

ANTIBODY-DRUG CONJUGATES FOR DELIVERING CYTOTOXIC AGENTS

FIELD OF THE INVENTION

The present invention relates to antibody-drug conjugates for delivering cytotoxic agents.

- 5 Specifically, the present invention relates to antibody-drug conjugates for delivering anti-mitotic agents to cancerous cells.

BACKGROUND OF THE INVENTION

- 10 Antibody-drug conjugates are useful for various purposes, e.g., as diagnostic reagents, therapeutics (e.g., antigen targeted therapeutics), etc. Existing drug antibody conjugation technologies can suffer from various challenges. For example, reactions conjugating moieties of interest (e.g., detection moieties, drug moieties, etc.) to target molecules (e.g., antibodies for antibody-drug conjugates) can be of low efficiency and/or have low selectivity (e.g., conjugation at various locations (e.g., various amino acid residues of antibodies) of target molecules), and product conjugate
- 15 compositions are often highly heterogeneous, including a number of individual conjugate types each independently having its own copy number of moieties of interest, conjugation locations (e.g., different amino acid residues of proteins), etc.

- Approved antibody drug conjugates for delivering for delivering cytotoxic agents to cancer cells include PADCEV (enfortumab vedotin) are ADCETRIS (brentuximab vedotin), both useful for delivering
- 20 monomethyl auristatin E (MMAE). Current drug antibody conjugation technologies include conjugation through lysine residues, conjugation through reduced interchain disulfide bonds, and conjugation through engineered cysteine residues. FIG. 1. Each of these techniques has shortcomings. Conjugation through lysine produces a broad range of drug anti-body ratios (DAR), with each lysine labeled at is statistically probability. The result being millions of possible drug antibody conjugates. High DAR
- 25 species are prone to CMC issues such as aggregation. Some species may easily release their conjugated drug, leading to toxicity. Conjugation through reduced inter-chain disulfide bonds also produces a variety of antibody conjugate species. The drug linkage can reverse over time, releasing free drug. Existing techniques for conjugation through engineered cysteines involved extensive antibody manipulation or engineering.

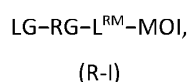
There exists a need for drug an antibody conjugates with predictable DAR and conjugation sites that do not “leak” the conjugated drug, and without the need for extensive antibody engineering. This disclosure fulfills that need and has additional advantages.

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SUMMARY OF THE INVENTION

The present invention is directed to site specific conjugation of antibodies to produce conjugates with high degree of homogeneity.

In an embodiment, provided is a compound having the structure of formula (R-I):

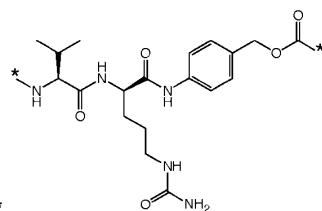


10

or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;

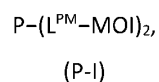


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L^{RM} is a linker including ; and

MOI is moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

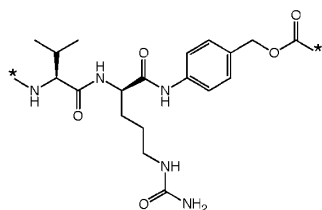
In another embodiment, provided is a method of preparing an agent having the structure of formula (P-I):



20

or a salt thereof, wherein:

P is a brentuximab moiety or a biosimilar analog thereof;

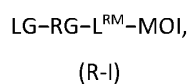


each L^{PM} is a linking group including ; and

each MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

The method may include the steps of:

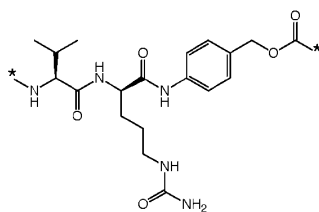
- 5 (1) contacting brentuximab or a biosimilar analog thereof with a reaction partner having the structure of formula (R-I):



or a salt thereof, wherein:

- 10 LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

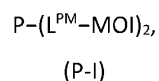
RG is a reactive group;



L^{RM} is a linking group including ;

- 15 MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF), and
- (2) forming an agent having the structure of formula (P-I).

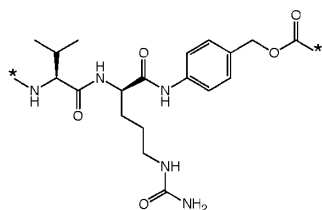
In another embodiment, provide is an agent having the structure of formula (P-I):



20

or a salt thereof, wherein:

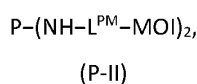
P is a brentuximab moiety or a biosimilar analog thereof;



each L^{PM} is a linking group including

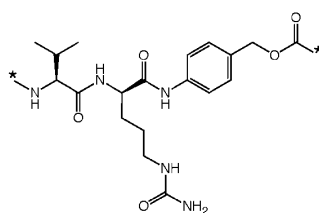
each MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

In another embodiment, provided is a method of preparing an agent having the structure of (P-II):



wherein:

P-NH is a brentuximab moiety or a biosimilar analog thereof including a lysine residue having a terminal group NH;



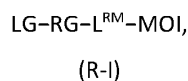
L^{PM} is a linking group including

MOI is or includes monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

The method may include the steps of:

(1) providing brentuximab or a biosimilar analog thereof having a structure $P-(NH_2)_2$, wherein each NH_2 is a terminal amino group of a lysine residue; and

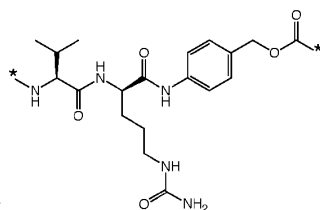
(2) contacting $P-(NH_2)_2$ with a reaction partner having a structure of formula (R-I):



or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;



L^{RM} is a linking group including ; and

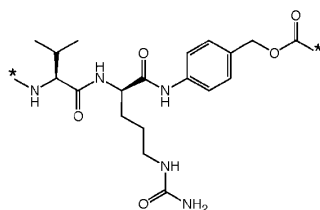
MOI is a moiety of interest monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

In another embodiment, provided is an agent having the structure of (P-II):



wherein:

P-NH is brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH;



10 L^{PM} is a linking group including ; and

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

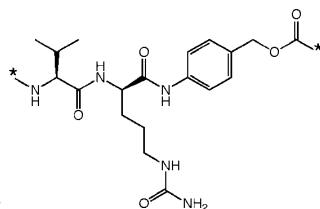
In an embodiment, provided is a compound having the structure of formula (R-I):



or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;

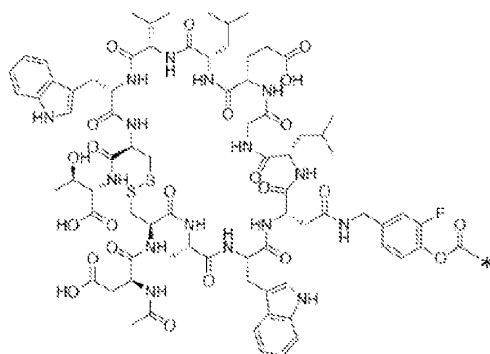


20 L^{RM} is a linking group including ; and

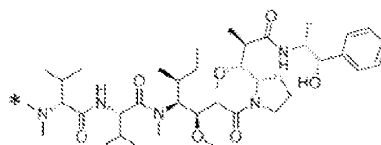
MOI is a moiety of interest including comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF),

wherein brentuximab includes an IgG heavy chain including K246 or K248, and

wherein the target binding moiety is configured to bind brentuximab so as to bring the reactive
 5 group in proximity with K246 or K248 of a brentuximab IgG heavy chain to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety including L^{RM}-MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.



LG-RG may be



MOI may include

10 In another embodiment, provided is a method of treating Hodgkin lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

In another embodiment, provided is a method of treating systemic anaplastic large cell lymphoma in a subject in need of such treatment, including administering to the subject a
 15 pharmaceutically effective amount of the agent described above.

In another embodiment, provided is a method of treating primary cutaneous anaplastic large cell lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

20 In another embodiment, provided is a method of treating CD30-expressing mycosis fungoides in a subject in need of such treatment, including administering to the subject a pharmaceutically effective

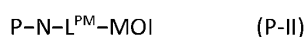
amount of the agent described above.

In another embodiment, provided is a method of treating cutaneous T cell lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

5 In another embodiment, provided is a method of selectively targeting tumor cells expressing the CD30 antigen in a subject, including administering to the subject the agent described above.

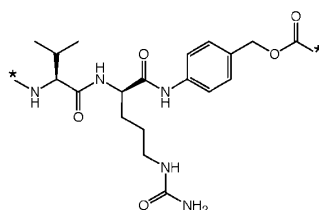
In another embodiment, provided is a composition including:

a first compound having the structure of formula (P-II):



10 wherein:

P-N is brentuximab or a biosimilar analog thereof including a lysine residue;



L^{PM} is a linking group including ; and

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

15 a second compound having the structure:



wherein LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof.

The composition may further include:

20 a third compound having the formula (R-I):



LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof, which is identical to LG in formula (LG-I);

RG is a reactive group;

25 L^{RM} is a linking group, which is identical to that in formula (P-II); and

MOI is a moiety of interest which is identical to that in formula (P-II);

a fourth compound having the formula (R-III):



or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

5 These and/or other aspects will become apparent and more readily appreciated from the following description of the embodiments, taken in conjunction with the accompanying drawings in which:

FIG. 1 illustrates spectral characterization of the conjugate of brentuximab with Reagent A, according to embodiments of the present invention;

10 FIG. 2 illustrates properties of the site-specific antibody-drug conjugates according to embodiments of the present invention;

FIG. 3 illustrates challenges of alternate antibody-drug conjugate protein engineering and chemistry;

15 FIG. 4 illustrates potential best-in-class antibody-drug conjugates according to embodiments of the present invention;

FIG. 5 illustrates pharmacokinetic (PK) studies of Adcetris® and antibody-drug conjugate according to embodiments of the present invention;

FIG. 6 illustrates potential for superior efficacy of an antibody-drug conjugate according to embodiments of the present invention compared to Adcetris®;

20 FIG. 7 illustrates improved survival in a mouse model with an antibody-drug conjugate according to embodiments of the present invention compared to Adcetris®; and

FIG. 8 illustrates results of the single dose 14-day mouse tumor xenograft study with an antibody-drug conjugate according to embodiments of the present invention.

25 DETAILED DESCRIPTION OF THE INVENTION

The following detailed description is provided to aid those skilled in the art in practicing the present invention. Exemplary embodiments will hereinafter be described in detail. However, these embodiments are only exemplary, and the present disclosure is not limited thereto but rather is defined by the scope of the appended claims. Those of ordinary skill in the art may make modifications and
30 variations in the embodiments described herein without departing from the spirit or scope of the present disclosure.

Accordingly, the embodiments are merely described below, by referring to structures and schemes, to explain aspects of the present description. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items. The term "or" means "and/or." Expressions such as "at least one of," when preceding a list of elements, modify the entire list of
5 elements and do not modify the individual elements of the list.

It will be understood that when an element is referred to as being "on" another element, it can be directly in contact with the other element or intervening elements may be present therebetween. In contrast, when an element is referred to as being "directly on" another element, there are no intervening elements present.

10 It will be understood that, although the terms first, second, third etc. may be used herein to describe various elements, components, regions, layers, and/or sections, these elements, components, regions, layers, and/or sections should not be limited by these terms. These terms are only used to distinguish one element, component, region, layer, or section from another element, component, region, layer, or section. Thus, a first element, component, region, layer, or section discussed below
15 could be termed a second element, component, region, layer, or section without departing from the teachings of the present embodiments.

It is understood that the terms "comprises" and/or "comprising," or "includes" and/or "including" when used in this specification, specify the presence of stated features, regions, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or
20 more other features, regions, integers, steps, operations, elements, components, and/or groups thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description is for describing particular embodiments only and is not intended to
25 be limiting. It will be further understood that the terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and the present disclosure, and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

As used in this application, except as otherwise expressly provided herein, each of the following
30 terms shall have the meaning set forth below. Additional definitions are set forth throughout the application. In instances where a term is not specifically defined herein, that term is given an art-

recognized meaning by those of ordinary skill applying that term in context to its use in describing the present invention.

The articles "a" and "an" refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article unless the context clearly indicates otherwise. By way of example, "an element" means one element or more than one element.

As used herein, when specific definition is not otherwise provided, the term "substituted" refers to a group substituted with deuterium, a halogen (-F, -Cl, -Br, -I), a hydroxy group (-OH), an amino group (-NH₂), a carboxyl group (-CO₂H), a substituted or unsubstituted C1-C10 amine group, a nitro group (-NO₂), a C1-C10 alkyl group, a C3-C10 cycloalkyl group, a C6-C12 aryl group, a C1-C10 alkoxy group, a C1 to C10 trifluoroalkyl group such as a trifluoromethyl group (-CF₃) and the like, or a cyano group (-CN) instead of at least one hydrogen of a substituting group or compound.

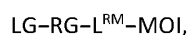
As used herein, the term "brentuximab" refers to a CD30-directed antibody, cAC10. As used herein, the term "biosimilar analogue of brentuximab" or "biosimilar analogue thereof" refers to a composition of matter that is almost an identical copy of brentuximab that is officially approved by a regulatory agency in any country or jurisdiction around the world.

Additional aspects will be set forth in part in the description which follows and, in part, will be apparent from the description.

The starting materials useful for making the pharmaceutical compositions of the present invention are readily commercially available or can be prepared by those skilled in the art.

Solvates are compounds formed by solvation, which is the combination of solvent molecules with molecules or ions of the solute. Solvation is an interaction of a solute with the solvent, which leads to stabilization of the solute species in the solution. One may also refer to the solvated state, whereby an ion in a solution is complexed by solvent molecules. The difference in the physical properties of different solvates and polymorphic forms thereof results from different orientation and intermolecular interactions of adjacent molecules in the solid. Polymorphic forms of compounds or solvates can be distinguished by X-ray diffraction and by other methods such as, infrared spectroscopy or Raman spectroscopy.

In an embodiment, provided is a compound having the structure of formula (R-I):

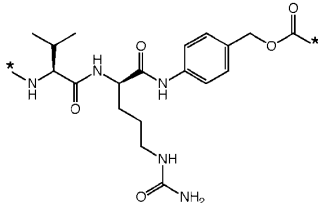


(R-I)

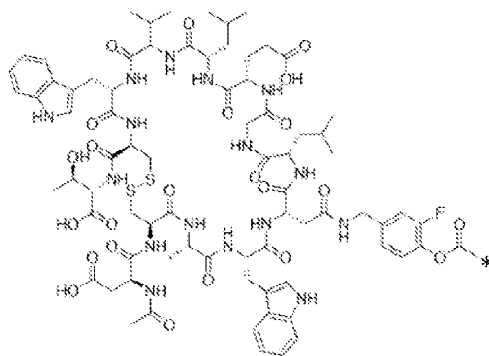
or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

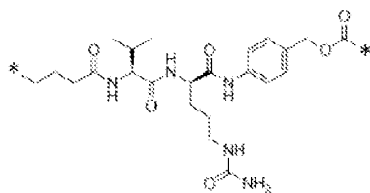
RG is a reactive group;

5 L^{RM} is a linking group including ; and

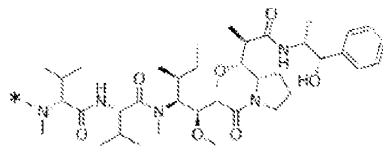
MOI is moiety of interest including monomethyl auristatin D, monomethyl auristatin E, or monomethyl auristatin F.



LG-RG may be or include



10 L^{RM} may be or include

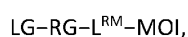


MOI may be or include

The target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that may result in attachment of a moiety including L^{RM}-MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

The target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety including L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

In another embodiment, provided is a compound having the structure of formula (R-I):

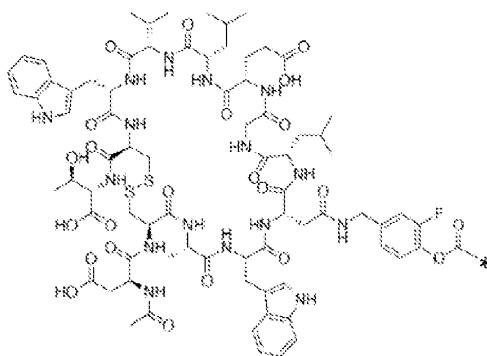


(R-I)

or a salt thereof, wherein:

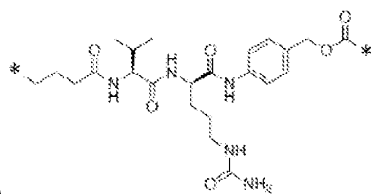
LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;



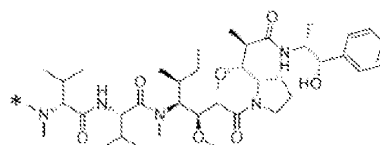
LG-RG may be or include

;



L^{RM} may be or include

; and



MOI may be or include

The target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that may result in attachment of a moiety including L^{RM} -MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

The target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that may result in attachment of a moiety including L^{RM} -MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

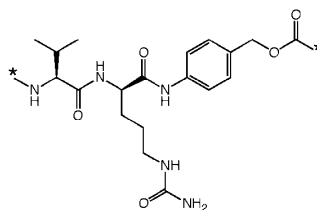
In another embodiment, provided is a method of preparing an agent having the structure of formula (P-I):

$P-(L^{PM}-MOI)_2$,

(P-I)

or a salt thereof, wherein:

P is brentuximab or a biosimilar analog thereof;



each L^{PM} is a linking group including

; and

each MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF),

the method including the steps of:

- (1) contacting brentuximab or a biosimilar analog thereof with a reaction partner having the structure of formula (R-I):

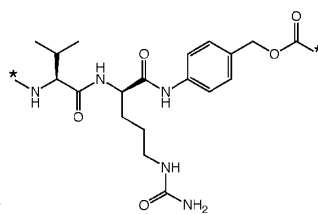
LG-RG-L^{RM}-MOI,

(R-I)

or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;

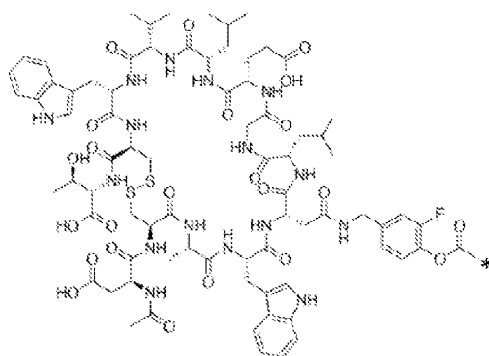


L^{RM} is a linking group including

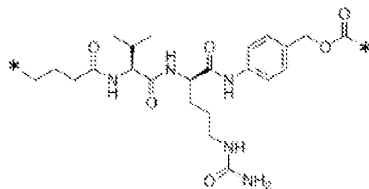
;

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF), and

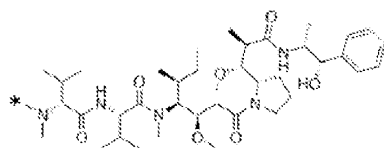
- (2) forming an agent having the structure of formula (P-I).



LG-RG may be or include



L^{RM} may be or include



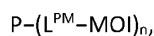
MOI may be or include

5 The target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that may result in attachment of a moiety including L^{RM} -MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

10 The target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety including L^{RM} -MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

15 The contacting and forming steps may be performed in one chemical reaction.

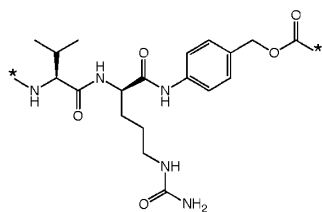
In another embodiment, provided is an agent having the structure of formula (P-I):



(P-I)

or a salt thereof, wherein:

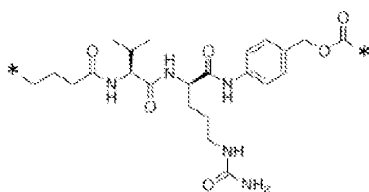
20 P is a brentuximab or a biosimilar analog thereof;



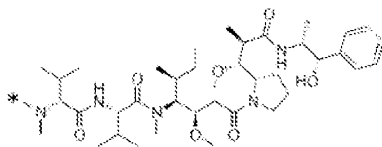
each L^{PM} is a linking group including

each MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

n is 1, 2, 3, or 4.

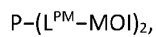


5 L^{RM} may be or include



MOI may include

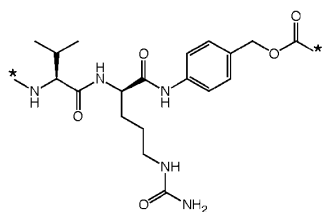
In another embodiment, provided is an agent having the structure of formula (P-I):



(P-1)

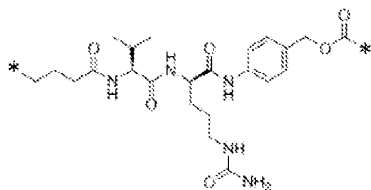
10 or a salt thereof, wherein:

P is a brentuximab or a biosimilar analog thereof;

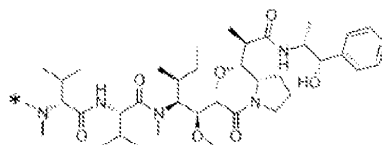


each L^{PM} is a linking group including

each MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).



L^{RM} may be or include

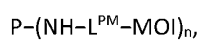


MOI may include

Each L^{PM} -MOI may be attached to K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

Each L^{PM} -MOI may be attached to K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

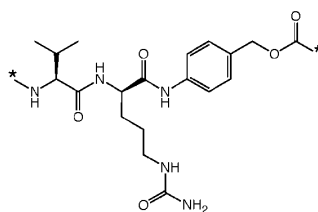
In another embodiment, provided is a method of preparing an agent having the structure of (P-II):



(P-II)

wherein:

P-NH is a brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH;



L^{PM} is a linking group including

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

n is 1, 2, 3, or 4,

the method including the steps of:

(1) providing brentuximab or a biosimilar analog thereof having a structure $P-(NH_2)_2$, wherein each NH_2 is a terminal amino group of a lysine residue; and

(2) contacting $P-(NH_2)_n$ with a reaction partner having a structure of formula (R-I):

$LG-RG-L^{RM}-MOI$,

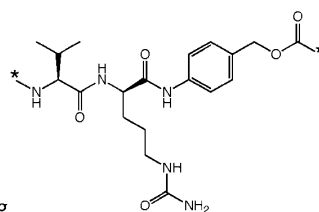
5 (R-I)

or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;

10



L^{RM} is a linking group including ;

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

n is 1, 2, 3, and 4.

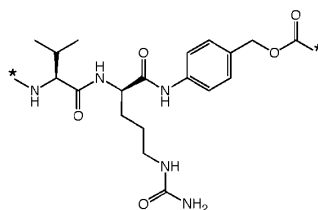
15 In another embodiment, provided is a method of preparing an agent having the structure of (P-II):

$P-(NH-L^{PM}-MOI)_2$,

(P-II)

wherein:

20 P-NH is a brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH_2 ;



L^{PM} is a linking group including ; and

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF),

the method including the steps of:

5 (1) providing brentuximab or a biosimilar analog thereof having a structure $P-(NH_2)_2$, wherein each NH_2 is a terminal amino group of a lysine residue; and

(2) contacting $P-(NH_2)_2$ with a reaction partner having a structure of formula (R-I):

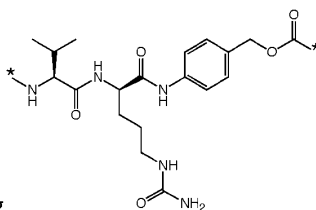
$LG-RG-L^{RM}-MOI$,

(R-I)

10 or a salt thereof, wherein:

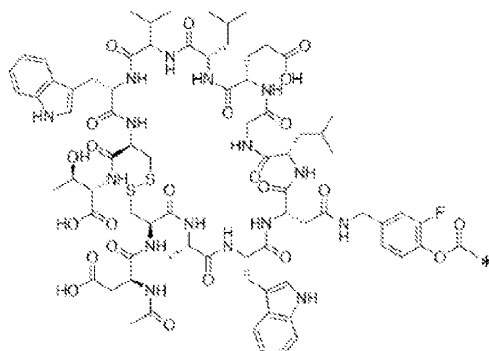
LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;

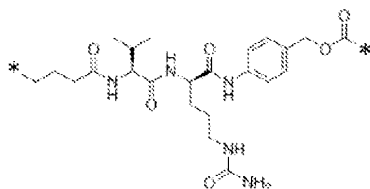


15 L^{RM} is a linking group including ; and

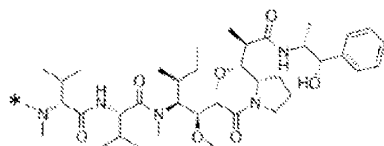
MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).



LG-RG may be or include



L^{RM} may be or include



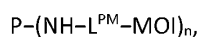
MOI may be or include

- The target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that may result in attachment of a moiety including L^{RM}-MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

- The target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety including L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

The contacting and forming steps may be performed in one chemical reaction.

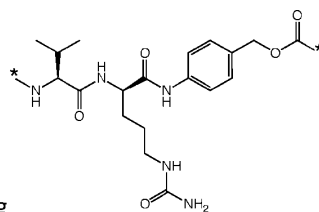
- In another embodiment, provided is an agent having the structure of (P-II):



(P-II)

wherein:

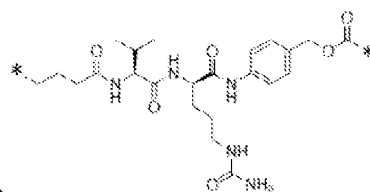
P-NH is a brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH;



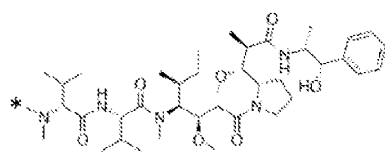
5 L^{PM} is a linking group including

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

n is 1, 2, 3, or 4.

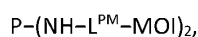


L^{RM} may be or include



10 MOI may be or include

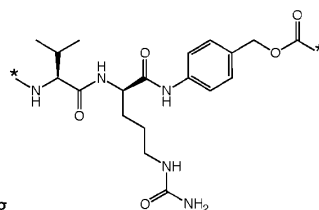
In another embodiment, provided is an agent having the structure of (P-II):



(P-II)

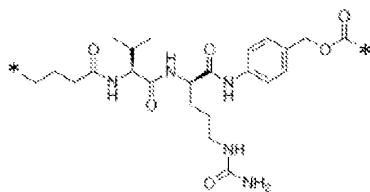
wherein:

15 P-NH is a brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH;

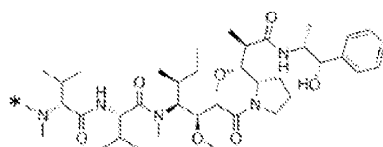


L^{PM} is a linking group including ; and

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).



L^{RM} may be or include



5 MOI may be or include

The lysine residue may be K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

Each lysine residue may be K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

In another embodiment, provided is a compound having the structure of formula (R-1):

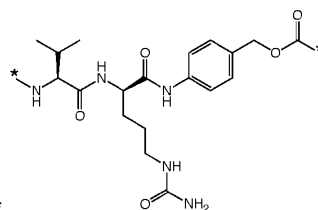
10 LG-RG-L^{RM}-MOI,

(R-1)

or a salt thereof, wherein:

LG may be a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

15 RG may be a reactive group;

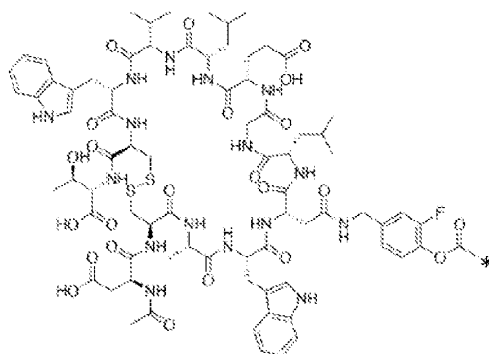


L^{RM} may be a linking group including ; and

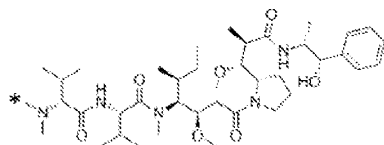
MOI may be a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF),

wherein brentuximab or a biosimilar analog thereof may include an IgG heavy chain including K246 or K248, and

wherein the target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety including L^{RM} -MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.



LG-RG may be or include .



MOI may be or include .

The target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that may result in attachment

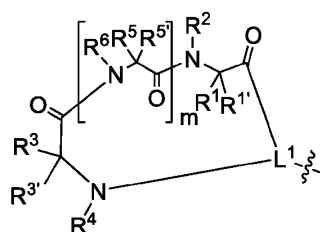
of a moiety including L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

TARGET BINDING MOIETY

5 In some embodiments, target binding moieties are antibody binding moieties. In some
embodiments, provided compounds and/or agents include antibody binding moieties. Various antibody
binding moieties can be utilized in accordance with the present disclosure. In some embodiments,
antibody binding moieties are universal antibody binding moieties which can bind to antibodies having
different Fab regions and different specificity. Among other things, compounds including such antibody
10 binding moieties may be utilized for conjugation with antibodies having different specificity. In some
embodiments, antibody binding moieties of the present disclosure, e.g., universal antibody binding
moieties, bind to Fc regions. In some embodiments, the antibody binding moieties are capable of
binding to brentuximab or a biosimilar analog thereof.

Various antibody binding moieties including universal antibody binding moieties can be utilized in accordance with the present disclosure. Certain antibody binding moieties and technologies for identifying and/or assessing antibody binding moieties are described in WO 2019/023501 A1 and WO 2019/136442 A1, and are incorporated herein by reference. Those skilled in the art appreciate that additional technologies in the art may be suitable for identifying and/or assessing antibody binding moieties in accordance with the present disclosure. In some embodiments, an antibody binding moiety includes one or more amino acid residues, each independently natural or unnatural.

In some embodiments, a target binding moiety, e.g., a protein binding moiety (e.g., an antibody binding moiety (e.g., a universal antibody binding moiety)), has the structure of



or a salt form thereof, wherein:

each of R¹, R³ and R⁵ is independently hydrogen or an optionally substituted group selected from C₁₋₆ aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially

unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or:

5

R^1 and $R^{1'}$ are optionally taken together with their intervening carbon atom to form a 3-8 membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8 membered saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

10

R^3 and $R^{3'}$ are optionally taken together with their intervening carbon atom to form a 3-8 membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8 membered saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

15

an R^5 group and the $R^{5'}$ group attached to the same carbon atom are optionally taken together with their intervening carbon atom to form a 3-8 membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8 membered saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or

20

two R^5 groups are optionally taken together with their intervening atoms to form a C_{1-10} optionally substituted bivalent straight or branched saturated or unsaturated hydrocarbon chain wherein 1-3 methylene units of the chain are independently and optionally replaced with $-S-$, $-SS-$, $-N(R)-$, $-O-$, $-C(O)-$, $-OC(O)-$, $-C(O)O-$, $-C(O)N(R)-$, $-N(R)C(O)-$, $-S(O)-$, $-S(O)_2-$, or $-Cy^1-$, wherein each $-Cy^1-$ is independently a 5-6 membered heteroarylenyl with 1-4 heteroatoms independently selected from nitrogen, oxygen or sulfur;

25

each of $R^{1'}$, $R^{3'}$ and $R^{5'}$ is independently hydrogen or optionally substituted C_{1-3} aliphatic;

each of R^2 , R^4 and R^6 is independently hydrogen, or optionally substituted C_{1-4} aliphatic, or:

R^2 and R^1 are optionally taken together with their intervening atoms to form a 4-8 membered, optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

30

R^4 and R^3 are optionally taken together with their intervening atoms to form a 4-8 membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or

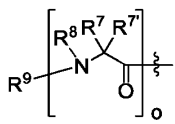
an R⁶ group and its adjacent R⁵ group are optionally taken together with their intervening atoms to form a 4-8 membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

5 L¹ is a trivalent linker moiety; and

each of m and n is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

In some embodiments, L¹ is an optionally substituted trivalent group selected from C₁-C₂₀ aliphatic or C₁-C₂₀ heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the
 10 group are optionally and independently replaced with -C(R')₂-, -Cy-, -O-, -S-, -S-S-, -N(R')-, -C(O)-, -C(S)-, -C(NR')-, -C(O)N(R')-, -N(R')C(O)N(R')-, -N(R')C(O)O-, -S(O)-, -S(O)₂-, -S(O)₂N(R')-, -C(O)S-, or -C(O)O-.

In some embodiments, a target binding moiety, e.g. a protein binding moiety (e.g., an antibody

binding moiety (e.g., a universal antibody binding moiety)), has the structure of  or a

15 salt form thereof, wherein:

each of R⁷ is independently hydrogen or an optionally substituted group selected from C₁₋₆ aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or:

25 an R⁷ group and the R^{7'} group attached to the same carbon atom are optionally taken together with their intervening carbon atom to form a 3-8 membered optionally substituted saturated or partially unsaturated spirocyclic carbocyclic ring or a 3-8 membered optionally substituted saturated or partially unsaturated spirocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

each of R^{7'} is independently hydrogen or optionally substituted C₁₋₃ aliphatic;

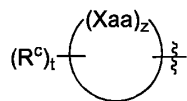
each of R^8 is independently hydrogen, or optionally substituted C_{1-4} aliphatic, or:

an R^8 group and its adjacent R^7 group are optionally taken together with their intervening atoms to form a 4-8 membered optionally substituted saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

R^9 is hydrogen, optionally substituted C_{1-3} aliphatic, or $-C(O)-$.

In some embodiments, an antibody binding moiety, e.g., a universal antibody binding moiety is or includes a peptide moiety, e.g., a moiety having the structure of $R^c-(Xaa)_z-$ or a salt form thereof, wherein each of R^c , z and Xaa is independently as described herein. In some embodiments, one or more Xaa are independently an unnatural amino acid residue. In some embodiments, side chains of two or more amino acid residues may be linked together to form bridges. For example, in some embodiments, side chains of two cysteine residues may form a disulfide bridge including $-S-S-$ (which, as in many proteins, can be formed by two $-SH$ groups).

In some embodiments, a target binding moiety, e.g. a protein binding moiety (e.g., an antibody binding moiety (e.g., a universal antibody binding moiety)), is or includes a cyclic peptide moiety, e.g., a



moiety having the structure of

or a salt form thereof, wherein:

each Xaa is independently a residue of an amino acid or an amino acid analog;

t is 0-50;

z is 1-50;

L is a linker moiety;

each R^c is independently $-L^a-R'$;

each L^a is independently a covalent bond, or an optionally substituted bivalent group selected from C_1-C_{20} aliphatic or C_1-C_{20} heteroaliphatic having 1-5 heteroatoms, wherein one or more methylene units of the group are optionally and independently replaced with $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, or $-C(O)O-$;

each $-Cy-$ is independently an optionally substituted bivalent monocyclic, bicyclic or polycyclic group wherein each monocyclic ring is independently selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms independently selected from oxygen,

nitrogen, sulfur, phosphorus and silicon, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon;

each R' is independently -R, -C(O)R, -CO₂R, or -SO₂R;

each R is independently -H, or an optionally substituted group selected from C₁₋₃₀ aliphatic, C₁₋₃₀ heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C₆₋₃₀ aryl, C₆₋₃₀ arylaliphatic, C₆₋₃₀ arylheteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, 5-30 membered heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and 3-30 membered heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, or

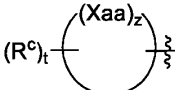
two R groups are optionally and independently taken together to form a covalent bond, or:

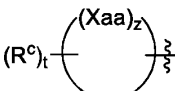
two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon; or

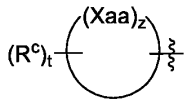
two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

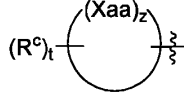
In some embodiments, a heteroatom is independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon.

In some embodiments, a target binding moiety is or includes R^c-(Xaa)_z- or a salt form thereof, wherein each variable is as described herein. In some embodiments, a protein binding moiety is or includes R^c-(Xaa)_z- or a salt form thereof, wherein each variable is as described herein. In some embodiments, an antibody binding moiety, e.g., a universal antibody binding moiety, is or includes R^c-(Xaa)_z- or a salt form thereof, wherein each variable is as described herein. In some embodiments, a

target binding moiety is or includes  or a salt form thereof, wherein each variable is as

described herein. In some embodiments, a protein binding moiety is or includes  or a salt form thereof, wherein each variable is as described herein. In some embodiments, an antibody

binding moiety, e.g., a universal antibody binding moiety, is or includes  or a salt form thereof, wherein each variable is as described herein. In some embodiments, an antibody binding

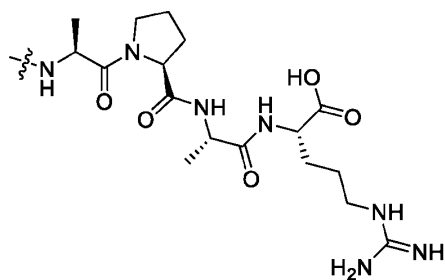
moiety, e.g., a universal antibody binding moiety is $R^c-(Xaa)_z-$ or , or a salt form thereof, and is or includes a peptide unit. In some embodiments, $-(Xaa)_z-$ is or includes a peptide unit.

- 5 In some embodiments, amino acid residues may form bridges, e.g., connections formed by side chains optionally through linker moieties (e.g., L); for example, as in many polypeptides, cysteine residues may form disulfide bridges. In some embodiments, a peptide unit includes an amino acid residue (e.g., at physiological pH about 7.4, “positively charged amino acid residue”, Xaa^P), e.g., a residue of an amino acid of formula **A-I** that has a positively charged side chain. In some embodiments, a peptide unit
- 10 includes R. In some embodiments, at least one Xaa is R. In some embodiments, a peptide unit is or includes APAR. In some embodiments, a peptide unit is or includes RAPA. In some embodiments, a peptide unit includes an amino acid residue, e.g., a residue of an amino acid of formula **A-I**, that has a side chain including an aromatic group (“aromatic amino acid residue”, Xaa^A). In some embodiments, a peptide unit includes a positively charged amino acid residue and an aromatic amino acid residue. In
- 15 some embodiments, a peptide unit includes W. In some embodiments, a peptide unit includes a positively charged amino acid residue and an aromatic amino acid residue. In some embodiments, a peptide unit is or includes $Xaa^AXaa^PXaa^P$. In some embodiments, a peptide unit is or includes $Xaa^PXaa^PXaa^A$. In some embodiments, a peptide unit is or includes $Xaa^PXaa^AXaa^P$. In some
- 20 embodiments, a peptide unit is or includes two or more $Xaa^PXaa^AXaa^P$. In some embodiments, a peptide unit is or includes $Xaa^PXaa^AXaa^PXaa^PXaa^AXaa^P$. In some embodiments, a peptide unit is or includes $Xaa^PXaa^PXaa^PXaa^A$. In some embodiments, a peptide unit is or includes two or more $Xaa^AXaa^AXaa^P$. In some embodiments, a peptide residue includes one or more proline residues.

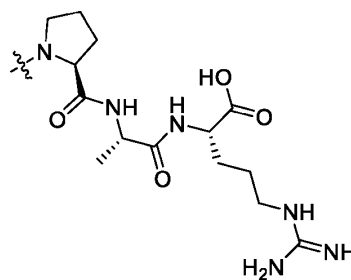
- In some embodiments, a target binding moiety is or includes optionally substituted moiety of
- 25 Table A-1. In some embodiments, a protein binding moiety is or includes optionally substituted moiety of Table A-1. In some embodiments, an antibody binding moiety, e.g., a universal antibody binding moiety, is or includes optionally substituted moiety of Table A-1. In some embodiments, a target binding moiety is selected from able A-1. In some embodiments, a protein binding moiety is selected from able

A-1. In some embodiments, an antibody binding moiety, e.g., a universal antibody binding moiety, is selected from able A-1. In some embodiments, C-terminus and/or N-terminus are optionally capped (e.g., for C-terminus, by converting $-\text{COOH}$ into $-\text{C}(\text{O})\text{N}(\text{R}')_2$ like $-\text{C}(\text{O})\text{NH}_2$; for N-terminus, by adding $\text{R}'\text{C}(\text{O})-$ like $\text{CH}_3\text{C}(\text{O})-$ to an amino group).

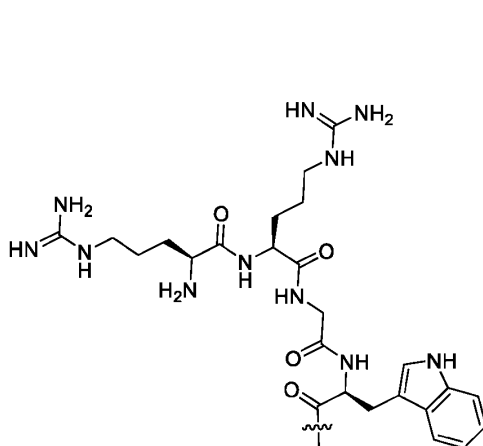
5 Table A-1. Exemplary antibody binding moieties.



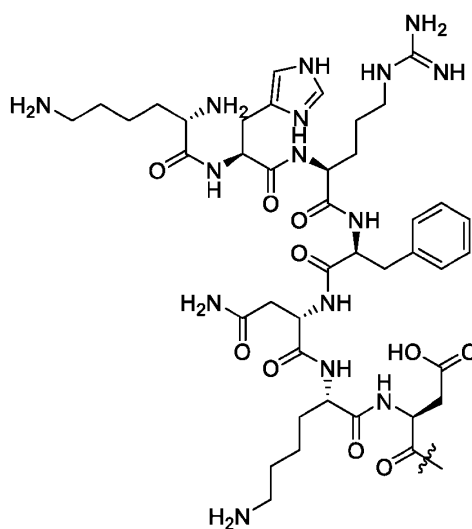
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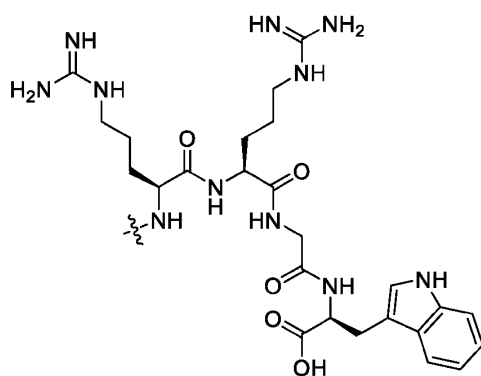
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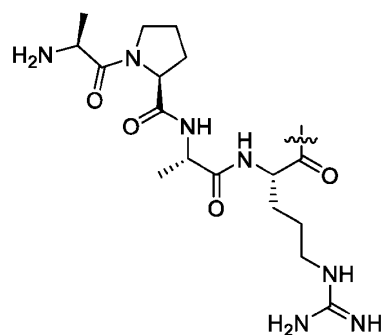
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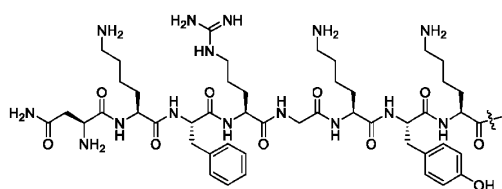
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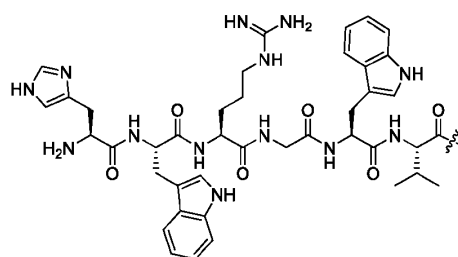
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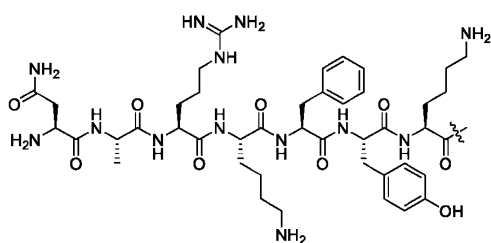
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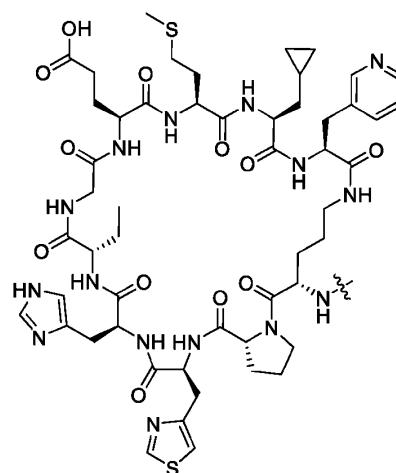
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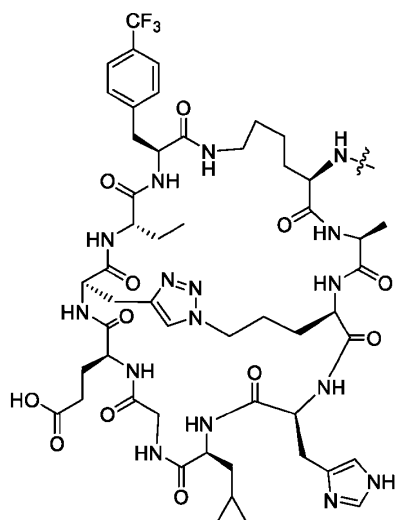
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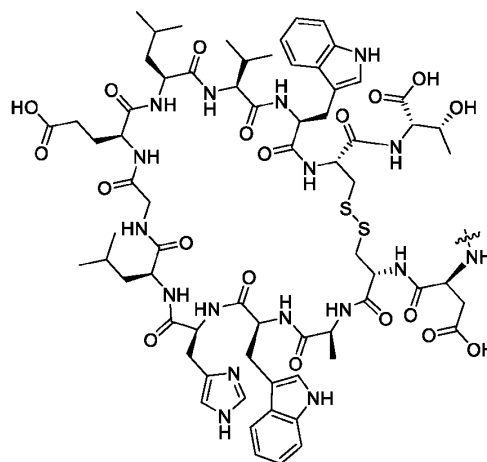
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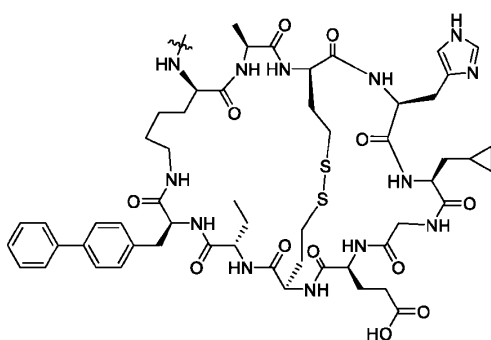
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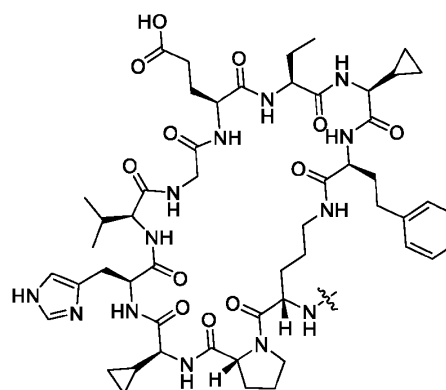
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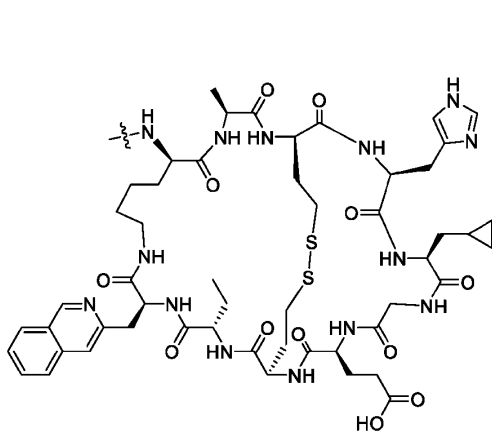
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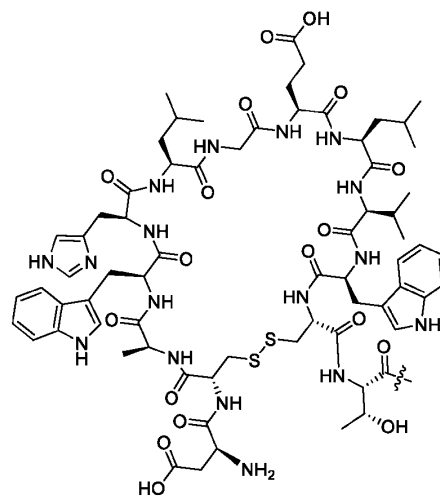
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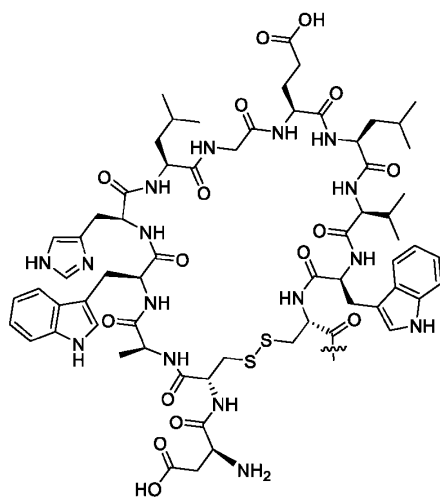
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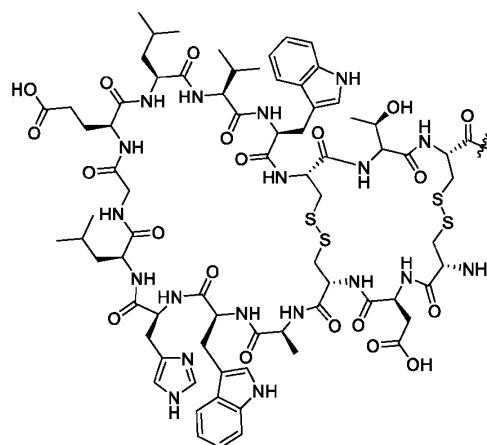
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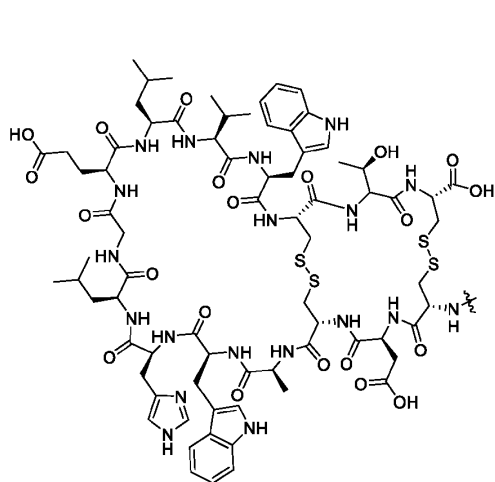
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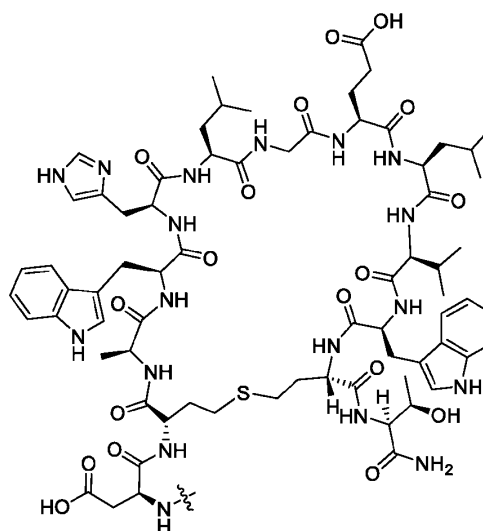
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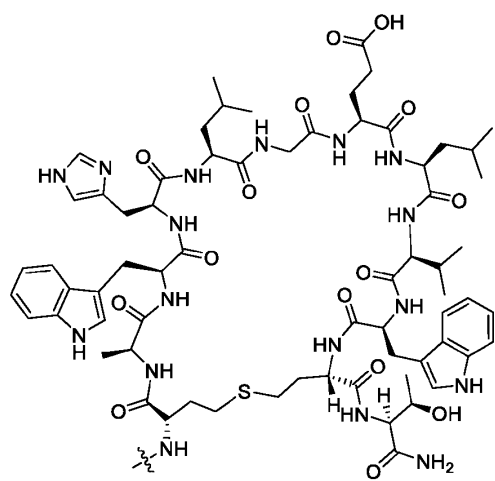
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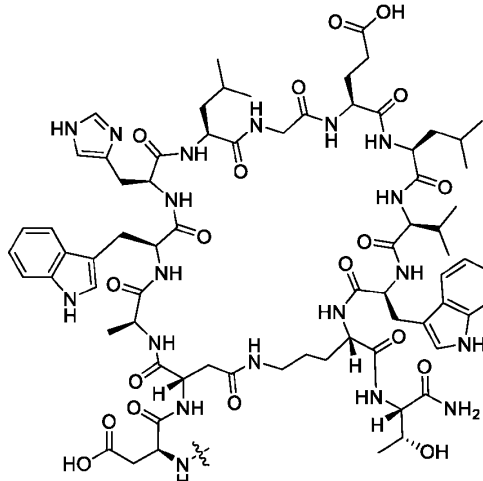
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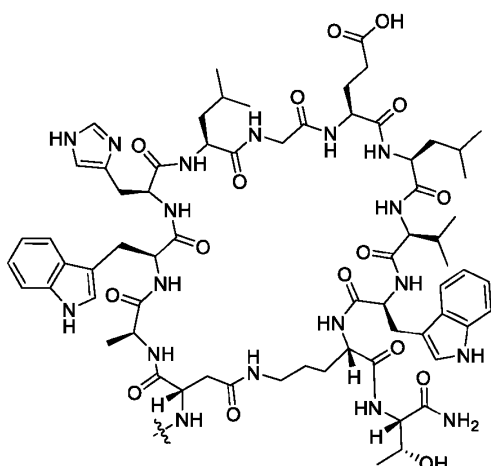
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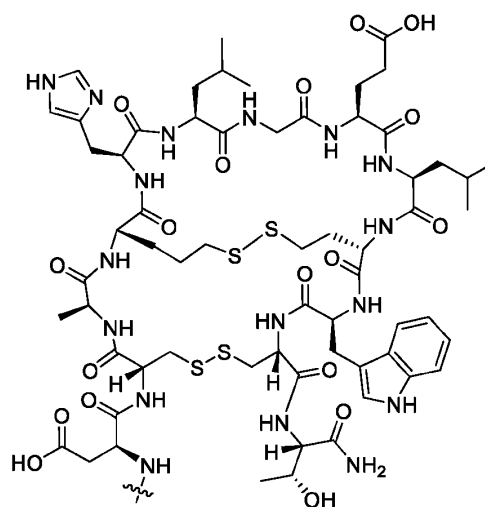
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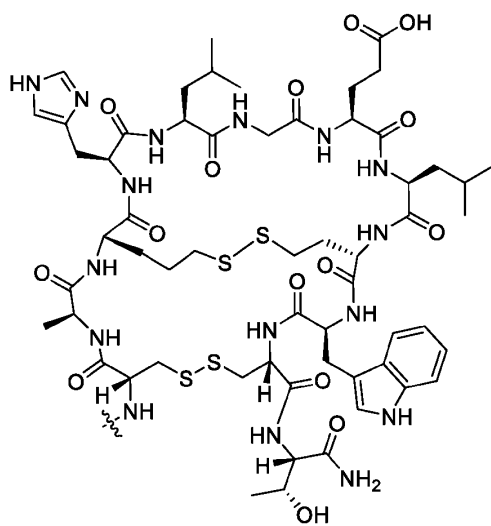
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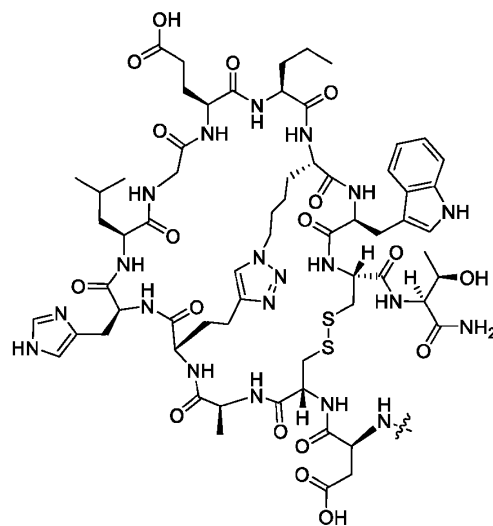
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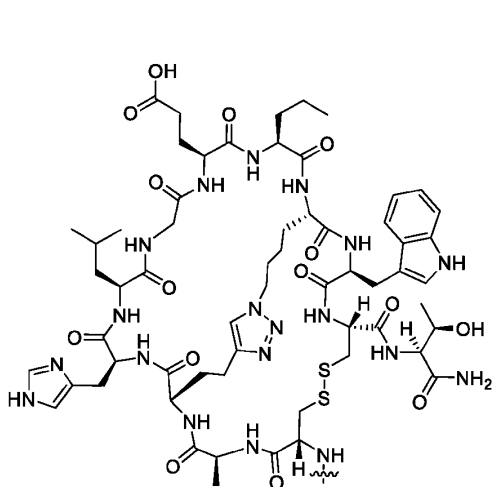
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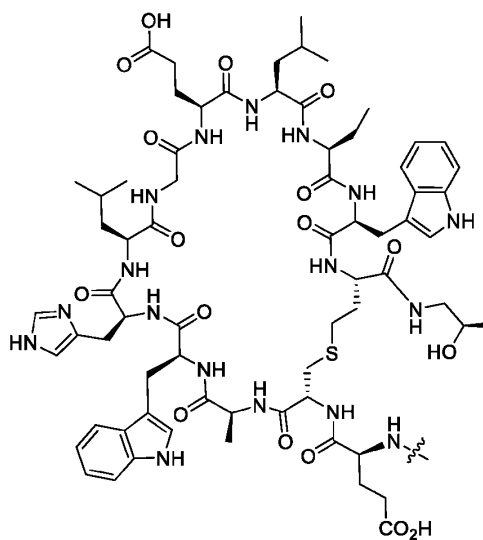
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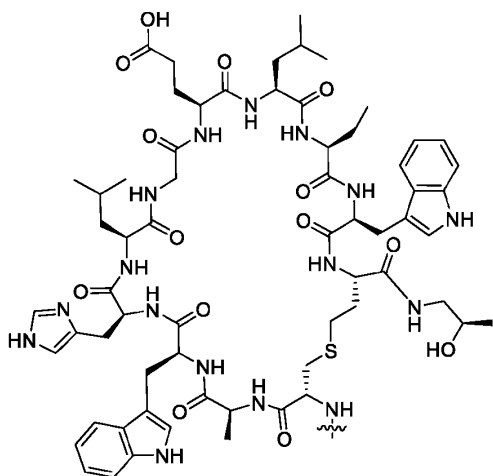
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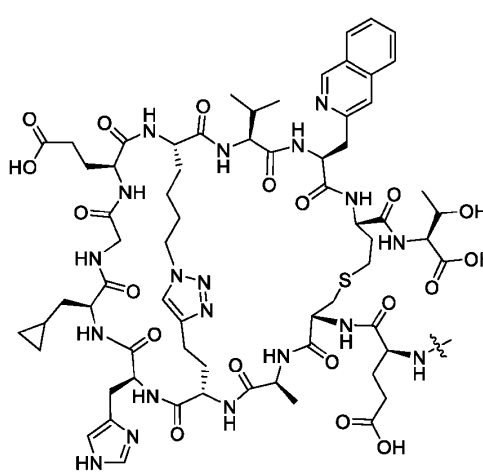
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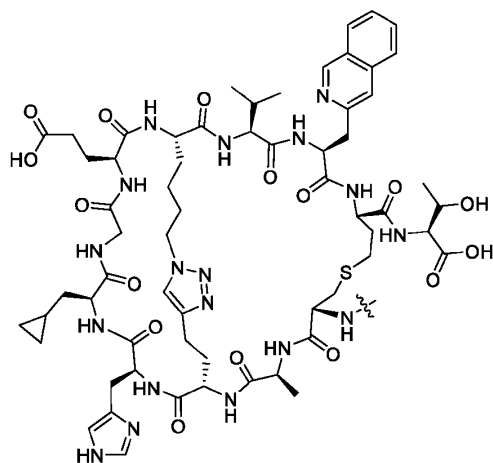
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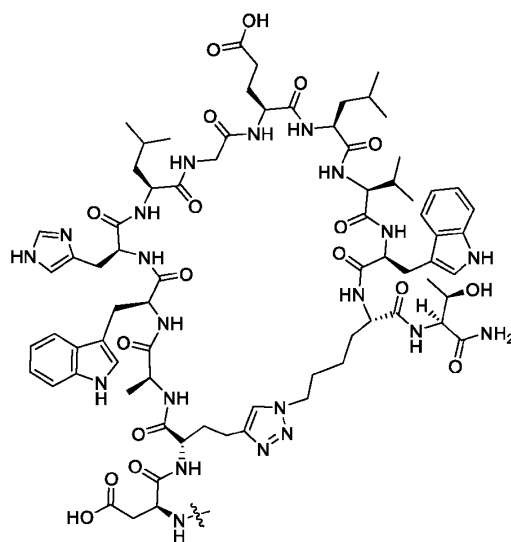
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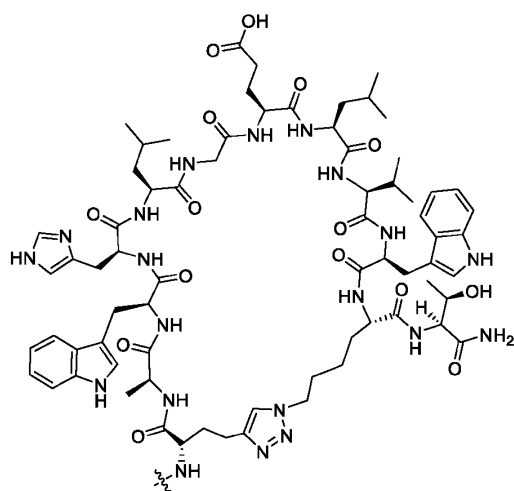
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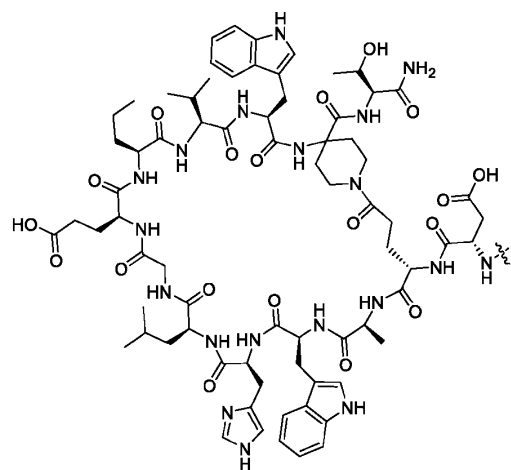
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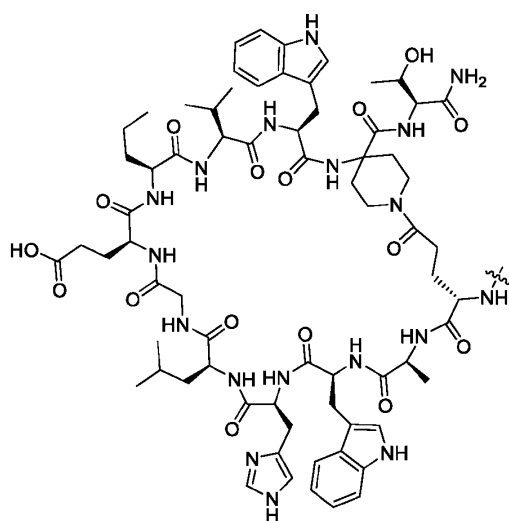
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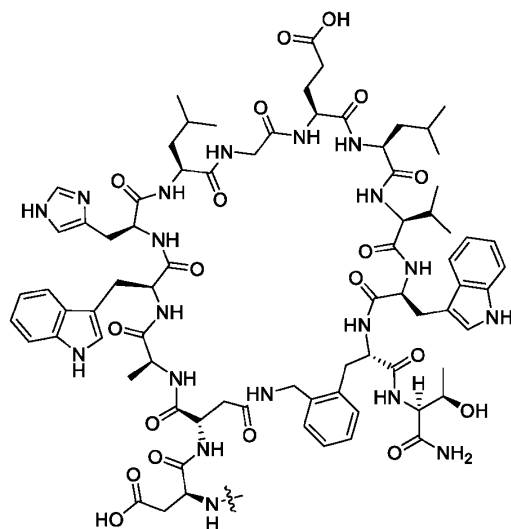
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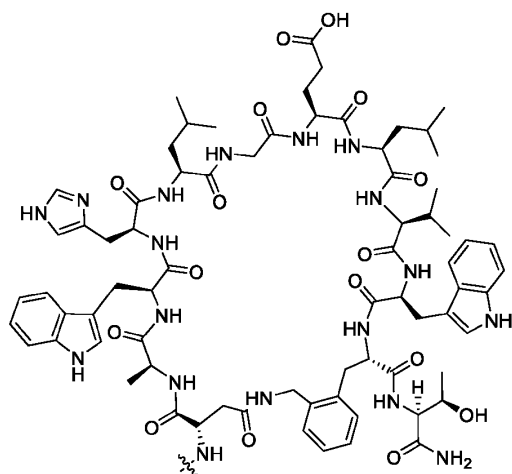
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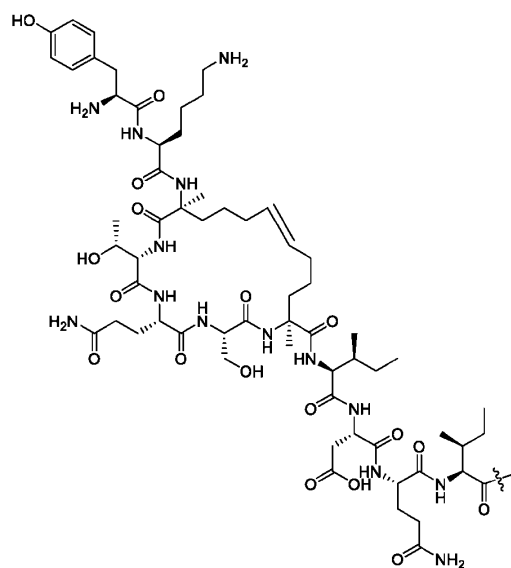
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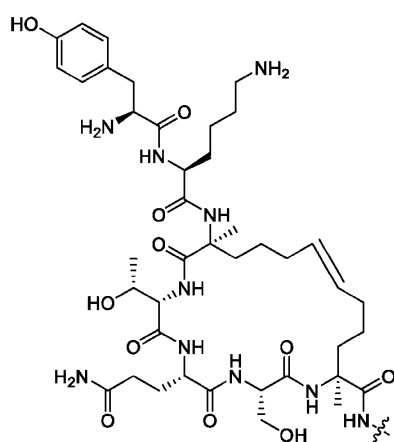
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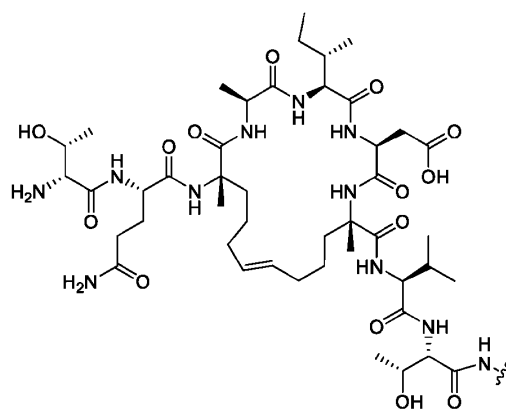
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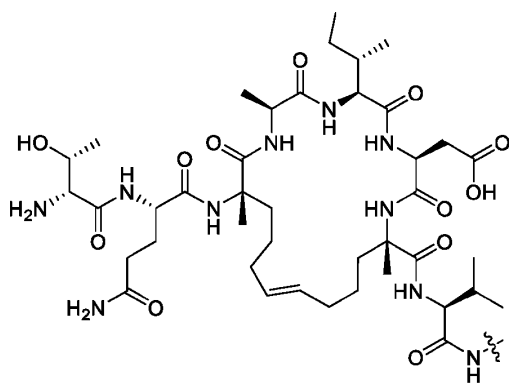
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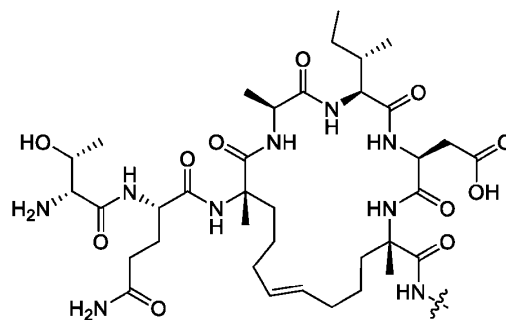
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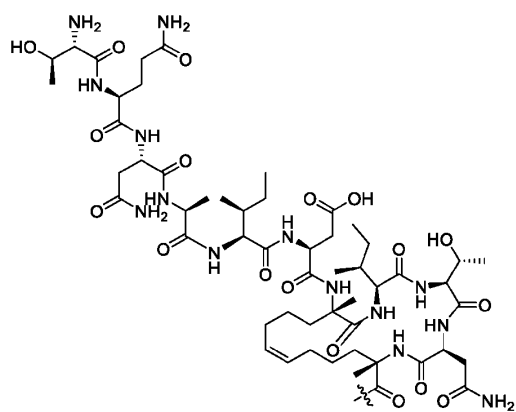
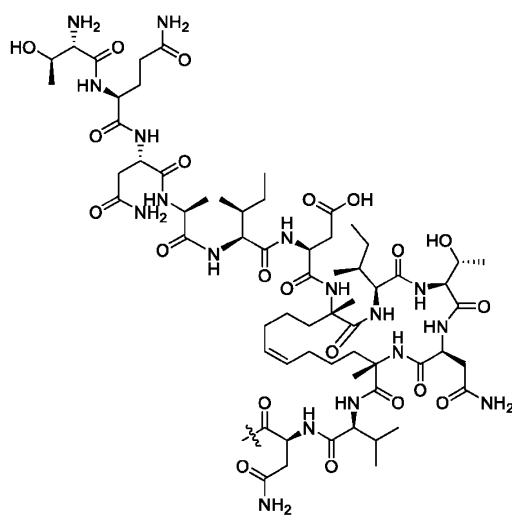
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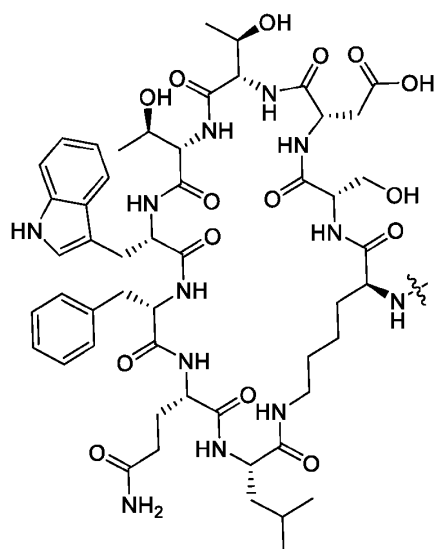
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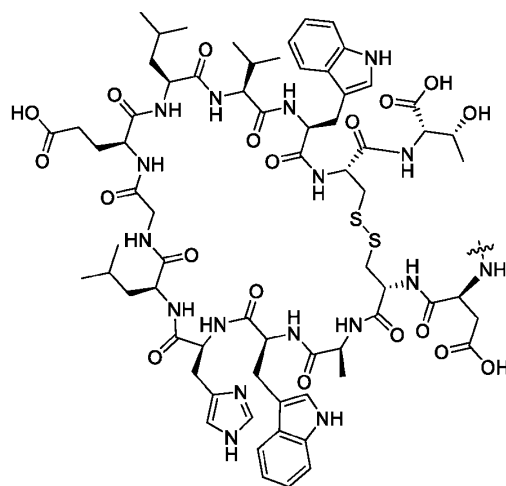
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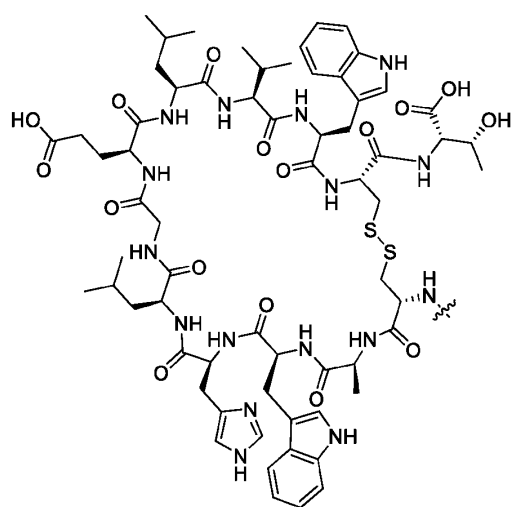
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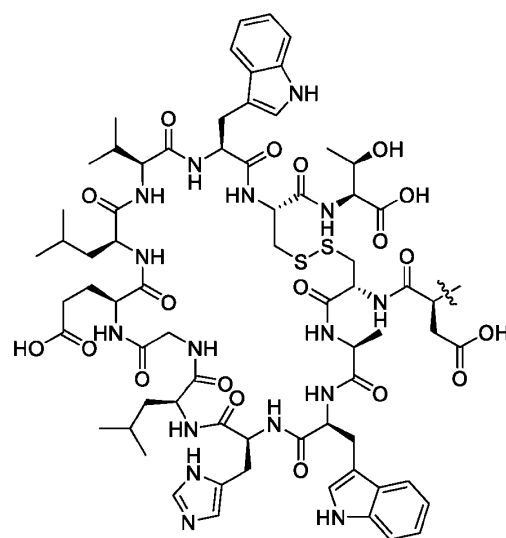
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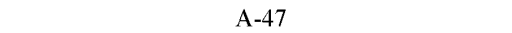
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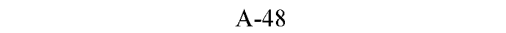
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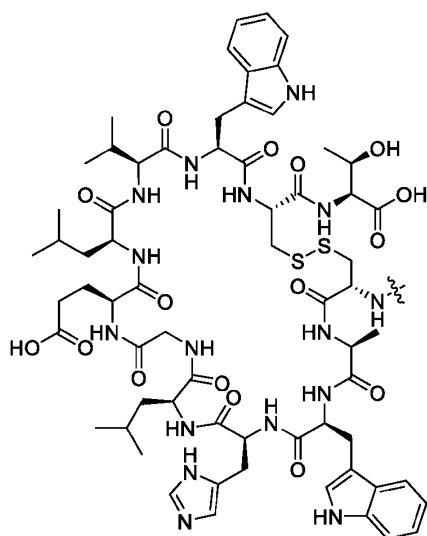


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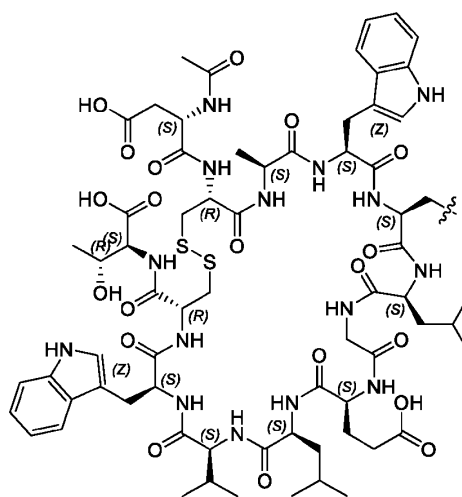


A-48





A-49



A-50

In some embodiments, a target binding moiety is an antibody binding moiety described herein. In some embodiments, a protein binding moiety is an antibody binding moiety described herein. In some embodiments, -COOH and/or amino groups of amino acid residues, e.g., those at the C-terminus or N-terminus, is optionally capped. For example, in some embodiments, a -COOH group (e.g., a C-terminus -COOH) is amidated (e.g., converted into -CON(R')_2 , e.g., -C(O)NHR (e.g., -C(O)NH_2)), and in some embodiments, an amino group, e.g. -NH_2 (e.g., a N-terminus -NH_2) is capped with R' or R'C(O)- (e.g., in some embodiments, by conversion -NH_2 into -NHR' (e.g., -NHC(O)R , (e.g., -NHC(O)CH_3)).

In some embodiments, a target binding moiety is or includes optionally substituted A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, A-21, A-22, A-23, A-24, A-25, A-26, A-27, A-28, A-29, A-30, A-31, A-32, A-33, A-34, A-35, A-36, A-37, A-38, A-39, A-40, A-41, A-42, A-43, A-44, A-45, A-46, A-47, A-48, A-49, or A-50, each of which is optionally substituted. In some embodiments, such a target binding moiety is an antibody binding moiety. In some embodiments, such a target binding moiety is a universal antibody binding moiety.

In some embodiments, a target binding moiety, e.g., a protein binding moiety (e.g., an antibody binding moiety (e.g., a universal antibody binding moiety)) includes a peptide unit, and is connected to a linker moiety through the C-terminus of the peptide unit. In some embodiments, it is connected to a linker moiety through the N-terminus of the peptide unit. In some embodiments, it is connected to a

linker through a side chain group of the peptide unit. In some embodiments, an antibody binding moiety, e.g., a universal antibody binding moiety includes a peptide unit, and is connected to a target binding moiety optionally through a linker moiety through the C-terminus of the peptide unit. In some embodiments, a target binding moiety, e.g., a protein binding moiety (e.g., an antibody binding moiety (e.g., a universal antibody binding moiety)) includes a peptide unit, and is connected to a target binding moiety optionally through a linker moiety through the N-terminus of the peptide unit. In some embodiments, In some embodiments, a target binding moiety, e.g., a protein binding moiety (e.g., an antibody binding moiety (e.g., a universal antibody binding moiety)) includes a peptide unit, and is connected to a target binding moiety optionally through a linker moiety through a side chain of the peptide unit.

REACTIVE GROUP

In some embodiments, provided compounds, e.g., those useful as reaction partners, include reactive groups (e.g., RG). As exemplified herein, in many embodiments, in provided compounds reactive groups (e.g., RG) are located between first groups (e.g., LG) and moieties of interest (e.g., MOI), and are optionally and independently linked to first groups and moieties of interest via linkers. In some embodiments, RG is a reaction group as described herein.

In some embodiments, as demonstrated herein, reactive groups when utilized in compounds that include no target binding moieties react slowly and provide low level of, in some embodiments, substantially no conjugation of moieties of interest with target agents. As demonstrated herein, combination of reactive groups with target binding moieties in the same compounds, e.g., as in compounds of formula R-I or salts thereof, can, among other things, promote reactions between reactive groups and target agents, enhance reaction efficiency, reduce side reactions, and/or improve reaction selectivity (e.g., in terms of target sites wherein conjugation of moieties of interest with target agents occurs).

Reactive groups in provided compounds can react with various types of groups in target agents. In some embodiments, reactive groups in provided compounds selectively react with amino groups of target agents, e.g., $-NH_2$ groups on side chains of lysine residues of proteins. In some embodiments, reactive groups when utilized in provided compounds, e.g., those of formula R-I or salts thereof, selectively react with particular sites of target agents, e.g., as shown in examples herein, one or more of K246, K248, K288, K290, K317, etc. of IgG1, K251, K 253, etc. for IgG2, K239, K241 for IgG4, etc. In some

embodiments, a site is K246 or K248 of an antibody heavy chain. In some embodiments, sites are K246 and/or K248 of an antibody heavy chain. In some embodiments, a site is K246 of an antibody heavy chain. In some embodiments, a site is K248 of an antibody heavy chain. In some embodiments, a site is K288 or K290 of an antibody heavy chain. In some embodiments, a site is K288 of an antibody heavy chain. In some embodiments, a site is K290 of an antibody heavy chain. In some embodiments, a site is K317. In some embodiments, a site is K414 of an antibody heavy chain. In some embodiments, a site is K185 of an antibody light chain. In some embodiments, a site is K187 of an antibody light chain. In some embodiments, sites are K251 and/or K253 of an IgG2 heavy chain. In some embodiments, a site is K251 of an IgG2 heavy chain. In some embodiments, a site is K253 of an IgG2 heavy chain. In some embodiments, sites are K239 and/or K241 of an IgG4 heavy chain. In some embodiments, a site is K239 of an IgG4 heavy chain. In some embodiments, a site is K241 of an IgG4 heavy chain. In some embodiments, conjugation selectively occurs at one or more heavy chain sites over light chain sites. In some embodiments, for technologies without target binding moieties, conjugation occurs at light chain sites more than heavy chain sites (e.g., see Figure 15).

15 In some embodiments, a reactive group, e.g., RG, is or includes an ester group. In some embodiments, a reactive group, e.g., RG, is or includes an electrophilic group, e.g., a Michael acceptor.

In some embodiments, a reactive group, e.g., RG, is or includes $-L^{RG1}-L^{RG2}-$, wherein each of L^{RG1} and L^{RG2} is independently L as described herein. In some embodiments, a reactive group, e.g., RG, is or includes $-L^{LG4}-L^{RG1}-L^{RG2}-$, wherein each variable is as described herein. In some embodiments, a reactive group, e.g., RG, is or includes $-L^{LG3}-L^{LG4}-L^{RG1}-L^{RG2}-$, wherein each variable is as described herein. In some embodiments, a reactive group, e.g., RG, is or includes $-L^{LG2}-L^{LG3}-L^{LG4}-L^{RG1}-L^{RG2}-$, wherein each variable is as described herein. In some embodiments, a reactive group, e.g., RG, is or includes $-L^{LG4}-L^{RG2}-$, wherein each variable is as described herein. In some embodiments, a reactive group, e.g., RG, is or includes $-L^{LG3}-L^{LG4}-L^{RG2}-$, wherein each variable is as described herein. In some embodiments, a reactive group, e.g., RG, is or includes $-L^{LG2}-L^{LG3}-L^{LG4}-L^{RG2}-$, wherein each variable is as described herein.

In some embodiments, as described herein, L^{LG4} is $-O-$. In some embodiments, L^{LG4} is $-N(R)-$. In some embodiments, L^{LG4} is $-NH-$.

In some embodiments, as described herein, L^{LG3} is or includes an optionally substituted aryl ring. In some embodiments, L^{LG3} is or includes a phenyl ring. In some embodiments, an aryl or phenyl ring is substituted. In some embodiments, a substituent is an electron-withdrawing group as described herein, e.g., $-NO_2$, $-F$, etc.

In some embodiments, L^{RG1} is a covalent bond. In some embodiments, L^{RG1} is not a covalent bond. In some embodiments, L^{RG1} is $-S(O)_2-$.

In some embodiments, L^{RG2} is $-C(O)-$. In some embodiments, a reactive group is or includes $-L^{LG4}-C(O)-$, wherein each variable is as described herein. In some embodiments, a reactive group is or includes $-L^{LG3}-L^{LG4}-C(O)-$, wherein each variable is as described herein. In some embodiments, a reactive group is or includes $-L^{LG2}-L^{LG3}-L^{LG4}-C(O)-$, wherein each variable is as described herein.

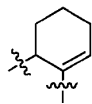
In some embodiments, L^{RG2} is $-L^{RG3}-C(=CR^{RG1}R^{RG2})-CR^{RG3}R^{RG4}-$, wherein each of R^{RG1} , R^{RG2} , R^{RG3} and R^{RG4} is independently $-L-R'$, and L^{RG3} is $-C(O)-$, $-C(O)O-$, $-C(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-P(O)(OR')-$, $-P(O)(SR')-$, or $-P(O)(N(R')_2)-$. In some embodiments, each of R^{RG1} , R^{RG2} , R^{RG3} and R^{RG4} is independently R' . In some embodiments, one or more of R^{RG1} , R^{RG2} , R^{RG3} and R^{RG4} is independently $-H$. In some embodiments, L^{RG3} is $-C(O)-$. In some embodiments, L^{RG3} is $-C(O)O-$. In some embodiments, $-O-$, $-N(R')-$, etc. of L^{RG3} is bonded to L^{PM} .

In some embodiments, R^{RG1} is $-H$. In some embodiments, R^{RG3} is $-H$.

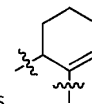
In some embodiments, L^{RG2} is optionally substituted $-L^{RG3}-C(=CHR^{RG2})-CHR^{RG4}-$, wherein each variable is as described herein.

In some embodiments, R^{RG2} and R^{RG4} are taken together with their intervening atoms to form an optionally substituted ring as described herein. In some embodiments, a formed ring is an optionally substituted 3-10 membered monocyclic or bicyclic ring having 0-5 heteroatoms. In some embodiments, a formed ring is an optionally substituted 3-10 membered cycloaliphatic ring. In some embodiments, a formed ring is an optionally substituted 3-8 membered cycloaliphatic ring. In some embodiments, a formed ring is an optionally substituted 5-8 membered cycloaliphatic ring. In some embodiments, a formed ring is an optionally substituted 5-membered cycloaliphatic ring. In some embodiments, a formed ring is an optionally substituted 6-membered cycloaliphatic ring. In some embodiments, a formed ring is an optionally substituted 7-membered cycloaliphatic ring. In some embodiments, a formed ring is substituted. In some embodiments, a formed ring is not substituted. In some embodiments, a formed ring contains no additional unsaturation in addition to the double bond in $C(=CHR^{RG2})$ or $C(=CR^{RG1}R^{RG2})$.

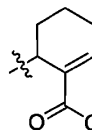
In some embodiments, $-\text{C}(=\text{CHR}^{\text{RG2}})-\text{CHR}^{\text{RG4}}$ or $-\text{C}(=\text{CR}^{\text{RG1}}\text{R}^{\text{RG2}})-\text{CR}^{\text{RG3}}\text{R}^{\text{RG4}}$ is optionally substituted



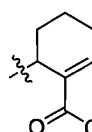
. In some embodiments, $-\text{C}(=\text{CHR}^{\text{RG2}})-\text{CHR}^{\text{RG4}}$ or $-\text{C}(=\text{CR}^{\text{RG1}}\text{R}^{\text{RG2}})-\text{CR}^{\text{RG3}}\text{R}^{\text{RG4}}$ is



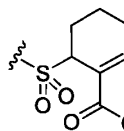
embodiments, $-\text{C}(=\text{CHR}^{\text{RG2}})-\text{CHR}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ or $-\text{C}(=\text{CR}^{\text{RG1}}\text{R}^{\text{RG2}})-\text{CR}^{\text{RG3}}\text{R}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ is optionally substituted



. In some embodiments, $-\text{C}(=\text{CHR}^{\text{RG2}})-\text{CHR}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ or $-\text{C}(=\text{CR}^{\text{RG1}}\text{R}^{\text{RG2}})-\text{CR}^{\text{RG3}}\text{R}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ is

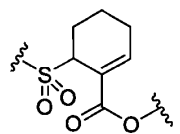


5. In some embodiments, $-\text{L}^{\text{RG1}}-\text{C}(=\text{CHR}^{\text{RG2}})-\text{CHR}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ or



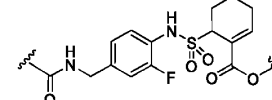
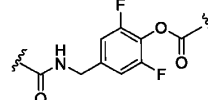
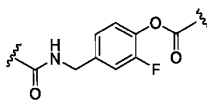
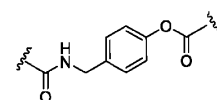
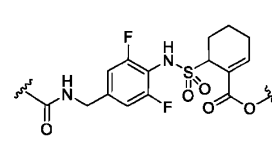
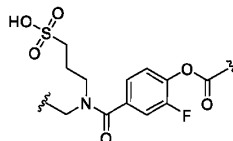
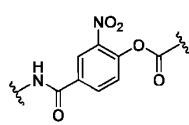
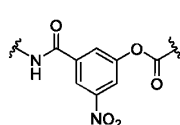
$-\text{L}^{\text{RG1}}-\text{C}(=\text{CR}^{\text{RG1}}\text{R}^{\text{RG2}})-\text{CR}^{\text{RG3}}\text{R}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ is optionally substituted

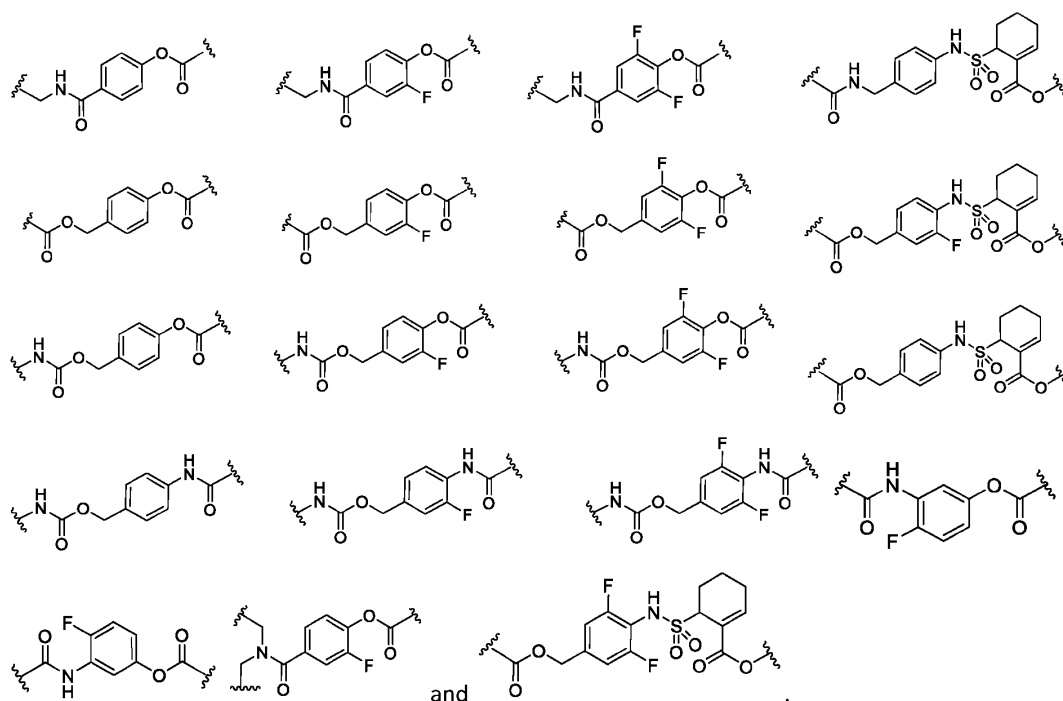
$-\text{L}^{\text{RG1}}-\text{C}(=\text{CHR}^{\text{RG2}})-\text{CHR}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ or $-\text{L}^{\text{RG1}}-\text{C}(=\text{CR}^{\text{RG1}}\text{R}^{\text{RG2}})-\text{CR}^{\text{RG3}}\text{R}^{\text{RG4}}-\text{L}^{\text{RG3}}-$ is optionally substituted



10 In some embodiments, a reactive group is a structure selected from the Table below. In some embodiments, $-\text{L}^{\text{LG2}}-\text{L}^{\text{LG3}}-\text{L}^{\text{LG4}}-\text{L}^{\text{RG1}}-\text{L}^{\text{RG2}}-$ is a structure selected from the Table below. In some embodiments, $-\text{L}^{\text{LG2}}-\text{L}^{\text{LG3}}-\text{L}^{\text{LG4}}-\text{RG}-$ is a structure selected from the Table below.

Table RG-1. Certain structures as examples.





In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-O-C(O)-$. In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-S-C(O)-$. In some embodiments, $-L^{LG4}-L^{RG1}-L^{RG2}-$ is $-S-C(O)-$.

In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-N(-)-C(O)-$, wherein N is a ring atom of an optionally substituted heteroaryl ring. In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-N(-)-C(O)-$, wherein N is a ring atom of L^{LG4} which is or includes an optionally substituted heteroaryl ring. In some embodiments, $-L^{LG4}-L^{RG2}-$ is $-N(-)-C(O)-O-$, wherein N is a ring atom of L^{LG4} which is or includes an optionally substituted heteroaryl ring.

In some embodiments, L^{RG2} is optionally substituted $-CH_2-C(O)-$, wherein $-CH_2-$ is bonded to an electron-withdrawing group including or connected to a target binding moiety. In some embodiments, L^{RG2} is optionally substituted $-CH_2-$ bonded to an electron-withdrawing group including or connected to a target binding moiety. In some embodiments, L^{RG1} is an electron-withdrawing group. In some embodiments, L^{RG1} is $-C(O)-$. In some embodiments, L^{RG1} is $-S(O)-$. In some embodiments, L^{RG1} is $-S(O)_2-$. In some embodiments, L^{RG1} is $-P(O(OR))-$. In some embodiments, L^{RG1} is $-P(O(SR))-$. In some embodiments, L^{RG1} is $-P(O(N(R)_2))-$. In some embodiments, L^{RG1} is $-OP(O(OR))-$. In some embodiments, L^{RG1} is $-OP(O(SR))-$. In some embodiments, L^{RG1} is $-OP(O(N(R)_2))-$.

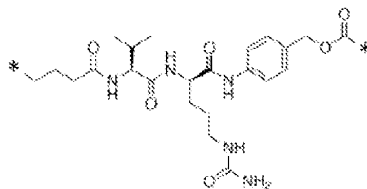
In some embodiments, L^{RG2} is optionally substituted $-CH_2-C(O)-$, wherein $-CH_2-$ is bonded to a leaving group including or connected to a target binding moiety. In some embodiments, L^{RG2} is optionally substituted $-CH_2-$ bonded to a leaving group including or connected to a target binding moiety. In some embodiments, L^{RG1} is $-O-C(O)-$. In some embodiments, L^{RG1} is $-OS(O)_2-$. In some embodiments, L^{RG1} is $-OP(O(OR))-$. In some embodiments, L^{RG1} is $-OP(O(SR))-$. In some embodiments, L^{RG1} is $-OP(O(N(R)_2))-$.

In some embodiments, a reactive group reacts with an amino group of a target agent. In some embodiments, an amino group is $-NH_2$ of the side chain of a lysine residue.

In some embodiments, a target agent is a protein agent. In some embodiments, a target agent is an antibody agent. In some embodiments, a reactive group reacts with an amino acid residue of such protein or antibody agent. In some embodiments, an amino acid residue is a lysine residue. In some embodiments, a reactive group reacts with $-NH_2$ of the side chain of a lysine residue. In some embodiments, a reactive group is or includes $-C(O)-O-$, it reacts with $-NH_2$ (e.g., of the side chain of a lysine residue), and forms an amide group $-C(O)-O-$ with the $-NH_2$.

LINKING GROUP

In some embodiments, the linking group L^{RM} may include a lysosomal-cleavable peptide linker. Lysosomal-cleavable peptide linkers are described, for example, in Balamkundu et al. "Lysosomal-Cleavable Peptide Linkers in Antibody-Drug Conjugates" *Biomedicines*, 2023, 11, 3080, which is incorporated herein in its entirety by reference. In some embodiments, the linking group L^{RM} may include a cathepsin-cleavable peptide linker. In some embodiments, the linking group L^{RM} may include a valine-citrulline-p-aminobenzyl carbamate (ValCitPABC) group, wherein the p-aminobenzyl group may be substituted or unsubstituted. The linking group L^{RM} may further include a connecting moiety having formula $*(CH_2)_n-C(=O)-*$, wherein n is an integer of 1 to 10, $*$ is a point of attachment to reactive group RG, and $*'$ is a point of attachment to the valine of the ValCitPABC group. In some embodiments,



the linking group L^{RM} may have the formula

In some embodiments, the linking group may include Val-Cit. In some embodiments, the linking group may include Phe-Lys. In some embodiments, the linking group may include Val-Lys. See, for

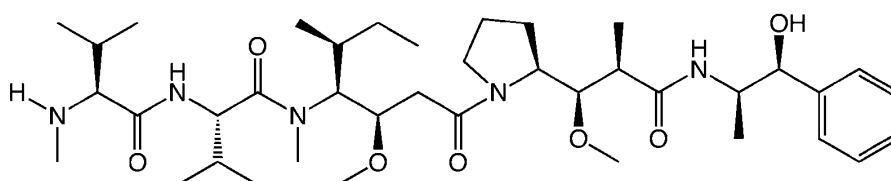
example, BinQing Wei et al. "Discovery of Peptidomimetic Antibody-Drug Conjugate Linkers with Enhanced Protease Specificity" J. Med. Chem. 2018, 61, 3, 989-1000, which is incorporated herein in its entirety by reference.

Various linking groups that may be present in antibody-drug conjugates according to
5 embodiments of the present invention are described, for example, in WO 2022/246086 A1, which is incorporated herein in its entirety by reference.

MOIETY OF INTEREST

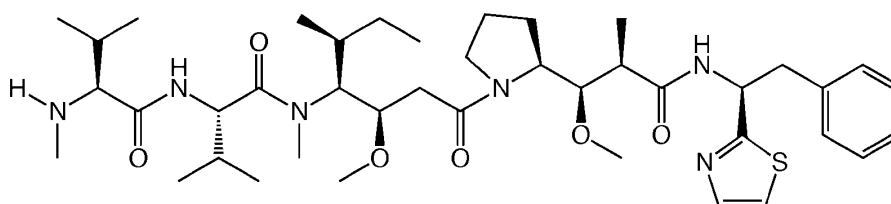
Those skilled in the art reading the present disclosure will appreciate that various types of
10 moieties of interest including monomethyl auristatin E (MMAE) or a close analog thereof can be utilized for various purposes in accordance with the present disclosure.

In some embodiments of this disclosure the moiety of interest is or includes MMAE. MMAE is an anti-neoplastic agent that is used in drug-antibody conjugates, *e.g.* a MAB-MMAE conjugate. The MMAE is joined to a monoclonal antibody through a linking group that can be cleaved once the drug-
15 antibody conjugate has attached to the tumor cell. The linking group will be discussed below.



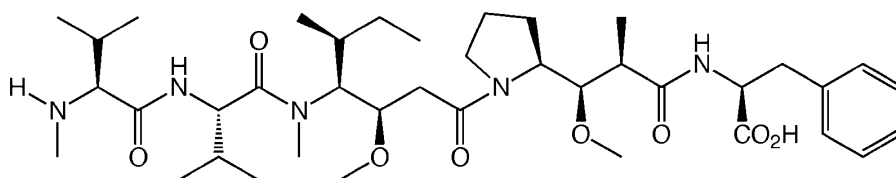
(MMAE)

In some embodiments of this disclosure, the moiety of interest is or includes monomethyl auristatin D (MMAD):



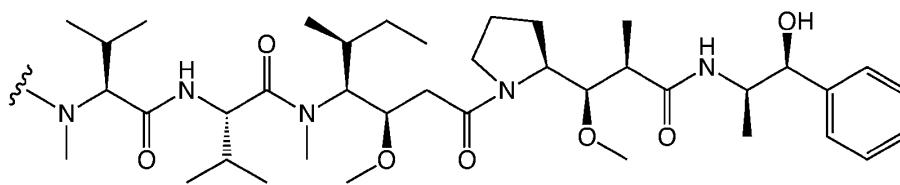
(MMAD)

In some embodiments of this disclosure, the moiety of interest is or includes MMAF, that is monomethyl auristatin F or desmethyl-auristatin F, shown below with its linking structure:



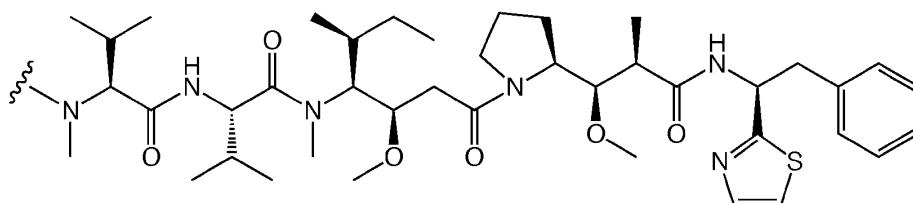
(MMAF)

- 5 In some embodiments, the moiety of interest that is or includes monomethyl auristatin E may be the following moiety:



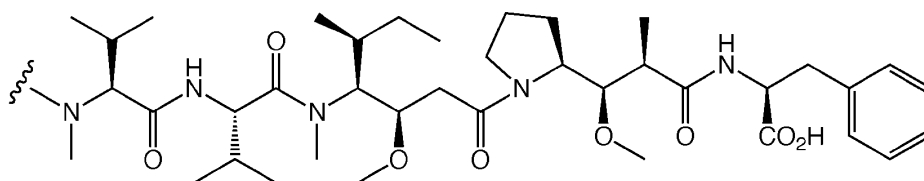
wherein the wavy line “” indicates the point of attachment to the linker.

- 10 In some embodiments, the moiety of interest that is or includes monomethyl auristatin D may be the following moiety:



wherein the wavy line “” indicates the point of attachment to the linker.

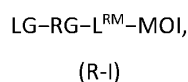
In some embodiments, the moiety of interest that is or includes monomethyl auristatin F may be the following moiety:



15

wherein the wavy line “” indicates the point of attachment to the linker.

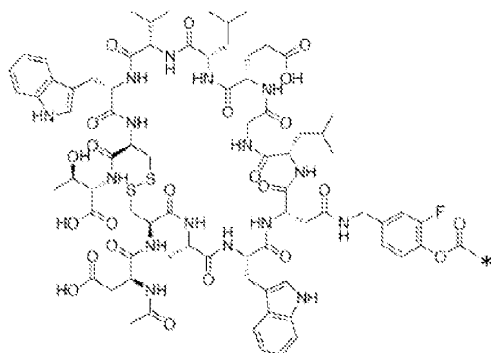
In an embodiment, provided is a compound having the structure of formula (R-I):



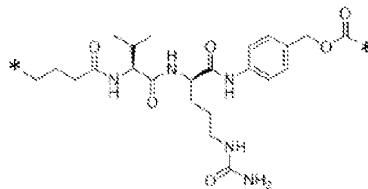
5 or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;

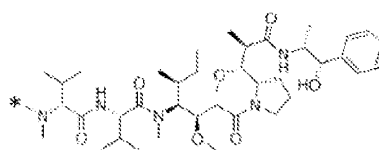


LG-RG is



10 L^{RM} is a linker having the formula

; and



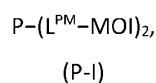
MOI is moiety of interest having the formula

In an aspect, the target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety including $\text{L}^{\text{RM}}\text{-MOI}$ to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

15

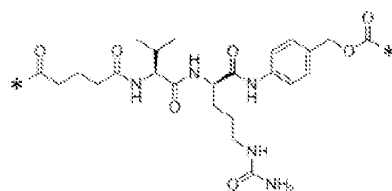
In another aspect, the target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety including L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

In an embodiment, provided is a method of preparing an agent having the structure of formula (P-I):



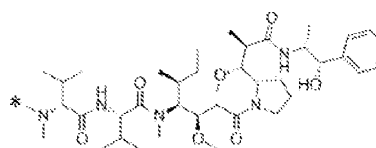
or a salt thereof, wherein:

P is brentuximab or a biosimilar analog thereof;



each L^{PM} is a linker having the formula

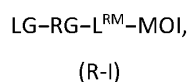
; and



each MOI is a moiety of interest having the formula

The method may include the steps of:

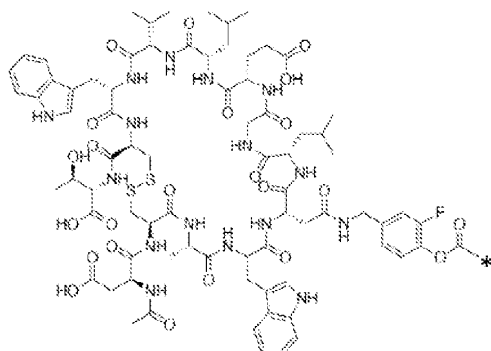
(1) contacting brentuximab or a biosimilar analog thereof with a reaction partner having the structure of formula (R-I):



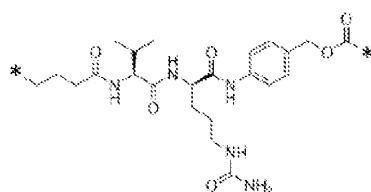
or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

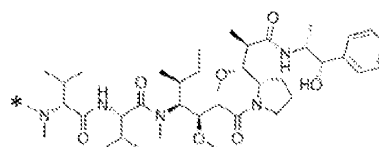
RG is a reactive group;



LG-RG is



L^{RM} is a linker having the formula



MOI is a moiety of interest having the formula

, and

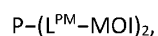
(2) forming an agent having the structure of formula (P-I).

5 In an aspect, the target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety including L^{RM} -MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

10 In another aspect, the target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety including L^{RM} -MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

15 In another aspect, the contacting and forming steps may be performed in one chemical reaction.

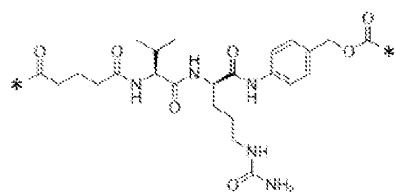
In another embodiment, provide is an agent having the structure of formula (P-I):



(P-I)

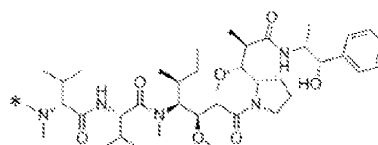
or a salt thereof, wherein:

P is brentuximab or a biosimilar analog thereof;



each L^{PM} is a linker having the formula

; and

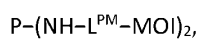


5 each MOI is a moiety of interest having the formula

In an aspect, each L^{PM} -MOI may be attached to K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

In another aspect, each L^{PM} -MOI may be attached to K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

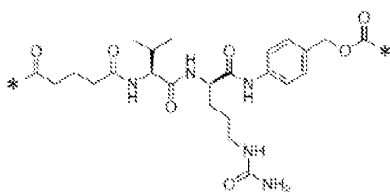
10 In another embodiment, provided is a method of preparing an agent having the structure of (P-II):



(P-II)

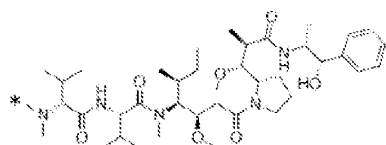
wherein:

15 P-NH is brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH;



L^{PM} is

; and



MOI is

The method may include the steps of:

(1) providing brentuximab or a biosimilar analog thereof having a structure $P-(NH_2)_2$, wherein each NH_2 is a terminal amino group of a lysine residue; and

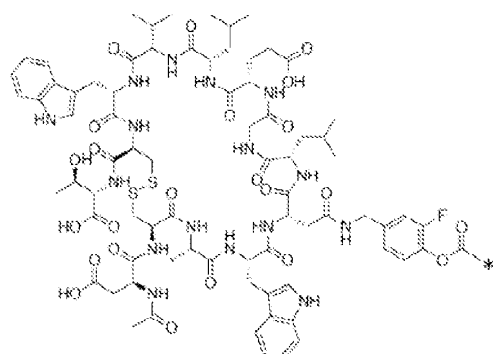
(2) contacting $P-(NH_2)_2$ with a reaction partner having a structure of formula (R-I):



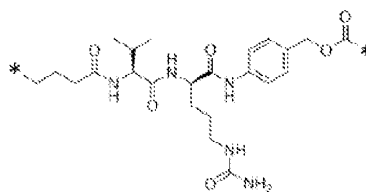
or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

10 RG is a reactive group;

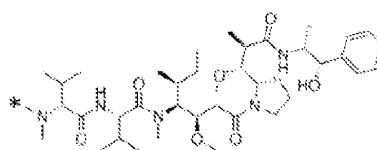


LG-RG is



L^{RM} is a linker having the formula

; and



MOI is a moiety of interest having the formula

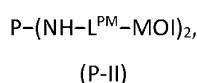
15 In an aspect, the target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety including $L^{RM}-MOI$ to K246 or

K248 and expulsion of the group containing a target binding moiety from the compound.

In another aspect, the target binding moiety of the reagent partner may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the
 5 reactive group that results in attachment of a moiety including L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

In an aspect, the contacting and forming steps may be performed in one chemical reaction.

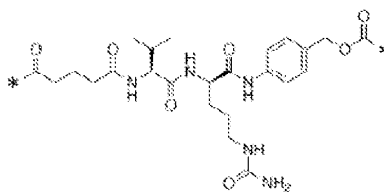
In another embodiment, provided is an agent having the structure of (P-II):



10

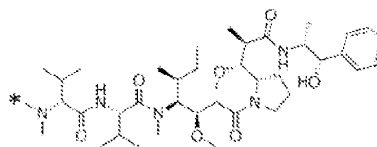
wherein:

P-NH is brentuximab or a biosimilar analog thereof including a lysine residue having a terminal group NH;



L^{PM} is a linker having the formula

; and



15

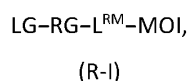
MOI is a moiety of interest having the formula

The lysine residue may be K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

Each lysine residue may be K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

20

In an embodiment, provided is a compound having the structure of formula (R-I):

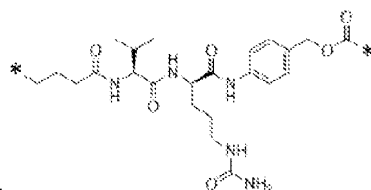


or a salt thereof, wherein:

LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog

thereof,

RG is a reactive group;

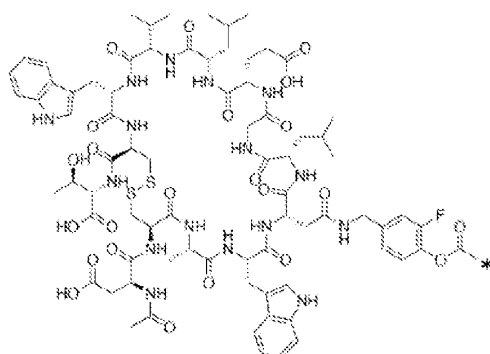


L^{RM} is a linker including ; and

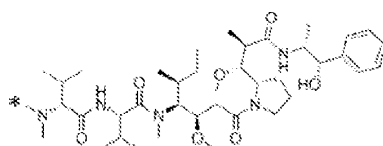
MOI is a moiety of interest including monomethyl auristatin E (MMAE),

5 wherein brentuximab or a biosimilar analog thereof includes an IgG heavy chain including K246 or K248, and

wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety including L^{RM} -MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.



LG-RG may be



MOI may include

15 In an aspect, the target binding moiety may be configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety including L^{RM} -MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

ANTIBODY

The antibodies that are used in accordance with the compositions and methods according to embodiments of the present invention may be a known antibody for the treatment or prevention of cancer. Antibodies immunospecific for a cancer cell antigen can 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 cancer cell antigen can be obtained, *e.g.* from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing. Examples of antibodies available for the treatment of cancer include, but are not limited to, HERCEPTIN (Trastuzumab; Genentech, CA) which is a humanized anti-HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer (Stebbing, J., Copson, E., and O'Reilly, S. "Herceptin (trastuzumab) in advanced breast cancer" *Cancer Treat Rev.* 26, 287-90, 2000); RITUXAN (rituximab; Genentech) which is a chimeric anti-CD20 monoclonal antibody for the treatment of patients with non-Hodgkin's lymphoma; OvaRex (AltaRex Corporation, MA) which is a murine antibody for the treatment of ovarian cancer; Panorex (Glaxo Wellcome, NC) which is a murine IgG_{2a} antibody for the treatment of colorectal cancer; BEC2 (ImClone Systems Inc., NY) which is murine IgG antibody for the treatment of lung cancer; IMC-C225 (Imclone Systems Inc., NY) which is a chimeric IgG antibody for the treatment of head and neck cancer; Vitaxin MedImmune, Inc., MD) which is a humanized antibody for the treatment of sarcoma; Campath I/H (Leukosite, MA) which is a humanized IgG₁ antibody for the treatment of chronic lymphocytic leukemia (CLL); Smart MI95 (Protein Design Labs, Inc., CA) which is a humanized IgG antibody for the treatment of acute myeloid leukemia (AML); LymphoCide (Immunomedics, Inc., NJ) which is a humanized IgG antibody for the treatment of non-Hodgkin's lymphoma; Smart ID10 (Protein Design Labs, Inc., CA) which is a humanized antibody for the treatment of non-Hodgkin's lymphoma; Oncolym (Techniclone, Inc., CA) which is a murine antibody for the treatment of non-Hodgkin's lymphoma; Allomune (BioTransplant, CA) which is a humanized anti-CD2 mAb for the treatment of Hodgkin's Disease or non-Hodgkin's lymphoma; anti-VEGF (Genentech, Inc., CA) which is humanized antibody for the treatment of lung and colorectal cancers; CEAcide (Immunomedics, NJ) which is a humanized anti-CEA antibody for the treatment of colorectal cancer; IMC-1C11 (ImClone Systems, NJ) which is an anti-KDR chimeric antibody for the treatment of colorectal cancer, lung cancers, and melanoma; and Cetuximab (ImClone, NJ) which is an anti-EGFR chimeric antibody for the treatment of epidermal growth factor positive cancers.

Other antibodies useful in the treatment of cancer include, but are not limited to, antibodies against the following antigens: CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA 242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE 4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MUC1-KLH (breast cancer), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), PSA (prostate), IL-2 receptor (T-cell leukemia and lymphomas), CD20 (non-Hodgkin's lymphoma), CD52 (leukemia), CD33 (leukemia), CD22 (lymphoma), human chorionic gonadotropin (carcinoma), CD38 (multiple myeloma), CD40 (lymphoma), mucin (carcinomas), P21 (carcinomas), MPG (melanoma), and Neu oncogene product (carcinomas). Some specific useful antibodies include, but are not limited to, BR96 mAb (Trail, P. A., Willner, D., Lasch, S. J., Henderson, A. J., Hofstead, S. J., Casazza, A. M., Firestone, R. A., Hellström, I., Hellström, K. E., "Cure of Xenografted Human Carcinomas by BR96-Doxorubicin Immunoconjugates" *Science* 1993, 261, 212-215), BR64 (Trail, Pa., Willner, D, Knipe, J., Henderson, A. J., Lasch, S. J., Zoeckler, M. E., Trailsmith, M. D., Doyle, T. W., King, H. D., Casazza, A. M., Braslawsky, G. R., Brown, J. P., Hofstead, S. J., (Greenfield, Ill. S., Firestone, R. A., Mosure, K., Kadow, D. F., Yang, M. B., Hellstrom, K E., and Hellstrom, I. "Effect of Linker Variation on the Stability, Potency, and Efficacy of Carcinoma-reactive BR64-Doxorubicin Immunoconjugates" *Cancer Research* 1997, 57, 100-105, mAbs against the CD40 antigen, such as S2C6 mAb (Francisco, J. A., Donaldson, K. L., Chace, D., Siegall, C. B., and Wahl, A. F. "Agonistic properties and in vivo antitumor activity of the anti-CD-40 antibody, SGN-14" *Cancer Res.* 2000, 60, 3225-3231), mAbs against the CD70 antigen, such as 1F6 mAb, and mAbs against the CD30 antigen, such as AC10 (Bowen, M. A., Olsen, K. J., Cheng, L., Avila, D., and Podack, E. R. "Functional effects of CD30 on a large granular lymphoma cell line YT" *J. Immunol.*, 151, 5896-5906, 1993). Many other internalizing antibodies that bind to tumor associated antigens can be used in this invention, and have been reviewed (Franke, A. E., Sievers, E. L., and Scheinberg, D. A., "Cell surface receptor-targeted therapy of acute myeloid leukemia: a review" *Cancer Biother Radiopharm.* 2000, 15, 459-76; Murray, J. L., "Monoclonal antibody treatment of solid tumors: a coming of age" *Semin Oncol.* 2000, 27, 64-70; Breitling, F., and Dubel, S., *Recombinant Antibodies*, John Wiley, and Sons, New York, 1998).

In some embodiments, the antibody is brentuximab or a biosimilar analog of brentuximab.

PHARMACEUTICAL COMPOSITIONS

The present disclosure also provides a composition including an effective amount of a compound according to embodiments of the present invention and a pharmaceutically acceptable carrier or vehicle. The compositions are suitable for veterinary or human administration.

5 The compositions can be in any form that allows for the composition to be administered to a human or animal. For example, the composition can be in the form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral, sublingual, rectal, vaginal, ocular, and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In some embodiments, the compositions
10 are administered parenterally. Pharmaceutical compositions can be formulated so as to allow a compound to be bioavailable upon administration of the composition to a human or animal. Compositions can take the form of one or more dosage units, where for example, a tablet can be a single dosage unit, and a container of a compound in aerosol form can hold a plurality of dosage units.

Materials used in preparing the pharmaceutical compositions can be non-toxic in the amounts
15 used. It will be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of an organism (e.g., human or animal), the particular form of the compound, the manner of administration, and the composition employed.

The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions
20 are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s) can be gaseous, so as to provide an aerosol composition useful in, e.g., inhalatory administration.

When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein
25 as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin;
30 excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate,

Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin, a flavoring agent such as peppermint, methyl salicylate or orange flavoring, and a coloring agent.

When the composition is in the form of a capsule, *e.g.*, a gelatin capsule, it can contain, in
5 addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

The composition can be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can include one or more of a sweetening agent, preservatives,
10 dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The liquid compositions according to embodiments of the present invention, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents
15 such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates,
20 citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. Physiological saline is a preferred adjuvant. An injectable composition is preferably sterile.

The amount of the compound that is effective in the treatment of a particular disorder or
25 condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

30 The compositions include an effective amount of a compound such that a suitable dosage will be obtained. Typically, this amount is at least about 0.01% of a compound by weight of the composition.

When intended for oral administration, this amount can be varied to range from about 0.1% to about 80% by weight of the composition. Preferred oral compositions can include from about 4% to about 50% of the compound by weight of the composition. Preferred compositions are prepared so that a parenteral dosage unit contains from about 0.01% to about 2% by weight of the compound.

5 For intravenous administration, the composition can include from about 1 to about 250 mg of a compound per kg of the animal's body weight. For example, the amount administered may be in the range from about 4 to about 25 mg/kg of body weight of the compound.

 Generally, the dosage of compound administered to a human or animal is typically about 0.1 mg/kg to about 250 mg/kg of the human's or animal's body weight. For example, the dosage
10 administered to a human or animal is between about 0.1 mg/kg and about 20 mg/kg of the human's or animal's body weight, for example, about 1 mg/kg to about 10 mg/kg of the human's or animal's body weight.

 The compounds or compositions can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa,
15 rectal and intestinal mucosa, etc.). Administration can be systemic or local. Various delivery systems are known, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer a compound or composition. In certain embodiments, more than one compound or composition is administered to a human or animal. Methods of administration include, but are not limited to, oral administration and parenteral administration; parenteral administration including, but
20 not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous; intranasal, epidural, sublingual, intranasal, intracerebral, intraventricular, intrathecal, intravaginal, transdermal, rectally, by inhalation, or topically to the ears, nose, eyes, or skin. The preferred mode of administration is left to the discretion of the practitioner, and will depend in-part upon the site of the medical condition (such as the site of cancer or autoimmune disease).

25 In some embodiments, the present compounds or compositions are administered parenterally.

 In some embodiments, the present compounds or compositions are administered intravenously.

 In some embodiments, it can be desirable to administer one or more compounds or compositions locally to the area in need of treatment. This can be achieved, for example, and not by way of limitation, by local infusion during surgery; topical application, *e.g.*, in conjunction with a wound
30 dressing after surgery; by injection; by means of a catheter, by means of a suppository; or by means of

an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In some embodiments, administration can be by direct injection at the site (or former site) of a cancer, tumor or neoplastic or pre-neoplastic tissue. In some other embodiments, administration can be by direct injection at the site (or former site) of a manifestation of
5 an autoimmune disease.

In some embodiments, it can be desirable to introduce one or more compounds or compositions into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection can be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

10 Pulmonary administration can also be employed, *e.g.* by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In some embodiments, the compounds or compositions can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

In some embodiments, the compounds can be delivered in a vesicle, in particular a liposome
15 (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In some embodiments, the compounds or compositions can be delivered in a controlled release system. In some embodiments, a pump can be used (see Langer, *supra*; Sefton, *CRC Crit. Ref Biomed.*
20 *Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In some embodiments, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During
25 et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In some embodiments, a controlled-release system can be placed in proximity of the target of the compounds or compositions, *e.g.*, the brain, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer (*Science* 249:1527-1533 (1990)) can be used.

30 The term "carrier" refers to a diluent, adjuvant or excipient, with which a compound is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of

petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In some embodiments, when administered to a human or animal, the compounds or
5 compositions and pharmaceutically acceptable carriers are sterile. Water may be a preferred carrier when the compounds are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim
10 milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In some embodiments, the
15 pharmaceutically acceptable carrier is a capsule (see *e.g.*, U.S. Pat. No. 5,698,155). Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

In some embodiments, the compounds are formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to animals, particularly human
20 beings. Typically, the carriers or vehicles for intravenous administration are sterile isotonic aqueous buffer solutions. Where necessary, the compositions can also include a solubilizing agent. Compositions for intravenous administration can optionally include a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically
25 sealed container such as an ampoule or sachette indicating the quantity of active agent. Where a compound is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the Compound of the Invention is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

30 Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered

compositions can contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compounds. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be used. Oral compositions can include standard carriers such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such carriers are preferably of pharmaceutical grade.

The compositions can be intended for topical administration, in which case the carrier may be in the form of a solution, emulsion, ointment or gel base. The base, for example, can include one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents can be present in a composition for topical administration. If intended for transdermal administration, the composition can be in the form of a transdermal patch or an iontophoresis device. Topical formulations can include a concentration of a Compound of the Invention of from about 0.1% to about 10% w/v (weight per unit volume of composition).

The composition can be intended for rectal administration, in the form, *e.g.*, of a suppository which will melt in the rectum and release the compound. The composition for rectal administration can contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

The composition can include various materials that modify the physical form of a solid or liquid dosage unit. For example, the composition can include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and can be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients can be encased in a gelatin capsule.

The compositions can consist of gaseous dosage units, *e.g.*, it can be in the form of an aerosol.

The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery can be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols of compounds can be delivered in single phase, bi-phasic, or tri-phasic systems in order to deliver the compound(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, spacers and the like, which together can form a kit. Preferred aerosols can be determined by one skilled in the art, without undue experimentation.

Whether in solid, liquid or gaseous form, the compositions according to embodiments of the present invention can include a pharmacological agent used in the treatment of cancer, an autoimmune disease or an infectious disease.

The pharmaceutical compositions can be prepared using methodology well-known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining a compound with water so as to form a solution. A surfactant can be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with a compound according to embodiments of the present invention so as to facilitate dissolution or homogeneous suspension of the active compound in the aqueous delivery system.

TREATMENT OF CANCER

The compounds according to embodiments of the present invention are useful for treating cancer. The compounds according to embodiments of the present invention are useful for inhibiting the multiplication of a tumor cell or cancer cell, or for treating cancer in a human or animal. The compounds can be used accordingly in a variety of settings for the treatment of human or animal cancers. The antibody-drug conjugates can be used to deliver a drug or drug unit to a tumor cell or cancer cell. Without being bound by theory, in some embodiments, the Moiety of Interest of a compound binds to or associates with a cancer-cell or a tumor-cell-associated antigen, and the compound can be taken up inside a tumor cell or cancer cell through receptor-mediated endocytosis. The antigen can be attached to a tumor cell or cancer cell or can be an extracellular matrix protein associated with the tumor cell or cancer cell. Once inside the cell, one or more specific peptide sequences within the linking group are hydrolytically cleaved by one or more tumor-cell or cancer-cell-associated proteases, resulting in release of a drug or drug unit. The released drug or drug unit is then

free to migrate in the cytosol and induce cytotoxic activities. In some alternative embodiments, the drug or drug unit is cleaved from the compound outside the tumor cell or cancer cell, and the drug or drug unit subsequently penetrates the cell.

In some embodiments, the Moiety of Interest binds to the tumor cell or cancer cell.

5 In another embodiment, the Moiety of Interest binds to a tumor cell or cancer cell antigen which is on the surface of the tumor cell or cancer cell.

In another embodiment, the Moiety of Interest binds to a tumor cell or cancer cell antigen which is an extracellular matrix protein associated with the tumor cell or cancer cell.

10 In some embodiments, the tumor cell or cancer cell is of the type of tumor or cancer that the human or animal needs treatment or prevention of.

The specificity of the Moiety of Interest for a particular tumor cell or cancer cell can be important for determining those tumors or cancers that are most effectively treated. For example, compounds having a BR96 Ligand unit can be useful for treating antigen positive carcinomas including those of the lung, breast, colon, ovaries, and pancreas. Compounds having an anti-CD30 or an anti-CD40
15 Ligand unit can be useful for treating hematologic malignancies.

Other particular types of cancers that can be treated with compounds include solid tumors, including but not limited to fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, colorectal cancer, kidney cancer, pancreatic cancer, bone cancer,
20 breast cancer, ovarian cancer, prostate cancer, esophageal cancer, stomach cancer, oral cancer, nasal cancer, throat cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer,
25 uterine cancer, testicular cancer, small cell lung carcinoma, bladder carcinoma, lung cancer, epithelial carcinoma, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, skin cancer, melanoma, neuroblastoma, retinoblastoma, blood-borne cancers, including but not limited to,
30 acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell

leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocytic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma, acute and
5 chronic leukemias: lymphoblastic, myelogenous, lymphocytic, myelocytic leukemias, Lymphomas: Hodgkin's disease, non-Hodgkin's Lymphoma, Multiple myeloma, Waldenström's macroglobulinemia, Heavy chain disease, and Polycythemia vera. The compounds according to embodiments of the present invention can also be used as chemotherapeutics in the untargeted form. For example, the compounds are useful for treating ovarian, CNS, renal, lung, colon, melanoma, or hematologic cancers or tumors.

10 The compounds provide conjugation specific tumor or cancer targeting, thus reducing general toxicity of these compounds. The linking groups stabilize the compounds in blood, yet are cleavable by tumor-specific proteases within the cell, liberating a drug or drug unit.

Cancer, including, but not limited to, a tumor, metastasis, or any disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration of a
15 compound disclosed herein.

Other embodiments provide methods for treating or preventing cancer, including administering to an animal in need thereof an effective amount of a compound and a chemotherapeutic agent. In some embodiments the chemotherapeutic agent is that with which treatment of the cancer has not been found to be refractory. In other embodiments, the chemotherapeutic agent is that with which the
20 treatment of cancer has been found to be refractory. The compounds disclosed herein can be administered to a human or animal that has also undergone surgery as treatment for the cancer.

In some embodiments, the additional method of treatment is radiation therapy.

In some embodiments, the Compound of the Invention is administered concurrently with the chemotherapeutic agent or with radiation therapy. In some embodiments, the chemotherapeutic agent
25 or radiation therapy is administered prior or subsequent to administration of a compound, for example, at least an hour, five hours, 12 hours, a day, a week, a month, or for example, several months (e.g. up to three months), prior or subsequent to administration of a compound.

A chemotherapeutic agent can be administered over a series of sessions, any one or a combination of the chemotherapeutic agents listed below can be administered. With respect to
30 radiation, any radiation therapy protocol can be used depending upon the type of cancer to be treated.

For example, but not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater than 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

5 Additionally, some embodiments provide methods of treatment of cancer with a compound as an alternative to chemotherapy or radiation therapy where the chemotherapy or the radiation therapy has proven or can prove too toxic, *e.g.*, results in unacceptable or unbearable side effects, for the subject being treated. The human or animal being treated can, optionally, be treated with another cancer treatment such as surgery, radiation therapy or chemotherapy, depending on which treatment is
10 found to be acceptable or bearable.

 The compounds can also be used in an *in vitro* or *ex vivo* fashion, such as for the treatment of certain cancers, including, but not limited to leukemias and lymphomas, such treatment involving autologous stem cell transplants. This can involve a multi-step process in which the animal's autologous hematopoietic stem cells are harvested and purged of all cancer cells, the patient's remaining bone-
15 marrow cell population is then eradicated via the administration of a high dose of a compound with or without accompanying high dose radiation therapy, and the stem cell graft is infused back into the animal. Supportive care is then provided while bone marrow function is restored and the human or animal recovers.

 Some embodiments include methods for treating cancer, including administering to an animal in
20 need thereof an effective amount of a compound and another therapeutic agent that is an anti-cancer agent. Suitable anticancer agents include, but are not limited to, methotrexate, taxol, L-asparaginase, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbazine, topotecan, nitrogen mustards, cytoxan, etoposide, 5-fluorouracil, BCNU, irinotecan, camptothecins, bleomycin, doxorubicin, idarubicin,
25 daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel. In some embodiments, the anti-cancer agent includes, but is not limited to, the following drug: alkylating agents, nitrogen mustards: cyclophosphamide, ifosfamide, trofosfamide, chlorambucil, nitrosoureas: carmustine (BCNU), lomustine (CCNU), alkylsulphonates: busulfan, treosulfan, triazenes: dacarbazine, platinum containing compounds: cisplatin, carboplatin,
30 plant alkaloids such as vinca alkaloids: vincristine, vinblastine, vindesine, vinorelbine, taxoids: paclitaxel, docetaxol, DNA topoisomerase inhibitors, epipodophyllins: etoposide, teniposide, topotecan, 9-

aminocamptothecin, camptothecin, crisnatol, mitomycins: Mitomycin C, anti-metabolites, anti-folates: DHFR inhibitors: methotrexate, trimetrexate, IMP dehydrogenase Inhibitors: mycophenolic acid, tiazofurin, ribavirin, EICAR, ribonucleotide reductase Inhibitors: hydroxyurea, deferoxamine, pyrimidine analogs: uracil analogs: 5-fluorouracil, floxuridine, doxifluridine, ratitrexed, cytosine analogs: cytarabine (ara C), cytosine arabinoside, fludarabine, purine analogs: mercaptopurine, thioguanine, hormonal therapies: receptor antagonists, anti-estrogen: tamoxifen, raloxifene, megestrol, LHRH agonists: goserelin, leuprolide acetate, anti-androgens: flutamide, bicalutamide, retinoids/deltoids, vitamin D3 analogs: EB 1089, CB 1093, KH 1060, photodynamic therapies: vertoporphin (BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A, (2BA-2-DMHA), cytokines: Interferon- α , Interferon- γ , tumor necrosis factor, and others such as isoprenylation inhibitors: Lovastatin, dopaminergic neurotoxins: 1-methyl-4-phenylpyridinium ion, cell cycle inhibitors: staurosporine, actinomycins: actinomycin D, dactinomycin, bleomycins: bleomycin A2, bleomycin B2, peplomycin, anthracyclines: daunorubicin, doxorubicin (adriamycin), idarubicin, epirubicin, pirarubicin, zorubicin, mitoxantrone, MDR inhibitors: verapamil, and Ca²⁺-ATPase inhibitors: thapsigargin.

15 In some embodiments, another therapeutic agent may be an anti-PD-1 antibody, for example, nivolumab, pembrolizumab and pidilizumab.

In some embodiments, another therapeutic agent may be an anti-PD-L1 antibody, for example, BMS-936559, durvalumab, atezolizumab, avelumab, and MDX-1105.

20 In some embodiments, another therapeutic agent may be an anti-CTLA-4 antibody, for example, ipilimumab and tremelimumab.

In some embodiments, provided is a method of treating Hodgkin lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

25 In another embodiment, provided is a method of treating systemic anaplastic large cell lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

In another embodiment, provided is a method of treating primary cutaneous anaplastic large cell lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

30 In another embodiment, provided is a method of treating CD30-expressing mycosis fungoides in

a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

In another embodiment, provided is a method of treating cutaneous T cell lymphoma in a subject in need of such treatment, including administering to the subject a pharmaceutically effective amount of the agent described above.

In another embodiment, provided is a method of selectively targeting tumor cells expressing the CD30 antigen in a subject, including administering to the subject the agent described above. The antibody-drug conjugates according to embodiments of the present invention can also be used to treat autoimmune and infectious disease. Such diseases are listed, for example, in WO 2004/010957 A2, which is incorporated herein in its entirety by reference.

PROCESS FINGERPRINT COMPOSITIONS

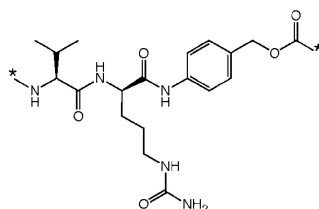
In another embodiment, provided is a composition including:

a first compound having the structure of formula (P-II):



wherein:

P-N is brentuximab or a biosimilar analog thereof including a lysine residue;



L^{PM} is a linking group including ; and

MOI is a moiety of interest including monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

a second compound having the structure:



wherein LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof.

The composition may further include:

a third compound having the formula (R-I):



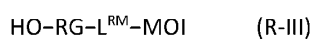
LG is a group including a target binding moiety that binds to brentuximab or a biosimilar analog thereof, which is identical to LG in formula (LG-I);

RG is a reactive group;

L^{RM} is a linking group, which is identical to that in formula (P-II); and

5 MOI is a moiety of interest which is identical to that in formula (P-II);

a fourth compound having the formula (R-III):



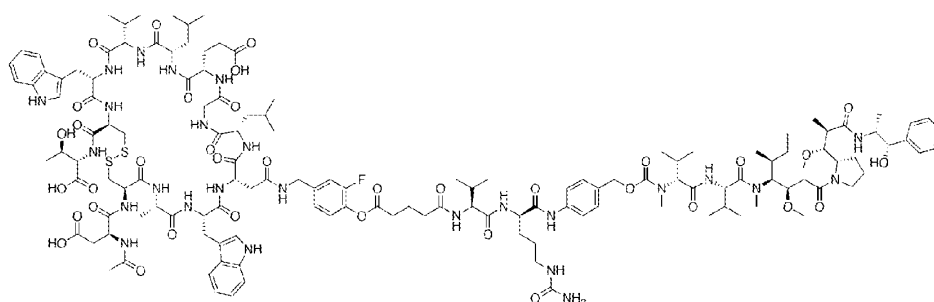
or a combination thereof.

10 The invention is further illustrated by the following non-limiting example.

EXAMPLES

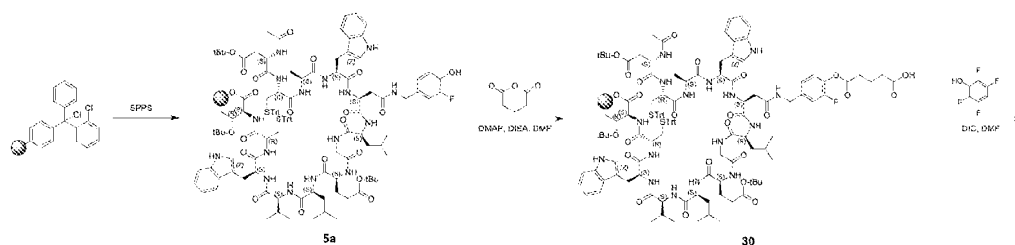
Synthesis of Reagent A

15

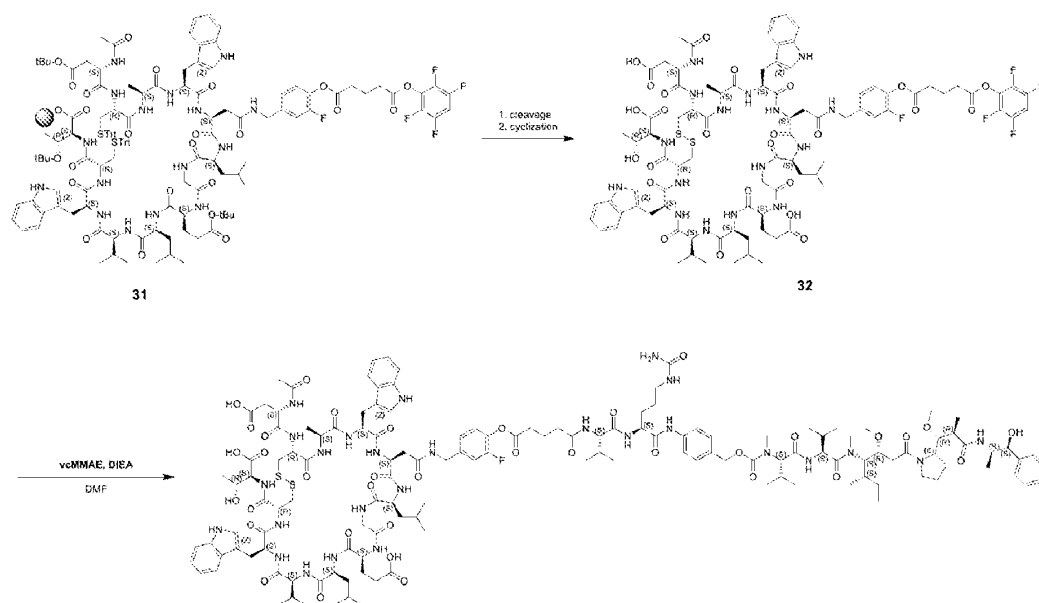


Reagent A

Route of Synthesis

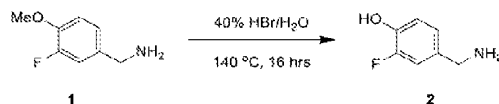


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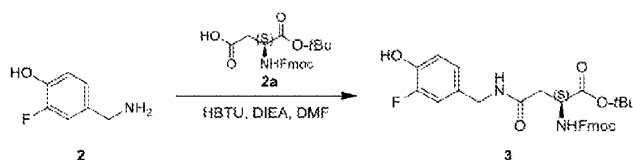


Synthesis and purification of 8H3S16

Synthesis of 3-Fluoro-4-hydroxybenzylamine-containing Reactive Group (Cmp. 4)

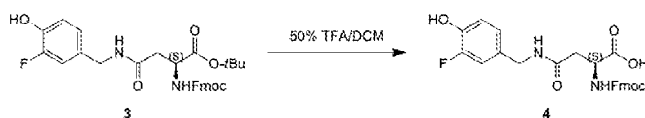


[0001] A mixture of intermediate 1 (10 g, 64.45 mmol) in HBr/H₂O (40% HBr, 300 mL in total) was stirred at 140 °C for 16 hrs. The solvent was removed at 70 °C under reduced pressure, the residue was triturated in MeCN (50 mL) for 10 mins. After filtration, the solid was dried under lyophilization to provide intermediate 2 (13.0 g, 58.5 mmol, 90.8% yield, HBr salt) as a brown solid. ¹H NMR: (400 MHz DMSO-*d*₆) δ ppm 10.04 (s, 1 H) 8.18 (s, 3 H) 7.32 (dd, *J* = 12.17, 1.88 Hz, 1 H) 7.11 (dd, *J* = 8.28, 1.51 Hz, 1 H) 6.96 - 7.03 (m, 1 H) 3.93 (q, *J* = 5.52 Hz, 2 H).



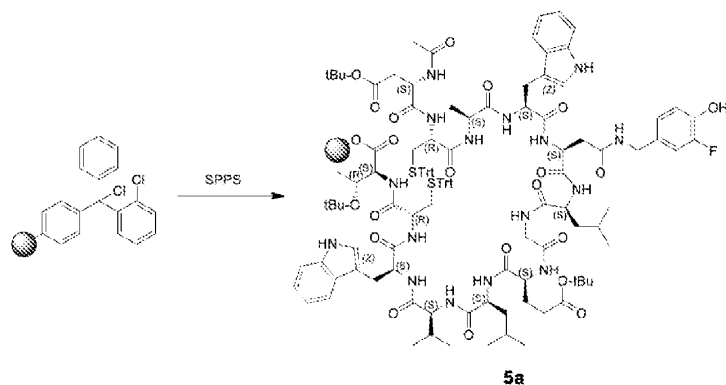
[0002] To a mixture of intermediate 2 (13.0 g, 58.5 mmol, 1 *eq*, HBr), intermediate 2a (24.1 g, 58.5 mmol, 1 *eq*), DIEA (3.78 g, 29.2 mmol, 5.10 mL, 0.5 *eq*) and HOBt (11.87 g, 87.8 mmol, 1.5 *eq*) in DMF (200 mL) was added EDCI (12.35 g, 64.4 mmol, 1.1 *eq*) at 15 °C, the mixture was stirred at 15 °C for 3 hr. The mixture was dropwise added to 0.5 M HCl (cold, 1 L) and white solid was precipitated. After filtration, the solid was dried under lyophilization to afford intermediate 3 (31 g, crude) as a white solid.

[0003] Alternatively, the reaction can be conducted with 60.0 g of compound 2 starting material at 20 °C. After precipitation with HCl and filtration the solid can be dissolved in DCM (2 L), washed with 0.5 M HCl (800 mL), H₂O (800 mL), brine (800 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by silica gel column (DCM/MeOH = from 1/0 to 20/1) to afford **Intermediate compound 3** (120.0 g, 90% purity, containing a small amount of DMF, 83.3% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.70 (s, 1 H) 8.34 (t, *J* = 5.77 Hz, 1 H) 7.90 (d, *J* = 7.53 Hz, 2 H) 7.71 (d, *J* = 7.53 Hz, 2 H) 7.61 (d, *J* = 8.28 Hz, 1 H) 7.39 - 7.47 (m, 2 H) 7.29 - 7.36 (m, 2 H) 7.02 (d, *J* = 12.30 Hz, 1 H) 6.85 - 6.92 (m, 2 H) 4.20 - 4.39 (m, 4 H) 4.11 - 4.19 (m, 2 H) 1.36 (s, 9 H).



[0004] A mixture of **compound 3** (30 g, 56.12 mmol, 1.0 eq) in TFA (300 mL) and DCM (300 mL) was stirred at 15 °C for 0.5 hr. The solvent was removed under reduced pressure. The residue was purified by flash C18 (ISCO®; 120 g SepaFlash® C18 Flash Column, Eluent of 0~90% MeCN/H₂O gradient @ 75 mL/min) directly to get **compound 4** (18 g, 37.6 mmol, 67.0% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.69 (s, 1 H) 8.34 (t, *J* = 5.90 Hz, 1 H) 7.90 (d, *J* = 7.28 Hz, 2 H) 7.71 (d, *J* = 7.53 Hz, 2 H) 7.54 (d, *J* = 6.53 Hz, 1 H) 7.42 (t, *J* = 7.40 Hz, 2 H) 7.27 - 7.37 (m, 1 H) 7.27 - 7.37 (m, 1 H) 7.02 (d, *J* = 12.05 Hz, 1 H) 6.82 - 6.93 (m, 2 H) 4.35 - 4.43 (m, 1 H) 4.20 - 4.31 (m, 3 H) 4.13 - 4.19 (m, 2 H).

Procedure for Preparation of Antibody Binding Moiety Coupled to Reactive Group (Intermediate Cmp. 5a)



[0005] Peptide was synthesized using standard Fmoc chemistry.

1) Resin preparation: To the vessel containing CTC Resin (3.0 mmol, 3.0 g, 1.00 mmol/g) and Fmoc-Thr(*t*Bu)-OH (1.19 g, 3.0 mmol, 1.00 eq) in DCM (30 mL) was added DIEA (4.00 eq) dropwise and mixed for 2 hrs with N₂ bubbling at 15 °C. Then MeOH (3.0 mL) was added and bubbled with N₂ for another 30 mins. The resin was washed with DMF (60 mL), followed by adding 20% piperidine in DMF (60 mL) and bubbled with N₂ for 30 mins at 15 °C for Fmoc deprotection.

The mixture was filtered, and the resin was washed with DMF (60 mL) before proceeding to next step. Alternatively, this reaction can be conducted at 20 °C.

2) Coupling: A solution of Fmoc-Cys(Trt)-OH (5.25 g, 3.00 eq), HBTU (3.24 g, 2.85 eq) in DMF (30 mL) was added to the resin with N₂ bubbling. Then DIEA (6.00 eq) was added to the mixture dropwise and bubbled with N₂ for 30 mins at 15 °C (or 20 °C). The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (60 mL).

3) Deprotection: 20% piperidine in DMF (60 mL) was added to the resin and the mixture was bubbled with N₂ for 30 mins at 15 °C. The deprotection reaction was monitored by ninhydrin test, if it showed blue or other brownish red, the reaction was completed. The resin was then washed with DMF (60 mL).

4) Steps 2 and 3 were repeated for amino acids (3-13 in table below).

5) Coupling for compound: A solution of compound 4 (2.87 g, 2.00 eq), DIC (0.76 g, 2.00 eq) and HOBt (0.82 g, 2.00 eq) in DMF (30 mL) was added to the resin with N₂ bubbling for 60 mins at 15 °C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (60 mL).

6) Repeat step 3 for Fmoc deprotection.

7) Steps 5 and 6 were repeated for amino acids (10-13 in table below).

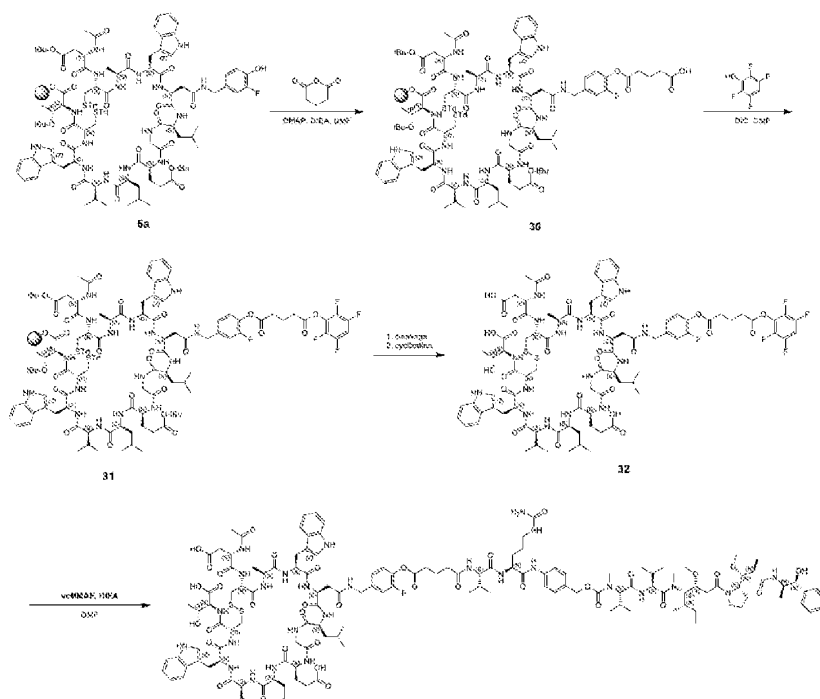
8) Acetylation: A solution of 10%Ac₂O/5%NMM/85%DMF (60 mL) was added to resin and the mixture was bubbled with N₂ for 20 mins. The acetylation reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (60 mL) to afford **Intermediate 5a**.

TABLE 1. List of amino acids and the corresponding reagents used on SPPS

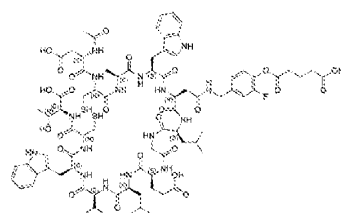
#	Materials	Coupling reagents
1	Fmoc-Thr(<i>t</i> Bu)-OH (1.00 eq)	DIEA (4.00 eq)
2	Fmoc-Cys(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Trp-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Glu(OtBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Intermediate Compound 4 (2.00 eq)	DIC (2.00 eq) and HOBt (2.00 eq)
10	Fmoc-Trp-OH (3.00 eq)	DIC (3.00 eq) and HOBt (3.00 eq)
11	Fmoc-Ala-OH (3.00 eq)	DIC (3.00 eq) and HOBt (3.00 eq)
12	Fmoc-Cys(Trt)-OH (3.00 eq)	DIC (3.00 eq) and HOBt (3.00 eq)

13	Fmoc-Asp(OtBu)-OH (3.00 eq)	DIC (3.00 eq) and HOBt (3.00 eq)
14	Acetylation	Ac ₂ O/NMM/DMF (10/5/85, 60 mL)

Procedure for Preparation of BH-0003816.

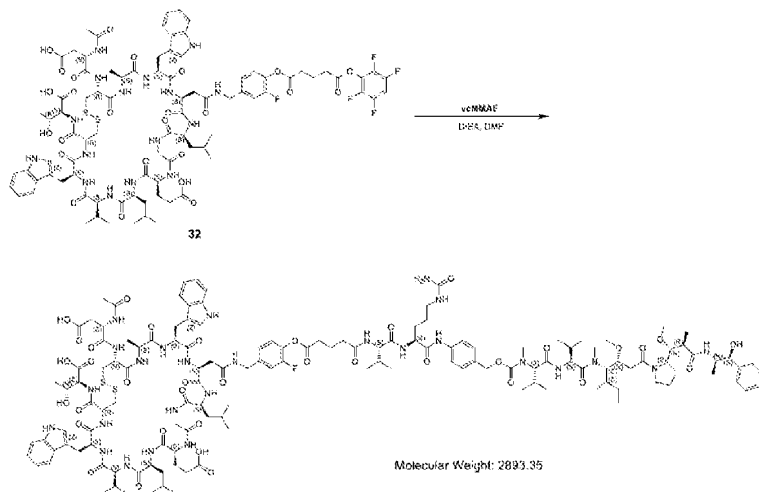


1) Coupling: To a mixture of **Compound 5a** (CTC resin, 0.50 mmol), DIEA (387.7 mg, 3.00 mmol, 522.53 μ L, 6.00 equiv.), DMAP (183.26 mg, 1.50 mmol, 3.00 equiv.) in anhydrous DMF (10 mL) was added dihydro-2H-pyran-2,6(3H)-dione (342.3 mg, 3.00 mmol, 6.00 equiv.) at 20 °C with N₂ bubbling. Then the mixture was bubbled by N₂ for 2 h. After a mini-cleavage test, LCMS showed the reaction was completed. The peptide resin (**Intermediate 30**) was washed with DMF (20 mL, 5x), used for next step directly. LCMS: RT = 1.21 min, MS cal.: M_{calc} = 1789.95, [M + 2H]²⁺ = 896.48.



Molecular Weight: 1789.96
30, after mini-cleavage

- 2) TFP ester formation: A solution of TFP (830.37 mg, 5.00 mmol, 10.00 equiv.) and DIC (631.00 mg, 5.00 mmol, 774.23 μ L, 10.00 equiv.) in anhydrous DMF (5 mL) was added to the resin-bound peptide (**Intermediate 30**) at 20 °C with N₂ bubbling. Then the mixture was bubbled by N₂ for 2 h. After a mini-cleavage test, LCMS showed the reaction was completed. The resin was washed with DMF (20 mL, 5x), 2-isopropoxypropane (20 mL, 3x), dried by N₂ bubbling to afford **Intermediate 31** (CTC resin, 0.5 mmol) as a bright yellow solid.
- 3) Cleavage: Cleavage solution (TFA/Tis/H₂O, 95/2.5/2.5, v/v/v, 20 mL) was added to the flask containing the side chain protected peptide at room temperature and stirred for 1h. After filtration, the filtrate was precipitated with isopropyl ether (cold, 100 mL). After filtration, the solid was washed with isopropyl ether (cold, 50 mL) for two additional times, and dried under reduced pressure for 2 h.
- 4) Cyclization: The crude peptide was dissolved in HOAc/MeCN/H₂O (4/3/3, v/v/v, 500 mL). Then the mixture was added 0.1 M I₂/AcOH dropwise until a yellow color persisted, then the mixture was stirred at 20 °C for 5 min. The mixture was quenched by addition of 0.1 M aq. Na₂S₂O₃ dropwise until the yellow color disappeared. After filtration, the filtrate was purified by prep-HPLC (A: 0.075% TFA/H₂O, B: MeCN), followed by lyophilization to afford **Intermediate 32** (73.0 mg, 89.2% purity, 6.7% yield) as a white solid. LCMS: RT = 1.31 min, MS cal.: M_{ov} = 1935.99, [M + 2H]²⁺ = 968.03.

[0006] Preparation

[0007] To a solution of **vcMMAE** (11.9 mg, 10.6 μmol , 1.00 equiv.) in DMSO (400 μL) was added **Intermediate 32** (20.0 mg, 10.6 μmol , 1.00 equiv.) and DIPEA (6.82 mg, 53.0 μmol , 5.00 equiv.) at 20 °C. The mixture was stirred at 20 °C for 2 h. LC-MS showed the starting material was consumed completely. The solvent was removed under reduced pressure. The residue was purified by prep-HPLC (TFA condition) to obtained (16.8 mg, 5.89 μmol , 55.8% yield, 96.8% purity) as a white solid. LCMS: RT = 1.118 min, MS cal.: M_{calc} = 2893.35, $[M + 2H]^{2+}$ = 1447.20, $[M + 3H]^{3+}$ = 965.15.

Preparative HPLC Conditions

Purification condition	
Dissolution condition	Dissolve in DMF
Instrument	GX-281C
Mobile Phase	A: H ₂ O (0.075% TFA in H ₂ O)
	B: CH ₃ CN
Gradient	44-64%B-40 min. Retention time: 26 min
Column	PN-C18, 250*30 mm, 5 um, 110Å
Flow Rate	20 mL/min
Wavelength	220/254 nm
Oven Tem.	Room temperature

5 Conjugation Method

Brentuximab ("Antibody A") was buffer exchanged for over 8 dilution volumes (DVs) with 50 mM HEPES buffer, pH 7.5. The target antibody concentration after buffer exchange was >14 mg/mL. A 10 mM stock solution in DMSO of Reagent A was then prepared for the conjugation (Reagent A: (S)-3-acetamido-4-(((4R,7S,10S,13S,16S,22S,25S,28S,31S,34R)-7,28-bis((1H-indol-3-yl)methyl)-25-(2-(((4-(((S)-1-(((S)-1-(((S)-1-(((4-(((5S,8S,11S,12R)-11-(((S)-sec-butyl)-12-(2-(((S)-2-(((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-5-oxopentanoyl)oxy)-3-fluorobenzyl)amino)-2-oxoethyl)-4-(((1S,2R)-1-carboxy-2-hydroxypropyl)carbamoyl)-16-(2-carboxyethyl)-13,22-diisobutyl-10-isopropyl-31-methyl-6,9,12,15,18,21,24,27,30,33-decaoxo-1,2-dithia-5,8,11,14,17,20,23,26,29,32-decaazacyclopentatriacontan-34-yl)amino)-4-oxobutanoic acid).

Conjugation of Reagent A to Antibody A (40 mg, 0.27 umol) was performed using 4 equivalents of Reagent A (1.07 umol, 3.12 mg) at an antibody concentration of 10 mg/mL in 50 mM HEPES buffer (pH 7.5) with 20%(v/v) DMSO at 25 °C and agitating for 48 hours.

20 The conjugation reaction was analyzed for drug to antibody ratio (DAR) by LC-MS every 24hrs. When the DAR reached >1.9, a buffer exchange by UFDF over 30 DVs to PBS, pH7.4 was performed to a

final concentration range of approximately 7 mg/mL. Reagent A was then analyzed for quality under various conditions. The results are shown in Table 1.

Table 1. Analytical Data.

5

Starting amount of antibody (mg)	Aggregation (%)	DAR	Yield (%)	Amount (mg)	Conc. (mg/mL)	Endotoxin (EU/mg)	Residual leaving group (% mol/mol of mAb)	Residual Reagent A (% mol/mol of total reagent)	Residual Payload (% mol/mol of total payload)
40	2.56	2.01	68.65	27.46	7.04	<0.085	<4.194	<2.908	<2.908

DAR Calculation:

$$DAR = \frac{1 \times Abundance_{DAR1} + 2 \times Abundance_{DAR2} + \dots + n \times Abundance_{DARn}}{Abundance_{DAR0} + \dots + Abundance_{DARn}}$$

10

Spectral characterization of the conjugate of brentuximab with Reagent A is provided in FIG. 1.

FIG. 2 illustrates properties of the site-specific antibody-drug conjugates according to embodiments of the present invention.

15 FIG. 3 illustrates challenges of alternate antibody-drug conjugate protein engineering and chemistry.

FIG. 4 illustrates potential best-in-class antibody-drug conjugates according to embodiments of the present invention.

FIG. 5 illustrates pharmacokinetic (PK) studies of Adcetris® and antibody-drug conjugate according to embodiments of the present invention.

20 FIG. 6 illustrates potential for superior efficacy of an antibody-drug conjugate according to embodiments of the present invention compared to Adcetris®.

FIG. 7 illustrates improved survival in a mouse model with an antibody-drug conjugate according to embodiments of the present invention compared to Adcetris®.

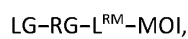
FIG. 8 illustrates results of the single dose 14-day mouse tumor xenograft study with an antibody-drug conjugate according to embodiments of the present invention.

5 Throughout this application, various publications are referenced by author name and date, or by patent number or patent publication number. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement
10 that such reference is prior art to the present invention.

 Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims. For example, pharmaceutically acceptable salts other than those specifically disclosed in the description and
15 Examples herein can be employed. Furthermore, it is intended that specific items within lists of items, or subset groups of items within larger groups of items, can be combined with other specific items, subset groups of items or larger groups of items whether or not there is a specific disclosure herein identifying such a combination.

CLAIMS

1. A compound having the structure of formula (R-I):



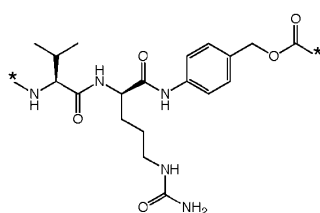
5

(R-I)

or a salt thereof, wherein:

LG is a group comprising a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;



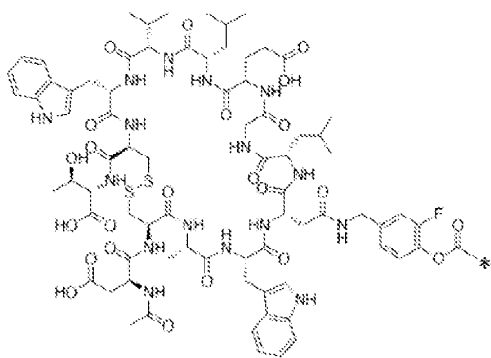
10

L^{RM} is a linking group comprising

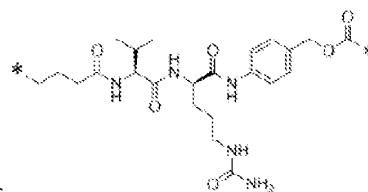
; and

MOI is moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

2. The compound of Claim 1, wherein LG-RG is or comprises

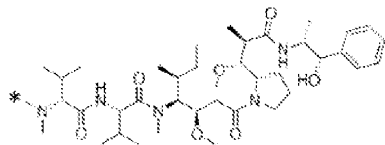


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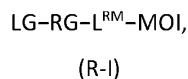
3. The compound of Claim 1 or 2, wherein L^{RM} is or comprises

4. The compound of any one of Claims 1 to 3, wherein MOI is or comprises



5. The compound of any one of Claims 1 to 4, wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety comprising L^{RM}-MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.
6. The compound of any one of Claims 1 to 5, wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety comprising L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

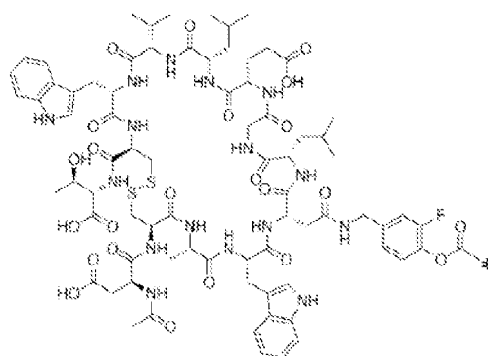
7. A compound having the structure of formula (R-I):



- or a salt thereof, wherein:

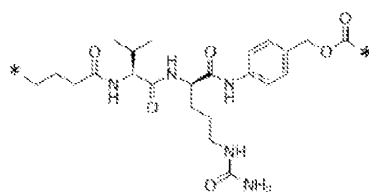
LG is a group comprising a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;



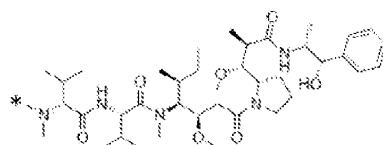
LG-RG is or comprises

;



L^{RM} is or comprises

; and

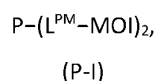


MOI is or comprises

8. The compound of Claim 7, wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety comprising L^{RM}-MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

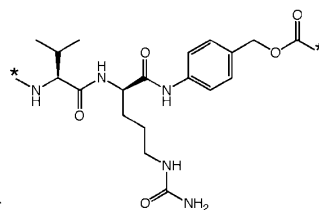
9. The compound of Claim 7 or 8, wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety comprising L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

10. A method of preparing an agent having the structure of formula (P-I):



or a salt thereof, wherein:

P is brentuximab or a biosimilar analog thereof;

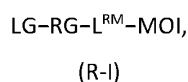


each L^{PM} is a linking group comprising ; and

each MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl
5 auristatin E (MMAE), or monomethyl auristatin F (MMAF),

the method comprising the steps of:

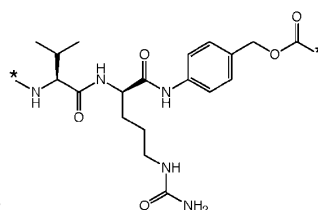
(1) contacting brentuximab or a biosimilar analog thereof with a reaction partner having the
structure of formula (R-I):



or a salt thereof, wherein:

LG is a group comprising a target binding moiety that binds to brentuximab or a biosimilar
analog thereof,

RG is a reactive group;

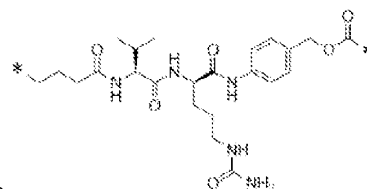
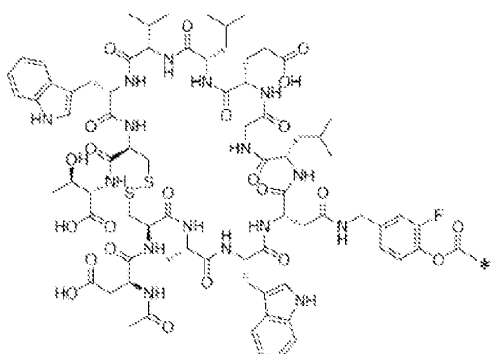


L^{RM} is a linking group comprising ;

MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl
auristatin E (MMAE), or monomethyl auristatin F (MMAF), and

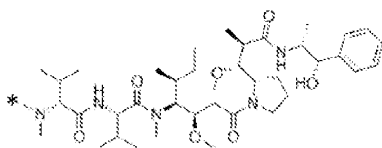
(2) forming an agent having the structure of formula (P-I).

11. The method of Claim 10, wherein LG-RG is or comprises



12. The method of Claim 10 or 11, wherein L^{RM} is or comprises

5 13. The method of any one of Claims 10 to 12, wherein MOI is or comprises



14. The method of any one of Claims 10 to 13, wherein the target binding moiety of the reagent partner is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety comprising L^{RM} -MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

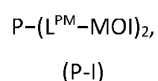
15 15. The method of any one of Claim 10 to 14, wherein the target binding moiety of the reagent partner is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety comprising

L^{RM} -MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

16. The method of any one of Claims 10 to 15, wherein the contacting and forming steps are performed in one chemical reaction.

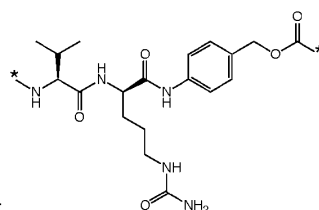
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17. An agent having the structure of formula (P-I):



or a salt thereof, wherein:

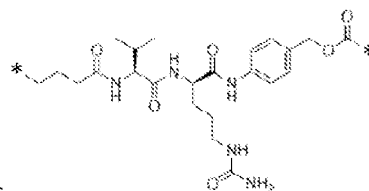
10 P is a brentuximab or a biosimilar analog thereof;



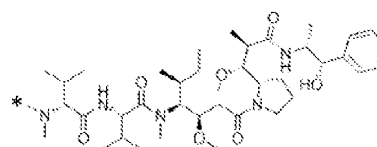
each L^{PM} is a linking group comprising

; and

each MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).



15 18. The agent of Claim 17, wherein L^{RM} is or comprises



19. The agent of Claim 17 or 18, wherein MOI is or comprises

20 20. The agent of any one of Claims 17 to 19, wherein each L^{PM} -MOI is attached to K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

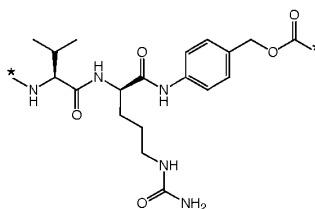
21. The agent of any one of Claims 17 to 20, wherein each L^{PM} -MOI is attached to K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

22. A method of preparing an agent having the structure of (P-II):



wherein:

P-NH is a brentuximab or a biosimilar analog thereof comprising a lysine residue having a terminal group NH;



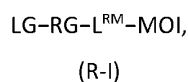
10 L^{PM} is a linking group comprising ; and

MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF),

the method comprising the steps of:

(1) providing brentuximab or a biosimilar analog thereof having a structure $P-(NH_2)_2$, wherein
15 each NH_2 is a terminal amino group of a lysine residue; and

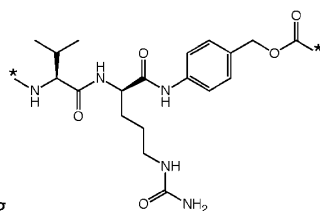
(2) contacting $P-(NH_2)_2$ with a reaction partner having a structure of formula (R-I):



or a salt thereof, wherein:

20 LG is a group comprising a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

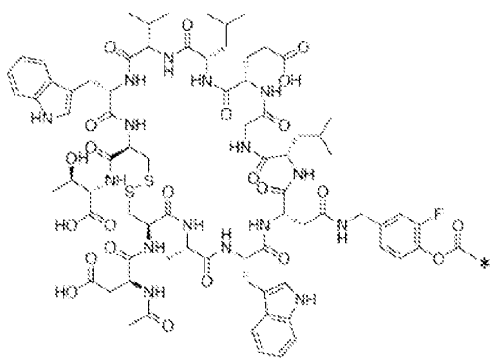
RG is a reactive group;



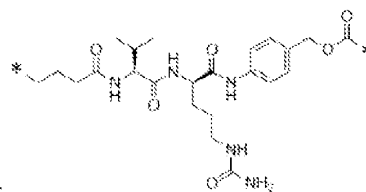
L^{RM} is a linking group comprising ; and

MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).

23. The method of Claim 22, wherein LG-RG is or comprises

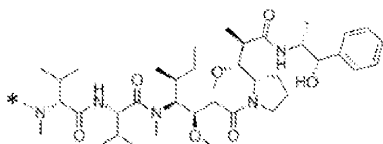


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24. The method of Claim 22 or 23, wherein L^{RM} is or comprises

25. The compound of any one of Claim 22 to 24, wherein MOI is or comprises



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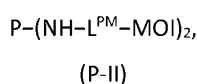
26. The method of any one of Claims 22 to 25, wherein the target binding moiety of the reagent partner is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety comprising L^{RM}-MOI to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

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27. The method of any one of Claims 22 to 26, wherein the target binding moiety of the reagent partner is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety comprising
- 5 L^{RM}-MOI to K248 and expulsion of the group containing a target binding moiety from the compound.

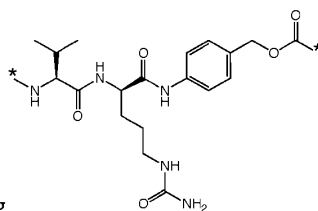
28. The method of any one of Claims 22 to 27, wherein the contacting and forming steps are performed in one chemical reaction.

- 10 29. An agent having the structure of (P-II):



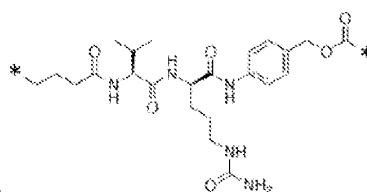
wherein:

- 15 P-NH is a brentuximab or a biosimilar analog thereof comprising a lysine residue having a terminal group NH;

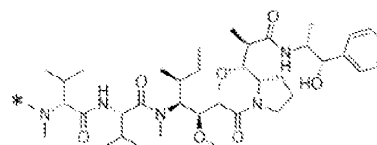


L^{PM} is a linking group comprising ; and

MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF).



- 20 30. The agent of Claim 29, wherein L^{RM} is or comprises

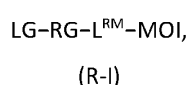


31. The agent of Claim 29 or 30, wherein MOI is or comprises

32. The agent of any one of Claims 29 to 31, wherein the lysine residue is K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

33. The agent of any one of Claims 28 to 32, wherein each lysine residue is K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof.

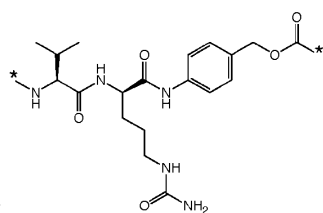
34. A compound having the structure of formula (R-I):



or a salt thereof, wherein:

LG is a group comprising a target binding moiety that binds to brentuximab or a biosimilar analog thereof,

RG is a reactive group;



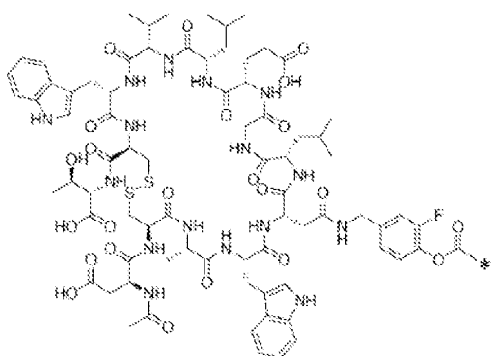
L^{RM} is a linking group comprising ; and

MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF),

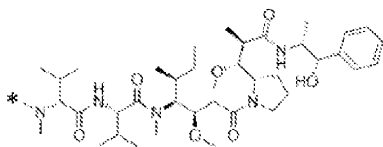
wherein brentuximab or a biosimilar analog thereof comprises an IgG heavy chain comprising K246 or K248, and

wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K246 or K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K246 or K248 and the reactive group that results in attachment of a moiety comprising $\text{L}^{\text{RM}}\text{-MOI}$ to K246 or K248 and expulsion of the group containing a target binding moiety from the compound.

35. The compound of Claim 34, wherein LG-RG is or comprises



36. The compound of Claim 34 or 35, wherein MOI is or comprises



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37. The compound of any one of Claims 34 to 36, wherein the target binding moiety is configured to bind brentuximab or a biosimilar analog thereof so as to bring the reactive group in proximity with K248 of a brentuximab IgG heavy chain or a biosimilar analog thereof to enable a reaction between K248 and the reactive group that results in attachment of a moiety comprising L^{RM}-MOI to K248 and expulsion of

10 the group containing a target binding moiety from the compound.

38. A method of treating Hodgkin lymphoma in a subject in need of such treatment, comprising administering to the subject a pharmaceutically effective amount of the agent according to any one of Claims 17 to 21 or 29 to 37.

15

39. A method of treating systemic anaplastic large cell lymphoma in a subject in need of such treatment, comprising administering to the subject a pharmaceutically effective amount of the agent according to any one of Claims 17 to 21 or 29 to 37.

20 40. A method of treating primary cutaneous anaplastic large cell lymphoma in a subject in need of such treatment, comprising administering to the subject a pharmaceutically effective amount of the

agent according to any one of Claims 17 to 21 or 29 to 37.

41. A method of treating CD30-expressing mycosis fungoides in a subject in need of such treatment, comprising administering to the subject a pharmaceutically effective amount of the agent according to
5 any one of Claims 17 to 21 or 29 to 37.

42. A method of treating cutaneous T cell lymphoma in a subject in need of such treatment, comprising administering to the subject a pharmaceutically effective amount of the agent according to any one of Claims 17 to 21 or 29 to 37.

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43. A method of selectively targeting tumor cells expressing the CD30 antigen in a subject, comprising administering to the subject the agent according to any one of Claims 17 to 21 or 29 to 37.

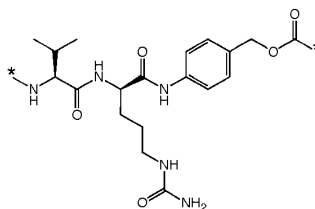
44. A composition comprising:

15 a first compound having the structure of formula (P-II):



wherein:

P-N is brentuximab or a biosimilar analog thereof comprising a lysine residue;



L^{PM} is a linking group comprising ; and

20

MOI is a moiety of interest comprising monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF); and

a second compound having the structure:



wherein LG is a group comprising a target binding moiety that binds to brentuximab or a
25 biosimilar analog thereof.

45. The composition of Claim 44, further comprising:

a third compound having the formula (R-I):



LG is a group comprising a target binding moiety that binds to brentuximab or a biosimilar analog thereof, which is identical to LG in formula (LG-I);

RG is a reactive group;

5 L^{RM} is a linking group, which is identical to that in formula (P-II); and

MOI is a moiety of interest which is identical to that in formula (P-II);

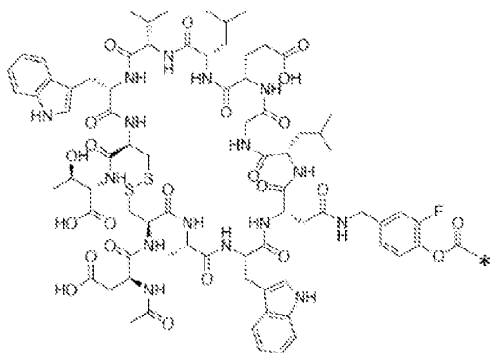
a fourth compound having the formula (R-III):



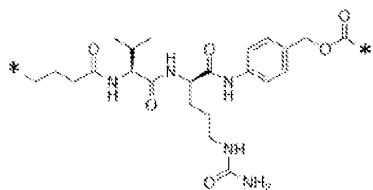
or a combination thereof.

10

46. The compound of Claim 44 or 45, wherein LG-RG is or comprises



47. The compound of any one of Claim 44 to 46, wherein L^{RM} is or comprises



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48. The compound of any one of Claims 44 to 47, wherein MOI is or comprises

