

ANTIBODY-DRUG CONJUGATE COMPRISING ANTIBODIES AGAINST HUMAN L1CAM AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to Korean Republic Application No. 10-2023-0032106, filed March 10, 2023, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND

Cancer is a disease caused by abnormal and uncontrolled cell growth in the tissues of the body and is the result of uncontrolled cell growth in a variety of tissues. Tumors in early-stage cancers may be removed through surgical and radiotherapeutic measures, and metastasized tumors are generally treated palliatively using chemotherapy. Most chemotherapy agents administered non-orally may induce unwanted adverse effects or serious toxicity as a result of systemic administration. Accordingly, the focus of development has been on developing novel chemotherapy agents to achieve improved efficacy and minimal toxicity/adverse effects through improved and selective action of chemotherapy agents on tumor cells or immediately adjacent tissues.

An antibody-drug conjugate (ADC) is a targeted technology where a toxin or drug is bonded to an antibody which in turn binds to an antigen, the toxin or drug released into a cell to cause cell death of tumor cells. The technology has superior efficacy over antibody drugs and is able to substantially reduce the risk of adverse effects compared to conventional anti-cancer agents, because it specifically delivers drugs to the target cancer cells with minimum impact to healthy cells, and only releases drugs under specific conditions.

The basic structure of an antibody-drug conjugate is “antibody-linker-small molecule drug (toxin)”. Here, the linker needs to play not only the functional role of linking the antibody and drug, but also ensure that the drug is released properly through antibody-drug dissociation (e.g. as a result of hydrolysis by enzyme) after being circulated through the body and reaching the target cells, and exhibits efficacy against the target cancer cells. In other words, the stability of the linker plays a very important role in the efficacy and systemic toxicity of an antibody-drug conjugate (*Discovery Medicine* 2010, 10(53): 329-39). 85-8 2018-08-14).

The use of monoclonal antibodies for cancer treatment is having substantial success. Monoclonal antibodies are suitable for target-oriented addressing of tumor tissue and tumor cells. Antibody-drug conjugates have become a novel and powerful option for therapy of lymphomas and solid cancers, and recently, immunoregulatory antibodies are seeing significant success in clinical trials. Development of therapeutic antibodies is based on a profound understanding of cancer serology, protein engineering technology, mechanisms of action and resistance, and interactions between immune systems and cancer cells.

Antigens which are expressed on the surface of human cancer cells refer to a broad range of targets which are over-expressed compared to normal tissues, mutated or selectively expressed. The key problem is identifying the appropriate antigens for antibody-based therapies. These therapeutic agents mediate changes in antigen or receptor function (i.e., as a stimulant or antagonist), regulate the immune system through Fc and T cell activation, and exhibit efficacy through the delivery of specific drugs that bind to antibodies targeting specific antigens. Molecular techniques that can alter antibody pharmacokinetics, function, size and immune stimulation are emerging as key elements in the development of novel antibody-based therapies. Evidence from clinical trials of therapeutic antibodies in cancer patients highlights the importance of approaches for selecting optimized antibodies, including affinity and binding of the target antigen and antibodies, selection of antibody structure, and therapeutic approaches (blocking signaling or immune function).

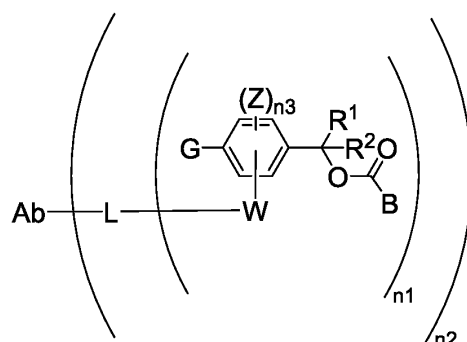
In view of the foregoing, there is an ongoing need for new antibody drug conjugates to treat diseases such as cancer.

SUMMARY OF THE INVENTION

The present invention provides, among other things, antibody-drug conjugates (ADCs) targeting L1CAM (Neural cell adhesion molecule L1), active metabolites of such ADCs, methods for preparation of such ADCs, uses for such ADCs in treatment and/or prevention of illnesses (e.g., proliferative and/or angiogenetic diseases, for example, cancer). More particularly, the present invention provides antibody-drug conjugates comprising an antibody binding to L1CAM or an antigen-binding fragment thereof, and a pharmaceutical composition comprising the same.

In one aspect, the present disclosure provides conjugates having a structure represented by Formula I:

[Formula I]



wherein

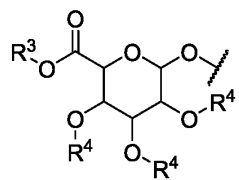
Ab is anti-human neural cell adhesion molecule L1 (L1CAM) antibody or an antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 8;
- (b) a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 10;
- (c) a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 14;
- (d) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 4;
- (e) a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5;

and

- (f) a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6,

each B is independently an active agent;



each G is independently a glucuronic acid moiety or

R³ is hydrogen or a carboxyl protecting group;

each R⁴ is independently hydrogen or a hydroxyl protecting group;

R¹ and R² are each independently hydrogen, C₁₋₈ alkyl, or C₃₋₈ cycloalkyl;

W is each independently -C(O)-, -C(O)NR', -C(O)O-, -SO₂NR', -P(O)R''NR', -SONR' or

-PO₂NR', wherein the C, S or P directly binds to the phenyl ring and the NR' binds to L;

R' and R'' are each independently hydrogen, C₁₋₈ alkyl, C₃₋₈ cycloalkyl, C₁₋₈ alkoxy, C₁₋₈

alkylthio, mono- or di-C₁₋₈ alkylamino, C₃₋₁₀ heteroaryl or C₆₋₁₀ aryl;

each Z is independently C₁₋₈ alkyl, halogen, cyano or nitro;

each L is independently a linker and comprises:

A) C₁₋₅₀ alkylene or 1-50 membered heteroalkylene comprising:

(i) one or more unsaturated bonds;

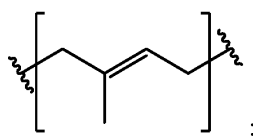
(ii) a heteroarylene (e.g., a heteroarylene in the alkylene or heteroalkylene chain);

or

(iii) a C₁₋₂₀ alkyl substituent; and/or

B) at least one isoprenyl unit having a structure represented by Formula II:

[Formula II]



n₁ and n₂ are each independently integers selected from 1 to 20; and

n₃ is an integer of 0 to 3.

In another aspect, the present disclosure provides pharmaceutical compositions comprising a conjugate disclosed herein and a pharmaceutically acceptable excipient.

In yet another aspect, the present disclosure provides methods of treating or preventing hyperplasia, cancer, or angiogenic disease in a subject, comprising administering a conjugate disclosed herein or a pharmaceutically acceptable salt thereof to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram showing the production method and structure of an exemplified antibody-drug conjugate (ADC1).

FIG. 2 is a schematic diagram showing the production method and structure of an exemplified antibody-drug conjugate (ADC2).

FIG. 3 is a schematic diagram showing the production method and structure of an exemplified antibody-drug conjugate (ADC3).

FIG. 4 is a graph showing the hydrophobic properties of antibody AFF4-CaaX, prenylated antibody AFF4-CaaX, and antibody-drug conjugates ADC1-ADC3 through HIC-HPLC.

FIG. 5 is a graph showing the molecular weight of antibody-drug conjugates ADC1 and ADC3 through intact mass.

FIG. 6 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a breast cancer cell line JIMT-1 heterograft model.

FIG. 7 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a colorectal cancer cell line SW480 heterograft model.

FIG. 8 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a melanoma cancer cell line MeWo heterograft model.

FIG. 9 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a non-small cell lung cancer cell line Calu-6 heterograft model.

FIG. 10 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in an ovarian cancer cell line SNU840 heterograft model.

FIG. 11 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a pancreatic cancer cell line Patu8988s heterograft model.

FIG. 12 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a small cell lung cancer cell line NCI-H146 heterograft model.

FIG. 13 is a graph showing the tumor growth suppression effect of antibody-drug conjugates in a small cell lung cancer cell line NCI-H69 heterograft model.

FIG. 14 is a graph showing the pharmacokinetics of antibody-drug conjugates.

FIG. 15 is a graph showing the lack of acute toxicity of antibody-drug conjugates.

DETAILED DESCRIPTION OF THE INVENTION

Human derived L1CAM (Neural cell adhesion molecule L1) is a 200-220 kDA transmembrane protein having 323 amino acids (1101 extracellular regions, 23 transmembrane regions, and 114 intracellular regions) and widely known as a neuronal cell adhesion molecule. L1CAM belongs to the L1 protein family that performs important roles in the transport of nerve cells, growth of neurites, axonal guidance, promotion of axons and dendrites, and myelination and synapse formation. L1CAM is expressed in areas where cancer stem cells exist in specific tumors and is widely known as serving a causal role in biological reactions closely related with cancer stem cells, such as endothelial-mesenchymal transition (EMT) and drug resistance. Additionally, L1CAM is abnormally expressed in malignant tumors, such as various neuroblasts, solenoma, gynecologic cancer (cervical cancer, ovarian cancer, uterine cancer, endometrial cancer), ovarian cancer, pancreatic cancer, lung cancer, colorectal cancer, cholangiocarcinoma, and melanoma, and

is widely known as being directly related with treatment resistance and the poor prognosis of malignant tumors.

In certain aspects, the present invention provides an antibody-linker-drug (e.g., toxin) system. The disclosed antibodies bind to L1CAM, which is abnormally expressed in various cancer tissue. Additionally, the antibody-linker-drug comprises an effective self-immolative group to strengthen the effect of the antibodies further. The effective self-immolative group is stable in the plasma and in circulation and allows a drug to be easily released and exhibit efficacy in a cancer cell. A linker comprising an effective self-immolative group is stable in the plasma and in circulation and allows a drug to be easily released and exhibit efficacy in a cancer cell (See US Patent No. 9,919,057, incorporated herein by reference). As a result, disclosed herein is a novel antibody-drug conjugate (ADC) that effectively targets L1CAM in the treatment and/or prevention of proliferative and/or metastatic cancers. By applying a linker including an effective self-immolative group, which is stable in the plasma and in circulation, yet allows a drug to be easily released and exhibit efficacy in a cancer cell, to an anti-L1CAM antibody to further reinforce the effect of the antibody, it is possible to provide a useful antibody-drug conjugate (ADC) that effectively targets L1CAM in the treatment and/or prevention of diseases (e.g., proliferative and/or metastatic cancers).

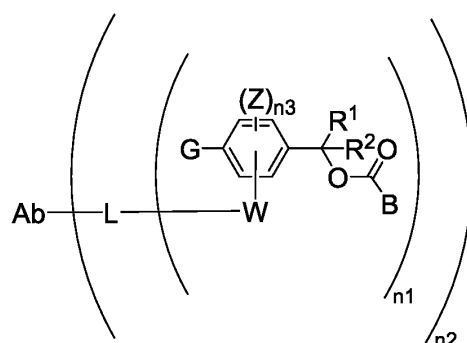
Antibody-drug conjugates disclosed herein, by comprising an anti-L1CAM antibody that more strongly binds specifically to the type of L1CAM mainly exhibited in tumors, is able to effectively, specifically, and selectively deliver drugs to cells expressing L1CAM. By having an antibody stably bonded to a drug to maintain *in vivo* stability while exhibiting the intended cytotoxicity, and in particular the stability in the serum, and even in circulation, and by employing linker technology which includes a self-immolative group which allows a drug to be easily released within a cancer cell to maximize efficacy, these conjugates exhibit an effect of providing an antibody-linker-drug system which allows a drug and/or toxin to safely reach a target cell and effectively exhibit efficacy while having significantly reduced toxicity.

Therefore, in certain embodiments, the present invention provides antibody-drug conjugates targeting L1CAM, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present invention provides pharmaceutical compositions comprising the antibody-drug conjugates disclosed herein or a pharmaceutically acceptable salt thereof.

In one aspect, the present disclosure provides conjugates having a structure represented by Formula I:

[Formula I]



wherein

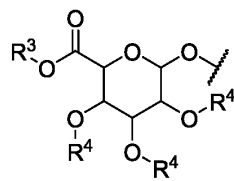
Ab is anti-human neural cell adhesion molecule L1 (L1CAM) antibody or an antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 8;
- (b) a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 10;
- (c) a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 14;
- (d) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 4;
- (e) a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5;

and

- (f) a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6,

each B is independently an active agent;



each G is independently a glucuronic acid moiety or

R³ is hydrogen or a carboxyl protecting group;

each R⁴ is independently hydrogen or a hydroxyl protecting group;

R¹ and R² are each independently hydrogen, C₁₋₈ alkyl, or C₃₋₈ cycloalkyl;

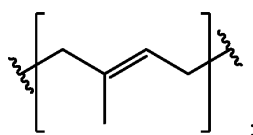
W is each independently -C(O)-, -C(O)NR'-, -C(O)O-, -SO₂NR'-, -P(O)R''NR'-, -SONR'- or -PO₂NR'-, wherein the C, S or P directly binds to the phenyl ring and the NR' binds to L;
 R' and R'' are each independently hydrogen, C₁₋₈ alkyl, C₃₋₈ cycloalkyl, C₁₋₈ alkoxy, C₁₋₈ alkylthio, mono- or di-C₁₋₈ alkylamino, C₃₋₁₀ heteroaryl or C₆₋₁₀ aryl;
 each Z is independently C₁₋₈ alkyl, halogen, cyano or nitro;
 each L is independently a linker and comprises:

A) C₁₋₅₀ alkylene or 1-50 membered heteroalkylene comprising:

- (i) one or more unsaturated bonds;
 - (ii) a heteroarylene (e.g., a heteroarylene in the alkylene or heteroalkylene chain);
- or
- (iii) a C₁₋₂₀ alkyl substituent; and/or

B) at least one isoprenyl unit having a structure represented by Formula II:

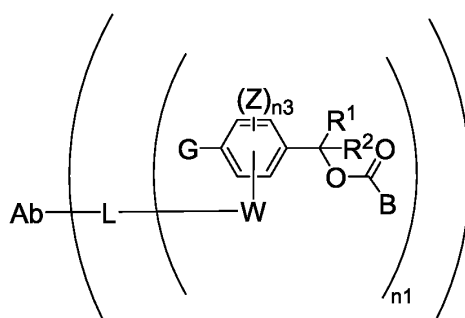
[Formula II]



n₁ and n₂ are each independently integers selected from 1 to 20; and
 n₃ is an integer of 0 to 3.

In yet another aspect, the present disclosure provides conjugates having a structure represented by Formula I:

[Formula I]

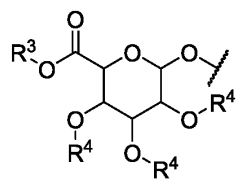


wherein

Ab is anti-human neural cell adhesion molecule L1 (L1CAM) antibody or an antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising an amino acid sequence (GYSITSDYX₁WN) of SEQ ID NO: 16; wherein the X₁ is A or T;
- (b) a heavy chain CDR2 comprising an amino acid sequence (YISYSGSX₁SYX₂PSLKS) of SEQ ID NO: 17; wherein the X₂ is H or N;
- (c) a heavy chain CDR3 comprising an amino acid sequence (SX₁SYX₂YGFAY) of SEQ ID NO: 18; wherein the X₁ is L or F, and the X₂ is G, S, or A;
- (d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
- (e) a light chain CDR2 comprising an amino acid sequence (SASYRYX₁) of SEQ ID NO: 19; wherein the X₁ is T or I; and
- (f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6;

each B is independently an active agent;



each G is independently a glucuronic acid moiety or

R³ is hydrogen or a carboxyl protecting group;

each R⁴ is independently hydrogen or a hydroxyl protecting group;

R¹ and R² are each independently hydrogen, C₁₋₈ alkyl, or C₃₋₈ cycloalkyl;

W is each independently -C(O)-, -C(O)NR', -C(O)O-, -SO₂NR', -P(O)R''NR', -SONR' or

-PO₂NR', wherein the C, S or P directly binds to the phenyl ring and the NR' binds to L;

R' and R'' are each independently hydrogen, C₁₋₈ alkyl, C₃₋₈ cycloalkyl, C₁₋₈ alkoxy, C₁₋₈

alkylthio, mono- or di-C₁₋₈ alkylamino, C₃₋₁₀ heteroaryl or C₆₋₁₀ aryl;

each Z is independently C₁₋₈ alkyl, halogen, cyano or nitro;

each L is independently a linker and comprises:

A) C₁₋₅₀ alkylene or 1-50 membered heteroalkylene comprising:

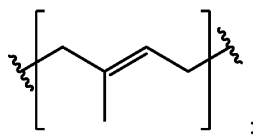
- (i) one or more unsaturated bonds;
- (ii) a heteroarylene (e.g., a heteroarylene in the alkylene or heteroalkylene chain);

or

- (iii) a C₁₋₂₀ alkyl substituent; and/or

B) at least one isoprenyl unit having a structure represented by Formula II:

[Formula II]



n1 and n2 are each independently integers selected from 1 to 20; and

n3 is an integer of 0 to 3.

In some embodiments, the conjugate comprises an antibody or antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8,;
- (b) a heavy chain CDR2 comprising an amino acid sequence (YISYSGSX₁SYX₂PSLKS) of SEQ ID NO: 17; wherein the X₁ is Y, and the X₂ is N;
- (c) a heavy chain CDR3 comprising an amino acid sequence (SX₁SYX₂YGFAY) of SEQ ID NO: 18; wherein X₁ is F, and X₂ is S;
- (d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
- (e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
- (f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6.

In some embodiments, the conjugate comprises an antibody or antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 1 and 8,;
- (b) a heavy chain CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 9, 10, and 11,;
- (c) a heavy chain CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 3, 12, 13, and 14;
- (d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
- (e) a light chain CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 5 and 15; and
- (f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6.

In some embodiments, the conjugate comprises an antibody or antigen-binding fragment thereof comprising:

- (i) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 10;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (ii) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 1;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (iii) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (iv) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 1;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 10;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (v) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 11;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 13;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and

- (f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (vi) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 13;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 15; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (vii) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 15; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (viii) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 1;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 10;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 12;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (ix) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 11;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 3;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6; or,
- (x) (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 1;
(b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 2;
(c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 3;
(d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
(e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
(f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6.

In some embodiments, the conjugate comprises an antibody or antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 8;
- (b) a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 10;
- (c) a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;
- (d) a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 4;
- (e) a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and
- (f) a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 6.

In some embodiments, variables X₁ or X₂ included in one SEQ ID NO may be an amino acid sequence independently selected from X₁ or X₂ included in another SEQ ID NO. For example, variables X₁ or X₂ included in SEQ ID NO: 17 may each be an amino acid sequence independently selected from variable X₁ included in SEQ ID NO: 16, variables X₁ or X₂ included in SEQ ID NO: 18, or variable X₁ included in SEQ ID NO: 19.

In some embodiments, the antibody may include mutations at the Fc site, specifically, 1 to 10 mutations (1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations). Each mutation may be introduced to one chain of the Fc site, or independently and symmetrically introduced to two chains of the Fc site.

In some embodiments, the antibody may include mutants at the CL site and/or CH1, specifically, 1 to 10 mutations (1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations).

In various embodiments, a mutation refers to a deletion (the deletion of one amino acid), an insertion (an insertion of one amino acid), or a substitution (a substitution of one amino acid for another different amino acid). In various embodiments including multiple mutations, each amino acid difference can be independently selected from, e.g., a deletion, an insertion, or a substitution.

In some embodiments, the antibody or antigen-binding fragment thereof may comprise a heavy chain variable region comprising the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 amino acid sequences listed in **Table 1**. Specifically, the antibody and antigen-binding fragment thereof may comprise H1 to H4 for a heavy chain variable domain of humanized antibody of mouse antibody OV549.20. AFF1 to AFF10 may have a further progressed affinity maturation process in humanized antibodies.

Table 1. Heavy Chain CDR Sequences

Antibody	CDRH1 (SEQ ID No.)	CDRH2 (SEQ ID No.)	CDRH3 (SEQ ID No.)
----------	--------------------	--------------------	--------------------

H1, H2, H3, H4 (heavy chain variable region)	GYSITSDYAWN (1)	YISYSGSFSYHPSLKS (2)	SFSYGYGFAY (3)
AFF1, AFF3, AFF7, AFF9	GYSITSDYAWN (1)		
AFF2, AFF4, AFF5, AFF6, AFF8, AFF10	GYSITSDYTWN (8)		
AFF1, AFF2, AFF6, AFF7, AFF8		YISYSGSFSYNPSLKS (9)	
AFF3, AFF4, AFF9		YISYSGSYNPSLKS (10)	
AFF5, AFF10		YISYSGSYHPSLKS (11)	
AFF5, AFF6			SLSYGYGFAY (13)
AFF10			SFSYGYGFAY (3)
AFF9			SFSYAYGFAY (12)
AFF1, AFF2, AFF3, AFF4, AFF7, AFF8			SFSYSYGFAY (14)
AFF4	GYSITSDYTWN (8)	YISYSGSYNPSLKS (10)	SFSYSYGFAY (14)
Humanized antibody (human IgG1)	GYSITSDYTWN (8)	YISYSGSYNPSLKS (10)	SFSYSYGFAY (14)
Consensus sequences	GYSITSDYX ₁ W N (16) X ₁ is A or T	YISYSGSX ₁ SYX ₂ PSLK S (17) X ₁ is F or Y X ₂ is H or N	SX ₁ SYX ₂ YGFAY (18) X ₁ is L or F, X ₂ is G, S, or A

In some embodiments, the antibody or antigen-binding fragment thereof may comprise a light chain variable region comprising light chain CDR1, light chain CDR2, and light chain CDR3 amino acid sequences listed in **Table 2**. Specifically, the antibody and antigen-binding fragment thereof may comprise L1 or L2 for a light chain variable domain of humanized antibody of mouse antibody OV549.20. The antibody may be combined with the H1 to H4 heavy chain variable domain, and L1 or L2 light chain variable domain shown in **Table 1**.

Table 2. Light Chain CDR Sequences

Antibody	CDRL1 (SEQ ID No.)	CDRL2 (SEQ ID No.)	CDRL3 (SEQ ID No.)
----------	-----------------------	-----------------------	-----------------------

L1, L2 (light chain variable region) [Combine with H1 to H4 of heavy chain site]	KASQDVSSAVA (4)	SASYRYT (5)	QQHYSTPWT (6)
AFF1, AFF2, AFF3, AFF4, AFF5, AFF6, AFF7, AFF8, AFF9, AFF10	KASQDVSSAVA (4)		QQHYSTPWT (6)
AFF1, AFF2, AFF3, AFF4, AFF5, AFF6, AFF9, AFF10		SASYRYT (5)	
AFF7, AFF8		SASYRYI (15)	
AFF4		SASYRYT (5)	QQHYSTPWT (6)
Humanized antibody (human IgG1)	KASQDVSSAVA (4)	SASYRYT (5)	QQHYSTPWT (6)
Consensus sequences	KASQDVSSAVA (4)	SASYRYX ₁ (19) X ₁ is T or I	QQHYSTPWT (6)

In some embodiments, the antibody or antigen-binding fragment thereof comprises one or more specific framework regions that are described herein.

In some embodiments, the antibody or antigen-binding fragment thereof comprises one or more types of arbitrary framework sequences. Specifically, the framework region sequence may be a human framework sequence.

In the present specification, the term “framework (FR) amino acid moiety” refers to an amino acid in the framework region of the immunoglobulin chain. In the present specification, the terms “framework” or “FR region” refer to amino acid moieties in variable regions that are not part of the CDRs. Each variable domain typically has four FRs, identified as FR1, FR2, FR3, and FR4. The framework region generally supports binding to an antigen by supporting the structure of an antibody (not contacting an antigen) or directly contacting an antigen. The method of producing an antibody having a CDR sequence is well known by a person skilled in the art, for example, and may comprise introducing an expression vector encoding the desired framework sequence of a nucleic acid sequence encoding the CDR.

In some embodiments, FR1 to FR4 of the heavy chain variable region may be independently selected from FR1 to FR4 of the light chain variable region. Specifically, an FR of the heavy chain variable region may comprise an amino acid sequence of **Table 3**, and an FR of the light chain variable region may comprise an amino acid sequence of **Table 4**.

In some embodiments, the heavy chain variable region may comprise a framework region including an amino acid sequence of SEQ ID NO: 39, and 41-46. Specifically, the heavy chain variable region of an antibody may comprise 1 to 4 framework regions (FR) selected from amino acid sequences of SEQ ID NO: 39, and 41-46. More specifically, the heavy chain variable region may comprise FR1, CDRH1, FR2, CDRH2, FR3, CDRH3, and FR4. More specifically, FR1 of a heavy chain variable region may comprise an amino acid sequence of SEQ ID NO: 39; FR2 of a heavy chain variable region may comprise an amino acid sequence selected from SEQ ID NO: 41 to 44; FR 3 of heavy chain variable region may comprise an amino acid sequence of SEQ ID NO: 45; and FR 4 of a heavy chain variable region may comprise an amino acid sequence of SEQ ID NO: 46.

Table 3. VH Framework Region Sequences

Antibody	VH FR1 (SEQ ID No.)	VH FR2 (SEQ ID No.)	VH FR3 (SEQ ID No.)	VH FR4 (SEQ ID No.)
H1-H4AFF1-AFF10 Humanized antibody (human IgG1)	QVQLQESGPG LVKPSQTLSLT CTVT (39)		RVTISRDTSKNQFS LKLSSVTAADTAV YYCAR (45)	
H1,AFF1-AFF10 Humanized antibody (human IgG1)		WIRQFPGKG LEWMG (41)		
H2		WIRQFPGNG LEWIG (42)		
H3		WIRQFPGNG LEWMG (43)		
H4		WIRQFPGNK VEWMG (44)		
H1-H4,AFF1-AFF10 Humanized antibody (human IgG1)				WGQGTLLVTV SS (46)

In some embodiments, the light chain variable region may comprise a framework region comprise an amino acid sequence selected from SEQ ID NO: 51-56 or SEQ ID NO: 60. Specifically, the light chain variable region of an antibody may comprise 1 to 4 framework regions

(FR) selected from amino acid sequences of SEQ ID NO: 51-56 and SEQ ID NO: 60. More specifically, the light chain variable region may comprise FR1, CDRL1, FR2, CDRL2, FR3, CDRL3, and FR4. More specifically, FR1 of a light chain variable region may comprise an amino acid sequence selected from SEQ ID NO: 51 and 55; FR2 of a light chain variable region may comprise an amino acid sequence selected from SEQ ID NO: 48 or 52; FR3 of a light chain variable region may comprise an amino acid sequence selected from SEQ ID NO: 53 and 56; and FR4 of a light chain variable region may comprise an amino acid sequence selected from SEQ ID NO: 50, 54, or 60.

Table 4. VL Framework Region Sequences

Antibody	VL FR1 (SEQ ID NO)	VL FR2 (SEQ ID NO)	VL FR3 (SEQ ID NO)	VL FR4 (SEQ ID NO)
L1, AFF1-AFF10 Humanized antibody (human IgG1)	DIQMTQSPSSLS ASVGDRVITC (51)	WYQQKPGK APKLLIY (52)	GVPSRFSGSGSGTDF TFTISSLQPEDFATYY C (53)	FGGGTKVE IK (54)
L2	DIVMTQSPSSLS ASVGDRVITC (55)	WYQQKPGQ SPKLLIY (48)	GVPDRFSGSGSGTDF TFTISSLQPEDFATYY C (56)	FGGGTKLE IK (50)

In some embodiments, an antibody or antigen-binding fragment thereof that specifically binds to human L1CAM (Neural cell adhesion molecule L1) may comprise a heavy chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 23-34, and may comprise a light chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 20-22. More specifically, as shown in **Table 5**, the heavy chain variable region may comprise an amino acid sequence selected from SEQ ID NOs: 23-34, and the light chain variable region may comprise an amino acid sequence selected from SEQ ID NOs: 20-22.

Table 5.VH and VL Sequences

Antibody	Heavy chain variable region	SEQ ID NO:	Light chain variable region	SEQ ID NO:

H1	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWN <u>WIRQFPGKGLEW</u> MGYISYSGSFSYHPSLKSRVTISR TSKNQFSLKLSSVTAADTAVYYCA RSFSYGYGFAYWGQGLTVTVSS	23	[L1 or L2]	
H2	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWN <u>WIRQFPGNGLEW</u> IGYISYSGSFSYHPSLKSRVTISRDT SKNQFSLKLSSVTAADTAVYYCAR SFSYGYGFAYWGQGLTVTVSS	24	[L1 or L2]	
H3	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWN <u>WIRQFPGNGLEW</u> MGYISYSGSFSYHPSLKSRVTISR TSKNQFSLKLSSVTAADTAVYYCA RSFSYGYGFAYWGQGLTVTVSS	25	[L1 or L2]	
H4	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWN <u>WIRQFPGNKVE</u> WMGYISYSGSFSYHPSLKSRVTISR DTSKNQFSLKLSSVTAADTAVYYC AR <u>SFSYGYGFAYWGQGLTVTVSS</u>	26	[L1 or L2]	
L1	[H1 to H4]		DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY SASYRYTGVP SRFSGSGSGT DFTFTISLQP EDFATYYCQQ HYSTPWTFG GGTKVEIK	20

L2	[H1 to H4]		DIVMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGQSPKLLIYS ASYRYTGVPD RFSGSGSGTD FTFTISSLQPE DFATYYCQQ HYSTPWTFG GGTKLEIK	22
AFF1	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWNWIRQFPGKGLEW MGYISYSGSFSYNPSLKSRVTISRD TSKNQFSLKLSSVTAADTAVYYCA RSFSYSYGFAYWGQGLVTVSS	27	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY SASYRYTGVP SRFSGSGSGT DFTFTISSLQP EDFATYYCQQ HYSTPWTFG GGTKVEIK	20
AFF2	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWNWIRQFPGKGLEW MGYISYSGSFSYNPSLKSRVTISRD TSKNQFSLKLSSVTAADTAVYYCA RSFSYSYGFAYWGQGLVTVSS	28	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY SASYRYTGVP SRFSGSGSGT DFTFTISSLQP EDFATYYCQQ	20

			<u>HYSTPWTFG</u> GGTKVEIK	
AFF3	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWN <u>WIRQ</u> FPKGLEW MGYISYSGSYSYNPSLK <u>SRVT</u> ISRD TSKNQFSLKLSSVTAADTAVYYCA <u>RSFSYSYG</u> FAYWGQGLTVTVSS	29	DIQMTQSPSS LSASVGDRVT <u>ITCKASQDVS</u> <u>SAVA</u> WYQQK PGKAPKLLIY <u>SASYRYT</u> GVP SRFSGSGSGT DFTFTISLQP EDFATYYC <u>QQ</u> <u>HYSTPWTFG</u> GGTKVEIK	20
AFF4	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWN <u>WIRQ</u> FPKGLEW MGYISYSGSYSYNPSLK <u>SRVT</u> ISRD TSKNQFSLKLSSVTAADTAVYYCA <u>RSFSYSYG</u> FAYWGQGLTVTVSS	30	DIQMTQSPSS LSASVGDRVT <u>ITCKASQDVS</u> <u>SAVA</u> WYQQK PGKAPKLLIY <u>SASYRYT</u> GVP SRFSGSGSGT DFTFTISLQP EDFATYYC <u>QQ</u> <u>HYSTPWTFG</u> GGTKVEIK	20
AFF5	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWN <u>WIRQ</u> FPKGLEW MGYISYSGSYSYHPSLK <u>SRVT</u> ISRD TSKNQFSLKLSSVTAADTAVYYCA <u>RSLSYGYG</u> FAYWGQGLTVTVSS	31	DIQMTQSPSS LSASVGDRVT <u>ITCKASQDVS</u> <u>SAVA</u> WYQQK PGKAPKLLIY <u>SASYRYT</u> GVP SRFSGSGSGT	20

			DFTFTISSLQP EDFATYYC <u>QQ</u> HYSTPWTFG GGTKVEIK	
AFF6	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWNWIRQFPGKGLEW MGYISYSGSFSYNPSLKSRVTISR TSKNQFSLKLSSVTAADTAVYYCA RSLSYGYGFAYWGQGLVTVSS	32	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY SASYRYTGVP SRFSGSGSGT DFTFTISSLQP EDFATYYC <u>QQ</u> HYSTPWTFG GGTKVEIK	20
AFF7	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWNWIRQFPGKGLEW MGYISYSGSFSYNPSLKSRVTISR TSKNQFSLKLSSVTAADTAVYYCA RSFSYSYGFAYWGQGLVTVSS	27	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY SASYRYIGVP SRFSGSGSGT DFTFTISSLQP EDFATYYC <u>QQ</u> HYSTPWTFG GGTKVEIK	21
AFF8	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWNWIRQFPGKGLEW MGYISYSGSFSYNPSLKSRVTISR TSKNQFSLKLSSVTAADTAVYYCA RSFSYSYGFAYWGQGLVTVSS	28	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY	21

			<u>SASYRYIGVP</u> SRFSGSGSGT DFTFTISLQP EDFATYYC <u>QQ</u> <u>HYSTPWTFG</u> GGTKVEIK	
AFF9	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYAWN <u>WIRQ</u> FPKGLEW MGYISYSGSYSYNPSLK <u>SRVTISRD</u> TSKNQFSLKLSSVTAADTAVYYCA RSFSYAYGFAYWGQGLTVTVSS	33	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY <u>SASYRYTGVP</u> SRFSGSGSGT DFTFTISLQP EDFATYYC <u>QQ</u> <u>HYSTPWTFG</u> GGTKVEIK	20
AFF10	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWN <u>WIRQ</u> FPKGLEW MGYISYSGSYSYHPSLK <u>SRVTISRD</u> TSKNQFSLKLSSVTAADTAVYYCA RSFSYGYGFAYWGQGLTVTVSS	34	DIQMTQSPSS LSASVGDRVT ITCKASQDVS SAVAWYQQK PGKAPKLLIY <u>SASYRYTGVP</u> SRFSGSGSGT DFTFTISLQP EDFATYYC <u>QQ</u> <u>HYSTPWTFG</u> GGTKVEIK	20
Humanized antibody	QVQLQESGPGLVKPSQTLSTCTV TGYSITSDYTWN <u>WIRQ</u> FPKGLEW MGYISYSGSYSYNPSLK <u>SRVTISRD</u>	30	DIQMTQSPSS LSASVGDRVT ITCKASQDVS	20

(human IgG1)	TSKNQFSLKLSSVTAADTAVYYCA		<u>SAVAWYQQK</u>	
	RSFSYSYGFAIWGQGT		PGKAPKLLIY	
			<u>SASYRYTGVP</u>	
			SRFSGSGSGT	
			DFTFTISLQP	
			EDFATYYC <u>QQ</u>	
			<u>HYSTPWTFG</u>	
			GGTKVEIK	

*The underlines in the above table represent CDR sequences.

In some embodiments, an antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 58 and 37, and may comprise a light chain comprising an amino acid sequence selected from SEQ ID NOs: 38 and 59. More specifically, as shown in **Table 6**, the heavy chain may comprise an amino acid sequence selected from SEQ ID NOs: 37 and 58, and the light chain may comprise an amino acid sequence selected from SEQ ID NOs: 38 or 59.

More specifically, an antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30. The antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain comprising an amino acid sequence of SEQ ID NO: 37.

More specifically, an antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30 and LALA mutations in a heavy chain constant region. The antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain comprising an amino acid sequence of SEQ ID NO: 58. For example, the LALA mutations may include substitutions of L236A/L237A.

More specifically, an antibody or antigen-binding fragment thereof disclosed herein may comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO: 20. The antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain comprising an amino acid sequence of SEQ ID NO: 38.

More specifically, an antibody or antigen-binding fragment thereof disclosed herein may comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO: 20 and a CaaX peptide moiety (GGGGGGGCVIM, SEQ ID NO: 7) at the C-terminus of a light chain.

The antibody or antigen-binding fragment thereof disclosed herein may comprise a heavy chain comprising an amino acid sequence of SEQ ID NO: 59.

In some embodiments, an antibody or antigen-binding fragment thereof may comprise a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 30, or an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 30.

More specifically, an antibody or antigen-binding fragment thereof may comprise a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 30; or an amino acid sequence having a sequence identity of at least 80% or more, 85% or more, 90% or more, or 95% or more to SEQ ID NO: 30.

Even more specifically, an antibody or antigen-binding fragment thereof may comprise a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 30; an amino acid sequence having a sequence identity of at least 90% or more to SEQ ID NO: 30; or an amino acid sequence having a sequence identity of at least 95% or more to SEQ ID NO: 30.

In some embodiments, an antibody or antigen-binding fragment thereof may comprise a light chain variable region comprising an amino acid sequence of SEQ ID NO: 20, or an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 20.

More specifically, an antibody or antigen-binding fragment thereof may comprise a light chain variable region comprising an amino acid sequence of SEQ ID NO: 20; or an amino acid sequence having a sequence identity of at least 80% or more, 85% or more, 90% or more, or 95% or more to SEQ ID NO: 20.

Even more specifically, an antibody or antigen-binding fragment thereof may include a light chain variable region including an amino acid sequence of SEQ ID NO: 20; an amino acid sequence having a sequence identity of at least 90% or more to SEQ ID NO: 20; or an amino acid sequence having a sequence identity of at least 95% or more to SEQ ID NO: 20.

In some embodiments, an antibody or antigen-binding fragment thereof may comprise a combination of a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 20; or a combination of a heavy chain variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 20; and the antibody or antigen-binding fragment thereof specifically binds to human L1CAM.

More specifically, an antibody or antigen-binding fragment thereof may comprise a combination of a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 20; or a combination of a heavy chain variable region comprising an amino acid sequence with at least 80% or more, 85% or more, 90% or more, or 95% or more sequence identity to SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence with at least 80% or more, 85% or more, 90% or more, or 95% or more sequence identity to SEQ ID NO: 20; and the antibody or antigen-binding fragment thereof specifically binds to human L1CAM.

Even more specifically, an antibody or antigen-binding fragment thereof may comprise a combination of a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 20; or a combination of a heavy chain variable region comprising an amino acid sequence with at least 80% or more, 81% or more, 82% or more, 83% or more, 84% or more, 85% or more, 86% or more, 87% or more, 88% or more, 89% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more, or 99.5% or more sequence identity to SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence with at least 80% or more, 81% or more, 82% or more, 83% or more, 84% or more, 85% or more, 86% or more, 87% or more, 88% or more, 89% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more, or 99.5% or more sequence identity to SEQ ID NO: 20; and the antibody or antigen-binding fragment thereof specifically binds to human L1CAM.

Table 6. Heavy Chain and Light Chain Amino Acid Sequences

Antibody	Heavy chain	SEQ ID NO:	Light chain	SEQ ID NO:
Humanized antibody “AFF4” (human IgG1;	QVQLQESGPGLVKPSQTL SLTCTVTGYSITSDYTWN WIRQFPGKGLEWMGYIS YSGSYSYNPSLKSRVTISR	37	DIQMTQSPSSLSASVGDRV TITCKASQDVSSAVAWYQ QKPGKAPKLLIYSASYRYT GVPSRFSGSGSGTDFTFTIS SLQPEDFATYYCQQHYSTP	38

G236A/S23 9D/ A330L/I332 E mutant O)	DTSKNQFSLKLSSVTAAD TAVYYCAR <u>SFSYSYG</u> <u>FAY</u> WGQGLTVTVSSAST KGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSL GTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPP CPAPELLAGPDVFLFPPKP KDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNG KEYKCKVSNKALPLPEE KTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQP ENNYKTTTPVLDSGSFF LYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQK SLSLSPG		WTFGGGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQ WKVDNALQSGNSQESVT EQDSKDSTYLSSTLTLSK ADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	
Humanized antibody “AFF4- LALA- CaaX”	QVQLQESGPGLVKPSQTL SLTCTVTGYSITSDYTWN WIRQFPGKGLEWMGYIS <u>YSGSYSYNPSLKS</u> RVTISR DTSKNQFSLKLSSVTAAD TAVYYCAR <u>SFSYSYG</u> <u>FAY</u> WGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSW	58	DIQMTQSPSSLSASVGDRV TITC <u>KASQDVSSA</u> VAWYQ QKPGKAPKLLIY <u>SASYRYT</u> GVPSRFGSGSGTDFFTIS SLQPEDFATYYC <u>QQHYSTP</u> <u>WTFGGGTKVEIKRTVAAP</u> SVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQ	59

	NSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVD KRVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTY RVVSVLTVLHQ DWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHN HYTQKSLSLSPG		WKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQG LSSPVTKSFNRGECGGGG GGGCVIM	
Humanized antibody “AFF4- LALA”	QVQLQESGPGLVKPSQTL SLTCTVTGYSITSDYTNW WIRQFPGKGLEWMGYIS YSGSYSYNPSLKSRTISR DTSKNQFSLKLSSVTAAD TAVYYCARSFYSYGFAY WGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVD KRVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPK	58	DIQMTQSPSSLSASVGDRV TITCKASQDVSSAVAWYQ QKPGKAPKLLIYSASYRYT GVPSRFGSGSGTDFTFTIS SLQPEDFATYYCQQHYSTP WTFGGGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQ WKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQG LSSPVTKSFNRGECGGGG GGG	61

	DTLMISRTPEVTCVVVDV		
	SHEDPEVKFNWYVDGVE		
	VHNAKTKPREEQYNSTY		
	RVVSVLTVLHQ		
	DWLNGKEYKCKVSNKA		
	LPAPIEKTISKAKGQPREP		
	QVYTLPPSREEMTKNQV		
	SLTCLVKGFYPSDIAVEW		
	ESNGQPENNYKTTPPVLD		
	SDGSFFLYSKLTVDKSRW		
	QQGNVFSCSVMHEALHN		
	HYTQKSLSLSPG		

*The underlines in the above table represent CDR sequences.

In some embodiments, the antibody or antigen-binding fragment thereof is a humanized antibody.

In some embodiments, the humanized antibody may comprise:

- (a) (i) a variable domain framework region from a heavy chain of a human antibody or a human consensus framework; and (ii) a variable domain framework region from a light chain of the human antibody or the human consensus framework,
- (b) (i) at least one amino acid in the variable domain framework region from the heavy chain is substituted with a corresponding amino acid from a heavy chain of a mouse antibody or mouse consensus framework; or (ii) the variable domain framework region from the light chain is substituted with a corresponding amino acid from a light chain of the mouse antibody or mouse consensus framework, and
- (c) (i) the amino acid substitutions in the heavy chain are selected from the following amino acid substitutions:
 - substitution to A (mouse) instead of T (human) at position 34;
 - substitution to F (mouse) instead of Y (human) at position 58;
 - substitution to H (mouse) instead of N (human) at position 61; and
 - substitution to G (mouse) instead of S (human) at position 103.

In some embodiments, the amino acid substitutions in the heavy chain are selected from the following amino acid substitutions:

substitution to G (mouse) instead of A (human) at position 238

substitution to S (human) instead of D (human) at position 241; and/or

substitution to I (human) instead of E (human) at position 335.

In some embodiments, the humanized antibody comprises:

(i) a variable heavy chain framework region from a heavy chain of a human antibody or a human consensus framework, wherein the variable heavy chain framework region comprises one or more of the following mutations: T34A, Y58F, N61H, and S103G; and

(ii) a variable light chain framework region from a light chain of the human antibody or the human consensus framework.

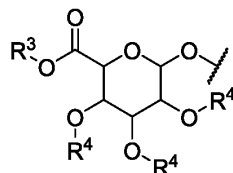
In some embodiments, the humanized antibody comprises LALA mutations in a heavy chain constant region. In some embodiments, the LALA mutations comprise substitutions of L236A/L237A.

In some embodiments, the antibody or antigen-binding fragment thereof may be selected from a monoclonal antibody, a domain antibody (dAb), a single chain antibody (scAb), a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a scFab fragment, a Fv fragment, a dsFv Fragment, a single chain variable fragment (scFv), a scFv-Fc fragment, a single domain heavy chain antibody, a single domain light chain antibody, a variant antibody, a multimeric antibodies, a minibody, a diabody, a bispecific antibody, and a multispecific antibody.

Antibody-drug conjugates disclosed herein, by comprising anti-L1CAM antibody that more strongly binds specifically to the type of L1CAM mainly exhibited in tumors, is able to effectively, specifically, and selectively deliver drugs to cells expressing L1CAM. By having an antibody stably bonded to a drug to maintain *in vivo* stability while exhibiting the intended cytotoxicity, and in particular the stability in the serum, and even in circulation, and by employing linker technology which includes a self-immolative group which allows a drug to be easily releases within a cancer cell to maximize efficacy, these conjugates exhibit an effect of providing an antibody-linker-drug system which allows a drug and/or toxin to safely reach a target cell and effectively exhibit efficacy while having significantly reduced toxicity.

In certain embodiments, n1 is 1, 2, 3, or 4. In certain preferred embodiments, n1 is 1. In other preferred embodiments, n1 is 2.

In certain embodiments, n2 is 1, 2, 3, or 4. In certain preferred embodiments, n2 is 1.



In certain preferred embodiments, each G is H. ; and R³ and R⁴ are each

In certain embodiments, R¹ and R² are each hydrogen.

In certain preferred embodiments, each W independently is -C(O)NR'-.

In certain embodiments, R' is H.

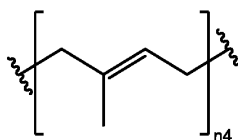
In certain embodiments, R'' is H.

In certain embodiments, Z is halo (*e.g.*, bromo) or cyano.

In certain embodiments, n3 is 1. In certain preferred embodiments, n3 is 0.

In certain embodiments, L comprises a C₁₋₅₀ alkylene. In other embodiments, L comprises a C₁₋₅₀ heteroalkylene. In certain embodiments, L comprises an unsaturated bond (*e.g.*, 1, 2, 3, 4 or 5 unsaturated bonds). In certain embodiments, L is substituted with a C₁₋₂₀ alkyl.

In certain preferred embodiments, L comprises an isoprenyl group having a structure represented by Formula IIIa:



IIIa

wherein n4 is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

In certain embodiments, L comprises a peptide. In certain embodiments, the peptide comprises at least one hydrophilic amino acid. In certain embodiments, the peptide comprises an amino acid having a side chain having a moiety that bears a charge at neutral pH in aqueous solution (*e.g.*, an amine, guanidine, or carboxyl moiety). In certain embodiments, the peptide comprises an amino acid selected from alanine, aspartate, asparagine, glutamate, glutamine, glycine, lysine, ornithine, proline, serine, and threonine.

In certain embodiments, the antibody comprises a cysteine residue and L is covalently connected to the antibody by a sulfur atom in the cysteine residue (*e.g.*, by a sulfur atom in the side chain of the cysteine residue) via a thioether bond. In certain embodiments, a C-terminus of the antibody comprises an amino acid motif that is recognized by a isoprenoid transferase. In certain embodiments, the isoprenoid transferase is farnesyl protein transferase (FTase) or geranylgeranyl transferase (GGTase). In certain embodiments, the amino acid motif comprises a cysteine residue and L is covalently connected to the antibody by a sulfur atom in the cysteine residue (*e.g.*, by a sulfur atom in the side chain of the cysteine residue) via a thioether bond. In certain preferred embodiments, the amino acid motif has the sequence:

CYYX

wherein:

C is cysteine;

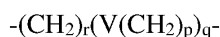
each Y is an aliphatic amino acid; and

X is selected from glutamine, glutamate, serine, cysteine, methionine, alanine, and leucine.

In certain embodiments, each Y is independently selected from alanine, isoleucine, leucine, methionine, and valine. In certain embodiments, at least one of 1 to 20 amino acids preceding the amino acid motif is glycine. In certain preferred embodiments, the amino acid motif has the sequence GGGGGGGCVIM.

In certain preferred embodiments, L comprises an oxime. In certain embodiments, the oxygen atom of the oxime is on the side of Y that is linked to the active agent and the carbon atom of the oxime is on the side of L that is linked to Ab. In certain embodiments, the carbon atom of the oxime is on the side of Y that is linked to the active agent and the oxygen atom of the oxime is on the side of L that is linked to Ab.

In certain preferred embodiments, L comprises a structure represented by formula IVa or IVb:



Formula IVa,



Formula IVb;

V is a single bond, -O-, -S-, -NR²¹-, -C(O)NR²²-, -NR²³C(O)-, -NR²⁴SO₂-, or -SO₂NR²⁵-, preferably -O-;

X is -O-, C₁₋₈ alkylene, or -NR²¹-, preferably -O-;

R²¹ to R²⁵ are each independently H, C₁₋₆ alkyl, C₁₋₆ alkyl C₆₋₂₀ aryl, or C₁₋₆ alkyl C₃₋₂₀ heteroaryl;

each r is independently an integer of 1 to 10, preferably 2;

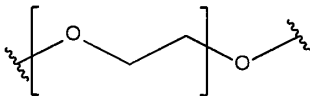
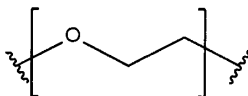
each p is independently an integer of 0 to 12, preferably 2;

q is an integer of 1 to 20, preferably 2, 5, or 11; and

w is an integer of 1 to 20, preferably 6 to 20.

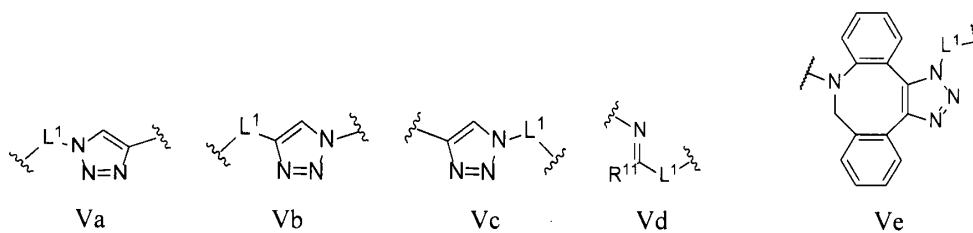
In certain embodiments, V is -O-. In certain embodiments, X is -O-. In certain embodiments, R²¹ is H. In certain embodiments, R²² is H. In certain embodiments, R²³ is H. In certain embodiments, R²⁴ is H. In certain embodiments, R²⁵ is H. In certain embodiments, r is 2. In certain embodiments, p is 2. In certain embodiments, q is 2, 5, or 11. In certain embodiments, w is 6 to 20.

In certain preferred embodiments, L comprises at least one polyethylene glycol unit

represented by  or . In certain embodiments, L comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 polyethylene glycol units. In even further preferred embodiments, L comprises an oxime and at least one polyethylene glycol unit covalently binds the oxime to the active agent.

In certain embodiments, L comprises a moiety formed by a reaction of alkyne with azide, or a reaction of an aldehyde or ketone group with hydrazine or hydroxylamine.

In certain preferred embodiments, L comprises a unit having a structure represented by Formula Va, Vb, Vc, Vd, or Ve:



L¹ is a single bond or C₁₋₃₀ alkylene; and

R^{11} is H or C_{1-10} alkyl.

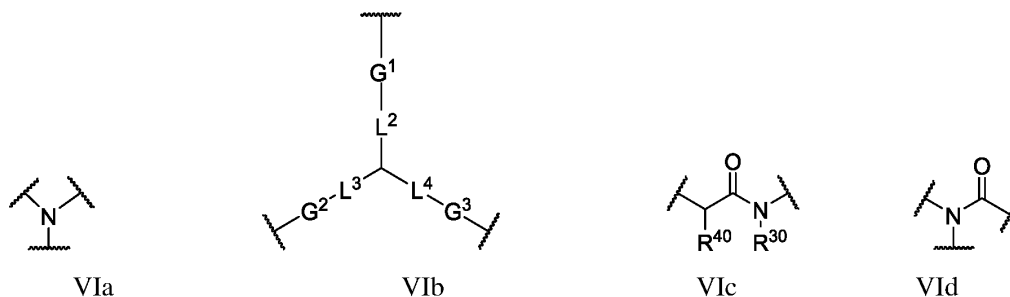
In certain embodiments, L^1 is a single bond. In certain embodiments, L is C_{1-30} alkylene.

In certain embodiments, R^{11} is H. In certain embodiments, R^{11} is C_{1-10} alkyl (*e.g.*, methyl).

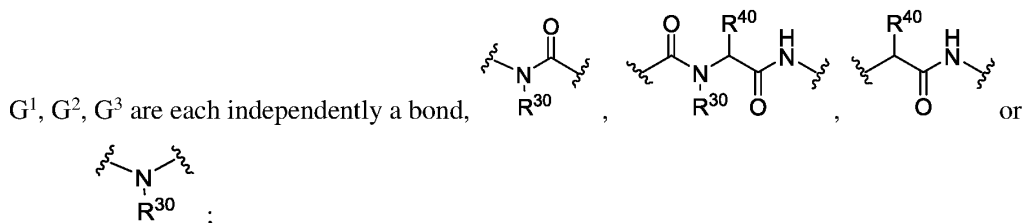
In certain preferred embodiments, L comprises:

- i) a branching unit covalently coupled to Ab by a primary linker;
- ii) a first branch which couples a first B to the branching unit; and
- iiia) a second branch which couples a second B to the branching unit; or
- iiib) a second branch comprising an alkyl or heteroalkyl (*e.g.*, a polyethylene glycol monomer or a polyethylene glycol oligomer) coupled the branching unit.

In certain embodiments, L comprises a second branch which couples a second active agent, via a second cleavage group, to the branching unit. In certain embodiments, L comprises a second branch comprising an alkyl or heteroalkyl (*e.g.*, a polyethylene glycol monomer or a polyethylene glycol oligomer) coupled the branching unit. In certain embodiments, the branching unit has a structure represented by formula VIa, VIb, VIc, or VId:



wherein



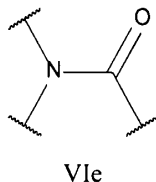
R^{30} is H or alkyl;

R^{40} is H, alkyl or $L_5-CO_2R^{50}$;

R^{50} is H or alkyl; and

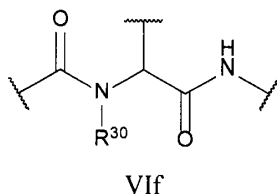
L^2, L^3, L^4 , and L^5 are each independently a bond or alkylene.

In certain embodiments, the branching unit has a structure represented by formula VIe:



wherein R^{30} is H or alkyl.

In certain embodiments, the branching unit has a structure represented by formula VIf:

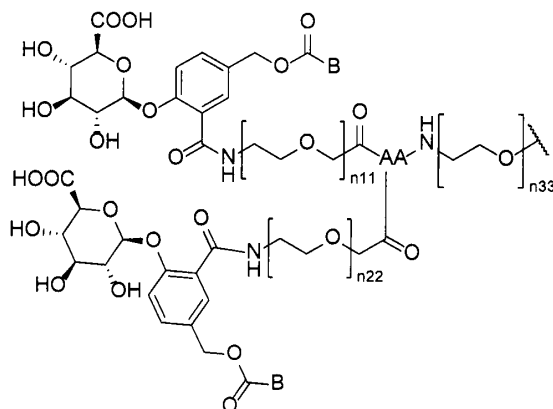


wherein R^{30} is H or alkyl.

In certain embodiments, the conjugate comprises 1, 2, 3, or 4 branched linkers. In certain embodiments, each branched linker comprises at least two active agents. In certain embodiments, each branched linker comprises two active agents. In certain embodiments, the branching unit is a lysine and the primary linker is bound to a C-terminus of lysine.

In certain embodiments, the conjugate is cleavable in a target cell (e.g., the conjugate cleaves to release one or more active agents).

In certain embodiments, the conjugate comprises a structure represented by:



$n11$, $n22$, and $n33$ are each independently integers of 0 to 30; and

AA represents an amino acid group.

In certain embodiments, each active agent is independently a chemotherapeutic agent or a toxin. In certain embodiments, each active agent is independently an immunomodulatory compound, an anticancer agent, an antiviral agent, an antibacterial agent, an antifungal agent, an antiparasitic agent, or a combination thereof. In certain embodiments, the active agent is independently an immunomodulatory compound, an anticancer agent, an antiviral agent, an antibacterial agent, an antifungal agent, or an antiparasitic agent. In certain embodiments, the active agent is independently selected from:

(a) erlotinib, bortezomib, fulvestrant, sunitinib, letrozole, imatinib mesylate, PTK787/ZK 222584, oxaliplatin, 5-fluorouracil, leucovorin, rapamycin, lapatinib, lonafarnib, sorafenib, gefitinib, AG1478, AG1571, thiotepa, cyclophosphamide, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, ethylenimine, altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, topotecan, bryostatin, callystatin, CC-1065, adozelesin, carzelesin, bizelesin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, KW-2189, CB1-TM1, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, calicheamicin gamma 1, calicheamicin omega 1, dynemicin, dynemicin A, clodronate, esperamicin, neocarzinostatin chromophore, alacinomysins, actinomycin, antrmycin, azaserine, bleomycins, cactinomycin, carabycin, carinomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin, deoxydoxorubicin, epirubicin, esorubicin, Marcelo marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, 5-fluorouracil, denopterin, methotrexate, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thiguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, calusterone,

dromostanolone, propionate, epitiostanol, mepitiothane, testolactone, aminogluthethimide, mitotane, trilostane, folinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansine, ansamitocins, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, 2-ethylhydrazide, procarbazine, polysaccharidek, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2''-trichlorotriethylamine, T-2 toxin, verracurin A, roridin A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside, cyclophosphamide, thiotepa, paclitaxel, albumin-engineered nanoparticle formulation of paclitaxel, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, carboplatin, vinblastine, platinum, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, CPT-11, SN-38, GI-147211C, 9-aminocamptothecin, 7-hydroxymethyl camptothecin, 7-aminomethyl camptothecin, 10-hydroxycamptothecin, (20S)-camptothecin, rubitecan, gimatecan, karenitecin, silatecan, lurtotecan, exatecan, diflomotecan, belotecan, lurtotecan, topoisomerase inhibitor RFS 2000, topoisomerase inhibitor S39625, difluoromethylornithine, retinoic acid, capecitabine, or a pharmaceutically acceptable salt, solvate or acid thereof;

- (b) monokine, lymphokine, traditional polypeptide hormone, parathyroid hormone, thyroxine, relaxin, prorelaxin, glycoprotein hormone, follicle stimulating hormone, thyroid stimulating hormone, luteinizing hormone, hepatic growth factor fibroblast growth factor, prolactin, placental lactogen, tumor necrosis factor, tumor necrosis factor- α , tumor necrosis factor- β , mullerian inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, vascular endothelial growth factor, thrombopoietin, erythropoietin, osteoinductive factor, interferon, interferon- α , interferon- β , interferon- γ , colony stimulating factor (CSF), macrophage-CSF, granulocyte-macrophage-CSF, granulocyte-CSF, interleukin (IL), IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, polypeptide factor, LIF, kit ligand, or combinations thereof;

- (c) diphtheria toxin, botulinum toxin, tetanus toxin, decentritoxin, cholera toxin, amanitin, α -amanitin, pyrrolbenzodiazepine, pyrrolbenzodiazepine derivatives, indolinbenzodiazepine, pyridinbenzodiazepine, tetrodotoxin, brevetoxin, ciguatoxin, ricin, AM toxin, auristatin, tubulysin, geldanamycin, maytansinoid, calicheamycin, daunomycin, doxorubicin, methotrexate, vindesine, SG2285, dolastatin, dolastatin analog, cryptophycin, camptothecin, rhizoxin, rhizoxin derivatives, CC-1065, CC-1065 analogs or derivatives, duocarmycin, enediyne antibiotic, esperamicin, epothilone, toxoid, or combinations thereof;
- (d) affinity ligands, wherein the affinity ligand is a substrate, an inhibitor, an activator, a neurotransmitter, a radioisotope, or a combination thereof;
- (e) a radioactive label, ^{32}P , ^{35}S , a fluorescent dye, an electron dense reagent, an enzyme, biotin, streptavidin, dioxigenin, hapten, an immunogenic protein, a nucleic acid molecule with a sequence complementary to a target, or a combination thereof;
- (f) an immunomodulatory compound, an anti-cancer agent, an anti-viral agent, an anti-bacterial agent, an anti-fungal agent, and an anti-parasitic agent, or combinations thereof;
- (g) tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone or toremifene;
- (h) 4(5)-imidazole, aminoglutethimide, megestrol acetate, exemestane, letrozole, or anastrozole;
- (i) flutamide, nilutamide, bicalutamide, leuprolide, goserelin, or troxacitabine;
- (j) an aromatase inhibitor;
- (k) a protein kinase inhibitor;
- (l) a lipid kinase inhibitor;
- (m) antisense oligonucleotide;
- (n) ribozyme;
- (o) vaccines;
- (p) an anti-angiogenic agent; and
- (q) a topoisomerase I inhibitor.

In certain embodiments, at least one active agent is a pyrrolbenzodiazepine dimer. In certain embodiments, the active agent is a pyrrolbenzodiazepine dimer; the pyrrolbenzodiazepine dimer is substituted with X at position N10 or X' at position N'10, wherein X or X' links the pyrrolbenzodiazepine dimer to the conjugate;

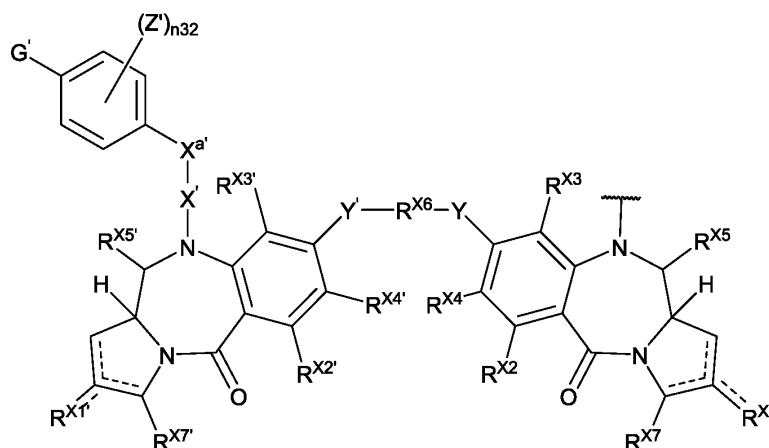
X and X' are each independently -C(O)O-*, -or -C(O)-*; and

* is a binding site between the pyrrolobenzodiazepine dimer and the linker.

In certain embodiments, at least one active agent has a structure represented by Formula

X:

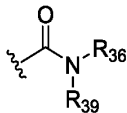
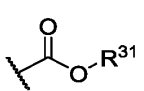
[Formula X]



wherein

each dotted line represents an optional double bond;

G' is a glucuronide group or a galactoside group;

Z' is selected from H, C₁₋₈ alkyl, halo, NO₂, CN, , and ;

X' is selected from -C(O)O-, -S(O)O-, -C(O)-, -C(O)NR³²-, -S(O)₂NR³²-, -P(O)R³³NR³²-, -S(O)NR³²-, and -PO₂NR³²-;

Xa' is a bond or substituted or unsubstituted C₁₋₆ alkylene, wherein the C₁₋₆ alkylene substituent is hydrogen, C₁₋₈ alkyl, or C₃₋₈ cycloalkyl;

R^{X1} and R^{X1'} are each independently selected from H, OH, =O, =CH₂, CN, R^m, OR^m, =CH-R^m, =C(R^m)₂, OSO₂-R^m, CO₂R^m, COR^m, halo, dihalo, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₂₋₆ alkenyl, C₅₋₇ aryl, C₃₋₆ heteroaryl, or C₅₋₇ aryl substituted with C₁₋₆ alkyl or C₁₋₆ alkoxy;

R^{X2}, R^{X2'}, R^{X3}, and R^{X3'} are each independently selected from H, R^m, OH, OR^m, NR^m, NO₂, and halo;

R^{X4} and $R^{X4'}$ are each independently selected from H, R^m , OH, OR^m , SH, SR^m , NH_2 , NHR^m , NR^{m2} , halo, and C_{1-6} alkyl;

R^{X5} and $R^{X5'}$ are each independently selected from H, R^m , OH, OR^m , SH, SR^m , NH_2 , NHR^m , NR^{m2} , $-NR^mR^{m'}$, NO_2 , $-NR^mC(O)R^{m'}$, $-NR^mC(O)OR^{m'}$, $-NR^mC(O)NR^mR^{m'}$, $-S(O)R^m$, $-S(O)_2R^m$, $-S(O)NR^mR^{m'}$, $-S(O)_2NR^mR^{m'}$, $-NR^mS(O)R^{m'}$, $-NR^mS(O)_2R^{m'}$, $-P(O)R^m$, $-P(O)_2R^m$, $-P(O)NR^mR^{m'}$, $-P(O)_2NR^mR^{m'}$, $-NR^mP(O)R^{m'}$, $-NR^mP(O)_2R^{m'}$, and halo;

Y and Y' are each independently O, S, or N(H);

R^{X6} is independently C_{3-12} alkylene, C_{3-12} alkenylene, or C_{3-12} heteroalkylene;

R^{X6} is unsubstituted or substituted with $-NH_2$, $-NHR^m$, $-NHC(O)R^m$, $-NHC(O)CH_2-[OCH_2CH_2]_{n31}-R^{XX}$, or $-[CH_2CH_2O]_{n31}-R^{XX}$;

the R^{XX} is H, OH, N_3 , CN, NO_2 , SH, NH_2 , ONH_2 , $NHNH_2$, halo, C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{1-8} alkoxy, C_{1-8} alkylthio, C_{3-20} heteroaryl, C_{5-20} aryl or mono- or di- C_{1-8} alkylamino, wherein each n_{31} is an integer of 1 to 6;

R^{X7} and $R^{X7'}$ are each independently H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-C(O)R^r$, $-C(O)OR^s$ or $-C(O)NR^rR^r$;

R^r , R^r , and R^s are each independently H, C_{1-7} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{3-13} cycloalkyl, 3 to 7-membered heterocycloalkyl, C_{5-10} aryl or 5 to 7-membered heteroaryl;

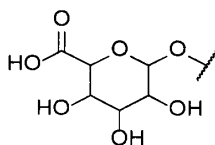
each $R^{m'}$ is independently selected from R^m , CO_2R^m , COR^m , CHO, CO_2H , and halo, and

each R^m is independently selected from H, OH, C_{1-12} alkyl, C_{1-12} alkoxy, C_{2-12} alkenyl, C_{2-12} alkynyl, C_{5-20} aryl, C_{5-20} heteroaryl, C_{3-6} cycloalkyl, 3 to 7-membered heterocyclyl, 3 to 7-membered heterocycloalkyl, and 5 to 7-membered heteroaryl, wherein one or more hydrogen atoms of C_{5-20} aryl, C_{5-20} heteroaryl, C_{3-6} cycloalkyl, 3 to 7-membered heterocyclyl, 3 to 7-membered heterocycloalkyl, and 5 to 7-membered heteroaryl may be substituted with OH, =O, C_{1-12} alkyl, or C_{1-12} alkoxy; and

R^{31} , R^{36} , and R^{39} are each independently selected from H, C_{1-8} alkyl, C_{2-6} alkenyl, C_{1-6} alkoxy, and alkyloxyalkyl;

R^{32} and R^{33} are each H, alkyl, alkoxy, or aralkyl; and

n^{32} is 0, 1, 2, 3 or 4.



In certain preferred embodiments, G' is

In certain embodiments, R^{X1} and R^{X1'} are each independently selected from =CH₂, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₂₋₆ alkenyl, C₅₋₇ aryl, C₃₋₆ heteroaryl, or C₅₋₇ aryl; further wherein the C₃₋₆ heteroaryl or C₅₋₇ aryl is substituted with C₁₋₆ alkyl or C₁₋₆ alkoxy.

In certain embodiments, R^{X2} is H or OH.

In certain embodiments, R^{X2'} is H or OH.

In certain embodiments, R^{X3} is H or OH.

In certain embodiments, R^{X3'} is H or OH.

In certain embodiments, R^{X5} is H, OH, -S(O)R^m, S(O)₂R^m, -P(O)R^m, and -P(O)₂R^m, wherein R^m is H, OH, C₁₋₁₂ alkyl, C₁₋₁₂ alkoxy, C₂₋₁₂ alkenyl, or C₂₋₁₂ alkynyl.

In certain embodiments, R^{X5'} is H, OH, -S(O)R^m, S(O)₂R^m, -P(O)R^m, and -P(O)₂R^m, wherein R^m is H, OH, C₁₋₁₂ alkyl, C₁₋₁₂ alkoxy, C₂₋₁₂ alkenyl, or C₂₋₁₂ alkynyl.

In certain embodiments, R^{X4} is C₁₋₆ alkoxy.

In certain embodiments, R^{X4'} is C₁₋₆ alkoxy.

In certain embodiments, Y is O.

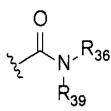
In certain embodiments, Y' is O.

In certain embodiments, R^{X6} is C₃₋₁₂ alkylene, C₃₋₁₂ alkenylene or C₃₋₁₂ heteroalkylene, and R^{X6} is substituted with -NH₂, -NHR^m, -NHC(O)R^m, -NHC(O)CH₂-[OCH₂CH₂]_n-R^{XX}, or -[CH₂CH₂O]_n-R^{XX};

R^{XX} is H, OH, N₃, CN, NO₂, SH, NH₂, ONH₂, NHHN₂, halo, C₁₋₈ alkyl, C₃₋₈ cycloalkyl, C₁₋₈ alkoxy, C₁₋₈ alkyl thio, C₃₋₂₀ heteroaryl, C₅₋₂₀ aryl or mono- or di-C₁₋₈ alkylamino; and n is an integer of 1 to 6.

In certain embodiments, R^{X6} is C₃₋₁₂ alkylene.

In certain embodiments, X' is -C(O)-.

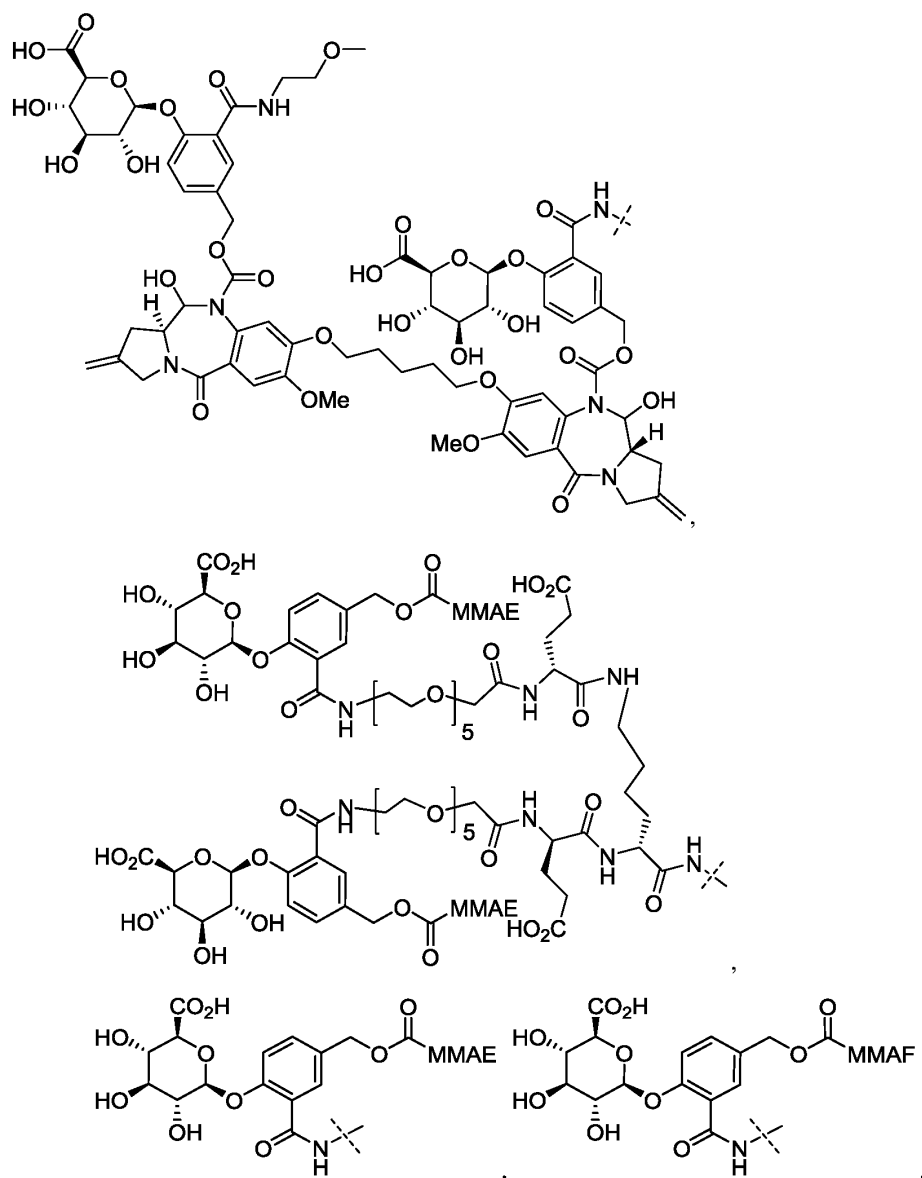


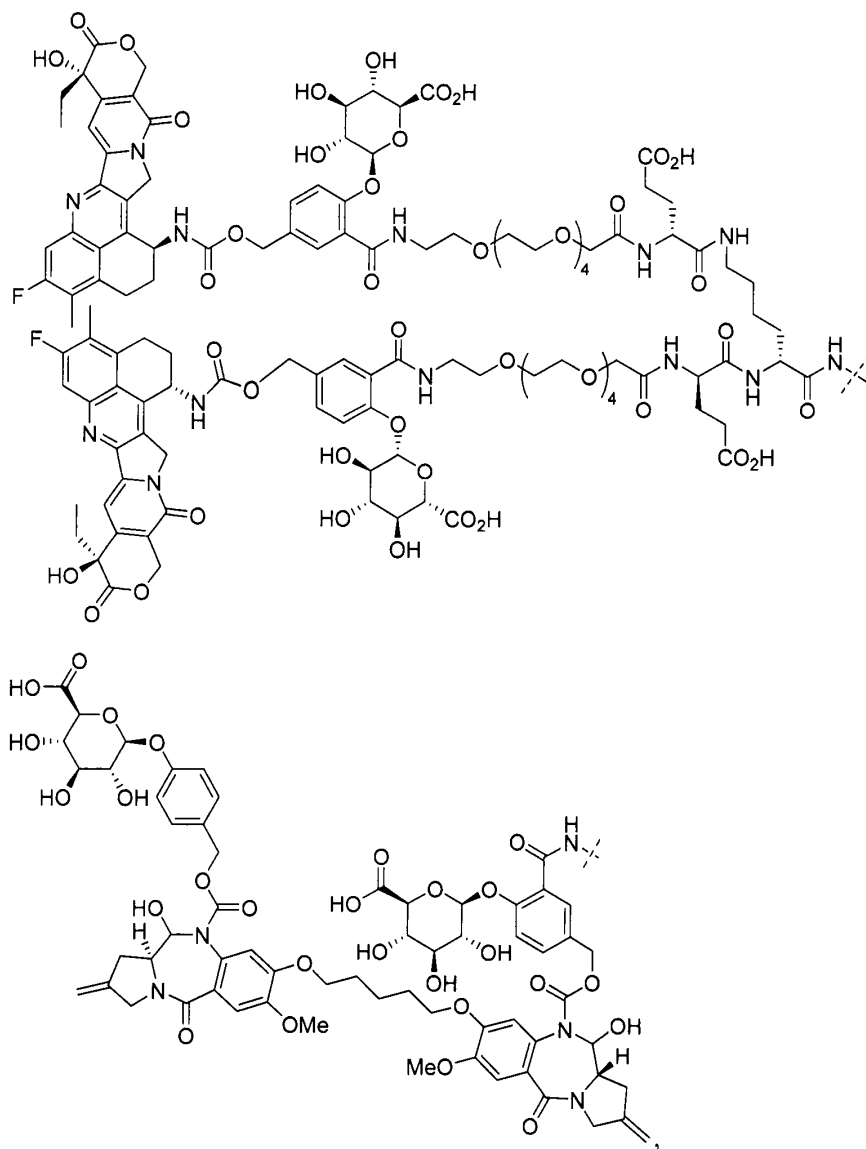
In certain embodiments, Z' is

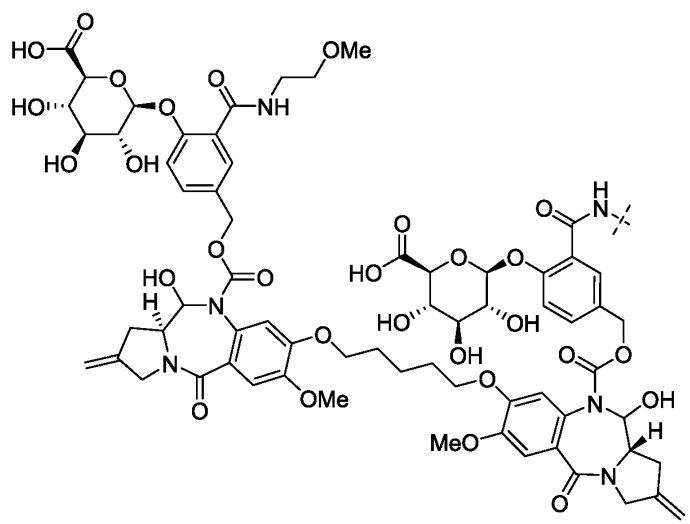
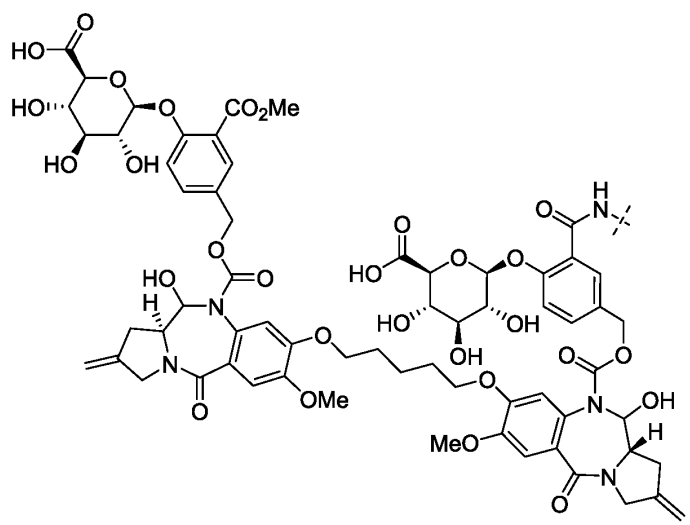
In certain embodiments, R₃₆ is alkyloxyalkyl.

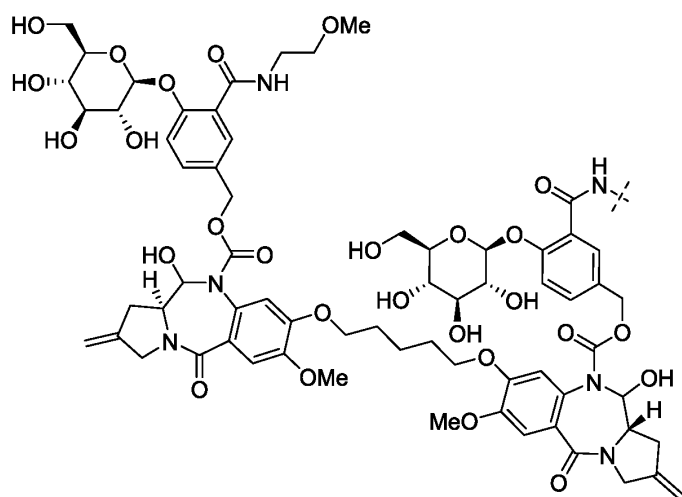
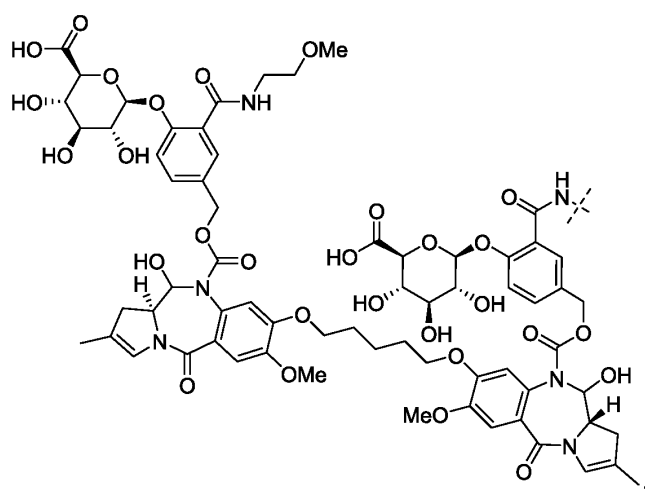
In certain embodiments, R₃₉ is H.

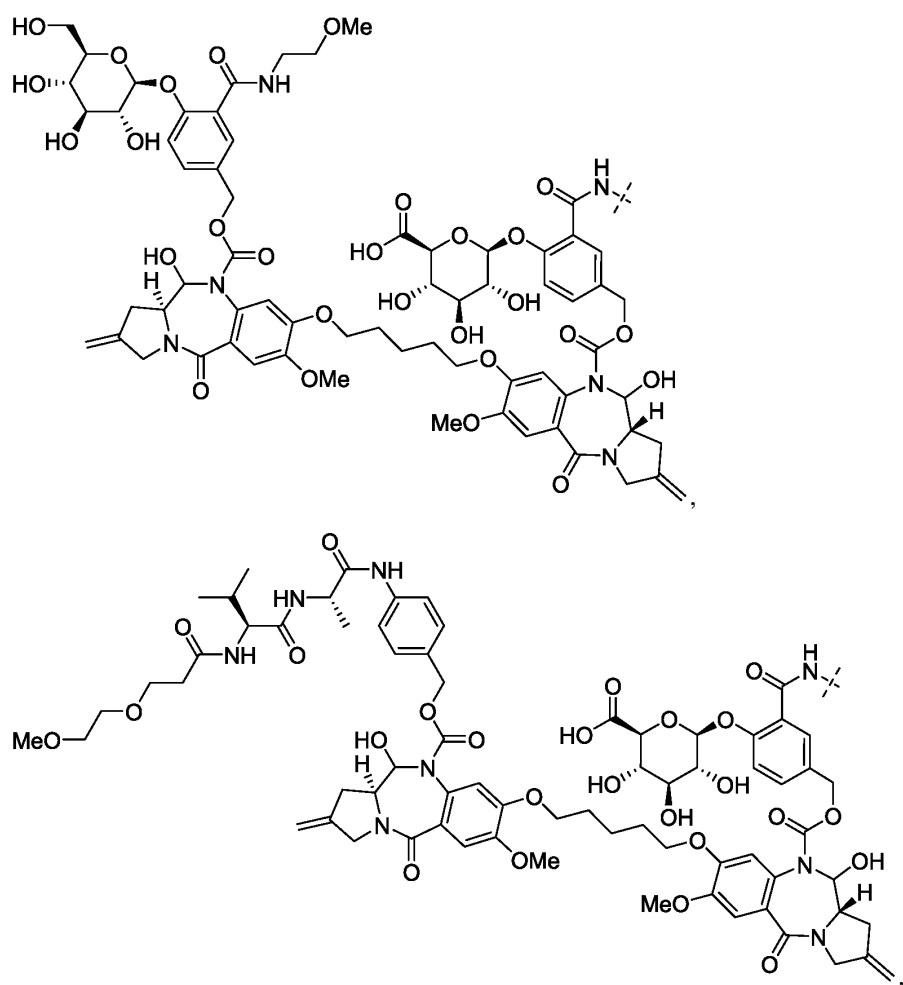
In certain embodiments, the conjugate comprises

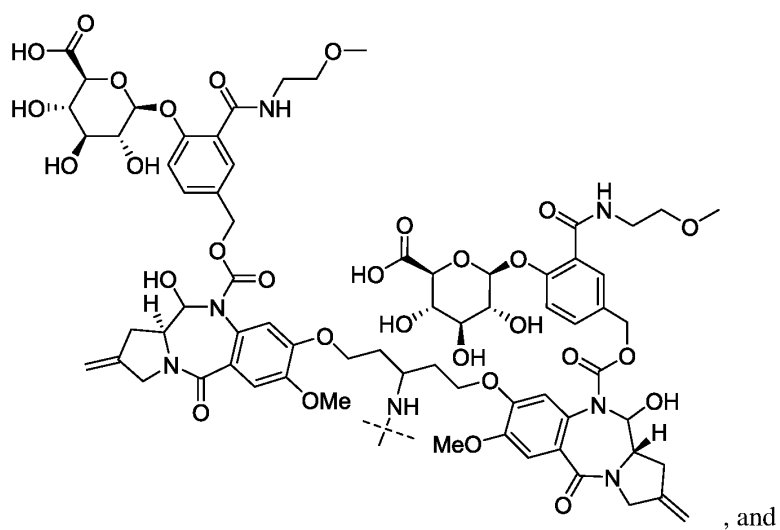
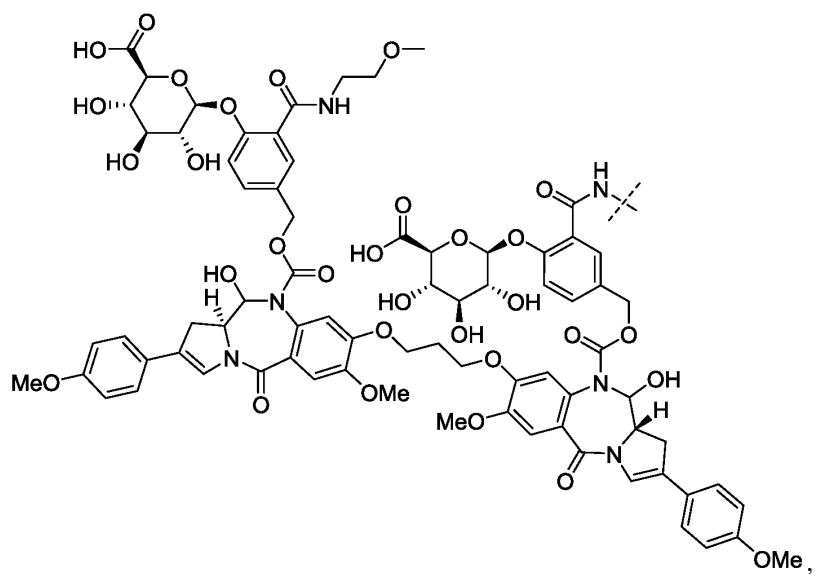


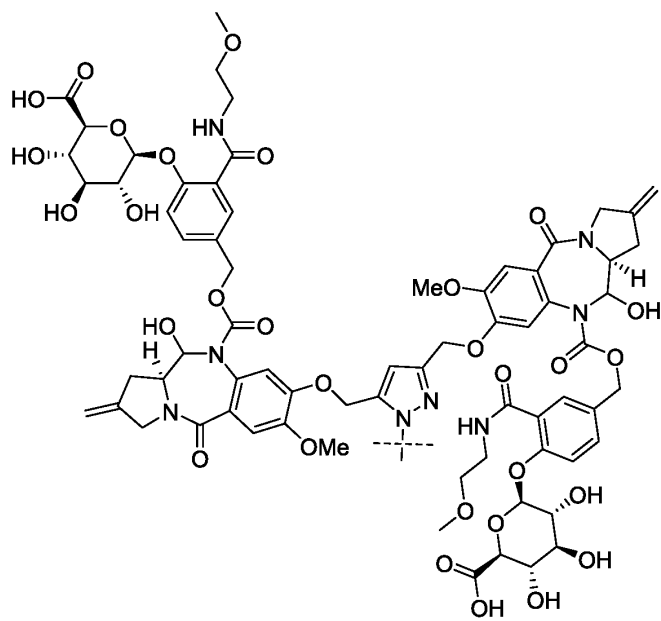








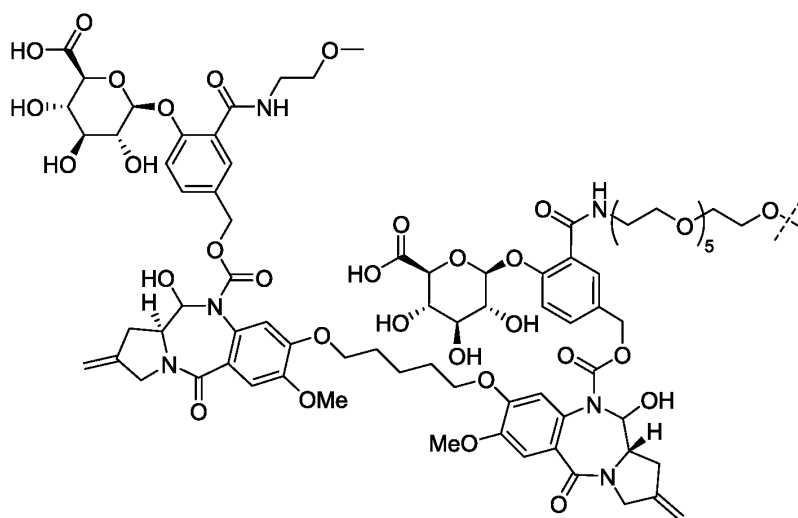


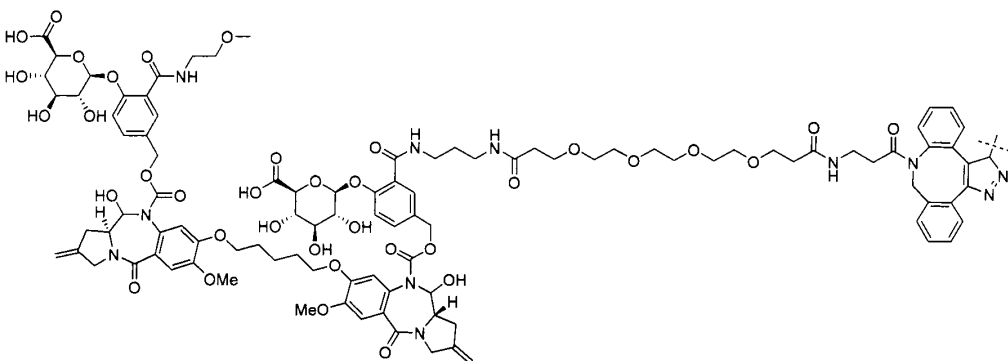
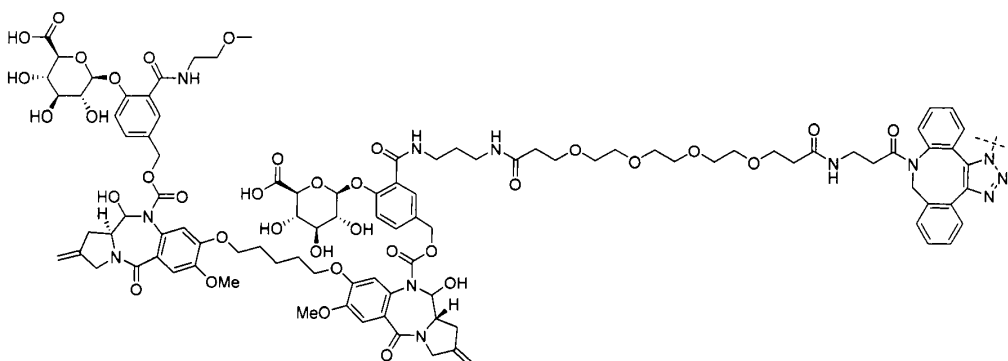
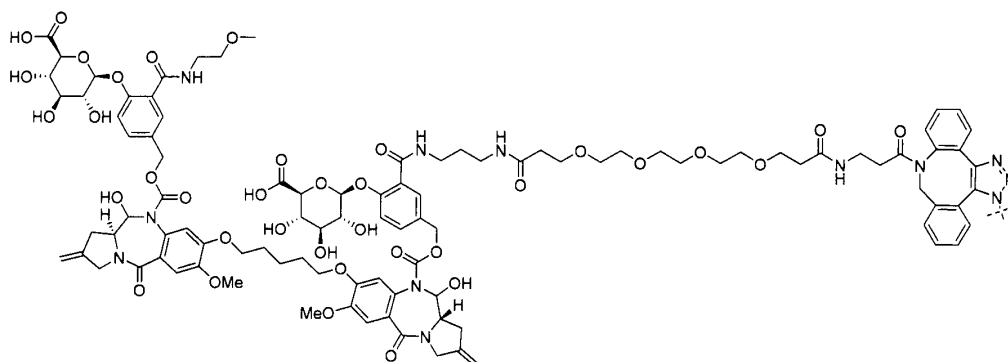


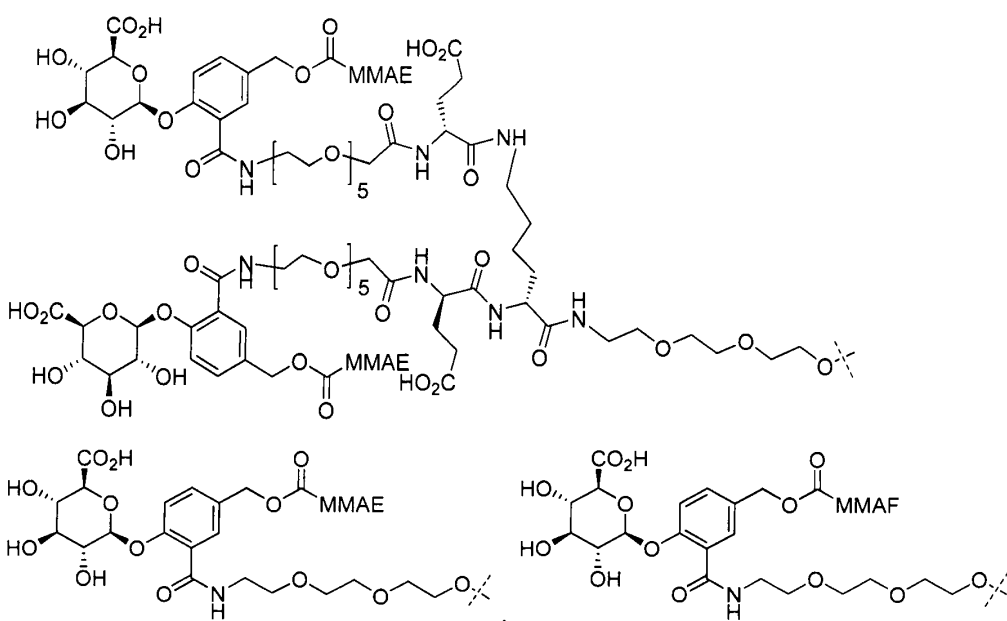
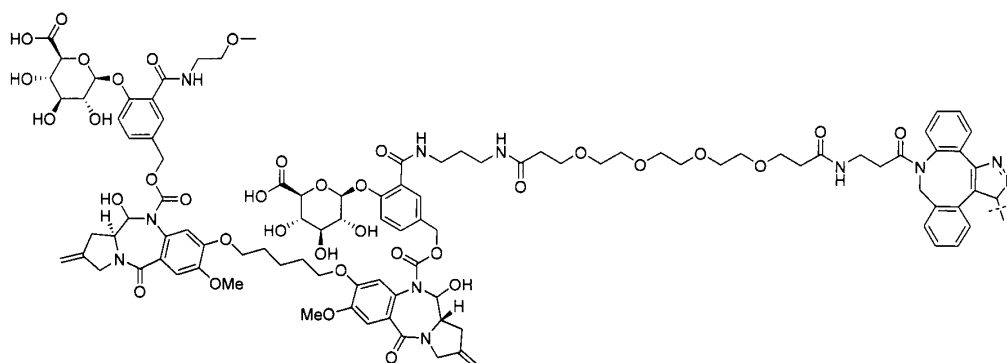
, wherein

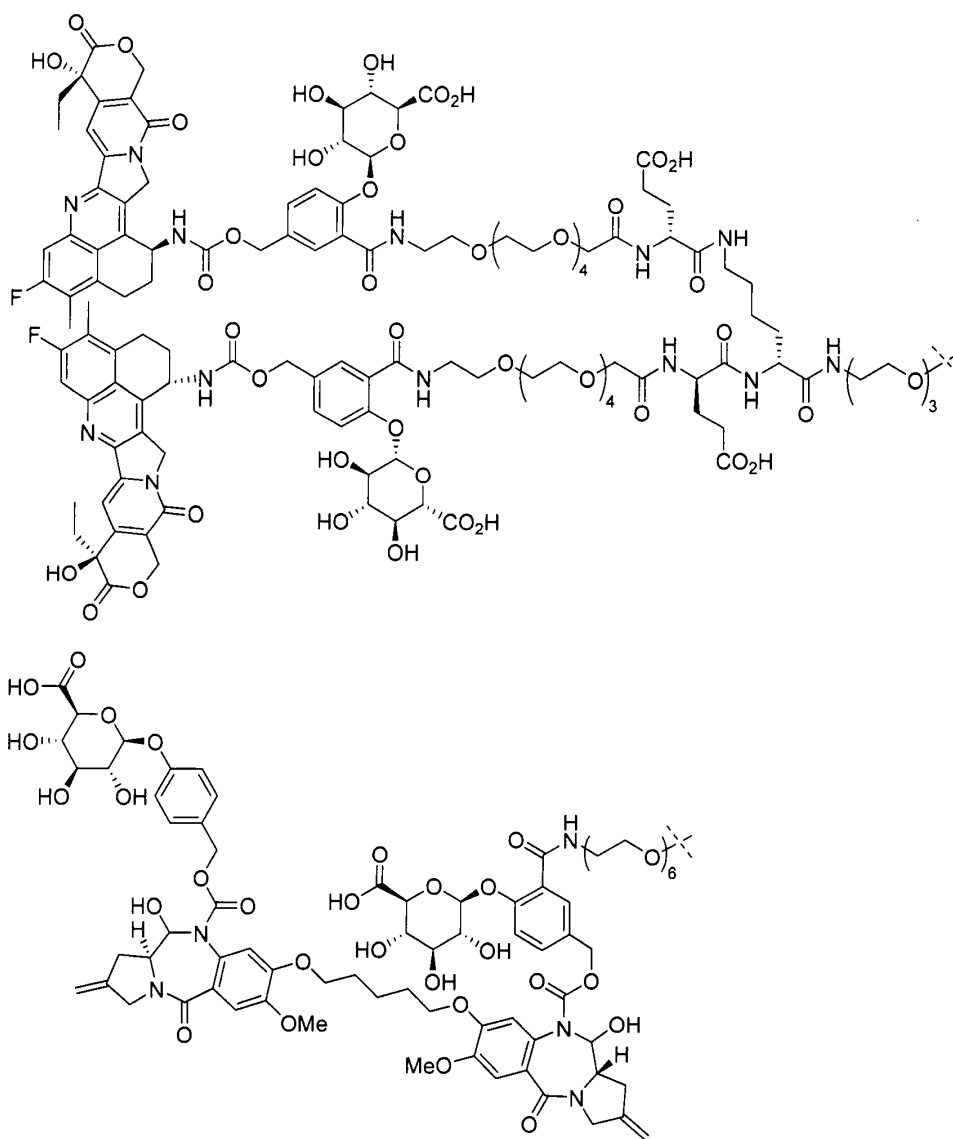
MMAE is monomethyl auristatin E, and MMAF is monomethyl auristatin F; and the dotted line represents a connect to the rest of the conjugate.

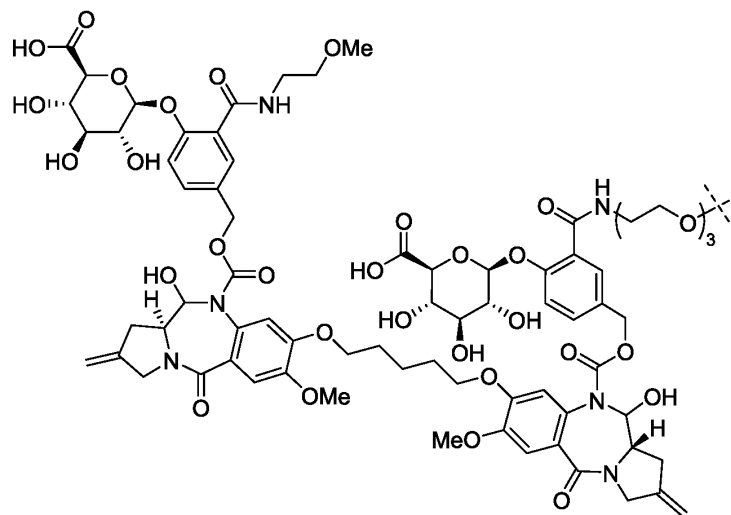
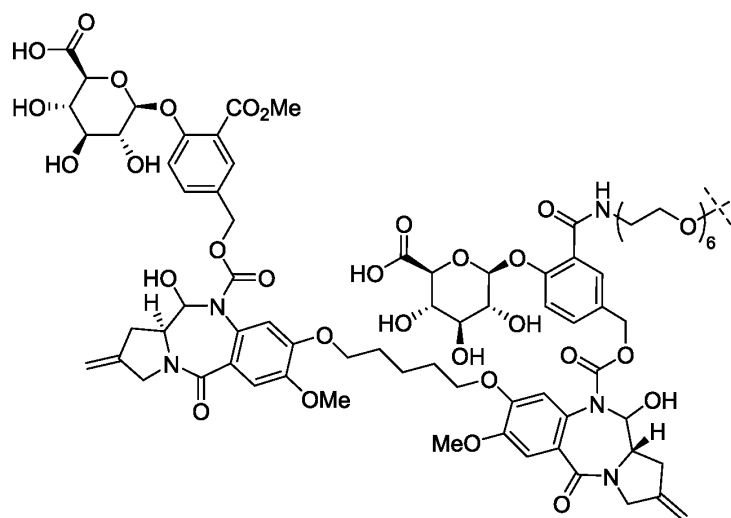
In certain embodiments, the conjugate comprises

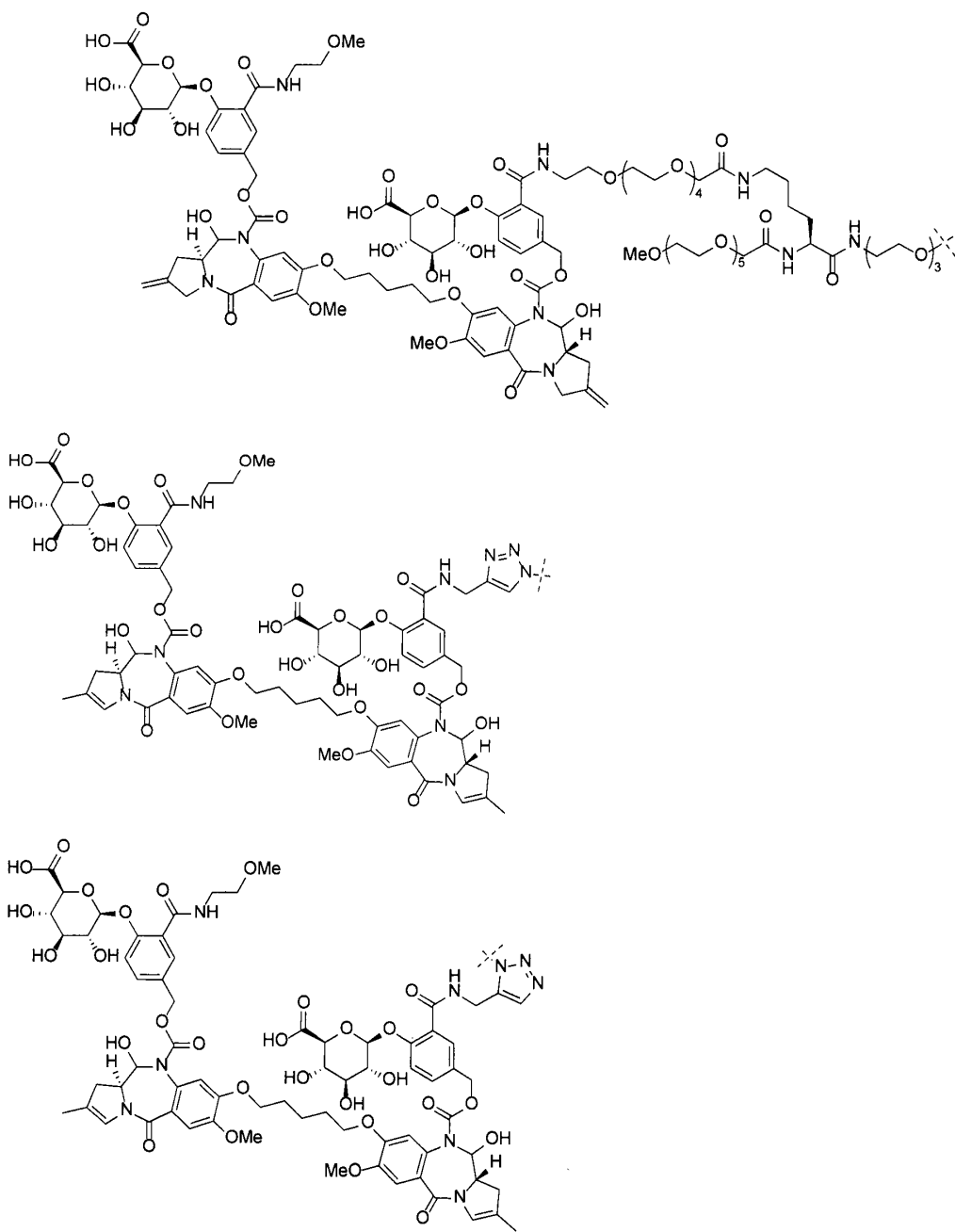




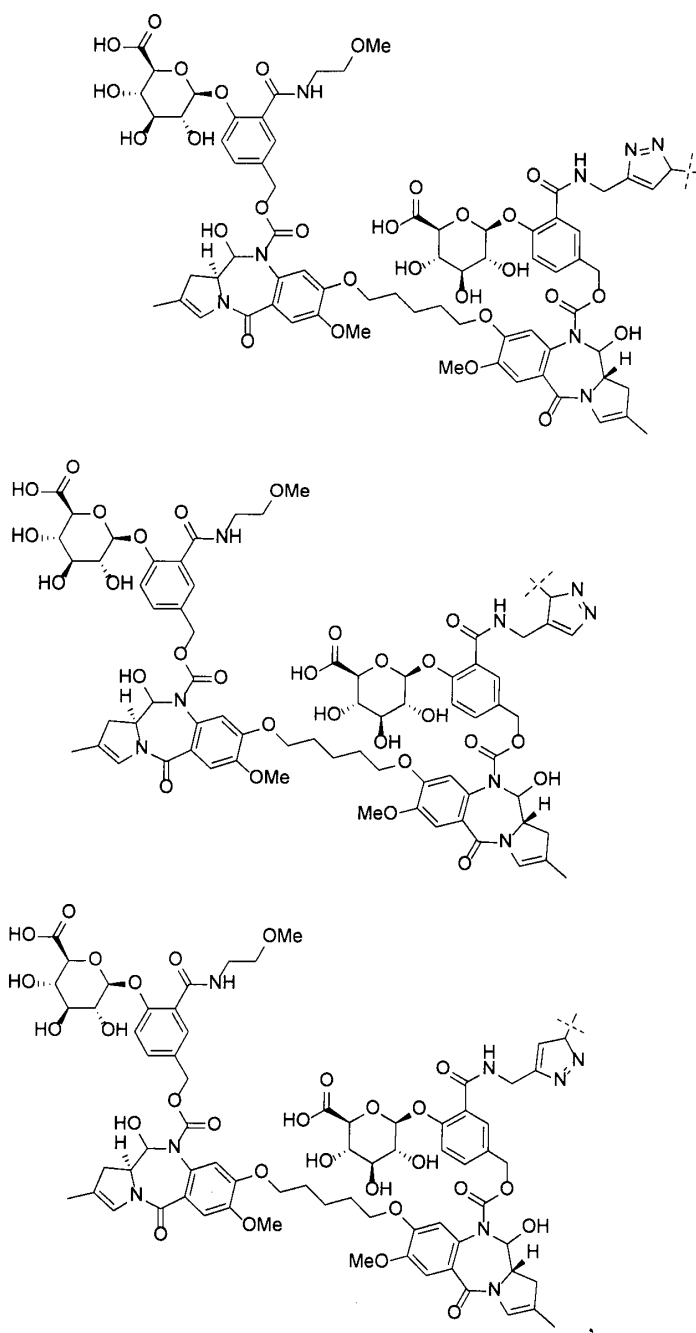




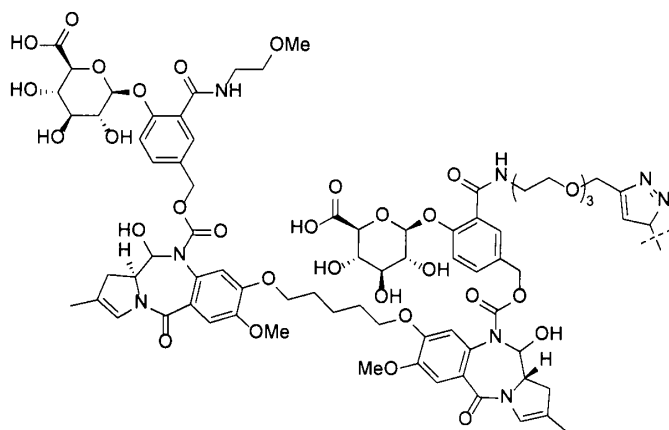
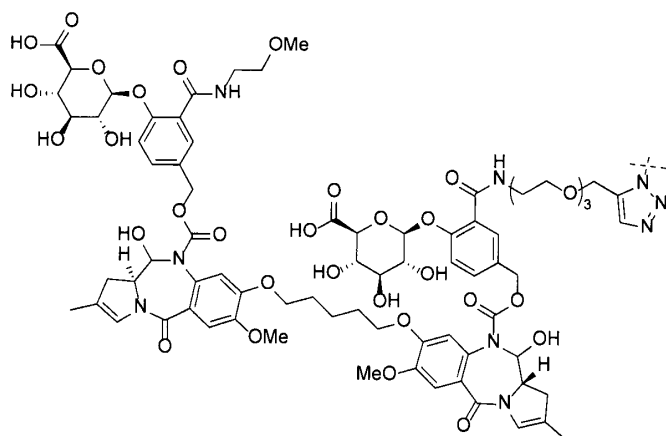
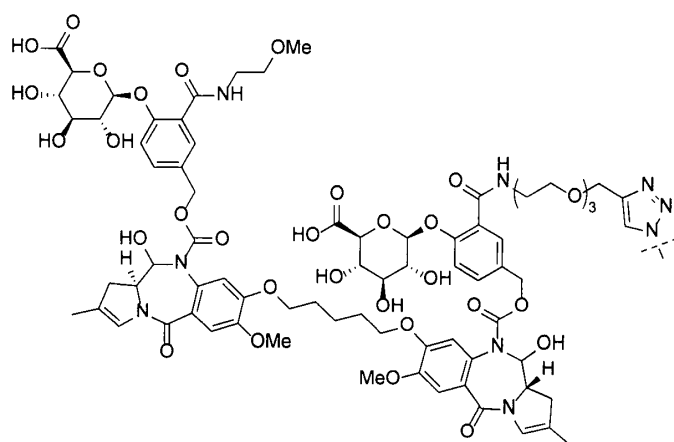




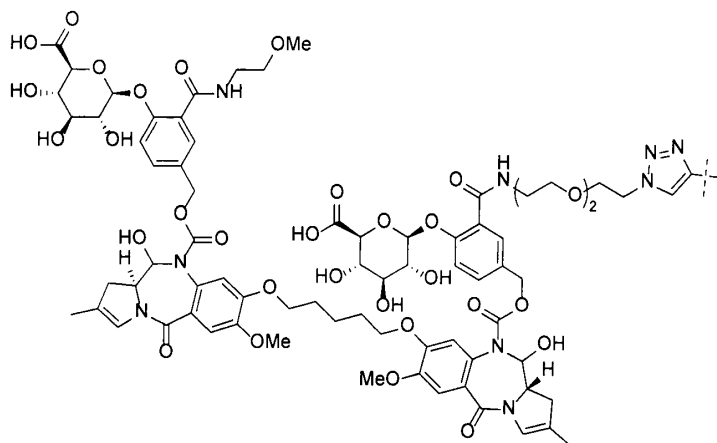
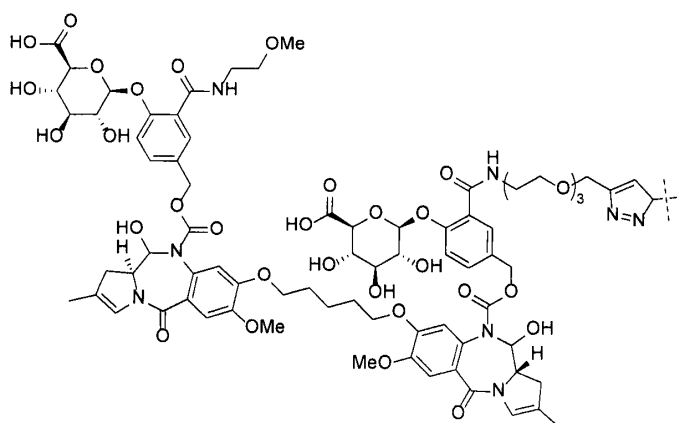
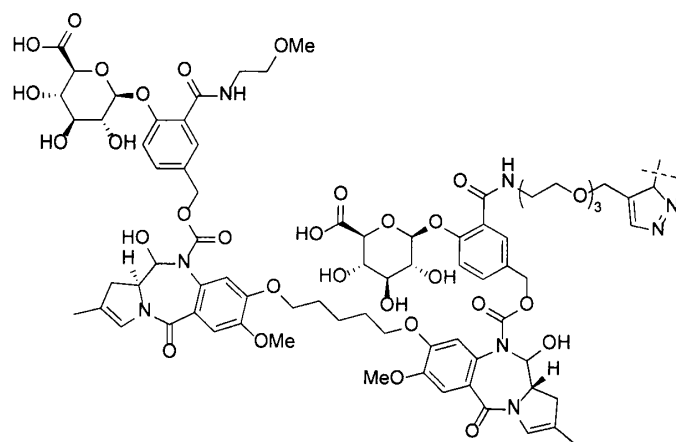
RECTIFIED SHEET (RULE 91) ISA/KR



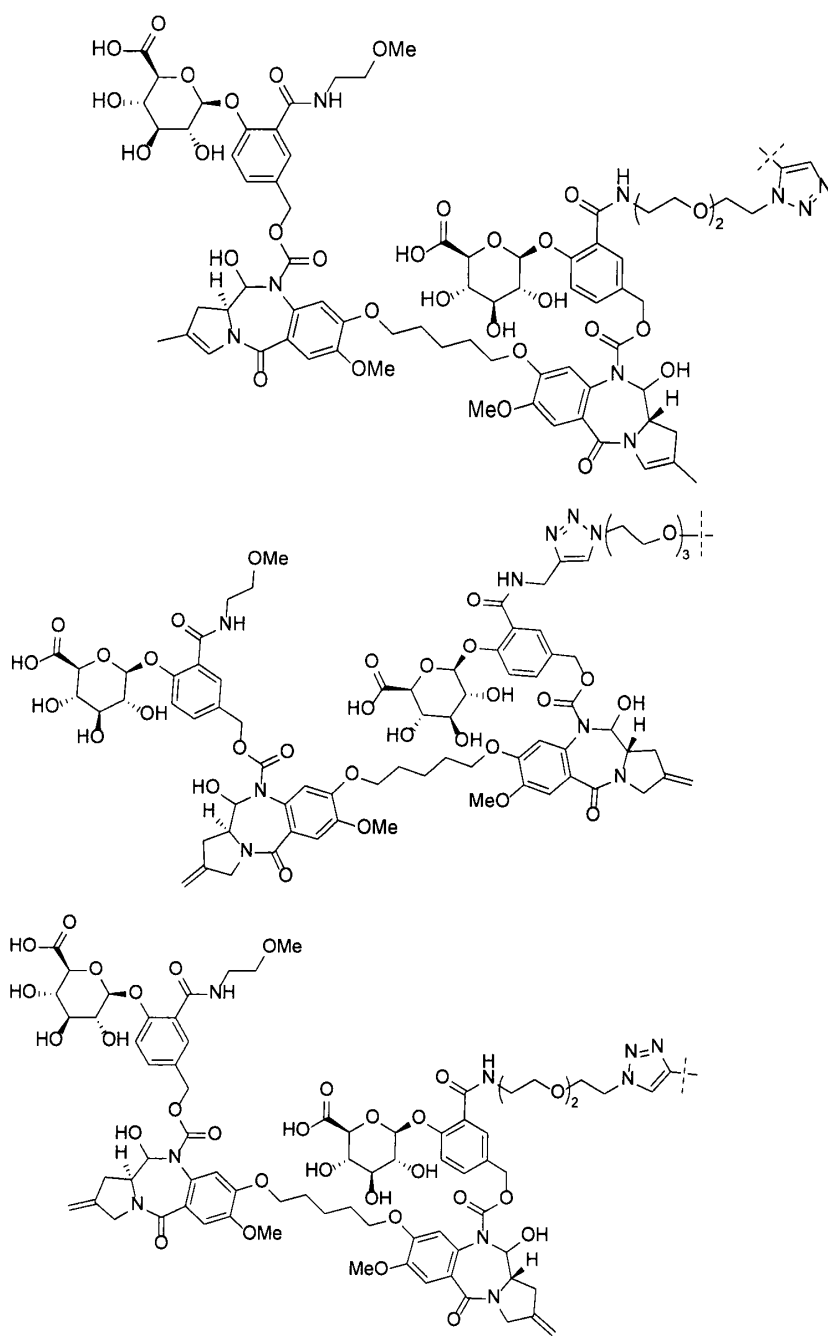
RECTIFIED SHEET (RULE 91) ISA/KR

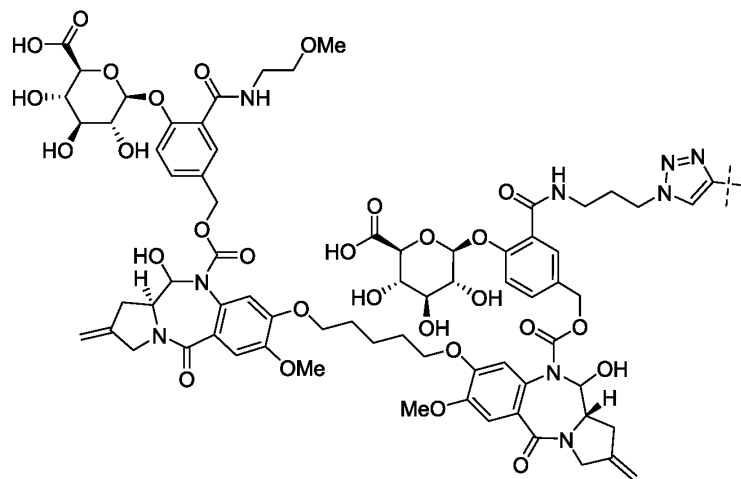
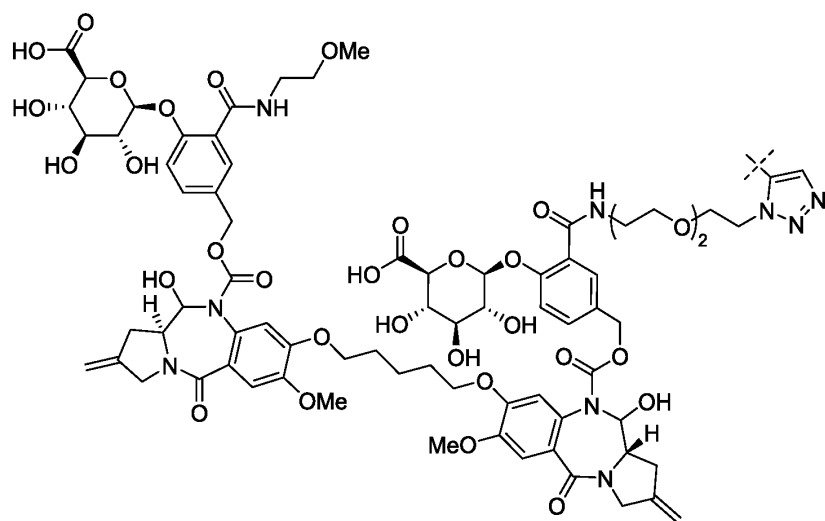


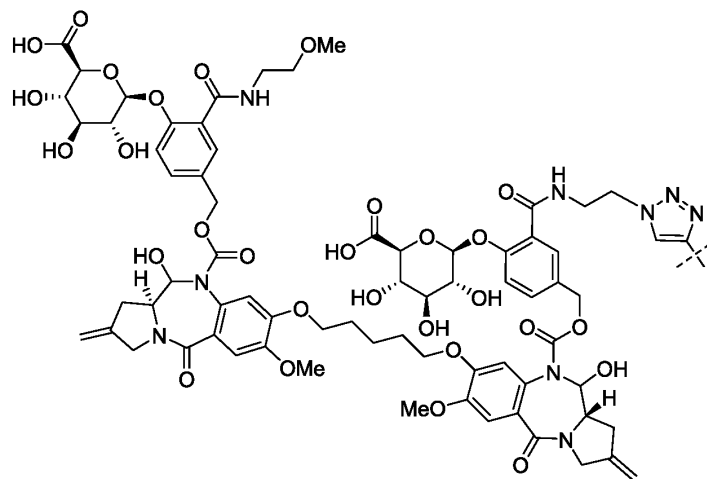
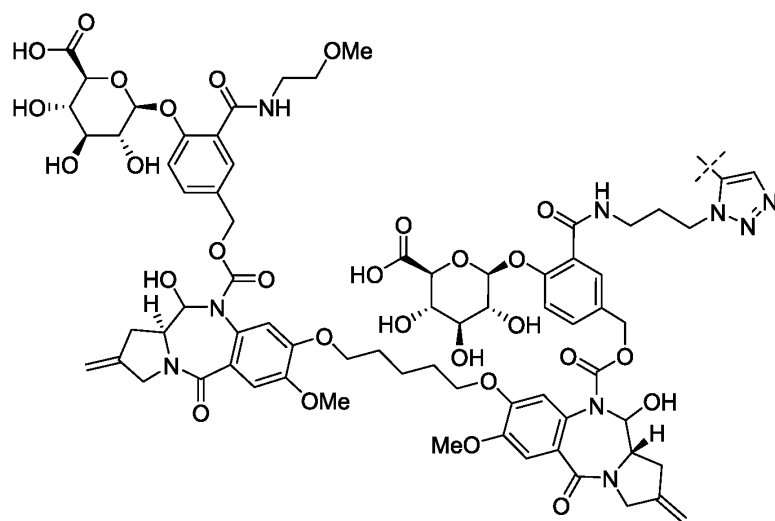
RECTIFIED SHEET (RULE 91) ISA/KR

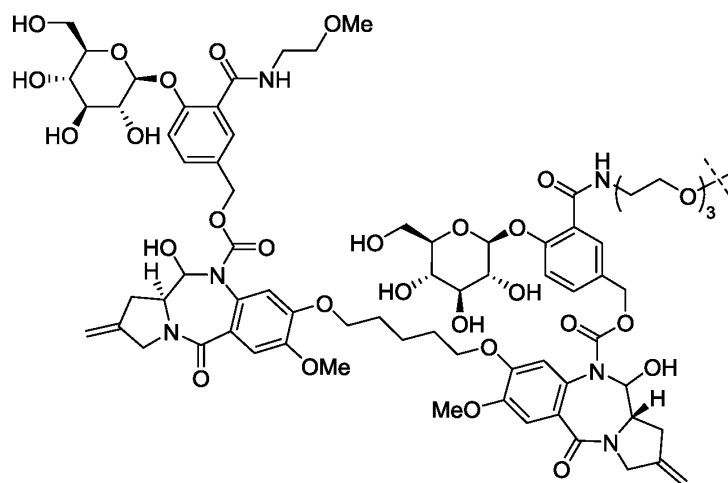
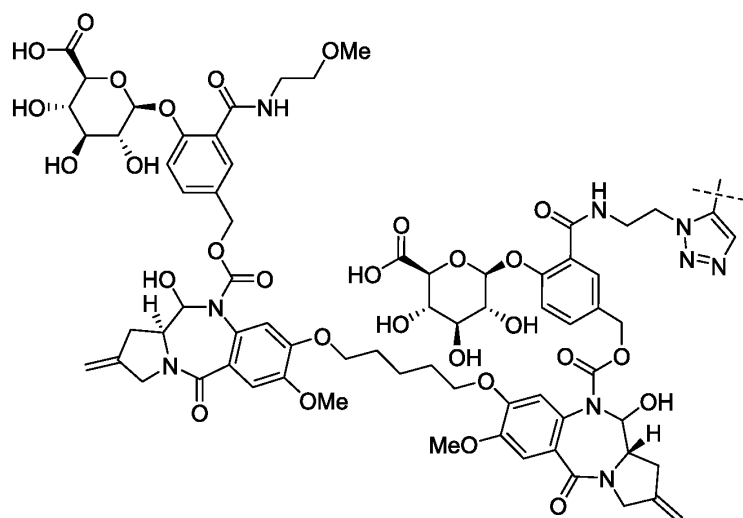


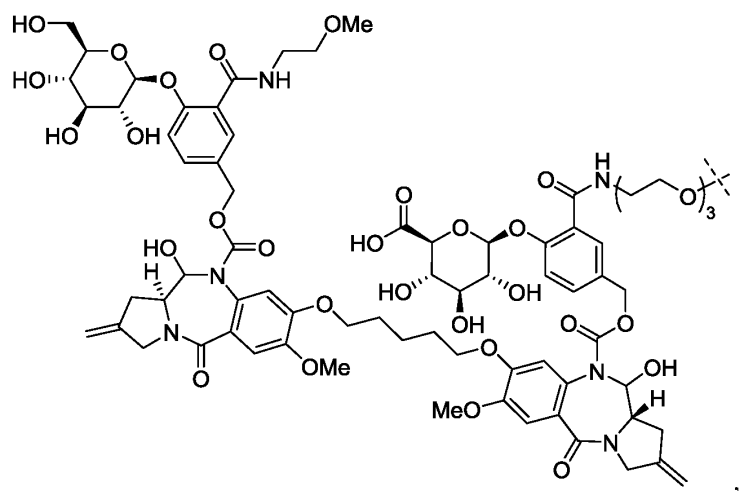
RECTIFIED SHEET (RULE 91) ISA/KR

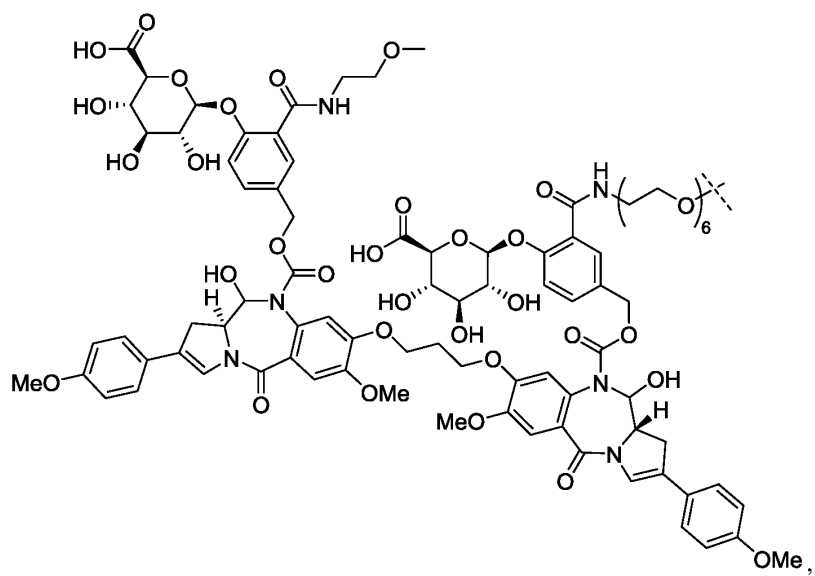
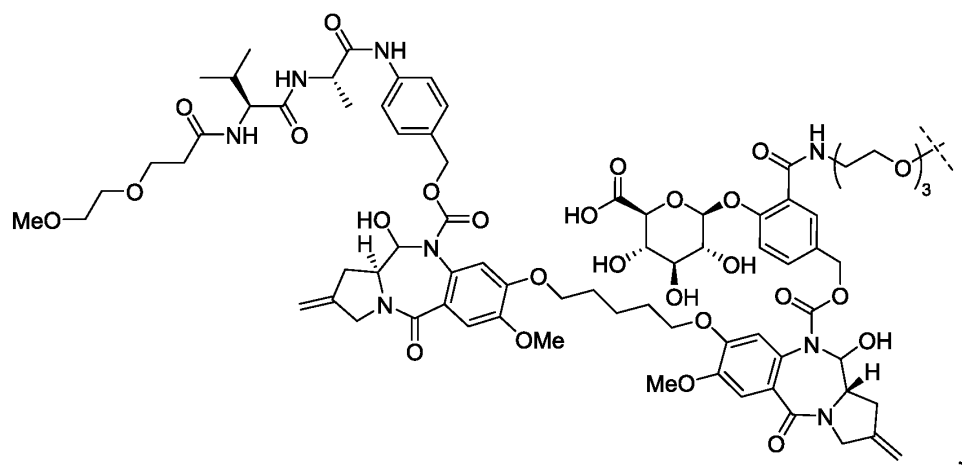


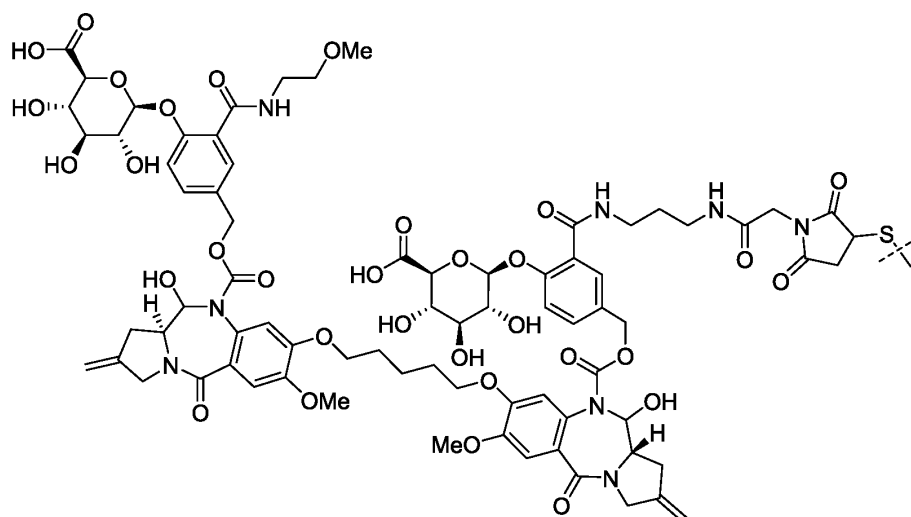
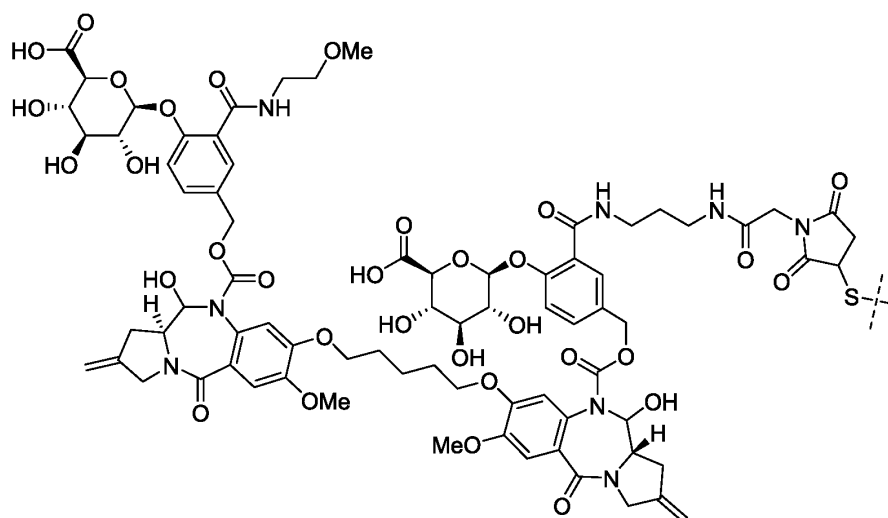


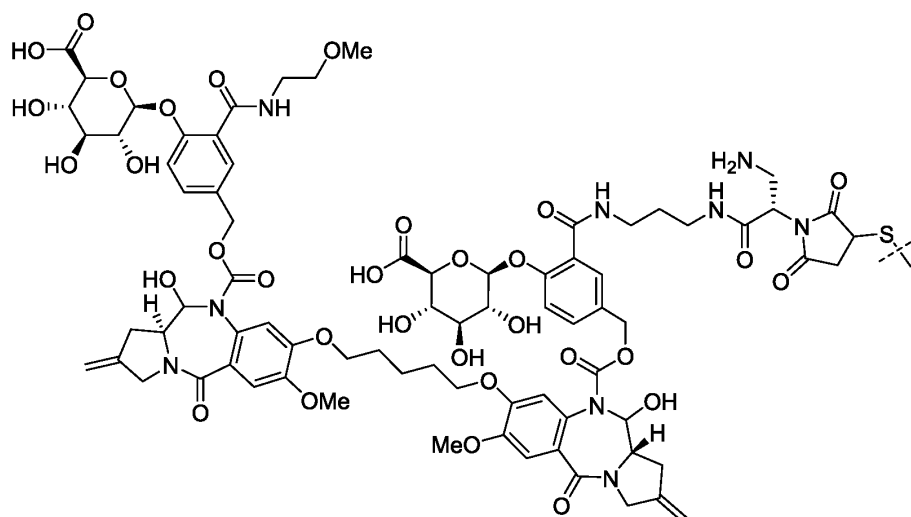
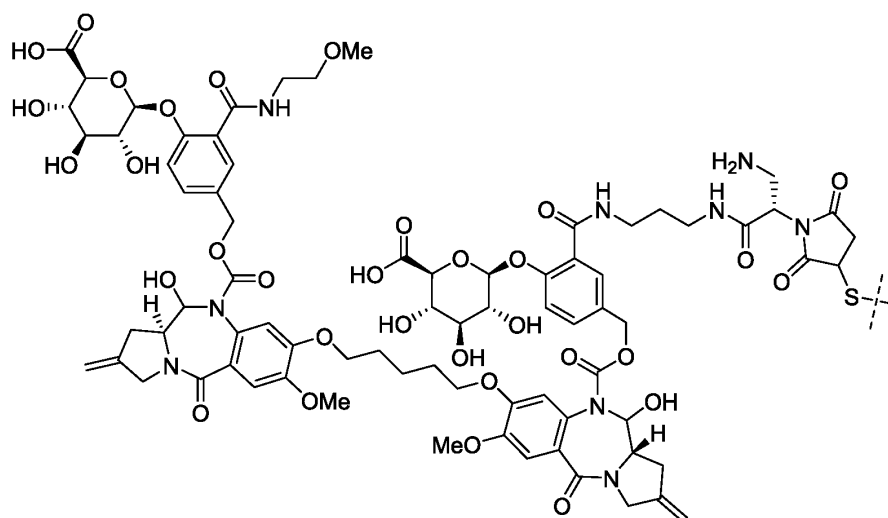


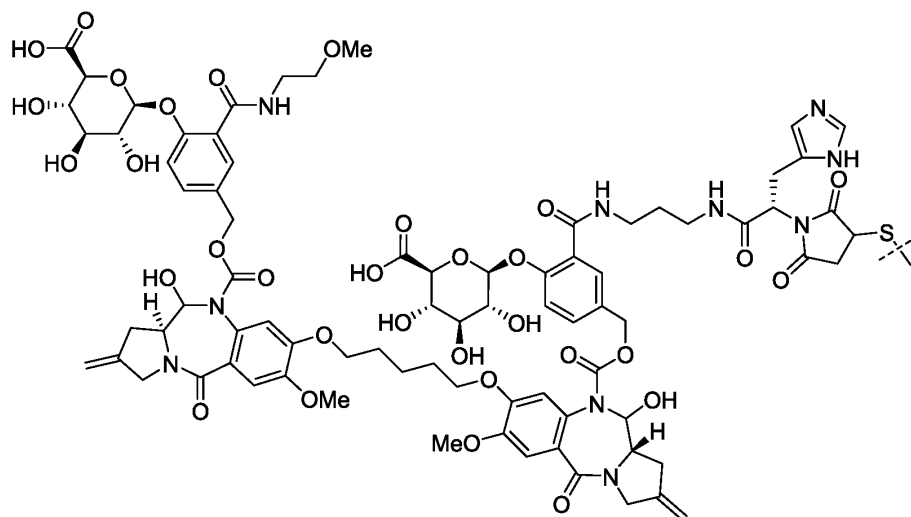
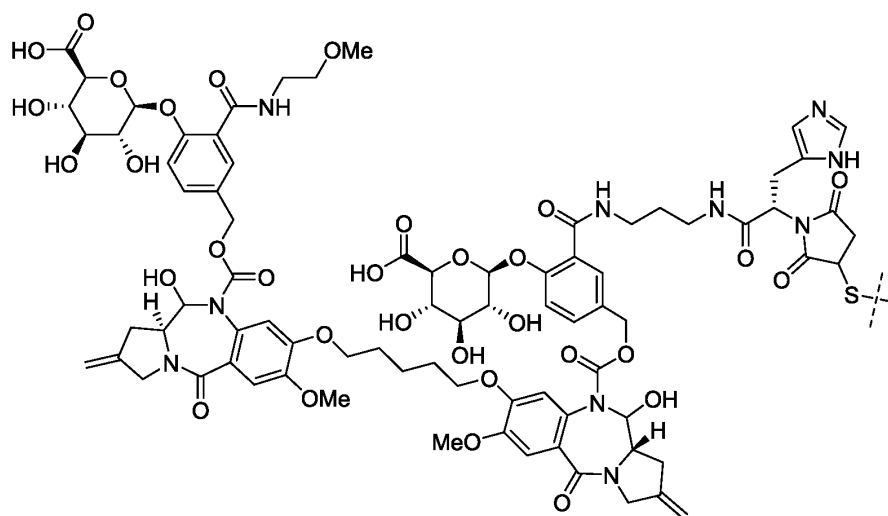


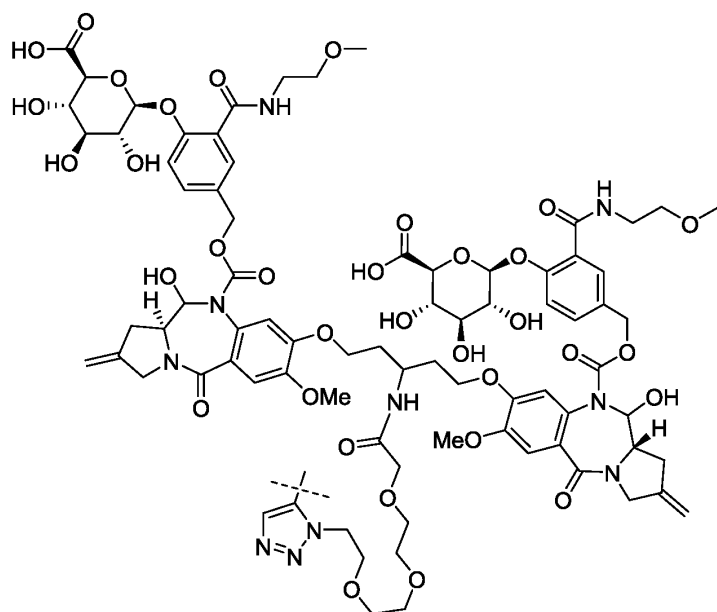
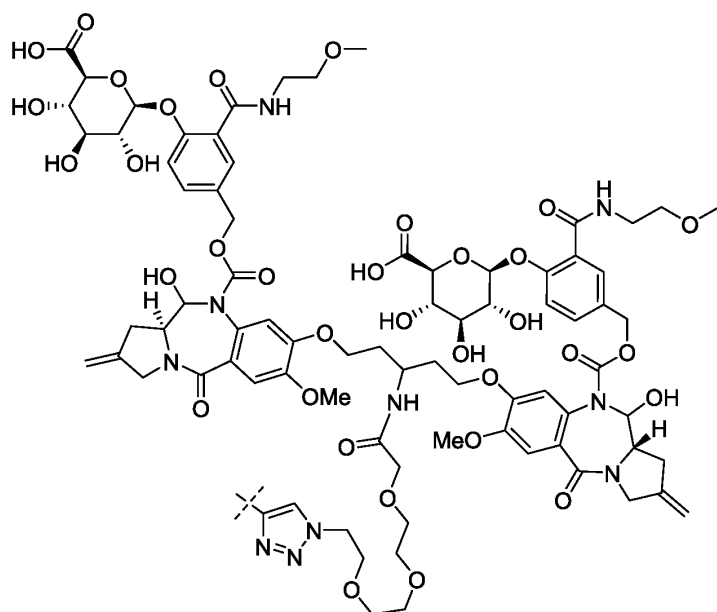


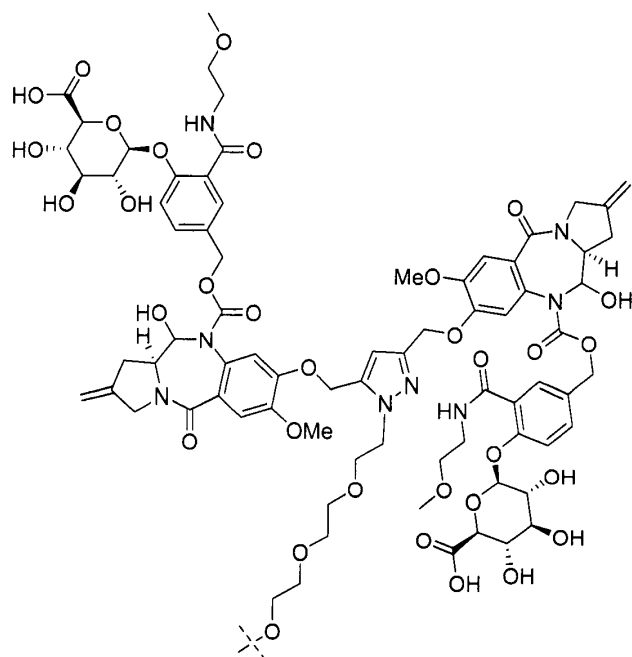










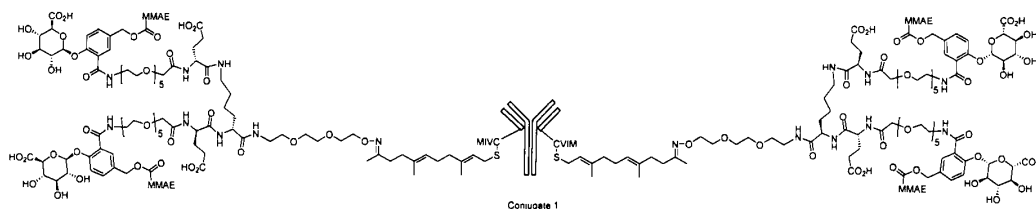


, wherein

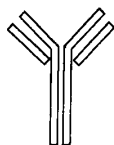
MMAE is monomethyl auristatin E, and MMAF is monomethyl auristatin F; and the dotted line represents a connect to the rest of the conjugate.

In certain preferred embodiments, the active agent is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

In certain preferred embodiments, the conjugate is:

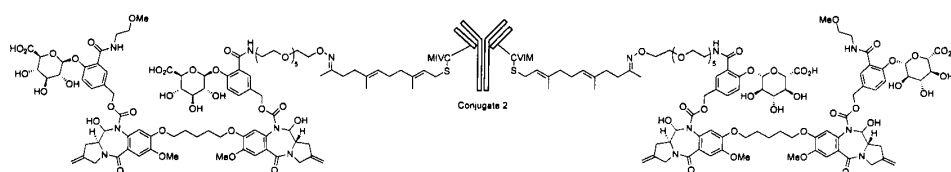


or a salt thereof, wherein:

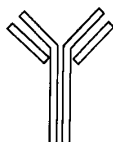


is the antibody.

In certain preferred embodiments, the conjugate is:

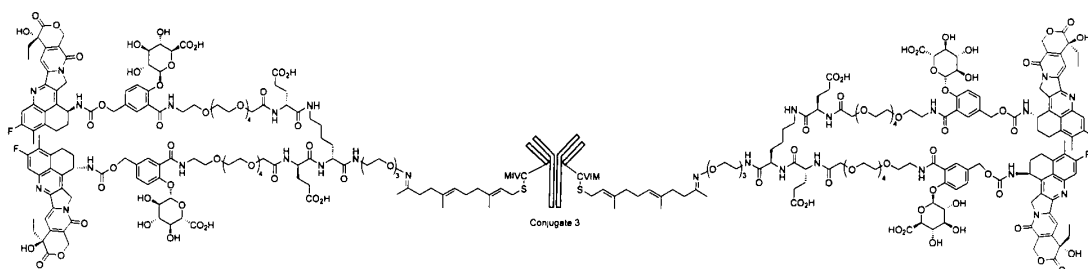


or a salt thereof, wherein:

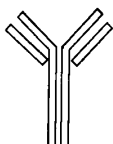


is the anti-human neural cell adhesion molecule L1 (L1CAM) antibody.

In certain preferred embodiments, the conjugate is:



or a salt thereof, wherein:



is the anti-human neural cell adhesion molecule L1 (L1CAM) antibody.

In another aspect, the present disclosure provides pharmaceutical compositions comprising a conjugate disclosed herein and a pharmaceutically acceptable excipient.

In yet another aspect, the present disclosure provides methods of treating or preventing hyperplasia, cancer, or angiogenic disease in a subject, comprising administering a conjugate disclosed herein or a pharmaceutically acceptable salt thereof to the subject. In certain preferred embodiments, the method treats cancer. In certain embodiments, the cancer is lung cancer, small-cell lung cancer, gastrointestinal cancer, colon cancer, intestinal cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreatic cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, or melanoma.

Pharmaceutical Compositions

The compositions and methods of the present invention may be utilized to treat an individual in need thereof. In certain embodiments, the individual is a mammal such as a human, or a non-human mammal. When administered to an animal, such as a human, the composition or the compound is preferably administered as a pharmaceutical composition comprising, for example, a compound of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters. In preferred embodiments, when such pharmaceutical compositions are for human administration, particularly for invasive routes of administration (i.e., routes, such as injection or implantation, that circumvent transport or diffusion through an epithelial barrier), the aqueous solution is pyrogen-free, or substantially pyrogen-free. The excipients can be chosen, for example, to effect delayed release of an agent or to selectively target one or more cells, tissues or organs. The pharmaceutical composition can be in dosage unit form such as tablet, capsule (including sprinkle capsule and gelatin capsule), granule, lyophile for reconstitution, powder, solution, syrup, suppository, injection or the like. The composition can also be present in a transdermal delivery system, e.g., a skin patch. The composition can also be present in a solution suitable for topical administration, such as a lotion, cream, or ointment.

A pharmaceutically acceptable carrier can contain physiologically acceptable agents that act, for example, to stabilize, increase solubility or to increase the absorption of a compound such as a compound of the invention. Such physiologically acceptable agents include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. The choice of a pharmaceutically acceptable carrier, including a physiologically acceptable agent, depends, for example, on the route of administration of the composition. The preparation or pharmaceutical composition can be a selfemulsifying drug delivery system or a selfmicroemulsifying drug delivery system. The pharmaceutical composition (preparation) also can be a liposome or other polymer matrix, which can have incorporated therein, for example, a compound of the invention. Liposomes, for example, which comprise phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

A pharmaceutical composition (preparation) can be administered to a subject by any of a number of routes of administration including, for example, orally (for example, drenches as in aqueous or non-aqueous solutions or suspensions, tablets, capsules (including sprinkle capsules and gelatin capsules), boluses, powders, granules, pastes for application to the tongue); absorption through the oral mucosa (e.g., sublingually); subcutaneously; transdermally (for example as a patch applied to the skin); and topically (for example, as a cream, ointment or spray applied to the skin). The compound may also be formulated for inhalation. In certain embodiments, a compound may be simply dissolved or suspended in sterile water. Details of appropriate routes of administration and compositions suitable for same can be found in, for example, U.S. Pat. Nos. 6,110,973, 5,763,493, 5,731,000, 5,541,231, 5,427,798, 5,358,970 and 4,172,896, as well as in patents cited therein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association an active compound, such as a compound of the invention, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules (including sprinkle capsules and gelatin capsules), cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), lyophile, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. Compositions or compounds may also be administered as a bolus, electuary or paste.

To prepare solid dosage forms for oral administration (capsules (including sprinkle capsules and gelatin capsules), tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds;

(7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; (10) complexing agents, such as, modified and unmodified cyclodextrins; and (11) coloring agents. In the case of capsules (including sprinkle capsules and gelatin capsules), tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules (including sprinkle capsules and gelatin capsules), pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms useful for oral administration include pharmaceutically acceptable emulsions, lyophiles for reconstitution, microemulsions, solutions, suspensions, syrups and elixirs.

In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, cyclodextrins and derivatives thereof, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. Pharmaceutical compositions suitable for parenteral administration comprise one or more active compounds in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

For use in the methods of this invention, active compounds can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Methods of introduction may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinaceous biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a compound at a particular target site.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound or combination of compounds employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound(s) being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By “therapeutically effective

amount” is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those skilled in the art (Isselbacher et al. (1996) Harrison's Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as one, two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain embodiments of the present invention, the active compound may be administered two or three times daily. In preferred embodiments, the active compound will be administered once daily.

The patient receiving this treatment is any animal in need, including primates, in particular humans; and other mammals such as equines, cattle, swine, sheep, cats, and dogs; poultry; and pets in general.

In certain embodiments, compounds of the invention may be used alone or conjointly administered with another type of therapeutic agent.

The present disclosure includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. In certain embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium,

triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, Na, Ca, K, Mg, Zn or other metal salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, l-ascorbic acid, l-aspartic acid, benzenesulfonic acid, benzoic acid, (+)-camphoric acid, (+)-camphor-10-sulfonic acid, capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, d-glucoheptonic acid, d-gluconic acid, d-glucuronic acid, glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, l-malic acid, malonic acid, mandelic acid, methanesulfonic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, phosphoric acid, propionic acid, l-pyroglutamic acid, salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, l-tartaric acid, thiocyanic acid, p-toluenesulfonic acid, trifluoroacetic acid, and undecylenic acid acid salts.

The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Definitions

Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular biology, cell and cancer biology, neurobiology, neurochemistry, virology, immunology, microbiology, pharmacology, genetics and protein and nucleic acid chemistry, described herein, are those well known and commonly used in the art.

The methods and techniques of the present disclosure are generally performed, unless otherwise indicated, according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout this specification. See, e.g. "Principles of Neural Science", McGraw-Hill Medical, New York, N.Y. (2000); Motulsky, "Intuitive Biostatistics", Oxford University Press, Inc. (1995); Lodish et al., "Molecular Cell Biology, 4th ed.", W. H. Freeman & Co., New York (2000); Griffiths et al., "Introduction to Genetic Analysis, 7th ed.", W. H. Freeman & Co., N.Y. (1999); and Gilbert et al., "Developmental Biology, 6th ed.", Sinauer Associates, Inc., Sunderland, MA (2000).

Chemistry terms used herein, unless otherwise defined herein, are used according to conventional usage in the art, as exemplified by "The McGraw-Hill Dictionary of Chemical Terms", Parker S., Ed., McGraw-Hill, San Francisco, C.A. (1985).

All of the above, and any other publications, patents and published patent applications referred to in this application are specifically incorporated by reference herein. In case of conflict, the present specification, including its specific definitions, will control.

The term "agent" is used herein to denote a chemical compound (such as an organic or inorganic compound, a mixture of chemical compounds), a biological macromolecule (such as a nucleic acid, an antibody, including parts thereof as well as humanized, chimeric and human antibodies and monoclonal antibodies, a protein or portion thereof, e.g., a peptide, a lipid, a carbohydrate), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Agents include, for example, agents whose structure is known, and those whose structure is not known.

The term "conjugates" as used herein refers to cell binding agents that are covalently bonded to one or more molecules of a cytotoxic compound. In this regard, "cell binding agent" is a molecule having affinity for a biological target, and may be, for example, an antibody,

particularly a monoclonal antibody, or an antibody fragment, and the binding agent functions to direct a biologically active compound to a biological target. In certain embodiments of the present disclosure, the conjugate may be designed to target tumor cells through cell surface antigens. The antigen may be a cell surface antigen that is overexpressed or expressed in an abnormal cell type. Specifically, the target antigen may be expressed only on proliferative cells (e.g., tumor cells). The target antigen may be selected on the basis of different expression, usually between proliferative tissues and normal tissues.

A “patient,” “subject,” or “individual” are used interchangeably and refer to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

“Treating” a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term “preventing” is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount.

“Administering” or “administration of” a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion),

intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age and/or the physical condition of the subject and the chemical and biological properties of the compound or agent (e.g., solubility, digestibility, bioavailability, stability and toxicity). In some embodiments, a compound or an agent is administered orally, e.g., to a subject by ingestion. In some embodiments, the orally administered compound or agent is in an extended release or slow release formulation, or administered using a device for such slow or extended release.

As used herein, the phrase “conjoint administration” refers to any form of administration of two or more different therapeutic agents such that the second agent is administered while the previously administered therapeutic agent is still effective in the body (e.g., the two agents are simultaneously effective in the patient, which may include synergistic effects of the two agents). For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic agents.

A “therapeutically effective amount” or a “therapeutically effective dose” of a drug or agent is an amount of a drug or an agent that, when administered to a subject will have the intended therapeutic effect. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. The precise effective amount needed for a subject will depend upon, for example, the subject’s size, health and age, and the nature and extent of the condition being treated, such as cancer or MDS. The skilled worker can readily determine the effective amount for a given situation by routine experimentation.

As used herein, the terms “optional” or “optionally” mean that the subsequently described event or circumstance may occur or may not occur, and that the description includes instances where the event or circumstance occurs as well as instances in which it does not. For example,

“optionally substituted alkyl” refers to the alkyl may be substituted as well as where the alkyl is not substituted.

It is understood that substituents and substitution patterns on the compounds of the present invention can be selected by one of ordinary skilled person in the art to result chemically stable compounds which can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials. If a substituent is itself substituted with more than one group, it is understood that these multiple groups may be on the same carbon or on different carbons, so long as a stable structure results.

As used herein, the term “optionally substituted” refers to the replacement of one to six hydrogen radicals in a given structure with the radical of a specified substituent including, but not limited to: hydroxyl, hydroxyalkyl, alkoxy, halogen, alkyl, nitro, silyl, acyl, acyloxy, aryl, cycloalkyl, heterocyclyl, amino, aminoalkyl, cyano, haloalkyl, haloalkoxy, -OCO-CH₂-O-alkyl, -OP(O)(O-alkyl)₂ or -CH₂-OP(O)(O-alkyl)₂. Preferably, “optionally substituted” refers to the replacement of one to four hydrogen radicals in a given structure with the substituents mentioned above. More preferably, one to three hydrogen radicals are replaced by the substituents as mentioned above. It is understood that the substituent can be further substituted.

In the present invention, the prefixes (e.g., C₁₋₁₂, C₃₋₈, etc.) refer to the number of ring atoms or a range for the number of ring atoms, regardless of whether they are carbon atoms or hetero atoms.

As used herein, the term “alkyl” refers to saturated aliphatic groups, including but not limited to C₁-C₁₀ straight-chain alkyl groups or C₁-C₁₀ branched-chain alkyl groups. Preferably, the “alkyl” group refers to C₁-C₆ straight-chain alkyl groups or C₁-C₆ branched-chain alkyl groups. Most preferably, the “alkyl” group refers to C₁-C₄ straight-chain alkyl groups or C₁-C₄ branched-chain alkyl groups. Examples of “alkyl” include, but are not limited to, methyl, ethyl, 1-propyl, 2-propyl, n-butyl, sec-butyl, tert-butyl, 1-pentyl, 2-pentyl, 3-pentyl, neo-pentyl, 1-hexyl, 2-hexyl, 3-hexyl, 1-heptyl, 2-heptyl, 3-heptyl, 4-heptyl, 1-octyl, 2-octyl, 3-octyl or 4-octyl and the like. The “alkyl” group may be optionally substituted.

The term “acyl” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term “acylamino” is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term “acyloxy” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term “alkoxy” refers to an alkyl group having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term “alkyl” refers to saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C₁₋₃₀ for straight chains, C₃₋₃₀ for branched chains), and more preferably 20 or fewer.

Moreover, the term “alkyl” as used throughout the specification, examples, and claims is intended to include both unsubstituted and substituted alkyl groups, the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc.

The term “alkenyl” refers to an alkyl having at least one carbon-carbon double bond. Examples of unsaturated alkenyl groups are ethenyl (vinyl, -CH=CH₂), 1-propenyl (-CH=CHCH₃), 2-propenyl, isopropenyl, butenyl, pentenyl and hexenyl, etc.

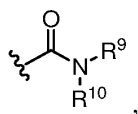
The term “alkynyl” refers to an alkyl group having at least one carbon-carbon triple bond, and examples of unsaturated alkynyl group include ethynyl and 2-propynyl, etc.

The term “C_{x-y}” or “C_x-C_y”, when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. C₀alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. A C₁₋₆alkyl group, for example, contains from one to six carbon atoms in the chain.

The term “alkylamino”, as used herein, refers to an amino group substituted with at least one alkyl group.

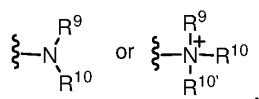
The term “alkylthio”, as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

The term “amido”, as used herein, refers to a group



wherein R^9 and R^{10} each independently represent a hydrogen or hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



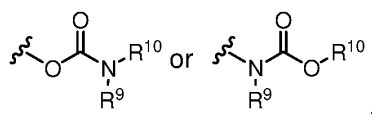
wherein R^9 , R^{10} , and $R^{10'}$ each independently represent a hydrogen or a hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term “aminoalkyl”, as used herein, refers to an alkyl group substituted with an amino group.

The term “aralkyl”, as used herein, refers to an alkyl group substituted with an aryl group.

The term “aryl” as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term “carbamate” is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group.

The term “carbocyclalkyl”, as used herein, refers to an alkyl group substituted with a carbocycle group.

The term “carbocycle” includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term “fused carbocycle” refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary “carbocycles” include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. “Carbocycles” may be substituted at any one or more positions capable of bearing a hydrogen atom.

The term “carbocyclalkyl”, as used herein, refers to an alkyl group substituted with a carbocycle group.

The term “carbonate” is art-recognized and refers to a group $-\text{OCO}_2-$.

The term “carboxy”, as used herein, refers to a group represented by the formula $-\text{CO}_2\text{H}$.

The term “cycloalkyl” includes substituted or unsubstituted non-aromatic single ring structures, preferably 4- to 8-membered rings, more preferably 4- to 6-membered rings. The term “cycloalkyl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is cycloalkyl and the substituent (e.g., R^{100}) is attached to the cycloalkyl ring, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, denzodioxane, tetrahydroquinoline, and the like.

The term “ester”, as used herein, refers to a group $-\text{C}(\text{O})\text{OR}^9$ wherein R^9 represents a hydrocarbyl group.

The term “ether”, as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include,

but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include “alkoxyalkyl” groups, which may be represented by the general formula alkyl-O-alkyl.

The term “formyl” refers to $-C(=O)H$.

The terms “halo” and “halogen” as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms “hetaralkyl” and “heteroaralkyl”, as used herein, refers to an alkyl group substituted with a hetaryl group.

The terms “heteroaryl” and “hetaryl” include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heteroaryl” and “hetaryl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term “heteroatom” as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The term “heterocyclylalkyl”, as used herein, refers to an alkyl group substituted with a heterocycle group.

The terms “heterocyclyl”, “heterocycle”, and “heterocyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heterocyclyl” and “heterocyclic” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term “hydrocarbyl”, as used herein, refers to a group that is bonded through a carbon atom that does not have a $=O$ or $=S$ substituent, and typically has at least one carbon-hydrogen

bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and even trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocycle, alkyl, alkenyl, alkynyl, and combinations thereof.

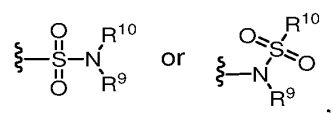
The term “hydroxyalkyl”, as used herein, refers to an alkyl group substituted with a hydroxy group.

The term “lower” when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer atoms in the substituent, preferably six or fewer. A “lower alkyl”, for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

The terms “polycyclyl”, “polycycle”, and “polycyclic” refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are “fused rings”. Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term “sulfate” is art-recognized and refers to the group $-\text{OSO}_3\text{H}$, or a pharmaceutically acceptable salt thereof.

The term “sulfonamido” is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl.

The term “sulfoxide” is art-recognized and refers to the group $-\text{S}(\text{O})-$.

The term “sulfonate” is art-recognized and refers to the group SO_3H , or a pharmaceutically acceptable salt thereof.

The term “sulfone” is art-recognized and refers to the group $-\text{S}(\text{O})_2-$.

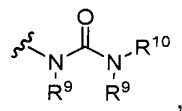
The term “substituted” refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It is understood that “substitution” or “substituted with” includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In some embodiments, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It is understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

The term “thioalkyl”, as used herein, refers to an alkyl group substituted with a thiol group.

The term “thioester”, as used herein, refers to a group $-\text{C}(\text{O})\text{SR}^9$ or $-\text{SC}(\text{O})\text{R}^9$ wherein R^9 represents a hydrocarbyl.

The term “thioether”, as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term “urea” is art-recognized and may be represented by the general formula



wherein R⁹ and R¹⁰ independently represent hydrogen or a hydrocarbyl.

The term “modulate” as used herein includes the inhibition or suppression of a function or activity (such as cell proliferation) as well as the enhancement of a function or activity.

The phrase “pharmaceutically acceptable” is art-recognized. In certain embodiments, the term includes compositions, excipients, adjuvants, polymers and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

“Pharmaceutically acceptable salt” or “salt” is used herein to refer to an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

The term “pharmaceutically acceptable acid addition salt” as used herein means any non-toxic organic or inorganic salt of any base compounds represented by Formula I. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of the appropriate salt is known to one skilled in the art. Other non-pharmaceutically acceptable salts, e.g., oxalates, may be used, for example, in the isolation of compounds of Formula I for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

The term “pharmaceutically acceptable basic addition salt” as used herein means any non-toxic organic or inorganic base addition salt of any acid compounds represented by Formula I or any of their intermediates. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium, or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic, or aromatic organic amines such as methylamine,

trimethylamine and picoline or ammonia. The selection of the appropriate salt is known to a person skilled in the art.

Many of the compounds useful in the methods and compositions of this disclosure have at least one stereogenic center in their structure. This stereogenic center may be present in a R or a S configuration, said R and S notation is used in correspondence with the rules described in Pure Appl. Chem. (1976), 45, 11-30. The disclosure contemplates all stereoisomeric forms such as enantiomeric and diastereoisomeric forms of the compounds, salts, prodrugs or mixtures thereof (including all possible mixtures of stereoisomers). See, e.g., WO 01/062726.

Furthermore, certain compounds which contain alkenyl groups may exist as Z (zusammen) or E (entgegen) isomers. In each instance, the disclosure includes both mixture and separate individual isomers.

“Prodrug” or “pharmaceutically acceptable prodrug” refers to a compound that is metabolized, for example hydrolyzed or oxidized, in the host after administration to form the compound of the present disclosure (e.g., compounds of formula I). Typical examples of prodrugs include compounds that have biologically labile or cleavable (protecting) groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, or dephosphorylated to produce the active compound. Examples of prodrugs using ester or phosphoramidate as biologically labile or cleavable (protecting) groups are disclosed in U.S. Patents 6,875,751, 7,585,851, and 7,964,580, the disclosures of which are incorporated herein by reference. The prodrugs of this disclosure are metabolized to produce a compound of Formula I. The present disclosure includes within its scope, prodrugs of the compounds described herein. Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in “Design of Prodrugs” Ed. H. Bundgaard, Elsevier, 1985.

The phrase “pharmaceutically acceptable carrier” as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filter, diluent, excipient, solvent or encapsulating material useful for formulating a drug for medicinal or therapeutic use.

The term “Log of solubility”, “LogS” or “logS” as used herein is used in the art to quantify the aqueous solubility of a compound. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. A low solubility often goes along with a poor

absorption. LogS value is a unit stripped logarithm (base 10) of the solubility measured in mol/liter.

The term “identity” refers to the similarity or relatedness of two or more polypeptide or nucleic acid sequences determined by aligning and comparing the sequences of two or more polypeptides or nucleic acids. Such inter-sequence identity is typically expressed as “percentage identity,” representing the proportion of identical amino acids or nucleotides between the compared molecules, calculated based on the smallest size molecule among the compared molecules. Methods for aligning nucleic acids or polypeptides to calculate identity between them are known in the art and may also be referenced herein.

The term “affinity” or “avidity” refers to the strength of interaction between an antibody or its antigen-binding fragment and an antigen, determined by characteristics of the antigen such as size, shape, and/or charge, and the CDR sequences of the antibody or antigen-binding fragment. Methods for determining such affinities are known in the art and may also be referenced herein.

Antibodies or their antigen-binding fragments used in the present invention are said to “specifically bind” to the target, such as the antigen, when the dissociation constant (KD) is $\leq 10^{-6}$ M. Antibodies bind “with high affinity” to the target when KD is $\leq 1 \times 10^{-8}$ M.

The term “antigen-binding fragment” of an antibody or immunoglobulin chain (light or heavy) used in the present invention refers to a portion of the antibody that includes some amino acids missing compared to the full-length chain but includes a portion of the antibody that can specifically bind to the target antigen. These fragments can be biologically active in terms of being able to specifically bind to target antigens or compete with other antibodies or antigen-binding fragments for binding to specific epitopes. In some embodiments, such fragments comprise at least one CDR in a full-length light chain or heavy chain, and comprise short heavy chain and/or light chains, or portions thereof in some embodiments. These biologically active fragments may be produced by recombinant DNA techniques or, for example, by enzymatic or chemical cleavage of intact antibodies. Immunologically functional immunoglobulin fragments include, but are not limited to, Fab, Fab', F(ab')₂, scFab, dsFv, Fv, scFV, scFV-Fc, diabody, minibody, scAb, and dAb and may be derived from any mammal including human, mouse, rat, camelid or rabbit. In this invention, functional portion of the antibody, such as one or more CDRs can be covalently linked to a second protein or small molecule compound and used as a targeted therapeutic agent for a specific target.

The “Fc” region is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions and in this invention includes two heavy chain fragments comprising the CH2 and CH3 domains of an antibody. These two heavy chain fragments are linked to each other by two or more disulfide bonds and hydrophobic interactions of the CH3 domain. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The Cterminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the present disclosure include human IgG1, IgG2, IgG3 and IgG4.

The “Fab fragment” in this invention consists of one light chain and one heavy chain containing only the variable region and CH1. The heavy chain of a Fab molecule cannot form disulfide bonds with other heavy chain molecules. scFab is Fab of two molecules linked by a flexible linker.

The “Fab' fragment” in this invention includes a region between the CH1 and CH2 domains of the heavy chain in addition to the Fab fragment, which forms an interchain disulfide bond between the two heavy chains of the two molecules of the Fab' fragment, thereby forming F(ab')₂ molecules.

As described above, the “F(ab')₂ fragment” in this invention includes two light chains and two heavy chains including a variable region, CH1, and a portion of the constant region between the CH1 and CH2 domains, which form 2 interchain disulfide bonds are between the heavy chains. Therefore, the F(ab')₂ fragment is composed of two Fab' fragments, and the two Fab' fragments are associated with each other by a disulfide bond between them.

The “Fv region” in this invention is a fragment of an antibody that includes the variable regions of the heavy chain and light chains, but does not include the constant region. sdFV is where heavy chains and light chains are linked by disulfide bonds. scFv is Fv linked by a flexible linker.

scFv-Fc is where Fc is linked to scFV. The minibody is where CH3 is linked to scFV. The diabody contains scFV of two molecules.

A “single chain Fv” or “scFv” antibody fragment in this invention comprises the VH and VL domains of an antibody, and these domains are in a single polypeptide chain. The Fv polypeptide may further include a polypeptide linker between the VH and VL domains that allows the scFv to form the desired structure for antigen binding.

A “single chain antibody” (scAb) in this invention is a single polypeptide chain containing one constant region of a heavy chain or a light chain constant region where the heavy chain and light chain variable regions are linked by a flexible linker. For single chain antibodies, see, for example, U.S. Pat. No. 5,260,203, which is disclosed in the present invention for reference.

A “domain antibody” (dAb) in this invention is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. For example, in one embodiment, two or more VH regions are covalently linked by a peptide linker to form a bivalent domain antibody. The two VH regions of these bivalent domain antibodies can target the same or different antigens.

As used herein, “complementarity determining region” (CDR; i.e., CDR1, CDR2, and CDR3) refers to amino acid moieties of an antibody variable domain that are required for antigen binding. Generally, antibodies comprise six CDRs; three in the VH (H1 CDR, H2 CDR, H3 CDR), and three in the VL (L1 CDR, L2 CDR, L3 CDR). In native antibodies, H3 and L3 display the most diversity of the six CDRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al., *Immunity* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, e.g., Hamers-Casterman et al., *Nature* 363:446-448 (1993) and Sheriff et al., *Nature Struct. Biol.* 3:733-736 (1996).

As used herein, “framework region” (FR) is variable domain moieties other than CDR moieties. Each variable domain typically has four FRs, identified as FR1, FR2, FR3 and FR4.

As used herein, a “bivalent antigen binding protein” or “bivalent antibody” includes two antigen binding sites. The two antigen binding sites included in such a bivalent antibody may have the same antigen specificity, or may be bispecific antibodies that each bind to different antigens.

As used herein, “multispecific antigen binding protein” or “multispecific antibody” targets two or more antigens or epitopes.

As used herein, a “chimeric antibody” refers to an antibody (immunoglobulin) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Nat’l Acad. Sci. USA, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with an antigen of interest.

“Humanized” forms of non-human (e.g., murine) antibodies, are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an CDR of the recipient are replaced by residues from an CDR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct.

Biol. 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

A “human antibody” is one that possesses an amino-acid sequence corresponding to that of an antibody, produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice (see, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al., *Proc. Nat’l Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

A “human consensus framework” is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat et al., *supra*. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat et al., *supra*.

An “affinity-matured” antibody is one with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al.,

Bio/Technology 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of CDR and/or framework residues is described by, for example: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147- 155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

EXAMPLES

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

Example 1: Production of Exemplified Human L1CAM (Neural Cell Adhesion Molecule L1) Specific Antibodies

L1CAM (Neural cell adhesion molecule L1) is widely expressed in tumors. An exemplified anti-L1CAM humanized antibody AFF4, which specifically binds to L1CAM, was produced by the method described in European Patent Application No. EP22196917.3, the entirety of which is herein included in the present specification by reference. The amino acid sequences of the antibody AFF4 are shown in **Tables 7 and 8** below.

Table 7. AFF4 Amino Acid Sequences

AFF4 heavy chain		SEQ ID NO:
FR1	QVQLQESGPGLVKPSQTLSTCTVT	39
CDR 1	GYSITSDYTWN	8
FR2	WIRQFPGKGLEWMG	41
CDR 2	YISYSGSYSNPSLKS	10
FR3	RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR	45

CDR 3	SFSYSYGFA Y	14
FR4	WGQGT LVT VSS	46
VH	QVQLQESGPGLVKPSQTL SLTCTVTGYSITSDYTWNWIRQFPGK GLEWMGYISYSGSYSNPSLKS RVTISRDTSKNQFSLKLSSVTAA DTAVYYCARSFSYSYGFAYWGQGT LVT VSS	30
HC	QVQLQESGPGLVKPSQTL SLTCTVTGYSITSDYTWNWIRQFPGK GLEWMGYISYSGSYSNPSLKS RVTISRDTSKNQFSLKLSSVTAA DTAVYYCARSFSYSYGFAYWGQGT LVT VSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDRKVEPKSC DKTHTCPPCPAPELLAGPDVFLFPPKPKDTLMISRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPLPEEKTISKAKGQPREPQVYT LPSSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG	37
AFF4 light chain		SEQ ID NO:
FR1	DIQMTQSPSSLSASVGDRV TITC	51
CDR 1	KASQDVSSAVA	4
FR2	WYQQKPGKAPKLLIY	52
CDR 2	SASYRYT	5
FR3	GVPSRFSGSGSGTDFTFTIS SLQPEDFATYYC	53
CDR 3	QQHYSTPWT	6
FR4	FGGGTKVEIK	54

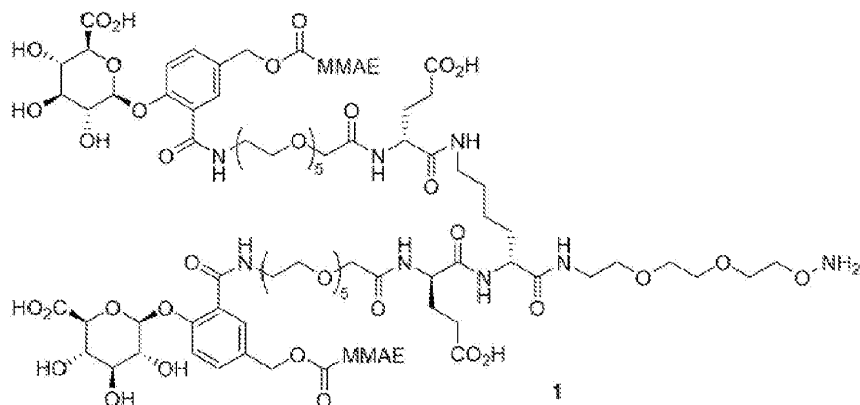
VL	DIQMTQSPSSLSASVGDRVTITCKASQDVSSAVAWYQQKPGKAP KLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLPEDFATYYCQQ HYSTPWTFGGGTKVEIK	20
LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSSAVAWYQQKPGKAP KLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLPEDFATYYCQQ HYSTPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	38

Additionally, for ADC synthesis, AFF4-CaaX was produced by introducing LALA mutations (L236A/L237A) into the heavy chain constant region of the antibody AFF4, and introducing a CaaX peptide moiety (GGGGGGGCVIM, SEQ ID NO: 7) to the C-terminus of the light chain. The antibody AFF4-CaaX produced was used in ADC synthesis. The amino acid sequences of the antibody AFF4-CaaX are shown in Table 8 below.

Table 8. AFF4-CaaX Amino Acid Sequences

AFF4-CaaX heavy chain		SEQ ID NO:
FR1	QVQLQESGPGLVKPSQTLSTCTVT	39
CDR1	GYSITSDYTWN	8
FR2	WIRQFPGKGLEWMG	41
CDR2	YISYSGSYSLK	10
FR3	RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR	45
CDR3	SFSYSYGFAY	14
FR4	WGQGLTVTVSS	46
VH	QVQLQESGPGLVKPSQTLSTCTVTGYSITSDYTWNWIRQFPGK GLEWMGYISYSGSYSLKSRVTISRDTSKNQFSLKLSSVTA ADTAVYYCARSFYSYGFAYWGQGLTVTVSS	30

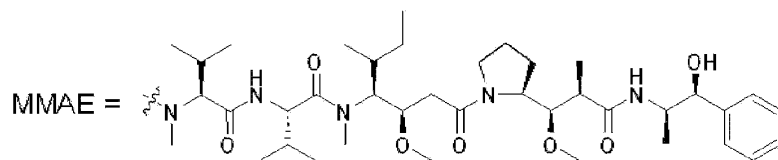
HC	QVQLQESGPGLVKPSQTLSTCTVTGYSITSDYTWNWIRQFPGK GLEWMGYISYSGSYSNPSLKSRVTISRDTSKNQFSLKLSSVTA ADTAVYYCARSFSYSYGFAYWGQGLTVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	58
AFF4-CaaX light chain		SEQ ID NO:
FR1	DIQMTQSPSSLSASVGDRVITITC	51
CDR1	KASQDVSSAVA	4
FR2	WYQQKPGKAPKLLIY	52
CDR2	SASYRYT	5
FR3	GVPSRFSGSGSGTDFTFTISSLQPEDFATYYC	53
CDR3	QHYSTPWT	6
FR4	FGGGTKVEIKRTV	60
VL	DIQMTQSPSSLSASVGDRVITITCKASQDVSSAVAWYQQKPGKAP KLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDFATYYCQ QHYSTPWTFGGGTKVEIK	20
LC	DIQMTQSPSSLSASVGDRVITITCKASQDVSSAVAWYQQKPGKAP KLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDFATYYCQ QHYSTPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG GGGCVIM	59

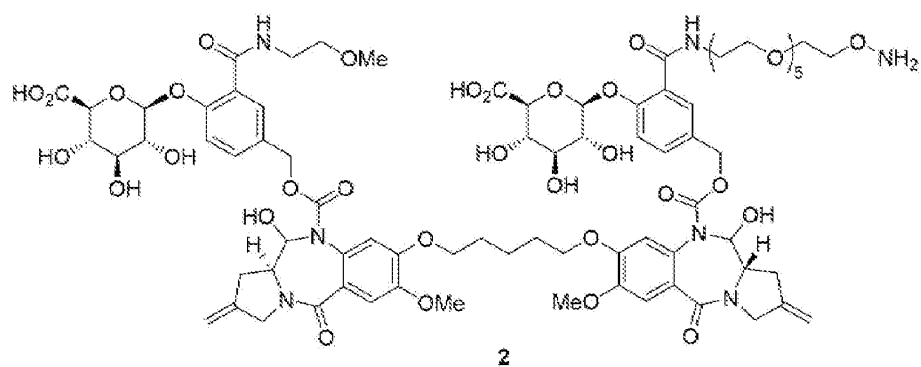
Example 2: Synthesis of Exemplified Compounds of the Disclosure**Production of Compound 1**

Compound 1 was produced with the method disclosed in US Patent No. 11,173,214, the entirety of which is herein included in the present specification by reference.

EI-MS m/z : $[\frac{1}{2}M+H]^+$ 1623.6, $[\frac{1}{3}M+H]^+$ 1082.8.

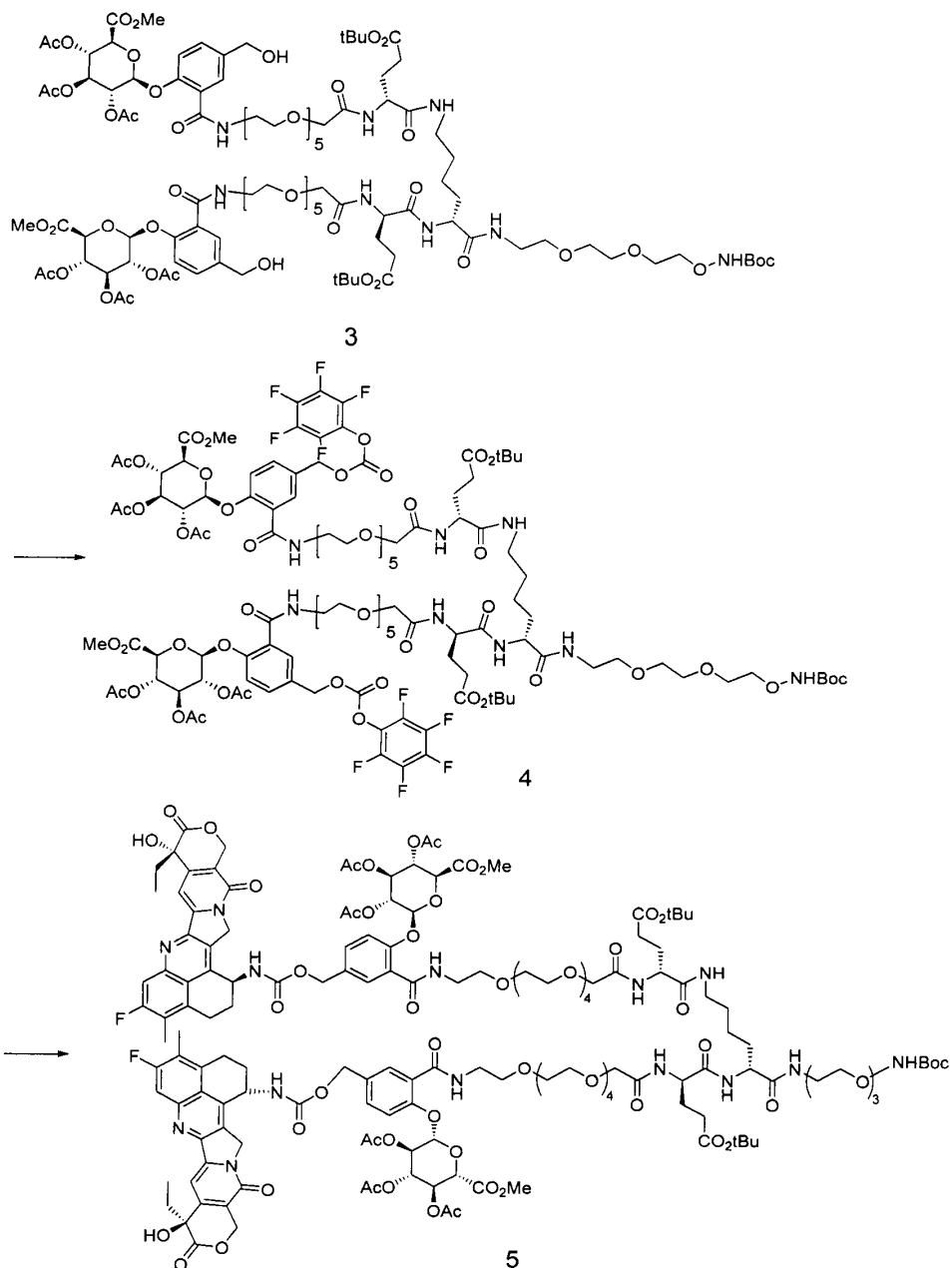
The structure of MMAE (Monomethyl auristatin E) in Compound 1 is as follows:

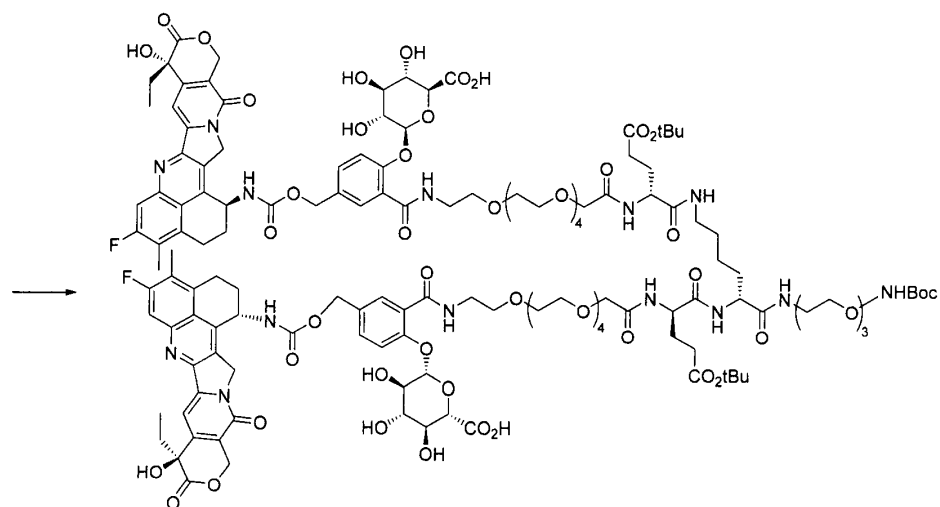
**Production of Compound 2**



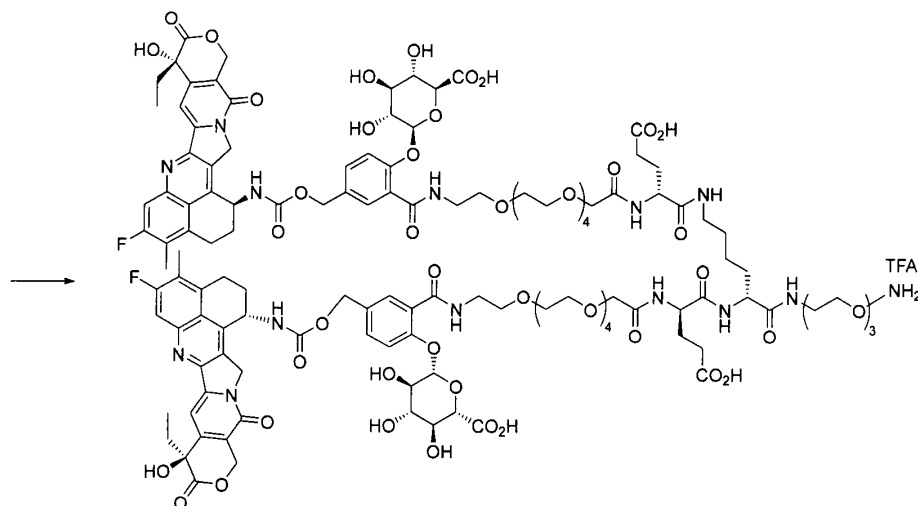
Compound 2 was produced with the method disclosed in US Patent No. 11,654,197, the entirety of which is herein included in the present specification by reference.

EI-MS m/z: [M+H]⁺ 1698.2, [1/2M+H]⁺ 849.6.

Production of Compound 4-7



6



7

Production of Compound 4

Compound 3 (PCT Application No. PCT/KR2023/018766, the entirety of which is incorporated herein by reference) (1.5 g, 0.67 mmol) was dissolved in (24 mL) of dichloromethane. Then, 0.22 mL of pyridine (2.68 mmol) and 0.79 g of bis (pentafluorophenyl) carbonate (2.01 mmol) were added at room temperature under a nitrogen atmosphere and stirred for 16 hours. The

reaction solution was diluted with 80 mL of ethyl acetate, rinsed with 50 mL x 2 of saturated aqueous sodium hydrogen carbonate, followed by 50 mL of brine, and dehydrated using anhydrous sodium sulfate. Compound 4 (2.06 g) was obtained after filtering and concentration under reduced pressure, without additional purification, and was subsequently utilized in the next process.

EI-MS m/z: $[M+H]^+$ 2670.3, $[\frac{1}{2}M+H]^+$ 1335.7, $[\frac{1}{3}M+H]^+$ 890.8.

Production of Compound 5

Compound 4 (100 mg, 0.04 mmol) was dissolved in N,N-dimethylformamide (3 mL), followed by sequential addition of exatecan mesylate (44 mg, 0.08 mmol), N,N-diisopropylethylamine (0.05 mL, 0.28 mmol), and 1-hydroxy-7-benzotriazole (HOAt, 2.55 mg, 0.02 mmol) at room temperature under nitrogen atmosphere with stirring for 3 hours. The reaction mixture was diluted with ethyl acetate (40 mL), washed successively with saturated ammonium chloride aqueous solution (30 mL), saturated sodium bicarbonate aqueous solution (30 mL), and brine (30 mL), then dried over anhydrous sodium sulfate. Compound 5 (140 mg) was obtained after filtering and concentration under reduced pressure, without additional purification.

EI-MS m/z: $[\frac{1}{2}M+H]^+$ 1587.5, $[\frac{1}{3}M+H]^+$ 1058.8.

Production of Compound 6

Compound 5 (550 mg, 0.17 mmol) was dissolved in methanol (7 mL), tetrahydrofuran (7 mL) and then a solution of lithium hydroxide (65.8 mg, 1.57 mmol) dissolved in distilled water (8 mL) was added gradually at -40°C, under nitrogen atmosphere. Then the mixture was agitated for 2 hours as the temperature of the reaction was incrementally increased to 0°C. The reaction mixture was neutralized using acetic acid, condensed under reduced pressure, purified via HPLC, and then freeze-dried to provide Compound 6 (236 mg, 44%) in the form of a yellow solid.

EI-MS m/z: $[\frac{1}{2}M+H]^+$ 1446.7, $[\frac{1}{3}M+H]^+$ 964.9.

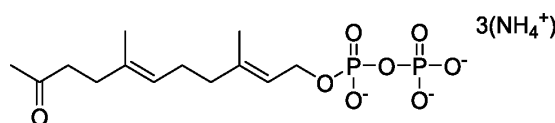
Production of Compound 7

Compound 6 (236 mg, 0.08 mmol) was dissolved in acetonitrile (10 mL) and phosphoric acid (85%, 5 mL) was added at 0°C. After stirring at room temperature for 4 hours, the reaction mixture was purified by HPLC, followed by freeze-drying to afford yellow solid Compound 7 (138 mg, 61%).

EI-MS m/z: $[\frac{1}{2}M+H]^+$ 1340.5, $[\frac{1}{3}M+H]^+$ 894.1.

Example 3: Synthesis of Exemplified ADCs of the Disclosure

Exemplified ADCs were produced with the below two steps. LCB14-0606 was produced with the method disclosed in US Patent No. 9,669,107, the entirety of which is herein included in the present specification by reference. The structural formula of LCB14-0606 is as follows:



LCB14-0606

Step 1: Production of Prenylated Antibody with LCB14-0606

AFF4-CaaX was prepared according to Example 1. A mixture comprising AFF4-CaaX for the prenylation reaction was prepared and reacted at 30°C for 16 hours. The reaction mixture contained a total of 24 μ M of the antibody, 600 mg or 400 nM FTase (Genscript), and 144 μ M LCB14-0606 in a buffer solution (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 10 μ M $ZnCl_2$, 0.5 mM DTT). After the reaction, the prenylated antibody was desalted using a G25 Sepharose column (AKTA purifier, GE healthcare) equilibrated with PBS buffer. As a result, a total of 544 mg of prenylated antibody was produced.

Step 2: Drug-Conjugation Method

Oxime Bond Reaction of ADC 1 (conjugation by oxime bond formation)

The oxime bond formation reaction between the prenylated antibody and the linker-drug was carried out by mixing 100 μ M sodium acetate buffer, pH 5.2, 10% DMSO, 350 mg of prenylated antibody (24 μ M in total), and 10 equivalents of the linker-drug (Compound 1 of Example 1) at 30°C with stirring at 500 to 600 rpm for 24 hours. After the reaction, excess low molecular weight compounds were removed by FPLC (AKTA purifier, GE healthcare), and the protein fraction was collected and concentrated. As a result, a total of 336 mg of ADC1 was obtained. The production process and structure thereof are shown in **FIG. 1**.

Oxime Bond Reaction of ADC 2 (conjugation by oxime bond formation)

The oxime bond formation reaction between the prenylated antibody and the linker-drug was carried out by mixing 100 μ M sodium acetate buffer, pH 5.2, 10% DMSO, 14.4 mg of prenylated antibody (24 μ M in total), and 10 equivalents of the linker-drug (Compound 2 of Example 2) at 30°C with stirring at 500 to 600 rpm for 6 hours. After the reaction, excess low molecular weight compounds were removed by FPLC (AKTA purifier, GE healthcare), and the protein fraction was collected and concentrated. As a result, a total of 13.5 mg of ADC2 was obtained. The production process and structure thereof are shown in **FIG. 2**.

Oxime Bond Reaction of ADC 3 (conjugation by oxime bond formation)

The oxime bond formation reaction mixture between the prenylated antibody and the linker-drug was carried out by mixing 100 μ M sodium acetate buffer, pH 5.2, 10% DMSO, 10 mg of prenylated antibody (48 μ M in total), and 4 equivalents of the linker-drug (Compound 7 of Example 3) at 30°C with stirring at 500 to 600 rpm for 6 hours. After the reaction, excess low molecular weight compounds were removed by FPLC (AKTA purifier, GE healthcare), and the protein fraction was collected and concentrated. As a result, a total of 7.7 mg of ADC3 was obtained. The production process and structure thereof are shown in **FIG. 3**.

To confirm the characteristics of the three ADCs produced above, analysis was conducted using an HPLC instrument with an HIC column, employing a mobile phase prepared with ammonium sulfate, acetonitrile, and potassium phosphate, and the results are shown in **FIG. 4**. Additionally, intact mass analysis was performed to confirm the molecular weights of ADC1 and ADC3, and the results are shown in **FIG. 5**.

As shown in **FIG. 4**, ADC1, ADC2, and ADC3 all exhibited different levels of hydrophobicity, with the hydrophobicity confirmed to be in the order of ADC1 > ADC2 > ADC3, and the purity for the same DAR (drug-antibody ratio) was confirmed to be 97% or higher.

As shown in **FIG. 5**, the molecular weight of ADC1 was 153,535.27 Da and the molecular weight of ADC3 was 152,404.38 Da.

The ADCs produced through the above two steps are summarized in **Table 9**.

Table 9. Exemplified ADCs

ADC	Antibody	Linker-toxin
ADC1	AFF4-CaaX	Compound 1
ADC2		Compound 2
ADC3		Compound 7

Example 4: Evaluation of Cancer Cell Binding Force of Anti-L1CAM Antibodies

The cell binding affinity of the AFF4-CaaX antibody produced in Example 1 was confirmed using pancreatic cancer cell lines (PANC-1, PATU-8988s), breast cancer cell lines (JIMT-1, MDA-MB-231, SK-BR-3, HCC1395, MCF7), ovarian cancer cell lines (SK-OV-3, OVCAR-3, SNU-840, SNU-8), colorectal cancer cell lines (HCT116, SW480, SNU-1197), lung cancer cell lines (H69, PC-9, NCI-H460, Calu-6), melanoma cell lines (A2058, MeWo, G361), and neuroblastoma cell lines (SK-N-F1).

Specifically, the above cancer cell lines were cultured, and the cells were divided into two groups: control antibody-treated group and anti-L1CAM antibody-treated group. Subsequently, the cells were incubated in a phosphate-buffered saline solution containing 2% fetal bovine serum at 4°C for 1 hour to remove nonspecific binding of antibodies. Next, the control antibody and anti-L1CAM antibody were incubated with each group of the cell lines at 4°C for 1 hour to allow the antibodies to bind to the L1CAM proteins on the cancer cell surface. Subsequently, antibodies that did not bind to the cell surface were removed using a phosphate-buffered saline solution containing 2% fetal bovine serum. Subsequently, the cells were incubated with fluorescent-labeled secondary antibodies that bound to control antibody and anti-L1CAM antibody at 4°C for 1 hour. Following the removal of secondary antibodies that did not bind to the antibodies using a phosphate-buffered saline solution, the fluorescence intensity of the cells was measured using a flow cytometer to calculate the fluorescence intensity of cell lines in the anti-L1CAM antibody group compared to those in the control group, confirming the binding affinity of the antibodies to the cancer cell lines. The results are presented in **Tables 10-15** below.

Table 10. Fluorescence Intensity of JIMT-1, MDA-MB-231, SK-BR-3 and HCC1395 cells

Median FITC-A (Fold Value)				
Sample	JIMT-1	MDA-MB-231	SK-BR-3	HCC1395
Anti-AFF4-CaaX/IgG	24	414	5	4

Table 11. Fluorescence Intensity of MCF7, SK-OV-3, OVCAR-3, and SNU-840 cells

Median FITC-A (Fold Value)				
Sample	MCF7	SK-OV-3	OVCAR-3	SNU-840
Anti-AFF4-CaaX/IgG	4	15	20	29

Table 12. Fluorescence Intensity of SNU-8, PANC-1, PATU-8988s, and H69 cells

Median FITC-A (Fold Value)				
Sample	SNU-8	PANC-1	PATU-8988s	H69
Anti-AFF4-CaaX/IgG	31	25	4	3

Table 13. Fluorescence Intensity of PC-9, NCI-H460, Calu-6, and HCT116 cells

Median FITC-A (Fold Value)				
Sample	PC-9	NCI-H460	Calu-6	HCT116
Anti-AFF4-CaaX/IgG	2	18	13	6

Table 14. Fluorescence Intensity of SW480, SNU-1197, A2058, and MeWo cells

Median FITC-A (Fold Value)				
Sample	SW480	SNU-1197	A2058	MeWo
Anti-AFF4-CaaX/IgG	13	20	14	61

Table 15. Fluorescence Intensity of G361 and SK-N-F1 cells

Median FITC-A (Fold Value)		
Sample	G361	SK-N-F1
Anti-AFF4-CaaX/IgG	13	20

As shown in **Tables 10-15**, the AFF4-CaaX antibody could strongly bind to all cancer cell lines.

Example 5: *In vitro* cell toxicity evaluation

The cell proliferation inhibitory activity of the three ADCs produced in Example 3 against cancer cell lines was measured.

Specifically, commercially available human breast cancer cell lines (JIMT-1, MDA-MB-231, SK-BR-3, HCC1395, MCF7), ovarian cancer cell lines (OVCAR-3, SNU-840, SNU-8), pancreatic cancer cell lines (PANC-1, PATU-8988s), lung cancer cell lines (NCI-H69, PC-9, NCI-H460, Calu-6), colorectal cancer cell lines (HCT116, SW480, SNU-1197), and melanoma cell lines (A2058, MeWo, G-361) were used as cancer cell lines, and commercially available human kidney cells (HK-2, HEK293T), skin cells (HaCaT), and liver cells (Fa2N-4) were used as control, normal cell lines.

Additionally, each cancer cell line was seeded at 500 to 9,000 cells per well in a 96-well plate and cultured for 24 hours. Subsequently, the cells were treated with ADCs at concentrations ranging from 0.256 pM to 100 nM (5-fold serial dilution). After 144 hours, the number of viable cells was quantified using SRB (Sulforhodamine B) dye or Cell Titer Glo, and the results are shown in **Tables 16-18** below.

Table 16. ADC 50% Cytotoxicity Concentrations in MT-1, MDA-MB-231, SK-BR-3, HCC1395, MCF7, OVCAR-3, and SNU-840 Cells

Test samples	CC50 (nM)						
	JIMT-1	MDA-MB-231	SK-BR-3	HCC1395	MCF7	OVCAR-3	SNU-840
ADC1	0.011	>100	0.170	18.37	15.48	0.013	0.057
ADC2	0.031	0.148	0.176	88.36	39.09	0.013	0.013

Table 17. ADC 50% Cytotoxicity Concentrations in SNU-8, PANC-1, PATU-8988s, H69, PC-9, NCI-H460, Calu-6, and HCT116 Cells

Test samples	CC50 (nM)							
	SNU-8	PANC-1	PATU-8988s	H69	PC-9	NCI-H460	Calu-6	HCT116
ADC1	0.238	0.964	18.76	12.26	57.04	49.18	0.019	54.79
ADC2		0.040		0.109	15.91	1.78	0.012	97.54

Table 18. ADC 50% Cytotoxicity Concentrations in SW480, SNU-1197, A2058, MeWo, and G-361 Cells

Test samples	CC50 (nM)				
	SW480	SNU-1197	A2058	MeWo	G-361
ADC1	9.325	9.356	3.969	0.029	0.113
ADC2	0.106		0.003	0.009	0.012
ADC3	15.04			>100	

As shown in **Tables 16 and 17**, the cytotoxicity assay results for ADC1 and ADC2 indicate that the pyrrolobenzodiazepine (PBD) antibody-drug conjugate (ADC2) exhibits significantly stronger cytotoxicity compared to the auristatin-based antibody-drug conjugate (ADC1) in most cancer cell lines except for the colorectal cancer cell line HCT116 and the breast cancer cell lines HCC1395 and MCF7. Additionally, as shown in **Table 18**, the cytotoxicity assay results for

ADC1, 2, and 3 indicate that in colorectal cancer cell line SW480 and melanoma cell line MeWo, the strength of cytotoxicity was shown in the order of ADC2 > ADC1 > ADC3.

Additionally, the cytotoxicity results in normal cells are shown in **Table 19**.

Table 19. ADC 50% Cytotoxicity Concentrations in HK-2, Fa2N-4, and HEK293T Cells

Test samples	CC50 (nM)		
	HK-2	Fa2N-4	HEK293T
ADC1	74.08	>500	38.49
ADC2	>100	>500	39.51

As shown in **Table 19**, ADCs exhibited weak cytotoxicity in normal cell lines. Specifically, ADC2 showed weaker cytotoxicity compared to ADC1 in normal cell lines, while ADC1 and ADC2 showed similar cytotoxicity in Fa2N-4 and HEK293T cells.

These results indicate that the exemplified ADCs exhibited weak cytotoxicity in normal cells while showing strong cytotoxicity in cancer cells. In particular, ADC2 demonstrated weaker cytotoxicity in normal cells and stronger cytotoxicity in cancer cells compared to other ADCs.

Example 6: *In vivo* anti-cancer efficacy assessment

The tumor growth inhibitory efficacy of ADCs produced in Example 3 was analyzed in a tumor xenograft mouse model.

Specifically, breast cancer cell lines (JIMT-1), colorectal cancer cell lines (SW480), melanoma cell lines (MeWo), colorectal cancer cell lines (HT29), small cell lung cancer cell lines (NCI-H146, NCI-H69), ovarian cancer cell lines (SNU-840), pancreatic cancer cell lines (PATU-8988s), and non-small cell lung cancer cell lines (Calu-6) were used for cell culture, and 2,000,000 to 5,000,000 cells were mixed with 100 µl of PBS solution and 100 µl of Matrigel. Subsequently, these mixtures were subcutaneously implanted into Balb/c-nude mice to generate tumor xenograft mouse models. ADCs were intravenously injected according to the dosage and administration schedule shown in **Table 20** when the tumor size reached 100 - 300 mm³. The tumor size was measured immediately before the initial administration (Day 1) and periodically for a certain period (up to 56 days), and the results are shown in **FIGs. 6-13**.

Table 20. ADC Administration Schedule

	JIMT-1	SW480	MeWo	Calu-6	SNU840	Patu8988s	NCI-H146	NCI-H69
ADC1	5 mg/kg (QD*1)	1, 3, 5 mg/kg (QD*1)	1, 2, 4 mg/kg (QD*1)	4 mg/kg (BIW*2)	4 mg/kg (QD*1)	4 mg/kg (BIW*2)	1, 2, 4, 8 mg/kg (BIW*4)	1, 2, 4, 8 mg/kg (BIW*4)
ADC2	1 mg/kg (QD*1)	0.3, 0.6, 0.9 mg/kg (QD*1)	0.2, 0.4, 0.6 mg/kg (QD*1)					
ADC3				4 mg/kg (QD*1) 4 mg/kg (BIW*2)	8 mg/kg (QD*1) 8 mg/kg (BIW*2)	4 mg/kg (BIW*2) 8 mg/kg (QW*2) 8 mg/kg (BIW*2)	16 mg/kg (BIW*4)	16 mg/kg (BIW*4)

As shown in **FIG. 6**, ADCs exhibited significant tumor growth inhibition in the JIMT-1 model of breast cancer cell lines. Specifically, the auristatin-based antibody-drug conjugate (ADC1) showed excellent tumor growth inhibition compared to the control group at 5 mg/kg, and the pyrrolobenzodiazepine-based antibody-drug conjugate (ADC2) showed tumor growth inhibition at 1 mg/kg.

Additionally, as shown in **FIG. 7**, in the SW480 model of colorectal cancer cell lines, both ADC1 and ADC2 exhibited concentration-dependent tumor growth inhibition compared to the control group. Further, ADC2 demonstrated significant tumor growth inhibition even at low doses.

Additionally, as shown in **FIG. 8**, in the MeWo model of melanoma cell lines, both ADC1 and ADC2 exhibited concentration-dependent tumor growth inhibition compared to the control group.

Moreover, as shown in **FIG. 9**, in the Calu-6 model of non-small cell lung cancer cell lines, both the 4 mg/kg (BIW*2) dose group of ADC1 and the 4 mg/kg (QD*1, BIW*2) dose group of ADC3 showed significant tumor growth inhibition compared to the control group.

Additionally, as shown in **FIG. 10**, in the SNU840 model of ovarian cancer cell lines, both 4 mg/kg of ADC1 and 8 mg/kg ADC3 (QD*1 and BIW*2) groups showed significant tumor growth inhibition compared to the control group.

Additionally, as shown in **FIG. 11**, in the Patu8988s model of pancreatic cancer cell lines, 4 mg/kg of ADC1 showed significant tumor growth inhibition compared to the control group.

Additionally, as shown in **FIG. 12**, in the NCI-H146 model of small cell lung cancer cell lines, 4 mg/kg of ADC1 and 16 mg/kg of ADC3 groups showed significant tumor growth inhibition compared to the control group.

Additionally, as shown in **FIG. 13**, in the NCI-H69 model of small cell lung cancer cell line, 2 mg/kg of ADC1 and 16 mg/kg of ADC3 showed significant tumor growth inhibition compared to the control group.

Example 7: Analysis of Pharmacokinetics in Mouse Model

To analyse the pharmacokinetics of ADCs, ADC1 and ADC3 were intravenously administered to nude mice at a single dose of 2 mg/kg and 8 mg/kg, respectively. Blood sample was collected at 0 minutes, 3 minutes, 3 hours, 1-, 2-, 3-, 7-, 9-, and 14-days post-administration to measure the serum concentrations of ADCs and antibody using LC-MS. Five nude mice were used for each time point.

FIG. 14 shows the results of pharmacokinetic analysis in the mouse model of ADC1 and ADC3 that ADC1 and ADC3 had excellent half-life. In particular, there was almost no difference in the half-life of ADCs and the antibody, which was used as control.

Example 8: Single Dose Acute Toxicity Study in Rats

To analyse the acute toxicity of ADCs, ADC1 (10 mg/kg, 20 mg/kg) and ADC3 (100 mg/kg, 150 mg/kg) were intravenously administered to tail vein of Sprague-Dawley rats (n=5) in a single dose, and rats were observed for 4 weeks.

FIG. 15 shows that ADC1 or ADC3 administration in rats did not result in significant toxic symptoms, measured as body weight changes, at the highest concentration, and there were no abnormal findings in the autopsy compared to the control group.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to

be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS

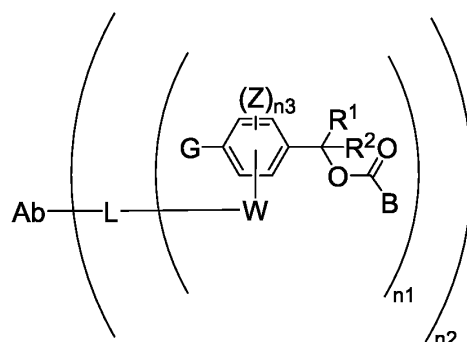
While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

CLAIMS

We claim:

1. A conjugate having a structure represented by Formula I:

[Formula I]

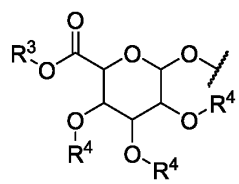


wherein

Ab is anti-human neural cell adhesion molecule L1 (L1CAM) antibody or an antigen-binding fragment thereof comprising:

- (a) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 8;
 - (b) a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 10;
 - (c) a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 14;
 - (d) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 4;
 - (e) a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5;
- and
- (f) a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6;

each B is independently an active agent;



each G is independently a glucuronic acid moiety or

R³ is hydrogen or a carboxyl protecting group;

each R⁴ is independently hydrogen or a hydroxyl protecting group;

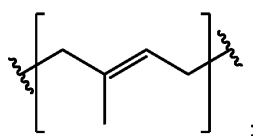
R^1 and R^2 are each independently hydrogen, C_{1-8} alkyl, or C_{3-8} cycloalkyl;
 W is each independently $-C(O)-$, $-C(O)NR'-$, $-C(O)O-$, $-SO_2NR'-$, $-P(O)R''NR'-$, $-SONR'-$ or $-PO_2NR'-$, wherein the C, S or P directly binds to the phenyl ring and the NR' binds to L;
 R' and R'' are each independently hydrogen, C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{1-8} alkoxy, C_{1-8} alkylthio, mono- or di- C_{1-8} alkylamino, C_{3-10} heteroaryl or C_{6-10} aryl;
each Z is independently C_{1-8} alkyl, halogen, cyano or nitro;
each L is independently a linker and comprises:

A) C_{1-50} alkylene or 1-50 membered heteroalkylene comprising:

- (i) one or more unsaturated bonds;
 - (ii) a heteroarylene (e.g., a heteroarylene in the alkylene or heteroalkylene chain);
- or
- (iii) a C_{1-20} alkyl substituent; and/or

B) at least one isoprenyl unit having a structure represented by Formula II:

[Formula II]



n_1 and n_2 are each independently integers selected from 1 to 20; and
 n_3 is an integer of 0 to 3.

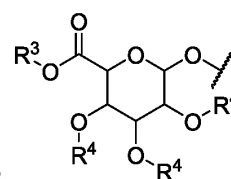
2. The conjugate of claim 1, wherein Ab comprises

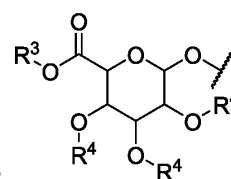
- (i) a variable heavy chain framework region from a heavy chain of a human antibody or a human consensus framework, wherein the variable heavy chain framework region comprises one or more of the following mutations: T34A, Y58F, N61H, and S103G; and
- (ii) a variable light chain framework region from a light chain of the human antibody or the human consensus framework.

3. The conjugate of claim 1 or 2, wherein Ab comprises LALA mutations in a heavy chain constant region.

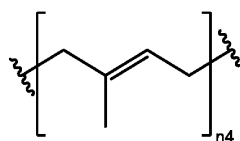
4. The conjugate of claim 3, wherein the LALA mutations comprise L236A and L237A.

5. The conjugate of any one of claims 1-4, wherein Ab is any one selected from a monoclonal antibody, a domain antibody (dAb), a single chain antibody (scAb), a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a scFab fragment, a Fv fragment, a dsFv Fragment, a single chain variable fragment (scFv), a scFv-Fc fragment, a single domain heavy chain antibody, a single domain light chain antibody, a variant antibody, a multimeric antibodies, a minibody, a diabody, a bispecific antibody, and a multispecific antibody.
6. The conjugate of any one of claims 1-5, wherein the antibody is a humanized antibody.
7. The conjugate of any one of claims 1-6, wherein n₁ is 1, 2, 3, or 4.
8. The conjugate of any one of claims 1-6, wherein n₁ is 1 or 2.
9. The conjugate of any one of claims 1-8, wherein n₂ is 1, 2, 3, or 4.
10. The conjugate of any one of claims 1-8, wherein n₂ is 1.



11. The conjugate of any one of claims 1-10, wherein each G is ; and R³ and R⁴ are each H.
12. The conjugate of any one of claims 1-11, wherein R¹ and R² are each hydrogen.
13. The conjugate of any one of claims 1-12, wherein each W independently is -C(O)NR'.
14. The conjugate of any one of claims 1-13, wherein R' is H.
15. The conjugate of any one of claims 1-14, wherein R'' is H.
16. The conjugate of any one of claims 1-15, wherein Z is halo (*e.g.*, bromo) or cyano.

17. The conjugate of any one of claims 1-16, wherein n_3 is 1.
18. The conjugate of any one of claims 1-17, wherein n_3 is 0.
19. The conjugate of any one of claims 1-18, wherein L comprises a C_{1-50} alkylene.
20. The conjugate of any one of claims 1-19, wherein L comprises a C_{1-50} heteroalkylene.
21. The conjugate of any one of claims 1-20, wherein L comprises an unsaturated bond (*e.g.*, 1, 2, 3, 4 or 5 unsaturated bonds).
22. The conjugate of any one of claims 1-21, wherein L is substituted with a C_{1-20} alkyl.
23. The conjugate of any one of claims 1-22, wherein L comprises an isoprenyl group having a structure represented by Formula IIIa:



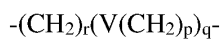
IIIa

wherein n_4 is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

24. The conjugate of any one of claims 1-23, wherein L comprises a peptide.
25. The conjugate of claim 24, wherein the peptide comprises at least one hydrophilic amino acid.
26. The conjugate of claim 24 or 25, wherein the peptide comprises an amino acid having a side chain having a moiety that bears a charge at neutral pH in aqueous solution (*e.g.*, an amine, guanidine, or carboxyl moiety).

27. The conjugate of claim any one of claims 24-26, wherein the peptide comprises an amino acid selected from alanine, aspartate, asparagine, glutamate, glutamine, glycine, lysine, ornithine, proline, serine, and threonine.
28. The conjugate of any one of claims 1-27, wherein the antibody comprises a cysteine residue and L is covalently connected to the antibody by a sulfur atom in the cysteine residue (*e.g.*, by a sulfur atom in the side chain of the cysteine residue) via a thioether bond.
29. The conjugate of claim any one of claims 1-28, wherein a C-terminus of the antibody comprises an amino acid motif that is recognized by an isoprenoid transferase.
30. The conjugate of claim 29, wherein the isoprenoid transferase is farnesyl protein transferase (FTase) or geranylgeranyl transferase (GGTase).
31. The conjugate of claim 29 or 30, wherein the amino acid motif comprises a cysteine residue and L is covalently connected to the antibody by a sulfur atom in the cysteine residue (*e.g.*, by a sulfur atom in the side chain of the cysteine residue) via a thioether bond.
32. The conjugate of claim 31, wherein the amino acid motif comprises the sequence:
CYYX
- wherein:
C is cysteine;
each Y is an aliphatic amino acid; and
X is selected from glutamine, glutamate, serine, cysteine, methionine, alanine, and leucine.
33. The conjugate of claim 32, wherein each Y is independently selected from alanine, isoleucine, leucine, methionine, and valine.
34. The conjugate of claim 32, wherein the amino acid motif has the sequence CVIM or CVLL.

35. The conjugate of any one of claims 29-34, wherein at least one of 1 to 20 amino acids preceding the amino acid motif is glycine.
36. The conjugate of any one of claims 29-35, wherein the amino acid motif comprises the sequence GGGGGGGCVIM.
37. The conjugate of any one of claims 1-36, wherein L comprises an oxime.
38. The conjugate of claim 37, wherein the oxygen atom of the oxime is on the side of L that is linked to the active agent and the carbon atom of the oxime is on the side of L that is linked to Ab.
39. The conjugate of claim 37, wherein the carbon atom of the oxime is on the side of Y that is linked to the active agent and the oxygen atom of the oxime is on the side of L that is linked to Ab.
40. The conjugate of any one of claims 1-39, wherein L comprises a structure represented by formula IVa or IVb:



Formula IVa,



Formula IVb;

V is a single bond, -O-, -S-, -NR²¹-, -C(O)NR²²-, -NR²³C(O)-, -NR²⁴SO₂-, or -SO₂NR²⁵-, preferably -O-;

X is -O-, C₁₋₈ alkylene, or -NR²¹-, preferably -O-;

R²¹ to R²⁵ are each independently H, C₁₋₆ alkyl, C₁₋₆ alkyl C₆₋₂₀ aryl, or C₁₋₆ alkyl C₃₋₂₀ heteroaryl;

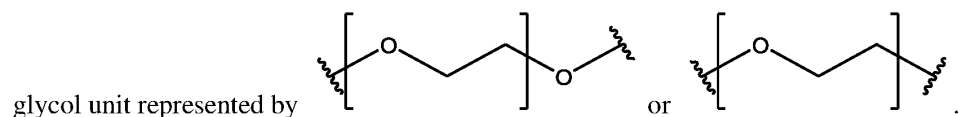
each r is independently an integer of 1 to 10, preferably 2;

each p is independently an integer of 0 to 12, preferably 2;

q is an integer of 1 to 20, preferably 2, 5, or 11; and

w is an integer of 1 to 20, preferably 6 to 20.

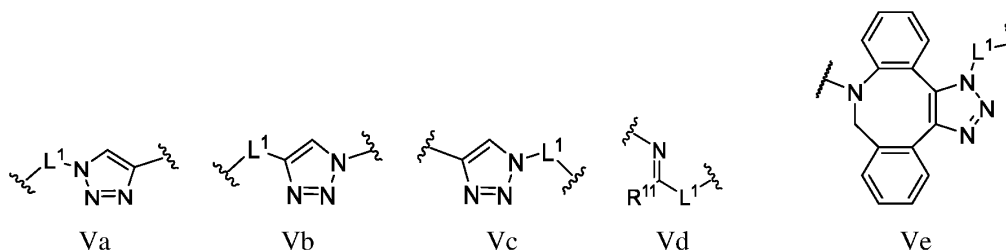
41. The conjugate of claim 40, wherein V is -O-.
42. The conjugate of claim 40 or 41, wherein X is -O-.
43. The conjugate of any one of claims 40-42, wherein R²¹ is H.
44. The conjugate of any one of claims 40-43, wherein R²² is H.
45. The conjugate of any one of claims 40-44, wherein R²³ is H.
46. The conjugate of any one of claims 40-45, wherein R²⁴ is H.
47. The conjugate of any one of claims 40-46, wherein R²⁵ is H.
48. The conjugate of any one of claims 40-47, wherein r is 2.
49. The conjugate of any one of claims 40-48, wherein p is 2.
50. The conjugate of any one of claims 40-49, wherein q is 2 or 5.
51. The conjugate of any one of claims 40-50, wherein w is 6 to 20.
52. The conjugate of any one of claims 40-51, wherein L comprises at least one polyethylene glycol unit represented by



53. The conjugate of claim 52, wherein L comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 polyethylene glycol units.
54. The conjugate of any one of claims 1-53, wherein L comprises an oxime and at least one polyethylene glycol unit covalently binds the oxime to the active agent.

55. The conjugate of any one of claims 1-54, wherein L comprises a moiety formed by a reaction of alkyne with azide, or a reaction of an aldehyde or ketone group with hydrazine or hydroxylamine.

56. The conjugate of any one of claims 1-55, wherein L comprises a unit having a structure represented by Formula Va, Vb, Vc, Vd, or Ve:



L^1 is a single bond or C_{1-30} alkylene; and

R^{11} is H or C_{1-10} alkyl.

57. The conjugate of claim 56, wherein L^1 is a single bond.

58. The conjugate of claim 56, wherein L is C_{1-30} alkylene.

59. The conjugate of any one of claims 56-58, wherein R^{11} is H.

60. The conjugate of any one of claims 56-58, wherein R^{11} is C_{1-10} alkyl (*e.g.*, methyl).

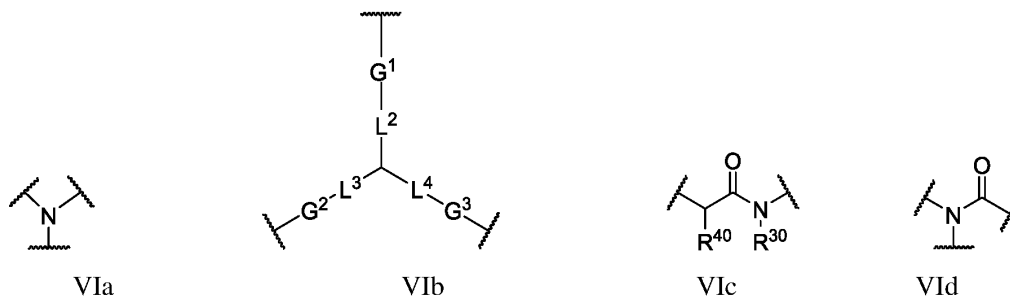
61. The conjugate of any one of claims 1-60, wherein L comprises:

- i) a branching unit covalently coupled to Ab by a primary linker;
- ii) a first branch which couples a first B to the branching unit; and
- iii) a second branch which couples a second B to the branching unit; or
- iii) a second branch comprising an alkyl or heteroalkyl (*e.g.*, a polyethylene glycol monomer or a polyethylene glycol oligomer) coupled the branching unit.

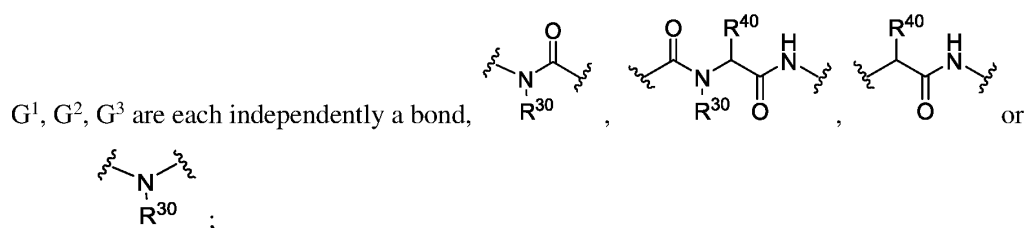
62. The conjugate of claim 61, wherein L comprises a second branch which couples a second B, via a second cleavage group, to the branching unit.

63. The conjugate of claim 61, wherein L comprises a second branch comprising an alkyl or heteroalkyl (*e.g.*, a polyethylene glycol monomer or a polyethylene glycol oligomer) coupled the branching unit.

64. The conjugate of any one of claims 61-63, wherein the branching unit has a structure represented by formula VIa, VIb, VIc, or VIId:



wherein



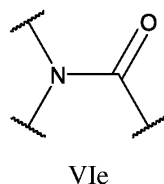
R^{30} is H or alkyl;

R^{40} is H, alkyl or L^5 -CO₂ R^{50} ;

R^{50} is H or alkyl; and

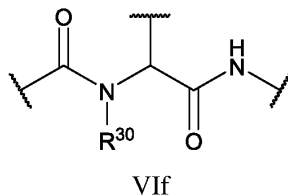
L^2, L^3, L^4 , and L^5 are each independently a bond or alkylene.

65. The conjugate of any one of claims 61-63, wherein the branching unit has a structure represented by formula VIe:



wherein R^{30} is H or alkyl.

66. The conjugate of any one of claims 61-63, wherein the branching unit has a structure represented by formula VI_f:



wherein R³⁰ is H or alkyl.

67. The conjugate of any one of claims 1-66, wherein the conjugate comprises 1, 2, 3, or 4 branched linkers.

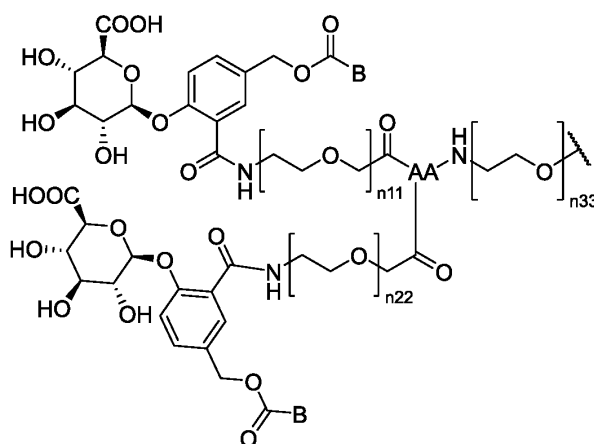
68. The conjugate of claim 67, wherein each branched linker comprises at least two active agents.

69. The conjugate of claim 67, wherein each branched linker comprises two active agents.

70. The conjugate of any one of claims 61-69, wherein the branching unit is a lysine and the primary linker is bound to a C-terminus of lysine.

71. The conjugate of any one of claims 1-70, wherein the conjugate is cleavable in a target cell (e.g., the conjugate cleaves to release one or more active agents).

72. The conjugate of any one of claims 1-71, wherein the conjugate comprises a structure represented by:



n11, n22, and n33 are each independently integers of 0 to 30; and

AA represents an amino acid group.

73. The conjugate of any one of claims 1-72, wherein each active agent is independently a chemotherapeutic agent or a toxin.

74. The conjugate of any one of claims 1-72, wherein each active agent is independently an immunomodulatory compound, an anticancer agent, an antiviral agent, an antibacterial agent, an antifungal agent, an antiparasitic agent, or a combination thereof.

75. The conjugate of any one of claims 1-72, wherein the active agent is independently an immunomodulatory compound, an anticancer agent, an antiviral agent, an antibacterial agent, an antifungal agent, or an antiparasitic agent.

76. The conjugate of any one of claims 1-72, wherein the active agent is independently selected from:

(a) erlotinib, bortezomib, fulvestrant, sutent, letrozole, imatinib mesylate, PTK787/ZK 222584, oxaliplatin, 5-fluorouracil, leucovorin, rapamycin, lapatinib, lonafarnib, sorafenib, gefitinib, AG1478, AG1571, thiotepa, cyclophosphamide, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, ethylenimine, altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, topotecan, bryostatin,

callystatin, CC-1065, adozelesin, carzelesin, bizelesin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, KW-2189, CB1-TM1, eleutherobin, pancratistatin, sarcodictyin, spongestatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, calicheamicin gamma 1, calicheamicin omega 1, dynemicin, dynemicin A, clodronate, esperamicin, neocarzinostatin chromophore, alacinomysins, actinomycin, antrmycin, azaserine, bleomycins, cactinomycin, carabycin, carninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin, deoxydoxorubicin, epirubicin, esorubicin, Marcelo marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, 5-fluorouracil, denopterin, methotrexate, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thiguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, calusterone, dromostanolone, propionate, epitio stanol, mepitio stanane, testolactone, aminoglutethimide, mitotane, trilostane, folinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansine, ansamitocins, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, 2-ethylhydrazide, procarbazine, polysaccharidek, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2''-trichlorotriethylamine, T-2 toxin, verracurin A, roridin A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside, cyclophosphamide, thiotepa, paclitaxel, albumin-engineered nanoparticle formulation of paclitaxel, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, carboplatin, vinblastine, platinum, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda,

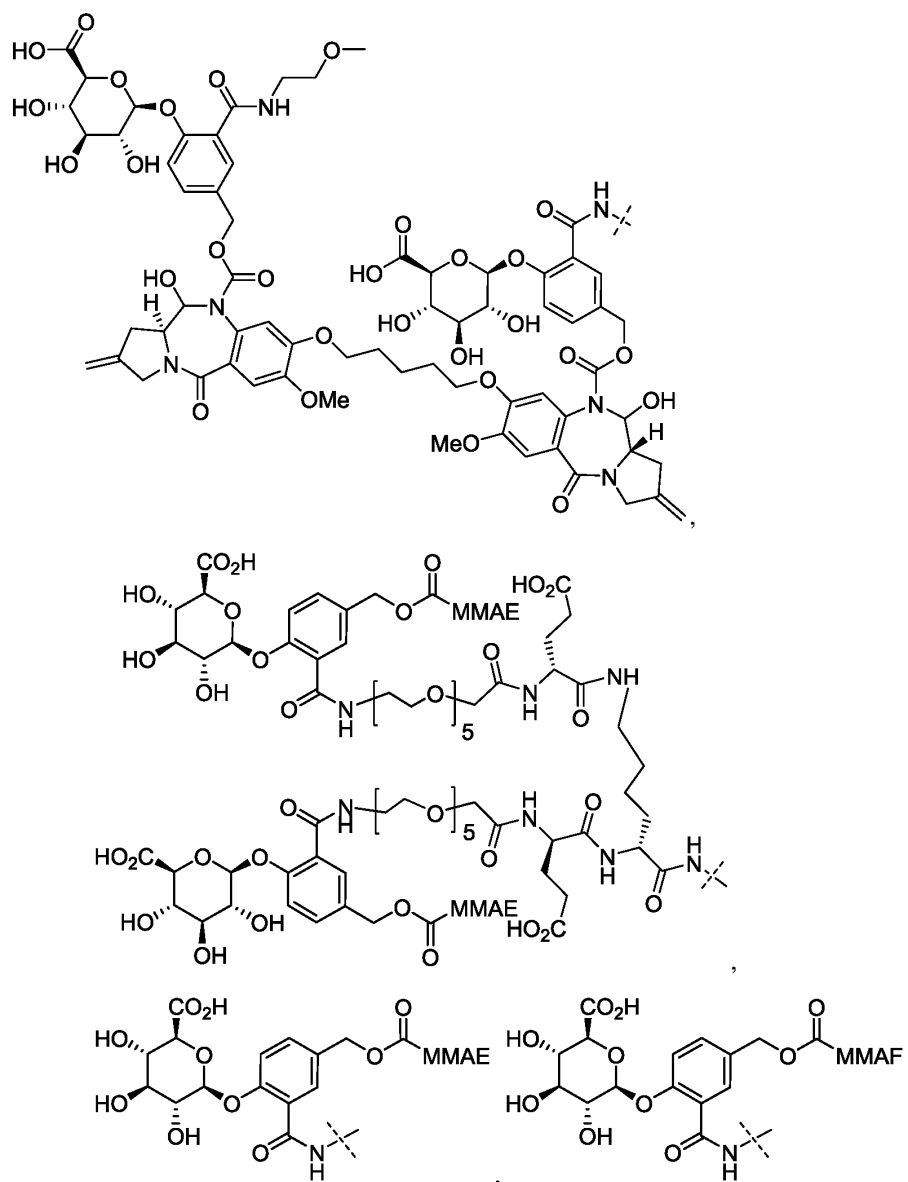
- ibandronate, CPT-11, SN-38, GI-147211C, 9-aminocamptothecin, 7-hydroxymethyl camptothecin, 7-aminomethyl camptothecin, 10-hydroxycamptothecin, (20S)-camptothecin, rubitecan, gimatecan, karenitecin, silatecan, lurtotecan, exatecan, diflomotecan, belotecan, lurtotecan, topoisomerase inhibitor RFS 2000, topoisomerase inhibitor S39625, difluoromethylornithine, retinoic acid, capecitabine, or a pharmaceutically acceptable salt, solvate or acid thereof;
- (b) monokine, lymphokine, traditional polypeptide hormone, parathyroid hormone, thyroxine, relaxin, prorelaxin, glycoprotein hormone, follicle stimulating hormone, thyroid stimulating hormone, luteinizing hormone, hepatic growth factor fibroblast growth factor, prolactin, placental lactogen, tumor necrosis factor, tumor necrosis factor- α , tumor necrosis factor- β , mullerian inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, vascular endothelial growth factor, thrombopoietin, erythropoietin, osteoinductive factor, interferon, interferon- α , interferon- β , interferon- γ , colony stimulating factor (CSF), macrophage-CSF, granulocyte-macrophage-CSF, granulocyte-CSF, interleukin (IL), IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, polypeptide factor, LIF, kit ligand, or combinations thereof;
- (c) diphtheria toxin, botulinum toxin, tetanus toxin, decentritoxin, cholera toxin, amanitin, α -amanitin, pyrrolbenzodiazepine, pyrrolbenzodiazepine derivatives, indolinbenzodiazepine, pyridinbenzodiazepine, tetrodotoxin, brevetoxin, ciguatoxin, ricin, AM toxin, auristatin, tubulysin, geldanamycin, maytansinoid, calicheamycin, daunomycin, doxorubicin, methotrexate, vindesine, SG2285, dolastatin, dolastatin analog, cryptophycin, camptothecin, rhizoxin, rhizoxin derivatives, CC-1065, CC-1065 analogs or derivatives, duocarmycin, enediyne antibiotic, esperamicin, epothilone, toxoid, or combinations thereof;
- (d) affinity ligands, wherein the affinity ligand is a substrate, an inhibitor, an activator, a neurotransmitter, a radioisotope, or a combination thereof;
- (e) a radioactive label, ^{32}P , ^{35}S , a fluorescent dye, an electron dense reagent, an enzyme, biotin, streptavidin, dioxigenin, hapten, an immunogenic protein, a nucleic acid molecule with a sequence complementary to a target, or a combination thereof;
- (f) an immunomodulatory compound, an anti-cancer agent, an anti-viral agent, an anti-bacterial agent, an anti-fungal agent, and an anti-parasitic agent, or combinations thereof;

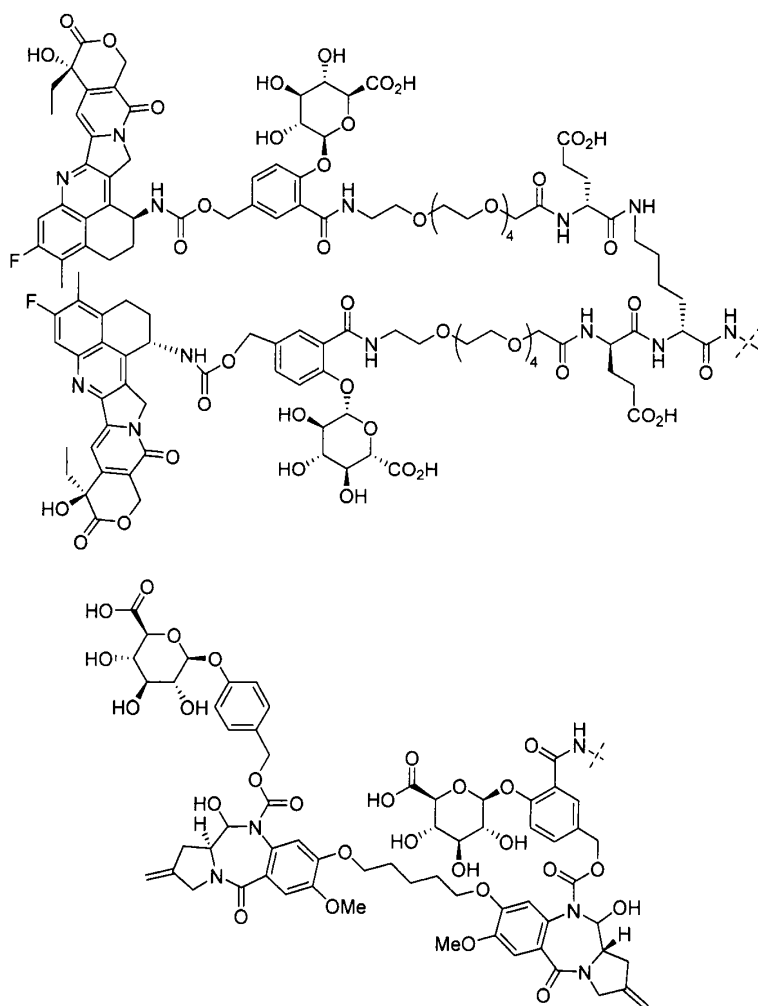
- (g) tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone or toremifene;
- (h) 4(5)-imidazole, aminoglutethimide, megestrol acetate, exemestane, letrozole, or anastrozole;
- (i) flutamide, nilutamide, bicalutamide, leuprolide, goserelin, or troxacitabine;
- (j) an aromatase inhibitor;
- (k) a protein kinase inhibitor;
- (l) a lipid kinase inhibitor;
- (m) antisense oligonucleotide;
- (n) ribozyme;
- (o) vaccines;
- (p) an anti-angiogenic agent; and
- (q) a topoisomerase I inhibitor.

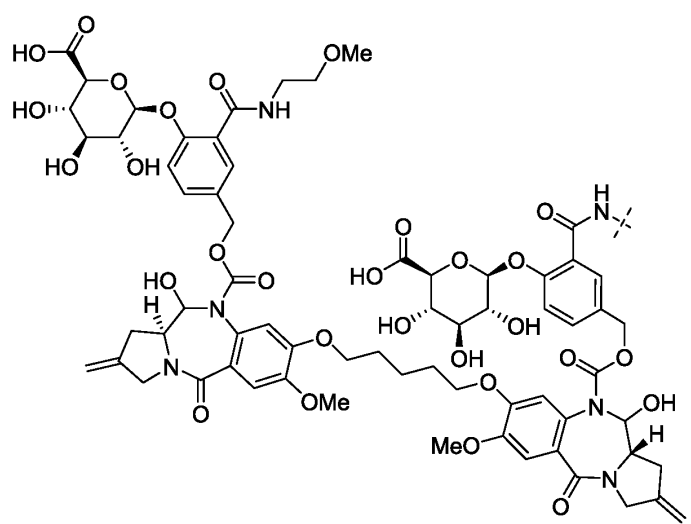
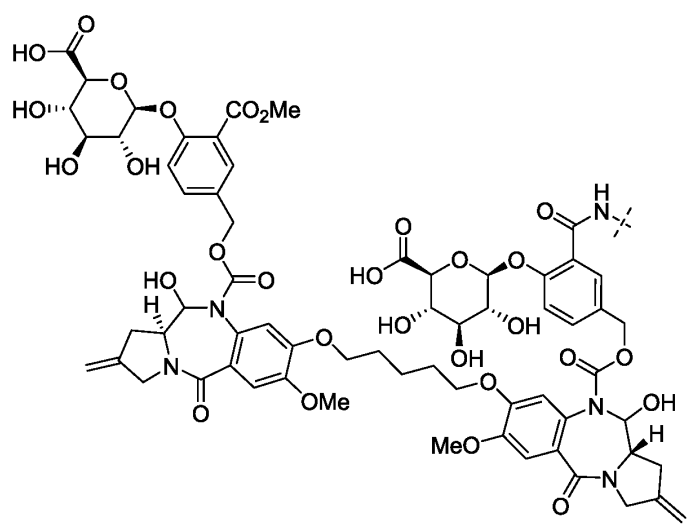
77. The conjugate of any one of claims 1-74, wherein at least one active agent is a pyrrolobenzodiazepine dimer.

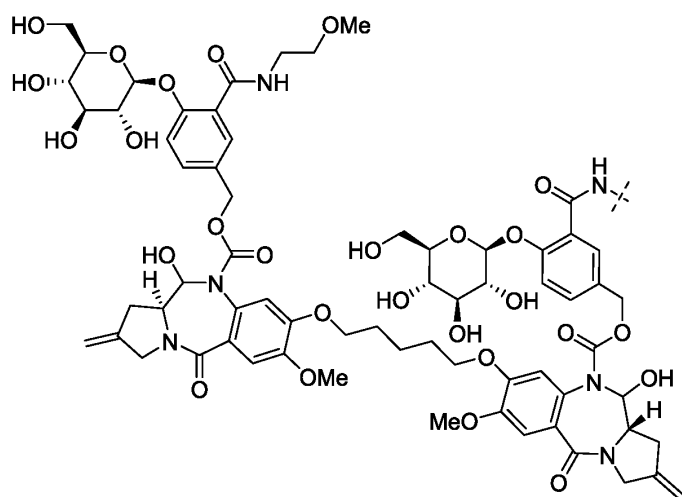
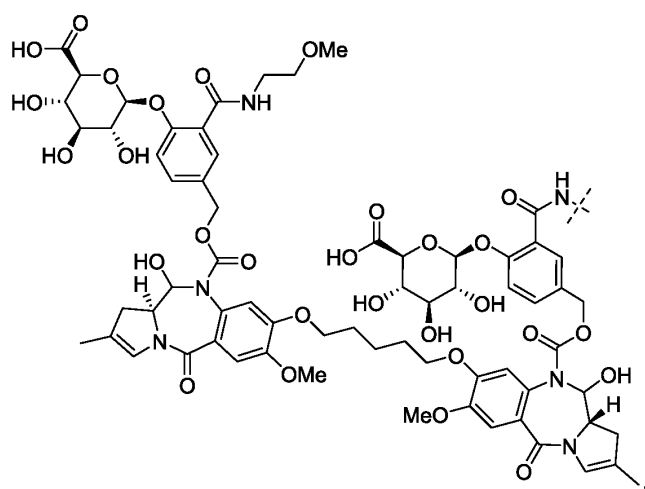
78. The conjugate of any one of claims 1-74, wherein the active agent is a pyrrolobenzodiazepine dimer;
the pyrrolobenzodiazepine dimer is substituted with X at position N10 or X' at position N'10,
wherein X or X' links the pyrrolobenzodiazepine dimer to L;
X and X' are each independently -C(O)O-*, -or -C(O)-*; and
* is a binding site between the pyrrolobenzodiazepine dimer and L.

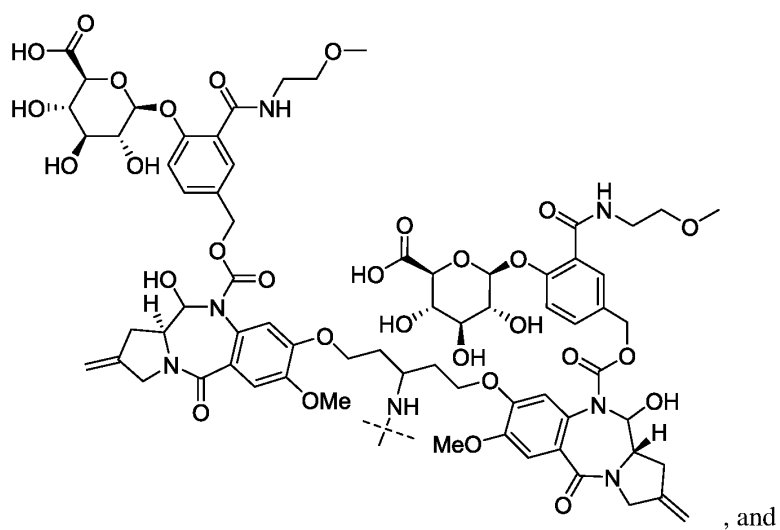
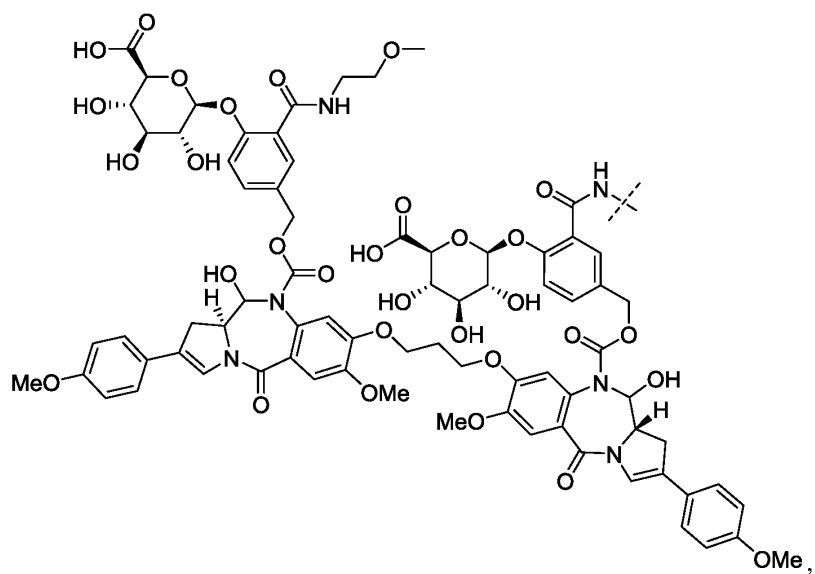
79. The conjugate of any one of claims 1-77, wherein the conjugate comprises

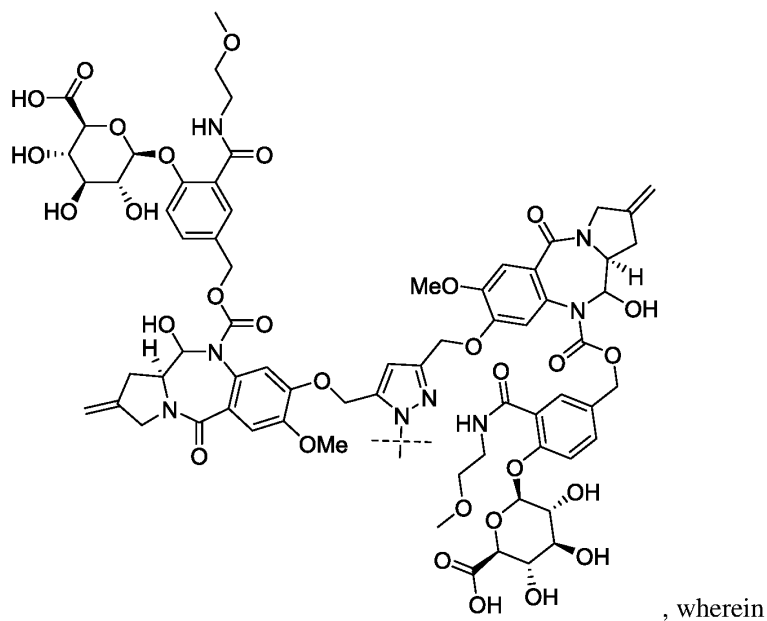






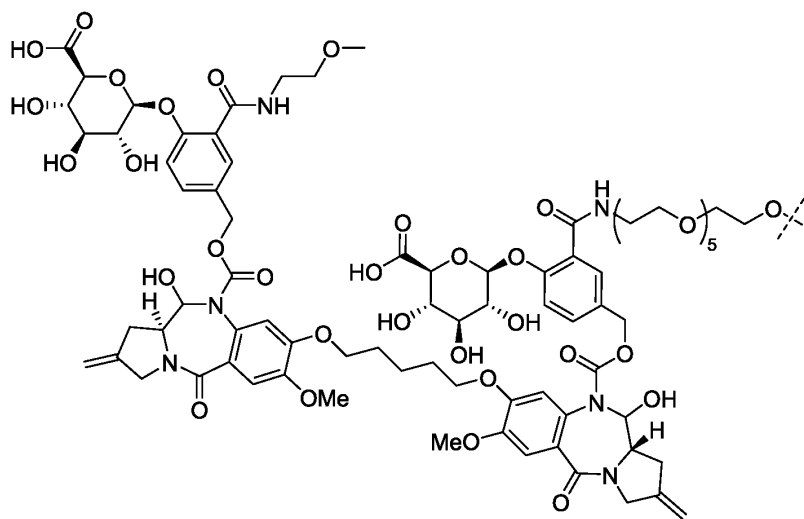


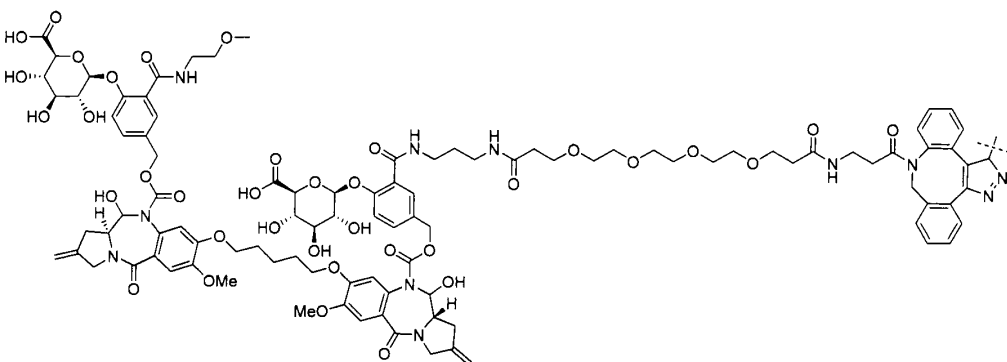
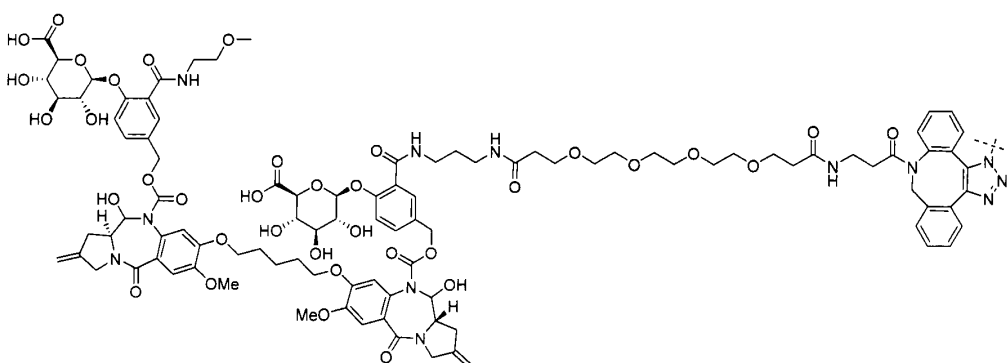
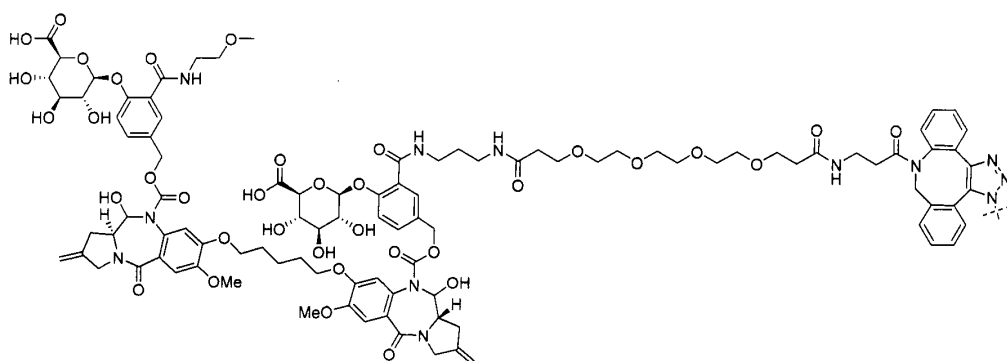


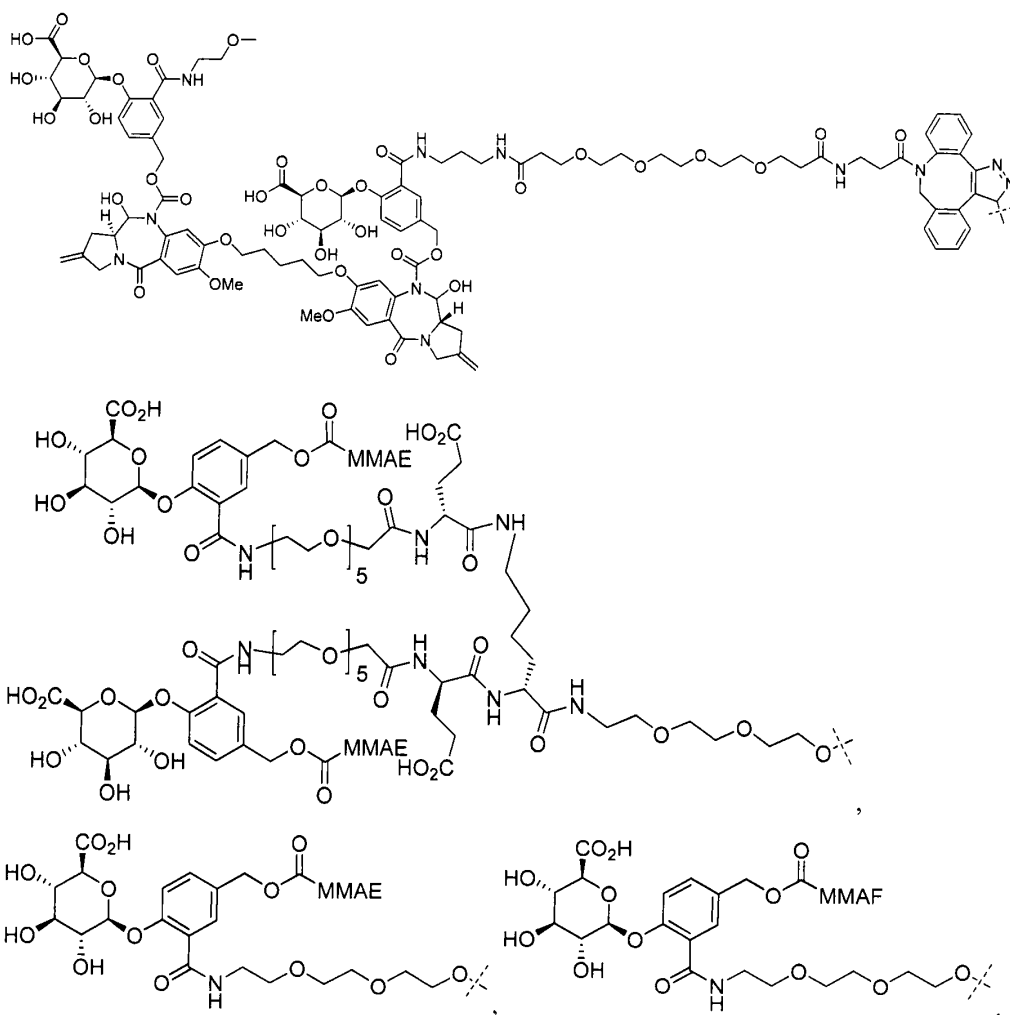


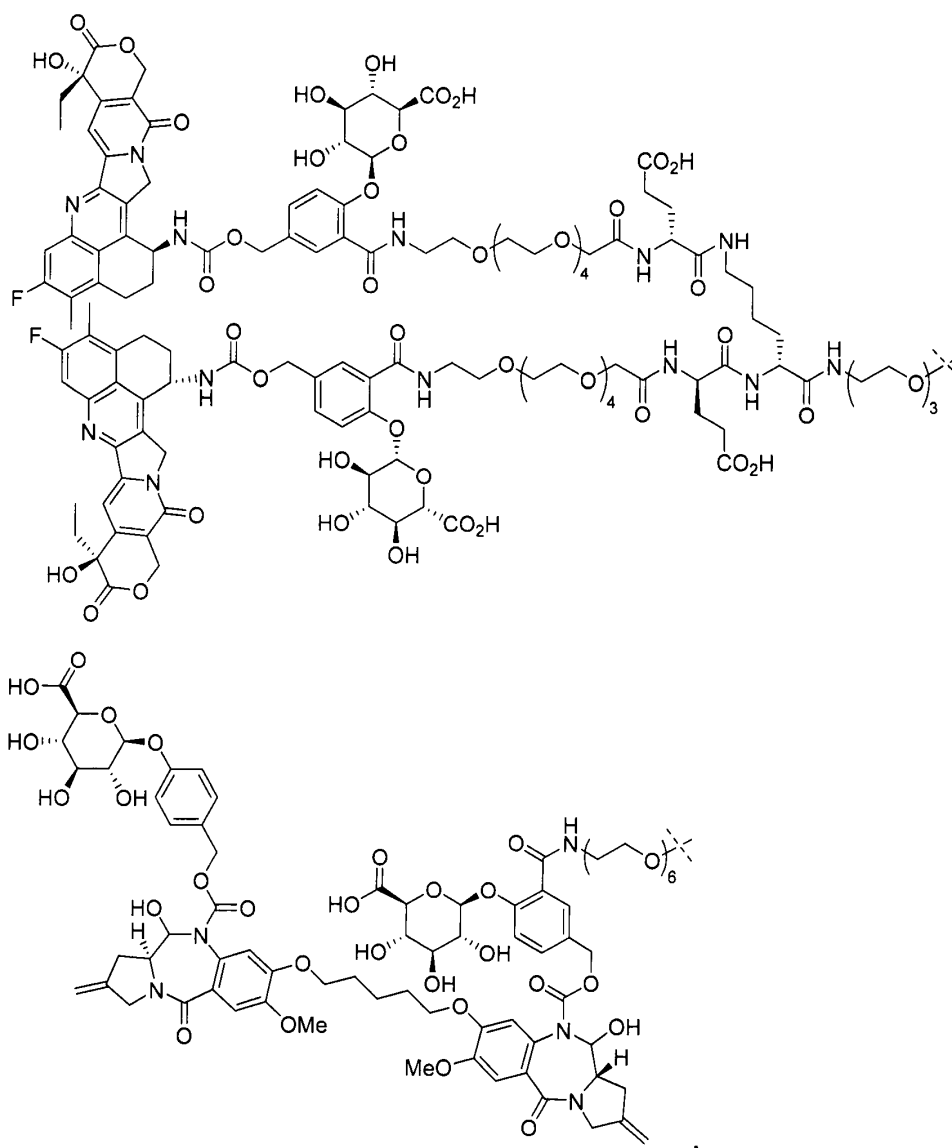
MMAE is monomethyl auristatin E, and MMAF is monomethyl auristatin F; and the dotted line represents a connect to the rest of the conjugate.

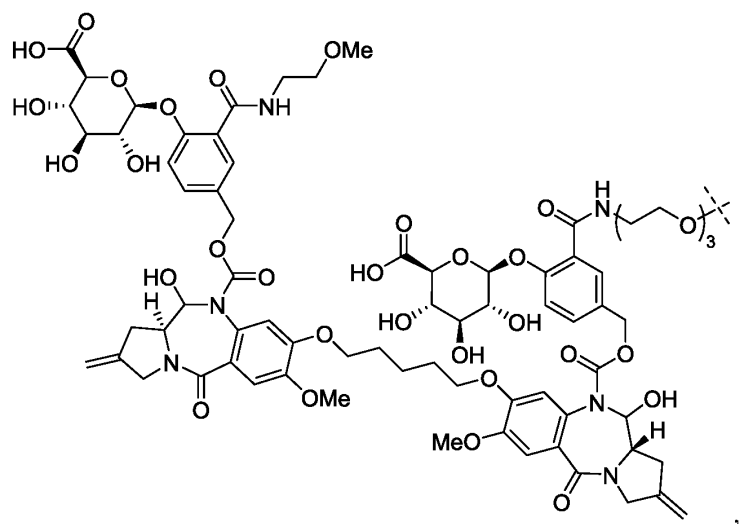
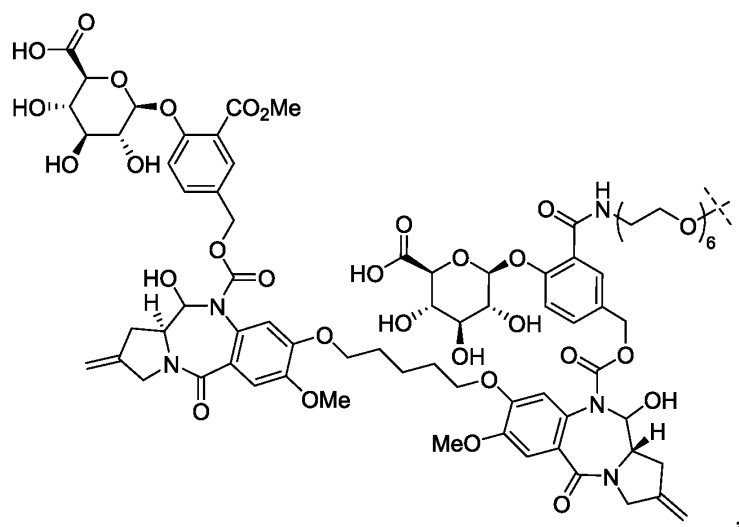
80. The conjugate of any one of claims 1-77, wherein the conjugate comprises

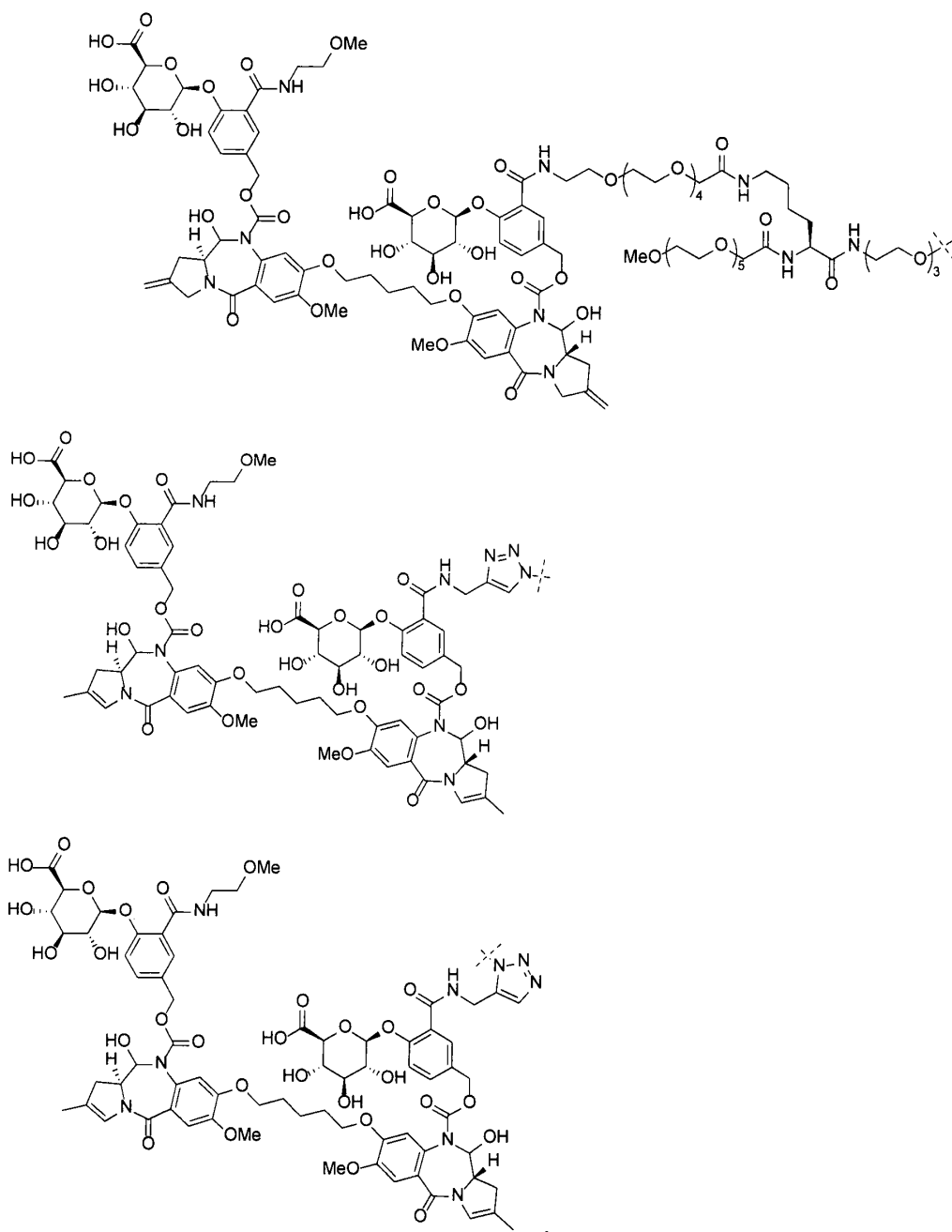


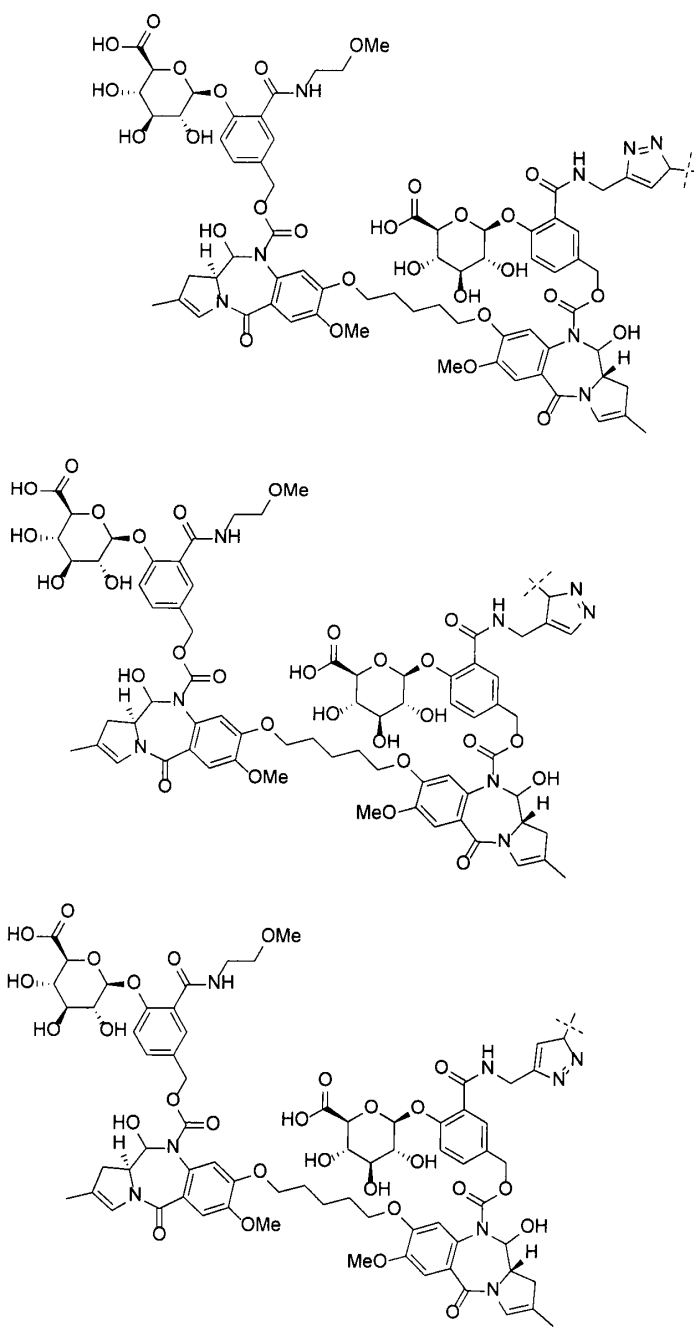




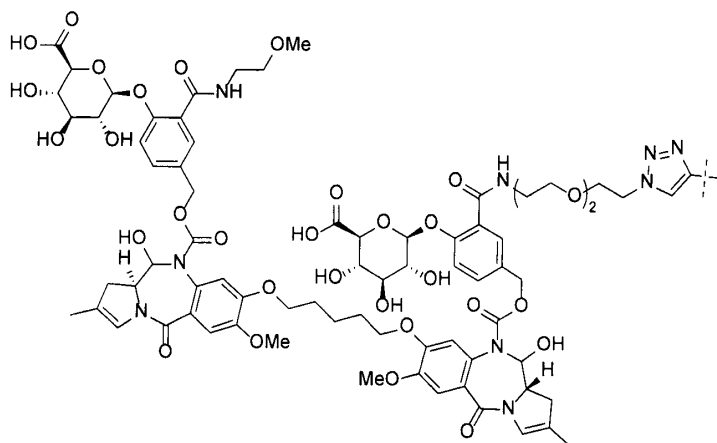
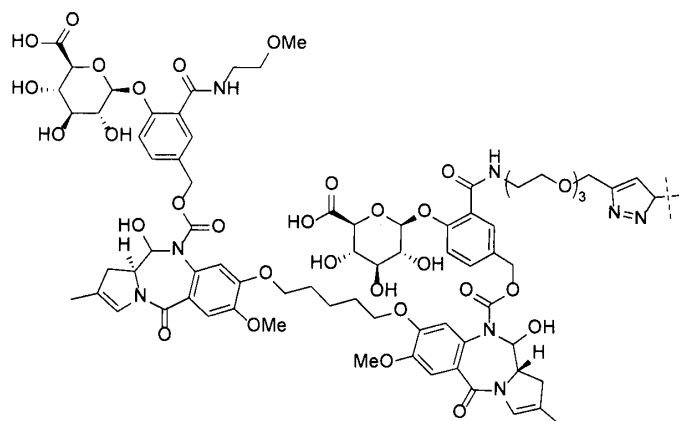
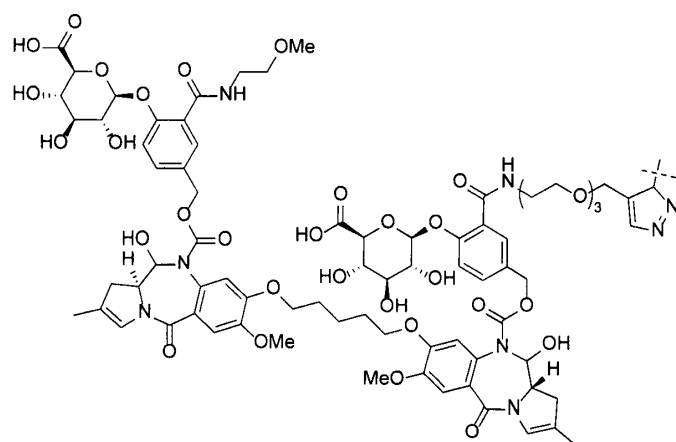




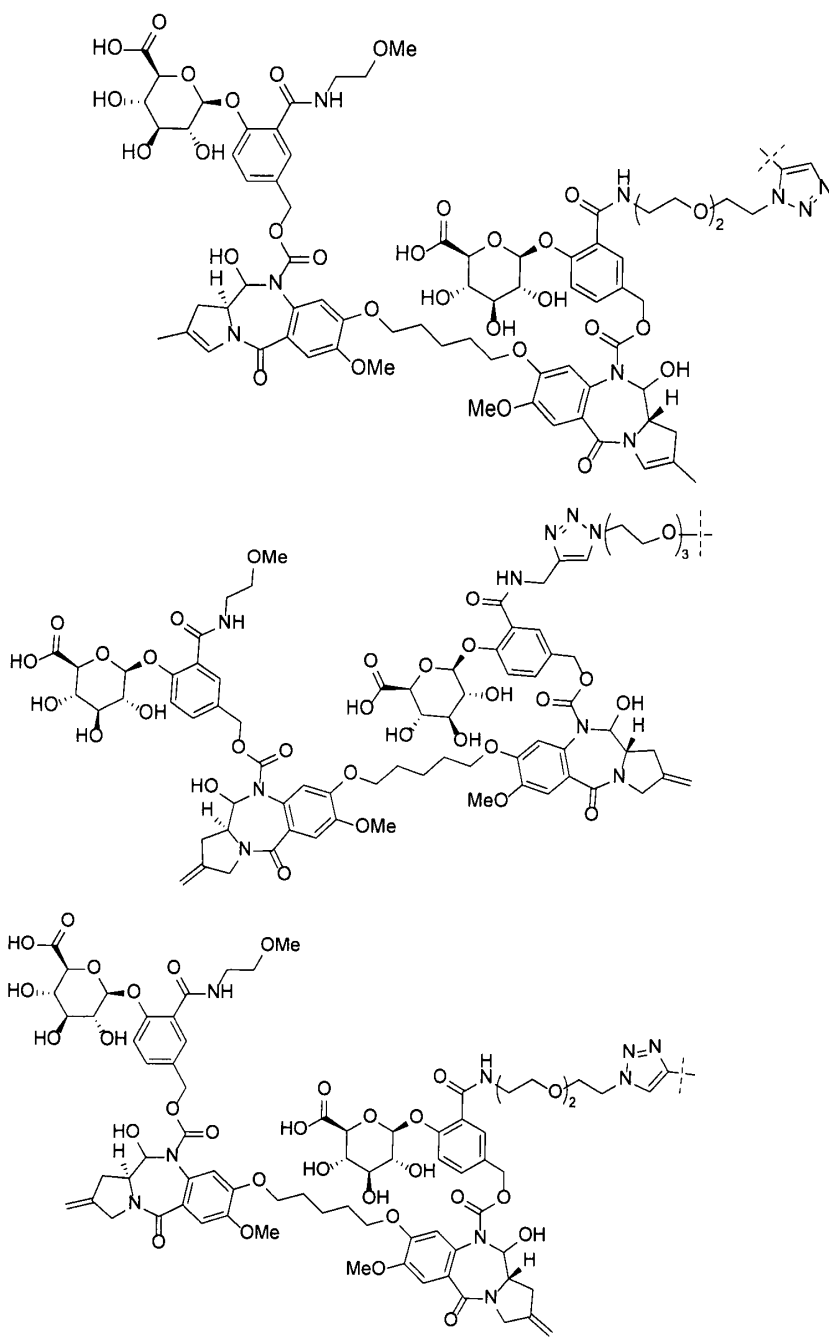




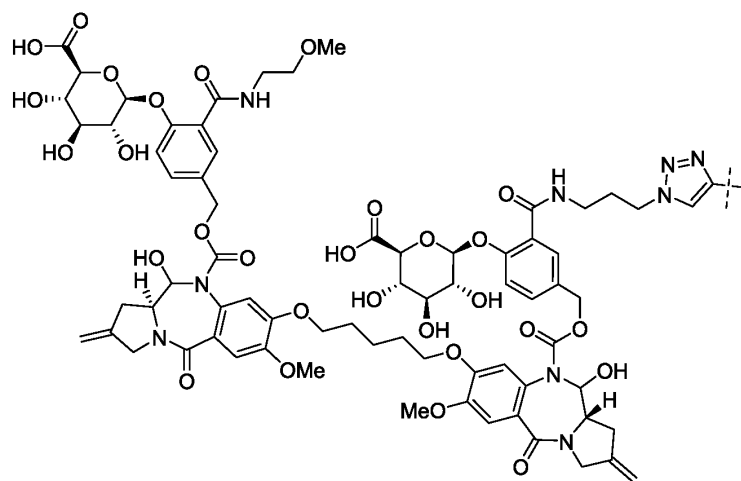
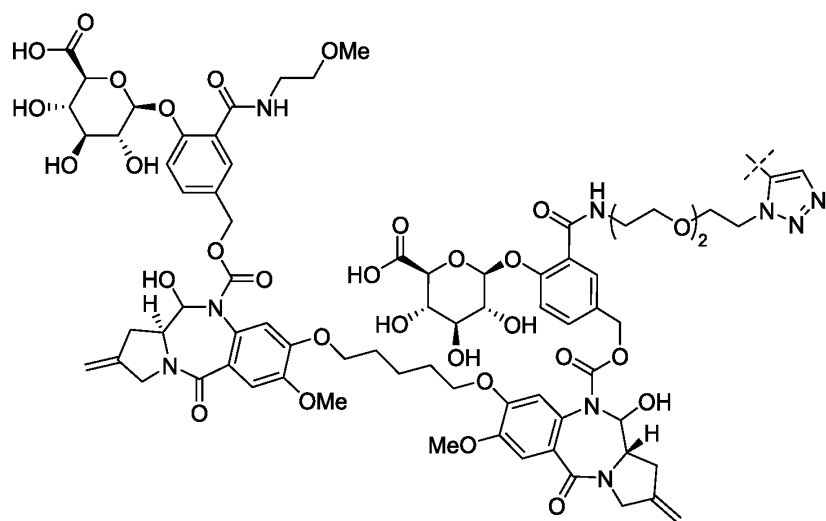
RECTIFIED SHEET (RULE 91) ISA/KR

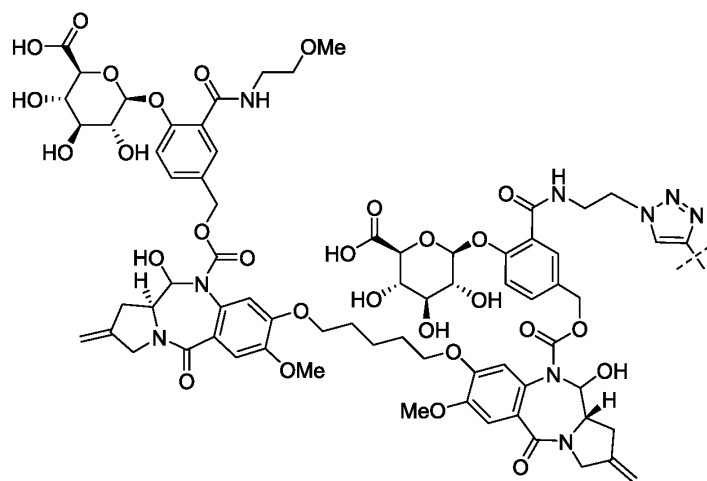
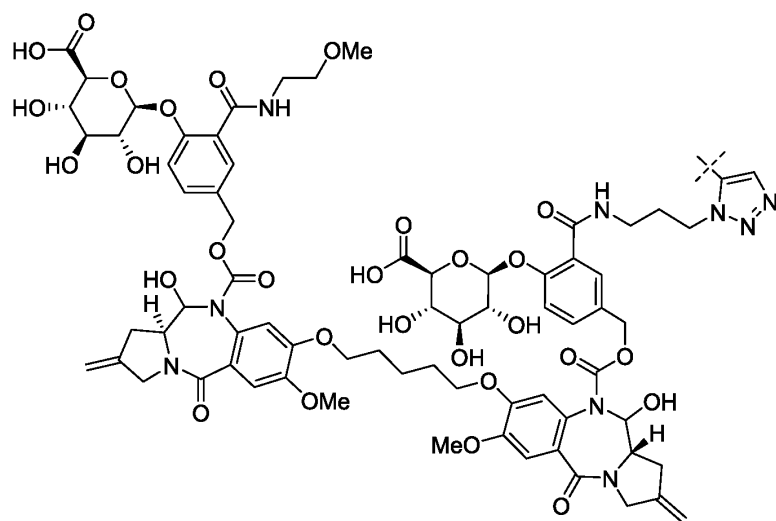


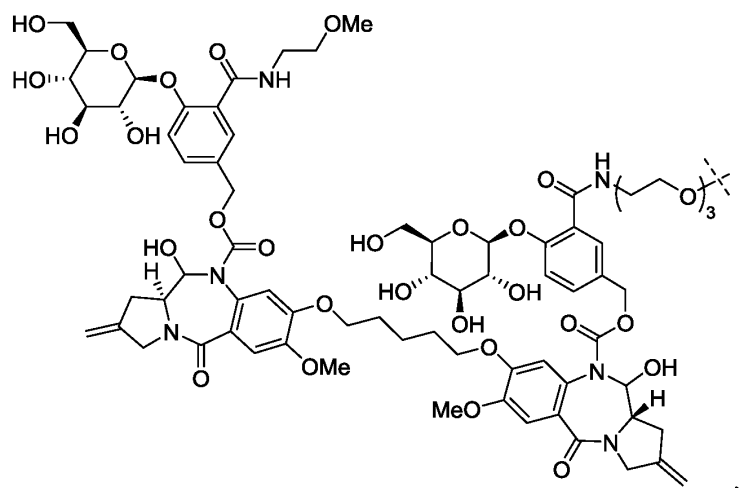
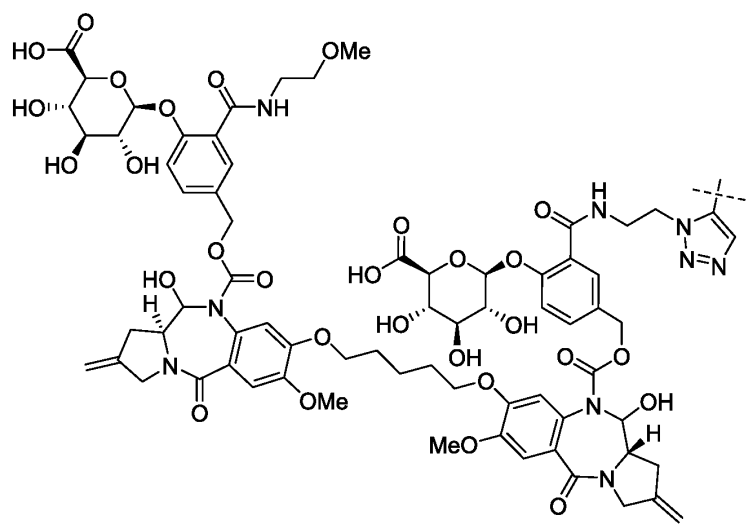
RECTIFIED SHEET (RULE 91) ISA/KR

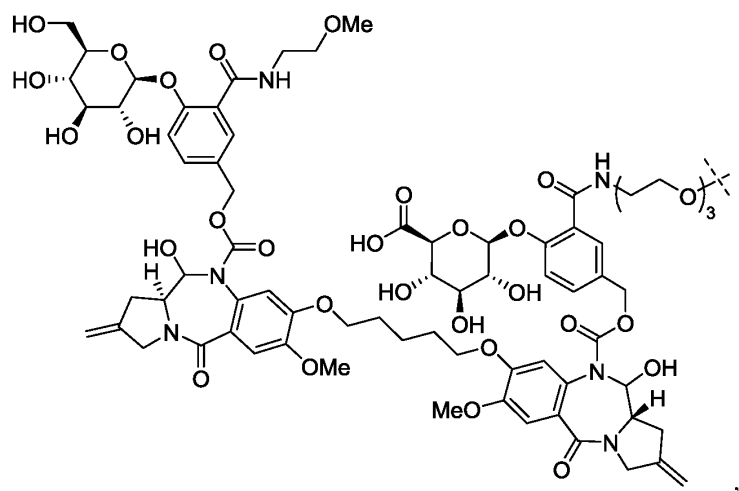


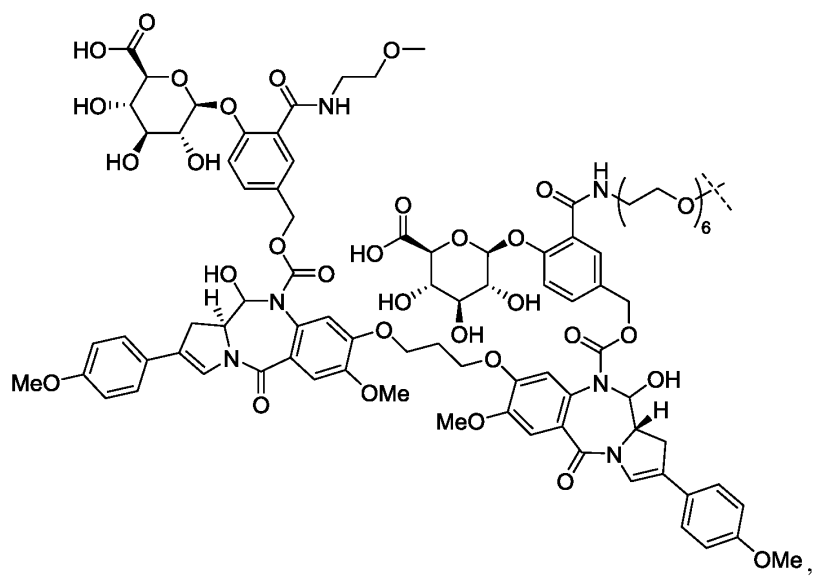
RECTIFIED SHEET (RULE 91) ISA/KR

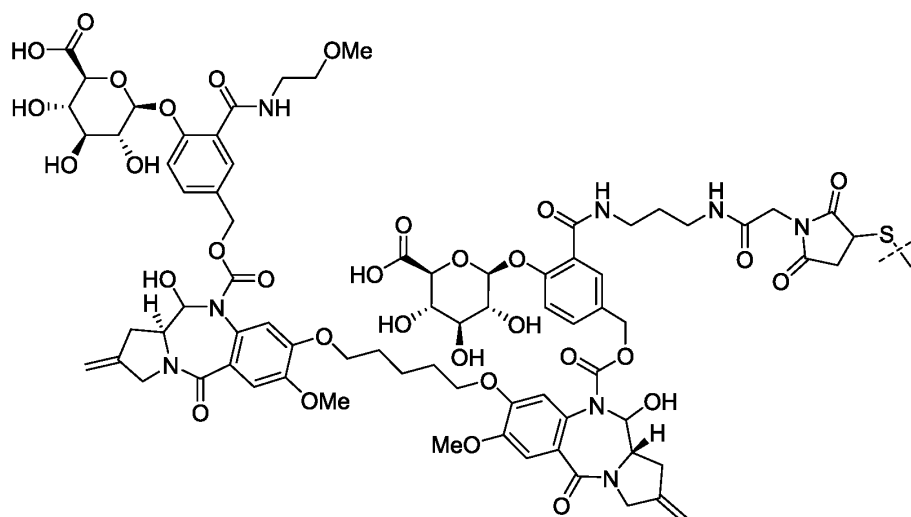
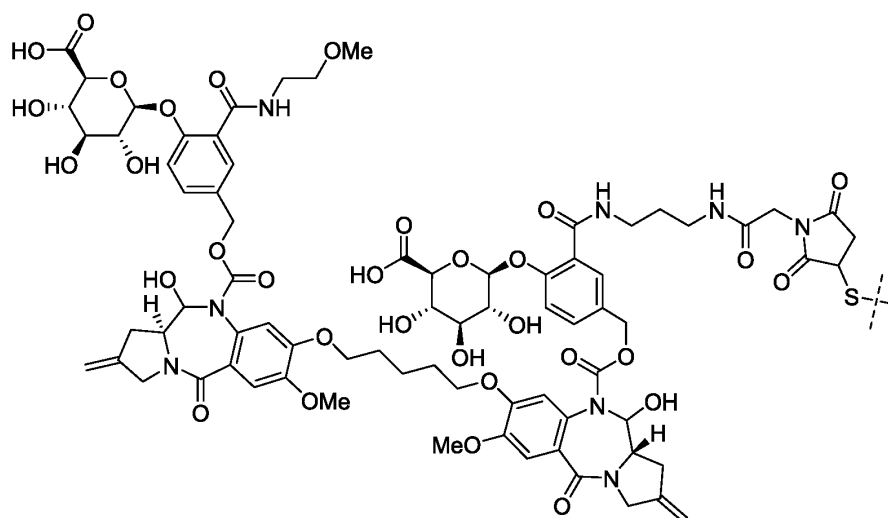


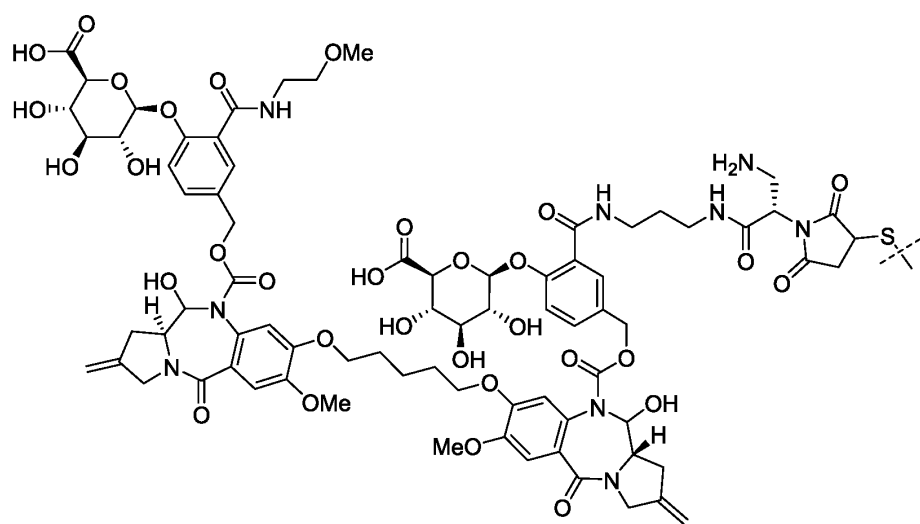
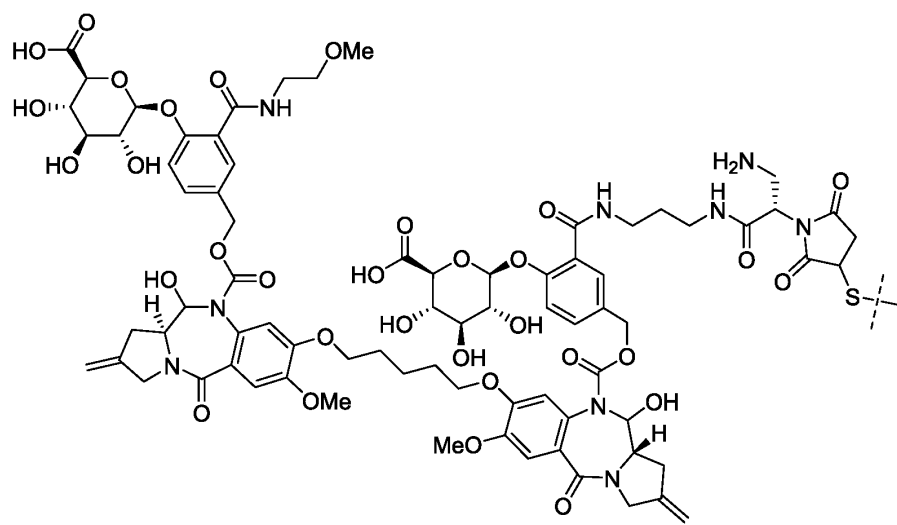


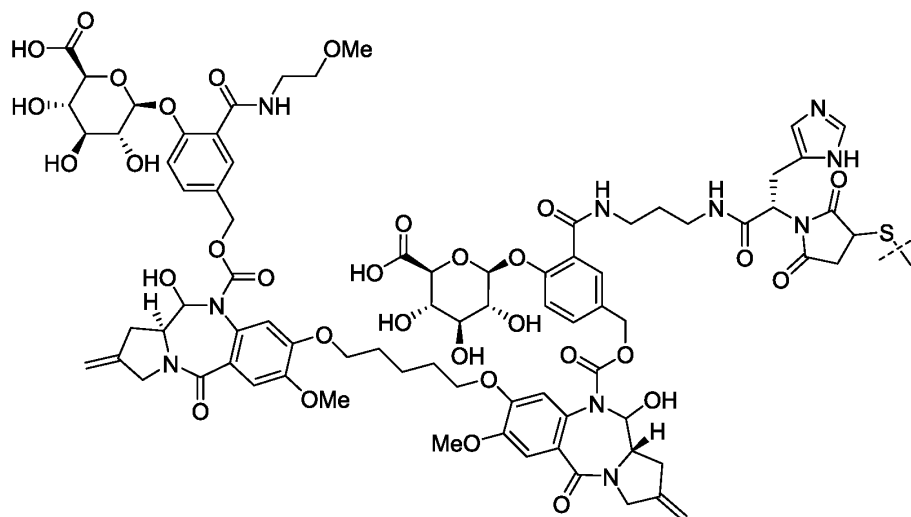
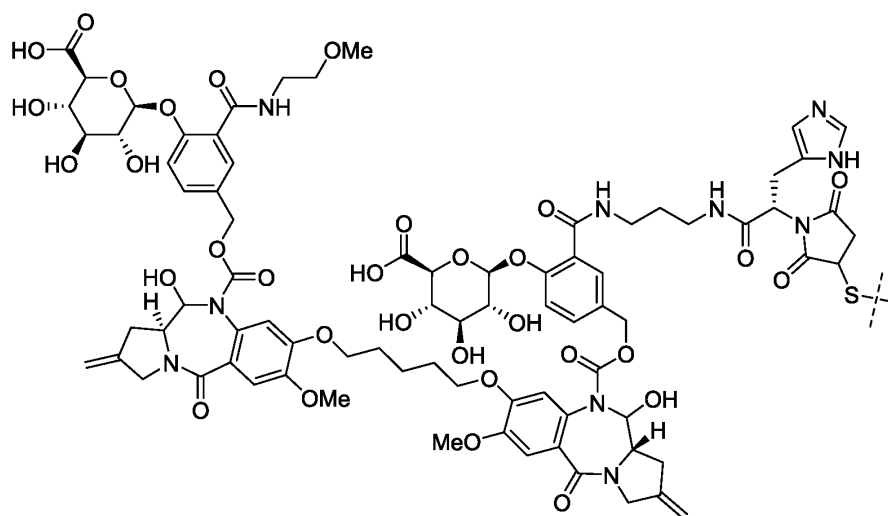


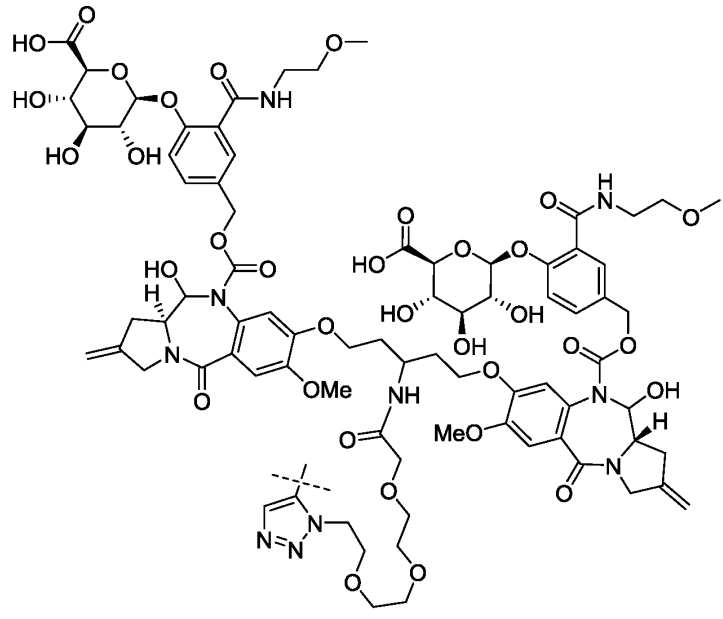
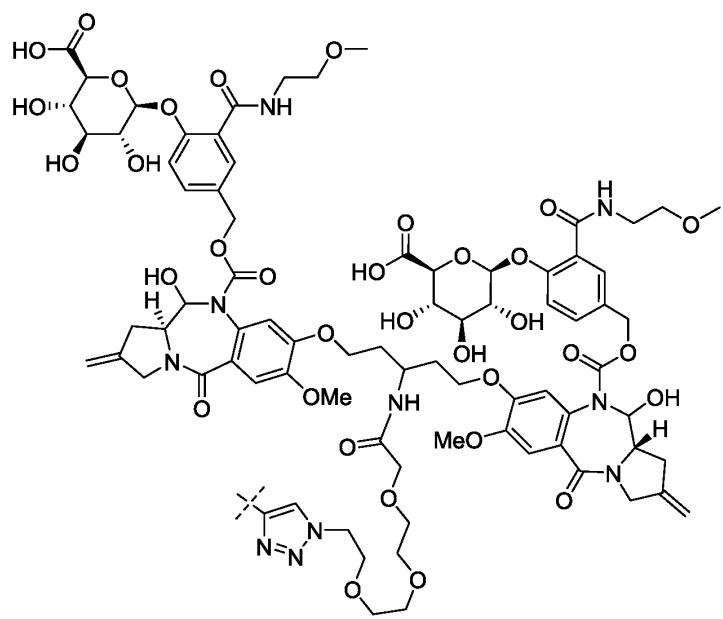


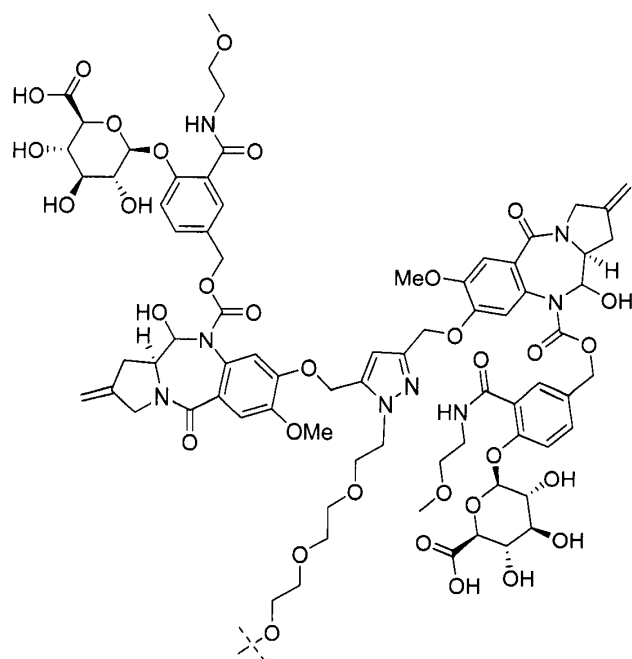










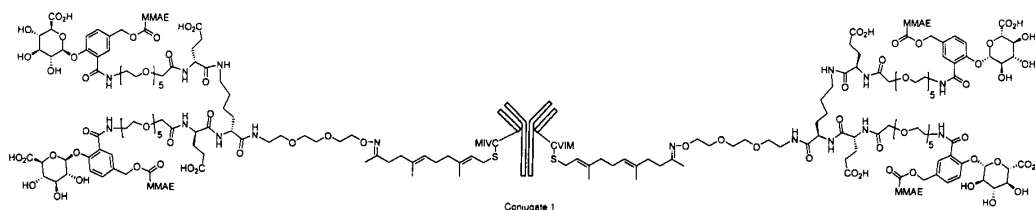


wherein

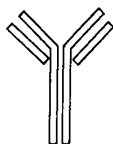
MMAE is monomethyl auristatin E, and MMAF is monomethyl auristatin F; and the dotted line represents a connect to the rest of the conjugate.

81. The conjugate of any one of claims 1-97, wherein the active agent is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

82. The conjugate of claim 1, wherein the conjugate is:

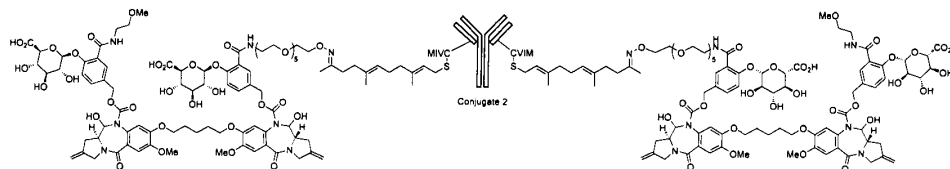


or a salt thereof, wherein:

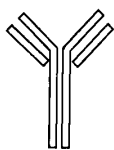


is the anti-human neural cell adhesion molecule L1 (L1CAM) antibody.

83. The conjugate of claim 1, wherein the conjugate is:

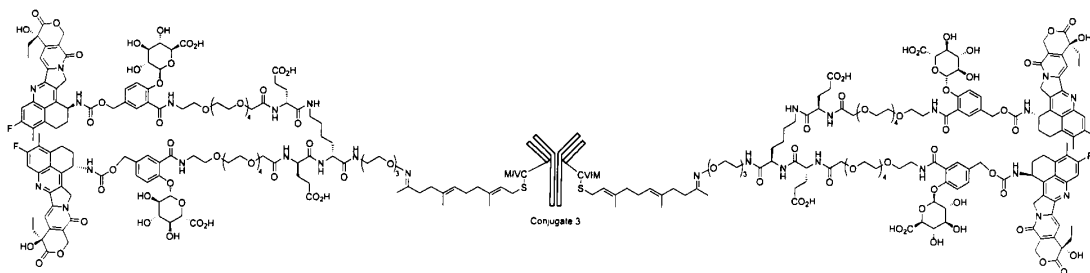


or a salt thereof, wherein:

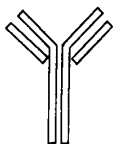


is the anti-human neural cell adhesion molecule L1 (L1CAM) antibody.

84. The conjugate of claim 1, wherein the conjugate is:



or a salt thereof, wherein:



is the anti-human neural cell adhesion molecule L1 (L1CAM) antibody.

85. The conjugate of any one of claims 82-84, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 58 and a light chain comprising the amino acid sequence of SEQ ID NO: 61.

86. A pharmaceutical composition comprising the conjugate of any one of claims 1-85 and a pharmaceutically acceptable excipient.

87. A method of treating or preventing hyperplasia, cancer, or angiogenic disease in a subject, comprising administering the conjugate of any one of claims 1-85 or a pharmaceutically acceptable salt thereof to the subject.
88. The method of claim 87, wherein the method treats cancer.
89. The method of claims 87 or 88, wherein the cancer is lung cancer, small-cell lung cancer, gastrointestinal cancer, colon cancer, intestinal cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreatic cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, or melanoma.