DLL3 ANTIBODY-DRUG CONJUGATES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/454,009, filed March 22, 2023, the disclosure of which is incorporated by reference herein it its entirety.

SEQUENCE LISTING

[0002] This application contains a computer readable Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled "14529-133-228_SEQ_LISTING.xml", was created on March 20, 2024, and is 49,227 bytes in size.

FIELD

[0003] The present disclosure relates generally to antibody-drug conjugates (ADCs) that comprise Delta-Like Canonical Notch Ligand (DLL3) antibodies and methods of their use.

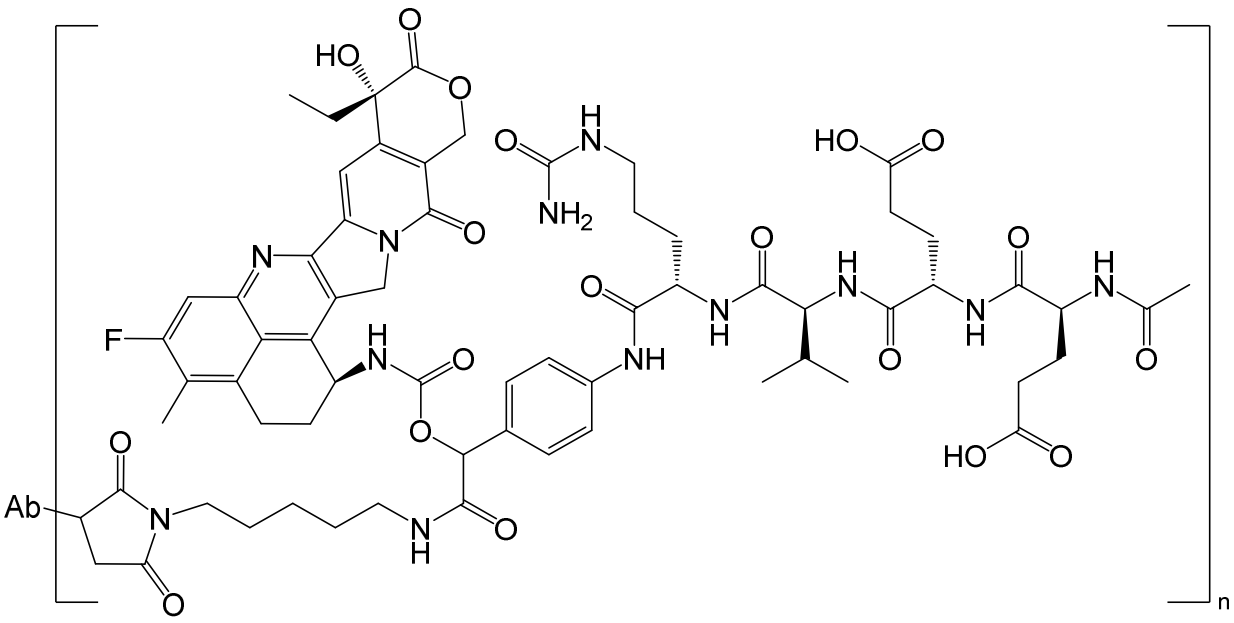
BACKGROUND

[0004] Delta Like Canonical Notch Ligand 3 (DLL3) is a type I transmembrane protein that belongs to the DSL family of Notch ligands. It is normally expressed exclusively on intracellular membranes, especially the Golgi apparatus. Additional Notch family ligands include Delta Like Canonical Notch Ligand 1 (D1), Delta Like Canonical Notch Ligand 4 (D4), Jagged Canonical Notch Ligand 1 (J1), and Jagged Canonical Notch Ligand 2 (J2). Except for DLL3, the other ligands can activate Notch signaling. DLL3 acts as an inhibitor of Notch signaling by interfering with the binding between Notch and its ligands. DLL3 is highly expressed on lung tumor cell surface, including small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC). While it is normally expressed exclusively on the intracellular membranes, DLL3 is a potential therapeutic tumor target for any tumors that express DLL3, including SCLC and LCNEC. In recent years, the establishment of DLL3 as a unique target in SCLC has accelerated the development of therapeutic agents. However, therapeutic success with antibodies and ADCs targeting DLL3 has not yet been achieved.

[0005] There remains a need in the art for ADCs that can target DLL3 to treat, prevent, or alleviate DLL3-mediated diseases, disorders, or conditions, including lung cancer, such as small cell lung cancer (SCLC) or large cell neuroendocrine carcinoma (LCNEC).

SUMMARY

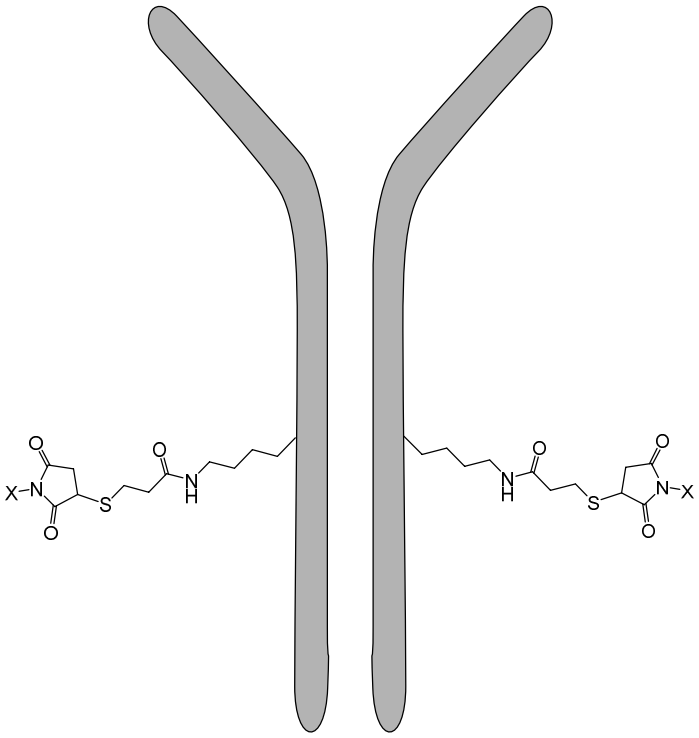
[0006] In one aspect, the present disclosure refers to an antibody-drug conjugate (ADC) of Formula (II):



(II)

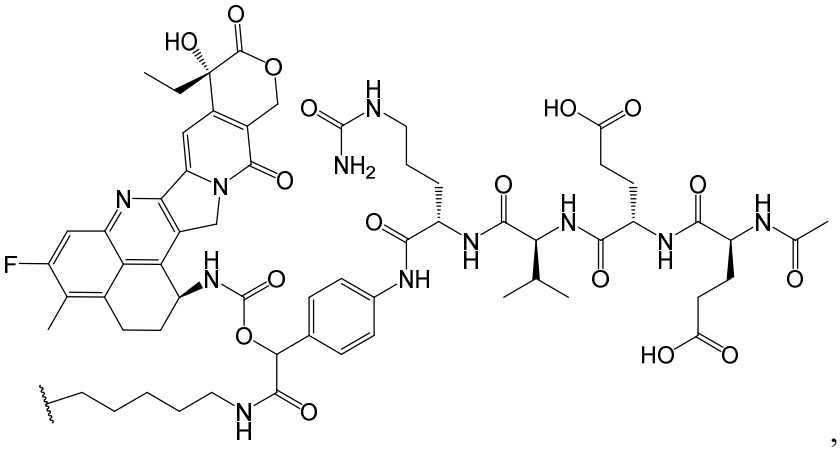
or a salt thereof, wherein: *n* is an integer from 1 to 8, and Ab represents an antibody that binds to DLL3 ("DLL3 antibody").

In another aspect, the present disclosure refers to an antibody-drug conjugate (ADC) of Formula (B):



(B),

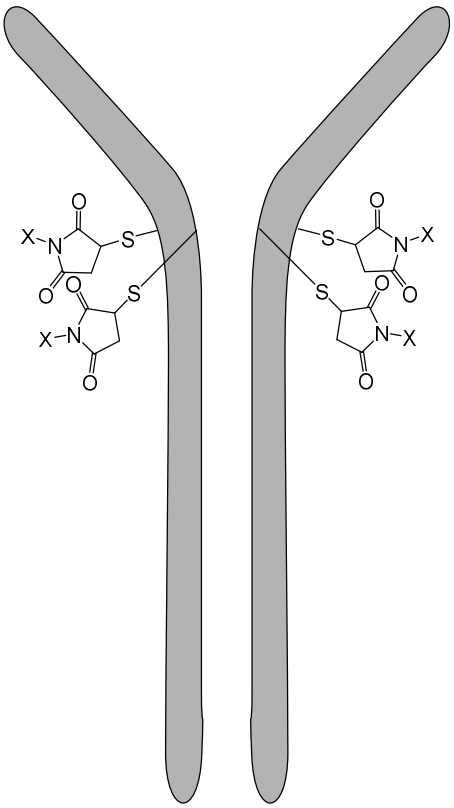
wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted $CH_2CH_2CH_2CH_2NH$ moiety is from a lysine residue (optionally any one or more of K246, K248, K288, K290, or K317 according to the EU numbering) of the CH2 domain of the DLL3 antibody, and X represents the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

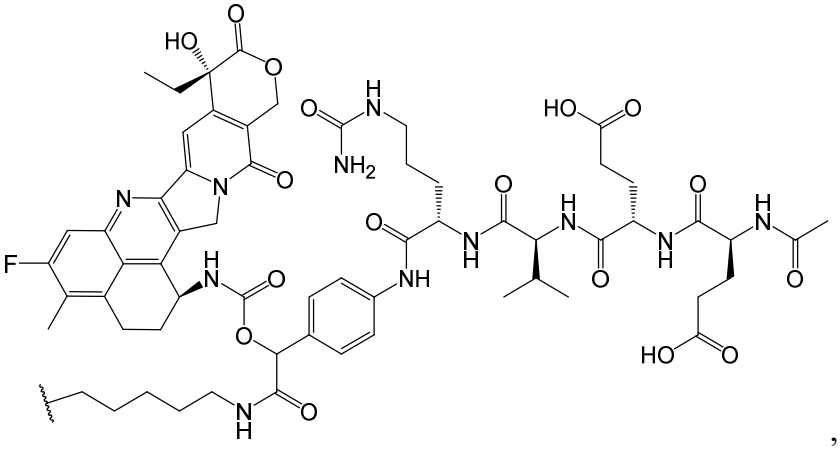
In another aspect, the present disclosure refers to an antibody-drug conjugate (ADC) of Formula (A):



(A),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted sulfurs are from cysteine residues (optionally C226 and/or C229) of the DLL3 antibody, and X represents

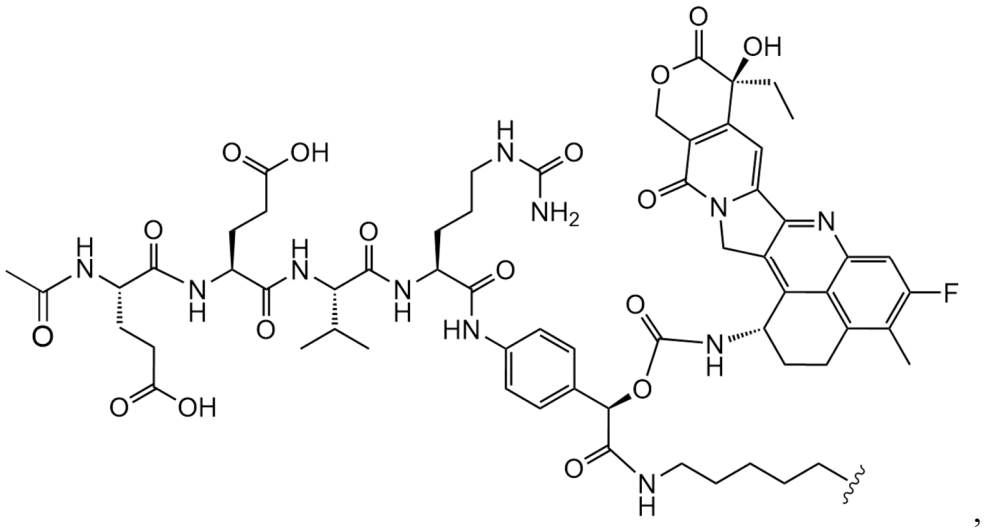
(i) the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A);

(ii) the structure below or a salt thereof:

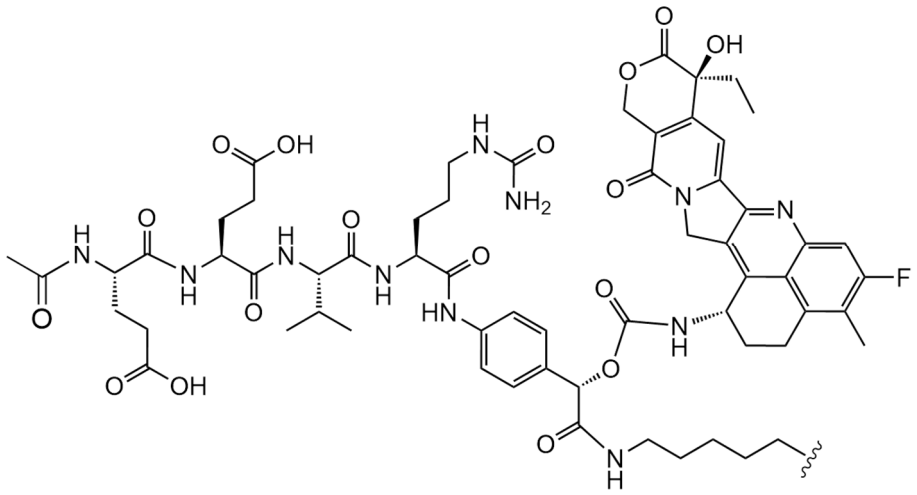


,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A),

or

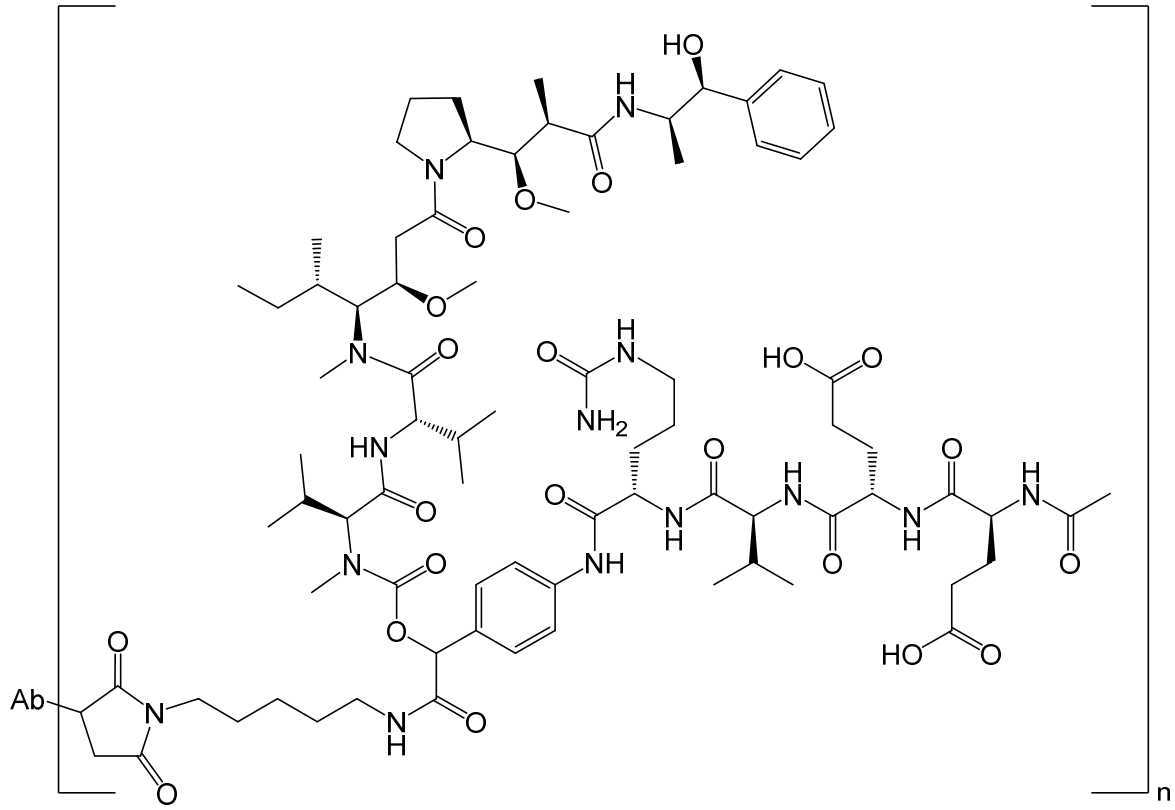
(iii) the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

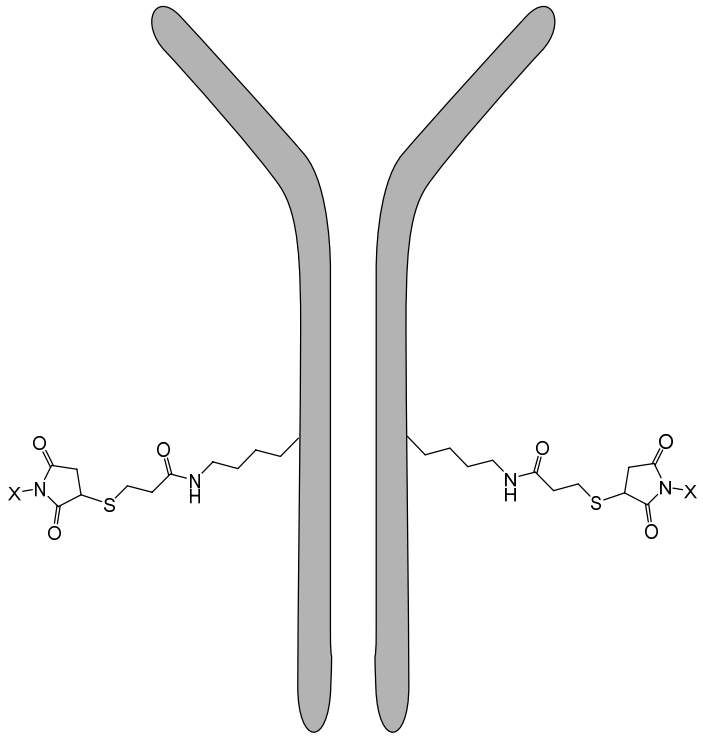
In another aspect, the present disclosure refers to an antibody-drug conjugate (ADC) of Formula (I):



(I)

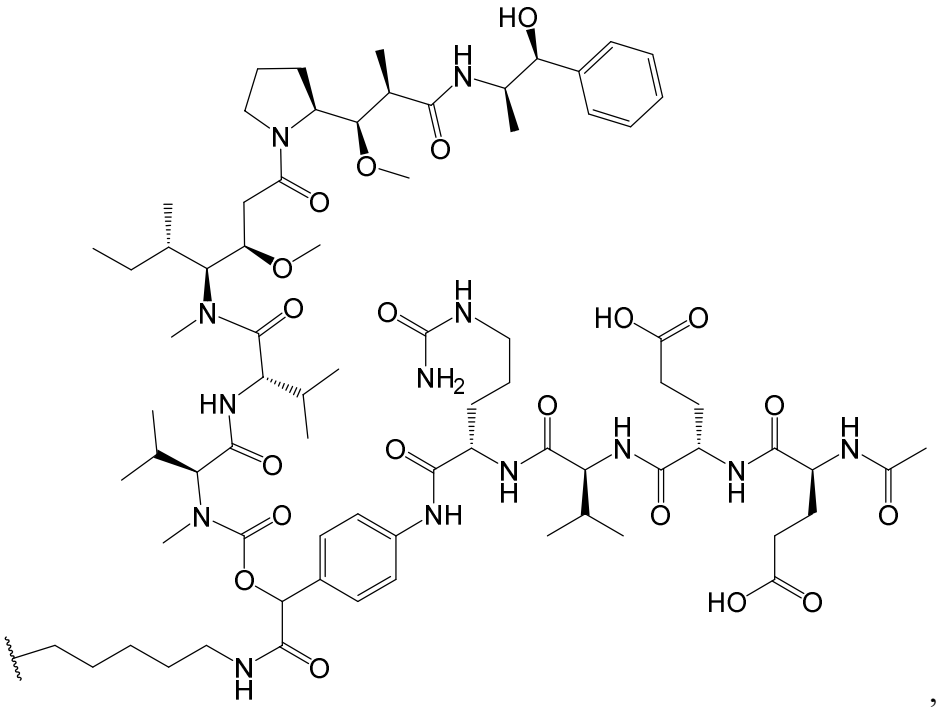
or a salt thereof, wherein: *n* is an integer from 1 to 8, and Ab represents an antibody that binds to DLL3 ("DLL3 antibody").

In another aspect, the present disclosure refers to an antibody-drug conjugate (ADC) of Formula (B):



(B),

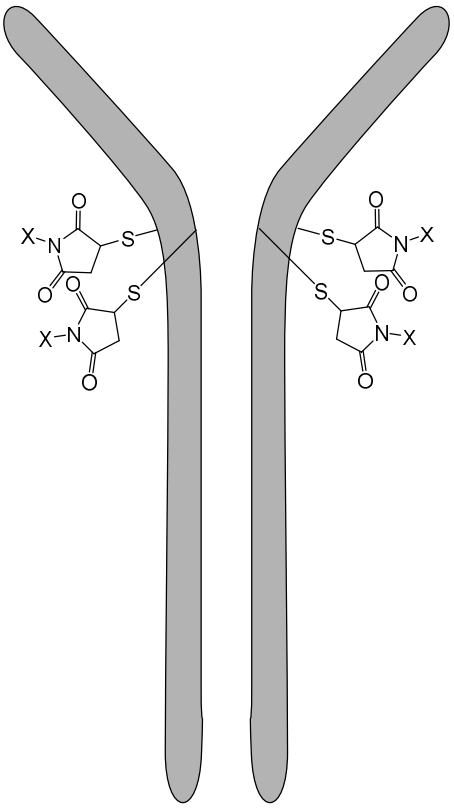
wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted $CH_2CH_2CH_2CH_2NH$ moiety is from a lysine residue (optionally any one or more of K246, K248, K288, K290, or K317 according to the EU numbering) of the DLL3 antibody, and X represents the structure below or a salt thereof:



,

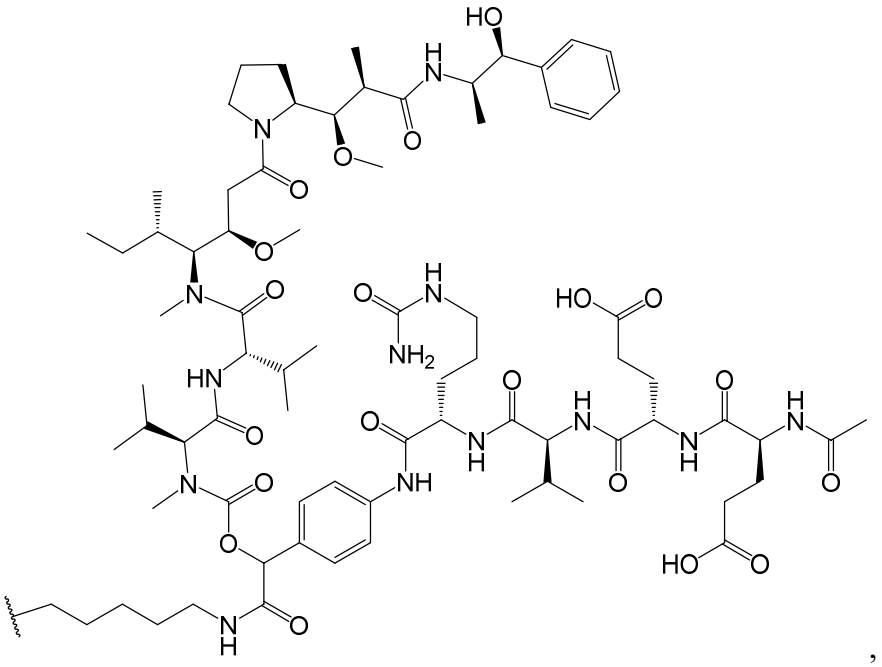
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

In another aspect, the present disclosure refers to an antibody-drug conjugate (ADC) of Formula (A):



(A),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted sulfurs are from cysteine residues (optionally C226 and/or C229) of the DLL3 antibody, and X represents the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

In another aspect, the present disclosure refers to a pharmaceutical composition comprising one or more of the ADCs disclosed herein and a pharmaceutically acceptable carrier; optionally the composition is characterized by a drug-to-antibody ratio ("DAR") of about 1 to about 8, about 1 to about 2, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2 to about 4, about 2, about 2.1, about 2.2, about 2.4, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9 or about 4.

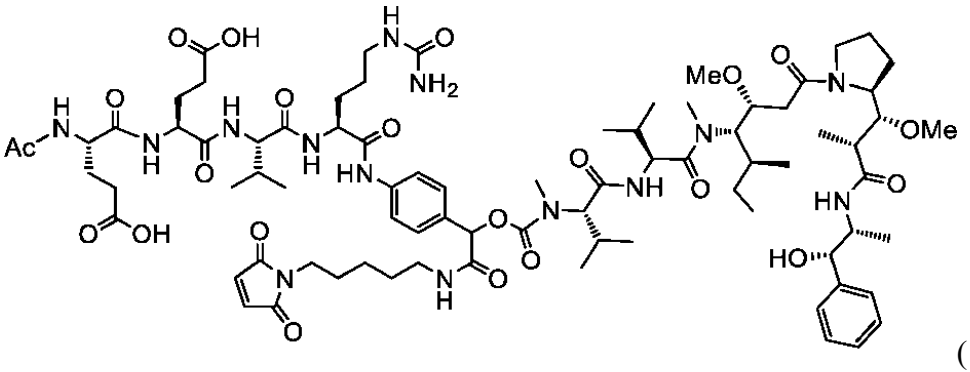
In another aspect, the present disclosure refers to use of the ADC disclosed herein in the manufacture of a medicament, wherein the medicament is used for modulating a DLL3 related immune response.

In another aspect, the present disclosure refers to use of the ADC disclosed herein in the manufacture of a medicament, wherein the medicament is used for treatment of a cancer, wherein the cancer is DLL3-positive or overexpressed.

In another aspect, the present disclosure refers to use of the ADC disclosed herein in the manufacture of a medicament for diagnosing or treating a DLL3 positive cancer.

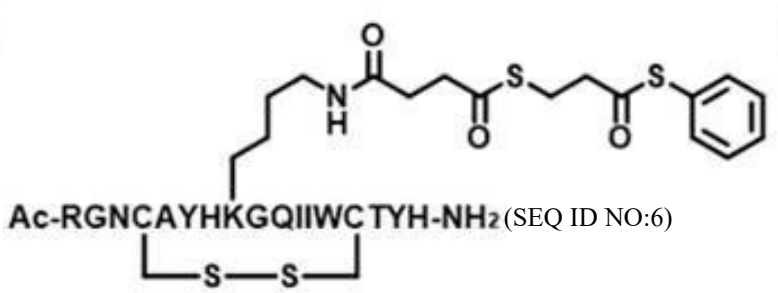
In another aspect, the present disclosure refers to the ADC disclosed herein for use in treating a DLL3 positive cancer.

In another aspect, the present disclosure refers to a method of making the ADC disclosed herein, comprising the steps of: (1) a) providing a solution comprising the DLL3 antibody; b) contacting the solution of a) with a reducing agent, optionally the reducing agent is tris(2 carboxyethyl)phosphine (TCEP); and c) contacting the solution of b) with a solution comprising Linker-Drug (1):



(1),

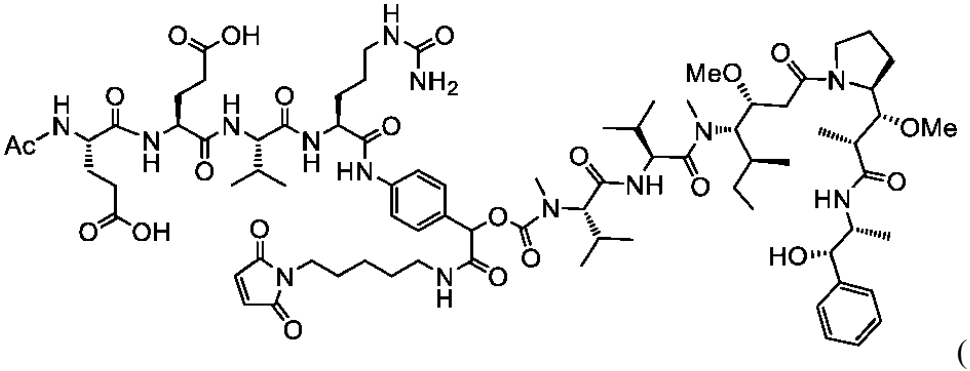
wherein the ADC is made, or (2) a) providing a solution comprising the DLL3 antibody; b) contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety, wherein the affinity peptide is a peptide of SEQ ID NO:6, and/or the affinity peptide conjugated to a thiophenol activation moiety is:



(SEQ ID NO:6)

; and

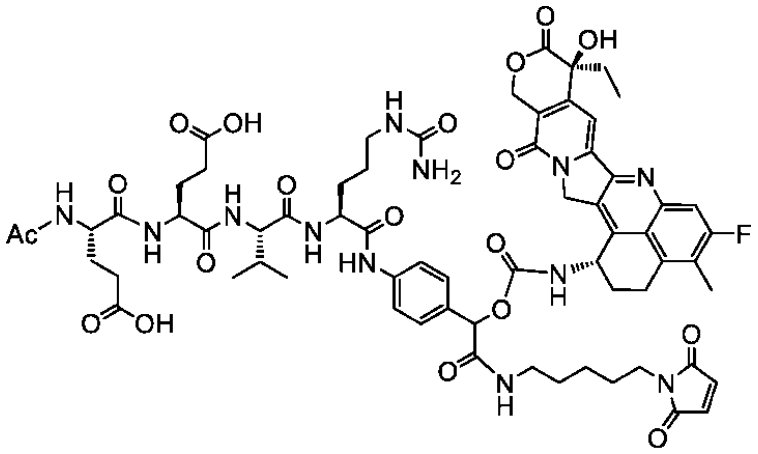
c) contacting the solution of b) with a solution comprising Linker-Drug (1):



(1),

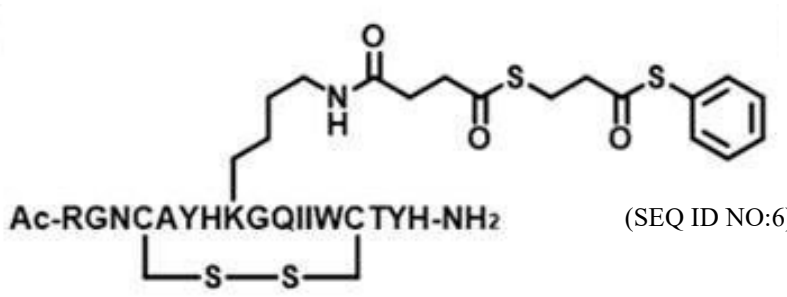
wherein the ADC is made.

In another aspect, the present disclosure refers to a method of making the ADC of any one of claims 1-6, comprising the steps of: (1) a) providing a solution comprising the DLL3 antibody; b) contacting the solution of a) with a reducing agent, optionally the reducing agent is tris(2 carboxyethyl)phosphine (TCEP); and c) contacting the solution of b) with a solution comprising Linker-Drug (2):



(2),

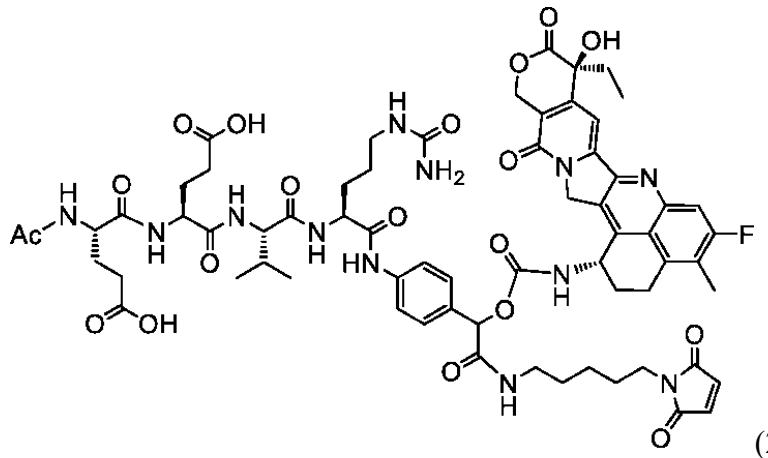
wherein the ADC is made, or (2) a) providing a solution comprising the DLL3 antibody; b) contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety, wherein the affinity peptide is a peptide of SEQ ID NO:6, and/or the affinity peptide conjugated to a thiophenol activation moiety is:



(SEQ ID NO:6)

; and

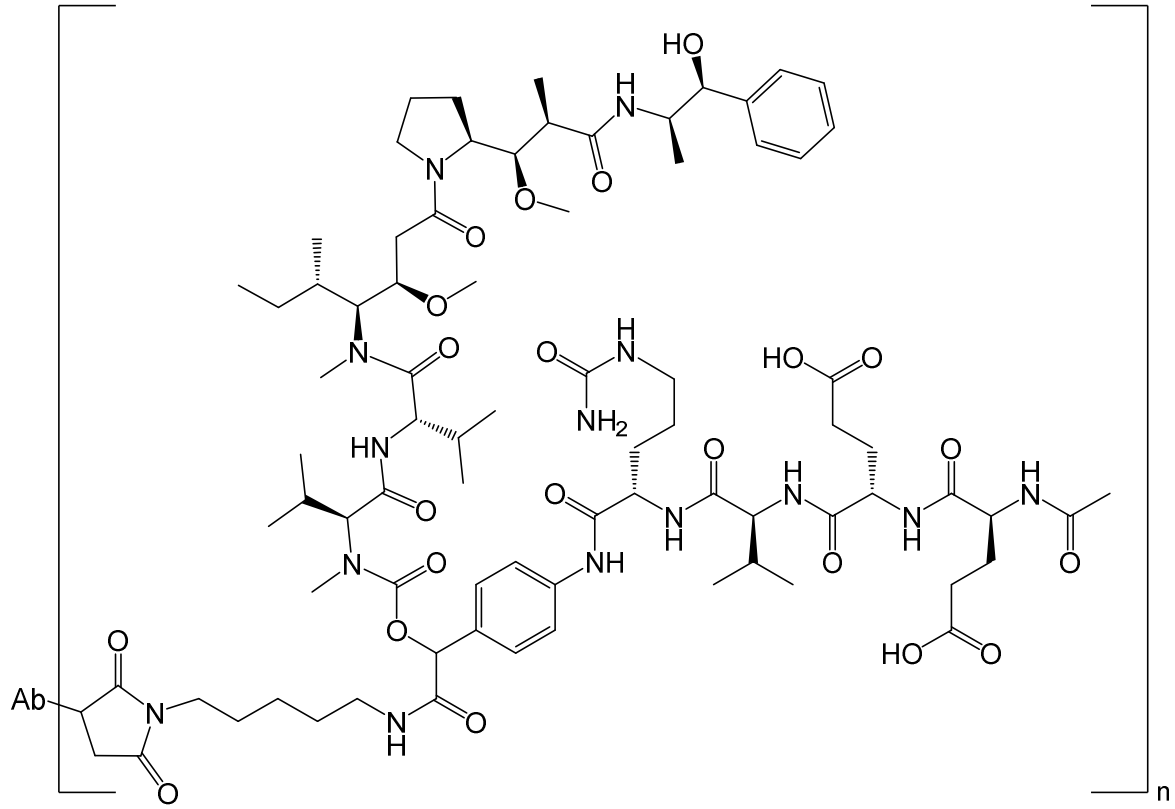
c) contacting the solution of b) with a solution comprising Linker-Drug (2):



(2),

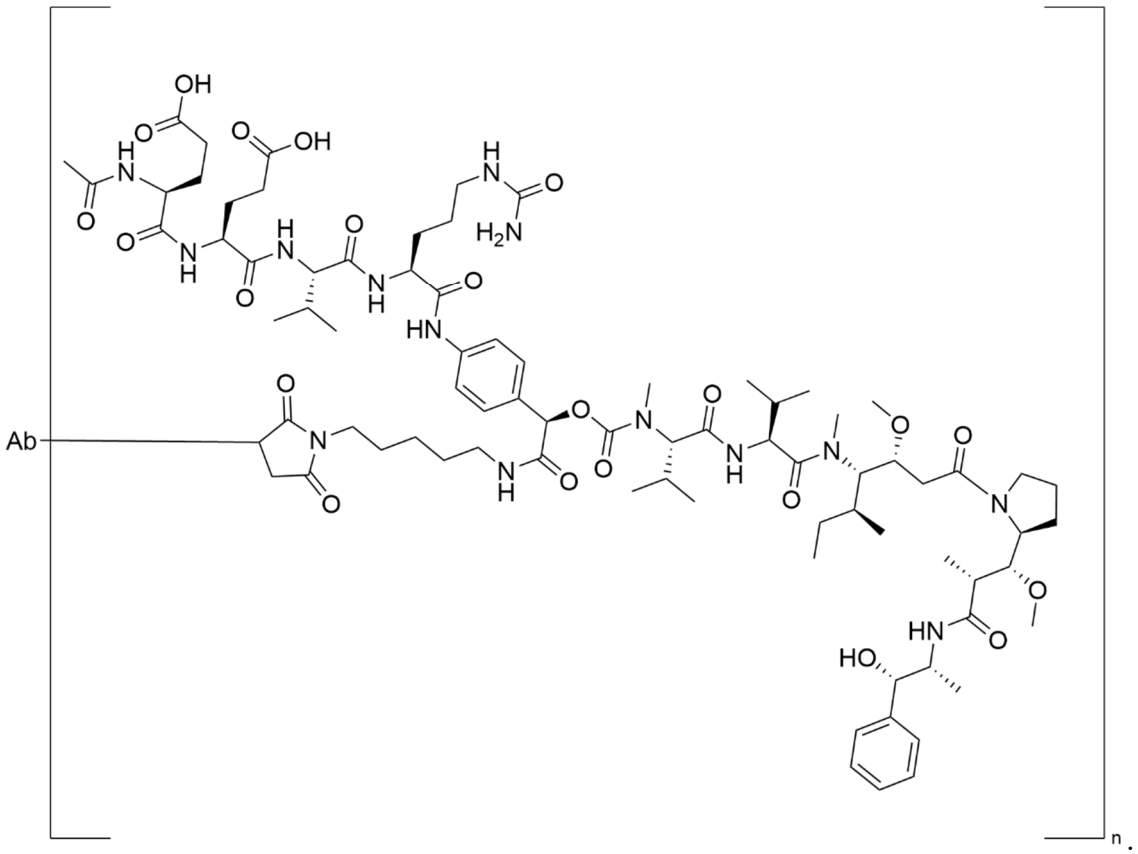
wherein the ADC is made.

In one example, provided herein is an antibody-drug conjugate (ADC) of Formula (I):



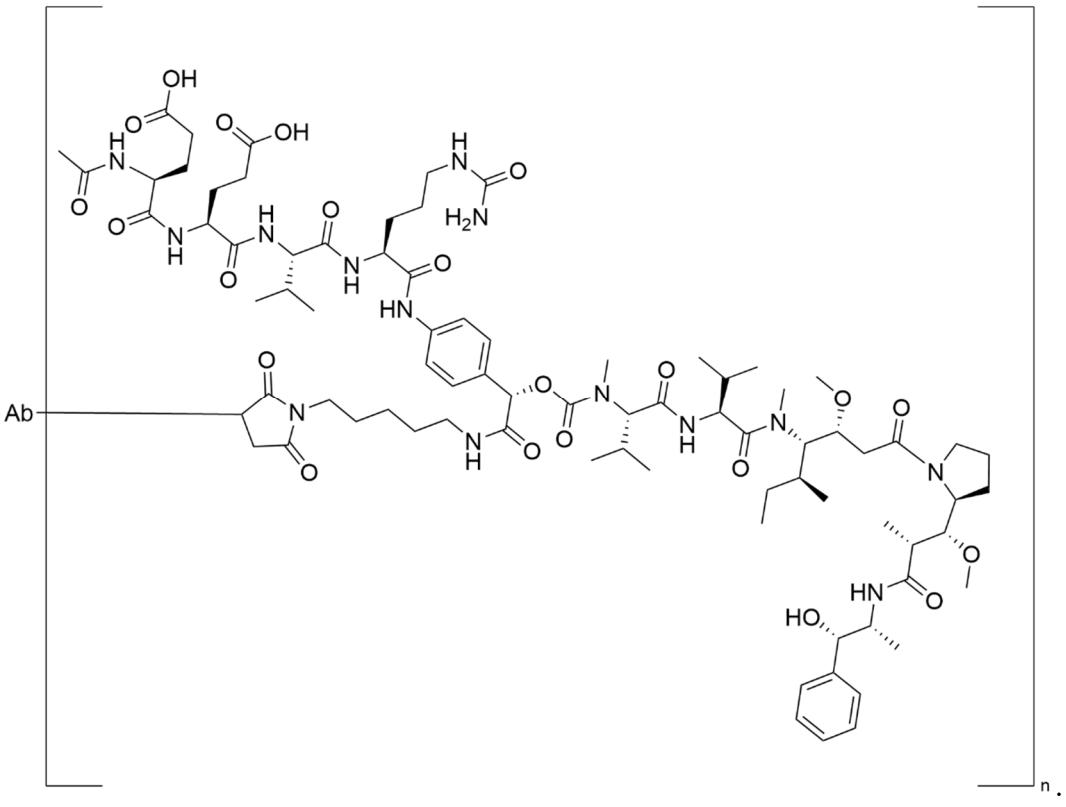
(I)

or a salt thereof, wherein *n* is an integer from 1 to 8, and Ab represents an antibody that binds to DLL3 ("DLL3 antibody"), wherein the point of attachment of the drug conjugate to the Ab is through a cysteine or a lysine residue of the DLL3 antibody. In certain examples, *n* is 2. In certain examples, *n* is 4. In some examples, the ADC is of Formula (I-A) or a salt thereof.



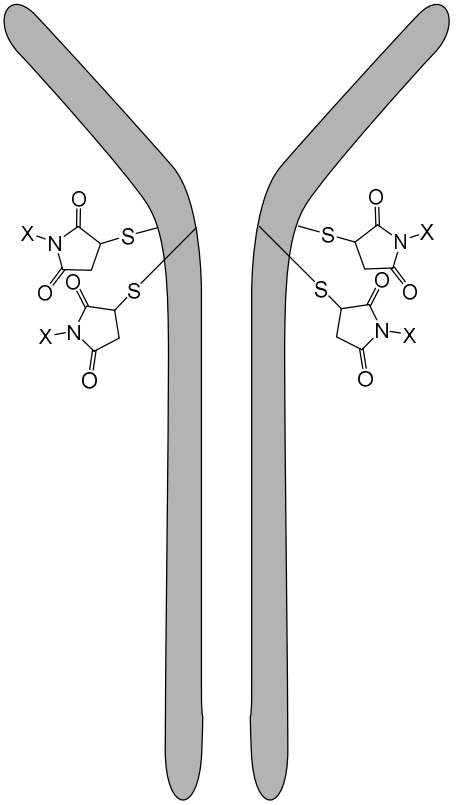
(I-A)

In other examples, the ADC is of Formula (I-B) or a salt thereof:



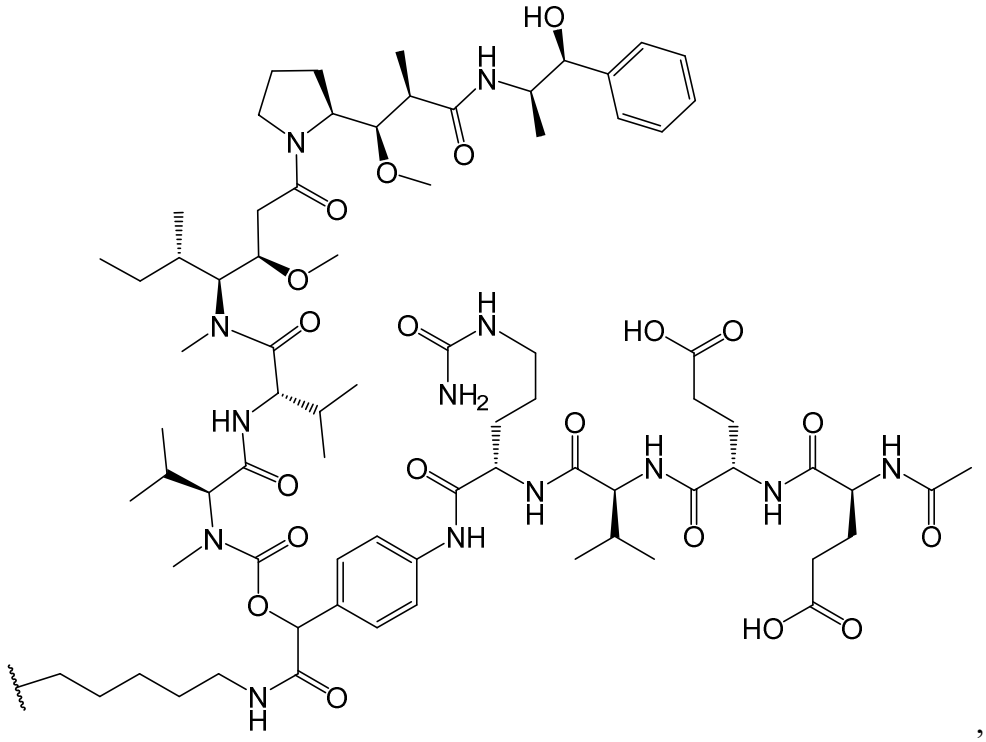
(I-B)

[0007] In another example, provided herein is an ADC of Formula (A):



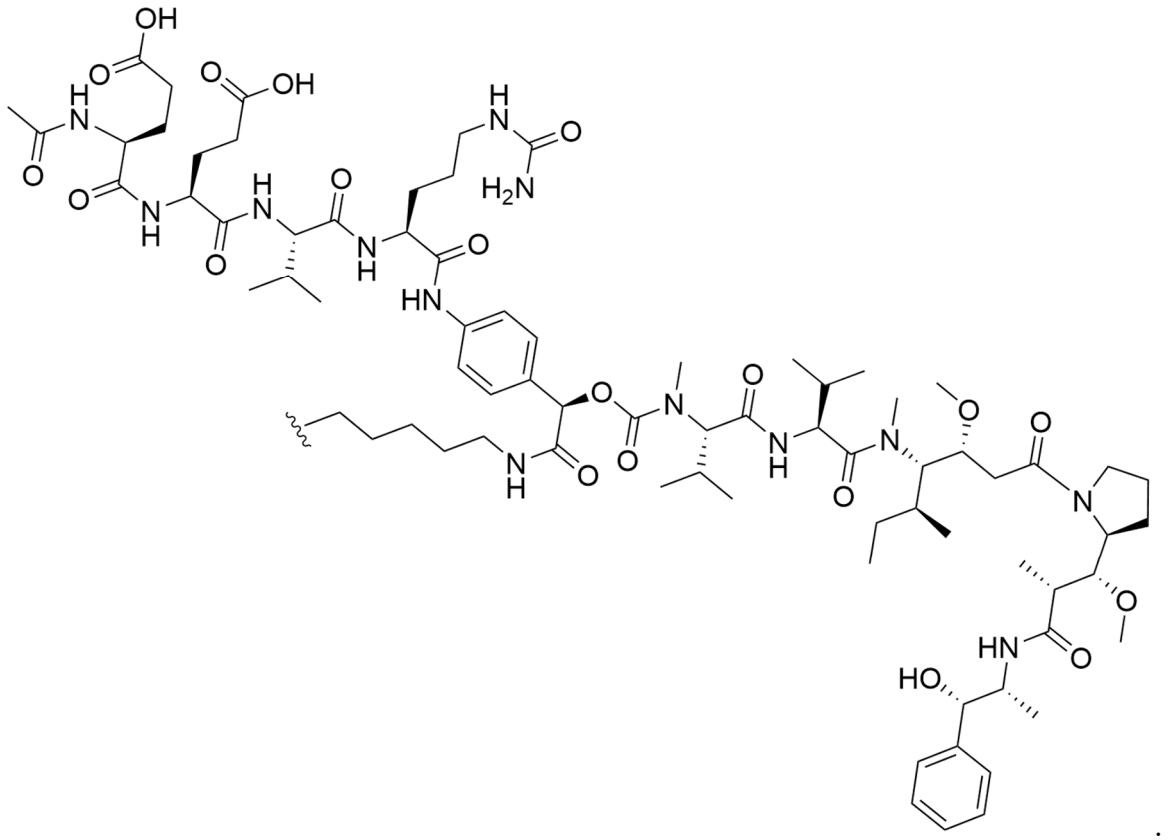
(A),

wherein each thick shaded line represents a chain of the DLL3 antibody, the depicted sulfurs are from cysteine residues of the DLL3 antibody, and X represents the structure below or a salt thereof:

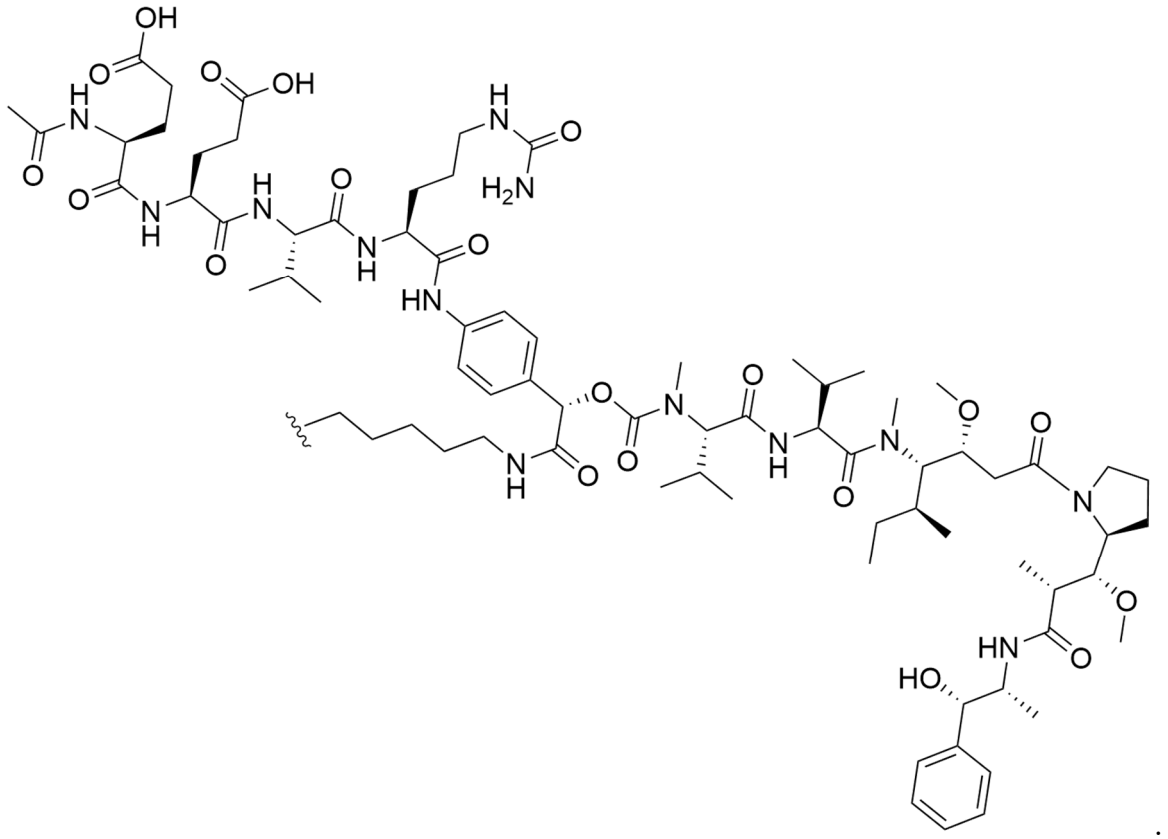


,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some examples, the cysteine residues are in the hinge region of the DLL3 antibody. In further examples, the cysteine residues form an interchain disulfide bridge in a DLL3 antibody not conjugated directly or indirectly to X, for example, between two heavy chains, between a heavy chain and a light chain, between two VHH chains, or between two VHH-Fc chains. In yet further examples, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively). In some examples, X represents the structure below or a salt thereof:

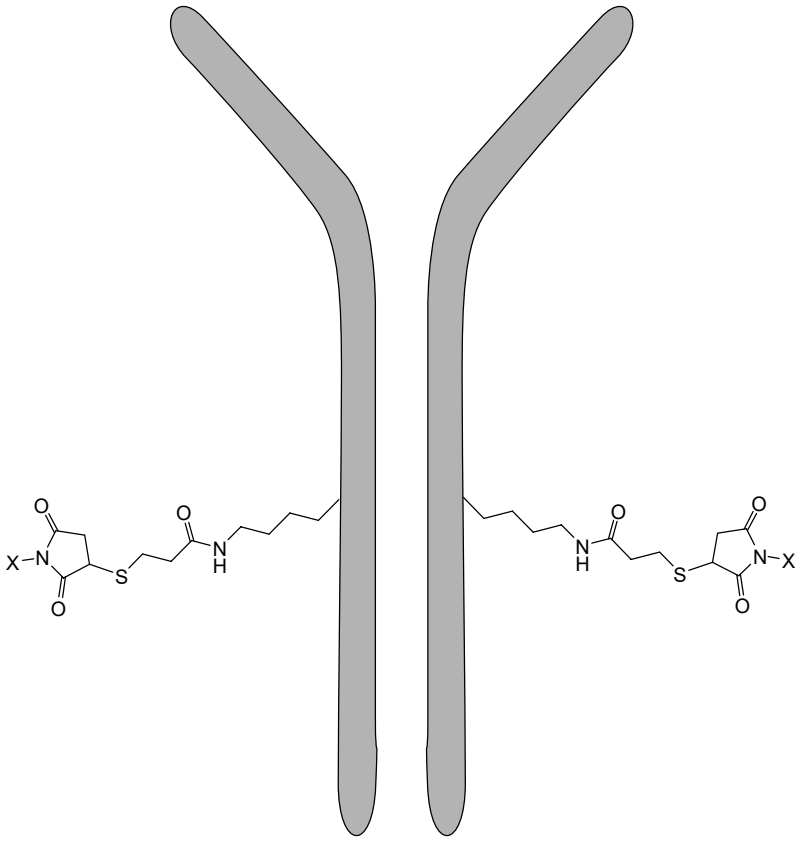


In other examples, X represents the structure below or a salt thereof:



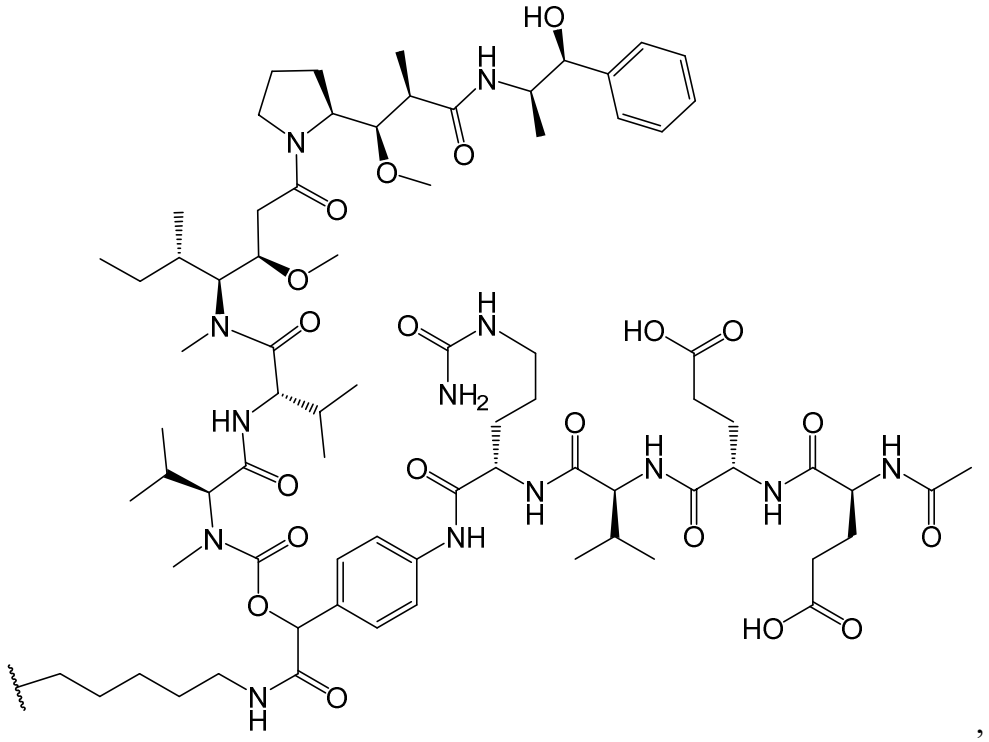
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[0008] In another example, provided herein is an ADC of Formula (B):



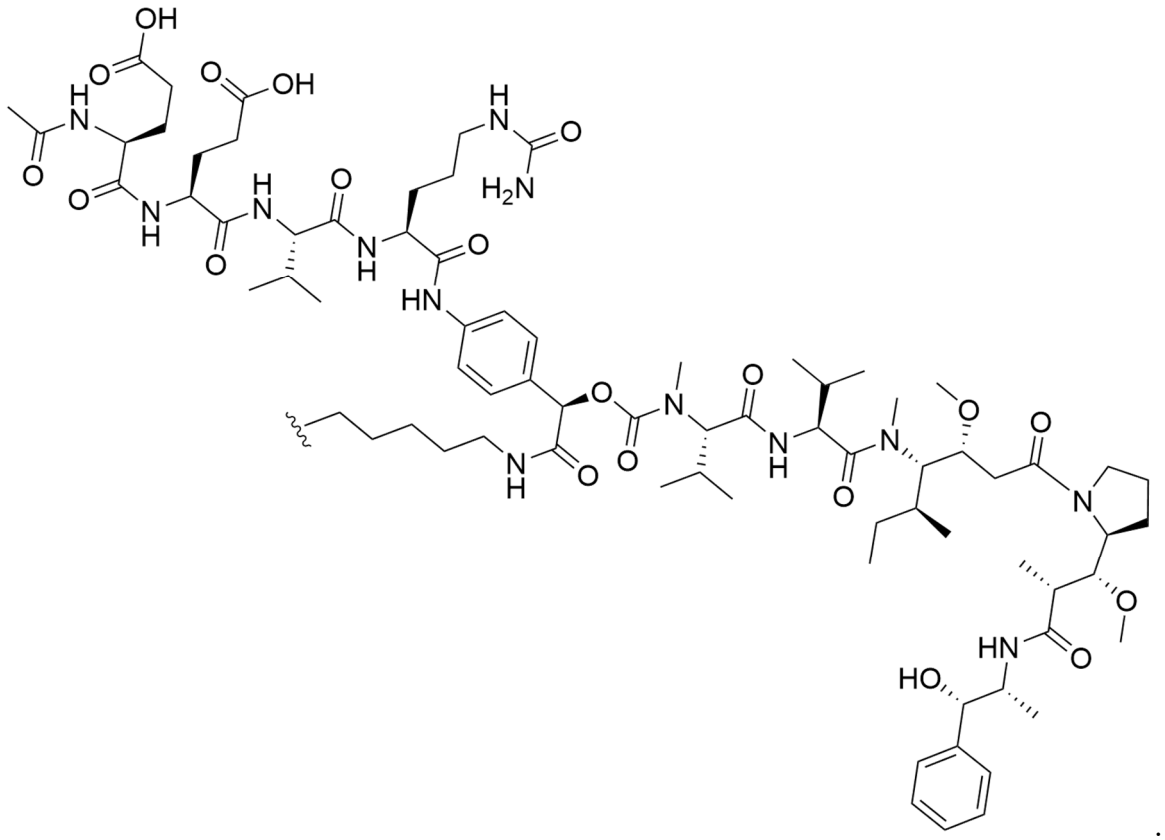
(B),

wherein each thick shaded line represents a chain of the DLL3 antibody, the depicted $CH_2CH_2CH_2CH_2NH$ moiety is from a lysine residue of the CH2 domain of the DLL3 antibody, and X represents the structure below:

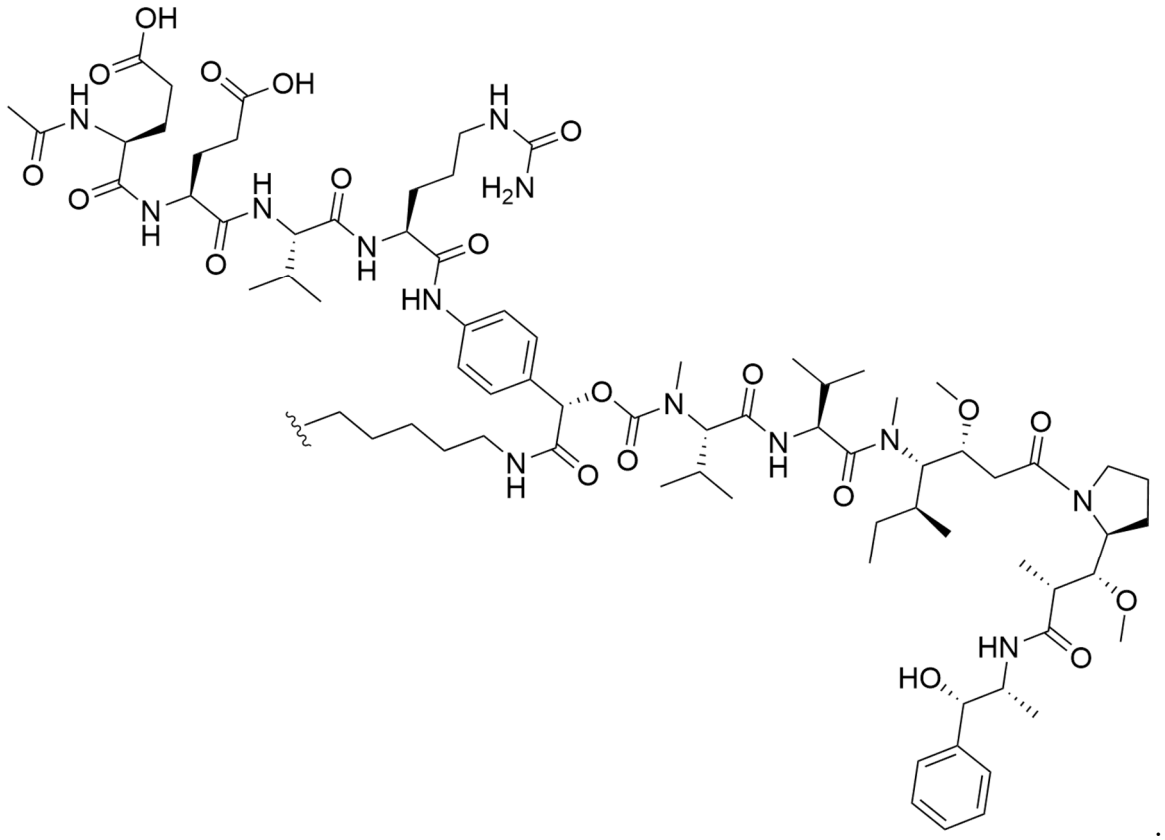


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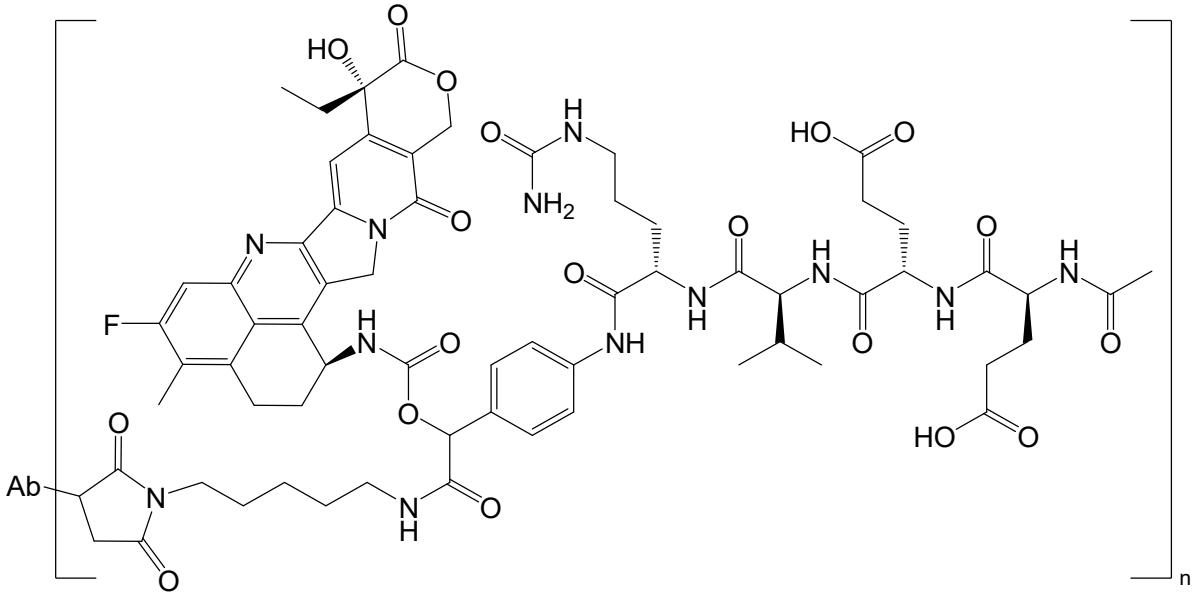
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some examples, the lysine residue is at any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In some examples, X represents the structure below or a salt thereof:



In other examples, X represents the structure below or a salt thereof:

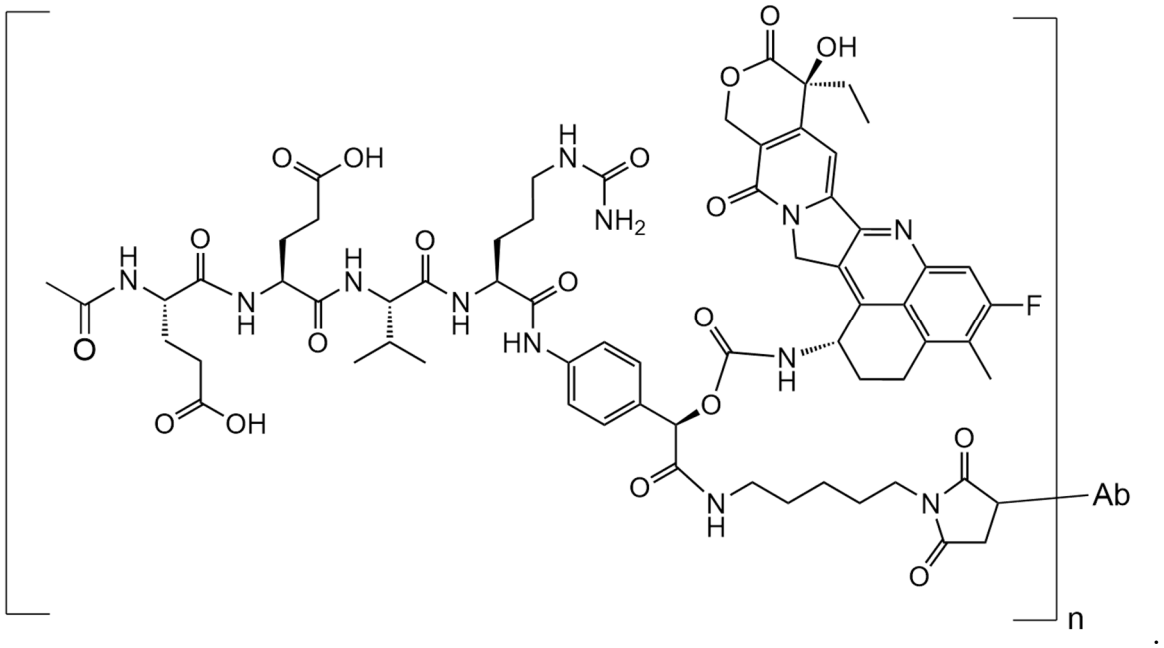


[0009] In another example, provided herein an ADC of Formula (II):



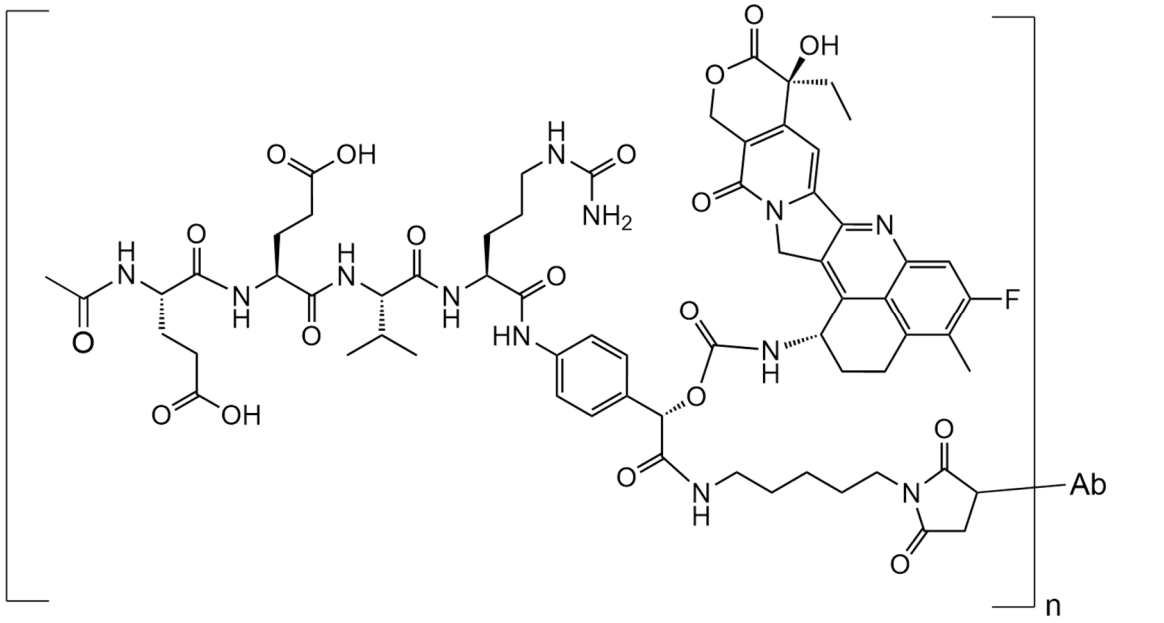
(II)

wherein *n* is an integer from 1 to 8, and Ab represents a DLL3 antibody. In some examples, the point of attachment of the drug conjugate to the Ab is through a cysteine or a lysine residue of the DLL3 antibody. In certain examples, *n* is 2. In certain examples, *n* is 4. In some examples, the ADC is of Formula (II-A) or a salt thereof:



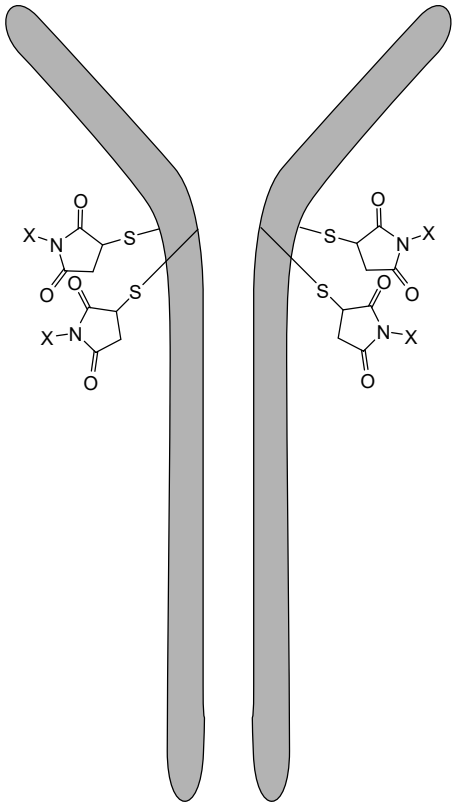
(II-A)

In other examples, the ADC is of Formula (II-B) or a salt thereof:



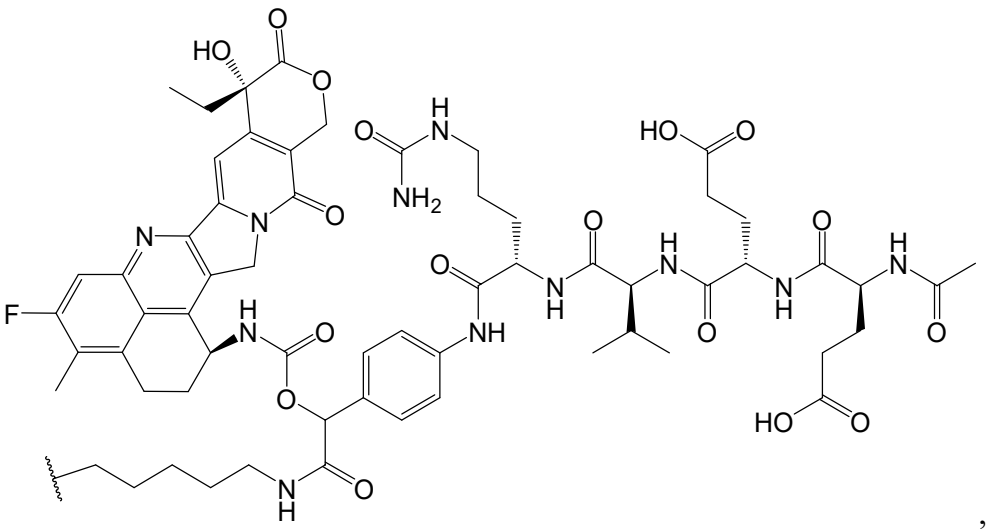
(II-B)

[0010] In another example, provided herein is an ADC of Formula (A):



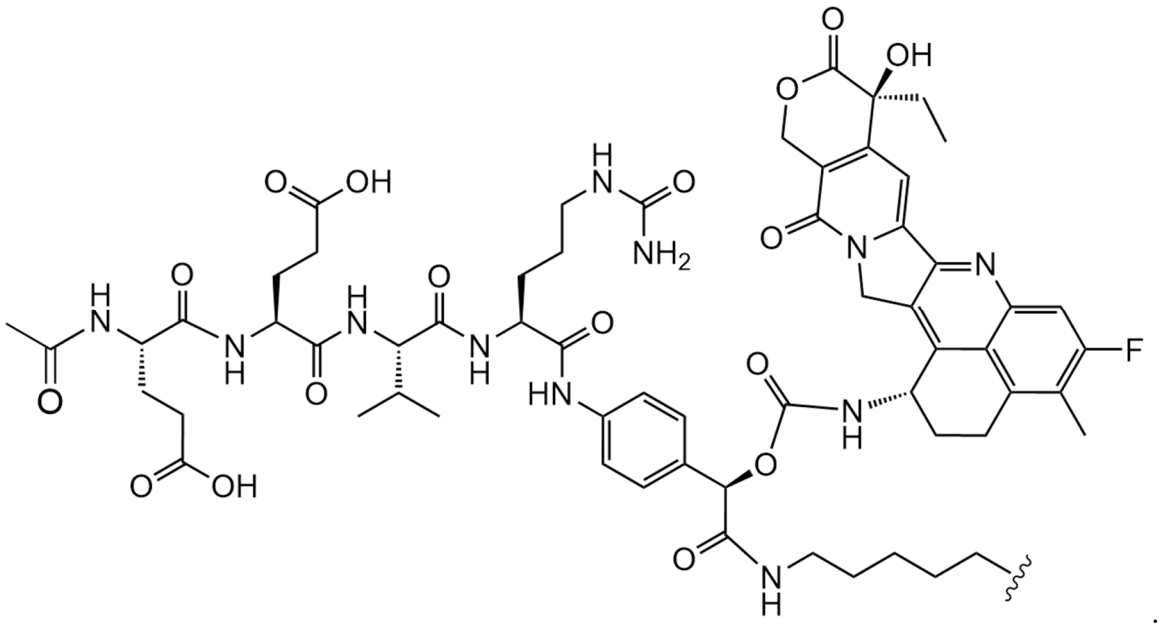
(A),

wherein each thick shaded line represents a chain of a DLL3 antibody, the depicted sulfurs are from cysteine residues of the DLL3 antibody, and X represents the structure below:



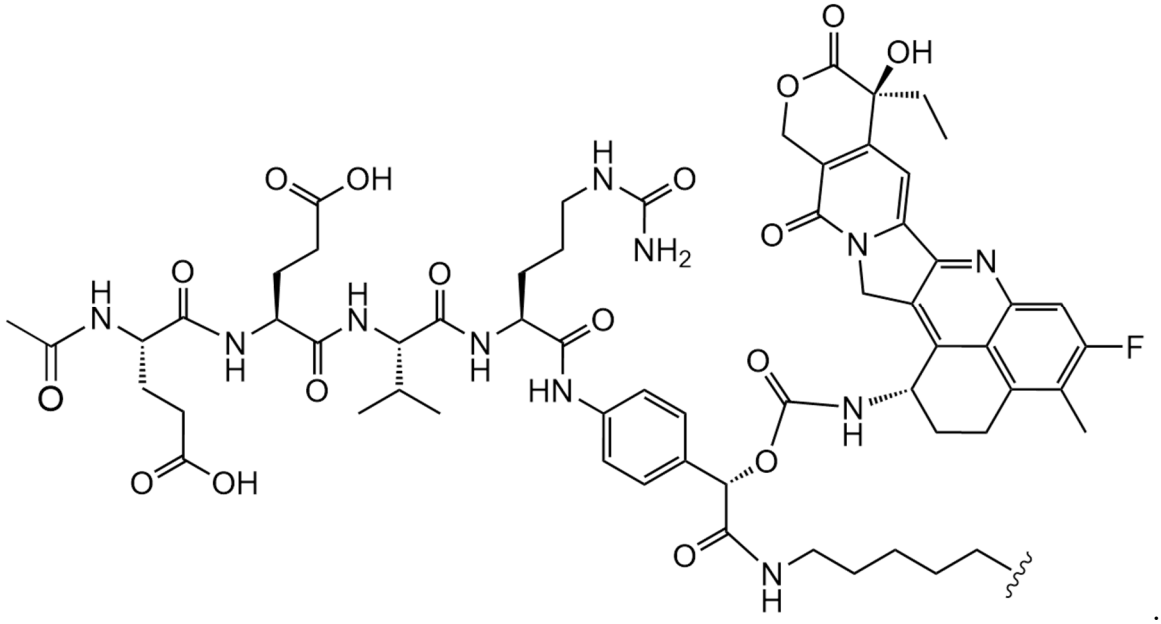
,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some examples, the cysteine residues are in the hinge region of the DLL3 antibody. In further examples, the cysteine residues form an interchain disulfide bridge in a DLL3 antibody not conjugated directly or indirectly to X, for example, between two heavy chains, between a heavy chain and a light chain, between two VHH chains, or between two VHH-Fc chains. In yet further examples, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively). In some examples, X represents the structure below or a salt thereof:

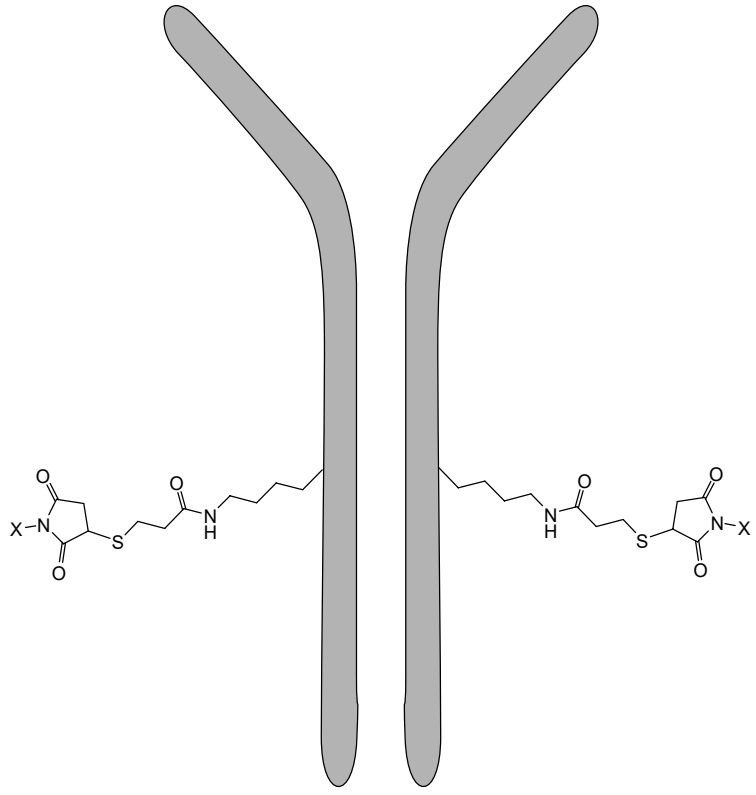


.

In some examples, X represents the structure below or a salt thereof:

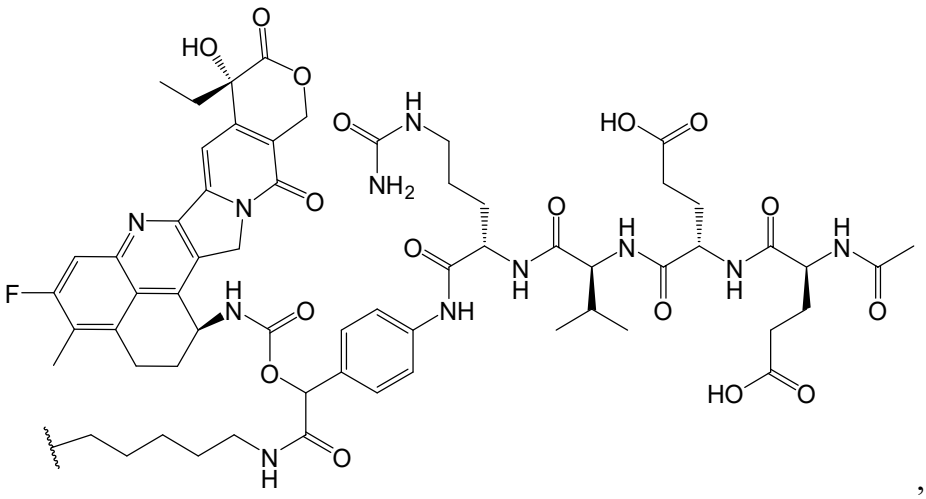


[0011] In another example, provided herein is an ADC of Formula (B):



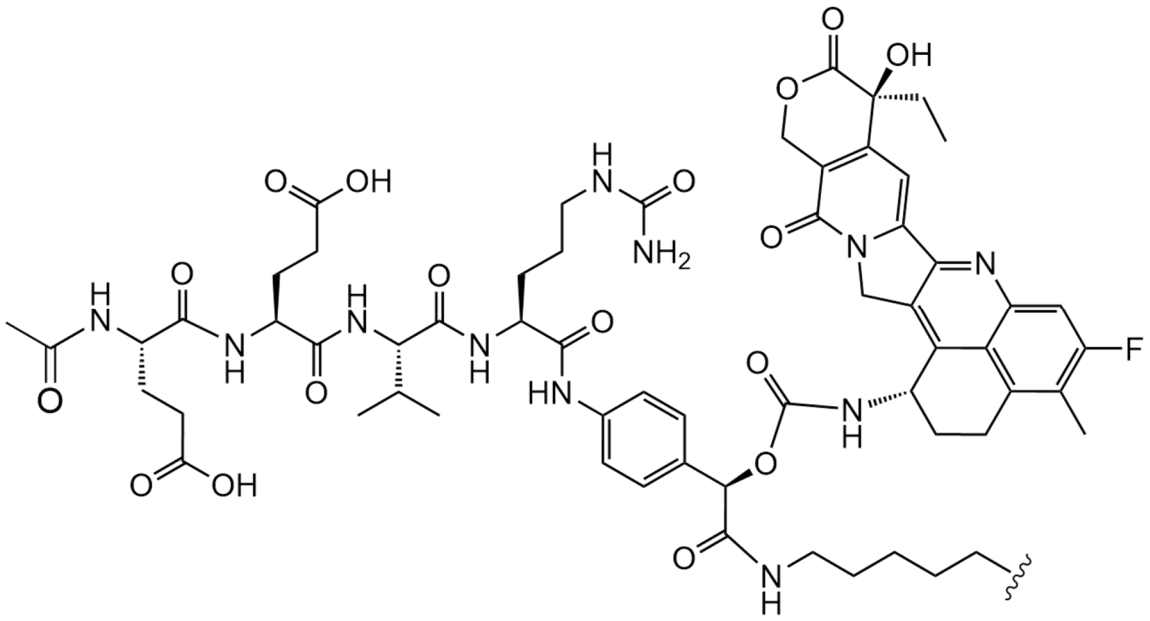
(B),

wherein each thick shaded line represents a chain of a DLL3 antibody, the depicted $CH_2CH_2CH_2CH_2NH$ moiety is from a lysine residue of the CH2 domain of the DLL3 antibody, and X represents the structure below:



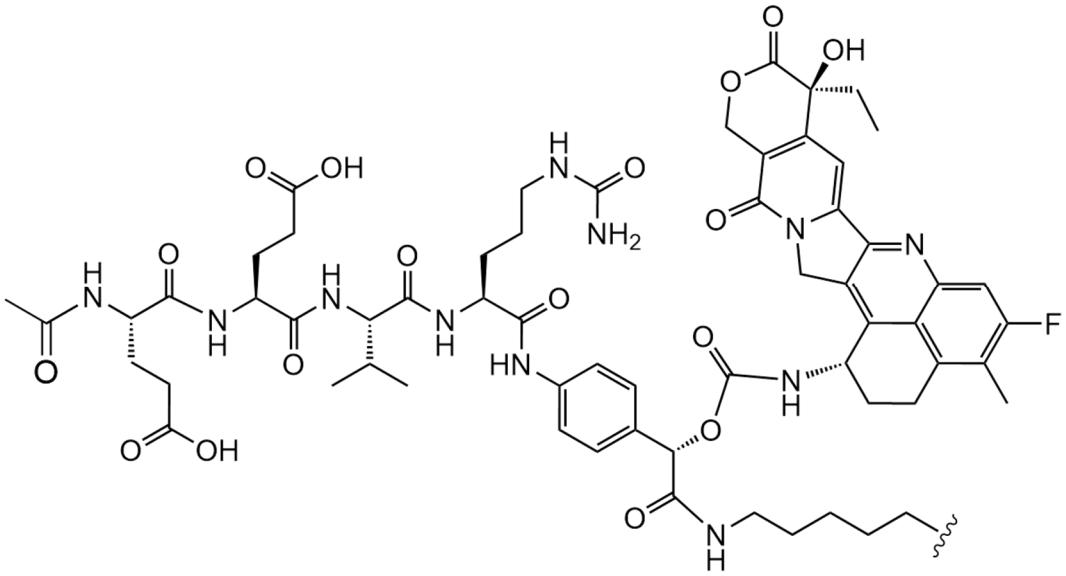
,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some examples, the lysine residue is at any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In some examples, X represents the structure below or a salt thereof:



.

In some examples, X represents the structure below or a salt thereof:



[0012] In some examples, the DLL3 antibody as used herein comprises a VHH. In further examples, the DLL3 antibody as used herein comprises a VHH-Fc fusion, for example, as depicted in **Figure 1A**. Additionally or alternatively, the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain variable domain (VH), wherein the VH comprises an amino acid sequence as set forth in SEQ ID NO:14, 15 or 16, for example, as those disclosed in **Table 1** and **Table 2**. In further examples, the DLL3 antibody comprises a VH as set forth in SEQ ID NO:14, 15 or 16. In yet further examples, the DLL3 antibody comprises a homodimer of a VHH-Fc chain as set forth in any one of SEQ ID NO:4, 5, or 10.

[0013] In another example, provided herein is a pharmaceutical composition comprising an ADC described herein, and a pharmaceutically acceptable carrier. In some examples, the pharmaceutical composition has a drug-to-antibody ratio (DAR) of about 1.5 to about 2.5. In further examples, the pharmaceutical composition has a DAR of about 1.9 to 2.1. In some examples, the pharmaceutical composition has a DAR of about 1 to about 4, for example, about 1 to about 3, about 1 to about 2, about 2 to about 4, or about 3 to about 4. In further examples, the pharmaceutical composition has a DAR of about 3.5 to about 4.0, such as about 3.6, about 3.7, about 3.8, about 3.9, or about 4.0.

[0014] In another example, provided herein is a method of modulating a DLL3 related immune response in a subject, comprising administering to the subject an ADC or a pharmaceutical composition described herein such that an immune response is modulated in the subject.

[0015] In another example, provided herein is a method for treating a cancer in a subject, comprising administering to the subject an ADC or a pharmaceutical composition described herein, wherein the cancer is DLL3-positive or overexpressed. In certain examples, the cancer is selected from lung cancer and neuroendocrine carcinoma. In certain examples, the cancer is small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC) or colorectal cancer.

[0016] In still further examples, provided herein are methods of making an ADC described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **Figure 1A** shows an antibody comprising VHH fused to an Fc. Two interchain disulfide bonds may be present between hinge regions of the two chains.

[0018] **Figure 1B** provides exemplary routes of synthesis for ADCs, wherein the antibody may be a conventional antibody comprising two heavy chains and two light chains, or a VHH-Fc fusion as illustrated in **Figure 1A**.

[0019] **Figure 2** provides an exemplary RP-HPLC analysis result of Linker-Drug (2-A), Linker-Drug (2-B), and Linker-Drug (2) (mixture of Linker-Drug (2-A) and Linker-Drug (2-B)), further described in Example 7.

[0020] **Figure 3** illustrates Linker-Drug (2-B).

[0021] **Figures 4A-4D** show exemplary results for mAb300, mAb301, mAb302, and relevant ADCs from developability assays, further described in **Example 15**. **Figure 4A** compares mAb302, ADC-013 and ADC-014 using size exclusion chromatography (SEC). **Figure 4B** compares mAb300 and mAb302 using hydrophobic interaction chromatography (HIC). **Figure 4C** compares mAb300, ADC-009, and ADC-010 using HIC. **Figure 4D** compares mAb302, ADC-013, and ADC-014 using HIC.

[0022] **Figure 5** shows exemplary results of assessing the binding characteristics of mAb300, mAb301 and mAb302 via a Biacore™ assay, further described in Example 16.

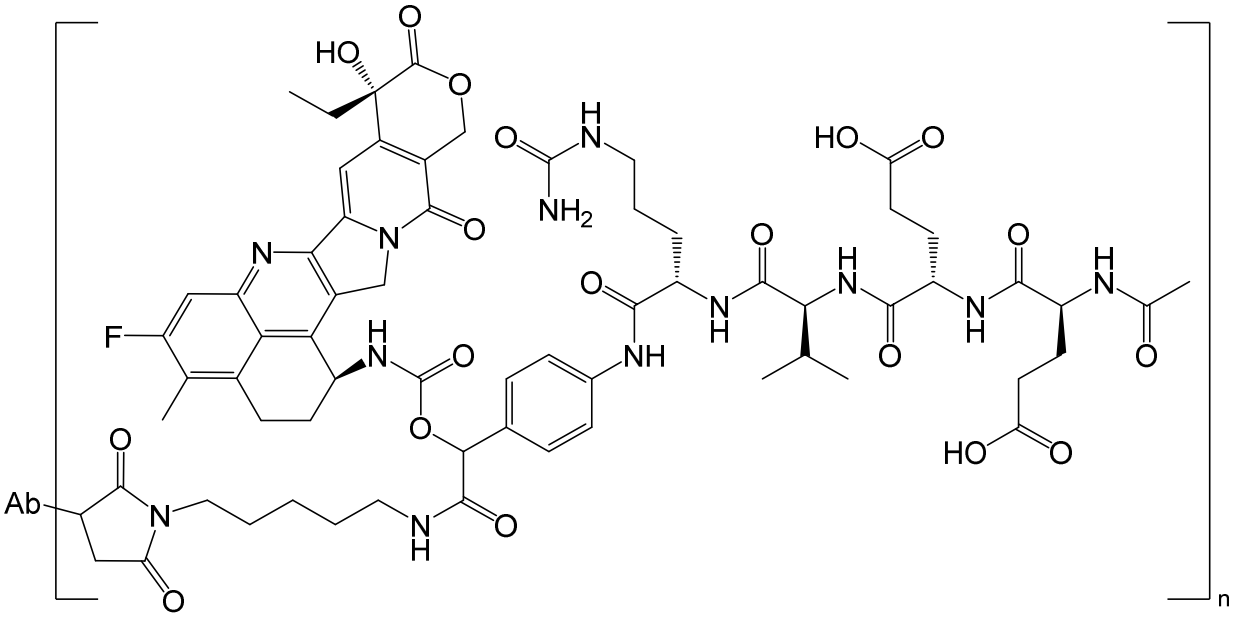
[0023] **Figure 6** shows exemplary results of assessing drug-to-antibody ratios (DARs) of ADC-015 (marked as mAb301-Linker-Drug (2) (Target DAR = 2.0)), ADC-016 (marked as mAb301-Linker-Drug (2) (Target DAR = 4.0)), and ADC-017 (marked as mAb301-Linker-Drug (1) (Target DAR = 4.0)), further described in Example 17.

[0024] **Figures 7A-7R** provide exemplary SEC-HPLC analysis results of the produced ADC-009-M (mAb300, Linker-Drug (2), DAR2, **Figure 7A**), ADC-009-A (mAb300, Linker-Drug (2-A), DAR2, **Figure 7B**), ADC-009-B (mAb300, Linker-Drug (2-B), DAR2, **Figure 7C**), ADC-015-M (mAb301, Linker-Drug (2), DAR2, **Figure 7D**), ADC-015-A (mAb301,

CLAIMS

What is claimed is:

1. An antibody-drug conjugate (ADC) of Formula (II):



(II)

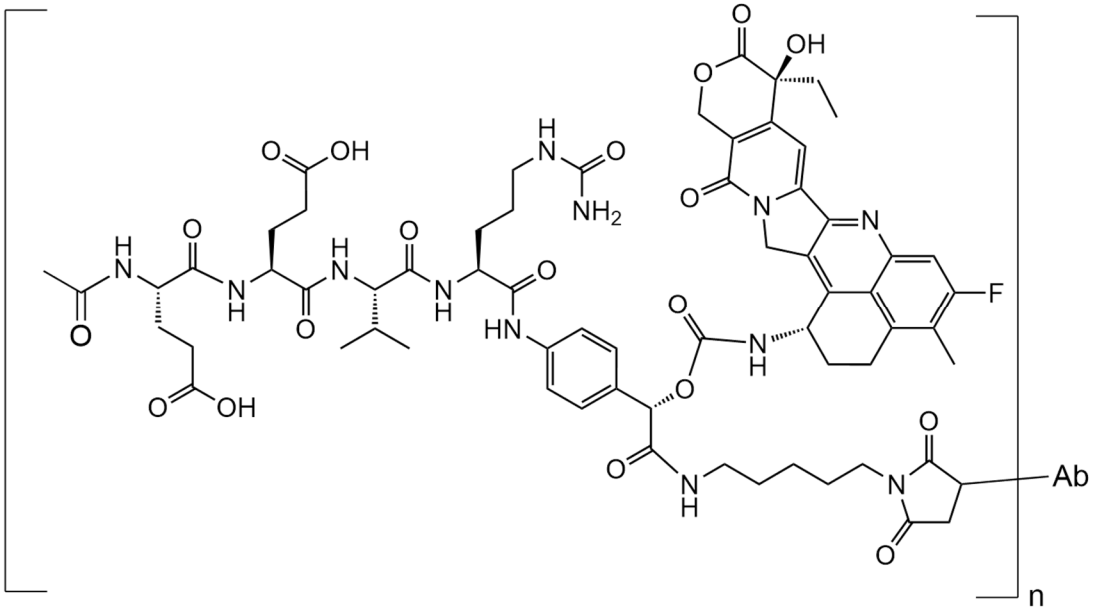
or a salt thereof, wherein:

n is an integer from 1 to 8, and

Ab represents an antibody that binds to DLL3 ("DLL3 antibody").

2. The ADC of claim 1, wherein the ADC is of

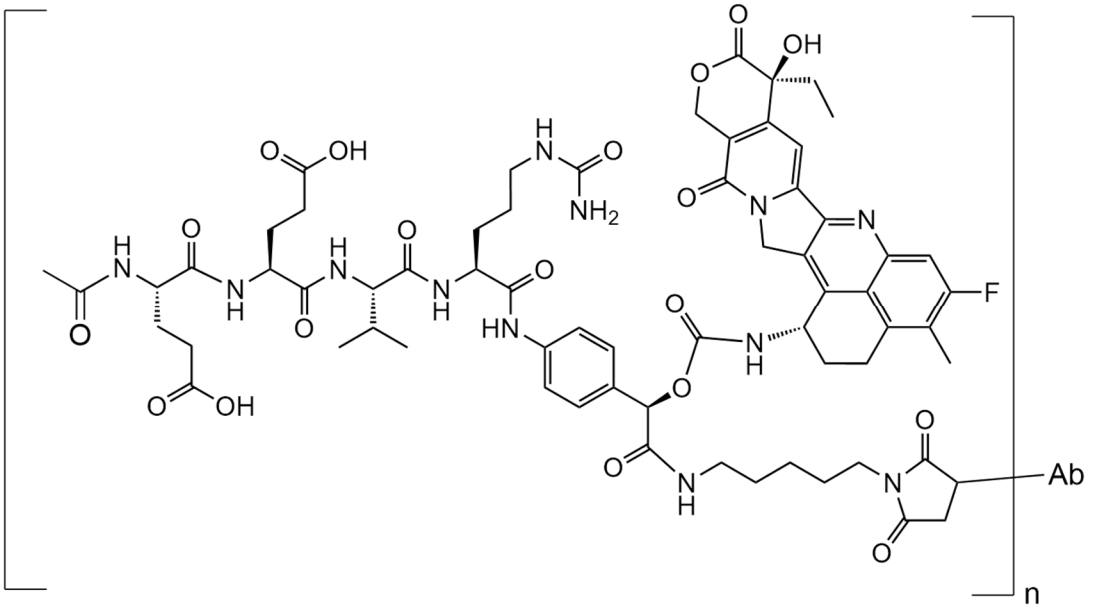
(1) Formula (II-B)



(II-B)

or a salt thereof,

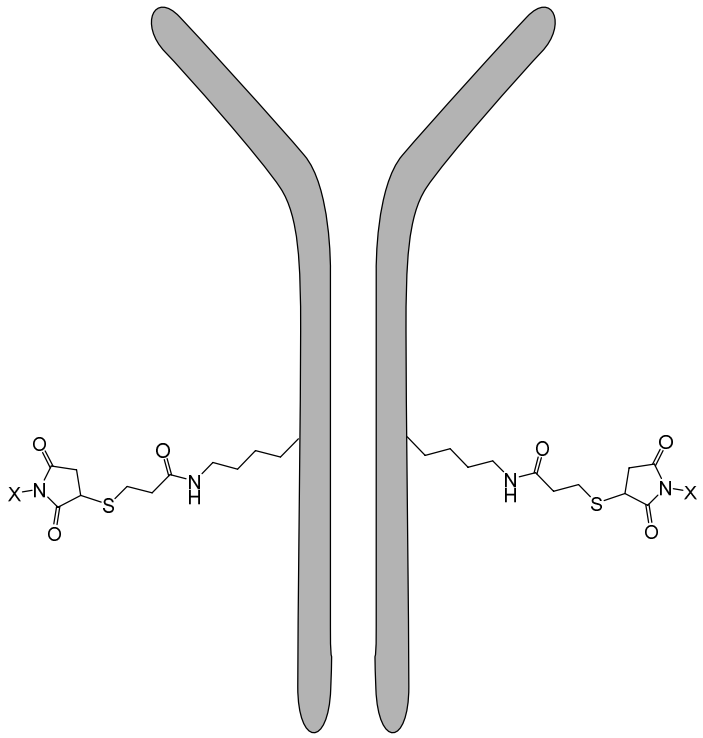
(2) Formula (II-A)



(II-A)

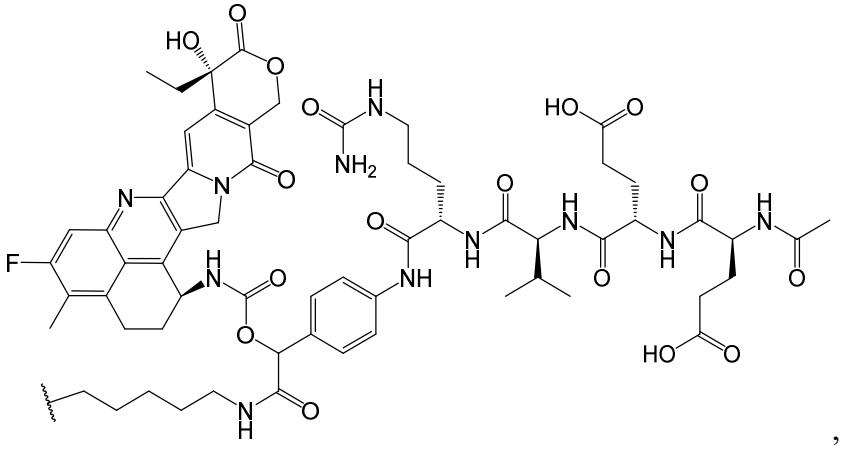
or a salt thereof.

3. An antibody-drug conjugate (ADC) of Formula (B):



(B),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted $CH_2CH_2CH_2CH_2NH$ moiety is from a lysine residue (optionally any one or more of K246, K248, K288, K290, or K317 according to the EU numbering) of the CH2 domain of the DLL3 antibody, and X represents the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

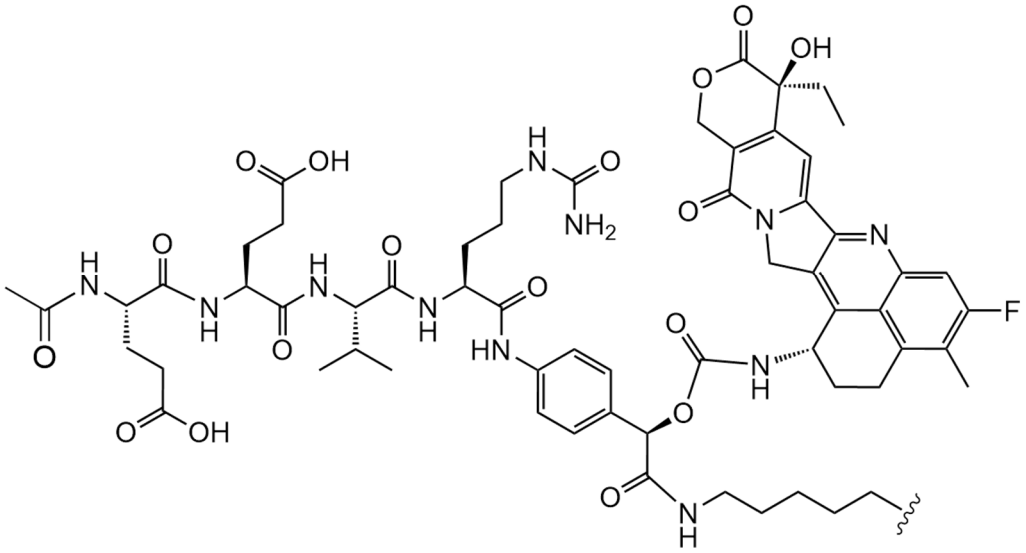
4. The ADC of claim 3, wherein

(1) the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:5 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:5;

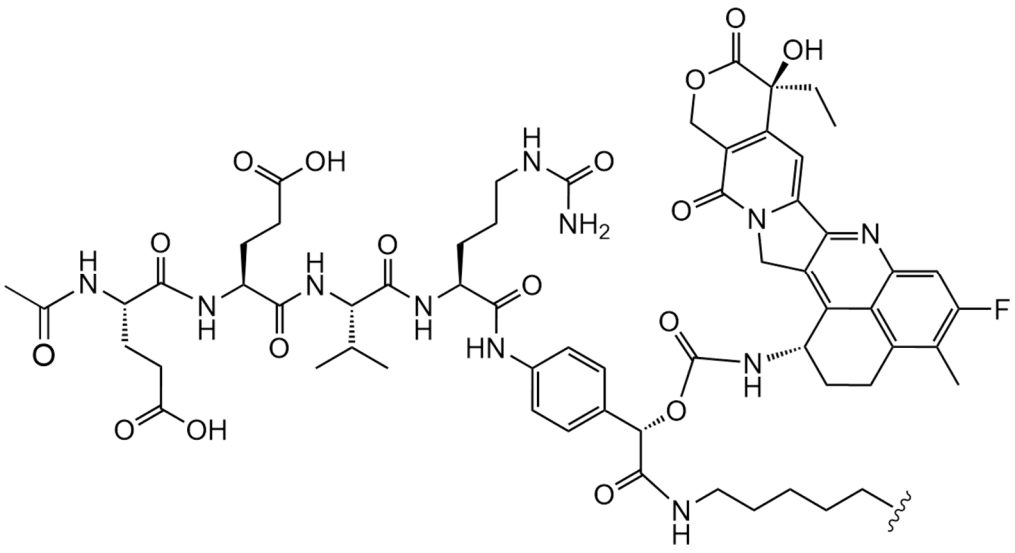
(2) The ADC of claim 4, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:4 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:4;

(3) The ADC of claim 4, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:10 and wherein the lysine residue is the 152nd amino acid residue of SEQ ID NO:10.

5. The ADC of claim 3, wherein X represents:



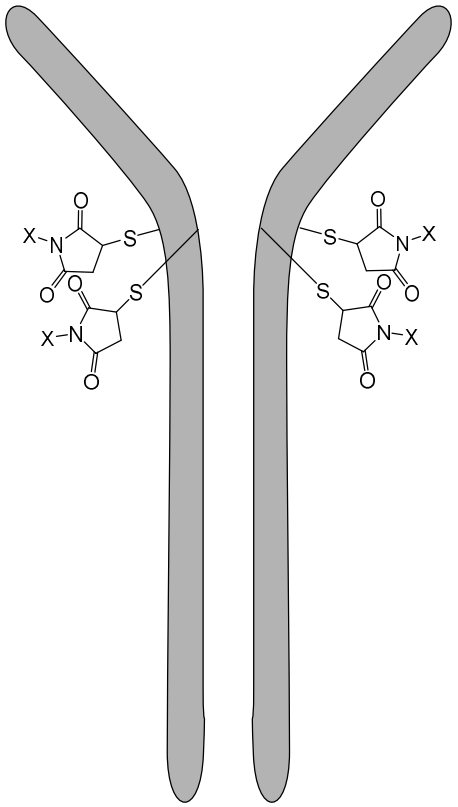
, or



,

or a salt of each thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

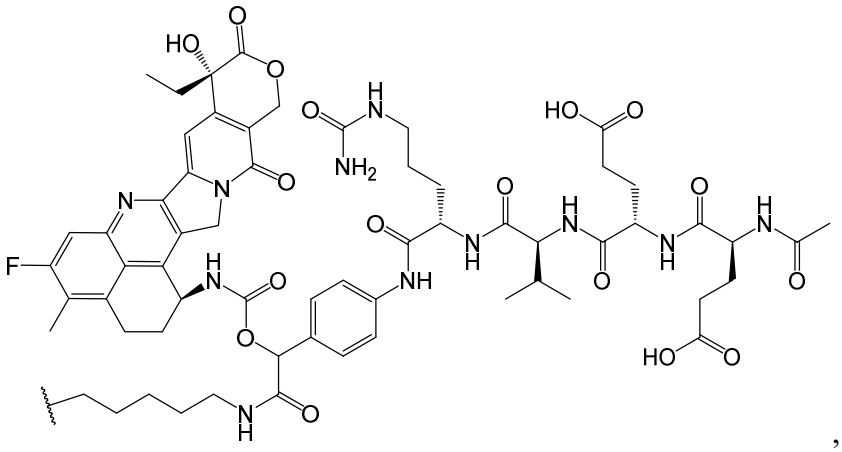
6. An antibody-drug conjugate (ADC) of Formula (A):



(A),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted sulfurs are from cysteine residues (optionally C226 and/or C229) of the DLL3 antibody, and X represents

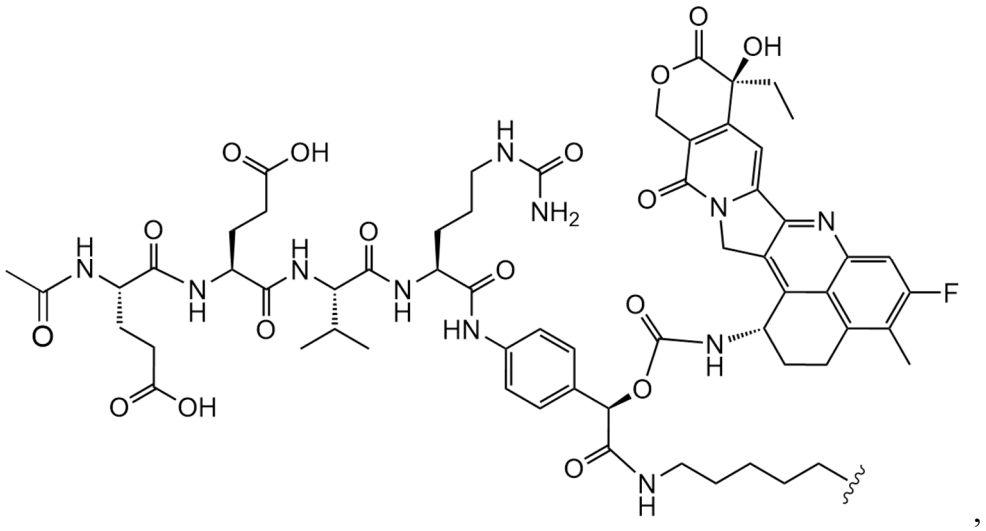
(i) the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A);

(ii) the structure below or a salt thereof:

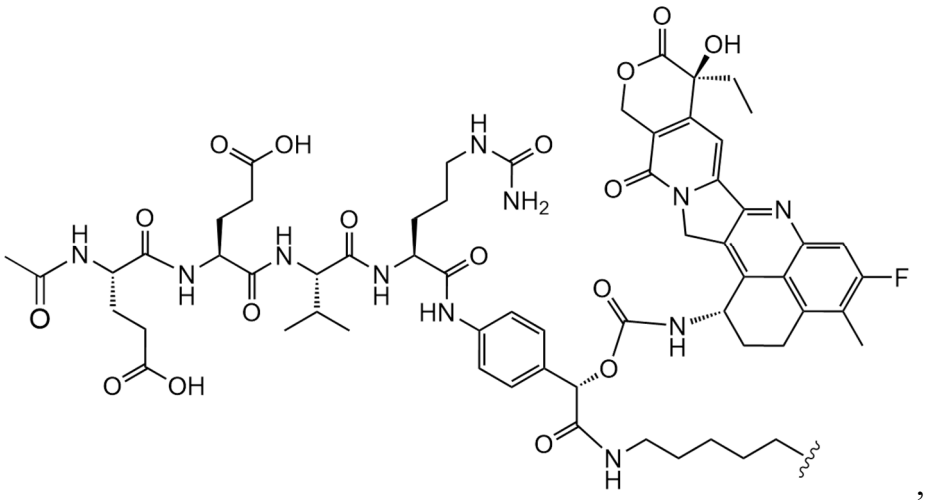


,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A),

or

(iii) the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

7. The ADC of any of claims 1-6, wherein:

(1) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:15;

(2) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:15, wherein the complementarity determining regions (CDRs) are defined according to the Exemplary, IMGT, Kabat, Chothia, Contact, or AbM numbering system;

(3) the DLL3 antibody comprises a VHH comprising:

(i) a CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:3;

(ii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:20, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:21, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:22;

(iii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:23, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(iv) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:24, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:25, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:26;

(v) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:27, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:56, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:29; or

(vi) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:30, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(4) the DLL3 antibody comprises a VHH comprising the amino acid sequence as set forth in SEQ ID NO:15;

(5) the DLL3 antibody further comprises a human IgG constant domain;

(6) the DLL3 antibody further comprises a human IgG1 constant domain;

(7) the DLL3 antibody further comprises an Fc fragment;

(8) the DLL3 antibody further comprises an Fc fragment that comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17;

(9) the DLL3 antibody comprises at least one VHH single domain;

(10) the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment; and/or

(11) the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:5.

8. The ADC of any of claims 1-6, wherein:

(1) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14;

(2) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14, wherein the complementarity determining regions (CDRs) are defined according to the Exemplary, IMGT, Kabat, Chothia, Contact, or AbM numbering system;

(3) the DLL3 antibody comprises a VHH comprising:

(i) a CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:3;

(ii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:20, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:21, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:22;

(iii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:23, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(iv) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:24, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:25, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:26;

(v) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:27, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:28, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:29; or

(vi) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:30, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(4) the DLL3 antibody comprises a VHH comprising the amino acid sequence as set forth in SEQ ID NO:14;

(5) the DLL3 antibody further comprises a human IgG constant domain;

(6) the DLL3 antibody further comprises a human IgG1 constant domain;

(7) the DLL3 antibody further comprises an Fc fragment;

(8) the DLL3 antibody further comprises an Fc fragment that comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17;

(9) the DLL3 antibody comprises at least one VHH single domain;

(10) the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment; and/or

(11) the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:4.

9. The ADC of any of claims 1-6, wherein:

(1) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:16;

(2) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:16, wherein the complementarity determining regions (CDRs) are defined according to the Exemplary, IMGT, Kabat, Chothia, Contact, or AbM numbering system;

(3) the DLL3 antibody comprises a VHH comprising:

(i) a CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:7, a CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:8, and a CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:9;

(ii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:42, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:43, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:44;

(iii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:45, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:8, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:9;

(iv) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:46, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:47,

and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:48;

(v) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:49, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:50, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:51; or

(vi) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:7, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:52, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:9;

(4) the DLL3 antibody comprises a VHH comprising the amino acid sequence as set forth in SEQ ID NO:16;

(5) the DLL3 antibody further comprises a human IgG constant domain;

(6) the DLL3 antibody further comprises a human IgG1 constant domain;

(7) the DLL3 antibody further comprises an Fc fragment;

(8) the DLL3 antibody further comprises an Fc fragment that comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17;

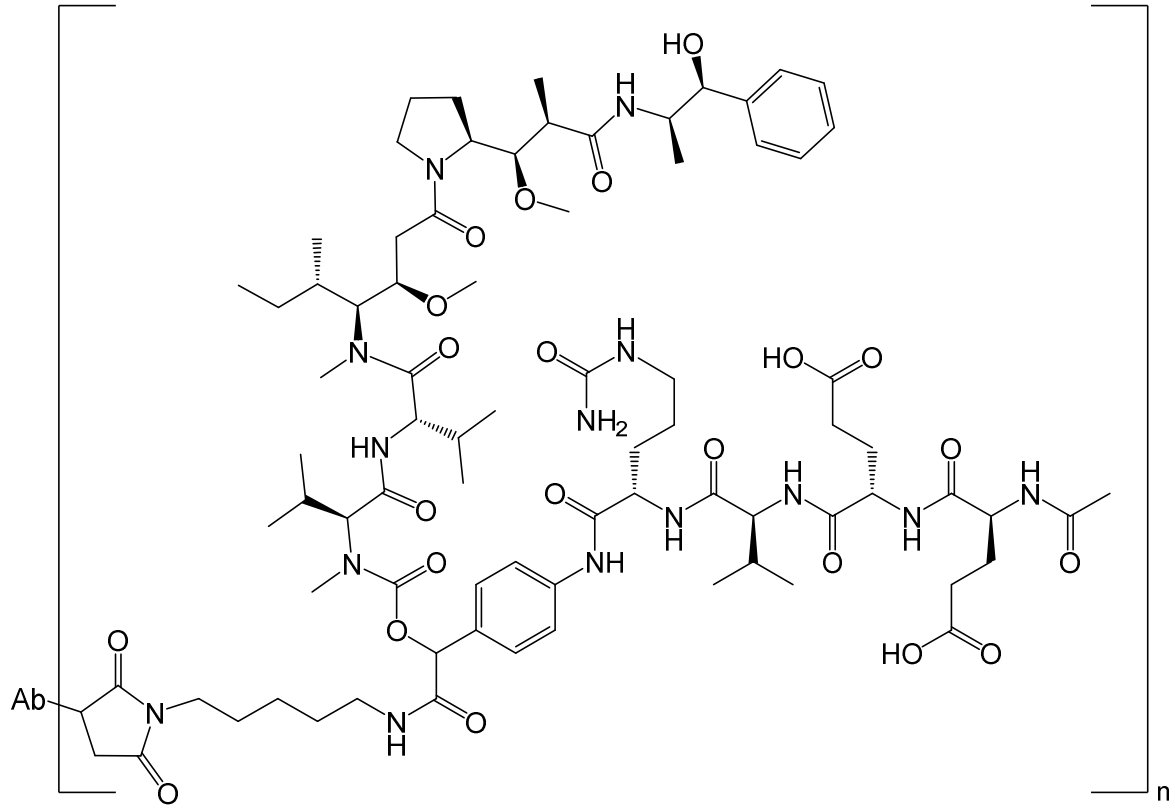
(9) the DLL3 antibody comprises at least one VHH single domain;

(10) the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment; and/or

(11) the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:10.

10. The ADC of any one of claims 1-2 or 7-9, wherein *n* is any one of 1, 2, 3, or 4.

11. An antibody-drug conjugate (ADC) of Formula (I):



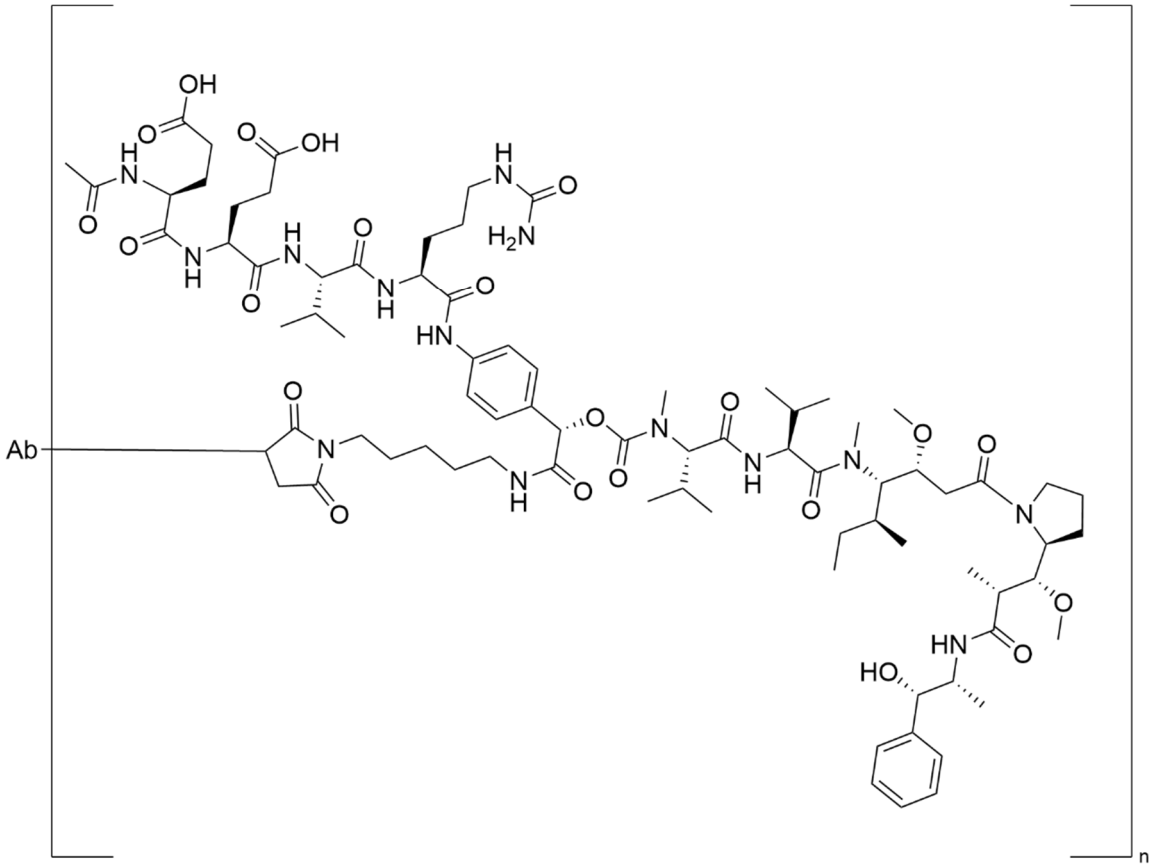
(I)

or a salt thereof, wherein:

n is an integer from 1 to 8, and

Ab represents an antibody that binds to DLL3 (“DLL3 antibody”).

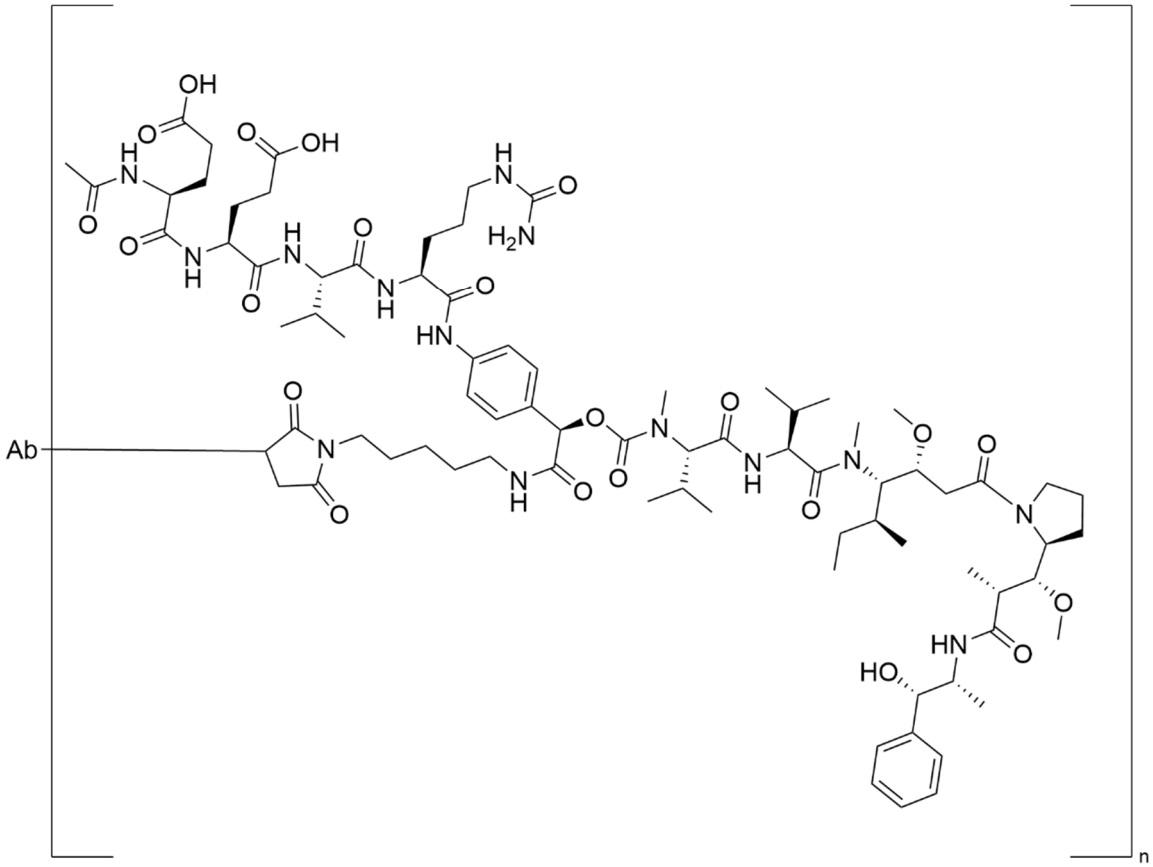
12. The ADC of claim 11, wherein the ADC is of Formula (I-B):



(I-B)

or a salt thereof.

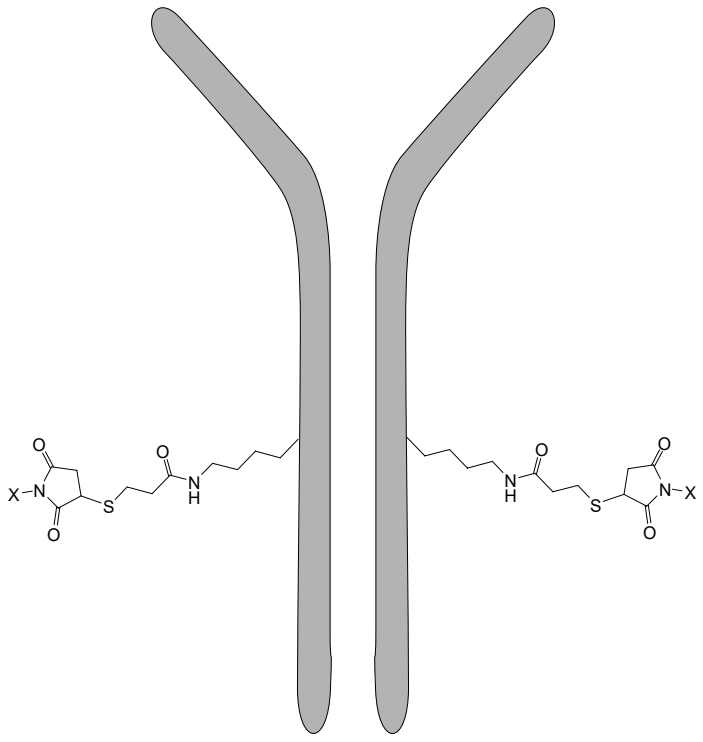
13. The ADC of claim 11, wherein the ADC is of Formula (I-A):



(I-A)

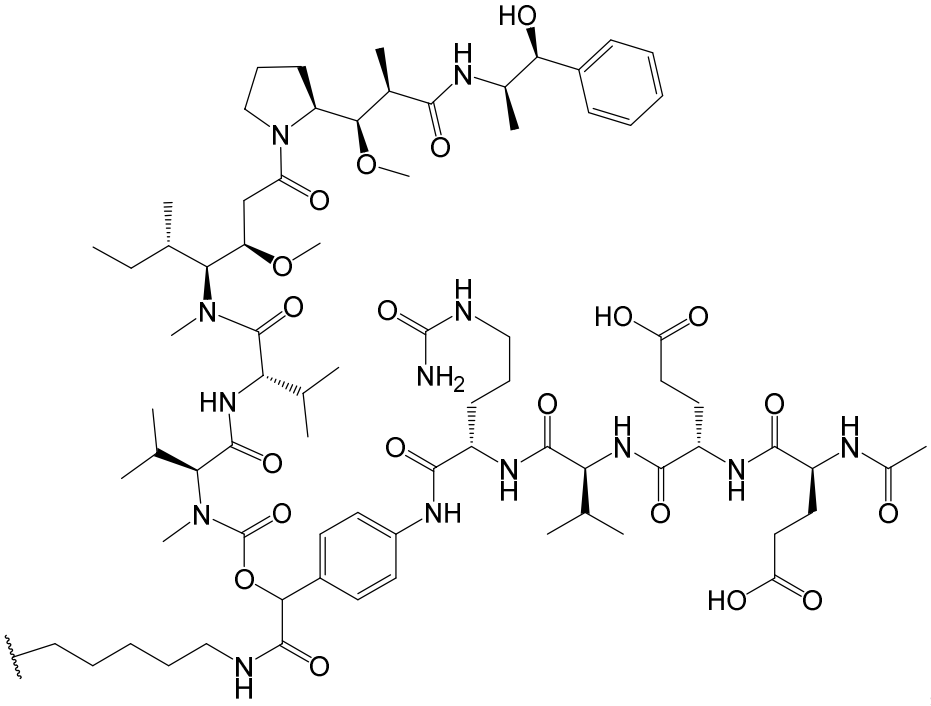
or a salt thereof.

14. An antibody-drug conjugate (ADC) of Formula (B):



(B),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted $CH_2CH_2CH_2CH_2NH$ moiety is from a lysine residue (optionally any one or more of K246, K248, K288, K290, or K317 according to the EU numbering) of the DLL3 antibody, and X represents the structure below or a salt thereof:



,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

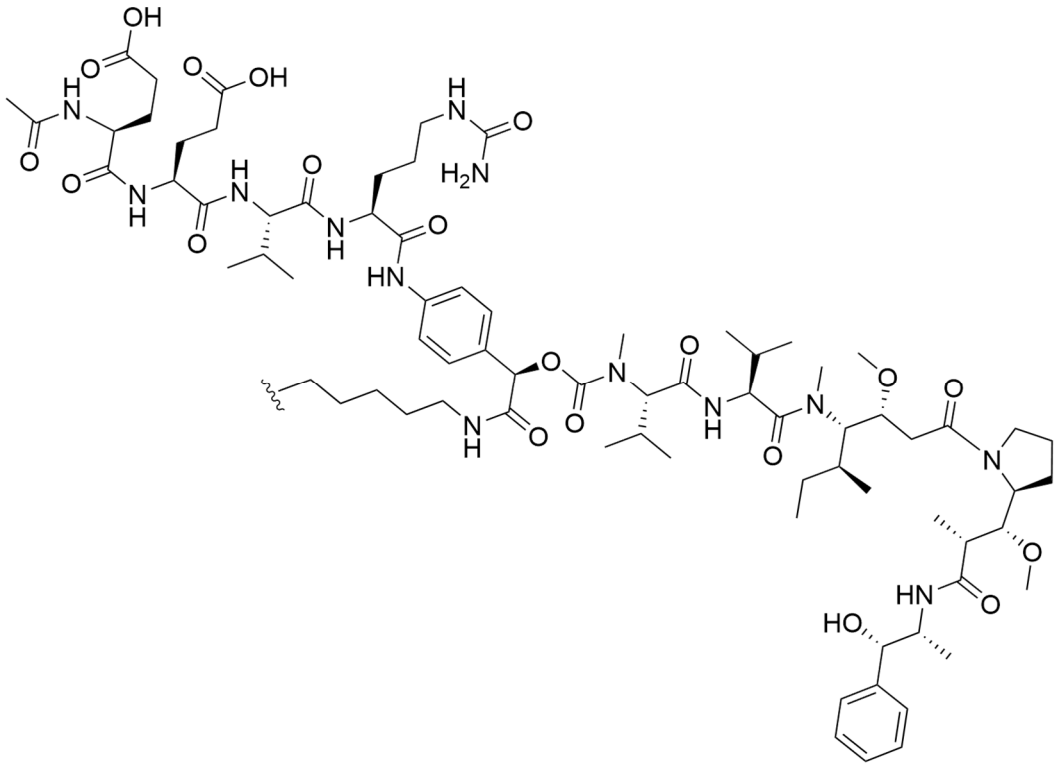
15. The ADC of claim 14, wherein the DLL3 antibody comprises an amino acid sequence as set forth in

(i) SEQ ID NO:5 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:5;

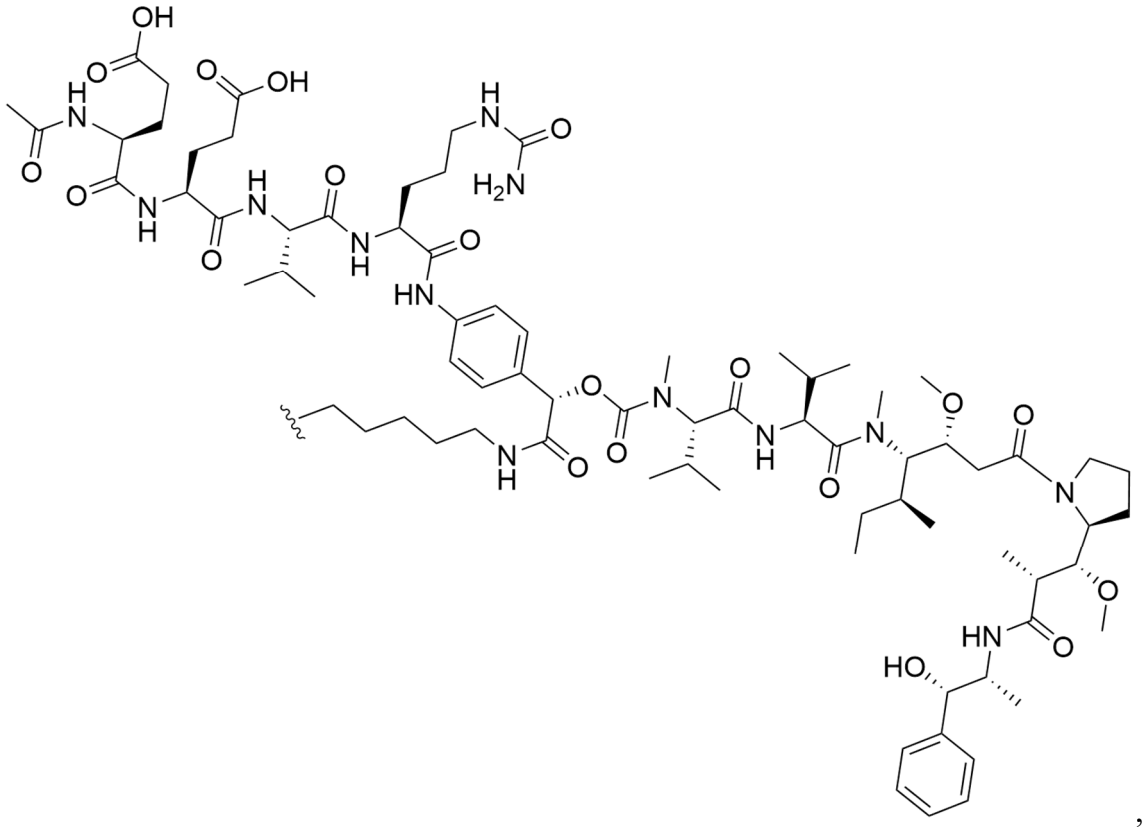
(ii) SEQ ID NO:4 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:4; or

(iii) SEQ ID NO:10 and wherein the lysine residue is the 152nd amino acid residue of SEQ ID NO:10.

16. The ADC of claim 14, wherein the X represents:



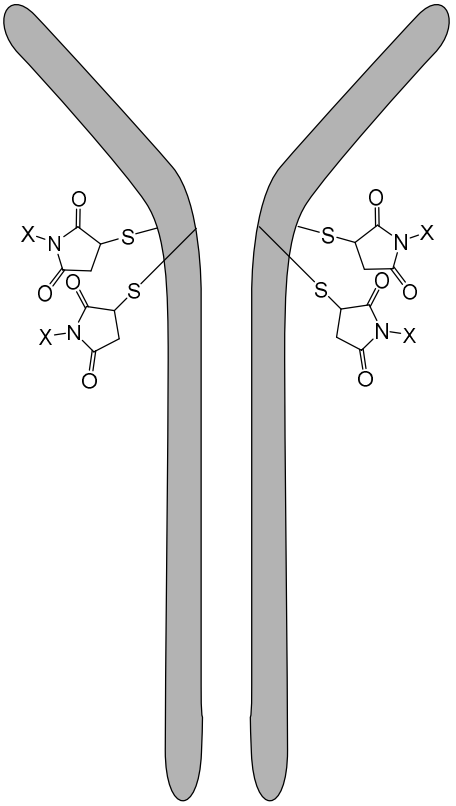
, or



,

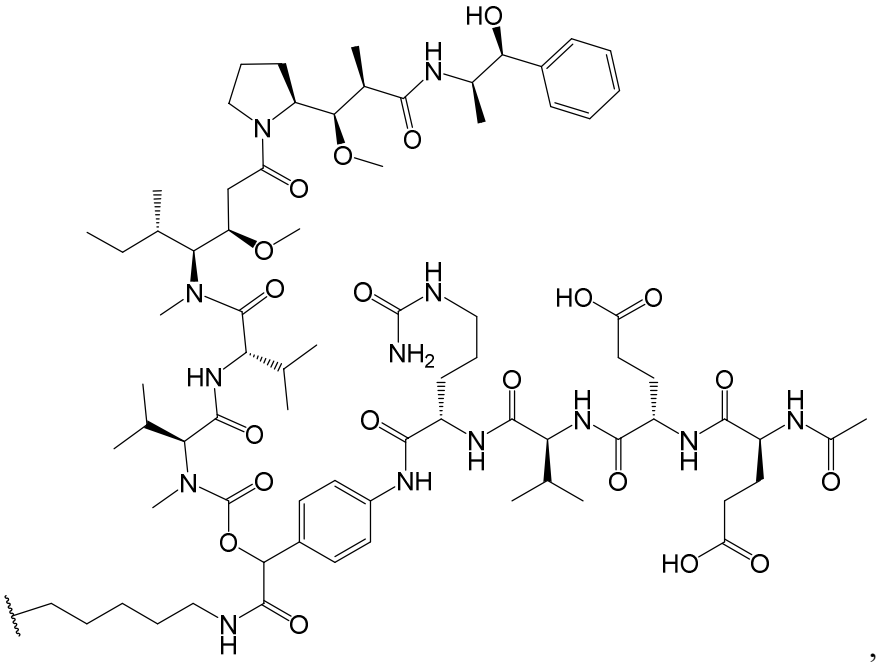
or a salt of each thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

17. An antibody-drug conjugate (ADC) of Formula (A):



(A),

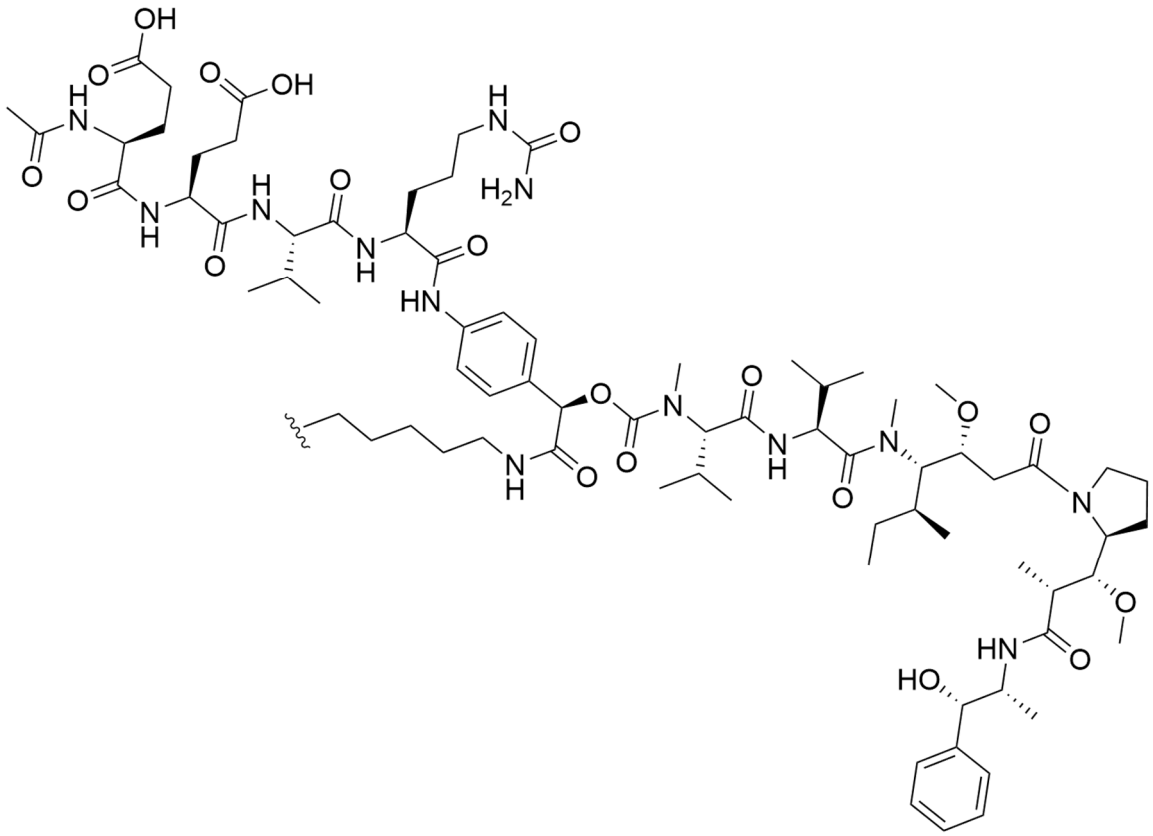
wherein each thick shaded line represents a chain of an antibody that binds to DLL3 ("DLL3 antibody"), the depicted sulfurs are from cysteine residues (optionally C226 and/or C229) of the DLL3 antibody, and X represents the structure below or a salt thereof:



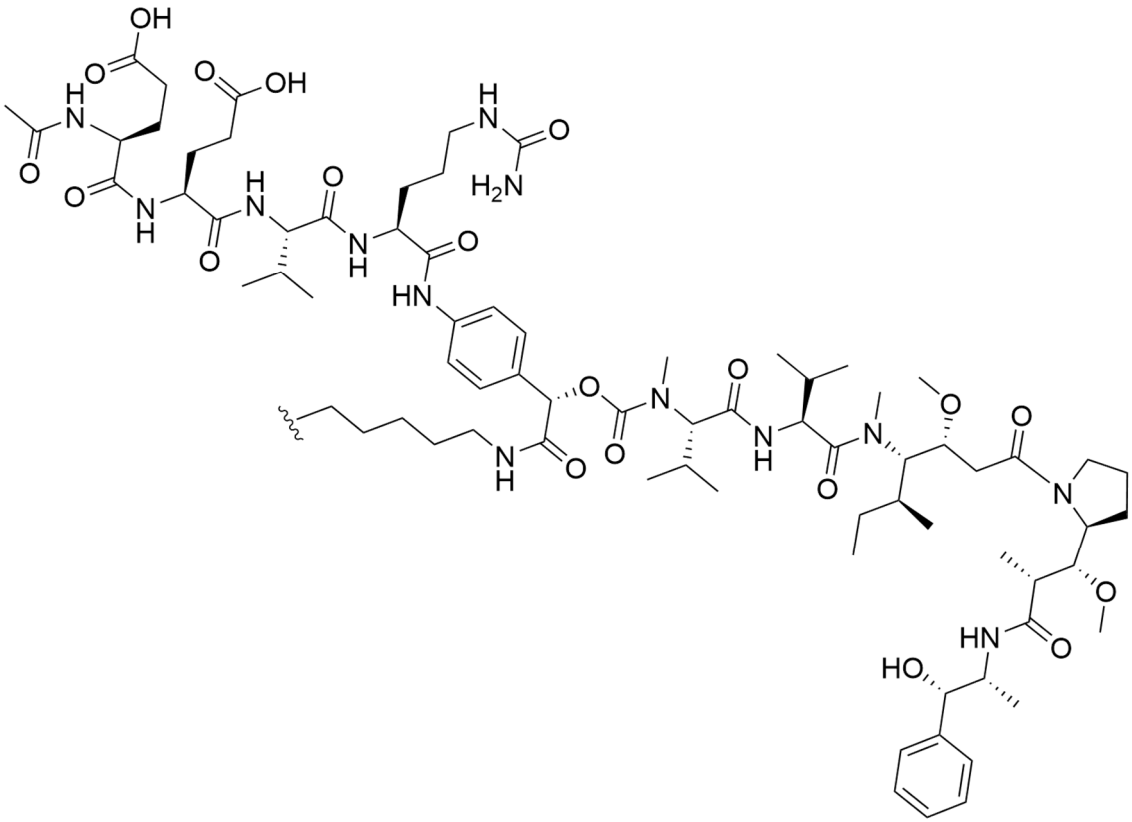
,

wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

18. The ADC of claim 17, wherein the X represents:



, or



, or a salt of each thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

19. The ADC of any of claims 11-18, wherein:

(1) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:15;

(2) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:15, wherein the complementarity determining regions (CDRs) are defined according to the Exemplary, IMGT, Kabat, Chothia, Contact, or AbM numbering system;

(3) the DLL3 antibody comprises a VHH comprising:

(i) a CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:3;

(ii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:20, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:21, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:22;

(iii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:23, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(iv) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:24, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:25, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:26;

(v) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:27, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:56, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:29; or

(vi) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:30, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(4) the DLL3 antibody comprises a VHH comprising the amino acid sequence as set forth in SEQ ID NO:15;

(5) the DLL3 antibody further comprises a human IgG constant domain;

(6) the DLL3 antibody further comprises a human IgG1 constant domain;

(7) the DLL3 antibody further comprises an Fc fragment;

(8) the DLL3 antibody further comprises an Fc fragment that comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17;

(9) the DLL3 antibody comprises at least one VHH single domain;

(10) the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment; and/or

(11) the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:5.

20. The ADC of any of claims 11-18, wherein:

(1) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14;

(2) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14, wherein the complementarity determining regions (CDRs) are defined according to the Exemplary, IMGT, Kabat, Chothia, Contact, or AbM numbering system;

(3) the DLL3 antibody comprises a VHH comprising:

(i) a CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:3;

(ii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:20, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:21, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:22;

(iii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:23, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(iv) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:24, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:25,

and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:26;

(v) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:27, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:28, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:29; or

(vi) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:30, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3;

(4) the DLL3 antibody comprises a VHH comprising the amino acid sequence as set forth in SEQ ID NO:14;

(5) the DLL3 antibody further comprises a human IgG constant domain;

(6) the DLL3 antibody further comprises a human IgG1 constant domain;

(7) the DLL3 antibody further comprises an Fc fragment;

(8) the DLL3 antibody further comprises an Fc fragment that comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17;

(9) the DLL3 antibody comprises at least one VHH single domain;

(10) the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment; and/or

(11) the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:4.

21. The ADC of any of claims 11-18, wherein:

(1) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:16;

(2) the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:16, wherein the complementarity determining regions (CDRs) are defined according to the Exemplary, IMGT, Kabat, Chothia, Contact, or AbM numbering system;

(3) the DLL3 antibody comprises a VHH comprising:

(i) a CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:7, a CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:8, and a CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:9;

(ii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:42, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:43, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:44;

(iii) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:45, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:8, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:9;

(iv) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:46, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:47, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:48;

(v) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:49, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:50, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:51; or

(vi) a CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:7, a CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:52, and a CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:9;

(4) the DLL3 antibody comprises a VHH comprising the amino acid sequence as set forth in SEQ ID NO:16;

(5) the DLL3 antibody further comprises a human IgG constant domain;

(6) the DLL3 antibody further comprises a human IgG1 constant domain;

(7) the DLL3 antibody further comprises an Fc fragment;

(8) the DLL3 antibody further comprises an Fc fragment that comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17;

(9) the DLL3 antibody comprises at least one VHH single domain;

(10) the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment; and/or

(11) the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:10.

22. The ADC of any one of claims 11-12 and 19-21, wherein *n* is 1, 2, 3, or 4.

23. A pharmaceutical composition comprising one or more of the ADCs of any one of claims 1-22 and a pharmaceutically acceptable carrier; optionally
the composition is characterized by a drug-to-antibody ratio ("DAR") of about 1 to about 8,
about 1 to about 2,
about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2,
about 2 to about 4,
about 2, about 2.1, about 2.2, about 2.4, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9 or about 4.

24. Use of the ADC of any one of claims 1-22 in the manufacture of a medicament, wherein the medicament is used for modulating a DLL3 related immune response.

25. Use of the ADC of any one of claims 1-22 in the manufacture of a medicament, wherein the medicament is used for treatment of a cancer, wherein the cancer is DLL3-positive or overexpressed.

26. Use of the ADC of any one of claims 1-22 in the manufacture of a medicament for diagnosing or treating a DLL3 positive cancer.

27. The ADC of any one of claims 1-22 for use in treating a DLL3 positive cancer.

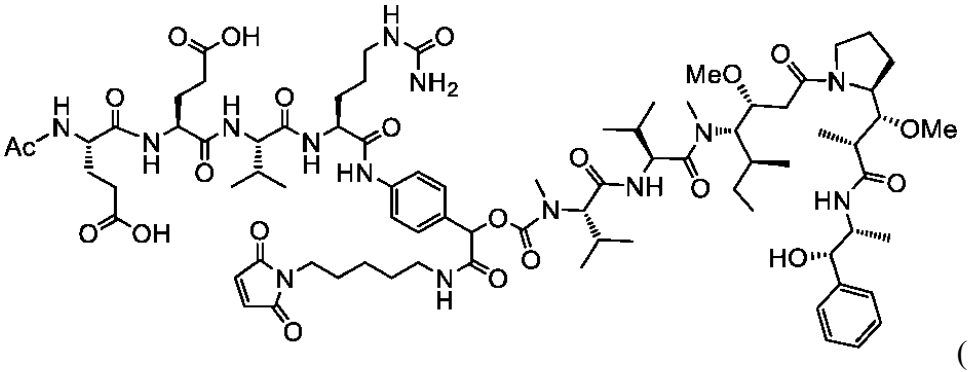
28. A method of making the ADC of any one of claims 11-18, comprising the steps of:

(1)

a) providing a solution comprising the DLL3 antibody;

b) contacting the solution of a) with a reducing agent, optionally the reducing agent is tris(2 carboxyethyl)phosphine (TCEP); and

c) contacting the solution of b) with a solution comprising Linker-Drug (1):



(1),

wherein the ADC is made,

or

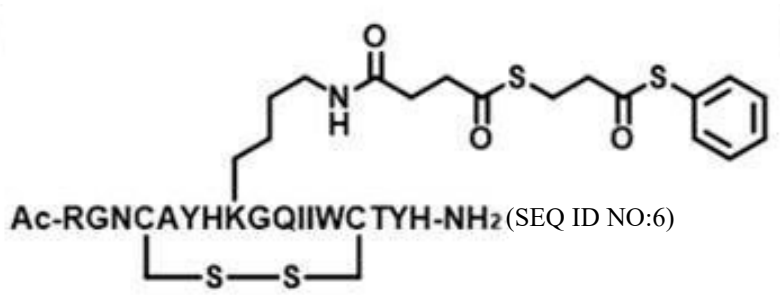
(2)

a) providing a solution comprising the DLL3 antibody;

b) contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety,

wherein the affinity peptide is a peptide of SEQ ID NO:6, and/or

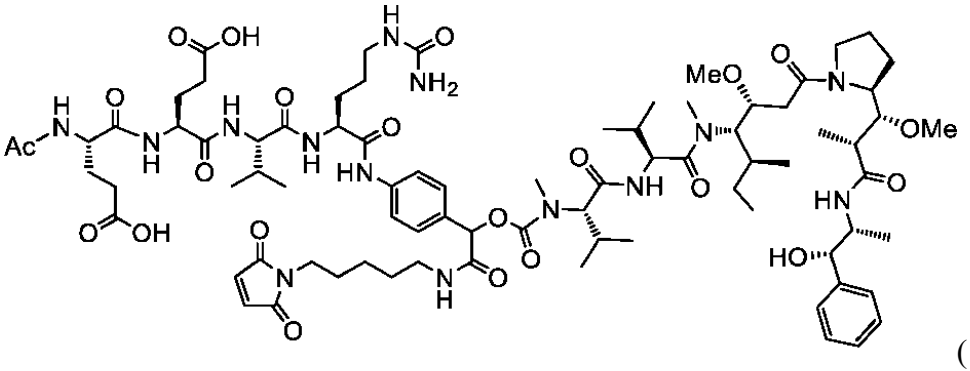
the affinity peptide conjugated to a thiophenol activation moiety is:



(SEQ ID NO:6)

; and

c) contacting the solution of b) with a solution comprising Linker-Drug (1):


(1),

wherein the ADC is made.

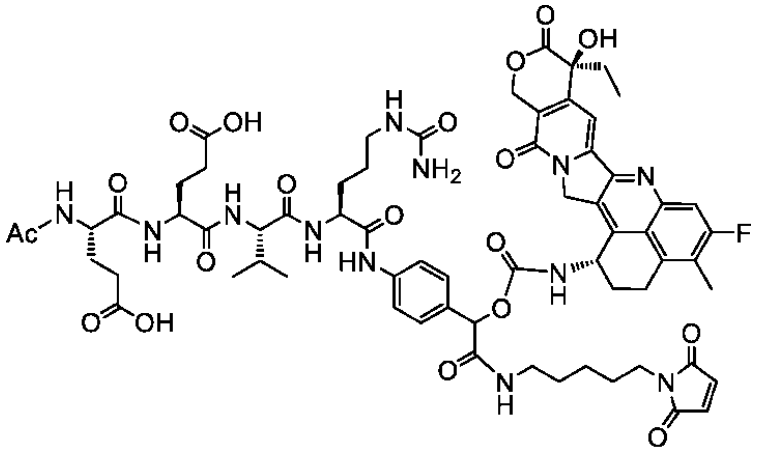
29. A method of making the ADC of any one of claims 1-6, comprising the steps of:

(1)

a) providing a solution comprising the DLL3 antibody;

b) contacting the solution of a) with a reducing agent, optionally the reducing agent is tris(2 carboxyethyl)phosphine (TCEP); and

c) contacting the solution of b) with a solution comprising Linker-Drug (2):


(2),

wherein the ADC is made,

or

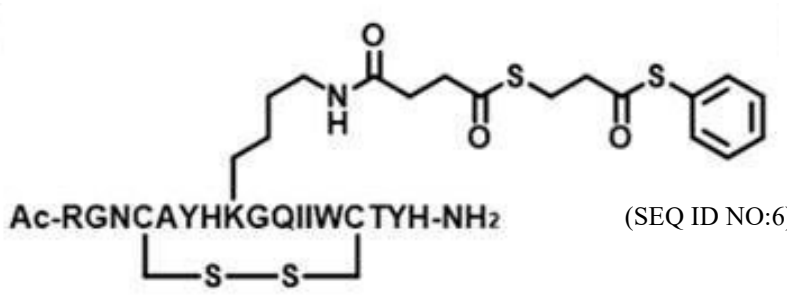
(2)

a) providing a solution comprising the DLL3 antibody;

b) contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety,

wherein the affinity peptide is a peptide of SEQ ID NO:6, and/or

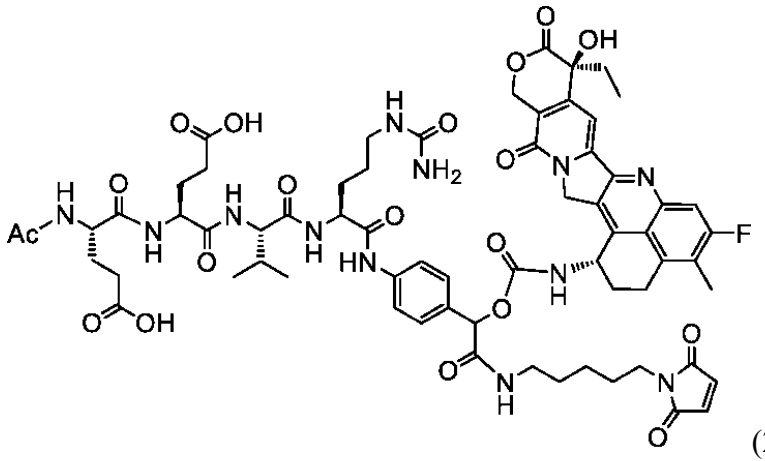
the affinity peptide conjugated to a thiophenol activation moiety is:



(SEQ ID NO:6)

; and

c) contacting the solution of b) with a solution comprising Linker-Drug (2):



(2),

wherein the ADC is made.