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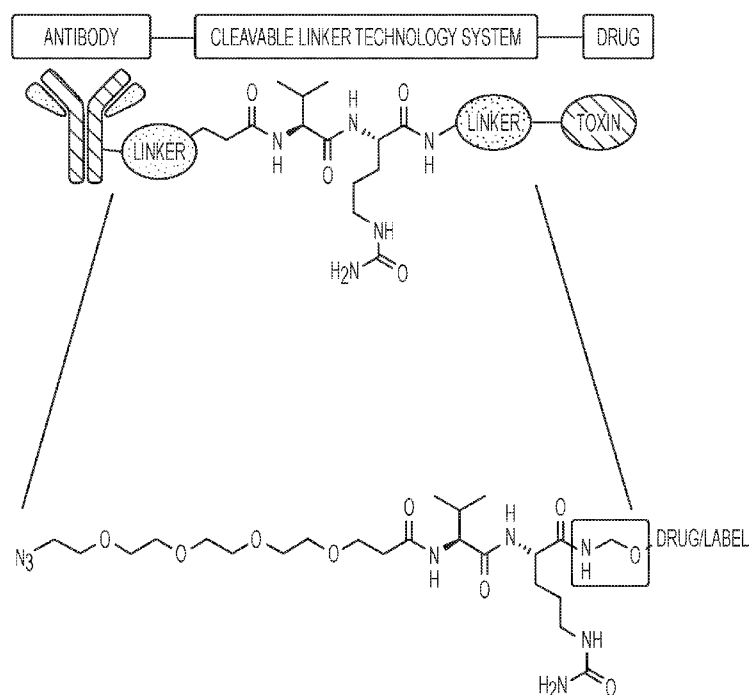


Figure 1

[Continued on next page]



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(57) Abstract: Provided herein are targeted conjugates and methods for their use. The conjugate comprises a targeting element, a cleavable linker comprising an activation element, a releasable linker comprising a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent.

TARGETED CONJUGATES

CROSS- REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Provisional Application Number
5 62/218,447, filed September 14, 2015. The entire content of the application referenced above is
hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

Antibody drug conjugates (ADCs) have emerged as a major new therapeutic strategy to
10 create new anti-cancer therapeutics after thirty years of development (Ducry, Laurent; Stump,
Bernhard (2010). "Antibody-Drug Conjugates: Linking Cytotoxic Payloads to Monoclonal
Antibodies". Bioconjugate Chemistry 21 (1): 5-13). Recently, two products have been
approved by US FDA: Adcetris® from SeattleGenetics to treat Hodgkin lymphoma (HL) and a
rare lymphoma known as systemic anaplastic large cell lymphoma (ALCL), and Kydcyla® from
15 Genentech/Immunogen to treat patients with HER2-positive, late-stage (metastatic) breast
cancer. As targeted therapy, ADC has the advantages of outstanding anticancer efficacy and
minimal side effects (Eric L. Sievers and Peter D. Senter (2013). "Antibody-Drug Conjugates in
Cancer Therapy". Annual Review of Medicine 64: 15-29. Vol. 64: 15-29).

Since the toxin suitable for ADC applications is highly potent (often with a half maximal
20 inhibitory concentration (IC50) in the range of picomoles), it is critically important to prevent
premature release of the drug before the ADC compound reaches target tissue. Once in the
cancer cell, the ADC should release the payload quickly and efficiently in order to achieve an
optimal cancer cell killing. It is essential that linkers used to conjugate the payload to the
antibody have these properties in order to ensure efficacy and minimal off-target activity.

The first ADC to enter the market was Mylotarg®, developed by Wyeth Co. It used a
25 plasma-sensitive hydrazone linkage to conjugate the toxin with the antibody (Hamann, Philip R.;
Berger, Mark S. (2002). "Mylotarg" Tumor Targeting in Cancer Therapy, Cancer Drug
Discovery and Development, Springer, 239-254). Due to the instability of the linker, it
prematurely released the toxin calicheamicin in the bloodstream and caused fatality in patients.
30 As a result, Pfizer, which had acquired Wyeth, withdrew Mylotarg® from the market at the
request of the US FDA in June 2010.

Kydcyla®, another approved ADC, has a permanent linker, succinimidyl-4-(N-
maleimidomethyl)cyclohexane-1-carboxylate (SMCC), that connects the drug to the remainder
of the construct. Intravenous delivery leads to a stable ADC during circulation (Chari, Ravi V.
35 et al., (1992). "Immunoconjugates containing novel maytansinoids: promising anticancer

drugs". Cancer Research 52 (1): 127–31). Once the drug enters the cancer cell it is trapped in lysosome where lysosomal enzymes degrade the antibody protein and leave behind a modified Maytansinoid DM1 molecule. The structure and activity of Maytansinoid DM1 is such that the modification, which is extensive, does not ablate the potency of the drug despite the presence of a residual lysine and SMCC linker appendage. However, this strategy is not general and works in this case because the toxin maintains its anticancer activity despite modification. In general, the biological activity of small molecule drugs is highly sensitive to modification.

Adcetris® employs a linker system with two parts: valine-citrulline dipeptide and aniline-based p-aminobenzyloxycarbonyl (PABC) (Senter, Peter D.; Sievers, Eric L. (2012).

“The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma”. Nature Biotechnology, 30(7): 631-637).

Valine-citrulline is a substrate for the lysosomal enzyme cathepsin B. Upon enzymatic cleavage of the dipeptide, 1,6-benzyl elimination of the PABC system will release the amine containing drug molecule. The drug molecule can keep its integrity during circulation and transportation within the body, and it will release the toxin payload monomethyl auristatin E (MMAE) inside the lysosome when the valine-citrulline dipeptide is cleaved by cathepsin B and trigger the PABC linker to regenerate the intact amino containing toxin molecule. There are clear limitations to this system as well. The PABS moiety is aromatic and hydrophobic, it may hinder and slow down the approach of cathepsin B enzyme and reduce the rate of valine-citrulline cleavage. Slow release of toxin inside the cancer cell may not produce a high enough concentration of toxin molecules required to kill the cancer cell, may prolong the survival of cancer, and thereby provides a mechanism by which the cancer cell can generate drug resistance. Thus resistance can emerge as a function of ADC properties and cancer type. Perhaps most importantly, this system is only applicable to amino containing toxins. Many drug molecules lack an amino group, or even a nitrogen atom. Current technologies are not applicable as a releasing agent to these drug molecules.

Up to now, there has only been one cleavable linker system that has been approved for use in humans – the aniline-based p-aminobenzyloxycarbonyl (PABC) system used in Adcetris®. However, this system has some serious limitations. There is a critical and unmet need for new linker systems that have all the desired features required for broadly applicable ADCs.

SUMMARY OF THE INVENTION

The present invention provides a targeted conjugate suitable for targeting a bioagent (e.g., an imaging agent or a therapeutic agent such as drug) to a target location (e.g., a target

cell) using a targeting element (e.g., an antibody). The conjugate comprises a targeting element, a cleavable linker comprising an activation element, a releasable linker comprising a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent. The activation element masks the amino methyl moiety (releasable linker). When the conjugate has been
5 targeted to a location (e.g., targeted and subsequently internalized into a cell), the activation element is cleaved (e.g., enzymatically cleaved) from the amino methyl releasable linker resulting in the degradation of the amino methyl moiety by spontaneous collapse which liberates the bioagent. The targeted conjugates described herein are stable under physiological conditions such as in plasma, and activate inside the targeted location such as a cell (for example, a cancer
10 cell).

Accordingly, one embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent.

One embodiment provides a pharmaceutical composition comprising a targeted
15 conjugate as described herein and a pharmaceutically acceptable carrier.

One embodiment provides a method to deliver a bioagent to an animal (e.g., a mammal such as a human) comprising administering a targeted conjugate described herein to the animal.

One embodiment provides a method to treat or diagnose a disease in an animal (e.g., a mammal such as a human) comprising administering a targeted conjugate as described herein to
20 the animal.

One embodiment provides a method to treat or diagnose cancer in an animal (e.g., a mammal such as a human) comprising administering a targeted conjugate as described herein to the animal.

One embodiment provides a method to treat cancer in an animal (e.g., a mammal such as
25 a human) comprising administering a targeted conjugate as described herein to the animal.

One embodiment provides a method to treat cancer in an animal (e.g., a mammal such as a human) in need thereof comprising administering to the animal a targeted conjugate as described herein.

One embodiment provides a targeted conjugate as described in herein for medical
30 therapy or medical diagnosis.

One embodiment provides a targeted conjugate as described herein for the therapeutic treatment of cancer.

One embodiment provides the use of a targeted conjugate as described herein for the preparation of a medicament to treat a disease such as cancer.

35 One embodiment provides intermediates and processes described herein.

DESCRIPTION OF FIGURES

Figure 1 is an exemplary antibody-drug-conjugate synthesized which can be prepared by the methods of Example 1 and which demonstrates a new releasing linker. The exemplary releasing linker is outlined with a box on the structural formula.

5 Figure 2 shows an exemplary ADC incorporating a releasing linker according to the present invention. Adjacent to the Figure is a schematic showing the mechanism of toxin cleavage from the releasing linker.

10 Figure 3A is a schematic illustrating the branched conjugate according to an embodiment of the present invention. In the exemplary 'branched' image, the targeting element (here an antibody) is directly attached to the releasing linker (a residue of a substituted or unsubstituted amino methyl moiety). The activation element and the residue of a bioagent are also directly attached to the releasing linker.

15 Figure 3B is a schematic illustrating a linear conjugate. The targeting element, activation element, releasing linker (a residue of a substituted or unsubstituted amino methyl moiety) and a residue of a bioagent (are connected in series).

Figure 4 shows natural products.

DETAILED DESCRIPTION

20 Recently, SeattleGenetics developed the first effective ADC: anti-CD30 Ab-Valine-citrilline-PABC-MMAE (Adcetris). The drug was granted accelerated approval by the FDA on August 19, 2011 for relapsed Hodgkin's lymphoma (HL) and relapsed or refractory systemic anaplastic large cell lymphoma (sALCL). The Adcetris technology has many limitations. The construct that SeattleGenetics has successfully assembled is a linear array of antibody attached to a linker system that is then attached to the drug, which may not be ideal. The linker system
25 has two parts: valine-citrulline dipeptide and aniline-based p-aminobenzyloxycarbonyl (PABC) system. Importantly, to have the desired properties for targeted delivery the drug must have a suitable nitrogen atom in its structure and this must be attached to the PABC. This is severely limiting, since the overwhelmingly common functionality of highly potent drugs is oxygen – not nitrogen. Valine-citrilline is a protease substrate, e.g. for the lysosomal enzyme cathepsin B.
30 Upon enzymatic cleavage of the dipeptide, 1,6-benzyl elimination of the PABC system releases the amine containing drug molecule. PABC is an aromatic moiety that does not mimic native peptides and may attenuate protease activity. The construct is not ideal because of the ADC conjugate structure, the cleavage mechanism, and the activation process. The PABC 1,6-elimination is not easily tuned while maintaining enzyme substrate viability.

The present invention provides a targeted conjugate comprising a targeting agent, an activation element, a releasing linker and a bioagent. The releasing linker comprises a residue of a substituted or unsubstituted amino methyl moiety. The targeting agent (such as an antibody) is used to target the bioagent (e.g., drug) to a specific cell type. The targeting agent recognizes a particular molecule on the cell, such as a receptor, and the conjugate is subsequently internalized into the cell. The releasing linker is masked by an activation element until the conjugate reaches a target cell. Within a cellular environment, such as a lysosome, the activation element is enzymatically cleaved away from the releasing linker and the releasing linker is subsequently degraded by spontaneous intramolecular collapse releasing the drug inside the target cell. The targeted conjugate may be an antibody-drug-conjugate (ADC).

The cleavable linker described herein is superior to PABC, including tunable activity to proteases (e.g., cathepsin B), as well as tunable, fast release of the drug following proteolysis, the highly enabling flexibility to be attached to oxygen, as well as other atoms other than nitrogen, but including nitrogen, and a construct structure that can be branched or linear.

For example, protease hydrolysis of a dipeptide (activation element) attached to the releasing linker, which is attached in turn to a drug (bioagent), would release the drug so it is free to act on the cell. This activation preferably takes place in a cellular compartment, such as the lysosome or endosome of a target cell. Liberation of the amino group by proteolysis triggers a cascade that results in disintegration of the new linker and releases the drug molecules within the cell. These linker systems are stable under physiological conditions such as in plasma, and only activate inside the targeted cells (for example, cancer cells). They are therefore especially suitable to be incorporated in antibody drug conjugates (ADCs).

The term “alkyl” as used herein refers to a straight or branched saturated hydrocarbon.

The term “alkenyl” as used herein refers to a straight or branched hydrocarbon comprising one or more double bonds.

The term “alkynyl” as used herein refers to a straight or branched hydrocarbon comprising one or more triple bonds.

The term “chain” includes any branched or unbranched arrangement of atoms that are bonded together but are not cyclic. Thus chains include by way of example but are not limited to alkyl, alkenyl, alkynyl and heteroalkyl groups. For example, a heteroalkyl group can comprise a straight or branched arrangement of atoms such as a hydrocarbon wherein one or more of the carbon atoms has been replaced by a heteroatom selected from nitrogen, oxygen and sulfur.

The term “halo” or “halogen” as used herein refers to fluoro, chloro, bromo and iodo.

The term “carbocycle” or “carbocyclyl” refers to a single saturated (i.e., cycloalkyl) or a single partially unsaturated (e.g., cycloalkenyl, cycloalkadienyl, etc.) all carbon ring having 3 to 7 carbon atoms (i.e. (C₃-C₇)carbocycle). “Carbocyclene” refers to an carbocycle group having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of the carbocycle.

The term “heterocyclyl” or “heterocycle” as used herein refers to a single saturated or partially unsaturated ring that has at least one atom other than carbon in the ring, wherein the atom is selected from the group consisting of oxygen, nitrogen and sulfur. Thus, the term includes 3, 4, 5, 6, 7 or 8-membered single saturated or partially unsaturated rings from about 1 to 7 carbon atoms and from about 1 to 4 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur in the ring. The ring may be substituted with one or more (e.g., 1, 2 or 3) oxo groups and the sulfur and nitrogen atoms may also be present in their oxidized forms. Such rings include but are not limited to azetidiny, tetrahydrofuranyl or piperidinyl.

The term “heteroaryl” as used herein refers to a single aromatic ring that has at least one atom other than carbon in the ring, wherein the atom is selected from the group consisting of oxygen, nitrogen and sulfur. Thus, the term includes 5 and 6-membered single aromatic rings from about 1 to 5 carbon atoms and from about 1 to 4 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur in the ring. The sulfur and nitrogen atoms may be present in their oxidized forms provided the ring is aromatic. Such rings include but are not limited to pyridine, tetrazole and triazole.

The term “cyclic group” includes any arrangement of atoms that are bonded together that form a cyclic structure. Thus cyclic groups include by way of example but are not limited to carbocycle, phenyl, heteroaryl and heterocycle.

The term “aryl” as used herein refers to a phenyl or an ortho-fused bicyclic carbocyclic having about nine to ten ring atoms in which at least one ring is aromatic. In one embodiment the aryl is phenyl.

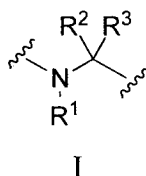
Residue of a substituted or unsubstituted amino methyl moiety (the releasable linker)

The releasable linker described herein is a residue of a substituted or unsubstituted amino methyl moiety which is connected (bonded) to both an activation element and a residue of a bioagent. When the residue of a substituted or unsubstituted amino methyl moiety is separated from the activation element such as by cleavage of the bond connecting the two, the amino methyl moiety readily degrades to release the bioagent. The amino methyl moiety, through substitution can be tuned in terms of both sterics and electronics. This feature allows easy access of potential proteases (for example) to cleave the activation element which enables rapid

disintegration of the amino methyl moiety to release the bioagent (e.g., a hydroxyl containing bioagent).

Once the activation element has been cleaved from the residue of the substituted or unsubstituted amino methyl moiety (releasable linker) it disintegrates spontaneously and rapidly within the cellular environment because of very low enthalpic barriers and highly favorable entropic gains. This is in contrast to previously known linkers that require de-aromatization, a large enthalpic barrier, or the formation of intermediates that are slow to liberate the drug.

In one embodiment the residue of a substituted or unsubstituted amino methyl moiety is a residue of formula I:



wherein:

R^1 is H or (C_1-C_6) alkyl wherein (C_1-C_6) alkyl is optionally substituted with one or more halogen

R^2 is H, (C_1-C_6) alkyl, $-O(C_1-C_6)$ alkyl, aryl or aryl (C_1-C_6) alkyl wherein any (C_1-C_6) alkyl or $-O(C_1-C_6)$ alkyl of R^2 is optionally substituted independently with one or more halogen, $-O(C_1-C_3)$ alkyl or $-OH$, and wherein any aryl or aryl (C_1-C_6) alkyl of R^2 is optionally substituted independently with one or more halogen, (C_1-C_6) alkyl, $-O(C_1-C_3)$ alkyl or $-OH$;

R^3 is H, (C_1-C_6) alkyl, $-O(C_1-C_6)$ alkyl, aryl or aryl (C_1-C_6) alkyl wherein any (C_1-C_6) alkyl or $-O(C_1-C_6)$ alkyl of R^3 is optionally substituted independently with one or more halogen, $-O(C_1-C_3)$ alkyl or $-OH$, and wherein any aryl or aryl (C_1-C_6) alkyl of R^3 is optionally substituted independently with one or more halogen, (C_1-C_6) alkyl, $-O(C_1-C_3)$ alkyl or $-OH$.

In one embodiment the activation element is bonded to the nitrogen atom attached to R^1 of the residue of formula I.

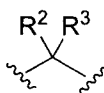
In one embodiment the residue of the bioagent is bonded to the carbon atom attached to R^2 and R^3 of the residue formula I.

It is to be understood that the term “residue of the substituted or unsubstituted amino methyl moiety” means that open valences have been created by removal of one or more atoms (e.g., hydrogen atoms) from the corresponding substituted or unsubstituted amino methyl moiety to allow for bonding between the moiety and various elements of the conjugate (e.g., activation element, residue of a bioagent, targeting element).

In one embodiment the residue of the substituted or unsubstituted amino methyl moiety is part of a second bioagent. In such cases aldehyde and ketone and imine containing bioagents

can be included into the residue of the substituted or unsubstituted amino methyl moiety. As a result, part of the functional group (e.g., aldehyde and ketone and imine) of the second bioagent would become part of the linker itself. Upon release from the activation element the aminomethyl functionality can decompose to provide the second bioagent in addition to the first bioagent (e.g., H-W).

Thus the portion of the residue of the substituted or unsubstituted amino methyl moiety of formula I depicted by the residue of formula Ia:



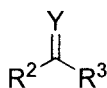
Ia

can be residue of a second bioagent.

In one embodiment the residue of the second bioagent depicted by formula Ia is derivable from a bioagent the comprises one or more groups selected from ketone, aldehyde and imine.

In one embodiment the residue of the second bioagent depicted by formula Ia is derivable from a bioagent the comprises one or more groups selected from ketone, aldehyde and imine. In one embodiment the residue of the second bioagent depicted by formula Ia is derived from a bioagent the comprises one or more groups selected from ketone, aldehyde and imine.

In one embodiment the residue of the second bioagent depicted by formula Ia is derivable from a bioagent of formula Ib:



Ib

wherein Y is O or N^{Ry'}; Ry' is H or (C₁-C₆)alkyl; and R² and R³ together with the remainder of the compound of formula Ib is a bioagent. In one embodiment the residue of the second bioagent depicted by formula Ia is derived from a bioagent of formula Ib. Examples of such bioagents include but are not limited to zaleplon, famciclovir, cetirizine, and peptides and peptide analogues, which contain or could be made to contain this functionality.

Activation element

The activation element is a masking (protective) group which masks the releasing linker. Cleavage of the activation element from the releasing linker results in a reactive releasing linker intermediate which is capable of undergoing spontaneous disintegration to release the bioagent. The activation element may be any element (e.g., molecular structure) that stabilizes (e.g., protects) the releasing linker (the substituted or unsubstituted amino methyl moiety) from

disintegration and release of the bioagent until it is desirable to release the bioagent. The activation element, once activated (e.g., through contact with an enzyme), results in the release of the bioagent (e.g., through breaking of the bond (cleavage) linking the activation element to the substituted or unsubstituted amino methyl moiety which moiety subsequently disintegrates to release the bioagent). In one embodiment the activation element is cleavable from the substituted or unsubstituted amino methyl moiety by an enzyme; the cleavage can take place at any appropriate location including but not limited to inside a cell, outside a cell or proximal to a cell (e.g., a cell such as a target cell). In one embodiment the activation element is cleaved from the substituted or unsubstituted amino methyl moiety by an enzyme; the cleavage can take place at any appropriate location including but not limited to inside a cell, outside a cell or proximal to a cell (e.g., a cell such as a target cell). The activation element may comprise any molecular structure that functions in this manner such as amino acid, peptide, polypeptide or protein (e.g., a complete or partial protein or any variant thereof). In one embodiment the activation element may comprise or consist of a polypeptide of 2-3, 2-5, 2-10 or 2-20 amino acids in length. In one embodiment the activation element may comprise or consist 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length (e.g., polypeptide, amino acid). The activation element may comprise or consist of an amino acid.

In one embodiment the activation element is cleaved within a target such as a cellular compartment (e.g., a lysosome or endosome of a target cell). In one embodiment the activation element is cleaved by an enzyme located within the target cell. The enzyme may be cathepsin B. The cleavage may be proteolysis. In one embodiment the activation element is cleavable within a target such as a cellular compartment (e.g., a lysosome or endosome of a target cell). In one embodiment the activation element is cleaved by an enzyme located within the target cell. In one embodiment the activation element is cleavable by an enzyme located within the target cell. The enzyme may be a proteolytic enzyme. The cleavage may be proteolysis. The enzyme may be cathepsin (e.g., cathepsin B).

In one embodiment the activation element is an amino acid or polypeptide.

In one embodiment the activation element is a polypeptide.

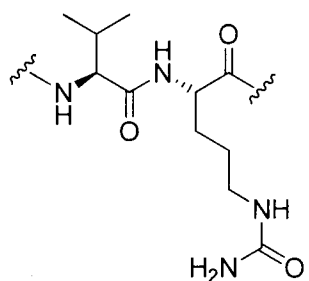
In one embodiment the activation element is a polypeptide comprising 2-10 amino acids.

In one embodiment the activation element is a polypeptide comprising 2-5 amino acids.

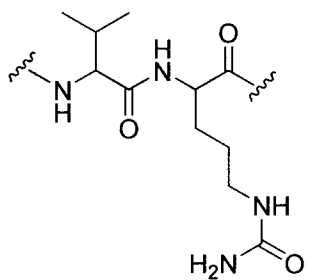
In one embodiment the activation element is a polypeptide comprising 2 amino acids.

In one embodiment the activation element is a polypeptide comprising valine and citrulline.

In one embodiment the activation element is:



In one embodiment the activation element is:



5 Targeting Element

The targeting element can be any element suitable for the targeting a bioagent; for example, to a specific cell or tissue, or to a specific location such as a location in a cell. For example, the targeting element may specifically bind an antigen on a target cell, for example, a tumor antigen on a cancer cell. The targeting element can be subsequently absorbed or internalized into the target cell, thus delivering the targeted conjugate into the target cell. Suitable targeting elements are known in the art and include polypeptides, linear or cyclic, such as an antibody (e.g., monoclonal antibodies), single chain antibody, Fab, small molecules (such as folate), or other binding scaffolds, such as centyrin, darpin, and small protein and peptide structures, or oligonucleotides, such as aptamers, that can be varied such that they can be made to target cells.

Antibodies (Abs) are excellent and selective cellular recognition elements that, due to their innate structure and function, have good stability and pharmacokinetic profiles. In one embodiment the targeting agent is an antibody. An antibody as discussed herein may be a whole antibody or antigen-binding fragment thereof including Fab and scFv fragments. The antibody may be a monoclonal antibody (mAb). Monoclonal antibodies that target a cell specific antigen include bevacizumab (humanized monoclonal antibody with a circulatory system target (VEGF-A)); cetuximab (chimeric monoclonal antibody with a tumor target (EGFR)); ipilimumab (fully human antibody with an immune system target (CTLA-4)); rituximab (targets CD20 on B cells); trastuzumab (targets the Her2/neu (ErbB2) receptor). Other monoclonal antibodies are known in the art and may be used as targeting elements.

Methods for making antibodies against a target antigen are known in the art. Examples of antigens which may be targeted by an antibody include HER2 (ERBB2/neu), EGFR (HER1/ERBB1), VEGF, CD30, CD20. Other target antigens are known in the art.

Since the function of the targeting element is independent of its point of attachment, this element can be connected to the targeted conjugate at any point that does not attenuate other modules of the construct. In one embodiment the targeting agent is directly connected to the activation element. In one embodiment the targeting agent is directly connected to the releasing linker. Methods for linking a targeting agent (such as an antibody) to a targeted conjugate are known and include for example an azido site exemplified in Example 1.

Bioagent

The term “bioagent” includes therapeutic agents that are useful for the treatment of a disease or a physiological condition in an animal (e.g., a mammal such as a human) and thus includes known drugs. Thus, the term “bioagent” includes “therapeutic agent” which includes but is not limited to known drugs and/or drugs that have been approved for sale in the United States. For example, therapeutic agents include but are not limited to chemotherapeutic (anticancer) agents, antibiotic agents, antifungal agents, antiparasitic agents and antiviral agents. The term “bioagent” also includes imaging agents (detectable agents) that are useful for example in diagnosing diseases.

The bioagent can be bonded to the remainder of the targeted conjugate as described herein by the removal of an atom such as a hydrogen atom from the bioagent (e.g., a residue of a bioactive agent). Removal of the atom (e.g., hydrogen) provides the open valency to be connected to the remainder of the targeted conjugate. In one embodiment the bioagent comprises one or more hydroxyl, thiol, or amine (primary or secondary) groups which can be bonded to the conjugate. In one embodiment the term W is the residue of a bioagent and the corresponding group H-W is the corresponding bioagent. Thus, when the residue of the bioagent (W) is released from the conjugate the bioagent (H-W) is provided. In a similar manner the group -Z-W^a is a residue of a bioagent and the corresponding group H-Z-W^a is the corresponding bioagent. In one embodiment the Z of -Z-W^a is an oxygen atom, sulfur atom, NH or NR (R can be any group such as alkyl). Thus, one embodiment provides bioagents comprising one or more hydroxyl (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-, -NH(C₁-C₆)alkyl) groups which groups can be connected to remainder of the targeted conjugate as described herein. In one embodiment the residue of the bioagent is derivable from a bioagent that comprises one or more groups selected from hydroxyl (OH), thiol (SH), primary amine (NH₂) and secondary amine (NH). In one embodiment the residue of the

bioagent is derivable from a bioagent that comprises one or more hydroxyl (OH). In one embodiment the residue of the bioagent is derived from a bioagent that comprises one or more groups selected from hydroxyl (OH), thiol (SH), primary amine (NH₂) and secondary amine (NH). In one embodiment the residue of the bioagent is derived from a bioagent that comprises

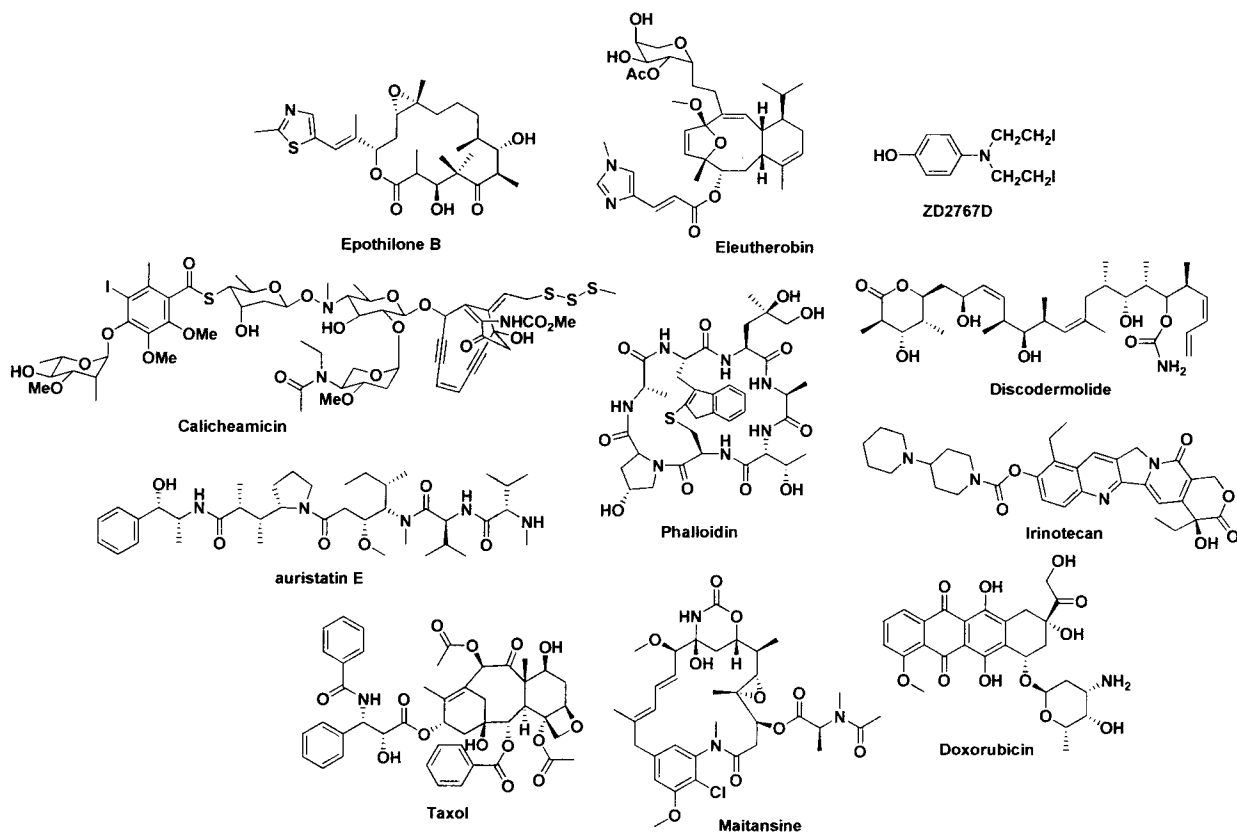
5 one or more hydroxyl (OH).

In one embodiment the bioagent is a therapeutic agent such as a chemotherapeutic agent, an antibiotic agent, an antifungal agent, an antiparasitic agent or an antiviral agent or a prodrug thereof.

In one embodiment the bioagent has one or more hydroxyl or thiol groups.

10 In one embodiment the bioagent has at least one hydroxyl group.

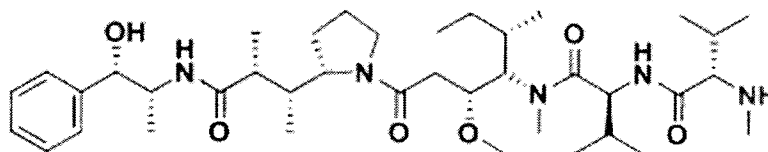
In one embodiment the bioagent has at least one hydroxyl group and is bonded to the residue of the substituted or unsubstituted amino methyl moiety through the oxygen atom of said hydroxyl group. On such group of bioagents include but are not limited to the dolastatins, pyrrolbenzodiazepines and structures of the formula:



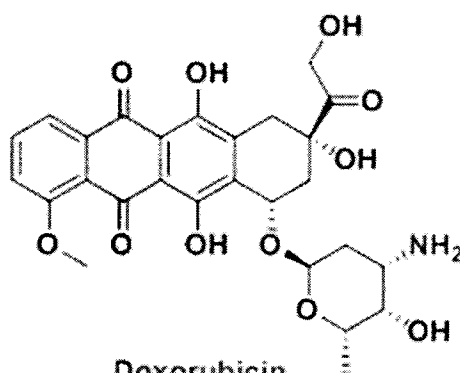
The bioagent described herein may be a therapeutic agent (drug) such as cell toxin or cytotoxin. Suitable therapeutic agents are known in the art and include monomethyl auristatin E (MMAE) toxin, auristatin and maytansinoids. The bioagent may also be selected from other

20 natural products (Figure 4).

Notably, existing amino containing bioagents such as auristatin and maytansinoids can be incorporated using the linker system described herein in their native form – without the need to remove a methyl group and reveal a derivatizable nitrogen in the case of auristatin and without the modification of an additional appendage in the case of the maytansinoids. The native drug molecule will be regenerated to achieve fully biological efficacy. Other examples of suitable bioagents include:

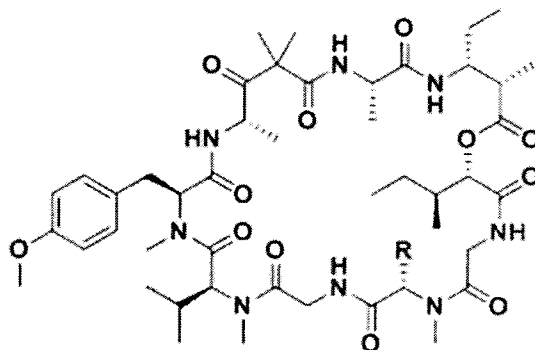


auristatin E



Doxorubicin

and



Dolastatin 11

The bioagent may be an imaging agent (detectable agent). Suitable imaging agents are known and include fluorescent or luminescent labels and radioactive isotopes such as $^{99\text{m}}\text{Tc}$ and ^{111}In . Other examples of suitable imaging agents include for example FITC, biotin and ^{18}F T807].

A targeted conjugate as described herein may comprise one or bioagents, which may be the same or different. A targeted conjugate may comprise one, two, three, or more bioagents.

For example, in one embodiment the targeted conjugate may be a multi-toxin conjugate which comprises two different types of therapeutic agents. In one embodiment the targeted conjugate may comprise a bioagent which is a therapeutic agent and a bioagent which is an imaging agent.

Methods for linking a bioagent to a conjugate are known and include for example a
5 linkage via a hydroxyl group as demonstrated in Example 1.

Linker.

As described herein, the targeting element can be bonded (connected) to the remainder of the targeted conjugate agent through an optional linker. In one embodiment the linker is absent
10 (e.g., the targeting element can be bonded (connected) directly to the remainder of the targeted conjugate). The linker can be variable provided the targeting conjugate functions as described herein. The linker can vary in length and atom composition and for example can be branched or non-branched or cyclic or a combination thereof. The linker may also modulate the properties of the targeted conjugate such as but not limited to solubility, stability and aggregation.

15 Since the linkers used in the targeted conjugates (e.g., linkers comprising polyethylene glycol (PEG)) can be highly variable, it is possible to use different sizes and types of targeting elements and still maintain the desired and/or optimal pharmacokinetic profile for the targeted conjugate.

In one embodiment the linker comprises about 3-5000 atoms. In one embodiment the
20 linker comprises about 3-4000 atoms. In one embodiment the linker comprises about 3-2000 atoms. In one embodiment the linker comprises about 3-1000 atoms. In one embodiment the linker comprises about 3-750 atoms. In one embodiment the linker comprises about 3-500 atoms. In one embodiment the linker comprises about 3-250 atoms. In one embodiment the linker comprises about 3-100 atoms. In one embodiment the linker comprises about 3-50atoms.
25 In one embodiment the linker comprises about 3-25 atoms.

In one embodiment the linker comprises about 10-5000 atoms. In one embodiment the linker comprises about 10-4000 atoms. In one embodiment the linker comprises about 10-2000 atoms. In one embodiment the linker comprises about 10-1000 atoms. In one embodiment the linker comprises about 10-750 atoms. In one embodiment the linker comprises about 10-500
30 atoms. In one embodiment the linker comprises about 10-250 atoms. In one embodiment the linker comprises about 10-100 atoms. In one embodiment the linker comprises about 10-50atoms. In one embodiment the linker comprises about 10-25 atoms.

In one embodiment the linker comprises atoms selected from H, C, N, S and O.

In one embodiment the linker comprises atoms selected from H, C, N, S, P and O.

In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from about 1 to 1000 (or 1-750, 1-500, 1-250, 1-100, 1-50, 1-25, 1-10, 1-5, 5-1000, 5-750, 5-500, 5-250, 5-100, 5-50, 5-25, 5-10 or 2-5 carbon atoms) wherein one or more of the carbon atoms is optionally replaced independently

- 5 by -O-, -S-, -N(R^a)-, 3-7 membered heterocycle, 5-6-membered heteroaryl or carbocycle and wherein each chain, 3-7 membered heterocycle, 5-6-membered heteroaryl or carbocycle is optionally and independently substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents selected from (C₁-C₆)alkyl, (C₁-C₆)alkoxy, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, (C₁-C₆)alkanoyloxy, (C₁-C₆)alkoxycarbonyl, (C₁-C₆)alkylthio, azido, cyano, nitro, halo, -N(R^a)₂,
 10 hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy, wherein each R^a is independently H or (C₁-C₆)alkyl. In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from about 1 to 1000 (or 1-750, 1-500, 1-250, 1-100, 1-50, 1-25, 1-10, 1-5, 5-1000, 5-750, 5-500, 5-250, 5-100, 5-50, 5-25, 5-10 or 2-5 carbon atoms) wherein one or more of the carbon atoms is optionally replaced
 15 independently by -O-, -S-, -N(R^a)-, , wherein each R^a is independently H or (C₁-C₆)alkyl.

In one embodiment the linker comprises a polyethylene glycol. In one embodiment the linker comprises a polyethylene glycol linked to the remainder of the targeted conjugate by a carbonyl group. In one embodiment the polyethylene glycol comprises about 1 to about 500 or about 5 to about 500 or about 3 to about 100 repeat (e.g., -CH₂CH₂O-) units (Greenwald, R.B.,
 20 et al., Poly (ethylene glycol) Prodrugs: Altered Pharmacokinetics and Pharmacodynamics, Chapter, 2.3.1., 283-338; Filpula, D., et al., Releasable PEGylation of proteins with customized linkers, Advanced Drug Delivery, 60, 2008, 29-49; Zhao, H., et al., Drug Conjugates with Poly(Ethylene Glycol), Drug Delivery in Oncology, 2012, 627-656).

In one embodiment the linker is -NH(CH₂CH₂O)₄CH₂CH₂C(=O)-. In one embodiment
 25 the linker is -NH(CH₂CH₂O)_nCH₂CH₂C(=O)- wherein n is 1-500, 5-500, 3-100, 5-50, 1-50, 1-20, 1-10, 1-5, 2-50, 2-20, 2-10, 2-5, 3-50, 3-20, 3-10, 3-5, 4-50, 4-20, 4-10, 4-5. In one embodiment the linker is -(CH₂CH₂O)₄CH₂CH₂C(=O)-.

Capped Linker.

30 As mentioned previously the linker may be used to modulate the properties of the targeted conjugate such as but not limited to solubility, stability and aggregation. Thus, it is possible to use different sizes and types of targeting elements and still maintain the desired and/or optimal pharmacokinetic profile for the targeted conjugate. Thus, it may be advantageous to incorporate a group that corresponds to the structure of the "linker" for the
 35 primary purpose to modulate properties of the targeted conjugate rather than to link moieties of

the targeted conjugate. Thus the term “capped linker” refers to a linker as described herein above that is capped with an atom or functional group for instance a hydrogen atom. In one embodiment the capped linker comprises a polyethylene glycol. In one embodiment the capped linker comprises a polyethylene glycol linked to the remainder of the targeted conjugate by a carbonyl group. In one embodiment the polyethylene glycol comprises about 1 to about 500 or about 5 to about 500 or about 3 to about 100 repeat (e.g., -CH₂CH₂O-) units. In one embodiment the polyethylene glycol is capped with a hydrogen, ethyl, methyl, ethoxy or methoxy group.

10 Branched targeted conjugate

In currently known ADCs the targeting element (e.g. antibody), activation element, releasing linker and targeted bioagent (e.g. drug or label) are connected in series (also known as a linear arrangement), i.e: antibody – activation element – releasing linker – drug.

In one embodiment a conjugate comprising a releasing linker as described herein may comprise a targeting element, activation element, releasing linker and bioagent that are connected in series, (i.e. in a linear arrangement).

However, it has been discovered that altering the configuration of these components to provide a branched, rather than linear arrangement provides several advantages over previously known ADCs including increased accessibility of the activation element to enzyme action; accelerated release of the targeted compound, tunable enhanced solubility and reduced aggregation and increased structural malleability. Accordingly, one embodiment provides a targeted conjugate comprising a targeting element, bioagent, releasing linker and activation element which are configured in a branched (parallel) arrangement. As the function of the targeting element is independent of its point of attachment the targeting element can be connected to the construct at any point that does not attenuate the activation element, releasing linker or bioagent and which results in the conjugate having a branched/parallel configuration. Thus, the targeting agent may be directly connected to the activation element, releasing linker or bioagent.

Targeted conjugates of the present invention also have tunable properties which can be modified as desired. For example, solubilizing/aggregation-suppressing elements can be added onto the activation element and/or between the targeting agent (e.g. antibody) and the releasing linker. The linker may also comprise solubilizing/aggregation-suppressing elements such as but not limited to polysorbate 80, 20, or polyethylene glycol chains of varying lengths such as PEG200, 300, or 400. Thus, a conjugate as described herein may further comprise one or more solubilizing elements and/or aggregation-suppressing elements.

Additional Embodiments.

It is to be understood that two or more of the embodiments provided herein may be combined.

5 In one embodiment the activation element is bonded to the substituted or unsubstituted amino methyl moiety.

 In one embodiment the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent.

10 In one embodiment the targeting element is bonded to the activation element through an optional linker, or the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.

 In one embodiment the targeting element is bonded to the activation element through an optional linker.

15 In one embodiment the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.

 One embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent wherein:

20 (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;

 (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and

25 (c) the targeting element is bonded to the activation element through an optional linker, or the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.

 One embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent wherein:

30 (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;

 (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and

 (c) the targeting element is bonded to the activation element through an optional linker.

One embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent wherein:

- (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;
- (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and
- (c) the targeting element is bonded to the activation element through a linker.

One embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent wherein:

- (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;
- (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and
- (c) the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.

One embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent wherein:

- (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;
- (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and
- (c) the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through a linker.

In one embodiment the residue of the substituted or unsubstituted amino methyl moiety is capable of releasing the bioagent from the targeted conjugate.

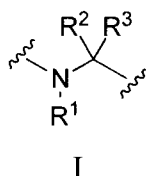
In one embodiment the residue of the substituted or unsubstituted amino methyl moiety releases the bioagent from the targeted conjugate.

In one embodiment the residue of the substituted or unsubstituted amino methyl moiety releases the bioagent from the targeted conjugate when the targeted conjugate is contacted with an enzyme. In one embodiment the enzyme interacts with the activation element. In one embodiment the enzyme contact results in the activation element being separated from the residue of the substituted or unsubstituted amino methyl moiety. In one embodiment the

enzyme contact breaks a bond connecting the activation element and the residue of the substituted or unsubstituted amino methyl moiety. In one embodiment the enzyme contact breaks an amide bond connecting the activation element and the residue of the substituted or unsubstituted amino methyl moiety. In one embodiment the enzyme is capable of cleaving amide bonds. In one embodiment the enzyme is a proteolytic enzyme. In one embodiment the enzyme is a cathepsin. In one embodiment the enzyme is a cathepsin B.

In one embodiment the residue of the substituted or unsubstituted amino methyl moiety is capable of releasing the bioagent from the targeted conjugate when the targeted conjugate is contacted with an enzyme. In one embodiment an enzyme is capable of interacting with the activation element of the targeted conjugate. In one embodiment an enzyme is capable of separating the activation element from the residue of the substituted or unsubstituted amino methyl moiety of the targeted conjugate. In one embodiment an enzyme is capable of breaking a bond connecting the activation element and the residue of the substituted or unsubstituted amino methyl moiety of the targeted conjugate. In one embodiment an enzyme is capable of breaking an amide bond connecting the activation element and the residue of the substituted or unsubstituted amino methyl moiety of the targeted conjugate.

One embodiment provides a targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent wherein, the residue of the substituted or unsubstituted amino methyl moiety is a residue of formula I:



wherein:

R^1 is H or (C_1-C_6) alkyl wherein (C_1-C_6) alkyl is optionally substituted with one or more halogen

R^2 is H, (C_1-C_6) alkyl, $-O(C_1-C_6)$ alkyl, aryl or aryl (C_1-C_6) alkyl wherein any (C_1-C_6) alkyl or $-O(C_1-C_6)$ alkyl of R^2 is optionally substituted independently with one or more halogen, $-O(C_1-C_3)$ alkyl or $-OH$, and wherein any aryl or aryl (C_1-C_6) alkyl of R^2 is optionally substituted independently with one or more halogen, (C_1-C_6) alkyl, $-O(C_1-C_3)$ alkyl or $-OH$;

R^3 is H, (C_1-C_6) alkyl, $-O(C_1-C_6)$ alkyl, aryl or aryl (C_1-C_6) alkyl wherein any (C_1-C_6) alkyl or $-O(C_1-C_6)$ alkyl of R^3 is optionally substituted independently with one or more halogen, $-O(C_1-C_3)$ alkyl or $-OH$, and wherein any aryl or aryl (C_1-C_6) alkyl of R^3 is optionally substituted independently with one or more halogen, (C_1-C_6) alkyl, $-O(C_1-C_3)$ alkyl or $-OH$.

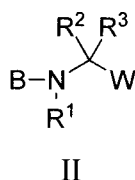
In one embodiment the activation element is bonded to the nitrogen atom attached to R¹ of the residue of formula I.

In one embodiment the residue of the bioagent is bonded to the carbon atom attached to R² and R³ of the residue of formula I.

5 In one embodiment the targeting element is bonded to the activation element through an optional linker.

In one embodiment the targeting element is bonded, through an optional linker, to R¹, R², or the carbon atom attached to R² and R³ of formula I or formula II or the nitrogen attached to R¹ of formula I or formula II.

10 One embodiment provides a targeted conjugate comprising a targeting element bonded to a compound formula II through an optional linker:



wherein:

15 R¹ is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

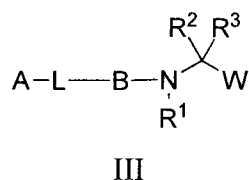
R² is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R² is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R² is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

20 R³ is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R³ is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R³ is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

25 B is an activation element; and

W is a residue of a bioagent.

One embodiment provides a targeted conjugate of formula III:



30 wherein:

R¹ is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

R^2 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

R^3 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^3 is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^3 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

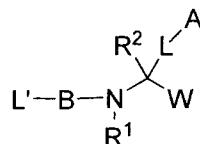
A is the targeting element;

L is absent or a linker;

B is an activation element; and

W is a residue of a bioagent.

One embodiment provides a conjugate of formula IV:



IV

wherein:

R^1 is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

R^2 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

A is the targeting element;

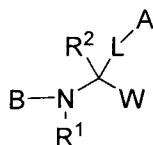
L is absent or a linker;

L' is absent or a capped linker;

B is an activation element; and

W is a residue of a bioagent.

One embodiment provides a conjugate of formula IV':



IV'

wherein:

R^1 is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

R^2 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any (C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more

5 halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

A is the targeting element;

L is absent or a linker;

B is an activation element; and

10 W is a residue of a bioagent.

In one embodiment R^1 is H.

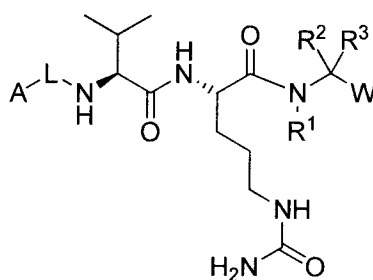
In one embodiment R^3 is H or (C₁-C₆)alkyl.

In one embodiment R^3 is H.

In one embodiment R^2 is H or (C₁-C₆)alkyl.

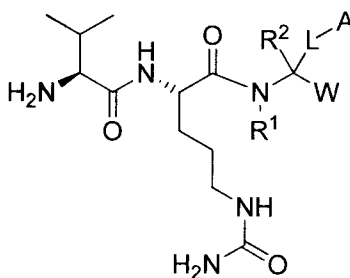
15 In one embodiment R^2 is H.

One embodiment provides a targeted conjugate of formula IIIa:



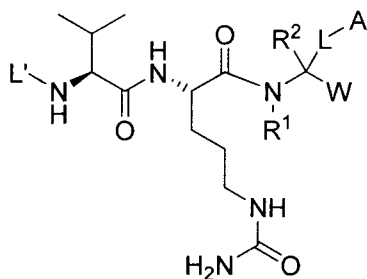
IIIa

20 One embodiment provides a targeted conjugate of formula IVa:



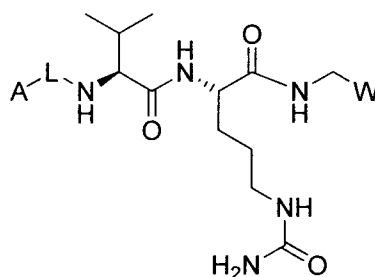
IVa

One embodiment provides a targeted conjugate of formula IVb:



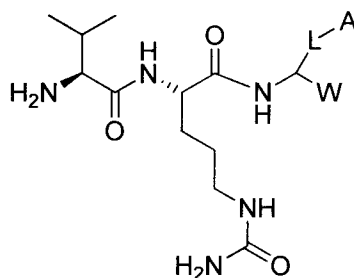
IVb

One embodiment provides a targeted conjugate of formula IIIa':



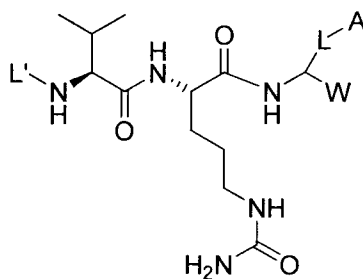
IIIa'

One embodiment provides a targeted conjugate of formula IVa':



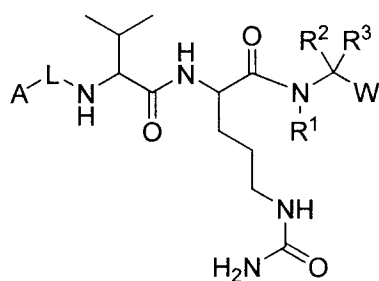
IVa'

One embodiment provides a targeted conjugate of formula IVb':



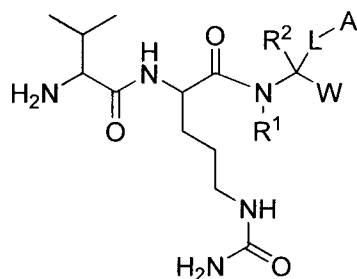
IVb'

One embodiment provides a targeted conjugate of formula IIIa'':



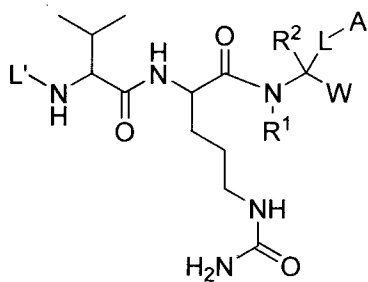
IIIa''

One embodiment provides a targeted conjugate of formula IVa'':



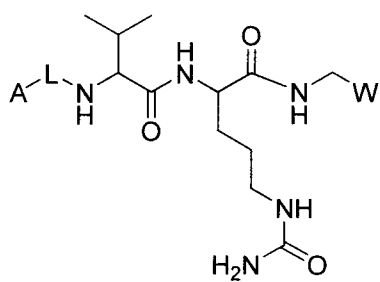
IVa''

One embodiment provides a targeted conjugate of formula IVb'':



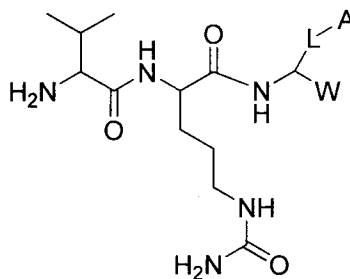
IVb''

One embodiment provides a targeted conjugate of formula IIIa*:



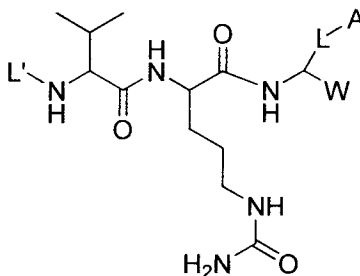
IIIa*

One embodiment provides a targeted conjugate of formula IVa*:



IVa*

One embodiment provides a targeted conjugate of formula IVb*:



IVb*

One embodiment provides a salt or pharmaceutically acceptable salt of any targeted conjugate described herein (e.g., a salt or pharmaceutically acceptable salt of any targeted conjugate of any formula (e.g., III, IV or any subformula thereof)).

10 Administration

The method of administering the targeted conjugate to the desired area for treatment and the dosage may be varied as needed. Some methods of administration include intravascular injection, intravenous injection, intraperitoneal injection, subcutaneous injection, and intramuscular injection. The n targeted conjugates may be formulated in an injectable format (*e.g.*, suspension, emulsion) in a medium such as, for example, water, saline, Ringer's solution, dextrose, dimethylsulfoxide, albumin solution, and oils. The targeted conjugate may also be administered to the patient through topical application via a salve or lotion, transdermally through a patch, orally ingested as a pill or capsule or suspended in a liquid or rectally inserted in suppository form. Targeted conjugates may also be suspended in an aerosol or pre-aerosol formulation suitable for inhalation via the mouth or nose. The targeted conjugates may also be delivered to the patient using other methods. For example, the targeted conjugate may be administered to the patient orally, or may be administered rectally. It is to be understood the targeted conjugate described herein may also be useful in diagnostics as well as studies in cells, tissues and animals. The targeted conjugates may also be in the form of a salt such as a pharmaceutically acceptable salt.

Medical use

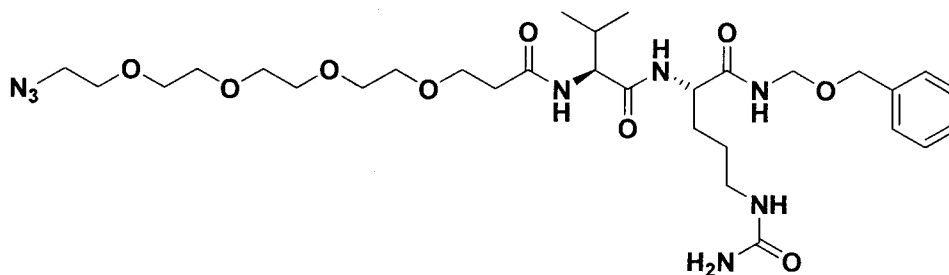
A targeted conjugate as described herein may be used in the treatment or prevention of a disease in a subject such as an animal (e.g., a mammal such as human). The present invention provides a targeted conjugate as described herein for use in therapy. The present invention provides a targeted conjugate as described herein for use in the treatment or prevention of cancer. For example, the cancer may be a solid tumor or blood malignancy such as carcinomas of the bladder, breast, colon and rectal, endometrial, kidney, leukemia, lung, melanoma, non-hodgkin lymphoma, pancreatic, prostate, or thyroid.

The present invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

EXAMPLES

Example 1: Synthesis of a construct containing a novel releasing linker (i.e., residue of a substituted or unsubstituted amino methyl moiety).

The following compound has all the key features of the design of a linear construct, including a site for targeting element (e.g., an antibody) which can be conjugated through the azido (left), the activation element (e.g., a site for proteolysis (dipeptide, middle), the aminomethoxy unit (middle right), and a moiety that, once released, is an alcohol containing compound that represents a bioagent attached to the linker system by a hydroxyl group. This construct is also shown in Figure 1 which highlights the releasing linker.



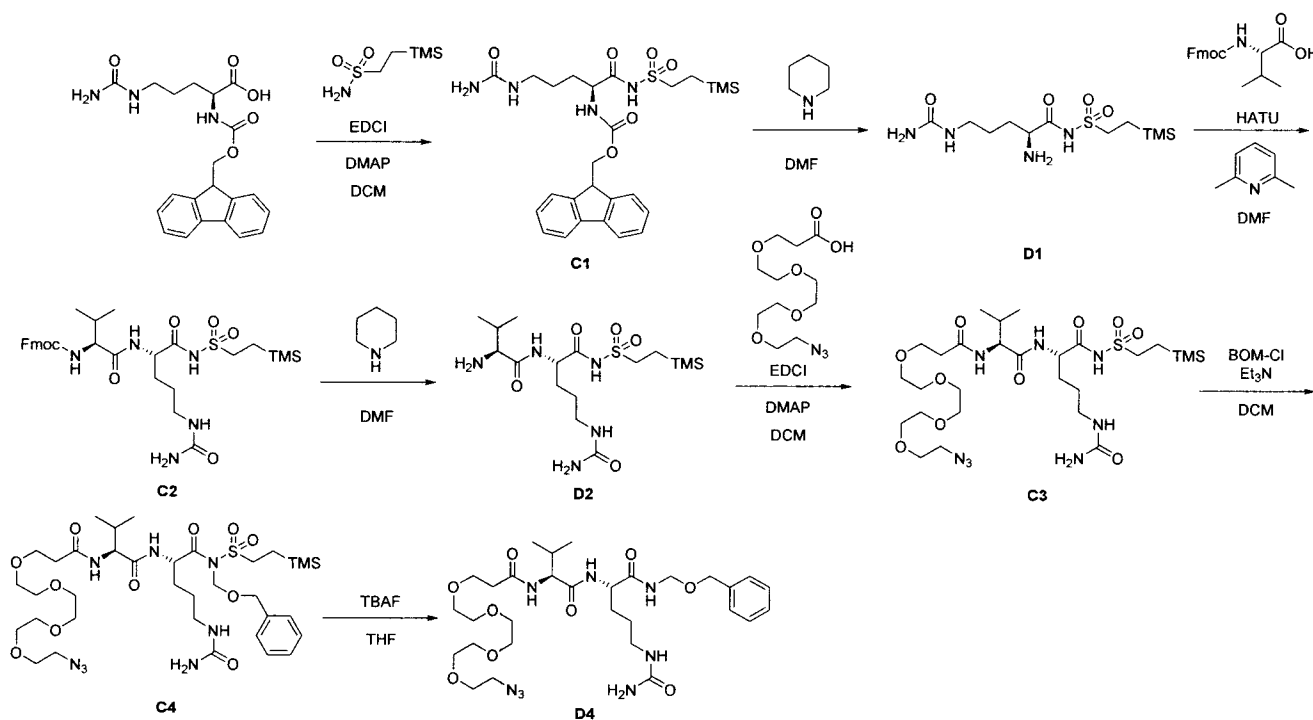
Results: The synthesized construct was found to be:

- Stable to storage (-20 C), 3+ months, no decomposition as assessed by HPLC
- Stable to pH 5 buffer (37 C), 7+ days, no decomposition as assessed by HPLC

- Construct was assessed for human Cathepsin B enzyme assay, including positive controls, and was shown to have $t_{1/2} = 8.5$ h at 37 C in pH 5 buffer.

These data support the concept for a new targeted conjugate design (e.g., new ADC design) and validate the conjugates described herein. Accordingly, the targeted conjugates described herein have considerably expand the scope of bioagents that can be potentially delivered because bioagents (including therapeutic agents and therapeutic agent candidates drug candidates) that have hydroxyl, thiol and amine functional groups as well as aldehyde, ketone, imine, and functional groups can serve as point of attachment point of the bioactive to the remainder of the targeted conjugate. This is very significant as numerous bioactive agents contain such groups. Thus, the targeted conjugates provided herein have broad applications for the design of therapeutic agents and imaging agents that can benefit from targeted delivery (e.g., antibody targeted delivery). These data which demonstrate the parallel connectivity design are also compatible with the branched design described herein.

The synthetic scheme shown below outlines the preparation of the compounds:



General Experimental Information

Starting materials, reagents, and solvents were purchased from commercial suppliers (Aldrich, Acros, and Fischer) and used as received unless otherwise noted. Proton nuclear

magnetic resonance (^1H NMR) spectra were recorded on Varian-500 instrument (500 MHz). Chemical shifts are reported in ppm relative to chloroform or methanol as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, m = multiplet, br = broad, app = apparent), coupling constants (Hz), and integration. Carbon nuclear magnetic resonance (^{13}C NMR) Varian-500 instrument (125 MHz). Chemical shifts are reported in ppm relative to chloroform or methanol as the internal standard. Mass spectra were recorded on either a Finnigan LCQ-DUO (ESI) system or a Waters Micromass ZQ Mass Spectrometer (ESI) system. HPLC data were recorded on Waters Alliance e2695 separations module with 50 mm C-18 reverse phase column.

Compound C1:

^1H NMR (500 MHz, Methanol- d_4) δ 7.75 (d, J = 7.5 Hz, 2H), 7.63 (dd, J = 12.8, 7.5 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.28 (t, J = 7.5 Hz, 2H), 4.34 (dd, J = 10.6, 7.0 Hz, 1H), 4.28 (dd, J = 10.5, 7.2 Hz, 1H), 4.22 – 4.09 (m, 2H), 3.38 (td, J = 14.1, 4.2 Hz, 1H), 3.26 (dd, J = 14.0, 4.3 Hz, 1H), 3.14 (dq, J = 28.1, 6.8 Hz, 2H), 1.80 (dt, J = 8.7, 4.5 Hz, 1H), 1.73 – 1.50 (m, 2H), 1.09 – 0.91 (m, 2H), -0.02 (s, 9H).

^{13}C NMR (126 MHz, Methanol- d_4) δ 173.47, 161.13, 157.36, 143.90, 141.39, 127.66, 127.02, 125.06, 119.81, 66.94, 55.44, 49.09, 47.18, 39.15, 28.57, 26.66, 9.96, -3.15.

ESIMS m/z 583.1 ($\text{M}+\text{Na}^+$)

Compound C2:

^1H NMR (500 MHz, Methanol- d_4) δ 7.75 (d, J = 7.7 Hz, 2H), 7.63 (t, J = 7.1 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.26 (t, J = 7.5 Hz, 2H), 4.31 (dh, J = 23.7, 6.5 Hz, 3H), 4.22 – 4.14 (m, 1H), 3.89 (d, J = 7.0 Hz, 1H), 3.39 – 3.28 (m, 1H), 3.23 – 3.07 (m, 2H), 3.04 (dt, J = 14.5, 7.0 Hz, 1H), 2.06 – 1.91 (m, 1H), 1.76 (d, J = 12.1 Hz, 1H), 1.65 (q, J = 11.1, 8.8 Hz, 1H), 1.50 (d, J = 19.5 Hz, 2H), 0.92 (m, 8H), -0.00 (d, J = 2.7 Hz, 7H).

^{13}C NMR (126 MHz, Methanol- d_4) δ 176.62, 175.58, 164.42, 160.72, 147.43, 144.71, 130.93, 128.31, 123.06, 108.29, 70.09, 64.00, 56.90, 52.45, 42.26, 34.09, 31.70, 29.86, 21.94, 20.95, 13.16, 0.04.

ESIMS m/z 658.1 ($\text{M}-\text{H}^+$)

Compound C3:

^1H NMR (500 MHz, Chloroform- d) δ 11.03 (s, 1H), 7.85 – 7.76 (m, 1H), 7.63 (s, 1H), 5.87 (s, 1H), 5.11 (s, 2H), 4.54 (d, J = 9.5 Hz, 1H), 4.21 (t, J = 7.2 Hz, 1H), 3.85 – 3.60 (m, 16H), 3.43 (t, J = 5.3 Hz, 2H), 3.36 – 3.28 (m, 2H), 3.19 (s, 2H), 2.64 (m, 1H), 2.56 (m, 1H), 2.17 – 2.10 (m, 1H), 1.90 (s, 1H), 1.74 (m, 1H), 1.58 – 1.52 (m, 2H), 1.07 – 0.89 (m, 8H), 0.05 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 174.56, 174.11, 162.20, 160.73, 72.18, 69.27, 55.67, 52.56, 51.79, 41.12, 38.32, 32.30, 30.46, 28.46, 21.43, 20.45, 11.81, 0.03.

ESIMS m/z 733.3 ($\text{M}+\text{Na}^+$)

Compound C4:

- 5 ^1H NMR (500 MHz, Chloroform-*d*) δ 7.83 (s, 1H), 7.71 (s, 1H), 7.33 (m, 5H), 5.72 – 5.47 (m, 2H), 5.32 – 5.09 (m, 1H), 4.97 – 4.75 (m, 2H), 4.73 – 4.57 (m, 2H), 3.81 – 3.72 (m, 2H), 3.64 (m, 16H), 3.39 (m, 2H), 3.08 (m, 2H), 2.61 (m, 1H), 2.52 (m, 2H), 2.14 (m, 1H), 1.89 – 1.64 (m, 3H), 1.57 – 1.49 (m, 2H), 1.04 – 0.82 (m, 8H), 0.05 – -0.00 (m, 9H).

HPLC-Retention Time: 5.2 min

- 10 ESIMS m/z 831.5 ($\text{M}+\text{H}^+$)

Compound D4:

HPLC-Retention Time: 3.7 min

ESIMS m/z 667.5 ($\text{M}+\text{H}^+$)

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be
5 understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

CLAIMS

1. A targeted conjugate comprising a targeting element, an activation element, a residue of a substituted or unsubstituted amino methyl moiety and a residue of a bioagent.
2. The targeted conjugate of claim 1, wherein the activation element is bonded to the substituted or unsubstituted amino methyl moiety.
3. The targeted conjugate of claim 1 or claim 2, wherein the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent.
4. The targeted conjugate of any one of claims 1-3, wherein the targeting element is bonded to the activation element through an optional linker, or the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.
5. The targeted conjugate of any one of claims 1-3, wherein the targeting element is bonded to the activation element through an optional linker.
6. The targeted conjugate of any one of claims 1-3, wherein the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.
7. The targeted conjugate of claim 1 wherein:
 - (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;
 - (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and
 - (c) the targeting element is bonded to the activation element through an optional linker, or the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.
8. The targeted conjugate of claim 1 wherein:
 - (a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;
 - (b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and

(c) the targeting element is bonded to the activation element through an optional linker.

9. The targeted conjugate of claim 1 wherein:

(a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;

(b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and

(c) the targeting element is bonded to the activation element through a linker.

10. The targeted conjugate of claim 1 wherein:

(a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;

(b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and

(c) the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an optional linker.

11. The targeted conjugate of claim 1 wherein:

(a) the activation element is bonded to the residue of the substituted or unsubstituted amino methyl moiety;

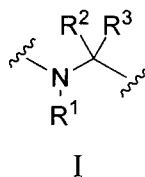
(b) the residue of the substituted or unsubstituted amino methyl moiety is bonded to the residue of the bioagent; and

(c) the targeting element is bonded to the residue of the substituted or unsubstituted amino methyl moiety through a linker.

12. The targeted conjugate of any one of claims 1-11, wherein the residue of the substituted or unsubstituted amino methyl moiety is capable of releasing the bioagent from the targeted conjugate.

13. The targeted conjugate of any one of claims 1-11, wherein the residue of the substituted or unsubstituted amino methyl moiety releases the bioagent from the targeted conjugate.

14. The targeted conjugate of any one of claims 1-11, wherein the residue of the substituted or unsubstituted amino methyl moiety is capable of releasing the bioagent from the targeted conjugate when the targeted conjugate is contacted with an enzyme.
15. The targeted conjugate of any one of claim 14, wherein the enzyme is capable of interacting with the activation element.
16. The targeted conjugate of any one of claims 14-15, wherein enzyme contact is capable of separating the activation element from the residue of the substituted or unsubstituted amino methyl moiety.
17. The targeted conjugate of any one of claims 14-16, wherein the enzyme contact is capable of breaking a bond connecting the activation element and the residue of the substituted or unsubstituted amino methyl moiety.
18. The targeted conjugate of any one of claims 14-17, wherein the enzyme contact is capable of breaking an amide bond connecting the activation element and the residue of the substituted or unsubstituted amino methyl moiety.
19. The targeted conjugate of any one of claims 14-18, wherein the enzyme is capable of cleaving amide bonds.
20. The targeted conjugate of any one of claims 14-19, wherein the enzyme is a cathepsin.
21. The targeted conjugate of any one of claims 1-20, wherein the residue of the substituted or unsubstituted amino methyl moiety is a residue of formula I:



wherein:

R^1 is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen

R^2 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more

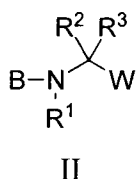
halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R² is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

R³ is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R³ is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R³ is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH.

22. The targeted conjugate of claim 21, wherein the activation element is bonded to the nitrogen atom attached to R¹ of formula I.

23. The targeted conjugate of any one of claims 21-22, wherein the residue of the bioagent is bonded to the carbon atom attached to R² and R³ of formula I.

24. The targeted conjugate of any one of claims 1-20, comprising a targeting element bonded to a compound of formula II through an optional linker:



wherein:

R¹ is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

R² is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R² is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R² is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

R³ is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R³ is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R³ is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

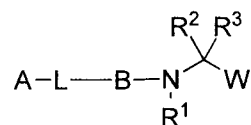
B is an activation element; and

W is a residue of a bioagent.

25. The targeted conjugate of any one of claims 21-24, wherein the targeting element is bonded to the activation element through an optional linker.

26. The targeted conjugate of any one of claims 21-24, wherein the targeting element is bonded to R^1 , R^2 , the carbon atom attached to R^2 and R^3 of formula I or formula II or the nitrogen attached to R^1 of formula I or formula II through an optional linker.

27. The targeted conjugate of claim 1, which is a conjugate of formula III:



III

wherein:

R^1 is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

R^2 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

R^3 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^3 is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^3 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

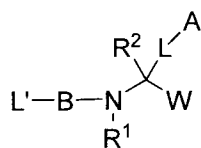
A is the targeting element;

L is absent or a linker;

B is an activation element; and

W is a residue of a bioagent.

28. The targeted conjugate of claim 1, which is a conjugate of formula IV:



IV

wherein:

R^1 is H or (C₁-C₆)alkyl wherein (C₁-C₆)alkyl is optionally substituted with one or more halogen;

R^2 is H, (C₁-C₆)alkyl, -O(C₁-C₆)alkyl, aryl or aryl(C₁-C₆)alkyl wherein any(C₁-C₆)alkyl or -O(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, -O(C₁-C₃)alkyl or -OH, and wherein any aryl or aryl(C₁-C₆)alkyl of R^2 is optionally substituted independently with one or more halogen, (C₁-C₆)alkyl, -O(C₁-C₃)alkyl or -OH;

A is the targeting element;

L is absent or a linker;

L' is absent or a capped linker;

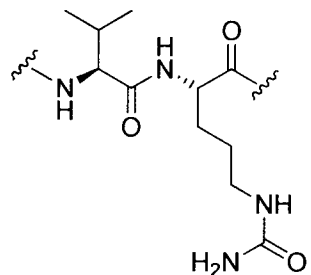
B is an activation element; and

W is a residue of a bioagent.

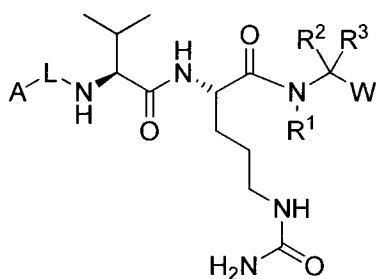
29. The targeted conjugate of any one of claims 21-28, wherein R^1 is H.
30. The targeted conjugate of any one of claims 21-27, wherein R^3 is H or (C₁-C₆)alkyl.
31. The targeted conjugate of any one of claims 21-27, wherein R^3 is H.
32. The targeted conjugate of any one of claims 21-31, wherein R^2 is H or (C₁-C₆)alkyl.
33. The targeted conjugate of any one of claims 21-31, wherein R^2 is H.
34. The targeted conjugate of any one of claims 1-33, wherein the activation element is an amino acid or polypeptide.
35. The targeted conjugate of any one of claims 1-33, wherein the activation element is a polypeptide.
36. The targeted conjugate of any one of claims 1-33, wherein the activation element is a polypeptide comprising 2-10 amino acids.
37. The targeted conjugate of any one of claims 1-33, wherein the activation element is a polypeptide comprising 2-5 amino acids.
38. The targeted conjugate of any one of claims 1-33, wherein the activation element is a polypeptide comprising 2 amino acids.

39. The targeted conjugate of any one of claims 1-33, wherein the activation element is a polypeptide comprising valine and citrulline.

40. The targeted conjugate of any one of claims 1-33, wherein the activation element is:

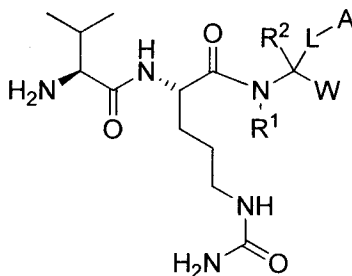


41. The targeted conjugate of claim 27, which is a conjugate of formula IIIa:



IIIa.

42. The targeted conjugate of claim 28, which is a conjugate of formula IVa:



43. The targeted conjugate of any one of claims 41-42, wherein R^1 is H.

44. The targeted conjugate of any one of claims 41-43, wherein R^3 is H or (C_1-C_6) alkyl.

45. The targeted conjugate of any one of claims 41-43, wherein R^3 is H.

46. The targeted conjugate of any one of claims 41-45, wherein R^2 is H or (C_1-C_6) alkyl.

47. The targeted conjugate of any one of claims 41-45, wherein R^2 is H.

48. The targeted conjugate of any one of claims 4-47, wherein the linker comprises about 3-200 atoms.
49. The targeted conjugate of any one of claims 4-47, wherein the linker comprises about 3-100 atoms.
50. The targeted conjugate of any one of claims 4-47, wherein the linker comprises about 3-50 atoms.
51. The targeted conjugate of any one of claims 4-50, wherein the linker comprises atoms selected from H, C, N, S and O.
52. The targeted conjugate of any one of claims 1-51, wherein the targeting element is a polynucleotide.
53. The targeted conjugate of any one of claims 1-51, wherein the targeting element is an antibody.
54. The targeted conjugate of any one of claims 1-53, wherein the residue of the bioagent is derivable from a bioagent that comprises one or more groups selected from hydroxyl (OH), thiol (SH), primary amine (NH₂) and secondary amine (NH).
55. The targeted conjugate of any one of claims 1-53, wherein the residue of the bioagent is derivable from a bioagent that comprises one or more hydroxyl (OH).
56. The targeted conjugate of any one of claims 1-55, wherein the residue of the bioagent is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an oxygen, sulfur or nitrogen atom.
57. The targeted conjugate of any one of claims 1-55, wherein the residue of the bioagent is bonded to the residue of the substituted or unsubstituted amino methyl moiety through an oxygen atom.
58. The targeted conjugate of any one of claims 1-54, wherein the residue of the bioagent is -Z-W^a wherein:

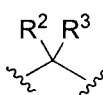
Z is -O-, -S- or -N(R^a)-;

R^a is H or (C₁-C₆)alkyl; and

W^a is the remainder of the residue of the bioagent.

59. The targeted conjugate of claim 58, wherein Z is -O- or -S-.
60. The targeted conjugate of any one of claims 1-54, wherein the residue of the bioagent is -O-W^a wherein W^a is the remainder of the residue of the therapeutic agent.
61. The targeted conjugate of any one of claims 1-60, wherein the residue of the bioagent is a residue of a therapeutic agent or a residue of an imaging agent.
62. The targeted conjugate of any one of claims 1-61, wherein the residue of the bioagent is a residue of an anti-cancer therapeutic agent.
63. The targeted conjugate of any one of claims 1-62 further comprising a second bioagent.
64. The targeted conjugate of claim 63, wherein the second bioagent is a therapeutic agent.
65. A pharmaceutical composition comprising a targeted conjugate as described in any one of claims 1-64 and a pharmaceutically acceptable carrier.
66. A method to deliver a bioagent to an animal comprising administering a targeted conjugate as described in any one of claims 1-64 to the animal.
67. A method to treat or diagnose a disease in an animal comprising administering a targeted conjugate as described in any one of claims 1-64 to the animal.
68. A method to treat or diagnose cancer in an animal comprising administering a targeted conjugate as described in any one of claims 1-64 to the animal.
69. A method to treat cancer in an animal comprising administering a targeted conjugate as described in any one of claims 1-64 to the animal.

70. A method to treat cancer in an animal in need thereof comprising administering to the animal a targeted conjugate as described in any one of claims 1-64.
71. A targeted conjugate as described in any one of claims 1-64 for medical therapy or medical diagnosis.
72. A targeted conjugate as described in any one of claims 1-64 for the therapeutic treatment of cancer.
73. The use of a targeted conjugate as described in any one of claims 1-64 for the preparation of a medicament for the treatment of a disease such as cancer.
74. The targeted conjugate of claim 28, wherein L' is absent.
75. The targeted conjugate of any one of claims 1, 2, 3, 4 or 6 further comprising a capped linker.
76. The targeted conjugate of claim 10 or 11 wherein a capped linker is attached to the activation element.
77. The targeted conjugate of any one of claims 21, 23 or 27 wherein the residue depicted by formula Ia:



Ia

is the residue of a second bioagent.

78. The targeted conjugate of any one of claims 4-47, wherein the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from about 1 to 1000 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by -O-, -S-, -N(R^a)-, 3-7 membered heterocycle, 5-6-membered heteroaryl or carbocycle and wherein each chain, 3-7 membered heterocycle, 5-6-membered heteroaryl or carbocycle is optionally and independently substituted with one or more substituents selected from (C₁-C₆)alkyl, (C₁-C₆)alkoxy, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, (C₁-C₆)alkanoyloxy, (C₁-C₆)alkoxycarbonyl, (C₁-C₆)alkylthio, azido, cyano, nitro, halo, -N(R^a)₂, hydroxy, oxo (=O),

carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy, wherein each R^a is independently H or (C₁-C₆)alkyl.

79. The targeted conjugate of any one of claims 4-47, wherein the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from about 5 to 300 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by -O-, -S-, -N(R^a)- and wherein each chain is optionally and independently substituted with one or more substituents selected from (C₁-C₆)alkyl, (C₁-C₆)alkoxy, (C₃-C₆)cycloalkyl, (C₁-C₆)alkanoyl, (C₁-C₆)alkanoyloxy, (C₁-C₆)alkoxycarbonyl, (C₁-C₆)alkylthio, azido, cyano, nitro, halo, -N(R^a)₂, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy, wherein each R^a is independently H or (C₁-C₆)alkyl.

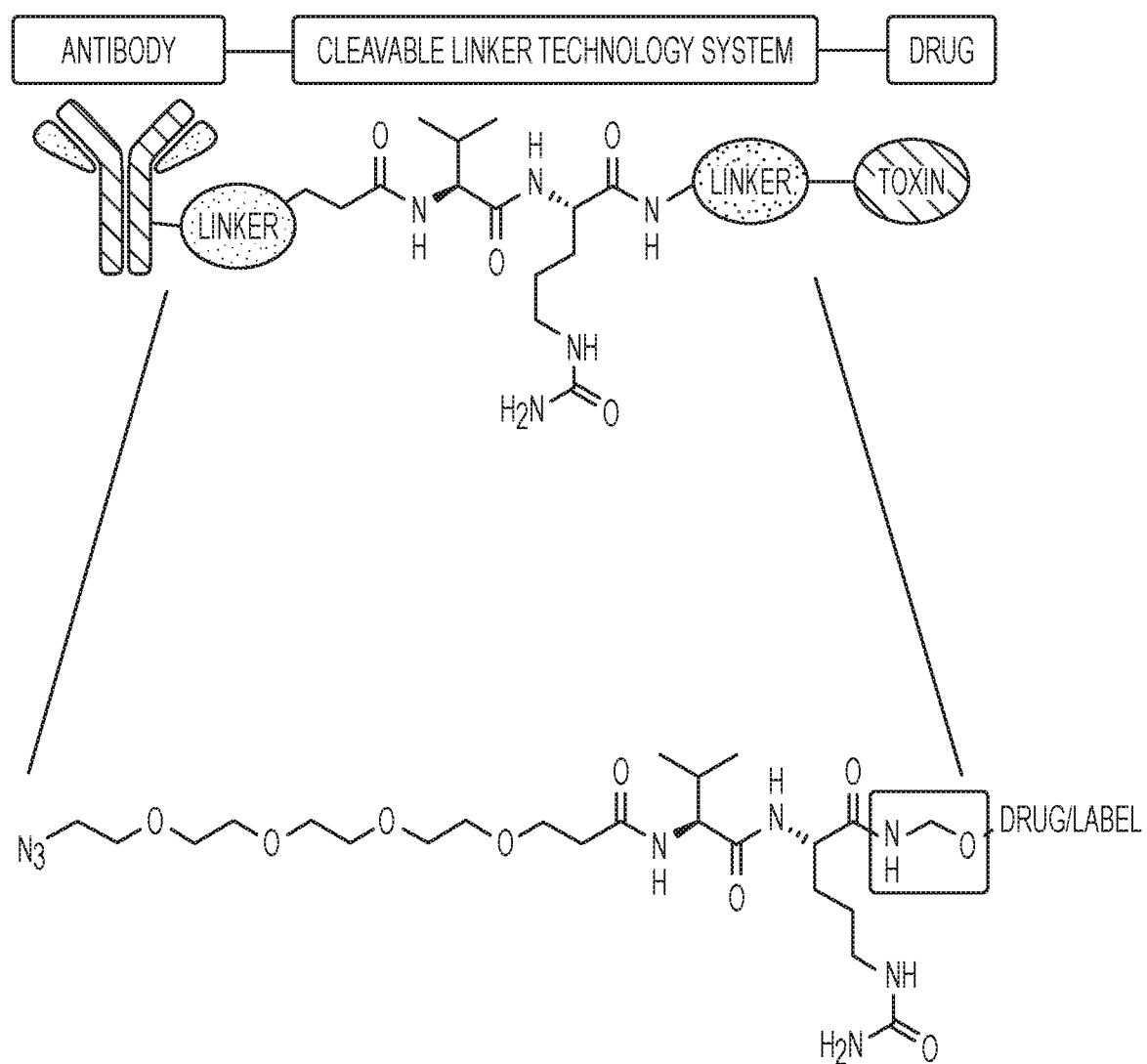


Figure 1

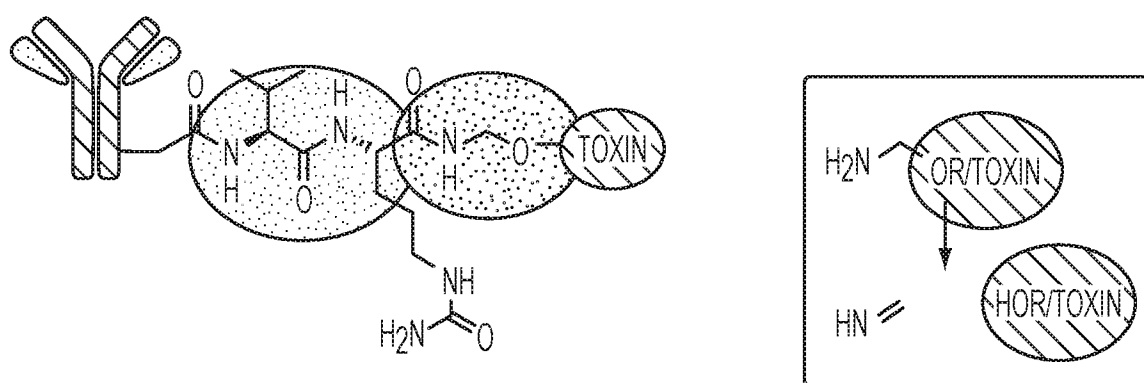


Figure 2

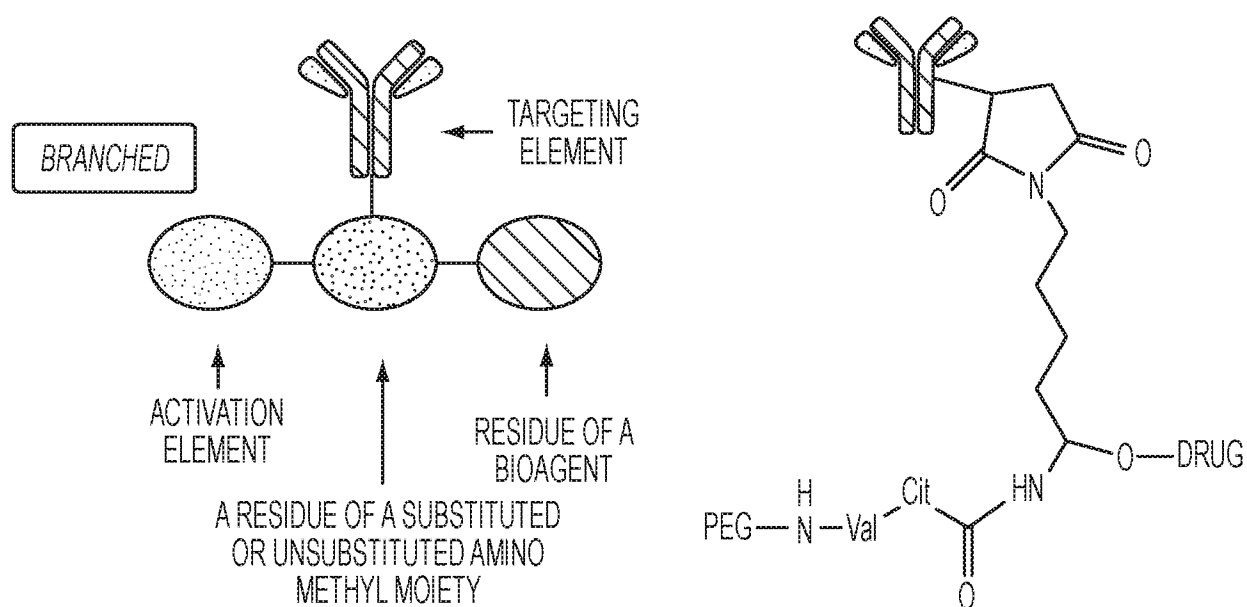


Figure 3A

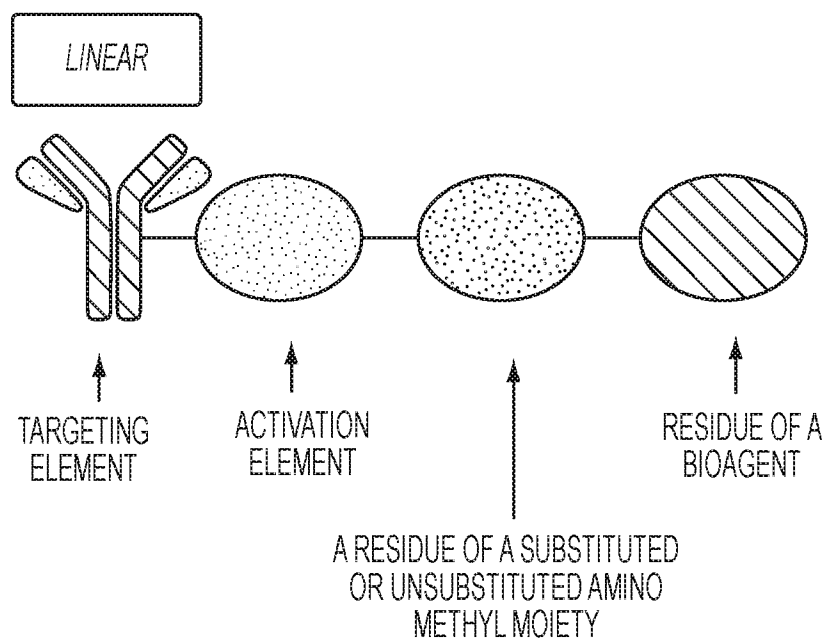
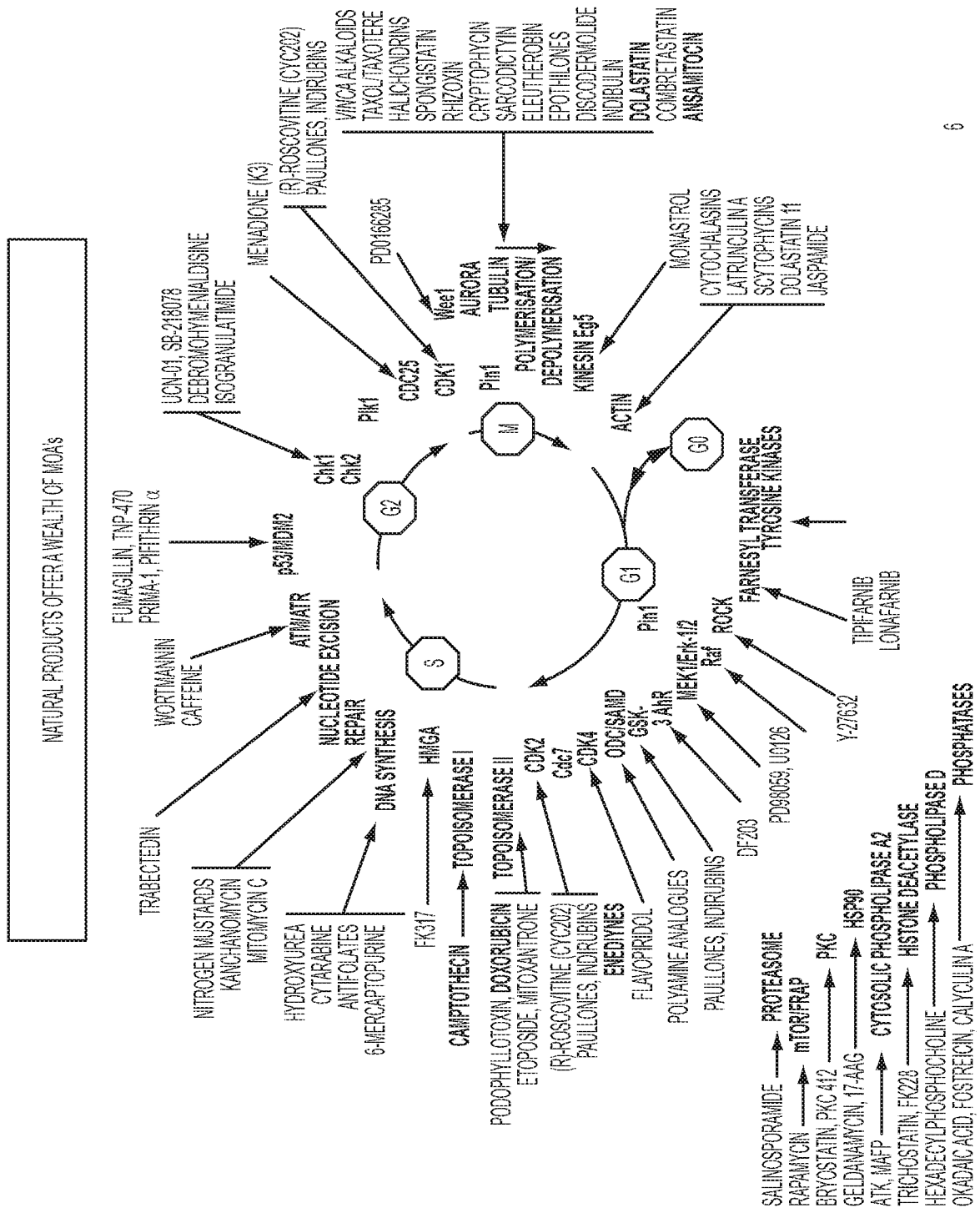


Figure 3B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/51546

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/704; A61K 38/04; A61K 47/48 (2016.01)

CPC - A61K47/48376; C07K16/32; A61K31/704

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61K 31/704; A61K 38/04; A61K 47/48 (2016.01)

CPC: A61K47/48376; C07K16/32; A61K31/704

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC 530/330; 536/6.4 (Keyword limited, terms below)Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar (NPL); Keywords: targeted conjugate, antibody-drug conjugate, antibody targeting agent, linker auristatin/doxorubicin/dolastatin/taxol/irinotecan/maytansinoids

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0145036 A1 (Sufi et al.) 10 June 2010 (10.06.2010) para [0004], [0015], [0019]-[0021], [0070]-[0072], [0076]-[0082], [0086], [0091], [0280]	1-3, 7-11, 27-28, 74, 76 ----- 41-43
Y	US 2011/0150908 A1 (Law et al.) 23 June 2011 (23.06.2011) para [0024], [0025], [0026], [0296]	41-43
A	US 2006/0182750 A1 (Chari et al.) 17 August 2006 (17.08.2006) entire document	1-3, 7-11, 27-28, 41-43, 74 and 76

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 October 2016

Date of mailing of the international search report

29 DEC 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/51546

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-6, 12-26, 29-40, 44-73, 75 and 77-79
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.