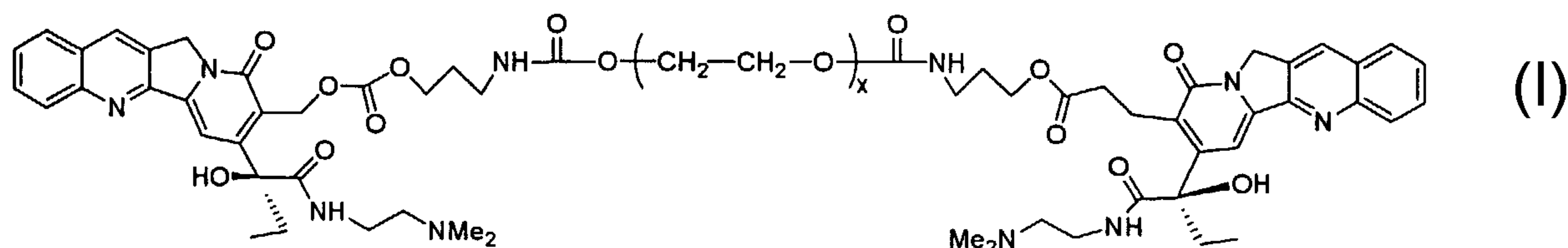




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(54) Title: CAMPTOTHECIN DERIVATIVES AND POLYMERIC CONJUGATES THEREOF



(57) **Abrégé/Abstract:**

Polymeric conjugates of camptothecin derivatives having increased circulating half-lives are disclosed. In preferred aspects, the E ring lactone of the camptothecin derivatives is opened and functionalized to allow attachment of a polymer such as PEG in the 17-or 20-position thereof. A representative example of such compound is (I) methods of preparing and using the same are also disclosed.

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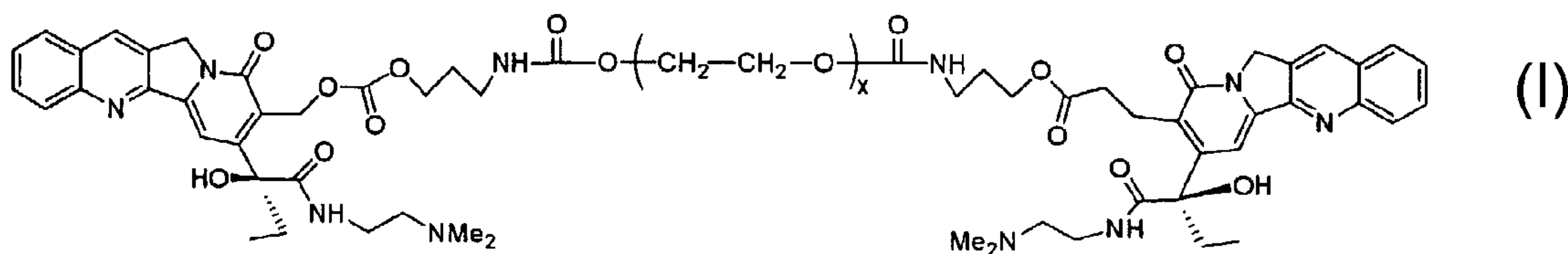
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(57) Abstract: Polymeric conjugates of camptothecin derivatives having increased circulating half-lives are disclosed. In preferred aspects, the E ring lactone of the camptothecin derivatives is opened and functionalized to allow attachment of a polymer such as PEG in the 17- or 20-position thereof. A representative example of such compound is (I) methods of preparing and using the same are also disclosed.



WO 03/097356 A1

CAMPTOTHECIN DERIVATIVES AND POLYMERIC CONJUGATES THEREOF

TECHNICAL FIELD

5 The present invention relates to polymeric derivatives of camptothecin and related topoisomerase inhibitor compounds. More particularly, the invention relates to camptothecin derivatives in which the E ring lactone has been modified to allow attachment of substantially non-antigenic polymers.

10 BACKGROUND OF THE INVENTION

 Camptothecin is a water-insoluble cytotoxic alkaloid produced by *Camptotheca accuminata* trees indigenous to China and *Nothapodytes foetida* trees indigenous to India. Camptothecin and related analogs are known to be potential anticancer agents and have demonstrated therapeutic activity *in vitro* and *in vivo*.

15 Over the years, various proposals have been made to increase the water solubility and/or therapeutic and pharmacokinetic properties of camptothecin analogs. One early attempt to increase the water solubility of camptothecin is disclosed in U.S. Patent No. 4,943,579 (hereinafter the '579 patent). The '579 patent discloses certain simple 20(S)-camptothecin amino acid esters in their salt
20 forms as water soluble prodrugs. As evidenced by the data provided in Table 2 of the '579 patent, hydrolysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body before full therapeutic effect can be achieved. A related effort was directed to developing a water-soluble camptothecin sodium salt. Unfortunately,
25 the water-soluble sodium salt of camptothecin remained too toxic for clinical application (Gottlieb et al., 1970 *Cancer Chemother, Rep.* 54, 461; Moertel et al., 1972 *ibid*, 56, 95; Gottlieb et al., 1972 *ibid*, 56, 103).

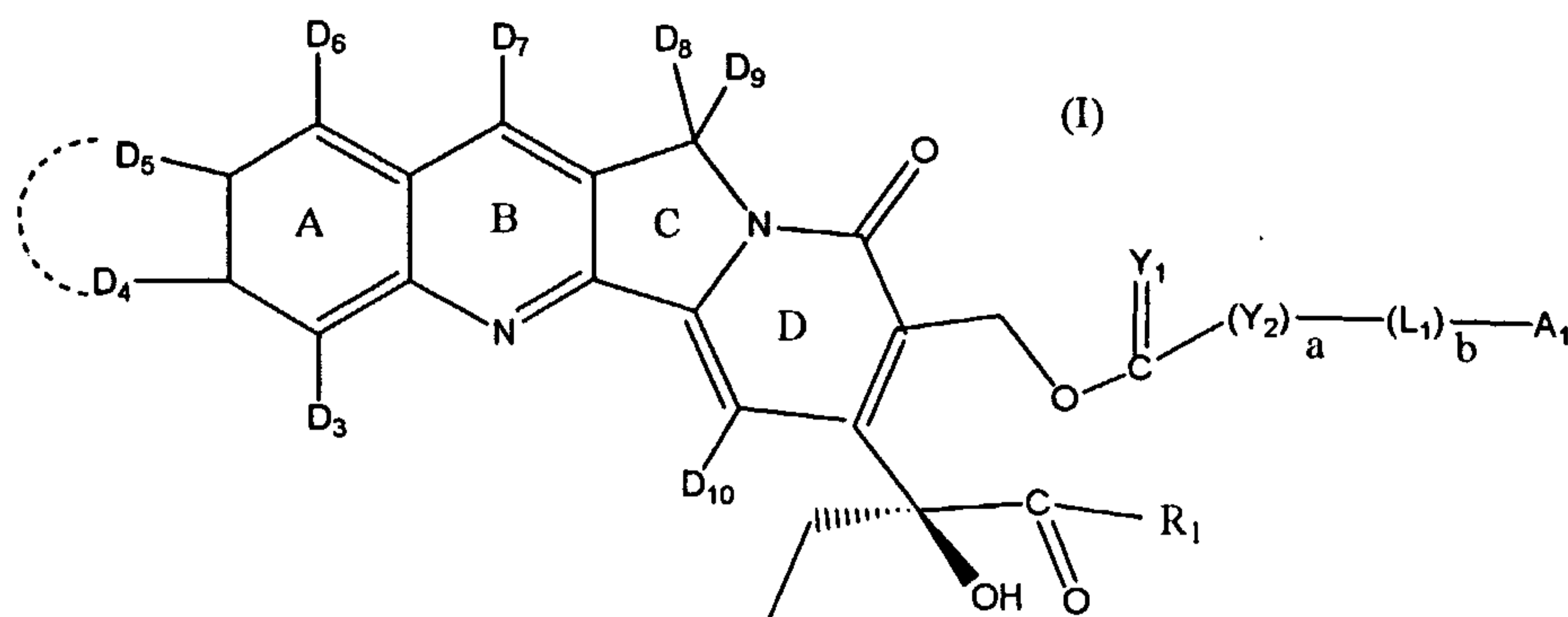
 Other attempts at improving the solubility of camptothecin analogs are provided by Greenwald et al. For example, commonly-assigned U.S. Patent No.
30 5,965,566 discloses camptothecin attached to a bifunctional PEG using an amino acid-based linkers. U.S. Patent No. 6,011,042 discloses polymeric derivatives of

10-hydroxycamptothecin. The PEG-derivatives provide the artisan with a water soluble prodrug which, upon administration, eventually liberate the parent compound in vivo. Prodrugs allow the artisan to modify the onset and/or duration of action of an agent in vivo and can modify the transportation, distribution or
5 solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations.

The E-lactone ring is thought to be essential for anti-cancer activity. Nonetheless, Sawada et al. have suggested in U.S. Patent No. 4,914,205 and
10 Chem. Pharm. Bull. 41(2) 310-313 (1993) prodrugs of camptothecin and the 7-ethyl derivative thereof having a modified E-lactone-ring. Specifically, the E-lactone ring is opened to provide 17-*O*-acyl derivatives and the authors demonstrated improved solubility of HCl salts thereof when compared to the native alkaloid. In spite of the increases in solubility demonstrated by the open
15 lactone E-ring derivatives, further improvements have been sought. For example, after administration, the prodrug must be converted back into its active form in order to have biological (anticancer) activity. Consequently, these derivatives are highly dependent upon the hydrolysis of the respective hydroxyacids to free the 17-hydroxyl group in order to regenerate the E-ring lactone. Such conversions,
20 however, are difficult to predict. It would be highly desirable to provide E-lactone ring modified camptothecin analogs with extended circulating half lives and more predictable rates of regenerating the closed lactone ring. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (I) are provided:



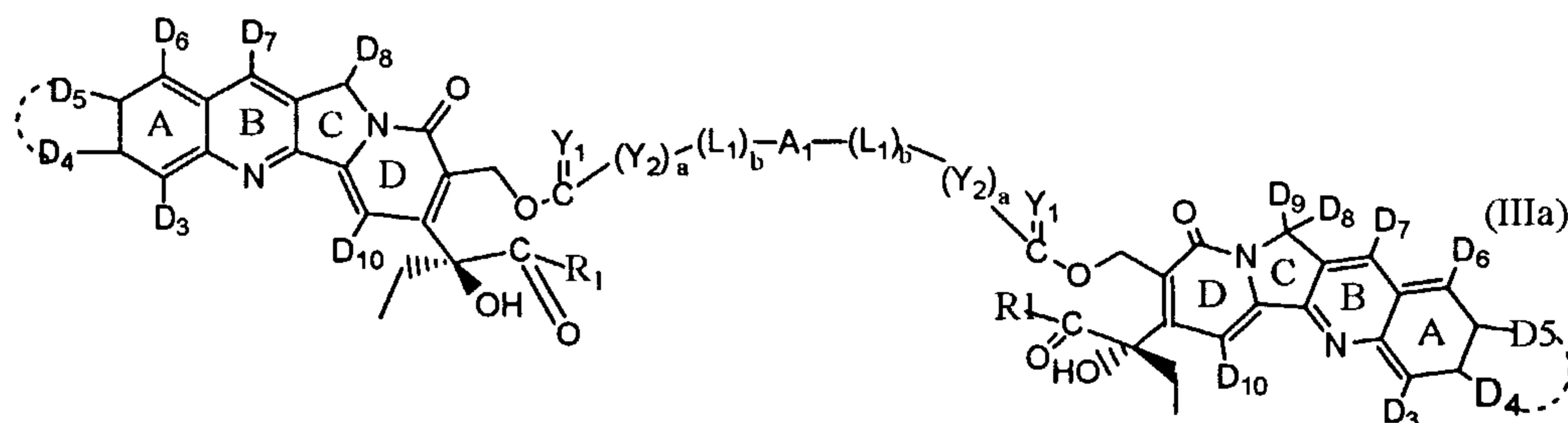
wherein:

- 5 R_1 is selected from the group consisting of amino acid residues, peptide residues containing from about 2 to about 10 amino acids, $Y_3-(L_2)_p-A_2$ and R_2 ;
 Y_3 is O, S or NR_3 ;
 p is zero or one;
 L_2 is a bifunctional linker;
- 10 Y_1 is O, S or NR_4 ;
 Y_2 is O, S, CR_5R_6 or NR_7 ;
 L_1 is a bifunctional linker;
 a and b are independently zero or one;
 A_1 and A_2 are independently selected from the group consisting of hydrogen,
- 15 amino protecting groups, NR_8R_9 , amino acid residues, peptide residues containing from about 2 to about 10 amino acids; polymeric residues, R_{10} , SR_{11} , $NC(O)R_{12}$;
 D_3 - D_7 are independently selected from the group consisting of H,
 C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls,
substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls, C_{1-8} alkoxys,
20 C_{1-8} hydroxyalkyls, C_{1-8} aminoalkoxy, aryloxys, gycals, CO_2R_{13} , R_{14} , nitro, cyano,
halo, hydroxyl, amino, SR_{15} , $NR_{16}R_{17}$ or OR_{18} , where D_4 and D_5 optionally, when
taken together, form a saturated 3-7 membered heterocyclic ring which may
contain O, S or NR_{19} groups, where R_{19} is hydrogen or a C_{1-6} alkyl;
 D_8 - D_{10} are independently selected from the group consisting of H,

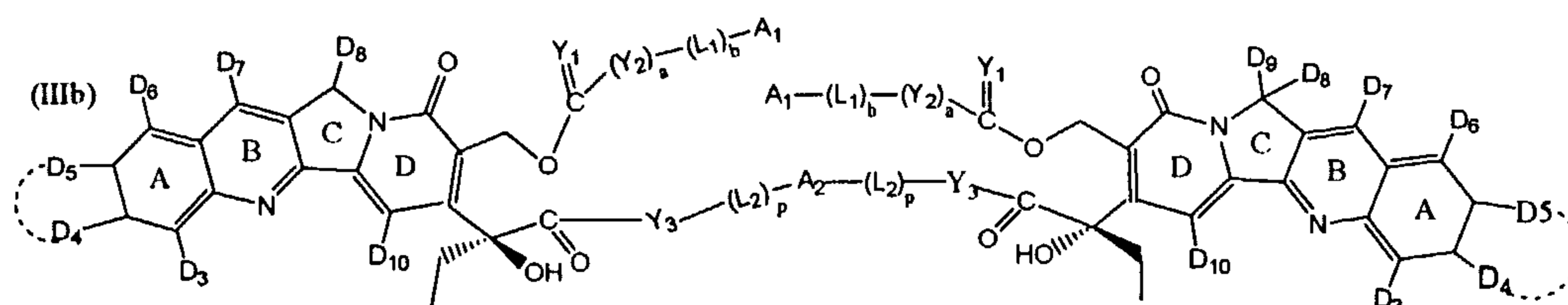
C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls and C₁₋₈ hydroxyalkyls; and

- R₂₋₁₈ are independently selected from the group consisting of hydrogen,
 5 C₁₋₆ alkyls, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy; except that at least one of A₁ and A₂ comprise a polymeric residue.

- In another aspect of the invention there is provided bifunctional compounds
 10 that are formed when at least one of A₁ and A₂ comprises a polymeric residue and the polymeric residue is functionalized on both the alpha and omega termini to allow two equivalents of the camptothecin to be delivered per equivalent of the polymer which is preferably PEG. Such preferred compositions correspond to formulae (IIIa) and (IIIb) below:



- 15 and



wherein all variables are as previously defined above.

- 20 For purposes of the present invention, the term "residue" shall be understood to mean that portion of a camptothecin derivative or bifunctional spacer which remains after it has undergone a substitution reaction.

Methods of preparing the compositions of the invention and methods of treatment using the same are also provided.

For purposes of the present invention, the term "polymeric residue" or "PEG residue" shall each be understood to mean that portion of the polymer or PEG which remains after it has undergone a reaction with a heteroaromatic amine-containing compound.

5 For purposes of the present invention, the term "alkyl" shall be understood to include straight, branched, substituted, e.g. halo-, alkoxy-, nitro-, C₁₋₁₂ alkyls, C₃₋₈ cycloalkyls or substituted cycloalkyls, etc.

For purposes of the present invention, the term "substituted" shall be understood to include adding or replacing one or more atoms contained within a
10 functional group or compound with one or more different atoms.

For purposes of the present invention, substituted alkyls include carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls and mercaptoalkyls; substituted alkenyls include carboxyalkenyls, aminoalkenyls, dialkenylaminos, hydroxyalkenyls and mercaptoalkenyls; substituted alkynyls include
15 carboxyalkynyls, aminoalkynyls, dialkynylaminos, hydroxyalkynyls and mercaptoalkynyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as naphthyl; substituted aryls include moieties such as 3-bromo-phenyl; aralkyls include moieties such as toluyl; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls
20 include moieties such as 3-methoxy-thiophene; alkoxy includes moieties such as methoxy; and phenoxy includes moieties such as 3-nitrophenoxy. Halo- shall be understood to include fluoro, chloro, iodo and bromo.

The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood
25 by those of ordinary skill in the art.

One advantage of the invention is that the artisan is provided with prodrugs of camptothecin derivatives with improved aqueous solubility.

Another advantage of the compounds of the invention is that in certain preferred embodiments, the releasable polymer not only extends the circulating life
30 of the camptothecin derivative, but it also provides a means for controlling conversion of the open E-ring lactone back to the biologically active form. This

result is achieved by virtue of the fact that the open E-ring camptothecin derivative cannot hydrolyze into the closed E lactone ring until the polymer is released.

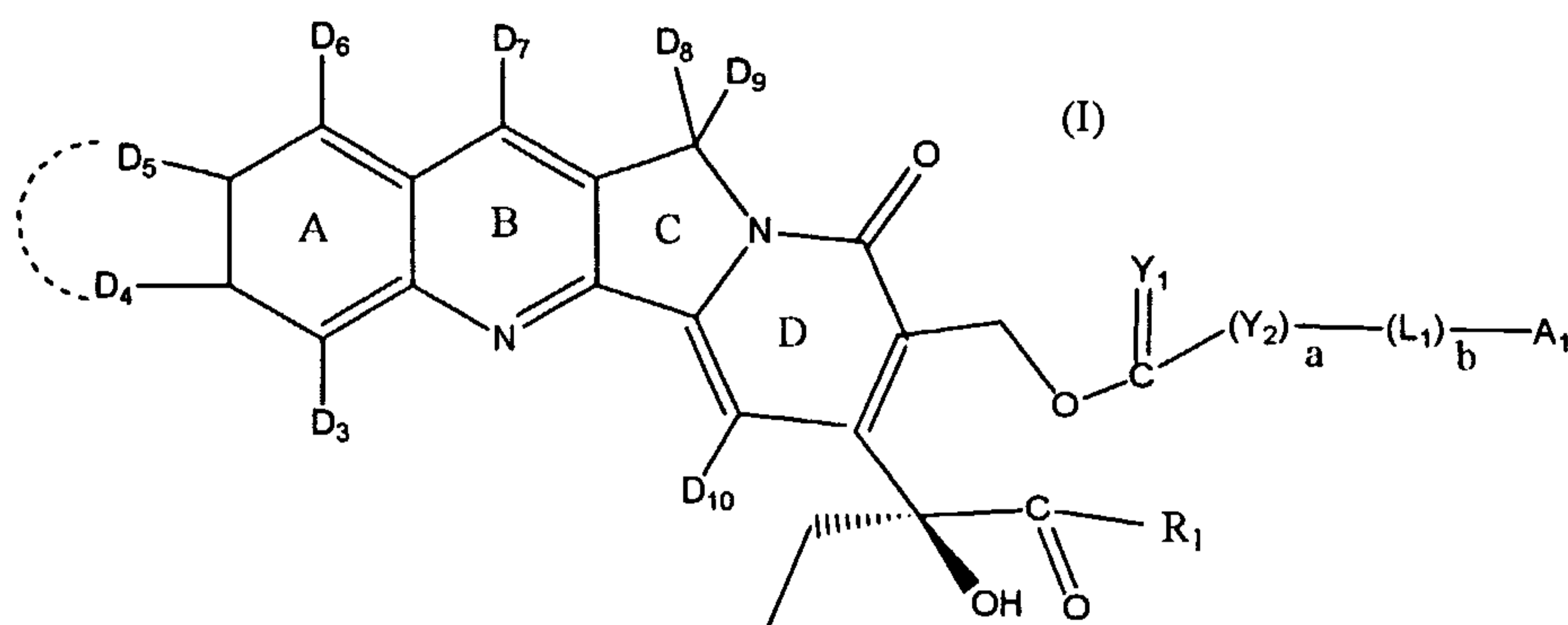
BRIEF DESCRIPTION OF THE DRAWINGS

- 5 Figures 1-6 schematically illustrate methods of forming compounds of the present invention which are described in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

A. FORMULA (I)

- 10 In one preferred embodiment of the invention, there are provided compounds of the Formula (I):



wherein:

- 15 R_1 is selected from the group consisting of amino acid residues, peptide residues containing from about 2 to about 10 amino acids, $Y_3-(L_2)_p-A_2$ and R_2 ;
 Y_3 is O, S or NR_3 ;
 p is zero or one;
 L_2 is a bifunctional linker;
20 Y_1 is O, S or NR_4 ;
 Y_2 is O, S, CR_5R_6 or NR_7 ;
 L_1 is a bifunctional linker;
 a and b are independently zero or one;

A_1 and A_2 are independently selected from the group consisting of hydrogen, amino protecting groups, NR_8R_9 , amino acid residues, peptide residues containing from about 2 to about 10 amino acids; polymeric residues, R_{10} , SR_{11} , $NC(O)R_{12}$;

D_3 - D_7 are independently selected from the group consisting of H,

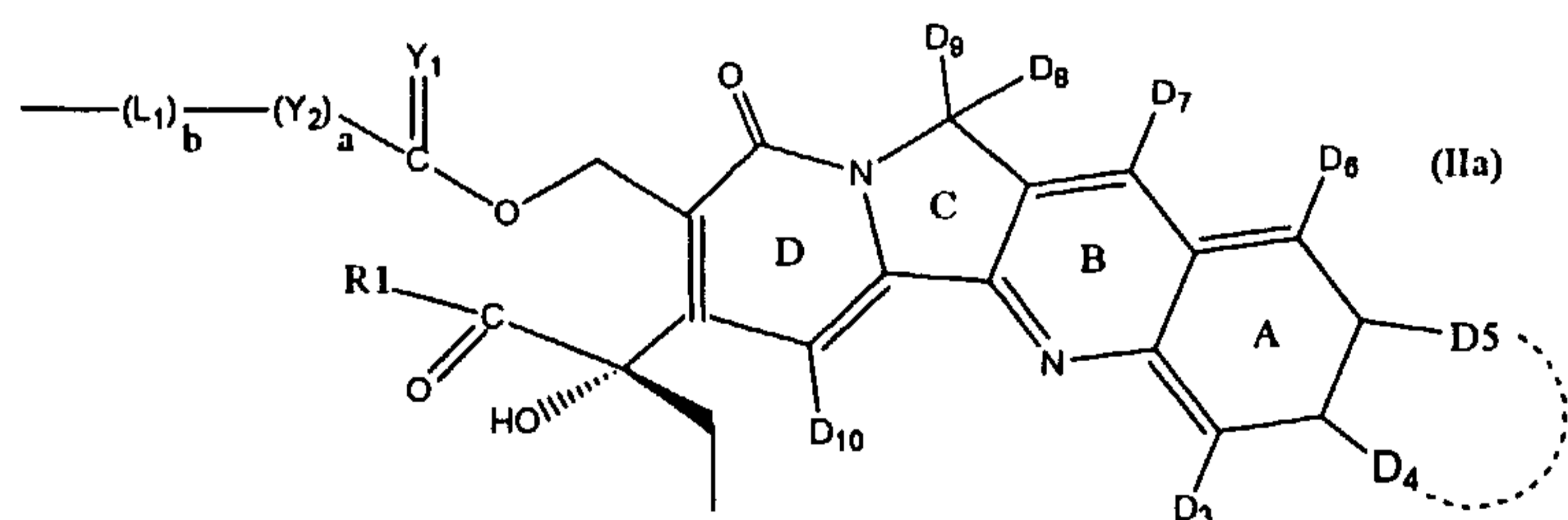
- 5 C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls, C_{1-8} alkoxys, C_{1-8} hydroxyalkyls, C_{1-8} aminoalkoxy, aryloxys, glycals, CO_2R_{13} , R_{14} , nitro, cyano, halo, hydroxyl, amino, SR_{15} , $NR_{16}R_{17}$ or OR_{18} , where D_4 and D_5 optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may
- 10 contain O, S or NR_{19} groups, where R_{19} is hydrogen or a C_{1-6} alkyl;

D_8 - D_{10} are independently selected from the group consisting of H,

C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls and C_{1-8} hydroxyalkyls; and

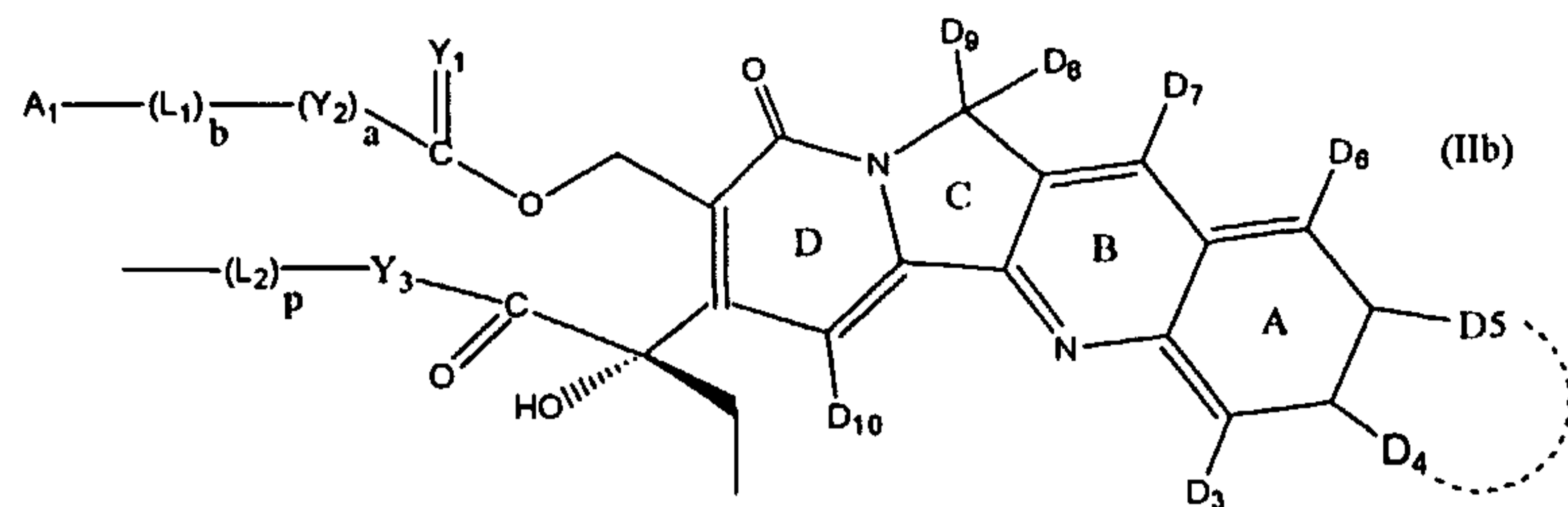
- 15 R_{2-18} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-19} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy; except that at least one of A_1 and A_2 comprise a polymeric residue.

- 20 In many preferred aspects of the invention, one of A_1 and A_2 includes a polymer residue. The polymer residue optionally includes a capping group designated J herein. Within this aspect of the invention, when A_1 is a polymer residue, the capping group J is selected from among, for example, OH, NH_2 , SH, CO_2H , C_{1-6} alkyl moieties and

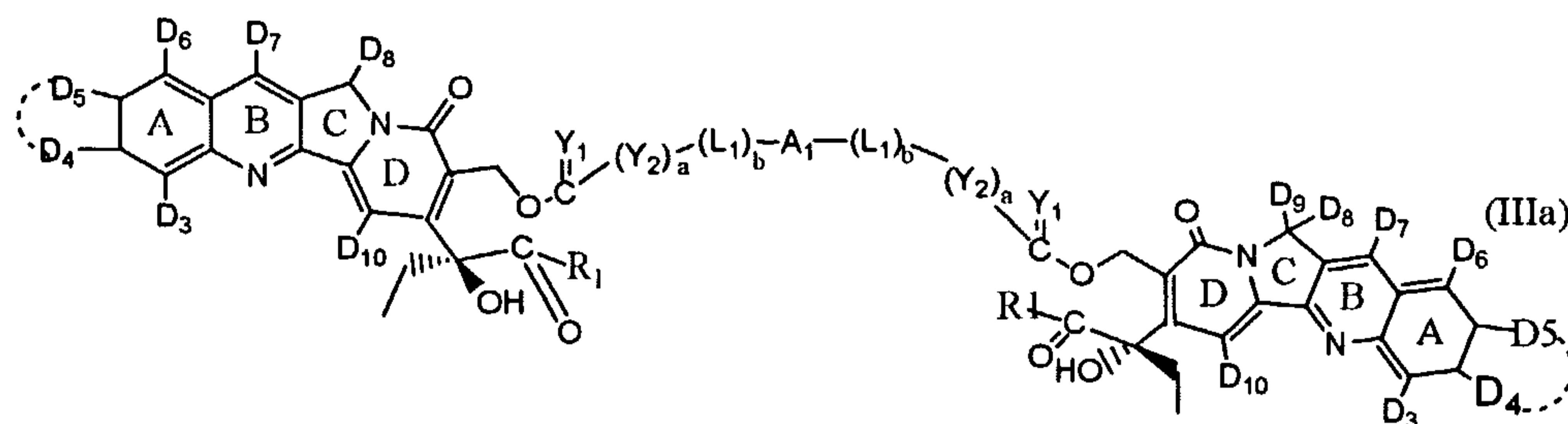


Alternatively, when R_1 is $Y_3-(L_2)_p-A_2$ in formula (I), and A_2 is a polymer residue, the capping group J is selected from among, for example, OH, NH_2 , SH-, CO_2H ,

C₁₋₆ alkyl moieties and

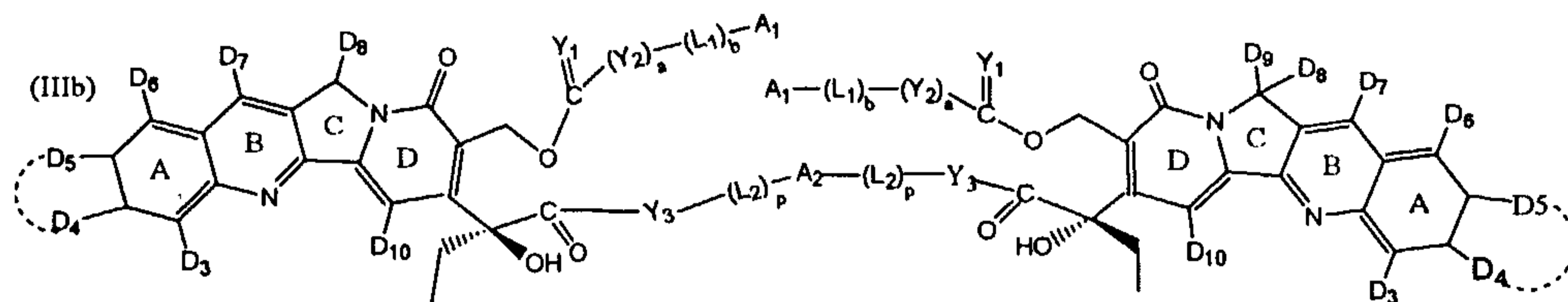


The preferred capping groups allow preferred compositions of (IIIa) and (IIIb), respectively,



5

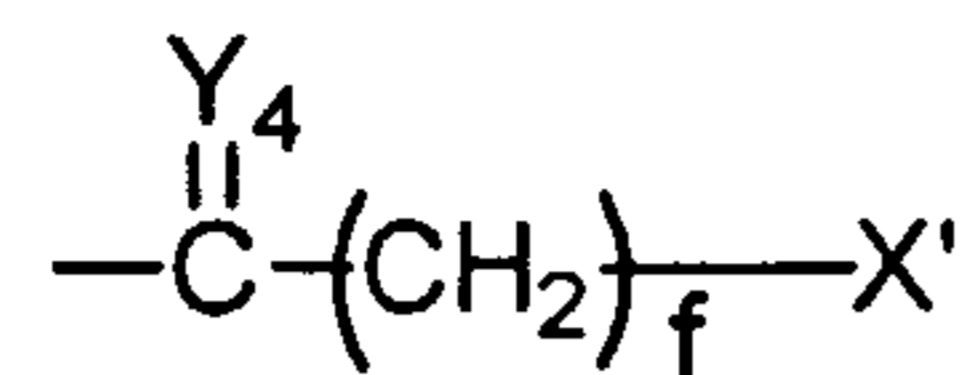
and



to be formed, wherein all variables are as previously defined.

- 10 When L₁ and/or L₂ include an amino acid residue, the amino acid can be selected from any of the known naturally-occurring L- amino acids is, e.g., alanine, valine, leucine, isoleucine, glycine, serine, threonine, methionine, cysteine, phenylalanine, tyrosine, tryptophan, aspartic acid, glutamic acid, lysine, arginine, histidine, proline, and/or a combination thereof, to name but a few. When L₁
- 15 and/or L₂ include a peptide, the peptide ranges in size, for instance, from about 2 to about 10 amino acid residues. In one preferred embodiment, the peptide is Gly-Phe-Leu-. Alternatively, glycine can be added to the aforementioned tripeptide after leucine to form a 4 residue peptide.

The amino acid residues are preferably of the formula



wherein X' is O, S or NR₃₄, Y₄ is O, S or NR₃₅, and f is a positive integer from about 1 to about 10, preferably 1; and R₃₄ and R₃₅ are independently selected
 5 from the same group as that which defines R₃₁ but each is preferably H or lower alkyl.

Derivatives and analogs of the naturally occurring amino acids, as well as various art-known non-naturally occurring amino acids (D or L), hydrophobic or non-hydrophobic, are also contemplated to be within the scope of the invention.

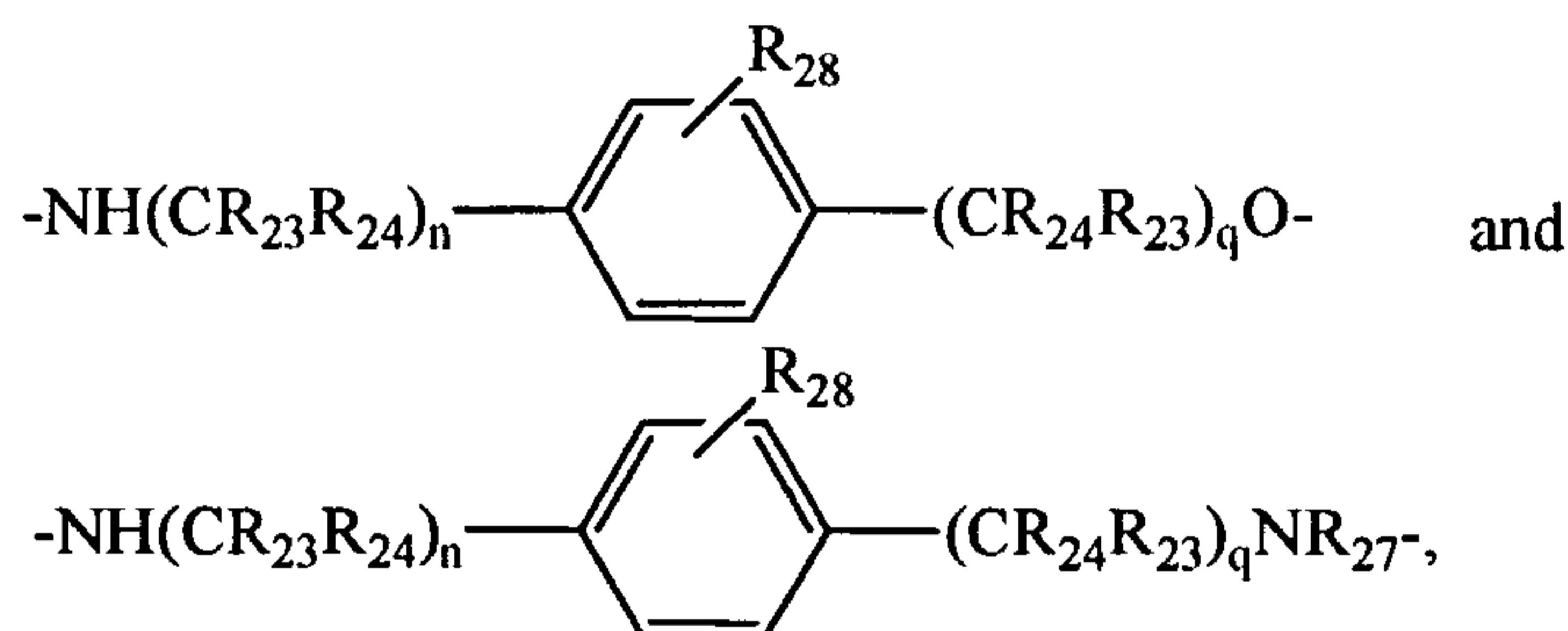
10 Simply by way of example, amino acid analogs and derivatives include:
 2-aminoadipic acid, 3-amino-adipic acid, beta-alanine, beta-aminopropionic acid, 2-aminobutyric acid, 4-amino-butyric acid, piperidinic acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4-diamino-butyric acid, desmosine, 2,2-diaminopimelic
 15 acid, 2,3-diaminopropionic acid, n-ethylglycine, N-ethylasparagine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, sarcosine, N-methylisoleucine, 6-N-methyl-lysine, N-methylvaline, norvaline, norleucine, ornithine, and others too numerous to mention, that are listed in 63 Fed. Reg., 29620, 29622, incorporated by reference
 20 herein.

Short peptides are, for example, peptides ranging from 2 to about 10, or more, amino acid residues, as mentioned supra.

Within most aspects of the invention, the following embodiments are preferred:

25 Y₁ and Y₂ are each oxygen,
 R₂-R₁₄ and D₃ - D₁₀ are each hydrogen,
 L₁ is selected from among

$(\text{CH}_2)_n$,
 $(\text{CH}_2)_3\text{NH}-\text{C}(\text{O})$
 $(\text{CH}_2)_3\text{NH}-$
 $-\text{NH}(\text{CH}_2\text{CH}_2\text{O})_n(\text{CH}_2)_n\text{NR}_{22}-$,
 $-\text{NH}(\text{CH}_2\text{CH}_2\text{O})_n-$,
 $-\text{NH}(\text{C}_{23}\text{R}_{24})_n\text{O}-$,
 $-\text{C}(\text{O})(\text{CR}_{23}\text{R}_{24})_n\text{NHC}(\text{O})(\text{CR}_{25}\text{R}_{26})_q\text{NR}_{27}-$,
 $-\text{C}(\text{O})\text{O}(\text{CH}_2)_n\text{O}-$,
 $-\text{C}(\text{O})(\text{CR}_{23}\text{R}_{24})_n\text{NR}_{27}-$,
 $-\text{C}(\text{O})\text{NH}(\text{CH}_2\text{CH}_2\text{O})_n(\text{CH}_2)_n\text{NR}_{27}-$,
 $-\text{C}(\text{O})\text{O}-(\text{CH}_2\text{CH}_2\text{O})_n\text{NR}_{27}-$,
 $-\text{C}(\text{O})\text{NH}(\text{CR}_{23}\text{R}_{24})_n\text{O}-$,
 $-\text{C}(\text{O})\text{O}(\text{CR}_{23}\text{R}_{24})_n\text{O}-$,
 $-\text{C}(\text{O})\text{NH}(\text{CH}_2\text{CH}_2\text{O})_n-$,



wherein

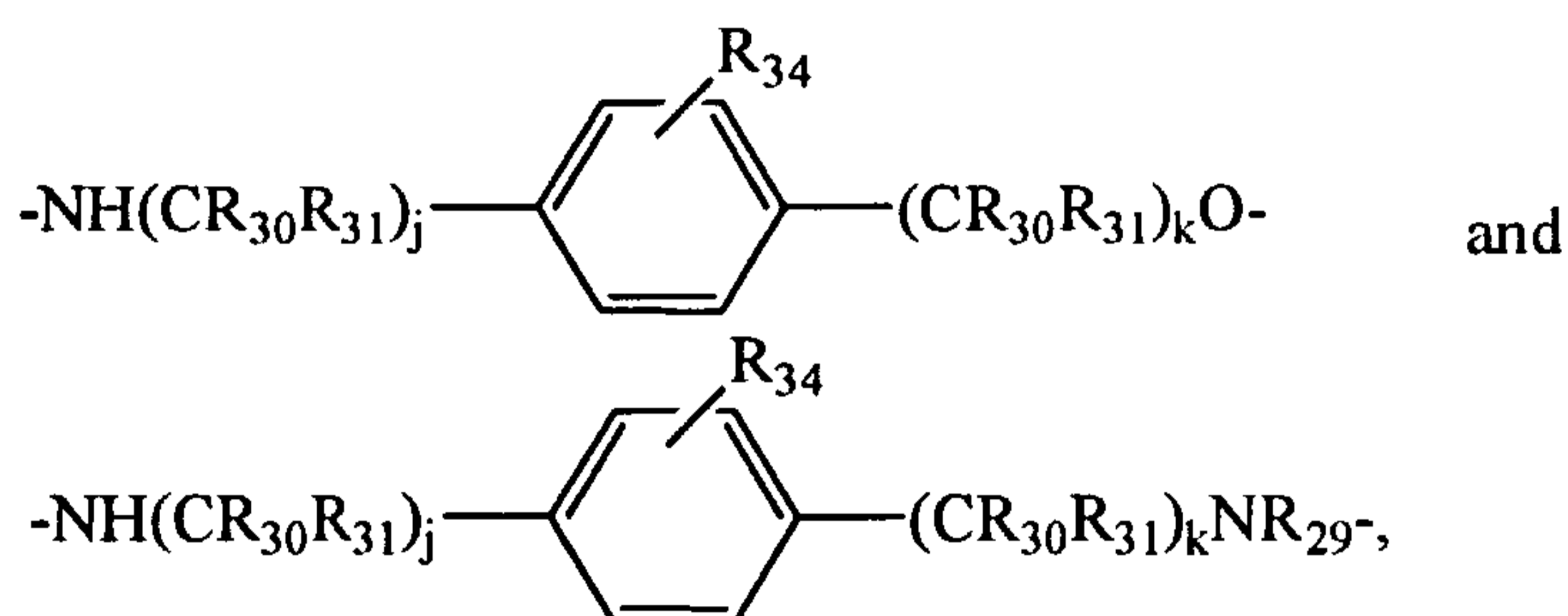
- R_{22-27} are independently selected from the group consisting of hydrogen,
 5 C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls,
 C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls,
 substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

- R_{28} is selected from the group consisting of hydrogen, C_{1-6} alkyls,
 C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted
 10 cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted
 C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy, NO_2 , haloalkyl and
 halogen; and

n and q are independently selected positive integers.

L_2 is preferably selected from among:

$(\text{CH}_2)_j$
 $(\text{CH}_2\text{CH}_2\text{O})_2(\text{CH}_2)_2\text{NH}$,
 $-\text{NH}(\text{CH}_2\text{CH}_2\text{O})_j(\text{CH}_2)_k\text{NR}_{29^-}$,
 $-\text{NH}(\text{CH}_2\text{CH}_2\text{O})_j^-$,
 $-\text{NH}(\text{CR}_{30}\text{R}_{31})_j\text{O}^-$,
 $-\text{C}(\text{O})(\text{CR}_{30}\text{R}_{31})_j\text{NHC}(\text{O})(\text{CR}_{32}\text{R}_{33})_j\text{NR}_{29^-}$,
 $-\text{C}(\text{O})\text{O}(\text{CH}_2)_k\text{O}^-$,
 $-\text{C}(\text{O})(\text{CR}_{30}\text{R}_{31})_j\text{NR}_{29^-}$,
 $-\text{C}(\text{O})\text{NH}(\text{CH}_2\text{CH}_2\text{O})_j(\text{CH}_2)_k\text{NR}_{29^-}$,
 $-\text{C}(\text{O})\text{O}-(\text{CH}_2\text{CH}_2\text{O})_j\text{NR}_{29^-}$,
 $-\text{C}(\text{O})\text{NH}(\text{CR}_{30}\text{R}_{31})_j\text{O}^-$,
 $-\text{C}(\text{O})\text{O}(\text{CR}_{30}\text{R}_{31})_j\text{O}^-$,
 $-\text{C}(\text{O})\text{NH}(\text{CH}_2\text{CH}_2\text{O})_j^-$,



wherein

- R_{29-33} are independently selected from the group consisting of hydrogen,
 5 C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls,
 C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls,
 substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

- R_{34} is selected from the group consisting of hydrogen, C_{1-6} alkyls,
 C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted
 10 cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted
 C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy, NO_2 , haloalkyl and
 halogen; and

j and k are independently selected positive integers.

15 B. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

As stated above, A_1 and A_2 can comprise polymer residues. The polymer residues are preferably water soluble and substantially non-antigenic. Particularly

preferred polymers useful in the compositions of the present invention include polyalkylene oxides such as polyethylene glycol. The polymer residues optionally the previously mentioned capping groups, designated J, which allows a bifunctional or bis-polymer system to be formed.

5 In those aspects of the invention where A₁ includes the polyethylene glycol residue, the PEG derivatives can be selected from the following non-limiting list:

- J-O-(CH₂CH₂O)_x-,
- J-O-(CH₂CH₂O)_x-CH₂C(O)-O-,
- J-O-(CH₂CH₂O)_x-CH₂CH₂ NR₂₀-,
- 10 J-O-(CH₂CH₂O)_x-CH₂CH₂ SH-,
- O-C(O)CH₂-O-(CH₂CH₂O)_x-CH₂C(O)-O-,
- NR₂₀CH₂CH₂-O-(CH₂CH₂O)_x-CH₂CH₂ NR₂₀-, and
- SHCH₂CH₂-O-(CH₂CH₂O)_x-CH₂CH₂ SH-,

wherein:

x is the degree of polymerization;

R₂₀ is selected from the group consisting of hydrogen, C₁₋₆ alkyls,

- 15 C₃₋₁₂ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₃₋₈ substituted cycloalkyls, aryls substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy and

J is a capping group.

- Similarly, in those aspects of the invention where A₂ includes the polyethylene glycol residue, the PEG derivatives can be selected from the following non-limiting list:

- of J-O-(CH₂CH₂O)_x -
- J-O-(CH₂CH₂O)_x-CH₂C(O)-O-,
- J-O-(CH₂CH₂O)_x-CH₂CH₂ NR₂₁-,
- 25 J-O-(CH₂CH₂O)_x-CH₂CH₂ SH-,
- O-C(O)CH₂-O-(CH₂CH₂O)_x-CH₂C(O)-O-,
- NR₂₁CH₂CH₂-O-(CH₂CH₂O)_x-CH₂CH₂ NR₂₁-, and
- SHCH₂CH₂-O-(CH₂CH₂O)_x-CH₂CH₂ SH-,

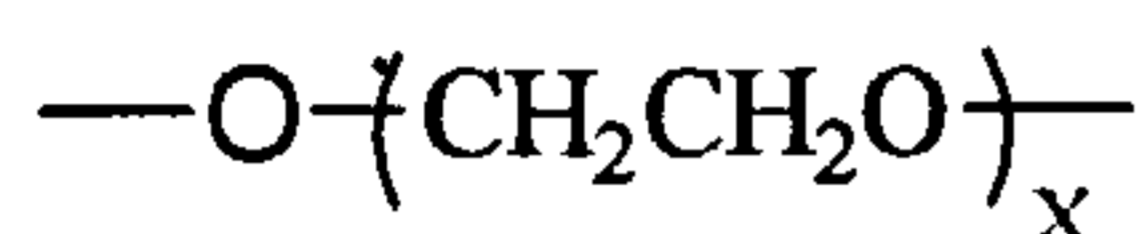
wherein:

x is the degree of polymerization;

R₂₁ is selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₃₋₁₂ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₃₋₈ substituted cyloalkyls, aryls substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy and

J is a capping group.

For the purpose of the present invention the structure:



10 wherein x is a positive integer, is referred to as PEG throughout the application.

The degree of polymerization for the polymer (x) can be from about 10 to about 2,300. This represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. In certain preferred aspects, the amount of polymerization is preferably sufficient to provide the polymer with a molecular weight of at least about 20,000 daltons. Since the ethylene glycol monomer has a molecular weight of 44, (x) is preferably at least about 454. The (J) moiety is a capping group as defined herein, i.e. a group which is found on the terminal of the polymer and, in some aspects, can be selected from any of NH₂, OH, SH-, CO₂H, C₁₋₆ alkyls or other PEG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Corporation's 2001 catalog "Polyethylene Glycol and Derivatives for Biomedical Application". The disclosure of each of the foregoing is incorporated herein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkage groups if required without undue experimentation.

In many aspects of the present invention, bis-activated polyethylene glycols are preferred when di- or multi-substituted polymer conjugates are desired. Alternatively, polyethylene glycols (PEG's), mono-activated, C₁₋₄ alkyl-terminated

polyalkylene oxides (PAO's) such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired.

In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono-
5 or di-PEG amines and mono- or di-PEG diols. Suitable PAO acids can be synthesized by first converting $\text{CH}_3\text{O-PEG-OH}$ (mPEG-OH) to an ethyl ester followed by saponification. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F.M., et al., J. Controlled Release 10: 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a
10 t-butyl ester followed by acid cleavage. See, for example, commonly assigned U.S. Patent No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Although PAO's and PEG's can vary substantially in average molecular weight, the polymer portion of the prodrug is at least about 20,000 Da average in
15 most aspects of the invention. Preferably, the polymer portion of the compositions has a weight average molecular weight of from about 20,000 Da to about 100,000 Da and more preferably from about 25,000 Da to about 60,000 Da. The average molecular weight of the polymer selected for inclusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug before hydrolysis of
20 the linker.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof,
25 provided that the water solubility of the block copolymers is maintained.

In a further embodiment, and as an alternative to PAO-based polymers, A_1 and A_2 can optionally comprise one or more other effectively non-antigenic materials such as dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmeth-acrylamide (HPMA), polyalkylene oxides, and/or copolymers
30 thereof. See also commonly-assigned U.S. Patent No. 6,153,655, the contents of which are incorporated herein by reference. It will be understood by those of

ordinary skill that the same type of activation is employed as described herein as for PAO's such as PEG. Those of ordinary skill in the art will further realize that the foregoing list is merely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention,

5 "effectively non-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in mammals.

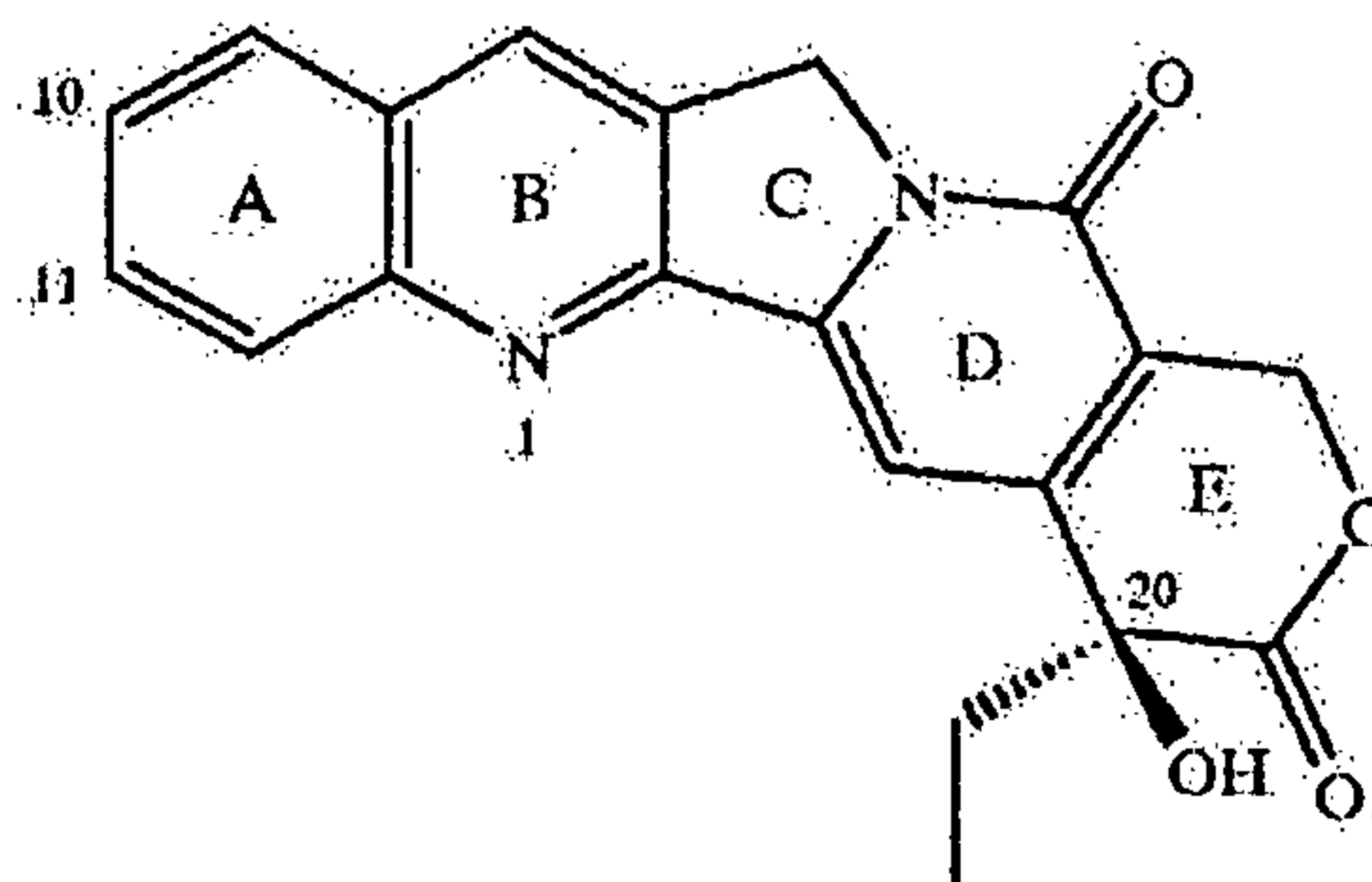
It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, etc., as well as other bi-

10 functional linking groups are also contemplated.

C. CAMPTOTHECIN AND CAMPTOTHECIN ANALOGS

Camptothecin and certain related analogues and derivatives share the structure:

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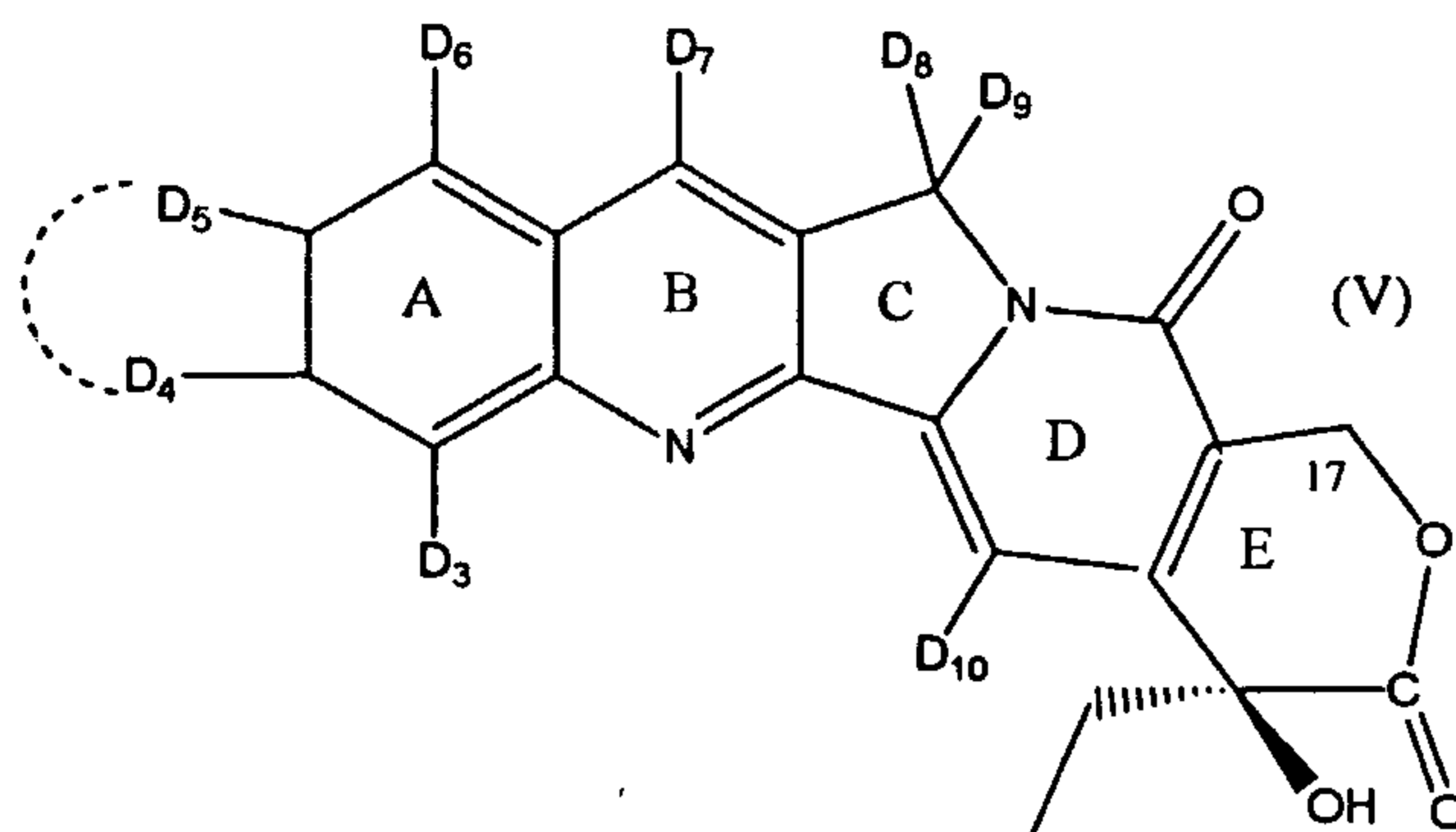
From this core structure, various derivatives are known. For example, the A ring in either or both of the 10- and 11- positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or branched C₁₋₃₀ alkyl or C₁₋₁₇ alkoxy, optionally linked to the ring by a heteroatom i.e. O or S. The B

25 ring can be substituted in the 7-position with a straight or branched C₁₋₃₀ alkyl or substituted alkyl-, C₅₋₈ cycloalkyl, C₁₋₃₀ alkoxy, phenyl alkyl, etc., alkyl carbamate, alkyl carbazides, phenyl hydrazine derivatives, amino, aminoalkyl, aralkyl, etc.

Other substitutions are possible in the C, D and E rings. See, for example, U.S. Pat. Nos. 5,004,758, 4,943,579, Re 32,518, 4,894,456, 5,225, 5,053,512, 4,981,968,

30 5,049,668, 5,106,742, 5,180,722, 5,244,903, 5,227,380, 5,122,606, 5,122,526, and 5,340,817, the contents of each of which are incorporated herein by reference.

In most aspects of the invention, the camptothecin derivatives which are employed in the formation of the compositions of the present invention are generally of the formula



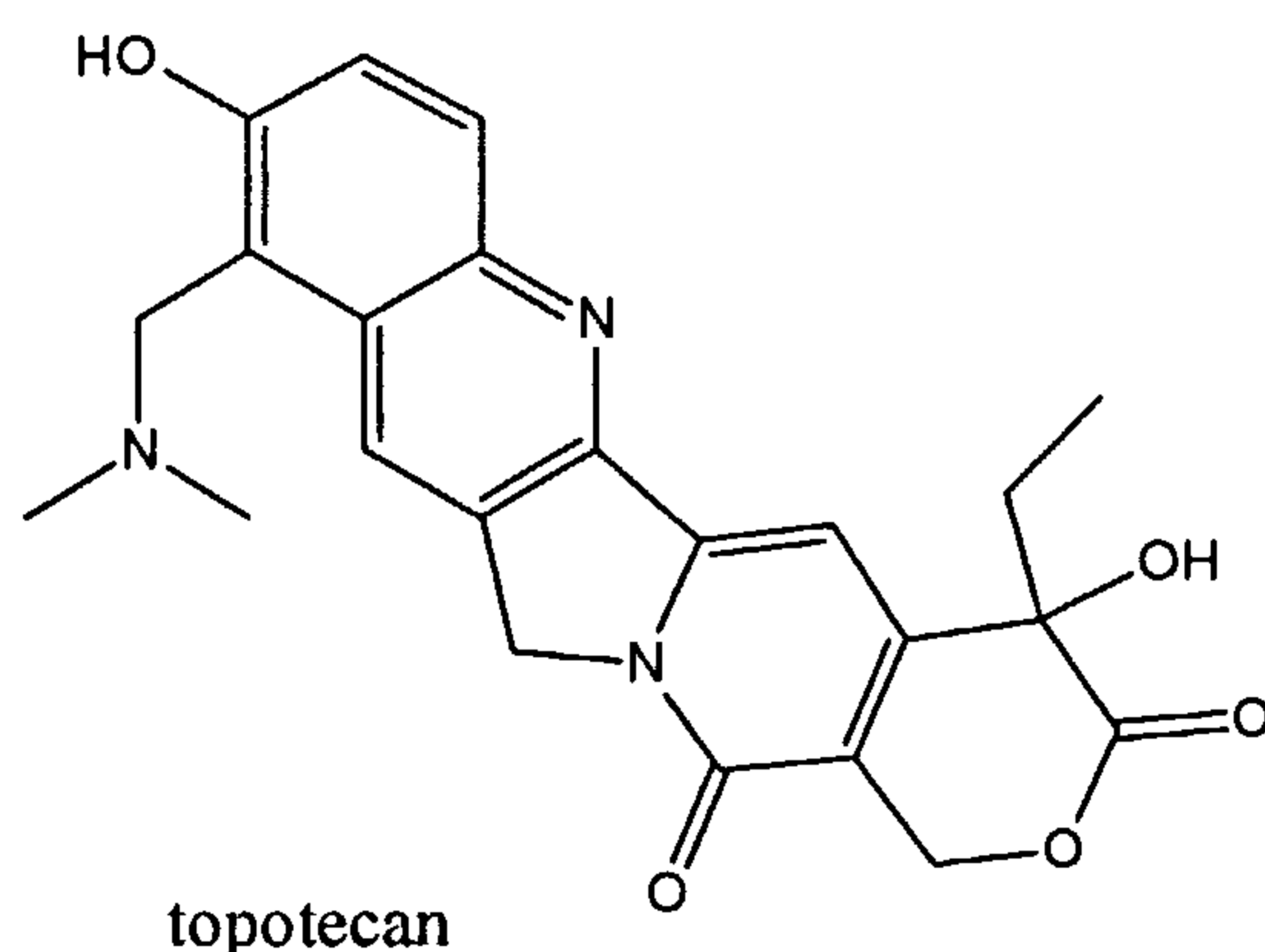
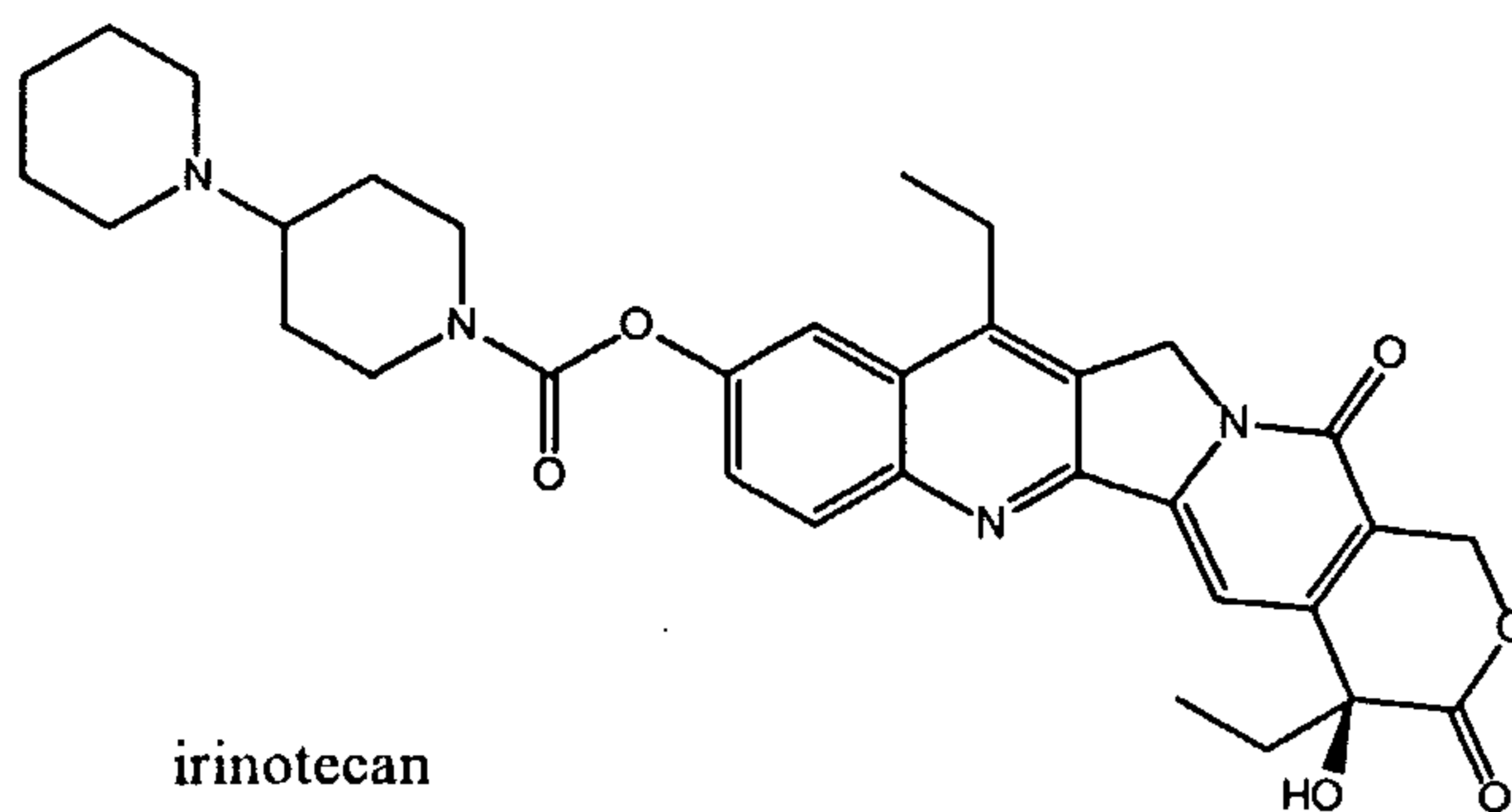
5 wherein:

D_3 - D_7 are independently selected from the group consisting of H, C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls, C_{1-8} alkoxy, C_{1-8} hydroxyalkyls, C_{1-8} aminoalkoxy, aryloxy, glycols, CO_2R_{13} , R_{14} , nitro, cyano, halo, hydroxyl, amino, SR_{15} , $NR_{16}R_{17}$ or OR_{18} , where D_4 and D_5 optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may contain O, S or NR_{19} groups, where R_{19} is hydrogen or a C_{1-6} alkyl;

D_8 - D_{10} are independently selected from the group consisting of H, C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls and C_{1-8} hydroxyalkyls; and

R_{8-18} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-19} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy.

In other preferred aspects of the invention, the following can be used:



Still further camptothecin analogs which can be used in the practice of the present invention include, 10,11-oxycamptothecin, 10,11-methylene dioxycamptothecin, 7-ethylcamptothecin, 9-amino-camptothecin, to name but a few.

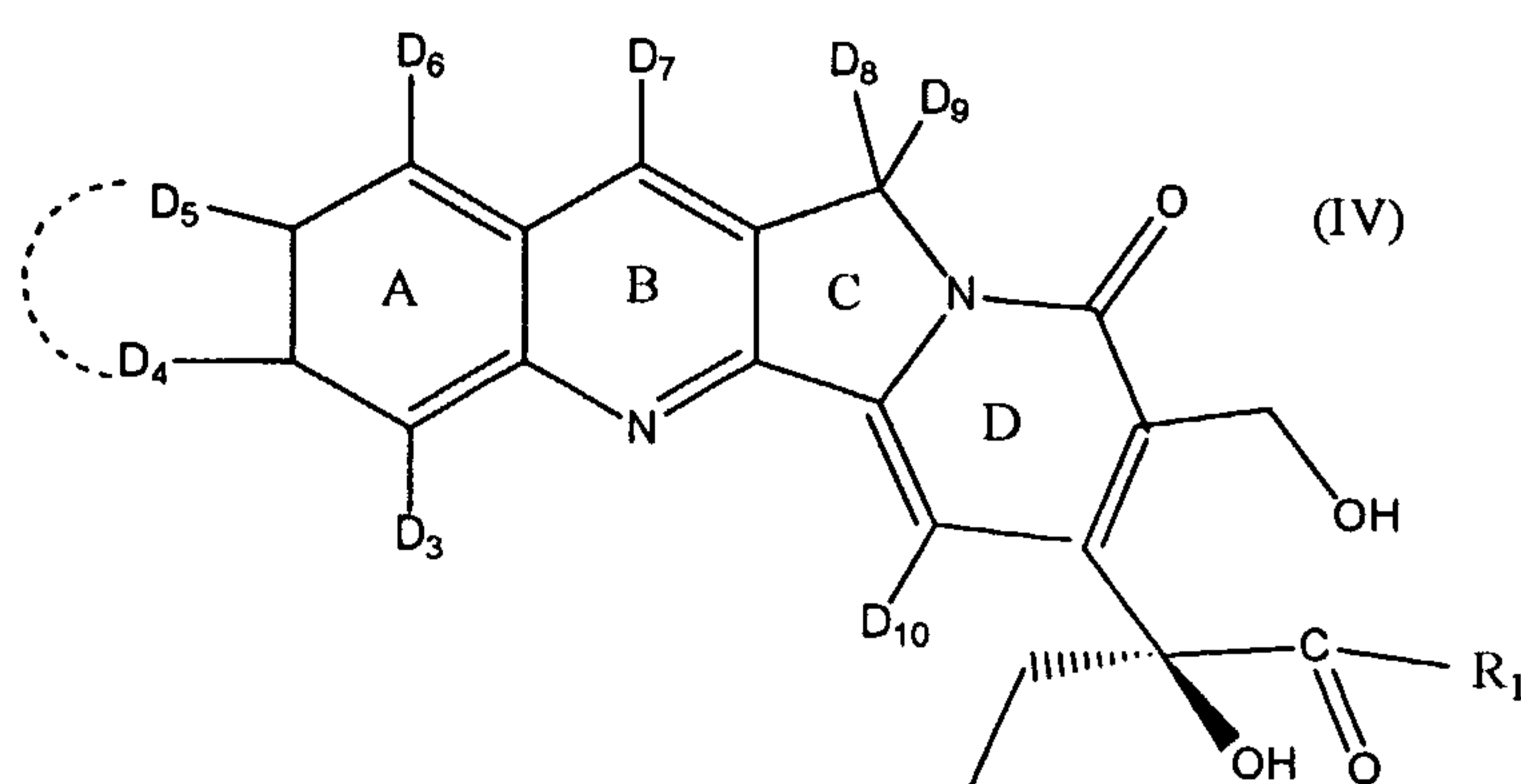
Those of ordinary skill will realize that the foregoing is merely illustrative of the camptothecin derivatives useful in practicing the present invention. The only limitations on the camptothecin derivatives useful in the invention is that the specific derivative must be capable of undergoing the opening of the E-lactone ring, allowing a polymer to be attached thereto and, after administration, allowing hydrolysis to occur which allows the E-lactone ring to be re-formed in vivo.

D. SYNTHESIS OF THE CAMPTOTHECIN-POLYMERIC PRODRUGS

Synthesis of specific representative polymer prodrugs is set forth in the Examples. Generally, however, in one preferred method of preparing the prodrug transport systems of the present invention, the E-lactone ring is opened, a capped

bifunctional linker is attached at the 17-position of the open lactone ring, the blocking group is removed and followed by PEGylation with appropriate solvent and coupling agent, if required. The lactone ring can be opened using known techniques such as by dissolving the camptothecin derivative in *N,N*-
 5 dimethylethylenediamine (**2** in the examples) or in isopropylamine (**9** in the examples) or other suitable reagent solvents containing a primary amine and heating the reaction solution for a sufficient amount of time to allow the ring to be opened.

Once the E ring has been opened, the artisan is provided with a
 10 camptothecin derivative of the formula:



wherein:

- R_1 is selected from the group consisting of amino acid residues, peptide residues containing from about 2 to about 10 amino acids, $Y_3-(L_2)_p-A_2$ and R_2 ;
 15 Y_3 is O, S or NR_3 ;
 p is zero or one;
 L_2 is a bifunctional linker;
 A_2 is selected from the group consisting of hydrogen, amino protecting groups, NR_8R_9 , amino acid residues, peptide residues containing from about 2 to about 10
 20 amino acids; polymeric residues, R_{10} , SCR_{11} , $NC(O)R_{12}$;

D_3 - D_7 are independently selected from the group consisting of H, C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls, C_{1-8} alkoxys,

C₁₋₈ hydroxyalkyls, C₁₋₈ aminoalkoxy, aryloxys, glycals, CO₂R₁₃, R₁₄, nitro, cyano, halo, hydroxyl, amino, SR₁₅, NR₁₆R₁₇ or OR₁₈, where D₄ and D₅ optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may contain O, S or NR₁₉ groups, where R₁₉ is hydrogen or a C₁₋₆ alkyl;

5 D₈-D₁₀ are independently selected from the group consisting of H, C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls and C₁₋₈ hydroxyalkyls; and

R₂₋₁₈ are independently selected from the group consisting of hydrogen,
10 C₁₋₆ alkyls, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy;

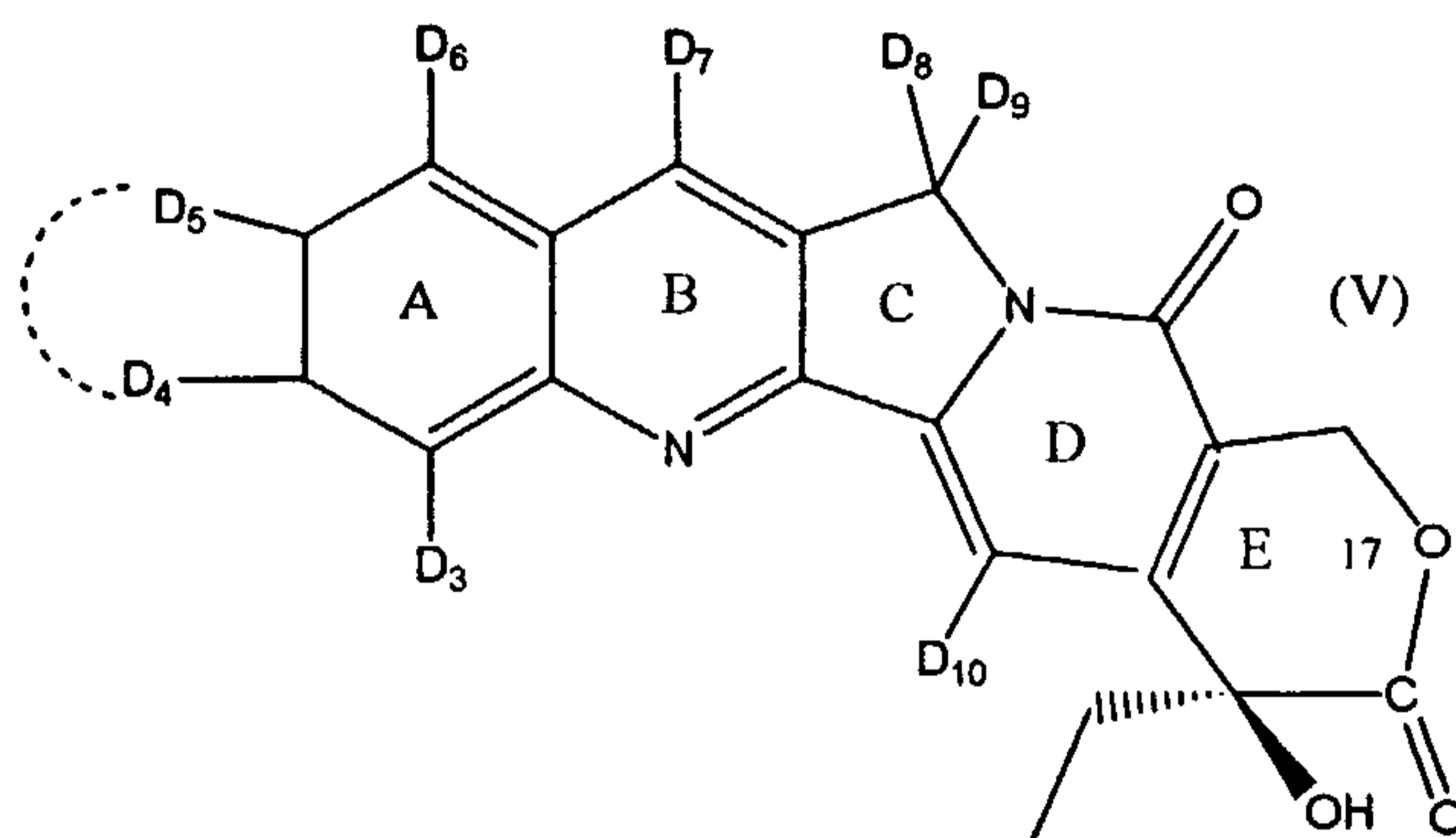
which is then reacted with a blocked bifunctional spacer to form a protected intermediate. This intermediate is then deblocked and reacted with an activated
15 polymer under conditions sufficient to cause a polymeric conjugate to be formed.

A non-limiting list of activated polymers include bis-succinimidyl carbonate activated PEG (SC-PEG), bis-thiazolidine-2-thione activated PEG (T-PEG), N-hydroxyphthalimidyl carbonate activated PEG (BSC-PEG), see
20 herein by reference, succinimidyl succinate activated PEG (SS-PEG), imidazole-activated PEG (PEG-IMD), bis-carboxylic acid- PEG (PEG-CO₂H) and mono-activated PEG's such as those found in, for example, in the aforementioned 2001 Shearwater Catalog.

Conjugation of the intermediate to the PEG residue can be carried out in the
25 presence of a coupling agent. A non-limiting list of suitable coupling agents include 1,3-diisopropylcarbodiimide (DIPC), any suitable dialkyl carbodiimide, 2-halo-1-alkyl-pyridinium halides (Mukaiyama reagents), 1-(3-dimethylamino-propyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophosphates, etc. which are available, for example
30 from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituents are reacted in an inert solvent such as tetrahydrofuran (THF), acetonitrile (CH₃CN), methylene chloride (DCM), chloroform (CHCl₃), dimethylformamide (DMF) or mixtures thereof. The reaction is preferably conducted in the presence of a base, such as dimethylaminopyridine (DMAP), diisopropyl ethylamine, pyridine, triethylamine, KOH, potassium t-butoxide and NaOH, etc. and at a temperature from 0° C up to about 22° C (room temperature).

In an alternative embodiment, the compositions of the present invention are prepared by using the blocked bifunctional spacer to open the E-lactone ring in an inert solvent, acylating the 17-position OH group with, for example acetic or butyric anhydride, deblocking the bifunctional spacer and attaching the polymer. Specifically, the reaction involves reacting a compound of the formula



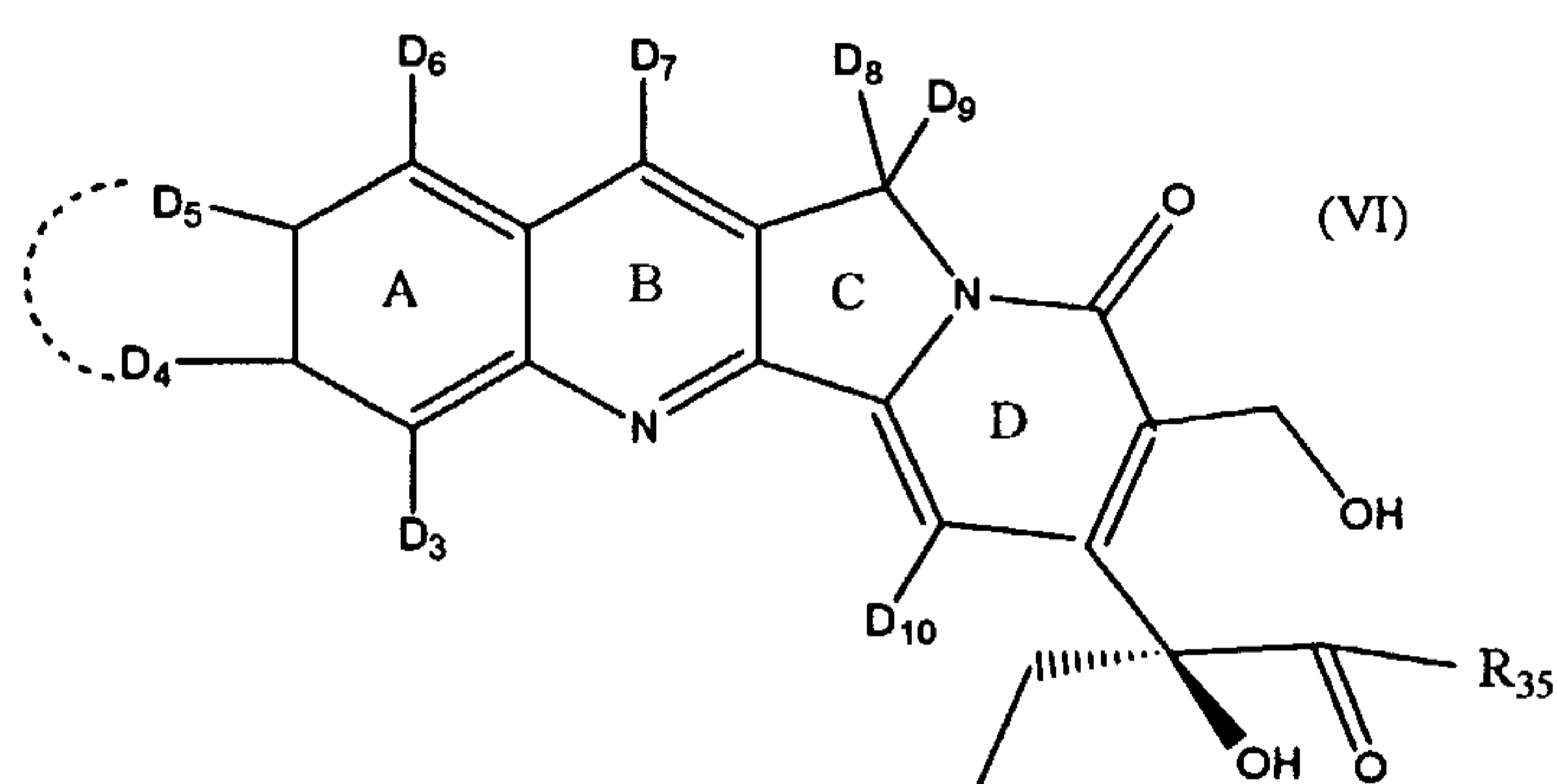
wherein:

D₃ -D₇ are independently selected from the group consisting of H, C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls, C₁₋₈ alkoxy, C₁₋₈ hydroxyalkyls, C₁₋₈ aminoalkoxy, aryloxy, glycols, CO₂R₁₃, R₁₄, nitro, cyano, halo, hydroxyl, amino, SR₁₅, NR₁₆R₁₇ or OR₁₈, where D₄ and D₅ optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may contain O, S or NR₁₉ groups, where R₁₉ is hydrogen or a C₁₋₆ alkyl;

D₈-D₁₀ are independently selected from the group consisting of H, C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls and C₁₋₈ hydroxyalkyls; and

R_{8-18} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-19} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

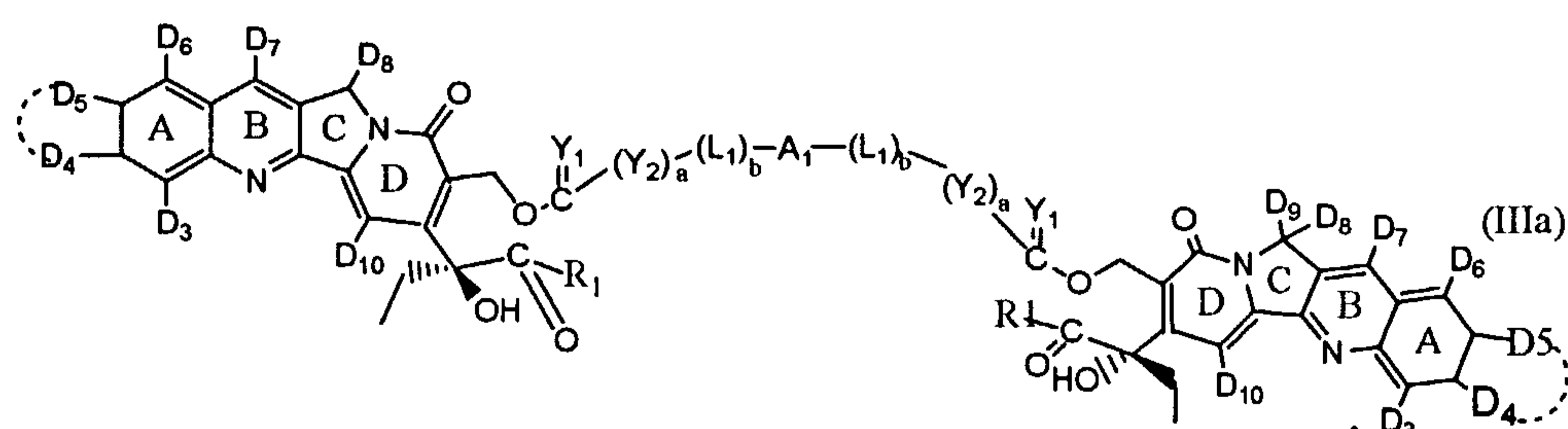
- 5 with a blocked bifunctional spacer in an inert solvent under conditions sufficient to open the E lactone and form a blocked intermediate of formula (VI)



wherein R_{35} is a residue of a blocked bifunctional spacer;

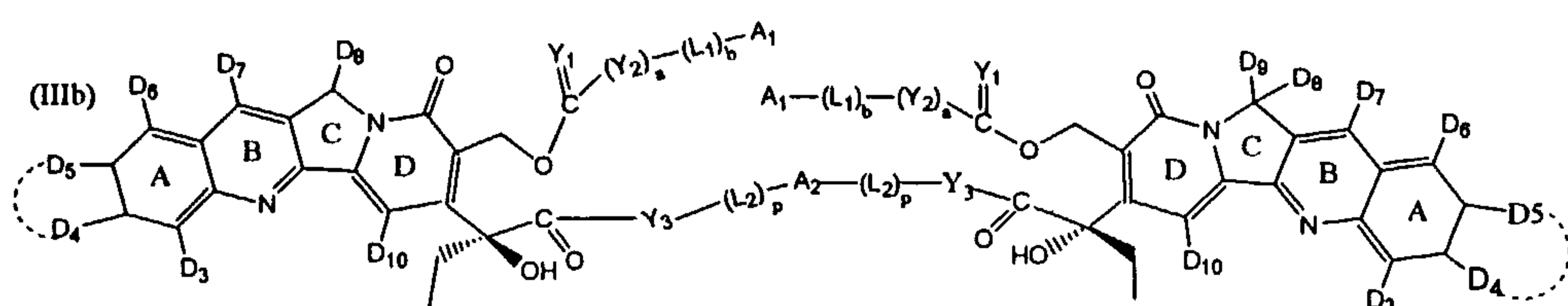
- 10 b) acylating the C_{17} hydroxyl of the blocked intermediate, and
c) deprotecting the blocked intermediate and reacting it with an activated polymer under conditions sufficient to cause a polymeric conjugate to be formed.

Regardless of the synthesis selected, some of the preferred compounds which result from the synthetic techniques described herein include:

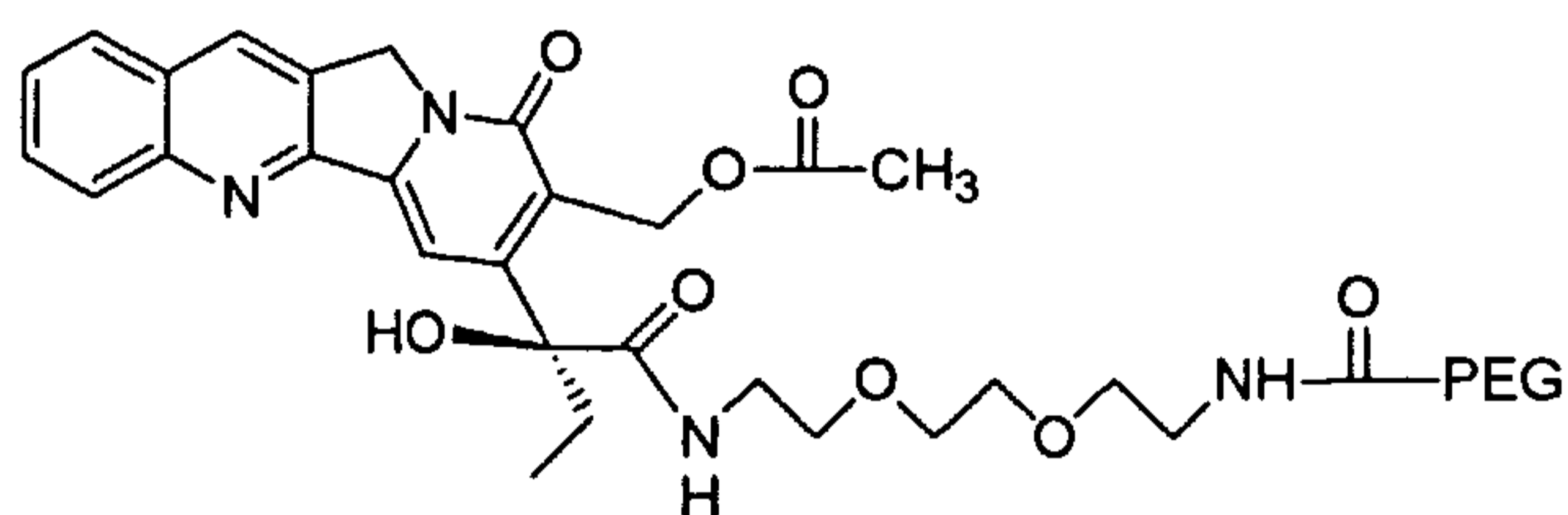
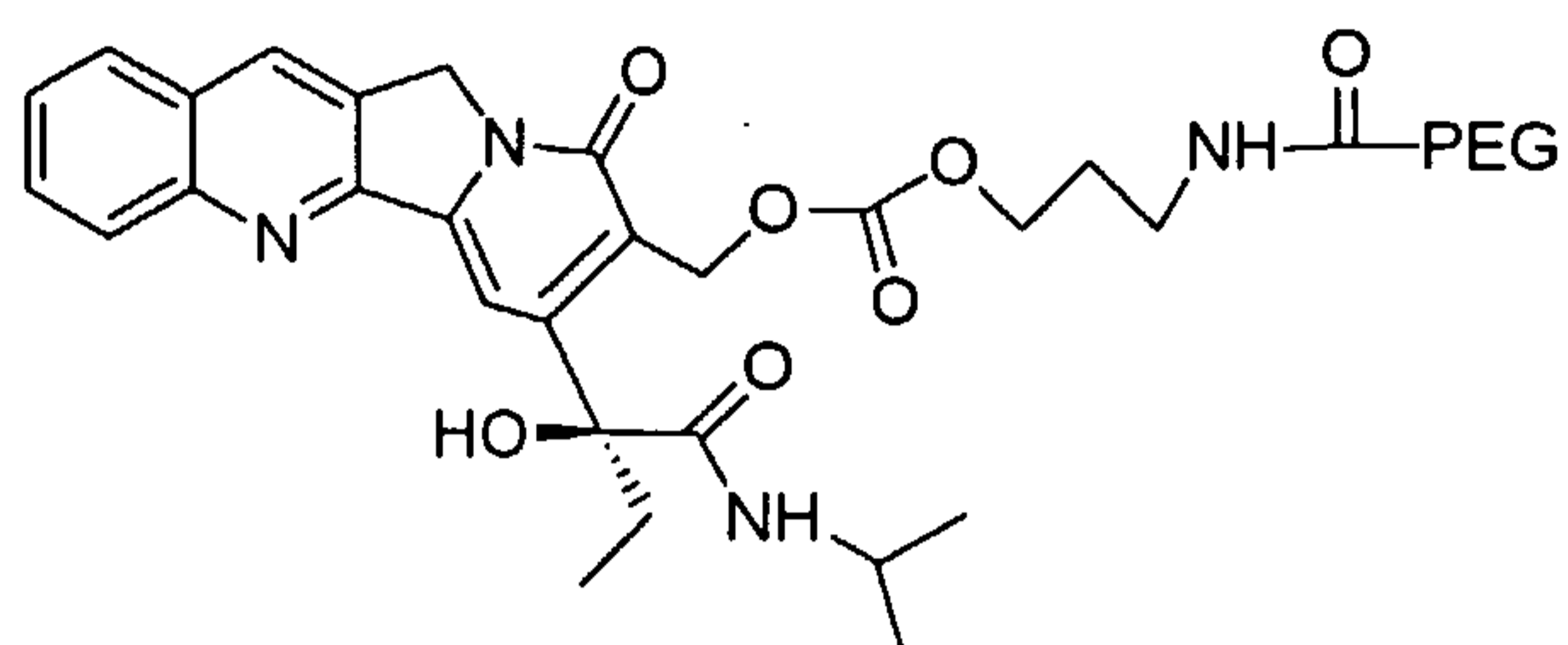
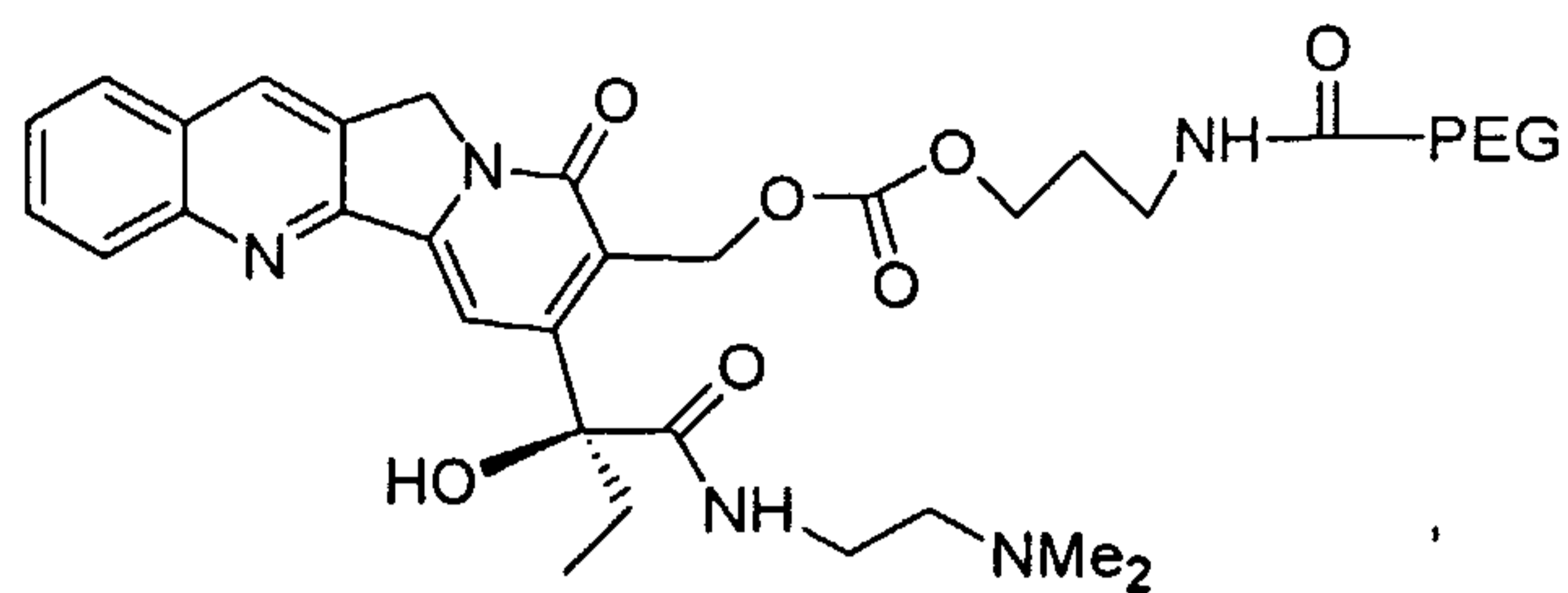


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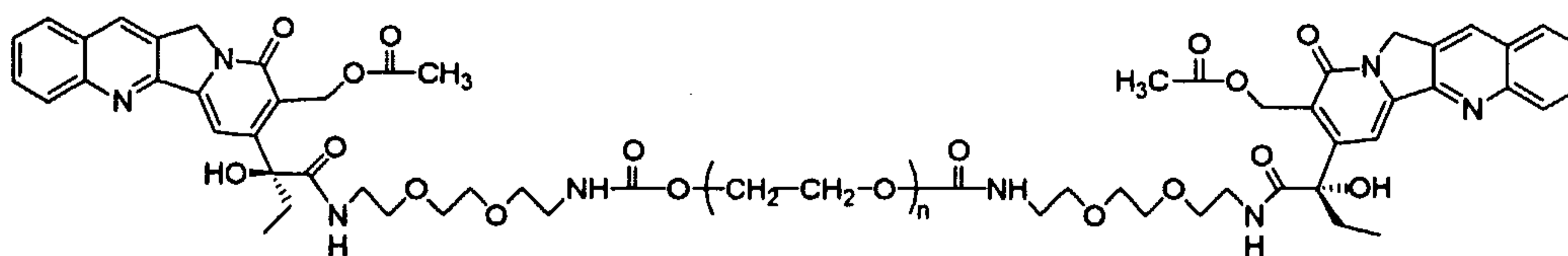
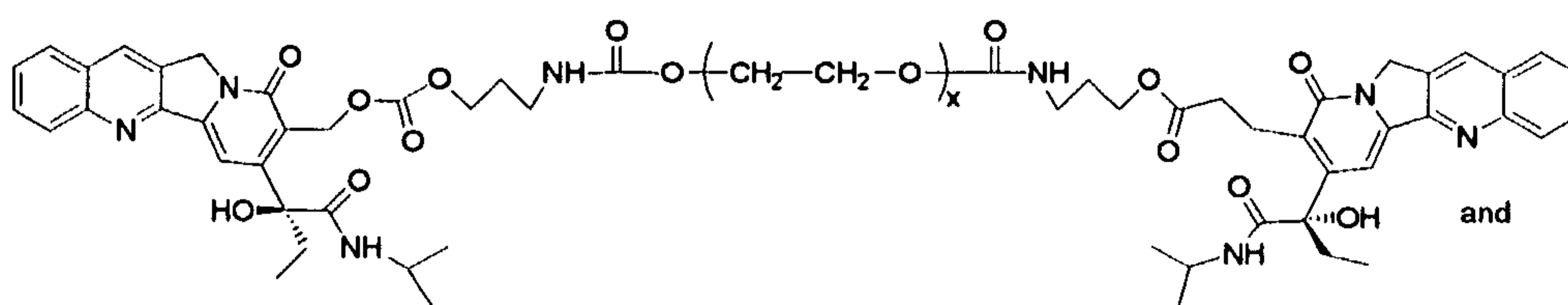
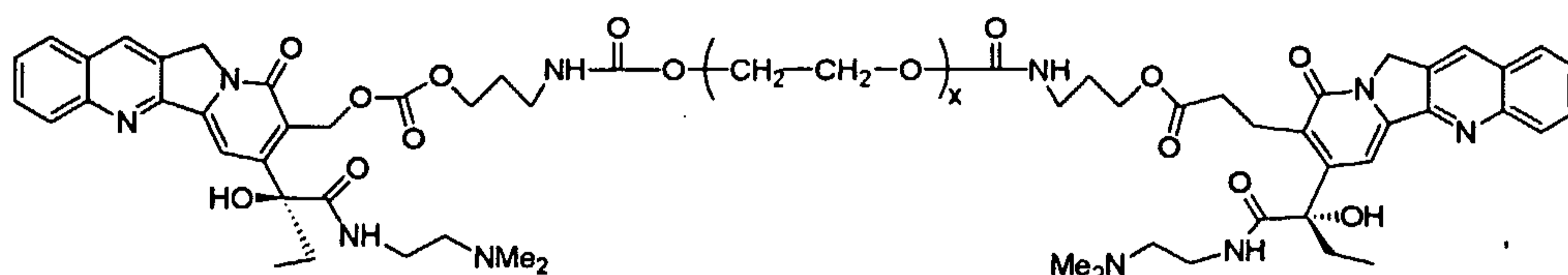
and



where all variables are as previously defined and



5



where PEG = Me-O-(CH₂CH₂O)_x- or -O-(CH₂CH₂O)_x-.

10

E. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various medical conditions, including topoisomerase inhibitor-related diseases in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, of a camptothecin, camptothecin derivative, camptothecin analog and/or a mixture thereof, such as, those described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor/neoplastic growths in mammals.

10 The amount of the inventive composition administered will depend upon several factors, including potency of the parent molecule included therein and specific disease being treated. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis of the open E ring, the molecular weight of the polymer used, if any, etc. In general, dosing is based upon the amount of native (unmodified E-lactone ring) camptothecin or derivative thereof. The camptothecin and derivatives of camptothecin are generally administered in amounts ranging from about 5 to 15 about 500 mg/m² per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication.

The compositions, including prodrugs, of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral, inhalation and/or parenteral routes depending upon the needs of the artisan. A solution and/or suspension of the composition may be utilized, for example, as a carrier vehicle for injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and 25 30

the like. Such administration may also be by infusion into a body space or cavity, as well as by inhalation and/or intranasal routes. In preferred aspects of the invention, however, the prodrugs are parenterally administered to mammals in need thereof.

5

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. All compounds mentioned in the following examples are numbered with reference to Figs 1-6.

10

General Procedures. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were 40kDa and were dried under vacuum or by azeotropic distillation from toluene prior to use. ¹³C NMR spectra were obtained at 75.46 MHz using a

15

Varian Mercury[®]300 NMR spectrometer and deuterated chloroform as the solvent unless otherwise specified. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). All PEG conjugated compounds were dissolved in saline for injection prior to in vivo drug treatments and were given as their camptothecin equivalents (absolute amount of camptothecin given).

20

HPLC method. The reaction mixtures and the purity of intermediates and final products were monitored by a Beckman Coulter System Gold[®] HPLC instrument employing a ZOBAX[®] 300 SB C-8 reversed phase column (150 x 4.6 mm) or a Phenomenex Jupiter[®] 300A C18 reversed phase column (150 x 4.6 mm) with a multiwavelength UV detector, using a gradient of 30-90 % of acetonitrile in 0.5 % trifluoroacetic acid (TFA) at a flow rate of 1 mL/min.

25

Example 1

Compound 5. Camptothecin (1) (1.44 g, 4.14 mmol) was dissolved in *N,N*-dimethylethylenediamine (2) (15 mL) and the reaction solution was heated at 50 °C for 2 hours. The solvent was removed under reduced pressure and the resulting solid washed with ether to give 3, which was suspended with dimethylamino-

30

pyridine (DMAP, 1.0 g, 8.88 mmol) and 4 (1.3 g, 4.44 mmol) in anhydrous

methylene chloride (DCM, 10 mL). The resulting reaction mixture was refluxed for 4 hours and then stirred at room temperature for 12 hours, washed with 0.1N HCl, dried with MgSO₄, filtered, and solvent evaporated under reduced pressure. The crude material was purified on silica gel column to give **5** (0.080 g, 0.13 mmol, 3 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 167.26, 157.15, 155.77, 153.69, 152.18, 148.76, 146.33, 145.71, 131.14, 130.65, 129.56, 128.37, 128.10, 128.02, 120.10, 95.82, 79.14, 77.83, 77.20, 67.01, 66.50, 50.03, 36.97, 31.84, 29.01, 28.37, 25.59, 7.73.

Example 2

Compound **6**. To a solution of **5** (0.080 g, 0.13 mmol) in DCM (1 mL) cooled in an ice bath, trifluoroacetic acid (TFA, 1 mL) was added drop-wise and stirred for 20 min. The solvents were then removed under reduced pressure to give **6** (0.080 g, 0.13 mmol, ~100 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 167.50, 157.01, 153.95, 146.42, 145.31, 143.81, 134.69, 132.80, 129.26, 129.08, 128.52, 126.56, 120.92, 98.92, 78.27, 77.21, 66.69, 65.27, 50.47, 37.15, 31.33, 26.55, 25.58, 7.53.

Example 3a

Compound **8a**. A solution of **6** (0.080 g, 0.13 mmol), **7a** (1.50 g, 0.037 mmol), and DMAP (0.031 g, 0.25 mmol) in DCM (8 mL) was stirred at room temperature for 12 hours. The reaction mixture was washed with 0.1N HCl (2x10 mL) and the organic layer evaporated under reduced pressure. The solid residue was crystallized from isopropyl alcohol (IPA, 30 mL) to yield **8a** (1.2 g, 0.029 mmol, 79 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 166.41, 156.36, 155.64, 152.92, 151.50, 148.01, 145.76, 144.89, 130.76, 129.90, 128.84, 127.95, 127.58, 127.29, 119.31, 94.90, 67.0-73.5 (PEG), 66.29, 65.78, 63.05, 49.46, 36.69, 31.12, 28.39, 7.14.

Example 3b

Compound **8b**. The procedure of Example 3a is followed, except that PEG-CO₂H **7b** (1.5 g, 0.037 mmol) and 2 equivalents of EDC is used in place of **7a** to form an amide-linked PEG conjugate.

Example 4

Compound **11**. **1** (1.44g, 4.14 mmol) was dissolved in isopropylamine **9** (35ml) and the solution refluxed for 1.5 hours. The solvent was removed under reduced

pressure and the resulting solid washed with ether to give **10**, which was suspended with DMAP (0.29 g, 2.36 mmol) and **4** (0.36 g, 1.18 mmol) in anhydrous chloroform (2 mL). The resulting reaction mixture was heated at 60 °C for 1 hour, washed with 0.1N HCl, dried (MgSO₄), filtered, and the solvent evaporated under reduced pressure. The crude material was purified by column chromatography to give **11** (0.068 g, 0.11 mmol, 3 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 171.26, 161.43, 156.41, 155.92, 155.33, 152.09, 148.46, 144.57, 130.66, 130.22, 129.37, 128.37, 127.90, 127.71, 123.90, 100.57, 79.17, 77.20, 75.92, 65.22, 62.13, 50.27, 41.72, 37.09, 32.87, 29.33, 28.46, 22.74, 22.44, 7.90.

10 **Example 5**

Compound **12**. To a solution of compound **11** (0.68 g, 0.11 mmol) in DCM (1 mL), cooled in an ice bath, was added TFA (1 mL) drop-wise and the solution stirred for 2 hours. The solvents were removed under reduced pressure to give **12** (0.067 g, 0.11 mmol, ~100 %). ¹³C NMR (67.8 MHz, CDCl₃/CD₃OD) δ 171.74, 161.75, 157.19, 154.76, 152.01, 148.27, 144.55, 131.41, 130.62, 128.90, 128.58, 128.08, 127.91, 123.53, 101.24, 78.50, 64.99, 62.33, 50.23, 41.55, 37.06, 32.19, 26.35, 22.32, 22.00, 7.55.

Example 6a

Compound **13a**. A solution of **12** (0.067 g, 0.11 mmol), **7a**, (1.30 g, 0.032 mmol), and DMAP (0.027 g, 0.22 mmol) in DCM (8 mL) was stirred at room temperature for 12 hours. The reaction mixture was washed with 0.1N HCl (2x10 mL) and the organic layer evaporated under reduced pressure. The solid residue was crystallized from IPA (30 mL) to yield **13a** (1.1 g, 0.028 mmol, 86 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 170.64, 160.74, 155.82, 154.54, 151.65, 147.90, 144.02, 134.37, 130.35, 129.57, 128.77, 128.03, 127.43, 127.20, 99.61, 67.0-73.5 (PEG), 64.63, 63.23, 61.49, 49.71, 41.07, 37.02, 32.11, 28.74, 22.20, 21.84, 7.41.

Example 6b

Compound **13b**. The procedure of Example **6a** is followed, except that PEG-CO₂H **7b** (1.5 g, 0.037 mmol) is used with 2 equivalents of EDC in place of **7a** to form an amide-linked PEG conjugate.

Example 7

Compound **16**. A mixture of **1** (0.34 g, 0.97 mmol), **14** (4.7 g, 19 mmol), and diisopropylethylamine (DIEA, 3.3 mL, 1.4 mmol) in dimethylformamide (DMF, 7 mL) was heated at 70 °C for 48 hours and a clear solution was obtained. The solvent was removed under reduced pressure, and the resulting solid washed with hexane and then ether to give **15** which was suspended in anhydrous DCM (10 mL) and cooled to 0 °C in an ice bath. Acetic anhydride (0.10 mL, 1.1 mmol) and DMAP (0.057 g, 0.47 mmol) were added to the above suspension and stirred for 3 hours at ambient temperature. The reaction mixture was washed with 0.1 N sodium bicarbonate (10 mL), and the organic layer evaporated under reduced pressure. The residue was purified on silica gel column to give pure **16** (0.25 g, 0.39 mmol, 40 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 172.19, 170.95, 161.38, 156.80, 155.91, 151.42, 148.04, 144.05, 130.42, 130.00, 128.92, 127.89, 127.68, 127.58, 127.26, 124.77, 100.60, 79.15, 78.55, 70.14, 69.37, 58.78, 50.23, 40.33, 39.04, 32.93, 28.40, 20.99, 7.92.

Example 8

Compound **17**. To a solution of **16** (0.12 g, 0.19 mmol) in DCM (1 mL) cooled in an ice bath was added TFA (1 mL) dropwise and stirred for 30 minutes. Solvent was removed under reduced pressure to give **6** (0.12 g, 0.19 mmol, ~100 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 173.01, 172.22, 161.33, 157.08, 149.97, 146.13, 142.92, 132.94, 131.47, 128.55, 128.40, 128.10, 127.67, 127.23, 125.13, 117.41, 113.59, 102.62, 78.65, 70.19, 69.37, 66.48, 58.63, 50.67, 40.06, 39.28, 32.68, 20.78, 7.62.

Example 9a

Compound **18a**. A solution of **17** (0.050 g, 0.090 mmol), **7a** (1.20 g, 0.030 mmol), and DMAP (0.022 g, 0.18 mmol) in DCM (6 mL) was stirred at room temperature for 12 hours. The reaction mixture was washed with 0.1N HCl (2x10 mL) and the organic layer evaporated under reduced pressure. The solid residue was crystallized from IPA (30 mL) to yield **18a** (1.1 g, 0.027 mmol, 89 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 171.82, 170.32, 160.71, 155.75, 155.23, 151.59, 147.82, 143.79, 134.41, 130.30, 129.55, 128.70, 127.90, 127.42, 127.11, 124.07, 99.59, 67.0-73.5 (PEG), 63.14, 58.50, 49.61, 40.17, 38.58, 32.14, 20.48, 7.38.

Example 9b

Compound **18b**. The procedure of Example **9a** is followed, except that PEG-CO₂H **7b** (1.5 g, 0.037 mmol) is used with 2 equivalents of EDC in place of **7a** to form an amide-linked PEG conjugate.

5 Example 10

The efficacy of open-form camptothecin analogs against a subcutaneous human mammary carcinoma (MX-1) grown in nude mice was determined as follows. Following at least one week of acclimation, tumors were established by implanting small tumor fragments from donor mice into a single subcutaneous site, on the left
10 axillary flank region of nude mice. The tumor implantation site was observed twice weekly and measured once palpable. The tumor volume for each mouse was determined by measuring two dimensions with calipers and calculated using the formula: tumor volume = (length x width²). When tumors reached the average volume of approximately 75 mm³, the mice were divided into their experimental
15 groups, which consists of untreated controls, PEG_{40,000}-ala-20(S)-Camptothecin, compound **8**, and compound **18**.

The mice were sorted to evenly distribute tumor size, grouped into 4 to 5 mice/cage, and ear punched for permanent identification. Drugs were dosed intravenously via the tail vein as a single dose (Qd1). Mouse weight and tumor
20 sizes were measured at the beginning of study and twice weekly through week 5. The overall growth of tumors was calculated as the mean tumor volume at one week following the end of the treatment. A percent treatment over control (T/C) value was also calculated when the control group's median tumor size reached approximately 800-1100 mm³ and again at one week following treatment. The T/C
25 value in percent is a non-quantitative indication of anti-tumor effectiveness. Treatments with PEG_{40,000}-ala-20(S)-Camptothecin, compounds **8** and **18** all caused significantly (P<0.05) smaller tumor volumes as compared to control mice. In addition, at equivalent doses, there was no significant (P< 0.05) difference in the antitumor activity of these three compounds.

30

Table 2. Efficacy Summary of PEG-Camptothecin Against a Human Mammary Carcinoma (MX-1) Xenograft in Nude Mice^a

Compound	Treatment Schedule (mg/kg/dose)	Tumor Volume (mean \pm sem) Day 21	T/C (%) ^b At 1000 mm ³	Tumor Regression at Day 25 (#/grp)
Control	0	2722 \pm 223	--	0/5
PEG _{40,000} -ala-20(S)-Campto.	Qd1 (24)	15 \pm 5	1.4	5/5
8	Qd1 (24)	21 \pm 6	3.0	5/5
18	Qd1 (24)	14 \pm 2	2.5	4/4

5 α Intravenous treatment in nude mice bearing established tumors (~ 75 mm³). N=4 to 5/group.

β The median tumor volume of treatment and control groups were measured and compared when the control group's median tumor volume reached approximately 1000 mm³ and one week after final dosage (Day 15). T/C < 42% at 1000 mm³ is
10 considered significant anti-tumor activity by the Drug Evaluation Branch of the NCI.

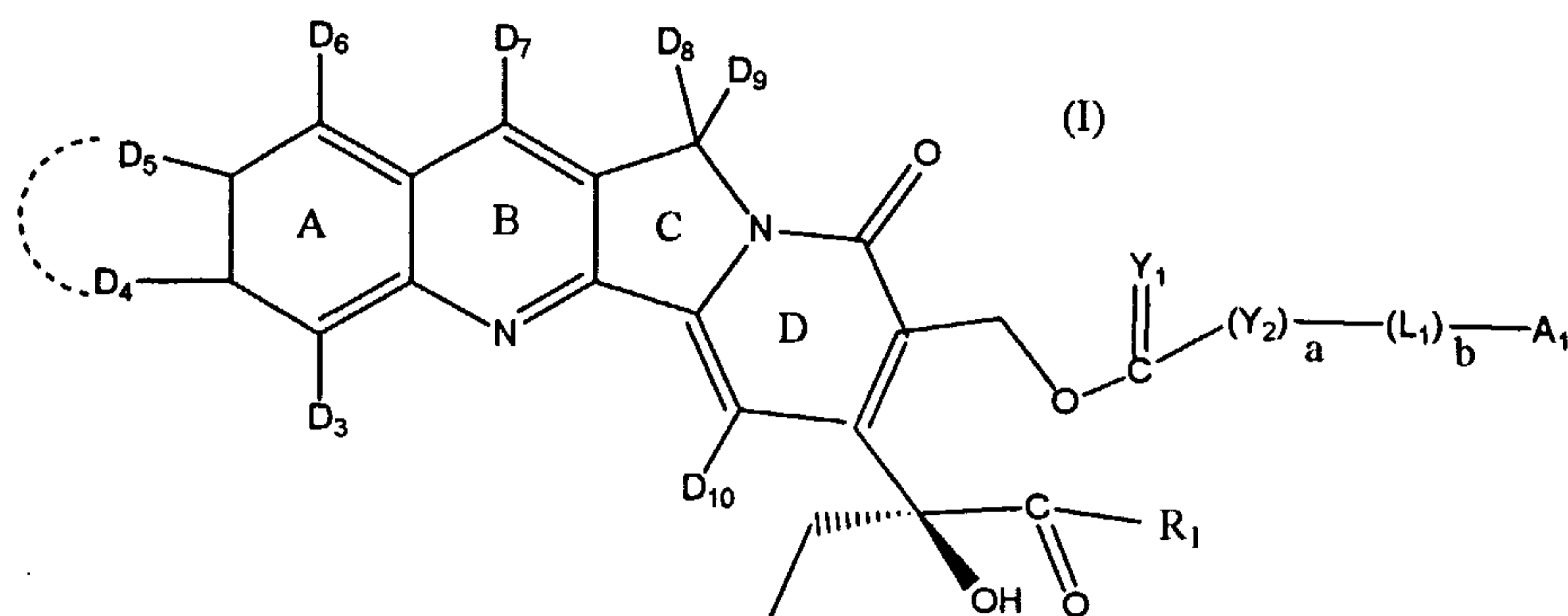
While applicants are not bound by theory, it is believed that significant conversion of the open lactone E ring into the closed lactone ring has occurred in order to achieve the observed tumor regression.

15 The various publications, patents, patent applications and published applications mentioned in this application are hereby incorporated by reference herein in their entireties.

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that
20 changes and modifications may be made without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

WE CLAIM:

1. A compound comprising Formula I:



wherein:

R_1 is selected from the group consisting of amino acid residues, peptide residues containing from about 2 to about 10 amino acids, $Y_3-(L_2)_p-A_2$ and R_2 ;

Y_3 is O, S or NR_3 ;

p is zero or one;

L_2 is a bifunctional linker;

Y_1 is O, S or NR_4 ;

Y_2 is O, S, CR_5R_6 or NR_7 ;

L_1 is a bifunctional linker;

a and b are independently zero or one;

A_1 and A_2 are independently selected from the group consisting of hydrogen, amino protecting groups, NR_8R_9 , amino acid residues, peptide residues containing from about 2 to about 10 amino acids; polymeric residues, R_{10} , SR_{11} , $NC(O)R_{12}$;

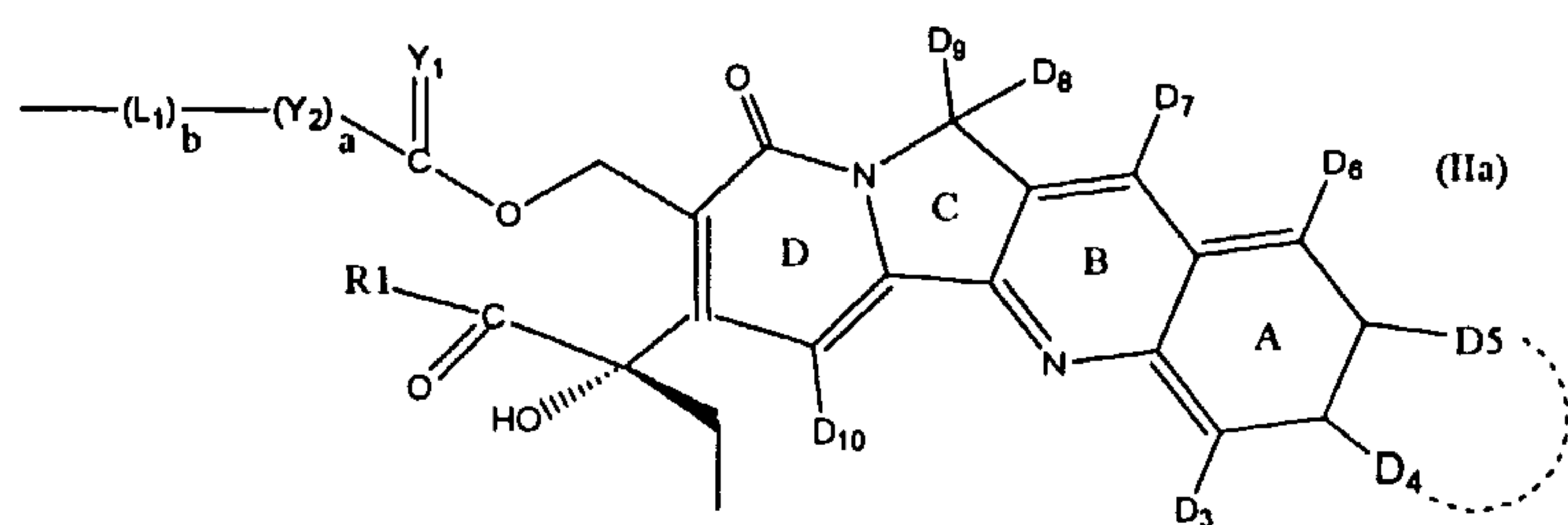
D_3 - D_7 are independently selected from the group consisting of H, C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls, C_{1-8} alkoxy, C_{1-8} hydroxyalkyls, C_{1-8} aminoalkoxy, aryloxy, glycals, CO_2R_{13} , R_{14} , nitro, cyano, halo, hydroxyl, amino, SR_{15} , $NR_{16}R_{17}$ or OR_{18} , where D_4 and D_5 optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may contain O, S or NR_{19} groups, where R_{19} is hydrogen or a C_{1-6} alkyl;

D_8 - D_{10} are independently selected from the group consisting of H,

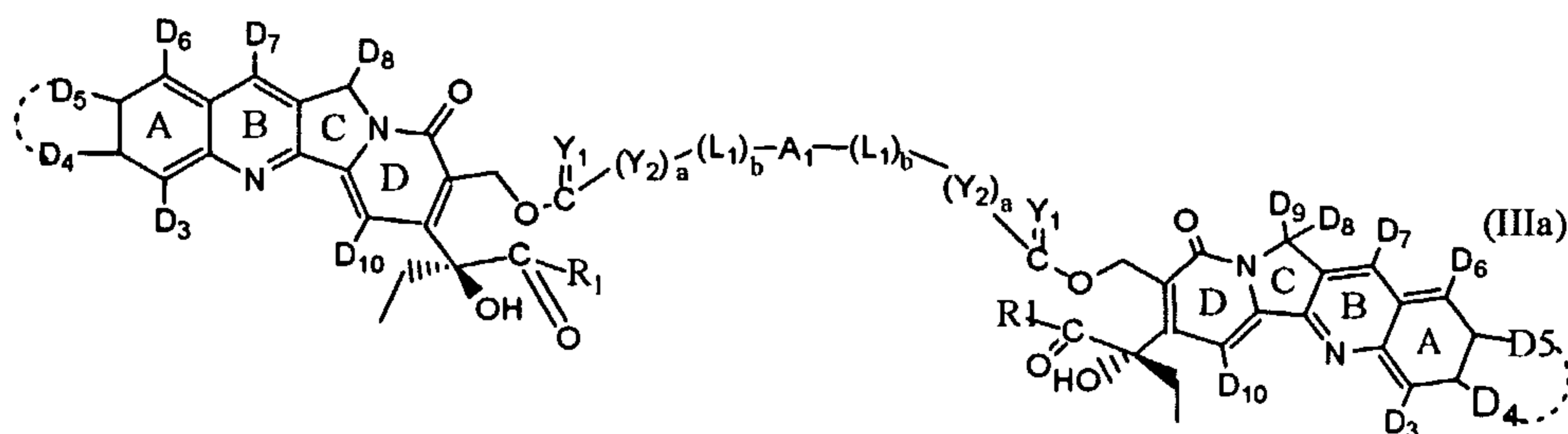
C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls and C₁₋₈ hydroxyalkyls; and

R₂₋₁₈ are independently selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy; provided that at least one of A₁ and A₂ comprise a polymeric residue.

2. The compound of claim 1, wherein Y₁ and Y₂ are each O.
3. The compound of claim 1, wherein R₂-R₁₄ and D₃ - D₁₀ are each hydrogen.
4. The compound of claim 1, wherein A₁ is a polymer residue.
5. The compound of claim 4, wherein said polymer residue further includes a capping group J, selected from the group consisting of OH, NH₂, SH, CO₂H, C₁₋₆ alkyl moieties and



6. A compound of claim 5, of the formula:



14. The compound of claim 6, wherein A_1 comprises a polyethylene glycol residue.

15. The compound of claim 9, wherein A_2 comprises a polyethylene glycol residue.

16. The compound of claim 12, wherein A_1 is selected from the group consisting of $J-O-(CH_2CH_2O)_x-$,
 $J-O-(CH_2CH_2O)_x-CH_2C(O)-O-$,
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 NR_{20}-$,
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 SH-$,
 $-O-C(O)CH_2-O-(CH_2CH_2O)_x-CH_2C(O)-O-$,
 $-NR_{20}CH_2CH_2-O-(CH_2CH_2O)_x-CH_2CH_2 NR_{20}-$, and
 $-SHCH_2CH_2-O-(CH_2CH_2O)_x-CH_2CH_2 SH-$,

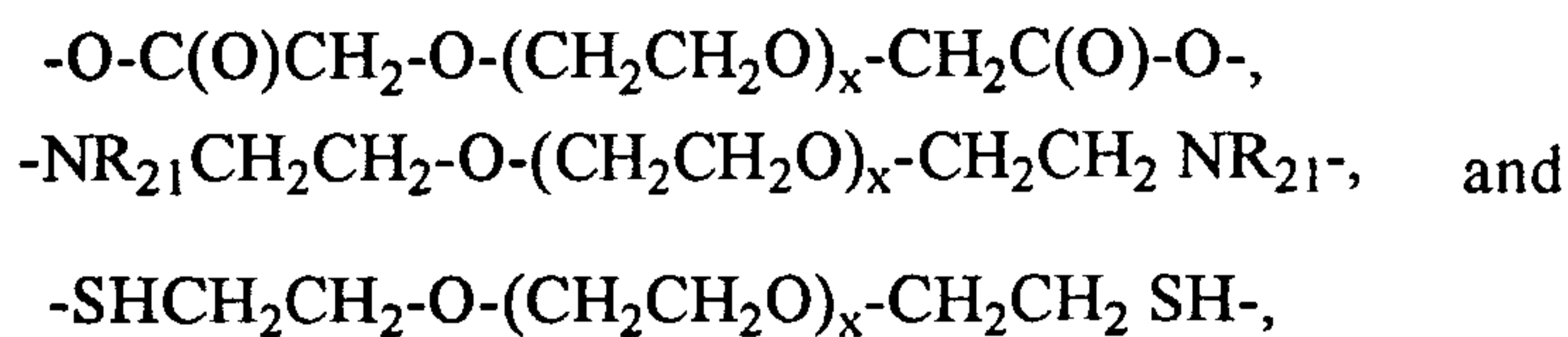
wherein:

x is the degree of polymerization;

R_{20} is selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy and

J is a capping group.

17. The compound of claim 13, wherein A_2 is selected from the group consisting of $J-O-(CH_2CH_2O)_x-$,
 $J-O-(CH_2CH_2O)_x-CH_2C(O)-O-$,
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 NR_{21}-$,
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 SH-$,



wherein:

x is the degree of polymerization;

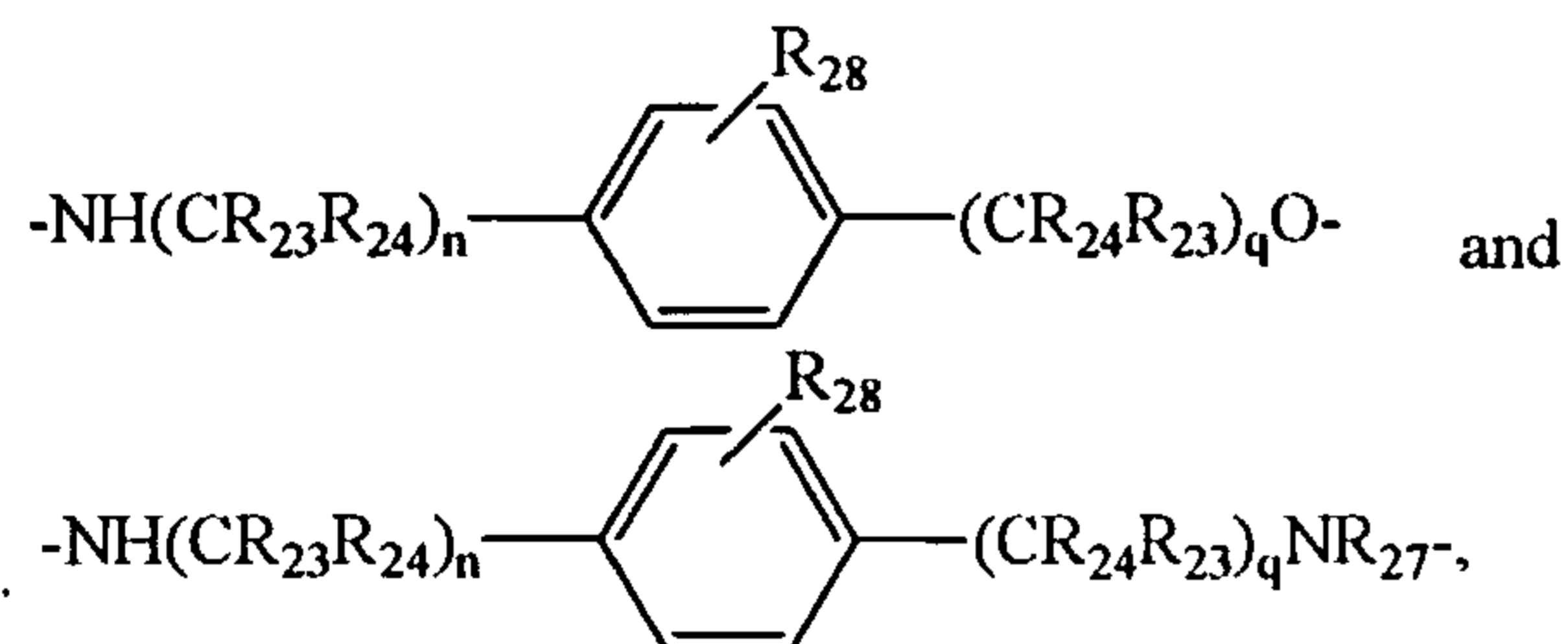
R_{21} is selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy and

J is a capping group.

18. The compound of claim 16, wherein A_1 comprises $-\text{O}-(\text{CH}_2\text{CH}_2\text{O})_x-$ and x is a positive integer selected so that the weight average molecular weight is at least about 20,000 Da.
19. The compound of claim 17, wherein A_2 comprises $-\text{O}-(\text{CH}_2\text{CH}_2\text{O})_x-$ and x is a positive integer selected so that the weight average molecular weight thereof is at least about 20,000 Da.
20. The compound of claim 16, wherein A_1 has a weight average molecular weight of from about 20,000 Da to about 100,000 Da.
21. The compound of claim 17, wherein A_2 has a weight average molecular weight of from about 20,000 Da to about 100,000 Da.
22. The compound of claim 16, wherein A_1 has a weight average molecular weight of from about 25,000 Da to about 60,000 Da.
23. The compound of claim 17, wherein A_2 has a weight average molecular weight of from about 25,000 Da to about 60,000 Da.

24. The compound of claim 1, wherein L_1 is selected from the group consisting of:

$(CH_2)_n$,
 $(CH_2)_3NH-C(O)$
 $(CH_2)_3NH-$
 $-NH(CH_2CH_2O)_n(CH_2)_nNR_{22}-$,
 $-NH(CH_2CH_2O)_n-$,
 $-NH(C_{23}R_{24})_nO-$,
 $-C(O)(CR_{23}R_{24})_nNHC(O)(CR_{25}R_{26})_qNR_{27}-$,
 $-C(O)O(CH_2)_nO-$,
 $-C(O)(CR_{23}R_{24})_nNR_{27}-$,
 $-C(O)NH(CH_2CH_2O)_n(CH_2)_nNR_{27}-$,
 $-C(O)O-(CH_2CH_2O)_nNR_{27}-$,
 $-C(O)NH(CR_{23}R_{24})_nO-$,
 $-C(O)O(CR_{23}R_{24})_nO-$,
 $-C(O)NH(CH_2CH_2O)_n-$,



wherein

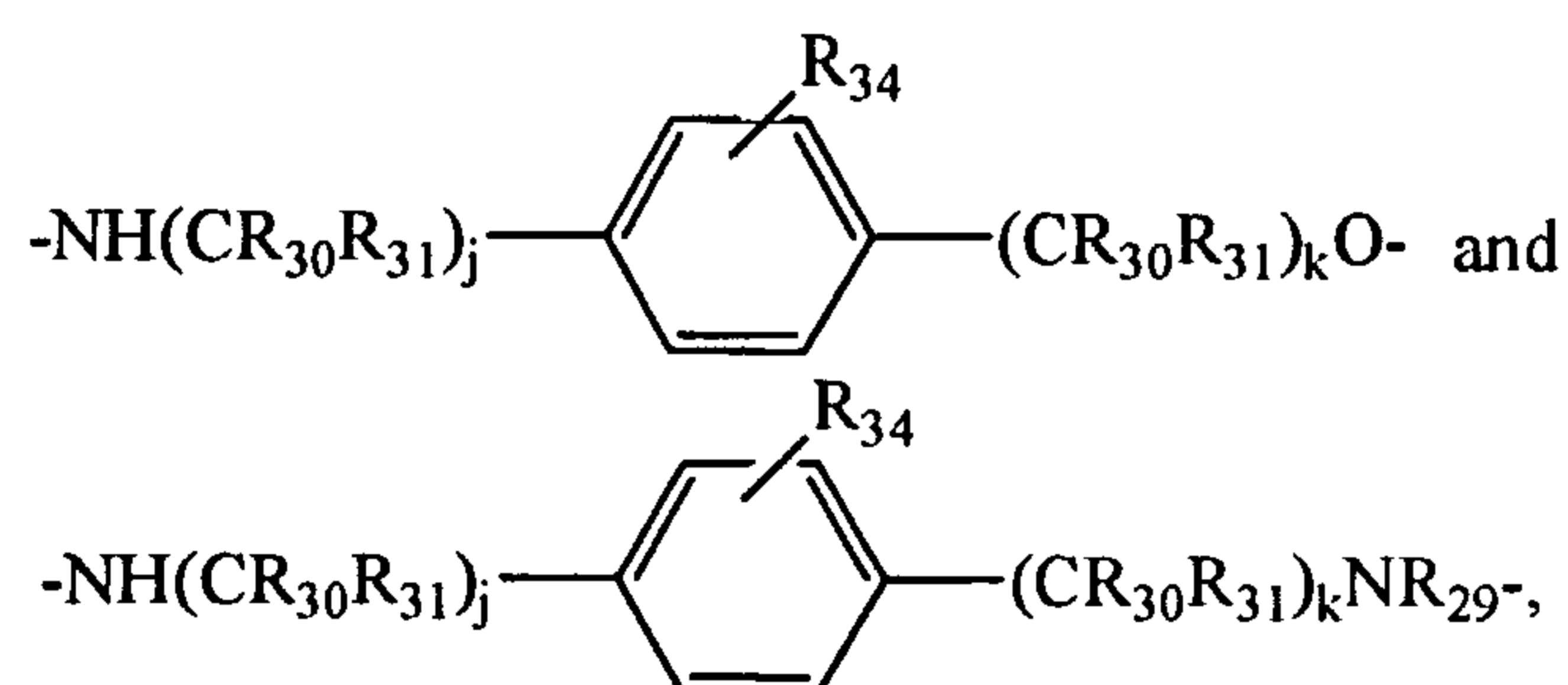
R_{22-27} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

R_{28} is selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy, NO_2 , haloalkyl and halogen; and

n and q are independently selected positive integers.

25. The compound of claim 1, wherein L_2 is selected from the group consisting of:

$(CH_2)_j$
 $(CH_2CH_2O)_2(CH_2)_2NH$,
 $-NH(CH_2CH_2O)_j(CH_2)_kNR_{29^-}$,
 $-NH(CH_2CH_2O)_j-$,
 $-NH(CR_{30}R_{31})_jO-$,
 $-C(O)(CR_{30}R_{31})_jNHC(O)(CR_{32}R_{33})_jNR_{29^-}$,
 $-C(O)O(CH_2)_kO-$,
 $-C(O)(CR_{30}R_{31})_jNR_{29^-}$,
 $-C(O)NH(CH_2CH_2O)_j(CH_2)_kNR_{29^-}$,
 $-C(O)O-(CH_2CH_2O)_jNR_{29^-}$,
 $-C(O)NH(CR_{30}R_{31})_jO-$,
 $-C(O)O(CR_{30}R_{31})_jO-$,
 $-C(O)NH(CH_2CH_2O)_j-$,



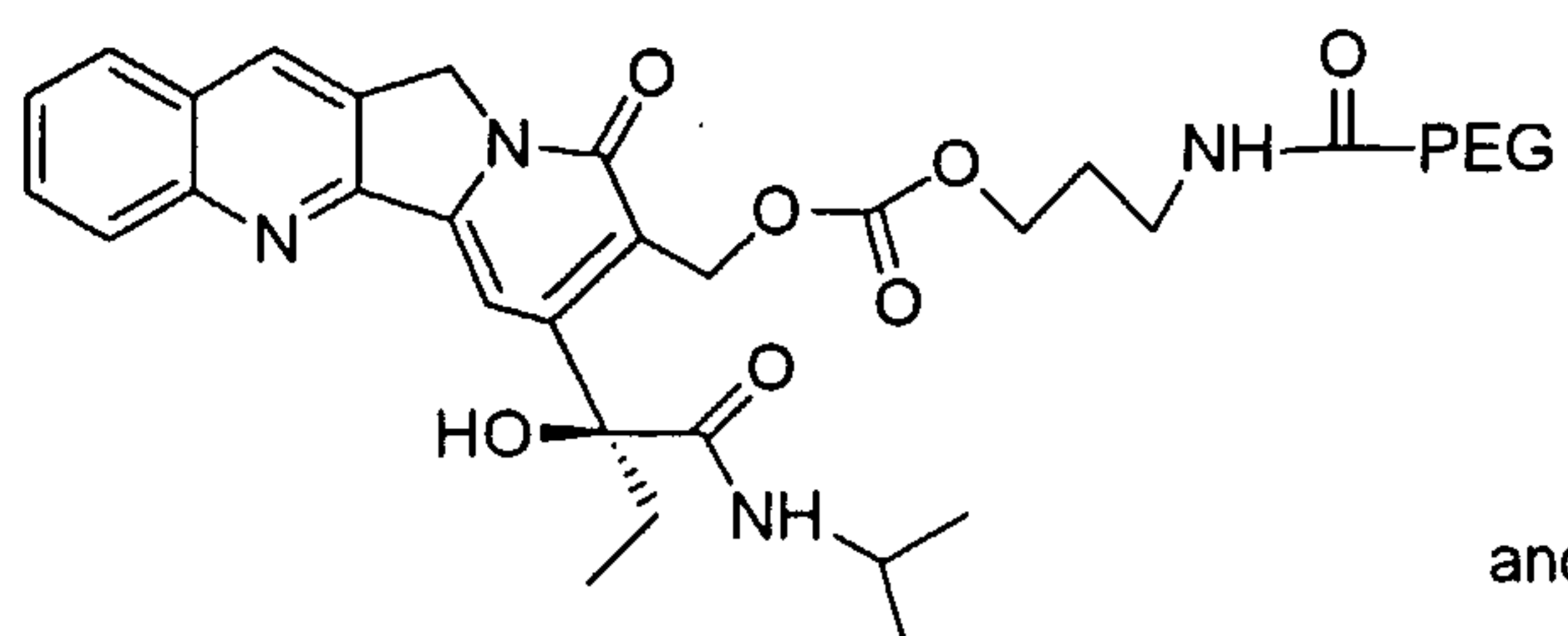
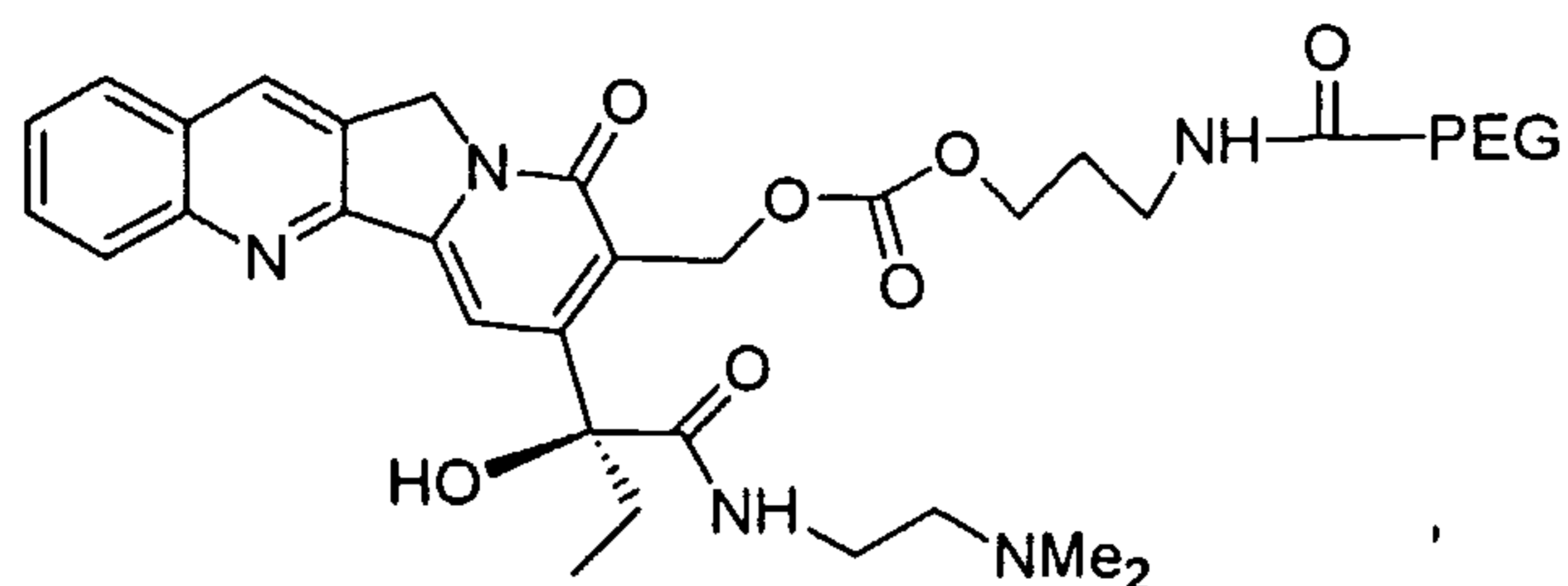
wherein

R_{29-33} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

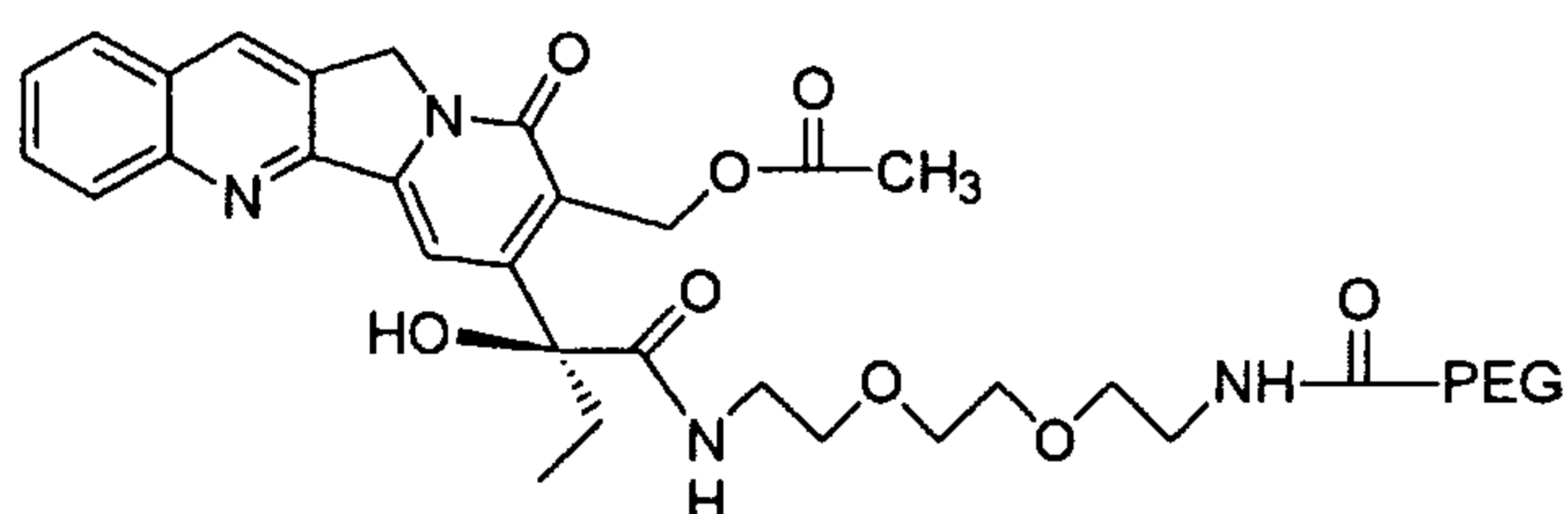
R_{34} is selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy, NO_2 , haloalkyl and halogen; and

j and k are independently selected positive integers.

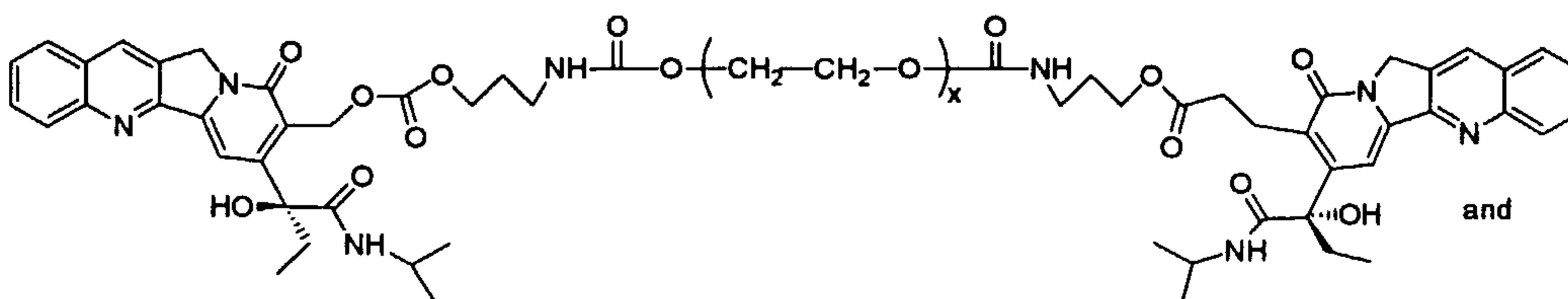
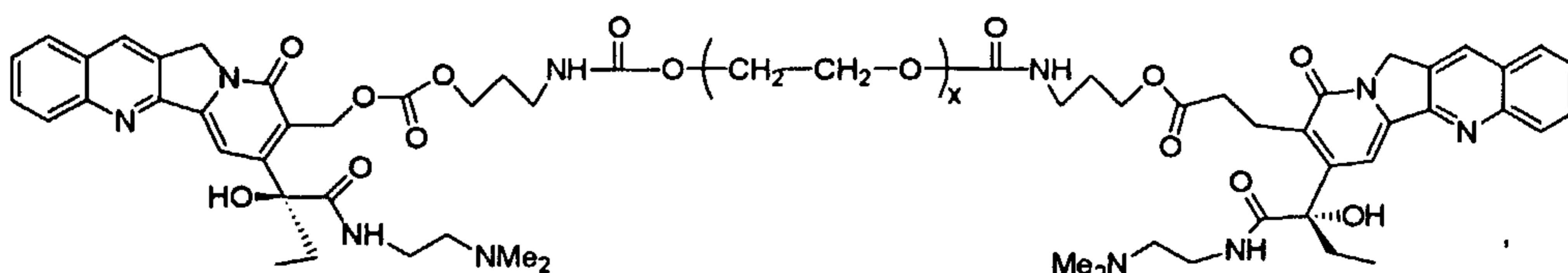
26. A compound of claim 1, selected from the group consisting of



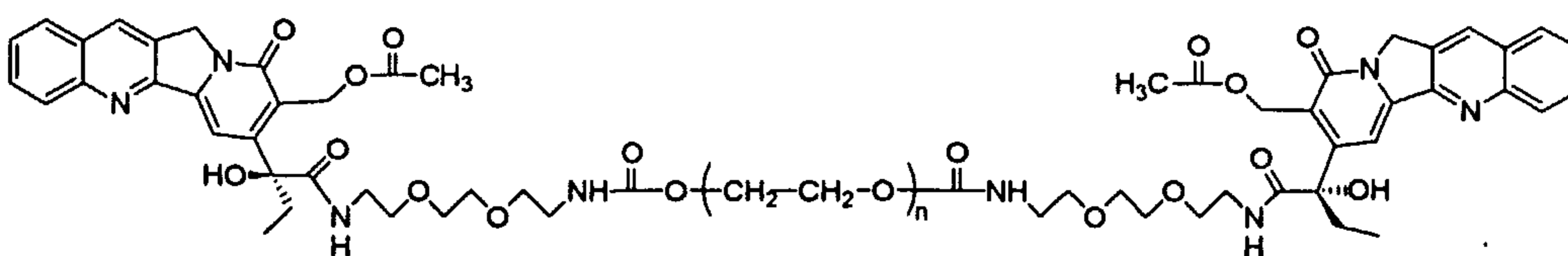
and



27. A compound of claim 1, selected from the group consisting of

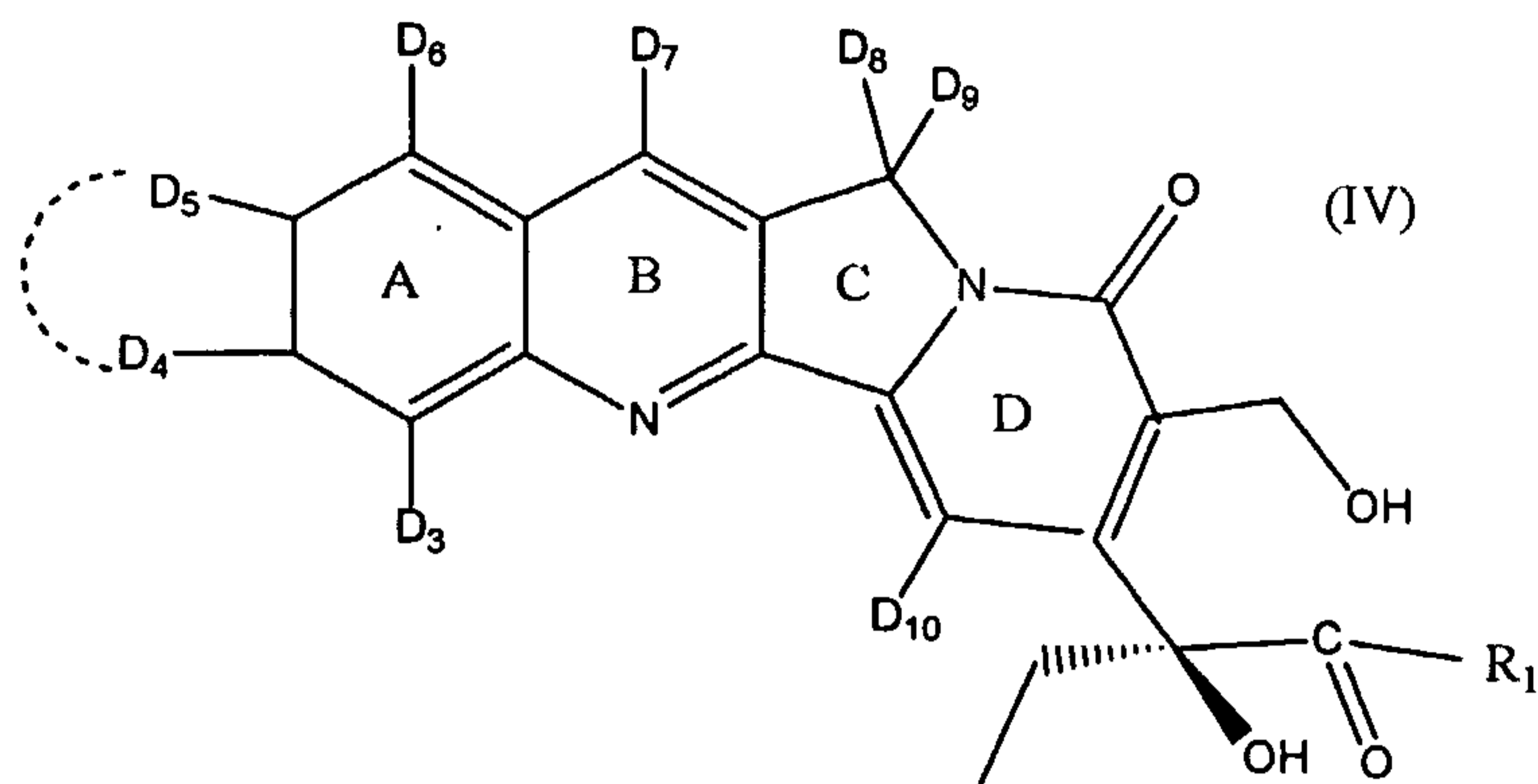


and



28. A method of preparing a camptothecin analog, comprising:

a) reacting a camptothecin derivative of the formula:



wherein:

R_1 is selected from the group consisting of amino acid residues, peptide residues containing from about 2 to about 10 amino acids, $Y_3-(L_2)_p-A_2$ and R_2 ;

Y_3 is O, S or NR_3 ;

p is zero or one;

L_2 is a bifunctional linker;

A_2 is selected from the group consisting of hydrogen, amino protecting groups, NR_8R_9 , amino acid residues, peptide residues containing from about 2 to about 10 amino acids; polymeric residues, R_{10} , SR_{11} , $NC(O)R_{12}$;

D_3 - D_7 are independently selected from the group consisting of H, C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls, C_{1-8} alkoxy, C_{1-8} hydroxyalkyls, C_{1-8} aminoalkoxy, aryloxy, glycols, CO_2R_{13} , R_{14} , nitro, cyano, halo, hydroxyl, amino, SR_{15} , $NR_{16}R_{17}$ or OR_{18} , where D_4 and D_5 optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may contain O, S or NR_{19} groups, where R_{19} is hydrogen or a C_{1-6} alkyl;

D_8 - D_{10} are independently selected from the group consisting of H, C_{1-8} straight or branched alkyls, substituted C_{1-8} straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C_{1-8} alkylaryls and C_{1-8} hydroxyalkyls; and

R_{2-18} are independently selected from the group consisting of hydrogen,

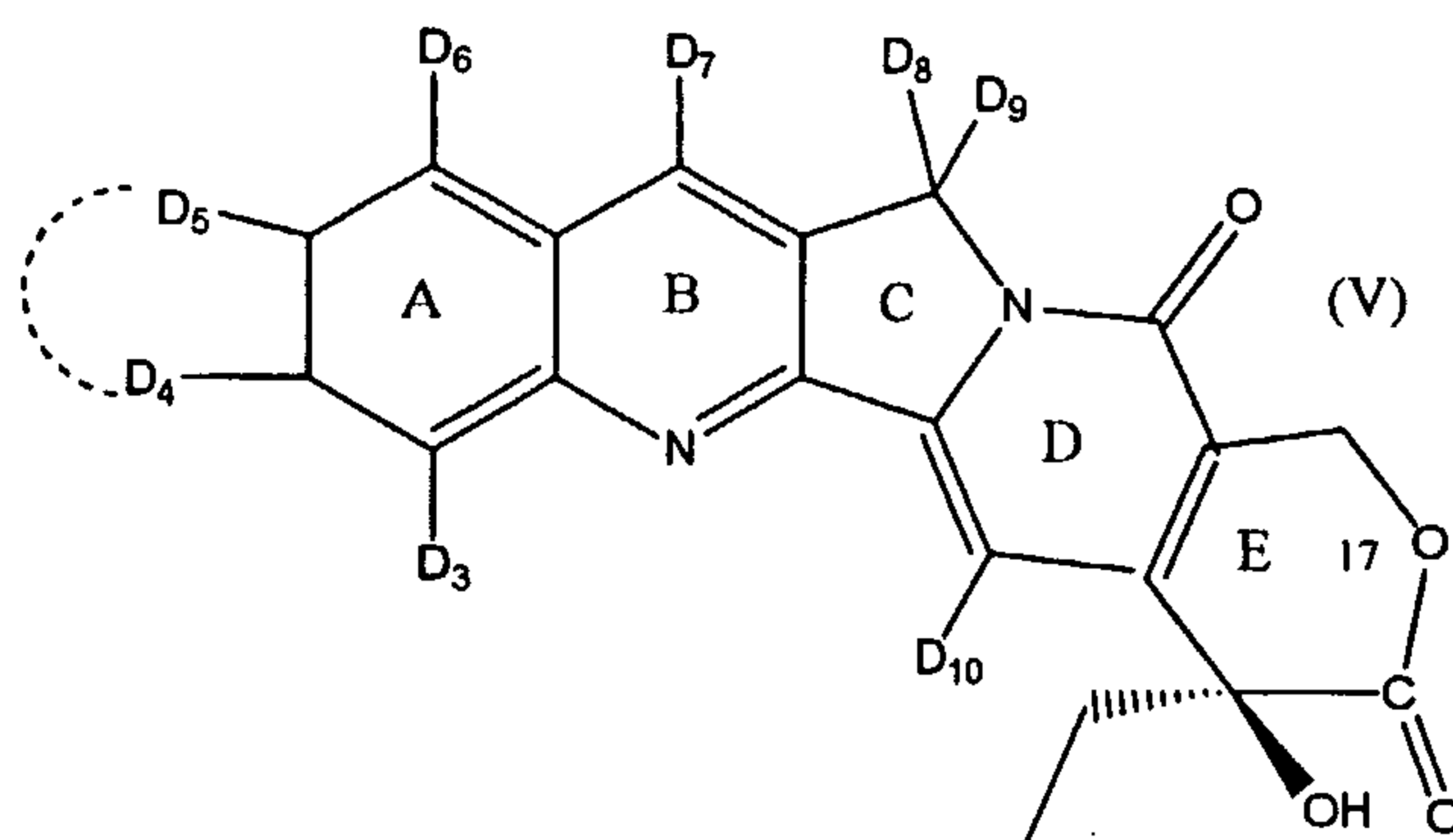
C₁₋₆ alkyls, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₃₋₈ substituted cyloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy;

with a blocked bifunctional spacer to form a protected intermediate, and

b) deprotecting said protected intermediate and reacting the resultant deprotected intermediate with an activated polymer under conditions sufficient to cause a polymeric conjugate to be formed.

29. A method of preparing a polymer conjugate, comprising

a) reacting a compound of the formula



wherein:

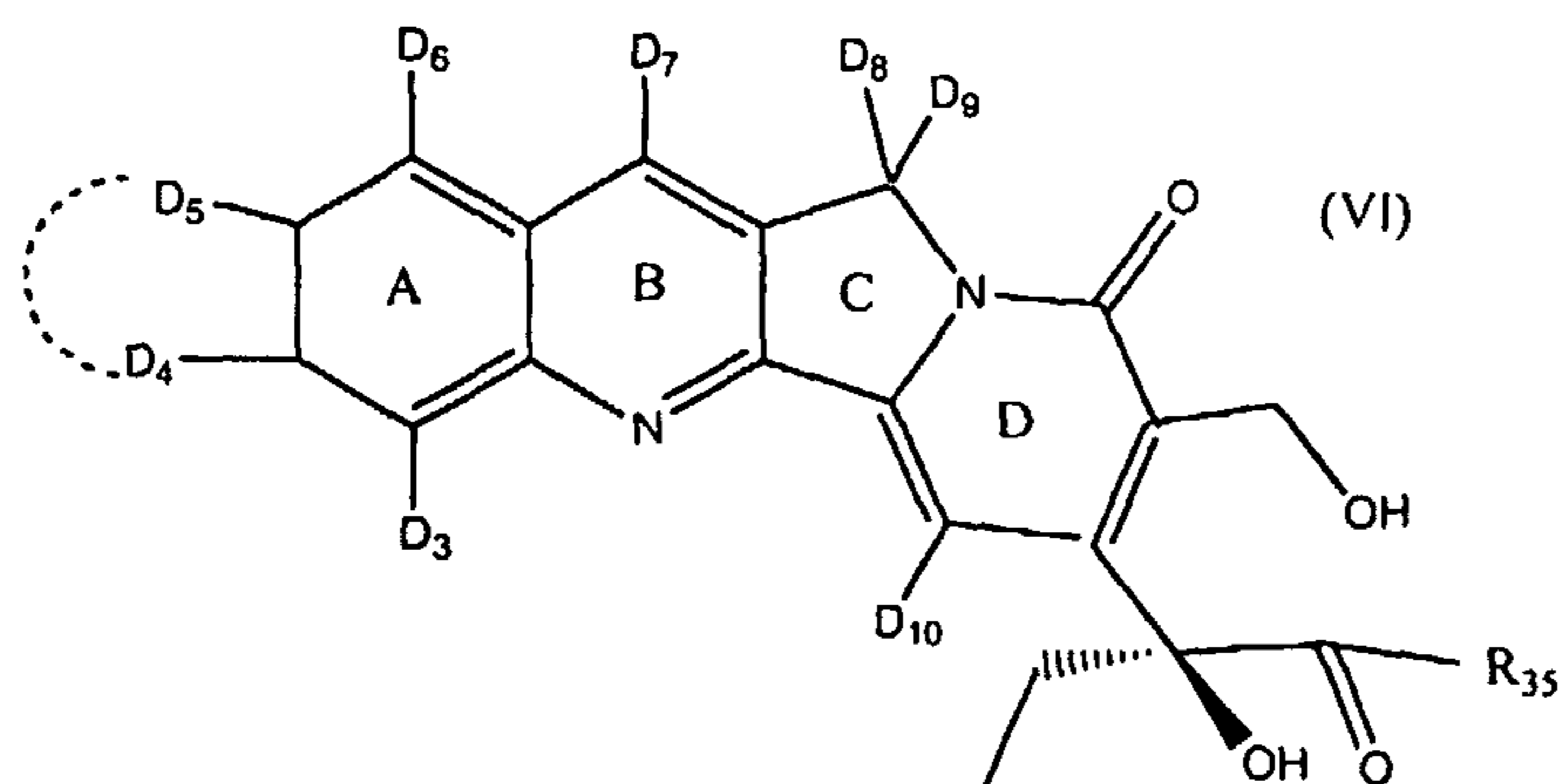
D₃ -D₇ are independently selected from the group consisting of H, C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls, C₁₋₈ alkoxys, C₁₋₈ hydroxyalkyls, C₁₋₈ aminoalkoxy, aryloxys, glycals, CO₂R₁₃, R₁₄, nitro, cyano, halo, hydroxyl amino, SR₁₅, NR₁₆R₁₇ or OR₁₈, where D₄ and D₅ optionally, when taken together, form a saturated 3-7 membered heterocyclic ring which may contain O, S or NR₁₉ groups, where R₁₉ is hydrogen or a C₁₋₆ alkyl;

D₈-D₁₀ are independently selected from the group consisting of H, C₁₋₈ straight or branched alkyls, substituted C₁₋₈ straight or branched alkyls, aryls, substituted aryls, arylalkyls, substituted arylalkyls, C₁₋₈ alkylaryls and C₁₋₈ hydroxyalkyls; and

R₈₋₁₈ are independently selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls,

C₃₋₈ substituted cyloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy;

with a blocked bifunctional spacer in an inert solvent under conditions sufficient to open the E lactone and form a protected intermediate of formula (VI)



wherein R₃₅ is a residue of a blocked bifunctional spacer;

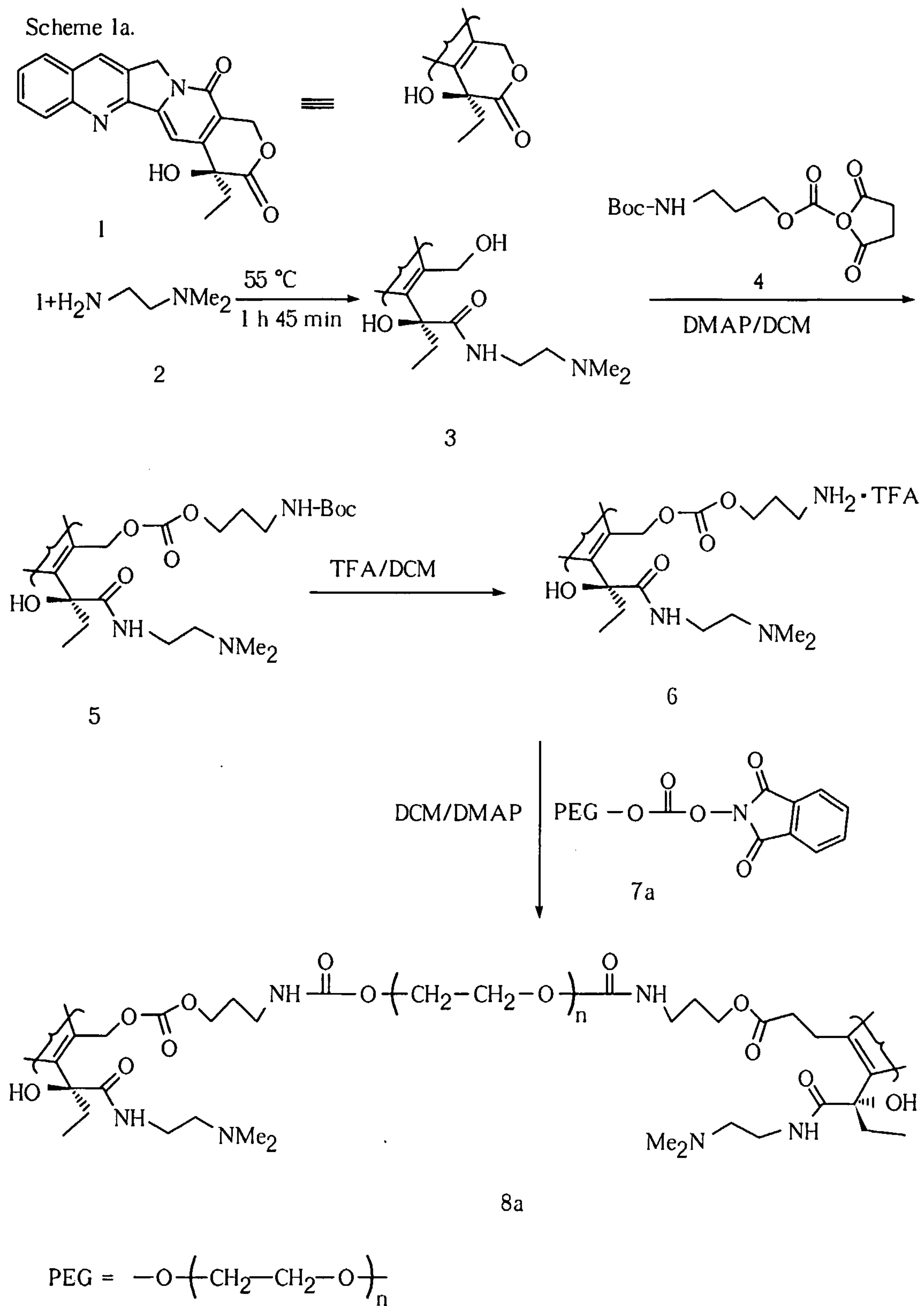
- b) acylating the C₁₇ OH of said protected intermediate, and
- c) deprotecting said protected intermediate and reacting at least about 2 equivalents

of the resultant deblocked intermediate with an activated polymer under conditions sufficient to cause a polymeric conjugate to be formed.

30. A method of treating a topoisomerase I inhibitor- related disease in mammals, comprising administering an effective amount of a compound of claim 1 to a mammal in need of such treatment.

1/6

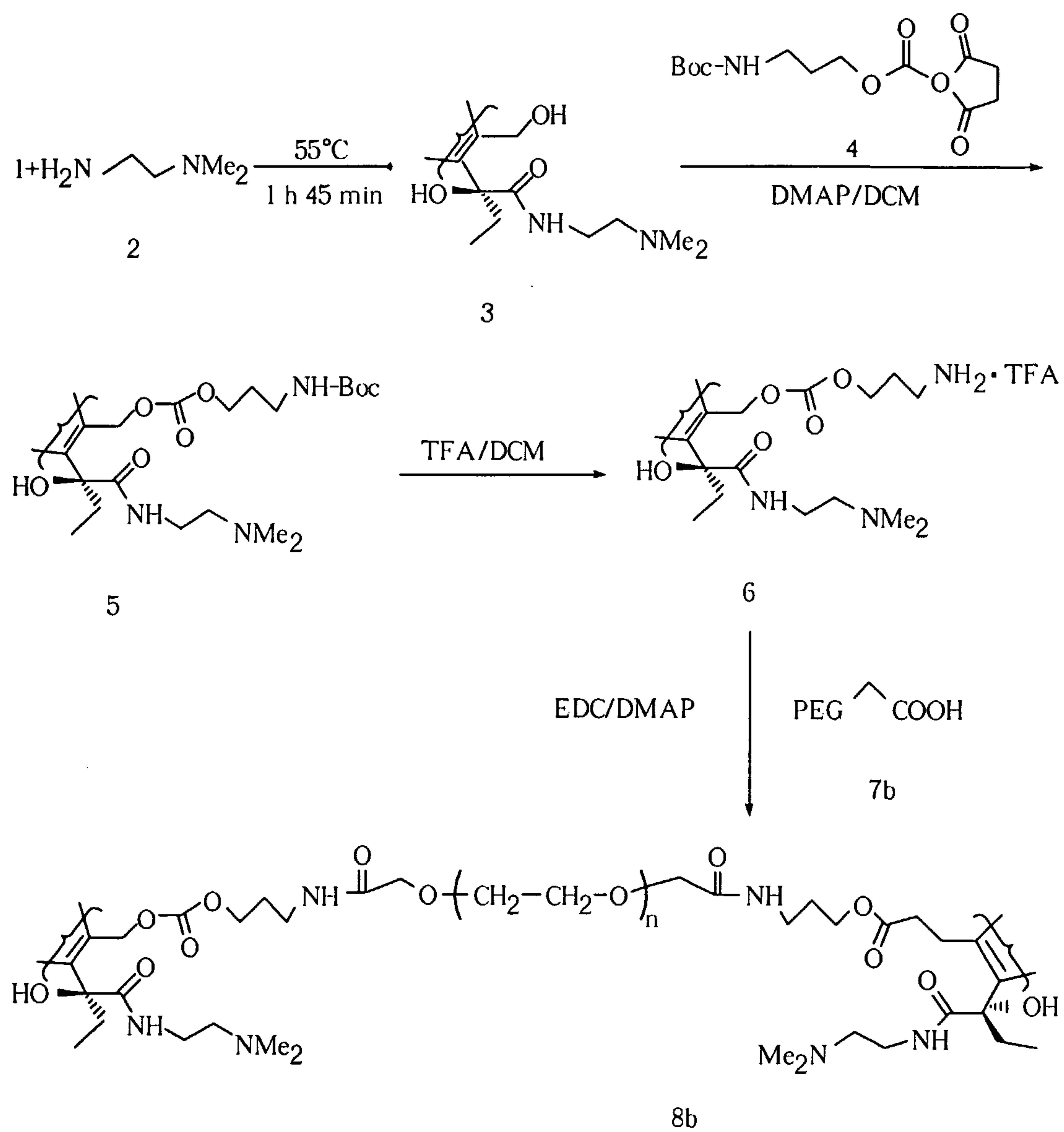
FIG. 1



2/6

FIG. 2

Scheme 1b.



3/6

FIG. 3

Scheme 2a.

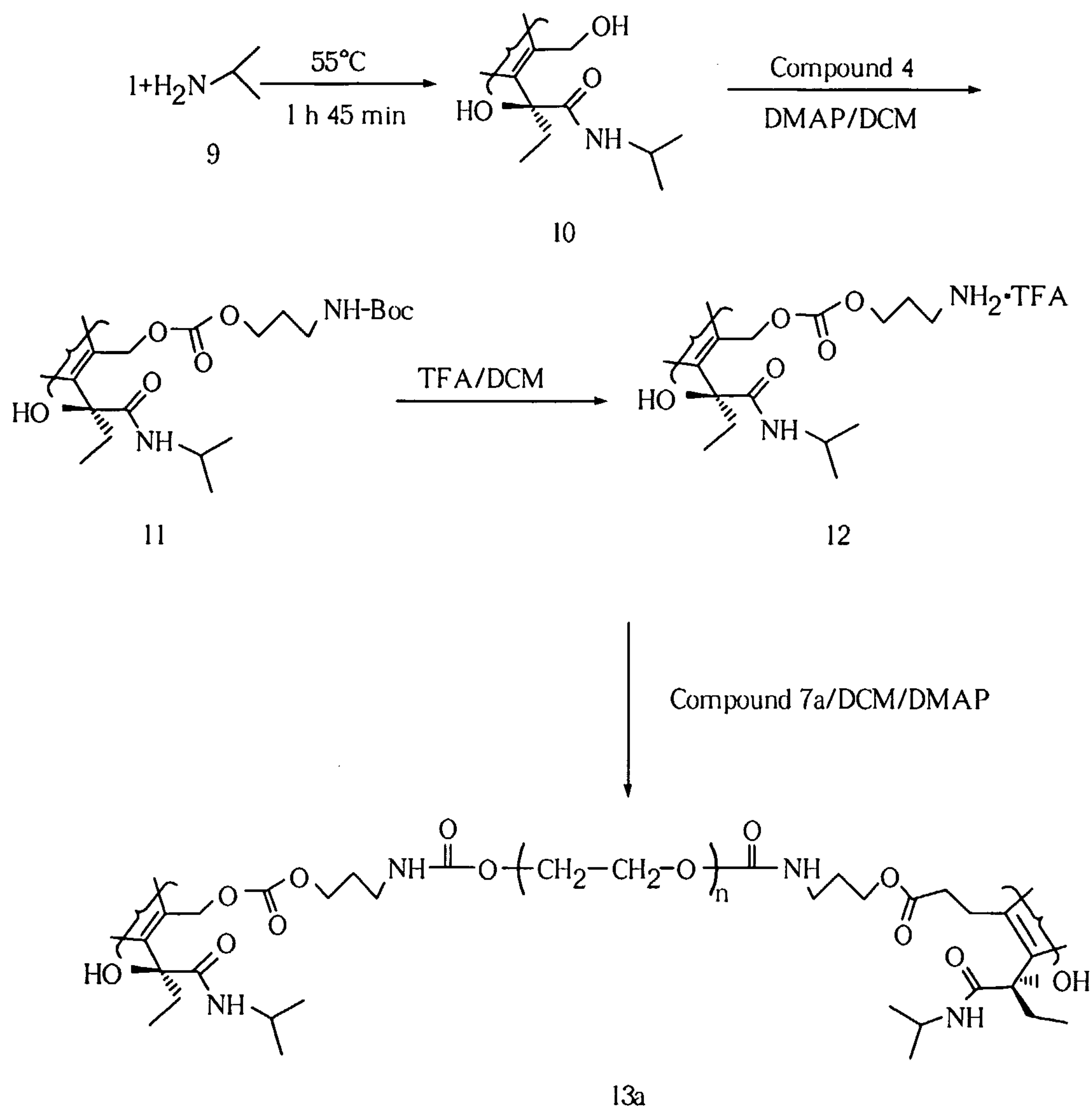


FIG. 4

Scheme 2b.

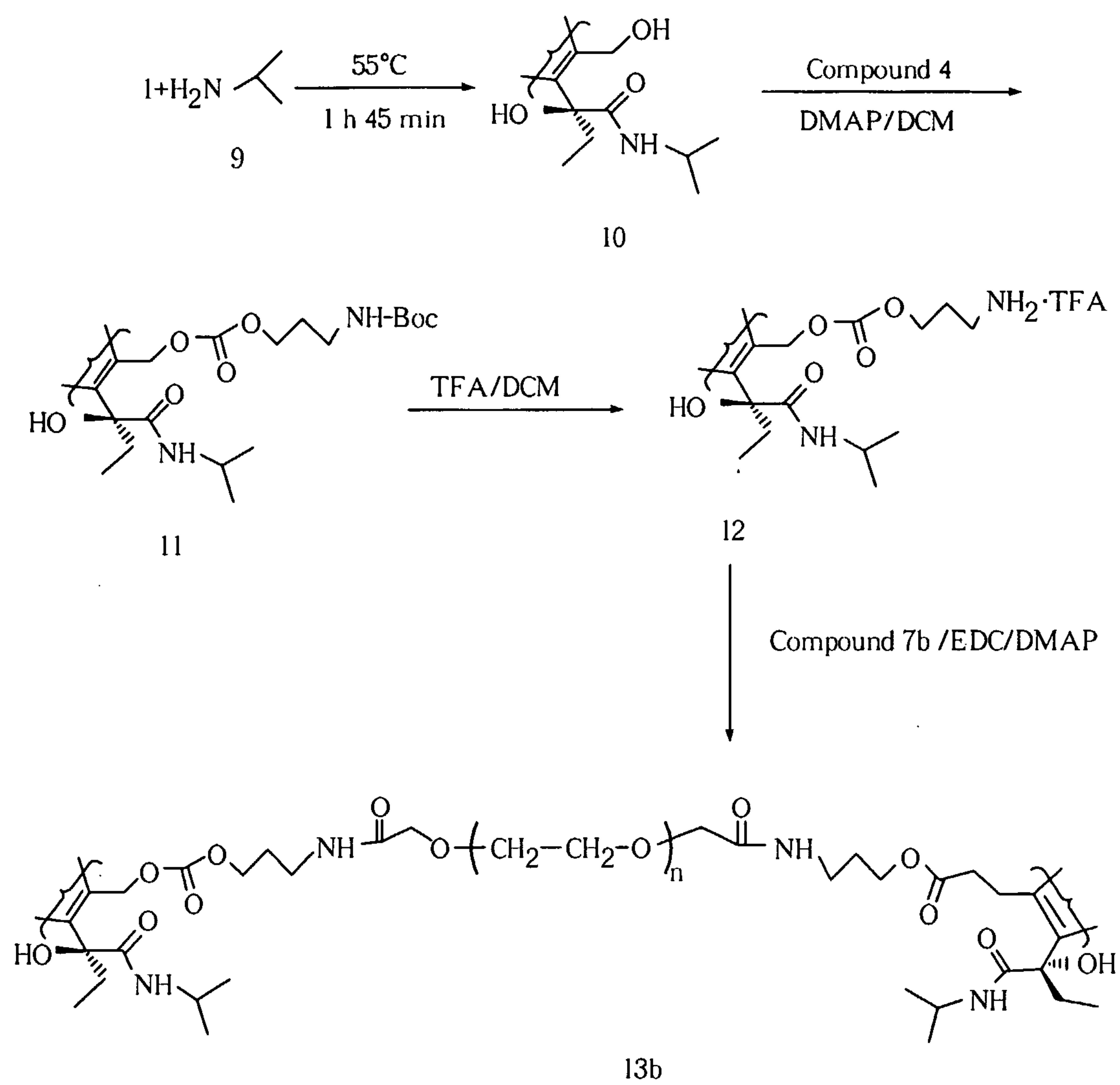


FIG. 5

Scheme 3a.

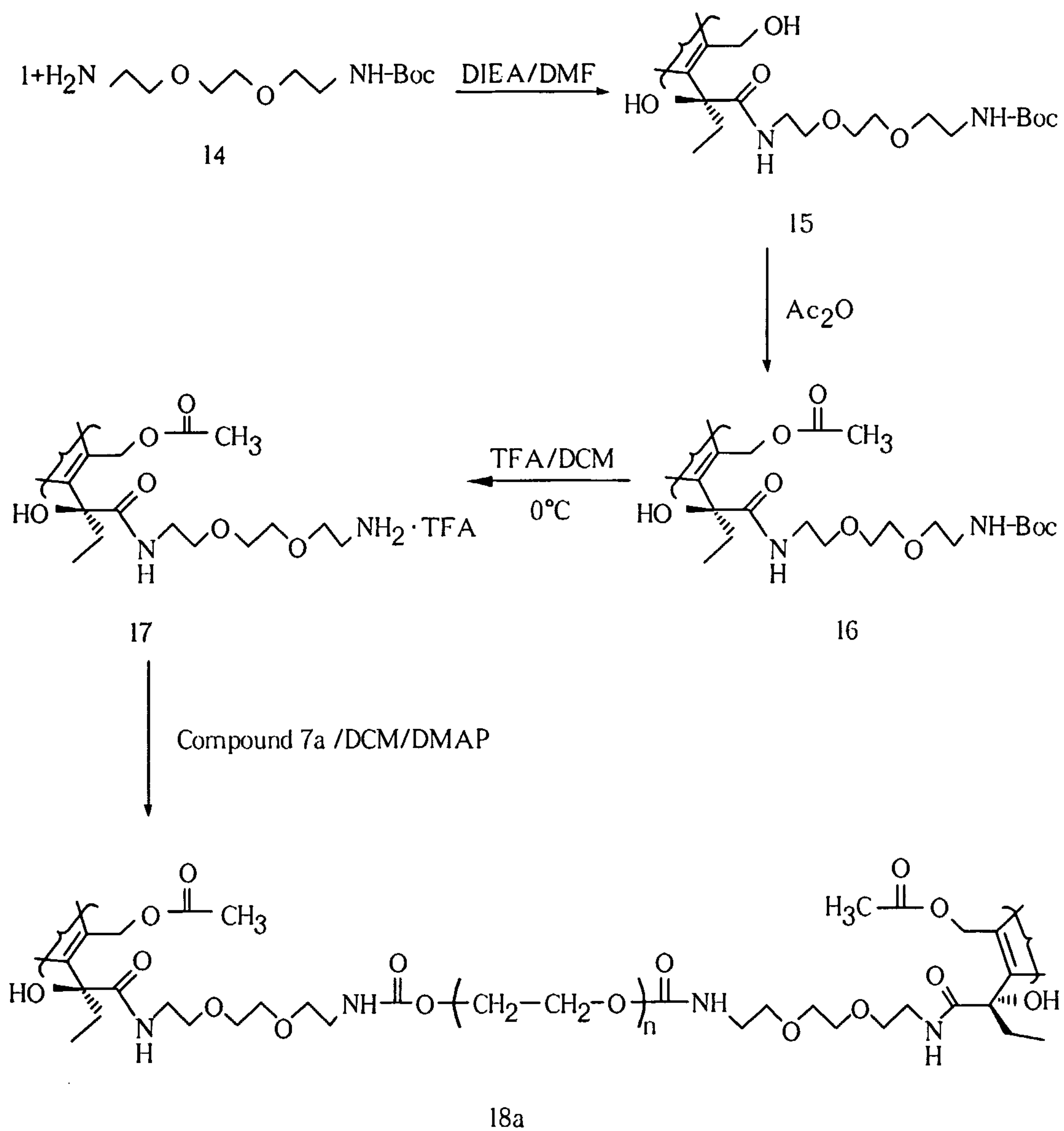


FIG. 6

Scheme 3a.

