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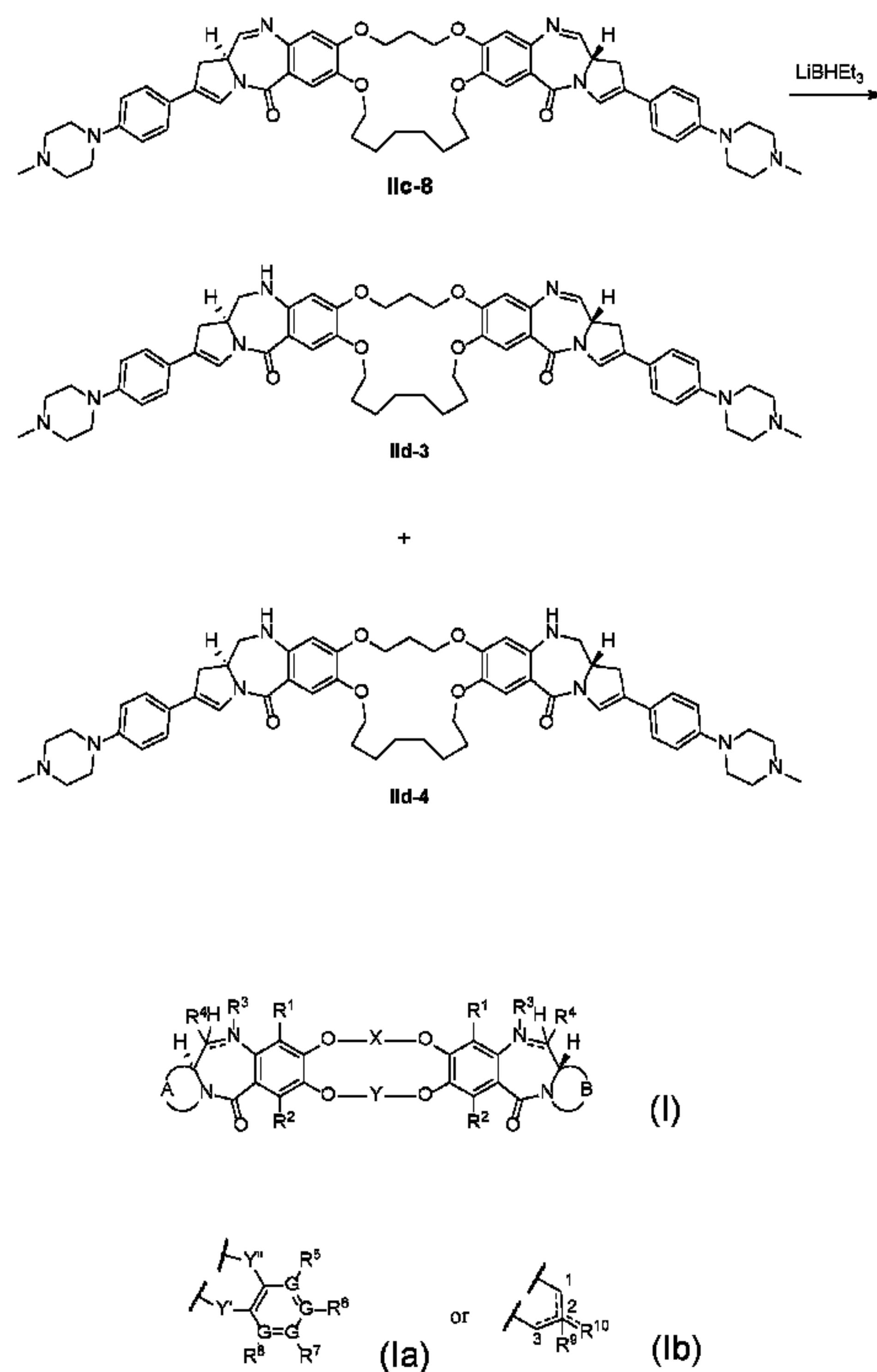
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(54) Titre : FORMES MACROCYCLIQUES DIMERES DES BENZODIAZEPINES, LEURS CONJUGUES, PREPARATION ET UTILISATIONS  
 (54) Title: MACROCYCLIC BENZODIAZEPINE DIMERS, CONJUGATES THEREOF, PREPARATION AND USES

**FIG. 10**



(57) Abrégé/Abstract:

Macrocyclic benzodiazepine dimers having a structure represented by formula (I) where A and B are independently according to formulae (Ia) or (Ib); and the other variables in formulae (I), (Ia), and (Ib) are as defined in the application. Such dimers are useful as anti-cancer agents, especially when used as the drug component in an antibody-drug conjugate (ADC).

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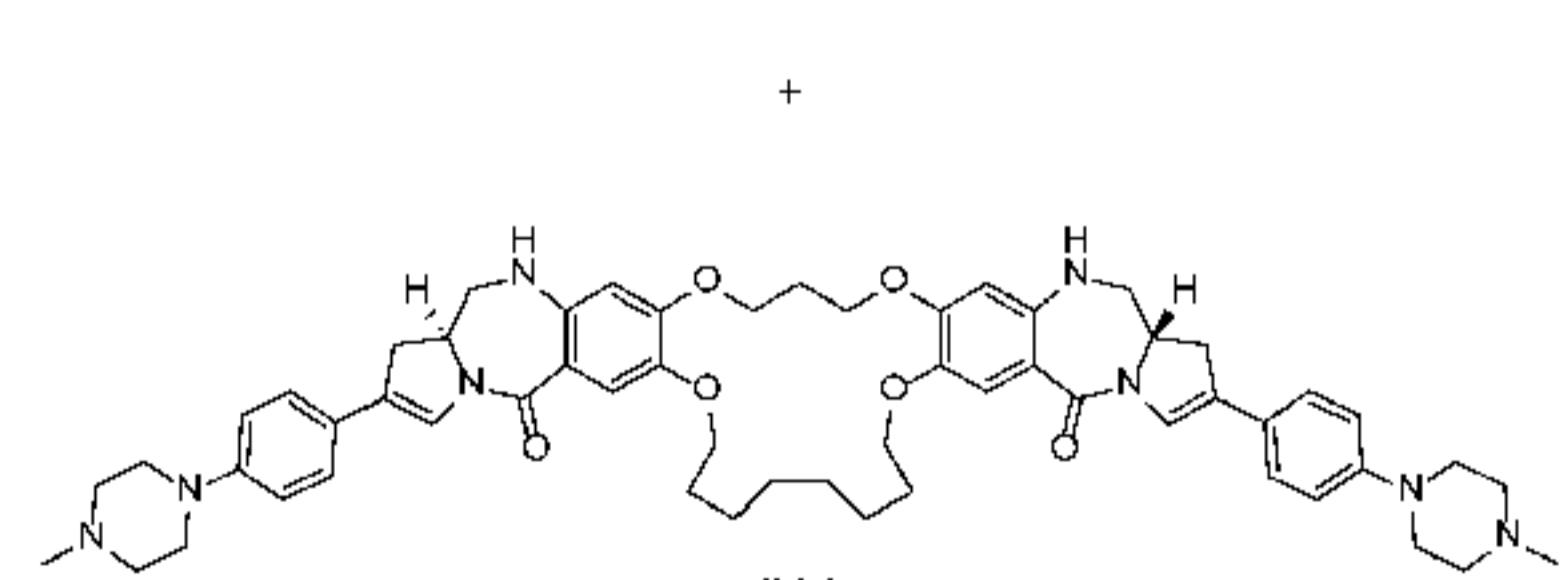
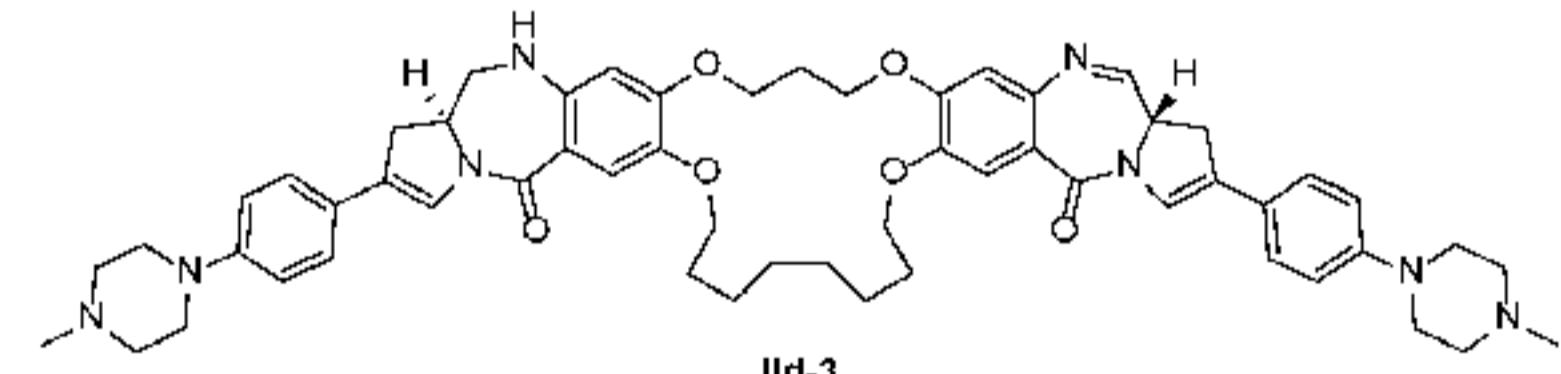
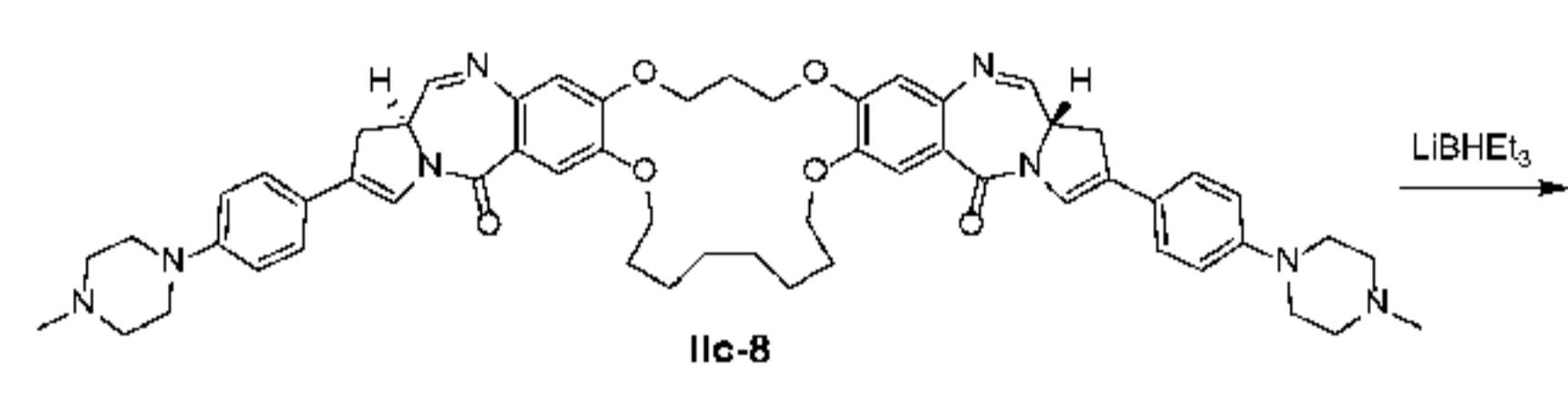
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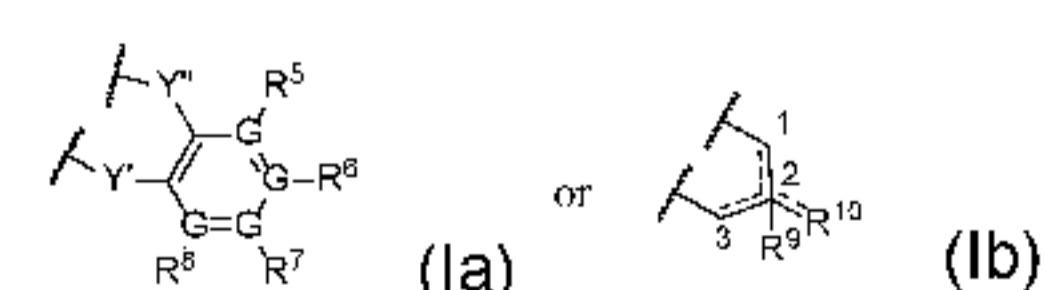
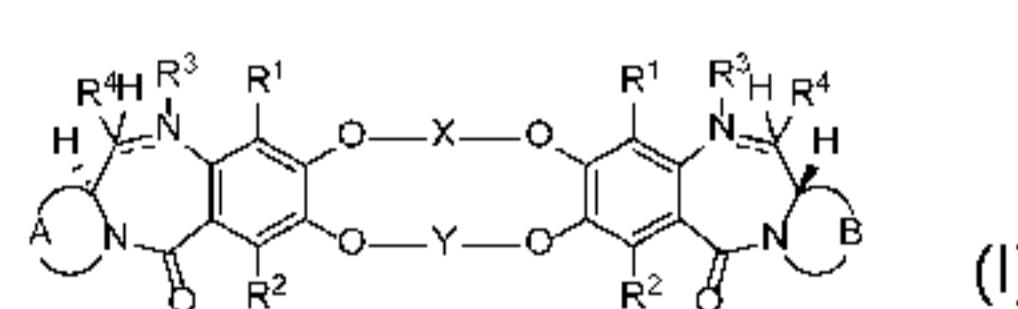
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(54) Title: MACROCYCLIC BENZODIAZEPINE DIMERS, CONJUGATES THEREOF, PREPARATION AND USES

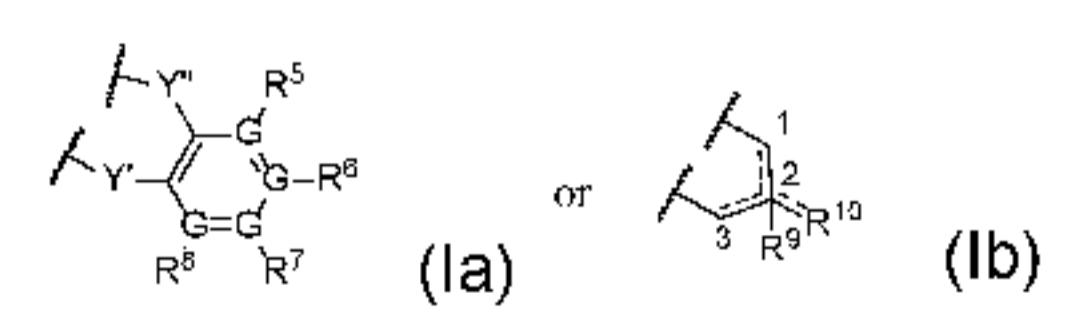
**FIG. 10**



(57) Abstract: Macrocylic benzodiazepine dimers having a structure represented by formula (I) where A and B are independently according to formulae (Ia) or (Ib); and the other variables in formulae (I), (Ia), and (Ib) are as defined in the application. Such dimers are useful as anti-cancer agents, especially when used as the drug component in an antibody-drug conjugate (ADC).



(Ia)



(Ib)

WO 2016/209951 A1

WO 2016/209951 A1



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**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

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MACROCYCLIC BENZODIAZEPINE DIMERS, CONJUGATES THEREOF,  
PREPARATION AND USES

CROSS-REFERENCE TO RELATED APPLICATIONS

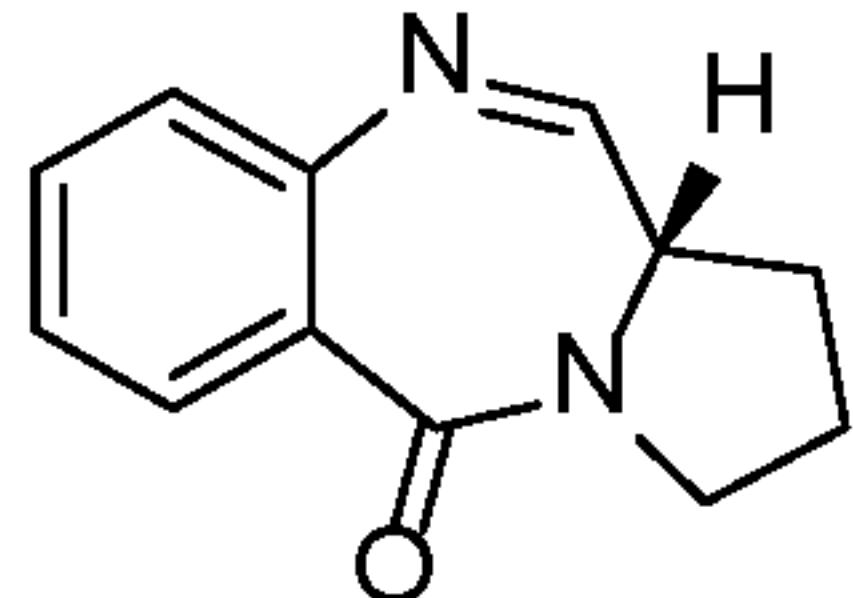
5 [0001] This application claims the benefit under 35 U.S.C. §119(e) of US Provisional Application Ser. No. 62/183,350; filed June 23, 2015; the disclosure of which is incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

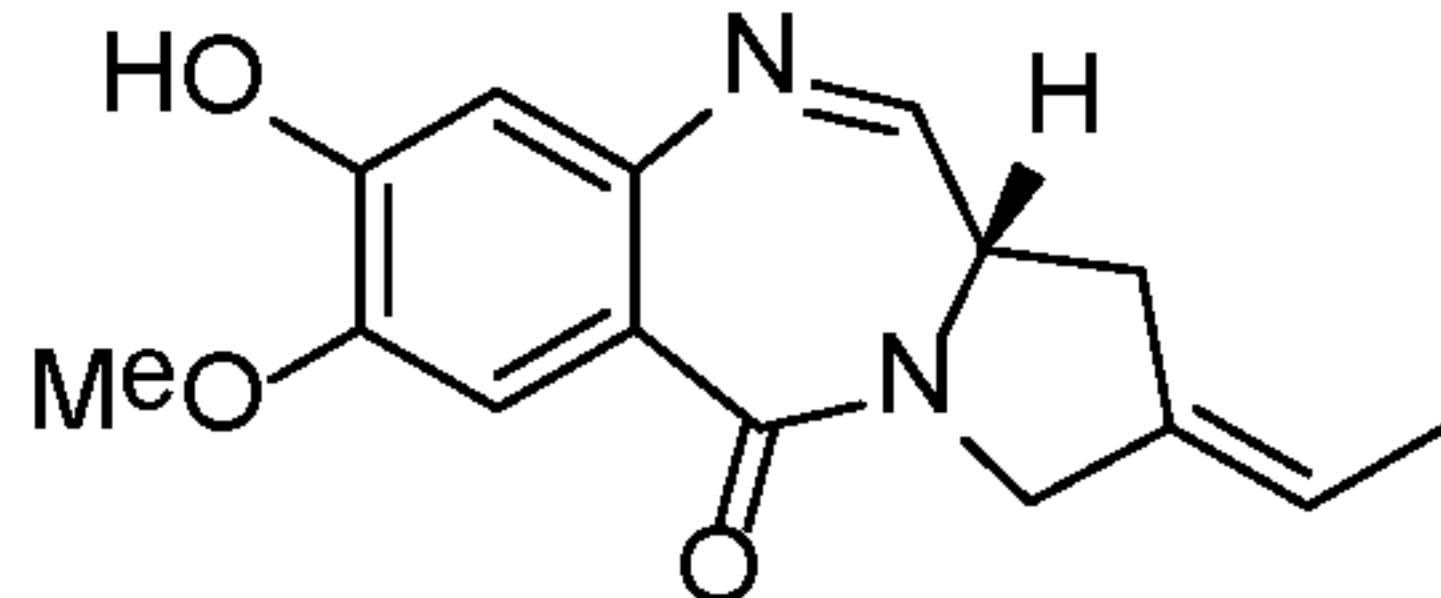
10 [0002] This invention relates to macrocyclic benzodiazepine dimers, dimer-linker compounds derived therefrom, and conjugates thereof, and methos for their preparation and use.

BACKGROUND OF THE INVENTION

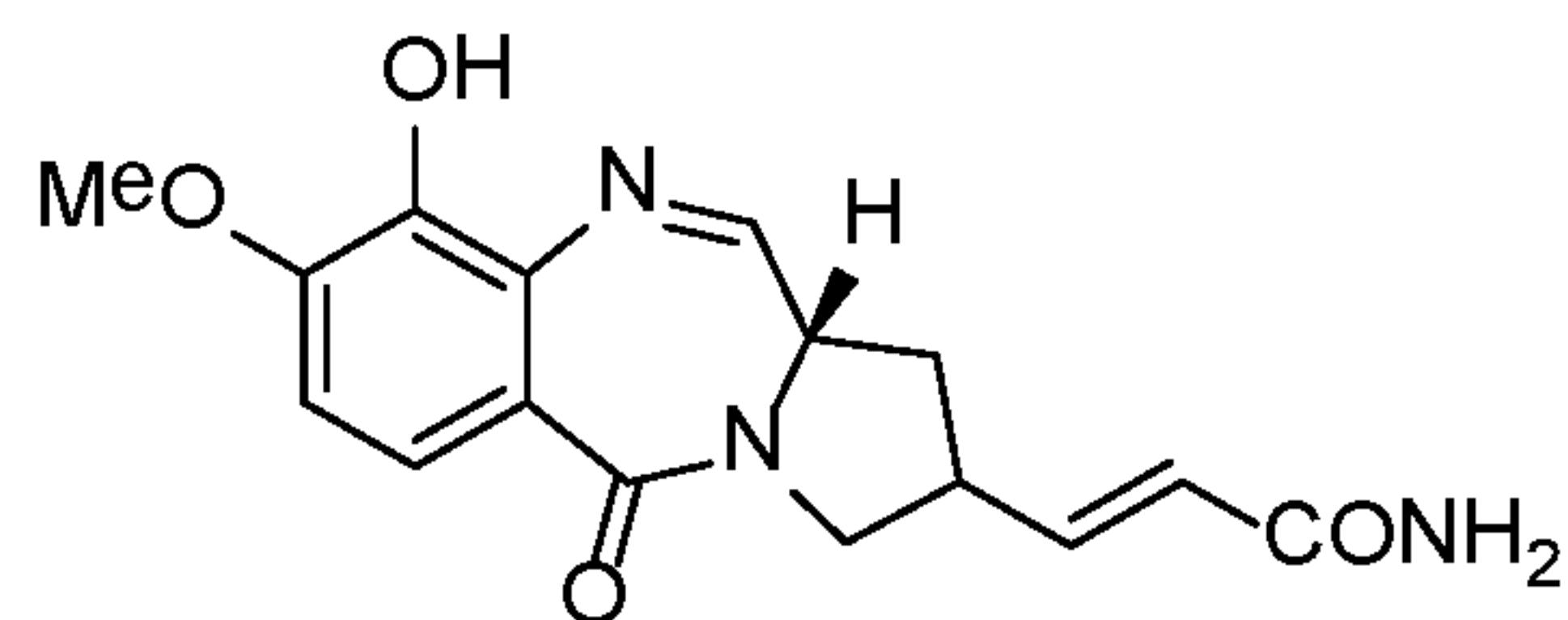
15 [0003] Some naturally occurring cytotoxins, such as tomaymycin and anthramycin, contain a benzodiazepine ring system. Reflecting the additional presence of a pyrrolidine ring fused to the diazepine ring, these compounds are often referred to as pyrrolobenzodiazepines, or PBDs.



PBD Scaffold



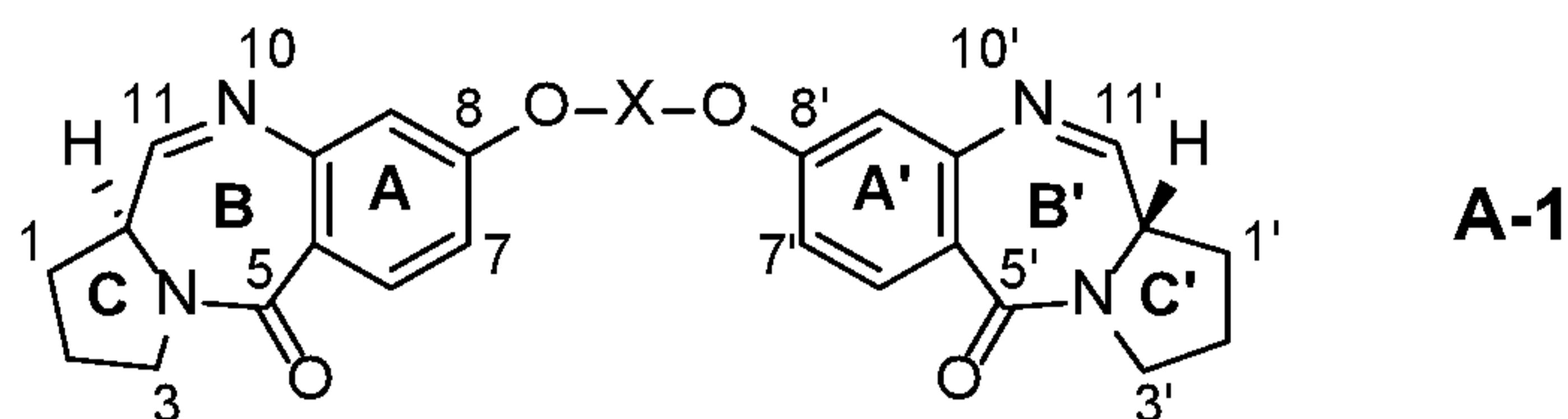
Tomaymycin



Anthramycin

20 [0004] PBDs possess antibiotic and antitumor activity, the latter trait leading to interest in them as anticancer drugs. Mechanistically, PBDs bind to the minor groove of DNA in a sequence selective manner and alkylate the DNA. The structure-activity relationship (SAR) of different substituents has been studied (Antonow *et al.* 2010; Thurston *et al.* 1999).

[0005] Additional studies have shown that PBD dimers also show special promise as anticancer agents. The core structure of a typical PBD dimer can be represented by formula A-1, where X is a bridging group connecting the two dimer halves.



[0006] As with monomeric PBDs, the dimers are DNA minor groove binder-alkylators. Being bifunctional, alkylation results in cross-linked DNA, making DNA repair more difficult. (DNA alkylation occurs via the imine group. PDBs having one of the imine groups reduced can still alkylate DNA, but cannot crosslink it. They are still biologically active, albeit generally less so.) For a review on the evolution of PBDs as antitumor agents, from naturally occurring monomers to synthetic monomers to synthetic dimers, see Hartley 2011.

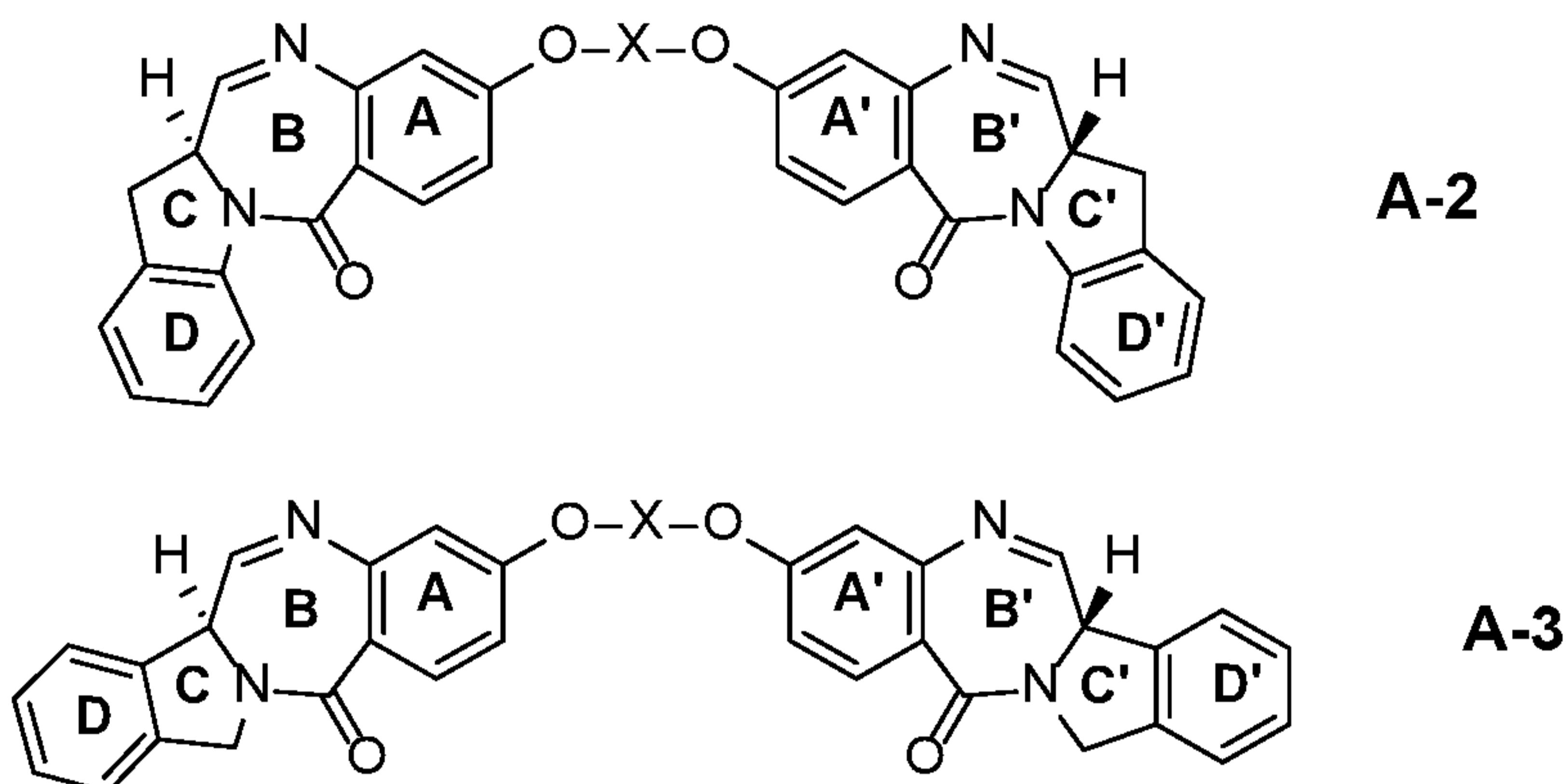
[0007] The SAR of PBD dimers has been explored via substituents on the A/A' and C/C' rings, unsaturation in the C/C' rings, the structure and length of the bridging group X, and the oxidation or reduction of the imine double bonds in rings B/B', and combinations of such features. See Bose *et al.* 1992, Gregson *et al.* 1999, Gregson *et al.* 2001a and 2001b, Gregson *et al.* 2004, Gregson *et al.* 2009, Hartley *et al.* 2012, Howard *et al.* 2007, Howard *et al.* 2009a, Howard *et al.* 2010, Howard *et al.* 2013a and 2013b, Liu *et al.* 2007, Thurston *et al.* 1996, Thurston *et al.* 2006, and Thurston *et al.* 2008. Most PBD dimers are joined via an 8/8' bridge as shown above, but a 7/7' bridge also has been disclosed (Howard *et al.* 2009b).

[0008] A type of anticancer agent that is generating strong interest is an antibody-drug conjugate (ADC, also referred to as an immunoconjugate). In an ADC, a therapeutic agent (also referred to as the drug, payload, or warhead) is covalently linked to an antibody whose antigen is expressed by a cancer cell (tumor associated antigen). The antibody, by binding to the antigen, delivers the ADC to the cancer site. There, cleavage of the covalent link (referred to as the linker) or degradation of the antibody leads to the release of the therapeutic agent. Conversely, while the ADC is circulating in the blood system, the therapeutic agent is held inactive because of its covalent linkage to the antibody. Thus, the therapeutic agent used in an ADC can be much more potent (*i.e.*, cytotoxic) than ordinary chemotherapy agents because of its localized release. For a review on ADCs, see Schrama *et al.* 2006.

[0009] PBD dimers have been proposed as the drug in an ADC. Attachment of the linker connecting to the antibody can be via a functional group located in a C/C' ring, the bridging group X, or by addition across the imine group in a B/B' ring. See Bouchard *et al.* 2013, Commercon *et al.* 2013a and 2013b, Flygare *et al.* 2013, Gauzy *et al.* 2012, Howard 2104a-

2014e, Howard *et al.* 2011, Howard *et al.* 2013c and 2013d, Howard *et al.* 2014a-2014d, Jeffrey *et al.* 2013, Jeffrey *et al.* 2014a and 2014b, and Zhao *et al.* 2014.

**[0010]** Another type of benzodiazepine dimer also has been proposed as a drug in ADCs. Structurally, this type may be viewed as a PBD dimer further having a phenyl ring fused to each of C/C' rings, as shown in formulae A-2 and A-3. See Chari *et al.* 2013, Li *et al.* 2013, Fishkin *et al.* 2014, Li *et al.* 2014.

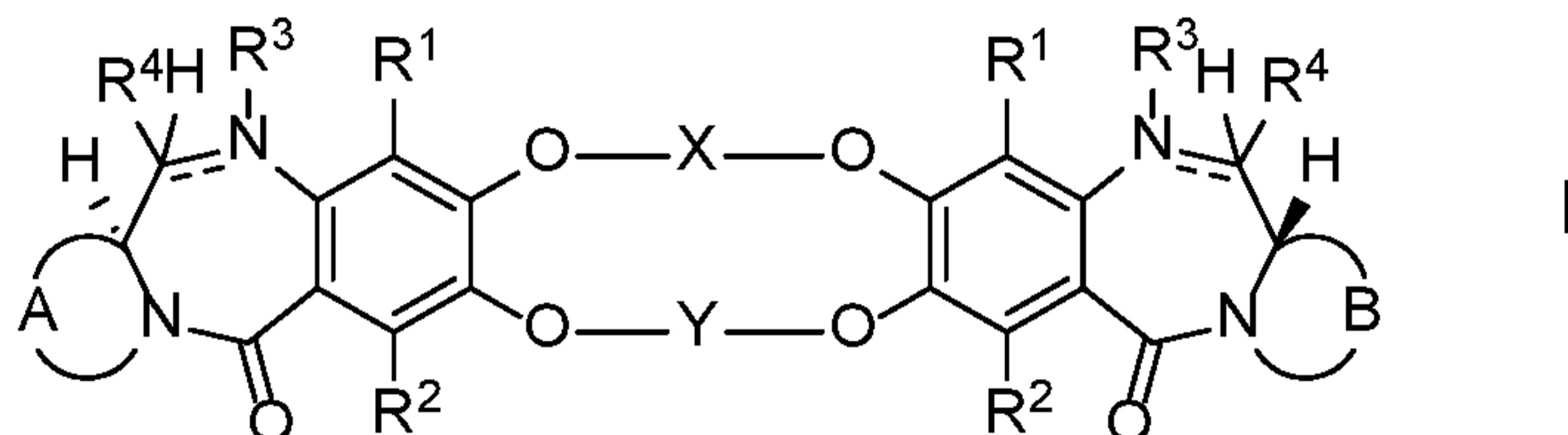


**[0011]** Benzodiazepine compounds having other ring systems, such as a tetrahydro-isoquinolino[2,1-c][1,4]benzodiazepine, also have been disclosed. Kothakonda *et al.* 2004.

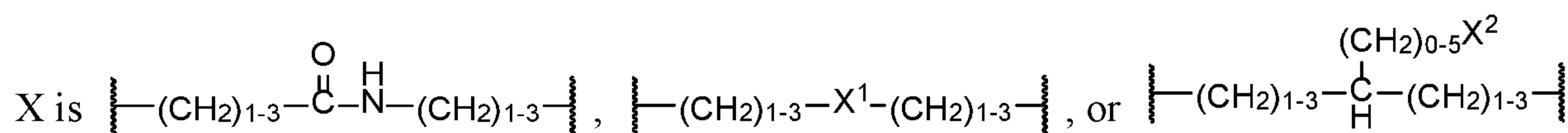
**[0012]** Full citations for the documents cited herein by first author or inventor and year are listed at the end of this specification.

#### BRIEF SUMMARY OF THE INVENTION

**[0013]** This invention provides novel benzodiazepine dimers, having both an 8/8' bridge and a 7/7' bridge, to form a macrocyclic ring structure, as represented by formula I:

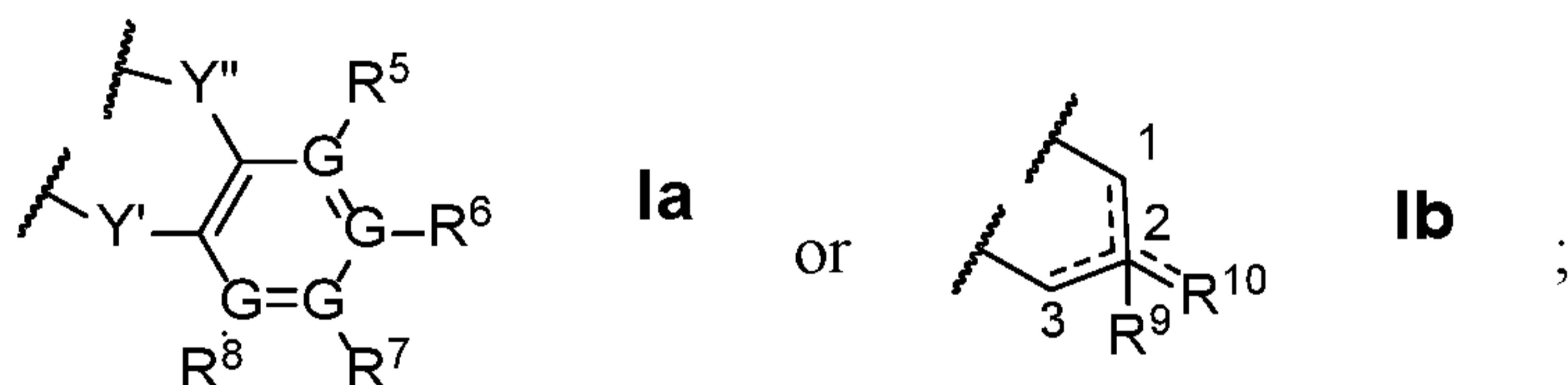


wherein



$X^1$  is  $CH_2$ ,  $O$ ,  $NH$ ,  $S(O)_{0-2}$ , 3- to 7-membered cycloalkylene or heterocycloalkylene unsubstituted or substituted with  $(CH_2)_{0-5}X^2$  or  $O(CH_2)_{2-5}X^2$ , or 5- to 6-membered arylene or heteroarylene unsubstituted or substituted with  $(CH_2)_{0-5}X^2$  or  $O(CH_2)_{2-5}X^2$ ; each  $X^2$  is independently  $Me$ ,  $CO_2H$ ,  $NH_2$ ,  $NH(C_1-C_5$  alkyl),  $N(C_1-C_5$  alkyl) $_2$ ,  $SH$ ,  $CHO$ , 5  $N(CH_2CH_2)_2N(C_1-C_3$  alkyl),  $N(CH_2CH_2)_2NH$ ,  $NHNH_2$ , or  $C(=O)NHNH_2$ ;  $Y$  is  $(CH_2)_{4-6}CH=CH(CH_2)_{4-6}$ ,  $(CH_2)_{4-6}X^1(CH_2)_{4-6}$ , or  $(CH_2)_2(OCH_2CH_2)_{2-3}$ ; each  $R^1$  and  $R^2$  is independently  $H$ ,  $F$ ,  $Cl$ ,  $Br$ ,  $OH$ ,  $C_1-C_3$  alkyl,  $O(C_1-C_3$  alkyl), cyano,  $(CH_2)_{0-5}NH_2$ , or  $NO_2$  (with both  $R^1$  and  $R^2$  preferably being  $H$ ); each double line  $---$  in a diazepine ring system independently represents a single bond or a 10 double bond; each  $R^3$  is  $H$  if the double line  $---$  to the  $N$  to which it is attached – *i.e.*, with which it is associated – is a single bond and is absent if the double line is a double bond; each  $R^4$  is  $H$ ,  $OH$ ,  $SO_3Na$ , or  $SO_3K$  if the double line  $---$  to the  $C$  to which it is attached – *i.e.*, with which it is associated – is a single bond and is absent if the double line is a double 15 bond;

$A$  and  $B$  are independently according to formula Ia or Ib



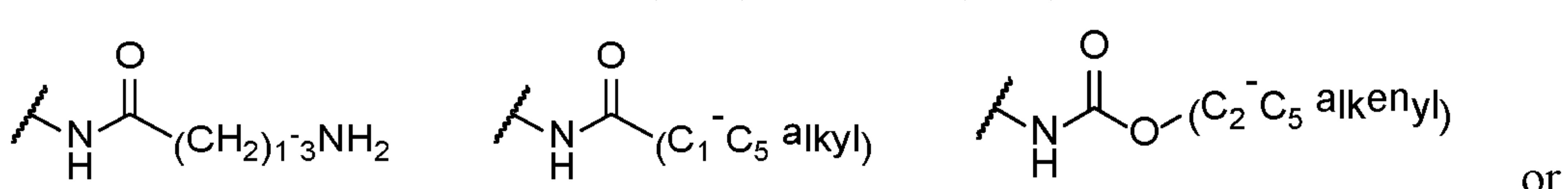
wherein, in formula Ia

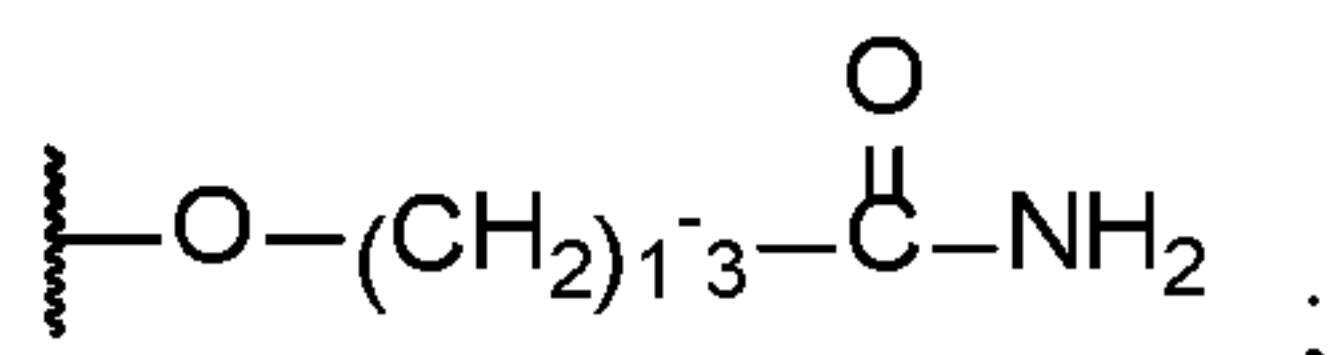
$Y'$  and  $Y''$  are independently absent,  $CH_2$ ,  $C=O$ , or  $CHR^{12}$ ; wherein each  $R^{12}$  is

20 independently  $F$ ,  $Cl$ ,  $Br$ , or  $C_1-C_3$  alkyl, with the proviso that  $Y'$  and  $Y''$  are not both absent;

each  $G$  is independently  $C$  or  $N$ , with the proviso that no more than two  $G$ s are  $N$ ; and each  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$  is independently  $H$ ,  $C_1-C_5$  alkyl,  $C\equiv C(CH_2)_{1-5}X^2$ ,  $OH$ ,  $O(C_1-C_5$  alkyl), cyano,  $NO_2$ ,  $F$ ,  $Cl$ ,  $Br$ ,  $O(CH_2CH_2O)_{1-8}(C_1-C_3$  alkyl),  $(CH_2)_{0-5}X^2$ ,  $O(CH_2)_{2-5}X^2$ , 3- to 7-membered cycloalkyl or heterocycloalkyl unsubstituted or substituted with  $(CH_2)_{0-5}X^2$  or  $O(CH_2)_{2-5}X^2$ , 5- to 6-membered aryl or heteroaryl unsubstituted or substituted with  $(CH_2)_{0-5}X^2$  or  $O(CH_2)_{2-5}X^2$ ,

25





or where a R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is attached to – *i.e.*, is associated with – a G that is N, such R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is absent;

and

5 wherein, in formula Ib,

the dotted lines indicate the optional presence of a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond;

R<sup>9</sup> is absent if a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond is present and otherwise is H; and

10 R<sup>10</sup> is H, =O, =CH<sub>2</sub>, =CH(C<sub>1</sub>-C<sub>5</sub> alkyl), CH=CH(CH<sub>2</sub>)<sub>1-5</sub>X<sup>2</sup>, C≡C(CH<sub>2</sub>)<sub>1-5</sub>X<sup>2</sup>,

C<sub>1</sub>-C<sub>5</sub> alkyl, OH, O(C<sub>1</sub>-C<sub>5</sub> alkyl), cyano, NO<sub>2</sub>, F, Cl, Br, O(CH<sub>2</sub>CH<sub>2</sub>O)<sub>1-8</sub>(C<sub>1-3</sub> alkyl), (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup>, 4- to 7-membered aryl, heteroaryl, cycloalkyl, or heterocycloalkyl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup>, O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, 3- to 7-membered cycloalkyl or heterocycloalkyl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, 5- to 6-membered aryl or heteroaryl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>;

15 or a pharmaceutically acceptable salt thereof.

**[0014]** In another embodiment, this invention provides a conjugate comprising a dimer of formula (I) covalently bonded to a targeting moiety that specifically or preferentially binds to a chemical entity on a target cell, which target cell preferably is a cancer cell. Preferably, the targeting moiety is an antibody – more preferably a monoclonal antibody; even more preferably a human monoclonal antibody – and the chemical entity is a tumor associated antigen. The tumor associated antigen can be one that is displayed on the surface of a cancer cell or one that is secreted by a cancer cell into the surrounding extracellular space. Preferably, the tumor associated antigen is one that is over-expressed by the cancer cell compared to normal cells or one that is expressed by cancer cells but not normal cells.

**[0015]** In another embodiment, there is provided a dimer according to formula (I) covalently bonded to a linker moiety having a reactive functional group, suitable for conjugation to a targeting moiety.

**[0016]** In another embodiment, there is provided a method for treating a cancer in a subject suffering from such cancer, comprising administering to the subject a therapeutically effective

amount of a dimer of this invention or a conjugate thereof with a targeting moiety. In another embodiment, there is provided the use of a dimer of this invention or a conjugate thereof with a targeting moiety for the preparation of a medicament for the treatment of cancer in a subject suffering from such cancer. A dimer of this invention or a conjugate thereof with a targeting moiety can also be used to inhibit the proliferation, *in vitro*, *ex vivo*, or *in vivo*, of cancer cells. 5 Especially, the cancer is lung, gastric, or ovarian cancer.

#### BRIEF DESCRIPTION OF THE DRAWING(S)

[0017] **FIGs. 1A-1B, 2, 3, 4A-4B, 5, 6, 7, 8, 9, and 10** show reaction schemes for the synthesis of dimers of this invention.

10 [0018] **FIGs. 11, 12, 13A-13B, 14, and 15** show reaction schemes for the preparation of dimer-linker compounds usable for the preparation of ADCs.

[0019] **FIGs. 16A-16B** show, in combination, the synthesis of another dimer of this invention.

[0020] **FIG. 17** shows the activity of two ADCs of this invention against cancer cells.

15 **DETAILED DESCRIPTION OF THE INVENTION**

#### DEFINITIONS

[0021] “Antibody” means whole antibodies and any antigen binding fragment (*i.e.*, “antigen-binding portion”) or single chain variants thereof. A whole antibody is a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (V<sub>H</sub>) and a heavy chain constant region comprising three domains, C<sub>H1</sub>, C<sub>H2</sub> and C<sub>H3</sub>. Each light chain comprises a light chain variable region (V<sub>L</sub> or V<sub>k</sub>) and a light chain constant region comprising one single domain, C<sub>L</sub>. The V<sub>H</sub> and V<sub>L</sub> regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with more conserved framework regions (FRs). Each V<sub>H</sub> and V<sub>L</sub> comprises three CDRs and four FRs, arranged from amino- to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions contain a binding domain that interacts with an antigen. The constant regions may mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system. An antibody is said to “specifically bind” to an antigen X if the 20 25 30

antibody binds to antigen X with a  $K_D$  of  $5 \times 10^{-8}$  M or less, more preferably  $1 \times 10^{-8}$  M or less, more preferably  $6 \times 10^{-9}$  M or less, more preferably  $3 \times 10^{-9}$  M or less, even more preferably  $2 \times 10^{-9}$  M or less. The antibody can be chimeric, humanized, or, preferably, human. The heavy chain constant region can be engineered to affect glycosylation type or extent, to extend antibody half-life, to enhance or reduce interactions with effector cells or the complement system, or to modulate some other property. The engineering can be accomplished by replacement, addition, or deletion of one or more amino acids or by replacement of a domain with a domain from another immunoglobulin type, or a combination of the foregoing.

**[0022]** “Antigen binding fragment” and “antigen binding portion” of an antibody (or simply “antibody portion” or “antibody fragment”) mean one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody, such as (i) a Fab fragment, a monovalent fragment consisting of the  $V_L$ ,  $V_H$ ,  $C_L$  and  $C_{H1}$  domains; (ii) a  $F(ab')_2$  fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fab’ fragment, which is essentially an Fab with part of the hinge region (see, for example, Abbas *et al.*, *Cellular and Molecular Immunology*, 6th Ed., Saunders Elsevier 2007); (iv) a Fd fragment consisting of the  $V_H$  and  $C_{H1}$  domains; (v) a Fv fragment consisting of the  $V_L$  and  $V_H$  domains of a single arm of an antibody, (vi) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a  $V_H$  domain; (vii) an isolated complementarity determining region (CDR); and (viii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Preferred antigen binding fragments are Fab,  $F(ab')_2$ , Fab’, Fv, and Fd fragments. Furthermore, although the two domains of the Fv fragment,  $V_L$  and  $V_H$ , are encoded by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the  $V_L$  and  $V_H$  regions pair to form monovalent molecules (known as single chain Fv, or scFv); see, e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also encompassed within the term “antigen-binding portion” of an antibody.

**[0023]** An “isolated antibody” means an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds antigen X is substantially free of antibodies that specifically bind antigens other than antigen X). An isolated antibody that specifically binds antigen X may, however, have cross-

reactivity to other antigens, such as antigen X molecules from other species. In certain embodiments, an isolated antibody specifically binds to human antigen X and does not cross-react with other (non-human) antigen X antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

5 [0024] “Monoclonal antibody” or “monoclonal antibody composition” means a preparation of antibody molecules of single molecular composition, which displays a single binding specificity and affinity for a particular epitope.

10 [0025] “Human antibody” means an antibody having variable regions in which both the framework and CDR regions (and the constant region, if present) are derived from human germline immunoglobulin sequences. Human antibodies may include later modifications, including natural or synthetic modifications. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, 15 “human antibody” does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

20 [0026] “Human monoclonal antibody” means an antibody displaying a single binding specificity, which has variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, human monoclonal antibodies are produced by a hybridoma that includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

25 [0027] “Aliphatic” means a straight- or branched-chain, saturated or unsaturated, non-aromatic hydrocarbon moiety having the specified number of carbon atoms (*e.g.*, as in “C<sub>3</sub> aliphatic,” “C<sub>1-5</sub> aliphatic,” “C<sub>1-C<sub>5</sub></sub> aliphatic,” or “C<sub>1</sub> to C<sub>5</sub> aliphatic,” the latter three phrases being synonymous for an aliphatic moiety having from 1 to 5 carbon atoms) or, where the number of carbon atoms is not explicitly specified, from 1 to 4 carbon atoms (2 to 4 carbons in the instance of unsaturated aliphatic moieties). A similar understanding is applied to the number of carbons in other types, as in C<sub>2-4</sub> alkene, C<sub>4-C<sub>7</sub></sub> cycloaliphatic, etc. In a similar vein, 30 a term such as “(CH<sub>2</sub>)<sub>1-3</sub>” is to be understood as shorthand for the subscript being 1, 2, or 3, so that such term represents CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>, and CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>.

**[0028]** “Alkyl” means a saturated aliphatic moiety, with the same convention for designating the number of carbon atoms being applicable. By way of illustration, C<sub>1</sub>-C<sub>4</sub> alkyl (or, synonymously, C<sub>1-4</sub> alkyl) moieties include, but are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl, *t*-butyl, 1-butyl, 2-butyl, and the like. “Alkylene” means a divalent counterpart of an alkyl group, such as CH<sub>2</sub>CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, and CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>.

**[0029]** “Alkenyl” means an aliphatic moiety having at least one carbon-carbon double bond, with the same convention for designating the number of carbon atoms being applicable. By way of illustration, C<sub>2</sub>-C<sub>4</sub> alkenyl moieties include, but are not limited to, ethenyl (vinyl), 2-propenyl (allyl or prop-2-enyl), *cis*-1-propenyl, *trans*-1-propenyl, *E*- (or *Z*-) 2-butenyl, 3-butenyl, 1,3-butadienyl (but-1,3-dienyl) and the like.

**[0030]** “Alkynyl” means an aliphatic moiety having at least one carbon-carbon triple bond, with the same convention for designating the number of carbon atoms being applicable. By way of illustration, C<sub>2</sub>-C<sub>4</sub> alkynyl groups include ethynyl (acetylenyl), propargyl (prop-2-ynyl), 1-propynyl, but-2-ynyl, and the like.

**[0031]** “Cycloaliphatic” means a saturated or unsaturated, non-aromatic hydrocarbon moiety having from 1 to 3 rings, each ring having from 3 to 8 (preferably from 3 to 6) carbon atoms. “Cycloalkyl” means a cycloaliphatic moiety in which each ring is saturated. “Cycloalkenyl” means a cycloaliphatic moiety in which at least one ring has at least one carbon-carbon double bond. “Cycloalkynyl” means a cycloaliphatic moiety in which at least one ring has at least one carbon-carbon triple bond. By way of illustration, cycloaliphatic moieties include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctyl, and adamantyl. Preferred cycloaliphatic moieties are cycloalkyl ones, especially cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. “Cycloalkylene” means a divalent counterpart of a cycloalkyl group.

**[0032]** “Heterocycloaliphatic” means a cycloaliphatic moiety wherein, in at least one ring thereof, up to three (preferably 1 to 2) carbons have been replaced with a heteroatom independently selected from N, O, or S, where the N and S optionally may be oxidized and the N optionally may be quaternized. Similarly, “heterocycloalkyl,” “heterocycloalkenyl,” and “heterocycloalkynyl” means a cycloalkyl, cycloalkenyl, or cycloalkynyl moiety, respectively, in which at least one ring thereof has been so modified. Exemplary heterocycloaliphatic moieties include aziridinyl, azetidinyl, 1,3-dioxanyl, oxetanyl, tetrahydrofuryl, pyrrolidinyl, piperidinyl, piperazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl

sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolanyl, tetrahydro-1,1-dioxothienyl, 1,4-dioxanyl, thietanyl, and the like. “Heterocycloalkylene” means a divalent counterpart of a heterocycloalkyl group.

**[0033]** “Alkoxy,” “aryloxy,” “alkylthio,” and “arylthio” mean –O(alkyl), -O(aryl), -S(alkyl), and -S(aryl), respectively. Examples are methoxy, phenoxy, methylthio, and phenylthio, respectively.

**[0034]** “Halogen” or “halo” means fluorine, chlorine, bromine or iodine.

**[0035]** “Aryl” means a hydrocarbon moiety having a mono-, bi-, or tricyclic ring system wherein each ring has from 3 to 7 carbon atoms and at least one ring is aromatic. The rings in the ring system may be fused to each other (as in naphthyl) or bonded to each other (as in biphenyl) and may be fused or bonded to non-aromatic rings (as in indanyl or cyclohexyl-phenyl). By way of further illustration, aryl moieties include, but are not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthracenyl, and acenaphthyl. “Arylene” means a divalent counterpart of an aryl group, for example 1,2-phenylene, 1,3-phenylene, or 1,4-phenylene.

**[0036]** “Heteroaryl” means a moiety having a mono-, bi-, or tricyclic ring system wherein each ring has from 3 to 7 carbon atoms and at least one ring is an aromatic ring containing from 1 to 4 heteroatoms independently selected from from N, O, or S, where the N and S optionally may be oxidized and the N optionally may be quaternized. Such at least one heteroatom containing aromatic ring may be fused to other types of rings (as in benzofuranyl or tetrahydroisoquinolyl) or directly bonded to other types of rings (as in phenylpyridyl or 2-cyclopentylypyridyl). By way of further illustration, heteroaryl moieties include pyrrolyl, furanyl, thiophenyl (thienyl), imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, pyridyl, N-oxopyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, quinolinyl, isoquinolynyl, quinazolinyl, cinnolinyl, quinozalinyl, naphthyridinyl, benzofuranyl, indolyl, benzothiophenyl, oxadiazolyl, thiadiazolyl, phenothiazolyl, benzimidazolyl, benzotriazolyl, dibenzofuranyl, carbazolyl, dibenzothiophenyl, acridinyl, and the like. “Heteroarylene” means a divalent counterpart of a heteroaryl group.

**[0037]** Where it is indicated that a moiety may be substituted, such as by use of “unsubstituted or substituted” or “optionally substituted” phrasing as in “unsubstituted or substituted C<sub>1</sub>-C<sub>5</sub> alkyl” or “optionally substituted heteroaryl,” such moiety may have one or

more independently selected substituents, preferably one to five in number, more preferably one or two in number. Substituents and substitution patterns can be selected by one of ordinary skill in the art, having regard for the moiety to which the substituent is attached, to provide compounds that are chemically stable and that can be synthesized by techniques known in the art as well as the methods set forth herein.

5 [0038] “Arylalkyl,” (heterocycloaliphatic)alkyl,” “arylalkenyl,” “arylalkynyl,” “biarylalkyl,” and the like mean an alkyl, alkenyl, or alkynyl moiety, as the case may be, substituted with an aryl, heterocycloaliphatic, biaryl, etc., moiety, as the case may be, with the open (unsatisfied) valence at the alkyl, alkenyl, or alkynyl moiety, for example as in benzyl, 10 phenethyl, N-imidazoylethyl, N-morpholinoethyl, and the like. Conversely, “alkylaryl,” “alkenylcycloalkyl,” and the like mean an aryl, cycloalkyl, etc., moiety, as the case may be, substituted with an alkyl, alkenyl, etc., moiety, as the case may be, for example as in methylphenyl (tolyl) or allylcyclohexyl. “Hydroxyalkyl,” “haloalkyl,” “alkylaryl,” “cyanoaryl,” and the like mean an alkyl, aryl, etc., moiety, as the case may be, substituted with 15 one or more of the identified substituent (hydroxyl, halo, etc., as the case may be).

20 [0039] For example, permissible substituents include, but are not limited to, alkyl (especially methyl or ethyl), alkenyl (especially allyl), alkynyl, aryl, heteroaryl, cycloaliphatic, heterocycloaliphatic, halo (especially fluoro), haloalkyl (especially trifluoromethyl), hydroxyl, hydroxyalkyl (especially hydroxyethyl), cyano, nitro, alkoxy, -O(hydroxyalkyl), -O(haloalkyl) (especially -OCF<sub>3</sub>), -O(cycloalkyl), -O(heterocycloalkyl), -O(aryl), alkylthio, arylthio, =O, =NH, =N(alkyl), =NOH, =NO(alkyl), -C(=O)(alkyl), -C(=O)H, -CO<sub>2</sub>H, -C(=O)NHOH, -C(=O)O(alkyl), -C(=O)O(hydroxyalkyl), -C(=O)NH<sub>2</sub>, -C(=O)NH(alkyl), -C(=O)N(alkyl)<sub>2</sub>, -OC(=O)(alkyl), -OC(=O)(hydroxyalkyl), -OC(=O)O(alkyl), -OC(=O)O(hydroxyalkyl), -OC(=O)NH<sub>2</sub>, -OC(=O)NH(alkyl), -OC(=O)N(alkyl)<sub>2</sub>, azido, -NH<sub>2</sub>, -NH(alkyl), -N(alkyl)<sub>2</sub>, 25 -NH(aryl), -NH(hydroxyalkyl), -NHC(=O)(alkyl), -NHC(=O)H, -NHC(=O)NH<sub>2</sub>, -NHC(=O)NH(alkyl), -NHC(=O)N(alkyl)<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -OSO<sub>2</sub>(alkyl), -SH, -S(alkyl), -S(aryl), -S(cycloalkyl), -S(=O)alkyl, -SO<sub>2</sub>(alkyl), -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH(alkyl), -SO<sub>2</sub>N(alkyl)<sub>2</sub>, and the like.

30 [0040] Where the moiety being substituted is an aliphatic moiety, preferred substituents are aryl, heteroaryl, cycloaliphatic, heterocycloaliphatic, halo, hydroxyl, cyano, nitro, alkoxy, -O(hydroxyalkyl), -O(haloalkyl), -O(cycloalkyl), -O(heterocycloalkyl), -O(aryl), alkylthio, arylthio, =O, =NH, =N(alkyl), =NOH, =NO(alkyl), -CO<sub>2</sub>H, -C(=O)NHOH, -C(=O)O(alkyl),

-C(=O)O(hydroxyalkyl), -C(=O)NH<sub>2</sub>, -C(=O)NH(alkyl), -C(=O)N(alkyl)<sub>2</sub>, -OC(=O)(alkyl), -OC(=O)(hydroxyalkyl), -OC(=O)O(alkyl), -OC(=O)O(hydroxyalkyl), -OC(=O)NH<sub>2</sub>, -OC(=O)NH(alkyl), -OC(=O)N(alkyl)<sub>2</sub>, azido, -NH<sub>2</sub>, -NH(alkyl), -N(alkyl)<sub>2</sub>, -NH(aryl), -NH(hydroxyalkyl), -NHC(=O)(alkyl), -NHC(=O)H, -NHC(=O)NH<sub>2</sub>, -NHC(=O)NH(alkyl), 5 -NHC(=O)N(alkyl)<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -OSO<sub>2</sub>(alkyl), -SH, -S(alkyl), -S(aryl), -S(=O)alkyl, -S(cycloalkyl), -SO<sub>2</sub>(alkyl), -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH(alkyl), and -SO<sub>2</sub>N(alkyl)<sub>2</sub>. More preferred substituents are halo, hydroxyl, cyano, nitro, alkoxy, -O(aryl), =O, =NOH, =NO(alkyl), -OC(=O)(alkyl), -OC(=O)O(alkyl), -OC(=O)NH<sub>2</sub>, -OC(=O)NH(alkyl), -OC(=O)N(alkyl)<sub>2</sub>, azido, -NH<sub>2</sub>, -NH(alkyl), -N(alkyl)<sub>2</sub>, -NH(aryl), -NHC(=O)(alkyl), -NHC(=O)H, 10 -NHC(=O)NH<sub>2</sub>, -NHC(=O)NH(alkyl), -NHC(=O)N(alkyl)<sub>2</sub>, and -NHC(=NH)NH<sub>2</sub>. Especially preferred are phenyl, cyano, halo, hydroxyl, nitro, C<sub>1</sub>-C<sub>4</sub>alkyoxy, O(C<sub>2</sub>-C<sub>4</sub> alkylene)OH, and O(C<sub>2</sub>-C<sub>4</sub> alkylene)halo.

**[0041]** Where the moiety being substituted is a cycloaliphatic, heterocycloaliphatic, aryl, or heteroaryl moiety, preferred substituents are alkyl, alkenyl, alkynyl, halo, haloalkyl, hydroxyl, 15 hydroxyalkyl, cyano, nitro, alkoxy, -O(hydroxyalkyl), -O(haloalkyl), -O(aryl), -O(cycloalkyl), -O(heterocycloalkyl), alkylthio, arylthio, -C(=O)(alkyl), -C(=O)H, -CO<sub>2</sub>H, -C(=O)NHOH, -C(=O)O(alkyl), -C(=O)O(hydroxyalkyl), -C(=O)NH<sub>2</sub>, -C(=O)NH(alkyl), -C(=O)N(alkyl)<sub>2</sub>, -OC(=O)(alkyl), -OC(=O)(hydroxyalkyl), -OC(=O)O(alkyl), -OC(=O)O(hydroxyalkyl), -OC(=O)NH<sub>2</sub>, -OC(=O)NH(alkyl), -OC(=O)N(alkyl)<sub>2</sub>, azido, -NH<sub>2</sub>, -NH(alkyl), -N(alkyl)<sub>2</sub>, 20 -NH(aryl), -NH(hydroxyalkyl), -NHC(=O)(alkyl), -NHC(=O)H, -NHC(=O)NH<sub>2</sub>, -NHC(=O)NH(alkyl), -NHC(=O)N(alkyl)<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -OSO<sub>2</sub>(alkyl), -SH, -S(alkyl), -S(aryl), -S(cycloalkyl), -S(=O)alkyl, -SO<sub>2</sub>(alkyl), -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH(alkyl), and -SO<sub>2</sub>N(alkyl)<sub>2</sub>. More preferred substituents are alkyl, alkenyl, halo, haloalkyl, hydroxyl, hydroxyalkyl, cyano, nitro, alkoxy, -O(hydroxyalkyl), -C(=O)(alkyl), -C(=O)H, -CO<sub>2</sub>H, 25 -C(=O)NHOH, -C(=O)O(alkyl), -C(=O)O(hydroxyalkyl), -C(=O)NH<sub>2</sub>, -C(=O)NH(alkyl), -C(=O)N(alkyl)<sub>2</sub>, -OC(=O)(alkyl), -OC(=O)(hydroxyalkyl), -OC(=O)O(alkyl), -OC(=O)O(hydroxyalkyl), -OC(=O)NH<sub>2</sub>, -OC(=O)NH(alkyl), -OC(=O)N(alkyl)<sub>2</sub>, -NH<sub>2</sub>, -NH(alkyl), -N(alkyl)<sub>2</sub>, -NH(aryl), -NHC(=O)(alkyl), -NHC(=O)H, -NHC(=O)NH<sub>2</sub>, -NHC(=O)NH(alkyl), -NHC(=O)N(alkyl)<sub>2</sub>, and -NHC(=NH)NH<sub>2</sub>. Especially preferred are C<sub>1</sub>- 30 C<sub>4</sub> alkyl, cyano, nitro, halo, and C<sub>1</sub>-C<sub>4</sub>alkoxy.

**[0042]** Where a range is stated, as in “C<sub>1</sub>-C<sub>5</sub> alkyl” or “5 to 10%,” such range includes the end points of the range, as in C<sub>1</sub> and C<sub>5</sub> in the first instance and 5% and 10% in the second instance.

**[0043]** Unless particular stereoisomers are specifically indicated (e.g., by a bolded or 5 dashed bond at a relevant stereocenter in a structural formula, by depiction of a double bond as having E or Z configuration in a structural formula, or by use stereochemistry-designating nomenclature), all stereoisomers are included within the scope of the invention, as pure compounds as well as mixtures thereof. Unless otherwise indicated, individual enantiomers, diastereomers, geometrical isomers, and combinations and mixtures thereof are all 10 encompassed by this invention.

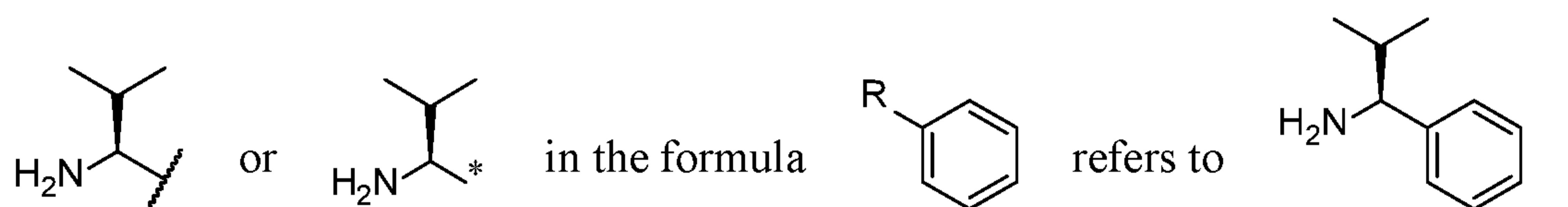
**[0044]** Those skilled in the art will appreciate that compounds may have tautomeric forms (e.g., keto and enol forms), resonance forms, and zwitterionic forms that are equivalent to those depicted in the structural formulae used herein and that the structural formulae encompass such tautomeric, resonance, or zwitterionic forms.

**[0045]** “Pharmaceutically acceptable ester” means an ester that hydrolyzes *in vivo* (for 15 example in the human body) to produce the parent compound or a salt thereof or has *per se* activity similar to that of the parent compound. Suitable esters include C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>2</sub>-C<sub>5</sub> alkenyl or C<sub>2</sub>-C<sub>5</sub> alkynyl esters, especially methyl, ethyl or n-propyl.

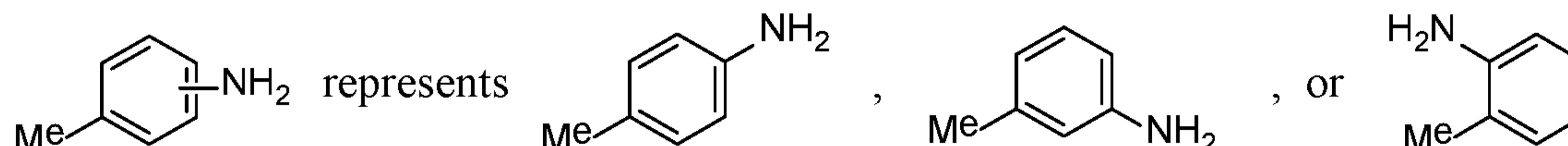
**[0046]** “Pharmaceutically acceptable salt” means a salt of a compound suitable for 20 pharmaceutical formulation. Where a compound has one or more basic groups, the salt can be an acid addition salt, such as a sulfate, hydrobromide, tartrate, mesylate, maleate, citrate, phosphate, acetate, pamoate (embonate), hydroiodide, nitrate, hydrochloride, lactate, methyl-sulfate, fumarate, benzoate, succinate, mesylate, lactobionate, suberate, tosylate, and the like. Where a compound has one or more acidic groups, the salt can be a salt such as a calcium salt, 25 potassium salt, magnesium salt, meglumine salt, ammonium salt, zinc salt, piperazine salt, tromethamine salt, lithium salt, choline salt, diethylamine salt, 4-phenylcyclohexylamine salt, benzathine salt, sodium salt, tetramethylammonium salt, and the like. Polymorphic crystalline forms and solvates are also encompassed within the scope of this invention.

**[0047]** In the formulae of this specification, a wavy line (~~~~) transverse to a bond or an 30 asterisk (\*) at the end of the bond denotes a covalent attachment site. For instance, a statement

that R is



[0048] In the formulae of this specification, a bond traversing an aromatic ring between two carbons thereof means that the group attached to the bond may be located at any of the 5 available positions of the aromatic ring. By way of illustration, the formula



## DIMERS

[0049] Preferably, in formula I, X is a C7/C7' bridge that is 3 or 5 atoms long and Y is a C8/C8' bridge that is 7, 8, 9, 10, 11, or 12 atoms long, where the atoms in each bridge are 10 selected from C, O, N, and S, especially where all the bridge atoms are C or all are C except for one O, N, or S. More preferably, where X is 3 atoms long, Y is 7, 8, 9, or 10 atoms long (even more preferably 8 to 10 atoms long) and where X is 5 atoms long, Y is 11 or 12 atoms long.

[0050] In a preferred embodiment, in formula I, X is  $(CH_2)_3$  or  $(CH_2)_5$ .

[0051] In another preferred embodiment, in formulae I, IIa, IIb, or IIc (the latter three 15 formulae shown hereinbelow), Y is  $(CH_2)_{7-12}$ , more preferably  $(CH_2)_8$ .

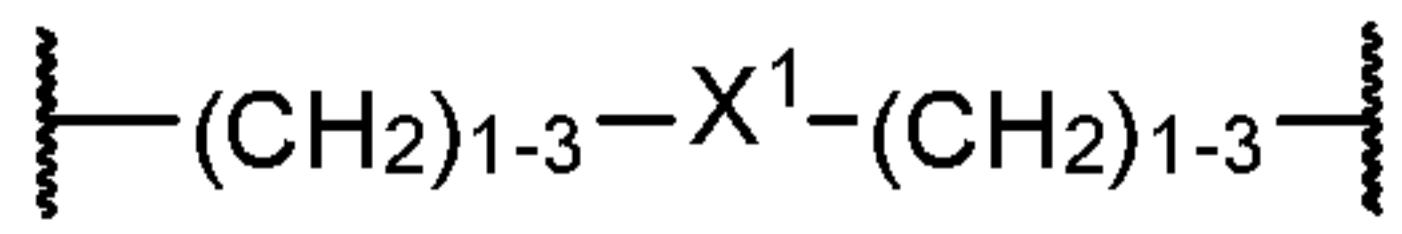
[0052] In formula I, preferred combinations of X and Y are X equals  $(CH_2)_3$  while Y equals  $(CH_2)_{8-10}$  and X equals  $(CH_2)_5$  while Y equals  $(CH_2)_{11-12}$ . A more preferred combination is X equals  $(CH_2)_3$  and Y equals  $(CH_2)_8$ .

[0053] In another preferred embodiment, in formulae I, IIa, IIb, or IIc, Y is 20  $(CH_2)_2(OCH_2CH_2)_{2-3}$ , especially  $(CH_2)_2(OCH_2CH_2)_2$ .

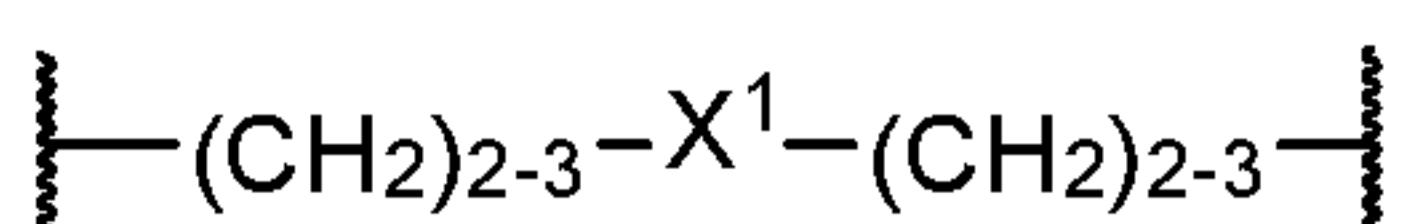
[0054] In another preferred embodiment, in formulae I, IIa, IIb, or IIc, Y is  $(CH_2)_4NH(CH_2)_4$  or  $(CH_2)_4N(CH_2(p.C_6H_4NH_2))(CH_2)_4$ .

[0055] In the formulae where it appears,  $X^2$  preferably is  $NH_2$ ,  $SH$ , or  $CO_2H$ .

[0056] Preferably, where X in formula I is



and  $X^1$  is O or NH, then X is



[0057] In a preferred embodiment, dimers of this invention have a structure represented by formula IIa, wherein x is 3 or 5 (preferably 3); Y is  $(CH_2)_{7-12}$ ,  $(CH_2)_2(OCH_2CH_2)_{1-3}$ ,  $(CH_2)_{2-4}NH(CH_2)_{2-4}$  or  $(CH_2)_{2-4}NH((CH_2)_{0-1}phenyl)(CH_2)_{2-4}$  where the phenyl group is optionally substituted with  $NH_2$ ; A is A1, A2, or A3 as depicted below, and B is B1, B2, or B3 as depicted below. Representative species are listed in Table 1.

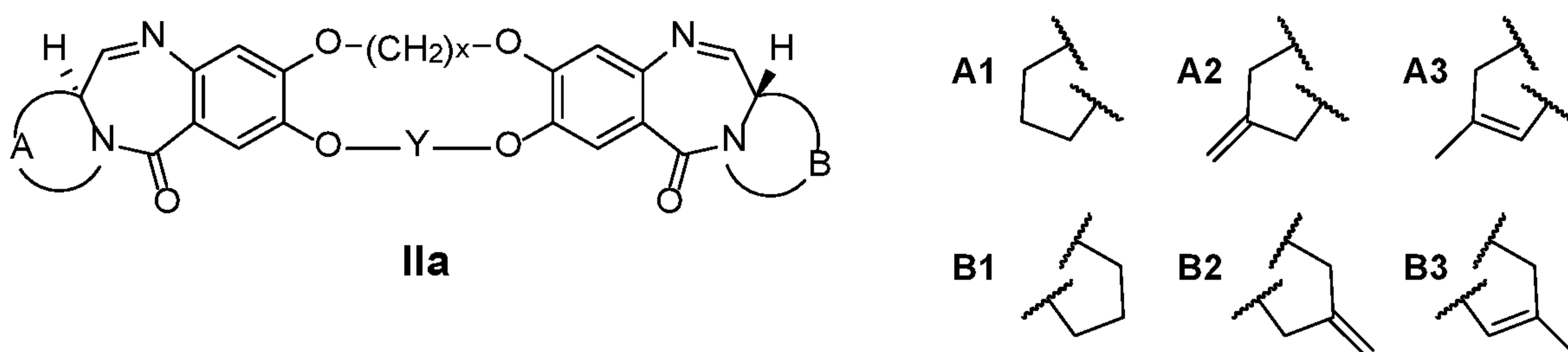


Table 1 – Examples of Dimers According to Formula IIa

Dimer	x	Y	A	B
IIa-1	3	$(CH_2)_7$	A1	B1
IIa-2	3	$(CH_2)_8$	A1	A1
IIa-3	3	$(CH_2)_9$	A1	A1
IIa-4	3	$(CH_2)_{10}$	A1	B1
IIa-5	3	$(CH_2)_{11}$	A1	A1
IIa-6	3	$(CH_2)_{12}$	A1	A1
IIa-7	5	$(CH_2)_{11}$	A1	B1
IIa-8	5	$(CH_2)_{12}$	A1	A1
IIa-9	3	$(CH_2)_4\text{---}\text{CH}_2\text{---}(CH_2)_4$	A1	A1
IIa-10	3	$(CH_2)_4\text{---}\text{CH}_2\text{---}(CH_2)_4$	A2	B2
IIa-11	3	$(CH_2)_{10}$	A3	B3
IIa-12	3	$(CH_2)_8$	A2	B2
IIa-13	3	$(CH_2)_2(OCH_2CH_2)_2$	A2	B2
IIa-14	3	$(CH_2)_4NH(CH_2)_4$	A2	B2

[0058] In another preferred embodiment, dimers of this invention have a structure represented by formula IIb, where x is 3 or 5 (preferably 3), each Y' is independently absent or CH<sub>2</sub>, Y is (CH<sub>2</sub>)<sub>7-12</sub>, (CH<sub>2</sub>)<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-3</sub>, (CH<sub>2</sub>)<sub>2-4</sub>NH(CH<sub>2</sub>)<sub>2-4</sub> or (CH<sub>2</sub>)<sub>2-4</sub>NH((CH<sub>2</sub>)<sub>0-1</sub>phenyl)(CH<sub>2</sub>)<sub>2-4</sub> where the phenyl group is optionally substituted with NH<sub>2</sub>; and R<sup>40</sup> and R<sup>41</sup> are independently H, Cl, Br, OH, O(C<sub>1-3</sub> alkyl), NH<sub>2</sub>, or C<sub>1-3</sub> alkyl. Representative species are listed in Table 2.

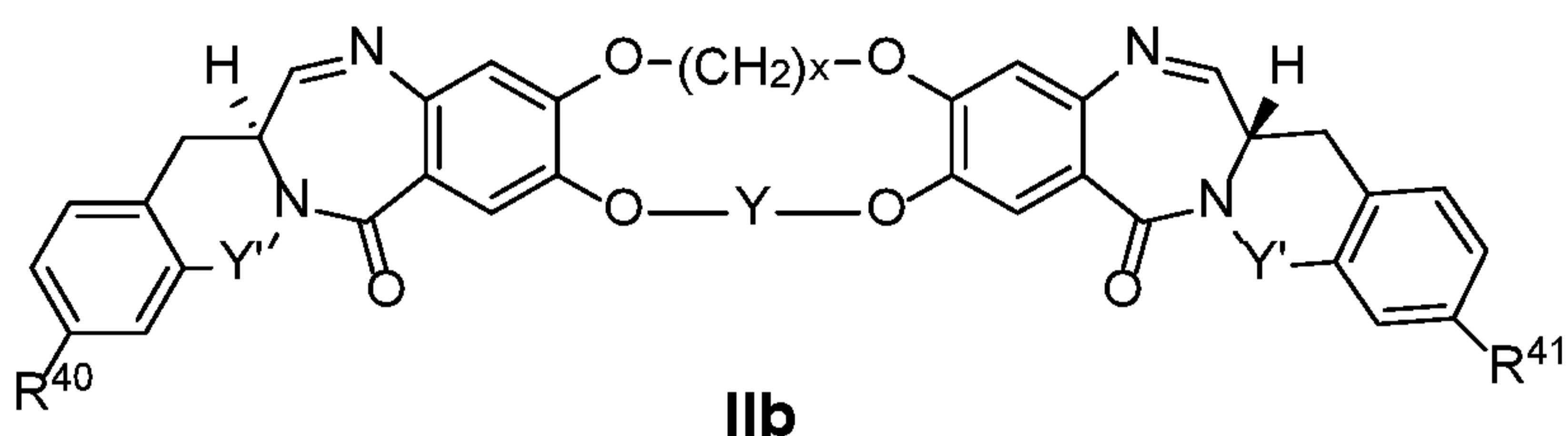


Table 2 – Examples of Dimers According to Formula IIb

Dimer	x	Y'	Y	R <sup>40</sup>	R <sup>41</sup>
IIb-1	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>8</sub>	H	H
IIb-2	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>10</sub>	H	H
IIb-3	3	absent	(CH <sub>2</sub> ) <sub>10</sub>	H	H
IIb-4	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> (OCH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub>	H	H
IIb-5	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>4</sub> NH(CH <sub>2</sub> ) <sub>4</sub>	H	H
IIb-6	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>8</sub>	H	NH <sub>2</sub>
IIb-7	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> (OCH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub>	H	NH <sub>2</sub>
IIb-8	3	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>4</sub> -N-(CH <sub>2</sub> ) <sub>4</sub> 	H	H

[0059] In another preferred embodiment, dimers of this invention have a structure represented by formula IIc, where x is 3 or 5 (preferably 3), Y is (CH<sub>2</sub>)<sub>7-12</sub>, (CH<sub>2</sub>)<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-3</sub>, (CH<sub>2</sub>)<sub>2-4</sub>NH(CH<sub>2</sub>)<sub>2-4</sub> or (CH<sub>2</sub>)<sub>2-4</sub>NH((CH<sub>2</sub>)<sub>0-1</sub>phenyl)(CH<sub>2</sub>)<sub>2-4</sub> where the phenyl group is optionally substituted with NH<sub>2</sub>; and R<sup>42</sup> and R<sup>43</sup> are independently H, OMe, NH<sub>2</sub>, OCH<sub>2</sub>CH<sub>2</sub>OMe, N(CH<sub>2</sub>CH<sub>2</sub>)O, N(CH<sub>2</sub>CH<sub>2</sub>)NMe, or N(CH<sub>2</sub>CH<sub>2</sub>)NH. Representative species are listed in Table 3.

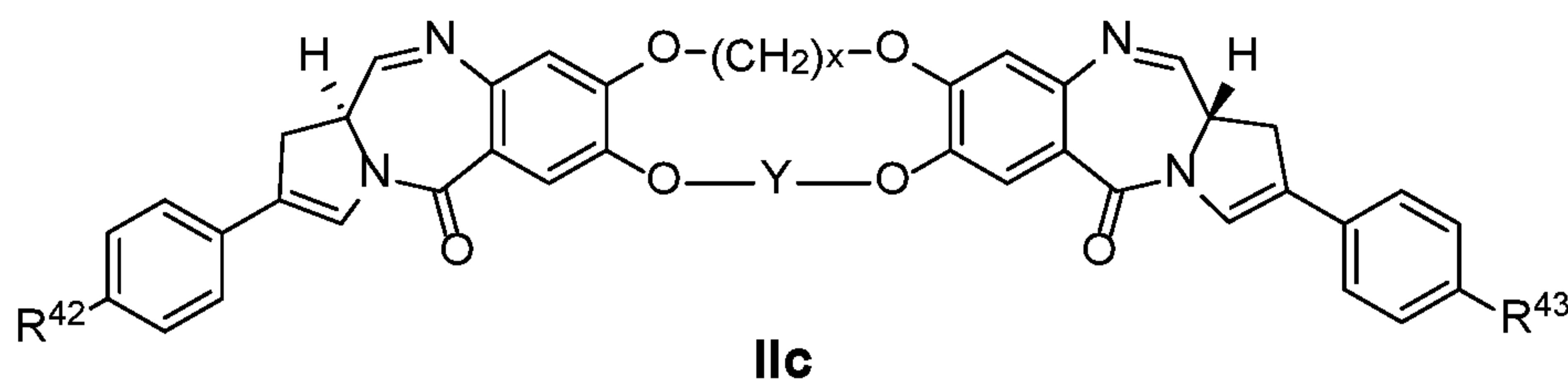
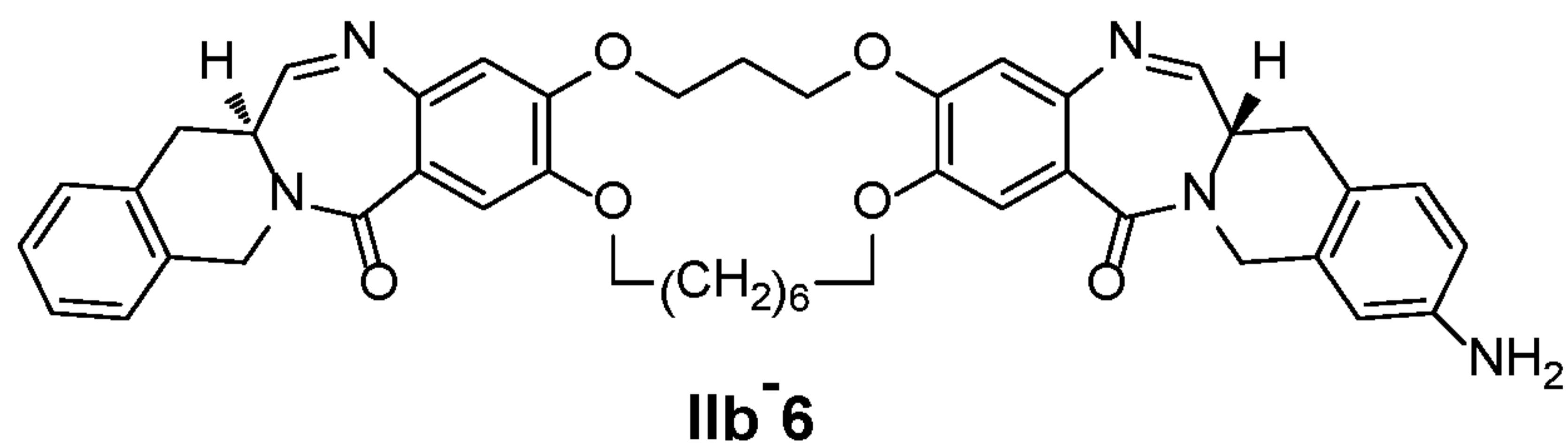
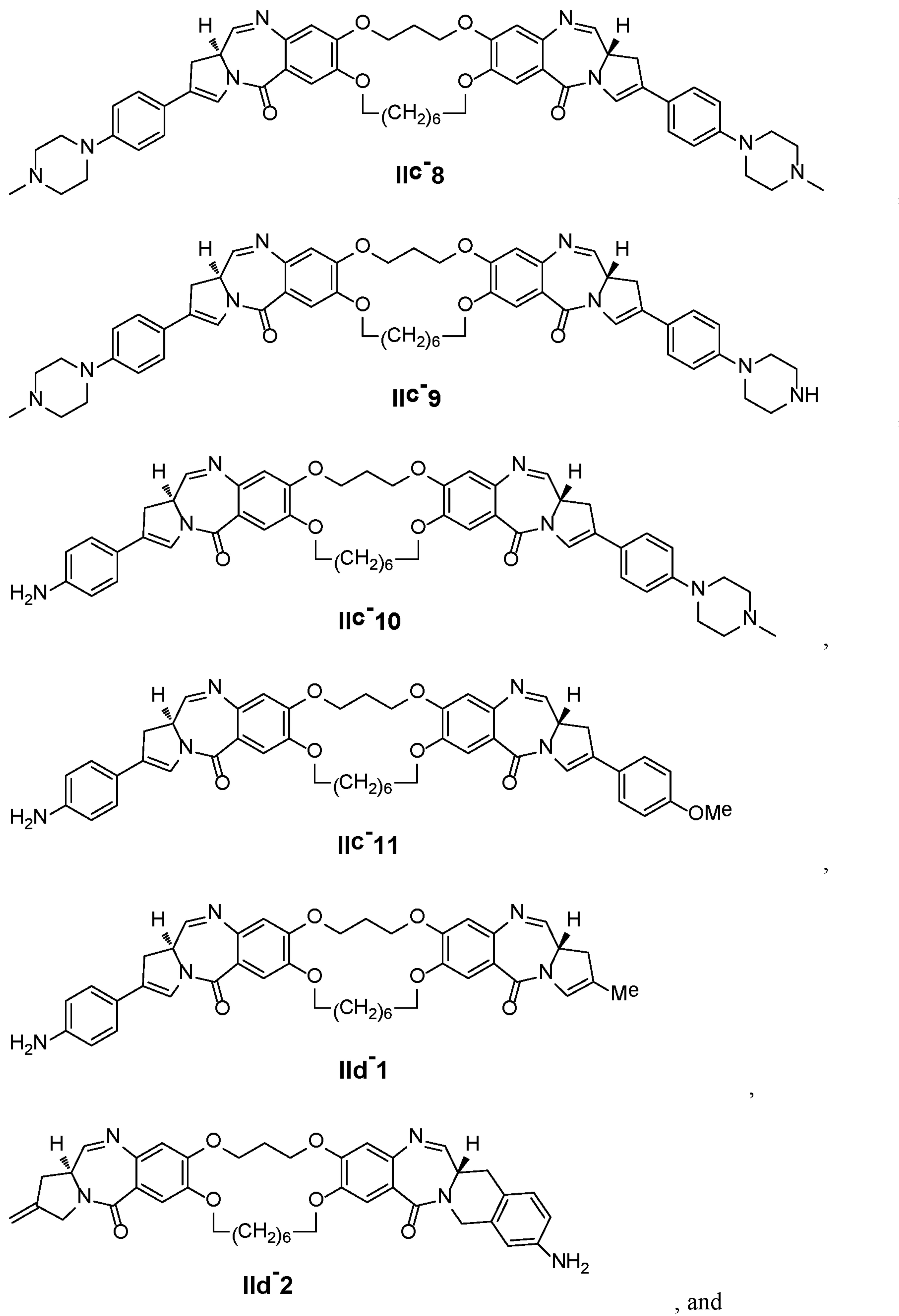


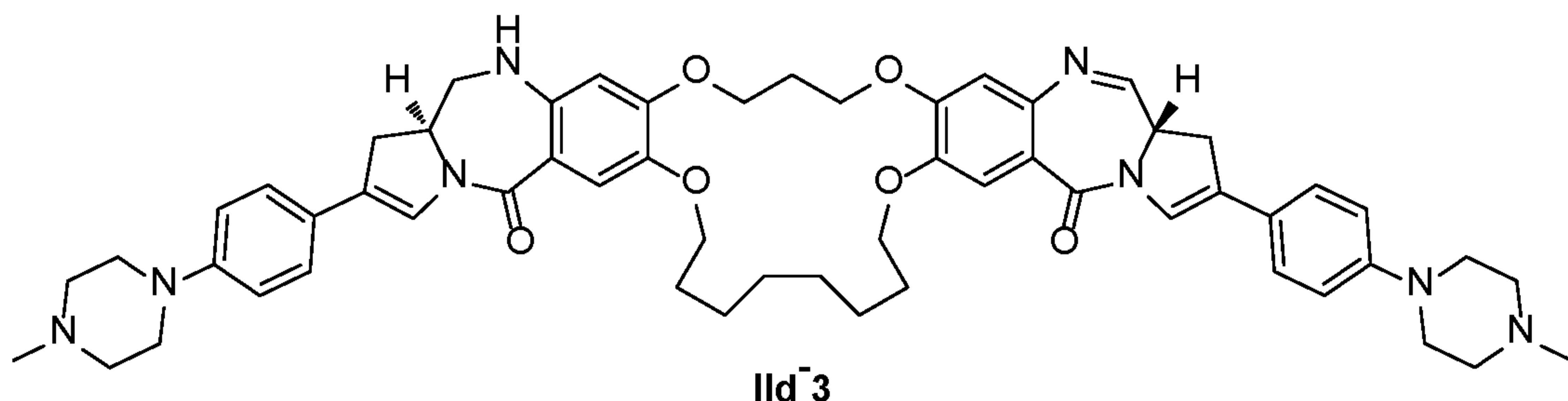
Table 3 – Examples of Dimers According to Formula IIc

Dimer	x	Y	R <sup>42</sup>	R <sup>43</sup>
IIc-1	3	(CH <sub>2</sub> ) <sub>10</sub>	H	H
IIc-2	3	(CH <sub>2</sub> ) <sub>12</sub>	OMe	OMe
IIc-3	3	(CH <sub>2</sub> ) <sub>12</sub>	OCH <sub>2</sub> CH <sub>2</sub> OMe	OCH <sub>2</sub> CH <sub>2</sub> OMe
IIc-4	3	(CH <sub>2</sub> ) <sub>12</sub>		
IIc-5	3	(CH <sub>2</sub> ) <sub>12</sub>		
IIc-6	3	(CH <sub>2</sub> ) <sub>2</sub> (OCH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub>	H	H
IIc-7	3	(CH <sub>2</sub> ) <sub>8</sub>	OMe	OMe
IIc-8	3	(CH <sub>2</sub> ) <sub>8</sub>		
IIc-9	3	(CH <sub>2</sub> ) <sub>8</sub>		
IIc-10	3	(CH <sub>2</sub> ) <sub>8</sub>		NH <sub>2</sub>
IIc-11	3	(CH <sub>2</sub> ) <sub>8</sub>	NH <sub>2</sub>	OMe

[0060] Preferably, dimers of this invention are selected from the group consisting of dimer 5 IIb-6, IIc-8, IIc-9, IIc-10, IIc-11, IId-1, IId-2, and IId-3:







## CONJUGATES

### *General*

**[0061]** Dimers of this invention can be used as therapeutic agents *per se*, but preferably are used as conjugates with a targeting moiety that specifically or preferentially binds to a chemical entity on a cancer cell. Preferably, the targeting moiety is an antibody or antigen binding portion thereof and the chemical entity is a tumor associated antigen.

**[0062]** Thus, another embodiment of this invention is a conjugate comprising dimer of this invention and a ligand, represented by formula (II)



where Z is a ligand, D is a dimer of this invention, and  $-(X^D)_a C (X^Z)_b-$  are collectively referred to as a “linker moiety” or “linker” because they link Z and D. Within the linker, C is a cleavable group designed to be cleaved at or near the site of intended biological action of dimer D;  $X^D$  and  $X^Z$  are referred to as spacer moieties (or “spacers”) because they space apart D and C and C and Z, respectively; subscripts a, b, and c are independently 0 or 1 (that is, the presence of  $X^D$ ,  $X^Z$  and C are optional). Subscript m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (preferably 1, 2, 3, or 4). D,  $X^D$ , C,  $X^Z$  and Z are more fully described hereinbelow.

**[0063]** Ligand Z – for example an antibody – performs a targeting function. By binding to a target tissue or cell where its antigen or receptor is located, ligand Z directs the conjugate there. (When ligand Z is an antibody, the conjugate is sometimes referred to as antibody-drug conjugate (ADC) or an immunoconjugate. Preferably, the target tissue or cell is a cancer tissue or cell and the antigen or receptor is a tumor-associated antigen, that is, an antigen that is uniquely expressed by cancerous cells or is overexpressed by cancer cells, compared to non-cancerous cells. Cleavage of group C at the target tissue or cell releases dimer D to exert its cytotoxic effect locally. In some instances, the conjugate is internalized into a target cell by endocytosis and cleavage takes place within the target cell. In this manner, precise delivery of

dimer D is achieved at the site of intended action, reducing the dosage needed. Also, dimer D is normally biologically inactive (or significantly less active) in its conjugated state, thereby reducing undesired toxicity against non-target tissue or cells. As anticancer drugs are often highly toxic to cells in general, this is an important consideration.

5 [0064] As reflected by the subscript m, each molecule of ligand Z can conjugate with more than one dimer D, depending on the number of sites ligand Z has available for conjugation and the experimental conditions employed. Those skilled in the art will appreciate that, while each individual molecule of ligand Z is conjugated to an integer number of dimers D, a preparation of the conjugate may analyze for a non-integer ratio of dimers D to ligand Z, reflecting a 10 statistical average. This ratio is referred to as the substitution ratio (SR) or, synonymously, the drug-antibody ratio (DAR).

#### *Ligand Z*

15 [0065] Preferably, ligand Z is an antibody. For convenience and brevity and not by way of limitation, the detailed subsequent discussion herein about the conjugation of ligand Z is written in the context of its being an antibody, but those skilled in the art will understand that other types of ligand Z can be conjugated, *mutatis mutandis*. For example, conjugates with folic acid as the ligand can target cells having the folate receptor on their surfaces (Leamon *et al.*, Cancer Res. 2008, 68 (23), 9839). For the same reason, the detailed discussion below is primarily written in terms of a 1:1 ratio of antibody Z to analog D (m = 1).

20 [0066] Preferably, ligand Z is an antibody against a tumor associated antigen, allowing the selective targeting of cancer cells. Examples of such antigens include: mesothelin, prostate specific membrane antigen (PSMA), CD19, CD22, CD30, CD70, B7H3, B7H4 (also known as O8E), protein tyrosine kinase 7 (PTK7), glypican-3, RG1, fucosyl-GM1, CTLA-4, and CD44. The antibody can be animal (*e.g.*, murine), chimeric, humanized, or, preferably, human. The 25 antibody preferably is monoclonal, especially a monoclonal human antibody. The preparation of human monoclonal antibodies against some of the aforementioned antigens is disclosed in Korman *et al.*, US 8,609,816 B2 (2013; B7H4, also known as O8E; in particular antibodies 2A7, 1G11, and 2F9); Rao-Naik *et al.*, 8,097,703 B2 (2012; CD19; in particular antibodies 5G7, 13F1, 46E8, 21D4, 21D4a, 47G4, 27F3, and 3C10); King *et al.*, US 8,481,683 B2 (2013; 30 CD22; in particular antibodies 12C5, 19A3, 16F7, and 23C6); Keler *et al.*, US 7,387,776 B2 (2008; CD30; in particular antibodies 5F11, 2H9, and 17G1); Terrett *et al.*, US 8,124,738 B2 (2012; CD70; in particular antibodies 2H5, 10B4, 8B5, 18E7, and 69A7); Korman *et al.*, US

6,984,720 B1 (2006; CTLA-4; in particular antibodies 10D1, 4B6, and 1E2); Vistica *et al.*, US 8,383,118 B2 (2013, fucosyl-GM1, in particular antibodies 5B1, 5B1a, 7D4, 7E4, 13B8, and 18D5) Korman *et al.*, US 8,008,449 B2 (2011; PD-1; in particular antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3, and 5F4); Huang *et al.*, US 2009/0297438 A1 (2009; PSMA, in particular antibodies 1C3, 2A10, 2F5, 2C6); Cardarelli *et al.*, US 7,875,278 B2 (2011; PSMA; in particular antibodies 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5, and 1C3); Terrett *et al.*, US 8,222,375 B2 (2012; PTK7; in particular antibodies 3G8, 4D5, 12C6, 12C6a, and 7C8); Terrett *et al.*, US 8,680,247 B2 (2014; glypican-3; in particular antibodies 4A6, 11E7, and 16D10); Harkins *et al.*, US 7,335,748 B2 (2008; RG1; in particular antibodies A, B, C, and D); Terrett *et al.*, US 8,268,970 B2 (2012; mesothelin; in particular antibodies 3C10, 6A4, and 7B1); Xu *et al.*, US 2010/0092484 A1 (2010; CD44; in particular antibodies 14G9.B8.B4, 2D1.A3.D12, and 1A9.A6.B9); Deshpande *et al.*, US 8,258,266 B2 (2012; IP10; in particular antibodies 1D4, 1E1, 2G1, 3C4, 6A5, 6A8, 7C10, 8F6, 10A12, 10A12S, and 13C4); Kuhne *et al.*, US 8,450,464 B2 (2013; CXCR4; in particular antibodies F7, F9, D1, and E2); and Korman *et al.*, US 7,943,743 B2 (2011; PD-L1; in particular antibodies 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4); the disclosures of which are incorporated herein by reference. Each of the aforementioned antibodies can be used in an ADC with d dimer of this invention.

**[0067]** Ligand Z can also be an antibody fragment or antibody mimetic, such as an 20 affibody, a domain antibody (dAb), a nanobody, a unibody, a DARPin, an anticalin, a versabody, a duocalin, a lipocalin, or an avimer.

**[0068]** Any one of several different reactive groups on ligand Z can be a conjugation site, including  $\epsilon$ -amino groups in lysine residues, pendant carbohydrate moieties, carboxylic acid groups, disulfide groups, and thiol groups. Each type of reactive group represents a trade-off, 25 having some advantages and some disadvantages. For reviews on antibody reactive groups suitable for conjugation, see, e.g., Garnett, *Adv. Drug Delivery Rev.* 53 (2001), 171-216 and Dubowchik and Walker, *Pharmacology & Therapeutics* 83 (1999), 67-123, the disclosures of which are incorporated herein by reference.

**[0069]** In one embodiment, ligand Z is conjugated via a lysine  $\epsilon$ -amino group. Most 30 antibodies have multiple lysine  $\epsilon$ -amino groups, which can be conjugated via amide, urea, thiourea, or carbamate bonds using techniques known in the art. However, it is difficult to control which and how many  $\epsilon$ -amino groups react, leading to potential batch-to-batch vari-

ability in conjugate preparations. Also, conjugation may cause neutralization of a protonated  $\epsilon$ -amino group important for maintaining the antibody's native conformation or may take place at a lysine near or at the antigen binding site, neither being a desirable occurrence.

[0070] In another embodiment, ligand Z can be conjugated via a carbohydrate side chain, 5 as many antibodies are glycosylated. The carbohydrate side chain can be oxidized with periodate to generate aldehyde groups, which in turn can be reacted with amines to form an imine group, such as in a semicarbazone, oxime, or hydrazone. If desired, the imine group can be converted to a more stable amine group by reduction with sodium cyanoborohydride. For 10 additional disclosures on conjugation via carbohydrate side chains, see, e.g., Rodwell *et al.*, *Proc. Nat'l Acad. Sci. USA* 83, 2632-2636 (1986); the disclosure of which is incorporated herein by reference. As with lysine  $\epsilon$ -amino groups, there are concerns regarding 15 reproducibility of the location of the conjugation site(s) and stoichiometry.

[0071] In yet another embodiment, ligand Z can be conjugated via a carboxylic acid group. In one embodiment, a terminal carboxylic acid group is functionalized to generate a 15 carbohydrazide, which is then reacted with an aldehyde-bearing conjugation moiety. See Fisch *et al.*, *Bioconjugate Chemistry* 1992, 3, 147-153.

[0072] In yet another embodiment, antibody Z can be conjugated via a disulfide group bridging a cysteine residue on antibody Z and a sulfur on the other portion of the conjugate. Some antibodies lack free thiol (sulphydryl) groups but have disulfide groups, for example in 20 the hinge region. In such case, free thiol groups can be generated by reduction of native disulfide groups. The thiol groups so generated can then be used for conjugation. See, e.g., Packard *et al.*, *Biochemistry* 1986, 25, 3548-3552; King *et al.*, *Cancer Res.* 54, 6176-6185 (1994); and Doronina *et al.*, *Nature Biotechnol.* 21(7), 778-784 (2003); the disclosures of 25 which are incorporated herein by reference. Again, there are concerns regarding conjugation site location and stoichiometry and the possible disruption of antibody native conformation.

[0073] A number of methods are known for introducing free thiol groups into antibodies without breaking native disulfide bonds, which methods can be practiced with a ligand Z of this invention. Depending on the method employed, it may be possible to introduce a predictable 30 number of free sulfhydryls at predetermined locations. In one approach, mutated antibodies are prepared in which a cysteine is substituted for another amino acid. See, for example, Eigenbrot *et al.*, US 7,521,541 B2 (2009); Chilkoti *et al.*, *Bioconjugate Chem.* 1994, 5, 504-507; Urnovitz *et al.*, US 4,698,420 (1987); Stimmel *et al.*, *J. Biol. Chem.*, 275 (39), 30445-30450 (2000); Bam

*et al.*, US 7,311,902 B2 (2007); Kuan *et al.*, *J. Biol. Chem.*, 269 (10), 7610-7618 (1994); Poon *et al.*, *J. Biol. Chem.*, 270 (15), 8571-8577 (1995). In another approach, an extra cysteine is added to the C-terminus. See, e.g. Cumber *et al.*, *J. Immunol.*, 149, 120-126 (1992); King *et al.*, *Cancer Res.*, 54, 6176-6185 (1994); Li *et al.*, *Bioconjugate Chem.*, 13, 985-995 (2002); Yang *et al.*, *Protein Engineering*, 16, 761-770 (2003); and Olafson *et al.*, *Protein Engineering Design & Selection*, 17, 21-27 (2004). A preferred method for introducing free cysteines is that taught by Liu *et al.*, WO 2009/026274 A1, in which a cysteine bearing amino acid sequence is added to the C-terminus of the heavy chain of an antibody. This method introduces a known number of cysteine residues (one per heavy chain) at a known location away from the antigen binding site. The disclosures of the documents cited in this paragraph are all incorporated herein by reference.

**[0074]** In yet another embodiment, lysine  $\epsilon$ -amino groups can be modified with reagents such as 2-iminothiolane or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), converting an  $\epsilon$ -amino group into a thiol or disulfide group – creating a cysteine surrogate, as it were. However, this method suffers from the same conjugation location and stoichiometry limitations associated with  $\epsilon$ -amino groups proper.

#### *Linker Components*

**[0075]** As noted above, the linker portion of a conjugate of this invention comprises up to three elements: a cleavable group C and optional spacers X<sup>Z</sup> and X<sup>D</sup>.

**[0076]** Cleavable group C is a group cleavable under physiological conditions, preferably selected such that it is relatively stable while the conjugate is in general circulation in the blood plasma, but is readily cleaved once the conjugate reaches its site of intended action, that is, near, at, or within the target cell. Preferably, the conjugate is internalized by a target cell upon binding of antibody Z to an antigen displayed on the surface of the target cell. Subsequently, cleavage of group C occurs in a vesicular body of the target cell (an early endosome, a late endosome, or, especially, a lysosome).

**[0077]** In one embodiment, group C is a pH sensitive group. The pH in blood plasma is slightly above neutral, while the pH inside a lysosome is acidic, circa 5. Thus, a group C whose cleavage is acid catalyzed will cleave at a rate several orders of magnitude faster inside a lysosome than in the blood plasma rate. Examples of suitable acid-sensitive groups include cis-aconityl amides and hydrazones, as described in Shen *et al.*, US 4,631,190 (1986); Shen *et al.*,

US 5,144,011 (1992); Shen *et al.*, *Biochem. Biophys. Res. Commun.* 102, 1048-1054 (1981) and Yang *et al.*, *Proc. Natl Acad. Sci (USA)*, 85, 1189-1193 (1988); the disclosures of which are incorporated herein by reference.

[0078] In another embodiment, group C is a disulfide. Disulfides can be cleaved by a thiol-disulfide exchange mechanism, at a rate dependent on the ambient thiol concentration. As the intracellular concentration of glutathione and other thiols is higher than their serum concentrations, the cleavage rate of a disulfide will be higher intracellularly. Further, the rate of thiol-disulfide exchange can be modulated by adjustment of the steric and electronic characteristics of the disulfide (e.g., an alkyl-aryl disulfide versus an alkyl-alkyl disulfide; substitution on the aryl ring, etc.), enabling the design of disulfide linkages that have enhanced serum stability or a particular cleavage rate. For additional disclosures relating to disulfide cleavable groups in conjugates, see, e.g., Thorpe *et al.*, *Cancer Res.* 48, 6396-6403 (1988); Santi *et al.*, US 7,541,530 B2 (2009); Ng *et al.*, US 6,989,452 B2 (2006); Ng *et al.*, WO 2002/096910 A1; Boyd *et al.*, US 7,691,962 B2; and Sufi *et al.*, US 2010/0145036 A1; the disclosures of which are incorporated herein by reference.

[0079] A preferred cleavable group is a peptide that is cleaved selectively by a protease inside the target cell, as opposed to by a protease in the serum. Typically, a cleavable peptide group comprises from 1 to 20 amino acids, preferably from 1 to 6 amino acids, more preferably from 1 to 3 amino acids. The amino acid(s) can be natural and/or non-natural  $\alpha$ -amino acids. Natural amino acids are those encoded by the genetic code, as well as amino acids derived therefrom, *e.g.*, hydroxyproline,  $\gamma$ -carboxyglutamate, citrulline, and O-phosphoserine. In this context, the term “amino acid” also includes amino acid analogs and mimetics. Analogs are compounds having the same general  $\text{H}_2\text{N}(\text{R})\text{CHCO}_2\text{H}$  structure of a natural amino acid, except that the R group is not one found among the natural amino acids. Examples of analogs include homoserine, norleucine, methionine-sulfoxide, and methionine methyl sulfonium. An amino acid mimetic is a compound that has a structure different from the general chemical structure of an  $\alpha$ -amino acid but functions in a manner similar to one. The amino acid can be of the “L” stereochemistry of the genetically encoded amino acids, as well as of the enantiomeric “D” stereochemistry.

[0080] Preferably, group C contains an amino acid sequence that is a cleavage recognition sequence for a protease. Many cleavage recognition sequences are known in the art. *See, e.g.*, Matayoshi *et al.* *Science* 247: 954 (1990); Dunn *et al.* *Meth. Enzymol.* 241: 254 (1994); Seidah

*et al. Meth. Enzymol.* 244: 175 (1994); Thornberry, *Meth. Enzymol.* 244: 615 (1994); Weber *et al. Meth. Enzymol.* 244: 595 (1994); Smith *et al. Meth. Enzymol.* 244: 412 (1994); and Bouvier *et al. Meth. Enzymol.* 248: 614 (1995); the disclosures of which are incorporated herein by reference.

5 [0081] For conjugates that are not intended to be internalized by a cell, a group C can be chosen such that it is cleaved by a protease present in the extracellular matrix in the vicinity of the target tissue, e.g., a protease released by nearby dying cells or a tumor-associated protease. Exemplary extracellular tumor-associated proteases are matrix metalloproteases (MMP), thimet oligopeptidase (TOP) and CD10.

10 [0082] For conjugates that are designed to be internalized by a cell, group C preferably comprises an amino acid sequence selected for cleavage by an endosomal or lysosomal protease, especially the latter. Non-limiting examples of such proteases include cathepsins B, C, D, H, L and S, especially cathepsin B. Cathepsin B preferentially cleaves peptides at a sequence -AA<sup>2</sup>-AA<sup>1</sup>- where AA<sup>1</sup> is a basic or strongly hydrogen bonding amino acid (such as lysine, arginine, or citrulline) and AA<sup>2</sup> is a hydrophobic amino acid (such as phenylalanine, valine, alanine, leucine, or isoleucine), for example Val-Cit (where Cit denotes citrulline) or Val-Lys. (Herein, amino acid sequences are written in the N-to-C direction, as in H<sub>2</sub>N-AA<sup>2</sup>-AA<sup>1</sup>-CO<sub>2</sub>H, unless the context clearly indicates otherwise.) Lys-Val-Ala, Asp-Val-Ala, Val-Ala, Lys-Val-Cit, and Asp-Val-Cit are also substrate peptide motifs for cathepsin B, although in some instances the cleavage rate may be slower. For additional information regarding cathepsin-cleavable groups, see Dubowchik *et al.*, *Bioorg. Med. Chem. Lett.* **8**, 3341-3346 (1998); Dubowchik *et al.*, *Bioorg. Med. Chem. Lett.*, **8** 3347-3352 (1998); and Dubowchik *et al.*, *Bioconjugate Chem.* **13**, 855-869 (2002); the disclosures of which are incorporated by reference. Another enzyme that can be utilized for cleaving peptidyl linkers is legumain, a lysosomal cysteine protease that preferentially cleaves at Ala-Ala-Asn.

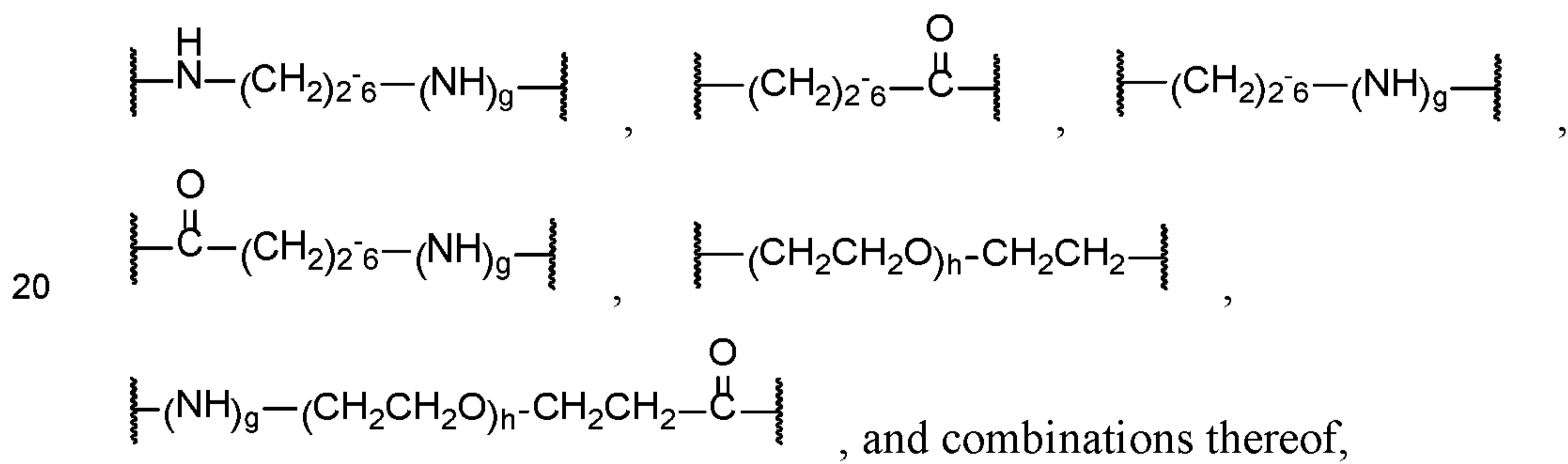
20 [0083] In one embodiment, Group C is a peptide comprising a two-amino acid sequence -AA<sup>2</sup>-AA<sup>1</sup>- wherein AA<sup>1</sup> is lysine, arginine, or citrulline and AA<sup>2</sup> is phenylalanine, valine, alanine, leucine or isoleucine. In another embodiment, C consists of a sequence of one to three amino acids, selected from the group consisting of Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Ala-Asn-Val, Val-Leu-Lys, Cit-Cit, Val-Lys, Ala-Ala-Asn, Lys, Cit, Ser, and Glu.

[0084] The preparation and design of cleavable groups C consisting of a single amino acid is disclosed in Chen *et al.*, US 8,664,407 B2 (2014), the disclosure of which is incorporated herein by reference.

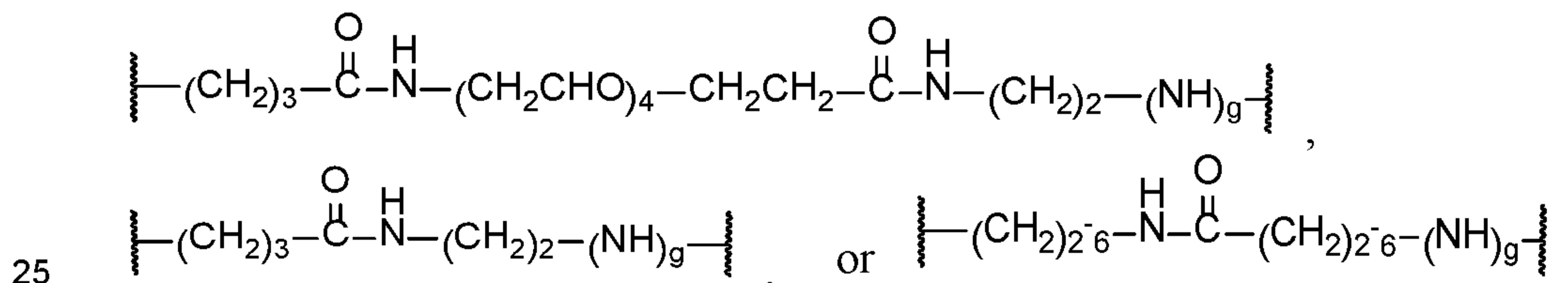
[0085] Group C can also be a photocleavable one, for example a nitrobenzyl ether that is 5 cleaved upon exposure to light.

[0086] Group C can be bonded directly to antibody Z or analog D; *i.e.* spacers X<sup>Z</sup> and X<sup>D</sup>, as the case may be, can be absent. For example, if group C is a disulfide, one of the two sulfurs can be a cysteine residue or its surrogate on antibody Z. Or, group C can be a hydrazone bonded to an aldehyde on a carbohydrate side chain of the antibody. Or, group C can be a 10 peptide bond formed with a lysine ε-amino group of antibody Z. In a preferred embodiment, dimer D is directly bonded to group C via a peptidyl bond to a carboxyl or amine group in dimer D.

[0087] When present, spacer X<sup>Z</sup> provides spatial separation between group C and antibody Z, lest the former sterically interfere with antigen binding by latter or the latter sterically 15 interfere with cleavage of the former. Further, spacer X<sup>Z</sup> can be used to confer increased solubility or decreased aggregation properties to conjugates. A spacer X<sup>Z</sup> can comprise one or more modular segments, which can be assembled in any number of combinations. Examples of suitable segments for a spacer X<sup>Z</sup> are:



where the subscript g is 0 or 1 and the subscript h is 1 to 24, preferably 2 to 4. These segments can be combined, such as illustrated below:



[0088] Spacer X<sup>D</sup>, if present, provides spatial separation between group C and dimer D, lest the latter interfere sterically or electronically with cleavage of the former. Spacer X<sup>D</sup> also can

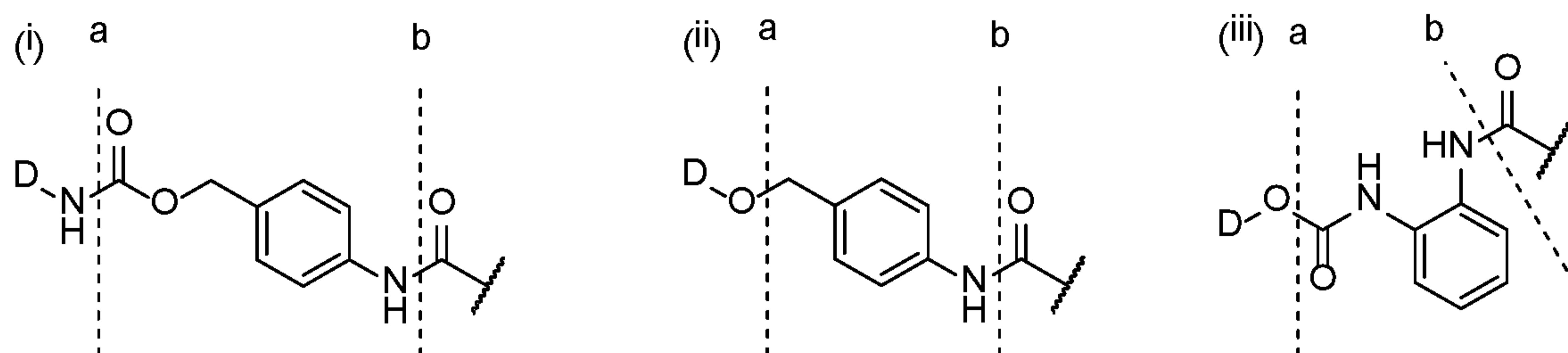
serve to introduce additional molecular mass and chemical functionality into a conjugate. Generally, the additional mass and functionality will affect the serum half-life and other properties of the conjugate. Thus, through judicious selection of spacer groups, the serum half-life of a conjugate can be modulated. Spacer  $X^D$  also can be assembled from modular segments, as described above in the context of spacer  $X^Z$ .

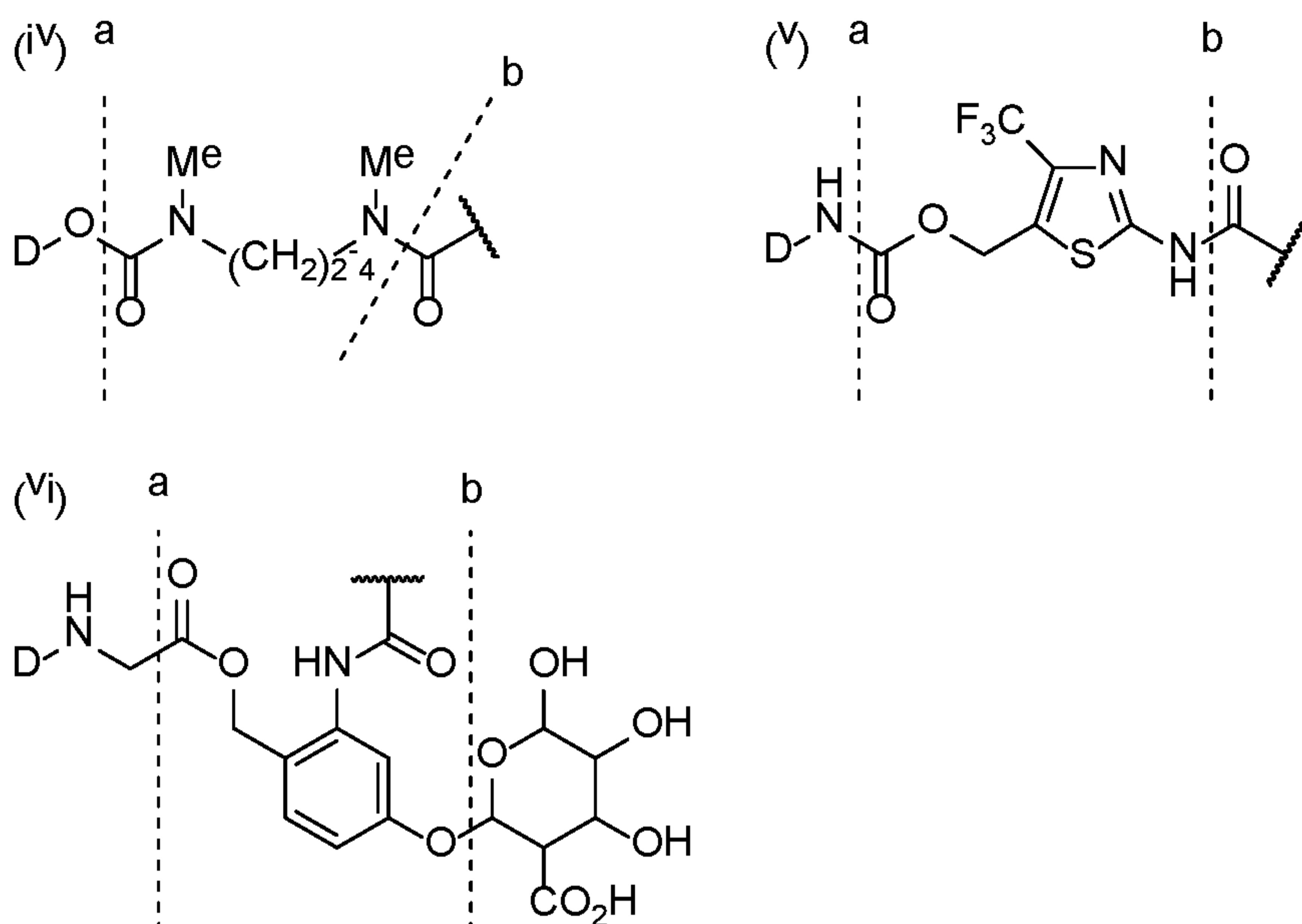
**[0089]** Spacers  $X^Z$  and/or  $X^D$ , where present, preferably provide a linear separation of from 4 to 25 atoms, more preferably from 4 to 20 atoms, between Z and C or D and C, respectively.

**[0090]** The linker can perform other functions in addition to covalently linking the antibody and the drug. For instance, the linker can contain poly(ethylene glycol) (PEG) groups, which enhance solubility either during the performance the conjugation chemistry or in the final ADC product. Where a PEG group is present, it may be incorporated into either spacer  $X^Z$  or  $X^D$ , or both. The number of repeat units in a PEG group can be from 2 to 20, preferably between 4 and 10.

**[0091]** Either spacer  $X^Z$  or  $X^D$ , or both, can comprise a self-immolating moiety. A self-immolating moiety is a moiety that (1) is bonded to group C and either antibody Z or dimer D and (2) has a structure such that cleavage from group C initiates a reaction sequence resulting in the self-immolating moiety disbonding itself from antibody Z or dimer D, as the case may be. In other words, reaction at a site distal from antibody Z or dimer D (cleavage from group C) causes the  $X^Z$ -Z or the  $X^D$ -D bond to rupture as well. The presence of a self-immolating moiety is desirable in the case of spacer  $X^D$  because, if, after cleavage of the conjugate, spacer  $X^D$  or a portion thereof were to remain attached to dimer D, the biological activity of the latter may be impaired. The use of a self-immolating moiety is especially desirable where cleavable group C is a polypeptide, in which instance the self-immolating moiety typically is located adjacent thereto.

**[0092]** Exemplary self-immolating moieties (i)-(v) bonded to a hydroxyl or amino group on a partner molecule D are shown below:





**[0093]** The self-immolating moiety is the structure between dotted lines a and b, with

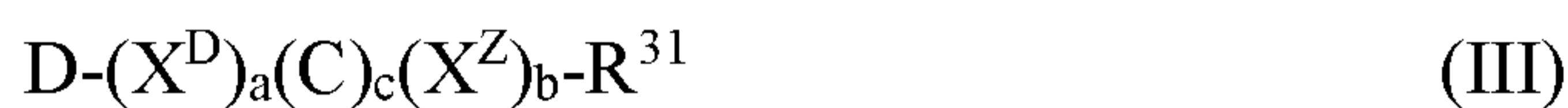
5 adjacent structural features shown to provide context. Self-immolating moieties (i) and (v) are bonded to a dimer D-NH<sub>2</sub> (i.e., dimer D is conjugated via an amino group), while self-  
 immolating moieties (ii), (iii), and (iv) are bonded to a dimer D-OH (i.e., dimer D is conjugated  
 via a hydroxyl or carboxyl group). Cleavage of the amide bond at dotted line b (e.g., by a  
 peptidase) releases the amide nitrogen as an amine nitrogen, initiating a reaction sequence that  
 10 results in the cleavage of the bond at dotted line a and the consequent release of D-OH or  
 D-NH<sub>2</sub>, as the case may be. Alternatively, the cleavage that triggers the self-immolating  
 reaction can be by a different type of enzyme, for example by a  $\beta$ -glucuronidase, as in the  
 instance of structure (vi). For additional disclosures regarding self-immolating moieties, see  
 Carl *et al.*, *J. Med. Chem.*, 24 (3), 479-480 (1981); Carl *et al.*, WO 81/01145 (1981);  
 15 Dubowchik *et al.*, *Pharmacology & Therapeutics*, 83, 67-123 (1999); Firestone *et al.*, US  
 6,214,345 B1 (2001); Toki *et al.*, *J. Org. Chem.* 67, 1866-1872 (2002); Doronina *et al.*, *Nature  
 Biotechnology* 21 (7), 778-784 (2003) (erratum, p. 941); Boyd *et al.*, US 7,691,962 B2; Boyd *et  
 al.*, US 2008/0279868 A1; Sufi *et al.*, WO 2008/083312 A2; Feng, US 7,375,078 B2; Jeffrey *et  
 al.*, US 8039,273; and Senter *et al.*, US 2003/0096743 A1; the disclosures of which are  
 20 incorporated by reference. A preferred self-immolating group is *p*-aminobenzyl oxycarbonyl  
 (PABC) group, as shown in structure (i).

**[0094]** In another embodiment, an antibody targeting moiety and the dimer D are linked by a non-cleavable linker, *i.e.*, element C is absent. Degradation of the antibody eventually

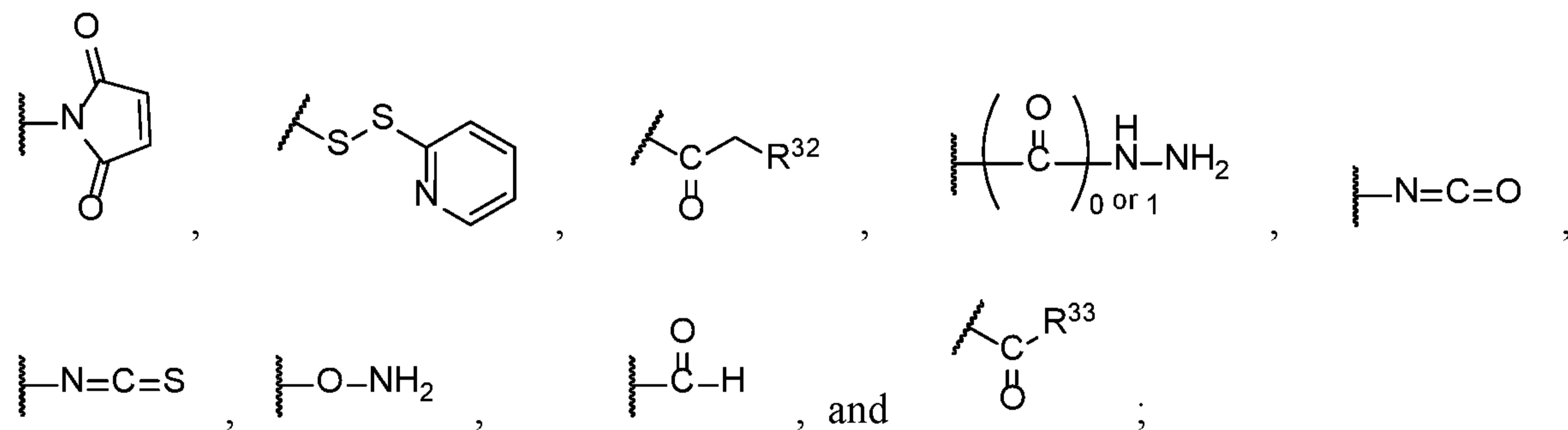
reduces the linker to a small appended moiety that does not interfere with the biological activity of dimer D.

*Conjugation Techniques*

**[0095]** Conjugates of this invention preferably are made by first preparing a compound comprising an analog of this invention (represented by D in the formulae below) and linker  $(X^D)_a(C)_c(X^Z)_b$  (where  $X^D$ , C,  $X^Z$ , a, b, and c are as defined for formula (II)) to form an analog-linker composition represented by formula (III):

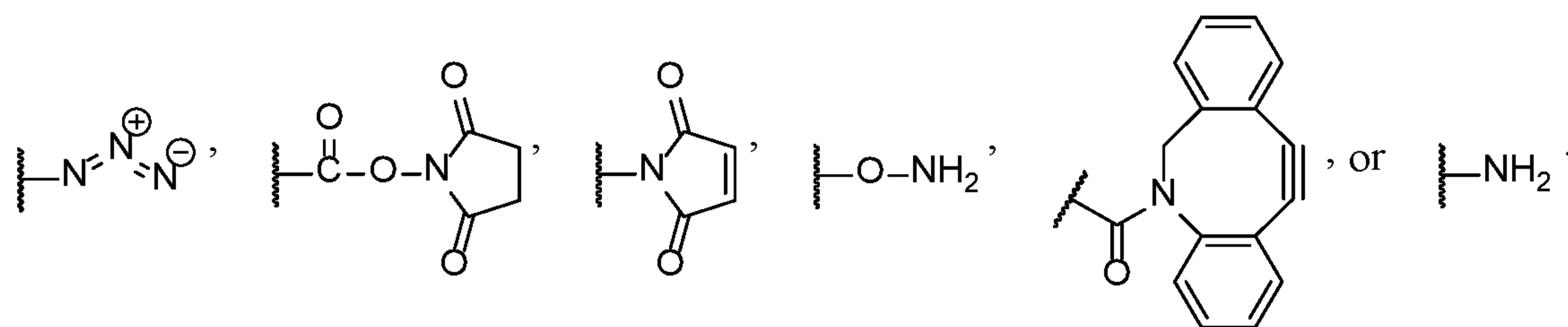


where  $R^{31}$  is a functional group suitable for reacting with a complementary functional group on antibody Z to form the conjugate. Examples of suitable groups  $R^{31}$  include amino, azide, cyclooctyne,



where  $R^{32}$  is Cl, Br, F, mesylate, or tosylate and  $R^{33}$  is Cl, Br, I, F, OH, -O-N-succinimidyl, -O-(4-nitrophenyl), -O-pentafluorophenyl, or -O-tetrafluorophenyl. Chemistry generally usable for the preparation of suitable moieties  $D-(X^D)_a(C)_c(X^Z)_b-R^{31}$  is disclosed in Ng *et al.*, US 7,087,600 B2 (2006); Ng *et al.*, US 6,989,452 B2 (2006); Ng *et al.*, US 7,129,261 B2 (2006); Ng *et al.*, WO 02/096910 A1; Boyd *et al.*, US 7,691,962 B2; Chen *et al.*, US 7,517,903 B2 (2009); Gangwar *et al.*, US 7,714,016 B2 (2010); Boyd *et al.*, US 2008/0279868 A1; Gangwar *et al.*, US 7,847,105 B2 (2010); Gangwar *et al.*, US 7,968,586 B2 (2011); Sufi *et al.*, US 2010/0145036 A1; and Chen *et al.*, US 2010/0113476 A1; the disclosures of which are incorporated herein by reference.

**[0096]** Preferably reactive functional group  $-R^{31}$  is  $-NH_2$ ,  $-OH$ ,  $-CO_2H$ ,  $-SH$ , maleimido, cyclooctyne, azido ( $-N_3$ ), hydroxylamino ( $-ONH_2$ ) or N-hydroxysuccinimido. Especially preferred functional groups  $-R^{31}$  are:



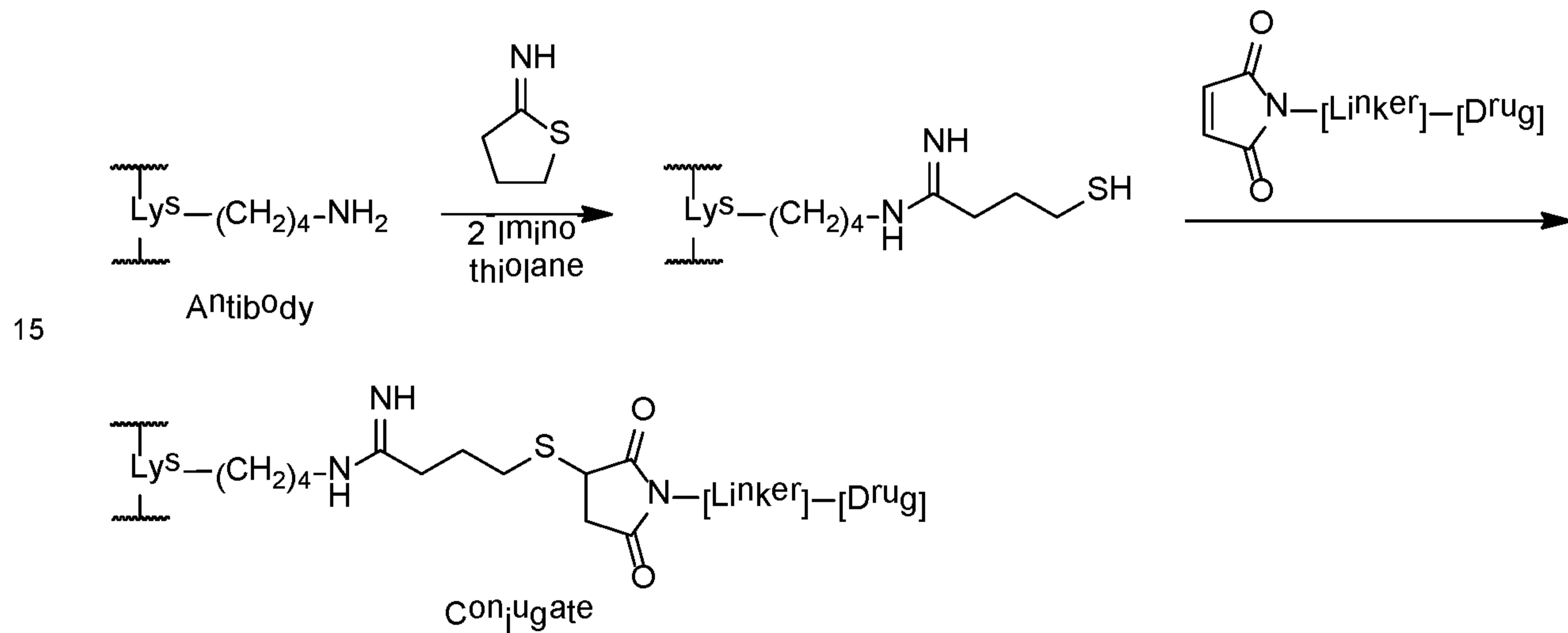
[0097] An -OH group can be esterified with a carboxy group on the antibody, for example, on an aspartic or glutamic acid side chain.

[0098] A -CO<sub>2</sub>H group can be esterified with a -OH group or amidated with an amino group (for example on a lysine side chain) on the antibody.

[0099] An N-hydroxysuccinimide group is functionally an activated carboxyl group and can conveniently be amidated by reaction with an amino group (e.g., from lysine).

[00100] A maleimide group can be conjugated with an -SH group on the antibody (e.g., from cysteine or from the chemical modification of the antibody to introduce a sulfhydryl functionality), in a Michael addition reaction.

[00101] Various techniques can be introducing an -SH group into an antibody. In a preferred one, an  $\varepsilon$ -amino group in the side chain of a lysine residue in the antibody is reacted with 2-iminothiolane to introduce a free thiol (-SH) group. The thiol group can react with a maleimide or other nucleophile acceptor group to effect conjugation:

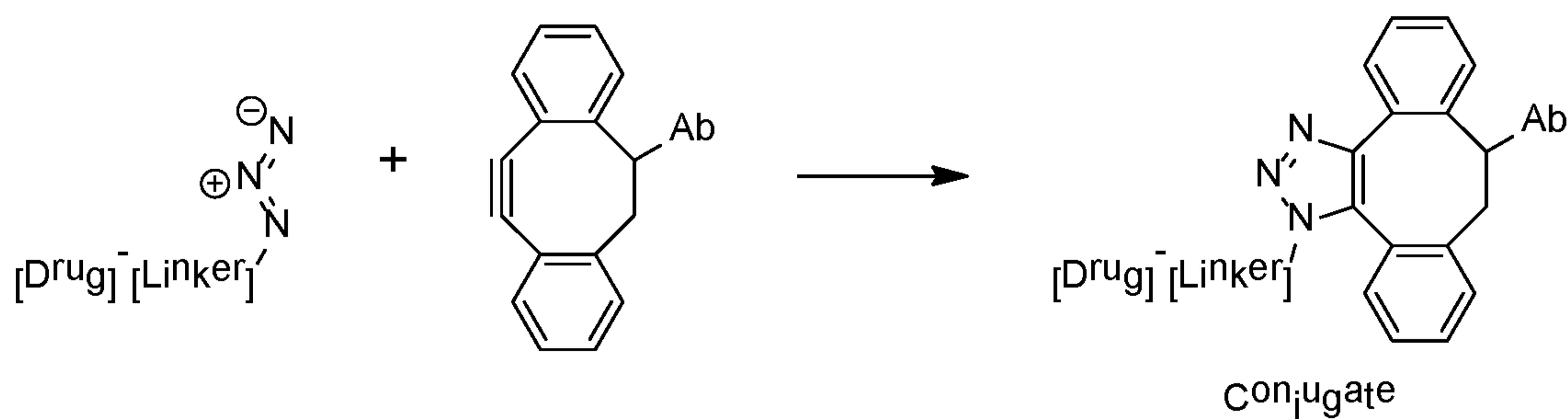


[00102] Typically, a thiolation level of two to three thiols per antibody is achieved. For a representative procedure, see Cong *et al.* 2014, the disclosure of which is incorporated herein by reference. Thus, in one embodiment, an antibody for conjugation to a dimer of this invention

has one or more lysine residues (preferably two or three) modified by reaction with iminothiolane.

[00103] An –SH group can also be used for conjugation where the antibody has been modified to introduce a maleimide group thereto, in a Michael addition reaction that is the 5 “mirror image” of that described above. Antibodies can be modified to have maleimide groups with N-succinimidyl 4-(maleimidomethyl)-cyclohexanecarboxylate (SMCC) or its sulfonated variant sulfo-SMCC, both reagents being available from Sigma-Aldrich.

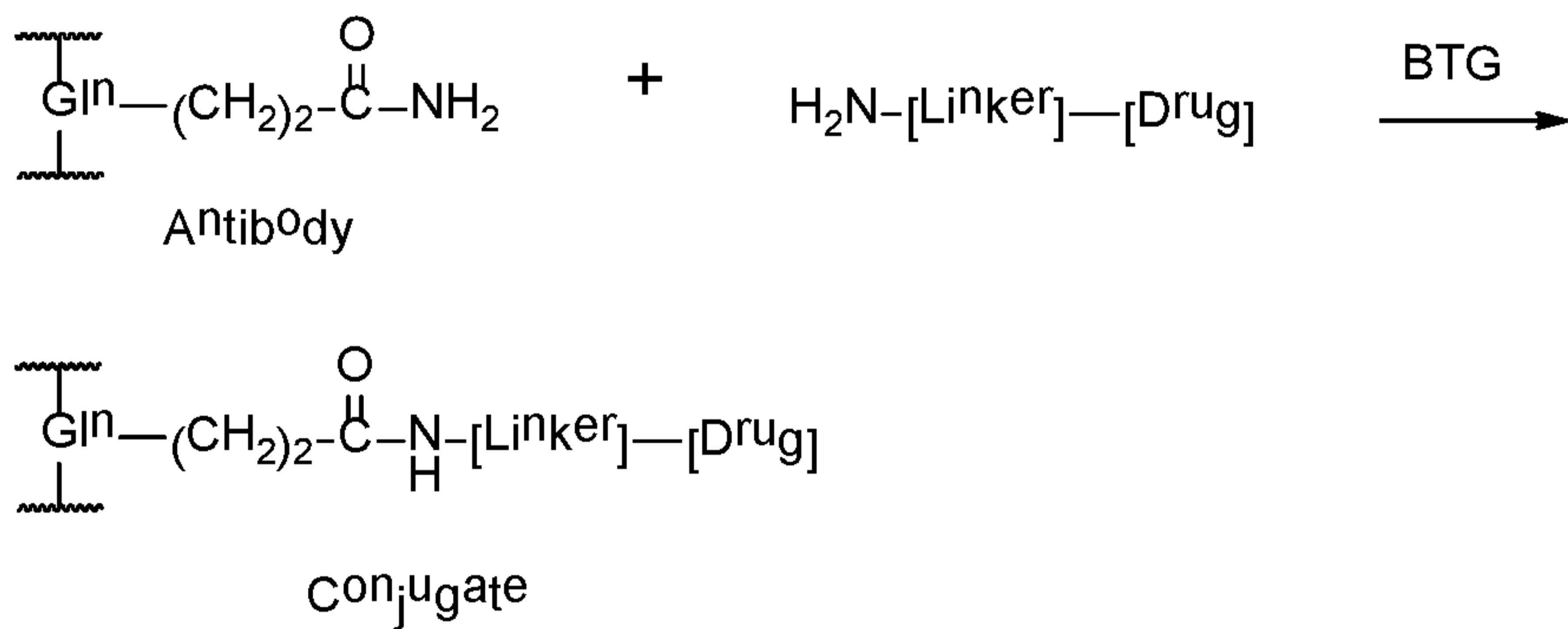
[00104] An alternative conjugation technique employs copper-free “click chemistry,” in which an azide group adds across the strained alkyne bond of a cyclooctyne to form an 1,2,3-10 triazole ring. See, *e.g.*, Agard *et al.*, *J. Amer. Chem. Soc.* **2004**, *126*, 15046; Best, *Biochemistry* **2009**, *48*, 6571, the disclosures of which are incorporated herein by reference. The azide can be located on the antibody and the cyclooctyne on the drug moiety, or vice-versa. A preferred cyclooctyne group is dibenzocyclooctyne (DIBO). Various reagents having a DIBO group are available from Invitrogen/Molecular Probes, Eugene, Oregon. The reaction below illustrates 15 click chemistry conjugation for the instance in which the DIBO group is attached to the antibody (Ab):



[00105] Yet another conjugation technique involves introducing a non-natural amino acid into an antibody, with the non-natural amino acid providing a functionality for conjugation with 20 a reactive functional group in the drug moiety. For instance, the non-natural amino acid p-acetylphenylalanine can be incorporated into an antibody or other polypeptide, as taught in Tian *et al.*, WO 2008/030612 A2 (2008). The ketone group in p-acetylphenylalanine can be a conjugation site via the formation of an oxime with a hydroxylamino group on the linker-drug moiety. Alternatively, the non-natural amino acid p-azidophenylalanine can be incorporated 25 into an antibody to provide an azide functional group for conjugation via click chemistry, as discussed above. Non-natural amino acids can also be incorporated into an antibody or other polypeptide using cell-free methods, as taught in Goerke *et al.*, US 2010/0093024 A1 (2010)

and Goerke *et al.*, *Biotechnol. Bioeng.* **2009**, 102 (2), 400-416. The foregoing disclosures are incorporated herein by reference. Thus, in one embodiment, an antibody that is used for making a conjugate with a dimer of this invention has one or more amino acids replaced by a non-natural amino acid, which preferably is p-acetylphenylalanine or p-azidophenylalanine, 5 more preferably p-acetylphenylalanine.

[00106] Still another conjugation technique uses the enzyme transglutaminase (preferably bacterial transglutaminase or BTG), per Jeger *et al.*, *Angew. Chem. Int. Ed.* **2010**, 49, 9995. BTG forms an amide bond between the side chain carboxamide of a glutamine (the amine acceptor) and an alkyleneamino group (the amine donor), which can be, for example, the  $\epsilon$ -10 amino group of a lysine or a 5-amino-n-pentyl group. In a typical conjugation reaction, the glutamine residue is located on the antibody, while the alkyleneamino group is located on the linker-drug moiety, as shown below:



15

[00107] The positioning of a glutamine residue on a polypeptide chain has a large effect on its susceptibility to BTG mediated transamidation. None of the glutamine residues on an antibody are normally BTG substrates. However, if the antibody is deglycosylated – the glycosylation site being asparagine 297 (N297) – nearby glutamine 295 (Q295) is rendered 20 BTG susceptible. An antibody can be deglycosylated enzymatically by treatment with PNGase F (Peptide-N-Glycosidase F). Alternatively, an antibody can be synthesized glycoside free by introducing an N297A mutation in the constant region, to eliminate the N297 glycosylation site. Further, it has been shown that an N297Q substitution in an antibody not only eliminates 25 glycosylation, but also introduces a second glutamine residue (at position 297) that too is an amine acceptor. Thus, in one embodiment, an antibody that is conjugated to a dimer of this invention is deglycosylated. In another embodiment, the antibody has an N297Q substitution. Those skilled in the art will appreciate that deglycosylation by post-synthesis modification or by introducing an N297A mutation generates two BTG-reactive glutamine residues per

antibody (one per heavy chain, at position 295), while an antibody with an N297Q substitution will have four BTG-reactive glutamine residues (two per heavy chain, at positions 295 and 297).

**[00108]** Conjugation can also be effected using the enzyme Sortase A, as taught in Levary *et al.*, *PLoS One* **2011**, 6(4), e18342; Proft, *Biotechnol. Lett.* **2010**, 32, 1-10; Ploegh *et al.*, WO 2010/087994 A2 (2010); and Mao *et al.*, WO 2005/051976 A2 (2005). The Sortase A recognition motif (typically LPXTG, where X is any natural amino acid) may be located on the ligand Z and the nucleophilic acceptor motif (typically GGG) may be the group R<sup>31</sup> in formula (III), or vice-versa.

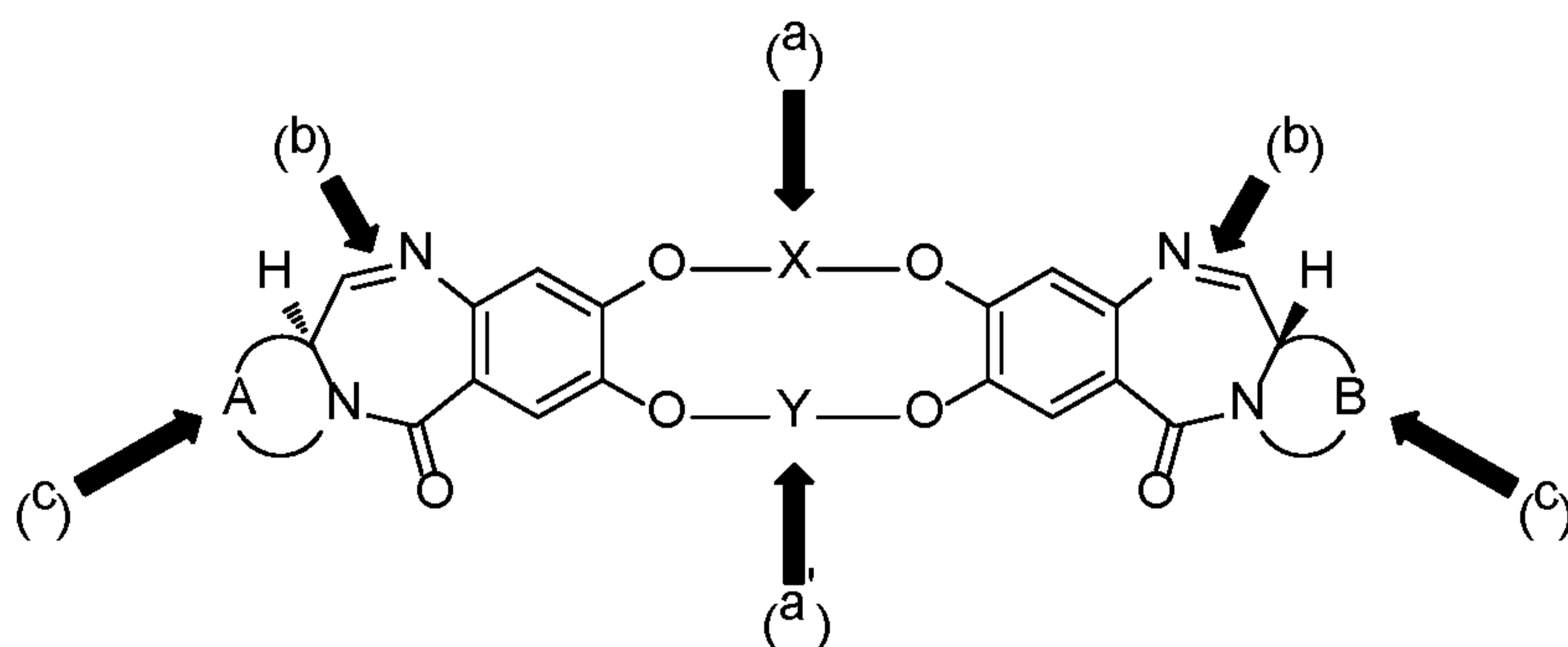
**[00109]** An antibody also can be adapted for conjugation by modifying its glycosyl group to introduce a keto group that serves as a conjugation site by oxime formation, as taught by Zhu *et al.*, *mAbs* **2014**, 6, 1. In another glycoengineering variation, an antibody's glycosyl group can be modified to introduce an azide group for conjugation by "click chemistry." See Huang *et al.*, *J. Am. Chem. Soc.* **2012**, 134, 12308 and Wang, US 8,900,826 B2 (2014) and US 7,807,405 B2 (2010).

**[00110]** Yet another conjugation technique can be generally referred to as disulfide bridging: the disulfide bonds in an antibody are cleaved, creating a pair of thiol (-SH) groups. The antibody is then treated with a drug-linker compound that contains two thiol-reactive sites. Reaction of the thiol groups with the two sites effects a re-bridging that re-creates, in a fashion, the original disulfide bridge, thus preserving the antibody tertiary structure and attaching a drug-linker moiety. See, *e.g.*, Burt *et al.*, WO 2013/190292 A2 (2013) and Jackson *et al.*, US 2013/0224228 A1 (2013).

#### *Dimer-Linker Compounds*

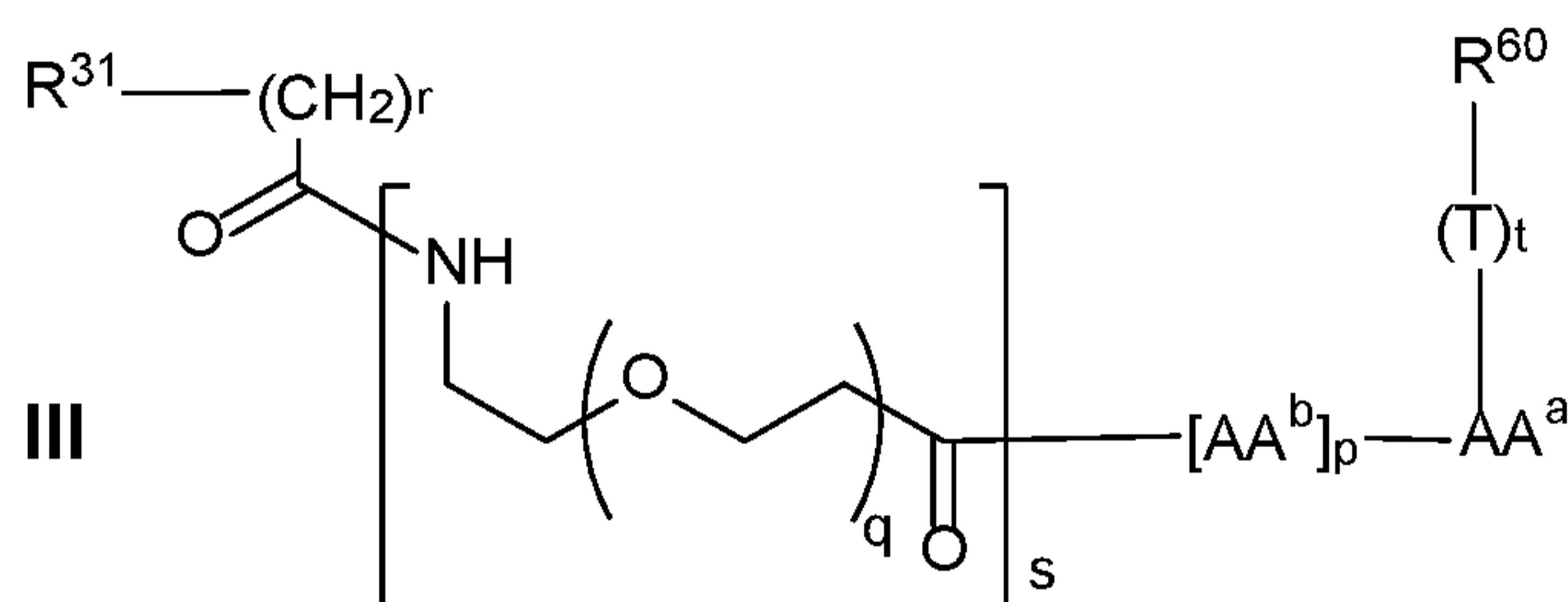
**[00111]** Generally, an ADC of a dimer of this invention comprises a linker attached to a functional group on the dimer, which linker is attached to the antibody. Reflecting the diversity of conjugation techniques available, the dimers of this invention can be elaborated into many different dimer-linker compounds suitable for conjugation to an antibody.

**[00112]** Generally, there are three different modes for attachment of the linker to a dimer of this invention, as illustrated in the figure below (which is a simplified version of formula I with some variables not shown for simplicity):



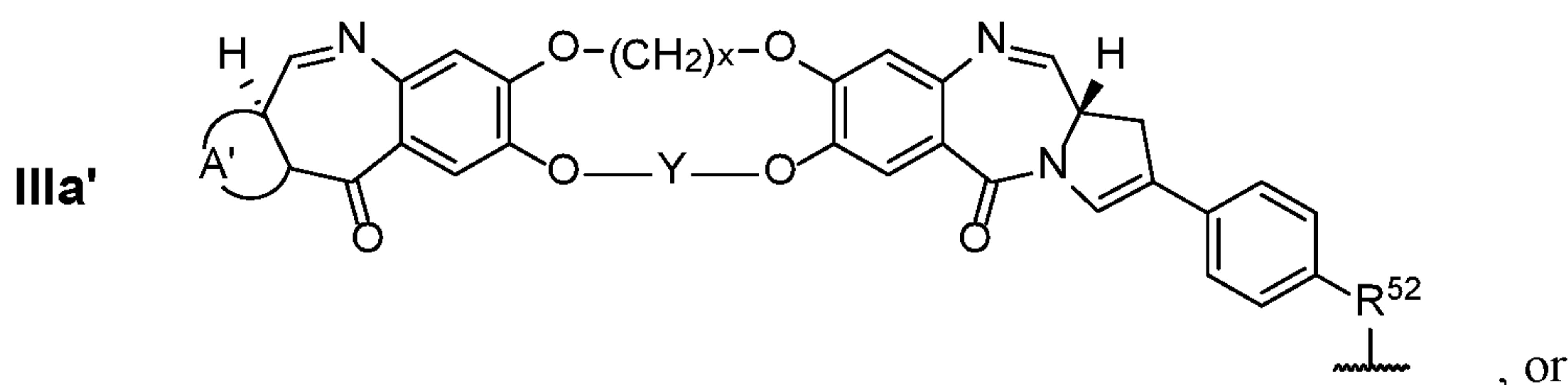
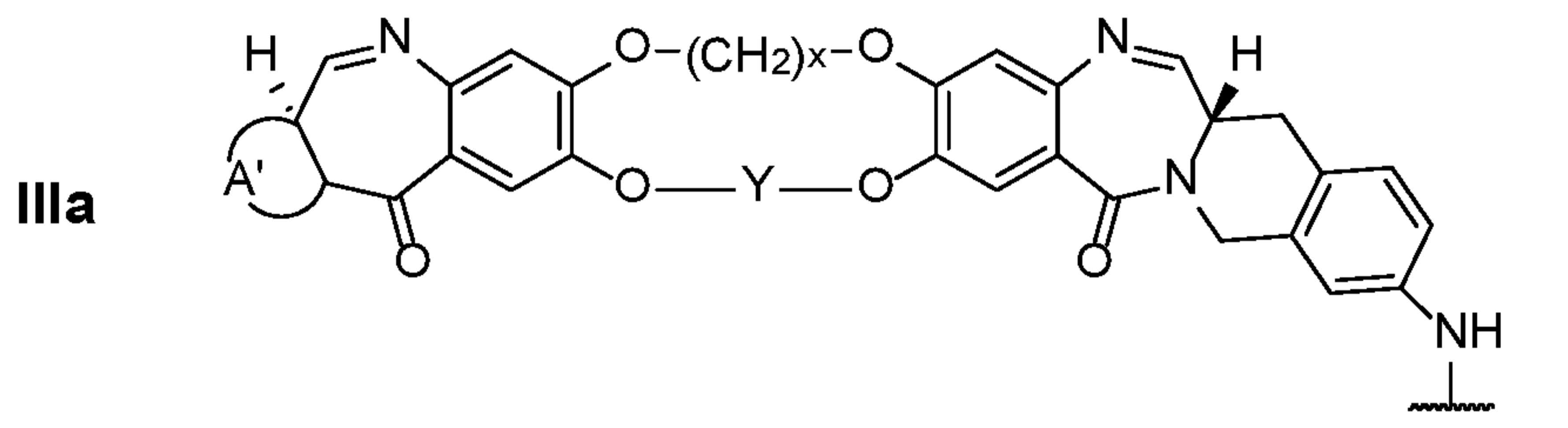
**[00113]** In type (a) and (a') dimer-linker compounds, a functional group for attachment of the linker is located in the bridge X or Y between the two dimer halves. In type (b) dimer-linker compounds, the linker is attached as an addition product across an imine double bond. In 5 type (c) dimer-linker compounds, a functional group for attachment of the linker is located at either A or B.

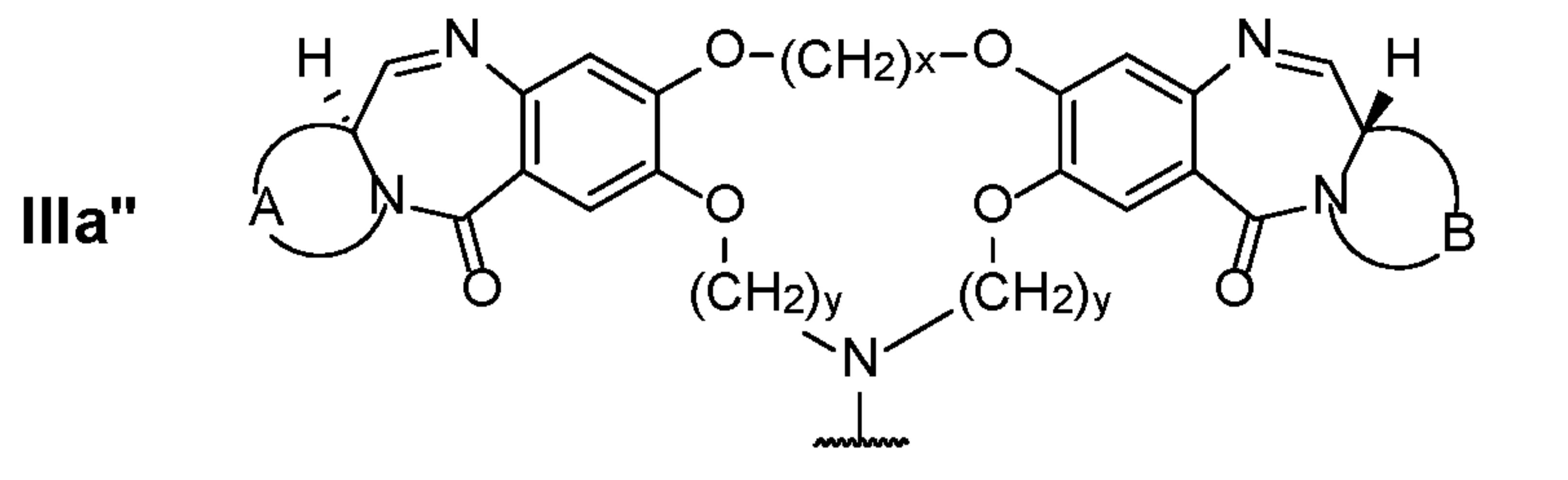
**[00114]** A preferred dimer-linker compound has a structure according to formula III:



wherein

10  $R^{60}$  is according to formula IIIa, IIIa', or IIIa''



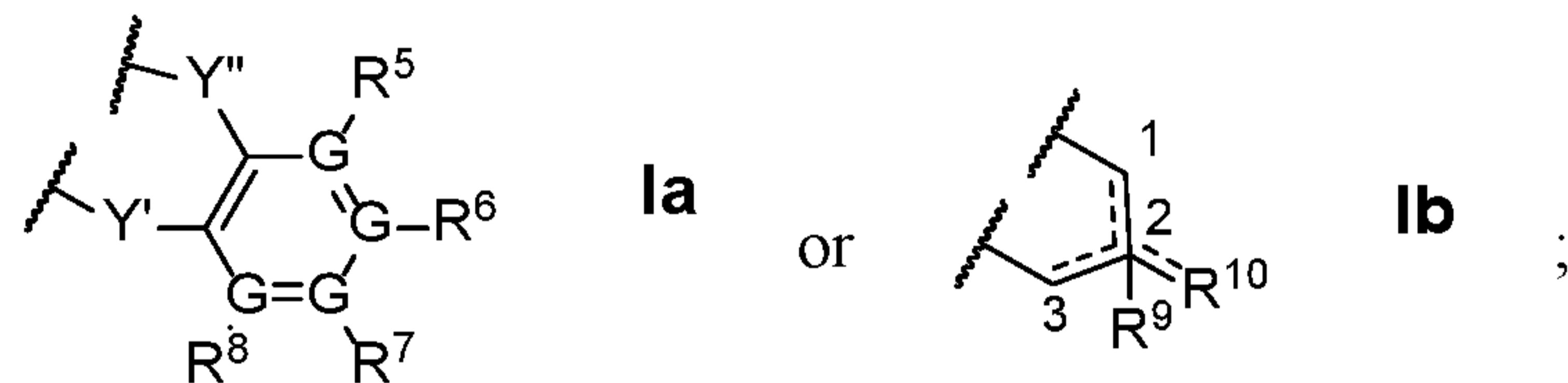


Y is (CH<sub>2</sub>)<sub>6-10</sub> (preferably (CH<sub>2</sub>)<sub>8</sub>);

x is 3 or 5 (preferably 3);

each y is independently 2, 3, or 4 (preferably both are 4);

5 A and B are independently according to formula Ia or Ib



wherein, in formula Ia

Y' and Y" are independently absent, CH<sub>2</sub>, C=O, or CHR<sup>12</sup>; wherein each R<sup>12</sup> is independently F, Cl, Br, or C<sub>1</sub>-C<sub>3</sub> alkyl, with the proviso that Y' and Y" are not both absent;

each G is independently C or N, with the proviso that no more than two Gs are N; and each R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, and R<sup>8</sup> is independently H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl) (preferably H);

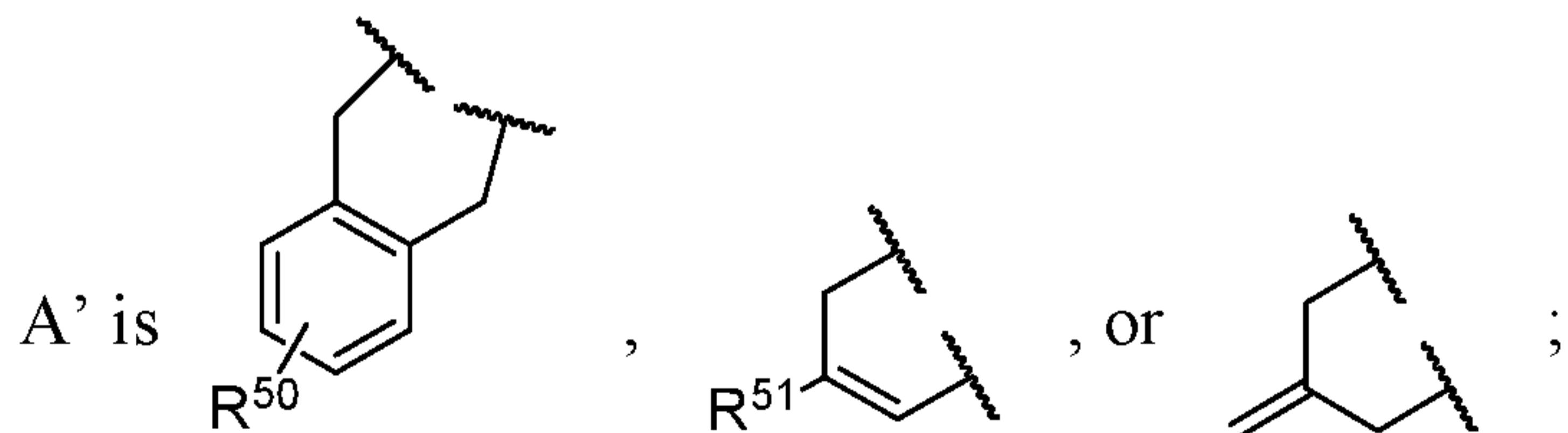
or where a R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is attached to – *i.e.*, is associated with – a G that is N, such R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is absent;

and

wherein, in formula Ib,

the dotted lines indicate the optional presence of a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond;

20 R<sup>9</sup> is absent if a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond is present and otherwise is H; R<sup>10</sup> is H, Cl, Br, =CH<sub>2</sub>, =CH(C<sub>1-5</sub> alkyl), C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, or NH<sub>2</sub> (preferably H);



$R^{50}$  is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl) (preferably H);

$R^{51}$  is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, or NH<sub>2</sub> (preferably H);

T is a self-immolating group;

5 t is 0 or 1;

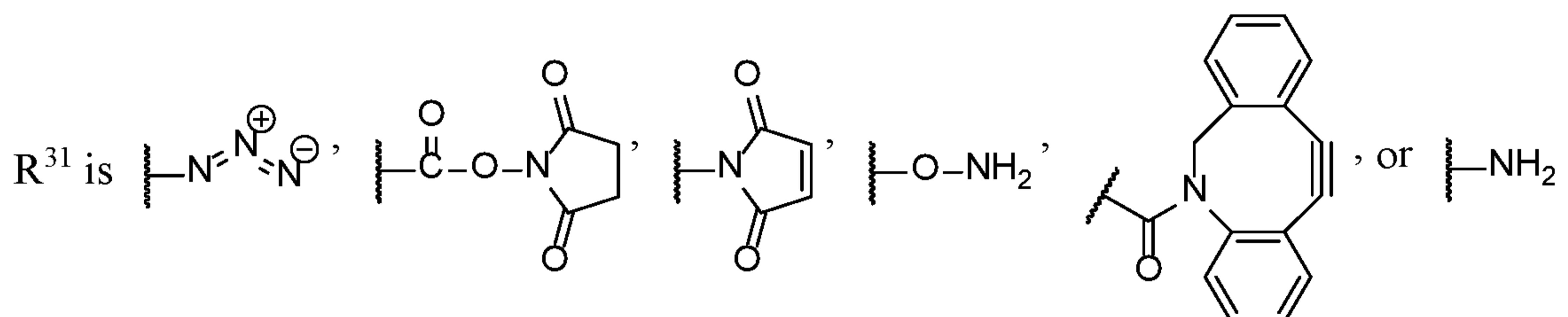
AA<sup>a</sup> and each AA<sup>b</sup> are independently selected from the group consisting of alanine,  $\beta$ -alanine,  $\gamma$ -aminobutyric acid, arginine, asparagine, aspartic acid,  $\gamma$ -carboxyglutamic acid, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, norvaline, ornithine, phenylalanine, proline, serine, 10 threonine, tryptophan, tyrosine, and valine;

p is 1, 2, 3, or 4;

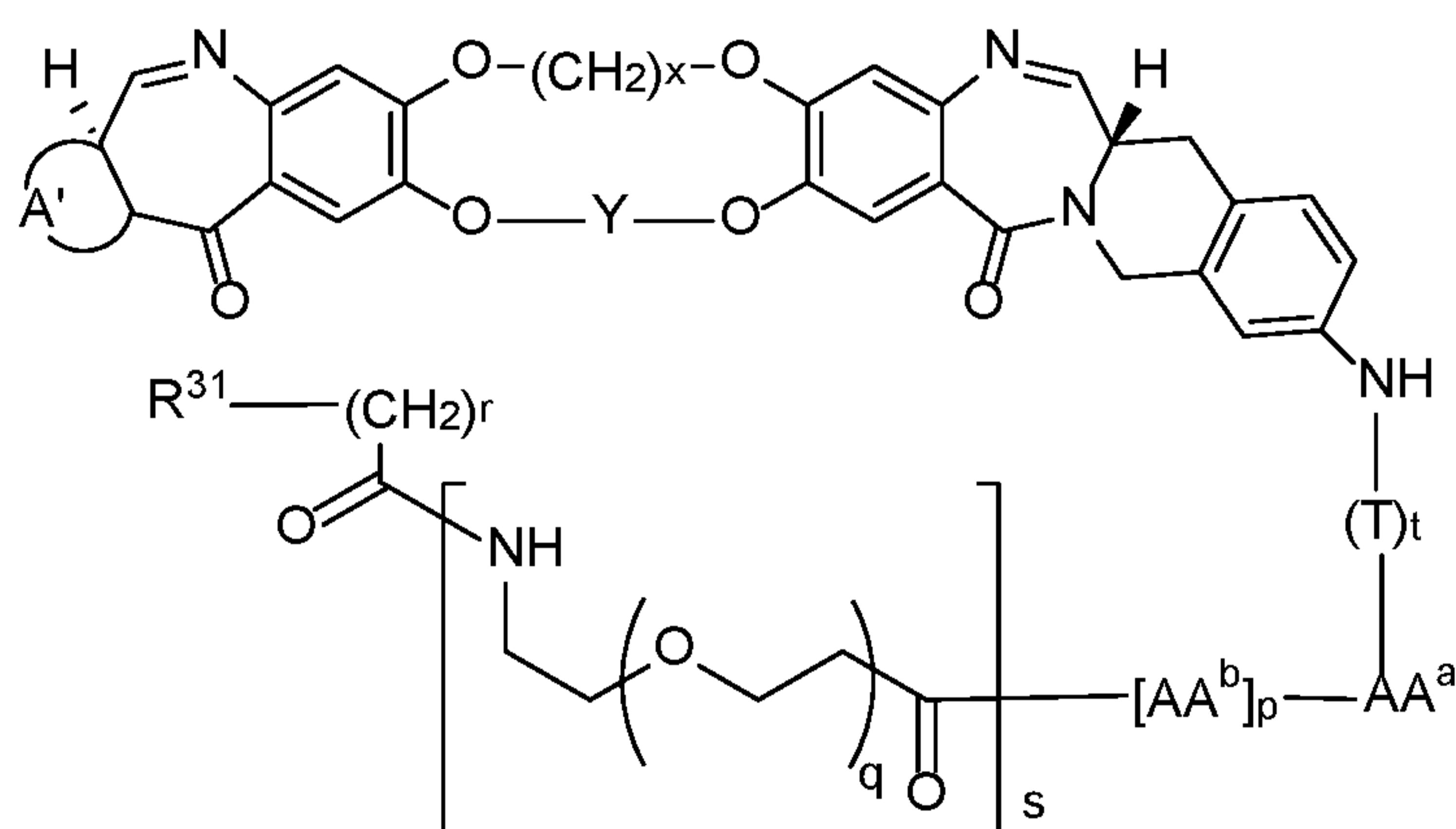
q is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (preferably 2, 3, 4, or 8);

r is 1, 2, 3, 4, or 5 (preferably 2, 3, 4, or 5);

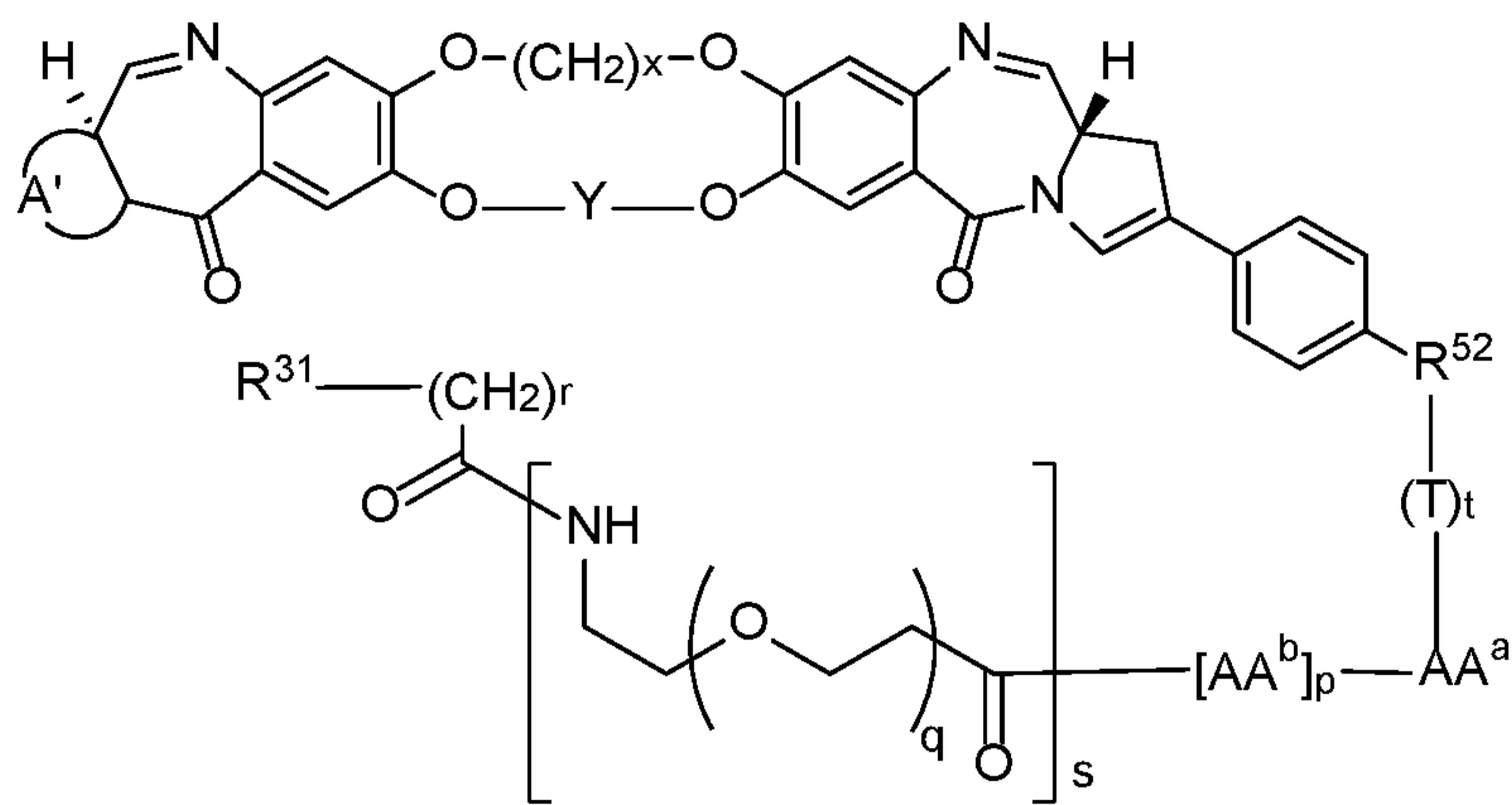
s is 0 or 1; and



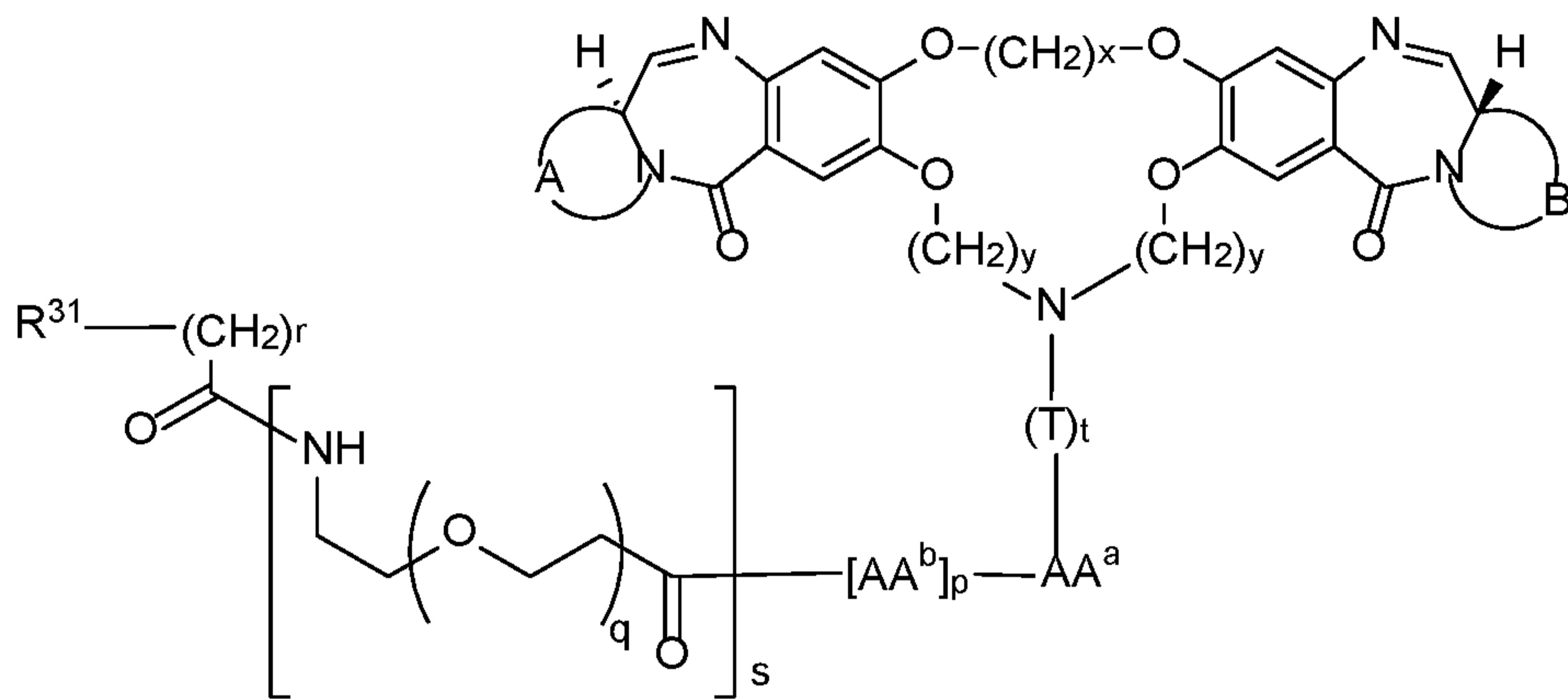
**[00115]** In a preferred dimer-linker compound according to formula III,  $R^{60}$  is IIIa, corresponding to a dimer-linker of the following structure:



**[00116]** In another preferred dimer-linker compound according to formula III,  $R^{60}$  is IIIa', corresponding to a dimer-linker of the following structure:

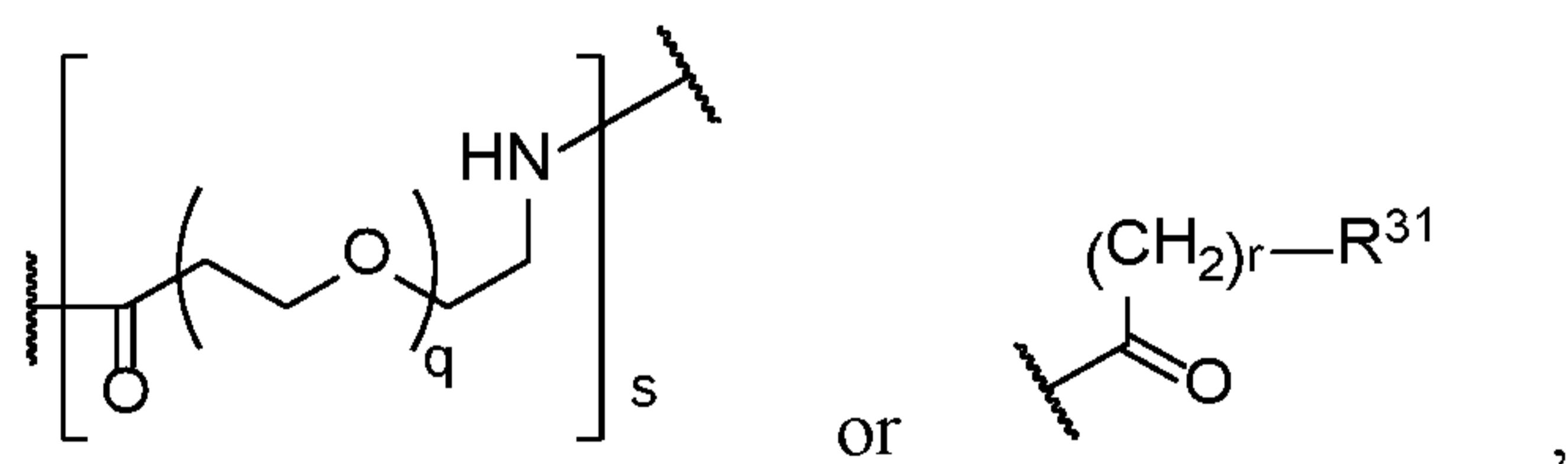


**[00117]** In yet another preferred dimer-linker compound according to formula III,  $R^{60}$  is IIIa", corresponding to a dimer-linker of the following structure:



5 **[00118]**  $R^{31}$  in formula III is a reactive functional group capable of reacting with a complementary functional group on the antibody to effect conjugation, as described above.

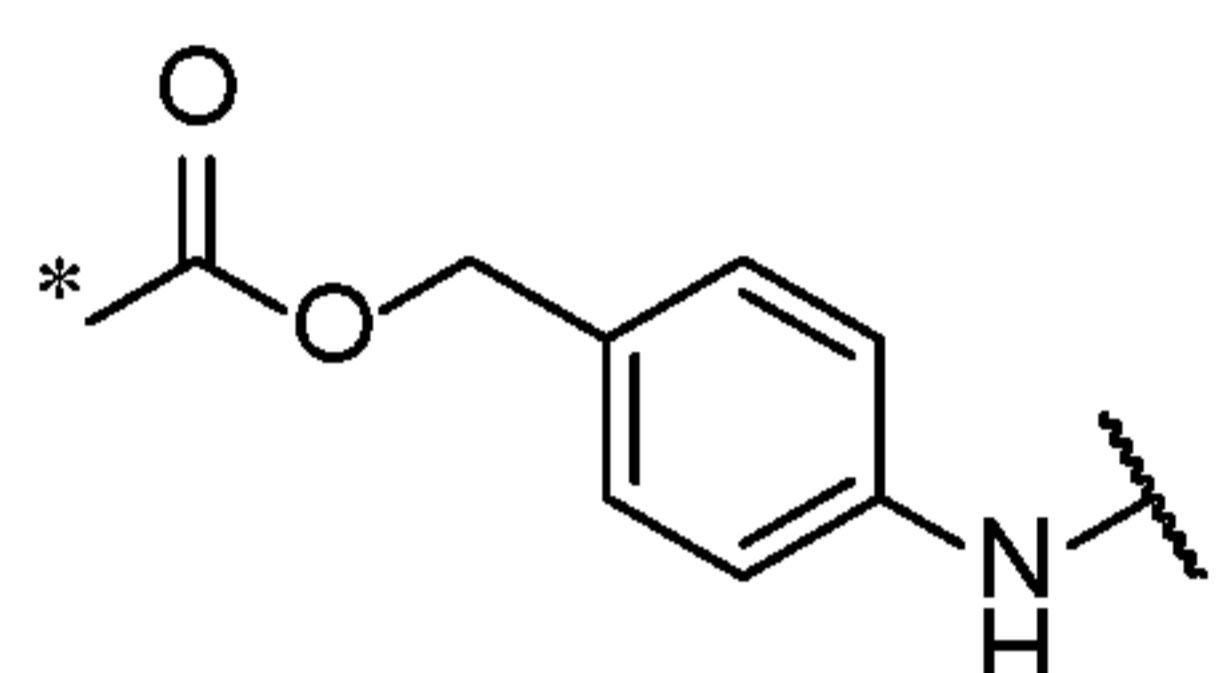
**[00119]** In formula III,  $-AA^a-[AA^b]_p-$  represents a polypeptide whose length is determined by the value of  $p$  (dipeptide if  $p$  is 1, tetrapeptide if  $p$  is 3, etc.).  $AA^a$  is at the carboxy terminus of the polypeptide and its carboxyl group forms a peptide (amide) bond with an amine nitrogen of the dimer. Conversely, the last  $AA^b$  is at the amino terminus of the polypeptide and its  $\alpha$ -amino group forms a peptide bond with



depending on whether  $s$  is 1 or 0, respectively. Preferred polypeptides  $-AA^a-[AA^b]_p-$  are Val-Cit, Val-Lys, Lys-Val-Ala, Asp-Val-Ala, Val-Ala, Lys-Val-Cit, Ala-Val-Cit, Val-Gly,

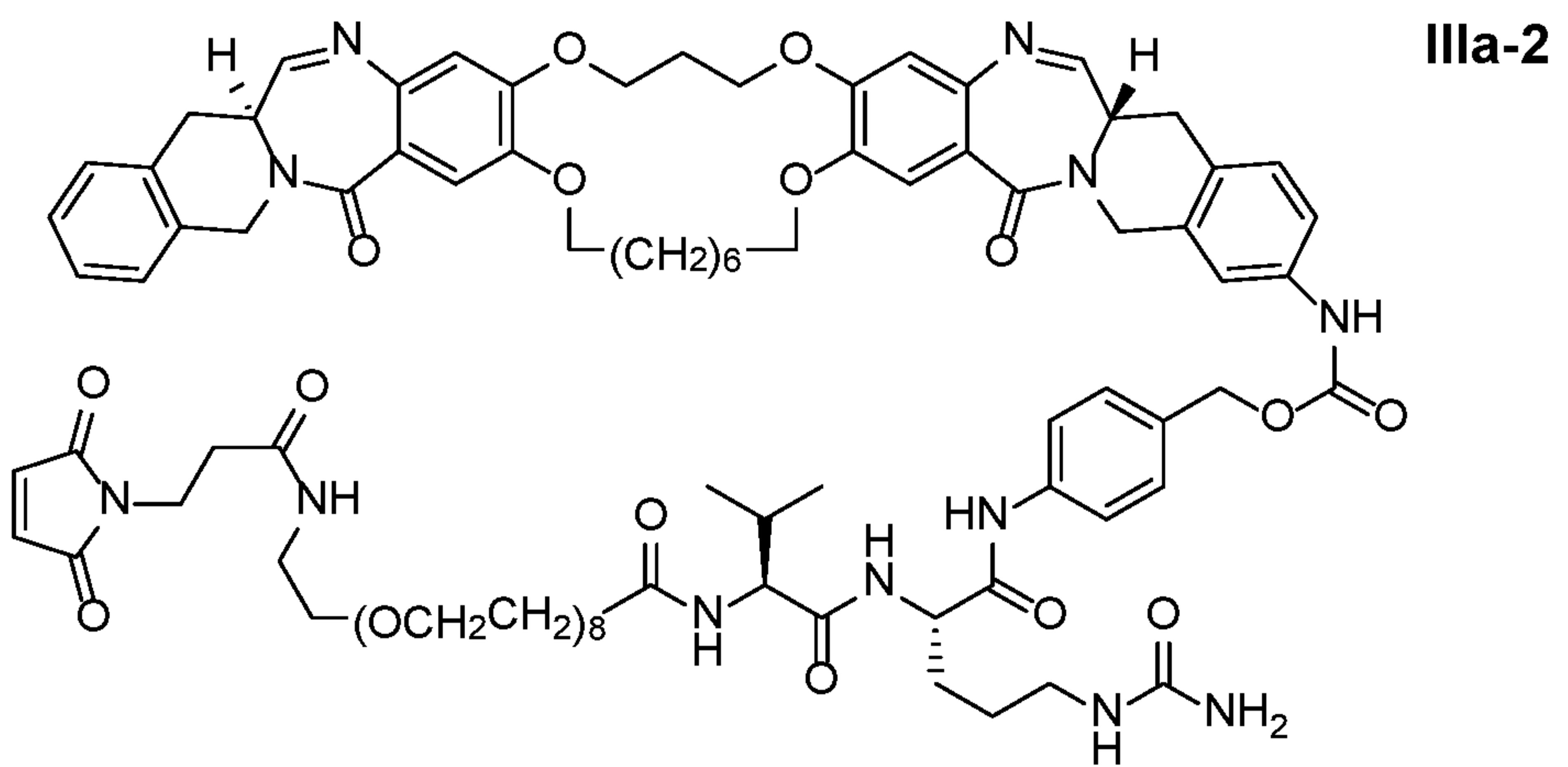
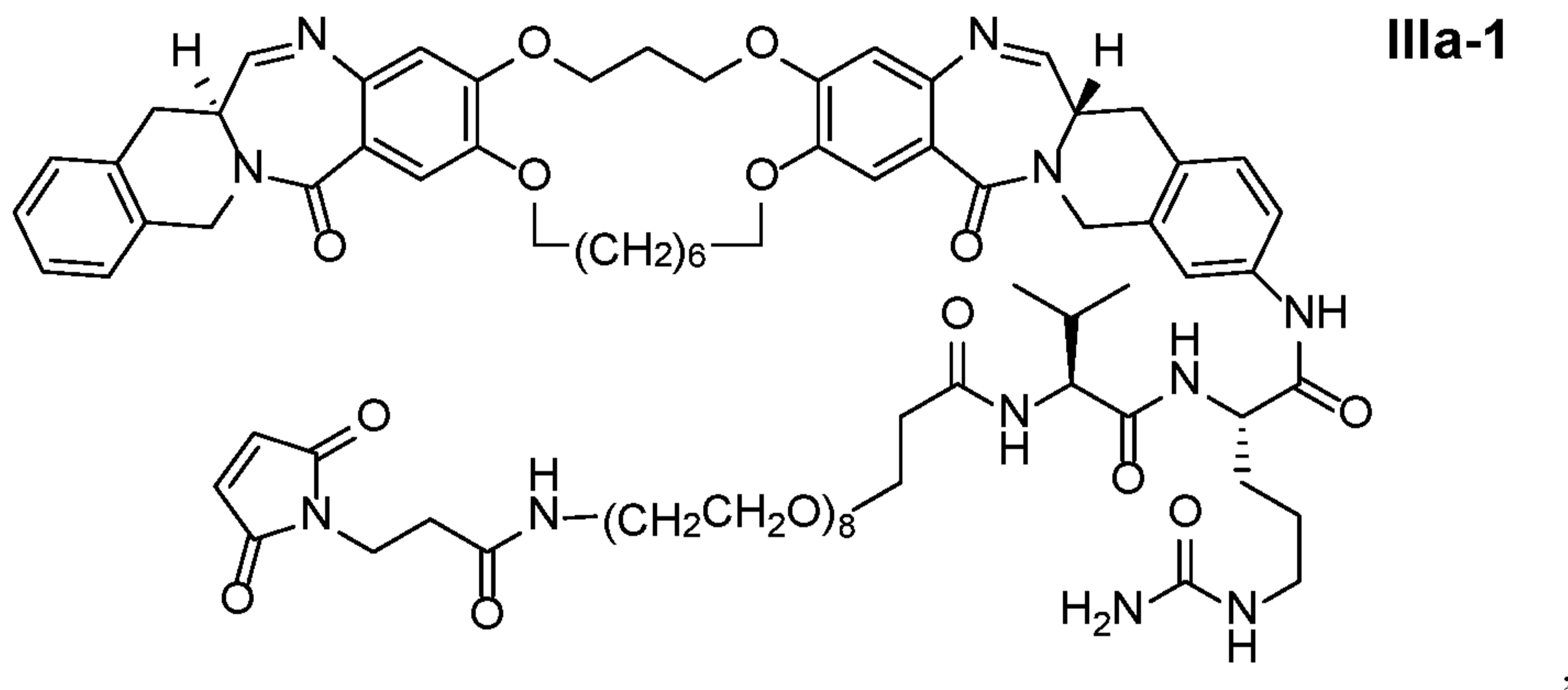
Val-Gln, and Asp-Val-Cit, written in the conventional N-to-C direction, as in H<sub>2</sub>N-Val-Cit-CO<sub>2</sub>H). More preferably, the polypeptide is Val-Cit, Val-Lys, or Val-Ala. Preferably, a polypeptide -AA<sup>a</sup>-[AA<sup>b</sup>]<sub>p</sub>- is cleavable by an enzyme found inside the target (cancer) cell, for example a cathepsin and especially cathepsin B.

5 [00120] As indicated by the subscript t equals 0 or 1, a self-immolating group T is optionally present in dimer-linker compounds of formula **III**. When present, the self-immolating group T preferably is a *p*-aminobenzyl oxycarbonyl (PABC) group, whose structure is shown below, with an asterisk (\*) denoting the end of the PABC bonded to an amine nitrogen of the dimer and a wavy line (~~~~) denoting the end bonded to the polypeptide -AA<sup>a</sup>-[AA<sup>b</sup>]<sub>p</sub>-.

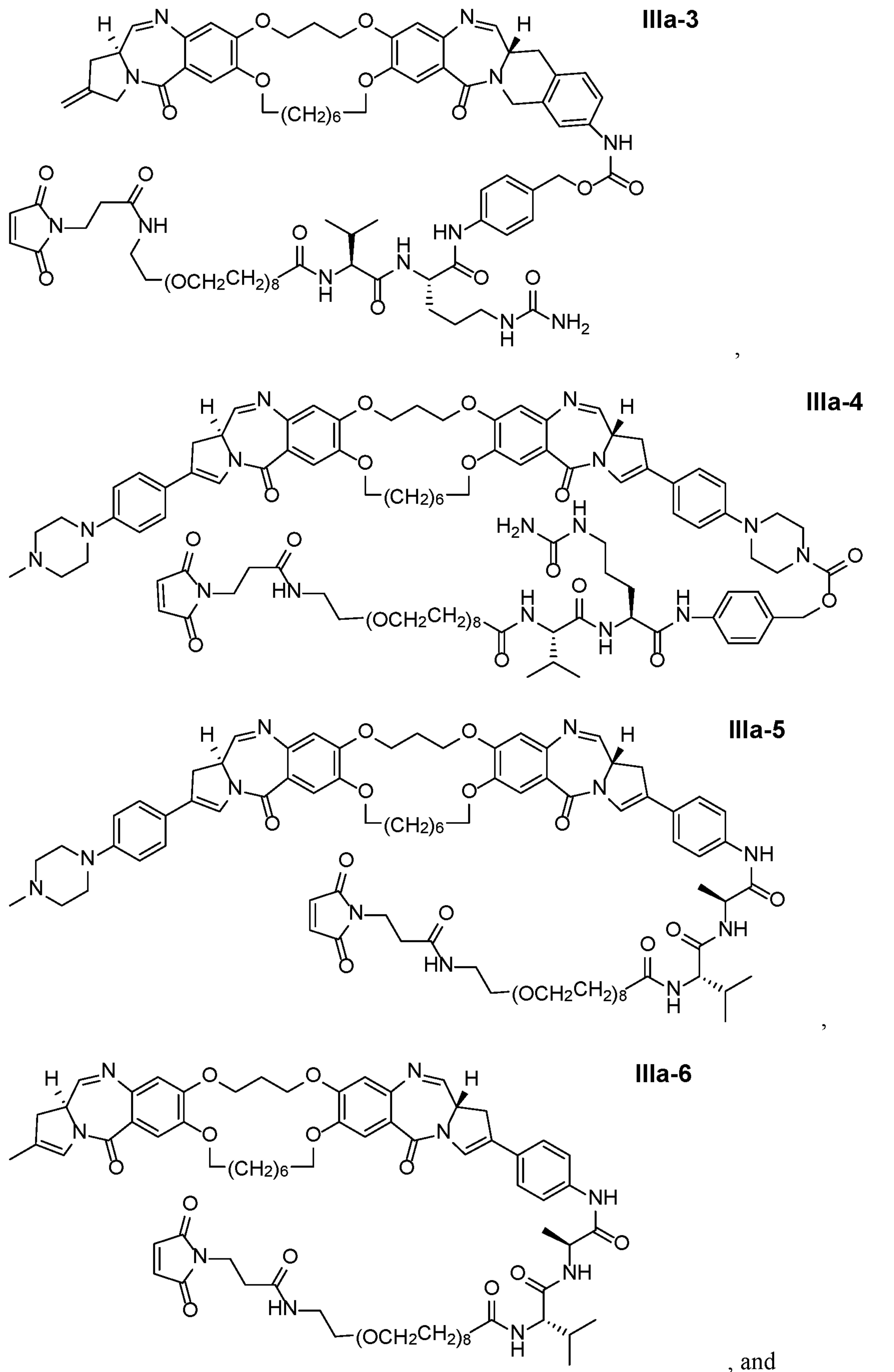


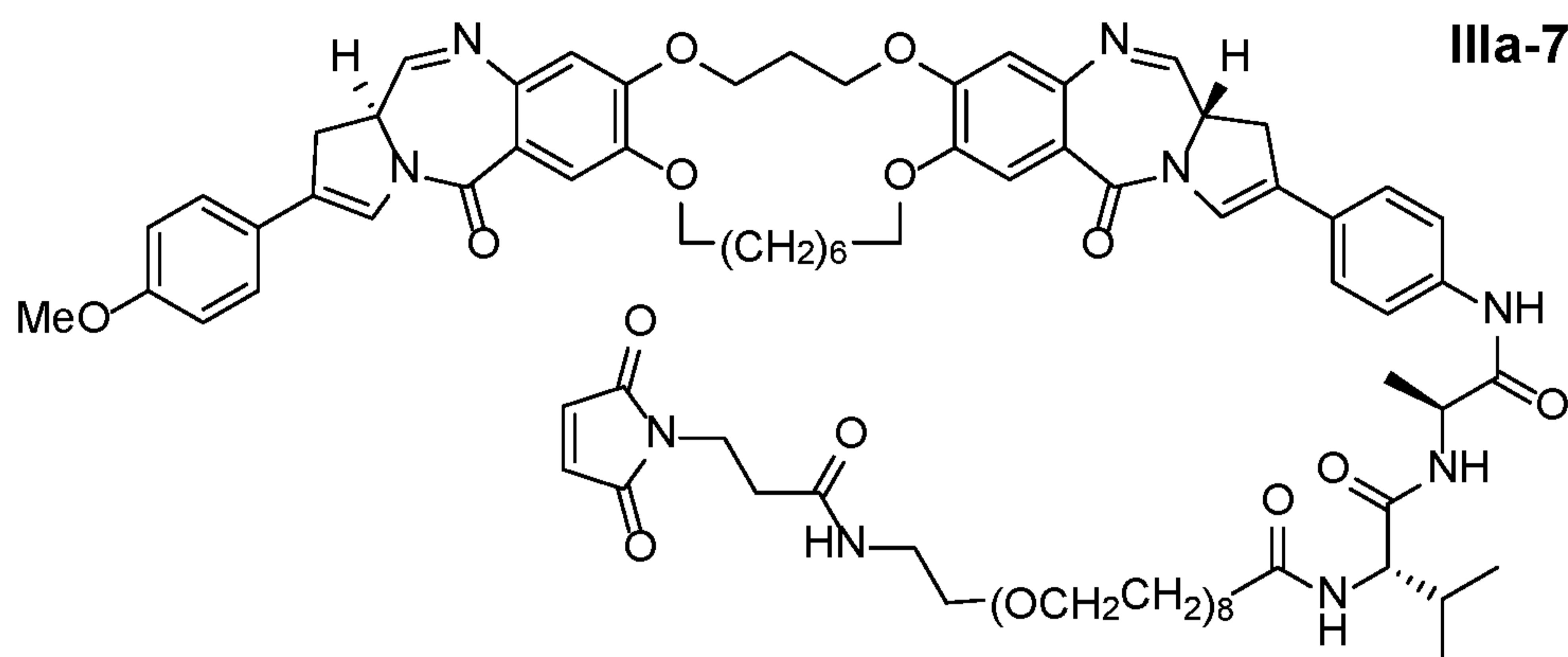
10

[00121] Preferred dimer linker compounds are selected from the group having structures represented by formulae **IIIa-1**, **IIIa-2**, **IIIa-3**, **IIIa-4**, **IIIa-5**, **IIIa-6**, and **IIIa-7**.



15





*Preparation of Conjugates*

**[00122]** This general procedure is based on introduction of free thiol groups into an antibody by reaction of lysine  $\epsilon$ -amino groups with 2-iminothiolane, followed by reaction with a

5 maleimide-containing drug-linker moiety, such as described above. Initially the antibody is buffer exchanged into 0.1 M phosphate buffer (pH 8.0) containing 50 mM NaCl and 2 mM diethylene triamine pentaacetic acid (DTPA) and concentrated to 5-10 mg/mL. Thiolation is achieved through addition of 2-iminothiolane to the antibody. The amount of 2-iminothiolane to be added can be determined by a preliminary experiment and varies from antibody to  
10 antibody. In the preliminary experiment, a titration of increasing amounts of 2-iminothiolane is added to the antibody, and following incubation with the antibody for 1 h at RT (room temperature, circa 25 °C), the antibody is desalted into 50 mM HEPES, 5 mM Glycine, 2 mM DTPA, pH 5.5 using a SEPHADEX™ G-25 column and the number of thiol groups introduced determined rapidly by reaction with dithiodipyridine (DTDP). Reaction of thiol groups with  
15 DTDP results in liberation of thiopyridine, which can be monitored spectroscopically at 324 nm. Samples at a protein concentration of 0.5-1.0 mg/mL are typically used. The absorbance at 280 nm can be used to accurately determine the concentration of protein in the samples, and then an aliquot of each sample (0.9 mL) is incubated with 0.1 mL DTDP (5 mM stock solution in ethanol) for 10 min at RT. Blank samples of buffer alone plus DTDP are also incubated  
20 alongside. After 10 min, absorbance at 324 nm is measured and the number of thiol groups is quantitated using an extinction coefficient for thiopyridine of 19,800 M<sup>-1</sup>.

**[00123]** Typically a thiolation level of about two to three thiol groups per antibody is desirable. For example, with some antibodies this can be achieved by adding a 15-fold molar excess of 2-iminothiolane followed by incubation at RT for 1 h. The antibody is then incubated  
25 with 2-iminothiolane at the desired molar ratio and then desalted into conjugation buffer (50

mM HEPES, 5 mM glycine, 2 mM DTPA, pH 5.5)). The thiolated material is maintained on ice while the number of thiols introduced is quantitated as described above.

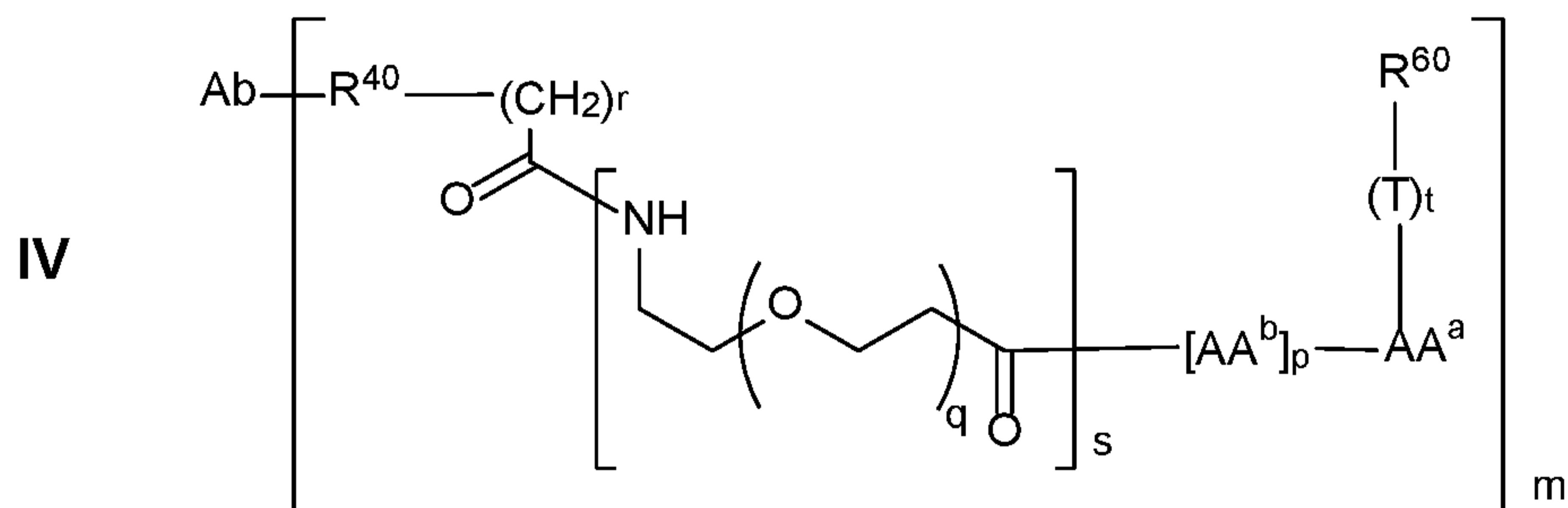
[00124] After verification of the number of thiols introduced, the drug (dimer)-linker moiety is added at a 2.5-fold molar excess per thiol. The conjugation reaction is allowed to proceed in 5 conjugation buffer containing a final concentration of 25% propylene glycol and 5% trehalose. Commonly, the drug-linker stock solution is dissolved in 100% DMSO. The stock solution is added directly to the thiolated antibody.

[00125] The conjugation reaction mixture is incubated at RT for 2 h with gentle stirring. A 10-fold molar excess of N-ethyl maleimide (100 mM Stock in DMSO) is then added to the 10 conjugation mixture and stirred for an additional hour to block any unreacted thiols.

[00126] The sample is then filtered via a 0.2  $\mu$  filter. The material is buffer exchanged via TFF VivaFlow 50 Sartorius 30 MWCO PES membrane into 10 mg/mL glycine, 20 mg/mL sorbitol, 15% acetonitrile (MeCN) pH 5.0 (5X TFF buffer exchange volume), to remove any unreacted drug. The final formulation is carried out by TFF into 20 mg/mL sorbitol, 10 mg/mL 15 glycine, pH 5.0.

[00127] Those skilled in the art will understand that the above-described conditions and methodology are exemplary and non-limiting and that other approaches for conjugation are known in the art and usable in the present invention.

[00128] A preferred conjugate of this invention has a structure represented by formula IV:

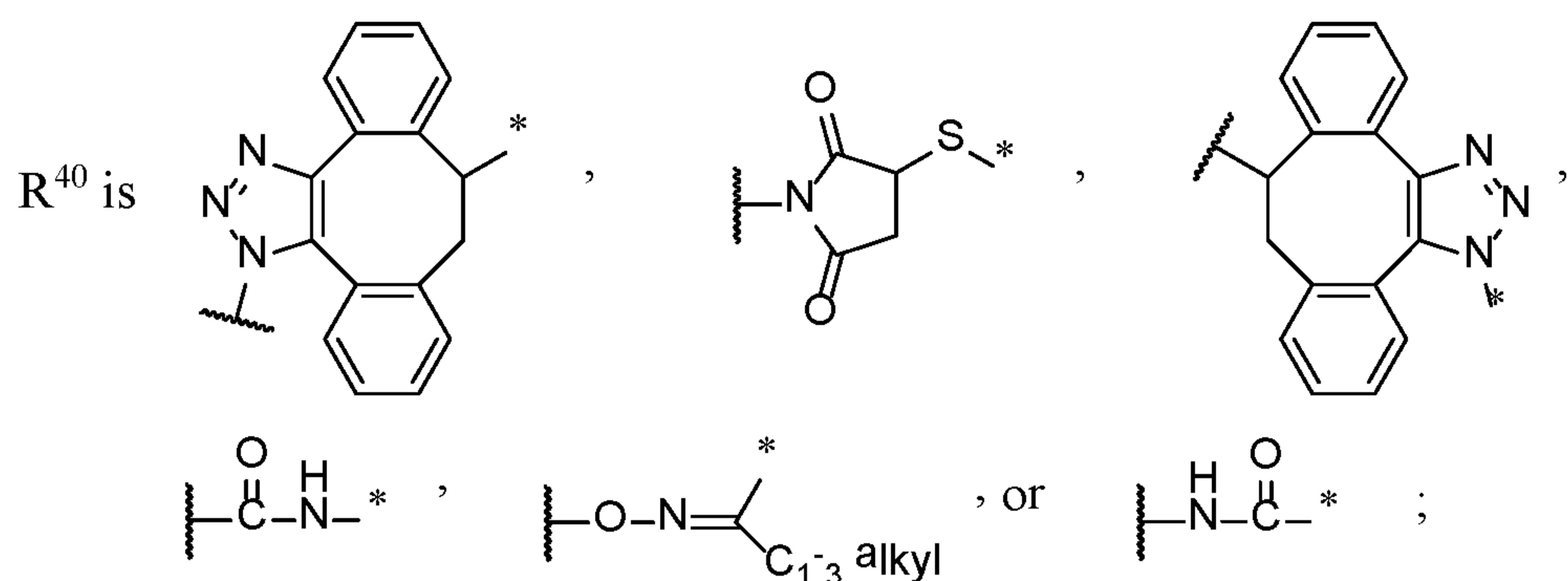


20

wherein

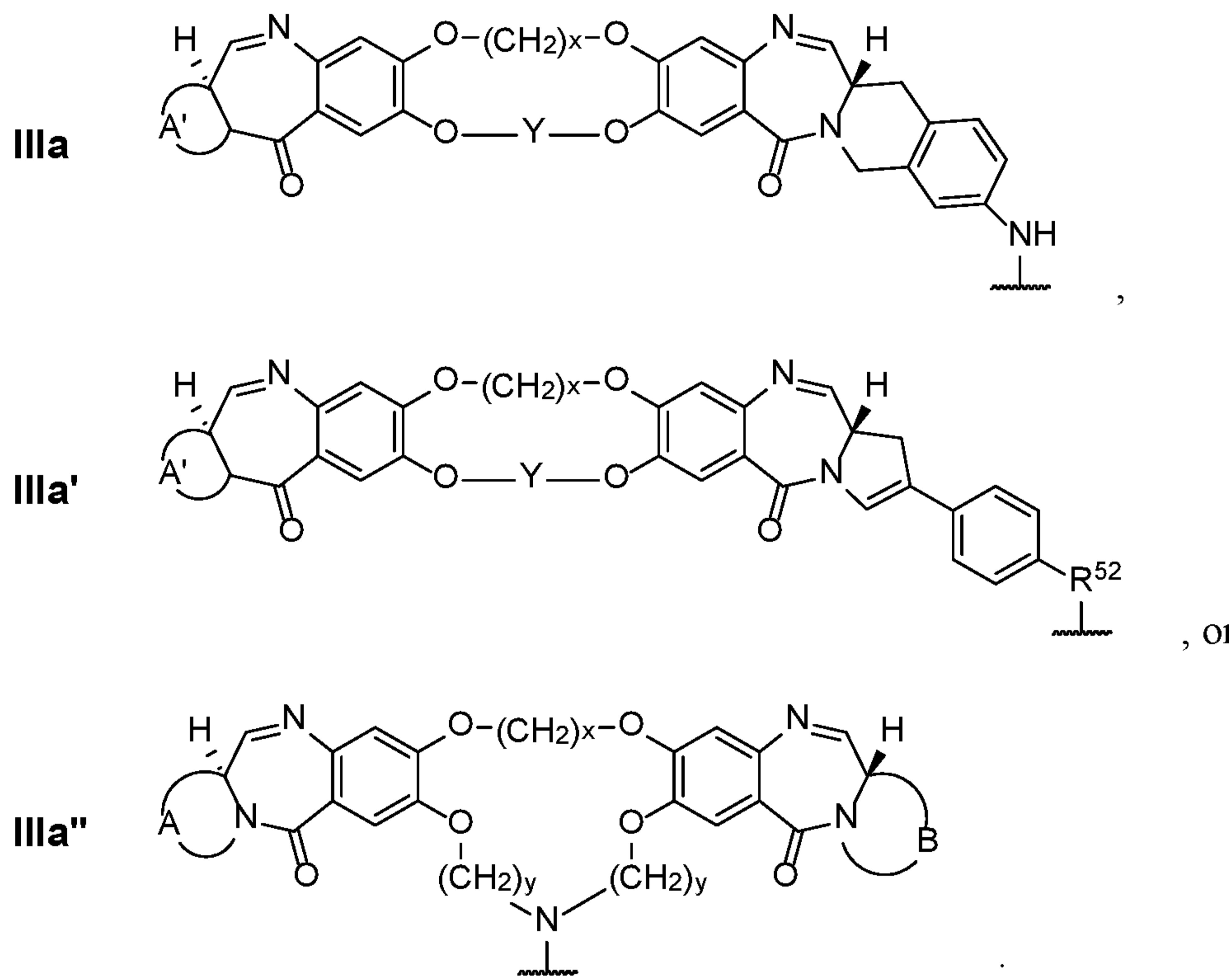
Ab is an antibody;

m is 1, 2, 3, or 4;



where the open valence of  $R^{40}$  that is bonded to  $Ab$  is denoted by an asterisk (\*) and the open valence of  $R^{40}$  that is bonded to  $(CH_2)_r$  is denoted by a wavy line (---);

5  $R^{60}$  is according to formula IIIa, IIIa', or IIIa"



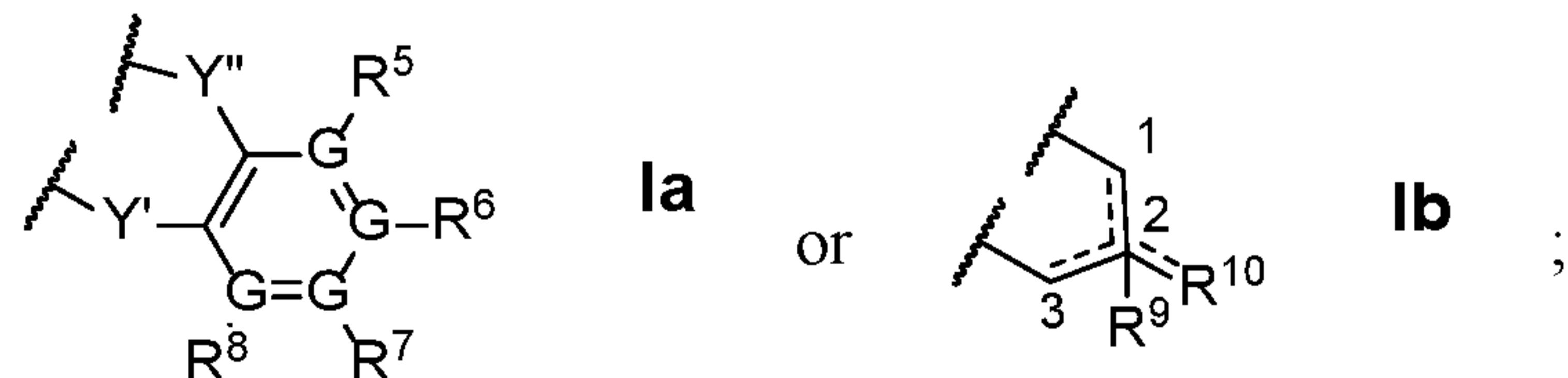
10

$Y$  is  $(CH_2)_{6-10}$  (preferably  $(CH_2)_8$ );

$x$  is 3 or 5 (preferably 3);

each  $y$  is independently 2, 3, or 4 (preferably both are 4);

$A$  and  $B$  are independently according to formula Ia or Ib



15

wherein, in formula Ia

Y' and Y" are independently absent, CH<sub>2</sub>, C=O, or CHR<sup>12</sup>; wherein each R<sup>12</sup> is independently F, Cl, Br, or C<sub>1</sub>-C<sub>3</sub> alkyl, with the proviso that Y' and Y" are not both absent;

5 each G is independently C or N, with the proviso that no more than two Gs are N; and each R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, and R<sup>8</sup> is independently H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl) (preferably H); or where a R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is attached to – *i.e.*, is associated with – a G that is N, such R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is absent;

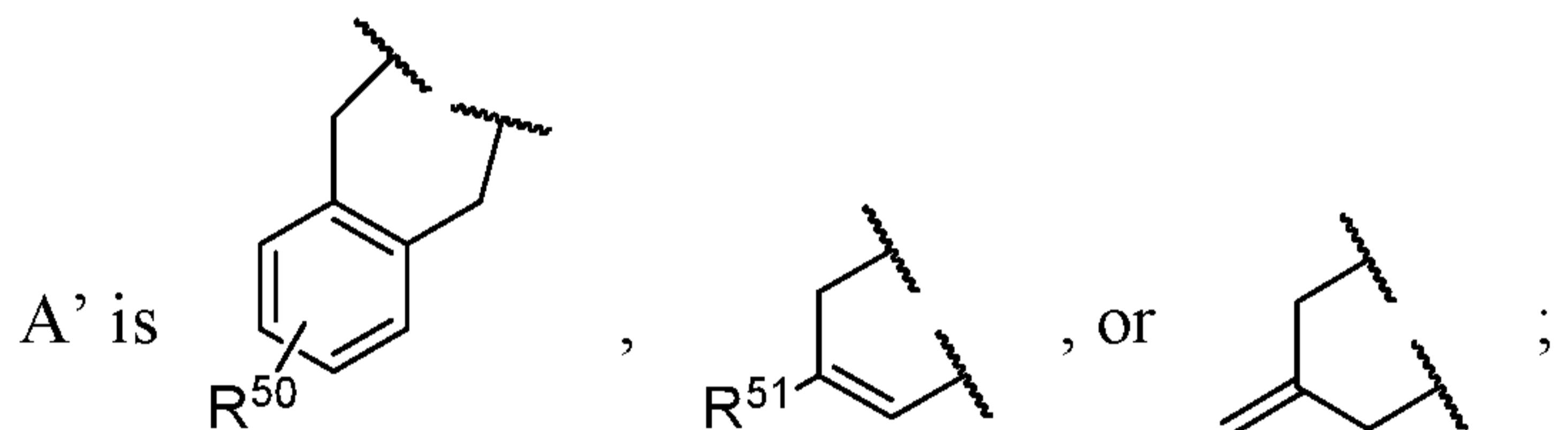
10 and

wherein, in formula Ib,

the dotted lines indicate the optional presence of a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond;

R<sup>9</sup> is absent if a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond is present and otherwise is H;

15 R<sup>10</sup> is H, Cl, Br, =CH<sub>2</sub>, =CH(C<sub>1-5</sub> alkyl), C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, or NH<sub>2</sub> (preferably H);



R<sup>50</sup> is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl) (preferably H);

R<sup>51</sup> is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub> (preferably H);

20 T is a self-immolating group;

t is 0 or 1;

AA<sup>a</sup> and each AA<sup>b</sup> are independently selected from the group consisting of alanine,  $\beta$ -alanine,  $\gamma$ -aminobutyric acid, arginine, asparagine, aspartic acid,  $\gamma$ -carboxyglutamic acid, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, norvaline, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine;

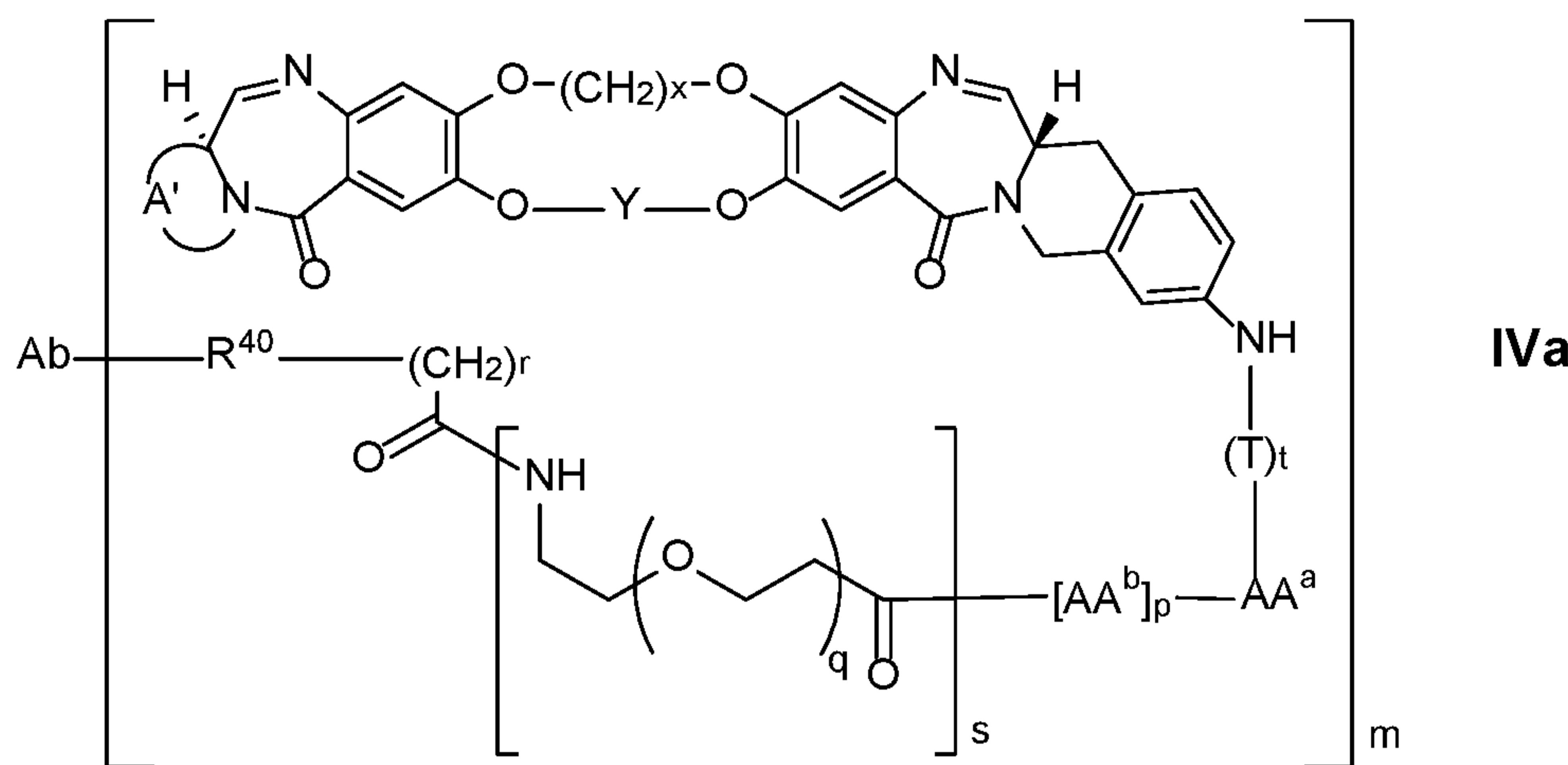
p is 1, 2, 3, or 4;

q is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (preferably 2, 3, 4, or 8);

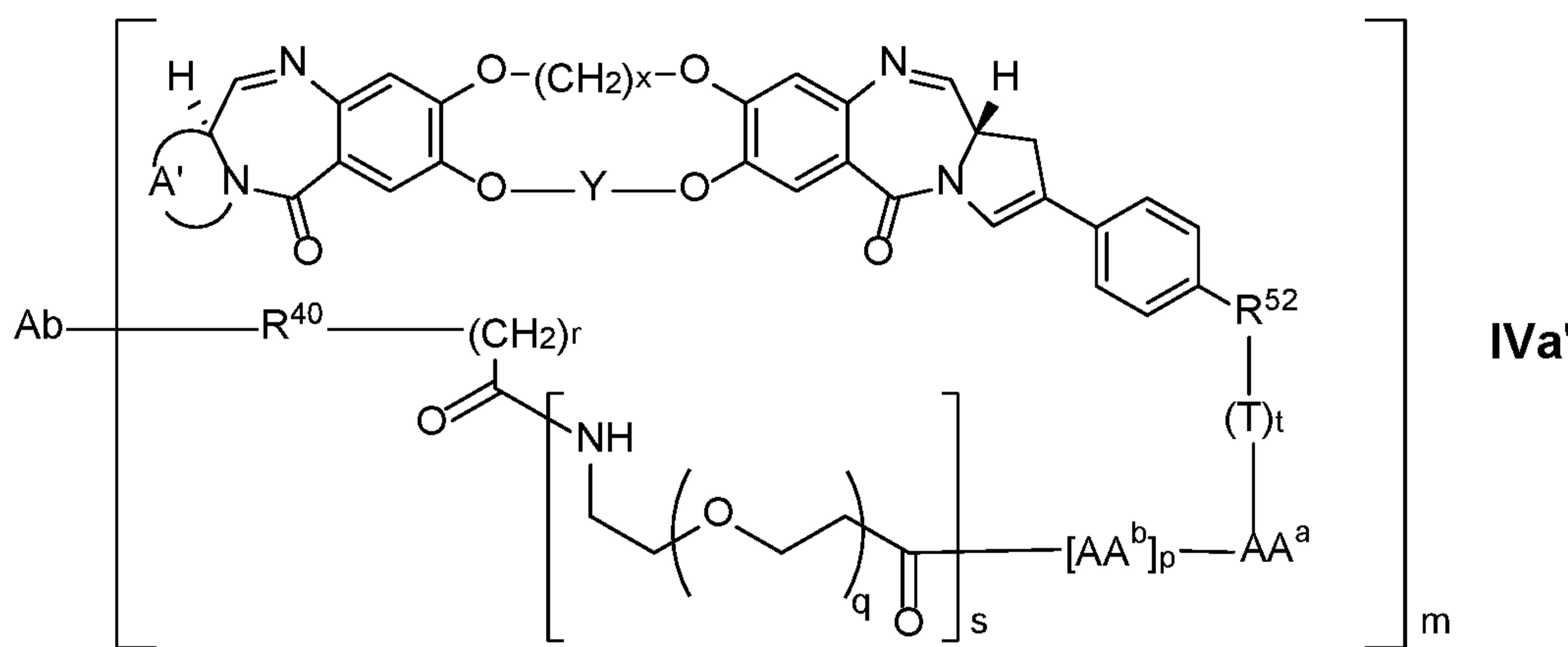
r is 1, 2, 3, 4, or 5 (preferably 2, 3, 4, or 5); and

30 s is 0 or 1.

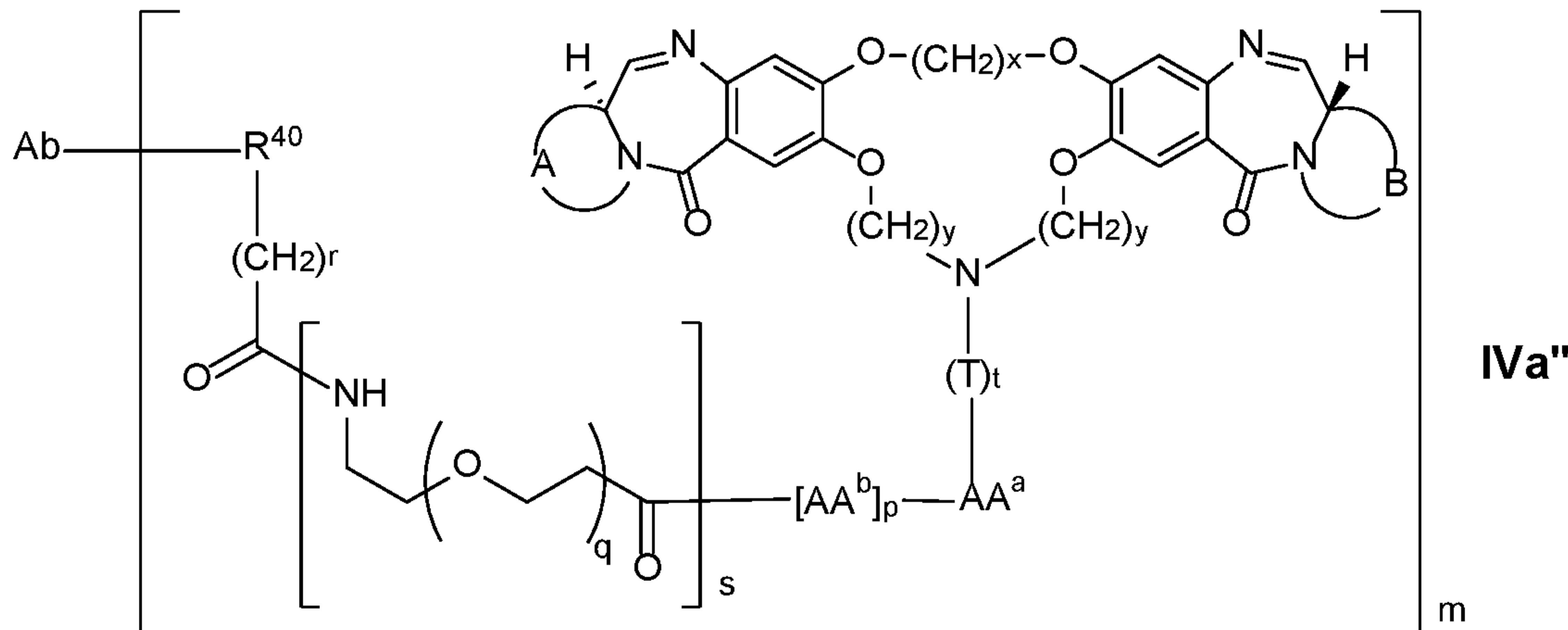
[00129] In a preferred conjugate according to formula IV, R<sup>60</sup> is IIIa, corresponding to a conjugate having a structure represented by formula IVa:



[00130] In another preferred conjugate according to formula IV, R<sup>60</sup> is IIIa', corresponding to a conjugate having a structure represented by formula IVa':



[00131] In another preferred conjugate according to formula IV, R<sup>60</sup> is IIIa'', corresponding to a conjugate having a structure represented by formula IVa'':



## PHARMACEUTICAL COMPOSITIONS

**[00132]** In another aspect, the present disclosure provides a pharmaceutical composition comprising a compound of the present invention, or of a conjugate thereof, formulated together with a pharmaceutically acceptable carrier or excipient. It may optionally contain one or more additional pharmaceutically active ingredients, such as an antibody or another drug. The pharmaceutical compositions can be administered in a combination therapy with another therapeutic agent, especially another anti-cancer agent.

**[00133]** The pharmaceutical composition may comprise one or more excipients. Excipients that may be used include carriers, surface active agents, thickening or emulsifying agents, solid binders, dispersion or suspension aids, solubilizers, colorants, flavoring agents, coatings, disintegrating agents, lubricants, sweeteners, preservatives, isotonic agents, and combinations thereof. The selection and use of suitable excipients is taught in Gennaro, ed., *Remington: The Science and Practice of Pharmacy*, 20th Ed. (Lippincott Williams & Wilkins 2003), the disclosure of which is incorporated herein by reference.

**[00134]** Preferably, a pharmaceutical composition is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound may be coated in a material to protect it from the action of acids and other natural conditions that may inactivate it. The phrase “parenteral administration” means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, the pharmaceutical composition can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

**[00135]** Pharmaceutical compositions can be in the form of sterile aqueous solutions or dispersions. They can also be formulated in a microemulsion, liposome, or other ordered structure suitable to achieve high drug concentration. The compositions can also be provided in the form of lyophilates, for reconstitution in water prior to administration.

**[00136]** The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration and will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range 5 from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

**[00137]** Dosage regimens are adjusted to provide a therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time, or the 10 dose may be proportionally reduced or increased as indicated by the exigencies of the situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic 15 response, in association with the required pharmaceutical carrier.

**[00138]** The dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, or alternatively 0.1 to 5 mg/kg. Exemplary treatment regimens are 20 administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months, or once every three to 6 months. Preferred dosage regimens include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body 25 weight every three weeks. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 µg/mL and in some methods about 25-300 µg /mL.

**[00139]** A "therapeutically effective amount" of a compound of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease 30 affliction. For example, for the treatment of tumor-bearing subjects, a "therapeutically effective amount" preferably inhibits tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at

least about 80% relative to untreated subjects. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject, which is typically a human but can be another mammal.

**[00140]** The pharmaceutical composition can be a controlled or sustained release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

**[00141]** Therapeutic compositions can be administered via medical devices such as (1) needleless hypodermic injection devices (e.g., US 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; and 4,596,556); (2) micro-infusion pumps (US 4,487,603); (3) transdermal devices (US 4,486,194); (4) infusion apparati (US 4,447,233 and 4,447,224); and (5) osmotic devices (US 4,439,196 and 4,475,196); the disclosures of which are incorporated herein by reference.

**[00142]** In certain embodiments, the pharmaceutical composition can be formulated to ensure proper distribution *in vivo*. For example, to ensure that the therapeutic compounds of the invention cross the blood-brain barrier, they can be formulated in liposomes, which may additionally comprise targeting moieties to enhance selective transport to specific cells or organs. See, *e.g.* US 4,522,811; 5,374,548; 5,416,016; and 5,399,331; V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685; Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038; Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180; Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134; Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090; Keinanen and Laukkonen (1994) *FEBS Lett.* 346:123; and Killion and Fidler (1994) *Immunomethods* 4:273.

## USES

**[00143]** Compounds of this invention or their conjugates can be used for treating diseases such as, but not limited to, hyperproliferative diseases, including: cancers of the head and neck which include tumors of the head, neck, nasal cavity, paranasal sinuses, nasopharynx, oral cavity, oropharynx, larynx, hypopharynx, salivary glands, and paragangliomas; cancers of the liver and biliary tree, particularly hepatocellular carcinoma; intestinal cancers, particularly

colorectal cancer; ovarian cancer; small cell and non-small cell lung cancer (SCLC and NSCLC); breast cancer sarcomas, such as fibrosarcoma, malignant fibrous histiocytoma, embryonal rhabdomyosarcoma, leiomyosarcoma, neurofibrosarcoma, osteosarcoma, synovial sarcoma, liposarcoma, and alveolar soft part sarcoma; leukemias such as acute promyelocytic 5 leukemia (APL), acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML); neoplasms of the central nervous systems, particularly brain cancer; multiple myeloma (MM), lymphomas such as Hodgkin's lymphoma, lymphoplasmacytoid lymphoma, follicular lymphoma, mucosa-associated lymphoid tissue lymphoma, mantle cell lymphoma, B-lineage large cell lymphoma, Burkitt's lymphoma, and T- 10 cell anaplastic large cell lymphoma. Clinically, practice of the methods and use of compositions described herein will result in a reduction in the size or number of the cancerous growth and/ or a reduction in associated symptoms (where applicable). Pathologically, practice of the method and use of compositions described herein will produce a pathologically relevant response, such as: inhibition of cancer cell proliferation, reduction in the size of the cancer or 15 tumor, prevention of further metastasis, and inhibition of tumor angiogenesis. The method of treating such diseases comprises administering a therapeutically effective amount of an inventive combination to a subject. The method may be repeated as necessary. Especially, the cancer can be renal, lung, gastric, or ovarian cancer.

**[00144]** Compounds of this invention or their conjugates can be administered in combination 20 with other therapeutic agents, including antibodies, alkylating agents, angiogenesis inhibitors, antimetabolites, DNA cleavers, DNA crosslinkers, DNA intercalators, DNA minor groove binders, enediynes, heat shock protein 90 inhibitors, histone deacetylase inhibitors, immuno-modulators, microtubule stabilizers, nucleoside (purine or pyrimidine) analogs, nuclear export inhibitors, proteasome inhibitors, topoisomerase (I or II) inhibitors, tyrosine kinase inhibitors, 25 and serine/threonine kinase inhibitors. Specific therapeutic agents include adalimumab, ansamitocin P3, auristatin, bendamustine, bevacizumab, bicalutamide, bleomycin, bortezomib, busulfan, callistatin A, camptothecin, capecitabine, carboplatin, carmustine, cetuximab, cisplatin, cladribin, cytarabin, cryptophycins, dacarbazine, dasatinib, daunorubicin, docetaxel, doxorubicin, duocarmycin, dynemycin A, epothilones, etoposide, floxuridine, fludarabine, 5- 30 fluorouracil, gefitinib, gemcitabine, ipilimumab, hydroxyurea, imatinib, infliximab, interferons, interleukins,  $\beta$ -lapachone, lenalidomide, irinotecan, maytansine, mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mitomycin C, nilotinib, oxaliplatin, paclitaxel, procarbazine,

suberoylanilide hydroxamic acid (SAHA), 6-thioguanidine, thiotepa, teniposide, topotecan, trastuzumab, trichostatin A, vinblastine, vincristine, and vindesine.

## EXAMPLES

**[00145]** The practice of this invention can be further understood by reference to the following examples, which are provided by way of illustration and not of limitation. The following general procedures are illustrative, with those skilled in the art understanding that alternative but equivalent methods can be used.

### *Example 1 – Dimer IIa-9*

**[00146]** This example pertains to **FIGs. 1A-1B** and the synthesis of dimer **IIa-9**.

**[00147]** 4-(Benzylxy)-5-methoxy-2-nitrobenzoyl chloride **1** was prepared from the corresponding methyl ester as follows: To a solution of methyl 4-(benzylxy)-5-methoxy-2-nitrobenzoate (Harve Chem, 15 g, 47.3 mmol) in tetrahydrofuran (THF, 350 mL) was added a solution of aq. NaOH (56.7 mL, 142 mmol, 2.5M). The reaction was stirred at 50 °C for 5 h. The reaction was cooled to room temperature (RT) and then concentrated *in vacuo* to remove the THF. The remaining aqueous layer was acidified with aq. HCl (6 N) to pH 2. The resulting yellow precipitate was filtered, washed with water, and dried under vacuum to give 4-(benzylxy)-5-methoxy-2-nitrobenzoic acid (14.32 g, 100% yield). LCMS (M+H) = 304.08; <sup>1</sup>H NMR (400MHz, METHANOL-d<sub>4</sub>) δ 7.60 (s, 1H), 7.53 - 7.45 (m, 2H), 7.45 - 7.31 (m, 3H), 7.29 (s, 1H), 5.23 (s, 2H), 3.98 (s, 3H).

**[00148]** To a solution of the above nitrobenzoic acid (1.2 g, 3.96 mmol) in THF (30 mL) was added dropwise oxalyl chloride (0.416 mL, 4.75 mmol), followed by N,N-dimethyl-formamide (DMF, 20 uL). The resulting solution was stirred at RT for 35 h before it was concentrated *in vacuo* to give acid chloride **1** as a yellow solid.

**[00149]** Acid chloride **1** was dissolved in THF (20 mL) and added dropwise to a solution of S-methyl pyrrolidine-2-carboxylate **2** hydrochloride (0.768 g, 4.75 mmol) and triethylamine (NEt<sub>3</sub>, 1.65 mL, 11.87 mmol) in THF (10 mL) at 0 °C. The reaction mixture was warmed to RT and stirred at RT for 1 h before quenching with aq. LiCl and concentrated to remove the THF. The resulting mixture was extracted with EtOAc (3x). The combined organic layers were washed with sat. aq. NaHCO<sub>3</sub> and then brine and dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude product mixture was purified using ISCO silica gel chromatography (80 g column, gradient from 0% to 100% EtOAc/dichloromethane (DCM) in 15 minutes) to give ester **3** (1.18

mg, 72% yield). LCMS (M+H) = 415.4; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.77 (s, 1H), 7.51 - 7.32 (m, 5H), 6.92 - 6.80 (m, 1H), 5.25 - 5.20 (m, 2H), 4.80 - 4.73 (m, 1H), 4.03 - 3.93 (m, 3H), 3.82 (s, 2H), 3.56 (s, 1H), 3.38 - 3.30 (m, 1H), 3.21 (s, 1H), 2.41 - 2.30 (m, 1H), 2.16 - 2.07 (m, 1H), 2.04 - 1.87 (m, 2H).

5 [00150] A suspension of ester **3** (900 mg, 2.172 mmol) and Pd(OH)<sub>2</sub> (20% on carbon, 90 mg) in EtOH (15 mL) was stirred under H<sub>2</sub> (50 psi) for 3 h. The reaction mixture was filtered through a pad of CELITE™ and washed with EtOAc. The combined filtrates were concentrated and dissolved in MeOH (10 mL). After a drop of AcOH was added, the reaction was heated at 80 °C for 5 h. The reaction was then cooled to RT and concentrated. The residue was purified 10 using ISCO silica gel chromatography (40 g column, 0-100% EtOAc/Hexane gradient) to give compound **4**. LCMS (M+H) = 263; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 10.18 (s, 1H), 9.89 (br. s., 1H), 7.22 (s, 1H), 6.55 (s, 1H), 4.17 - 3.96 (m, 1H), 3.77 (s, 3H), 3.63 - 3.37 (m, 2H), 1.96 - 1.63 (m, 4H).

15 [00151] A suspension of compound **4** (0.8 g, 3.05 mmol) and 1,3-bromopropane **4a** (0.308 g, 1.525 mmol) and K<sub>2</sub>CO<sub>3</sub> (527 mg, 3.81 mmol) in DMSO (8 mL) were stirred at RT for 12 h. The reaction mixture was diluted with aq. NH<sub>4</sub>Cl and extracted with chloroform (3x). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The crude product mixture was purified using ISCO silica gel chromatography (gradient from 0% to 10% MeOH/DCM) to give compound **5** (670 mg, 78% yield). LCMS (M+H) = 20 565; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.45 (s, 2H), 6.54 (s, 2H), 4.19 (d, *J*=7.5 Hz, 4H), 4.03 (d, *J*=5.9 Hz, 2H), 3.89 (s, 6H), 3.82 - 3.72 (m, 2H), 3.64 - 3.55 (m, 2H), 2.73 (br. s., 2H), 2.37 - 2.32 (m, 2H), 2.07 - 1.95 (m, 6H).

25 [00152] To a solution of compound **5** (650 mg, 1.151 mmol) in DCM (2 mL) at -78 °C was added dropwise a solution of boron tribromide (10.04 mL, 10.4 mmol, 1M in DCM). The reaction was slowly warmed to -5 °C and stirred for 30 min. The reaction was then quenched with aq. potassium phosphate dibasic buffer (5 mL), and then concentrated to remove DCM. The remaining slurry was filtered to give a grey solid, which was purified using ISCO silica gel chromatography (120 g column, gradient from 0% to 10% MeOH/DCM) to give compound **6** (202 mg, 33% yield). LCMS (M+H) = 537; <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>) δ 10.10 (br. s., 2H), 9.44 - 9.01 (m, 2H), 7.17 (s, 2H), 6.66 (s, 2H), 4.13 (br. s., 5H), 4.03 (d, *J*=6.4 Hz, 2H), 30 3.51 (br. s., 6H), 2.46 (br. s., 1H), 2.32 - 2.16 (m, 2H), 2.06 - 1.66 (m, 7H).

[00153] A suspension of compound **6** (86mg, 0.160 mmol), 6-bromohex-1-ene **6a** (52.3 mg, 0.321 mmol), and K<sub>2</sub>CO<sub>3</sub> (66.5 mg, 0.481 mmol) in DMF (1.5 mL) was stirred at RT for 15 h. The reaction mixture was filtered and purified using ISCO silica gel chromatography (40 g column, gradient from 0% to 10% MeOH/DCM) to give compound **7** (55 mg, 49% yield).

5 LCMS (M+H) = 701.7.

[00154] A vial was charged with compound **7** (52 mg, 0.074 mmol). A solution of Grubbs-II catalyst (6.30 mg, 7.42  $\mu$ mol) in DCE (10 mL) was then added. The resulting solution was degassed and heated to 75 °C for 1h. The reaction mixture was then concentrated and purified using ISCO silica gel chromatography (12 g column, gradient 0% to 10% MeOH/DCM) to give 10 the 21-membered macrocycle **8** (44 mg, 88%, along with ~10% 20-membered macrocycle by-product **9**). LCMS (M+H) = 673.7.

[00155] To a solution of the mixture of compounds **8** and **9** (18mg, 0.027 mmol) in DMF (1 mL) at 0 °C was added NaH (4.01 mg, 0.067 mmol). The resulting suspension was stirred at 0 °C for 30 min before 2-(chloromethoxy)ethyltrimethylsilane (SEM-Cl, 0.014 mL, 0.080 mmol) was added. The reaction was then stirred at 0 °C for 1h before quenching with brine. 15 The mixture was extracted with EtOAc (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and purified using ISCO silica gel chromatography (12 g column, gradient from 0 % to 100% MeOH/DCM) to give compound **10**. LCMS (M+H) = 933; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  7.37 (s, 2H), 7.22 (s, 2H), 5.48 (m, 2H), 5.43 (t, *J*=3.5 Hz, 2H), 4.70 (m, 2H), 4.29 - 4.21 (m, 4H), 4.14 - 4.03 (m, 6H), 3.84 - 3.65 (m, 7H), 3.62 - 20 3.50 (m, 2H), 2.79 - 2.67 (m, 2H), 2.35 (m, 2H), 2.12 - 1.96 (m, 10H), 1.80 (m, 4H), 1.58 - 1.47 (m, 2H), 0.98 (m, 4H), 0.04 - 0.02 (s, 18H).

[00156] To a solution of compound **10** in THF/EtOH (1:1, 1 mL) at 0 °C was added a 25 solution of lithium borohydride (0.268 mL, 0.535 mmol, 2M in THF). The resulting solution was stirred at 0 °C for 1 h before it was warmed to RT and stirred for 5 min. The reaction was then quenched with brine and extracted with CHCl<sub>3</sub> (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was then taken up in CHCl<sub>3</sub>/ EtOH (1:1, 2 mL). Silica gel (0.9 g) was added, followed by water (1 mL). The resulting suspension was stirred at RT for 48 h and then filtered, washing with 10% MeOH/CHCl<sub>3</sub>. The filtrate was 30 concentrated and purified using reverse phase HPLC (Column: Phenomenex Luna C18 20x100mm; Mobile Phase A: 10:90 MeCN:water with 0.1% trifluoroacetic acid (TFA); Mobile Phase B: 90:10 MeCN:water with 0.1% TFA acid; Gradient: 0-80% B over 15 minutes; Flow:

20 mL/min; Detection: UV at 220 nm) to giveThe fractions containing desired fractions were combined and neutralized with aq. NaHCO<sub>3</sub>, then extracted with chloroform, and dried and concentrated to give dimer **IIa-9**. (7.1 mg, 39% over two steps). LCMS (M+H) = 641.3; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.68 (d, *J*=4.4 Hz, 2H), 7.53 (s, 2H), 6.85 (s, 2H), 5.46 (m, 2H), 4.36 - 4.23 (m, 4H), 4.20 - 4.12 (m, 2H), 4.11 - 4.03 (m, 2H), 3.87- 3.82 (m, 2H), 3.75 (dt, *J*=7.5, 4.0 Hz, 2H), 3.60 (dt, *J*=11.8, 7.8 Hz, 2H), 2.41 - 2.30 (m, 6H), 2.14 - 2.01 (m, 8H), 1.88 - 1.77 (m, 4H), 1.67 - 1.58 (m, 4H).

*Example 2 – Dimers IIa-3 and IIa-4*

[00157] This example pertains to **FIG. 2** and the synthesis of dimers **IIa-3** and **IIa-4**.

[00158] A suspension of a mixture of compounds **8** and **9** (24 mg, 0.036 mmol) and 10% Pd/C (6 mg) in MeOH (3 mL) was stirred under a balloon of H<sub>2</sub> for 3h. The reaction mixture was purged with N<sub>2</sub> and filtered through a pad of CELITE™, washing with EtOAc. The combined filtrates were concentrated to give 21-membered macrocycle **11** (24 mg, 100% yield, alon with ~10% macrocycle **12**). LCMS (M+H) = 675.4.

[00159] To a solution of the mixture of macrocycle **11** and **12** (24mg, 0.036 mmol) in DMF (1 mL) at 0 °C was added NaH (5.33 mg, 0.089 mmol). The resulting suspension was stirred at 0 °C for 30 min before SEM-Cl (0.019 mL, 0.107 mmol) was added. The reaction was stirred at 0 °C for 1h before it was quenched with brine. The mixture was extracted with EtOAc (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and purified using ISCO 20 silica gel chromatography (12 g column, gradient from 0 % to 100% MeOH/DCM) to give a mixture of SEM-macrocycles (21-membered macrocycle: LCMS (M+H) = 935 . 20-membered macrocycle: LCMS (M+H) = 921).

[00160] To a solution of the mixture of the above SEM-macrocycles in THF/EtOH (1:1, 1 mL) at 0 °C was added a solution of LiBH<sub>4</sub> (0.356 mL, 0.71 mmol, 2M in THF). The resulting solution was stirred at 0 °C for 1 h before warming to RT and stirring for 15 min. The reaction was quenched with brine and extracted with CHCl<sub>3</sub> (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was taken up in CHCl<sub>3</sub>/EtOH (1:1, 2 mL). Silica gel (0.9 g) was added, followed by water (1 mL). The resulting suspension was stirred at RT for 48 h and filtered, washing with 10% MeOH/CHCl<sub>3</sub>. The filtrate was concentrated and purified using reverse phase HPLC to give dimers **IIa-4** and **IIa-3** (Column: Phenomenex Luna C18 20x100mm; Mobile Phase A: 10:90 MeCN:water with 0.1% trifluoroacetic acid (TFA);

Mobile Phase B: 90:10 MeCN:water with 0.1% TFA acid; Gradient: 0-80% B over 15 minutes; Flow: 20 mL/min; Detection: UV at 220 nm).

[00161] Dimer **IIa-4**: (7.5 mg, 31% yield); LCMS (M+H) = 643.4; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.68 (d, *J*=4.4 Hz, 2H), 7.53 (s, 2H), 6.84 (s, 2H), 4.33 - 4.16 (m, 6H), 4.13 - 4.06 (m, 2H), 3.88 - 3.80 (m, 2H), 3.79 - 3.71 (m, 2H), 3.64 – 3.57 (m, 2H), 2.44 - 2.30 (m, 6H), 2.15 - 2.04 (m, 4H), 1.85 - 1.77 (m, 4H), 1.61 - 1.52 (m, 4H), 1.44 - 1.38 (m, 8H).

[00162] Dimer **IIa-3**: (1 mg, 4% yield); LCMS (M+H) = 629.4; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.68 (d, *J*=4.4 Hz, 2H), 7.55 (s, 2H), 6.86 (s, 2H), 4.37 - 4.24 (m, 4H), 4.21 - 4.14 (m, 2H), 4.12 - 4.06 (m, 2H), 3.87-3.80 (m, 2H), 3.79 - 3.70 (m, 4H), 3.63 - 3.56 (m, 2H), 2.42 - 2.31 (m, 6H), 2.14 - 2.06 (m, 4H), 1.83-1.78 (m, 4H), 1.61 - 1.52 (m, 4H), 1.43 - 1.33 (m, 6H).

*Example 3 – Dimer IIa-1*

[00163] This example pertains to **FIG. 3** and the synthesis of dimer **IIa-1**.

[00164] To a suspension of compound **6** (29mg, 0.054 mmol) and K<sub>2</sub>CO<sub>3</sub> (7.47 mg, 0.054 mmol) in DMF (1.5 mL) was added 1,7-dibromoheptane (14.64 mg, 0.057 mmol). The mixture was heated at 50 °C for 2h. The reaction was cooled to RT. The reaction mixture was filtered and purified using ISCO silica gel chromatography (12 g column, gradient from 0 to 10% MeOH/DCM) to give macrocycle **13**. LCMS (M+H) = 633.5.

[00165] To a solution of macrocycle **13** in DMF (0.8 mL) at 0 °C was added NaH (4.32 mg, 0.108 mmol). The resulting suspension was stirred at 0 °C for 30 min before SEM-Cl (0.019 mL, 0.11 mmol) was added. The reaction was stirred at 0 °C for 1h before it was quenched with brine. The mixture was extracted with EtOAc (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and purified using ISCO silica gel chromatography (12 g column, gradient from 0 % to 100% MeOH/DCM) to give SEM-macrocycle **14** (9 mg, 10.08 μmol, 18.6 % yield over two steps). LCMS (M+H) = 893.4.

[00166] To a solution of SEM-macrocycle **14** (9 mg, 10.08 μmol) in THF/EtOH (1:1, 1 mL) at 0 °C was added a solution of LiBH<sub>4</sub> (101 μL, 0.202 mmol, 2M in THF). The resulting solution was stirred at 0 °C for 1 h before warming to RT and stirring for 15 min. The reaction was quenched with brine and extracted with CHCl<sub>3</sub> (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was taken up in CHCl<sub>3</sub>/EtOH (1:1, 2 mL). Silica gel (0.7 g) was added, followed by water (0.6 mL). The resulting suspension was stirred

at RT for 24 h and filtered and washed with 10% MeOH/CHCl<sub>3</sub>. The filtrate was concentrated and purified using reverse phase HPLC (Column: Phenomenex Luna C18 20x100mm; Mobile Phase A: 10:90 MeCN:H<sub>2</sub>O with 0.1% trifluoroacetic acid (TFA); Mobile Phase B: 90:10 MeCN:water with 0.1% TFA acid; Gradient: 0-70% B over 15 min; Flow: 20 mL/min; 5 Detection: UV at 220 nm) to give dimer **IIa-1** (1.8 mg, 2.70  $\mu$ mol, 26.8 % yield). LCMS (M+H) = 601.2; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  7.68 (d, *J*=4.4 Hz, 2H), 7.54 (s, 2H), 6.90 (s, 2H), 4.35 - 4.25 (m, 4H), 4.18 (dt, *J*=9.5, 5.7 Hz, 2H), 4.15 – 4.05 (m, 2H), 3.86 – 3.78 (m, 2H), 3.77 - 3.72 (m, 2H), 3.65 - 3.54 (m, 2H), 2.42 - 2.28 (m, 6H), 2.14 - 2.03 (m, 4H), 1.91 - 1.80 (m, 4H), 1.70 - 1.61 (m, 4H), 1.49 - 1.41 (m, 2H).

10 *Example 4 – Dimer IIb-5*

[00167] This example pertains to **FIGs. 4A-4B** and the synthesis of dimer **IIb-5**.

[00168] A suspension of methyl 4-hydroxy-3-methoxybenzoate **14** (18 g, 99 mmol), K<sub>2</sub>CO<sub>3</sub> (20.48 g, 148 mmol) and 1,3-dibromopropane **15** (5.04 ml, 49.4 mmol) in DMSO (300 mL) was stirred at RT for 16 hours. To the reaction mixture was added water, and the resulting 15 solution stirred at RT for 20 min. The resulting precipitate was filtered, washed with water and dried under vacuum. The resulting white solid was triturated with EtOAc/DCM and filtered to give compound **16** (10.45 g, 52.4%) and a dark brown filtrate. The filtrate was purified by ISCO (0 % - 50 % of EtOAC/DCM in 15 minutes, 120 g column) to provide additional compound **16** (3.55 g, 17.7%). LCMS (M+H) = 405; <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$  7.58 (dd, *J*=8.4, 2.0 Hz, 2H), 7.46 (d, *J*=2.0 Hz, 2H), 7.12 (d, *J*=8.6 Hz, 2H), 4.22 (t, *J*=6.2 Hz, 4H), 20 3.83 (s, 6H), 3.81 (s, 6H), 2.24 (t, *J*=6.2 Hz, 2H);

[00169] To a solution of tin (IV) chloride (19.91 mL, 19.91 mmol, 1M in DCM) at 0°C was added dropwise concentrated nitric acid (1.375 mL, 27.7 mmol). The resulting mixture was added dropwise to a solution of compound **16** (3.5 g, 8.65 mmol) in DCM (15 mL) at -25°C. 25 The reaction was stirred at -25 °C for 30 min before it was quenched with water (100 mL). The organic layer was separated. The aq. layer was extracted with EtOAc (2x). The combined organic layers were concentrated to give a crude product, which was recrystallized from hot DCM/Hexane to give compound **17** (3.5 g, 82 % yield) as off white crystals. LCMS (M+H) = 495; <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$  7.68 (s, 2H), 7.32 (s, 2H), 4.29 (t, *J*=6.2 Hz, 4H), 3.91 30 (s, 6H), 3.83 (s, 6H), 2.35 - 2.13 (m, 2H).

[00170] A flask was charged with compound **17** (3.1 g, 6.27 mmol) and aq. NaOH (25.08 mL, 62.7 mmol, 2.5 M). The reaction mixture was heated at 100 °C for 16 h. The heterogenous mixture became reddish solution at the end of the reaction. The reaction mixture was cooled to RT and acidified with aq. HCl to pH2. The resulting precipitate was filtered, washed with water and dried to give compound **18** (2.65 g, 6.05 mmol, 96 % yield). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>) δ 13.41 (br. s., 2H), 10.62 (br. s., 2H), 7.59 (s, 2H), 7.10 (s, 2H), 4.31 (t, *J*=6.2 Hz, 4H), 2.25 (quin, *J*=6.1 Hz, 2H).

[00171] To a solution of compound **18** (2.9 g, 6.62 mmol) in THF (5 mL) at RT was added oxalyl chloride (1.390 mL, 15.88 mmol), followed by 2 drops of DMF. The reaction was stirred at RT for 2 h before it was concentrated and dissolved in MeOH (20 mL). The resulting solution was stirred at RT for 30 min and concentrated. The crude product was triturated with water, filtered, and dried to give compound **19** (3 g, 6.43 mmol, 97 % yield) LCMS (M+H) = 467; <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>) δ 10.80 (br. s., 2H), 7.65 (s, 2H), 7.10 (s, 2H), 4.33 (t, *J*=5.9 Hz, 4H), 3.80 (s, 6H), 2.39 - 2.15 (m, 2H).

[00172] To a suspension of compound **19** (1g, 2.144 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.889 g, 6.43 mmol) in DMF (1 mL) was added 1,4-dibromobutane **19a** (3.70 g, 17.15 mmol). The reaction mixture was heated to 80 °C for 2 h before it was cooled to RT, diluted with water, and extracted with EtOAc (3x). The combined organic layers were concentrated and purified using ISCO silica gel chromatography (40 g column, gradient from 0% to 50% EtOAC/Hexane) to give compound **20** (0.95 g, 60.2 % yield). LCMS (M+H) = 521; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.47 (s, 2H), 7.04 (s, 2H), 4.31 (s, 5H), 4.11 (s, 4H), 3.89 (s, 6H), 3.51 (t, *J*=6.3 Hz, 4H), 2.42 (s, 2H), 2.14 - 1.92 (m, 8H).

[00173] A suspension of compound **20** (0.95 g, 1.290 mmol), 2-nitrobenzenesulfonamide **21a** (0.261 g, 1.290 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.535 g, 3.87 mmol) in DMF (20 mL) was heated at 80 °C for 2 h. The reaction was diluted with water and extracted with EtOAc (3x). The combined organic layers were concentrated and purified using ISCO silica gel chromatography (80 g column, gradient from 0% to 80% EtOAC/Hexane) to give macrocycle **21** (330 mg, 32.9 % yield). LCMS (M+H) = 777.5; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.97 (dd, *J*=7.6, 1.7 Hz, 1H), 7.74 - 7.64 (m, 2H), 7.63 - 7.56 (m, 1H), 7.51 (s, 2H), 7.07 (s, 2H), 4.36 - 4.28 (m, 4H), 4.17 - 4.07 (m, 4H), 3.90 (s, 6H), 3.36 (br. s., 4H), 2.31 (t, *J*=6.1 Hz, 2H), 1.86 – 1.80 (m., 8H)

[00174] To a solution of macrocycle **21** (320mg, 0.412 mmol) in DMF (3mL) was added 2-mercaptoethanol (322 mg, 4.12 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 310  $\mu$ L, 2.060 mmol). The mixture was stirred at RT for 2 h, diluted with DCM, washed with water and then brine, dried over sodium sulfate, and concentrated. The residue was taken up in DCM (4 mL) and cooled to 0 °C. Triethylamine (115  $\mu$ l, 0.824 mmol) was then added, followed by chloro allylformate (Alloc-Cl, 99 mg, 0.824 mmol). The mixture was stirred at 0 °C for 30 min before it was quenched with water, extracted with DCM, dried, and concentrated. The crude product was purified using ISCO silica gel chromatography (40 g column, gradient from 0% to 70% EtOAc/Hexane ) to give macrocycle **22** (140 mg, 0.207 mmol, 50.3 % yield). LCMS 10 (M+H) = 676.2

[00175] To a suspension of macrocycle **22** in MeOH (1mL) and THF (6 mL) was added aq. NaOH (1M, 1 mL). The resulting mixture was stirred at RT for 12 h. The reaction mixture was concentrated to remove THF and MeOH. The residue was acidified with aq. HCL (1N) and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried, and 15 concentrated to give acid **23** (135 mg, 0.208 mmol, 97 % yield). LCMS (M+H) = 485.

[00176] To a solution of acid **23** (135 mg, 0.208 mmol) in THF (2 mL) was added oxalyl chloride (45.6  $\mu$ L, 0.521 mmol), followed by DMF (5 uL). The reaction mixture was stirred at RT for 2 h and concentrated. The residue was dissolved in THF (10 mL) and cooled to 0 °C. A solution of (S)-benzyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate p-toluenesulfonic acid salt 20 **23a** (Accela, 275 mg, 0.625 mmol) and NEt<sub>3</sub> (0.29 mL, 2.09 mmol) in THF ( 5 mL) was added dropwise. The reaction mixture was slowly warmed to RT and stirred for 15 min before quenching with water. The resulting mixture was extracted with EtOAc (3x). The combined organic layers were dried, concentrated, and purified using ISCO silica gel chromatography (12 g column, gradient from 0% to 100% EtOAc/hexane) to give compound **24** (190 mg, 0.166 25 mmol, 80 % yield). LCMS (M+H) = 1146.8 .

[00177] A suspension of compound **24** (190mg, 0.166 mmol), zinc powder (108 mg, 1.658 mmol), and NH<sub>4</sub>Cl (133 mg, 2.486 mmol) in MeOH (4 mL) was heated to 50 °C and stirred for 8 h. The reaction mixture was cooled to RT, diluted with MeOH, and filtered through a pad of CELITE™, washing with MeOH followed by EtOAc. The combined filtrates were 30 concentrated and purified using ISCO silica gel chromatography (24 g column, gradient from 0% to10% MeOH/DCM,) to give compound **25** (125 mg, 0.144 mmol, 87 % yield). LCMS (M+H) = 870.1; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  7.40-7.36 (m, 3H), 7.35 - 7.21 (m,

9H), 6.52 (br. s., 2H), 6.01 - 5.76 (m, 1H), 5.25 (dd,  $J=17.2, 1.5$  Hz, 1H), 5.16 (dd,  $J=10.5, 1.4$  Hz, 1H), 5.06 (d,  $J=15.2$  Hz, 2H), 4.55 (d,  $J=5.5$  Hz, 2H), 4.45 (d,  $J=15.4$  Hz, 2H), 4.24 - 4.05 (m, 4H), 4.00 (br. s., 2H), 3.49 (dd,  $J=15.4, 7.0$  Hz, 2H), 3.31 (br. s., 4H), 3.01 (dd,  $J=15.4, 6.4$  Hz, 2H), 2.19 (d,  $J=5.5$  Hz, 2H), 1.84-1.76 (m., 8H).

5 [00178] To a solution of compound **25** (120mg, 0.138 mmol) in DMF (5 mL) at 0 °C was added NaH (9.93 mg, 0.414 mmol). The resulting suspension was stirred for 30 min before SEM-Cl (0.073 mL, 0.414 mmol) was added. The reaction mixture was then slowly warmed to RT and stirred for 2 h before quenching with brine. The resulting mixture was extracted with EtOAc (3x). The combined organic layers were dried, concentrated and purified using ISCO 10 silica gel chromatography (24 g column, gradient from 0% to 100% EtOAc/hexane) to give an intermediate SEM-macrocycle (82 mg, 0.073 mmol, 52.6 % yield).

15 [00179] The preceding intermediate SEM macrocycle (82 mg, 0.073 mmol) was dissolved in DCM (5 mL). The solution was purged with N<sub>2</sub> before Pd(Ph<sub>3</sub>P)<sub>4</sub> (7.97 mg, 6.90 µmol) and morpholine (0.060 mL, 0.690 mmol) were added sequentially. The reaction mixture was stirred at RT overnight before it was concentrated and purified using ISCO silica gel chromatography ( 12 g column, gradient from 0% to 20% MeOH/DCM) to give macrocycle **26** (56 mg, 0.054 mmol, 38.8 % yield). LCMS (M+H) = 1046.3; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.40 - 7.27 (m, 12H), 5.51 (d,  $J=10.1$  Hz, 2H), 5.11 (d,  $J=15.4$  Hz, 2H), 4.66 (d,  $J=10.1$  Hz, 2H), 4.45 (d,  $J=15.4$  Hz, 2H), 4.35 - 4.24 (m, 6H), 4.21 - 4.14 (m, 2H), 4.08 20 - 3.99 (m, 2H), 3.79 (d,  $J=6.8$  Hz, 2H), 3.73 - 3.62 (m, 2H), 3.54 (s, 2H), 3.32 - 3.13 (m, 4H), 3.04 (s, 2H), 2.30 (br. s., 2H), 2.18 (d,  $J=6.2$  Hz, 4H), 1.95 (br. s., 4H), 1.65 (br. s., 4H), 0.97 (dd,  $J=4.2, 3.1$  Hz, 4H), 0.03 (s, 18H).

25 [00180] To a solution of macrocycle **26** ( 46 mg, 44 µmol) in THF (1 mL) at -78 °C was added a solution of lithium triethylborohydride (SUPER-HYDRIDE®, 0.13 mL, 0.123 mmol, 1M in THF). The resulting solution was stirred at -78 °C for 2 h before quenching with brine and extracted with CHCl<sub>3</sub> (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was then taken up in CHCl<sub>3</sub>/EtOH (1:1, 2 mL). Silica gel (0.8 g ) was added, followed by water (0.6 mL). The resulting suspension was stirred at RT for 24 h and then filtered, washing with 10% MeOH/ CHCl<sub>3</sub>. The filtrate was concentrated and purified 30 using reverse phase HPLC (Column: Phenomenex Luna C18 20x100mm; Mobile Phase A: 10:90 MeCN:water with 0.1% TFA; Mobile Phase B: 90:10 MeCN:water with 0.1% TFA; Gradient: 10-70% B over 15 min; Flow: 20 mL/min; Detection: UV at 220 nm). to give dimer

**IIb-5** (20 mg, 24  $\mu$ mol, 54.3 % yield). LCMS (M+H) = 754.2;  $^1$ H NMR (400MHz, CHLOROFORM-d)  $\delta$  7.54 (s, 2H), 7.49 (d,  $J$ =5.3 Hz, 2H), 7.42 - 7.30 (m, 8H), 6.84 (s, 2H), 5.03 (d,  $J$ =15.6 Hz, 2H), 4.56 (d,  $J$ =15.4 Hz, 2H), 4.37 - 4.20 (m, 4H), 4.15 - 4.07 (m, 2H), 4.02 - 3.94 (m, 2H), 3.36 - 3.25 (m, 2H), 3.23 - 3.13 (m, 2H), 3.05 - 2.95 (m, 6H), 2.42 - 2.30 (m, 2H), 1.96 (br. s., 8H).

*Example 5 – Intermediate Compound 48*

[00181] This example pertains to **FIG. 5** and the synthesis of intermediate compound **48**.

[00182] To a RT solution of compound **19** (10.36 g, 22.21 mmol) in DMF (178 mL) was added  $K_2CO_3$  (12.28 g, 89 mmol). The mixture was heated to 100 °C, then a solution of 1,8-diiodooctane **19b** (5.30 mL, 26.7 mmol) in DMF (44.4 mL) was added dropwise over 1.5 h. The reaction mixture was stirred at 100 °C for 3 h more, cooled to RT, and slowly added to a stirred flask of  $H_2O$  (2000 mL). The resulting precipitate was collected by vacuum filtration (washed with  $H_2O$ ). The crude material was purified by flash chromatography (220 g silica gel; linear gradient 0-10% EtOAc-DCM) to provide macrocycle **43** (5.994 g, 47%) as a white solid. LC-MS  $m/z$  594 [M+18] $^+$ ;  $^1$ H NMR (400MHz,  $CDCl_3$ )  $\delta$  7.53 (s, 2H), 7.08 (s, 2H), 4.33 (t,  $J$ =6.1 Hz, 4H), 4.15 (t,  $J$ =5.3 Hz, 4H), 3.91 (s, 6H), 2.36 (quin,  $J$ =6.0 Hz, 2H), 1.86 - 1.78 (m, 4H), 1.61 - 1.52 (m, 4H), 1.49 - 1.42 (m, 4H).

[00183] To a RT suspension of macrocycle **43** (5.994 g, 10.40 mmol) in THF (78 mL) was added MeOH (26.0 mL) followed by 1 M aq. NaOH (104 mL, 104 mmol). The yellow suspension was stirred at 50 °C for 4 h, gradually becoming a clear yellow solution. The reaction mixture was cooled to RT, partially concentrated, and acidified with 1 M aq. HCl. The resulting solids were collected by vacuum filtration (washed with  $H_2O$ ) to provide macrocycle **44** (5.41 g, 95%) as a yellow solid. LC-MS  $m/z$  566 [M+18] $^+$ ;  $^1$ H NMR (400MHz,  $DMSO-d_6$ )  $\delta$  13.58 (br s, 2H), 7.64 (s, 2H), 7.30 (s, 2H), 4.27 (t,  $J$ =6.2 Hz, 4H), 4.16 (t,  $J$ =5.1 Hz, 4H), 2.20 (quin,  $J$ =6.1 Hz, 2H), 1.74 - 1.65 (m, 4H), 1.53 - 1.44 (m, 4H), 1.42 - 1.32 (m, 4H).

[00184] To a RT solution of macrocycle **44** (5.144 g, 9.38 mmol) in THF (94 mL) was added oxalyl chloride (2.140 mL, 22.51 mmol) followed by DMF (7.29  $\mu$ L, 0.094 mmol). The reaction mixture was stirred at RT for 1 h then concentrated *in vacuo*. The residue was taken up in THF (94 mL) and cooled to 0 °C.  $NEt_3$  (7.84 mL, 56.3 mmol) and compound **44a** (5.84 g, 22.51 mmol) were added. The cooling bath was removed and the reaction mixture was stirred at RT for 3 h. The reaction was quenched by the addition of a mixture of sat. aq.  $NH_4Cl$  (250

mL) and H<sub>2</sub>O (250 mL) and extracted with EtOAc (2 × 250 mL). The combined organic layers were washed with sat. aq. NaCl (250 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (120 g silica gel; linear gradient 0-100% EtOAc-hexanes) to provide compound **45** (9.274 g, 96%) as a yellow foam.

5 LC-MS *m/z* 1031 [M+H]<sup>+</sup>.

**[00185]** To a 0 °C solution of compound **45** (9.274 g, 8.99 mmol) in MeOH (56.2 mL) and THF (56.2 mL) was added NH<sub>4</sub>Cl (9.62 g, 180 mmol) and zinc dust (11.76 g, 180 mmol). The resulting suspension was stirred at 50 °C for 22 h. The reaction was cooled to RT and filtered through CELITE™ (washed with 300 mL EtOAc). The filtrate was concentrated *in vacuo*.

10 The crude material was taken up in DCM (400 mL), washed with H<sub>2</sub>O (400 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo* to provide compound **46** (8.2 g, quantitative yield) as a yellow foam. LC-MS *m/z* 907 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>) δ 10.25 (s, 2H), 7.27 (s, 2H), 6.73 (s, 2H), 4.46 (quin, *J*=5.2 Hz, 2H), 4.24 - 4.12 (m, 6H), 4.06 - 3.96 (m, 4H), 3.60 - 3.53 (m, 2H), 3.50 - 3.43 (m, 2H), 2.69 - 2.56 (m, 2H), 2.26 - 2.16 (m, 2H), 1.98 - 1.89 (m, 2H), 1.72 - 1.62 (m, 4H), 1.54 - 1.43 (m, 4H), 1.41 - 1.32 (m, 4H), 0.87 - 0.83 (m, 18H), 0.08 (s, 12H).

**[00186]** To a 0 °C solution of compound **46** (8.16 g, 8.99 mmol) in DMF (90 mL) was added NaH (1.798 g, 60% *w/w* in mineral oil, 45.0 mmol). The reaction mixture was stirred at 0 °C for 30 min and SEM-Cl (6.38 mL, 36.0 mmol) was added dropwise. The reaction mixture was 20 stirred at 0 °C for 30 min. Reaction was quenched by the dropwise addition of saturated aq. NH<sub>4</sub>Cl, followed by warming to RT, dilution with EtOAc (400 mL), washing with H<sub>2</sub>O (2 x 400 mL) and sat. aq. NaCl (200 mL), drying over Na<sub>2</sub>SO<sub>4</sub>, filtering, and concentrating *in vacuo*. The crude material was purified by flash chromatography (220 g silica gel; linear gradient 0-100% EtOAc-hexanes) to provide compound **47** (7.980 g, 76%) as a white foam.

25 LC-MS *m/z* 1168[M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.36 (s, 2H), 7.24 (s, 2H), 5.48 (d, *J*=10.0 Hz, 2H), 4.69 (d, *J*=9.9 Hz, 2H), 4.58 (quin, *J*=5.7 Hz, 2H), 4.31 - 4.21 (m, 6H), 4.19 - 4.06 (m, 4H), 3.82 - 3.63 (m, 6H), 3.56 (dd, *J*=11.9, 5.6 Hz, 2H), 2.90 - 2.81 (m, 2H), 2.33 (quin, *J*=6.0 Hz, 2H), 2.07 - 1.98 (m, 2H), 1.84 - 1.75 (m, 4H), 1.62 - 1.54 (m, 4H), 1.50 - 1.40 (m, 4H), 1.01 - 0.95 (m, 4H), 0.88 (s, 18H), 0.10 (s, 12H), 0.04 (s, 18H).

30 **[00187]** To a RT solution of compound **47** (7.979 g, 6.83 mmol) in THF (68.3 mL) was added tetrabutylammonium fluoride (TBAF, 17.08 mL, 1 M solution in THF, 17.08 mmol). The clear yellow solution was stirred at RT for 15 h. The reaction mixture was diluted with

DCM (400 mL), washed with H<sub>2</sub>O (400 mL) and sat. aq. NaCl (400 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (120 g silica gel with 25 g prepacked load cartridge; linear gradient 0-10% MeOH-CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **48** (5.360 g) as a white foam. The combined aqueous layers from the 5 workup were extracted with DCM (250 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. This material was purified by flash chromatography (3 x) (80 g silica gel with 25 g prepacked load cartridge; linear gradient 0-10% MeOH-CH<sub>2</sub>Cl<sub>2</sub>) to provide an additional 0.617 g compound **48**. The two isolates were combined to provide 10 compound **48** (5.997 g, 93%) as a white foam. LC-MS *m/z* 939 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.37 (s, 2H), 7.25 (s, 2H), 5.48 (d, *J*=10.0 Hz, 2H), 4.70 (d, *J*=10.0 Hz, 2H), 4.69 - 4.64 (m, 2H), 4.33 - 4.25 (m, 6H), 4.16 - 4.03 (m, 4H), 3.85 (dd, *J*=12.7, 2.1 Hz, 2H), 3.78 (td, *J*=9.6, 7.1 Hz, 2H), 3.72 - 3.63 (m, 4H), 2.97 (dt, *J*=13.6, 5.5 Hz, 2H), 2.32 (quin, *J*=6.1 Hz, 2H), 2.16 - 2.07 (m, 2H), 1.95 - 1.87 (m, 2H), 1.84 - 1.74 (m, 4H), 1.63 - 1.53 (m, 4H), 1.48 - 1.42 (m, 4H), 0.99 (ddd, *J*=9.5, 6.9, 2.3 Hz, 4H), 0.08 - -0.01 (m, 18H).

15 *Example 6 – Dimers IIc-7 and IIc-8*

[00188] This example pertains to **FIG. 6** and the synthesis of dimers **IIc-7** and **IIc-8**.

[00189] To a 0 °C solution of compound **48** (5.997 g, 6.38 mmol) in DCM (31.9 mL) and DMSO (31.9 mL) was added NEt<sub>3</sub> (8.90 mL, 63.8 mmol) followed by SO<sub>3</sub>-pyridine complex (4.06 g, 25.5 mmol). The reaction was allowed to warm to RT as it was stirred for 16 h. The 20 reaction was diluted with DCM (400 mL), washed with sat. aq. NH<sub>4</sub>Cl (400 mL), H<sub>2</sub>O (2 × 400 mL), and sat. aq. NaHCO<sub>3</sub> (400 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (120 g silica gel with 25 g prepacked 25 load cartridge; linear gradient 0-100% EtOAc-CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **54** (4.822 g, 81%) as a white foam. LC-MS *m/z* 935 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.36 (s, 2H), 7.28 (s, 2H), 5.52 (d, *J*=10.1 Hz, 2H), 4.76 (d, *J*=10.0 Hz, 2H), 4.65 (dd, *J*=9.8, 3.0 Hz, 2H), 4.36 - 4.28 (m, 4H), 4.28 - 4.20 (m, 2H), 4.18 - 4.06 (m, 4H), 3.94 - 3.86 (m, 2H), 3.78 (td, *J*=9.8, 6.7 Hz, 2H), 3.69 (td, *J*=9.8, 6.6 Hz, 2H), 3.58 (dd, *J*=19.1, 2.9 Hz, 2H), 2.85 - 2.73 (m, 2H), 2.39 - 2.31 (m, 2H), 1.86 - 1.77 (m, 4H), 1.64 - 1.53 (m, 4H), 1.50 - 1.42 (m, 4H), 0.99 (ddd, *J*=9.8, 6.6, 4.7 Hz, 4H), 0.04 (s, 18H).

30 [00190] To a -78 °C solution of compound **54** (4.822 g, 5.16 mmol) in DCM (129 mL) was added 2,6-lutidine (3.72 mL, 32.0 mmol) and trifluoromethanesulfonic anhydride (Tf<sub>2</sub>O, 30.9 mL, 1 M solution in DCM, 30.9 mmol) dropwise over 30 min. The bright yellow solution was

allowed to warm to -20 °C over 1.5 h, then it was stirred at -20 °C for an additional 1 h. The reaction was diluted with sat. aq. NaHCO<sub>3</sub> (400 mL) and extracted with DCM (2 × 200 mL). The combined organic layers were washed with H<sub>2</sub>O (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (2x) (120 g silica gel with 25 g prepacked load cartridge; linear gradient 0-100% EtOAc-hexanes) to provide compound **55** (4.018 g, 65%) as an orange foam. <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>) δ 7.38 (t, *J*=2.0 Hz, 2H), 7.28 (s, 2H), 7.25 (s, 2H), 5.33 - 5.26 (m, 2H), 5.21 (d, *J*=10.5 Hz, 2H), 4.91 (dd, *J*=10.8, 3.5 Hz, 2H), 4.31 - 4.18 (m, 4H), 4.10 - 4.03 (m, 4H), 3.65 - 3.57 (m, 2H), 3.53 - 3.38 (m, 4H), 3.18 (ddd, *J*=16.3, 11.0, 2.1 Hz, 2H), 2.26 - 2.18 (m, 2H), 1.75 - 1.66 (m, 4H), 1.55 - 1.46 (m, 4H), 1.44 - 1.35 (m, 4H), 0.85 - 0.70 (m, 4H), -0.08 (s, 18H).

**[00191]** A mixture of compound **55** (65 mg, 0.054 mmol), (4-methoxyphenyl)boronic acid (18.12 mg, 0.119 mmol), and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (PdCl<sub>2</sub>(dppf), 1.983 mg, 2.71 μmol) was evacuated and backfilled with N<sub>2</sub>, then THF (1084 μL) and tribasic potassium phosphate (542 μL, 0.5 M solution in H<sub>2</sub>O, 0.271 mmol) were added. The mixture was sparged with N<sub>2</sub> for 5 min then stirred at RT for 1.5 h. The reaction was diluted with EtOAc (50 mL) and washed with sat. aq. NaHCO<sub>3</sub> (50 mL) and sat. aq. NaCl (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (24 g silica gel with 5 g prepacked load cartridge; linear gradient 0-100% EtOAc-hexanes) to provide compound **56a** (50.3 mg, 83%) as a white foam.

LC-MS *m/z* 1116 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.42 (s, 2H), 7.40 - 7.36 (m, 4H), 7.34 - 7.32 (m, 2H), 7.29 (s, 2H), 6.91 - 6.87 (m, 4H), 5.53 (d, *J*=10.1 Hz, 2H), 4.76 (d, *J*=10.1 Hz, 2H), 4.65 (dd, *J*=10.5, 3.4 Hz, 2H), 4.32 (t, *J*=6.2 Hz, 4H), 4.19 - 4.06 (m, 4H), 3.98 - 3.91 (m, 2H), 3.83 (s, 6H), 3.84 - 3.77 (m, 2H), 3.71 (td, *J*=9.6, 7.0 Hz, 2H), 3.15 (ddd, *J*=16.1, 10.7, 2.1 Hz, 2H), 2.35 (quin, *J*=5.9 Hz, 2H), 1.85 - 1.77 (m, 4H), 1.64 - 1.55 (m, 4H), 1.50 - 1.42 (m, 4H), 1.00 (ddd, *J*=9.5, 7.0, 2.2 Hz, 4H), 0.04 (s, 18H).

**[00192]** To a -78 °C solution of compound **56a** (50.3 mg, 0.045 mmol) in THF (1503 μL) was added lithium triethylborohydride (225 μL, 1 M solution in THF, 0.225 mmol) dropwise. The reaction mixture was stirred at -78 °C for 1 h. The reaction mixture was diluted with H<sub>2</sub>O (10 mL) and extracted with DCM (2 × 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was taken up in CHCl<sub>3</sub> (1.0 mL) and EtOH (1.0 mL), then silica gel (0.50 g) and H<sub>2</sub>O (0.50 mL) were added. The reaction was stirred at RT for 3 days. The mixture was filtered through CELITE™ (washed with CHCl<sub>3</sub>)

and the filtrate was concentrated *in vacuo*. The crude material was purified by preparative HPLC (3 injections, each in 2 mL of DMSO; Phenomenex Luna C18 21.2 × 100 mm; linear gradient 42-90% MeCN-H<sub>2</sub>O with 0.1% *v/v* TFA over 12 min; 20 mL/min; 220 nm detection). The product-containing fractions were immediately diluted with sat. aq. NaHCO<sub>3</sub> (100 mL) and extracted with CHCl<sub>3</sub> (2 × 50 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to provide dimer **IIc-7** (6.0 mg, 15%) as a orange solid. LC-MS *m/z* 823 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.89 (d, *J*=4.0 Hz, 2H), 7.54 (s, 2H), 7.40 (s, 2H), 7.37 - 7.33 (m, 4H), 6.94 - 6.89 (m, 4H), 6.88 (s, 2H), 4.47 - 4.40 (m, 2H), 4.39 - 4.25 (m, 4H), 4.22 - 4.16 (m, 2H), 4.14 - 4.05 (m, 2H), 3.84 (s, 6H), 3.59 (ddd, *J*=16.3, 11.5, 1.9 Hz, 2H), 3.43 - 3.34 (m, 2H), 2.42 - 2.29 (m, 2H), 1.86 - 1.76 (m, 4H), 1.64 - 1.55 (m, 4H), 1.49 - 1.43 (m, 4H).

**[00193]** Dimer **IIc-8** was prepared analogously according to the synthetic procedures described above for dimer **IIc-7**. The analytical data for dimer **IIc-8** were: LC-MS *m/z* 960 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.88 (d, *J*=4.0 Hz, 2H), 7.55 - 7.52 (m, 2H), 7.39 - 7.36 (m, 2H), 7.34 - 7.30 (m, 4H), 6.94 - 6.90 (m, 4H), 6.89 - 6.87 (m, 2H), 4.45 - 4.38 (m, 2H), 4.37 - 4.25 (m, 4H), 4.21 - 4.15 (m, 2H), 4.14 - 4.05 (m, 2H), 3.62 - 3.53 (m, 2H), 3.42 - 3.33 (m, 2H), 3.29 - 3.22 (m, 8H), 2.62 - 2.56 (m, 8H), 2.37 (s, 6H), 2.38 - 2.30 (m, 2H), 1.84 - 1.76 (m, 4H), 1.62 - 1.52 (m, 4H), 1.48 - 1.40 (m, 4H).

*Example 7 – Dimers IIc-9, IIc-10, IIc-11, and IId-1*

**[00194]** This example pertains to **FIG. 7** and the synthesis of dimers **IIc-9**, **IIc-10**, **IIc-11** and **IId-1**.

**[00195]** A mixture of compound **55** (0.51g, 0.425 mmol), (4-aminophenyl)boronic acid (58 mg, 0.425mmol), and P<sub>c</sub>Cl<sub>2</sub>(dppf) (16 mg, 21  $\mu$ mol) was evacuated and backfilled with N<sub>2</sub>. THF (8.5 mL) and tribasic potassium phosphate (4.25 mL, 0.5 M solution in H<sub>2</sub>O, 2.126 mmol) were added. The mixture was sparged with N<sub>2</sub> for 5 min then stirred at RT for 1 h. The reaction mixture was diluted with DCM (30 mL), washed with sat. aq. NaCl (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (40 g column; linear gradient 0-100% EtOAc-hexanes) to provide compound **57b** (205 mg, 42%) as a yellow foam. LC-MS *m/z* 1142 [M+H]<sup>+</sup>.

**[00196]** A mixture of compound **57b** (70 mg, 0.061 mmol), (4-methoxyphenyl)boronic acid (12.1 mg, 0.080 mmol), and P<sub>c</sub>Cl<sub>2</sub>(dppf) (2.24 mg, 3.1  $\mu$ mol) was evacuated and backfilled

with N<sub>2</sub>. THF (1226  $\mu$ L) and tribasic potassium phosphate (613  $\mu$ L, 0.5 M solution in H<sub>2</sub>O, 0.306 mmol) were added. The mixture was sparged with N<sub>2</sub> for 5 min then stirred at RT for 30 min. The reaction was diluted with EtOAc (30 mL), washed with sat. aq. NaCl (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (40 g column; linear gradient 0-100% EtOAc-hexanes) to provide compound 58a (60 mg, 89%) as a yellow foam. LC-MS *m/z* 1100.8 [M+H]<sup>+</sup>.

[00197] Alternatively to using a boronic acid, a Grignard reagent can be used, as illustrated by the following synthesis of compound 58d from compound 57b: To a -30 °C solution of compound 57b (112 mg, 0.098 mmol) and iron(III) acetylacetone (3.46 mg, 9.80  $\mu$ mol) in THF (1334  $\mu$ L) and NMP (66.7  $\mu$ L) was added methylmagnesium bromide (131  $\mu$ L, 3.0 M solution in Et<sub>2</sub>O, 0.392 mmol), dropwise slowly. The reaction was stirred at -30 °C for 10 min, then it was quenched by the addition of sat. aq. NH<sub>4</sub>Cl (30 mL) and extracted with EtOAc (2  $\times$  30 mL). The combined organic layers were washed with sat. aq. NaCl (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (12 g silica gel with 5 g prepacked load cartridge; linear gradient 0-100% EtOAc-CH<sub>2</sub>Cl<sub>2</sub>) to provide compound 58d (50 mg, 51%) as a yellow foam. LC-MS *m/z* 1008 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  7.42 - 7.38 (m, 2H), 7.29 - 7.24 (m, 5H), 6.70 - 6.64 (m, 3H), 5.55 - 5.48 (m, 2H), 4.77 - 4.71 (m, 2H), 4.62 (dd, *J*=10.6, 3.2 Hz, 1H), 4.48 (dd, *J*=10.4, 3.3 Hz, 1H), 4.35 - 4.27 (m, 4H), 4.18 - 4.05 (m, 4H), 3.95 - 3.88 (m, 1H), 3.84 - 3.64 (m, 6H), 3.49 - 3.42 (m, 1H), 3.12 (ddd, *J*=16.1, 10.5, 2.0 Hz, 1H), 2.83 - 2.72 (m, 1H), 2.34 (quin, *J*=6.0 Hz, 2H), 1.84 (d, *J*=1.1 Hz, 3H), 1.83 - 1.76 (m, 4H), 1.63 - 1.56 (m, 4H), 1.49 - 1.43 (m, 4H), 0.99 (ddd, *J*=9.6, 6.9, 2.4 Hz, 4H), 0.04 (s, 18H).

[00198] To a -78 °C solution of the crude compound 58a (30 mg, 0.027 mmol) in THF (1 mL) was added lithium triethylborohydride (273  $\mu$ L, 1 M solution in THF, 0.273 mmol) dropwise. The reaction was stirred at -78 °C for 1 h. The reaction was diluted with brine and extracted with EtOAc (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was taken up in EtOH/THF (1:1, 2 mL) and aq. formic acid (0.055, 1 mL). The resulting solution was stirred at RT for 2h before it was neutralized with aq. NaHCO<sub>3</sub>. The resulting mixture was extracted with chloroform (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude product was then purified by flash chromatography (12 g column, 0-10% MeOH/DCM) to give dimer IIc-11 (11 mg, 45%). LC-MS *m/z* 808.4 [M+H]<sup>+</sup>.

[00199] Dimers **IIc-9**, **IIc-10**, and **IId-1** were analogously prepared according to the synthetic procedures described for above for dimer **IIc-11**. Their analytical data is provided below.

[00200] Dimer **IIc-9**: LC-MS *m/z* 876 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.90 - 7.86 (m, 2H), 7.56 - 7.52 (m, 2H), 7.39 - 7.36 (m, 1H), 7.35 - 7.33 (m, 1H), 7.31 (d, *J*=8.6 Hz, 2H), 7.22 (d, *J*=8.6 Hz, 2H), 6.92 (d, *J*=9.0 Hz, 2H), 6.88 (s, 2H), 6.68 (d, *J*=8.6 Hz, 2H), 4.45 - 4.37 (m, 2H), 4.36 - 4.25 (m, 4H), 4.22 - 4.15 (m, 2H), 4.13 - 4.06 (m, 2H), 3.79 - 3.69 (m, 2H), 3.63 - 3.52 (m, 2H), 3.41 - 3.32 (m, 2H), 3.31 - 3.23 (m, 4H), 2.65 - 2.57 (m, 4H), 2.38 (s, 3H), 2.41 - 2.30 (m, 2H), 1.84 - 1.76 (m, 4H), 1.62 - 1.54 (m, 4H), 1.49 - 1.40 (m, 4H).

[00201] Dimer **IIc-10**: LC-MS *m/z* 946 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.88 (d, *J*=4.0 Hz, 2H), 7.56 - 7.51 (m, 2H), 7.39 - 7.36 (m, 2H), 7.35 - 7.29 (m, 4H), 6.94 - 6.90 (m, 4H), 6.89 - 6.86 (m, 2H), 4.45 - 4.38 (m, 2H), 4.37 - 4.24 (m, 4H), 4.22 - 4.15 (m, 2H), 4.13 - 4.05 (m, 2H), 3.62 - 3.52 (m, 2H), 3.42 - 3.33 (m, 2H), 3.29 - 3.22 (m, 4H), 3.22 - 3.15 (m, 4H), 3.07 - 3.02 (m, 4H), 2.61 - 2.55 (m, 4H), 2.39 - 2.29 (m, 6H), 1.84 - 1.76 (m, 4H), 1.64 - 1.51 (m, 4H), 1.48 - 1.40 (m, 4H).

[00202] Dimer **IId-1**: LC-MS 716 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.87 (d, *J*=4.0 Hz, 1H), 7.80 (d, *J*=4.0 Hz, 1H), 7.55 - 7.50 (m, 2H), 7.35 - 7.32 (m, 1H), 7.24 - 7.20 (m, 2H), 6.88 - 6.84 (m, 2H), 6.76 - 6.73 (m, 1H), 6.70 - 6.66 (m, 2H), 4.44 - 4.36 (m, 1H), 4.35 - 4.22 (m, 5H), 4.22 - 4.14 (m, 2H), 4.13 - 4.05 (m, 2H), 3.84 - 3.69 (m, 2H), 3.61 - 3.48 (m, 1H), 3.40 - 3.31 (m, 1H), 3.23 - 3.11 (m, 1H), 3.00 - 2.91 (m, 1H), 2.34 (quin, *J*=6.1 Hz, 2H), 1.84 (d, *J*=1.1 Hz, 3H), 1.82 - 1.75 (m, 4H), 1.63 - 1.53 (m, 4H), 1.48 - 1.40 (m, 4H).

#### Example 8 – Dimer **IIb-6**

[00203] This example pertains to **FIG. 8** and the synthesis of dimer **IIb-6**.

[00204] To a RT solution of compound **44** (1.006 g, 1.834 mmol) in DMF (12.23 mL) was added *N,N,N',N'*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU, 1.743 g, 4.59 mmol) followed by (S)-ethyl 7-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate **44b** (0.459 g, 1.834 mmol) portionwise, and then *N,N*-diisopropylethylamine (DIEA, 1.917 mL, 11.00 mmol) dropwise. The resulting clear brown solution was stirred at RT for 30 min, then (S)-benzyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate 4-methylbenzenesulfonate **44c** (0.806 g, 1.834 mmol) was added. The reaction was stirred at RT for an additional 1 h. The reaction mixture was slowly added to a stirred flask of H<sub>2</sub>O (125 mL) at 0 °C and the resulting

precipitate was collected by vacuum filtration (washed with H<sub>2</sub>O). The solids were dissolved in EtOAc (200 mL), washed with sat. aq. NaCl (150 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (220 g RediSep Gold silica gel with 25 g prepacked load cartridge; linear gradient 0-100% EtOAc-hexanes) to provide compound **67** (538 mg, 29%) as a brown foam. LC-MS *m/z* 1030 [M+H]<sup>+</sup>.

5 [00205] To a RT solution of compound **67** (538 mg, 0.522 mmol) in MeOH (3264  $\mu$ L) and THF (3264  $\mu$ L) was added NH<sub>4</sub>Cl (559 mg, 10.45 mmol), zinc dust (683 mg, 10.45 mmol), and HOAc (1 drop). The resulting suspension was stirred at 60 °C for 18 h. The reaction was 10 cooled to RT and filtered through CELITE™ (washed with EtOAc, DCM, MeOH, and 5% v/v Et<sub>3</sub>N in DCM). The filtrate was concentrated *in vacuo*. The crude material was taken up in DCM (200 mL), washed with sat. aq. NaHCO<sub>3</sub> (200 mL) and H<sub>2</sub>O (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo* to provide compound **68** (250 mg, 61%) as an orange solid. LC-MS *m/z* 786 [M+H]<sup>+</sup>.

15 [00206] To a 0 °C solution of compound **68** (250 mg, 0.318 mmol) in DMF (3181  $\mu$ L) was added NaH (63.6 mg, 60% *w/w* in mineral oil, 1.591 mmol). The reaction was stirred at 0 °C for 30 min, then SEM-Cl (226  $\mu$ L, 1.272 mmol) was added. The reaction was stirred at 0 °C for 30 min. The reaction was quenched by the addition of sat. aq. NH<sub>4</sub>Cl, then it was warmed to RT, diluted with EtOAc (100 mL), washed with H<sub>2</sub>O (100 mL) and sat. aq. NaCl (100 mL), 20 dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (40 g silica gel with 5 g prepacked load cartridge; linear gradient 0-10% MeOH-CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **69** (289 mg, 87%) as a yellow foam. LC-MS *m/z* 1047 [M+H]<sup>+</sup>.

25 [00207] To a -78 °C solution of compound **69** (43.9 mg, 0.042 mmol) in THF (1398  $\mu$ L) was added lithium triethylborohydride (210  $\mu$ L, 1 M solution in THF, 0.210 mmol) dropwise. The reaction was stirred at -78 °C for 1.5 h. The reaction was diluted with H<sub>2</sub>O (10 mL) and extracted with CHCl<sub>3</sub> (2  $\times$  10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was taken up in CHCl<sub>3</sub> (1 mL) and EtOH (1 mL), then silica gel (0.5 g) and H<sub>2</sub>O (0.5 mL) were added. The reaction was stirred at RT for 2 30 days. The mixture was filtered through CELITE™ (washed with acetone, CHCl<sub>3</sub>, and 10% MeOH-CHCl<sub>3</sub>) and the filtrate was concentrated *in vacuo*. This material was taken up in CHCl<sub>3</sub> and H<sub>2</sub>O and the layers were separated. The aqueous layer was extracted with CHCl<sub>3</sub>,

dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo*. The crude material was purified by preparative HPLC (2 injections, each in 2 mL of 1:1 MeCN-DMSO; Phenomenex Luna C18 21.2 x 100 mm; linear gradient 26-90% MeCN-H<sub>2</sub>O with 0.1% *v/v* TFA over 12 min; 20 mL/min; 220 nm detection). The product-containing fractions were immediately diluted with 5 sat. aq.  $\text{NaHCO}_3$  (100 mL) and extracted with  $\text{CHCl}_3$  (2 x 50 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo* to provide dimer **IIb-6** (6.6 mg, 19%) as an off-white solid. LC-MS *m/z* 754 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  7.54 - 7.52 (m, 2H), 7.49 - 7.45 (m, 2H), 7.39 - 7.29 (m, 4H), 7.13 (d, *J*=8.1 Hz, 1H), 6.86 - 6.83 (m, 2H), 6.66 - 6.62 (m, 2H), 5.01 (d, *J*=15.6 Hz, 1H), 4.90 (d, *J*=15.6 Hz, 1H), 4.56 (d, *J*=15.6 Hz, 10 1H), 4.44 (d, *J*=15.4 Hz, 1H), 4.35 - 4.23 (m, 4H), 4.22 - 4.15 (m, 2H), 4.08 (dt, *J*=9.6, 4.9 Hz, 2H), 3.98 - 3.93 (m, 1H), 3.92 - 3.87 (m, 1H), 3.78 - 3.65 (m, 2H), 3.31 - 3.24 (m, 1H), 3.20 - 3.12 (m, 2H), 3.05 - 2.99 (m, 1H), 2.33 (quin, *J*=6.1 Hz, 2H), 1.84 - 1.74 (m, 4H), 1.62 - 1.50 (m, 4H), 1.48 - 1.39 (m, 4H).

*Example 9 – Dimer II<sup>d</sup>-2*

15 [00208] This example pertains to **FIG. 9** and the synthesis of dimer **II<sup>d</sup>-2**.

[00209] Following the reaction scheme shown in **FIG. 9**, dimer **II<sup>d</sup>-2** was synthesized analogously to the procedures of the previous example. LC-MS *m/z* 704 [M+H]<sup>+</sup>.

*Example 10 – Dimers II<sup>d</sup>-3 and II<sup>d</sup>-4*

[00210] This example pertains to **FIG. 10** and the synthesis of dimers **II<sup>d</sup>-3** and **II<sup>d</sup>-4**.

20 [00211] To a -78 °C suspension of dimer **IIc-8** (46.2 mg, 0.048 mmol) in THF (482  $\mu$ L) was added LiBHEt<sub>3</sub> (48.2  $\mu$ L, 1 M solution in THF, 0.048 mmol), dropwise. The reaction mixture was stirred at -78 °C for 15 min. Then it was quenched by the addition of H<sub>2</sub>O, warmed to RT, diluted with sat. aq.  $\text{NaHCO}_3$  (25 mL) and H<sub>2</sub>O (25 mL), and extracted with 10% MeOH-CHCl<sub>3</sub> (2 x 50 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and 25 concentrated *in vacuo*. The crude material was purified by preparative HPLC (4 1-mL injections in DMSO; Phenomenex Luna C18 21.2 x 100 mm; linear gradient 0-50% MeCN-H<sub>2</sub>O w/ 0.05% *v/v* HCO<sub>2</sub>H over 25 min; 20 mL/min; 220 nm detection). The fractions containing the mono- and bis-reduced products were separately lyophilized then repurified by preparative HPLC to provide dimer **II<sup>d</sup>-3** (2.7 mg, 6%) as a light yellow solid and dimer **II<sup>d</sup>-4** 30 (1.24 mg, 3%), also as a light yellow solid. Their analytical data is presented below.

[00212] Dimer **IIId-3**: LC-MS *m/z* 961 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.87 (d, *J*=4.0 Hz, 1H), 7.56 (s, 1H), 7.54 - 7.52 (m, 1H), 7.52 - 7.50 (m, 1H), 7.39 - 7.37 (m, 1H), 7.34 - 7.29 (m, 4H), 6.94 - 6.86 (m, 5H), 6.13 (s, 1H), 4.41 (dt, *J*=11.1, 4.7 Hz, 1H), 4.37 - 4.22 (m, 5H), 4.18 (dt, *J*=9.8, 5.1 Hz, 1H), 4.13 - 3.96 (m, 3H), 3.62 - 3.52 (m, 3H), 3.41 - 3.32 (m, 2H), 5 3.31 - 3.24 (m, 8H), 2.75 - 2.69 (m, 1H), 2.70 - 2.64 (m, 8H), 2.41 (s, 6H), 2.35 - 2.28 (m, 2H), 1.84 - 1.70 (m, 4H), 1.62 - 1.51 (m, 4H), 1.47 - 1.38 (m, 4H).

[00213] Dimer **IIId-4**: LC-MS *m/z* 963 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.56 (s, 2H), 7.51 - 7.50 (m, 2H), 7.30 - 7.26 (m, 4H), 6.90 (d, *J*=8.8 Hz, 4H), 6.10 (s, 2H), 4.36 - 4.29 (m, 2H), 4.24 - 4.17 (m, 4H), 4.10 - 3.97 (m, 4H), 3.59 - 3.50 (m, 4H), 3.40 - 3.32 (m, 2H), 3.27 - 10 3.21 (m, 8H), 2.72 (dd, *J*=16.0, 3.4 Hz, 2H), 2.62 - 2.55 (m, 8H), 2.36 (s, 6H), 2.34 - 2.26 (m, 2H), 1.77 - 1.68 (m, 4H), 1.64 - 1.50 (m, 4H), 1.43 - 1.36 (m, 4H).

*Example 11 – Dimer-linker **IIIa-1***

[00214] This example pertains to **FIG. 11** and the synthesis of dimer-linker **IIIa-1**.

[00215] To a 0 °C mixture of dipeptide **69a** (70.4 mg, 0.142 mmol) and HATU (53.9 mg, 0.142 mmol) was added DMF (945 μL). The mixture was stirred at 0 °C for 10 min and 2,6-lutidine (22.02 μL, 0.189 mmol) was added. This mixture was added dropwise to compound **69** (98.9 mg, 0.095 mmol) in a vial at 0 °C. The reaction was allowed to warm to RT as it was stirred for 22 h. Then it was added dropwise to a stirred flask of H<sub>2</sub>O (20 mL) in a 0 °C bath. The precipitate was collected by vacuum filtration (washed with H<sub>2</sub>O), taken up in DCM (50 mL), and washed with H<sub>2</sub>O (50 mL). The aqueous layer was extracted with DCM (50 mL). 15 The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (24 g silica gel with 5 g prepacked load cartridge; linear gradient 0–10% MeOH–DCM). The mixed fractions were repurified by flash chromatography (24 g RediSep Gold silica gel with 5 g prepacked load cartridge; linear gradient 0–10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>) and the products of the two columns were combined to 20 provide compound **70** (68.8 mg, 48%). LC-MS *m/z* 1525 [M+H]<sup>+</sup>.

[00216] To a RT solution of compound **70** (39.9 mg, 0.026 mmol) in DMF (1047 μL) was added silica-supported piperazine (575 mg, 0.91 mmol/g loading, 0.523 mmol). The suspension was stirred at RT for 18 h, then it was filtered (washed with ~2 mL DMF), and the 25 filtrate was concentrated *in vacuo*. This crude compound 71 was combined with crude 30

compound **71** from another batch (0.019 mmol scale) and used without further purification. LC-MS *m/z* 1303 [M+H]<sup>+</sup>.

[00217] To a -78 °C solution of crude compound **71** in THF (1356 µL) was added LiBH<sub>3</sub> (203 µL, 1 M solution in THF, 0.203 mmol), dropwise. The reaction was stirred at -78 °C for 5 1.5 h, then it was diluted with sat. aq. NaCl (50 mL) and extracted with DCM (2 x 50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was taken up in CHCl<sub>3</sub> (1 mL) and EtOH (1 mL), then silica gel (0.5 g) and H<sub>2</sub>O (0.5 mL) were added. The reaction mixture was stirred at RT for 2 days, then it was filtered through CELITE™ (washed with 10% EtOH–CHCl<sub>3</sub>) and the filtrate was concentrated *in vacuo*. The 10 crude material was purified by preparative HPLC (2 injections, each in 2 mL DMSO; Phenomenex Luna C18 21.2 x 100 mm; linear gradient 18–90% MeCN–H<sub>2</sub>O with 0.1% v/v TFA over 12 min; 20 mL/min; 220 nm detection). The product-containing fractions were filtered under gravity through an SPE cartridge packed with PL-HCO<sub>3</sub> MP resin (Agilent, 500 15 mg, 1.8 mmol/g loading) (washed with 3 mL of 1:1 MeCN–H<sub>2</sub>O), and the eluent was lyophilized to provide compound **72** (4.9 mg, 12%) as a white solid. LC-MS *m/z* 1011 [M+H]<sup>+</sup>.

[00218] To a RT solution of compound **72** (4.9 mg, 4.85 µmol) in DMSO (129 µL) was added a solution of compound **71a** (6.69 mg, 9.70 µmol) in DMSO (64.7 µL), followed by 2,6-lutidine (1.130 µL, 9.70 µmol). The clear colorless solution was stirred at RT for 4 h. The 20 reaction was purified by preparative HPLC (1 injection; Phenomenex Luna C18 21.2 × 100 mm; linear gradient 18–90% MeCN–H<sub>2</sub>O with 0.1% v/v TFA over 12 min; 20 mL/min; 220 nm detection). The product-containing fraction was filtered under gravity through an SPE cartridge packed with PL-HCO<sub>3</sub> MP resin (Agilent, 200 mg, 1.8 mmol/g loading) (washed with 2 mL of 1:1 MeCN–H<sub>2</sub>O), and the eluent was lyophilized to provide dimer-linker compound 25 **IIIa-1** (2.4 mg, 31%) as a white solid. LC-MS *m/z* 1585 [M+H]<sup>+</sup>.

*Example 12 – Dimer-linkers IIIa-2, IIIa-3, and IIIa-4*

[00219] This example pertains to **FIG. 12** and the synthesis of dimer-linkers **IIIa-2**, **IIIa-3**, and **IIIa-4**.

[00220] To a RT solution of dimer **IIb-6** (7 mg, 9.29 µmol) in DMF (93 µL) was added a 30 solution of compound **72a** (12.47 mg, 0.011 mmol) in DMF (93 µL), followed by DIEA (4.85 µL, 0.028 mmol). The clear orange solution was stirred at RT for 15 mi; then 1-hydroxy-7-

azabenzotriazole (1.517 mg, 0.011 mmol) was added. The reaction mixture was stirred at RT for 29 h, then diluted with DMSO and purified by preparative HPLC (1 injection; Phenomenex Luna C18 21.2 x 100 mm; linear gradient 18–90% MeCN–H<sub>2</sub>O with 0.1% v/v TFA over 15 min; 20 mL/min; 220 nm detection). The product-containing fraction was filtered under gravity through an SPE cartridge packed with PL-HCO<sub>3</sub> MP resin (Agilent, 200 mg, 1.8 mmol/g loading) (washed with 2 mL of 1:1 MeCN–H<sub>2</sub>O), and the eluent was lyophilized to provide dimer-linker **IIIa-2** (4.43 mg, 28%) as a white solid. LC-MS *m/z* 1734 [M+H]<sup>+</sup>.

[00221] Dimer-linkers **IIIa-3** and **IIIa-4** were analogously prepared. Dimer-linker **IIIa-3**: LC-MS *m/z* 1684 [M+H]<sup>+</sup>. Dimer-linker **IIIa-4**: LC-MS *m/z* 1925 [M+H]<sup>+</sup>.

10 *Example 13 – Dimer-linker IIIa-11*

[00222] This example pertains to **FIGs. 13A-13B** and the synthesis of dimer-linker **IIIa-11**.

[00223] To a 0 °C solution of compound **44e** (2.065 g, 8.09 mmol) in DMF (25.7 mL) was added compound **44** (2.114 g, 3.85 mmol), followed by DIEA (5.37 mL, 30.8 mmol) and HATU (3.22 g, 8.48 mmol), portionwise. The reaction was stirred at 0 °C for 5 min and RT for 15 1 h. Then it was slowly added to a stirred flask of H<sub>2</sub>O (200 mL) in a 0 °C bath. The resulting precipitate was collected by vacuum filtration (H<sub>2</sub>O wash), dissolved in EtOAc (200 mL), washed with sat. aq. NaCl (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (40 g RediSep Gold silica gel with 25 g prepacked load cartridge; linear gradient 0–100% EtOAc–CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **76** 20 (3.1 g, quantitative) as an orange film. LC-MS *m/z* 795 [M+H]<sup>+</sup>.

[00224] To a 0 °C solution of compound **76** (3.1 g, 3.90 mmol) in THF (39.0 mL) was added LiBH<sub>4</sub> (5.85 mL, 2.0 M solution in THF, 11.70 mmol), dropwise. The reaction was stirred at 0 °C for 30 min, then was allowed to warm to RT and stirred for an additional 3 h. The reaction was cooled to 0 °C and quenched by the addition of 1 M aq. HCl (100 mL), diluted with H<sub>2</sub>O (400 mL), and extracted with 10% MeOH-EtOAc (400 mL) and EtOAc (400 mL). The combined organic layers were washed with sat. aq. NaCl (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude product **77** was used without further purification. LC-MS *m/z* 739 [M+H]<sup>+</sup>.

[00225] To a 0 °C solution of the crude product **77** in DCM (77 mL) was added NEt<sub>3</sub> (1.607 30 mL, 11.53 mmol), followed by acetyl chloride (0.713 mL, 9.99 mmol). The reaction was allowed to warm to RT as it was stirred for 20 h, diluted with DCM (400 mL), washed with

$\text{H}_2\text{O}$  (400 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (80 g silica gel with 25 g prepacked load cartridge; linear gradient 0–100%  $\text{EtOAc}$ –hexanes) to provide compound **78** (1.59 g, 50%) as a white foam. LC-MS  $m/z$  823  $[\text{M}+\text{H}]^+$ .

5 [00226] To a RT solution of compound **78** (1.59 g, 1.932 mmol) in  $\text{EtOH}$  (61.8 mL) was added  $\text{AcOH}$  (15.46 mL), followed by zinc dust (3.79 g, 58.0 mmol). The reaction was stirred at reflux for 1 h, then it was cooled to RT and filtered through CELITE<sup>TM</sup> (washed with 400 mL DCM). The filtrate was washed with  $\text{H}_2\text{O}$  (400 mL), sat. aq.  $\text{NaHCO}_3$  (400 mL), and  $\text{H}_2\text{O}$  (400 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo*. The crude material was 10 purified by flash chromatography (40 g silica gel with 2.5 g prepacked load cartridge; linear gradient 0–10%  $\text{MeOH}$ – $\text{CH}_2\text{Cl}_2$ ). The mixed fractions were repurified by flash chromatography (12 g silica gel with 2.5 g prepacked load cartridge; linear gradient 0–10%  $\text{MeOH}$ –DCM) and the products from both columns were combined to provide compound **79** (1.162 g, 79%) as a white foam. LC-MS  $m/z$  763  $[\text{M}+\text{H}]^+$ ;  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  6.80 (s, 2H), 6.27 (s, 2H), 5.04 - 5.00 (m, 2H), 4.99 - 4.95 (m, 2H), 4.86 - 4.75 (m, 2H), 4.37 (br s, 4H), 4.24 - 4.08 (m, 12H), 3.94 (t,  $J=5.5$  Hz, 4H), 2.83 - 2.72 (m, 2H), 2.50 - 2.42 (m, 2H), 2.31 (quin,  $J=6.4$  Hz, 2H), 2.04 (s, 6H), 1.76 - 1.65 (m, 4H), 1.61 - 1.52 (m, 4H), 1.44 - 1.37 (m, 4H).

20 [00227] To a 0 °C solution of triphosgene (117 mg, 0.396 mmol) and  $\text{NEt}_3$  (487  $\mu\text{L}$ , 3.49 mmol) in THF (3695  $\mu\text{L}$ ) was added a solution of compound **79** (444 mg, 0.582 mmol) in THF (3695  $\mu\text{L}$ ), dropwise. The cloudy mixture was stirred at 0 °C for 10 min, then a suspension of compound **79a** (231 mg, 0.611 mmol) in THF (5543  $\mu\text{L}$ ) was added dropwise, followed by allyl alcohol (41.7  $\mu\text{L}$ , 0.611 mmol). The suspension was stirred at 0 °C for 1 h, then it was allowed to warm to RT as it was stirred for 5 h. The reaction was diluted with  $\text{H}_2\text{O}$  (125 mL) 25 and extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 125$  mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (80 g RediSep Gold silica gel with 5 g prepacked load cartridge; linear gradient 0–10%  $\text{MeOH}$ –DCM) to provide compound **80** (214 mg, 29%) as an off-white solid. LC-MS  $m/z$  1250  $[\text{M}+\text{H}]^+$ ;  $^1\text{H}$  NMR (400MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.98 (s, 1H), 9.13 - 9.02 (m, 2H), 30 8.14 (d,  $J=6.8$  Hz, 1H), 7.58 (d,  $J=8.6$  Hz, 2H), 7.31 (d,  $J=8.7$  Hz, 2H), 7.23 (d,  $J=8.7$  Hz, 1H), 7.20 - 7.12 (m, 2H), 6.99 - 6.89 (m, 2H), 5.98 - 5.85 (m, 2H), 5.36 - 5.26 (m, 2H), 5.22 - 5.13 (m, 2H), 5.06 - 4.93 (m, 6H), 4.62 - 4.50 (m, 4H), 4.50 - 4.46 (m, 2H), 4.45 - 4.38 (m, 1H),

4.22 - 3.80 (m, 17H), 2.78 - 2.67 (m, 2H), 2.44 - 2.35 (m, 2H), 2.21 - 2.13 (m, 2H), 2.04 - 1.90 (m, 7H), 1.69 - 1.62 (m, 4H), 1.54 - 1.46 (m, 4H), 1.40 - 1.35 (m, 4H), 1.30 (d,  $J$ =7.0 Hz, 3H), 0.88 (d,  $J$ =6.8 Hz, 3H), 0.83 (d,  $J$ =6.6 Hz, 3H).

**[00228]** To a RT solution of compound **80** (213 mg, 0.170 mmol) in MeOH (3097  $\mu$ L) was added H<sub>2</sub>O (310  $\mu$ L) and K<sub>2</sub>CO<sub>3</sub> (118 mg, 0.852 mmol). The reaction was stirred at RT for 1 h, diluted with H<sub>2</sub>O (100 mL), and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with sat. aq. NaCl (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (40 g silica gel with 5 g prepacked load cartridge; linear gradient 0–10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **81** (159 mg, 80%) as a white solid. LC-MS *m/z* 1166 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>)  $\delta$  9.99 (s, 1H), 9.17 - 8.86 (m, 2H), 8.15 (d,  $J$ =7.0 Hz, 1H), 7.58 (d,  $J$ =8.6 Hz, 2H), 7.32 (d,  $J$ =8.7 Hz, 2H), 7.27 - 7.16 (m, 3H), 7.03 - 6.96 (m, 2H), 5.98 - 5.85 (m, 2H), 5.35 - 5.26 (m, 2H), 5.23 - 5.14 (m, 2H), 5.07 - 4.76 (m, 8H), 4.56 - 4.52 (m, 2H), 4.50 - 4.46 (m, 2H), 4.45 - 4.39 (m, 1H), 4.37 - 4.30 (m, 1H), 4.18 - 4.11 (m, 4H), 4.08 - 3.80 (m, 10H), 3.57 - 3.46 (m, 1H), 3.39 - 3.26 (m, 2H), 3.22 - 3.03 (m, 1H), 2.65 - 2.46 (m, 4H), 2.21 - 2.12 (m, 2H), 2.03 - 1.93 (m, 1H), 1.70 - 1.61 (m, 4H), 1.55 - 1.46 (m, 4H), 1.42 - 1.34 (m, 4H), 1.30 (d,  $J$ =7.0 Hz, 3H), 0.88 (d,  $J$ =6.8 Hz, 3H), 0.84 (d,  $J$ =6.7 Hz, 3H).

**[00229]** To a RT solution of compound **81** (157 mg, 0.135 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3365  $\mu$ L) was added Dess–Martin periodinane (DMP, 120 mg, 0.283 mmol). The reaction was stirred at RT for 4 h, diluted with sat. aq. NaHCO<sub>3</sub> (50 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (40 g silica gel with 5 g prepacked load cartridge; linear gradient 0–10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>). The mixed fractions were repurified by flash chromatography (40 g RediSep silica gel with 5 g prepacked load cartridge; linear gradient 0–10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>) and the products from both columns were combined to provide compound **82** (115 mg, 74%) as a white solid. LC-MS *m/z* 1162 [M+H]<sup>+</sup>.

**[00230]** To a RT suspension of compound **82** (96.5 mg, 0.083 mmol) in DCM (1661  $\mu$ L) was added morpholine (36.5  $\mu$ L, 0.415 mmol) and tetrakis(triphenylphosphine)palladium(0) (4.80 mg, 4.15  $\mu$ mol). The reaction was stirred at RT for 2 h, was concentrated under a stream of N<sub>2</sub>, and purified by flash chromatography (12 g silica gel; linear gradient 0–10% MeOH–DCM). The mixed fractions were repurified by flash chromatography (12 g silica gel; linear

gradient 0–10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>) and the products from both columns were combined to provide compound **83** (54.9 mg, 68%). LC-MS *m/z* 976 [M+H]<sup>+</sup>.

[00231] To a RT solution of compound **83** (42.3 mg, 0.043 mmol) in DMF (867  $\mu$ L) was added 2,6-lutidine (15.14  $\mu$ L, 0.130 mmol), followed by compound **83a** (20.04 mg, 0.065 mmol). The reaction was stirred at RT for 2 days, diluted with DMSO, and purified by preparative HPLC (5 1-mL injections; Phenomenex Luna C18 21.2  $\times$  100 mm; linear gradient 20–80% MeCN–H<sub>2</sub>O with 0.05% v/v HCO<sub>2</sub>H over 25 min; 20 mL/min; 220 nm detection). The product-containing fractions were lyophilized and purified by flash chromatography (12 g RediSep Gold silica gel; linear gradient 0–20% MeOH–DCM) to provide dimer-linker **IIIa-11** (3.67 mg, 7%) as an off-white solid. LC-MS *m/z* 1170 [M+H]<sup>+</sup>.

*Example 14 – Dimer-linker IIIa-5*

[00232] This example pertains to **FIG. 14** and the synthesis of dimer-linker **IIIa-5**.

[00233] A mixture of compound **57a** (82.5 mg, 0.067 mmol), compound **84** (45.3 mg, 0.074 mmol), PdCl<sub>2</sub>(dppf) (2.463 mg, 3.37  $\mu$ mol), and Na<sub>2</sub>CO<sub>3</sub> (35.7 mg, 0.337 mmol) was evacuated and backfilled with N<sub>2</sub>. THF (898  $\mu$ L) and H<sub>2</sub>O (449  $\mu$ L) were added. The mixture was sparged with N<sub>2</sub> for 5 min and stirred at RT for 30 min. The reaction mixture was diluted H<sub>2</sub>O (50 mL) and extracted with DCM (2  $\times$  50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (24 g silica gel with 5 g prepacked load cartridge; linear gradient 0–20% MeOH–DCM) to provide compound **85** (98.9 mg, 94%) as an orange film. LC-MS *m/z* 1561 [M+H]<sup>+</sup>.

[00234] To a -78 °C solution of compound **85** (98.9 mg, 0.063 mmol) in THF (2112  $\mu$ L) was added LiBHEt<sub>3</sub> (317  $\mu$ L, 1 M solution in THF, 0.317 mmol), dropwise. The reaction mixture was stirred at -78 °C for 1 h, diluted with H<sub>2</sub>O (50 mL) and extracted with CHCl<sub>3</sub> (50 mL) and 10% MeOH–CHCl<sub>3</sub> (50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was taken up in a mixture of THF (4217  $\mu$ L), MeCN w/ 0.05% v/v HCO<sub>2</sub>H (2109  $\mu$ L), and H<sub>2</sub>O w/ 0.05% v/v HCO<sub>2</sub>H (2109  $\mu$ L), and stirred at RT for 1 h. The reaction was quenched by the addition of sat. aq. NaHCO<sub>3</sub> (50 mL) and extracted with CHCl<sub>3</sub> (2  $\times$  50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography (24 g basic

alumina; linear gradient 0–20% MeOH–CHCl<sub>3</sub>) to provide compound **86** (54.6 mg, 68%) as an orange solid. LC-MS *m/z* 1286 [M+18]<sup>+</sup>.

**[00235]** To a RT solution of compound **86** (29.0 mg, 0.023 mmol) in THF (457  $\mu$ L) was added piperidine (45.3  $\mu$ L, 0.457 mmol). The clear orange solution was stirred at RT for 45 min and then concentrated *in vacuo*. The crude material was taken up in a mixture of MeCN (2 mL) and MeOH (2 mL) and washed with heptane (4  $\times$  2 mL). The MeCN-MeOH layer was concentrated *in vacuo*. The residue was taken up in CHCl<sub>3</sub> and concentrated (2x), to give compound **87**, which was used without further purification. LC-MS *m/z* 1065 [M+H]<sup>+</sup>.

**[00236]** To a RT solution of crude **87** and compound **71a** (23.65 mg, 0.034 mmol) in DMSO (457  $\mu$ L) was added 2,6-lutidine (6.66  $\mu$ L, 0.057 mmol). The clear yellow solution was stirred at RT for 1.5 h, diluted with DMSO, and purified by preparative HPLC (3 1-mL injections; Phenomenex Luna C18 21.2 x 100 mm; linear gradient 20–60% MeCN–H<sub>2</sub>O w/ 0.05% v/v HCO<sub>2</sub>H over 25 min; 20 mL/min; 220 nm detection). The product-containing fractions were lyophilized to provide dimer-linker **IIIa-5** (6.97 mg, 19%) as a light yellow solid. LC-MS *m/z* 1621 [M+H]<sup>+</sup>.

*Example 15 – Dimer-linker IIIa-6*

**[00237]** This example pertains to **FIG. 15** and the synthesis of dimer-linker **IIIa-6**.

**[00238]** To a 0 °C solution of compound **58d** (50.0 mg, 0.050 mmol) and compound **88** (24.42 mg, 0.060 mmol) in DMF (496  $\mu$ L) was added HATU (22.62 mg, 0.060 mmol), followed by 2,6-lutidine (14.44  $\mu$ L, 0.124 mmol). The reaction mixture was stirred at 0 °C for 10 min and RT for 2 h and added dropwise to a stirred flask of H<sub>2</sub>O (20 mL) in a 0 °C bath. The resulting precipitate was collected by vacuum filtration (washed with H<sub>2</sub>O), then taken up in DCM (50 mL) and sat. aq. NaHCO<sub>3</sub> (50 mL). The layers were separated and the aqueous layer was extracted with DCM (50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (12 g silica gel; linear gradient 0–10% MeOH–DCM) to provide compound **89** (61.5 mg, 89%) as a yellow solid. LC-MS *m/z* 1401 [M+H]<sup>+</sup>.

**[00239]** To a –78 °C solution of compound **89** (61.5 mg, 0.044 mmol) in THF (1463  $\mu$ L) was added LiBHEt<sub>3</sub> (220  $\mu$ L, 1 M solution in THF, 0.220 mmol), dropwise. The reaction mixture was stirred at –78 °C for 1 h, quenched by the addition of H<sub>2</sub>O, warmed to RT, diluted with a mixture of sat. aq. NaHCO<sub>3</sub> (25 mL) and H<sub>2</sub>O (25 mL), and extracted with 10% MeOH–

CHCl<sub>3</sub> (2 x 50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. This residue was taken up in a mixture of THF (5867 μL), EtOH (5867 μL), and H<sub>2</sub>O w/ 0.05% v/v HCO<sub>2</sub>H (2933 μL) and stirred at RT for 2 h. The reaction was diluted with sat. aq. NaHCO<sub>3</sub> (50 mL) and extracted with CHCl<sub>3</sub> (50 mL), followed by 10% MeOH–CHCl<sub>3</sub> (50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (12 g silica gel; linear gradient 0–20% MeOH–DCM) to provide compound **90** (49 mg, quant.) as a yellow solid. LC-MS *m/z* 1109 [M+H]<sup>+</sup>.

**[00240]** To a RT solution of compound **90** (49 mg, 0.044 mmol) in THF (884 μL) was added piperidine (88 μL, 0.884 mmol). The clear orange solution was stirred at RT for 1 h and concentrated *in vacuo*. The crude product was taken up in a mixture of MeCN (2 mL) and MeOH (2 mL) and washed with heptane (4 x 2 mL). The MeCN–MeOH layer was concentrated *in vacuo*. This product **91** was taken up in CHCl<sub>3</sub> and concentrated (2x) and then used without further purification. LC-MS *m/z* 887 [M+H]<sup>+</sup>.

**[00241]** To a RT solution of crude product **91** and compound **71a** (22.76 mg, 0.033 mmol) in DMSO (440 μL) was added 2,6-lutidine (6.41 μL, 0.055 mmol). The clear, yellow solution was stirred at RT for 30 min. An additional solution of compound **71a** (3.2 μL, 0.027 mmol) in DMSO (0.220 mL) was added, and the reaction mixture was stirred for an additional 1.5 h, diluted with DMSO and purified by preparative HPLC (3 1-mL injections; Phenomenex Luna C18 21.2 × 100 mm; linear gradient 20–60% MeCN–H<sub>2</sub>O w/ 0.05% v/v HCO<sub>2</sub>H over 25 min; 20 mL/min; 220 nm detection). The product-containing fractions were lyophilized to provide dimer-linker **IIIa-6** (6.51 mg, 20%) as a yellow solid. LC-MS *m/z* 1461 [M+H]<sup>+</sup>.

*Example 16 – Dimer IIb-8*

**[00242]** This example pertains to **FIGs. 16A** and **16B** and the synthesis of dimer **IIb-8**.

**[00243]** To a solution of compound **19** (10 g, 21.44 mmol) in acetone (80 mL) was added benzyl bromide (5.23 ml, 44.0 mmol, Aldrich) followed by K<sub>2</sub>CO<sub>3</sub> (11.85 g, 86 mmol, Aldrich). The resulting bright yellow reaction mixture was stirred at 80 °C overnight. The yellow reaction mixture was poured into 200 mL of cold water. The solid precipitate was collected by filtration, washed with water and ether, and dried under vaccume to give a light yellow solid (12.8 g, 92%).

[00244] To a suspension of the precipitate from the previous step (12.8 g, 19.80 mmol) in THF (75 ml) and MeOH (25 mL) was added NaOH (39.6 ml, 119 mmol, 3.0 N). The reaction mixture was stirred at RT overnight to give a light brown homogeneous solution. The reaction mixture was concentrated *in vacuo* to remove most of the organic solvents. The residue was neutralized with 1.0 N HCl to pH 2-3. The solid formed was collected by filtration, washed with water and ether to give an off-white solid. The solid was dried under vacuum overnight to give compound **92** as a white solid (10.21 g, 83%).  $^1\text{H}$  NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  7.47 (s, 2H), 7.42 - 7.36 (m, 10H), 7.16 (s, 2H), 5.18 (s, 4H), 4.31 (t,  $J$  = 5.9 Hz, 4H), 2.41 (t,  $J$  = 5.9 Hz, 2H). MS (ESI<sup>+</sup>) m/z 619.5 (M + H)<sup>+</sup>.

[00245] Compound **92** was converted to compound **95**, proceeding via compounds **93** and **94**, following the procedures described in Example 14 and **FIG. 4A**.

[00246] To a pressure flask containing Pd(OH)<sub>2</sub> on carbon (0.060 g, 0.085 mmol, Aldrich) was added a solution of compound **95** (0.94 g, 0.853 mmol) in MeOH (10 mL) and EtOAc (10 mL). The resulting reaction mixture was stirred under H<sub>2</sub> at 20 psi pressure for 2 h and at 40 psi pressure for another 2 h. The reaction mixture was filtered through a pad of CELITE<sup>TM</sup>, washing with EtOAc. The filtrate was concentrated in vacuo to give 650 mg of compound **96** as a faint orange solid (650 mg, 83%). MS (ESI<sup>+</sup>) m/z 921.6 (M + H)<sup>+</sup>.

[00247] To a solution of compound **96** (101 mg, 0.110 mmol) and 1,4-dibromobutane **96a** (189 mg, 0.877 mmol, Aldrich) in DMF (1 mL) was added K<sub>2</sub>CO<sub>3</sub> (45.5 mg, 0.329 mmol). The reaction mixture was stirred at RT overnight. The reaction was diluted with water. The solid formed was collected by filtration, and purified by flash chromatography to give compound **97**, as a semi solid (110 mg, 84%). MS (ESI<sup>+</sup>) m/z 1191.6 (M + H)<sup>+</sup>.

[00248] To a solution of compound **97** (470 mg, 0.395 mmol) and tert-butyl (4-(aminomethyl)phenyl)carbamate **97a** (88 mg, 0.395 mmol, Aldrich) in DMF (4 mL) was added K<sub>2</sub>CO<sub>3</sub> (164 mg, 1.184 mmol). The reaction was heated at 85 °C for 3 h. The reaction was diluted with water and extracted with DCM (3x). The combined organic extracts were dried and concentrated, and purified by flash chromatography to give compound **98**, as a semi solid (175 mg, 35%). MS (ESI<sup>+</sup>) m/z 1251.5 (M + H)<sup>+</sup>.

[00249] To a solution of compound **98** (78.5 mg, 0.063 mmol) and 2,6-lutidine (0.022 mL, 0.188 mmol, Aldrich) in DCM (1.0 mL) at RT was added trimethylsilyl trifluoromethane sulfonate (TMS-OTf, 0.034 mL, 0.188 mmol, Aldrich). The reaction mixture was stirred at RT

for 1 h. The reaction mixture was diluted with DCM and washed with aq. sat. NaHCO<sub>3</sub>. The organic phase was dried, concentrated, and purified by flash chromatography to give compound **99**, as a semi solid (23 mg, 32%). MS (ESI<sup>+</sup>) m/z 1152.6 (M + H)<sup>+</sup>.

[00250] To a -78 °C solution of compound **99** (46 mg, 0.040 mmol) in THF (1 mL) was 5 added a solution of SUPER-HYDRIDE® (0.399 mL, 0.399 mmol, 1M in THF, Aldrich). The reaction was stirred at -78 °C for 1 h. The reaction was quenched with water and extracted with chloroform (2x), then 10% MeOH in chloroform (2x). The combined organic extracts were dried, concentrated and purified by preparative HPLC. Fractions containing the product were neutralized with NaHCO<sub>3</sub>, and extracted with chloroform (2x) and 10% MeOH in chloroform 10 (2x). The combined organic extracts were dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The product was then placed under high vacuum over a weekend to give dimer **IIb-8** a white solid (12 mg, 32%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.61 - 7.55 (m, 4H), 7.49 (d, *J*=7.9 Hz, 2H), 7.42 - 7.31 (m, 6H), 7.09 (d, *J*=7.9 Hz, 2H), 6.86 - 6.82 (m 2H), 6.55 (s, 2H), 5.03 (d, *J*=15.5 Hz, 2H), 4.57 (d, *J*=15.5 Hz, 2H), 4.37 - 4.29 (m, 8H), 4.01 - 3.88 (m, 4H), 3.37 - 3.07 15 (m, 4H), 2.52 (t, *J* = 5.9 Hz, 2H), 1.93 - 1.85 (m 6H), 1.71 - 1.60 (m, 8H). MS (ESI<sup>+</sup>) m/z 859.2 (M + H)<sup>+</sup>.

*Example 17 – Additional dimers and dimer-linkers*

[00251] Following the synthetic principles described hereinabove, the following additional dimers and dimer linkers were prepared:

[00252] Dimer **IIa-2**: LCMS (M+H) = 615.2 <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 20 7.68 (d, *J*=4.4 Hz, 2H), 7.54 (s, 2H), 6.88 (s, 2H), 4.39 - 4.25 (m, 4H), 4.23 - 4.15 (m, 2H), 4.14 - 4.06 (m, 2H), 3.83 (ddd, *J*=11.7, 7.2, 4.3 Hz, 2H), 3.76 (dt, *J*=7.6, 4.0 Hz, 2H), 3.65 - 3.55 (m, 2H), 2.40 - 2.29 (m, 6H), 2.13 - 2.01 (m, 4H), 1.84 - 1.7 (m, 4H), 1.64-1.52 (m., 4H), 1.48 - 1.42 (m, 4H).

[00253] Dimer **IIa-5**: LCMS (M+H) = 657.4 <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 25 7.69 (d, *J*=4.4 Hz, 2H), 7.55 (s, 2H), 6.84 (s, 2H), 4.36 - 4.17 (m, 6H), 4.15 - 4.08 (m, 2H), 3.84 (ddd, *J*=11.7, 7.2, 4.3 Hz, 2H), 3.78 - 3.72 (m, 2H), 3.67 - 3.56 (m, 2H), 2.46 - 2.30 (m, 6H), 2.16 - 2.03 (m, 4H), 1.88 - 1.78 (m, 4H), 1.65 - 1.50 (m, 6H), 1.48 - 1.34 (m, 8H).

[00254] Dimer **IIa-6**: LCMS (M+H) = 671.4 <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 30 7.68 (d, *J*=4.4 Hz, 2H), 7.53 (s, 2H), 6.84 (s, 2H), 4.34 - 4.15 (m, 6H), 4.12 - 4.05 (m, 2H), 3.84

(ddd,  $J=11.7, 7.2, 4.3$  Hz, 2H), 3.78 - 3.73 (m, 2H), 3.66 - 3.56 (m, 2H), 2.52 - 2.30 (m, 6H), 2.17 - 2.02 (m, 4H), 1.92 - 1.77 (m, 4H), 1.65 - 1.50 (m, 6H), 1.43 - 1.27 (m, 10 H).

**[00255]** Dimer **IIa-7**: LCMS (M+H) = 685.3  $^1$ H NMR (400MHz, CHLOROFORM-d)  $\delta$  7.68 (d,  $J=4.4$  Hz, 2H), 7.54 (s, 2H), 6.83 (s, 2H), 4.22 - 4.03 (m, 10H), 3.84 (ddd,  $J=11.7, 7.1, 4.5$  Hz, 3H), 3.76 (dt,  $J=7.5, 4.0$  Hz, 2H), 3.61 (dt,  $J=11.8, 7.8$  Hz, 2H), 2.34 (td,  $J=6.7, 2.6$  Hz, 4H), 2.08 (d,  $J=5.1$  Hz, 4H), 1.99 - 1.92 (m, 5H), 1.88 - 1.76 (m, 8H), 1.59 - 1.50 (m, 14H), 1.47 - 1.38 (m, 7H).

**[00256]** Dimer **IIa-8**. LCMS (M+H) = 699.5  $^1$ H NMR (400MHz, CHLOROFORM-d)  $\delta$  7.68 (d,  $J=4.4$  Hz, 2H), 7.52 (s, 2H), 6.81 (s, 2H), 4.23 - 4.01 (m, 10H), 3.84 (ddd,  $J=11.7, 7.2, 4.3$  Hz, 2H), 3.78 - 3.71 (m, 2H), 3.61 (dt,  $J=11.9, 7.6$  Hz, 2H), 2.34 (td,  $J=6.7, 2.9$  Hz, 4H), 2.09 (dd,  $J=6.9, 4.7$  Hz, 4H), 2.00 - 1.92 (m, 5H), 1.88 - 1.72 (m, 8H), 1.58 (br. s., 13H), 1.39 (d,  $J=6.2$  Hz, 12H).

**[00257]** Dimer **IIa-10**: LC-MS  $m/z$  665 [M+H] $^+$ ;  $^1$ H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  7.68 (d,  $J=4.4$  Hz, 2H), 7.50 (s, 2H), 6.84 (s, 2H), 5.47 - 5.43 (m, 2H), 5.23 - 5.14 (m, 4H), 4.34 - 4.20 (m, 8H), 4.18 - 4.11 (m, 2H), 4.10 - 4.02 (m, 2H), 3.93 - 3.86 (m, 2H), 3.18 - 3.08 (m, 2H), 2.99 - 2.90 (m, 2H), 2.40 - 2.29 (m, 2H), 2.09 - 2.01 (m, 4H), 1.87 - 1.76 (m, 4H), 1.64 - 1.56 (m, 4H).

**[00258]** Dimer **IIa-11**: LC-MS  $m/z$  667 [M+H] $^+$ .

**[00259]** Dimer **IIa-12**: LC-MS  $m/z$  639 [M+H] $^+$ ;  $^1$ H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  7.68 (d,  $J=4.4$  Hz, 2H), 7.51 (s, 2H), 6.86 (s, 2H), 5.22 - 5.15 (m,  $J=4.0$  Hz, 4H), 4.36 - 4.23 (m, 8H), 4.21 - 4.14 (m, 2H), 4.12 - 4.05 (m, 2H), 3.93 - 3.87 (m, 2H), 3.17 - 3.07 (m, 2H), 2.99 - 2.90 (m, 2H), 2.34 (quin,  $J=6.2$  Hz, 2H), 1.84 - 1.75 (m, 4H), 1.63 - 1.54 (m, 4H), 1.48 - 1.40 (m, 4H).

**[00260]** Dimer **IIa-13**: LC-MS  $m/z$  643 [M+H] $^+$ ;  $^1$ H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  7.67 (d,  $J=4.4$  Hz, 2H), 7.49 (s, 2H), 6.86 (s, 2H), 5.22 - 5.13 (m, 4H), 4.40 - 4.25 (m, 10H), 4.23 - 4.17 (m, 2H), 3.94 - 3.85 (m, 6H), 3.84 - 3.77 (m, 4H), 3.18 - 3.05 (m, 2H), 2.97 - 2.89 (m, 2H), 2.32 (quin,  $J=6.0$  Hz, 2H).

**[00261]** Dimer **IIa-14**: LC-MS  $m/z$  672.5 [M+H<sub>2</sub>O+H] $^+$ .

**[00262]** Dimer **IIb-1**: LC-MS  $m/z$  739 [M+H] $^+$ ;  $^1$ H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  7.55 (s, 2H), 7.49 (d,  $J=5.3$  Hz, 2H), 7.41 - 7.30 (m, 8H), 6.87 (s, 2H), 5.02 (d,  $J=15.6$  Hz, 2H), 4.57 (d,

*J*=15.6 Hz, 2H), 4.38 - 4.24 (m, 4H), 4.24 - 4.16 (m, 2H), 4.10 (dt, *J*=9.7, 5.1 Hz, 2H), 4.01 - 3.94 (m, 2H), 3.34 - 3.24 (m, 2H), 3.22 - 3.11 (m, 2H), 2.35 (quin, *J*=6.1 Hz, 2H), 1.91 - 1.74 (m, 4H), 1.59 (d, *J*=5.9 Hz, 6H), 1.50 - 1.40 (m, 4H), 0.97 - 0.82 (m, 2H).

[00263] Dimer **IIb-2**: LC-MS *m/z* 767 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.53 (s, 2H), 7.47 (d, *J*=5.3 Hz, 2H), 7.39 - 7.29 (m, 8H), 6.81 (s, 2H), 5.01 (d, *J*=15.6 Hz, 2H), 4.56 (d, *J*=15.6 Hz, 2H), 4.33 - 4.14 (m, 6H), 4.11 - 4.04 (m, 2H), 3.99 - 3.93 (m, 2H), 3.31 - 3.24 (m, 2H), 3.19 - 3.13 (m, 2H), 2.39 (quin, *J*=6.7 Hz, 2H), 1.83 - 1.75 (m, 4H), 1.62 - 1.52 (m, 4H), 1.41 - 1.32 (m, 8H).

[00264] Dimer **IIb-3**: LC-MS *m/z* 739 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J*=7.9 Hz, 2H), 7.88 (d, *J*=4.4 Hz, 2H), 7.56 (s, 2H), 7.32 - 7.20 (m, 4H), 7.14 - 7.08 (m, 2H), 6.86 (s, 2H), 4.50 (dt, *J*=10.9, 4.2 Hz, 2H), 4.38 - 4.05 (m, 8H), 3.78 - 3.67 (m, 2H), 3.55 - 3.46 (m, 2H), 2.47 - 2.36 (m, 2H), 1.83 - 1.76 (m, 4H), 1.59 (s, 4H), 1.42 - 1.31 (m, 8H).

[00265] Dimer **IIb-4**: LC-MS *m/z* 743.2 (M + H)<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d) δ 7.53 (s, 2H), 7.47 (d, *J*= 5.1 Hz, 2H), 7.40 - 7.29 (m, 8H), 6.86 (s, 2H), 5.01 (d, *J*= 15.6 Hz, 2H), 4.54 (d, *J*= 15.6 Hz, 2H), 4.37 - 4.27 (m, 6H), 4.24 - 4.18 (m, 2H), 3.97 - 3.88 (m, 6H), 3.82 (d, *J*= 1.1 Hz, 4H), 3.31 - 3.23 (m, 2H), 3.19 - 3.11 (m, 2H), 2.37 - 2.29 (m, 2H).

[00266] Dimer **IIb-7**: LC-MS *m/z* 758.6 (M + H)<sup>+</sup>; <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>) δ 7.48 (s, 2H), 7.43 (d, *J*= 5.3 Hz, 2H), 7.38 (d, *J*= 5.3 Hz, 2H), 7.36 - 7.26 (m, 4H), 7.04 (d, *J*= 8.4 Hz, 1H), 6.87 (s, 2H), 6.52 (br. s., 2H), 4.91 (d, *J*= 15.3 Hz, 2H), 4.53 (d, *J*= 15.0 Hz, 2H), 4.30 - 4.20 (m, 6H), 4.20 - 4.16 (m, 2H), 3.91 - 3.88 (m, 6H), 3.65 (s, 4H), 3.46 - 3.45 (m, 2H), 3.10 - 3.06 (m, 2H), 2.25 - 2.17 (m, 2H).

[00267] Dimer **IIc-1**: LC-MS *m/z* 791 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.91 (d, *J*=3.7 Hz, 2H), 7.56 - 7.51 (m, 4H), 7.49 - 7.32 (m, 10H), 6.86 (s, 2H), 4.51 - 4.42 (m, 2H), 4.37 - 3.84 (m, 8H), 3.68 - 3.58 (m, 2H), 3.48 - 3.38 (m, 2H), 2.49 - 2.36 (m, 2H), 1.87 - 1.74 (m, 4H), 1.69 - 1.20 (m, 12H).

[00268] Dimer **IIc-2**: LC-MS *m/z* 907 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.89 (d, *J*=4.0 Hz, 2H), 7.53 (s, 2H), 7.41 - 7.39 (m, 2H), 7.37 - 7.33 (m, 4H), 6.94 - 6.89 (m, 4H), 6.82 (s, 2H), 4.47 - 4.38 (m, 2H), 4.21 - 3.96 (m, 8H), 3.84 (s, 6H), 3.59 (ddd, *J*=16.2, 11.4, 1.9 Hz, 2H), 3.39 (ddd, *J*=16.4, 5.2, 1.5 Hz, 2H), 2.03 - 1.92 (m, 4H), 1.86 - 1.70 (m, 6H), 1.62 - 1.51 (m, 4H), 1.45 - 1.30 (m, 12H).

[00269] Dimer **IIc-3**: LC-MS *m/z* 996 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 7.89 (d, *J*=4.0 Hz, 2H), 7.52 (s, 2H), 7.42 - 7.38 (m, 2H), 7.35 - 7.32 (m, 4H), 6.97 - 6.91 (m, 4H), 6.82 (s, 2H), 4.46 - 4.38 (m, 2H), 4.20 - 4.03 (m, 12H), 3.80 - 3.75 (m, 4H), 3.63 - 3.54 (m, 2H), 3.48 - 3.47 (m, 6H), 3.42 - 3.34 (m, 2H), 2.04 - 1.92 (m, 4H), 1.86 - 1.71 (m, 6H), 1.60 - 1.51 (m, 4H), 1.45 - 1.29 (m, 12H).

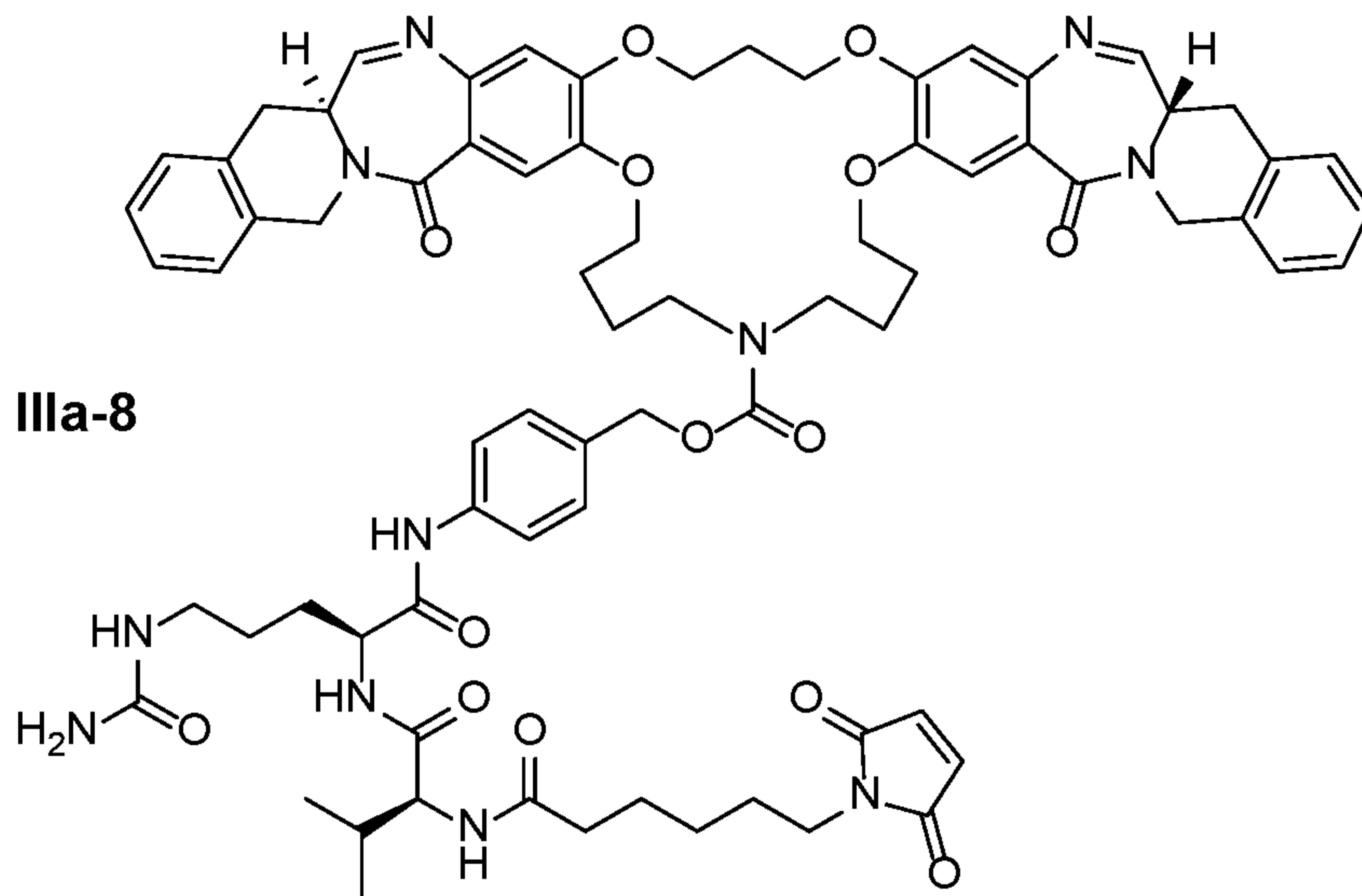
[00270] Dimer **IIc-4**: LC-MS *m/z* 1017 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.88 (d, *J*=4.0 Hz, 2H), 7.52 (s, 2H), 7.39 (s, 2H), 7.33 (d, *J*=8.6 Hz, 4H), 6.91 (d, *J*=9.0 Hz, 4H), 6.82 (s, 2H), 4.46 - 4.37 (m, 2H), 4.21 - 4.01 (m, 8H), 3.91 - 3.85 (m, 8H), 3.63 - 3.53 (m, 2H), 3.42 - 3.35 (m, 2H), 3.23 - 3.16 (m, 8H), 1.97 (quin, *J*=6.9 Hz, 4H), 1.86 - 1.72 (m, 6H), 1.56 (s, 4H), 1.44 - 1.22 (m, 12H).

[00271] Dimer **IIc-5**: LC-MS *m/z* 1061 [M+H<sub>2</sub>O]<sup>+</sup>; <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ 7.88 (d, *J*=4.0 Hz, 2H), 7.55 - 7.51 (m, 2H), 7.38 (s, 2H), 7.31 (d, *J*=8.8 Hz, 4H), 6.92 (d, *J*=8.8 Hz, 4H), 6.81 (s, 2H), 4.45 - 4.37 (m, 2H), 4.20 - 4.01 (m, 8H), 3.62 - 3.52 (m, 2H), 3.43 - 3.33 (m, 2H), 3.30 - 3.23 (m, 8H), 2.63 - 2.57 (m, 8H), 2.37 (s, 6H), 1.97 (quin, *J*=7.0 Hz, 4H), 1.87 - 1.71 (m, 6H), 1.66 - 1.48 (m, 4H), 1.43 - 1.22 (m, 12H).

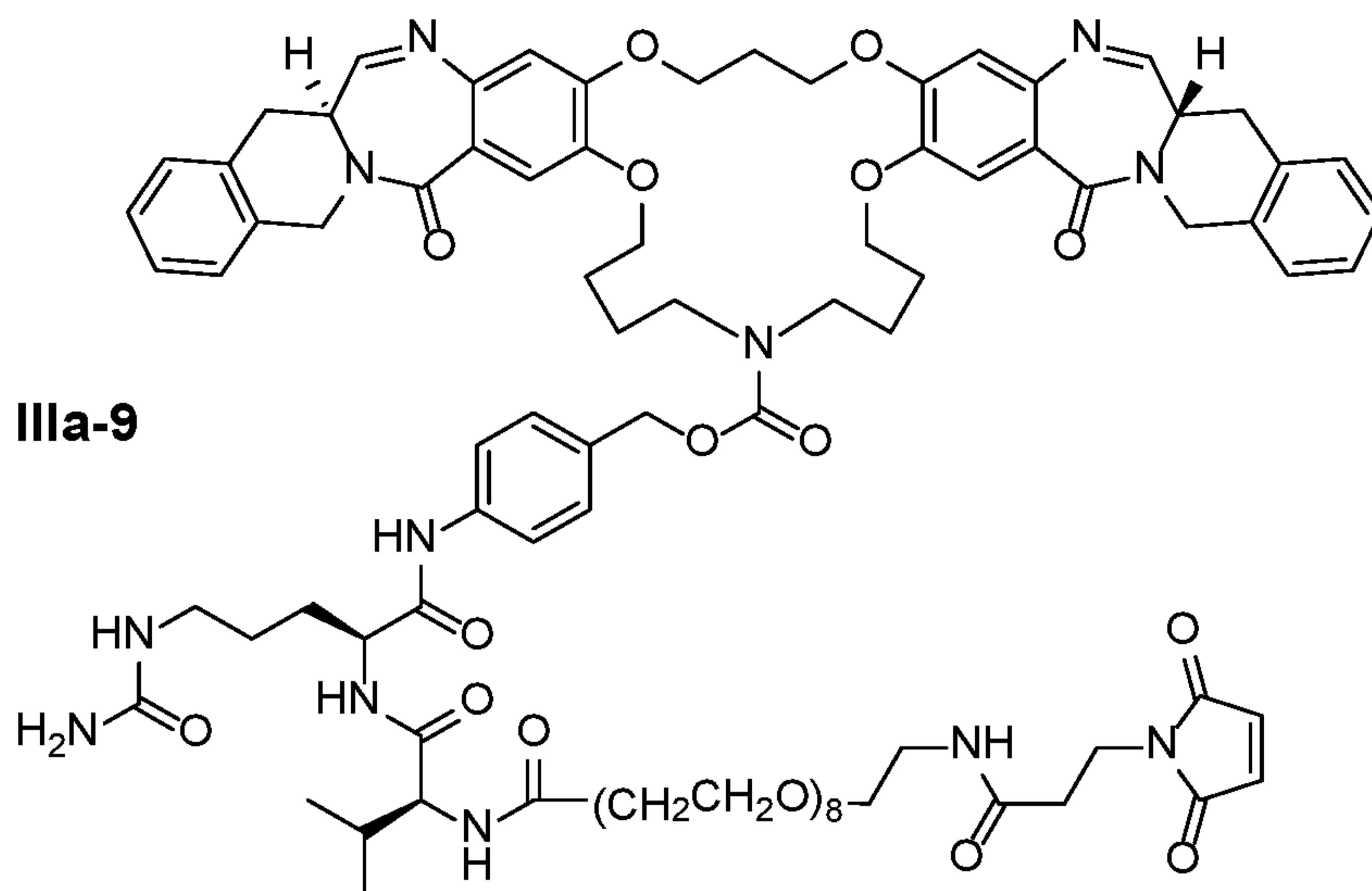
[00272] Dimer **IIc-6**: LC-MS *m/z* 767 [M+H]<sup>+</sup>.

[00273] Dimer-linker **IIIa-7**: LC-MS *m/z* 777 [M+2H]<sup>+</sup>.

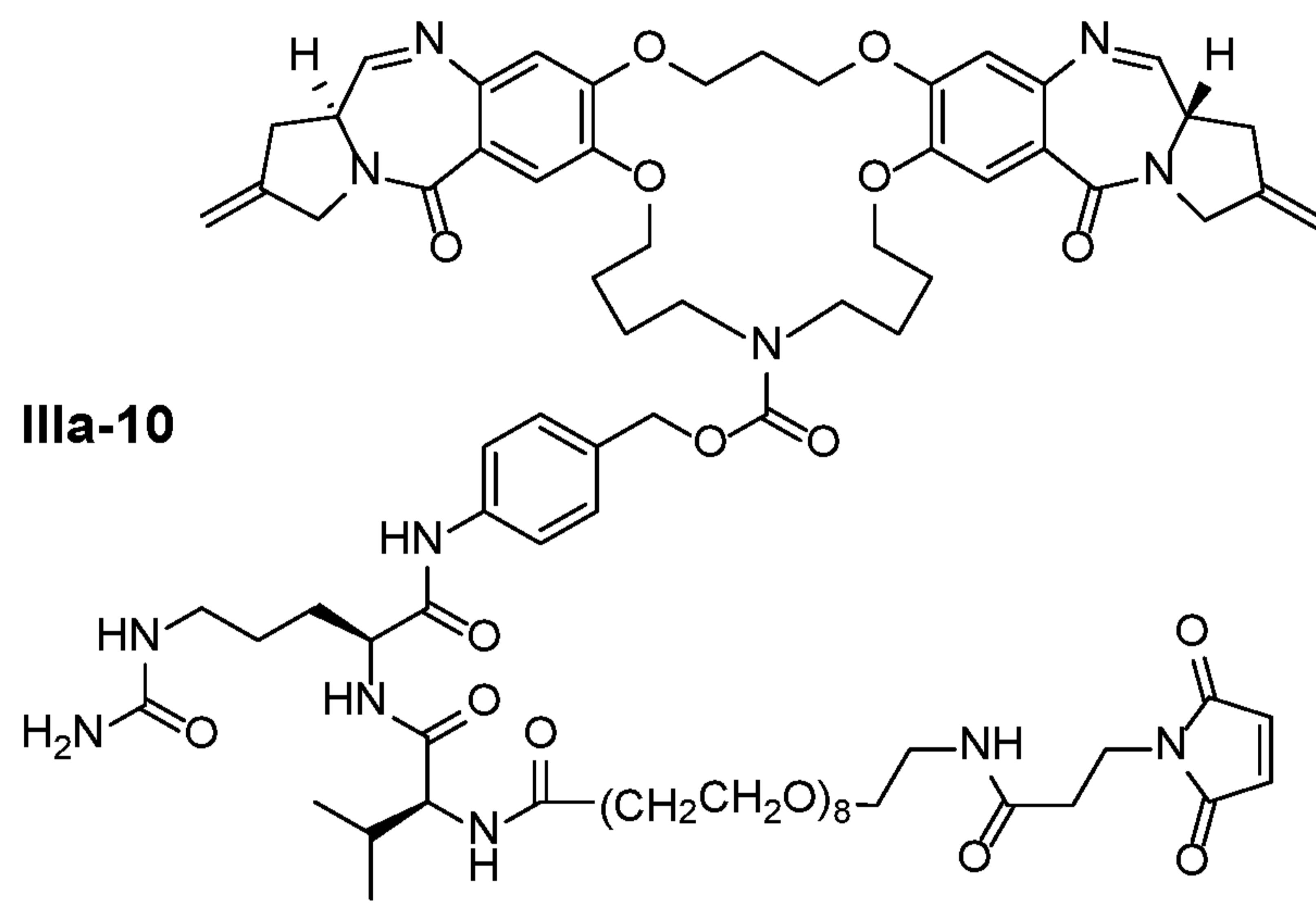
[00274] Dimer-linker **IIIa-8**: LC-MS *m/z* 1352.8 [M+H]<sup>+</sup>.



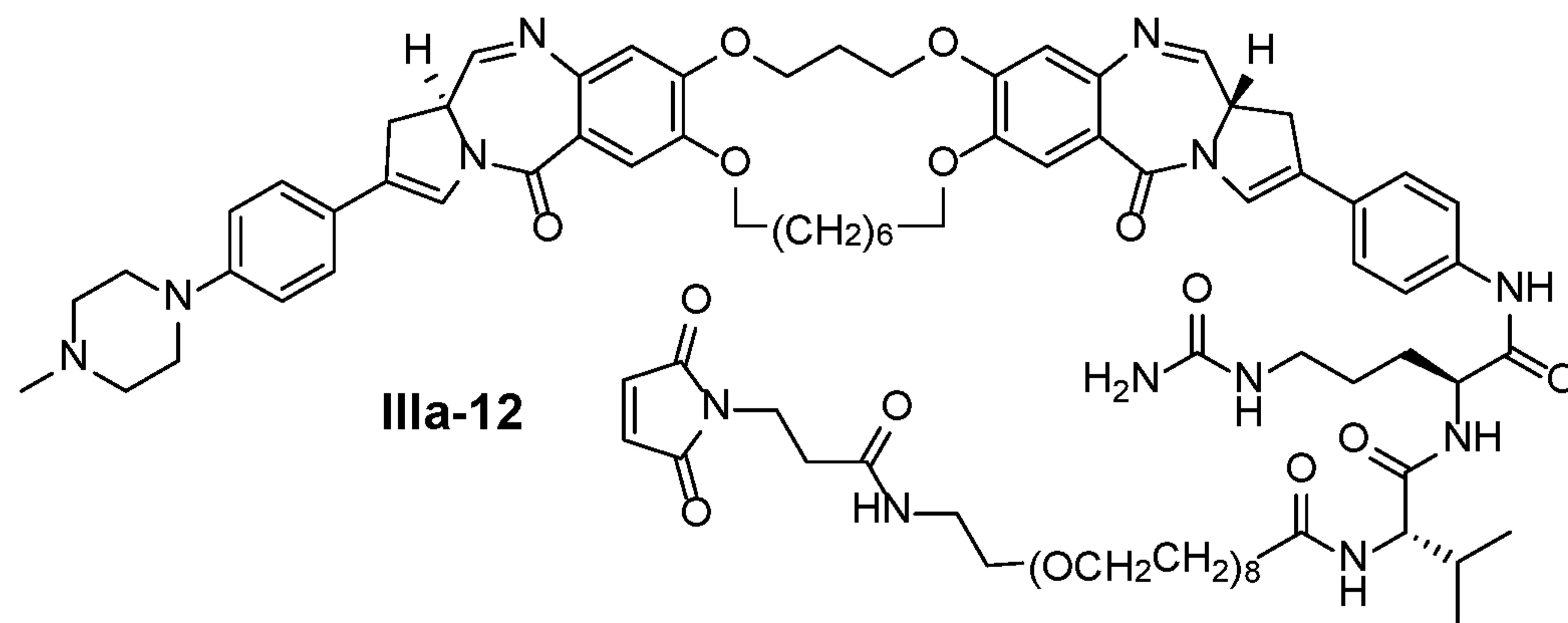
[00275] Dimer-linker **IIIa-9**: LC-MS *m/z* 1734.2 [M+H]<sup>+</sup>.



[00276] Dimer-linker IIIa-10: LC-MS *m/z* 1633 [M+H]<sup>+</sup>.



[00277] Dimer-linker IIIa-12: LC-MS *m/z* 1707 [M+H]<sup>+</sup>.



*Example 18 – Biological Activity (dimers)*

**[00278]** The cytotoxic activity of dimers of this invention against various different cancer cell lines is shown in Table 4. H226 is a human lung cancer cell line. N87 is a human gastric cancer cell line. OVCAR3 is a human ovarian cancer cell line. HCT116 is a human colon cancer cell line. HCT116/VM46 is a human colon cancer cell line that is multi-drug and paclitaxel resistant.

Table 4 – Cytotoxic Activity of Dimers

Dimer	Cell Line (IC <sub>50</sub> , nM)				
	H226	N87	OVCAR3	HCT116	HCT116/ VM46
IIa-1	8.1	4.6	3.4	—	74
IIa-2	1.6	2.7	1.2	—	—
IIa-3	8.7	8.3	7.3	—	—
IIa-4	12	22	20	—	—
IIa-5	69	150	78	—	—
IIa-6	110	310	140	—	—
IIa-7	12	87	14	—	—
IIa-8	10	15	9.9	—	—
IIa-9	12	13	12	—	—
IIa-10	2.0	2.3	0.47	—	11
IIa-11	13	18	8.6	—	48
IIa-12	0.32	0.89	0.52	—	4.8
IIa-13	29	40	20	32	85
IIa-14	21	23	50	38	170
IIb-1	0.22	0.53	0.33	—	1.3
IIb-2	3.0	2.5	1.3	—	17
IIb-3	5.9	11	3.5	—	13
IIb-4	0.15	0.14	0.20	0.068	2.0
IIb-5	0.45	0.35	0.60	0.077	7.3
IIb-6	0.21	0.25	0.21	0.016	0.66
IIb-7	0.56	0.40	0.56	0.19	3.5
IIb-8	0.13	0.27	0.25	0.015	1.2
IIc-1	12	12	7.6	—	30
IIc-2	220	230	>250	48	>250
IIc-3	32	29	104	13	58
IIc-4	—	—	—	—	—
IIc-5	—	—	—	—	—
IIc-6	1.1	1.5	1.7	0.13	6.4

Table 4 (continued) – Cytotoxic Activity of Dimers

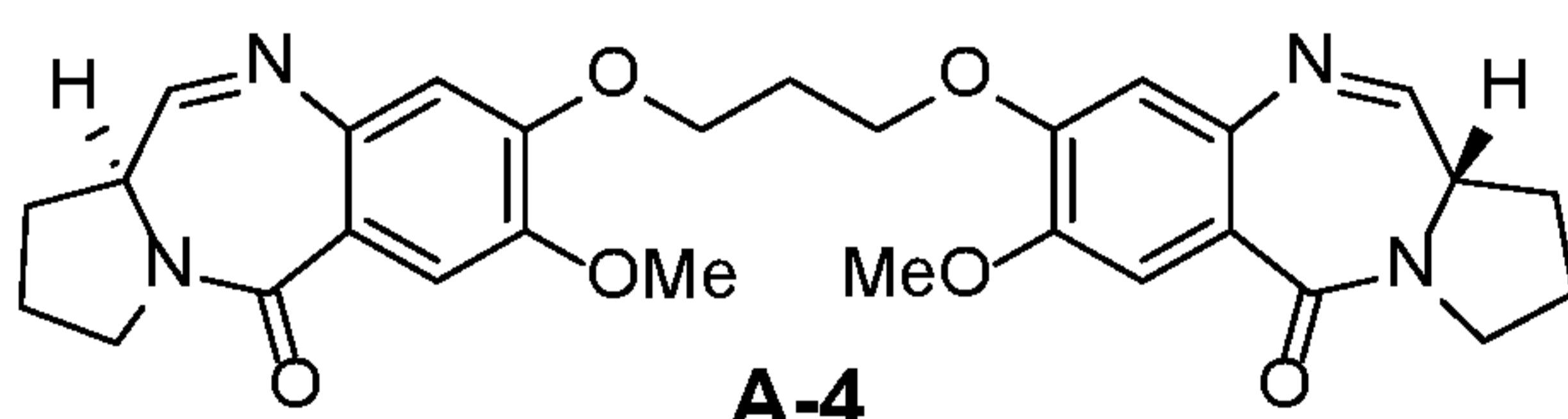
Dimer	Cell Line (IC <sub>50</sub> , nM)				
	H226	N87	OVCAR3	HCT116	HCT116/ VM46
IIC-7	0.99	0.62	1.8	0.17	1.1
IIC-8	0.047	0.034	0.35	0.006	0.076
IIC-9	0.33	0.68	1.0	0.063	0.16
IIC-10	0.044	0.36	0.36	0.022	0.071
IIC-11	0.36	0.56	0.31	0.063	0.36
IID-1	0.66	1.4	1.2	0.14	1.2
IID-2	0.56	1.1	0.71	0.11	2.8
IID-3	0.035	0.072	0.26	0.045	0.033
IID-4	8.1	12	20	2.0	4.8

*Example 19 – Biological Activity (ADCs)*

[00279] FIG. 17 shows the activity of two ADCs made with dimer-linker **IIIa-7**, one with an anti-CD70 antibody and one with an anti-mesothelin antibody. The ADCs were prepared following the procedure generally described above. Each had drug-antibody ratio of about 2. Activity was measured using a <sup>3</sup>H thymidine incorporation assay, where a decrease in the incorporation of the radiolabeled thymidine indicates inhibition of cell proliferation (Cong *et al.*, US 8,980,824 B2 (2015)). As can be seen from the figure, both ADCs were active, with EC<sub>50</sub> values in the range of nanomolar or less.

*Example 20 – Comparative Activity*

[00280] Table 5 compares the cytotoxic activities of dimers of this invention against that of a non-macrocyclic PBD having a structure shown by formula A-4. It is noteworthy that the macrocyclic ring does not appear to introduce conformational constraints that interfere with the ability of both benzodiazepine rings to slide into the DNA minor groove, as evidenced by the comparable, and in some cases, superior cytotoxic potency.



Dimer	Table 5 –Comparative Activities		
	Cell line (EC <sub>50</sub> , nM)		
	H226	N87	OVCAR3
A-4	70	63	56
IIa-1	8.1	4.6	3.4
IIa-2	1.6	2.7	1.2
IIa-3	8.7	8.3	7.3
IIa-4	12	22	20
IIa-5	69	150	78
IIa-6	110	310	140
IIa-7	12	87	14
IIa-8	10	15	9.9
IIa-9	12	13	12

[00281] The foregoing detailed description of the invention includes passages that are chiefly or exclusively concerned with particular parts or aspects of the invention. It is to be understood that this is for clarity and convenience, that a particular feature may be relevant in more than just the passage in which it is disclosed, and that the disclosure herein includes all the appropriate combinations of information found in the different passages. Similarly, although the various figures and descriptions herein relate to specific embodiments of the invention, it is to be understood that where a specific feature is disclosed in the context of a particular figure or embodiment, such feature can also be used, to the extent appropriate, in the context of another figure or embodiment, in combination with another feature, or in the invention in general.

[00282] Further, while the present invention has been particularly described in terms of certain preferred embodiments, the invention is not limited to such preferred embodiments. Rather, the scope of the invention is defined by the appended claims.

[00283] Full citations for the following references, cited in abbreviated fashion by first author (or inventor) and date earlier in this specification, are provided below. Each of these references is incorporated herein by reference for all purposes.

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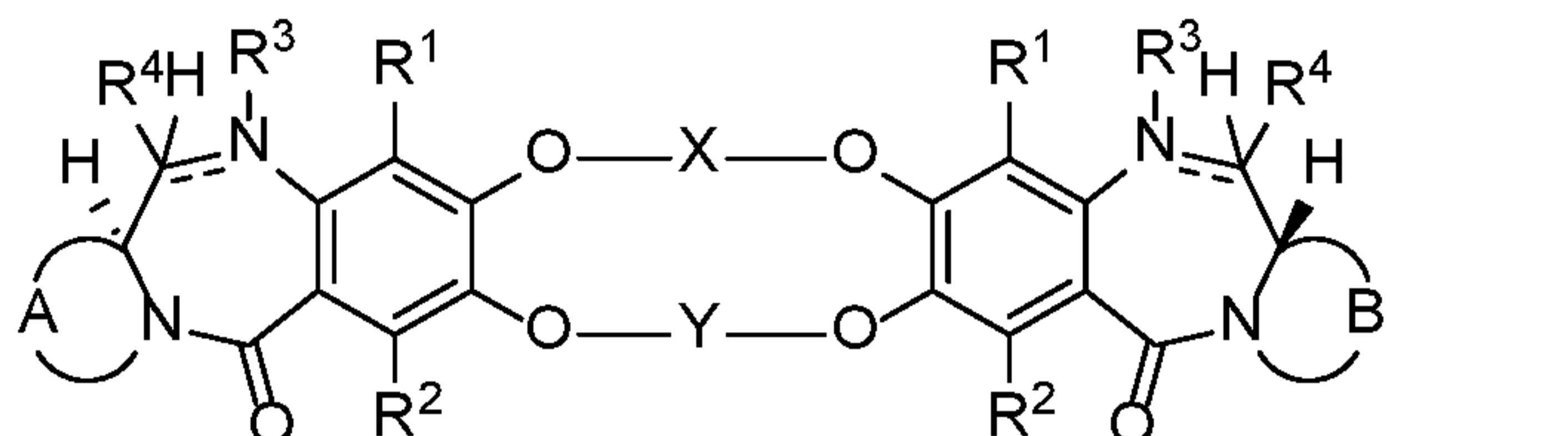
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## CLAIMS

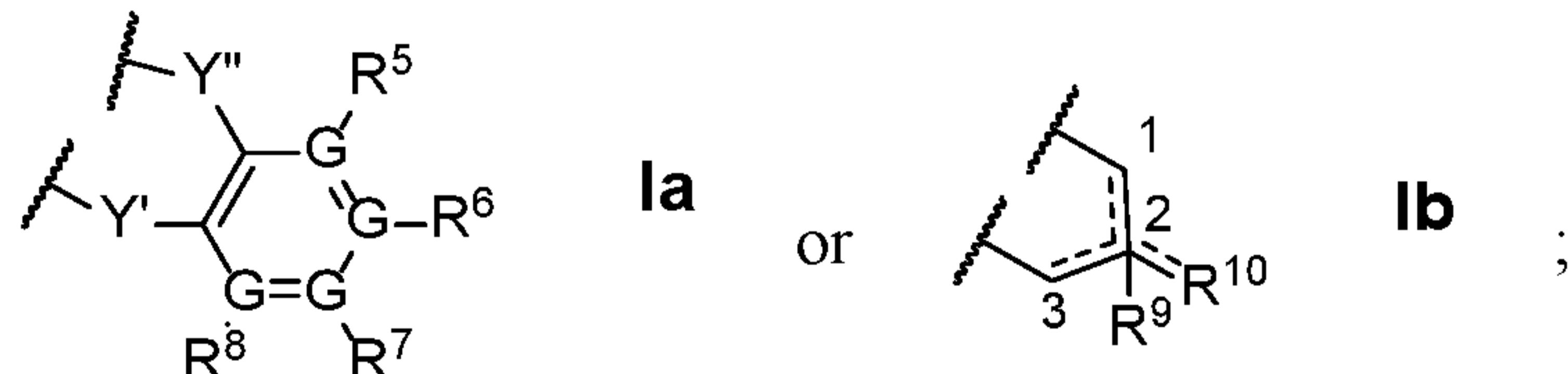
What is claimed is:

1. A benzodiazepine dimer having a structure represented by formula I



5 X is  $-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-$ ,  $-\text{CH}_2-\text{X}^1-\text{CH}_2-$ , or  $-\text{CH}_2-\text{C}(=\text{O})-\text{CH}_2-$   
 ;

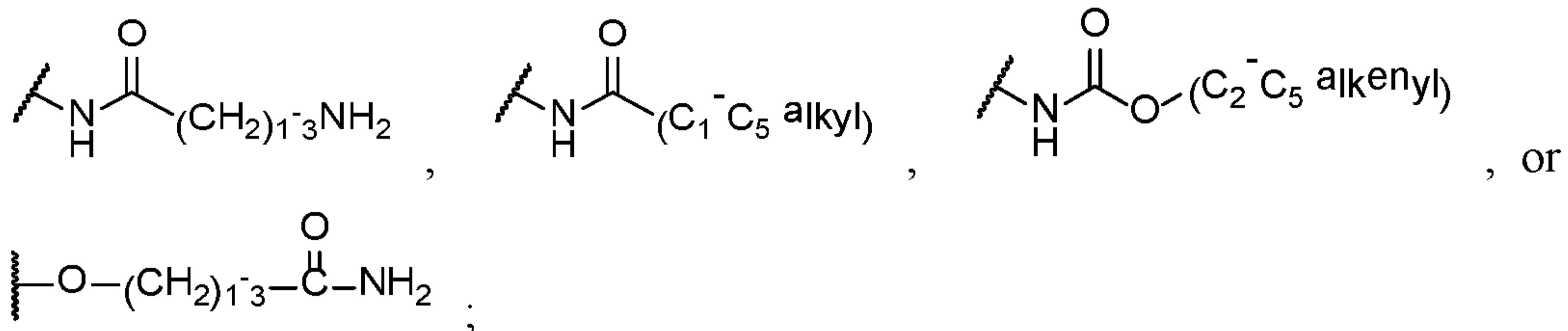
X<sup>1</sup> is CH<sub>2</sub>, O, NH, S(O)<sub>0-2</sub>, 3- to 7-membered cycloalkylene or heterocycloalkylene unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, or 5- to 6-membered arylene or heteroarylene unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>;  
 10 each X<sup>2</sup> is independently Me, CO<sub>2</sub>H, NH<sub>2</sub>, NH(C<sub>1</sub>-C<sub>5</sub> alkyl), N(C<sub>1</sub>-C<sub>5</sub> alkyl)<sub>2</sub>, SH, CHO, N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N(C<sub>1</sub>-C<sub>3</sub> alkyl), N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>NH, NHNNH<sub>2</sub>, or C(=O)NHNNH<sub>2</sub>;  
 Y is (CH<sub>2</sub>)<sub>4-6</sub>CH=CH(CH<sub>2</sub>)<sub>4-6</sub>, (CH<sub>2</sub>)<sub>4-6</sub>X<sup>1</sup>(CH<sub>2</sub>)<sub>4-6</sub>, or (CH<sub>2</sub>)<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>2-3</sub>;  
 each R<sup>1</sup> and R<sup>2</sup> is independently H, F, Cl, Br, OH, C<sub>1</sub>-C<sub>3</sub> alkyl, O(C<sub>1</sub>-C<sub>3</sub> alkyl), cyano, (CH<sub>2</sub>)<sub>0-5</sub>NH<sub>2</sub>, or NO<sub>2</sub>;  
 15 each double line == in a diazepine ring system independently represents a single bond or a double bond;  
 each R<sup>3</sup> is H if the double line == to the N to which it is attached is a single bond and is absent if the double line is a double bond;  
 each R<sup>4</sup> is H, OH, SO<sub>3</sub>Na, or SO<sub>3</sub>K if the double line == to the C to which it is attached is a single bond and is absent if the double line is a double bond;  
 20 A and B are independently according to formula Ia or Ib



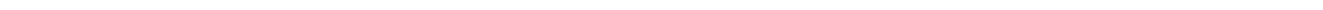
wherein, in formula Ia

Y' and Y" are independently absent, CH<sub>2</sub>, C=O, or CHR<sup>12</sup>; wherein each R<sup>12</sup> is independently F, Cl, Br, or C<sub>1</sub>-C<sub>3</sub> alkyl, with the proviso that Y' and Y" are not both absent; each G is independently C or N, with the proviso that no more than two Gs are N; and each R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, and R<sup>8</sup> is independently H, C<sub>1</sub>-C<sub>5</sub> alkyl, C≡C(CH<sub>2</sub>)<sub>1-5</sub>X<sup>2</sup>, OH, O(C<sub>1</sub>-C<sub>5</sub> alkyl), cyano, NO<sub>2</sub>, F, Cl, Br, O(CH<sub>2</sub>CH<sub>2</sub>O)<sub>1-8</sub>(C<sub>1</sub>-C<sub>3</sub> alkyl), (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup>, O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, 3- to 7-membered cycloalkyl or heterocycloalkyl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, 5- to 6-membered aryl or heteroaryl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>,

10



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or where a R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is attached to a G that is N, such R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is absent;

and

wherein, in formula Ib,

the dotted lines indicate the optional presence of a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond;

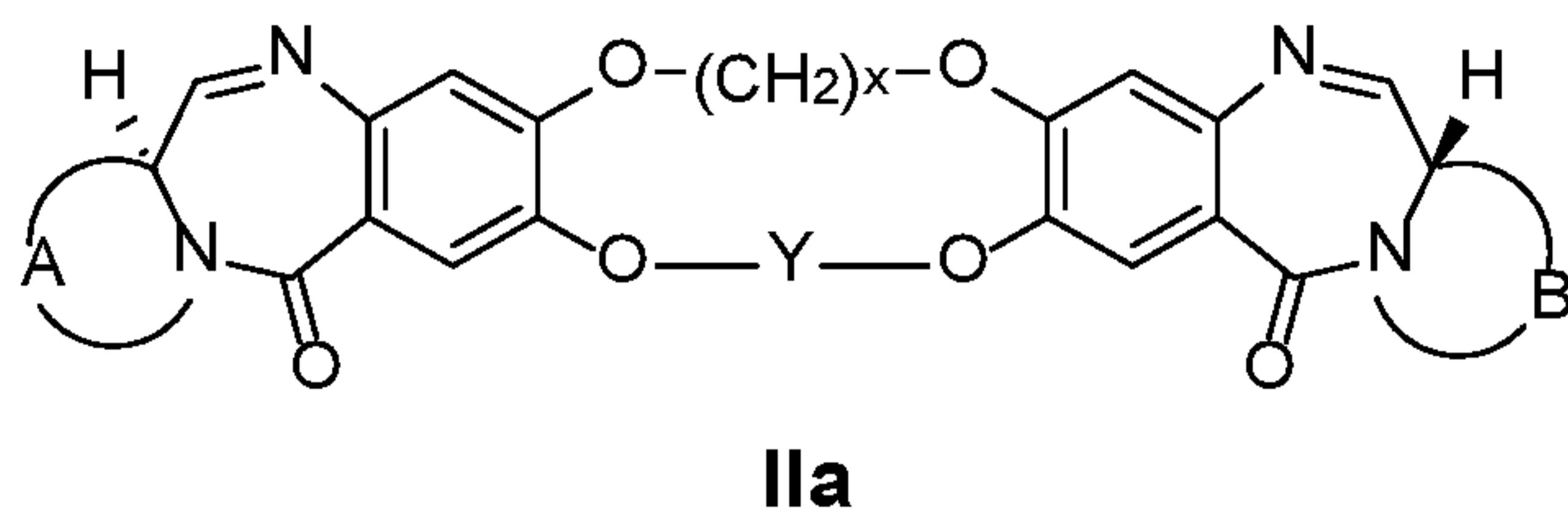
R<sup>10</sup> is H, =O, =CH<sub>2</sub>, =CH(C<sub>1</sub>-C<sub>5</sub> alkyl), CH=CH(CH<sub>2</sub>)<sub>1-5</sub>X<sup>2</sup>, C≡C(CH<sub>2</sub>)<sub>1-5</sub>X<sup>2</sup>, C<sub>1</sub>-C<sub>5</sub> alkyl, OH, O(C<sub>1</sub>-C<sub>5</sub> alkyl), cyano, NO<sub>2</sub>, F, Cl, Br, O(CH<sub>2</sub>CH<sub>2</sub>O)<sub>1-8</sub>(C<sub>1</sub>-C<sub>3</sub> alkyl), (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup>, 4- to 7-membered aryl, heteroaryl, cycloalkyl, or heterocycloalkyl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup>, O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, 3- to 7-membered cycloalkyl or heterocycloalkyl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>, 5- to 6-membered aryl or heteroaryl unsubstituted or substituted with (CH<sub>2</sub>)<sub>0-5</sub>X<sup>2</sup> or O(CH<sub>2</sub>)<sub>2-5</sub>X<sup>2</sup>; and

20

25

R<sup>9</sup> is absent if a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond is present and otherwise is H; or a pharmaceutically acceptable salt thereof.

2. A benzodiazepine dimer according to claim 1, having a structure represented by formula IIa:



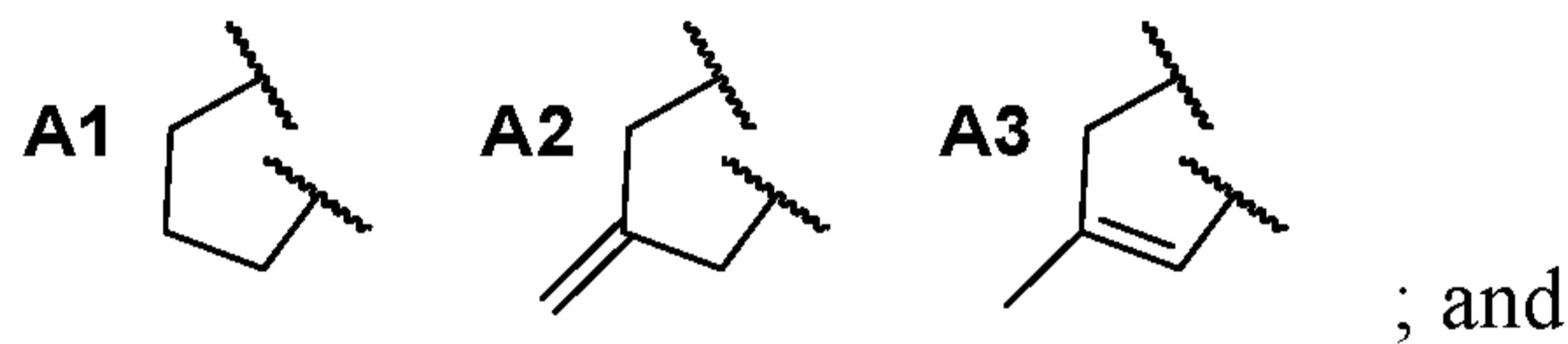
wherein

x is 3 or 5;

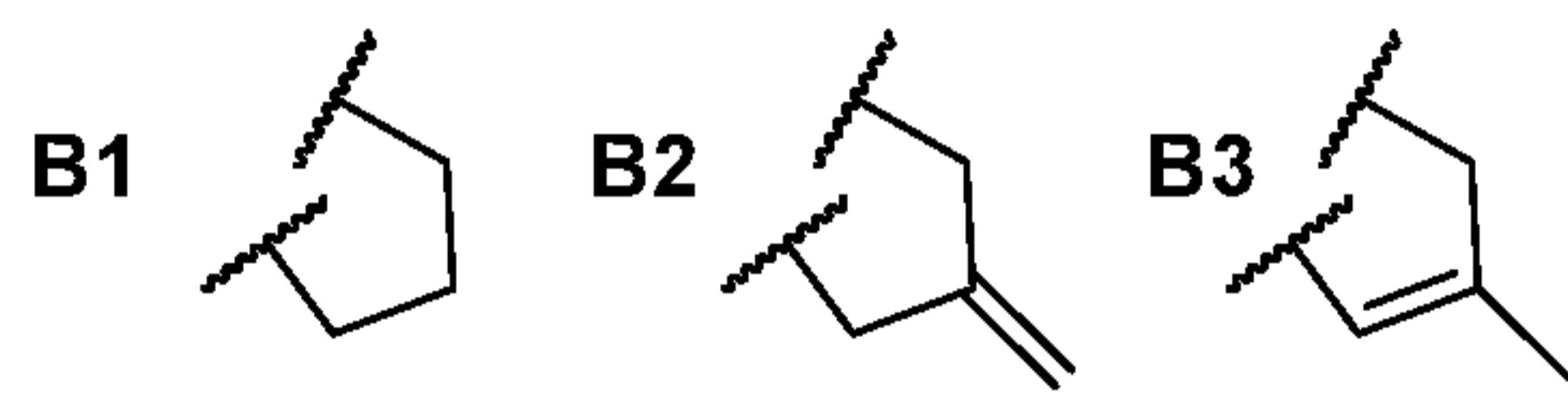
Y is  $(CH_2)_{7-12}$ ,  $(CH_2)_2(OCH_2CH_2)_{1-3}$ ,  $(CH_2)_{2-4}NH(CH_2)_{2-4}$  or

5  $(CH_2)_{2-4}NH((CH_2)_{0-1}phenyl)(CH_2)_{2-4}$  where the phenyl group is optionally substituted with  $NH_2$ ;

A is A1, A2, or A3

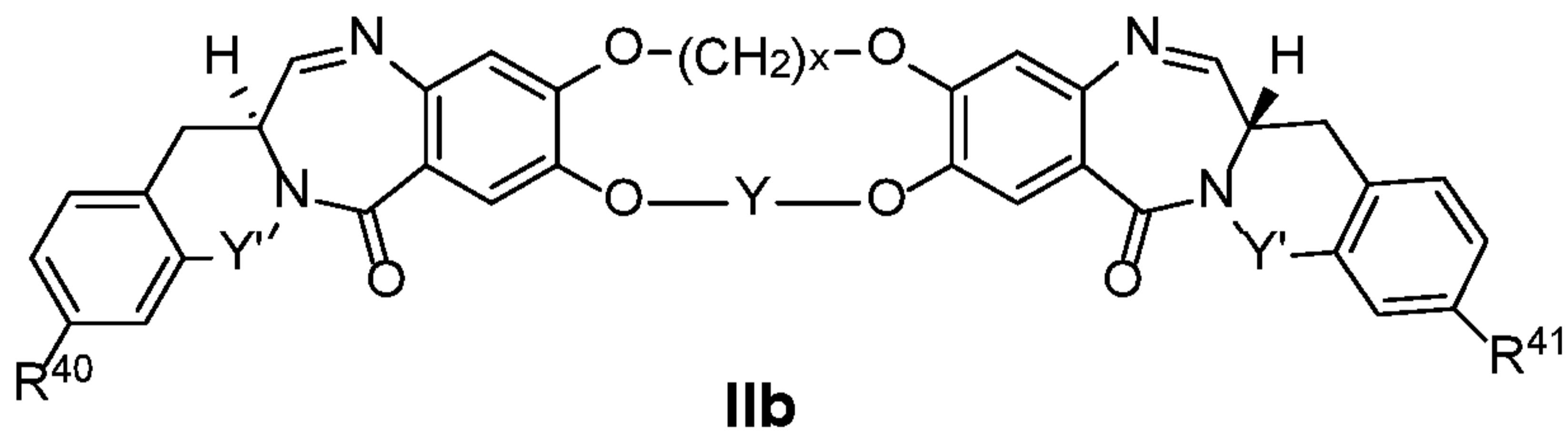


B is B1, B2, or B3



10

3. A benzodiazepine dimer according to claim 1, having a structure represented by formula IIb:



wherein

15 x is 3 or 5;

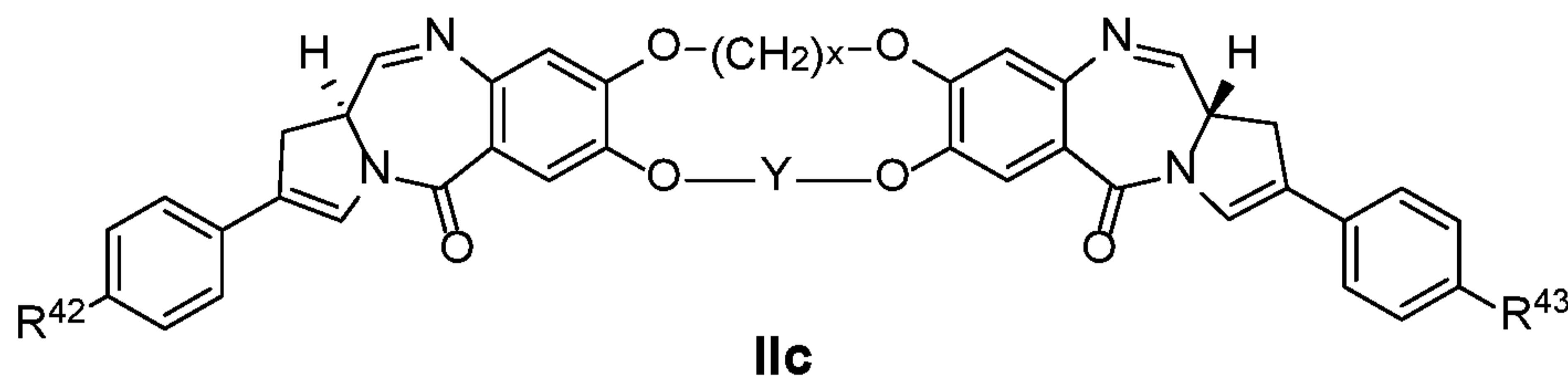
each Y' is independently absent or  $CH_2$ ;

Y is  $(CH_2)_{7-12}$ ,  $(CH_2)_2(OCH_2CH_2)_{1-3}$ ,  $(CH_2)_{2-4}NH(CH_2)_{2-4}$  or

$(CH_2)_{2-4}NH((CH_2)_{0-1}phenyl)(CH_2)_{2-4}$  where the phenyl group is optionally substituted with  $NH_2$ ; and

20 R<sup>40</sup> and R<sup>41</sup> are independently H, Cl, Br, OH, O(C<sub>1-3</sub> alkyl), NH<sub>2</sub>, or C<sub>1-3</sub> alkyl.

4. A benzodiazepine dimer according to claim 1, having a structure represented by formula IIc



wherein

5 x is 3 or 5;

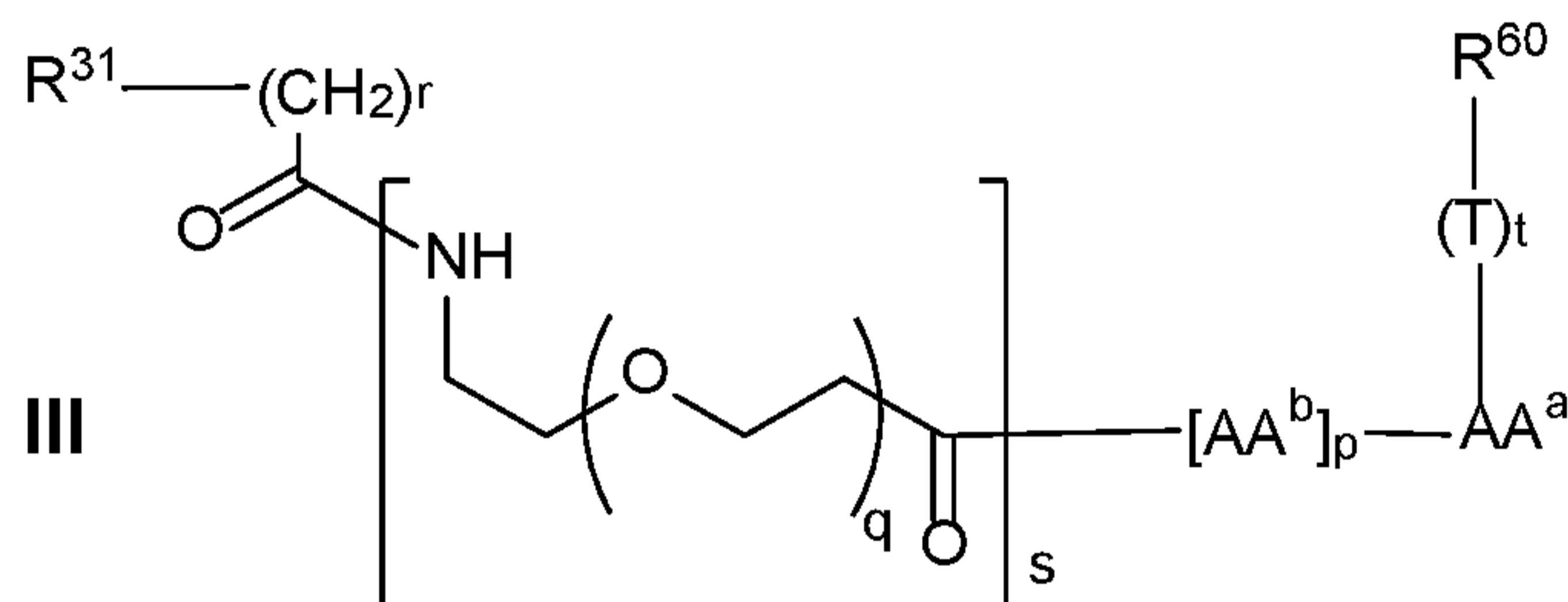
Y is  $(CH_2)_{7-12}$ ,  $(CH_2)_2(OCH_2CH_2)_{1-3}$ ,  $(CH_2)_{2-4}NH(CH_2)_{2-4}$  or

$(CH_2)_{2-4}NH((CH_2)_{0-1}phenyl)(CH_2)_{2-4}$  where the phenyl group is optionally substituted with  $NH_2$ ; and

10 R<sup>42</sup> and R<sup>43</sup> are independently H, OMe, NH<sub>2</sub>, OCH<sub>2</sub>CH<sub>2</sub>OMe, N(CH<sub>2</sub>CH<sub>2</sub>)O, N(CH<sub>2</sub>CH<sub>2</sub>)NMe, or N(CH<sub>2</sub>CH<sub>2</sub>)NH.

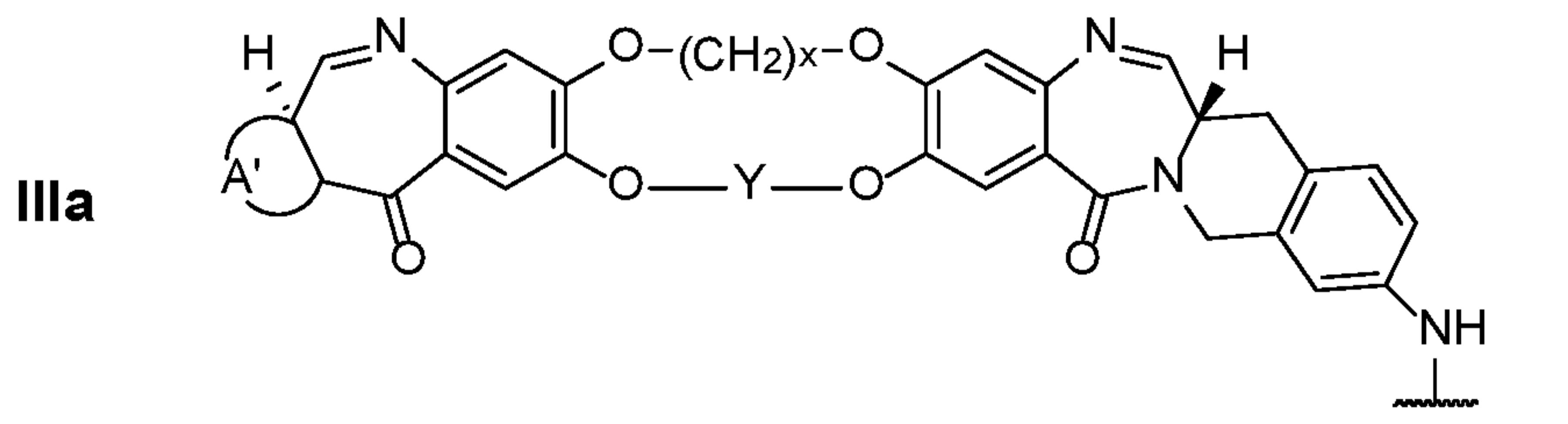
5. A benzodiazepine dimer according to claim 1, having a structure represented by formula IIb-6, IIc-8, IIc-9, IIc-10, IIc-11, IId-1, IId-2, or IId-3.

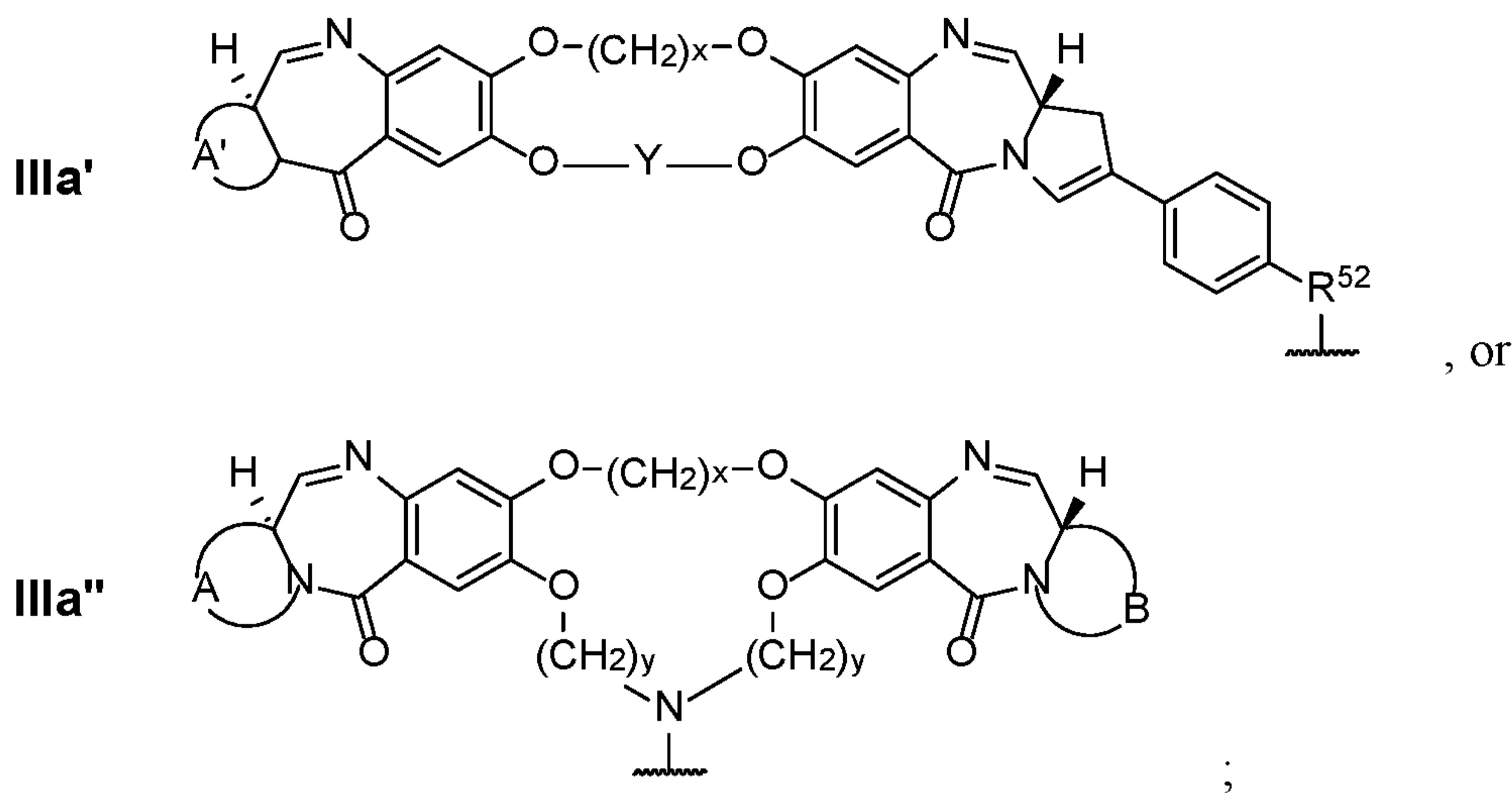
6. A benzodiazepine dimer-linker compound having a structure represented by formula III:



15 wherein

R<sup>60</sup> is according to formula IIIa, IIIa', or IIIa''



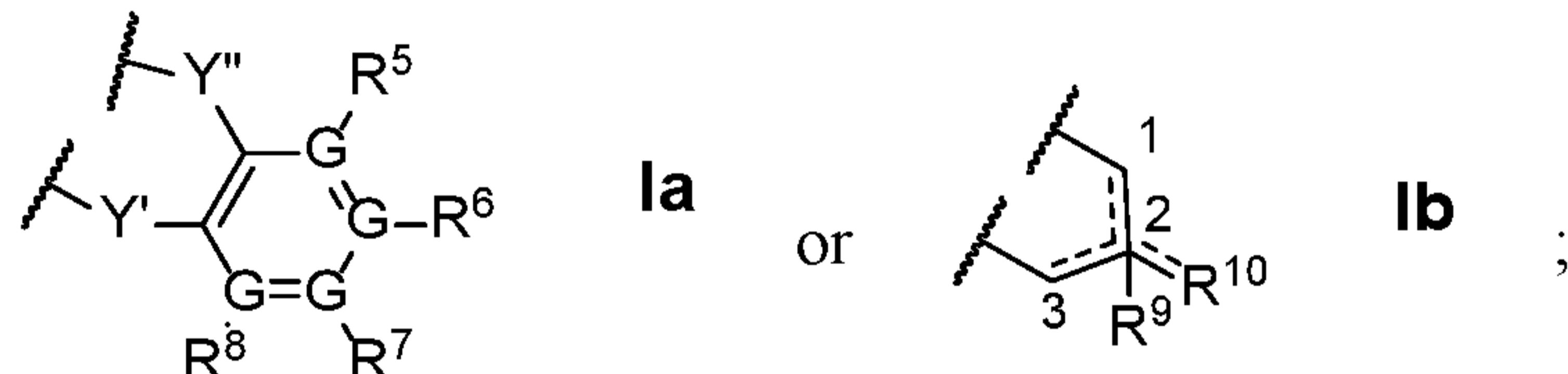


Y is  $(CH_2)_{6-10}$ ;

5 x is 3 or 5;

each y is independently 2, 3, or 4;

A and B are independently according to formula Ia or Ib



wherein, in formula Ia

10 Y' and Y'' are independently absent,  $CH_2$ ,  $C=O$ , or  $CHR^{12}$ ; wherein each  $R^{12}$  is independently F, Cl, Br, or  $C_1-C_3$  alkyl, with the proviso that Y' and Y'' are not both absent; each G is independently C or N, with the proviso that no more than two Gs are N; and each  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$  is independently H, Cl, Br,  $C_{1-3}$  alkyl,  $NO_2$ , CN,  $NH_2$ ,  $O(C_{1-3}$  alkyl), or  $(OCH_2CH_2)_{1-2}O(C_{1-3}$  alkyl); or where a  $R^5$ ,  $R^6$ ,  $R^7$ , or  $R^8$  is attached to a G that is N, such  $R^5$ ,  $R^6$ ,  $R^7$ , or  $R^8$  is absent;

15

alkyl), or  $(OCH_2CH_2)_{1-2}O(C_{1-3}$  alkyl);

or where a  $R^5$ ,  $R^6$ ,  $R^7$ , or  $R^8$  is attached to a G that is N, such  $R^5$ ,  $R^6$ ,  $R^7$ , or  $R^8$  is absent;

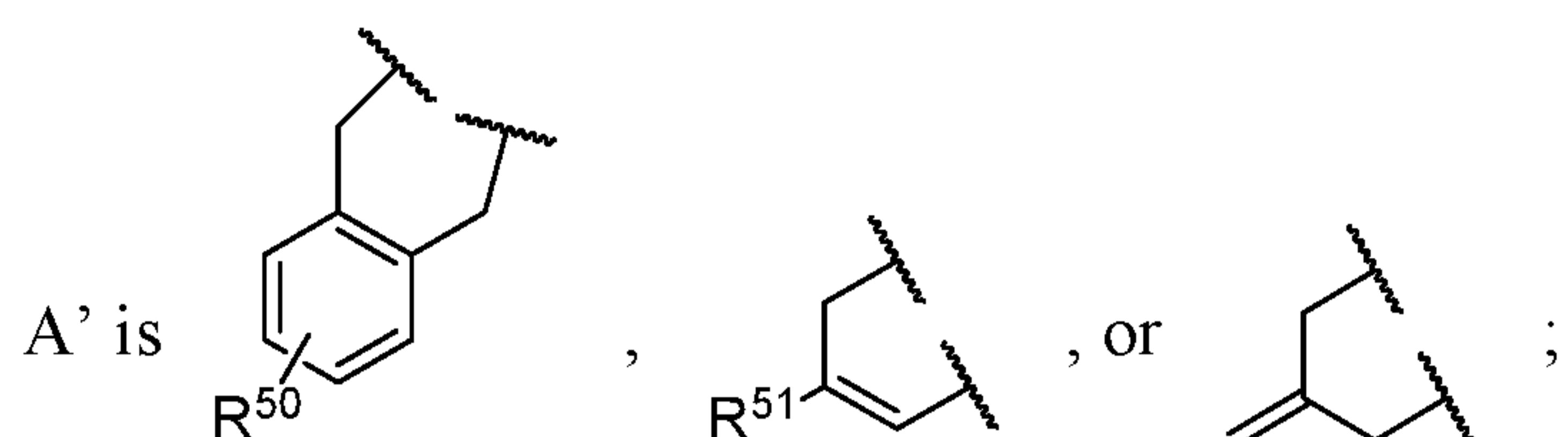
and

wherein, in formula Ib,

20 the dotted lines indicate the optional presence of a  $C_1-C_2$ ,  $C_2-C_3$ , or  $C_2-R^{10}$  double bond;

$R^9$  is absent if a  $C_1-C_2$ ,  $C_2-C_3$ , or  $C_2-R^{10}$  double bond is present and otherwise is H;

$R^{10}$  is H, Cl, Br,  $=CH_2$ ,  $=CH(C_{1-5}$  alkyl),  $C_{1-3}$  alkyl,  $NO_2$ , CN, or  $NH_2$ ;



R<sup>50</sup> is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl) (preferably H);

R<sup>51</sup> is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, or NH<sub>2</sub>;

5 T is a self-immolating group;

t is 0 or 1;

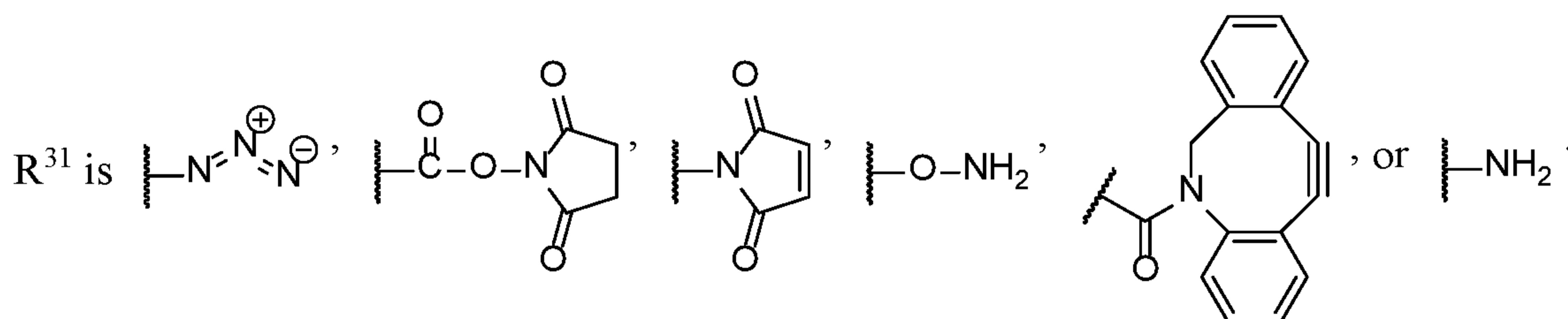
AA<sup>a</sup> and each AA<sup>b</sup> are independently selected from the group consisting of alanine,  $\beta$ -alanine,  $\gamma$ -aminobutyric acid, arginine, asparagine, aspartic acid,  $\gamma$ -carboxyglutamic acid, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, 10 lysine, methionine, norleucine, norvaline, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine;

p is 1, 2, 3, or 4;

q is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

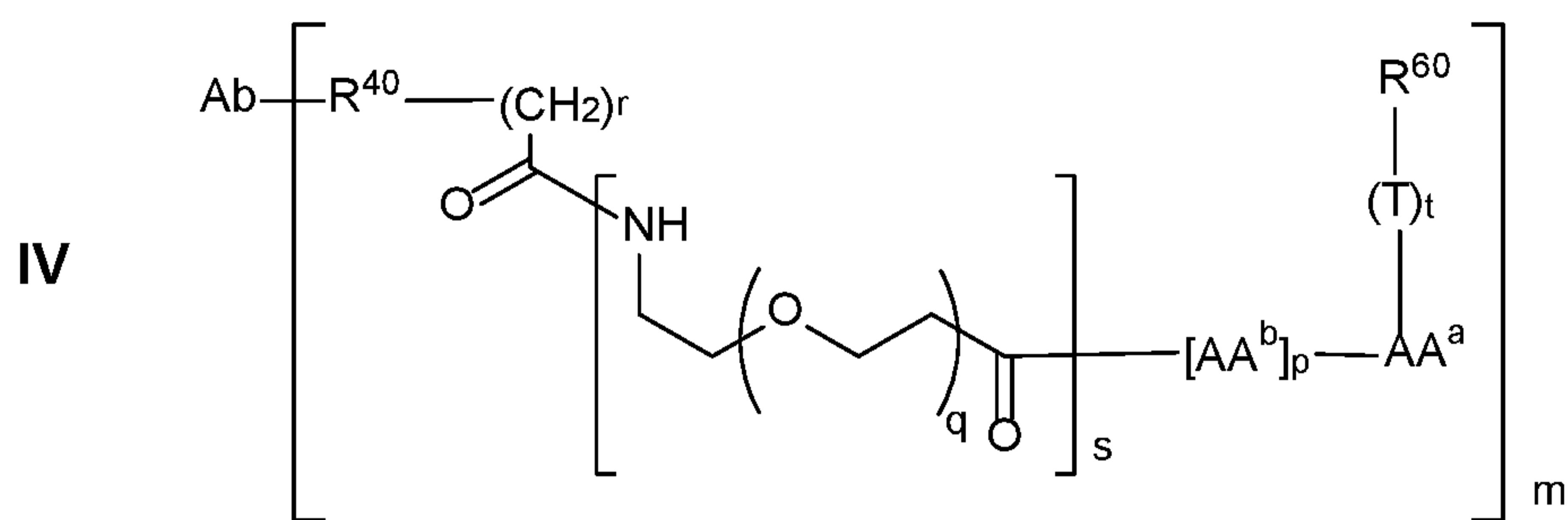
r is 1, 2, 3, 4, or 5;

15 s is 0 or 1; and



7. A benzodiazepine dimer-linker compound according to claim 6, wherein R<sup>60</sup> is IIIa.
8. A benzodiazepine dimer-linker compound according to claim 6, wherein R<sup>60</sup> is IIIa'.
9. A benzodiazepine dimer-linker compound according to claim 6, wherein R<sup>60</sup> is IIIa''.
- 20 10. A benzodiazepine dimer linker compound according to claim 6, having a structure represented by formula IIIa-1, IIIa-2, IIIa-3, IIIa-4, IIIa-5, IIIa-6, or IIIa-7.
11. A conjugate comprising a benzodiazepine dimer according to claim 1 conjugated to an antibody.

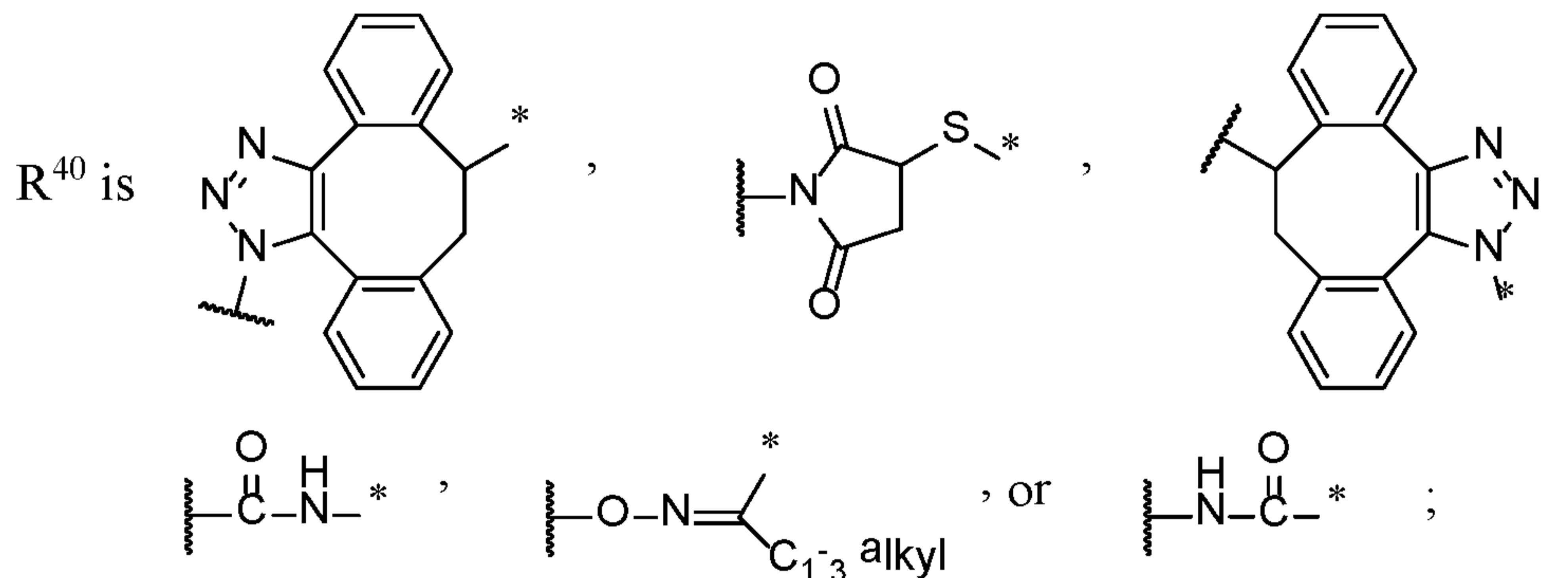
12. A conjugate according to claim 11, having a structure represented by formula IV:



wherein

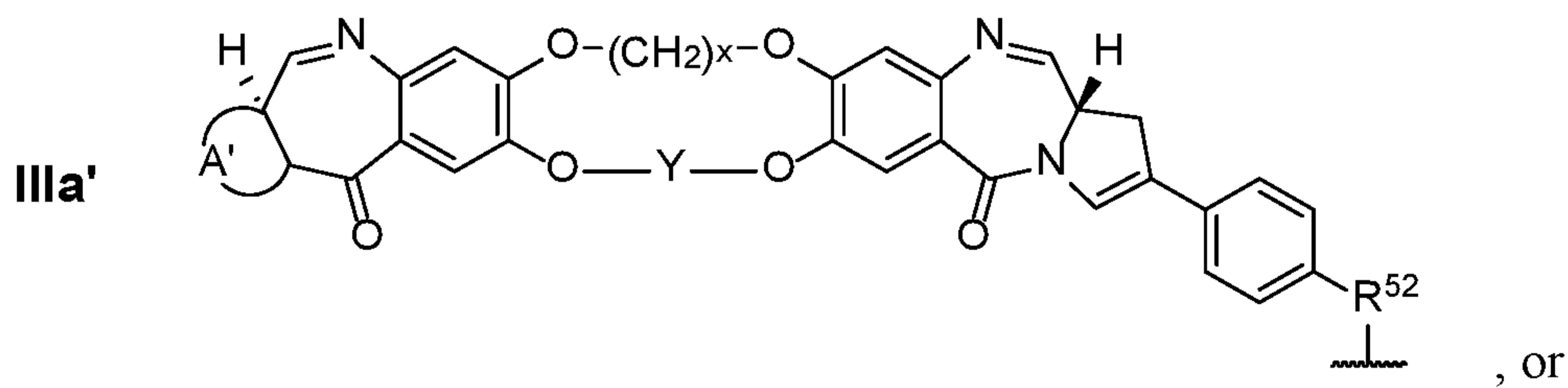
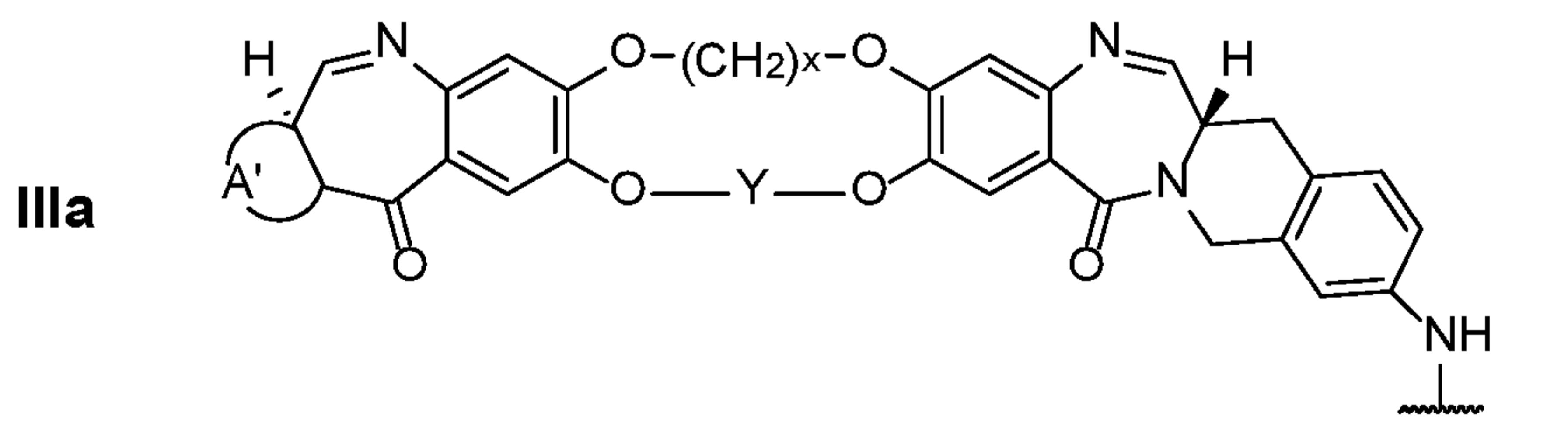
Ab is an antibody;

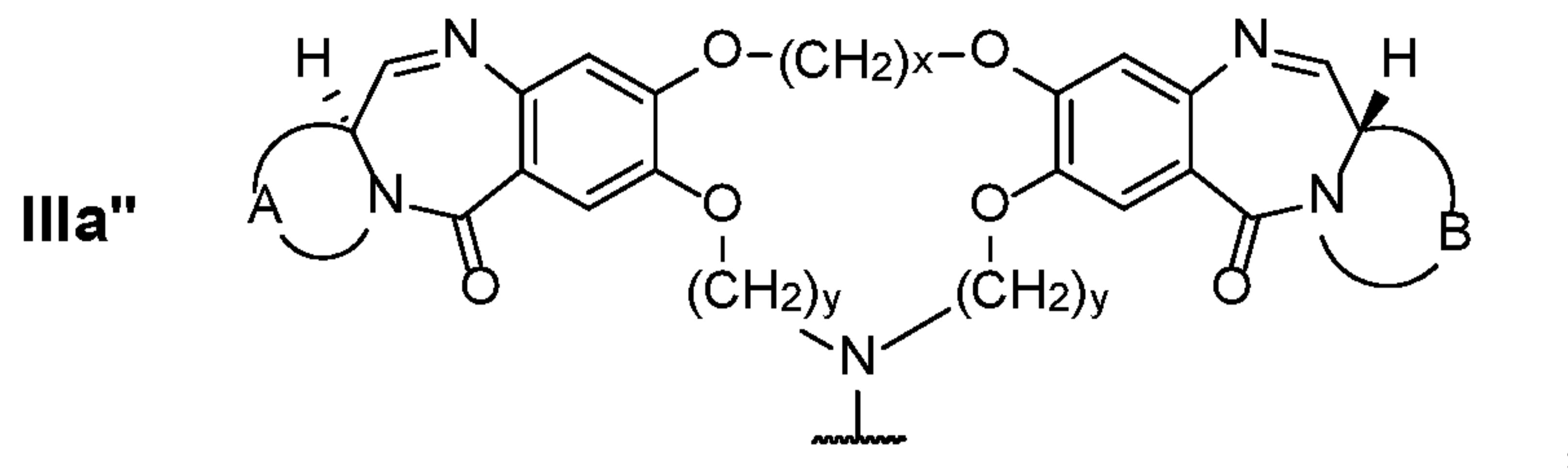
5 m is 1, 2, 3, or 4;



where the open valence of R<sup>40</sup> that is bonded to Ab is denoted by an asterisk (\*) and the open valence of R<sup>40</sup> that is bonded to (CH<sub>2</sub>)<sub>r</sub> is denoted by a wavy line (----);

10 R<sup>60</sup> is according to formula IIIa, IIIa', or IIIa"



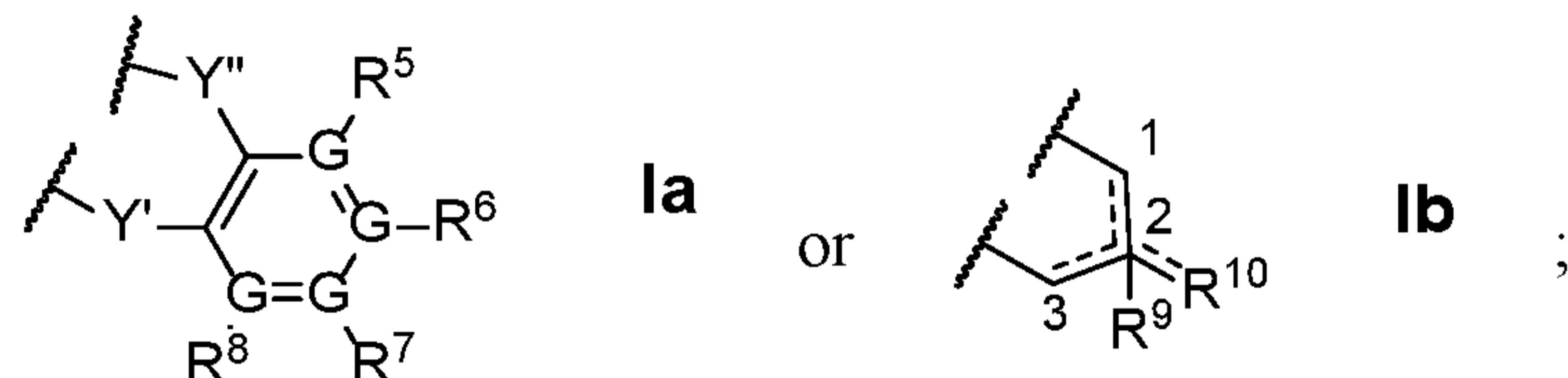


Y is (CH<sub>2</sub>)<sub>6-10</sub>;

x is 3 or 5;

each y is independently 2, 3, or 4;

5 A and B are independently according to formula Ia or Ib



wherein, in formula Ia

Y' and Y" are independently absent, CH<sub>2</sub>, C=O, or CHR<sup>12</sup>; wherein each R<sup>12</sup> is independently F, Cl, Br, or C<sub>1</sub>-C<sub>3</sub> alkyl, with the proviso that Y' and Y" are not both absent;

each G is independently C or N, with the proviso that no more than two Gs are N; and each R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, and R<sup>8</sup> is independently H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl) (preferably H);

or where a R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is attached to a G that is N, such R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, or R<sup>8</sup> is absent;

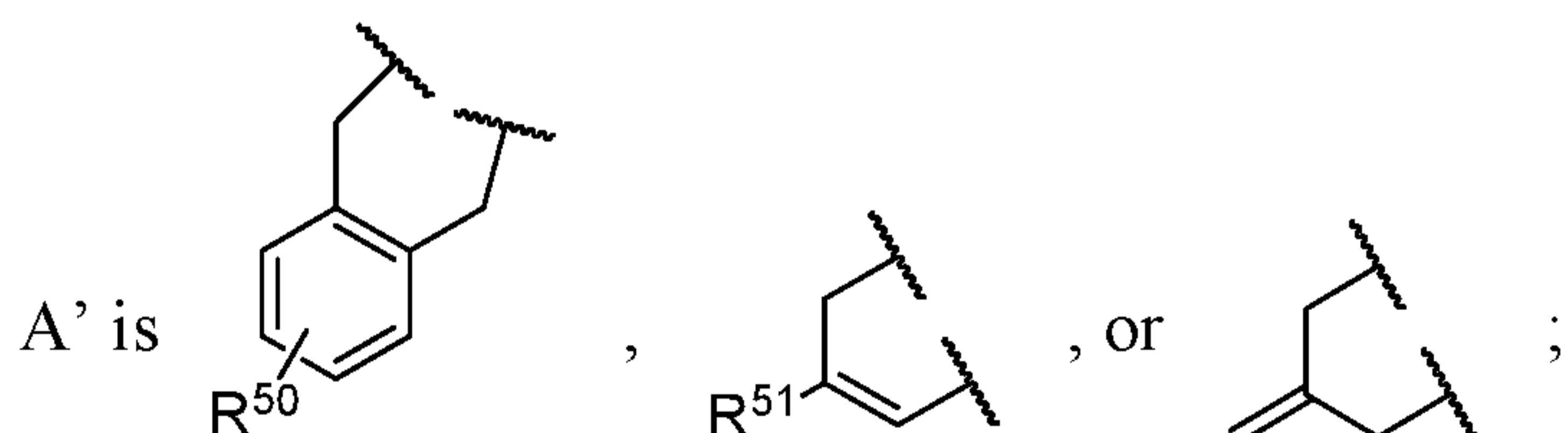
and

wherein, in formula Ib,

the dotted lines indicate the optional presence of a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond;

20 R<sup>9</sup> is absent if a C1-C2, C2-C3, or C2-R<sup>10</sup> double bond is present and otherwise is H;

R<sup>10</sup> is H, Cl, Br, =CH<sub>2</sub>, =CH(C<sub>1-5</sub> alkyl), C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, or NH<sub>2</sub>;



R<sup>50</sup> is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>, O(C<sub>1-3</sub> alkyl), or (OCH<sub>2</sub>CH<sub>2</sub>)<sub>1-2</sub>O(C<sub>1-3</sub> alkyl);

$R^{51}$  is H, Cl, Br, C<sub>1-3</sub> alkyl, NO<sub>2</sub>, CN, NH<sub>2</sub>;

T is a self-immolating group;

t is 0 or 1;

AA<sup>a</sup> and each AA<sup>b</sup> are independently selected from the group consisting of alanine,  $\beta$ -alanine,

5  $\gamma$ -aminobutyric acid, arginine, asparagine, aspartic acid,  $\gamma$ -carboxyglutamic acid, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, norvaline, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine;

p is 1, 2, 3, or 4;

10 q is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

r is 1, 2, 3, 4, or 5; and

$s$  is 0 or 1.

13. A conjugate according to claim 12, wherein R<sup>60</sup> is IIIa.

14. A conjugate according to claim 12, wherein R<sup>60</sup> is IIIa'.

15 15. A conjugate according to claim 12, wherein R<sup>60</sup> is IIIa".

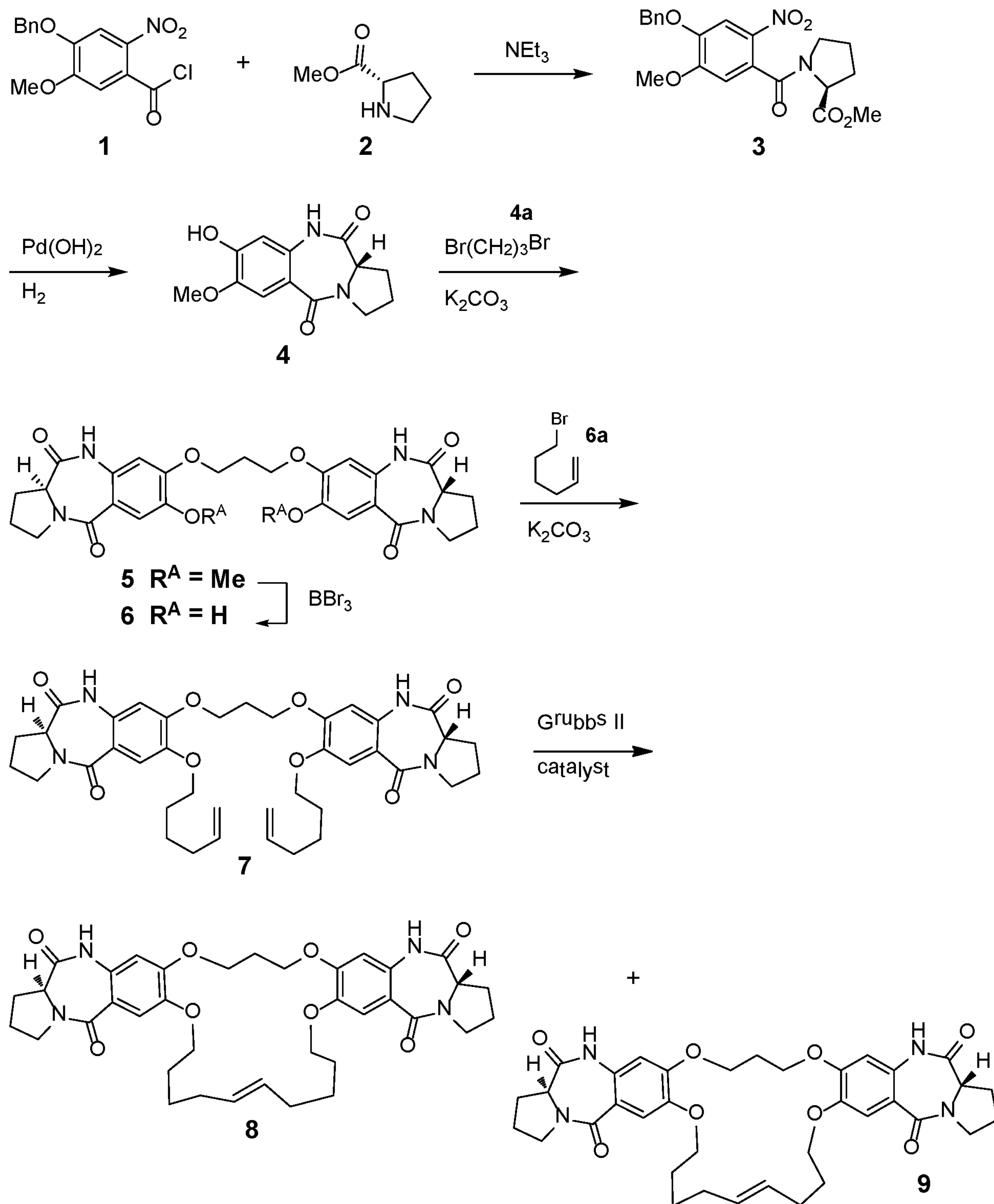
16. A pharmaceutical formulation comprising a conjugate according to claim 11 or 12 and a pharmaceutically acceptable excipient.

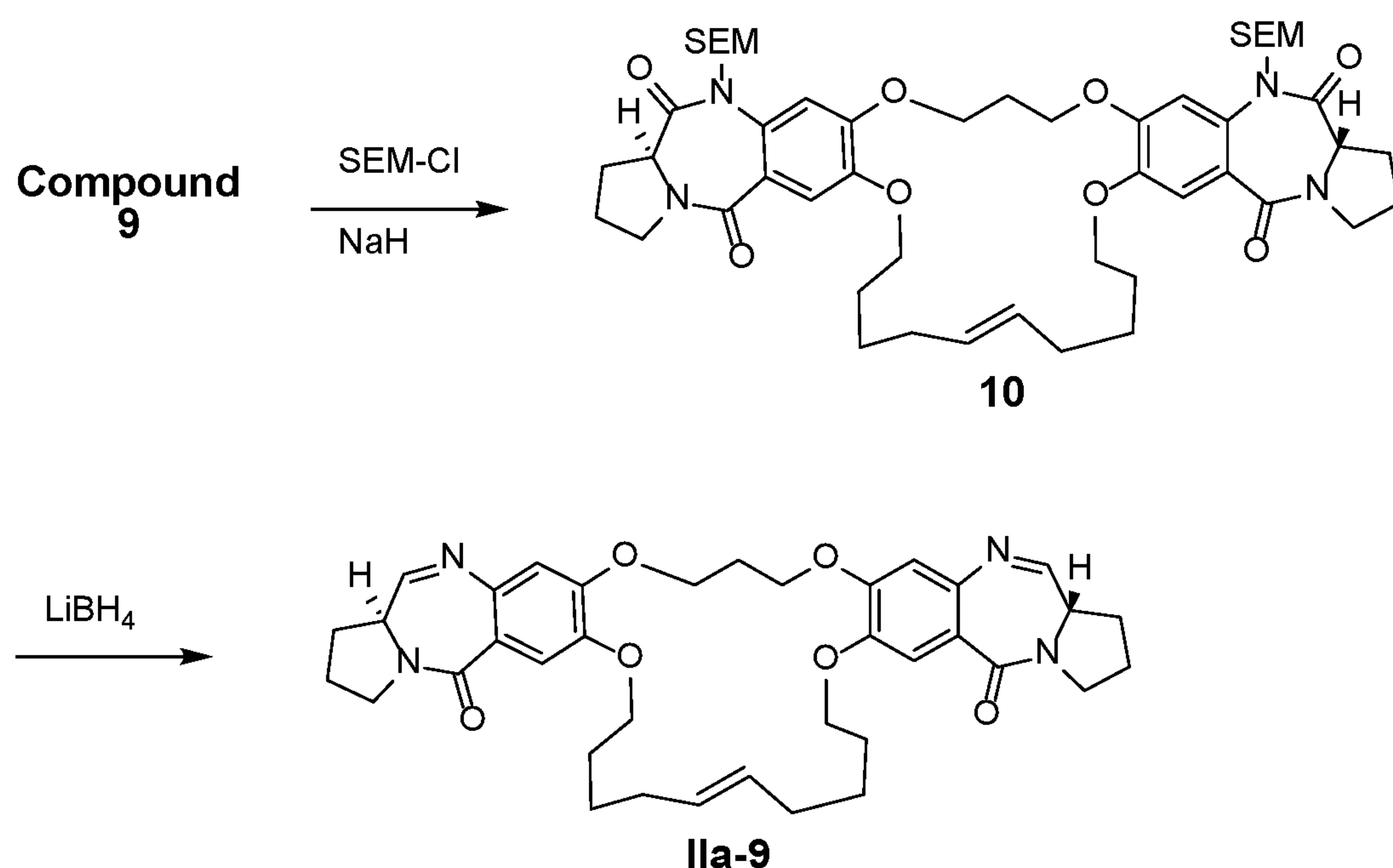
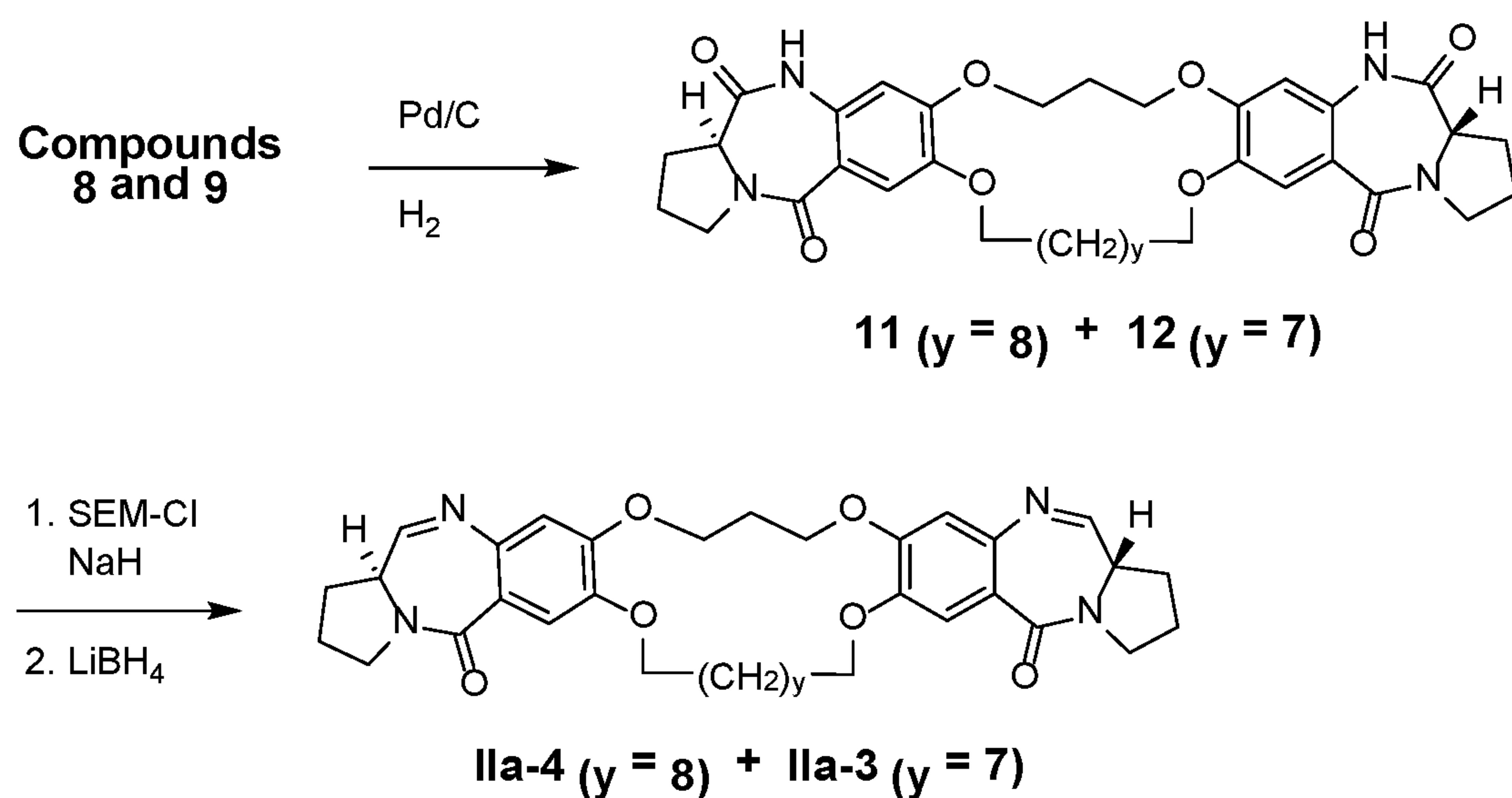
17. A method of treating a cancer in a subject suffering from such cancer, comprising  
administering to the subject a therapeutically effective amount of a benzodiazepine dimer  
according to claim 1, or a conjugate thereof according to claim 11 or 12.

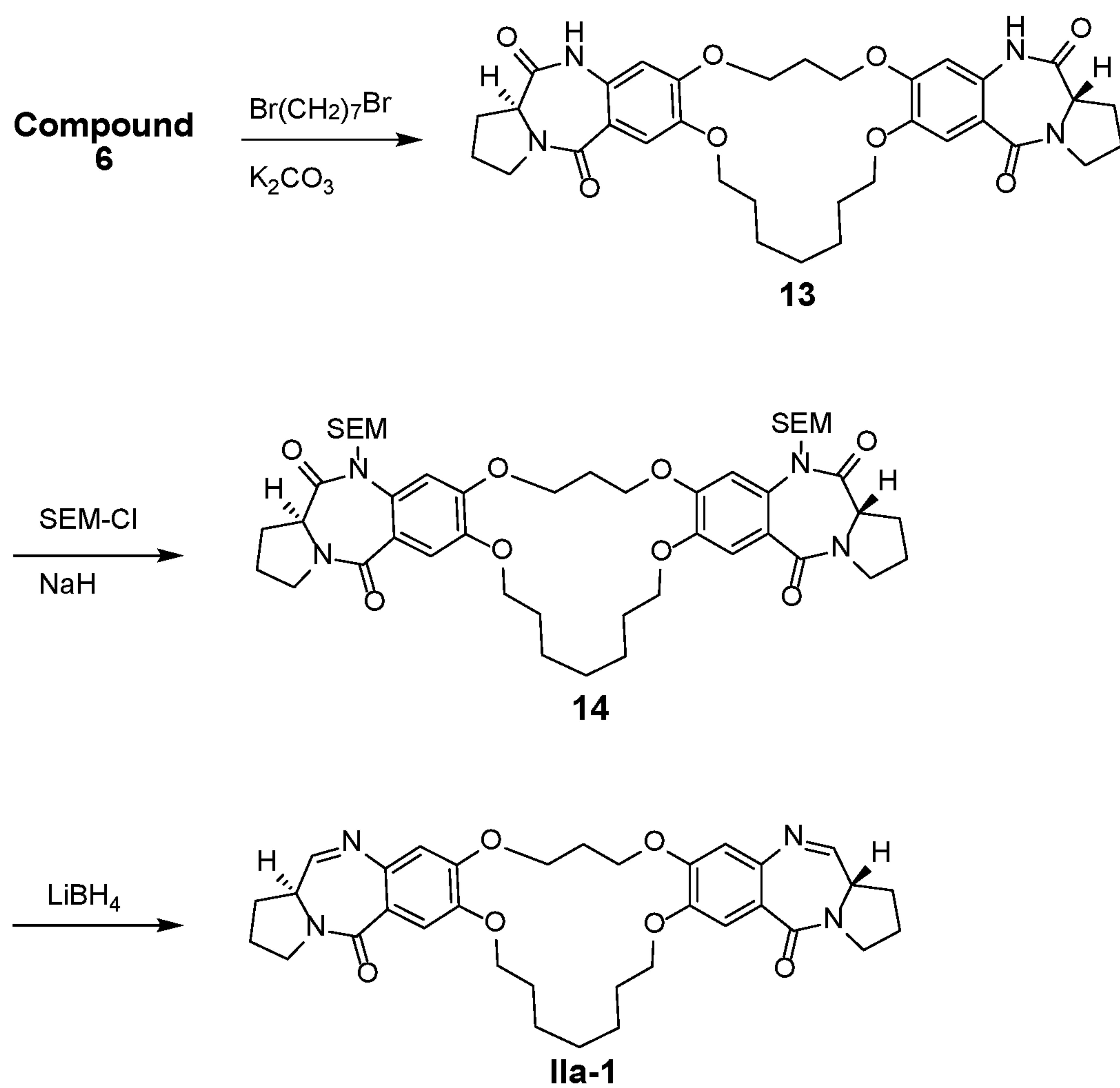
18. A method according to claim 17, wherein a conjugate of a benzodiazepine dimer according to claim 12 is administered.

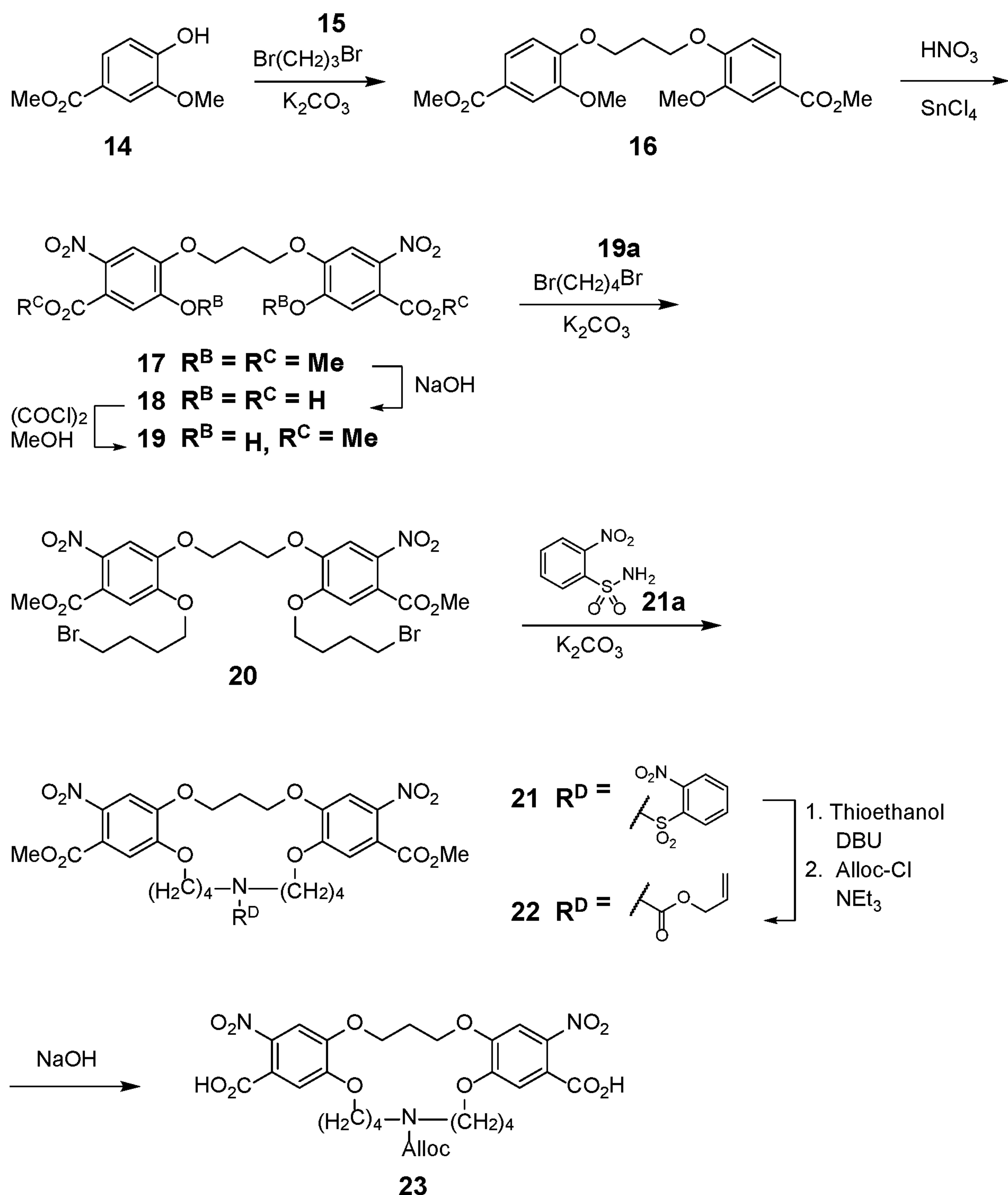
19. A method according to claim 18, wherein the cancer is lung, gastric or ovarian cancer.

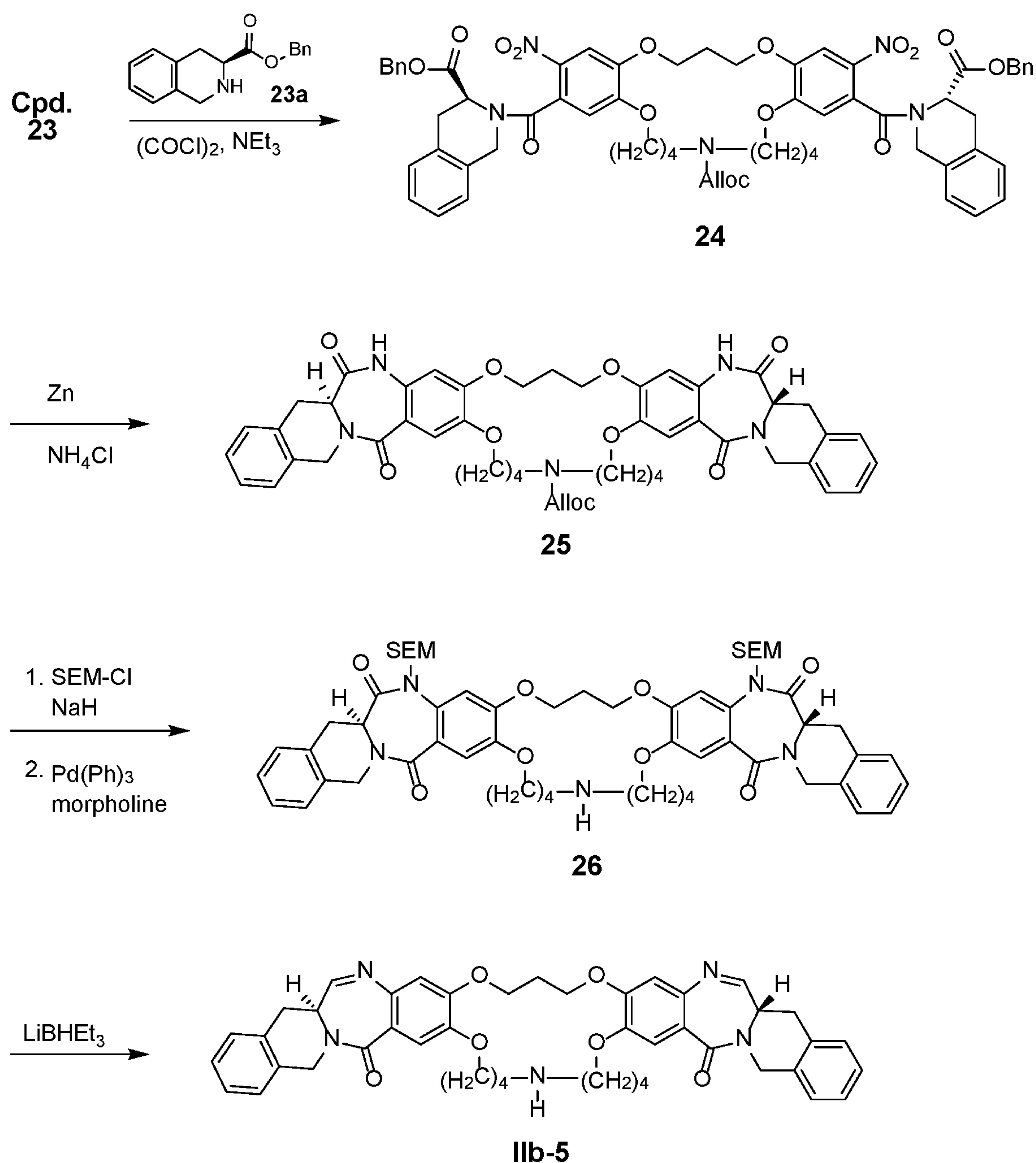
\* \* \* \* \*

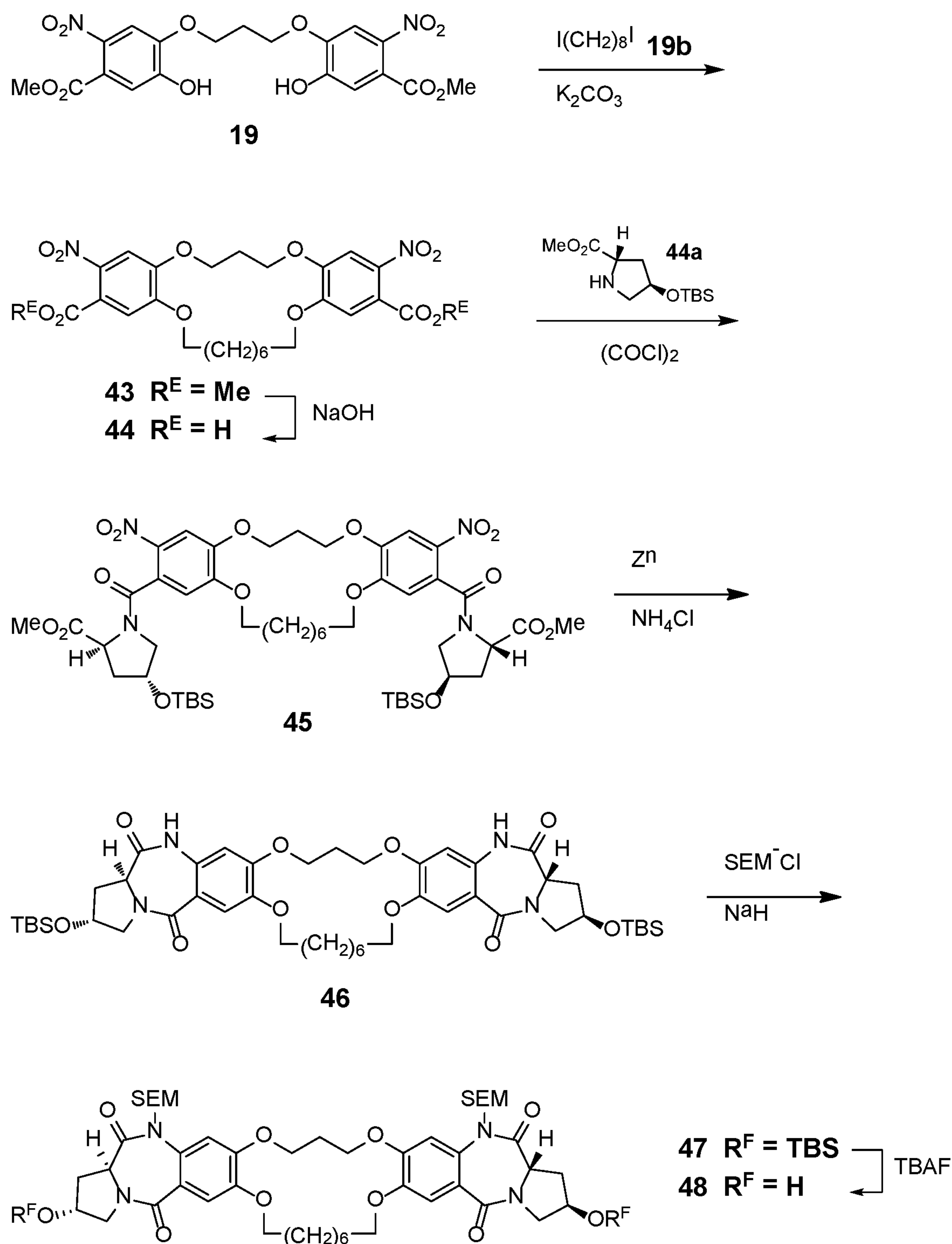
**FIG. 1A**

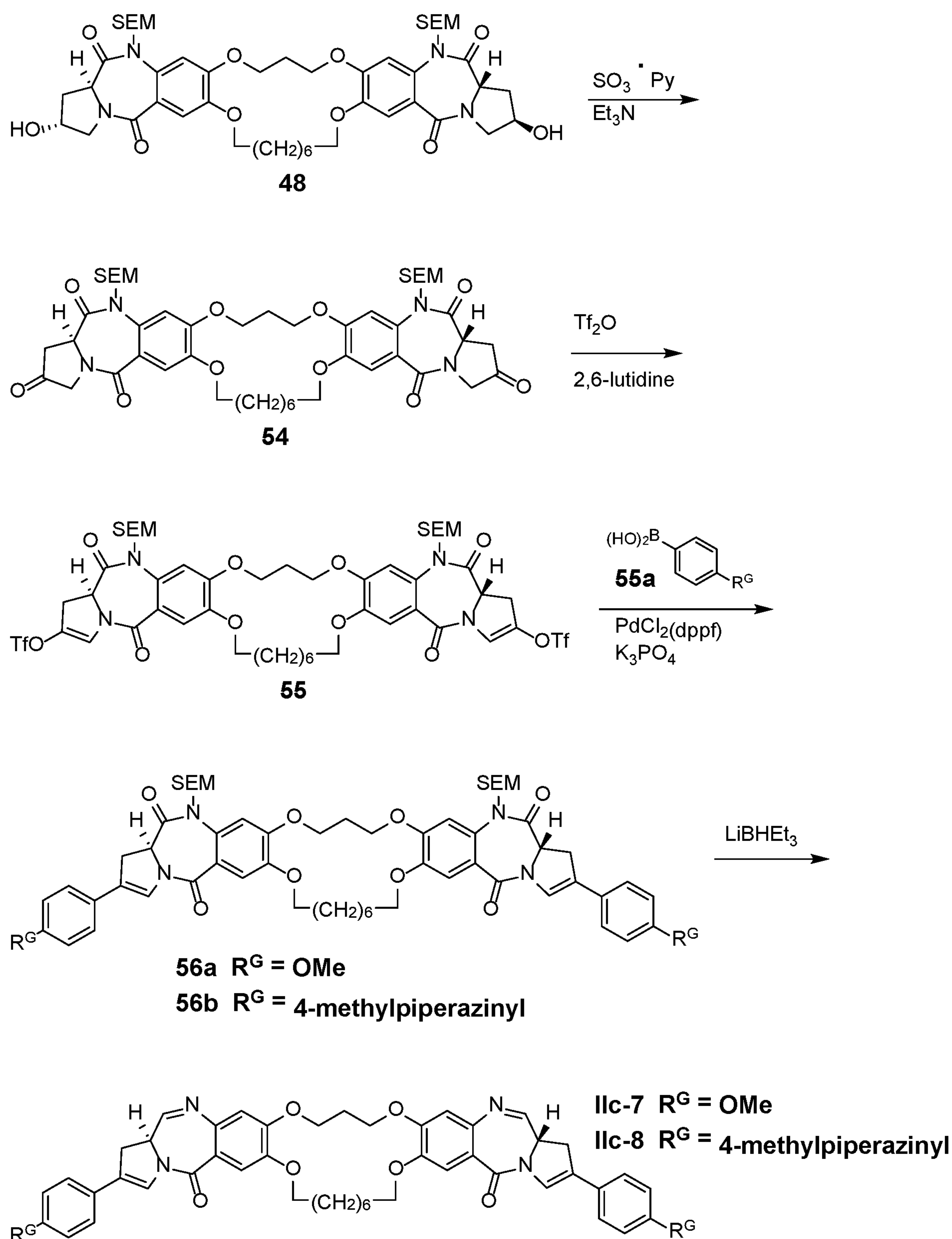
**FIG. 1B****FIG. 2**

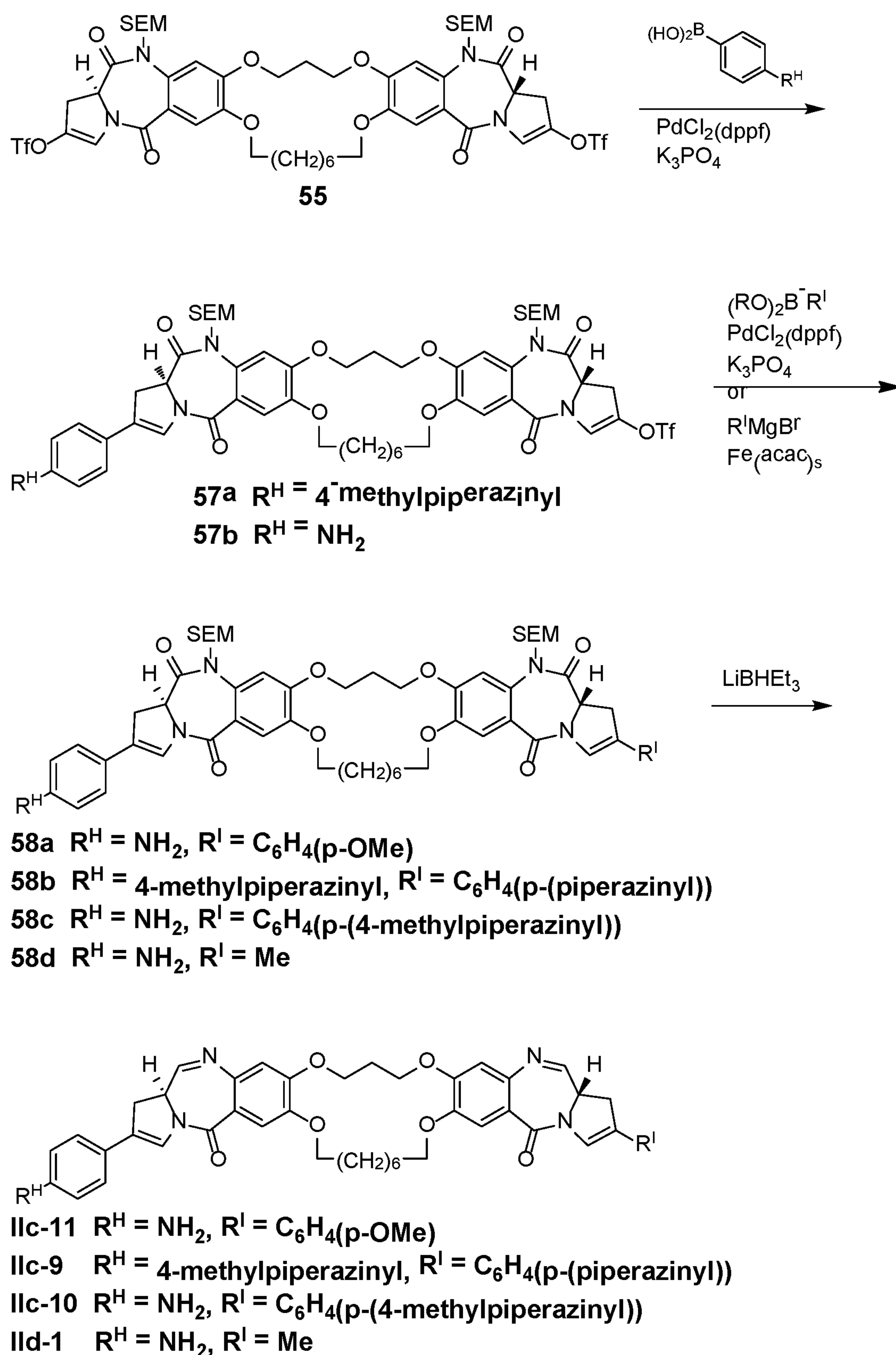
**FIG. 3**

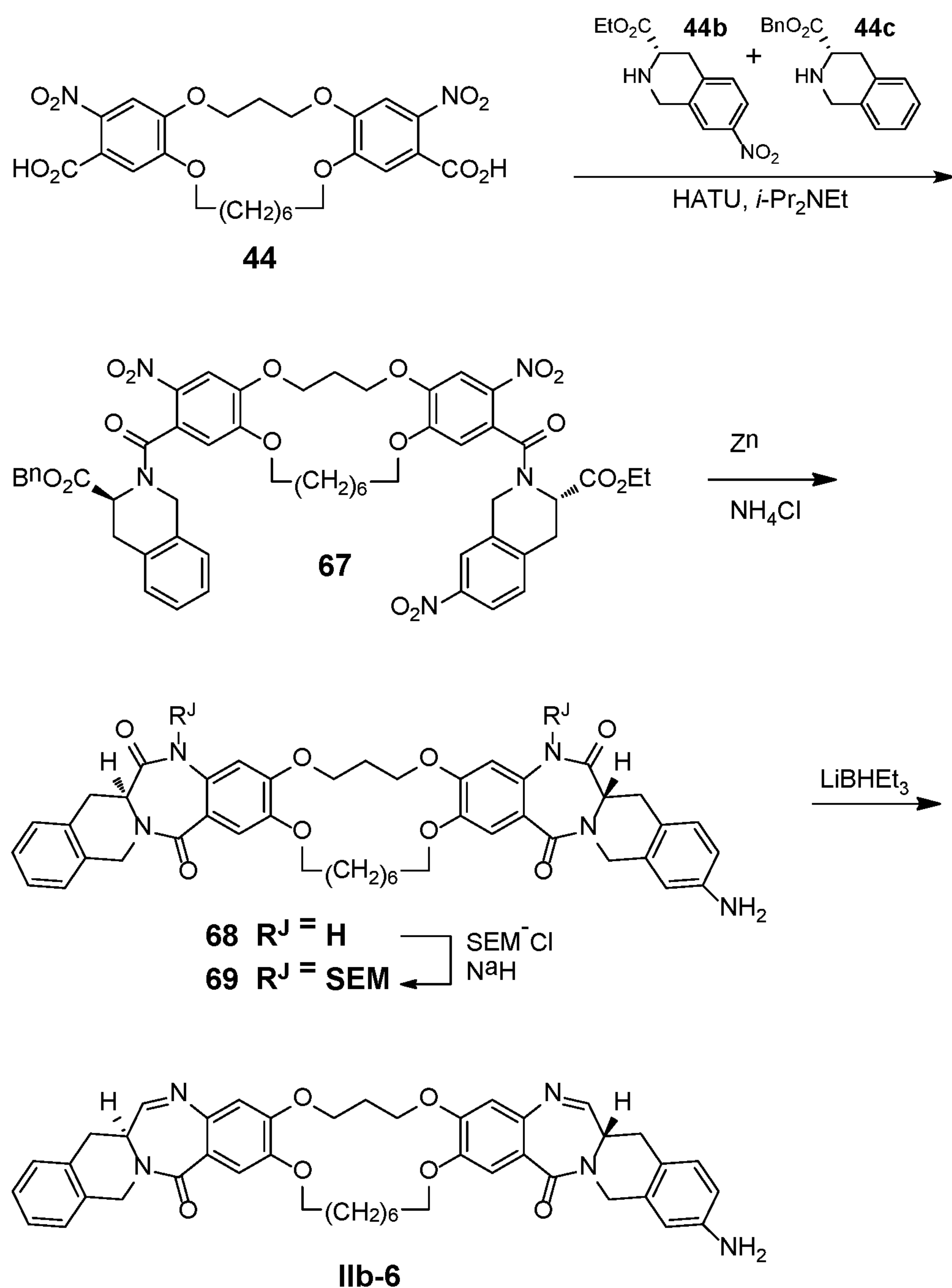
**FIG. 4A**

**FIG. 4B**

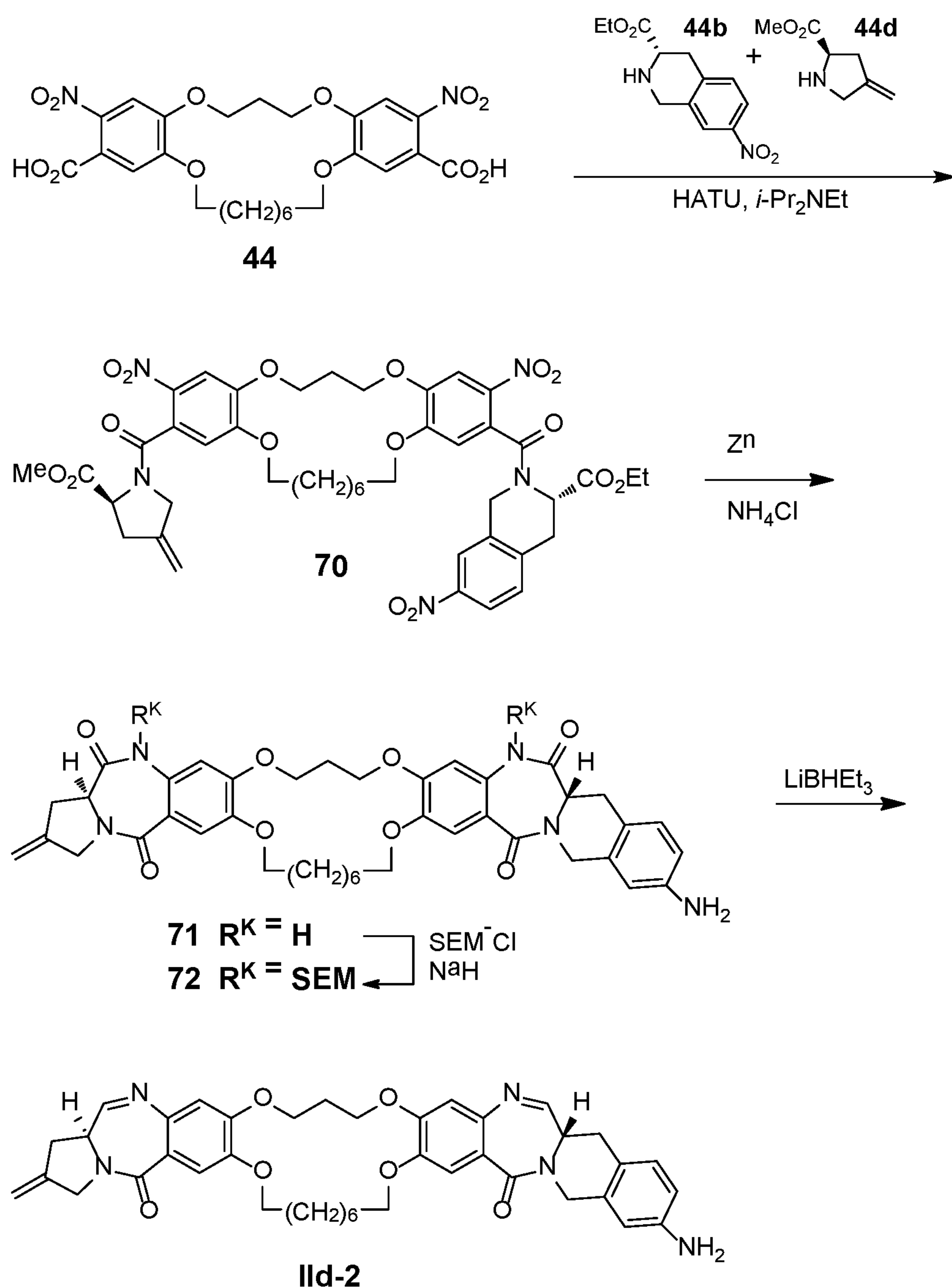
**FIG. 5**

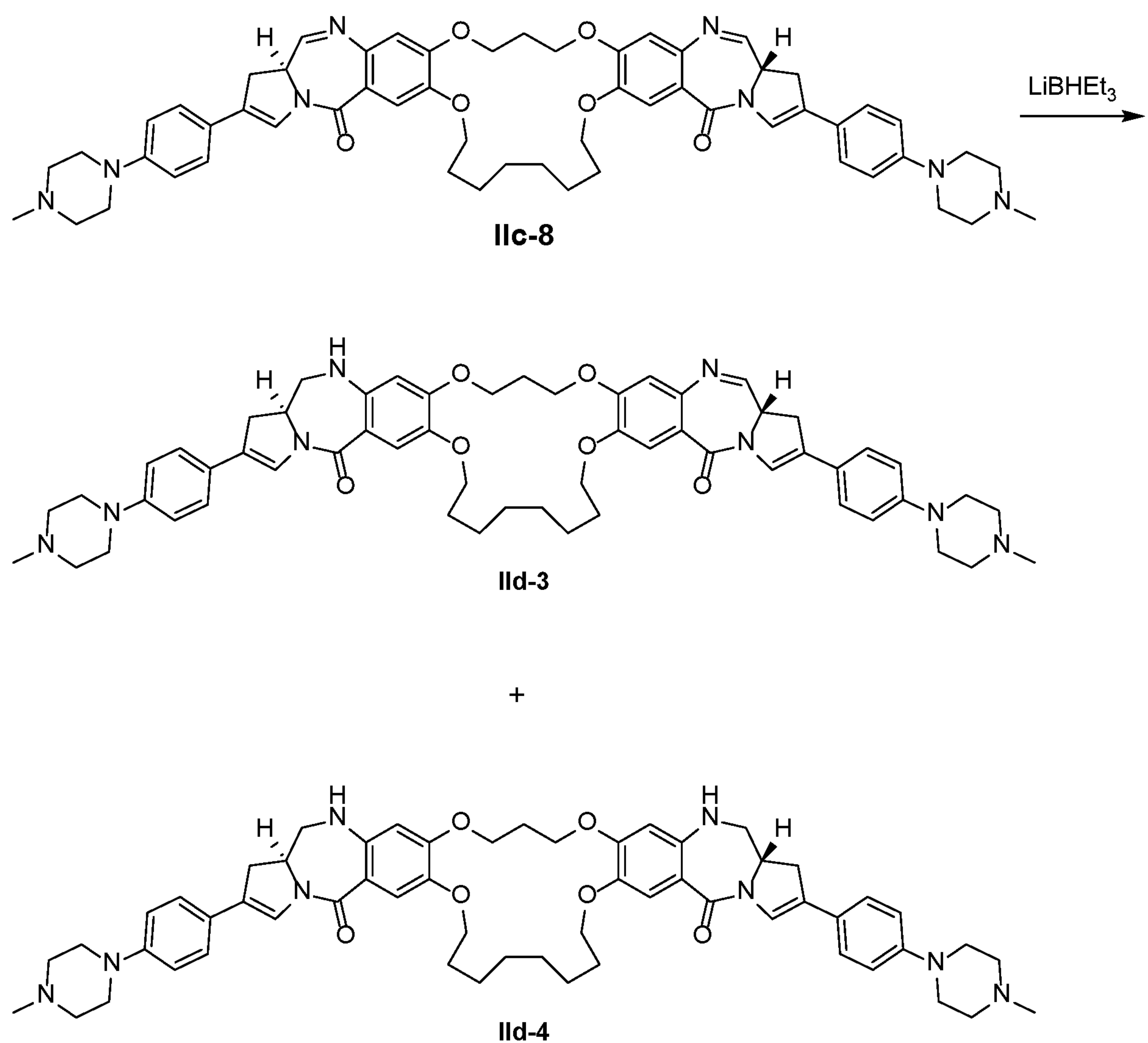
**FIG. 6**

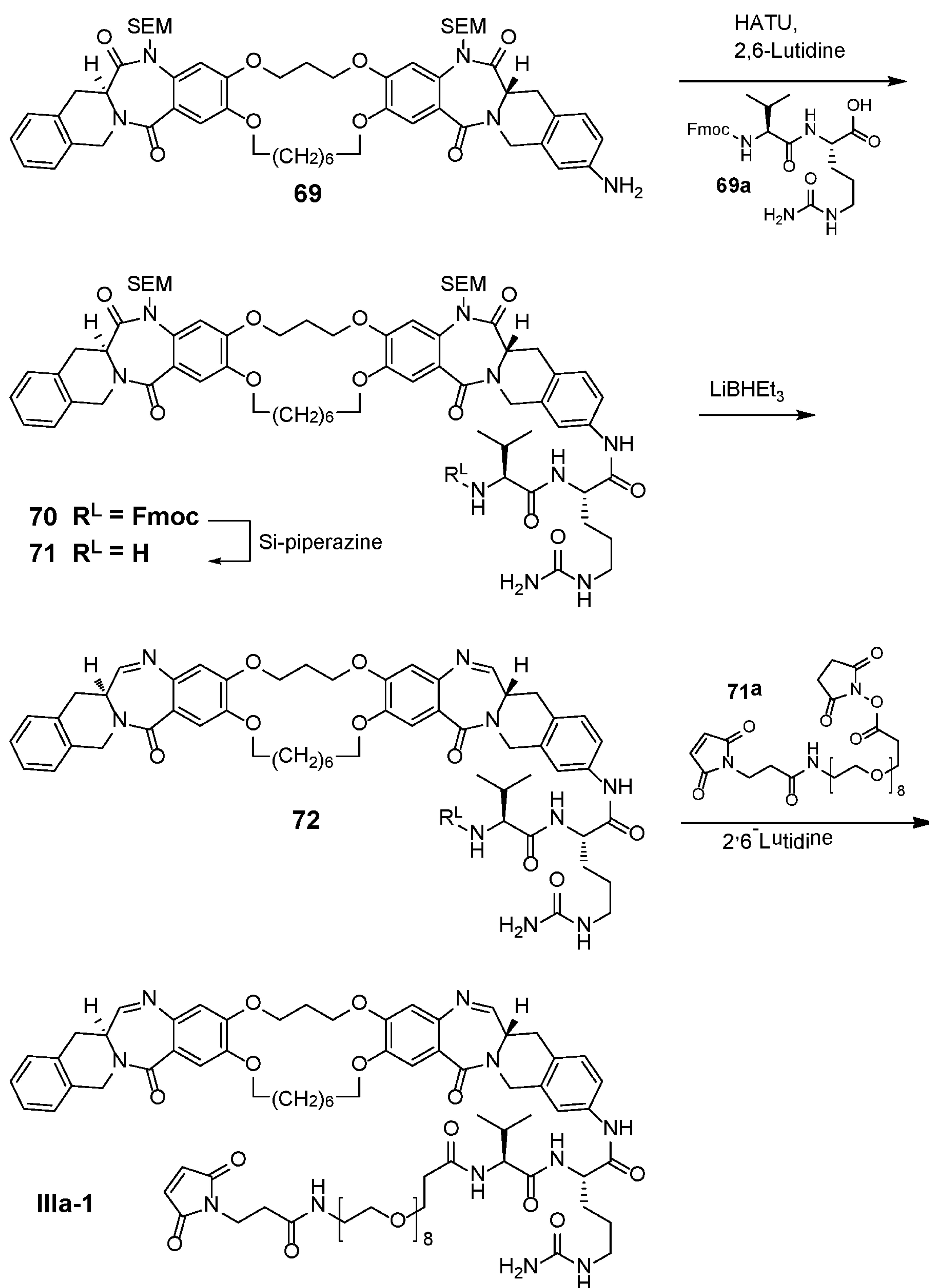
**FIG. 7**

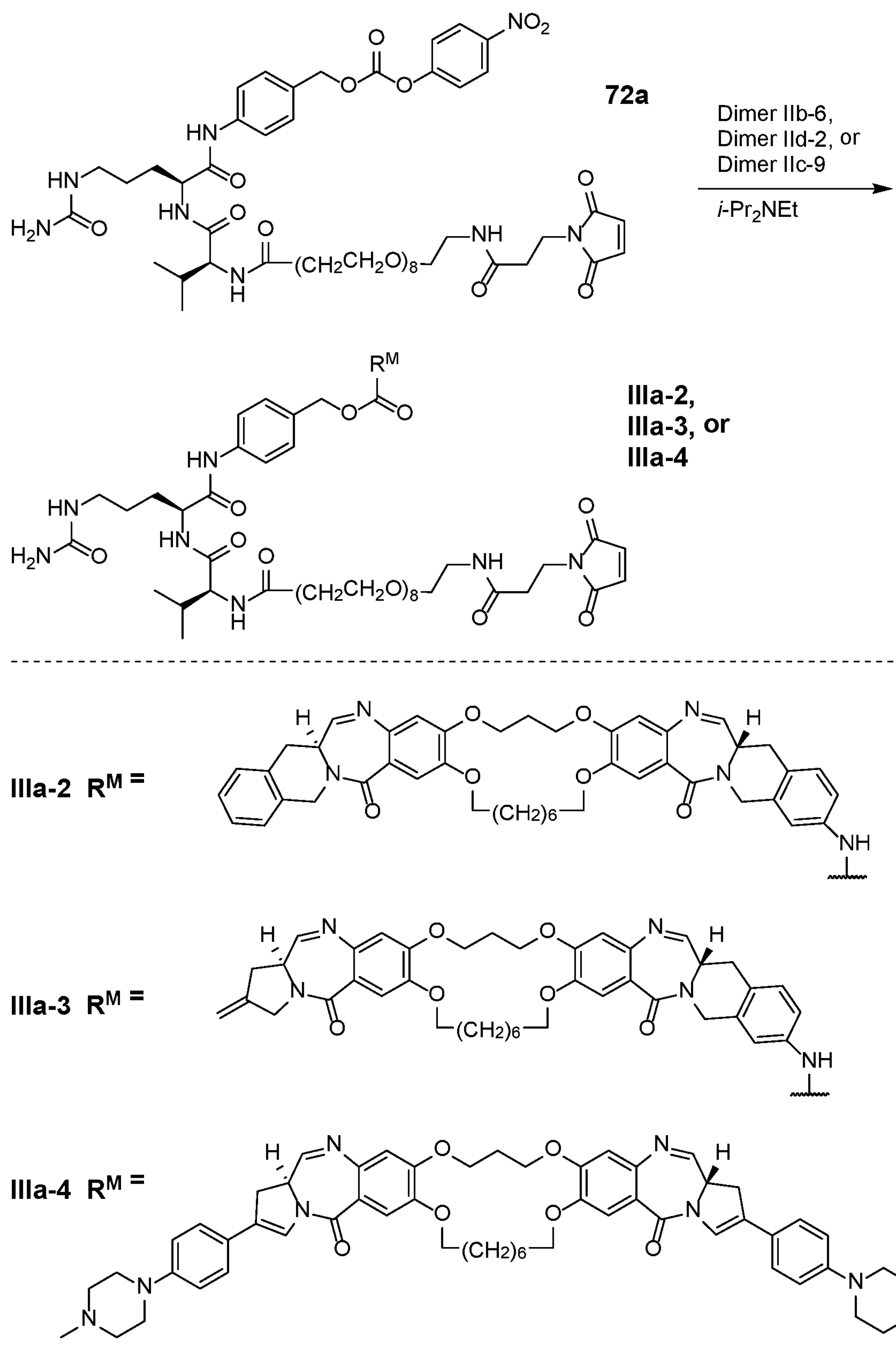
**FIG. 8**

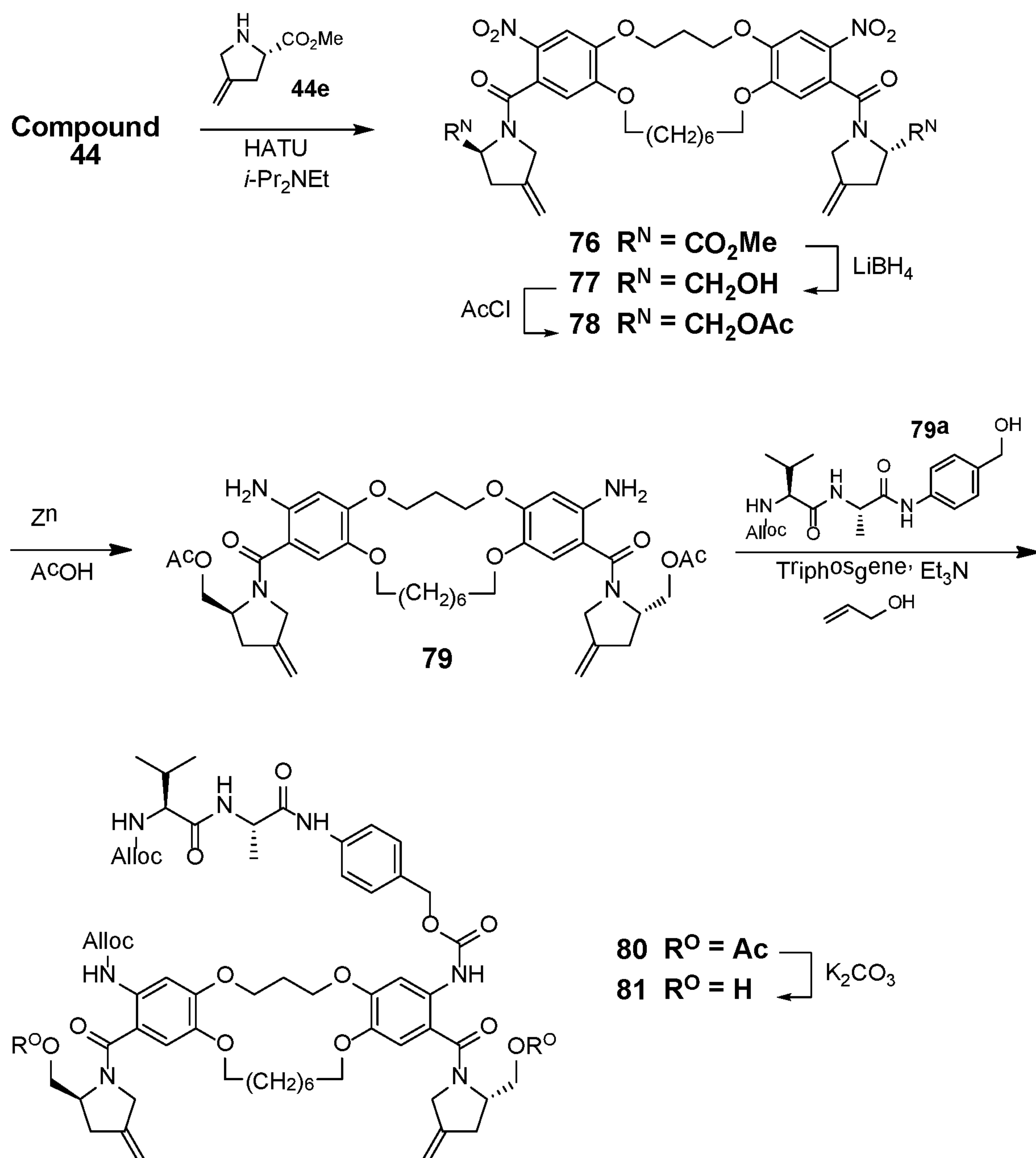
**FIG. 9**



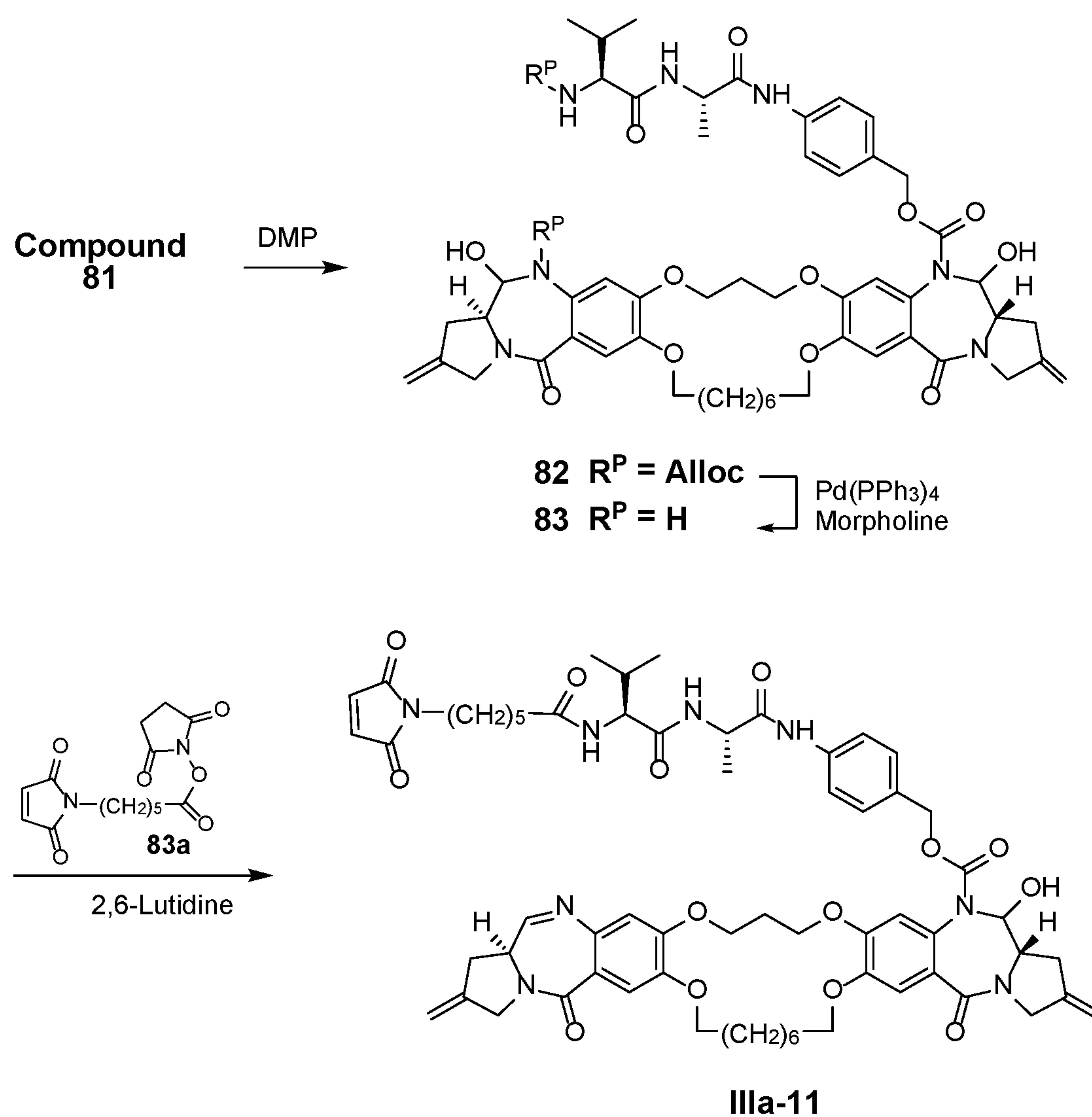
**FIG. 10**

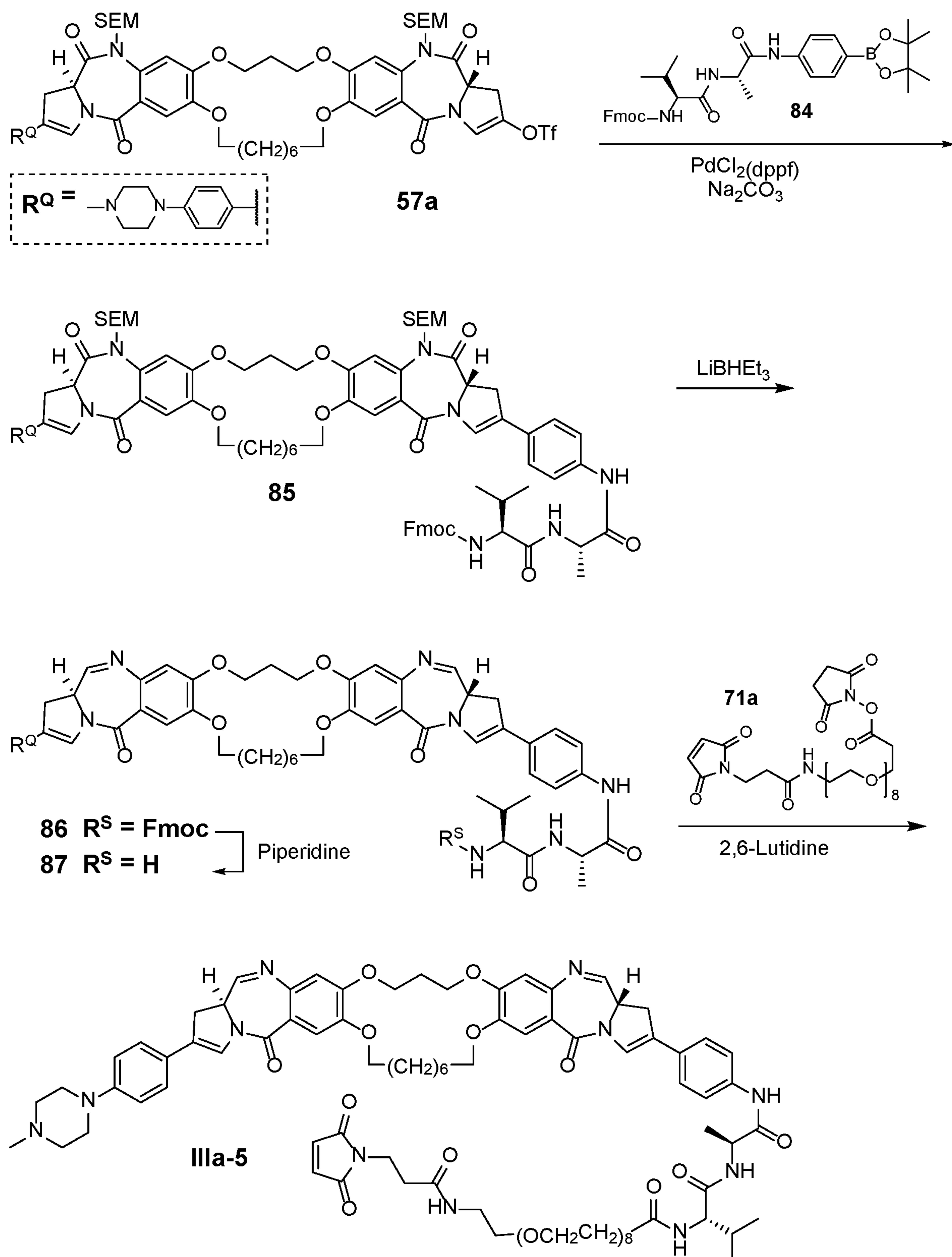
**FIG. 11**

**FIG. 12**

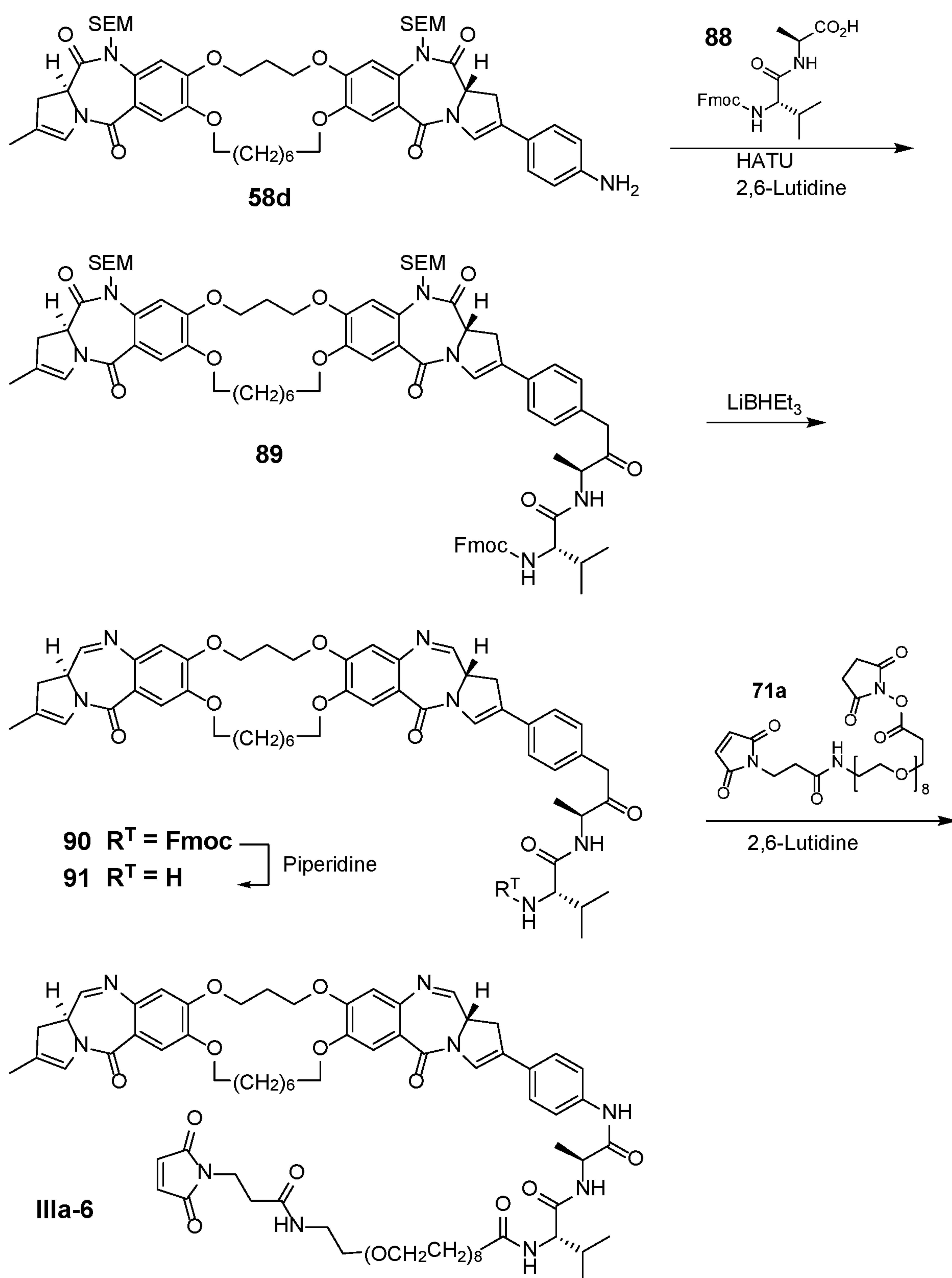
**FIG. 13A**

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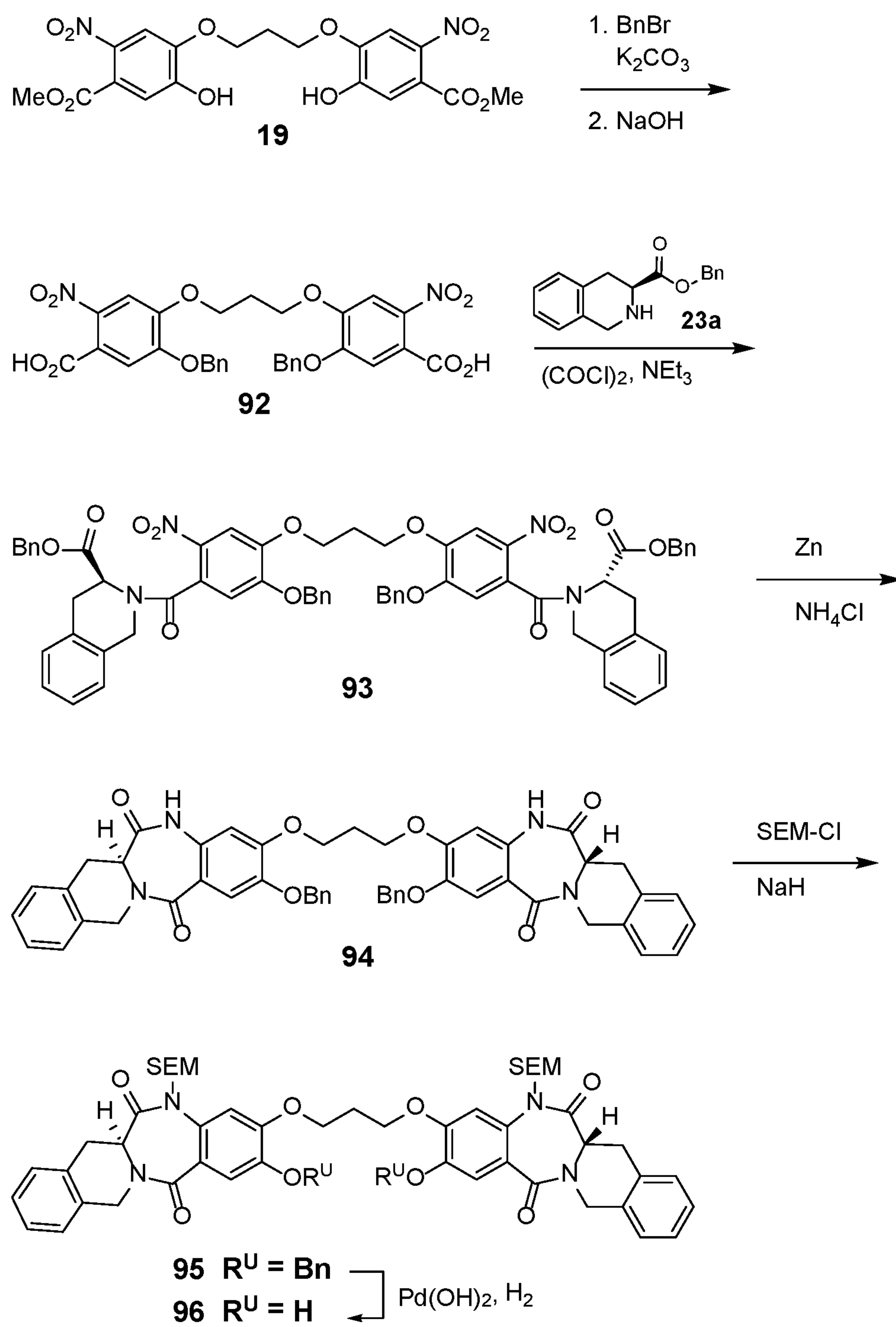
**FIG. 13B**

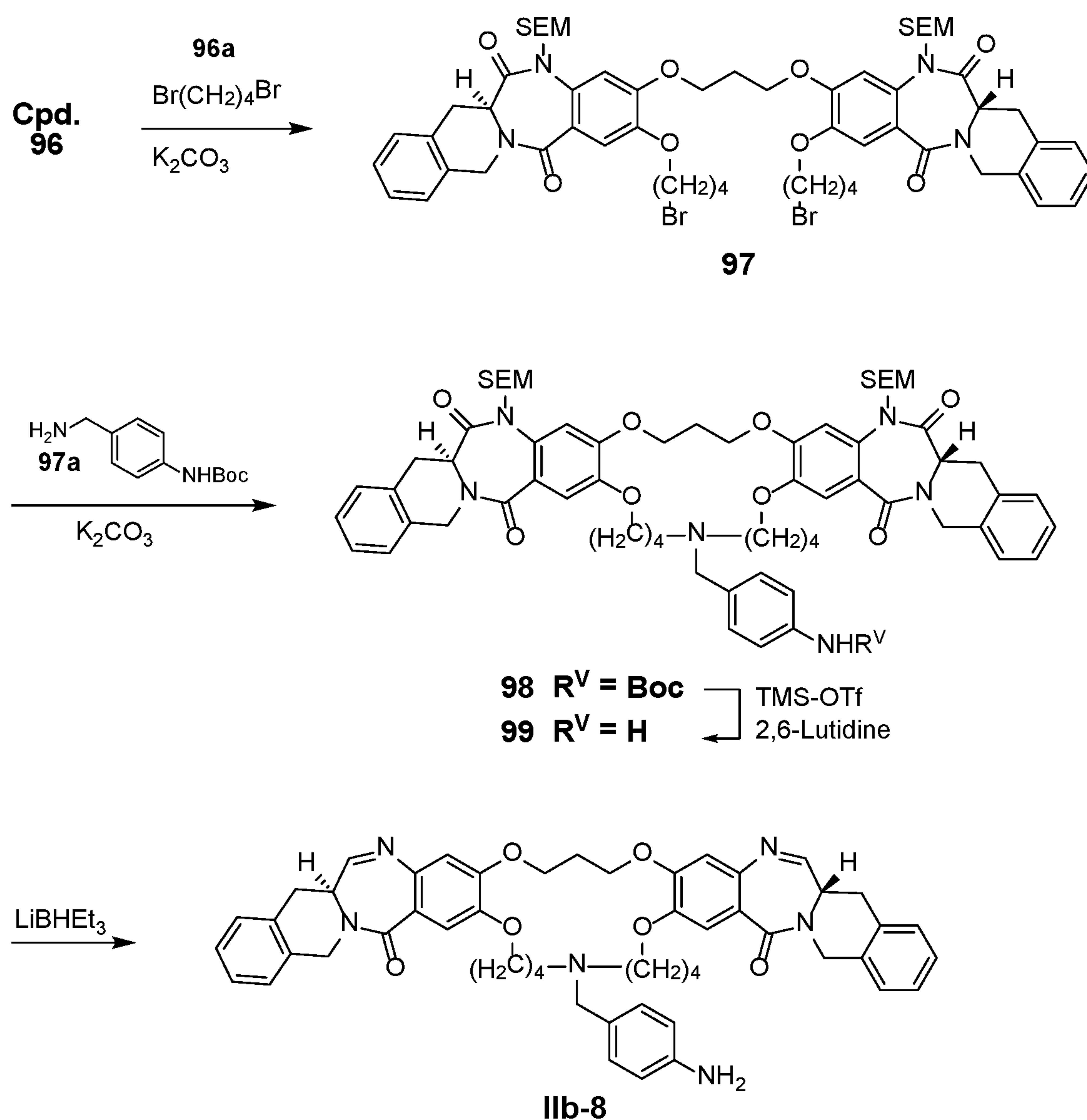
**FIG. 14**

**FIG. 15**

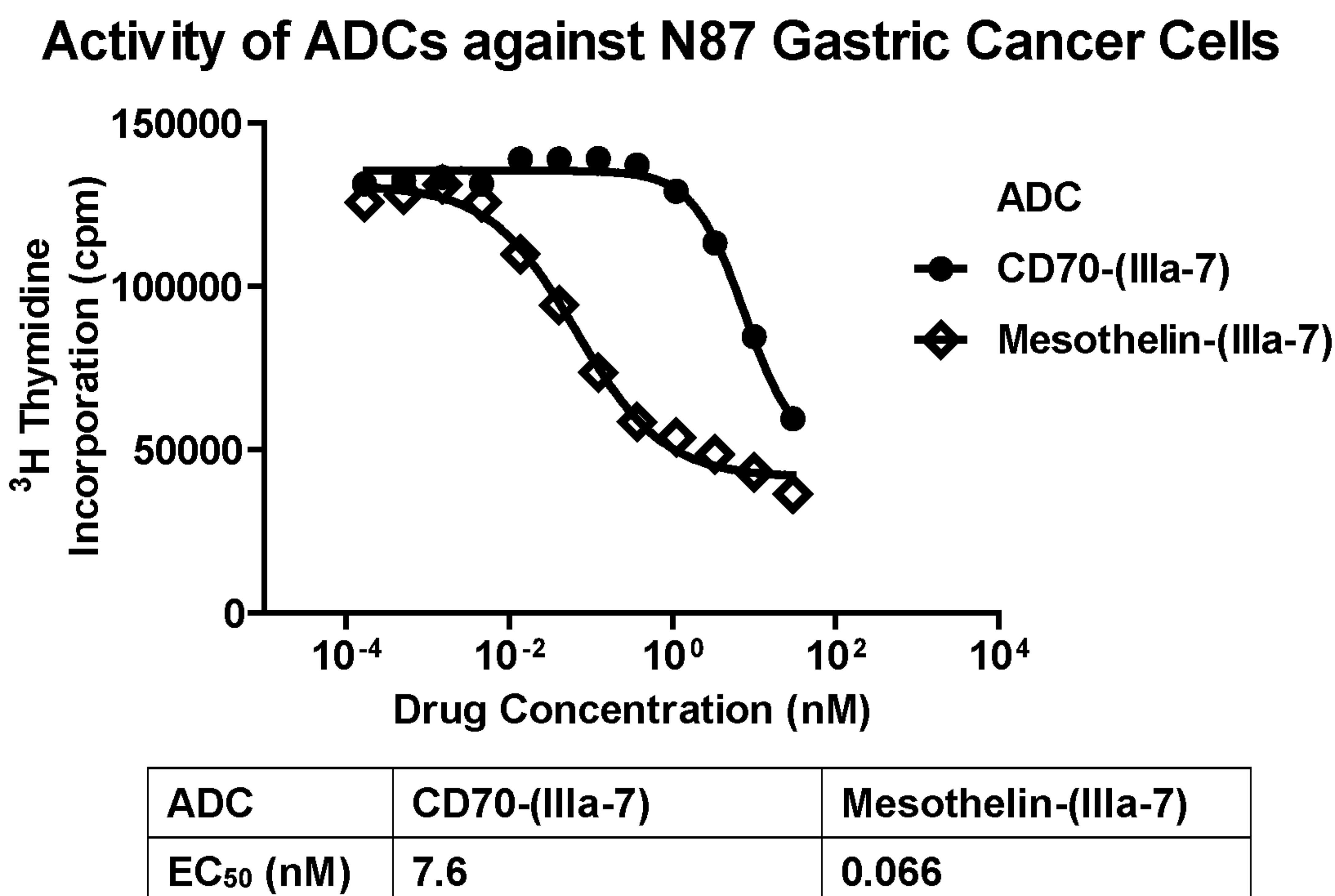


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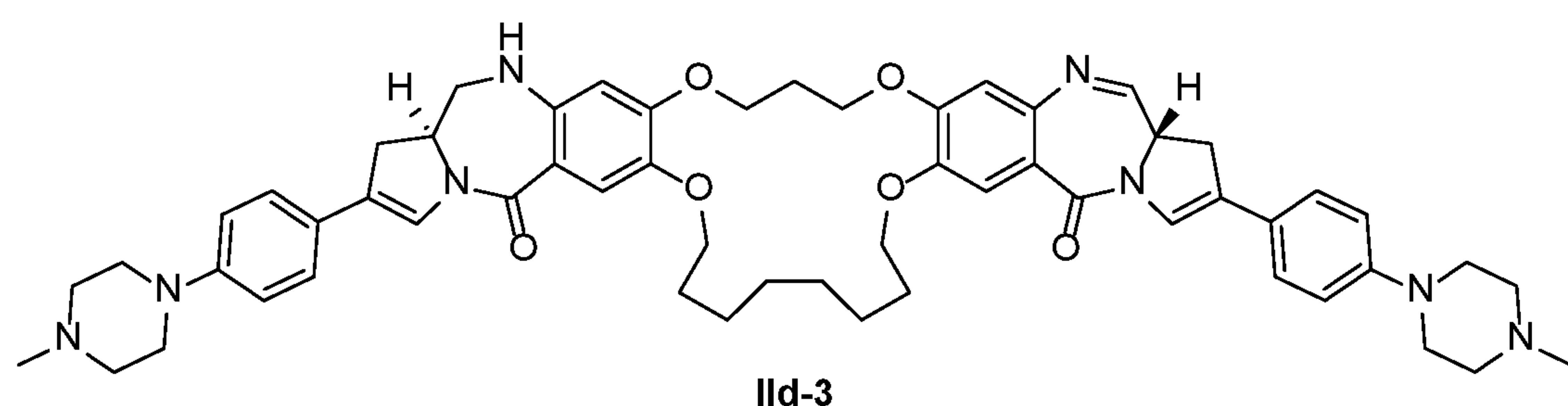
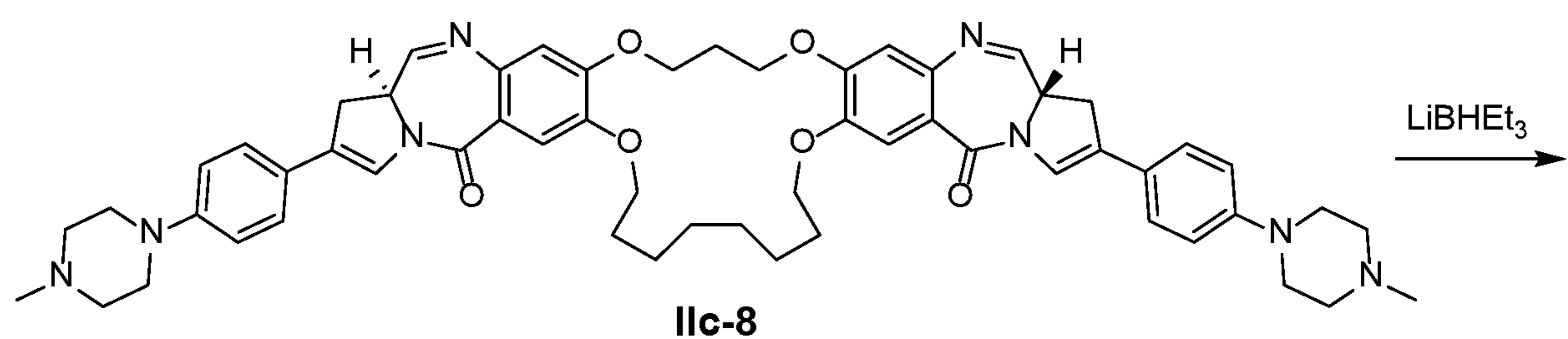
**FIG. 16A**

**FIG. 16B**

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**FIG. 17**

**FIG. 10**



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