IMMUNOMODULATORY ANTIBODY-DRUG CONJUGATES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos. 63/138,360, filed January 15, 2021, and 63/292,779 filed December 22, 2021, both entitled "IMMUNOMODULATORY ANTIBODY-DRUG CONJUGATES", both of which are incorporated herein by reference in their entirety.

BACKGROUND

Field of the Invention

[0002] The present invention relates to the fields of chemistry and medicine. More particularly, the present invention relates to antibody-drug conjugates, compositions, their preparation, and their use as therapeutic agents.

Description of the Related Art

[0003] The cGAS-STING pathway is an innate immune pathway that recognizes intracellular DNA and triggers a type I interferon and inflammatory cytokine response that is important for both anti-viral and anti-tumor immunity. Upon DNA binding, cGMP-AMP synthase (cGAS) produces cGAMP, which is the endogenous ligand of STING. *See, e.g.,* Villanueva, *Nat. Rev. Drug Disc.* 2019: 18; 15. At the molecular level, upon activation by cGAMP, the transmembrane STING dimer translocates from the endoplasmic reticulum to the Golgi apparatus, ultimately recruiting TANK-binding kinase 1 (TBK1) and the transcription factor interferon regulatory factor 3 (IRF3), leading to induction of type I interferons (IFNs) and an inflammatory response. *See* Konno, et al., *Cell* 2013: 155; 688–698. This innate immune pathway must be tightly regulated as excessive cGAS-STING activity has been linked to various autoimmune and inflammatory disorders. *See* Barber, *Nat. Rev. Immunol.* 2015: 15; 760-770; *see also,* Liu, et al., *N. Engl. J. Med.* 2014: 371; 507-518.

[0004] Exogenous STING agonists can help to overcome the immunosuppressive tumor microenvironment by activating an immune response against a tumor, resulting in tumor regression. *See* Sun, et al., *Science* 2013: 6121; 786-791; *see also,* Corrales and Gajewski, *Clinc.*

Cancer Res. 2015: 21; 4774-4779. Examples include nucleotide-based STING agonists, which are, like the endogenous ligands, cyclic di-nucleotides. These compounds are typically charged and hydrophilic, susceptible to enzymatic degradation, and have poor bioavailability and pharmacokinetics. Thus, there remains a need for STING agonists with improved pharmacological properties that avoid systemic cytokine induction.

SUMMARY

[0005] Some embodiments described herein relate to antibody-drug conjugates (ADCs) that can elicit a localized immune response to target cells, and hence, exhibit reduced off-target toxicity, such as that observed with systemically administered immunostimutory compounds.

[0006] Some embodiments provide an antibody-drug conjugate (ADC) comprising:

an antibody;

a linker, as described herein; and

a compound of Formula (I) as described herein;

wherein the compound of Formula (I) is conjugated to the linker; and

wherein each linker is conjugated to the antibody via a succinimide or hydrolyzed succinimide covalently linked to a sulfur atom of a cysteine residue of the antibody.

[0007] Some embodiments provide an antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^{*}\text{-}M^{1}\text{-}(D))_{p}}$$

wherein:

Ab is an antibody;

each S* is a sulfur atom from a cysteine residue of the antibody;

M^1 is a succinimide or a hydrolyzed succinimide;

subscript p is an integer from 2 to 8; and

each (D) is a Drug Unit of Formula (I), as described herein.

[0007a] Disclosed herein is an antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^{*}\text{-}M^{1}\text{-}(D))_{p}}$$

wherein:

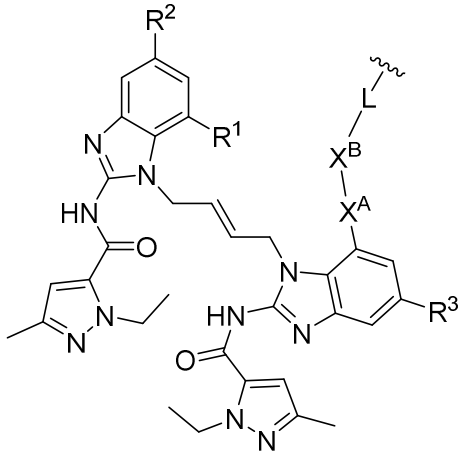
Ab is an antibody;

each S* is a sulfur atom from a cysteine residue of the antibody;

M^1 is a succinimide or a hydrolyzed succinimide;

subscript p is an integer from 2 to 8; and

each (D) is a Drug Unit of Formula (I):



(I)

wherein:

~~~ represents covalent attachment of L to M^1;

R^1 is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl)C_{1-6} alkoxy, $–(CH_2)_n-NR^AR^B$, or PEG2 to PEG4;

R^2 and R^3 are independently $–CO_2H$, $–(C=O)_m-NR^CR^D$, or $–(CH_2)_q-NR^ER^F$;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or C_{1-3} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript m is independently 0 or 1;

each subscript q is an integer from 0 to 6;

X^A is $–CH_2–$, –O–, –S–, –NH–, or $–N(CH_3)–$;

X^B is absent or a 2-16 membered heteroalkylene;

L has the formula $–(A)_a-(W)_w-(Y)_y–$, wherein:

subscript a is 0 or 1;

subscript y is 0 or 1;

subscript w is 0 or 1;

A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};
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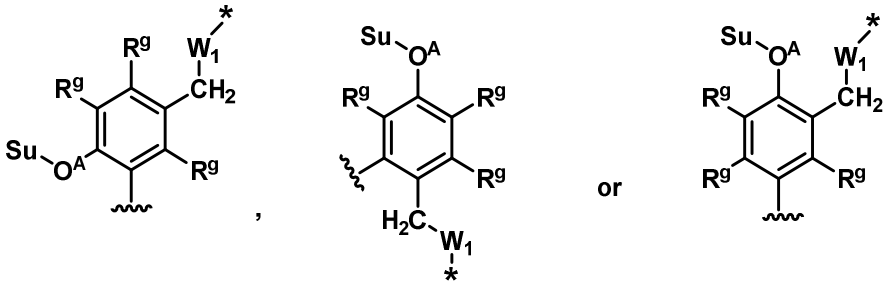
each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

W is from 1-12 amino acids or has the structure:



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

W^1 is absent or –O-C(=O)–;

~~~ represents covalent attachment to A or M^1;

* represents covalent attachment to Y, X^B, or X^A;

Y is self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety; and

X^B and L are each independently optionally substituted with a PEG Unit from PEG1 to PEG 72.

[0008] In some embodiments, Formula (I) has the structure:
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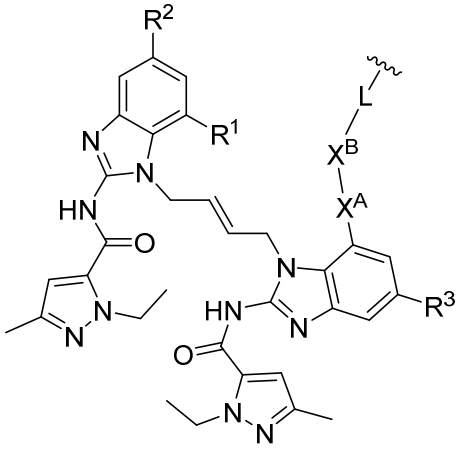
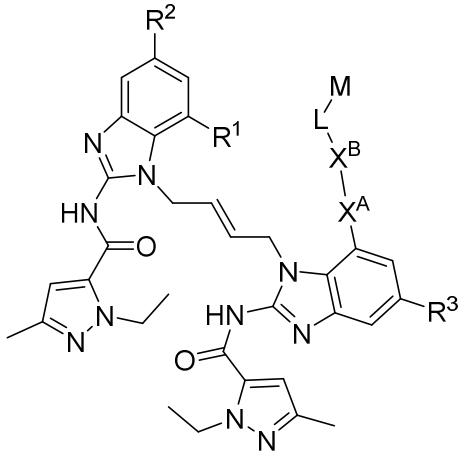


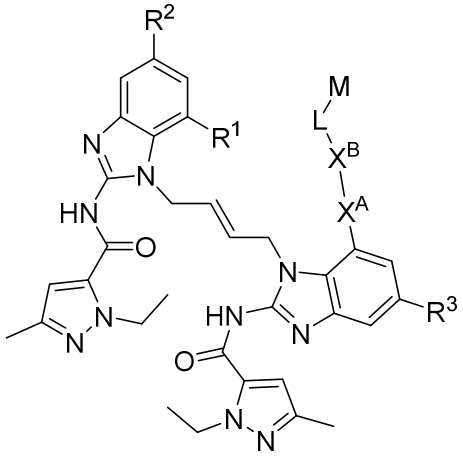
(I).

[0009] Some embodiments provide a compound of Formula (II):



(II).

[0009a] Disclosed herein is a compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

each R^2 and R^3 are independently –CO_2H, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or C_{1-3} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript m is independently 0 or 1;

each subscript q is independently an integer from 0 to 6;

X^A is –CH_2–, –O–, –S–, –NH–, or –$N(CH_3)$–;

X^B is absent or a 2-16 membered heteroalkylene;

L is a linker having the formula –$(A)_a$-$(W)_w$-$(Y)_y$–, wherein:

subscript a is 0 or 1;

subscript y is 0 or 1;

subscript w is 0 or 1;

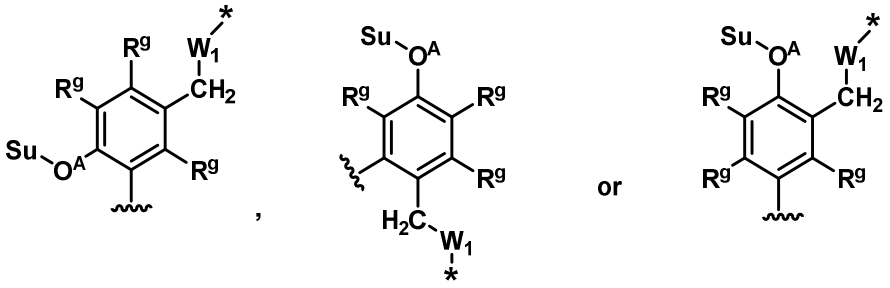
A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};

each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, -$NR^{d1}R^{e1}$, -$C(O)NR^{d1}R^{e1}$, -$C(O)(C_{1-6}$ alkyl), and -$C(O)O(C_{1-6}$ alkyl);

each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, -$NR^{d1}R^{e1}$, -$C(O)NR^{d1}R^{e1}$, -$C(O)(C_{1-6}$ alkyl), and -$C(O)O(C_{1-6}$ alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

W is from 1-12 amino acids or has the structure:



wherein Su is a Sugar moiety;

-O^A- represents a glycosidic bond;

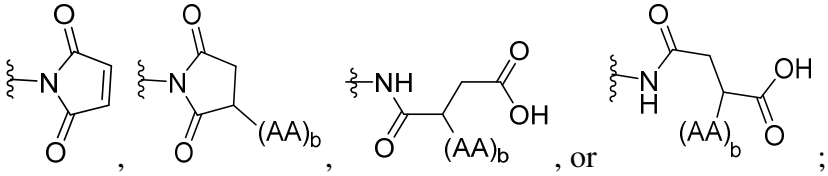
each R^g is independently hydrogen, halogen, -CN, or -NO_2;

W^1 is absent or –O-C(=O)–;

 represents covalent attachment to A or M;

* represents covalent attachment to Y, X^A, or X^B; and

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

M is ;

each AA is an independently selected amino acid, wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom;

each subscript b is independently an integer from 1 to 6; and

X^B and L are each independently optionally substituted with a PEG Unit from PEG1 to PEG 72.

[0010] Disclosed herein is an antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^*\text{-}(D'))_p}$$

wherein:

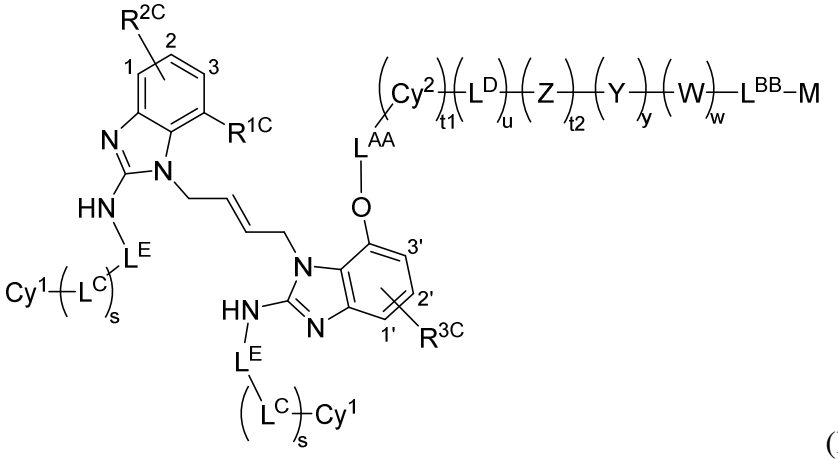
Ab is an antibody;

each S* is a sulfur atom from a cysteine residue of the antibody;

D' is a drug unit that is a radical of the compound of Formula (IV), as described herein; and

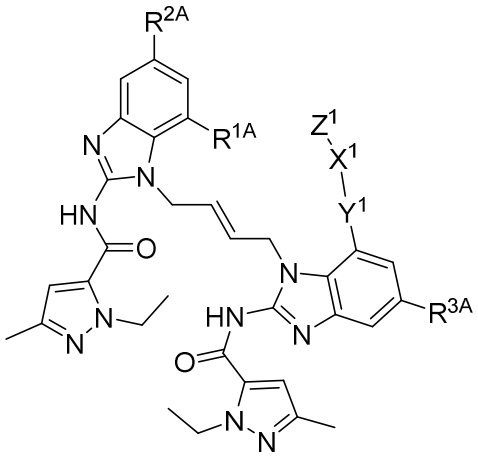
subscript p is an integer from 2 to 8.

[0011] In some embodiments, Formula (IV) has the structure:



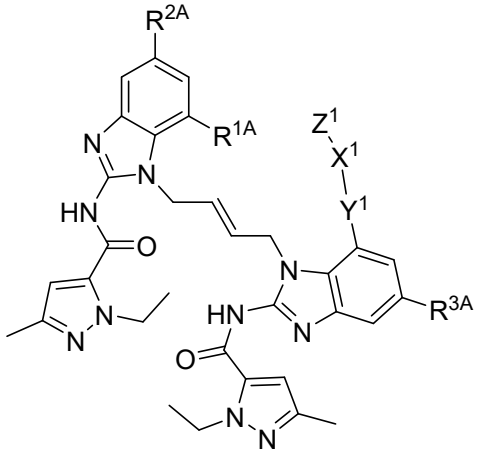
(IV).

[0012] Some embodiments provide a compound of Formula (III):



(III).

[0012a] Disclosed herein is a compound of Formula (III):



(III)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} is hydrogen, hydroxyl, C_{1-6} alkoxy, $-(C_{1-6}$ alkyl)C_{1-6} alkoxy, $-(CH_2)_{nn}$-$NR^{AA}R^{BB}$;

R^{2A} and R^{3A} are independently $-CO_2H$, $-(C=O)_{mm}$-$NR^{CC}R^{DD}$, or $-(CH_2)_q$-$NR^{EE1}R^{FF1}$;

each subscript nn is independently an integer from 0 to 6;

each subscript mm is independently 0 or 1;

each subscript qq is an integer from 0 to 6;

Y^1 is $-CH_2-$, $-O-$, $-S-$, $-NH-$, or $-N(CH_3)-$;

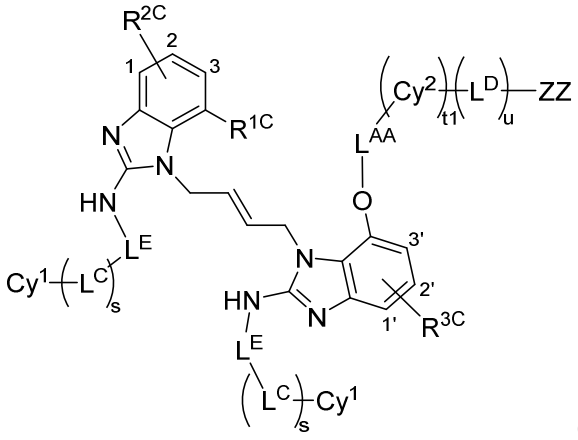
X^1 is a C_{2-6} alkylene;

Z^1 is $-NR^{EE}R^{FF}$, $-C(=O)NR^{GG}R^{HH}$, or $-CO_2H$;

each R^{AA}, R^{BB}, R^{CC}, and R^{DD}, R^{EE1}, and R^{FF1} are independently hydrogen or C_{1-3} alkyl; and

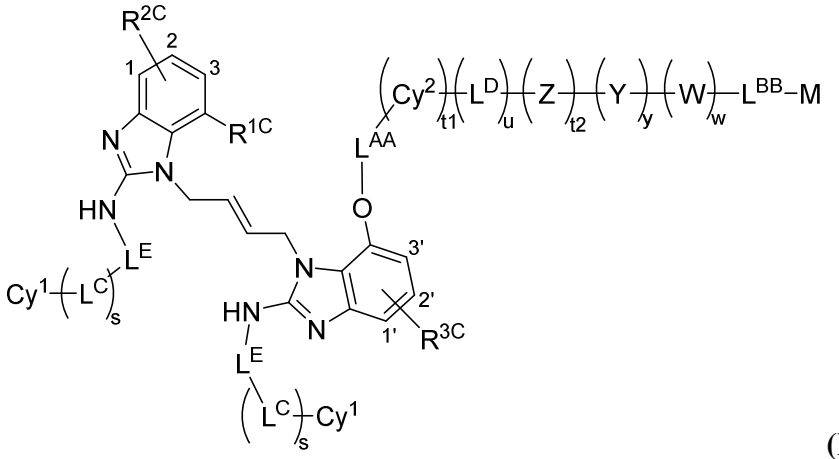
each R^{EE}, R^{FF}, R^{GG}, and R^{HH} are independently hydrogen or C_{1-6} alkyl.

[0013] Some embodiments provide a compound having the structure of Formula (V):



(V).

[0013a] Disclosed herein is a compound having the structure of Formula (IV):



(IV)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1C} is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, $–(CH_2)_n-NR^AR^B$, or PEG2 to PEG4;

R^{2C} is $–CO_2R^M$, $–(C=O)NR^CR^D$, $–S(O)_2NR^CR^D$, $–S(O)_2R^M$, $–(CH_2)_q-NR^ER^F$, $–(CH_2)_q-OR^M$, $–O(C=O)-NR^ER^F$, or $–NR^M(C=O)-NR^ER^F$, wherein R^{2C} is attached at any one of positions labeled 1, 2, or 3;

R^{3C} is $–CO_2R^M$, $–(C=O)NR^CR^D$, $–S(O)_2NR^CR^D$, $–S(O)_2R^M$, $–(CH_2)_q-NR^ER^F$, $–(CH_2)_q-OR^M$, $–O(C=O)-NR^ER^F$, or $–NR^M(C=O)-NR^ER^F$, wherein R^{3C} is attached at any one of positions labeled 1', 2', or 3';

each R^A, R^B, R^C, R^D, R^E, R^F, and R^M are independently hydrogen or C_{1-6} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript q is independently an integer from 0 to 6;

L^E is –(C=O)– or $–S(O)_2–$;

L^C is $–(CR^IR^J)_{1-3}–$

each R^I and R^J are independently hydrogen or C_{1-3} alkyl;

subscript s is 0 or 1;

each Cy^1 is independently a 4-6 membered heterocycle, a 5-6 membered heteroaryl, or a C_{3-6} cycloalkyl, each optionally substituted with one or more R^K;

each R^K is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d2}R^{e2}$, $-C(O)NR^{d2}R^{e2}$, -C(O)(C_{1-6} alkyl), and -C(O)O(C_{1-6} alkyl);

each R^{d2} and R^{e2} are independently hydrogen or C_{1-3} alkyl;

L^{AA} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, $–C(O)NR^L(CH_2)_{1-6}–$, $–(CH_2)_{1-6}O–$, $–C(O)(CH_2)_{1-6}O–$, or $–C(O)NR^L(CH_2)_{1-6}O–$;

R^L is hydrogen or C_{1-3} alkyl;

Cy^2 is C_{3-6} cycloalkyl, 4-6 membered heterocycle, 5-6 membered heteroaryl, or phenyl, each optionally substituted with one or more R^U;

each R^U is independently selected from the group consisting of $–CO_2R^{j1}$, $–(C=O)NR^{d3}R^{e3}$, $–S(O)_2NR^{d3}R^{e3}$, $–(CH_2)_{q1}$-$NR^{g1}R^{h1}$, $–(CH_2)_{q1}$-OR^{j1}, and $–(CH_2)_{q1}$-$(OCH_2CH_2)_{1-8}OH$;

each R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or C_{1-6} alkyl;

subscript q1 is an integer from 0 to 6;

subscripts t1 and t2 are independently 0 or 1, wherein at least one of t1 and t2 is 1;

L^D is $–(CH_2)_{1-6}–$;

subscript u is 0 or 1;

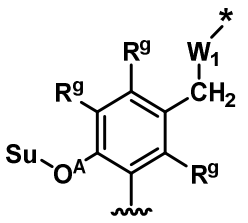
Z is $–N(R^{HH})–$ or $–N^+(C_{1-6}$ alkyl$)(R^{HH})–$;

R^{HH} is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl;

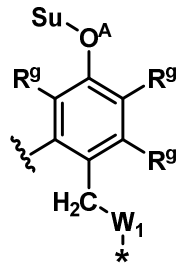
Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

subscript y is 0 or 1;

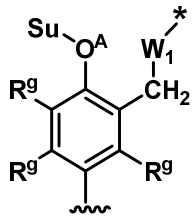
W is a chain of 1-12 amino acids or has the structure:



,



or



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

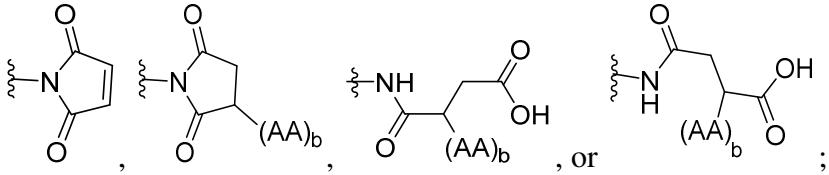
W^1 is absent or –O-C(=O)–;

~~~ represents covalent attachment to L^{BB};
~~~

* represents covalent attachment to Y, L^D, NR^{HH}, or Cy^2;

subscript w is 0 or 1;

L^{BB} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–[NHC(O)(CH_2)_{1-4}]_{1-3}–$; and

M is ; each AA is an independently selected amino acid, wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom; and

each subscript b is independently an integer from 1 to 6.

[0014] Disclosed herein is a composition comprising a distribution of ADCs as described herein.

[0015] Some embodiments provide a method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC composition, as described herein, to the subject.

[0015a] Disclosed herein is the use of the composition as defined herein in the manufacture of a medicament for treating cancer in a subject in need thereof.

[0016] Some embodiments provide a method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC, as described herein, to the subject.

[0016a] Disclosed herein is the use of the ADC as defined herein in the manufacture of a medicament for treating cancer in a subject in need thereof.

[0017] Some embodiments provide a method of inducing an anti-tumor immune response in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC composition, as described herein, to the subject.

[0017a] Disclosed herein is the use of the composition as defined herein in the manufacture of a medicament for inducing an anti-tumor immune response in a subject in need thereof.

[0018] Some embodiments provide a method of inducing an anti-tumor immune response in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC, as described herein, to the subject.

[0018a] Disclosed herein is the use of the ADC as defined herein in the manufacture of a medicament for inducing an anti-tumor immune response in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 illustrates the response of THP1-Dual™ cells (also referred to as THP1 dual reporter cells) to various small molecule STING agonists.

[0020] Figure 2 illustrates the response of wild type (WT) and STING-deficient murine bone marrow-derived macrophages to various small molecule STING agonists.

[0021] Figure 3 illustrates the response of THP1 dual reporter cells to ADCs comprising a non-targeted or targeted antibody conjugated to either compound **11** (cleavable linker with compound **1**), compound **12** (non-cleavable linker with compound **12a**), or compounds **13** or **14** (cleavable linkers with compound **12a**).

[0022] Figure 4 illustrates the response of THP1 dual reporter cells to compound **12** (non-cleavable linker with compound **12a**) and compound **16** (cysteine adduct of compound **12** and free drug released from ADCs containing compound **12**).

[0023] Figure 5 illustrates the response of THP1 dual reporter cells to compounds **12a** and **15b** as a free drug or conjugated to a non-binding or targeted antibody (ADC of compounds **12** and **15**) following incubation for 48 hours.

[0024] Figures 6A and 6B illustrate the response of SU-DHL-1 lymphoma cells to ADCs comprising a non-targeted, antigen C-targeted or PD-L1-targeted antibody conjugated to compound **11** (cleavable linker with compound **1**). Both cytokine production (MIP-1α) (Figure 6A) and viability (Figure 6B) are plotted.

[0025] Figure 7 illustrates the response of THP1 dual reporter cells cultured alone or co-cultured with HEK 293T cells engineered to express target antigen C to ADCs comprising an antigen C-targeted mAb with a hIgG1 LALAPG backbone conjugated to compounds **12**, **13**, or **14**.

[0026] Figure 8 illustrates the bystander activity of ADCs comprising either an EphA2-targeted mAb or a non-binding mAb with a mIgG2a WT or LALAPG backbone conjugated to compound **12** using Renca cancer cells and THP1 dual reporter cells.

[0027] Figures 9A and 9B illustrate the response to q7dx3 ADC dosing (3 weekly doses) in a Renca tumor mouse model to evaluate various ADCs comprising a non-binding or

EphA2-targeted mAb with a mIgG2a LALAPG backbone conjugated to compound 11 (dosed intraperitoneally), or compound 1 or (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-(3-morpholinopropoxy)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxamide tris(2,2,2-trifluoroacetate) (Compound A, a reference compound, dosed intravenously). FIG. 9A: tumor growth; FIG. 9B: % weight change.

[0028] Figures 10A and 10B illustrate the response to q7dx3 ADC dosing (3 weekly doses) in a Renca tumor mouse model to evaluate various ADCs comprising a non-binding or EphA2-targeted mAb with a mIgG2a LALAPG backbone conjugated to compounds **11** or **12** (dosed intraperitoneally). FIG. 10A: tumor growth; FIG. 10B: % weight change.

[0029] Figures 11A and 11B illustrate the response to q7dx3 ADC dosing (3 weekly doses) in a Renca tumor mouse model, which is engineered to express a human protein, to evaluate various ADCs comprising a non-binding or EphA2-targeted mAb with either a mIgG2a wild type (WT) or a mIgG2a LALAPG backbone conjugated to compounds **12** or **15**. FIG. 11A: tumor growth; FIG. 11B: % weight change.

[0030] Figure 12 illustrates the response to q7dx3 dosing (3 weekly doses, intraperitoneally) in a Renca tumor mouse model to evaluate the ADC comprising an EphA2-targeted mAb with a mIgG2a LALAPG backbone conjugated to compound **12** or unconjugated compound **12a**.

[0031] Figure 13 illustrates the response to q7dx3 dosing (3 weekly doses) of various compounds in a Renca tumor model to evaluate PD-L1-targeted mAb, and various ADCs comprising a non-binding, PD-L1-targeted or antigen C-targeted mAb conjugated to compound **11**.

[0032] Figure 14 illustrates the response to q7dx3 dosing (3 weekly doses) of various compounds in a CT26 tumor model to evaluate unconjugated compound **1**, a PD-L1-targeted mAb, and various ADCs comprising a non-binding, antigen C, PD-L1, or EphA2-targeted mAb conjugated to compound **11**.

[0033] Figures 15A-D illustrate the response to q7dx3 (3 weekly doses) or a single dose of ADC, as indicated, in a MC38 tumor model to evaluate various ADCs comprising a non-binding or EphA2-targeted mAb with a mIgG2a LALAPG backbone conjugated to compound **12**. Mice that achieved complete tumor regression in response to ADC treatment were rechallenged

with MC38 tumor cells and tumor growth was monitored. Figure 15A: tumor growth (WT mice); Figure 15B: weight loss (WT mice); Figure 15C: tumor growth (STING-deficient *Tmem173*^{gt} mice); Figure 15D: tumor growth following MC38 tumor rechallenge.

[0034] Figure 16A and 16B illustrate the response to q7dx3 mAb or ADC dosing (3 weekly doses indicated by the arrow heads) in a 4T1 tumor model to evaluate various ADCs comprising a non-binding or EphA2-targeted mAb with a mIgG2a LALAPG backbone conjugated to compound **12**. Figure 16A: tumor growth; Figure 16B: % weight change.

[0035] Figure 17 illustrates the pharmacokinetic profile of an ADC comprising a [deglycosylated] non-binding mAb conjugated to compound **12** following administration to male C57BL/6 mice.

DETAILED DESCRIPTION

[0036] Provided herein are antibody-drug conjugates (ADCs) that can elicit a localized immune response to target cells, and hence, reduced off-target toxicity, for example, as compared to the toxicity often observed with systemic administration of immunostimutory compounds, such as STING agonists. The *in vivo* toxicity of such compounds is often linked to systemic cytokine activation, resulting in both on- and off-target immune responses. The ADCs described herein include STING agonists as the drug payload to provide localized, selective induction of cytokines. *See, e.g.,* Milling, et al., *Adv. Drug Deliv. Rev.* 2017: 114; 79-101; *see also,* Hu, et al., *EBioMedicine* 2019: 41; 497-508. This approach can deliver specific STING activation, as well as localized immune cell recruitment, while reducing systemic cytokine release and its concomitant adverse effects.

Definitions

[0037] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present application; other, suitable methods and materials known in the art in some aspects of this disclosure are also used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entireties. In case of conflict, the present

specification, including definitions, will control. When trade names are used herein, the trade name includes the product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product, unless otherwise indicated by context.

[0038] The terms "a," "an," or "the" as used herein not only include aspects with one member, but also include aspects with more than one member. For instance, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a linker" includes reference to one or more such linkers, and reference to "the cell" includes reference to a plurality of such cells.

[0039] The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation, for example, within experimental variability and/or statistical experimental error, and thus the number or numerical range may vary up to ±10% of the stated number or numerical range. In reference to an ADC composition comprising a distribution of ADCs as described herein, the average number of conjugated STING agonist compounds to an antibody in the composition can be an integer or a non-integer, particularly when the antibody is to be partially loaded. Thus, the term "about" recited prior to an average drug loading value is intended to capture the expected variations in drug loading within an ADC composition.

[0040] The term "antibody" as used herein covers intact monoclonal antibodies, polyclonal antibodies, monospecific antibodies, multispecific antibodies (*e.g.,* bispecific antibodies), including intact antibodies and antigen binding antibody fragments, and reduced forms thereof in which one or more of the interchain disulfide bonds are disrupted, that exhibit the desired biological activity and provided that the antigen binding antibody fragments have the requisite number of attachment sites for the desired number of attached groups, such as a linker (L), as described herein. In some aspects, the linkers are attached via a succinimide or hydrolyzed succinimide to the sulfur atoms of cysteine residues of reduced interchain disulfide bonds and/or cysteine residues introduced by genetic engineering. The native form of an antibody is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light chain and one heavy chain. In each pair, the light and heavy chain variable domains (VL and VH) are together primarily responsible for binding to an antigen. The light chain and heavy chain variable domains consist of a framework region interrupted by three hypervariable regions, also called "complementarity determining regions" or "CDRs." The light chain and heavy chains also contain

constant regions that may be recognized by and interact with the immune system. (*see*, *e.g.*, Janeway *et al.*, 2001, *Immuno. Biology, 5th Ed.*, Garland Publishing, New York). An antibody includes any isotype (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or subclass (*e.g.,* IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) thereof. The antibody is derivable from any suitable species. In some aspects, the antibody is of human or murine origin, and in some aspects the antibody is a human, humanized or chimeric antibody. Antibodies can be fucosylated to varying extents or afucosylated.

[0041] An "intact antibody" is one which comprises an antigen-binding variable region as well as light chain constant domains (C_L) and heavy chain constant domains, C_H1, C_H2, C_H3 and C_H4, as appropriate for the antibody class. The constant domains are either native sequence constant domains (*e.g.,* human native sequence constant domains) or amino acid sequence variants thereof.

[0042] An "antibody fragment" comprises a portion of an intact antibody, comprising the antigen-binding or variable region thereof. Antibody fragments of the present disclosure include at least one cysteine residue (natural or engineered) that provides a site for attachment of a linker and/or linker-drug compound. In some embodiments, an antibody fragment includes Fab, Fab′, or $F(ab')_2$.

[0043] As used herein the term "engineered cysteine residue" or "eCys residue" refers to a cysteine amino acid or a derivative thereof that is incorporated into an antibody. In those aspects one or more eCys residues can be incorporated into an antibody, and typically, the eCys residues are incorporated into either the heavy chain or the light chain of an antibody. Generally, incorporation of an eCys residue into an antibody is performed by mutagenizing a nucleic acid sequence of a parent antibody to encode for one or more amino acid residues with a cysteine or a derivative thereof. Suitable mutations include replacement of a desired residue in the light or heavy chain of an antibody with a cysteine or a derivative thereof, incorporation of an additional cysteine or a derivative thereof at a desired location in the light or heavy chain of an antibody, as well as adding an additional cysteine or a derivative thereof to the N- and/or C-terminus of a desired heavy or light chain of an amino acid. Further information can be found in U.S. Pat. No. 9,000,130, the contents of which are incorporated herein in its entirety. Derivatives of cysteine (Cys) include but are not limited to beta-2-Cys, beta-3-Cys, homocysteine, and N-methyl cysteine.

[0044] In some embodiments, the antibodies of the present disclosure include those having one or more engineered cysteine (eCys) residues. In some embodiments, derivatives of

cysteine (Cys) include, but are not limited to beta-2-Cys, beta-3-Cys, homocysteine, and N-methyl cysteine.

[0045] An "antigen" is an entity to which an antibody specifically binds.

[0046] The terms "specific binding" and "specifically binds" mean that the antibody or antibody fragment thereof will bind, in a selective manner, with its corresponding target antigen and not with a multitude of other antigens. Typically, the antibody or antibody fragment binds with an affinity of at least about $1x10^{-7}$ M, for example, 10^{-8} M to 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen.

[0047] The term "amino acid" as used herein, refers to natural and non-natural, and proteogenic amino acids. Exemplary amino acids include, but are not limited to alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, proline, tryptophan, valine, cysteine, methionine, ornithine, β-alanine, citrulline, serine methyl ether, aspartate methyl ester, glutamate methyl ester, homoserine methyl ether, and N,N-dimethyl lysine.

[0048] A "sugar moiety" as used herein, refers to a monovalent radical of monosaccharide, for example, a pyranose or a furanose. A sugar moiety may comprise a hemiacetal or a carboxylic acid (from oxidation of the pendant $–CH_2OH$ group). In some embodiments, the sugar moiety is in the β-D conformation. In some embodiments, the sugar moiety is a glucose, glucuronic acid, or mannose group.

[0049] The term "inhibit" or "inhibition of" means to reduce by a measurable amount, or to prevent entirely (e.g., 100% inhibition).

[0050] The term "therapeutically effective amount" refers to an amount of an ADC as described herein that is effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the ADC provides one or more of the following biological effects: reduction of the number of cancer cells; reduction of tumor size; inhibition of cancer cell infiltration into peripheral organs; inhibition of tumor metastasis; inhibition, to some extent, of tumor growth; and/or relief, to some extent, of one or more of the symptoms associated with the cancer. For cancer therapy, efficacy, in some aspects, is measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

[0051] Unless otherwise indicated or implied by context, the term "substantial" or "substantially" refers to a majority, *i.e.* >50% of a population, of a mixture, or a sample, typically more than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97 %, 98%, or 99%.

[0052] The terms "intracellularly cleaved" and "intracellular cleavage" refer to a metabolic process or reaction occurring inside a cell, in which the cellular machinery acts on the ADC or a fragment thereof, to intracellularly release free drug from the ADC, or other degradant products thereof. The moieties resulting from that metabolic process or reaction are thus intracellular metabolites.

[0053] The terms "cancer" and "cancerous" refer to or describe the physiological condition or disorder in mammals that is typically characterized by unregulated cell growth. A "tumor" comprises multiple cancerous cells.

[0054] "Subject" as used herein refers to an individual to which an ADC is administered. Examples of a "subject" include, but are not limited to, a mammal such as a human, rat, mouse, guinea pig, non-human primate, pig, goat, cow, horse, dog, cat, bird and fowl. Typically, a subject is a rat, mouse, dog, non-human primate, or human. In some aspects, the subject is a human.

[0055] The terms "treat" or "treatment," unless otherwise indicated or implied by context, refer to therapeutic treatment and prophylactic measures to prevent relapse, wherein the object is to inhibit an undesired physiological change or disorder, such as, for example, the development or spread of cancer. For purposes of the present disclosure, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.,* not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" in some aspects also means prolonging survival as compared to expected survival if not receiving treatment.

[0056] In the context of cancer, the term "treating" includes any or all of: inhibiting growth of cancer cells or of a tumor; inhibiting replication of cancer cells, lessening of overall tumor burden or decreasing the number of cancer cells, and ameliorating one or more symptoms associated with the disease.

[0057] The term "salt," as used herein, refers to organic or inorganic salts of a compound, such as a Drug Unit (D), a linker such as those described herein, or an ADC. In some aspects, the compound contains at least one amino group, and accordingly acid addition salts can be formed with the amino group. Exemplary salts include, but are not limited to, sulfate, trifluoroacetate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion, or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a salt has one or more than one charged atom in its structure. In instances where there are multiple charged atoms as part of the salt, multiple counter ions can be present. Hence, a salt can have one or more charged atoms and/or one or more counterions. A "pharmaceutically acceptable salt" is one that is suitable for administration to a subject as described herein and in some aspects includes salts as described by P. H. Stahl and C. G. Wermuth, editors, Handbook of Pharmaceutical Salts: Properties, Selection and Use, Weinheim/Zürich:Wiley-VCH/VHCA, 2002, the list for which is specifically incorporated by reference in its entirety.

[0058] The term "tautomer," as used herein refers to compounds whose structures differ markedly in arrangement of atoms, but which exist in easy and rapid equilibrium, and it is to be understood that compounds provided herein may be depicted as different tautomers, and when compounds have tautomeric forms, all tautomeric forms are intended to be within the scope of the disclosure, and the naming of the compounds does not exclude any tautomer.

[0059] The term "halo" or "halogen" refers to fluoro, chloro, bromo, or iodo.

[0060] The term "alkyl" refers to an unsubstituted straight chain or branched, saturated hydrocarbon having the indicated number of carbon atoms (e.g., "C_1-C_4 alkyl," "C_1-C_6 alkyl," "C_1-C_8 alkyl," or "C_1-C_{10}" alkyl have from 1 to 4, to 6, 1 to 8, or 1 to 10 carbon atoms, respectively) and is derived by the removal of one hydrogen atom from the parent alkane. Representative straight chain "C_1-C_8 alkyl" groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl,

n-pentyl, n-hexyl, n-heptyl and n-octyl; while branched C_1-C_8 alkyls include, but are not limited to, isopropyl, *sec*-butyl, isobutyl, *tert*-butyl, isopentyl, and 2-methylbutyl.

[0061] The term "alkylene" refers to a bivalent unsubstituted saturated branched or straight chain hydrocarbon of the stated number of carbon atoms (e.g., a C_1- C_6 alkylene has from 1 to 6 carbon atoms) and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of the parent alkane. Alkylene groups can be substituted with 1-6 fluoro groups, for example, on the carbon backbone (as –CHF– or $–CF_2–$) or on terminal carbons of straight chain or branched alkylenes (such as $–CHF_2$ or $–CF_3$). Alkylene radicals include but are not limited to: methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), n-propylene ($-CH_2CH_2CH_2-$), n-propylene ($-CH_2CH_2CH_2-$), n-butylene ($-CH_2CH_2CH_2CH_2-$), difluoromethylene ($-CF_2-$), tetrafluoroethylene ($-CF_2CF_2-$), and the like.

[0062] The term "alkenyl" refers to an unsubstituted straight chain or branched, hydrocarbon having at least one carbon-carbon double bond and the indicated number of carbon atoms (*e.g.*, "C_2-C_8 alkenyl" or "C_2-C_{10}" alkenyl have from 2 to 8 or 2 to 10 carbon atoms, respectively). When the number of carbon atoms is not indicated, the alkenyl group has from 2 to 6 carbon atoms.

[0063] The term "alkynyl" refers to an unsubstituted straight chain or branched, hydrocarbon having at least one carbon-carbon triple bond and the indicated number of carbon atoms (*e.g.*, "C_2-C_8 alkynyl" or "C_2-C_{10}" alkynyl have from 2 to 8 or 2 to 10 carbon atoms, respectively). When the number of carbon atoms is not indicated, the alkynyl group has from 2 to 6 carbon atoms.

[0064] The term "heteroalkyl" refers to a stable straight or branched chain saturated hydrocarbon having the stated number of total atoms and at least one (e.g., 1 to 15) heteroatom selected from the group consisting of O, N, Si and S. The carbon and heteroatoms of the heteroalkyl group can be oxidized (e.g., to form ketones, N-oxides, sulfones, and the like) and the nitrogen atoms can be quaternized. The heteroatom(s) can be placed at any interior position of the heteroalkyl group and/or at the position at which the heteroalkyl group is attached to the remainder of the molecule. Heteroalkyl groups can be substituted with 1-6 fluoro groups, for example, on the carbon backbone (as –CHF– or $–CF_2–$) or on terminal carbons of straight chain or branched heteroalkyls (such as $–CHF_2$ or $–CF_3$). Examples of heteroalkyl groups include, but are not limited to, $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)_2$, $-C(=O)-NH-CH_2-CH_2-NH-CH_3$,

-C(=O)-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$, -C(=O)-NH-CH_2-CH_2-NH-C(=O)-CH_2-CH_3, -C(=O)-N(CH_3)-CH_2-CH_2-N(CH_3)-C(=O)-CH_2-CH_3, -O-CH_2-CH_2-CH_2-NH(CH_3), -O-CH_2-CH_2-CH_2-N(CH_3)$_2$, -O-CH_2-CH_2-CH_2-NH-C(=O)-CH_2-CH_3, -O-CH_2-CH_2-CH_2-N(CH_3)-C(=O)-CH_2-CH_3, -CH_2-CH_2-CH_2-NH(CH_3), -O-CH_2-CH_2-CH_2-N(CH_3)$_2$, -CH_2-CH_2-CH_2-NH-C(=O)-CH_2-CH_3, -CH_2-CH_2-CH_2-N(CH_3)-C(=O)-CH_2-CH_3, -CH_2-S-CH_2-CH_3, -CH_2-CH_2-S(O)-CH_3, -NH-CH_2-CH_2-NH-C(=O)-CH_2-CH_3, -CH_2-CH_2-S(O)$_2$-CH_3, -CH_2-CH_2-O-CF_3, and -Si(CH_3)$_3$. Up to two heteroatoms may be consecutive, such as, for example, -CH_2-NH-OCH_3 and –CH_2-O-Si(CH_3)$_3$. A terminal polyethylene glycol (PEG) moiety is a type of heteroalkyl group.

[0065] The term "heteroalkylene" refers to a bivalent unsubstituted straight or branched group derived from heteroalkyl (as defined herein). Examples of heteroalkylene groups include, but are not limited to, -CH_2-CH_2-O-CH_2-, -CH_2-CH_2-O-CF_2-, -CH_2-CH_2-NH-CH_2-, -C(=O)-NH-CH_2-CH_2-NH-CH_2- -C(=O)-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-, -C(=O)-NH-CH_2-CH_2-NH-C(=O)-CH_2-CH_2-, -C(=O)-N(CH_3)-CH_2-CH_2-N(CH_3)-C(=O)-CH_2-CH_2-, -O-CH_2-CH_2-CH_2-NH-CH_2-, -O-CH_2-CH_2-CH_2-N(CH_3)-CH_2-, -O-CH_2-CH_2-CH_2-NH-C(=O)-CH_2-CH_2-, -O-CH_2-CH_2-CH_2-N(CH_3)-C(=O)-CH_2-CH_2-, -CH_2-CH_2-CH_2-NH-CH_2-, -CH_2-CH_2-CH_2-N(CH_3)-CH_2-, -CH_2-CH_2-CH_2-NH-C(=O)-CH_2-CH_2-, -CH_2-CH_2-CH_2-N(CH_3)-C(=O)-CH_2-CH_2-, -CH_2-CH_2-NH-C(=O)-, -CH_2-CH_2-N(CH_3)-CH_2-, -CH_2-CH_2-N^+(CH_3)$_2$-, -NH-CH_2-CH_2(NH_2)-CH_2-, and -NH-CH_2-CH_2($NHCH_3$)-CH_2-. A bivalent polyethylene glycol (PEG) moiety is a type of heteroalkylene group.

[0066] The term "alkoxy" refers to an alkyl group, as defined herein, which is attached to a molecule via an oxygen atom. For example, alkoxy groups include, but are not limited to methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, sec-butoxy, tert-butoxy, n-pentoxy and n-hexoxy.

[0067] The term "alkylthio" refers to an alkyl group, as defined herein, which is attached to a molecule via a sulfur atom. For example, alkythio groups include, but are not limited to thiomethyl, thioethyl, thio-n-propyl, thio-iso-propyl, and the like.

[0068] The term "haloalkyl" refers to an unsubstituted straight chain or branched, saturated hydrocarbon having the indicated number of carbon atoms (*e.g.*, "C_1-C_4 alkyl," "C_1-C_6 alkyl," "C_1-C_8 alkyl," or "C_1-C_{10}" alkyl have from 1 to 4, to 6, 1 to 8, or 1 to 10 carbon atoms, respectively) wherein at least one hydrogen atom of the alkyl group is replaced by a halogen (e.g., fluoro, chloro, bromo, or iodo). When the number of carbon atoms is not indicated, the haloalkyl

group has from 1 to 6 carbon atoms. Representative C_{1-6} haloalkyl groups include, but are not limited to, trifluoromethyl, 2,2,2-trifluoroethyl, and 1-chloroisopropyl.

[0069] The term "haloalkoxy" refers to a haloalkyl group, as defined herein, which is attached to a molecule via an oxygen atom. For example, haloalkoxy groups include, but are not limited to trifluoromethoxy, 2,2,2-trifluoroethoxy, and 1,1,1-trifluoro2-methylpropoxy.

[0070] The term "cycloalkyl" refers to a cyclic, saturated or partially unsaturated hydrocarbon having the indicated number of carbon atoms (e.g., "C_{3-8} cycloalkyl" or "C_{3-6}" cycloalkyl have from 3 to 8 or 3 to 6 carbon atoms, respectively). When the number of carbon atoms is not indicated, the cycloalkyl group has from 3 to 6 carbon atoms. Cycloalkyl groups include bridged, fused, and spiro ring systems, and bridged bicyclic systems where one ring is aromatic and the other is unsaturated. Representative "C_{3-6} cycloalkyl" groups include, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

[0071] The term "aryl" refers to an unsubstituted monovalent carbocyclic aromatic hydrocarbon radical of 6-10 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Aryl groups include, but are not limited to, phenyl, naphthyl, anthracenyl, biphenyl, and the like.

[0072] The term "heterocycle" refers to a saturated or partially unsaturated ring or a multiple condensed ring system, including bridged, fused, and spiro ring systems. Heterocycles can be described by the total number of atoms in the ring system, for example a 3-10 membered heterocycle has 3 to 10 total ring atoms. The term includes single saturated or partially unsaturated rings (e.g., 3, 4, 5, 6 or 7-membered rings) from about 1 to 6 carbon atoms and from about 1 to 3 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur in the ring. The ring may be substituted with one or more (e.g., 1, 2 or 3) oxo groups and the sulfur and nitrogen atoms may also be present in their oxidized forms. Such rings include but are not limited to azetidinyl, tetrahydrofuranyl and piperidinyl. The term "heterocycle" also includes multiple condensed ring systems (e.g., ring systems comprising 2, 3 or 4 rings) wherein a single heterocycle ring (as defined above) can be condensed with one or more heterocycles (e.g., decahydronapthyridinyl), carbocycles (e.g., decahydroquinolyl) or aryls. The rings of a multiple condensed ring system can be connected to each other via fused, spiro and bridged bonds when allowed by valency requirements. It is to be understood that the point of attachment of a multiple condensed ring system (as defined above for a heterocycle) can be at any position of the multiple condensed ring

system including a heterocycle, aryl and carbocycle portion of the ring. It is also to be understood that the point of attachment for a heterocycle or heterocycle multiple condensed ring system can be at any suitable atom of the heterocycle or heterocycle multiple condensed ring system including a carbon atom and a heteroatom (e.g., a nitrogen). Exemplary heterocycles include, but are not limited to aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl, homopiperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, tetrahydrofuranyl, dihydrooxazolyl, tetrahydropyranyl, tetrahydrothiopyranyl, 1,2,3,4-tetrahydroquinolyl, benzoxazinyl, dihydrooxazolyl, chromanyl, 1,2-dihydropyridinyl, 2,3-dihydrobenzofuranyl, 1,3-benzodioxolyl, and 1,4-benzodioxanyl.

[0073] The term "heteroaryl" refers to an aromatic hydrocarbon ring system with at least one heteroatom within a single ring or within a fused ring system, selected from the group consisting of O, N and S. The ring or ring system has 4n +2 electrons in a conjugated π system where all atoms contributing to the conjugated π system are in the same plane. In some embodiments, heteroaryl groups have 5-10 total ring atoms and 1, 2, or 3 heteroatoms (referred to as a "5-10 membered heteroaryl"). Heteroaryl groups include, but are not limited to, imidazole, triazole, thiophene, furan, pyrrole, benzimidazole, pyrazole, pyrazine, pyridine, pyrimidine, and indole.

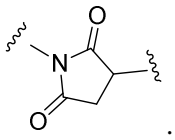
[0074] The term "hydroxyl" refers to an –OH radical.

[0075] The term "cyano" refers to a –CN radical.

[0076] The term "carboxy" refers to a –C(=O)OH radical.

[0077] The term "oxo" refers to a =O radical.

[0078] The term "succinimide" as used as part of an antibody-drug conjugate (ADC) refers to:



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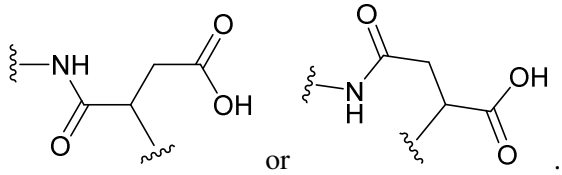
[0079] The term "hydrolyzed succinimide" as used as part of an antibody-drug conjugate (ADC) refers to:



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[0080] It will be appreciated by those skilled in the art that compounds of this disclosure having a chiral center may exist in and be isolated in optically active and racemic forms.

[0081] As used herein, the term "free drug" refers to a biologically active species that is not covalently attached to an antibody. Accordingly, free drug refers to any unconjugated compound, including a compound as it exists immediately upon cleavage from the ADC. The release mechanism can be via a cleavable linker in the ADC, or via intracellular conversion or metabolism of the ADC. In some aspects, the free drug will be protonated and/or may exist as a charged moiety. The free drug is a pharmacologically active species which is capable of exerting the desired biological effect. In some embodiments, the pharamacologically active species is the parent drug alone. In some embodiments, the pharamacologically active species is the parent drug bonded to a component or vestige of the ADC (e.g., a component of the linker, succinimide, hydrolyzed succinimide, and/or antibody that has not undergone subsequent intracellular metabolism). In some embodiments, free drug refers to a compound of Formula (I), as described herein, for example, wherein one or more of X^B, Y, W, A, and M^1 are absent. In some embodiments, free drug refers to a compound of Formula (II), as described herein. In some embodiments, free drug refers to a compound of Formula (II-A), as described herein. In some embodiments, free drug refers to a compound of Formula (III), as described herein. In some embodiments, free drug refers to a compound of Formula (IV), as described herein. In some embodiments, free drug refers to a compound of Formula (V), as described herein.

[0082] As used herein, the term "Drug Unit" refers to the free drug that is conjugated to an antibody in an ADC, as described herein.

Antibody-Drug Conjugate (ADC) Compounds

[0083] Some embodiments provide an antibody-drug conjugate (ADC) comprising:

an antibody;

a linker, as described herein; and

a compound of Formula (I) as described herein;

wherein the compound of Formula (I) is conjugated to the linker; and

wherein each linker is conjugated to the antibody via a succinimide or hydrolyzed succinimide covalently linked to a sulfur atom of a cysteine residue.

[0084] Some embodiments provide an antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^*\text{-}M^1\text{-}(D))_p}$$

wherein:

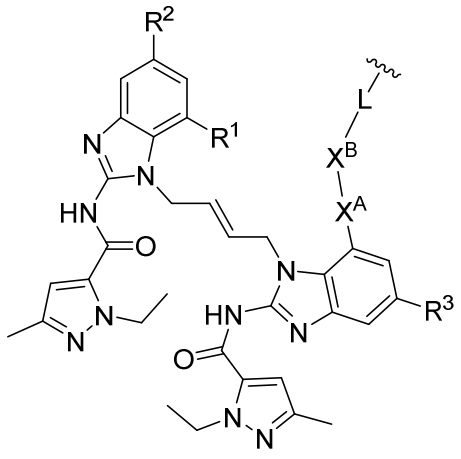
Ab is an antibody;

each S* is a sulfur atom from a cysteine residue of the antibody;

M^1 is a succinimide or a hydrolyzed succinimide;

subscript p is an integer from 2 to 8; and

each (D) is a Drug Unit of Formula (I):



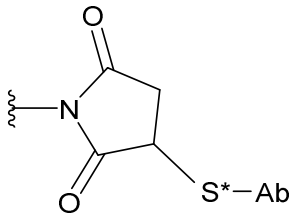
(I)

wherein:

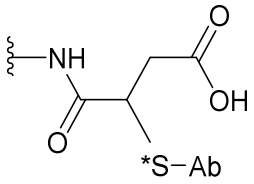
~~~ represents covalent attachment of L to M^1;

R^1 is hydrogen, hydroxyl, $C_{1\text{-}6}$ alkoxy, –($C_{1\text{-}6}$ alkyl) $C_{1\text{-}6}$ alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

each R^2 and R^3 are independently –CO_2H, –$(C{=}O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or $C_{1\text{-}3}$ alkyl;

each subscript n is independently an integer from 0 to6;

each subscript m is independently 0 or 1;

each subscript q is an integer from 0 to 6;

X^A is –CH_2–, –O–, –S–, –NH–, or –$N(CH_3)$–;

X^B is absent or a 2-16 membered heteroalkylene;

X^B, M^1, and L are each independently optionally substituted with a PEG Unit from PEG1 to PEG72; and
~~~

L is an optional linker as described herein. When present, L is linked via a covalent bond to X^B, or X^A if X^B is absent, as depicted in Formula (I). When L is absent, M^1 is linked via a covalent bond to X^B, or X^A if X^B is absent, as depicted in Formula (I).

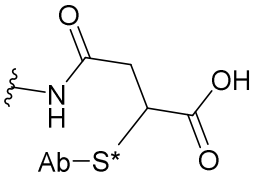
[0085] In some embodiments, M^1 is a succinimide. In some embodiments, M^1 is a hydrolyzed succinimide. It will be understood that a hydrolyzed succinimide may exist in two regioisomeric form(s). Those forms are exemplified below for hydrolysis of M^1 bonded to *S-Ab, wherein the structures representing the regioisomers from that hydrolysis are formula M^1a and M^1b; wherein the wavy lines adjacent to the bonds represent the covalent attachment to Formula (I).



Ab-S*-M^1



Ab-S*-M^1a



Ab-S*-M^1b

[0086] The M or M^1 groups, when present, are capable of linking an antibody to an A group, when present (or a W, Y, or X^B group if subscript a and/or subscript w and/or subscript y are 0). In this regard an antibody has a functional group that can form a bond with a functional group of M or M^1. Useful functional groups that can be present on an antibody, either naturally or via chemical manipulation include, but are not limited to, sulfhydryl (-SH), amino, hydroxyl, carboxy, and the anomeric hydroxyl group of a carbohydrate. In one aspect, the antibody functional groups are sulfhydryl and amino. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of an antibody. Alternatively, sulfhydryl groups can be generated by reaction of an amino group of a lysine moiety of an antibody using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent. In some embodiments, M or M^1 forms a bond with a sulfur atom of the antibody. The sulfur atom can be derived from a sulfhydryl group of the antibody.

[0087] In some embodiments, L has the formula $–(A)_a\text{-}(W)_w\text{-}(Y)_y–$, wherein:

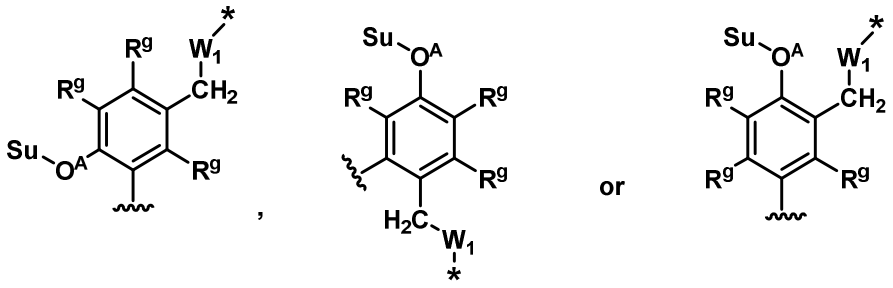
A is a $C_{2\text{-}20}$ alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};

each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

W is from 1-12 amino acids or has the structure:



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

W_1 is absent or –O-C(=O)–;

represents covalent attachment to A or M^1;

* represents covalent attachment to Y, X^A, or X^B in Formula (I);

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

subscript a is 0 or 1;

subscript y is 0 or 1; and

subscript w is 0 or 1.

[0088] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is hydroxyl. In some embodiments, R^1 is C_{1-6} alkoxy. In some embodiments, R^1 is methoxy. In some embodiments, R^1 is $-(C_{1-6}$ alkyl)C_{1-6} alkoxy. In some embodiments, R^1 is methoxyethyl. In some embodiments, R^1 is PEG2 to PEG4.

[0089] In some embodiments, R^1 is $-(CH_2)_n-NR^AR^B$. In some embodiments, R^A and R^B are both hydrogen. In some embodiments, R^A and R^B are independently C_{1-3} alkyl. In some

embodiments, one of R^A and R^B is hydrogen and the other of R^A and R^B is C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, each subscript n is 0. In some embodiments, each subscript n is 1. In some embodiments, each subscript n is 2. In some embodiments, each subscript n is 3, 4, 5, or 6.

[0090] In some embodiments, each R^2 and R^3 are independently $–CO_2H$, $–(C=O)_m$-NR^CR^D, or $–(CH_2)_q$-NR^ER^F; and R^2 and R^3 are the same. In some embodiments, each R^2 and R^3 are independently $–CO_2H$, $–(C=O)_m$-NR^CR^D, or $–(CH_2)_q$-NR^ER^F; and R^2 and R^3 are different.

[0091] In some embodiments, R^2 is $–(C=O)_m$-NR^CR^D. In some embodiments, R^3 is $–(C=O)_m$-NR^CR^D. In some embodiments, R^C and R^D are both hydrogen. In some embodiments, R^C and R^D are each independently C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, one of R^C and R^D is hydrogen and the other of R^C and R^D is C_{1-3} alkyl. In some embodiments, each subscript m is 0. In some embodiments, each subscript m is 1.

[0092] In some embodiments, R^2 is $–(CH_2)_q$-NR^ER^F. In some embodiments, R^3 is $–(CH_2)_q$-NR^ER^F. In some embodiments, R^E and R^F are both hydrogen. In some embodiments, R^E and R^F are each independently C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, one of R^E and R^F is hydrogen and the other of R^E and R^F is C_{1-3} alkyl. In some embodiments, each subscript q is 0. In some embodiments, each subscript q is an integer from 1 to 6. In some embodiments, each subscript q is 1. In some embodiments, each subscript q is 2. In some embodiments, each subscript q is 3, 4, 5, or 6.

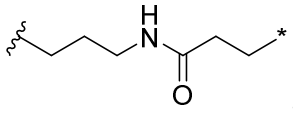
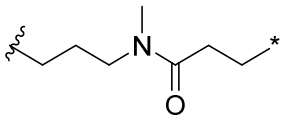
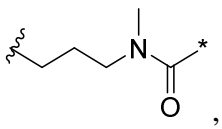
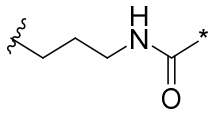
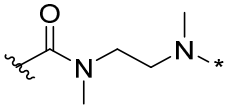
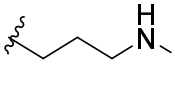
[0093] In some embodiments, R^3 is $–CO_2H$. In some embodiments, R^2 is $–CO_2H$.

[0094] In some embodiments, X^A is $–CH_2–$. In some embodiments, X^A is –O–. In some embodiments, X^A is –S–. In some embodiments, X^A is –NH–. In some embodiments, X^A is $–N(CH_3)–$.

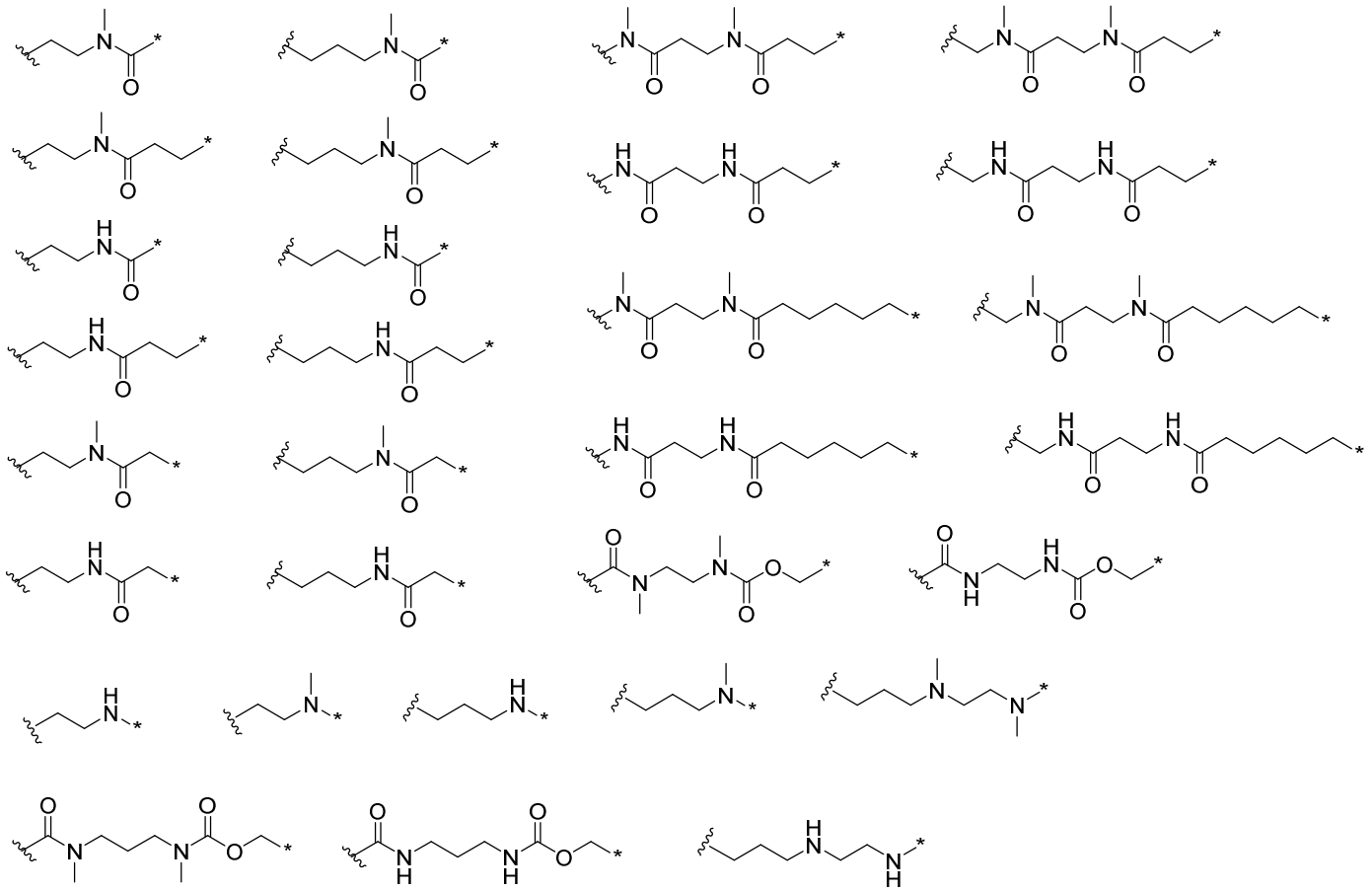
[0095] In some embodiments, X^B is a 2-16 membered heteroalkylene. In some embodiments, X^B is a 2-12 membered heteroalkylene. In some embodiments, X^B is a 2-10 membered heteroalkylene. In some embodiments, X^B is a 2-8 membered heteroalkylene. In some embodiments, X^B is a 4-8 membered heteroalkylene. In some embodiments, the heteroalkylene is straight chained. In some embodiments, the heteroalkylene is branched. In some embodiments, the heteroalkylene is branched, having 1-4 methyl groups. In some embodiments, the heteroalkylene is branched, having 1 or 2 methyl groups. In some embodiments, the heteroalkylene is substituted with 1-3 fluoro groups. In some embodiments, X^B comprises one or

two nitrogen atoms. In some embodiments, X^B comprises one or two oxo groups. In some embodiments, X^B comprises one nitrogen atom and one oxo group. In some embodiments, X^B comprises two nitrogen atoms and two oxo groups. In some embodiments, X^B comprises a carbamate.

[0096] In some embodiments, the covalent attachment of Y and X^B comprises an amide. In some embodiments, the covalent attachment of Y and X^B comprises a carbamate. In some embodiments, the covalent attachment of Y and X^B comprises an ether.

[0097] In some embodiments, X^B is , wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1. In some embodiments, X^B is , wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1. In some embodiments, X^B is , wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1. In some embodiments, X^B is , wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1. In some embodiments, X^B is , wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1. In some embodiments, X^B is , wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1.

[0098] In some embodiments, X^B is selected from the group consisting of the structures below, wherein  represents covalent attachment to X^A, and * represents covalent attachment to L, when present, or M^1.

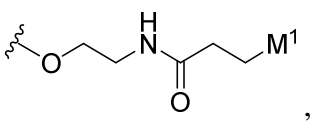


[0099] In some embodiments, one of X^B and L is substituted with a PEG Unit from PEG1 to PEG72, as described herein. In some embodiments, X^B and L are each substituted with an independently selected PEG Unit from PEG2 to PEG72, as described herein. In some embodiments, each PEG Unit from PEG 1 to PEG72 can range from PEG8 to PEG12, PEG12 to PEG24, or PEG36 to PEG72. In some embodiments, each PEG Unit from PEG 1 to PEG72 is PEG8 to PEG24.

[0100] In some embodiments, X^B and L are unsubstituted.

[0101] In some embodiments, R^1 is methoxy; R^2 and R^3 are both $–C(=O)NH_2$; and X^A is –O–.

[0102] In some embodiments, L is absent and X^A-X^B-M^1 is selected from the group consisting of: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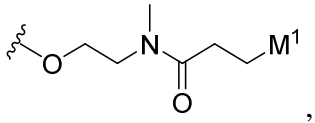
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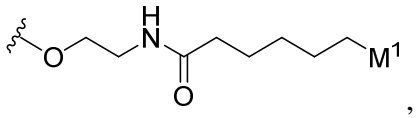
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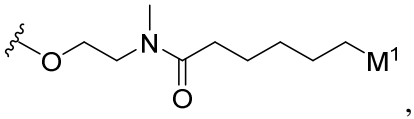
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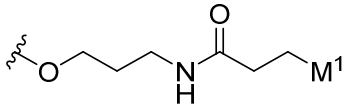
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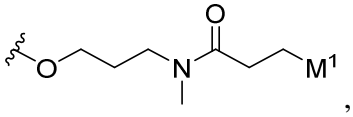
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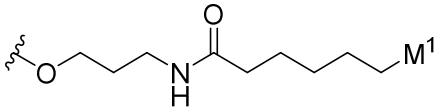
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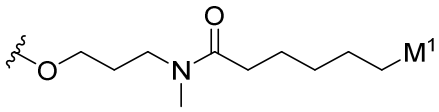
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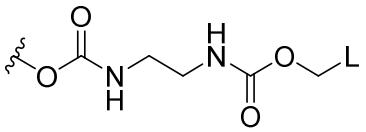
wherein  represents covalent attachment to the remainder of Formula (I).

[0103] In some embodiments, X^A-X^B-L is selected from: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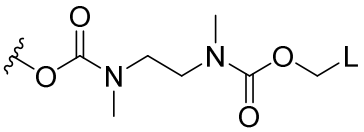
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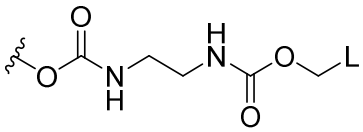
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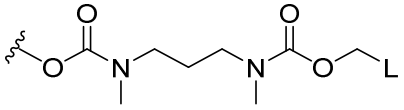
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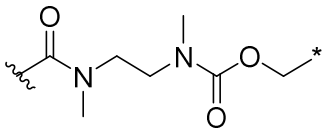
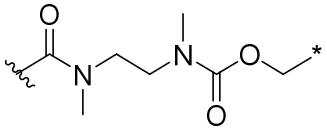
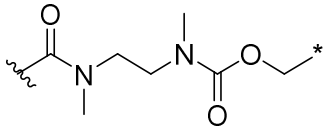
wherein  represents covalent attachment to the remainder of Formula (I).

[0104] In some embodiments, R^1 is methoxy and R^2 and R^3 are both $–C(=O)NH_2$. In some embodiments, X^A is –O– and X^B is , wherein  represents covalent attachment to X^A and * represents covalent attachment to L, when present, or M^1. In some embodiments, R^1 is methoxy; R^2 and R^3 are both $–C(=O)NH_2$; X^A is –O–; and X^B is ; wherein  represents covalent attachment to X^A and * represents covalent attachment to L, when present, or M^1. In some embodiments, R^1 is methoxy; R^2 and R^3 are both $–C(=O)NH_2$; X^A is –O–; X^B is ;  represents covalent attachment to X^A and * represents covalent attachment to L; and subscript a and subscript y are both 0.

[0105] In some embodiments, X^B is absent.

[0106] In some embodiments, subscript p is an integer from 2 to 8, from 2 to 6, from 2 to 4, from 4 to 8, or from 6 to 8. In some embodiments, subscript p is 2, 4, 6, or 8. In some embodiments, subscript p is 2. In some embodiments, subscript p is 4. In some embodiments, subscript p is 6. In some embodiments, subscript p is 8.

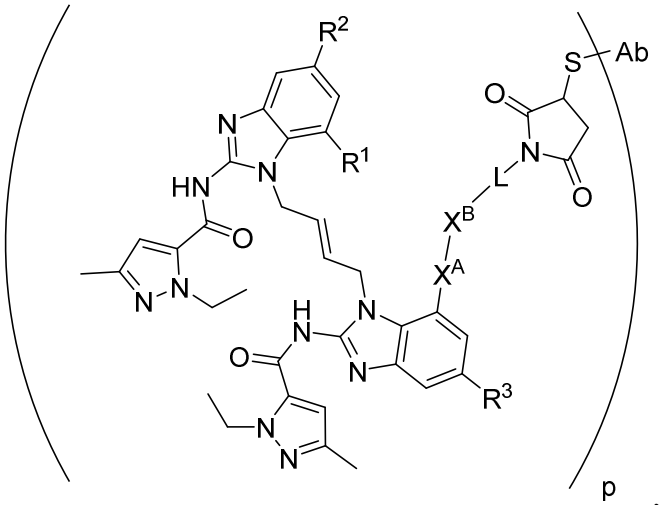
[0107] In some embodiments, X^B is absent and L is covalently attached to X^A. In some embodiments, X^B is absent and Y is covalently attached to X^A. In some embodiments, X^B is absent and Y is absent, and W is covalently attached to X^A. In some embodiments, X^B is absent, Y is absent, W is absent, and A is covalently attached to X^A.

[0108] In some embodiments, X^B is 2-16 membered heteroalkylene and L is covalently attached to X^B. In some embodiments, X^B is 2-16 membered heteroalkylene and Y is covalently attached to X^B. In some embodiments, X^B is 2-16 membered heteroalkylene, Y is absent, and W is covalently attached to X^B. In some embodiments, X^B is 2-16 membered heteroalkylene, Y is absent, W is absent, and A is covalently attached to X^B.

[0109] In some embodiments, W_1 is -OC(=O)- and subscript y is 1. In some embodiments, X^A is -O- and X^B and W_1 are absent. In some embodiments, X^A is NH or -O-, X^B is absent, and W_1 is -OC(=O). In some embodiments, X^A is $–N(CH_3)–$, X^B is absent, and W_1 is -OC(=O). In some embodiments, X^A is -S-, X^B is absent, and W_1 is -OC(=O). In some embodiments, W_1 is -OC(=O)- and X^B is covalently attached to W via -O- or -NH-.

[0110] In some embodiments, A is covalently attached to M^1. In some embodiments, when subscript a is 0, W is covalently attached to M^1. In some embodiments, when subscript a is 0 and subscript w is 0, Y is covalently attached to M^1. In some embodiments, when subscripts a, y, and w, are each 0, X^B is covalently attached to M^1.

[0111] In some embodiments, the ADC has the formula:



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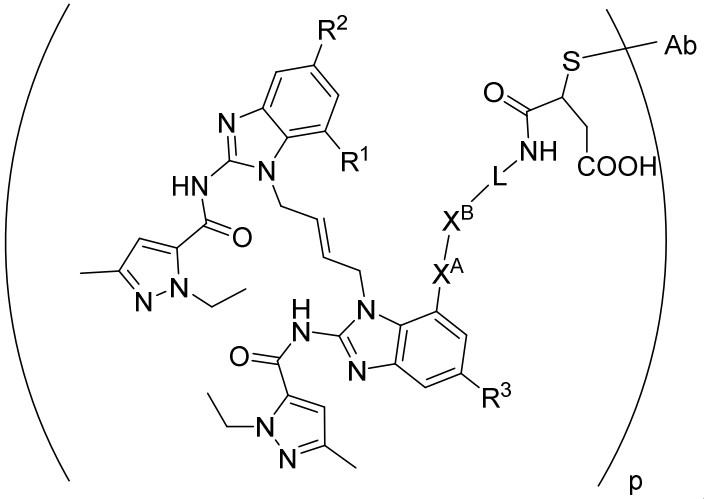

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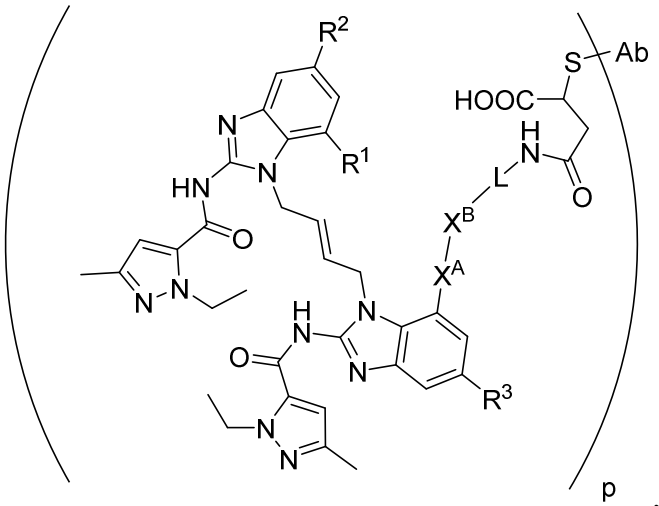


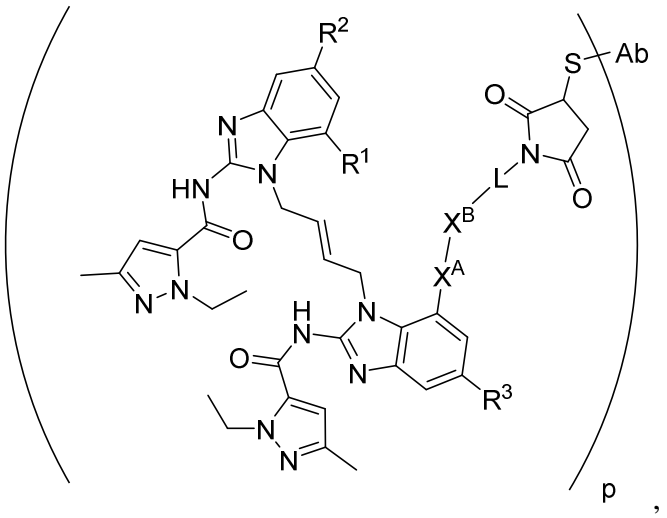
,

wherein:

Ab is an antibody;

R^1, R^2, R^3, X^A, X^B, and L are as defined above in connection with Formula (I); and each subscript p is independently an integer from 2 to 8.

[0112] In some aspects, the ADC has the formula:

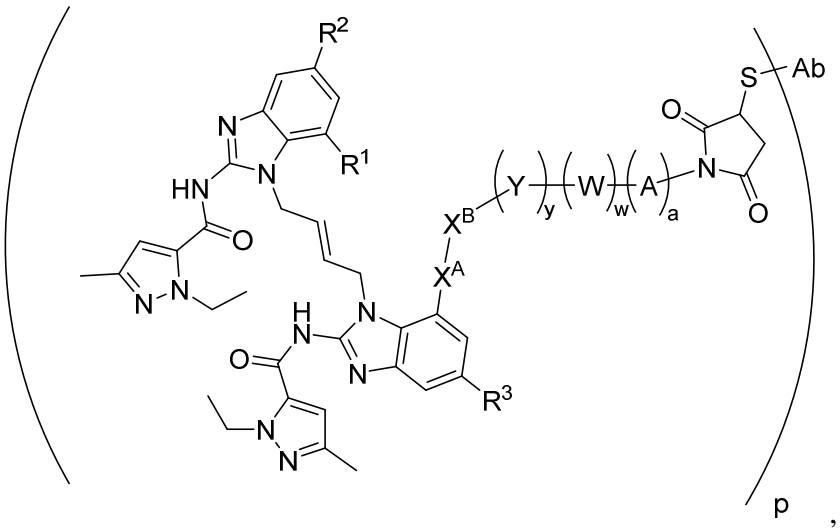


wherein:

Ab is an antibody;

R^1, R^2, R^3, X^A, X^B, and L are as defined above in connection with Formula (I); and each subscript p is independently an integer from 2 to 8.

[0113] In some aspects, the ADC has the formula:



wherein:

Ab is an antibody;

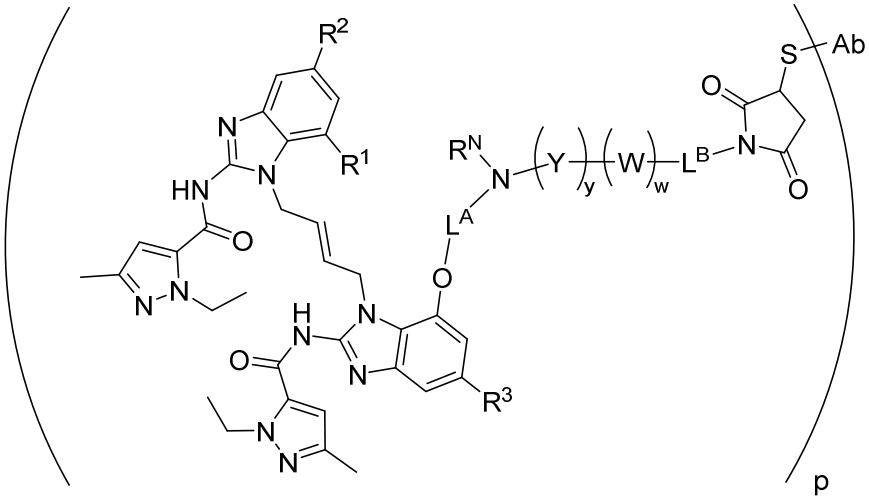
R^1, R^2, R^3, X^A, X^B, Y, W, and A are as defined above in connection with Formula (I); each subscript y is independently 0 or 1;

each subscript w is independently 0 or 1;

each subscript a is independently 0 or 1; and

each subscript p is independently an integer from 2 to 8.

[0114] In some embodiments, the ADC has the formula:



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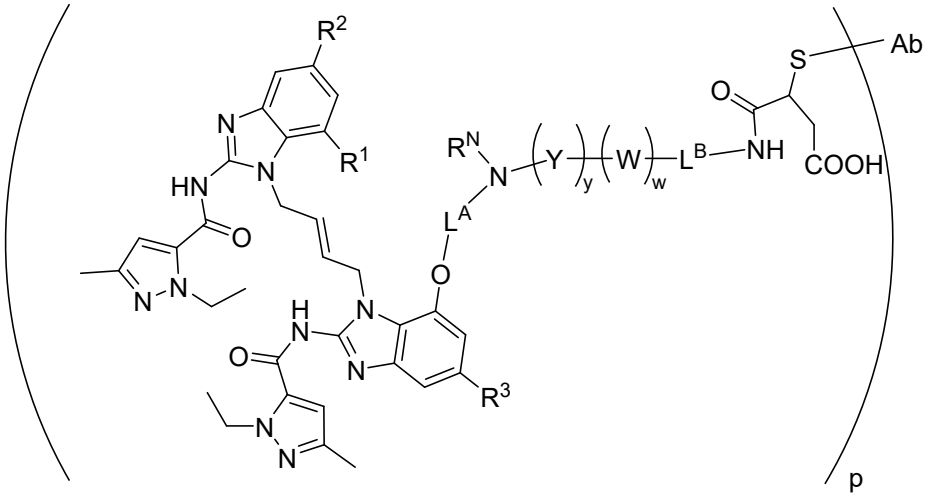
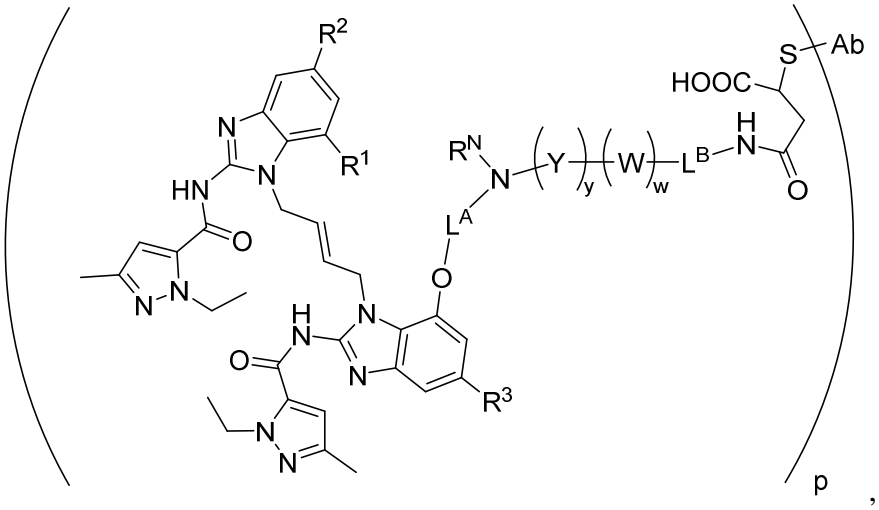


, or



wherein:

Ab is an antibody;

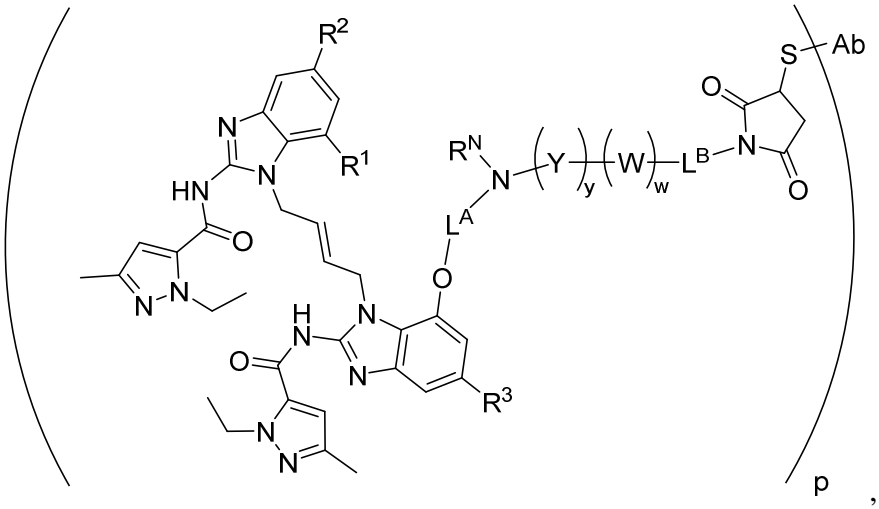
R^1, R^2, R^3, L^A, R^N, Y, W, and L^B are as defined below in connection with Formula (II-A);

each subscript y is independently 0 or 1;

each subscript w is independently 0 or 1; and

each subscript p is independently an integer from 2 to 8.

[0115] In some aspects, the ADC has the formula:



wherein:

Ab is an antibody;

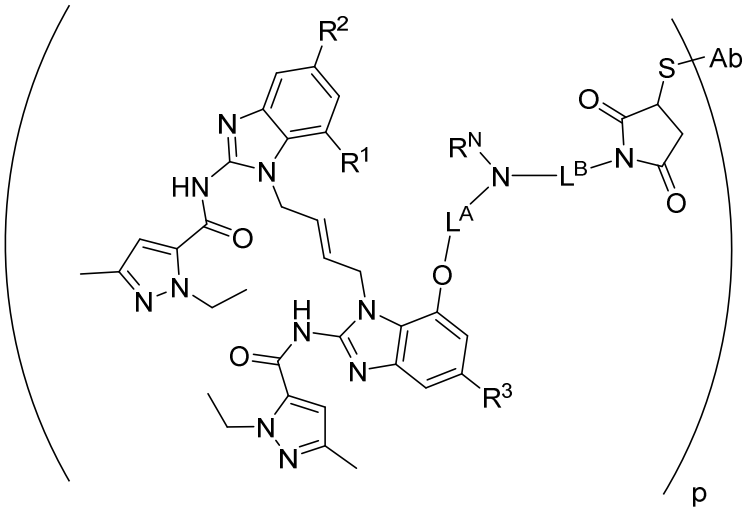
R^1, R^2, R^3, L^A, R^N, Y, W, and L^B are as defined below in connection with Formula (II-A);

each subscript y is independently 0 or 1;

each subscript w is independently 0 or 1; and

each subscript p is independently an integer from 2 to 8.

[0116] In some embodiments, the ADC has the formula:



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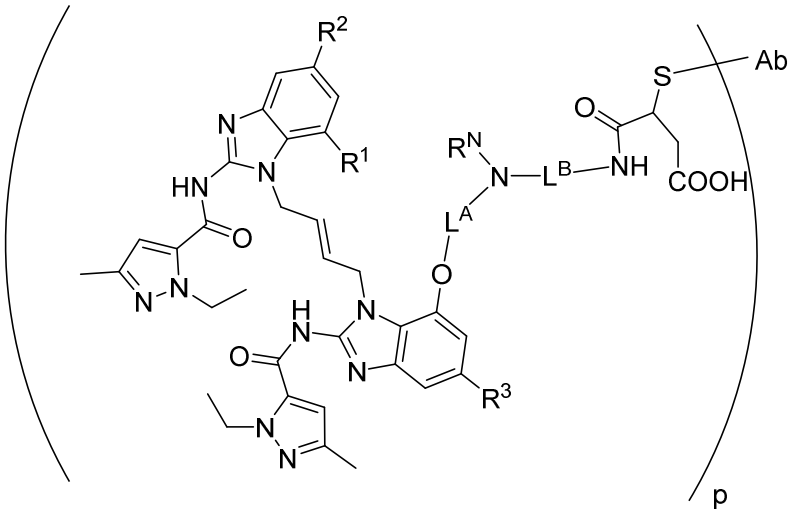
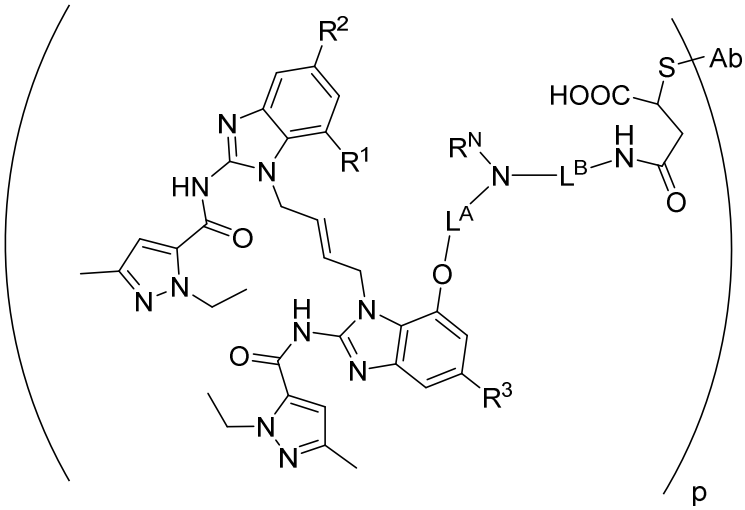


p , or



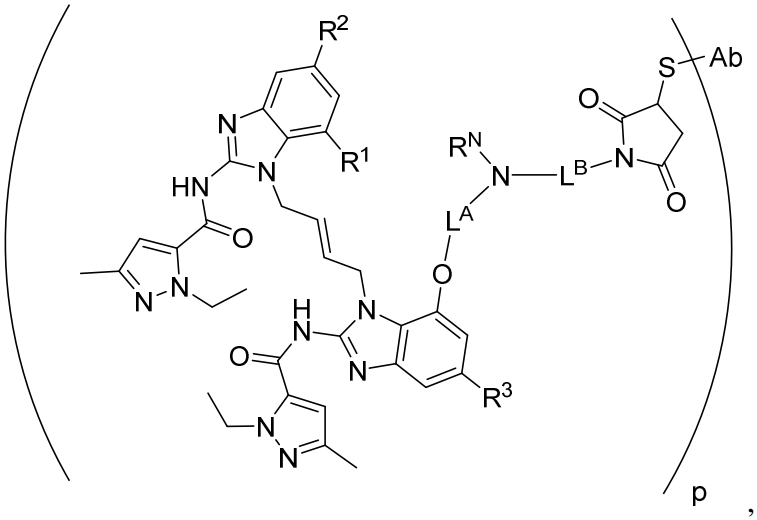
p ,

wherein:

Ab is an antibody;

R^1, R^2, R^3, L^A, R^N, and L^B are as defined below in connection with Formula (II-B); and each subscript p is independently an integer from 2 to 8.

[0117] In some aspects, the ADC has the formula:



,

wherein:

Ab is an antibody;

R^1, R^2, R^3, L^A, R^N, and L^B are as defined below in connection with Formula (II-B); and each subscript p is independently an integer from 2 to 8.

[0118] Some embodiments provide an antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^*\text{-}(D'))_p}$$

wherein:

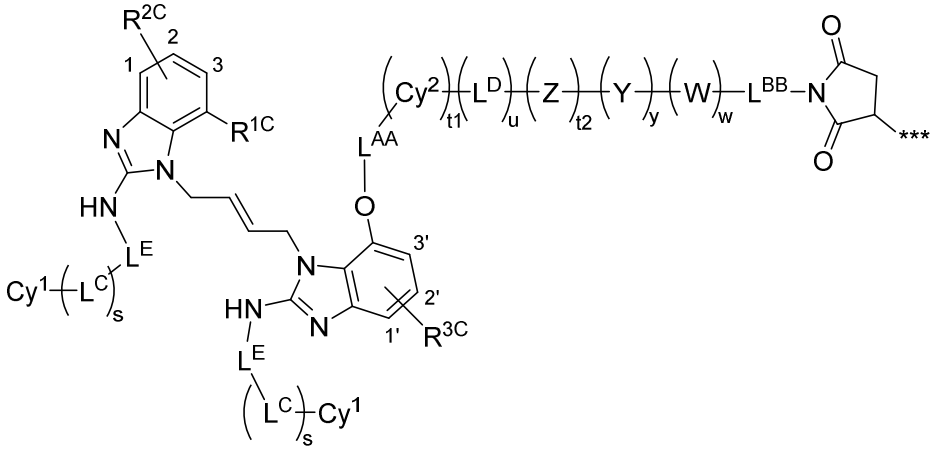
Ab is an antibody;

each S* is a sulfur atom from a cysteine residue of the antibody;

D' is a drug unit that is a radical of the compound of Formula (IV), as described below; and

subscript p is an integer from 2 to 8.

[0119] In some embodiments, the radical of the compound of Formula (IV) comprises a radical in substituent M within Formula (IV). In some embodiments, the drug unit D' has the structure:



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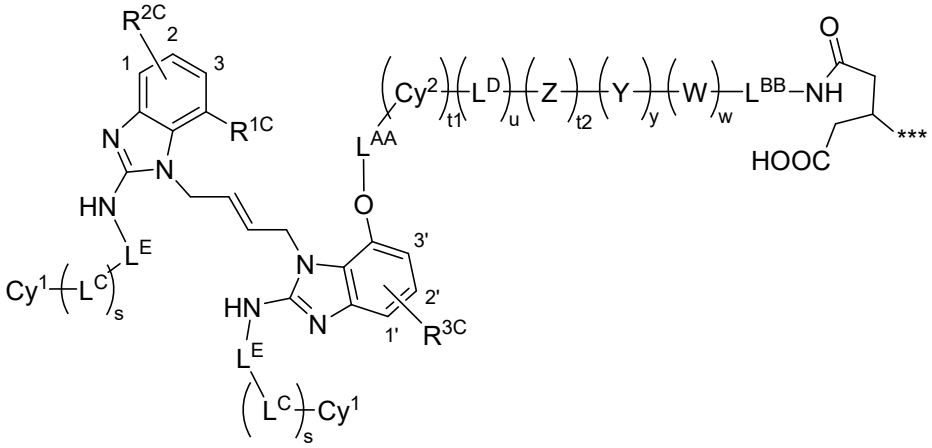

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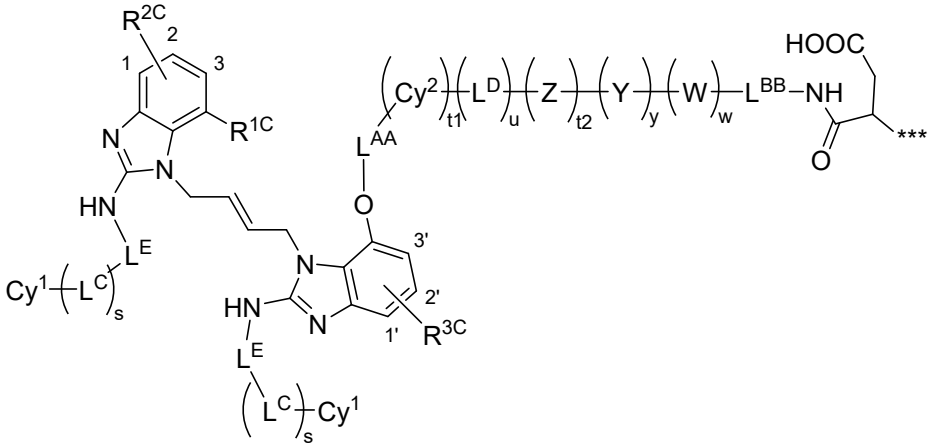
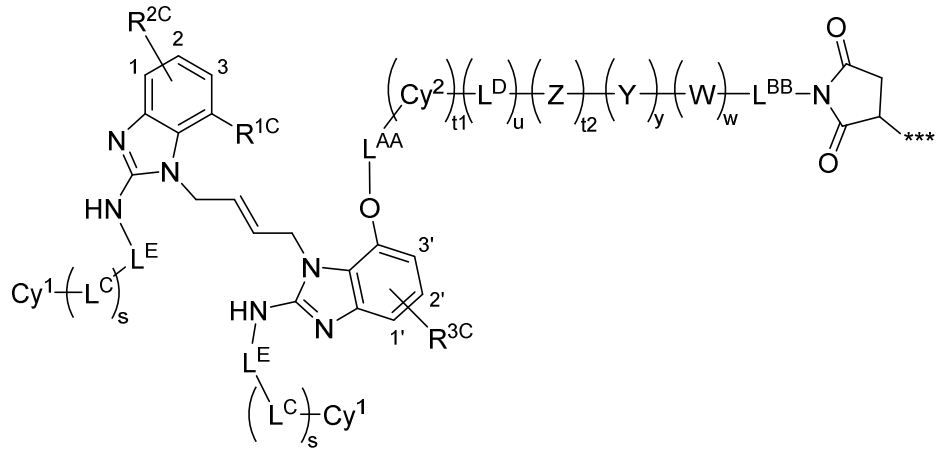


,

where *** indicates attachment to S* and the remaining variables are as defined below in connection with Formula (IV).

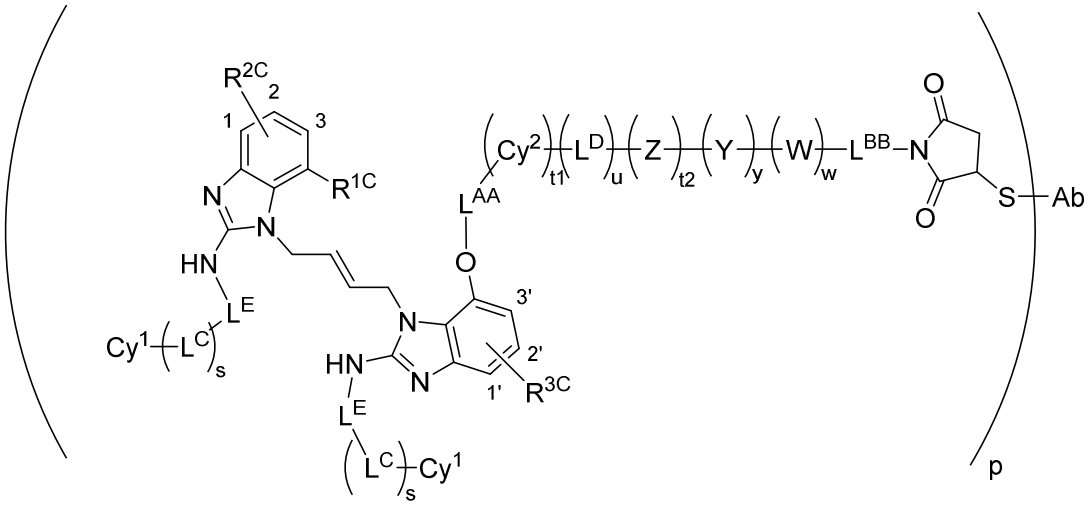
[0120] In some aspects, the drug unit D' has the structure:



,

where *** indicates attachment to S* and the remaining variables are as defined below in connection with Formula (IV).

[0121] In some embodiments, the ADC has the formula:



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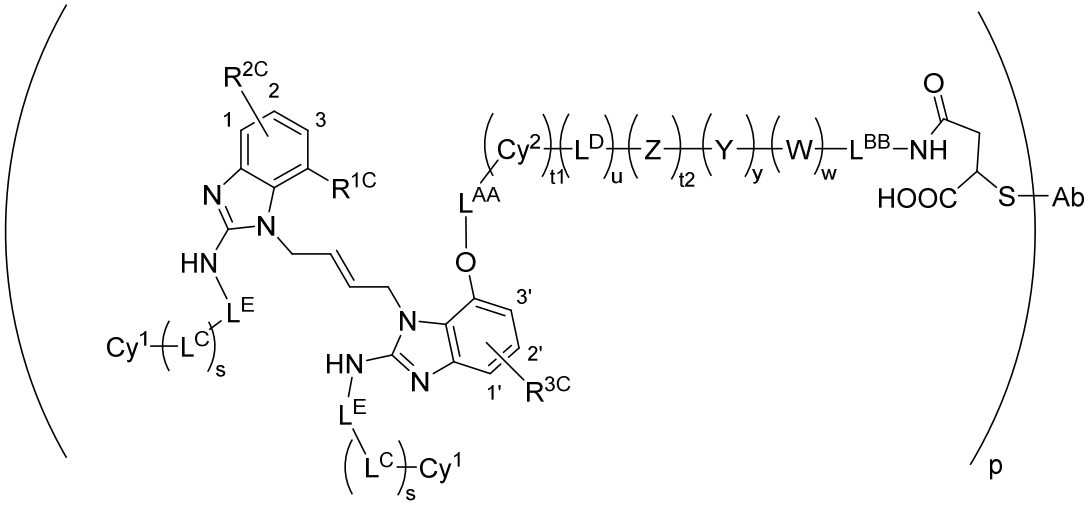

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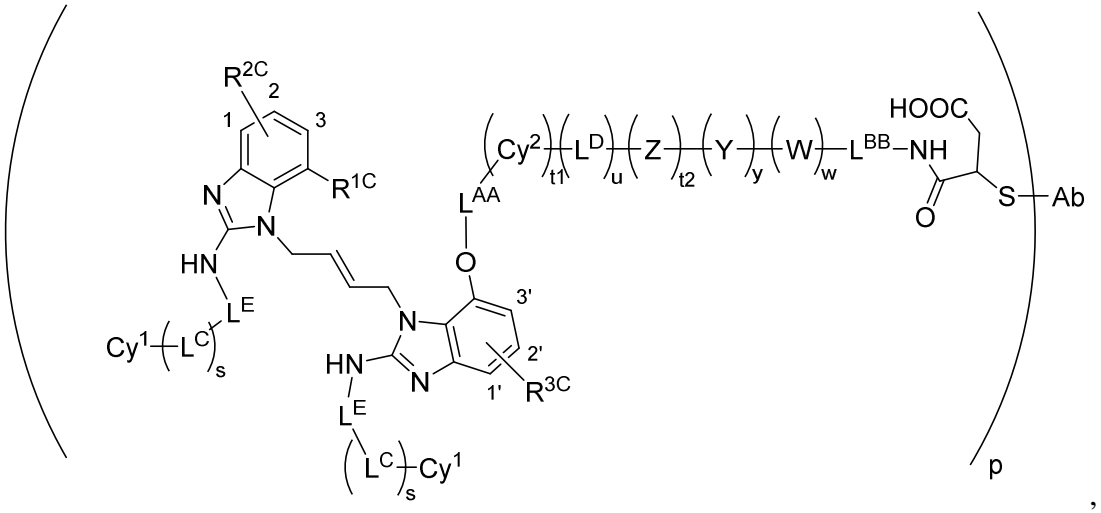


,

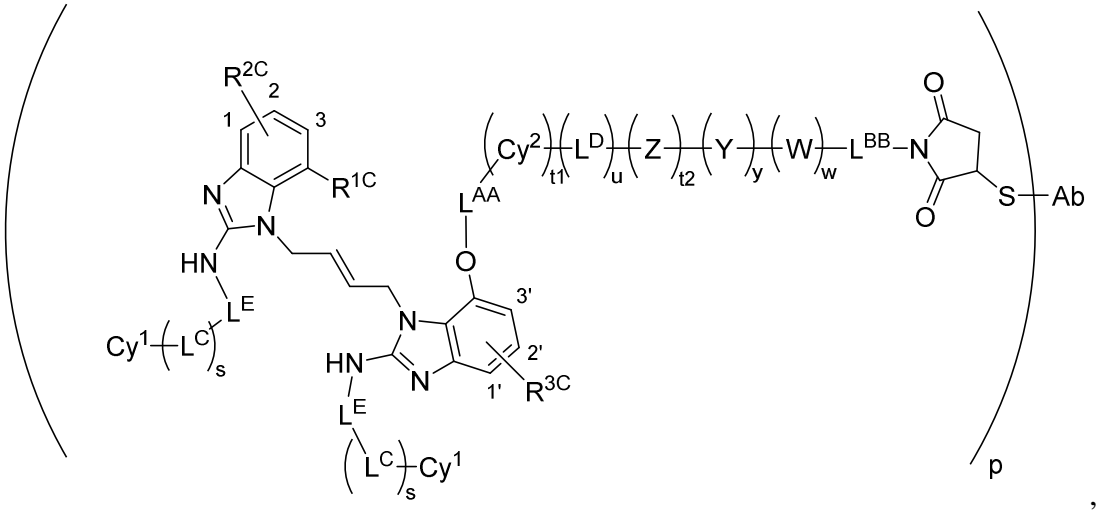
wherein:

Ab is an antibody;

each subscript p is independently an integer from 2 to 8; and

the remaining variables are as defined below in connection with Formula (IV).

[0122] In some aspects, the ADC has the formula:



,

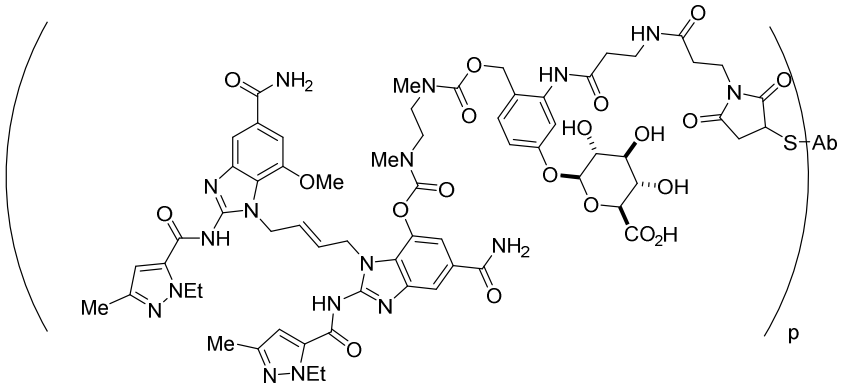
wherein:

Ab is an antibody;

each subscript p is independently an integer from 2 to 8; and

the remaining variables are as defined below in connection with Formula (IV).

[0123] Some embodiments provide an antibody-drug conjugate (ADC) selected from the group consisting of:



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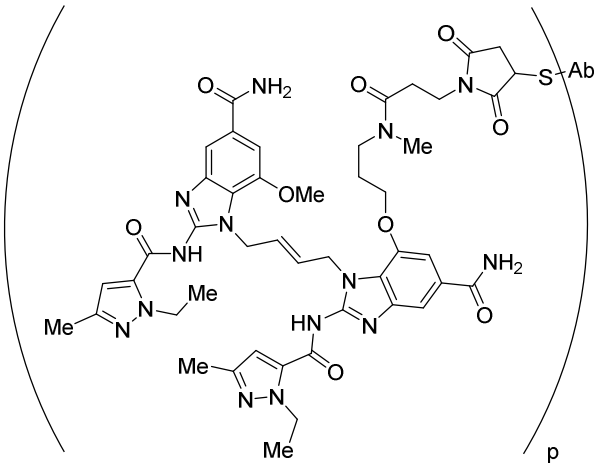

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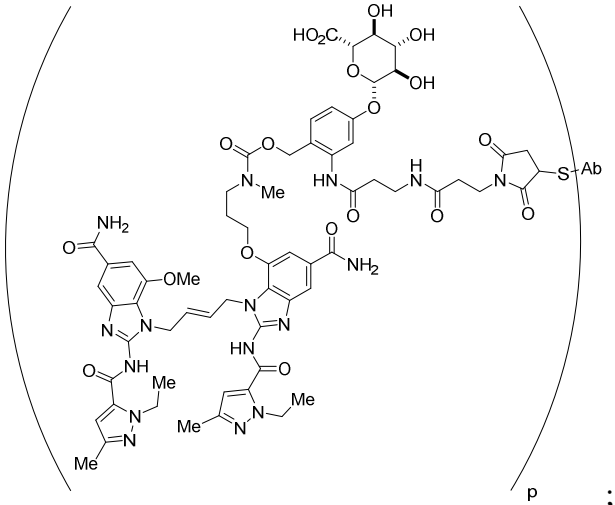

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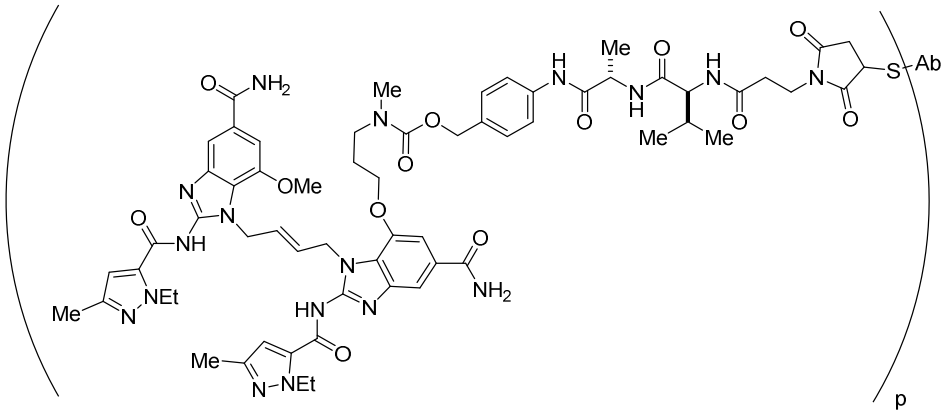

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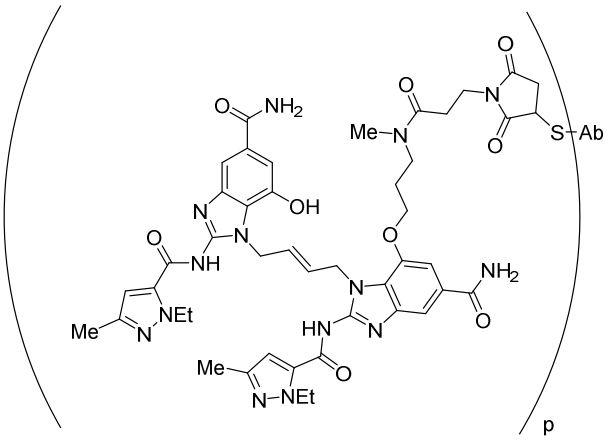
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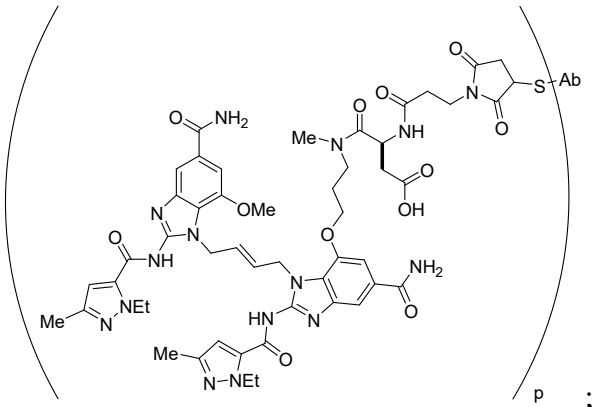
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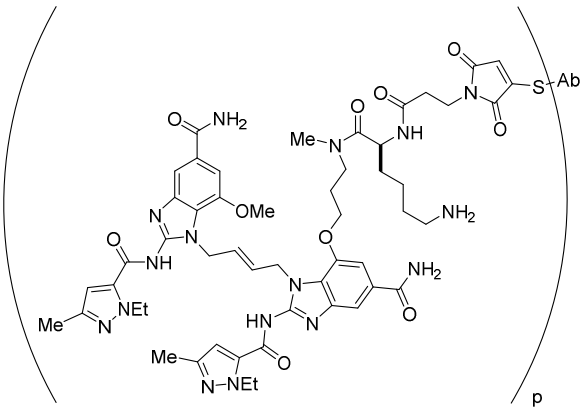
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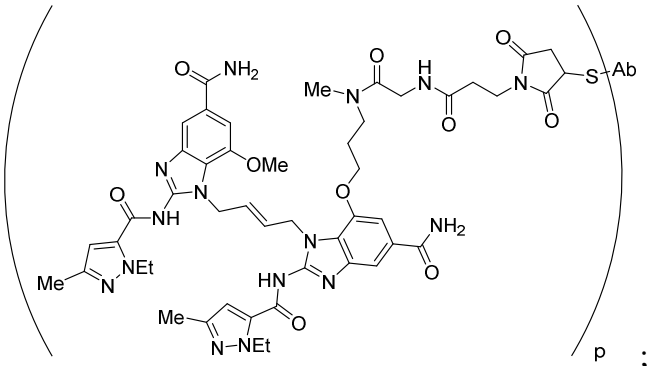
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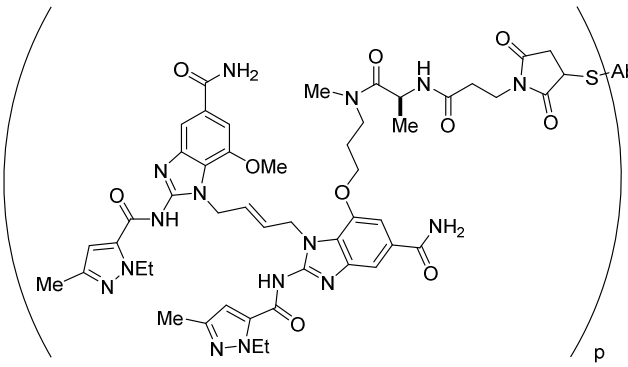
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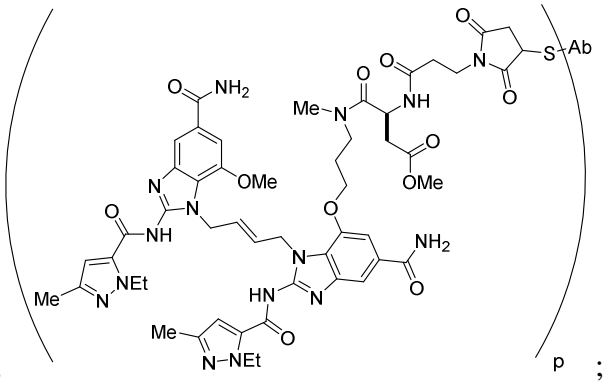
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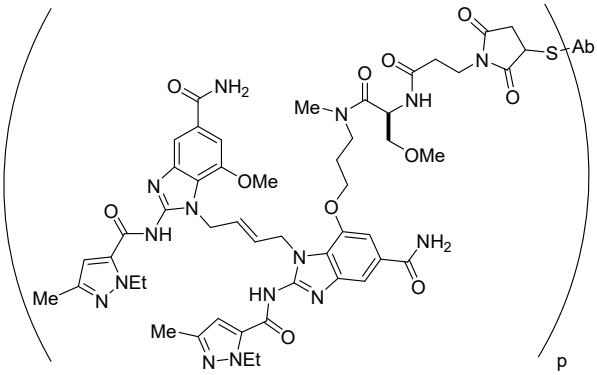
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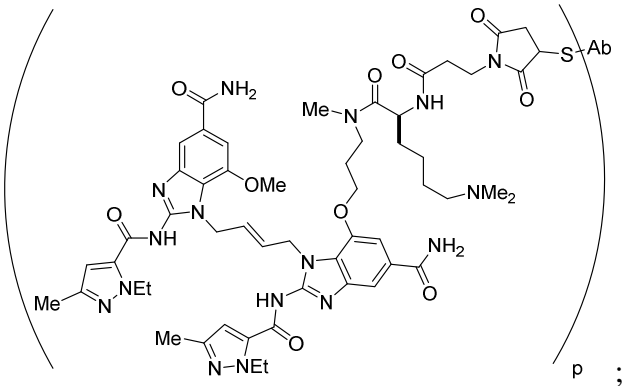
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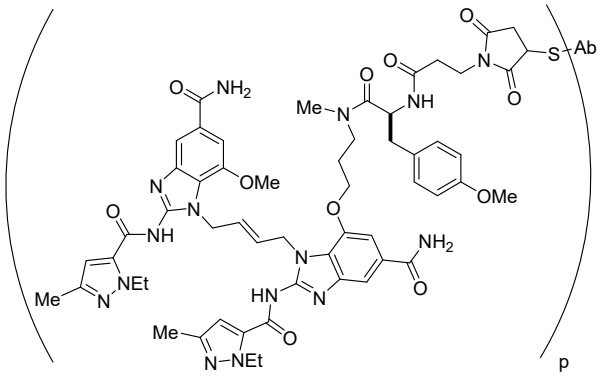
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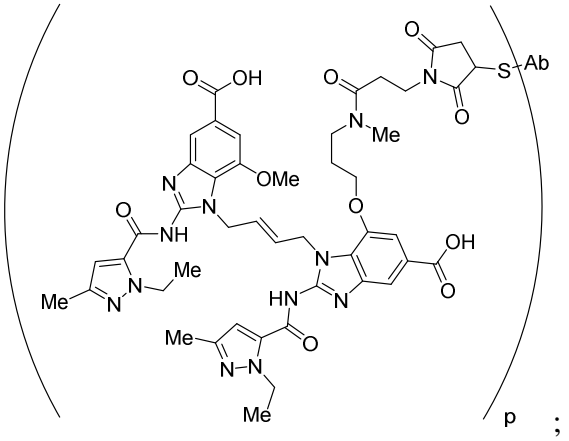
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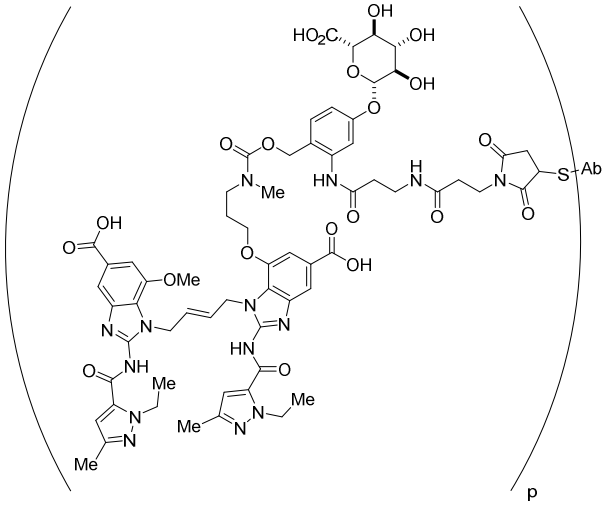
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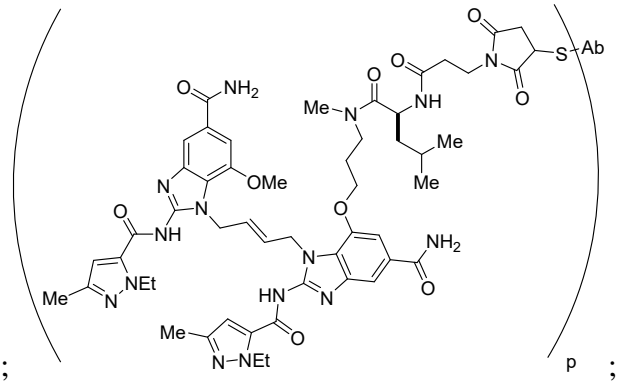
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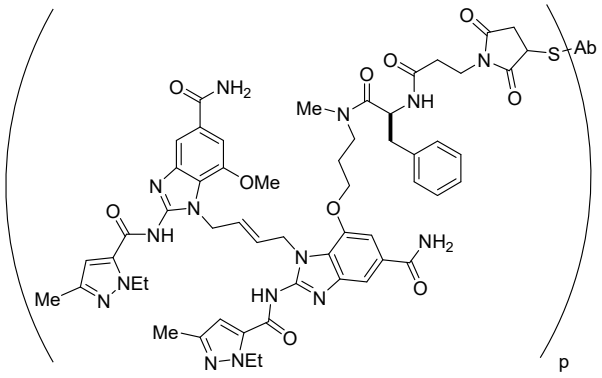
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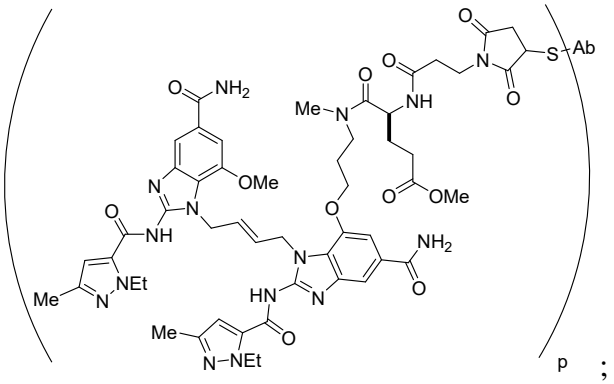
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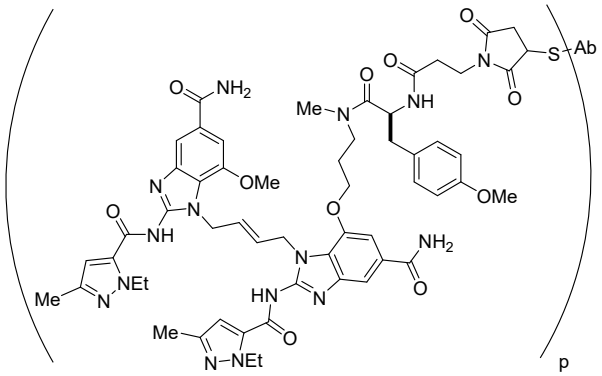
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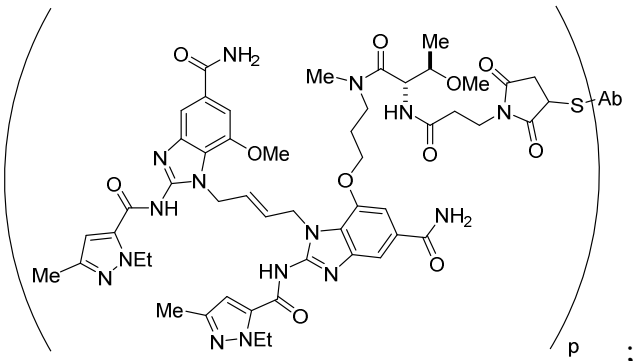
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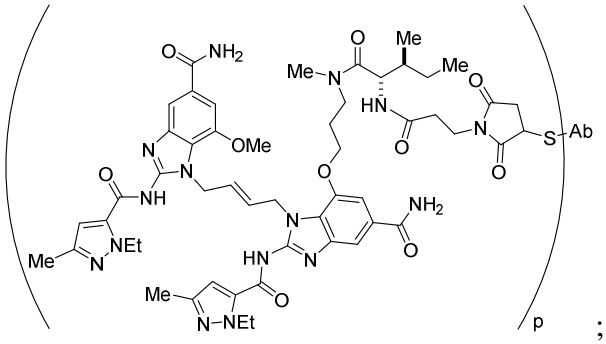
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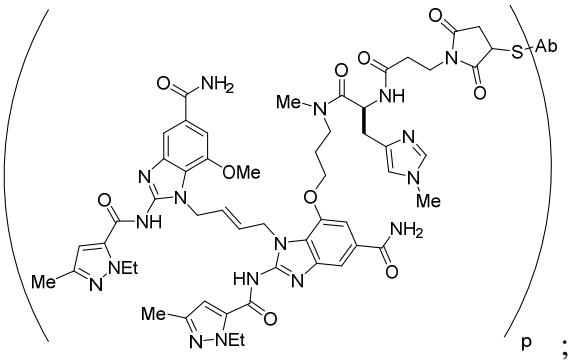
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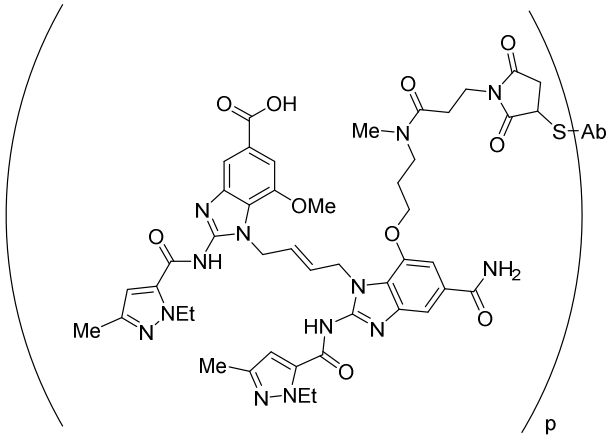
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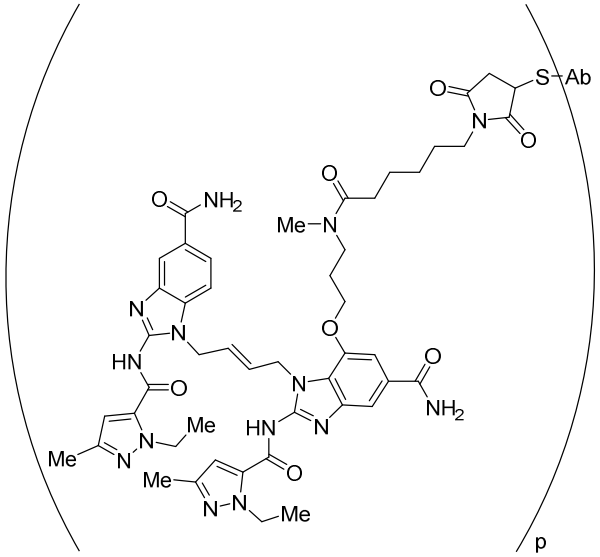
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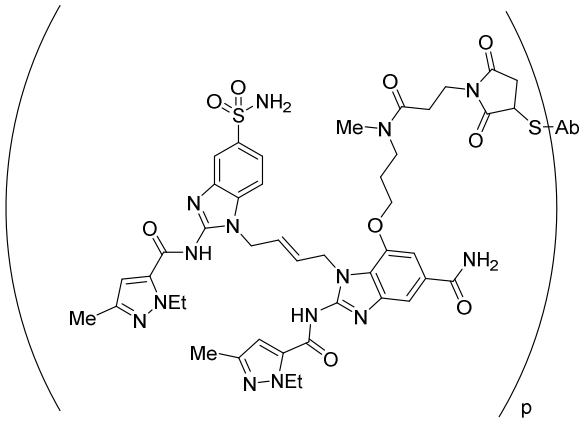
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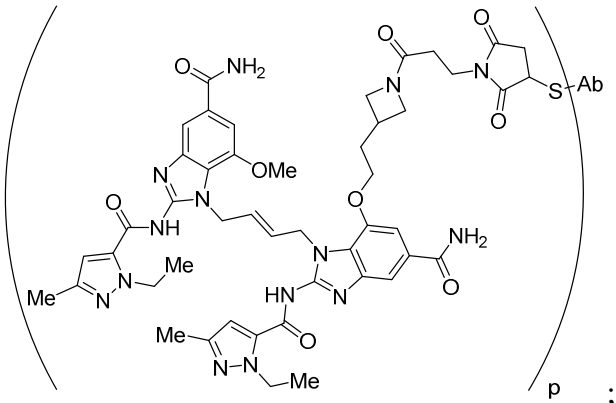
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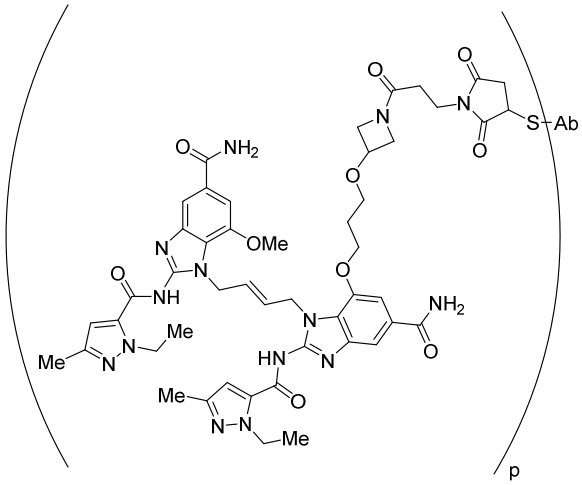
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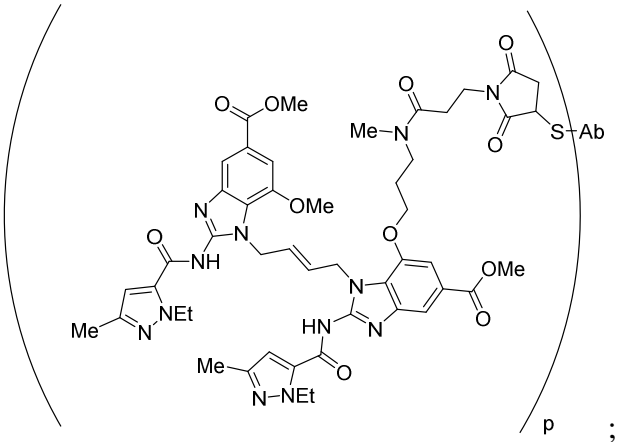
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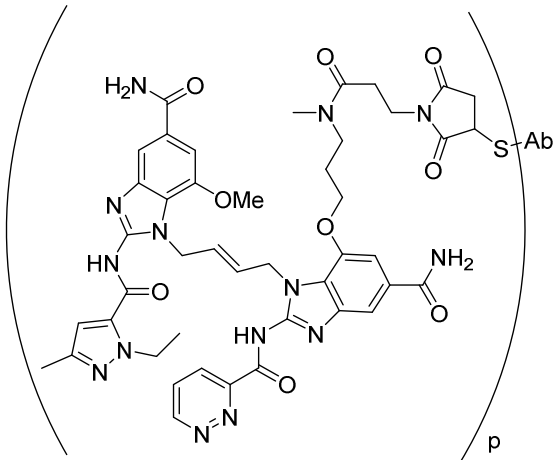
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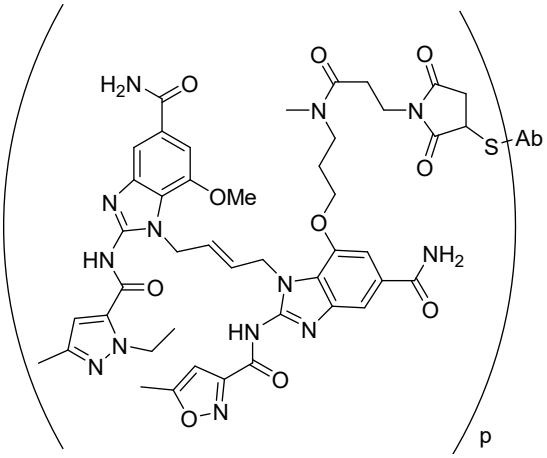
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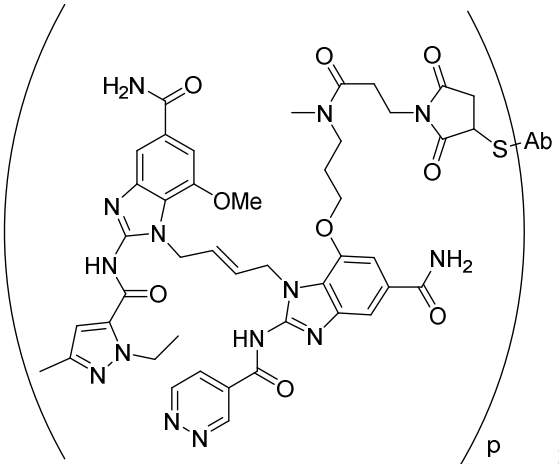
H2N
O
OMe
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NH2
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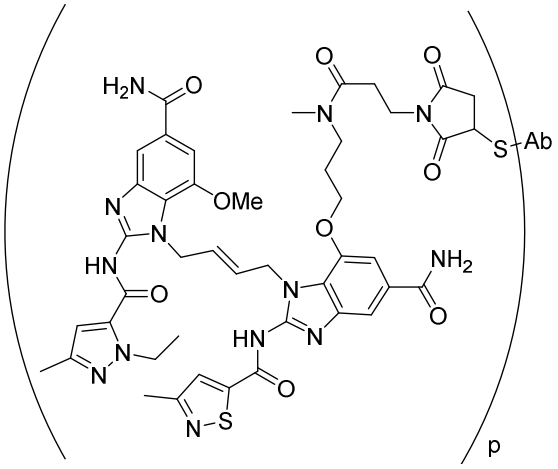
p
;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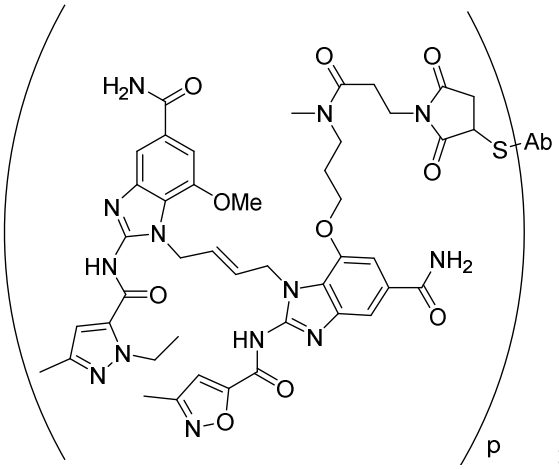
OMe
N
HN
NH2
Ab
S
p
;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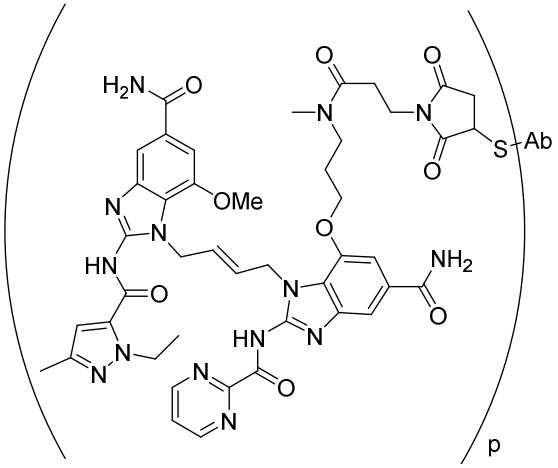
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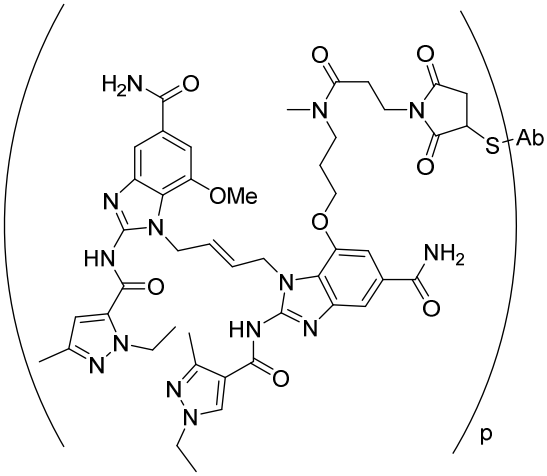
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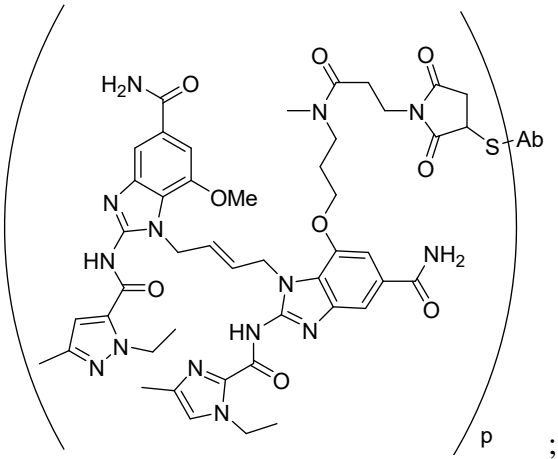
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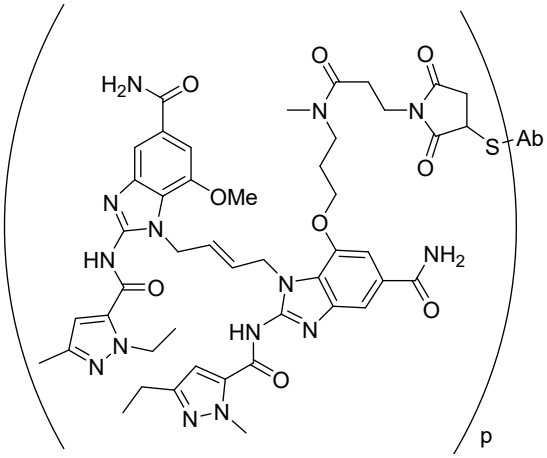
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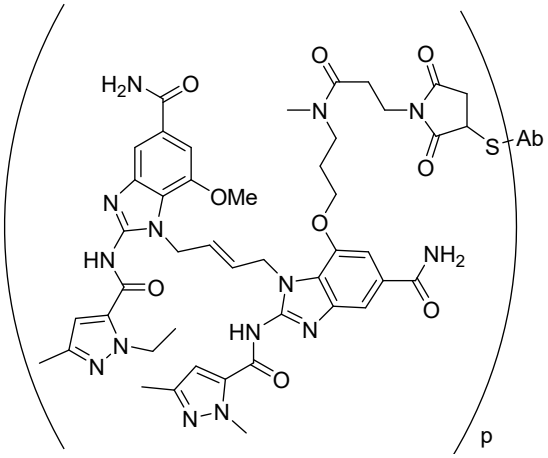
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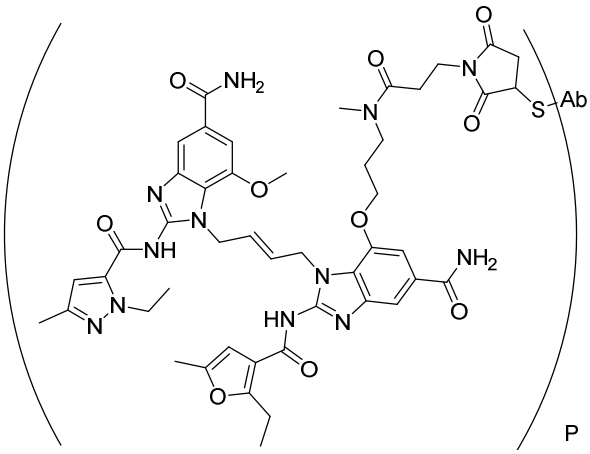
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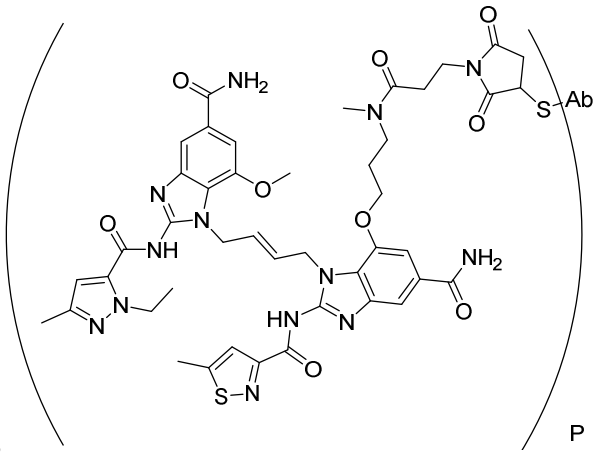
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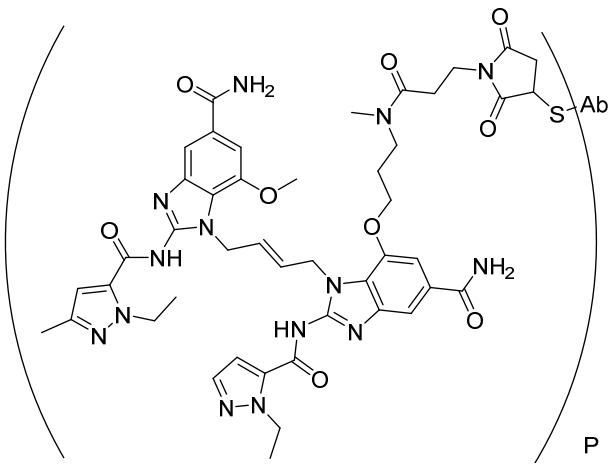
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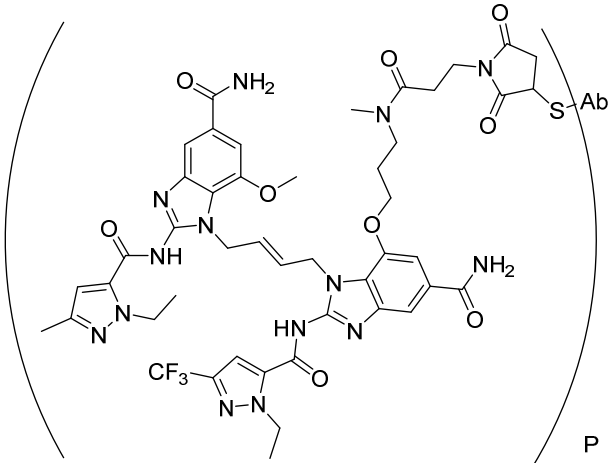
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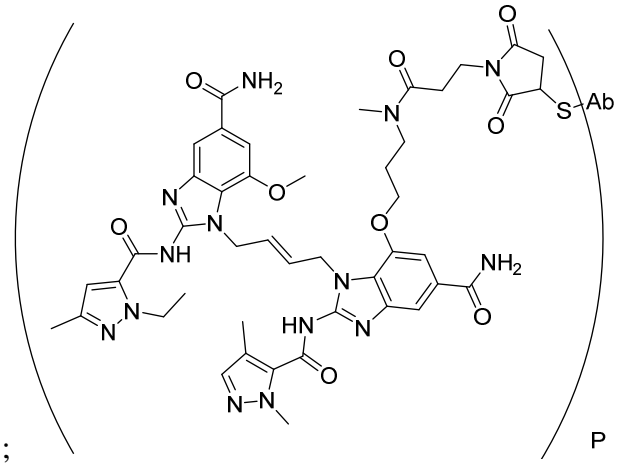
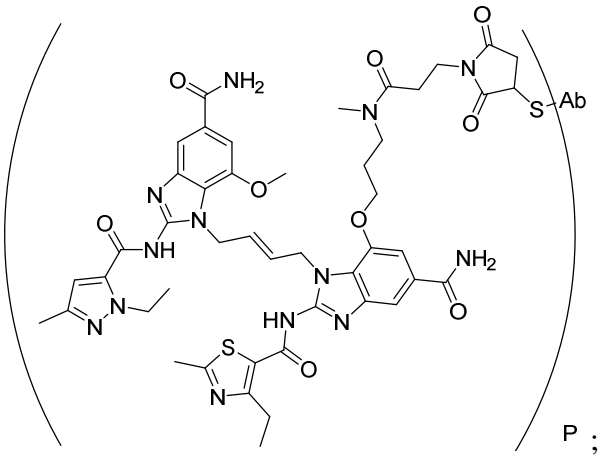

O
NH2
N
O
NH
N
N
O
N
O
S
Ab
O
NH2
HN
N
O
N
N

P ;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NH2
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S
Ab
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NH2
HN
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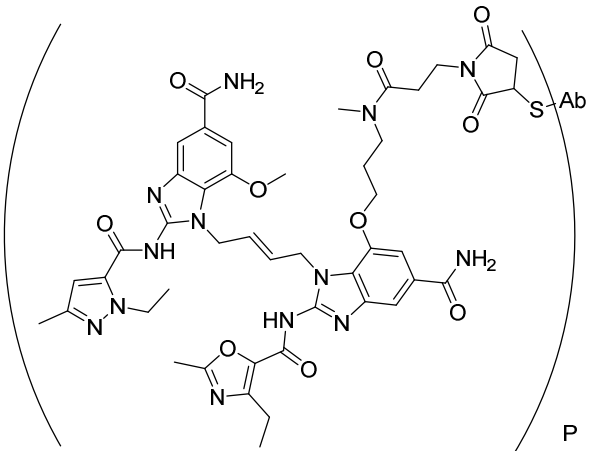
P ;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NH2
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NH
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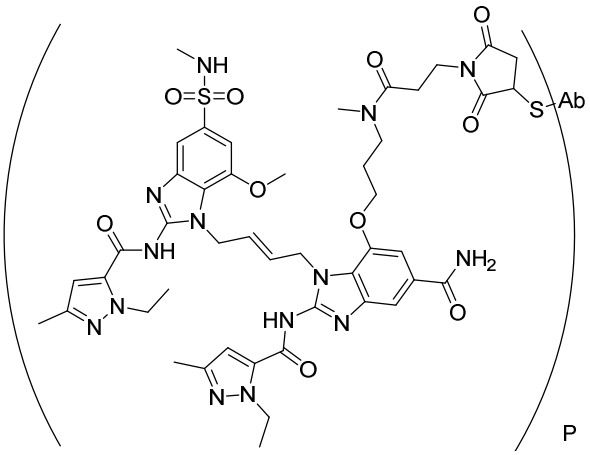
O
S
Ab
O
NH2
HN
N
O
O
N

P ;


NH
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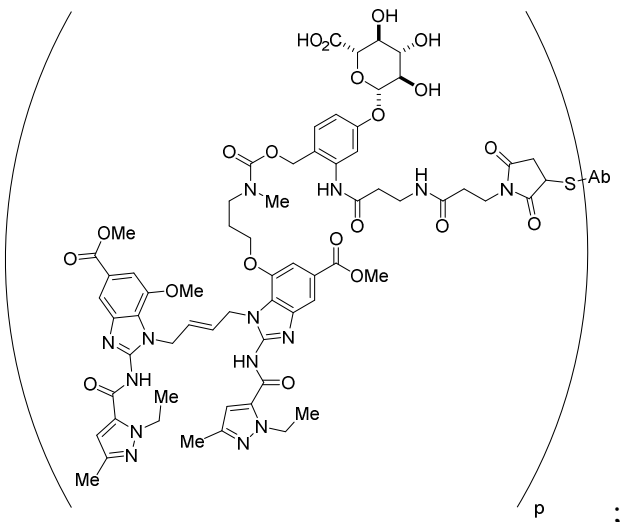
N
O
NH
N
N
O
N
O
S
Ab
O
NH2
HN
N
O
N–N

P ;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HO2C
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OH
O
O
O
N
Me
HN
H
N
O
S
Ab
OMe
O
OMe
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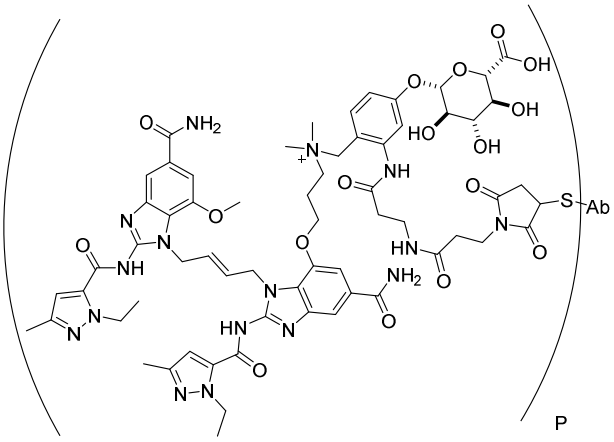
HN
Me
Me
Me
Me

p ;


O
OH
O
O
OH
HO
OH
NH
O
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Ab
O
NH2
N
O
NH
N
N
O
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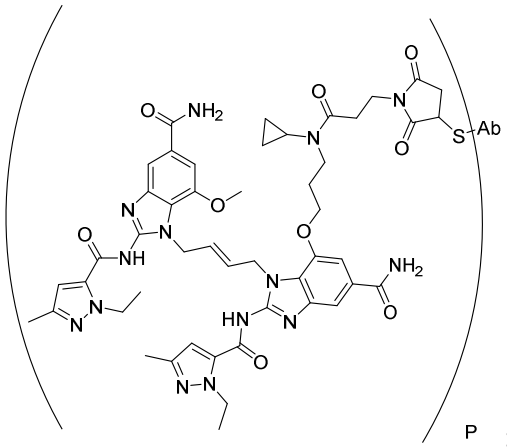
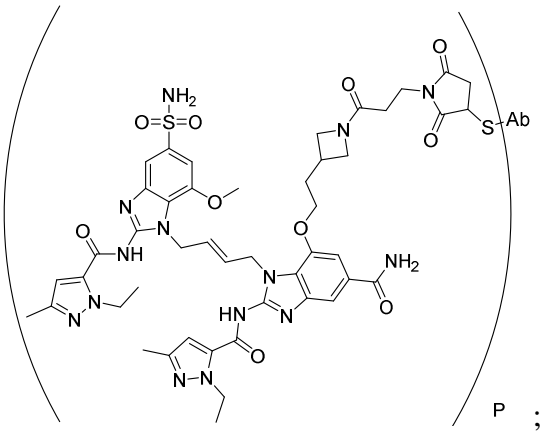
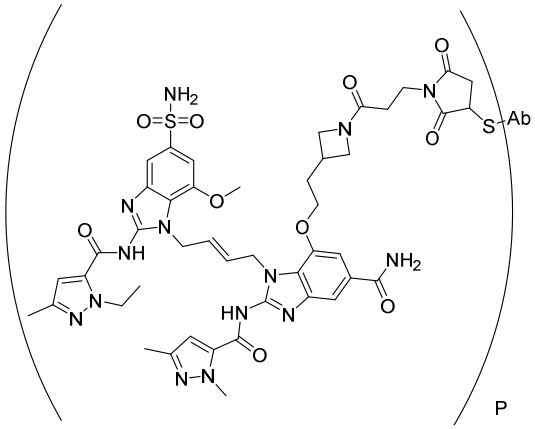
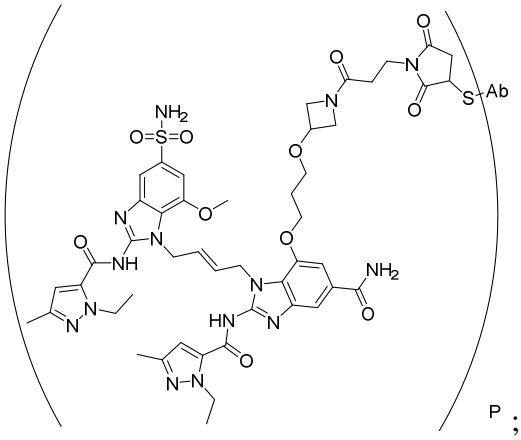
HN
N
O
N–N

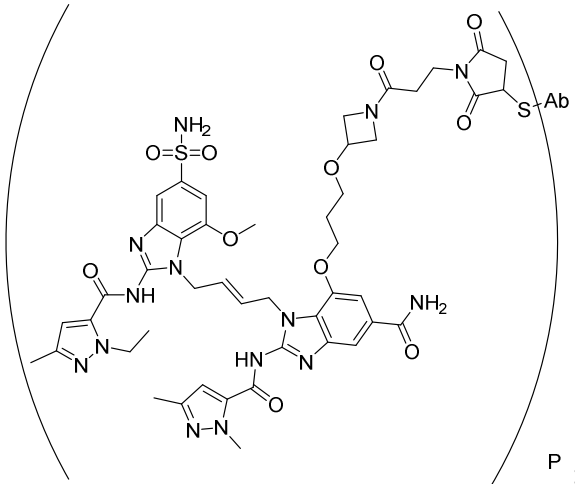
P ;

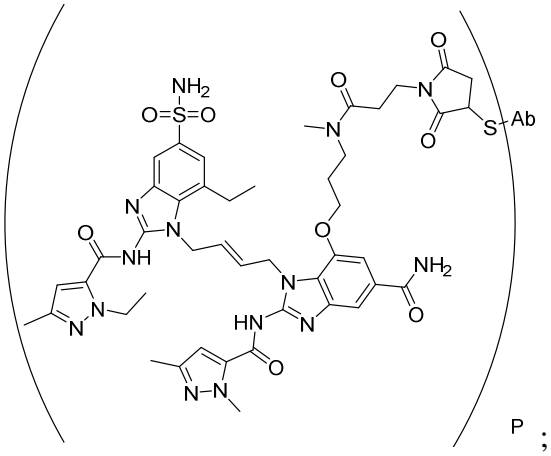












and pharmaceutically acceptable salts thereof,

wherein:

Ab is an antibody; and

each subscript p is independently an integer from 2 to 8.

Antibodies

[0124] In some embodiments, an antibody is a polyclonal antibody. In some embodiments, an antibody is a monoclonal antibody. In some embodiments, an antibody is chimeric. In some embodiments, an antibody is humanized. In some embodiments, an antibody is fully human. In some embodiments, an antibody is an antigen binding fragment.

[0125] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method.

[0126] Useful polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Useful monoclonal antibodies are homogeneous populations of antibodies to a particular antigenic determinant (*e.g.*, a cancer cell antigen, a protein, a peptide, a carbohydrate, a chemical, nucleic acid, or fragments thereof). A monoclonal antibody (mAb) to an antigen-of-interest can be prepared by using any technique known in the art which provides for the production of antibody molecules by continuous cell lines in culture.

[0127] Useful monoclonal antibodies include, but are not limited to, human monoclonal antibodies, humanized monoclonal antibodies, or chimeric human-mouse (or other species) monoclonal antibodies. The antibodies include full-length antibodies and antigen binding fragments thereof. Human monoclonal antibodies may be made by any of numerous techniques known in the art (*e.g*., Teng *et al.*, 1983, *Proc. Natl. Acad. Sci. USA*. 80:7308-7312; Kozbor *et al.*, 1983, *Immunology Today* 4:72-79; and Olsson *et al.*, 1982, *Meth. Enzymol*. 92:3-16).

[0128] In some embodiments, an antibody includes a functionally active fragment, derivative or analog of an antibody that binds specifically to target cells (e.g., cancer cell antigens) or other antibodies bound to cancer cells or matrix. In this regard, "functionally active" means that the fragment, derivative or analog is able to bind specifically to target cells. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences are typically used in binding assays with the antigen by any binding assay method known in the art

(e.g., the Biacore assay) (*See*, *e.g.*, Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md; Kabat E *et al.*, 1980, *J. Immunology* 125(3):961-969).

[0129] Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which are typically obtained using standard recombinant DNA techniques, are useful antibodies. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as for example, those having a variable region derived from a murine monoclonal and a constant region derived from a human immunoglobulin. *See*, *e.g.*, U.S. Patent No. 4,816,567; and U.S. Patent No. 4,816,397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species having one or more CDRs from the non-human species and a framework region from a human immunoglobulin molecule. *See*, *e.g.*, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Publication No. WO 87/02671; European Patent Publication No. 0 184 187; European Patent Publication No. 0 171 496; European Patent Publication No. 0 173 494; International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Publication No. 012 023; Berter *et al.*, 1988, *Science* 240:1041-1043; Liu *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol*. 139:3521-3526; Sun *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.*, 1987, *Cancer. Res.* 47:999-1005; Wood *et al.*, 1985, *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl. Cancer Inst*. 80:1553-1559; Morrison, 1985, *Science* 229:1202-1207; Oi *et al.*, 1986, *BioTechniques* 4:214; U.S. Patent No. 5,225,539; Jones *et al.*, 1986, *Nature* 321: 522-525; Verhoeyan *et al.*, 1988, *Science* 239:1534; and Beidler *et al.*, 1988, *J. Immunol.* 141:4053-4060; each of which is incorporated herein by reference in its entirety.

[0130] In some embodiments, an antibody is a completely human antibody. In some embodiments, an antibody is produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which are capable of expressing human heavy and light chain genes.

[0131] In some embodiments, an antibody is an intact or fully-reduced antibody. The term 'fully-reduced' is meant to refer to an antibody in which all four inter-chain disulfide linkages have been reduced to provide eight thiols that can be attached to a linker (L).

[0132] Attachment to an antibody can be via thioether linkages from native and/or engineered cysteine residues, or from an amino acid residue engineered to participate in a cycloaddition reaction (such as a click reaction) with the corresponding linker intermediate. *See, e.g.,* Maerle, et al., *PLOS One* 2019: 14(1); e0209860. In some embodiments, an antibody is an intact or fully-reduced antibody, or is an antibody bearing engineered an cysteine group that is modified with a functional group that can participate in, for example, click chemistry or other cycloaddition reactions for attachment of other components of the ADC as described herein (e.g., Diels-Alder reactions or other [3+2] or [4+2] cycloadditions).

[0133] Antibodies that bind specifically to a cancer cell antigen are available commercially or produced by any method known to one of skill in the art such as, *e.g.*, chemical synthesis or recombinant expression techniques. The nucleotide sequences encoding antibodies that bind specifically to a cancer cell antigen are obtainable, *e.g.*, from the GenBank database or similar database, literature publications, or by routine cloning and sequencing.

[0134] In some embodiments, the antibody can be used for the treatment of a cancer (e.g., an antibody approved by the FDA and/or EMA). Antibodies that bind specifically to a cancer cell antigen are available commercially or produced by any method known to one of skill in the art such as, *e.g.*, recombinant expression techniques. The nucleotide sequences encoding antibodies that bind specifically to a cancer cell antigen are obtainable, *e.g.*, from the GenBank database or similar database, literature publications, or by routine cloning and sequencing.

[0135] In some embodiments, an antibody can bind specifically to a receptor or a receptor complex expressed on lymphocytes. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member, a TNF receptor superfamily member, an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin, or a complement control protein.

[0136] In some embodiments, an antibody can bind specifically to a cancer cell antigen. It will be understood that the antibody component in an ADC is an antibody in residue form such that "Ab" in the ADC structures described herein incorporates the structure of the antibody.

[0137] Non-limiting examples of antibodies that can be used for treatment of cancer and antibodies that bind specifically to tumor associated antigens are disclosed in Franke, A. E., Sievers, E. L., and Scheinberg, D. A., "Cell surface receptor-targeted therapy of acute myeloid leukemia: a review" *Cancer Biother Radiopharm*. 2000,15, 459-76; Murray, J. L., "Monoclonal antibody treatment of solid tumors: a coming of age" *Semin Oncol*. 2000, 27, 64-70; Breitling, F., and Dubel, S., *Recombinant Antibodies*, John Wiley, and Sons, New York, 1998, each of which is hereby incorporated by reference in its entirety.

[0138] Embodiments of antibodies that bind to one or more of cancer cell antigens and immune cell antigens are provided below.

[0139] Non-limiting examples of target antigens and associated antibodies useful for the treatment of cancer and antibodies that bind specifically to cancer cell antigens (also called tumor antigens), include B7-DC (e.g., Catalog #PA5-20344); BCMA; B7-H3 (e.g., enoblituzumab, omburtamab, MGD009, MGC018, DS-7300); B7-H4 (e.g., Catalog #14-5949-82); B7-H6 (e.g., Catalog #12-6526-42); B7-H7; C5 complement (e.g., BCD-148; CAN106); CA-125; CA9 (e.g., girentuximab); CCR8 (e.g., JTX-1811); CLEC12A (e.g., tepoditamab); CSPG4 (e.g., U.S. Patent No. 10,822,427); CCNB1; DDR1; de2-7 EGFR (e.g., MAb 806); DPEP1; DR4 (e.g., mapatumumab); endosialin (e.g., ontuxizumab); ENPP1; EPCAM (e.g., adecatumumab); EPHA2; ERBB2 (e.g., trastuzumab); ERBB3; ERVMER34_1; FAP(e.g., sibrotuzumab); FasL; FGFR2 (e.g., aprutumab); FGFR4 (e.g., MM-161); FLT3 (e.g., 4G8SDIEM); FBP; FucGM1 (e.g., BMS-986012); FZD8; G250; GAGE; GD2 (e.g., dinutuximab); gpNMB (e.g., glembatumumab); GPR87; GUCY2C (e.g., indusatumab); HAVCR2; IDO1; ITGB6; ITGB8; L1CAM (e.g., JCAR023); MRC1 (e.g., ThermoFisher Catalog #12-2061-82); ML-IAP (e.g., 88C570, ThermoFisher Catalog #40958); NT5E (e.g., 7G2, ThermoFisher Catalog #41-0200); OY-TES1; p53; p53mutant; PAX5; PDPN (e.g., ThermoFisher Catalog #14-5381-82); VSIR (e.g., ThermoFisher Catalog #PA5-52493); Dectin2 (e.g., ThermoFisher Catalog #MA5-16250); PAX3 (e.g., GT1210, ThermoFisher Catalog #MA5-31583); Sialyl-Thomsen-nouveau-antigen (e.g., Eavarone et al. *PLoS One*, 2018; 13(7): e0201314); PDGFR-B (e.g., rinucumab); ADAM12 (e.g., Catalog #14139-1-AP); ADAM9 (e.g., IMGC936); AFP (e.g., ThermoFisher Catalog #PA5-25959); AGR2 (e.g., ThermoFisher Catalog #PA5-34517); AKAP-4 (e.g., Catalog #PA5-52230); androgen receptor (e.g., ThermoFisher Catalog #MA5-13426); ALPP (e.g., Catalog #MA5-15652); CD44 (e.g., RG7356); AMHR2 (e.g., ThermoFisher Catalog #PA5-13902); ANTXR1

(e.g., Catalog #MA1-91702); ARTN (e.g., ThermoFisher Catalog #PA5-47063); αVβ6; CA19-9 (e.g., AbGn-7; MVT-5873); carcinoembryonic antigen (e.g., arcitumomab; cergutuzumab; amunaleukin; labetuzumab); CD115 (e.g., axatilimab; cabiralizumab; emactuzumab); CD137 (e.g., ADG106; CTX-471); CD147 (e.g., gavilimomab; metuzumab); CD155 (e.g., U.S. Publication No. 2018/0251548); CD274 (e.g., adebrelimab; atezolizumab; garivulimab); CDCP1 (e.g., RG7287); CDH3 (e.g., PCA062); CDH6 (e.g., HKT288); CEACAM1; CEACAM6; CLDN18.1 (e.g., zolbetuximab); CLDN18.2 (e.g., zolbetuximab); CLPTM1L; CS-1 (e.g., tigatuzumab); GD3 (e.g., mitumomab); HLA-G (e.g., TTX-080); IL1RAP (e.g., nidanilimab); LAG-3 (e.g., encelimab); LY6G6D (e.g., PA5-23303); LYPD1 (e.g., ThermoFisher Catalog #PA5-26749); MAD-CT-2; MAGEA3 (e.g., ThermoFisher Catalog #60054-1-IG); MAGEA4 (e.g., Catalog #MA5-26117); MAGEC2 (e.g., ThermoFisher Catalog #PA5-64010); MLANA (e.g., Catalog #MA5-15237); MELTF (e.g., ThermoFisher Catalog #H00004241-M04A); MSLN (e.g., 5B2, Catalog #MA5-11918); MUC1 (e.g., MH1 (CT2), ThermoFisher Catalog #MA5-11202); MUC5AC (e.g., 45M1, Catalog #MA5-12178); MYCN (e.g., NCM-II 100, ThermoFisher Catalog #MA1-170); NCAM1 (e.g., ThermoFisher Catalog #MA5-11563); Nectin-4 (e.g., enfortumab); NY-BR-1 (e.g., NY-BR-1 No. 2, Catalog #MA5-12645); PSMA (e.g., BAY 2315497); PSA (e.g., ThermoFisher Catalog #PA1-38514; Daniels-Wells et al. *BMC Cancer*, 2013; 13:195); PSCA (e.g., AGS-1C4D4); PTK7 (e.g., cofetuzumab); PVRIG; Ras mutant (e.g., Shin et al. *Sci Adv.* 2020; 6(3):eaay2174); RET (e.g., WO2020210551); RGS5 (e.g., TF-TA503075); RhoC (e.g., ThermoFisher Catalog PA5-77866); ROR2 (e.g., BA3021); ROS1 (e.g., WO2019107671); SART3 (e.g., TF 18025-1-AP); SLC12A2 (e.g., ThermoFisher Catalog #13884-1-AP); SLC38A1 (e.g., ThermoFisher Catalog #12039-1-AP); SLC39A6 (e.g., ladiratuzumab); SLC44A4 (e.g., ASG-5ME); SLC7A11 (e.g., ThermoFisher Catalog #PA1-16893); SLITRK6 (e.g., sirtratumab); SSX2 (e.g., ThermoFisher Catalog #MA5-24971); survivin (e.g., PA1-16836); TACSTD2 (e.g., PA5-47074); TAG-72 (e.g., MA1-25956); TIGIT (e.g., etigilimab); TM4SF5 (e.g., 18239-1-AP); TMPRSS11D (e.g., PA5-30927); TNFRSF12 (e.g., BAY-356); TRAIL (e.g., Catalog #12-9927-42); Trem2 (e.g., PY314); TRP-2 (e.g., PA5-52736); uPAR (e.g., ATN-658); UPK1B (e.g., ThermoFisher Catalog #PA5-56863); UPK2 (e.g., ThermoFisher Catalog #PA5-60318); UPK3B (e.g., ThermoFisher Catalog #PA5-52696); VEGF (e.g., GNR-011); VEGFR2 (e.g., gentuximab); CD44 (e.g., RG7356); WT1 (e.g., ThermoFisher Catalog #MA5-32215); XAGE1 (e.g., ThermoFisher Catalog #PA5-46413); CTLA4 (e.g.,

ipilimumab); Sperm protein 17 (e.g., BS-5754R); TLR2/4/1 (e.g., tomaralimab); B7-1 (e.g., galiximab); ANXA1 (e.g., Catalog #71-3400); BCR-ABL; CAMPATH-1 (e.g., alemtuzumab; ALLO-647; ANT1034); CD123 (e.g., BAY-943; CSL360); CD19 (e.g., ALLO-501); CD20 (e.g., divozilimab; ibritumomab); CD30 (e.g., iratumumab); CD33 (e.g., lintuzumab; BI 836858; AMG 673); CD352 (e.g., SGN-CD352A); CD37 (e.g., lilotomab; GEN3009); CD40 (e.g., dacetuzumab; lucatumumab); CD45 (e.g., apamistamab); CD48 (e.g., SGN-CD48A); CXCR4 (e.g., ulocuplumab); ETV6-AML (e.g., Catalog #PA5-81865); ROR1 (e.g., cirmtuzumab); CD74 (e.g., milatuzumab); SIT1 (e.g., PA5-53825); SLAMF7 (e.g., Elotuzumab); Axl (e.g., BA3011; tilvestamab); Siglecs 1-16 (see, e.g., Angata et al. Trends Pharmacol Sci. 2015; 36(10): 645–660); SIRPa (e.g., Catalog #17-1729-42); SIRPg (e.g., PA5-104381); OX40 (e.g., ABM193); PROM1 (e.g., Catalog #14-1331-82); TMEM132A (e.g., Catalog #PA5-62524); TMEM40 (e.g., PA5-60636); PD-1 (e.g., balstilimab; budigalimab; geptanolimab); ALK (e.g., DLX521); CCR4 (e.g., AT008; mogamulizumab-kpkc); CD27 (e.g., varlilumab); CD278 (e.g., feladilimab; vopratelimab); CD32 (e.g., mAb 2B6); CD47 (e.g., letaplimab; magrolimab); and CD70 (e.g., cusatuzumab).

[0140] In some embodiments, an antibody can bind specifically to a cancer cell antigen associated with a solid tumor and/or a hematological cancer. Non-limiting examples of target antigens and associated antibodies that bind specifically to cancer cell antigens associated with a solid tumor and/or a hematological cancer target antigen include Axl (e.g., BA3011; tilvestamab); B7-H3 (e.g., enoblituzumab, omburtamab, MGD009, MGC018, DS-7300); B7-H4 (e.g., Catalog #14-5949-82); B7-H6 (e.g., Catalog #12-6526-42); B7-H7; Siglecs 1-16 (see, e.g., Angata et al. *Trends Pharmacol Sci.* 2015; 36(10): 645–660); SIRPa (e.g., Catalog #17-1729-42); SIRPg (e.g., PA5-104381); OX40 (e.g., ABM193); PROM1 (e.g., Catalog #14-1331-82); TMEM132A (e.g., Catalog #PA5-62524); TMEM40 (e.g., PA5-60636); PD-1 (e.g., balstilimab; budigalimab; geptanolimab); ALK (e.g., DLX521); CCR4 (e.g., AT008; mogamulizumab-kpkc); CD27 (e.g., varlilumab); CD278 (e.g., feladilimab; vopratelimab); CD32 (e.g., mAb 2B6); CD47 (e.g., letaplimab; magrolimab); and CD70 (e.g., cusatuzumab).

[0141] In some embodiments, an antibody can bind specifically to a cancer cell antigen associated with a solid tumor. Non-limiting examples of target antigens and associated antibodies that bind specifically to solid-tumor-associated target antigens include PAX3 (e.g., GT1210, ThermoFisher Catalog #MA5-31583); Sialyl-Thomsen-nouveau-antigen (e.g., Eavarone et al.

PLoS One. 2018; 13(7): e0201314); PDGFR-B (e.g., rinucumab); ADAM12 (e.g., Catalog #14139-1-AP); ADAM9 (e.g., IMGC936); AFP (e.g., ThermoFisher Catalog #PA5-25959); AGR2 (e.g., ThermoFisher Catalog #PA5-34517); AKAP-4 (e.g., Catalog #PA5-52230); androgen receptor (e.g., ThermoFisher Catalog #MA5-13426); ALPP (e.g., Catalog #MA5-15652); CD44 (e.g., RG7356); AMHR2 (e.g., ThermoFisher Catalog #PA5-13902); ANTXR1 (e.g., Catalog #MA1-91702); ARTN (e.g., ThermoFisher Catalog #PA5-47063); αVβ6; CA19-9 (e.g., AbGn-7; MVT-5873); carcinoembryonic antigen (e.g., arcitumomab; cergutuzumab; amunaleukin; labetuzumab); CD115 (e.g., axatilimab; cabiralizumab; emactuzumab); CD137 (e.g., ADG106; CTX-471); CD147 (e.g., gavilimomab; Metuzumab); CD155 (e.g., U.S. Publication No. 2018/0251548); CD274 (e.g., adebrelimab; atezolizumab; garivulimab); CDCP1 (e.g., RG7287); CDH3 (e.g., PCA062); CDH6 (e.g., HKT288); CEACAM1; CEACAM6); CLDN18.1 (e.g., zolbetuximab); CLDN18.2 (e.g., zolbetuximab); CLPTM1L; CS-1 (e.g., tigatuzumab); GD3 (e.g., mitumomab); HLA-G (e.g., TTX-080); IL1RAP (e.g., nidanilimab); LAG-3 (e.g., encelimab); LY6G6D (e.g., PA5-23303); LYPD1 (e.g., ThermoFisher Catalog #PA5-26749); MAD-CT-2; MAGEA3 (e.g., ThermoFisher Catalog #60054-1-IG); MAGEA4 (e.g., Catalog #MA5-26117); MAGEC2 (e.g., ThermoFisher Catalog #PA5-64010); MLANA (e.g., Catalog #MA5-15237); MELTF (e.g., ThermoFisher Catalog #H00004241-M04A); MSLN (e.g., 5B2, Catalog #MA5-11918); MUC1 (e.g., MH1 (CT2), ThermoFisher Catalog #MA5-11202); MUC5AC (e.g., 45M1, Catalog #MA5-12178); MYCN (e.g., NCM-II 100, ThermoFisher Catalog #MA1-170); NCAM1 (e.g., ThermoFisher Catalog #MA5-11563); Nectin-4 (e.g., enfortumab); NY-BR-1 (e.g., NY-BR-1 No. 2, Catalog #MA5-12645); PSMA (e.g., BAY 2315497); PSA (e.g., ThermoFisher Catalog #PA1-38514; Daniels-Wells et al. *BMC Cancer* 2013; 13:195); PSCA (e.g., AGS-1C4D4); PTK7 (e.g., cofetuzumab); PVRIG; Ras mutant (e.g., Shin et al. *Sci Adv.* 2020; 6(3):eaay2174); RET (e.g., WO2020210551); RGS5 (e.g., TF-TA503075); RhoC (e.g., ThermoFisher Catalog PA5-77866); ROR2 (e.g., BA3021); ROS1 (e.g., WO2019107671); SART3 (e.g., TF 18025-1-AP); SLC12A2 (e.g., ThermoFisher Catalog #13884-1-AP); SLC38A1 (e.g., ThermoFisher Catalog #12039-1-AP); SLC39A6 (e.g., ladiratuzumab); SLC44A4 (e.g., ASG-5ME); SLC7A11 (e.g., ThermoFisher Catalog #PA1-16893); SLITRK6 (e.g., sirtratumab); SSX2 (e.g., ThermoFisher Catalog #MA5-24971); survivin (e.g., PA1-16836); TACSTD2 (e.g., PA5-47074); TAG-72 (e.g., MA1-25956); TIGIT (e.g., etigilimab); TM4SF5 (e.g., 18239-1-AP); TMPRSS11D (e.g., PA5-30927); TNFRSF12 (e.g., BAY-356); TRAIL (e.g., Catalog #12-9927-

42); Trem2 (e.g., PY314); TRP-2 (e.g., PA5-52736); uPAR (e.g., ATN-658); UPK1B (e.g., ThermoFisher Catalog #PA5-56863); UPK2 (e.g., ThermoFisher Catalog #PA5-60318); UPK3B (e.g., ThermoFisher Catalog #PA5-52696); VEGF (e.g., GNR-011); VEGFR2 (e.g., gentuximab); CD44 (e.g., RG7356); WT1 (e.g., ThermoFisher Catalog #MA5-32215); XAGE1 (e.g., ThermoFisher Catalog #PA5-46413); and CTLA4 (e.g., ipilimumab).

[0142] In some embodiments, an antibody can bind specifically to a cancer cell antigen associated with a hematological cancer. Non-limiting examples of target antigens and associated antibodies that bind specifically to hematological cancer cell target antigens include Sperm protein 17 (e.g., BS-5754R); TLR2/4/1 (e.g., Tomaralimab); B7-1 (e.g., galiximab); ANXA1 (e.g., Catalog #71-3400); BCR-ABL; CAMPATH-1 (e.g., alemtuzumab; ALLO-647; ANT1034); CD123 (e.g., BAY-943; CSL360); CD19 (e.g., ALLO-501); CD20 (e.g., divozilimab; ibritumomab); CD30 (e.g., iratumumab); CD33 (e.g., lintuzumab; BI 836858; AMG 673); CD352 (e.g., SGN-CD352A); CD37 (e.g., lilotomab; GEN3009); CD40 (e.g., dacetuzumab; lucatumumab); CD45 (e.g., apamistamab); CD48 (e.g., SGN-CD48A); CXCR4 (e.g., ulocuplumab); ETV6-AML (e.g., Catalog #PA5-81865); ROR1 (e.g., cirmtuzumab); CD74 (e.g., milatuzumab); SIT1 (e.g., PA5-53825); and SLAMF7 (e.g., elotuzumab).

[0143] In some embodiments, an antibody can be used that binds specifically to a target antigen (e.g., an antigen associated with a disease or disorder). Antibodies that bind specifically to a target antigen (e.g., an antigen associated with a disease or disorder) are available commercially or can be produced by any method known to one of skill in the art such as, e.g., recombinant expression techniques. The nucleotide sequences encoding antibodies that bind specifically to a target antigen (e.g., an antigen associated with a disease or disorder) are obtainable, e.g., from the GenBank database or similar database, literature publications, or by routine cloning and sequencing.

[0144] Non-limiting examples of target antigens and associated antibodies that bind specifically to target antigens (e.g., an antigen associated with a disease or disorder, or an antigen associated with an immune cell) include CD163 (e.g., TBI 304H); TIGIT (e.g., etigilimab); DCSIGN (see, e.g., International Publication No. WO2018134389); IFNAR1 (e.g., faralimomab); ASCT2 (e.g., idactamab); ULBP1/2/3/4/5/6 (e.g., PA5-82302); CLDN1 (e.g., INSERM anti-Claudin-1); CLDN2 (see, e.g., International Publication No. WO2018123949); IL-21R (e.g., PF-05230900); DCIR; DCLK1 (see, e.g., International Publication No. WO2018222675); Dectin1

(see, e.g., U.S. Patent No. 9,045,542); GITR (e.g., ragifilimab); ITGAV (e.g., abituzumab); LY9 (e.g., PA5-95601); MICA (e.g., 1E2C8, Catalog #66384-1-IG); MICB (e.g., Catalog #MA5-29422); NOX1 (e.g., Catalog #PA5-103220); CD2 (e.g., BTI-322; siplizumab); CD247 (e.g., AFM15); CD25 (e.g., basiliximab); CD28 (e.g., REGN5668); CD3 (e.g., otelixizumab; visilizumab); CD38 (e.g., felzartamab; AMG 424); CD3E (e.g., foralumab; teplizumab); CD5 (e.g., MAT 304; zolimomab aritox); ALPPL2 (e.g., Catalog #PA5-22336); B7-2 (e.g., Catalog #12-0862-82); B7-H3 (e.g., enoblituzumab, omburtamab, MGD009, MGC018, DS-7300); B7-H4 (e.g., Catalog #14-5949-82); B7-H6 (e.g., Catalog #12-6526-42); B7-H7; BAFF-R (e.g., Catalog #14-9117-82); BMPR2; BORIS; CD112 (see, e.g., U.S. Publication No. 20100008928); CD24 (see, e.g., U.S. Patent No. 8,614,301); CD244 (e.g., R&D AF1039); CD30L (see, e.g., U.S. Patent No. 9926373); CD3D; CD3G; CD79A (see, e.g., International Publication No. WO 2020252110); CD83 (e.g., CBT004); CD97; CDH17 (see, e.g., International Publication No. WO 2018115231); CLDN16; CLDN19; CYP1B1; DPEP3; DPP4; DSG2 (see, e.g., U.S. Patent No. 10,836,823); EPHA receptors; epidermal growth factor; FAS; FGFR1 (e.g., RG7992); FGFR3 (e.g., vofatamab); FN1; FOLR1 (e.g., farletuzumab); FSHR; FZD5; GM2 (e.g., BIW-8962); GM3 (e.g., racotumomab); GPA33 (e.g., KRN330); GPC3 (e.g., codrituzumab); HAS3; HLA-E; HLA-F; HLA-DR; ICAM1; IFNAR2; IL13Ra2; IL-5R (e.g., benralizumab); KISS1R; LAMP1; LAYN; LCK; legumain; LILRB2; LILRB4; LMP2; MAD-CT-1; MAGEA1 (e.g., Catalog #MA5-11338); MerTk (e.g., DS5MMER, Catalog #12-5751-82); MFSD13A; hTERT; gp100; Fas-related antigen 1; a metalloproteinase; Mincle (e.g., OTI2A8, Catalog #TA505101); NA17; NY-ESO-1 (e.g., E978m, Catalog #35-6200); polysialic acid (see, e.g., Watzlawik et al. *J Nat Sci.* 2015; 1(8):e141); PR1; Sarcoma translocation breakpoints; SLC10A2 (e.g., ThermoFisher Catalog #PA5-18990); SLC17A2 (e.g., ThermoFisher Catalog #PA5-106752); SLC39A5 (e.g., ThermoFisher Catalog #MA5-27260); SLC6A15 (e.g., ThermoFisher Catalog #PA5-52586); SLC6A6 (e.g., ThermoFisher Catalog #PA5-53431); SLC7A5; and CALCR (see, e.g., International Publication No. WO 2015077826).

[0145] In some embodiments, an antibody can bind specifically to an antigen associated with anemia. A non-limiting example of an antibody that binds specifically to an antigen associated with anemia includes CD163 (e.g., TBI 304H).

[0146] In some embodiments, an antibody can bind specifically to an antigen associated with a viral infection. Non-limiting examples of target antigens and associated

antibodies that binds specifically to an antigen associated with a viral infection include DCSIGN (see, e.g., International Publication No. WO2018134389) IFNAR1 (e.g., faralimomab); ASCT2 (e.g., idactamab); ULBP1/2/3/4/5/6 (e.g., PA5-82302); and CLDN1 (e.g., INSERM anti-Claudin-1).

[0147] In some embodiments, an antibody can bind specifically to an antigen associated with an autoimmune disease. Non-limiting examples of target antigens and associated antibodies that bind specifically to an antigen associated with an autoimmune disease include CLDN2 (see, e.g., International Publication No. WO 2018123949); IL-21R (e.g., PF-05230900); DCIR; DCLK1 (see, e.g., WO2018222675); Dectin1 (see, e.g., U.S. Patent No. 9,045,542); GITR (e.g., ragifilimab); ITGAV (e.g., abituzumab); LY9 (e.g., PA5-95601); MICA (e.g., 1E2C8, Catalog #66384-1-IG); MICB (e.g., Catalog #MA5-29422); NOX1 (e.g., Catalog #PA5-103220); CD2 (e.g., BTI-322; siplizumab); CD247 (e.g., AFM15); CD25 (e.g., basiliximab); CD28 (e.g., REGN5668); CD3 (e.g., otelixizumab; visilizumab); CD38 (e.g., felzartamab; AMG 424); CD3E (e.g., foralumab; teplizumab); and CD5 (e.g., MAT 304; zolimomab aritox).

[0148] In some embodiments, the antibody is a non-targeted antibody, for example, a non-binding or control antibody.

[0149] In some embodiments, the antigen is CD30. In some embodiments, the antibody is an antibody or antigen-binding fragment that binds to CD30, such as described in International Patent Publication No. WO 02/43661. In some embodiments, the anti-CD30 antibody is cAC10, which is described in International Patent Publication No. WO 02/43661. cAC10 is also known as brentuximab. In some embodiments, the anti-CD30 antibody comprises the CDRs of cAC10. In some embodiments, the CDRs are as defined by the Kabat numbering scheme. In some embodiments, the CDRs are as defined by the Chothia numbering scheme. In some embodiments, the CDRs are as defined by the IMGT numbering scheme. In some embodiments, the CDRs are as defined by the AbM numbering scheme. In some embodiments, the anti-CD30 antibody comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively. In some embodiments, the anti-CD30 antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 7 and a light chain variable region comprising an amino acid sequence that is at least 95% at least 96%, at least 97%, at least 98%, at least 99%, or 100%

identical to the amino acid sequence of SEQ ID NO: 8. In some embodiments, the anti-CD30 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10 and a light chain comprising the amino acid sequence of SEQ ID NO: 11.

[0150] In some embodiments, an antibody provided herein binds to EphA2. In some embodiments, the antibody comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 12, 13, 14, 15, 16, and 17, respectively. In some embodiments, the anti-EphA2 antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18 and a light chain variable region comprising an amino acid sequence that is at least 95% at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 19. In some embodiments, the anti-EphA2 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 21 and a light chain comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the anti-EphA2 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24 and a light chain comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the anti-EphA2 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26 or SEQ ID NO: 27 and a light chain comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the antibody is h1C1 or 1C1.

Table of Sequences

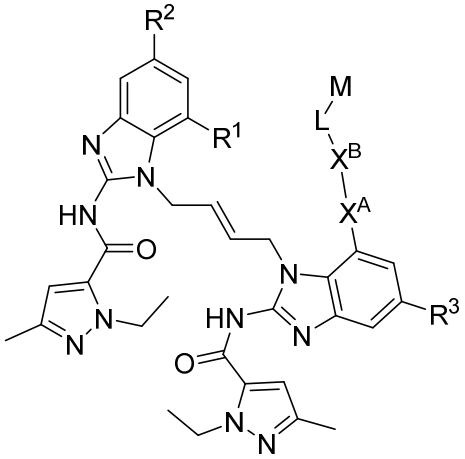
SEQ ID NO	Description	Sequence
1	cAC10 CDR-H1	DYYIT
2	cAC10 CDR-H2	WIYPGSGNTKYNEKFKG
3	cAC10 CDR-H3	YGNYWFAY
4	cAC10 CDR-L1	KASQSVDFDGDSYMN
5	cAC10 CDR-L2	AASNLES
6	cAC10 CDR-L3	QQSNEDPWT
7	cAC10 VH	QIQLQQSGPEVVKPGASVKISCKASGYTFTDYYITWVKQKPGQGLEWIGWIYPGSGNTKYNE KFKGKATLTVDTSSSTAFMQLSSLTSEDTAVYFCANYGNYWFAYWGQGTQVTVSA
8	cAC10 VL	DIVLTQSPASLAVSLGQRATISCKASQSVDFDGDSYMNWYQQKPGQPPKVLIYAASNLESGIP ARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPWTFGGGTKLEIK
9	cAC10 HC	QIQLQQSGPEVVKPGASVKISCKASGYTFTDYYITWVKQKPGQGLEWIGWIYPGSGNTKYNE KFKGKATLTVDTSSSTAFMQLSSLTSEDTAVYFCANYGNYWFAYWGQGTQVTVSAAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE

		LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
10	cAC10 HC v2	QIQLQQSGPEVVKPGASVKISCKASGYTFTDYYITWVKQKPGQGLEWIGWIYPGSGNTKYNE KFKGKATLTVDTSSSTAFMQLSSLTSEDTAVYFCANYGNYWFAYWGQGTQVTVSAAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
11	cAC10 LC	DIVLTQSPASLAVSLGQRATISCKASQSVDFDGDSYMNWYQQKPGQPPKVLIYAASNLES GIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPWTFGGGTKLEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
12	h1C1 CDR-H1	HYMMA
13	h1C1 CDR-H2	RIGPSGGPTHYADSVKG
14	h1C1 CDR-H3	YDSGYDYVAVAGPAEYFQH
15	h1C1 CDR-L1	RASQSISTWLA
16	h1C1 CDR-L2	KASNLHT
17	h1C1 CDR-L3	QQYNSYSRT
18	h1C1 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSS
19	h1C1 VL	DIQMTQSPSSLSASVGDRVTITCRASQSISTWLAWYQQKPGKAPKLLIYKASNLHTGVPSRFSG SGSGTEFSLTISGLQPDDFATYYCQQYNSYSRTFGQGTKVEIK
20	h1C1 HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
21	h1C1 HC v2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
22	h1C1 LC	DIQMTQSPSSLSASVGDRVTITCRASQSISTWLAWYQQKPGKAPKLLIYKASNLHTGVPSRFSG SGSGTEFSLTISGLQPDDFATYYCQQYNSYSRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
23	h1C1 mIgG2a HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGP SVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRV VSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCS VVHEGLHNHHTTKSFSRTPGK
24	h1C1 mIgG2a HC v2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGP SVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRV VSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCS VVHEGLHNHHTTKSFSRTPG

25	h1C1 mIgG2a LC	DIQMTQSPSSLSASVGDRVTITCRASQSISTWLAWYQQKPGKAPKLLIYKASNLHTGVPSRFSG SGSGTEFSLTISGLQPDDFATYYCQQYNSYSRTFGQGTKVEIKRADAAPTVSIFPPSSEQLTSGG ASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNS YTCEATHKTSTSPIVKSFNRNEC
26	h1C1 mIgG2a LALAPG HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRV VSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVT LTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSC SVVHEGLHNHHTTKSFSRTPGK
27	h1C1 mIgG2a LALAPG HC v2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYMMAWVRQAPGKGLEWVSRIGPSGGPTHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGYDSGYDYVAVAGPAEYFQHWGQG TLVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRV VSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVT LTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSC SVVHEGLHNHHTTKSFSRTPG
28	h1C1 mIgG2a LALAPG LC	DIQMTQSPSSLSASVGDRVTITCRASQSISTWLAWYQQKPGKAPKLLIYKASNLHTGVPSRFSG SGSGTEFSLTISGLQPDDFATYYCQQYNSYSRTFGQGTKVEIKRADAAPTVSIFPPSSEQLTSGG ASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNS YTCEATHKTSTSPIVKSFNRNEC

Compounds of Formula (II)

[0151] Some embodiments provide compounds of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof, wherein:

M is a succinimide or a hydrolyzed succinimide;

R^1 is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl)C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

each R^2 and R^3 are independently –CO_2H, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or C_{1-3} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript m is independently 0 or 1;

each subscript q is independently an integer from 0 to 6;

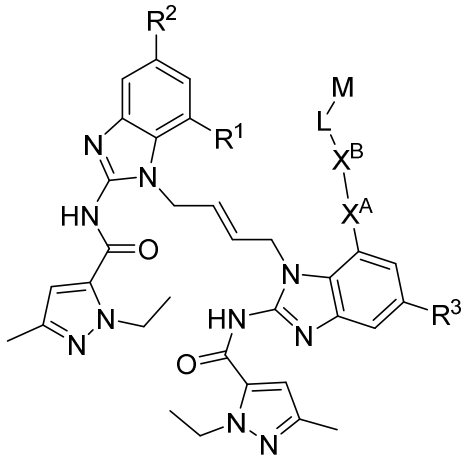
X^A is –CH_2–, –O–, –S–, –NH–, or –$N(CH_3)$–;

X^B is absent or a 2-16 membered heteroalkylene;

X^B, M, and L are each independently optionally substituted with a PEG Unit from PEG1 to PEG 72; and

L is an optional linker as described herein.

[0152] In some embodiments, the compound of Formula (II) has the structure:



(II)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl)C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

each R^2 and R^3 are independently –CO_2H, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or C_{1-3} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript m is independently 0 or 1;

each subscript q is independently an integer from 0 to 6;

X^A is $–CH_2–$, –O–, –S–, –NH–, or $–N(CH_3)–$;

X^B is absent or 2-16 membered heteroalkylene;

L is a linker having the formula $–(A)_a-(W)_w-(Y)_y–$, wherein:

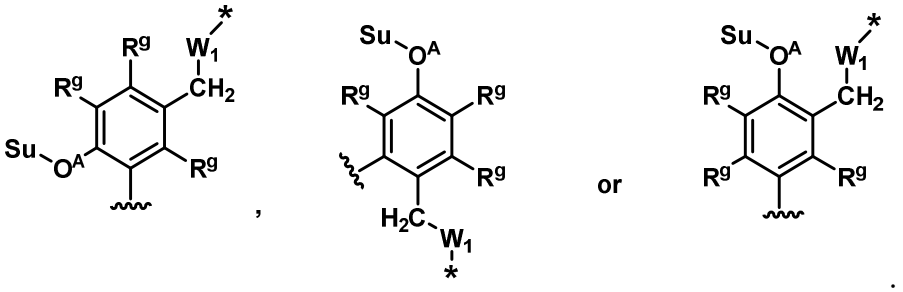
A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};

each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

W is from 1-12 amino acids or has the structure:



;

wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

W_1 is absent or –O-C(=O)–;

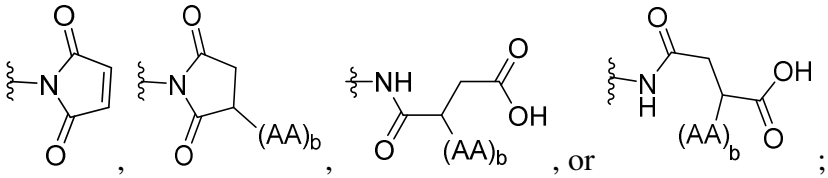
~~~ represents covalent attachment to A or M; and * represents covalent attachment to Y, X^A, or X^B.

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

subscript a is 0 or 1;

subscript y is 0 or 1;

subscript w is 0 or 1;
~~~

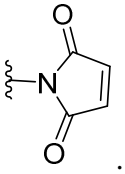
M is ;

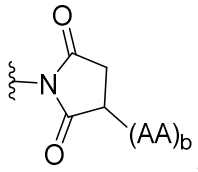
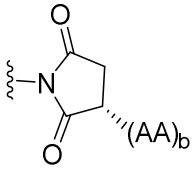
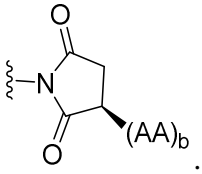
each AA is an independently selected amino acid, wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom;

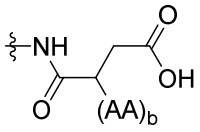
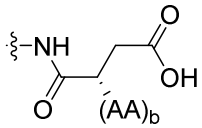
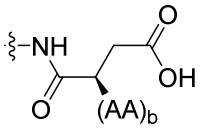
each subscript b is independently an integer from 1 to 6; and

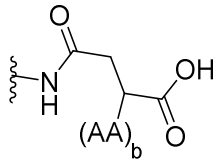
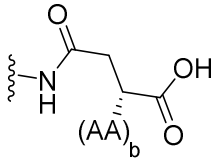
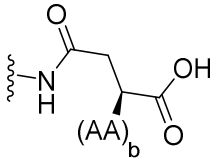
X^B and L are each independently optionally substituted with a PEG Unit from PEG2 to PEG 72.

[0153] As used herein, A, when present is covalently attached to M or M^1, and Y, when present is attached to X^B or to X^A (when X^B is absent).

[0154] In some embodiments, M is .

[0155] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

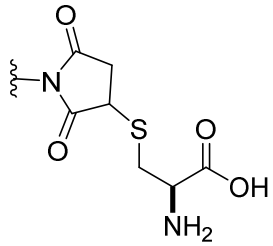
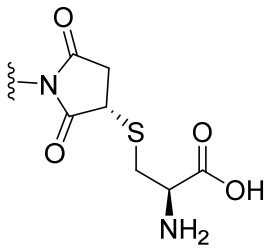
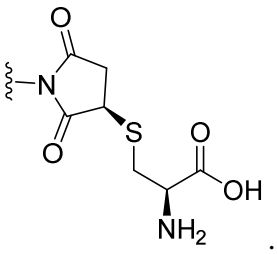
[0156] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

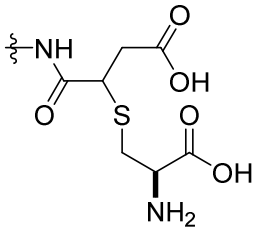
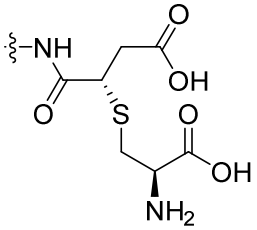
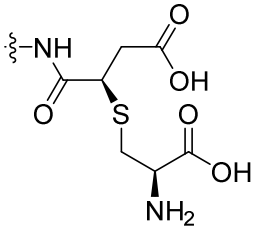
[0157] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

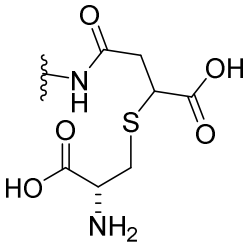
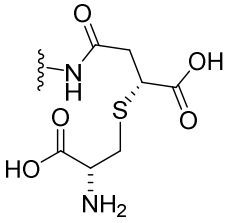
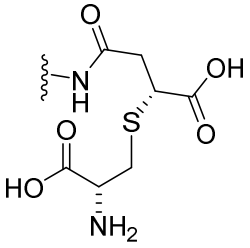
[0158] In some embodiments, each AA is independently a natural amino acid; wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom. In some embodiments, each AA is independently a natural amino acid; wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom of a cysteine residue.

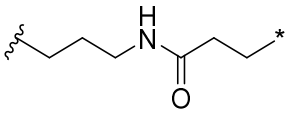
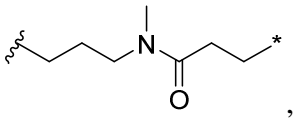
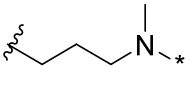
[0159] In some embodiments, each AA is independently a natural amino acid; wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a nitrogen atom. In some embodiments, each AA is independently a natural amino acid; wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via the ϵ-nitrogen atom of a lysine residue.

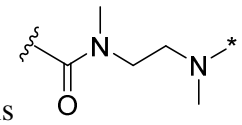
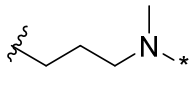
[0160] In some embodiments, each subscript b is 1, 2, or 3. In some embodiments, each subscript b is 1. In some embodiments, each subscript b is 2. In some embodiments, each subscript b is 3. In some embodiments, each subscript b is 3, 4, 5, or 6. In some embodiments, each subscript b is 4. In some embodiments, each subscript b is 5. In some embodiments, each subscript b is 6.

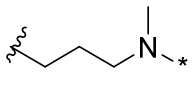
[0161] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

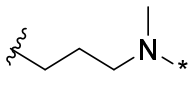
[0162] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

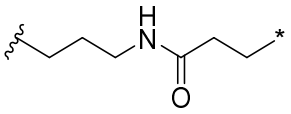
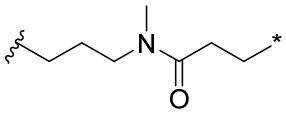
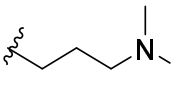
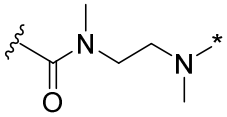
[0163] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

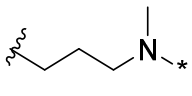
[0164] In some embodiments, R^1 is methoxy and R^2 and R^3 are both $–C(=O)NH_2$. In some embodiments, X^A is –O– and X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L, when present, or M. In some embodiments, R^1 is methoxy; R^2 and R^3 are both $–C(=O)NH_2$; X^A is –O–; and X^B is , wherein represents covalent linkage to X^A, and * represents covalent linkage to L, when present, or M. In some such embodiments, L is absent. In some embodiments, R^1 is methoxy; R^2 and R^3 are both $–C(=O)NH_2$; X^A is –O–; X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L; and

subscript a and subscript y are both 0 (i.e., X^B is covalently attached to W). In some embodiments, X^A is –O–; X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L. In some embodiments, R^1 is methoxy; R^2 and R^3 are both –$C(=O)NH_2$; X^A is –O–; and X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L; and subscript a and subscript w are both 0.

[0165] In some embodiments, R^1 is methoxy; R^2 and R^3 are both –$C(=O)NH_2$; X^A is –O–; and X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L; and subscript y and subscript w are both 0.

[0166] In some embodiments, R^1 is methoxy; R^2 and R^3 are both –$C(=O)NH_2$; X^A is –O–; and X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L; and subscript y is 0.

[0167] In some embodiments, R^1 is methoxy and R^2 and R^3 are both –$C(=O)NH_2$. In some embodiments, X^A is –CH_2–; and X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L, when present, or M. In some embodiments, R^1 is methoxy; R^2 and R^3 are both –$C(=O)NH_2$; X^A is –CH_2–; and X^B is , wherein represents covalent linkage to X^A, and * represents covalent linkage to L, when present, or M. In some embodiments, R^1 is methoxy; R^2 and R^3 are both –$C(=O)NH_2$; X^A is –CH_2–; and X^B is  wherein represents covalent linkage to X^A, and * represents covalent linkage to L; and subscript a and subscript y are both 0 (i.e., X^B is covalently attached to W). In some embodiments, X^A is –CH_2–; and X^B is  wherein represents covalent linkage to X^A, and * represents covalent

linkage to L. In some embodiments, R^1 is methoxy; R^2 and R^3 are both $–C(=O)NH_2$; X^A is $–CH_2–$; and X^B is  wherein  represents covalent linkage to X^A, and * represents covalent linkage to L; and subscript a and subscript w are both 0 (i.e., X^B is covalently bound to Y).

[0168] In some such embodiments, L is a linker having the formula $–(A)_a-(W)_w-(Y)_y–$.

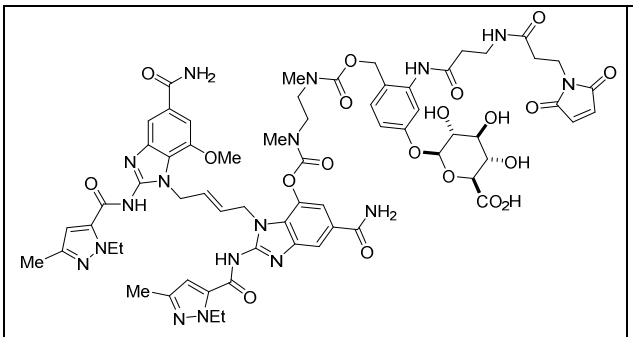
[0169] In some embodiments: X^B is absent and L is covalently attached to X^A. In some embodiments: X^B is absent and Y is covalently attached to X^A. In some embodiments: X^B is absent and Y is absent, and W is covalently attached to X^A. In some embodiments: X^B is absent, Y is absent, W is absent, and A is covalently attached to X^A.

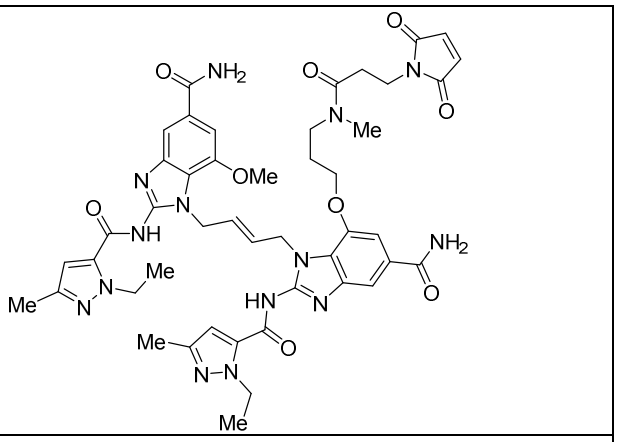
[0170] In some embodiments: X^B is a 2-16 membered heteroalkylene and L is covalently attached to X^B. In some embodiments: X^B is a 2-16 membered heteroalkylene and Y is covalently attached to X^B. In some embodiments: X^B is a 2-16 membered heteroalkylene, Y is absent, and W is covalently attached to X^B. In some embodiments: X^B is a 2-16 membered heteroalkylene, Y is absent, W is absent, and A is covalently attached to X^B.

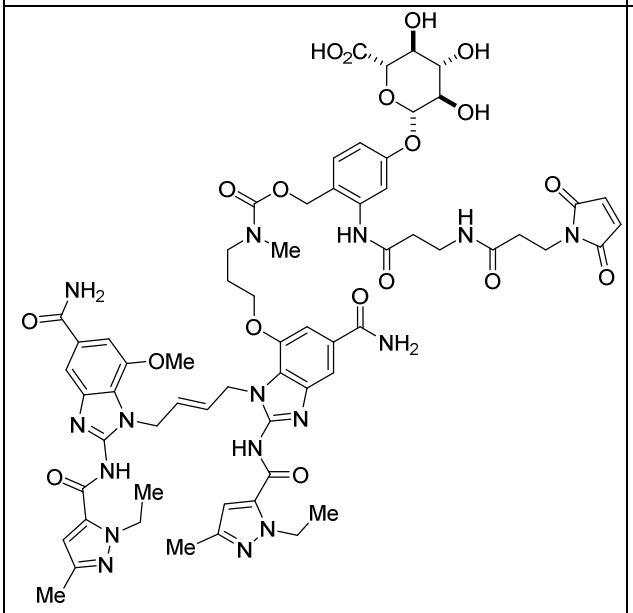
[0171] In some embodiments, W_1 is -OC(=O)- and subscript y is 1. In some embodiments, X^A is -O- and X^B and W are absent. In some embodiments, X^A is NH or -O-, X^B is absent, and W_1 is -OC(=O). In some embodiments, X^A is $–N(CH_3)–$, X^B is absent, and W_1 is -OC(=O). In some embodiments, X^A is -S-, X^B is absent, and W_1 is -OC(=O). In some embodiments, W_1 is -OC(=O)- and X^B is covalently attached to W via -O- or -NH-.

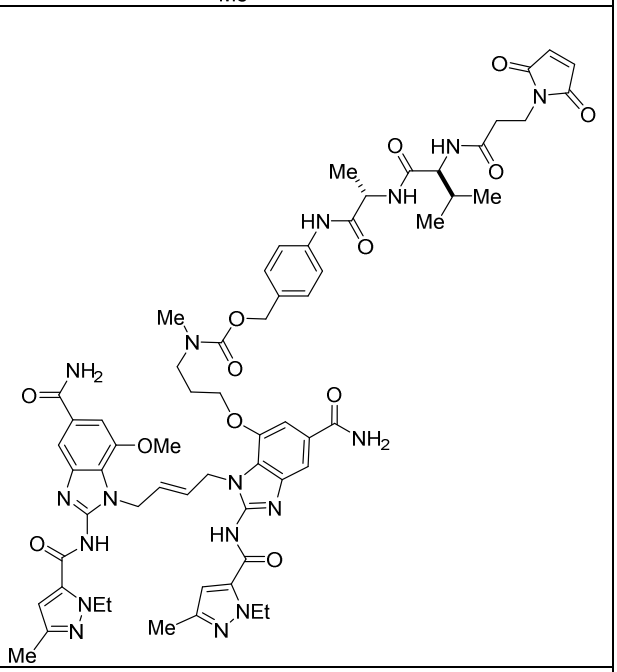
[0172] In some embodiments, A is covalently attached to M. In some embodiments, when subscript a is 0 and subscript w is 0, Y is covalently attached to M. In some embodiments, when subscripts a, y, and w, are each 0, X^B is covalently attached to M.

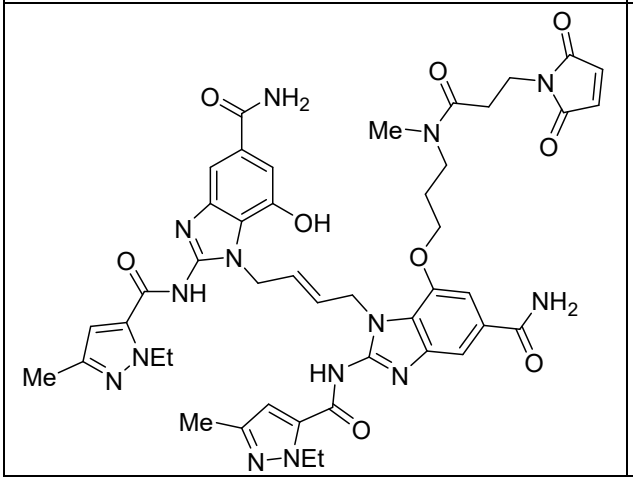
[0173] In some embodiments, the compound of Formula (II) is selected from the group consisting of:

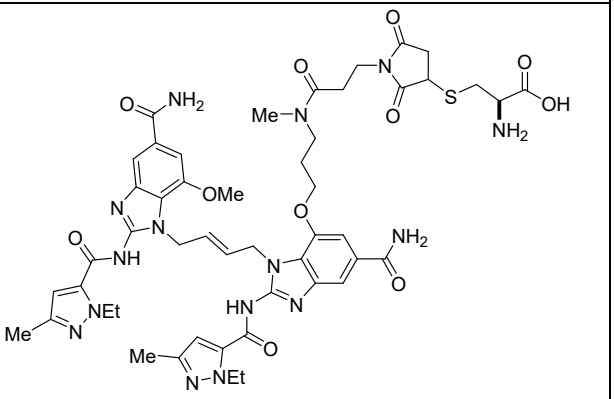


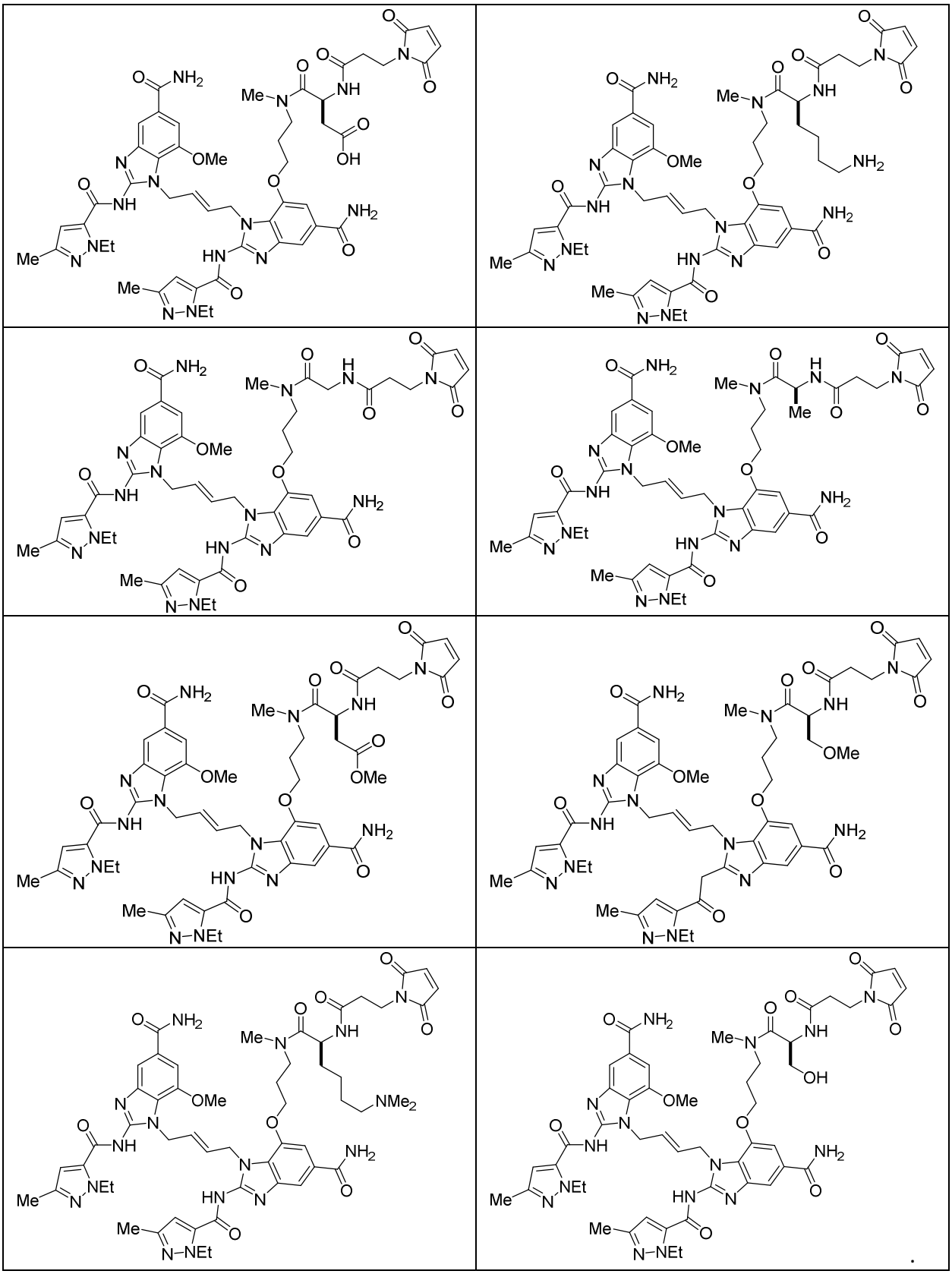






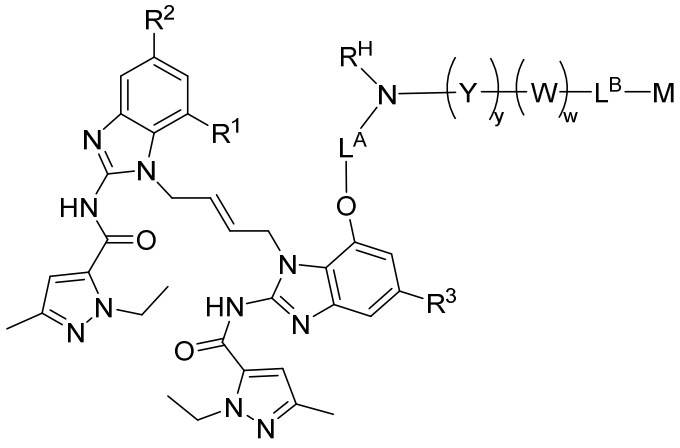






Compounds of Formula (II-A)

[0174] In some embodiments, the compound of Formula (II) has the structure of Formula (II-A):

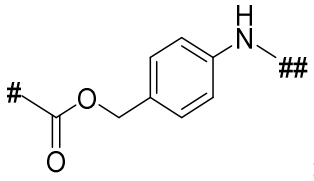


(II-A)

or a pharmaceutically acceptable salt thereof, wherein:

L^A is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–C(O)NR^H(CH_2)_{1-6}–$;

each R^H is independently hydrogen or C_{1-3} alkyl;

Y is  ;

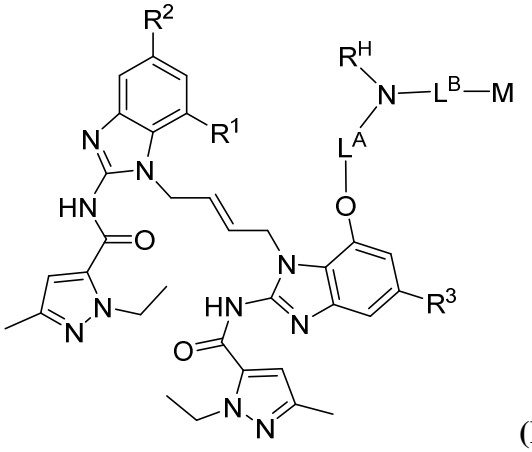
represents covalent attachment to $–NR^HL^A$;

represents covalent attachment to W or L^B;

L^B is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–[NHC(O)(CH_2)_{1-4}]_{1-3}–$; and

the remaining variables are as defined above in connection of Formula (II).

[0175] In some embodiments, R^H is methyl. In some embodiments, L^A is $–(CH_2)_{2-6}–$. In some embodiments, L^A is $–(CH_2)_3–$. In some embodiments, subscript y is 0. In some embodiments, subscript y is 1. In some embodiments, subscript w is 0. In some embodiments, subscript w is 1. In some embodiments, subscript y and subscript w are both 1. In some embodiments, subscript y and subscript w are both 0. When subscript y and subscript w are both 0, the compound of Formula (II) has the structure of Formula (II-B):



(II-B)

or a pharmaceutically acceptable salt thereof, wherein:

L^A is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–C(O)NR^H(CH_2)_{1-6}–$;

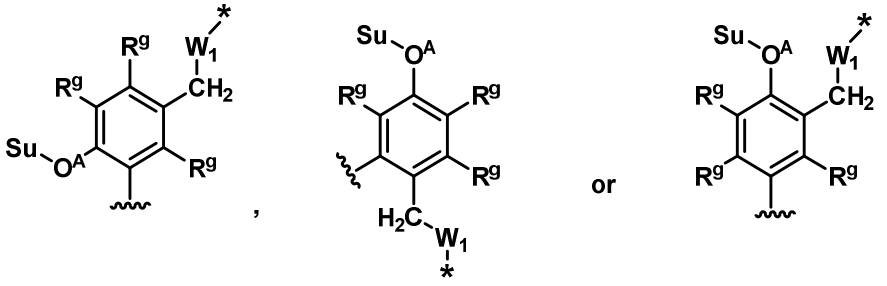
each R^H is independently hydrogen or C_{1-3} alkyl;

L^B is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–[NHC(O)(CH_2)_{1-4}]_{1-3}–$; and

the remaining variables are as defined above in connection of Formula (II).

[0176] In some embodiments, W is a chain of 1-6 amino acids. In some embodiments, W is a chain of 1-4 amino acids. In some embodiments, W is a chain of 1-3 amino acids. In some embodiments, each amino acid of W is independently selected from the group consisting of alanine, valine, isoleucine, leucine, aspartic acid, glutamic acid, lysine, histidine, arginine, glycine, serine, threonine, phenylalanine, O-methylserine, O-methylaspartic acid, O-methylglutamic acid, N-methyllysine, O-methyltyrosine, O-methylhistidine, and O-methylthreonine.

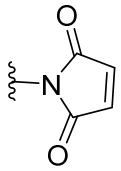
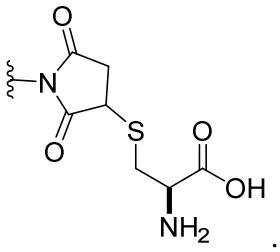
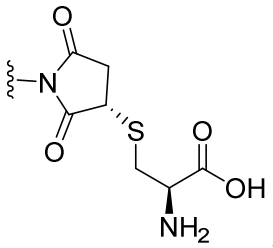
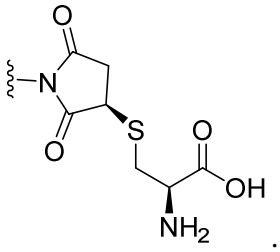
[0177] In some embodiments, W is:



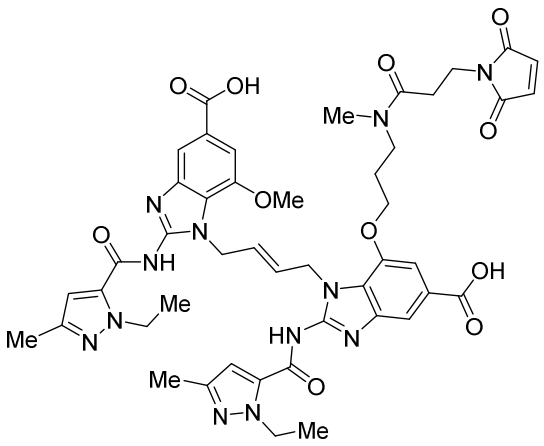
, wherein:

~~~ represents covalent attachment to L^B; and

* represents covalent attachment to Y or NR^H.
~~~

[0178] In some embodiments, L^B is $–C(O)(CH_2)_2–$. In some embodiments, L^B is $–[NHC(O)(CH_2)_2]_2–$. In some embodiments, M is . In some embodiments, M is . In some aspects, M is . In some aspects, M is .

[0179] In some embodiments, the compound of Formula (II-A) is selected from the group consisting of: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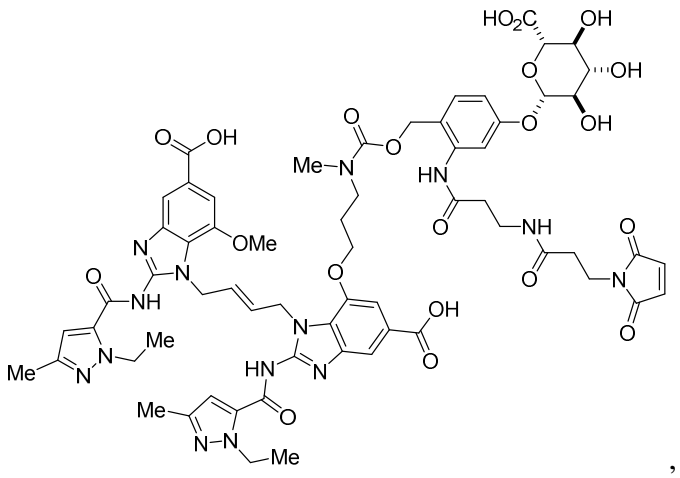
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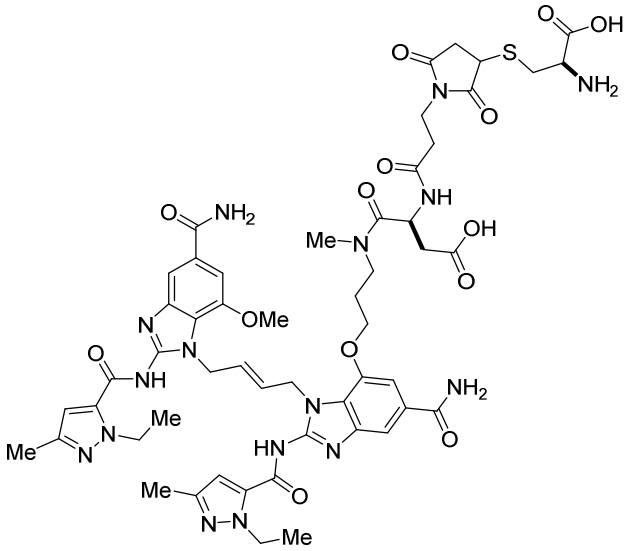
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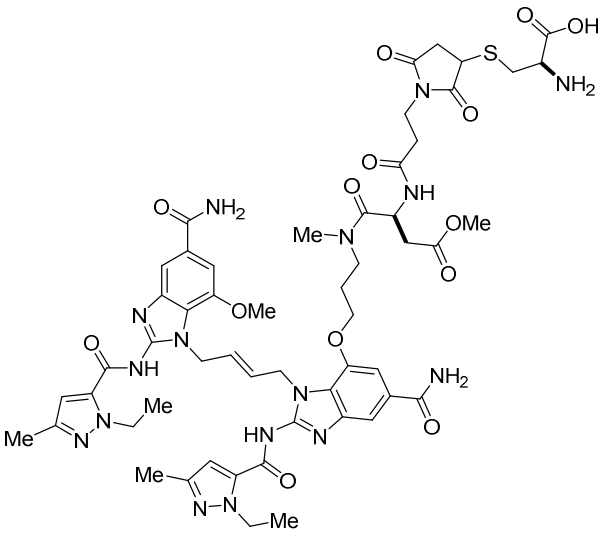
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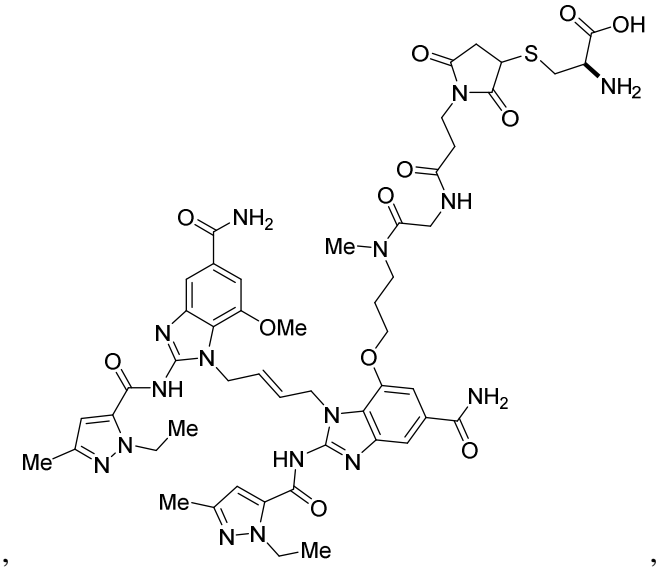
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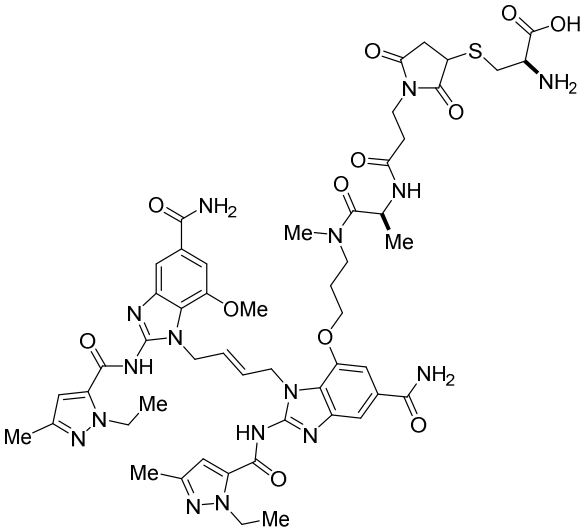
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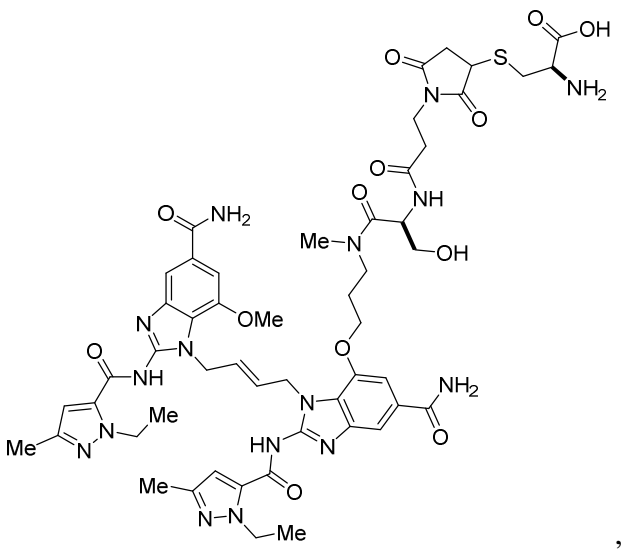

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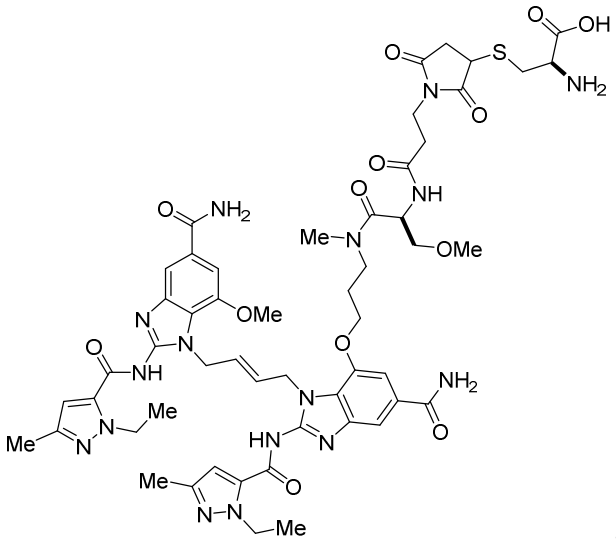

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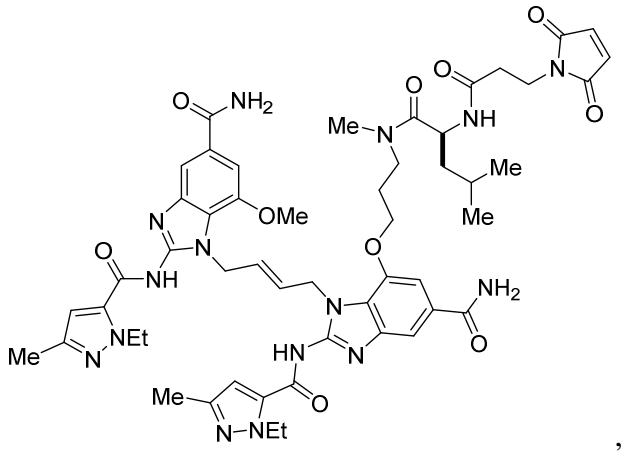

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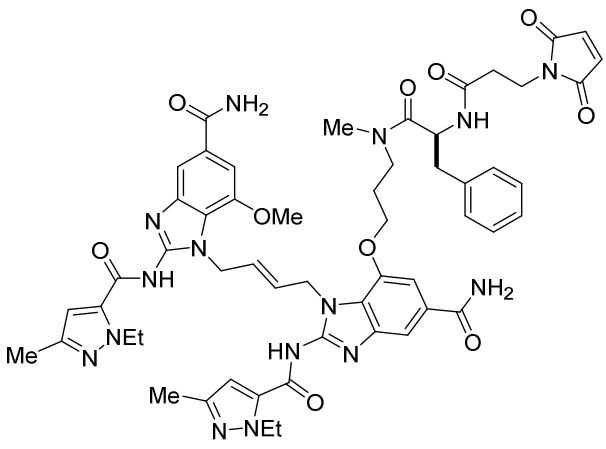

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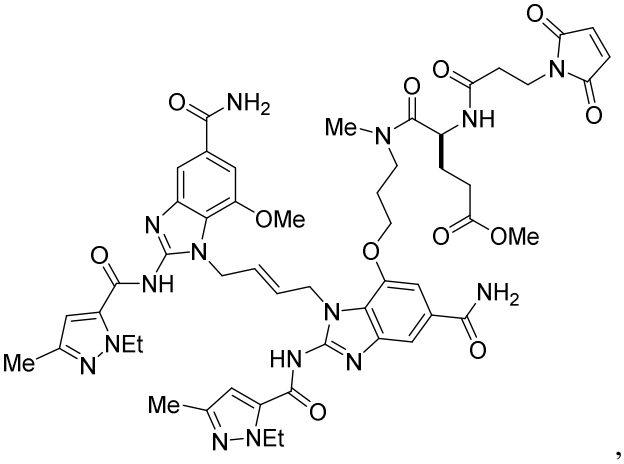

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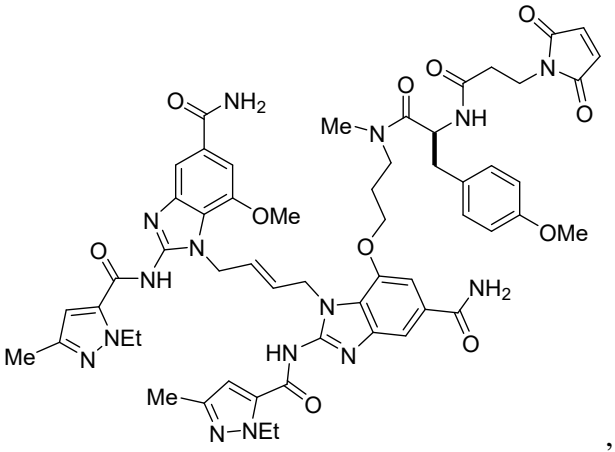
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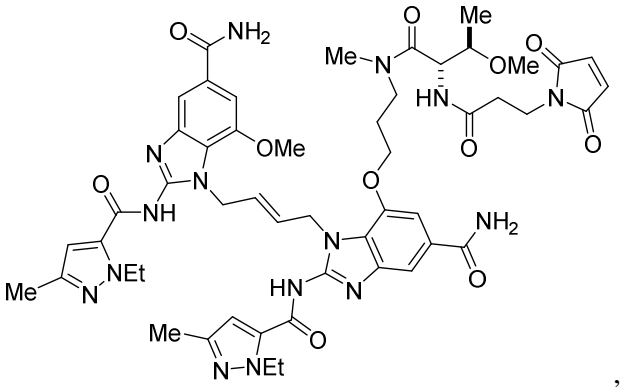
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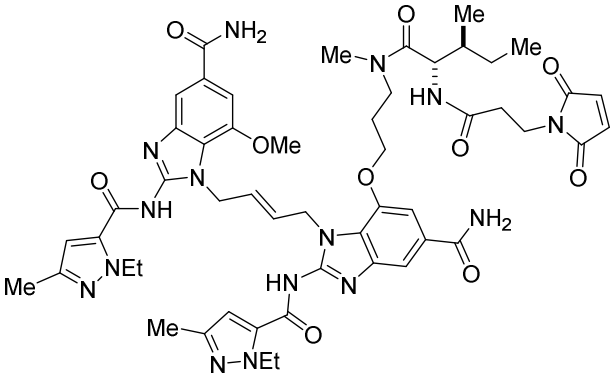
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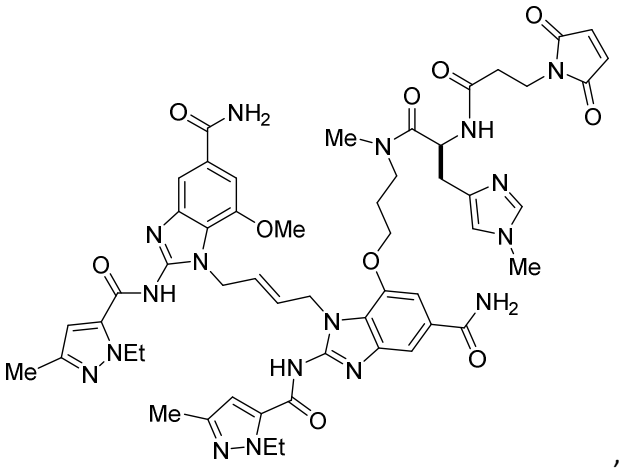
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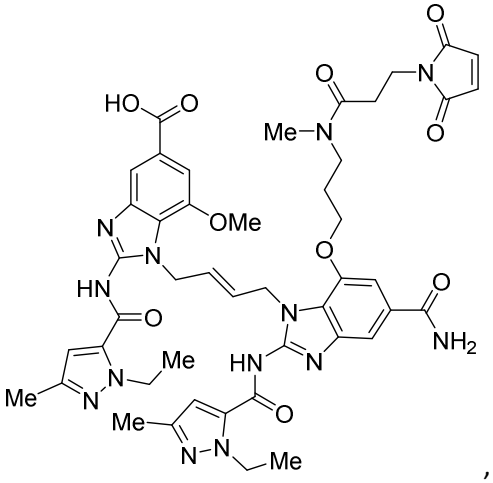
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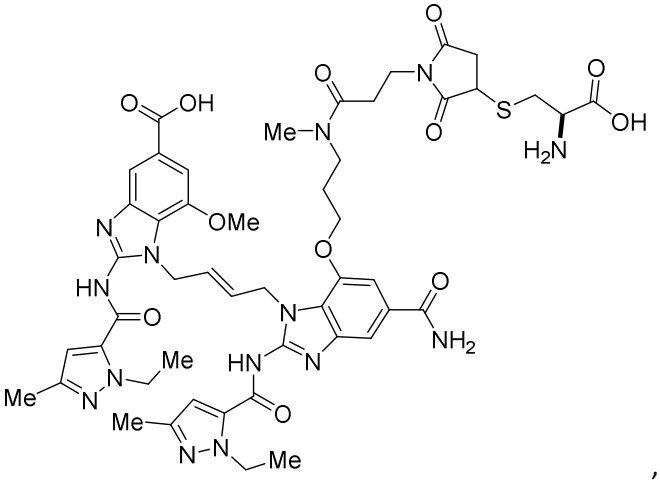
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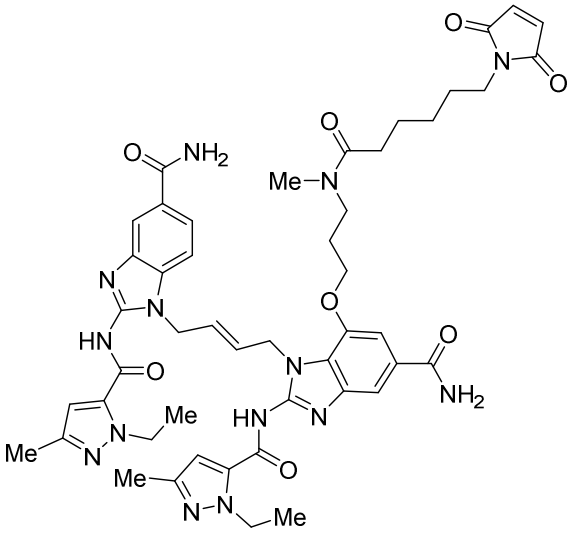

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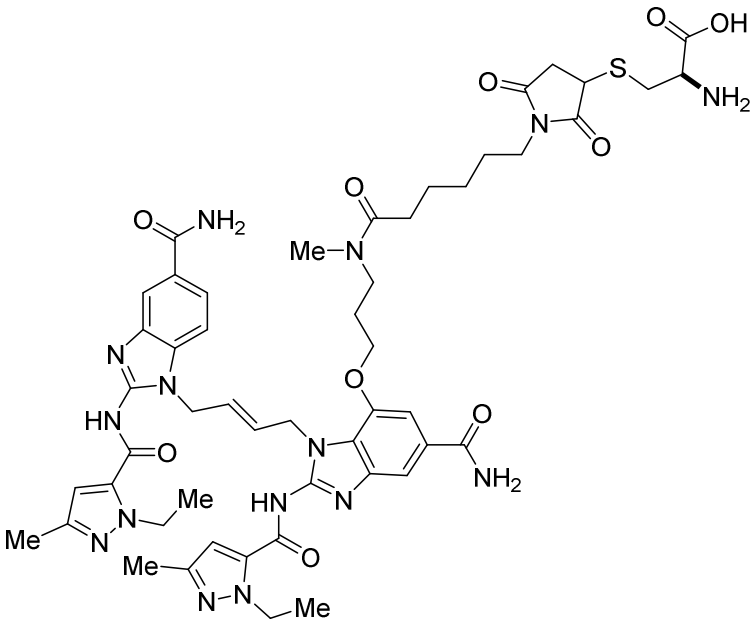
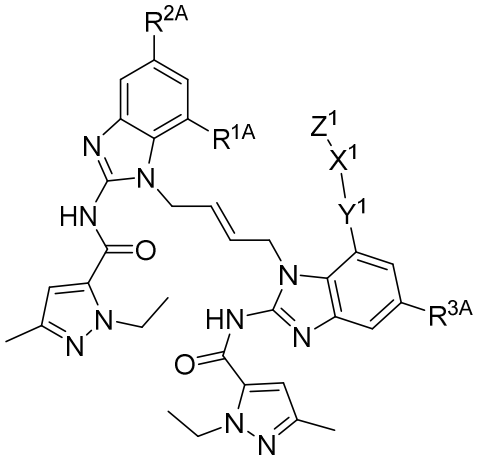


, and pharmaceutically acceptable salts thereof.

Compounds of Formula (III)

[0180] Some embodiments provide compounds of Formula (III):



(III)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl)C_{1-6} alkoxy, –$(CH_2)_{nn}$-$NR^{AA}R^{BB}$;

each R^{2A} and R^{3A} are independently $–CO_2H$, –$(C=O)_{mm}$-$NR^{CC}R^{DD}$, or –$(CH_2)_{qq}$-$NR^{EE1}R^{FF1}$;

each subscript nn is independently an integer from 0 to 6;

each subscript mm is independently 0 or 1;

each subscript qq is independently an integer from 0 to 6;

Y^1 is $–CH_2–$, –O–, –S–, –NH–, or $–N(CH_3)–$;

X^1 is a C_2-C_6 alkylene;

Z^1 is $–NR^{EE}R^{FF}$, $–C(=O)NR^{GG}R^{HH}$, or $–CO_2H$;

each R^{AA}, R^{BB}, R^{CC}, R^{DD}, R^{EE1}, and R^{FF1} are independently hydrogen or C_{1-3} alkyl; and

each R^{EE}, R^{FF}, R^{GG}, and R^{HH} are independently hydrogen or C_{1-6} alkyl.

[0181] In some embodiments, R^{1A} is hydrogen. In some embodiments, R^{1A} is hydroxyl. In some embodiments, R^{1A} is C_{1-6} alkoxy. In some embodiments, R^1 is methoxy. In some embodiments, R^{1A} is –(C_{1-6} alkyl)C_{1-6} alkoxy. In some embodiments, R^{1A} is methoxyethyl.

[0182] In some embodiments, R^1 is –$(CH_2)_{nn}$-$NR^{AA}R^{BB}$. In some embodiments, R^{AA} and R^{BB} are both hydrogen. In some embodiments, R^{AA} and R^{BB} are independently C_{1-3} alkyl. In some embodiments, one of R^{AA} and R^{BB} is hydrogen and the other of R^{AA} and R^{BB} is C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, each subscript nn is 0. In some embodiments, each subscript nn is 1. In some embodiments, each subscript nn is 2. In some

embodiments, each subscript nn is 3. In some embodiments, each subscript nn is 3, 4, 5, or 6. In some embodiments, each subscript nn is 4. In some embodiments, each subscript nn is 5. In some embodiments, each subscript nn is 6.

[0183] In some embodiments, each R^{2A} and R^{3A} are independently $–CO_2H$, $–(C=O)_{mm}$-$NR^{CC}R^{DD}$, or $–(CH_2)_{qq}$-$NR^{EE1}R^{FF1}$; and R^{2A} and R^{3A} are the same. In some embodiments, each R^{2A} and R^{3A} are independently $–CO_2H$, $–(C=O)_{mm}$-$NR^{CC}R^{DD}$, or $–(CH_2)_{qq}$-$NR^{EE1}R^{FF1}$; and R^{2A} and R^{3A} are different.

[0184] In some embodiments, R^{2A} is $–(C=O)_{mm}$-$NR^{CC}R^{DD}$. In some embodiments, R^{3A} is $-(C=O)_{mm}$-$NR^{CC}R^{DD}$. In some embodiments, each R^{CC} and each R^{DD} is hydrogen. In some embodiments, each R^{CC} and each R^{DD} is independently C_{1-3} alkyl. In some embodiments, one of each R^{CC} and R^{DD} is hydrogen and the other of each R^{CC} and R^{DD} is C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, each subscript mm is 0. In some embodiments, each subscript mm is 1.

[0185] In some embodiments, R^{2A} is $–(CH_2)_{qq}$-$NR^{EE1}R^{FF1}$. In some embodiments, R^{3A} is $-(CH_2)_{qq}$-$NR^{EE1}R^{FF1}$. In some embodiments, each R^{EE1} and each R^{FF1} is hydrogen. In some embodiments, each R^{EE1} and each R^{FF1} is independently C_{1-3} alkyl. In some embodiments, one of each R^{EE1} and R^{FF1} is hydrogen and the other of each R^{EE1} and R^{FF1} is C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, each subscript q is 0. In some embodiments, each subscript q is an integer from 1 to 6. In some embodiments, each subscript qq is 1. In some embodiments, each subscript qq is 2. In some embodiments, each subscript qq is 3, 4, 5, or 6.

[0186] In some embodiments, R^{3A} is $–CO_2H$. In some embodiments, R^{2A} is $–CO_2H$.

[0187] In some embodiments, Y^1 is $–CH_2–$. In some embodiments, Y^1 is –O–. In some embodiments, Y^1 is –S–. In some embodiments, Y^1 is –NH–. In some embodiments, Y^1 is $-N(CH_3)–$.

[0188] In some embodiments, X^1 is a C_2-C_5 alkylene. In some embodiments, X^1 is a C_2-C_4 alkylene. In some embodiments, X^1 is ethylene or n-propylene. In some embodiments, X^1 is ethylene. In some embodiments, X^1 is n-propylene.

[0189] In some embodiments, Z^1 is $–NR^{E1}R^{F1}$. In some embodiments, R^{EE} and R^{FF} are both hydrogen. In some embodiments, R^{EE} and R^{FF} are independently C_{1-6} alkyl. In some

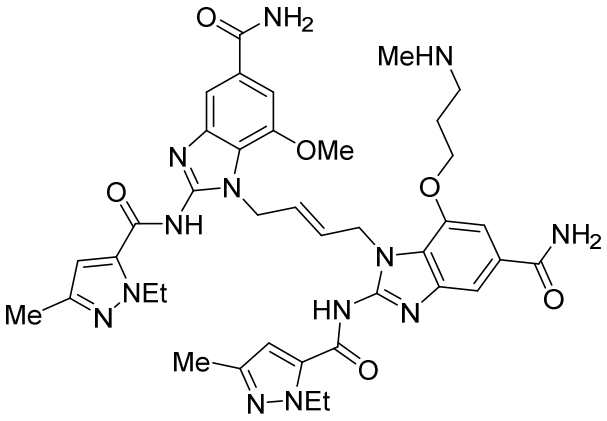
embodiments, one of R^{EE} and R^{FF} is hydrogen and the other of R^{EE} and R^{FF} is C_{1-6} alkyl. In some embodiments, the C_{1-6} alkyl is a C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl.

[0190] In some embodiments, Z^1 is $–C(=O)NR^{GG}R^{HH}$. In some embodiments, R^{GG} and R^{HH} are both hydrogen. In some embodiments, R^{GG} and R^{HH} are independently C_{1-6} alkyl. In some embodiments, one of R^{GG} and R^{HH} is hydrogen and the other of R^{GG} and R^{HH} is C_{1-6} alkyl. In some embodiments, the C_{1-6} alkyl is a C_{1-3} alkyl. In some embodiments, the C_{1-3} alkyl is methyl. In some embodiments, Z^1 is $–CO_2H$. In some embodiments, Z^1 is $–NR^{EE}R^{FF}$. In some embodiments, R^{EE} is hydrogen and R^{FF} is methyl.

[0191] In some embodiments, R^{1A} is methoxy and R^{2A} and R^{3A} are both $–C(=O)NH_2$. In some embodiments, Y^1 is –O– and X^1 is a C_3 alkylene. In some embodiments, Y^1 is –O– and X^1 is n-propylene. In some embodiments, Y^1 is –O–, X^1 is n-propylene, and Z^1 is $–NH_2$. In some embodiments, Y^1 is –O–, X^1 is n-propylene, and Z^1 is $–NHCH_3$. In some embodiments, Y^1 is –O–, X^1 is n-propylene, and Z^1 is $–N(CH_3)_2$.

[0192] In some embodiments, R^{1A} is methoxy; R^{2A} and R^{3A} are both $–C(=O)NH_2$; Y^1 is –O–; X^1 is n-propylene; and Z^1 is $–NH_2$. In some embodiments, R^{1A} is methoxy; R^{2A} and R^{3A} are both $–C(=O)NH_2$; Y^1 is –O–; X^1 is n-propylene; and Z^1 is $–NHCH_3$. In some embodiments, R^{1A} is methoxy; R^{2A} and R^{3A} are both $–C(=O)NH_2$; Y^1 is –O–; X^1 is n-propylene; and Z^1 is $–N(CH_3)_2$.

[0193] In some embodiments, the compound of Formula (III) is



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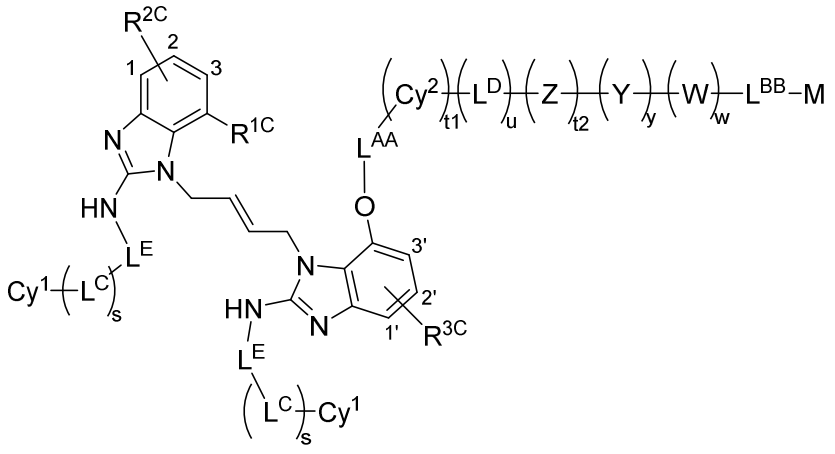
Compounds of Formula (IV)

[0194] Some embodiments include a compound of Formula (IV):



(IV)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1C} is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

R^{2C} is –CO_2R^M, –$(C=O)NR^CR^D$, –$S(O)_2NR^CR^D$, –$S(O)_2R^M$, –$(CH_2)_q$-NR^ER^F, –$(CH_2)_q$-OR^M, –O(C=O)-NR^ER^F, or –NR^M(C=O)-NR^ER^F, wherein R^{2C} is attached at any on of positions labeled 1, 2, or 3;

R^{3C} is –CO_2R^M, –$(C=O)NR^CR^D$, –$S(O)_2NR^CR^D$, –$S(O)_2R^M$, –$(CH_2)_q$-NR^ER^F, –$(CH_2)_q$-OR^M, –O(C=O)-NR^ER^F, or –NR^M(C=O)-NR^ER^F, wherein R^{3C} is attached at any one of positions labeled 1', 2', or 3';

each R^A, R^B, R^C, R^D, R^E, R^F, and R^M are independently hydrogen or C_{1-6} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript q is independently an integer from 0 to 6;

L^E is –(C=O)– or –$S(O)_2$–;

L^C is –$(CR^IR^J)_{1-3}$–

each R^I and R^J are independently hydrogen or C_{1-3} alkyl;

subscript s is 0 or 1;

each Cy^1 is independently a 4-6 membered heterocycle, a 5-6 membered heteroaryl, or a C_{3-6} cycloalkyl, each optionally substituted with one or more R^K;

each R^K is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, -$NR^{d2}R^{e2}$, -$C(O)NR^{d2}R^{e2}$, -$C(O)(C_{1-6}$ alkyl), and -$C(O)O(C_{1-6}$ alkyl);

each R^{d2} and R^{e2} are independently hydrogen or C_{1-3} alkyl;

L^{AA} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, $–C(O)NR^L(CH_2)_{1-6}–$, $–(CH_2)_{1-6}O–$, $–C(O)(CH_2)_{1-6}O–$, or $–C(O)NR^L(CH_2)_{1-6}O–$;

R^L is hydrogen or C_{1-3} alkyl;

Cy^2 is C_{3-6} cycloalkyl, 4-6 membered heterocycle, 5-6 membered heteroaryl, or phenyl, each optionally substituted with one or more R^U;

each R^U is independently selected from the group consisting of $–CO_2R^{j1}$, $–(C=O)NR^{d3}R^{e3}$, $–S(O)_2NR^{d3}R^{e3}$, $–(CH_2)_{q1}$-$NR^{g1}R^{h1}$, $–(CH_2)_{q1}$-OR^{j1}, and $–(CH_2)_{q1}$-$(OCH_2CH_2)_{1-8}OH$;

each R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or C_{1-6} alkyl;

subscript q1 is an integer from 0 to 6;

subscripts t1 and t2 are independently 0 or 1, wherein at least one of t1 and t2 is 1;

L^D is $–(CH_2)_{1-6}–$;

subscript u is 0 or 1;

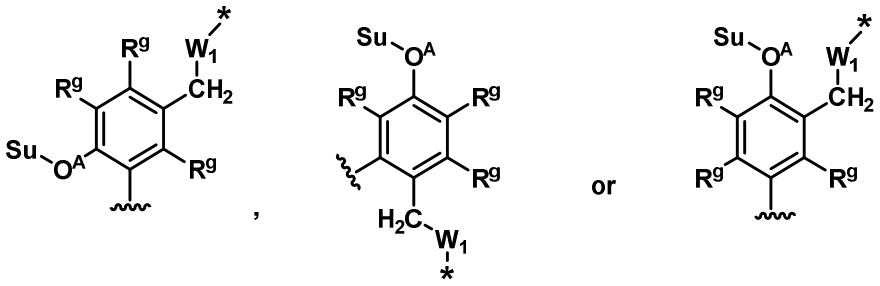
Z is $–N(R^{HH})–$ or $–N^+(C_{1-6}$ alkyl$)(R^{HH})–$;

R^{HH} is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl;

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

subscript y is 0 or 1;

W is a chain of 1-12 amino acids or has the structure:



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

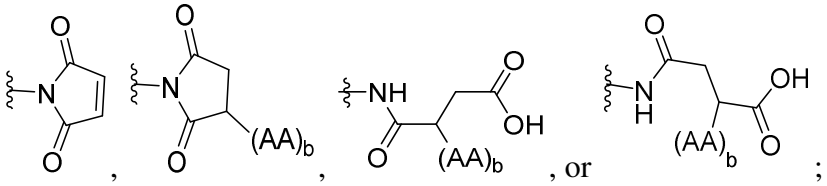
W^1 is absent or –O-C(=O)–;

~~~ represents covalent attachment to L^{BB};
~~~

* represents covalent attachment to Y, L^D, NR^{HH}, or Cy^2;

subscript w is 0 or 1;

L^{BB} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–[NHC(O)(CH_2)_{1-4}]_{1-3}–$; and

M is ;

each AA is an independently selected amino acid, wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom; and

each subscript b is independently an integer from 1 to 6.

[0195] In some embodiments, R^{1C} is hydrogen. In some embodiments, R^{1C} is hydroxyl. In some embodiments, R^{1C} is C_{1-6} alkoxy. In some embodiments, R^{1C} is methoxy. In some embodiments, R^{1C} is $–(C_{1-6}$ alkyl)C_{1-6} alkoxy. In some embodiments, R^{1C} is methoxyethyl. In some embodiments, R^{1C} is PEG2 to PEG4. In some embodiments, R^{1C} is $–(CH_2)_n$-NR^AR^B.

[0196] In some embodiments, R^A and R^B are both hydrogen. In some embodiments, R^A and R^B are independently C_{1-3} alkyl. In some embodiments, one of R^A and R^B is hydrogen and the other of R^A and R^B is C_{1-3} alkyl.

[0197] In some embodiments, each subscript n is 0. In some embodiments, each subscript n is 1. In some embodiments, each subscript n is 2. In some embodiments, each subscript n is 3, 4, 5, or 6.

[0198] In some embodiments, R^{2C} and R^{3C} are independently $–CO_2H$, $–(C=O)_m$-NR^CR^D, or $–(CH_2)_q$-NR^ER^F; and R^{2C} and R^{3C} are the same. In some embodiments, R^{2C} and R^{3C} are independently $–CO_2H$, $–(C=O)_m$-NR^CR^D, or $–(CH_2)_q$-NR^ER^F; and R^{2C} and R^{3C} are different. In some embodiments, R^{2C} is $–(C=O)_m$-NR^CR^D. In some embodiments, R^{3C} is $–(C=O)_m$-NR^CR^D. In some embodiments, R^C and R^D are both hydrogen. In some embodiments, R^C and R^D are each independently C_{1-3} alkyl. In some embodiments, one of R^C and R^D is hydrogen and the other of R^C and R^D is C_{1-3} alkyl. In some embodiments, each subscript m is 0. In some embodiments, each subscript m is 1.

[0199] In some embodiments, R^{2C} is $–(CH_2)_q$-NR^ER^F. In some embodiments, R^{3C} is $–(CH_2)_q$-NR^ER^F. In some embodiments, R^E and R^F are both hydrogen. In some embodiments, R^E and R^F are each independently C_{1-3} alkyl. In some embodiments, one of R^E and R^F is hydrogen and

the other of R^E and R^F is C_{1-3} alkyl. In some embodiments, each subscript q is 0. In some embodiments, each subscript q is an integer from 1 to 6.

[0200] In some embodiments, R^{2C} is $–CO_2R^M$. In some embodiments, R^{3C} is $–CO_2R^M$. In some embodiments, R^M is hydrogen. In some embodiments, R^M is C_{1-3} alkyl.

[0201] In some embodiments, R^{2C} is $–(CH_2)_q$-OR^M.

[0202] In some embodiments, R^{3C} is $–(CH_2)_q$-OR^M. In some embodiments, R^M is hydrogen. In some embodiments, q is 0. In some embodiments, q is 1.

[0203] In some embodiments, R^{2C} is $–O(C=O)$-NR^ER^F. In some embodiments, R^{3C} is $–O(C=O)$-NR^ER^F. In some embodiments, R^E and R^F are both hydrogen. In some embodiments, R^E and R^F are each independently C_{1-3} alkyl. In some embodiments, R^E and R^F is hydrogen and the other of R^E and R^F is C_{1-3} alkyl.

[0204] In some embodiments, R^{2C} is $–NR^M(C=O)$-NR^ER^F. In some embodiments, R^{3C} is $–NR^M(C=O)$-NR^ER^F. In some embodiments, R^E, R^F, and R^M are all hydrogen. In some embodiments, R^E, R^F, and R^M are each independently C_{1-3} alkyl. In some embodiments, one of R^E, R^F, and R^M is C_{1-3} alkyl and the rest of R^E, R^F, and R^M is hydrogen.

[0205] In some embodiments, R^{2C} is $–S(O)_2NR^CR^D$. In some embodiments, R^{3C} is $–S(O)_2NR^CR^D$. In some embodiments, R^C and R^D are both hydrogen. In some embodiments, R^C and R^D are each independently C_{1-3} alkyl. In some embodiments, one of R^C and R^D is hydrogen and the other of R^C and R^D is C_{1-3} alkyl.

[0206] In some embodiments, R^{2C} is $–S(O)_2R^M$. In some embodiments, R^{3C} is $–S(O)_2R^M$. In some embodiments, R^M is hydrogen. In some embodiments, R^M is C_{1-3} alkyl.

[0207] In some embodiments, R^{2C} is attached at position 1. In some embodiments, R^{2C} is attached at position 2. In some embodiments, R^{2C} is attached at position 3. In some embodiments, R^{3C} is attached at position 1'. In some embodiments, R^{3C} is attached at position 2'. In some embodiments, R^{3C} is attached at position 3'.

[0208] In some embodiments, L^E is $–(C=O)–$. In some embodiments, L^E is $–S(O)_2–$.

[0209] In some embodiments, each R^I and R^J is hydrogen. In some embodiments, each R^I and R^J is C_{1-3} alkyl. In some embodiments, one of R^I and R^J is hydrogen and the other of R^I and R^J is C_{1-3} alkyl.

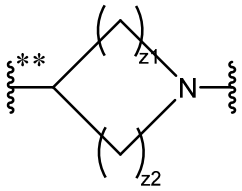
[0210] In some embodiments, L^C is $–(CR^IR^J)–$.

[0211] In some embodiments, s is 0. In some embodiments, s is 1.

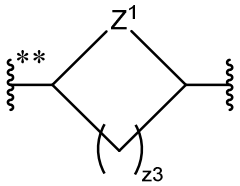
[0212] In some embodiments, each Cy^1 is independently a 5-6 membered heteroaryl. In some embodiments, each Cy^1 is pyrazole optionally substituted with one or more R^K. In some embodiments, each Cy^1 is independently selected from the group consisting of pyrazole, imidazole, furan, thiophene, thiazole, isothiazole, oxazole, isoxazole, pyrrole, pyridazine, pyridine, pyrimidine, and pyrazine, each optionally substituted with one or more R^K. In some embodiments, each Cy^1 is independently selected from the group consisting of imidazole, furan, thiophene, thiazole, isothiazole, oxazole, isoxazole, pyrrole, pyridazine, pyridine, pyrimidine, and pyrazine, each optionally substituted with one or more R^K. In some embodiments, each Cy^1 is independently a C_{4-5} cycloalkyl optionally substituted with one or more R^K. In some embodiments, each R^K is independently selected from the group consisting of C_{1-3} alkyl, C_{1-3} haloalkyl, and halogen. In some embodiments, each R^K is independently selected from the group consisting of methyl, ethyl, $–CF_3$, and halogen.

[0213] In some embodiments, each Cy^1 is the same. In some embodiments, each Cy^1 is different.

[0214] In some embodiments, L^{AA} is $–(CH_2)_{1-6}–$. In some embodiments, L^{AA} is $–(CH_2)_{1-3}–$. In some embodiments, L^{AA} is $–(CH_2)_{1-6}O–$. In some embodiments, L^{AA} is $–(CH_2)_{1-3}O–$.

[0215] In some embodiments, Cy^2 is a 4-6 membered heterocycle. In some embodiments, Cy^2 has the structure: 

, wherein each of subscripts z1 and z2 is independently an integer from 1 to 3 and ** indicates attachment to L^{AA}. In some embodiments, z1 and z2 are 1. In some embodiments, z1 and z2 are 2. In some embodiments, z1 is 1 and z2 is 2.

[0216] In some embodiments, Cy^2 has the structure: 

, wherein

Z^1 is selected from the group consisting of –O–, –S–, $–CR^NR^O–$, and $–NR^P–$;

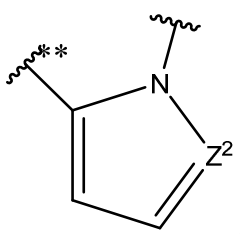
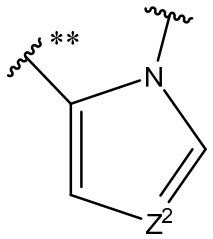
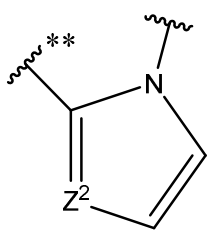
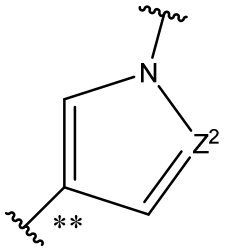
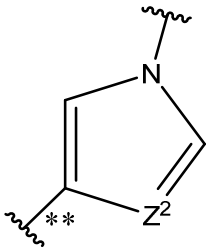
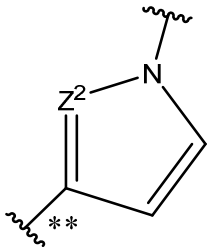
R^N, R^O, and R^P are independently hydrogen or C_{1-6} alkyl;

subscript z3 is an integer from 1 to 3; and

** indicates attachment to L^{AA}.

[0217] In some embodiments, R^N and R^O are hydrogen. In some embodiments, R^P is hydrogen. In some embodiments, R^P is methyl.

[0218] In some embodiments, Cy^2 is a 5-6 membered heteroaryl. In some embodiments, Cy^2 is selected from the group consisting of:

, and , wherein

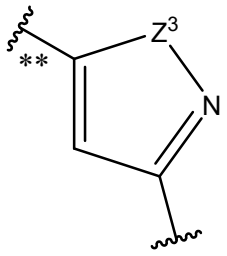
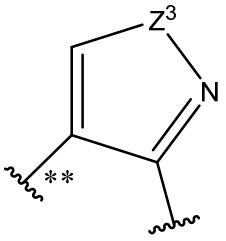
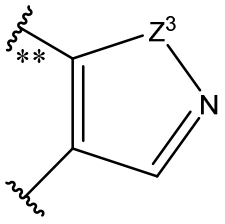
Z^2 is $=CR^N-$ or $=N$;

R^N is hydrogen or C_{1-6} alkyl; and

** indicates attachment to L^{AA}.

[0219] In some embodiments, Z^2 is $=CR^N$ and R^N is hydrogen. In some embodiments, Z^2 is $=N-$.

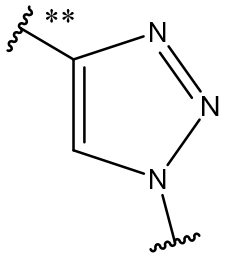
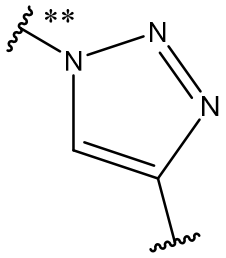
[0220] In some embodiments, Cy^2 is selected from the group consisting of:

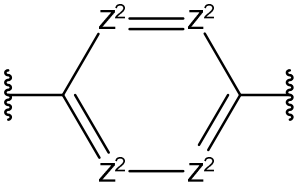
, , and , wherein Z^3 is $-O-$ or $-S-$ and ** indicates attachment to L^{AA}, L^D, NR^{HH}, Y, W, or L^{BB}.

[0221] In some embodiments, ** indicates attachment to L^{AA}. In some embodiments, ** indicates attachment to L^D, NR^{HH}, Y, W, or L^{BB}.

[0222] In some embodiments, Cy^2 is selected from the group consisting of:



and , wherein ** indicates attachment to L^{AA}.

[0223] In some embodiments, Cy^2 is selected from the group consisting of: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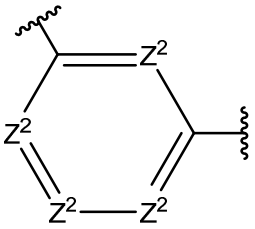
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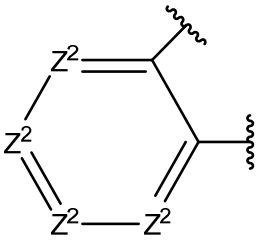

, wherein

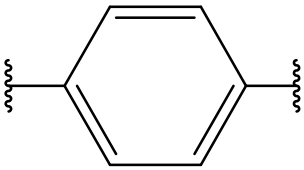
each Z^2 is independently $=CR^N-$ or $=N-$; and

each R^N is hydrogen or $C_{1\text{-}6}$ alkyl.

[0224] In some embodiments, at least one Z^2 is $=N-$. In some embodiments, one Z^2 is $=N-$ and the remaining Z^2 are $=CR^N-$. In some embodiments, two Z^2 are $=N-$ and the remaining Z^2 are $=CR^N-$.

[0225] In some embodiments, R^N is hydrogen.

[0226] In some embodiments, Cy^2 is selected from the group consisting of: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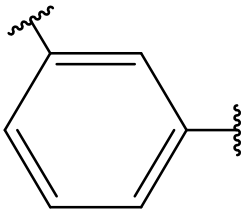
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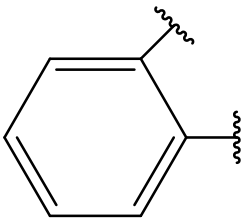

.

[0227] In some embodiments, Cy^2 is cyclobutyl.

[0228] In some embodiments, each R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or $-CH_3$.

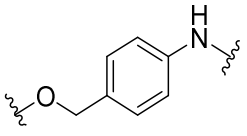
[0229] In some embodiments, each R^U is independently selected from $-CO_2H$, $-(C=O)NH_2$, $-S(O)_2NH_2$, $-CH_2NH_2$, and $-CH_2OH$.

[0230] In some embodiments, t1 is 0 and t2 is 1. In some embodiments, t1 is 1 and t2 is 0. In some embodiments, t1 is 1 and t2 is 1.

[0231] In some embodiments, u is 1 and L^D is $–(CH_2)_{1-3}$. In some embodiments, u is 0.

[0232] In some embodiments, t2 is 1 and R^{HH} is hydrogen. In some embodiments, t2 is 1 and R^{HH} is C_{1-3} alkyl. In some embodiments, t2 is 1 and R^{HH} is C_{3-4} cycloalkyl. In some embodiments, t2 is 1 and R^{HH} is $–(CH_2)$ C_{3-4} cycloalkyl. In some embodiments, t2 is 1 and R^{HH} is $–(CH_2)$ 4-5 membered heterocycle. In some embodiments, t2 is 1 and R^{HH} is $–(CH_2)$ 5-membered heteroaryl.

[0233] In some embodiments, Z is $–N(R^{HH})–$. In other embodiments, Z is $–N^+(C_{1-6}$ alkyl$)(R^{HH})$-.

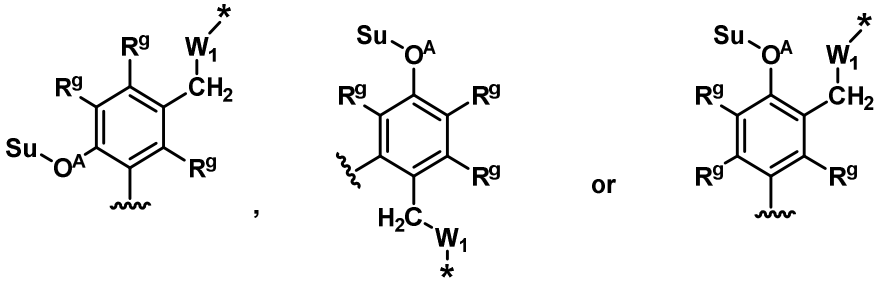
[0234] In some embodiments, Y is .

[0235] In some embodiments, Y is a cyclohexanecarboxyl, undecanoyl, caproyl, hexanoyl, butanoyl or propionyl group. In some embodiments, Y is PEG4 to PEG12. In some embodiments, y is 0. In some embodiments, y is 1.

[0236] In some embodiments, W is a chain of 1-12 amino acids. In some embodiments, W is a chain of 1-6 amino acids. In some embodiments, W is a chain of 1-3 amino acids.

[0237] In some embodiments, W is independently selected from the group consisting of alanine, valine, isoleucine, leucine, aspartic acid, glutamic acid, lysine, histidine, arginine, glycine, serine, threonine, phenylalanine, O-methylserine, O-methylaspartic acid, O-methylglutamic acid, N-methyllysine, O-methyltyrosine, O-methylhistidine, and O-methylthreonine. In some embodiments, each amino acid in W is independently selected from the group consisting of alanine, glycine, lysine, serine, aspartic acid, aspartate methyl ester, N,N-dimethyl-lysine, phenylalanine, citrulline, valine-alanine, valine-citrulline, phenylalanine-lysine or homoserine methyl ether.

[0238] In some embodiments, W has the structure:



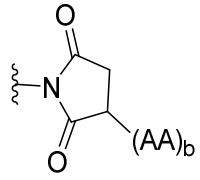
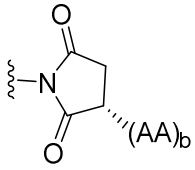
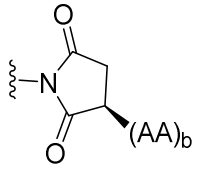
.

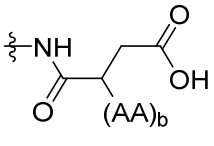
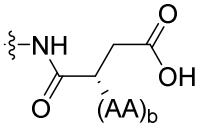
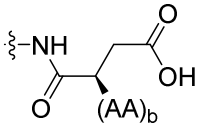
[0239] In some embodiments, W^1 is –O-C(=O)–. In some embodiments, one R^g is halogen, –CN, or $–NO_2$, and the remaining R^G are hydrogen. In some embodiments, each R^g is hydrogen.

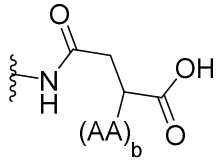
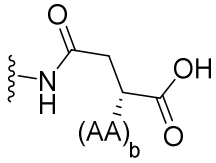
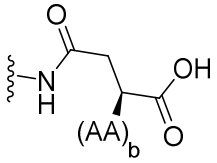
[0240] In some embodiments, w is 0. In some embodiments, w is 1.

[0241] In some embodiments, L^{BB} is $–(CH_2)_{1\text{-}3}–$. In some embodiments, L^{BB} is $–C(O)(CH_2)_{1\text{-}2}–$.

[0242] In some embodiments, L^{BB} is $–C(O)(CH_2)_2–$. In some embodiments, L^{BB} is $–[NHC(O)(CH_2)_2]_{1\text{-}2}–$. In some embodiments, L^{BB} is $–[NHC(O)(CH_2)_2]_2–$.

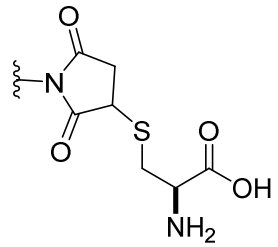
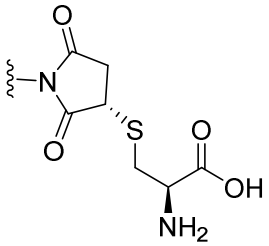
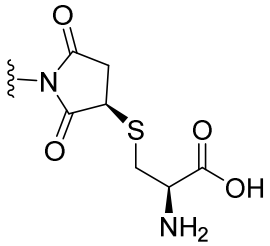
[0243] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

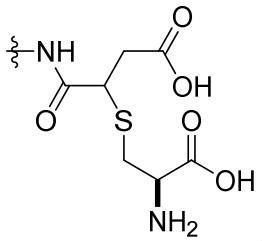
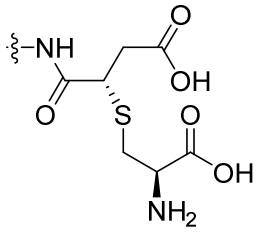
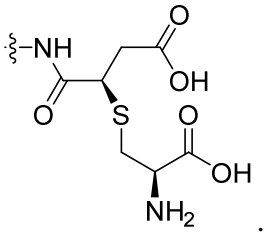
[0244] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

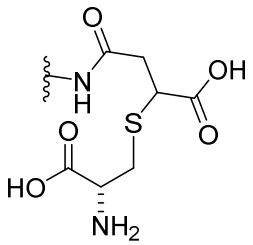
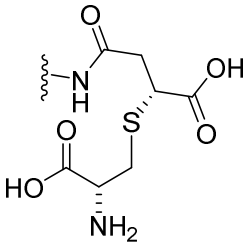
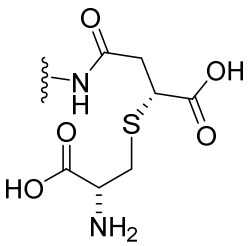
[0245] In some embodiments, M is . n some aspects, M is . In some aspects, M is .

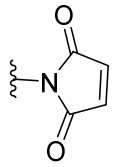
[0246] In some embodiments, each AA is independently a natural amino acid; wherein (AA)b is connected to the succinimide or hydrolyzed succinimide via a sulfur atom. In some embodiments, each AA is independently a natural amino acid; wherein $(AA)_b$ is connected to the

succinimide or hydrolyzed succinimide via a nitrogen atom. In some embodiments, each subscript b is 1. In some embodiments, each subscript b is 2. In some embodiments, each subscript b is 3, 4, 5, or 6.

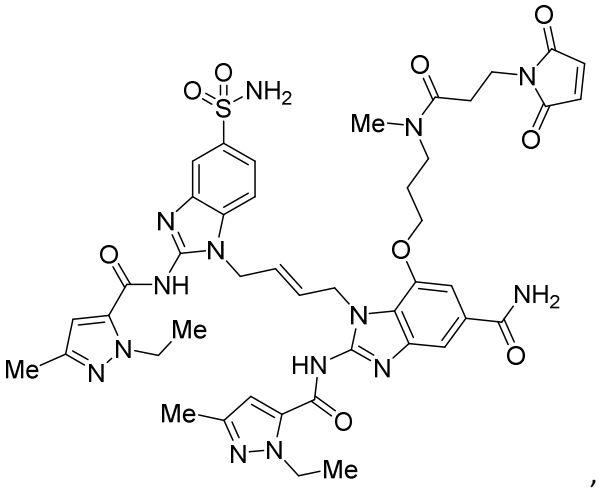
[0247] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

[0248] In some embodiments, M is . n some aspects, M is . In some aspects, M is .

[0249] In some embodiments, M is . In some aspects, M is . In some aspects, M is .

[0250] In some embodiments, M is .

[0251] Some embodiments of the compound of Formula (IV) include a compound selected from the group consisting of: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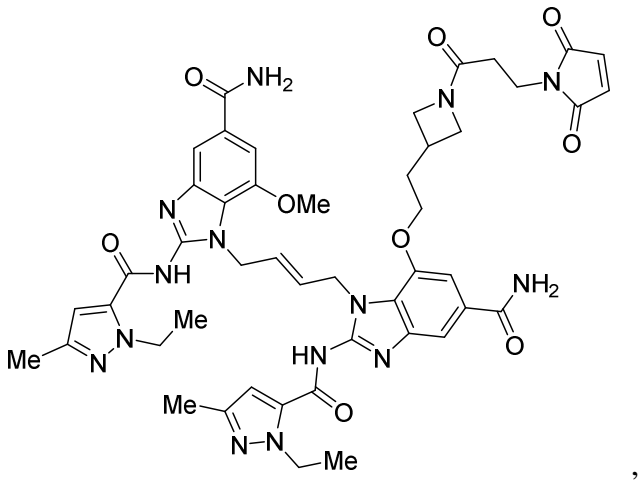
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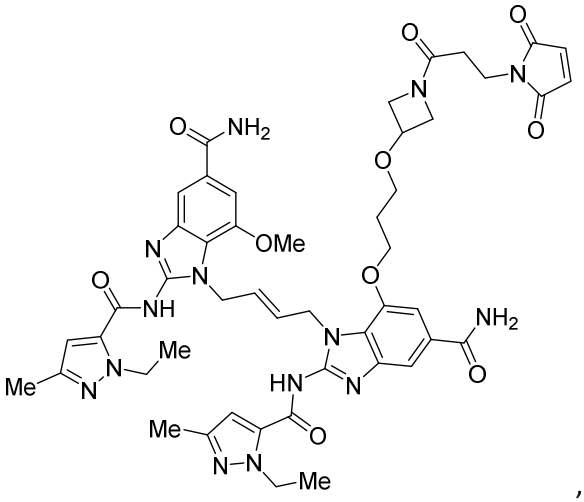
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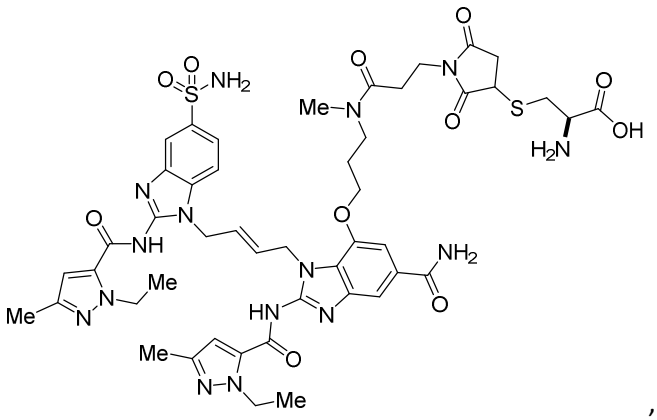
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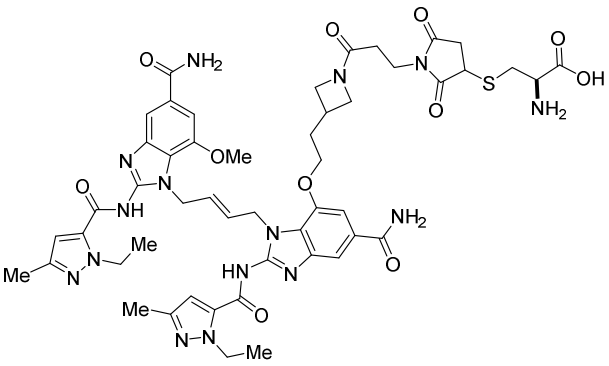
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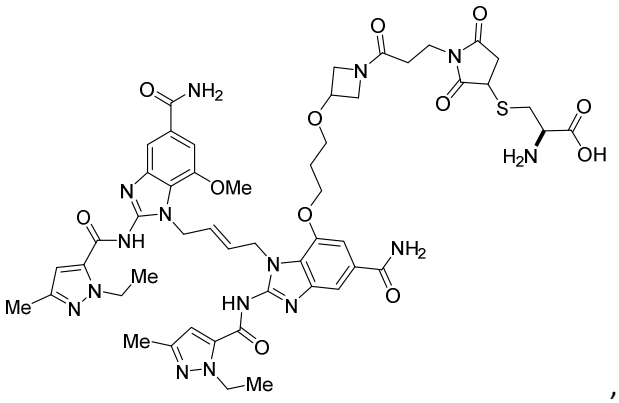
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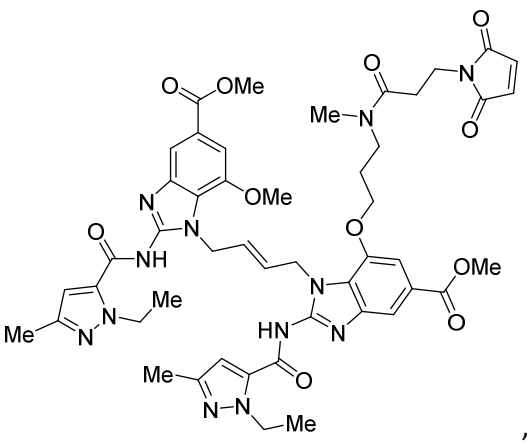
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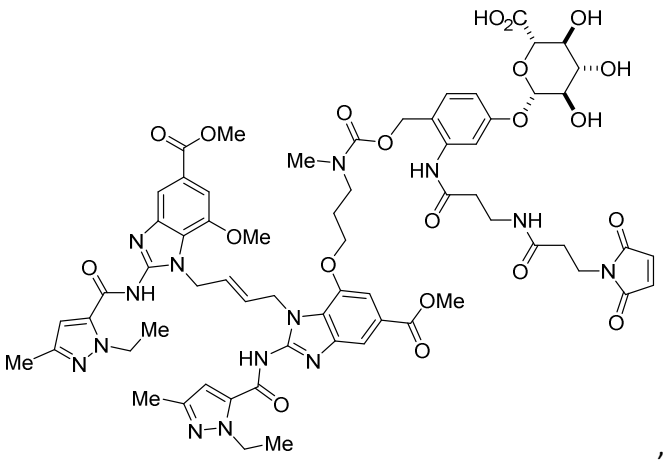
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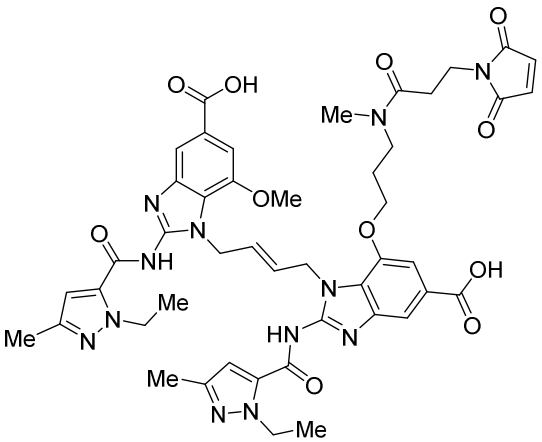
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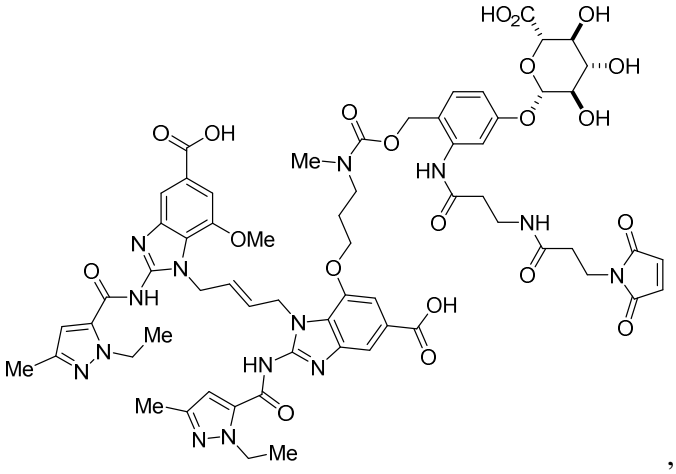
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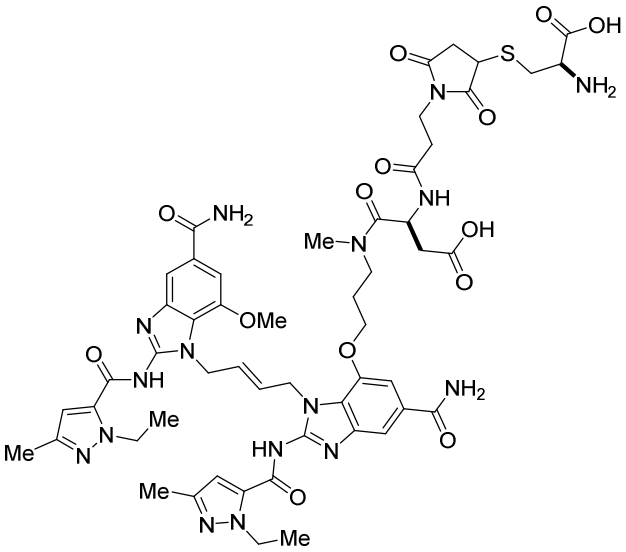
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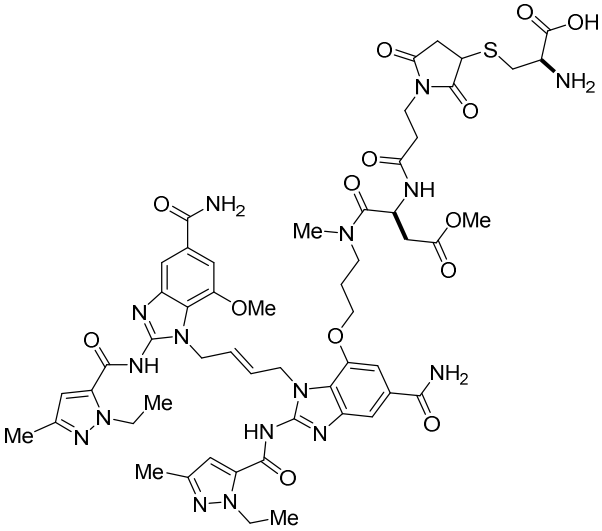

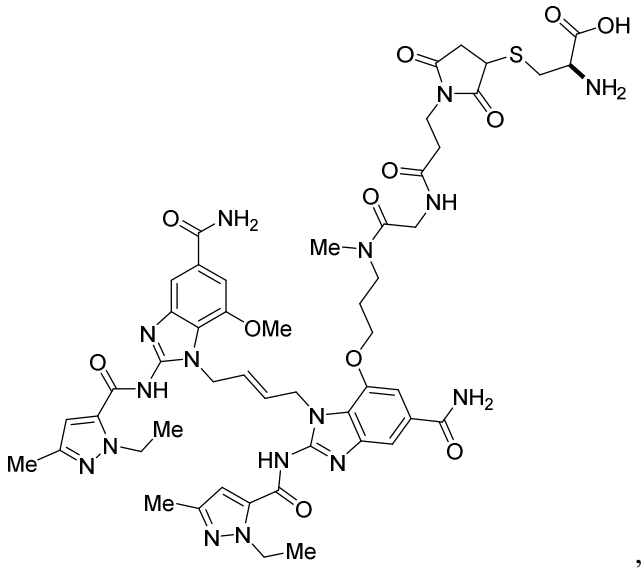

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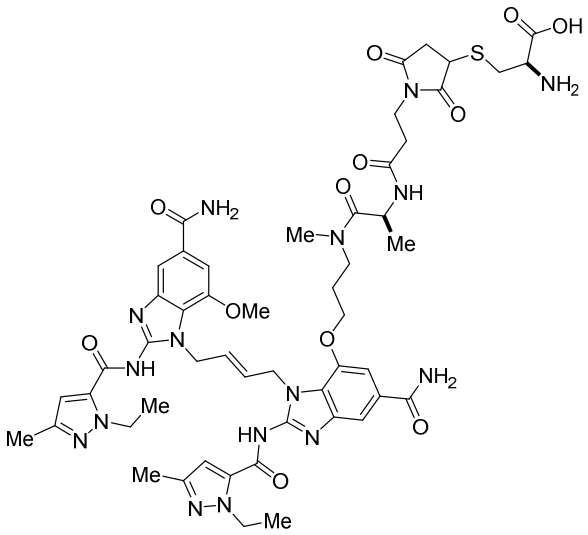

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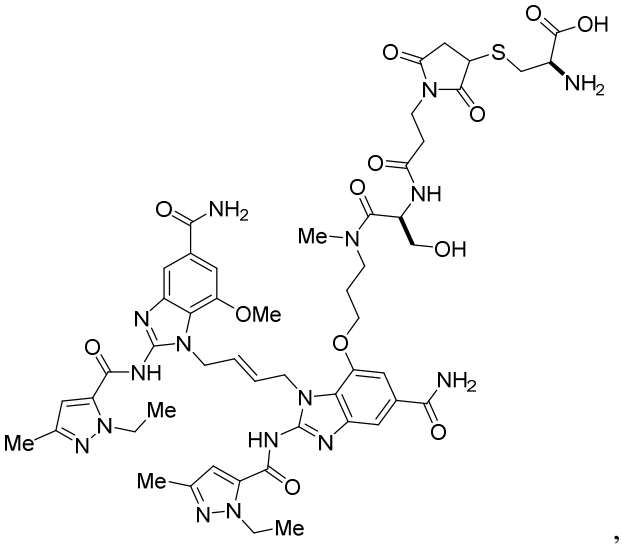

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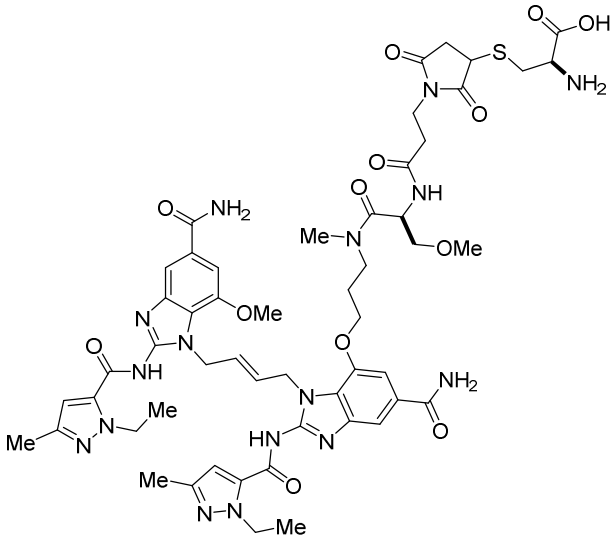

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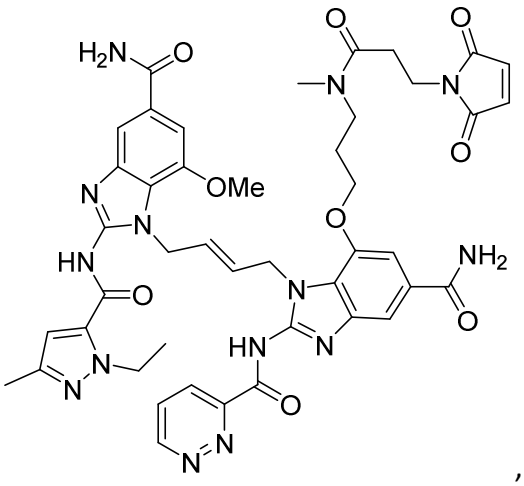

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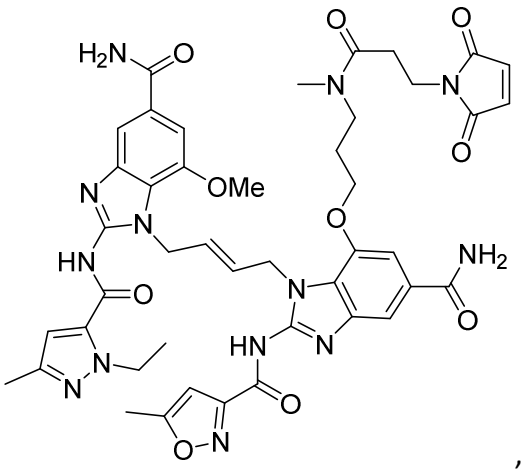
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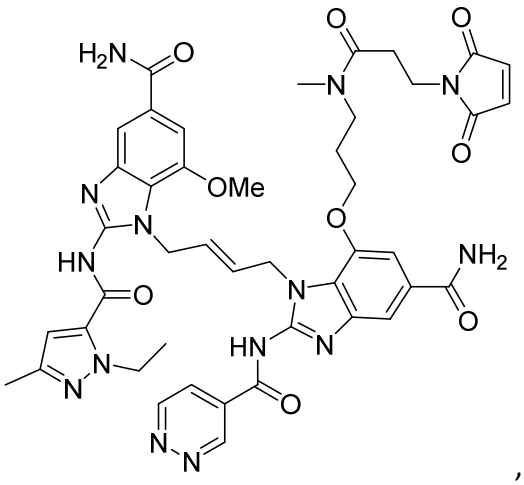
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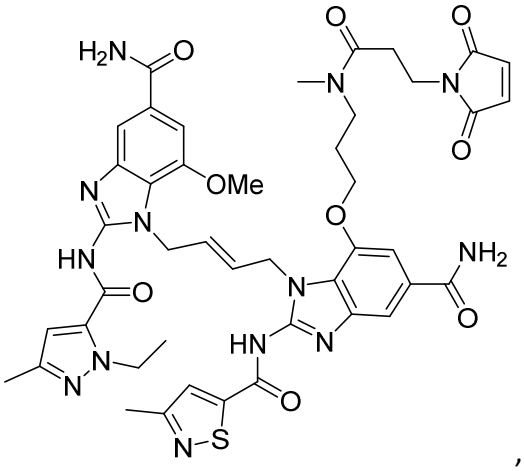
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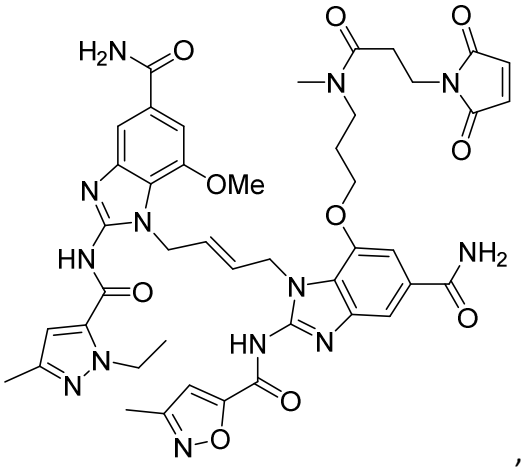
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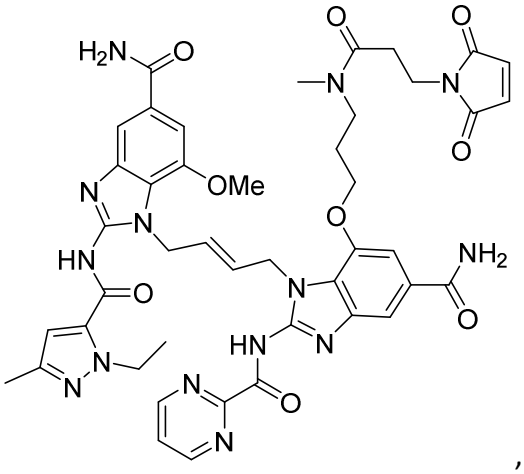
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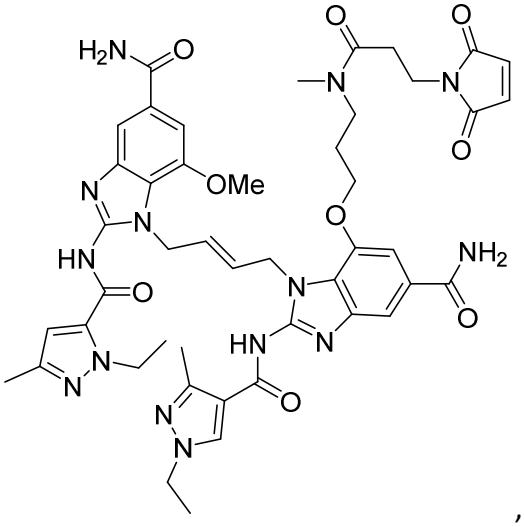
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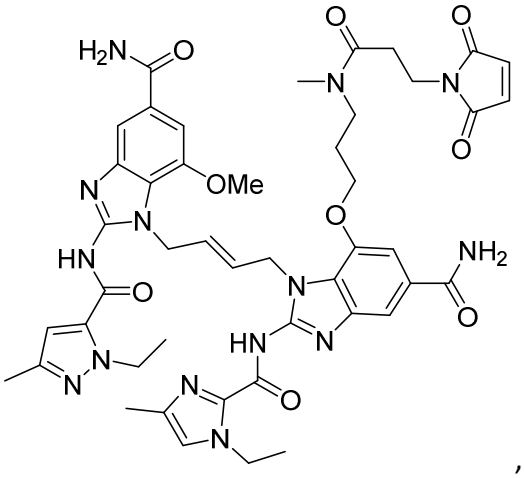
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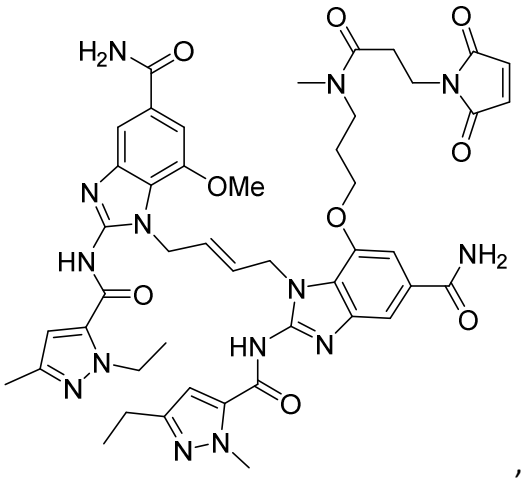
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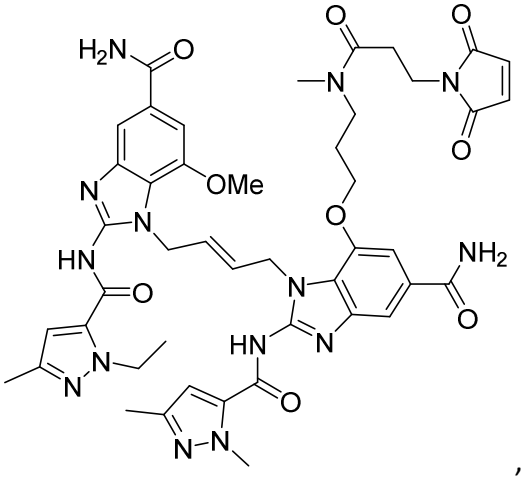
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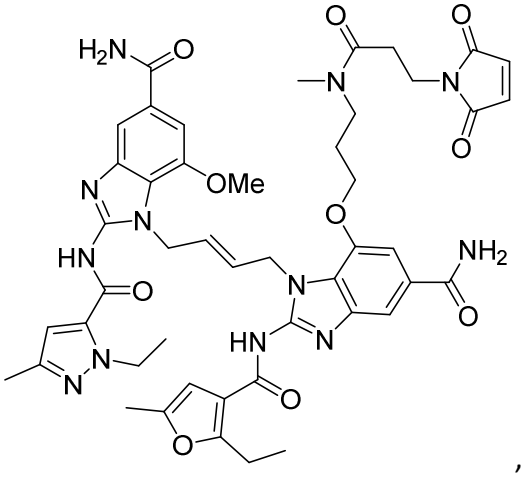
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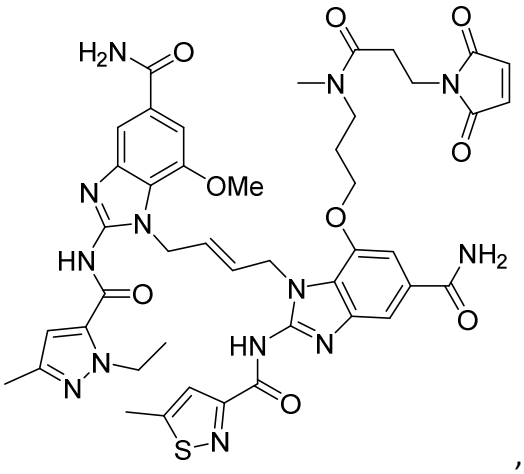
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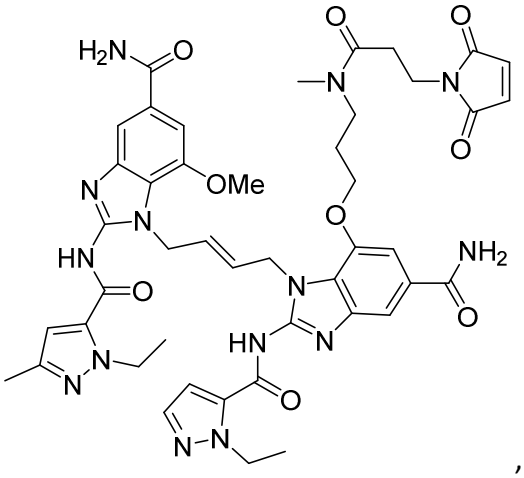
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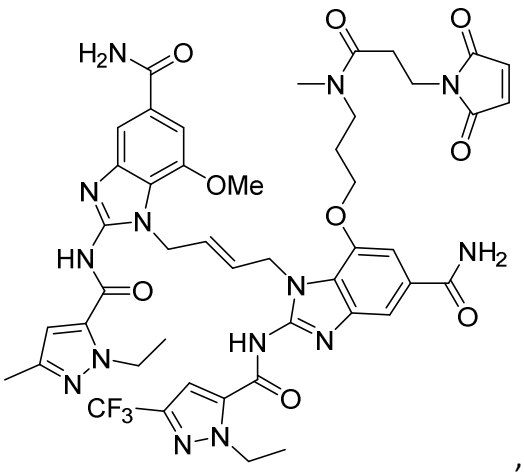
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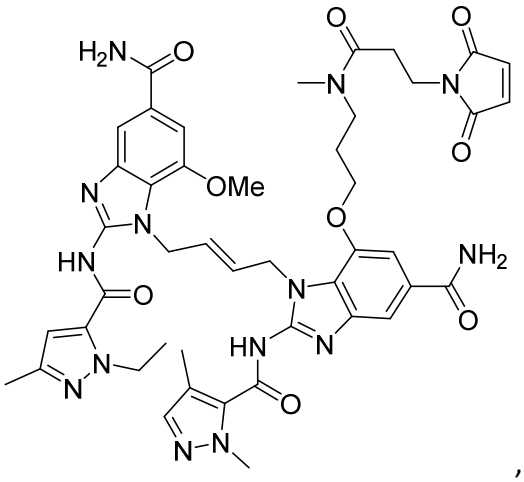
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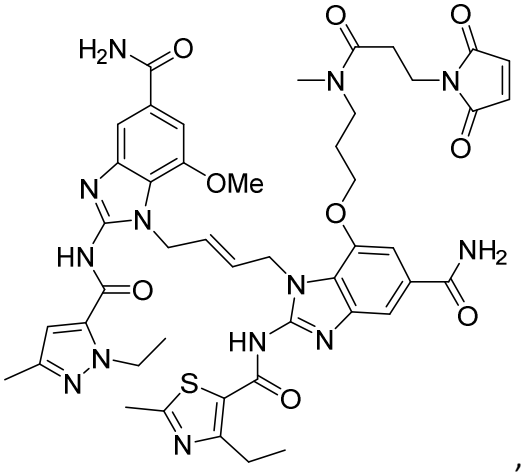
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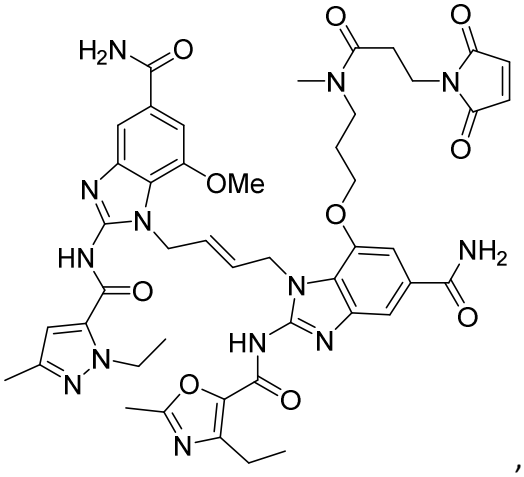
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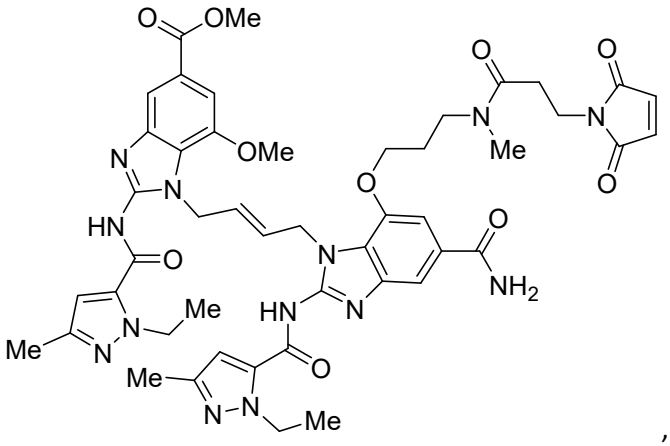
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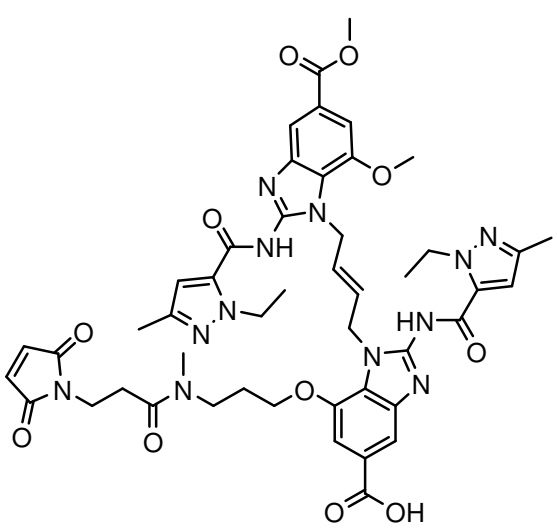
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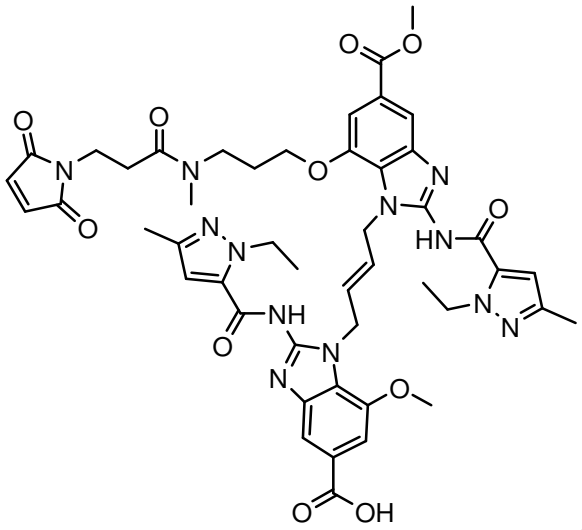
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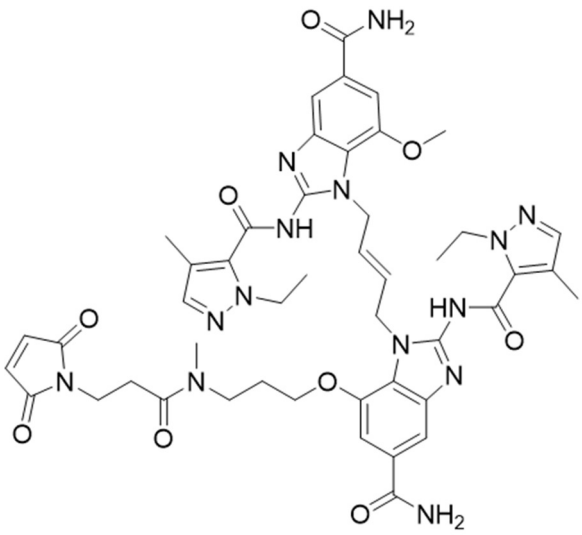
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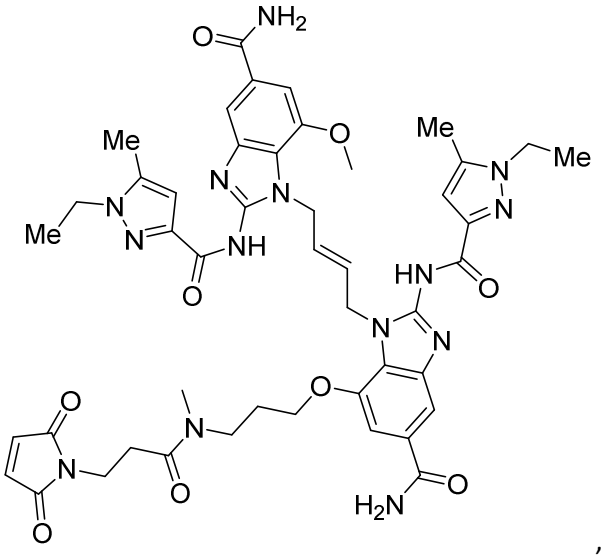
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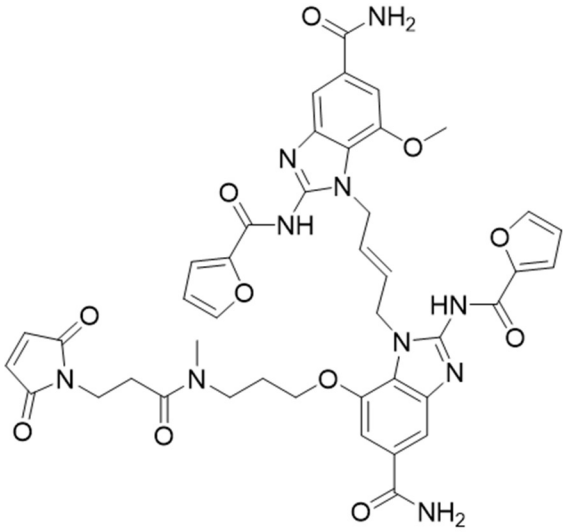
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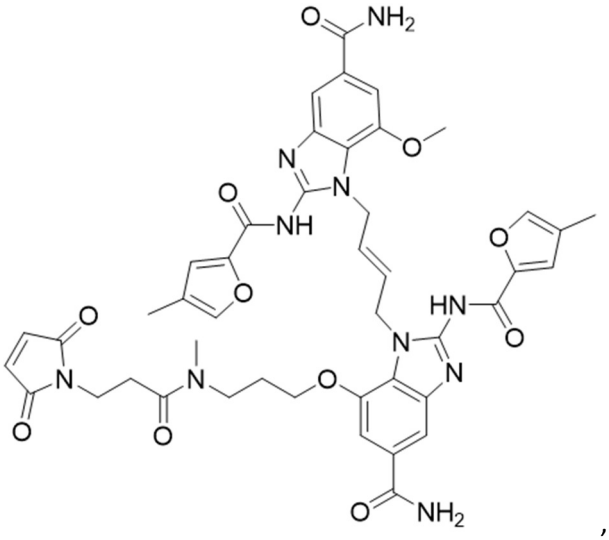
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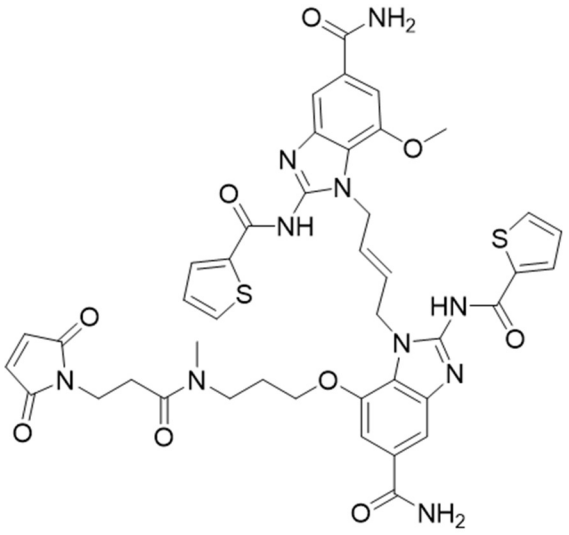
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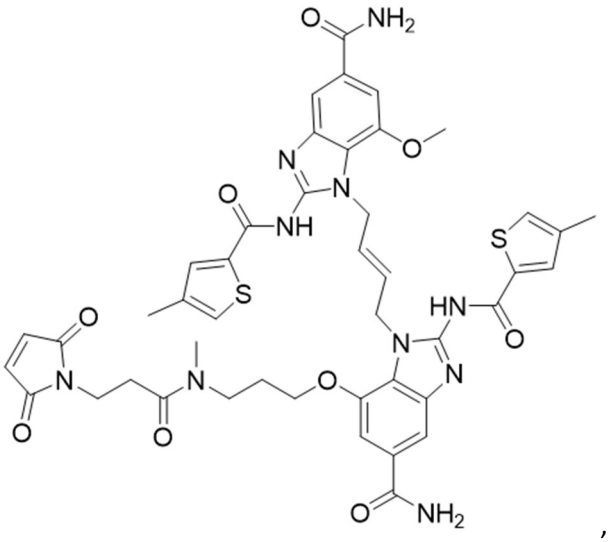
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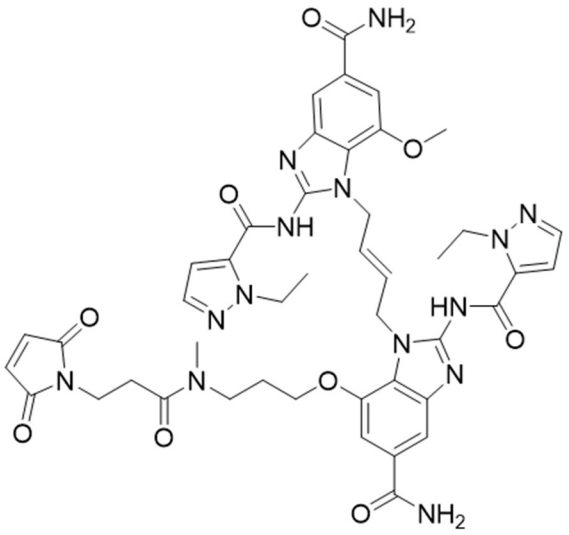
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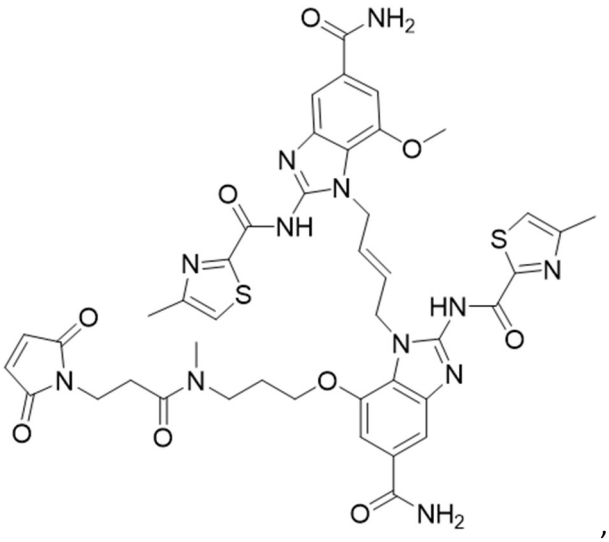
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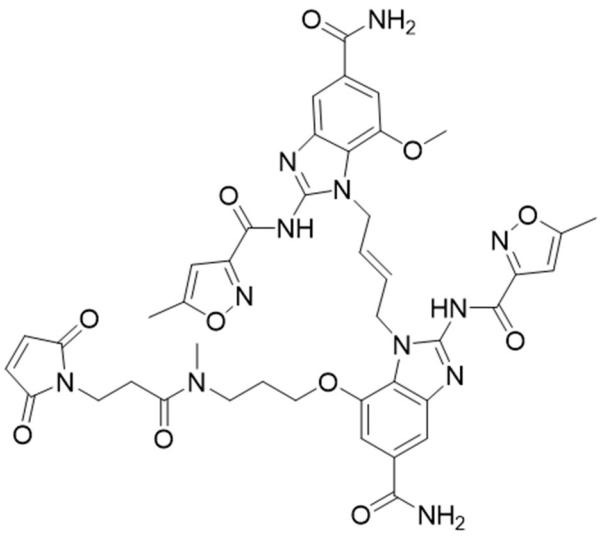
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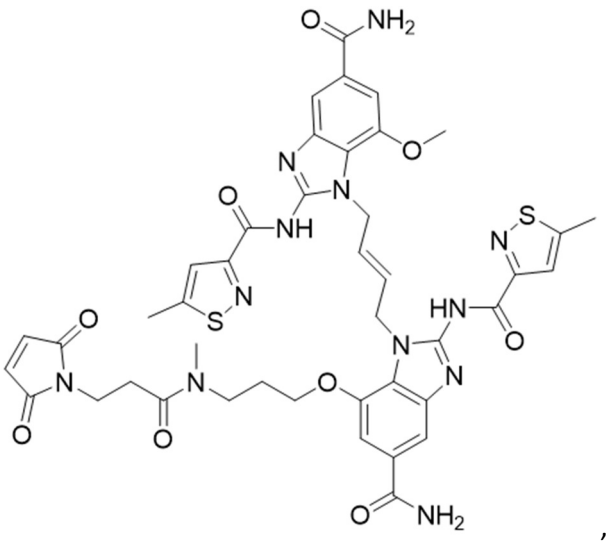
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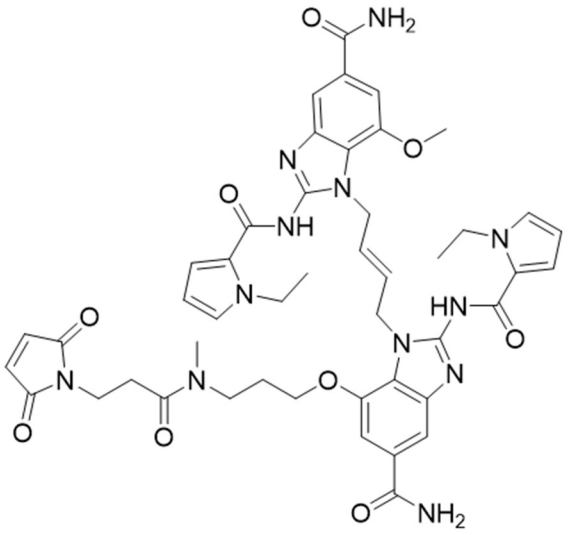
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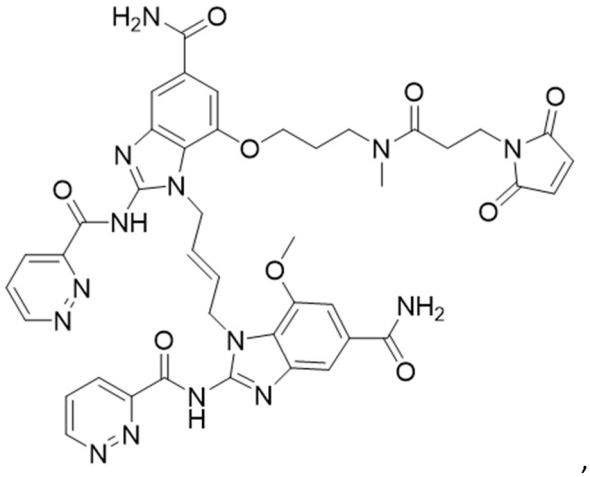
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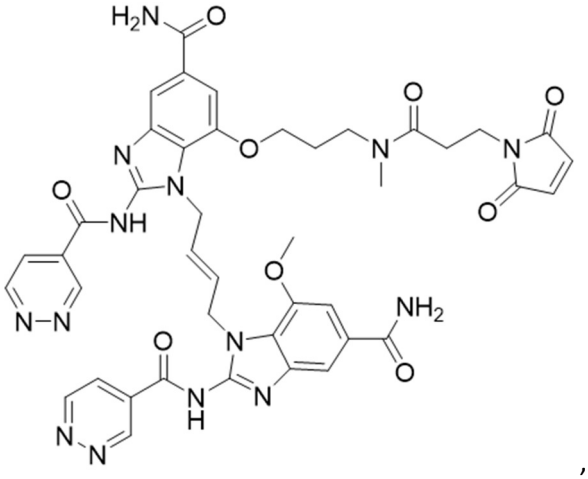
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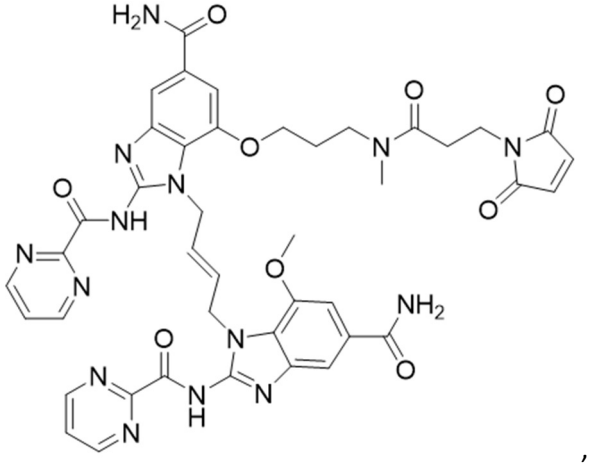
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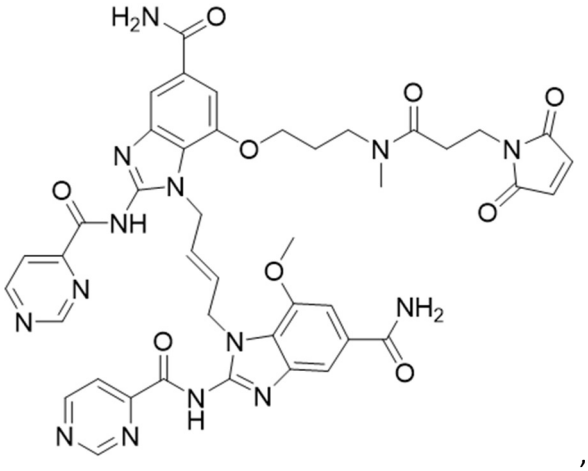
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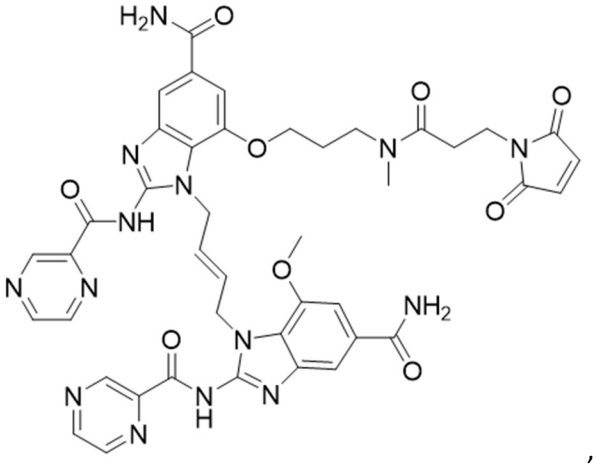
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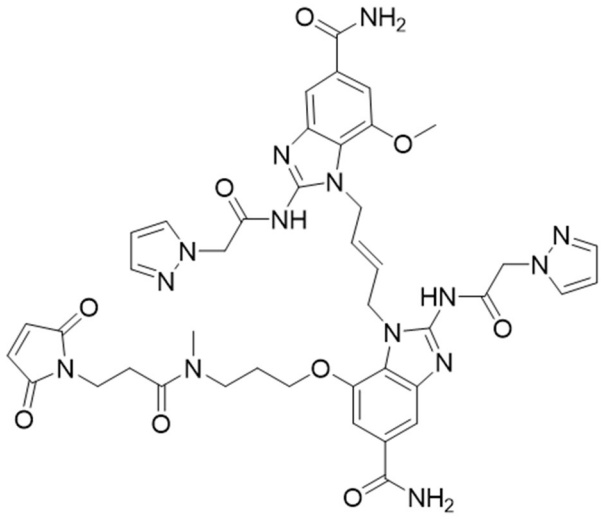
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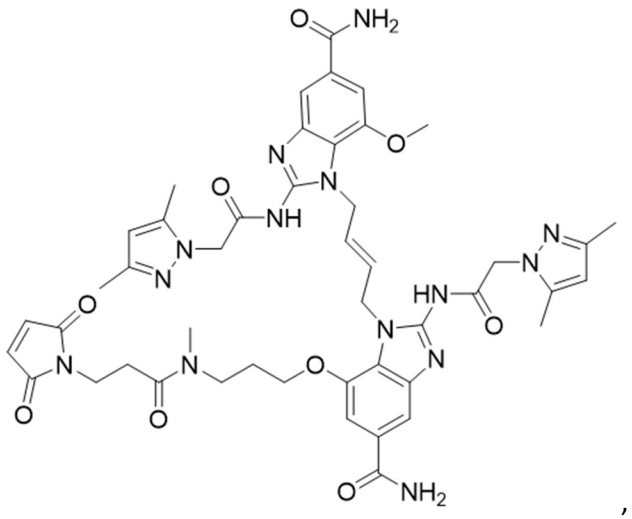
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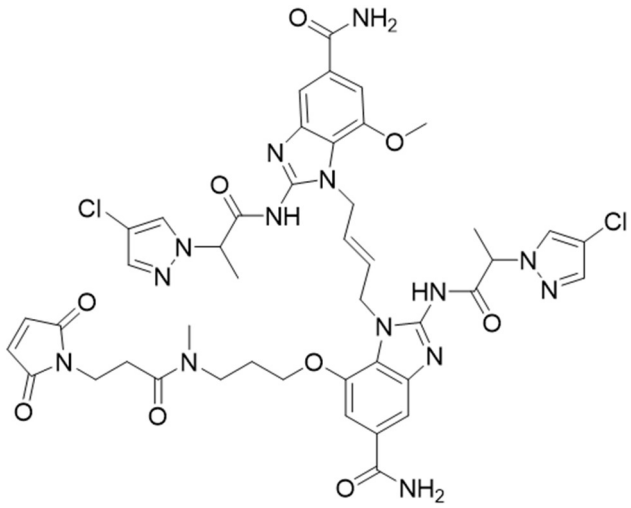
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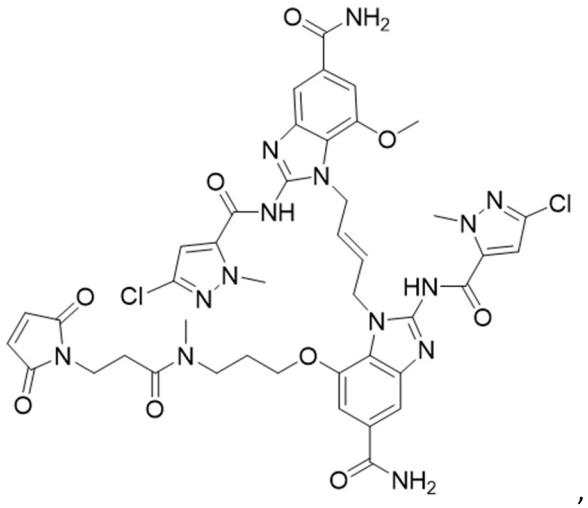
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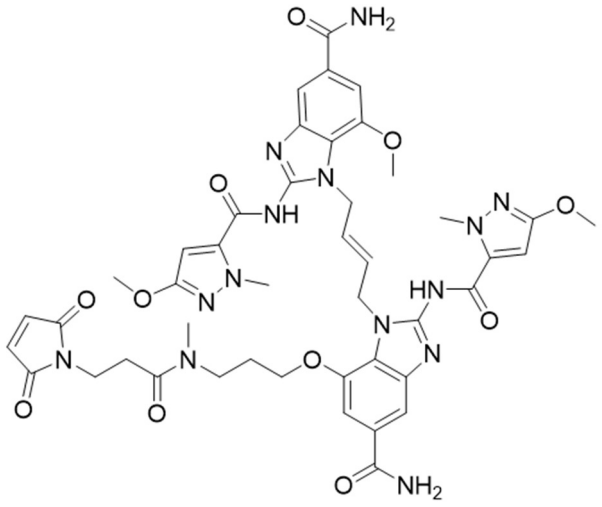
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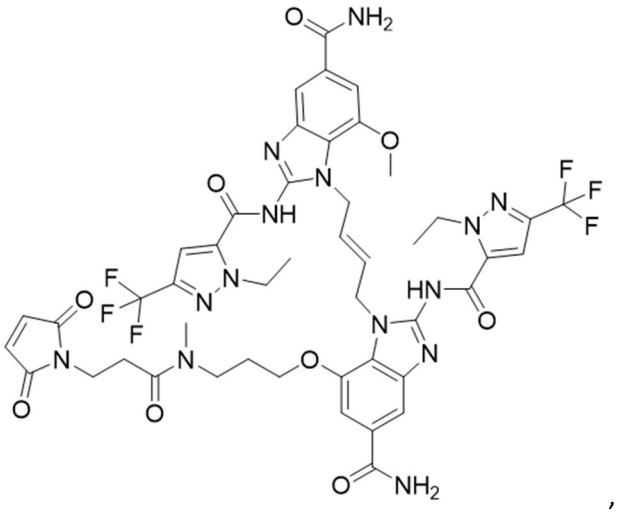
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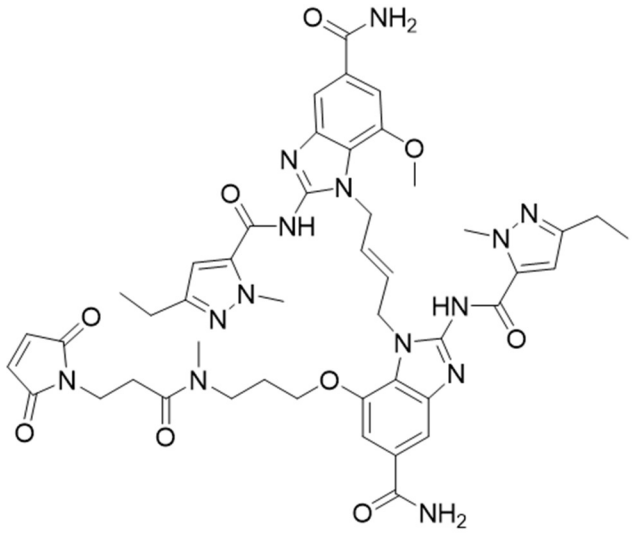
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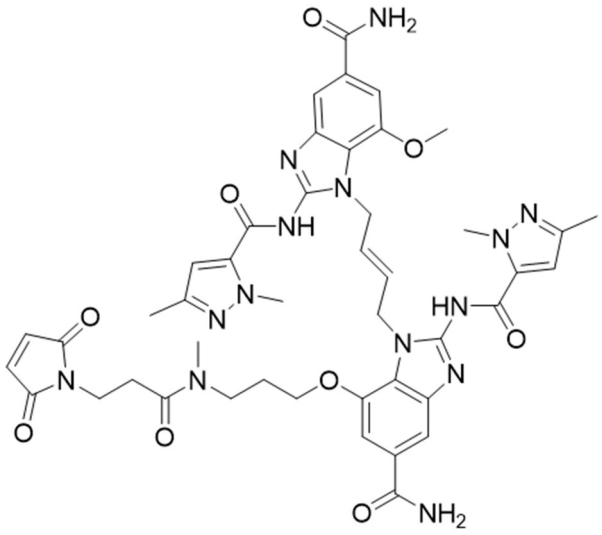
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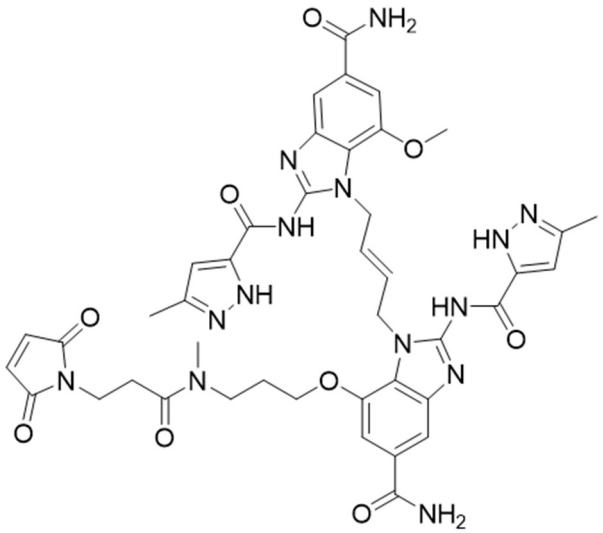
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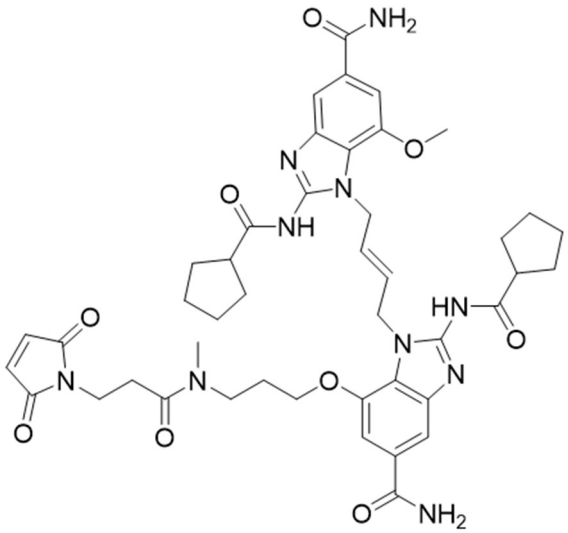
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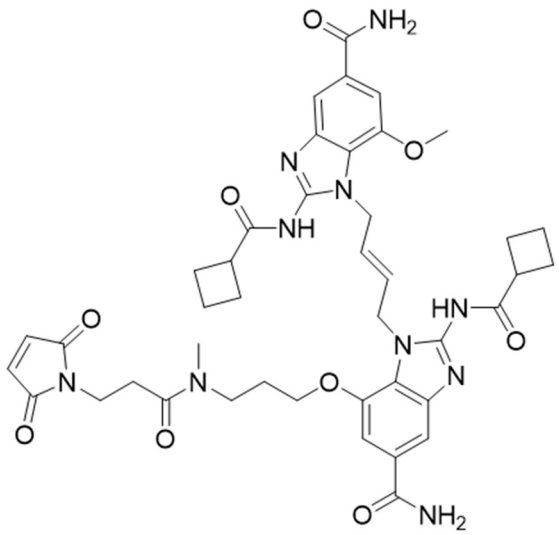
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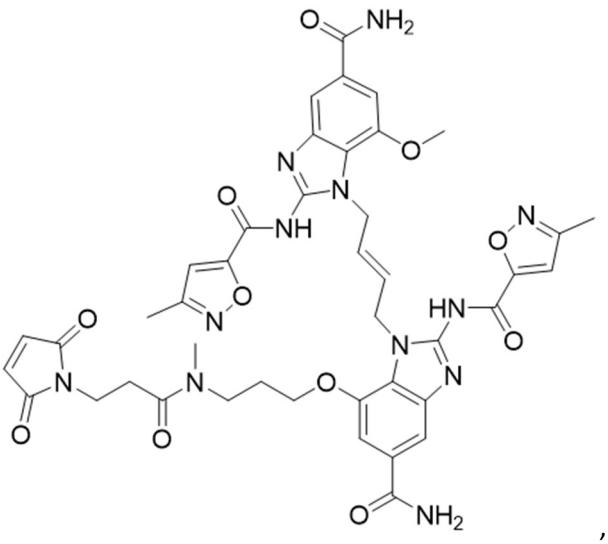
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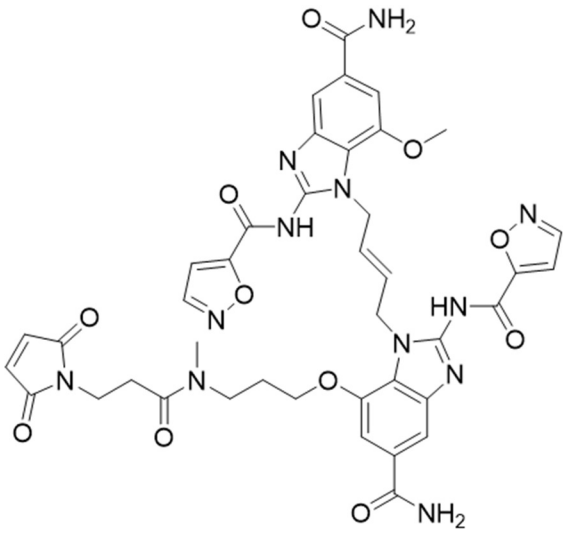
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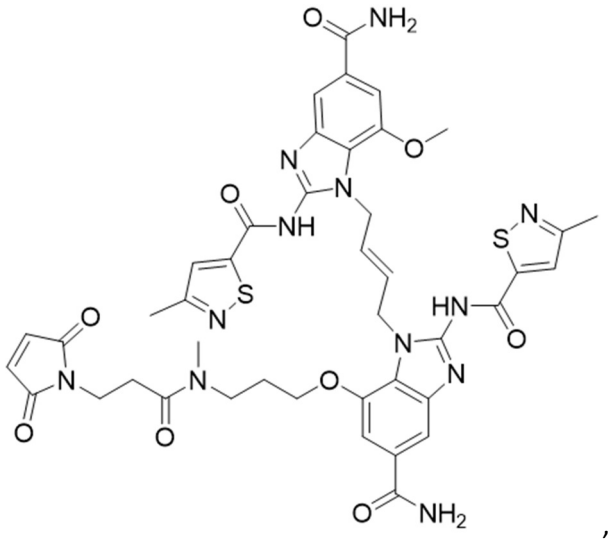
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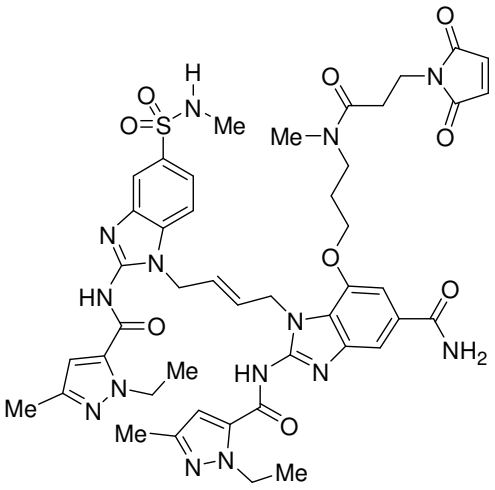
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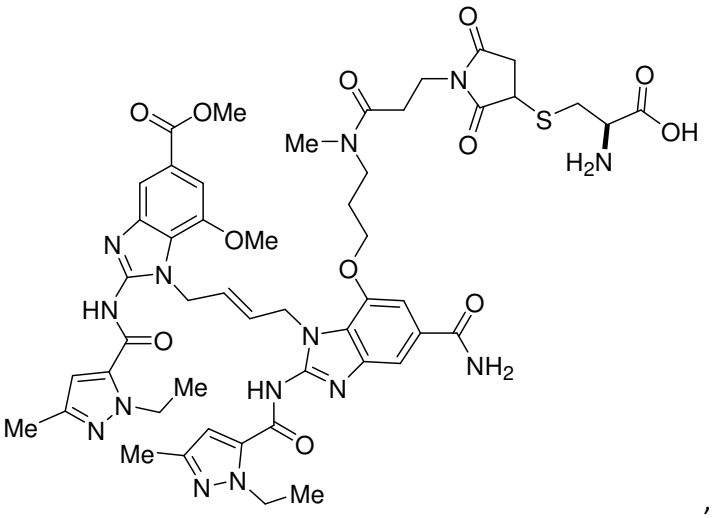
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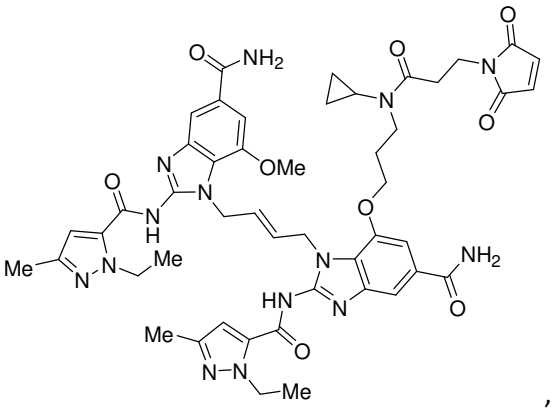
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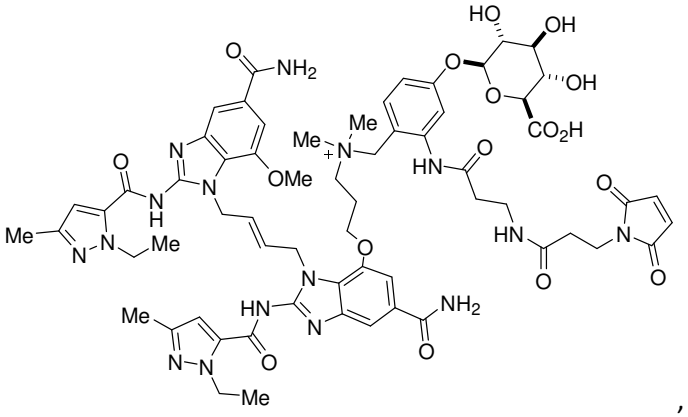
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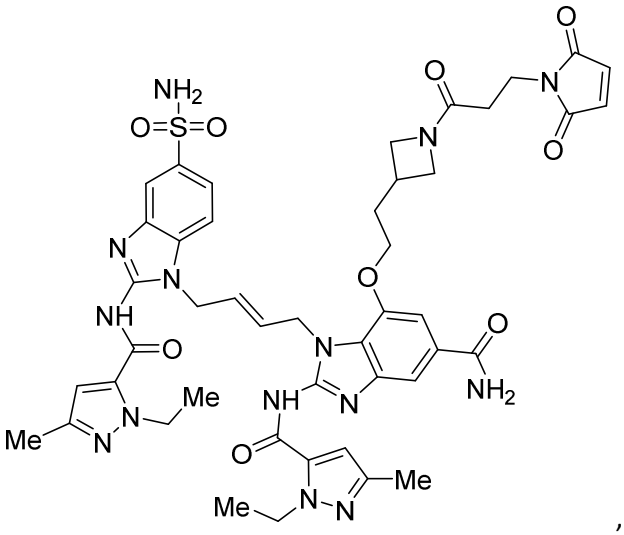
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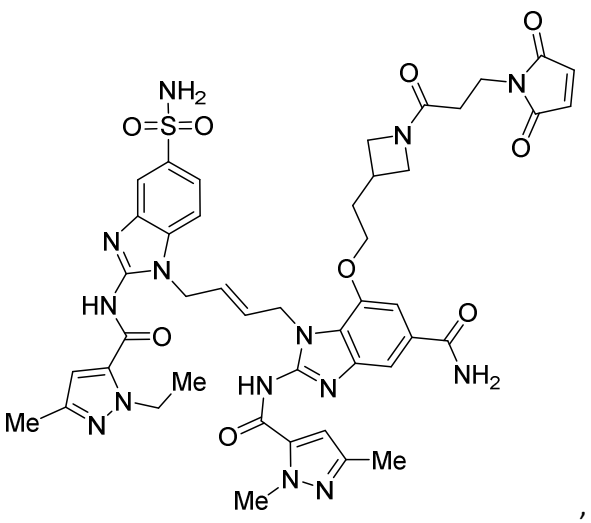
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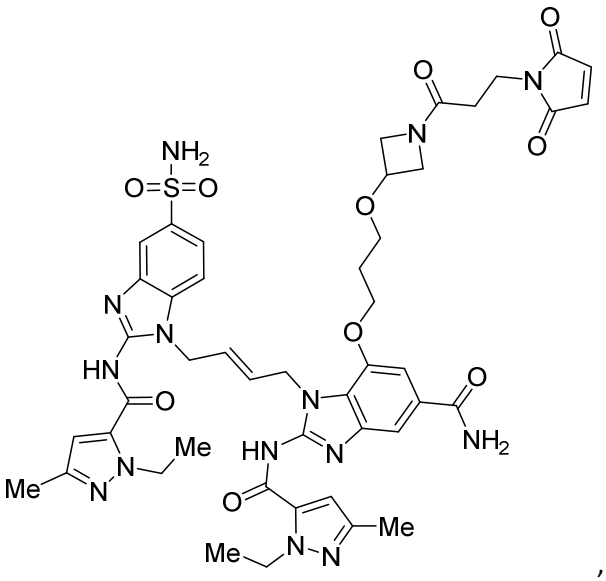
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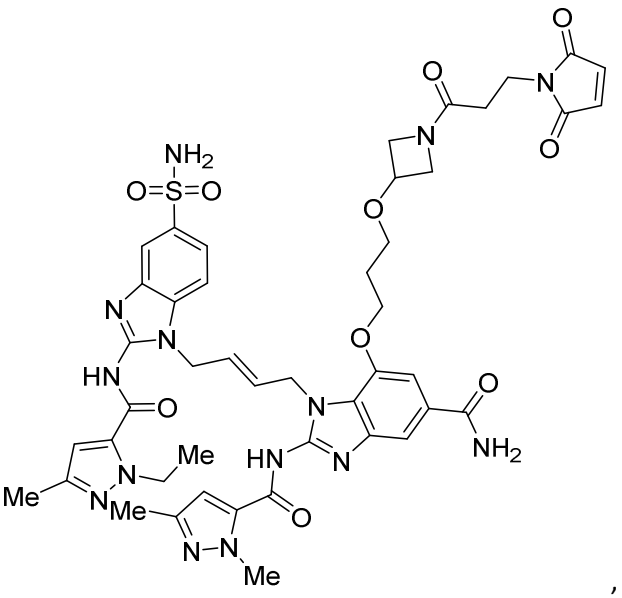
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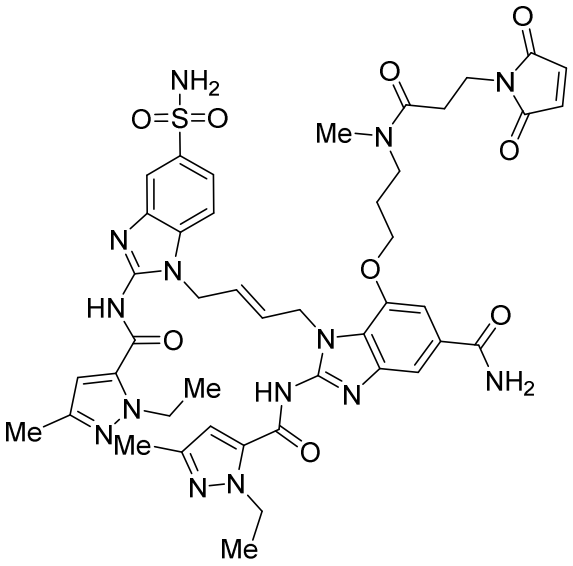

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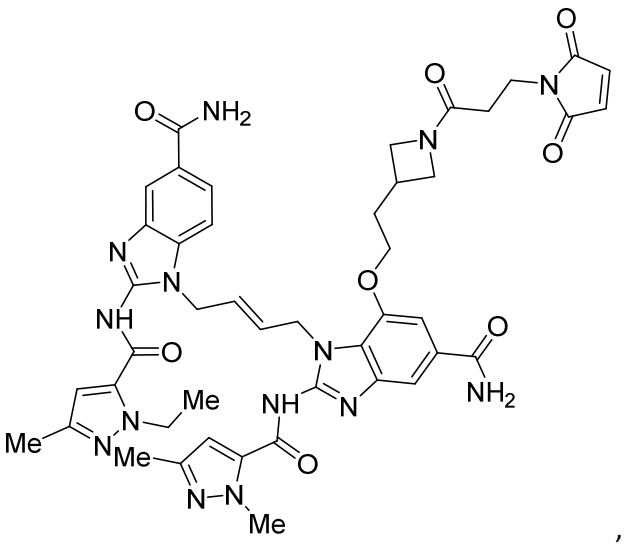

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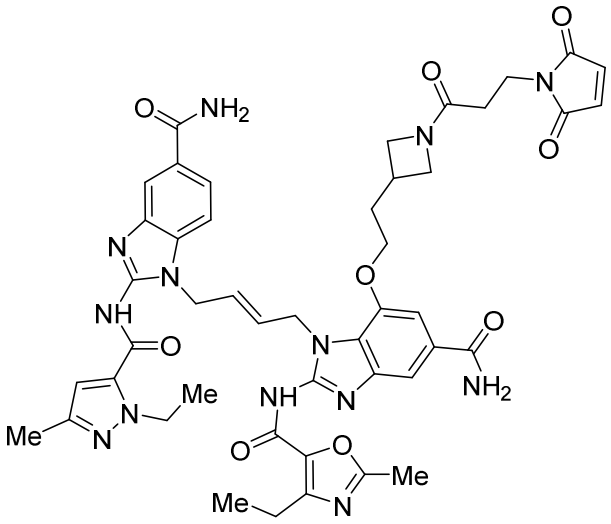

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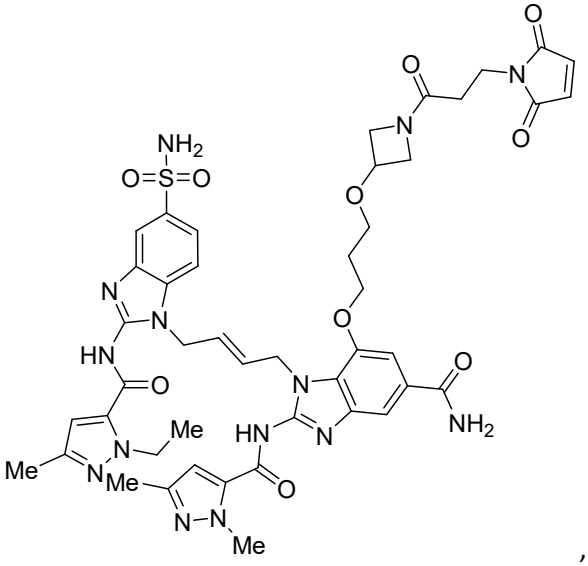

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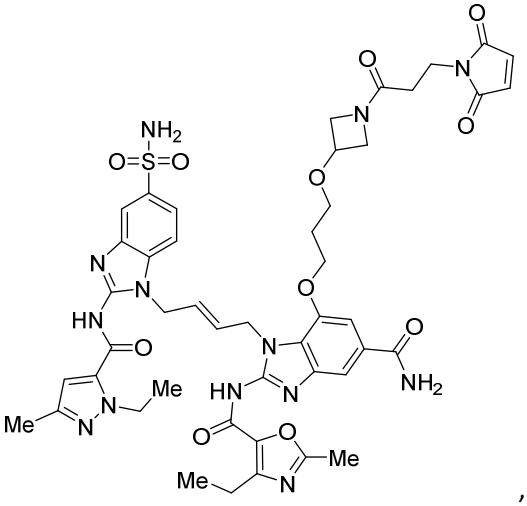

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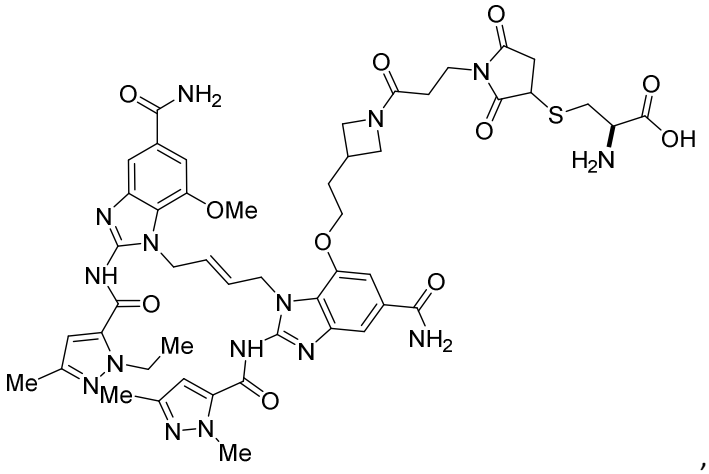

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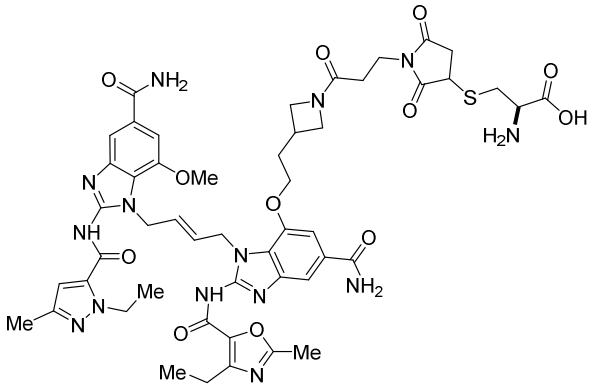
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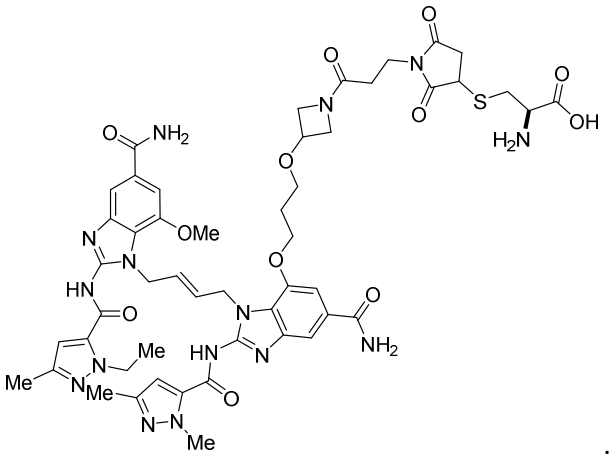
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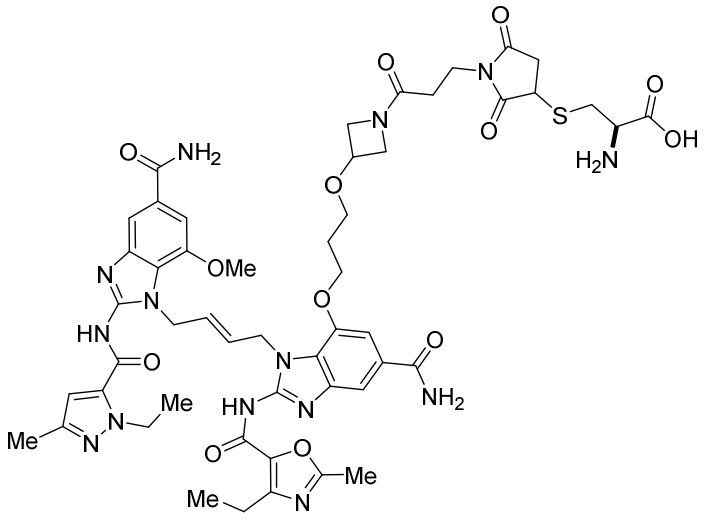
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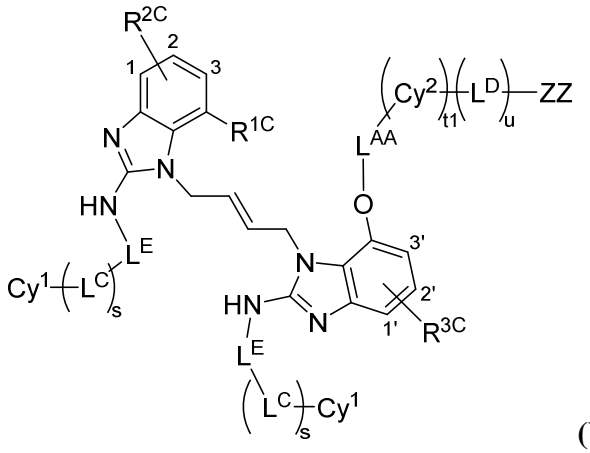
and pharmaceutically acceptable salts thereof.

Compounds of Formula (V)

[0252] Some embodiments include a compound of Formula (V):



(V)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1C} is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, $–(CH_2)_n-NR^AR^B$, or PEG2 to PEG4;

R^{2C} is $–CO_2R^M$, $–(C=O)NR^CR^D$, $–S(O)_2NR^CR^D$, $–S(O)_2R^M$, $–(CH_2)_q-NR^ER^F$, $–(CH_2)_q-OR^M$, $–O(C=O)-NR^ER^F$, or $–NR^M(C=O)-NR^ER^F$, wherein R^{2C} is attached at any one of positions labeled 1, 2, or 3;

R^{3C} is $–CO_2R^M$, $–(C=O)NR^CR^D$, $–S(O)_2NR^CR^D$, $–S(O)_2R^M$, $–(CH_2)_q-NR^ER^F$, $–(CH_2)_q-OR^M$, $–O(C=O)-NR^ER^F$, or $–NR^M(C=O)-NR^ER^F$, wherein R^{3C} is attached at any one of positions labeled 1', 2', or 3';

each R^A, R^B, R^C, R^D, R^E, R^F, and R^M are independently hydrogen or C_{1-6} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript q is independently an integer from 0 to 6;

L^E is $–(C=O)–$ or $–S(O)_2–$;

L^C is $–(CR^IR^J)_{1-3}–$

each R^I and R^J are independently hydrogen or C_{1-3} alkyl;

subscript s is 0 or 1;

each Cy^1 is independently a 4-6 membered heterocycle, a 5-6 membered heteroaryl, or a C_{3-6} cycloalkyl, each optionally substituted with one or more R^K;

each R^K is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d2}R^{e2}$, $-C(O)NR^{d2}R^{e2}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{d2} and R^{e2} are independently hydrogen or C_{1-3} alkyl;

L^{AA} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, $–C(O)NR^L(CH_2)_{1-6}–$, $–(CH_2)_{1-6}O–$, $-C(O)(CH_2)_{1-6}O–$, or $–C(O)NR^L(CH_2)_{1-6}O–$;

R^L is hydrogen or C_{1-3} alkyl;

Cy^2 is C_{3-6} cycloalkyl, 4-6 membered heterocycle, 5-6 membered heteroaryl, or phenyl, each optionally substituted with one or more R^U;

each R^U is independently selected from the group consisting of $–CO_2R^{j1}$, $–(C=O)NR^{d3}R^{e3}$, $–S(O)_2NR^{d3}R^{e3}$, $–(CH_2)_{q1}-NR^{g1}R^{h1}$, $–(CH_2)_{q1}-OR^{j1}$, and $–(CH_2)_{q1}-(OCH_2CH_2)_{1-8}OH$;

each R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or C_{1-6} alkyl;

subscript q1 is an integer from 0 to 6;

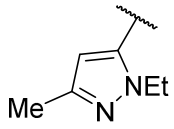
subscript t1 is 0 or 1;

L^D is $–(CH_2)_{1-6}–$;

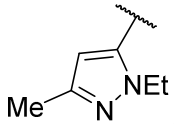
subscript u is 0 or 1;

when t1 is 0, ZZ is $–NR^QR^R$, $–N^+(C_{1-6}$ alkyl$)R^QR^R$, $–C(=O)N^SR^T$, $-C(O)O(C_{1-6}$ alkyl$)$,$–CO_2H$, or an amino acid, or when t1 is 1, ZZ is hydrogen, $–NR^QR^R$, $–N^+(C_{1-6}$ alkyl$)R^QR^R$; $–C(=O)N^SR^T$, $-C(O)O(C_{1-6}$ alkyl$)$,$–CO_2H$, or an amino acid;

R^Q is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl, provided that

if t1 is 0 and both Cy^1 are 

, then R^Q is C_{2-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl, and

if t1 is 0 and at least one Cy^1 is not 

, then ZZ is $–NR^QR^R$, $–N^+(C_{1-6}$ alkyl$)R^QR^R$, or $–C(=O)N^SR^T$, and R^Q is C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl; and

each R^R, R^S, and R^T are independently hydrogen or C_{1-6} alkyl.

[0253] In some embodiments, R^{1C} is hydrogen. In some embodiments, R^{1C} is hydroxyl. In some embodiments, R^{1C} is C_{1-6} alkoxy. In some embodiments, R^{1C} is methoxy. In some embodiments, R^{1C} is $–(C_{1-6}$ alkyl$)C_{1-6}$ alkoxy. In some embodiments, R^{1C} is methoxyethyl. In some embodiments, R^{1C} is PEG2 to PEG4. In some embodiments, R^{1C} is $–(CH_2)_n$-NR^AR^B. In some embodiments, R^A and R^B are both hydrogen. In some embodiments, R^A and R^B are independently C_{1-3} alkyl. In some embodiments, one of R^A and R^B is hydrogen and the other of R^A and R^B is C_{1-3} alkyl. In some embodiments, each subscript n is 0. In some embodiments, each subscript n is 1. In some embodiments, each subscript n is 2. In some embodiments, each subscript n is 3, 4, 5, or 6.

[0254] In some embodiments, R^{2C} and R^{3C} are–CO_2H, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F; and R^{2C} and R^{3C} are the same. In some embodiments, R^{2C} and R^{3C} are independently –CO_2H, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F; and R^{2C} and R^{3C} are different.

[0255] In some embodiments, R^{2C} is –$(C=O)_m$-NR^CR^D. In some embodiments, R^{3C} is –$(C=O)_m$-NR^CR^D. In some embodiments, R^C and R^D are both hydrogen. In some embodiments, R^C and R^D are each independently C_{1-3} alkyl. In some embodiments, one of R^C and R^D is hydrogen and the other of R^C and R^D is C_{1-3} alkyl. In some embodiments, each subscript m is 0. In some embodiments, each subscript m is 1.

[0256] In some embodiments, R^{2C} is –$(CH_2)_q$-NR^ER^F. In some embodiments, R^{3C} is –$(CH_2)_q$-NR^ER^F. In some embodiments, R^E and R^F are both hydrogen. In some embodiments, R^E and R^F are each independently C_{1-3} alkyl. In some embodiments, one of R^E and R^F is hydrogen and the other of R^E and R^F is C_{1-3} alkyl.

[0257] In some embodiments, each subscript q is 0. In some embodiments, each subscript q is an integer from 1 to 6.

[0258] In some embodiments, R^{2C} is –CO_2R^M. In some embodiments, R^{3C} is –CO_2R^M.

[0259] In some embodiments, R^M is hydrogen. In some embodiments, R^M is C_{1-3} alkyl.

[0260] In some embodiments, R^{2C} is –$(CH_2)_q$-OR^M. In some embodiments, R^{3C} is –$(CH_2)_q$-OR^M.

[0261] In some embodiments, R^M is hydrogen. In some embodiments, subscript q is 0. In some embodiments, subscript q is 1.

[0262] In some embodiments, R^{2C} is –O(C=O)-NR^ER^F. In some embodiments, R^{3C} is –O(C=O)-NR^ER^F. In some embodiments, R^E and R^F are both hydrogen. In some embodiments, R^E and R^F are each independently C_{1-3} alkyl. In some embodiments, one of R^E and R^F is hydrogen and the other of R^E and R^F is C_{1-3} alkyl.

[0263] In some embodiments, R^{2C} is –NR^M(C=O)-NR^ER^F. In some embodiments, R^{3C} is –NR^M(C=O)-NR^ER^F. In some embodiments, R^E, R^F, and R^M are all hydrogen. In some embodiments, R^E, R^F, and R^M are each independently C_{1-3} alkyl. In some embodiments, one of R^E, R^F, and R^M is C_{1-3} alkyl and the rest of R^E, R^F, and R^M is hydrogen.

[0264] In some embodiments, R^{2C} is –$S(O)_2NR^CR^D$.

[0265] In some embodiments, R^{3C} is $–S(O)_2NR^CR^D$. In some embodiments, R^C and R^D are both hydrogen. In some embodiments, R^C and R^D are each independently C_{1-3} alkyl. In some embodiments, one of R^C and R^D is hydrogen and the other of R^C and R^D is C_{1-3} alkyl.

[0266] In some embodiments, R^{2C} is $–S(O)_2R^M$. In some embodiments, R^{3C} is $–S(O)_2R^M$. In some embodiments, R^M is hydrogen. In some embodiments, R^M is C_{1-3} alkyl.

[0267] In some embodiments, R^{2C} is attached at position 1. In some embodiments, R^{2C} is attached at position 2. In some embodiments, R^{2C} is attached at position 3. In some embodiments, R^{3C} is attached at position 1'. In some embodiments, R^{3C} is attached at position 2'. In some embodiments, R^{3C} is attached at position 3'.

[0268] In some embodiments, L^E is $–(C=O)–$. In some embodiments L^E is $–S(O)_2–$.

[0269] In some embodiments, each R^I and R^J is hydrogen. In some embodiments, each R^I and R^J is C_{1-3} alkyl. In some embodiments, one of R^I and R^J is hydrogen and the other of R^I and R^J is C_{1-3} alkyl.

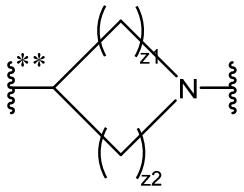
[0270] In some embodiments, L^C is $–(CR^IR^J)–$.

[0271] In some embodiments, subscript s is 0. In some embodiments, subscript s is 1.

[0272] In some embodiments, each Cy^1 is independently a 5-6 membered heteroaryl. In some embodiments, each Cy^1 is pyrazole optionally substituted with one or more R^K. In some embodiments, each Cy^1 is independently selected from the group consisting of pyrazole, imidazole, furan, thiophene, thiazole, isothiazole, oxazole, isoxazole, pyrrole, pyridazine, pyridine, pyrimidine, and pyrazine, each optionally substituted with one or more R^K. In some embodiments, each Cy^1 is independently selected from the group consisting of imidazole, furan, thiophene, thiazole, isothiazole, oxazole, isoxazole, pyrrole, pyridazine, pyridine, pyrimidine, and pyrazine, each optionally substituted with one or more R^K. In some embodiments, each Cy^1 is independently a C_{4-5} cycloalkyl optionally substituted with one or more R^K. In some embodiments, each R^K is independently selected from the group consisting of C In some embodiments, each R^K is independently selected from the group consisting of methyl, ethyl, $–CF_3$, and halogen.

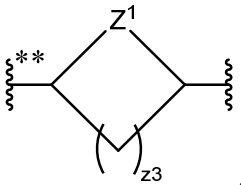
[0273] In some embodiments, each Cy^1 is the same. In some embodiments, each Cy^1 is different.

[0274] In some embodiments, L^{AA} is $–(CH_2)_{1-6}–$. In some embodiments, L^{AA} is $–(CH_2)_{1-3}–$. In some embodiments, L^{AA} is $–(CH_2)_{1-6}O–$. In some embodiments, L^{AA} is $–(CH_2)_{1-3}O-$.

[0275] In some embodiments, Cy^2 is a 4-6 membered heterocycle. In some embodiments, Cy^2 has the structure: , wherein each of subscripts z1 and z2 is independently an integer from 1 to 3 and ** indicates attachment to L^{AA}.

[0276] In some embodiments, subscript z1 and subscript z2 are 1. In some embodiments, subscript z1 and subscript z2 are 2.

[0277] In some embodiments, subscript z1 is 1 and subscript z2 is 2.

[0278] In some embodiments, Cy^2 has the structure: , wherein

Z^1 is selected from the group consisting of –O–, –S–, $–CR^NR^O–$, and $–NR^P–$;

R^N, R^O, and R^P are independently hydrogen or C_{1-6} alkyl;

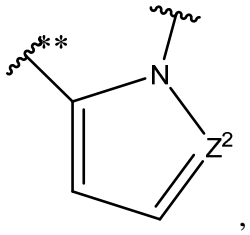
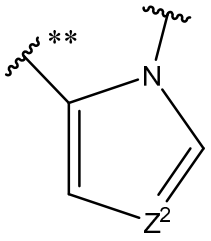
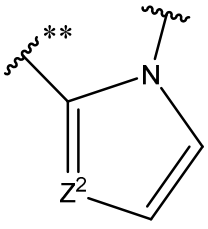
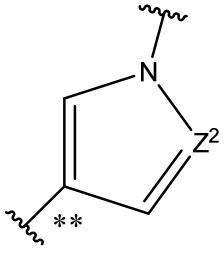
subscript z3 is an integer from 1 to 3; and

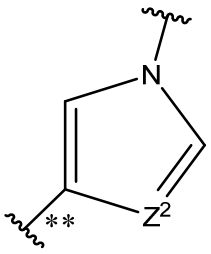
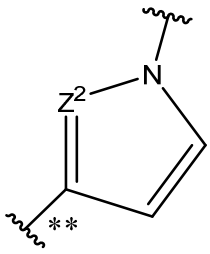
** indicates attachment to L^{AA}.

[0279] In some embodiments, R^N and R^O are hydrogen. In some embodiments, R^P is hydrogen. In some embodiments, R^P is methyl.

[0280] In some embodiments, Cy^2 is a 5-6 membered heteroaryl.

[0281] In some embodiments, Cy^2 is selected from the group consisting of:

, , , ,

, and , wherein

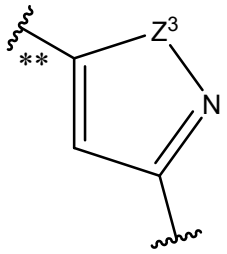
Z^2 is $=CR^N-$ or $=N-$;

R^N is hydrogen or C_{1-6} alkyl; and

** indicates attachment to L^{AA}.

[0282] In some embodiments, Z^2 is $=CR^N-$ and R^N is hydrogen. In some embodiments, Z^2 is =N-.

[0283] In some embodiments, Cy^2 is selected from the group consisting of:



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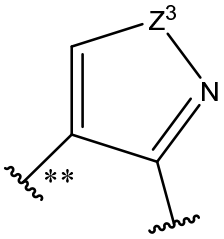

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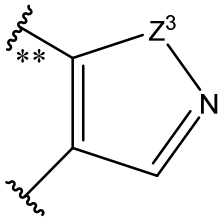
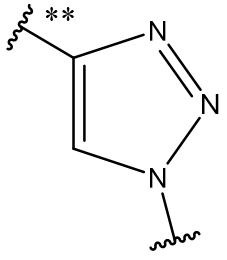
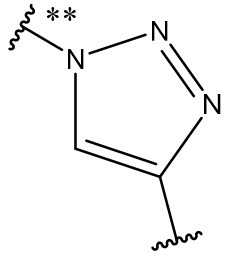


, wherein Z^3 is –O– or –S– and ** indicates attachment to L^{AA}, L^D, NR^{HH}, Y, W, or L^{BB}.

[0284] In some embodiments, ** indicates attachment to L^{AA}. In some embodiments, ** indicates attachment to L^D, NR^{HH}, Y, W, or L^{BB}.

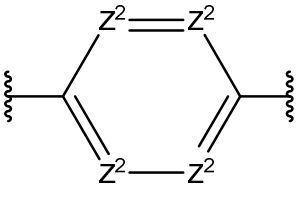
[0285] In some embodiments, Cy^2 is selected from the group consisting of:



and


, wherein ** indicates attachment to L^{AA}.

[0286] In some embodiments, Cy^2 is selected from the group consisting of:



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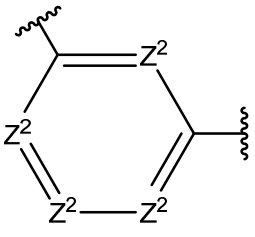

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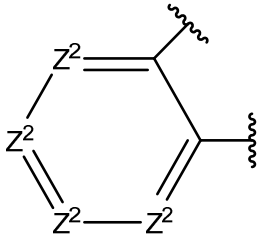


, wherein

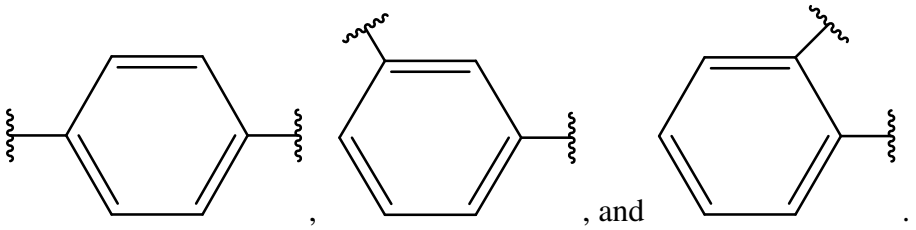
each Z^2 is independently $=CR^N-$ or $=N-$; and

each R^N is hydrogen or C_{1-6} alkyl.

[0287] In some embodiments, at least one Z^2 is =N–. In some embodiments, one Z^2 is =N– and the remaining Z^2 are =CR^N–. In some embodiments, two Z^2 are –NR^P– and the remaining Z^2 are =CR^N–.

[0288] In some embodiments, R^N is hydrogen.

[0289] In some embodiments, Cy^2 is selected from the group consisting of:



[0290] In some embodiments, Cy^2 is cyclobutyl.

[0291] In some embodiments, R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or $–CH_3$.

[0292] In some embodiments, ach R^U is independently selected from $–CO_2H$, $–(C=O)NH_2$, $–S(O)_2NH_2$, $–CH_2NH_2$, and $–CH_2OH$.

[0293] In some embodiments, t1 is 0. In some embodiments, t1 is 1.

[0294] In some embodiments, u is 1 and L^D is $–(CH_2)_{1-3}$. In some embodiments, u is 0.

[0295] In some embodiments, ZZ is –NR^QR^R. In some embodiments, R^Q is C_{1-6} alkyl, In some embodiments, R^Q is C_{3-6} cycloalkyl. In some embodiments, R^Q is cyclopropyl. In some embodiments, R^Q is $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl. In some embodiments, R^R is hydrogen.

[0296] In some embodiments, ZZ is $–N^+(C_{1-6}$ alkyl$)R^QR^R$.

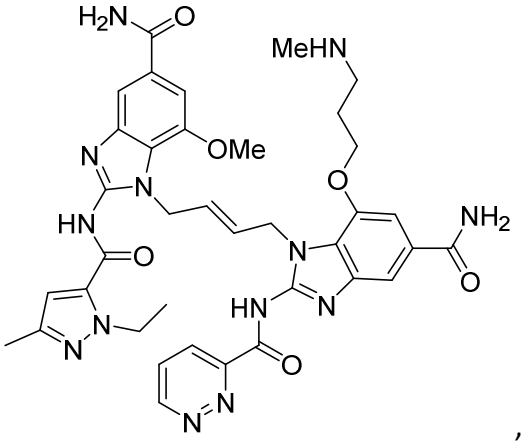
[0297] In some embodiments, ZZ is –$C(=O)N^SR^T$.

[0298] In some embodiments, ZZ is -C(O)O(t-butyl).

[0299] In some embodiments, ZZ is $–CO_2H$.

[0300] In some embodiments, ZZ is an amino acid selected from the group consisting of alanine, valine, isoleucine, leucine, aspartic acid, glutamic acid, lysine, histidine, arginine, glycine, serine, threonine, phenylalanine, O-methylserine, O-methylaspartic acid, O-methylglutamic acid, N-methyllysine, O-methyltyrosine, O-methylhistidine, and O-methylthreonine.

[0301] Some embodiments of Formula (V) include compounds selected from the group consisting of: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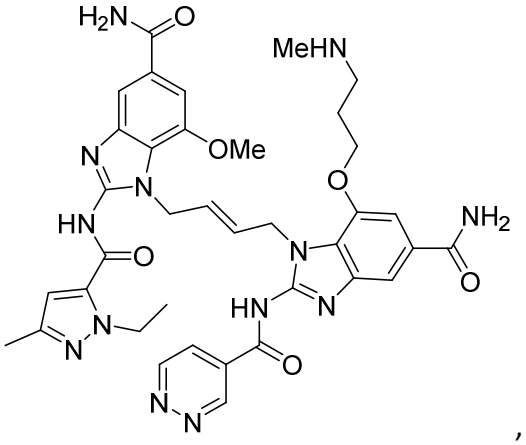
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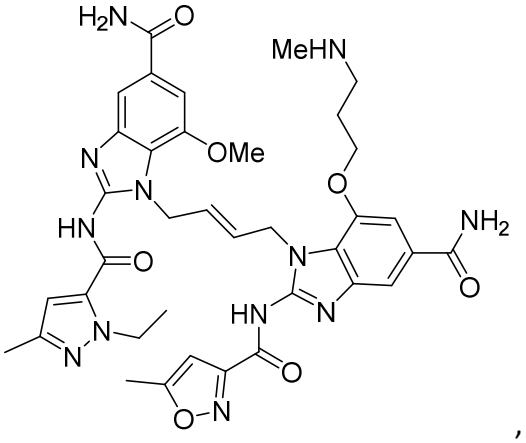
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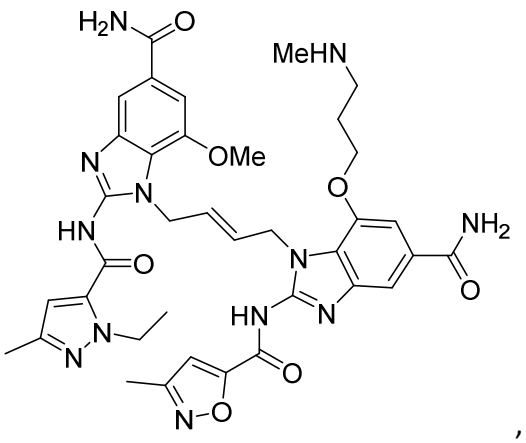
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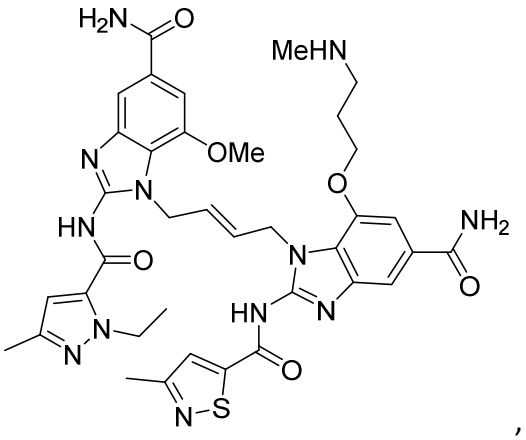
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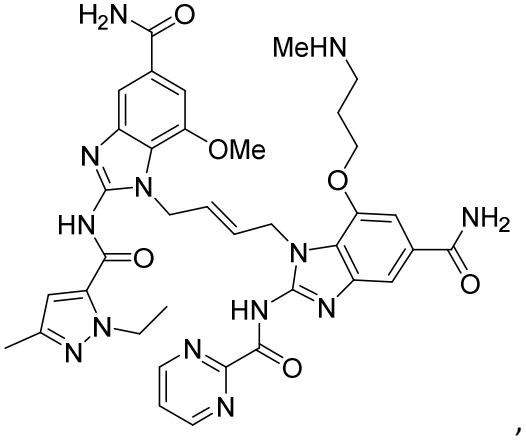
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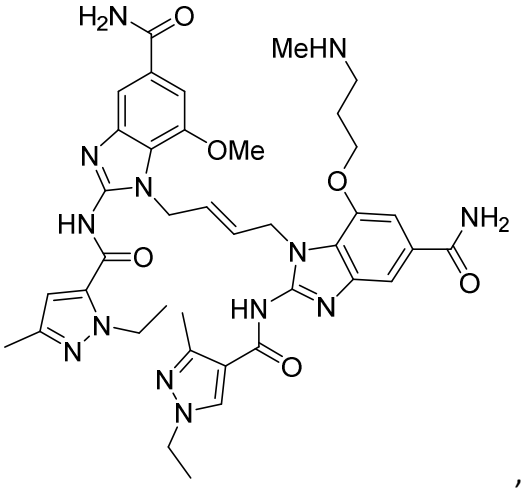
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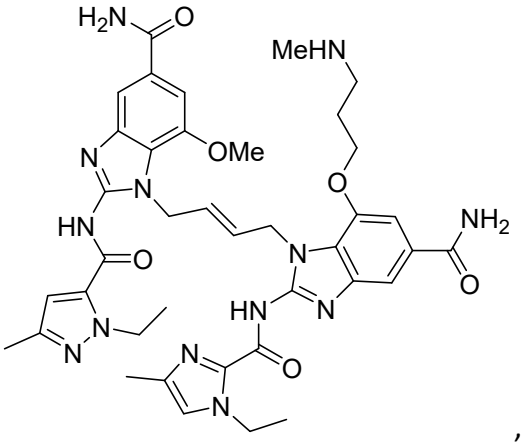
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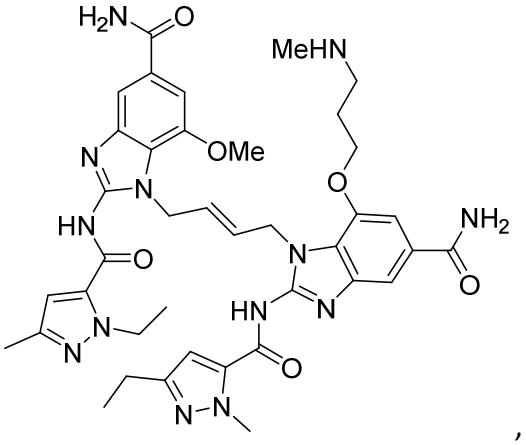
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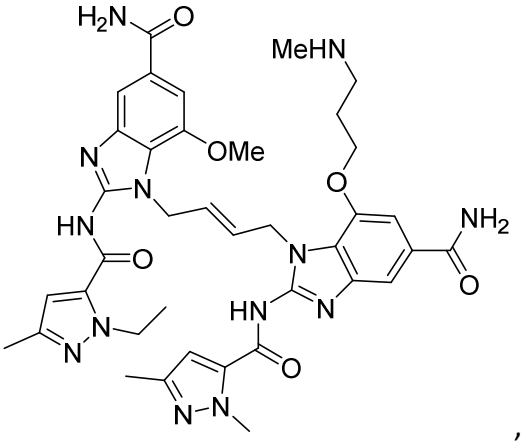
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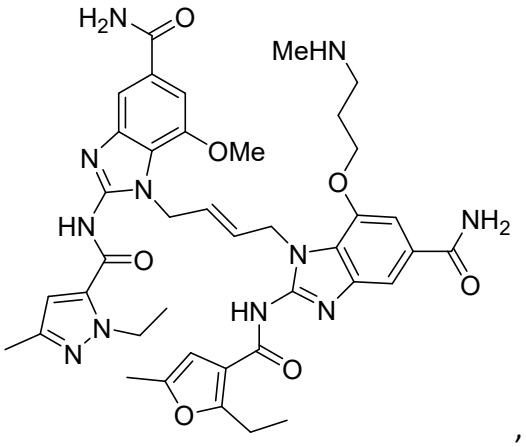
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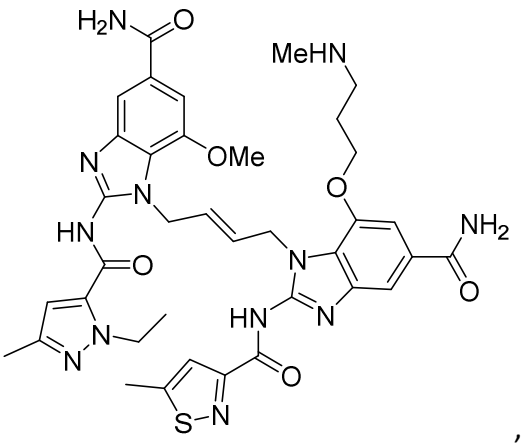
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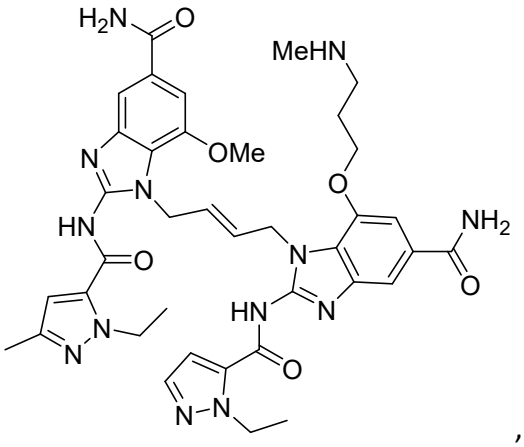
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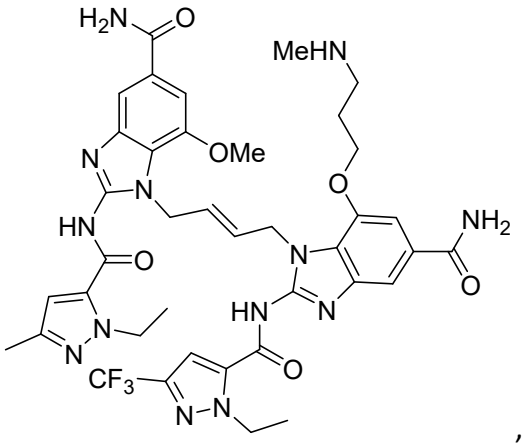
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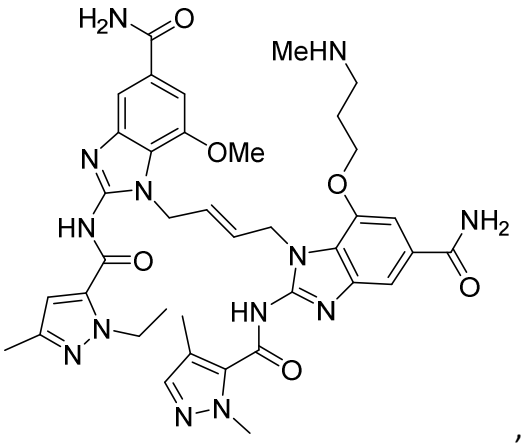
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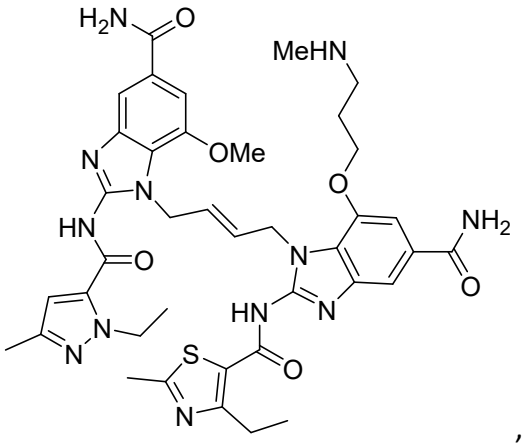
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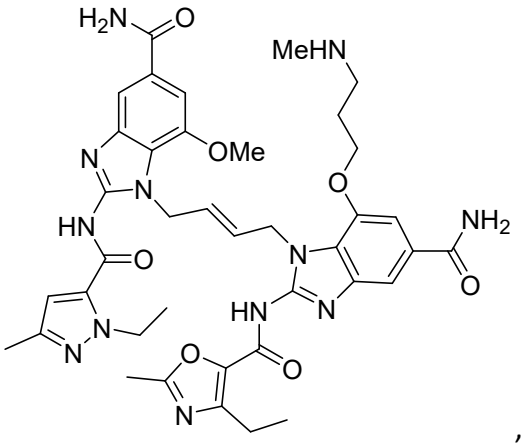
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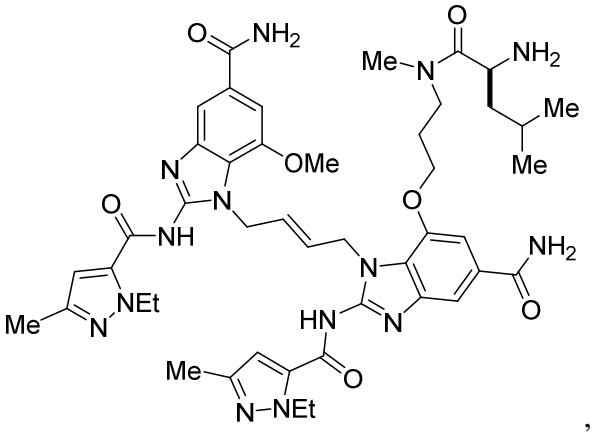
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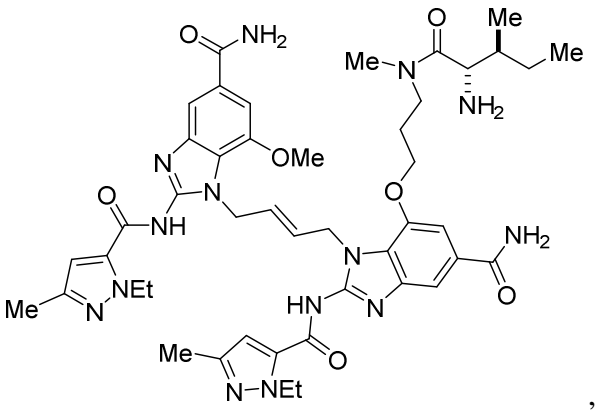
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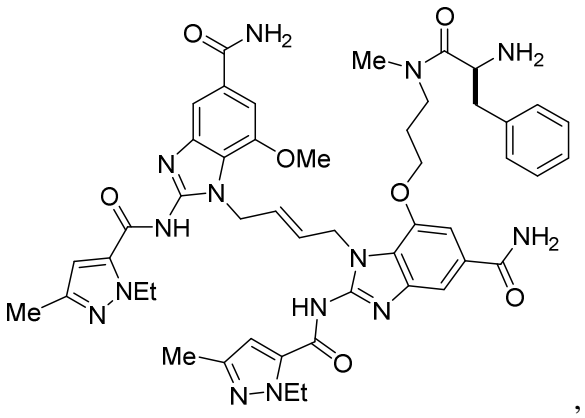
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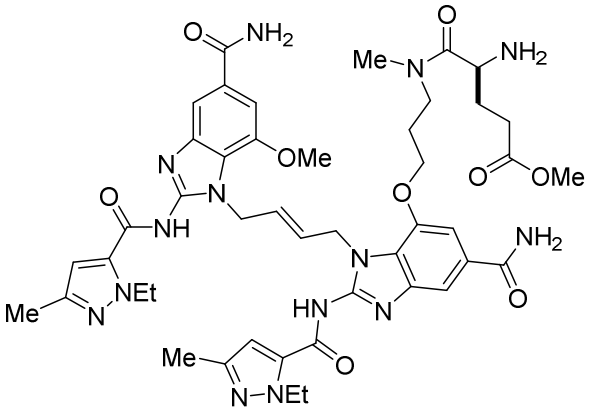
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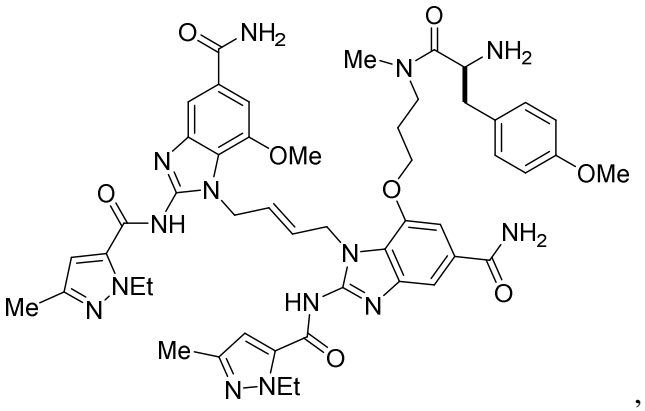
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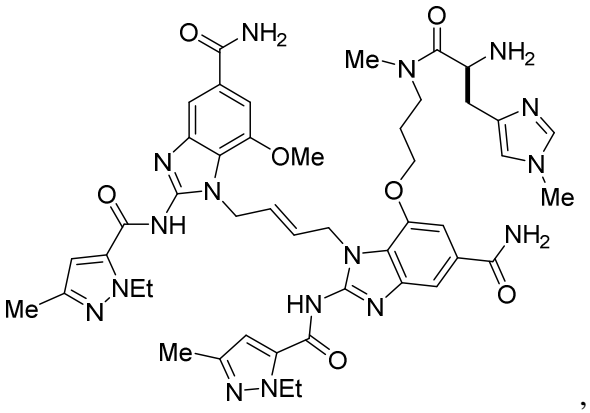
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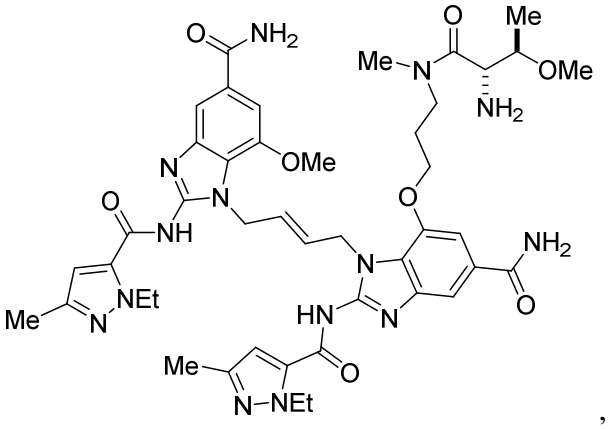
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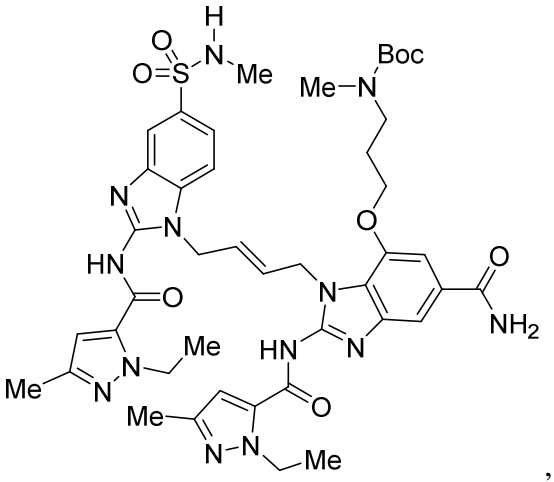
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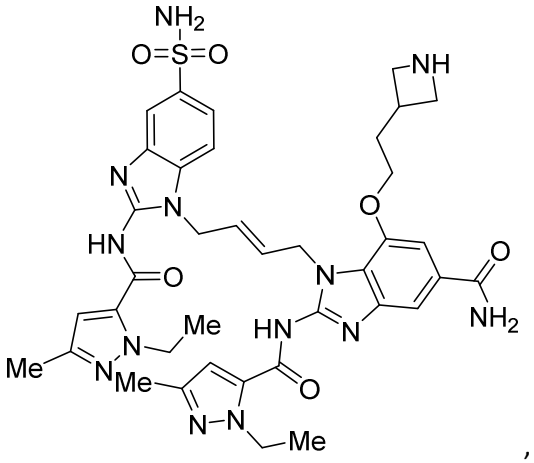
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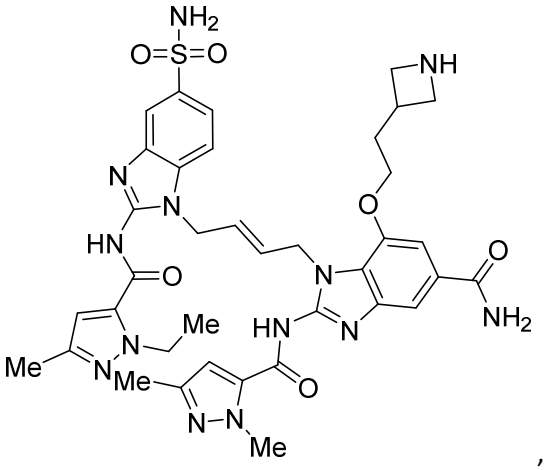
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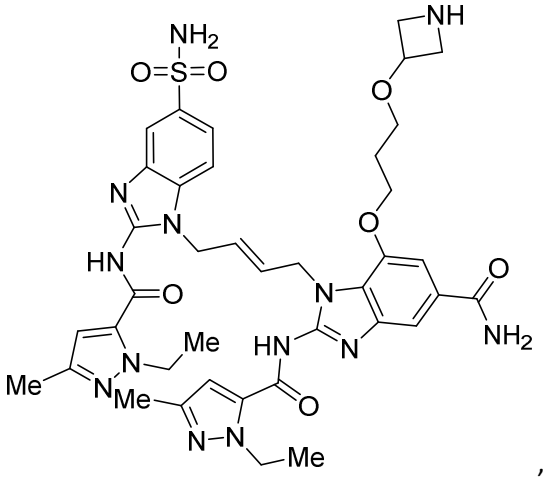
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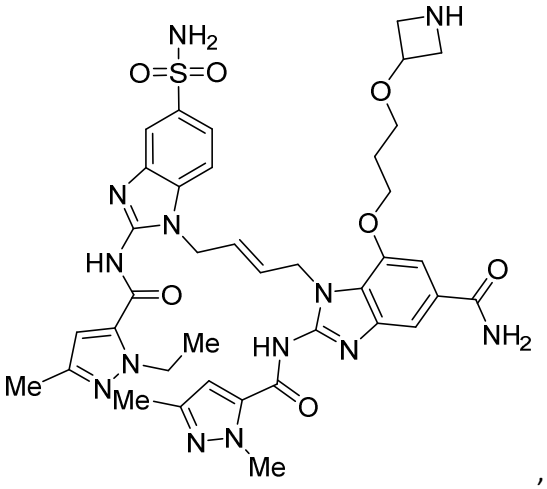
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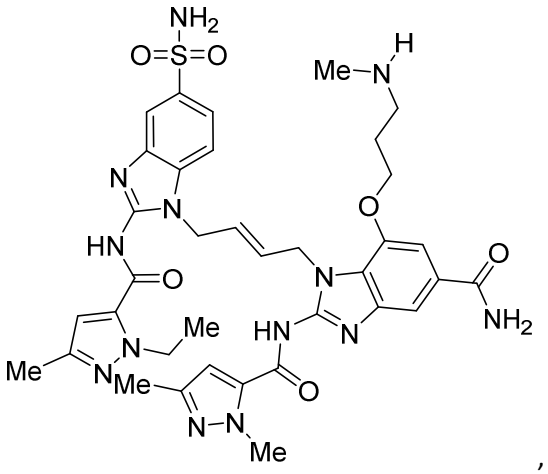
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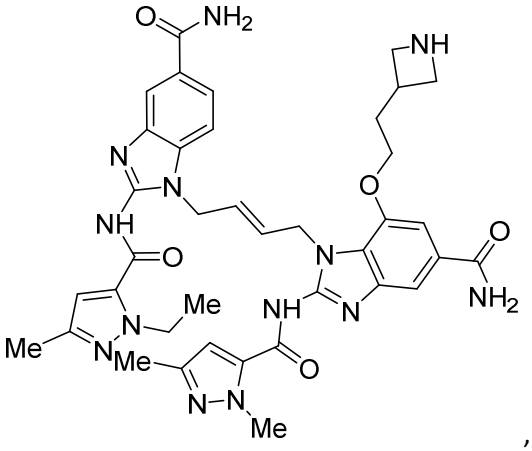
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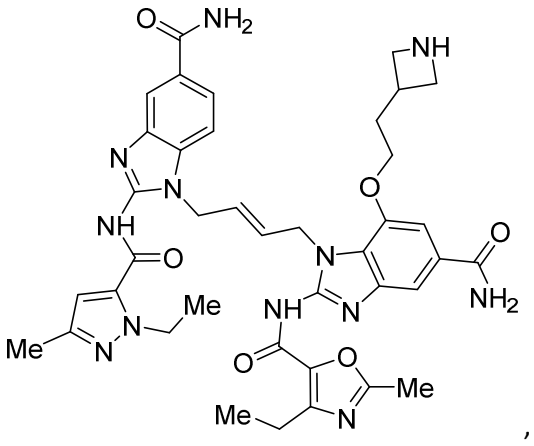
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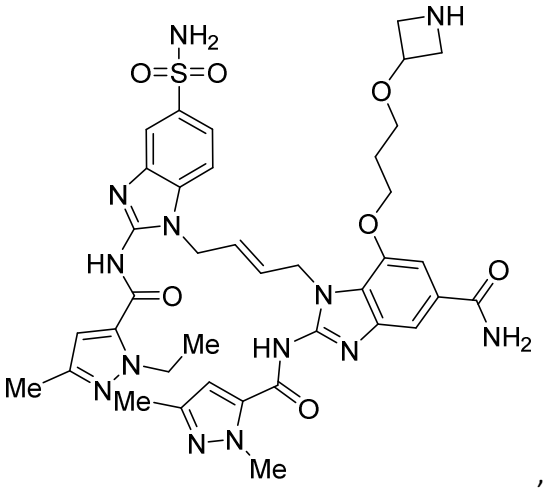
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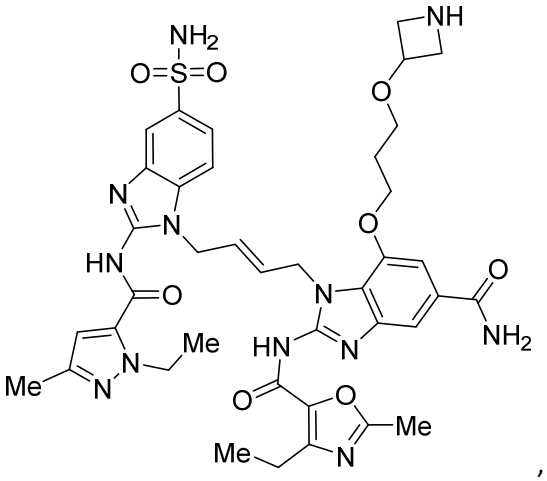

, and pharmaceutically acceptable salts thereof.

Linkers

[0302] As described herein, linkers (L) as defined in connection with Formulae (I), (II), and (II-A) are optional groups that connect X^A or X^B, when present, with M or M^1. For example, A, when present, is covalently attached to M or M^1, and Y, when present, is attached to X^B or to X^A (when X^B is absent). In some embodiments, the linker (L) has the formula $–(A)_a-(W)_w-(Y)_y$, wherein:

A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};

each R^{a1} is independently selected from the group consisting of:

C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

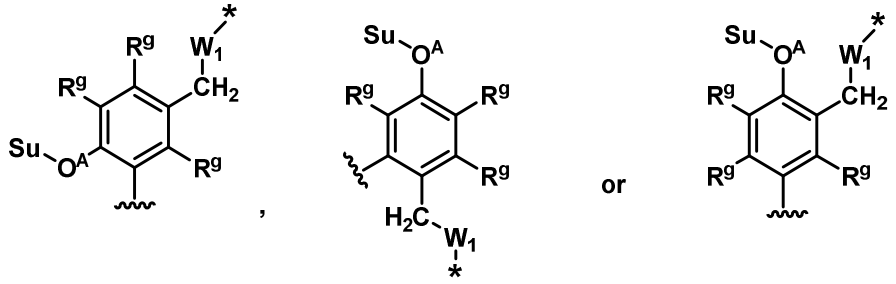
each R^{b1} is independently selected from the group consisting of:

C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, -$NR^{d1}R^{e1}$, -$C(O)NR^{d1}R^{e1}$, -$C(O)(C_{1-6}$ alkyl), and -$C(O)O(C_{1-6}$ alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

a is 0 or 1;

W is from 1-12 amino acids or has the structure:



wherein Su is a Sugar moiety;

-O^A- represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or -NO_2;

W_1 is absent or –O-C(=O)–;

~~~ represents covalent attachment to A, when present, or M in compounds of Formula (II) and covalent attachment to A, M, or M^1 in the ADCs and compounds described herein;

* represents covalent attachment to Y, X^A, or X^B in compounds of Formula (II) and to Y, X^A, or X^B in the ADCs described herein;

w is 0 or 1;

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety; and

y is 0 or 1.

[0303] In some embodiments, -O^A- represents a glycosidic bond. In some embodiments, the glycosidic bond provides a β-glucuronidase or a β-mannosidase-cleavage site. In some embodiments, the β-glucuronidase-cleavage site is cleavable by human lysosomal β-glucuronidase. In some embodiments, the β-mannosidase-cleavage site is cleavable by human lysosomal β-mannosidase.
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[0304] In some embodiments, a is 0. In some embodiments, a is 1. In some embodiments, w is 0. In some embodiments, w is 1. In some embodiments, y is 0. In some embodiments, y is 1. In some embodiments, a + y + w = 1. In some embodiments, a + y + w = 2. In some embodiments, a + y + w = 3. In some embodiments, a + y + w = 0 (i.e., the linker (L) is absent).

[0305] In some embodiments, A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}. In some embodiments, A is a C_{2-10} alkylene optionally substituted with 1-3 R^{a1}. In some embodiments, A is a C_{4-10} alkylene optionally substituted with 1-3 R^{a1}. In some embodiments, A is a C_{2-20} alkylene substituted with R^{a1}. In some embodiments, A is a C_{2-10} alkylene substituted with R^{a1}. In some embodiments, A is a C_{2-10} alkylene substituted with R^{a1}.

[0306] In some embodiments, each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl). In some embodiments, each R^{a1} is C_{1-6} alkyl. In some embodiments, each R^{a1} is C_{1-6} haloalkyl. In some embodiments, each R^{a1} is C_{1-6} alkoxy. In some embodiments, each R^{a1} is C_{1-6} haloalkoxy. In some embodiments, each R^{a1} is halogen. In some embodiments, each R^{a1} is –OH. In some embodiments, each R^{a1} is =O. In some embodiments, each R^{a1} is $-NR^{d1}R^{e1}$. In some embodiments, each R^{a1} is $C(O)NR^{d1}R^{e1}$. In some embodiments, each R^{a1} is $-C(O)(C_{1-6}$ alkyl). In some embodiments, each R^{a1} is $-C(O)O(C_{1-6}$ alkyl). In some embodiments, one occurrence of R^{a1} is $–NR^{d1}R^{e1}$. In some embodiments, A is a C_{2-20} alkylene substituted with 1 or 2 R^{a1}, each of which is =O.

[0307] In some embodiments, R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl. In some embodiments, one of R^{d1} and R^{e1} is hydrogen, and the other of R^{d1} and R^{e1} is C_{1-3} alkyl. In some embodiments, R^{d1} and R^{e1} are both hydrogen or C_{1-3} alkyl. In some embodiments, R^{d1} and R^{e1} are both C_{1-3} alkyl. In some embodiments, R^{d1} and R^{e1} are both methyl.

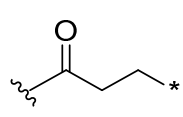
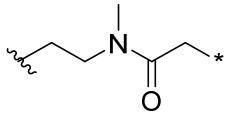
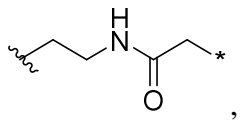
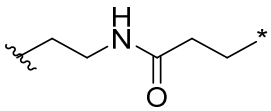
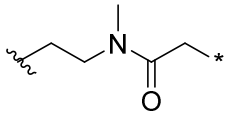
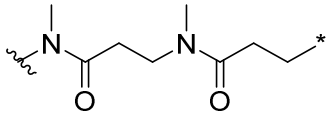
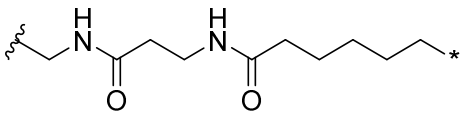
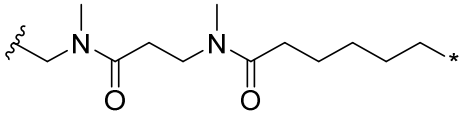
[0308] In some embodiments, A is a C_{2-20} alkylene. In some embodiments, A is a C_{2-10} alkylene. In some embodiments, A is a C_{2-10} alkylene. In some embodiments, A is a C_{2-6} alkylene. In some embodiments, A is a C_{4-10} alkylene.

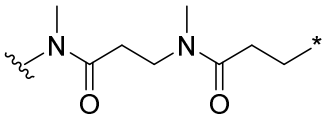
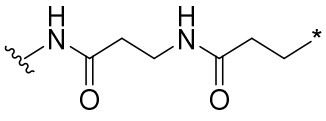
[0309] In some embodiments, A is a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1}. In some embodiments, A is a 2 to 20 membered heteroalkylene optionally substituted with 1-3 R^{b1}. In some embodiments, A is a 2 to 12 membered heteroalkylene optionally substituted with 1-3 R^{b1}. In some embodiments, A is a 4 to 12 membered heteroalkylene optionally

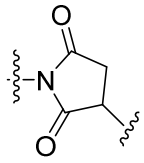
substituted with 1-3 R^{b1}. In some embodiments, A is a 4 to 8 membered heteroalkylene optionally substituted with 1-3 R^{b1}. In some embodiments, A is a 2 to 40 membered heteroalkylene substituted with R^{b1}. In some embodiments, A is a 2 to 20 membered heteroalkylene substituted with R^{b1}. In some embodiments, A is a 2 to 12 membered heteroalkylene substituted with R^{b1}. In some embodiments, A is a 4 to 12 membered heteroalkylene substituted with R^{b1}. In some embodiments, A is a 4 to 8 membered heteroalkylene substituted with R^{b1}.

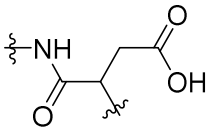
[0310] In some embodiments, each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl). In some embodiments, each R^{b1} is C_{1-6} alkyl. In some embodiments, each R^{b1} is C_{1-6} haloalkyl. In some embodiments, each R^{b1} is C_{1-6} alkoxy. In some embodiments, each R^{b1} is C_{1-6} haloalkoxy. In some embodiments, each R^{b1} is halogen. In some embodiments, each R^{b1} is –OH. In some embodiments, each R^{b1} is $-NR^{d1}R^{e1}$. In some embodiments, each R^{b1} is $C(O)NR^{d1}R^{e1}$. In some embodiments, each R^{b1} is $-C(O)(C_{1-6}$ alkyl). In some embodiments, each R^{b1} is $-C(O)O(C_{1-6}$ alkyl). In some embodiments, one occurrence of R^{b1} is $–NR^{d1}R^{e1}$.

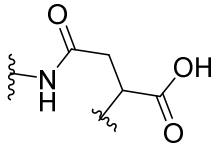
[0311] In some embodiments, R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl. In some embodiments, one of R^{d1} and R^{e1} is hydrogen, and the other of R^{d1} and R^{e1} is C_{1-3} alkyl. In some embodiments, R^{d1} and R^{e1} are both hydrogen or C_{1-3} alkyl. In some embodiments, R^{d1} and R^{e1} are both C_{1-3} alkyl. In some embodiments, R^{d1} and R^{e1} are both methyl.

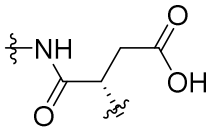
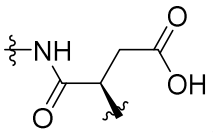
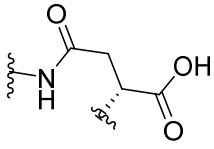
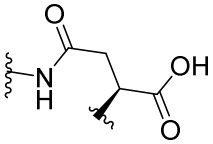
[0312] In some embodiments, A is a 2 to 40 membered heteroalkylene. In some embodiments, A is a 2 to 20 membered heteroalkylene. In some embodiments, A is a 2 to 12 membered heteroalkylene. In some embodiments, A is a 4 to 12 membered heteroalkylene. In some embodiments, A is a 4 to 8 membered heteroalkylene. In some embodiments, A is selected from the group consisting of: , , , , , , , ,

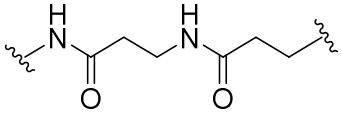
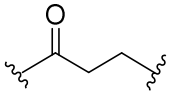
 and , wherein  represents covalent attachment to W or Y, and * represents covalent linkage to M^1 or M (e.g., in compounds of Formula (I) or (II), respectively). In some embodiments, M is a succinimide. In some embodiments, M is a hydrolyzed succinimide. In some embodiments, M^1 is a succinimide. In some embodiments, M^1 is a hydrolyzed succinimide. It will be understood that a hydrolyzed succinimide may exist in two regioisomeric form(s). Those forms are exemplified below for hydrolysis of M, wherein the structures representing the regioisomers from that hydrolysis are formula M' and M''; wherein the wavy lines adjacent to the bonds are as defined for A.


M


M'


M''

[0313] In some embodiments, M' is . In some embodiments, M' is . In some embodiments, M'' is . In some embodiments, M'' is .

[0314] In some embodiments, A is a PEG4 to PEG12. In some embodiments, A is a PEG4 to PEG8. Representative A groups include, but are not limited to:  and 

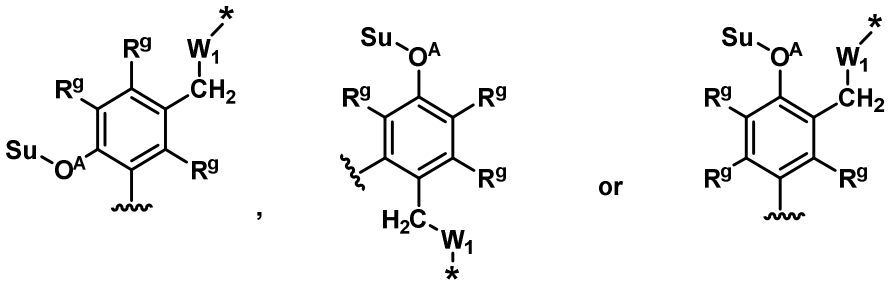
[0315] In some embodiments, w is 0. In some embodiments w is 1.

[0316] In some embodiments, W is a single amino acid. In some embodiments, W is a single natural amino acid. In some embodiments, W is a peptide including from 2-12 amino

acids, wherein each amino acid is independently a natural or unnatural amino acid. In some embodiments, the natural or unnatural amino acid is a D or L isomer. In some embodiments, each amino acid is independently a natural amino acid. In some embodiments, each W is independently an alpha, beta, or gamma amino acid that is natural or unnatural. In some embodiments, W comprises a natural amino acid linked to an unnatural amino acid. In some embodiments, W comprises a natural or unnatural amino acid linked to a D-isomer of a natural or unnatural amino acid. In some embodiments, W is a dipeptide. In some embodiments, W is a tripeptide. In some embodiments, W is a tetrapeptide. In some embodiments, W is a pentapeptide. In some embodiments, W is a hexapeptide. In some embodiments, W is 7, 8, 9, 10, 11, or 12 amino acids. In some embodiments, each amino acid of W is independently selected from the group consisting of valine, alanine, β-alanine, glycine, lysine, leucine, phenylalanine, proline, aspartic acid, serine, glutamic acid, homoserine methyl ether, aspartate methyl ester, N,N-dimethyl lysine, arginine, valine-alanine, valine-citrulline, phenylalanine-lysine, and citrulline. In some embodiments, W is an aspartic acid. In some embodiments, W is a lysine. In some embodiments, W is a glycine. In some embodiments, W is an alanine. In some embodiments, W is aspartate methyl ester. In some embodiments, W is a N,N-dimethyl lysine. In some embodiments, W is a homoserine methyl ether. In some embodiments, W is a serine. In some embodiments, W is a valine-alanine.

[0317] In some embodiments, w is 1; W is from 1-12 amino acids; and the bond between W and the X^B or between W and Y is enzymatically cleavable by a tumor-associated protease. In some embodiments, the tumor-associated protease is a cathepsin. In some embodiments, the tumor-associated protease is cathepsin B, C, or D.

[0318] In some embodiments, w is 1; and W has the structure of:



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

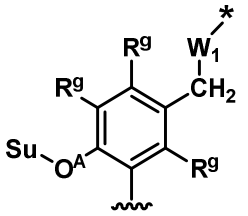
each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

W_1 is absent or –O-C(=O)–;

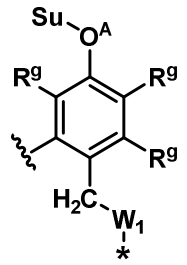
~~~ represents covalent attachment to A or M in compounds of Formula (II); and

the * represents covalent attachment to Y, X^A, or X^B in compounds of Formula (II);

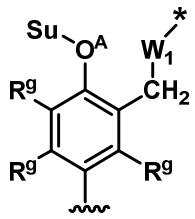
[0319] In some embodiments, w is 1; and W has the structure of:



,



or



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

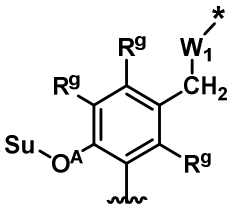
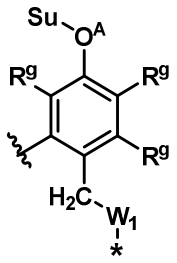
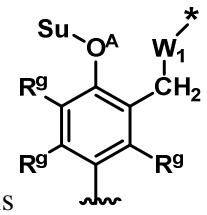
each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

W_1 is absent or –O-C(=O)–;

~~~ represents covalent attachment to A or M in the ADCs described herein; and

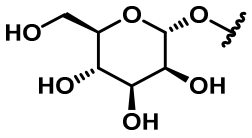
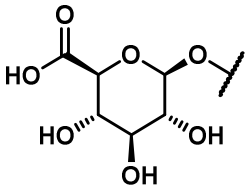
the * represents covalent attachment to Y, X^A, or X^B in the ADCs described herein;

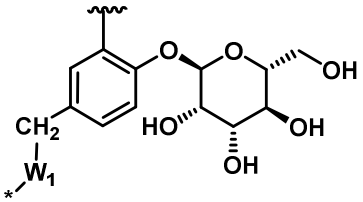
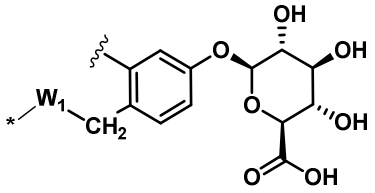
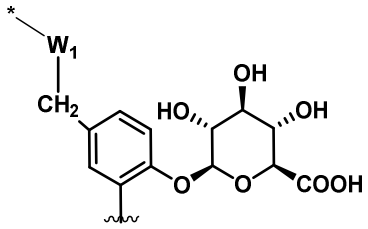
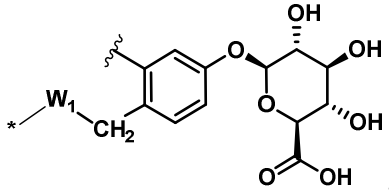
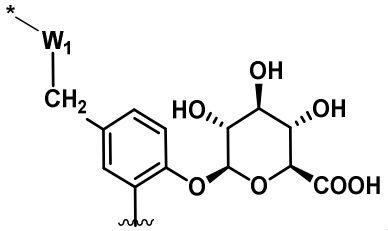
[0320] In some embodiments, $-O^A-$ represents a glycosidic bond. In some embodiments, the glycosidic bond provides a β-glucuronidase or a β-mannosidase-cleavage site. In some embodiments, the β-glucuronidase or a β-mannosidase-cleavage site is cleavable by human lysosomal β-glucuronidase or by human lysosomal β-mannosidase.

[0321] In some embodiments, W is . In some embodiments, W is . In some embodiments, W is .

[0322] In some embodiments, each R^g is hydrogen. In some embodiments, one R^g is hydrogen, and the remaining R^g are independently halo, -CN, or $-NO_2$. In some embodiments, two R^g are hydrogen, and the remaining R^g is halo, -CN, or $-NO_2$.

[0323] In some embodiments, one R^g is halogen, -CN, or $-NO_2$, and the other R^g are hydrogen. In some embodiments, each R^g is hydrogen.

[0324] In some embodiments, O^A-Su is charged neutral at physiological pH. In some embodiments, O^A-Su is mannose. In some embodiments, O^A-Su is . In some embodiments, O^A-Su comprises a carboxylate moiety. In some embodiments, O^A-Su is glucuronic acid. In some embodiments, O^A-Su is .

[0325] In some embodiments, W is . In some embodiments, W is  or . In some embodiments, W is . In some embodiments, W is .

[0326] In some embodiments, a is 0.

[0327] In some embodiments, y is 0. In some embodiments y is 1.

[0328] In some embodiments, Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety. In some embodiments, Y is a self-immolative moiety or a non-self-immolative releasable moiety. In some embodiments, Y is a self-immolative moiety. In some embodiments, Y is a non-self-immolative moiety.

[0329] A non-self-immolative moiety is one which requires enzymatic cleavage, and in which part or all of the group remains bound to the Drug Unit after cleavage from the ADC, thereby forming free drug. Examples of a non-self-immolative moiety include, but are not limited to: -glycine-; and -glycine-glycine. When an ADC having Y is -glycine- or -glycine-glycine- undergoes enzymatic cleavage (for example, via a cancer-cell-associated protease or a lymphocyte-associated protease), the Drug Unit is cleaved from the ADC such that the free drug includes the glycine or glycine-glycine group from Y. In some embodiments, an independent hydrolysis reaction takes place within, or in proximity to, the target cell, further cleaving the

glycine or glycine-glycine group from the free drug. In some embodiments, enzymatic cleavage of the non-self-immolative moiety, as described herein, does not result in any further hydrolysis step(s).

[0330] A self-immolative moiety refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a normally stable tripartite molecule. The self-immolative group will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved. For example, a self-immolative moiety includes a p-aminobenzyl alcohol (PAB) optionally substituted with one or more alkyl, alkoxy, halogen, cyano, or nitro groups. Other examples of self-immolative moieties include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (*see, e.g.,* Hay et al., 1999*, Bioorg. Med. Chem. Lett.* 9:2237), ortho or para-aminobenzylacetals, substituted and unsubstituted 4-aminobutyric acid amides (*see, e.g.,* Rodrigues et al., 1995*, Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (*see, e.g.,* Storm et al., 1972*, J. Amer. Chem. Soc.* 94:5815), 2-aminophenylpropionic acid amides (see, e.g., Amsberry et al., 1990*, J. Org. Chem.* 55:5867), and elimination of amine-containing drugs that are substituted at the α-position of glycine (*see, e.g.,* Kingsbury et al., 1984*, J. Med. Chem.* 27:1447).

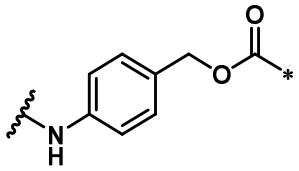
[0331] In some embodiments, Y is a PAB group, optionally substituted with one or more alkyl, alkoxy, halogen, cyano, or nitro groups; a para-aminobenzyloxy-carbonyl (PABC) group optionally substituted with a sugar moiety; -glycine-; -glycine-glycine-; or a branched bis(hydroxymethyl)styrene (BHMS) unit, which is capable of incorporating (and releasing) multiple Drug Units.

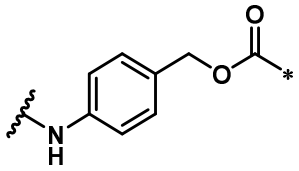
[0332] In some embodiments, $–(A)_a-(W)_w-(Y)_y$ comprises a non-self-immolative releasable linker, which provides release of the free drug once the ADC has been internalized into the target cell. In some embodiments, $–(A)_a-(W)_w-(Y)_y$ comprises a releasable linker, which provides release of the free Drug with, or in the vicinity, of targeted cells. Releasable linkers possess a recognition site, such as a peptide cleavage site, sugar cleavage site, or disulfide cleavage side. In some embodiments, each releasable linker is a di-peptide. In some embodiments, each releasable linker is a disulfide. In some embodiments, each releasable linker is a hydrazone. In some embodiments, each releasable linker is independently Val-Cit-, -Phe-Lys-, or -Val-Ala-. In some embodiments, each releasable linker, when bound to a succinimide or hydrolyzed

succinimide, is independently succinimido-caproyl (mc), succinimido-caproyl-valine-citrulline (sc-vc), succinimido-caproyl-valine-citrulline-paraaminobenzyloxycarbonyl (sc-vc-PABC), or SDPr-vc (where "S" refers to succinimido).

[0333] In some embodiments, $-(A)_a-(W)_w-(Y)_y$ comprises a non-cleavable linker. Non-cleavable linkers are known in the art and can be adapted for use with the ADCs described herein as the "Y" group. A non-cleavable linker is capable of linking a Drug Unit to an antibody in a generally stable and covalent manner and is substantially resistant to acid-induced cleavage, light-induced cleavage, peptidase- or esterase-induced cleavage, and disulfide bond cleavage. The free drug can be released from the ADCs containing non-cleavable linkers via alternative mechanisms, such as proteolytic antibody degradation. In some embodiments, the Drug Unit can exert a biological effect as a part of the ADC (i.e., while still conjugated to the antibody via a linker).

[0334] Reagents that form non-cleavable linker-maleimide and non-cleavable linker-succinimide compounds are known in the art and can adapted for use herein. Exemplary reagents comprise a maleimido or haloacetyl-based moiety, such as 6-maleimidocaproic acid N-hydroxy succinimide ester (MCC), N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ-maleimidobutyric acid N-succinimidyl ester (GMBS), c-maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(α-maleimidoacetoxy)-succinimide ester [AMAS], succinimidyl-6-(β-maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)-butyrate (SMPB), and N-(p-maleimidophenyl)isocyanate (PMPI), N-succinimidyl-4-(iodoacetyl)-aminobenzoate (STAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA) and N-succinimidyl 3-(bromoacetamido)propionate (SBAP). Additional "A-M" and "A-M^1" groups for use in the ADCs described herein can be found, for example, in U.S. Pat. No. 8,142,784, incorporated herein by reference in its entirety.

[0335] In some embodiments, y is 1; and Y is , wherein ~~~ represents connection to W, A, or M in compounds of Formula (II); and the * represents connection to X^A or X^B, in compounds of Formula (II).

[0336] In some embodiments, y is 1; and Y is , wherein ~~~ represents connection to W, A, M or M^1 in the ADCs described herein; and the * represents connection to X^A or X^B, in the ADCs described herein.

[0337] In some embodiments, $-(A)_a-(W)_w-(Y)_y-$ comprises a non-releasable linker, wherein the Drug is released after the ADC has been internalized into the target cell and degraded, liberating the Drug.

[0338] In some embodiments, the linker (L) is substituted with a polyethylene glycol moiety selected from the group consisting of PEG2 to PEG20. In some embodiments, L is substituted with a polyethylene glycol moiety selected from the group consisting of PEG2, PEG4, PEG6, PEG8, PEG10, PEG12, PEG16, and PEG20. In some embodiments, L is not substituted with a polyethylene glycol moiety selected from the group consisting of PEG2 to PEG20.

[0339] Polydisperse PEGs, monodisperse PEGs and discrete PEGs can be used to make the ADCs and intermediates thereof. Polydisperse PEGs are a heterogeneous mixture of sizes and molecular weights whereas monodisperse PEGs are typically purified from heterogeneous mixtures and therefore provide a single chain length and molecular weight. Discrete PEGs are synthesized in step-wise fashion and not via a polymerization process. Discrete PEGs provide a single molecule with defined and specified chain length. The number of $-CH_2CH_2O-$ subunits of a PEG Unit ranges, for example, from 8 to 24 or from 12 to 24, referred to as PEG8 to PEG24 and PEG12 to PEG24, respectively.

[0340] The PEG moieties provided herein, which are also referred to as PEG Units, comprise one or multiple polyethylene glycol chains. The polyethylene glycol chains are linked together, for example, in a linear, branched or star shaped configuration. Typically, at least one of the polyethylene glycol chains of a PEG Unit is derivatized at one end for covalent attachment to

an appropriate site on a component of the ADC (e.g., L). Exemplary attachments to ADCs are by means of non-conditionally cleavable linkages or via conditionally cleavable linkages. Exemplary attachments are via amide linkage, ether linkages, ester linkages, hydrazone linkages, oxime linkages, disulfide linkages, peptide linkages or triazole linkages. In some embodiments, attachment to the Formula (I) ADC is by means of a non-conditionally cleavable linkage. In some embodiments, attachment to the ADC is not via an ester linkage, hydrazone linkage, oxime linkage, or disulfide linkage. In some embodiments, attachment to the ADC is not via a hydrazone linkage.

[0341] A conditionally cleavable linkage refers to a linkage that is not substantially sensitive to cleavage while circulating in plasma but is sensitive to cleavage in an intracellular or intratumoral environment. A non-conditionally cleavable linkage is one that is not substantially sensitive to cleavage in any biologically relevant environment in a subject that is administered the ADC. Chemical hydrolysis of a hydrazone, reduction of a disulfide bond, and enzymatic cleavage of a peptide bond or glycosidic bond of a Glucuronide Unit as described by WO 2007/011968 (which is incorporated by reference in its entirety) are examples of conditionally cleavable linkages.

[0342] In some embodiments, the PEG Unit is directly attached to the ADC (or an intermediate thereof) at L. In those embodiments, the other terminus (or termini) of the PEG Unit is free and untethered (i.e., not covalently attached), and in some embodiments, is a methoxy, carboxylic acid, alcohol or other suitable functional group. The methoxy, carboxylic acid, alcohol or other suitable functional group acts as a cap for the terminal polyethylene glycol subunit of the PEG Unit. By untethered, it is meant that the PEG Unit will not be covalently attached at that untethered site to a Drug Unit, to an antibody, or to a linking component to a Drug Unit and/or an antibody. Such an arrangement can allow a PEG Unit of sufficient length to assume a parallel orientation with respect to the drug in conjugated form, i.e., as a Drug Unit (D). For those embodiments in which the PEG Unit comprises more than one polyethylene glycol chain, the multiple polyethylene glycol chains are independently chosen, e.g., are the same or different chemical moieties (e.g., polyethylene glycol chains of different molecular weight or number of -CH_2CH_2O- subunits). A PEG Unit having multiple polyethylene glycol chains is attached to the ADC at a single attachment site. The skilled artisan will understand that the PEG Unit, in addition to comprising repeating polyethylene glycol subunits, may also contain non-PEG material (e.g., to

facilitate coupling of multiple polyethylene glycol chains to each other or to facilitate coupling to the ADC). Non-PEG material refers to the atoms in the PEG Unit that are not part of the repeating $–CH_2CH_2O-$ subunits. In some embodiments provided herein, the PEG Unit comprises two monomeric polyethylene glycol chains attached to each other via non-PEG elements. In other embodiments provided herein, the PEG Unit comprises two linear polyethylene glycol chains attached to a central core that is attached to the ADC (i.e., the PEG Unit itself is branched).

[0343] There are a number of PEG attachment methods available to those skilled in the art: see, for example: Goodson, et al. (1990) *Bio/Technology* 8:343 (PEGylation of interleukin-2 at its glycosylation site after site-directed mutagenesis); EP 0 401 384 (coupling PEG to G-CSF); Malik, et al., (1992) *Exp. Hematol*. 20:1028-1035 (PEGylation of GM-CSF using tresyl chloride); ACT Pub. No. WO 90/12874 (PEGylation of erythropoietin containing a recombinantly introduced cysteine residue using a cysteine-specific mPEG derivative); U.S. Pat. No. 5,757,078 (PEGylation of EPO peptides); U.S. Pat. No. 5,672,662 (Poly(ethylene glycol) and related polymers monosubstituted with propionic or butanoic acids and functional derivatives thereof for biotechnical applications); U.S. Pat. No. 6,077,939 (PEGylation of an N-terminal α-carbon of a peptide); Veronese et al., (1985) *Appl. Biochem. Bioechnol* 11:141-142 (PEGylation of an N-terminal α-carbon of a peptide with PEG-nitrophenylcarbonate ("PEG-NPC") or PEG-trichlorophenylcarbonate); and Veronese (2001) *Biomaterials* 22:405-417 (Review article on peptide and protein PEGylation).

[0344] For example, a PEG Unit may be covalently bound to an amino acid residue via reactive groups of a polyethylene glycol-containing compound and the amino acid residue. Reactive groups of the amino acid residue include those that are reactive to an activated PEG molecule (e.g., a free amino or carboxyl group). For example, N-terminal amino acid residues and lysine (K) residues have a free amino group; and C-terminal amino acid residues have a free carboxyl group. Thiol groups (e.g., as found on cysteine residues) are also useful as a reactive group for forming a covalent attachment to a PEG. In addition, enzyme-assisted methods for introducing activated groups (e.g., hydrazide, aldehyde, and aromatic-amino groups) specifically at the C-terminus of a polypeptide have been described. *See* Schwarz, et al. (1990) *Methods Enzymol.* 184:160; Rose, et al. (1991) *Bioconjugate Chem*. 2:154; and Gaertner, et al. (1994) *J. Biol. Chem*. 269: 7224.

[0345] In some embodiments, a polyethylene glycol-containing compound forms a covalent attachment to an amino group using methoxylated PEG ("mPEG") having different reactive moieties. Non-limiting examples of such reactive moieties include succinimidyl succinate (SS), succinimidyl carbonate (SC), mPEG-imidate, para-nitrophenylcarbonate (NPC), succinimidyl propionate (SPA), and cyanuric chloride. Non-limiting examples of such mPEGs include mPEG-succinimidyl succinate (mPEG-SS), $mPEG_2$-succinimidyl succinate ($mPEG_2$-SS); mPEG-succinimidyl carbonate (mPEG-SC), $mPEG_2$-succinimidyl carbonate ($mPEG_2$-SC); mPEG-imidate, mPEG-para-nitrophenylcarbonate (mPEG-NPC), mPEG-imidate; $mPEG_2$-para-nitrophenylcarbonate ($mPEG_2$-NPC); mPEG-succinimidyl propionate (mPEG-SPA); $mPEG_2$-succinimidyl propionate (mPEG--SPA); mPEG-N-hydroxy-succinimide (mPEG-NHS); $mPEG_2$-N-hydroxy-succinimide ($mPEG_2$--NHS); mPEG-cyanuric chloride; $mPEG_2$-cyanuric chloride; $mPEG_2$-Lysinol-NPC, and $mPEG_2$-Lys-NHS.

[0346] Generally, at least one of the polyethylene glycol chains that make up the PEG is functionalized to provide covalent attachment to the ADC. Functionalization of the polyethylene glycol-containing compound that is the precursor to the PEG includes, for example, via an amine, thiol, NHS ester, maleimide, alkyne, azide, carbonyl, or other functional group. In some embodiments, the PEG further comprises non-PEG material (i.e., material not comprised of $–CH_2CH_2O-$) that provides coupling to the ADC or in constructing the polyethylene glycol-containing compound or PEG facilitates coupling of two or more polyethylene glycol chains.

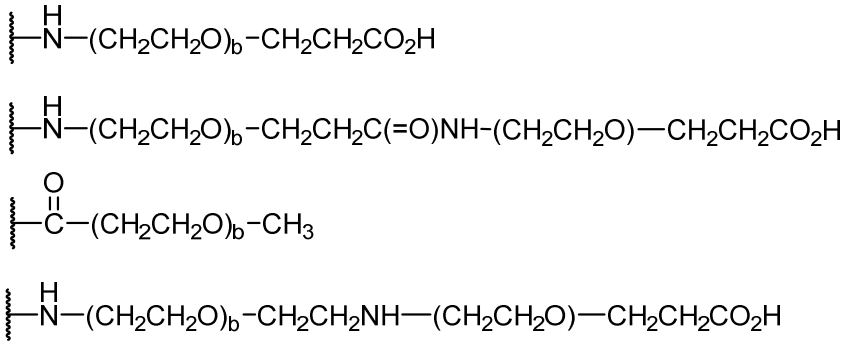
[0347] In some embodiments, the presence of the PEG Unit in an ADC is capable of having two potential impacts upon the pharmacokinetics of the resulting ADC. One impact is a decrease in clearance (and consequent increase in exposure) that arises from the reduction in non-specific interactions induced by the exposed hydrophobic elements of the Drug Unit. The second impact is a decrease in volume and rate of distribution that sometimes arises from the increase in the molecular weight of the ADC. Increasing the number of polyethylene glycol subunits increases the hydrodynamic radius of a conjugate, typically resulting in decreased diffusivity. In turn, decreased diffusivity typically diminishes the ability of the ADC to penetrate into a tumor. *See* Schmidt and Wittrup, *Mol Cancer Ther* 2009; 8:2861-2871. Because of these two competing pharmacokinetic effects, it can be desirable to use a PEG Unit that is sufficiently large to decrease the ADC clearance thus increasing plasma exposure, but not so large as to greatly diminish its diffusivity to an extent that it interferes with the ability of the ADC to reach the intended target

cell population. *See, e.g.,* Examples 1, 18, and 21 of US 2016/0310612, which is incorporated by reference herein (e.g., for methodology for selecting an optimal size of a PEG Unit for a particular Drug Unit, Linker, and/or drug-linker compound).

[0348] In one group of embodiments, the PEG Unit comprises one or more linear polyethylene glycol chains each having at 8 subunits, at least 9 subunits, at least 10 subunits, at least 11 subunits, at least 12 subunits, at least 13 subunits, at least 14 subunits, at least 15 subunits, at least 16 subunits, at least 17 subunits, at least 18 subunits, at least 19 subunits, at least 20 subunits, at least 21 subunits, at least 22 subunits, at least 23 subunits, or at least 24 subunits. In some embodiments, the PEG comprises a combined total of at least 8 subunits, at least 10 subunits, or at least 12 subunits. In some such embodiments, the PEG comprises no more than a combined total of about 72 subunits. In some such embodiments, the PEG comprises no more than a combined total of about 36 subunits. In some embodiments, the PEG comprises about 8 to about 24 subunits (referred to as PEG8 to PEG24).

[0349] In another group of embodiments, the PEG Unit comprises a combined total of from 8 to 72, 8 to 60, 8 to 48, 8 to 36 or 8 to 24 subunits, from 9 to 72, 9 to 60, 9 to 48, 9 to 36 or 9 to 24 subunits, from 10 to 72, 10 to 60, 10 to 48, 10 to 36 or 10 to 24 subunits, from 11 to 72, 11 to 60, 11 to 48, 11 to 36 or 11 to 24 subunits, from 12 to 72, 12 to 60, 12 to 48, 12 to 36 or 12 to 24 subunits, from 13 to 72, 13 to 60, 13 to 48, 13 to 36 or 13 to 24 subunits, from 14 to 72, 14 to 60, 14 to 48, 14 to 36 or 14 to 24 subunits, from 15 to 72, 15 to 60, 15 to 48, 15 to 36 or 15 to 24 subunits, from 16 to 72, 16 to 60, 16 to 48, 16 to 36 or 16 to 24 subunits, from 17 to 72, 17 to 60, 17 to 48, 17 to 36 or 17 to 24 subunits, from 18 to 72, 18 to 60, 18 to 48, 18 to 36 or 18 to 24 subunits, from 19 to 72, 19 to 60, 19 to 48, 19 to 36 or 19 to 24 subunits, from 20 to 72, 20 to 60, 20 to 48, 20 to 36 or 20 to 24 subunits, from 21 to 72, 21 to 60, 21 to 48, 21 to 36 or 21 to 24 subunits, from 22 to 72, 22 to 60, 22 to 48, 22 to 36 or 22 to 24 subunits, from 23 to 72, 23 to 60, 23 to 48, 23 to 36 or 23 to 24 subunits, or from 24 to 72, 24 to 60, 24 to 48, 24 to 36 or 24 subunits.

[0350] Illustrative linear PEGs that can be used in any of the embodiments provided herein are as follows:



wherein the wavy line indicates the site of attachment to the ADC, and each subscript b is independently selected from the group consisting of 7 to 72, 8 to 72, 10 to 72, 12 to 72, 6 to 24, or 8 to 24. In some embodiments, each subscript b is about 8, about 12, or about 24.

[0351] As described herein, the PEG Unit can be selected such that it improves clearance of the resultant ADC but does not significantly impact the ability of the ADC to penetrate into the tumor.

[0352] In some embodiments, the PEG is from about 300 daltons to about 5 kilodaltons; from about 300 daltons to about 4 kilodaltons; from about 300 daltons to about 3 kilodaltons; from about 300 daltons to about 2 kilodaltons; from about 300 daltons to about 1 kilodalton; or any value in between. In some embodiments, the PEG has at least 8, 10 or 12 subunits. In some embodiments, the PEG Unit is PEG8 to PEG72, for example, PEG8, PEG10, PEG12, PEG16, PEG20, PEG24, PEG28, PEG32, PEG36, PEG48, or PEG72.

[0353] In some embodiments, apart from the PEGylation of the ADC, there are no other PEG subunits present in the ADC (i.e., no PEG subunits are present as part of any of the other components of the conjugates and linkers provided herein, such as A and X^B). In some embodiments, apart from the PEG, there are no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2 or no more than 1 other polyethylene glycol (-CH_2CH_2O-) subunits present in the ADC, or intermediate thereof (i.e., no more than 8, 7, 6, 5, 4, 3, 2, or 1 other polyethylene glycol subunits in other components of the ADCs (or intermediates thereof) provided herein).

[0354] It will be appreciated that when referring to polyethylene glycol subunits of a PEG Unit, and depending on context, the number of subunits can represent an average number, e.g., when referring to a population of ADCs or intermediates thereto and/or using polydisperse PEGs.

Methods of Use

[0355] In some embodiments, the ADCs described herein, or pharmaceutically acceptable salts thereof, are used to deliver the conjugated drug to a target cell. Without being bound by theory, in some embodiments, an ADC associates with an antigen on the surface of a target cell. The Drug Unit can then be released as free drug to induce its biological effect (such as an immunostimulatory effect). The Drug Unit can also remain attached to the antibody, or a portion of the antibody and/or linker, and induce its biological effect.

[0356] Some embodiments provide a method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC described herein, or a pharmaceutically acceptable salt thereof, to the subject.

[0357] Some embodiments provide a method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of a composition comprising an ADC described herein, or a pharmaceutically acceptable salt thereof, to the subject.

[0358] Some embodiments provide a method of inducing an anti-tumor immune response in a subject in need thereof, comprising administering a therapeutically effective amount of a composition comprising a ADC described herein, or a pharmaceutically acceptable salt thereof, to the subject.

[0359] Some embodiments provide a method of inducing an anti-tumor immune response in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC described herein, or a pharmaceutically acceptable salt thereof, to the subject.

[0360] Some embodiments provide a method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC as described herein, or a pharmaceutically acceptable salt thereof, to the subject in combination with another anticancer therapy (e.g., surgery and radiation therapy) and/or anticancer agent (e.g., an immunotherapy such as nivolumab or pembrolizumab). The ADCs described herein can be administered before, during, or after administration of the anticancer therapy and/or anticancer agent to the subject. In some embodiments, the ADCs described herein can be administered to the subject following treatment with radiation and/or after surgery.

[0361] Some embodiments provide a method for delaying or preventing acquired resistance to an anticancer agent, comprising administering a therapeutically effective amount of an ADC as described herein, or a pharmaceutically acceptable salt thereof, to a patient at risk for

developing or having acquired resistance to an anticancer agent. In some embodiments, the patient is administered a dose of the anticancer agent (e.g., at substantially the same time as a dose of an ADC as described herein, or a pharmaceutically acceptable salt thereof is administered to the patient).

[0362] Some embodiments provide a method of delaying and/or preventing development of cancer resistant to an anticancer agent in a subject, comprising administering to the subject a therapeutically effective amount of an ADC as described herein, or a pharmaceutically acceptable salt thereof, before, during, or after administration of a therapeutically effective amount of the anticancer agent.

[0363] The ADCs described herein are useful for inhibiting the multiplication of a cancer cell, causing apoptosis in a cancer cell, for increasing phagocytosis of a cancer cell, and/or for treating cancer in a subject in need thereof. The ADCs can be used accordingly in a variety of settings for the treatment of cancers. The ADCs can be used to deliver a drug to a cancer cell. Without being bound by theory, in some embodiments, the antibody of an ADC binds to or associates with a cancer-cell-associated antigen. The antigen can be attached to a cancer cell or can be an extracellular matrix protein associated with the cancer cell. The drug can be released in proximity to the cancer cell, thus recruiting/activating immune cells to attack the cancer cell. In some embodiments, the Drug Unit is cleaved from the ADC outside the cancer cell. In some embodiments, the Drug Unit remains attached to the antibody bound to the antigen.

[0364] In some embodiments, the antibody binds to the cancer cell. In some embodiments, the antibody binds to a cancer cell antigen which is on the surface of the cancer cell. In some embodiments, the antibody binds to a cancer cell antigen which is an extracellular matrix protein associated with the tumor cell or cancer cell. In some embodiments, the antibody of an ADC binds to or associates with a cancer-associated cell or an antigen on a cancer-associated cell. In some embodiments, the cancer-associated cell is a stromal cell in a tumor, for example, a cancer-associated fibroblast (CAF).

[0365] In some embodiments, the antibody of an ADC binds to or associates with an immune cell or an immune-cell-associated antigen. The antigen can be attached to an immune cell or can be an extracellular matrix protein associated with the immune cell. The drug can be released in proximity to the immune cell, thus recruiting/activating the immune cell to attack a cancer cell. In some embodiments, the Drug Unit is cleaved from the ADC outside the immune cell. In some

embodiments, the Drug Unit remains attached to the antibody bound to the antigen. In some embodiments, the immune cell is a lymphocyte, an antigen-presenting cell, a natural killer (NK) cell, a neutrophil, an eosinophil, a basophil, a mast cell, innate lymphoid cells or a combination of any of the foregoing. In some embodiments, the immune cell is selected from the group consisting of B cells, plasma cells, T cells, NKT cells, gamma delta T (γδT) cells, monocytes, macrophages, dendritic cells, natural killer (NK) cells, neutrophils, eosinophils, basophils, mast cells, innate lymphoid cells and a combination of any of the foregoing.

[0366] The specificity of the antibody for a particular cancer cell can be important for determining those tumors or cancers that are most effectively treated. For example, ADCs that target a cancer cell antigen present on hematopoietic cancer cells in some embodiments treat hematologic malignancies. In some embodiments, ADCs target a cancer cell antigen present on abnormal cells of solid tumors for treating such solid tumors. In some embodiments an ADC are directed against abnormal cells of hematopoietic cancers such as, for example, lymphomas (Hodgkin Lymphoma and Non-Hodgkin Lymphomas) and leukemias.

[0367] Cancers, including, but not limited to, a tumor, metastasis, or other disease or disorder characterized by abnormal cells that are characterized by uncontrolled cell growth in some embodiments are treated or inhibited by administration of an ADC.

[0368] In some embodiments, the subject has previously undergone treatment for the cancer. In some embodiments, the prior treatment is surgery, radiation therapy, administration of one or more anticancer agents, or a combination of any of the foregoing.

[0369] In any of the methods described herein, the cancer is selected from the group consisting of: adenocarcinoma, adrenal gland cortical carcinoma, adrenal gland neuroblastoma, anus squamous cell carcinoma, appendix adenocarcinoma, bladder urothelial carcinoma, bile duct adenocarcinoma, bladder carcinoma, bladder urothelial carcinoma, bone chordoma, bone marrow leukemia lymphocytic chronic, bone marrow leukemia non-lymphocytic acute myelocytic, bone marrow lymph proliferative disease, bone marrow multiple myeloma, bone sarcoma, brain astrocytoma, brain glioblastoma, brain medulloblastoma, brain meningioma, brain oligodendroglioma, breast adenoid cystic carcinoma, breast carcinoma, breast ductal carcinoma in situ, breast invasive ductal carcinoma, breast invasive lobular carcinoma, breast metaplastic carcinoma, cervix neuroendocrine carcinoma, cervix squamous cell carcinoma, colon adenocarcinoma, colon carcinoid tumor, duodenum adenocarcinoma, endometrioid tumor,

esophagus adenocarcinoma, esophagus and stomach carcinoma, eye intraocular melanoma, eye intraocular squamous cell carcinoma, eye lacrimal duct carcinoma, fallopian tube serous carcinoma, gallbladder adenocarcinoma, gallbladder glomus tumor, gastroesophageal junction adenocarcinoma, head and neck adenoid cystic carcinoma, head and neck carcinoma, head and neck neuroblastoma, head and neck squamous cell carcinoma, kidney chromophore carcinoma, kidney medullary carcinoma, kidney renal cell carcinoma, kidney renal papillary carcinoma, kidney sarcomatoid carcinoma, kidney urothelial carcinoma, kidney carcinoma, leukemia lymphocytic, leukemia lymphocytic chronic, liver cholangiocarcinoma, liver hepatocellular carcinoma, liver carcinoma, lung adenocarcinoma, lung adenosquamous carcinoma, lung atypical carcinoid, lung carcinosarcoma, lung large cell neuroendocrine carcinoma, lung non-small cell lung carcinoma, lung sarcoma, lung sarcomatoid carcinoma, lung small cell carcinoma, lung small cell undifferentiated carcinoma, lung squamous cell carcinoma, upper aerodigestive tract squamous cell carcinoma, upper aerodigestive tract carcinoma, lymph node lymphoma diffuse large B cell, lymph node lymphoma follicular lymphoma, lymph node lymphoma mediastinal B-cell, lymph node lymphoma plasmablastic lung adenocarcinoma, lymphoma follicular lymphoma, lymphoma, non-Hodgkins, nasopharynx and paranasal sinuses undifferentiated carcinoma, ovary carcinoma, ovary carcinosarcoma, ovary clear cell carcinoma, ovary epithelial carcinoma, ovary granulosa cell tumor, ovary serous carcinoma, pancreas carcinoma, pancreas ductal adenocarcinoma, pancreas neuroendocrine carcinoma, peritoneum mesothelioma, peritoneum serous carcinoma, placenta choriocarcinoma, pleura mesothelioma, prostate acinar adenocarcinoma, prostate carcinoma, rectum adenocarcinoma, rectum squamous cell carcinoma, skin adnexal carcinoma, skin basal cell carcinoma, skin melanoma, skin Merkel cell carcinoma, skin squamous cell carcinoma, small intestine adenocarcinoma, small intestine gastrointestinal stromal tumors (GISTs), large intestine/colon carcinoma, large intestine adenocarcinoma, soft tissue angiosarcoma, soft tissue Ewing sarcoma, soft tissue hemangioendothelioma, soft tissue inflammatory myofibroblastic tumor, soft tissue leiomyosarcoma, soft tissue liposarcoma, soft tissue neuroblastoma, soft tissue paraganglioma, soft tissue perivascular epitheliod cell tumor, soft tissue sarcoma, soft tissue synovial sarcoma, stomach adenocarcinoma, stomach adenocarcinoma diffuse-type, stomach adenocarcinoma intestinal type, stomach adenocarcinoma intestinal type, stomach leiomyosarcoma, thymus carcinoma, thymus thymoma lymphocytic, thyroid papillary carcinoma, unknown primary adenocarcinoma, unknown primary carcinoma, unknown primary

malignant neoplasm, lymphoid neoplasm, unknown primary melanoma, unknown primary sarcomatoid carcinoma, unknown primary squamous cell carcinoma, unknown undifferentiated neuroendocrine carcinoma, unknown primary undifferentiated small cell carcinoma, uterus carcinosarcoma, uterus endometrial adenocarcinoma, uterus endometrial adenocarcinoma endometrioid, uterus endometrial adenocarcinoma papillary serous, and uterus leiomyosarcoma.

[0370] In some embodiments, the subject is concurrently administered one or more additional anticancer agents with the ADCs described herein, or a pharmaceutically acceptable salt thereof. In some embodiments, the subject is concurrently receiving radiation therapy with the ADCs described herein, or a pharmaceutically acceptable salt thereof. In some embodiments, the subject is administered one or more additional anticancer agents after administration of the ADCs described herein, or a pharmaceutically acceptable salt thereof. In some embodiments, the subject receives radiation therapy after administration of the ADCs described herein, or a pharmaceutically acceptable salt thereof.

[0371] In some embodiments, the subject has discontinued a prior therapy, for example, due to unacceptable or unbearable side effects, wherein the prior therapy was too toxic, or wherein the subject developed resistance to the prior therapy.

[0372] Some embodiments provide a method for delaying or preventing a disease or disorder, comprising administering a therapeutically effective amount of an ADC as described herein, or a pharmaceutically acceptable salt thereof, and a vaccine against the disease or disorder, to a patient at risk for developing the disease or disorder. In some embodiments, the disease or disorder is cancer, as described herein. In some embodiments, the disease or disorder is a viral pathogen. In some embodiments, the vaccine is administered subcutaneously. In some embodiments, the vaccine is administered intramuscularly. In some embodiments, the ADC and the vaccine are administered via the same route (for example, the ADC and the vaccine are both administered subcutaneously). In some embodiments, the ADC, or a pharmaceutically acceptable salt thereof, and the vaccine are administered via different routes. In some embodiments, the vaccine and the ADC, or a pharmaceutically acceptable salt thereof, are provided in a single formulation. In some embodiments, the vaccine and the ADC, or a pharmaceutically acceptable salt thereof, are provided in separate formulations.

Compositions and Methods of Administration

[0373] Some embodiments provide a composition comprising a distribution of ADCs, as described herein. In some embodiments, the composition comprises a distribution of ADCs, as described herein and at least one pharmaceutically acceptable carrier. In some embodiments, the route of administration is parenteral. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In some embodiments, the compositions are administered parenterally. In one of those embodiments, the ADCs are administered intravenously. Administration is typically through any convenient route, for example by infusion or bolus injection.

[0374] Compositions of an ADC are formulated so as to allow the ADC to be bioavailable upon administration of the composition to a subject. Compositions can be in the form of one or more injectable dosage units.

[0375] Materials used in preparing the compositions can be non-toxic in the amounts used. It will be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the composition will depend on a variety of factors. Relevant factors include, without limitation, the type of animal (*e.g.*, human), the particular form of the compound, the manner of administration, and the composition employed.

[0376] In some embodiments, the ADC composition is a solid, for example, as a lyophilized powder, suitable for reconstitution into a liquid prior to administration. In some embodiments, the ADC composition is a liquid composition, such as a solution or a suspension. A liquid composition or suspension is useful for delivery by injection and a lyophilized solid is suitable for reconstitution as a liquid or suspension using a diluent suitable for injection. In a composition administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent is typically included.

[0377] In some embodiments, the liquid compositions, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or digylcerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers

such as amino acids, acetates, citrates or phosphates; detergents, such as nonionic surfactants, polyols; and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition is typically enclosed in ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. In some embodiments, the sterile diluent comprises physiological saline. In some embodiments, the sterile diluent is physiological saline. In some embodiments, the composition described herein are liquid injectable compositions that are sterile.

[0378] The amount of the ADC that is effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, which is usually determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays are sometimes employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of parenteral administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances.

[0379] In some embodiments, the compositions comprise an effective amount of an ADC such that a suitable dosage will be obtained. Typically, this amount is at least about 0.01% of the ADC by weight of the composition.

[0380] In some embodiments, the compositions dosage of an ADC administered to a subject is from about 0.01 mg/kg to about 100 mg/kg, from about 1 to about 100 mg of a per kg or from about 0.1 to about 25 mg/kg of the subject's body weight. In some embodiments, the dosage administered to a subject is about 0.01 mg/kg to about 15 mg/kg of the subject's body weight. In some embodiments, the dosage administered to a subject is about 0.1 mg/kg to about 15 mg/kg of the subject's body weight. In some embodiments, the dosage administered to a subject is about 0.1 mg/kg to about 20 mg/kg of the subject's body weight. In some embodiments, the dosage administered is about 0.1 mg/kg to about 5 mg/kg or about 0.1 mg/kg to about 10 mg/kg of the subject's body weight. In some embodiments, the dosage administered is about 1 mg/kg to about 15 mg/kg of the subject's body weight. In some embodiments, the dosage administered is about 1 mg/kg to about 10 mg/kg of the subject's body weight. In some embodiments, the dosage administered is about 0.1 to about 4 mg/kg, about 0.1 to about 3.2 mg/kg, or about 0.1 to about 2.7 mg/kg of the subject's body weight over a treatment cycle.

[0381] The term "carrier" refers to a diluent, adjuvant or excipient, with which a compound is administered. Such pharmaceutical carriers are liquids. Water is an exemplary carrier

when the compounds are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are also useful as liquid carriers for injectable solutions. Suitable pharmaceutical carriers also include glycerol, propylene, glycol, or ethanol. The present compositions, if desired, will in some embodiments also contain minor amounts of wetting or emulsifying agents, and/or pH buffering agents.

[0382] In some embodiments, the ADCs are formulated in accordance with routine procedures as a composition adapted for intravenous administration to animals, particularly human beings. Typically, the carriers or vehicles for intravenous administration are sterile isotonic aqueous buffer solutions. In some embodiments, the composition further comprises a local anesthetic, such as lignocaine, to ease pain at the site of the injection. In some embodiments, the ADC and the remainder of the formulation are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where an ADC is to be administered by infusion, it is sometimes dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the ADCs are administered by injection, an ampoule of sterile water for injection or saline is typically provided so that the ingredients can be mixed prior to administration.

[0383] The compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

EXAMPLES

General Methods:

[0384] All commercially available anhydrous solvents were used without further purification. All commercially available reagents were used without further purification unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 aluminum sheets or glass plates (EMD Chemicals, Gibbstown, NJ). Flash column chromatography was performed on a Biotage Isolera One™ flash purification system 20 or Biotage Selekt™ flash purification system (Charlotte, NC). UPLC-MS analysis was performed on one of four systems. UPLC-MS system 1: Waters single quad detector mass spectrometer interfaced to a Waters

Acquity UPLC system equipped with a Waters Acquity UPLC BEH C18 2.1 x 50 mm, 1.7 µm, reversed-phase column. UPLC-MS system 2: Waters Xevo G2 TOF mass spectrometer interfaced to a Waters Acquity H-class Ultra Performance LC equipped with a C8 Phenomenex Synergi 2.0 x 150 mm, 4 µm, 80 Å reversed-phase column with a Waters 2996 Photodiode Array Detector. UPLC-MS system 3 (C18): Shimadzu LC-20 AD & MS 2020 interfaced with a diode array detector (DAD) and positive ESI mass spectrometer equipped with either a Luna-C18 2.0x30 mm, 3 µm particle size reversed-phase column maintained at 40 ºC or a Kinetex-C18 2.1x30 mm, 5 µm reversed-phase column maintained at 40 ºC. UPLC-MS system 4 (C18): Agilent 1200 series LC system interfaced a diode array detector (DAD) and Agilent 6110B positive ESI quadrapole mass spectrometer equipped with a Kinetex-C18 2.1x50 mm, 5 µm reversed-phase column maintained at 40 °C.

[0385] Compounds were eluted using one of Methods A-E, as described herein.

[0386] Method A – a linear gradient of 5-95% acetonitrile in water (1 mL/min) over 1.0 min, followed by isocratic flow of 95% acetonitrile to 1.80 min (1.0 mL/min) and column equilibration back to 5% acetonitrile to 2.20 min (1.2 mL/min). The water contained 0.037% TFA (v/v) and the acetonitrile contained 0.018% TFA (v/v). The column used was a Phenomenex Luna C18 2.0x30mm, 3 µm reversed-phase column.

[0387] Method B – a linear gradient of 5-95% acetonitrile in water (1 mL/min) over 1.0 min, followed by isocratic flow of 95% acetonitrile to 1.80 min (1.0 mL/min) and column equilibration back to 5% acetonitrile to 2.20 min (1.2 mL/min). The water contained 0.05% TFA (v/v) and the acetonitrile contained 0.05% TFA (v/v). The column used was a Phenomenex Kinetex C18 2.1x30mm, 5 µm reversed-phase column.

[0388] Method C – isocratic flow of 5% acetonitrile in water for 0.4 min, followed by a linear gradient of 5-95% acetonitrile in water to 3.0 min, followed by isocratic flow for 95% acetonitrile to 4.0 min and column equilibration back to 5% acetonitrile to 4.5 min. The flow rate was 1.0 mL/min and the water contained 0.05% TFA (v/v) and the acetonitrile contained 0.05% TFA (v/v). The column used was a Phenomenex Kinetex C18 2.1x30mm, 5 µm reversed-phase column.

[0389] Method D – a linear gradient of 3- 60% acetonitrile over 1.7 min, then 60-95% acetonitrile to 2.0 min, followed by isocratic flow of 95% acetonitrile to 2.5 min followed by column equilibration back to 3% acetonitrile. The flow rate was 0.6 mL/min and the water

contained 0.1% (v/v) formic acid and the acetonitrile contained 0.1% (v/v) formic acid. The column used was either a Waters Acquity UPLC BEH C18 2.1 x 50 mm, 1.7 µm, reversed-phase column or a C8 Phenomenex Synergi 2.0 x 150 mm, 4 µm, reversed-phase column.

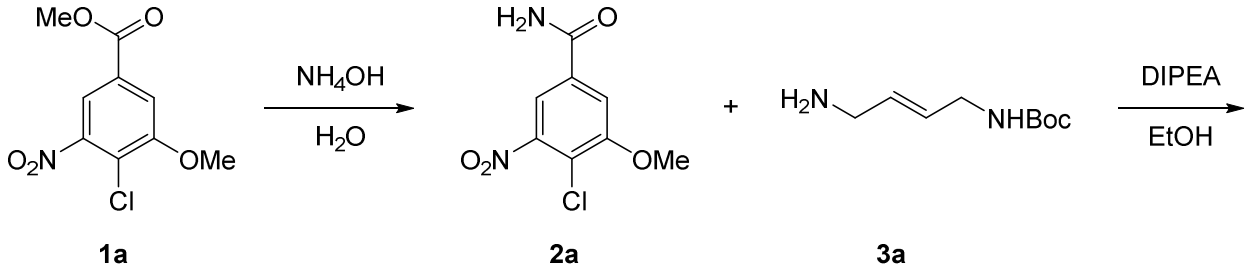
[0390] Method E – a linear gradient of 3 – 95% acetonitrile over 1.5 min, followed by isocratic elution of 95% acetonitrile to 2.4 min, followed by equilibration back to 3% acetonitrile. The flow rate was 0.6 mL/min and the water contained 0.1% (v/v) formic acid and the acetonitrile contained 0.1% (v/v) formic acid. The column used was either a Waters Acquity UPLC BEH C18 2.1 x 50 mm, 1.7 µm, reversed-phase column or a C8 Phenomenex Synergi 2.0 x 150 mm, 4 µm, reversed-phase column.

[0391] Unless otherwise specified, preparatory HPLC (PrepHPLC) was performed on one of two instruments using the procedures listed herein: (Method F) a Shimadzu LC-8a preparative HPLC with a Phenomenex Luna C-18 250x50 mm, 10 µm using water / acetonitrile mobile phase with 0.09% (v/v) TFA at a flow rate of 80 mL/min or on a Teledyne ISCO ACCQPrep HP150 equipped with one of three Phenomemex preparatory HPLC columns: (i) (Method G) 10 x 250 mm Synergi C12, 4 µm, Max-RP 80 Å LC Column, (ii) (Method H) 21.2 x 250 mm Synergi C12, 4 µm, Max-RP 80 Å LC Column or (iii) (Method I) 30 x 250 mm Synergi C12, 4 µm, Max-RP 80 Å LC Column using acetonitrile / water mobile phases containing either 0.05% (v/v) trifluoroacetic acid or 0.1% (v/v) formic acid as additives.

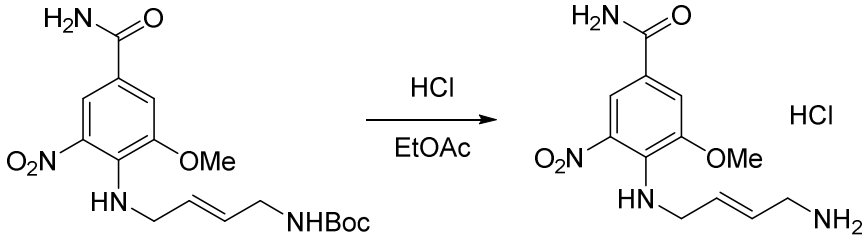
[0392] NMR spectra were recorded on one of three instruments: Bruker Avance III HD (400 MHz), Varian 400-MR (400 MHz) or Bruker Avance NEO (400 MHz).

EXAMPLE 1:
SYNTHETIC PROCEDURES FOR STING AGONISTS AND LINKERS

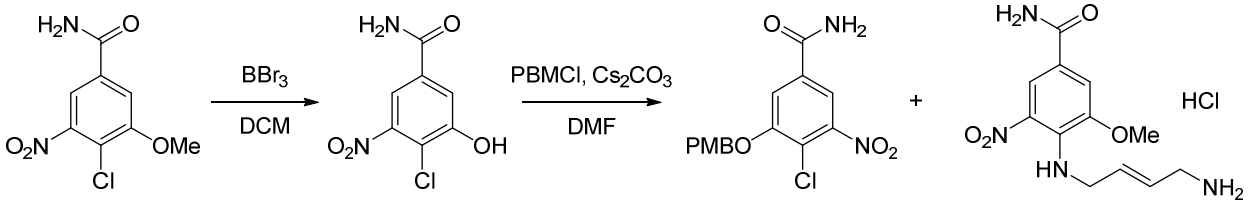
Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-hydroxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxamide (Compound 1)



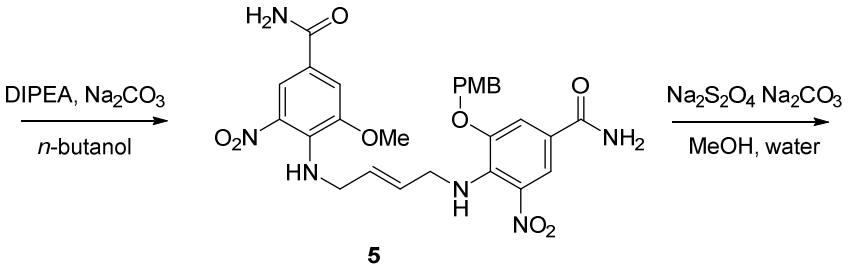
1a **2a** **3a**



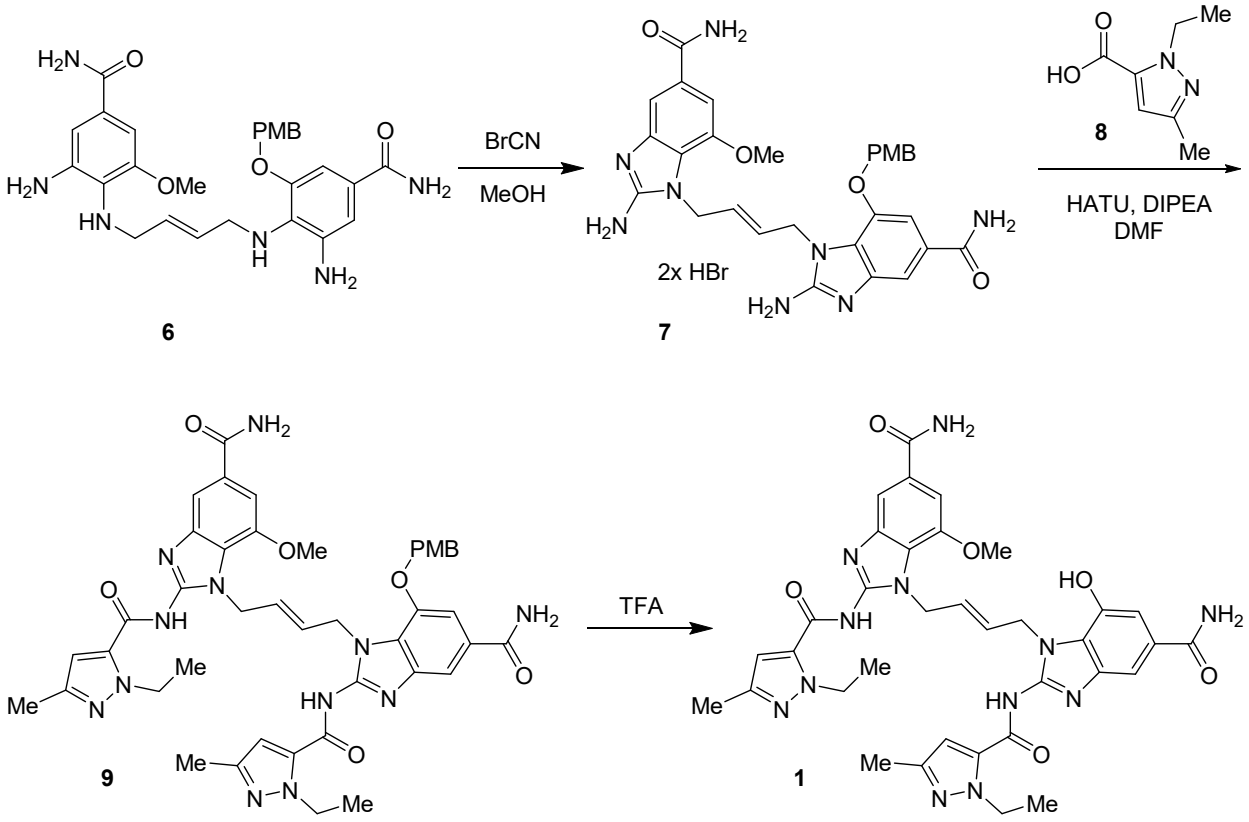
4a **5a**



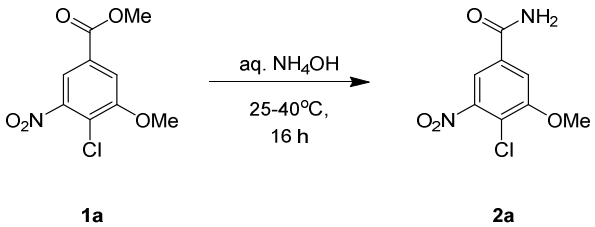
2a **2b** **3b** **5a**



5

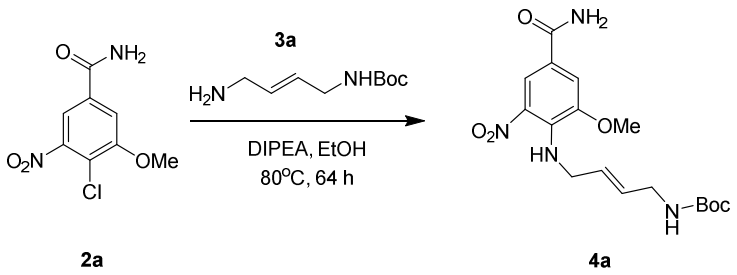


Synthesis of 4-chloro-3-methoxy-5-nitrobenzamide (Compound 2a)



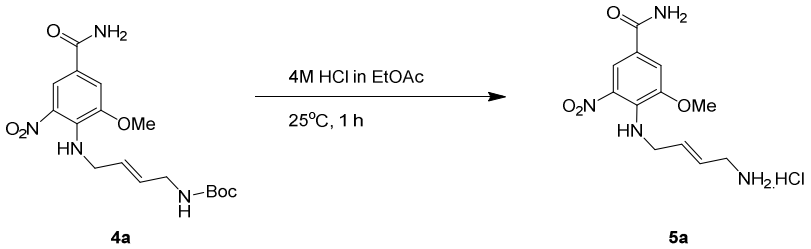
[0393] Compound **1a** (methyl 4-chloro-3-methoxy-5-nitrobenzoate, 18 g, 73 mmol, 1 equiv.) was added into aqueous NH_4OH solution (300mL, 28% NH_3 in H_2O) at 25°C. The reaction mixture was stirred at 40°C for 16 hrs, during which time a precipitate was formed. The precipitate was collected by filtration, washed with water and dried *in vacuo* to give **2a** (13 grams, 56 mmols, 77% yield) as a yellow solid. This product was used in subsequent steps without further purification. UPLC-MS (Method A, ESI+): m/z $(M+H)^+$ 231.0 (theoretical); 231.2 (observed). HPLC retention time: 0.93 min. ^{1}H NMR (DMSO-d_6, 400MHz): δ = 8.29 (br s, 1H), 8.05 (d, *J*=2.0 Hz, 1H), 7.88 (d, *J*=1.6 Hz, 1H), 7.79 (br s, 1H), 4.02 (s, 3H).

Synthesis of tert-butyl (E)-(4-((4-carbamoyl-2-methoxy-6-nitrophenyl)amino)but-2-en-1-yl)carbamate (Compound 4a)



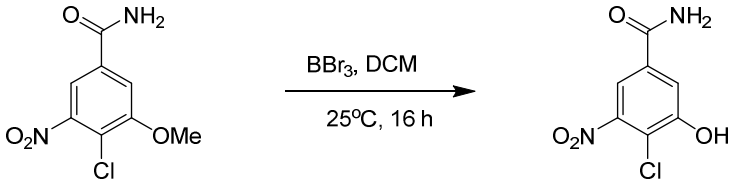
[0394] To a solution of **2a** (10 g, 43.4 mmol, 1 equiv.) in ethanol (EtOH, 200 mL) was added **3a** (tert-butyl (E)-(4-aminobut-2-en-1-yl)carbamate, 9.69 g, 52.0 mmol, 1.2 equiv.) and N,N-diisopropylethylamine (DIPEA, 16.8 g, 130 mmol, 3 equiv.) at 25°C. The reaction mixture was stirred at 80°C for 64 hours which point the precipitate was collected by filtration, washed with ethanol, and dried under high vacuum to give **4a** (8 grams, 21 mmols, 48% yield) as a red solid. This product was used in subsequent steps without further purification. **^{1}H NMR** (DMSO-d_6, 400MHz): δ = 8.18 (s, 1H), 8.01 (br s, 1H), 7.74 (br t, *J*=5.6 Hz, 1H), 7.55 (s, 1H), 7.31 (br s, 1H), 6.92 (br s, 1H), 5.53 (br s, 2H), 4.08 (br s, 2H), 3.87 (s, 3H), 3.47 (br s, 2H), 1.35 (s, 9H).

Synthesis of Compound 5a



[0395] Compound **4a** (8 g, 21.0 mmol, 1 equiv.) was added into a 4M solution of HCl in ethyl acetate (200 mL, 800 mmol HCl) at 25°C. The reaction mixture was stirred at 25°C for 1 h. The precipitate was collected by filtration, washed with EtOAc and dried under high vacuum to give **5a** as HCl salt (7.2 g, quantitative yield) as a red solid. This product was used in subsequent steps without further purification. **^{1}H NMR** (DMSO-d_6, 400MHz): δ = 8.21 (d, *J*=1.6 Hz, 1H), 8.02 (br s, 4H), 7.59 (d, *J*=2.0 Hz, 1H), 7.34 (br s, 1H), 5.87 (td, *J*=5.6, 15.6 Hz, 1H), 5.67 - 5.56 (m, 1H), 4.17 (br d, *J*=5.6 Hz, 2H), 3.89 (s, 3H), 3.39 (br t, *J*=5.6 Hz, 2H).

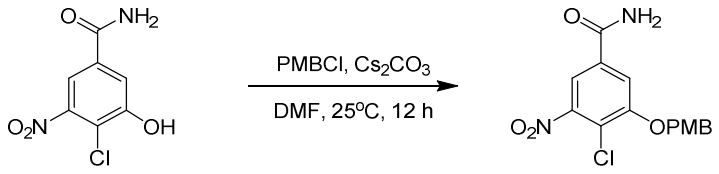
Synthesis of Compound 2b



2a **2b**

[0396] To a solution of compound **2a** (4-chloro-3-methoxy-5-nitrobenzamide, 16 g, 69.4 mmol, 1 equiv.) in dichloromethane (DCM, 500 mL) was added a solution of boron tribromide (BBr_3, 1 M in DCM, 275 mL, 4 equiv.) dropwise at 20°C under nitrogen. The reaction mixture was stirred at 20°C for 16 h, upon which LC-MS analysis (Method B) showed the reaction was complete. The reaction mixture was poured into ice water (2 L) and stirred vigorously for 20 min. The resulting suspension was filtered and the filtrate was extracted with ethyl acetate (2 x 300 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give a crude product. The crude product (9 g) was dissolved in DMF (30 mL) and purified by reversed-phase flash chromatography on a Biotage Isolera One (330 gram Agela C18 column (20 – 35 µm particle size), utilizing water / acetonitrile with 0.09% (v/v) TFA eluting with a gradient of 20-40% acetonitrile over 20 min followed by 40 – 45% acetonitrile at 35 min to give **2b** (6 grams, 27.7 mmols, 40% yield) as an off-white solid**. LCMS** (Method B, ESI+): m/z $[M+H]^+$ 217.0 (theoretical); 217.2 (observed). HPLC retention time: 0.84 min.

Synthesis of Compound 3b

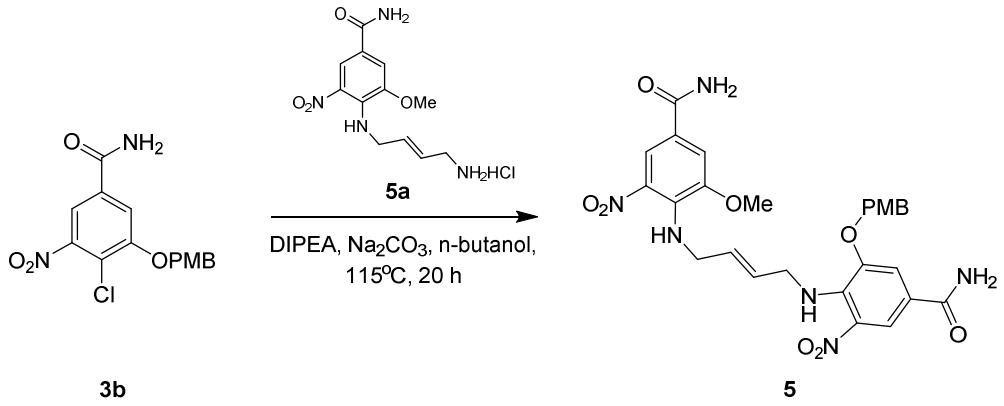


2b **3b**

[0397] To a solution of **2b** (4.5 g, 20.8 mmol, 1 equiv.) in dimethylformamide (DMF, 20 mL) was added 1-(chloromethyl)-4-methoxybenzene (PMBCl, 3.42 g, 21.8 mmol, 1.05 equiv.) and cesium carbonate (Cs_2CO_3, 7.45 g, 22.9 mmol, 1.1 equiv.), the reaction mixture was stirred at 25°C for 12 h, upon which LC-MS analysis (Method B) showed the reaction was complete. The reaction mixture was poured into ice water, and the precipitate was filtered and dried to give **3b** (6.4 grams, 19.0 mmols, 91% yield)) as a light yellow solid. This product was used in subsequent

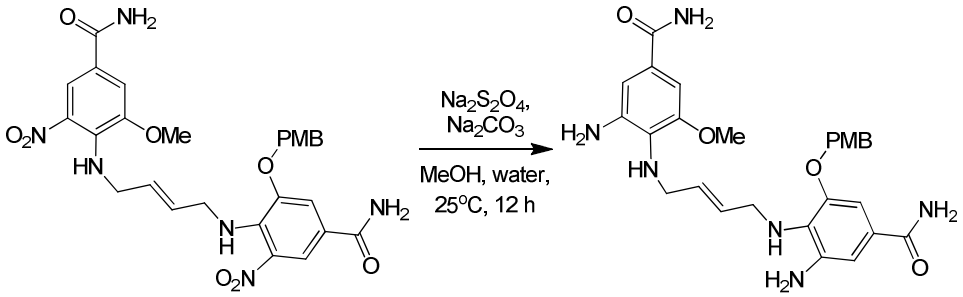
steps without further purification. **LC-MS** (Method B, ESI+): m/z $[M+H]^+$: 337.1 (theoretical); 337.2 (observed). HPLC Retention Time: 1.11 min.

Synthesis of Compound 5



[0398] A solution of **5a** (762 mg, 2.16 mmol, 1.2 equiv.) in *n*-butanol (10 mL) was added to a vial, followed by the addition of DIPEA (1.11 g, 8.62 mmol, 4.8 equiv.) and sodium bicarbonate (457 mg, 4.31 mmol, 2 equiv.). The vial was sealed and the reaction mixture was stirred at 20°C for 10 min. This was followed by the addition of **3b** (600 mg, 1.78 mmol, 2.4 equiv.), and the reaction mixture was stirred at 115°C for 20 hours upon which time UPLC-MS analysis (Method B) showed the reaction was complete. Four additional vials were set up as described above. All five reaction mixtures were combined at the end of the reaction. The resulting combined reaction mixture was cooled to 20°C and diluted with MeCN (180 mL). The solid material in the reaction mixture was filtered and rinsed with MeCN (80 mL) to give a dark red solid. The solid was then washed with water and dried under high vacuum to give **5** (2.7 grams, 4.65 mmols, 52% yield) as a brick-red solid. This product was used in subsequent steps without further purification. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.17 (dd, *J*=1.9, 7.8 Hz, 2H), 8.08 – 7.96 (m, 2H), 7.77 – 7.63 (m, 3H), 7.51 (d, *J*=1.8 Hz, 1H), 7.37 (d, *J*=8.6 Hz, 2H), 7.33 (br s, 2H), 6.92 (d, *J*=8.6 Hz, 2H), 5.57 – 5.42 (m, 2H), 5.04 (s, 2H), 4.01 (q, *J*=5.8 Hz, 4H), 3.79 (s, 3H), 3.74 (s, 3H).

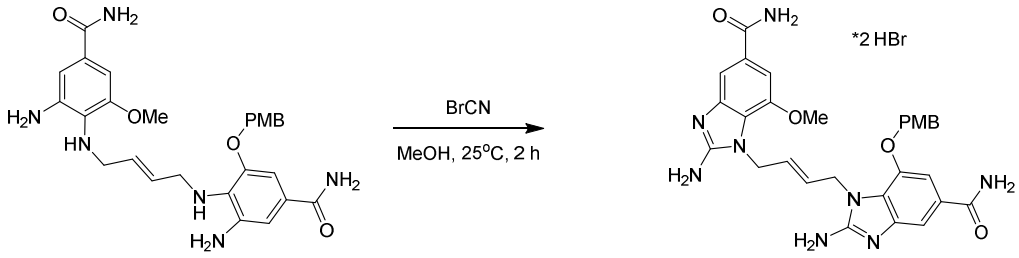
Synthesis of Compound 6



5 **6**

[0399] To a solution of **5** (2 g, 3.45 mmol, 1 equiv.) in a 1:1 (v/v) mixture of methanol and water (160 mL) was added Na_2CO_3 (10.95 g, 103 mmol, 30 equiv.) and sodium dithionite ($Na_2S_2O_4$, 8.40 g, 48.2 mmol, 14 equiv.). The resulting red reaction mixture was stirred at 25°C for 12 h, upon which the red mixture turned into a pale yellow color, and UPLC-MS analysis (Method B) showed the reaction was complete. The reaction mixture was filtered, and the filtrate was concentrated and diluted with water. The mixture was extracted with EtOAc and the organic layer was concentrated to give **6** (1.0 grams, 1.81 mmols, 52% yield) as an off-white solid. This product was used in subsequent steps without further purification. **^{1}H NMR** (400MHz, DMSO-d_6): δ = 7.61 (br s, 2H), 7.37 (d, *J*=8.6 Hz, 2H), 6.97 (br s, 2H), 6.94 (s, 1H), 6.93 - 6.90 (m, 2H), 6.86 (s, 2H), 6.77 (d, *J*=1.8 Hz, 1H), 5.71 - 5.53 (m, 2H), 4.98 (s, 2H), 4.65 (br d, *J*=12.6 Hz, 4H), 3.74 (s, 3H), 3.71 (s, 3H), 3.49 (br s, 4H).

Synthesis of Compound 7

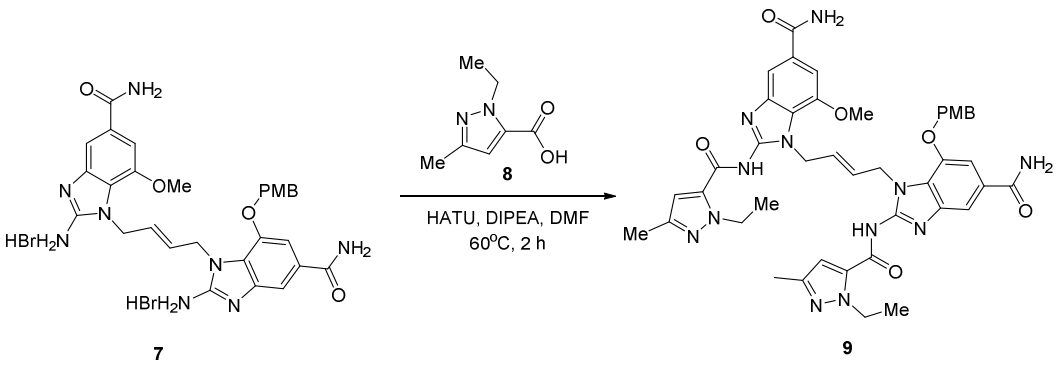


6 **7**

[0400] To a solution of **6** (1.4 g, 2.69 mmol, 1 equiv.) in methanol (20 mL) was added cyanogen bromide (BrCN, 1.71 g, 16.1 mmol, 6 equiv.). The reaction mixture was stirred at 25°C for 2 h, during which time a precipitate was observed. LC-MS analysis (Method C) showed the

reaction was complete. The solid was collected by filtration, washed with ethanol and petroleum ether to give **7** (1.2 g, 1.64 mmols,61% yield) as a light yellow solid. This product was used in subsequent steps without further purification. **LC-MS** (Method C, ESI+): m/z $[M+H]^+$ 571.2 (theoretical); 571 (observed). HPLC retention time: 1.634 min. **^{1}H NMR** (400MHz, DMSO-d_6): δ = 12.94 (br s, 2H), 8.63 (br d, *J*=12.8 Hz, 4H), 8.08 (br s, 2H), 7.62 – 7.52 (m, 3H), 7.47 (br s, 2H), 7.38 (s, 1H), 7.24 (d, *J*=8.6 Hz, 2H), 6.84 (d, *J*=8.6 Hz, 2H), 5.81 – 5.69 (m, 1H), 5.57 (td, *J*=5.4, 15.5 Hz, 1H), 5.07 (s, 2H), 4.80 (br t, *J*=6.6 Hz, 4H), 3.74 (s, 3H), 3.69 (s, 3H).

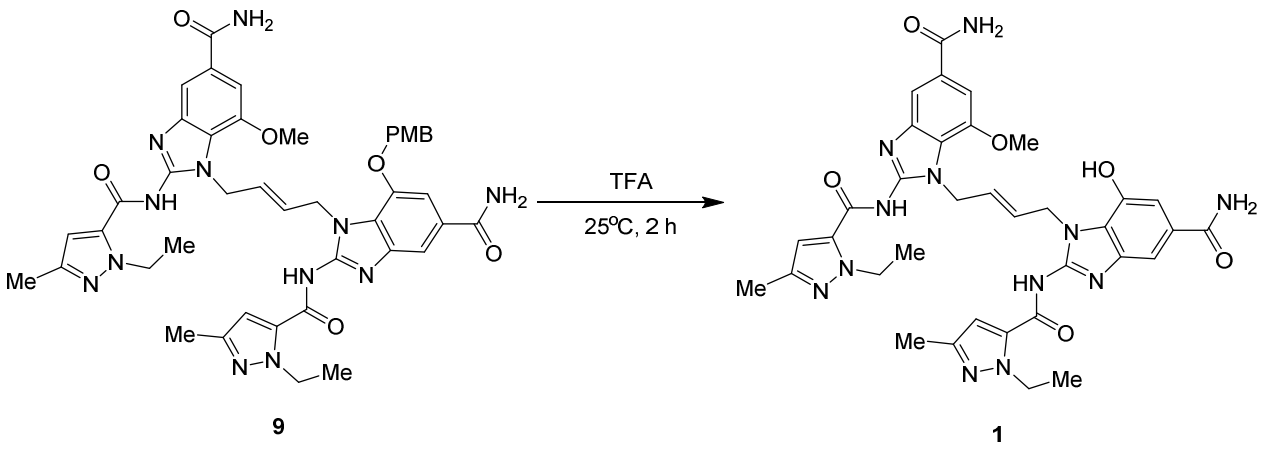
Synthesis of Compound 9



7 8 9

[0401] To a solution of compound **8** (1-ethyl-3-methyl-1H-pyrazole-5-carboxylic acid, 331 mg, 2.15 mmol, 2.1 equiv.) in dimethylformamide (DMF, 3 mL) was added 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU, 973 mg, 2.56 mmol, 2.5 equiv.) and the reaction mixture was stirred at 60°C for 10 min. A solution of DIPEA (596 mg, 4.61 mmol, 4.5 equiv.) and **7** (750 mg, 1.02 mmol, 1 equiv.) in DMF (1 mL) was then added to the reaction mixture, which was stirred at 60°C for 2 h, upon which LC-MS analysis (Method B,) showed the reaction was complete. The reaction mixture was poured into ice water, the solid was collected by filtration and dried to give a crude product. The crude product was used in the next step without further purification. **LC-MS** (Method B, ESI+): m/z $[M+H]^+$ 843.4 (theoretical); 843.4 (observed). HPLC Retention Time: 1.062 min.

Synthesis of Compound 1



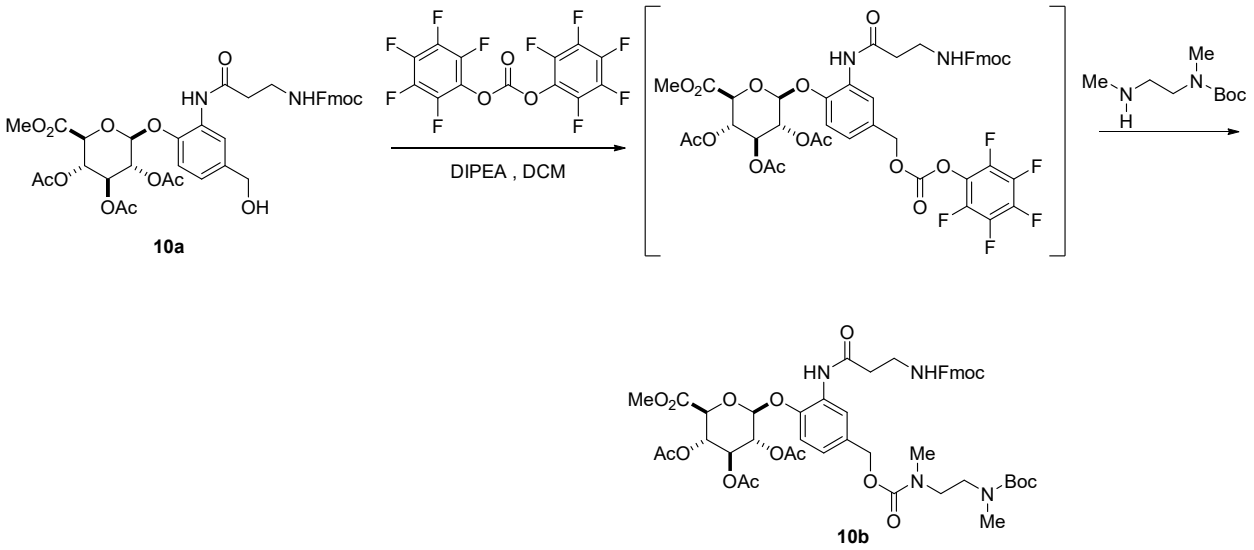
9 **1**

[0402] Compound **9** (700 mg, 0.83 mmol) was added to a glass vial containing trifluoroacetic acid (TFA, 3 mL), and the resulting mixture was stirred at 25°C for 2 h, upon which LC-MS analysis showed the reaction was complete. The TFA was removed in vacuo and the residue was dissolved in DMSO and acetonitrile and purified by preparatory HPLC (Method F,) to give **1** (40 mg, 0.055 mmols, 7% yield over 2 steps) as an off-white solid. **LCMS** (Method B, ESI+): m/z $[M+H]^+$ 723.3 (theoretical); 723.1 (observed); $[M+H]^+$, HPLC retention time:: 2.04 min. **^{1}H NMR** (400MHz, DMSO-d_6): δ = 13.00 - 12.51 (m, 2H), 10.41 (s, 1H), 7.96 (br s, 1H), 7.81 (br s, 1H), 7.63 (s, 1H), 7.43 (s, 1H), 7.37 - 7.28 (m, 2H), 7.22 (br s, 1H), 7.14 - 7.07 (m, 1H), 6.51 (br d, *J*=11.0 Hz, 2H), 5.97 - 5.75 (m, 2H), 4.91 (br dd, *J*=3.5, 16.3 Hz, 4H), 4.51 (br d, *J*=3.3 Hz, 4H), 3.77 (s, 3H), 2.10 (d, *J*=6.0 Hz, 6H), 1.25 (dt, *J*=3.6, 6.9 Hz, 6H).

Synthesis of (2S,3S,4S,5R,6S)-6-(4-((((2-((((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)carbonyl)(methyl)amino)ethyl)(methyl)carbamoyl)oxy)methyl)-3-(3-(3-(2,5-dioxo-2,5-

dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (Compound 11)

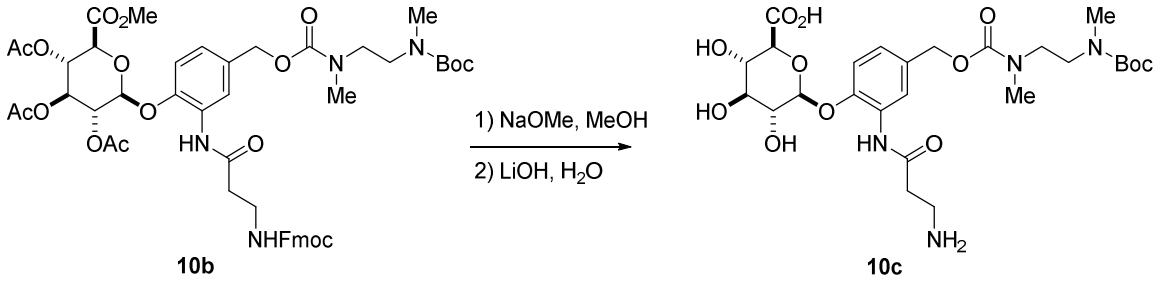
Synthesis of Compound 10b



[0403] Compound **10a** was prepared as previously reported (*ACS Med. Chem. Lett.* **2010**, 1, 6, 277–280).

[0404] An oven-dried 4 mL glass vial was charged with **10a** (150 mg, 0.20 mmol, 1 equiv.) and pentafluorophenyl carbonate (88 mg, 0.22 mmol, 1.1 equiv.), DMF (1 mL) and DIPEA (0.15 mL, 0.86 mmol, 4.3 equiv.). The reaction mixture was stirred at room temperature for 30 minutes upon which a light pink homogenous solution was observed. *Tert*-butyl methyl(2-(methylamino)ethyl)carbamate (50 uL, 0.27 mmol, 1.3 equiv.) was added to the solution, which resulted in the reaction mixture turning to a light yellow color. The reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with water (50 mL), transferred to a separatory funnel and extracted with EtOAc (3x50 mL). The organic layers were collected and combined, washed with 1M HCl, dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. The resulting solid was purified by flash column chromatography (25g SiO_2 column, eluting with 0 - 25% MeOH in DCM) to yield **10b** as a light yellow solid (70.4 mg, 0.073 mmol, 36% yield). UPLC-MS (Method E, ESI+) m/z $[(M\text{-Boc})+2H]^+$: 863.33 (theoretical); 863.14 (observed). HPLC retention time: 1.54 min.

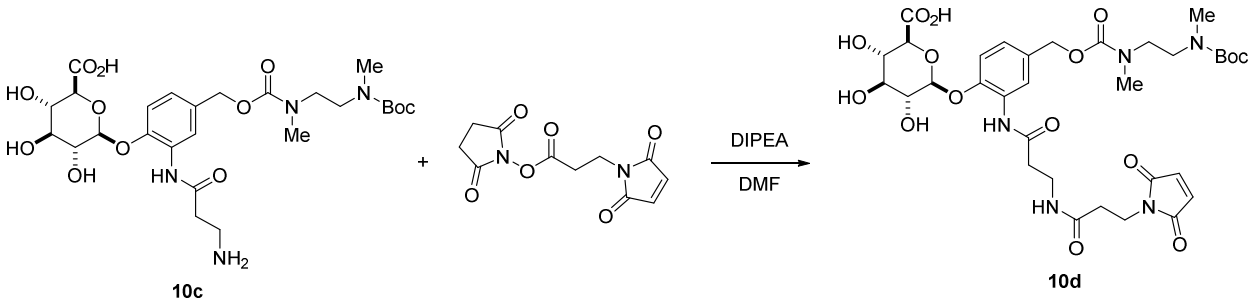
Synthesis of Compound 10c



10b 10c

[0405] Compound **10b** (70.4 mg, 0.073 mmol, 1 equiv.) was transferred as a solution in MeOH to an oven-dried 4 mL glass vial equipped with a magnetic stir bar. The MeOH was removed under vacuum and the vial back-filled with argon. To the vial, under Ar, was added MeOH (0.5 mL) and the resulting solution was cooled to 0 °C and sodium methoxide (0.5 M solution in MeOH, 150 uL, 0.075 mmol, 1 equiv.) was added. The reaction was monitored by LC-MS (Method D) and upon complete removal of all three acetate groups, lithium hydroxide (1M in water, 0.225 mL, 0.225 mmol, 3 equiv.) was added and the reaction mixture was stirred at room temperature for 30 min. DMSO (0.5 mL) and glacial acetic acid (50 uL) were added to the reaction mixture, yielding a homogenous solution. The crude product was purified by preparatory HPLC (Method H, 5 – 40% MeCN in water with 0.05% TFA as mobile phase additive) to give **10c** as a white solid (16.8 mg, 0.028 mmol, 38% yield). UPLC-MS (Method D, ESI+): m/z $[M+H]^+$ 601.26 (theoretical); 601.15 (observed). HPLC retention time: 1.09 min.

Synthesis of Compound 10d

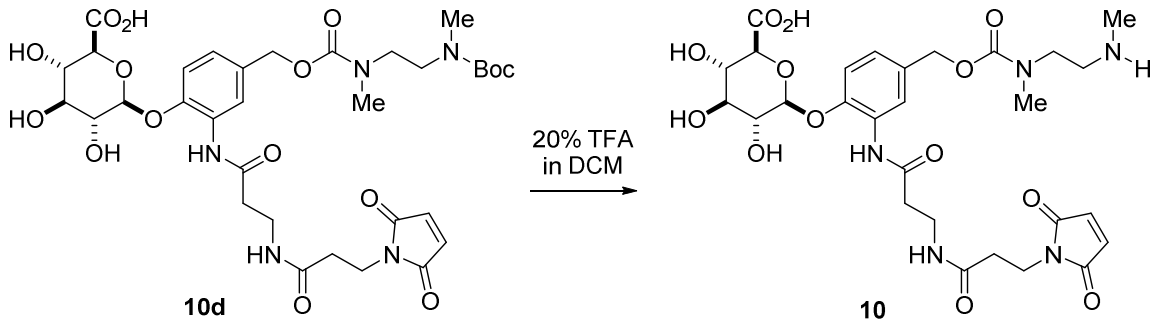


10c 10d

[0406] Compound **10c** (16.8 mg, 0.028 mmol, 1 equiv.) was added to an oven-dried 4 mL glass vial equipped with a magnetic stir bar as a solution in MeOH. The MeOH was removed under vacuum and the vial filled with argon. To the vial was added 3-(maleimido)propionic acid N-hydroxysuccinimide ester (MP-OSu, 16 mg, 0.06 mmol, 2 equiv.) followed by DMF (0.5 mL)

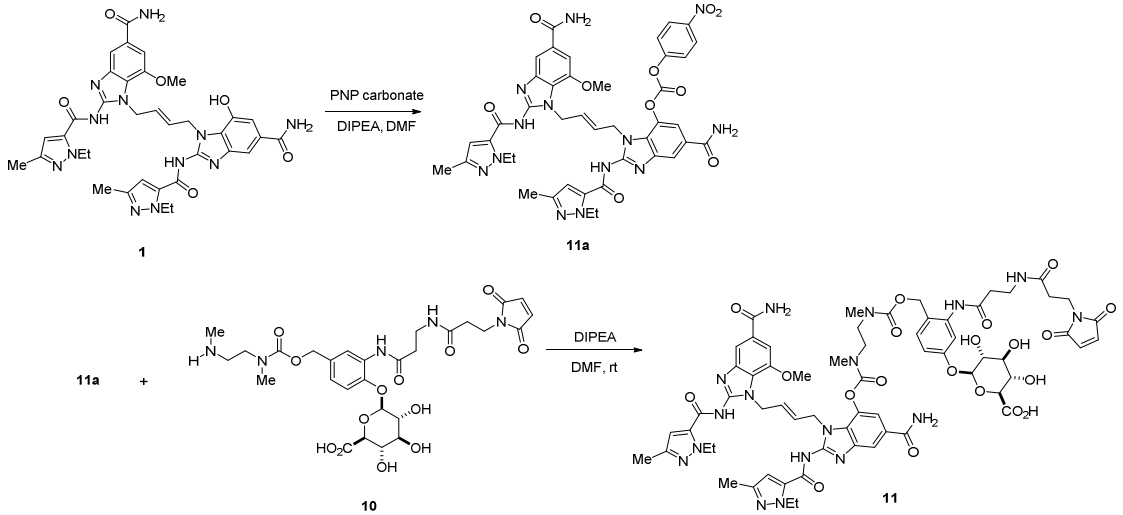
and DIPEA (50 uL, 0.28 mmol, 10 equiv.). After 15 minutes, DMSO (0.5 mL) and glacial acetic acid (100 uL) were added and the crude product purified by preparatory HPLC (Method H, 10 – 60% MeCN in water with 0.05% TFA as mobile phase additive) to give **10d** as a white solid (15 mg, 0.020 mmol, 71% yield). UPLC-MS (Method A, ESI+): m/z $[M+H]^+$: 752.29 (theoretical); 752.26 (observed). HPLC retention time: 1.27 min.

Synthesis of Compound 10



[0407] Compound **10d** (15 mg, 0.020 mmols, 1 equiv.) was dissolved in 20% (v/v) TFA in DCM (1 mL) and transferred to a 4 mL glass vial equipped with a magnetic stir bar. The vial was left uncapped and the reaction progress was monitored by LC-MS. After 2h, the solvent was removed *in vacuo* to give **10** as a white solid (13 mg, 0.02 mmol, quantitative yield) which was used in subsequent steps without any further purification. UPLC-MS (Method D, ESI+): m/z $[M+H]^+$: 652.24 (theoretical); 652.45 (observed). HPLC retention time: 0.69 min.

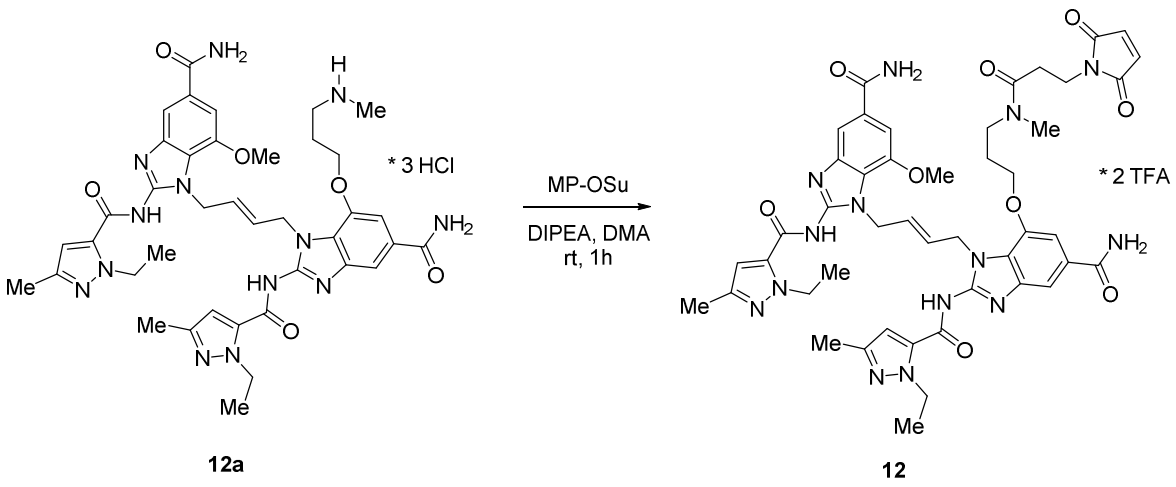
Synthesis of Compound 11



[0408] To an oven-dried 4 mL glass vial was added Compound **1** (9.5 mg, 0.010 mmol, 1 equiv.) followed by DMF (0.5 mL), *p*-nitrophenyl carbonate (9.0 mg, 0.030 mmol, 3 equiv.) and DIPEA (20 uL, 0.115 mmol, 11.5 equiv.). The reaction mixture was stirred at room temperature for 1 hour at which point full conversion to **11a** was confirmed by UPLC-MS analysis (Method D). Compound **10** (20 mg, 0.031 mmol, 3.1 equiv.) was added in a single portion to the reaction mixture which was stirred at room temperature for 2 h. Glacial acetic acid (20 uL) was added and the crude product purified by preparatory HPLC (Method H, 0 – 45% MeCN in water with 0.05% TFA as mobile phase additive). The fractions containing **11** were combined and the solvent was removed via lyophilization to give **11** (6.31 mg, 0.0039 mmol, 39% yield). Compound **1** was also recovered (2.81 mg, 0.0030 mmol, 30% recovery) as a white fluffy solid. UPLC-MS (Method D, ESI+): m/z $[M+H]^+$: 1400.52 (theoretical); 1400.25 (observed) & $[M+2H]^{2+}$ = 701.43 (observed). HPLC retention time: 1.28 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-*pyrrol-*

1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 12)



12a **12**

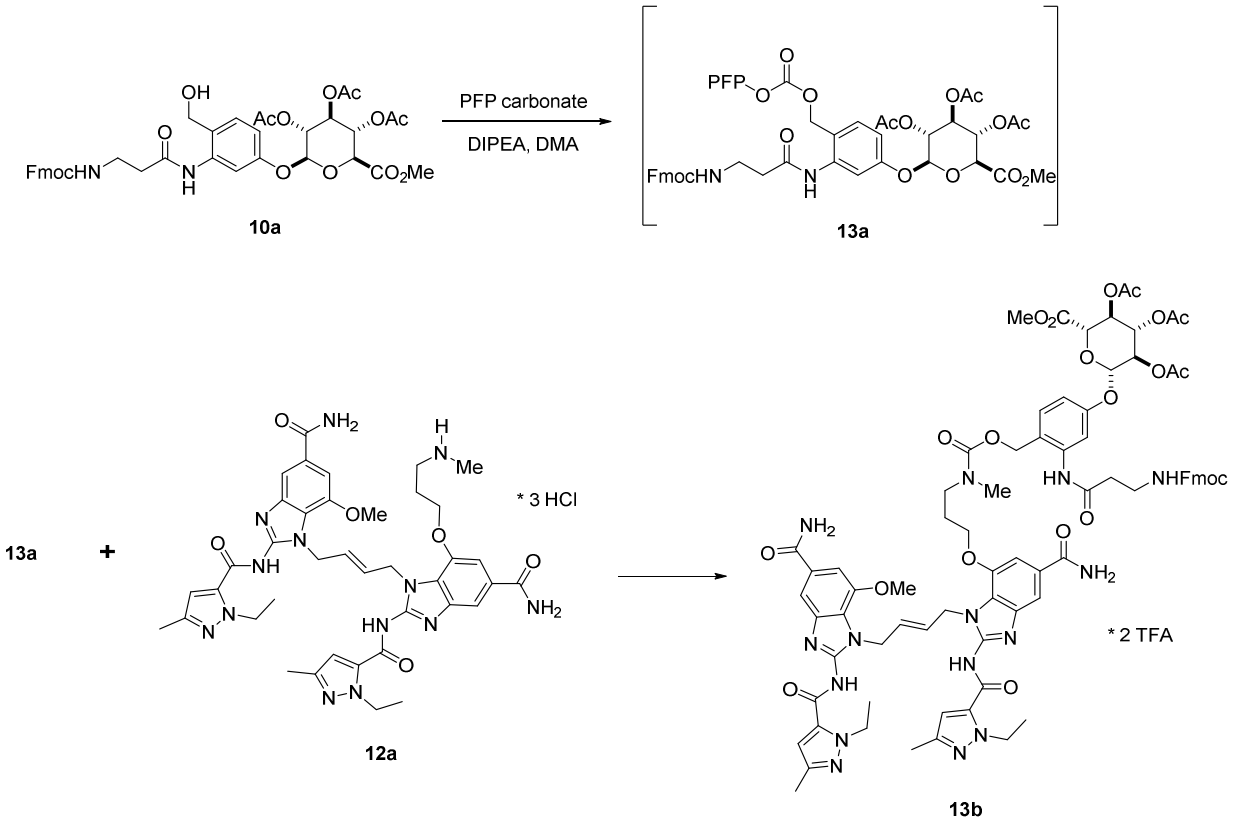
[0409] Compound **12a** was prepared as previously reported (WO2017/175147, example 40, page 292).

[0410] To a solution of **12a** (28.7 mg, 0.032 mmol, 1.0 equiv.) in DMA (635 µL) was added MP-OSu (15.9 mg, 0.0596 mmol, 1.9 equiv.), and DIPEA (35 µL, 0.199 mmol, 6.2 equiv.). The reaction mixture was stirred for 1 h at room temperature. Upon completion, the solution was concentrated under reduced pressure and the crude product was purified by preparatory HPLC (Method G, 20-50-95% MeCN in water with 0.1% formic acid as mobile phase additive) to yield **12** (46% yield, 17.8 mg, 0.0152 mmol). UPLC-MS (Method D, ESI+): m/z $[M+H]^+$ 945.40 (theoretical); 945.72 (observed). HPLC retention time: 1.79 min.

Synthesis of (2S,3S,4S,5R,6S)-6-(4-((((3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)carbamoyl)oxy)methyl)-3-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-

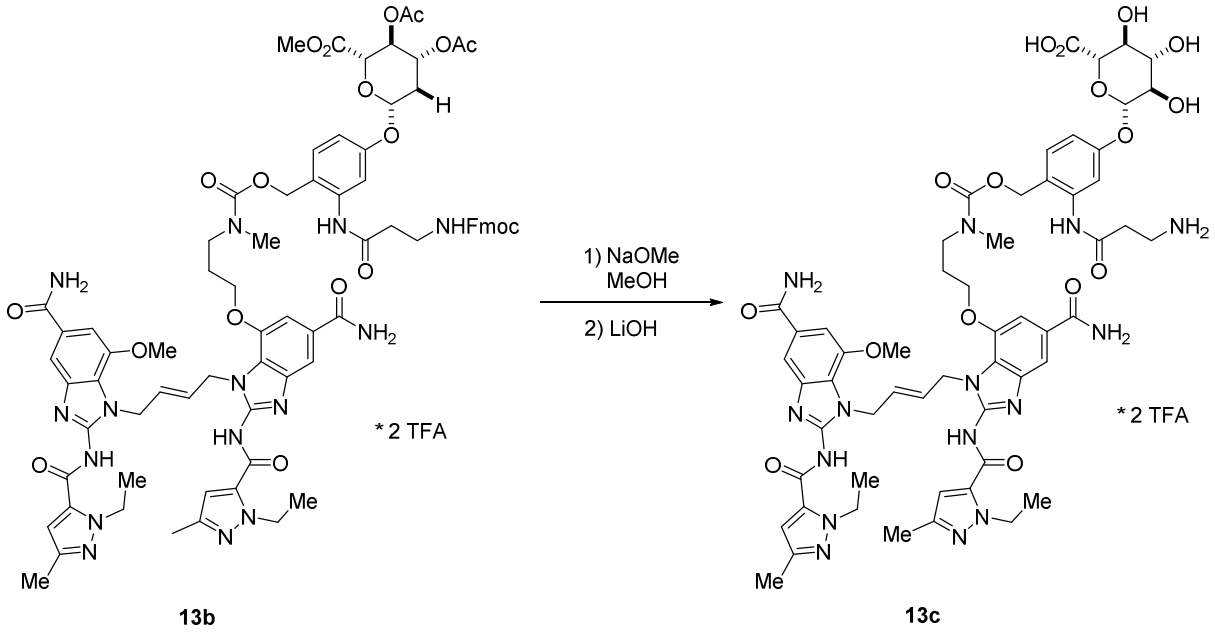
yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (Compound 13)

Synthesis of Compounds 13a and 13b



[0411] Compound **10a** (13 mg, 0.017 mmol) was dissolved in DMA (87 µL). To this solution was added pentafluorophenyl carbonate (13.7 mg, 0.035 mmol), and DIPEA (14 µL, 0.078 mmol). The mixture was stirred for 30 min at room temperature. Upon full conversion to intermediate **13a**, this solution was transferred to a second vial containing **12a** (10.6 mg, 0.012 mmol). The reaction mixture was stirred for 18 h at room temperature. The reaction was then diluted with water and extracted three times with EtOAc (20 mL x 3). The combined organic layers were then washed with 1M HCl. The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated in vacuo. The product was purified by preparatory HPLC (Method H, 5-50-95% MeCN in water using 0.05% TFA as mobile phase additive) to yield compound **13b** as a trifluoroacetate salt (10.0 mg, 0.0056 mmol, 48% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1568.60 (theoretical); 1568.95 (observed). HPLC retention time: 1.70 min.

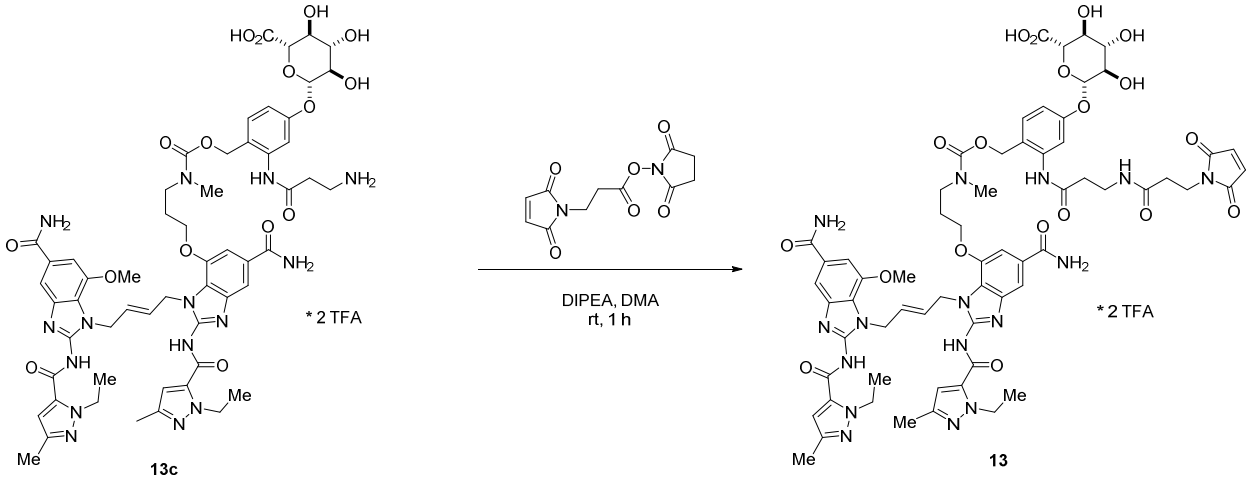
Synthesis of Compound 13c



13b 13c

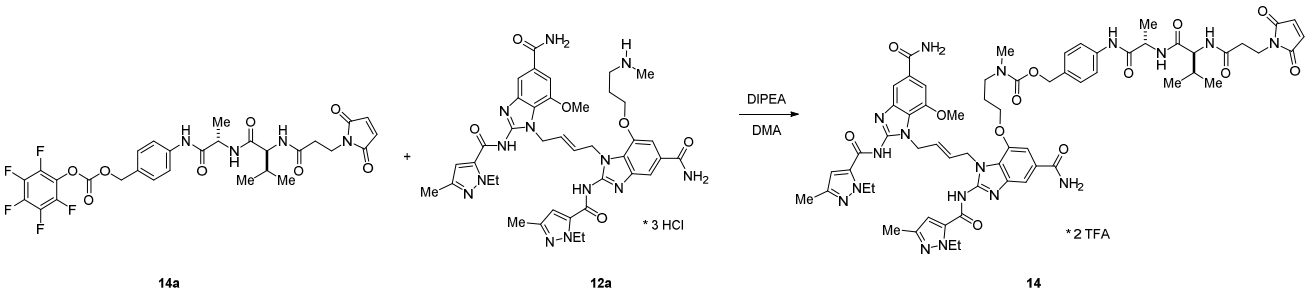
[0412] To a dry, well purged glass vial was added compound **13b** (10.0 mg, 0.0056 mmol) in anhydrous methanol (40 µL). The solution was cooled in an ice bath, and NaOMe (0.5 M in MeOH, 11.13 µL) was added. After about 1 h, 1 M aqueous LiOH (17 µL, 0.017 mmols, 3 equiv.) solution was added. Significant white precipitate formed upon the addition of the LiOH solution. After 1 hr, glacial acetic acid (12 µL) was added, and the solvents were removed in vacuo. The crude product was purified by preparatory HPLC (Method G, 20-60-95% MeCN in water, with 0.05% TFA as mobile phase additive) to yield compound **13c** as trifluoroacetate salt (4.13 mg, 0.0029 mmol, 52% yield). UPLC-MS (Method D, ESI+): m/z $[M+H]^+$ = 1206.49 (theoretical); 1206.50 (observed). HPLC retention time: 1.45 min.

Synthesis of Compound 13



[0413] Compound **13c** (4.13 mg, 0.00342 mmol, 1.0 equiv.) was dissolved in DMA (68 µL) in a glass vial under argon. MP-OSu (1.82 mg, 0.00685 mmol, 2 equiv.) and DIPEA (3.0 µL, 0.0171 mmol, 5 equiv.) were added and the reaction mixture was stirred for 1 h at RT. Glacial acetic acid (3.0 µL) was added, and the crude product purified by preparatory HPLC (Method G, 10-60-95% MeCN in water using 0.1% formic acid as mobile phase additive) to yield **13** as trifluoroacetate salt (5.43 mg, 0.0034 mmol, 93% yield). UPLC-MS (Method E, ESI+): m/z $[M+H]^+$ = 1357.52 (theoretical); 1357.82 (observed). HPLC retention time: 1.54 min.

Synthesis of 4-((S)-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-methylbutanamido)propanamido)benzyl (3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)carbamate (Compound 14)

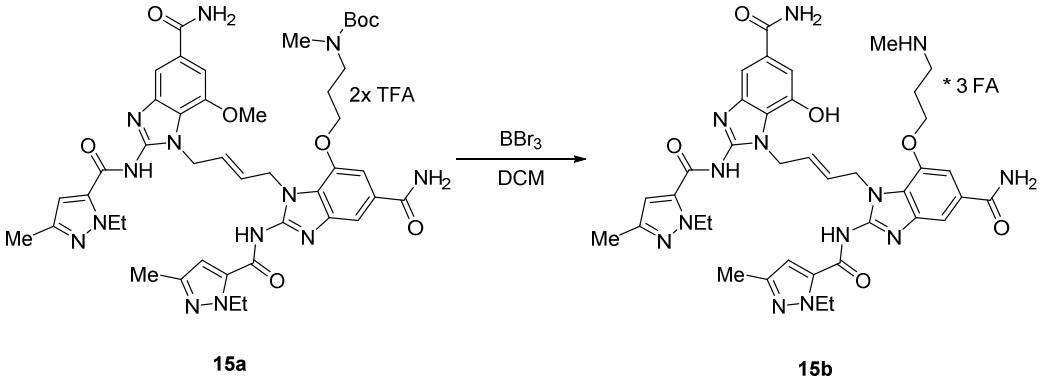


[0414] To a dry glass vial charged with compound **12a** (2.6 mg, 0.0033 mmol) was added DMA (66 µL) followed by MP-Val-Ala-PAB-Opfp (**14a**, 3.2 mg, 0.049 mmol, 15 equiv.)

and DIPEA (2.8 µL, 0.016 mmol, 4.9 equiv.). The reaction mixture was stirred for 30 minutes at RT and then glacial acetic acid (2.85 µL) was added, and the crude product purified by preparatory HPLC (Method G, 30-60-95% MeCN in water, with 0.1% formic acid as mobile phase additive), to yield compound **14** as trifluoroacetate salt (4.0 mg, 0.0027 mmol, 82% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1264.56 (theoretical); 1264.85 (observed). HPLC retention time: 1.75 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-hydroxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 15)

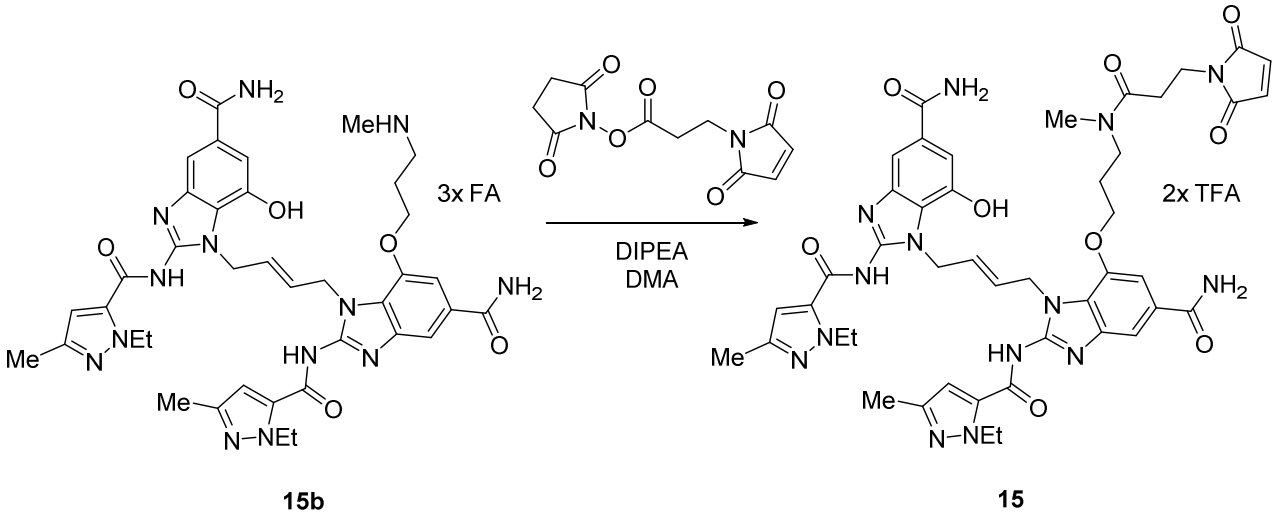
Synthesis of Compound 15b



[0415] Compound **15a** was prepared as previously reported (WO2017/175147, page 292)

[0416] To a dry glass vial containing compound **15a** (31.4 mg, 0.0280 mmol) in DCM (280 µL) was added boron tribromide (BBr_3, 1M in DCM, 168 µL, 0.168 mmol, 6 equiv.) dropwise. The reaction mixture was stirred at 40 ºC for 18 h. The reaction mixture was cooled to RT and cold water (170 µL) was slowly added. The resulting mixture was concentrated *in vacuo* and purified by preparatory HPLC (20-50-95%, 0.1% formic acid in acetonitrile, Method G). Fractions containing the desired product were combined and solvent removed via lyophilization to yield compound **15b** as the formate salt (17% yield, 4.36 mg, 0.0047 mmol). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 780.36 (theoretical); 780.38 (observed). HPLC retention time: 1.33 min.

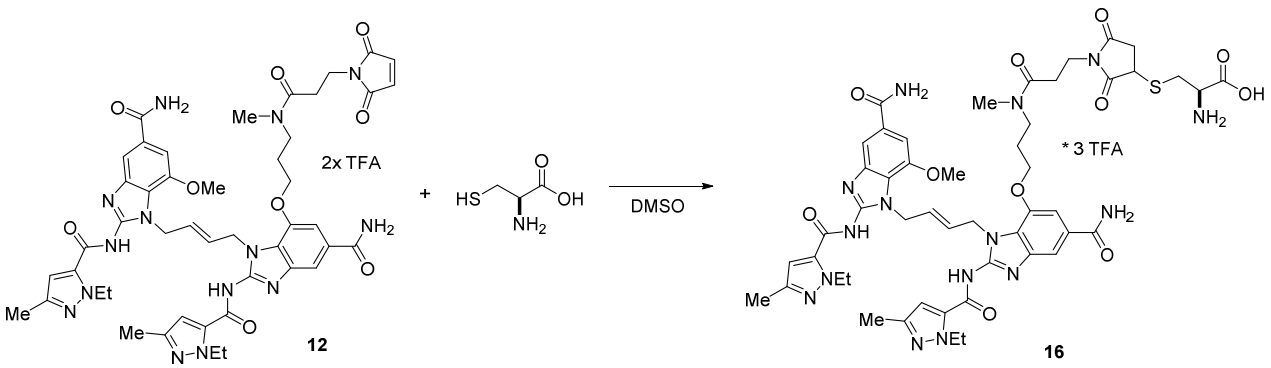
Synthesis of Compound 15



[0417] To a dry 4 mL vial containing 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (MP-OSu, 1.7 mg, 0.0063 mmol) was added compound **15b** (3.9 mg, 0.0042 mmol) as a solution in DMA (423 µL). To the mixture was added DIPEA (3.7 µL, 0.0211 mmol, 5 equiv.) and the reaction mixture was stirred for 30 min at RT, after which glacial acetic acid (3.68 µL) was added, and the product was purified via preparatory HPLC (10-40-95%, 0.05% TFA in acetonitrile, Method G). Fractions containing the desired product were combined and solvents removed via lyophilization to yield compound **15** as trifluoroacetate salt (20% yield, 1.0 mg, 0.0009 mmol). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 931.39 (theoretical); 931.41 (observed). HPLC retention time: 1.62 min.

Synthesis of S-(1-(3-((3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-

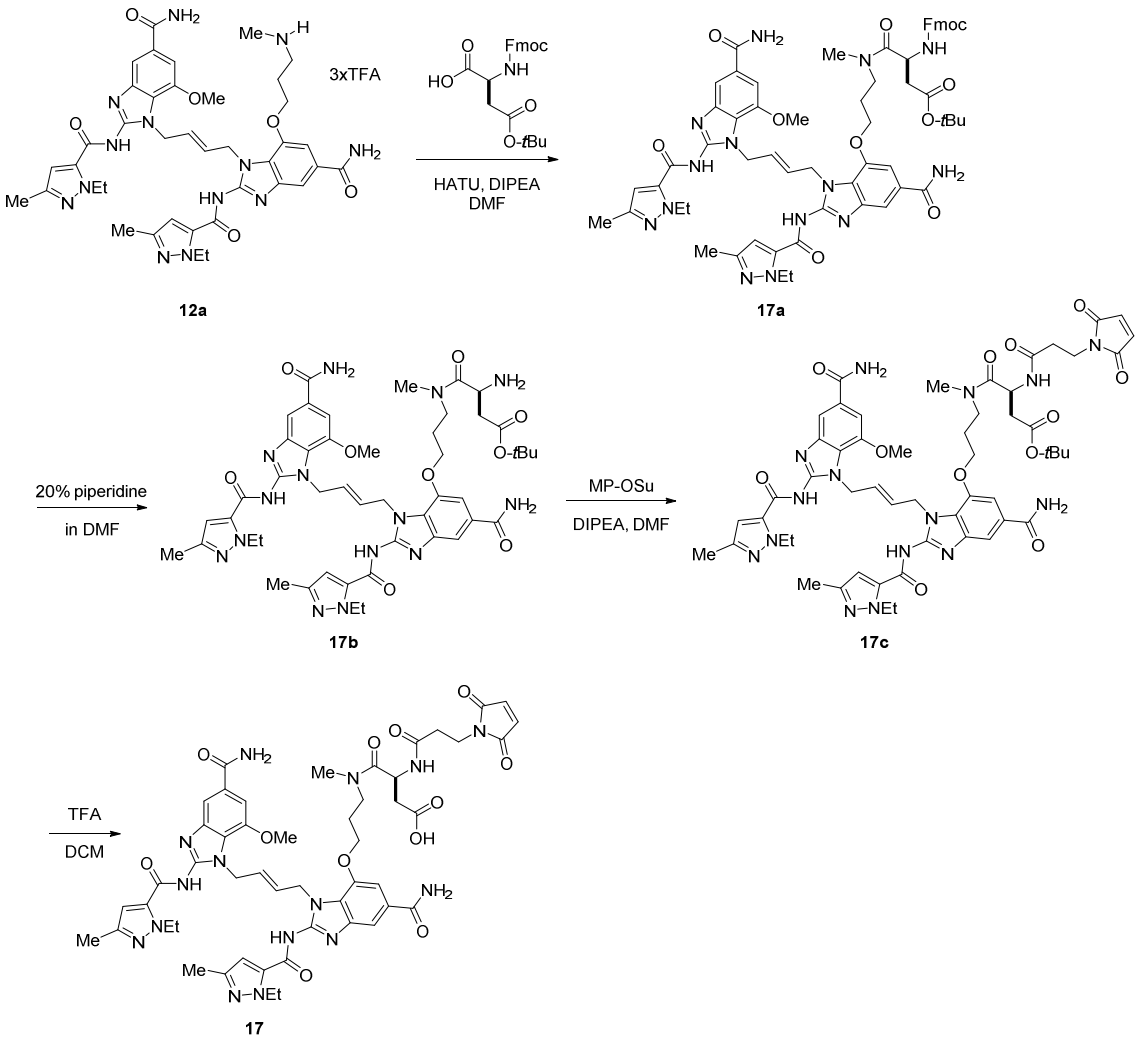
methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)amino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 16)



[0418] Compound **12** (1.5 mg, 0.0015 mmol, 1 equiv.) was dissolved in DMSO (50 µL). L-cysteine (1 M, 2.2 µL, 0.0022 mmols, 1.5 equiv.) was added as a solution in water. The reaction mixture was stirred at 30 ºC for 30 min, and subsequently purified directly via preparatory HPLC (30-70-95%, 0.05% TFA in acetonitrile, Method G). Fractions containing the desired product were combined and frozen. The solvents were removed via lyophilization to yield compound **16** as the trifluoroacetate salt (49% yield, 1.03 mg, 0.0007 mmol). UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1066.42 (theoretical); 1066.44 (observed). HPLC retention time: 1.65 min.

Synthesis of (S,E)-4-((3-((5-carbamoyl-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl1H-

pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)amino)-3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-4-oxobutanoic acid (Compound 17)



Synthesis of Compound 17a

[0419] An oven dried 4 mL vial equipped with a stir bar was charged with compound **12a** (10 mg, 0.011 mmol, 1.0 equiv.), Fmoc-aspartate 4-tert-butyl ester (9.1 mg, 0.022 mmol, 2.0 equiv.) and HATU (8.4 mg, 0.022 mmol, 2.0 equiv.), followed by DMF (0.5 mL) and DIPEA (9.6 uL, 0.055 mmol, 5.0 equiv.). The reaction mixture was stirred at room temperature overnight and full conversion to the amide was observed. Solvent was removed in vacuo, and the resulting crude oil was dissolved in DCM and the desired product isolated by flash chromatography (10g SiO_2, 0 - 40% MeOH in DCM) to give **17a** (12 mg, 0.0104 mmol, 94% yield) as a light brown solid. The isolated material still contained some impurities, but was used

in subsequent steps without further purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1187.54 (theoretical); 1187.53 (observed). HPLC retention time: 2.40 min.

Synthesis of Compound 17b

[0420] An oven dried 4 mL vial equipped with a stir bar was charged with **17a** (12 mg, 0.0104 mmol, 1.0 equiv.) and 20% piperidine in DMF (1 mL). The reaction mixture was stirred for 1 hour, solvent removed *in vacuo* and product purified by prepHPLC (Method G, 5 - 95% acetonitrile in water) to yield **17b** (9.3 mg, 0.0096 mmol, 93 % yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 965.47 (theoretical); 965.48 (observed). HPLC retention time: 1.68 min.

Synthesis of Compound 17c

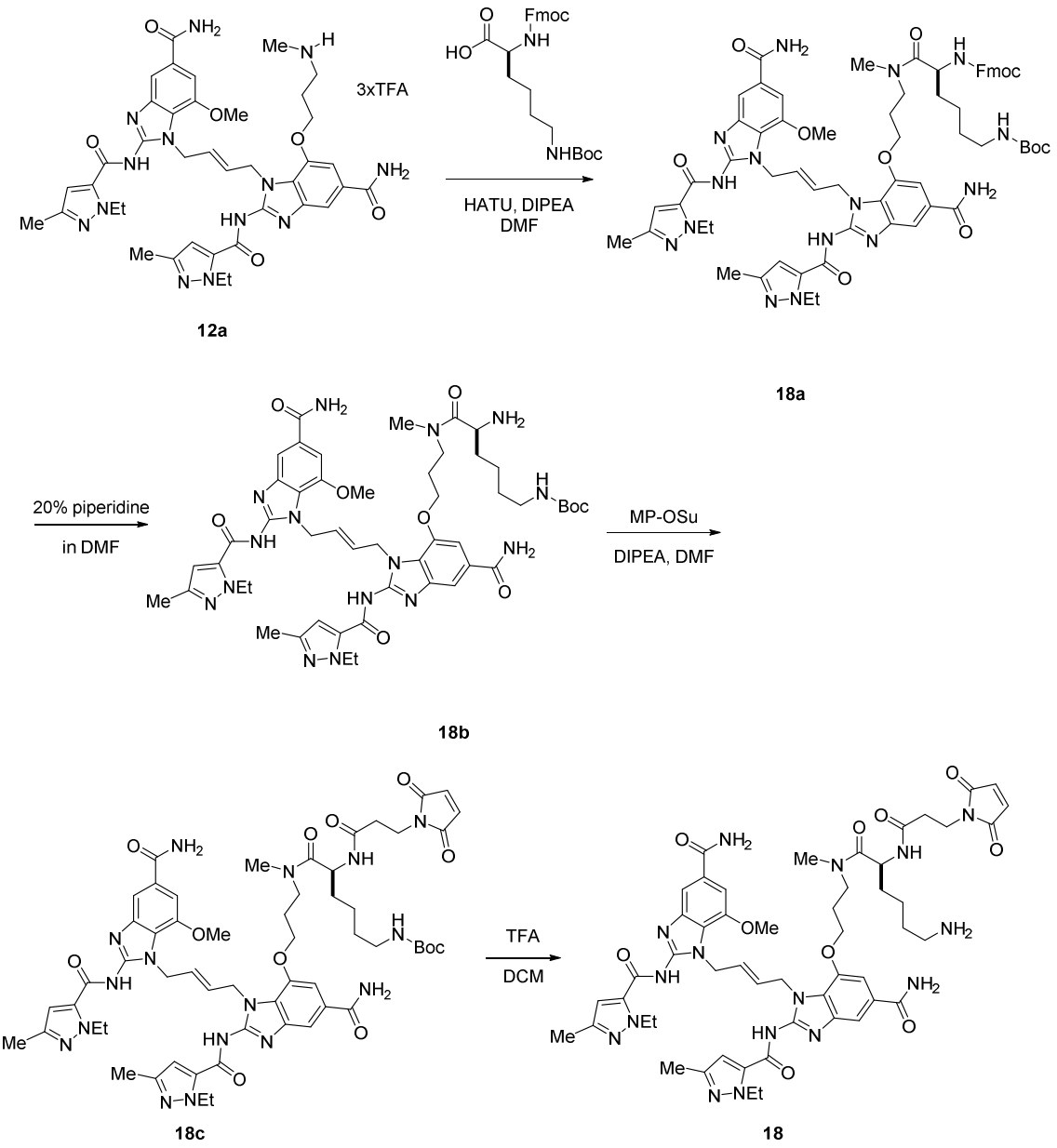
[0421] A stock solution of MP-OSu and DIPEA was prepared by dissolving 7.7 mg of MP-OSu and 10 µL of DIPEA in 1.0 mL of DMF. An oven dried 4 mL vial equipped with a stir bar was charged with **17b** (9.3 mg, 0.0096 mmol, 1.0 equiv.) and 0.5 mL of the stock solution containing MP-OSu (3.8 mg, 0.014 mmol, 1.5 equiv.) and DIPEA (0.029 mmol, 3 equiv.) was added to the vial. The reaction mixture was stirred at room temperature for 2 hours and solvent removed *in vacuo* to yield crude **17c**, which was used in the next step without any further purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1116.50 (theoretical); 1116.80 (observed). HPLC retention time: 1.51 min.

Synthesis of Compound 17

[0422] A 4 mL vial was charged with compound **17c** (10.7 mg, 0.0096 mmol, 1 equiv.) dissolved in 20% (v/v) TFA in DCM (1 mL) and the reaction mixture was stirred at room temperature for 3 hours. Solvent was subsequently removed *in vacuo*, and the crude product was dissolved in DMSO (0.75 mL) and purified by prepHPLC (Method G, 5 - 50% MeCN in water) to give Compound **17** (5.4 mg, 0.0051 mmol, 53% yield) as a white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1060.44 (theoretical); 1061.12 (observed). HPLC retention time: 1.28 min.

Synthesis of (S,E)-7-(3-(6-amino-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-N-methylhexanamido)propoxy)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-

carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 18)



Synthesis of Compound 18a

[0423] An oven dried 4 mL vial equipped with a stir bar was charged with HATU (7.8 mg, 0.021 mmol, 2.0 equiv.) and Fmoc-lysine N-epsilon-Boc (9.6 mg, 0.021 mmol, 2.0 equiv.); to which was added a solution of compound **12a** (9.3 mg, 0.0103 mmol, 1.0 equiv.) and DIPEA (9 uL, 0.051 mmol, 5 equiv.) in DMF (0.5 mL). The vial was capped and sealed with parafilm and

the mixture was stirred at RT overnight. Full conversion was observed by UPLC-MS (Method D). Solvent was removed *in vacuo* and product was purified by flash chromatography (10g SiO_2, 0 – 40% MeOH in DCM) to give **18a** (12 mg, 0.0097 mmol, 95%) as a tan solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1244.60 (theoretical); 1244.61 (observed). HPLC retention time: 2.40 min.

Synthesis of Compound 18b

[0424] An oven-dried 4 mL vial equipped with a stir bar was charged with **18a** (12 mg, 0.0096 mmol) and 20% (v/v) piperidine in DMF (1 mL) was added to the reaction. The reaction mixture was stirred until full conversion was observed by UPLC-MS (Method D), which took approximately 1 hour. Solvent was removed *in vacuo* and product was purified by preparatory HPLC (Method G, 5 - 95% MeCN in water with 0.1% (v/v) formic acid). The HPLC solvents were removed *in vacuo* to give **18b** (4.2 mg, 0.0041 mmol, 36%) as an off-white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1022.53 (theoretical); 1022.80 (observed). HPLC retention time: 1.30 min.

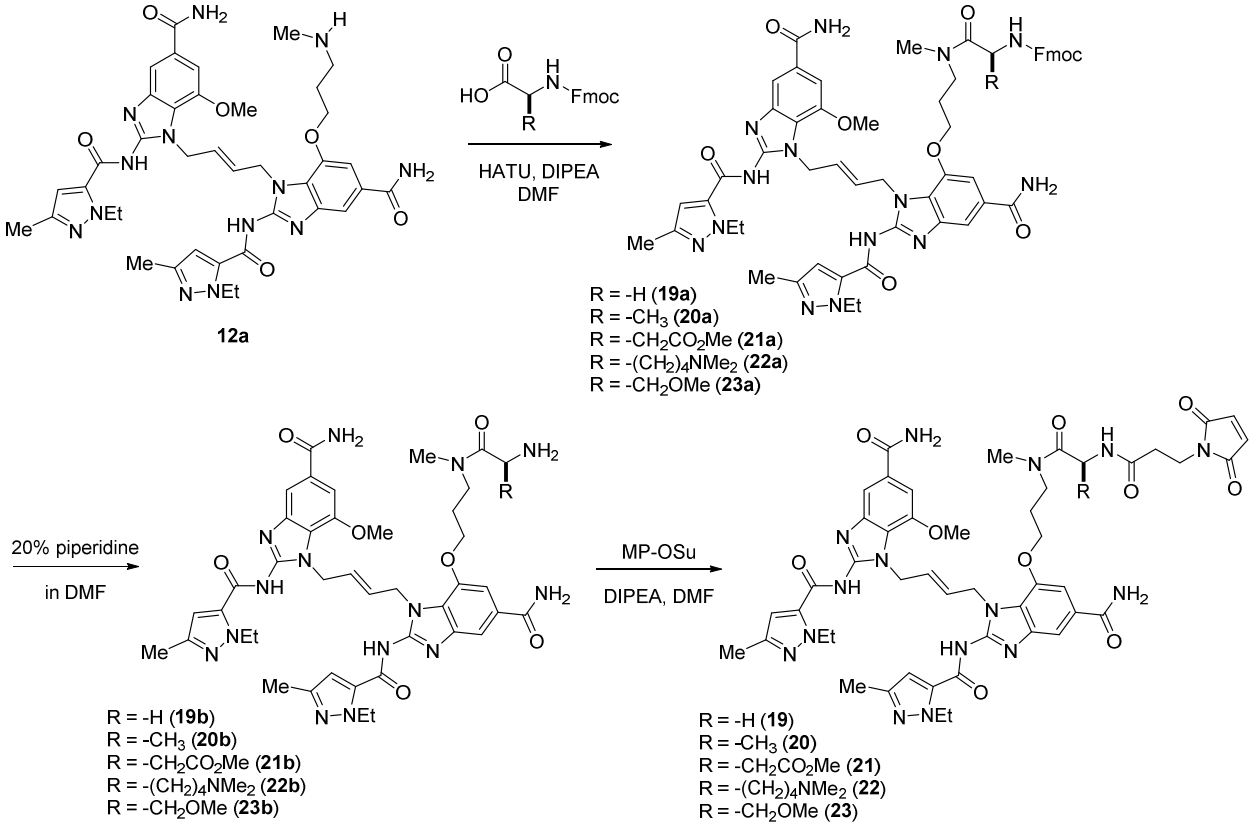
Synthesis of Compound 18c

[0425] An oven-dried 4 mL vial equipped with a stir bar was charged with **18b** (4.2 mg, 0.0034 mmol, 1 equiv.), followed by MP-OSu (1.8 mg, 0.0068 mmol, 2.0 equiv.), DIPEA (5.9 µL, 0.034 mmol, 10 equiv.) and DMF (0.8 mL). The reaction mixture was stirred at room temperature for 3 hours at which point UPLC-MS (Method D) analysis showed full conversion. Solvent was removed *in vacuo* to yield the crude product **18c**, which was used in the next step without purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1173.56 (theoretical); 1173.94 (observed). HPLC retention time: 1.54 min.

Synthesis of Compound 18

[0426] An oven-dried 4 mL vial containing a stir bar was charged with crude **18c** from the previous step (0.0034 mmol,) and 20% (v/v) TFA in DCM (1 mL) was added. The reaction mixture was stirred for one hour and the product was subsequently purified by preparatory HPLC (Method G, 5 – 50% MeCN in water with 0.1% (v/v) formic acid). The HPLC solvents were removed *in vacuo* to give **18c** (4.2 mg, 0.0035 mmol, 56% yield over 2 steps) as a white solid.

UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1073.51 (theoretical); 1073.73 (observed). HPLC retention time: 1.15 min.



General Methods for HATU coupling, Fmoc deprotection, and MP coupling

[0427] HATU coupling (Method 1): An oven-dried 4 mL vial equipped with a stir bar was charged with compound **12a** (1.0 equiv.), HATU (2.0 equiv.), DIPEA (5 equiv.) and DMF (20 mM in **12a**) and the reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and product purified via chromatography.

[0428] Fmoc deprotection (Method 2): An oven-dried 4 mL vial equipped with a stir bar was charged with the HATU coupled product from above, which was dissolved in 20% (v/v) piperidine in DMF (1 mL). The reaction mixture was stirred at room temperature for 1 hour, solvent removed *in vacuo*, and product purified via chromatography.

[0429] MP coupling (Method 3): An oven-dried 4 mL vial equipped with a stir bar was charged with the product from the previous reaction, to which was added MP-OSu (2 equiv.) and DIPEA (10 equiv.) and DMF (10 mM in Fmoc-deprotected amine starting material). The reaction

mixture was stirred at room temperature for 3 hours, solvent removed *in vacuo* and product purified by preparatory HPLC.

[0430] Compound **19a** was prepared according to General Method 1(8.0 mg, 0.0075 mol, 85% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1073.47 (theoretical); 1074.03 (observed). HPLC retention time: 1.76 min.

[0431] Compound **19b** was prepared according to General Method 2 (6.1 mg, 0.0072 mol, 95% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 851.41 (theoretical); 851.69 (observed). HPLC retention time: 1.15 min.

[0432] Compound **19** was prepared according to General Method 3 (4.3 mg, 0.0043 mol, 60% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1002.43 (theoretical); 1002.72 (observed). HPLC retention time: 1.31 min.

[0433] Compound **20a** was prepared according to General Method 1(8.7 mg, 0.0080 mol, 91% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1087.49 (theoretical); 1087.90 (observed). HPLC retention time: 1.75 min.

[0434] Compound **20b** was prepared according to General Method 2 (5.6 mg, 0.0065 mol, 81% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 865.42 (theoretical); 865.66 (observed). HPLC retention time: 1.12 min.

[0435] Compound **20** was prepared according to General Method 3 (3.4 mg, 0.0034 mol, 52% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1016.45 (theoretical); 1017.08 (observed). HPLC retention time: 1.33 min.

[0436] Compound **21a** was prepared according to General Method 1 (14 mg, 0.0119, mmol). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1145.50 (theoretical); 1145.42 (observed). HPLC retention time: 1.74 min.

[0437] Compound **21b** was prepared according to General Method 2 (7.2 mg, 0.0078 mol, 76% yield over 2 steps). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 923.43 (theoretical); 923.67 (observed). HPLC retention time: 1.13 min.

[0438] Compound **21** was prepared according to General Method 3 (1.5 mg, 0.0014 mols, 22% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1074.45 (theoretical); 1074.90 (observed). HPLC retention time: 1.36 min.

[0439] Compound **22a** was prepared according to General Method 1 (7.6 mg, 0.0065 mols, 63% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1172.58 (theoretical); 1172.59 (observed). HPLC retention time: 1.84 min.

[0440] Compound **22b** was prepared according to General Method 2 (6.1 mg, 0.0064 mmols, 57% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 950.51 (theoretical); 950.83 (observed). HPLC retention time: 0.99 min.

[0441] Compound **22** was prepared according to General Method 1 (2.6 mg, 0.0023 mols, 37% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1101.54 (theoretical); 1101.96 (observed). HPLC retention time: 1.18 min.

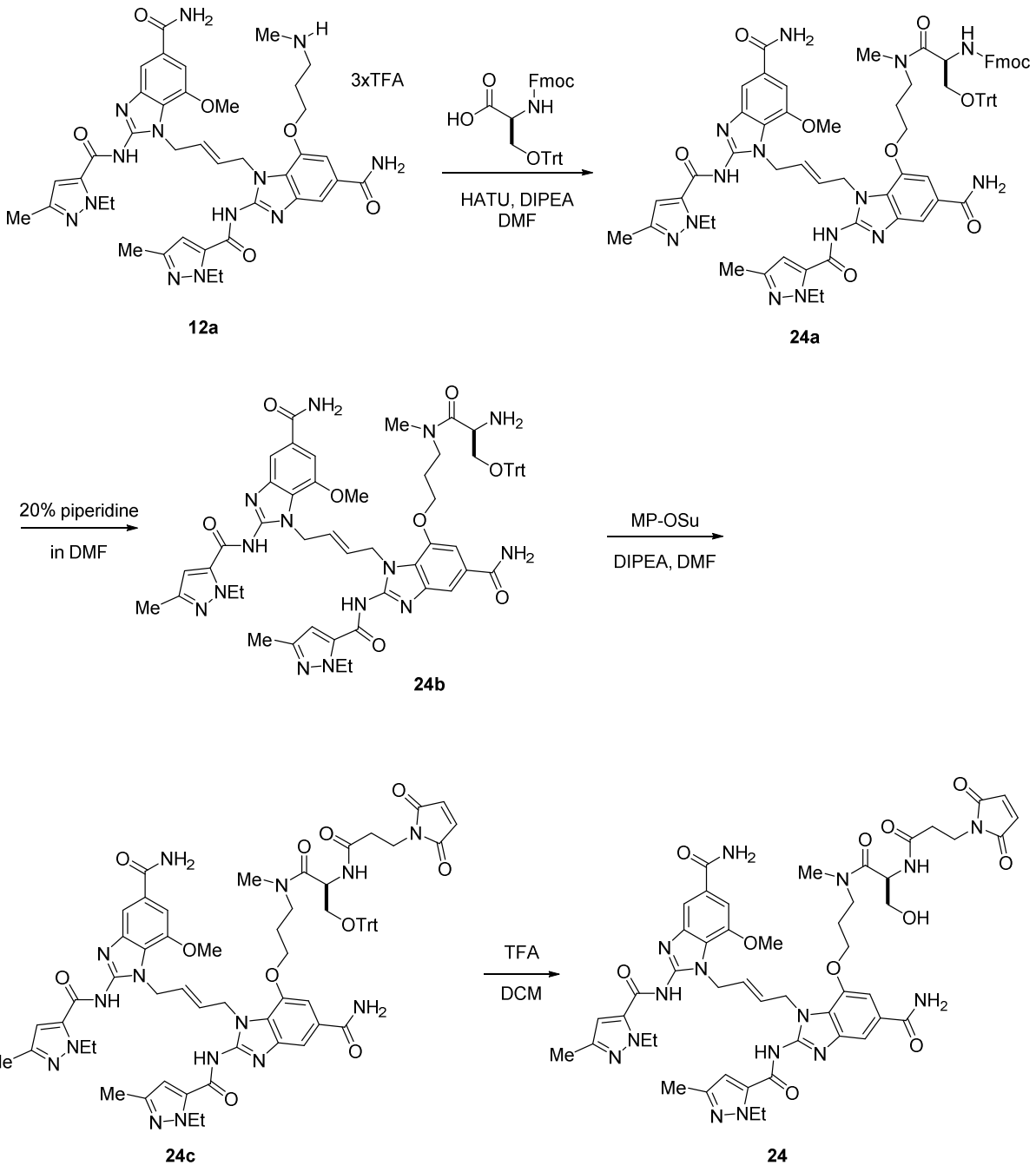
[0442] Compound **23a** was prepared according to General Method 1 (12 mg, 0.0105 mmol). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1117.50 (theoretical); 1117.77 (observed). HPLC retention time: 1.75 min.

[0443] Compound **23b** was prepared according to General Method 2 (7.2 mg, 0.00804 mmol, 91% over 2 steps). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 895.43 (theoretical); 895.73 (observed). HPLC retention time: 1.12 min.

[0444] Compound **23** was prepared according to General Method 3 (8.4 mg, 0.0047, 58% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1046.46 (theoretical); 1047.06 (observed). HPLC retention time: 1.36 min.

Synthesis of (S,E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(2-(3-(2,5-dioxo-2,5-dihydro-1H-

pyrrol-1-yl)propanamido)-3-hydroxy-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 24)



Synthesis of Compound 24a

[0445] An oven dried 4 mL vial equipped with a stir bar was charged with HATU (6.7 mg, 0.018 mmol, 2.0 equiv.) and 2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methoxy-

propanoic acid (6.0 mg, 0.018 mmol, 2.0 equiv.), and a solution of compound **12a** (8 mg, 0.0088 mmols, 1.0 equiv.) and DIPEA (8 uL, 0.044 mmols, 5 equiv.) in DMF (0.5 mL) was added to the vial. The vial was capped and sealed with parafilm and the reaction mixture was stirred at room temperature overnight, upon which full conversion was observed by UPLC-MS (Method D). Solvent was removed *in vacuo* and product purified by flash chromatography (10g SiO_2, 0 – 40% MeOH in DCM) to give **24a** (15 mg), which was used in the next reaction without further purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1345.59 (theoretical); 1346.12 (observed). HPLC retention time: 2.23 min.

Synthesis of Compound 24b

[0446] An oven-dried 4 mL vial equipped with a stir bar was charged with **24a** (15 mg, 0.011 mmol) and 20% (v/v) piperidine in DMF (1 mL) was added to it. The reaction mixture was stirred until full conversion was observed by UPLC-MS (Method D), which took approximately 1 hour. Solvent was removed *in vacuo* and the crude product was purified by preparatory HPLC (Method G, 5 - 95% MeCN in water with 0.1% (v/v) formic acid); the HPLC solvents were removed *in vacuo* to give **24b** (8.4 mg, 0.0075 mmol, 94% over 2 steps) as an off-white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1123.53 (theoretical); 1123.98 (observed). HPLC retention time: 1.47 min.

Synthesis of Compound 24c

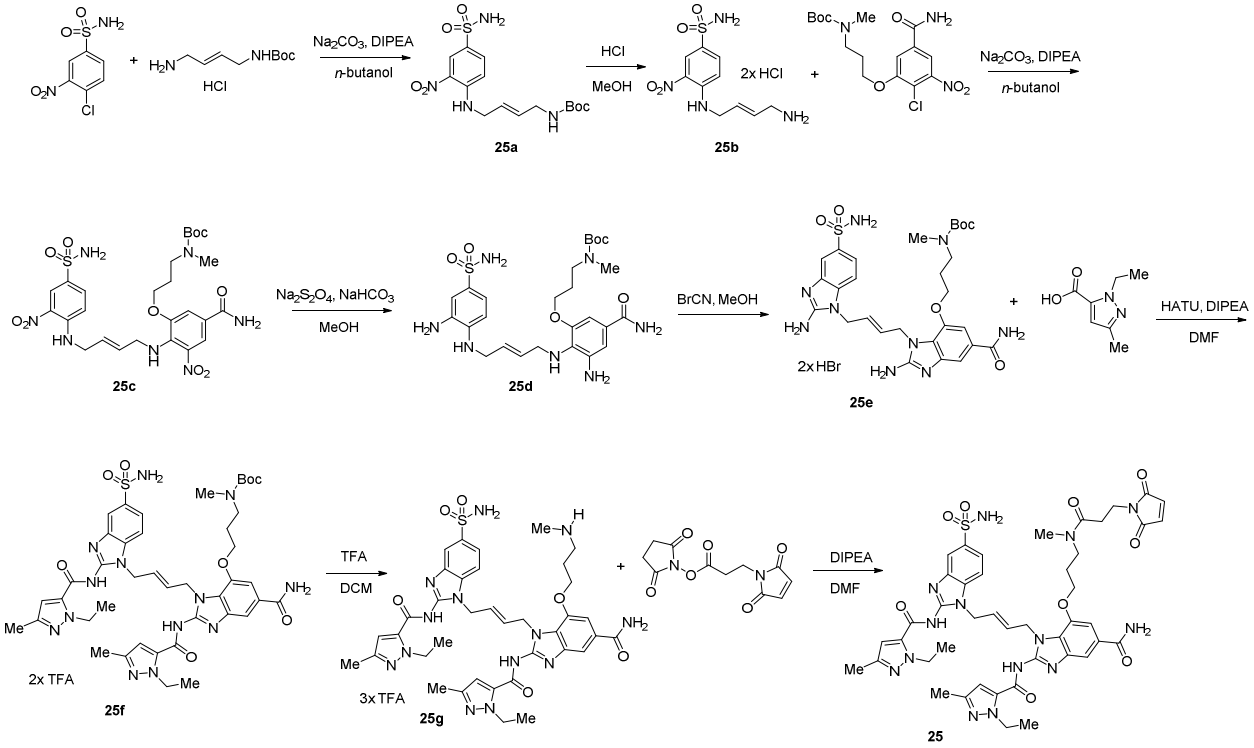
[0447] An oven-dried 4 mL vial equipped with a stir bar was charged with **24b** (8.4 mg, 0.0075 mmol, 1 equiv.), followed by MP-OSu (3.0 mg, 0.011 mmol, 1.5 equiv.), DIPEA (3.9 µL, 0.022 mmol, 3 equiv.) and DMF (0.5 mL). The reaction mixture was stirred at room temperature for 3 hours at which point UPLC-MS (Method D) analysis showed full conversion. Solvent was removed *in vacuo* and the resulting crude product was used in the next step without purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1274.55 (theoretical); 1275.21 (observed). HPLC retention time: 1.89 min.

Synthesis of Compound 24

[0448] An oven-dried 4 mL vial containing a stir bar was charged with crude **24c** (0.0075 mmol,) and 20% (v/v) TFA in DCM (1 mL) was added to the vial. The reaction mixture

was stirred for 20 minutes, and solvent removed *in vacuo*. The resulting crude product was dissolved in DMSO (0.5 mL) and purified by preparatory HPLC (Method G, 5 – 50% MeCN in water with 0.1% (v/v) formic acid) and solvent removed *in vacuo* to give **24** (4.0 mg, 0.0031 mmol, 42% yield over 2 steps) as a white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 1032.44 (theoretical); 1033.09 (observed). HPLC retention time: 1.28 min.

Synthesis of (E)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 25).



Synthesis of 25a

[0449] A 5 mL oven-dried microwave vial with stir bar was charged with 4-chloro-3-nitro-benzenesulfonamide (250 mg, 1.06 mmol, 1 equiv.), tert-butyl N-[(E)-4-aminobut-2-enyl]carbamate hydrochloride (353 mg, 1.6 mmol, 1.5 equiv.) and sodium carbonate (336 mg, 3.2 mmol, 3 equiv.). To the vial was added 1-butanol (3 mL) followed by DIPEA (1.1 mL, 6.34 mmol,

6 equiv.) and additional 1-butanol to bring the total volume of the reaction up to 5 mL. The vial was sealed and heated to 140 ºC in the microwave reactor for 120 minutes.

[0450] The crude product was poured into brine (100 mL) and extracted with EtOAc (3x200 mL), organics combined, washed with brine (2x100 mL), dried with $MgSO_4$, filtered and solvent removed in vacuo to give a bright red oil. This material was purified by flash chromatography (dry loaded on celite, 25g Sfar, HC Duo, SiO2 column, 0 - 40% MeOH in DCM) to give **25a** (295 mg, 0.763 mmol, 72% yield) as a bright yellow solid. UPLC-MS (Method D, ESI+): m/z $[M + H - Boc]^+$ = 287.1 (theoretical); 287.4 (observed). HPLC retention time: 1.53 min.

Synthesis of 25b

[0451] A 20 mL vial was charged with **25a** (295 mg, 0.763 mmol, 1 equiv.) which was dissolved in methanol (7.5 mL) and 4M HCl in 1,4-dioxane (40 eq, 7.5 mL, 30.0 mmol). The solution was stirred at 40 ºC for 30 minutes and solvent removed in vacuo to give **25b** as the 2x HCl salt (274 mg, 0.764 mmol, quantitative yield) as a bright red solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 287.1 (theoretical); 287.6 (observed). HPLC retention time: 0.52 min.

Synthesis of 25c

[0452] An oven dried 5 mL microwave vial with stir bar was charged with **25b** (135 mg, 0.376 mmol, 1 equiv.), tert-butyl N-[3-(5-carbamoyl-2-chloro-3-nitro-phenoxy)propyl]carbamate (211 mg, 0.564 mmol, 1.5 equiv., prepared as described below) and sodium carbonate (119 mg, 1.13 mmol, 3 equiv.) which was followed by addition of *n*-butanol (3.75 mL) and DIPEA (0.39 mL, 2.25 mmol, 6 equiv.). The vial was sealed and heated to 140 ºC for 3 hours in a microwave reactor to give a bright red heterogenous mixture. This solution was filtered over celite washing with 1:1 DCM:MeOH (100 mL), solvent removed in vacuo and crude product was loaded onto celite and purified by flash chromatography (25g SiO2 column, 0 - 40% MeOH in DCM) to give **25c** (245 mg, 0.384 mmol) as a mixture of product and starting material (3:2). Product mixture was used in the next step without any further purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 638.2 (theoretical); 638.5 (observed). HPLC retention time: 1.75 min.

Synthesis of 25d

[0453] A 20 mL vial with stir bar was charged with **25c** (245 mg, 0.384 mmol, 1 equiv.) and sodium bicarbonate (580 mg, 6.90 mmol, 18 equiv.) and methanol (4mL) was added. To the vial was then added sodium hydrosulfite (1.20 g, 6.90 mmol, 18 equiv. in 4 mL water) and the vial was heated to 50 ^{o}C for 60 minutes. The reaction was cooled to room temperature, filtered over celite washing with MeOH (50 mL) and DCM (50 mL) and the crude product loaded onto celite. The product was purified by flash chromatography (25g Sfar HC Duo, SiO2 column, 0 - 40% 10:1 MeOH:NH_4OH in DCM) to give **25d** (89 mg, 0.154 mmol, 41% yield over 2 steps) as a mixture of inseparable rotational conformers. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 578.3 (theoretical); 578.5 (observed). HPLC retention time: 0.98 & 1.18 min.

Synthesis of 25e

[0454] Two identical reactions were setup side by side. An oven dried 4 mL vial with stir bar was charged with **25d** (45 mg, 0.156 mmol, 1 equiv.), dissolved in methanol (1 mL) and cyanogen bromide (200 uL, 1.20 mmol, 8 equiv.) was added. Reaction was stirred overnight, and solvent removed *in vacuo* and two reactions combined to give **25e** as the 2x HBr salt (120 mg, 0.15 mmol, 97 % yield) as a light gray solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 628.3 (theoretical); 628.4 (observed). HPLC retention time: 0.79 min.

Synthesis of 25f

[0455] An oven dried 4 mL vial with stir bar was charged with **25e** (120 mg, 0.152 mmol, 1 equiv.), 2-ethyl-5-methyl-pyrazole-3-carboxylic acid (94 mg, 0.61 mmol, 4.0 equiv.) and HATU (231 mg, 0.61 mmol, 4 equiv.). The solids were dissolved in DMF (1 mL) and DIPEA (0.22 mL, 1.2 mmol, 8 equiv.) was added. The reaction was stirred at room temperature overnight, acetic acid was added (100 uL) and product purified by prepHPLC (Method I, 5 - 95% MeCN in water with 0.05% TFA) and solvent removed in vacuo to give **25f** (107 mg, 0.12 mmol, 78% yield) as an off-white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 900.4 (theoretical); 900.6 (observed). HPLC retention time: 1.69 min.

Synthesis of 25g

[0456] Compound **25f** (107 mg, 0.12 mmol, 1 equiv.) was added to a 20 mL vial with stir bar and dissolved in 20% TFA in DCM (5 mL). Reaction was stirred at room temperature for

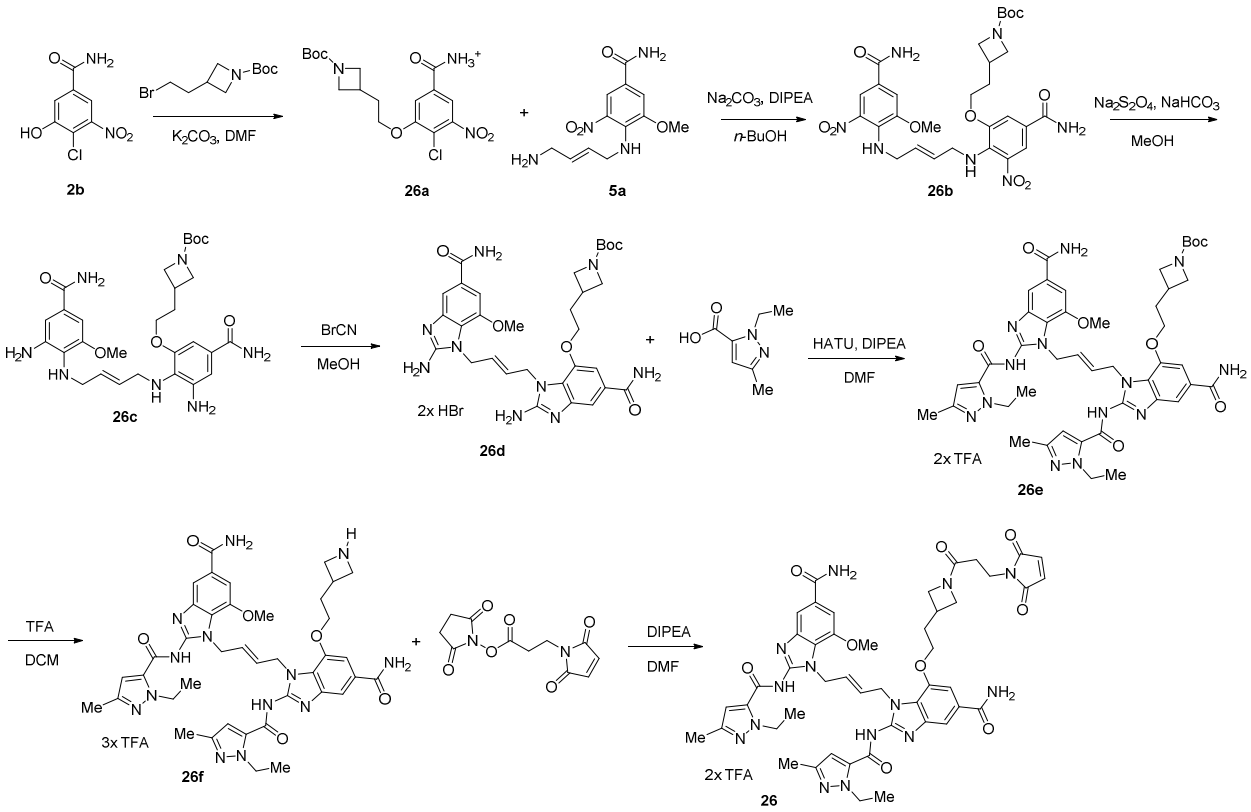
20 minutes and then solvent removed *in vacuo* to give **25g** as the 3x TFA salt and an off-white solid (70 mg, 0.0615 mmol, 52% yield). A sample of analytical purity was obtained by prepHPLC purification (Method G, 5 - 95% MeCN in water with 0.05% TFA). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 800.3 (theoretical); 800.6 (observed). HPLC retention time: 1.12 min.

Synthesis of 25

[0457] An oven dried 4 mL vial with stir bar was charged with **25g** (12 mg, 0.011 mmol, 1 equiv.) which was dissolved in DMF (1 mL) and then both DIPEA (15 uL, 0.087 mmol, 8 equiv.) and MP-OSu (4.3 mg, 0.0163 mmol, 1.5 equiv.) were added to the reaction. The solution was stirred at room temperature for 30 minutes, quenched with 20% TFA in DCM (100 uL) and purified by prepHPLC (Method G, 5 - 95% MeCN in water with 0.05% TFA) to **25** as the 2x TFA salt (5.7 mg, 0.0048 mmol, 45% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 951.4 (theoretical); 951.2 (observed). HPLC retention time: 2.18 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(2-(1-(3-(2,5-dioxo-2,5-dihydro-1H-

pyrrol-1-yl)propanoyl)azetidin-3-yl)ethoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 26).



Synthesis of 26a

[0458] An oven dried 8 mL vial with stir bar was charged with **2b** (100 mg, 0.462 mmol, 1 equiv.) and potassium carbonate (191 mg, 1.39 mmol, 3 equiv.) followed by addition of tert-butyl 3-(2-bromoethyl)azetidine-1-carboxylate (152 mg, 0.577 mmol, 1.25 equiv.). The starting materials were dissolved in DMF (3mL), vial sealed with parafilm and stirred at 70 ^{o}C for 24 hours. The crude material was poured into a separatory funnel containing saturated ammonium chloride (100 mL) and EtOAc (100 mL each), shaken, layers separated, and aqueous layer extracted with EtOAc (2x100 mL). The combined organic fractions were washed with brine (2x50 mL), dried with MgSO4, filtered and solvent removed in vacuo to give crude product as a light-yellow solid. The crude product was purified by flash chromatography (25g Sfar HC Duo SiO_2 column, 0 - 20% MeOH in DCM) to give **26a** as a yellow solid (86 mg, 0.215 mmol, 47 % yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 400.1 (theoretical); 400.5 (observed). HPLC retention time: 1.79 min.

Synthesis of 26b

[0459] An oven-dried 2 mL microwave vial was charged with **25a** (35 mg, 0.0875 mmol, 1 equiv.), **5a** (62 mg, 0.175 mmol, 2 equiv.) and sodium carbonate (28 mg, 0.263 mmol, 3 equiv.) and to this vial was added *n*-butanol (1 mL) and DIPEA (0.1 mL, 0.5 mmol, 6 equiv.). The vial was sealed and heated to 140 ºC for 3 hours in a microwave reactor. The reaction was then filtered over celite washing with 1:1 MeOH:DCM (100 mL), solvent removed in vacuo and crude material loaded onto celite. The product was purified by flash chromatography (25g Sfar HC Duo SiO_2 column, 0 - 20% MeOH in DCM) to give **25b** as a bright red solid (38 mg, 0.0592 mmol, 68 % yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 644.3 (theoretical); 644.6 (observed). HPLC retention time: 1.72 min.

Synthesis of 26c

[0460] An oven-dried 4 mL vial was charged with **25b** (38 mg, 0.0592 mmol, 1 equiv.) which was dissolved in methanol (1mL) and sodium bicarbonate (90 mg, 1.1 mmol, 18 equiv.) was added followed by sodium hydrosulfite (186 mg, 1.07 mmol, 18 equiv.) as a solution in water (1 mL). The reaction was heated to 50 ºC for 1 hour and filtered over celite washing with 1:1 DCM:MeOH (50 mL). The crude product was loaded onto celite and purified by flash chromatography (25g Sfar HC Duo, SiO_2 column, 0 - 40% 10:1 MeOH:NH_4OH in DCM) to give **25c** (10 mg, 0.017 mmol, 29% yield) as a light yellow solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 584.3 (theoretical); 584.6 (observed). HPLC retention time: 1.18 min.

Synthesis of 26d

[0461] An oven dried 4 mL vial with stir bar was charged with **25c** (10 mg, 0.017 mmol, 10 equiv.) which was dissolved in methanol (0.5 mL) and cyanogen bromide (0.050 mL, 0.150 mmol, 3M in DCM, 8.7 equiv.) was added. The reaction was stirred for 18 hours and solvent removed in vacuo to give the **25d** as a light grey solid and the 2x HBr salt (13 mg, 0.0165 mmol, 95 % yield) which was used without any further purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 634.3 (theoretical); 634.6 (observed). HPLC retention time: 0.98 min.

Synthesis of 26e

[0462] An oven dried 4 mL vial with stir bar was charged with **25d** (13 mg, 0.0165 mmol, 1 equiv.), HATU (25 mg, 0.066 mmol, 4 equiv.) and 2-ethyl-5-methyl-pyrazole-3-

carboxylic acid (10 mg, 0.066 mmol, 4 equiv.) which were dissolved in DMF (0.5 mL) and then DIPEA (0.050 mL, 0.20 mmol, 17 equiv.) was added. The reaction was stirred at room temperature for 24 hours. The reaction was quenched with acetic acid (100 uL) and product purified by via prepHPLC (Method H, 5 - 95% MeCN in water with 0.05% TFA) to give **25e** as the 2x TFA salt (14 mg, 0.016 mmol, 95% yield) as a light tan solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 906.4 (theoretical); 906.3 (observed). HPLC retention time: 2.44 min.

Synthesis of 26f

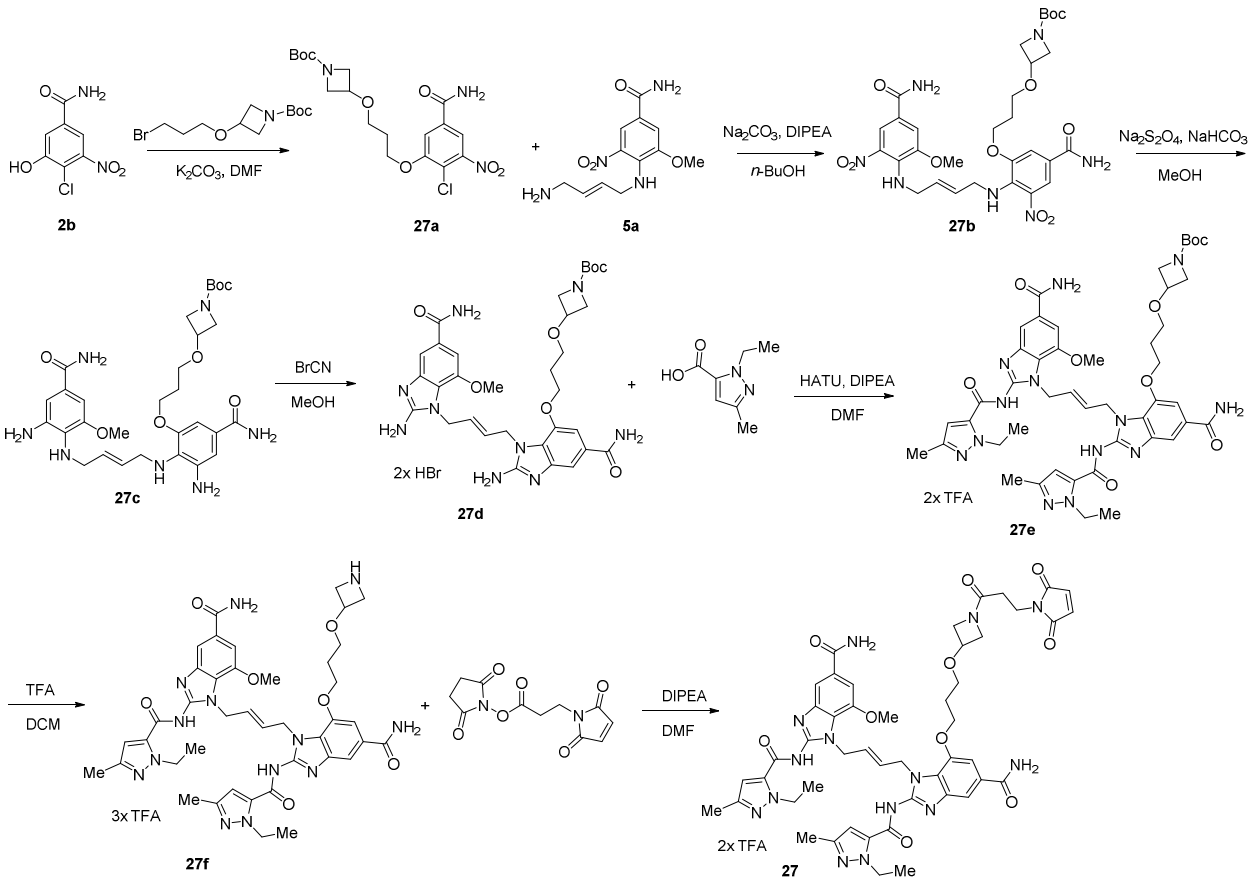
[0463] An oven dried 4 mL vial with stir bar was charged with **25e** (14 mg, 0.016 mmol, 1 equiv.) which was dissolved in 20% TFA in DCM (1 mL) and stirred at room temperature for 15 minutes. Solvent was removed in vacuo to give **25f** as the 3x TFA salt (15 mg, 0.013 mmol , 82 % yield) as a white solid and the product used without any further purification. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 806.4 (theoretical); 806.6 (observed). HPLC retention time: 1.25 min.

Synthesis of 26

[0464] An oven dried 4 mL vial with stir bar was charged with **25f** (5.7 mg, 0.0050 mmol, 1 equiv.) in DMSO (0.5 mL) and MP-OSu (2.0 mg, 0.00750 mmol, 1.5 equiv.) and DIPEA (5 uL, 0.030 mmol, 6 equiv.) was added. The reaction was stirred at room temperature for 1 hour. The reaction was quenched added 20% TFA in DCM (100 uL) and product purified by prepHPLC (Method G, 5 - 95% MeCN in water with 0.05% TFA) to give **25** as the 2x TFA salt (3.8 mg, 0.00321 mmol, 64 % yield) as a white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 957.4 (theoretical); 957.3 (observed). HPLC retention time: 2.19 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-((1-(3-(2,5-dioxo-2,5-dihydro-1H-

pyrrol-1-yl)propanoyl)azetidin-3-yl)oxy)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 27).



Synthesis of 27a

[0465] An oven dried 8 mL vial with stir bar was charged with **2b** as the TFA salt (150 mg, 0.454 mmol, 1 equiv.), tert-butyl 3-(3-bromopropoxy)azetidine-1-carboxylate (133 mg, 0.454 mmol, 1 equiv.) and potassium carbonate (141 mg, 1.02 mmol, 2.3 equiv.) which were dissolved in DMF (4.5mL) and heated to 55 ºC for 24 hours. The reaction was poured into a separatory funnel containing sat. $NaHCO_3$ (100 mL) and EtOAc (100 mL), shaken, layers separated, and aqueous layer extracted with EtOAc (3x50 mL). The organic fractions were combined and further washed with sat. $NaHCO_3$ (3x50 mL) and brine (2x50 mL). They were then dried with MgSO4, filtered and solvent removed in vacuo to **27a** (194 mg, 0.353 mmol, 78% yield) as a light yellow solid in a 4:1 ratio of starting material to product and used without further purification. MS (Method D, ESI+): m/z $[M + H]^+$ = 430.1 (theoretical); 430.6 (observed). HPLC retention time: 1.82 min.

Synthesis of 27b

[0466] An oven-dried 5 mL microwave vial was charged with Sodium carbonate (144 mg, 1.36 mmol, 3.00 eq), **5a** as the 2x HCL salt (240 mg, 0.678 mmol, 1.50 eq) and **27a** (194 mg, 0.452 mmol, 1 equiv.) and then 1-butanol (4mL) and DIPEA (0.5 mL, 2.7 mmol, 6 equiv.) were added. The vial was sealed and heated to 140 ºC for 3 hours in a microwave reactor. The reaction was cooled to room temperature and solution was filtered over celite washing with 1:1 MeOH:DCM (100 mL). The crude product was loaded onto celite and purified by flash chromatography (25g Sfar HC Duo, SiO_2 column, 0 - 20% MeOH in DCM) to give **27b** (95 mg, 0.141 mmol, 31% yield) as a bright red solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 674.3 (theoretical); 674.6 (observed). HPLC retention time: 1.73 min.

Synthesis of 27c

[0467] A 20 mL vial was charged **27b** (95 mg, 0.141 mmol, 1 equiv.) and sodium bicarbonate (442 mg, 5.3 mmol, 37 equiv.) and starting material dissolved in methanol (4mL). To the vial was added sodium hydrosulfite (442 mg, 2.54 mmol, 18 equiv.) as solution in water (4 mL) and reaction was heated, open to the atmosphere, to 50 ºC for 1 hour. The solution went from bright red to light yellow over the course of an hour. The reaction was filtered, filter cake washed with 1:1 MeOH:DCM (3x50 mL), solvent removed in vacuo, crude product redissolved in 1:1 MeOH:DCM (100 mL) and filtered over celite. The crude product was loaded onto celite and purified by flash chromatography (25g Sfar HC Duo, SiO_2 column, 0 - 40% 10:1 MeOH:NH_4OH in DCM) to give **27c** (42 mg, 0.0689 mmol, 49% yield) as an off-white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 614.3 (theoretical); 614.5 (observed). HPLC retention time: 0.78 min.

Synthesis of 27d

[0468] An oven-dried 4 mL vial was charged with **27c** (42 mg, 0.0689 mmol, 1 equiv.) which was dissolved in methanol (1.3mL) and then cyanogen bromide (3M in DCM, 0.14 mL, 0.414 mmol, 6 equiv.) was added. The vial was stirred at room temperature for 24 hours and solvent removed in vacuo to give **27d** as the 2x HBr salt (57 mg, 0.0694 mmol, quantitative yield) as an off-white solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 664.3 (theoretical); 664.7 (observed). HPLC retention time: 0.95 min.

Synthesis of 27e

[0469] An oven dried 4 mL vial with stir bar was charged with **27d** (57 mg, 0.0694 mmol, 1 equiv.), 2-ethyl-5-methyl-pyrazole-3-carboxylic acid (43 mg, 0.278 mmol, 4 equiv.) and HATU (106 mg, 0.278 mmol, 4 equiv.) which were dissolved in DMF (1 mL) and then DIPEA (0.097 mL, 0.555 mmol, 8 equiv.) was added. The reaction was stirred at room temperature for 24 hours, quenched with 20% TFA in MeCN (200 uL) and product purified by prepHPLC (Method I, 5 - 95% MeCN in water with 0.05% TFA), solvent removed via lyophilization to give **27e** as the 2x TFA salt (35 mg, 0.0302 mmol, 43% yield) as a tan solid. A sample of analytical purity was prepared via a second prepHPLC purification (Method G, 5 - 60% MeCN in water with 0.05% TFA). MS (Method D, ESI+): m/z $[M + H]^+$ = 936.4 (theoretical); 936.3 (observed). HPLC retention time: 2.37 min.

Synthesis of 27f

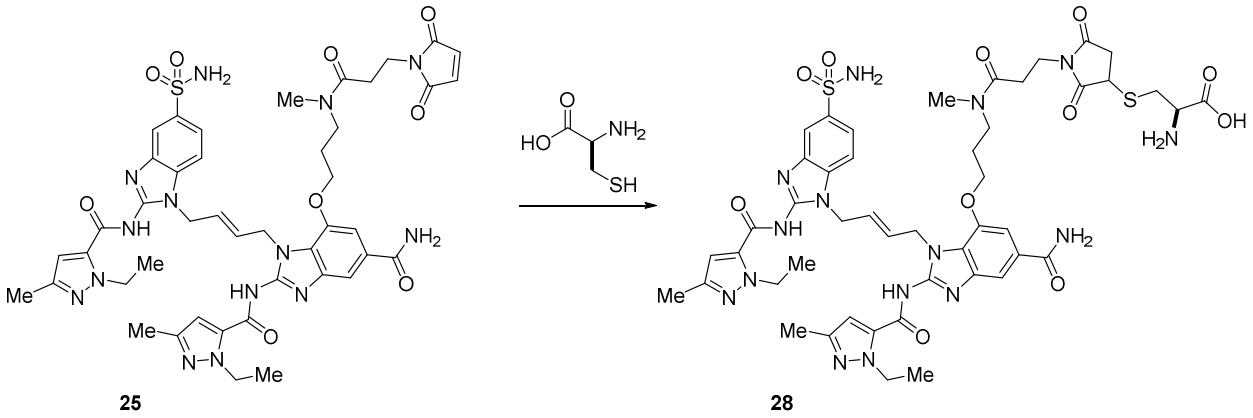
[0470] A 20 mL vial was charged with **27e** (31 mg, 0.0266 mmol. 1 equiv.) which was dissolved in 20% TFA in DCM (2 mL) and stirred at room temperature for 15 minutes. Solvent was removed in vacuo and crude product purified by prepHPLC (Method H, 5 - 95% MeCN in water with 0.05% TFA) to give **27f** as the 3x TFA salt (7.2 mg, 0.0061 mmol, 23% yield) as a white solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 836.4 (theoretical); 836.3 (observed). HPLC retention time: 2.02 min.

Synthesis of 27

[0471] An oven-dried 4 mL vial was charged with **27f** (10 mM in DMSO, 0.50 mL, 0.0050 mmol, 1 equiv.) and then MP-OSu (2.0 mg, 0.0075 mmol, 1.5 equiv.) and DIPEA (20 uL, 0.12 mmol, 23 equiv.) was added. The reaction was stirred at room temperature for 90 minutes, quenched with 20% TFA in MeCN (100 uL) and crude product was purified by prepHPLC (Method G, 5 - 95% MeCN in water with 0.05% TFA) to 27 as the 3x TFA salt (3.6 mg, 0.0029 mmol, 58% yield) as a white solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 987.4 (theoretical); 987.2 (observed). HPLC retention time: 2.23 min.

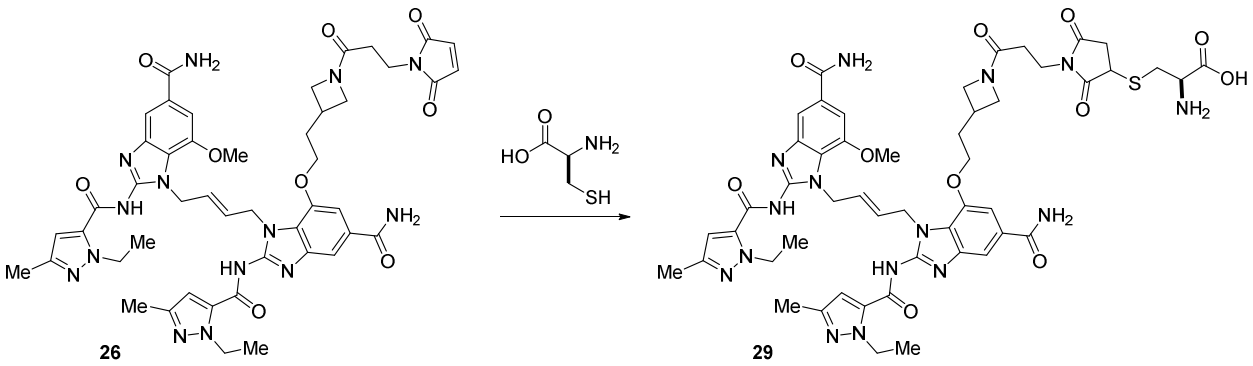
Synthesis of S-(1-(3-((3-((5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-((E)-4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-

yl)but-2-en-1-yl)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)amino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 28).



[0472] A 1.7 mL eppendorf tube was charged with **25** (10 mM in DMSO, 100 uL, 0.00100 mmol, 1 equiv.) and L-cysteine (15 mM in 4:1 DMSO:water, 150 uL, 0.00300 mmol, 3 equiv.) was added. The reaction was heated to 37 ºC for 90 minutes and the crude product was then purified by prepHPLC (Method G, 5 - 95% MeCN in water with 0.05% TFA) to give **28** as the 2x TFA salt (1.1 mg, 0.000861 mmol, 86% yield) as a white solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 1072.4 (theoretical); 1072.2 (observed). HPLC retention time: 1.98 min.

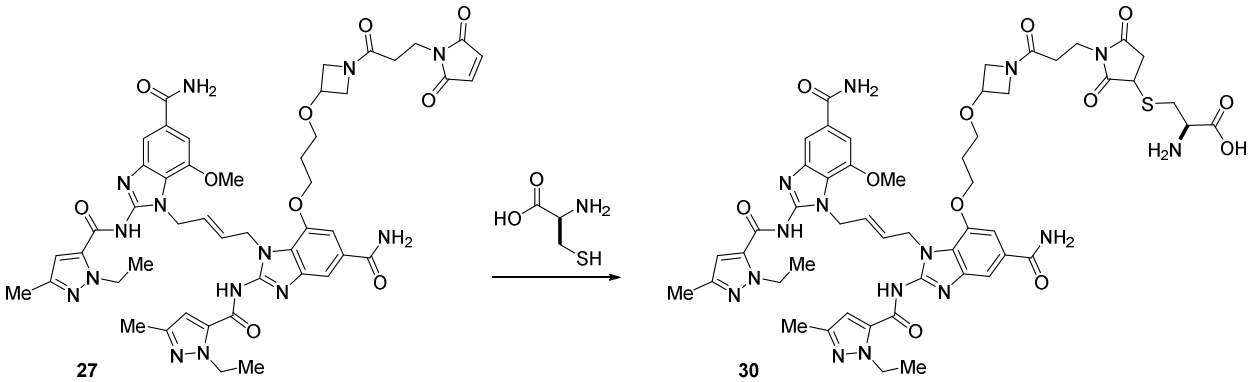
Synthesis of S-(1-(3-(3-(2-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)ethyl)azetidin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 29).



[0473] A 1.7 mL eppendorf tube was charged with **26** (10 mM in DMSO, 100 uL, 0.00100 mmol, 1 equiv.) and L-cysteine (15 mM in 4:1 DMSO:water, 150 uL, 0.00300 mmol, 3 equiv.) was added. The reaction was heated to 37 ºC for 2 hours and the crude product was then

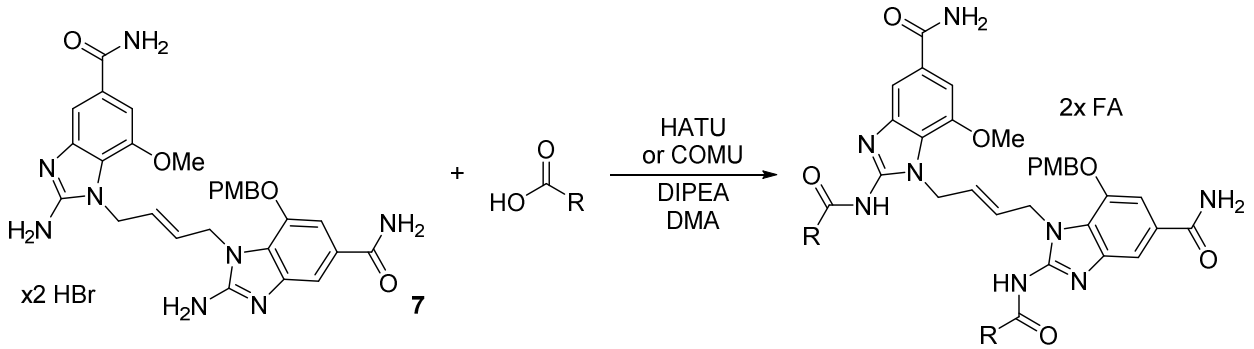
purified by prepHPLC (Method G, 5 - 95% MeCN in water with 0.05% TFA) to give **29** as the 2x TFA salt (0.91 mg, 0.000697 mmol, 70% yield) as a white solid. MS (Method D, ESI+): m/z [M + H]$^+$ = 1078.4 (theoretical); 1078.3 (observed). HPLC retention time: 2.03 min.

S-(1-(3-(3-(3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propoxy)azetidin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 30).



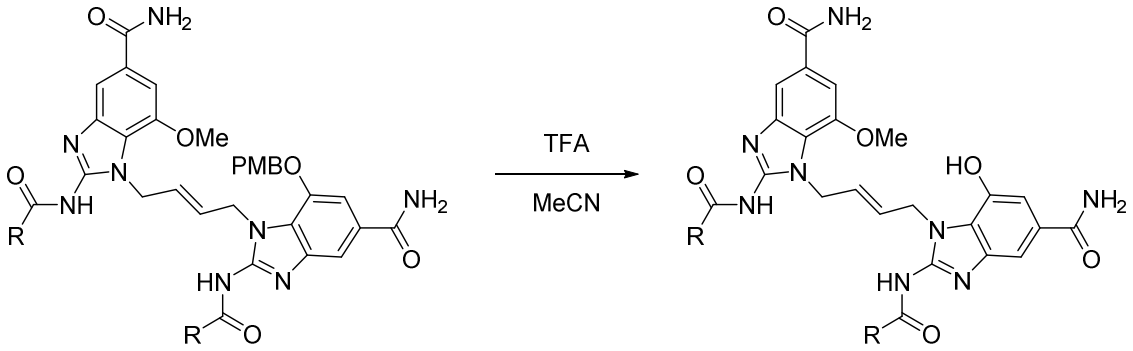
[0474] A 1.7 mL eppendorf tube was charged with **27** (10 mM in DMSO, 100 uL, 0.00100 mmol, 1 equiv.) and L-cysteine (100 mM in DMSO, 30 uL, 0.00300 mmol, 3 equiv.) and the solution incubated at 37 ^{o}C for 30 minutes. The crude product was purified by prepHPLC (Method G, 5 - 95% MeCN in water with 0.05%) to give **30** as the 2x TFA salt (1.2 mg, 0.000913 mmol, 61% yield) as a white solid. MS (Method D, ESI+): m/z [M + H]$^+$ = 1108.4 (theoretical); 1108.5 (observed). HPLC retention time: 2.08 min.

Library synthesis of amide analogs. Scheme and general methods. Compounds 31 – 60.



[0475] **HATU Couplings (General Method 4A)** To a solution of carboxylic acid (4 equiv.) in DMA (400 µL) was added HATU (6.2 mg, 0.016 mmol, 4 equiv.) and DIPEA (4.3 µL, 0.025 mmol, 6 equiv.). The mixture was stirred at room temperature for 30 minutes and then compound **7** (3 mg, 0.0041 mmol, 1 equiv.) was added to the mixture, and was heated to 70 °C for 18 hr. At which point, acetic acid (4.3 µL) was added, and resulting products were purified by prepHPLC (20-50-95% MeCN in water with 0.1% FA). All molecules were characterized using LC-MS Method D with ESI+ ionization.

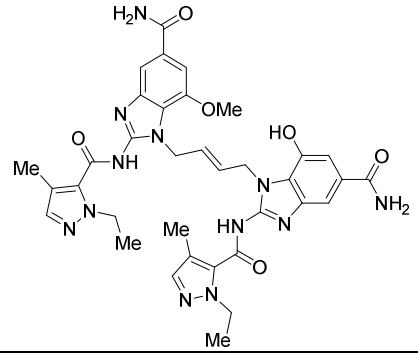
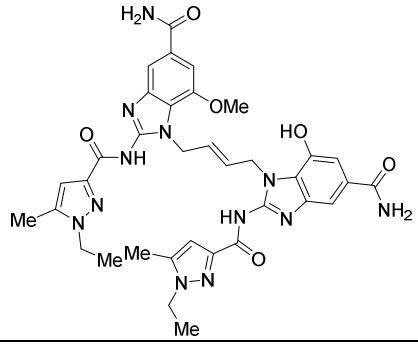
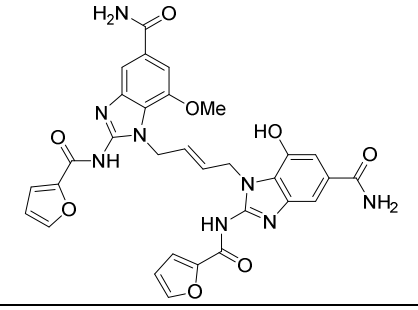
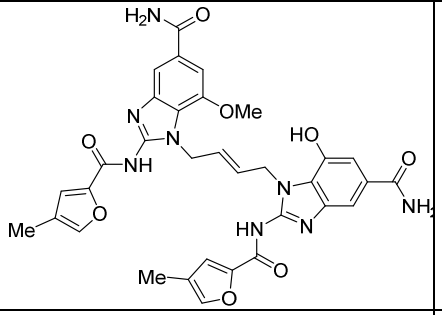
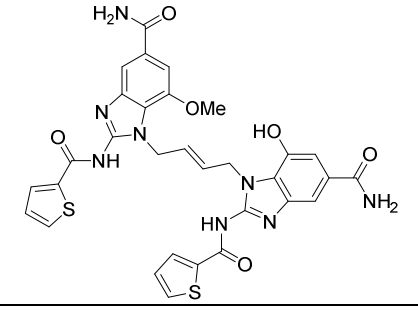
[0476] **COMU Couplings (General Method 4B)** To a solution of carboxylic acid (4 equiv.) in DMA (400 µL) was added COMU (7 mg, 0.016 mmol, 4 equiv.) and DIPEA (4.3 µL, 0.025 mmol, 6 equiv.). The mixture was stirred at room temperature for 30 min and then compound **7** (3 mg, 0.0041 mmol, 1 equiv.) was added to the mixture, and the solution was heated to 40 °C for 18 hr. At which point, acetic acid was added (4.3 µL), and the resulting products were purified by prepHPLC (20-50-95% MeCN in water with 0.1% FA).

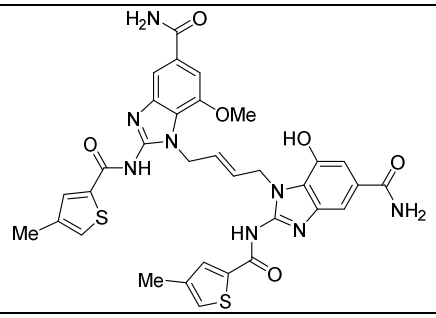
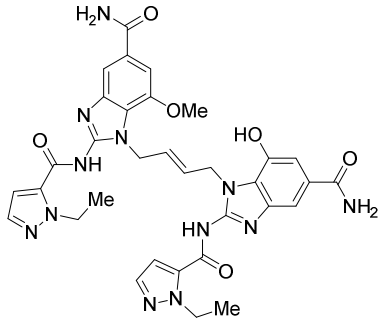
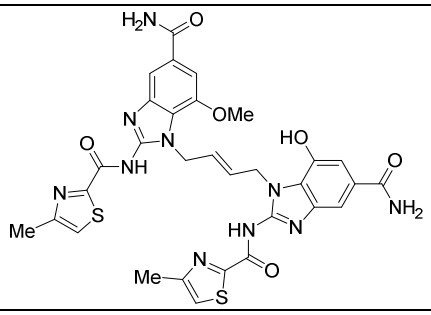
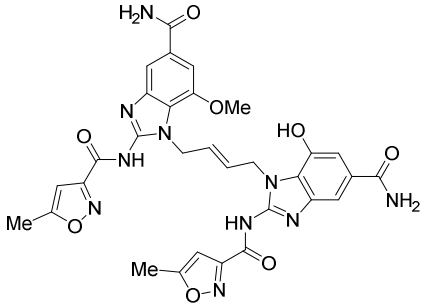
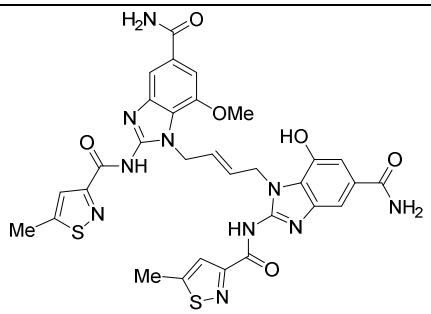


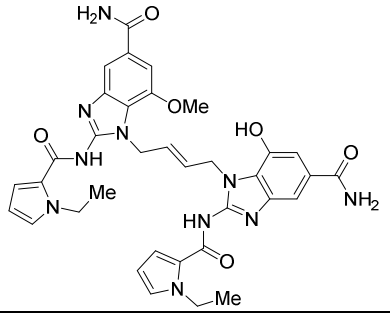
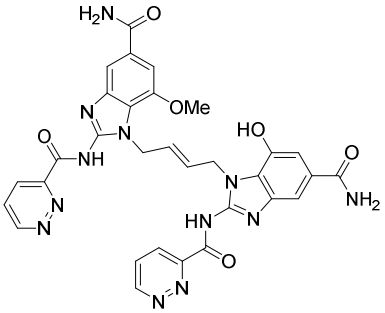
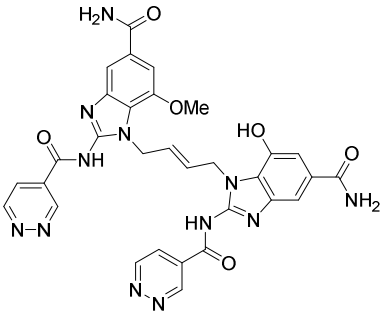
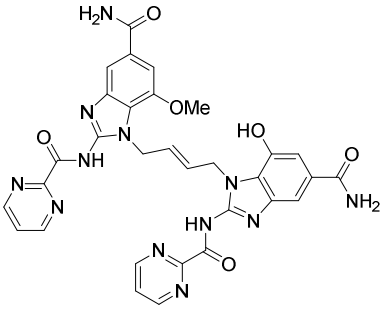
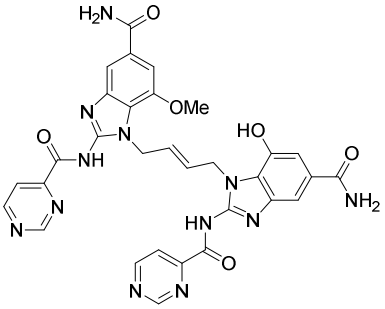
[0477] **PMB deprotection (General Method 5)** The resulting amide from the previous step was dissolved in 50% TFA in MeCN (0.01 M) and stirred at 30 °C for 30 min. Upon completion, the mixture was concentrated, and the product purified by prep-HPLC (20-50-95% water/acetonitrile 0.1% TFA).

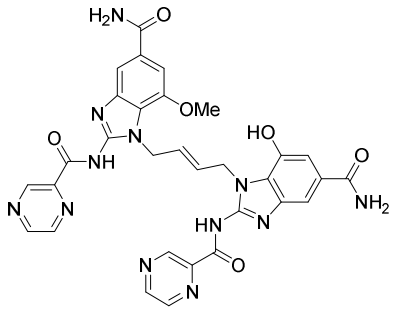
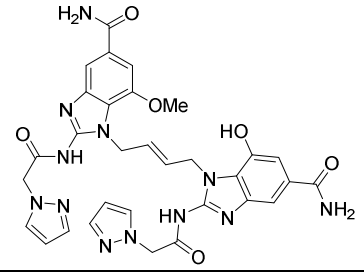
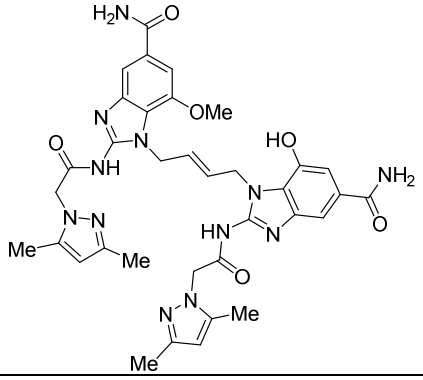
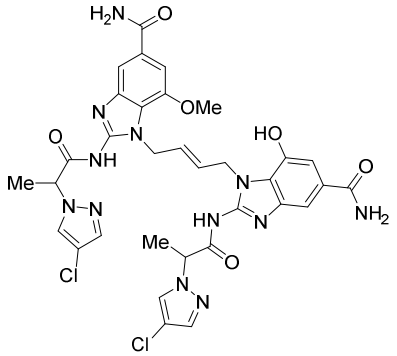
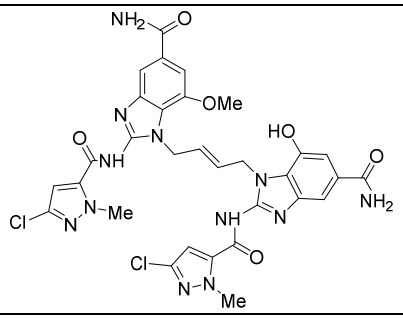
[0478] Examples below were prepared using the general methods specified above.

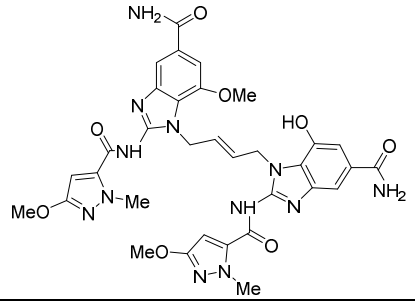
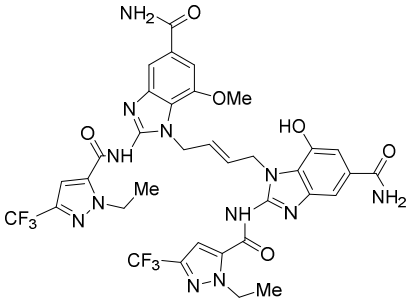
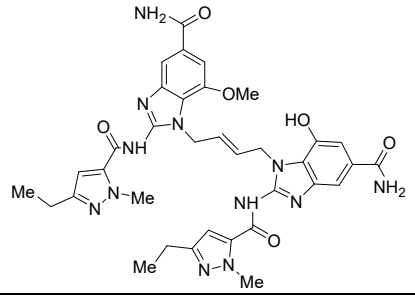
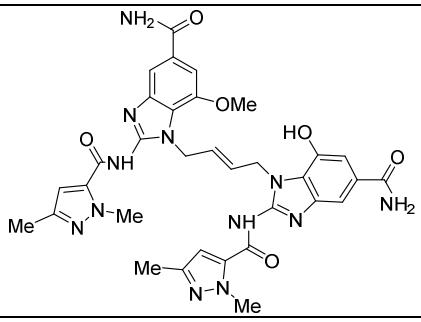
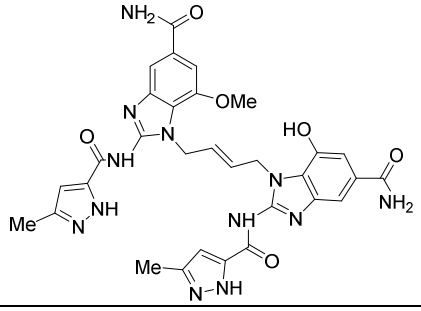
Cmpd.	Structure	Method	Yield (over 2 steps)	PMB LC-MS data	Phenol LC-MS data

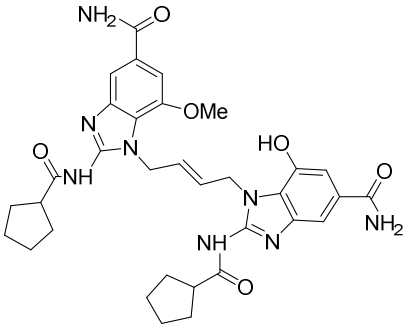
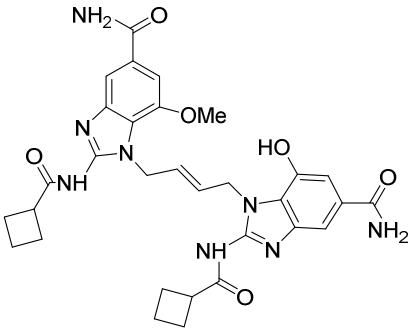
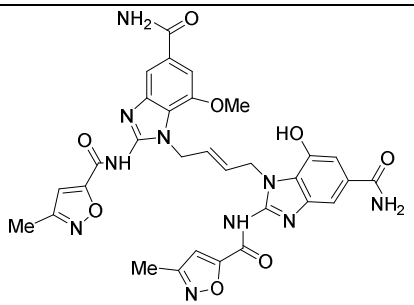
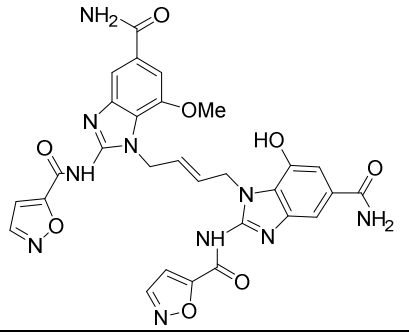
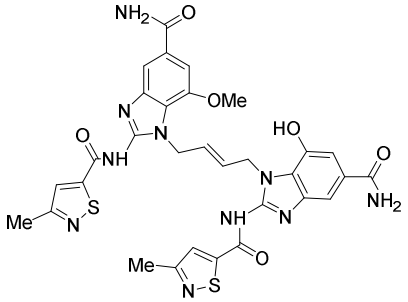
31		HATU - Method 4a	45% 1.74 mg 0.00183 mmol	RT: 1.49 Theoretical: 843.4 Observed: 843.5	RT: 1.25 Theoretical: 723.3 Observed: 723.5
32		HATU - Method 4a	58% 2.25 mg 0.00237 mmol	RT: 1.67 Theoretical: 843.4 Observed: 843.5	RT: 1.42 Theoretical: 723.3 Observed: 723.5
33		HATU - Method 4a	56% 2.00 mg 0.00231 mmol	RT: 1.78 Theoretical: 759.2 Observed: 759.4	RT: 1.60 Theoretical: 639.2 Observed: 639.3
34		HATU - Method 4a	61% 2.24 mg 0.00251 mmol	RT: 1.92 Theoretical: 787.3 Observed: 787.4	RT: 1.74 Theoretical: 667.2 Observed: 667.4
35		HATU - Method 4a	59% 2.19 mg 0.00244 mmol	RT: 2.00 Theoretical: 791.2 Observed: 791.3	RT: 1.85 Theoretical: 671.1 Observed: 671.3

36		HATU - Method 4a	40% 1.52 mg 0.00164 mmol	RT: 2.14 Theoretical: 819.2 Observed: 819.4	RT: 2.01 Theoretical: 699.2 Observed: 699.3
37		COMU – Method 4b	35% 1.32 mg 0.00143 mmol	RT: 2.02 Theoretical: 815.3 Observed: 815.5	RT: 1.63 Theoretical: 695.3 Observed: 695.4
38		COMU – Method 4b	39% 1.47 mg 0.00158 mmol	RT: 2.02 Theoretical: 821.2 Observed: 821.4	RT: 1.63 Theoretical: 701.2 Observed: 701.3
39		COMU – Method 4b	57% 2.08 mg 0.00232 mmol	RT: 1.86 Theoretical: 789.3 Observed: 789.5	RT: 1.65 Theoretical: 669.2 Observed: 669.4
40		COMU – Method 4b	36% 1.37 mg 0.00148 mmol	RT: 2.00 Theoretical: 821.2 Observed: 821.4	RT: 1.61 Theoretical: 701.2 Observed: 701.3

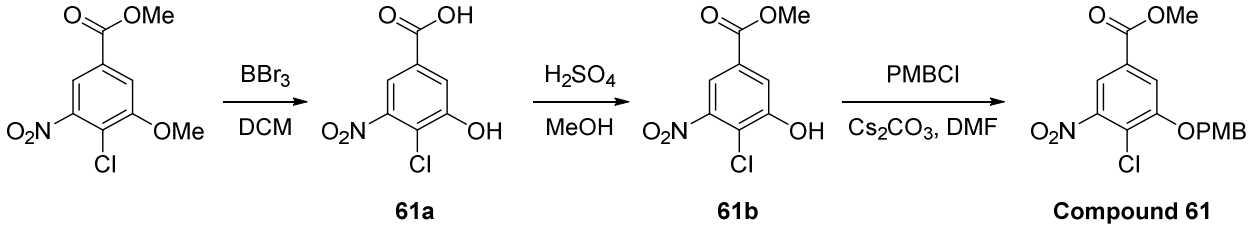
41		HATU - Method 4a	66% 2.50 mg 0.00272 mmol	RT: 2.24 Theoretical: 813.3 Observed: 813.5	RT: 1.84 Theoretical: 693.3 Observed: 693.5
42		COMU – Method 4b	45% 1.64 mg 0.00184 mmol	RT: 1.59 Theoretical: 783.3 Observed: 783.4	RT: 1.31 Theoretical: 663.2 Observed: 663.4
43		COMU – Method 4b	29% 1.07 mg 0.00120 mmol	RT: 1.63 Theoretical: 783.3 Observed: 783.4	RT: 1.36 Theoretical: 663.2 Observed: 663.4
44		COMU – Method 4b	55% 2.00 mg 0.00225 mmol	RT: 1.47 Theoretical: 783.3 Observed: 783.4	RT: 1.19 Theoretical: 663.2 Observed: 663.4
45		COMU – Method 4b	37% 1.36 mg 0.00153 mg	RT: 1.61 Theoretical: 783.3 Observed: 783.4	RT: 1.34 Theoretical: 663.2 Observed: 663.4

46		COMU – Method 4b	63% 2.30 mg 0.00258 mmol	RT: 1.62 Theoretical: 783.3 Observed: 783.4	RT: 1.34 Theoretical: 663.2 Observed: 663.4
47		COMU – Method 4b	46% 1.67 mg 0.00187 mmol	RT: 1.74 Theoretical: 787.3 Observed: 787.5	RT: 1.46 Theoretical: 667.2 Observed: 667.4
48		COMU – Method 4b	60% 2.35 mg 0.00247 mmol	RT: 1.79 Theoretical: 843.4 Observed: 843.5	RT: 1.52 Theoretical: 723.3 Observed: 723.5
49		COMU – Method 4b	60% 2.42 mg 0.00244 mmol	RT: 2.18 Theoretical: 883.3 Observed: 883.5	RT: 1.90 Theoretical: 763.2 Observed: 763.4
50		COMU – Method 4b	55% 2.17 mg 0.00225 mmol	RT: 2.17 Theoretical: 855.2 Observed: 855.4	RT: 1.59 Theoretical: 735.2 Observed: 735.3

51		COMU – Method 4b	71% 2.76 mg 0.00289 mmol	RT: 1.89 Theoretical: 847.3 Observed: 847.5	RT: 1.31 Theoretical: 727.3 Observed: 727.6
52		COMU – Method 4b	51% 2.23 mg 0.00211 mmol	RT: 2.50 Theoretical: 951.3 Observed: 951.6	RT: 1.99 Theoretical: 831.3 Observed: 831.6
53		COMU – Method 4b	51% 2.00 mg 0.00210 mmol	RT: 2.01 Theoretical: 843.4 Observed: 843.5	RT: 1.44 Theoretical: 723.3 Observed: 723.6
54		COMU – Method 4b	52% 1.97 mg 0.00214 mmol	RT: 1.86 Theoretical: 815.3 Observed: 615.5	RT: 1.28 Theoretical: 695.3 Observed: 695.5
55		HATU - Method 4a	56% 2.04 mg 0.00228 mmol	RT: 1.88 Theoretical: 1027.4 Observed: 1028.0	RT: 1.35 Theoretical: 667.2 Observed: 667.8

56		COMU – Method 4b	63% 2.24 mg 0.00257 mmol	RT: 1.78 Theoretical: 763.4 Observed: 763.5	RT: 1.11 Theoretical: 643.3 Observed: 643.9
57		HATU - Method 4a	60% 2.08 mg 0.00247 mmol	RT: 1.31 Theoretical: 735.3 Observed: 735.7	RT: 0.99 Theoretical: 615.3 Observed: 615.6
58		HATU - Method 4a	55% 2.02 mg 0.00225 mmol	RT: 1.88 Theoretical: 789.3 Observed: 789.4	RT: 1.60 Theoretical: 669.2 Observed: 669.3
59		HATU - Method 4a	56% 2.00 mg 0.00230 mmol	RT: 1.81 Theoretical: 761.2 Observed: 761.4	RT: 1.56 Theoretical: 641.2 Observed: 641.3
60		HATU - Method 4a	48% 1.82 mg 0.00196 mmol	RT: 1.98 Theoretical: 821.2 Observed: 821.4	RT: 1.68 Theoretical: 701.2 Observed: 701.4

Synthesis of methyl 4-chloro-3-((4-methoxybenzyl)oxy)-5-nitrobenzoate (Compound 61).



Synthesis of 61a

[0479] To a solution of methyl 4-chloro-3-methoxy-5-nitrobenzoate (15 g, 61 mmol, 1 equiv.) in DC (60 mL) at 0°C under nitrogen was added BBr_3 (1 M in DCM, 153 mL, 153 mmols, 2.5 equiv.) dropwise over 20 min. The reaction mixture was stirred at 0°C for 30 min and then allowed to warm to 25°C and stirred for a further 12 h. The reaction mixture was cooled to 0°C, quenched with methanol, and concentrated *in vacuo* to give **61a** (12.3 g, 56.5 mmols, 93% yield) as dark brown oil. LC-MS (Method C, ESI+): m/z $[M + H]^+$ = 218.0 (theoretical); 217.9 (observed). HPLC retention time: 0.21 min.

Synthesis of 61b

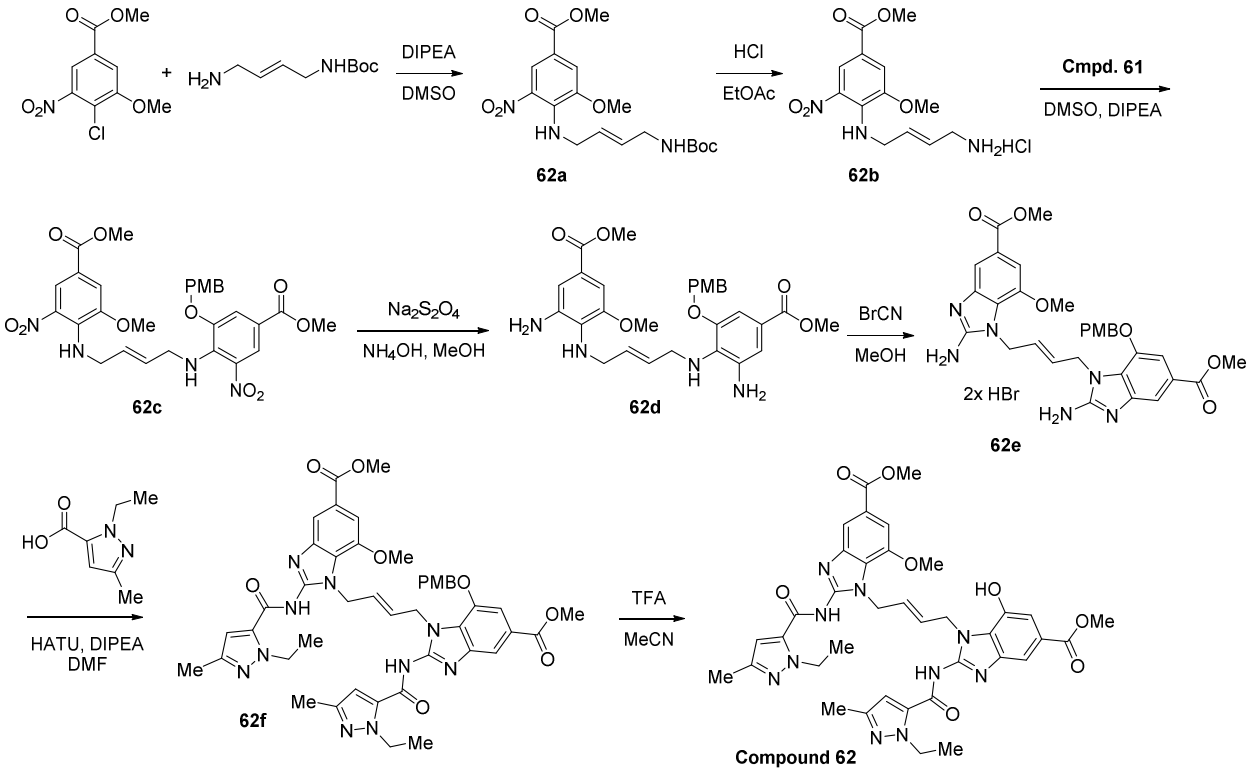
[0480] To a solution of **61a** (26.6 g, 122 mmol, 1 equiv.) in methanol (800 mL), was added concentrated H_2SO_4 (600 mg, 6.11 mmol, 0.05 equiv.), the mixture was stirred at 60°C for 12 h. LCMS analysis (Method C) showed the reaction was completed. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude residue was diluted with water (50 mL) and saturated $NaHCO_3$ (50 mL) was carefully added to achieve a pH > 7. The resultant solid was collected by filtration, washed with water (25 mL) and dried under vacuum to give **61b** (25 g, 88% yield) as a brown solid. LC-MS (Method C, ESI+): m/z $[M + H]^+$ = 232.0 (theoretical); 231.9 (observed). HPLC retention time: 0.92 min.

Synthesis of 61

[0481] To a solution of **61b** (18 g, 78 mmol, 1 equiv.) in DMF (200 mL) was added Cs_2CO_3 (27.9 g, 86 mmol, 1.1 equiv.) and 1-(chloromethyl)-4-methoxybenzene (12.8 g, 82 mmol, 1.05 equiv.) and the mixture was stirred at 25°C for 16 h. LCMS analysis (Method C) showed the reaction was completed. The reaction was poured into water, filtered and dried under high vacuum to give **61** (22.3 g, 82% yield) as a light-yellow solid. **^{1}H NMR** (400MHz, DMSO-d_6): δ =8.11 (d,

J=1.4 Hz, 1H), 7.97 (d, *J*=1.4 Hz, 1H), 7.43 (d, *J*=8.5 Hz, 2H), 6.99 (d, *J*=8.5 Hz, 2H), 5.33 (s, 2H), 3.92 (s, 3H), 3.77 (s, 3H).

Synthesis of methyl (E)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-hydroxy-5-(methoxycarbonyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 62).



Synthesis of 62a

[0482] To a solution of tert-butyl (E)-(4-aminobut-2-en-1-yl)carbamate (12.5 g, 67.2 mmol, 1.1 equiv.) in DMSO (150 mL) was added methyl 4-chloro-3-methoxy-5-nitrobenzoate (15 g, 61.1 mmol, 1 equiv.) and DIPEA (39.5 g, 305 mmol, 5 equiv.) the mixture was stirred at 100 °C for 12 h. The mixture was poured into water, extracted with EtOAc and concentrated *in vacuo* to give **62a** (16.4 g, 41.4 mmols, 68% yield) as a dark red solid. LC-MS (Method C, ESI+): m/z [M – *t*Bu]$^+$ = 340.1 (theoretical); 340.1 (observed). HPLC retention time: 1.08 min.

Synthesis of 62b

[0483] **62a** (21 g, 53.1 mmol, 1 equiv.) was added to a solution of HCl in ethyl acetate (4 M, 350 mL, 1400 mmols, 26 equiv.) and the mixture was stirred at 25°C for 2 h. The mixture was concentrated *in vacuo* and crude solid washed with EtOAc to give **62b** as the HCl salt (14.5 g, 43.7 mmols, 82% yield) as a dark red solid. **^{1}H NMR** (400MHz, DMSO-d_6): δ = 8.19 (d, *J*=1.8 Hz, 1H), 8.12 (br s, 1H), 8.01 (br s, 3H), 7.46 (d, *J*=1.6 Hz, 1H), 5.87 (td, *J*=5.8, 15.5 Hz, 1H), 5.71 - 5.55 (m, 1H), 4.21 (br s, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 3.42 - 3.35 (m, 2H).

Synthesis of 62c

[0484] To a solution of **61** (4.5 g, 12.8 mmol) in DMSO (70 mL) was added **62b** (4.67 g, 14.1 mmol, HCl salt) and DIPEA (8.3 g, 64 mmol, 5 equiv.) and the reaction was stirred at 80°C for 10 h. The mixture was poured into ice water, extracted with EtOAc and concentrated *in vacuo*. The residue was recrystallized (ethyl acetate, 20V, reflux) to give **62c** (6.4 g, 10.5 mmols, 82% yield) as a dark red solid. MS (Method C, ESI+): m/z $[M + H]^+$ = 611.2 (theoretical); 611.2 (observed). HPLC retention time: 1.34 min. **^{1}H NMR** (400MHz, DMSO-d_6): δ = 8.06 (dd, *J*=1.5, 9.5 Hz, 2H), 7.96 (br d, *J*=2.9 Hz, 2H), 7.44 (s, 1H), 7.36 (d, *J*=8.5 Hz, 2H), 7.30 (s, 1H), 6.94 (d, *J*=8.5 Hz, 2H), 5.53 - 5.29 (m, 2H), 5.00 (s, 2H), 4.03 (br t, *J*=5.4 Hz, 4H), 3.84 (s, 6H), 3.76 (d, *J*=3.5 Hz, 6H).

Synthesis of 62d

[0485] To a solution of **62c** (6.0 g, 9.83 mmol, 1 equiv.) in MeOH (300 mL) was added NH_4OH (60 mL, 28% NH_3 in H_2O) and $Na_2S_2O_4$ (20.5 g, 118 mmol, 12 equiv.). The mixture was stirred at 25°C for 16 h and went from bright red to a light yellow / nearly colorless heterogenous mixture. The mixture was filtered, concentrated to remove MeOH and the remaining aqueous solution was extracted with EtOAc. The organic phases were combined, dried with Na_2SO_4 and concentrated in vacuo to give **62d** (4.0 g, 7.25 mmols, 74% yield) as an off white solid. MS (Method B, ESI+): m/z $[M + H]^+$ = 551.25 (theoretical); 551.2 (observed). HPLC retention time: 1.29 min.

Synthesis of 62e

[0486] To a solution of **62d** (4.0 g, 7.25 mmol, 1 equiv.) in MeOH (200 mL) was added BrCN (4.62 g, 43.6 mmol, 6 equiv.). The mixture was stirred at 25°C for 2 h at which point LC-

MS analysis (Method C) showed full conversion. The reaction mixture was concentrated *in vacuo* and the crude product washed with ethanol and petroleum ether to give **62e** as the 2x HBr salt (2.6 g, 3.53 mmols 49% yield) as an off white solid. MS (Method C, ESI+): m/z $[M + H]^+$ = 601.2 (theoretical); 601.3 (observed). HPLC retention time: 2.73 min. **^{1}H NMR** (400MHz, DMSO-d_6): δ = 12.87 (br s, 1H), 8.72 (br d, *J*=17.0 Hz, 4H), 7.59 (s, 2H), 7.42 (s, 1H), 7.26 - 7.16 (m, 3H), 6.82 (d, *J*=8.6 Hz, 2H), 5.70 (br d, *J*=15.7 Hz, 1H), 5.57 - 5.48 (m, 1H), 5.00 (s, 2H), 4.83 - 4.73 (m, 4H), 3.88 (s, 6H), 3.71 (s, 3H), 3.65 (s, 3H).

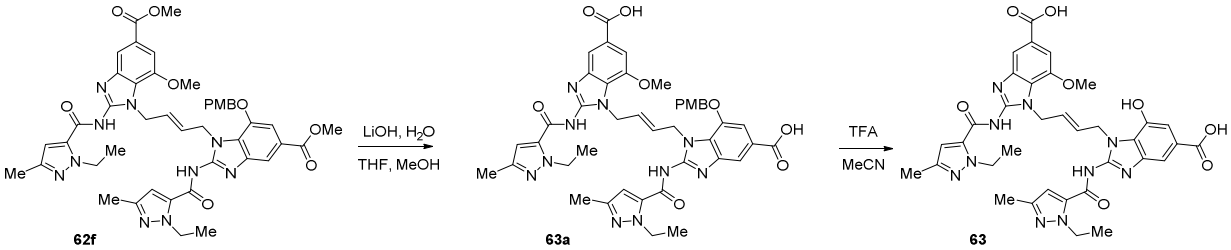
Synthesis of 62f

[0487] To a solution of 1-ethyl-3-methyl-1H-pyrazole-5-carboxylic acid (3.15 g, 20.5 mmol, 2.6 equiv.) in DMF (30 mL) was added HATU (8.38 g, 22.0 mmol, 2.8 equiv.) and the solution was stirred at 60°C for 10 min. A second solution containing DIPEA (5.09 g, 39 mmol, 5 equiv.) and **62e** (6.0 g, 7.87 mmol, 1 equiv. 2x HBr salt) in DMF, 30 mL) was prepared and added to the activated acid. The reaction was then stirred at 60°C for 2 h. The solution was poured into water, filtered and triturated with acetonitrile to give **62f** (2.54 g, 2.91 mmols, 37% yield) as an off-white solid. MS (Method C, ESI+): m/z $[M + H]^+$ = 873.4 (theoretical); 873.4 (observed). HPLC retention time: 3.44 min. **^{1}H NMR** (400MHz, DMSO-d_6) δ = 12.88 (br s, 2H), 7.74 (br s, 2H), 7.22 (br s, 1H), 7.16 - 6.97 (m, 3H), 6.66 (br d, *J*=7.9 Hz, 2H), 6.57 - 6.36 (m, 2H), 5.87 - 5.37 (m, 2H), 4.78 (br s, 6H), 4.51 (br dd, *J*=7.0, 17.3 Hz, 4H), 3.85 (s, 6H), 3.59 (s, 3H), 3.52 (br s, 3H), 2.10 (br d, *J*=11.1 Hz, 6H), 1.26 (td, *J*=6.8, 18.8 Hz, 6H).

Synthesis of 62

[0488] An oven-dried 4 mL vial with stir bar was charged with **62f** (9 mg, 0.010 mmols, 1 equiv.) which was dissolved in 1:1 MeCN:TFA (1 mL) and stirred for 1 hour at room temperature. Solvent was removed in vacuo and product dried on high-vac overnight to give **62** (7.5 mg, 0.0099 mmols, quant. yield) as a tan solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 753.3 (theoretical); 753.7 (observed). HPLC retention time: 1.99 min.

Synthesis of (E)-1-(4-(5-carboxy-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-hydroxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylic acid (Compound 63).



Synthesis of 63a

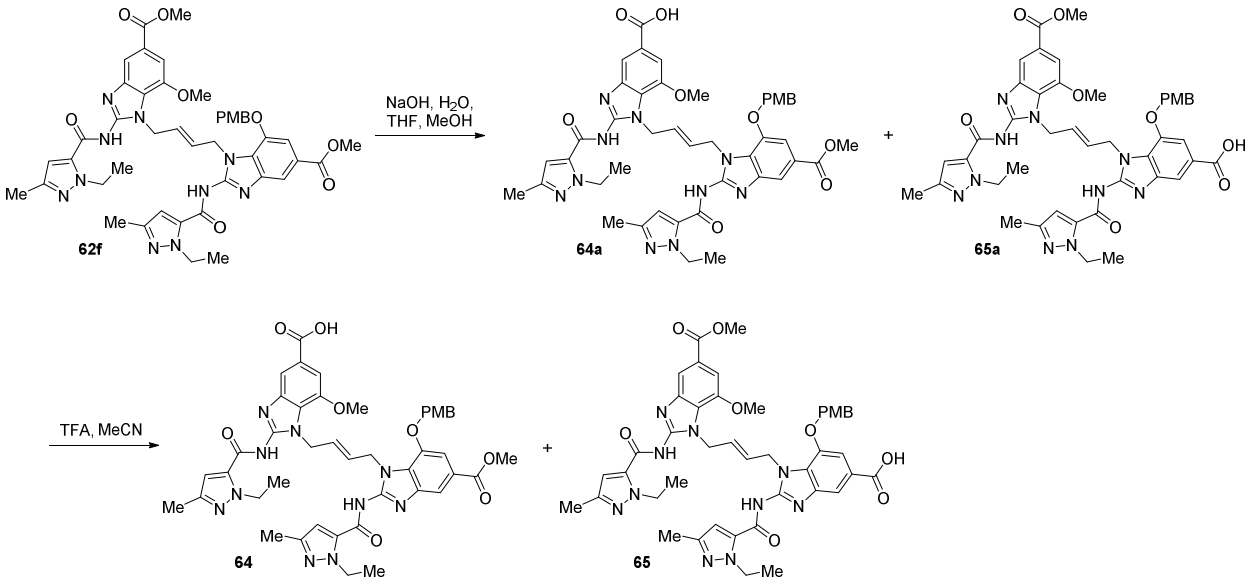
[0489] Compound **62f** (100 mg, 0.115 mmols, 1 equiv.) was dissolved in acetonitrile (1 mL), 1M LiOH (1 mL, 1 mmol, 9 equiv.) was added and the solution was heated to 80 ^{o}C for 1 hour. The vial was cooled, solvent removed *in vacuo* and product purified by prepHPLC (Method I, 5 – 95% MeCN in water with 0.1% TFA) to give **63a** (78 mg, 0.092 mmols, 97% yield) as a white solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 845.3 (theoretical); 845.8 (observed). HPLC retention time: 1.95 min.

Synthesis of 63

[0490] Compound **63** was prepared as previously described (see "Synthesis of 62"). MS (Method D, ESI+): m/z $[M + H]^+$ = 725.3 (theoretical); 725.4 (observed). HPLC retention time: 1.83 min.

Synthesis of (E)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-hydroxy-5-(methoxycarbonyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-methoxy-1H-benzo[d]imidazole-5-carboxylic acid (Compound 64) and (E)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-

carboxamido)-7-methoxy-5-(methoxycarbonyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-hydroxy-1H-benzo[d]imidazole-5-carboxylic acid Compound 65).



Synthesis of 64a and 65a

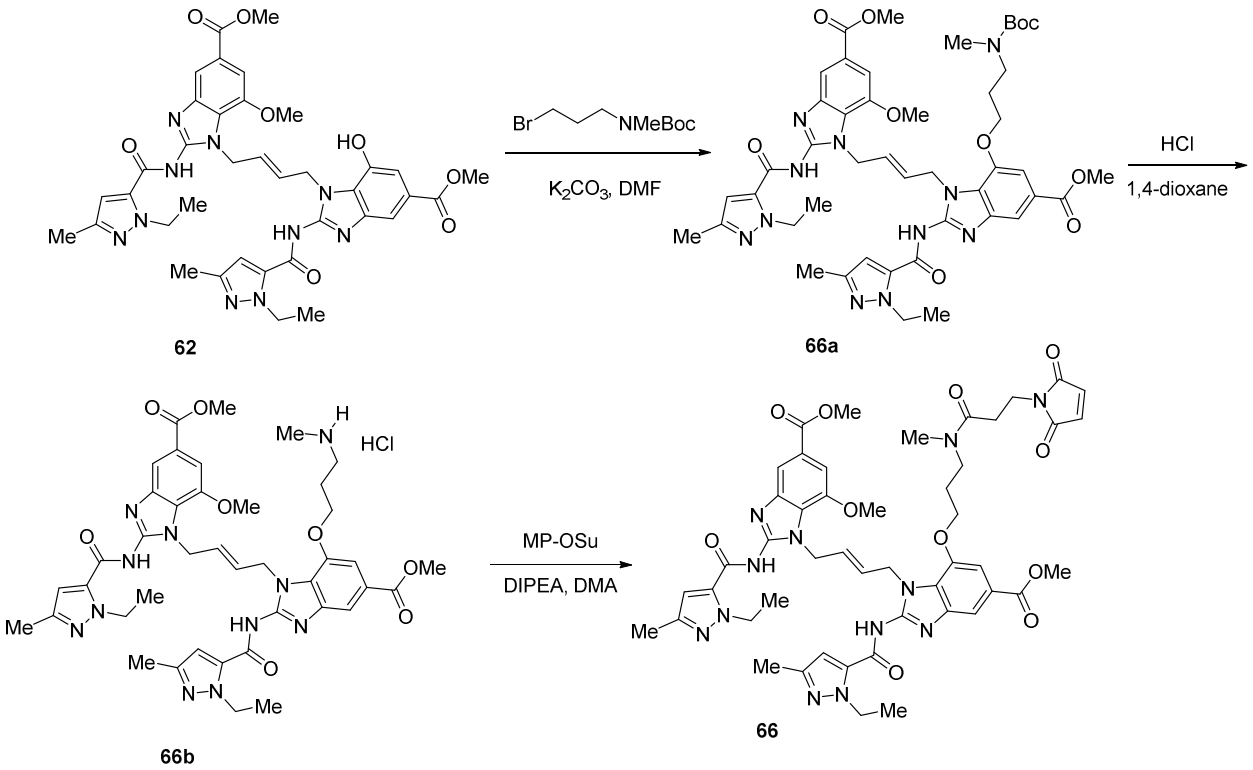
[0491] An inseparable 1:1 mixture of compounds 64a and 65a was prepared as previously described (see "Synthesis of 65a") substituting sodium hydroxide for lithium hydroxide and quenching the reaction at 50% conversion followed by purification via prepHPLC (Method H with 0.1% FA). MS (Method D, ESI+): m/z $[M + H]^+$ = 859.4 (theoretical); 859.5 (observed). HPLC retention time: 2.46 min.

Synthesis of 64 and 65

[0492] An inseparable 1:1 mixture of compounds 64a and 65a was prepared as previously described (see "Synthesis of 65a"). MS (Method D, ESI+): m/z $[M + H]^+$ = 739.4 (theoretical); 739.4 (observed). HPLC retention time: 1.99 and 2.04 min.

Synthesis of methyl (E)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-

ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-5-(methoxycarbonyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxylate (Compound 66)



Synthesis of 66a

[0493] Compound **62** (397 mg, 0.527 mmol, 1 equiv.), tert-butyl (3-bromopropyl)(methyl)carbamate (146 mg, 0.580 mmol, 1.1 equiv.) and potassium carbonate (218 mg, 1.58 mmol, 3 equiv.) were dissolved in DMF (5.3 mL) in a 20 mL vial. The reaction was stirred at 55 ^{o}C for 18 hours and then the mixture was filtered washing with methanol and the filtrate concentrated *in vacuo*. To the crude solid was added cold water and the precipitate isolated via filtration to give **66a** (232 mg, 0.251 mmol, 48% yield). MS (Method E, ESI+): m/z $[M + H]^+$ = 924.4 (theoretical); 924.9 (observed). HPLC retention time: 2.42 min.

Synthesis of 66b

[0494] Compound **66a** (232 mg, 0.251 mmol, 1 equiv.) was dissolved in methanol (2.5 mL) and 4M HCl in dioxane was added (0.5 mL, 2.01 mmol, 8 equiv.). The reaction stirred at 30 ^{o}C for 90 minutes. The solvent was in vacuo and the crude product purified by prepHPLC (Method

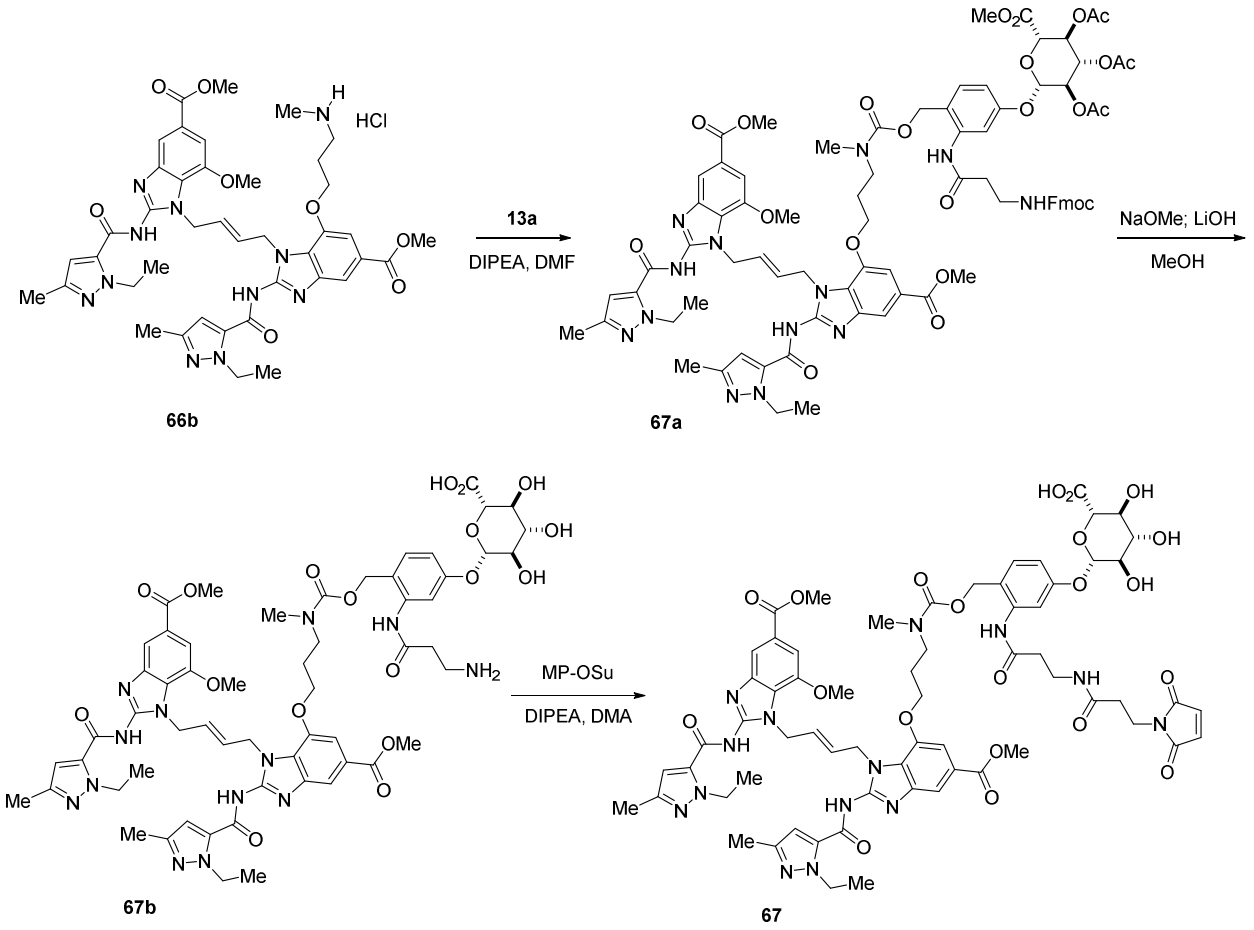
I with 0.05% TFA) to afford **66b** (206 mg, 0.24 mmol, 96% yield). MS (Method E, ESI+): m/z $[M + H]^+$ = 824.4 (theoretical); 824.9 (observed). HPLC retention time: 1.56 min.

Synthesis of 66

[0495] Compound **66** (25 mg, 0.0291 mmol, 1 equiv.) and MP-OSu (11.6 mg, 0.0436 mmol, 1.5 equiv.) were dissolved in DMA (0.58 mL) and DIPEA (20 µL, 0.116 mmol) was added. The reaction was stirred at room temperature for 1 hour. The mixture was directly purified by prepHPLC (Method H, with 0.05% TFA) to afford **66** as a white solid (10.88 mg, 0.0112 mmol, 38% yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 975.4 (theoretical); 975.4 (observed). HPLC retention time: 2.24 min.

Synthesis of (2S,3S,4S,5R,6S)-6-(3-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-((((3-((2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-((E)-4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-5-(methoxycarbonyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-5-(methoxycarbonyl)-1H-benzo[d]imidazol-7-

yl)oxy)propyl)(methyl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (Compound 67)



Synthesis of 67a

[0496] Compound **13a** (65 mg, 0.0868 mmol, 1.4 equiv.) and bis(pentafluorophenyl) carbonate (120 mg, 0.304 mmo, 5 equiv.) were dissolved in DMA (0.43 mL) and DIPEA (70 µL, 0.404 mmol, 6.7 equiv.) was added. The reaction was stirred for 30 minutes and then **66b** (52 mg, 0.0607 mmol, 1 equiv.) was added. The reaction was stirred at room temperature for 18 hours. The solution was diluted with H_2O and extracted with EtOAc (3x), and the combined organics were washed with 1M HCl (3x), dried with MgSO4, filtered and solvent removed in vacuo to give a crude solid. This material was dissolved in DMSO and purified by prepHPLC (Method H, with 0.05% TFA) to give **67a** as a white solid (33.1 mg, 0.0207 mmol, 34% yield). **LCMS** (Method D, ESI+) m/z $[M+H]^+$ 1598.6 (theoretical), 1598.6 (observed). LCMS retention time 2.65 min.

MS (Method D, ESI+): m/z $[M + H]^+$ = 1598.6 (theoretical); 1598.6 (observed). HPLC retention time: 2.65 min.

Synthesis of 67b

[0497] Compound **67a** (33.1 mg, 0.0207 mmol) was dissolved in dry methanol (0.21 mL), cooled in an ice bath, and 0.5M NaOMe in MeOH (41.5 µL, 0.0414 mmol, 2 equiv.) was added. The reaction was monitored by LCMS (Method D) and upon full acetate deprotection, 1M LiOH (62µL, 0.0621 mmol, 3 equiv.) was added. The reaction stirred at room temperature for 1h monitoring by LCMS (Method E). Upon full conversion, acetic acid (62µL) was added, solvent removed in vacuo and crude product purified via prepHPLC (Method H, with 0.05% TFA) to give **67b** as a white solid (10.1 mg, 0.0075 mmol, 36% yield). **LCMS** (Method D, ESI+) m/z $[M+H]^+$ 1236.5 (theoretical), 1236.5 (observed). LCMS retention time 2.31 min.
MS (Method D, ESI+): m/z $[M + H]^+$ = 1236.5 (theoretical); 1236.5 (observed). HPLC retention time: 2.31 min.

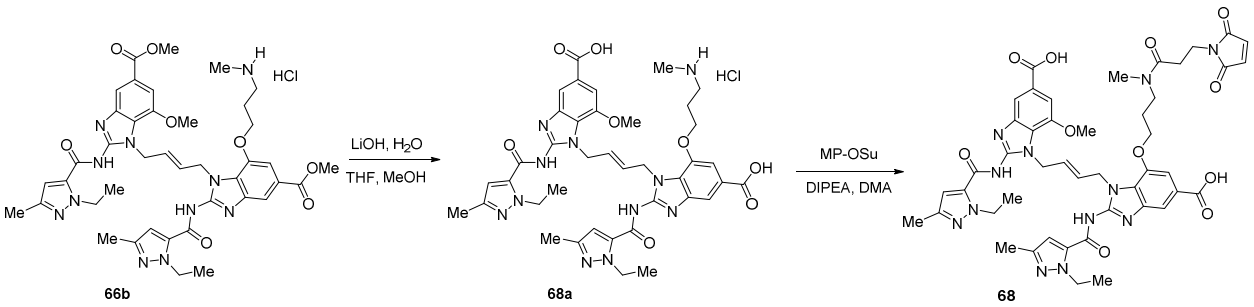
Synthesis of 67

[0498] Compound **67b** (10.1 mg, 0.0075 mmol, 1 equiv.) and MP-OSu (3.0 mg, 0.0112 mmol, 1.5 equiv.) were dissolved in DMA (150 µL), DIPEA (4µL, 0.0224 mmol) was added. The reaction was stirred for 30 min at room temperature at which point acetic acid (4 µL) was added, and the mixture was purified via prepHPLC (Method G, with 0.05% TFA) to obtain **67** as a white solid (3.3 mg, 0.0024mmol, 32% yield). **LCMS** (Method E, ESI+) m/z $[M+H]^+$ 1387.5 (theoretical), 1387.5 (observed). LCMS retention time 1.92 min.

[0499] MS (Method E, ESI+): m/z $[M + H]^+$ = 1387.5 (theoretical); 1387.5 (observed). HPLC retention time: 1.92 min.

Synthesis of (E)-1-(4-(5-carboxy-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-

methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxylic acid (Compound 68)



Synthesis of 68a

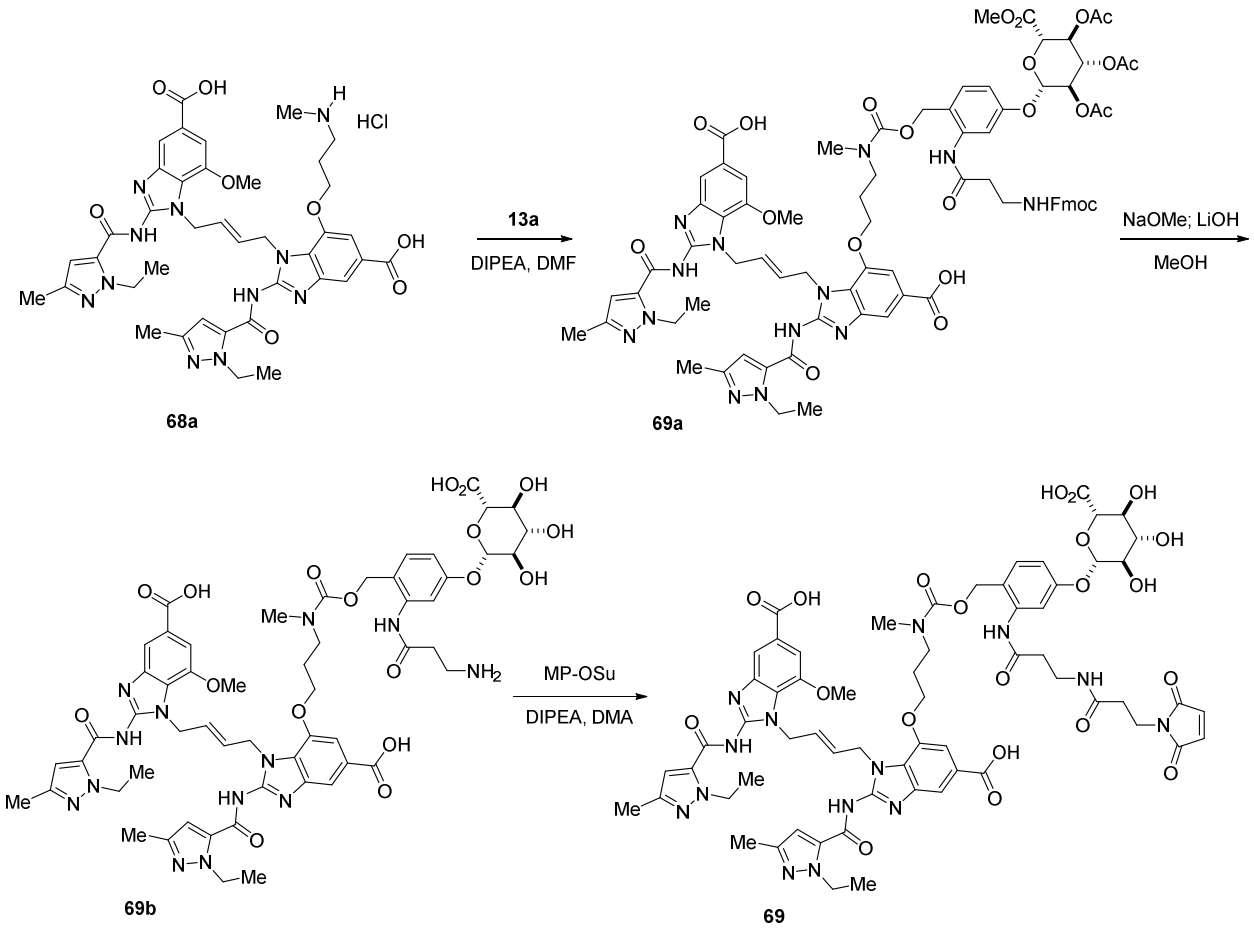
[0500] Compound **66b** (30 mg, 0.032 mmol) was dissolved in methanol (0.32 mL) and 1M LiOH (0.256 mL, 0.256 mmols, 8 equiv.) was added. The mixture was stirred at 80ºC for 1h. The mixture was concentrated in vacuo and purified via prepHPLC (Method H with 0.05% TFA) to afford **68a** as a white solid (17.4 mg, 0.0191 mmol, 60% yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 796.4 (theoretical); 796.4 (observed). HPLC retention time: 1.83 min.

Synthesis of 68

[0501] Compound **68a** (16.7 mg, 0.0183 mmol, 1 equiv.) and MP-OSu (7.3 mg, 0.0275 mmol, 1.5 equiv.) were dissolved in DMA (0.37 mL) and DIPEA (10 µL, 0.0574 mmol, 2 equiv.) was added. The reaction was stirred at room temperature for 1 hour, AcOH (10µL) was added and the crude product was purified via prepHPLC (Method H with 0.05% TFA) to afford **68** as a white solid (7.6 mg, 0.0080 mmol, 44% yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 974.4 (theoretical); 974.4 (observed). HPLC retention time: 2.42 min.

Synthesis of 1-((E)-4-(5-carboxy-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-((((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-

yl)propanamido)propanamido)benzyl)oxy)carbonyl)(methyl)amino)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxylic acid (Compound 69)



Synthesis of 69a

[0502] Compound **69a** was prepared as previously described (see "Synthesis of 67a"). MS (Method E, ESI+): m/z $[M + H]^+$ = 1570.6 (theoretical); 1570.4 (observed). HPLC retention time: 1.95 min.

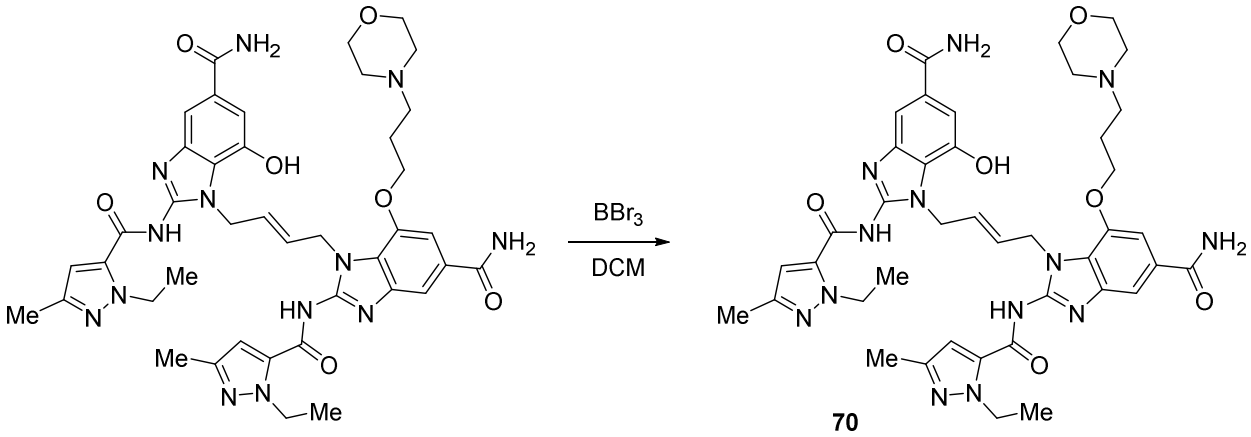
Synthesis of 69b

[0503] Compound **69b** was prepared as previously described (see "Synthesis of 67b"). MS (Method E, ESI+): m/z $[M + H]^+$ = 1208.5 (theoretical); 1208.3 (observed). HPLC retention time: 1.48 min.

Synthesis of 69

[0504] Compound **69** was prepared as previously described (see "Synthesis of 67"). MS (Method E, ESI+): m/z $[M + H]^+$ = 1359.5 (theoretical); 1359.4 (observed). HPLC retention time: 1.68 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-(3-morpholinopropoxy)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxamide (Compound 70)

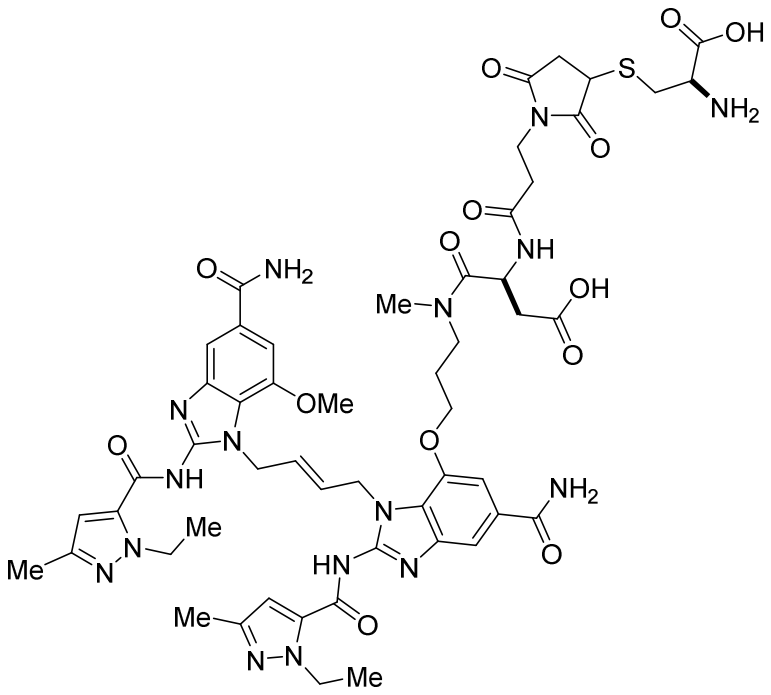
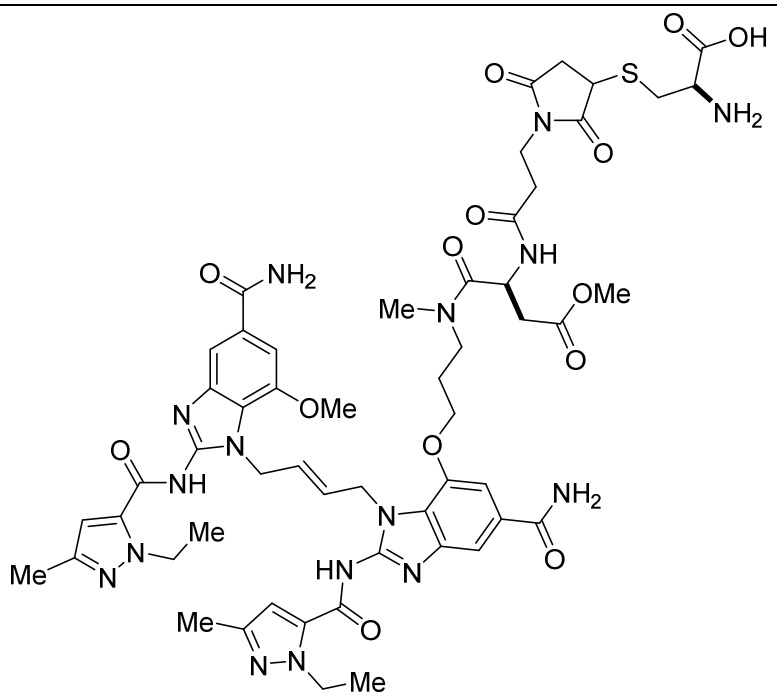


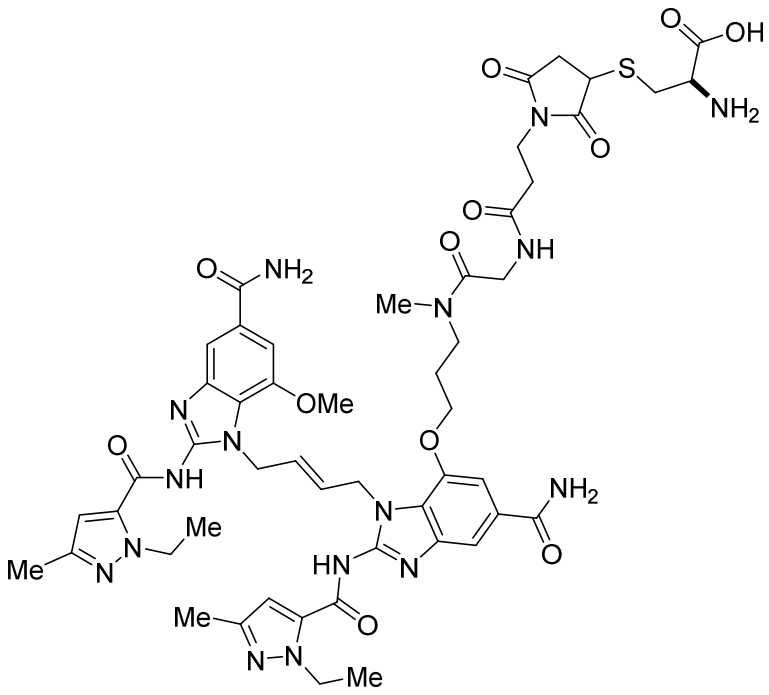
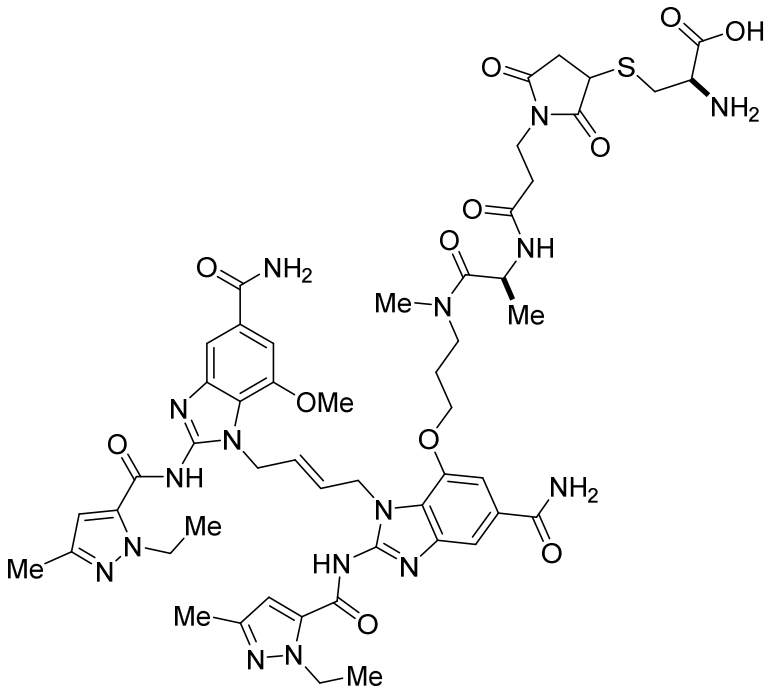
Synthesis of 70

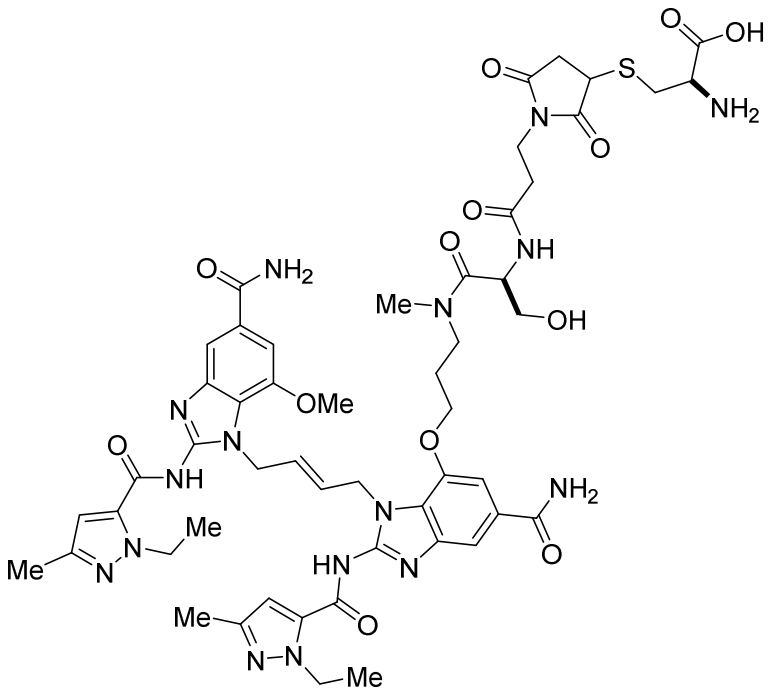
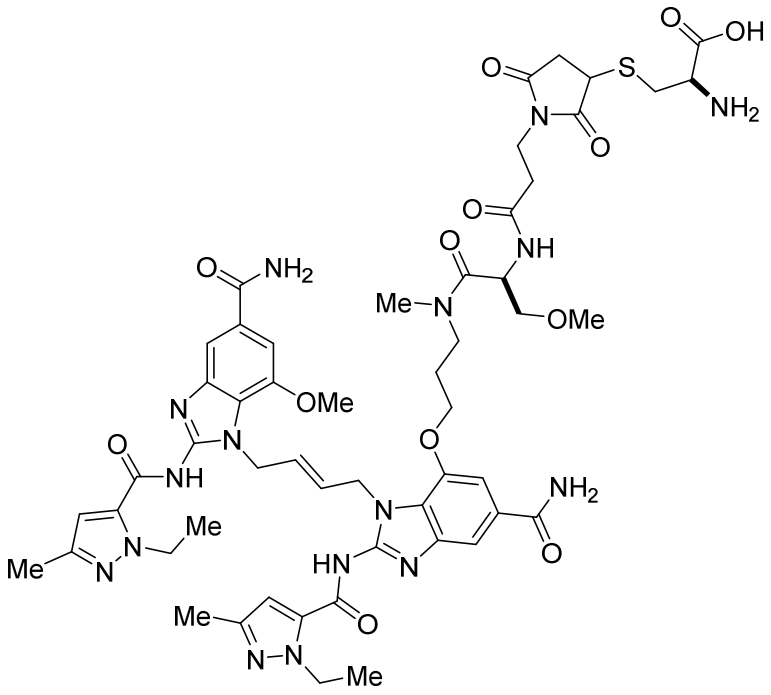
[0505] To a solution of compound **A** (6 mg, 0.00706 mmol) in dry DCM (0.10 mL) was added BBr_3 (0.04 mL, 1M in DCM) dropwise. The slurry that formed was stirred overnight at 30°C under argon. The reaction was monitored by UPLC-MS. Upon completion, cold water (0.10 mL) was added and the mixture was stirred vigorously. After 30 min., the solvent was evaporated, and the product purified by prepHPLC (Method G) using formic acid as the additive. Pure fractions were collected, frozen, and lyophilized to afford compound **70** (5.14 mg, 0.00528 mmol, 75% yield) as a white solid. UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 836.9 (theoretical), 836.6 (observed). HPLC retention time: 1.34 min.

[0506] The cysteine adducts of compounds 17-24 were prepared using the following method.

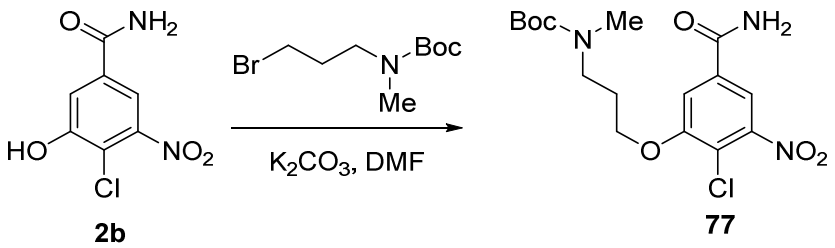
[0507] General Method 6. A 10 mM solution of maleimide was incubated with 1 equiv. of L-cysteine (100 mM in water) at 37 °C for 1 hour and the product used without any further purification.

Compound	Structure	LC-MS data
71		RT: 1.17 Theoretical: 1181.5 Observed: 1182.1
72		RT: 1.21 Theoretical: 1195.5 Observed: 1195.7

Compound	Structure	LC-MS data
73		RT: 1.17 Theoretical: 1123.5 Observed: 1124.0
74		RT: 1.19 Theoretical: 1137.5 Observed: 1137.9

Compound	Structure	LC-MS data
75		RT: 1.16 Theoretical: 1153.5 Observed: 1153.8
76		RT: 1.19 Theoretical: 1167.5 Observed: 1168.0

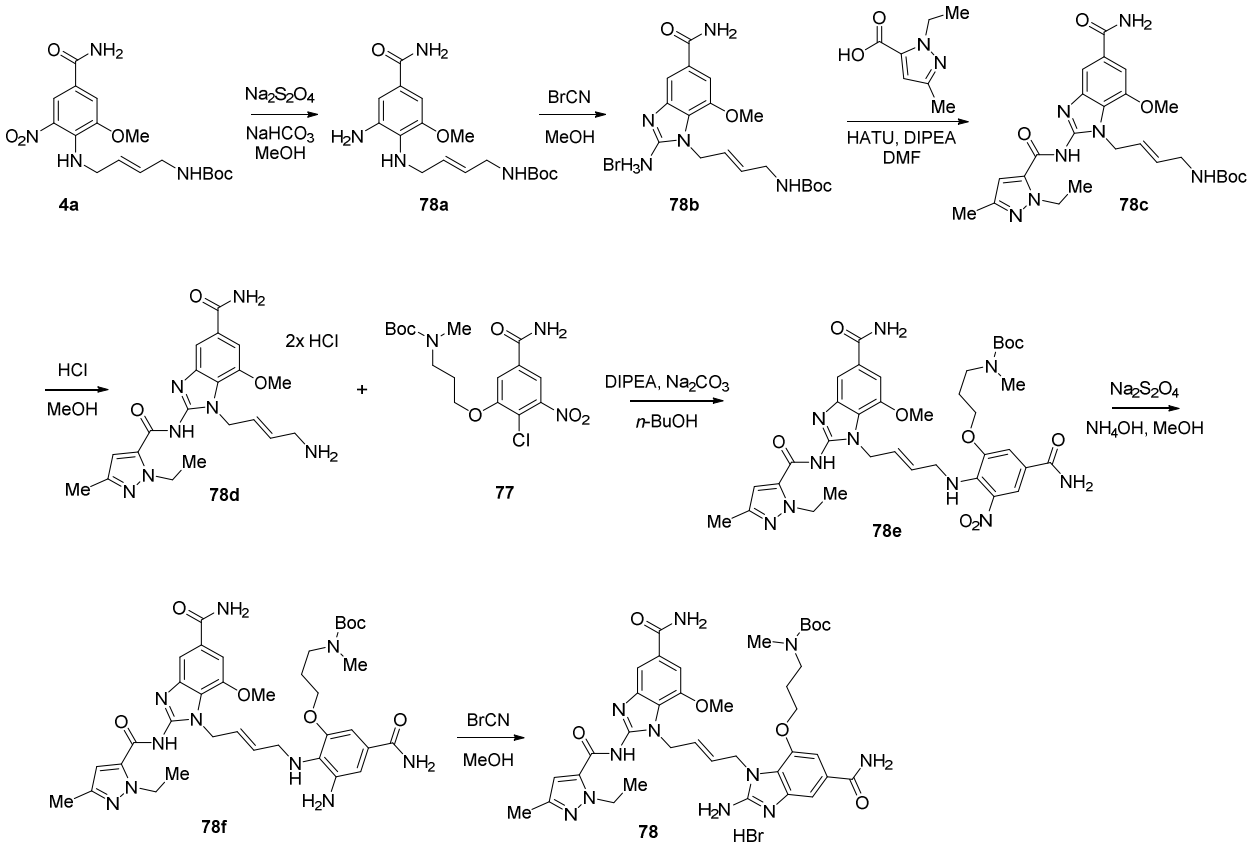
Synthesis of tert-butyl (3-(5-carbamoyl-2-chloro-3-nitrophenoxy)propyl)(methyl)carbamate (Compound 77)



2b **77**

[0508] A flame-dried 100 mL RB with stir bar was charged with a solution of **2b** (1.0 g, 4.62 mmol, 1 equiv.) in DMF (10 mL), potassium carbonate (830 mg, 6.00 mmol, 1.3 equiv.) and a solution of tert-butyl N-(3-bromopropyl)-N-methyl-carbamate (1.20 eq, 1.40 g, 5.54 mmol, 1.20 equiv.) in DMF (5 mL). Additional DMF was added to bring the total volume to 45 mL and the reaction was heated to 70 ºC for 24 hours. The reaction was cooled to room temperature and filtered over celite washing with DMF (3x10 mL). This solution was poured into ice water (900 mL), stirred for 90 minutes and crude product isolated via filtration. Finally, the filtrate was washed with cold water (300 mL) and dried in vacuo overnight to give **77** (1.23 g, 3.16 mmol, 68% yield).

Synthesis of tert-butyl (E)-(3-((2-amino-5-carbamoyl-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)carbamate (Compound 78)



Synthesis of 78a

[0509] A 500 mL round bottom flask with stir bar was charged with **4a** (3.0 g, 7.9 mmol, 1 equiv.) and sodium bicarbonate (12.5 g, 148 mmol, 19 equiv.) and ethanol (105 mL) was added to give a heterogenous solution. This solution was cooled in an ice-bath and a solution of sodium hydrosulfite (25.8 g, 148 mmol, 19 equiv.) in 105 mL water was added dropwise at such a rate to keep the internal temperature below 10 °C. The mixture was heated open to the atmosphere to 45 °C for 1 hour and cooled to room temperature. The mixture was filtered over celite, washing with EtOH (100 mL) and solvent removed in vacuo. The crude material was redissolved in 1:1 DCM:MeOH (200 mL), filtered over celite and solvent removed in vacuo. This procedure was repeated once more and then the crude product was loaded onto celite and purified by flash chromatography (50g Sfar HC Duo, SiO_2 column, 0 - 40% 10:1 NH_4OH:MeOH in DCM) to

give **78a** (1.45 g, 4.13 mmol, 52% yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 351.2 (theoretical); 351.1 (observed). HPLC retention time: 1.53 min.

Synthesis of 78b

[0510] An oven-dried 200 mL round bottom flask was charged with **78a** (1.95 g, 5.58 mmol, 1 equiv.) which was dissolved in methanol (45mL) and cyanogen bromide (3M in DCM, 5.6 mL, 16.7 mmol, 3 equiv.) was added to give a yellow homogenous solution. The reaction was stirred at room temperature for 48 hours and solvent removed *in vacuo* to give **78b** as the HBr salt (2.48 g, 5.44 mmol, 98% yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 376.2 (theoretical); 376.1 (observed). HPLC retention time: 0.71 min.

Synthesis of 78c

[0511] A flame dried 40 mL vial with stir bar was charged with **78b** HBr (867 mg, 1.90 mmol, 1 equiv.), 2-ethyl-5-methyl-pyrazole-3-carboxylic acid (879 mg, 5.70 mmol, 3 equiv.), and HATU (2.17 g, 5.70 mmol, 3 equiv.). The solids were dissolved in DMF (15mL) and then DIPEA (2.0 mL, 11.4 mmol, 6 equiv.) was added. The vial was sealed and stirred at room temperature for 48 hours. The solution was poured into ice-cold water (450 mL) with NH4OH (28% NH_3 in water, 10 mL) and allowed to precipitate at 4 ^oC overnight. The white precipitate was isolated via filtration and dried in vacuo overnight to give **78c** (658 mg, 1.29 mmol, 68% yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 512.3 (theoretical); 512.2 (observed). HPLC retention time: 2.35 min.

Synthesis of 78d

[0512] An oven dried 8 mL vial with stir bar was charged with **78c** (800 mg, 1.56 mmol, 3 equiv.) which was stirred in 3M HCl in MeOH (5.2 mL, 15.6 mmol HCl, 10 equiv.) for 1 hour. The solvent removed *in vacuo* to give **78d** as the 2xHCl salt (700 mg, 1.56 mmol, quantitative yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 412.2 (theoretical); 412.5 (observed). HPLC retention time: 0.73 min.

Synthesis of 78e

[0513] An oven-dried 20 mL microwave vial was charged with **78e** (700 mg, 1.56 mmol, 1 equiv.), **77** (909 mg, 2.34 mmol, 1.5 equiv.) and sodium carbonate (497 mg, 4.69 mmol,

3 equiv.) and to the mixture was added 1-butanol (15mL) and DIPEA (1.6 mL, 9.38 mmol, 6 equiv.). The vial was sealed and heated in a microwave reactor at 140 °C for 3 hours to give a bright red heterogenous mixture. This mixture was poured into DCM (100 mL) and filtered over celite washing with DCM (50 mL) and MeOH (50 mL). The crude product was loaded onto celite and purified via flash chromatography (50g Sfar HC Duo, SiO2 column, 0 - 20% MeOH in DCM) to give **78e** (569 mg, 0.746 mmol, 48% yield) as a bright red solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 763.4 (theoretical); 763.4 (observed). HPLC retention time: 2.17 min.

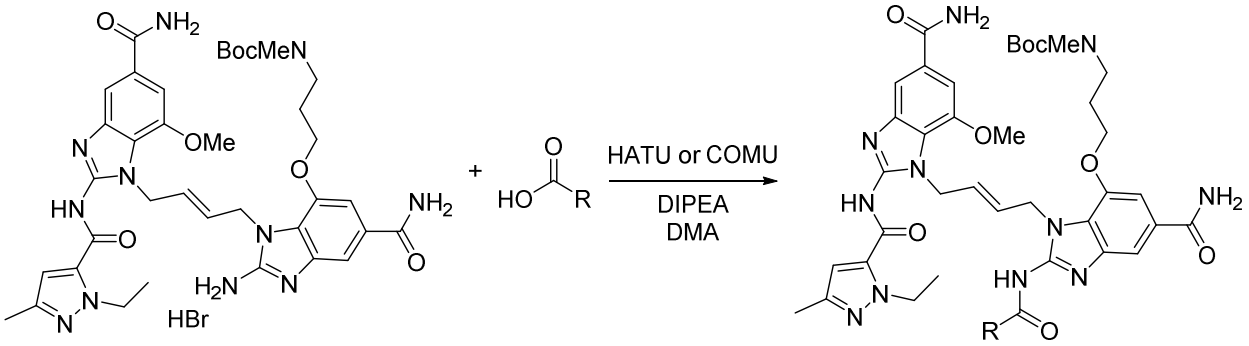
Synthesis of 78f

[0514] To a mixture of **78e** (569 mg, 0.746 mmol, 1 equiv.) in methanol (8mL) and NH_4OH (2.0 mL, 28% NH_3 in water) was added a solution of sodium hydrosulfite (2.34 g, 13.4 mmol, 18 equiv.) in water (8 mL). This solution was heated at 50 °C for 1 hour. The reaction was poured into a separatory funnel containing water (250 mL) and EtOAc (250 mL). The mixture was shaken, layers separated and the aqueous layer was further extracted with EtOAc (3x100 mL). The organics were combined, washed with brine (2x100 mL), dried with MgSO4, filtered and solvent removed *in vacuo* to give **78f** (400 mg, 0.546 mmol, 73% yield) as a tan solid. MS (Method D, ESI+): m/z $[M + H]^+$ = 733.4 (theoretical); 733.6 (observed). HPLC retention time: 1.39 min.

Synthesis of 78

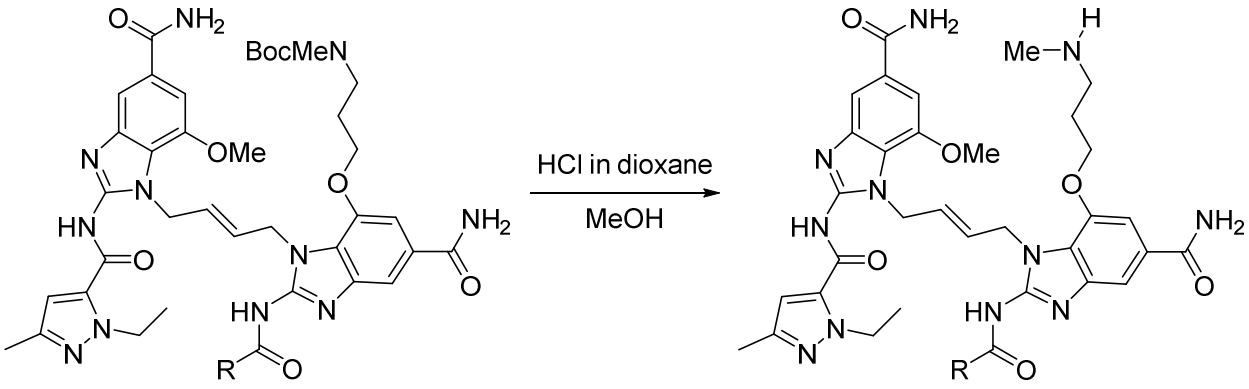
[0515] To a solution of **78f** (1.00 eq, 400 mg, 0.546 mmol) in methanol (5.5 mL) was added cyanogen bromide (3M in DCM, 0.55 mL, 1.65 mmol, 3 equiv.) and the mixture was stirred at room temperature for 24 hours. The solvent was removed *in vacuo* to give **78** as the HBr salt (456 mg, 0.544 mmol, quantitative yield). MS (Method D, ESI+): m/z $[M + H]^+$ = 758.4 (theoretical); 758.6 (observed). HPLC retention time: 1.19 min.

Library synthesis of amide analogs, group #2. Scheme and general methods. Compounds 71 – 95.

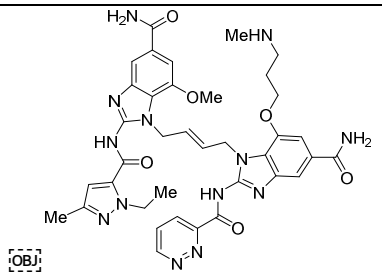
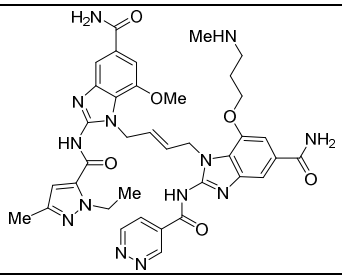
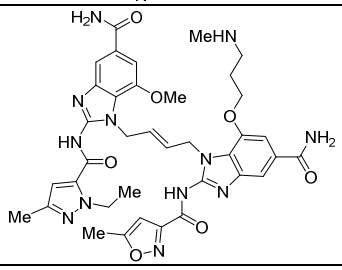


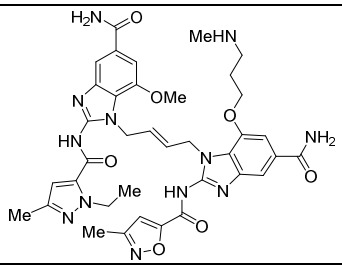
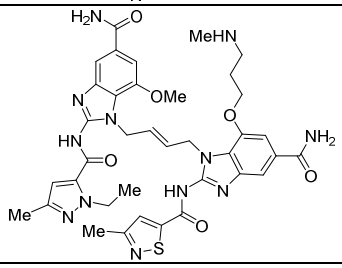
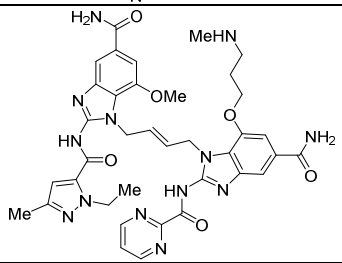
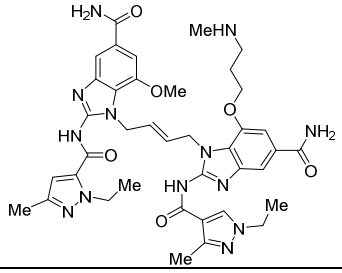
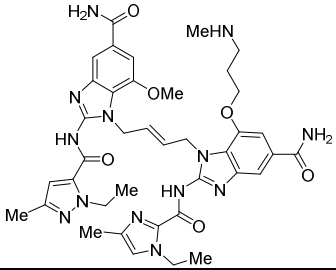
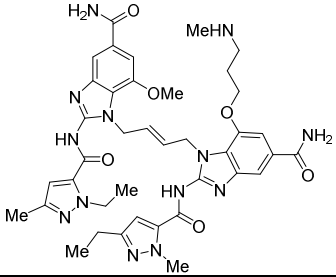
[0516] COMU Couplings (General Method 7A): A 2 mL microwave vial was charged with a solution of compound **78** (20 mg, 0.0238 mmol, 1 equiv.) in DMA (0.50 mL). The respective carboxylic acid (2 equiv.), COMU (20.4 mg, 0.0477 mmol, 2 equiv.) and DIPEA (20.8 µL, 0.119 mmol, 5 equiv.) were added. The vial was sealed and heated to 80 ºC in a microwave reactor for 1h. The reaction was monitored via UPLC-MS (Method E, ESI+). Upon completion, acetic acid (20 µL) was added and the resulting product was purified by prepHPLC (Method H) using 0.05% TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford product as a white solid.

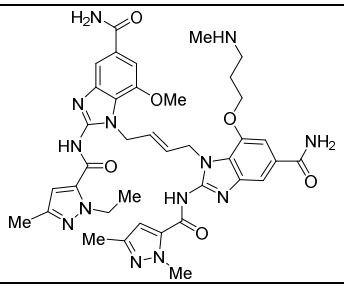
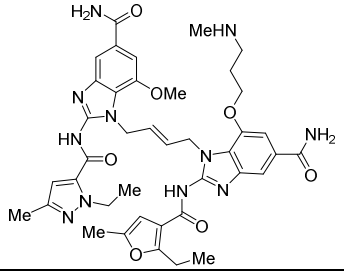
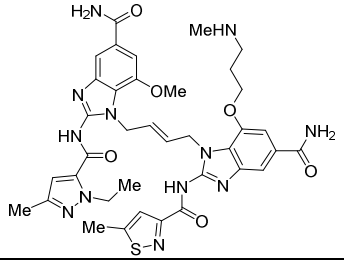
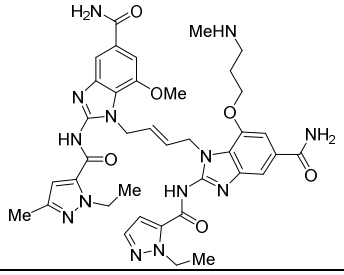
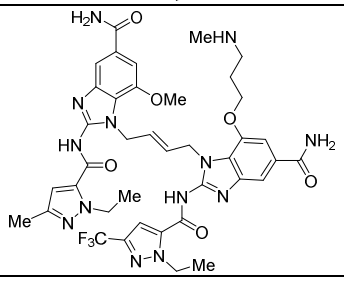
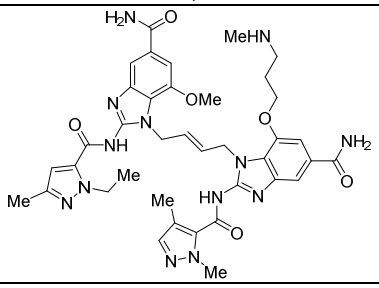
[0517] HATU Couplings (General Method 7B): A 2 mL microwave vial was charged with a solution of compound **78** (20 mg, 0.0238 mmol, 1 equiv.) in DMA (0.50 mL). The respective carboxylic acid (4 equiv.), HATU (36.3 mg, 0.0954 mmol, 2 equiv.) and DIPEA (20.8 µL, 0.119 mmol, 5 equiv.) were added. The vial was sealed and heated to 80 ºC a microwave reactor for 1h. The reaction was monitored via UPLC-MS (Method E, ESI+). Upon completion, acetic acid (20 µL) was added and the resulting product was purified by prepHPLC (Method H) using 0.05% TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford product as a white solid.

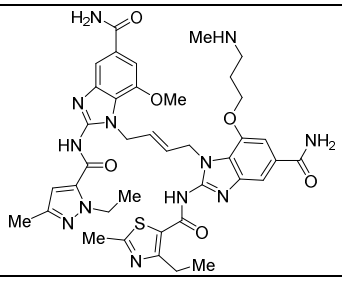
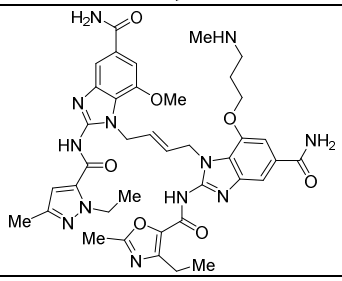


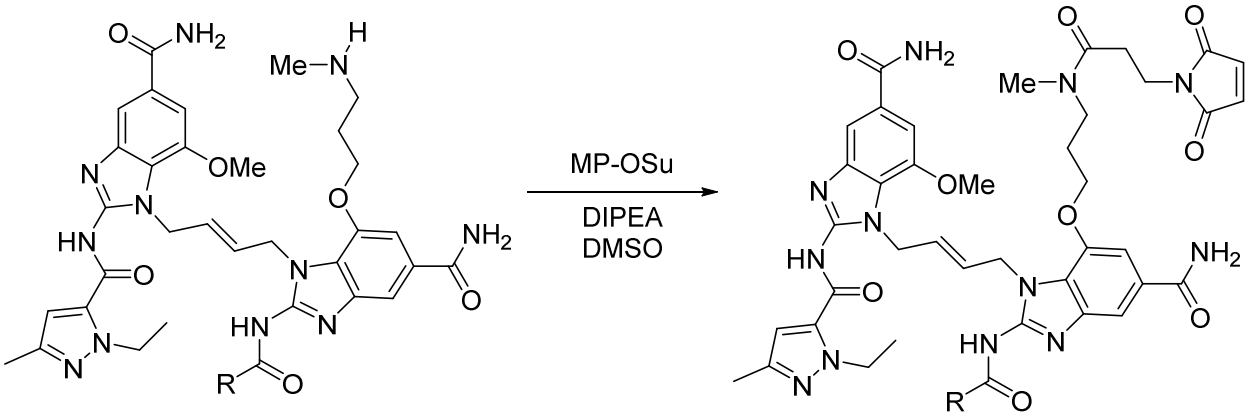
[0518] Boc Deprotection (General Method 8): The resulting product general method **7A** or **7B** was dissolved in MeOH (0.01 M), to which 4M HCl in dioxane (8 equiv.) was added. The solution stirred at room temperature for 30 min. The reaction was monitored via UPLC-MS (Method E, ESI+). Upon completion, the solution was concentrated, redissolved in DMSO, and purified via prepHPLC (Method G or H) using TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford product as a white solid.

Cmpd.	Structure	Method	Boc LC-MS data	Amine LC-MS data	Yield (over 2 steps)
79		7A	RT: 1.57 Theoretical: 864.4 Observed: 864.7	RT: 1.32 Theoretical: 764.3 Observed: 764.4	17% 4.5 mg 0.00406 mmol
80		7A	RT: 2.18 Theoretical: 864.4 Observed: 864.4	RT: 1.24 Theoretical: 764.3 Observed: 764.4	19% 5.0 mg 0.00455 mmol
81		7A	RT: 1.79 Theoretical: 867.4 Observed: 867.7	RT: 1.55 Theoretical: 767.3 Observed: 767.3	18% 4.8 mg 0.00436 mmol

82		7A	RT: 1.83 Theoretical: 867.4 Observed: 967.4	RT: 1.37 Theoretical: 767.3 Observed: 767.3	9% 2.4 mg 0.00216 mmol
83		7B	RT: 1.85 Theoretical: 883.4 Observed: 883.4	RT: 1.35 Theoretical: 783.3 Observed: 783.3	10% 2.8 mg 0.00247 mmol
84		7A	RT: 1.69 Theoretical: 864.4 Observed: 864.4	RT: 1.18 Theoretical: 764.3 Observed: 764.4	5% 1.2 mg 0.00110 mmol
85		7B	RT: 1.75 Theoretical: 894.4 Observed: 894.5	RT: 1.31 Theoretical: 794.4 Observed: 794.4	3% 0.88 mg 0.00077 mmol
86		7B	RT: 1.59 Theoretical: 894.4 Observed: 894.5	RT: 1.19 Theoretical: 794.4 Observed: 794.4	11% 3.1 mg 0.00270 mmol
87		7B	RT: 1.70 Theoretical: 894.4 Observed: 894.5	RT: 1.38 Theoretical: 794.4 Observed: 794.4	13% 3.5 mg 0.00307 mmol

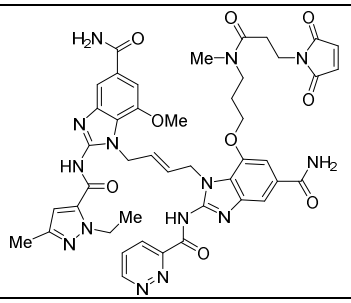
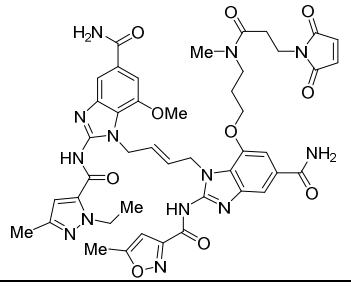
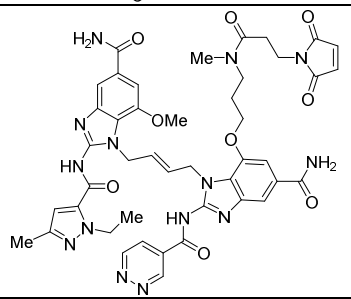
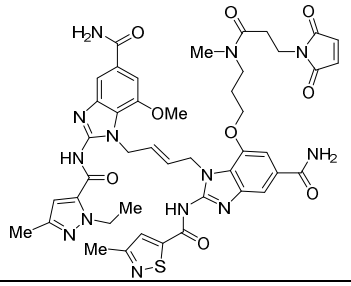
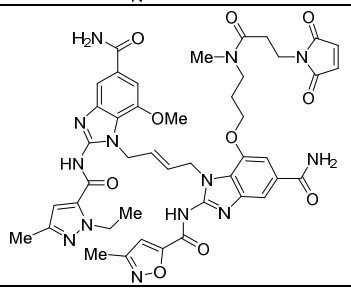
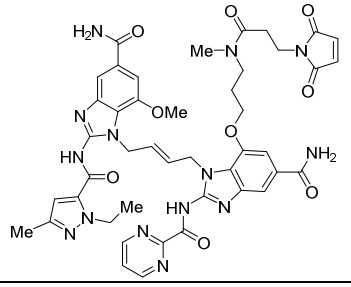
88		7B	RT: 1.63 Theoretical: 880.4 Observed: 880.5	RT: 1.28 Theoretical: 780.4 Observed: 780.4	16% 4.4 mg 0.00391 mmol
89		7B	RT: 1.77 Theoretical: 894.4 Observed: 894.5	RT: 1.46 Theoretical: 794.4 Observed: 794.4	9% 2.4 mg 0.00211 mmol
90		7B	RT: 1.70 Theoretical: 883.4 Observed: 883.4	RT: 1.30 Theoretical: 783.3 Observed: 783.4	17% 4.7 mg 0.00416 mmol
91		7B	RT: 1.65 Theoretical: 880.4 Observed: 880.5	RT: 1.32 Theoretical: 780.4 Observed: 780.4	6% 1.7 mg 0.00153 mmol
92		7B	RT: 1.83 Theoretical: 948.4 Observed: 948.5	RT: 1.49 Theoretical: 848.4 Observed: 848.4	10% 2.7 mg 0.00229 mmol
93		7B	RT: 1.74 Theoretical: 880.4 Observed: 880.5	RT: 1.37 Theoretical: 780.4 Observed: 780.4	22% 5.9 mg 0.00529 mmol

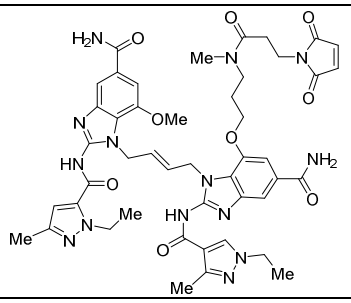
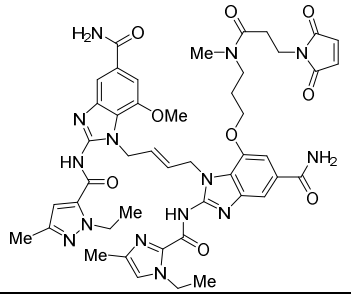
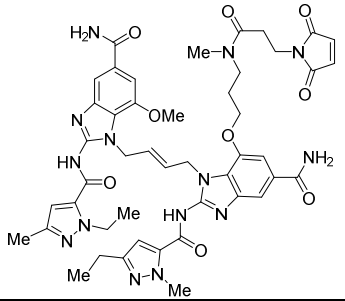
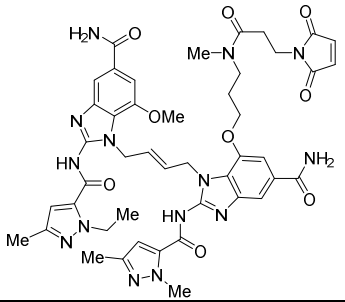
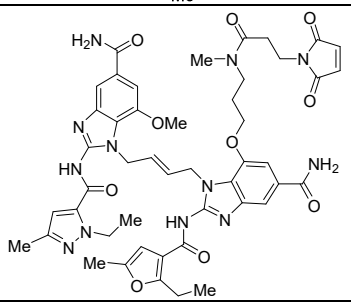
94		7B	RT: 1.88 Theoretical: 911.4 Observed: 911.5	RT: 1.38 Theoretical: 811.3 Observed: 811.4	30% 8.1 mg 0.00703 mmol
95		7B	RT: 1.86 Theoretical: 895.4 Observed: 895.5	RT: 1.41 Theoretical: 795.4 Observed: 795.4	8% 2.1 mg 0.0018 mmol

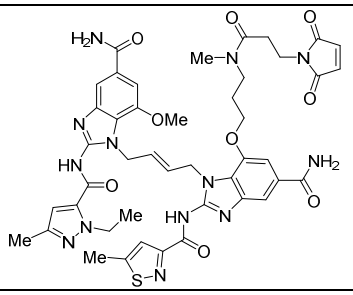
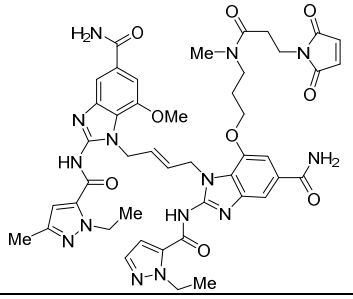
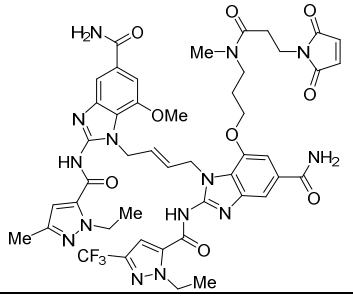
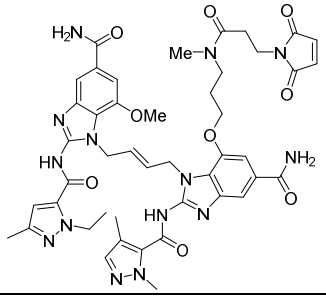
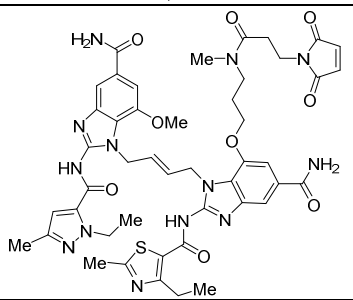


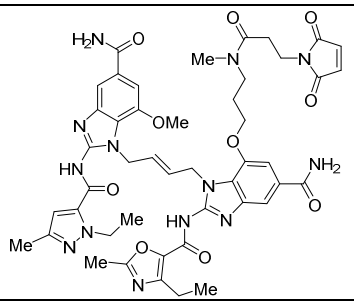
[0519] Maleimide Couplings (General Method 9): The resulting amine from the previous reaction (compounds **79 – 95**) was dissolved in DMSO (0.01M), to which was added MP-OSu (2 equiv.) and DIPEA (5 equiv.). The mixture was stirred at 30ºC overnight, and monitored by UPLC-MS (Method E, ESI+). Upon completion, the resulting product was purified via prepHPLC (Method G) using 0.05% TFA as the additive.

Cmpd.	**Structure**	**LC-MS data $[M+H]^+$**	**Yield**

96		RT: 1.38 Theoretical: 915.4 Observed: 915.4	68% 3.14 mg 0.0027 mmol
97		RT: 1.65 Theoretical: 918.4 Observed: 918.4	62% 3.11 mg 0.0027 mmol
98		RT: 1.42 Theoretical: 915.4 Observed: 915.4	84% 4.38 mg 0.0038 mmol
99		RT: 1.47 Theoretical: 934.3 Observed: 934.4	31% 0.89 mg 0.0008 mmol
100		RT: 1.50 Theoretical: 918.4 Observed: 918.4	53% 1.3 mg 0.0011 mmol
101		RT: 1.35 Theoretical: 915.4 Observed: 915.4	86% 5.88 mg 0.0051 mmol

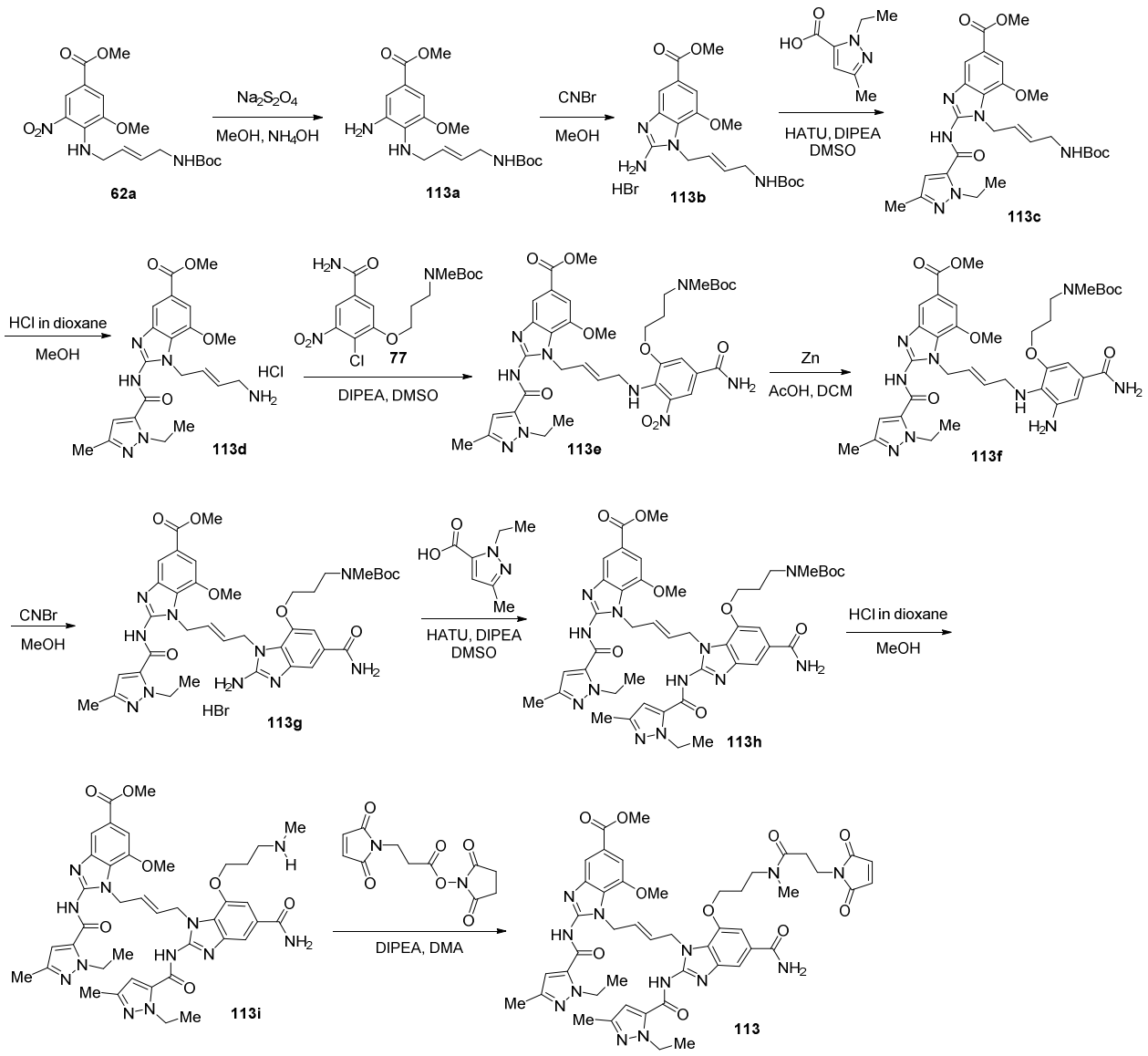
102		RT: 1.45 Theoretical: 945.4 Observed: 945.4	40% 0.36 mg 0.0003 mmol
103		RT: 1.37 Theoretical: 945.4 Observed: 945.4	41% 1.29 mg 0.0011 mmol
104		RT: 1.67 Theoretical: 945.4 Observed: 945.5	52% 1.87 mg 0.0016 mmol
105		RT: 1.48 Theoretical: 931.4 Observed: 931.4	88% 4.00 mg 0.0035 mmol
106		RT: 1.61 Theoretical: 945.4 Observed: 945.4	31% 0.76 mg 0.0006 mmol

107		RT: 1.45 Theoretical: 934.3 Observed: 934.4	51% 2.46 mg 0.0021 mmol
108		RT: 1.49 Theoretical: 931.4 Observed: 931.4	53% 0.94 mg 0.0008 mmol
109		RT: 1.83 Theoretical: 999.4 Observed: 999.4	51% 1.43 mg 0.0012 mmol
110		RT: 1.50 Theoretical: 931.4 Observed: 931.4	63% 3.86 mg 0.0033 mmol
111		RT: 1.50 Theoretical: 962.4 Observed: 962.4	40% 3.39 mg 0.0028 mmol

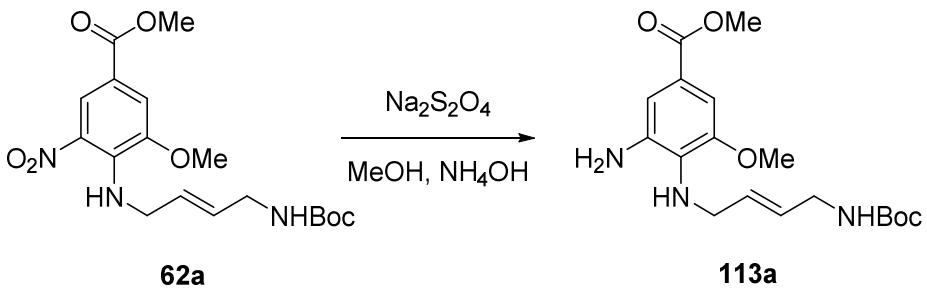
112		RT: 1.59 Theoretical: 946.4 Observed: 946.4	31% 0.67 mg 0.0006 mmol

Synthesis of methyl (E)-1-(4-(5-carbamoyl-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-

benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113).



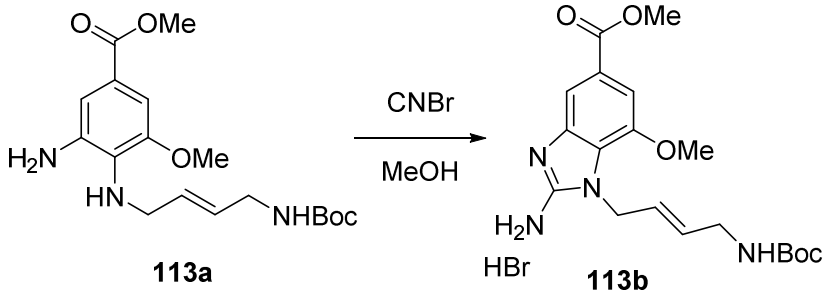
Synthesis of methyl (E)-3-amino-4-((4-((tert-butoxycarbonyl)amino)but-2-en-1-yl)amino)-5-methoxybenzoate (Compound 113a)



62a **113a**

[0520] Compound **62a** (500 mg, 1.26 mmol, 1 equiv.) was dissolved in MeOH (20 mL) and NH_4OH (6 mL). $Na_2S_2O_4$ (1.10 g, 6.32 mmol, 5 equiv.) in H_2O (5 mL) was slowly added and the mixture stirred at room temperature for 30 min. The reaction was monitored by UPLC-MS (Method E, ESI+). Upon completion, the mixture was filtered and concentrated. The resulting product was redissolved in EtOAc and washed with H_2O (x3). The organics were collected, dried with $MgSO_4$, filtered, and concentrated to afford compound **113a** (343 mg, 0.938 mmol, 74% yield) as a yellow solid. The resulting product was used without further purification. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 366.2 (theoretical), 366.2 (observed). HPLC retention time: 1.54 min.

Synthesis of methyl (E)-2-amino-1-(4-((tert-butoxycarbonyl)amino)but-2-en-1-yl)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate hydrobromide (Compound 113b)

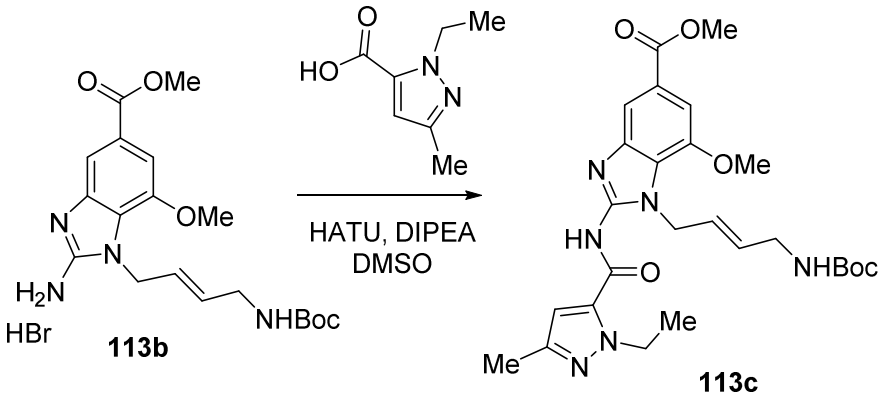


113a **113b**

[0521] Compound **113a** (343 mg, 0.938 mmol, 1 equiv.) was dissolved in MeOH (9.3 mL) to which CNBr (3 M in MeCN, 0.374 mL, 1.2 equiv.) was added. The reaction stirred for 18 h at room temperature, and monitored by UPLC-MS (Method E, ESI+). Upon completion, the solution was concentrated to afford compound **113b** (402 mg, 0.853 mmol, 91% yield), which was

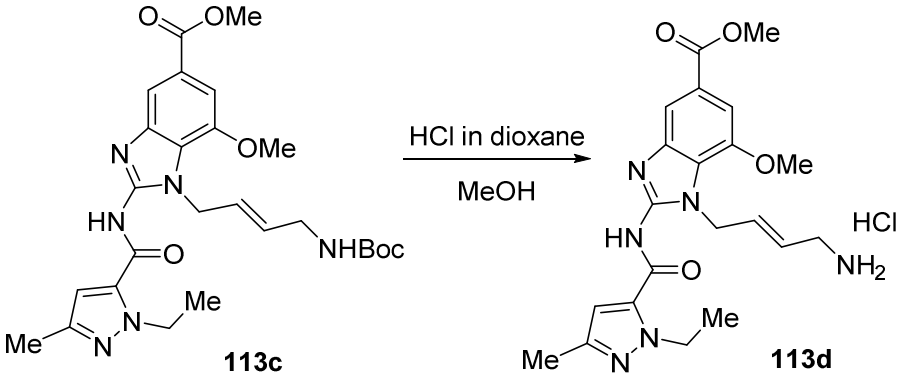
used without further purification. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 391.2 (theoretical), 391.1 (observed). HPLC retention time: 1.51 min.

Synthesis of methyl (E)-1-(4-((tert-butoxycarbonyl)amino)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113c)



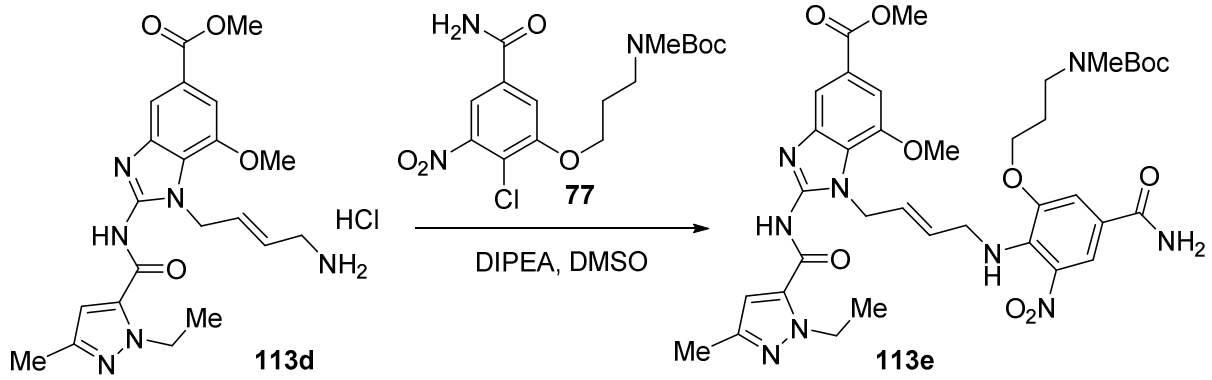
[0522] Compound **113b** (402 mg, 0.853 mmol, 1 equiv.), 1-ethyl-3-methyl-1H-pyrazole-5-carboxylic acid (394 mg, 2.56 mmol, 3 equiv.) and HATU (973 mg, 2.56 mmol, 3 equiv.) were dissolved in DMA (1.7 mL) in a 5 mL microwave vial. DIPEA (0.74 mL, 4.26 mmol, 5 equiv.) was added, and the reaction was heated to 80°C in a microwave reactor for 1 h. The reaction was monitored via UPLC-MS (Method E, ESI+). Upon completion, the reaction mixture was slowly added to ice-cold water (300 mL) to precipitate compound **113c** (295 mg, 0.560 mmol, 66% yield), which was used without further purification. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 527.3 (theoretical), 527.1 (observed). HPLC retention time: 2.30 min.

Synthesis of methyl (E)-1-(4-aminobut-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113d)



[0523] Compound **113c** (319 mg, 0.606 mmol, 1 equiv.) was dissolved in MeOH (1 mL), to which HCl in dioxane (4 M, 1.2 mL, 4.85 mmol, 8 equiv.) was added. The reaction was stirred at room temperature for 30 min. and was monitored by UPLC-MS (Method E, ESI+). Upon completion, the solution was concentrated and compound **113d** (280 mg, 0.605 mmol, quantitative yield) was used without further purification. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 427.2 (theoretical), 427.2 (observed). HPLC retention time: 1.54 min.

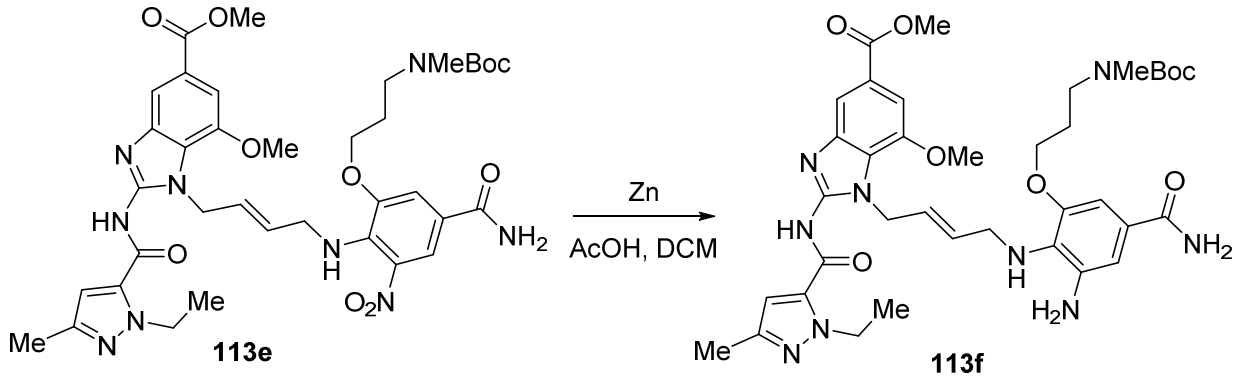
Synthesis of methyl (E)-1-(4-((2-(3-((tert-butoxycarbonyl)(methyl)amino)propoxy)-4-carbamoyl-6-nitrophenyl)amino)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113e)



Compound **113d** (280 mg, 0.605 mmol, 1 equiv.) and compound **77** (305 mg, 0.787 mmol, 1.3 equiv.) were dissolved in DMSO (3.0 mL) to which DIPEA (0.316 mL, 1.82 mmol, 3 equiv.) was added. The reaction stirred at 80°C for 18 h and monitored via UPLC-MS (Method E, ESI+).

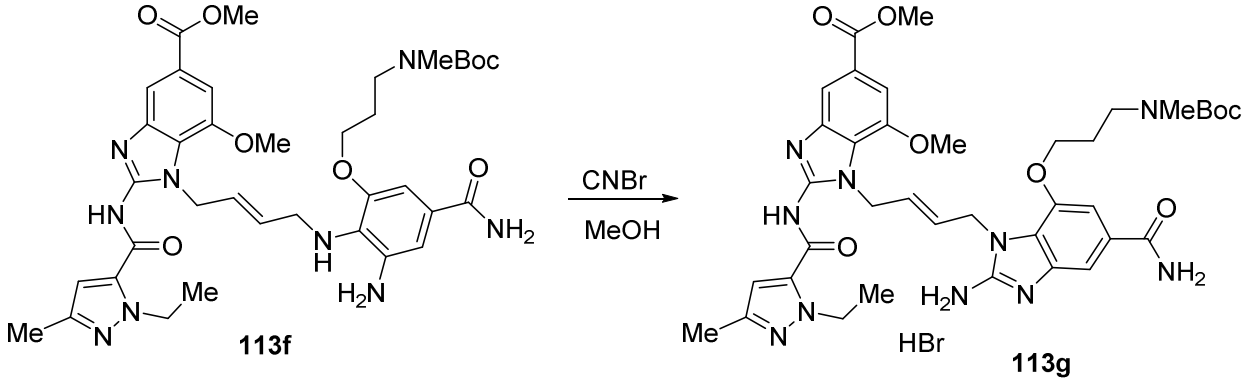
Upon completion, AcOH (0.30 mL) was added, and the product was purified by prepHPLC (Method I) using 0.05% TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford compound **113e** (58.6 mg, 0.0753 mmol, 12% yield) as an orange solid. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 778.3 (theoretical), 778.4 (observed). HPLC retention time: 1.88 min.

Synthesis of methyl (E)-1-(4-((2-amino-6-(3-((tert-butoxycarbonyl)(methyl)amino)propoxy)-4-carbamoylphenyl)amino)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113f)



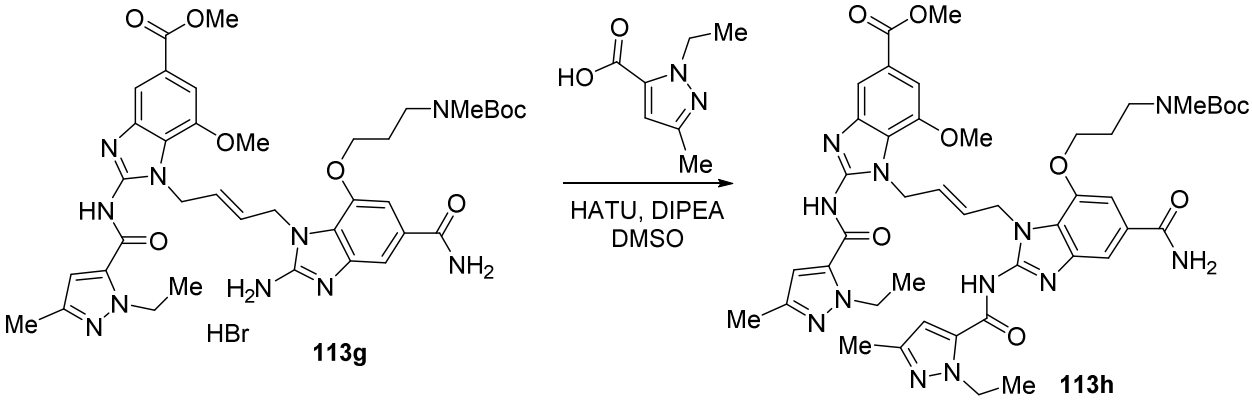
[0524] Compound **113e** (58.6 mg, 0.0753 mmol, 1 equiv.) was dissolved in a 1:1 mixture of AcOH/DCM (0.75 mL) and cooled to 0°C. Zn (49.2 mg, 0.753 mmol, 10 equiv.) was added and the mixture was allowed to warm to room temperature while stirring for 30 min. The reaction was monitored via UPLC-MS (Method E, ESI+). Upon completion, the solution was concentrated and redissolved in DCM to be purified by flash chromatography (25g SiO2 column, 0 – 40% MeOH:NH_4OH (10:1) in DCM) to afford compound **113f** (28.3 mg, 0.378 mmol, 50% yield). UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 748.4 (theoretical), 748.4 (observed). HPLC retention time: 1.84 min.

Synthesis of methyl (E)-1-(4-(2-amino-7-(3-((tert-butoxycarbonyl)(methyl)amino)propoxy)-5-carbamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113g)



[0525] Compound **113f** (28.3 mg, 0.378 mmol, 1 equiv.) was dissolved in MeOH (0.38 mL) to which CNBr (3 M in MeCN, 15 µL, 0.0454 mmol, 1.2 equiv.) was added. The reaction stirred at room temperature for 18 h and was monitored via UPLC-MS (Method E, ESI+). Upon completion, the solution was concentrated to afford product **113g** (30.7 mg, 0.360 mmol, quantitative yield), which was used without further purification. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 773.4 (theoretical), 773.4 (observed). HPLC retention time: 1.53 min.

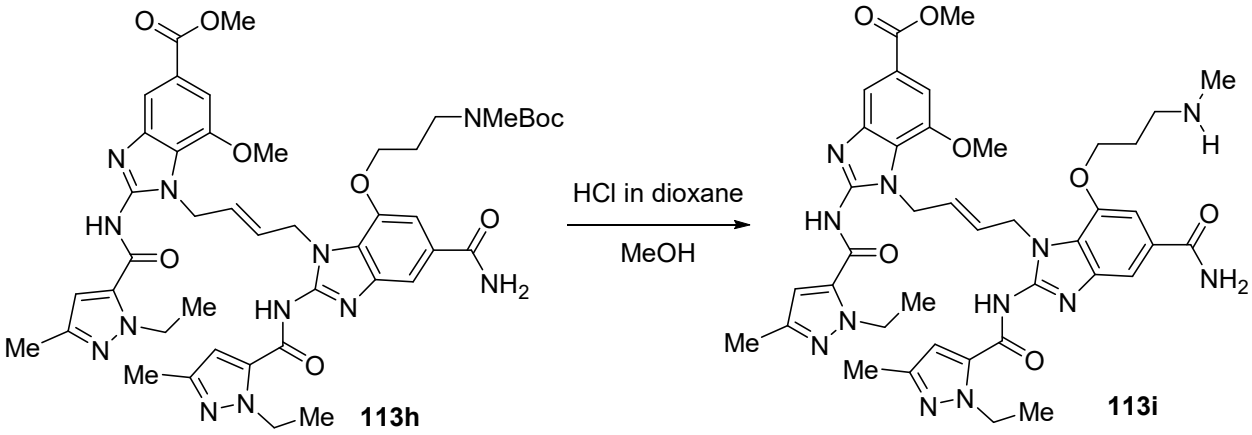
Synthesis of methyl (E)-1-(4-(7-(3-((tert-butoxycarbonyl)(methyl)amino)propoxy)-5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound H)



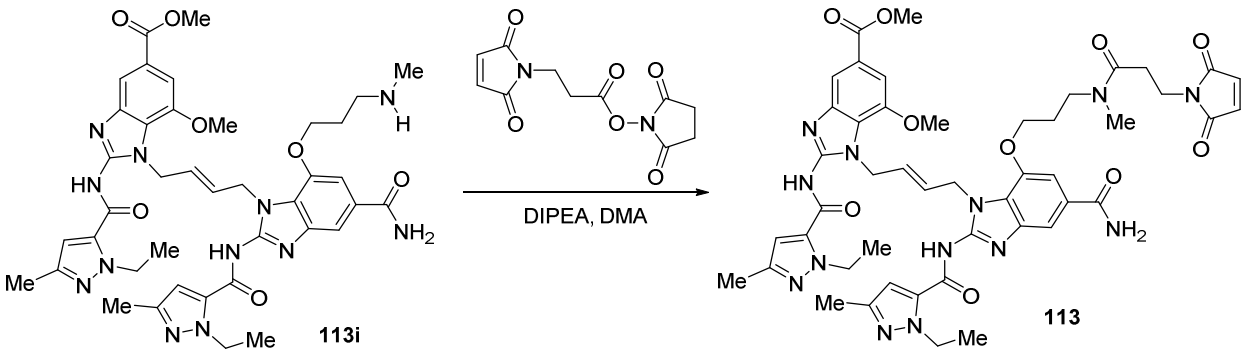
[0526] Compound **113g** (30.7 mg, 0.0360 mmol, 1 equiv.), 1-ethyl-3-methyl-1H-pyrazole-5-carboxylic acid (22.1 mg, 0.144 mmol, 4 equiv.), and HATU (54.6 mg, 0.144 mmol, 4

equiv.) were dissolved in DMA (0.50 mL) in a 2 mL microwave vial. DIPEA (0.025 mL, 0.144 mmol, 4 equiv.) was added, and the reaction was heated in a microwave reactor at 80 °C for 1 h. The reaction was monitored via UPLC-MS (Method E, ESI+). Upon completion, the product was purified by prepHPLC (Method H) using 0.05% TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford compound **113h** (6.52 mg, 0.0064 mmol, 18% yield) as a white solid. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 909.4 (theoretical), 909.5 (observed). HPLC retention time: 1.90 min.

Synthesis of methyl (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-(3-(methylamino)propoxy)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylate (Compound 113i)

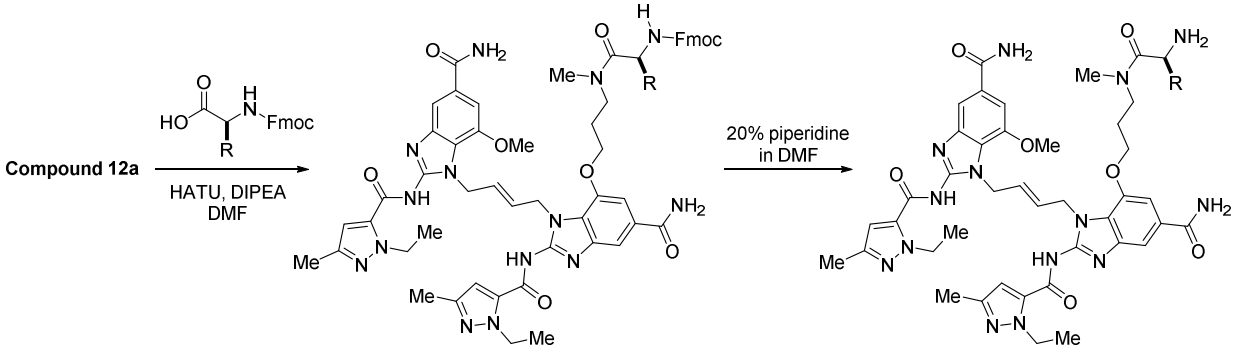


[0527] Compound **113h** (3.02 mg, 0.0030 mmol, 1 equiv.) was dissolved in MeOH (0.30 mL) to which HCl in dioxane (4 M, 6.00 µL, 0.0236 mmol, 8 equiv.) was added. The reaction stirred for 30 min at room temperature and monitored via UPLC-MS (Method E, ESI+). Upon completion, the product was purified via prepHPLC (Method G) using 0.05% TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford compound **113i** (1.35 mg, 0.0013 mmol, 44% yield) as a white solid. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 809.4 (theoretical), 809.4 (observed). HPLC retention time: 1.57 min.



[0528] Compound **113i** (7.53 mg, 0.0085 mmol, 1 equiv.) and MP-OSu (4.55 mg, 0.0171 mmol, 2 equiv.) were dissolved in DMA (0.854 mL), and DIPEA (42.7 µL, 0.0074 mmol, 5 equiv.) was added. The reaction stirred at room temperature for 18 h and monitored by UPLC-MS (Method E, ESI+). Upon completion, AcOH (42 µL) was added, and the product was purified via prepHPLC (Method G) using 0.05% TFA as the additive. Pure fractions were collected, frozen, and lyophilized to afford compound **113** (4.43 mg, 0.0041 mmol, 48% yield) as a white solid. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 960.4 (theoretical), 960.5 (observed). HPLC retention time: 1.79 min.

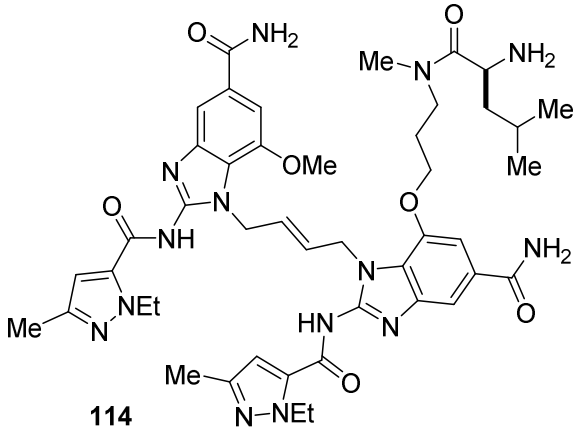
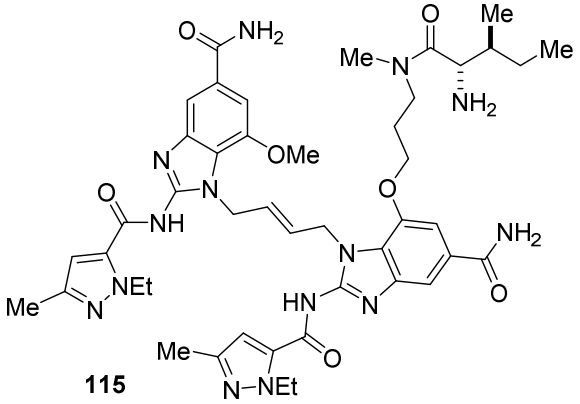
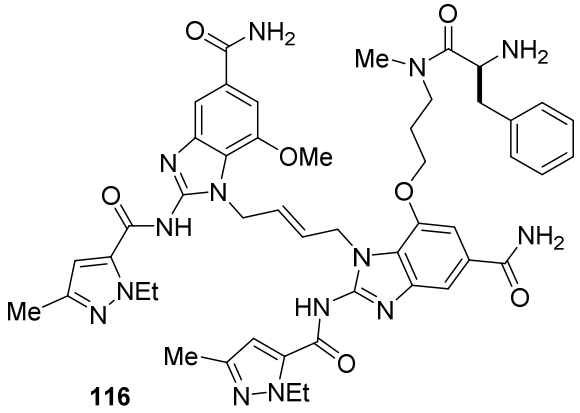
Linker library synthesis (compounds 114 – 124).

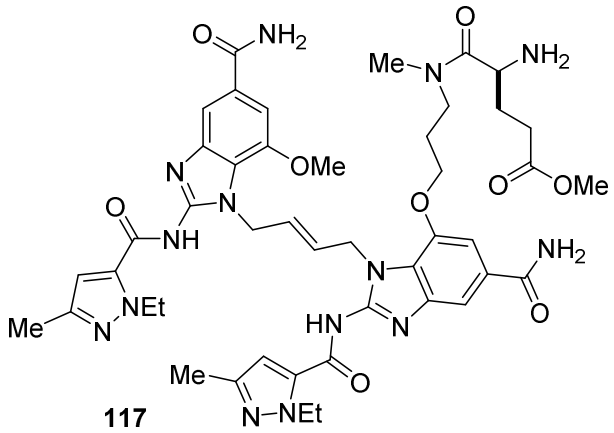
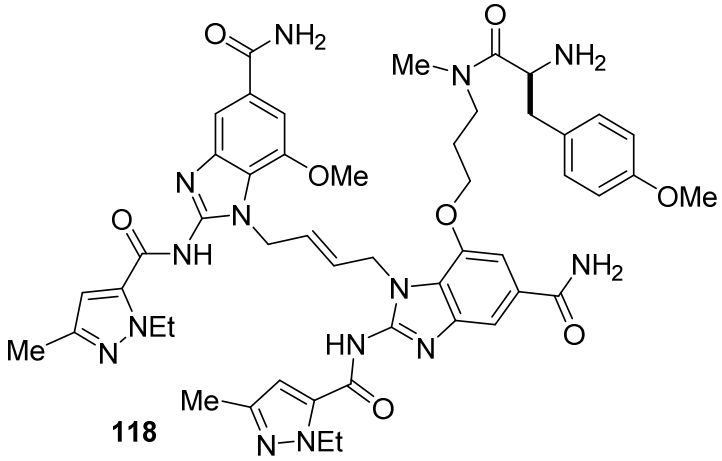
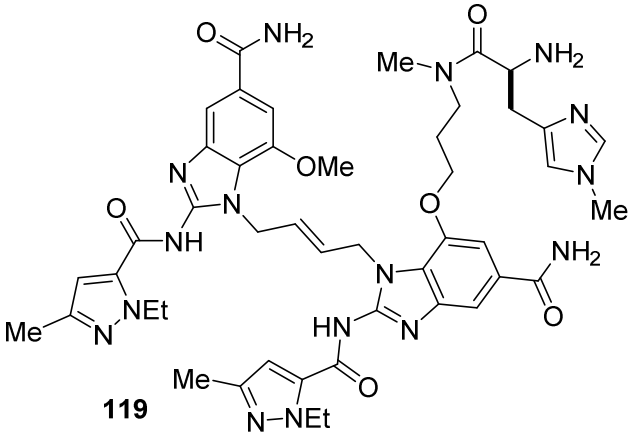


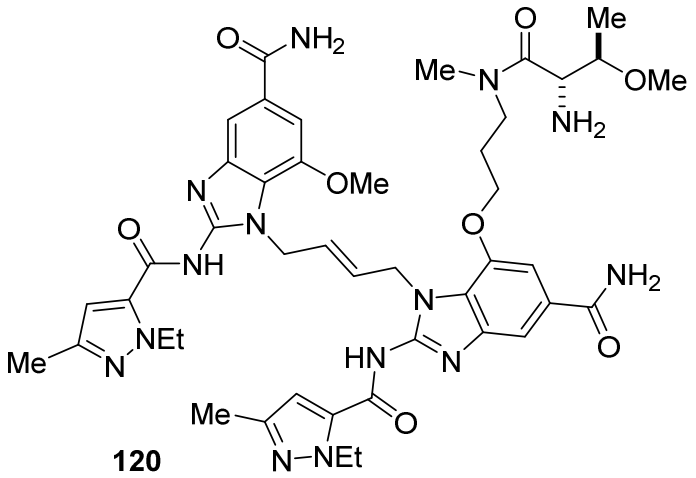
[0529] **Amide coupling (General Method 10)**: A mixture of Compound **12a** (1 equiv.), HATU (2 equiv.), DIPEA (5 equiv.), the appropriate L-amino acid (2 equiv.) was prepared in DMF (0.02 M in **12a**) and stirred at room temperature overnight. The solvent was removed *in vacuo*, and resulting product used in the next step without further purification.

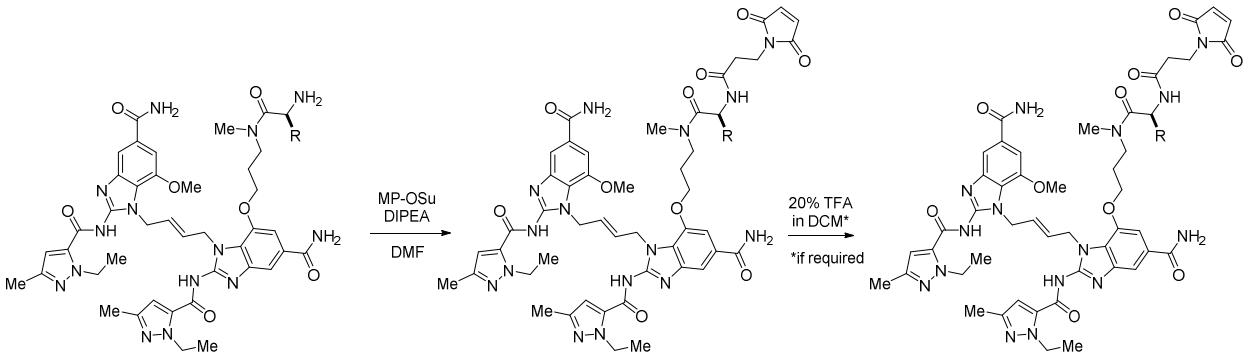
[0530] **Fmoc deprotection (General Method 11)**: The resulting Fmoc-protected amine was dissolved in 20% piperidine in DMF (1 mL) and stirred at room temperature for 15

minutes. The solvent was removed *in vacuo* and the product purified via prep HPLC (Method H, 5 - 95% in MeCN in H_2O in 0.05% TFA).

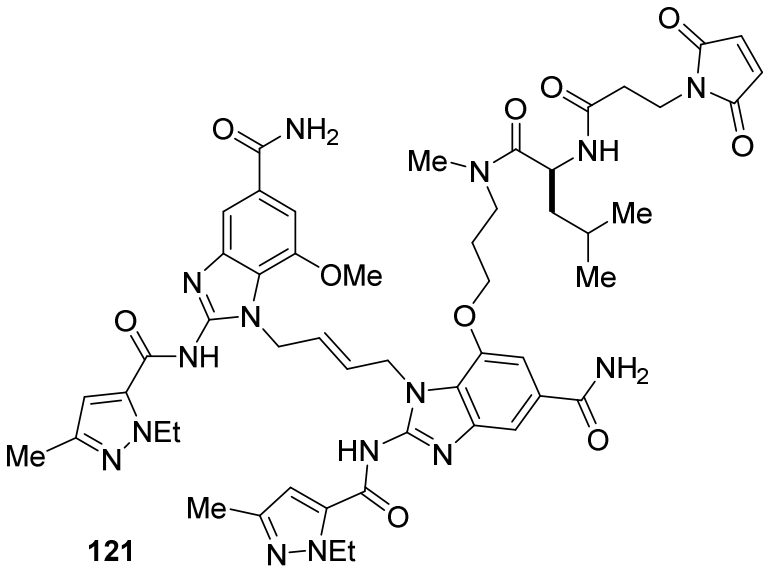
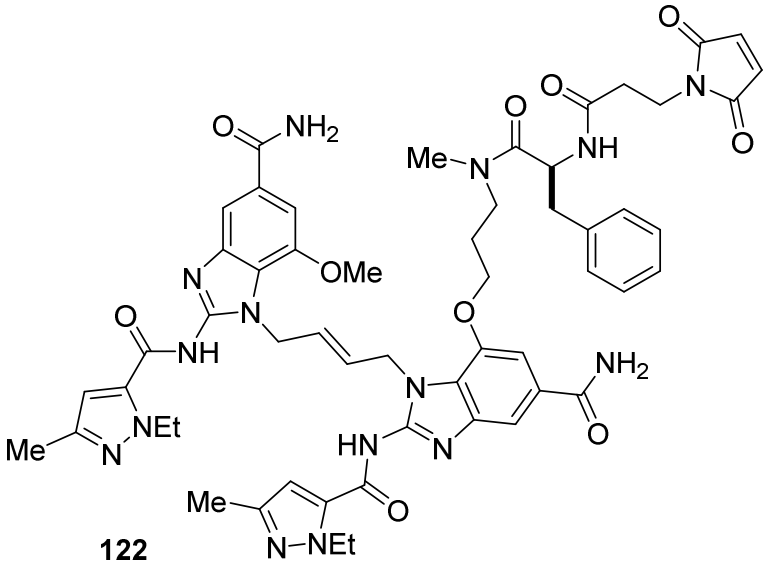
Compound	UPLC-MS $[M+H]^+$	Yield
 **114**	RT: 1.83 min Theoretical: 907.5 Observed: 907.5	18.6 mg (58%)
 **115**	RT: 1.77 Theoretical: 907.5 Observed: 907.5	24.9 mg (78%)
 **116**	RT: 1.76 Theoretical: 941.5 Observed: 941.5	13.5 mg (28%)

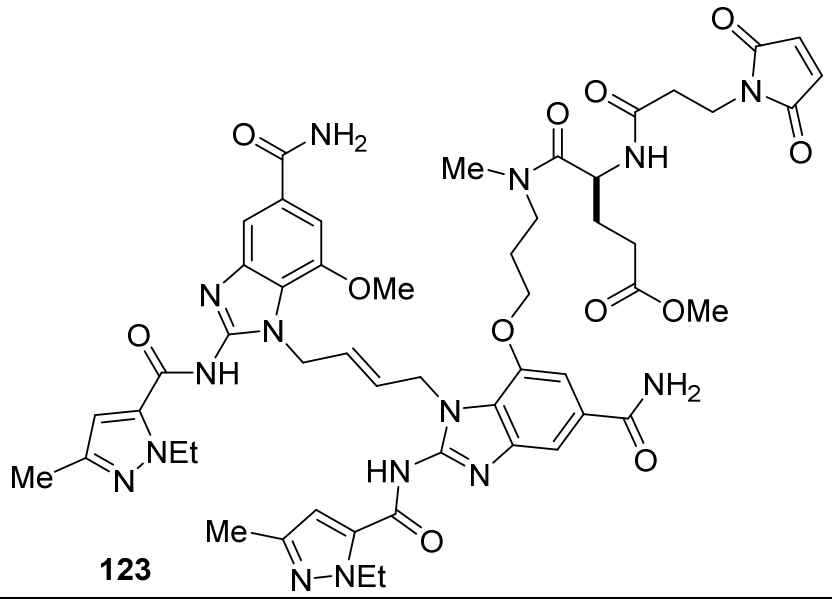
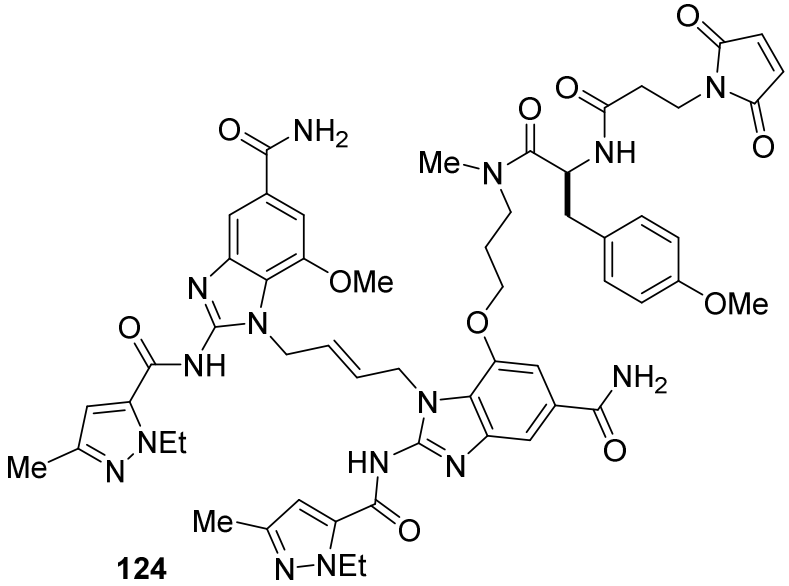
Compound	UPLC-MS [M+H]+	Yield
 **117**	RT: 2.05 Theoretical: 937.4 Observed: 937.5	9.8 mg (35%)
 **118**	RT: 1.93 Theoretical: 971.5 Observed: 971.5	18.6 mg (64%)
 **119**	RT: 1.93 Theoretical: 945.5 Observed: 945.5	19.4 (64%)

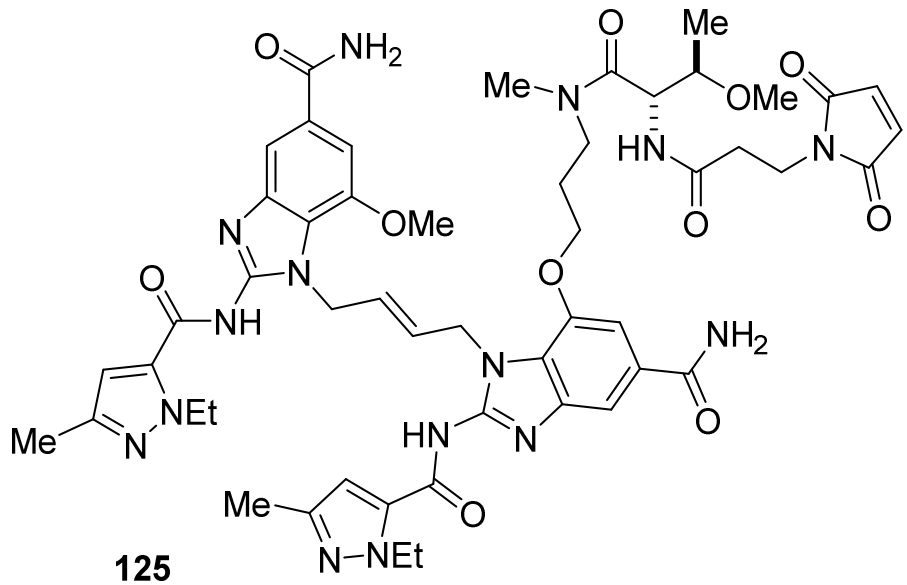
Compound	UPLC-MS $[M+H]^+$	Yield
 **120**	RT: 2.05 Theoretical: 909.4 Observed: 909.5	24.5 mg (76.4%)



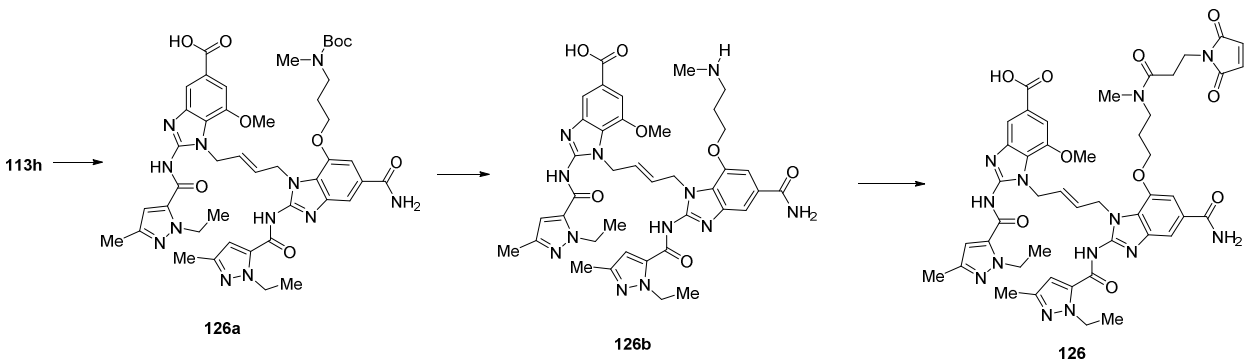
[0531] Synthesis of maleimide containing drug-linkers (compounds **121** – **125**) was performed according to **General Method 9**.

Compound	UPLC-MS [M+H]+	Yield
 **121**	RT: 2.16 Theoretical: 1058.5 Observed: 1058.5	1.5 mg (42%)
 **122**	RT: 2.00 Theoretical: 1092.5 Observed: 1092.5	0.5 mg (21%)

Compound	UPLC-MS [M+H]+	Yield
 **123**	RT: 2.21 Theoretical: 1088.5 Observed: 1088.5	1.1 mg (32%)
 **124**	RT: 1.81 Theoretical: 1122.5 Observed: 1122.6	1.1 mg (32%)

Compound	UPLC-MS $[M+H]^+$	Yield
 125	RT: 1.79 Theoretical: 1060.5 Observed: 1060.5	0.3 mg (8.6%)

(E)-1-(4-(5-carbamoyl-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylic acid (Compound 126).



Synthesis of compound 126a

[0532] Compound **113h** (25.44 mg, 0.0249 mmol, 1 equiv.) was dissolved in MeOH (166 µL). An aqueous solution of 1M LiOH (200 µL, 8 equiv.) was added and the reaction was stirred at 80°C for 2h. Upon completion, the solution was concentrated under reduced pressure and purified by prepHPLC (Method H) using 0.05% TFA as the additive. Pure fractions were collected,

frozen, and lyophilized to afford compound **126a** (7.1 mg, 0.0071 mmol, 28% yield) as a white solid. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 895.4 (theoretical), 895.6 (observed). HPLC retention time: 1.97 min.

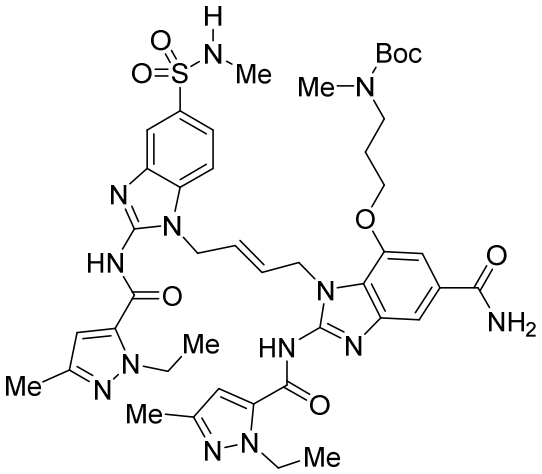
Synthesis of compound 126b

[0533] Compound **126b** was prepared following the same procedure used to prepare compound **113i**. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 795.4 (theoretical), 795.6 (observed). HPLC retention time: 1.40 min.

Synthesis of compound 126

[0534] Compound **126** was prepared following the same procedure used to prepare compound **113.** UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 946.4 (theoretical), 946.6 (observed). HPLC retention time: 1.68 min.

Synthesis of tert-butyl (E)-(3-((5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-(N-methylsulfamoyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)carbamate (Compound 127).

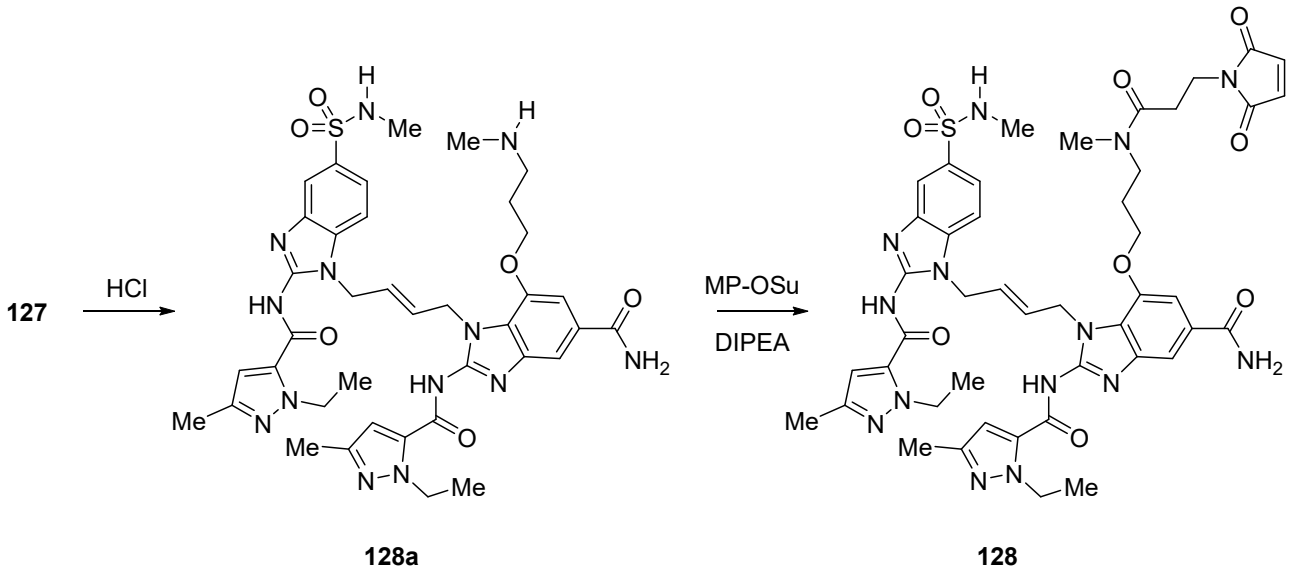


127

[0535] Compound **127** was prepared following the same procedures as compound **25f** substituting 4-chloro-N-methyl-3-nitrobenzenesulfonamide for 4-chloro-3-

nitrobenzenesulfonamide. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 914.4 (theoretical), 914.6 (observed). HPLC retention time: 1.80 min.

Synthesis of (E)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-(N-methylsulfamoyl)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 128).



128a **128**

Synthesis of 128a

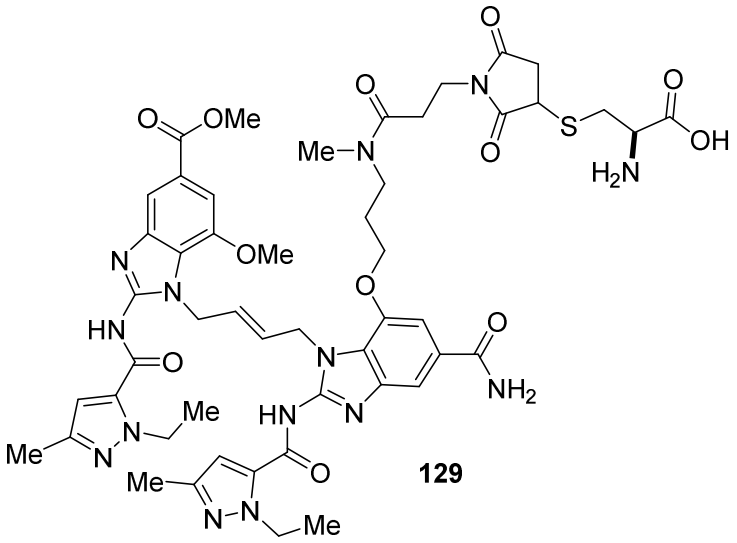
[0536] Compound **128a** was prepared following the same procedure used to prepare compound **66b**. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 814.4 (theoretical), 814.5 (observed). HPLC retention time: 1.53 min.

Synthesis of 128

[0537] Compound **128** was prepared following the same procedure used to prepare compound **12**. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 965.4 (theoretical), 965.6 (observed). HPLC retention time: 1.60 min.

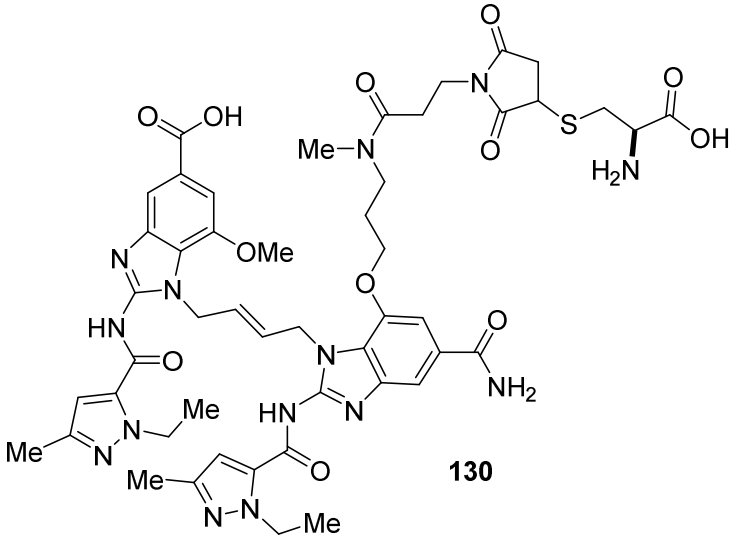
Synthesis of S-(1-(3-((3-((5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-((E)-4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-5-(methoxycarbonyl)-1H-

benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)amino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 129)



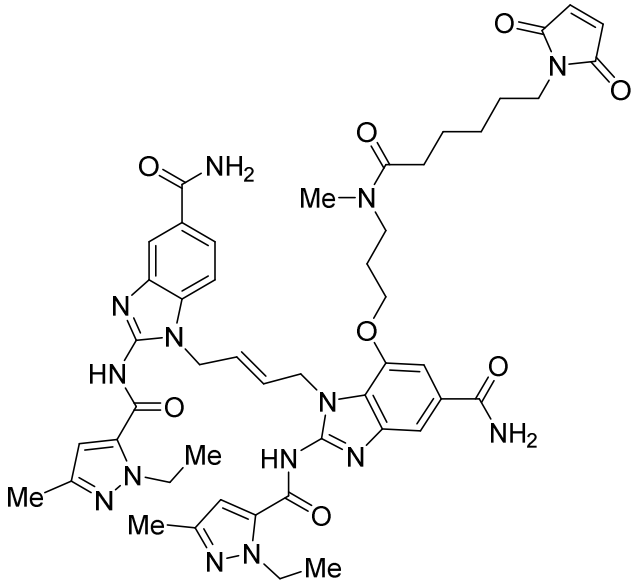
[0538] Compound **129** was prepared following General Method 6. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1081.4 (theoretical), 1081.6 (observed). HPLC retention time: 1.88 min.

Synthesis of 1-((E)-4-(7-(3-(3-(3-(((R)-2-amino-2-carboxyethyl)thio)-2,5-dioxopyrrolidin-1-yl)-N-methylpropanamido)propoxy)-5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxylic acid (Compound 130).



[0539] Compound **130** was prepared following General Method 6. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1067.4 (theoretical), 1067.6 (observed). HPLC retention time: 1.49 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylhexanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 131).

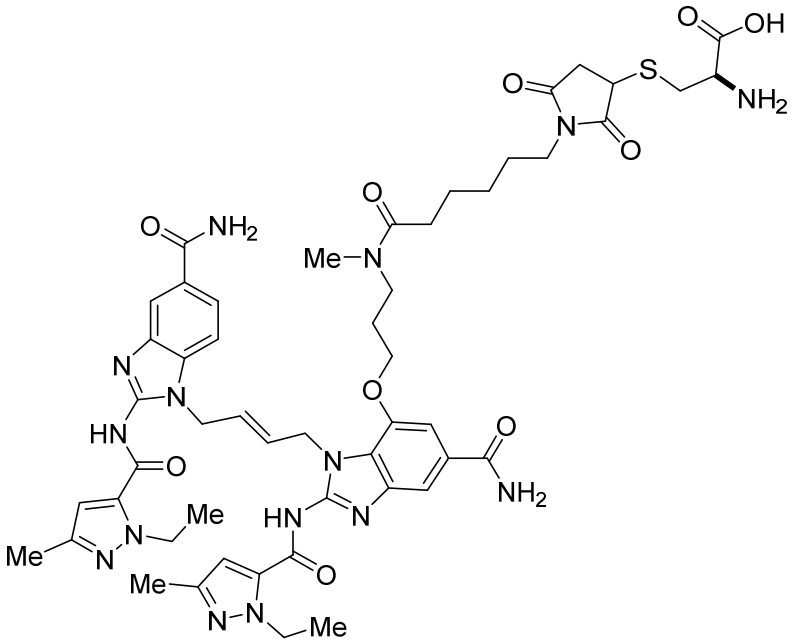


131

[0540] Compound **131** was prepared following the same procedure as compound **12** substituting 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate for 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 987.5 (theoretical), 987.7 (observed). HPLC retention time: 1.85 min.

Synthesis of S-(1-(6-((3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-

pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propyl)(methyl)amino)-6-oxohexyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 132).

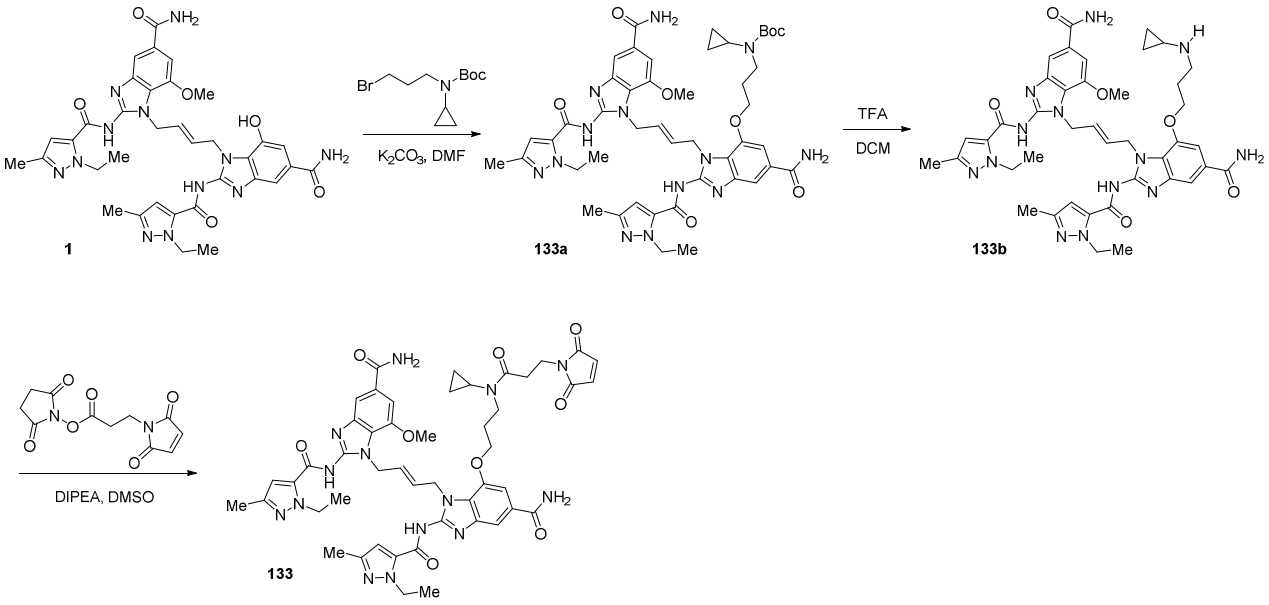


132

[0541] Compound **132** was prepared following General Method 6. UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1108.5 (theoretical), 1108.7 (observed). HPLC retention time: 1.42 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(N-cyclopropyl-3-(2,5-dioxo-2,5-

dihydro-1H-pyrrol-1-yl)propanamido)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 133).



Synthesis of 133a

[0542] An oven-dried 4 mL vial was charged with **1** (10 mg, 0.0105 mmol, 1 equiv), potassium carbonate (7.3 mg, 0.0526 mmol, 5 equiv.), and tert-butyl N-(3-bromopropyl)-N-cyclopropyl-carbamate (0.49 mL of a 9 mg/mL solution in DMF, 0.0158 mmol, 1.50 equiv.) and starting materials were dissolved in DMF (0.50 mL). The reaction was stirred overnight at 55 ^{o}C and purified by preparatory HPLC (Method B), after which it was frozen and lyophilized to afford compound **133a** (8.8 mg, 0.0077 mmol, 73% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 920.45 (theoretical), 920.64 (observed). HPLC retention time: 2.32 min.

Synthesis of 133b

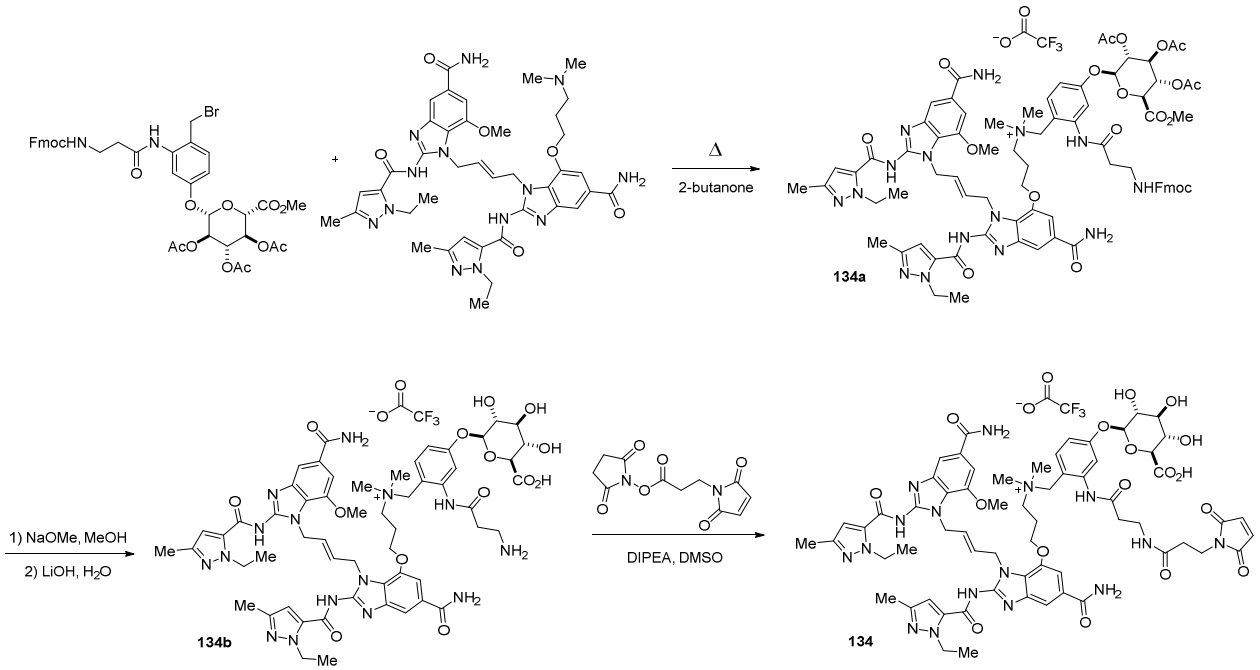
[0543] An oven-dried 4 mL vial was charged with **133a** (8.8 mg, 0.0077 mmol) and 20% TFA in DCM (100 μL). The reaction was stirred for 30 minutes at room temperature and purified by preparatory HPLC (Method B), after which it was frozen and lyophilized to afford compound **133b** (5.0 mg, 0.0043 mmol, 56% yield). UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 820.40 (theoretical), 820.49 (observed). HPLC retention time: 1.29 min.

Synthesis of 133

[0544] An oven-dried 8 mL vial was charged with **133b** (3.3 mg, 0.0085 mmols, 1 equiv.) which was dissolved in DMSO (1 mL) and a solution of 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate in DMSO (10mM in DMSO, 0.43 mL, 0.0043 mmol, 1.5 equiv.) and DIPEA (1.5 µL, 0.00851 mmol, 3 equiv.). The reaction was heated to 30 ^{o}C overnight, quenched with acetic acid and purified by preparatory HPLC (Method B), after which the compound was frozen and lyophilized to afford **133** (1.9 mg, 0.00158 mmol, 56 % yield).

[0545] UPLC-MS (Method D, ESI+): m/z $[M + H]^+$ = 971.43 (theoretical), 971.48 (observed). HPLC retention time: 1.99 min.

Synthesis of 3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)-N-(4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)benzyl)-N,N-dimethylpropan-1-aminium 2,2,2-trifluoroacetate (Compound 134).



Synthesis of 134a

[0546] An oven-dried 8 mL vial was charged with (E)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(dimethylamino)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (20 mg, 0.0248 mmol, 1 equiv., prepared as previously described WO2017/175147, example 39, page 291) and (2S,3R,4S,5S,6S)-2-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(bromomethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (60.3 mg, 0.0743 mmol, 3 equiv, prepared as previously described, *Mol Cancer Ther* **2016** 15(5), 938-945) and azeotroped with anhydrous acetonitrile. To the vial was added 2-butanone (2.5 mL) and the solution was heated to 100 ^{o}C overnight. The compound was directly purified by preparatory HPLC (Method B), frozen and lyophilized to afford **134a** (11.3 mg, 0.0070 mmol, 28% yield). UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ =1538.64 (theoretical), 1538.83 (observed). HPLC retention time: 2.55 min

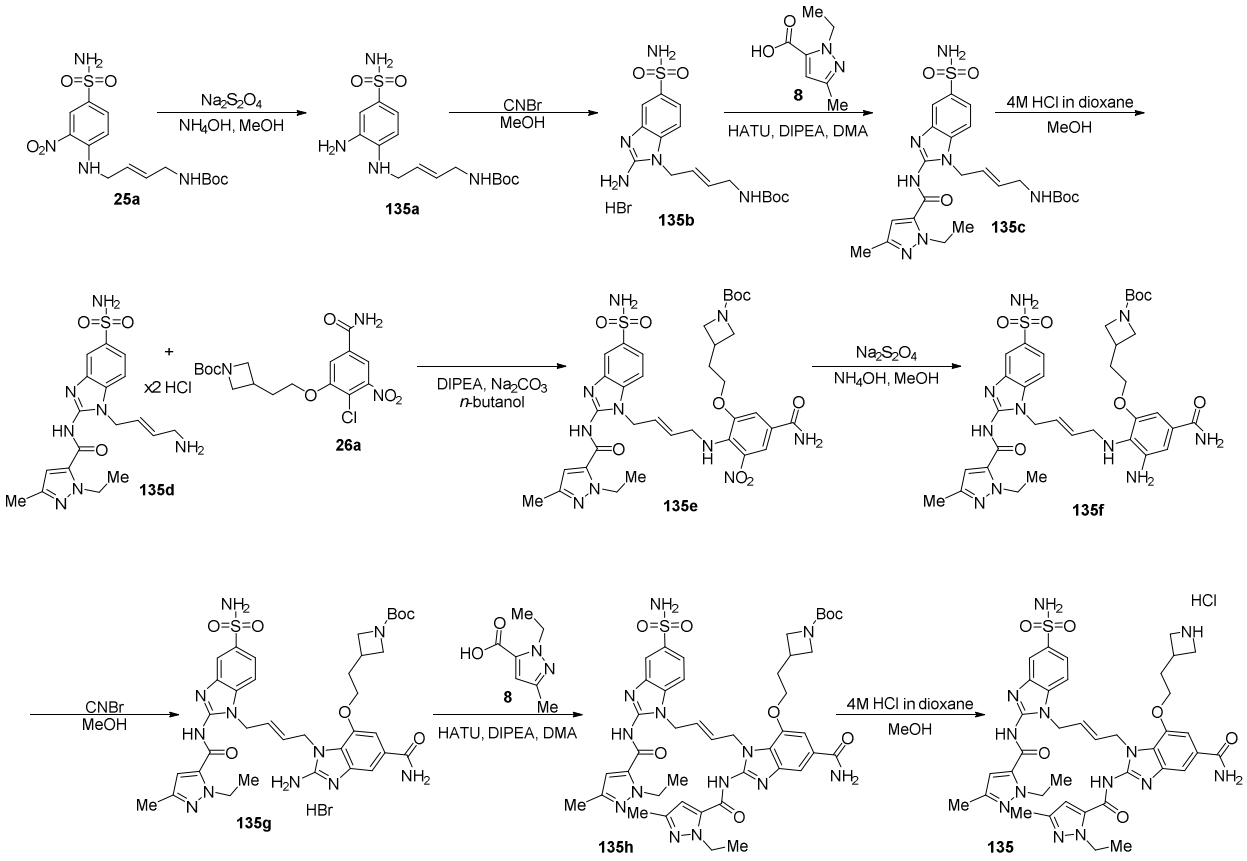
Synthesis of 134b

[0547] An oven-dried 4 mL vial was charged with **134a** (4.5 mg, 0.0094 mmol, 1 equiv.) and dissolved in anhydrous MeOH (0.5 mL). The vial was cooled in an acetonitrile / dry-ice bath at -40 ^{o}C and 0.5 M NaOMe (19 μL, 0.0094 mmol, 1 equiv) was added. The reaction was stirred for 1 hour before it was warmed to room temperature and LiOH (1 M in H_2O, 31 μL, 0.031 mmols, 3 equiv.) was added. The reaction was stirred at room temperature for 1 hour and then directly purified by preparatory HPLC (Method B) then frozen and lyophilized to afford **134b** (5.8 mg, 0.0049 mmol, 48% yield). UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1176.52 (theoretical), 1176.76 (observed). HPLC retention time: 1.29 min

Synthesis of 134

[0548] **134b** (5.8 mg, 0.0038 mmols, 1 equiv.) was added to an oven-dried 4 mL vial and dissolved in DMSO (1 mL) and then MP-OSu (10 mM in DMSO, 0.57 mL, 0.0057 mL, 1.5 equiv.) and DIPEA (2 μL, 0.0115 mmol, 3 equiv.) were added. The solution was stirred for 30 min, quenched with acetic acid and purified by preparatory HPLC (Method B), then frozen and lyophilized to afford **134** (3.6 mg, 0.0023 mmol, 61% yield). UPLC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1327.55 (theoretical), 1327.77 (observed). HPLC retention time: 1.38 min.

Synthesis of (E)-7-(2-(azetidin-3-yl)ethoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 135)



Synthesis of 135a

[0549] To a solution of **25a** (1.61 g, 4.18 mmol, 1 equiv.) in MeOH (63 mL) and aq. NH_4OH (21 mL) was added aq. $Na_2S_2O_4$ (1 M, 21 mL, 21 mmol, 5 equiv.). The mixture was stirred for 1 hour at 30 ºC, and the reaction was monitored by UPLC-MS (Method E, ESI+). Upon completion, the solution was filtered over celite and washed with MeOH. The filtrate was concentrated and the product was purified by flash chromatography (dry loaded on celite, Sfar HC Duo SiO_2 column, 10:1 MeOH:NH_4OH gradient in DCM) to yield **135a** (774 mg, 2.17 mmol, 52% yield). LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 357.2 (theoretical), 357.3 (observed). HPLC retention time: 1.44 min.

Synthesis of 135b

[0550] To a solution of compound **135a** (774 mg, 2.17 mmol, 1 equiv.) in MeOH (4 mL) was added cyanogen bromide in MeCN (3 M, 1.5 mL, 4.35 mmol, 2 equiv.). The solution stirred for 18 hours at 30 °C and was monitored via UPLC-MS (Method E, ESI+). Upon completion, solvent was removed in vacuo to yield **135b** (1.0 g, 2.25 mmol, quantitative yield). LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 382.2 (theoretical), 382.2 (observed). HPLC retention time: 1.12 min.

Synthesis of 135c

[0551] A microwave vial was charged with a solution of **135b** (1.0 g, 2.25 mmol, 1 equiv.) in DMA (11 mL), to which was added compound **8** (1.0 g, 6.74 mmol, 3 equiv.), HATU (2.6 g, 6.74 mmol, 3 equiv.) and DIPEA (1.2 mL, 6.74 mmol, 3 equiv.). This mixture was heated to 80 °C for 1 hour in a microwave reactor. Upon completion, **135c** was isolated by precipitation with cold water (1.0 g, 1.93 mmol, 86% yield). LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 518.2 (theoretical), 518.3 (observed). HPLC retention time: 1.60 min.

Synthesis of 135d

[0552] To a solution of **135c** (1.0g, 1.93 mmol, 1 equiv.) in MeOH (3.3 mL) was added HCl in dioxane (4 M, 5.3 mL, 21 mmol, 8 equiv.). The mixture stirred for 1 hour at 30 °C. Upon completion, solvent was removed in vacuo and **135d** (1.2 g, 2.65 mmol, quantitative yield) was used without further purification. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 418.2 (theoretical), 418.2 (observed). HPLC retention time: 1.09 min.

Synthesis of 135e

[0553] Compounds **135d** (200 mg, 0.408 mmol, 1 equiv.) and **26a** (245 mg, 0.612 mmol, 1.5 equiv.) were dissolved in n-butanol (2.0 mL) in a 5 mL microwave vial to which Na_2CO_3 (130 mg, 1.22 mmol, 3 equiv.) and DIPEA (0.36 mL, 2.04 mmol, 5 equiv.) were added. The reaction was heated via microwave reactor at 140 °C for 3 hours. The resulting product was filtered and washed with MeOH and DCM. The filtrate was concentrated and purified via flash chromatography (dry loaded on celite, Sfar HC Duo SiO_2 column, 10:1 MeOH:NH_4OH gradient in DCM) to yield **135e** (51 mg, 0.0651 mmol, 16% yield). LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 781.3 (theoretical), 781.4 (observed). HPLC retention time: 1.72 min.

Synthesis of 135f

[0554] Compound **135f** (30 mg, 0.0402 mmol, 62% yield) was prepared using the same procedure as **135a**, using **135e** (51 mg, 0.0651 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 751.3 (theoretical), 751.4 (observed). HPLC retention time: 1.46 min.

Synthesis of 135g

[0555] Compound **135g** (34 mg, 00394 mmol, quantitative yield) was prepared using the same procedure as **135b**, using **135f** (30 mg, 0.0402 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 776.3 (theoretical), 776.4 (observed). HPLC retention time: 1.54 min.

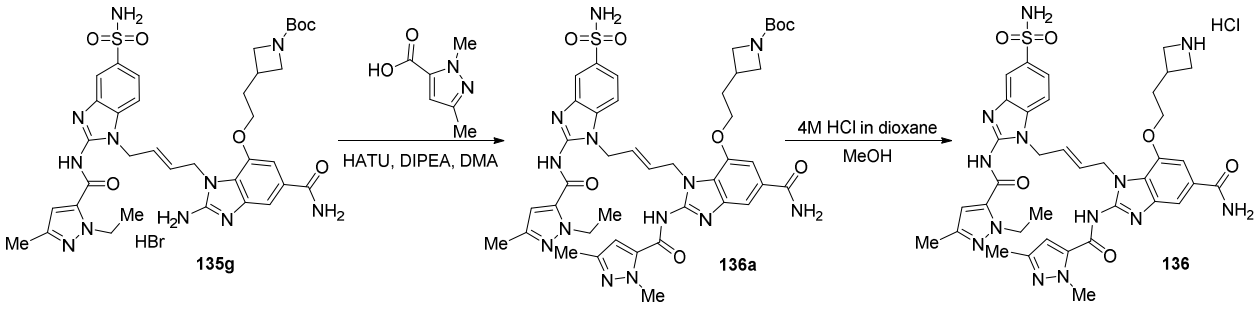
Synthesis of 135h

[0556] Compound **135h** was prepared using the same procedure as **135c**, using **135g** (17 mg, 0.0197 mmol, 1 equiv.) as the starting material. Upon completion, the product was purified by preparatory HPLC (Method H). Pure fractions were collected, frozen and lyophilized to afford **135h** (2.34 mg, 0.0021 mmol, 10% yield) as a white powder. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 912.4 (theoretical), 912.5 (observed). HPLC retention time: 1.65 min.

Synthesis of 135

[0557] Compound **135h** (2.34 mg, 0.0021 mmol, 1 equiv.) was dissolved in MeOH (0.21 mL) and HCl in dioxane (4 M, 4.1 µL, 0.0164 mmol, 8 equiv.) was added. The solution was heated to 40 ºC for 1 hour. Then solvent was removed in vacuo and **135** (1.86 mg, 0.0020 mmol, quantitative yield) was used without further purification. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 812.3 (theoretical), 812.4 (observed). HPLC retention time: 1.26 min.

Synthesis of (E)-7-(2-(azetidin-3-yl)ethoxy)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 136)



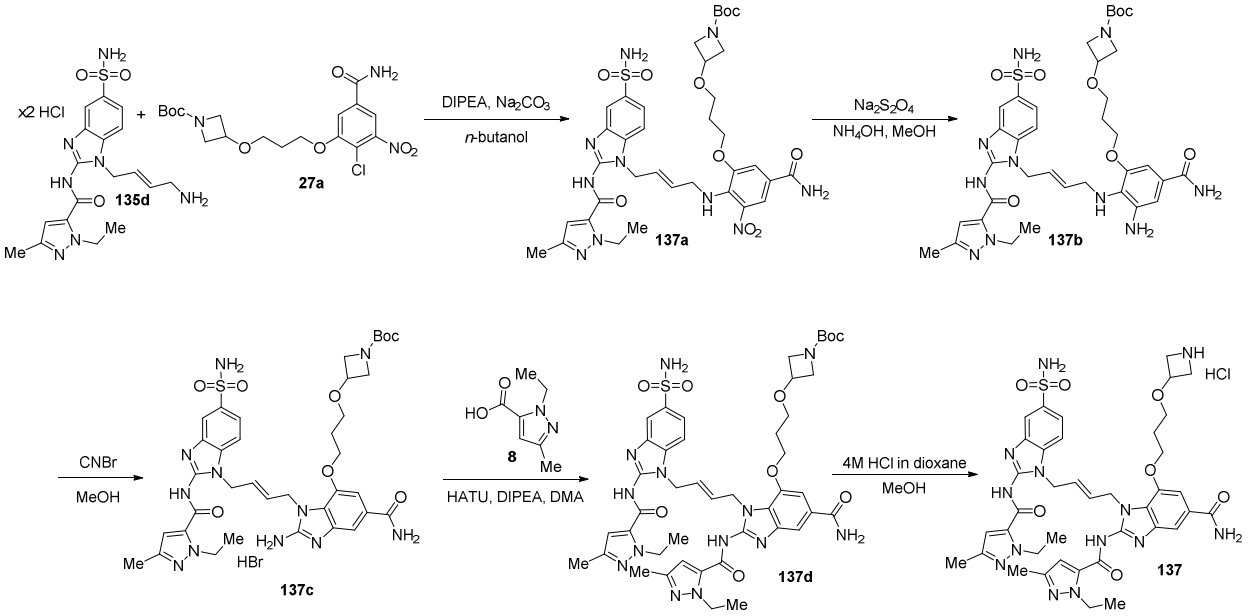
Synthesis of 136a

[0558] Compound **136a** (3.15 mg, 0.0028 mmol, 14% yield) was prepared using the same procedure as **135h**, using **135g** (17 mg, 0.0197 mmol, 1 equiv.) and 1, 3-dimethyl-1*H*-pyrazole-5-carboxyllic acid as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 898.4 (theoretical), 898.5 (observed). HPLC retention time: 1.62 min.

Synthesis of 136

[0559] Compound **136** (2.09 mg, 0.0023 mmol, 82% yield) was prepared using the same procedure as **135**, using **136a** (3.15 mg, 0.0028 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 798.3 (theoretical), 798.4 (observed). HPLC retention time: 1.24 min.

Synthesis of (E)-7-(3-(azetidin-3-yloxy)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 137)



Synthesis of 137a

[0560] Compound **137a** (72 mg, 0.0893 mmol, 22% yield) was prepared using the same procedure as **135e**, using **135d** (200 mg, 0.408 mmol, 1 equiv.) and **27a** (263 mg, 0.612 mmol, 1.5 equiv.) as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 811.3 (theoretical), 811.4 (observed). HPLC retention time: 1.72 min.

Synthesis of 137b

[0561] Compound **137b** (30 mg, 0.0386 mmol, 43% yield) was prepared using the same method as **135a**, using **137a** (72 mg, 0.0893 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 781.3 (theoretical), 781.4 (observed). HPLC retention time: 1.46 min.

Synthesis of 137c

[0562] Compound **137c** (34 mg, 0.0387 mmol, quantitative yield) was prepared using the same procedure as **135b**, using **137b** (30 mg, 0.03896 mmol, 1 equiv.) as the starting material.

LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 806.3 (theoretical), 806.4 (observed). HPLC retention time: 1.53 min.

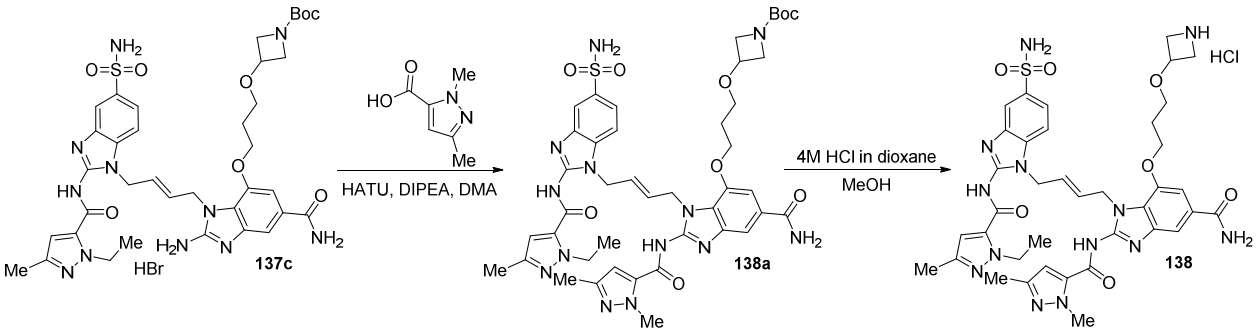
Synthesis of 137d

[0563] Compound **137d** (4.21 mg, 0.0036 mmol, 19% yield) was prepared using the same procedure as **135h**, using **137c** (17 mg, 0.0194 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 942.4 (theoretical), 942.5 (observed). HPLC retention time: 1.65 min.

Synthesis of Compound 137

[0564] Compound **137** (3.35 mg, 0.0035 mmol, quantitative yield), was prepared using the same procedure as **135**, using **137d** (4.21 mg, 0.0036 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 842.3 (theoretical), 842.4 (observed). HPLC retention time: 1.29 min.

Synthesis of (E)-7-(3-(azetidin-3-yloxy)propoxy)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 138)



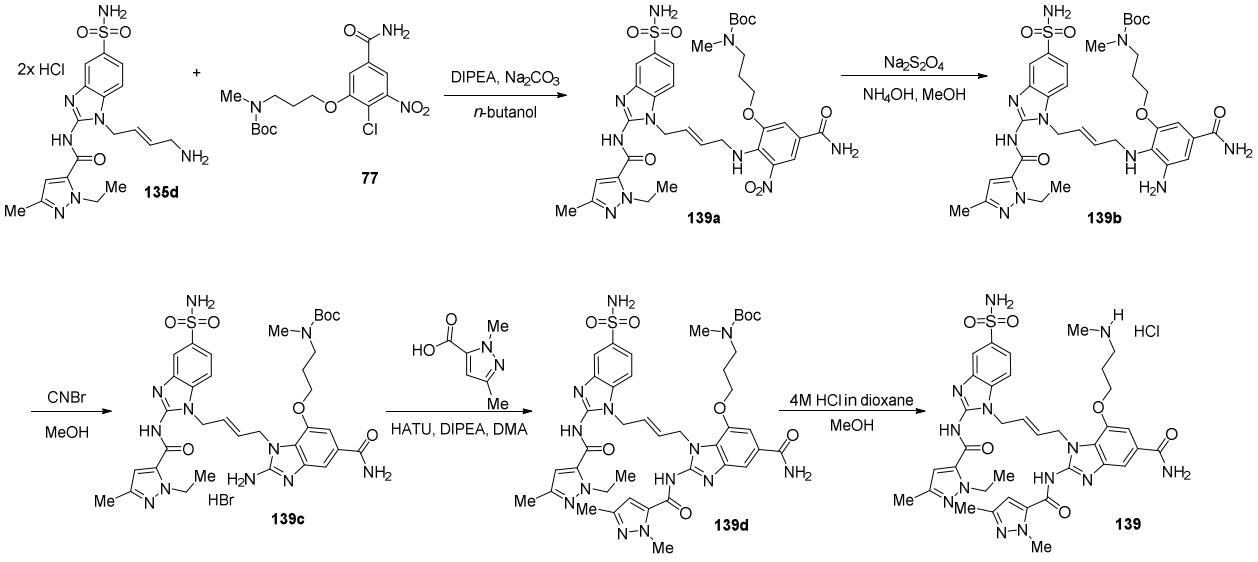
Synthesis of 138a

[0565] Compound **138a** (3.00 mg, 0.0026 mmol, 13% yield) was prepared using the same procedure as **135h**, using **137c** (17 mg, 0.0197 mmol, 1 equiv.) and 1, 3-dimethyl-1*H*-pyrazole-5-carboxyllic acid as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 928.4 (theoretical), 928.5 (observed). HPLC retention time: 1.62 min.

Synthesis of 138

[0566] Compound **138** (2.35 mg, 0.0025 mmol, quantitative yield), was prepared using the same procedure as **135**, using **138a** (3.00 mg, 0.0026 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 828.3 (theoretical), 828.4 (observed). HPLC retention time: 1.26 min.

Synthesis of (E)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-(methylamino)propoxy)-1H-benzo[d]imidazole-5-carboxamide (Compound 139)



Synthesis of 139a

[0567] Compound **139a** (125 mg, 0.162 mmol, 29% yield) was prepared using the same procedure as **135e**, using **135d** (250 mg, 0.551 mmol, 1 equiv.) and **77** (320 mg, 0.826 mmol, 1.5 equiv.) as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 769.3 (theoretical), 769.4 (observed). HPLC retention time: 1.67 min.

Synthesis of 139b

[0568] Compound **139b** (51 mg, 0.0686 mmol, 42% yield) was prepared using the same procedure as **135a**, using **139a** (125 mg, 0.162 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 739.3 (theoretical), 739.4 (observed). HPLC retention time: 1.45 min.

Synthesis of 139c

[0569] Compound **139c** (57 mg, 0.0670 mmol, quantitative yield) was prepared using the same procedure as **135b**, using **139b** (51 mg, 0.0686 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 764.3 (theoretical), 764.4 (observed). HPLC retention time: 1.31 min.

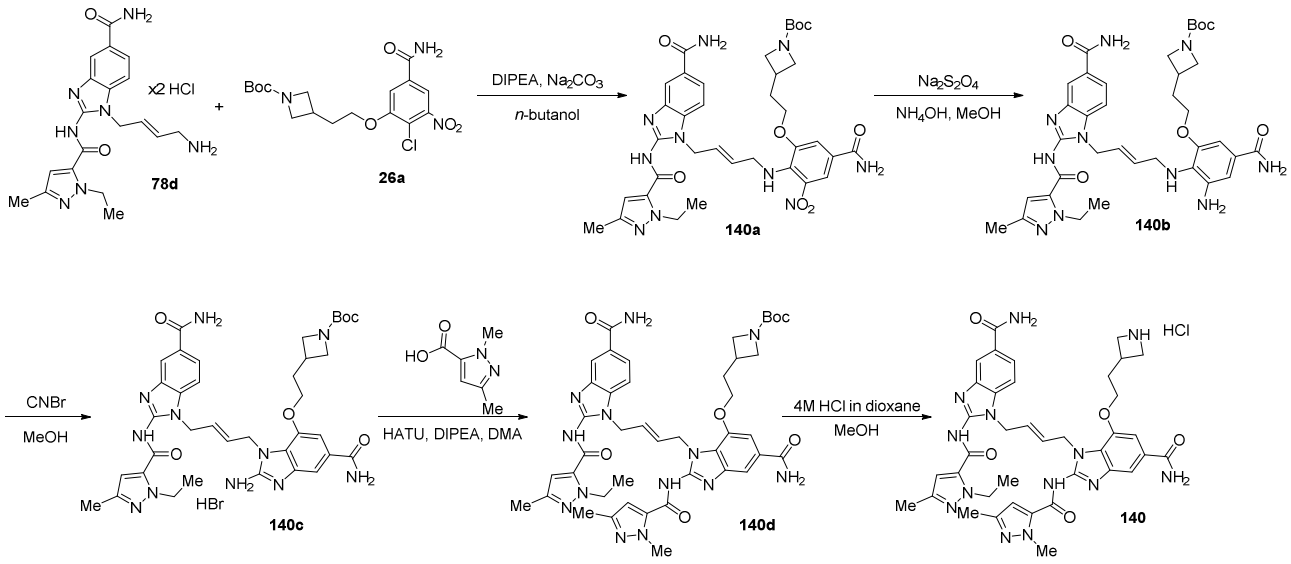
Synthesis of 139d

[0570] Compound **139d** (34 mg, 0.0303 mmol, 45% yield) was prepared following the same procedure as **135h** using **139c** (57 mg, 0.0670 mmol, 1 equiv.) and 1, 3-dimethyl-1*H*-pyrazole-5-carboxyllic acid as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 886.4 (theoretical), 886.5 (observed). HPLC retention time: 1.61 min.

Synthesis of 139

[0571] Compound **139** (27 mg, 0.0291, quantitative yield) was prepared using the same procedure as **135**, using **139d** (34 mg, 0.0303 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 786.3 (theoretical), 786.4 (observed). HPLC retention time: 1.23 min.

Synthesis of (E)-7-(2-(azetidin-3-yl)ethoxy)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 140)



Synthesis of 140a

[0572] Compound **140a** (380 mg, 0.490 mmol, 78% yield) was prepared using the same procedure as **135e**, using **26a** (250 mg, 0.625 mmol, 1 equiv.) and **78d** (420 mg, 0.938 mmol, 1.5 equiv.) as the starting materials. The product was precipitated in cold water and used without further purification. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 775.3 (theoretical), 775.4 (observed). HPLC retention time: 1.66 min.

Synthesis of 140b

[0573] Compound **140b** (193 mg, 0.260 mmol, 53% yield) was prepared using the same procedure as **135a**, using **140a** (380 mg, 0.490 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 745.4 (theoretical), 745.5 (observed). HPLC retention time: 1.44 min.

Synthesis of 140c

[0574] Compound **140c** (212 mg, 0.249 mmol, quantitative yield) was prepared using the same procedure as **135b**, using **140b** (193 mg, 0.260 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 770.4 (theoretical), 770.5 (observed). HPLC retention time: 1.60 min.

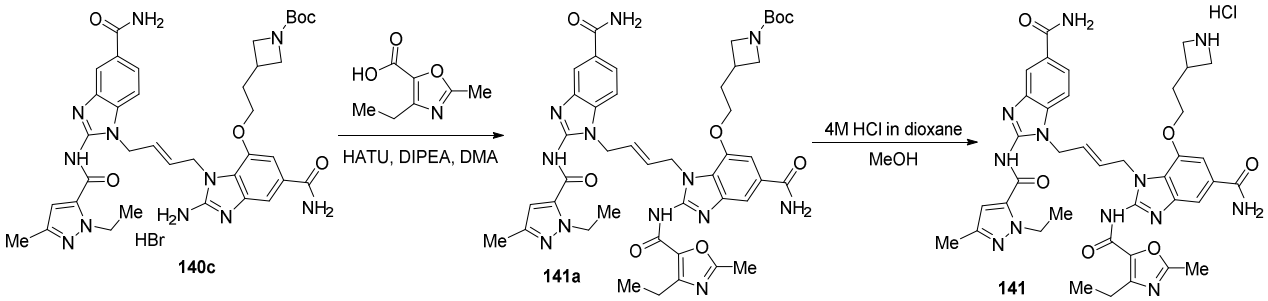
Synthesis of 140d

[0575] Compound **140d** (38 mg, 0.0339 mmol, 27% yield) was prepared using the same procedure as **135h**, using **140c** (106 mg, 0.124mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 892.4 (theoretical), 892.5 (observed). HPLC retention time: 1.59 min.

Synthesis of 140

[0576] Compound **140** (30 mg, 0.0334 mmol, quantitative yield) was prepared using the same procedure as **135**, using **140d** (38 mg, 0.0339 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 792.4 (theoretical), 792.5 (observed). HPLC retention time: 1.28 min.

Synthesis of (E)-N-(7-(2-(azetidin-3-yl)ethoxy)-5-carbamoyl-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazol-2-yl)-4-ethyl-2-methyloxazole-5-carboxamide (Compound 141)



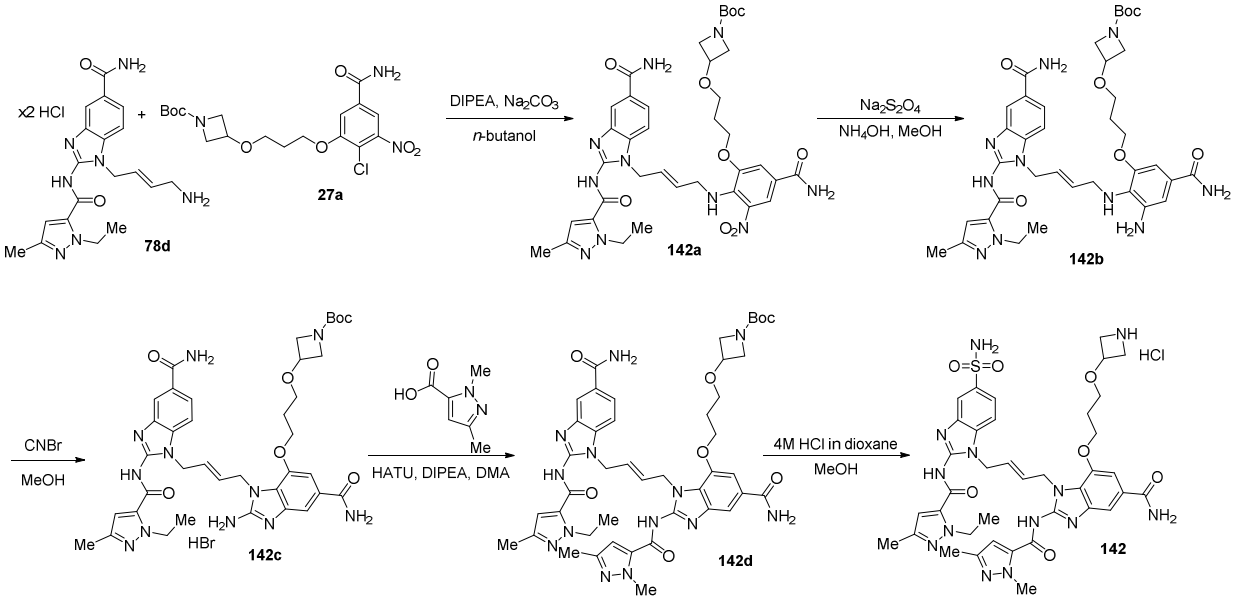
Synthesis of 141a

[0577] Compound **141a** (27 mg, 0.0237 mmol, 19% yield) was prepared using the same procedure as **135h**, using **140c** (106 mg, 0.124 mmol, 1 equiv.) and 4-ethyl-2-methyl-oxazole-5-carboxyllic acid as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 907.4 (theoretical), 907.5 (observed). HPLC retention time: 1.57 min.

Synthesis of 141

[0578] Compound **141** (21 mg, 0.0230 mmol, quantitative yield), was prepared using the same procedure as **135**, using **141a** (27 mg, 0.0237 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 807.4 (theoretical), 807.5 (observed). HPLC retention time: 1.26 min.

Synthesis of (E)-7-(3-(azetidin-3-yloxy)propoxy)-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazole-5-carboxamide (Compound 142)



Synthesis of 142a

[0579] Compound **142a** was prepared using the same procedure as **135e**, using **27a** (250 mg, 0.582 mmol, 1 equiv.) and **78d** (391 mg, 0.872 mmol, 1.5 equiv.) as the starting materials. The product was precipitated with cold water and used without further purification. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 805.4 (theoretical), 805.4 (observed). HPLC retention time: 1.66 min.

Synthesis of 142b

[0580] Compound **142b** (193 mg, 0.250 mmol, 37% yield over 2 steps) was prepared using the same procedure as **135a**, using **142a** (548 mg, 0.681 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 775.4 (theoretical), 775.5 (observed). HPLC retention time: 1.50 min.

Synthesis of 142c

[0581] Compound **142c** (164 mg, 0.186 mmol, 75% yield) was prepared using the same procedure as **135b**, using **142b** (193 mg, 0.260 mmol, 1 equiv.) as the starting material. LC-MS

(Method E, ESI+): m/z $[M + H]^+$ = 800.4 (theoretical), 800.5 (observed). HPLC retention time: 1.33 min.

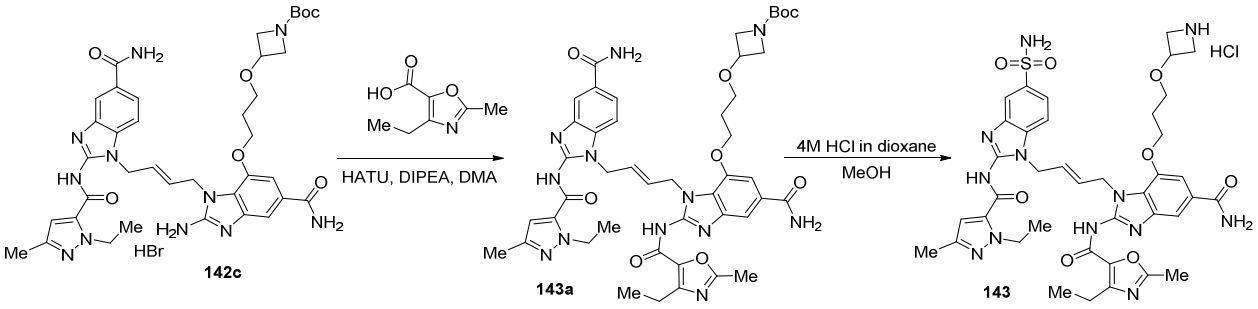
Synthesis of 142d

[0582] Compound **142d** (40 mg, 0.0345 mmol, 37% yield) was prepared using the same procedure as **135h**, using **142c** (48 mg, 0.373 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 922.4 (theoretical), 922.5 (observed). HPLC retention time: 1.58 min.

Synthesis of 142

[0583] Compound **142** (32 mg, 0.0323 mmol, quantitative yield) was prepared using the same procedure as **135**, using **142d** (40 mg, 0.0345 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 822.4 (theoretical), 822.5 (observed). HPLC retention time: 1.29 min.

Synthesis of (E)-N-(7-(3-(azetidin-3-yloxy)propoxy)-5-carbamoyl-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazol-2-yl)-4-ethyl-2-methyloxazole-5-carboxamide (Compound 143)



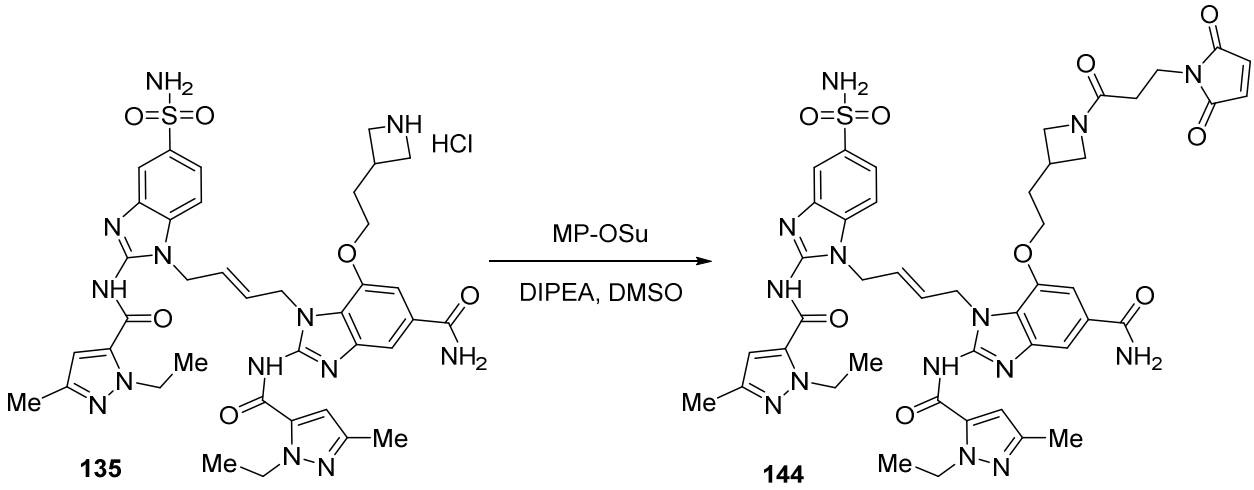
Synthesis of 143a

[0584] Compound **143a** (31 mg, 0.0263 mmol, 28% yield) was prepared using the same procedure as **135h**, using **142c** (82 mg, 0.0.0931 mmol, 1 equiv.) and 4-ethyl-2-methyl-oxazole-5-carboxyllic acid as the starting materials. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 937.4 (theoretical), 937.5 (observed). HPLC retention time: 1.56 min.

Synthesis of 143

[0585] Compound **143** (25 mg, 0.0261 mmol, quantitative yield), was prepared using the same procedure as **135**, using **141a** (31 mg, 0.0263 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 837.4 (theoretical), 837.5 (observed). HPLC retention time: 1.29 min.

Synthesis of (E)-7-(2-(1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)ethoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 144)

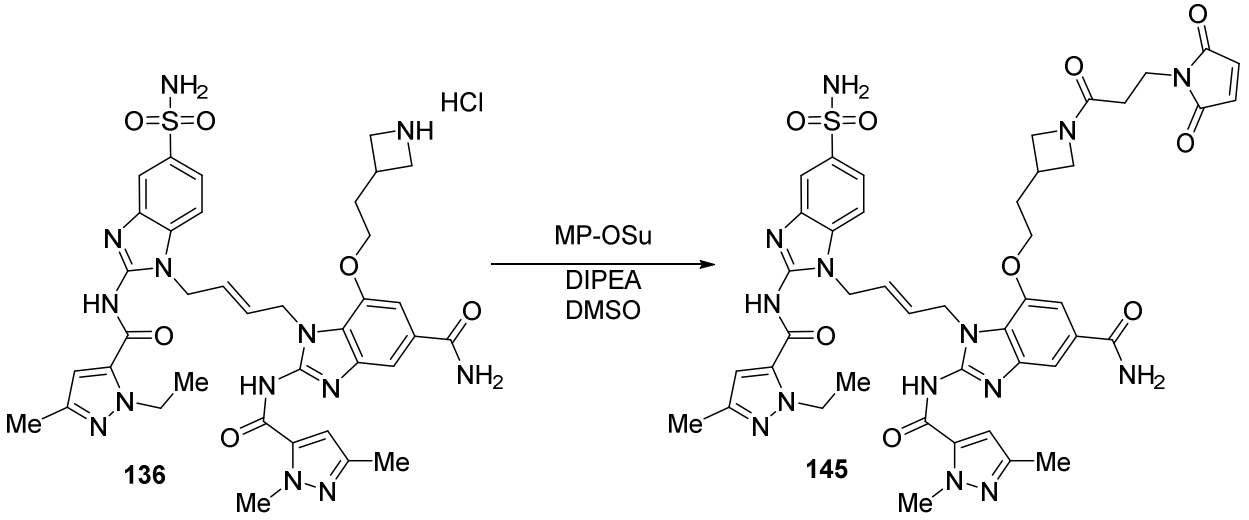


135 144

[0586] The x2 TFA salt of compound **144** (0.59 mg, 0.0005 mmol, 24% yield) was prepared according to general method 9, using compound **135** (1.86 mg, 0.0020 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 963.4 (theoretical), 963.5 (observed). HPLC retention time: 1.44 min.

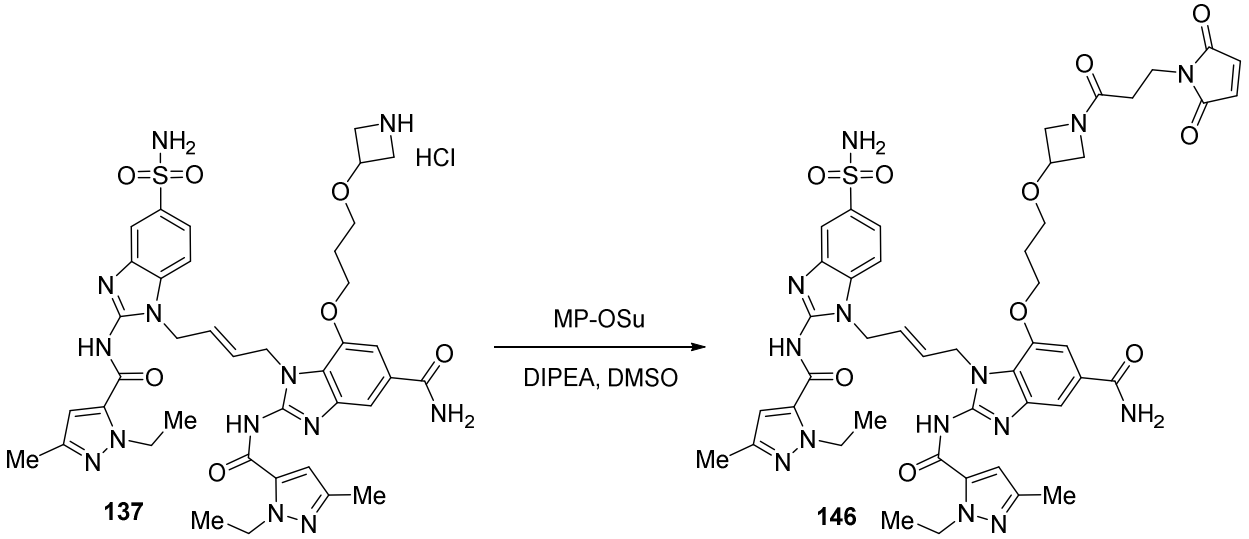
Synthesis of (E)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-7-(2-(1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)ethoxy)-1-(4-(2-(1-ethyl-3-methyl-1H-

pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 145)



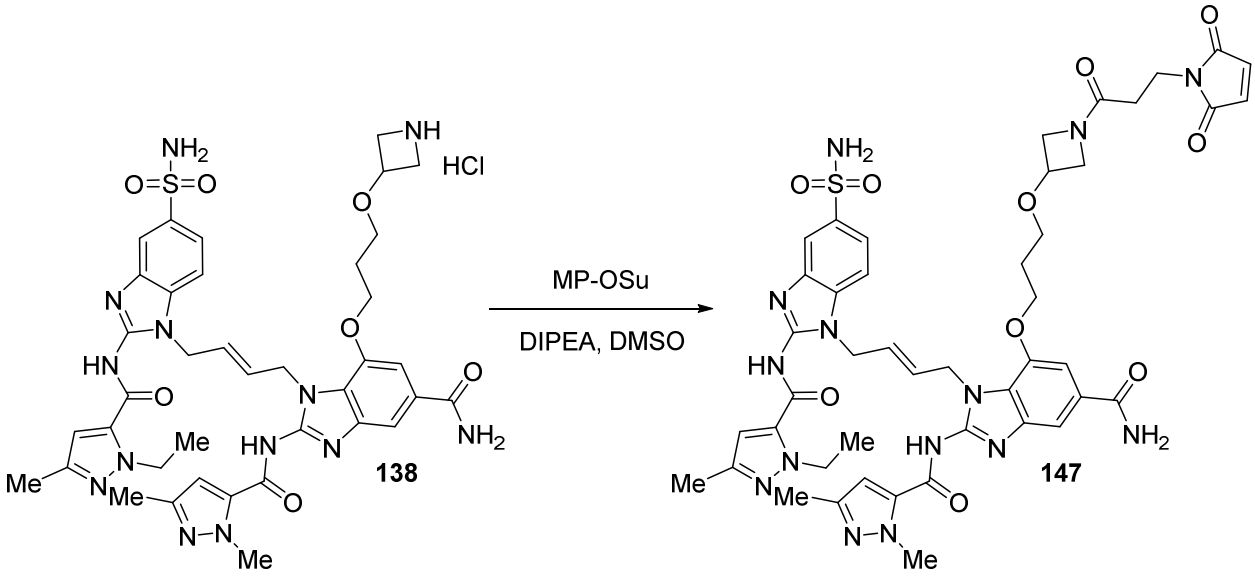
[0587] The x2 TFA salt of compound **145** (0.31 mg, 0.0003 mmol, 12% yield) was prepared according to general method 9, using compound **136** (2.09 mg, 0.0023 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 949.3 (theoretical), 949.5 (observed). HPLC retention time: 1.40 min.

Synthesis of (E)-7-(3-((1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)oxy)propoxy)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 146)



[0588] The x2 TFA salt of compound **146** (0.92 mg, 0.0008 mmol, 21% yield) was prepared according to general method 9, using compound **137** (3.35 mg, 0.0035 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 993.4 (theoretical), 993.5 (observed). HPLC retention time: 1.43 min.

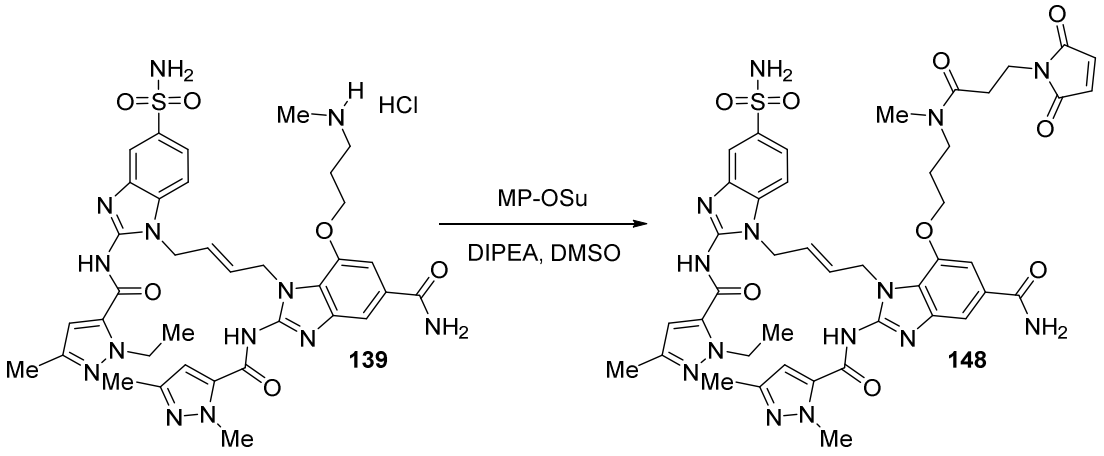
Synthesis of (E)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-7-(3-((1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)oxy)propoxy)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 147)



[0589] The x2 TFA salt of compound **147** (0.36 mg, 0.0003 mmol, 12% yield) was prepared according to general method 9, using compound **136** (2.83 mg, 0.0024 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 979.4 (theoretical), 979.5 (observed). HPLC retention time: 1.41 min.

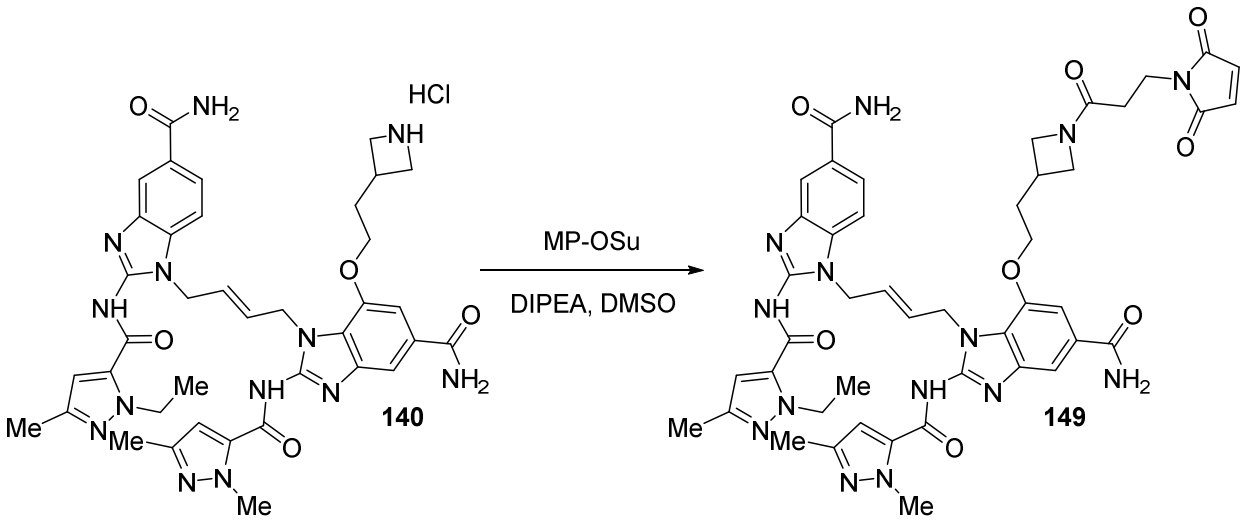
Synthesis of (E)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-7-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylpropanamido)propoxy)-1-(4-(2-(1-ethyl-3-methyl-1H-pyrazole-5-

carboxamido)-5-sulfamoyl-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-1H-benzo[d]imidazole-5-carboxamide (Compound 148)



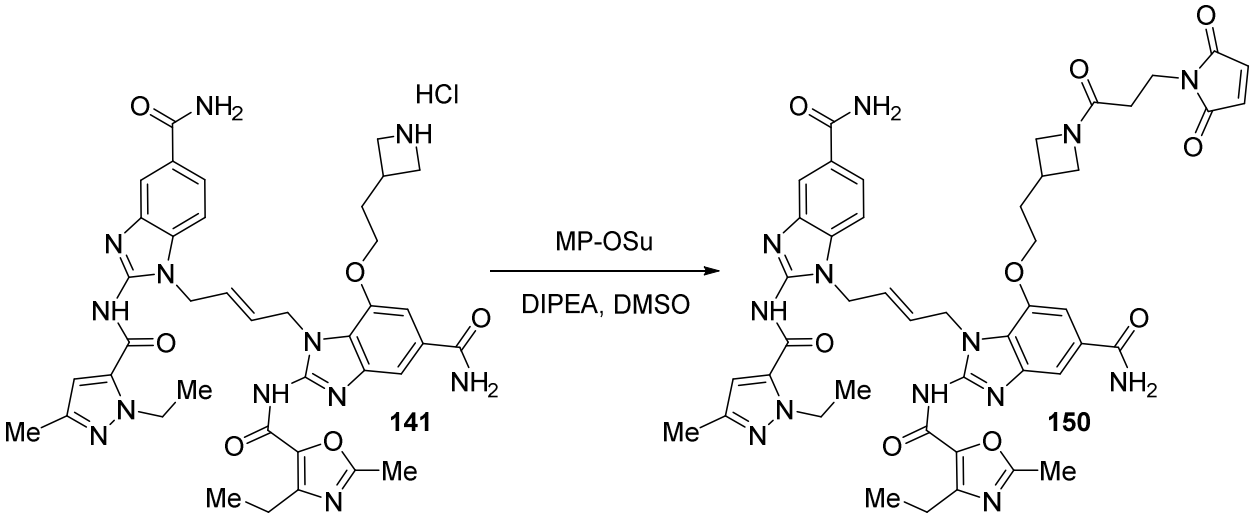
[0590] The x2 TFA salt of compound **148** (15 mg, 0.0129 mmol, 44% yield) was prepared according to general method 9, using compound **139** as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 937.3 (theoretical), 937.4 (observed). HPLC retention time: 1.42 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-7-(2-(1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)ethoxy)-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxamide (Compound 149)



[0591] The x2 TFA salt of compound **149** (16 mg, 0.0136 mmol, 41% yield) was prepared according to general method 9, using compound **140** (30 mg, 0.0334 mmol, 1 equiv). as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 943.4 (theoretical), 943.5 (observed). HPLC retention time: 1.41 min.

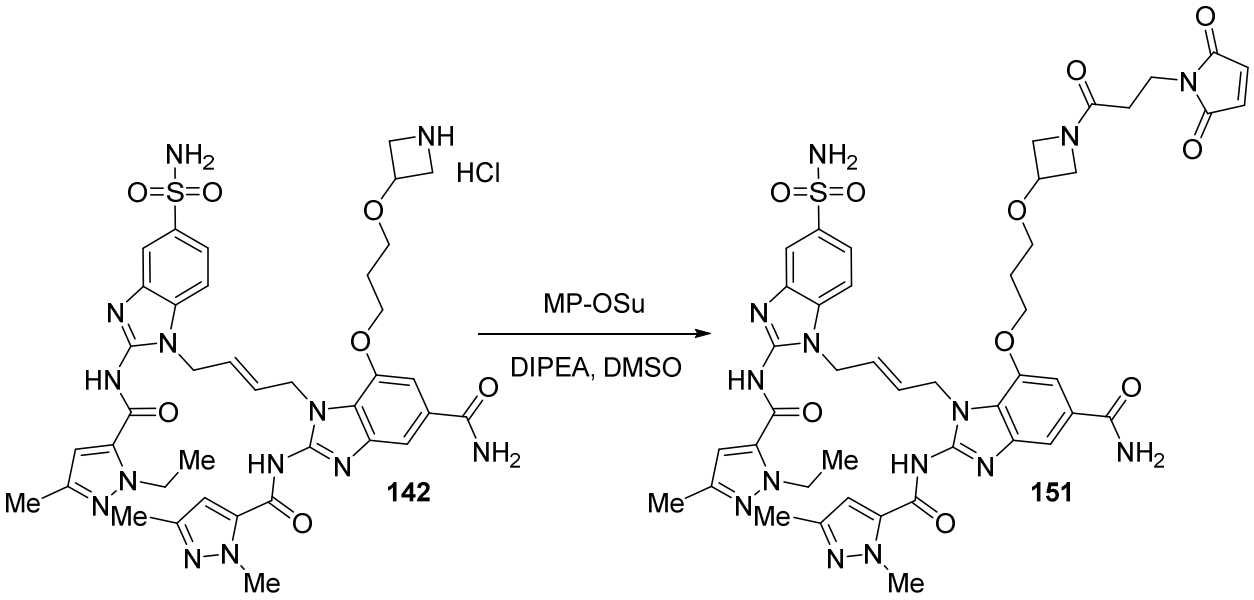
Synthesis of (E)-N-(5-carbamoyl-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(2-(1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)ethoxy)-1H-benzo[d]imidazol-2-yl)-4-ethyl-2-methyloxazole-5-carboxamide (Compound 150)



[0592] The x2 TFA salt of compound **150** (15 mg, 0.0123 mmol, 53% yield) was prepared according to general method 9, using compound **141** (21 mg, 0.0230 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 943.4 (theoretical), 943.5 (observed). HPLC retention time: 1.41 min.

Synthesis of (E)-1-(4-(5-carbamoyl-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-7-(3-((1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)oxy)propoxy)-1H-

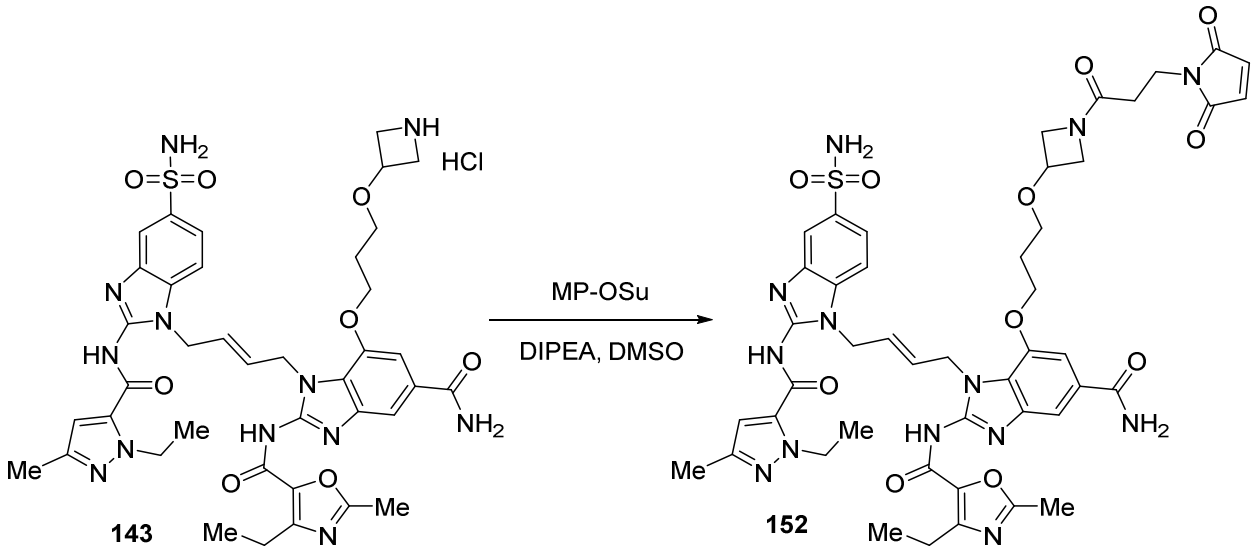
benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazole-5-carboxamide (Compound 151)



[0593] The x2 TFA salt of compound **151** (22 mg, 0.0182 mmol, 56% yield) was prepared according to general method 9, using compound **142** (30 mg, 0.0323 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 973.4 (theoretical), 973.5 (observed). HPLC retention time: 1.42 min.

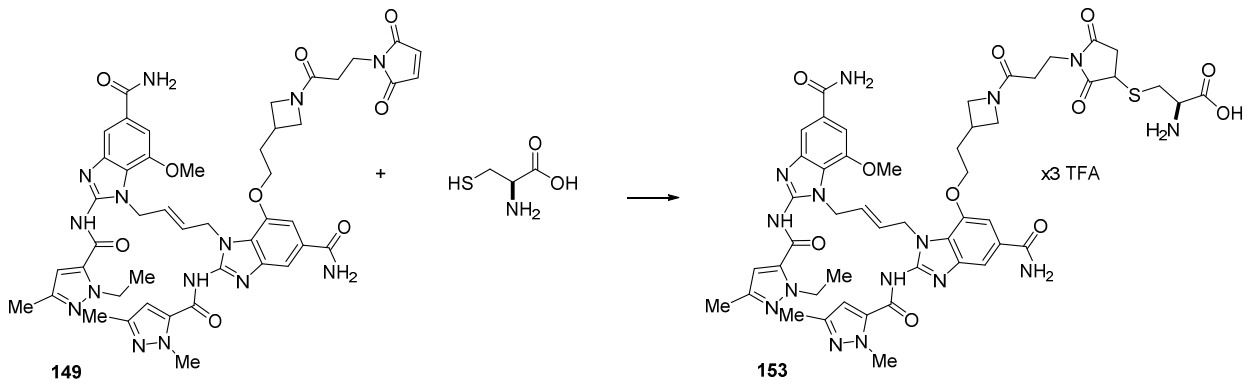
Synthesis of (E)-N-(5-carbamoyl-1-(4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-7-(3-((1-(3-(2,5-dioxo-2,5-

dihydro-1H-pyrrol-1-yl)propanoyl)azetidin-3-yl)oxy)propoxy)-1H-benzo[d]imidazol-2-yl)-4-ethyl-2-methyloxazole-5-carboxamide (Compound 152)



[0594] The x2 TFA salt of compound **152** (20 mg, 0.0168 mmol, 43% yield) was prepared according to general method 9, using compound **143** (37 mg, 0.0388 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 988.4 (theoretical), 988.5 (observed). HPLC retention time: 1.40 min.

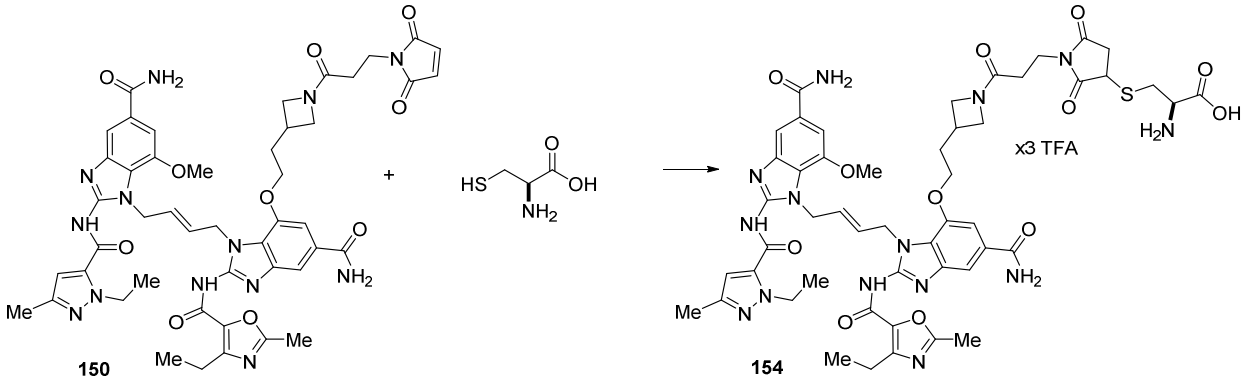
Synthesis of S-(1-(3-(3-(2-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1,3-dimethyl-1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)ethyl)azetidin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 153)



[0595] To a solution of compound **149** (10 mM in DMSO, 0.42 mL, 0.0042 mmol, 1 equiv.) was added l-cysteine (0.1 M H_2O, 63 µL, 0.063 mmol, 1.5 equiv.). The reaction stirred at

30 ºC for 1 h and was monitored by UPLC-MS. Upon completion, the reaction mixture was purified directly by preparatory HPLC (method G). Pure fractions were collected, frozen, and lyophilized to yield compound **153** (2.17 mg, 0.0015 mmol, 36% yield). LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1079.4 (theoretical), 1079.5 (observed). HPLC retention time: 1.28 min.

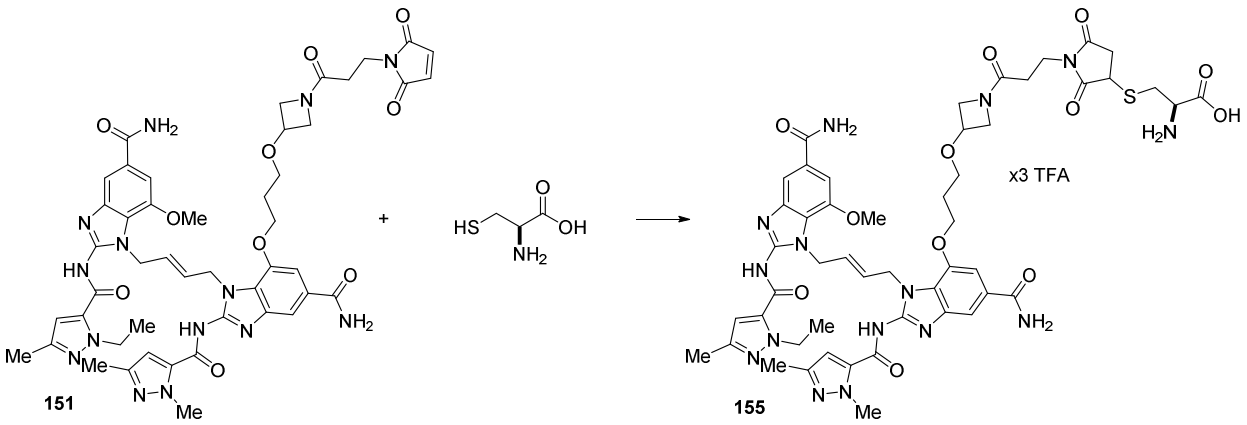
Synthesis of S-(1-(3-(3-(2-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(4-ethyl-2-methyloxazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)ethyl)azetidin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 154)



[0596] Compound **150** (2.35 mg, 0.0017 mmol, 39% yield) was prepared using the same procedure as compound **153**, using compound **145** (10 mM in DMSO, 0.43 mL, 0.0043 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1064.4 (theoretical), 1064.5 (observed). HPLC retention time: 1.29 min.

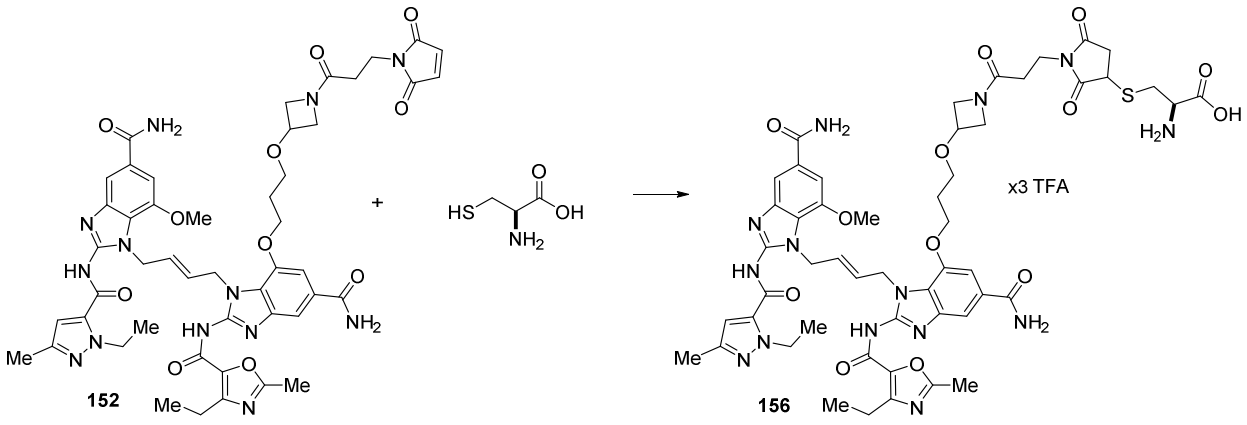
Synthesis of S-(1-(3-(3-(3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(1,3-dimethyl-

1H-pyrazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propoxy)azetidin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 155)



[0597] Compound **155** (2.34 mg, 0.0016 mmol, 39% yield) was prepared using the same procedure as compound **153**, using compound **151** (10 mM in DMSO, 0.41 mL, 0.0041 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ = 1109.4 (theoretical), 1109.5 (observed). HPLC retention time: 1.30 min.

Synthesis of S-(1-(3-(3-(3-((5-carbamoyl-1-((E)-4-(5-carbamoyl-2-(1-ethyl-3-methyl-1H-pyrazole-5-carboxamido)-7-methoxy-1H-benzo[d]imidazol-1-yl)but-2-en-1-yl)-2-(4-ethyl-2-methyloxazole-5-carboxamido)-1H-benzo[d]imidazol-7-yl)oxy)propoxy)azetidin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine (Compound 156)



[0598] Compound **156** (2.31 mg, 0.0016 mmol, 39% yield) was prepared using the same procedure as compound **153**, using compound **152** (10 in mM DMSO, 0.42 mL, 0.0042 mmol, 1 equiv.) as the starting material. LC-MS (Method E, ESI+): m/z $[M + H]^+$ =1094.4 (theoretical), 1094.5 (observed). HPLC retention time: 1.32 min.

General Procedures for the Preparation of ADCs:

[0599] ADCs were prepared as described previously (*Methods Enzymol.* **2012**, 502, 123-138). Briefly, DAR (drug-to-antibody ratio) 4 conjugates were prepared by partial reduction of the antibody inter-chain disulfide bonds using a sub-stoichiometric amount of tris(2-carboxyethyl)phosphine (TCEP). TCEP was added at approximately 2.2 molar equivalents relative to the antibody (TCEP:antibody) to a pre-warmed (37 °C) antibody stock solution in phosphate buffered saline, (PBS,Gibco, PN 10010023) and 1 M EDTA. The reduction reaction mixture was incubated at 37°C for approximately 60 minutes. Conjugation of the partially-reduced antibody with maleimide drug-linker was carried out by adding 6 molar equivalents of the drug-linker as a DMSO stock solution. Additional DMSO was added as necessary to achieve a final reaction concentration of 10% (v/v) DMSO to keep the drug-linker remain in solution during the conjugation reaction. The conjugation reaction was allowed to proceed for 30 minutes at room temperature or until all available antibody cysteine thiols had been alkylated by drug-linker as indicated by reversed-phase HPLC (Method G). Removal of excess drug-linker was achieved by incubating the reaction mixture with 100% molar excess QuadraSil® MP resin (Millipore Sigma, PN 679526) for 30 minutes at room temperature. Buffer exchange into formulation buffer (PBS, Gibco, PN 10010023) was achieved by gel filtration chromatography using a prepacked PD-10 column (GE Life Sciences, PN 17043501) according to manufacturer's instructions. Further removal of residual drug-linker was achieved by repeated diafiltration (5-10 times) of the reaction mixture containing the ADCs in formulation buffer using a 30 kilodalton molecular weight cutoff centrifugal filter (Millipore Sigma, PN Z717185), until there was no detectable free drug-linker remaining, as indicated by HPLC analysis (Method K).

General Procedures for the Characterization of ADCs:

[0600] ADCs were characterized using the following methods:

[0601] Method I: Size-exclusion chromatography (SEC) was performed with a Waters ACQUITY UPLC system and an Acquity UPLC Protein BEH SEC Column, (200 Å, 1.7µm, 4.6 x 150mm, PN: 186005225). The mobile phase used was 7.5% isopropanol in 92.5% aqueous (25

mM sodium phosphate, 350mM NaCl, pH 6.8), v/v. Elution was performed isocratically at a flow rate of 0.4 mL/min at ambient temperature.

[0602] Method J: Reversed-phase chromatography (RP-HPLC) was performed on a Waters 2695 HPLC system and an Agilent PLRP-S column (1000 Å, 8µm 50x2.1mm, PN: PL1912-1802). ADCs were treated with 10 mM DTT to reduce disulfide bonds prior to analysis. Sample elution was done using Mobile Phase A (0.05% (v/v) TFA in water) and Mobile Phase B (0.01% (v/v) TFA in MeCN) with a gradient of 25-44% B over 12.5 minutes at 80°C. The drug-to-antibody ratio (DAR) was calculated based on the integrated peak area measured at UV 280 nm.

Calculations of Molar Ratios

[0603] The average drug loading per antibody light-chain (MR_{DLC}) or antibody heavy-chain (MR_{DHC}) was calculated using the equations below:

$$MR_{DLC} = \frac{\sum(LC\%area_n \times MR_n)}{100}$$

where MR_{DLC} = average drug-to-light chain ratio

$LC\%area_n$ = % area of the *n*th loaded light chain species

% areas based on light chain peaks only

MR_n = drug-to-antibody ratio of the *n*th loaded species

AND

$$MR_{DHC} = \frac{\sum(HC\%area_n \times MR_n)}{100}$$

where MR_{DHC} = average drug-to-heavy chain ratio

$HC\%area_n$ = % area of the *n*th loaded heavy chain species

% areas based on heavy chain peaks only

MR_n = drug-to-antibody ratio of the *n*th loaded species

[0604] The average drug loading per antibody (MR_D) was calculated using the equation below:

$$MR_D = 2 \times (MR_{DLC} + MR_{DHC})$$

where MR_D = average drug-to-antibody ratio

MR_{DLC} = average drug-to-light chain ratio

MR_{DHC} = average drug-to-heavy chain ratio

[0605] Method K: Residual unconjugated drug linker was measured on a Waters ACQUITY UPLC system using an ACQUITY UPLC BEH C18 Column (130Å, 1.7 µm, 2.1 mm X 50 mm, PN: 186002350). ADC samples were treated with 2x volumes of ice-cold MeOH to induce precipitation and pelleted by centrifugation. The supernatant, containing any residual, unconjugated drug-linker, was injected onto the system. Sample elution was done using Mobile Phase A (0.05% (v/v) TFA in Water) and Mobile Phase B (0.01% TFA (v/v) in MeCN) with a gradient of 1-95% B over 2 minutes at 50°C. Detection was performed at 215 nm and quantitation of the residual drug-linker compound was achieved using an external standard of the corresponding linker.

EXAMPLE 2:
IN VITRO POTENCY EVALUATION OF STING AGONISTS AND CORRESPONDING ADCS

Experimental Procedures of in vitro biological assays

THP1-Dual™ Cell Reporter Assay

[0606] Potency of compounds and ADCs was evaluated using the THP1-Dual™ cells (InvivoGen PN: thpd-nfis [also referred to as THP1 dual reporter cells]), which contain an IRF-Lucia luciferase reporter. Cells were cultured in RPMI-1640 (Gibco) with 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/mL-100µg/mL, Gibco), HEPES (10mM, Gibco)), sodium pyruvate (1mM, Gibco), MEM non-essential amino acids (1x, Gibco), GlutaMAX (1x, Gibco), and beta-mercaptoethanol (55µM, Gibco). Cells were plated in a 96-well flat bottom tissue culture-treated clear polystyrene plate (Corning Costar #3596) at ~100,000 cells per well in 200 µL with the indicated concentration of the compound or ADC. The supernatant was harvested at 24 hours (compounds) or 48 hours (ADC) post plating for the reporter assay, or as indicated. To measure Lucia reporter signal, 10 µL of the supernatant was combined with 40 µL of QUANTI-Luc™ Luminescence assay reagent (Invivogen PN: rep-qlc1) in a 96-well clear flat bottom tissue culture-

treated black polystyrene plate (Corning Costar #3603) and read on a Perkin Elmer Envision plate reader.

Bone Marrow-derived Macrophage Assay

[0607] Potency of the compounds described herein was evaluated using mouse bone-marrow derived macrophages cultured from wild type (C57BL/6J, the Jackson Laboratory #000664) or STING-deficient (C57BL/6J-*Sting1*^{gt}/J, the Jackson Laboratory #017537) mice. Briefly, mouse bone marrow cells were cultured for 7-10 days in RPMI-1640 (Gibco) with 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/mL-100 μg/mL, Gibco), HEPES (10mM, Gibco)), sodium pyruvate (1 mM, Gibco), GlutaMAX (1x, Gibco), beta-mercaptoethanol (55 μM, Gibco) and 20-40 ng/mL murine M-CSF (Peprotech, #315-02). Cells were plated in a 96-well flat bottom tissue culture-treated clear polystyrene plate (Corning Costar #3596) at ~100,000 cells per well in 200 μL with the indicated concentration of the compound. The supernatant was harvested at 24 hours and cytokines were measured using a Milliplex MAP mouse cytokine/chemokine magnetic bead panel assay kit (MCYTOMAG-70k custom 11-plex kit: MCP1, MIP1α, MIP1β, TNFα, IFNγ, IL-10, IL-12p70, IL-1β, IL-6, IP10, RANTES) and analyzed using a Luminex™ MAGPIX™ Instrument System.

Bystander Activity Assay

[0608] Bystander activity of ADCs was evaluated using Renca cancer cells and THP1-Dual™ cells (InvivoGen) which contain an IRF-Lucia luciferase reporter. Cells were cultured in RPMI-1640 (Gibco) with 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100μg/ml, Gibco), HEPES (10mM, Gibco), sodium pyruvate (1mM, Gibco), MEM non-essential amino acids (1x, Gibco), GlutaMAX (1x, Gibco), and beta-mercaptoethanol (55μM, Gibco). Renca cells were plated in a 96-well flat bottom tissue culture-treated clear polystyrene plate (Corning Costar #3596) at 50,000 cells per well in 100 μL. On the day following the initial plating, 50,000 THP1-Dual™ cells were added to each well with the indicated concentration of ADC in a total volume of 200 μL. Supernatant was harvested at 48 hours post addition of the THP1-Dual™ cells. To measure Lucia reporter signal, 10μL of supernatant was combined with 40μL of QUANTI-Luc™ Luminescence assay reagent (Invivogen PN: rep-qlc1) in a 96-well clear flat bottom tissue culture-treated black polystyrene plate (Corning Costar PN: 3603) and read on a

Perkin Elmer Envision plate reader. In some experiments, HEK 293T cells engineered to express a murine protein typically expressed by immune cells (target antigen C— an immune cell antigen) were plated as above instead of Renca tumor cells.

Cancer Cell Direct Cytotoxicity Assay

[0609] Cancer cells were counted and plated in 40 µL complete growth media in 384-well, white-walled tissue culture treated plates (Corning). Cell plates were incubated at 37°C and with 5% CO_2 overnight to allow the cells to equilibrate. Stock solutions containing ADCs or free drugs were serially diluted in RPMI-1640 + 20% fetal bovine serum (FBS). 10 µL of each concentration were then added to each cell plate in duplicate. Cells were then incubated at 37°C and with 5% CO_2 for 96 hours, upon which, the cell plates were removed from the incubator and allowed to cool to room temperature for 30 minutes prior to analysis. CellTiter-Glo® luminescent assay reagent (Promega Corporation, Madison, WI) was prepared according to Promega's protocol. 10 µL of CellTiter-Glo® were added to assay plates using a Formulatrix Tempest liquid handler (Formulatrix) and the plates were protected from light for 30 minutes at room temperature. The luminescence of the samples was measured using an EnVision Multimode plate reader (Perkin Elmer, Waltham, MA). Raw data were analyzed in Graphpad Prism (San Diego, CA) using a nonlinear, 4-parameter curve fit model [Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))]. Results are reported as X50 values, which are defined as the concentration of ADC or free drug required to reduce cell viability to 50%.

SU-DHL-1 assay

[0610] Potency of ADCs was evaluated using the SU-DHL-1 lymphoma cells. Cells were cultured in RPMI-1640 (Gibco) with 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/mL-100µg/mL, Gibco), HEPES (10mM, Gibco)), sodium pyruvate (1mM, Gibco), MEM non-essential amino acids (1x, Gibco), GlutaMAX (1x, Gibco), and beta-mercaptoethanol (55µM, Gibco). Cells were plated in a 96-well flat bottom tissue culture-treated clear polystyrene plate (Corning Costar #3596) at ~100,000 cells per well in 200 µL with the indicated concentration of ADC. After 48 hours, the 50 µL supernatant was harvested and cytokine production was evaluated using a MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead panel (HCYTOMAG-60K custom 8-plex kit: IL-6, IL-8, MCP1, TNFα, GRO, IP-10, MIP1α, and

MIP1β). Cell viability was evaluated by adding 100 µL CellTiter-Glo® luminescent assay reagent (Promega Corporation, Madison, WI) to remaining 150 µL of cells in the plate and transferring the mixture to a 96-well black-walled plate (Corning Costar #3603). Plates were protected from light for 30 minutes at room temperature, and the luminescence of the samples was measured using an EnVision Multimode plate reader (Perkin Elmer, Waltham, MA).

Results from in vitro biological assays

[0611] STING agonist compounds were assessed for their ability to activate THP1-Dual™ reporter cells, a human monocytic cell line in which type I interferon (IRF) signaling can be monitored via a secreted luciferase reporter protein (Lucia). THP1-Dual™ cells were treated with increasing concentrations of the agonists for 24h, then supernatants were harvested and the Lucia reporter signal was quantified using QUANTI-Luc™ Luminescence assay reagent. Compound **A** and compound **1** were significantly more potent than (2',3')-Rp,Rpc-diAMPS disodium (Compound **B**) and activated the Lucia reporter with EC50 values of 3 and 5 nM respectively. Compound **12a** was less potent than compound **1** and compound **A** (**Figure 1**, EC_{50} value of 21 nM). Both compound **1** and **12a** induced cytokine production when used to stimulate wild type (WT), but not STING-deficient, murine bone marrow-derived macrophages, indicating the activity of these compounds is STING-dependent (**Figure 2**).

[0612] The STING agonist compounds were conjugated to both targeted and non-binding antibodies and the resulting ADCs were assessed for their ability to activate THP1-Dual™ reporter cells. Compound **1** was conjugated using a cleavable glucuronide-based linker (**11**). Compound **12a** was conjugated using a non-cleavable, cleavable peptide-based, and cleavable glucuronide-based linker (Compounds **12**, **14** and **13**, respectively). THP1-Dual™ cells were treated with increasing concentrations of ADCs with a non-binding or targeted mAb conjugated to a compound for 48h, then supernatants were harvested, and the Lucia reporter signal was quantified using QUANTI-Luc™ Luminescence assay reagent. Although compound **12a** was less potent than compound **1** as a free drug (**Figure 1**), compound **12a** was more potent when conjugated to a targeted mAb via a cleavable glucuronide linker (**13**) than the similar compound **1** conjugate (**11**). Furthermore, compound **12a** was more potent when conjugated to a targeted mAb via a non-cleavable linker (**12**) than either cleavable linker **13** or **14** (**Figure 3**), demonstrating that conjugation of STING agonist small molecules to an antibody can increase their potency.

[0613] Compound **12** and the cysteine adduct (compound **16**) that is released upon cleavage of the mAb conjugate in the endo-lysosome were assessed for their ability to activate THP1-Dual™ reporter cells. THP1-Dual™ cells were treated with increasing concentrations of the compounds for 24h, then supernatants were harvested and the Lucia reporter signal was quantified using QUANTI-Luc™ Luminescence assay reagent. Both compound **12** and compound **16** were active with EC50 values (37 nM and 34 nM, respectively) similar to the parent free drug **12a** (21nM, **Figure 4** and **Figure 1**).

[0614] Compound **15b** was also evaluated, both as a free drug and when conjugated to a targeted antibody using a non-cleavable linker (**15**). THP1-Dual™ cells were treated with increasing concentrations of free drug or ADCs with a non-binding or targeted mAb conjugated to a compound for 48h; then supernatants were harvested, and the Lucia reporter signal was quantified using QUANTI-Luc™ Luminescence assay reagent. Compound **15b** was more potent than **12a**, while the potency of the ADC of **15** was similar to that of the ADC of **12** when linked to the same targeted mAb (**Figure 5**).

[0615] Compound **12a** was conjugated to both targeted and non-binding antibodies using a variety of non-cleavable linkers (**12**, **17**, **19-24**) and the resulting ADCs were assessed for their ability to activate THP1-Dual™ reporter cells. All conjugates with the targeted mAb were active with EC50 values ranging from ~1.7-7.3 ng/mL (**Table 1**). We also evaluated the ability of these linkers to directly kill cancer cells when conjugated to targeted mAbs binding tumor antigen A or antigen B (CD30). All conjugates were active in a subset of cancer cell lines (regardless of target antigen expression), indicating target-independent killing of some cancer cells; compounds **1**, **12a**, and **16** also demonstrated direct cytotoxic activity on a subset of cancer cell lines (**Table 2**; targeted mAb A conjugates comprise a mAb targeting the tumor antigen A conjugated to various drug linker compounds; targeted mAb B conjugates comprise the cAC10 mAb targeting CD30 conjugated to various drug linkers).

Table 1: Activity of target STING agonist ADCs in THP1-Dual™ reporter cells.

Compound	EC_{50} (ng/mL)**
12	4.2
17	2.2
19	7.3
20	1.7

21	4.2
22	4.7
23	2.4
24	2.1
25	307
26	14.4
27	10.4
66	>10,000
67	>10,000
68	>10,000
69	>10,000
96	>10,000
97	*
98	>10,000
99	*
100	*
101	>10,000
102	>10,000
103	>10,000
104	>10,000
105	52.5
106	>10,000
107	>10,000
108	63.5
109	>10,000
110	>10,000
111	13.2
112	2
121	3.0
122	5.6
123	1.6
124	9.1
125	1.4
126	>10,000
128	>10,000
131	3.6
133	12.2
134	10.3
144	>10,000
145	>10,000
146	>10,000

147	>10,000
148	>10,000
149	>10,000
150	7.9
151	>10,000
152	6.5

*ADCs with >10% aggregate were not evaluated

**For some compounds, EC50 values comprise the average value from multiple experiments

Table 2: Direct cytotoxicity data of various ADCs and compounds on human cancer cell lines.

Target A expression	+	+	+	+	+	+	+	+	+	+	+
Target B expression	-	-	-	+	+	+	+	-	-	-	-
	ADC X50 (ng/mL)										
	786-O	**A2058**	**BxPC3**	**DEL**	**DELBVR**	**Karpas299**	**L540cy**	**Ls174T**	**MDAMB231**	**MOLM-13**	**SU-DHL-4**
Targeted mAb A **12** (8 load)	>1K	>1K	>1K	0.01	0.04	1	1	>1K	>1K	1	>1K
Targeted mAb A **12** (4 load)	>1K	>1K	>1K	0.012	0.03	3	2	>1K	>1K	12	>1K
Targeted mAb B **12** (8 load)	>1K	>1K	>1K	<0.004	0.004	1	1	>1K	>1K	2	>1K
Targeted mAb B **12** (4 load)	>1K	>1K	>1K	0.01	0.1	2	1	>1K	>1K	7	>1K
Targeted mAb A **17** (8 load)	>1K	>1K	>1K	<0.004	0.004	1	1	>1K	>1K	2	>1K
Targeted mAb A **17** (4 load)	>1K	>1K	>1K	0.1	0.03	5	2	>1K	>1K	26	>1K
Targeted mAb B **17** (8 load)	>1K	>1K	>1K	<0.004	<0.004	1	0.2	>1K	>1K	2	>1K
Targeted mAb B **17** (4 load)	>1K	>1K	>1K	0.004	0.01	1	1	>1K	>1K	25	>1K
Targeted mAb A **21** (8 load)	>1K	>1K	>1K	0.01	0.03	4	1	>1K	>1K	2	>1K
Targeted mAb A **21** (4 load)	>1K	>1K	>1K	0.1	0.2	7	2	>1K	>1K	20	>1K
Targeted mAb B **21** (8 load)	>1K	>1K	>1K	<0.004	0.01	1	0.4	>1K	>1K	2	>1K
Targeted mAb A **22** (6 load)	>1K	>1K	>1K	0.02	0.1	2	1	>1K	>1K	2	>1K
Targeted mAb A **22** (2 load)	>1K	>1K	>1K	0.03	0.2	29	4	>1K	>1K	29	>1K
Targeted mAb B **22** (6 load)	>1K	>1K	>1K	0.01	0.1	1	1	>1K	>1K	3	>1K
Targeted mAb A **19** (8 load)	>1K	>1K	>1K	<0.004	0.02	1	1	>1K	>1K	1	>1K
Targeted mAb A **19** (4 load)	>1K	>1K	>1K	0.01	0.1	3	5	>1K	>1K	13	>1K
Targeted mAb B **19** (8 load)	>1K	>1K	>1K	<0.004	0.01	0.3	0.3	>1K	>1K	0.4	>1K
Targeted mAb B **19** (4 load)	>1K	>1K	>1K	<0.004	0.02	0.5	1	>1K	>1K	1	>1K
Targeted mAb A **20** (8 load)	>1K	>1K	>1K	0.01	0.02	0.2	0.3	>1K	>1K	1	>1K
Targeted mAb A **20** (4 load)	>1K	>1K	>1K	0.01	0.02	1	1	>1K	>1K	10	>1K
Targeted mAb B **20** (8 load)	>1K	>1K	>1K	<0.004	<0.004	0.1	0.1	>1K	>1K	0.2	>1K
Targeted mAb B **20** (4 load)	>1K	>1K	>1K	<0.004	0.02	0.1	0.5	>1K	>1K	1	>1K
Targeted mAb A **24** (8 load)	>1K	>1K	>1K	<0.004	0.01	0.3	1	>1K	>1K	1	>1K
Targeted mAb A **24** (4 load)	>1K	>1K	>1K	0.01	0.01	1	4	>1K	>1K	11	>1K
Targeted mAb B **24** (8 load)	>1K	>1K	>1K	<0.004	0.01	0.03	0.5	>1K	>1K	1	>1K
Targeted mAb B **24** (4 load)	>1K	>1K	>1K	<0.004	0.01	0.2	2	>1K	>1K	4	>1K
Targeted mAb A **23** (8 load)	>1K	>1K	>1K	0.01	0.03	1	1	>1K	>1K	1	>1K
Targeted mAb A **23** (4 load)	>1K	>1K	>1K	0.02	0.05	4	5	>1K	>1K	8	>1K
Targeted mAb B **23** (8 load)	>1K	>1K	>1K	<0.004	0.01	0.1	0.2	>1K	>1K	0.3	>1K
Targeted mAb B **23** (4 load)	>1K	>1K	>1K	0.002	0.02	0.3	0.3	>1K	>1K	2	>1K
	Compound X50 (nM))										
Compound **1**	>1K	>1K	>1K	2	16	>1K	31	>1K	>1K	42	>1K
Compoud **12a**	>1K	>1K	>1K	3	111	>1K	83	>1K	>1K	87	>1K
Compound **16**	>1K	>1K	>1K	4	7	>1K	29	>1K	>1K	27	>1K

[0616] Multiple additional compounds were synthesized and evaluated for their ability activate THP1-Dual™ reporter cells. Several compounds were active with EC50 values ranging from 1.3 nM (compound **27e**) to 6337 nM (compound **126a**, **Table 3**). Compounds with minimal activity up to 10 µM are listed in **Table 3** as having an EC50 value of >10,000 nM. Several compounds were conjugated to targeted (**Table 1**) and non-binding antibodies (not shown) via cleavable or non-cleavable drug linkers and the resulting ADCs were assessed for their ability to activate THP1-Dual™ reporter cells. Conjugates with drug linkers **25-27, 105, 108, 111-112** and **121-125** were active with EC50 values ranging from 1.4 to 307 ng/mL (**Table 1**). All other conjugates tested were not active up to 10 µg/mL in this assay, including conjugates with drug linkers derived from active small molecules (**Table 3**, **Table 1**) thus highlighting the challenges of developing active ADCs targeting the STING pathway.

Table 3: Activity of STING agonist small molecules in THP1-Dual™ reporter cells.

Compound	EC_{50} (nM)**	Compound	EC_{50} (nM)**	Compound	EC_{50} (nM)**
A	3	**49**	>10,000	**93**	>10,000
1	5	**50**	>10,000	**94**	132.8
12	37	**51**	>10,000	**95**	12.9
12a	21	**52**	>10,000	**114**	9.4
15b	5.8	**53**	>10,000	**115**	17.4
16	52 / 34	**54**	>10,000	**116**	11.6
17	66.28	**55**	>10,000	**117**	14.6
19	190.2	**56**	>10,000	**118**	23.6
20	53.91	**57**	>10,000	**119**	49.9
21	2632	**58**	>10,000	**120**	5.2
23	62.55	**59**	>10,000	**126a**	6337
24	83.29	**60**	>10,000	**126b**	>10,000
25f	12	**62**	>10,000	**127**	301
25g	1576	**63**	2508	**128a**	>10,000
26e	5	**64/65**	388.3	**129**	836
26f	69	**66b**	1159	**130**	2260
27e	1.3	**68a**	2238	**132**	37.4
27f	38	**70**	1.6	**135**	2011
28	954	**71**	70.11	**136**	>5,000
29	52	**72**	275.3	**137**	1685
30	87	**73**	216.25	**138**	>5,000
31	175.6	**74**	35.98	**139**	>4,000
32	44.5	**75**	57.73	**140**	1793

33	>10,000	**76**	58.8	**141**	159
		79	>10,000	**142**	1123
35	>10,000	**80**	>10,000	**143**	155
37	>10,000	**81**	>10,000	**154**	>10,000
38	>10,000	**82**	>10,000	**155**	94
39	>10,000	**83**	>10,000	**156**	1561
40	>10,000	**84**	>10,000	**157**	51
41	>10,000	**85**	>10,000		
42	>10,000	**86**	2465		
43	>10,000	**87**	>10,000		
44	>10,000	**88**	394		
45	>10,000	**89**	1324		
46	>10,000	**90**	>10,000		
47	>10,000	**91**	350		
48	>10,000	**92**	1190		

**For some compounds, EC50 values comprise the average value from multiple experiments

[0617] Compound **1** was conjugated to a non-binding antibody as well as antigen C and PD-L1-targeted mAbs using the cleavable linker **11** and the resulting ADCs were assessed for their ability to induce cytokine production and direct cytotoxicity by SU-DHL-1 cells. Conjugates targeting antigen C and PD-L1, but not the non-binding conjugate, induced robust production of the cytokine MIP-1α and led to SU-DHL-1 cell death (**Figure 6A** and **Figure 6B**).

[0618] The ability of conjugates to activate THP1 dual reporter immune cells in a bystander manner was evaluated. Conjugates consisting of an antibody targeting antigen C with a hIgG1 LALAPG Fc backbone conjugated to compound **12**, **13**, and **14** demonstrated some bystander activity when THP1 dual cells were co-cultured with HEK 293T cells engineered to express antigen C (**Figure 7**). Conjugates consisting of the h1C1 antibody targeting EphA2 with a mIgG2a WT or LALAPG Fc backbone (*see, e.g.,* Schlothauer *et al*., Protein Engineering, Design and Selection, 2016, 29(10):457-466; and Hezareh *et al*., Journal of Virology, 2001, 75(24):12161-12168, each of which is incorporated herein by reference in its entirety) conjugated to compound **12** also demonstrated bystander activity when THP1 dual cells were co-cultured with murine Renca tumor cells. Markedly enhanced bystander activity was observed with conjugates with an intact WT Fc backbone (**Figure 8**).

EXAMPLE 3
IN VIVO EVALUATION OF ANTI-TUMOR IMMUNE RESPONSES INDUCED BY STING AGONIST ADCS

Experimental Procedures for in vivo Studies

In vivo Cytokine Assay

[0619] Cytokines were measured in mouse plasma harvested at 3, 6, 24, or 48 hours after treatment with compounds or ADCs using a Milliplex MAP mouse cytokine/chemokine magnetic bead panel assay kit (MCYTOMAG-70k custom 11-plex kit: MCP1, MIP1α, MIP1β, TNFα, IFNγ, IL-10, IL-12p70, IL-6, IL-1β, IP10, RANTES) and analyzed using a Luminex™ MAGPIX™ Instrument System. Values that were outside of the standard curve range (< 3.2 or > 10,000 pg/mL) were excluded from calculation of the mean values.

In vivo Anti-tumor Activity Studies

Renca cancer cells

[0620] Renca cancer cells (ATCC) were cultured in RPMI-1640 (ATCC) with 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/mL-100 µg/mL), MEM non-essential amino acids (1x), sodium pyruvate (1 mM), and L-glutamine (2 mM). Renca cancer cells were implanted ($2*10^6$ cells in 200 µL 25% Matrigel) subcutaneously into Balb/c female mice. In some experiments, Renca tumor cells were engineered to express the indicated murine or human target antigen.

[0621] When tumor volumes reached 100 mm^3, the mice were dosed with either compounds or ADCs by intraperitoneal or intravenous injection at the indicated dosing schedule and tumor volumes were monitored twice weekly. Compounds were formulated in 40% PEG400 in saline.

CT26 cancer cells

[0622] CT26 cancer cells (ATCC) were cultured in RPMI 1640 modified with 1mM Sodium Pyruvate, 10mM HEPES, 2.8mL 45% Glucose (1.25g) and supplemented with 10% fetal

bovine serum and 1% Pen/Strep/Glutamine. CT26 cancer cells were implanted (0.5*106 cells in 200uL serum-free RPMI 1640) subcutaneously into Balb/c mice.

MC38 cancer cells

[0623] MC38 cancer cells (Kerafast) were cultured in DMEM with 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/mL-100μg/mL), MEM non-essential amino acids (1x), sodium pyruvate (1mM), and L-glutamine (2mM). MC38 cancer cells were implanted ($1*10^6$ cells in 100uL 25% Matrigel) subcutaneously into C57BL/6 mice.

[0624] In some experiments, tumor-bearing mice that achieved complete tumor regression following ADC treatment were "rechallenged" with MC38 tumor cells; MC38 cancer cells were implanted ($1*10^6$ cells in 100uL 25% Matrigel) subcutaneously into the opposite flank of C57BL/6 mice.

4T1 cancer cells

[0625] 4T1 cancer cells (ATCC) were cultured in RPMI with 10% heat-inactivated fetal bovine serum and implanted ($0.02*10^6$ cells in 200uL plain RPMI) subcutaneously into Balb/c mice.

Results from in vivo studies

Renca cancer cells

[0626] A syngeneic system was used to assess the ability of the STING agonist ADCs to induce immune responses *in vivo* and drive an anti-tumor immune response. The Renca system is a subcutaneous, mouse renal adenocarcinoma model. Female Balb/c mice were implanted with $2x10^6$ Renca cells subcutaneously in the flank on day 0. When mean tumor size of 100 mm^3 (measured by using the formula Volume (mm^3) = 0.5 * Length * $Width^2$ where length is the longer dimension) was reached mice were randomized into treatment groups of ≥ 5 mice per group. Animals were then treated intraperitoneally (ADCs or compounds) or intravenously (compounds) with the indicated treatment every 7 days, for 3 doses total (or as indicated). Tumor length and width and the weight of the animals was measured throughout the study and tumor volume was calculated using the formula above. Animals were followed until the tumor volume reached ~ 1000 mm^3; animals were then euthanized.

[0627] The anti-tumor activity of compound **1** compared to the cleavable linker **11** conjugated to a non-binding or EphA2-targeted mAb (mIgG2a LALAPG backbone; *see, e.g.,* Schlothauer *et al*., Protein Engineering, Design and Selection, 2016, 29(10):457-466; and Hezareh *et al*., Journal of Virology, 2001, 75(24):12161-12168, each of which is incorporated herein by reference in its entirety) was evaluated; note that all EphA2-targeted mAb conjugates described herein consist of the h1C1 mIgG2a mAb conjugated to various drug linker compounds. When animals were treated with the Compound **1** or the non-binding mAb conjugate of **11**, some tumor growth delay was observed; however, tumor growth delay was significantly enhanced with the EphA2-targeted mAb conjugate of **11**, especially at the higher 12 mg/kg dose (**Figure 9A**), clearly demonstrating the anti-tumor benefit of delivering STING agonists using a targeted ADC.

[0628] In the next *in vivo* study, the anti-tumor activity of the non-cleavable linker compound **12** conjugated to a non-binding or EphA2-targeted mAb (mIgG2a LALAPG backbone) was evaluated. EphA2-targeted mAb conjugate of **12** exhibited robust anti-tumor activity and was surprisingly more active than the ADC of **11** conjugated to the same EphA2-targeted mAb (**Figure 10A**). In the next *in vivo* study, the anti-tumor activity of the non-cleavable linker **15** conjugated to a non-binding or EphA2-targeted mAb (mIgG2a WT backbone) was evaluated. EphA2-targeted mAb conjugates of **15** exhibited robust anti-tumor activity that was similar to the corresponding ADC of **12** (**Figure 11A**). In this study, the activity of **12** conjugated to an EphA2-targeted antibody with a mIgG2a WT and LALAPG backbone was also evaluated, and both conjugates were similarly active. This was a surprising finding, given that the *in vitro* bystander assay indicates that an intact WT Fc backbone significantly enhances bystander immune cell activation compared to LALAPG Fc backbone (**Figure 8**).

[0629] Compound **1** and all antibody conjugates of **11** and **12** on a mIgG2a LALAPG backbone were well tolerated – average weight loss was <~5% after the 1st and 2nd dose of the treatment. The STING agonist compound **A** was less well tolerated – with mice exhibiting on average 6.2% weight loss after the 2nd dose (**Figure 9B, 10B,** and **11B**). Moreover, EphA2-targeted mAb conjugates of **12** and **15** with a mIgG2a WT backbone at the 3 mg/kg dose level were less well tolerated than the conjugate of **12** with a LALAPG backbone – with mice treated with targeted WT backbone ADCs exhibiting ~8% weight loss (**Figure 11B**).

[0630] In the next *in vivo* study, the anti-tumor activity of the non-cleavable linker compound **12** conjugated to an EphA2-targeted mAb (mIgG2a LALAPG backbone) as well as

unconjugated Compound **12a** was evaluated. The EphA2-targeted mAb conjugates of **12** exhibited robust anti-tumor activity at doses of 1 mg/kg and 3 mg/kg, while Compound **12a** had limited anti-tumor efficacy (**Figure 12**). Collectively, this suggests that STING agonist compounds (e.g., compounds **1** and **12a**) that are inactive *in vivo* in tumor models can be converted into active therapeutics by conjugation to an antibody (e.g., targeted mAb conjugates of **11** and **12**).

[0631] Systemic cytokine production in response to the free drugs and conjugates was measured as a proxy for systemic activity. Compound **1** and all antibody conjugates of **11**, **12** and **15** induced very little pro-inflammatory cytokine (IL-6 and TNF) production. On the other hand, compound **A** and compound **12a** induced robust production of IL-6 and TNF (**Table 4**, **Table 5**, and **Table 6**). Moreover, EphA2-targeted conjugates of **11** and **12** with a WT Fc backbone induced more systemic MIP1α, MIP1β, and MCP-1 expression than the conjugate of **12** with a LALAPG Fc backbone. This indicates that, in the Renca tumor model with the specific EphA2-targeted antibodies described in **Figures 10-12** dosed at 3 mg/kg q7dx3, a LALAPG Fc backbone may reduce on-target toxicity (systemic cytokines/weight loss), without impacting anti-tumor efficacy. This also indicates that conjugation of a STING agonist compound (e.g., compound **12a** vs. the targeted mAb conjugate of **12)** may improve both efficacy and safety (reduce systemic cytokines).

Table 4: Cytokine production in peripheral blood (plasma) in Renca tumor-bearing mice upon treatment with various ADCs comprising a non-binding or EphA2-targeted mAb with a mIgG2a LALAPG backbone conjugated to compound **11** or **12**, or compound **1** or Compound A.

	Time (h)	control	3mg/kg non-binding mAb **11**	3mg/kg non-binding mAb **12**	3mg/kg targeted mAb **11**	3mg/kg targeted mAb **12**	control	2.4mg/kg non-binding mAb **11**	2.4mg/g targeted mAb **11**	12mg/kg targeted mAb **11**	1.48mg/kg Compound **1**	1.86mg/kg Compound **A**
IFNγ	**3**	< 3.2	1.1	0.4	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	0.3
	6	< 3.2	15.6	7.1	8.5	22.6	< 3.2	< 3.2	1.3	4.3	0.2	71.8
	24	< 3.2	< 3.2	1.4	< 3.2	3.0	< 3.2	< 3.2	< 3.2	0.9	< 3.2	< 3.2
IL1β	**3**	3.2	5.3	4.3	4.3	5.3	0.4	0.3	0.5	0.3	0.7	1.5
	6	3.2	4.3	4.3	5.3	6.4	0.3	1.7	0.3	1.8	1.5	0.6
	24	3.2	4.3	4.8	4.3	5.8	0.3	0.3	0.3	0.3	0.8	0.3
IL-6	**3**	9.2	120.1	23.8	150.5	109.5	154.7	14.3	5.5	37.7	95.7	3553.0
	6	26.9	472.1	176.6	305.6	404.4	0.7	162.2	81.9	303.4	6.8	731.9
	24	33.2	12.0	58.6	28.0	75.3	7.7	19.6	4.8	27.0	13.5	16.4
IL-10	**3**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	1.5	48.8	0.5
	6	< 3.2	3.9	4.3	4.8	6.4	< 3.2	< 3.2	< 3.2	< 3.2	0.8	< 3.2
	24	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	0.8	34.6	< 3.2
IL12p70	**3**	< 3.2	5.9	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	17.6	174.5	0.2
	6	< 3.2	< 3.2	< 3.2	< 3.2	3.1	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	0.2
	24	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	23.4	140.0	< 3.2
IP10	**3**	235.3	973.2	402.4	1069.7	1094.1	59.8	176.7	80.5	295.4	702.8	4785.0
	6	179.7	2976.0	2783.5	2963.1	3037.2	37.0	3977.4	4205.6	7374.2	3391.5	4976.1
	24	192.3	1129.0	2329.0	1019.3	2564.0	71.5	559.7	358.1	1524.0	551.1	889.6
MCP-1	**3**	50.2	138.6	97.6	152.5	166.5	67.8	21.6	10.0	65.2	167.8	14494.4
	6	35.8	1168.1	615.0	1727.0	7542.0	19.7	3822.7	1811.8	10211.9	795.6	10776.8
	24	50.2	157.0	2374.8	284.9	7777.1	26.9	238.0	161.9	1486.0	332.6	539.5
MIP1α	**3**	< 3.2	26.1	< 3.2	26.1	< 3.2	24.1	3.0	13.9	10.0	55.6	1300.3
	6	< 3.2	36.4	44.5	38.4	67.1	10.0	49.1	24.0	108.5	38.9	208.8
	24	< 3.2	< 3.2	62.9	62.9	36.4	33.9	10.0	49.4	29.9	69.9	41.7
MIP1β	**3**	< 3.2	78.2	40.4	66.6	53.3	6.4	11.3	6.4	45.4	362.3	6365.1
	6	< 3.2	736.5	697.0	657.3	1267.3	< 3.2	469.0	197.9	1170.5	168.7	2153.8
	24	< 3.2	61.8	439.4	35.9	705.5	< 3.2	35.6	26.4	270.7	70.7	35.5
RANTES	**3**	4.4	6.4	1.3	7.8	5.3	2.9	2.2	1.7	3.4	10.6	91.6
	6	< 3.2	14.9	3.4	13.2	34.1	2.7	19.9	11.9	57.3	18.4	736.9
	24	4.3	25.5	186.5	22.3	239.1	3.5	18.2	11.9	90.6	15.6	113.2
TNFa	**3**	< 3.2	3.2	< 3.2	< 3.2	< 3.2	1.6	1.4	1.3	2.5	3.6	57.0
	6	< 3.2	12.9	7.2	10.2	4.6	0.8	3.8	2.1	6.2	1.9	17.3
	24	< 3.2	< 3.2	3.2	< 3.2	8.2	1.3	1.4	1.3	3.1	2.0	1.3

Table 5: Cytokine production in peripheral blood (plasma) in engineered Renca tumor-bearing mice upon treatment with various ADCs comprising a non-binding or EphA2-targeted mAb with either a mIgG2a wild type (WT) or a mIgG2a LALAPG backbone conjugated to compounds **12** or **15**.

	Time (h)	control	3mg/kg non-targeted mAb **12** (LALAPG)	3mg/kg non-targeted mAb **12** (WT)	3mg/kg targeted mAb **12** (LALAPG)	3mg/kg targeted mAb **12** (WT)	3mg/kg non-targeted mAb **15** (WT)	3mg/kg targeted mAb **15** (WT)
IFNγ	**3**	1.1	1.2	2.2	0.7	1.2	1.3	1.8
	6	0.8	8.9	5.2	12.1	19.3	8.5	17.5
	24	1.3	7.1	6.1	18.8	27.0	6.6	21.1
	48	2.0	1.5	2.0	2.1	2.0	1.8	2.0
IL1β	**3**	8.8	12.7	26.0	19.8	7.8	15.9	30.3
	6	8.8	35.5	14.7	< 3.2	27.0	27.3	19.0
	24	10.7	12.2	22.7	4.9	10.7	10.8	7.8
	48	< 3.2	4.9	22.7	7.8	2.1	13.7	10.7
IL-6	**3**	5.1	12.7	12.6	71.6	61.6	12.0	105.8
	6	5.5	55.3	47.3	327.4	279.5	82.4	475.4
	24	3.5	33.4	25.2	61.1	80.8	41.3	63.0
	48	2.3	5.0	5.8	29.9	15.6	11.5	17.9
IL-10	**3**	2.6	4.0	4.4	4.1	2.2	5.9	5.1
	6	2.5	8.2	4.8	5.2	9.6	8.4	6.3
	24	1.6	3.2	2.7	4.9	5.5	4.9	7.3
	48	0.6	0.6	2.7	1.7	1.0	2.6	2.1
IL12p70	**3**	12.1	5.0	31.9	26.6	< 3.2	23.3	12.5
	6	0.9	48.9	13.7	< 3.2	23.5	29.0	10.3
	24	0.9	10.5	7.1	4.0	0.9	10.5	4.0
	48	< 3.2	0.9	20.1	< 3.2	0.9	7.1	0.9
IP10	**3**	99.4	134.0	104.9	335.5	371.9	185.4	637.3
	6	71.0	2469.9	2651.6	2757.9	3219.0	2885.3	3422.1
	24	86.3	1798.9	1652.7	2219.9	2004.1	2616.0	2324.1
	48	127.6	738.5	822.9	824.4	819.2	1052.2	935.8
MCP-1	**3**	63.2	120.4	165.9	135.5	96.9	142.7	276.1
	6	72.1	1368.6	1195.0	915.2	4492.9	1764.0	6594.8
	24	57.0	2083.8	1878.0	6406.5	8018.1	9546.4	15026.1
	48	42.1	285.6	599.9	794.1	934.2	1629.3	2234.6

MIP1α	**3**	137.3	142.9	205.0	166.4	101.3	187.3	163.7
	6	101.3	178.8	130.0	61.4	284.3	133.8	271.9
	24	133.8	120.7	153.3	101.3	227.0	193.7	205.2
	48	< 3.2	101.3	140.2	101.3	101.3	140.2	166.4
MIP1β	**3**	< 3.2	< 3.2	78.1	< 3.2	349.4	49.8	942.0
	6	< 3.2	578.4	850.5	735.3	2898.1	677.8	4342.5
	24	< 3.2	425.4	456.2	661.8	1493.2	1561.0	1387.7
	48	< 3.2	178.9	227.9	221.2	309.4	567.1	378.3
RANTES	**3**	5.6	8.0	11.0	9.1	6.7	10.1	11.0
	6	6.6	24.5	18.9	14.4	168.0	37.5	206.6
	24	5.6	77.1	52.5	338.9	174.8	335.7	772.2
	48	6.6	21.3	43.9	69.6	59.2	140.4	190.4
TNFα	**3**	4.6	6.2	6.8	7.7	4.3	11.0	9.8
	6	2.7	15.0	5.8	3.5	16.5	9.9	22.9
	24	5.4	7.7	7.7	6.6	8.8	13.3	11.0
	48	1.9	5.4	6.5	5.4	5.4	6.6	7.6

Table 6: Cytokine production in peripheral blood (plasma) in Renca tumor-bearing mice upon treatment with ADCs comprising an EphA2-targeted mAb with mIgG2a LALAPG backbone conjugated to compound **12** or compound **12a**.

	Dose #	Time (h)	Untreated	Vehicle	0.2mg/kg Compound 12a	0.6mg/kg Compound 12a	1.8mg/kg Compound 12a	0.3mg/kg targeted mAb 12 (LALAPG)	1.0mg/kg targeted mAb 12 (LALAPG)	3.0mg/kg targeted mAb 12 (LALAPG)
IFNg	**1**	**3**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2
	1	**6**	< 3.2	< 3.2	4.6	18.3	36.4	< 3.2	< 3.2	9.4
	1	**24**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	5.8	5.3
	1	**48**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2
	2	**6**	< 3.2	< 3.2	16.5	41.5	80.4	0.0	1.7	20.1
IL-1b	**1**	**3**	0.7	0.7	0.7	0.7	7.5	3.2	0.7	0.7
	1	**6**	0.7	< 3.2	0.7	5.7	0.7	3.2	11.0	5.7
	1	**24**	7.0	3.2	3.2	1.6	3.2	2.4	0.7	3.2
	1	**48**	3.2	5.7	0.7	5.7	3.2	3.2	3.2	5.8
	2	**6**	< 3.2	< 3.2	2.7	5.8	4.6	2.7	2.7	< 3.2
IL-6	**1**	**3**	3.3	16.6	780.0	1899.7	4314.6	5.8	13.9	17.8
	1	**6**	0.4	4.9	266.1	424.0	543.0	25.0	28.3	152.1
	1	**24**	2.8	1.5	4.1	6.1	6.8	8.6	22.5	74.1
	1	**48**	0.9	0.9	5.6	1.8	1.5	1.9	1.8	26.3
	2	**6**	2.2	5.1	410.9	516.9	1681.6	13.6	53.2	130.1
IL-10	**1**	**3**	< 3.2	< 3.2	15.6	30.4	27.3	< 3.2	< 3.2	< 3.2
	1	**6**	< 3.2	< 3.2	< 3.2	3.8	9.9	< 3.2	< 3.2	3.8
	1	**24**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	3.8
	1	**48**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2
	2	**6**	< 3.2	< 3.2	1.2	< 3.2	7.7	< 3.2	1.2	< 3.2
IL12p70	**1**	**3**	< 3.2	9.4	< 3.2	< 3.2	3.1	< 3.2	< 3.2	9.4
	1	**6**	< 3.2	< 3.2	< 3.2	3.1	< 3.2	< 3.2	< 3.2	< 3.2
	1	**24**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	3.1
	1	**48**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2
	2	**6**	< 3.2	< 3.2	< 3.2	28.4	28.4	< 3.2	< 3.2	12.1
IP10	**1**	**3**	88.9	124.3	1989.6	4826.2	8222.7	122.1	200.3	278.0
	1	**6**	95.4	116.3	8985.4	8828.3	7589.2	4294.0	5279.6	6945.6
	1	**24**	102.2	78.1	257.9	381.8	595.6	1123.5	1703.4	4251.8
	1	**48**	80.8	85.1	172.8	218.4	223.5	317.8	566.2	1631.2
	2	**6**	139.5	191.4	> 10000	3996.6	4151.5	1451.7	2590.8	2963.6

MCP-1	**1**	**3**	17.3	65.0	333.3	1547.8	5805.3	21.8	48.1	50.2
	1	**6**	11.9	62.8	7790.8	12898.6	13861.5	518.9	311.8	1080.9
	1	**24**	24.4	2.0	53.0	141.5	143.9	283.5	694.9	5104.5
	1	**48**	29.3	19.4	56.8	40.0	2.0	118.0	108.0	530.5
	2	**6**	51.7	87.7	5244.6	9761.2	17185.9	175.5	338.2	592.4
MIP1a	**1**	**3**	< 3.2	< 3.2	10.3	251.5	1052.6	< 3.2	< 3.2	10.3
	1	**6**	< 3.2	10.3	101.9	137.2	172.2	10.3	10.3	100.5
	1	**24**	10.3	< 3.2	< 3.2	< 3.2	10.3	< 3.2	< 3.2	92.5
	1	**48**	< 3.2	< 3.2	10.3	10.3	< 3.2	10.3	< 3.2	< 3.2
	2	**6**	32.6	32.6	104.9	115.5	209.9	52.2	32.6	99.2
MIP1b	**1**	**3**	< 3.2	< 3.2	727.2	2471.0	7438.6	< 3.2	< 3.2	< 3.2
	1	**6**	< 3.2	< 3.2	670.7	1050.0	1162.7	155.7	256.7	695.2
	1	**24**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	497.5
	1	**48**	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	< 3.2	157.8
	2	**6**	< 3.2	< 3.2	718.6	837.6	1407.7	< 3.2	216.1	449.7
RANTES	**1**	**3**	< 3.2	< 3.2	< 3.2	20.1	51.2	< 3.2	5.0	< 3.2
	1	**6**	20.3	< 3.2	130.3	255.0	526.2	14.7	17.1	23.2
	1	**24**	< 3.2	< 3.2	3.2	46.2	48.8	11.3	34.5	131.7
	1	**48**	9.2	< 3.2	< 3.2	5.0	< 3.2	7.9	17.1	79.4
	2	**6**	8.2	2.9	133.8	621.6	892.1	12.5	15.6	45.8
TNFa	**1**	**3**	< 3.2	4.3	9.1	21.0	46.4	3.2	3.2	4.5
	1	**6**	< 3.2	3.2	8.1	15.6	21.2	3.2	10.6	7.3
	1	**24**	7.3	3.2	3.2	5.2	3.2	5.2	5.9	15.2
	1	**48**	< 3.2	5.4	4.3	< 3.2	3.2	5.2	7.3	8.9
	2	**6**	< 3.2	< 3.2	10.0	16.7	46.4	3.2	3.2	3.2

[0632] The anti-tumor activity of the cleavable linker **11** conjugated to a non-binding mAb, PD-L1-targeted mAb (tumor and/or immune cell-targeted), or antigen C-targeted mAb (immune cell-targeted) was also evaluated in Renca tumor-bearing mice. All conjugates demonstrated tumor growth delay compared to untreated tumors. The PD-L1-targeted mAb conjugate of **11** demonstrated enhanced anti-tumor activity compared to an unconjugated PD-L1-targeted mAb. This demonstrates the anti-tumor benefit of delivering STING agonists using an ADC targeting antigens C and PD-L1 (**Figure 13**). The anti-tumor activity of the non-cleavable linker **12** conjugated to a PD-L1-targeted mAb was also evaluated in Renca tumor-bearing mice; these conjugates were efficacious at reducing tumor volume, though less well tolerated than PD-L1 targeted mAb conjugates of **11**.

CT26 cancer cells

[0633] The anti-tumor activity of compound **1** compared to the cleavable linker **11** conjugated to a non-binding mAb, antigen C-targeted mAb, PD-L1-targeted mAb, or EphA2-targeted mAb was evaluated in CT26 tumor-bearing mice. When animals were treated with compound **1** or the unconjugated PD-L1-targeted mAb, minimal tumor growth delay was observed. Modest tumor growth delay was observed with the non-binding mAb conjugate of **11**. In contrast, significant tumor growth delay was observed following treatment with all three targeted mAb conjugates of **11**. This demonstrates the anti-tumor benefit of delivering STING agonists using an ADC targeting a variety of antigens, including an immune cell-targeted conjugate (antigen C), immune and/or tumor-targeted conjugate (PD-L1), and tumor-targeted conjugate (EphA2) (**Figure 14**). Results of cytokine production in peripheral blood plasma is presented in Table 7.

Table 7: Cytokine production in peripheral blood (plasma) in CT26 tumor-bearing mice upon treatment with various ADCs comprising a mAb conjugated to compound **11**.

	Time (h)	Untreated			1.86 mg/kg Compound 1			12 mg/kg EphA2-targeted mAb 11 (mIgG2a LALAPG)			2.4 mg/kg EphA2-targeted mAb 11 (mIgG2a LALAPG)			2.4 mg/kg Antigen C-targeted mAb 11 (mIgG2a LALAPG)			2.4 mg/kg Non-binding mAb 11 (mIgG2a LALAPG)			2.4 mg/kg PD-L1-targeted mAb 11			2.4 mg/kg PD-L1-targeted mAb		
IL-6	3	0.5	3.2	3.2	622.1	781.4	1128.3	185.8	0.5	217.7	17.8	27.7	23.4	483.9	182.4	442.5	1.4	100.6	56.5	18.1	56.9	3.3	25.6	0.6	5.8
	6	2.7	3.2	0.2	2652.5	2393.5	2412.7	2677.9	1790.2	2777.7	168.7	303.4	304.8	1821.4	2769.8	2324.8	123.7	151.3	266.9	119.4	72.6	162.5	3.2	3.2	1
	24	5.7	5.4	0.6	44.5	31.8	36.5	72.8	0.2	67.6	16.2	143.2	16.1	9.4	34.4	126.9	5	16.9	3.2	23.8	21.8	5.2	21.2	6.5	216.8
	48	26.5	262.8	7.6	47.8	142.7	29.2	214.1	61.7	80.4	6.5	39.1	7.8	48.9	32.2	19.5	2.9	2.9	2.3	26.6	26.8	89.7	1.4	3.2	12.2
IL-10	3	3.2	3.2	3.2	0.2	2.3	1.2	3.2	3.2	1.2	3.2	3.2	3.2	0.9	3.2	0.4	3.2	3.2	3.2	3.2	3.2	3.2	33.8	3.2	3.2
	6	3.2	3.2	3.2	7.8	9	4.4	7.2	2	4.3	4	1.2	1.6	1.2	3.7	4.3	0.4	0.4	2	0.4	0	0	3.2	3.2	3.2
	24	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	1.5	3.2	3.2	3.2	0.4	3.2	3.2	3.1	3.2	3.2	3.2	3.2	3.2	26.2	3.2	3.2
	48	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	0.4	3.2	3.2	3.2
IP-10	3	242.4	109.7	200.2	1439.4	1501.7	1160.7	592.6	125.9	377.2	198.1	190.9	196.9	1271.3	629.1	627.8	109.4	450	308.7	163.9	154.4	178.3	105.7	190.4	96.1
	6	174.7	117.4	188.2	2610.8	2133.6	2473.4	2039.3	2711.3	2008.6	2327.7	2740.5	1949.2	2062.9	2328	2713.5	1779.6	1376.6	2092	1814.4	1744	1389.4	130.6	151.9	110.7
	24	272.5	134.4	201.1	1121.6	1025.3	696.2	1468.5	145	1900.5	1189.9	1094.8	1089	2565	1994.5	1329.8	246.4	693.7	3.2	717.5	1002.6	833.1	247.7	689.9	166.7
	48	122.9	155.8	74.7	560.1	398.8	560	1293.4	541.3	716.9	307.2	167.2	250.2	448.8	273.5	378	107.3	235.9	319.2	748.1	599.3	580.4	128.1	183.3	135.9
MCP-1	3	2.7	3.2	3.2	73.7	78.6	110.3	36.4	2.7	23.1	3.2	10.5	2.7	393.2	69.9	78.6	3.2	46.2	2.7	18.9	13.8	3.2	169.8	3.2	3.2
	6	7.2	7.2	3.2	3185.3	2816.3	1634.8	6186.6	3754.5	3698.6	656.5	578.3	382.2	10207.3	4960.7	37687.1	162	556.2	425	143.3	133	252	3.1	7.5	7.5
	24	3.2	3.2	3.2	397.7	294.7	305.9	765.3	3.2	821	99.1	99.1	99.1	1009.2	637.8	194	3.2	55.1	3.2	129.2	191.4	98	129.2	7.5	3.2
	48	13.5	18.6	3.2	93.1	20.9	41.4	207.4	85.1	108.7	27.2	10.5	13.5	39.8	34.7	365.9	3.2	2.7	20.9	135.9	127.3	253.1	7.5	13.8	16.4
MIP-1a	3	3.2	3.2	3.2	3.9	3.2	3.2	3.9	3.2	3.2	3.2	3.2	3.2	217.7	103.1	139.8	3.2	3.2	3.2	3.2	3	3.2	3	3	3
	6	3.9	3.2	3.2	94.6	93.6	73.9	222.1	127.3	261.8	12.7	3.9	3.2	144	152.7	82.1	17.7	12.7	3.9	11.2	3	3	3	3	11.2
	24	3.2	3.2	3.2	12.7	25.1	3.2	21.7	3.2	21.7	3.2	3.2	3.2	17.7	21.7	12.7	3.2	3.2	3.2	3	3	3	3	11.2	24
	48	3.2	3.2	3.2	3.9	12.7	3.2	3.9	3.2	3.9	3.2	3.2	3.2	3.2	3.2	17.7	3.2	3.2	3.2	11.2	11.2	3	3	7.9	3
MIP1b	3	3.2	1.6	3.2	297.7	336.8	224	311.6	3.2	185.9	26.9	19.7	5.4	1600.5	682.1	991.5	3.2	31.2	7.2	3.2	5.4	9.9	3.2	3.2	3.2
	6	3.2	3.2	3.2	925.8	369.7	1161.4	1679.2	1460.7	1237.9	367.2	264.6	356.3	1377.1	1236.6	1740.4	137.2	215.4	203.6	70	61.7	85.7	3.2	3.2	3.2
	24	3.2	3.2	3.2	127.6	125.3	55.3	268.8	3.2	356.8	84.9	63.2	51.3	197.4	142.2	127.5	3.2	26.9	3.2	3.2	19.3	32	3.2	3.2	46.1
	48	3.2	14.8	3.2	61.1	10.8	57.6	66.7	26.9	56.9	11.4	3.2	8.8	13.8	13.2	69	3.2	3.2	3.2	26.5	23.5	30	3.2	3.2	3.2
TNFa	3	3.2	3.2	3.2	3.2	4.9	4.3	1.4	3.2	3.2	3.2	3.2	3.2	31	25.6	27.6	3.2	3.2	3.2	5.8	3.2	3.2	75.5	3.2	3.2
	6	3.2	3.2	3.2	16.9	16.6	10	29.1	26.2	34.8	6.2	3.8	2.6	31	45.5	39.5	3.2	4.3	3.2	3.2	3.2	3.2	3.2	3.2	3.2
	24	3.2	3.2	3.2	0.4	0.7	3.2	4.3	3.2	3.2	3.2	2.9	8.5	2	7	3.8	3.2	1.4	3.2	6.4	3.2	3.2	58.6	3.2	3.2
	48	3.2	3.2	3.2	1.1	3.2	3.2	1.4	3.2	3.2	3.2	3.2	3.2	3.2	3.2	1.7	3.2	3.2	3.2	3.6	3.2	3.2	3.2	3.2	3.2

MC38 cancer cells

[0634] The anti-tumor activity of the cleavable linker **12** conjugated to a non-binding mAb or EphA2-targeted mAb with a LALAPG mIgG2a Fc backbone was evaluated in MC38-tumor bearing wild type (WT) or STING-deficient (*Tmem173*^{gt}) mice. Animals treated with 3 weekly doses of 1 mg/kg non-binding conjugates of **12** or 0.1 mg/kg targeted conjugates of **12** demonstrated modest and minimal tumor growth delay, respectively, in WT but not STING-deficient tumor bearing mice. Animals treated with 3 weekly doses of 1 mg/kg targeted conjugates of **12** demonstrated robust tumor growth delay in WT but not STING-deficient tumor bearing mice. This demonstrates that in MC38 tumor-bearing mice STING signaling is required in non-tumor cells in the tumor microenvironment for anti-tumor activity (**Figures 15A** and **15C**).

[0635] Animals treated with a single dose of 1 mg/kg EphA2-targeted conjugates of **12** also demonstrated robust tumor growth delay in WT tumor bearing mice, demonstrating that a single dose of EphA2-targeted conjugates of **12** is sufficient to drive complete tumor regression (**Figure 15A**).

[0636] Mice that achieved complete tumor regression in response to a single dose or 3 weekly doses of ADC were rechallenged with MC38 tumor cells on the opposite flank and tumor growth was monitored. All rechallenged mice – but not all naïve untreated mice challenged with MC38 tumor cells – were protected from rechallenge, suggesting that targeted conjugates of **12** elicit immune memory (**Figure 15D**).

4T1 cancer cells

[0637] The anti-tumor activity of the cleavable linker **12** conjugated to a non-binding or EphA2-targeted mAb with a LALAPG mIgG2a Fc backbone was evaluated in 4T1 tumor-bearing mice. All conjugates of compound **12** led to significant tumor growth delay at the doses tested, with the targeted mAb conjugate of compound **12** demonstrating enhanced tumor growth delay compared to the non-binding conjugate, with minimal weight loss observed (**Figure 16B**). This demonstrates that EphA2-targeted mAb conjugates of compound **12** are active in multiple tumor models (**Figures 12-16**).

Rat tolerability study:

[0638] The nonclinical safety profile of compound **12** conjugated to non-binding antibodies with a WT Fc backbone, non-binding antibodies with an Fc null backbone, targeted antibodies with a WT Fc backbone, and targeted antibodies with an Fc null backbone was evaluated in non-GLP rat toxicology studies. All conjugates with the compound **12** drug linker (both non-binding and targeted, WT and null Fc backbone) were tolerated in rat at doses higher than the minimally efficacious dose in mouse tumor models.

EXAMPLE 4
IN VIVO PHARMACOKINETIC STUDY

Methods

[0639] Pharmacokinetic profiles were analyzed following administration of two weekly 1 mg/kg doses of an ADC comprising a [deglycosylated] non-binding mAb conjugated to compound **12** to male C57BL/6 mice. Plasma was collected and analyzed for generic total antibody (gTAb) by immunoassay. TAb concentrations in mouse K_2EDTA plasma were determined by a Gyros flow-through immunoassay platform. Samples and standards were diluted in assay buffer and incubated with a solution containing biotinylated murine anti-human kappa light chain antibody and fluorescent goat anti-human IgG Fcg F(ab')$_2$ antibody fragment in a sandwich format. The resulting immunocomplexes were bound to the streptavidin-coated beads in the affinity column of the compact disc (CD). The CD was read by a laser that excites the fluorescent detection reagent, producing a signal that is directly proportional to the concentration of test article in the C57BL/6 male mouse plasma sample. Non-compartmental analysis was applied to pooled animal plasma concentration data (sparse sampling) using Phoenix WinNonlin 8.2 (Certara, USA). Concentration values below the limit of quantitation (BLQ) were treated as zero for analysis. Nominal doses and sampling times were used.

Results

[0640] Pharmacokinetic profiles were analyzed following administration of two weekly 1 mg/kg doses of an ADC comprising a [deglycosylated] non-binding mAb conjugated to compound **12** to male C57BL/6 mice. The maximum observed concentration (Cmax) after the first and second dose was 40500 and 52400 ng/mL, respectively. The area under the concentration-time

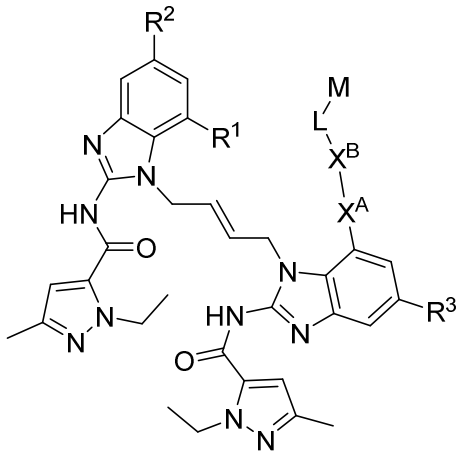
curve from time 0 through 7 days (AUC0-7d) was 85600 d*ng/mL. This suggests that the total antibody exposure for the non-binding conjugate of compound **12** was higher than the small molecule exposure of published small molecule STING agonists (**Figure 17**) (*See*, *e.g.*, Ramanjulu et al., 2018, Nature 564, 439-443).

[0641] The contents of each of the references cited in the present disclosure are hereby incorporated by reference in their entirety.

[0642] A number of embodiments of the present disclosure have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the present disclosure. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

each R^2 and R^3 are independently –CO_2H, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or C_{1-3} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript m is independently 0 or 1;

each subscript q is independently an integer from 0 to 6;

X^A is –CH_2–, –O–, –S–, –NH–, or –$N(CH_3)$–;

X^B is absent or a 2-16 membered heteroalkylene;

L is a linker having the formula –$(A)_a$-$(W)_w$-$(Y)_y$–, wherein:

subscript a is 0 or 1;

subscript y is 0 or 1;

subscript w is 0 or 1;

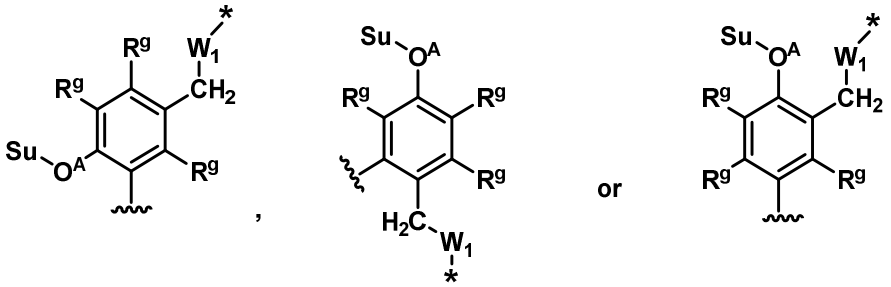
A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};

each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, -$NR^{d1}R^{e1}$, -$C(O)NR^{d1}R^{e1}$, -$C(O)(C_{1-6}$ alkyl), and -$C(O)O(C_{1-6}$ alkyl);

each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

W is from 1-12 amino acids or has the structure:

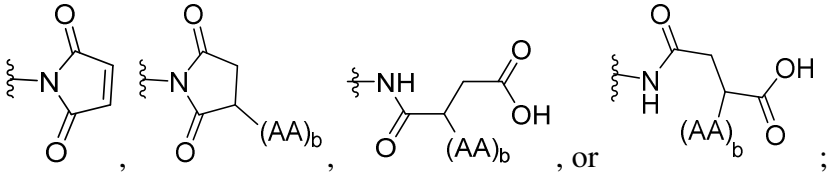


wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

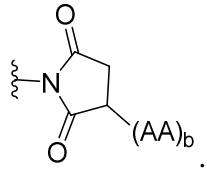
each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

W^1 is absent or –O-C(=O)–;

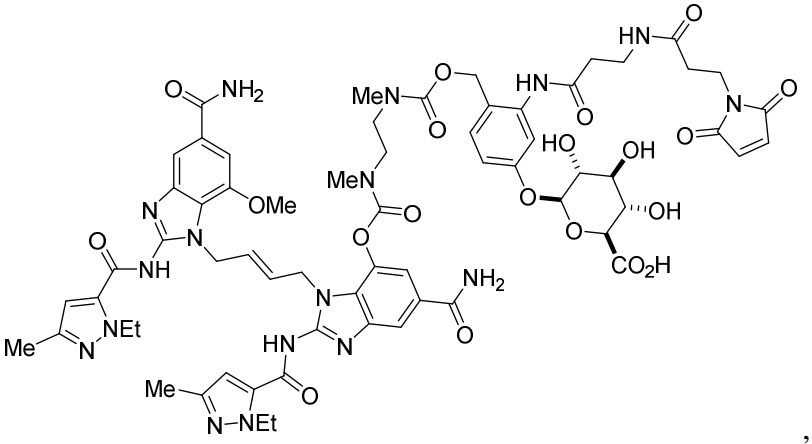
~~~ represents covalent attachment to A or M;

* represents covalent attachment to Y, X^A, or X^B; and

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

M is ;

each AA is an independently selected amino acid, wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom;

each subscript b is independently an integer from 1 to 6; and

X^B and L are each independently optionally substituted with a PEG Unit from PEG1 to PEG 72.

2. The compound of Claim 1, wherein X^A is –O–.

3. The compound of Claim 1 or 2, wherein each amino acid in W is independently selected from the group consisting of alanine, glycine, lysine, serine, aspartic acid, aspartate
~~~

methyl ester, N,N-dimethyl-lysine, phenylalanine, citrulline, valine-alanine, valine-citrulline, phenylalanine-lysine or homoserine methyl ether.

4. The compound of any one of Claims 1-3, wherein A is a 4 to 12 membered heteroalkylene.

5. The compound of any one of Claims 1-4, wherein M is .

6. The compound of Claim 1, selected from the group consisting of:



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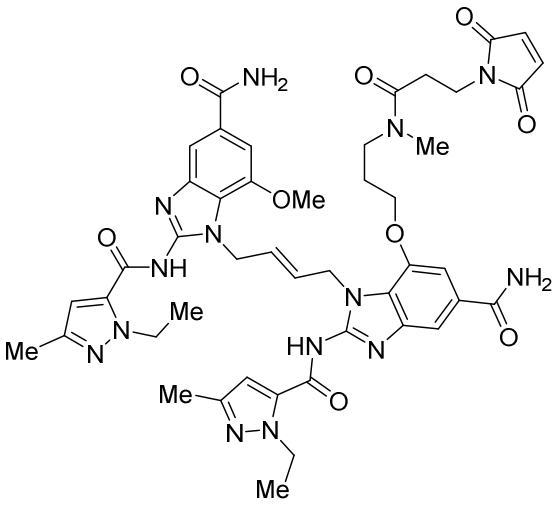

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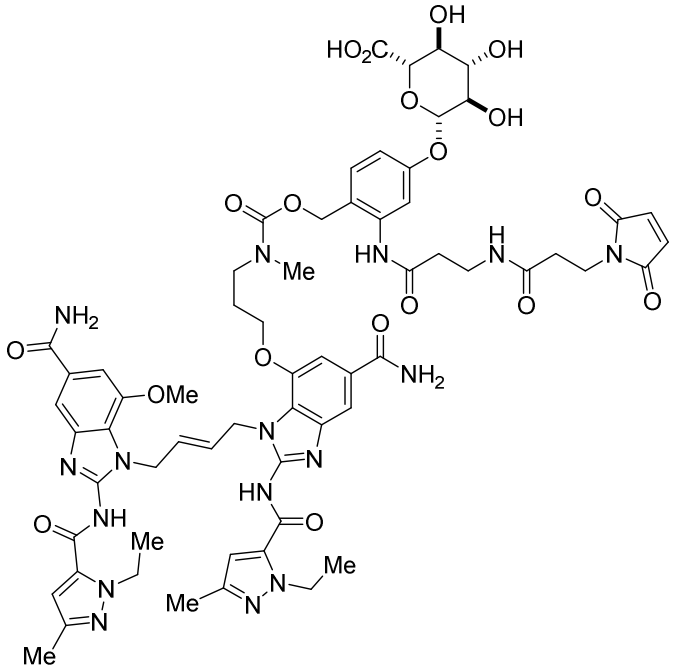

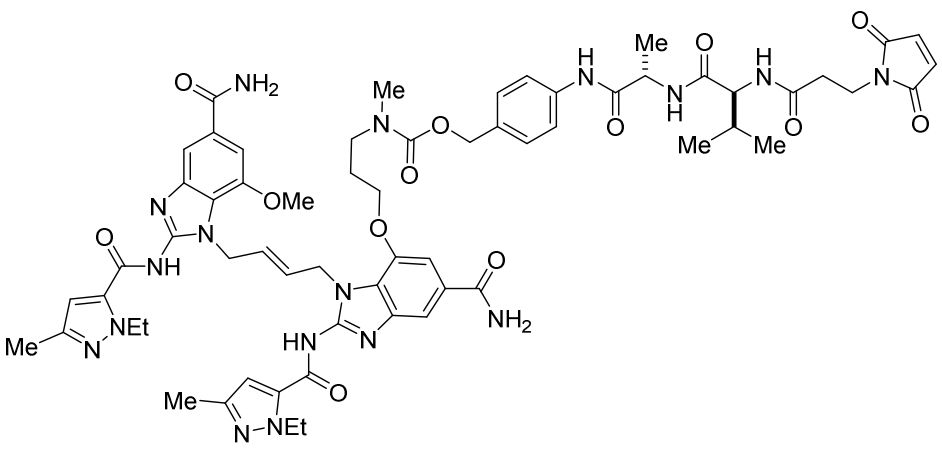

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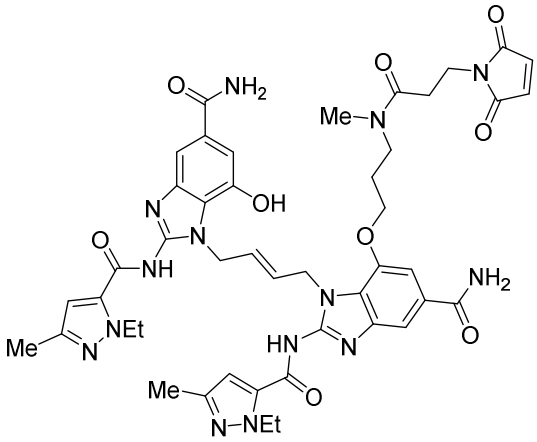

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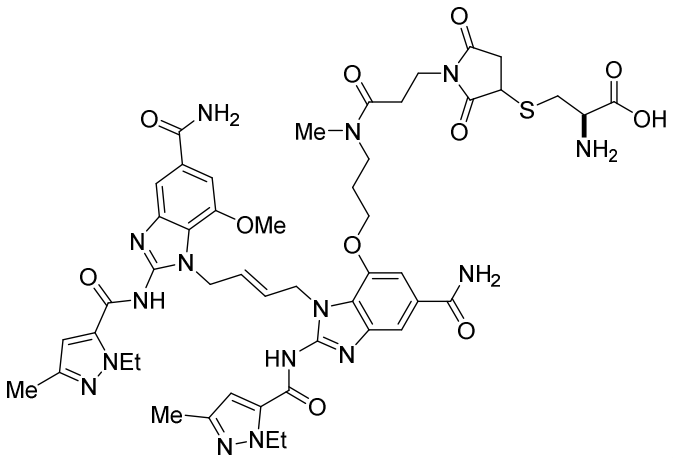

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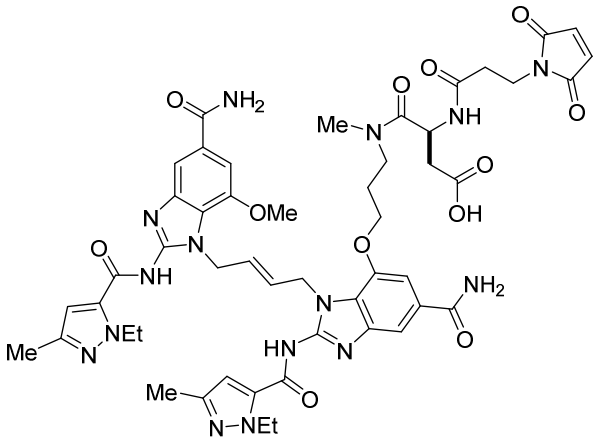

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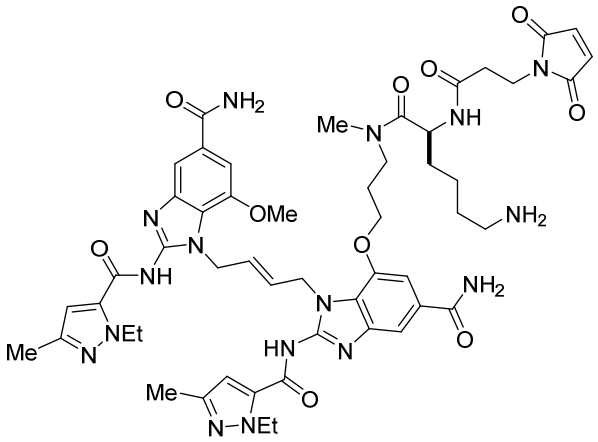

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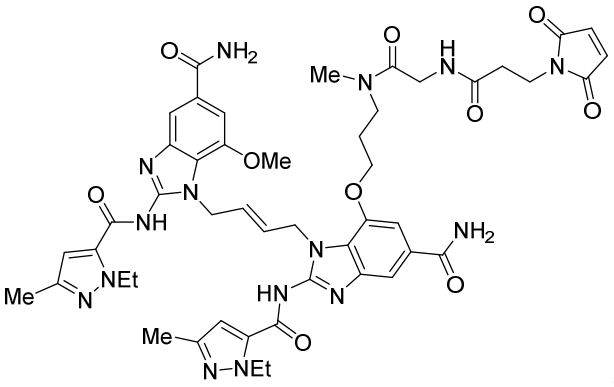
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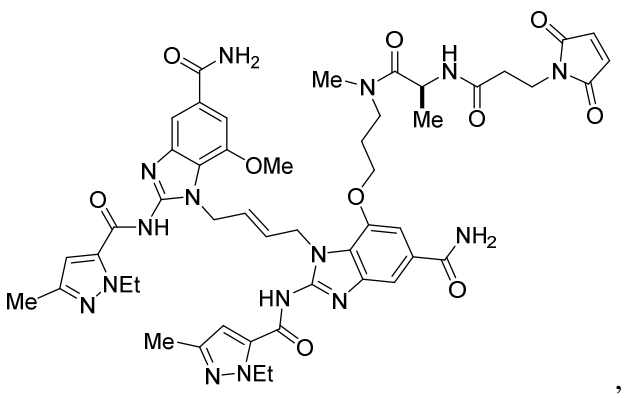
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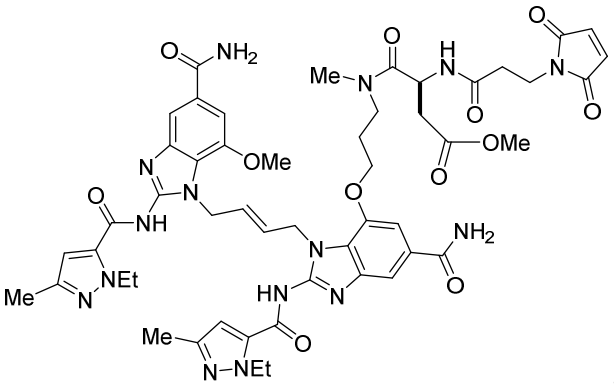
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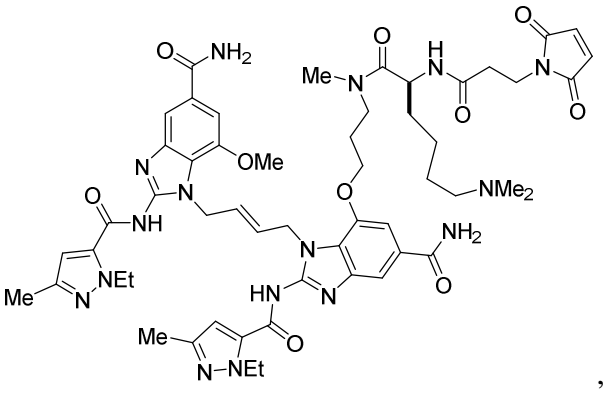
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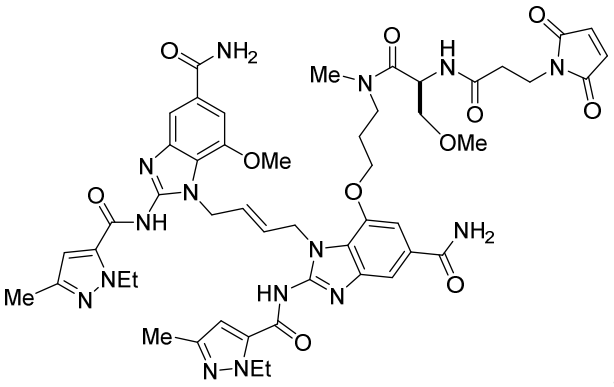
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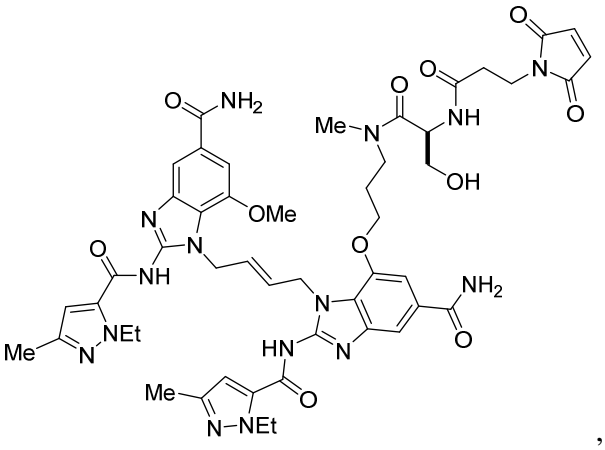
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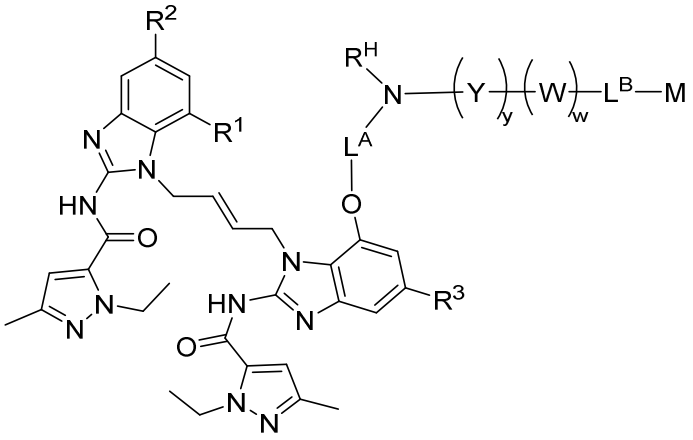
and pharmaceutically acceptable salts thereof.

7. The compound of Claim 1, having the structure of Formula (II-A):

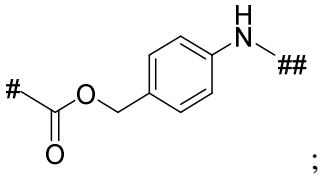


(II-A)

or a pharmaceutically acceptable salt thereof, wherein:

L^A is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–C(O)NR^H(CH_2)_{1-6}–$;

each R^H is independently hydrogen or C_{1-3} alkyl;

Y is  ;

represents covalent attachment to $–NR^HL^A$;

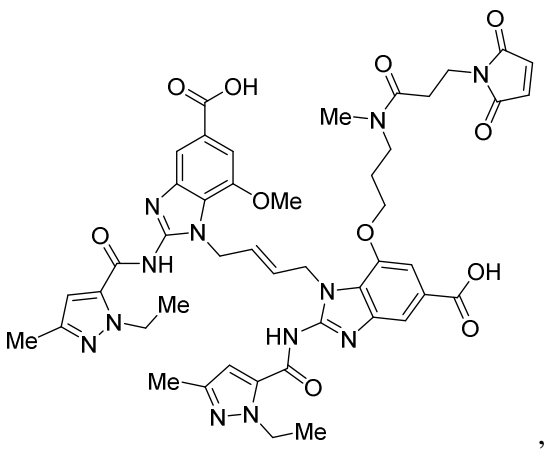
represents covalent attachment to W or L^B; and

L^B is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–[NHC(O)(CH_2)_{1-4}]_{1-3}–$.

8. The compound of Claim 7, wherein R^H is methyl.

9. The compound of Claim 7, wherein each amino acid of W is independently selected from the group consisting of alanine, valine, isoleucine, leucine, aspartic acid, glutamic acid, lysine, histidine, arginine, glycine, serine, threonine, phenylalanine, O-methylserine, O-methylaspartic acid, O-methylglutamic acid, N-methyllysine, O-methyltyrosine, O-methylhistidine, and O-methylthreonine.

10. The compound of Claim 7, selected from the group consisting of: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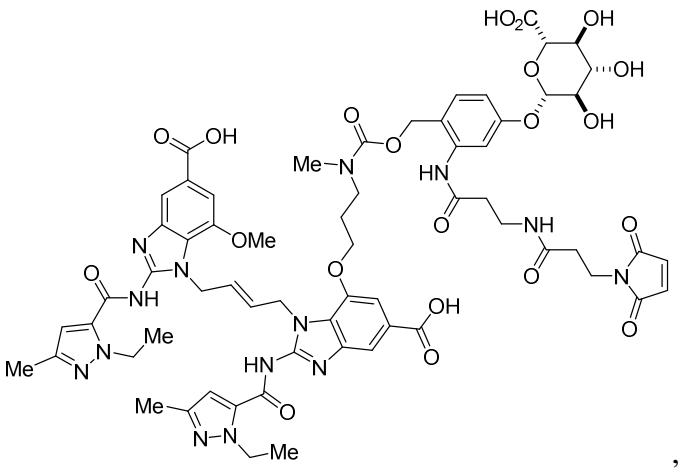
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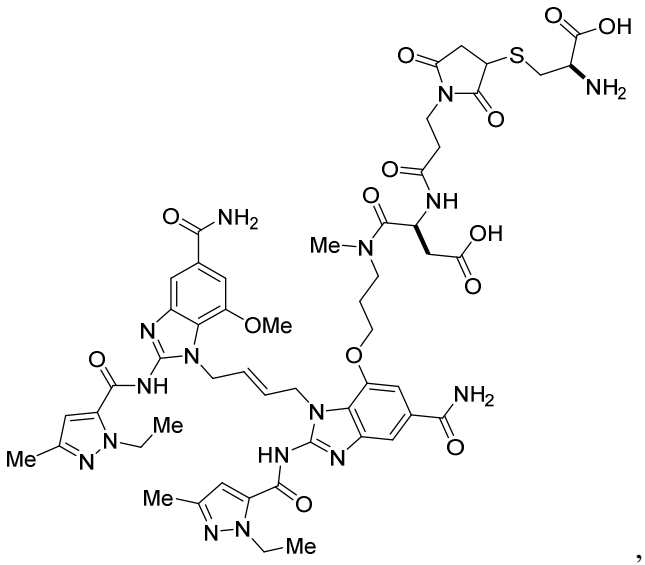
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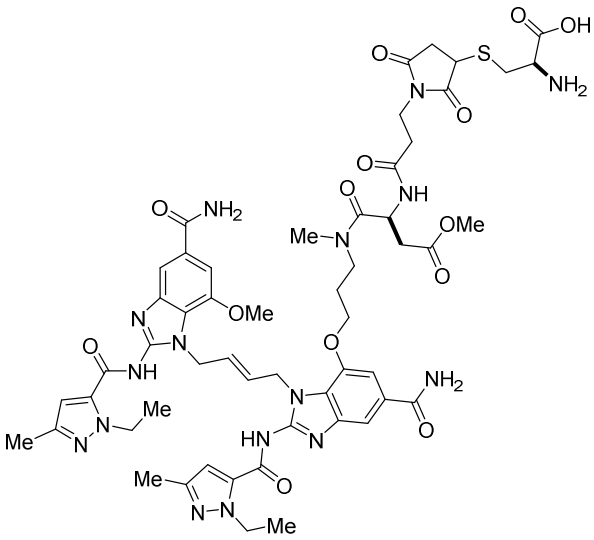
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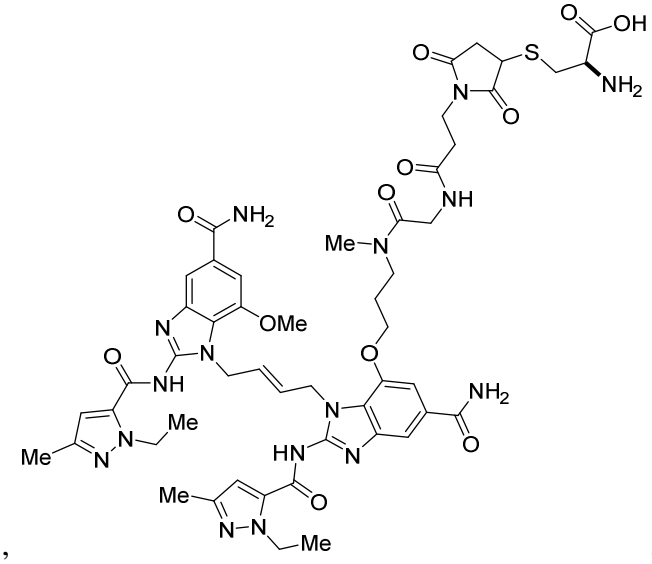
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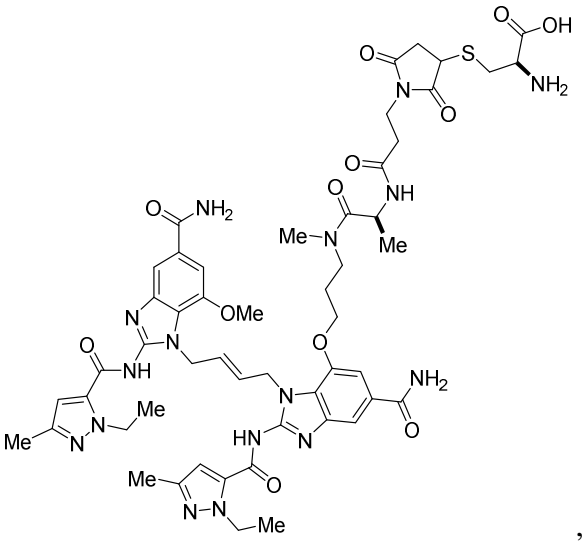
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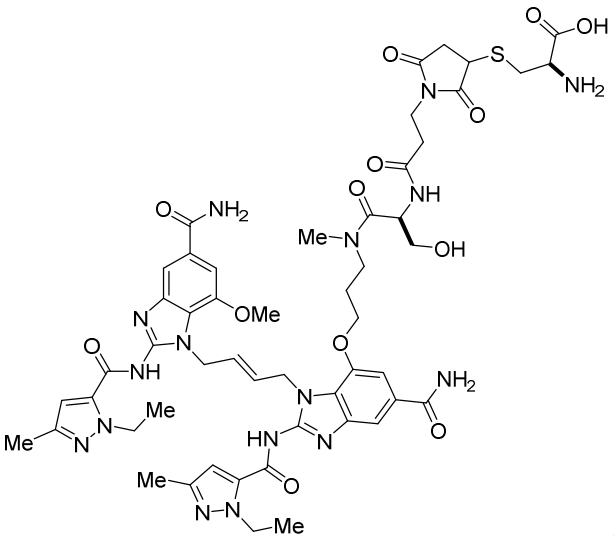
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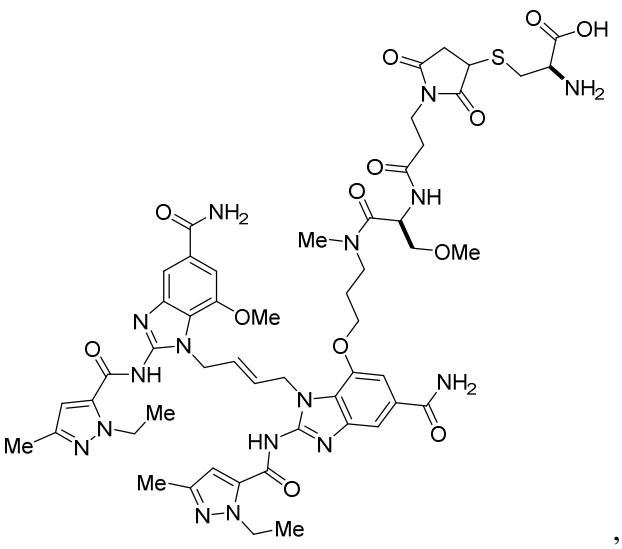
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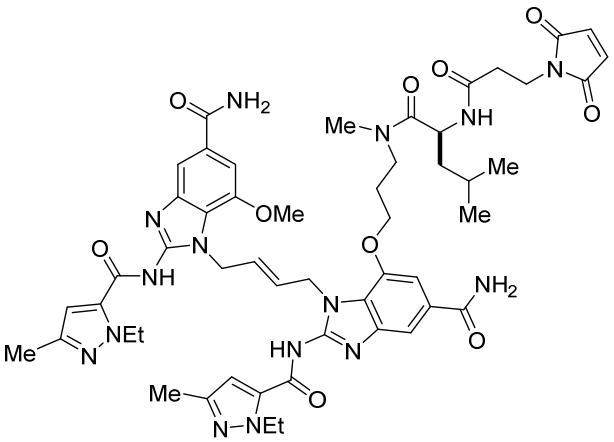
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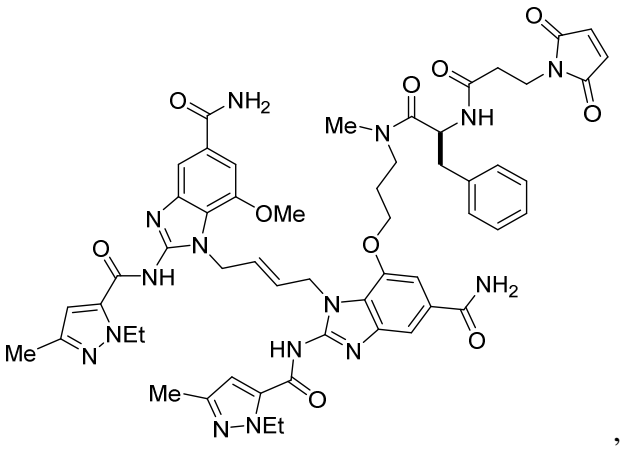
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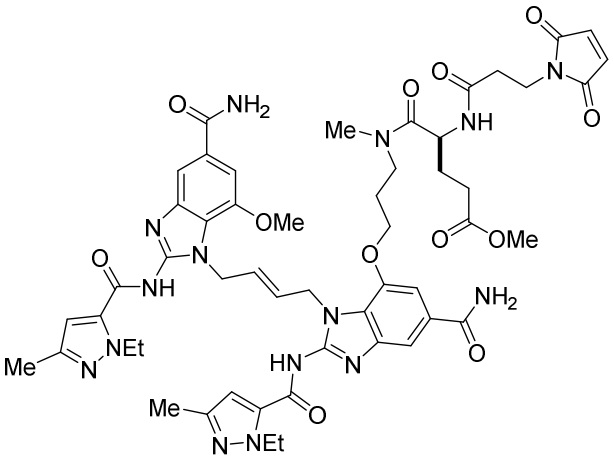
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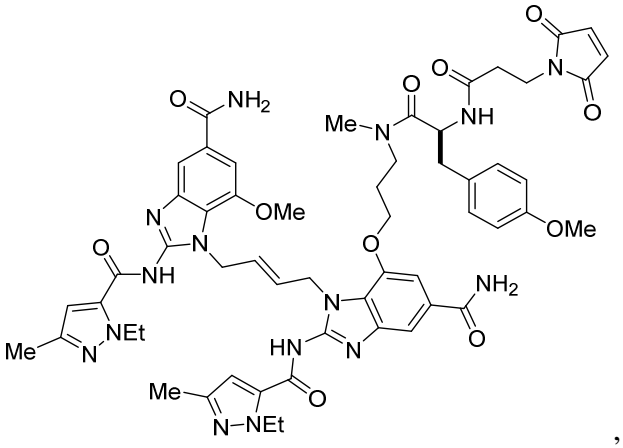
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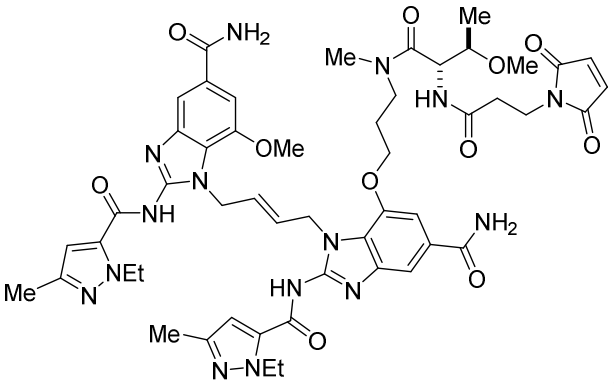
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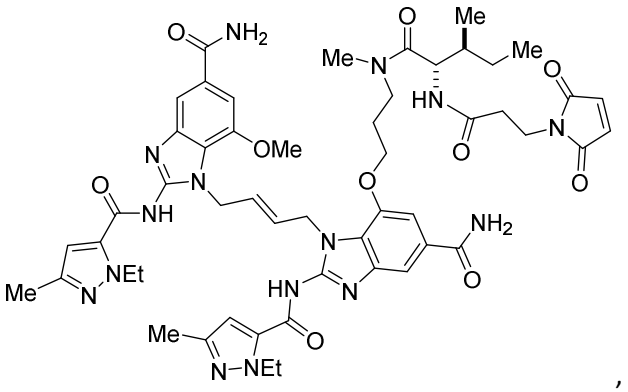
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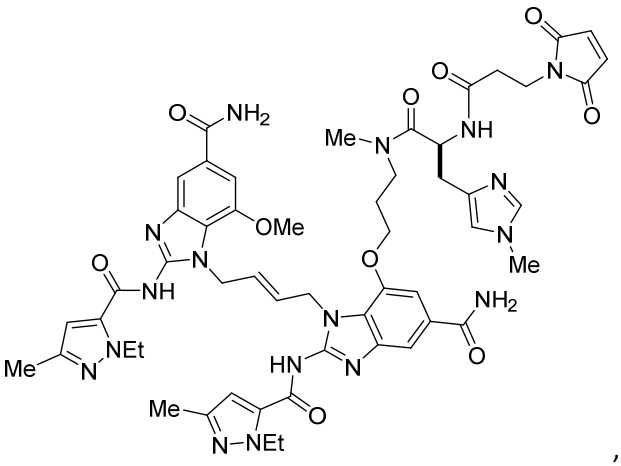
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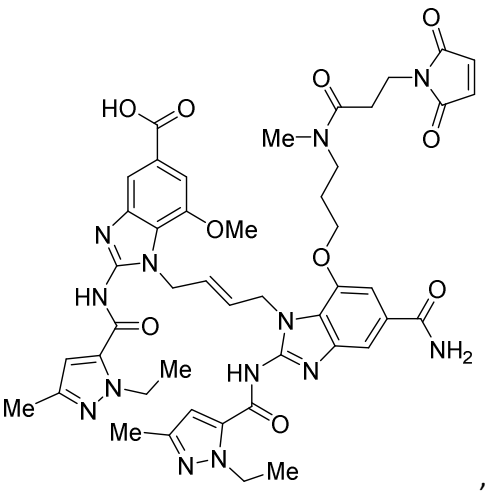
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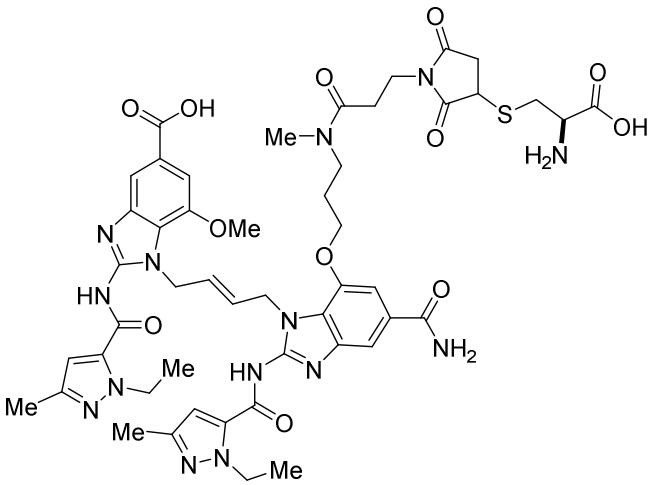
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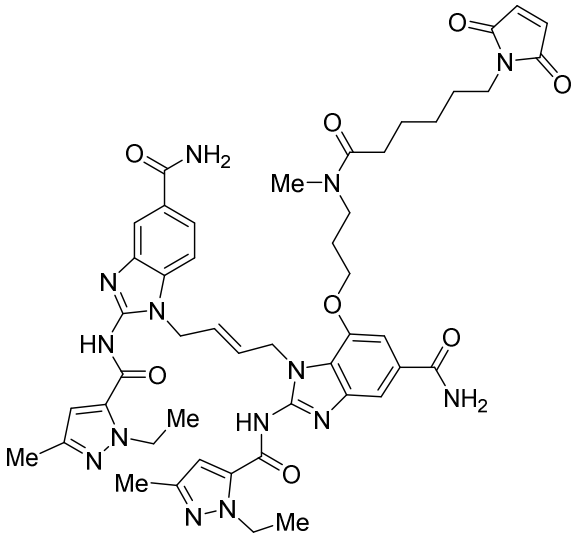
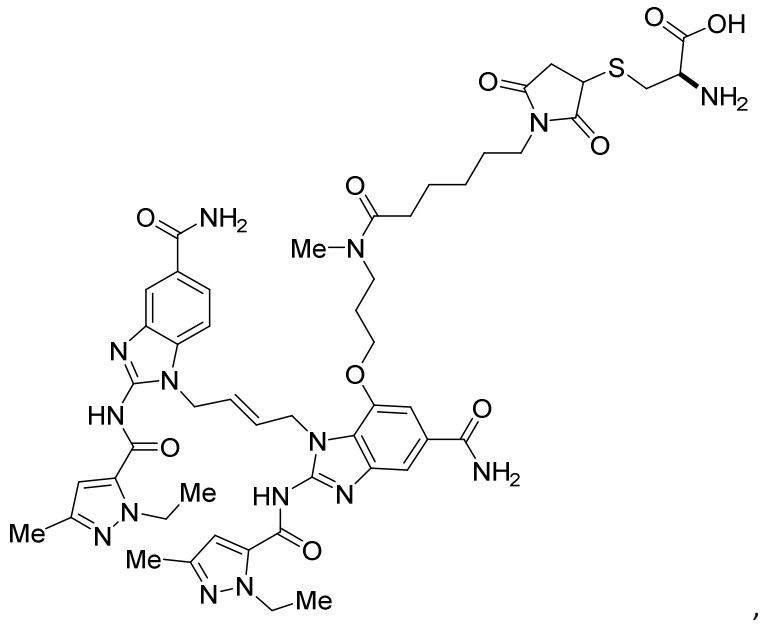




, , and pharmaceutically acceptable salts thereof.

11. An antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^{*}\text{-}M^{1}\text{-}(D))_{p}}$$

wherein:

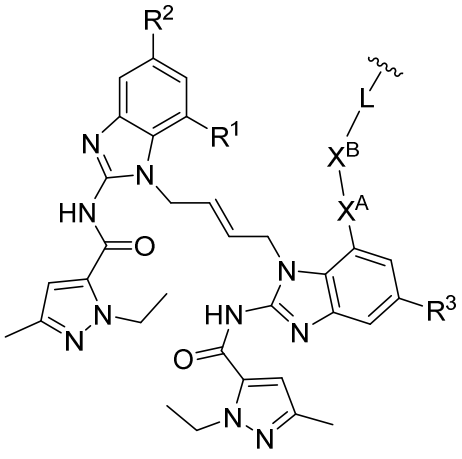
Ab is an antibody;

each S* is a sulfur atom from a cysteine residue of the antibody;

M^1 is a succinimide or a hydrolyzed succinimide;

subscript p is an integer from 2 to 8; and

each (D) is a Drug Unit of Formula (I):



(I)

wherein:

~~~ represents covalent attachment of L to M^1;
~~~

R^1 is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl)C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

R^2 and R^3 are independently $–CO_2H$, –$(C=O)_m$-NR^CR^D, or –$(CH_2)_q$-NR^ER^F;

each R^A, R^B, R^C, R^D, R^E, and R^F are independently hydrogen or C_{1-3} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript m is independently 0 or 1;

each subscript q is an integer from 0 to 6;

X^A is $–CH_2–$, –O–, –S–, –NH–, or $–N(CH_3)–$;

X^B is absent or a 2-16 membered heteroalkylene;

L has the formula –$(A)_a$-$(W)_w$-$(Y)_y$–, wherein:

subscript a is 0 or 1;

subscript y is 0 or 1;

subscript w is 0 or 1;

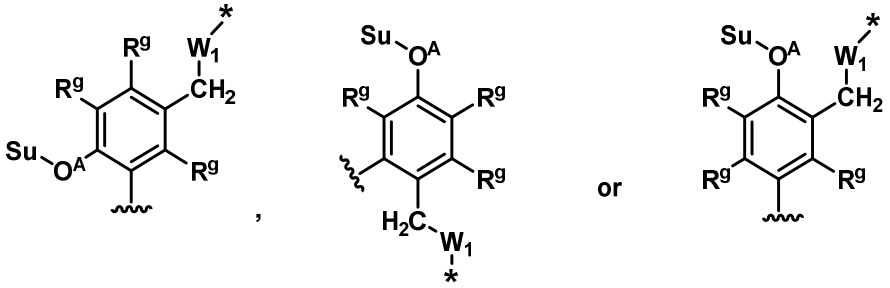
A is a C_{2-20} alkylene optionally substituted with 1-3 R^{a1}; or a 2 to 40 membered heteroalkylene optionally substituted with 1-3 R^{b1};

each R^{a1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, -C(O)(C_{1-6} alkyl), and -C(O)O(C_{1-6} alkyl);

each R^{b1} is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, $-NR^{d1}R^{e1}$, $-C(O)NR^{d1}R^{e1}$, -C(O)(C_{1-6} alkyl), and -C(O)O(C_{1-6} alkyl);

each R^{d1} and R^{e1} are independently hydrogen or C_{1-3} alkyl;

W is from 1-12 amino acids or has the structure:



wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or -NO_2;

W^1 is absent or –O-C(=O)–;

~~~ represents covalent attachment to A or M^1;

* represents covalent attachment to Y, X^B, or X^A;

Y is self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety; and

X^B and L are each independently optionally substituted with a PEG Unit from PEG1 to PEG 72.

12. The ADC of Claim 11, wherein X^A is –O–.

13. The ADC of Claim 11 or 12, wherein each amino acid in W is selected from the group consisting of alanine, glycine, lysine, serine, aspartic acid, aspartate methyl ester, N,N-dimethyl-lysine, phenylalanine, citrulline, valine-alanine, valine-citrulline, phenylalanine-lysine or homoserine methyl ether.

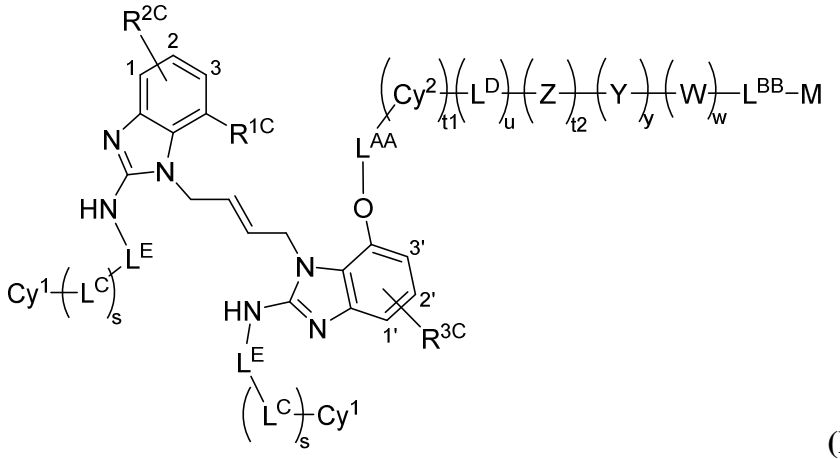
14. The ADC of any one of Claims 11-13, wherein A is C_{4-10} alkylene optionally substituted with 1-3 R^{a1}.

15. The ADC of any one of Claims 11-14, wherein the linker is a cleavable linker.

16. The ADC of any one of Claims 11-15, wherein the linker is cleavable by one or more of cathepsin B, C, or D; β-glucuronidase; and β-mannosidase.

17. The ADC of Claims 11-14, wherein the linker is a non-cleavable linker.

18. A compound having the structure of Formula (IV):



(IV)

or a pharmaceutically acceptable salt thereof, wherein:
~~~

R^{1C} is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, –$(CH_2)_n$-NR^AR^B, or PEG2 to PEG4;

R^{2C} is –CO_2R^M, –(C=O)NR^CR^D, –$S(O)_2NR^CR^D$, –$S(O)_2R^M$, –$(CH_2)_q$-NR^ER^F, –$(CH_2)_q$-OR^M, –O(C=O)-NR^ER^F, or –NR^M(C=O)-NR^ER^F, wherein R^{2C} is attached at any one of positions labeled 1, 2, or 3;

R^{3C} is –CO_2R^M, –(C=O)NR^CR^D, –$S(O)_2NR^CR^D$, –$S(O)_2R^M$, –$(CH_2)_q$-NR^ER^F, –$(CH_2)_q$-OR^M, –O(C=O)-NR^ER^F, or –NR^M(C=O)-NR^ER^F, wherein R^{3C} is attached at any one of positions labeled 1', 2', or 3';

each R^A, R^B, R^C, R^D, R^E, R^F, and R^M are independently hydrogen or C_{1-6} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript q is independently an integer from 0 to 6;

L^E is –(C=O)– or –$S(O)_2$–;

L^C is –$(CR^IR^J)_{1-3}$–

each R^I and R^J are independently hydrogen or C_{1-3} alkyl;

subscript s is 0 or 1;

each Cy^1 is independently a 4-6 membered heterocycle, a 5-6 membered heteroaryl, or a C_{3-6} cycloalkyl, each optionally substituted with one or more R^K;

each R^K is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, -$NR^{d2}R^{e2}$, -C(O)$NR^{d2}R^{e2}$, -C(O)(C_{1-6} alkyl), and -C(O)O(C_{1-6} alkyl);

each R^{d2} and R^{e2} are independently hydrogen or C_{1-3} alkyl;

L^{AA} is –$(CH_2)_{1-6}$–, –C(O)$(CH_2)_{1-6}$–, –C(O)$NR^L(CH_2)_{1-6}$–, –$(CH_2)_{1-6}$O–, –C(O)$(CH_2)_{1-6}$O–, or –C(O)$NR^L(CH_2)_{1-6}$O–;

R^L is hydrogen or C_{1-3} alkyl;

Cy^2 is C_{3-6} cycloalkyl, 4-6 membered heterocycle, 5-6 membered heteroaryl, or phenyl, each optionally substituted with one or more R^U;

each R^U is independently selected from the group consisting of –CO_2R^{j1}, –(C=O)$NR^{d3}R^{e3}$, –$S(O)_2NR^{d3}R^{e3}$, –$(CH_2)_{q1}$-$NR^{g1}R^{h1}$, –$(CH_2)_{q1}$-OR^{j1}, and –$(CH_2)_{q1}$-$(OCH_2CH_2)_{1-8}$OH;

each R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or C_{1-6} alkyl;

subscript q1 is an integer from 0 to 6;

subscripts t1 and t2 are independently 0 or 1, wherein at least one of t1 and t2 is 1;

L^D is $–(CH_2)_{1-6}–$;

subscript u is 0 or 1;

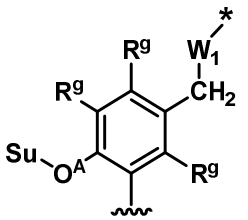
Z is $–N(R^{HH})–$ or $–N^+(C_{1-6}$ alkyl$)(R^{HH})–$;

R^{HH} is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl;

Y is a self-immolative moiety, a non-self-immolative releasable moiety, or a non-cleavable moiety;

subscript y is 0 or 1;

W is a chain of 1-12 amino acids or has the structure:



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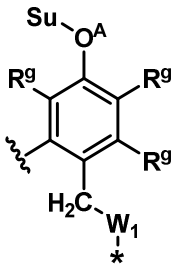

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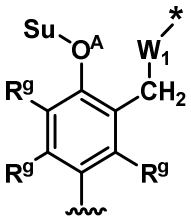


wherein Su is a Sugar moiety;

$-O^A-$ represents a glycosidic bond;

each R^g is independently hydrogen, halogen, -CN, or $-NO_2$;

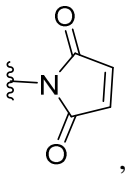
W^1 is absent or –O-C(=O)–;

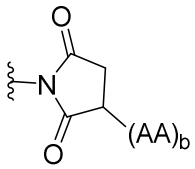
~~~ represents covalent attachment to L^{BB};

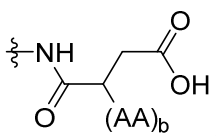
* represents covalent attachment to Y, L^D, NR^{HH}, or Cy^2;

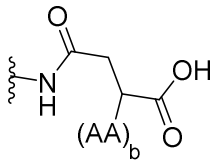
subscript w is 0 or 1;

L^{BB} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, or $–[NHC(O)(CH_2)_{1-4}]_{1-3}–$; and

M is 

, 

, 

, or 

;

each AA is an independently selected amino acid, wherein $(AA)_b$ is connected to the succinimide or hydrolyzed succinimide via a sulfur atom; and

each subscript b is independently an integer from 1 to 6.
~~~

19. The compound of Claim 18, wherein Cy^2 is a 4-6 membered heterocycle.

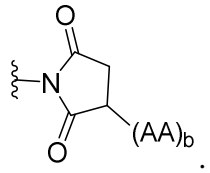
20. The compound of Claim 18 or 19, wherein t1 is 0 and t2 is 1.

21. The compound of Claim 18 or 19, wherein t1 is 1 and t2 is 0.

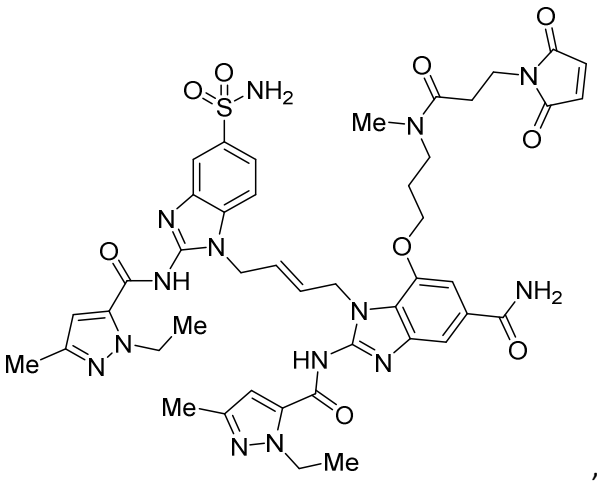
22. The compound of any one of Claims 18-20, wherein t2 is 1 and R^{HH} is C_{1-3} alkyl.

23. The compound of any one of Claims 18-22, wherein each amino acid of W is independently selected from the group consisting of alanine, valine, isoleucine, leucine, aspartic acid, glutamic acid, lysine, histidine, arginine, glycine, serine, threonine, phenylalanine, O-methylserine, O-methylaspartic acid, O-methylglutamic acid, N-methyllysine, O-methyltyrosine, O-methylhistidine, and O-methylthreonine.

24. The compound of any one of Claims 18-23, wherein L^{BB} is $-(CH_2)_{1-3}-$.

25. The compound of any one of Claims 18-24, wherein M is .

26. The compound of Claim 18, selected from the group consisting of: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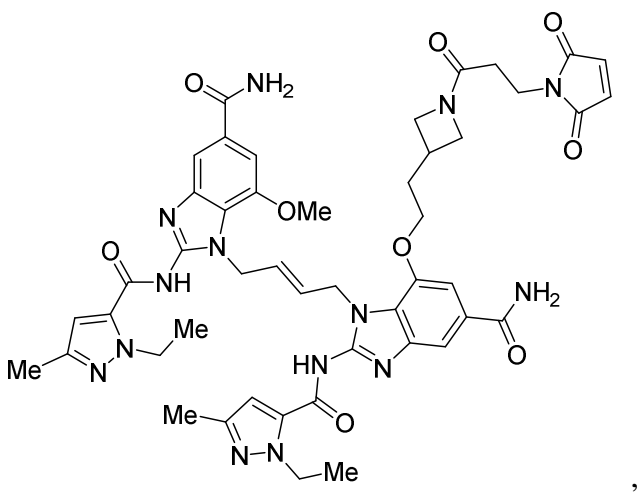
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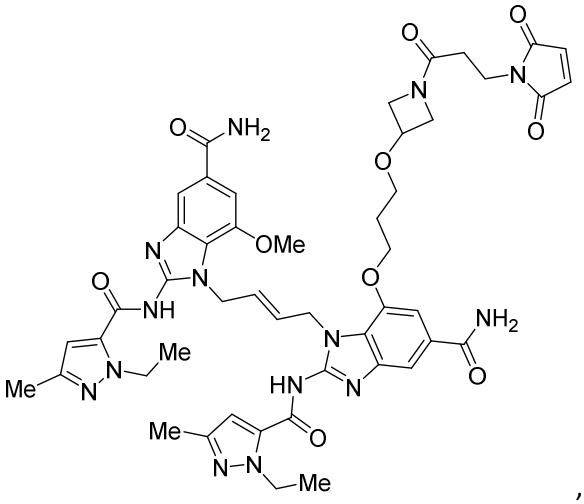
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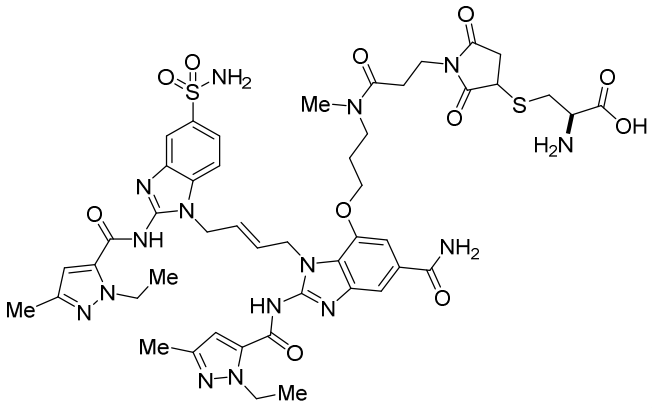
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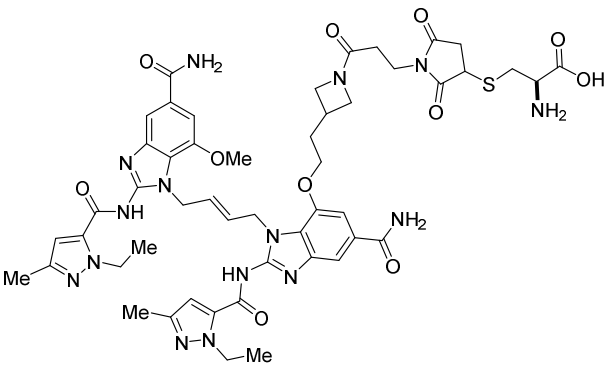
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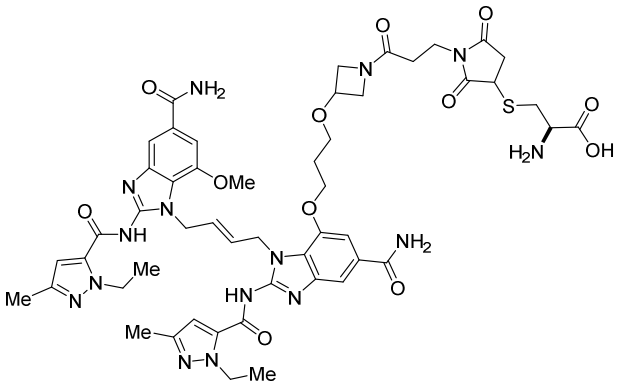
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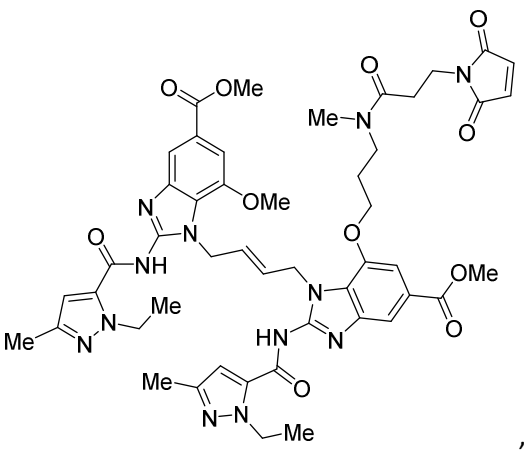
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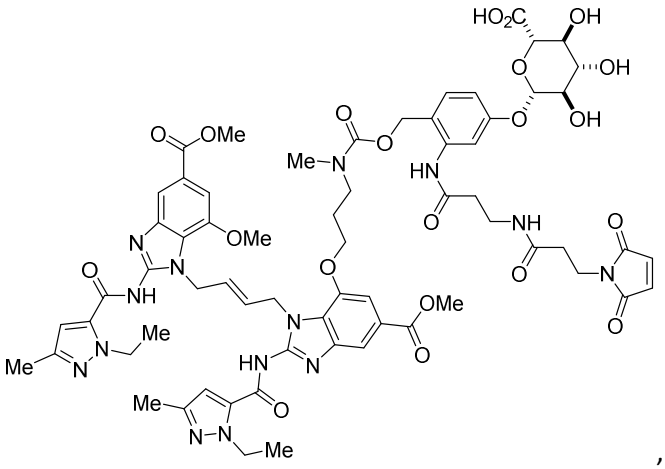
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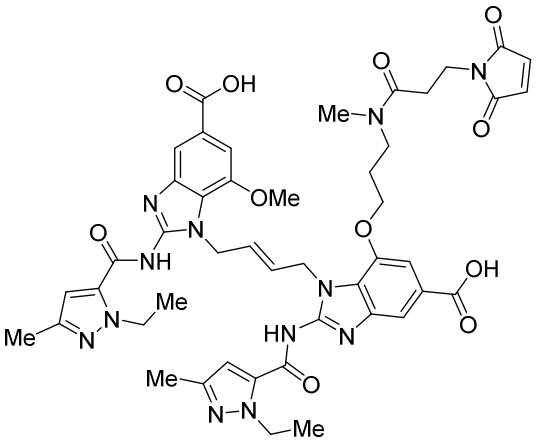
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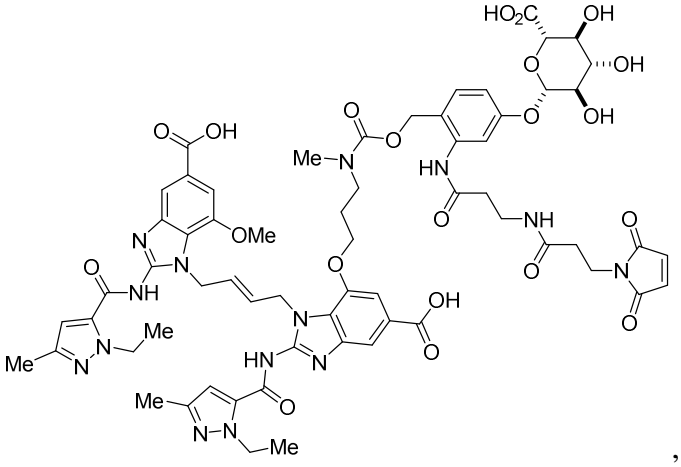
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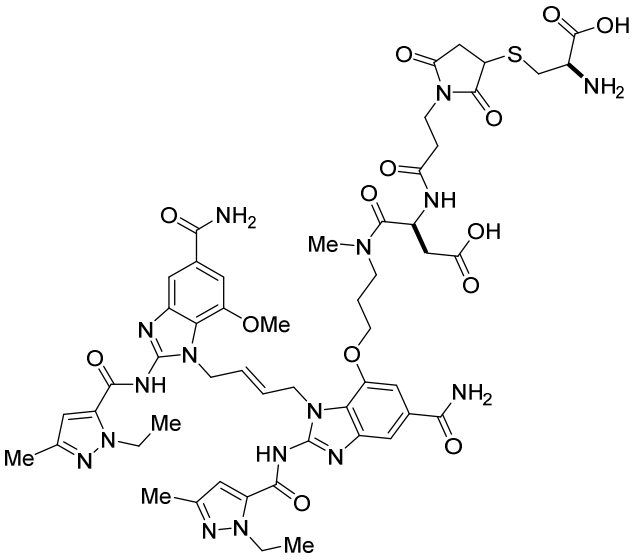
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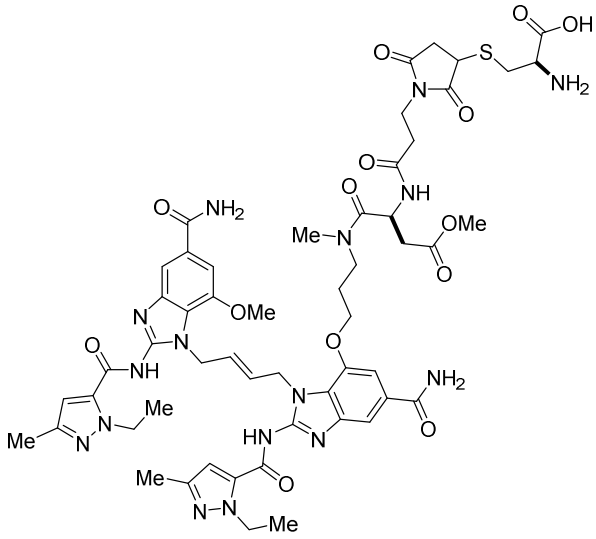

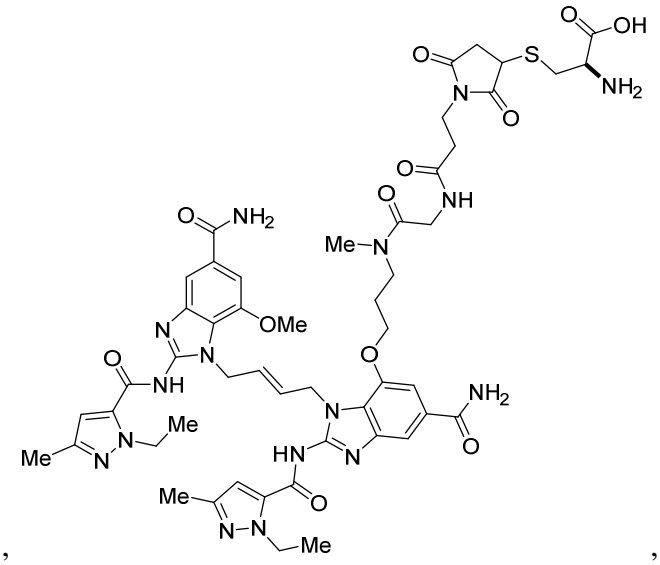
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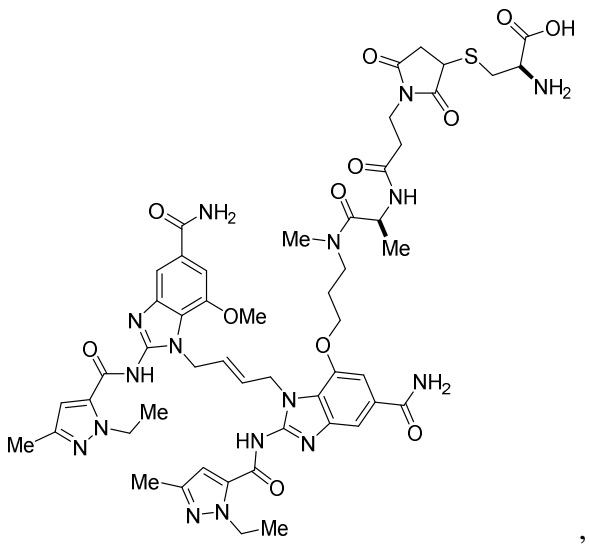
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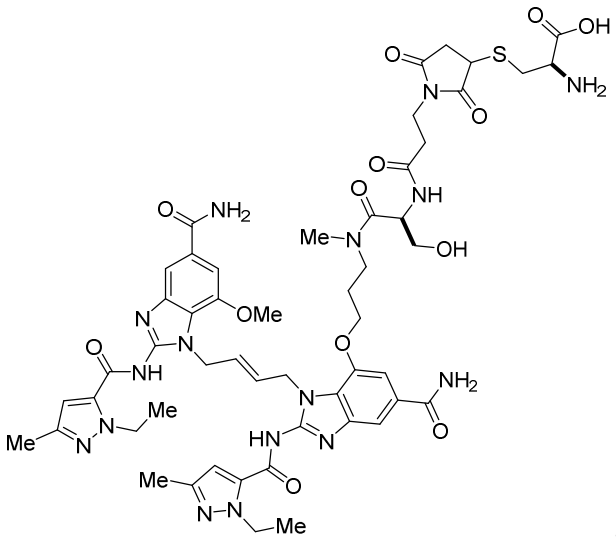
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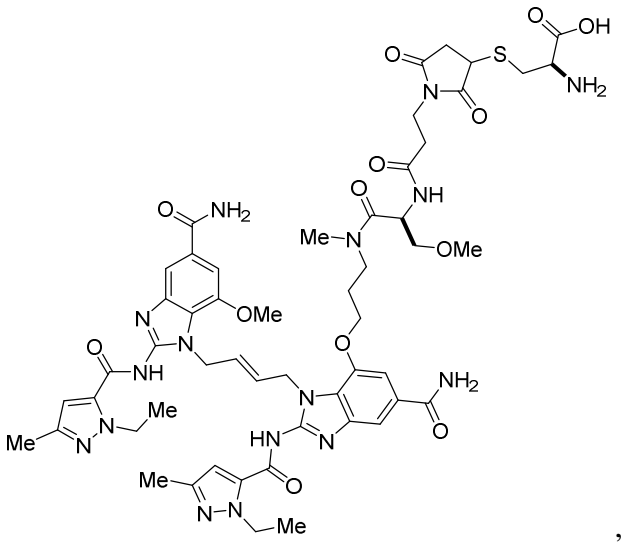
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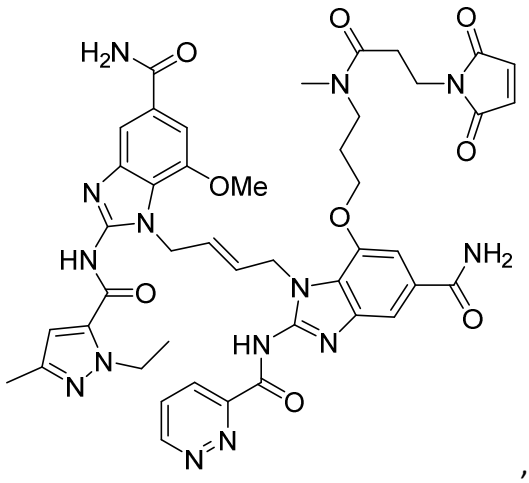
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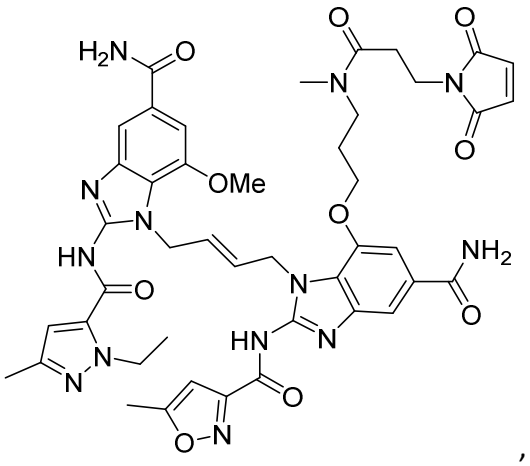
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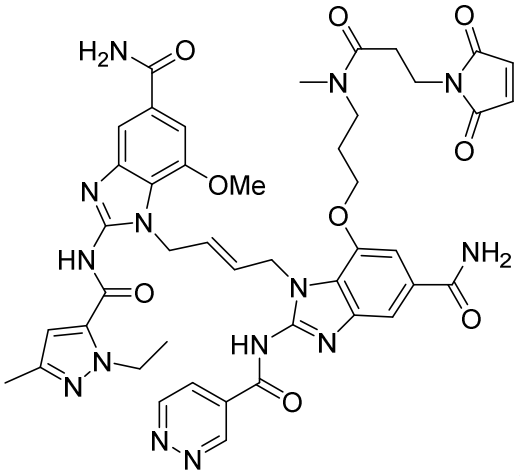
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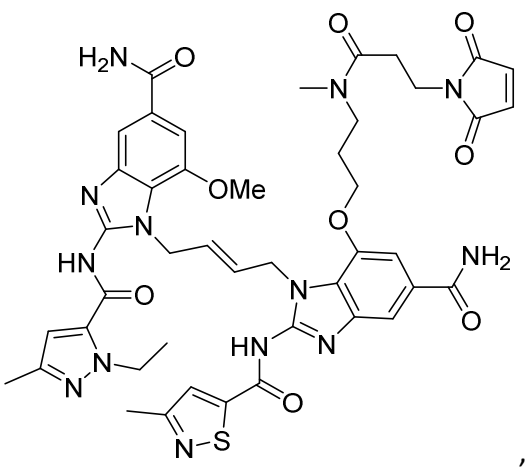
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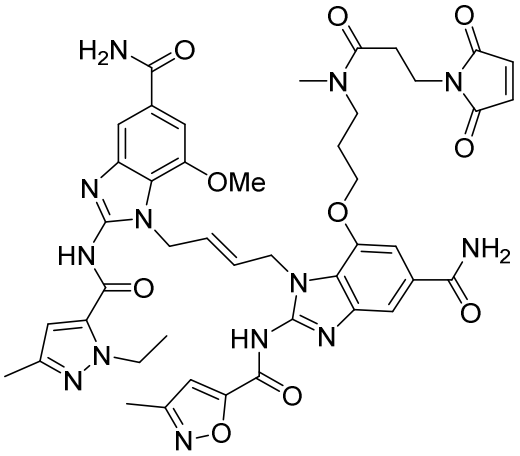
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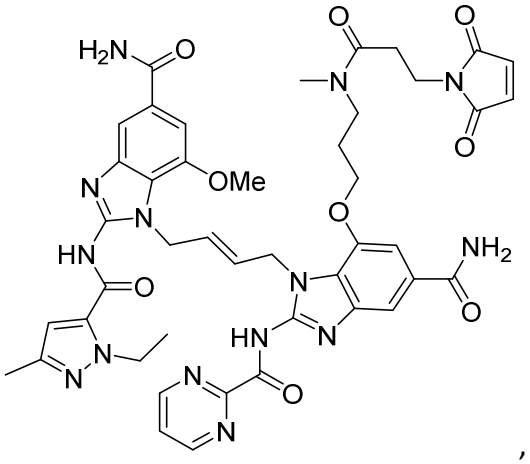
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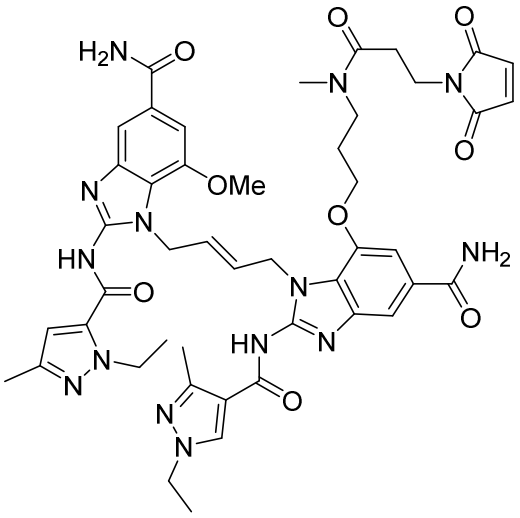
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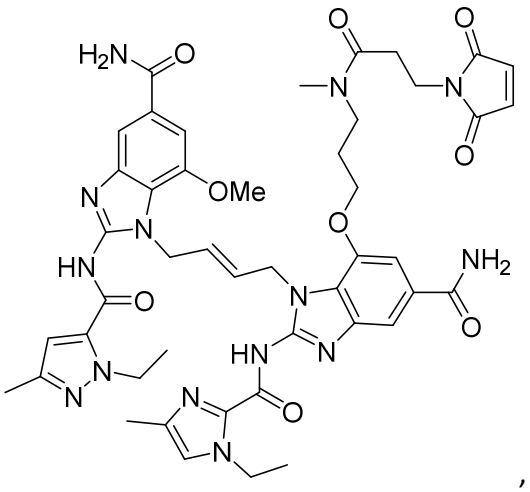
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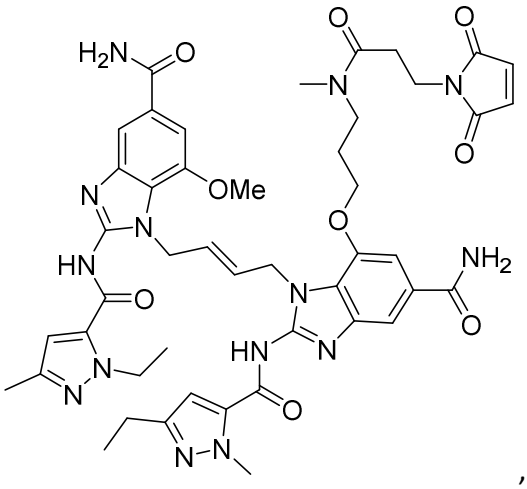
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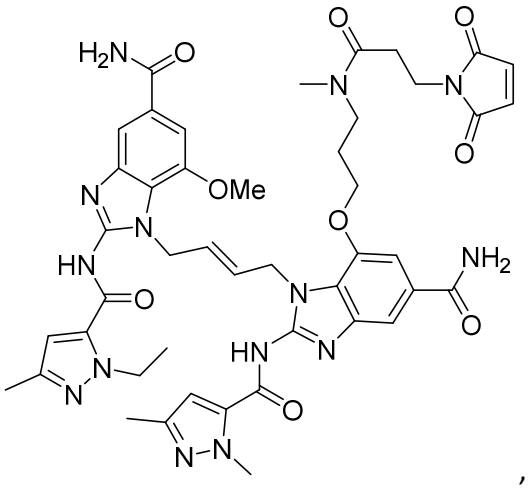
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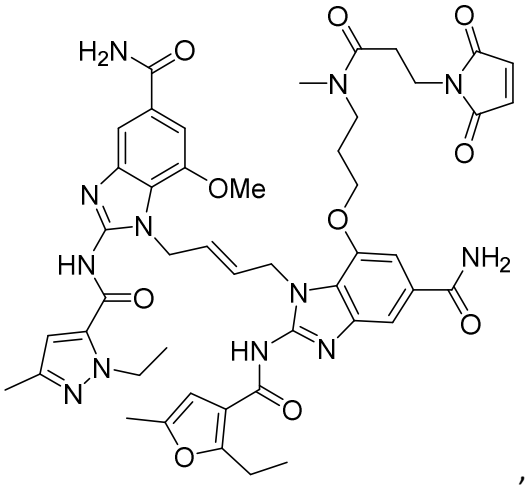
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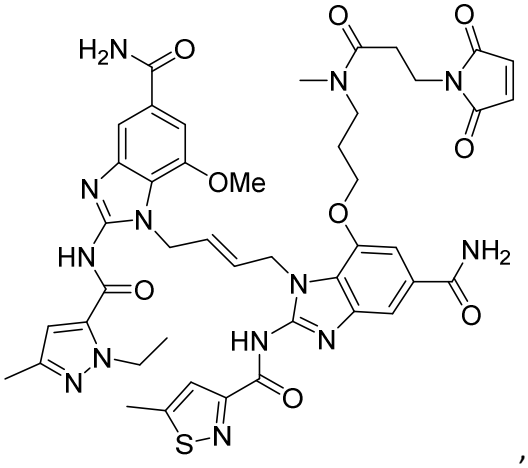
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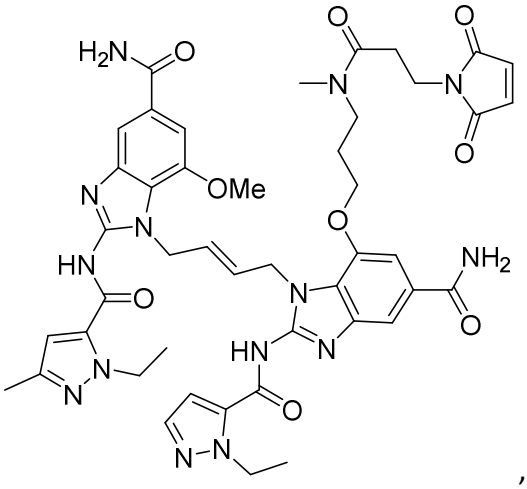
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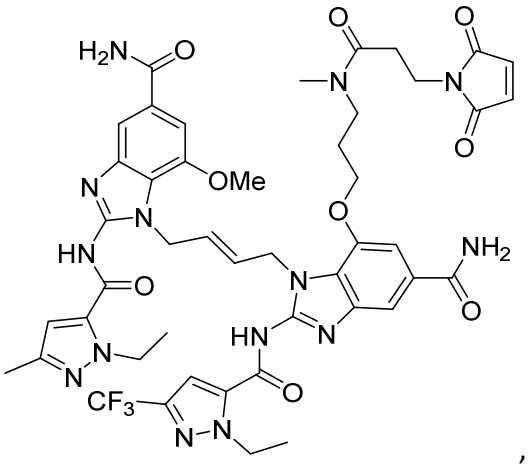
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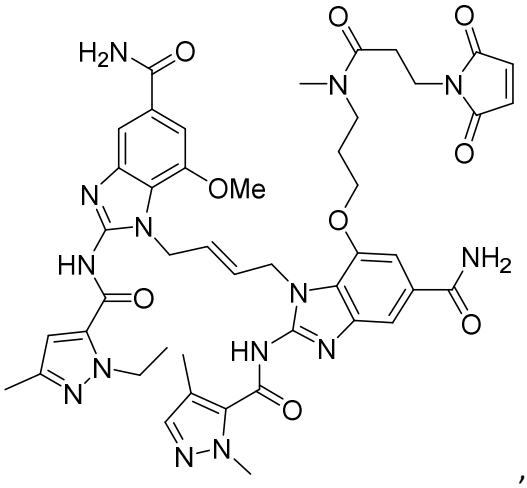
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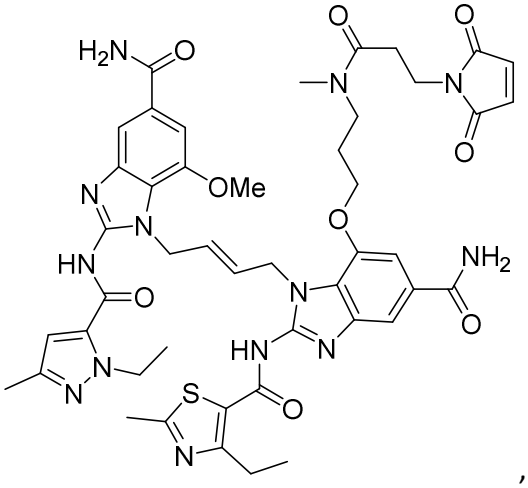
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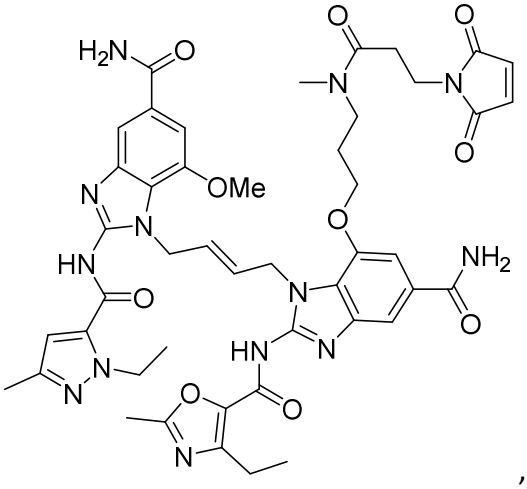
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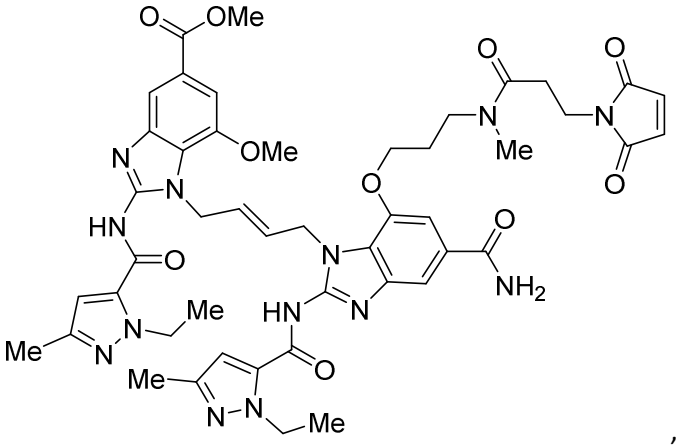
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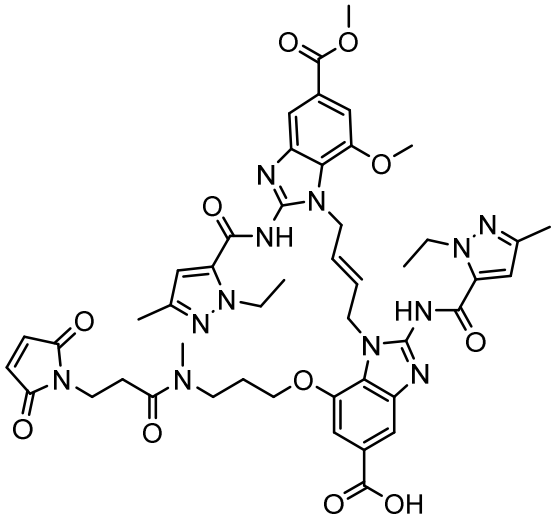
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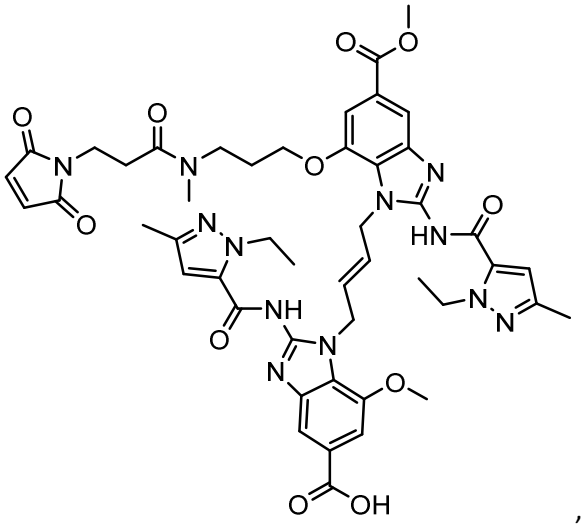
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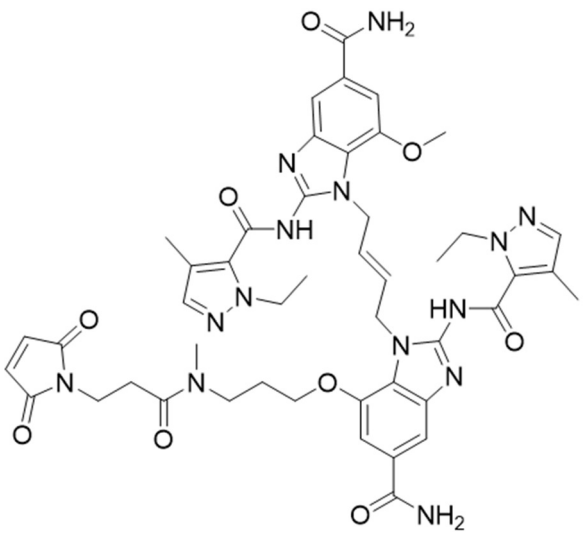
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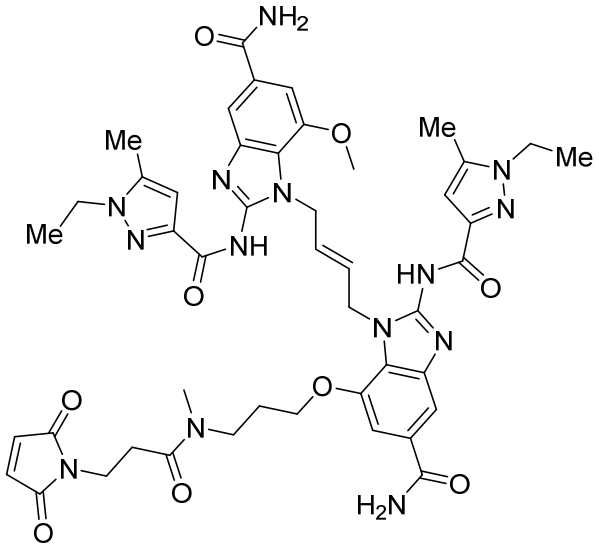
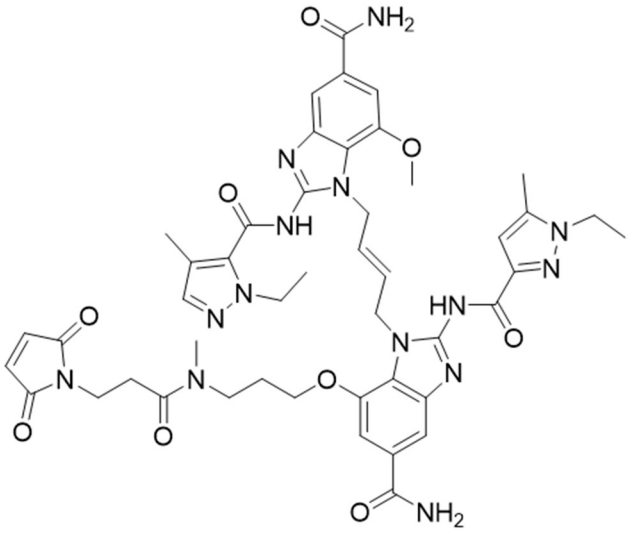
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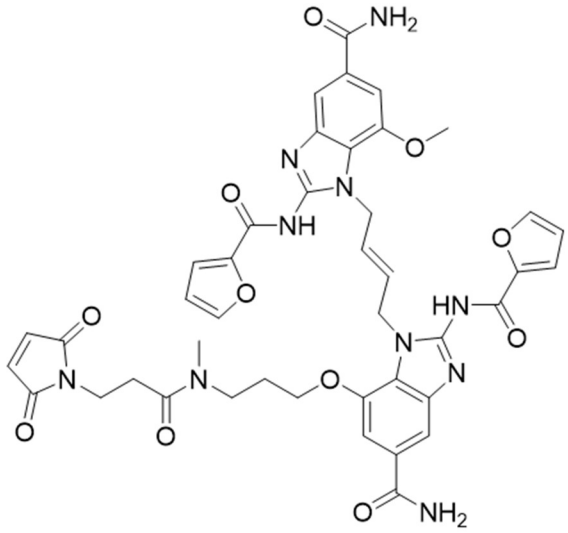
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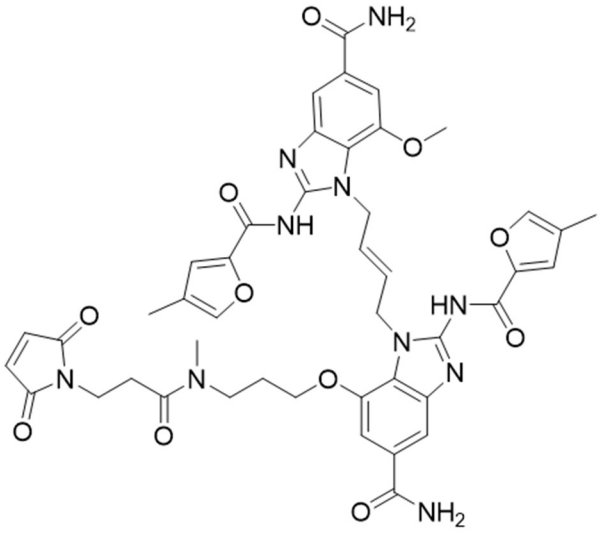
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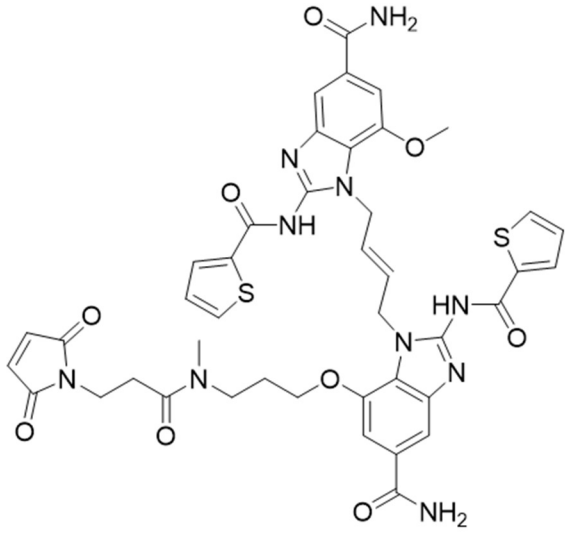
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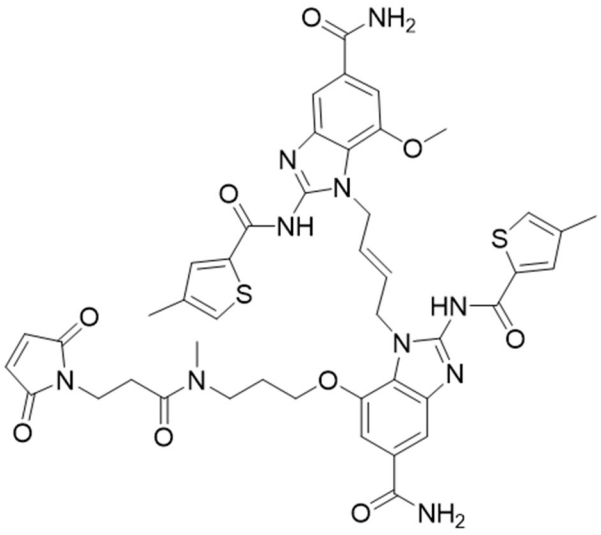
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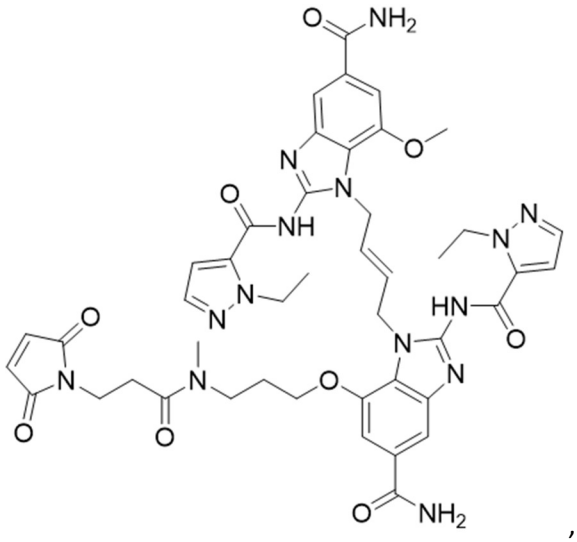
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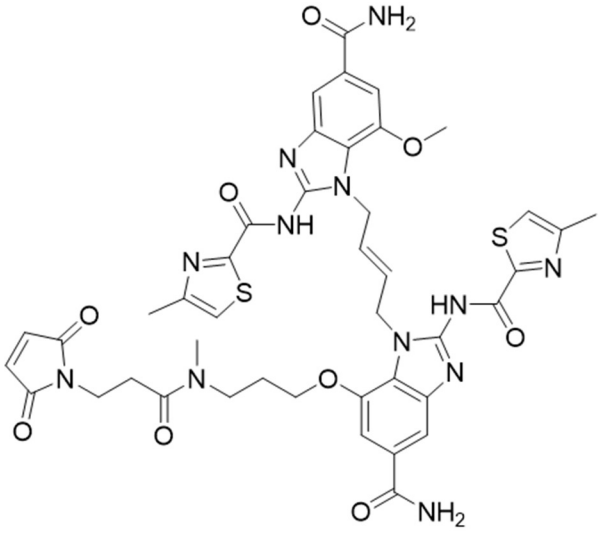
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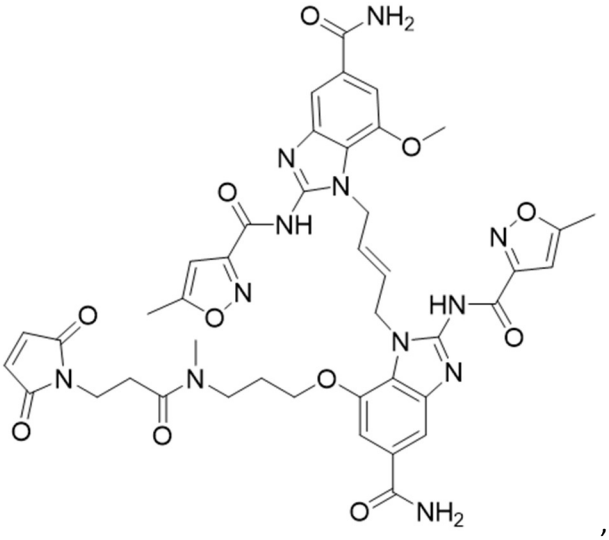
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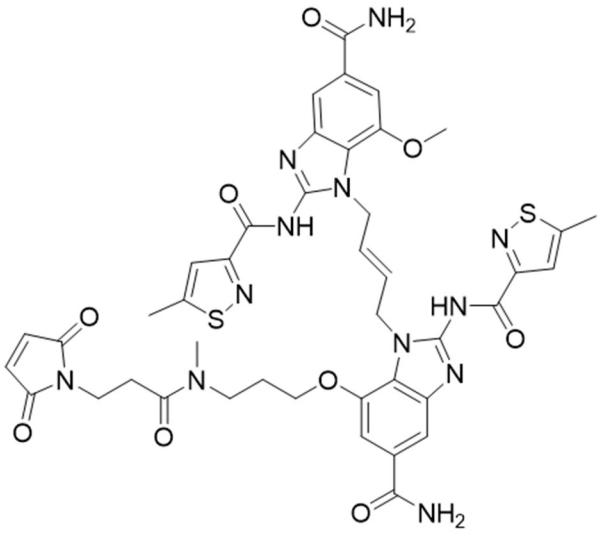
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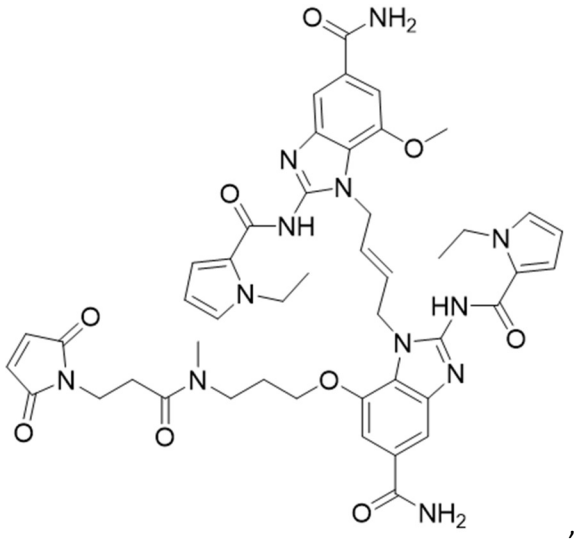
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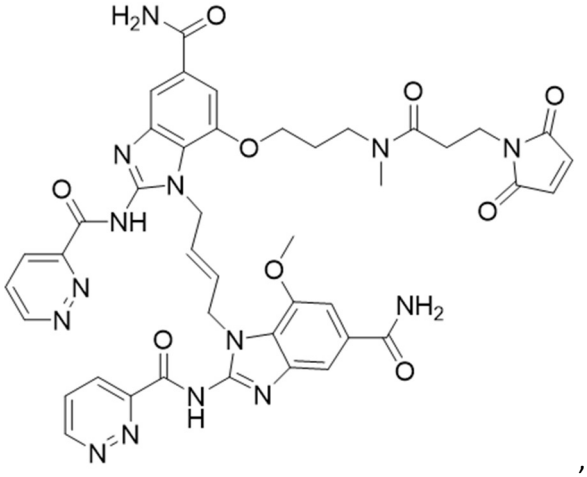
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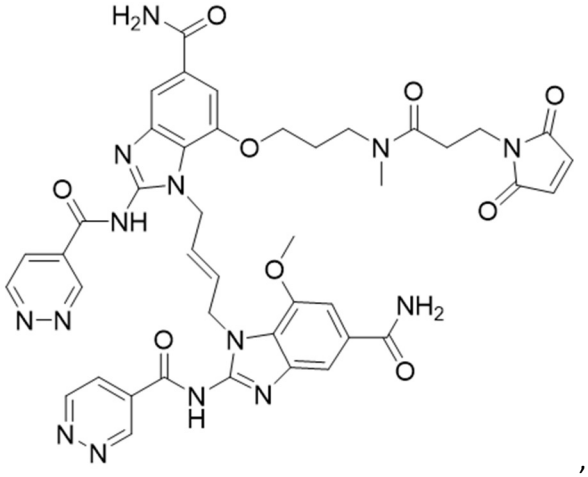
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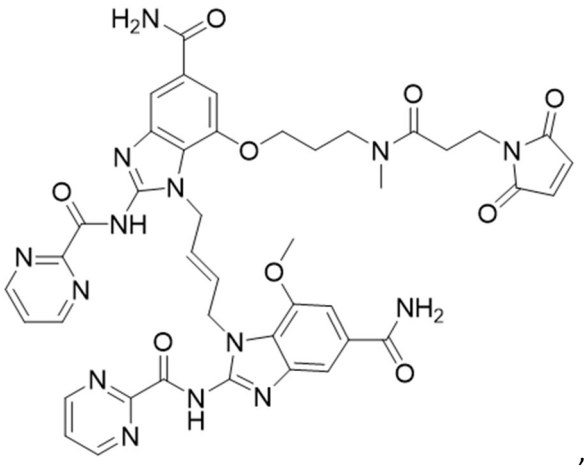
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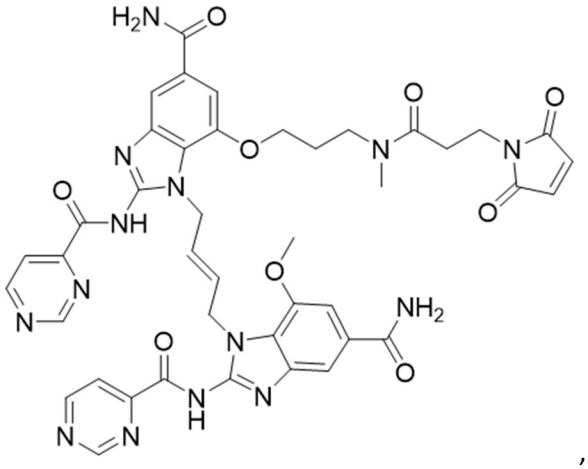
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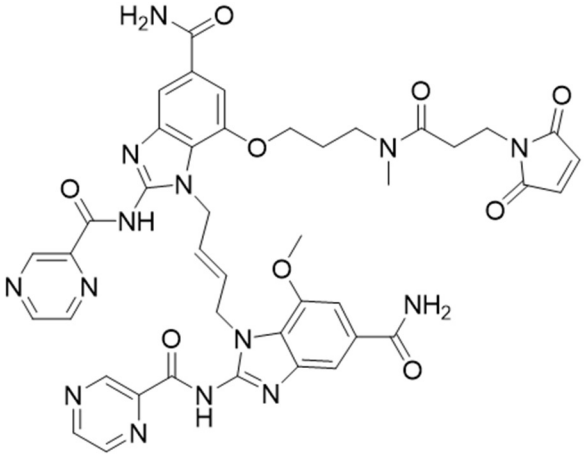
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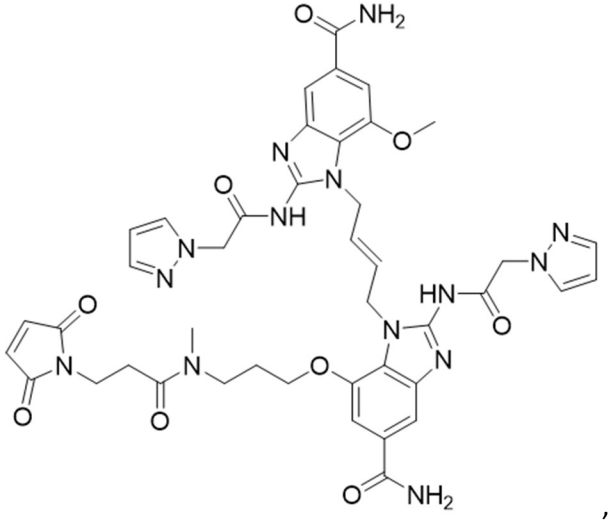
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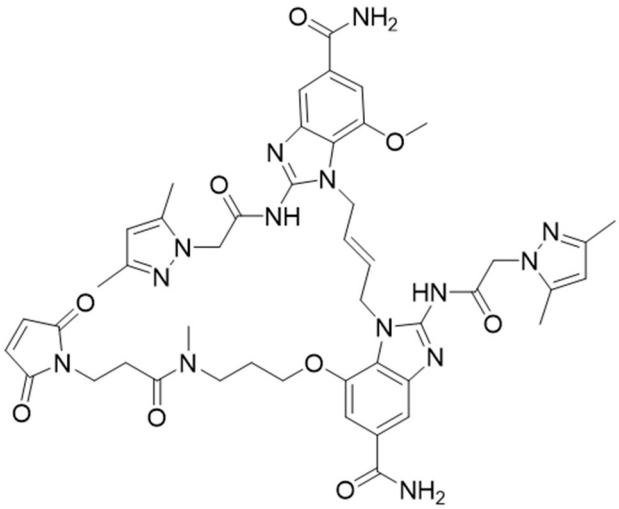

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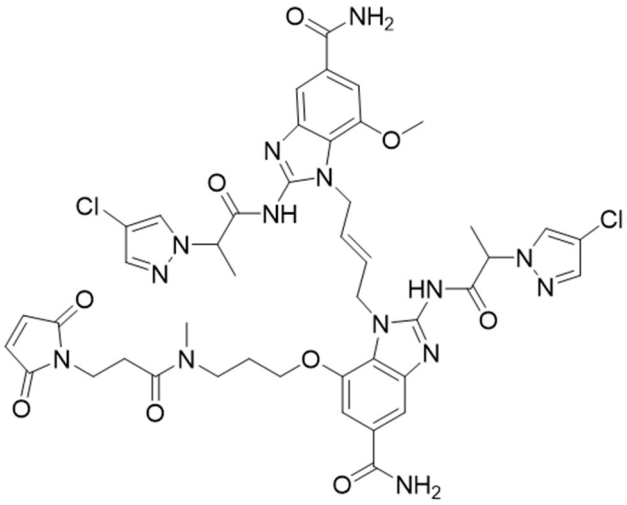

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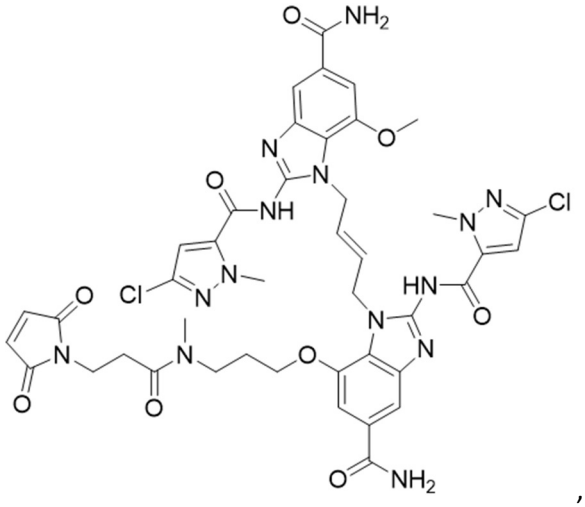

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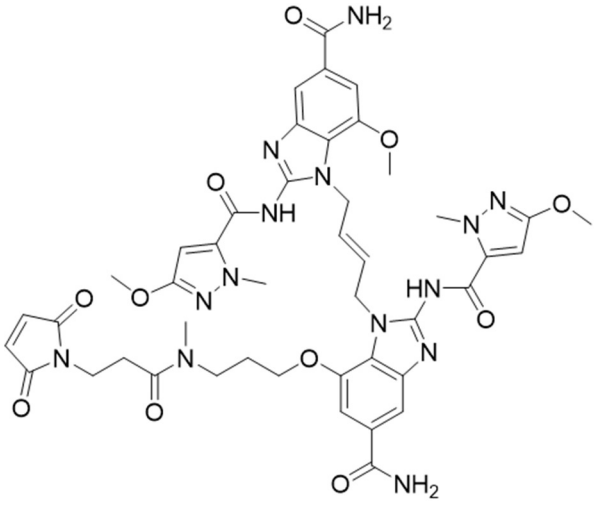

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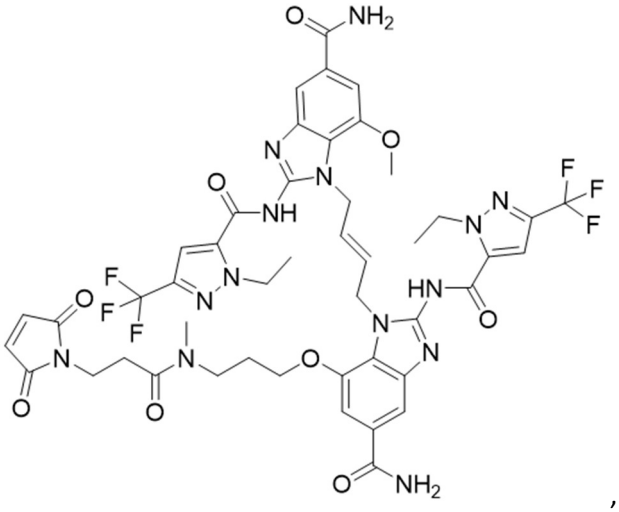

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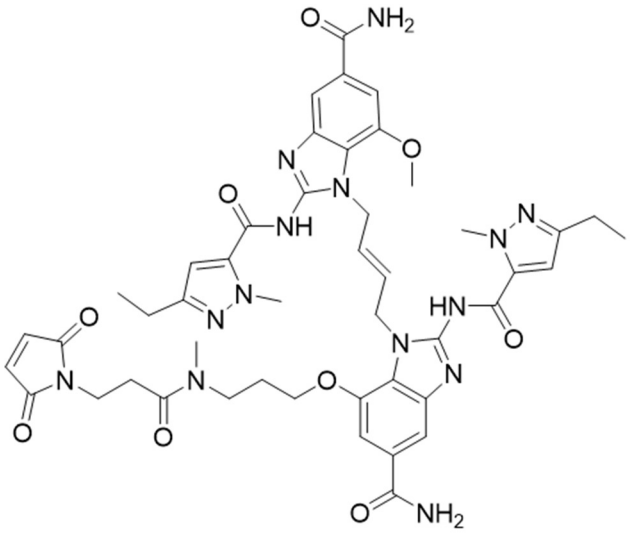
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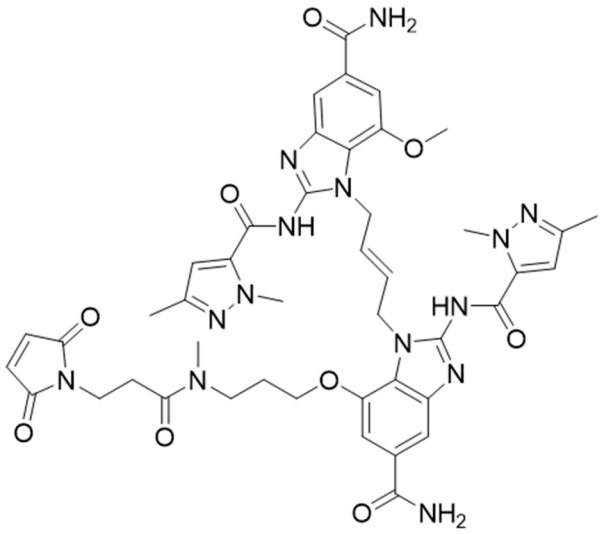
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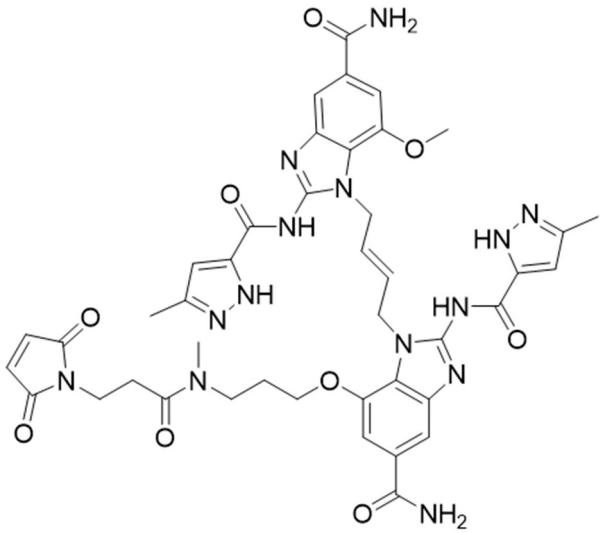
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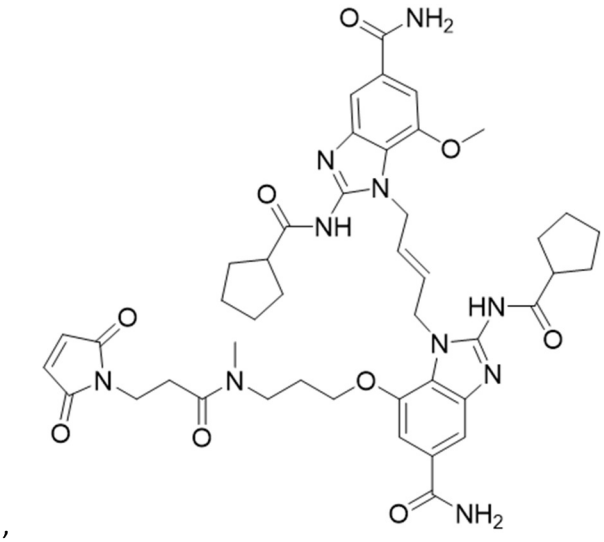
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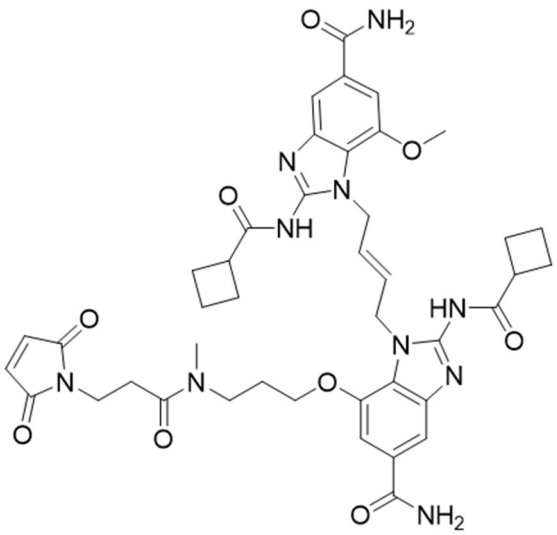
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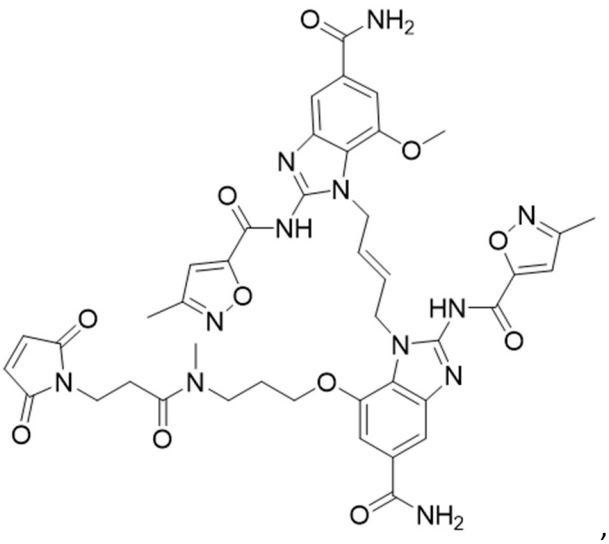
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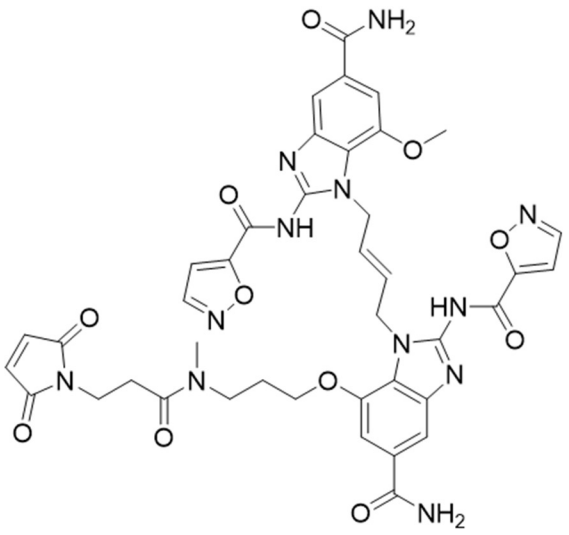
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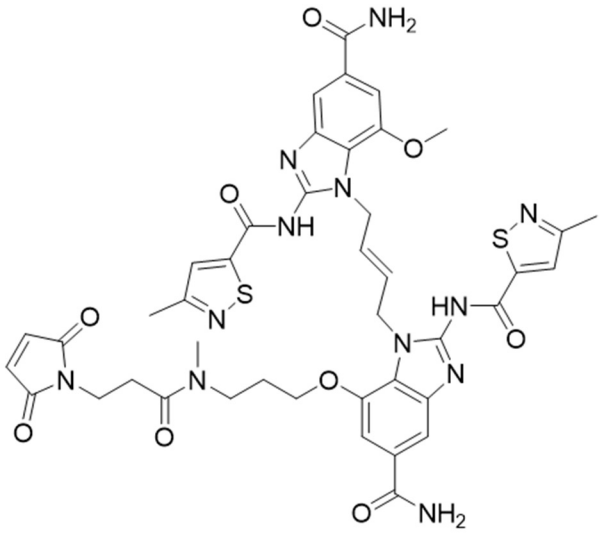
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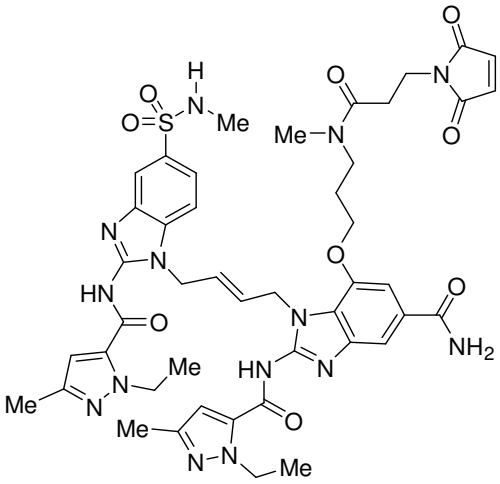
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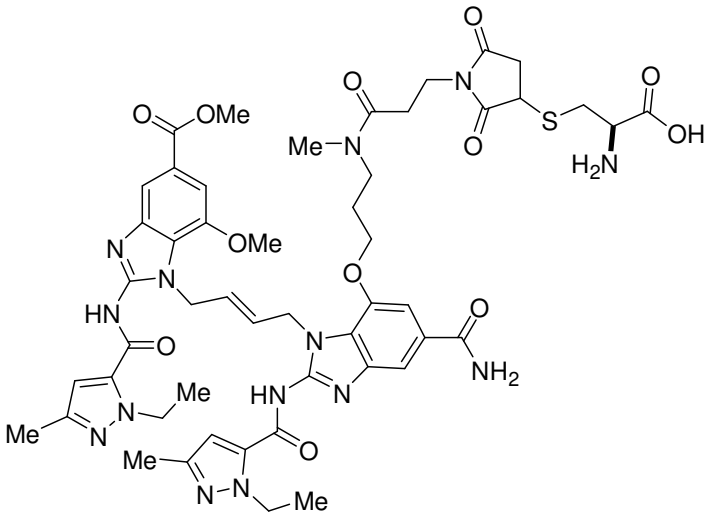
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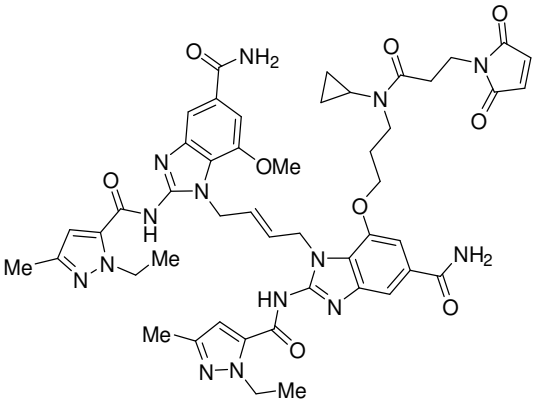
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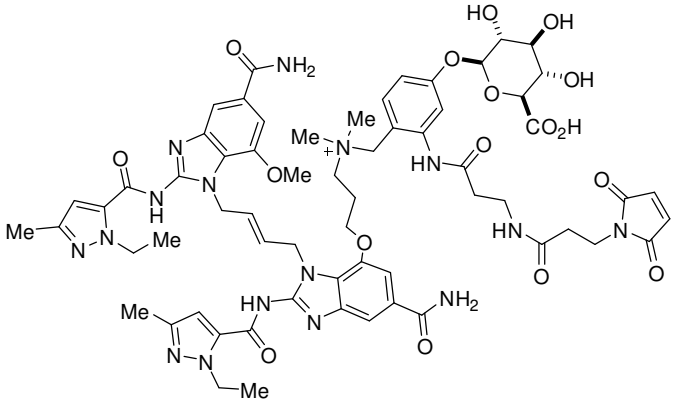
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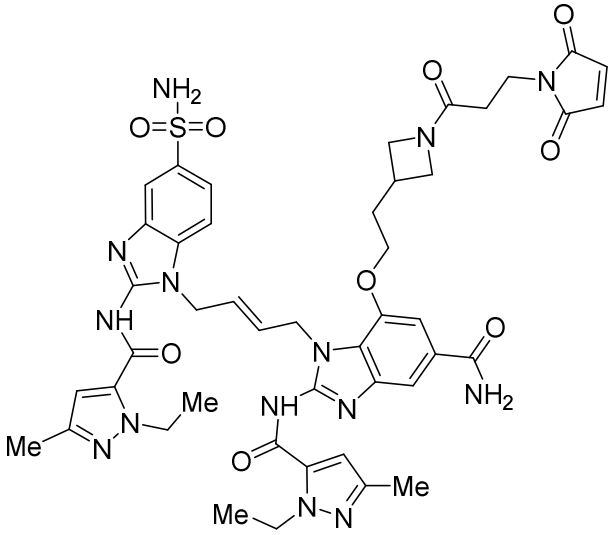
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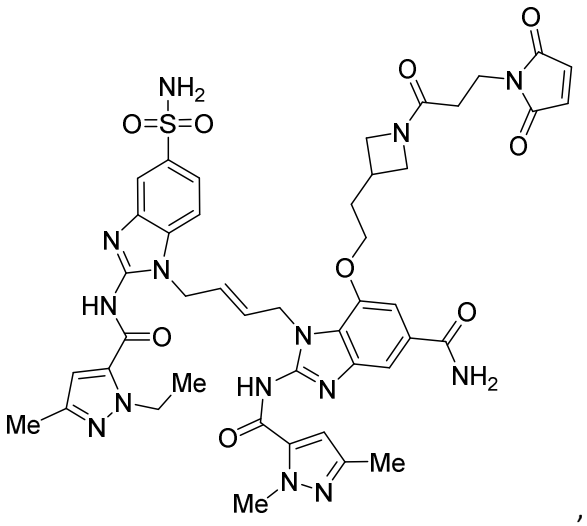
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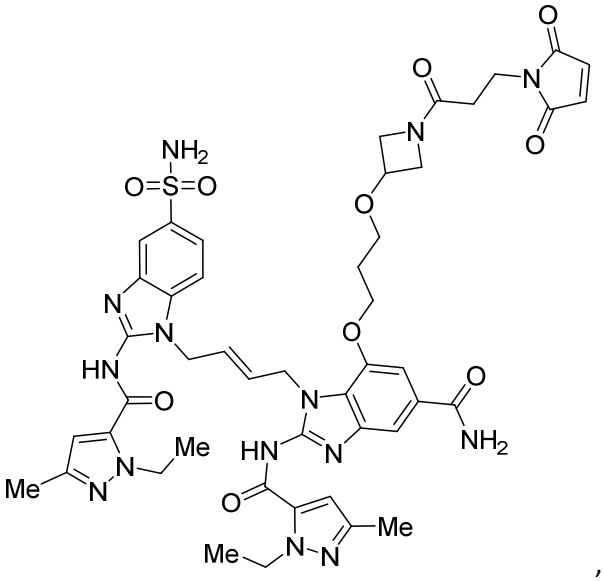
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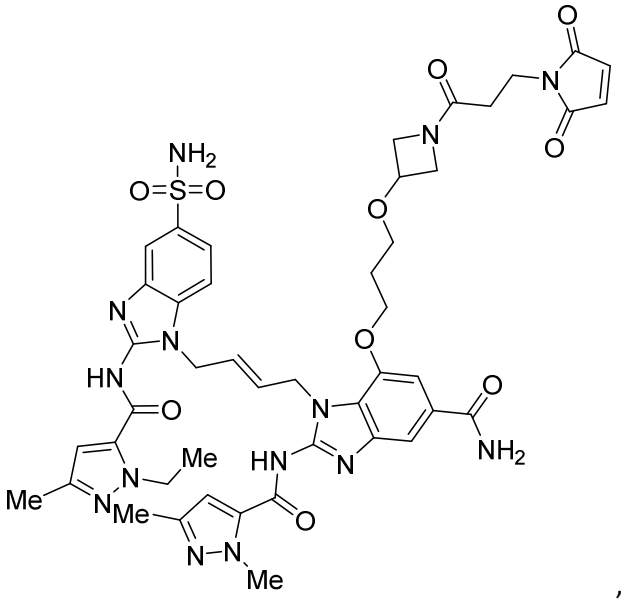
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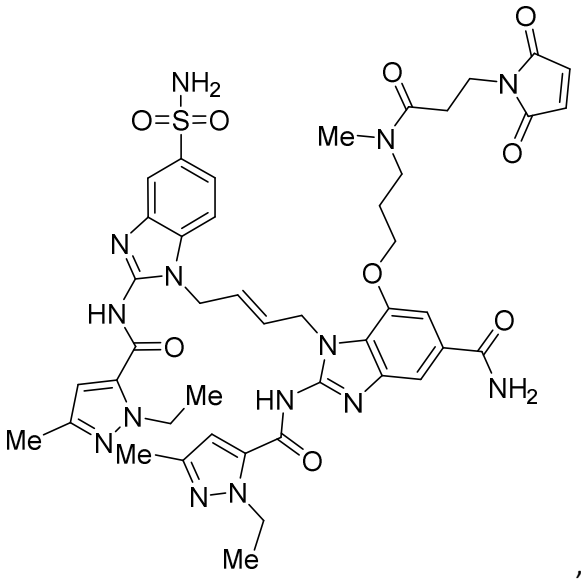

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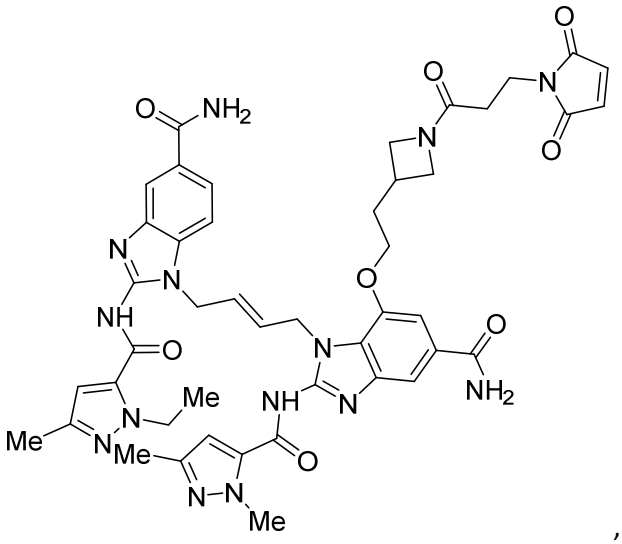

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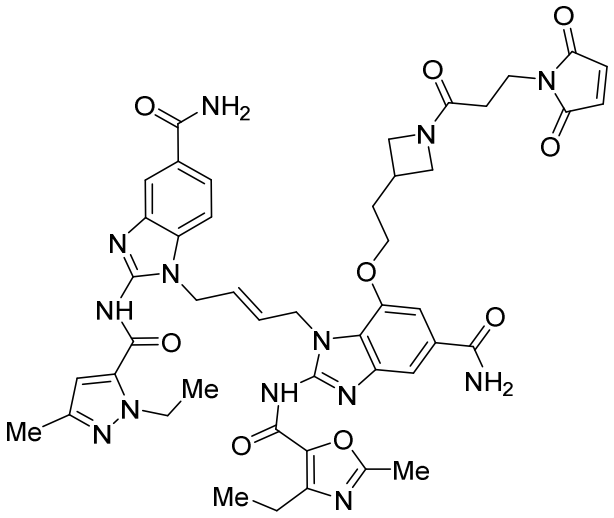

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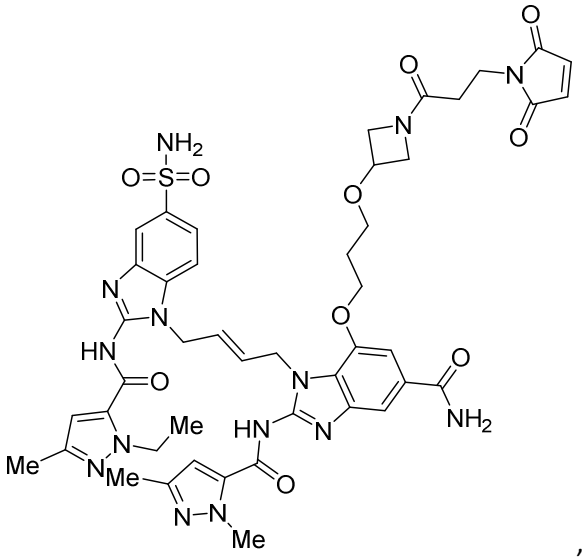

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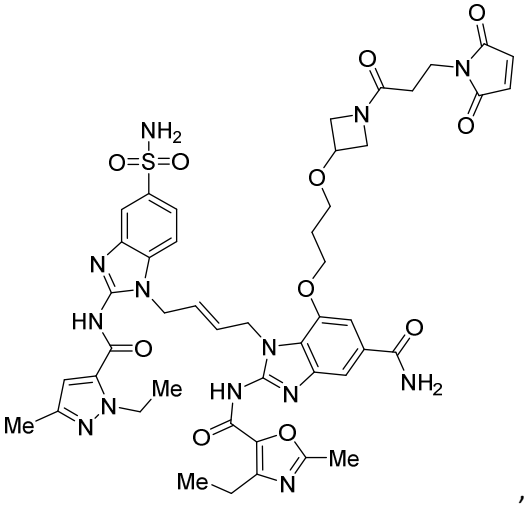

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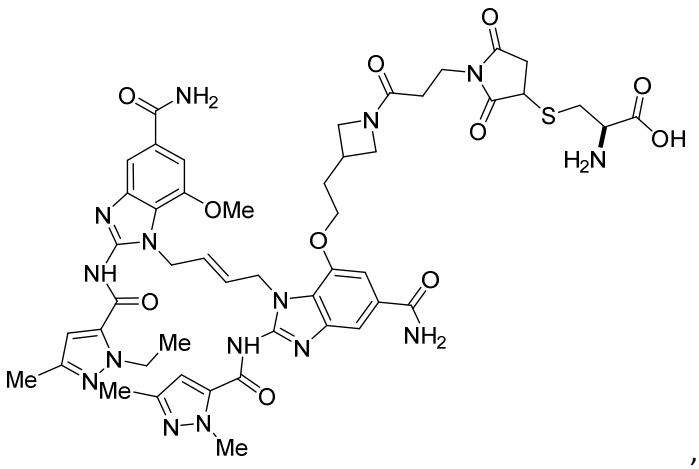

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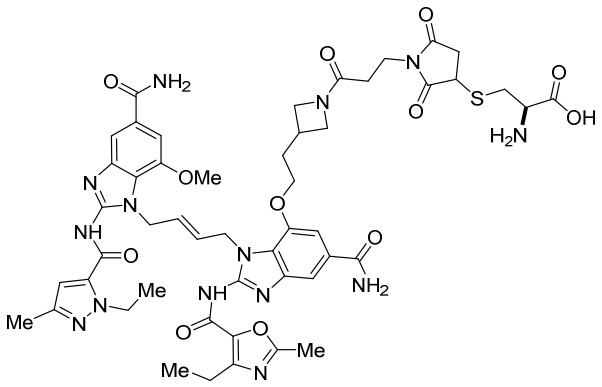
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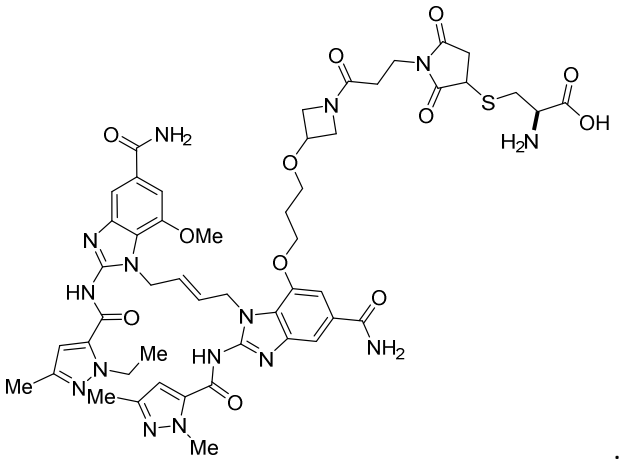
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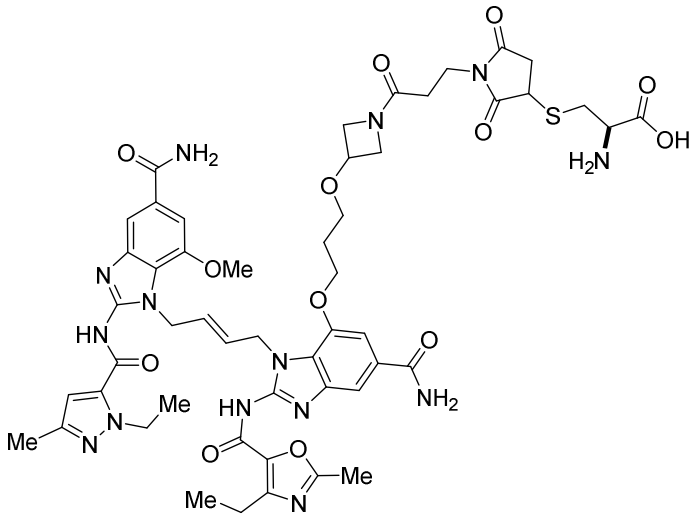
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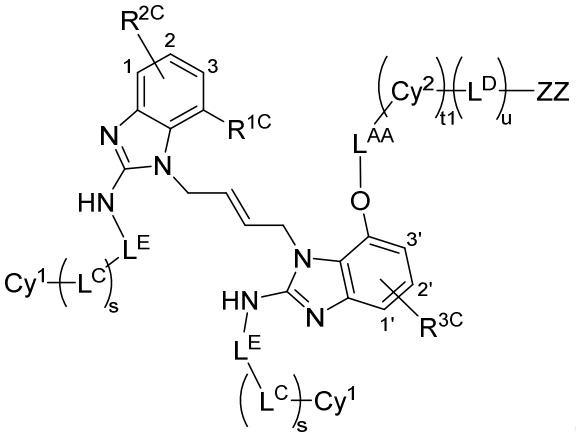
and pharmaceutically acceptable salts thereof.

27. A compound having the structure of Formula (V):



(V)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1C} is hydrogen, hydroxyl, C_{1-6} alkoxy, –(C_{1-6} alkyl) C_{1-6} alkoxy, $–(CH_2)_n-NR^AR^B$, or PEG2 to PEG4;

R^{2C} is $–CO_2R^M$, $–(C=O)NR^CR^D$, $–S(O)_2NR^CR^D$, $–S(O)_2R^M$, $–(CH_2)_q-NR^ER^F$, $–(CH_2)_q-OR^M$, $–O(C=O)-NR^ER^F$, or $–NR^M(C=O)-NR^ER^F$, wherein R^{2C} is attached at any one of positions labeled 1, 2, or 3;

R^{3C} is $–CO_2R^M$, $–(C=O)NR^CR^D$, $–S(O)_2NR^CR^D$, $–S(O)_2R^M$, $–(CH_2)_q-NR^ER^F$, $–(CH_2)_q-OR^M$, $–O(C=O)-NR^ER^F$, or $–NR^M(C=O)-NR^ER^F$, wherein R^{3C} is attached at any one of positions labeled 1', 2', or 3';

each R^A, R^B, R^C, R^D, R^E, R^F, and R^M are independently hydrogen or C_{1-6} alkyl;

each subscript n is independently an integer from 0 to 6;

each subscript q is independently an integer from 0 to 6;

L^E is $–(C=O)–$ or $–S(O)_2–$;

L^C is $–(CR^IR^J)_{1-3}–$

each R^I and R^J are independently hydrogen or C_{1-3} alkyl;

subscript s is 0 or 1;

each Cy^1 is independently a 4-6 membered heterocycle, a 5-6 membered heteroaryl, or a C_{3-6} cycloalkyl, each optionally substituted with one or more R^K;

each R^K is independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen, -OH, =O, $-NR^{d2}R^{e2}$, $-C(O)NR^{d2}R^{e2}$, $-C(O)(C_{1-6}$ alkyl), and $-C(O)O(C_{1-6}$ alkyl);

each R^{d2} and R^{e2} are independently hydrogen or C_{1-3} alkyl;

L^{AA} is $–(CH_2)_{1-6}–$, $–C(O)(CH_2)_{1-6}–$, $–C(O)NR^L(CH_2)_{1-6}–$, $–(CH_2)_{1-6}O–$, $–C(O)(CH_2)_{1-6}O–$, or $–C(O)NR^L(CH_2)_{1-6}O–$;

R^L is hydrogen or C_{1-3} alkyl;

Cy^2 is C_{3-6} cycloalkyl, 4-6 membered heterocycle, 5-6 membered heteroaryl, or phenyl, each optionally substituted with one or more R^U;

each R^U is independently selected from the group consisting of $–CO_2R^{j1}$, $–(C=O)NR^{d3}R^{e3}$, $–S(O)_2NR^{d3}R^{e3}$, $–(CH_2)_{q1}-NR^{g1}R^{h1}$, $–(CH_2)_{q1}-OR^{j1}$, and $–(CH_2)_{q1}-(OCH_2CH_2)_{1-8}OH$;

each R^{d3}, R^{e3}, R^{g1}, R^{h1}, and R^{j1} are independently hydrogen or C_{1-6} alkyl;

subscript q1 is an integer from 0 to 6;

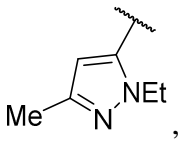
subscript t1 is 0 or 1;

L^D is $–(CH_2)_{1-6}–$;

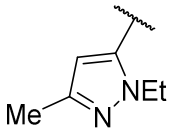
subscript u is 0 or 1;

when t1 is 0, ZZ is $–NR^QR^R$, $–N^+(C_{1-6}$ alkyl)R^QR^R, $–C(=O)N^SR^T$, $-C(O)O(C_{1-6}$ alkyl),$–CO_2H$, or an amino acid, or when t1 is 1, ZZ is hydrogen, $–NR^QR^R$, $–N^+(C_{1-6}$ alkyl)R^QR^R; $–C(=O)N^SR^T$, $-C(O)O(C_{1-6}$ alkyl),$–CO_2H$, or an amino acid;

R^Q is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl, provided that

if t1 is 0 and both Cy^1 are 

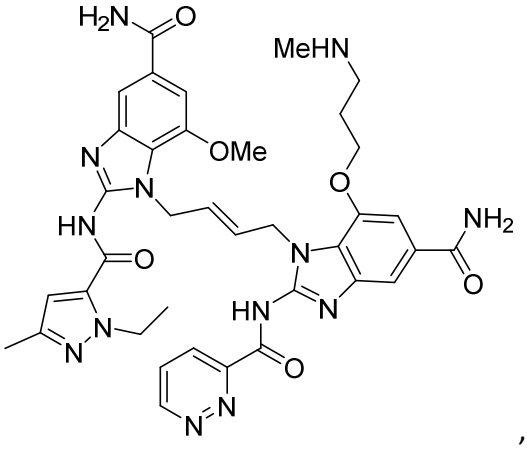
, then R^Q is C_{2-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl, and

if t1 is 0 and at least one Cy^1 is not 

, then ZZ is $–NR^QR^R$, $–N^+(C_{1-6}$ alkyl)R^QR^R, or $–C(=O)N^SR^T$, and R^Q is C_{1-6} alkyl, C_{3-6} cycloalkyl, $–(CH_2)_{1-3}C_{3-6}$ cycloalkyl, $–(CH_2)_{1-3}C_{1-3}$ alkoxy, $–(CH_2)_{1-3}$ 4-6 membered heterocycle, or $–(CH_2)_{1-3}$ 5-6 membered heteroaryl; and

each R^R, R^S, and R^T are independently hydrogen or C_{1-6} alkyl.

28. The compound of Claim 27, selected from the group consisting of: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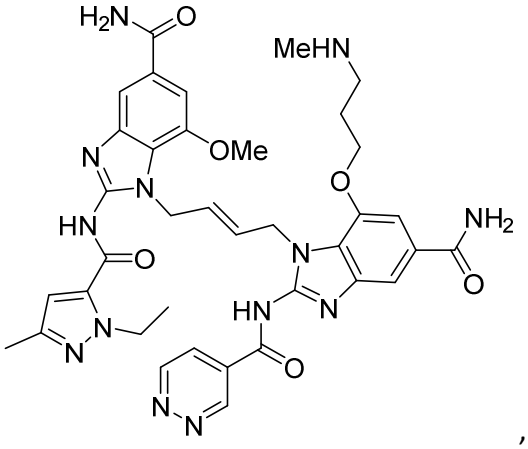
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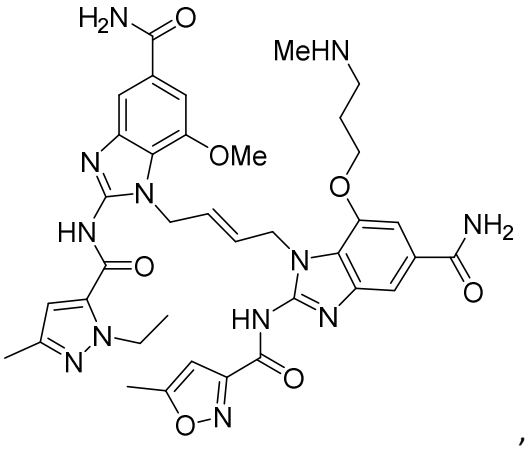
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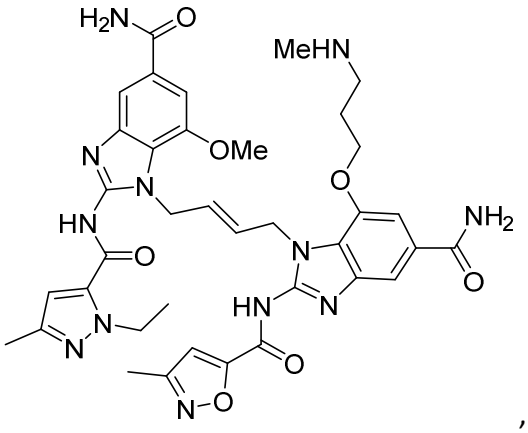
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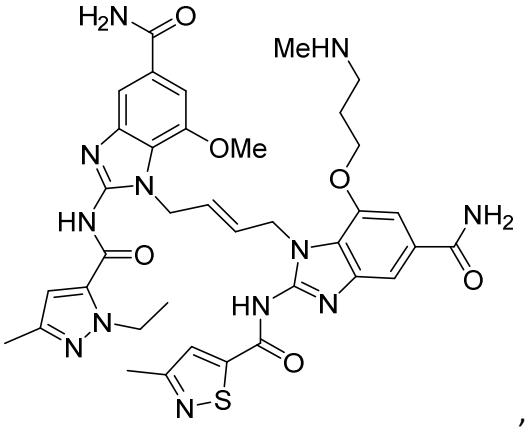
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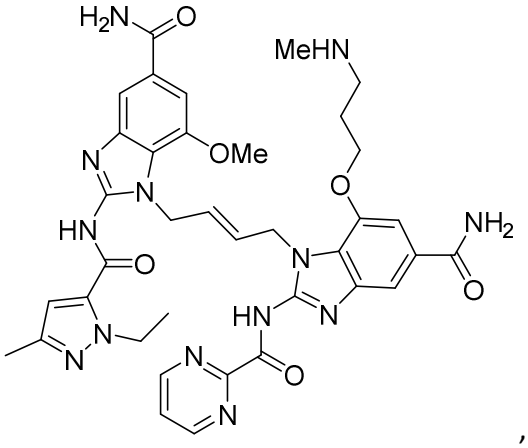
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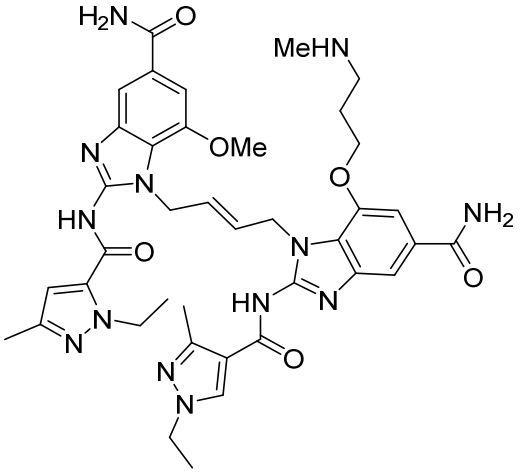
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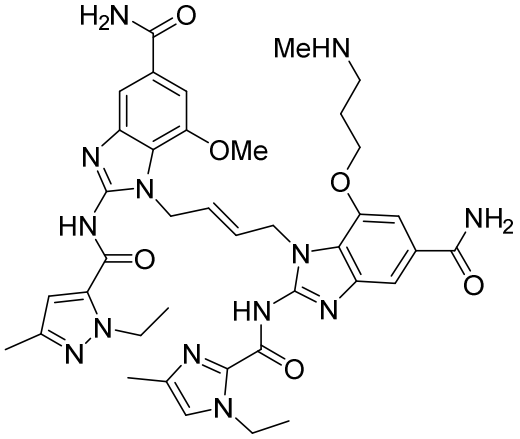
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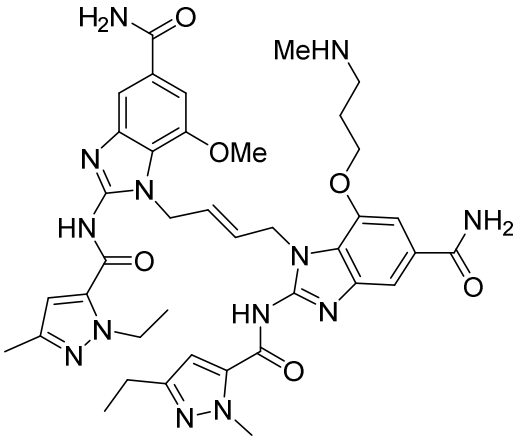
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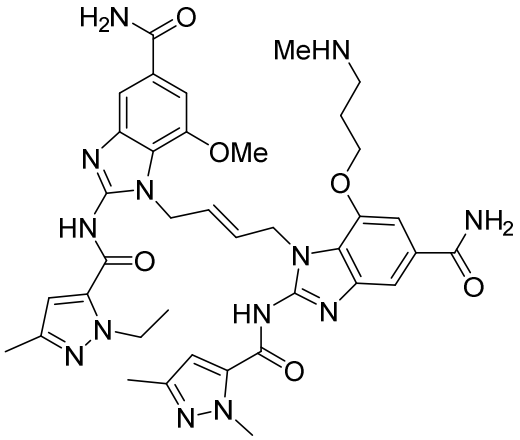
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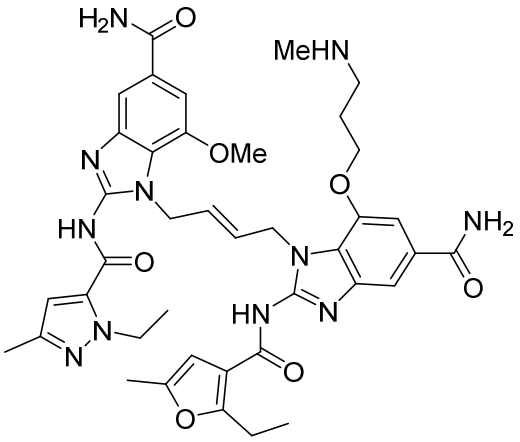
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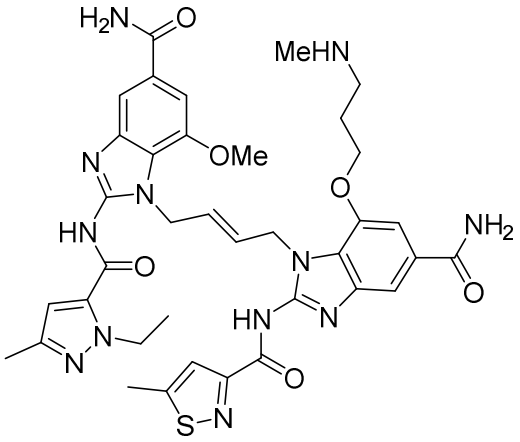
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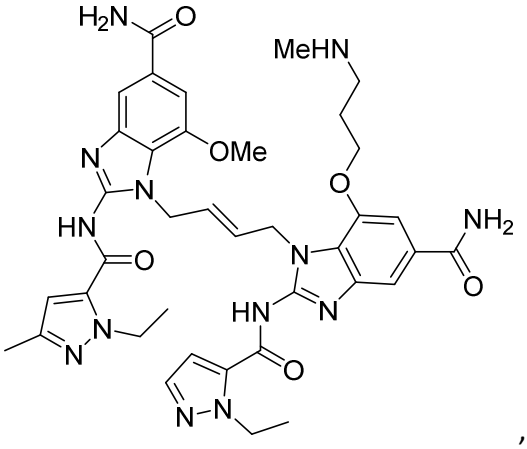
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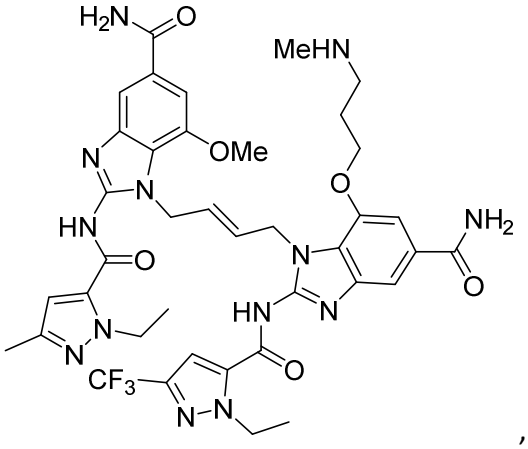
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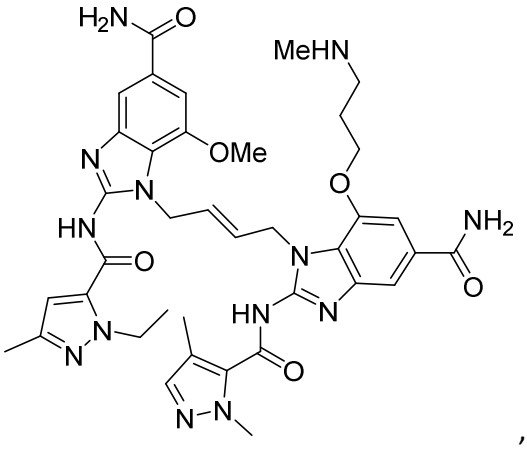
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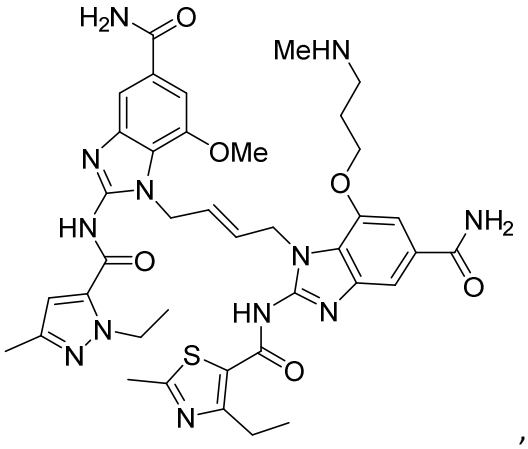
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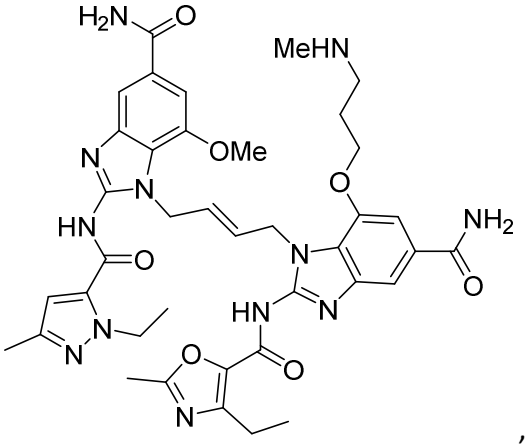
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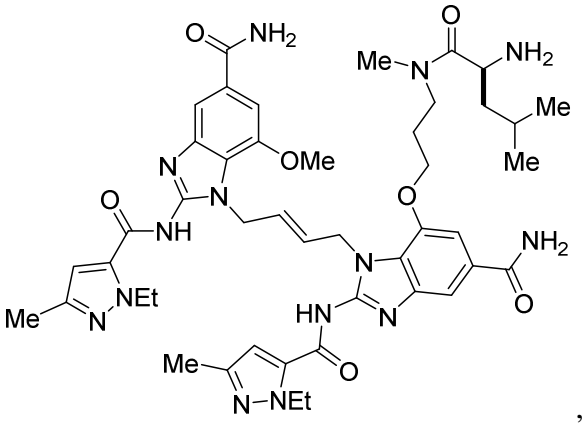
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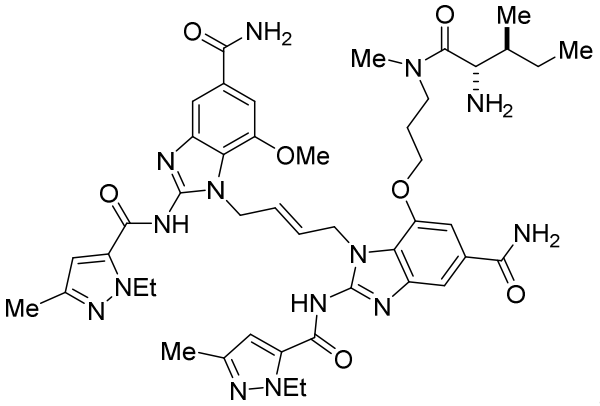
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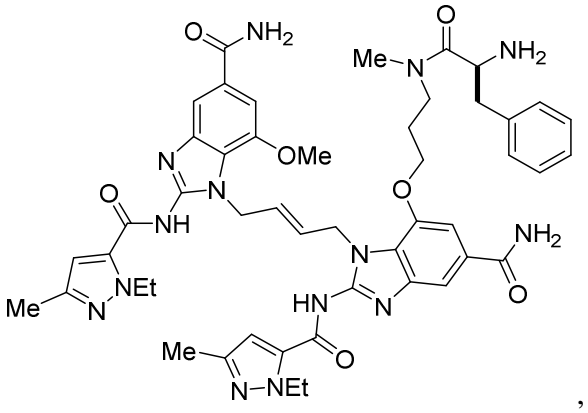
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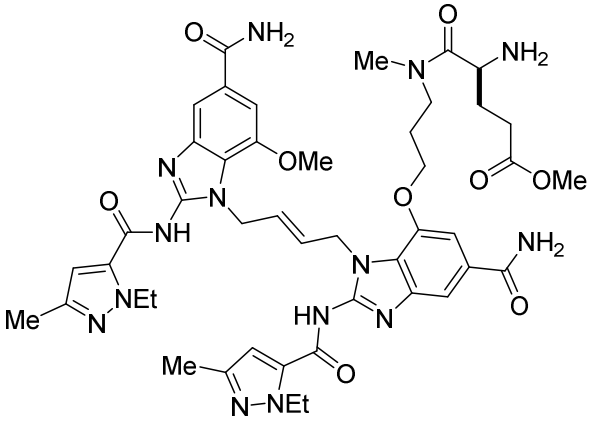
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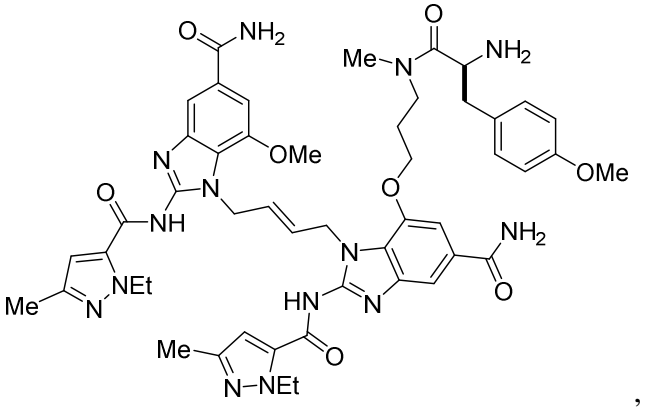
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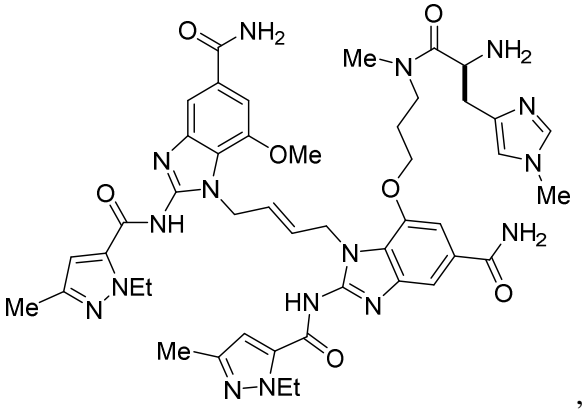
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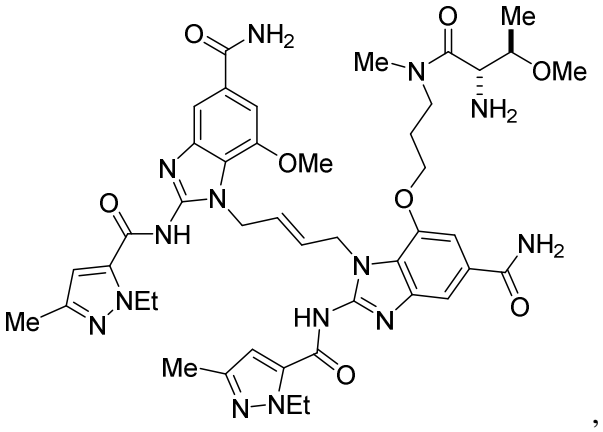
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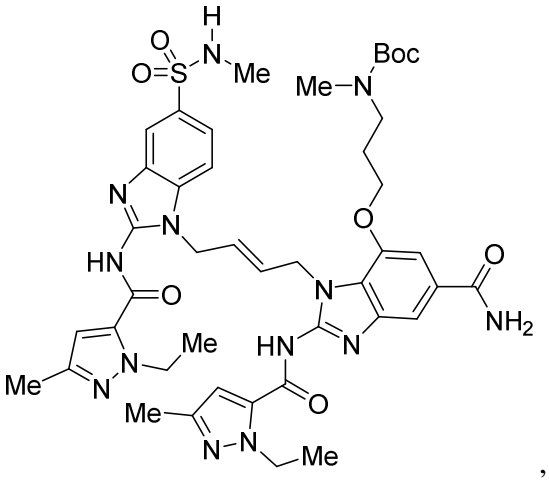
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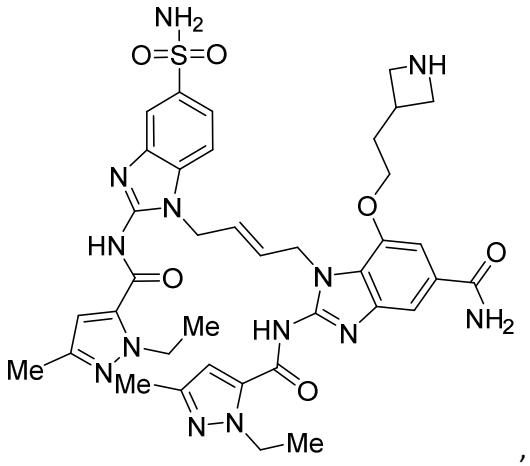
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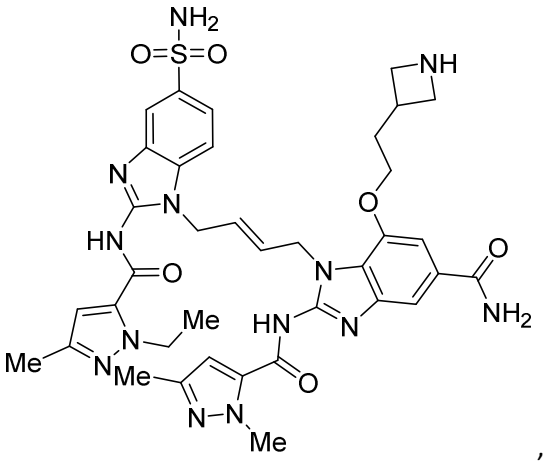
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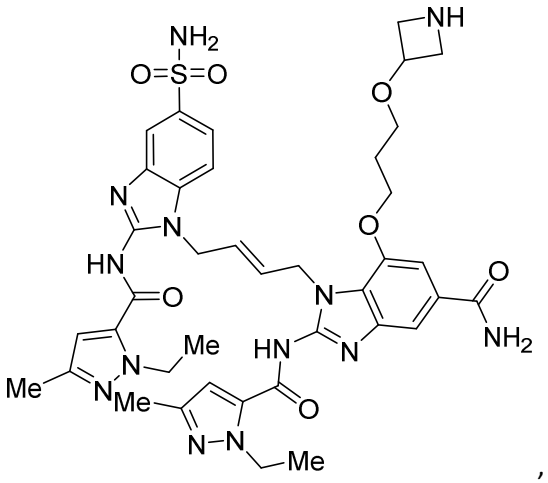
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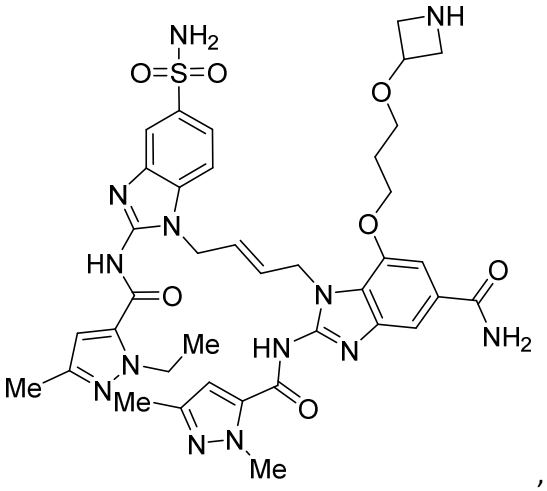
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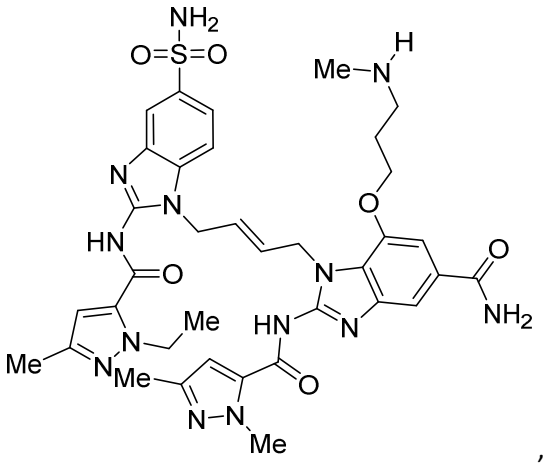
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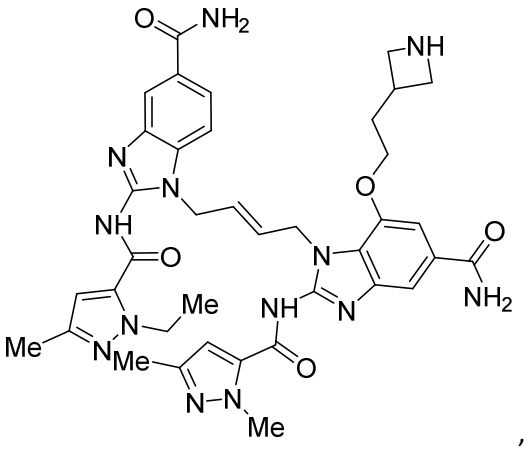
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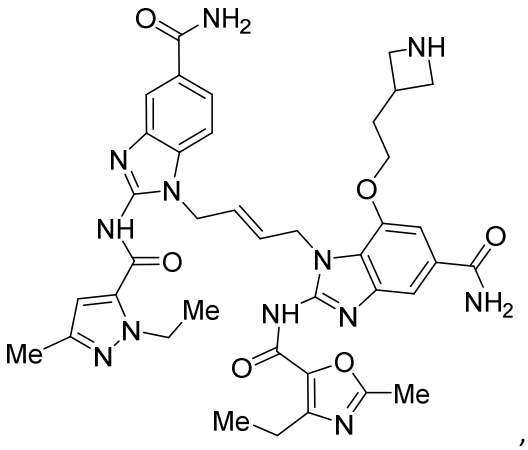
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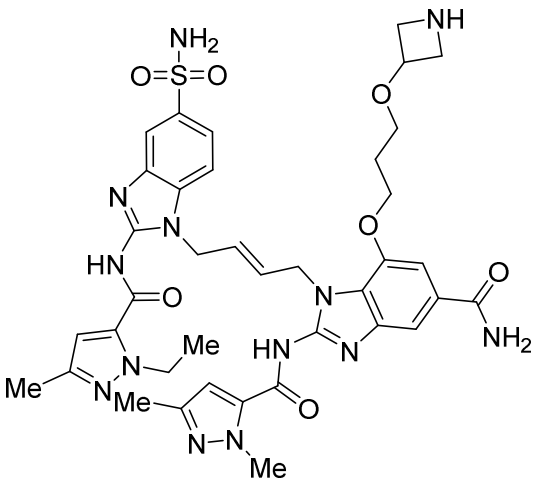
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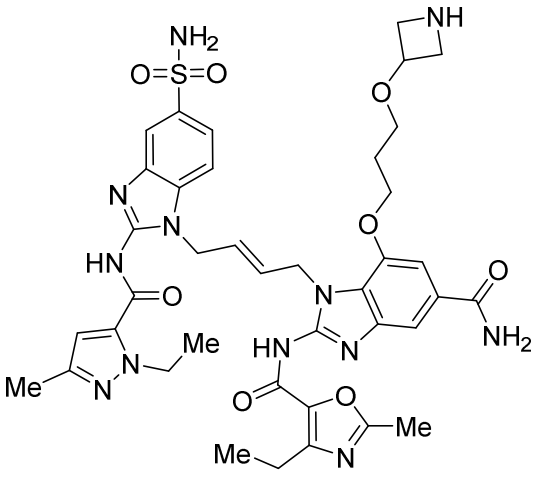

, and

pharmaceutically acceptable salts thereof.

29. An antibody-drug conjugate (ADC) having the formula:

$$\mathbf{Ab\text{-}(S^*\text{-}(D'))_p}$$

wherein:

Ab is an antibody;

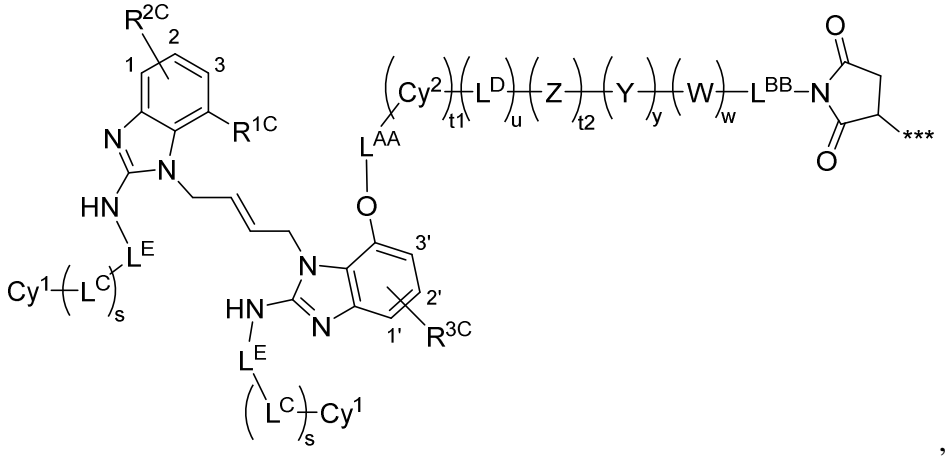
each S* is a sulfur atom from a cysteine residue of the antibody;

D' is a drug unit that is a radical of the compound of Formula (IV) according to any one of Claims 18-26; and

subscript p is an integer from 2 to 8.

30. The ADC of Claim 29, wherein the radical of the compound of Formula (IV) comprises a radical in substituent M.

31. The ADC of Claim 30, wherein D' has the structure:



, where *** indicates attachment to S*.

32. A composition comprising a distribution of the ADCs of any one of Claims 11-17 and 29-31.

33. The composition of Claim 32, further comprising and at least one pharmaceutically acceptable carrier.

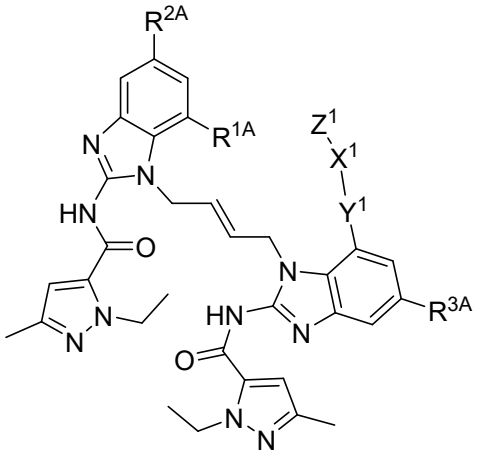
34. Use of the composition of Claim 32 or 33 in the manufacture of a medicament for treating cancer in a subject in need thereof.

35. Use of the ADC of any one of Claims 11-17 and 29-31 in the manufacture of a medicament for treating cancer in a subject in need thereof.

36. Use of the composition of Claim 32 or 33 in the manufacture of a medicament for inducing an anti-tumor immune response in a subject in need thereof.

37. Use of the ADC of any one of Claims 11-17 and 29-31 in the manufacture of a medicament for inducing an anti-tumor immune response in a subject in need thereof.

38. A compound of Formula (III):



(III)

or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} is hydrogen, hydroxyl, C_{1-6} alkoxy, $-(C_{1-6}$ alkyl)C_{1-6} alkoxy, $-(CH_2)_{nn}$-$NR^{AA}R^{BB}$;

R^{2A} and R^{3A} are independently $-CO_2H$, $-(C=O)_{mm}$-$NR^{CC}R^{DD}$, or $-(CH_2)_q$-$NR^{EE1}R^{FF1}$;

each subscript nn is independently an integer from 0 to 6;

each subscript mm is independently 0 or 1;

each subscript qq is an integer from 0 to 6;

Y^1 is $-CH_2-$, $-O-$, $-S-$, $-NH-$, or $-N(CH_3)-$;

X^1 is a C_{2-6} alkylene;

Z^1 is $-NR^{EE}R^{FF}$, $-C(=O)NR^{GG}R^{HH}$, or $-CO_2H$;

each R^{AA}, R^{BB}, R^{CC}, and R^{DD}, R^{EE1}, and R^{FF1} are independently hydrogen or C_{1-3} alkyl; and

each R^{EE}, R^{FF}, R^{GG}, and R^{HH} are independently hydrogen or C_{1-6} alkyl.