EGFR), Zanolimumab (HuMax-CD4, anti-CD4), Ziralimumab (anti-CD147 (basigin)), Zolimomab (anti-CD5), Etanercept (Enbrel®), Alefacect (Amevive®), Abatacept (Orencia®), Rilonacept (Arcalyst), 14F7 [anti-IRP-2 (Iron Regulatory Protein 2)], 14G2a (anti-GD2 ganglioside, from Nat. Cancer Inst. for melanoma and solid tumors), J591 (anti-PSMA, Weill Cornell Medical School for prostate cancers), 225.28S [anti-HMW-MAA (High molecular weight-melanoma-associated antigen), Sorin Radiofarmaci S.R.L. (Milan, Italy) for melanoma], COL-1 (anti-CEACAM3, CGM1, from Nat. Cancer Inst. USA for colorectal and gastric cancers), CYT-356 (Oncolitad®, for prostate cancers), HNK20 (OraVax Inc. for respiratory syncytial virus), ImmuRAIT (from Immunomedics for NHL), Lym-1 (anti-HLA-DR10, Peregrine Pharm. for Cancers), MAK-195F [anti-TNF (tumor necrosis factor; TNFA, TNF-alpha; TNFSF2), from Abbott / Knoll for Sepsis toxic shock], MEDI-500 [T10B9, anti-CD3, TRαβ (T cell receptor alpha/beta), complex, from MedImmune Inc for Graft-versus-host disease], RING SCAN [ anti-TAG 72 (tumour associated glycoprotein 72), from Neoprobe Corp. for Breast, Colon and Rectal cancers], Avicidin (anti-EPCAM (epithelial cell adhesion molecule), anti-TACSTD1 (Tumor-associated calcium signal transducer 1), anti-GA733-2 (gastrointestinal tumor-associated protein 2), anti-EGP-2 (epithelial glycoprotein 2); anti-KSA; KS1/4 antigen; M4S; tumor antigen 17-1A; CD326, from NeoRx Corp. for Colon, Ovarian, Prostate cancers and NHL]; LymphoCide (Immunomedics, NJ), Smart ID10 (Protein Design Labs), Oncolym (Techniclone Inc, CA), Allomune (BioTransplant, CA), anti-VEGF (Genentech, CA); CEAcide (Immunomedics, NJ), IMC-1C11 (ImClone Systems, NJ) and Cetuximab (ImClone, NJ).
Other antibodies as 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 glycolipid, melanoma), GD3 idiotype (cancers), Heat shock proteins (cancers), HER1 (lung, stomach cancers), HER2 (breast, lung and ovarian cancers), HLA-DR10 (NHL), HLA-DRB (NHL, B cell leukemia), human chorionic gonadotropin (carcinoma), IGF1R (insulin-like growth factor 1 receptor, solid tumors, blood cancers), IL-2 receptor (interleukin 2 receptor, T-cell leukemia and lymphomas), IL-6R (interleukin 6 receptor, multiple myeloma, RA, Castleman’s disease, IL6 dependent tumors), Integrins (αβ3, α5β1, α6β4, α1β3, α5β5, αvβ5, for various cancers), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE 4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A member 1, Non-Hodgkin’s B cell lymphoma, leukemia), MUC1 or MUC1-KLH (breast, ovarian, cervix,
bronchus and gastrointestinal cancer), MUC16 (CA125) (Ovarian cancers), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), MPG (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A, small cell lung cancers, NHL), Nucleolin, Neu oncogene product (carcinomas), P21 (carcinomas), Paratope of anti-(N-glycolylneuraminic acid, Breast, Melanoma cancers), PLAP-like testicular alkaline phosphatase (ovarian, testicular cancers), PSMA (prostate tumors), PSA (prostate), ROBO4, TAG 72 (tumour associated glycoprotein 72, AML, gastric, colorectal, ovarian cancers), T cell transmembrane protein (cancers), Tie (CD202b), TNFRSF10B (tumor necrosis factor receptor superfamily member 10B, cancers), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B, multiple myeloma, NHL, other cancers, RA and SLE), TPBG (trophoblast glycoprotein, Renal cell carcinoma), TRAIL-R1 (Tumor necrosis 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); 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 melanocytes. 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, 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 WAIIF1), 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 (Factor D), Ch4D5, Cholecystokinin 2 (CCK2R), CLDN18 (Claudin-18), Clumping factor A, CRIPTO, FCS1R (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 (folute 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 idiotype, GloboH, Glycpican 3, N-glycolylneuraminic acid, GM3, GMCSF receptor α-chain, Growth differentiation factor 8, GP100, GPNNMB (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 (α4, α5β1, αβ3, α4β1, α5β4, α7β7, αβ3, α5β5, α6β1), 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-TE51, P21, p53 nonmutant, P97, Page4, PAP, Paratope of anti-(N-glycolyneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1, CD279), PDGF-Rα (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 (Rhe polypeptide 1 (RhP1), 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-β1, TGF-β2 (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 apoprosis 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 bridge 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, Extrabiliary Bile Duct Cancer, Ewings Family of Tumors (PNET), Extracranial Germ Cell Tumor, Eye Cancer, Intraocular Melanoma, Gallbladder Cancer, Gastric Cancer (Stomach), Germ Cell Tumor, Extranodal, 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 bridge likers 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, Behcets Syndrome, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous Pemphigoid, Castleman's disease, Chagas disease, Chronic Fatigue Immune Dysfunction Syndrome, Chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal ostomyelitis, Chronic lyme disease, Chronic obstructive pulmonary disease, Churg-Strauss syndrome, Cicatricial Pemphigoid, Coeliac Disease, Cogan syndrome, Cold agglutinin disease, Component 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 I, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, Discoid lupus erythematosus, Eczema, Endometriosis, Enthesitis-related arthritis, Eosinophilic fasciitis, Epidermolysis bullosa acquisita, Erythema nodosum, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibromyalgia, Fibromyositis, Fibrosing aevolitis, Gastritis, Gastrointestinal pemphigoid, Giant cell arteritis, Glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Haemolytic anaemia, Henoch-Schonlein purpura, Herpes gestationis, Hidradenitis suppurativa, Hughes syndrome (See Antiphospholipid syndrome), Hypogammaglobulinemia, Idiopathic Inflammatory Demyelinating Diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura (See Autoimmune thrombocytopenic
purpura), IgA nephropathy (Also Berger's disease), Inclusion body myositis, Inflammatory
demyelinating 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, Majeed syndrome, Ménière's disease, Micrasclerotic
polyangiitis, Miller-Fisher syndrome, Mixed Connective Tissue Disease, Morphea, Mucha-
Habermann disease, Muckle–Wells syndrome, Multiple Myeloma, Multiple Sclerosis,
Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's Disease),
Neuromyotonia, Occular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord
thyroiditis, Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric
Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Parox-
ysmal nocturnal hemoglobinuria, Parry Romberg syndrome, Parsonnage-Turner syndrome,
Pars planitis, Pemphigus, Pemphigus vulgaris, Pernicious anaemia, Perivenous encephalo-
myelitis, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis,
Primary biliary cirrhosis, Primary sclerosing cholangitis, Progressive inflammatory neu-
ropathy, Psoriasis, Psoriatic Arthritis, Pyoderma gangrenosum, Pure red cell aplasia,
Rasmussen's encephalitis, Raynaud phenomenon, Relapsing polychondritis, Reiter's syn-
drome, Restless leg syndrome, Retroperitoneal fibrosis, Rheumatoid arthritis, Rheumatoid
fever, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis,
Scleroderma, Sjögren's syndrome, Spondyloarthropathy. Sticky blood syndrome, Still's
Disease, Stiff person syndrome, Subacute bacterial endocarditis, Susac's syndrome, Sweet
syndrome, Sydenham Chorea, Sympathetic ophthalmia, Takayasu's arteritis, Temporal
arteritis (giant cell arteritis), Tolosa-Hunt syndrome, Transverse Myelitis, Ulcerative
Colitis (a type of idiopathic inflammatory bowel diseases), Undifferentiated connective
tissue disease, Undifferentiated spondyloarthropathy, Vasculitis, Vitiligo, Wegener's
granulomatosis, Wilson's syndrome, Wiskott-Aldrich syndrome

In another specific embodiment, a binding molecule used for the conjugate via the
bridge 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 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-Desmogin 3 antibody; Anti-p62 antibody; Anti-sp100 antibody; Anti-Mitochondrial(M2) antibody; Rheumatoid factor antibody; Anti-MCV antibody; Anti-topoisomerase antibody; Anti-neutrophil cytoplasmic(cANCA) antibody.

In certain preferred embodiments, the binding molecule for the conjugate in the present invention, can bind to both a receptor and a receptor complex expressed on an activated lymphocyte which is associated with an autoimmune disease. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member (e.g. CD2, CD3, CD4, CD8, CD19, CD20, CD22, CD28, CD30, CD33, CD37, CD38, CD56, CD70, CD79, CD79b, CD90, CD125, CD147, CD152/CTLA-4, PD-1, or ICOS), a TNF receptor superfamily member (e.g. CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, INF-R1, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3), an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin (C-type, S-type, or I-type), or a complement control protein.

In another specific embodiment, useful 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 neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g. gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. As used herein, the term "microbial antigen" includes, but is not limited to, any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., a bacteria, fungi, pathogenic protozoa, or yeast 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 bridge 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, Aspergillus, 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, Chlamyphila pneumoniae infection, Cholera, Chromoblastomycosis, Clonorchiasis, Clostridium difficile infection, Coccidiodomycosis, Colorado tick fever, Common cold (Acute viral rhinopharyngitis; Acute coryza), Creutzfeldt-Jakob disease, Crimean-Congo hemorrhagic fever, Cryptococcosis, Cryptosporidiosis, Cutaneous larva migrans, Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue fever, Dientamoebiasis, Diphtheria, Diphyllobothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), Enterococcus infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum (Fifth disease), Exanthem subitum, Fasciolopsiasis, Fasciolosis, Fatal familial insomnia, Filariasis, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fusobacterium infection, Gas gangrene (Clostridial myonecrosis), Geotrichosis, Gerstmann-Sträussler-Scheinker syndrome, Giardiasis, Glanders, Gnathostomiasis, Gonorrhea, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group B streptococcal infection, Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hantavirus Pulmonary Syndrome, Helicobacter pylori infection, Hémolytic-uremic syndrome, Hemorrhagic fever with renal syndrome, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Herpes simplex, Histoplasmosis, Hookworm infection, Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis, Human metapneumovirus infection, Human monocytic ehrlichiosis, Human papillomavirus infection, Human parainfluenza virus infection, Hymenolepiasis, Epstein-Barr Virus Infectious Mononucleosis (Mono), Influenza, Isosporiasis, Kawasaki disease, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Legionellosis (Legionnaires’
disease), Legionellosis (Pontiac fever), Leishmaniasis, Leprosy, Leptospirosis, Listeriosis, Lyme disease (Lyme borreliosis), Lymphatic filariasis (Elephantiasis), Lymphocytic choriomeningitis, Malaria, Marburg hemorrhagic fever, Measles, Melioidosis (Whitmore’s disease), Meningitis, Meningococcal disease, Metagonimiasis, Microsporidiosis, Molluscum contagiosum, Mumps, Murine typhus (Endemic typhus), Mycoplasma pneumonia, Mycetoma, Myiasis, Neonatal conjunctivitis (Ophthalmia neonatorum), (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), Nocardiosis, Onchocerciasis (River blindness), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Pasteurellosis, Pediculosis capitis (Head lice), Pediculosis corporis (Body lice), Pediculosis pubis (Pubic lice, Crab lice), Pelvic inflammatory disease, Pertussis (Whooping cough), Plague, Pneumococcal infection, Pneumocystis pneumonia, Pneumonia, Poliomyelitis, Prevotella infection, Primary amoebic meningoencephalitis, Progressive multifocal leukoencephalopathy, Psittacosis, Q fever, Rabies, Rat-bite fever, Respiratory syncytial virus infection, Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsial-pox, 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, Strongyloides, Syphilis, Taeniasis, Tetanus (Lockjaw), Tinea barbae (Barber’s itch), Tinea capitis (Ringworm of the Scalp), Tinea corporis (Ringworm of the Body), Tinea cruris (Jock itch), Tinea manuum (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 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 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
Microsporidia phylum, Molluscum contagiosum virus (MCV), Mumps virus, Rickettsia typhi, Mycoplasma pneumoniae, numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma), parasitic dipterous fly larvae, Chlamydia trachomatis and Neisseria gonorrhoeae, vCJD prion, Nocardia asteroides and other Nocardia species, Onchocerca volvulus, Paracoccidioides braziliensis, Paragonimus westermani and other Paragonimus species, Pasteurella genus, Pediculus humanus capitis, Pediculus humanus corporis, Pthirus pubis, Bordetella pertussis, Yersinia pestis, Streptococcus pneumoniae, Pneumocystis jirovecii, Poliovirus, Prevotella genus, Naegleria fowleri, JC virus, Chlamydophila psittaci, Coxiella burnetii, Rabies virus, Streptobacillus moniliformis and Spirillum minus, Respiratory syncytial virus, Rhinosporidium seeberi, Rhinovirus, Rickettsia genus, Rickettsia akari, Rift Valley fever virus, Rickettsia rickettsii, Rotavirus, Rubella virus, Salmonella genus, SARS coronavirus, Sarcoptes scabiei, Schistosoma genus, Shigella genus, Varicella zoster virus, Variola major or Variola minor, Sporothrix schenckii, Staphylococcus genus, Staphylococcus aureus, Streptococcus pyogenes, Strongyloides stercoralis, Treponema pallidum, Taenia genus, Clostridium tetani, Trichophyton genus, Trichophyton tonsurans, Trichophyton genus, Epidermophyton floccosum, Trichophyton rubrum, and Trichophyton mentagrophytes, Trichophyton rubrum, Hortaea werneckii, Trichophyton genus, Malassezia genus, Toxocara canis or Toxocara cati, Toxoplasma gondii, Trichinella spiralis, Trichomonas vaginalis, Trichuris trichiura, Mycobacterium tuberculosis, Franciscella tularensis, Ureaplasma urealyticum, Venezuelan equine encephalitis virus, Vibrio cholerae, Guaranito virus, West Nile virus, Trichosporon beigelii, Yersinia pseudotuberculosis, Yersinia enterocolitica, Yellow fever virus, Mucoraales order (Mucormycosis) and Entomophthorales order (Entomophthora-mycosis), Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Aeromonas hydrophila, Edwardsiella tarda, Yersinia pestis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Treponema pertenue, Treponema carateneum, Borrelia vincentii, Borrelia burgdorferi, Leptospiraicterohemorrhagiae, Pneumocystis carinii, Brucella abortus, Brucella suis, Brucella melitensis, Mycoplasma spp., Rickettsia prowazekii, Rickettsia tsutsugamushi, Clamydia spp.; pathogenic fungi (Aspergillus fumigatus, Candida albicans, Histoplasma capsulatum); protozoa (Entamoeba histolytica, Trichomonas tenas, Trichomonas hominis, Trypanosoma gambiense, Trypanosoma rhodesiense, Leishmania donovani, Leishmania tropica, Leishmania braziliensis, Pneumocystis pneumonia, Plasmodium vivax, Plasmodium falciparum, Plasmodium malaria); 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: Poxyviridae, Herpesviridae, Adenoviridae,
Papovaviridae, Enteroviridae, Picornaviridae, Paroviridae, Reoviridae, Retroviridae,
influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus,
rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Non-A/Non-B Hepatitis virus,
Rhinoviridae, Coronaviridae, Rotoviridae, Oncovirus [such as, HBV (Hepatocellular
carcinoma), HPV (Cervical cancer, Anal cancer), Kaposi’s sarcoma-associated herpesvirus
(Kaposi’s sarcoma), Epstein-Barr virus (Nasopharyngeal carcinoma, Burkitt’s lymphoma,
Primary central nervous system lymphoma), MCPyV (Merkel cell cancer), SV40 (Simian
virus 40), HCV (Hepatocellular carcinoma), HTLV-I (Adult T-cell leukemia/lymphoma)],
Immune disorders caused virus: [such as Human Immunodeficiency Virus (AIDS)]; Cen-
tral nervous system virus: [such as, JCV (Progressive multifocal leukoencephalopathy),
MeV (Subacute sclerosing panencephalitis), LCV (Lymphocytic choriomeningitis),
Arbovirus encephalitis, Orthomyxoviridae (probable) (Encephalitis lethargica), RV (Ra-
bies), Chandipura virus, Herpesviral meningitis, Ramsay Hunt syndrome type II; Po-
liovirus (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 mononuc-
cleosis), 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, Coro-
navirus; 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 bridge linkers of the invention together with
a pharmaceutically acceptable carrier, diluent, or excipient for treatment of cancers, infec-
tions or autoimmune disorders. The method for treatment of cancers, infections and auto-
immune disorders can be practiced in vitro, in vivo, or ex vivo. Examples of in vitro uses
include treatments of cell cultures in order to kill all cells except for desired variants that
do not express the target antigen; or to kill variants that express undesired antigen. Examples of ex vivo uses include treatments of hematopoietic stem cells (HSC) prior to the performance of the transplantation (HSCT) into the same patient in order to kill diseased or malignant cells. For instance, clinical ex vivo treatment to remove tumour cells or lymphoid cells from bone marrow prior to autologous transplantation in cancer treatment or in treatment of autoimmune disease, or to remove T cells and other lymphoid cells from allogeneic bone marrow or tissue prior to transplant in order to prevent graft-versus-host disease, can be carried out as follows. Bone marrow is harvested from the patient or other individual and then incubated in medium containing serum to which is added the conjugate of the invention, concentrations range from about 1 pM to 0.1 mM, for about 30 minutes to about 48 hours at about 37 °C. The exact conditions of concentration and time of incubation (=dose) are readily determined by the skilled clinicians. After incubation, the bone marrow cells are washed with medium containing serum and returned to the patient by i.v. infusion according to known methods. In circumstances where the patient receives other treatment such as a course of ablative chemotherapy or total-body irradiation between the time of harvest of the marrow and reinfusion of the treated cells, the treated marrow cells are stored frozen in liquid nitrogen using standard medical equipment.

For clinical in vivo use, the conjugate 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 given weekly for 8-20 weeks as an i.v. bolus. Bolus doses are given in 50 to 500 ml of normal saline to which human serum albumin (e.g. 0.5 to 1 mL of a concentrated solution of human serum albumin, 100 mg/mL) can be added. Dosages will be about 50 μg to 20 mg/kg of body weight per week, i.v. (range of 10 μg to 200 mg/kg per injection). 4-20 weeks after treatment, the patient may receive a second course of treatment. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, etc., can be determined by the skilled clinicians.

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

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

In general terms, the conjugates 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 μg/kg to 0.1 g/kg of body weight per day; a preferred dose range is from 0.01 mg/kg to 20 mg/kg of body weight per day, or per week, or an equivalent dose in a human child. The preferred dosage of drug to be administered is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound, the route of administration (intravenous, intramuscular, or other), the pharmacokinetic properties of the conjugates 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 week, and even more preferably from 1 mg to 100 mg, once a week. 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). Alkylation agents: such as Nitrogen mustards: chlorambucil, cloromaphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxde hydrochloride, mannomustine, mitobronitol, melphanal, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiopeta, trofosfamide, uracil must; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); Duocarmycin (including the synthetic analogues, KW-2189 and CBITM1); Benzodiazepine dimers (e.g., dimers of pyrrolobenzodiazepine (PBD) or tomaymycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines); Nitrosoureas: (carmustine, lomustine, chlorozotocin, fotemustine, nimustine, ranimustine); Alkylsulphonates: (bursulan, treosulfan, imposulfan and piposulfan); Triazenes: (dacarbazine); Platinum containing compounds: (carboplatin, cisplatin, oxaliplatin); Aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylalamines including altretamine, triethylenemelamine, trietylenephosphoramides, triethylenemethylamino, 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, maytansine and ansamitocins) and their analogs, cryptophycins (particularly cryptophycin 1 and cryptophycin 8); epothilones, eleutherobin, discodermolide, bryostatins, dolastatins, auristatins, tubulysins, cephalostatins; pancretatstatin; a sarcodictyin; spargarstatin; c). DNA Topoisomerase Inhibitors: such as [Epipodophyllins: (9-aminocamptothecin, camptothecin, crinsolate, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantron, 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: (methylphenolic acid, tiazofurin, ribavirin, EICAR); Ribonucleotide reductase Inhibitors: (hydroxyurea, deferoxamine)]; [Pyrimidine analogs: Uracil analogs:
(ancitabine, azacitidine, 6-azuuridine, capecitabine (Xeloda), carmustin, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-Flourouracil, floxuridine, ratitrexed

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(Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, fludarabine, mercaptopurine, thiopurine, thioguanine)]; folic acid replenisher, such as folinic 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, meptiostane, 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, pnonatinib (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 γ1, δ1, α1 and β1, see, e.g. J. Med. Chem., 39 (11), 2103–2117 (1996), Angew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabcin, carinomycin, 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), Ca$^{2+}$ ATPase inhibitors (such as thapsigargin), Histone deacetylase inhibitors (Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A); Thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, Disulfiram, Salinosporamide A.; Anti-adrenals, such as aminogluthethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine (DFMO), elfomithine; elliptinium acetate, etogolucid; gallium nitrate; gacytosine, hydroxyurea; ibandronate, lentinan; lonidamine; mitoguazone; mtoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK$^\circledR$; razoxane; rhizoxin; sizofiran; spirogermanium; tenuasonic acid; triaziquone; 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, fluorocortolone danazol, dexamethasone, Triamcinolone acetonide, beclometasone dipropionate), DHEA, enanercept, hydroxycytoquinone, infliximab, meloxicam, methotrexate, mofetil, mycophenolate, 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, bekamycin, 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, cefadroxil, cefalonium, cefaloridine, cefalotin or cefalothin, cefalexin, cefaloglycin, cefamandole, cefapirin, cefazidine, cefazalexin, cefazolin, cefbuperazone, cefcapene, cefdaloxime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftezole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonicid,
cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cephalexin, cefpimizole,
cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime,
cefteram, ceftrubuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime,
cefuzonam, *cephamycin* (cefoxitin, cefotetan, cefmetazole), oxacephem (flomoxef,
latamoxef); l). Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin),
teicoplanin (dalbavancin), ramoplanin; g). Glycyclines: 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,
cephromycin), midecamycin, moxycamycin, oleandomycin, rifamycins (rifampicin, rifampin,
rifabutin, rifapentine), rokitamycin, roxithromycin, spectinomycin, spiramycin, tacroliimus
(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 phenoxyethyl-penicillin, clometocillin, procaine
benzylpenicillin, carbenicillin (carindacillin), cloxacillin, dicloxacillin, epicillin,
flucloxacillin, mecillinam (pivmecillinam), mezlocillin, meticillin, nafcillin, oxacillin,
penicillin, penicillin, penicillin, pheneticillin, phenoxyethylpenicillin, piperacillin, propicillin,
sulbenicillin, temocillin, ticarcillin; o). Polypeptides: bacitracin, colistin, polymyxin B; p).

*Quinolones*: alatrofloxacain, balofloxacin, ciprofloxacain, clinafloxacain, danofloxacain,
difloxacain, enoxacin, enrofloxacain, floxin, garenxocain, gatifloxacain, gemifloxacain,
grepafloxacain, kano trovafoxacain, levofloxacain, lomefloxacain, marbofloxacain,
moxifloxacain, nadifloxacain, norfloxacain, orbifloxacain, ofloxicain, pefloxacin, trovafloxacain,
grepafloxacain, sitafloxacain, sparfoxcain, temafloxacain, tosufloxcain, trovafloxacain; q).

*Streptogramins*: pristinamycin, quinupristin/dalfopristin; r). **Sulfonamides**: mafenide,
prontosil, sulfacetamide, sulfamethizole, sulfanilamide, sulfasalazine, sulfisoxazole, trimethoprim,
trimethoprim-sulfamethoxazole (co-trimoxazole); s). **Steroid antibacterials**: e.g.
fusidic acid; t). Tetracyclines: doxycycline, chlorotetracycline, clomocycline,
demeclocycline, lymecycline, meclocycline, metacycline, minocycline, oxytetracycline,
penimepicycline, rolitetracycline, tetracycline, glyclyclines (e.g. tigecycline); u).

Other types of antibiotics: annonacin, arsphenamine, bactoprenol inhibitors (Bacitracin),
DADAL/AR inhibitors (cyclosorine), dietyostatin, discodermolide, eleutherobin,
epothilone, ethambutol, etoposide, faropenem, fusidic acid, furazolidone, isoniazid,
lauinaldehyde, 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, vimecon; d). Neuraminidase inhibitors: oseltamivir, zanamivir, peramivir; e). Nucleosides & nucleotides: abacavir, aciclovir, adeovir, 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, aterviridine, capravirine, diarylpyrimidines (etravirine, rilpivirine), delavirdine, docosanol, emivirine, efavirenz, foscarinet (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), foscarinet, griffithsin, taribavirin (viramidine), hydroxyurea, KP-1461, miltefosine, pleconaril, portmanteau inhibitors, ribavirin, seliciclib.

5). The drugs used for conjugates via a bridge linker of the present invention also include radioisotopes. Examples of radioisotopes (radionuclides) are ³H, ¹¹C, ¹³C, ¹⁸F, ³²P, ³⁵S, ⁶⁴Cu, ⁶⁸Ga, ⁶⁷Y, ⁹⁹Tc, ¹¹¹In, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I, ¹³³Xe, ¹⁷⁷Lu, ²¹¹At, or ²¹²Bi. 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 bridge 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, Pubs. (1991). Chelating ligands which may complex a metal ion include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocycles, Dallas, Tex. USA).

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 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, florescent light, IR light, near IR light, visual light; A chromatophore 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, thiacarbocyanine, 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 benzoaxadiazole); 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). Tetrapyrrolo 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), FluorProbes (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);

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, Chromomycosin A3, CyTRAK Orange (Biostatus, red excitation dark), DAPI, DRAQ5, DRAQ7, Ethidium Bromide, Hoechst33258, Hoechst33342, LDS 751, Mithramycin, PropidiumIodide (PI), SYTOX Blue, 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′Dichlorodihydrofluorescein, 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, HeRed1, J-Red, Katusha, Kusabira Orange (monomer, MBL), mCFP, mChart, mCitrine, Midoriishii 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 another embodiment, the drug in the Formula (II) and (IV) can be a polyalkylene glycols that are used for extending the half-life of the cell-binding molecule when administered to a mammal. Polyalkylene glycols include, but are not limited to, poly(ethylene glycols) (PEGs), poly(propylene glycol) and copolymers of ethylene oxide and propylene oxide; particularly preferred are PEGs, and more particularly preferred are monofunctionally activated hydroxyPEGs (e.g., hydroxyPEGs activated at a single terminus, including reactive esters of hydroxyPEG-monocarboxylic acids, hydroxyPEG-monoaldehydes, hydroxyPEG-monoamines, hydroxyPEG-monohydrazides, hydroxyPEG-monocarbazates, hydroxyPEG-monoiodoacetamides, hydroxyPEG-monomaleimides, hydroxyPEG-monoorthopyridyl disulfides, hydroxyPEG-monooximes, hydroxyPEG-mono phenyl carbonates, hydroxyPEG-mono phenyl glyoxals, hydroxyPEG-monothiazolidine-2-thiones, hydroxyPEG-monothioesters, hydroxyPEG-mono thiols, hydroxyPEG-monotriazines and hydroxyPEG-monovinylsulfones).

In certain such embodiments, the polyalkylene glycol has a molecular weight of from about 10 Daltons to about 200 kDa, preferably about 88 Da to about 40 kDa; two branches each with a molecular weight of about 88 Da to about 40 kDa; and more preferably two branches, each of about 88 Da to about 20 kDa. In one particular embodiment, the polyalkylene glycol is poly(ethylene glycol) and has a molecular weight of about 10 kDa; about 20 kDa, or about 40 kDa. In specific embodiments, the PEG is a PEG 10 kDa (linear or branched), a PEG 20 kDa (linear or branched), or a PEG 40 kDa (linear or branched). A number of US patents have disclosed the preparation of linear or branched "non-antigenic" PEG polymers and derivatives or conjugates thereof, e.g., U.S. Pat. Nos. 5,428,128; 5,621,039; 5,622,986; 5,643,575; 5,728,560; 5,730,990; 5,738,846; 5,811,076; 5,824,701; 5,840,900; 5,880,131; 5,900,402; 5,902,588; 5,919,455; 5,951,974; 5,965,119; 5,965,566; 5,969,040; 5,981,709; 6,011,042; 6,042,822; 6,113,906; 6,127,355; 6,132,713; 6,177,087, and 6,180,095. The structure of the conjugates of the antibody-polyalkylene glycols via the bridge linker is as following Pg01:
Wherein mAb is an antibody; n is 1-30; R’ and R” are independently H or CH₃; m₃ and m₄ are independently 0-5000; “—”, “—”, X₁, X₂, R₁, R₂, and R₃ are the same defined in Formula (I) and (II); R₄ is OH, H, or R₁, or R₃ that is defined as in Formula (I).

In yet another embodiment, the preferred cytotoxic agents that conjugated to a cell-binding molecule via a bridge linker of this patent are tubulysins, maytansinoids, taxanoids (taxanes), CC-1065 analogs, daunorubicin and doxorubicin compounds, benzodiazepine dimers (e.g., dimers of pyrrolobenzodiazepine (PBD), tomaymycin, anthramycin, indolobenzodiazepines, imidazobenzothiadiazepines, or oxazolidino-benzodiazepines), calicheamicins and the ediniey antibiotic, actinomycins, 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, thiopeta, vinceristines, hemiasterlins, nazumamides, microginins, radiosumins, alterobactins, microsclerodermins, theonellamides, esperamicins, PNU-159682, and their analogues and derivatives above thereof.


Examples of the structures of the conjugates of the antibody-tubulysin analogs via the bridge linker are T01, T02, T03, T04, T05, T06 and T07 as following:
Wherein mAb is an antibody; Z_3 and Z'_3 are independently H, OP(O)(OM_1)(OM_2), \( \text{OCH}_2\text{OP(O)(OM}_1\text{)}(\text{OM}_2) \), \( \text{OSO}_3\text{M}_1 \), \( \text{R}_1 \), or \( \text{O}-\text{glycoside (glucoside, galactoside, mannoside, glucuronoside, alloside, fructoside, etc.)} \), NH-glycoside, S-glycoside or CH_2-glycoside; \( \text{M}_1 \) and \( \text{M}_2 \) are independently H, Na, K, Ca, Mg, NH_4, \( \text{NR}_1\text{R}_2\text{R}_3 \); \( n \) is 1~30; “—”, “=”; \( X_1 \), \( X_2 \), \( \text{R}_1 \), \( \text{R}_2 \) and \( \text{R}_3 \) are the same defined in Formula (I) and (II).

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; 8,153,768. An Example of the structure of the conjugate of the antibody-Calicheamicin analog via the bridge linker is C01 as the following:

Wherein mAb is an antibody; \( n \) is 1~30; “—”, “=”; \( X_1 \), \( X_2 \), \( \text{R}_1 \), \( \text{R}_2 \) and \( \text{R}_3 \) are the same defined in Formula (I) and (II).
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, 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. An example of the structure of the conjugate of the antibody- Maytansinoids via the bridge linker is as the following M01:

![Diagram of M01 conjugate]

Wherein mAb is an antibody; n is 1~30; “-”, “-”, X1, X2, R1, R2 and R3 are the same defined in Formula (I) and (II).


Examples of the structures of the conjugate of the antibody- taxanes via the bridge linker are as the following Tx01, Tx02 and Tx03.

![Diagram of Tx01 conjugate]
Wherein mAb is an antibody; n is 1~30; “—”, “—”, X₁, X₂, R₁ and R₂ are the same defined in Formula (I) and (II).

CC-1065 analogues and dourcarmycin analogs are also preferred to be used for a conjugate with the bridge linkers of the present patent. The examples of the CC-1065 analogues and dourcarmycin 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, 5660820, 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, 6103226, 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. Examples of the structures of the conjugate of the antibody-CC-1065 analogs via the bridge linker are as the following CC01, CC02, and CC03.

Wherein mAb is an antibody; n is 1~30; Z₄ and Z’₄ are independently H, PO(OM₁)(OM₂), CH₂PO(OM₁)(OM₂), SO₃M₁, CH₃N(CH₂CH₂)₂NC(O)⁻, O(CH₂CH₂)₂NC(O)⁻, R₁, or glycoside; X₃ and X’₃ are independently O, NH, NHC(O), OC(O), -C(O)O, R₁, or absent; “—”, “—”, X₁, X₂, R₁, R₂, M₁, and M₂ are the same defined in Formula (I) and (II).
Wherein mAb is an antibody; n is 1~30; X₃ and X'₃ are independently H, O, NH, NHC(O), NHC(O)NH, C(O), R₁, or OC(O); “—”, “—”, X₁, X₂, R₁, and R₂ are the same defined in Formula (I) and (II).

Auristatins and dolastatins are preferred in conjugation via the bridge linkers of this patent. The auristatins (e.g. auristain 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,
Examples of the structures of the conjugate of the antibody-auristatins via the bridge linker are as the following Au01, Au02, Au03, Au04, and Au05.

Wherein mAb is an antibody; n is 1-30; X_3 and X'_3 are independently CH_2, O, NH, NHC(O), NHC(O)NH, C(O), OC(O) R_1, or absent; X_4 and X'_4 are independently CH_2,
C(O), C(O)NH, C(O)N(R_1), R_1, NHR_1, NR_1, C(O)R_1 or C(O)O; Z_3 and Z'_3 are independently H, R_1, OP(O)(OM_1)(OM_2), NHR_1, OCH_2OP(O)(OM_1)(OM_2), OSO_3M_1, or O-glycoside (glucoside, galactoside, mannoside, glucuronoside, alloside, fructoside), NH-glycoside, S-glycoside, or CH_2-glycoside; M_1 and M_2 are independently H, Na, K, Ca, Mg, NH_4, NR_1R_2R_3; “—”, “—”, X_1, X_2, R_1, R_2 and R_3 are the same defined in Formula (I) and (II).

The benzodiazepine dimers (e.g. dimmers of pyrrolobenzodiazepine (PBD) or (tomaymycin), indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines) 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. Examples of the structures of the conjugate of the antibody-benzodiazepine dimers via the bridge linker are as the following PB01, PB02, PB03, PB04, PB05, PB06, PB07, PB08, PB09, PB10 and PB11.
Wherein mAb is an antibody; n is 1~30; X₃ and X'₃ are independently CH₂, O, NH, NHC(O), NHC(O)NH, C(O), OC(O), OC(O)(NR₃), R₁, NHR, NR, C(O)R₁ or absent; X₄ and X'₄ are independently CH₂, C(O), C(O)NH, C(O)N(R₁), R₁, NHR, NR, C(O)R₁ or C(O)O; M₁ and M₂ are independently H, Na, K, Ca, Mg, NH₄, NR₂R₃; “—”, “—”, X₁, X₂, R₁, R₂ and R₃ are the same defined in Formula (I) and (II). In addition, R₁ and/or R₂ can be absent.

In yet another embodiment, two or more different cytotoxic agents are preferred conjugated to a cell-binding molecule via a bridge linker of this patent. The two or more different cytotoxic agents can be selected from any combinations of tubulysins, maytansinoids, taxanoids (taxanes), CC-1065 analogs, daunorubicin and doxorubicin compounds, benzodiazepine dimers (e.g., dimers of pyrrolobenzodiazepine (PBD), tomatamycin, 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, MMAP, auristatin PYE, auristatin TP, Auristatins 2-AQ, 6-AQ, EB (AEB), and EFP (AEFP)), duocarmycins, thiopeta, vincristines, hemiasterlins, nazarinamides, microginins, radiosumins, alterobactins, microsclerodermins, theonellamides, esperamicins, PNU-159682, and their analogues and derivatives above thereof. Examples of the structures of the conjugates containing two or more different cytotoxic agents via the bridge linker are as the following Z01, Z02, Z04, Z05, Z06, Z07, Z08, Z09, Z10, Z12, Z13, Z14, Z15, Z16, Z17 and Z18:
Wherein mAb is an antibody; n is 1~30; X₃ and X'₃ are independently CH₂, O, NH, NHC(O), NHC(O)NH, C(O), OC(O), OC(O)(NR₃), R₁, NHR₁, NR₁, C(O)R₁ or absent; X₄ and X'₄ are independently H, CH₂, OH, O, C(O), C(O)NH, C(O)N(R₁), R₁, NHR₁, NR₁, C(O)R₁ or C(O)O; M₁ and M₂ are independently H, Na, K, Ca, Mg, NH₄, NR₁R₂R₃; “—”, “—”, X₁, X₂, R₁, R₂ and R₃ are the same defined in Formula (I) and (II). In addition, R₁ and/or R₂ can be absent.

In yet another embodiment, cell-binding ligands or receptors can be conjugated to a cell-binding molecule via a bridge linker of this patent. These conjugated cell-binding ligands or receptors, in particular, antibody-receptor conjugates, can be not only to work as a targeting conductor/director to deliver the conjugate to malignant cells, but also be used to modulate or co-stimulate a desired immune response or altering signaling pathways. In the immunotherapy, the cell-binding ligands or receptors are preferred to conjugate to an antibody of TCR (T cell receptors) T cell, or of CARs (chimeric antigen receptors) T cells, or of B cell receptor (BCR), or the cytotoxic cells. The cell-binding ligands or receptors are selected, but not limited, from: Folate derivatives (binding to the folate receptor, a protein over-expressed in ovarian cancer and in other malignancies) (Low, P. S. et al 2008, Acc.

The cell-binding ligands or receptors can be Ig-based and non-Ig-based protein scaffold molecules. The Ig-Based scaffolds can be selected, but not limited, from Nanobody (a derivative of VHH (camelid Ig)) (Muylrdemans S., 2013 Annu Rev Biochem. 82, 775–797); Domain antibodies (dAb, a derivative of VH or VL domain) (Holt, L. J, et al, 2003, Trends Biotechnol. 21, 484–490); Bispecific T cell Engager (BiTE, a bispecific diabody) (Baeuerle, P. A, et al, 2009, Curr. Opin. Mol. Ther. 11, 22–30); Dual Affinity ReTargeting

Examples of the structures of the conjugate of the antibody-cell-binding ligands or receptors via the bridge linker are as the following: LB01 (PMSA ligand conjugate), LB02 (Folate receptor conjugate), LB03 (Somatostatin receptor conjugate), LB04 (Octreotide, a Somatostatin analog receptor conjugate), LB05 (Lanreotide, a Somatostatin analog receptor conjugate), LB06 (CAIX receptor conjugate), LB07(CAIX receptor conjugate), LB08 (luteinizing hormone-releasing hormone (LH-RH) ligand and GnRH conjugate), LB09 (luteinizing hormone-releasing hormone (LH-RH) and GnRH ligand conjugate), LB10 (GnRH antagonist, Abarelix conjugate), LB11 (cobalamin, VB12 analog conjugate), LB12 (Gastrin releasing peptide receptor (GRPr), MBA conjugate), LB13 (α,β3 integrin receptor, cyclic RGD pentapeptide conjugate), LB14 (hetero-bivalent peptide ligand for VEGF receptor conjugate), LB15 (Neuromedin B conjugate), LB 16 (a G-protein coupled receptor, bombesin conjugate) and LB17 (a Toll-like receptor, TLR2 conjugate).
LB09

LB10

LB11

R₇ = 5'-deoxyadenosyl, Me, OH, or CN,
The drugs/ cytotoxic agents used for conjugation via a bridge 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 Varian 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 spect equipped with Waters Acquity UPLC separations module and Acquity TUV detector.

**Example 1:** tert-Butyl 3-(2-(2-hydroxyethoxy)ethoxy)propanoate (84)

To 350 mL of anhydrous THF was added 80 mg (0.0025 mol) of sodium metal and diethylene glycol 83 (150.1 g, 1.41 mol) with stirring. After the sodium had completely dissolved, tert-butyl acrylate (24 mL, 0.33 mol) was added. The solution was stirred for 20 h at room temperature and neutralized with 8 mL of 1.0 M HCl. The solvent was removed in vacuo and the residue was suspended in brine (250 mL) and extracted with ethyl acetate (3 x 125 mL). The combined organic layers were washed with brine (100 mL) then water (100 mL), dried over sodium sulfate, and the solvent was removed. The resulting colorless oil was dried under vacuum to give 60.27 g (78% yields) of product 84. \(^1\)H NMR: 1.41 (s, 9H), 2.49 (t, 2H, J=6.4 Hz), 3.59-3.72 (m, 10H). ESI MS m/z C\(_{11}\)H\(_{21}\)O\(_3\) (M-H), calcd. 233.15, found 233.40.

**Example 2:** tert-Butyl 3-(2-(2-(tosyloxy)ethoxy)ethoxy)propanoate (85)

A solution of 84 (10.0 g, 42.70 mmol) in dichloromethane (50.0 mL) was treated with pyridine (20.0 mL). A solution of methanesulfonyl chloride (7.50 g, 65.81 mmol) in 50 mL dichloromethane was added dropwise via an addition funnel over 30 minutes. After 5 h TLC analysis revealed that the reaction was complete. The pyridine hydrochloride that had formed was filtered off and the solvent was removed. The residue was purified on silica gel by eluting from with 20% ethyl acetate in hexane to with neat ethyl acetate to give 10.39 g (76% yield) of compound 85. \(^1\)H NMR: 1.40 (s, 9H), 3.23 (s, 3H), 2.45 (t, 2H, J=6.4 Hz), 3.54-3.70 (m, 10H); ESI MS m/z C\(_{12}\)H\(_{25}\)O\(_7\) (M+) calcd. 313.10, found 313.30.
**Example 3.** tert-Butyl 3-(2-(azidoethoxy)ethoxy)propanoate (86)

![Chemical Structure](image)

To 50 mL of DMA was added tert-butyl 3-(2-(Mesyloxy)ethoxy)ethoxy)ethoxy)propanoate 85 (4.0 g, 12.81 mmol) and sodium azide (0.90 g, 13.84 mmol) with stirring. The reaction was heated to 80 °C. After 4 h TLC analysis revealed that the reaction was complete. The reaction was cooled to room temperature and quenched with water (25 mL). The aqueous layer was separated and extracted into ethyl acetate (3 x 35 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, concentrated in vacuo and purified on silica gel by eluting from with 15% ethyl acetate in hexane to with neat ethyl acetate to give 2.88 g (87% yield) of compound 86. \(^1\)H NMR (CDCl3): 1.40 (s, 9H), 2.45 (t, 2H, J=6.4 Hz), 3.33 (t, 2H, J=5.2 Hz), 3.53-3.66 (m, 8H). ESI MS m/z+ C\(_{11}\)H\(_{22}\)N\(_{3}\)O\(_{7}\) (M+H), calcld. 260.13, found 260.20.

**Example 4.** 3-(2-(azidoethoxy)ethoxy)propanoic acid (87).

![Chemical Structure](image)

The azide compound 86 (2.51 g, 9.68 mmol) dissolved in 1.4-dioxane (30 mL) and was added 10 ml of HCl (conc.). The mixture was stirred for 35 min, diluted with EtOH (30 ml) and toluene (30 ml) and evacuated under vacuum. The crude mixture was purified on silica gel using a mixture of methanol (from 5% to 10%) and 1% formic acid in methylene chloride as the eluant to give title compound 87 (1.63 g, 83% yield), ESI MS m/z- C\(_{7}\)H\(_{12}\)N\(_{3}\)O\(_{4}\) (M-H), calcld. 202.06, found 202.30.

**Example 5.** 2,5-dioxopyrrolidin-1-yl 3-(2-(azidoethoxy)ethoxy)propanoate (88).

![Chemical Structure](image)

To compound 87 (1.60 g, 7.87 mmol) in 30 mL of dichloromethane was NHS (1.08 g, 9.39 mmol) and EDC (3.60 g, 18.75 mmol) with stirring. After 8 h TLC analysis revealed that the reaction was complete, the reaction mixture was evaporated and purified on silica gel using a mixture of ethyl acetate (from 5% to 10%) in methylene chloride as the eluant to give title compound 88 (1.93 g, 82% yield). ESI MS m/z+ C\(_{11}\)H\(_{17}\)N\(_{4}\)O\(_{6}\) (M+H), calcld. 301.11, found 301.20.

**Example 6.** (4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-
5-(3-(3-(2-(azidoethoxy)ethoxy)propanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (94)

To a solution of (4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-5-(3-amino-4-hydroxyphenyl)-2-methylpentanoic acid, 93 (Huang Y. et al, Med Chem. #44, 249th ACS National Meeting, Denver, CO, Mar. 22–26, 2015; WO2014009774) (100 mg, 0.131 mmol) in the mixture of DMA (10 ml) and NaH2PO4 buffer (5 ml, 1.0 M, pH 7.5) was added compound 88 (80.0 mg, 0.266 mmol) in four portions in 2 h. The mixture was stirred overnight, concentrated and purified on C-18 preparative HPLC (3.0 x 25 cm, 25 ml/min) eluted with from 80% water/20% methanol to 10% water/90% methanol in 45 min to afford the title compound (101.5 mg, 82% yield). LC-MS (ESI) m/z calcd. for C45H70N9O11S [M+H]+: 944.48, found: 944.70.

Example 7. (4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-5-(3-(2-(2-aminoethoxy)ethoxy)propanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (95).

Compound 94 (100.0 mg, 0.106 mmol) in methanol (25 ml) containing 0.1% HCl in a hydrogenation vessel was added Pd/C (25 mg, 10% Pd, 50% wet). After air was vacuumed out in the vessel, 35 psi H2 was conducted in. The mixture was shaken for 4 h, filtered through celite, concentrated, and purified on C-18 preparative HPLC (3.0 x 25 cm, 25 ml/min) eluted with from 85% water/15% methanol to 15% water/85% methanol in 45 min to afford the title compound (77.5 mg, 79% yield). LC-MS (ESI) m/z calcd. for C45H72N9O11S [M+H]+: 918.49, found: 918.60.

Example 8. 4-(benzyloxy)-3-methoxybenzoic acid
4-Hydroxy-3-methoxybenzoic acid (50.0 g, 297.5 mmol) in the mixture of ethanol (350 ml) and NaOH solution (2.0 M, 350 ml) was added BnBr (140.0 g, 823.5 mmol). The mixture was stirred at 65 °C for 8 h, concentrated, co-evaporated with water (2 x 400 ml) to ~400 ml, acidified with 6 M HCl to pH 3.0, filtered the solid, crystallized with EtOH, dried over the oven at 45 °C with vacuum to afford the title compound (63.6 g, 83% yield). ESI MS m/z+ 281.2 (M + Na).

**Example 9.** 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid

4-(Benzyloxy)-3-methoxybenzoic acid (63.5 g, 246.0 mmol) in the mixture of CH$_2$Cl$_2$ (400 ml) and HOAc (100 ml) was added HNO$_3$ (fuming, 25.0 ml, 528.5 mmol). The mixture was stirred for 6 h, concentrated, crystallized with EtOH, dried over the oven at 40 °C with vacuum to afford the title compound (63.3 g, 85% yield). ESI MS m/z+ 326.1 (M + Na).

**Example 10.** (2S,4R)-methyl 4-hydroxypyrrolidine-2-carboxylate, hydrochloric salt.

Trans-4-hydroxy-L-proline (15.0 g, 114.3 mmol) in dry methanol (250 mL) at 0 ~ 4 °C, was added dropwise thionyl chloride (17 mL, 231 mmol). The resulting mixture was stirred for at RT overnight, concentrated, crystallized with EtOH/hexane to provide the title compound (18.0 g, 87% yield), ESI MS m/z+ 168.2 (M + Na).

**Example 11.** (2S,4R)-1-tert-butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate

To a solution of trans-4-hydroxy-L-proline methyl ester (18.0 g, 107.0 mmol) in the mixture of MeOH (150 ml) and sodium bicarbonate solution (2.0 M, 350 ml) was added (BOC)$_2$O (30.0 g, 137.6 mmol) in three portions in 4 h. After stirring for an additional 4 h, the reaction was concentrated to ~350 ml and extracted with EtOAc (4 x 80 mL). The combined organic layers were washed with brine (100 mL), dried (MgSO$_4$), filtered, concentrated and purified by SiO$_2$ chromatography (1:1 hexanes/EtOAc) to give the title compound (22.54 g, 86% yield). ESI MS m/z+ 268.2 (M + Na).
Example 12. (S)-1-tert-butyl 2-methyl 4-oxopyrrolidine-1,2-dicarboxylate

\[
\text{O} \quad \text{COOMe} \\
\text{Boc}
\]

The title compound prepared through Dess-Martin oxidation was described in: Franco Manfre et al. J. Org. Chem. 1992, 57, 2060-2065. Alternatively Swern oxidation procedure is as following: A solution of (COCl)\textsubscript{2} (13.0 ml, 74.38 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (350 ml) cooled to -78 °C was added dry DMSO (26.0 mL). The solution was stirred at -78 °C for 15 min and then (2S,4R)-1-tert-butyl 2-methyl 4-hydroxypyrrrolidine-1,2-dicarboxylate (8.0 g, 32.63 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (100 ml). After stirred at -78 °C for 2 h, triethylamine (50 ml, 180.3 mmol) was added dropwise, and the solution was warmed to room temperature. The mixture was diluted with NaH\textsubscript{2}PO\textsubscript{4} (400 ml, 1.0 M) solution and separated. The aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (2 x 60 ml). The organic layers were combined, dried over MgSO\textsubscript{4}, filtered, concentrated and purified by Si\textsubscript{2}O chromatography (7:3 hexanes/EtOAc) to give the title compound (6.73 g, 85% yield). ESI MS m/z+ 266.2 (M + Na).

Example 13. (S)-1-tert-butyl 2-methyl 4-methyleneypyrrrolidine-1,2-dicarboxylate

\[
\begin{align*}
\text{O} & \quad \text{COOMe} \\
\text{Boc} & \quad \longrightarrow & \quad \text{O} \quad \text{COOMe} \\
\text{Boc} & \quad \longrightarrow & \quad \text{N} \quad \text{COOMe}
\end{align*}
\]

A solution of methyltriphenylphosphonium bromide (19.62 g, 55.11 mmol) in THF (150 mL) at 0°C was potassium-t-butoxide (6.20 g, 55.30 mmol) in anhydrous THF (80 mL). After stirred at 0°C for 2 h, the resulting yellow ylide suspension was added the solution of (S)-1-tert-butyl 2-methyl 4-oxopyrrolidine-1,2-dicarboxylate (6.70 g, 27.55 mmol) in THF (40 mL). After stirring at RT for 1 h, the reaction mixture was concentrated, diluted with EtOAc (200 mL), washed with H\textsubscript{2}O (150 mL), brine (150 mL), dried over MgSO\textsubscript{4}, concentrated purified on Si\textsubscript{2}O flash chromatography (9:1 hexanes/EtOAc) to yield the title compound (5.77 g, 87% yield). EIMS m/z+ 264 (M + Na).

Example 14. (S)-methyl 4-methyleneypyrrrolidine-2-carboxylate

\[
\begin{align*}
\text{N} & \quad \text{COOMe} \\
\text{Boc} & \quad \longrightarrow & \quad \text{N} \quad \text{COOMe}
\end{align*}
\]

(S)-1-tert-butyl 2-methyl 4-methyleneypyrrrolidine-1,2-dicarboxylate (5.70 g, 23.63 mmol) in EtOAc (40 ml) at 4 °C was added HCl (10 ml, 12 M). The mixture was stirred

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for 1 h, diluted with toluene (50 ml), concentrated, and crystallized with EtOH/hexane to yield the title compound as HCl salt (3.85 g, 92% yield). EIMS m/z+ 142.2 (M +H).

Example 15. (S)-methyl 1-(4-(benzyloxy)-5-methoxy-2-nitrobenzoyl)-4-methyleneypyrrrolidine-2-carboxylate

A catalytic amount of DMF (30 μl) was added to a solution of 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid (2.70 g, 8.91 mmol) and oxalyl chloride (2.0 mL, 22.50 mmol) in anhydrous CH₂Cl₂ (70 mL) and the resulting mixture was stirred at room temperature (RT) for 2 h. Excess CH₂Cl₂ and oxalyl chloride was removed with rotavap. The acetyl chloride was resuspended in fresh CH₂Cl₂ (70 mL) and was added dropwise to a solution of 4-methylene-L-proline methyl ester HCl salt (1.58 g, 8.91 mmol), Et₃N (6 mL) at 0°C under argon atmosphere. The reaction mixture was allowed to warm to RT and stirring was continued for 8 h. After removal of CH₂Cl₂ and Et₃N, the residue was partitioned between H₂O and EtOAc (70/70 mL). The aqueous layer was further extracted with EtOAc (2 x 60 mL). The combined organic layers were washed with brine (40 mL), dried (MgSO₄) and concentrated. Purification of the residue with flash chromatography (silica gel, 2:8 hexanes/EtOAc) yielded (S)-methyl 1-(4-(benzyloxy)-5-methoxy-2-nitrobenzoyl)-4-methyleneypyrrrolidine-2-carboxylate (2.88 g, 76.1% yield); EIMS m/z 449.1 ([M]+Na).

Example 16. (S)-1-(4-(benzyloxy)-5-methoxy-2-nitrobenzoyl)-4-methyleneypyrrrolidine-2-carbaldehyde

To a vigorously stirred solution of (S)-methyl 1-(4-(benzyloxy)-5-methoxy-2-nitrobenzoyl)-4-methyleneypyrrrolidine-2-carboxylate (2.80 g, 6.57 mmol) in anhydrous CH₂Cl₂ (60 mL) was added dropwise solution of DIBAL-H (10 mL of a 1M solution in CH₂Cl₂) at -78 °C under argon atmosphere. After the mixture was stirred for an additional 90 min, excess reagent was decomposed by addition of 2 ml of methanol followed by 5% HCl (10 mL). The resulting mixture was allowed to warm to 0°C. Layers were separated and the aqueous layer was further extracted with CH₂Cl₂ (3 x 50 mL). Combined organic layers were washed with brine, dried (MgSO₄) and concentrated. Purification of the residue with flash chromatography (silica gel, 95:5 CHCl₃/MeOH) yielded (S)-1-(4-
(benzyloxy)-5-methoxy-2-nitrobenzoyl)-4-methyleneypyrrolidine-2-carbaldehyde (2.19 g, 84% yield). EIMS m/z 419.1 ([M]++Na).

Example 17. (S)-8-(benzyloxy)-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]-pyrrolo[1,2-a]azepin-5(11aH)-one

(S)-1-(4-(benzyloxy)-5-methoxy-2-nitrobenzoyl)-4-methyleneypyrrolidine-2-carbaldehyde (2.18 g, 5.50 mmol) and Na₂S₂O₄ (8.0 g, 45.97 mmol) in the mixture of THF (60 ml) and H₂O (40 ml) were stirred at RT for 20 h. Solvents were removed under high vacuum. The residue was re-suspended in MeOH (60 mL), and HCl (6M) was added dropwise until pH ~ 2. The resulting mixture was stirred at RT for 1 h. The reaction was work-up by removing most of MeOH, then diluted with EtOAc (100 mL). The EtOAc solution was washed with sat. aq. NaHCO₃, brine, dried (MgSO₄), and concentrated. Purification of the residue with flash chromatography (silica gel, 97:3 CHCl₃/MeOH) yielded (S)-8-(benzyloxy)-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a]azepin-5(11aH)-one (1.52 g, 80%). EIMS m/z 372.1 ([M]⁺+Na).

Example 18. (S)-8-hydroxy-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]-pyrrolo[1,2-a]azepin-5(11aH)-one

(S)-8-(benzyloxy)-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a]azepin-5(11aH)-one (1.50 g, 4.32 mmol) in 70 ml of CH₂Cl₂ at 0°C was added 25 ml of CH₂SO₃H. The mixture was stirred at 0°C for 10 min then RT for 2 h, diluted with CH₂Cl₂ , neutralized with cold 1.0 M NaHCO₃ to pH 4, filtered. The aqueous layer was extracted with CH₂Cl₂ (3x 60 ml). The organic layers were combined, dried over Na₂SO₄, filtered, evaporated and purified on SiO₂ chromatography eluted with CH₃OH/CH₂Cl₂ (1:15) to afford 811 mg (73% yield) of the title product. EIMS m/z 281.1 ([M]⁺+Na).

Example 19. (11aS,11a'S)-8,8′-(pentane-1,5-diylbis(oxy))bis(7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one) (97)
To a stirred suspended solution of Cs₂CO₃ (0.761 g, 2.33 mmol) in butanone (8 ml) were added (S)-8-hydroxy-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one (401 mg, 1.55 mmol) and 1,5-diiodopentane (240 mg, 0.740 mmol). The mixture was stirred at RT overnight, concentrated, and purified on SiO₂ chromatography eluted with EtOAc/CH₂Cl₂ (1:10) to afford 337 mg (78% yield) of the title product. EIMS m/z 607.2 ([M]+Na).

**Example 20.** (S)-7-methoxy-8-(5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one (98)

(11aS,11a'S)-8,8'-(pentane-1,5-diyl)bis(oxy))bis(7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one) (150 mg, 0.256 mmol) in anhydrous dichloromethane (1 mL) and absolute ethanol (1.5 mL) was added sodium borohydride in methoxyethyl ether (85µl, 0.5 M, 0.042mmol) at 0 °C. The ice bath was removed after 5 minutes and the mixture was stirred at room temperature for 3 hours, then cooled to 0 °C, quenched with saturated ammonium chloride, diluted with dichloromethane, and separated. The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and filtered through Celite and concentrated. The residue was purified by reverse phase HPLC (C18 column, acetonitrile/ water). The corresponding fractions were extracted with dichloromethane and concentrated to afford the title compound (98), (S)-7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one, (64.7 mg, 43%), MS m/z+ 609.2 (M + Na), 625.3 (M + K), 627.2 (M + Na+ H₂O); the full reduced compound, (11aS,11a'S)-8,8'-(pentane-1,5-diyl)bis(oxy))bis(7-methoxy-2-methylene-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(10H)-one), (16.5 mg, 11.1%), MS m/z+ 611.2 (M + Na), 627.2 (M + K), 629.2 (M + Na+ H₂O); and the unreacted starting material (10.2 mg, 6.8%), MS m/z+ 607.2 (M + Na), 625.2 (M + Na+ H₂O).
**Example 21.** (S)-8-(((S)-10-(3-(2-pentyl)oxy)propanoyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one, (99)

To the mixture of compound 98 (60.0 mg, 0.102 mmol) and compound 88 (40.5 mg, 0.134 mmol) in dichloromethane (5 ml) was added EDC (100.5 mg, 0.520 mmol). The mixture was stirred at RT overnight, concentrated, and purified on SiO2 chromatography eluted with EtOAc/CH2Cl2 (1:6) to afford 63.1 mg (81% yield) of the title product 99. ESI MS m/z+ C40H50N7O9 (M+H), calcd. 772.36, found 772.30.

**Example 22.** (S)-8-(((S)-10-((3-(2-aminooxy)ethoxy)propanoyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one, (100).

To the compound 99 (60 mg, 0.078 mmol) in the mixture of THF (5 ml) and NaH2PO4 buffer (50 mM, pH 5.0, 1 ml) was added PPh3 (70 mg, 0.267 mmol). The mixture was stirred at RT overnight, concentrated, and purified on C-18 chromatography eluted with water/CH3CN (from 90% water to 35% water in 35 min) to afford 45.1 mg (79% yield) of the title product 100 after dried with high vacuum pump. ESI MS m/z+ C40H52N2O9 (M+H), calcd. 746.37, found 746.50.

**Example 23.** (S)-tert-butyl 2-(hydroxymethyl)pyrrolidine-1-carboxylate

Boc-L-proline (10.0 g, 46.4 mmol) dissolved in 50 mL THF was cooled to 0 °C, to which BH3 in THF (1.0 M, 46.4 mL) was added carefully. The mixture was stirred at 0 °C for 1.5 h then poured onto ice water and extracted with ethyl acetate. The organic layer
was washed with brine (50 mL), dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure to give the title compound (8.50 g, 91% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 3.94 (dd, J = 4.9, 2.7 Hz, 2H), 3.60 (ddd, J = 18.7, 11.9, 9.3 Hz, 2H), 3.49 – 3.37 (m, 1H), 3.34 – 3.23 (m, 1H), 2.06 – 1.91 (m, 1H), 1.89 – 1.69 (m, 2H), 1.65 – 1.51 (m, 1H), 1.49 – 1.40 (m, 9H).

**Example 24.** (S)-tert-butyl 2-formylpyrrolidine-1-carboxylate

![Chemical Structure](image)

To a solution of (S)-tert-butyl 2-(hydroxymethyl)pyrrolidine-1-carboxylate (13.0 g, 64.6 mmol) in dimethyl sulfoxide (90 mL) was added triethylamine (40 mL) and the stirring was continued for 15 min. The mixture was cooled over ice bath and sulfur trioxide-pyridine complex (35.98 g, 226 mmol) was added in portions over a 40 min period. The reaction was warmed to r.t. and stirred for 2.5 h. After addition of ice (250 g), the mixture was extracted with dichloromethane (150 mL × 3). The organic phase was washed with 50% citric acid solution (150 mL), water (150 mL), saturated sodium bicarbonate solution (150 mL), and brine (150 mL), dried over anhydrous Na$_2$SO$_4$. Removal of solvent in vacuo yielded the title compound (10.4 g, 81% yield) as dense oil which was used without further purification. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.45 (s, 1H), 4.04 (s, 1H), 3.53 (dd, J = 14.4, 8.0 Hz, 2H), 2.00 – 1.82 (m, 4H), 1.44 (d, J = 22.6 Hz, 9H).

**Example 25.** (4R,5S)-4-methyl-5-phenyl-3-propionylazolidin-2-one

![Chemical Structure](image)

$n$-Butyllithium in hexane (21.6 mL, 2.2 M, 47.43 mmol) was added dropwise at -78°C to a stirred solution of 4-methyl-5-phenyloxazolidin-2-one (8.0 g, 45.17 mmol) in THF (100 mL) under N$_2$. The solution was maintained at -78°C for 1 h then propionyl chloride (4.4 mL, 50.59 mmol) was added slowly. The reaction mixture was warmed to -50°C, stirred for 2 h then quenched by addition of a saturated solution of ammonium chloride (100 mL). The organic solvent was removed in vacuo and the resultant solution was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with saturated sodium bicarbonate solution (100 mL) and brine (100 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography (20% ethyl acetate/hexanes) to afford the title compound as dense oil (10.5 g, 98% yield). 1H
NMR (500 MHz, CDCl₃) δ 7.45 − 7.34 (m, 3H), 7.30 (d, J = 7.0 Hz, 2H), 5.67 (d, J = 7.3 Hz, 1H), 4.82 − 4.70 (m, 1H), 2.97 (dd, J = 19.0, 7.4 Hz, 2H), 1.19 (t, J = 7.4 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H).

**Example 26.** (S)-tert-butyl 2-((1R,2R)-1-hydroxy-2-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenylxazolidin-3-yl)-3-oxopropyl)pyrrolidine-1-carboxylate.

![Chemical Structure](image)

To a solution of (4R,5S)-4-methyl-5-phenyl-3-propionyloxazolidin-2-one (9.40 g, 40.4 mmol) in dichloromethane (60 mL) was added Et₃N (6.45 mL, 46.64 mmol) at 0 °C, followed by 1M dibutylboron triflate in dichloromethane (42 mL, 42 mmol). The mixture was stirred at 0 °C for 45 min, cooled to -70 °C, (S)-tert-butyl 2-formylpyrrolidine-1-carboxylate (4.58 g, 22.97 mmol) in dichloromethane (40 mL) was then added slowly over a 30 min period. The reaction was stirred at -70 °C for 2 h, 0 °C 1 h, and r.t. 15 min, and then quenched with phosphate buffer solution (pH 7, 38 mL). After the addition of MeOH-30% H₂O₂ (2:1, 100 mL) at below 10 °C and stirring for 20 min, water (100 mL) was added and the mixture was concentrated in vacuo. More water (200 mL) was added to the residue and the mixture was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with 1N KHSO₄ (100 mL), sodium bicarbonate solution (100 mL) and brine (100 mL), dried over anhydrous Na₂SO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (10% - 50% ethyl acetate/hexanes) to afford the title compound as a white solid (7.10 g, 71% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.39 (dt, J = 23.4, 7.1 Hz, 3H), 7.30 (d, J = 7.5 Hz, 2H), 5.67 (d, J = 7.1 Hz, 1H), 4.84 − 4.67 (m, 1H), 4.08 − 3.93 (m, 3H), 3.92 − 3.84 (m, 1H), 3.50 (d, J = 9.0 Hz, 1H), 3.24 (d, J = 6.7 Hz, 1H), 2.15 (s, 1H), 1.89 (dd, J = 22.4, 14.8 Hz, 3H), 1.48 (d, J = 21.5 Hz, 9H), 1.33 (d, J = 6.9 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H).

**Example 27.** (S)-tert-butyl 2-((1R,2R)-1-methoxy-2-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenylxazolidin-3-yl)-3-oxopropyl)pyrrolidine-1-carboxylate

![Chemical Structure](image)

To a mixture of (S)-tert-butyl 2-((1R,2R)-1-hydroxy-2-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenylxazolidin-3-yl)-3-oxopropyl)pyrrolidine-1-carboxylate. (5.1 g 11.9 mmol)
and molecular sieves (4 Å, 5 g) was added anhydrous dichloroethane (30 mL) under N₂. The mixture was stirred at room temperature for 20 min and cooled to 0 °C. Proton sponge (6.62 g, 30.9 mmol) was added, followed by trimethyloxonium tetrafluoroborate (4.40 g, 29.7 mmol). Stirring was continued for 2 h at 0 °C and 48 h at r.t. The reaction mixture was filtrated and the filtrate was concentrated and purified by column chromatography (20-70% ethyl acetate/hexanes) to afford the title compound as a colorless solid (1.80 g, 35% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.46 – 7.27 (m, 5H), 5.65 (s, 1H), 4.69 (s, 1H), 3.92 (s, 1H), 3.83 (s, 1H), 3.48 (s, 3H), 3.17 (s, 2H), 2.02 – 1.68 (m, 5H), 1.48 (d, J = 22.3 Hz, 9H), 1.32 (t, J = 6.0 Hz, 3H), 0.91 – 0.84 (m, 3H).

**Example 28.** (2R,3R)-3-((S)-1-(tert-butoxycarbonyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanoic acid

![Reaction scheme for Example 28](image)

To a solution of (S)-tert-butyl 2-((1R,2R)-1-methoxy-2-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenylazolidin-3-yl)-3-oxopropyl)pyrrolidine-1-carboxylate (1.80 g, 4.03 mmol) in THF (30 mL) and H₂O (7.5 mL), 30% H₂O₂ (1.44 mL, 14.4 mmol) was added over a 5 min period at 0 °C, followed by a solution of LiOH (0.27 g, 6.45 mmol) in water (5 mL). After stirring at 0 °C for 3 h, 1 N sodium sulfite (15.7 mL) was added and the mixture was allowed to warm to r.t. and stirred overnight. THF was removed in vacuo and the aqueous phase was wash with dichloromethane (3 x 50 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidified to pH 3 with 1N HCl and extracted with ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to afford the title compound as colorless oil (1.15 g, 98% yield). ¹H NMR (500 MHz, CDCl₃) δ 3.99 – 3.74 (m, 2H), 3.44 (d, J = 2.6 Hz, 3H), 3.23 (s, 1H), 2.60 – 2.45 (m, 1H), 1.92 (tt, J = 56.0, 31.5 Hz, 3H), 1.79 – 1.69 (m, 1H), 1.58 – 1.39 (m, 9H), 1.30 – 1.24 (m, 3H).

**Example 29.** (4S,5S)-ethyl 4-((tert-butoxycarbonyl)amino)-5-methyl-3-oxoheptanoate

![Reaction scheme for Example 29](image)
To an ice-cooled solution of (2S,3S)-2-((tert-butoxycarbonyl)amino)-3-methylpentanoic acid (4.55 g, 19.67 mmol) in THF (20 mL) was added 1,1'-carbonyldiimidazole (3.51 g, 21.63 mmol). After evolution of gas ceased, the resultant mixture was stirred at r.t. for 3.5 h. A solution of freshly prepared isopropylmagnesium bromide in THF (123 mmol, 30 mL) was added dropwise to a pre-cooled (0 °C) solution of ethyl hydrogen malonate (6.50 g, 49.2 mmol) at such a rate to keep the internal temperature below 5 °C. The mixture was stirred at r.t. for 1.5 h. This solution of the magnesium enolate was then cooled over an ice-water bath, followed by the gradual addition of the imidazolide solution over a 1 h period via a double-ended needle at 0 °C. The resultant mixture was stirred at 0 °C for 30 min then r.t. 64 h. The reaction mixture was quenched by addition of 10% aqueous citric acid (5 mL), and acidified to pH 3 with an additional 10% aqueous citric acid (110 mL). The mixture was extracted with ethyl acetate (150 mL x 3). The organic extracts were washed with water (50 mL), saturated aqueous sodium hydrogen carbonate (50 mL), and saturated aqueous sodium chloride (50 mL), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane (1:4) as an eluent to give title compound (5.50 g, 93% yield).

1H NMR (500 MHz, CDCl₃) δ 5.04 (d, J = 7.8 Hz, 1H), 4.20 (p, J = 7.0 Hz, 3H), 3.52 (t, J = 10.7 Hz, 2H), 1.96 (d, J = 3.7 Hz, 1H), 1.69 (s, 2H), 1.44 (s, 9H), 1.28 (dd, J = 7.1, 2.9 Hz, 3H), 0.98 (t, J = 6.9 Hz, 3H), 0.92 – 0.86 (m, 3H).

Example 30. (3R,4S,5S)-ethyl 4-((tert-butoxycarbonyl)amino)-3-hydroxy-5-methylheptanoate

\[
\begin{align*}
\text{Boc} & \quad \text{N} & \quad \text{H} & \quad \text{O} & \quad \text{O} & \quad \text{NaBH}_4 & \quad \text{EtOH} & \quad \text{Boc} & \quad \text{N} & \quad \text{H} & \quad \text{O} \\
\text{OH} & \quad \text{O} & \quad & \quad & \quad & \quad & \quad & & \quad & \quad & \quad
\end{align*}
\]

To a solution of (4S,5S)-ethyl 4-((tert-butoxycarbonyl)amino)-5-methyl-3-oxoheptanoate (5.90 g, 19.83 mmol) in ethanol (6 mL) at -60 °C was added sodium borohydride (3.77 g, 99.2 mmol) in one portion. The reaction mixture was stirred for 5.5 h below -55 °C then quenched with 10% aqueous citric acid (100 mL). The resultant solution was acidified to pH 2 with an additional 10% aqueous citric acid, followed by extraction with ethyl acetate (100 mL x 3). The organic extracts were washed with saturated aqueous sodium chloride (100 mL), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by column chromatography (10-50% ethyl acetate/hexane) to give pure diastereomer (3R,4S,5S)-ethyl 4-((tert-butoxycarbonyl)amino)-3-hydroxy-5-methylheptanoate (2.20 g, 37% yield) and a mixture of (3R,4S,5S)-ethyl 4-((tert-butoxycarbonyl)
amino)-3-hydroxy-5-methyl-heptanoate and (3S,4S,5S)-ethyl 4-((tert-butoxycarbonyl)-amino)-3-hydroxy-5-methylheptanoate (2.0 g, 34% yield, about 9:1 ratio). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.41 (d, \(J = 9.3\) Hz, 1H), 4.17 (tt, \(J = 7.1, 3.6\) Hz, 2H), 4.00 (t, \(J = 6.9\) Hz, 1H), 3.55 (dd, \(J = 11.7, 9.3\) Hz, 1H), 2.56 – 2.51 (m, 2H), 2.44 (dd, \(J = 16.4, 9.0\) Hz, 1H), 1.79 (d, \(J = 3.8\) Hz, 1H), 1.60 – 1.53 (m, 1H), 1.43 (s, 9H), 1.27 (dd, \(J = 9.3, 5.0\) Hz, 3H), 1.03 – 0.91 (m, 7H).

**Example 31.** (3R,4S,5S)-4-((tert-butoxycarbonyl)amino)-3-hydroxy-5-methyl-heptanoic acid

\[
\begin{align*}
\text{Boc} & \quad \text{N} & \quad \text{OH} & \quad \text{O} & \quad \text{H} & \quad \text{EtOH} & \quad 1\text{ N NaOH} & \quad \text{Boc} & \quad \text{N} & \quad \text{OH} & \quad \text{O} & \quad \text{H}
\end{align*}
\]

To a solution of compound (3R,4S,5S)-ethyl 4-((tert-butoxycarbonyl)amino)-3-hydroxy-5-methyl-heptanoate (2.20 g, 7.20 mmol) in ethanol (22 mL) was added 1 N aqueous sodium hydroxide (7.57 mL, 7.57 mmol). The mixture was stirred at 0 °C for 30 min then r.t. 2 h. The resultant solution was acidified to pH 4 by addition of 1 N aqueous hydrochloric acid, which was then extracted with ethyl acetate (50 mL x 3). The organic extracts were washed with 1 N aqueous potassium hydrogen sulfate (50 mL), and saturated aqueous sodium chloride (50 mL), dried over Na\(_2\)SO\(_4\), and concentrated in vacuo to give the title compound (1.90 g, 95% yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.50 (d, \(J = 8.7\) Hz, 1H), 4.07 (d, \(J = 5.5\) Hz, 1H), 3.59 (d, \(J = 8.3\) Hz, 1H), 2.56 – 2.45 (m, 2H), 1.76 – 1.65 (m, 1H), 1.56 (d, \(J = 7.1\) Hz, 1H), 1.45 (s, 9H), 1.26 (t, \(J = 7.1\) Hz, 3H), 0.93 (dd, \(J = 14.4, 7.1\) Hz, 6H).

**Example 32.** (3R,4S,5S)-4-((tert-butoxycarbonyl)(methyl)amino)-3-methoxy-5-methylheptanoic acid

\[
\begin{align*}
\text{Boc} & \quad \text{N} & \quad \text{OH} & \quad \text{O} & \quad \text{H} & \quad \text{THF} & \quad 0^\circ\text{C} & \quad \text{Mel, NaH} & \quad \text{Boc} & \quad \text{N} & \quad \text{OH} & \quad \text{O} & \quad \text{H}
\end{align*}
\]

To a solution of (3R,4S,5S)-4-((tert-butoxycarbonyl)amino)-3-hydroxy-5-methyl-heptanoic acid (1.90 g, 6.9 mmol) in THF (40 mL) was added sodium hydride (60% oil suspension, 1.93 g, 48.3 mmol) at 0 °C. After stirring for 1h, methyl iodide (6.6 mL, 103.5 mmol) was added. The stirring was continued at 0 °C for 40 h before saturated aqueous sodium hydrogen carbonate (50 mL) was added, followed by water (100 mL). The mixture was washed with diethyl ether (50 mL x 2) and the aqueous layer was acidified to pH 3 by 1 N aqueous potassium hydrogen sulfate, then extracted with ethyl acetate (50 mL x 3).
The combined organic extracts were washed with 5% aqueous sodium thiosulfate (50 mL) and saturated aqueous sodium chloride (50 mL), dried over Na$_2$SO$_4$, and concentrated in vacuo to give the title compound (1.00 g, 48% yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 3.95 (d, $J = 75.4$ Hz, 2H), 3.42 (d, $J = 4.4$ Hz, 3H), 2.71 (s, 3H), 2.62 (s, 1H), 2.56 – 2.47 (m, 2H), 1.79 (s, 1H), 1.47 (s, 1H), 1.45 (d, $J = 3.3$ Hz, 9H), 1.13 – 1.05 (m, 1H), 0.96 (d, $J = 6.7$ Hz, 3H), 0.89 (td, $J = 7.2$, 2.5 Hz, 3H).

**Example 33.** General procedure for the removal of the Boc function with trifluoroacetic acid.

To a solution of the $N$-Boc amino acid (1.0 mmol) in methylene chloride (2.5 mL) was added trifluoroacetic acid (1.0 mL). After being stirred at room temperature for 1-3 h, the reaction mixture was concentrated in vacuo. Co-evaporation with toluene gave the deprotected product, which was used without any further purification.

**Example 34.** (S)-tert-butyl 2-((1R,2R)-1-methoxy-3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-2-methyl-3-oxopropyl)pyrrolidine-1-carboxylate

![Chemical structure](image)

To a solution of (2R,3R)-3-((S)-1-((tert-butoxycarbonyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanoic acid (100 mg, 0.347 mmol) and L-Phenylalanine methyl ester hydrochloride (107.8 mg, 0.500 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (75.6 μL, 0.451 mmol), followed by Et$_3$N (131 μL, 0.94 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was then diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford the title compound (130 mg, 83% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.28 (dd, $J = 7.9$, 6.5 Hz, 2H), 7.23 (t, $J = 7.3$ Hz, 1H), 7.16 (s, 2H), 4.81 (s, 1H), 3.98 – 3.56 (m, 5H), 3.50 (s, 1H), 3.37 (d, $J = 2.9$ Hz, 3H), 3.17 (dd, $J = 13.9$, 5.4 Hz, 2H), 3.04 (dd, $J = 14.0$, 7.7 Hz, 1H), 2.34 (s, 1H), 1.81 – 1.69 (m, 2H), 1.65 (s, 3H), 1.51 – 1.40 (m, 9H), 1.16 (d, $J = 7.0$ Hz, 3H).

**Example 35.** (S)-methyl 2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((tert-butoxycarbonyl)-(methyl)amino)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoate
To a solution of the deprotected product from (S)-tert-butyl 2-((1R,2R)-1-methoxy-3-((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-2-methyl-3-oxopropylpyrrolidine-1-carboxylate (0.29 mmol) and (3R,4S,5S)-4-((tert-butoxycarbonyl)(methyl)amino)-3-methoxy-5-methylheptanoic acid (96.6 mg, 0.318 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (58 µL, 0.347 mmol), followed by Et$_3$N (109 µL, 0.78 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford the title compound (150 mg, 81% yield) as a white solid. LC-MS (ESI) m/z calc. for C$_{34}$H$_{55}$N$_3$O$_8$ [M+H]$^+$: 634.40, found: 634.40.

Example 36. (S)-methyl 2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((tert-butoxycarbonyl)amino)-N,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoate

To a solution of the deprotected product from (S)-methyl 2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((tert-butoxycarbonyl)-(methyl)amino)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoate (0.118 mmol) and Boc-Val-OH (51.8 mg, 0.236 mmol) in DCM (5 mL) at 0 °C was added bromotris(dimethylamino)- phosphonium hexafluorophosphate (BroP, 70.1 mg, 0.184 mmol), followed by diisopropylethylamine (70 µL, 0.425 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at r.t. for 2 days. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford the title compound (67 mg, 77% yield) as a white solid. LC-MS (ESI) m/z calc. for C$_{39}$H$_{64}$N$_8$O$_9$ [M+H]$^+$: 733.47, found: 733.46.
**Example 37.** Preparation of compound Boc-N-Me-Val-OH

\[
\text{Boc} \quad \overset{\text{MeI, NaH, THF}}{\rightarrow} \quad \text{Boc}
\]

To a solution of Boc-L-Val-OH (2.00 g, 9.2 mmol) and methyl iodide (5.74 mL, 92 mmol) in anhydrous THF (40 mL) was added sodium hydride (3.68 g, 92 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h, then warmed to r.t. and stirred for 24 h. The reaction was quenched by ice water (50 mL). After addition of water (100 mL), the reaction mixture was washed with ethyl acetate (50 mL x 3) and the aqueous solution was acidified to pH 3 then extracted with ethyl acetate (50 mL x 3). The combined organic phase was dried over Na₂SO₄ and concentrated to afford Boc-N-Me-Val-OH (2.00 g, 94% yield) as a white solid. 

\(^1\)H NMR (500 MHz, CDCl₃) δ 4.10 (d, J = 10.0 Hz, 1H), 2.87 (s, 3H), 2.37 – 2.13 (m, 1H), 1.44 (d, J = 26.7 Hz, 9H), 1.02 (d, J = 6.5 Hz, 3H), 0.90 (t, J = 8.6 Hz, 3H).

**Example 38.** (S)-methyl 2-((2R,3R)-3-(((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapentadecan-15-yl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoate

\[
\overset{\text{DECP/MMP/DMF, 0 °C to r.t.}}{\text{Ph}} \quad \overset{\text{Boc}}{\text{N}} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{CO₂Me}}{\text{Ph}}
\]

To a solution of the deprotected product from (S)-methyl 2-((2R,3R)-3-(((3R,4S,5S)-4-(((S)-2-((tert-butoxycarbonylamino)-N,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoate (0.091 mmol) and Boc-N-Me-Val-OH (127 mg, 0.548 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (18.2 μL, 0.114 mmol), followed by 4-methylmorpholine (59 μL, 0.548 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford the title compound (30 mg, 39% yield) as a white solid. LC-MS (ESI) m/z calcd. for C₄₅H₇₅N₅O₁₀ [M+H]⁺: 846.55, found: 846.56.

(S)-methyl 2-((2R,3R)-3-(((S)-1-((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapenta-decan-15-oyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoate (30 mg, 0.035 mmol) in THF (1.0 ml) was added LiOH in water (1.0 ml, 0.8 ml). The mixture was stirred at RT for 30 min, neutralized with 0.5 M H₃PO₄ to pH 6, concentrated and purified on SiO₂ column eluted with CH₃OH/CH₂Cl₂/IOAc (1:10:0.01) to afford the title compound (25.0 mg, 85% yield). LC-MS (ESI) m/z calc'd. for C₄₄H₇₄N₅O₁₀ [M+H]^+: 832.54, found: 832.60.

Example 40. (S)-2-((2R,3R)-3-(((S)-1-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamido)-3-methoxy-5-methyleneheptanoyl)-pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoic acid (101)

(S)-2-((2R,3R)-3-(((S)-1-((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapenta-decan-15-oyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoic acid (25 mg, 0.030 mmol) in the mixture of HCl (conc. 0.3 ml) and 1,4-dioxane (0.9 ml) was stirred at RT for 35 min. The mixture was diluted with EtOH (1.0 ml) and toluene (1.0 ml), concentrated and re-evaporated with EtOH/toluene (2:1) to afford the title compound as a white solid (22 mg, ~100% yield) for the next step without further purification. LC-MS (ESI) m/z calc'd. for C₃₉H₆₆N₂O₈ [M+H]^+: 732.48, found: 732.60.


101
To the crude compound 101 (22 mg, 0.030 mmol) in the mixture of DMA (0.8 ml) and NaH₂PO₄ buffer (0.7 ml, 1.0 M, pH 7.5) was added compound 88 (18.0 mg, 0.060 mmol) in four portions in 2 h. The mixture was stirred overnight, concentrated and purified on SiO₂ column eluted with CH₃OH/CH₂Cl₂/HOAc (1:8:0.01) to afford the title compound (22.5 mg, 82% yield). LC-MS (ESI) m/z calcd. for C₄₀H₇₉N₆O₁₁ [M+H]+: 917.56, found: 917.60.


![Chemical Structure](image)

Compound 102 (22.0 mg, 0.024 mmol) in methanol (5 ml) in a hydrogenation vessel was added Pd/C (5 mg, 10% Pd, 50% wet). After air was vacuumed out in the vessel, 25 psi H₂ was conducted in. The mixture was shaken for 4 h, filtered through celite, concentrated to afford the crude title product (~20 mg, ~92% yield) for the next step without further purification. ESI MS m/z+ C₄₀H₇₉N₆O₁₁ (M+H), calcd. 891.57, found 891.60.

Example 43. 2,3-dibromosuccinic anhydride (70)

![Chemical Structure](image)

2,3-dibromosuccinic acid (10.00 g, 36.51 mmol) in dry CH₂Cl₂ (100 ml) at 0°C was added phosphorus pentoxide (12.21 g, 85.84 mmol). The mixture was stirred at 0°C for 2 h and then RT for 5 h, filtered through short SiO₂ column, and rinsed the column with EtOAc/CH₂Cl₂ (1:6). The filtered solutions were combined, evaporated and solidified with EtOAc/Hexane to afford the title compound (6.63 g, 71% yield). ESI MS m/z+ C₄₄H₂₉Br₅O₃ (M+H), calcd. 256.85, found 256.70.

Example 44. 2,3-dibromo-4-(((2-((2-3-((S)-7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)pentyl)oxy)-2-methylen-5-oxo-2,3,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-3-oxopropoxy)ethoxy)ethyl)amino)-4-oxobutanoic acid (124)
To the compound 100 (40.0 mg, 0.068 mmol) in the mixture of DCM (4 ml) and DIPEA (12 uL, 0.069 mmol) was added 2,3-dibromosuccinic anhydride (38.0 mg, 0.148 mmol) at 0°C. The mixture was stirred at 0°C for 2 h and then RT 5 h. The mixture was concentrated and purified on SiO₂ column eluted with CH₃OH/CH₂Cl₂/HOAc (1:6:0.01) to afford the title compound (56.5 mg, 83% yield). LC-MS (ESI) m/z calcd. for C₄₄H₃₁Br₂N₃O₁₂ [M+H]^+: 1002.21, found: 1002.40, 1004.40 (M+2+H).

**Example 45.** 2,5-dioxopyrrolidin-1-yl 2,3-dibromo-4-(((2-(2-(3-((S)-7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]-diazepin-8-yl)oxy)pentyl)oxy)-2-methylene-5-oxo-2,3,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-3-oxopropoxy)ethoxy)ethyl)amino)-4-oxobutanoate (125)

Compound 125 (55.0 mg, 0.054 mmol) in CH₂Cl₂ (3 ml) was added NHS (10.0 mg, 0.086 mmol) and EDC (30.5 mg, 0.158 mmol). The mixture was stirred at RT overnight, concentrated and purified on SiO₂ column eluted with EtOAc/CH₂Cl₂ (1:5) to afford the title compound (50.5 mg, 85% yield). LC-MS (ESI) m/z calcd. for C₄₈H₅₆Br₂N₆O₁₄ [M+H]^+: 1099.22, found: 1099.40, 1101.40 (M+2+H), 1119.50 (M+2+H+H₂O).

Compound 103 (~20 mg, 0.022 mmol) in the mixture of DMA (1 ml) and NaH₂PO₄ buffer (0.6 ml, 0.15 M, pH 7.5) was added compound 125 (30.0 mg, 0.027 mmol). The mixture was stirred for 7 h, concentrated, and purified on C-18 chromatography (Φ 2.0 cm x 25 cm) eluted with water/CH₃CN (from 90% water to 15% water in 50 min at 10 ml/min) to afford the title product 126 (26.1 mg, 63% yield) after dried with high vacuum pump. ESI MS m/z+ C₉₀H₁₃₀Br₂N₁₁O₂₂ (M+H), cacld. 1874.77, found 1874.50.

Example 47. Conjugated compound 126 to an antibody for 127.

To a mixture of 2.0 mL of 10 mg/ml Herceptin 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 126 (14 µL, 20 mM in DMA). The mixture was incubated at RT for 4~16 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 16.5~17.7 mg of the conjugate compound 127 (82%~88% yield) in 13.1~15.0 ml buffer. The drug/antibody ratio (DAR) (the combination of PBD dimer and MMAF per antibody) was 3.85, which was determined via 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.

Example 48. (4R)-4-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-5-(3-(12,13-dibromo-24-((S)-7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-
methylen-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[c]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-11,14,24-trioxa-4,7,18,21-tetraoxa-10,15-diazatetracosanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (128)

Compound 95 (20 mg, 0.021 mmol) in the mixture of DMA (1 ml) and NaH₂PO₄ buffer (0.6 ml, 0.15 M, pH 7.5) was added compound 125 (30.0 mg, 0.027 mmol). The mixture was stirred for 8 h, concentrated, and purified on C-18 chromatography (Φ 2.0 cm x 25 cm) eluted with water/CH₃CN (from 90% water to 20% water in 50 min at 10 ml/min) to afford the title product 128 (26.6 mg, 64% yield) after dried with high vacuum pump. ESI MS m/z+C₈₀H₁₂₃Br₂N₁₂O₂₂S (M+H), cacl. 1901.69, found 1901.90.

**Example 49.** Conjugated compound 128 to an antibody for 129.

To a mixture of 2.0 mL of 10 mg/ml Herceptin 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 128 (14 μL, 20 mM in DMA). The mixture was incubated at RT for 4~16 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 16.4~17.6 mg of the conjugate compound 129 (82%~88% yield) in 13.2~15.1 ml buffer. The drug/antibody ratio (DAR) (the combination of PBD dimer and Tubulysin analog per antibody) was 3.9, which was determined via UPLC-Qtolf 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.
Example 50. Bis(2,5-dioxopyrrolidin-1-yl) 2,3-dibromosuccinate (9)

The solution of 2,3-dibromosuccinic acid (5.0 g, 18.25 mmol), N-hydroxysuccinimide (NHS) (5.01 g, 43.56 mmol) and EDC (12.02 g, 62.60 mmol) in dichloromethane (100 ml) was stirred at RT overnight, concentrated and purified on SiO₂ column eluted with EtOAc/CH₂Cl₂ (1:6) to afford the title compound (6.74 g, 79% yield). LC-MS (ESI) m/z calcd. for C₁₂H₁₁Br₂N₂O₈ [M+H]⁺: 468.88, [M+H+2]⁺:470.88, found: 468.70, 470.70.


Compound 95 (40 mg, 0.042 mmol) in the mixture of DMA (1 ml) and NaH₂PO₄ buffer (0.6 ml, 0.15 M, pH 7.5) was added Bis(2,5-dioxopyrrolidin-1-yl) 2,3-dibromosuccinate (9) (18.0 mg, 0.038 mmol). The mixture was stirred for 8 h, concentrated, and purified on C-18 chromatography (Φ 2.0 cm x 25 cm) eluted with water/CH₃CN (from 90% water/10% CH₃CN to 20% water/90% CH₃CN in 50 min at 10 ml/min) to afford the title product 141 (38.5 mg, 49% yield) after dried with high vacuum pump. ESI MS m/z C₉₄H₆₃Br₂N₁₂O₂₄S₂ (M+H), calcd. 2073.81, found 2073.60.

Example 52. Conjugated compound 141 to an antibody for 142.
To a mixture of 2.0 mL of 10 mg/ml Herceptin 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 141 (14 μL, 20 mM in DMA). The mixture was incubated at RT for 4~16 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 16.4~17.6 mg of the conjugate compound 92 (82%~88% yield) in 13.1~15.2 ml buffer. The drug/antibody ratio (DAR) was 3.9, which was determined via 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.

**Example 53.** *In vitro* cytotoxicity evaluation of conjugates 127, 129 and 142 in comparison with T-DM1:

The cell lines used in the cytotoxicity assays were HL-60, a human promyelocytic leukemia cell line; NCI-N87, a human gastric carcinoma cell line; BT-474, a human invasive ductal carcinoma cell line; and SKOV3, a human ovarian carcinoma cell line. For HL-60, NCI-N87, and BT-474 cells, the cells were grown in RPMI-1640 with 10% FBS. For SKOV3 cells, the cells were grown in McCoy’s 5A Medium with 10% FBS. To run the assay, the cells (180 μl, 6000 cells) were added to each well in a 96-well plate and incubated for 24 hours at 37°C with 5% CO₂. Next, the cells were treated with test compounds (20 μl) at various concentrations in appropriate cell culture medium (total volume, 0.2 mL). The control wells contain cells and the medium but lack the test compounds. The plates were incubated for 120 hours at 37°C with 5% CO₂. MTT (5 mg/ml) was then added to the wells (20 μl) and the plates were incubated for 1.5hr at 37°C. The medium was carefully removed and DMSO (180 μl) was added afterward. After it was shaken for 15min, the absorbance was measured at 490 nm and 570 nm with a reference filter of 620nm. The inhibition% was calculated according to the following equation: inhibition% = [1-(assay-blank)/(control-blank)] × 100.

The cytotoxicity results:
<table>
<thead>
<tr>
<th>IC₅₀ (nM)</th>
<th>N87 cell (Ag+)</th>
<th>SK-OV-3 cell (Ag+)</th>
<th>HL60 cell (Ag-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugate 127</td>
<td>0.009 nM</td>
<td>0.010 nM</td>
<td>&gt;8 nM</td>
</tr>
<tr>
<td>Conjugate 129</td>
<td>0.012 nM</td>
<td>0.015 nM</td>
<td>&gt;8 nM</td>
</tr>
<tr>
<td>Conjugate 142</td>
<td>0.097 nM</td>
<td>0.083 nM</td>
<td>&gt;15 nM</td>
</tr>
<tr>
<td>T-DM1</td>
<td>0.263 nM</td>
<td>0.187 nM</td>
<td>&gt;15 nM</td>
</tr>
</tbody>
</table>

Specificity of conjugate 127 for N87 cell was over 889 (IC₅₀> 8/ IC₅₀= 0.009), and for SK-OV-3 cell was over 800; Specificity of conjugate 129 for N87 cell was over 666 (IC₅₀> 8/ IC₅₀= 0.012), and for SK-OV-3 cell was over 533; Specificity of conjugate 142 for N87 cell was over 155 (IC₅₀> 15/ IC₅₀= 0.097), and for SK-OV-3 cell was over 180; Specificity of conjugate T-DM1 for N87 cell was over 57 (IC₅₀> 15/ IC₅₀= 0.263), and for SK-OV-3 cell was over 80.

The three new conjugates 127, 129 and 142 were extremely more potent than the commercial conjugate T-DM1.

**Example 54. Antitumor Activity *In vivo.***

The *in vivo* efficacy of conjugates 127, 129 and 142 along with T-DM1 were evaluated in a human gastric carcinoma N-87 cell line tumor xenograft models. Five-week-old female BALB/c Nude mice (30 animals) were inoculated subcutaneously in the area under the right shoulder with N-87 carcinoma cells (5 × 10⁶ cells/mouse) in 0.1 mL of serum-free medium. The tumors were grown for 8 days to an average size of 133 mm³. The animals were then randomly divided into 5 groups (6 animals per group). The first group of mice served as the control group and was treated with the phosphate-buffered saline vehicle. The remaining three groups were treated with conjugates 127, 129, 142 and T-DM1 respectively at dose of 3 mg/Kg administered intravenously. Three dimensions of the tumor were measured every 4 days and the tumor volumes were calculated using the formula tumor volume =1/2 (length × width × height). The weight of the animals was also measured at the same time. A mouse was sacrificed when any one of the following criteria was met: (1) loss of body weight of more than 20% from pretreatment weight, (2) tumor volume larger than 1500 mm³, (3) too sick to reach food and water, or (4) skin necrosis. A mouse was considered to be tumor-free if no tumor was palpable.
The results were plotted in Figure 16. All the four conjugates did not cause the animal body weight loss. And the animals at control group were sacrificed at day 37 due to the tumor volume larger than 1500 mm$^3$ and they were too sick. All 6/6 animals at the groups of compound 127 and 129 had completely no tumor measurable at day 13 till day 60 (the end of experiment). All 6/6 animals at the group of Compound 142 group had no tumor measurable at day 21 and 2/6 animals had tumor growth (measurable) back at days 48. In contrast T-DM1 at dose of 3 mg/Kg was not able to eradicate the tumors and it only inhibited the tumor growth for 28 days.
CLAIMS

What is claimed is:

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

   \[
   Z_1 \text{-} \text{R}_1 \text{-} X_1 \underset{\text{U}}{\text{-}} \underset{\text{U'}}{\text{-}} X_2 \text{-} \text{R}_2 \text{-} Z_2
   \]

   (I)

   Wherein:

   \[ \text{---} \] represents an optional single bond;

   \[ \text{-----} \] represents either a single bond or a double bond;

   It provided that when \[ \text{-----} \] represents a single bond, both \text{U} and \text{U'} are not H; when \[ \text{-----} \] represents a double bond, either \text{U} or \text{U'} can be H, but are not H at the same time.

   Wherein the component:

   \[
   \underset{\text{U}}{\text{O}} \text{-} \underset{\text{U'}}{\text{O}} \text{-} \text{O}
   \]

   which can be 2,3-disubstituted succinic group, or 2-monosubstituted or 2,3-disubstituted fumaric group, or 2-monosubstituted or 2,3-disubstituted maleic group, is capable of reacting with a pair of sulfur atoms of the cell-binding agent; The sulfur atoms are preferred pairs of thiols reduced from the interchain disulfide bonds of the cell-binding agent by a reducing agent, such as dithiothreitol (DTT), dithioerythritol (DTE), L-glutathione (GSH) and tris (2-carboxyethyl) phosphine (TCEP), or/and beta mercaptoethanol (β-ME, 2-ME).

   \text{U} and \text{U'} represent the same or different leaving group that can be substituted by a thiol.

   Such leaving groups are, but are not limited to, a halide (e.g., fluoride, chloride, bromide, and iodide), methanesulfonil (mesyl), \( p \)-toluenesulfonyl (tosyl), trifluoromethyl-sulfonyl (triflate), trifluoromethylsulfonate, nitrophenol, \( N \)-hydroxysuccinimide (NHS), phenol; dinitrophenol; pentfluorophenol, tetrafuorophenol, difluorophenol, monofluorophenol, pentachlorophenol, imidazole, dichlorophenol, tetrachlorophenol, 1-hydroxybenzotriazole, 2-ethyl-5-phenylisoxazolium-3'-sulfonate, or an intermediate molecule generated with a condensation reagent for Mitsunobu reactions.

   \text{R}_1 and \text{R}_2 are the same or different, and are absent, 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 \((\text{OCH}_2\text{CH}_2)_p\), or polypropyleneoxy unit of formula \((\text{OCH}_2\text{(CH}_3\text{CH}_2)_p\), wherein \( p \) is an integer from 0 to about 1000, or combination thereof.

   Additionally \text{R}_1 and \text{R}_2 are respectively a chain of atoms selected from C, N, O, S, Si, and P, preferably having 0~500 atoms, which covalently connects to \text{X}_1 or \text{X}_2 and \text{Z}_1 or \text{Z}_2. The atoms
used in forming the $R_1$ and $R_2$ 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, peptides, acyloxyamines, hydroxamic acids, or combination thereof.

$X_1$ and $X_2$ are independently selected from NH, N(R$_3$), O, S or CH$_2$; Wherein R$_3$ is 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$_2$CH$_2$)$_p$, wherein $p$ is an integer from 0 to about 1000, or combination thereof.

Z$_1$ and Z$_2$ are the same or different a function group that enables to react with a cytotoxic drug, to form a disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, amine (secondary, tertiary, or quarter), imine, cycloheteroalkylene, heteroaromatic, alkoxime or amide bond; The function groups of Z$_1$ and Z$_2$ are displayed below:
2. A cell-binding agent-drug conjugate compound of Formula (II)

\[
\text{Drug}_1 - R_1 - X_1 \quad \text{O} \quad \text{O} \quad X_2 - R_2 - \text{Drug}_2
\]

Wherein:

Cb represents a cell-binding agent, preferably an antibody, which conjugates to Drug$_1$ and Drug$_2$ via a pair of sulfur atoms (thiols). The conjugatable thiol groups can generally be generated from dithiothreitol (DTT), dithioerythritol (DTE), L-glutathione (GSH) and tris (2-carboxyethyl) phosphine (TCEP), or/and beta mercaptoethanol (β-ME, 2-ME) reduction of pairs of disulfide bonds on the surface of cell-binding molecule.

Drug$_1$ and Drug$_2$ represent the same or different cytotoxic agents, linked to the cell-binding agent via the bridge linker through an alkyl, alkenyl, alkenylene, alkynylene, ether, polyoxyalkylene, ester, amine, imine, polyamine, hydrazine, hydrazone, amide, urea, semicarbazide, carbazide, alkoxymine, urethanes, amino acid, peptide, acyloxyamine, hydroxamic acid, disulfide, thioether, thioester, carbamate, carbonate, heterocyclic ring, heteroalkyl, heteroaromatic, or alkoxime bond, or combination thereof.

n is 1 ~ 30; “”, “”, R$_1$, R$_2$, X$_1$ and X$_2$ are described the same previously in Claim 1.
3. A compound of Formula (III):

\[
\begin{array}{c}
\text{Z}_1 - \text{R}_1 - \text{X}_1 - \text{O} - \text{S} - \text{Cb} \quad \text{O} - \text{S} - \text{X}_2 - \text{R}_2 - \text{Z}_2 \\
\end{array}
\]

(III)

Wherein:
“−”, “_”, Cb, Z₁, Z₂, n, R₁, R₂, X₁, and X₂ are defined the same as in Claims 1 and 2.

4. A compound of Formula (IV):

\[
\begin{array}{c}
\text{Drug}_1 - \text{R}_1 - \text{X}_1 - \text{U} - \text{O} - \text{U}' - \text{X}_2 - \text{R}_2 - \text{Drug}_2 \\
\end{array}
\]

(IV)

Wherein:
“−”, “_”, U, U’, Drug₁, Drug₂, R₁, R₂, X₁, and X₂ are defined the same as in Claims 1 and 2.

5. The bridge linker compound of Formula (I) of claims 1, wherein the 2,3-disubstituted succinic group, or 2-monosubstituted or 2,3-disubstituted fumaric group, or 2-monosubstituted or 2,3-disubstituted maleic group is synthesized through the condensation of the 2,3-disubstituted succinic acid, or 2-monosubstituted or 2,3-disubstituted fumaric acid, or 2-monosubstituted or 2,3-disubstituted maleic acid, or its acid derivatives, with the other components containing an amine (1° or 2° amines), alcohol, or thiol on their terminal as shown in the following scheme (Ia):

\[
\begin{array}{c}
\text{Lv}_1 - \text{O} - \text{U} - \text{U}' - \text{Lv}_2 \\
\text{X} - \text{R} - \xi \\
\xi - \text{R} - \text{X} - \text{U} - \text{U}' - \text{X} - \text{R} - \xi \\
\end{array}
\]

(Ia)

Wherein X is X₁ or X₂ as described in Claim 1 as NH, N(R₃), O, or S; R is R₁ and/or R₂; R₁, R₂ and R₃ are the same described in Claim 1;

Lv₁ and Lv₂ are the same or independently OH; F; Cl; Br; I; nitrophenol; N-hydroxysuccinimide (NHS); phenol; dinitrophenol; pentafluorophenol; tetrafluorophenol; difluorophenol; monofluorophenol; pentachlorophenol; triflate; imidazole; dichlorophenol; tetrachlorophenol; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3'-sulfonate, anhydrides formed its self, or formed with the other anhydride, e.g. acetyl anhydride, formyl anhydride; or a intermediate molecule generated with a condensation reagent for peptide coupling reactions, or for Mitsunobu reactions, e.g. condensation reagents are: EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide), DCC (Dicyclohexyl-carbodiimide), N,N'-Diisopropylcarbodiimide (DIC), N-Cyclohexyl-N’-(2-morpholinoethyl)carbodiimide metho-p-
toluenesulfonate (CMC, or CME-CDI), 1,1’-Carbonyldimidazole (CDI), TBTU (O-(Benzotriazol-1-yl)-N,N,N′,N″-tetramethyluronium tetrafluoroborate), N,N,N′,N″-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (Benzotriazol-1-yloxy)tripyrrrolidinophosphonium hexafluorophosphate (PyBOP), Diethyl cyanophosphonate (DEPC), Chloro-N,N,N′,N″-tetramethylformamidinium hexafluorophosphate, 1-[Bis(dimethylamino)-methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 1-[(Dimethylamino)(morpholino) methylene]-1H-[1,2,3]triazolo[4,5-b]pyridine-1-ium 3-oxide hexafluorophosphate (HMDA), 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP), Chlorotripyrrrolidinophosphonium hexafluorophosphate (PyCloP), Fluoro-N,N,N′,N″-bis(tetramethylene)formamidinium hexafluorophosphate (BTFFH), N,N,N′,N″-Tetramethyl-S-(1-oxid-2-pyridyl)thiuronium hexafluorophosphate, O-(2-Oxo-1(2H)pyridyl)-N,N,N′,N″-tetramethyluronium tetrafluoroborate (TPTU), S-(1-Oxido-2-pyridyl)-N,N,N′,N″-tetramethylthiuronium tetrafluoroborate, O-[(Ethoxycarbonyl) cyano-methylenamino]-N,N,N′,N″-tetrakis(1-methylpyridinium hexafluorophosphate (HOTU), (1-Cyano-2-ethoxy-2-oxoethylidenamino-xyldimethylamino-morpholino-carbenium hexafluorophosphate(COMU), O-(Benzotriazol-1-yl)-N,N,N′,N″-bis(tetramethylene) uronium hexafluorophosphate (HBPyU), N-Benzyl-N′-cyclohexylcarbodiimide (with, or without polymer-bound), Dipyrrrolidino(N-succinimidylxylo)-carbenium hexafluoro-phosphate (HSPyU), Chlorodipyrrrolidinocarbenium hexafluorophosph (PyCIU), 2-Chloro-1,3-dimethylimidazolidinium tetrafluoroborate(CIB), (Benzotriazol-1-ylxy) dipiperidinocarbenium hexafluorophosphate (HBPipU), O-(6-Chlorobenzotriazol-1-yl)-N,N,N′,N″-tetramethyluronium tetrafluoroborate (TCTU), Bromotris(dimethylamino)phosphonium hexafluorophosphate (BroP), Propylphosphonic anhydride (PPACA, T3P®), 2-Morpholinoethyl isocyanide (MEI), N,N,N′,N″-Tetramethyl-O-(N-succinimidyl)uronium hexafluorophosphate (HSTU), 2-Bromo-1-ethyl-pyridinium tetrafluoroborate (BEP), O-[(Ethoxycarbonyl)cyano-methylenamino]-N,N,N′,N″-tetrakis(1-methylpyridinium hexafluorophosphate (TOTU), 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpheolin chloride (MMTM, DMTMM), N,N,N′,N″-Tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU), O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N′,N″-tetramethyluronium tetrafluoro-borate (TDBTU), 1,1’-(Azodicarboxyl) diiperidine (ADD), Di-(4-chlorobenzyl) azodicarboxylate (DCAD), Di-tert-butyl azodicarboxylate (DBAD), Diisopropyl azodicarboxylate (DIAD), Diethyl azodicarboxylate (DEAD).

6. The Formula (II) and (IV) of claims 2, and 4 wherein the Drug₁ and Drug₂ are the same or independently selected from:
1). Chemotherapeutic agents: a). Alkylating agents: such as Nitrogen mustards: chlorambucil, cloraphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, mannomustine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiopeta, 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 uredop; ethylpenamines and methylamelamines including altrettamine, triethylenemelamine, triethylene phosphoramide, triethylenethiophosphoroamide 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 sarcodicitin; 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-nitcamptothecin (RFS 2000)); mitomycins: (mitomycin C)]; d). Antimetabolites: 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, defereroxamine)]; [Pyrimidine analogs: Uracil analogs: (ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifuridirine, enocitabine, 5-Fluorouracil, flouxuridine, ratitrexed (Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine)]; folic acid replenisher, such as folicin acid}; e). Hormonal therapies: such as {Receptor antagonists: [Anti-estrogen: (megestrol, raloxifene, tamoxifen]; LHRH agonists: (goscrlcin, leuprolide acetate); Anti-androgens: (bicalutamide, flutamide, calusterone, dromostanolone propionate, epitiostanol, goserelin, leuprolide, mepitiostane, nilutamide, testolactone, trilostane and other androgens inhibitors]); Retinoids/Deltoids: [Vitamin D3 ana-
logs: (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 \(\gamma_1\), \(\delta_1\), \(\alpha_1\) and \(\beta_1\); dynemicin, including dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomycins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabine, 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, nitromycins, 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, Provence, 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), \(\text{Ca}^{2+}\)ATPase inhibitors (such as thapsigargin), Histone deacetylase inhibitors (Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A); Thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, Disulfiram, Salinosporamide A.; Anti-adrenals, such as aminogluthethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine (DFMO), elfomithine; elliptinium acetate, etoglucid; gallium nitrate; gacytosine, hydroxyurea, ibandronate, lenitnan; lonidamine;
mitoguazone; mitoxantrone; mopidamol; nitacrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®, razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2', 2''-trichloro triethylamine; trichotheccenes (especially T-2 toxin, verrucarin A, 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, flucortolone danazol, dexamethasone, Triamcinolone acetonide, beclometasone dipropionate), DHEA, enanercept, hydroxychloroquine, infliximab, meloxicam, methotrexate, mofetil, mycophenylate, prednisone, sirolimus, tacrolimus.

3). Anti-infectious disease agents, a). Aminoglycosides: amikacin, astromicin, gentamicin (netilmicin, sisomicin, isepamicin), hygromycin B, kanamycin (amikacin, arbekacin, bekanamycin, dibekacin, tobramycin), neomycin (framicetin, paromomycin, ribostamycin), netilmicin, spectinomycin, streptomycin, tobramycin, verdamicin; b). Amphenicols: azidamfenicol, chloramphenicol, florfenicol, thiampenicol; c). Ansamycins: geldanamycin, herbimycin; d). Carbapenems: biapenem, doripenem, ertapenem, imipenem/cilastatin, meropenem, panipenem; e). Cepheps: carbacephem (loracarbef), cefacetrile, cefaclor, cefradine, cefadroxil, cefalonium, cefaloridine, cefalotin or cefalothin, cefalexin, cefaloglycin, cefamandole, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcapene, cefdaloxime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftezole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cephalaxin, cefpiromize, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, ceferam, ceftibuten, ceftiolene, ceftizoxime, cefotibprole, ceftriaxone, cefuroxime, cefuzonam, cephalexin (cefotixin, cefotetan, cefmetazole), oxacephem (flomoxef, latamoxef); f). Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin; g). Glycycyclines: 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, ampi-
cillin (pivampicillin, hetacillin, bacampicillin, metampicillin, talampicillin), azidocillin, azlocillin, benzylpenicillin, benzathine benzylpenicillin, benzathine phenoxyethylpenicillin, clometocillin, procaine benzylpenicillin, carbenicillin (carindacillin), cloxacillin, dicloxacillin, epicillin, flucloxacillin, mecillinam (pivmecillinam), mezlocillin, meticillin, nafcillin, oxacillin, penamecillin, penicillin, pheneticillin, phenoxyethylpenicillin, pipercillin, propicillin, sulbenicillin, temocillin, ticarcillin; o). Polypeptides: bacitracin, colistin, polymyxin B; p). Quinolones: alatrofloxacina, balofloxacina, ciprofloxacina, clinafloxacina, danofloxacina, difloxacina, enoxacin, enrofloxacina, floxina, garenoxacin, gatifloxacina, gemifloxacina, grepafloxacina, kano trovafloxacina, levofloxacina, lomefloxacina, marbofloxacina, moxifloxacina, nadifloxacina, norfloxacina, orbifloxacina, ofloxacina, trovafloxacina, grepafloxacina, sitafloxacina, sparfloxacina, temafloxacina, tosufloxacina, trovafloxacina; q). Streptograminas: pristinamicina, quinupristina/dalfopristina; r). Sulfonamidas: mafenedina, prontosil, sulfacetamina, sulfametihizol, sulfanilimida, sulfadiazina, sulfisoxazole, trimetoprim, trimetoprim-sulfamethoxazol (cotrimoxazol); s). Steroid antibacterias: e.g. fusidico ácido; t). Tetraciclinas: doxiciclina, chlortetraciclina, clomociclina, demeclociclina, lymeciclina, meclocyclina, metacyclina, minociclina, oxtetraciclina, penimpecyclina, rolitetraciclina, tetraciclina, glycyclinas (e.g. tigecyclina); u). Other types of antibiotics: anonacina, arsphenamina, bactoprenol inhibitors (Bacitracina), DADAL/AR inhibitors (closersina), dictyostatin, discodermolida, eleutherobina, epothilona, ethambutol, etoposida, faropenem, fusidico ácido, furazolidona, isoniazida, laulimalida, metronidazol, mupirocina, mycolactona, NAM synthesis inhibitors (e.g. fosfomicina), nitrofurantoína, paclitaxela, platensimicina, pyrazinamida, quinupristina/dalfopristina, rifampicina (rifampicina), tazobactam tinidazol, uvaricina;  

4). Anti-viral drugs: a). Entry/fusion inhibitors: aplaviroc, maraviroc, vicriviroc, gp41 (enfuvirtida), 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, apricitabina, bruvudina, cidofovir, clevudina, dexecluvicabina, didanosina (ddI), elvcucitabina, emtricitabina (FTC), entecavir, famciclovir, fluorouracil (5-FU), 3’-fluoro-substituted 2’, 3’-dideoxynucleoside analogues (e.g. 3’-fluoro-2’,3’-dideoxythymidina (FLT) and 3’-fluoro-2’,3’-dideoxyguanosina (FLG), fomivirsena, ganciclovir, idoxuridina, lamivudina (3TC), l-nucleosidas (e.g. β-l-thymidina and β-l-2’-deoxycytidina), penciclovir, racivir, ribavirina, stanpivirina, stavudina (d4T), taribavirina (virmadina), telbivudina, tenofovir, trifluridina valaciclovir, valganciclovir, zalitabina (ddC), zidovudina (AZT); f). Non-nucleosides: amantadina, aterviridina, capravirina, diarylpyrimidinas (etravirina, rilpivirina), delavirdina,
docosanol, emivirine, efavirenz, foscarbem (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, osaprenavir, indinavir, lopinavir, neflinavir, 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, ¹¹C, ¹⁴C, ¹⁸F, ³²P, ³⁵S, ⁶⁴Cu, ⁶⁸Ga, ⁶⁸Y, ⁹⁹Tc, ¹¹¹In, ¹²⁴I, ¹²⁵I, ¹³¹I, ¹³³Xe, ¹⁷⁷Lu, ₂¹¹At, or ²¹³Bi.

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 mercocyanine); 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), FluorProbes (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 (Phycobiotec), Allophycoceyanin (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, Methoxy coumarin, 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′Dichorodihydro-fluorescein, oxidized form), DHR (Dihydorhodamine 123, oxidized form, light catalyzes oxidation), Fluo-3 (AM ester. pH > 6), Fluoro-4 (AM ester. pH 7.2), Indo-1 (AM ester, low/high calcium (Ca2+)), SNARF (pH 6/9), Allophycocyanin(APC), AmCy51 (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), GPPuv, 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.

7. The compound of Formula (II) and (IV) of claims 2 and 4, wherein “Drug1” and “Drug2” are a chromophore molecule, the conjugate compounds of Formula (II) and (IV) of claims 2, and 4, can be used for detecting, monitoring, or studying the interactions and/or functions of the cell binding molecule, and/or of the conjugate with a target, particularly, a targeted cell.
8. The compound of Formula (II) and (IV) of claims 2 and 4, wherein “Drug1” and “Drug2” can be a polyalkylene glycols or a polyalkylene glycol analogs, which are used for extending the half-life of the cell-binding molecule when administered to a mammal. Polyalkylene glycols are poly(ethylene glycols) (PEGs), poly(propylene glycol), copolymers of ethylene oxides or propylene oxides that has a molecular weight of from about 10 Daltons to about 200 kDa.

9. The compound of Formula (II) and (IV) of claims 2 and 4, wherein “Drug1” and “Drug2” can be a cell-binding ligands or receptors, or receptor analogs, so that the conjugates can work as a targeting conductor/director to deliver the conjugate to malignant cells, and also can modulate or co-stimulate a desired immune response or altering signaling pathways.

10. The conjugate compound of claims 2 and 4, wherein “Drug1” and “Drug2” are preferred selected from tubulysins, calicheamicins, auristatins, maytansinoids, CC-1065 analogs, daunorubicin and doxorubicin compounds, taxanoids (taxanes), cryptophycins, epothilones, benzodiazepine dimers (e.g., dimmers of pyrrolobenzodiazepine (PBD), tomaymycin, anthramycin, indolobenzodiazepines, 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, thiotapec, vincristine, hemiasterlins, nazumamides, microginins, radiosumins, alterobactins, microscleodermins, theonellamides, esperamicins, siRNA, nucleolytic enzymes, and/or pharmaceutically acceptable salts, acids, or/and their analogues derivatives of any of the above molecules.

11. The conjugate compounds of claims 2 and 3 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 molecule above coating with cell-binding ligands, or a combination of above thereof.

12. The cell-binding molecule/agent according to claims 2, 3, and 11 is preferred an antibody, full-length antibodies (polyclonal antibodies, monoclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies); 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, anti-idiotypic (anti-Id) antibodies, CDR's, diabody, triabody, tetrabody, miniantibody, small immune proteins (SIP), a lymphokine, a hormone, a vitamin, a growth factor, a colony stimulating factor, a nutrient-transport molecule, or large molecular weight proteins.

13. The cell-binding molecule/agent according to claims 2, 3, and 11 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.

14. The cell-binding molecule/agent according to claims 2, 3, and 11 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, CD137, CD138, CD141, CD142, CD143, CD144, CD147, CD151, 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 WAF1), 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 (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 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, EGF/L7 (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 glyvolipid), GD3 idiootype, GloboH, Glypican 3, N-glycolyneuraminic acid, GM3, GMCSF receptor a-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 (ERBB3), IgG4, HGF/SF (Hepatocyte growth factor/scatter factor), HHGFR, HIV-1, Histone complex, HLA-DR (human leukocyte antigen), HLA-DR10, HLA-DRB1, HMWMAA, Human chorionic gonadotropin, HNGF, Human scatter factor receptor kinase, HPV E6/E7, Hsp90, hTERT, ICAM-1 (Intercellular Adhesion Molecule 1), Idiotype, IGFI-R (IGF-1, insulin-like growth factor 1 receptor), IGHE, IFN-γ, Influenza hemagglutinin, IgE, IgE Fe 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 (α4, α5β3, αvβ3, αvβ7, α5β1, α6β4, α7β7, αvβ3, αβ5, αvβ5), 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 A4, 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 (granuloctye 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-glycolyneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1,CD279), PDGF-Rα (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 (Phosphatidylinerine), Prostatic carcinoma cells, Pseudomonas aeruginosa, PSMA, PSA, PSCA, Rabies virus glycoprotein, RHD (Rh polypeptide 1 (RhP1), 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-β1, TGF-β2 (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 apoposis 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.

15. The tumor cell according to claim 13 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, malignant cells, or any cells that grow and divide at an unregulated, quickened pace to cause cancers.

16. The linkage components R1 and/or R2 according to claims 1, 2, 3, and/or 4, 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-aminobenzyloxy carbonyl (PAB), 4-thio-pentanoate (SPP), 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (MCC), 4-thio-butyrate (SPDB), maleimidoethyl (ME), 4-thio-2-
hydroxysulfanyl-butyrate (2-Sulfo-SPDB), pyridinyl-dithiol (PySS), alkoxy amino (AOA), ethyleneoxy (EO), 4-methyl-4-dithio-pentanoic (MPDP), azido (N₃), alkynyl, dithio, peptides, and/or (4-acetyl)aminobenzoate (SIAB).

17. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are a Tubulysin analog, the conjugate compound of Formula (II) is preferred structures of T01, T02, T03, T04, T05, T06 and T07 as following:
Wherein mAb is an antibody; Z₃ and Z′₃ are independently H, OP(O)(OM₁)(OM₂), OCH₂OP(O)(OM₁)(OM₂), OSO₂M₁, R₁, or O-glycoside (glucoside, galactoside, mannoside, glucuronoside, alloside, fructoside, etc), NH-glycoside, S-glycoside or CH₂-glycoside; M₁ and M₂ are independently H, Na, K, Ca, Mg, NH₄, NR₁R₂R₃; n is 1-30; “—”, “—”, X₁, X₂, R₁, R₂ and R₃ are the same defined in Claims 1 and 2.

18. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are a Calicheamicin analog, the conjugate compound of Formula (II) is preferred structures of C01 as following:
Wherein mAb is an antibody; \( n \) is 1~30; “-”, “—”, \( X_1, X_2, R_1, R_2 \) and \( R_3 \) are the same defined in Claims 1 and 2.

19. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are a Maytansinoid analog, the conjugate compound of Formula (II) is preferred structures of M01 as following:

Wherein mAb is an antibody; \( n \) is 1~30; “-”, “—”, \( X_1, X_2, R_1, R_2 \) and \( R_3 \) are the same defined in Claims 1 and 2.

20. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are a Taxane analog, the conjugate compound of Formula (II) is preferred structures of Tx01, Tx02 and Tx03 as following:
Wherein mAb is an antibody; n is 1~30; "", ", X_1, X_2, R_1 and R_2 are the same defined in Claims 1 and 2.

21. The conjugate compound of claim 2, wherein “Drug_1” and “Drug_2” are a CC-1065 analogue and/or doucarmycin analog, the conjugate compound of Formula (II) is preferred structures of CC01, CC02, and CC03 as following:
Wherein mAb is an antibody; n is 1~30; Z4 and Z′4 are independently H, PO(OM1)(OM2), CH2PO(OM1)(OM2), SO3M1, CH3N(CH2CH2)2NC(O)-, O(CH2CH2)2NC(O)-, R1, or glycoside; X3 and X′3 are independently O, NH, NHC(O), OC(O), -C(O)O, R1, or absent; “---”, “-----”, X1, X2, R1, R2, M1, and M2 are the same defined in Claims 1 and 2.

22. The conjugate compound of claim 2, wherein “Drug1” and “Drug2” are a Daunorubicin or Doxorubicin analogue, the conjugate compound of Formula (II) is preferred structures of Da01, Da02, Da03 and Da04 as following:
Wherein mAb is an antibody; n is 1~30; X₃ and X₃' are independently H, O, NH, NH(C)(O), NH(C)(O)NH, C(O), R₁, or OC(O); "-"; "-", X₁, X₂, R₁, and R₂ are the same defined in Claims 1 and 2.

23. The conjugate compound of claim 2, wherein "Drug₁" and "Drug₂" are an Auristatin and dolastatin analogue, the conjugate compound of Formula (II) is preferred structures of Au01, Au02, Au03, Au04, and Au05 as following:
Wherein mAb is an antibody; n is 1~30; X3 and X'3 are independently CH2, O, NH, NHC(O), NHC(O)NH, C(O), OC(O)R1, or absent; X4 and X'4 are independently CH2, C(O), C(O)NH, C(O)N(R1), R1, NHR1, NR1, C(O)R1 or C(O)O; Z3 and Z'3 are independently H, R1, OP(O)(OM1)(OM2), NHR1, OCH2OP(O)(OM1)(OM2), OSO3M1, or O-glycoside (glucoside, galactoside, mannoside, glucuronoside, alloside, fructose), NH-glycoside, S-glycoside, or CH2-glycoside; M1 and M2 are independently H, Na, K, Ca, Mg, NH4, NR1R2R3; “—”, “=”, X1, X2, R1, R2 and R3 are the same defined in Claims 1 and 2.

24. The conjugate compound of claim 2, wherein “Drug1” and “Drug2” are a benzodiazepine dimer analogues, the conjugate compound of Formula (II) is preferred structures of PB01, PB02, PB03, PB04, PB05, PB06, PB07, PB08, PB09, PB10 and PB11.
Wherein mAb is an antibody; n is 1~30; X₃ and X’₃ are independently CH₂, O, NH, NHC(O), NHC(O)NH, C(O), OC(O), OC(O)(NR₃), R₁, NHR₁, NR₁, C(O)R₁ or absent; X₄ and X’₄ are independently CH₂, C(O), C(O)NH, C(O)N(R₁), R₁, NHR₁, NR₁, C(O)R₁ or C(O)O; M₁ and M₂ are independently H, Na, K, Ca, Mg, NH₄, NR₁R₂R₃; “——”, “——”, X₁, X₂, R₁, R₂ and R₃ are the same defined in Claims 1 and 2. In addition, R₁ and/or R₂ can be absent.

25. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are preferred two different cytotoxic agents selected from any combinations of tubulysins, maytansinoids, taxanoids (taxanes), CC-1065 analogs, daunorubicin and doxorubicin compounds, benzodiazepine dimers (e.g., dimers of pyrrolobenzodiazepine (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. The more preferred structures of the conjugates containing two or more different cytotoxic agents via the bridge linker are as the following Z01, Z02, Z04, Z05, Z06, Z07, Z08, Z09, Z10, Z12, Z13, Z14, Z15, Z16, Z17 and Z18:
Wherein mAb is an antibody; n is 1~30; X₃ and X'₃ are independently CH₂, O, NH, NHC(O), NHC(O)NH, C(O), OC(O), OC(O)(NR₃), R₁, NHR₁, NR₁, C(O)R₁ or absent; X₄ and X'₄ are independently H, CH₂, OH, O, C(O), C(O)NH, C(O)N(R₁), R₁, NHR₁, NR₁, C(O)R₁ or C(O)O; M₁ and M₂ are independently H, Na, K, Ca, Mg, NH₄, NR₁R₂R₃; “—”, “—”, X₁, X₂, R₁, R₂ and R₃ are the same defined in Claims 1 and 2. In addition, R₁ and/or R₂ can be absent.

26. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are a polyalkylene glycol analog, the conjugate compound of Formula (II) is preferred structures of Pg01:
Wherein mAb is an antibody; n is 1-30; R’ and R” are independently H or CH₃; m₃ and m₄ are independently 1-5000; “—”, “—”, X₁, X₂, R₁, R₂, and R₃ are the same defined in Claims 1 and 2, R₄ is OH, H, or R₃ or R₃ that is defined as in Claim 1.

27. The conjugate compound of claim 2, wherein “Drug₁” and “Drug₂” are a cell-binding ligand or receptor analog, the conjugate compound of Formula (II) is preferred structures: LB01 (PMSA ligand conjugate), LB02 (Folate receptor conjugate), LB03 (Somatostatin receptor conjugate), LB04 (Octreotide, a Somatostatin analog receptor conjugate), LB05 (Lanreotide, a Somatostatin analog receptor conjugate), LB06 (CAIX receptor conjugate), LB07(CAIX receptor conjugate), LB08 (luteinizing hormone-releasing hormone (LH-RH) ligand and GnRH conjugate), LB09 (luteinizing hormone-releasing hormone (LH-RH) and GnRH ligand conjugate), LB10 (GnRH antagonist, Aparelix conjugate), LB11 (cobalamin, VB12 analog conjugate), LB12 (Gastrin releasing peptide receptor (GRPr), MBA conjugate), LB13 (α,β₃ integrin receptor, cyclic RGD pentapeptide conjugate), LB14 (hetero-bivalent peptide ligand for VEGF receptor conjugate), LB15 (Neuromedin B conjugate), LB 16 (a G-protein coupled receptor, bombesin conjugate) and LB17 (a Toll-like receptor, TLR₂ conjugate).
LB09

LB10

R₇ = 5'-deoxyadenosyl, Me, OH, or CN,

LB11

LB12
Wherein mAb is an antibody; \( n \) is 1~30; \( X_3 \) and \( X'_3 \) are independently \( \text{CH}_2, \text{O}, \text{NH}, \text{NHC(O)}, \text{NHC(O)NH}, \text{C(O)}, \text{OC(O)}, \text{OC(O)(NR)}_3, \text{R}_1, \text{NHR}_1, \text{NR}_1, \text{C(O)R}_1 \) or absent; \( X_4 \) and \( X'_4 \) are independently \( \text{H}, \text{CH}_2, \text{OH}, \text{O}, \text{C(O)}, \text{C(O)NH}, \text{C(O)N(R)}_1, \text{R}_1, \text{NHR}_1, \text{NR}_1, \text{C(O)R}_1 \) or \( \text{C(O)O}; \text{M}_1 \) and
M₂ are independently H, Na, K, Ca, Mg, NH₄, NR₁R₂R₃; m₃ and m₄ is 0~5000, “—”, “—”, X₁, X₂, R₁, R₂ and R₃ are the same defined in Claims 1 and 2. In addition, R₁ and/or R₂ can be absent.

28. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate compounds of claims 2, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and/or 27, and a pharmaceutically acceptable salt, carrier, diluent, or excipient therefore, or a combination thereof, for the treatment or prevention of a cancer, or an autoimmune disease, or an infectious disease.

29. The conjugate of claim 2, 17, 18, 19, 20, 21, 22, 23 24, 25, 26, and/or 27, having in vitro, in vivo or ex vivo cell killing activity.

30. The conjugate compound of claim 2, 17, 18, 19, 20, 21, 22, 23 24, 25, 26, and/or 27, can comprise either a peptides of 1~20 units of natural or unnatural amino acids, or a p-aminobenzyl unit, or a 6-maleimidoacryloyl unit, or a disulfide unit, or a thioether unit, or a hydrozone unit, a triazole unit, or an alkoxime unit, among the linkage components R₁ and/or R₂.

31. The conjugate of claim 2, 17, 18, 19, 20, 21, 22 23, 24, 25, 26, and/or 27, wherein the linkage components R₁ and/or R₂ can be cleavable by a protease.

32. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate of claim 2, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, and/or 28, 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.

33. The synergistic agents according to claim 32 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 (Erbitux®), Ciclosporin, Cinacalcet, crizotinib (Xalkori®), Dabigatran, dabrafenib (Tafinlar®), Darbepoetin alfa, Darunavir, imatinib mesylate (Gleevec®, dasatinib (Sprycel®), denileukin diftitox (Ontak®), Denosumab (Xgeva®), Depakote, Dextansoprazole, Dexamethasone, Dinutuximab (Unituxin™), Doxyecycline, Du-
loxetine, 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), Ibrutinomab
tiuxetan (Zevalin®), ibritinib (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), lenalidomide (Revlimid®), lenvatinib mesylate (Lenvima™), letrozole
(Femara®), Levothyroxine, Levothyroxine, Lidocaine, Linezolid, Liraglutide,
Lisdexamfetamine, 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, candetani (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 thereof.
Figure 1.
Figure 2.
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
Figure 10
Figure 12
Figure 13.
Figure 14
Figure 15.
Figure 16