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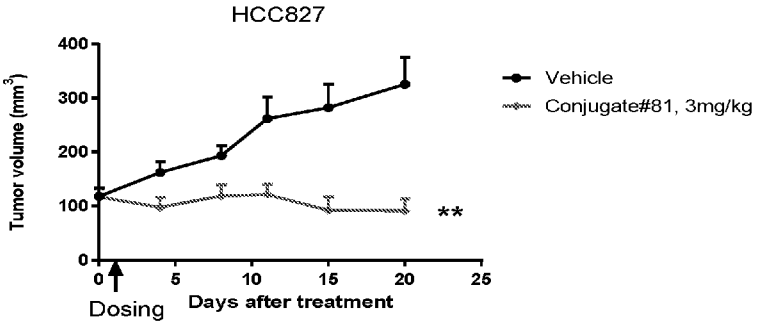
(54) **Title**: CALICHEAMICIN ANTIBODY DRUG CONJUGATES LINKING AN AMIDOACETYL GROUP TO A SUGAR MOIETY ON CALICHEAMICIN

FIG. 1



(57) **Abstract**: There is disclosed a calicheamicin antibody drug conjugate comprising a linking amidoacetyl group covalently bound to a sugar moiety on calicheamicin or linking to sulfur atom on calicheamicin through disulfide bond.

WO 2017/172907 A1

Calicheamicin Antibody Drug Conjugates Linking an Amidoacetyl Group to a Sugar Moiety on Calicheamicin

Related Applications

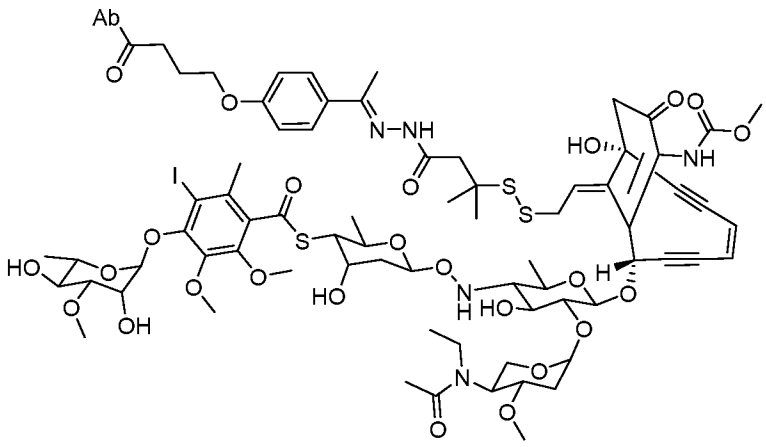
This application claims priority to U.S. Provisional Application No. 62/314,799, filed March 29, 2016, the entire contents of which are incorporated herein by reference.

Technical Field

The present disclosure provides a calicheamicin antibody drug conjugate comprising a linking amidoacetyl group covalently bound to a sugar moiety on calicheamicin or linking to sulfur atom on calicheamicin through disulfide bond.

Background

Calicheamicin is described in WO 03/092623 as an ADC (antibody drug conjugate). The synthesis of calicheamicin was published in 2002 (*Bioconjugate Chem.* 2002, 13, 47-58). ADCs (antibody drug conjugates) that used calicheamicin as the drug moiety linked an acid sensitive hydrazone linker attached to N-acetyl gamma calicheamicin via a disulfide bond to attach calicheamicin to the rest of the conjugate. As shown in formula I below of N-acetyl gamma calicheamicin linked an antibody that is shown as "Ab."



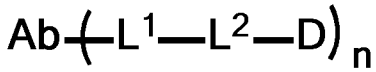
(I)

However, because of the instability of this hydrazone linker, the calicheamicin toxin was released from the linker at a high rate of 6% in 24 h at 37 °C. This high rate of release caused large amounts of non-specific cytotoxicity with such calicheamicin ADCs. Therefore, there is a need in the art to find a better way to link calicheamicin to a linker and a targeting

antibody such that the calicheamicin will be released from the linker at a much slower rate to lower non-specific cytotoxicity. The present disclosure provides a solution to utilizing calicheamicin in an ADC construct while significantly lowering the rate of calicheamicin release from the linker to significantly improve non-specific cytotoxity side effects.

Summary

The present disclosure provides a calicheamicin antibody drug conjugate (ADC) comprising a linking amidoacetyl group covalently bound to a sugar moiety on calicheamicin or linking to sulfur atom on calicheamicin through disulfide bond. More specifically, the present disclosure provides an ADC comprising a structure of Formula II

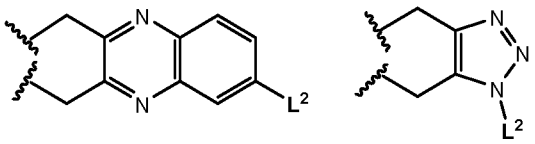


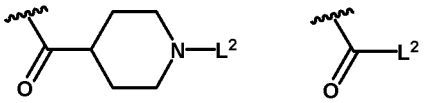
(II)

or a pharmaceutically acceptable salt thereof, wherein:

Ab is a monoclonal antibody;

$\mathbf{L^1}$ - $\mathbf{L^2}$ together are a linker selected from the group consisting of:





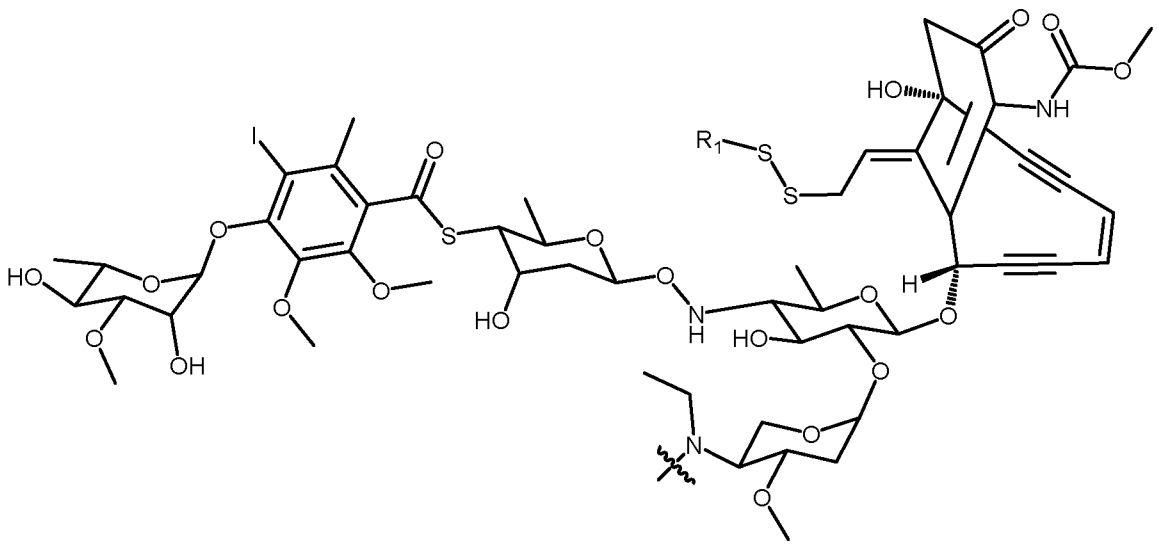
, wherein the wavy line indicates a point of attachment to an Ab;

$\mathbf{L^2}$ is a linker; wherein $\mathbf{L^2}$ is selected from the group consisting of an amino acid, a peptide, $-(CH_2)_m-$, $-(CH_2CH_2O)_m-$, PAB (p-aminobenzyl), Val (Valine)-Citrulline-PAB, Val-Ala (Alanine)-PAB, Ala-Ala-Asn-PAB, and combinations thereof, wherein m is an integer from 0 to 10;

D is calicheamicin; and

n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

Preferably, D has the structure of Formula III

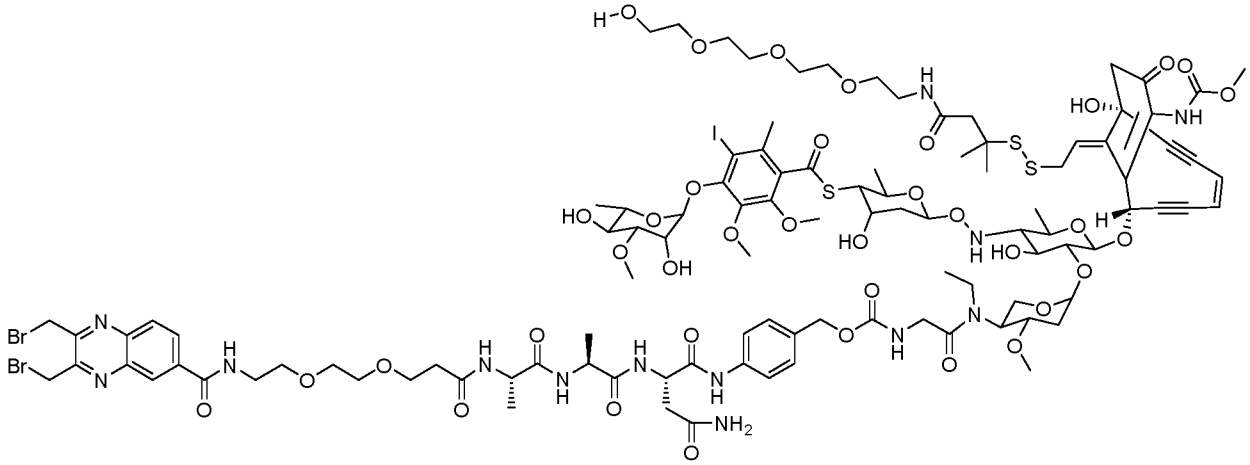


(III)

wherein the wavy line indicates the point of attachment to L^2;

wherein R1 is selected from the group consisting of C1-C8 alkyl, $-(CH_2CH_2O)_n-$, isopropyl, glucose, galactose, mannose, glucosamine, C1-C8 alkyl-OH, and combinations thereof.

More preferably the D and C components of the ADC is the structure of Formula IV:



(IV).

The linker was attached via a stable amide bond or a carbamate bond. A hydrophilic group (e.g., PEG4) was incorporated via a disulfide bond to improve the solubility in aqueous buffer and mitigate the issue of aggregation.

Brief Description of the Figures

FIG 1 illustrates the tumor volume from 3 mg/kg IV, single dose of Compound **81a** in HCC827 tumor in nude mice.

FIG 2 illustrates the tumor volume from 3 mg/kg IV, single dose of Compound **81a** in H292 tumor in nude mice.

FIG 3 illustrates the tumor volume from 3 mg/kg IV, one weekly for three treatments of Compound **81a** in H292 tumor in nude mice.

FIG 4 illustrates the tumor volume from 3 mg/kg IV, one weekly for three treatments of Compound **81a** in H1993 tumor in nude mice.

FIG 5 illustrates the tumor volume from 3 mg/kg IV, one weekly for three treatments of Compound **81a** in U87MG tumor in nude mice.

FIG 6 illustrates the tumor volume from 3 mg/kg IV, single dose of Compound **8a** in HCC827 tumor in nude mice.

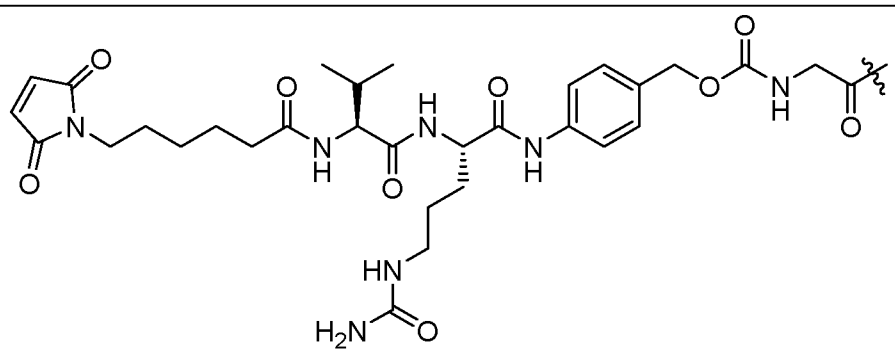
FIG 7 illustrates the tumor volume from 3 mg/kg IV, single dose of Compound **8a** in H292 tumor in nude mice.

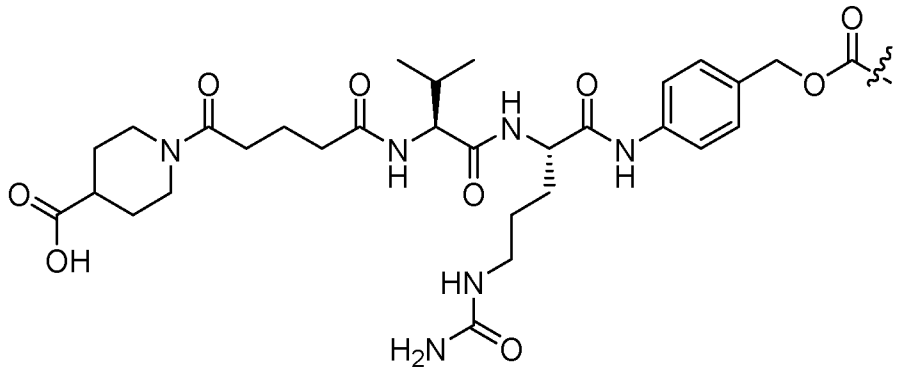
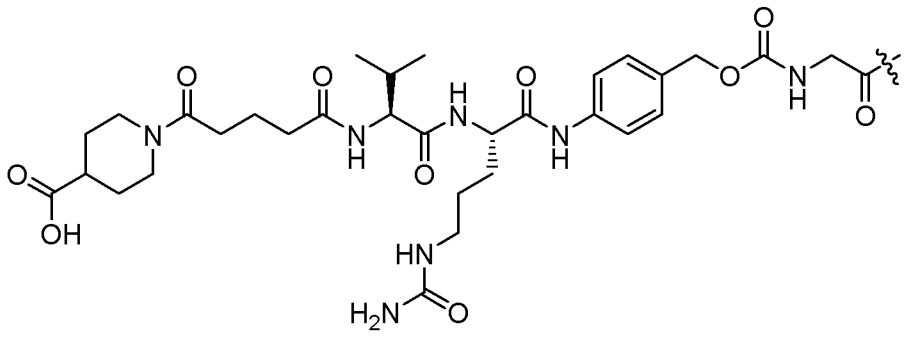
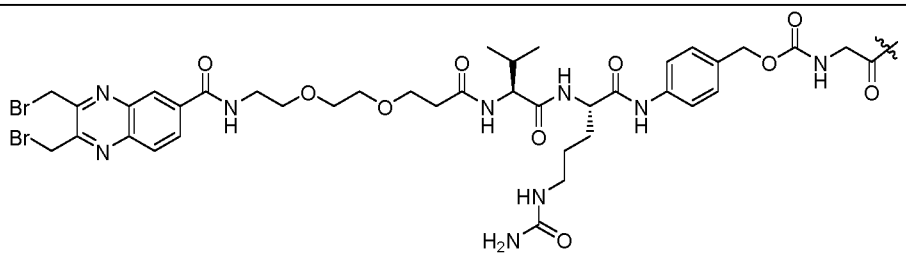
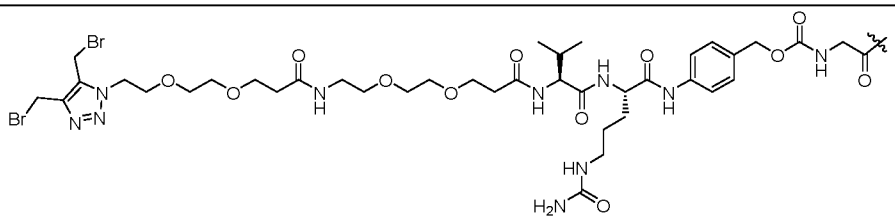
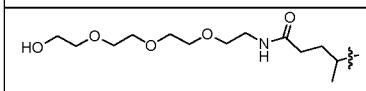
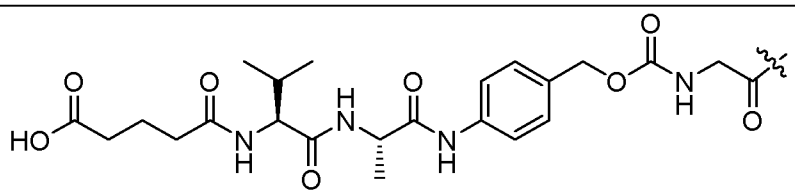
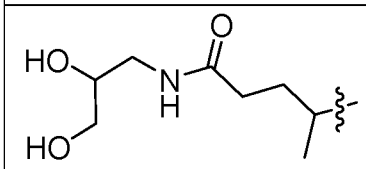
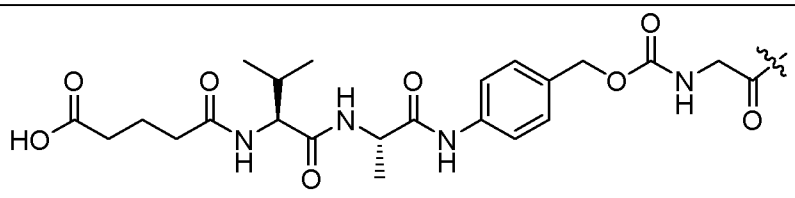
FIG 8 illustrates the tumor volume from 3 mg/kg IV, single dose of Compound **8a** in U87 tumor in nude mice.

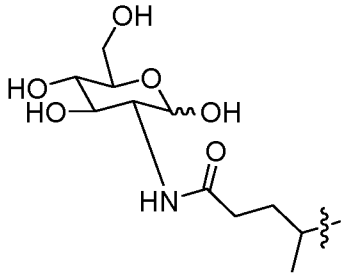
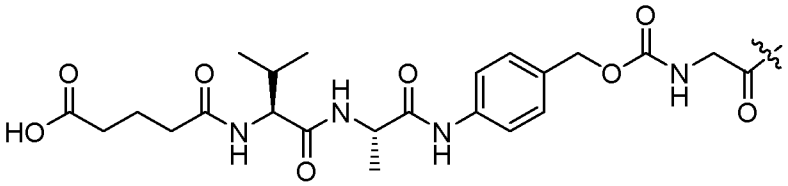
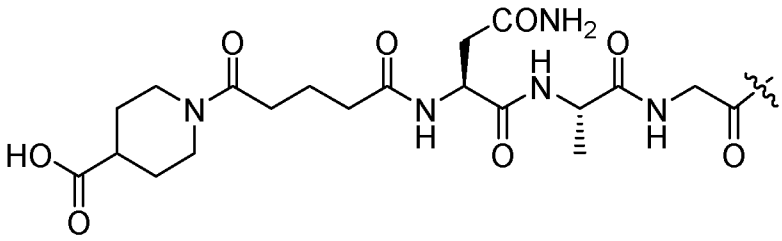
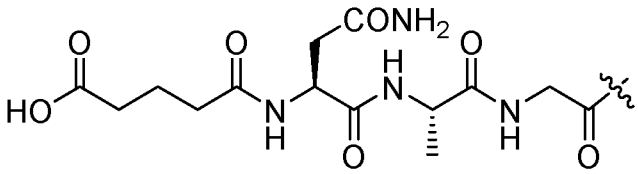
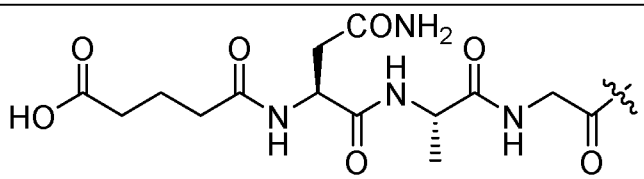
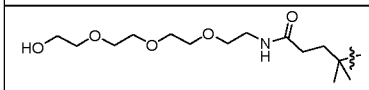
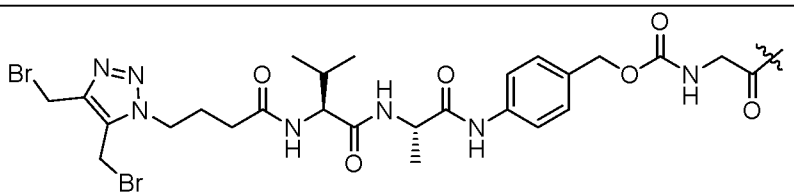
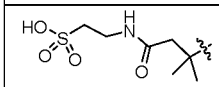
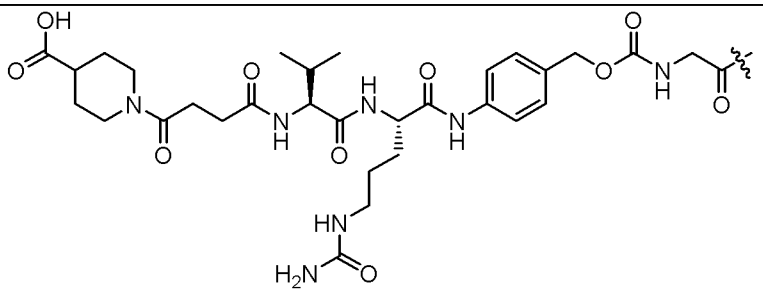
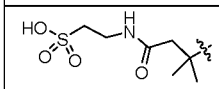
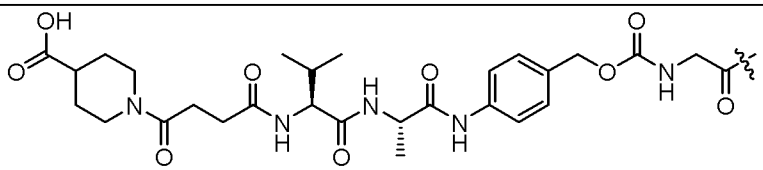
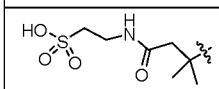
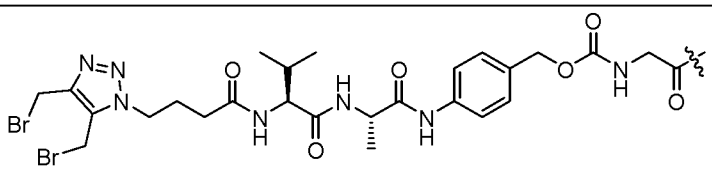
FIG 9 illustrates the tumor volume from 3 mg/kg IV, single dose of Compound **8a** in H1975 tumor in nude mice.

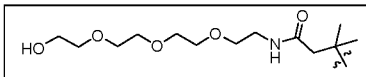
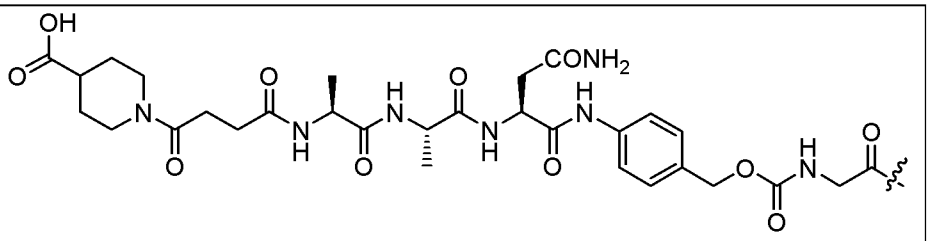
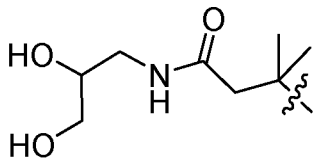
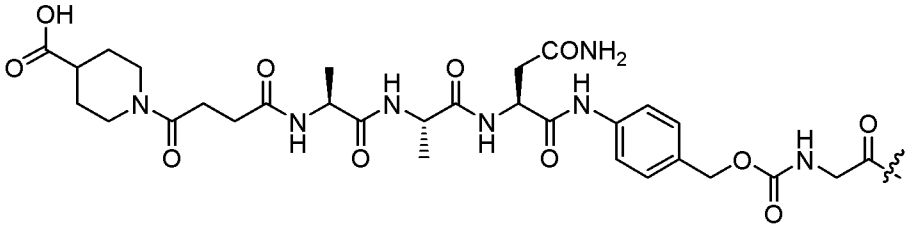
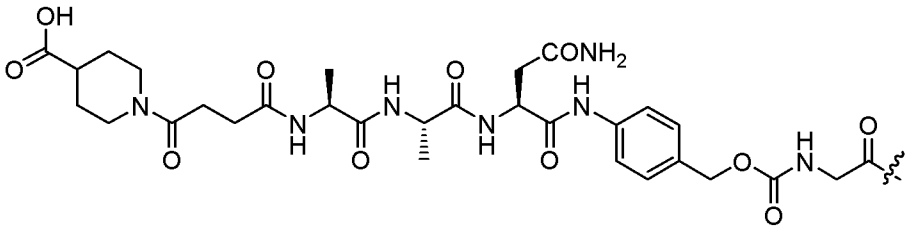
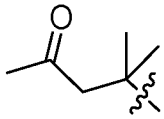
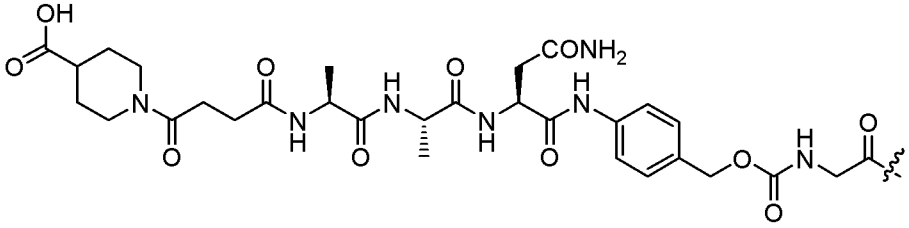
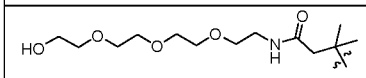
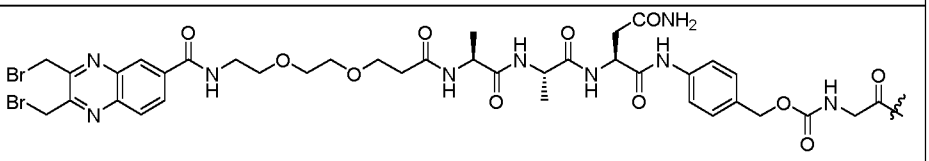
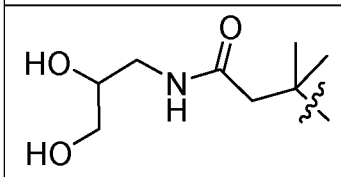
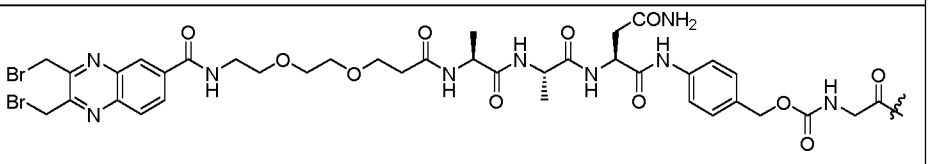
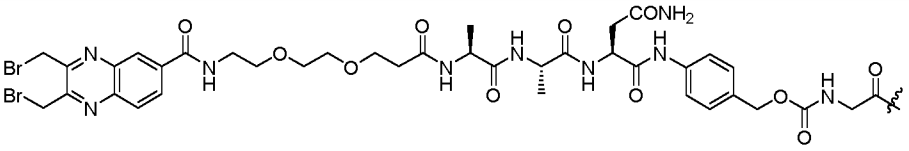
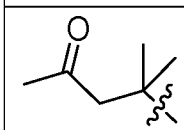
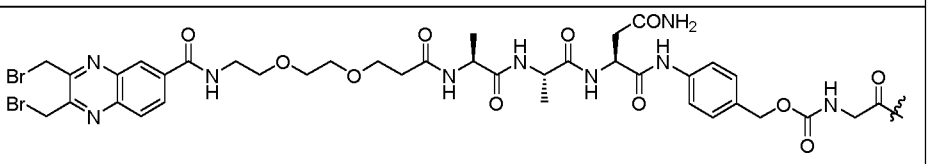
Detailed Description

Examples of Formula III, where R1 and L^1-L^2 are listed below (wavy line indicates point of attachment to Formula III):

R1	L^1-L^2
isopropyl	

isopropyl	
isopropyl	
isopropyl	
isopropyl	
	
	

	
tert-butyl	
isopropyl	
tert-butyl	
	
	
	
	

	
	
tert-butyl	
	
	
	
tert-butyl	
	

Antibody Component

The present disclosure provides a fully human antibody of an IgG class that binds to a c-Met epitope with a binding affinity of at least 10^{-6}M, which has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 33, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 37, SEQ ID NO. 40, SEQ ID NO. 42, SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO. 47, SEQ ID NO. 49, SEQ ID NO. 51, SEQ ID NO. 53, SEQ ID NO. 56, SEQ ID NO. 58, SEQ ID NO. 59, SEQ ID NO. 61, SEQ ID NO. 63, SEQ ID NO. 65, SEQ ID NO. 69, SEQ ID NO. 71, SEQ ID NO. 74, SEQ ID NO. 75, SEQ ID NO. 76, SEQ ID NO. 79, SEQ ID NO. 80, SEQ ID NO. 82, SEQ ID NO. 84, SEQ ID NO. 86, SEQ ID NO. 88, SEQ ID NO. 90, SEQ ID NO. 92, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 35, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 41, SEQ ID NO. 43, SEQ ID NO. 46, SEQ ID NO. 48, SEQ ID NO. 50, SEQ ID NO. 52, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 57, SEQ ID NO. 60, SEQ ID NO. 62, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 68, SEQ ID NO. 70, SEQ ID NO. 72, SEQ ID NO. 73, SEQ ID NO. 77, SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 83, SEQ ID NO. 85, SEQ ID NO. 87, SEQ ID NO. 89, SEQ ID NO. 91, SEQ ID NO. 93, and combinations thereof.

In one embodiment, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called A1 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called A2 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called A8 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called B12 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called D6 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called E1 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called E6 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called F3 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called H6 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called H8 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called H8-9 herein), SEQ ID NO. 21/SEQ ID NO. 23 (called H8-9EE8L3 herein),

SEQ ID NO. 24/SEQ ID NO. 22 (called H8-G3S herein), SEQ ID NO. 25/SEQ ID NO. 26 (called H8-A2 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called H8-B6 herein), SEQ ID NO. 29/SEQ ID NO. 23 (called H8-C1 herein), SEQ ID NO. 24/SEQ ID NO. 30 (called H8-D4 herein), SEQ ID NO. 31/SEQ ID NO. 23 (called H8-D5 herein), SEQ ID NO. 24/SEQ ID NO. 23 (called H8-D6 herein), SEQ ID NO. 32/SEQ ID NO. 23 (called H8-D10 herein), SEQ ID NO. 33/SEQ ID NO. 22 (called H8-E5 herein), SEQ ID NO. 34/SEQ ID NO. 22 (called H8-G7 herein), SEQ ID NO. 24/SEQ ID NO. 35 (called H8-G9 herein), SEQ ID NO. 36/SEQ ID NO. 26 (called H8-H6 herein), SEQ ID NO. 29/SEQ ID NO. 22 (called H8-2A2 herein), SEQ ID NO. 37/SEQ ID NO. 38 (called H8-2B1 herein), SEQ ID NO. 34/SEQ ID NO. 23 (called H8-2B2 herein), SEQ ID NO. 37/SEQ ID NO. 23 (called H8-2B4 herein), SEQ ID NO. 32/SEQ ID NO. 39 (called H8-2B7 herein), SEQ ID NO. 32/SEQ ID NO. 22 (called H8-A7P herein), SEQ ID NO. 40/SEQ ID NO. 41 (called GCE-A10 herein), SEQ ID NO. 42/SEQ ID NO. 43 (called GCE-A11 herein), SEQ ID NO. 44/SEQ ID NO. 41 (called GCE-A13 herein), SEQ ID NO. 45/SEQ ID NO. 46 (called GCE-A14 herein), SEQ ID NO. 47/SEQ ID NO. 48 (called GCE-A16 herein), SEQ ID NO. 49/SEQ ID NO. 50 (called GCE-A18 herein), SEQ ID NO. 51/SEQ ID NO. 52 (called GCE-B2 herein), SEQ ID NO. 53/SEQ ID NO. 54 (called GCE-B9 herein), SEQ ID NO. 45/SEQ ID NO. 55 (called GCE-B11 herein), SEQ ID NO. 56/SEQ ID NO. 57 (called GCE-B13 herein), SEQ ID NO. 58/SEQ ID NO. 57 (called GCE-B19 herein), SEQ ID NO. 59/SEQ ID NO. 60 (called GCE-BR1 herein), SEQ ID NO. 61/SEQ ID NO. 62 (called GCE-B20 herein), SEQ ID NO. 63/SEQ ID NO. 64 (called GCE-A19 herein), SEQ ID NO. 65/SEQ ID NO. 66 (called GCE-B10 herein), SEQ ID NO. 58/SEQ ID NO. 67 (called GCE-B5 herein), SEQ ID NO. 61/SEQ ID NO. 68 (called GCE-B4 herein), SEQ ID NO. 69/SEQ ID NO. 70 (called GCE-A26 herein), SEQ ID NO. 71/SEQ ID NO. 72 (called GCE-L1A-9 herein), SEQ ID NO. 49/SEQ ID NO. 73 (called GCE-H34-36 herein), SEQ ID NO. 74/SEQ ID NO. 73 (called GCE-H13-1 herein), SEQ ID NO. 61/SEQ ID NO. 73 (called GCE-H13-2 herein), SEQ ID NO. 44/SEQ ID NO. 73 (called GCE-H13-3 herein), SEQ ID NO. 40/SEQ ID NO. 73 (called GCE-H13-4 herein), SEQ ID NO. 75/SEQ ID NO. 73 (called GCE-H13-5 herein), SEQ ID NO. 69/SEQ ID NO. 73 (called GCE-H13-6 herein), SEQ ID NO. 76/SEQ ID NO. 73 (called GCE-H13-8 herein), SEQ ID NO. 21/SEQ ID NO. 77 (called H8-9EH11L herein), SEQ ID NO. 21/SEQ ID NO. 78 (called H8-9EG11L herein), SEQ ID NO. 79/SEQ ID NO. 20 (called H8-6AG2H3 herein), SEQ ID NO. 80/SEQ ID NO. 81 (called A1-2 herein), SEQ ID NO. 82/SEQ ID NO. 83 (called A1-4 herein), SEQ ID NO. 84/SEQ ID NO. 85 (called A1-6 herein), SEQ ID NO. 86/SEQ ID NO. 87 (called A1-8 herein), SEQ ID NO. 88/SEQ ID NO. 89 (called A1-9 herein), SEQ ID NO.

90/SEQ ID NO. 91 (called A1-24 herein), SEQ ID NO. 92/SEQ ID NO. 93 (called A1-32 herein), and combinations thereof.

The present disclosure also provides a fully human antibody Fab fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 33, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 37, SEQ ID NO. 40, SEQ ID NO. 42, SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO. 47, SEQ ID NO. 49, SEQ ID NO. 51, SEQ ID NO. 53, SEQ ID NO. 56, SEQ ID NO. 58, SEQ ID NO. 59, SEQ ID NO. 61, SEQ ID NO. 63, SEQ ID NO. 65, SEQ ID NO. 69, SEQ ID NO. 71, SEQ ID NO. 74, SEQ ID NO. 75, SEQ ID NO. 76, SEQ ID NO. 79, SEQ ID NO. 80, SEQ ID NO. 82, SEQ ID NO. 84, SEQ ID NO. 86, SEQ ID NO. 88, SEQ ID NO. 90, SEQ ID NO. 92, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 35, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 41, SEQ ID NO. 43, SEQ ID NO. 46, SEQ ID NO. 48, SEQ ID NO. 50, SEQ ID NO. 52, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 57, SEQ ID NO. 60, SEQ ID NO. 62, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 68, SEQ ID NO. 70, SEQ ID NO. 72, SEQ ID NO. 73, SEQ ID NO. 77, SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 83, SEQ ID NO. 85, SEQ ID NO. 87, SEQ ID NO. 89, SEQ ID NO. 91, SEQ ID NO. 93, and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 21/SEQ ID NO. 23, SEQ ID NO. 24/SEQ ID NO. 22, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO.

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The present disclosure also provides a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 33, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 37, SEQ ID NO. 40, SEQ ID NO. 42, SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO. 47, SEQ ID NO. 49, SEQ ID NO. 51, SEQ ID NO. 53, SEQ ID NO. 56, SEQ ID NO. 58, SEQ ID NO. 59, SEQ ID NO. 61, SEQ ID NO. 63, SEQ ID NO. 65, SEQ ID NO. 69, SEQ ID NO. 71, SEQ ID NO. 74, SEQ ID NO. 75, SEQ ID NO. 76, SEQ ID NO. 79, SEQ ID NO. 80, SEQ ID NO. 82, SEQ ID NO. 84, SEQ ID NO. 86, SEQ ID NO.

88, SEQ ID NO. 90, SEQ ID NO. 92, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 35, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 41, SEQ ID NO. 43, SEQ ID NO. 46, SEQ ID NO. 48, SEQ ID NO. 50, SEQ ID NO. 52, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 57, SEQ ID NO. 60, SEQ ID NO. 62, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 68, SEQ ID NO. 70, SEQ ID NO. 72, SEQ ID NO. 73, SEQ ID NO. 77, SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 83, SEQ ID NO. 85, SEQ ID NO. 87, SEQ ID NO. 89, SEQ ID NO. 91, SEQ ID NO. 93, and combinations thereof. In one embodiment, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 21/SEQ ID NO. 23, SEQ ID NO. 24/SEQ ID NO. 22, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 23, SEQ ID NO. 24/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 23, SEQ ID NO. 24/SEQ ID NO. 23, SEQ ID NO. 32/SEQ ID NO. 23, SEQ ID NO. 33/SEQ ID NO. 22, SEQ ID NO. 34/SEQ ID NO. 22, SEQ ID NO. 24/SEQ ID NO. 35, SEQ ID NO. 36/SEQ ID NO. 26, SEQ ID NO. 29/SEQ ID NO. 22, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 34/SEQ ID NO. 23, SEQ ID NO. 37/SEQ ID NO. 23, SEQ ID NO. 32/SEQ ID NO. 39, SEQ ID NO. 32/SEQ ID NO. 22, SEQ ID NO. 40/SEQ ID NO. 41, SEQ ID NO. 42/SEQ ID NO. 43, SEQ ID NO. 44/SEQ ID NO. 41, SEQ ID NO. 45/SEQ ID NO. 46, SEQ ID NO. 47/SEQ ID NO. 48, SEQ ID NO. 49/SEQ ID NO. 50, SEQ ID NO. 51/SEQ ID NO. 52, SEQ ID NO. 53/SEQ ID NO. 54, SEQ ID NO. 45/SEQ ID NO. 55, SEQ ID NO. 56/SEQ ID NO. 57, SEQ ID NO. 58/SEQ ID NO. 57, SEQ ID NO. 59/SEQ ID NO. 60, SEQ ID NO. 61/SEQ ID NO. 62, SEQ ID NO. 63/SEQ ID NO. 64, SEQ ID NO. 65/SEQ ID NO. 66, SEQ ID NO. 58/SEQ ID NO. 67, SEQ ID NO. 61/SEQ ID NO. 68, SEQ ID NO. 69/SEQ ID NO. 70, SEQ ID NO. 71/SEQ ID NO. 72, SEQ ID NO. 49/SEQ ID NO. 73, SEQ ID NO. 74/SEQ ID NO. 73, SEQ ID NO. 61/SEQ ID NO. 73, SEQ ID NO. 44/SEQ ID NO. 73, SEQ ID NO. 40/SEQ ID NO. 73, SEQ ID NO. 75/SEQ

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Definitions

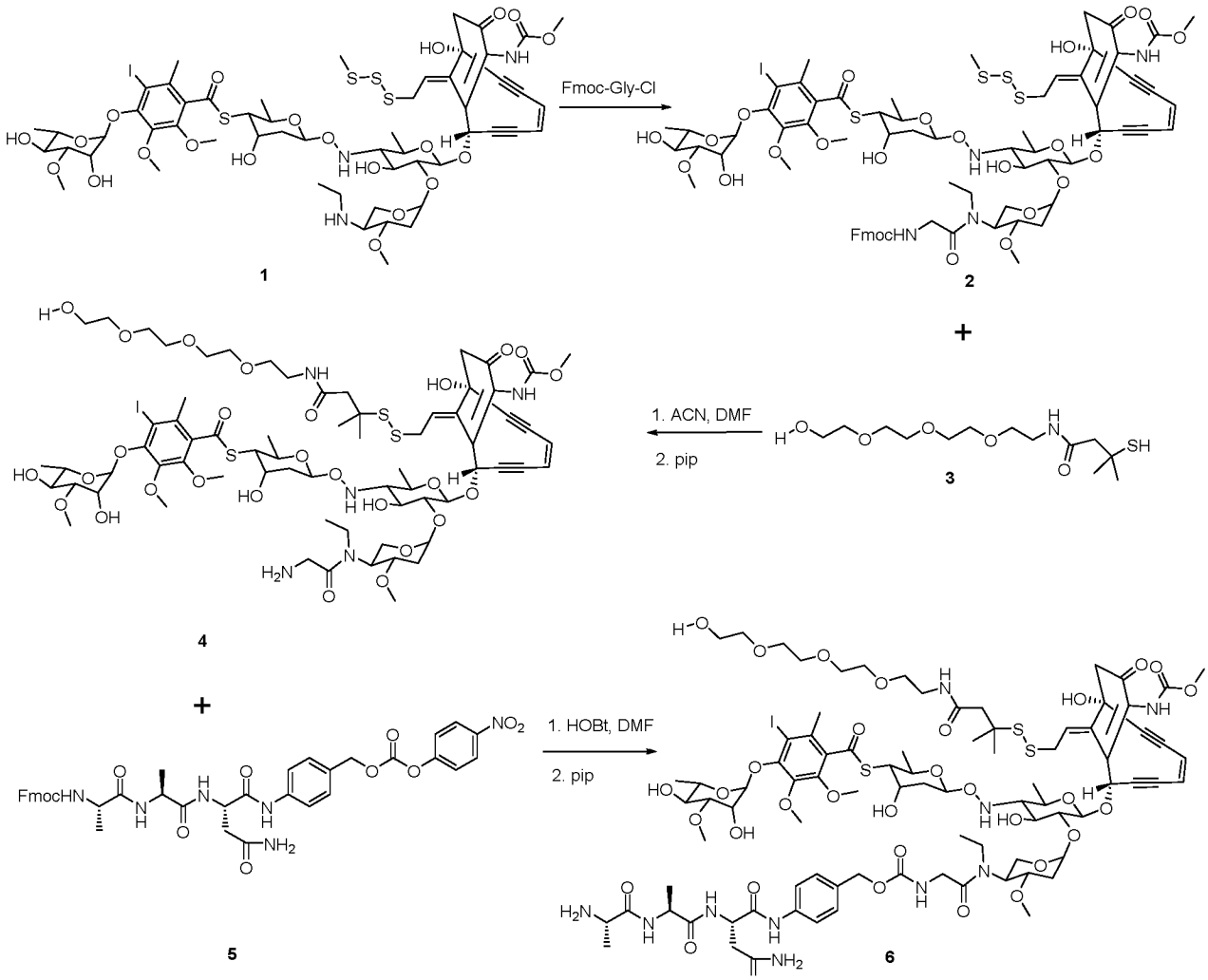
As used herein, common organic abbreviations are defined as follows:

Ac	Acetyl
ACN	Acetonitrile
Ala	Alanine
Asn	Asparagine
aq.	Aqueous
BOC or Boc	tert-Butoxycarbonyl
°C	Temperature in degrees Centigrade
Cit	Citrulline
DCM	dichloromethane
DIEA	Diisopropylethylamine
DMF	*N*,*N*'-Dimethylformamide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	Ethyl
EtOAc	Ethyl acetate
Eq	Equivalents
Fmoc	9-Fluorenylmethoxycarbonyl
g	Gram(s)
h	Hour (hours)
HATU	2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HOBt	N-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
LC/MS	Liquid chromatography-mass spectrometry
Me	Methyl
mg	milligrams
MeOH	Methanol

mL	Milliliter(s)
µL / µL	Microliter(s)
mol	moles
mmol	millimoles
µmol/umol	micromoles
MS	mass spectrometry
NHS	N-Hydroxysuccinimide
PAB	p-aminobenzyl
Pip	piperidine
RP-HPLC	reverse phase HPLC
rt	room temperature
t-Bu	tert-Butyl
Tert, t	tertiary
TFA	Trifluoracetic acid
THF	Tetrahydrofuran
Val	Valine

Synthesis Example 1

Preparation of compound **8** and **8a**:



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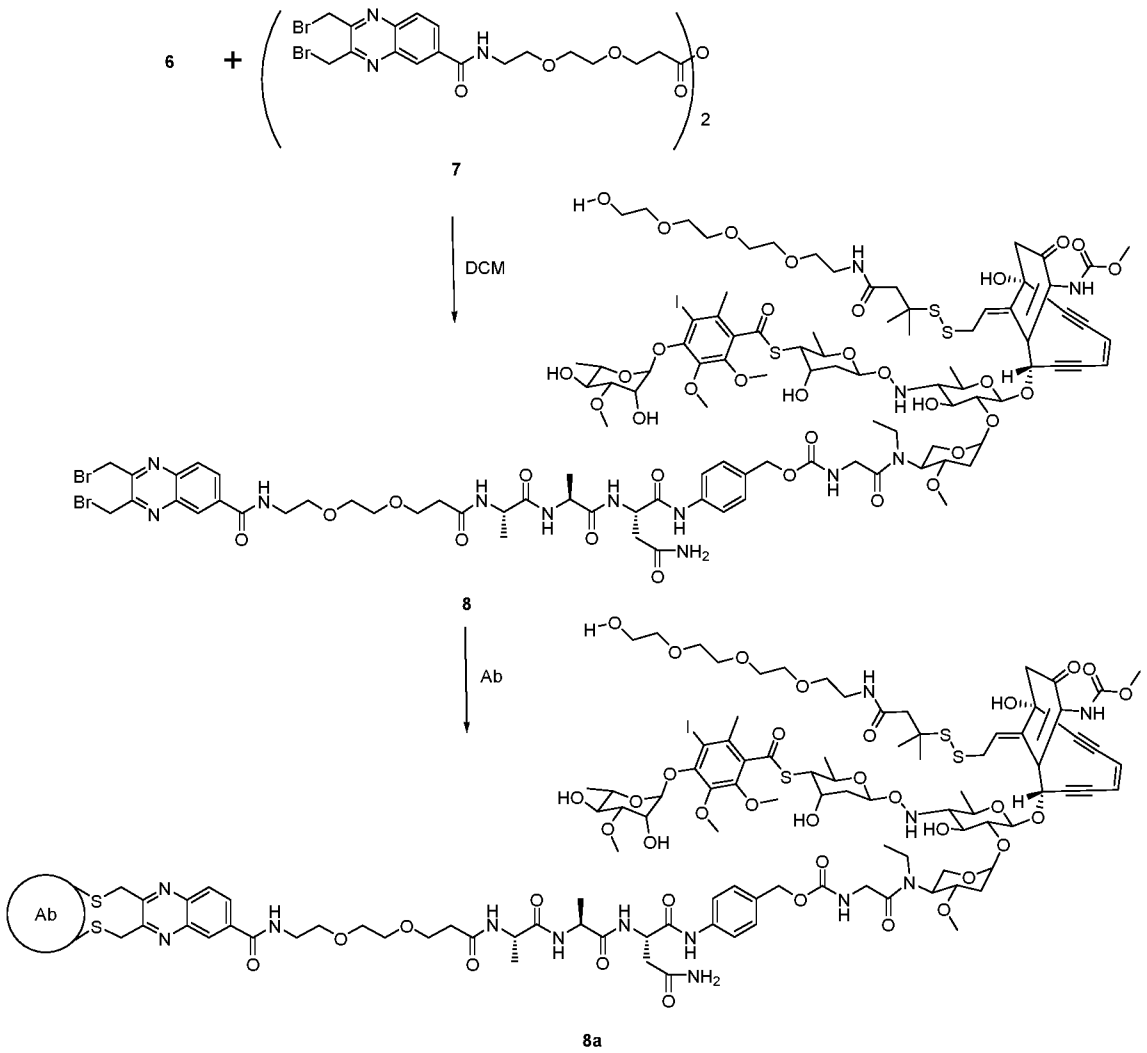


Preparation of compound **2**:

To calicheamicin γ1 (**1**) (880 mg, 0.54 mmol) in 25 mL of dimethylformamide (DMF) was added (9H-fluoren-9-yl)methyl (2-chloro-2-oxoethyl)carbamate (256 mg, 0.81 mmol) and diisopropylethylamine (DIEA, 173 µL, 1 mmol). The mixture was stirred for 2 h, then evaporated and purified by HPLC to give compound **2** (300 mg). MS m/z 1647.3 (M+H).

Preparation of compound **4**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **3** (75 mg, 0.24 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **4** (60 mg). MS m/z 1654.4 (M+H).

Preparation of compound **6**:

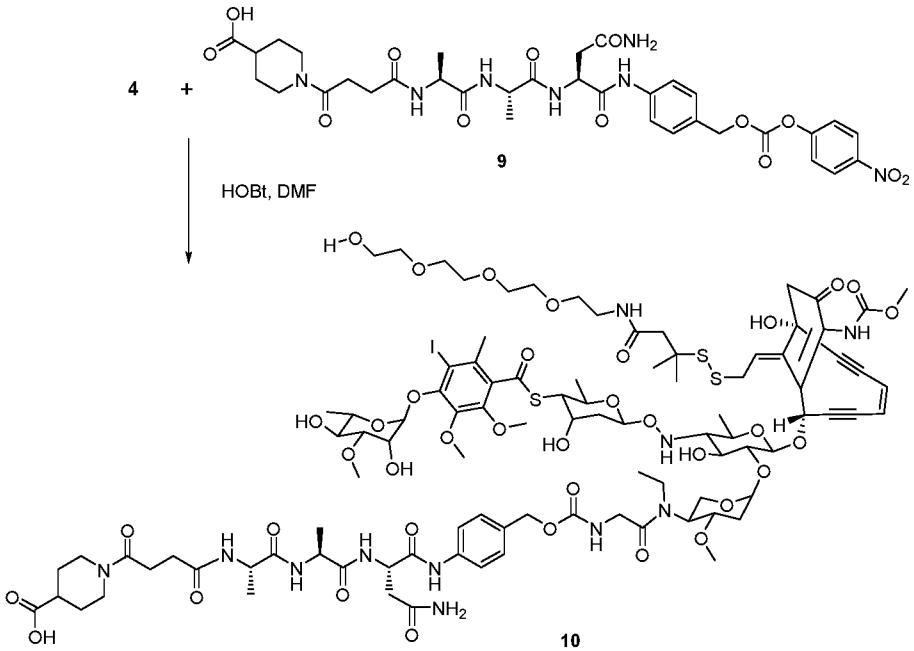
To compound **4** (20 mg, 12 µmol) in 2 mL of DMF was added compound **5** (11 mg, 14 µmol), N-Hydroxybenzotriazole (HOBt, 2 mg), and 5 µL of DIEA. The mixture was

stirred for 1 h, then 40 µL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **6** (21 mg). MS m/z 2059.6 (M+H).

Preparation of compound **8**:

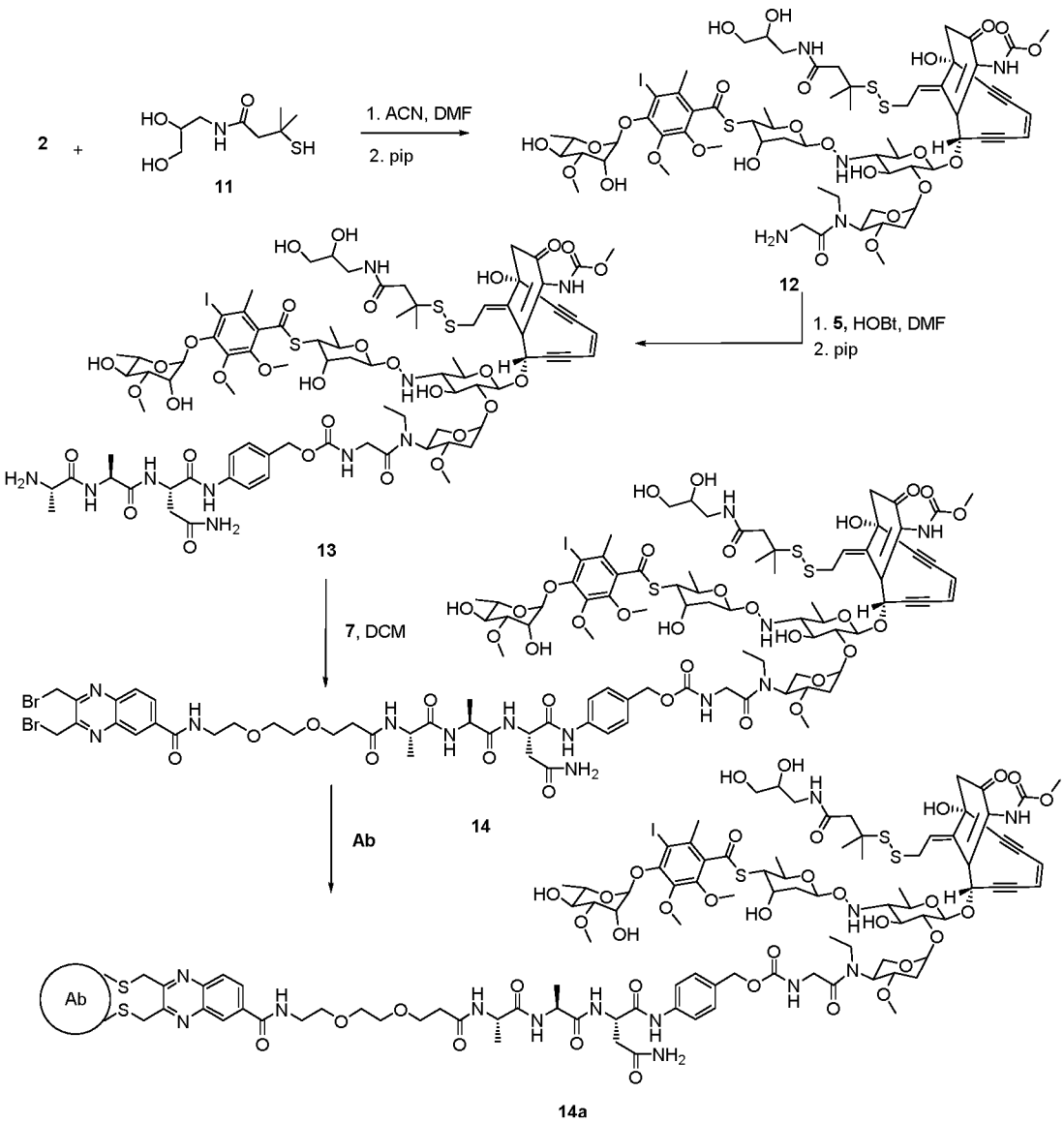
To compound **6** (21 mg, 10 µmol) in 2 mL of dichloromethane (DCM) was added compound **7** (13 mg, 13 µmol), and 3 µL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **8** (11 mg). MS m/z 2558.6 (M+H).

Preparation of compound **10**:



To compound **4** (12 mg, 7.3 µmol) in 1 mL of DMF was added compound **9** (7.1 mg, 9.4 µmol), N-Hydroxybenzotriazole (1 mg), and 5 µL of DIEA. The mixture was stirred for 1 h, then purified by HPLC to give compound **10** (12 mg). MS m/z 2270.6 (M+H).

Preparation of compound **14** and **14a**:



Preparation of compound **12**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **11** (118 mg, 0.57 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **12** (40 mg). MS m/z 1552.4 (M+H).

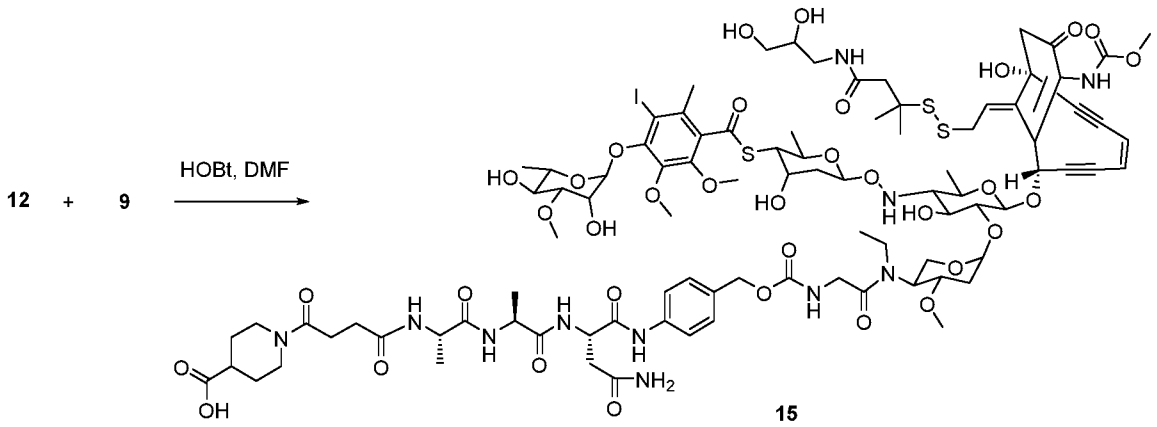
Preparation of compound **13**:

To compound **12** (28 mg, 18 mmol) in 1 mL of DMF was added compound **5** (17 mg, 22 mmol), HOBt (2 mg), and 5 µL of DIEA. The mixture was stirred for 1 h, then 20 µL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **13** (23 mg). MS m/z 1957.6 (M+H).

Preparation of compound **14**:

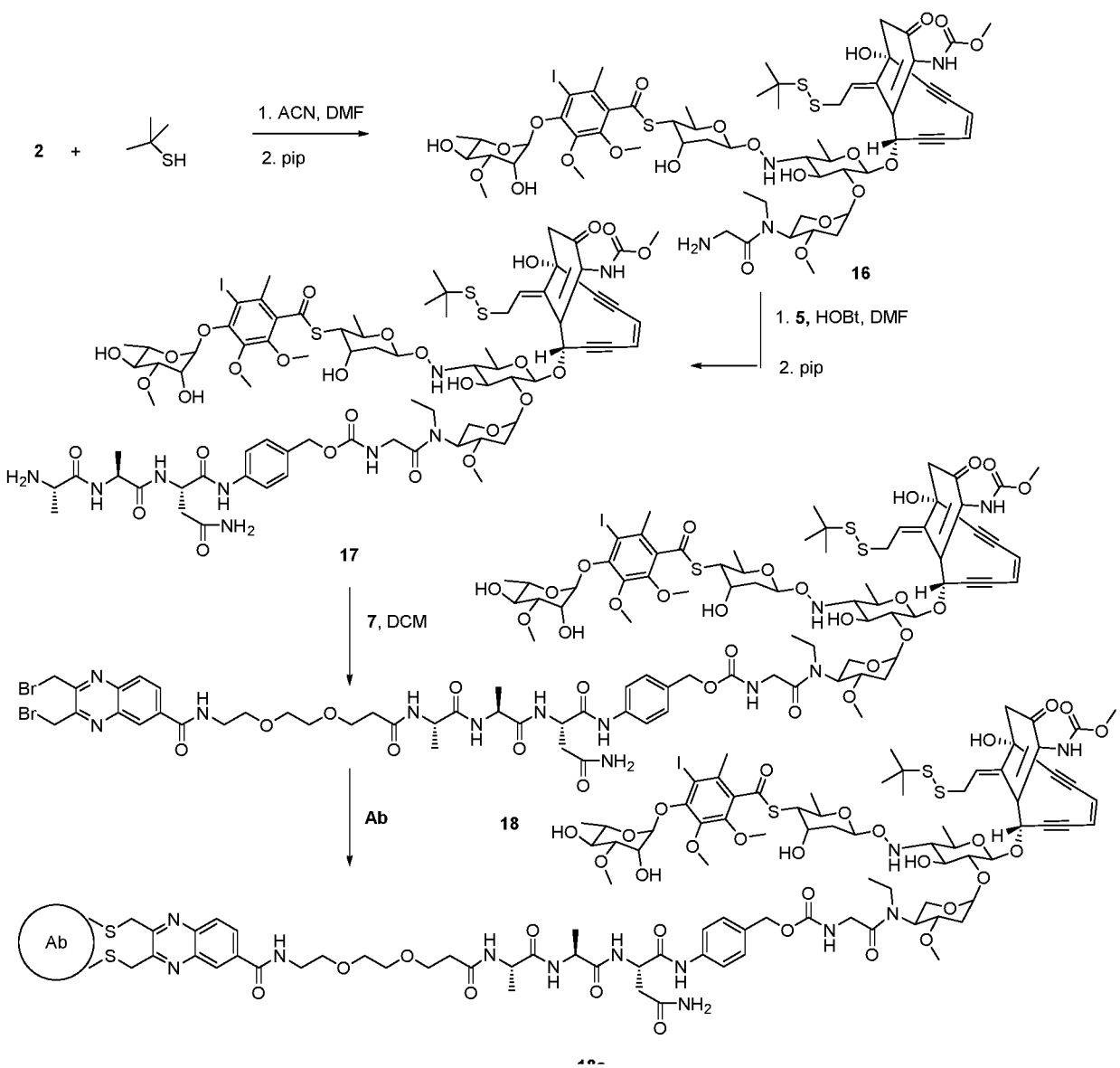
To compound **13** (23 mg, 12 μmol) in 2 mL of DCM was added compound **7** (17 mg, 17 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **14** (16 mg). MS m/z 2558.6 (M+H).

Preparation of compound **15**:



To compound **12** (10 mg, 6.5 μmol) in 1 mL of DMF was added compound **9** (7.1 mg, 9.4 μmol), HOBt (1 mg), and 5 μL of DIEA. The mixture was stirred for 1 h, then purified by HPLC to give compound **15** (11 mg). MS m/z 2168.6 (M+H).

Preparation of compound **18** and **18a**:



Preparation of compound **16**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added tert-butyl thiol (54 mg, 0.6 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **16** (55 mg). MS m/z 1435.4 (M+H).

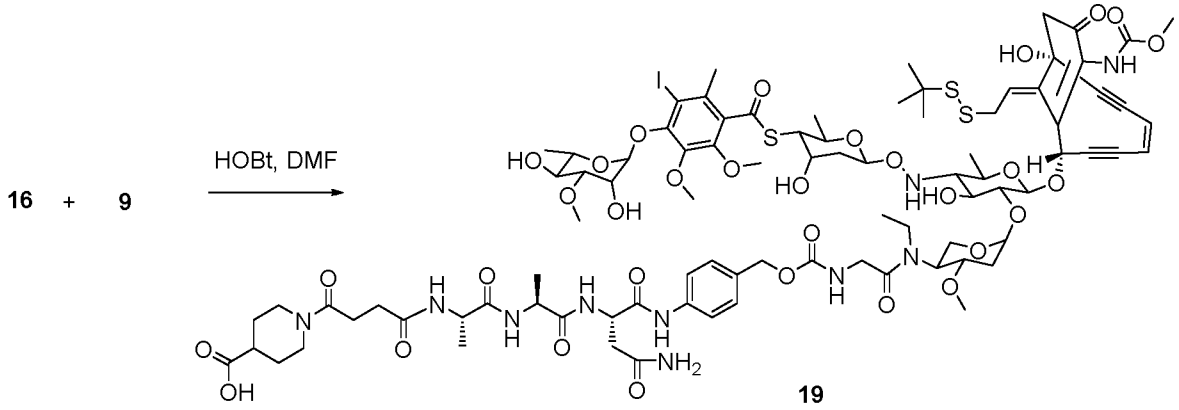
Preparation of compound **17**:

To compound **16** (20 mg, 14 µmol) in 2 mL of DMF was added compound **5** (13 mg, 17 µmol), HOBt (2 mg), and 5 µL of DIEA. The mixture was stirred for 1 h, then 40 µL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **17** (20 mg). MS m/z 1840.6 (M+H).

Preparation of compound **18**:

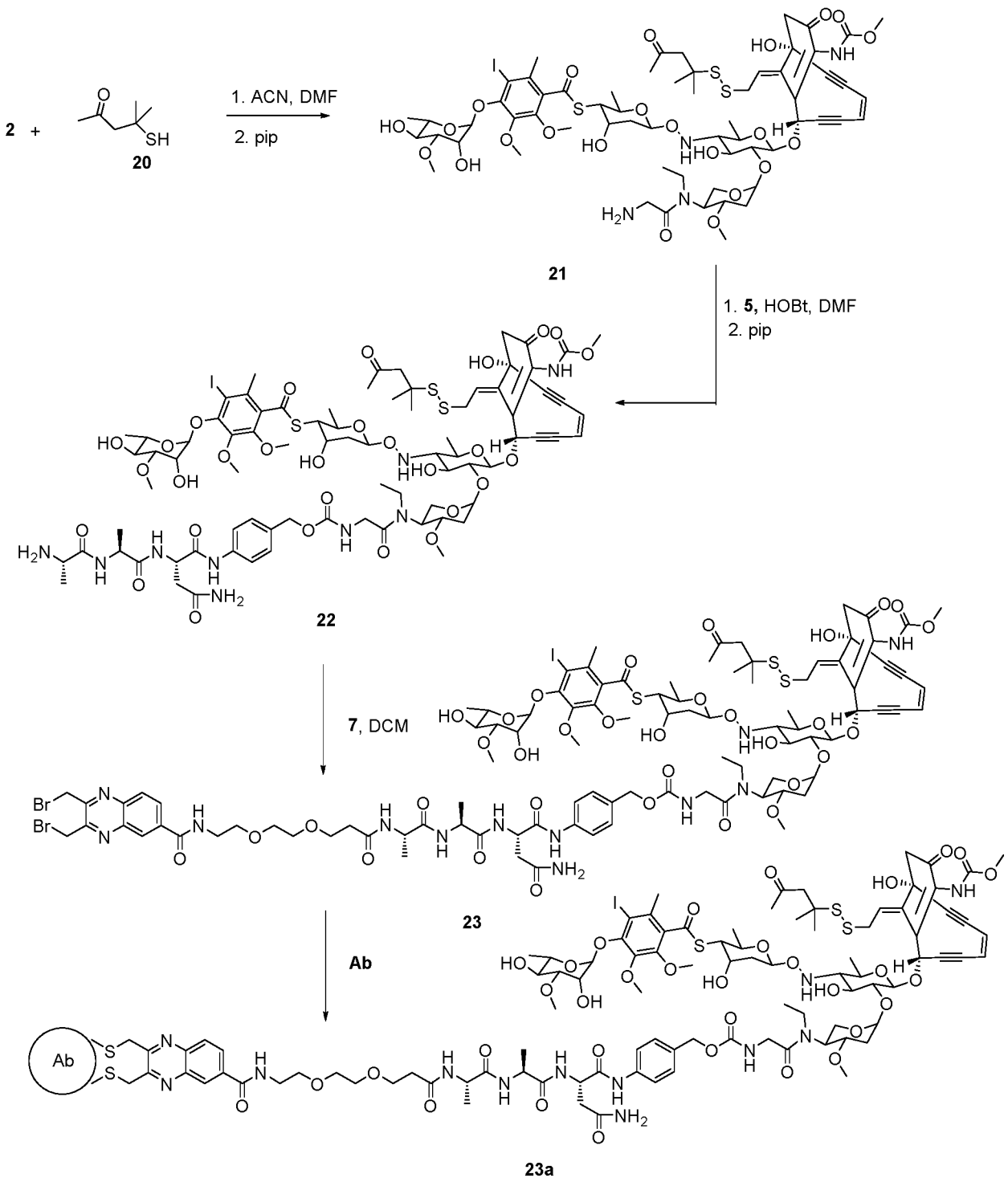
To compound **17** (20 mg, 11 μmol) in 2 mL of DCM was added compound **7** (14 mg, 14 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **18** (16 mg). MS m/z 2339.6 (M+H).

Preparation of compound **19**:



To compound **16** (23 mg, 16 μmol) in 1 mL of DMF was added compound **9** (13 mg, 17 μmol), HOBt (2 mg), and 7 μL of DIEA. The mixture was stirred for 5 h, then purified by HPLC to give compound **19** (21 mg). MS m/z 2051.6 (M+H).

Preparation of compound **23** and **23a**:



Preparation of compound **21**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **20** (24 mg, 0.18 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **21** (50 mg). MS m/z 1477.4 (M+H).

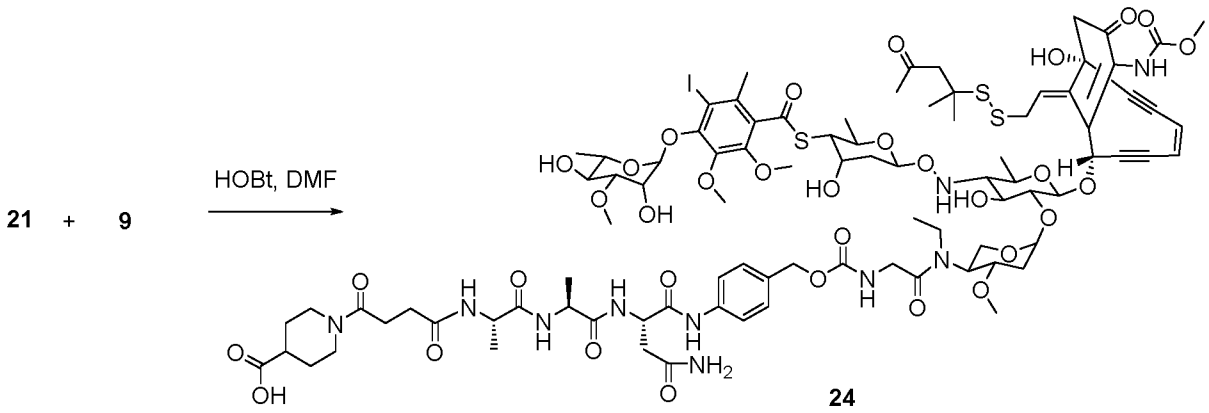
Preparation of compound **22**:

To compound **21** (20 mg, 14 μmol) in 2 mL of DMF was added compound **5** (13 mg, 17 μmol), HOBt (2 mg), and 5 μL of DIEA. The mixture was stirred for 1 h, then 40 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **22** (8 mg). MS m/z 1882.6 (M+H).

Preparation of compound **23**:

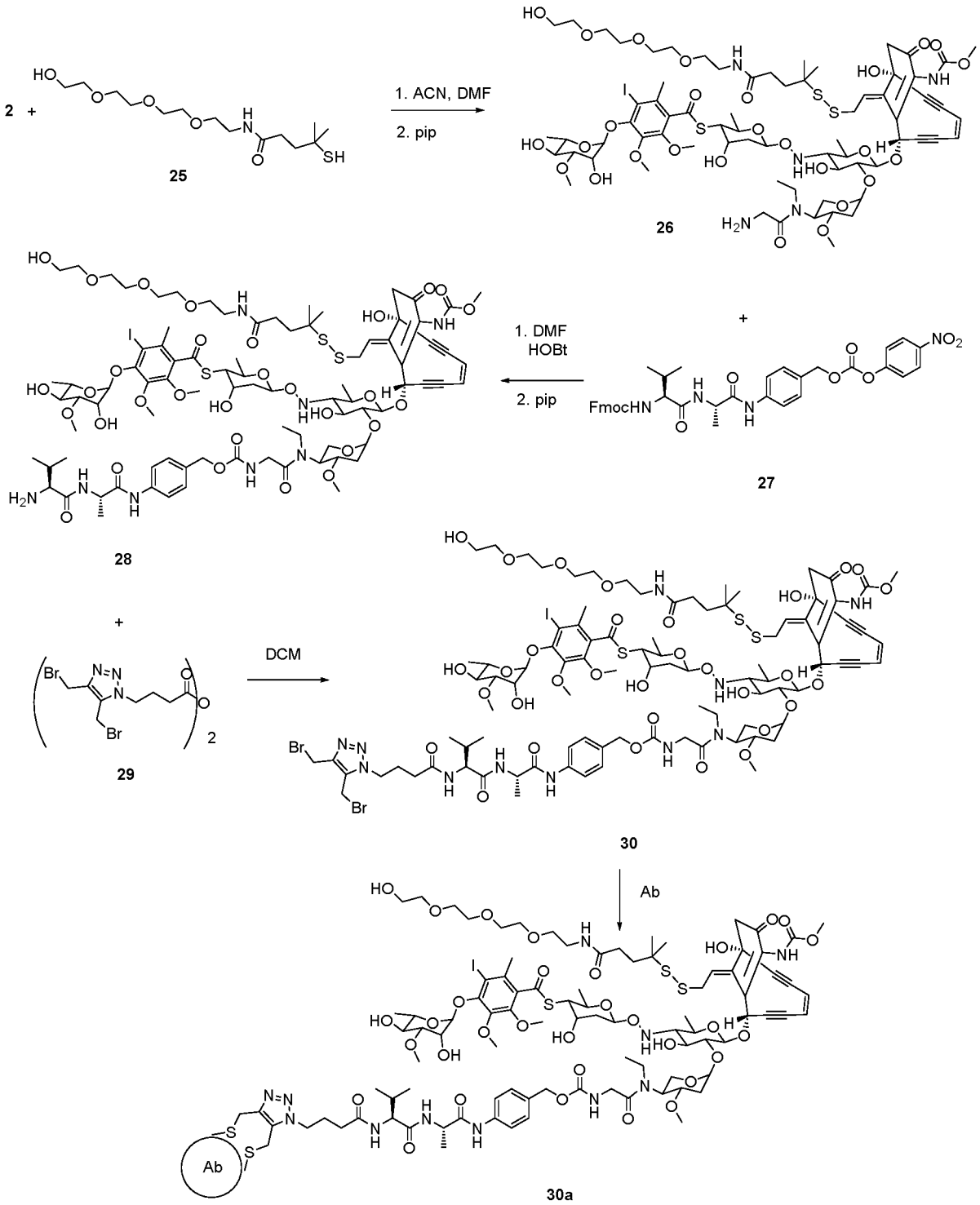
To compound **22** (8 mg, 4 μmol) in 2 mL of DCM was added compound **7** (6 mg, 6 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **23** (6 mg). MS m/z 2381.6 (M+H).

Preparation of compound **24**:



To compound **16** (23 mg, 16 μmol) in 1 mL of DMF was added compound **9** (13 mg, 17 μmol), HOBt (2 mg), and 7 μL of DIEA. The mixture was stirred for 5 h, then purified by HPLC to give compound **19** (21 mg). MS m/z 2093.6 (M+H).

Preparation of compound **30** and **30a**:



Preparation of compound **26**:

To compound **2** (313 mg, 0.19 mmol) in 3 mL of acetonitrile and 1 mL of DMF was added compound **25** (184 mg, 0.57 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 20 min the mixture was purified by HPLC to give compound **26** (160 mg). MS m/z 1668.4 (M+H).

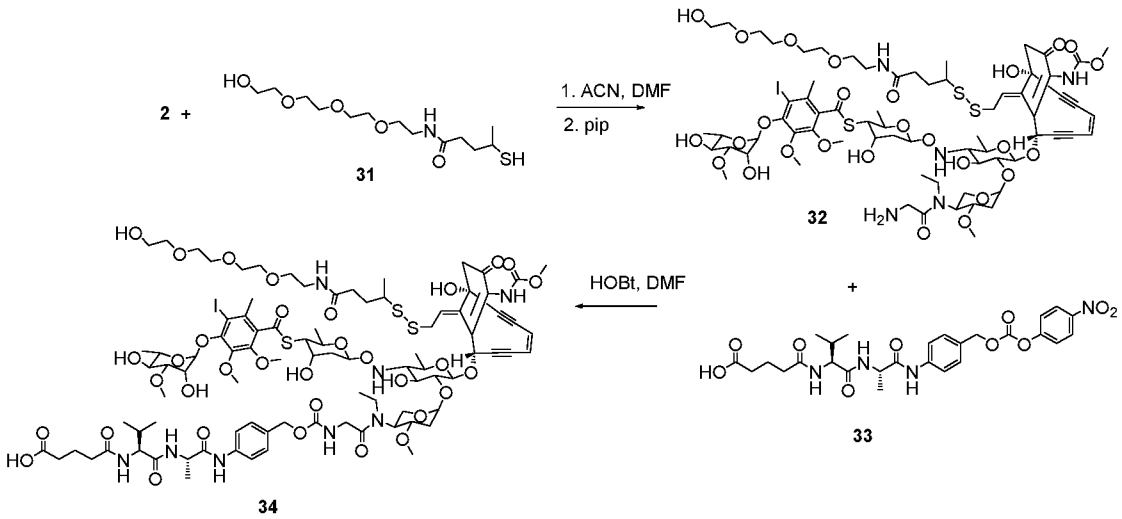
Preparation of compound **28**:

To compound **26** (140 mg, 84 μmol) in 3 mL of DMF was added compound **27** (69 mg, 101 μmol), HOBt (11 mg), and 43 μL of DIEA. The mixture was stirred for 1 h, then 150 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **28** (112 mg). MS m/z 1987.6 (M+H).

Preparation of compound **30**:

To compound **28** (22 mg, 11 μmol) in 2 mL of DCM was added compound **29** (8.6 mg, 13 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **30** (12 mg). MS m/z 2308.6 (M+H).

Preparation of compound **34**:



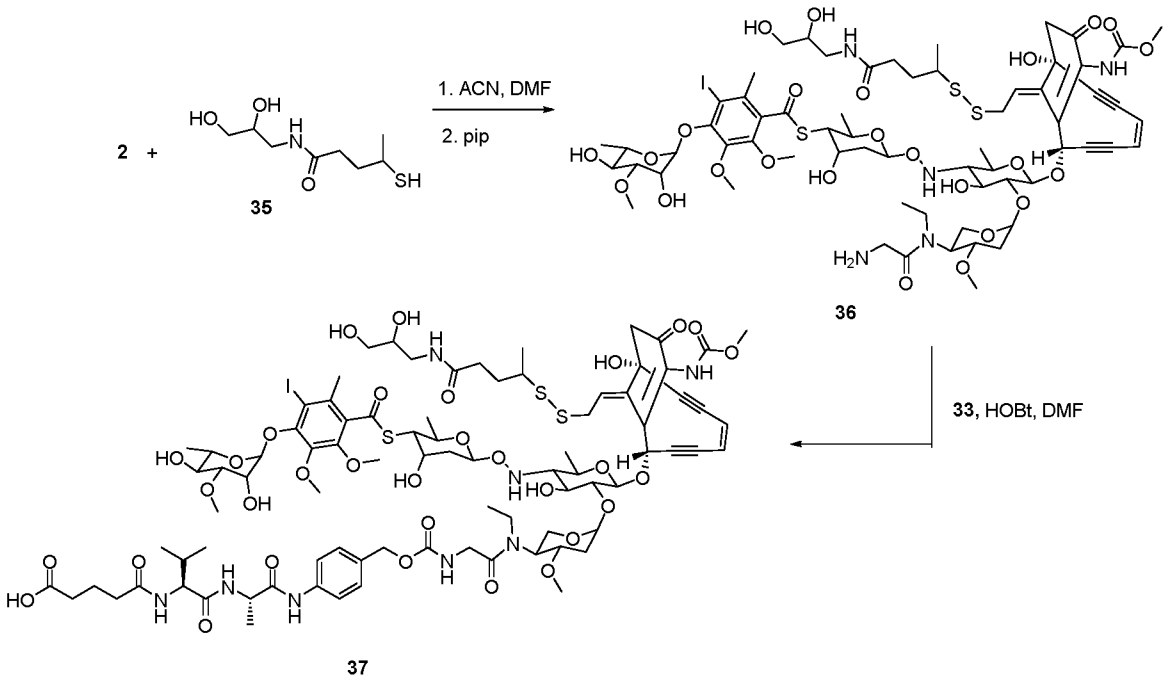
Preparation of compound **32**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **31** (75 mg, 0.24 mmol). The mixture was stirred for 16 h, then 120 μL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **32** (50 mg). MS m/z 1654.4 (M+H).

Preparation of compound **34**:

To compound **32** (20 mg, 12 μmol) in 1 mL of DMF was added compound **33** (10 mg, 17 μmol), HOBt (2 mg), and 5 μL of DIEA. The mixture was stirred for 5 h, then purified by HPLC to give compound **34** (18 mg). MS m/z 2087.6 (M+H).

Preparation of compound **37**:



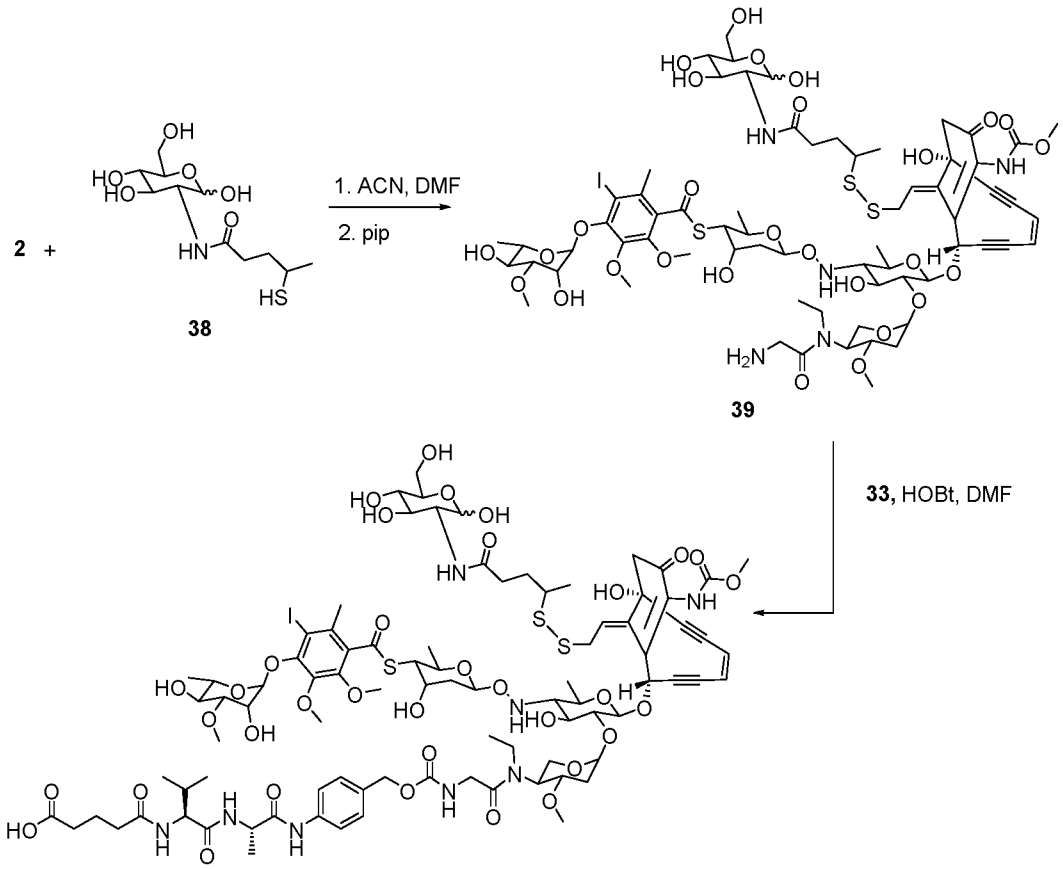
Preparation of compound **36**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **35** (37 mg, 0.18 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **36** (45 mg). MS m/z 1552.4 (M+H).

Preparation of compound **37**:

To compound **32** (20 mg, 13 µmol) in 1 mL of DMF was added compound **33** (10 mg, 17 µmol), HOBt (2 mg), and 5 µL of DIEA. The mixture was stirred for 5 h, then purified by HPLC to give compound **37** (19 mg). MS m/z 1985.6 (M+H).

Preparation of compound **40**:



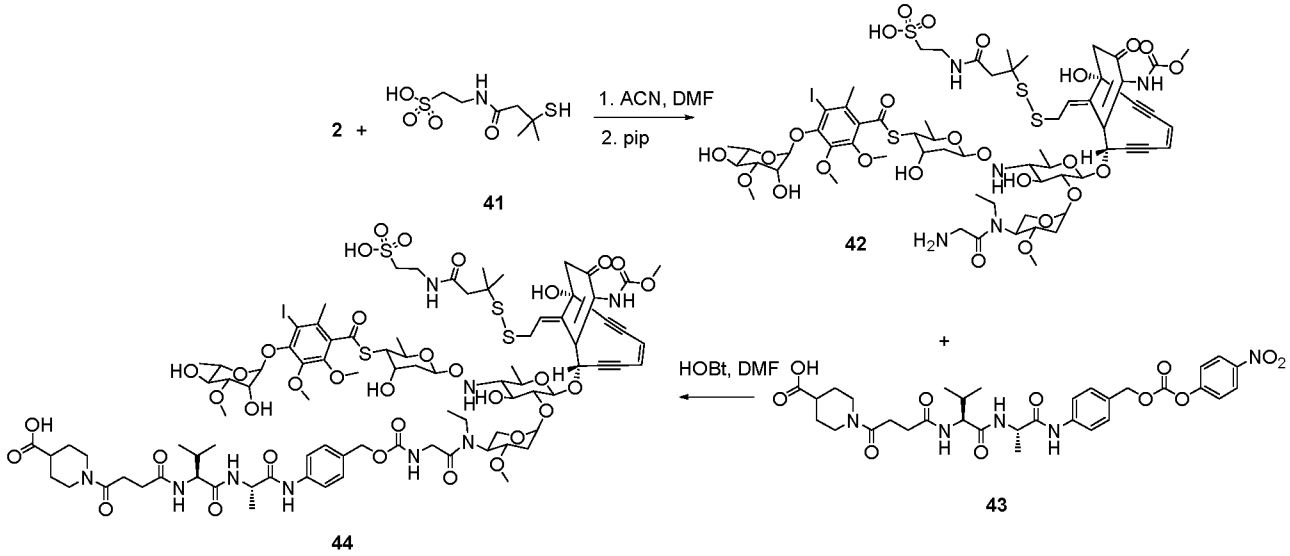
Preparation of compound **39**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **38** (53 mg, 0.18 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **39** (43 mg). MS m/z 1640.4 (M+H).

Preparation of compound **40**:

To compound **39** (20 mg, 12 µmol) in 1 mL of DMF was added compound **33** (10 mg, 17 µmol), HOBt (2 mg), and 5 µL of DIEA. The mixture was stirred for 5 h, then purified by HPLC to give compound **40** (16 mg). MS m/z 2073.6 (M+H).

Preparation of compound **44**:



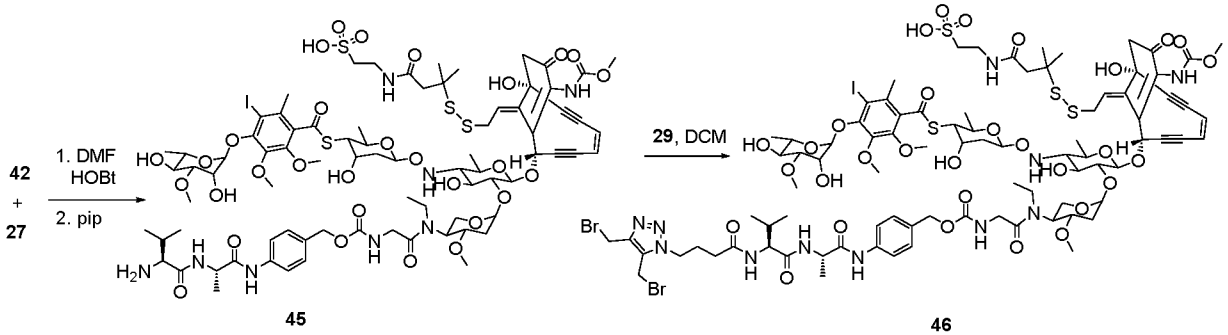
Preparation of compound **42**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added compound **41** (88 mg, 0.36 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **42** (53 mg). MS m/z 1586.4 (M+H).

Preparation of compound **44**:

To compound **42** (19 mg, 12 µmol) in 1 mL of DMF was added compound **43** (10 mg, 15 µmol), HOBt (2 mg), and 8 µL of DIEA. The mixture was stirred for 5 h, then purified by HPLC to give compound **44** (25 mg). MS m/z 2116.6 (M+H).

Preparation of compound **46**:



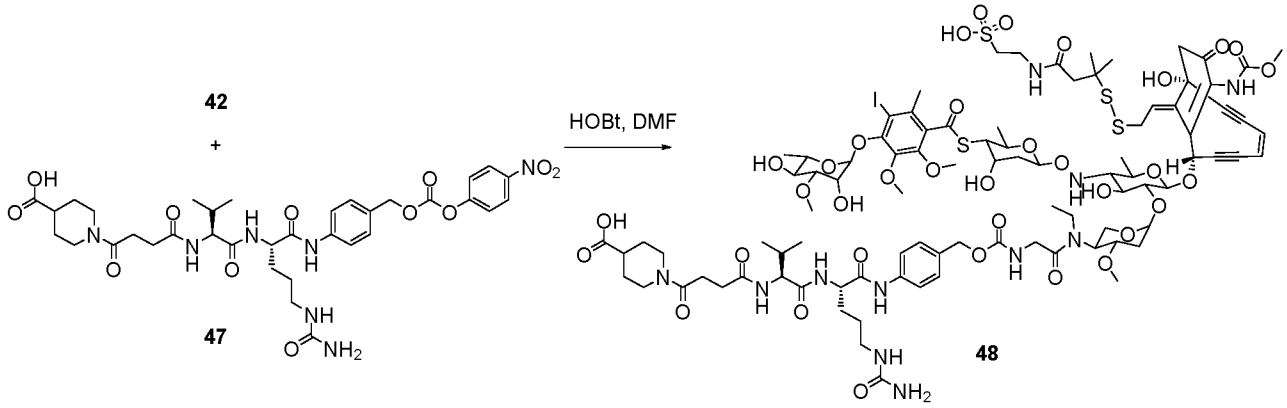
Preparation of compound **45**:

To compound **42** (30 mg, 19 umol) in 2 mL of DMF was added compound **27** (16 mg, 23 µmol), HOBt (5 mg), and 13 µL of DIEA. The mixture was stirred for 1 h, then 40 µL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **45** (22 mg). MS m/z 1905.5 (M+H).

Preparation of compound **46**:

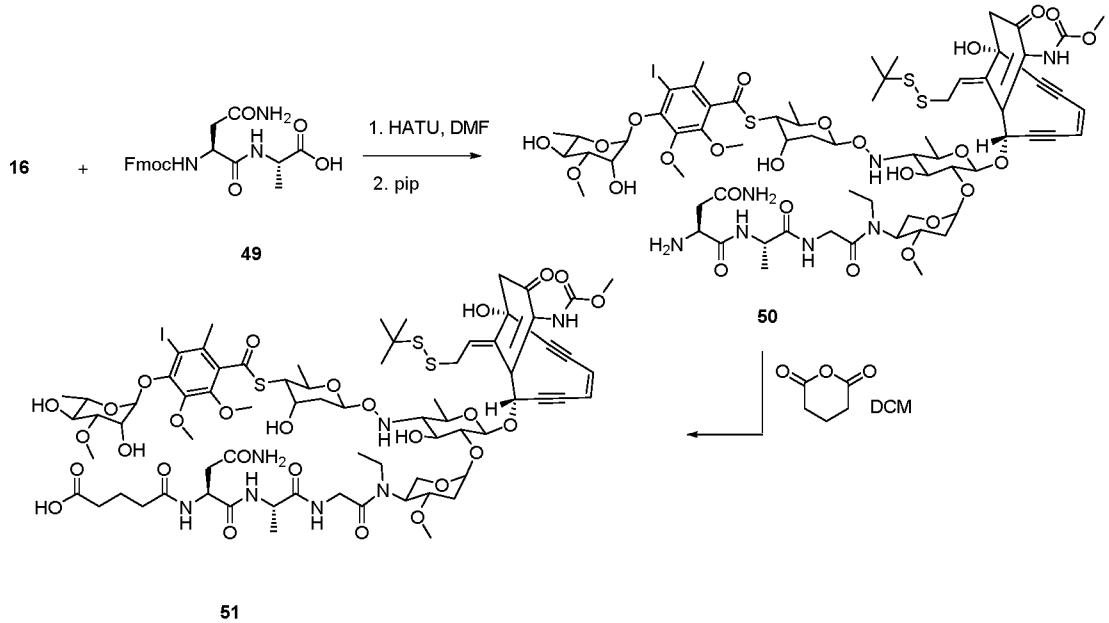
To compound **45** (18 mg, 10 μmol) in 2 mL of DCM was added compound **29** (8.6 mg, 13 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **46** (20 mg). MS m/z 2226.6 (M+H).

Preparation of compound **48**:



To compound **42** (6.2 mg, 3.9 μmol) in 1 mL of DMF was added compound **47** (3.1 mg, 4.2 μmol), HOBt (1 mg), and 3 μL of DIEA. The mixture was stirred for 1 h, then purified by HPLC to give compound **48** (5 mg). MS m/z 2202.6 (M+H).

Preparation of compound **51**:



Preparation of compound **50**:

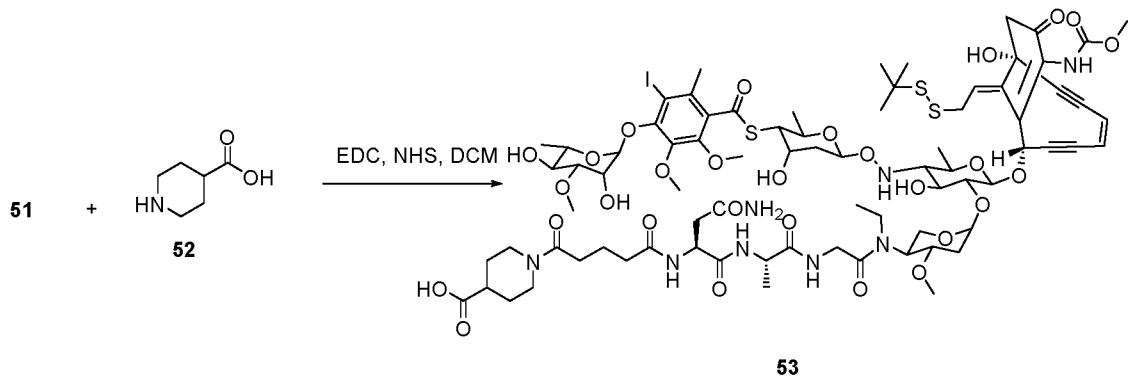
To compound **16** (14 mg, 10 μmol) in 2 mL of DMF was added compound **49** (8 mg, 19 μmol), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate

(HATU, 7 mg, 19 μmol), and 7 μL of DIEA. The mixture was stirred for 1 h, then 50 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **50** (12 mg). MS m/z 1620.5 (M+H).

Preparation of compound **51**:

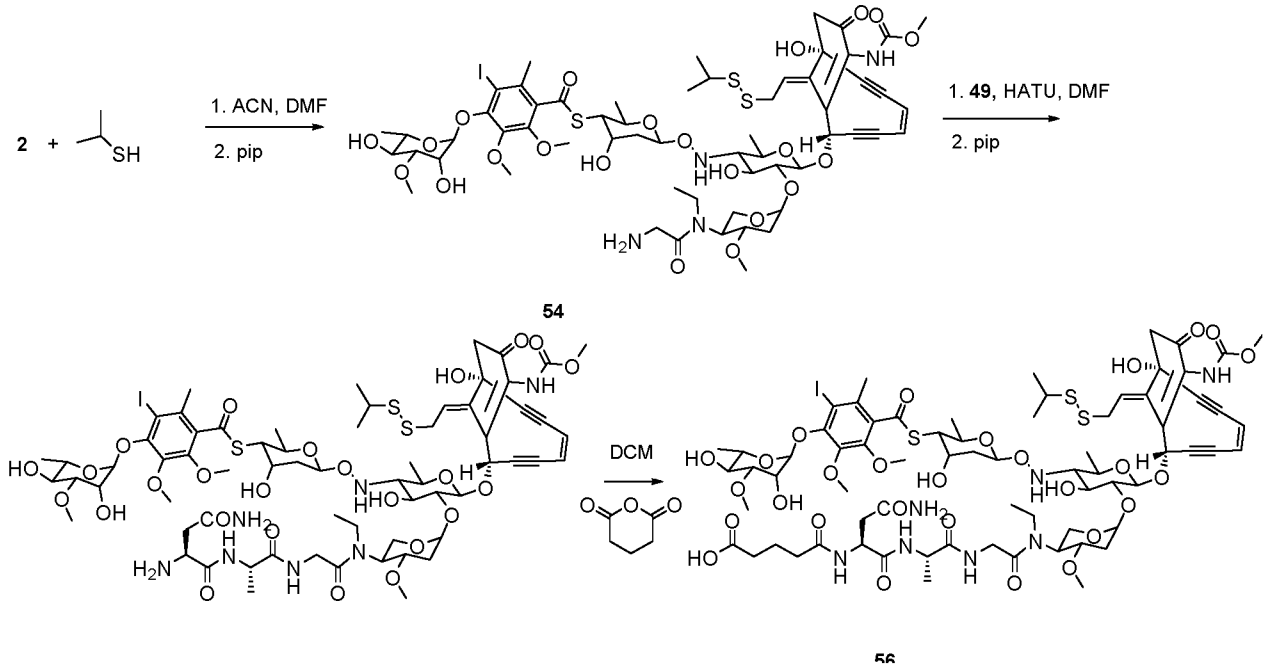
To compound **50** (12 mg, 8 μmol) in 2 mL of DCM was added glutaric anhydride (1 mg, 9 μmol), and 2 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **51** (10 mg). MS m/z 1734.5 (M+H).

Preparation of compound **53**:



To compound **51** (14 mg, 8.1 μmol) in 2 mL of DCM was added N-hydroxylsuccinimide (NHS, 3 mg, 26 μmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 50 mg, 260 μmol) and the mixture was stirred for 20 min. Then the mixture was washed with water (2 mL), evaporated and the residue was dissolved in 1.5 mL of acetonitrile, 0.5 mL of sat. $NaHCO_3$ aq. Then compound **52** (10 mg, 80 μmol) was added and the mixture was stirred for 30 min, then the mixture was purified by HPLC to give compound **53** (13 mg). MS m/z 1845.6 (M+H).

Preparation of compound **56**:



Preparation of compound **54**:

To compound **2** (100 mg, 0.06 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added isopropyl thiol (46 mg, 0.6 mmol). The mixture was stirred for 16 h, then 120 µL of piperidine was added. After 30 min the mixture was purified by HPLC to give compound **54** (50 mg). MS m/z 1421.4 (M+H).

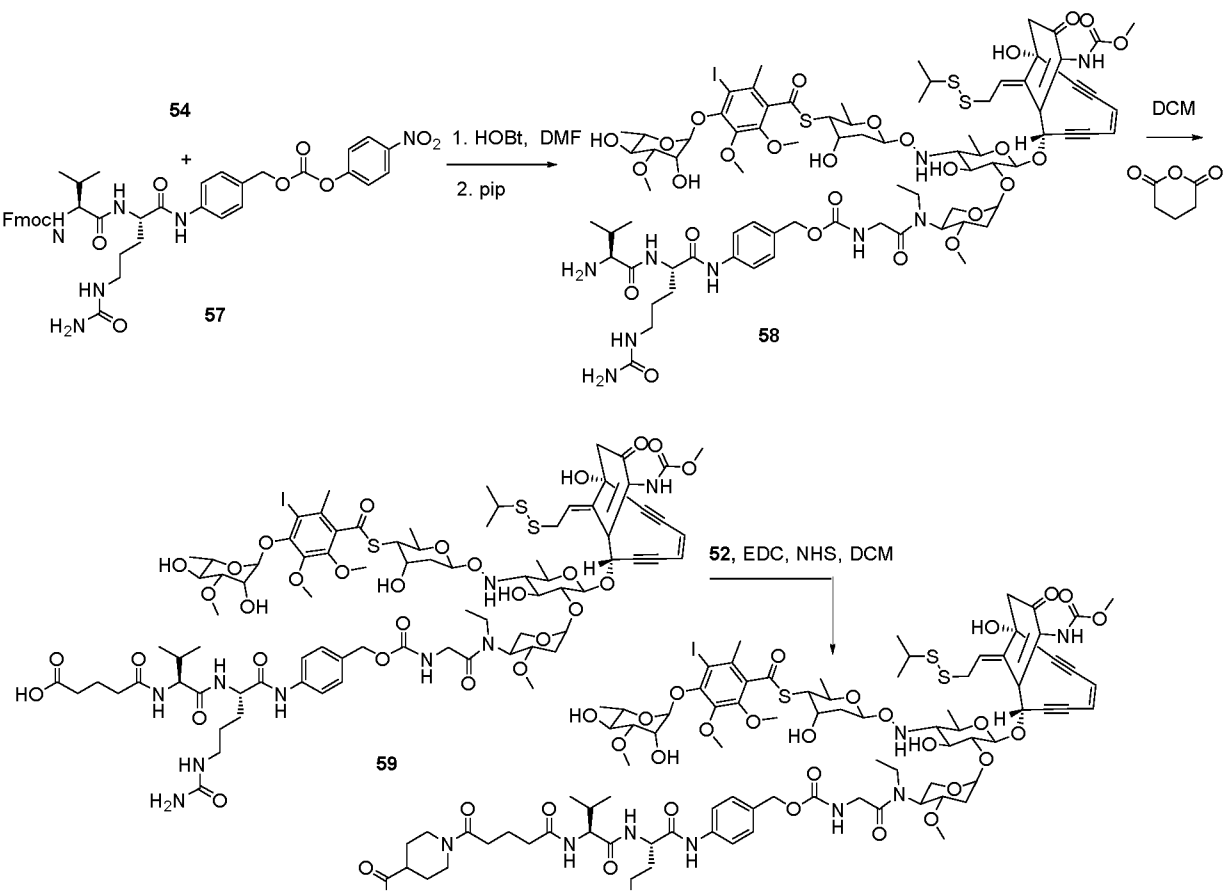
Preparation of compound **55**:

To compound **54** (15 mg, 10 µmol) in 2 mL of DMF was added compound **49** (8 mg, 19 µmol), HATU (7 mg, 19 umol), and 7 µL of DIEA. The mixture was stirred for 1 h, then 50 µL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **55** (9 mg). MS m/z 1606.5 (M+H).

Preparation of compound **56**:

To compound **55** (9 mg, 5.6 µmol) in 2 mL of DCM was added glutaric anhydride (1 mg, 9 µmol), and 2 µL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **56** (8 mg). MS m/z 1720.5 (M+H).

Preparation of compound **60**:



Preparation of compound **58**:

To compound **54** (23mg, 16 μmol) in 2 mL of DMF was added compound **57** (15 mg, 20 μmol), HOBt (2.5 mg), and 10 μL of DIEA. The mixture was stirred for 1 h, then 40 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **58** (21 mg). MS m/z 1826.5 (M+H).

Preparation of compound **59**:

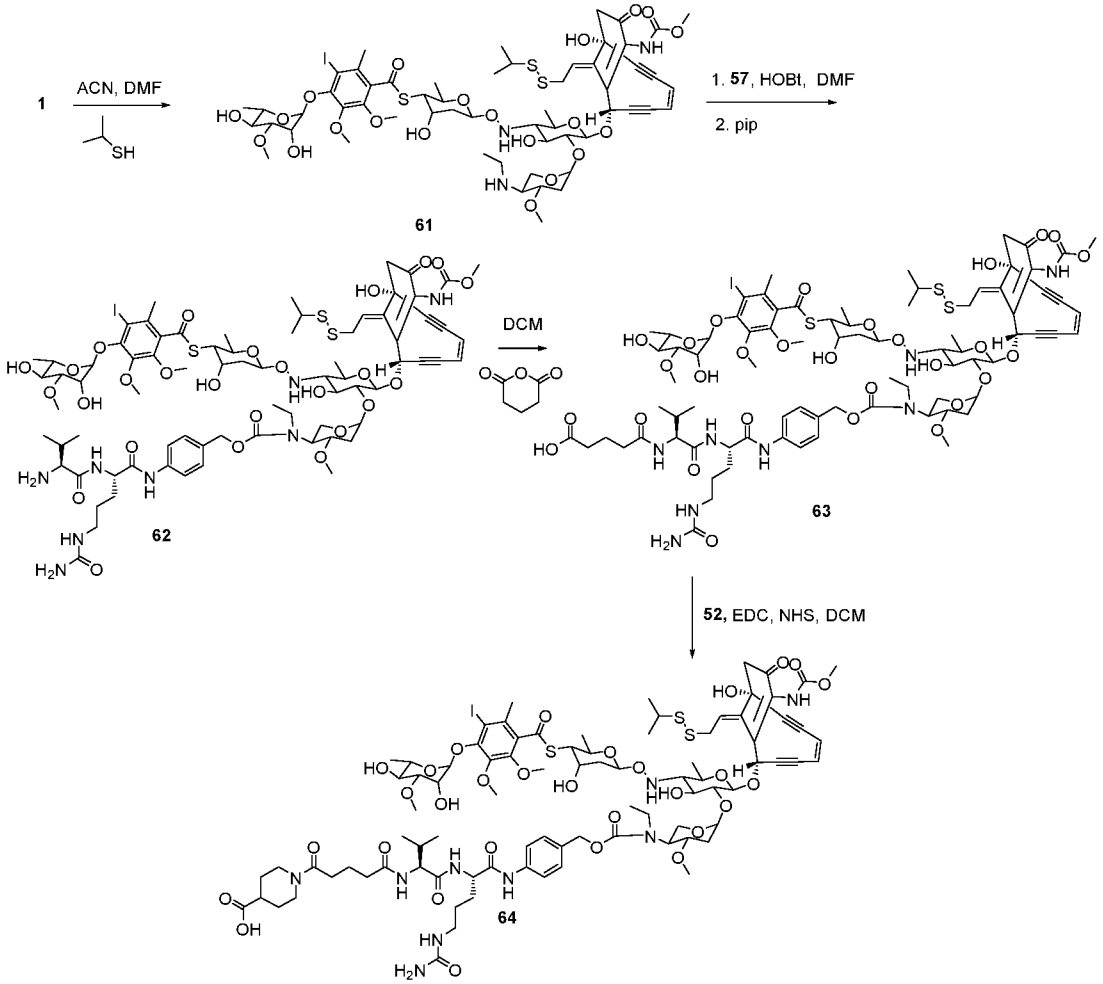
To compound **58** (21 mg, 12 μmol) in 2 mL of DCM was added glutaric anhydride (2 mg, 18 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **59** (18 mg). MS m/z 1940.5 (M+H).

Preparation of compound **60**:

To compound **59** (16 mg, 8 umol) in 2 mL of DCM was added NHS (3 mg, 26 μmol), EDC (50 mg, 260 μmol) and the mixture was stirred for 20 min. Then the mixture was washed with water (2 mL), evaporated and the residue was dissolved in 2 mL of acetonitrile, 0.5 mL of sat. $NaHCO_3$ aq. Then compound **52** (10 mg, 80 μmol) was added and the mixture

was stirred for 30 min, then the mixture was purified by HPLC to give compound **60** (13 mg). MS m/z 2051.6 (M+H).

Preparation of compound **64**:



Preparation of compound **61**:

To compound **1** (86 mg, 0.063 mmol) in 4 mL of acetonitrile and 0.3 mL of DMF was added isopropyl thiol (46 mg, 0.6 mmol). The mixture was stirred for 16 h, then the mixture was purified by HPLC to give compound **61** (25 mg). MS m/z 1364.3 (M+H).

Preparation of compound **62**:

To compound **61** (22mg, 16 μmol) in 2 mL of DMF was added compound **57** (15 mg, 20 μmol), HOBt (2.5 mg), and 10 μL of DIEA. The mixture was stirred for 16 h, then 40 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **62** (11 mg). MS m/z 1769.5 (M+H).

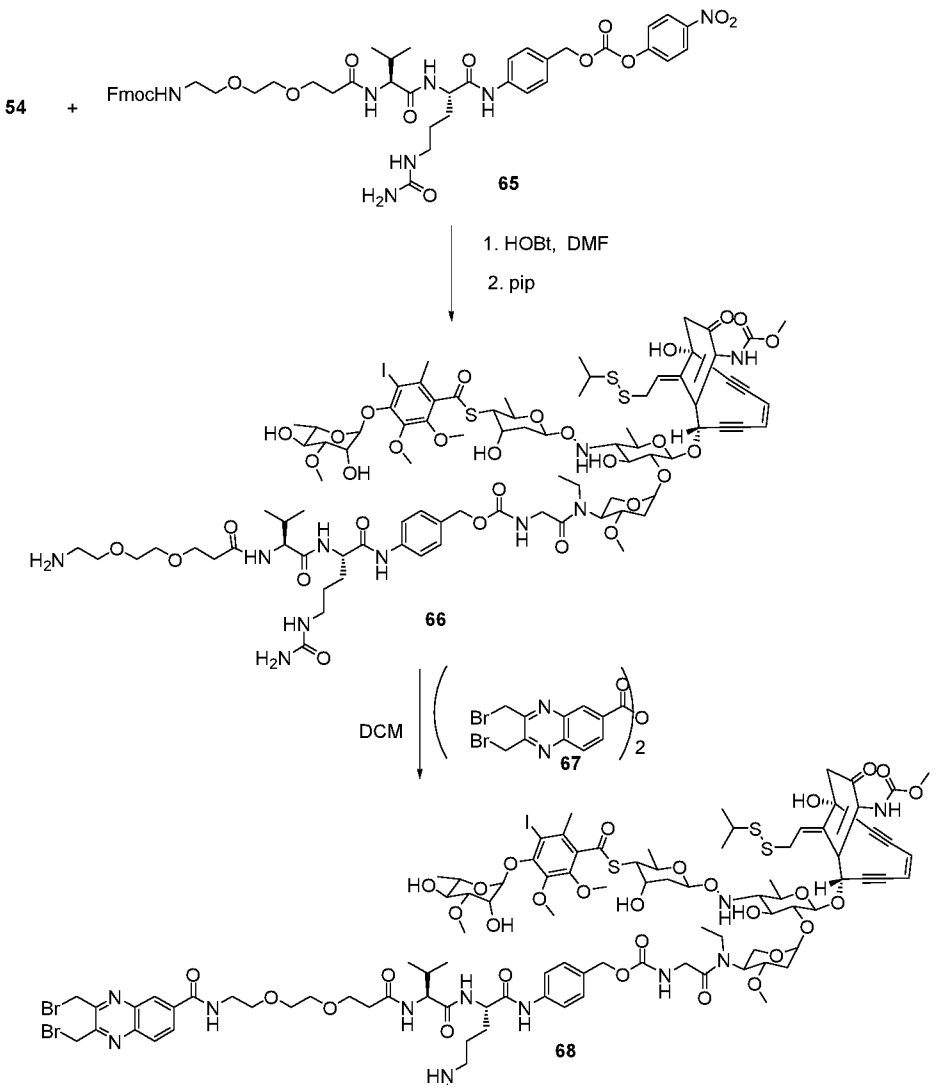
Preparation of compound **63**:

To compound **62** (11 mg, 6 μmol) in 2 mL of DCM was added glutaric anhydride (1 mg, 9 μmol), and 2 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **63** (9 mg). MS m/z 1883.5 (M+H).

Preparation of compound **64**:

To compound **63** (9 mg, 4.8 μmol) in 2 mL of DCM was added NHS (3 mg, 26 μmol), EDC (50 mg, 260 μmol) and the mixture was stirred for 20 min. Then the mixture was washed with water (2 mL), evaporated and the residue was dissolved in 2 mL of acetonitrile, 0.5 mL of sat. $NaHCO_3$ aq. Then compound **52** (5 mg, 40 μmol) was added and the mixture was stirred for 30 min, then the mixture was purified by HPLC to give compound **64** (8 mg). MS m/z 1994.6 (M+H).

Preparation of compound **68**:



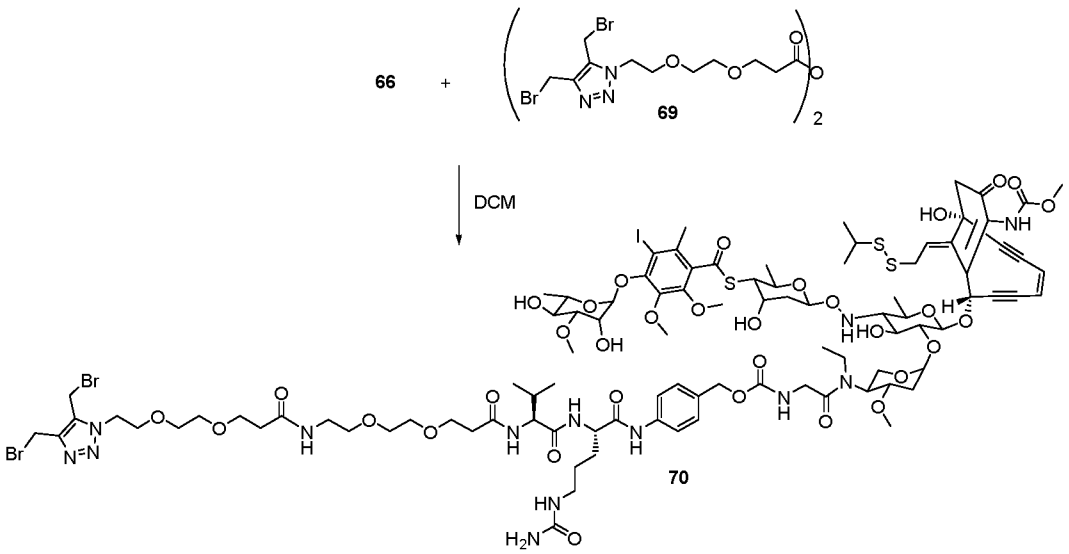
Preparation of compound **66**:

To compound **54** (10 mg, 7 μmol) in 1 mL of DMF was added compound **65** (8 mg, 8.4 μmol), HOBt (1 mg), and 5 μL of DIEA. The mixture was stirred for 2 h, then 40 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **66** (8.6 mg). MS m/z 1985.5 (M+H).

Preparation of compound **68**:

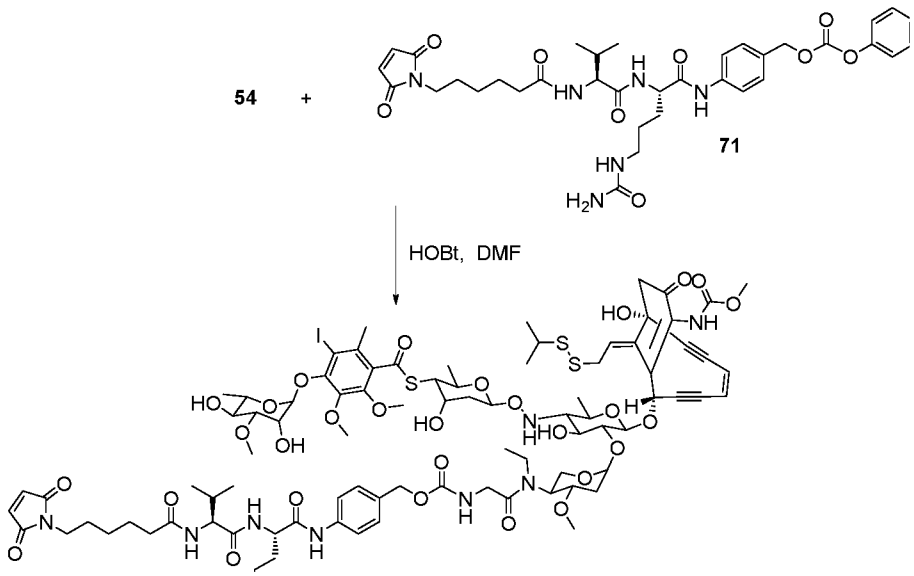
To compound **66** (8.6 mg, 4.3 μmol) in 2 mL of DCM was added compound **67** (2.1 mg, 3 μmol), and 2 μL of DIEA. The mixture was stirred for 10 min, then evaporated and purified by HPLC to give compound **68** (5 mg). MS m/z 2325.5 (M+H).

Preparation of compound **70**:



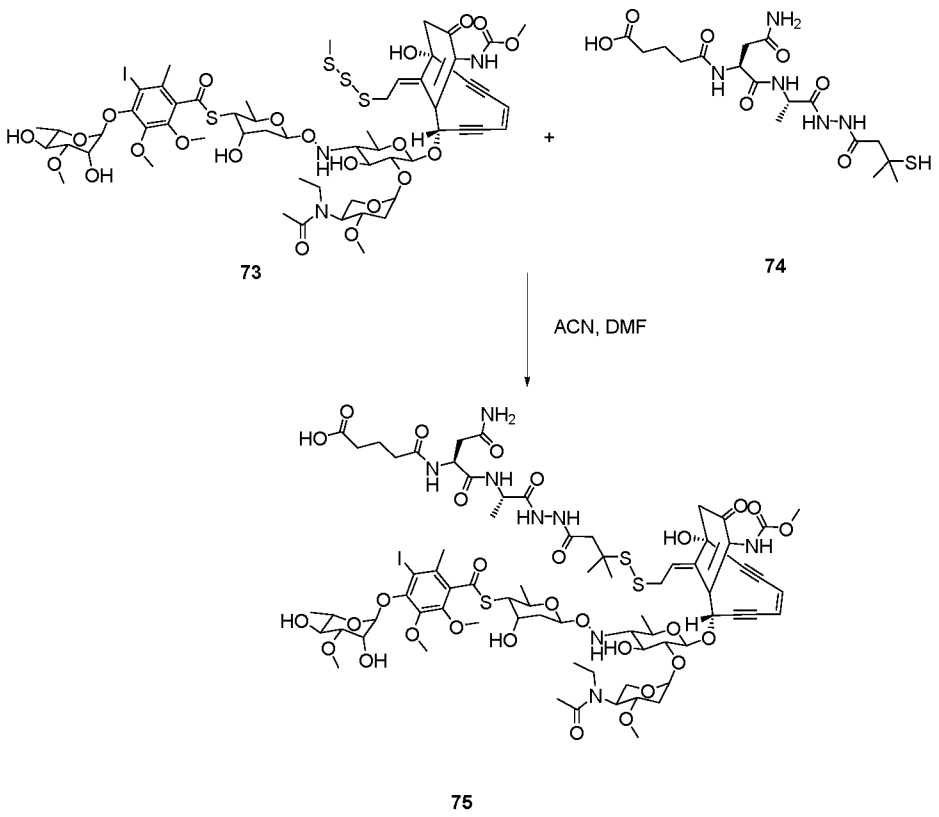
To compound **66** (4.5 mg, 2.3 μmol) in 2 mL of DCM was added compound **69** (3.3 mg, 5 μmol), and 2 μL of DIEA. The mixture was stirred for 10 min, then evaporated and purified by HPLC to give compound **70** (4.5 mg). MS m/z 2380.6 (M+H).

Preparation of compound **72**:



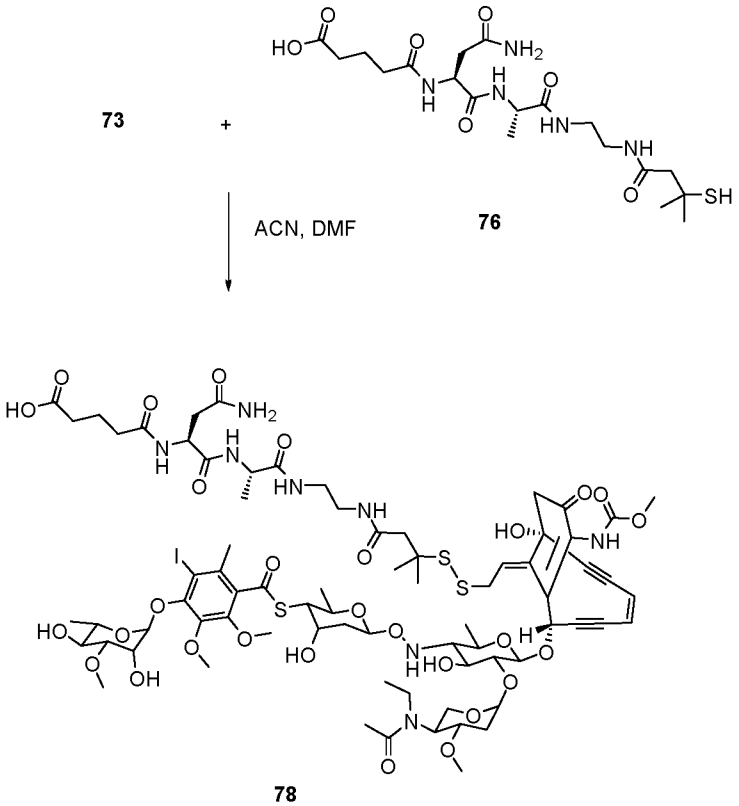
To compound **54** (10 mg, 7 μmol) in 1 mL of DMF was added compound **71** (6 mg, 8.4 μmol), HOBt (1 mg), and 5 μL of DIEA. The mixture was stirred for 2 h, then purified by HPLC to give compound **72** (8.6 mg). MS m/z 2019.6 (M+H).

Preparation of compound **75**:



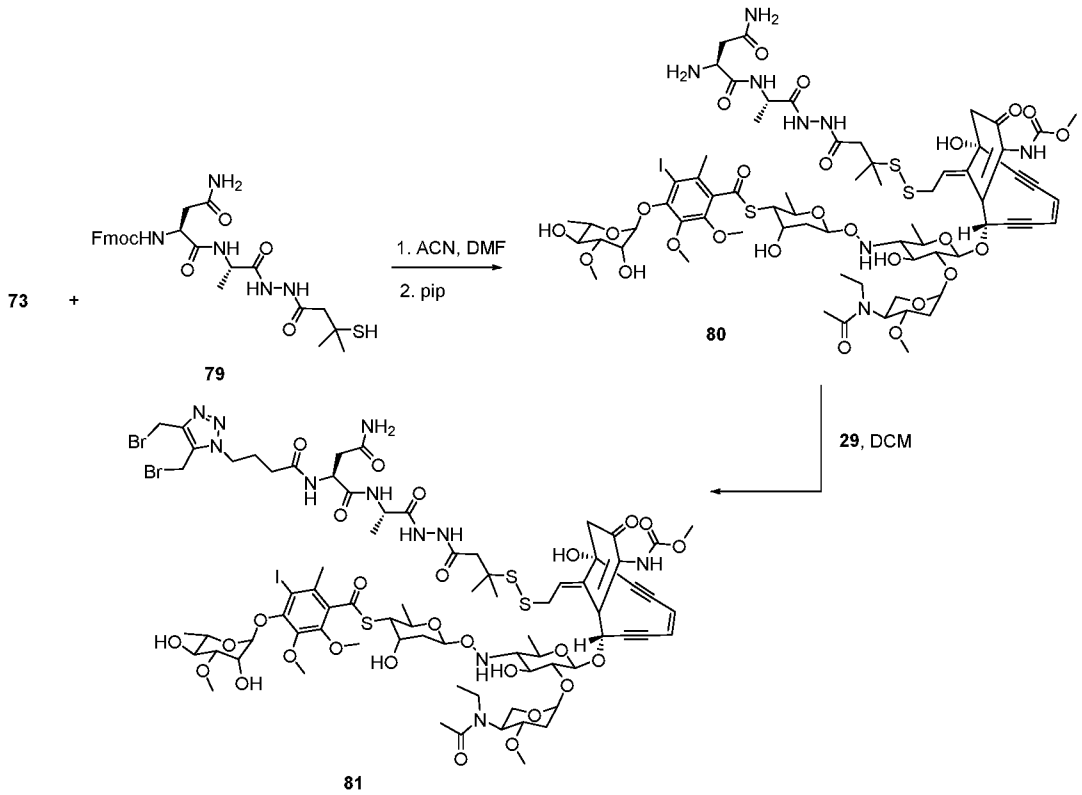
To compound **73** (50 mg, 35 μmol) in 2 mL of DMF and 1 mL of acetonitrile was added compound **74** (32 mg, 71 μmol). The mixture was stirred for 16 h, then purified by HPLC to give compound **75** (25 mg). MS m/z 1777.5 (M+H).

Preparation of compound **78**:



To compound **73** (50 mg, 35 μmol) in 2 mL of DMF and 1 mL of acetonitrile was added compound **76** (34 mg, 71 μmol). The mixture was stirred for 16 h, then purified by HPLC to give compound **78** (23 mg). MS m/z 1805.5 (M+H).

Preparation of compound **81** and **81a**:



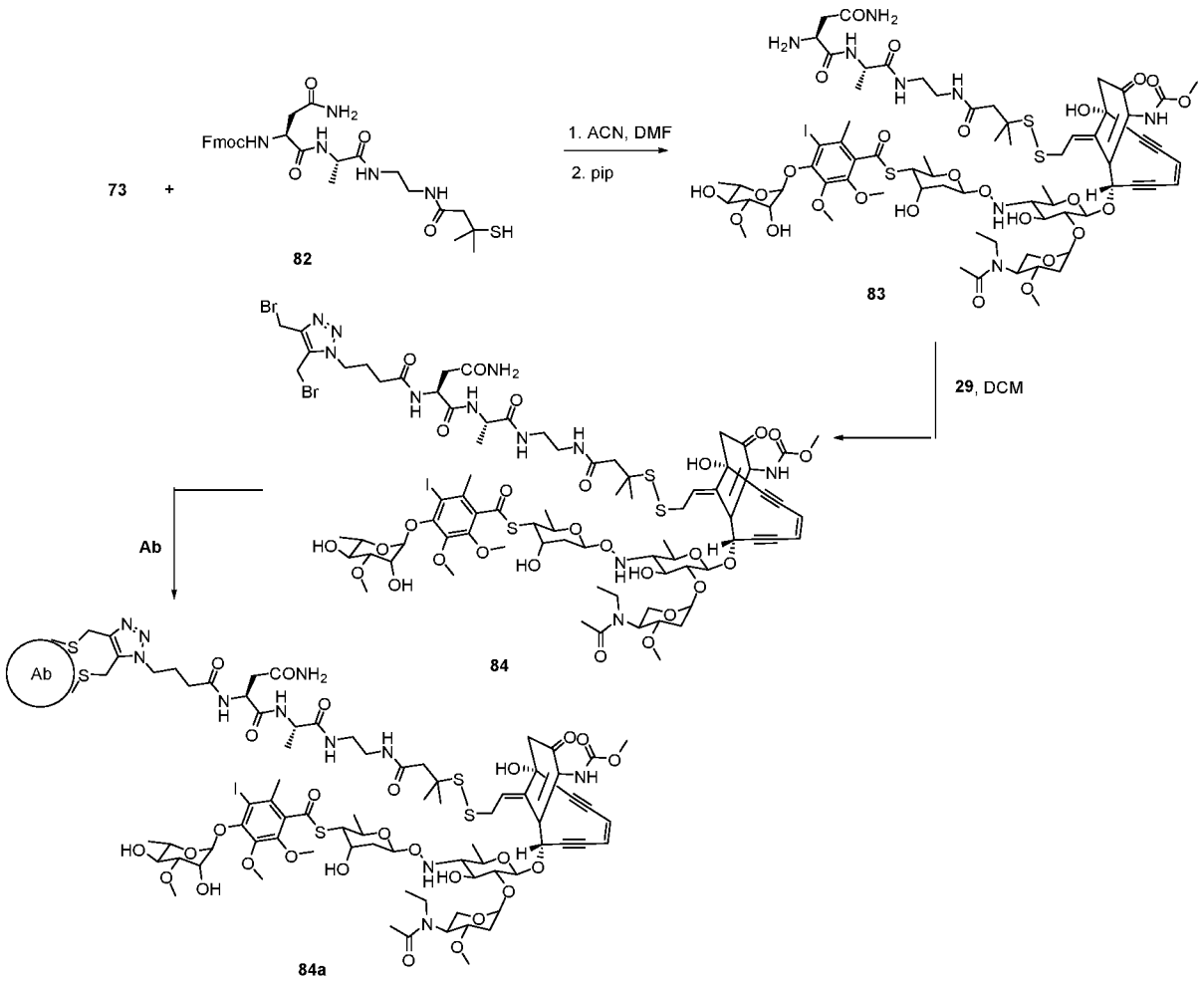
Preparation of compound **80**:

To compound **73** (50 mg, 35 μmol) in 2 mL of DMF and 1 mL of acetonitrile was added compound **79** (39 mg, 71 μmol). The mixture was stirred for 1 h, then 60 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **80** (18 mg). MS m/z 1663.5 (M+H).

Preparation of compound **81**:

To compound **80** (18 mg, 11 μmol) in 2 mL of DCM was added compound **29** (8.6 mg, 13 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **81** (12 mg). MS m/z 1984.4 (M+H).

Preparation of compound **84** and **84a**:



Preparation of compound **83**:

To compound **73** (50 mg, 35 μmol) in 2 mL of DMF and 1 mL of acetonitrile was added compound **82** (41 mg, 71 μmol). The mixture was stirred for 1 h, then 60 μL of piperidine was added. After 10 min the mixture was purified by HPLC to give compound **83** (20 mg). MS m/z 1691.5 (M+H).

Preparation of compound **84**:

To compound **80** (19 mg, 11 μmol) in 2 mL of DCM was added compound **29** (8.6 mg, 13 μmol), and 3 μL of DIEA. The mixture was stirred for 20 min, then evaporated and purified by HPLC to give compound **84** (13 mg). MS m/z 2012.4 (M+H).

Preparation of cMet-ADC (Compounds **8a** and **81a**)

Anti-cMet antibody was reduced by TCEP (tris(2-carboxyethyl)phosphine), up to 20 mM. The excess of TCEP was removed by gel-filtration chromatography or centrifugal filtration. Added organic solvent (up to 50%) to antibody solution. Compound **8** or **81** was

dissolved in Acetonitrile/water solution and added to the reduced antibody with compound **8** or **81**/antibody ratio from 3.5 to 6. After few hours' incubation at room temperature, unconjugated compound **8** or **81** was removed by gel-filtration chromatography or centrifugal filtration. The cMet-ADC was characterized by HPLC. The drug antibody ratio (DAR) was calculated based on UV-VIS or HIC-HPLC.

Example 2

This example provides the results of EC50 assays (nM) of the designated drug conjugated antibodies measured *in vitro* in specified cells. The antibody used was an anti-HER2 IgG class of antibody. Seven breast cancer cell lines with various level of Her2 expression as indicated with plus or minus signs in the table below were plated in 96 well plate. The ADCs as listed under Drug-Linker ID were serial diluted and added onto cells for treatment for 5 days. At the end of the study, cell proliferation was measured by Promega's CellTitreGlo. EC50 (in nM) was shown below and determined as the concentration of 50% cell growth inhibition. The selection criteria for a successful compound includes high efficacy, such as killing cell lines with high expression of the target receptor, with EC50 less than 2 nM. Also, the successful candidate should have low toxicity and good therapeutic window, as determined by relatively low killing of the control cell line (MDA468) with low expression of the target receptor. Compounds **15, 30, 34, 37, 40, 56, 75, 78, 81,** and **84**, were selected as successful candidates with high efficacy and good therapeutic window**.** Compounds **44** and **51** have low toxicity, but failed in efficacy in some cell lines. Compound **72** has high toxicity and low efficacy in several cell lines.

Drug-Linker ID	**SKBR-3 (Her2+++)**	**BT474 (Her2+++)**	**MDA-453 (Her2++)**	**JIMT-1 (Her2+++)**	**MCF-7 (Her2+/-)**	**MDA468 (Her2-)**	**HCC1954 (Her2+++)**
15	0.17	1.46		0.17	>100		0.064
30	0.065	1.41	0.05	0.09	0.5	24	
34	0.057	0.57	0.162	0.022	0.033	5.12	
37	0.234	1.53	0.33	0.141	0.33	16.9	
40	0.657	0.9	0.1	0.35	90	41.7	
44	0.223	2.49	0.08	>100	>100	>100	
51	0.127	0.639	0.1	>100	>100	>100	
56	0.04	0.42	0.05	0.238	22	32	
72	0.171	22.4	5.4	0.084		2	0.09

75	0.015	0.237	0.02	0.021	0.026	36.7	
78	0.134	0.4	0.01	0.44	>100	>100	
81	0.023	0.418	0.1	0.025	0.041	12.98	0.01
84	0.07	0.96	0.041	0.119	12	63.8	

Example 3

This example provides the results of EC50 assays (nM) of designated ADCs described herein measured *in vitro* in specified cells. The antibody used targets a receptor tyrosine kinase on cell surface. Eight cancer cell lines with various level of receptor expression as indicated with plus or minus signs in the table below were plated in 96 well plate. The ADCs as listed under Drug-Linker ID were serial diluted and added onto cells for treatment for 5 days. At the end of the study, cell proliferation was measured by Promega's CellTitreGlo. EC50 (in nM) was shown below and determined as the concentration of 50% cell growth inhibition. The selection criteria for a successful compound includes high efficacy, such as killing cell lines with high expression of the target receptor, with EC50 less than 2 nM. Also, the successful candidate should have low toxicity and good therapeutic window, as determined by relatively low killing of the control cell lines (T-47D and H520) with low expression of the target receptor. Compounds **8a, 14a, 30a, 81a,** and **84a** were selected as successful candidates with high efficacy and good therapeutic window. Compounds **23a** and **18a** have low toxicity, but did not show efficacy in some cell lines.

Drug-Linker ID	**H1993 (+)**	**H292 (+)**	**HCC827 (+)**	**Hs746T (+)**	**SNU-5 (+)**	**T-47D (-)**	**A549 (+)**	**H520 (-)**
8a	0.05	1.4	0.01		0.121	80	0.46	30
23a	0.306	3.5	40	0.21	1.23	20		
14a	0.231	0.5						>100
18a	0.265	>100					10	10
30a	0.055		0.36			>100	0.428	
81a	0.002	1	0.055	2	0.057	>100	0.015	80
84a	0.08		0.36			>100	0.154	

Example 4

This example provides the results for the in vivo efficacy of compounds **8a** and **81a** on a variety of Human Xenograft Tumour Growth in Nude Mice

Animals:

Female Nu/Nu mice at 5-7 weeks of age (from Charles River) were used in the studies. Upon receipt, mice were housed 5 mice per cage in a room with a controlled environment. Rodent chow was provided and water *ad libitum*. Mice were acclimated to laboratory conditions for 72 hours before the start of dosing. Animals' health status was determined during the acclimation period. Each cage was identified by group number and study number, and mice were identified individually by ear tags.

Study Design and Dosing Regimen for Conjugate #81a: refer to Table 1.

Table 1

Tumor model	Groups	Animals per Group	Treatment volume/route	Dose / Frequency
HCC827	1	7	PBS / 0.2 ml, IV	0 mg/kg / once
	2	7	Compound **81a** / 0.2 ml. IV	3 mg/kg / once
H292	1	7	PBS / 0.2 ml, IV	0 mg/kg / once
	2	7	Compound **81a** / 0.2 ml. IV	3 mg/kg / once
H292	1	9	PBS / 0.2 ml, IV	0 mg/kg / Q1W x 3
	2	10	Compound **81a** / 0.2 ml. IV	3 mg/kg / Q1W x 3
U87MG	1	10	PBS / 0.2 ml, IV	0 mg/kg / Q1W x 3
	2	10	Compound **81a** / 0.2 ml. IV	3 mg/kg / Q1W x 3
H1993	1	10	PBS / 0.2 ml, IV	0mg/kg / Q1W x 3
	2	10	Compound **81a** / 0.2 ml. IV	3 mg/kg / Q1W x 3

Study Design and Dosing Regimen for Conjugate #8a: refer to Table 2.

Table 2

Tumor model	Groups	Animals per Group	Treatment volume/route	**Dose** / Frequency
HCC827	1	7-10	PBS / 0.2 ml, IV	0mg/kg once

	3	7-10	Compound **8a** / 0.2 ml. IV	3 mg/kg once
H292	1	7-10	PBS / 0.2 ml, IV	0mg/kg once
	3	7-10	Compound **8a** / 0.2 ml. IV	3 mg/kg once
U87MG	1	7-10	PBS / 0.2 ml, IV	0mg/kg once
	2	7-10	Compound **8a** / 0.2 ml. IV	3 mg/kg once
H1975	1	7-10	PBS / 0.2 ml, IV	0mg/kg once
	3	7-10	Compound **8a** / 0.2 ml. IV	3mg/kg once

Procedures

1. Tumor cell inoculation and establishments of tumors:

   a. U87MG, H292, H1993, H1975 and HCC827 cell lines were obtained from ATCC (Manassas, Virginia). Human NSCLC cell lines HCC827, H292 H1993 and H1975 were cultured and expanded with 10% FBS RPMI medium, U87MG with DMEM medium at 37°C in a 5% carbon dioxide humidified environment. The cells were cultured, and passaged as needed for a period of 2 weeks and then harvested with 0.25% trypsin (Corning 25-050-CI). 7 million cells of HCC827, 5 million cells of H292, H1993 and U87MG in a total of 0.2 ml 1:1 ratio of mixture of HBSS (Hank's balanced salt solution) and matrigel (Corning 354234) were injected subcutaneously into the upper right flank of each mouse respectively. All mice were ear tagged for identification.

   b. Tumor growth was monitored by tumor volume measurement using a digital caliper starting day 5-7 after inoculation, and followed 2 times per week until tumor volume reaches ~150-250 mm^3.

2. Treatments:

   a. Once tumors were staged to the desired volume, animals were randomized and mice with very large or small tumors were culled. Mice were divided into treatment groups with animal numbers per group as indicated in the study design (Table 1 and Table 2) for each tumor model.

b. Mice were then treated with either vehicle (PBS, 0.2 ml IV) or ADC Compound **81a** or Compound **8a** according to the study design.

3. Tumor volume, body weight measurement and study end points

a. Tumor volumes were measured by using a digital caliper twice weekly through the whole experiment period. The volume was calculated using the formula: Volume (mm^3) = [Length (mm) x Width $(mm)^2$] / 2. TGI (tumor growth inhibition %) was calculated using the formula: TGI = [(Last Volume Measurement of vehicle Group – Volume of Treatment group at the same last day of vehicle control)/(Last Volume Measurement of Vehicle Group] x 100.

b. Body weight of each mouse was weighed twice weekly by an electric balance.

c. Tumor growth responses were monitored until tumor load reached IACUC protocol limits (2000 mm^3) or when animal body weight loss reaches 20%.

Data analysis

Raw data of tumor volume and body weight were recorded in an Excel file with Microsoft Office. Tumor volume and body weight graphs were generated by GraphPad Prism 6.0. Data statistical analysis was done by unpaired t-test or one-way ANOVA compared to PBS treated control group.

Results

As shown by **FIG**s **1** and **2**, compound **81a** significantly inhibited the growth of HCC827 and H292 tumor in nude mice at 3 mg/kg IV, single dose. Bars represent group averages ± SEM, N=7/group. ** $P < 0.01$ vs. Vehicle (unpaired t test).

FIGs **3**, **4**, and **5** show that compound **81a** significantly inhibited the tumor growth of H292, H1993, and U87MG tumor in nude mice at 3 mg/kg IV, once weekly for three treatment. Bars represent group averages ± SEM, N=9/group. ** $P < 0.01$ vs. Vehicle (unpaired t test).

Tumor growth inhibition (TGI) is show in Table 3 below.

Table 3

Tumor model	**Treatment**	**TGI%**
H292	Compound **81a**, 3mg/kg, IV single dose	75.30
H292	Compound **81a**, 3mg/kg, IV. Q1W x3	98.64%
H1993	Compound **81a**, 3mg/kg, IV. Q1W x3	85.04%
HCC827	Compound **81a**, 3mg/kg, IV. single dose	71.91%
U87MG	Compound **81a**, 3mg/kg,IV Q1Wx3	96.62%

As shown by **FIG**s **6**, **7**, **8**, and **9** compound **8a** significantly inhibited the growth of HCC827, H292, U87, and H1975 tumor in nude mice at 3 mg/kg IV, single dose. Bars represent group averages ± SEM, N=10/group. ** $P < 0.05$ vs. Vehicle (one way ANOVA, Dunnett's multiple comparison test).

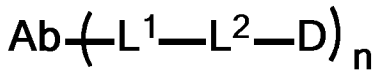
Tumor growth inhibition (TGI) is show in Table 4 below.

Table 4

Tumor model	Treatment	TGI%
H292	Compound **8a**, 3mg/kg, IV single dose	69.49% (Day38)
HCC827	Compound **8a**, 3mg/kg, IV. single dose	69.5% (Day48)
U87MG	Compound **8a**, 3mg/kg, IV. single dose	77.40% (Day17)
H1975	Compound **8a**, 3mg/kg, IV. Single dose	83.58% (Day40)

We claim:

1. An ADC comprising a structure of Formula II

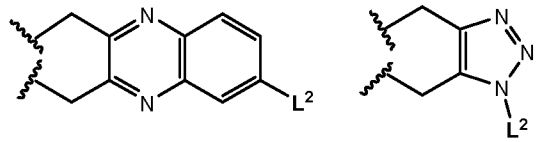


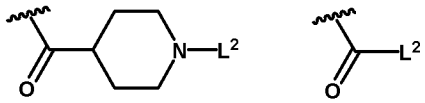
(**II**)

or a pharmaceutically acceptable salt thereof, wherein:

Ab is a monoclonal antibody;

$\mathbf{L^1}$ - $\mathbf{L^2}$ together are a linker selected from the group consisting of:





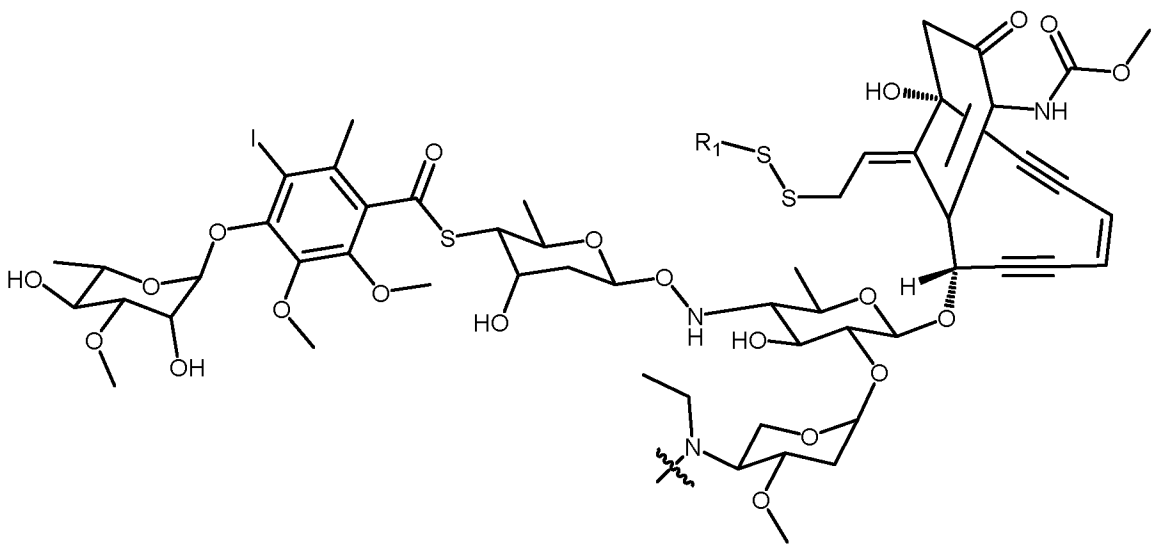
, wherein the wavy line indicates a point of attachment to an Ab;

$\mathbf{L^2}$ is a linker; wherein $\mathbf{L^2}$ is selected from the group consisting of an amino acid, a peptide, $-(CH_2)_m-$, $-(CH_2CH_2O)_m-$, PAB, Val-Cit-PAB, Val-Ala-PAB, Ala-Ala-Asn-PAB, and combinations thereof, wherein m is an integer from 0 to 10;

D is calicheamicin; and

n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

2. The ADC of claim 1, wherein D has the structure of Formula III



(III)

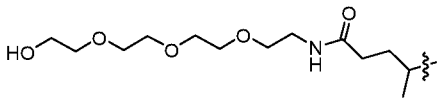
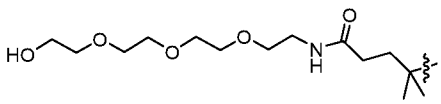
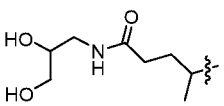
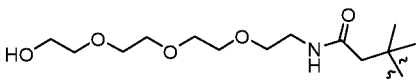
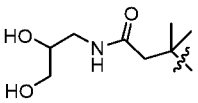
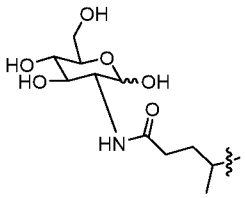
or a pharmaceutically acceptable salt thereof;

wherein the wavy line indicates the point of attachment to L^2; and

wherein R1 is selected from the group consisting of C1-C8 alkyl, $-(CH_2CH_2O)_n-$, isopropyl, glucose, galactose, mannose, glucosamine, C1-C8 alkyl-OH, and combinations thereof, and

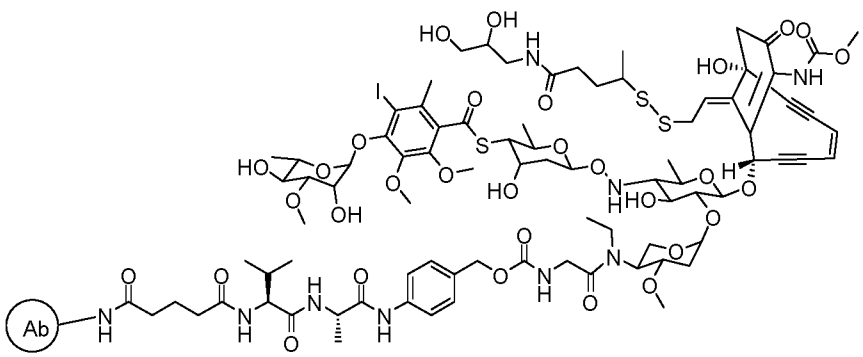
where n = 1-30

3. The ADC of claim 1, or a pharmaceutically acceptable salt thereof, wherein R1 is selected from the group consisting of: isopropyl,

wherein the wavy line indicates a point of attachment to the sulfur on calicheamicin.

4. The ADC of claim 1, wherein the structure of Formula II has a structure selected from the group consisting of: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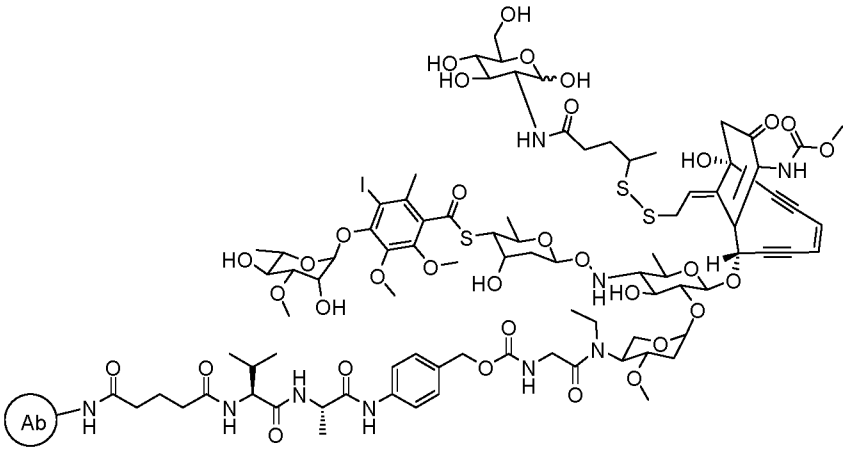

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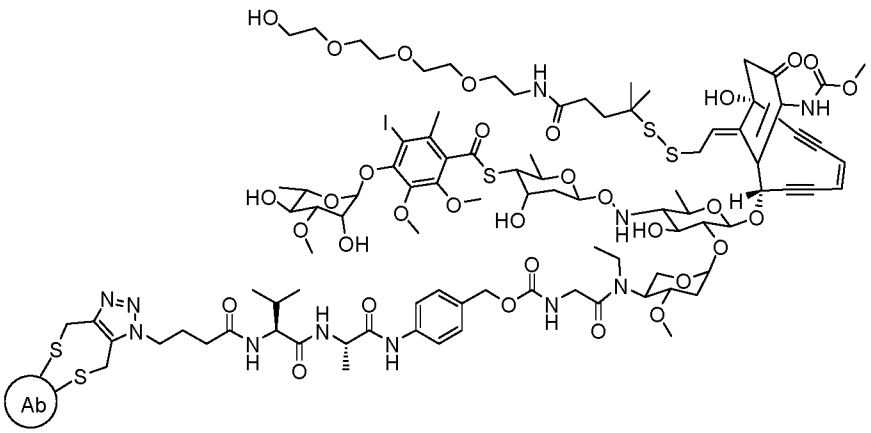

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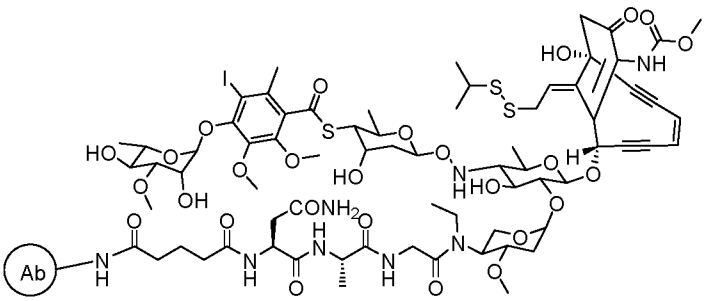

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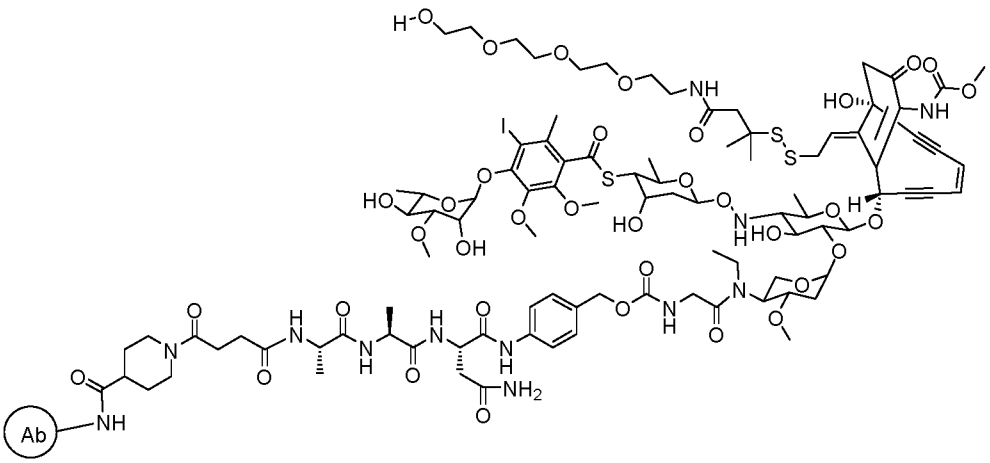

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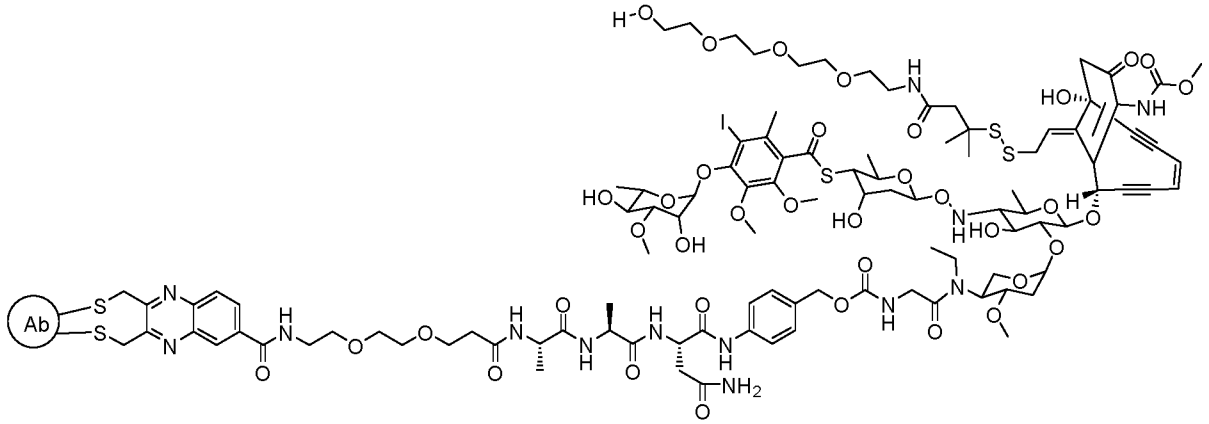

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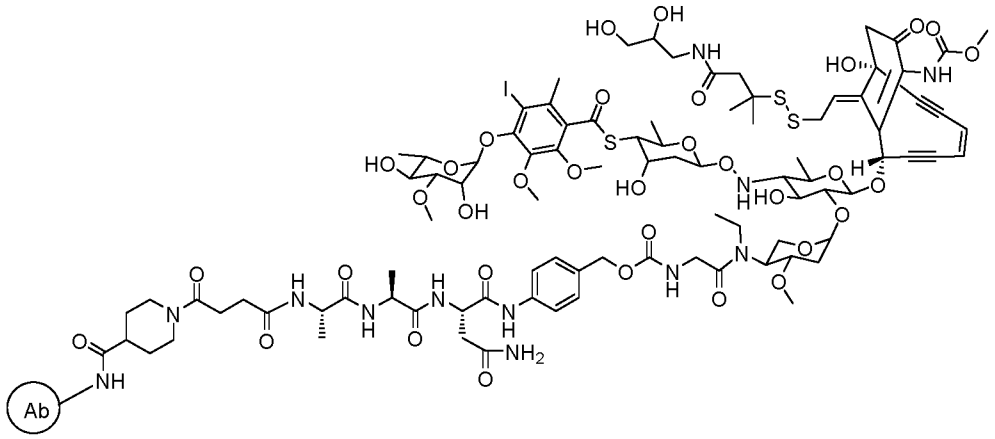

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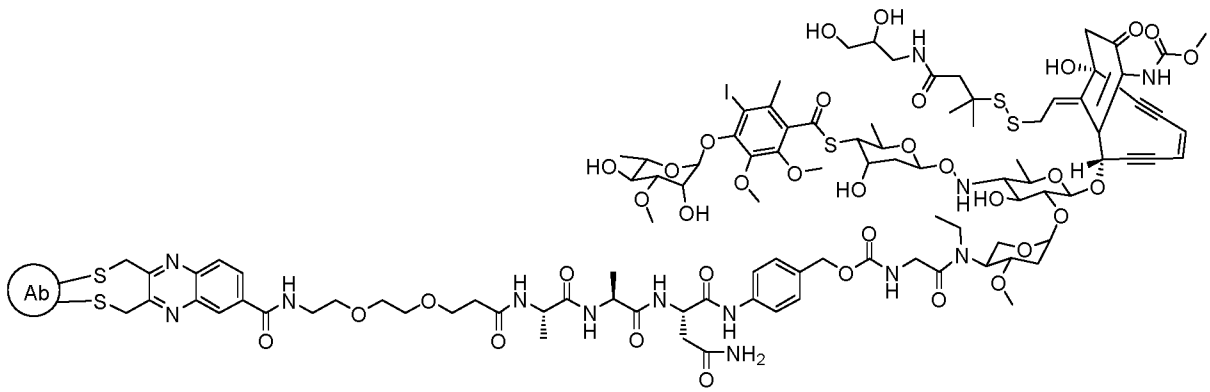

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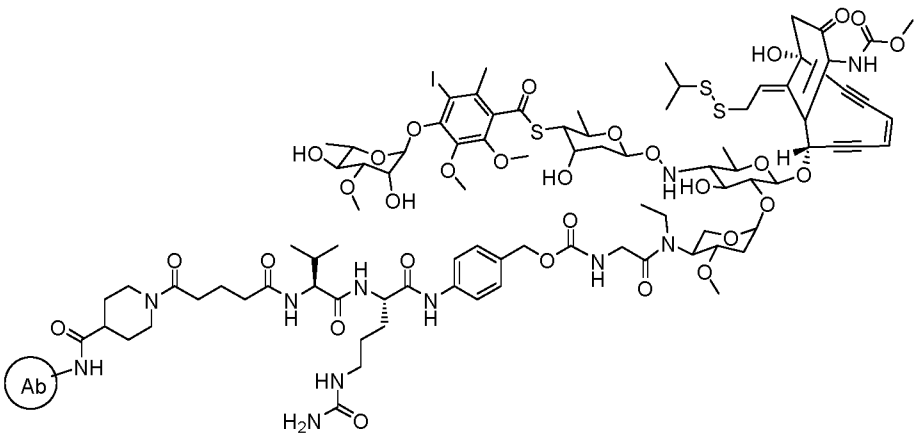

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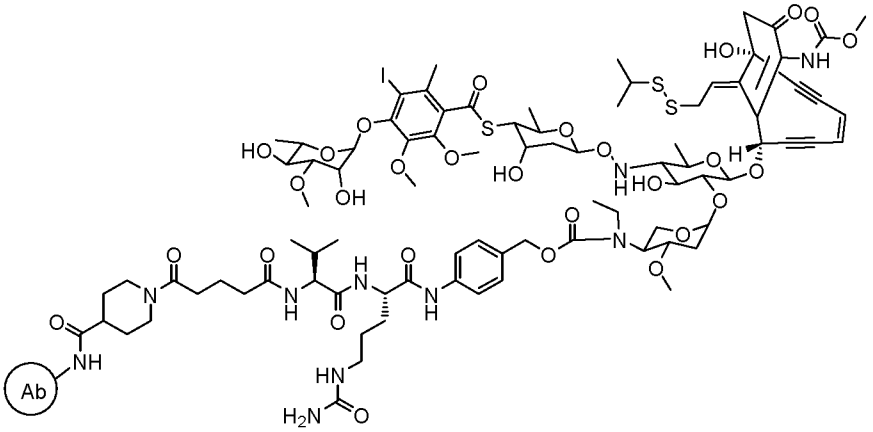

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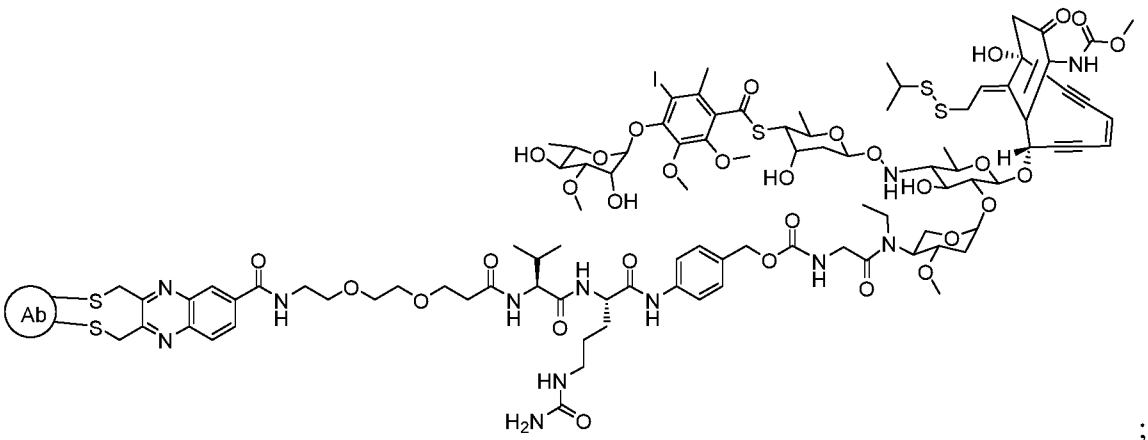

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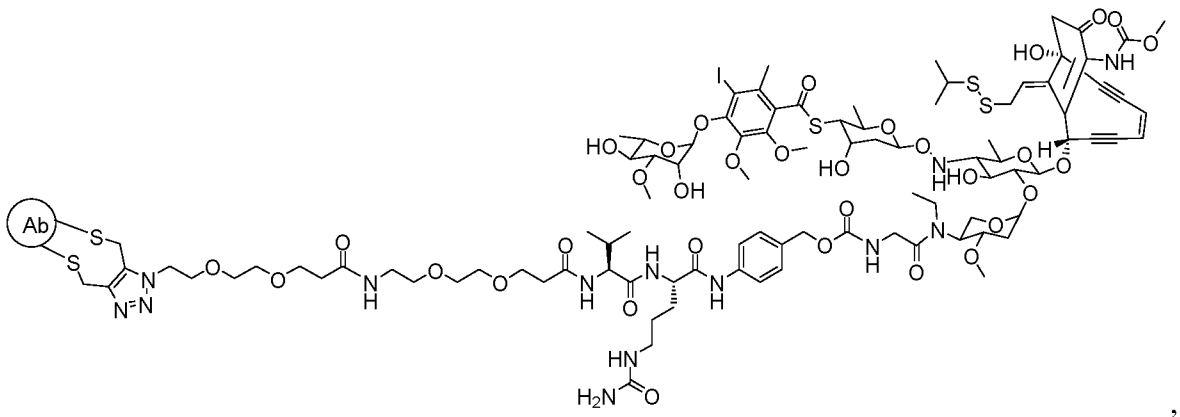
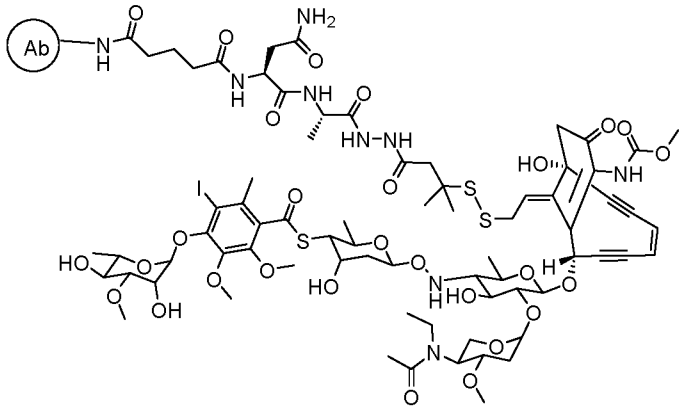


,

or a pharmaceutically acceptable salt thereof.

5. The ADC of claim 1, wherein the structure of Formula II has a structure selected from the group consisting of:



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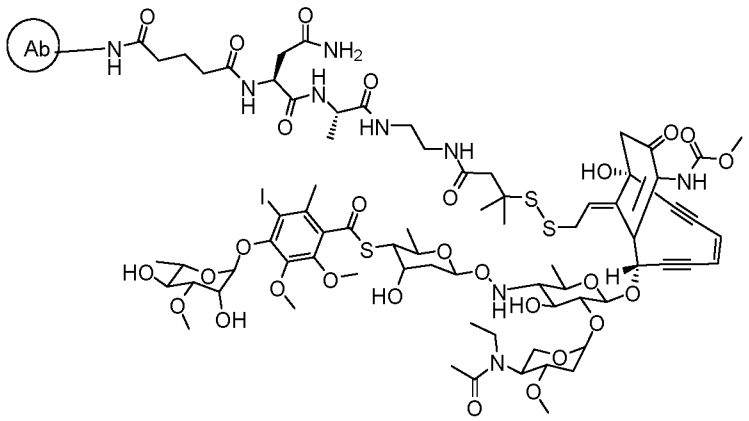
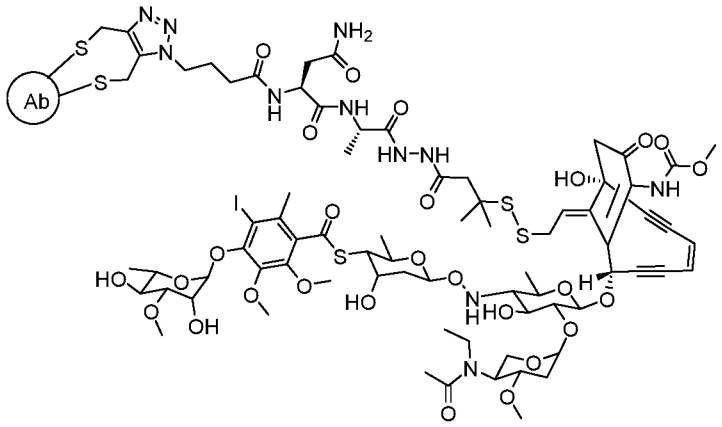
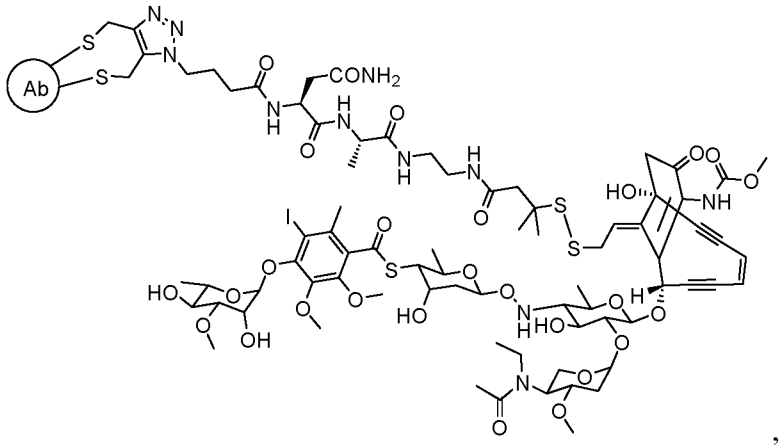




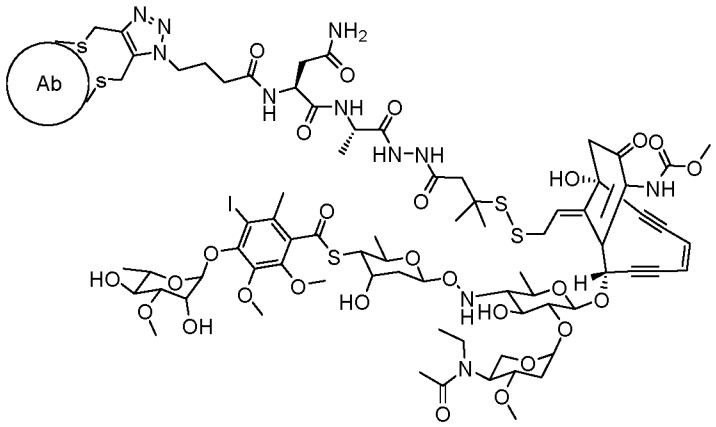
; and



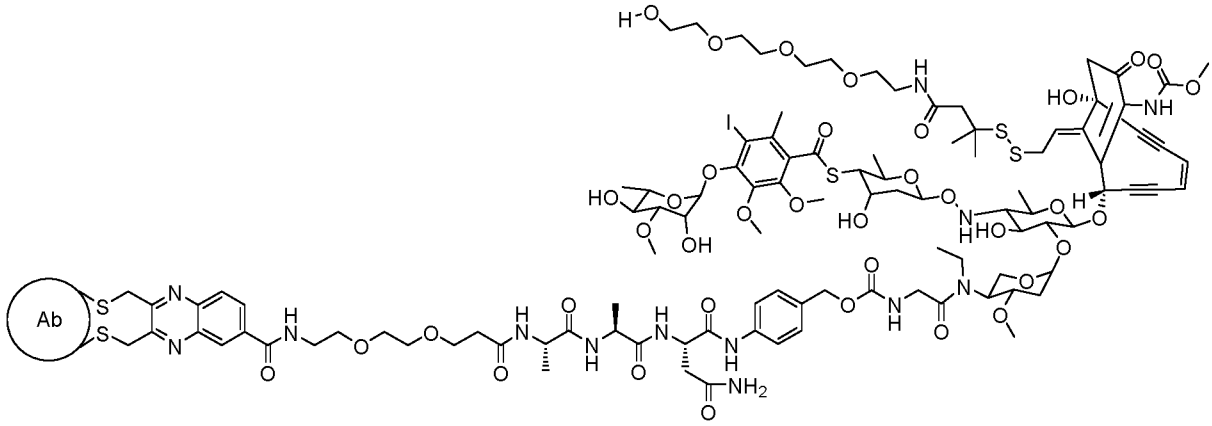
,

or a pharmaceutically acceptable salt thereof.

6. A compound having the structure:



; or



;

or a pharmaceutically acceptable salt thereof.

7. The compound of claim 6, wherein Ab is antibody of an IgG class that binds to a c-Met epitope with a binding affinity of at least 10^{-6}M.

FIG. 1

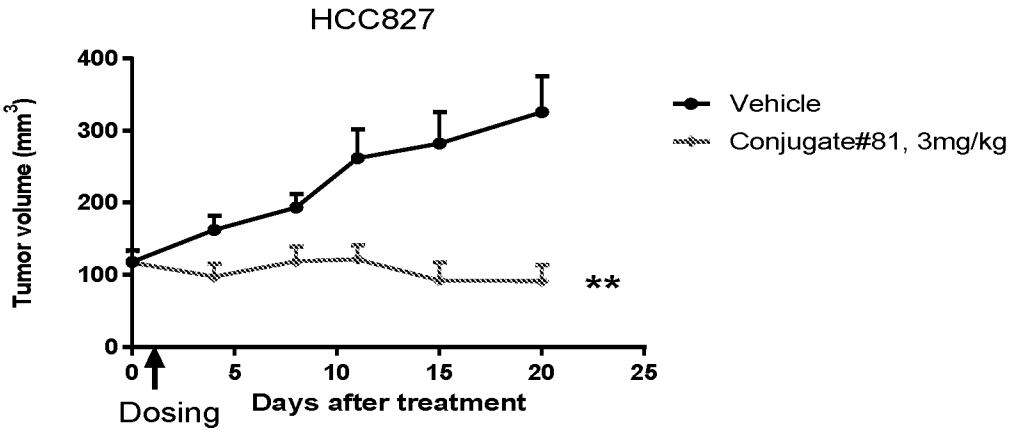

HCC827
Tumor volume (mm3)
400
300
200
100
0
0
5
10
15
20
25
Dosing
Days after treatment
**
Vehicle
Conjugate#81, 3mg/kg

FIG. 2

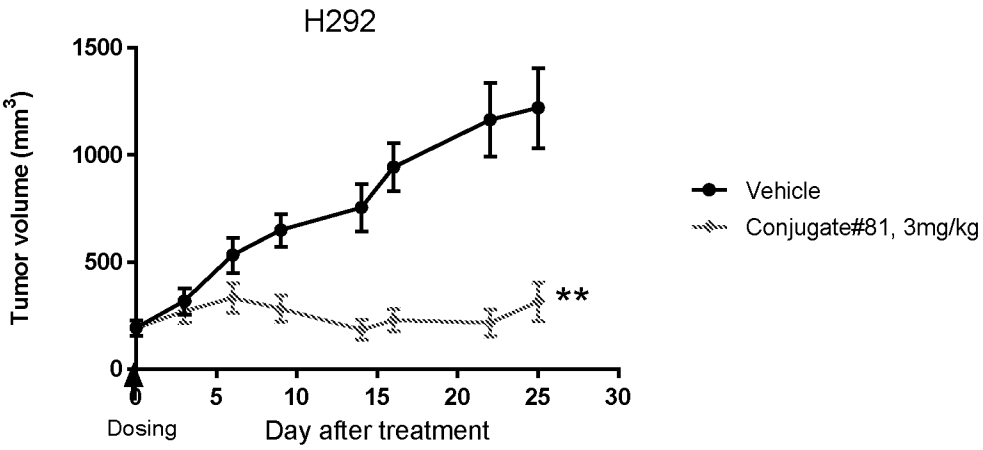

H292
Tumor volume (mm3)
1500
1000
500
0
0
5
10
15
20
25
30
Dosing
Day after treatment
**
Vehicle
Conjugate#81, 3mg/kg

FIG. 3

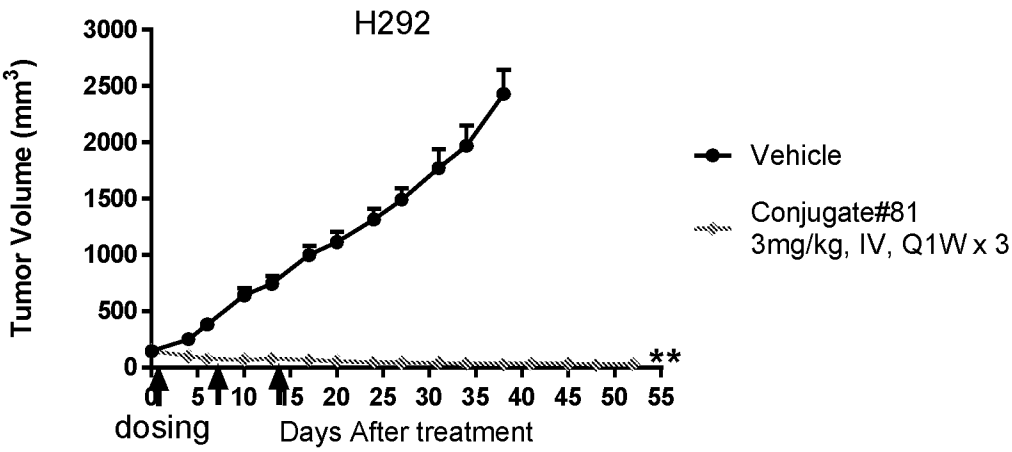

H292
Tumor Volume (mm3)
3000
2500
2000
1500
1000
500
0
**
0 5 10 15 20 25 30 35 40 45 50 55
dosing
Days After treatment
Vehicle
Conjugate#81
3mg/kg, IV, Q1W x 3

FIG. 4

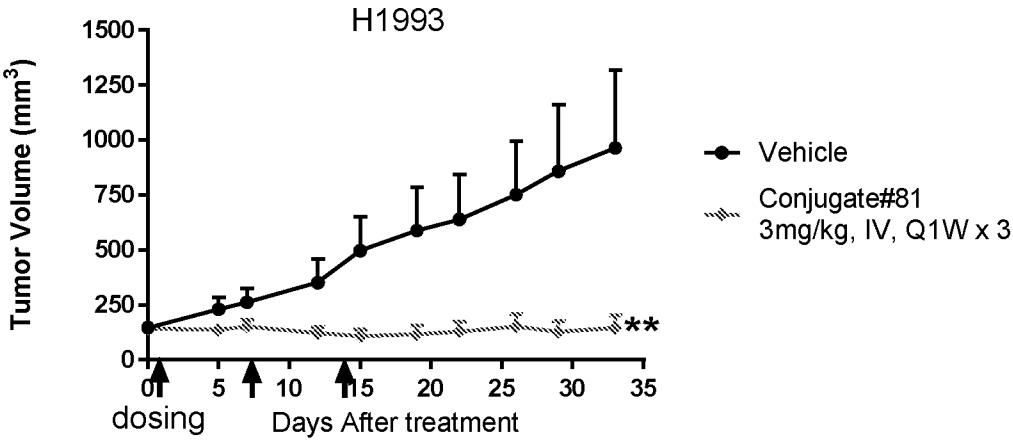

H1993
Tumor Volume (mm3)
1500
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35
dosing
Days After treatment
**
Vehicle
Conjugate#81
3mg/kg, IV, Q1W x 3

FIG. 5

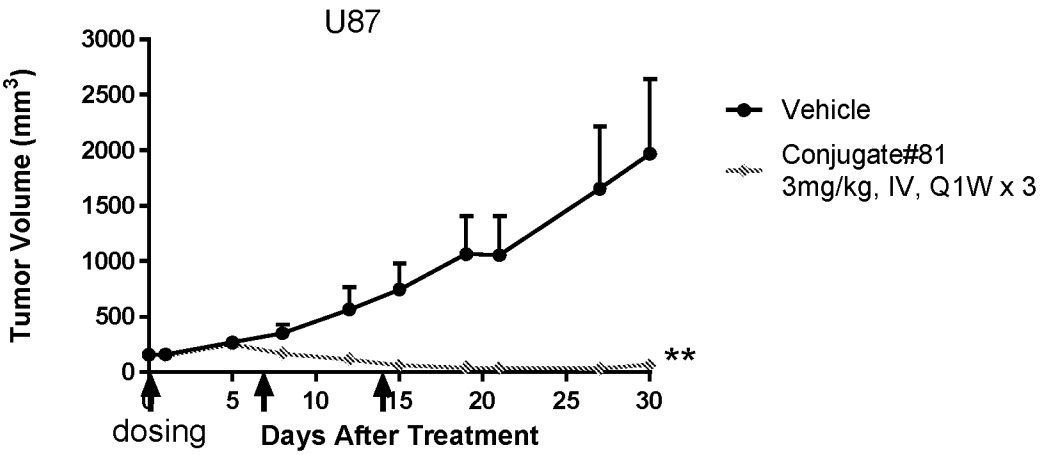

U87
Tumor Volume (mm3)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
dosing
Days After Treatment
**
Vehicle
Conjugate#81
3mg/kg, IV, Q1W x 3

FIG. 6

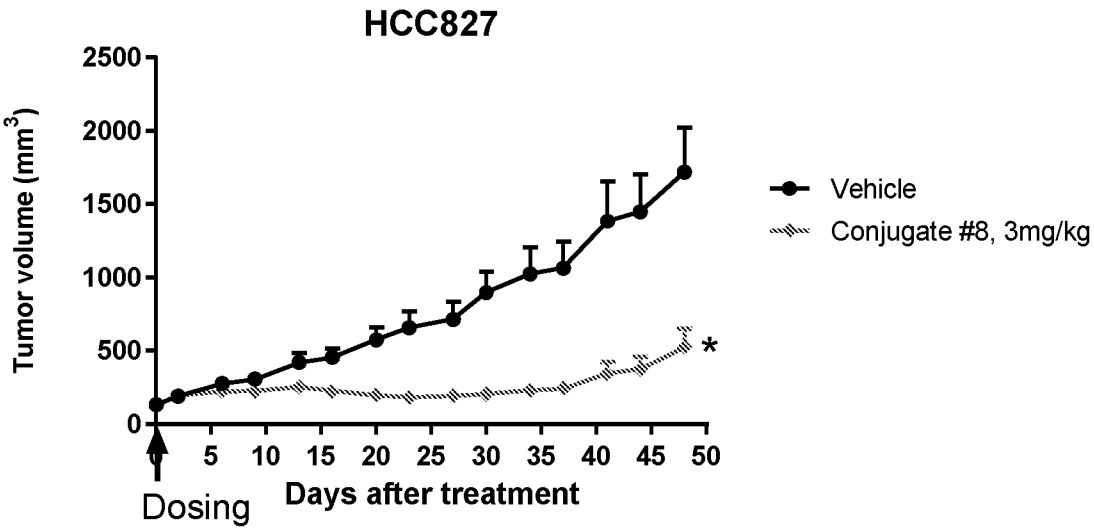

HCC827
Tumor volume (mm3)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
50
Dosing
Days after treatment
*
Vehicle
Conjugate #8, 3mg/kg

FIG. 7

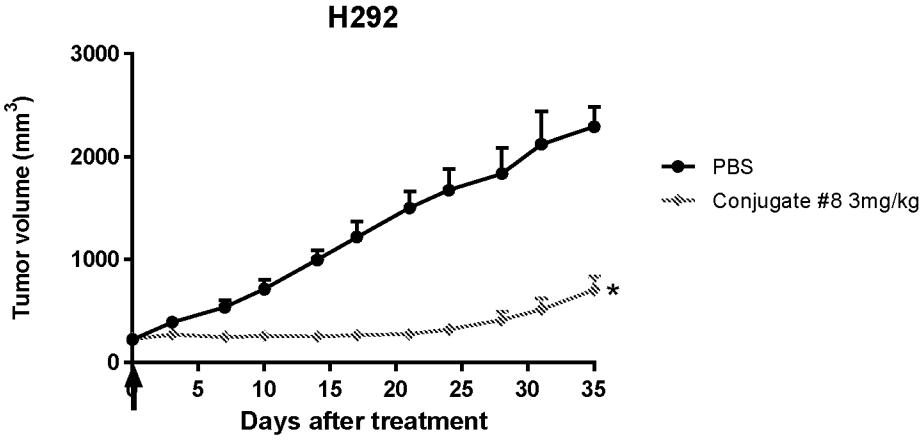

H292
Tumor volume (mm3)
3000
2000
1000
0
0
5
10
15
20
25
30
35
Days after treatment
*
PBS
Conjugate #8 3mg/kg

FIG. 8

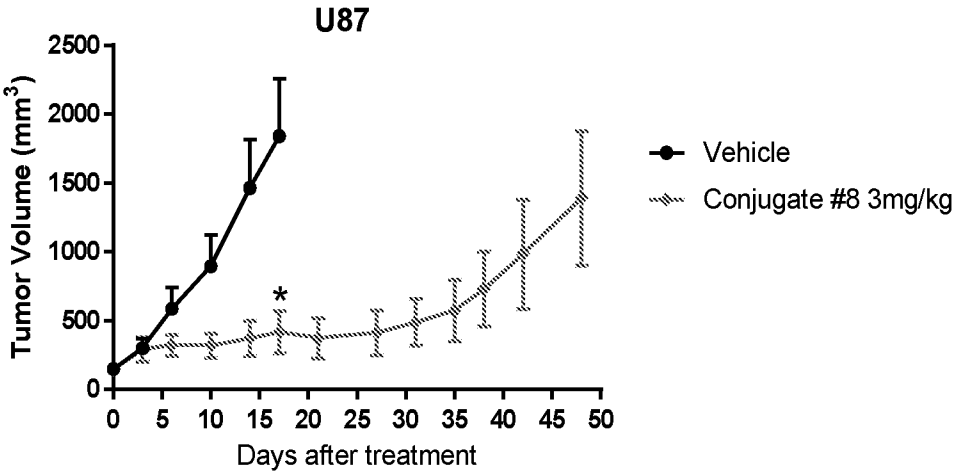
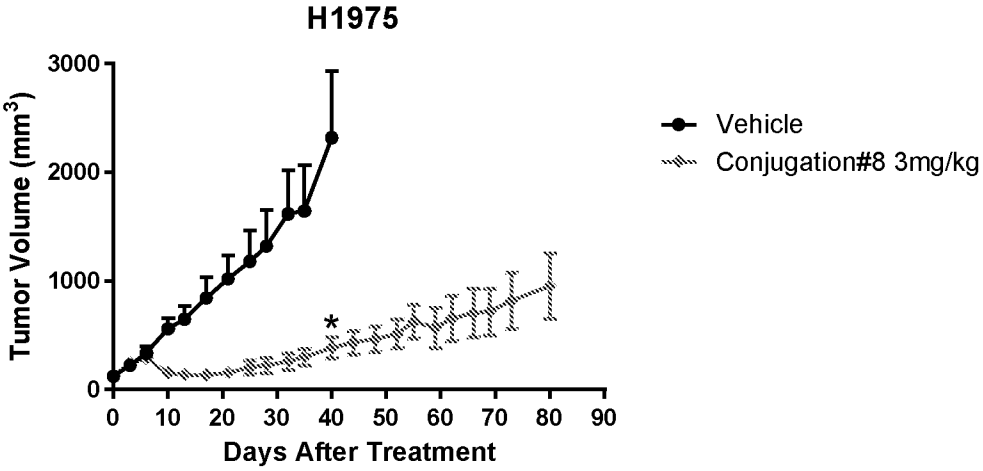

U87
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0 5 10 15 20 25 30 35 40 45 50
Days after treatment
*
Vehicle
Conjugate #8 3mg/kg

FIG. 9


H1975
Tumor Volume (mm3)
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
90
Days After Treatment
*
Vehicle
Conjugation#8 3mg/kg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/024736

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7034; A61K 39/395; A61K 45/00; A61K 47/48; A61P 35/00; A61P 37/00 (2017.01)

CPC - A61K 31/704; A61K 39/395; A61K 47/48384; A61K 47/48407; A61K 47/48507; A61K 47/48561; C07K 14/47; C07K 16/2803; C07K 16/46; C07K 2317/21; C07K 2317/24; C07K 2317/54; C07K 2317/55; C07K 2317/565; C07K 2317/622; C07K 2317/76; C12N 15/63 (2017.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/130.1; 424/133.1; 424/155.1; 424/174.1; 424/181.1; 424/183.1 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006/0088522 A1 (BOGHAERT et al) 27 April 2006 (27.04.2006) entire document	1-4
A	US 2015/0141646 A1 (CONCORTIS BIOSYSTEMS CORP A WHOLLY OWNED SUBSIDARY OF SORRENTO THERAPEUTICS INC) 21 May 2015 (21.05.2015) entire document	1-4
A	DAMELIN et al. "Anti-EFNA4 Calicheamicin Conjugates Effectively Target Triple-Negative Breast and Ovarian Tumor-Initiating Cells to Result in Sustained Tumor Regressions," Clinical Cancer Research, 26 May 2015 (26.05.2015), Vol. 21, Iss. 18, Pgs. 4165-4173. entire document	1-4
A	US 5,739,116 A (HAMANN et al) 14 April 1998 (14.04.1998) entire document	1-4
A	US 8,153,768 B2 (KUNZ et al) 10 April 2012 (10.04.2012) entire document	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2017	14 AUG 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/024736

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

ISA/225 mailed on 06 April 2017. No approved electronic sequence listing was submitted in response to the ISA/225.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/024736

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4 restricted to the first antibody-drug conjugate shown in claim 4.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

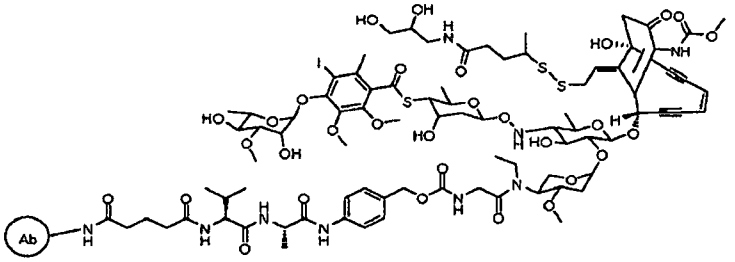
☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

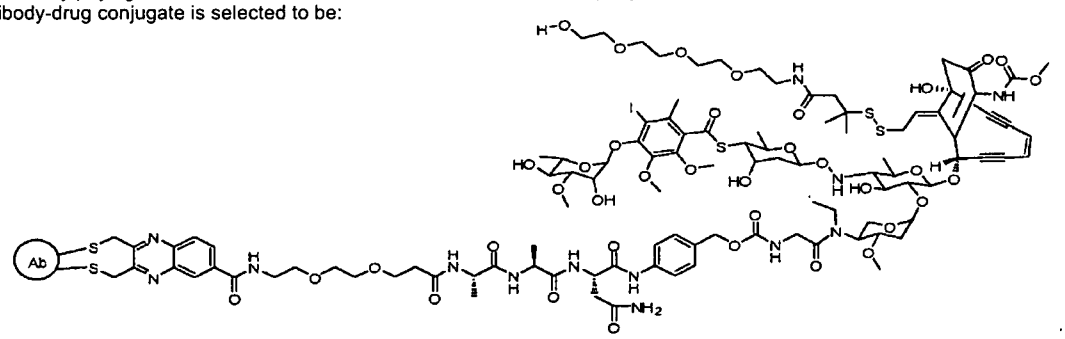
Group I+: claims 1-7 are drawn to antibody-drug conjugates for Formula II.

The first invention of Group I+ is restricted to an antibody-drug conjugate, wherein the antibody-drug conjugate is selected to be:



It is believed that claims 1-4 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above antibody-drug conjugate embodiment.

Applicant is invited to elect additional antibody-drug conjugates, each with specified chemical structure, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be an antibody-drug conjugate, wherein the antibody-drug conjugate is selected to be:



Additional antibody-drug conjugates will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for linking calicheamicin to a linker and a targeting antibody, such that the calicheamicin will be released from the linker at a much slower rate to lower non-specific cytotoxicity, requiring the selection of alternatives for the linkers, L1 and L2, and D, the calicheamicin group for each antibody-drug conjugate, where "[a]n ADC comprising a structure of Formula II Ab-(-L1-L2-D)n or a pharmaceutically acceptable salt thereof, wherein: Ab is a monoclonal antibody; L1- L2 together are a linker selected from the group consisting of: (see structures in claim 1 of the instant application), wherein the wavy line indicates a point of attachment to an Ab; 10 L2 is a linker; wherein L2 is selected from the group consisting of an amino acid, a peptide, -(CH2)m-, -(CH2CH2O)m-, PAB, Val-Cit-PAB, Val-Ala-PAB, Ala-Ala-Asn-PAB, and combinations thereof, wherein m is an integer from 0 to 10; D is calicheamicin; and n is 1, 2, 3,4, 5, 6, 7, 8, 9, or 10."

The Groups I+ share the technical features of an antibody-drug conjugate comprising a structure of Formula II: Ab-(-L1-L2-D)n; or a pharmaceutically acceptable salt thereof, and a compound. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2015/0141646 A1 to Concortis Biosystems, Corp discloses an ADC comprising a structure of Formula II Ab-(-L1-L2-D)n or a pharmaceutically acceptable salt thereof (compound-conjugates and uses thereof are Provided, Abstract; Also provided herein are the compounds described above conjugated to a targeting moiety with a linker., Para. [0040]; the compound may be included in a conjugate including a linker and a targeting moiety, such as antibody, Para. [0174]; Some embodiments provide a compound-conjugate having the structure of Formula V (A1-L1-(-L2-D)n-B1), Para. [0270]; or a pharmaceutically acceptable salt thereof, wherein: A1 may be a targeting moiety; B1 is an auxiliary moiety that optionally includes a second targeting moiety, or B1 is null; L1 includes a group including a N (nitrogen) atom or a group including a 2- to 5-carbon bridge and at least one sulfur atom; each D is independently selected, where each D includes a compound; each L2 is independently a linker, wherein at least one L2 links to L 1 ; and n is 1, 2, 3, 4, 5, 6, 7, 8,

9, or 10. In some embodiments, A1 may be a monoclonal antibody (mAB), Para. [0271[]), and a compound (Some embodiments provide a compound-conjugate having the structure of Formula V, Para. [0269]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.