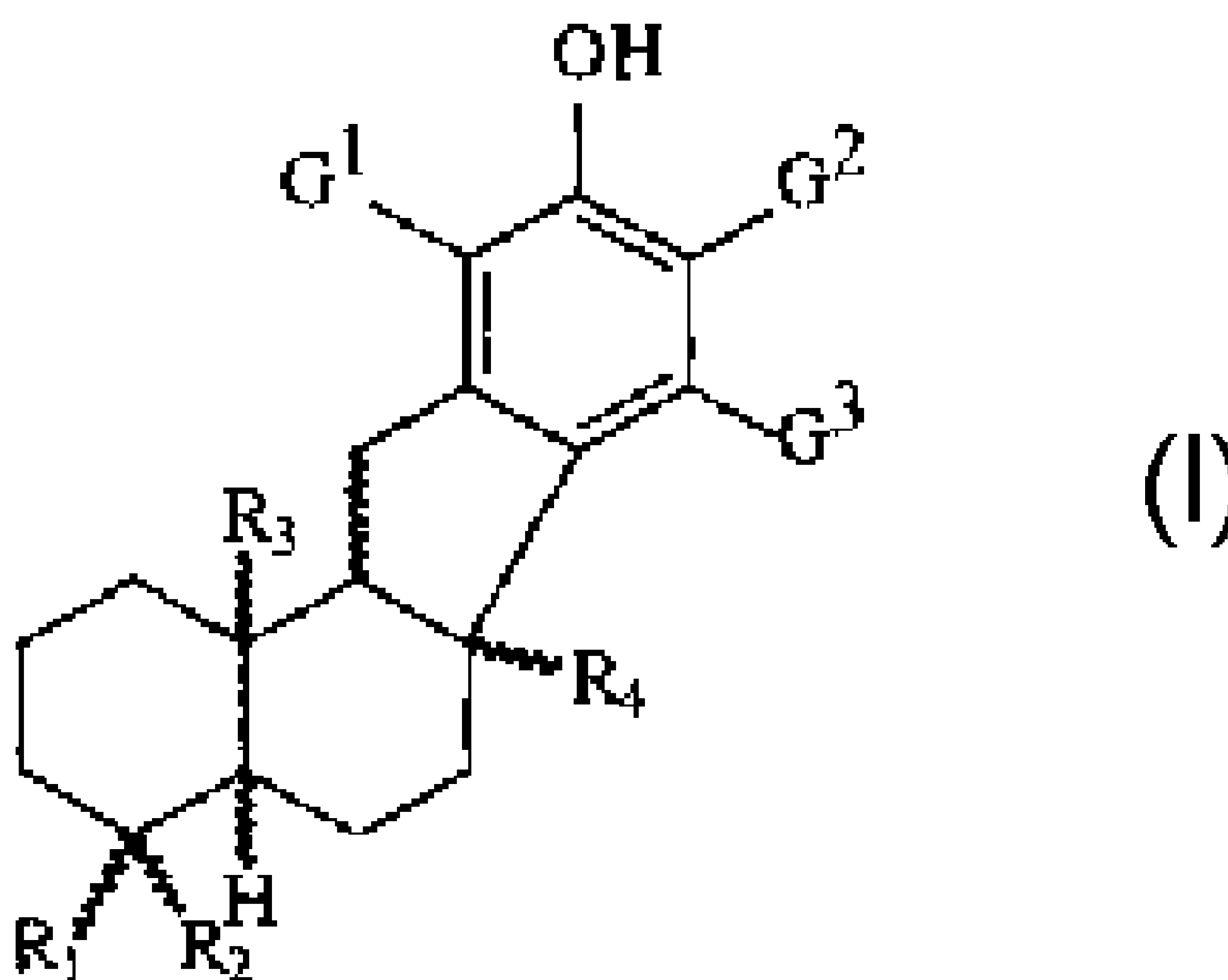




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(54) Titre : COMPOSES MODULATEURS DE LA SHIP 1
(54) Title: SHIP 1 MODULATOR COMPOUNDS



(57) Abrégé/Abstract:

The present invention provides the use of pelorol analogs of Formula, (I) and pharmaceutical compositions thereof as modulators of SHIP 1 activity. A compound or a pharmaceutical composition of the present invention may be used for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition in addition to other disorders and conditions.



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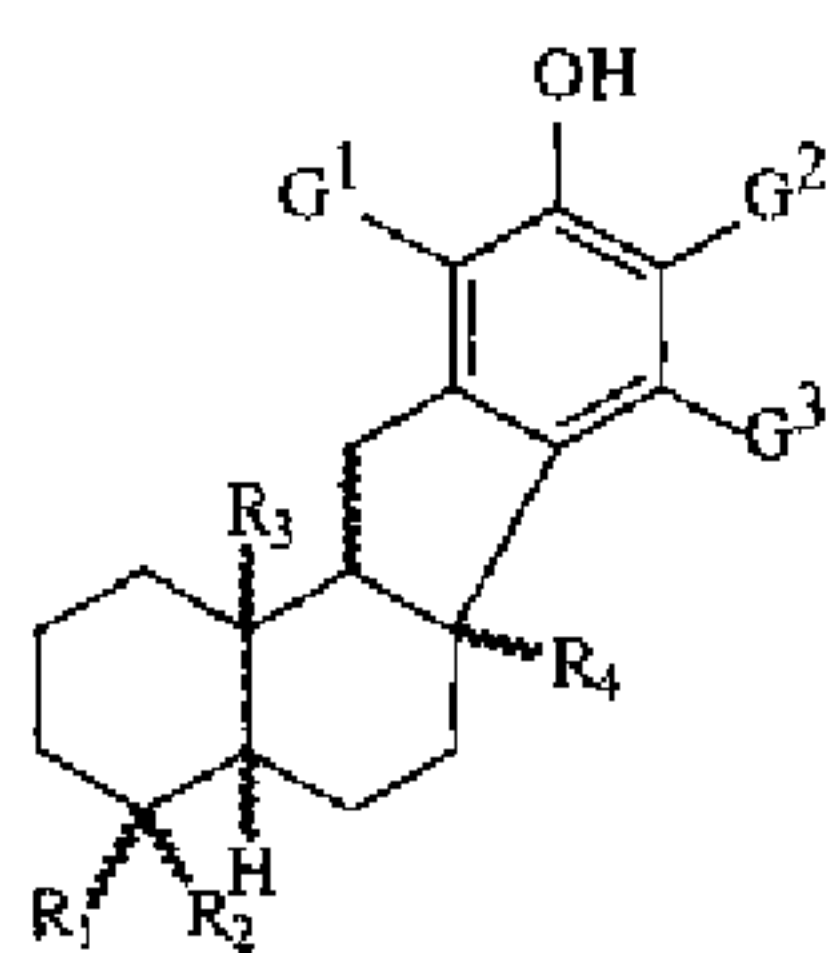
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(54) Title: SHIP 1 MODULATOR COMPOUNDS



(I)

(57) **Abstract:** The present invention provides the use of pelorol analogs of Formula, (I) and pharmaceutical compositions thereof as modulators of SHIP 1 activity. A compound or a pharmaceutical composition of the present invention may be used for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition in addition to other disorders and conditions.

WO 2007/147251 A1

SHIP 1 MODULATOR COMPOUNDS

Technical Field

The present invention relates to SHIP 1, a negative regulator of cell proliferation and survival
5 and immune cell activation.

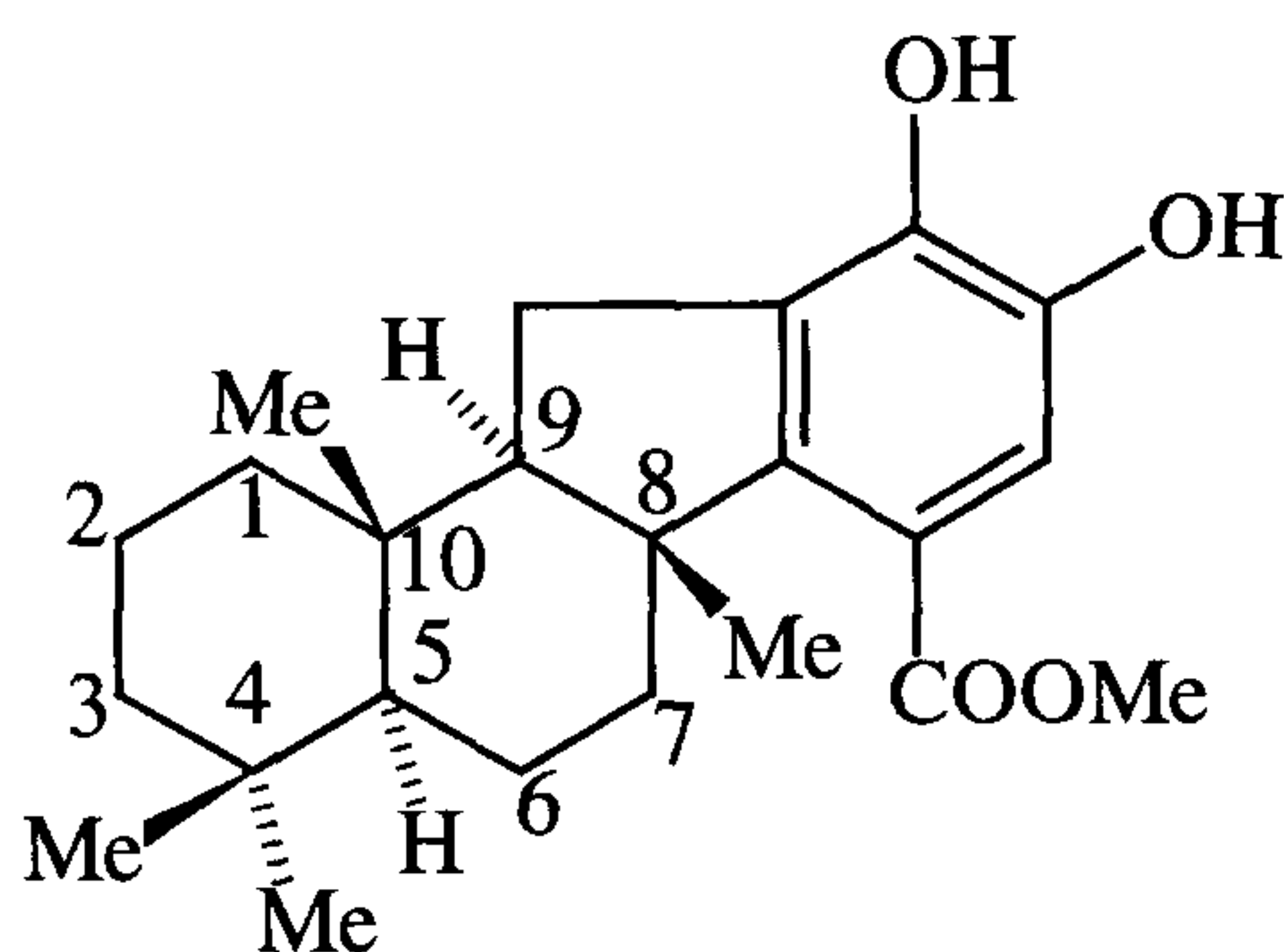
Background of the Invention

SH₂-containing inositol 5-phosphatase (SHIP 1), selectively hydrolyzes the 5-phosphate from inositol 1,3,4,5-tetraphosphate (IP₄) and phosphatidylinositol 3,4,5-triphosphate (PIP₃).

10 United States Patent No. 6,238,903 discloses that SHIP 1 is an enzyme regulator of signaling pathways that control gene expression, cell proliferation, differentiation, activation, and metabolism, particularly of the Ras and phospholipid signaling pathways. SHIP 1 plays an important role in cytokine and immune receptor signal transduction. SHIP 1 disrupted (SHIP 1^{-/-}) mice exhibit a myeloproliferative phenotype characterized by overproduction of
15 granulocytes and macrophages. (Huber, M. et al. (1999) *Prog Biophys Mol Biol* 71:423) SHIP 1^{-/-} mast cells are more prone to IgE and Steel factor induced degranulation, while SHIP 1^{-/-} B cells are resistant to negative regulation by Fc RIIB. SHIP 1 is also involved in the pathogenesis of chronic myelogenous leukemia. (Sattler, M. et al. (1999) *Mol Cell Biol* 19:7473)

20 SHIP 1 is expressed only in blood cells and is an important negative regulator of hemopoietic cell growth/survival and immune cell activation. The specialized function of SHIP 1 has been studied in mouse and man.

25 Various agonists of SHIP 1 activity are known from WO 2004/035601. An example of an agonist is the sesquiterpene compound pelorol, which was first obtained from marine sponge species. Its synthesis is described in WO 2004/035601. The precise structure of pelorol is as follows, with Me representing a methyl group and relative configuration of chiral atoms (C-5, 8, 9 and 10) shown.



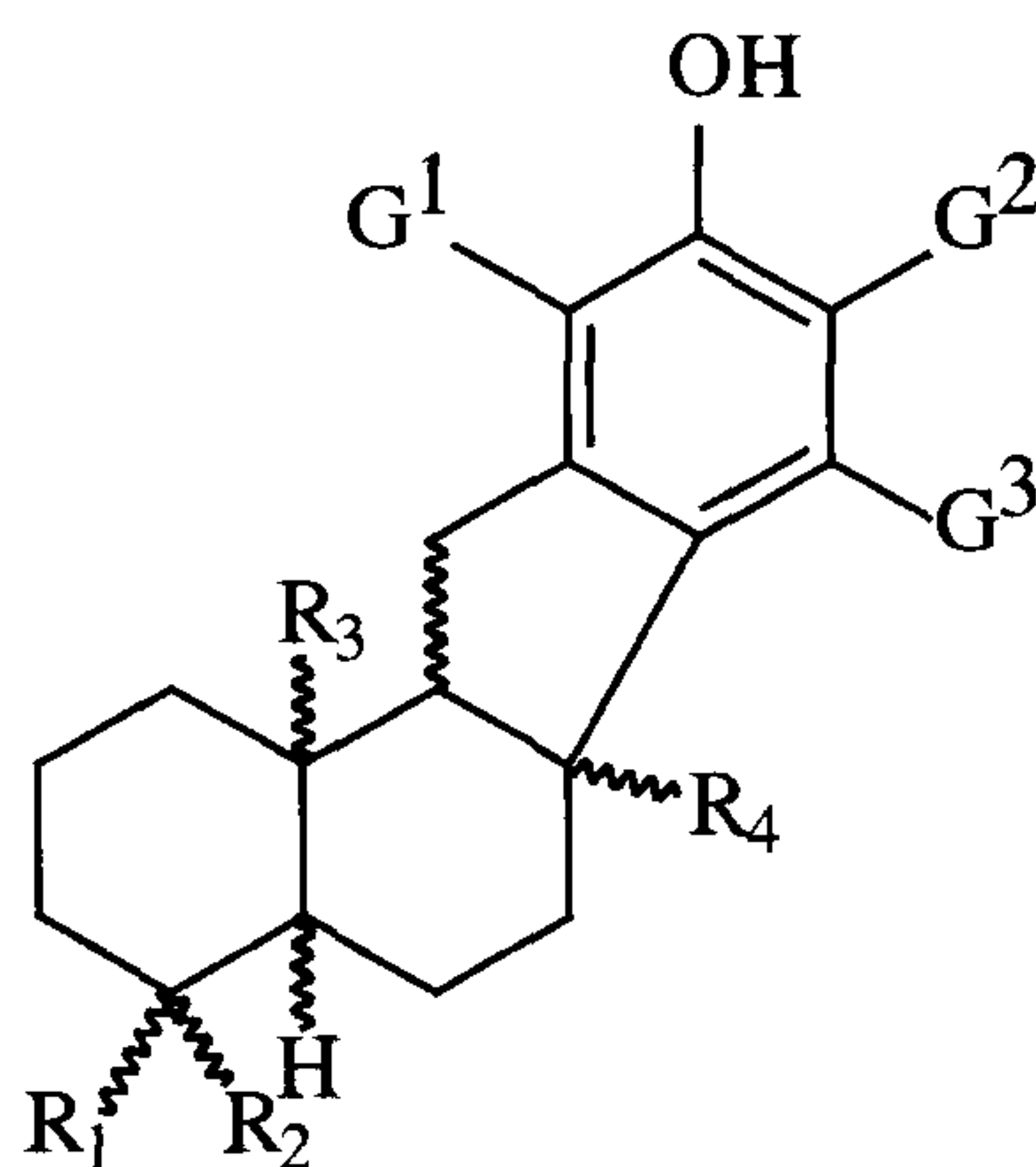
Pelorol

Summary

- 5 This invention is based, in part, on the discovery that increased SHIP modulating activity is provided by having an -OH moiety on the carbon atom at position 14 of SHIP 1 modulator compounds derived from pelorol.

In illustrative embodiments of this invention there is provided a compound of Formula 1 and

10 salts thereof:



Formula 1

wherein;

- R₁ and R₂ are independently selected from the group consisting of: -CH₃, -CH₂CH₃,
 15 -CH₂OH, -CH₂OR₁', -CHO, -CO₂H, and -CO₂R₂';

R₃ and R₄ are independently selected from the group consisting of: H, -CH₃,
 -CH₂CH₃, -CH₂OH, -CH₂OR₃', -CHO, -CO₂H, and -CO₂R₄';

R₁', R₂', R₃', and R₄', are independently a linear, branched, or cyclic, saturated or
 unsaturated one to ten carbon alkyl group that is unsubstituted or substituted with one or more

of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR₁'', -N(R₂'')₂, NO₂ and -CO₂H where R₁' and R₂' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group;

G₁ is selected from the group consisting of: O-(C₁-C₁₀ alkyl) and H;

G₂ is H or C₁-C₁₀ alkyl; and

5 G₃ is selected from the group consisting of: H, -OH, C₁-C₁₀ alkyl and O-(C₁-C₁₀ alkyl).

In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein one or both of R₁ and R₂ are selected from the group consisting of: methyl, ethyl, -CH₂OH, -CH₂OR₁', or -CH₂OR₃'.

10

In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein R₁', R₂', R₃', and/or R₄', in R₁ are selected from the group consisting of: methyl, ethyl, propyl or butyl.

15 In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein R₁', R₂', R₃', and/or R₄', in R₂ are selected from the group consisting of: methyl, ethyl, propyl or butyl.

In other illustrative embodiments of this invention there is provided a compound of any
20 formula described herein wherein R₁ is methyl or ethyl.

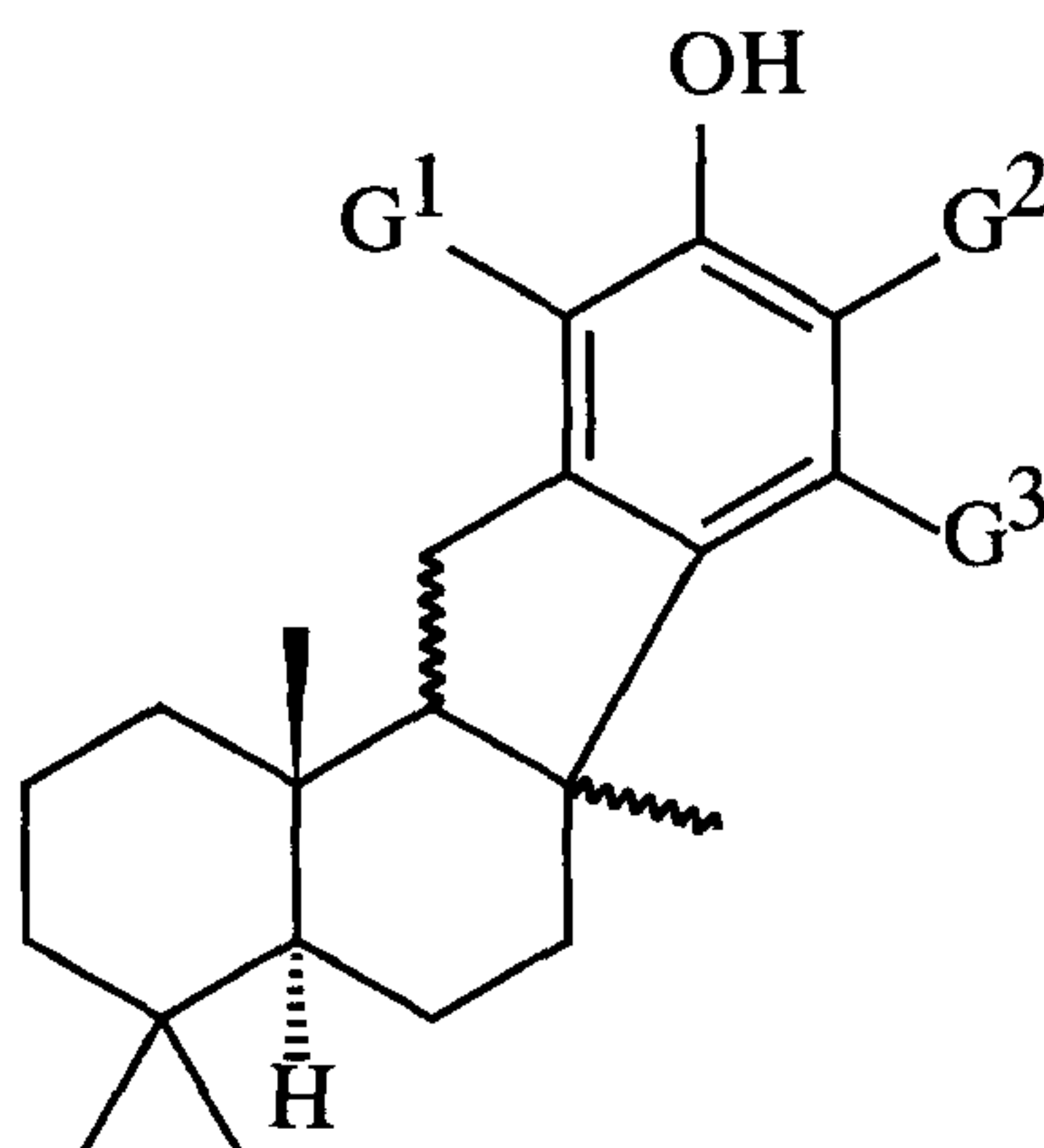
In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein R₂ is methyl or ethyl.

25 In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein R₁ is methyl.

In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein R₂ is methyl.

30

In other illustrative embodiments of this invention there is provided a compound of Formula 2 and salts thereof:



Formula 2

5 wherein;

G_1 is selected from the group consisting of: O-(C₁-C₁₀ alkyl) and H;

G_2 is H or C₁-C₁₀ alkyl; and

G_3 is selected from the group consisting of: H, -OH, C₁-C₁₀ alkyl and O-(C₁-C₁₀ alkyl).

10 In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein G_1 is selected from the group consisting of -O-methyl and H; G_2 is H or methyl; and G_3 is selected from the group consisting of: H, methyl and O-methyl.

In other illustrative embodiments of this invention there is provided a compound of any
15 formula described herein wherein only one of G_1 , G_2 and G_3 is -O-methyl.

In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein at least one of G_1 , G_2 and G_3 is H.

20 In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein G_3 is methyl.

In other illustrative embodiments of this invention there is provided a compound of any formula described herein wherein all of G_1 , G_2 and G_3 are H.

25

In other illustrative embodiments of this invention there is provided a pharmaceutical composition comprising a compound of any formula described herein and a pharmaceutically acceptable excipient.

- 5 In other illustrative embodiments of this invention there is provided a compound of any formula described herein or a pharmaceutical composition described herein for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.

- 10 In other illustrative embodiments of this invention there is provided a compound of any formula described herein for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition. The use may be for the preparation of a medicament.

- 15 In other illustrative embodiments of this invention there is provided a method of prophylaxis or treatment of an immune, hematopoietic, inflammatory or neoplastic disorder or condition comprising administering to a patient in need of said prophylaxis or treatment, an effective amount of a pharmaceutical composition described herein.

- 20 In other illustrative embodiments of this invention there is provided a use or method described herein wherein the neoplastic condition is a blood cancer, multiple myeloma, chronic myeloid leukemia or acute myelogenous leukemia.

- In other illustrative embodiments of this invention there is provided a use or method described herein wherein the immune disorder is an autoimmune disorder.

25

In other illustrative embodiments of this invention there is provided a pharmaceutical composition comprising a compound as described above and a pharmaceutically acceptable carrier.

- 30 In other illustrative embodiments of this invention there is provided a method of prophylaxis or treatment of an immune, hematopoietic, inflammatory or neoplastic disorder or condition comprising administering to a patient in need of said prophylaxis or treatment, an effective amount of a pharmaceutical composition as described herein. Such compositions may

comprise previously known compounds of any one of Formulas 1 and 2 which have not been known as particularly efficacious or advantageous.

5 In other illustrative embodiments of this invention there is provided the use of a compound described herein or pharmaceutically acceptable salt thereof for modulation of SHIP 1 activity and for preparation of agents and medicaments for the modulation of SHIP 1 activity. Such modulation may be *ex vivo*, *in vitro* or *in vivo*. Agents for *in vivo* use include a pharmaceutical composition of this invention as well as agents adapted for *in vitro* use. The modulation may be for a treatment or prophylaxis of an immune, inflammatory, or neoplastic condition or
10 disorders as described herein.

Brief Description of the Drawings

Figure 1: is a graph depicting the results of a cell based assay to test relative inhibition of TNF α by a prodrug compound, Compound 103, compared to a non-prodrug compound,
15 Compound 100.

Figure 2: is a graph depicting the results of a cell based assay to test the inhibition of macrophage TNF α production by varying concentrations of a prodrug, Compound 106.

20 **Figure 3:** is a graph depicting the results of a cell based assay to test the inhibition of calcium influx in mast cells by a prodrug, Compound 106.

Figure 4: is a graph depicting the results of a cell based assay to test the inhibition of TNF α production in wild type (WT) and knock-out (KO) macrophages by a prodrug, Compound 108.
25

Figure 5A: is a graph depicting the results of the ability of Compound 100 at varying concentrations to reduce tumor cell survival in multiple myeloma (MM) cell lines.

Figure 5B: is a graph depicting the results of the ability of Compound 100 at varying
30 concentrations to reduce tumor cell survival in multiple myeloma (MM) cell lines.

Figure 5C: is a graph depicting the results of the ability of AQX-016A at varying concentrations to reduce tumor cell survival in multiple myeloma (MM) cell lines.

Figure 6A: is a graph depicting the results of the ability of compound 100 at varying concentrations to inhibit growth of OPM2 MM cell lines.

5 **Figure 6B:** is a graph depicting the results of the ability of compound 100 at varying concentrations to inhibit growth of MM.1S MM cell lines.

Figure 6C: is a graph depicting the results of the ability of AQX-016A at varying concentrations to inhibit growth of RPMI 8226 MM cell lines.

10

Figure 6D: is a graph depicting the results of the ability of AQX-016A at varying concentrations to inhibit growth of U266 MM cell lines.

15 **Figure 6E:** is a graph depicting the results of the ability of AQX-016A at varying concentrations to inhibit growth of LCC6-Her2 MM cell lines.

Figure 7A: is a graph depicting the results of the activation of SHIP enzyme *in vitro* of Compound 100, AQX-16A and Compound 103.

20 **Figure 7B:** is a graph depicting the results of the activation of SHIP enzyme *in vitro* of Compound 100 and AQX-16A.

Figure 7C: is a graph depicting the results of Compound 100 inhibiting TNF α production from LPS stimulated SHIP^{+/+} but not ^{-/-} BMm ϕ s.

25

Figure 7D: is a graph depicting the results of Compound 100 inhibiting LPS-induced plasma TNF α levels in mice.

30 **Figure 8A:** is a graph depicting the results of SHIP^{+/+} (▨) and SHIP^{-/-} (■) macrophages pretreated with AQX-016A or carrier 30 min prior to stimulation with 10 ng/mL of LPS at 37°C for 2 h and TNF α production determination by ELISA. Absolute TNF α levels for SHIP^{+/+} and SHIP^{-/-} cells were 623 +/- 30 and 812 +/- 20 pg/ml, respectively. Data are expressed as mean +/- SEM and are representative of three independent experiments.

Figure 8B: is a graph depicting the results of SHIP^{+/+} and SHIP^{-/-} mast cells pre-loaded with IgE and Fura-2 and treated for 30 min with 15 μ M AQX-016A or carrier. Cells were then stimulated (as indicated by the arrow) with 0 (.....) or 10 (—) ng/mL DNP-HSA and intracellular calcium levels monitored over time by spectrofluorometry.

Figure 9: is a graph depicting the results of mice administered 20 mg/kg AQX-016A or 0.4 mg/kg dexamethasone orally 30 min prior to an IP injection of 2 mg/kg LPS. Blood was collected 2 h later for TNF α determination by ELISA. Each symbol indicates one mouse and data are representative of three independent experiments.

Figure 10A: is a graph depicting the results of Compound 100 inhibiting DNFB-induced neutrophil-specific myeloperoxidase (MPO) in sensitized mice. *P*-value <0.02 for the Compound 100 vs the vehicle treated groups. All data are representative of three independent experiments. Data are representative of three independent experiments.

Figure 10B: is a graph depicting the results of AQX-016A inhibiting mast cell degranulation in CD1 mice sensitized to hapten DNP by cutaneous application.

Figure 11A: is a graph depicting the results of SHIP enzyme initial velocities at the indicated concentration of inositol-1,2,4,5-tetrakisphosphate (IP₄) substrate.

Figure 11B: is a graph depicting the results of the ability of product PI-3,4-P₂ (20 μ M) or Compound 100 (3 μ M) to activate wild-type (WT) and C2 domain deleted (Δ C2) SHIP enzyme at 30 μ M IP₄.

Figure 11C: is a graph depicting the results of a protein overlay assay in which recombinant C2 domain was pre-incubated for 30 min at 23°C with 4. μ M of Compound 100 or EtOH control and allowed to bind to PI-3,4-P₂ immobilized on membrane strips.

Figure 11D: is a graph depicting the results of bead associated radioactivity obtained from recombinant C2 domain (10 nM) coated onto Copper chelate (His-Tag) YSi SPA Scintillation

Beads in the presence of 0.25% BSA and incubated with 5 μ Ci of [^{3} H]-Compound 100. Data are expressed as mean $\pm$ SEM and are representative of at least three independent experiments.

Figure 11E: is a graph depicting the results of bead associated radioactivity obtained from copper chelate (His-Tag) YSi SPA Scintillation Beads coated with either wild-type (WT) or C2 domain deleted (Δ C2) SHIP enzyme in the presence of 0.25% BSA aliquoted into 96 well plates and incubated with 5 μ Ci of [^{3} H]-Compound 100 (42 Ci/mmol) with shaking at 23°C in the dark. The amount of [^{3} H]-Compound 100 interacting with the protein coated beads was quantified on a plate scintillation counter.

Figure 12A: is a graph depicting the results of the activity of the enzymes in the presence of Compound 100 compared to that in the vehicle control and expressed as a % change in activity relative to that observed in the vehicle control. Changes in activity of $<25\%$ were not considered significant.

Figure 12B: is a graph depicting the results of the activity of enzymes affected by Compound 100 by more the 25% as shown in Figure 12A.

Figure 13: is a graph depicting the results of the effect of Compound 100 and vehicle control on tumour size in mice.

Figure 14: is a graph depicting the results of the effect of Compound 100 and vehicle control on tumour volume over time in mice.

Detailed Description

As used herein the phrase “alkyl” refers to a molecule comprising hydrogen and carbon having the general formula C_nH_{2n+1} . A “ C_x to C_y alkyl” or a “ C_x - C_y alkyl” refers to an alkyl having a number of carbons, the number being from x to y carbons. For example, “ C_1 to C_6 alkyl” denotes that the alkyl may have 1, 2, 3, 4, 5 or 6 carbons.

All possible stereoisomers, epimers, diastereomers and enantiomers and mixtures thereof are specifically included by formulas described herein that have one or more chiral centers with a

“  ” bond (hereinafter termed a stereo-bond). Stereo-bonds denote that any one or more of the possible orientations of the bond is/are specifically included or specifically excluded from a particular embodiment and all of the embodiments, when considered together, include all such combinations of inclusion and exclusion of the possible bond orientations.

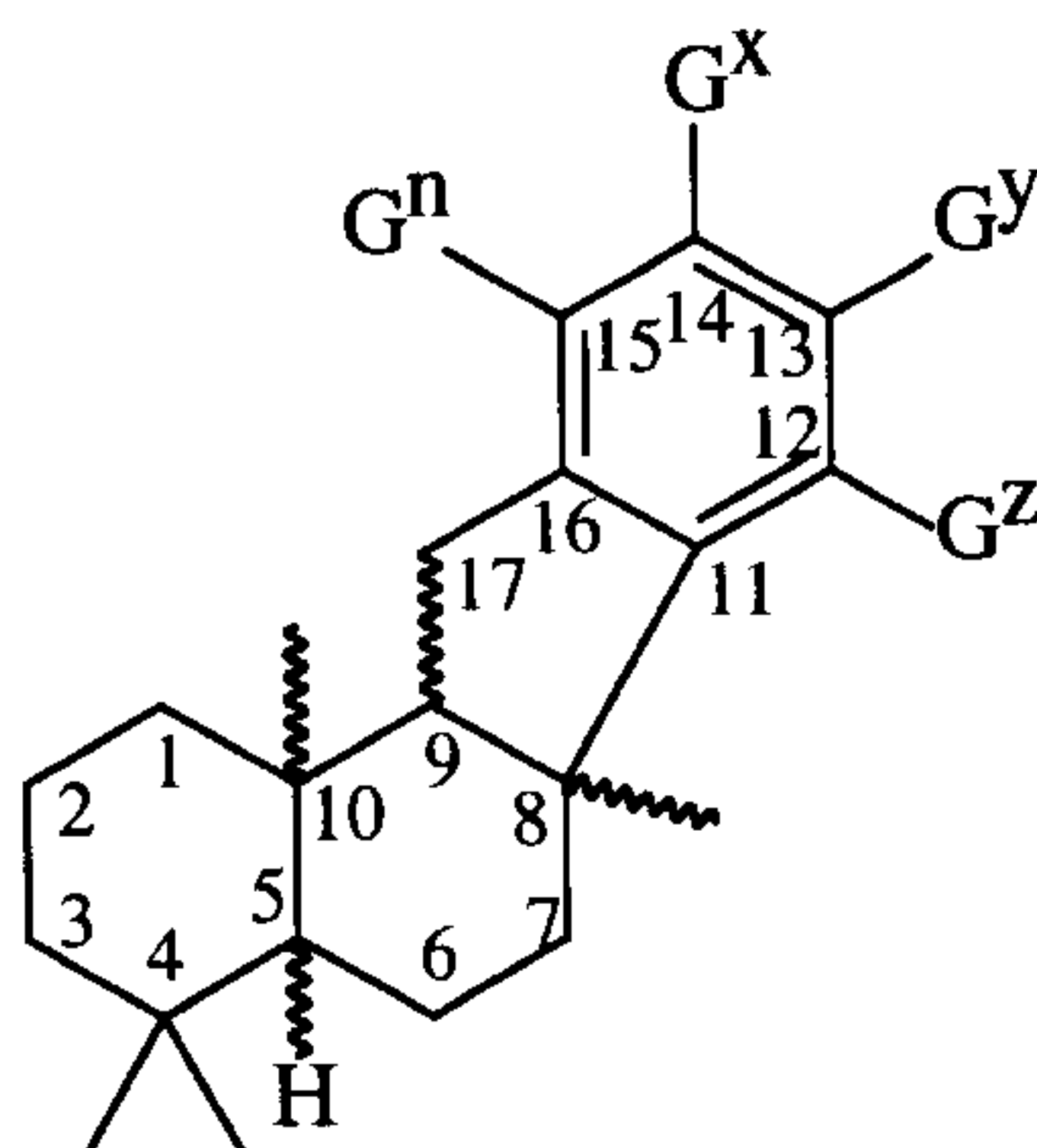
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The phrase “stereo-mixture” as used herein may be a mixture of equal quantities or unequal quantities of two or more different stereoisomers. Stereo-mixtures may comprise any particular stereoisomer from 0% to 100% (and all values in between) as a component of the stereo-mixture, provided that at least 2 different stereoisomers are present in the mixture. A
10 “racemic mixture” is a stereo-mixture that has equal quantities of each of the stereoisomers contained in the mixture.

The phrase “stereo-pure compound” as used herein refers to a compound having one or more chiral centers wherein each and every molecule of the compound has the same stereochemical
15 structure. The phrase “substantially stereo-pure compound” refers to a compound that may be a stereo-pure compound or may be a compound wherein at least 97% of the molecules have the same stereochemical structure. Substantially stereo-pure compounds may be compounds wherein at least 98% of the molecules have the same stereochemical structure or may be compounds wherein at least 99% of the molecules have the same stereochemical structure.
20 Substantially stereo-pure compounds may be compounds wherein at least 99.5% of the molecules have the same stereochemical structure or may be compounds wherein at least 99.9% of the molecules have the same stereochemical structure.

SHIP 1 Modulating Compounds and Prodrugs

25 Compounds of the invention comprise a pelorol analog having an –OH moiety attached to the carbon atom at positions 14, which have better activity. In some embodiments of the present invention, the –OH on the carbon at position 14 may be replaced by a prodrug moiety that is cleavable such that it provides an –OH moiety on the carbon at position 14 when the prodrug moiety is cleaved. Carbon atom position numbering of molecules described herein is
30 exemplified by the following:



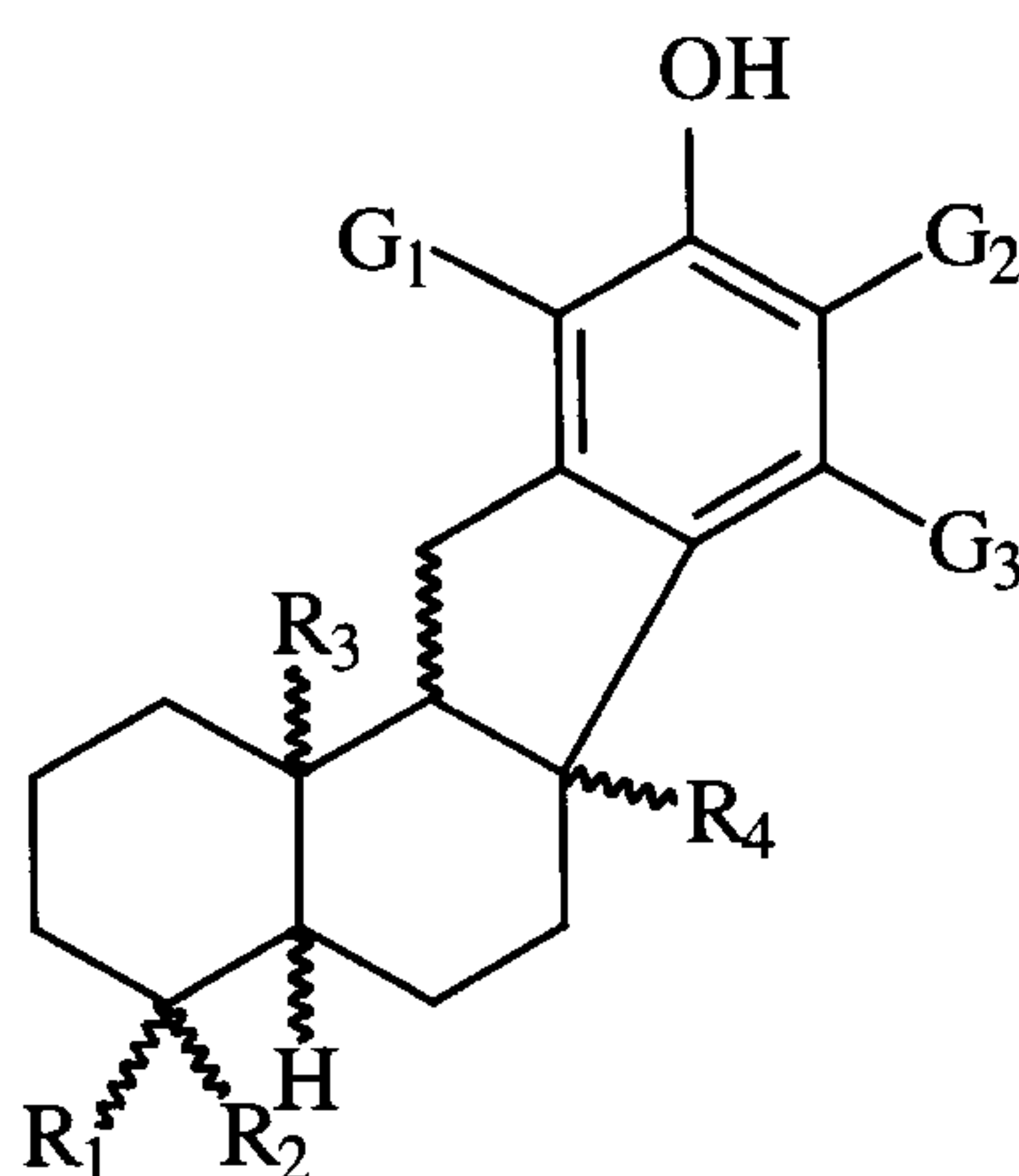
Compounds having more than one –OH moiety, do not have the second –OH moiety in a position that is ortho to the carbon atom at position 14. Due to the position of the carbon atom at position 14, this means that compounds having two –OH moieties comprise –OH moieties that have a meta configuration. Some compound of the present invention comprise a second –OH moiety substituted on the carbon atom at position 12.

Compounds having two –OH moieties that are meta to each other and compounds having a total of a single –OH or in other words, exactly one –OH have improved stability with respect to compounds having two or more –OH moieties that have a para configuration. For example, replacement of a catechol functionality with a single phenol removes the possibility of air oxidation to an orthoquinone. Orthoquinones are highly coloured and very reactive Michael acceptors. Compounds having exactly one –OH cannot readily form an orthoquinone via air oxidation thereby providing improved chemical stability. Furthermore, enzymatic oxidation to an orthoquinone is also reduced. Orthoquinones may react with nucleophilic functionalities on proteins leading to covalent linkages. Hence, *in vivo*, a portion of those compounds having 2 or more –OH moieties administered are more likely to bind to proteins. Consequently, compounds having only a single –OH provide similar activity at lower concentrations.

The synthesis of compounds having a single –OH moiety on the carbon atom at position 14 may use a Barton deoxygenation step. Such a step is more efficient for removing a benzylic alcohol generated in a coupling step that may occur earlier in the synthesis. This step may replace a hydrogenolysis that is often used in the synthesis of compounds having an –OH moiety on both the carbon atom at position 14 and the carbon atom at position 15.

Additionally, compounds having a total of a single OH moiety may provide additional advantages when making prodrug versions thereof. An OH moiety on a SHIP modulating pelorol analog may be substituted by a prodrug moiety in the preparation of prodrugs. In those instances where there is more than one OH moiety on the SHIP modulating pelorol analog, selectively providing the prodrug moiety in the desired position often requires additional steps, including but not limited to protecting and deprotecting -OH moieties. In those instances where there is a total of a single -OH moiety, then the chemical synthesis of prodrug derivatives of SHIP modulating pelorol analogs may be made simpler.

Some embodiments of this invention provide compounds of Formula 1 and salts thereof:



Formula 1

wherein;

R_1 and R_2 are independently selected from the group consisting of: $-CH_3$, $-CH_2CH_3$, $-CH_2OH$, $-CH_2OR_1'$, $-CHO$, $-CO_2H$, and $-CO_2R_2'$;

R_3 and R_4 are independently selected from the group consisting of: H , $-CH_3$, $-CH_2CH_3$, $-CH_2OH$, $-CH_2OR_3'$, $-CHO$, $-CO_2H$, and $-CO_2R_4'$;

R_1' , R_2' , R_3' , and R_4' , are independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or substituted with one or more of: OH , $=O$, SH , F , Br , Cl , I , NH_2 , $-NHR_1''$, $-N(R_2'')$, NO_2 and $-CO_2H$ where R_1'' and R_2'' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group;

G_1 is selected from the group consisting of: $O-(C_1-C_{10} \text{ alkyl})$ and H ;

G_2 is H or $C_1-C_{10} \text{ alkyl}$; and

G_3 is selected from the group consisting of: H , $-OH$, $C_1-C_{10} \text{ alkyl}$ and $O-(C_1-C_{10} \text{ alkyl})$.

In other embodiments of Formula 1, G_1 is selected from the group consisting of –O-methyl and H; G_2 is H or methyl; and G_3 is selected from the group consisting of: H, methyl and O-methyl.

5 Compounds of Formula 1 have chiral centres at C-5, C-8, C-9 and C-10 and may be chiral at C-4 depending upon whether R_1 and R_2 are different. Some embodiments have the same relative configuration of chiral centres as does pelorol or are enantiomers thereof, namely: S, R, R, S; or R, S, S, R (at C-5, 8, 9 and 10 respectively). Some embodiments have the same absolute configuration as pelorol at chiral centres. Some embodiments have the same relative
10 configuration as pelorol at C-5 and C-10 with independently variable configurations at C-8 and C-9. Some embodiments have the same relative configuration as pelorol at C-5, C-8, and C-10 with variable configuration at C-9. In all cases, the configuration at C-4 (if chiral) may be variable or may be the same relative configuration to the remaining chiral centres as is shown in examples of structures of compounds of Formula 1 illustrated herein.

15 In various embodiments the pelorol analog may have more specific limitations with respect to substituents R_1 , R_2 , R_3 , and R_4 . Any combination of the following limitations is encompassed by this invention.

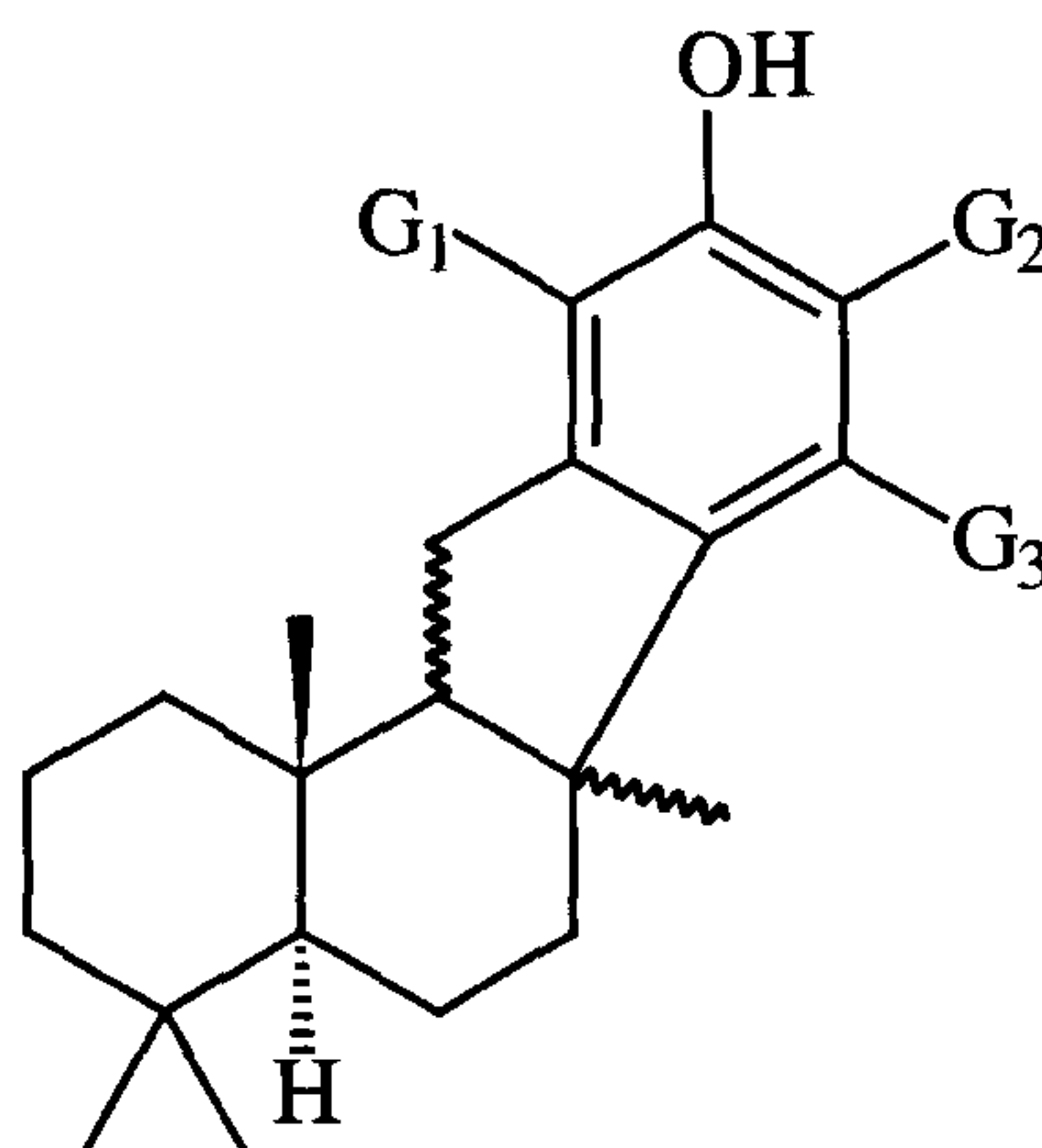
(a) one or both of R_1 and R_2 may be limited to methyl, ethyl, $-\text{CH}_2\text{OH}$, $-\text{CH}_2\text{OR}_1'$,
20 or $-\text{CH}_2\text{OR}_3'$;

(b) R_1' , R_2' , R_3' , and/or R_4' , in one or both of R_1 and R_2 according to Formula 1, or in the limitation of paragraph (a) above, may be limited to methyl, ethyl, propyl or butyl;

(c) one or both of R_1 and R_2 may be limited to methyl or ethyl;

(d) one or both of R_1 and R_2 may be limited to methyl;

Some embodiments of this invention provide compounds of Formula 2 and salts thereof:



Formula 2

wherein;

- 5 G_1 is selected from the group consisting of: O-(C₁-C₁₀ alkyl) and H;
 G_2 is H or C₁-C₁₀ alkyl; and
 G_3 is selected from the group consisting of: H, -OH, C₁-C₁₀ alkyl and O-(C₁-C₁₀ alkyl).

10 In other embodiments of Formula 2, G_1 is selected from the group consisting of -O-methyl and H; G_2 is H or methyl; and G_3 is selected from the group consisting of: H, methyl and O-methyl.

15 In various embodiments of formulas 1 and/or 2, the pelorol analog may have more specific limitations with respect to substituents G_1 , G_2 , and G_3 . Any combination of the following limitations is encompassed by this invention. Any combination of the following with paragraphs (a), (b), (c), and/or (d) above is also encompassed by this invention.

- (e) only one of G_1 , G_2 and/or G_3 is -O-methyl.
- (f) at least one of G_1 , G_2 and/or G_3 are H.
- (g) all of G_1 , G_2 and/or G_3 are H.
- (h) G_3 is methyl.

20

Shown below in Table 1 are non-limiting examples of the stereoisomers that are specifically encompassed by any one of Formulas 1 and/or 2 as depicted above. Stereo-mixtures and racemic mixtures of any two or more of the stereoisomers of Table 1, substantially stereo-pure compounds and stereo-pure compounds are also included by Formulas 1 and 2 as depicted

above. R_1 , R_2 , R_3 , R_4 , G_1 , G_2 , and G_3 as used below in Table 1 may as defined for the respective Formula or as by any of the limitations of paragraphs (a) to (h) above.

Table 1	

Table 1

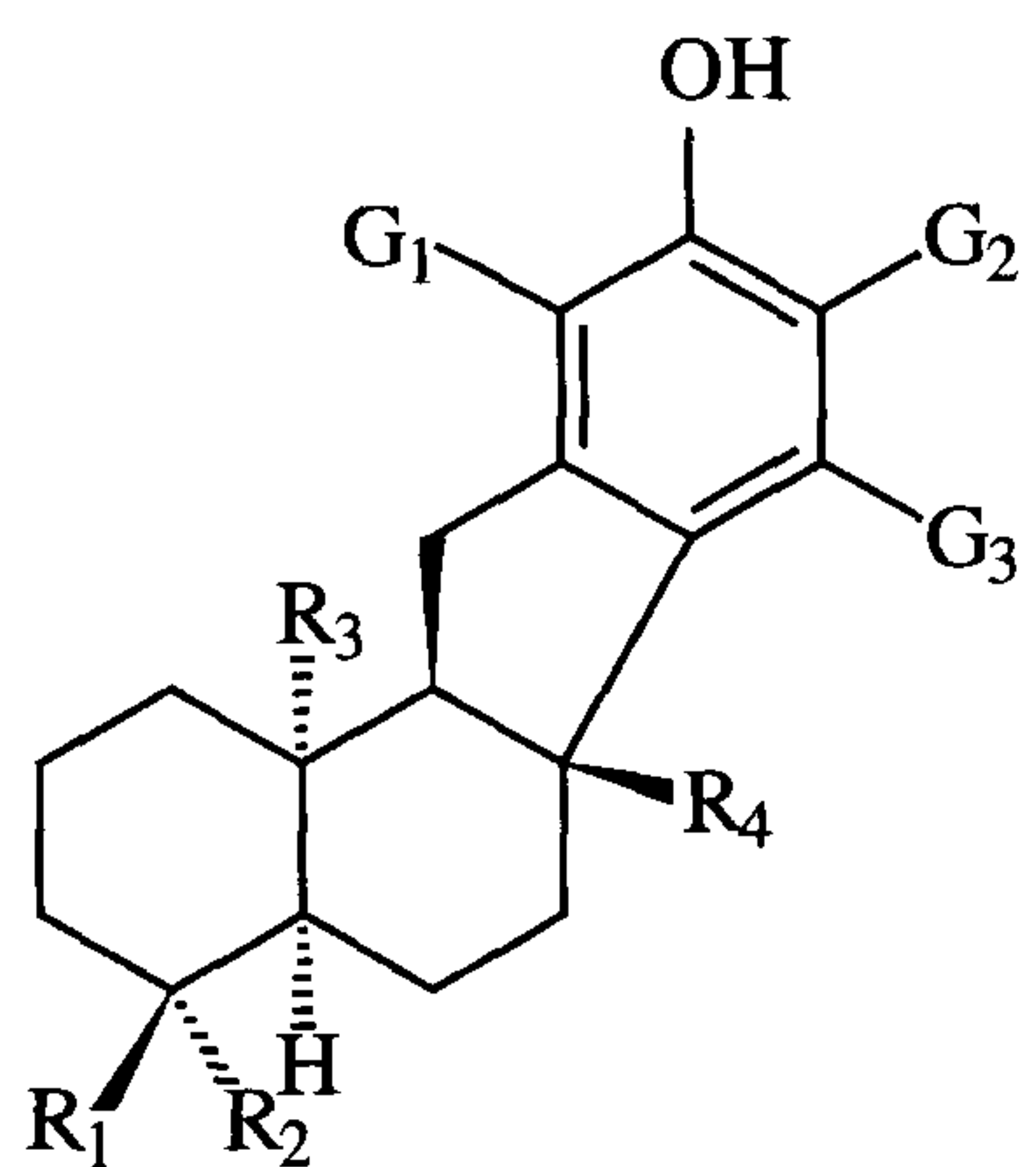
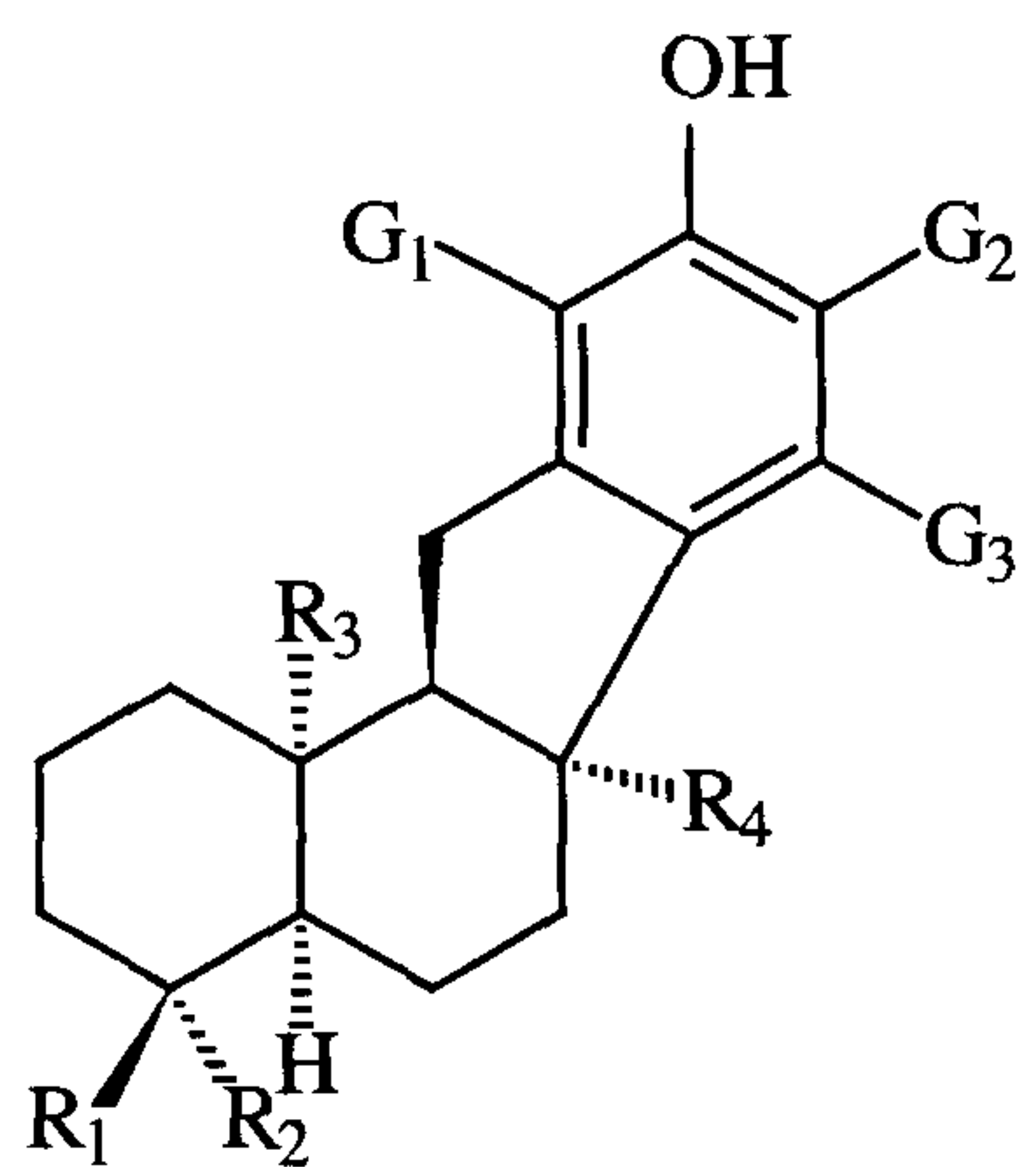
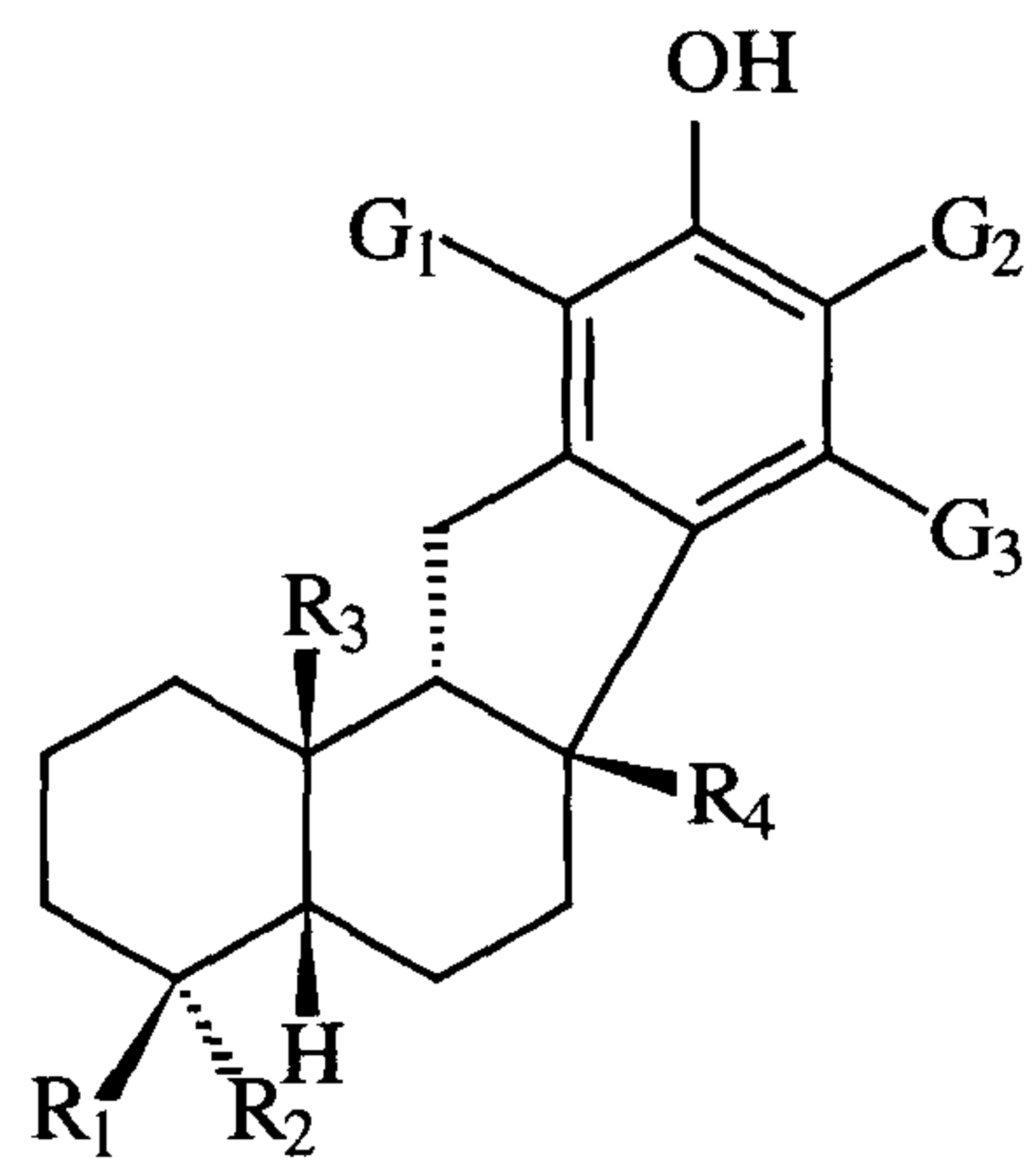
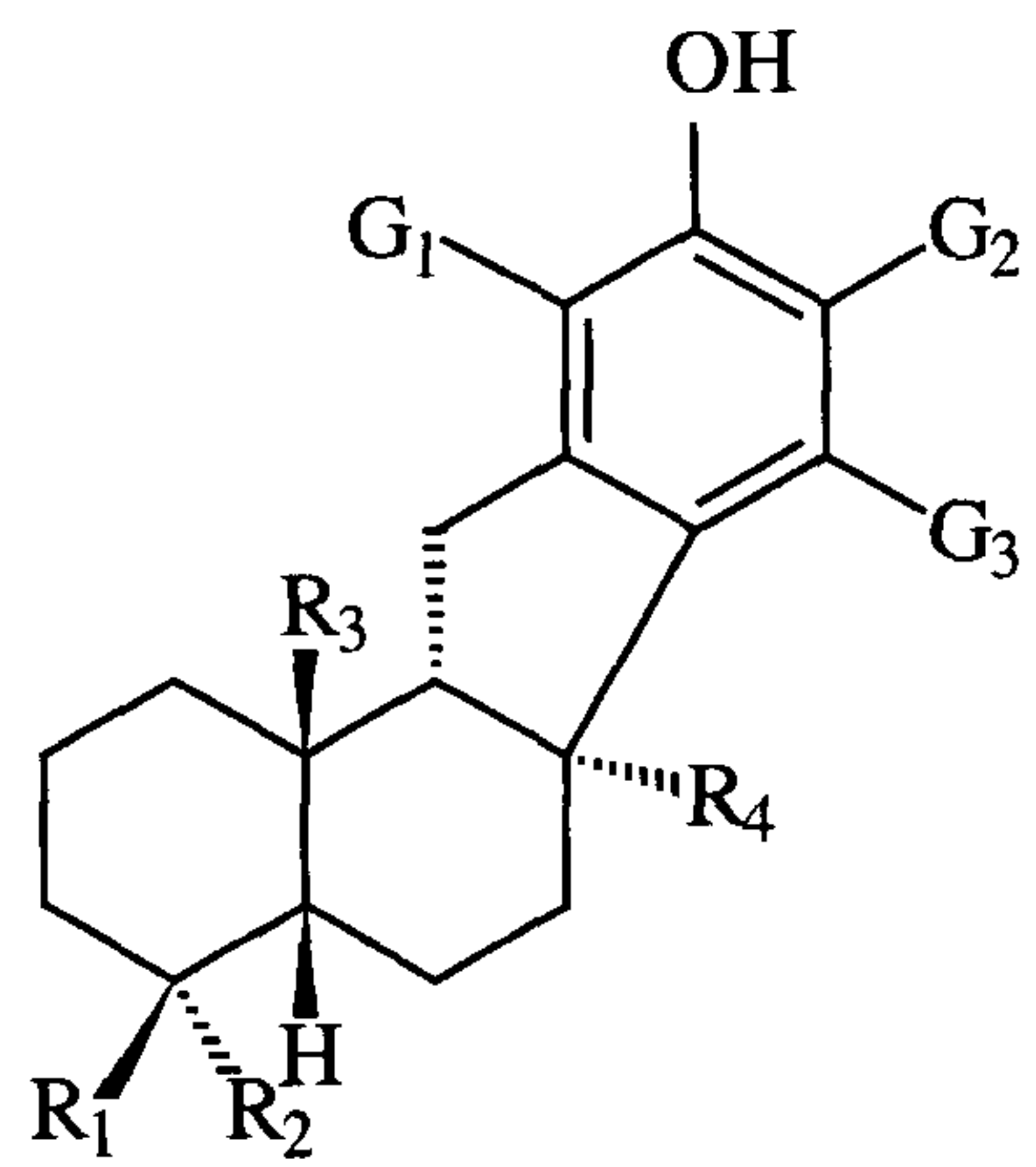
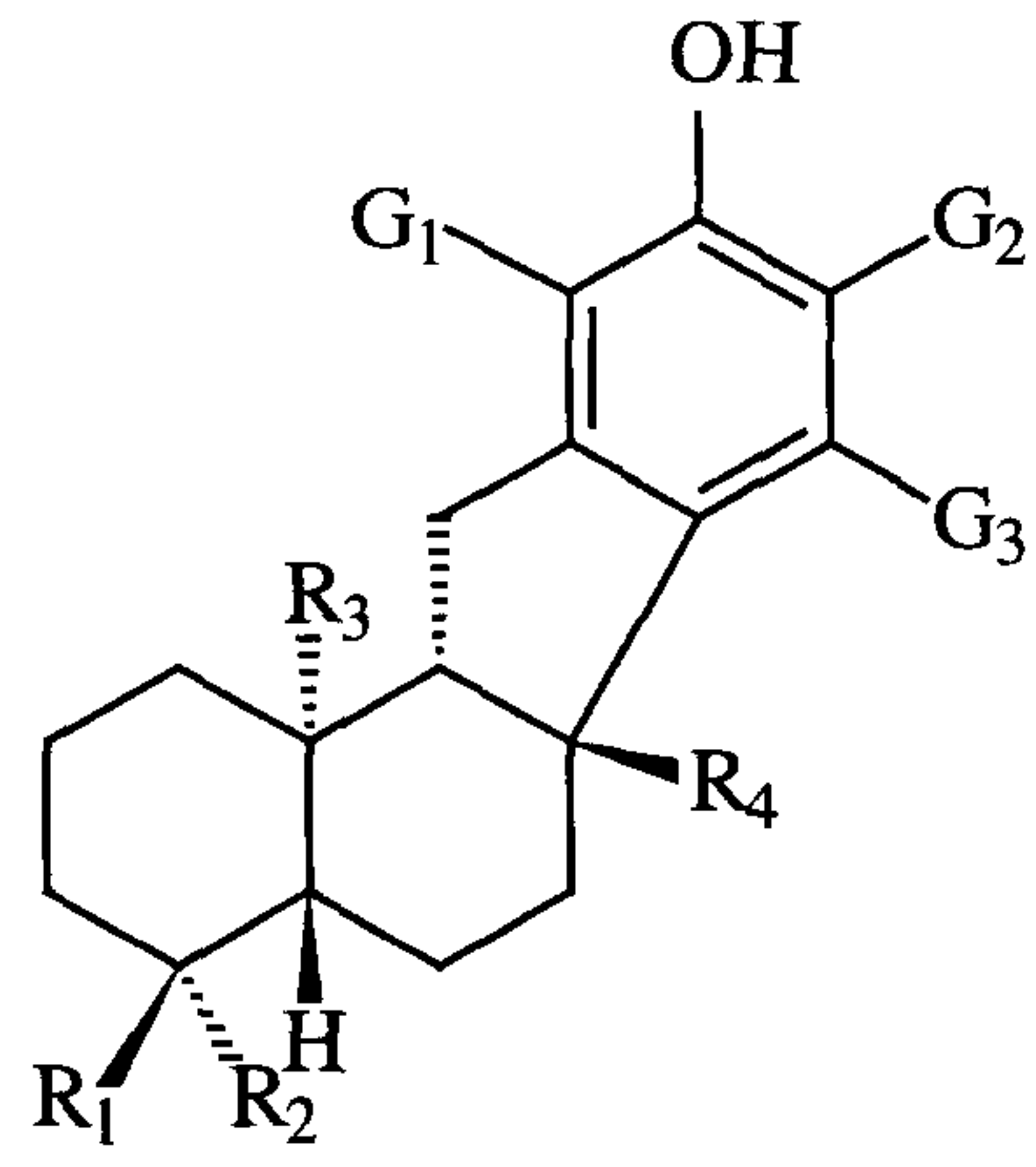
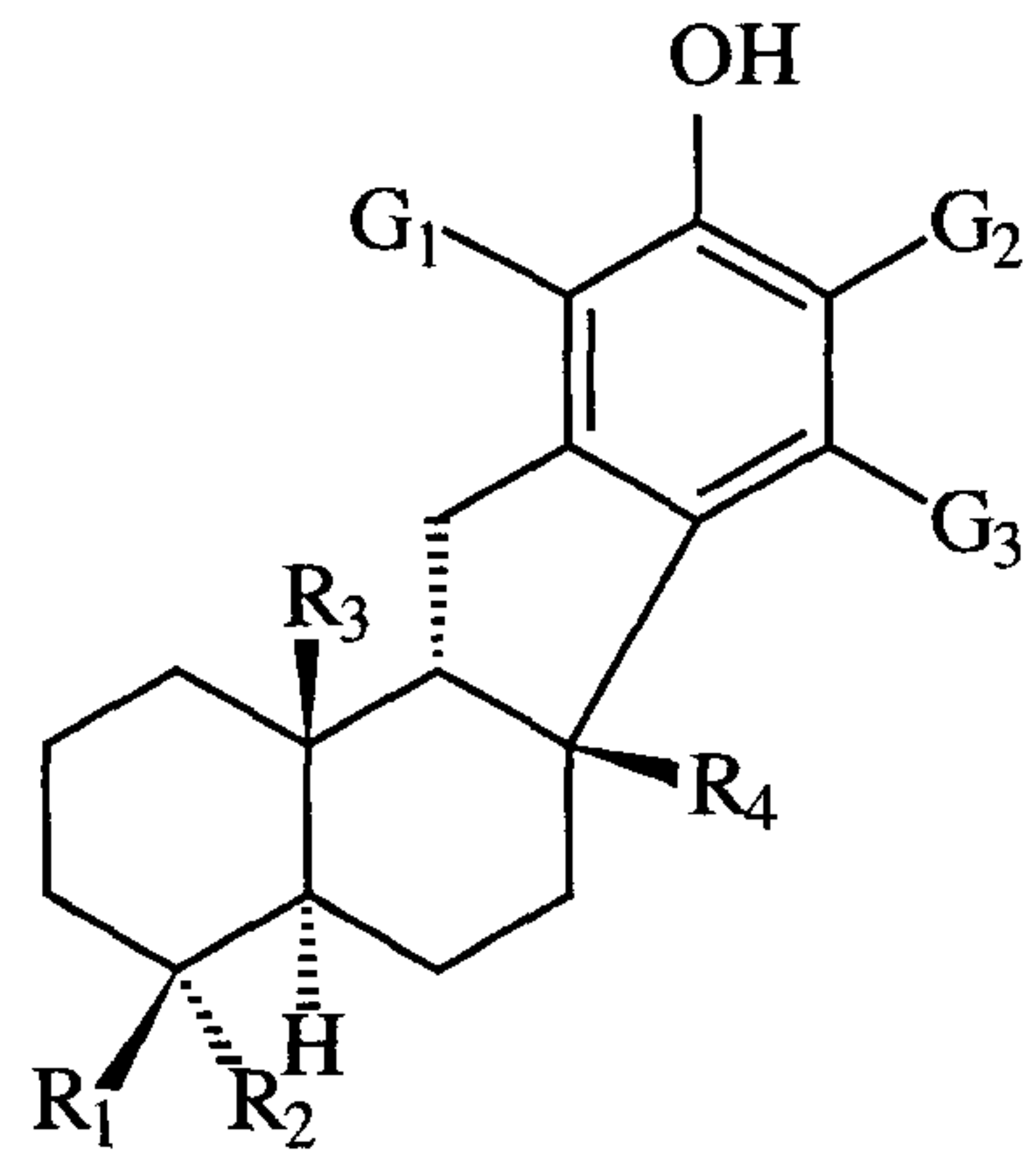
	
	
	

Table 1

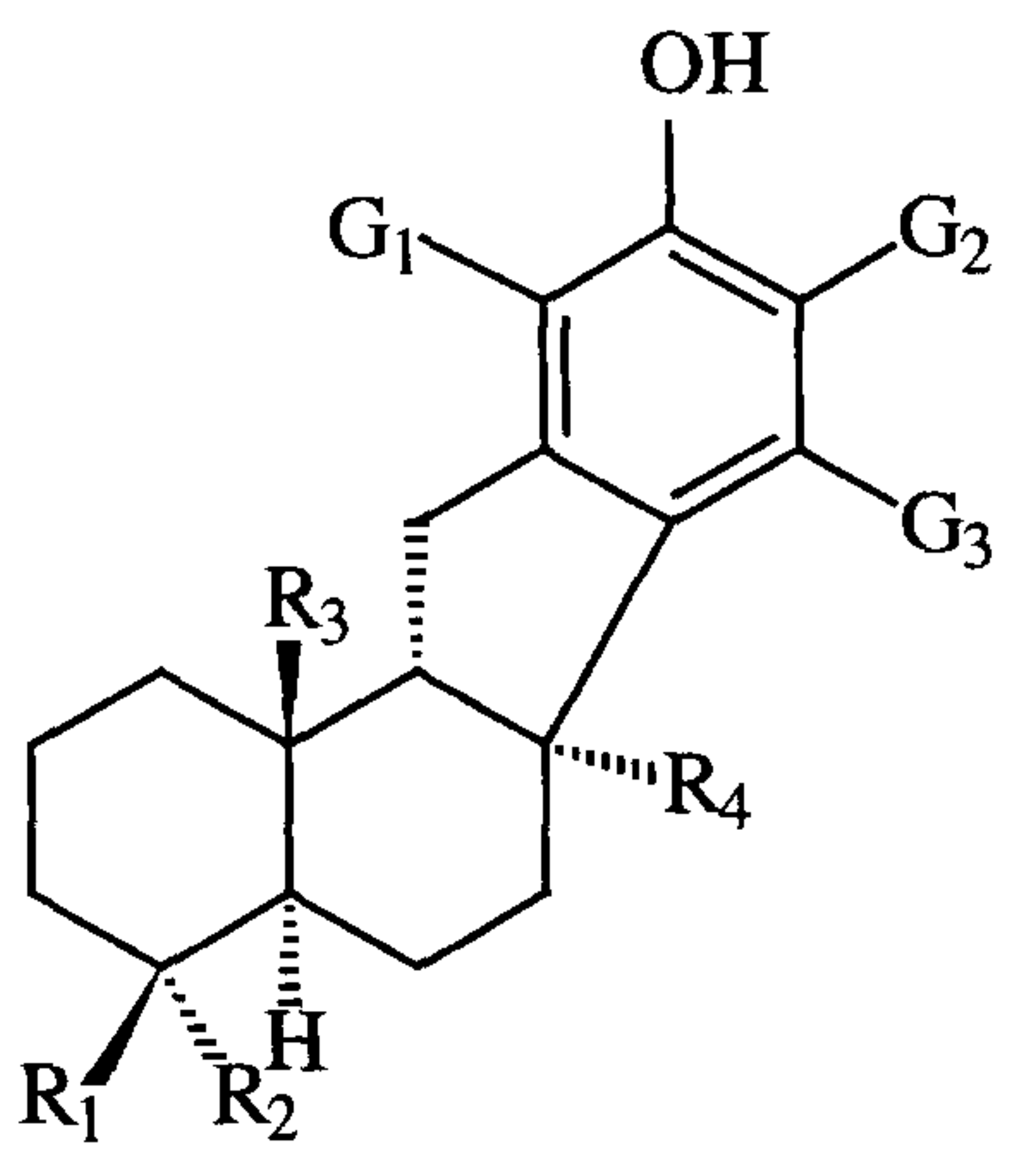
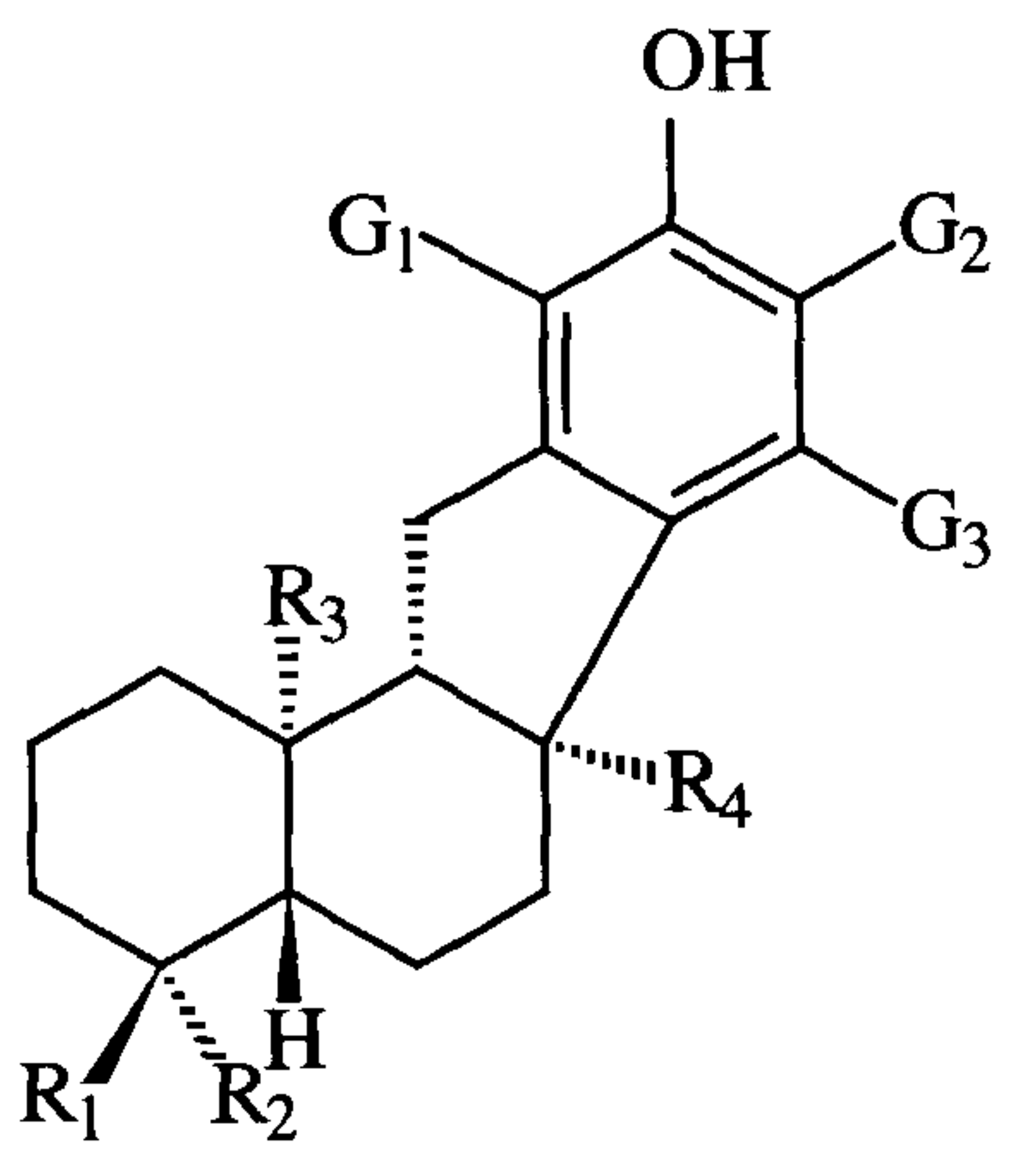
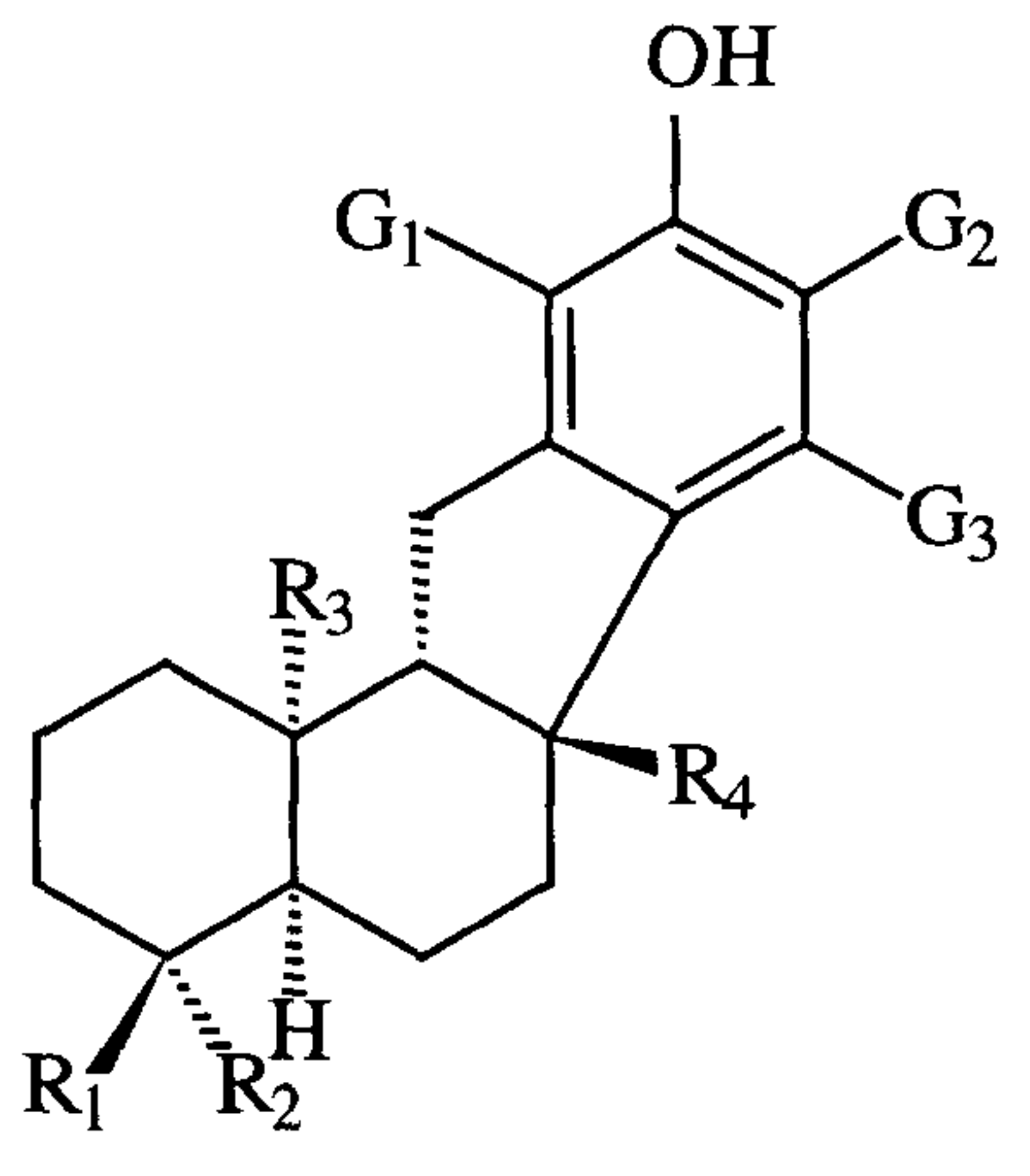
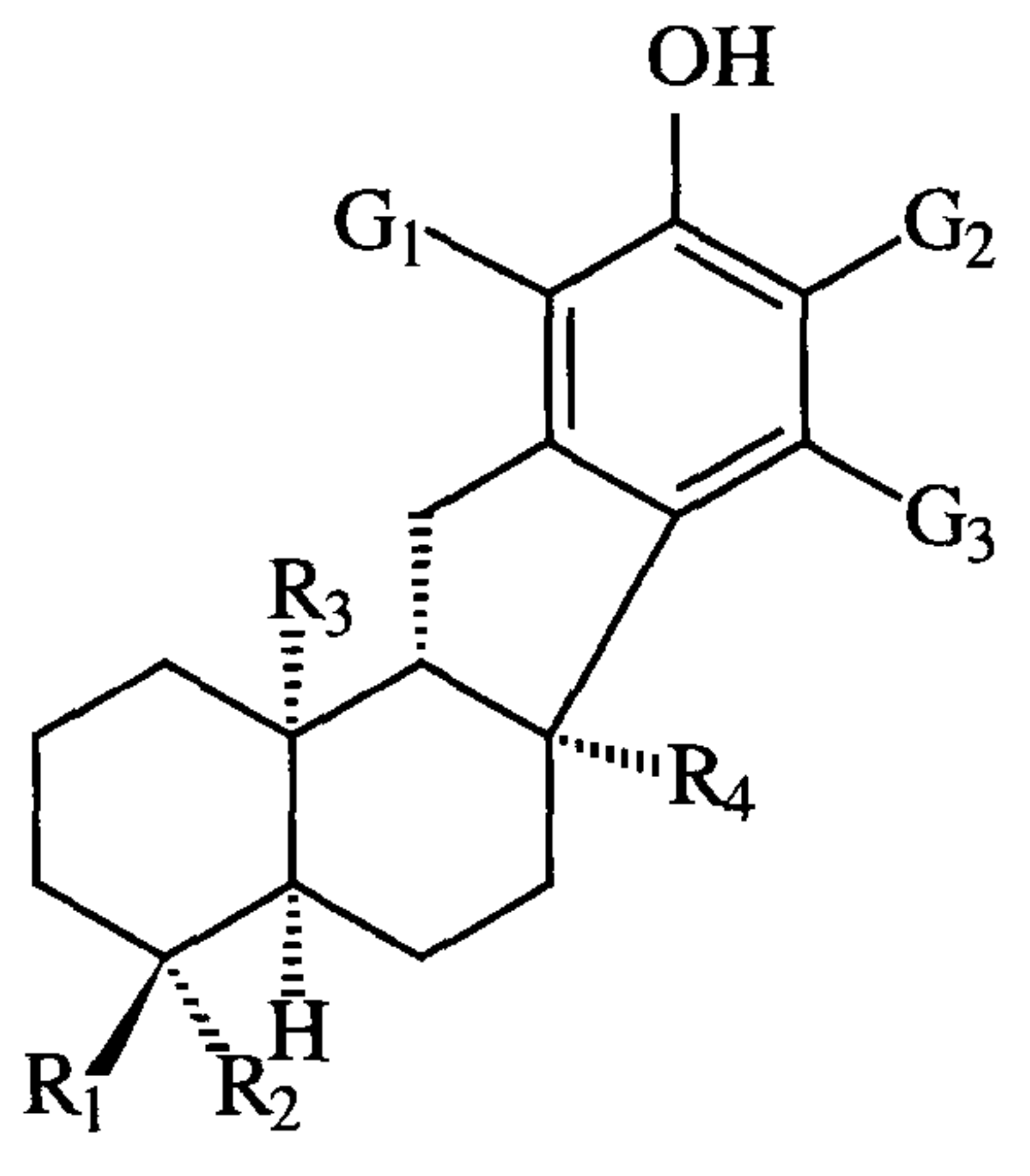
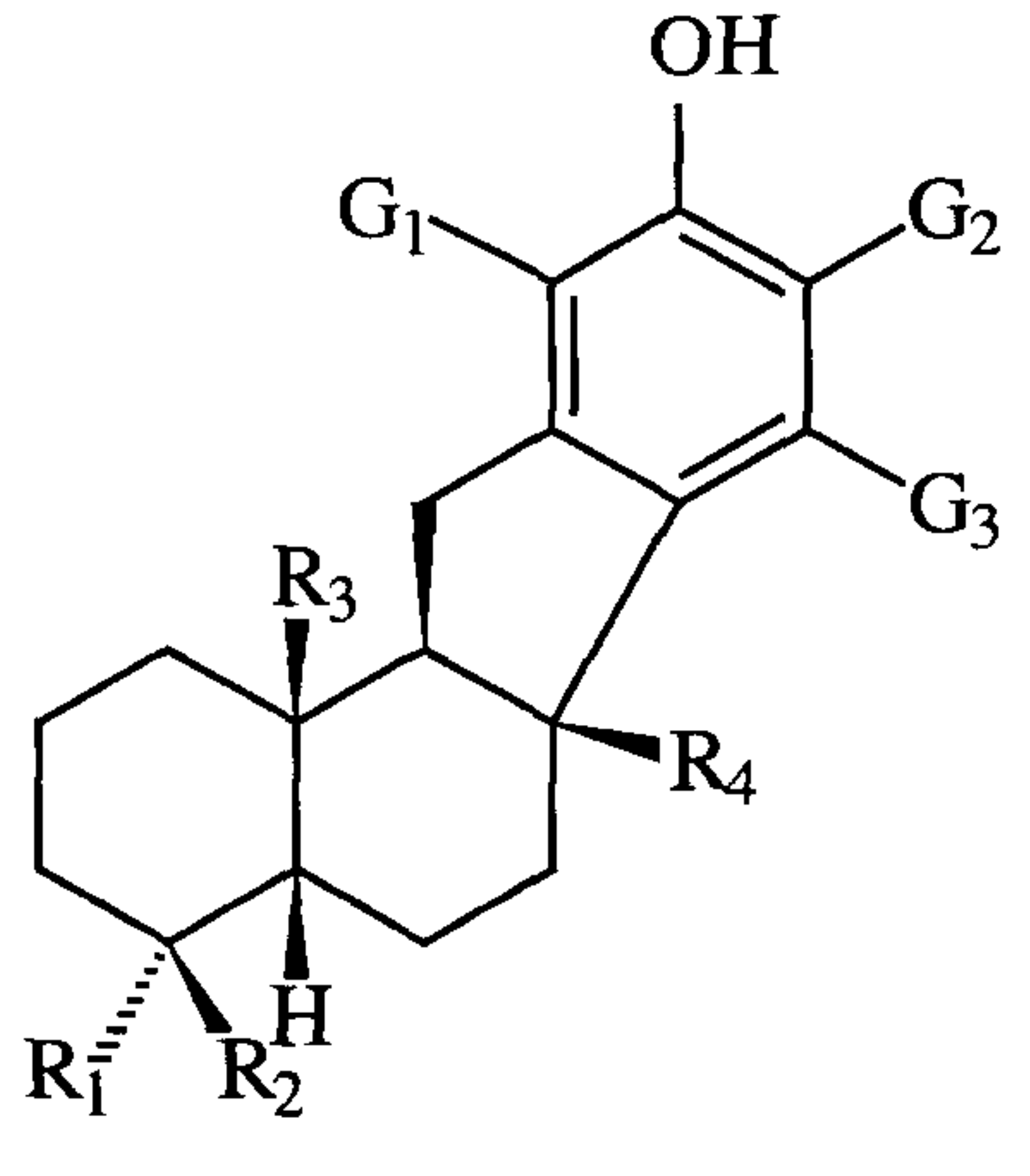
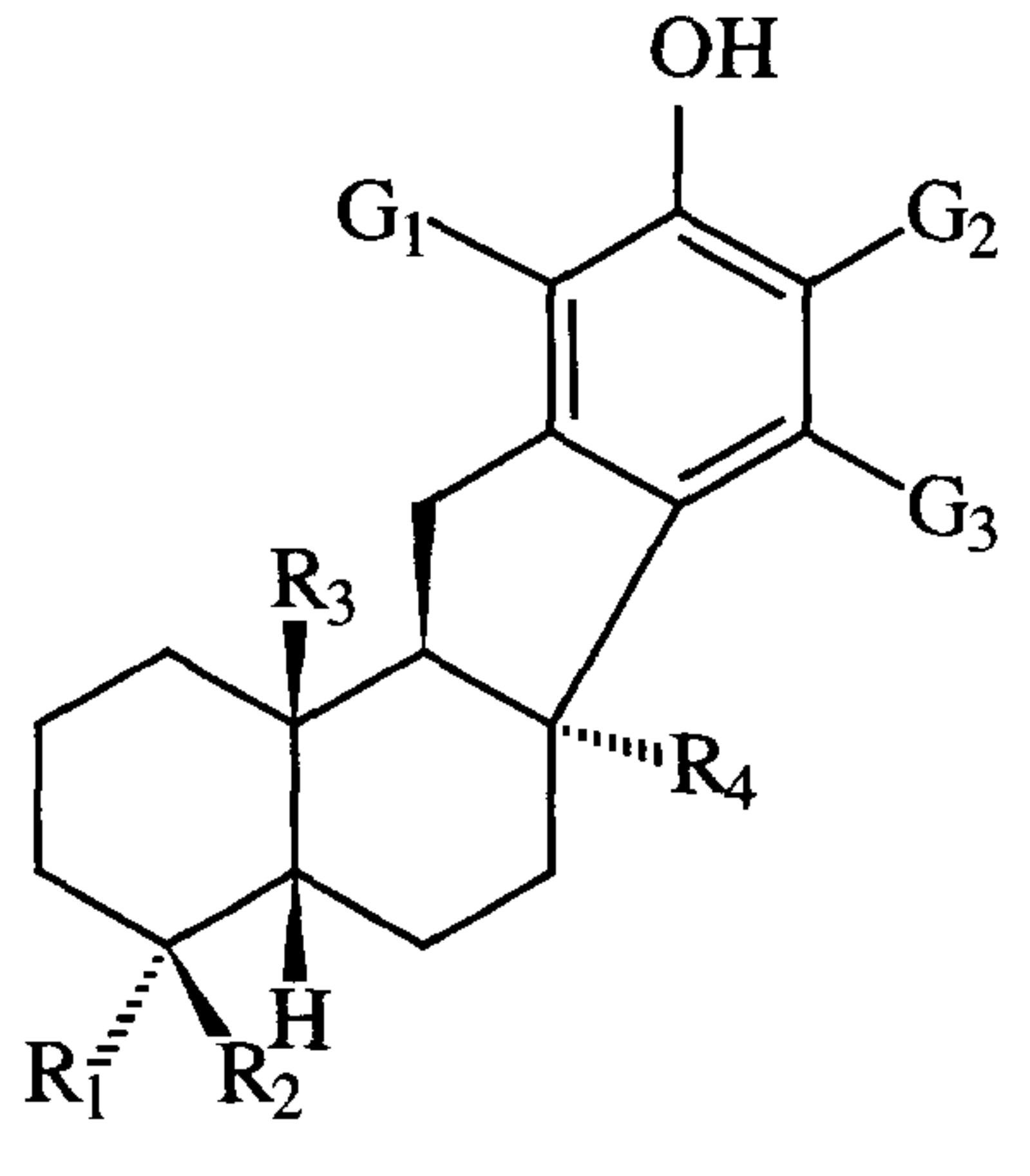
	
	
	

Table 1

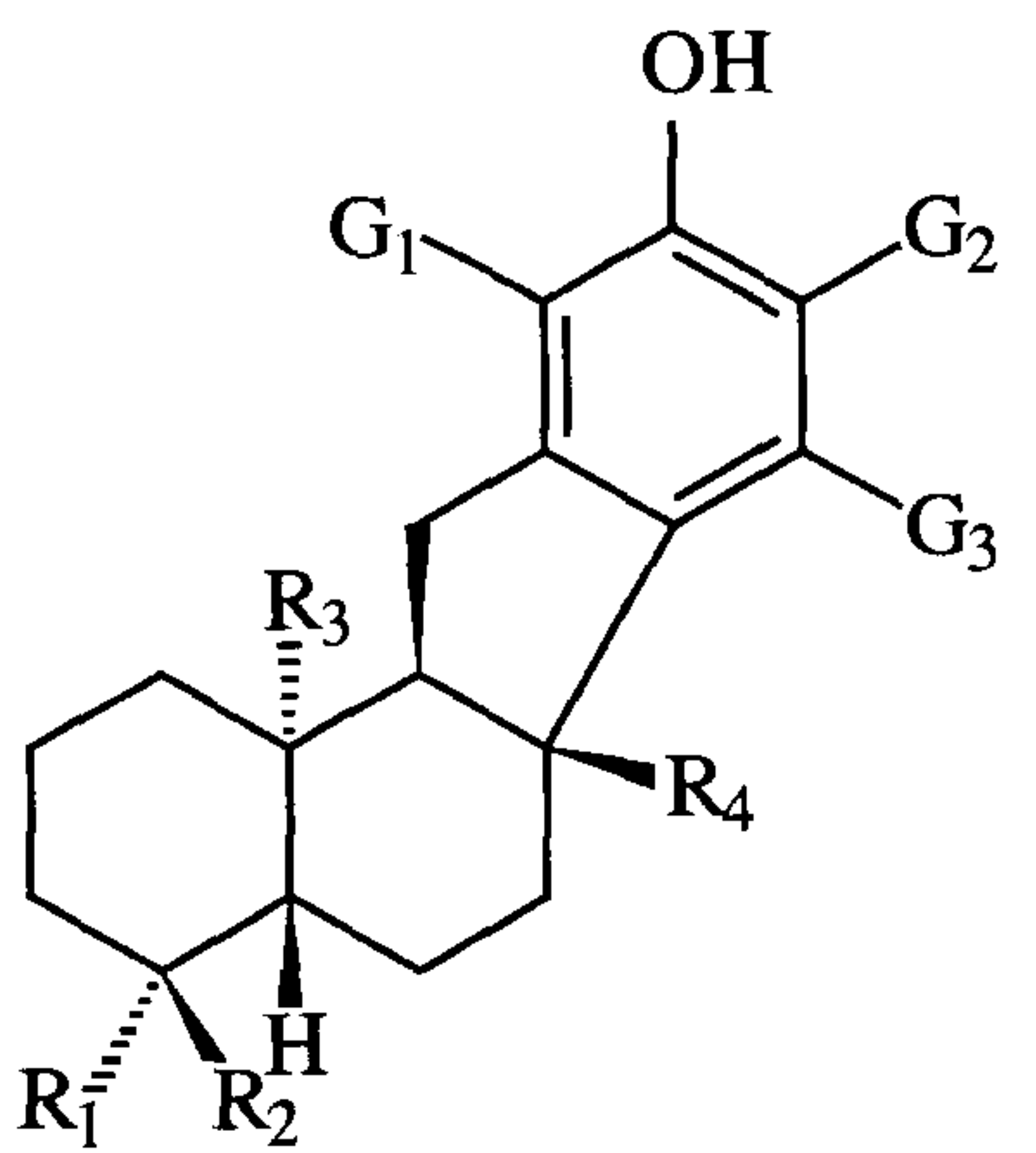
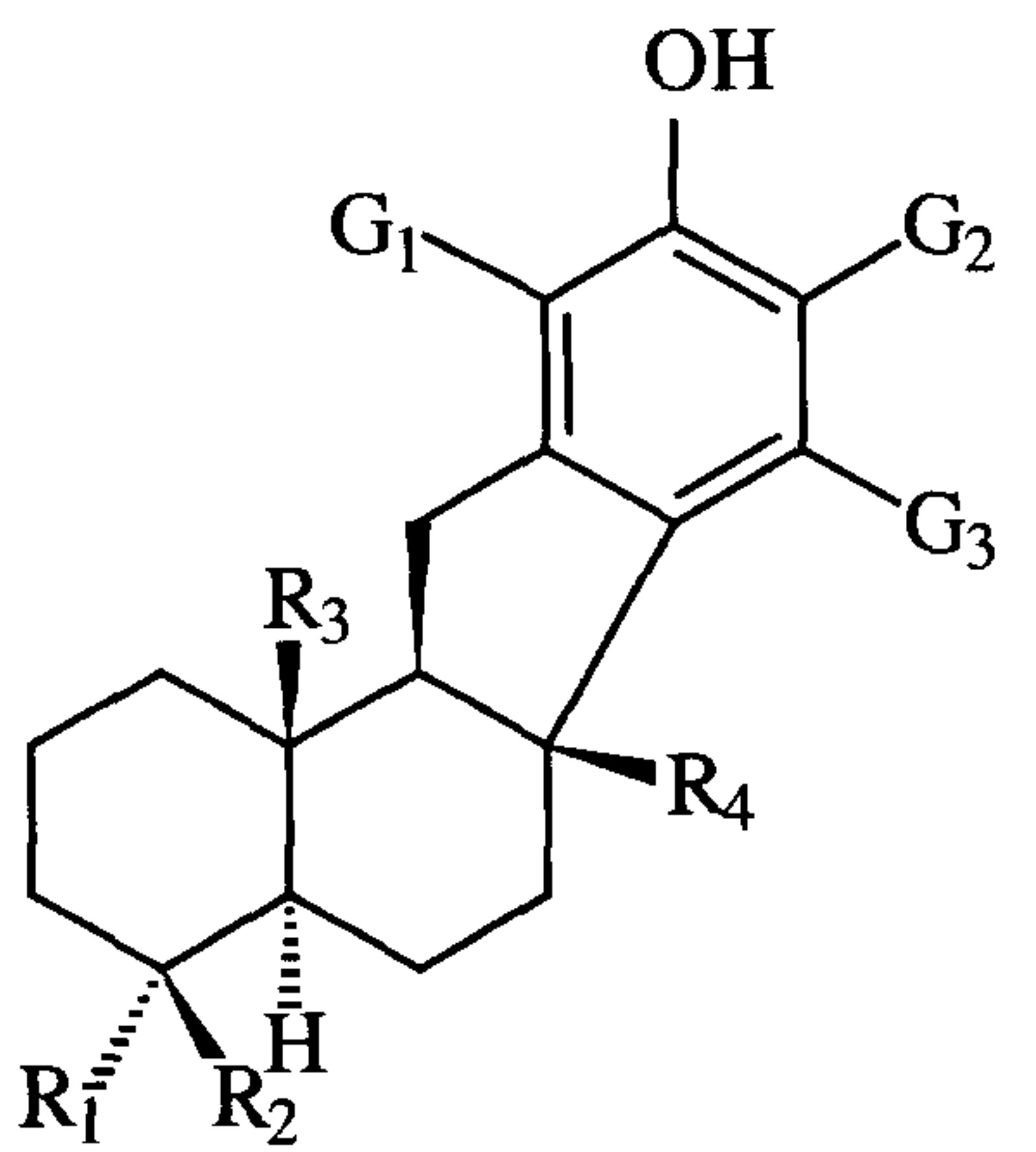
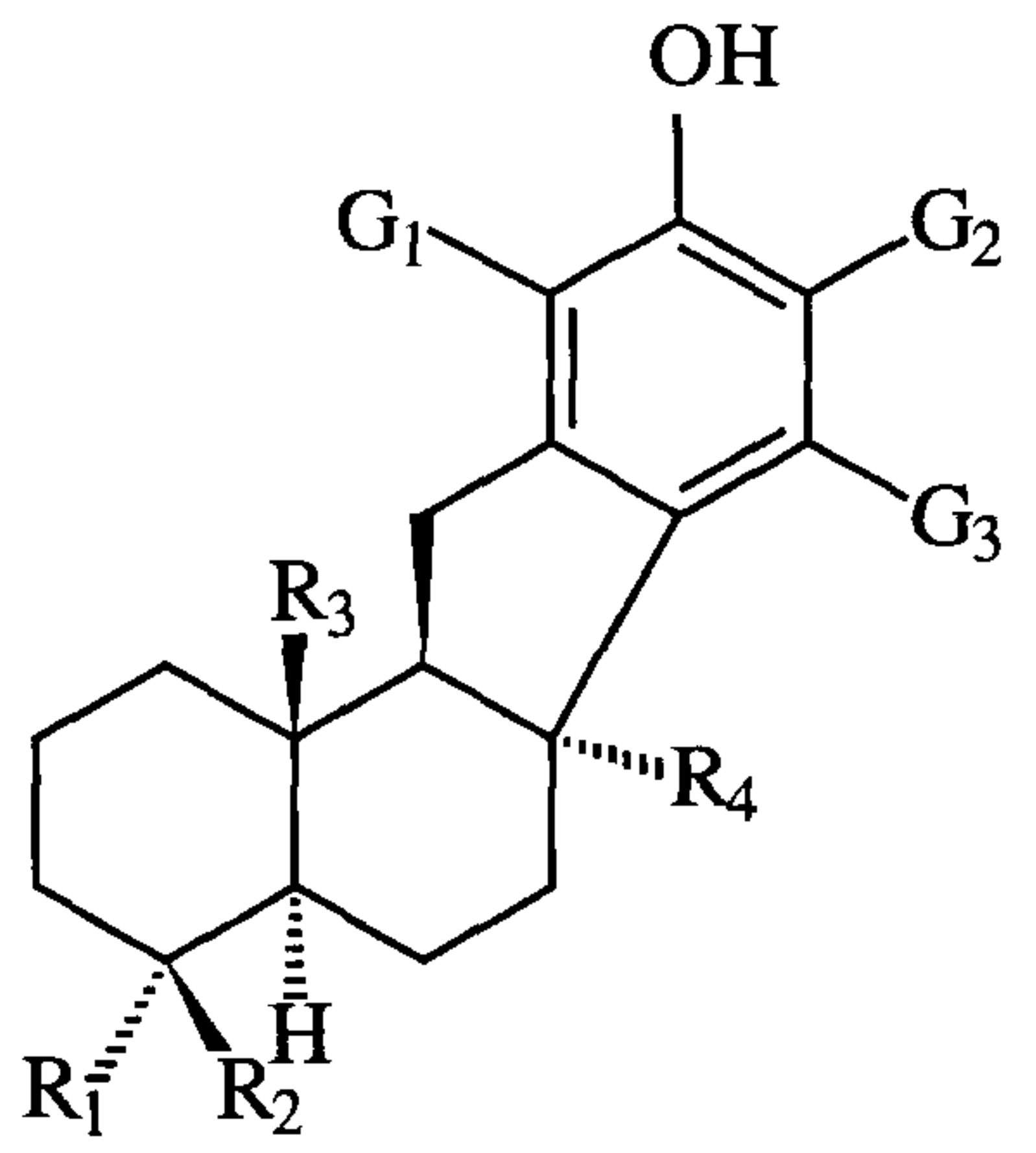
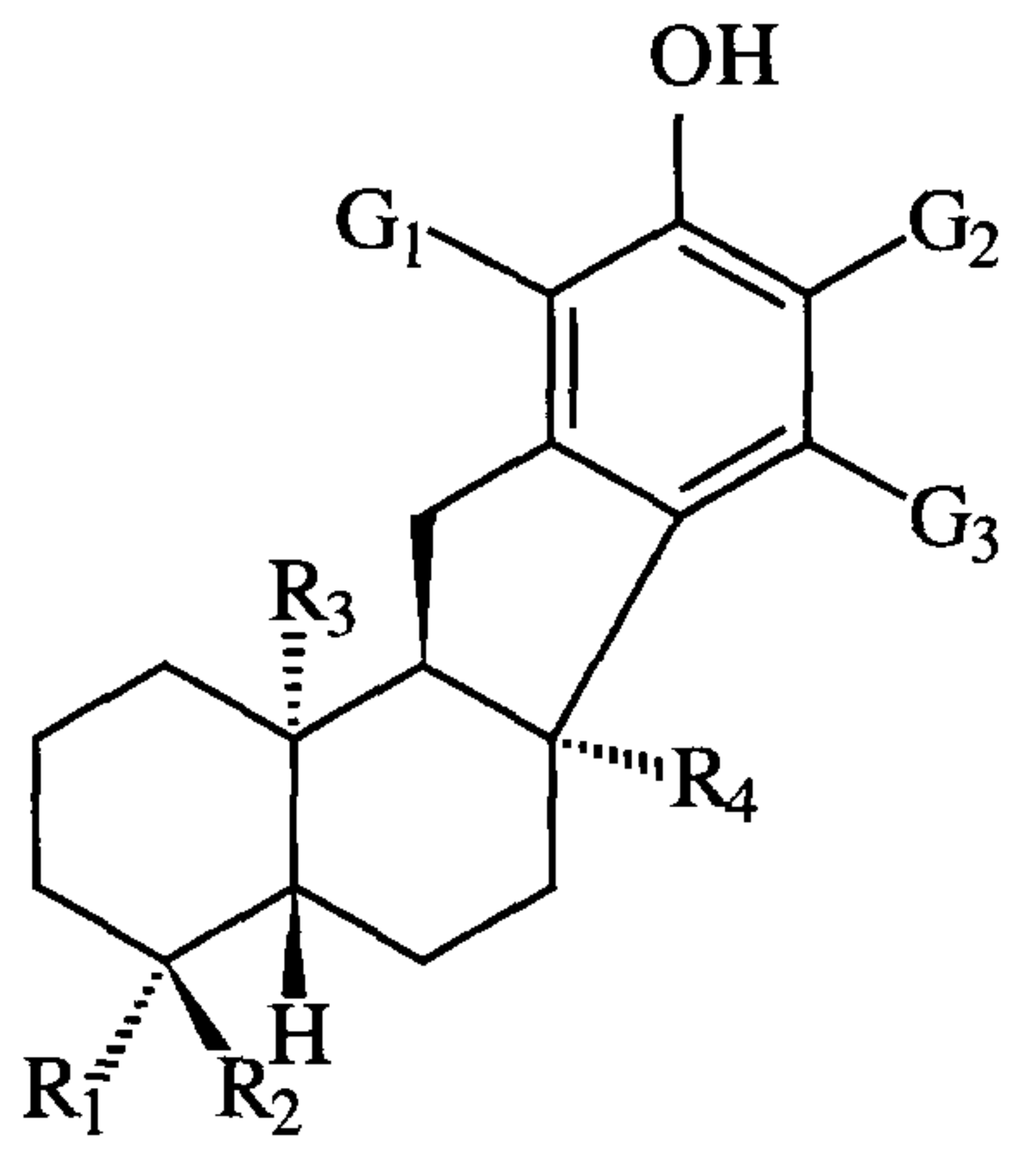
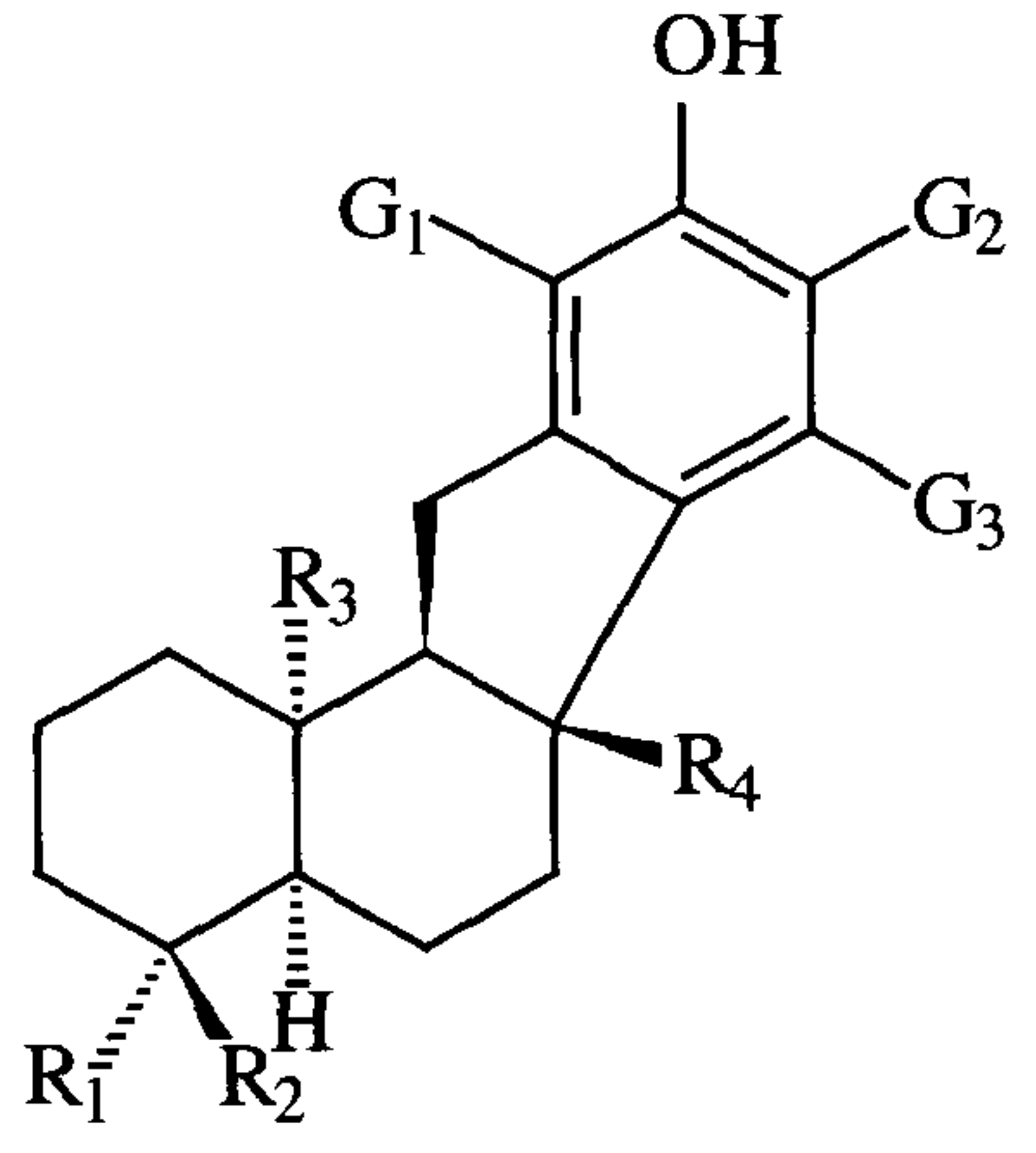
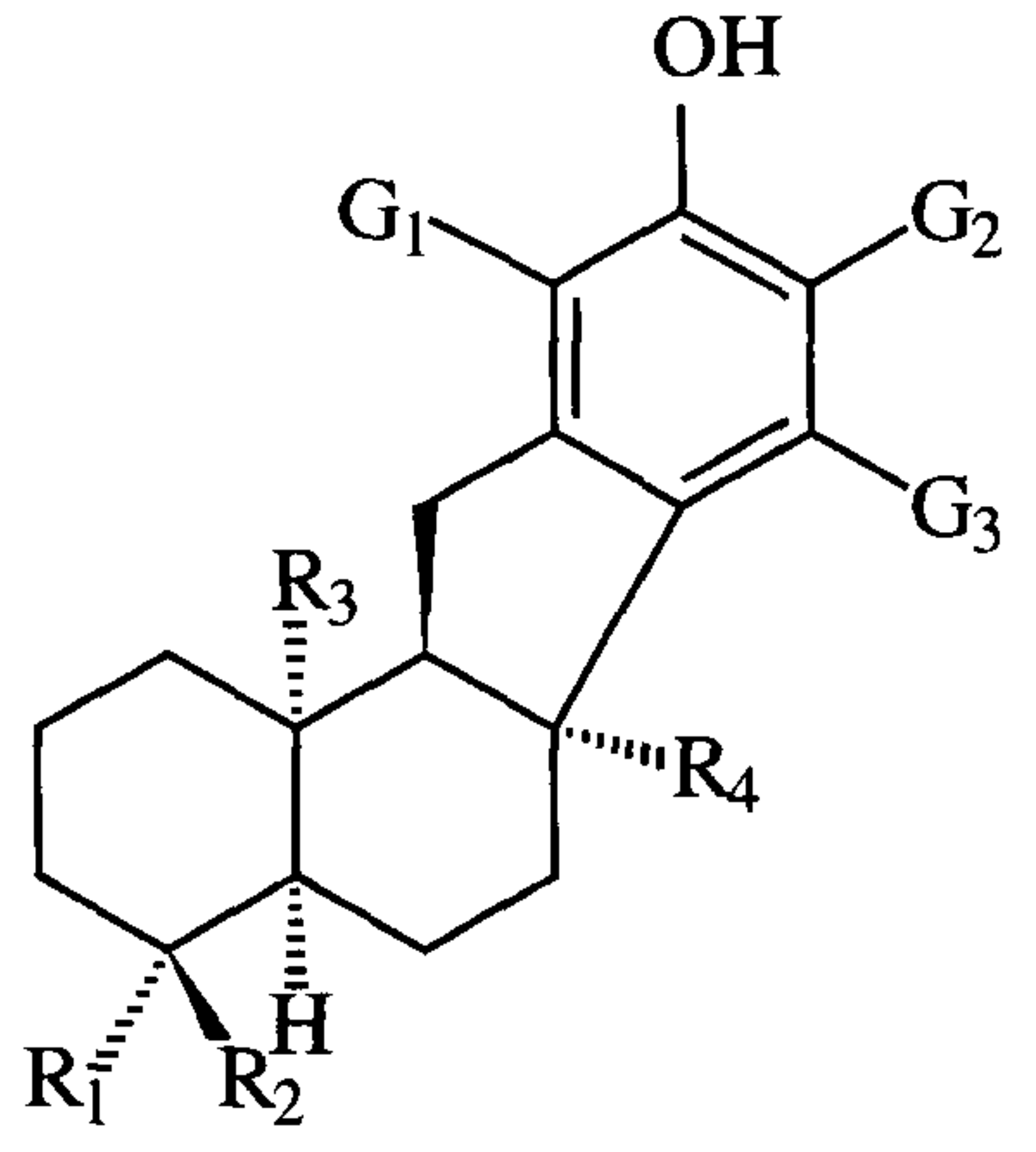
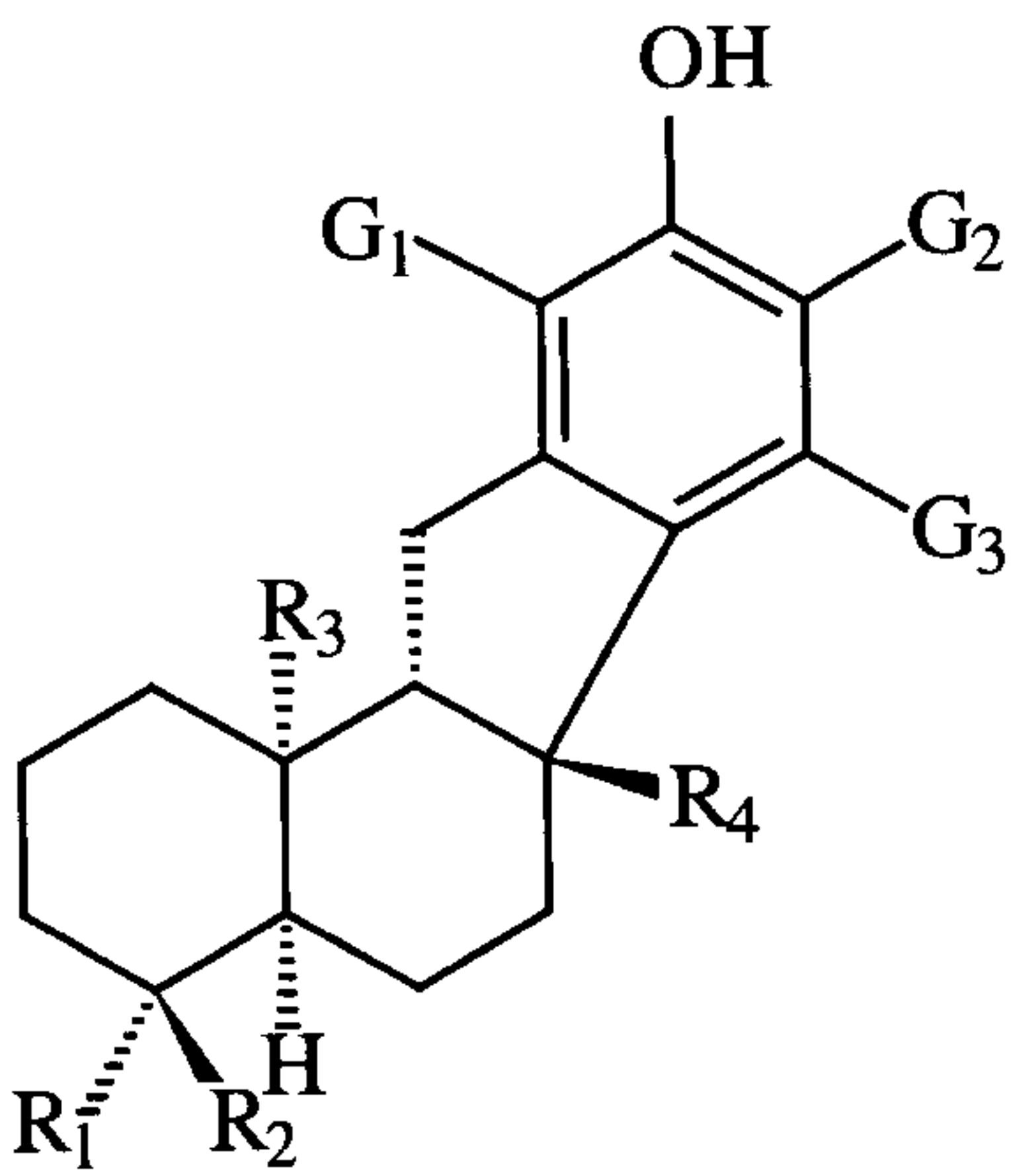
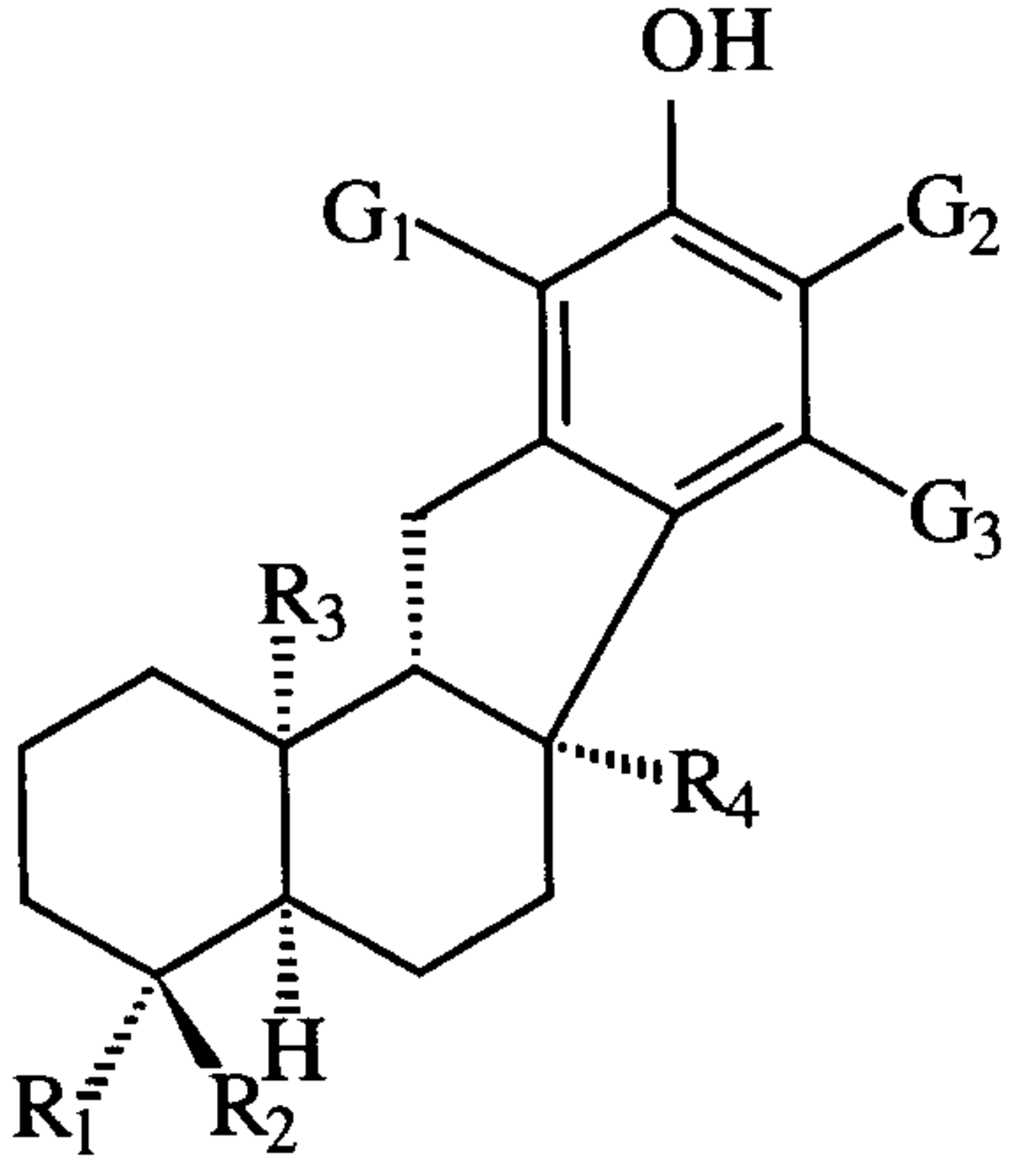
	
	
	

Table 1

Table 1	
	

Non-limiting examples of compounds of the present invention are provided below in Table 1A

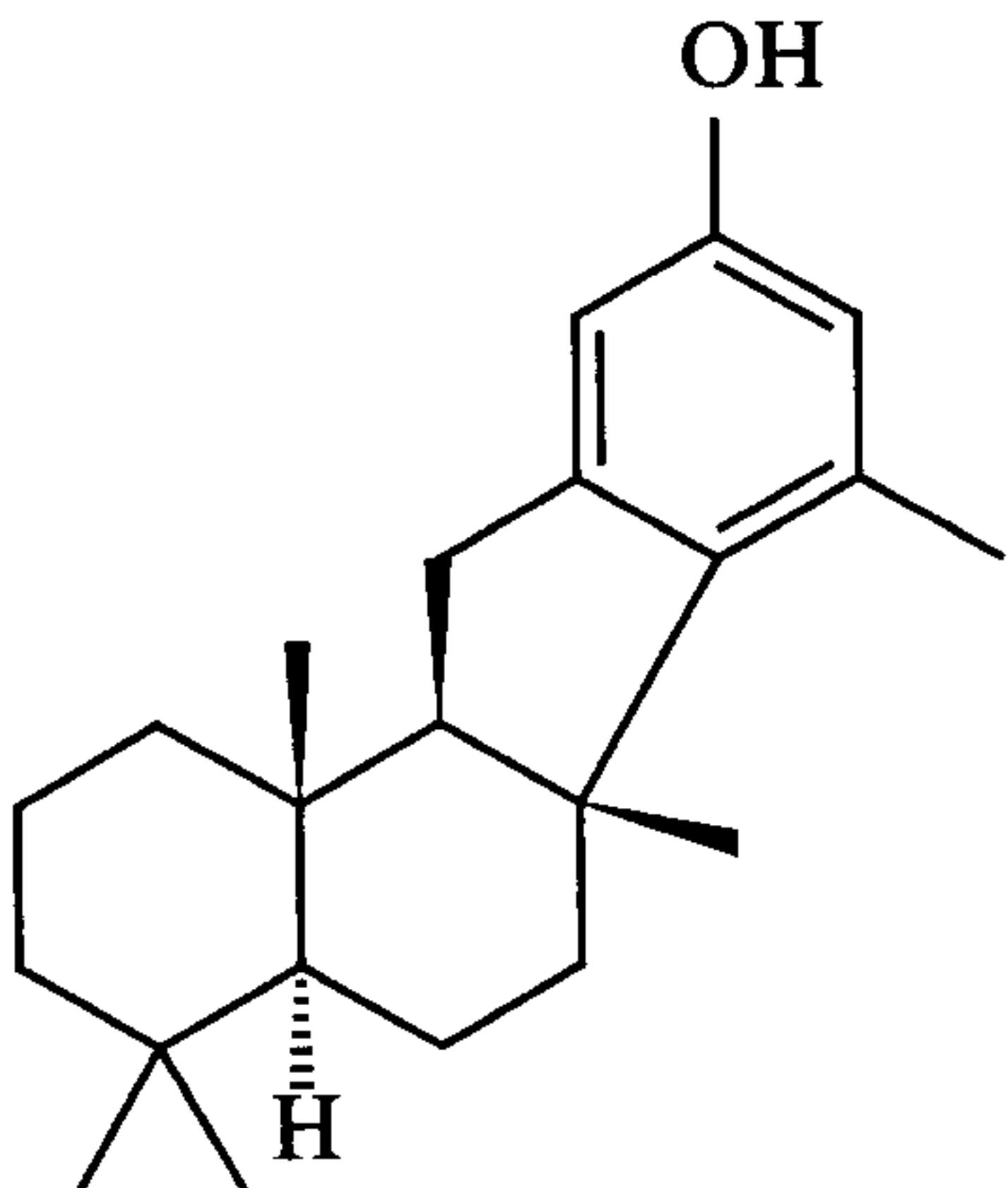
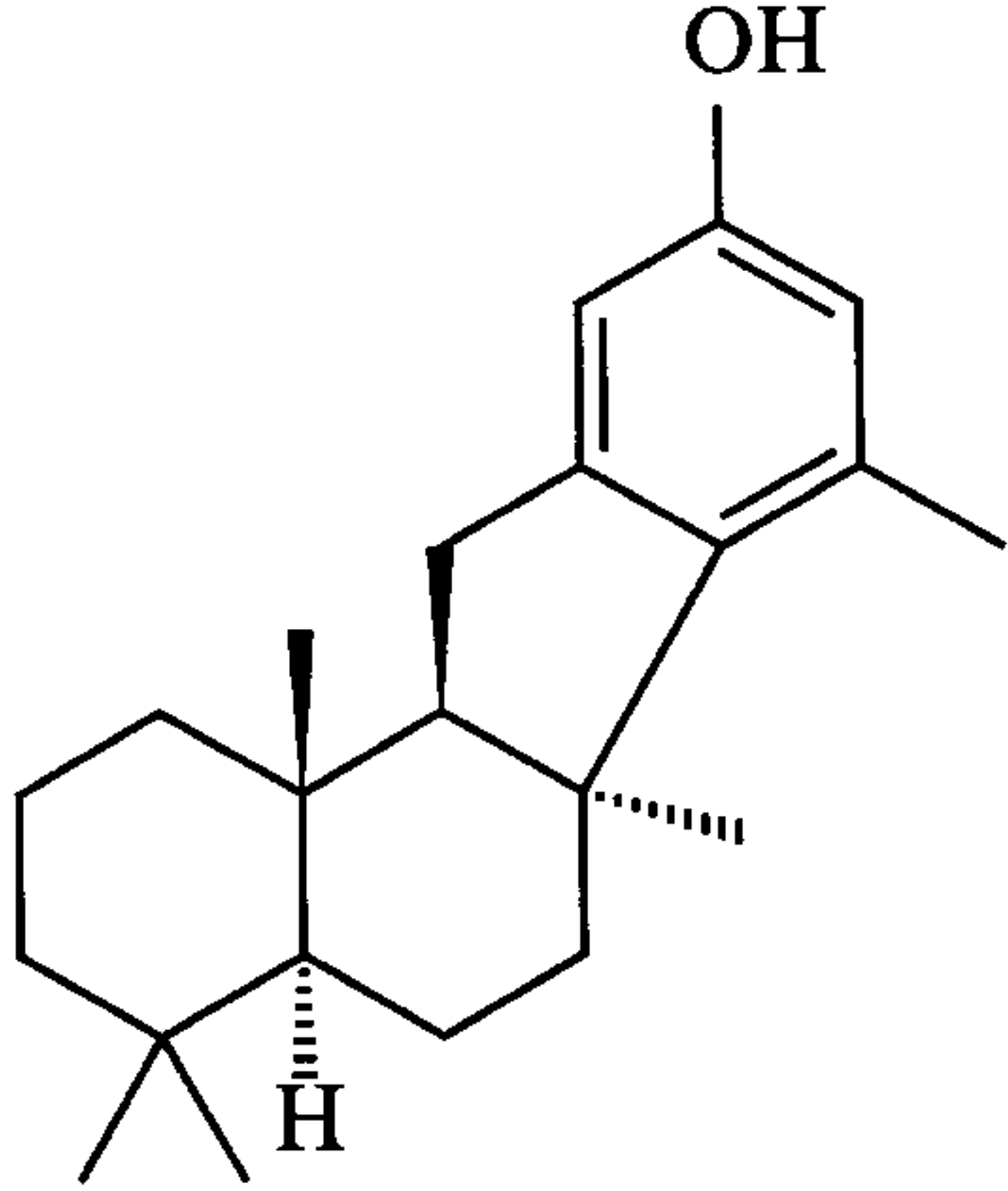
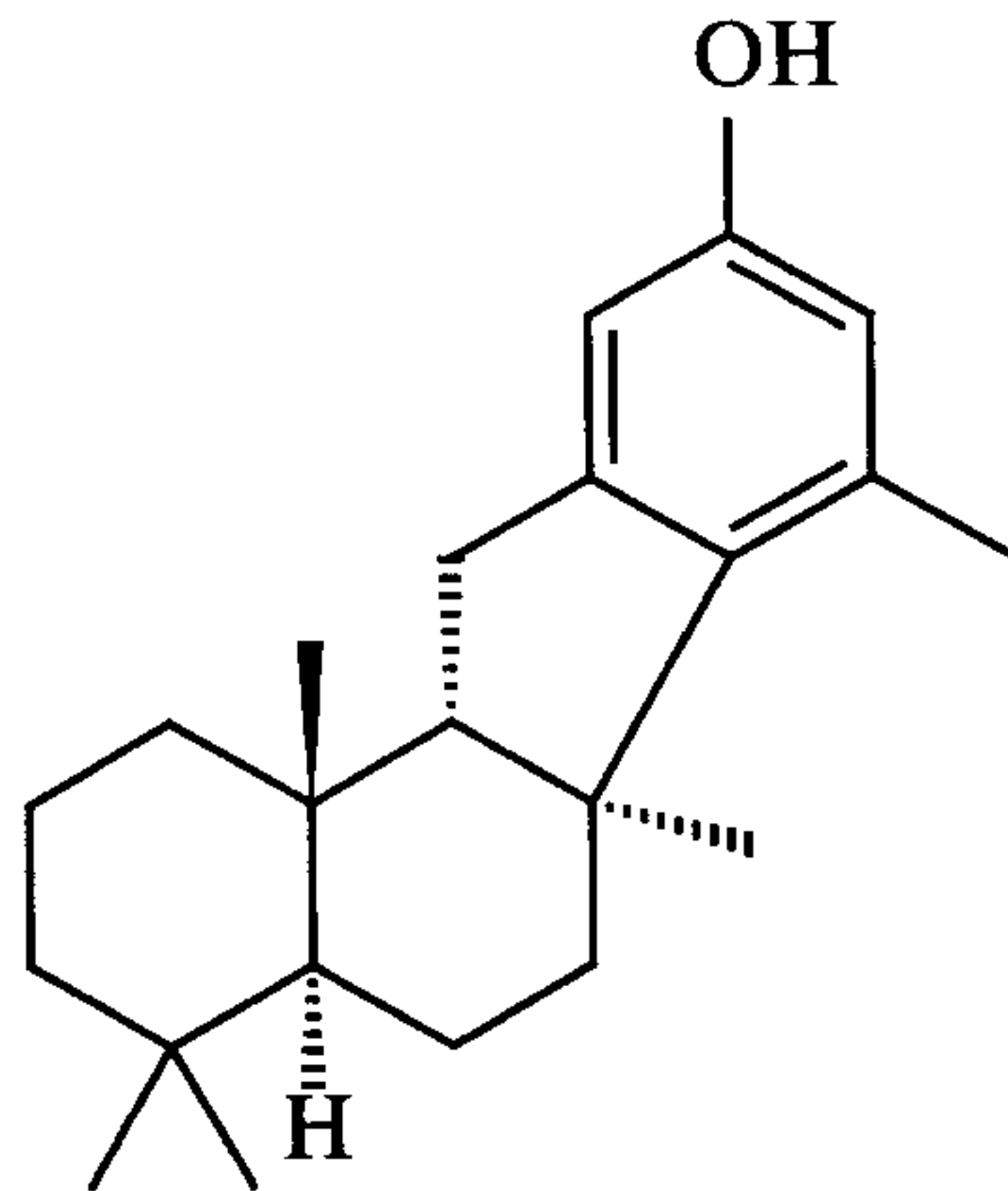
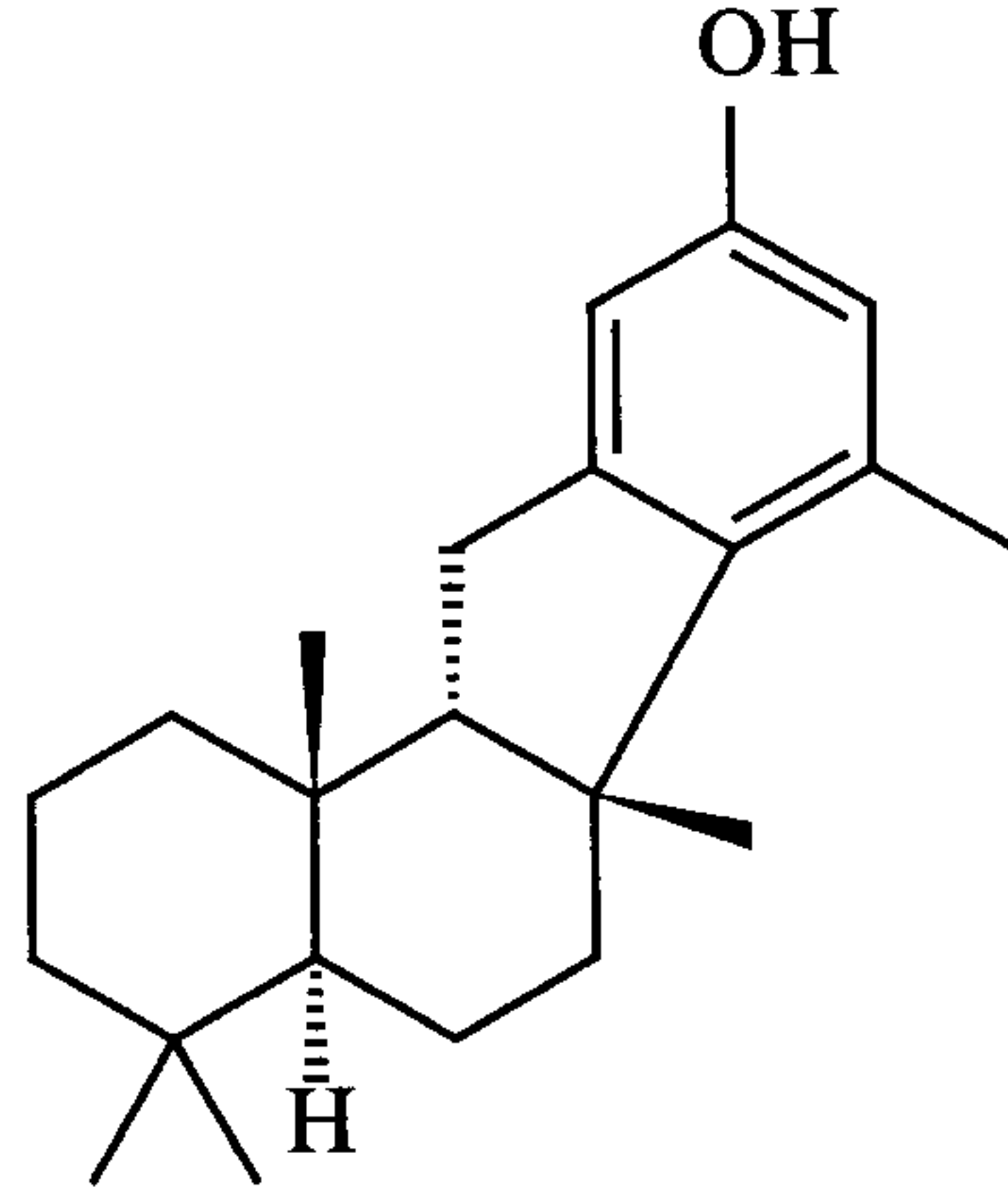
Table 1A	
	
	

Table 1A

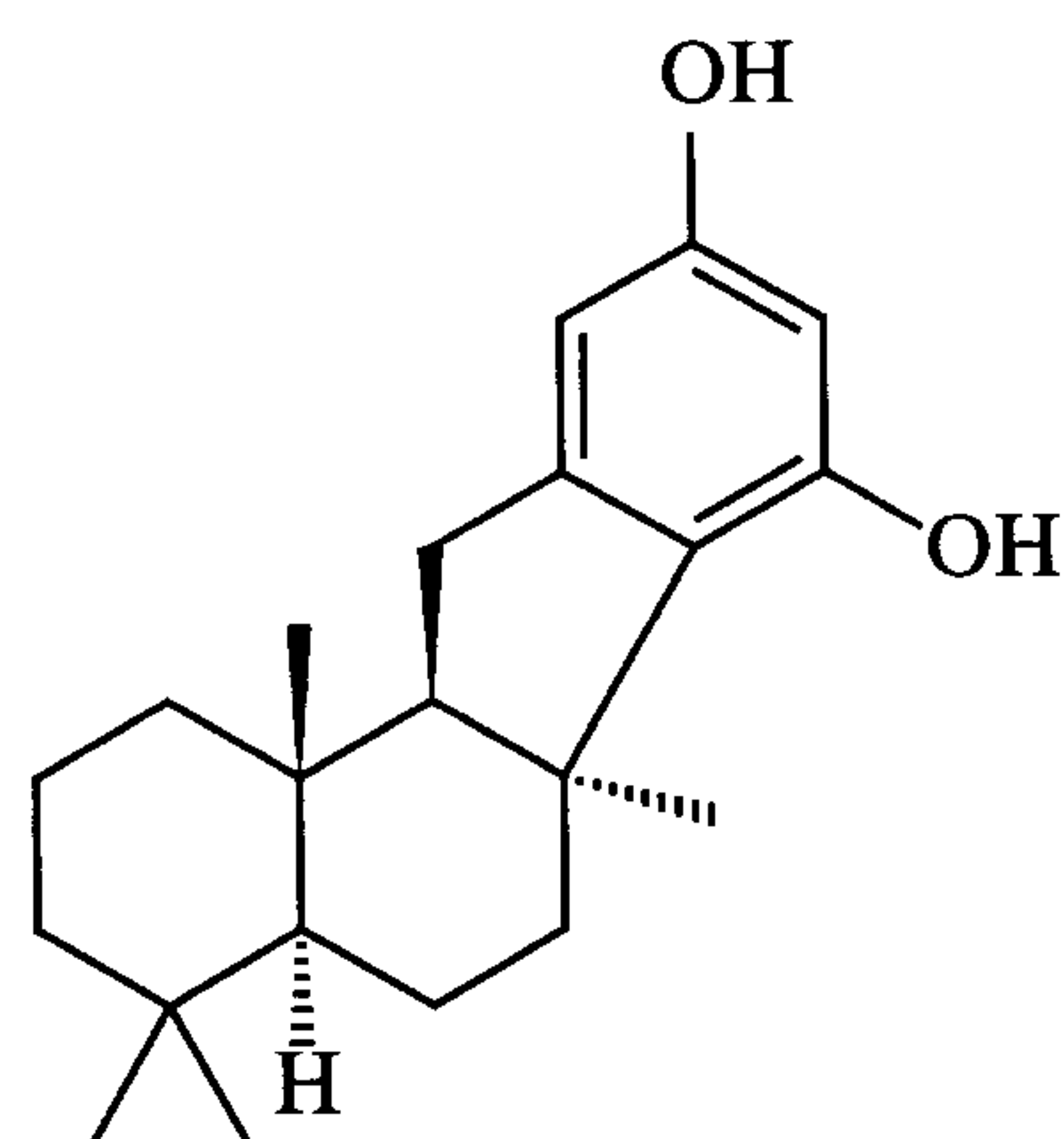
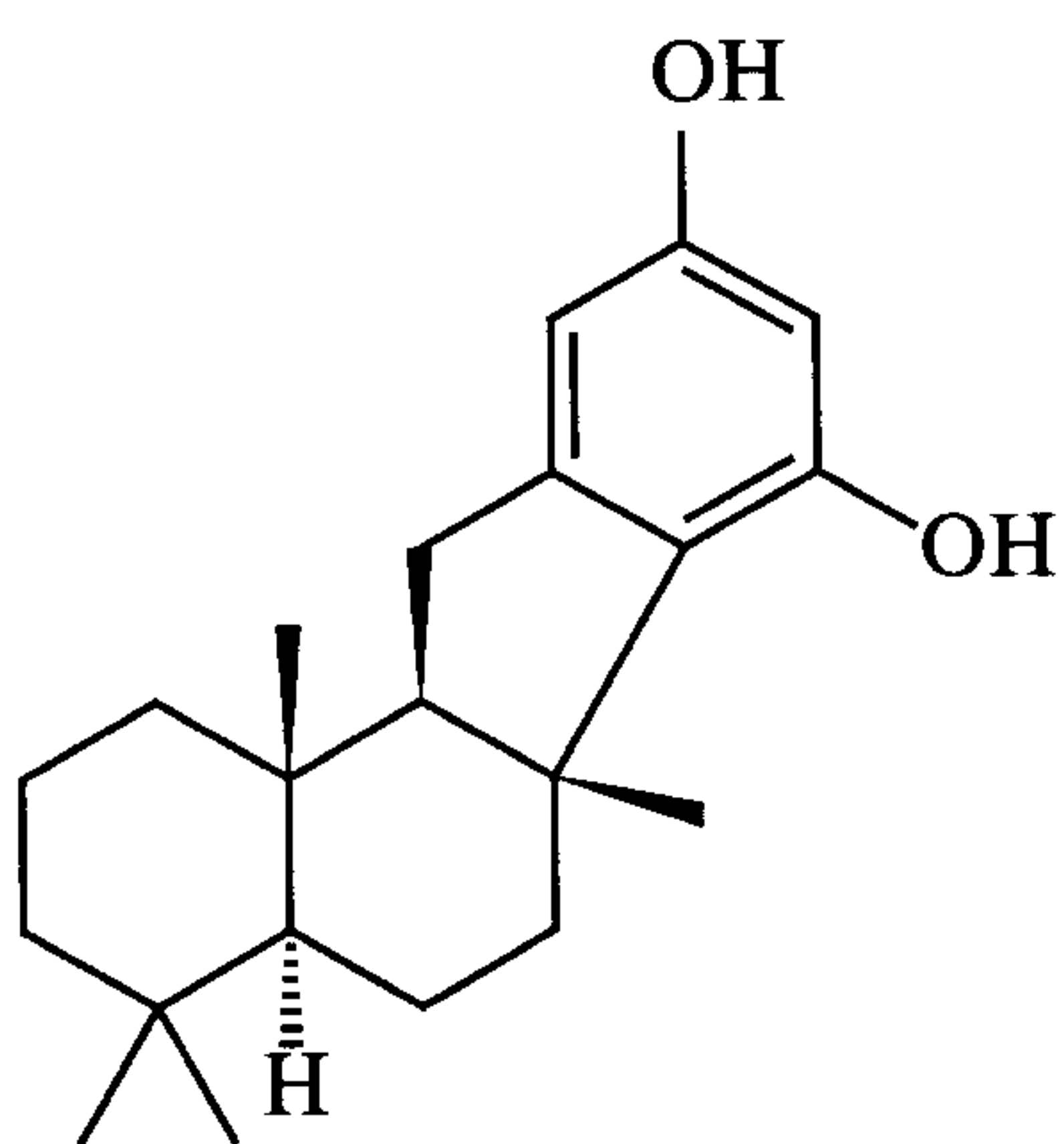
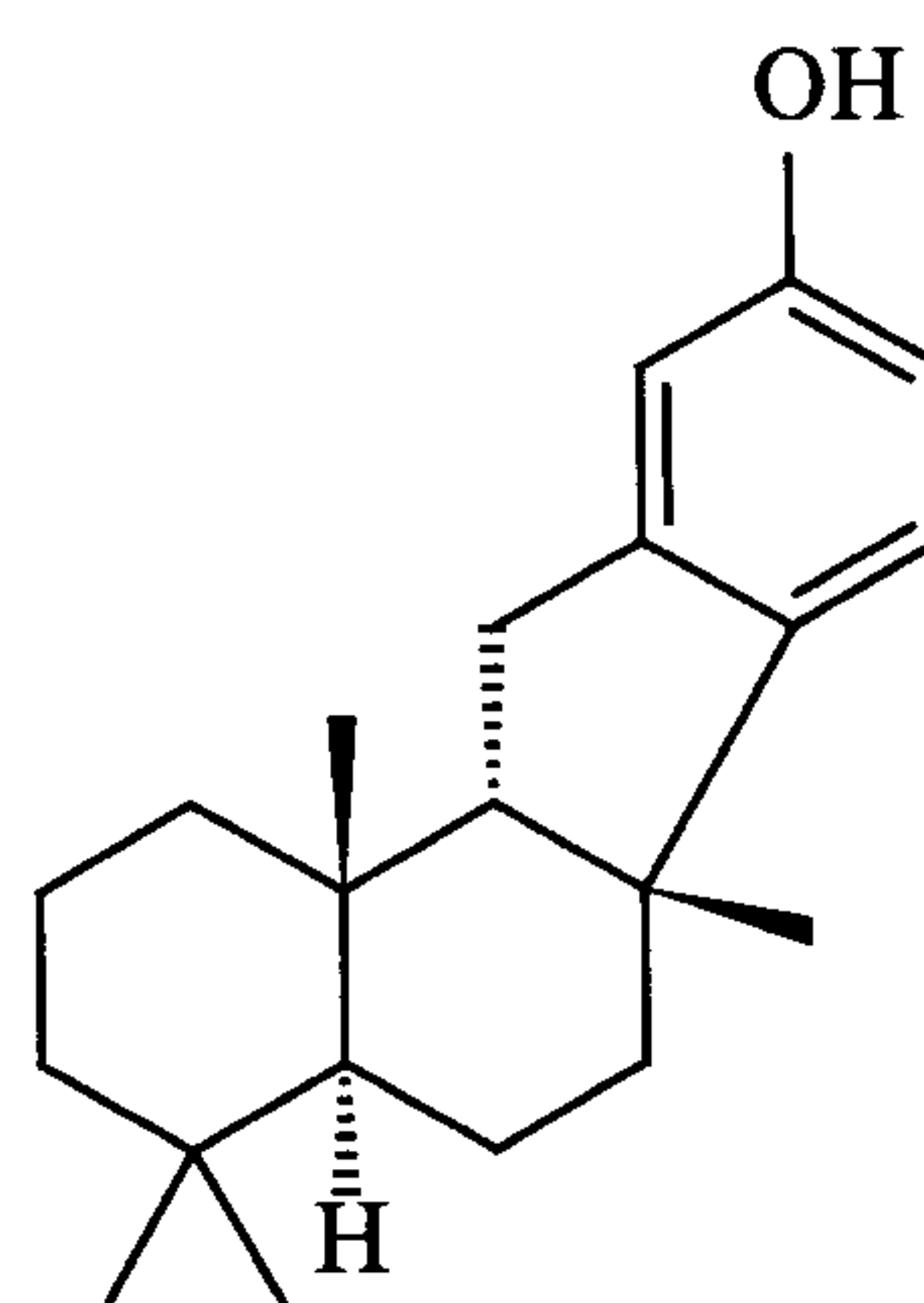
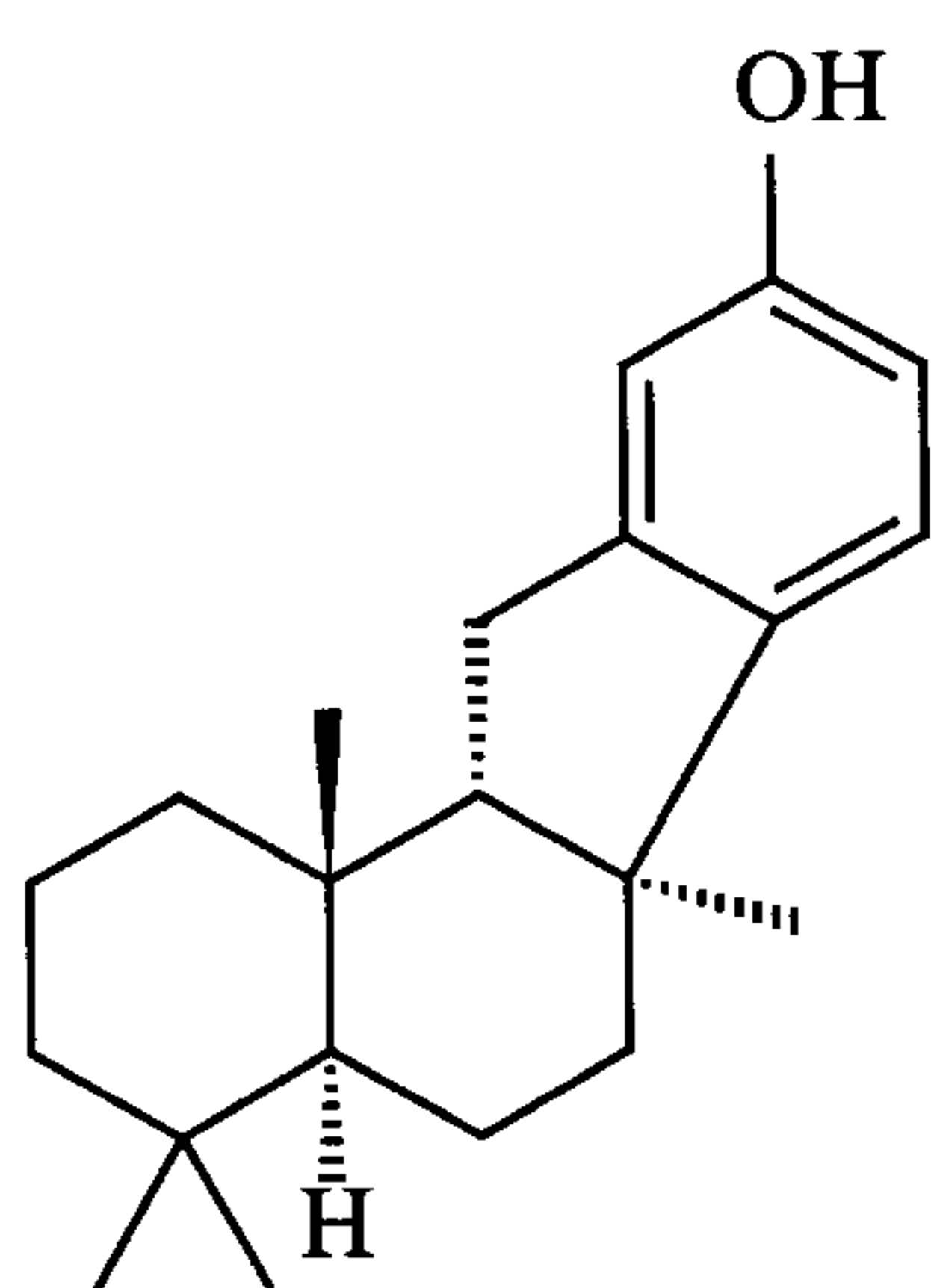
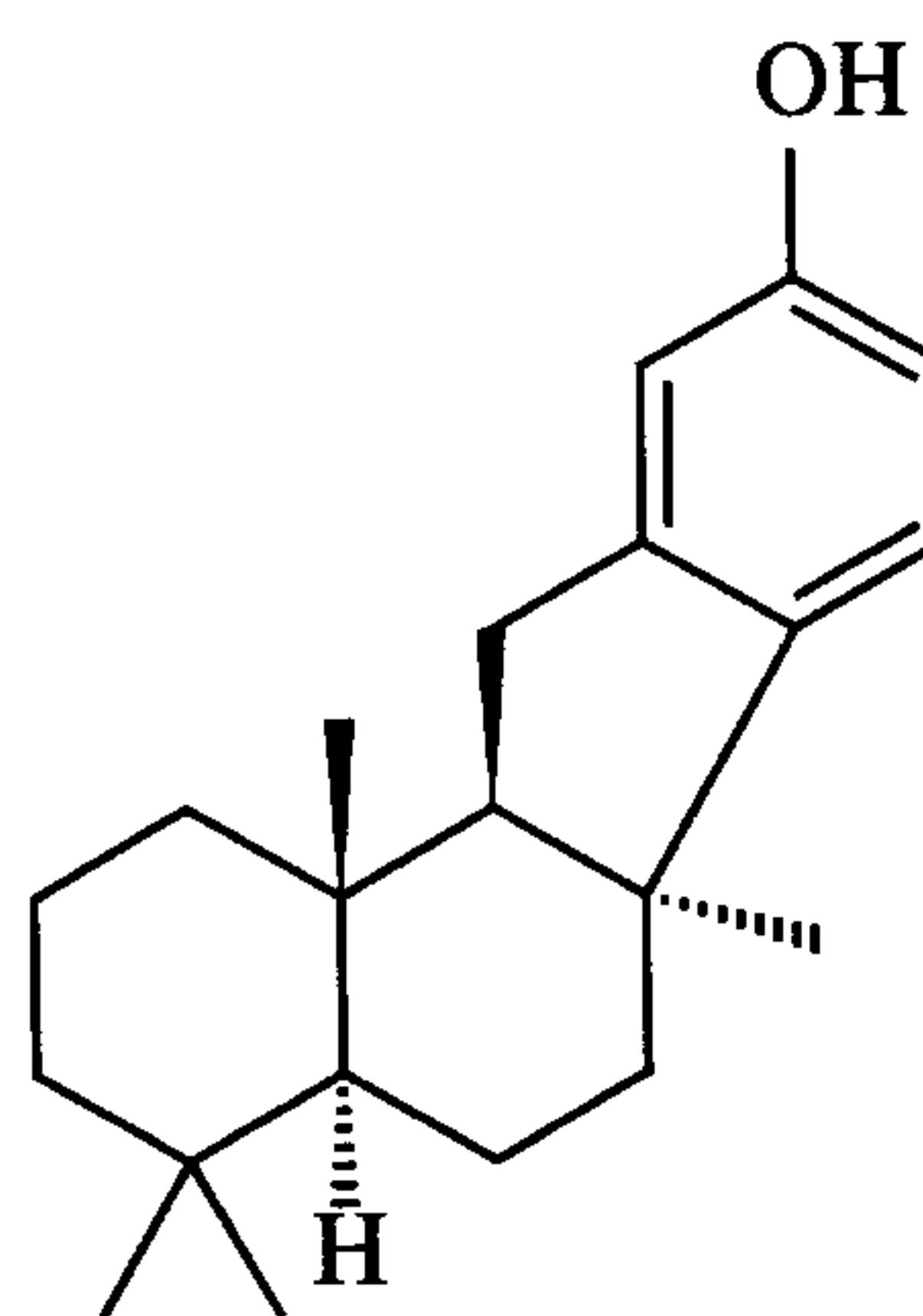
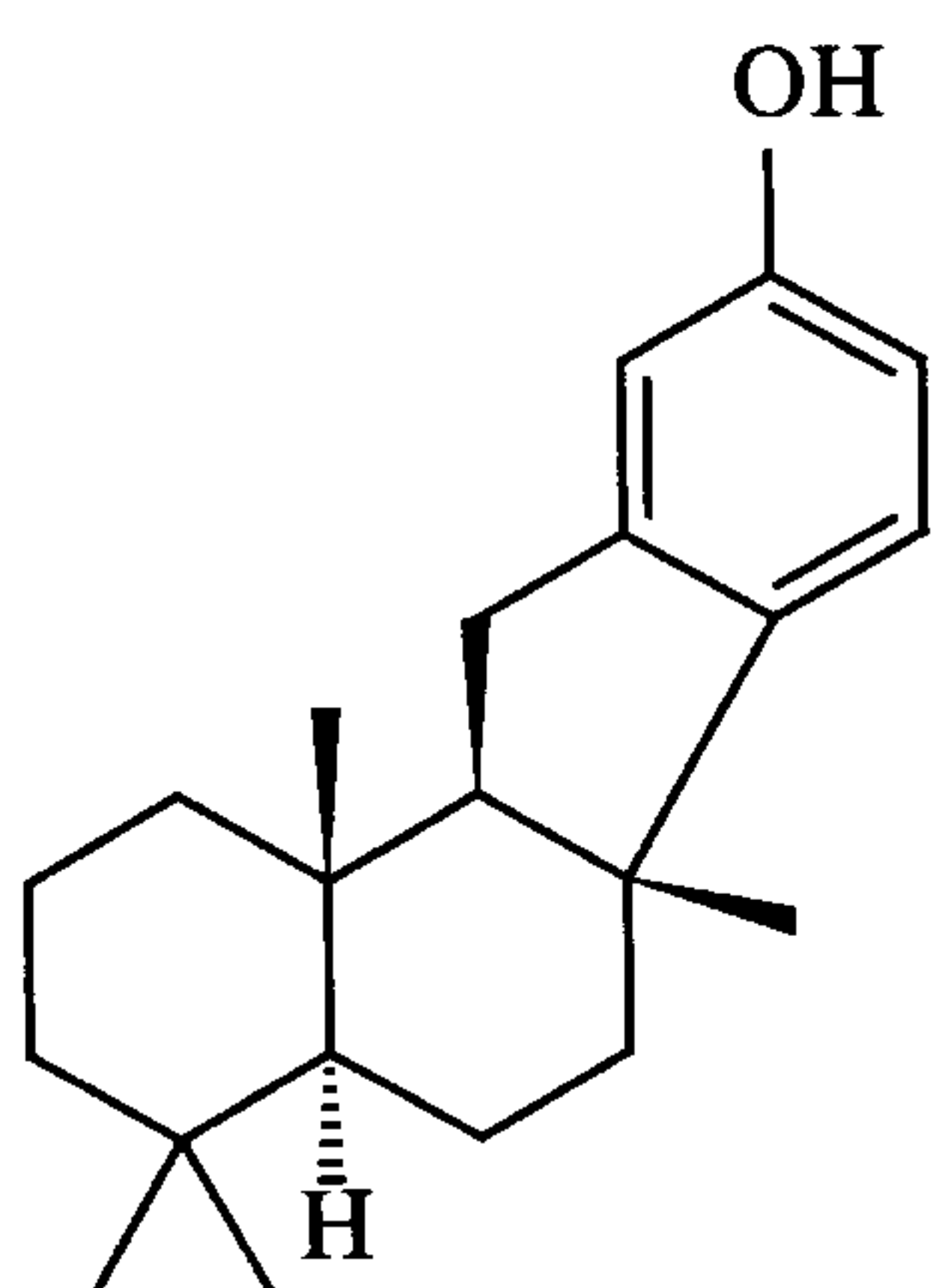


Table 1A

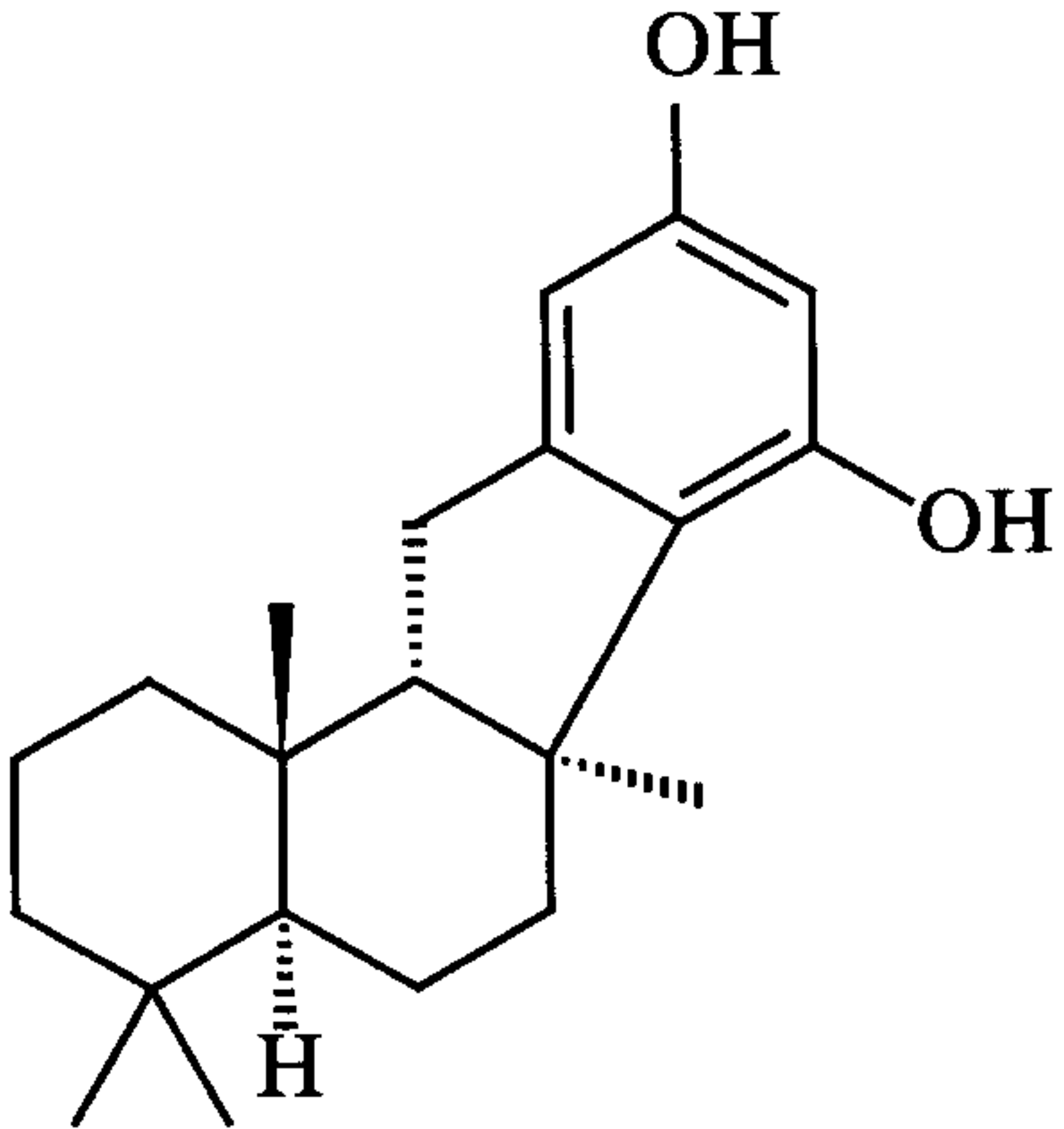
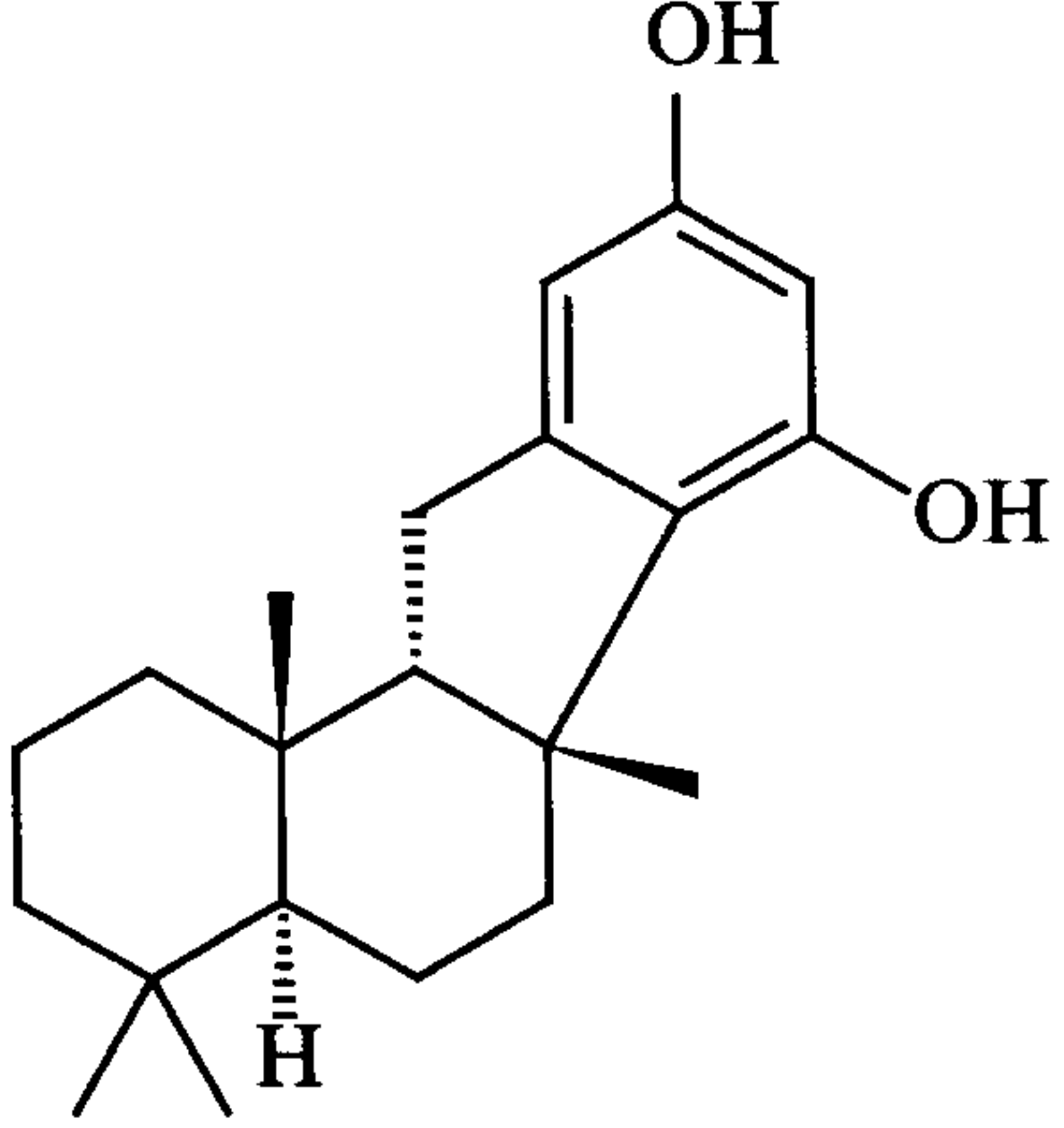
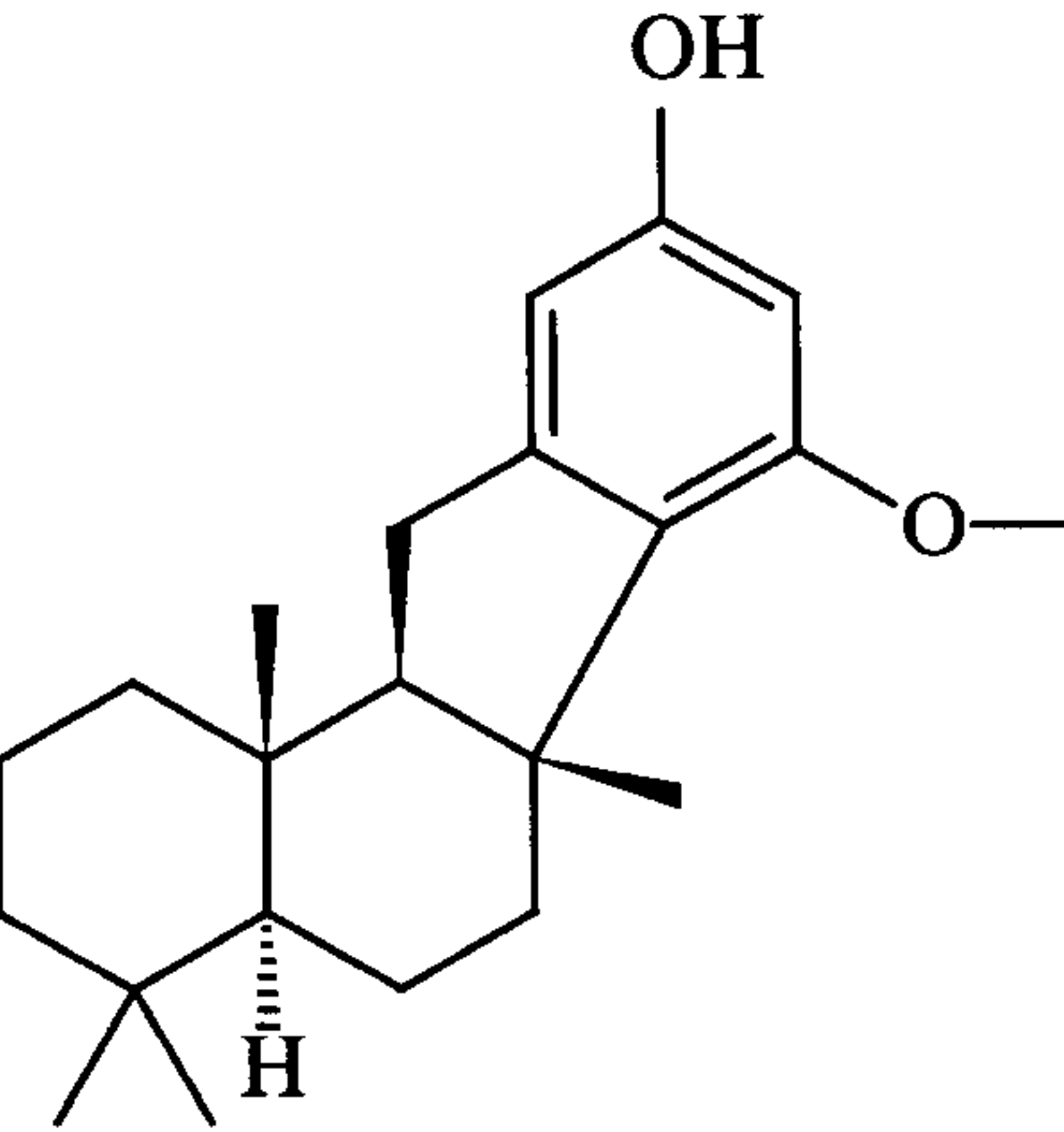
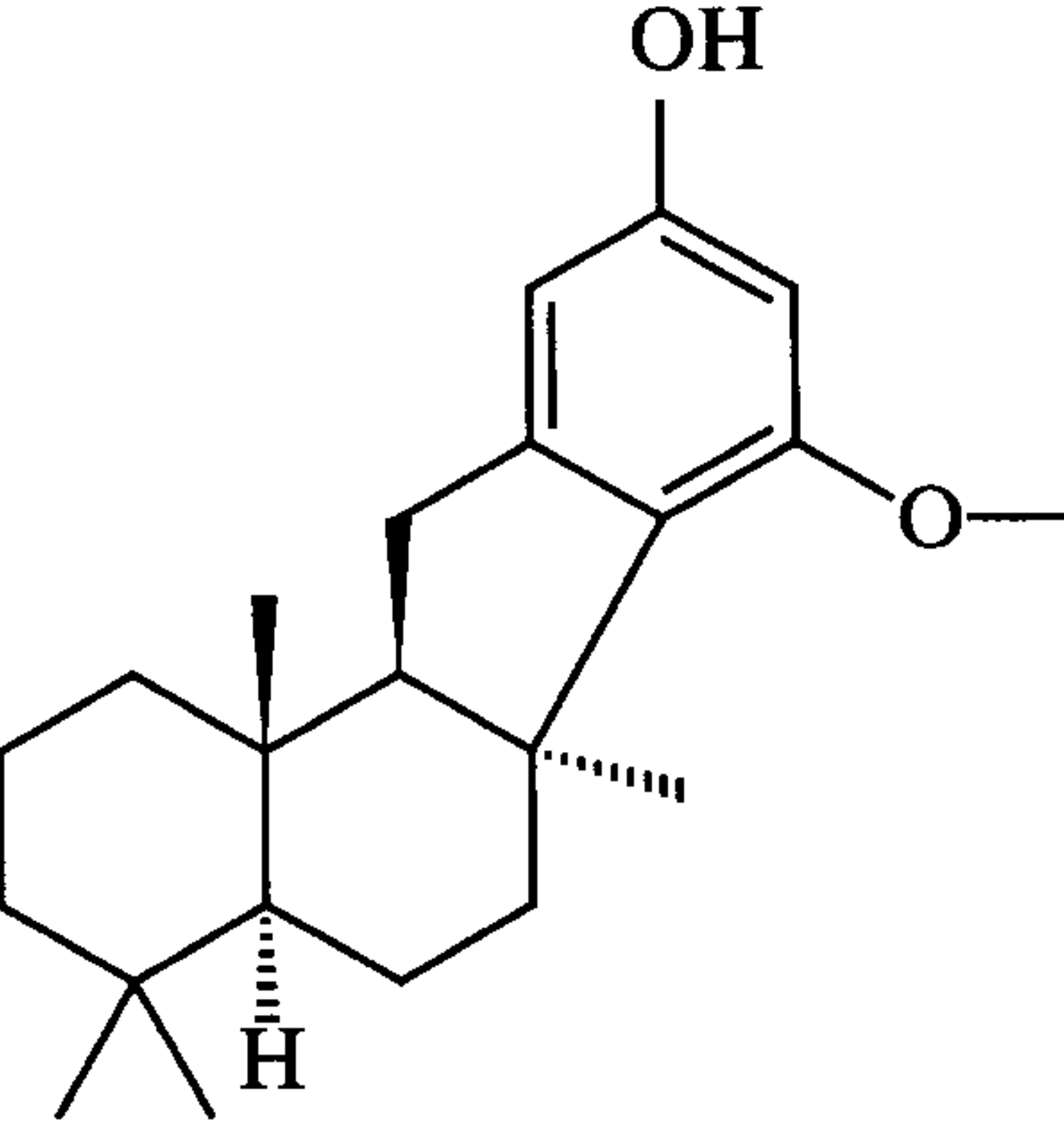
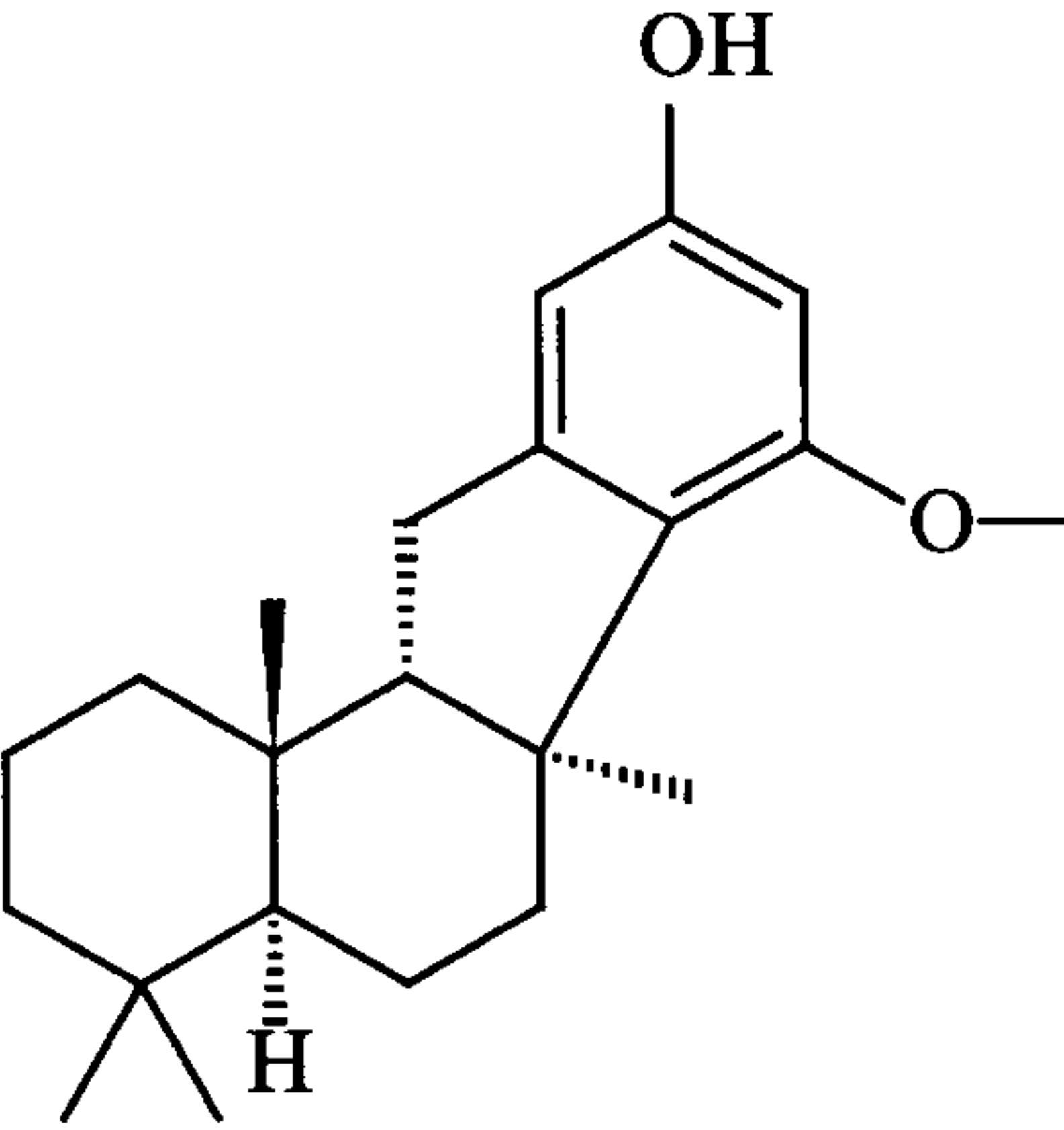
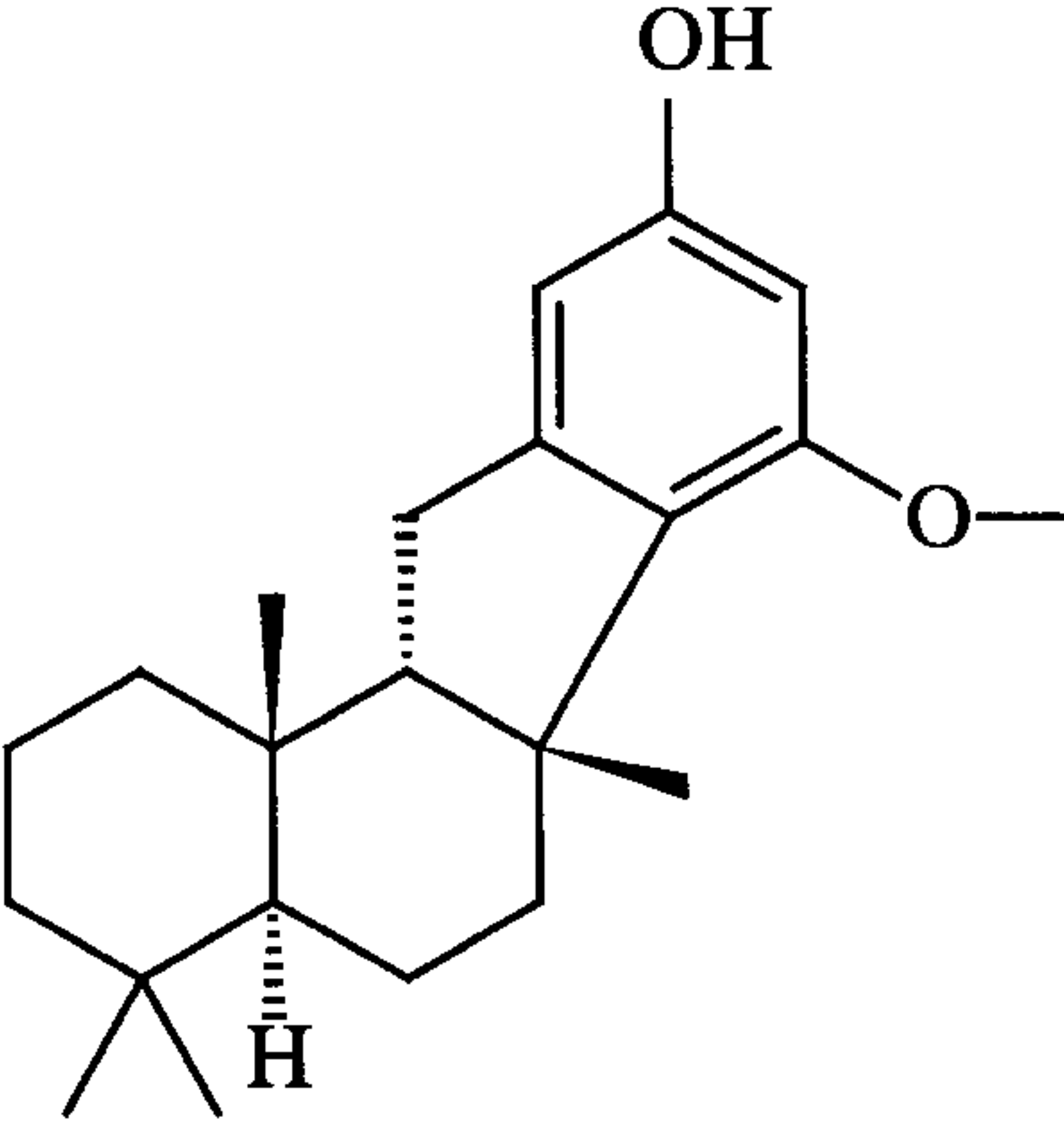
 Chemical structure of a pentacyclic compound. The core consists of a decalin system with a quaternary carbon at C-10 bearing two methyl groups. A five-membered ring is fused to the decalin at C-5 and C-8. This five-membered ring is further fused to a benzene ring at C-1 and C-4. The benzene ring has hydroxyl groups at positions 2 and 3. Stereochemistry: C-13 has a wedged methyl group, C-14 has a dashed hydrogen, and the bond from C-5 to the five-membered ring is dashed.	 Chemical structure of a pentacyclic compound, identical to the one in the first cell. Stereochemistry: C-13 has a wedged methyl group, C-14 has a dashed hydrogen, and the bond from C-8 to the five-membered ring is wedged.
 Chemical structure of a pentacyclic compound. The aromatic ring has a hydroxyl group at position 2 and a methoxy group at position 3. Stereochemistry: C-13 has a wedged methyl group, C-14 has a dashed hydrogen, and the bond from C-5 to the five-membered ring is wedged.	 Chemical structure of a pentacyclic compound, identical to the one in the third cell. Stereochemistry: C-13 has a wedged methyl group, C-14 has a dashed hydrogen, and the bond from C-8 to the five-membered ring is dashed.
 Chemical structure of a pentacyclic compound, identical to the one in the third cell. Stereochemistry: C-13 has a wedged methyl group, C-14 has a dashed hydrogen, and the bond from C-5 to the five-membered ring is dashed.	 Chemical structure of a pentacyclic compound, identical to the one in the third cell. Stereochemistry: C-13 has a wedged methyl group, C-14 has a dashed hydrogen, and the bond from C-8 to the five-membered ring is wedged.

Table 1A

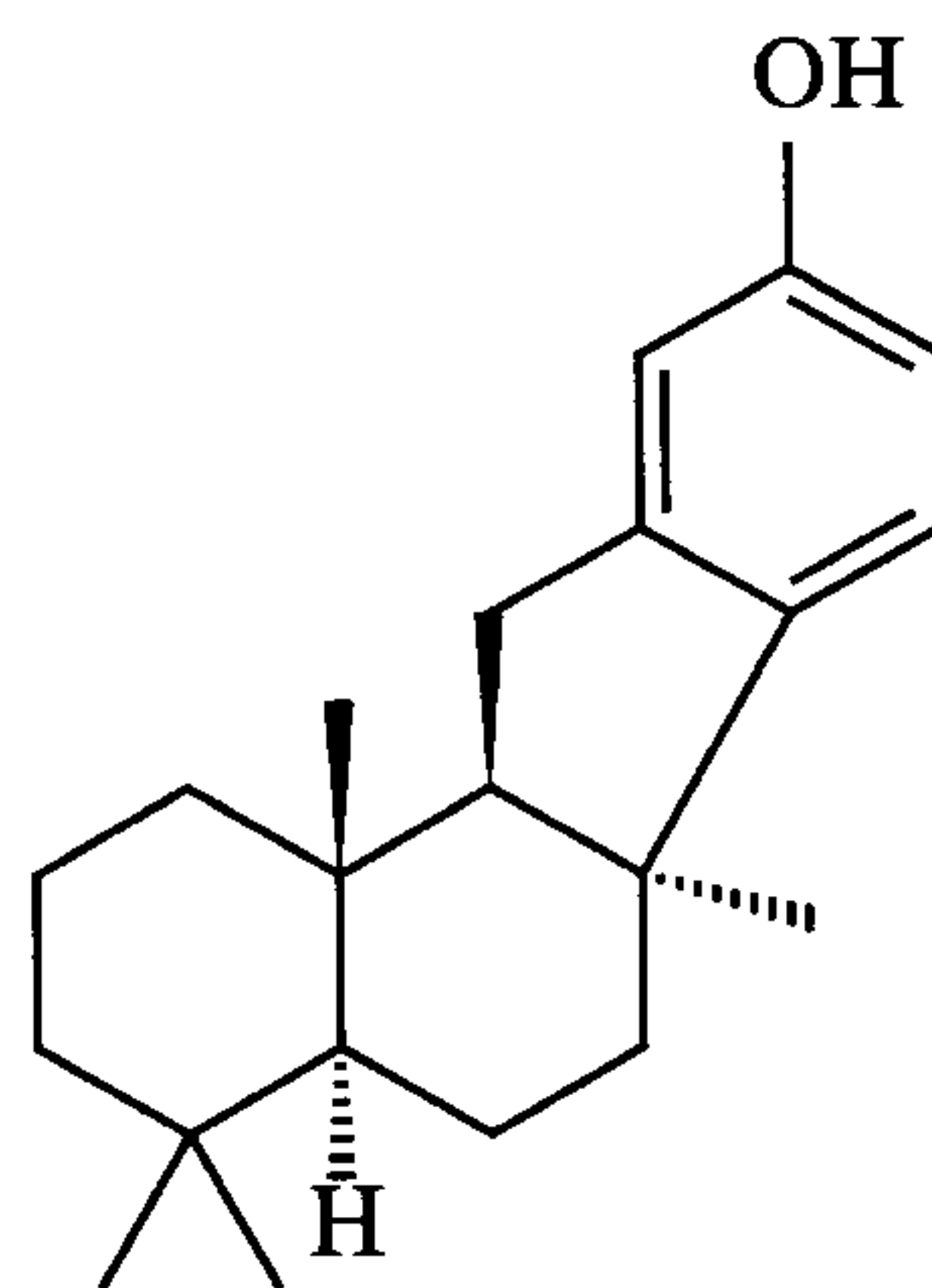
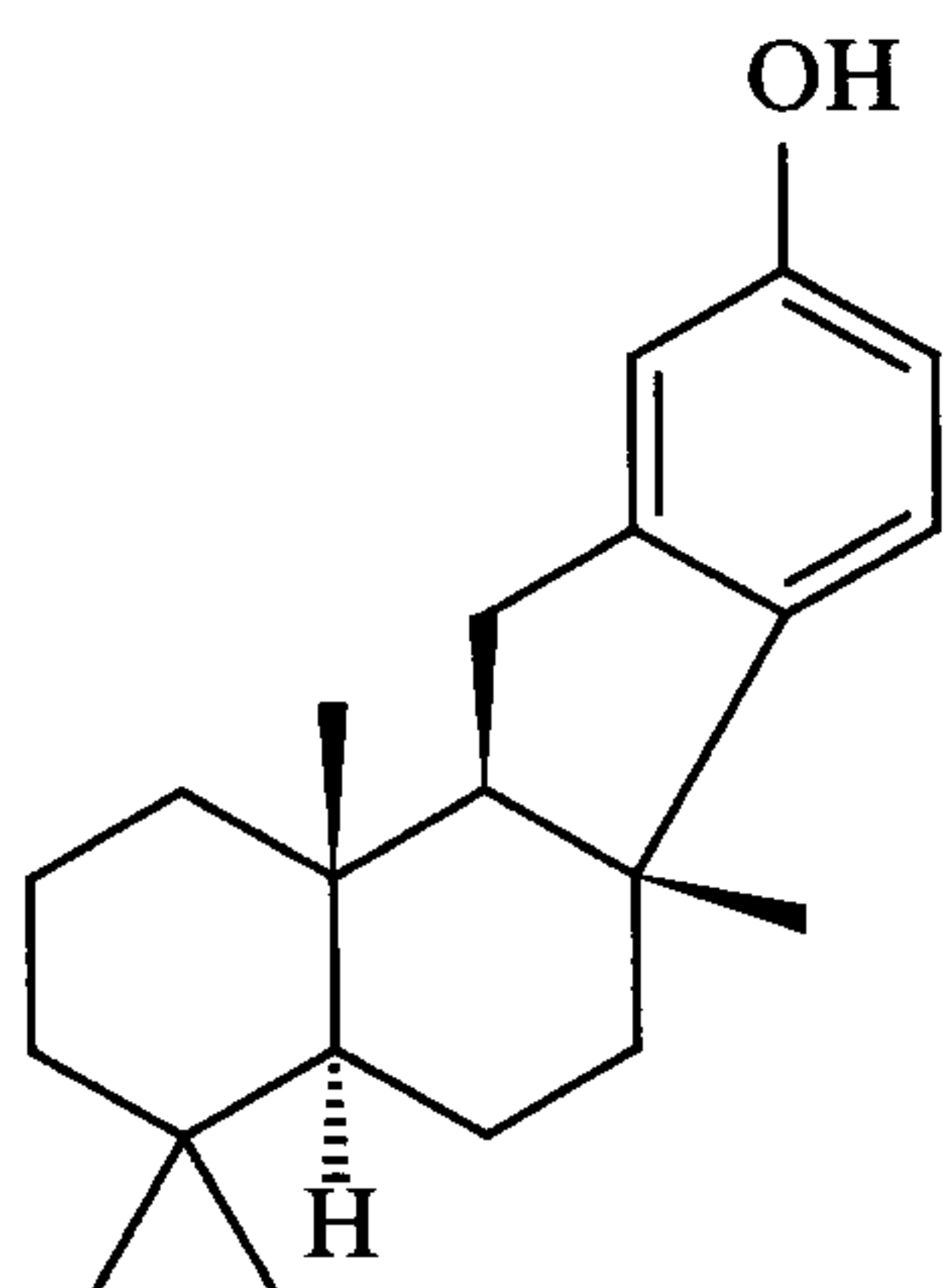
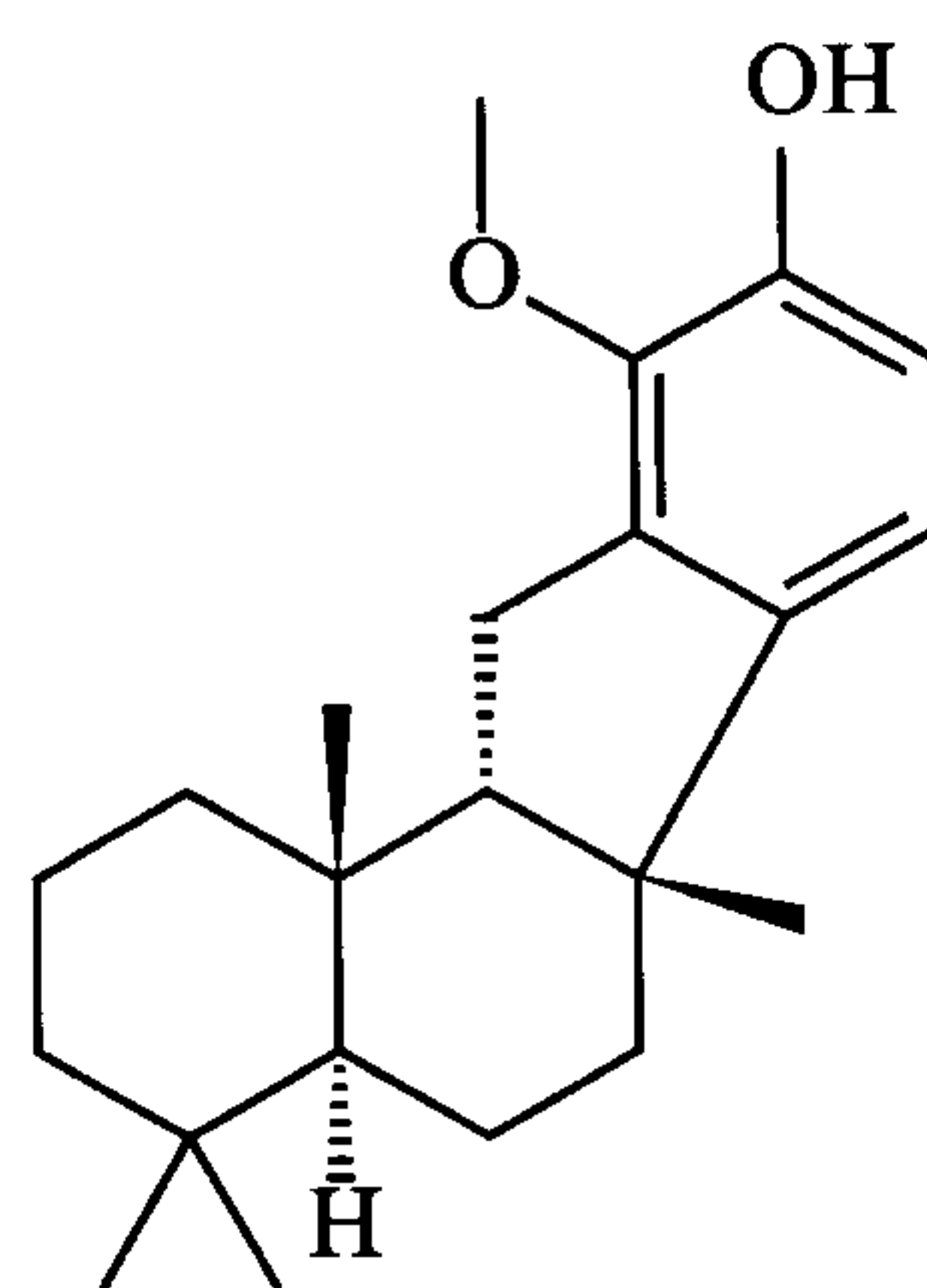
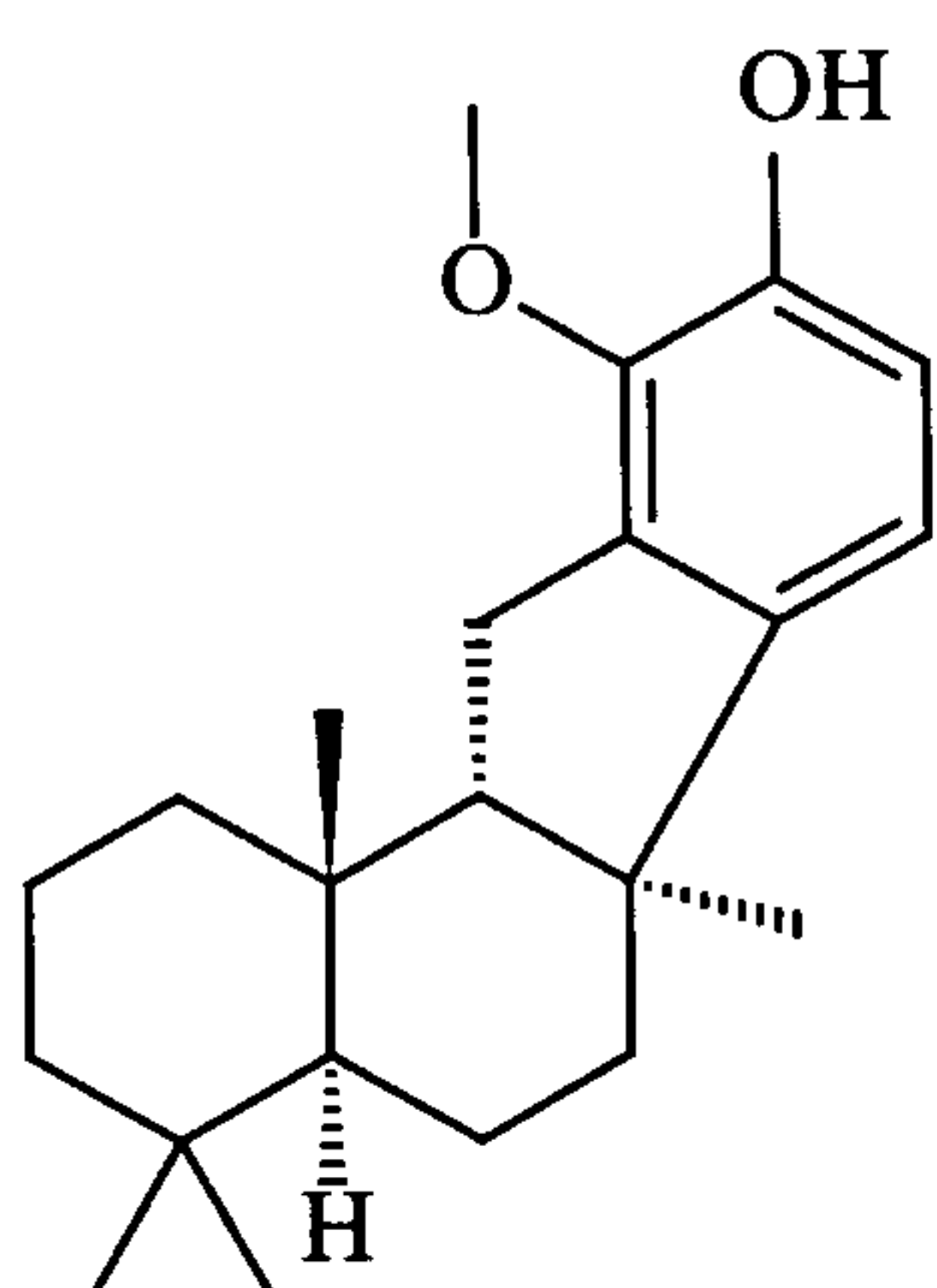
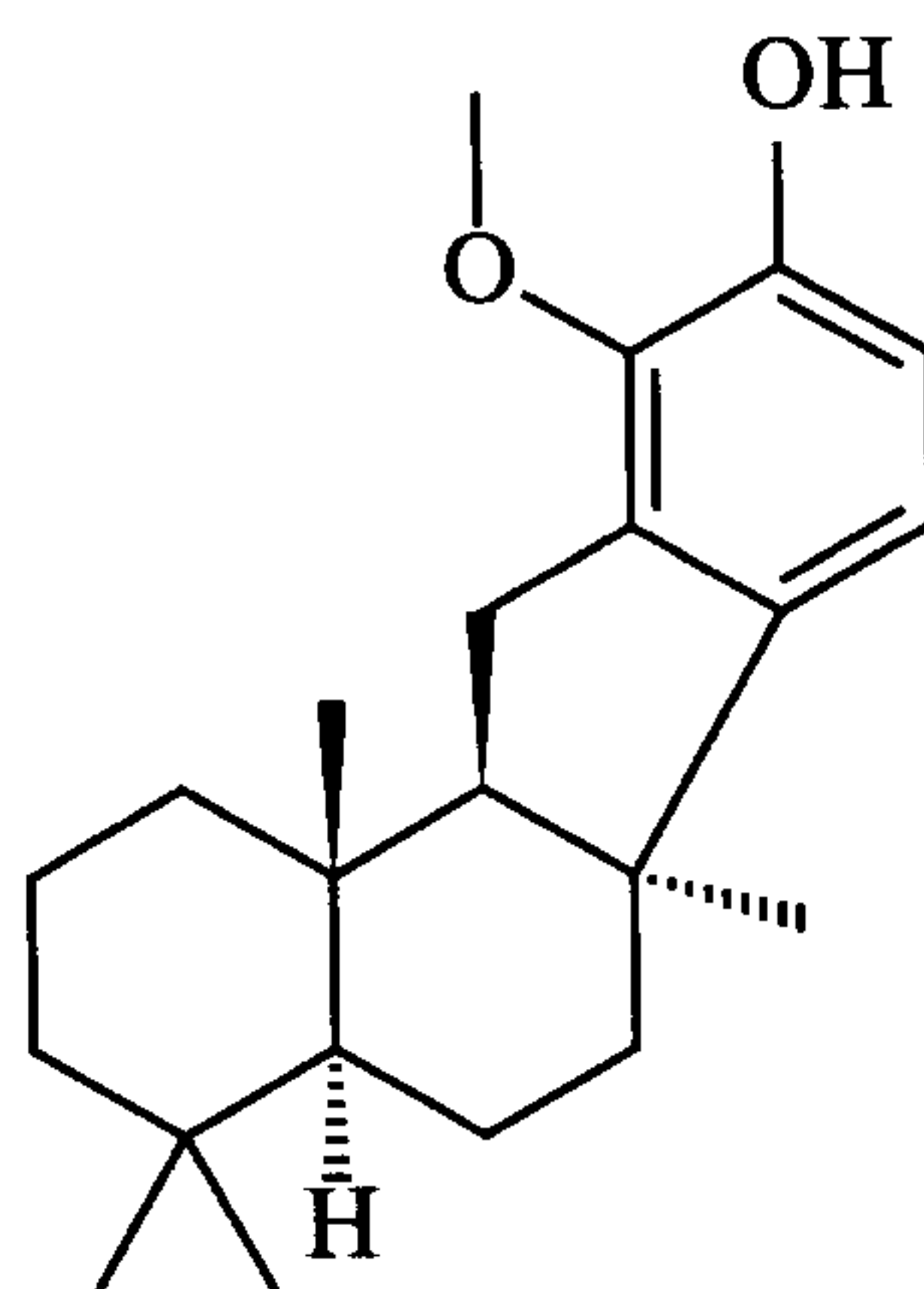
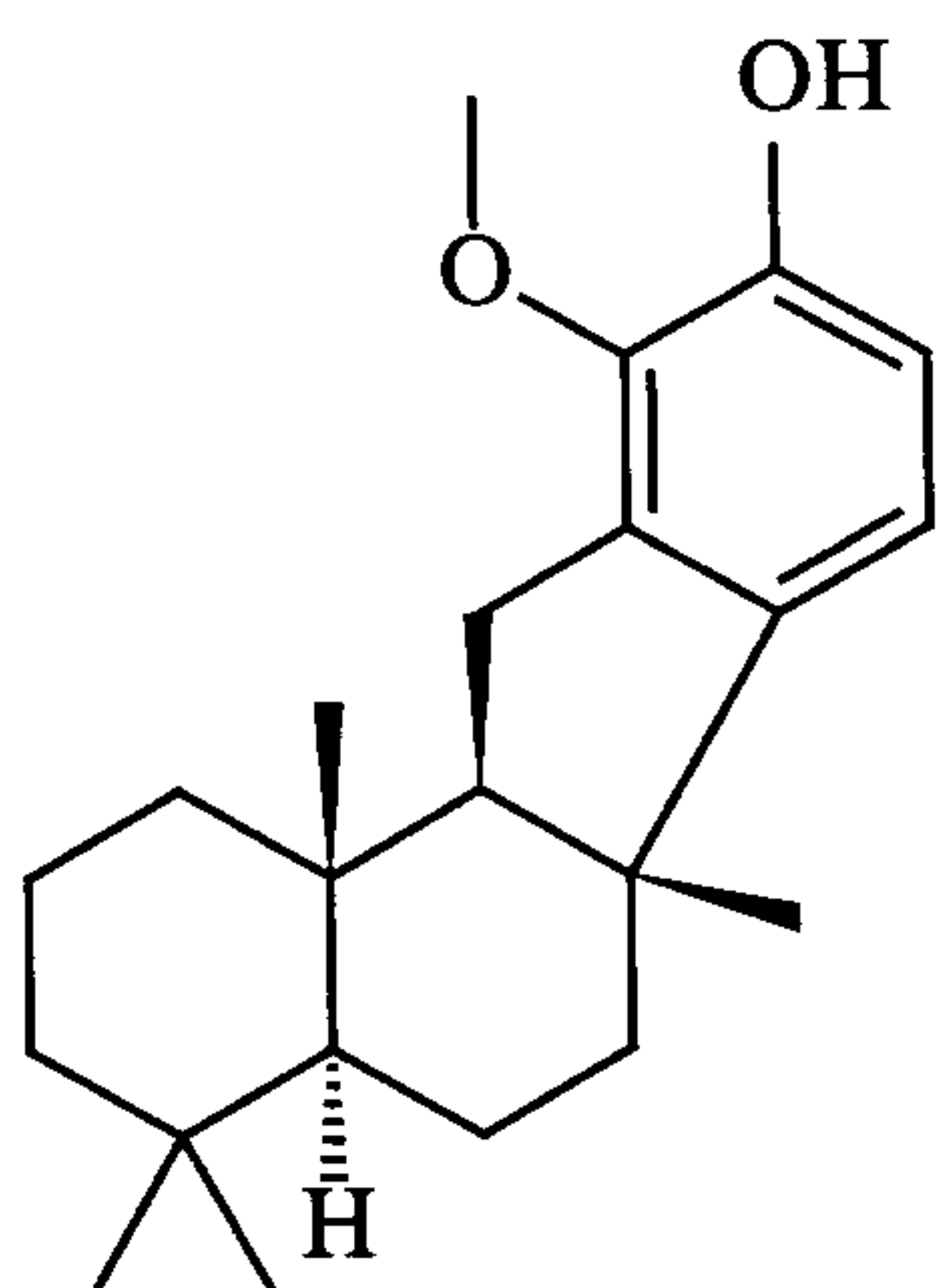
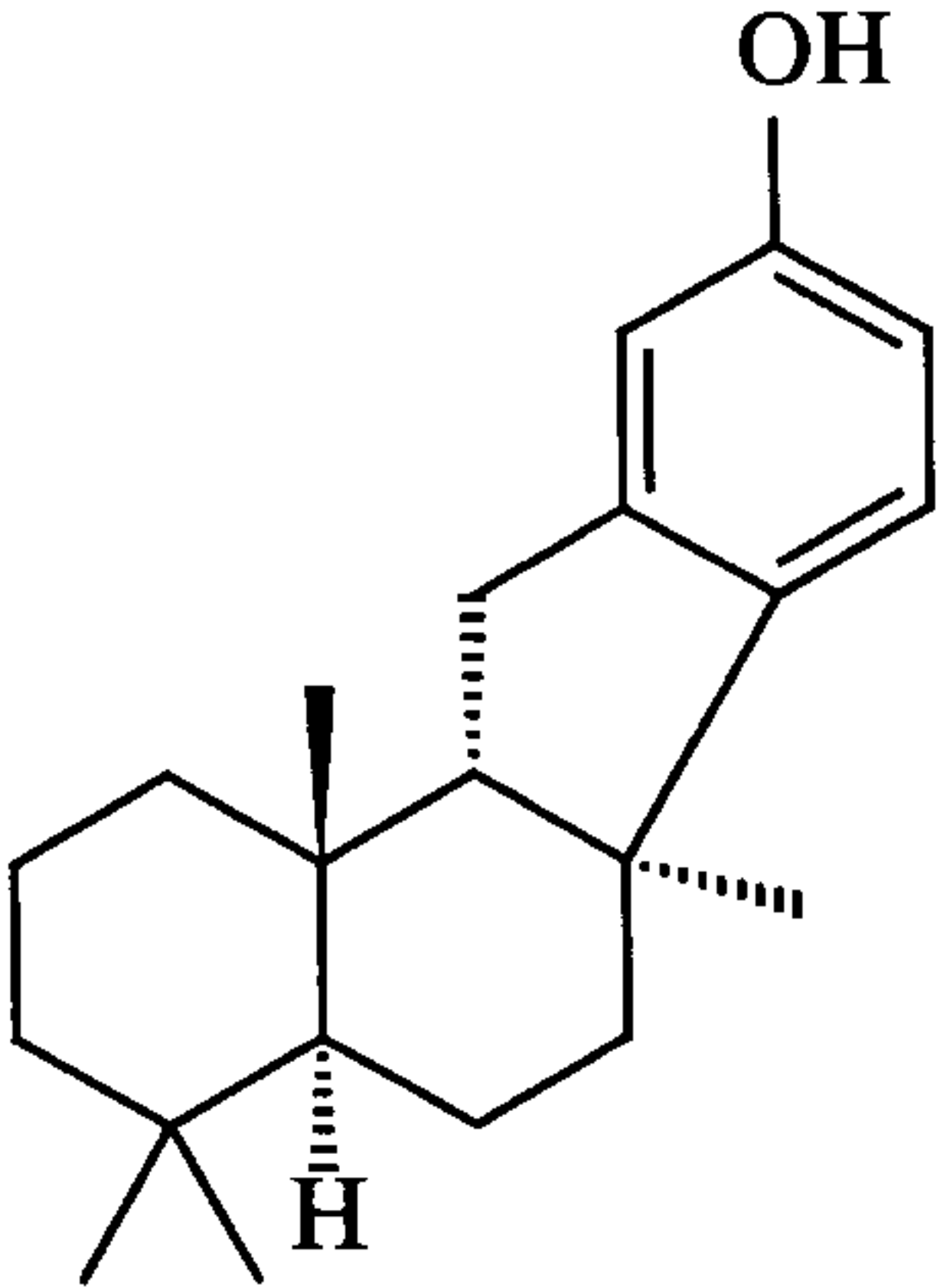
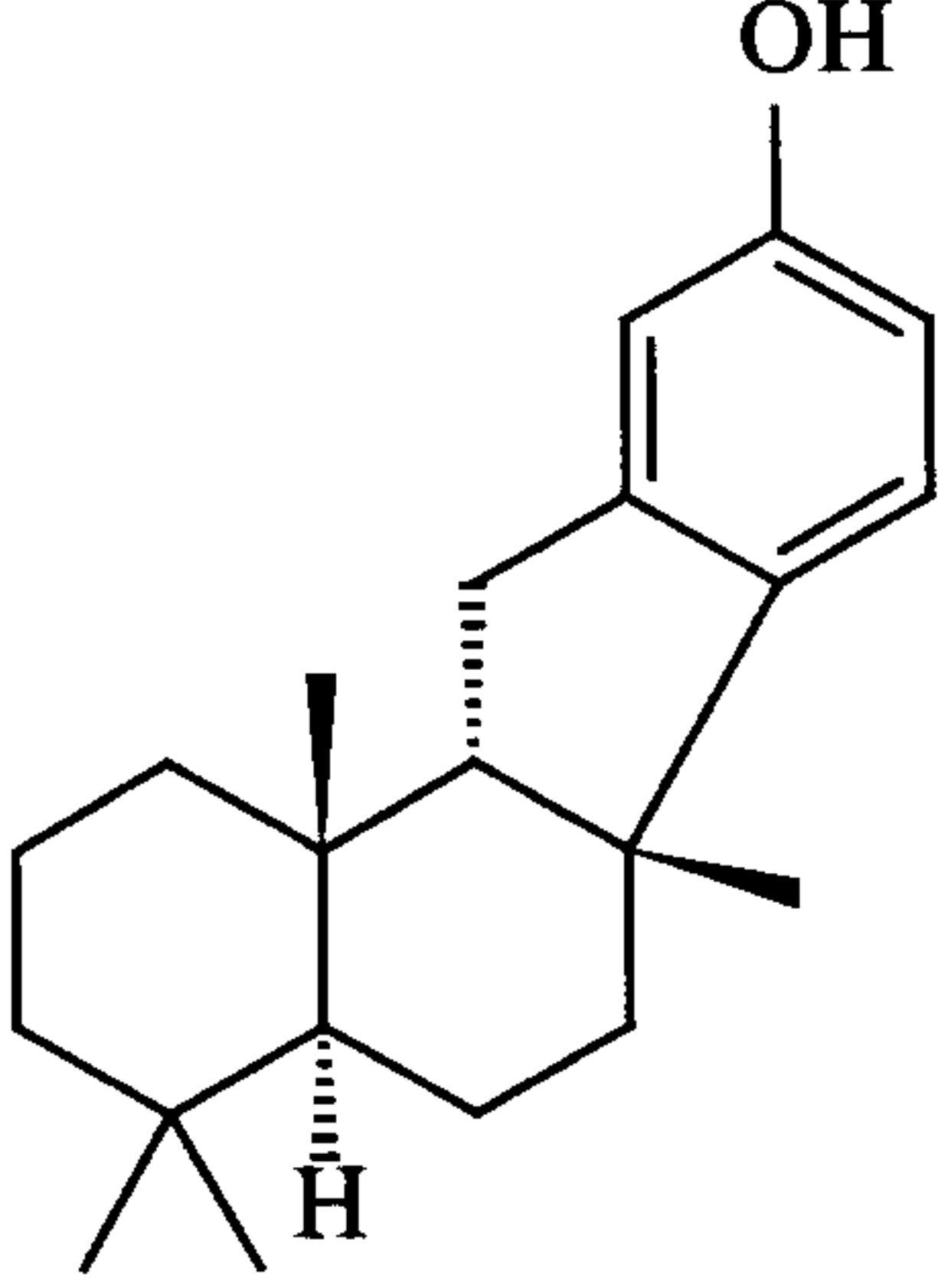
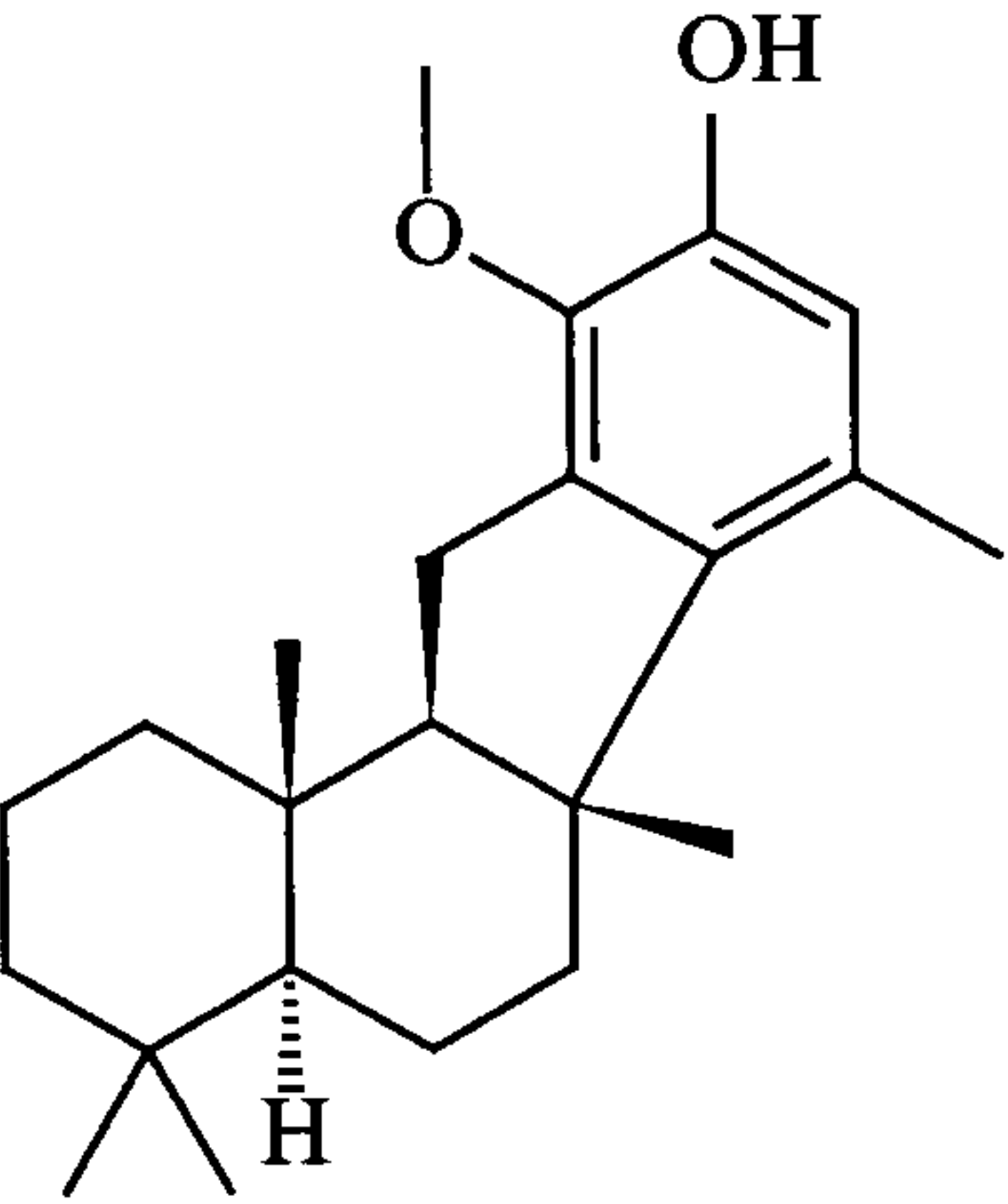
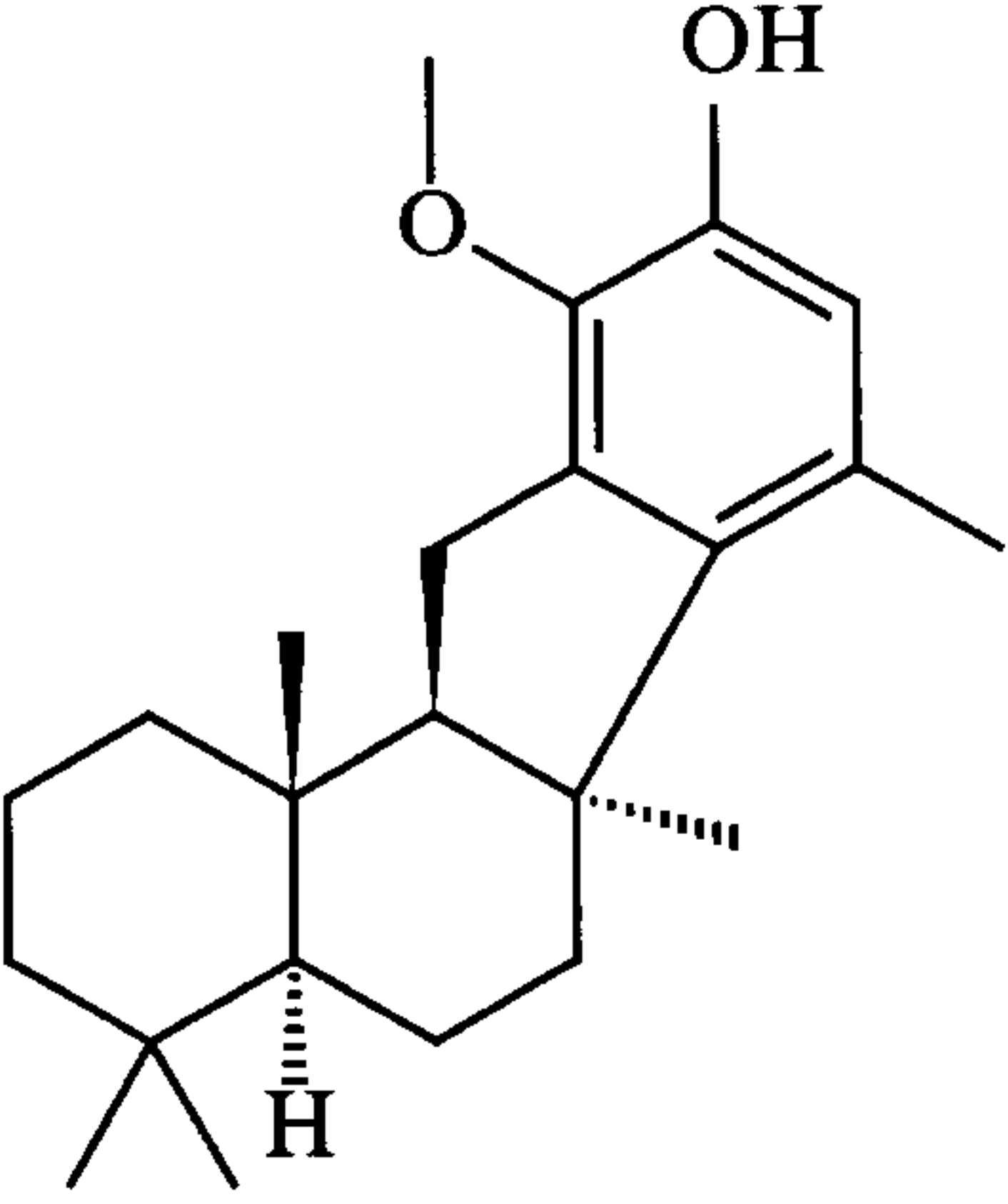
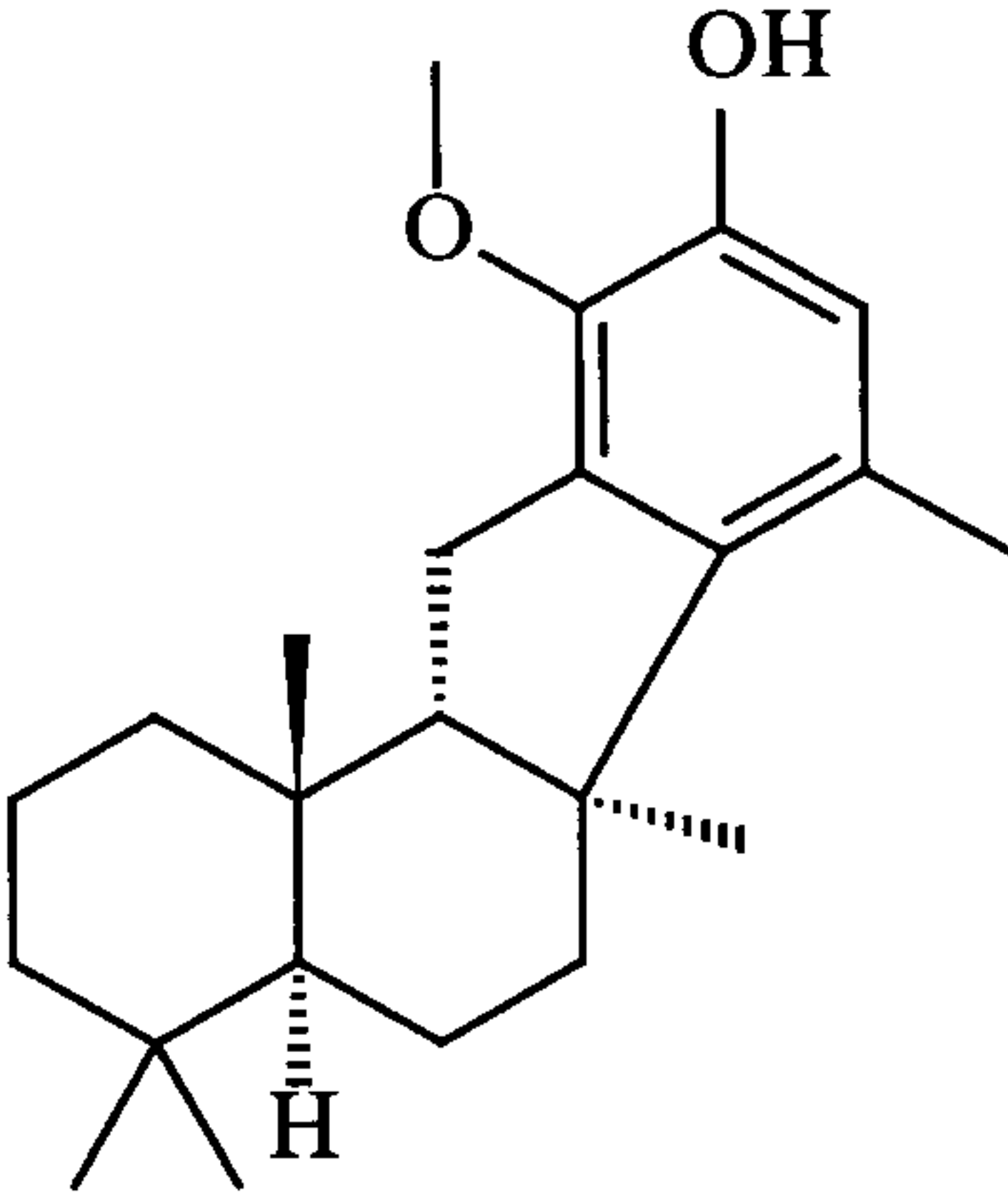
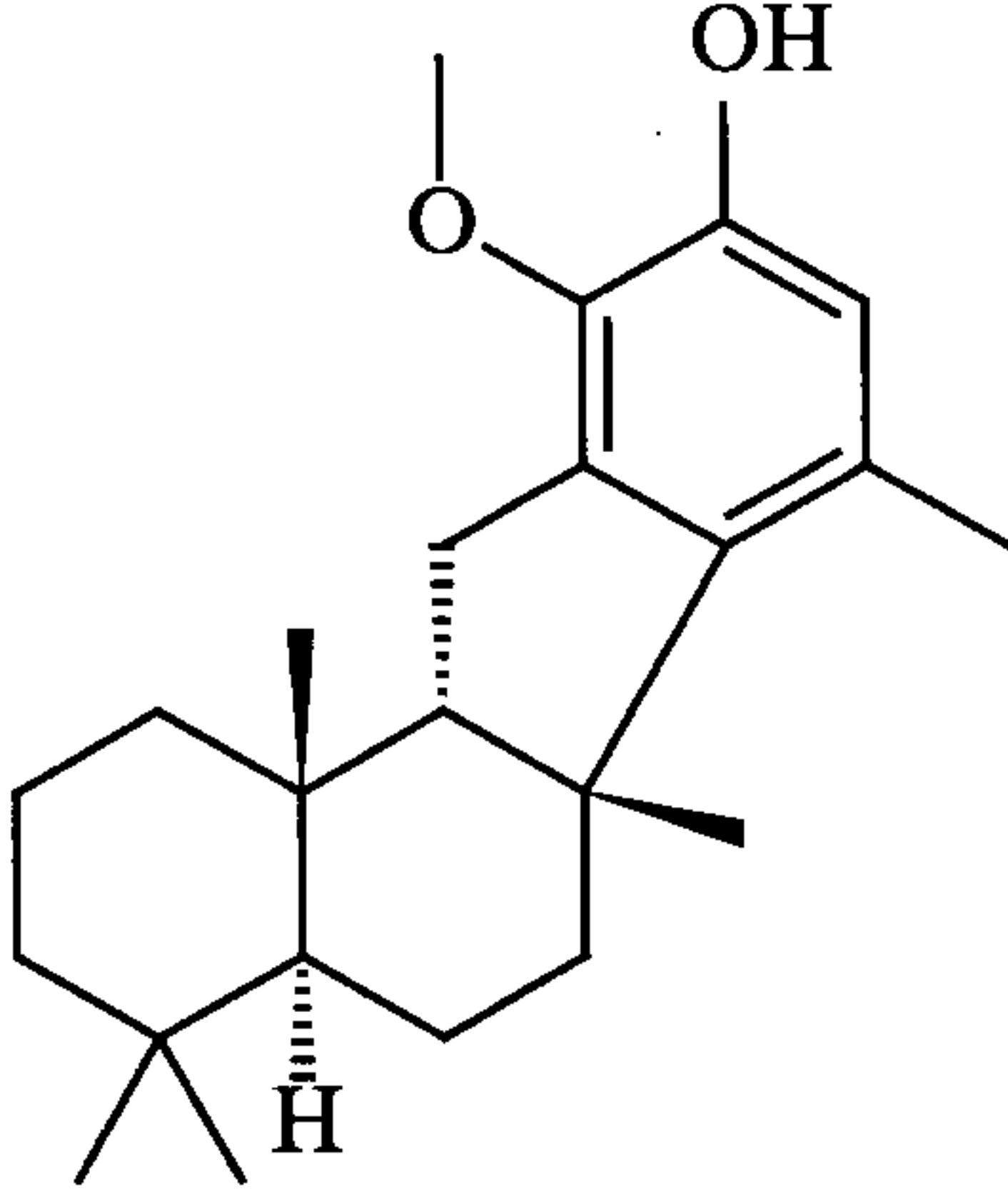


Table 1A

In all embodiments described herein –OH moieties may be replaced by prodrug moieties that are cleavable such that when the prodrug is cleaved an –OH moiety is provided in its place.

Phosphate prodrugs and solubilizing moieties linked with an ester linking moiety often provide

5 –OH moieties on the core compound when cleaved from the core compound. As used herein

X₅ is termed a prodrug moiety. In all instances where an –OH moiety is described by a

compound of this invention, the –OH moiety may be substituted with an X₅ moiety.

In particular the following structures in Table I are non-limiting examples of solubilizing moieties. In the following table, each R is independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or is substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR', -NR'₂, NO₂, -CO₂H, -CO₂R', and epoxide;

and R' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR'', -NR''₂, NO₂ and -CO₂H where R'' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group.

TABLE I - SOLUBILIZING MOIETIES

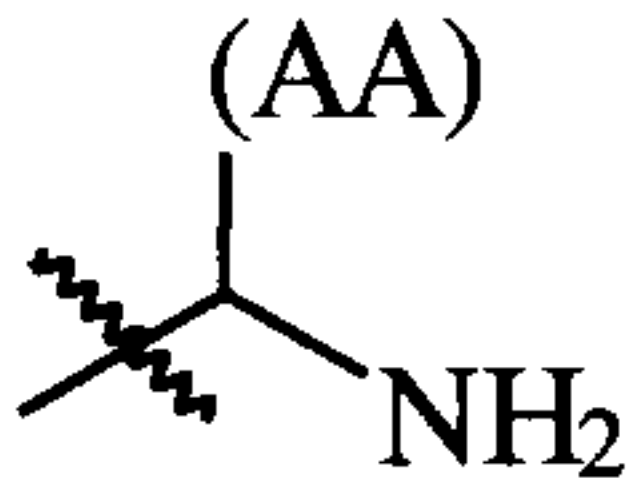
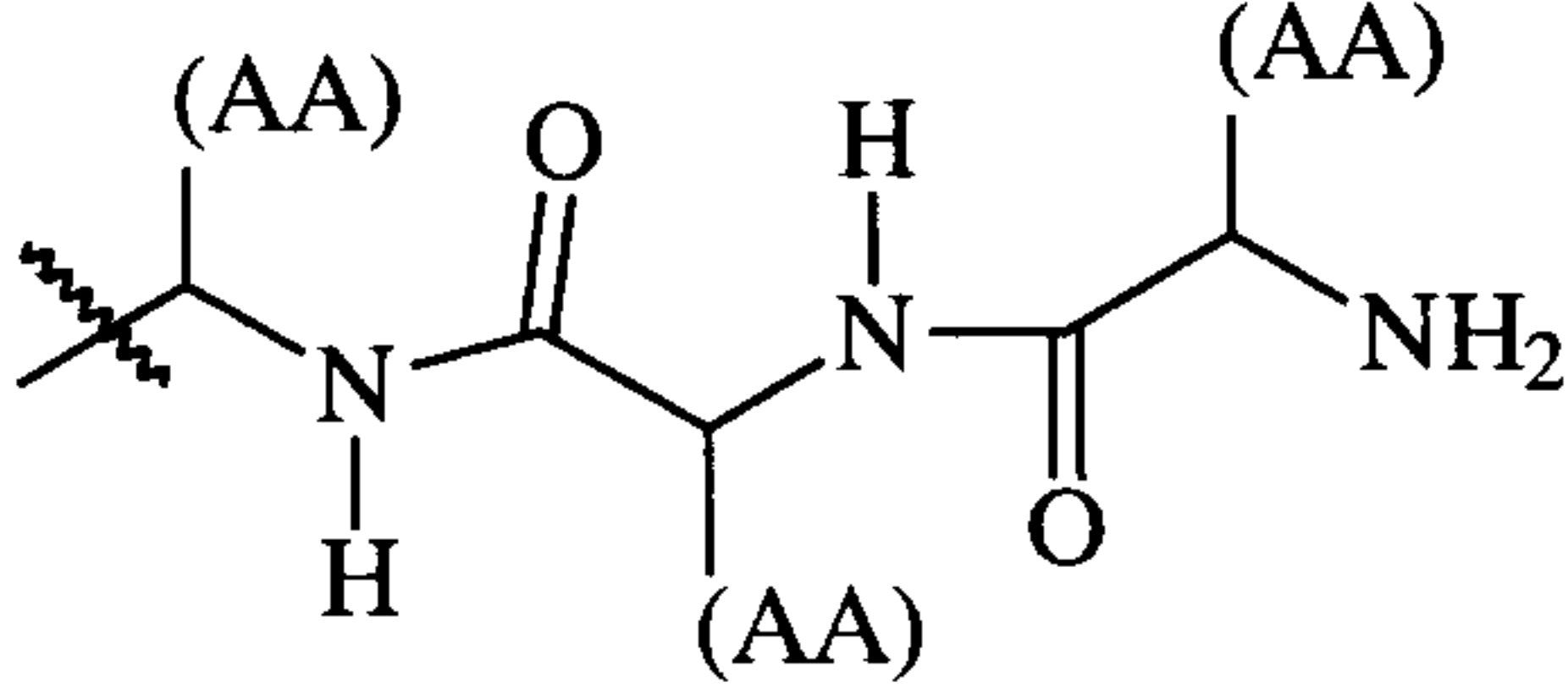
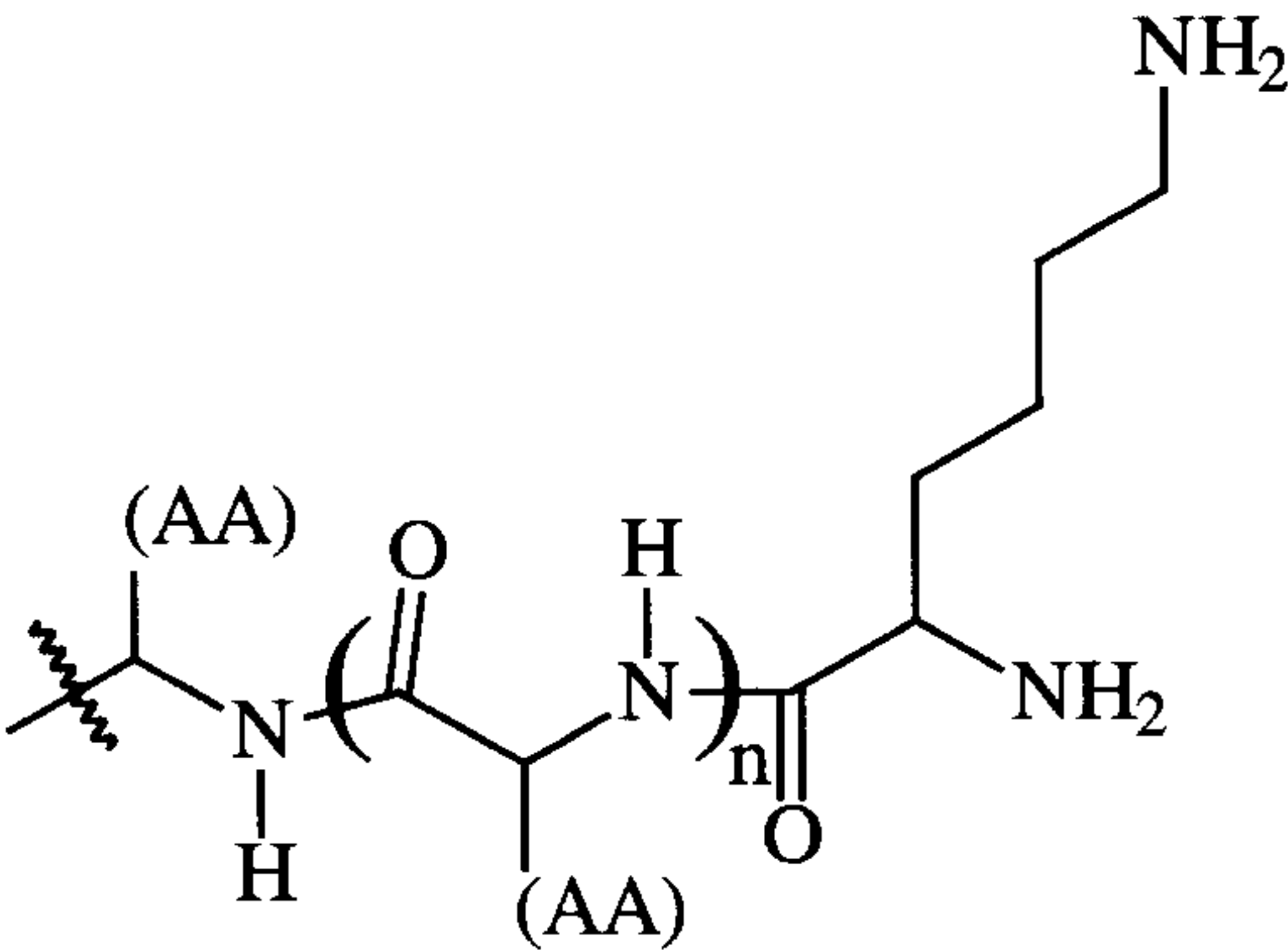
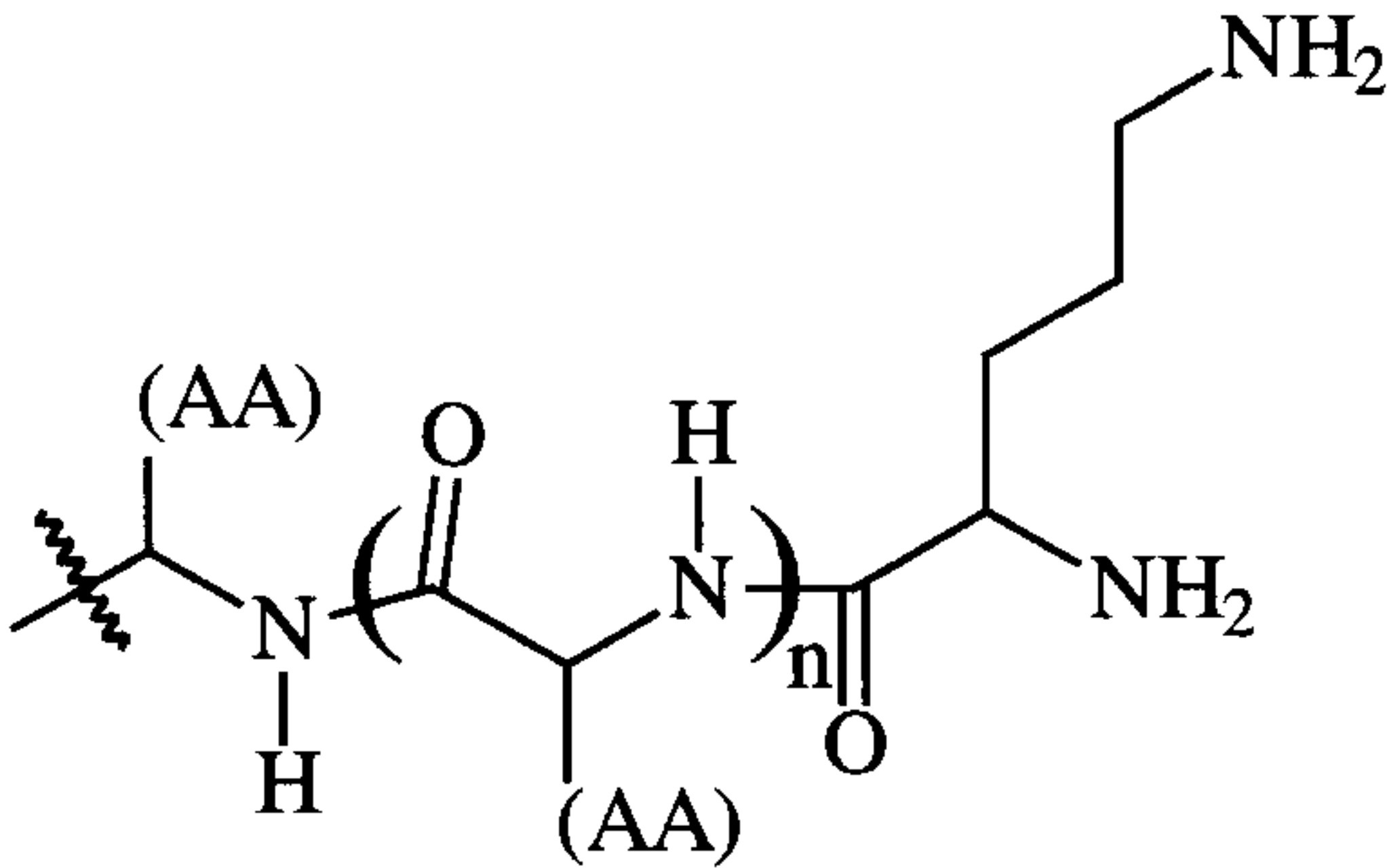
 Wherein (AA) is any amino acid side chain	 Wherein each (AA) is independently any amino acid side chain
 Wherein each (AA) is independently any neutral amino acid side chain; and n is 1 to 10	 Wherein each (AA) is independently any neutral amino acid side chain; and n is 1 to 10

TABLE I - SOLUBILIZING MOIETIES

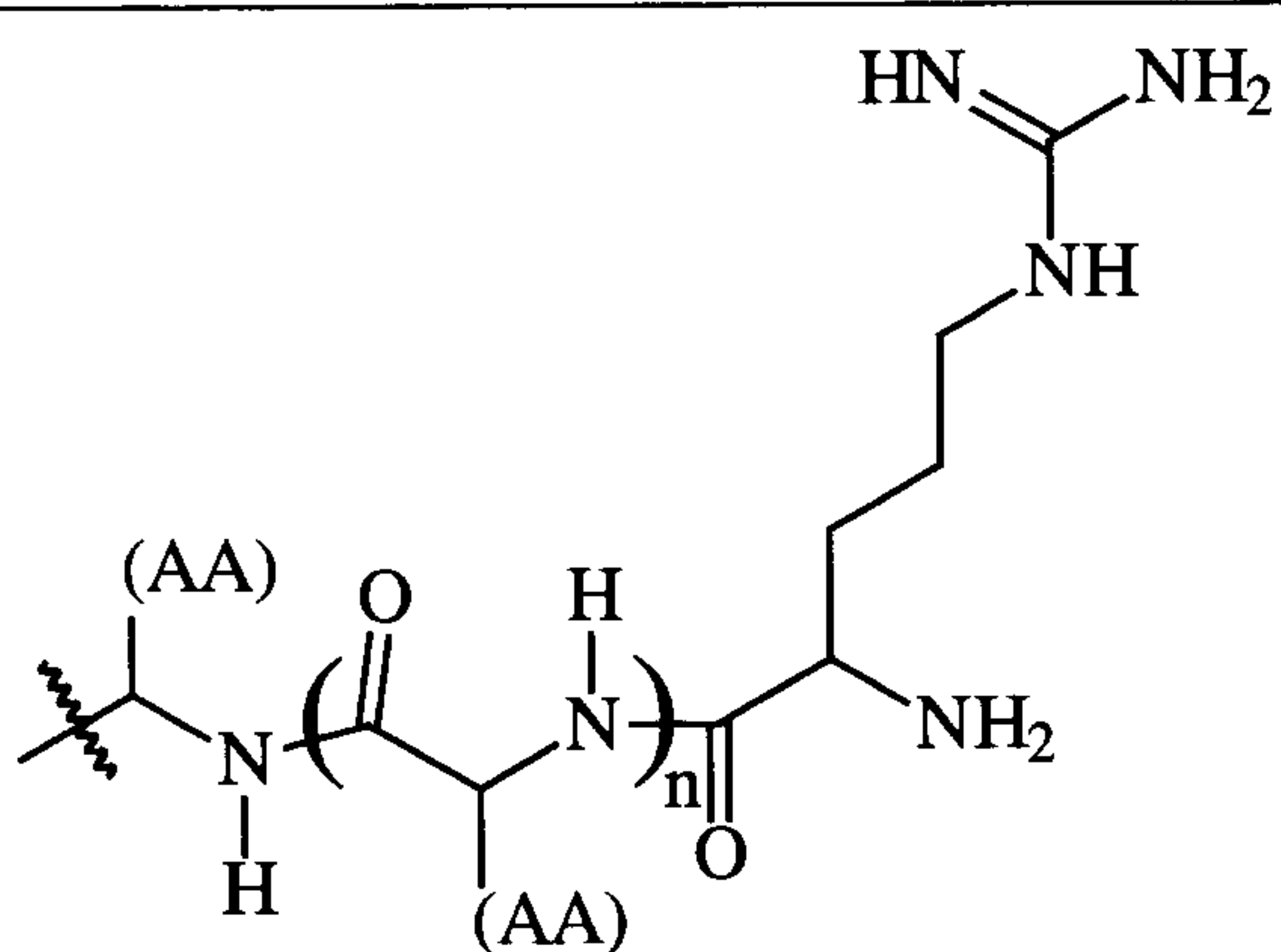
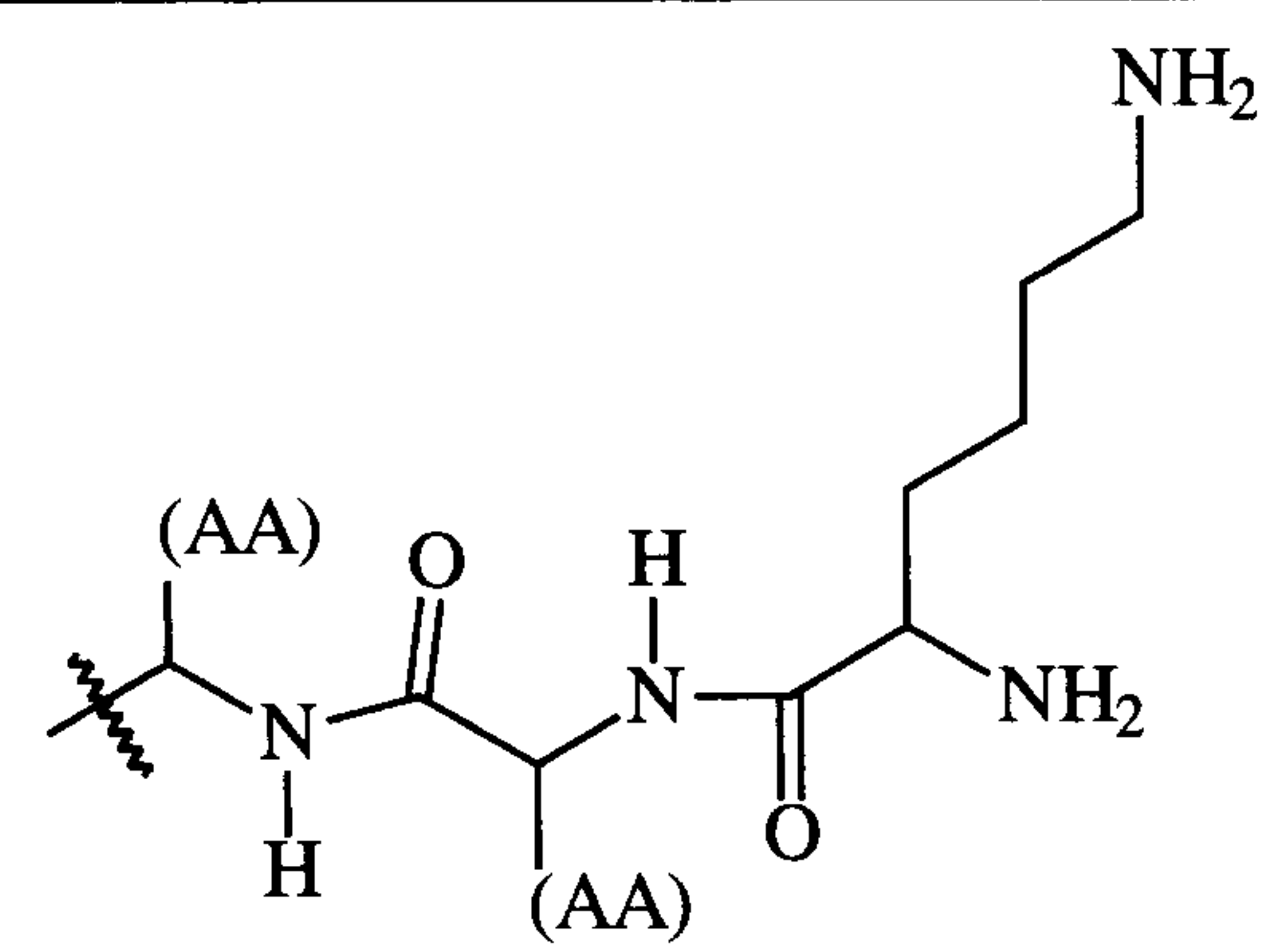
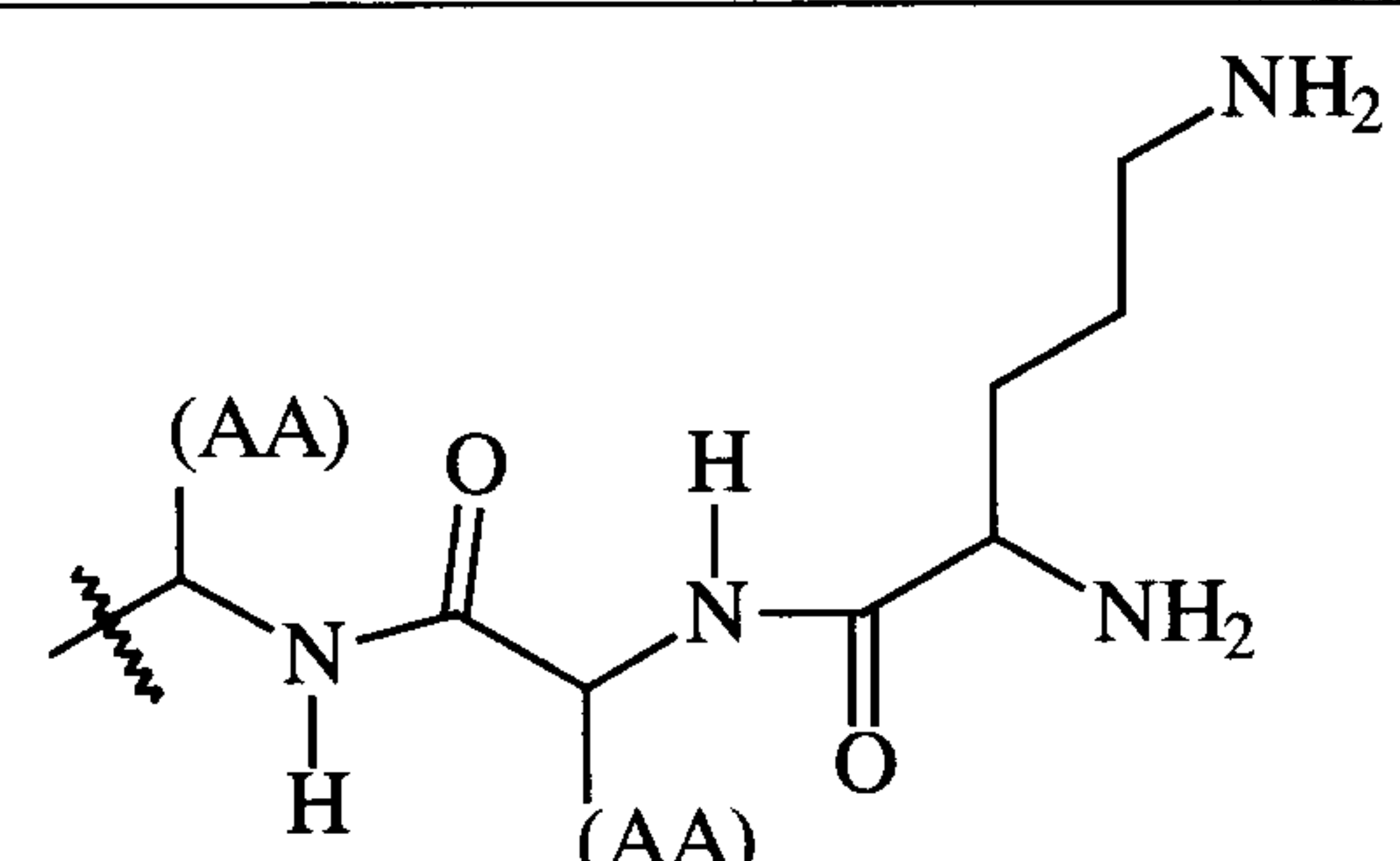
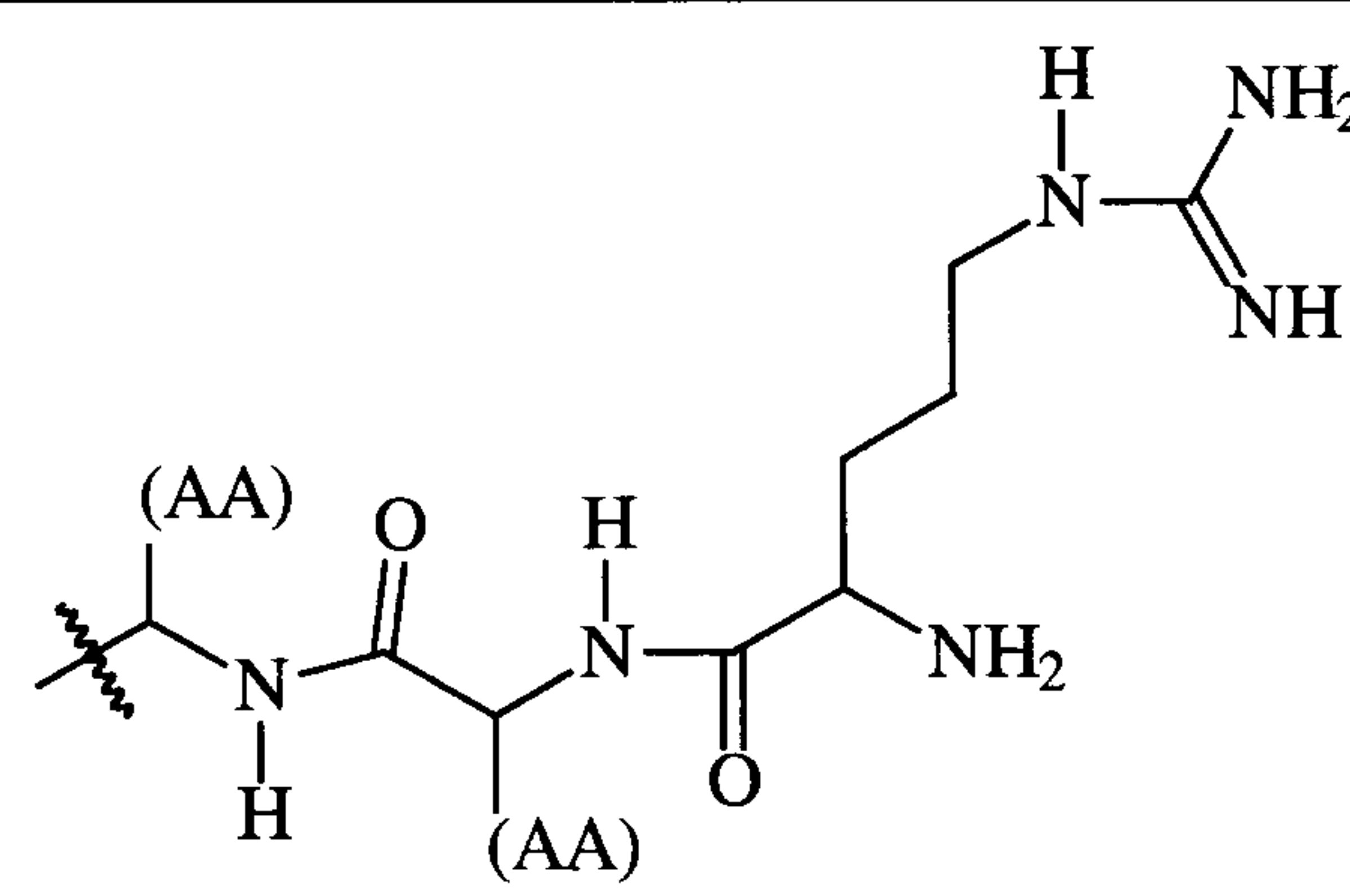
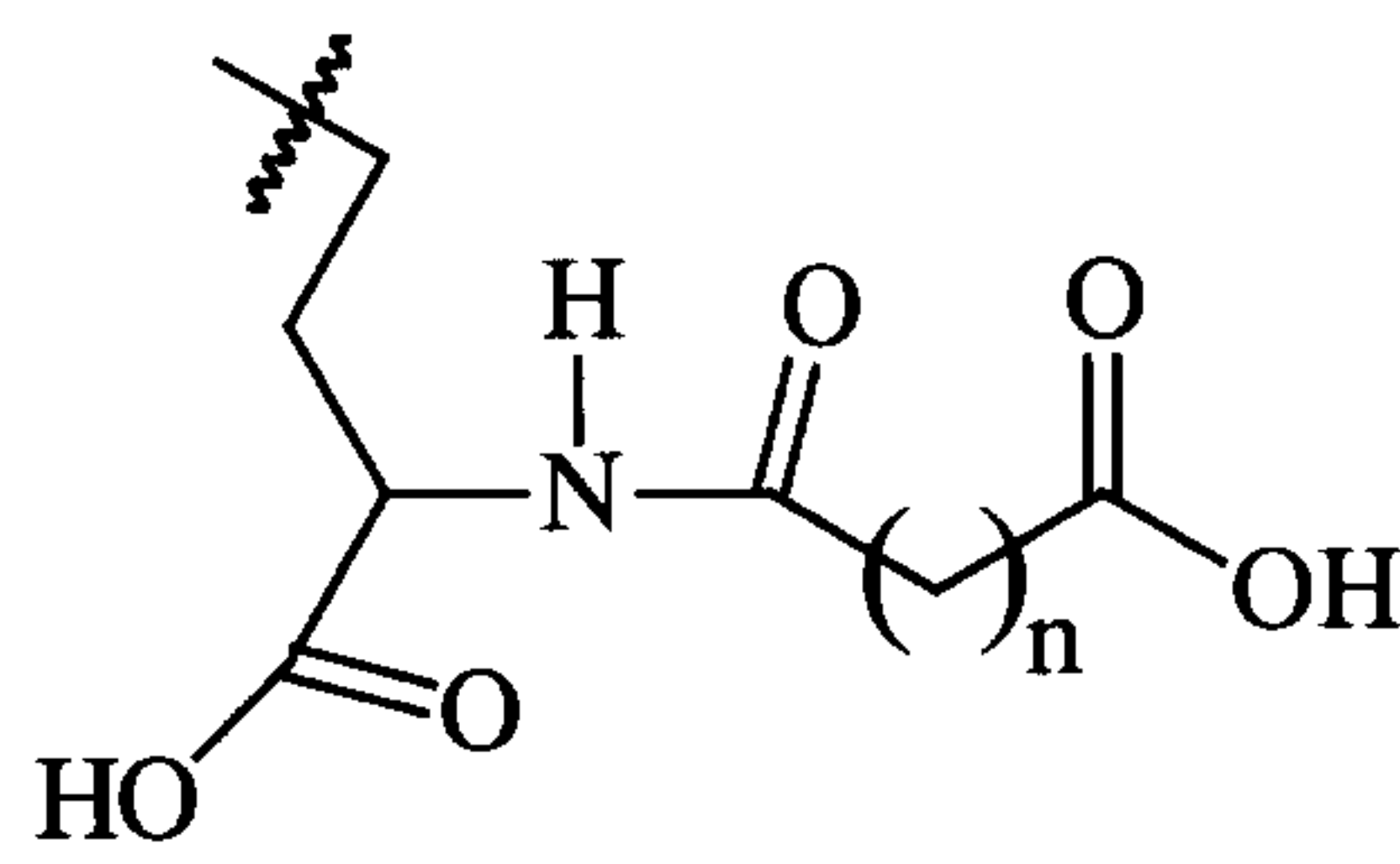
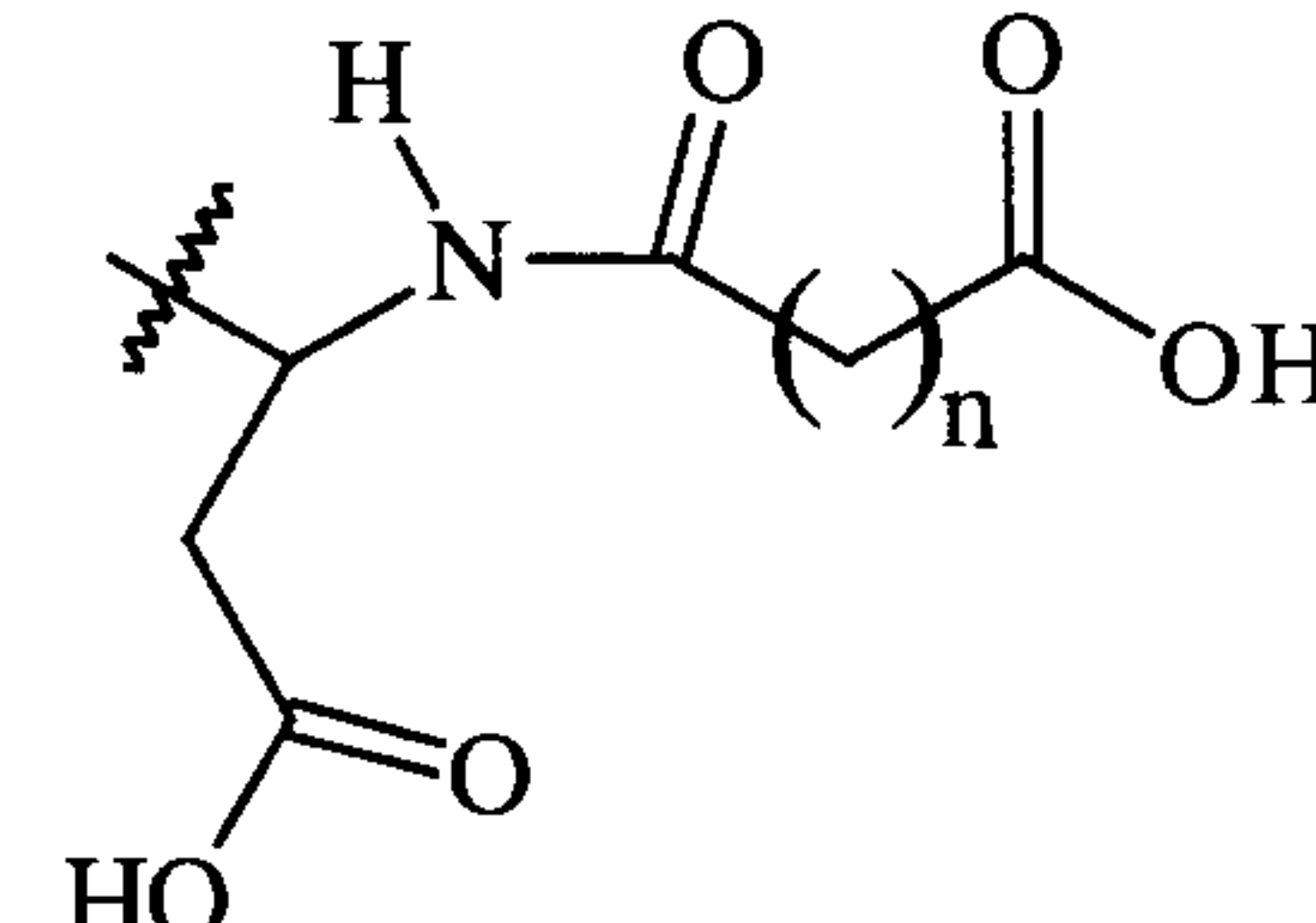
 Wherein each (AA) is independently any neutral amino acid side chain; and n is 1 to 10	 Wherein each (AA) is independently any neutral amino acid side chain
 Wherein each (AA) is independently any neutral amino acid side chain	 Wherein each (AA) is independently any neutral amino acid side chain
 Wherein n = 0, 1, 2, 3, 4, 5 or 6	 Wherein n = 0, 1, 2, 3, 4, 5 or 6

TABLE I - SOLUBILIZING MOIETIES

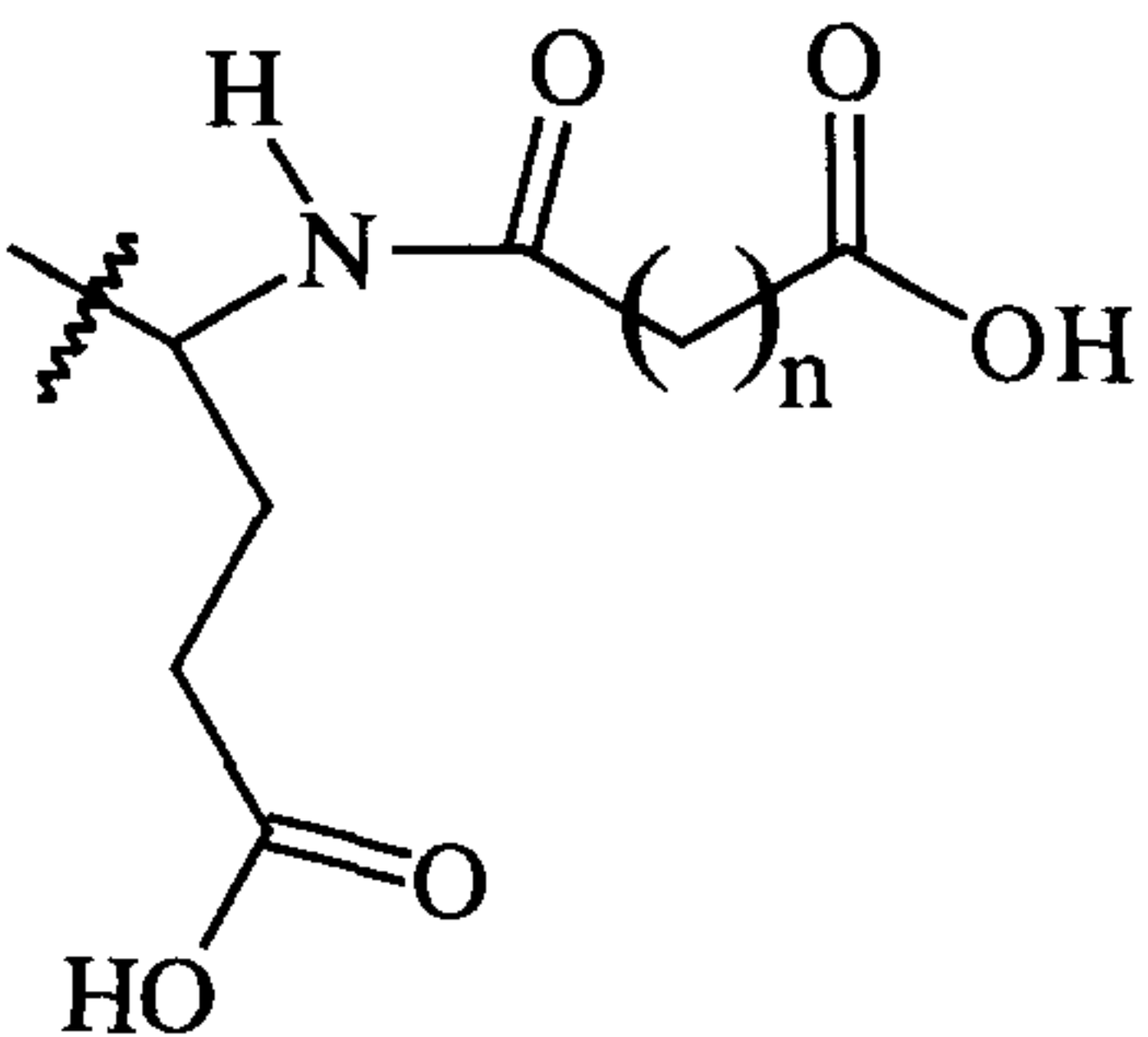
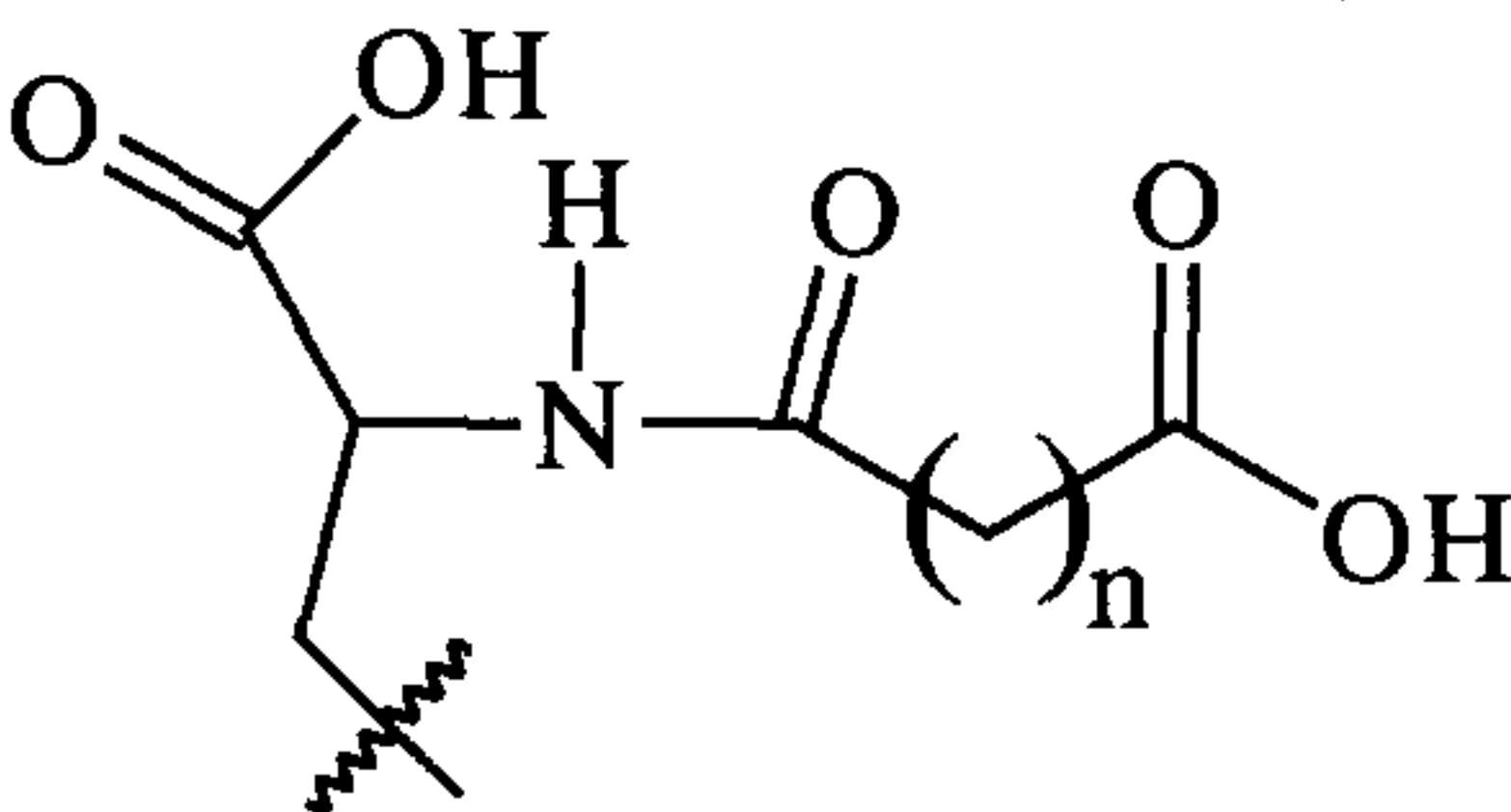
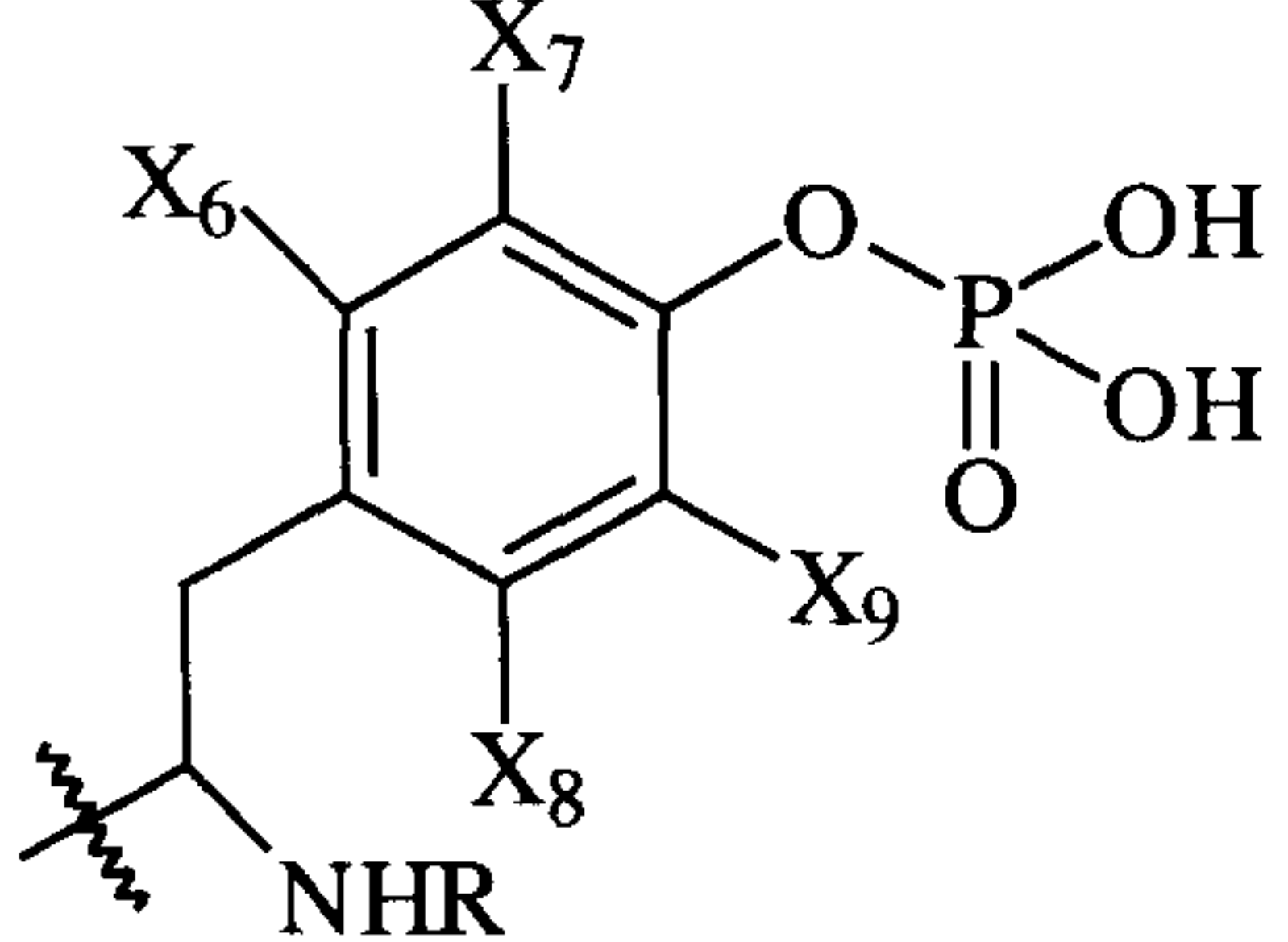
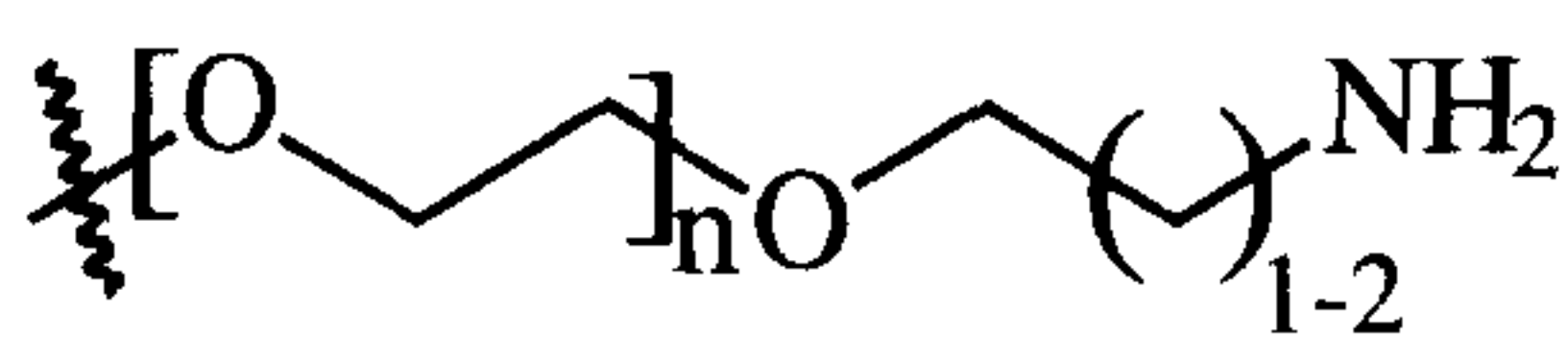
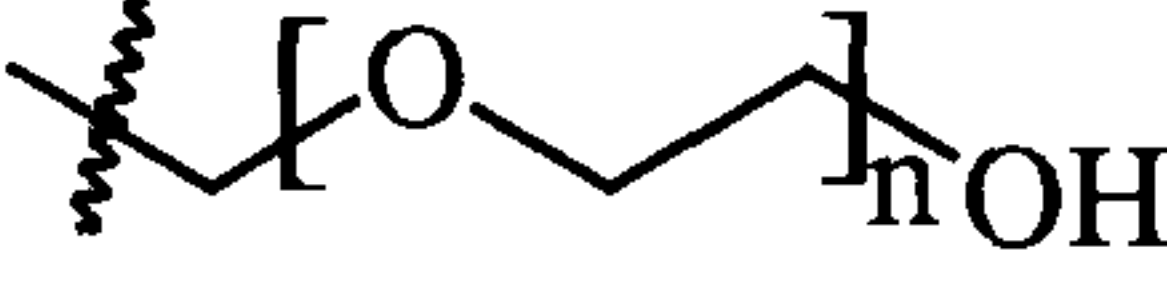
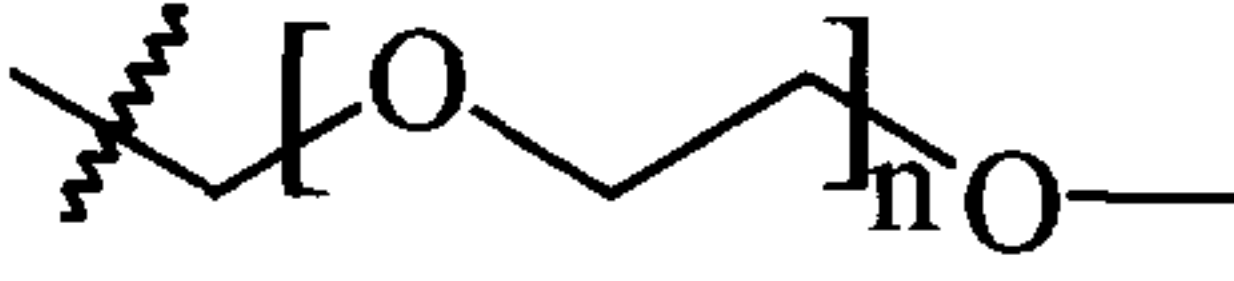
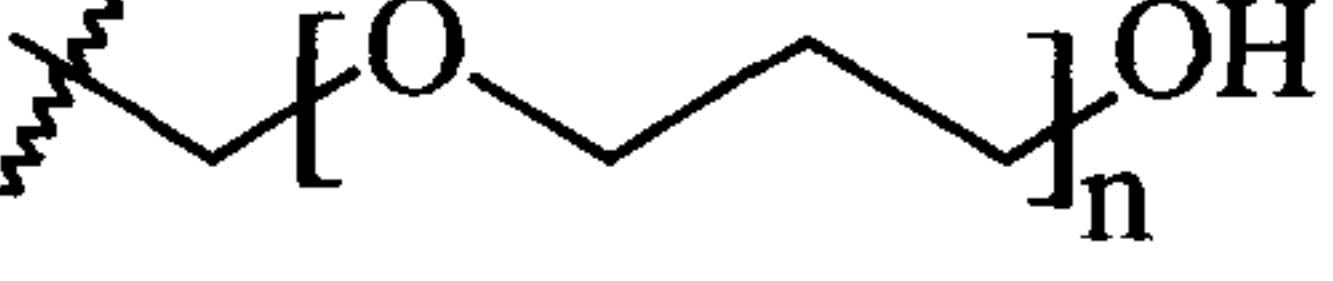
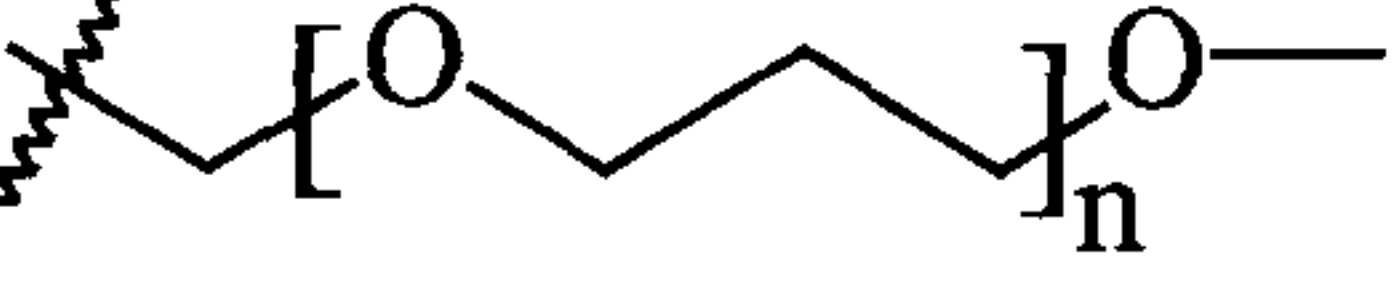
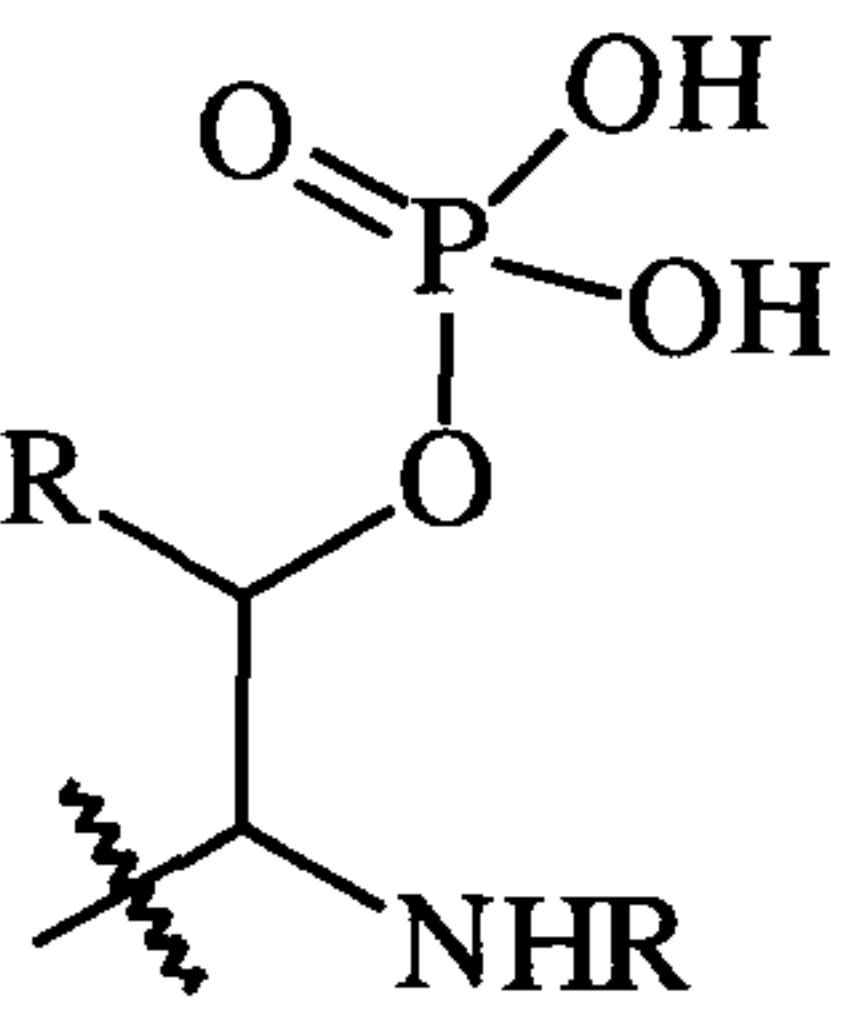
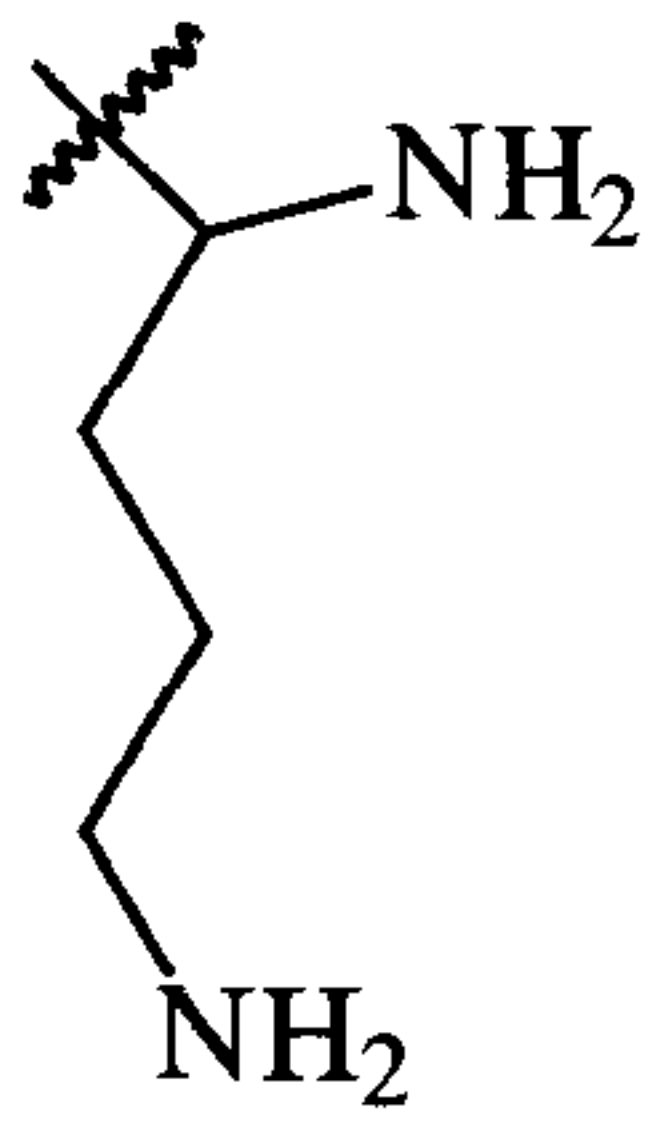
 Wherein n = 0, 1, 2, 3, 4, 5 or 6	 Wherein n = 0, 1, 2, 3, 4, 5 or 6
 Where X₆, X₇, X₈ and X₉ are as defined herein for X₁	 Where n = 1 to 450
 n is equal to number of repeating ethylene groups in a PEG	 n is equal to number of repeating ethylene groups in a PEG
 n is equal to number of repeating propylene groups in a PPG	 n is equal to number of repeating propylene groups in a PPG
	

TABLE I - SOLUBILIZING MOIETIES

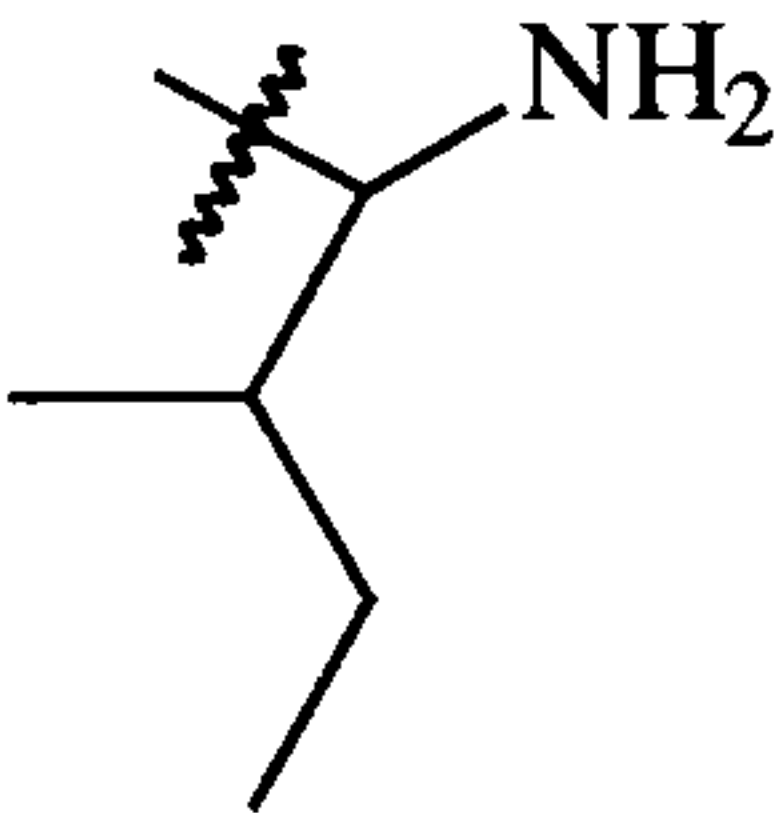
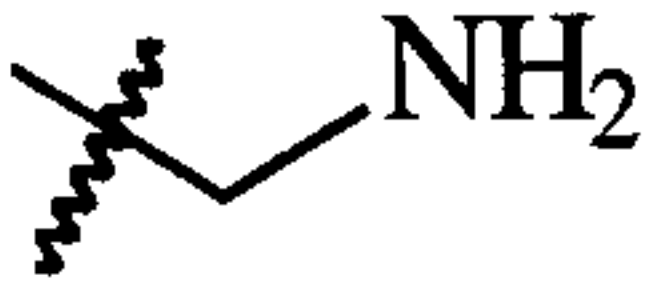
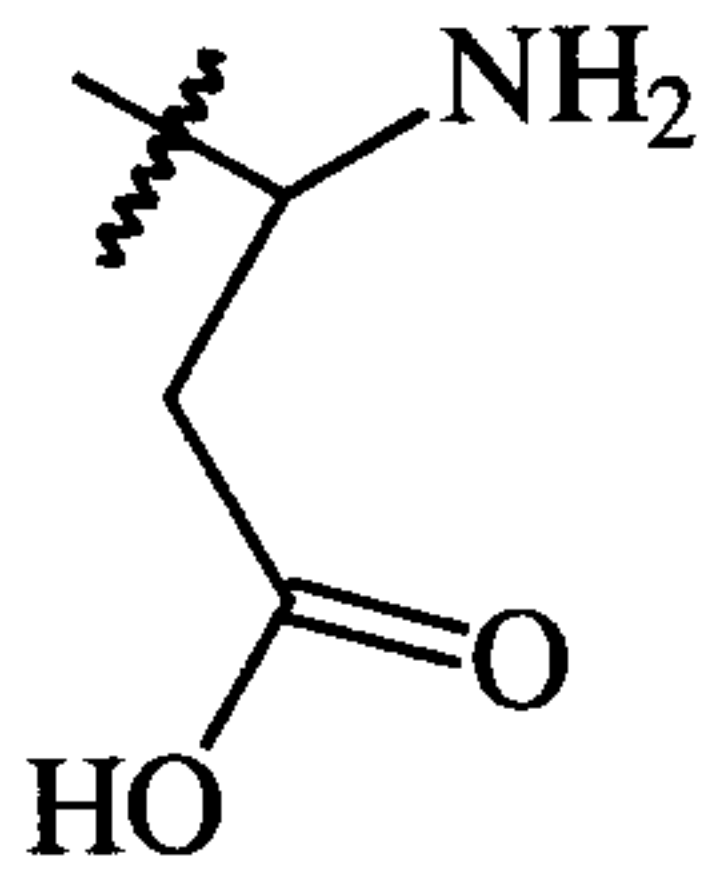
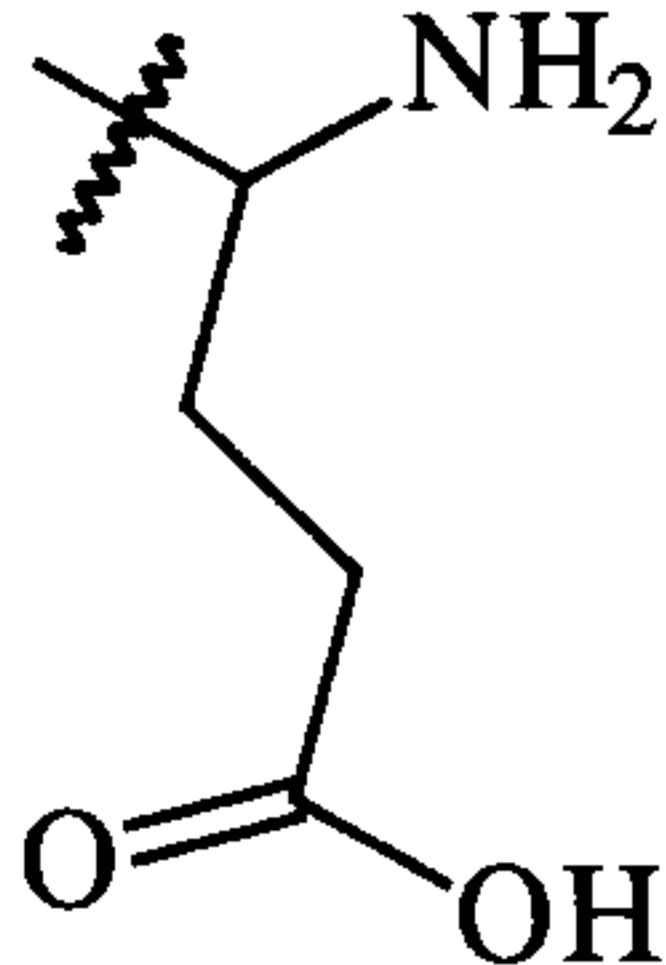
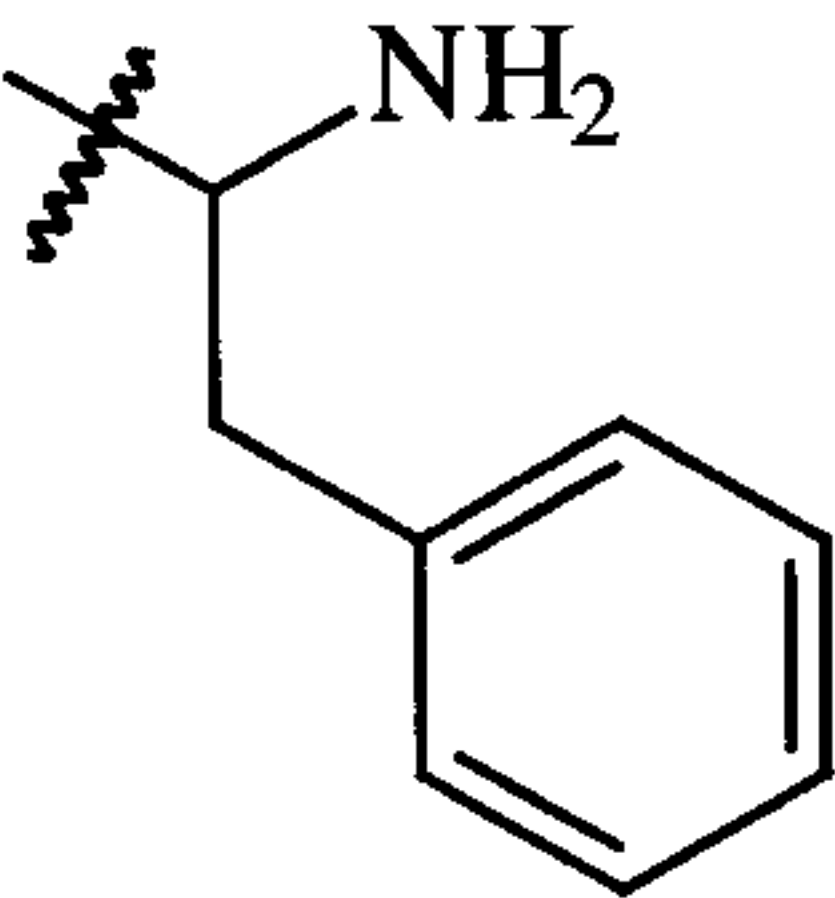
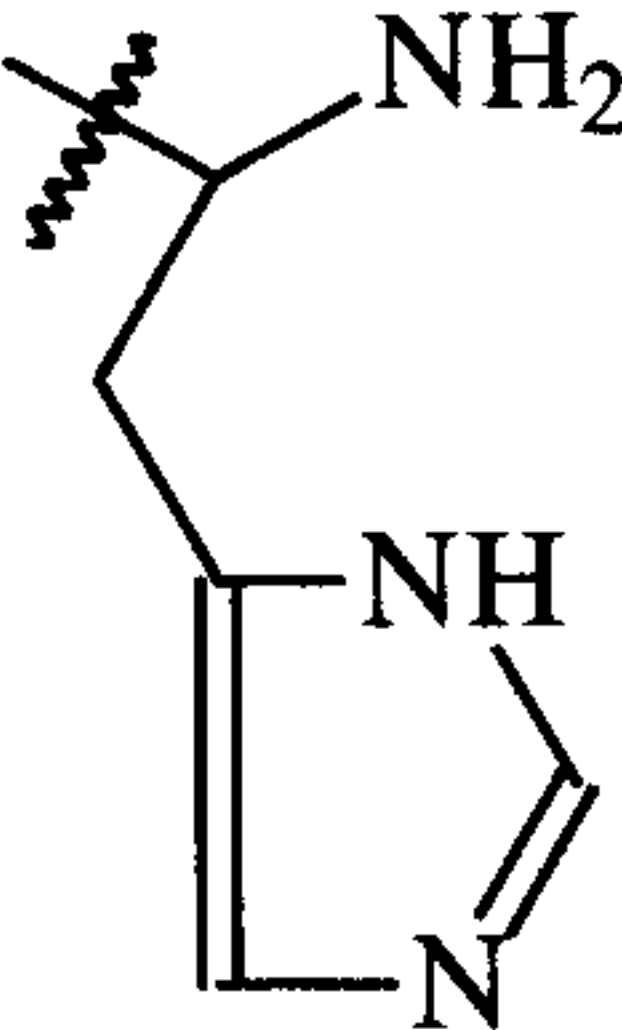
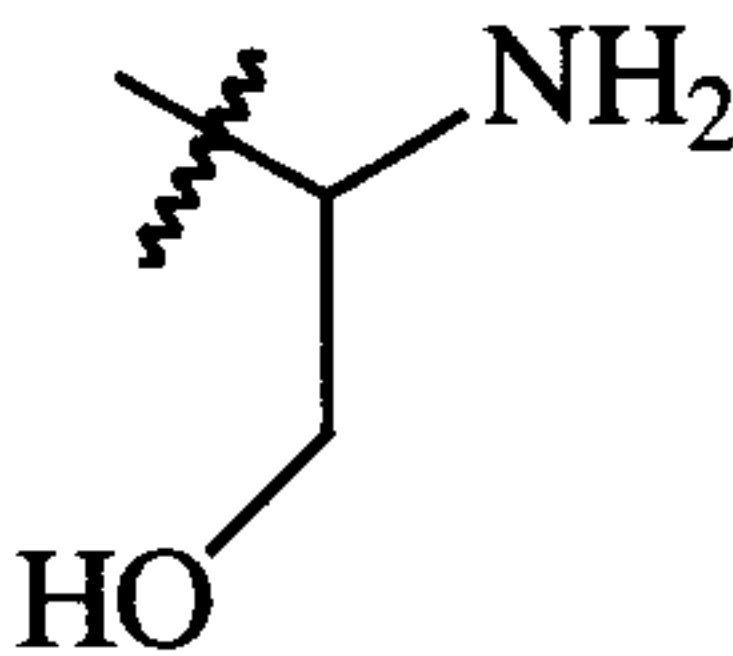
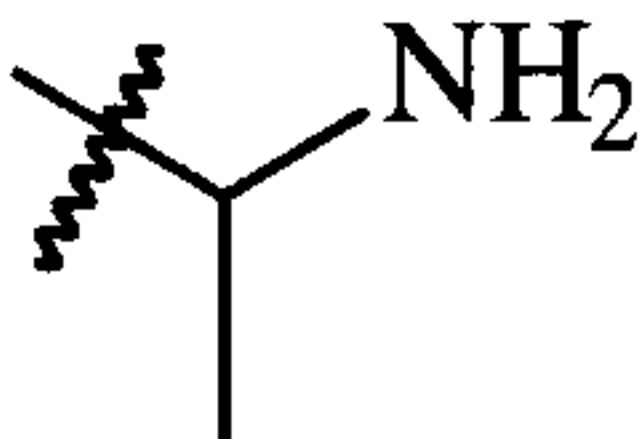
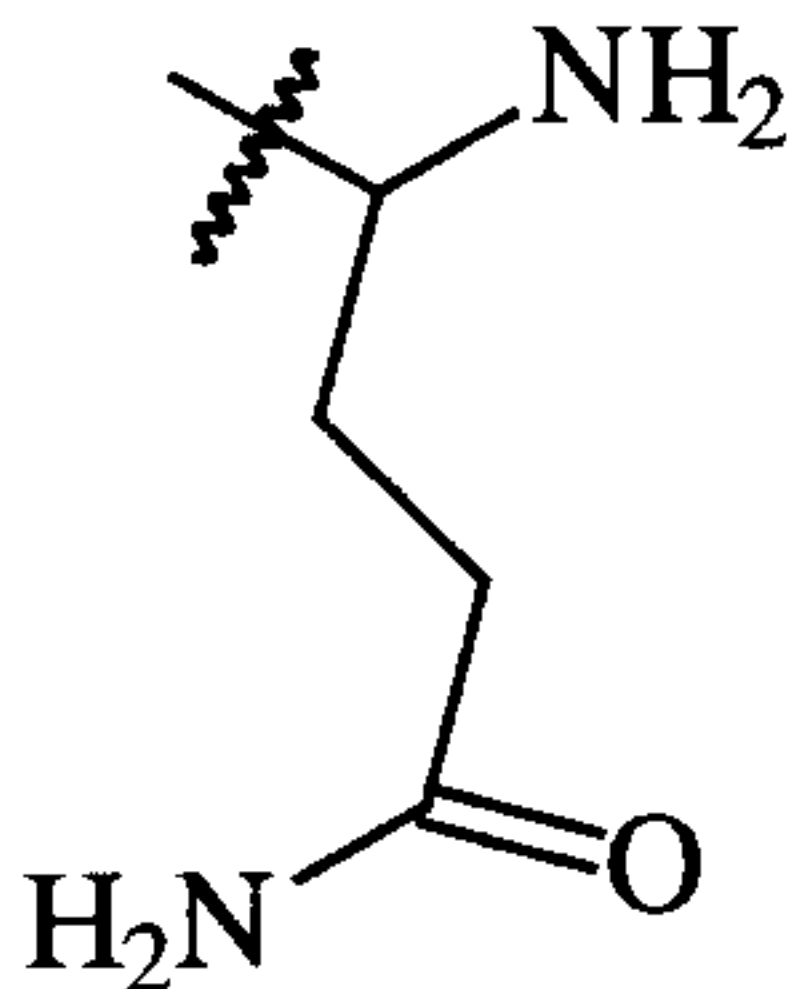
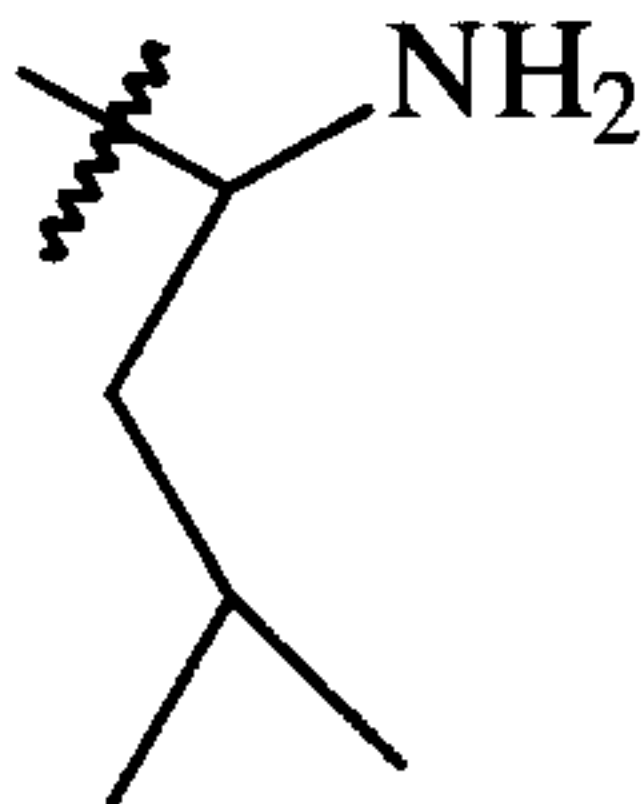
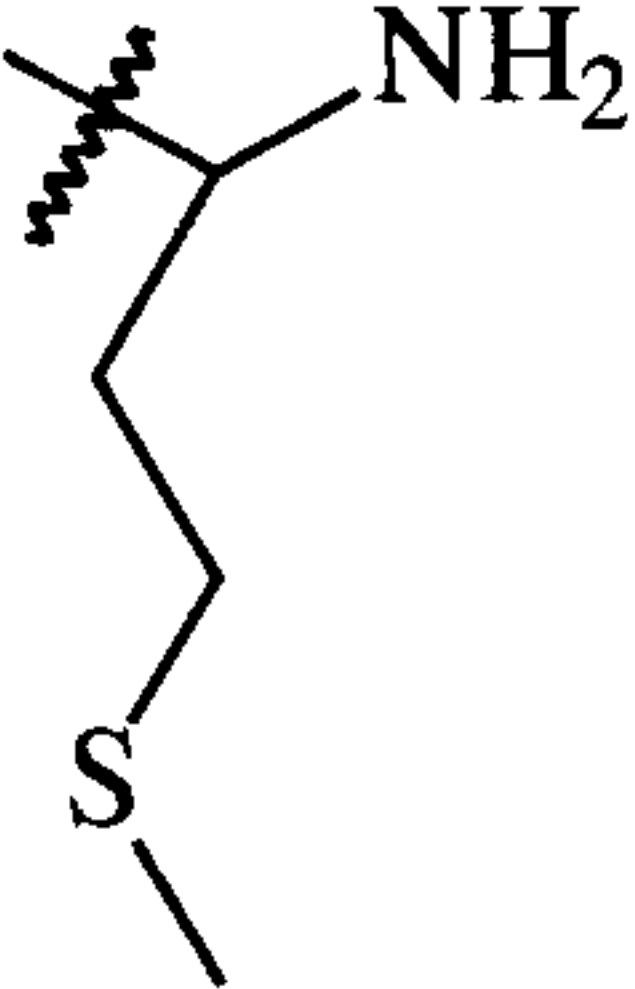
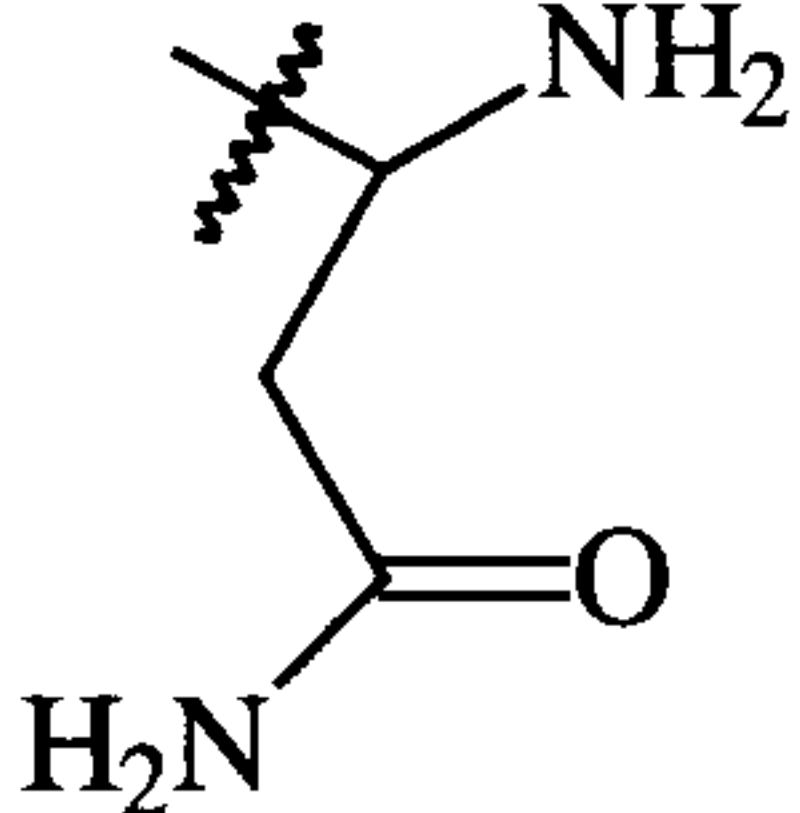
	
	
	
	
	
	

TABLE I - SOLUBILIZING MOIETIES

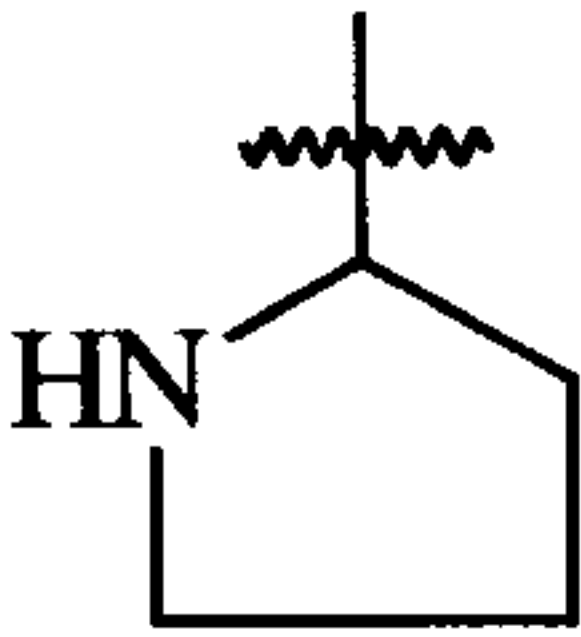
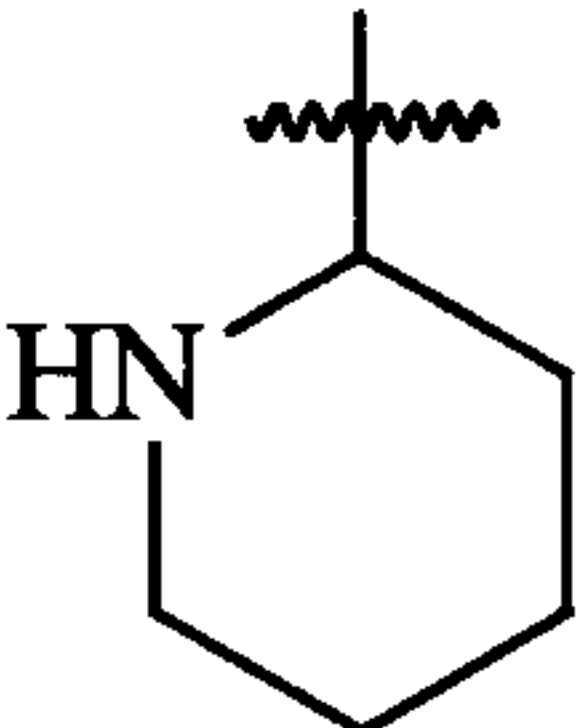
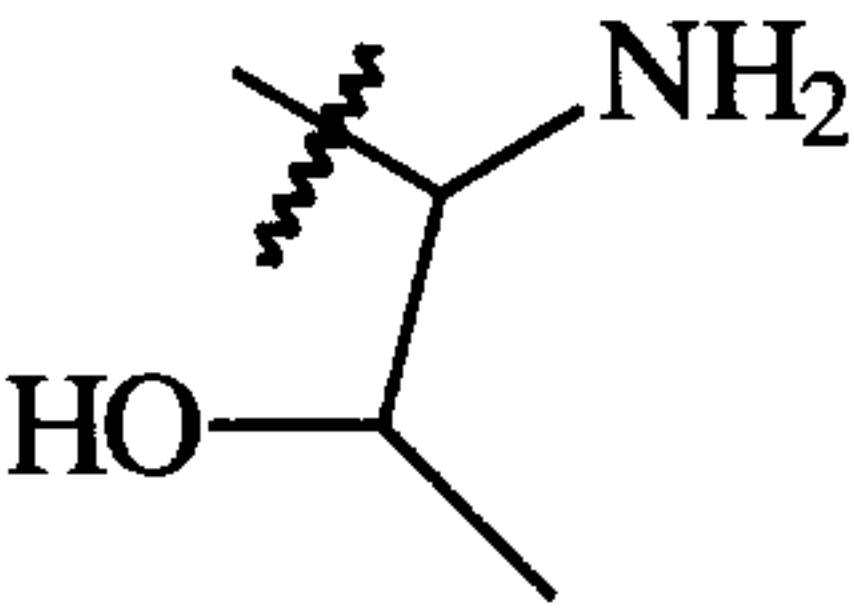
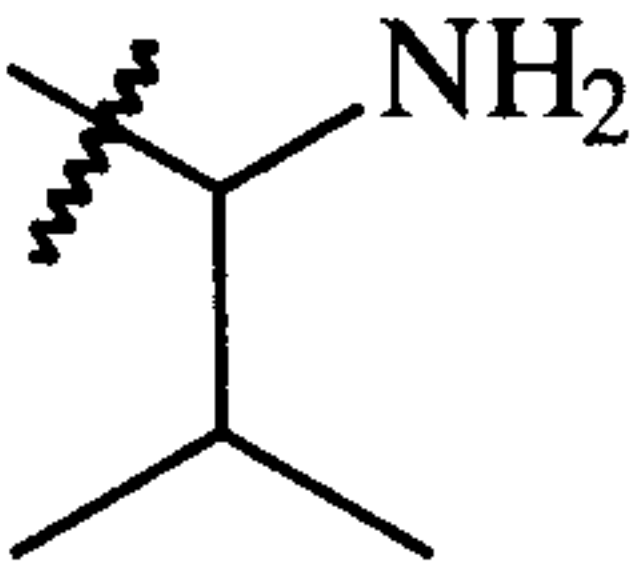
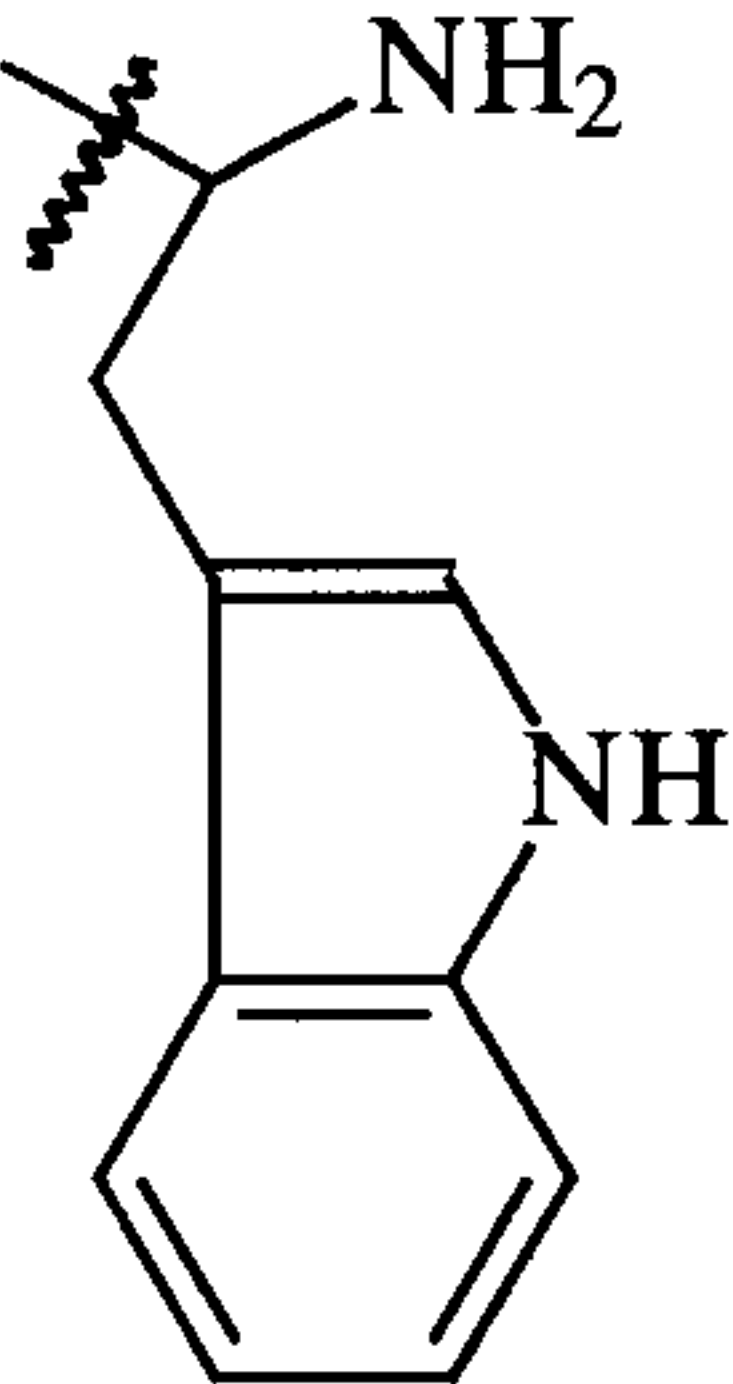
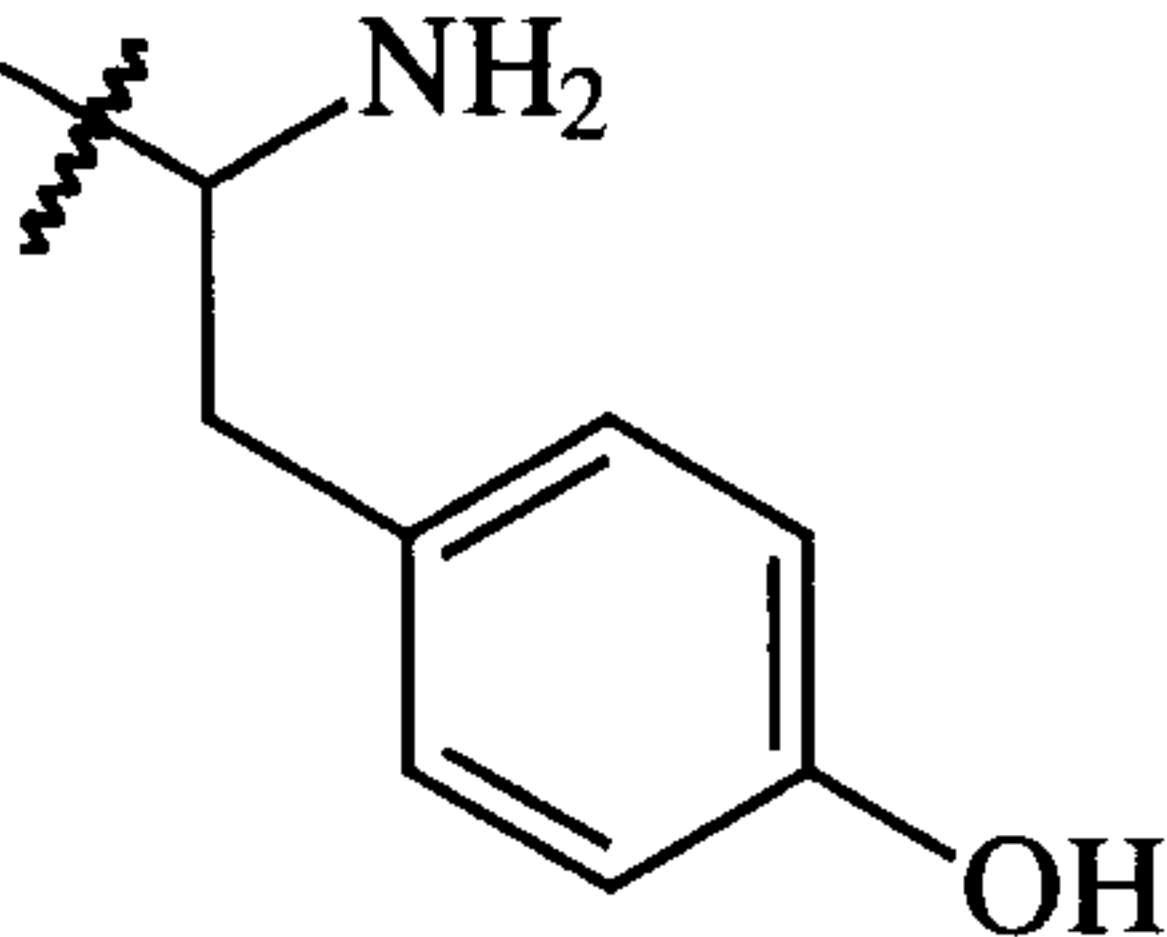
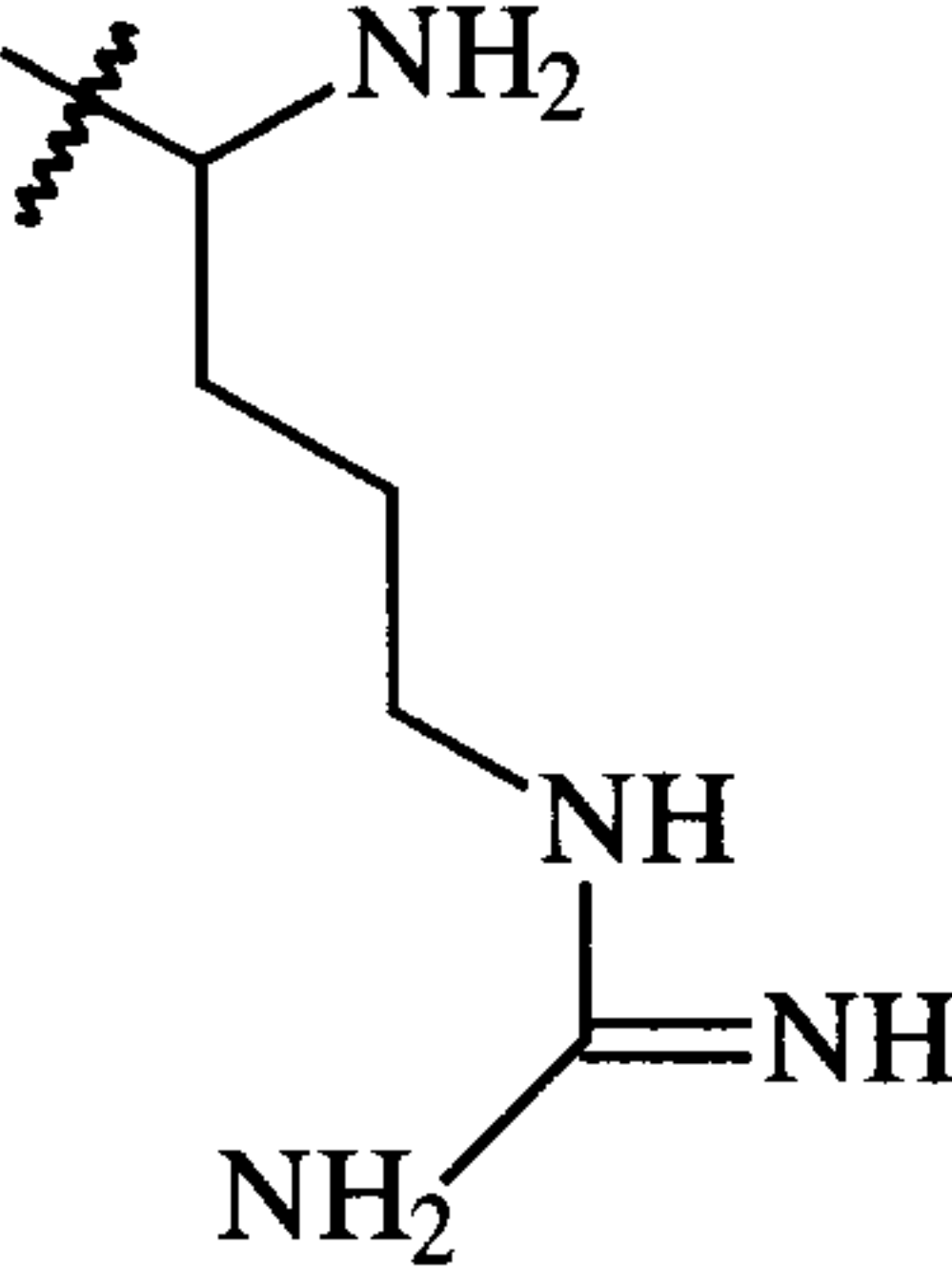
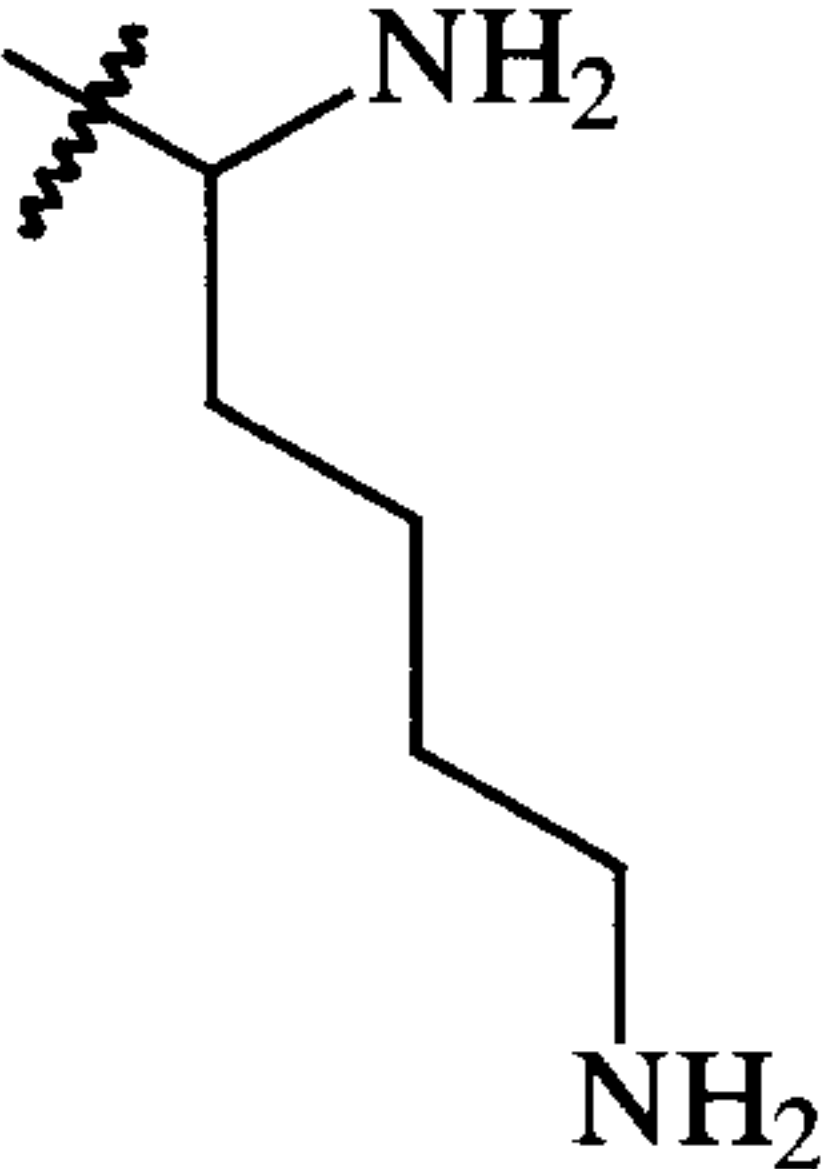
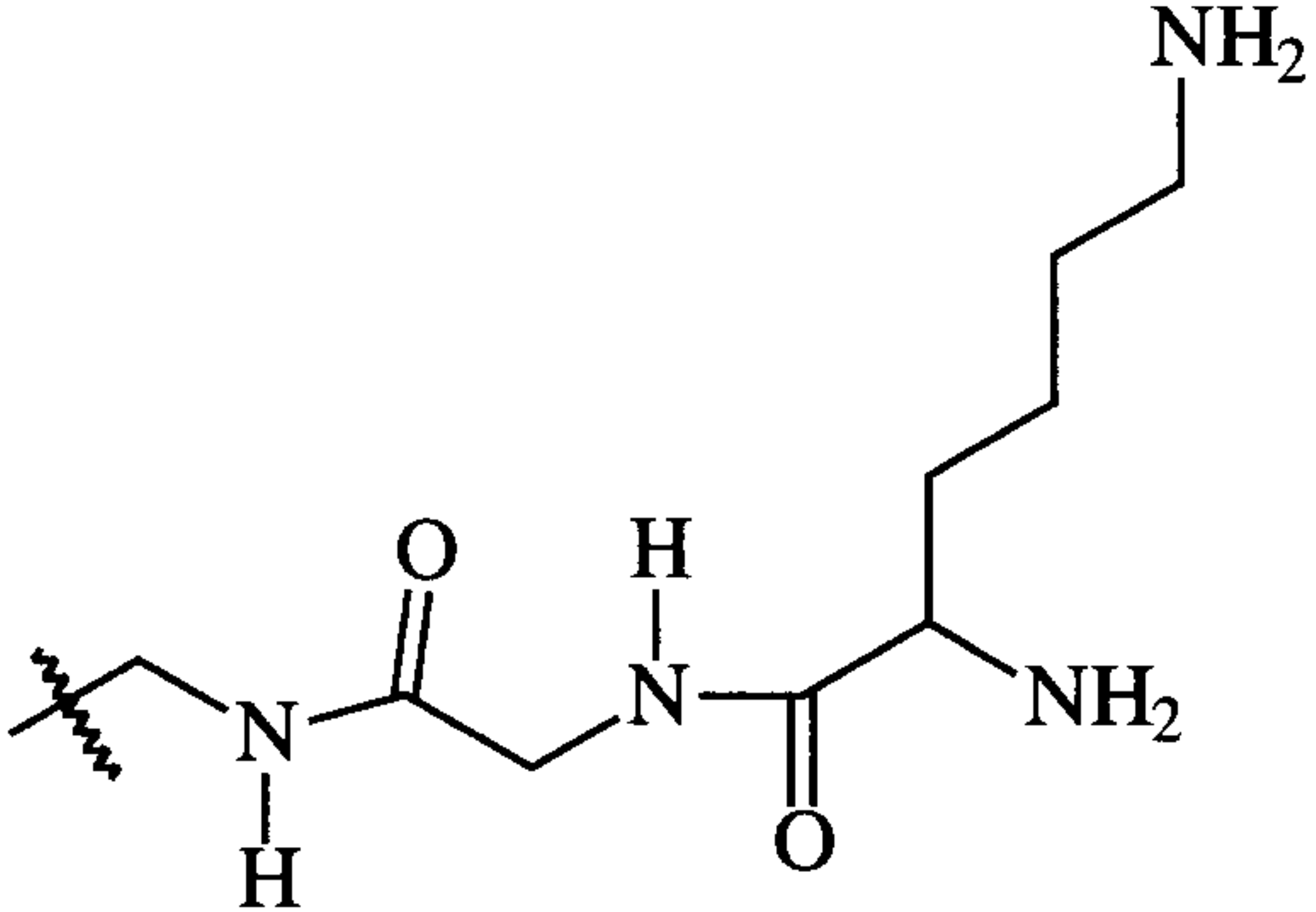
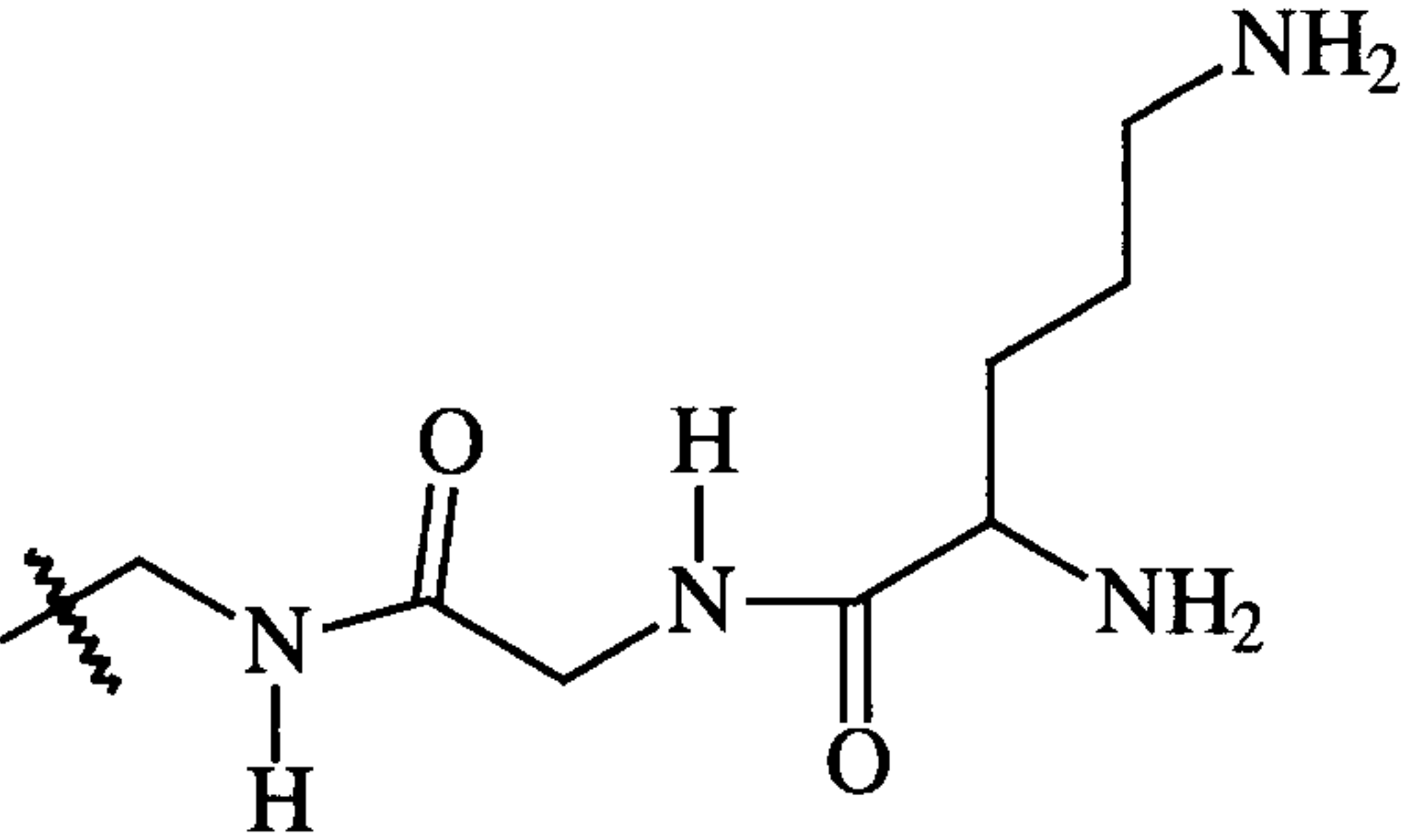
	
	
	
	
	

TABLE I - SOLUBILIZING MOIETIES

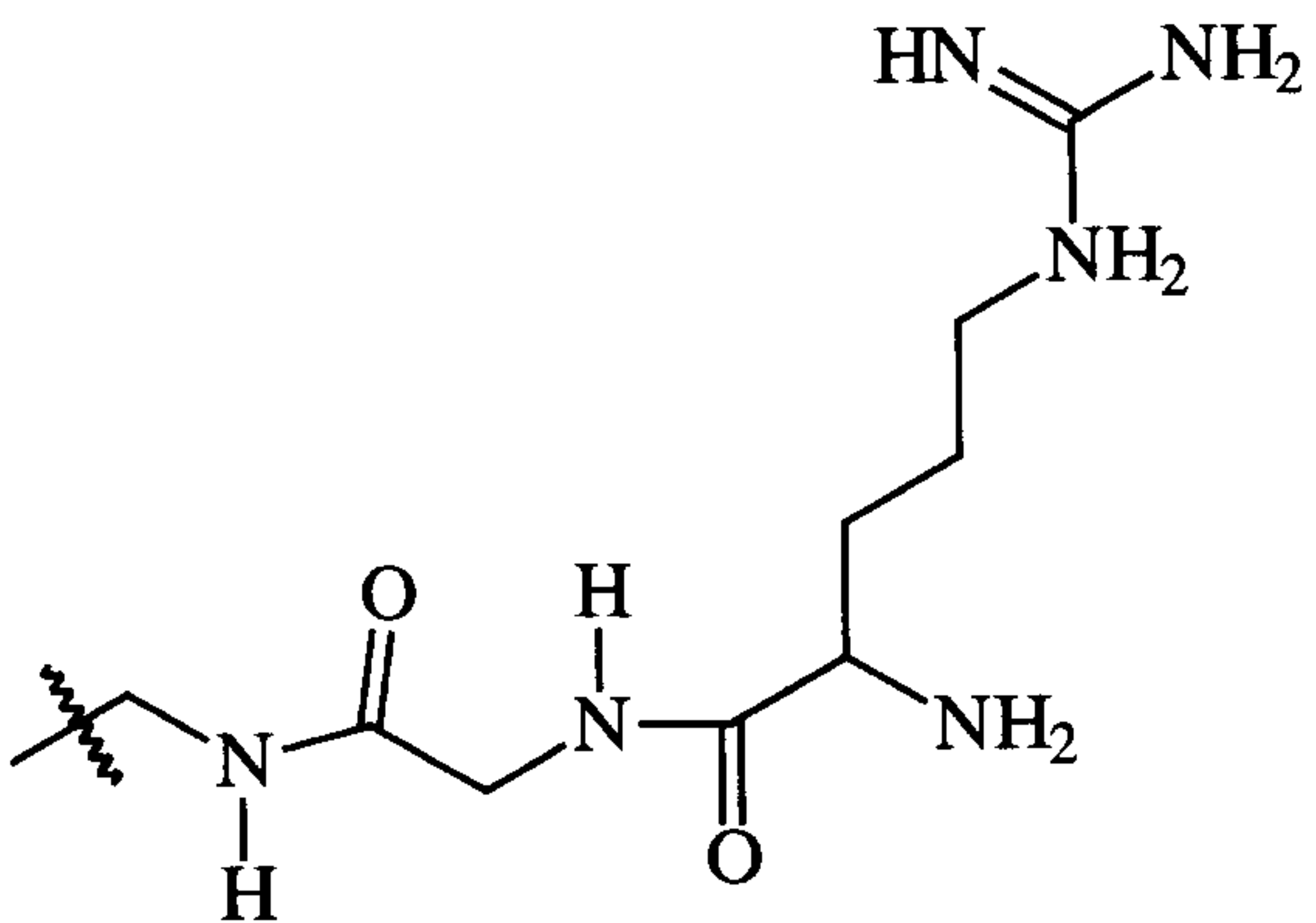
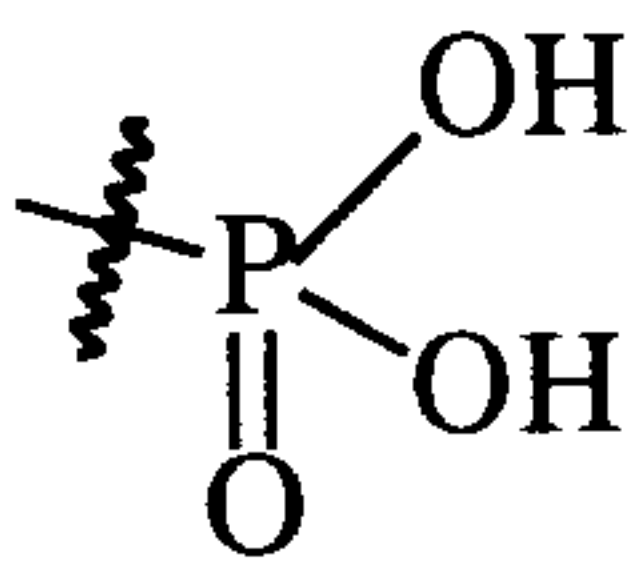
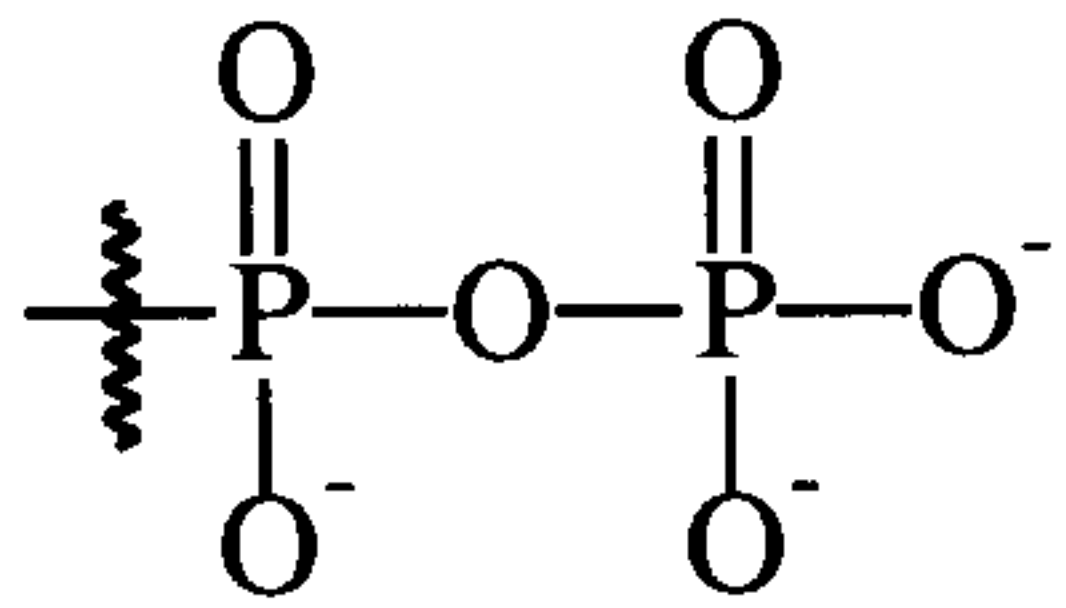
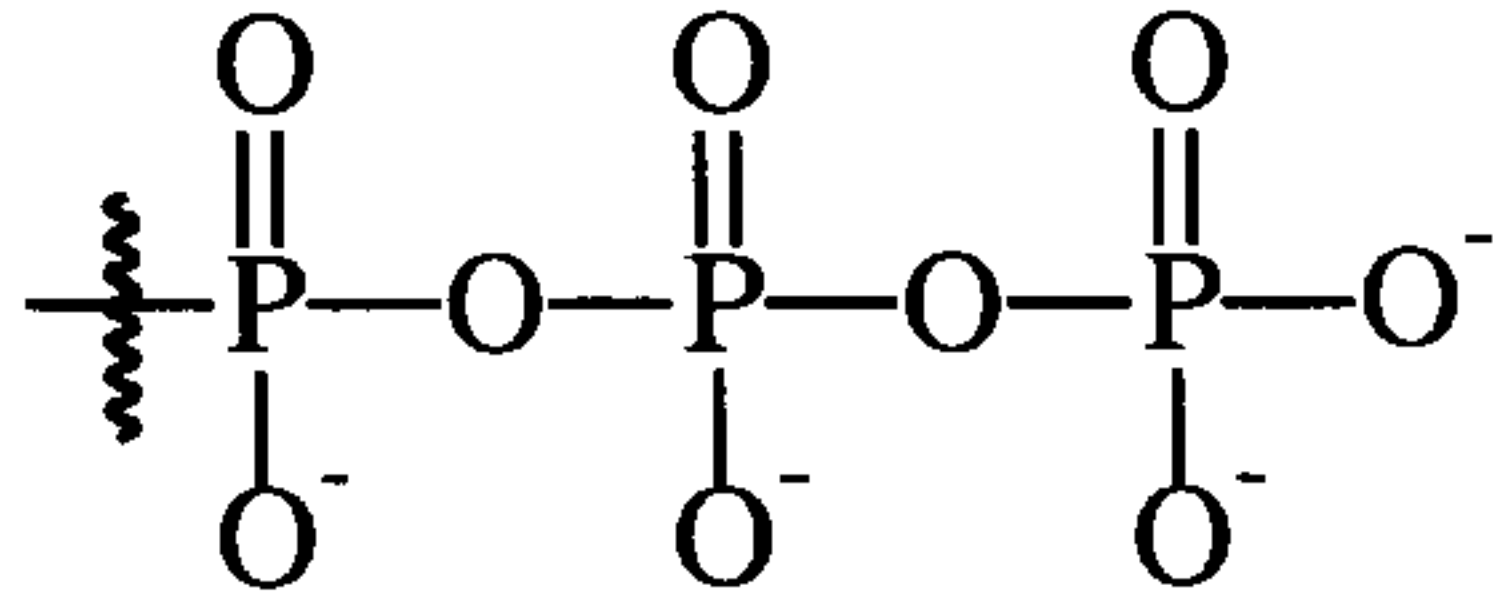
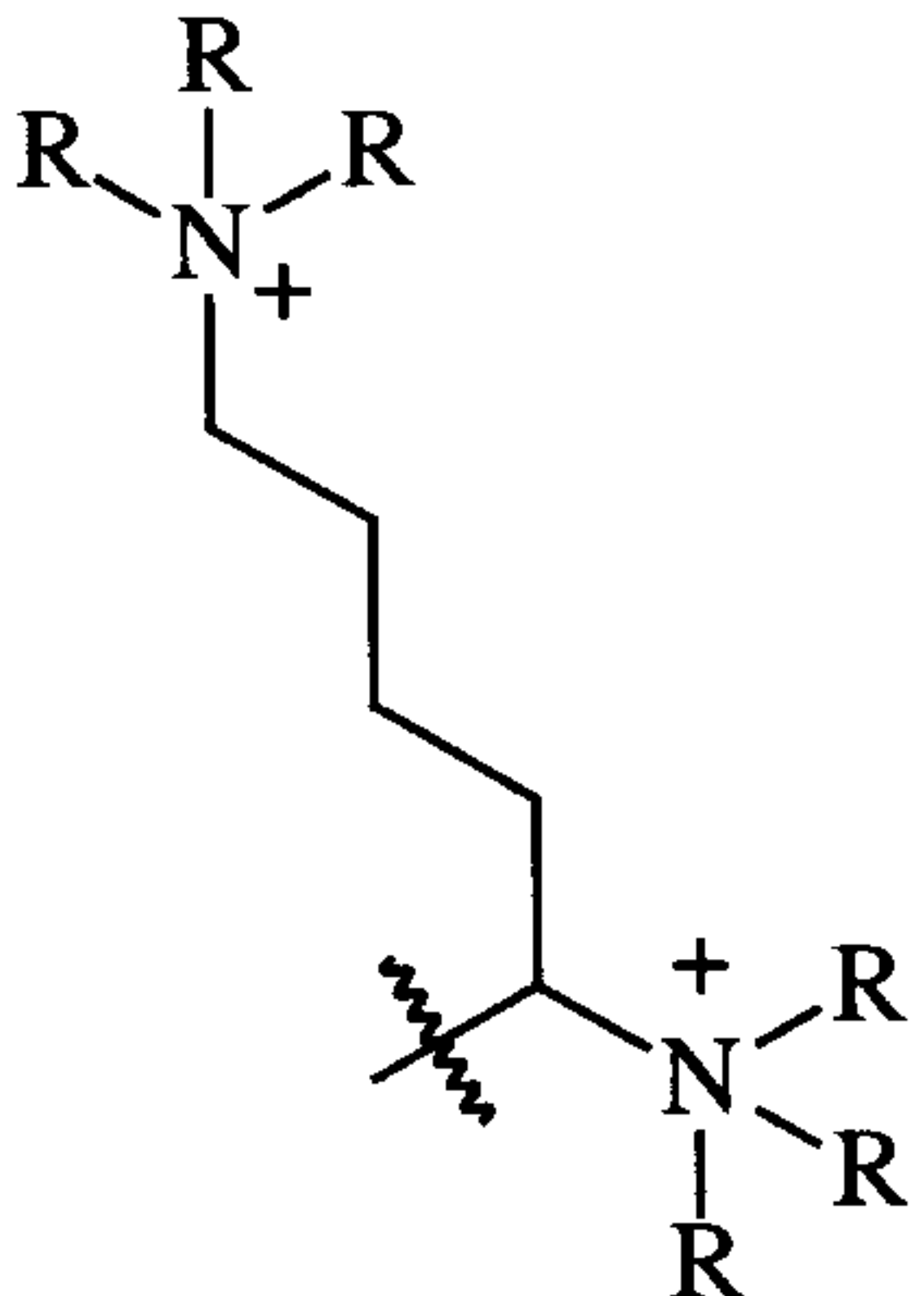
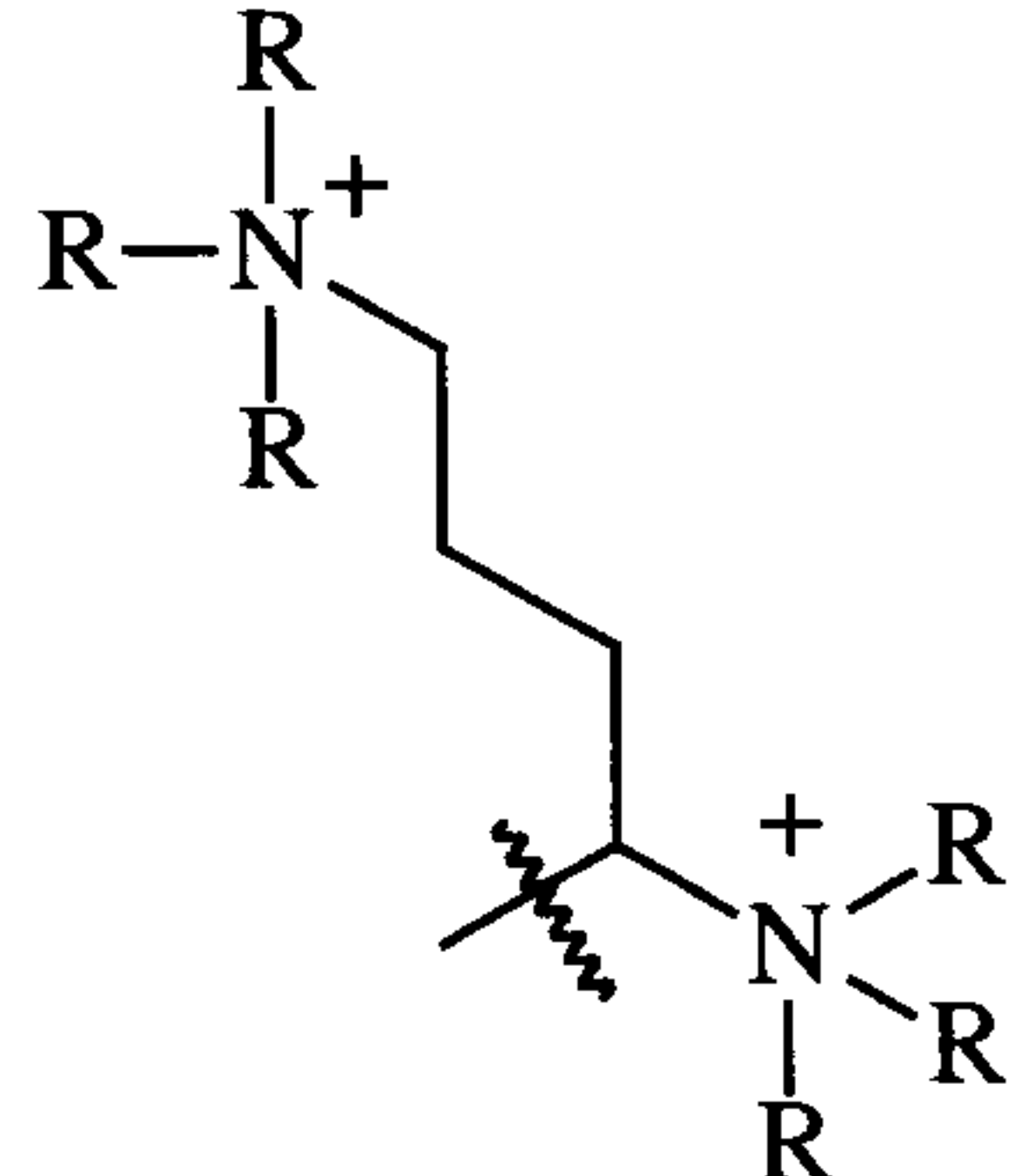
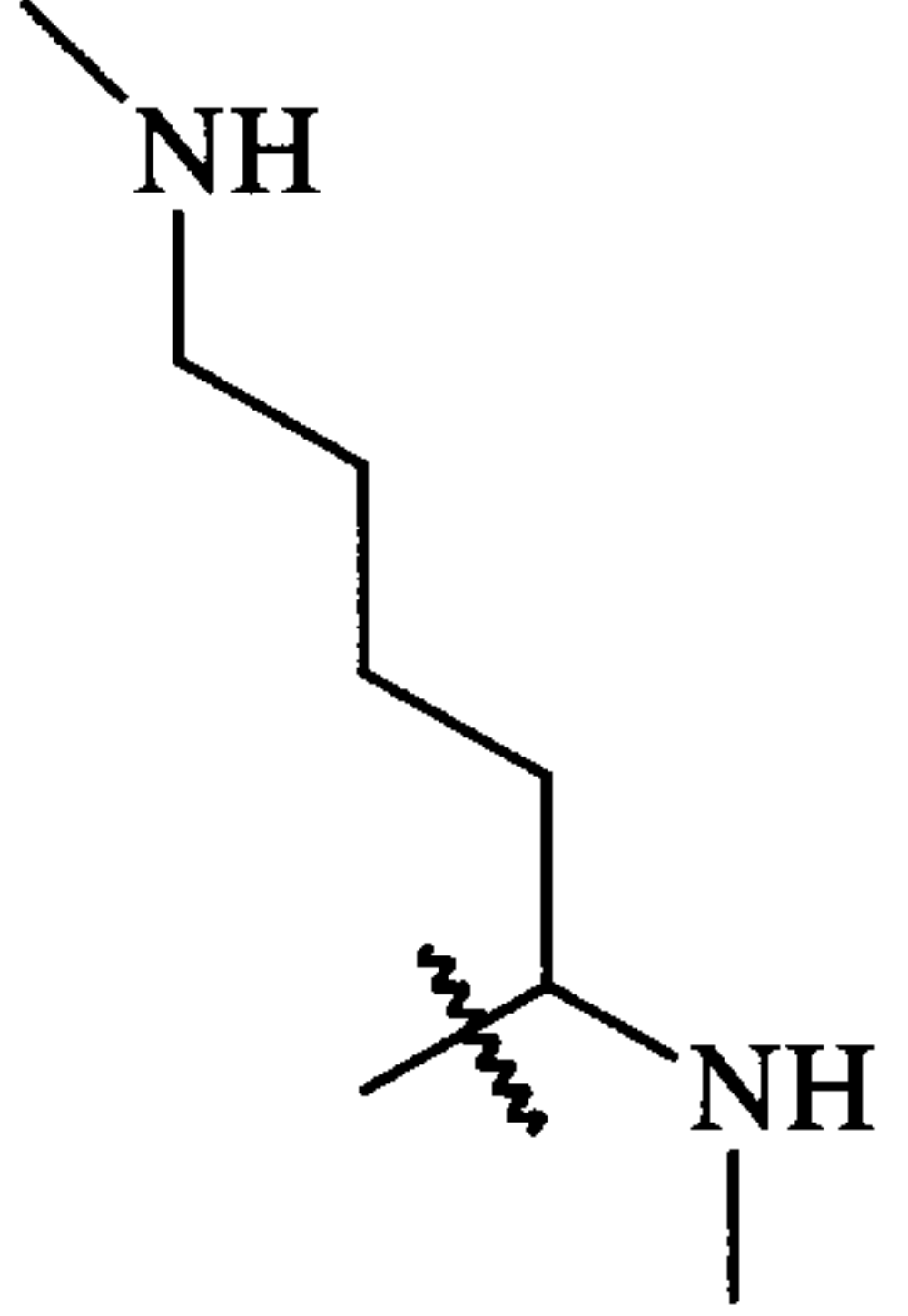
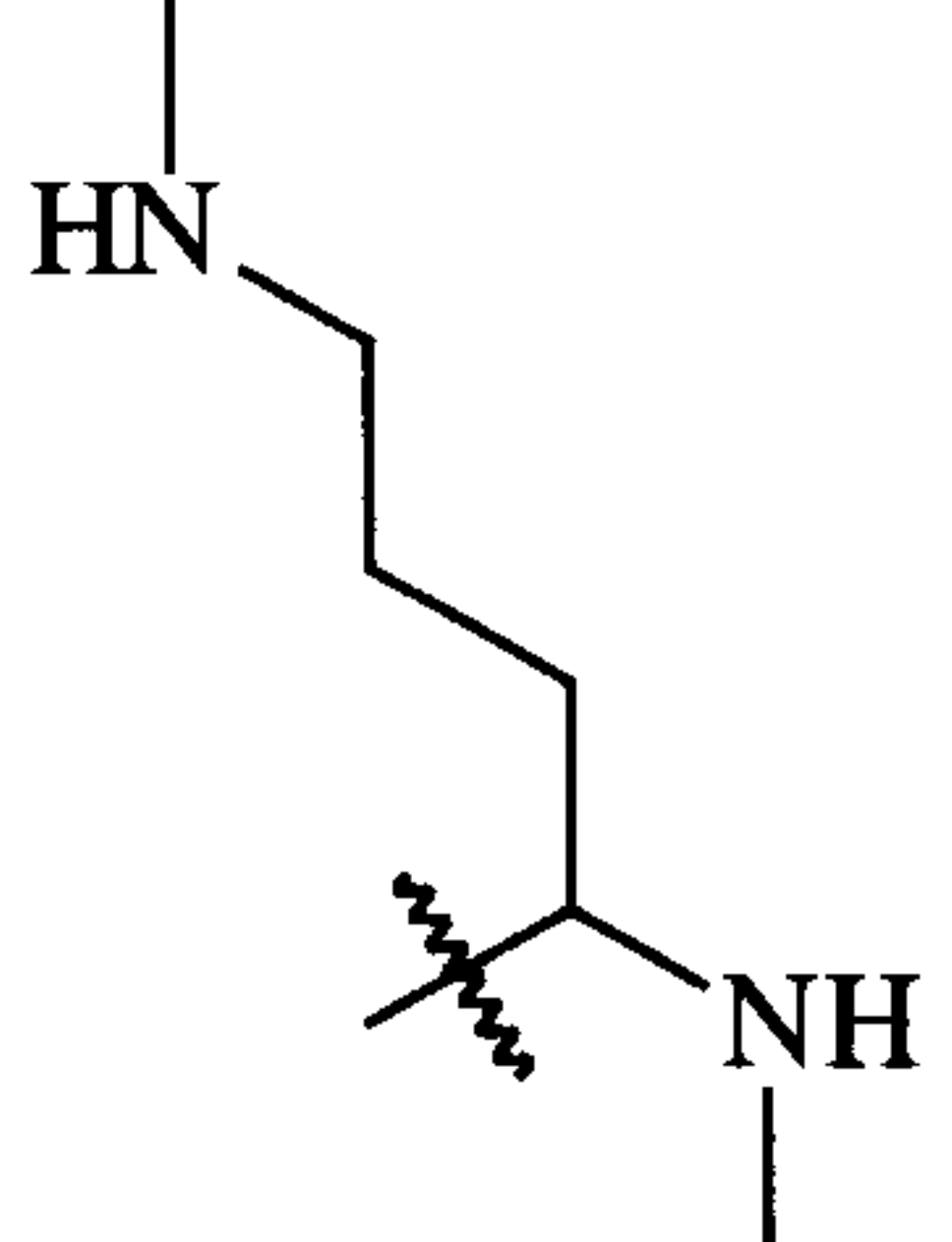
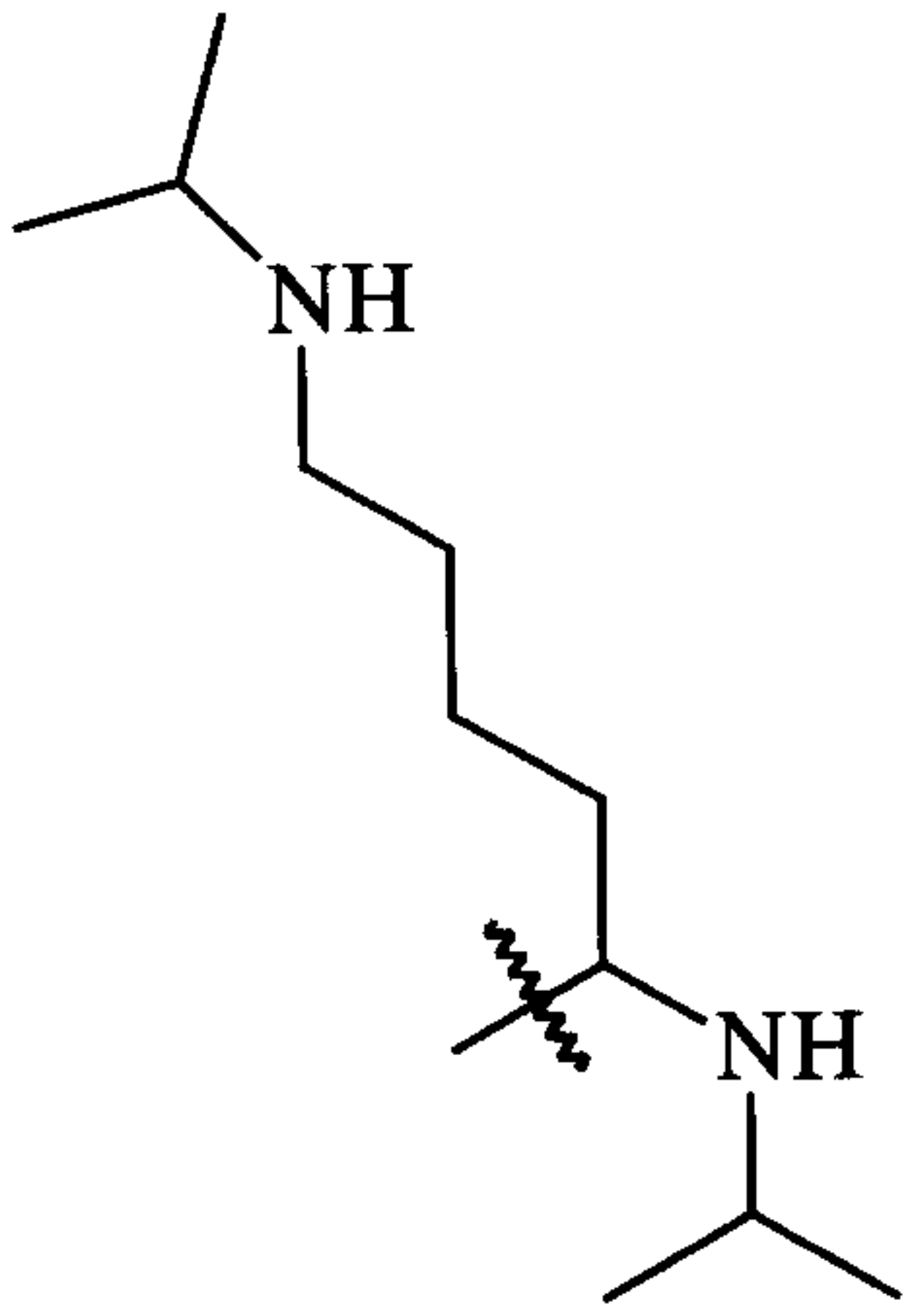
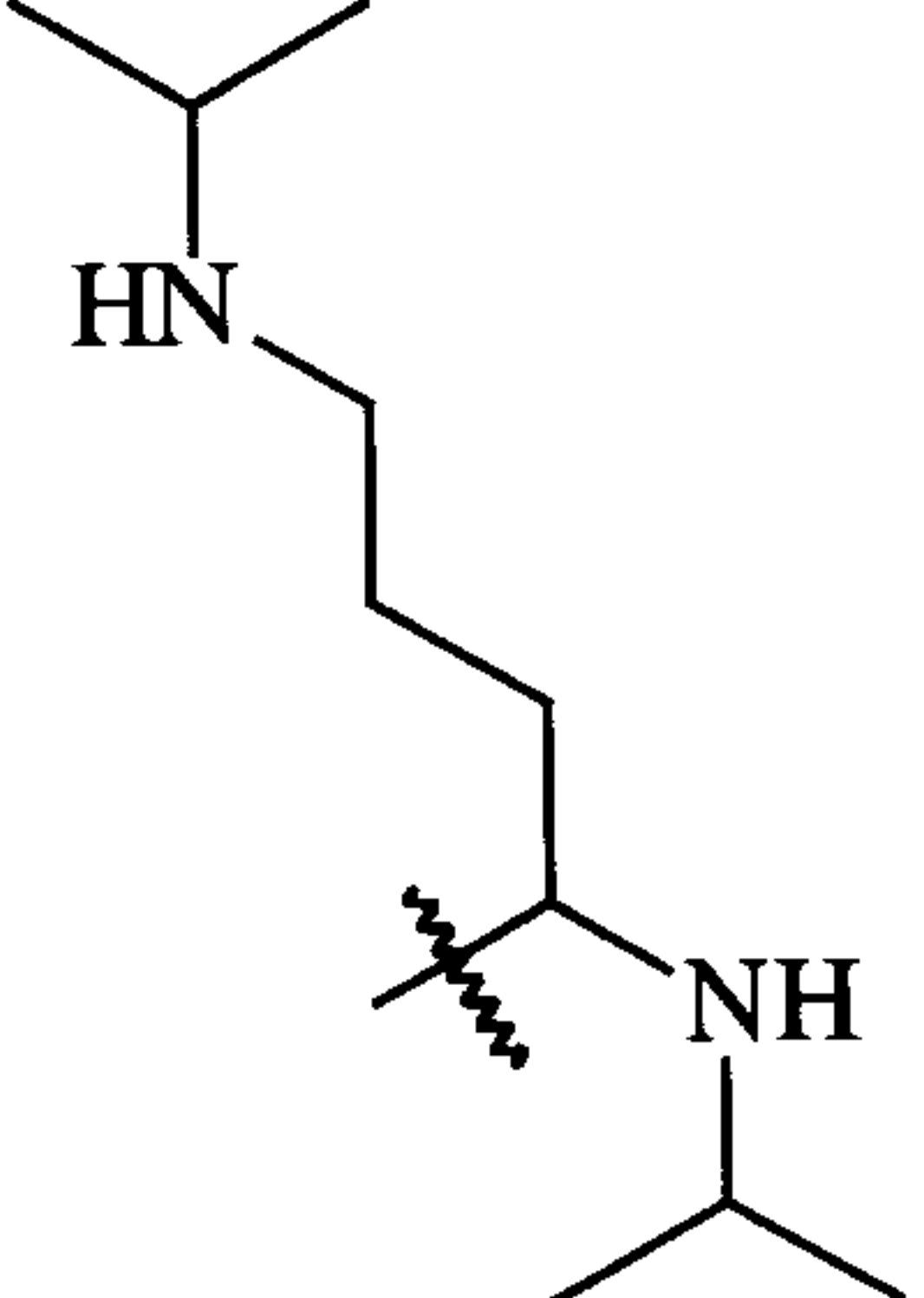
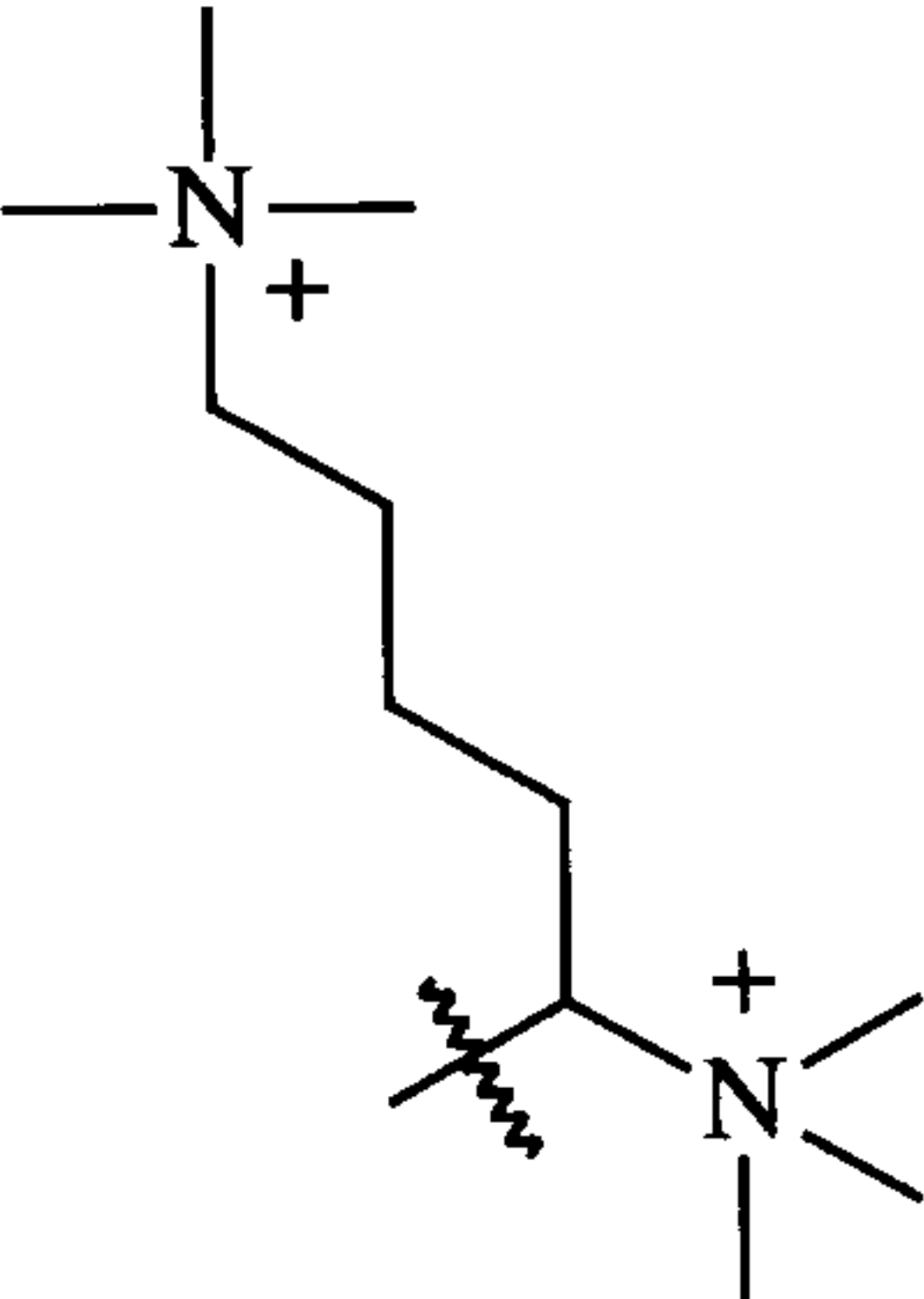
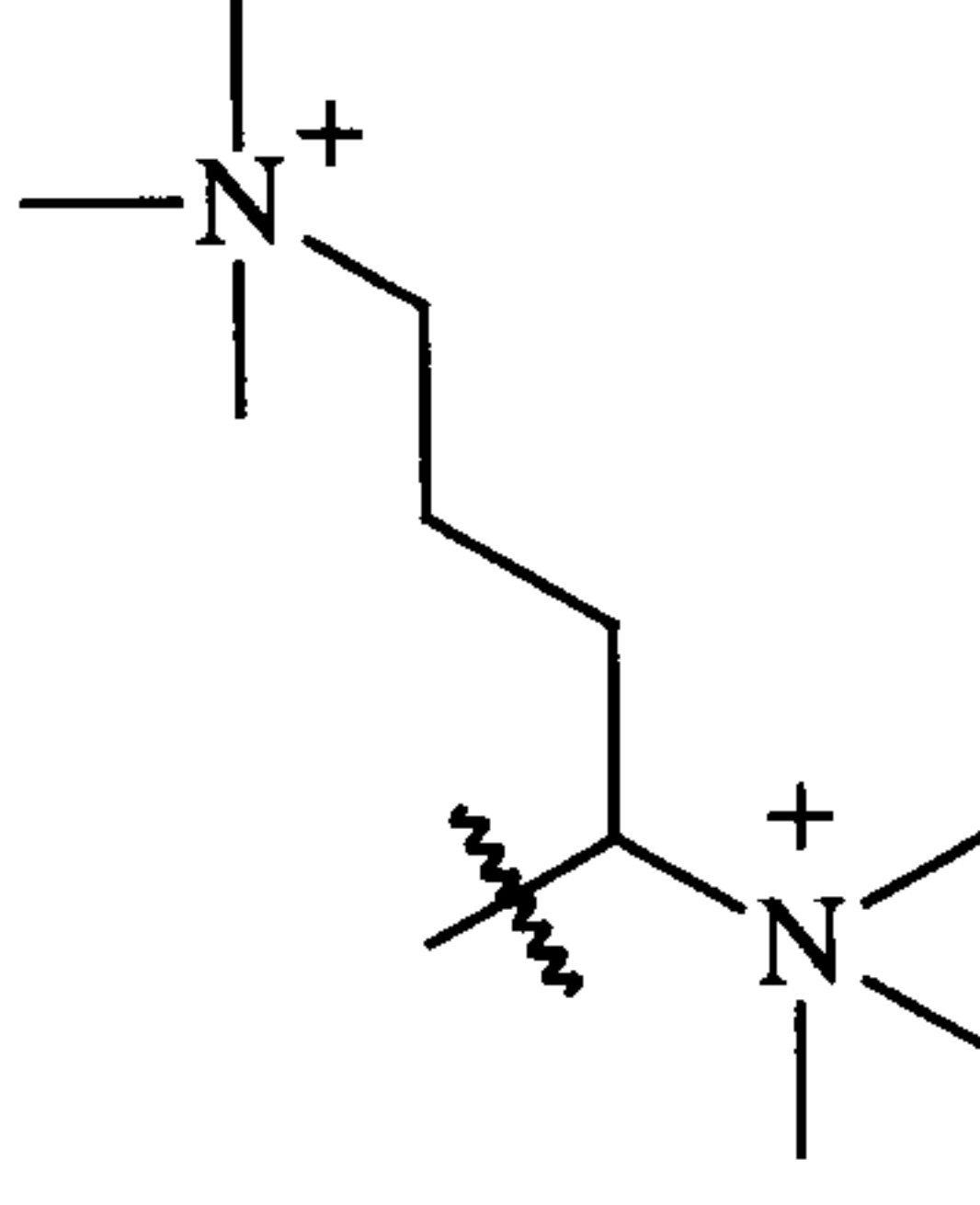
	
	
 Wherein each R is independent H or C₁ to C₁₀ alkyl	 Wherein each R is independent H or C₁ to C₁₀ alkyl
	

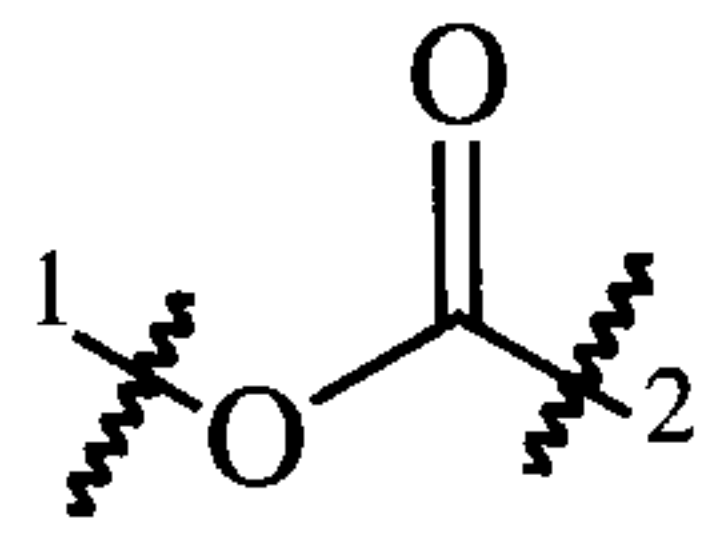
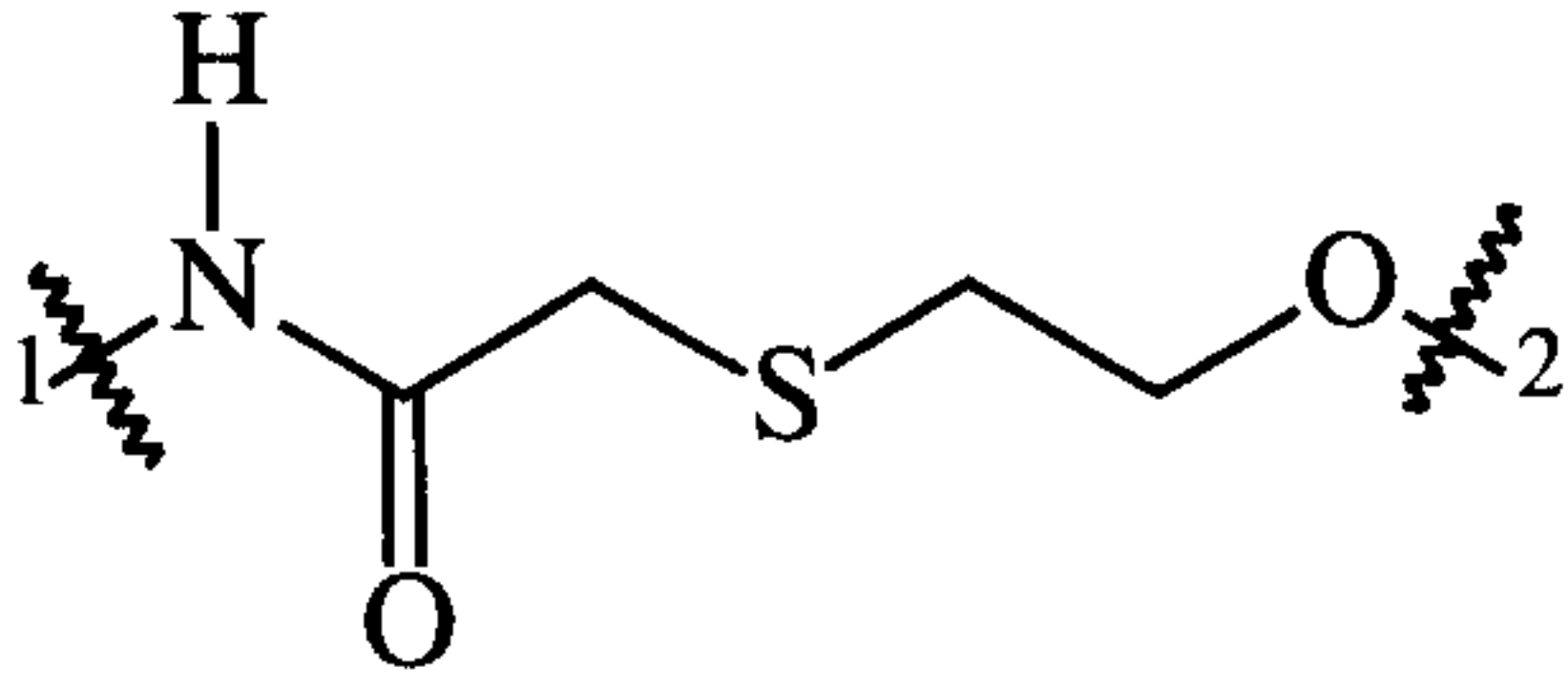
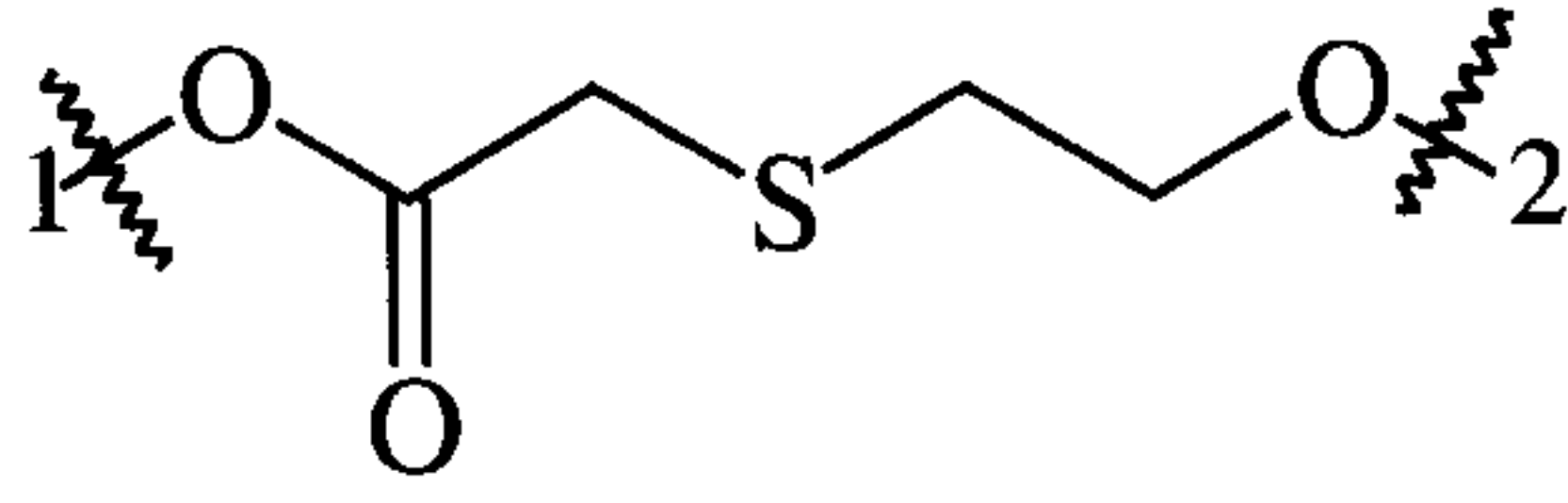
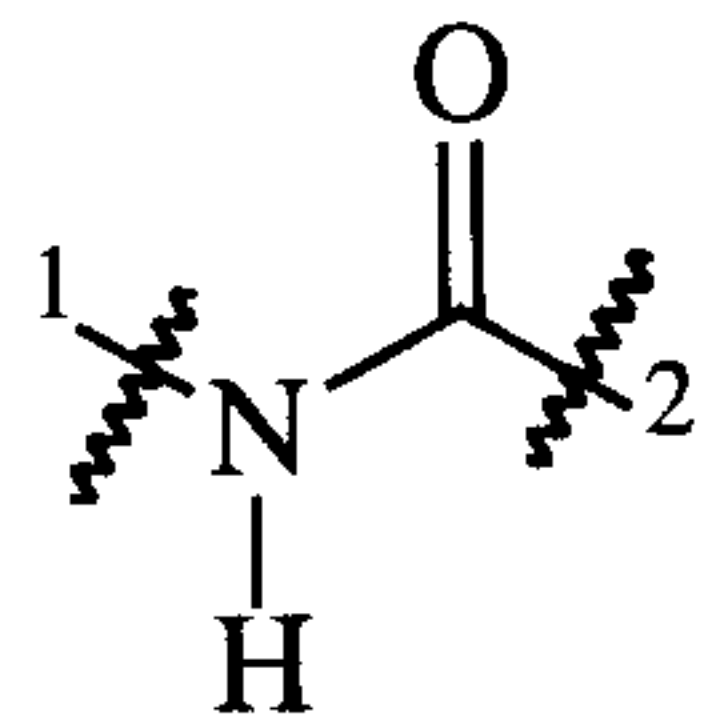
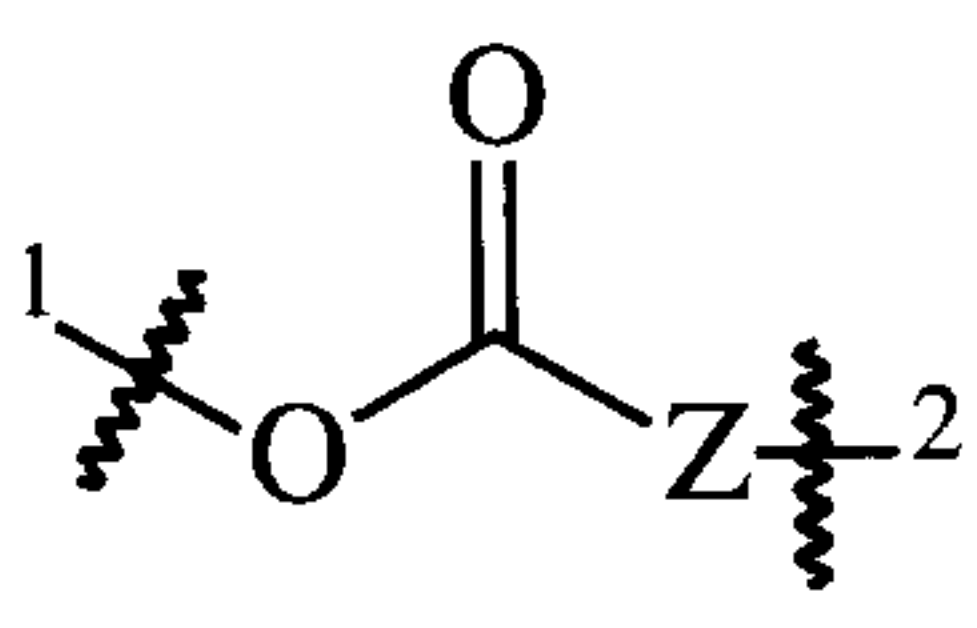
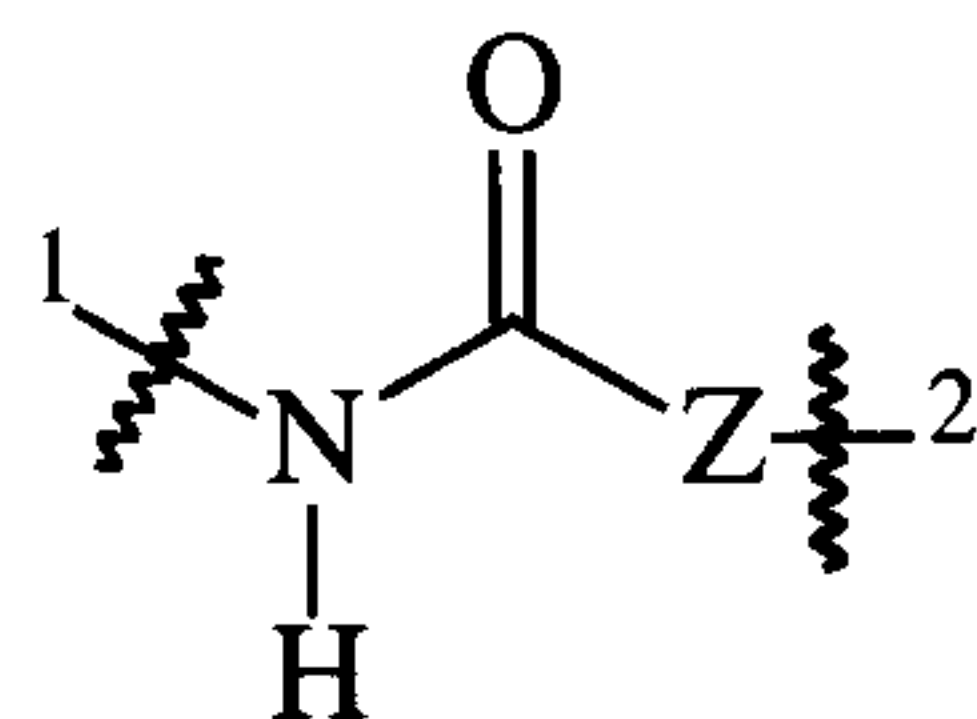
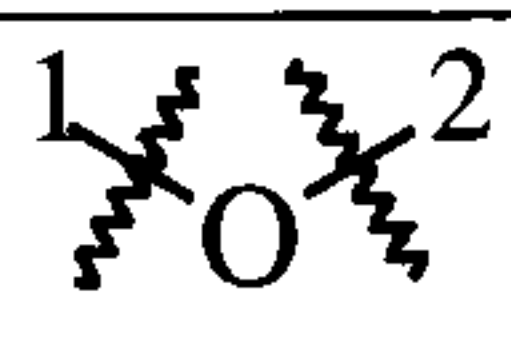
TABLE I - SOLUBILIZING MOIETIES	
	
	

In some particular embodiments, each R as set out in Table I may be independently selected from H, methyl or acyl.

- 5 Linking moieties may connect the core to a solubilizing moiety. A linking moiety is a moiety that is cleaved *in vivo* such that a compound of the core is produced via cleavage of the linking moiety from the core. In some embodiments, cleavage of the linking moiety may be related to the stability of the linking moiety under physiological conditions. In some embodiments, the linking moiety may be cleaved *in vivo* enzymatically. In some embodiments, cleavage of the
- 10 linking moiety in vivo results in the formation of a core comprising an OH moiety where the linking moiety was bonded to the core prior to cleavage. Linking moieties comprising an ester moiety may provide formation of a core comprising an OH moiety where the ester linking moiety was bonded to the core prior to cleavage. A Linking moiety may be selected from the following moieties: -O-C(=O)-Z-, -NH-C(=O)-Z-, -CH₂OC(=O)-, -C(=O)O-, and -C(=O)HN-;
- 15 where Z is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or is substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂,

-NHR', -NR'₂, NO₂, -CO₂H, -CO₂R', and epoxide and individual carbon atoms may be replaced by S, O, N, NR', or NR'₂ atoms; and wherein each R' is independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or is substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR'', -NR''₂, NO₂,
 5 -CO₂H, -CO₂R'', and epoxide; and R'' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group. In the case of phosphates, -O- is also a suitable linking moiety. Specific non-limiting examples of linking moieties are described below In Table II, where 1 represents the point of attachment to the core and 2 represents the point of attachment to a solubilizing moiety:

10

TABLE II - LINKING MOIETIES	
	
	
 Where Z is as defined above	 Where Z is as defined above
 Phosphates only	

The linking moiety and the solubilizing moiety may also be described as a single structure, termed a prodrug moiety or X₅. The prodrug moiety comprises all that is added to the core such that a compound of this invention is formed. Any combination of any linking moiety as
 15 described herein bonded to any solubilizing moiety as described herein may comprise a

prodrug moiety. In some embodiments, a prodrug moiety is stable and difficult to remove from the core. In some embodiments, prodrug moieties may be moieties that may be cleaved *in vivo* such that a compound of the core is produced via cleavage at the linking moiety thereby separating the prodrug moiety or the solubilizing moiety from the core. In some embodiments, the linking moiety may be cleaved enzymatically. In some embodiments, *in vivo* cleavage of the linking moiety to separate the prodrug moiety or solubilizing moiety from the core results in the formation of a core comprising an OH moiety where the prodrug moiety was bonded to the core prior to cleavage. Prodrug moieties comprising an ester moiety may provide formation of a core comprising an OH moiety where the ester prodrug moiety was bonded to the core prior to cleavage. Specific, non-limiting examples of prodrug moieties are described below in Tables III and IV. In the following Table III, each R is independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or is substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR', -NR'₂, NO₂, -CO₂H, -CO₂R', and epoxide;

and R' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR'', -NR''₂, NO₂ and -CO₂H where R'' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group.

TABLE III - PRODRUG MOIETIES

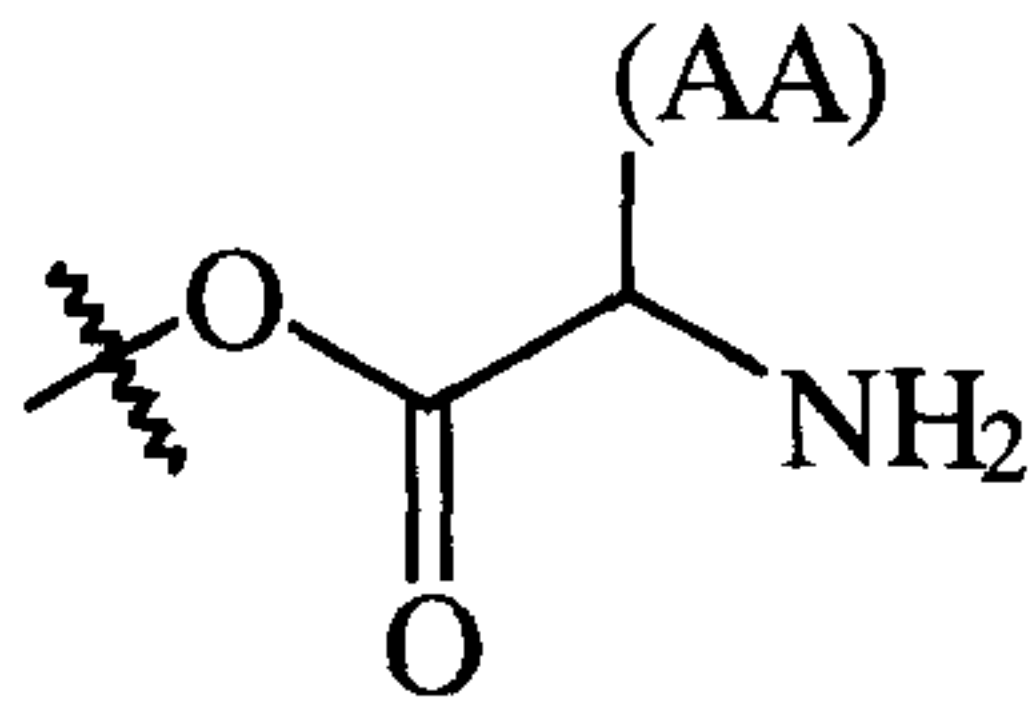
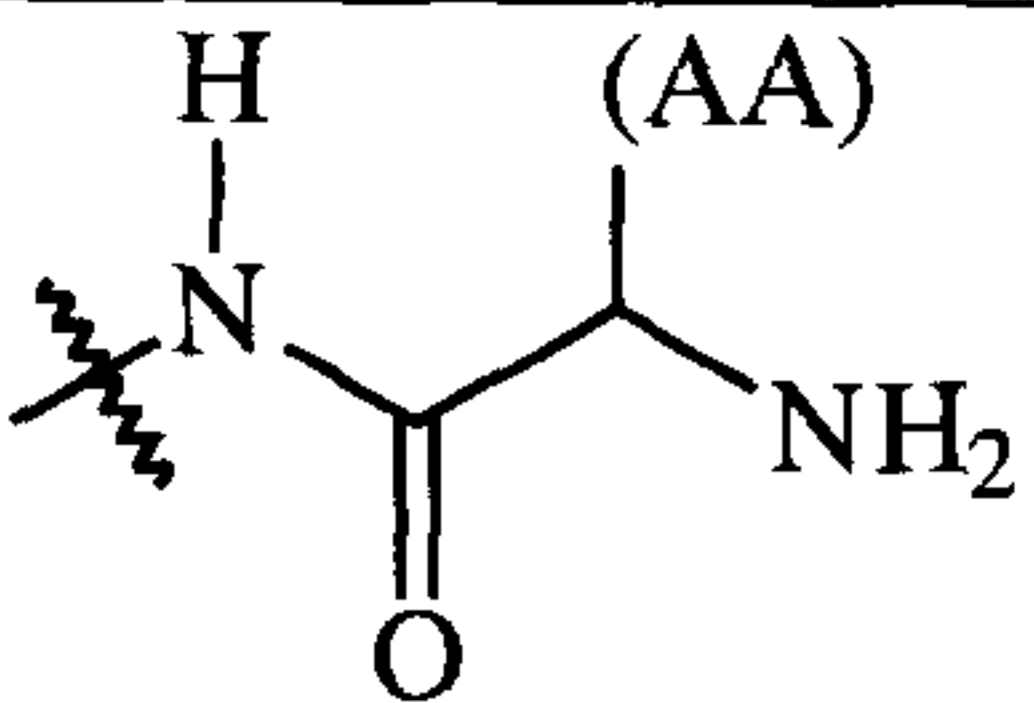
 Wherein (AA) is any amino acid side chain	 Wherein (AA) is any amino acid side chain
--	--

TABLE III – PRODRUG MOIETIES

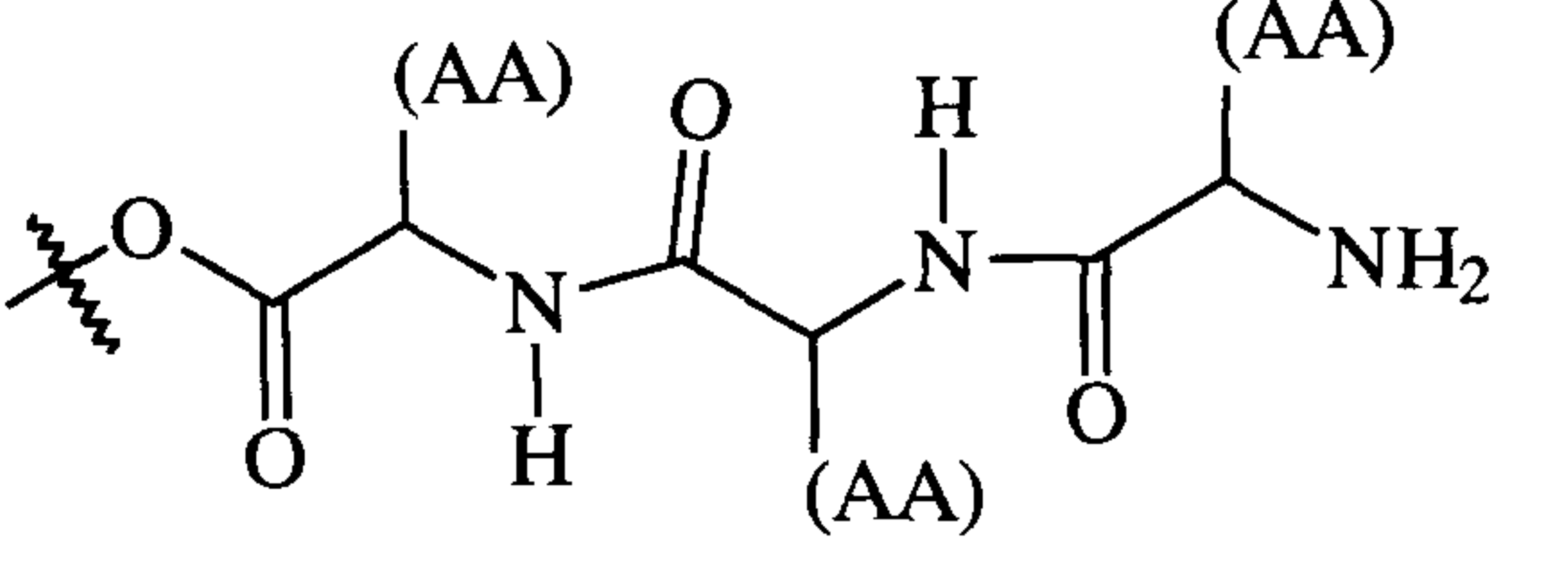
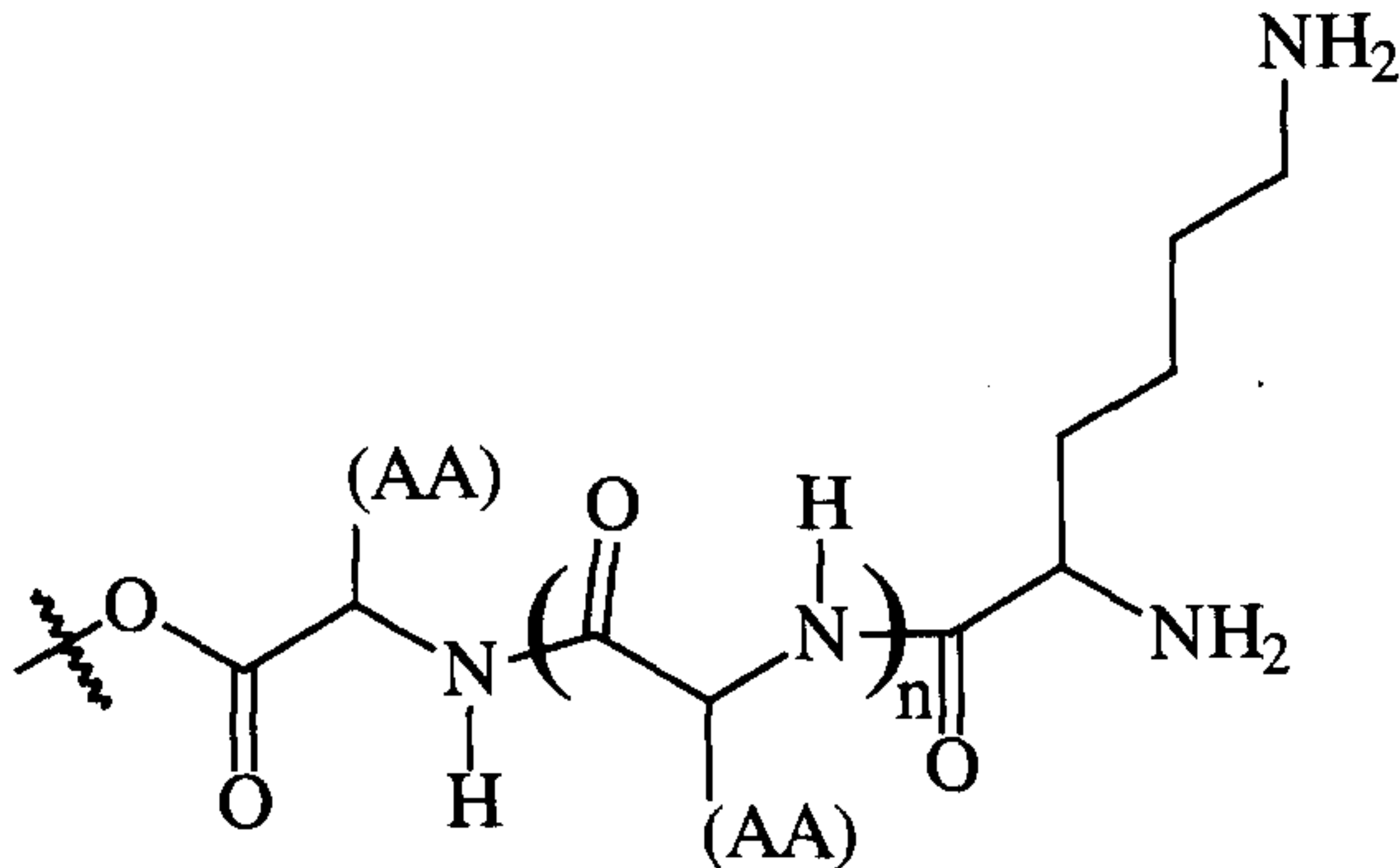
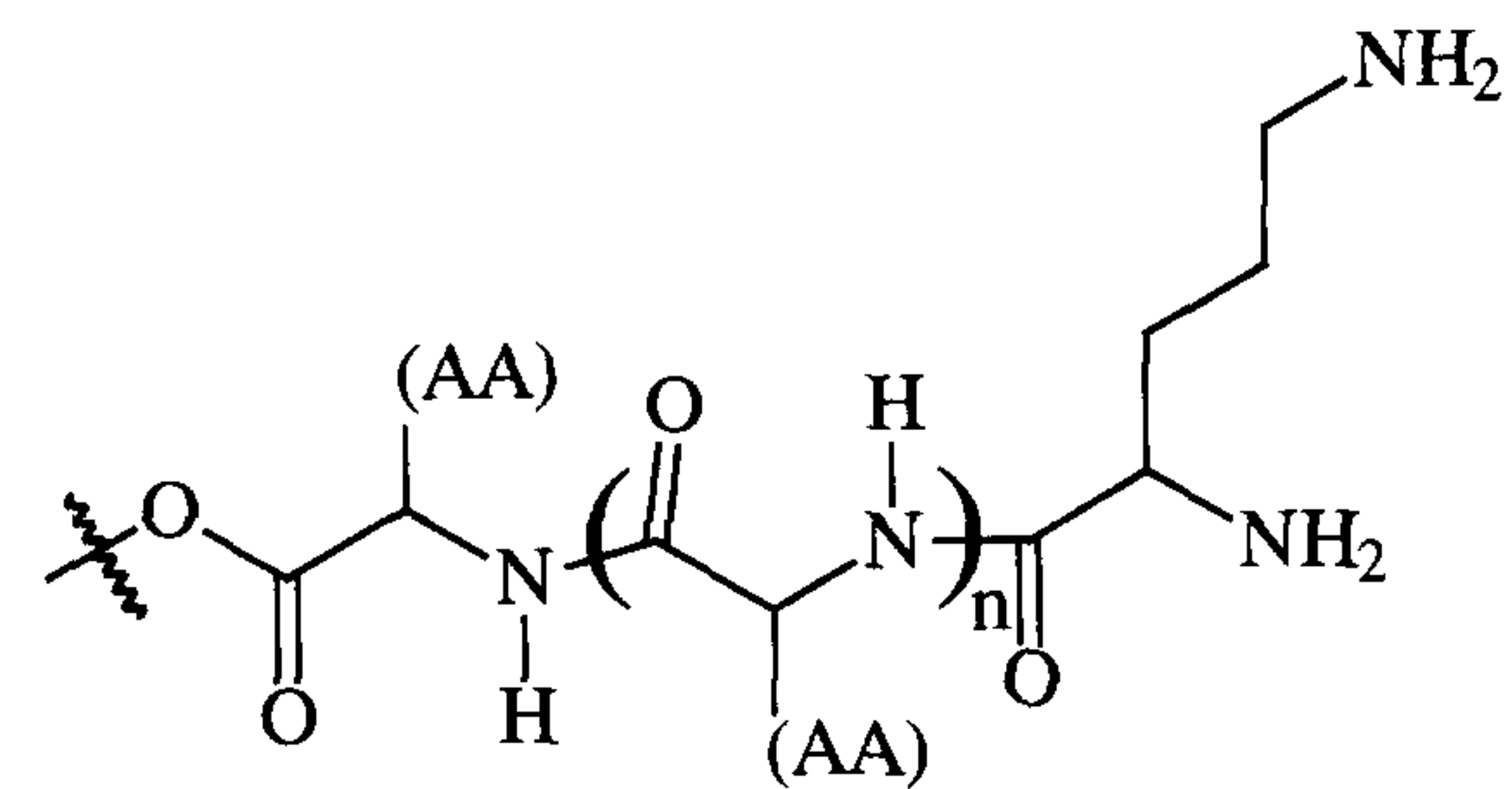
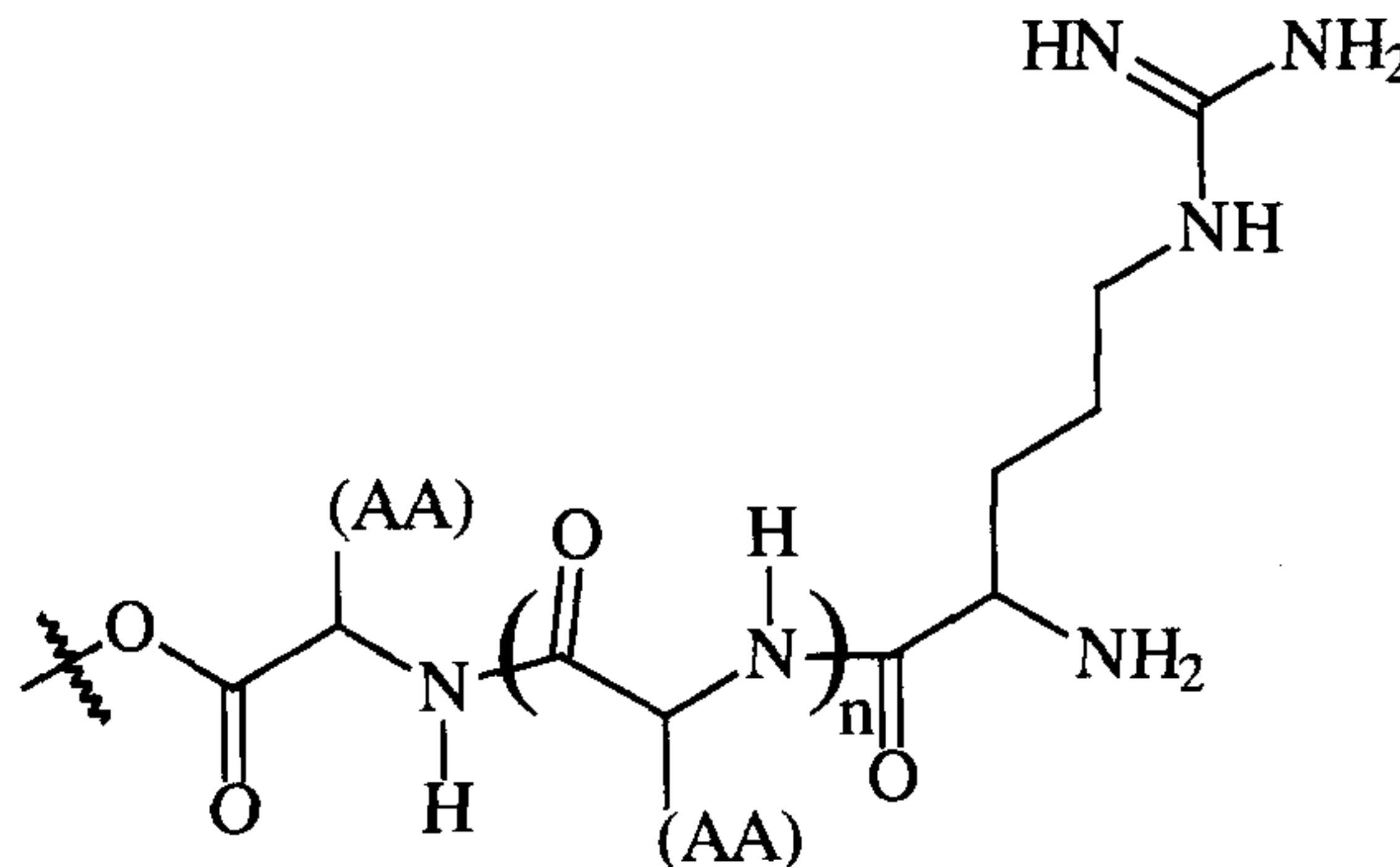
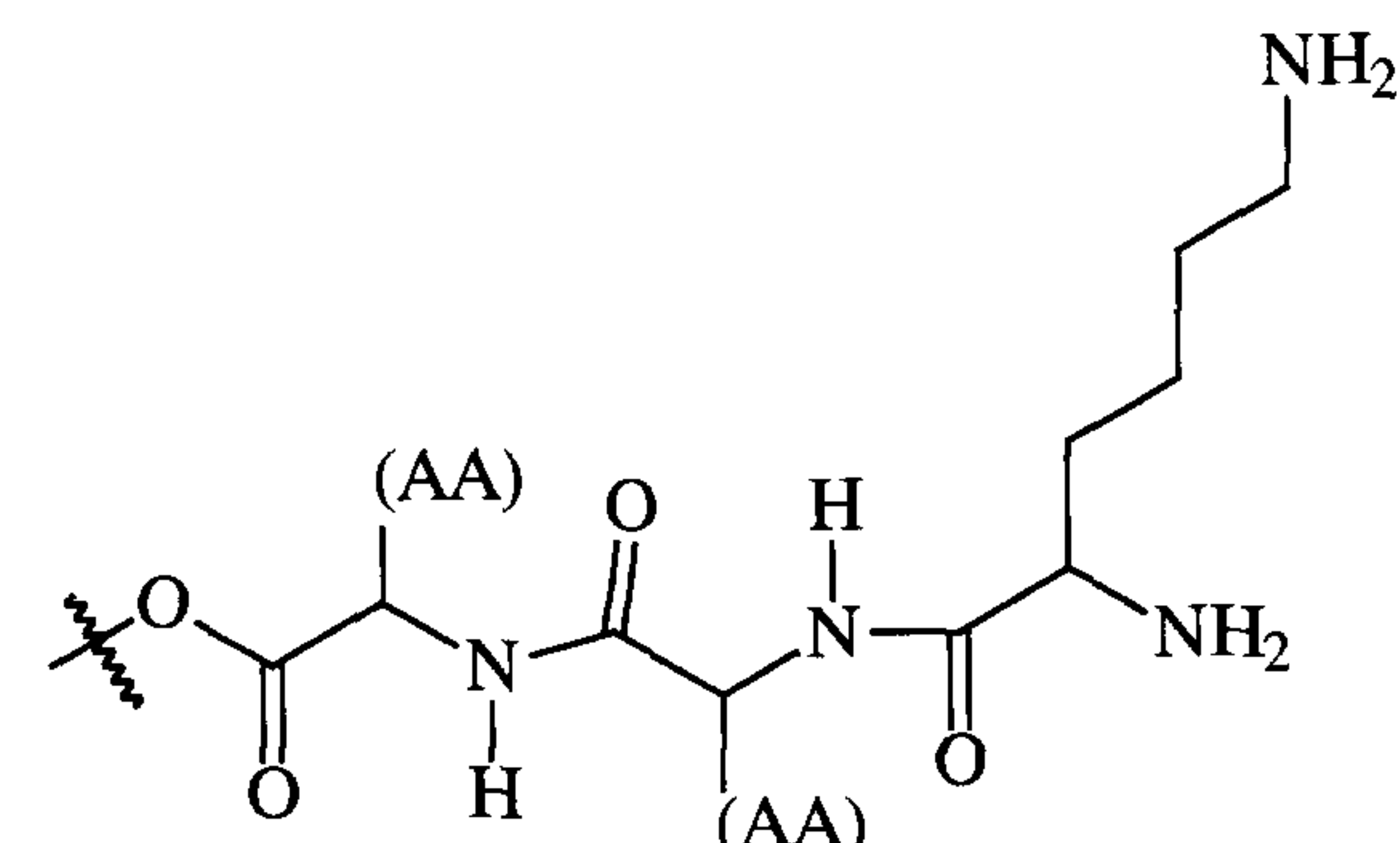
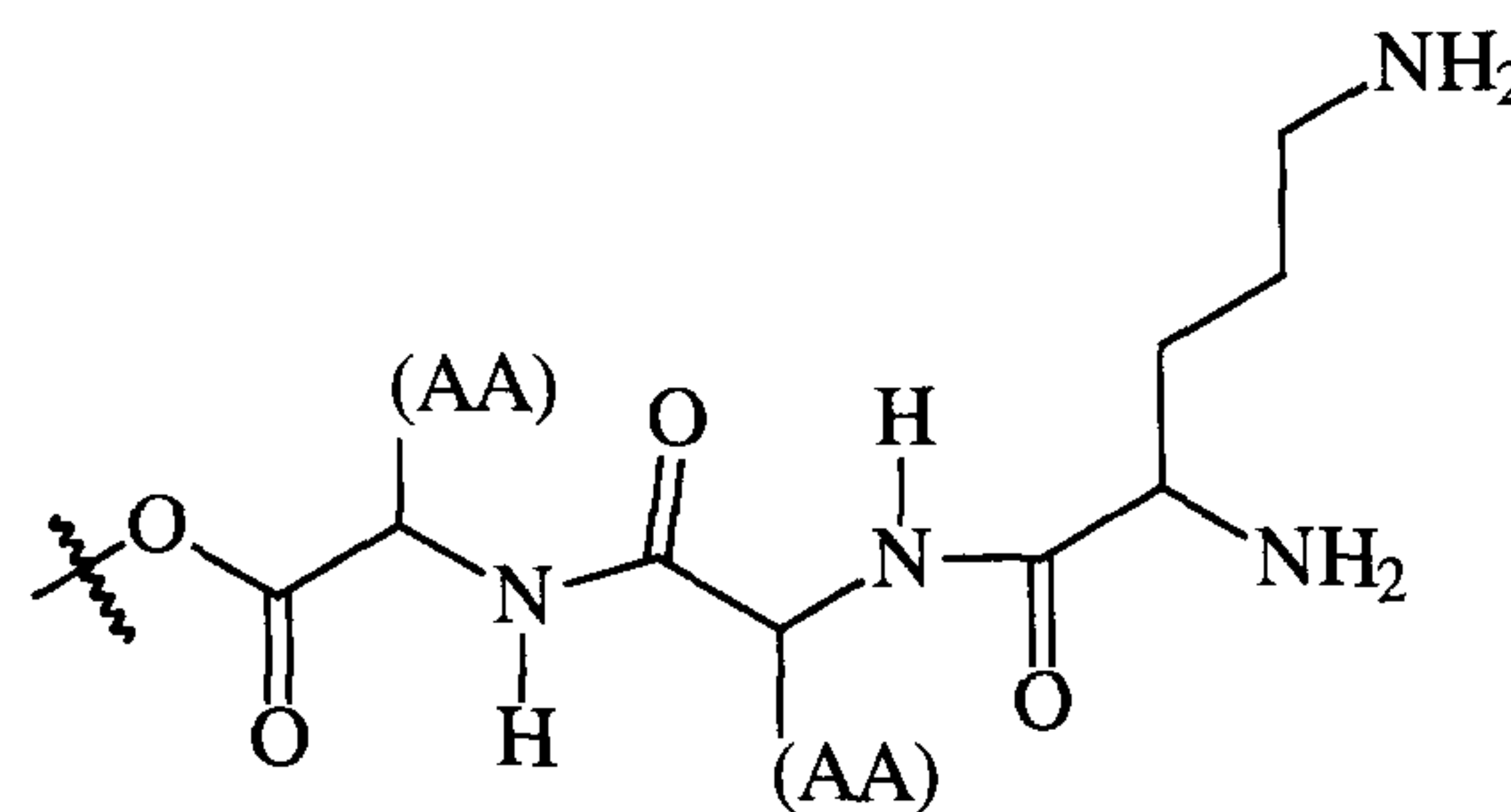
 Wherein each (AA) is independently any amino acid side chain	 Wherein each (AA) is independently any neutral amino acid side chain; and n is 1 to 10
 Wherein each (AA) is independently any neutral amino acid side chain; and n is 1 to 10	 Wherein each (AA) is independently any neutral amino acid side chain; and n is 1 to 10
 Wherein each (AA) is independently any neutral amino acid side chain	 Wherein each (AA) is independently any neutral amino acid side chain

TABLE III - PRODRUG MOIETIES

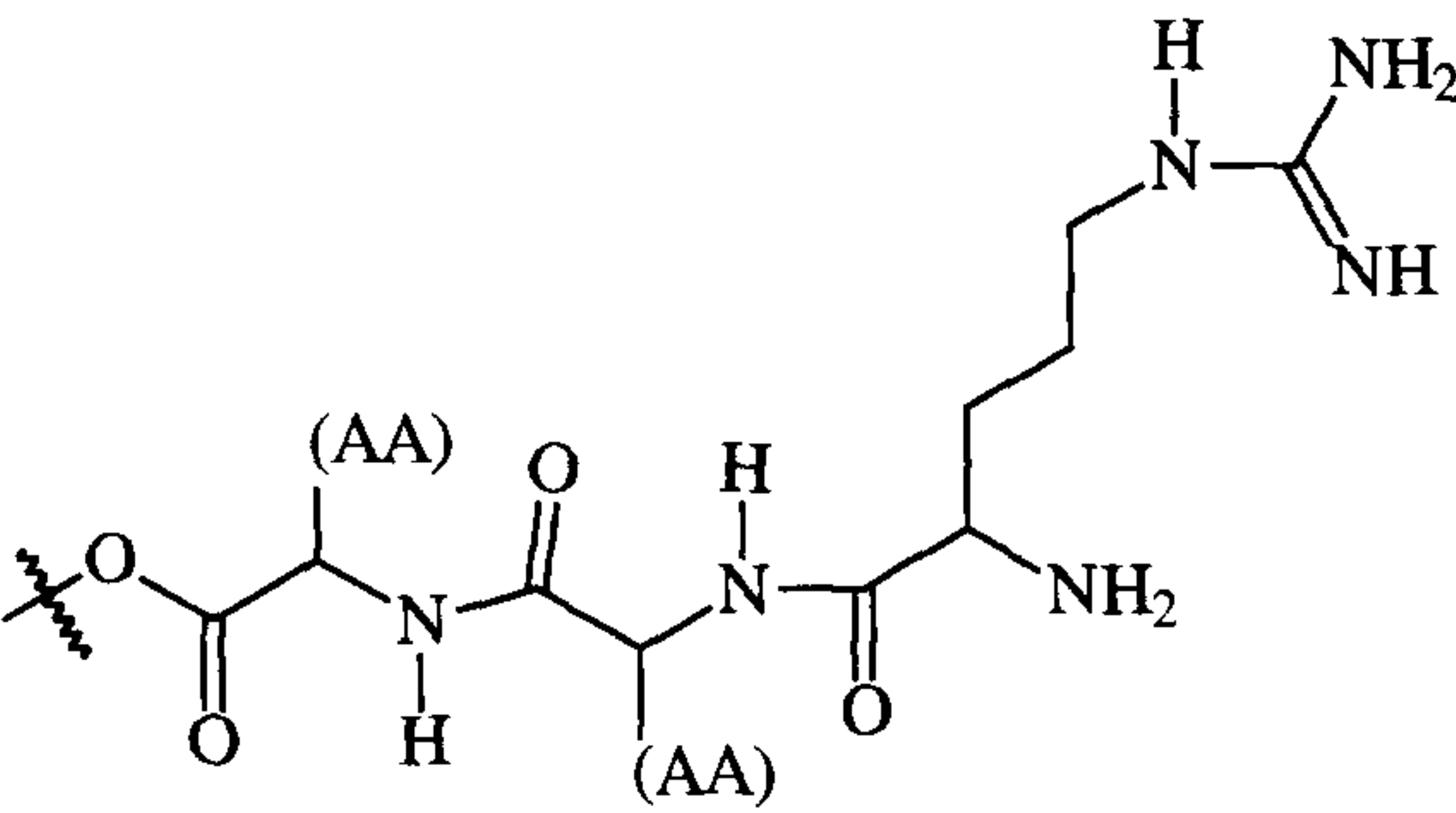
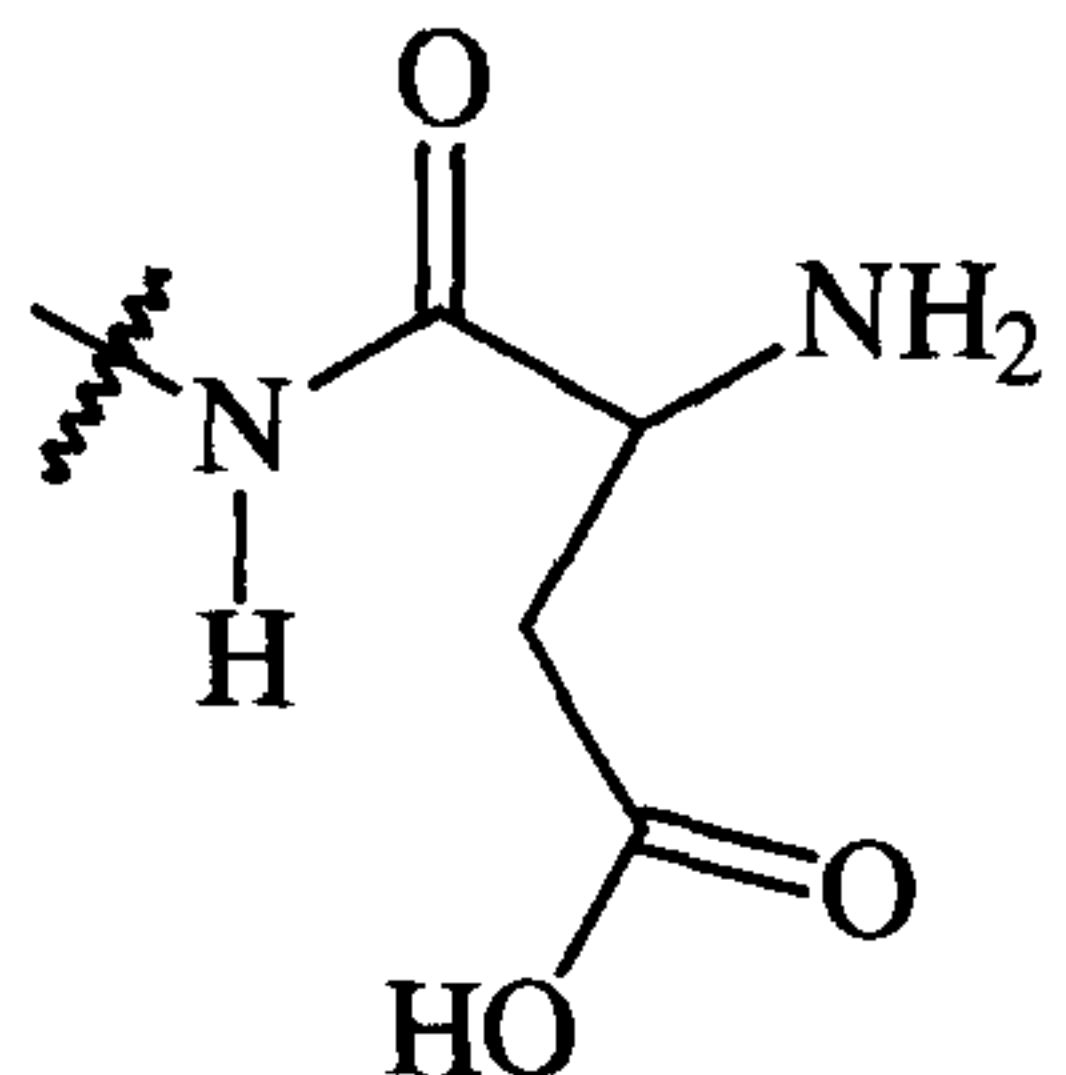
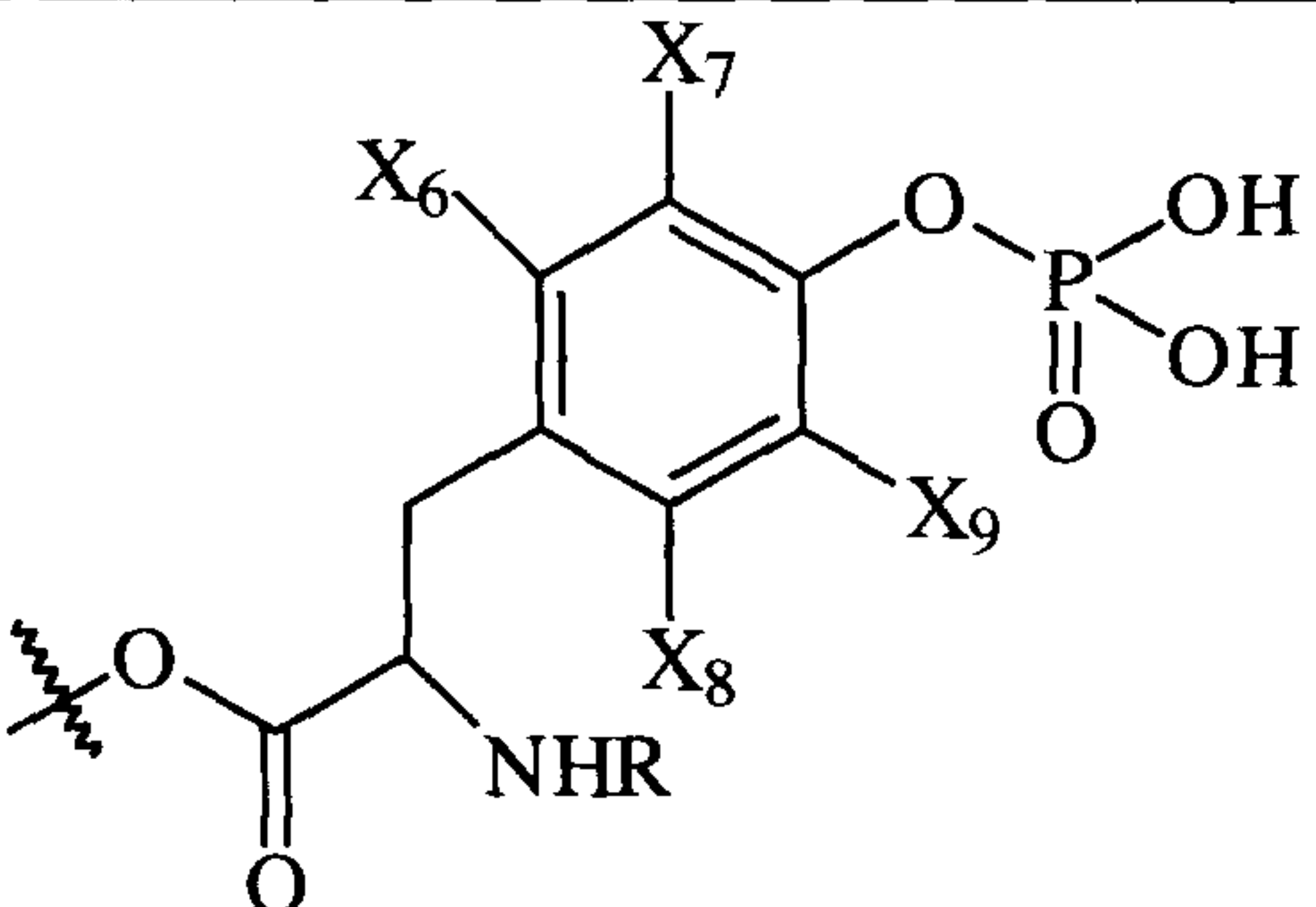
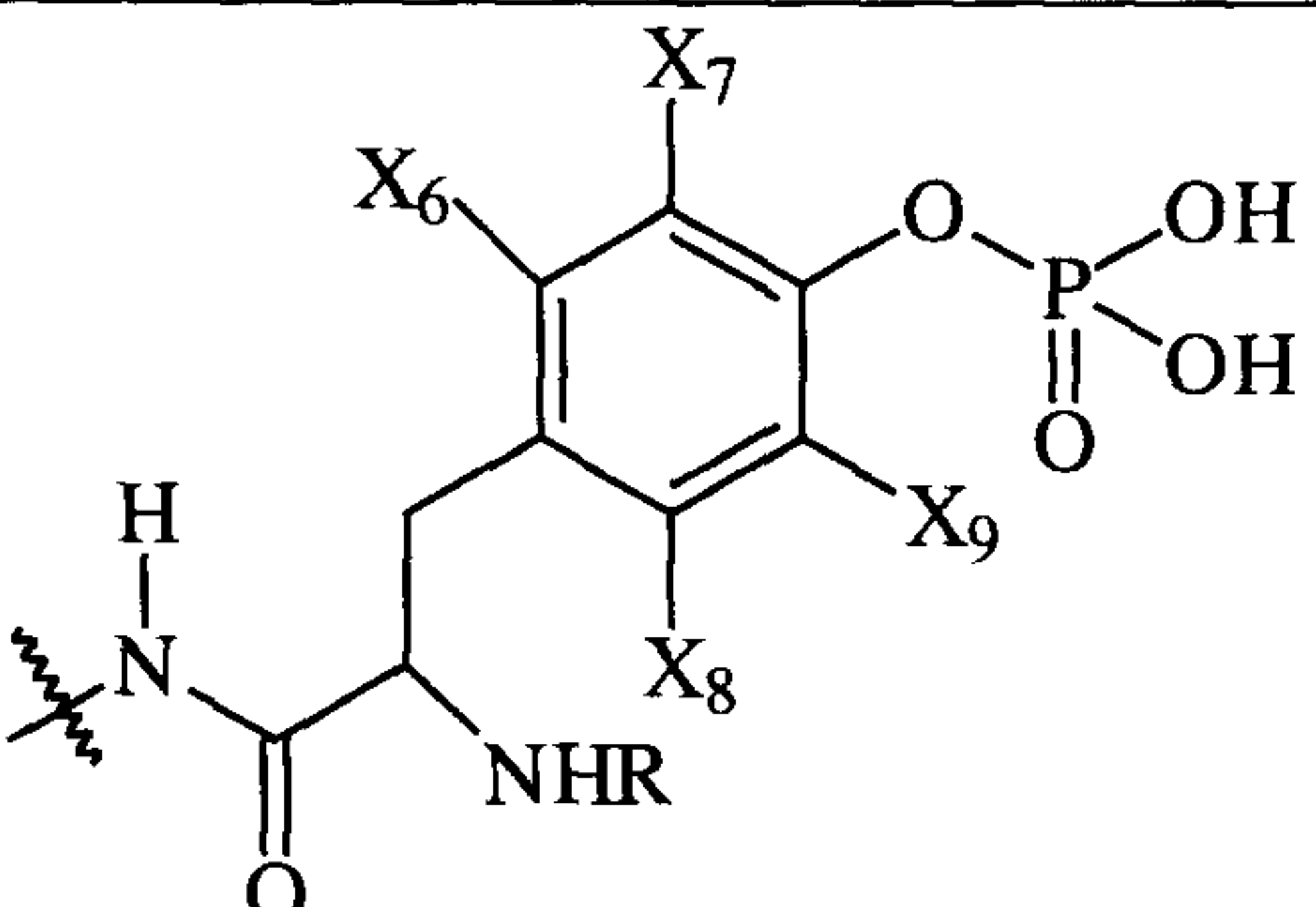
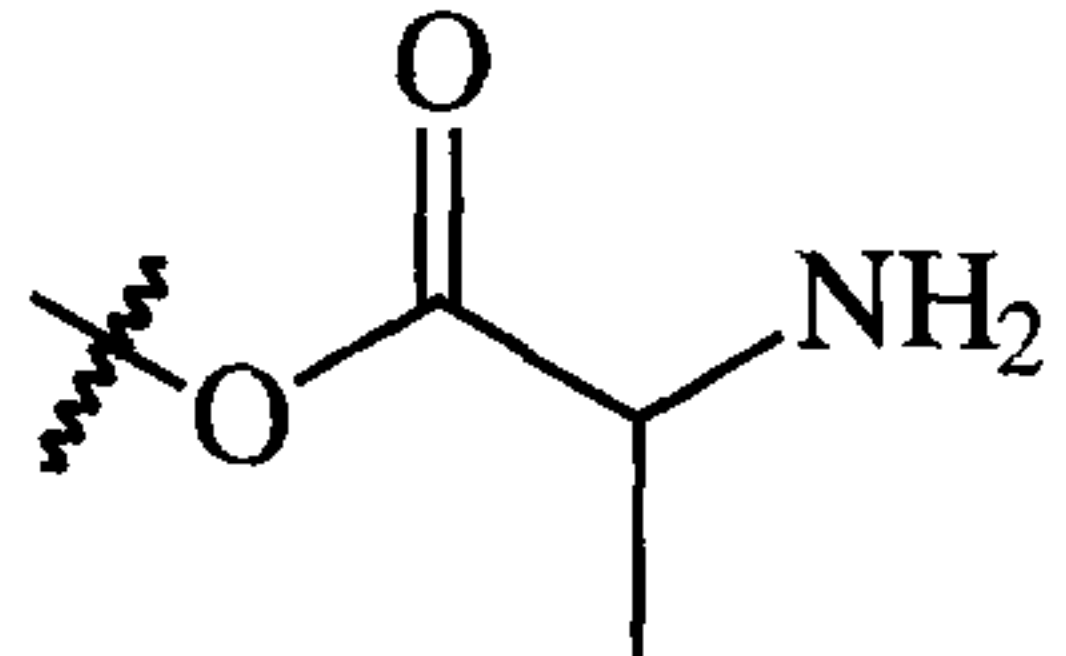
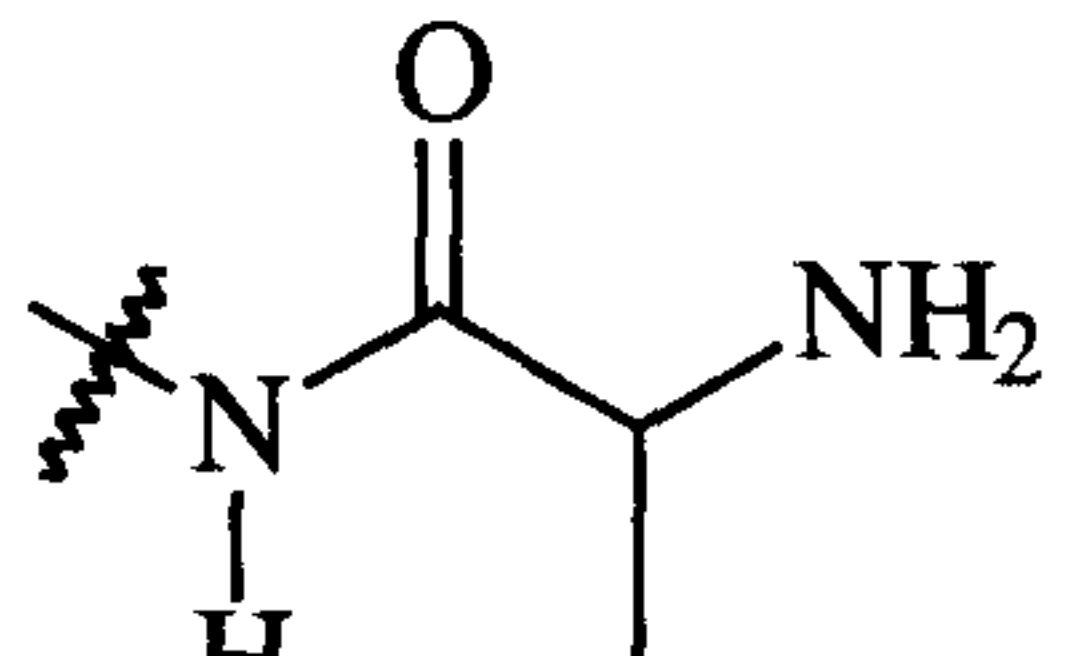
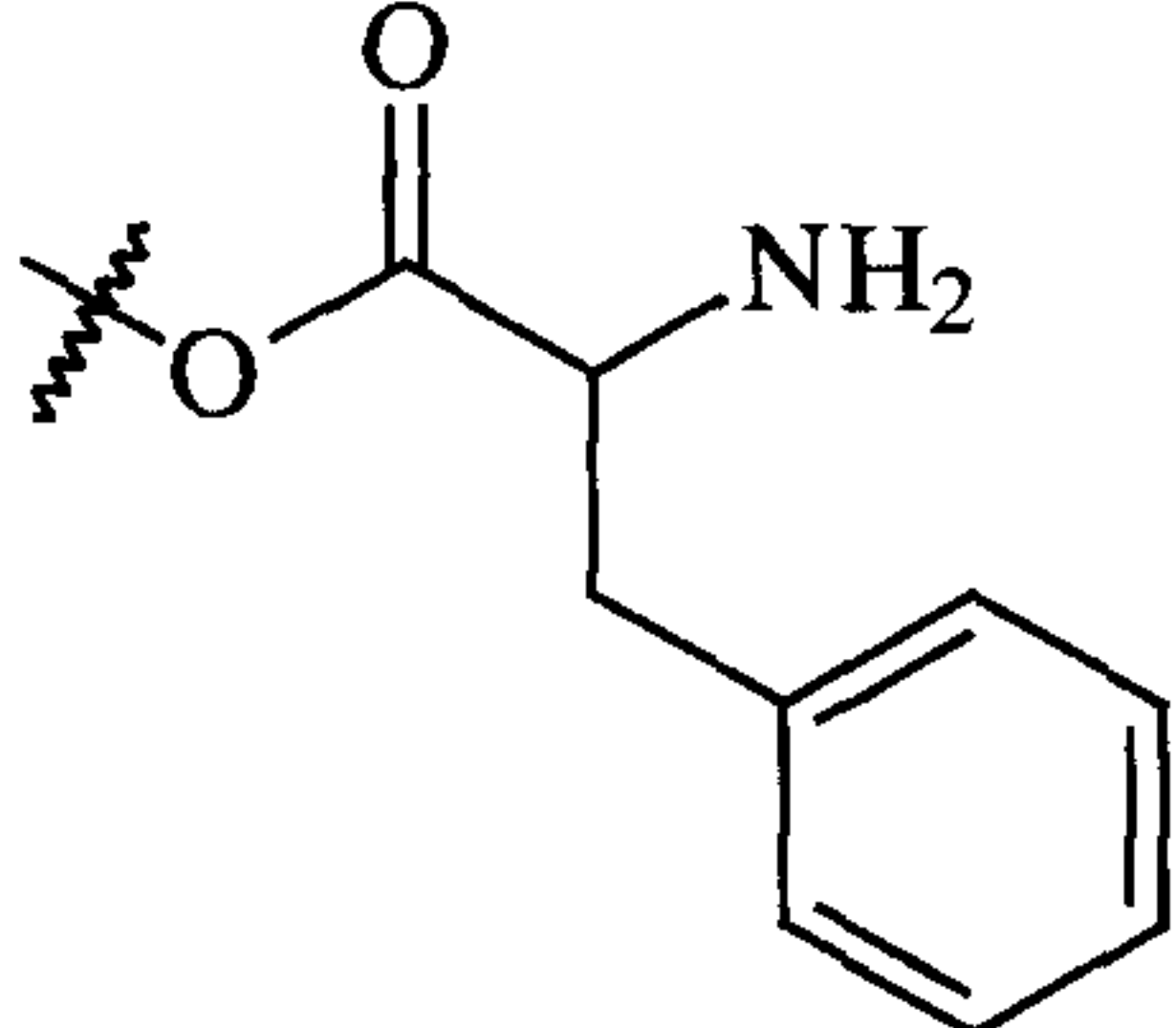
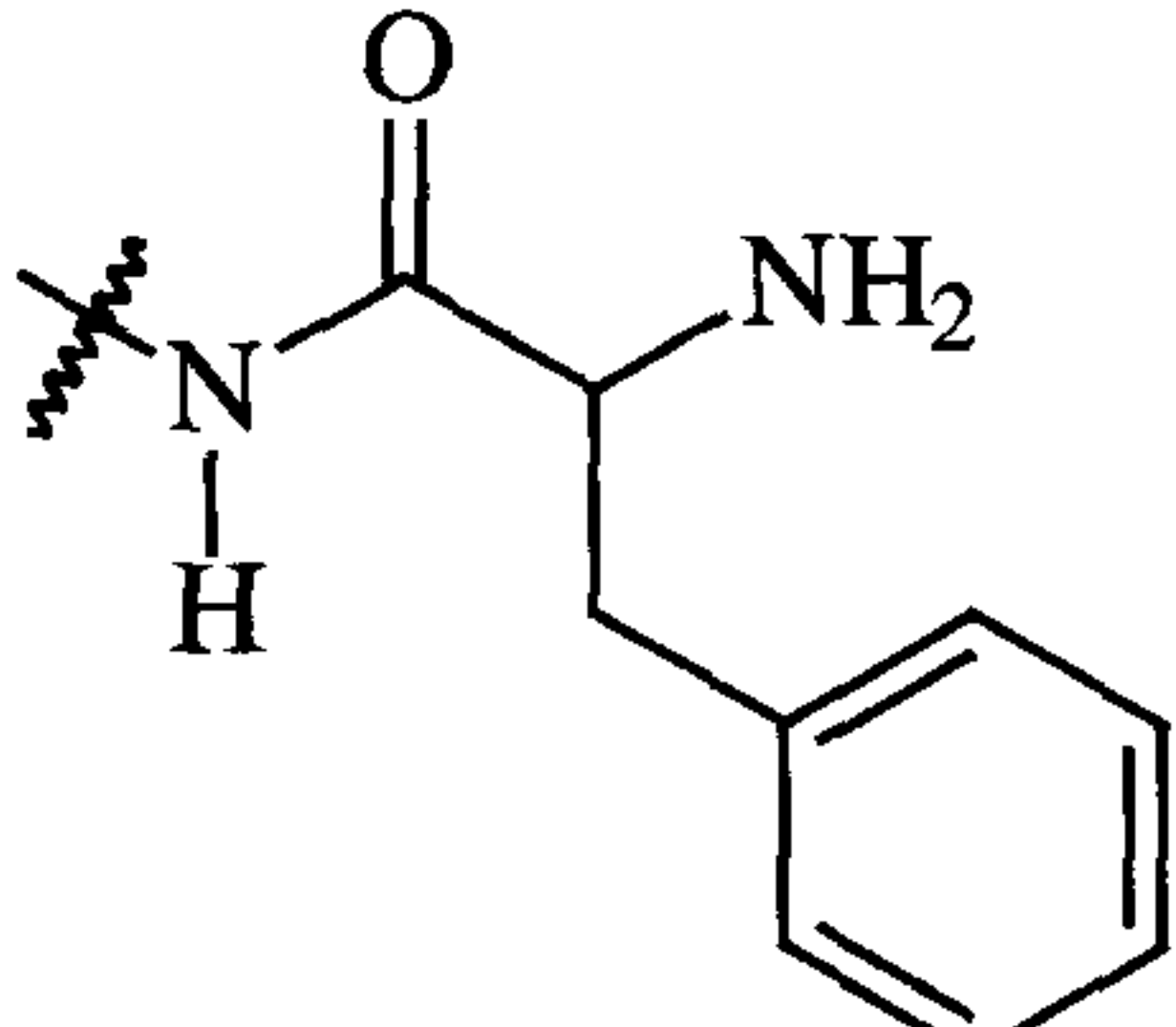
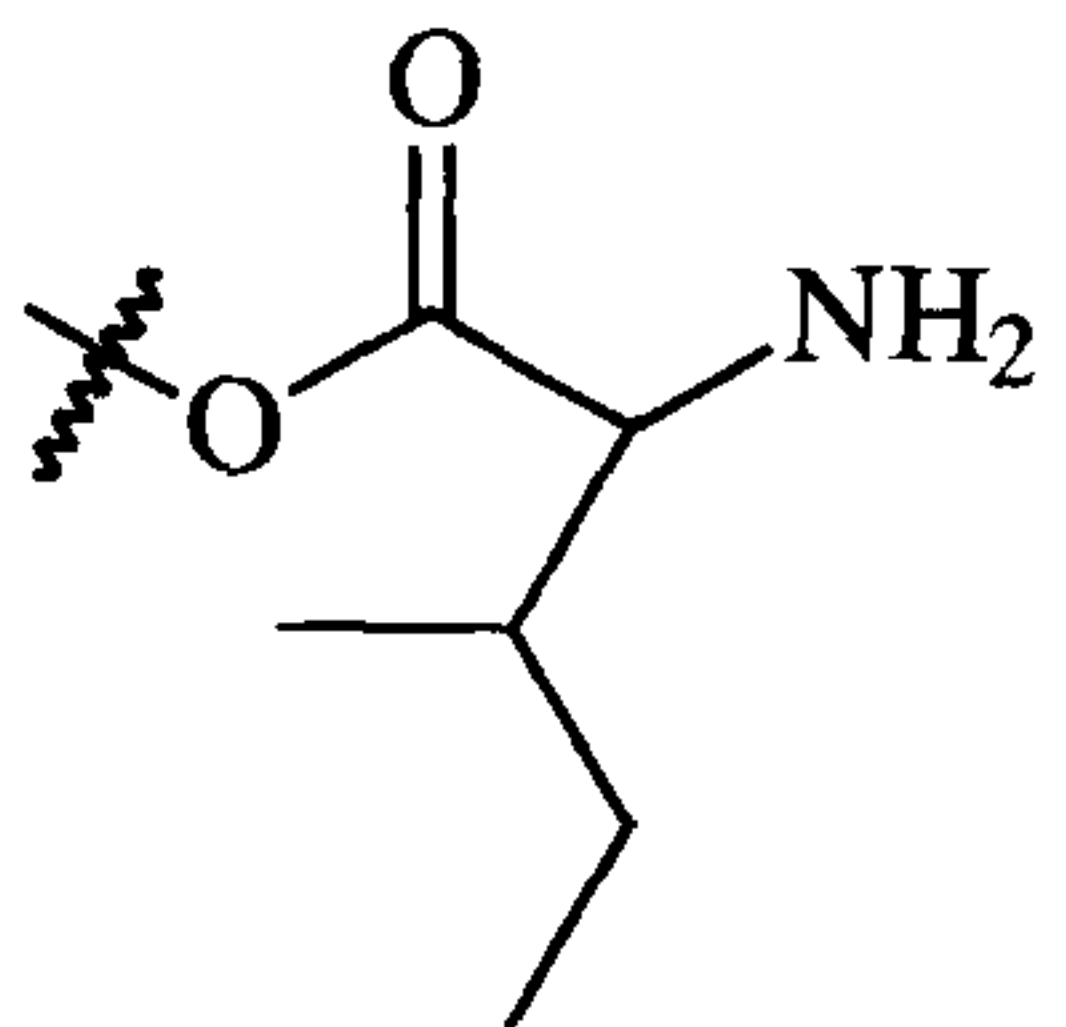
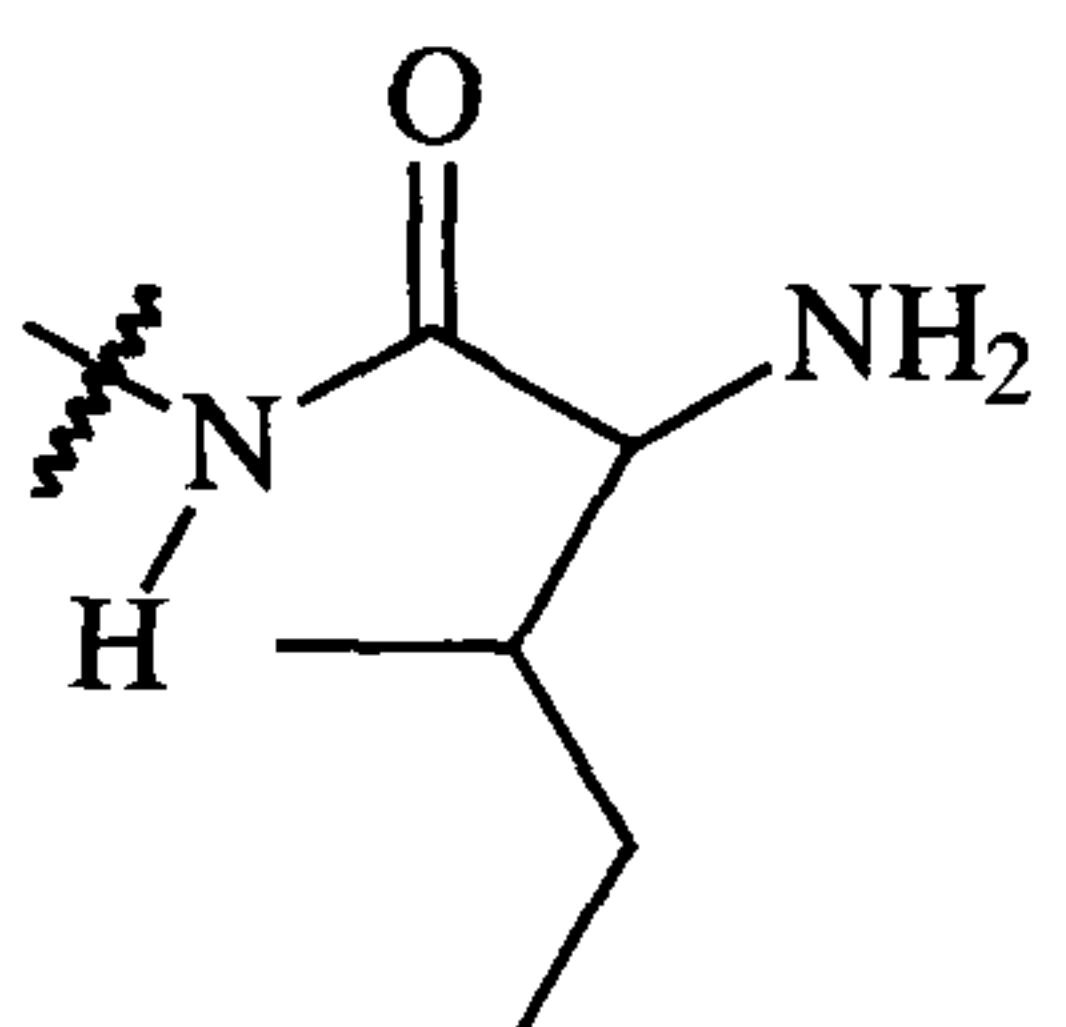
 Wherein each (AA) is independently any neutral amino acid side chain	
 Where X₆, X₇, X₈ and X₉ are as defined herein for X₁	 Where X₆, X₇, X₈ and X₉ are as defined herein for X₁
	
	
	

TABLE III – PRODRUG MOIETIES

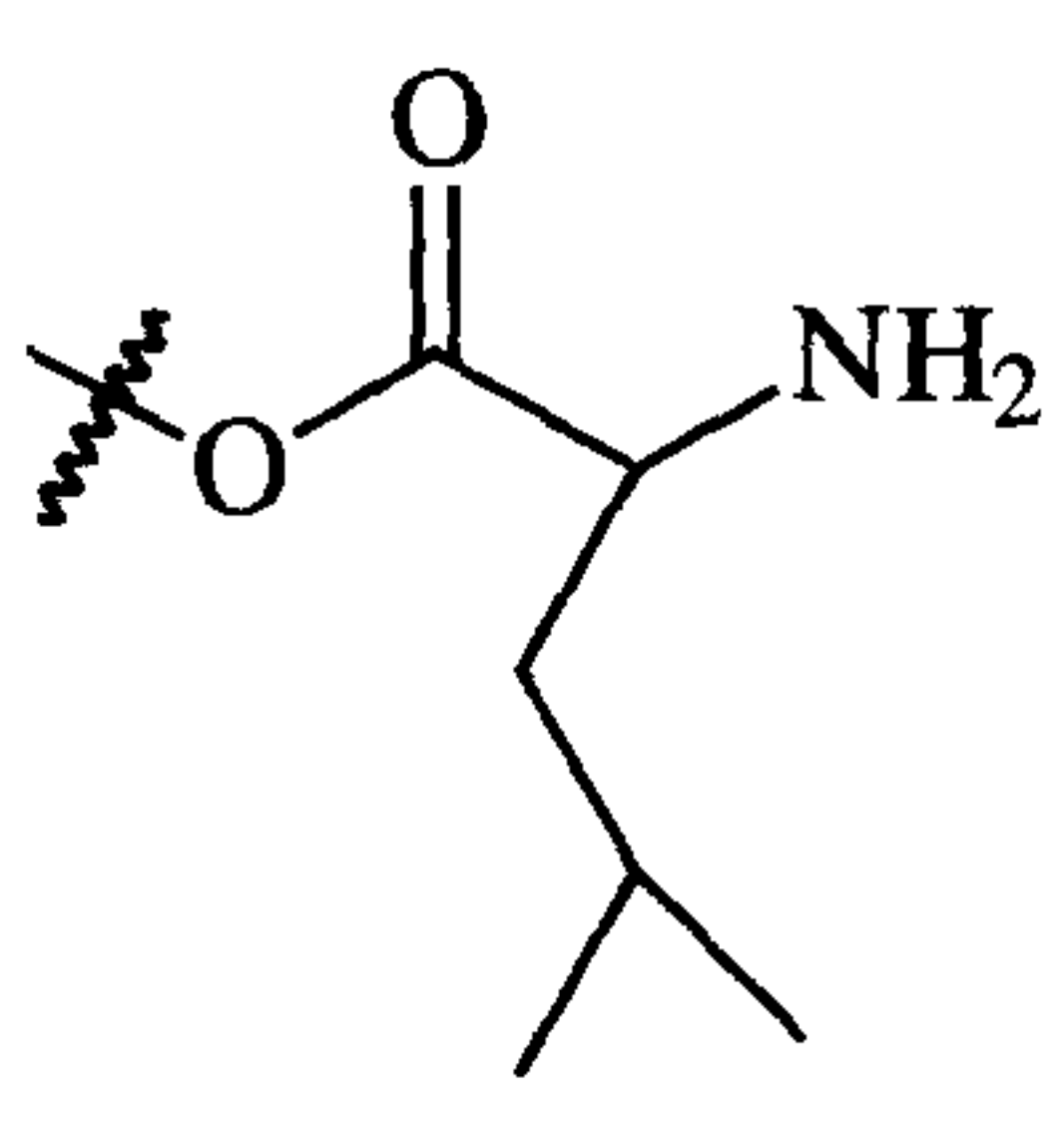
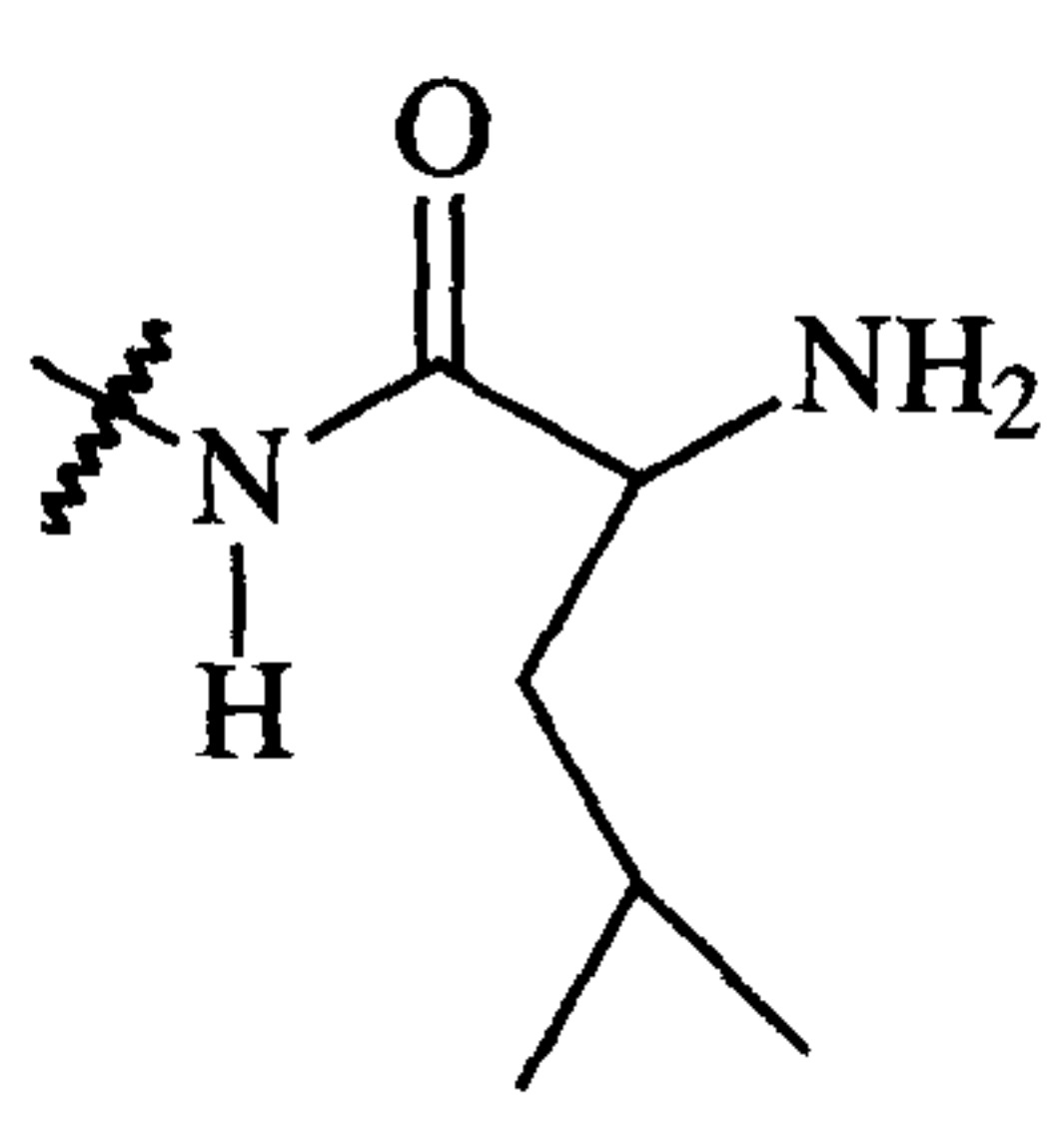
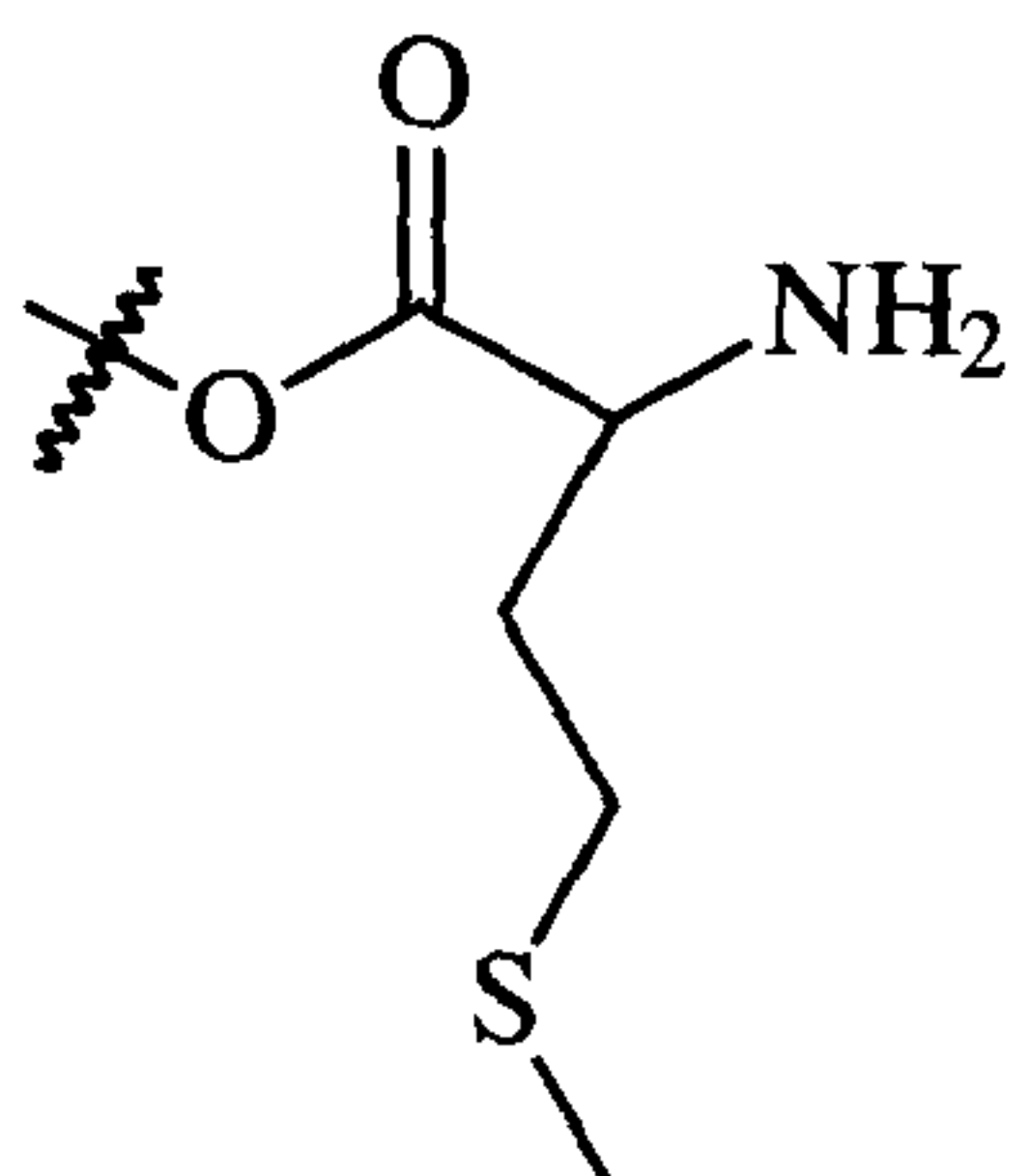
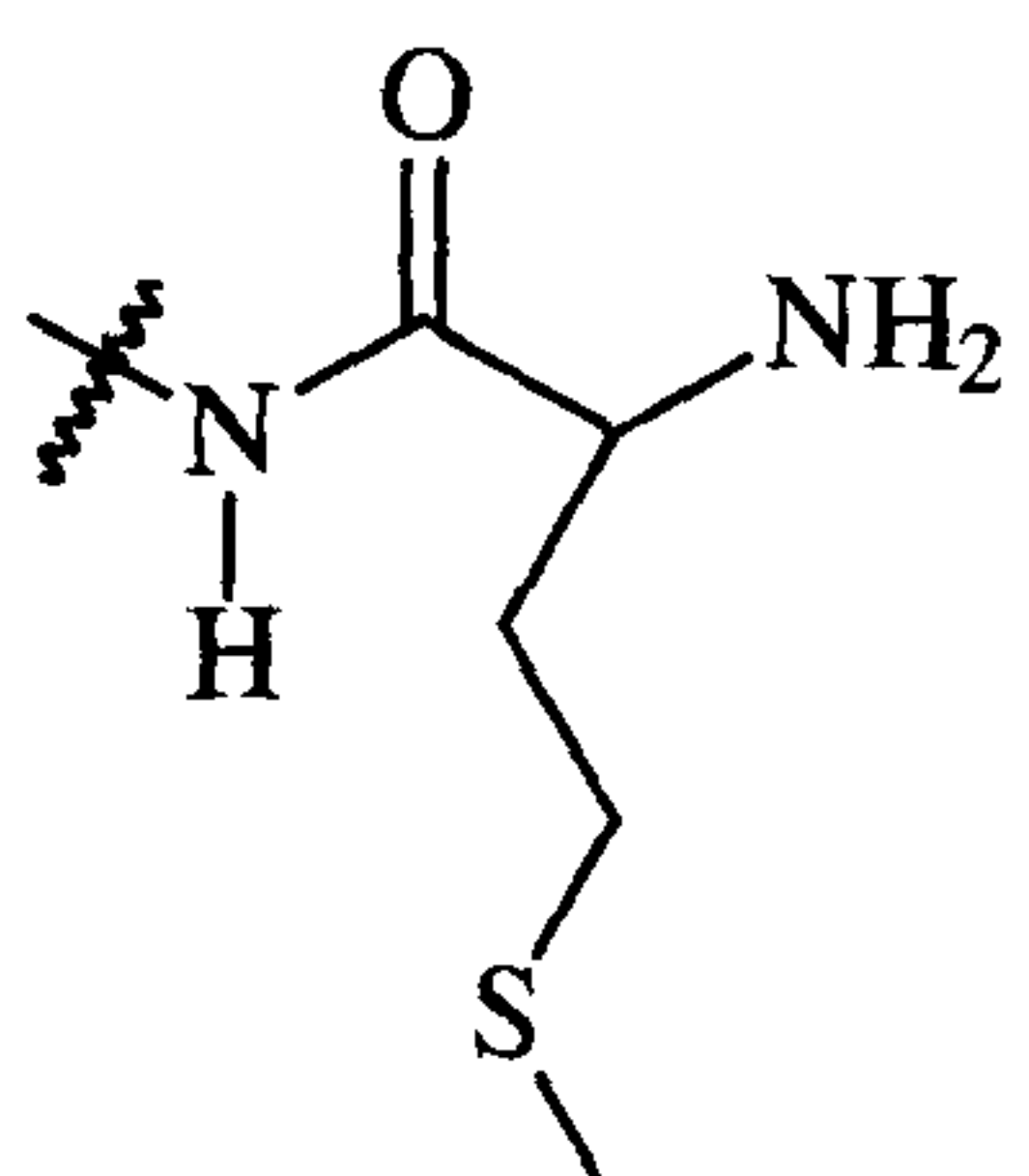
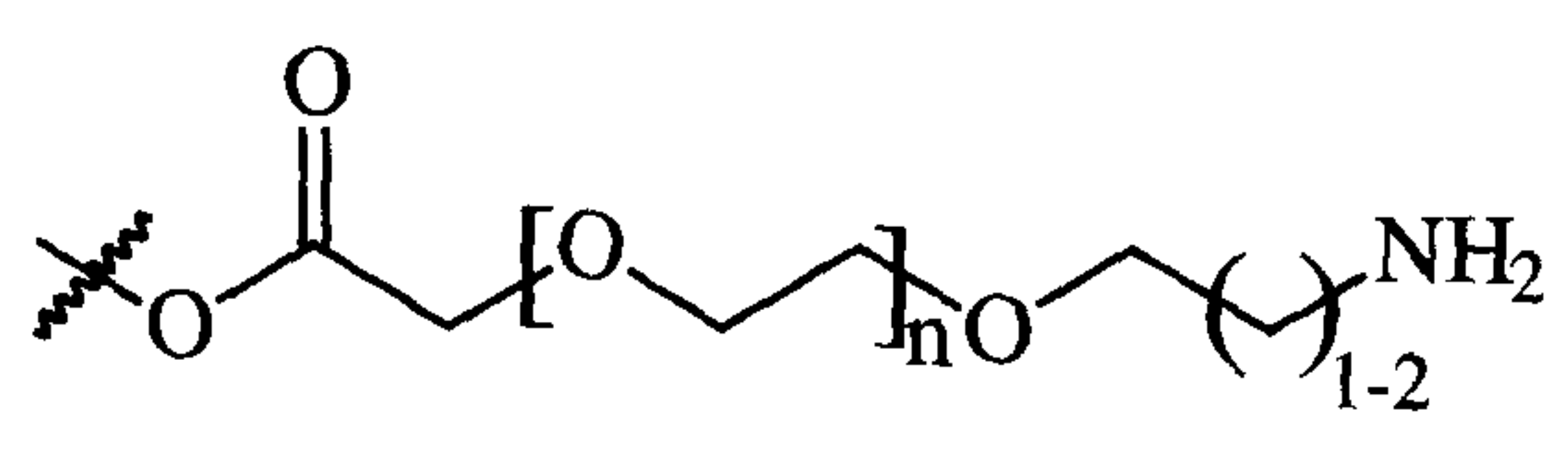
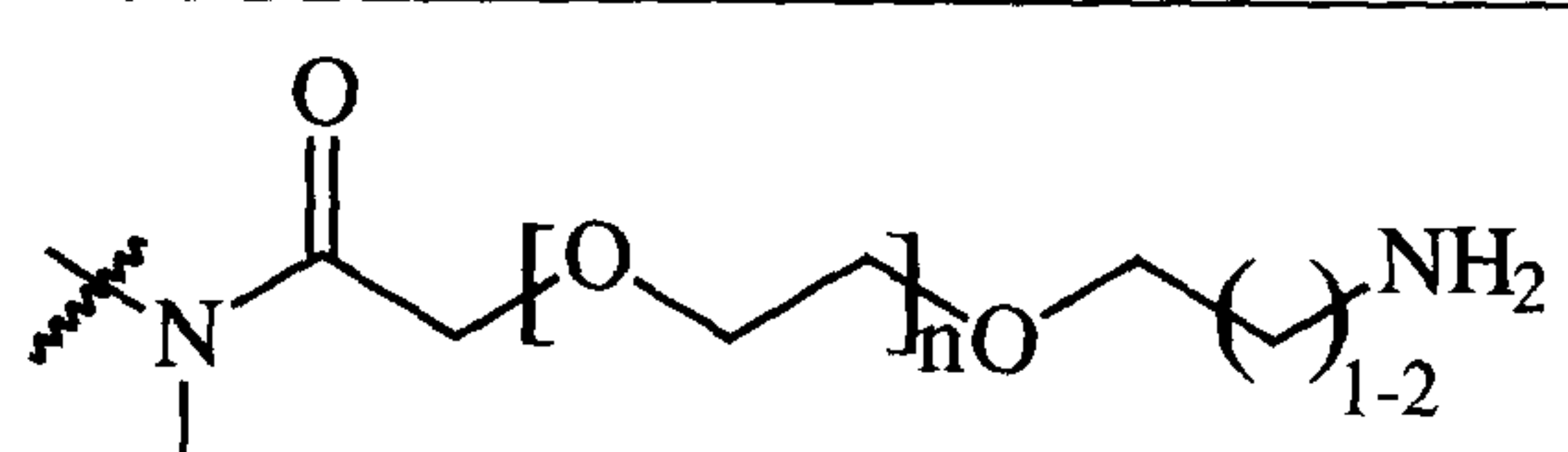
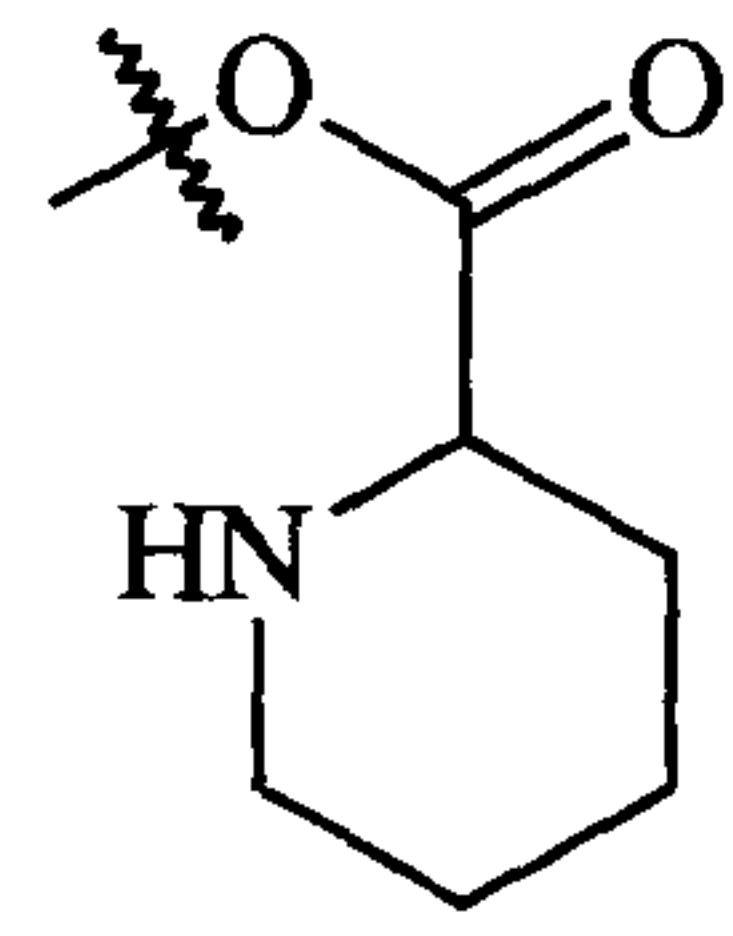
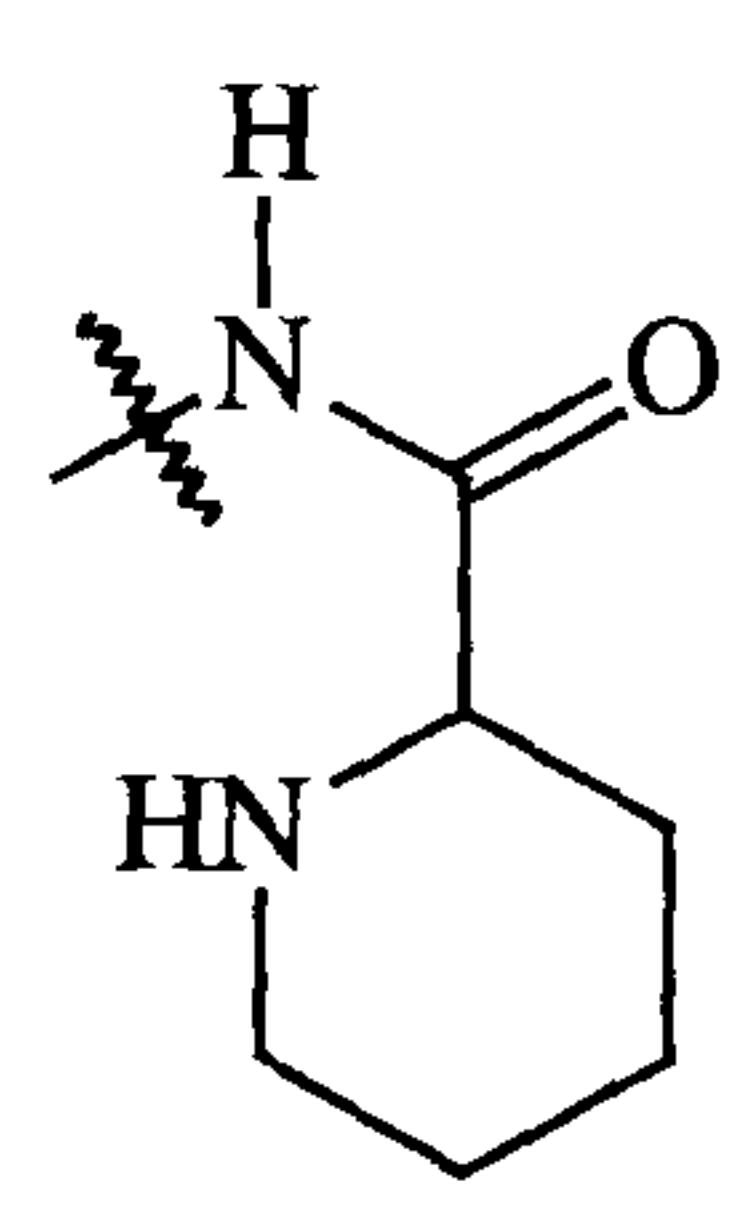
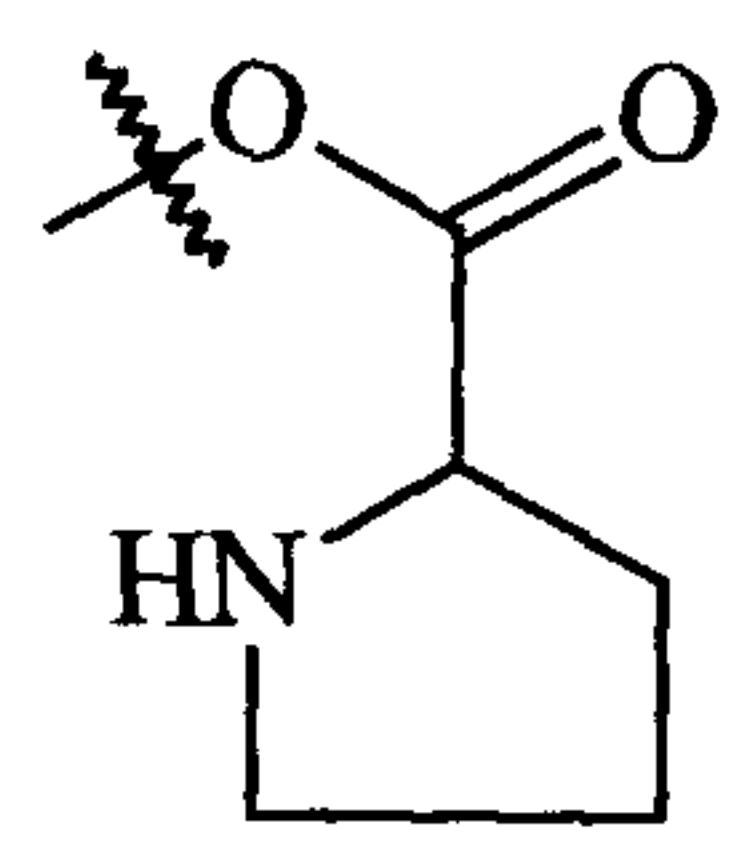
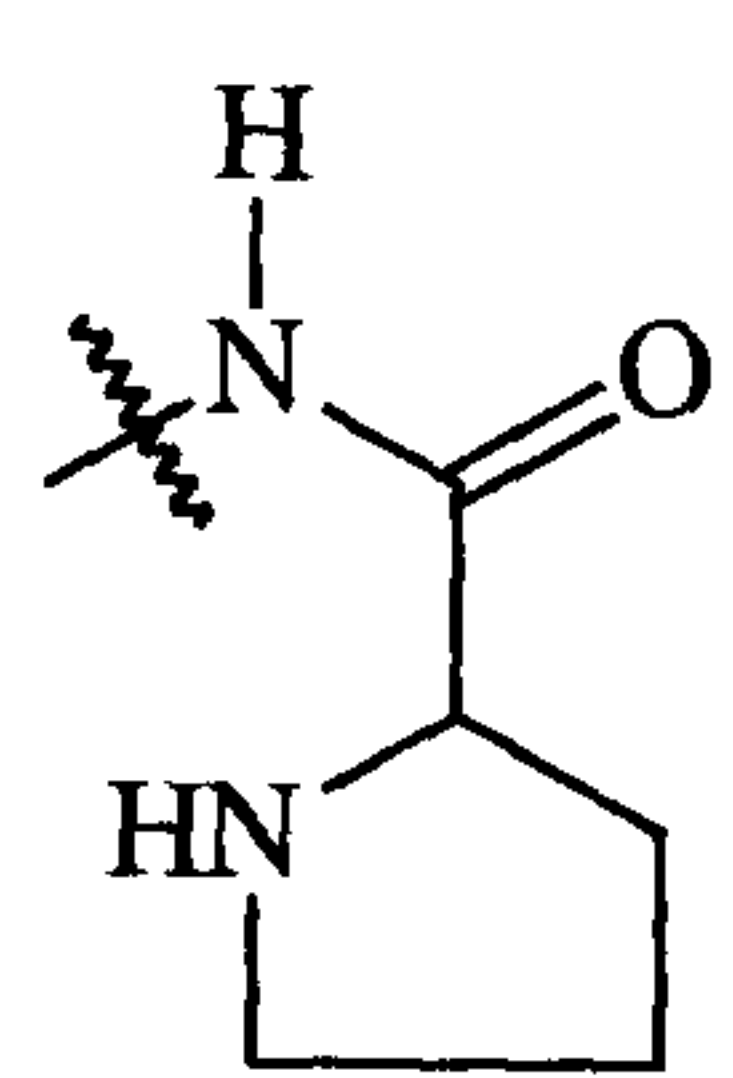
	
	
 Where n = 1 to 450	 Where n = 1 to 450
	
	

TABLE III – PRODRUG MOIETIES

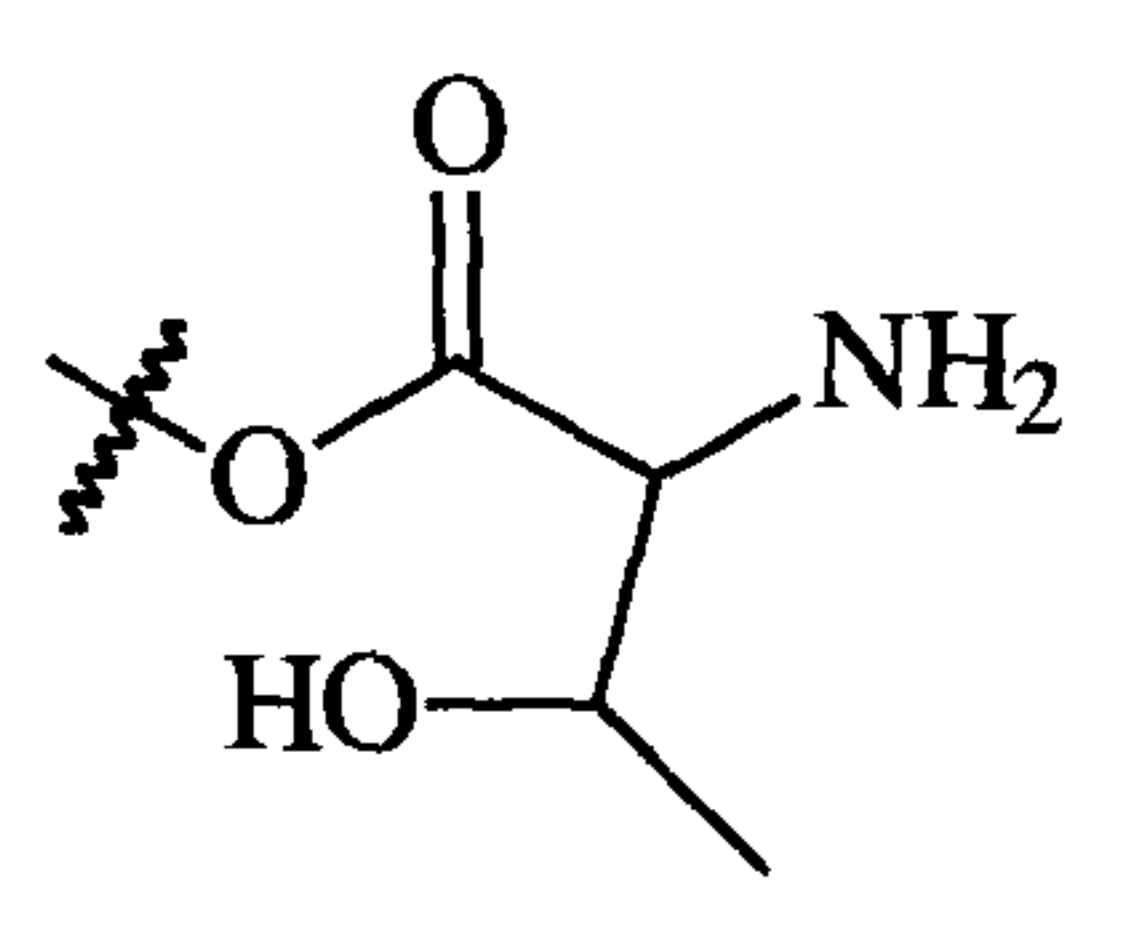
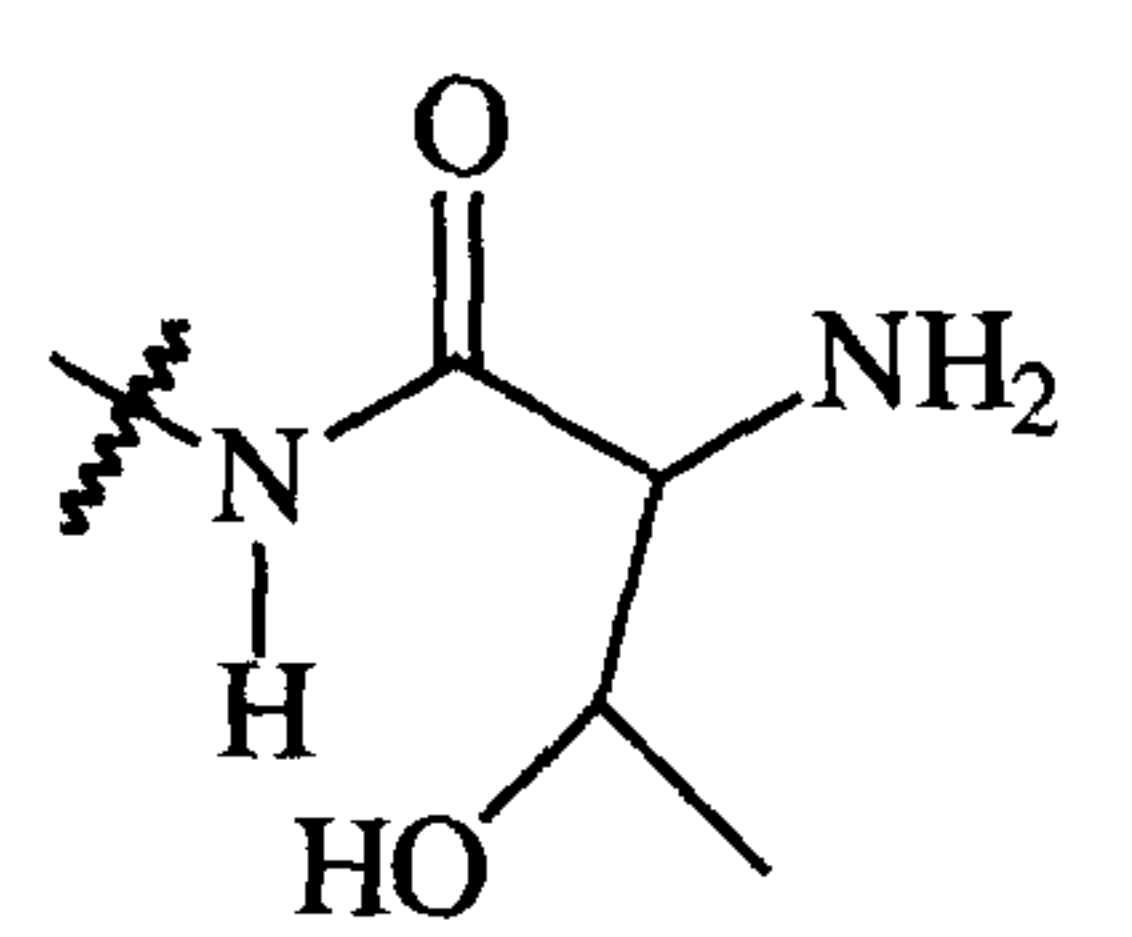
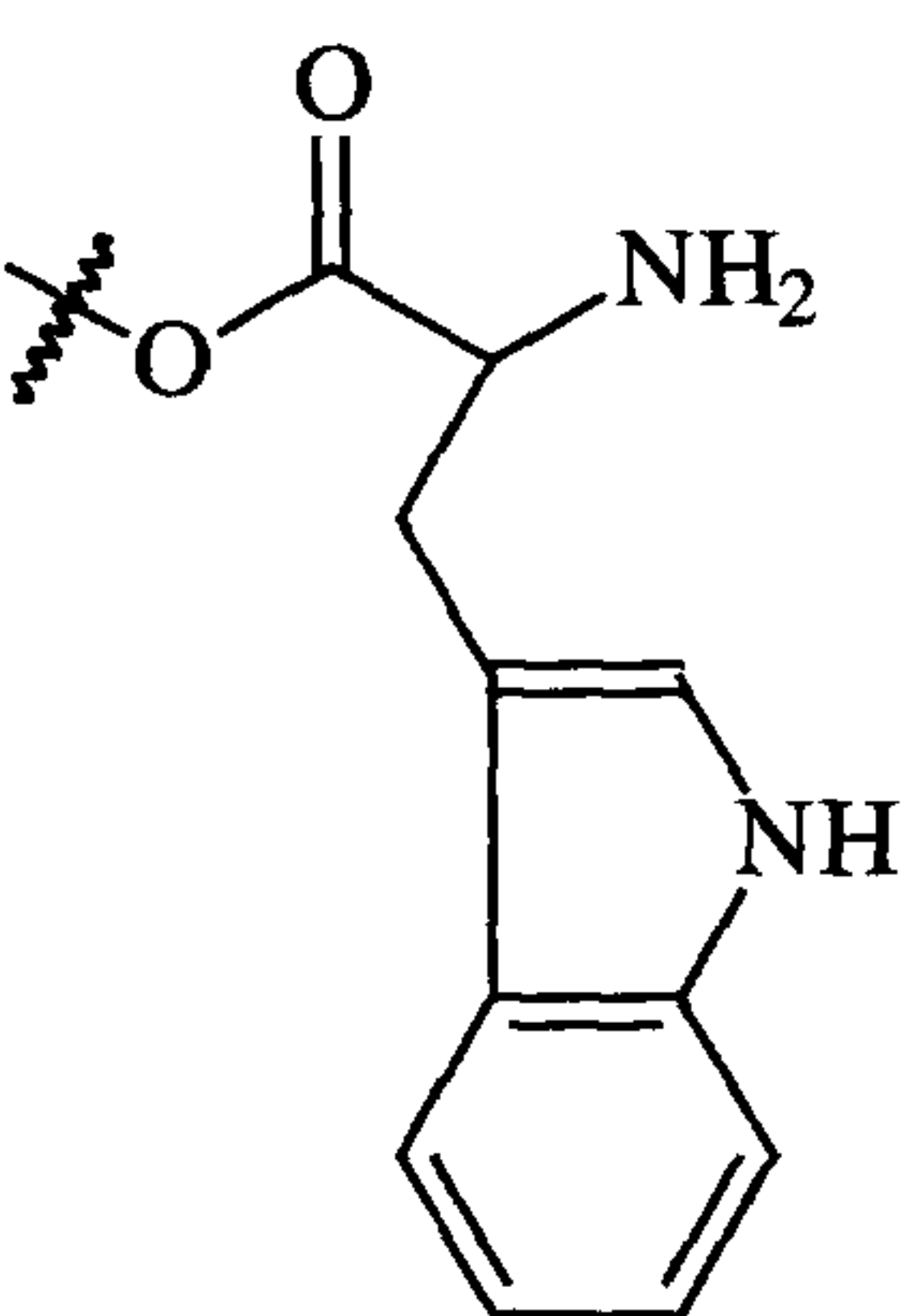
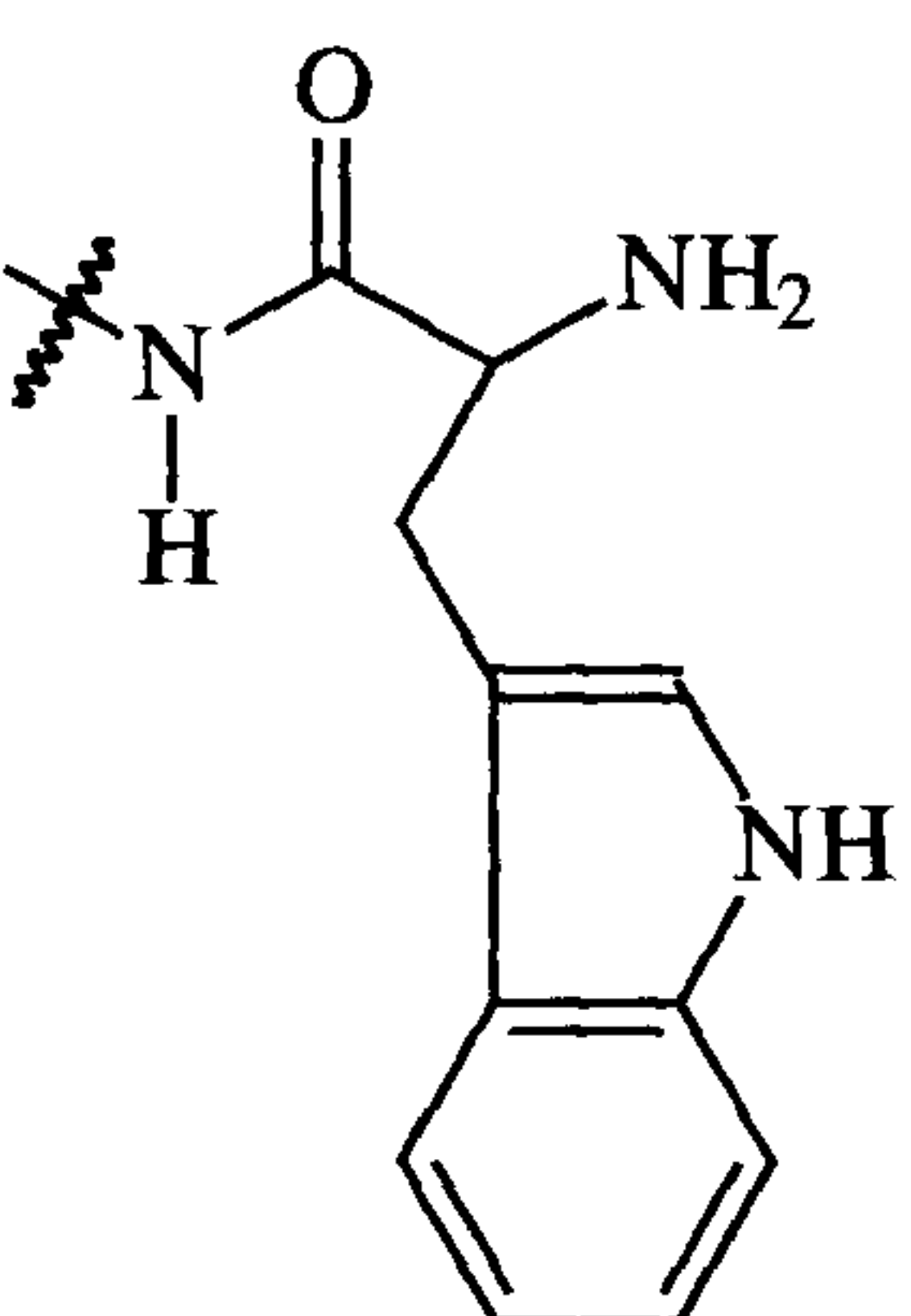
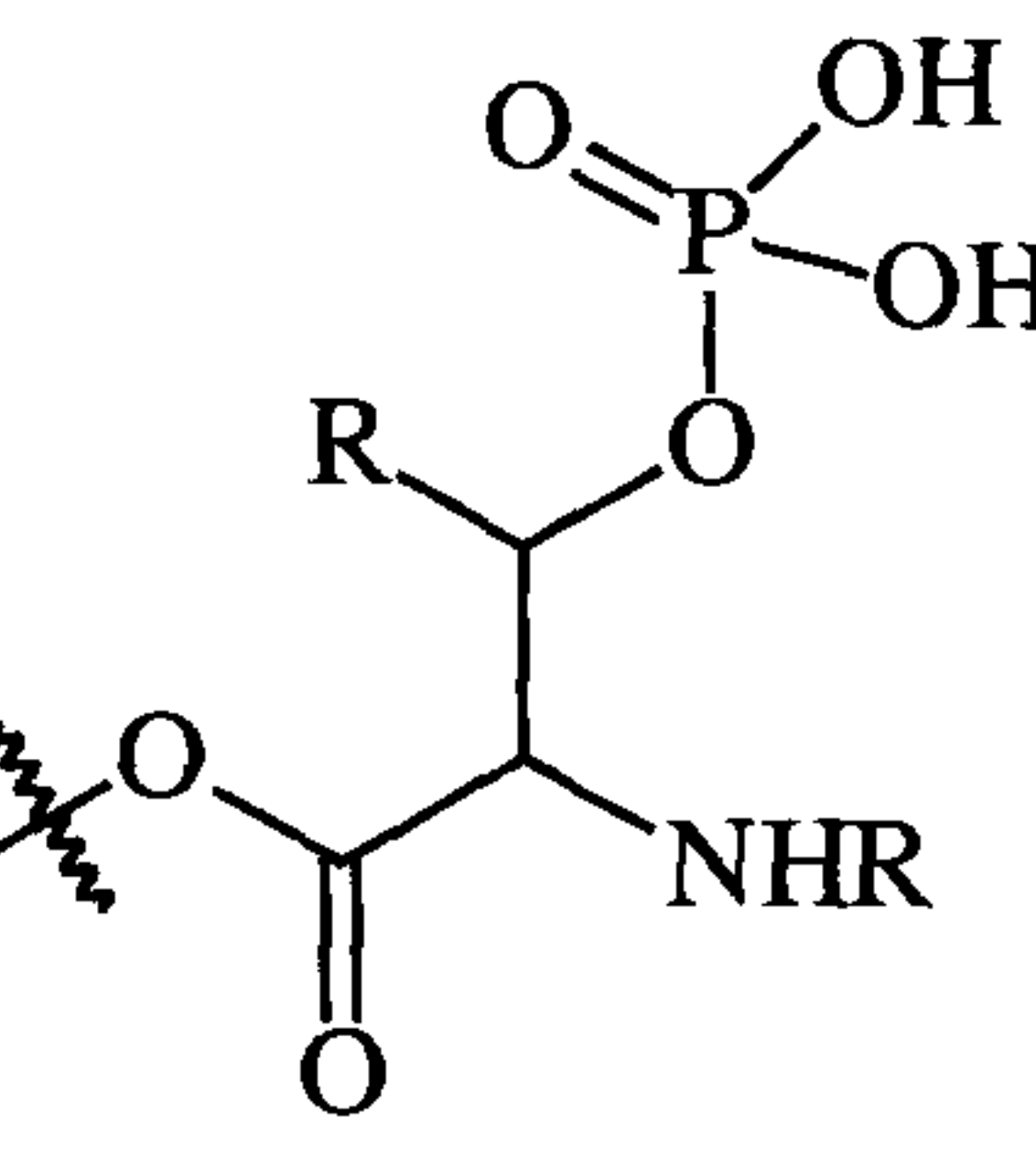
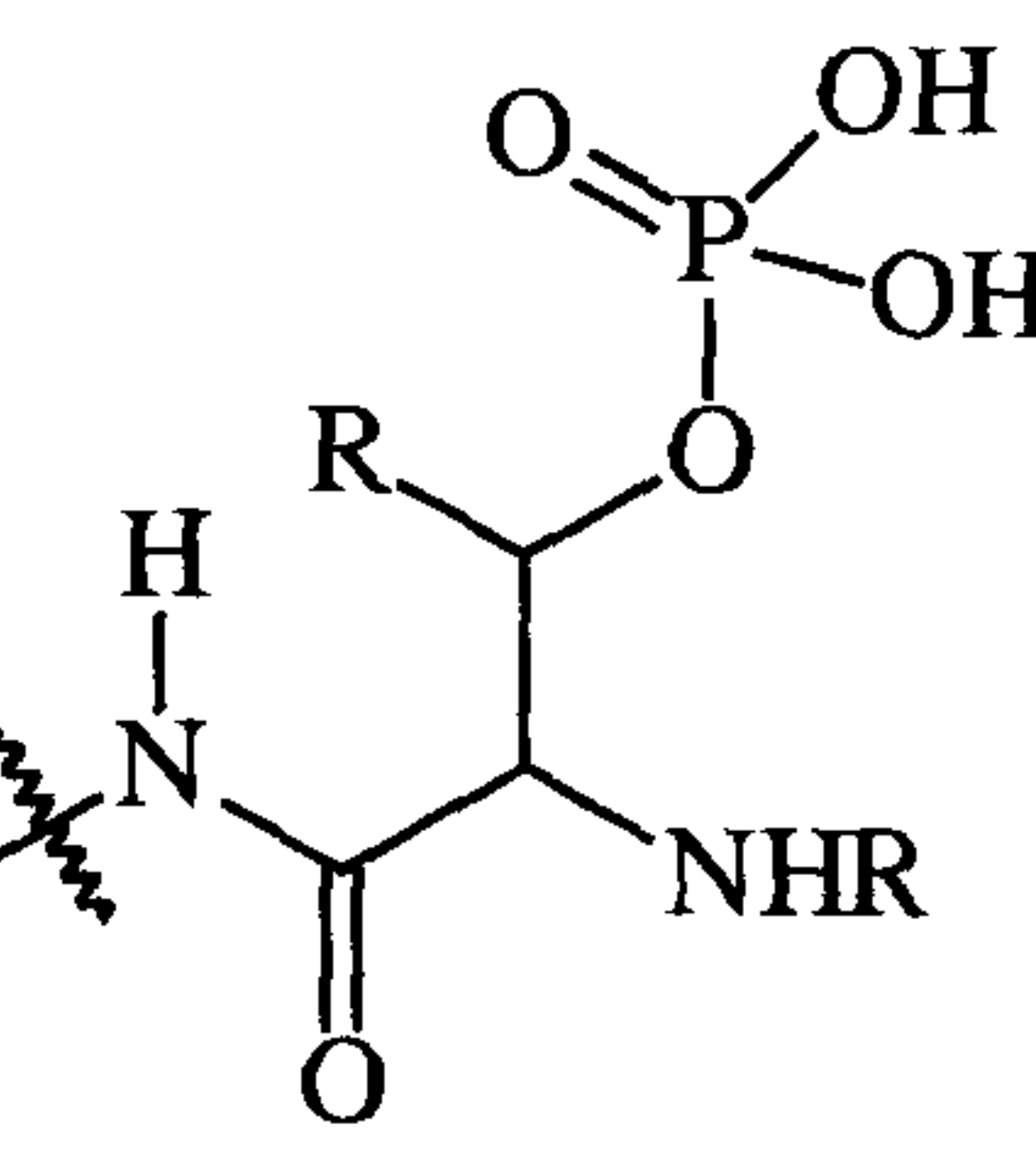
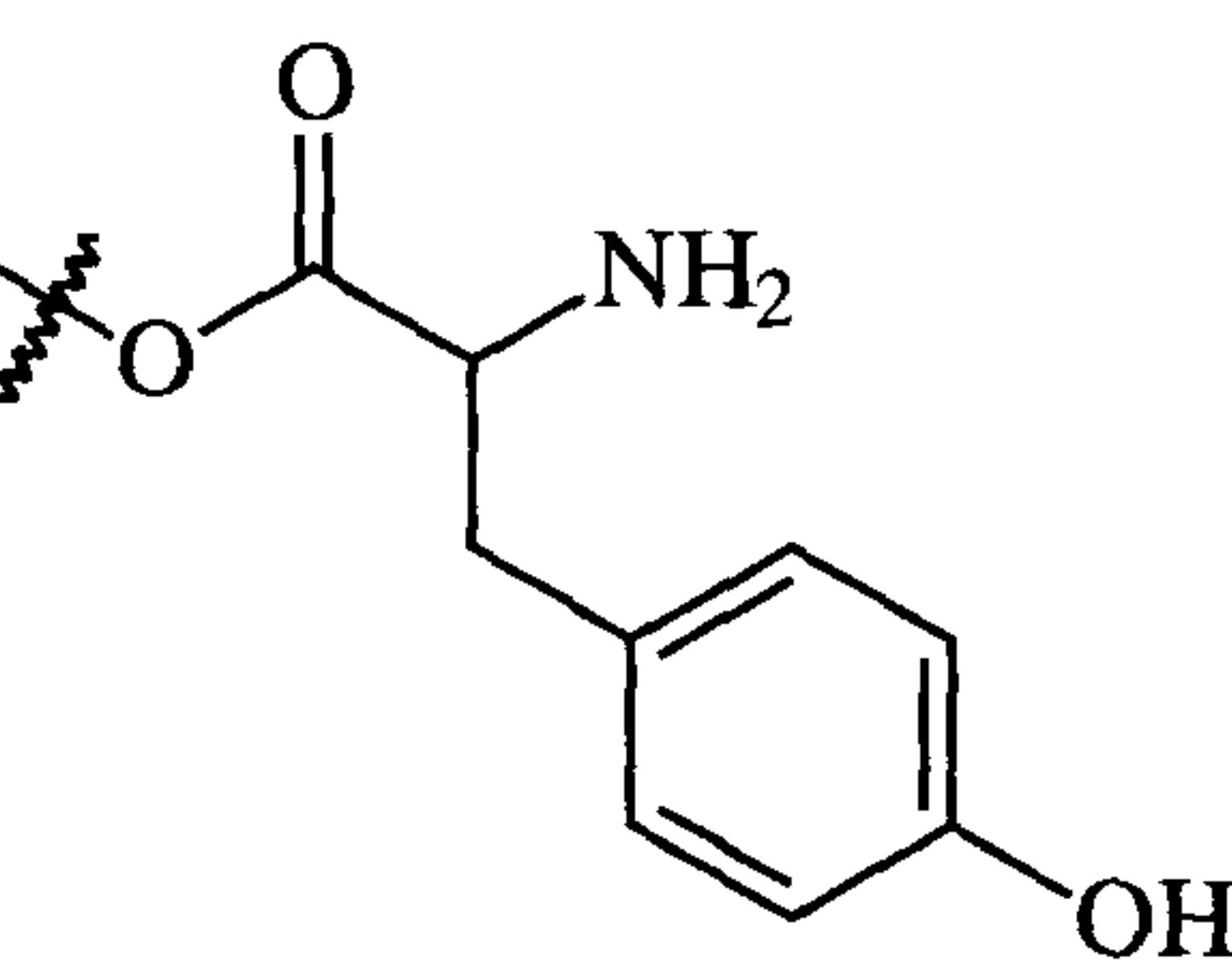
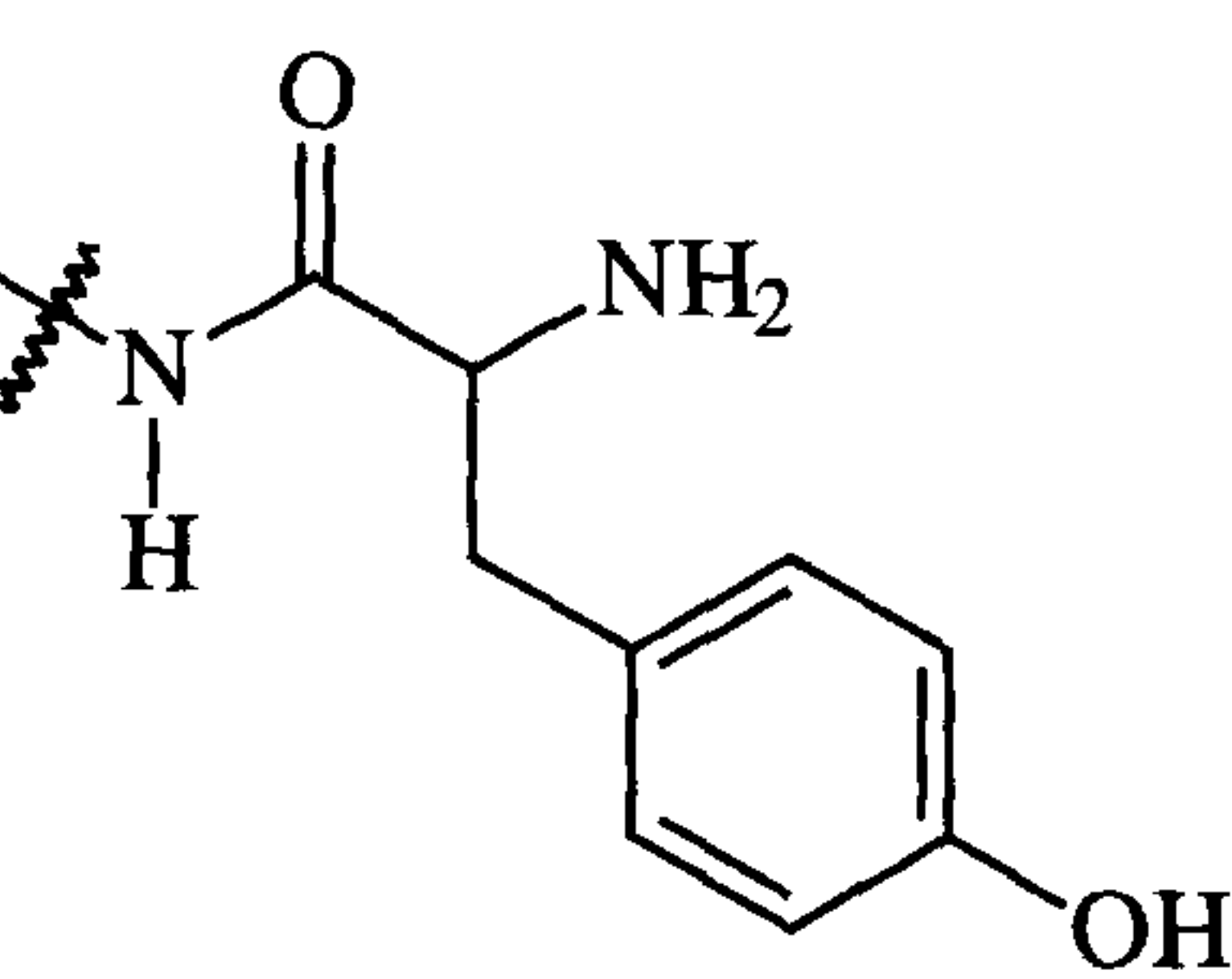
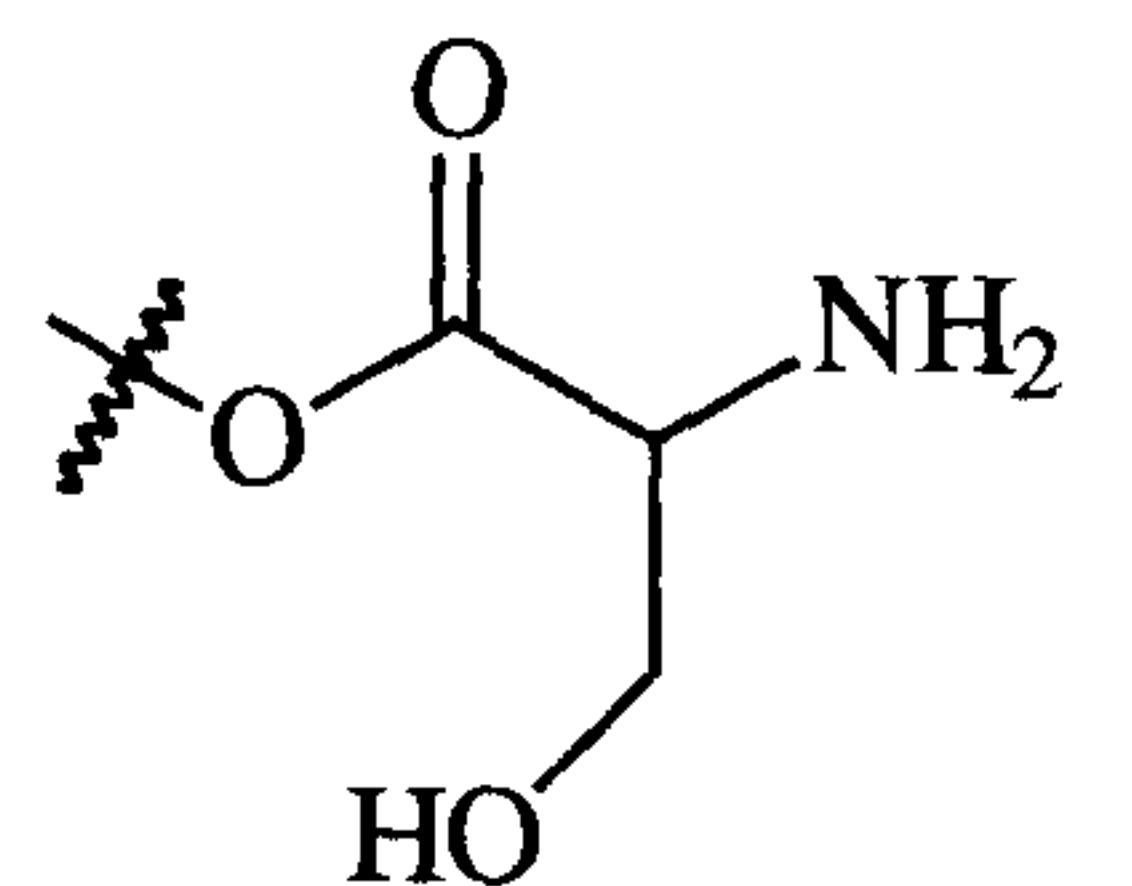
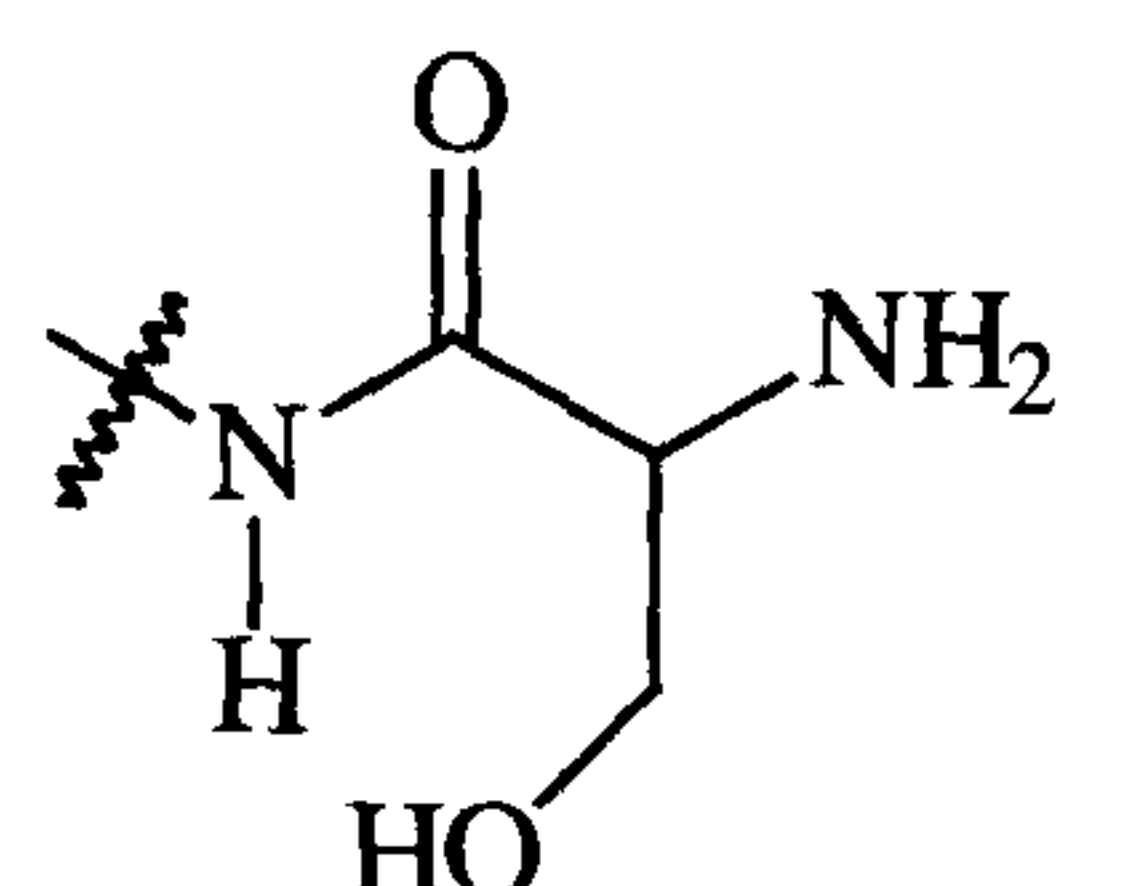
	
	
	
	
	

TABLE III – PRODRUG MOIETIES

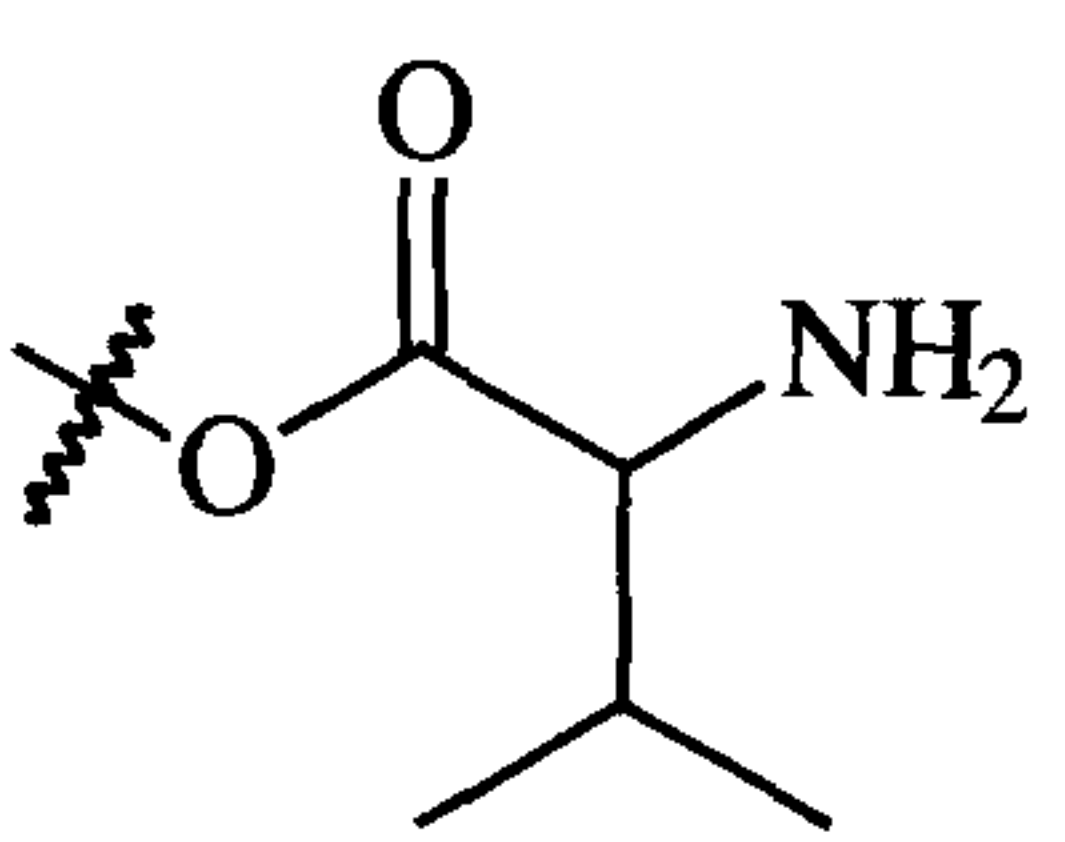
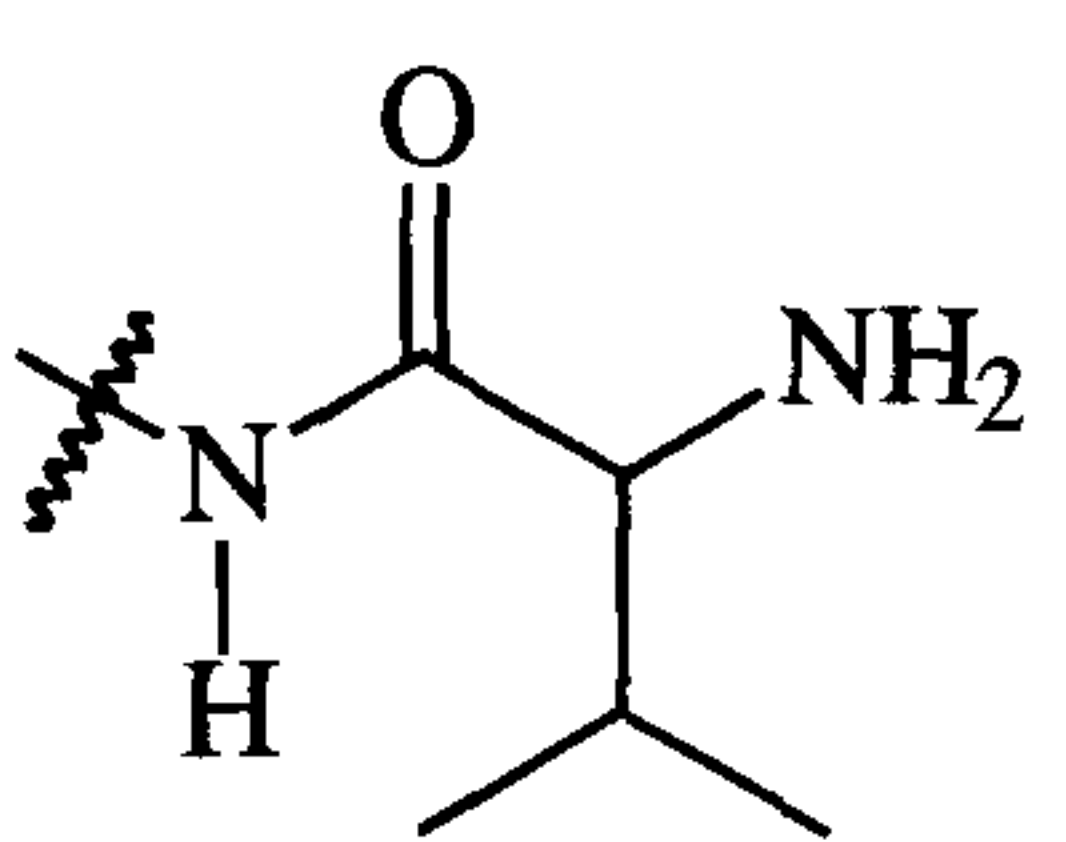
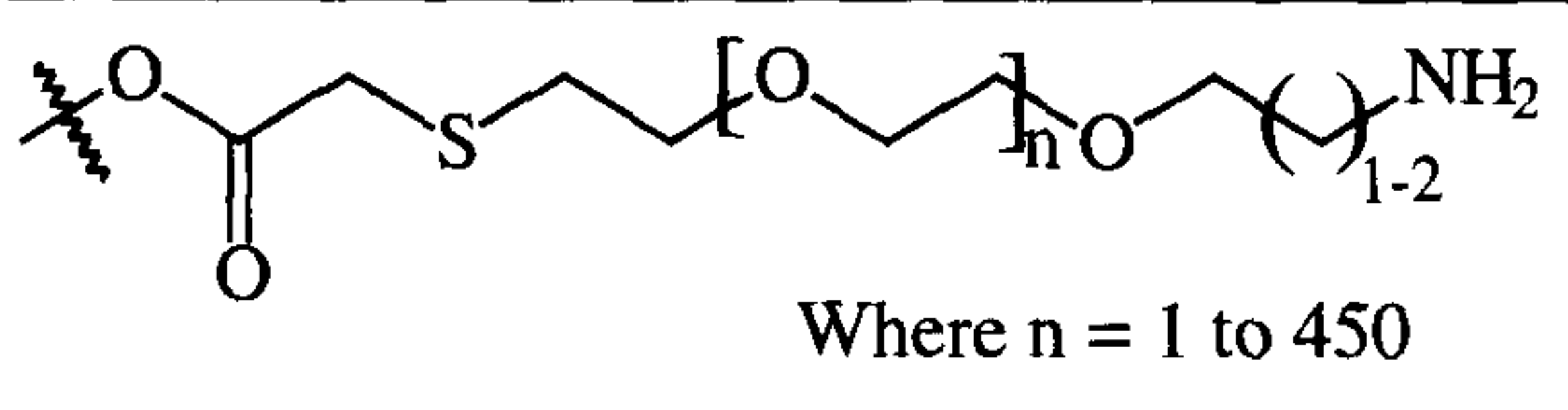
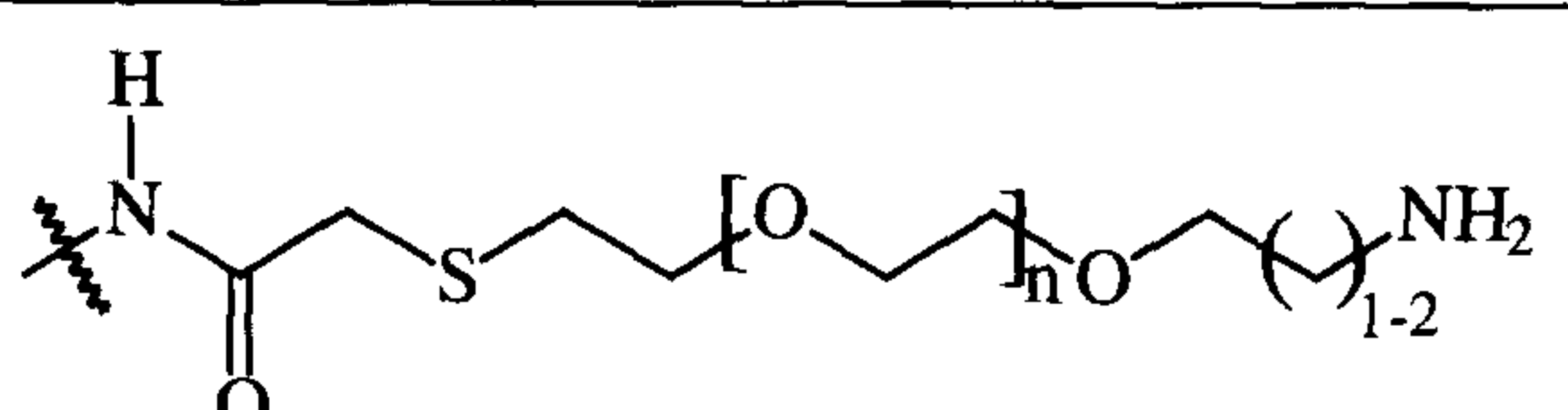
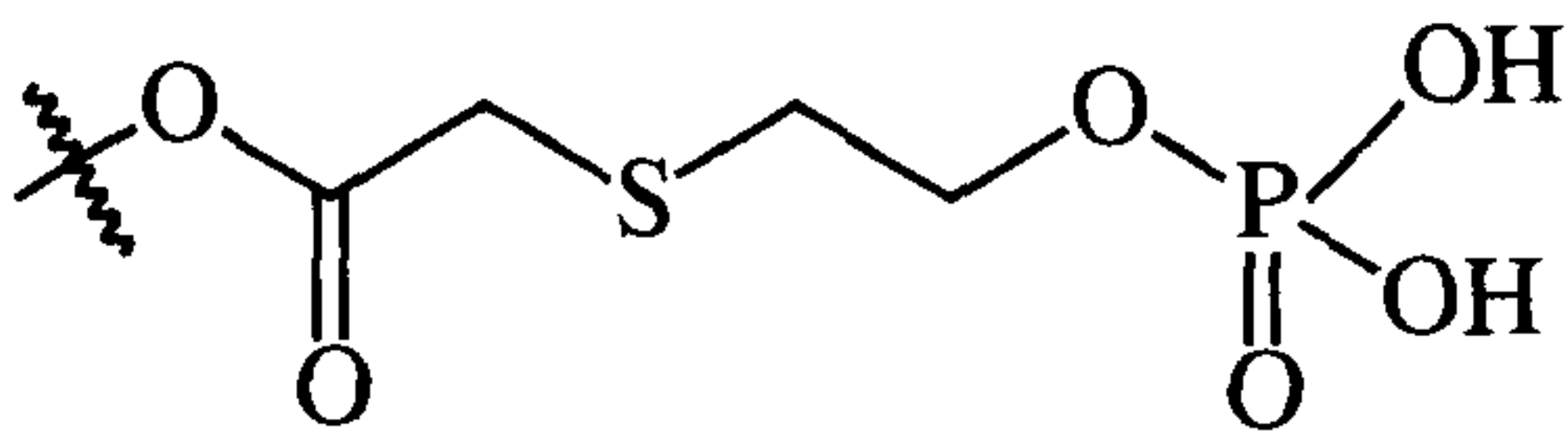
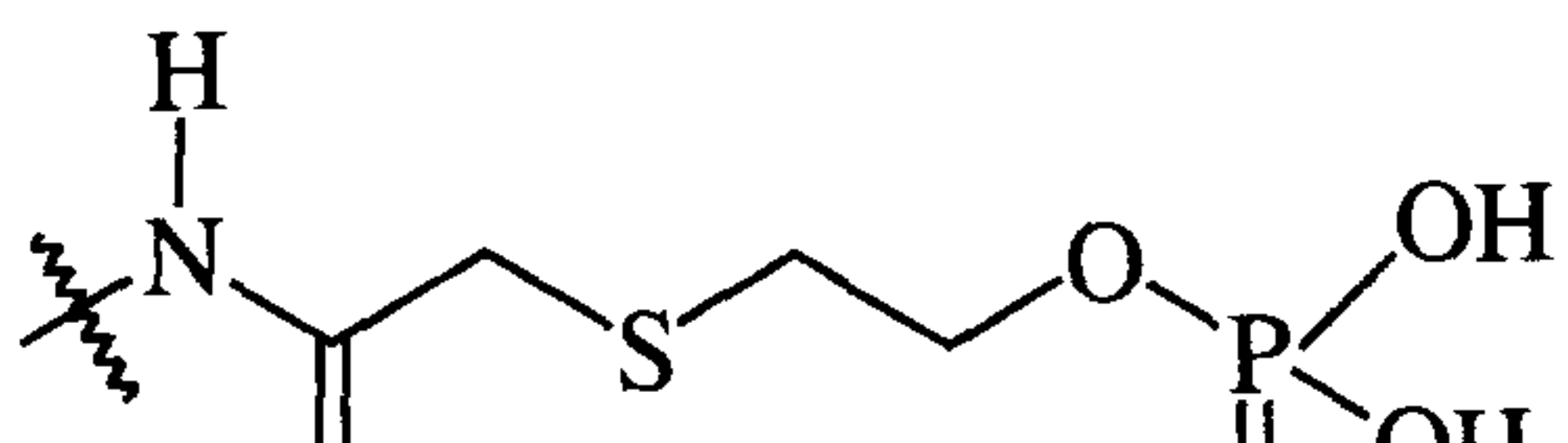
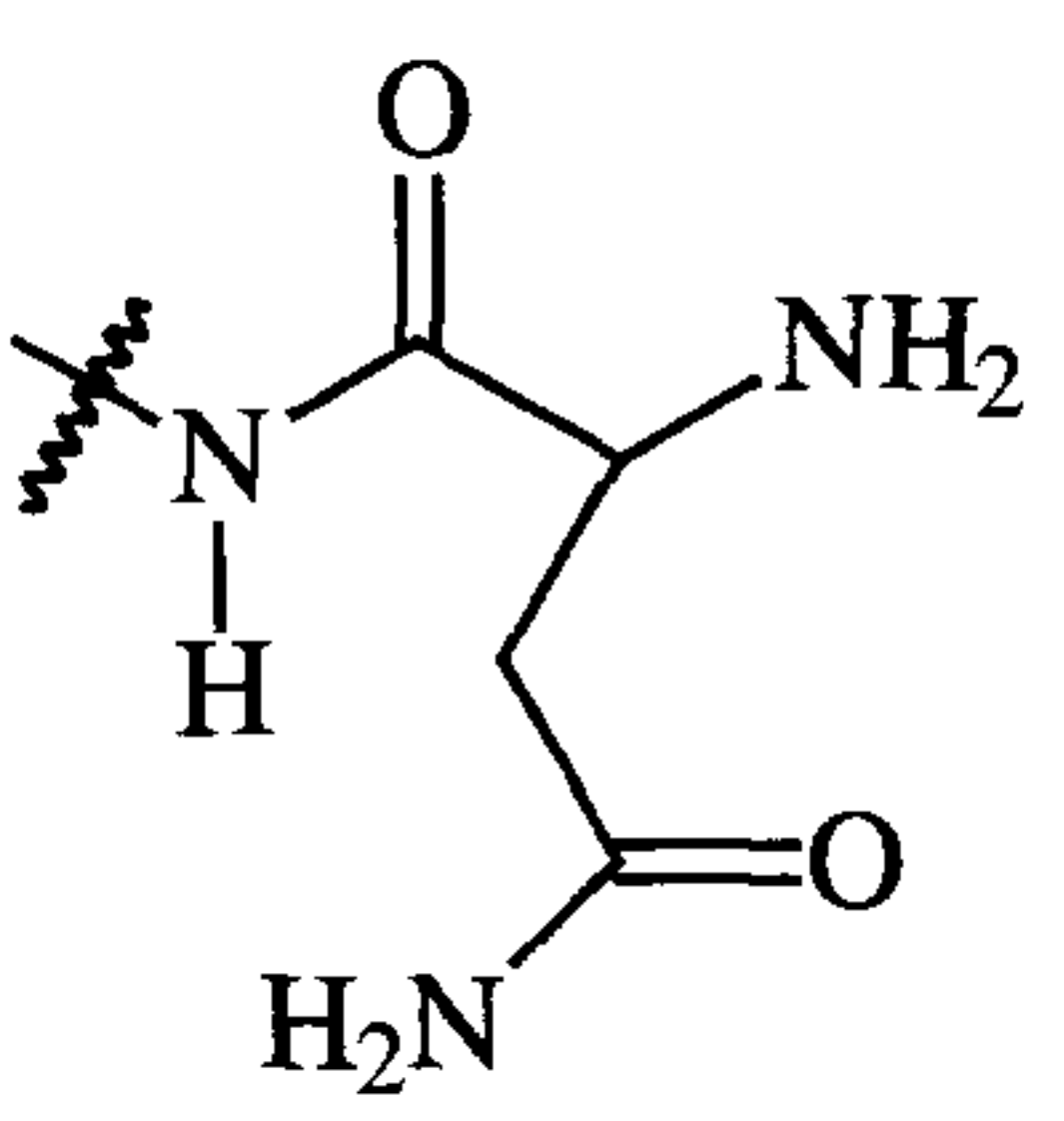
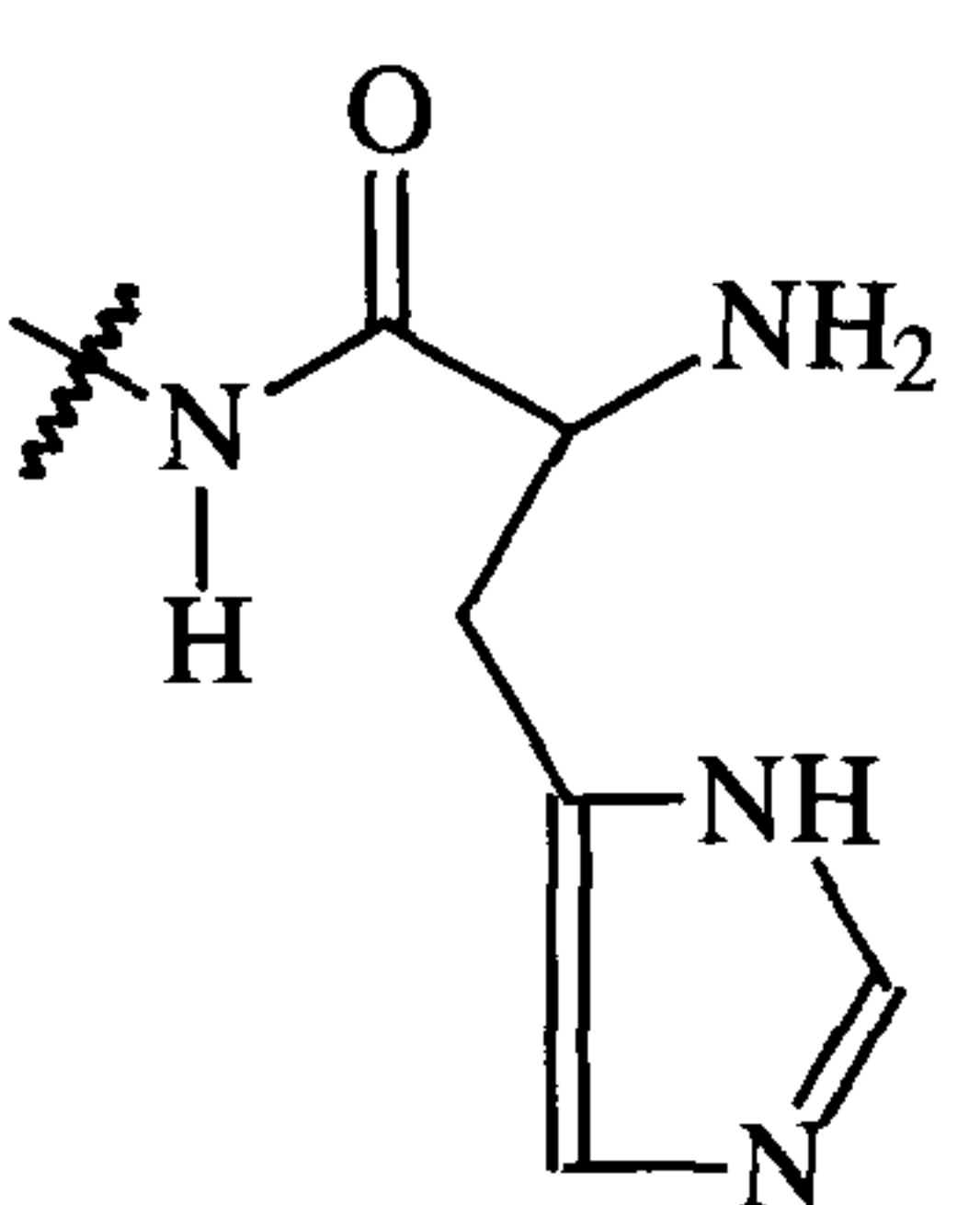
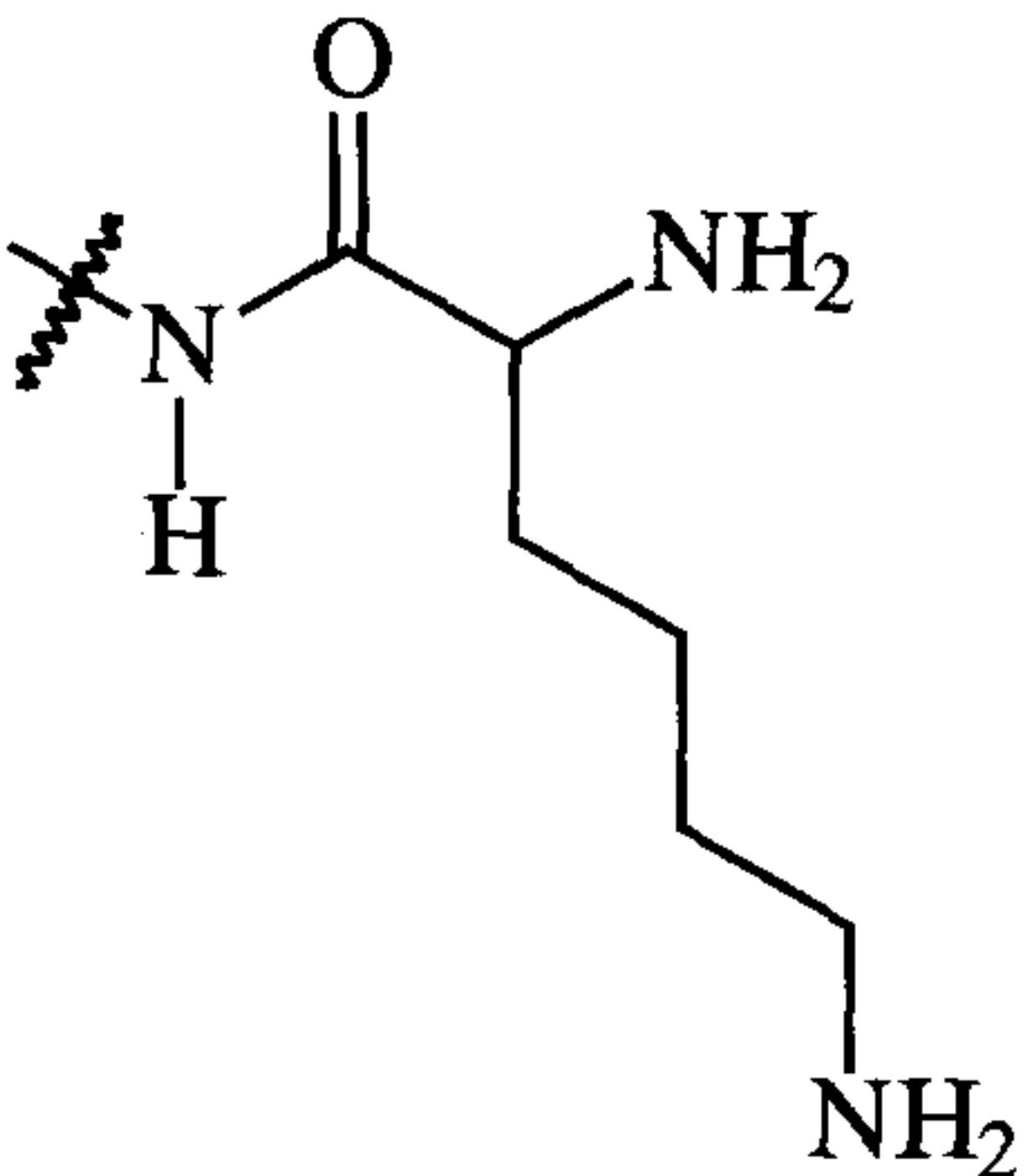
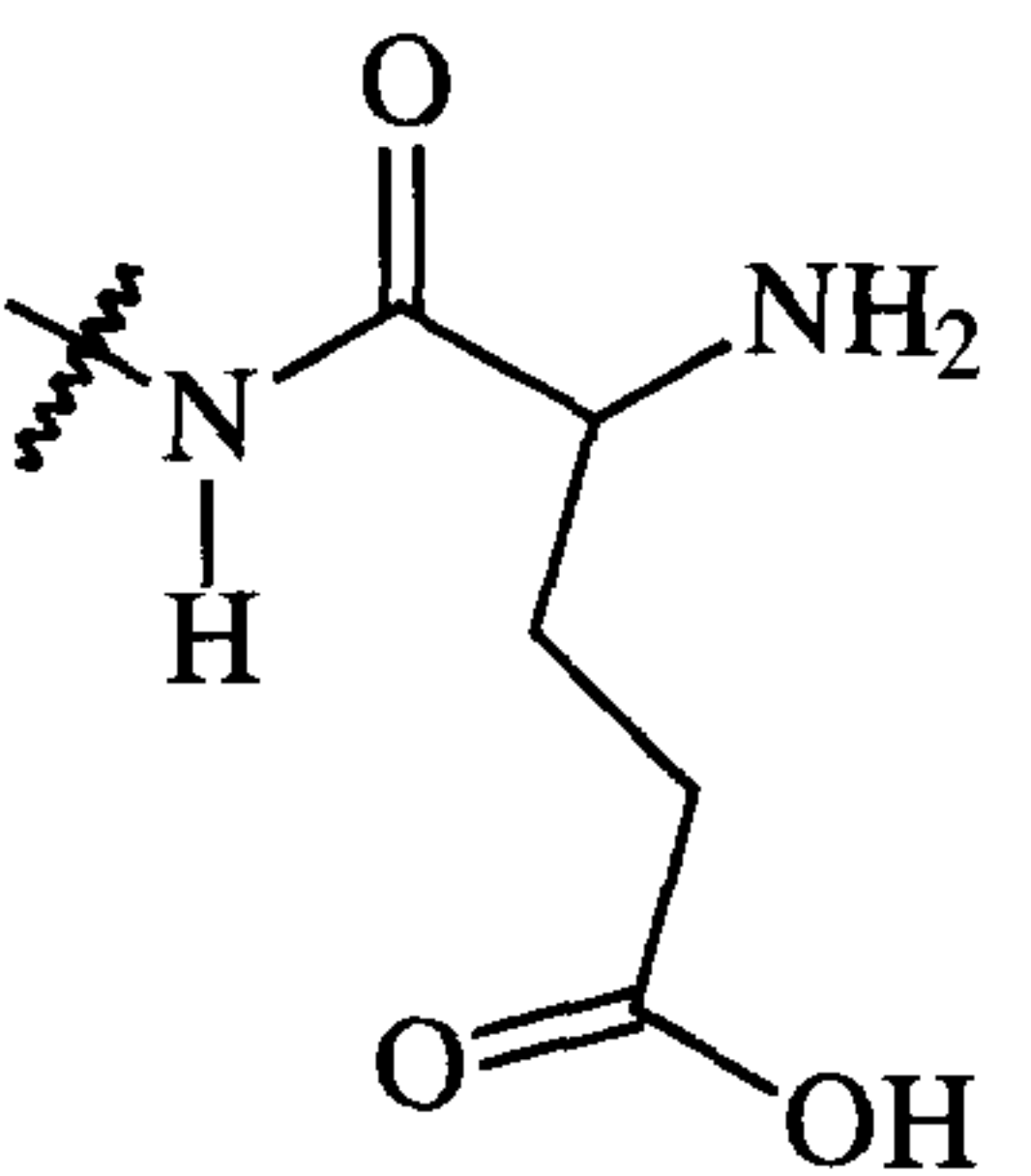
	
 Where n = 1 to 450	 Where n = 1 to 450
	
	
	

TABLE III - PRODRUG MOIETIES

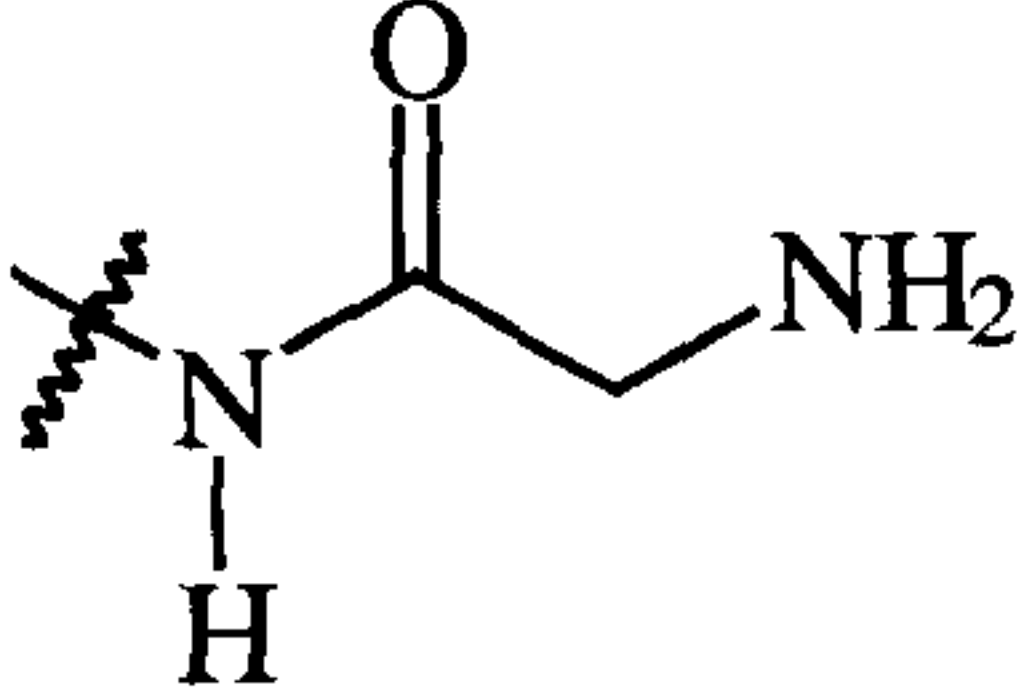
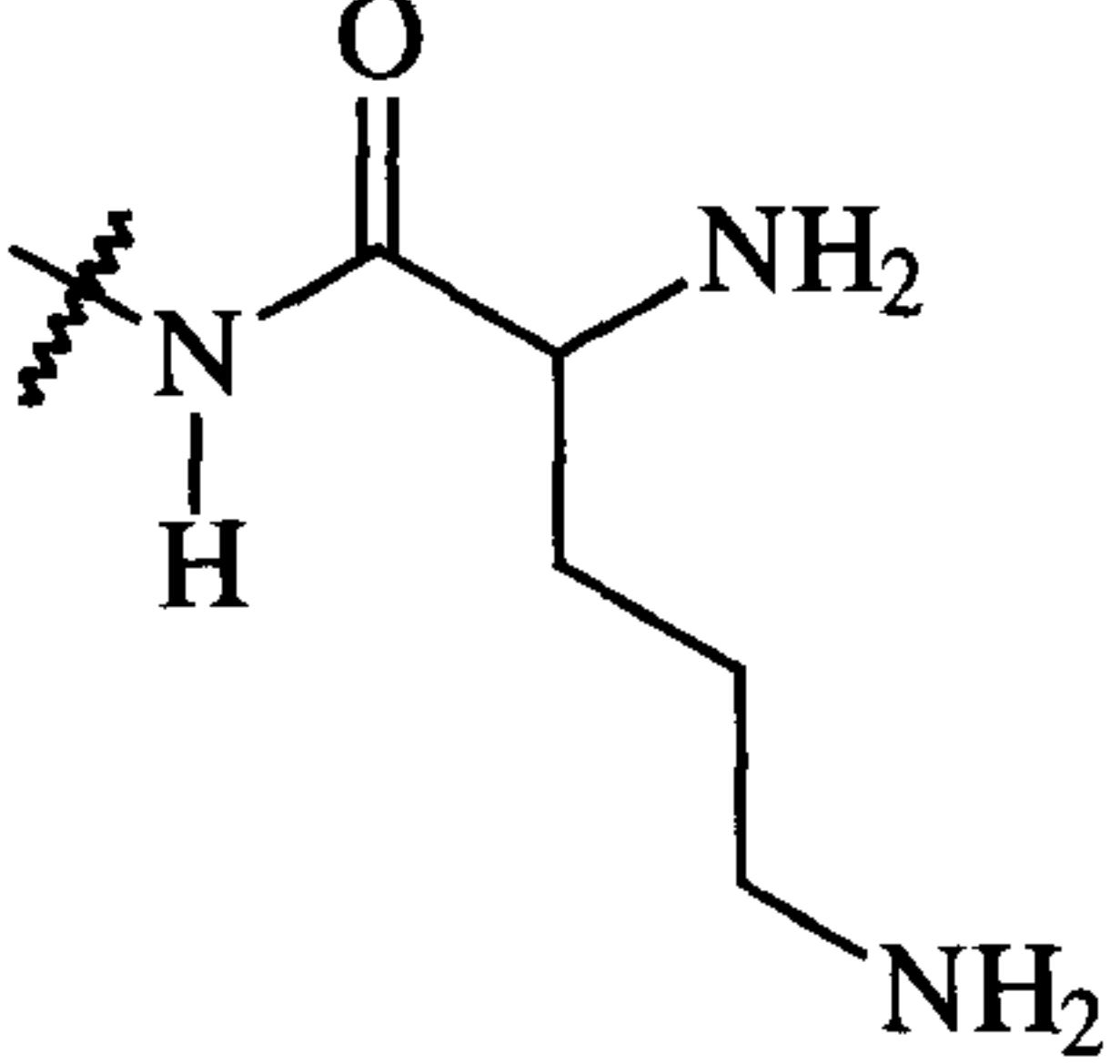
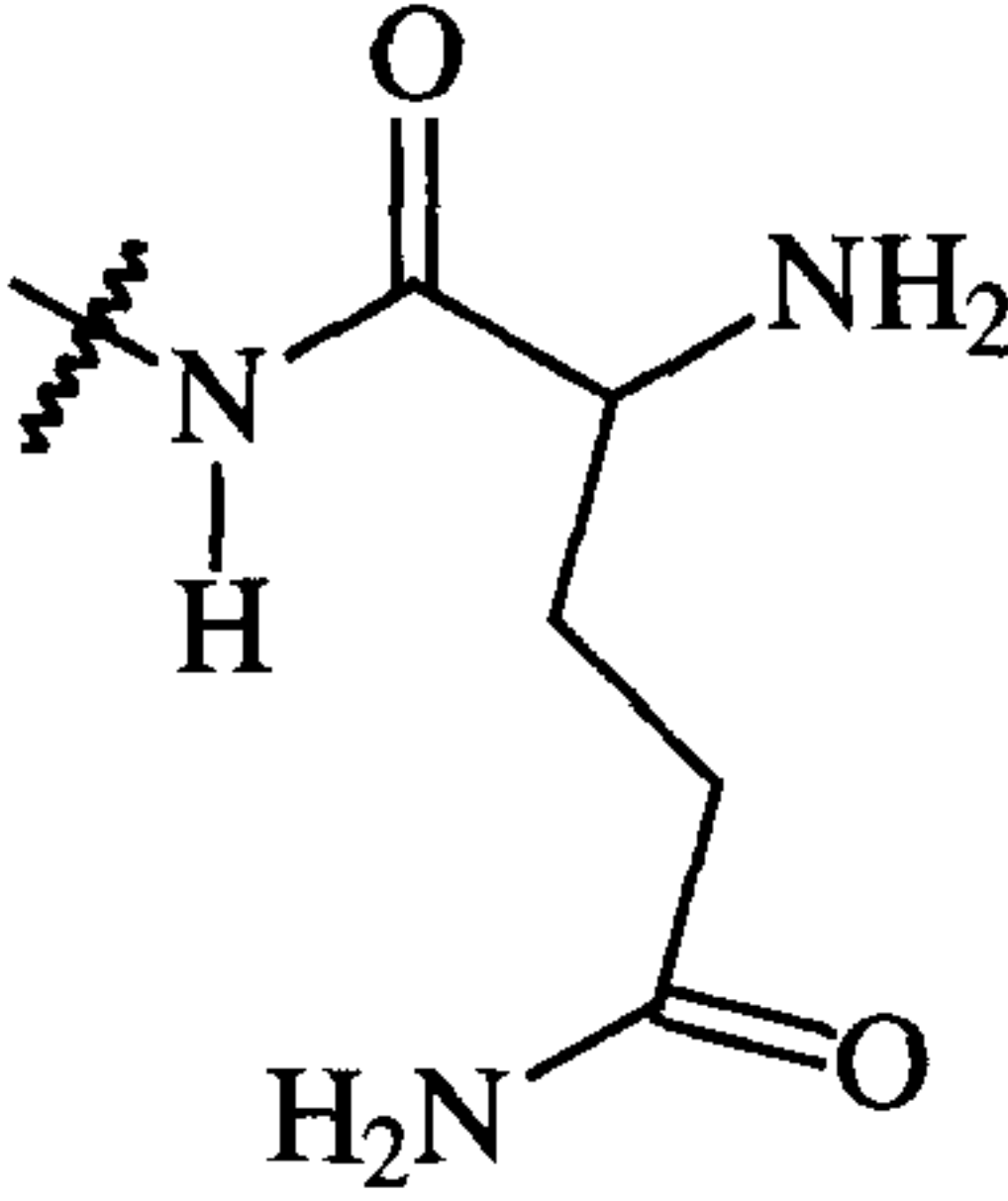
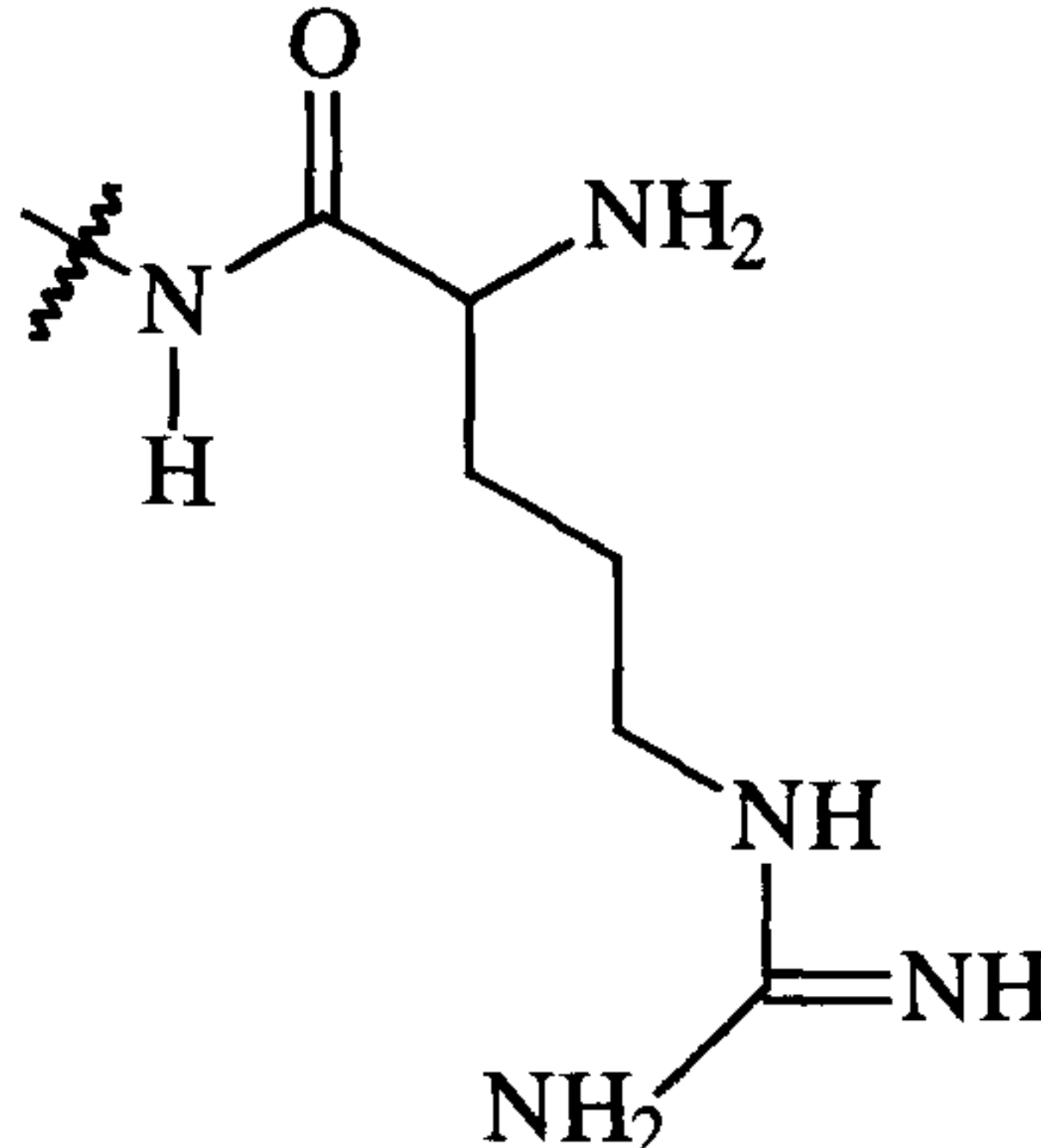
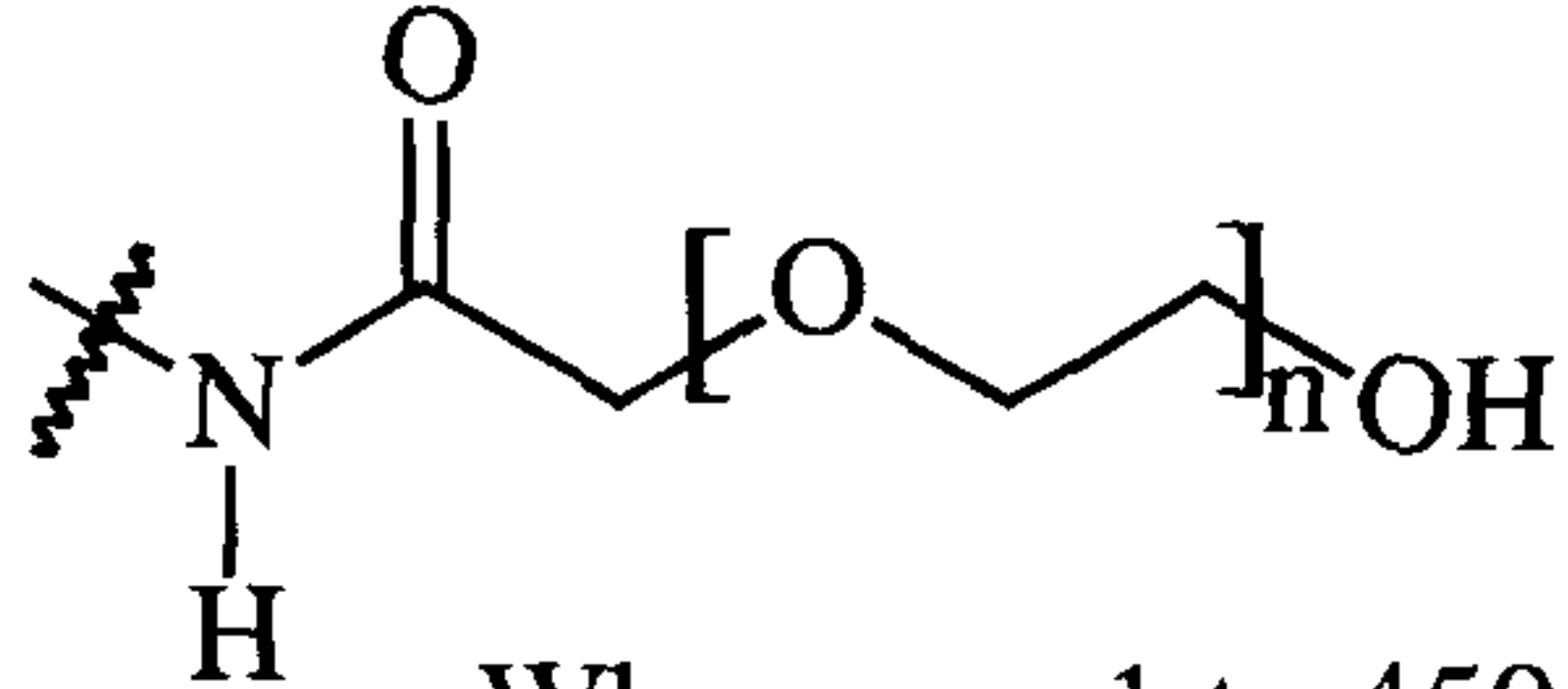
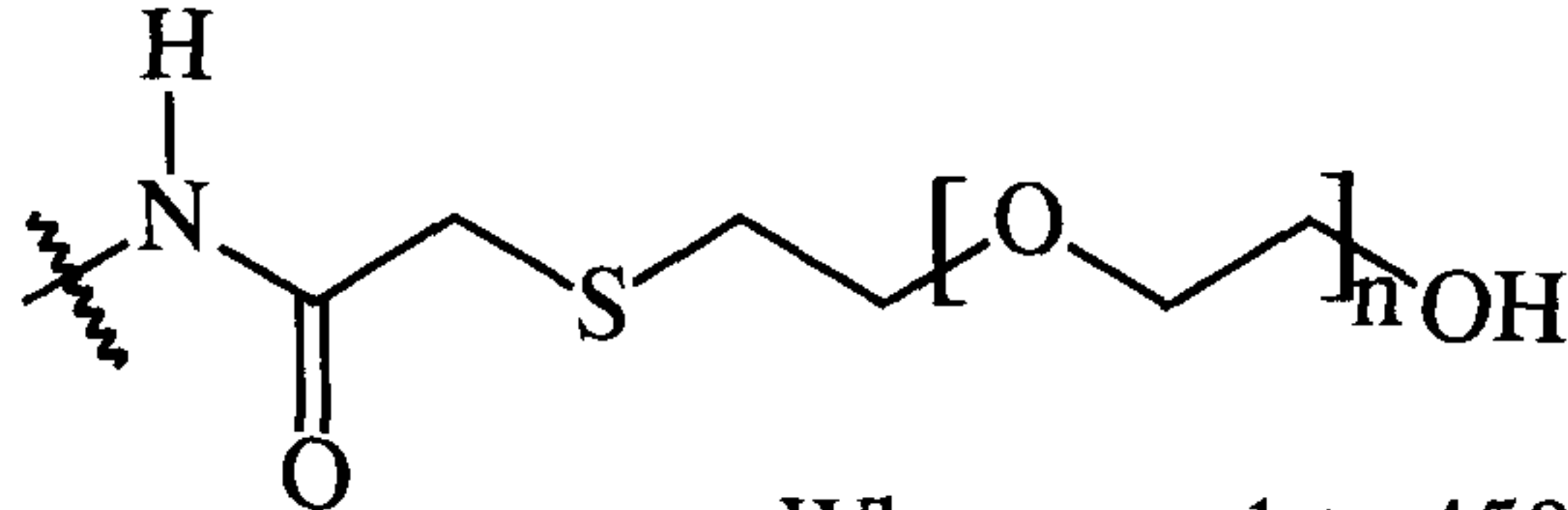
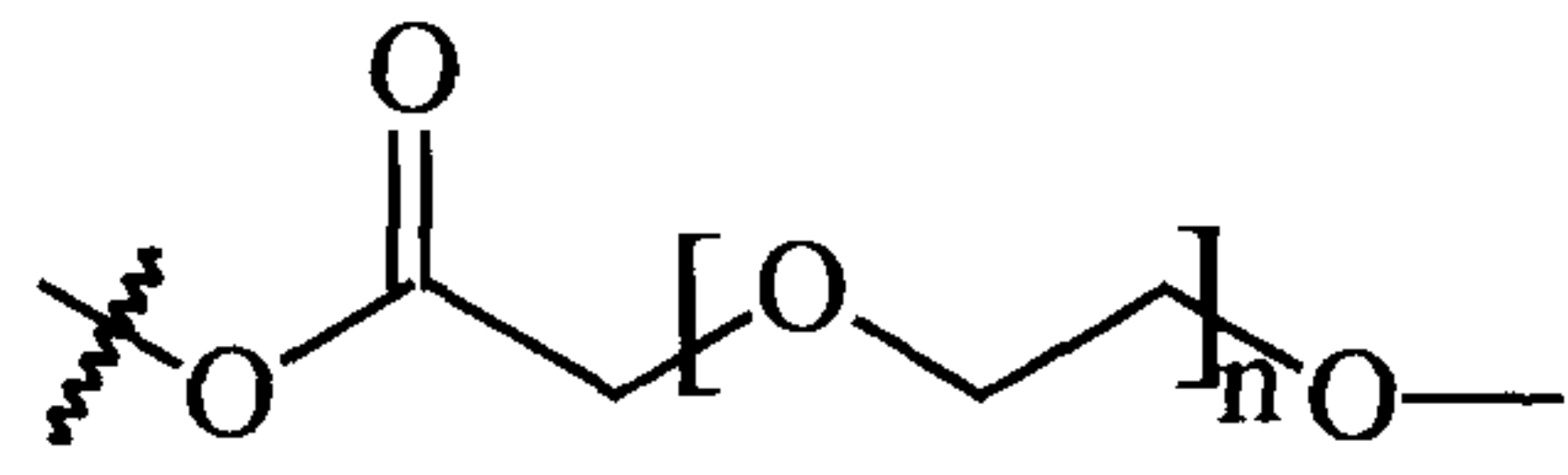
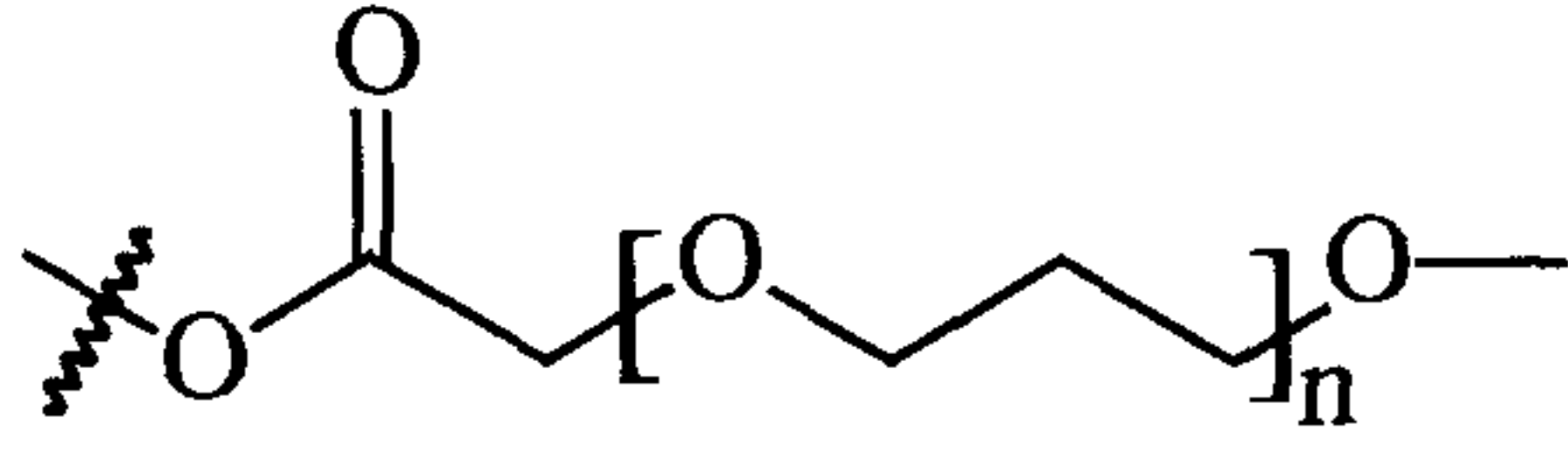
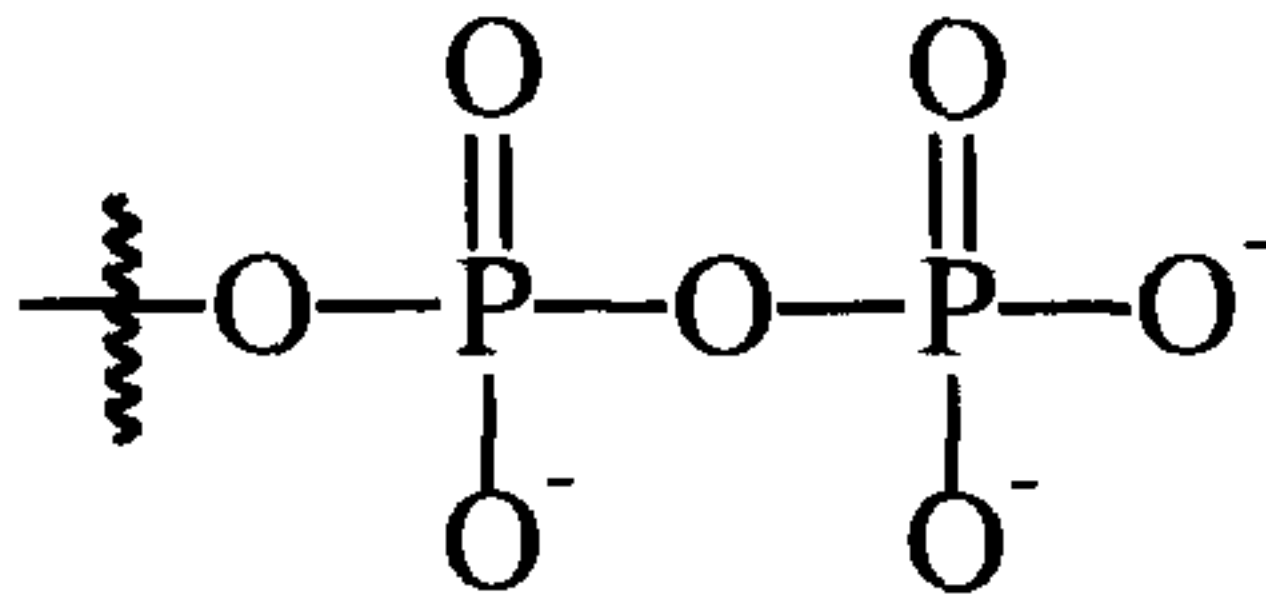
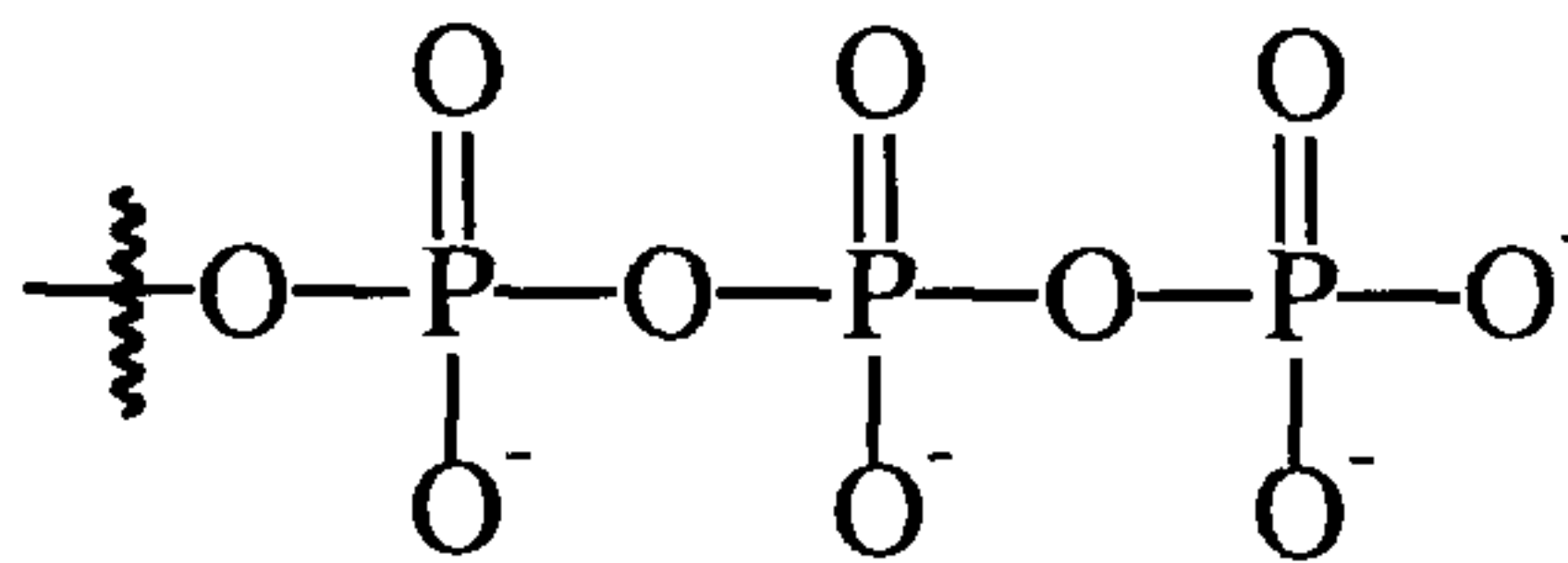
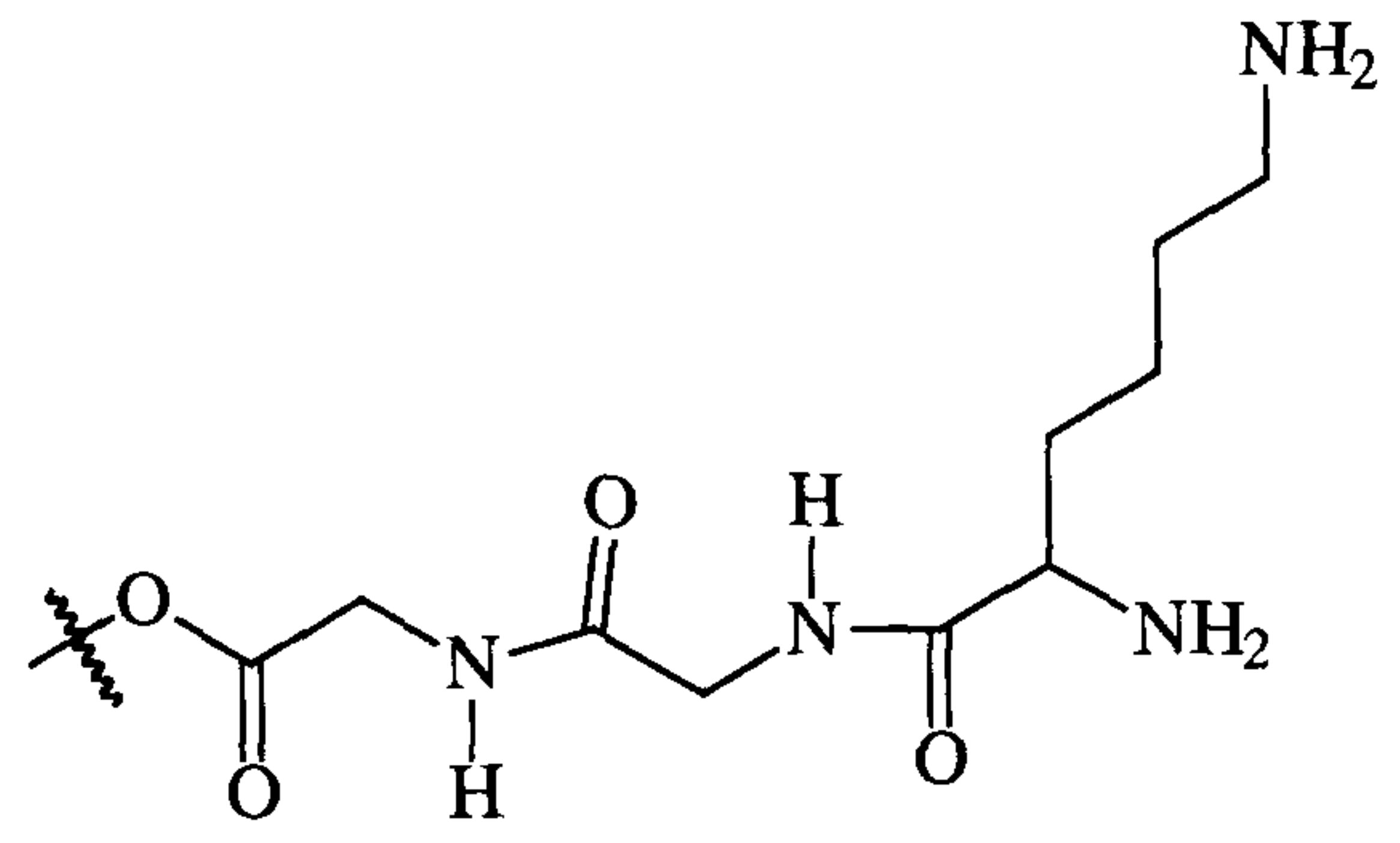
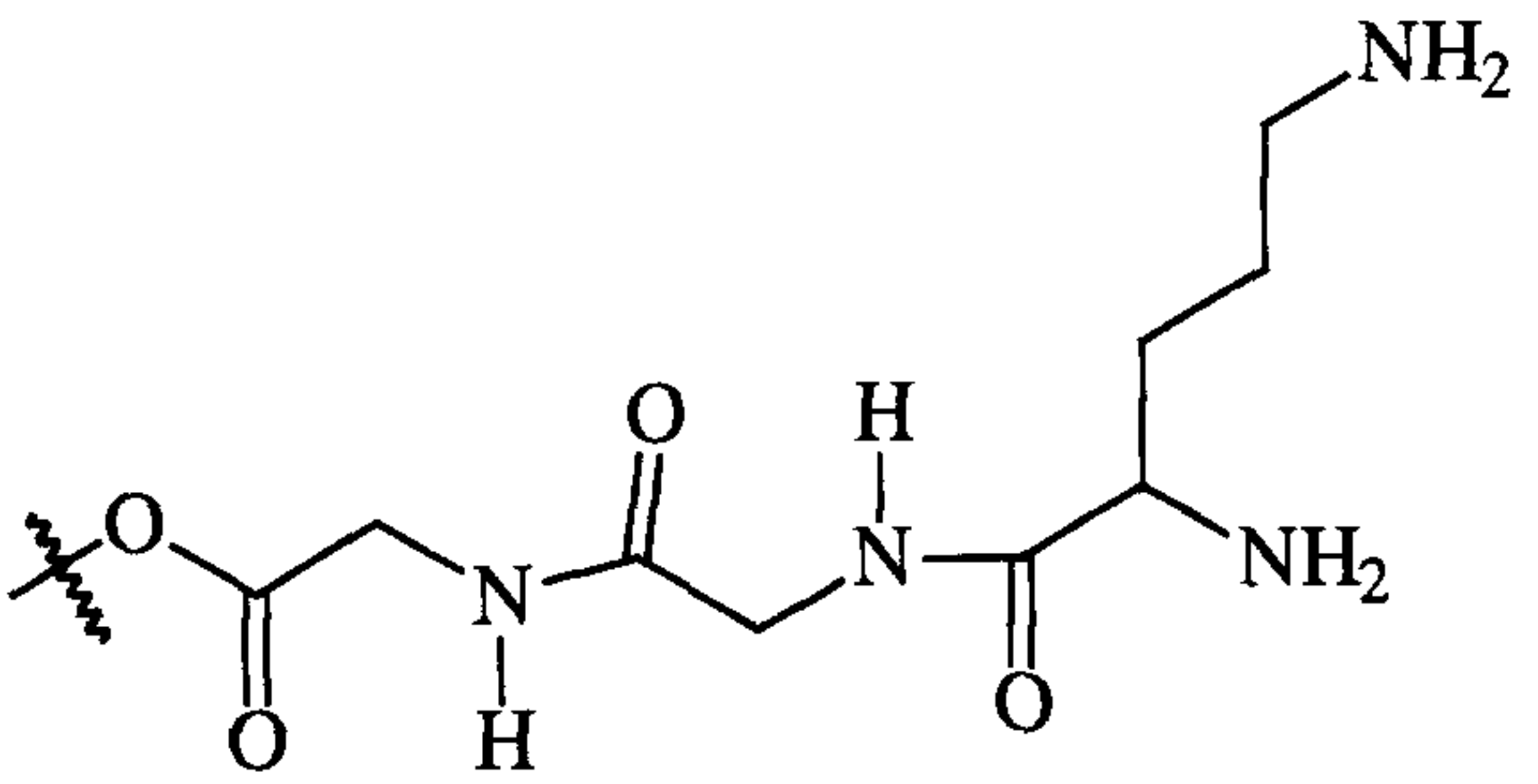
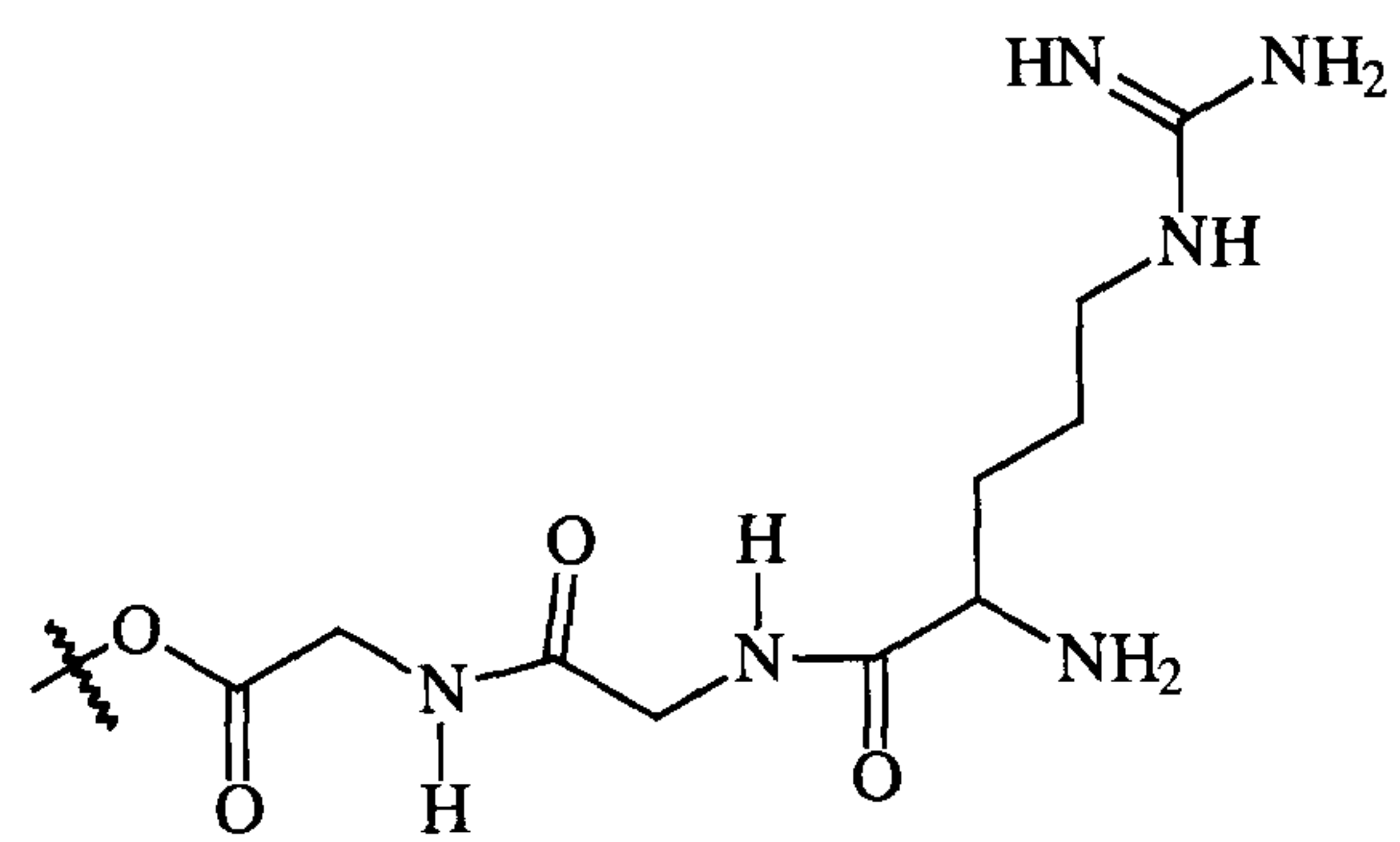
	
	
 Where n = 1 to 450	 Where n = 1 to 450
 Where n = 1 to 450	 Where n = 1 to 450
	

TABLE III – PRODRUG MOIETIES	
	
	

In some particular embodiments, each R as set out in Table III may be independently selected from H, methyl or acyl.

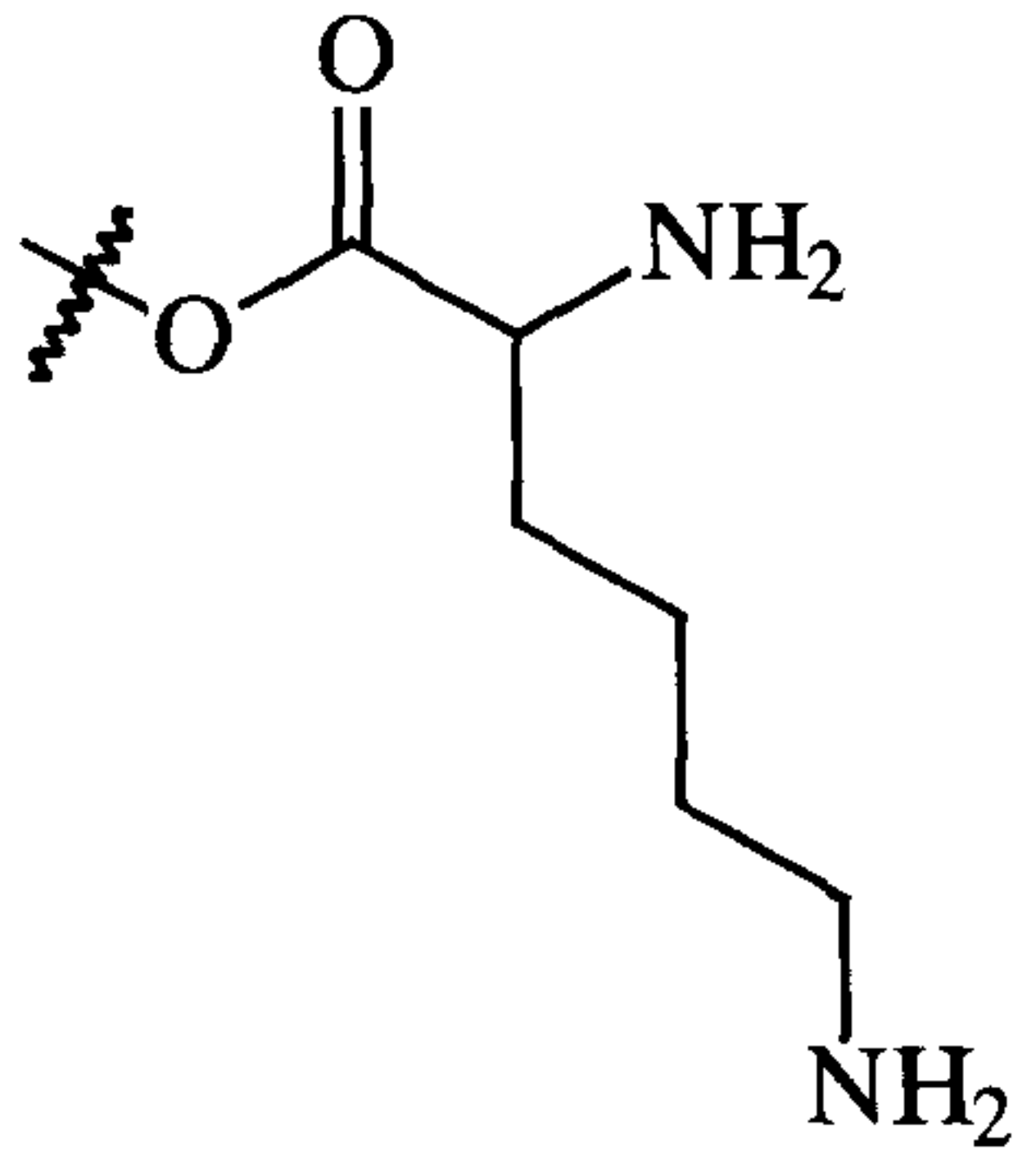
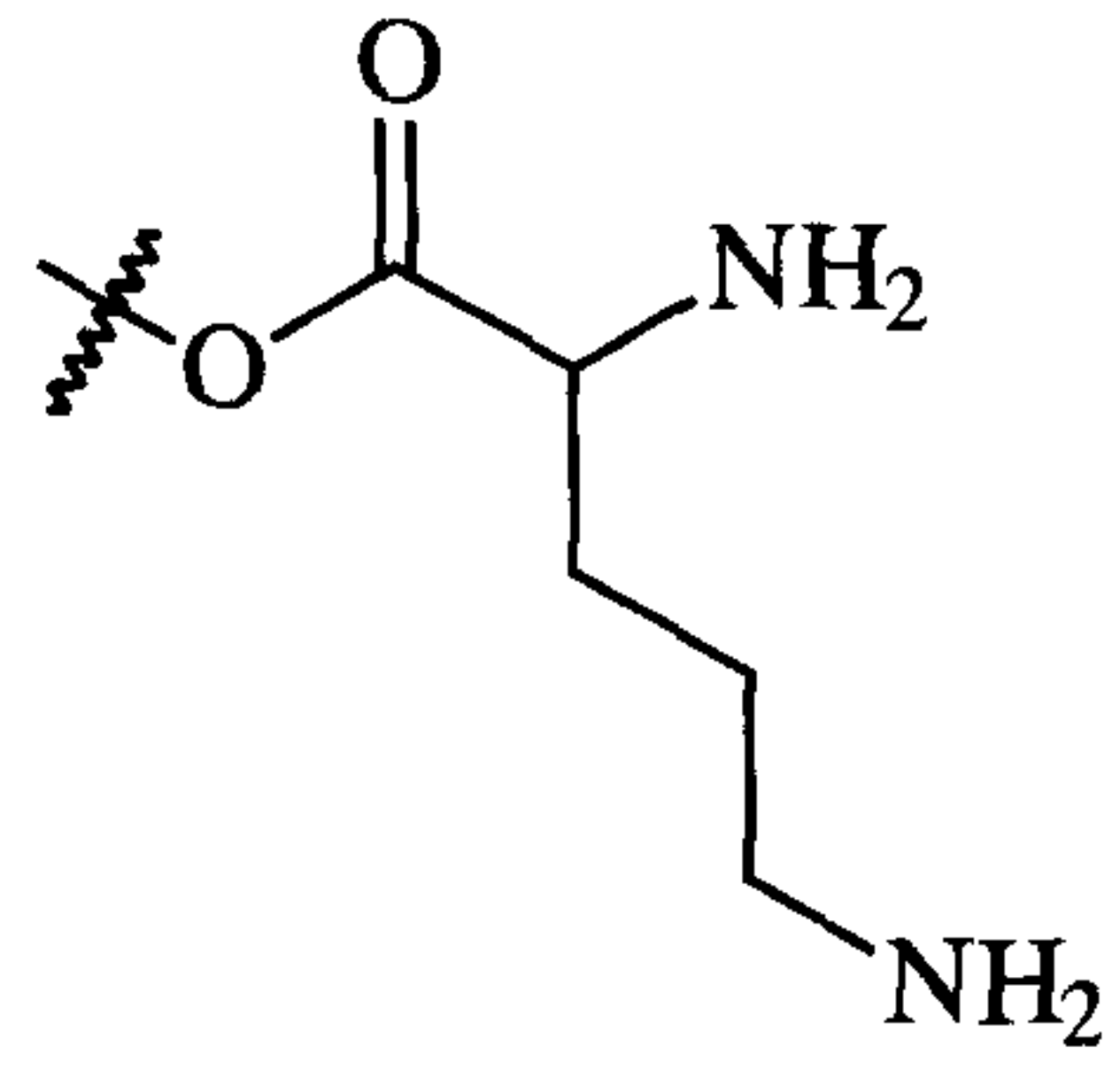
TABLE IV – ESTER PRODRUG MOIETIES	
	

TABLE IV – ESTER PRODRUG MOIETIES

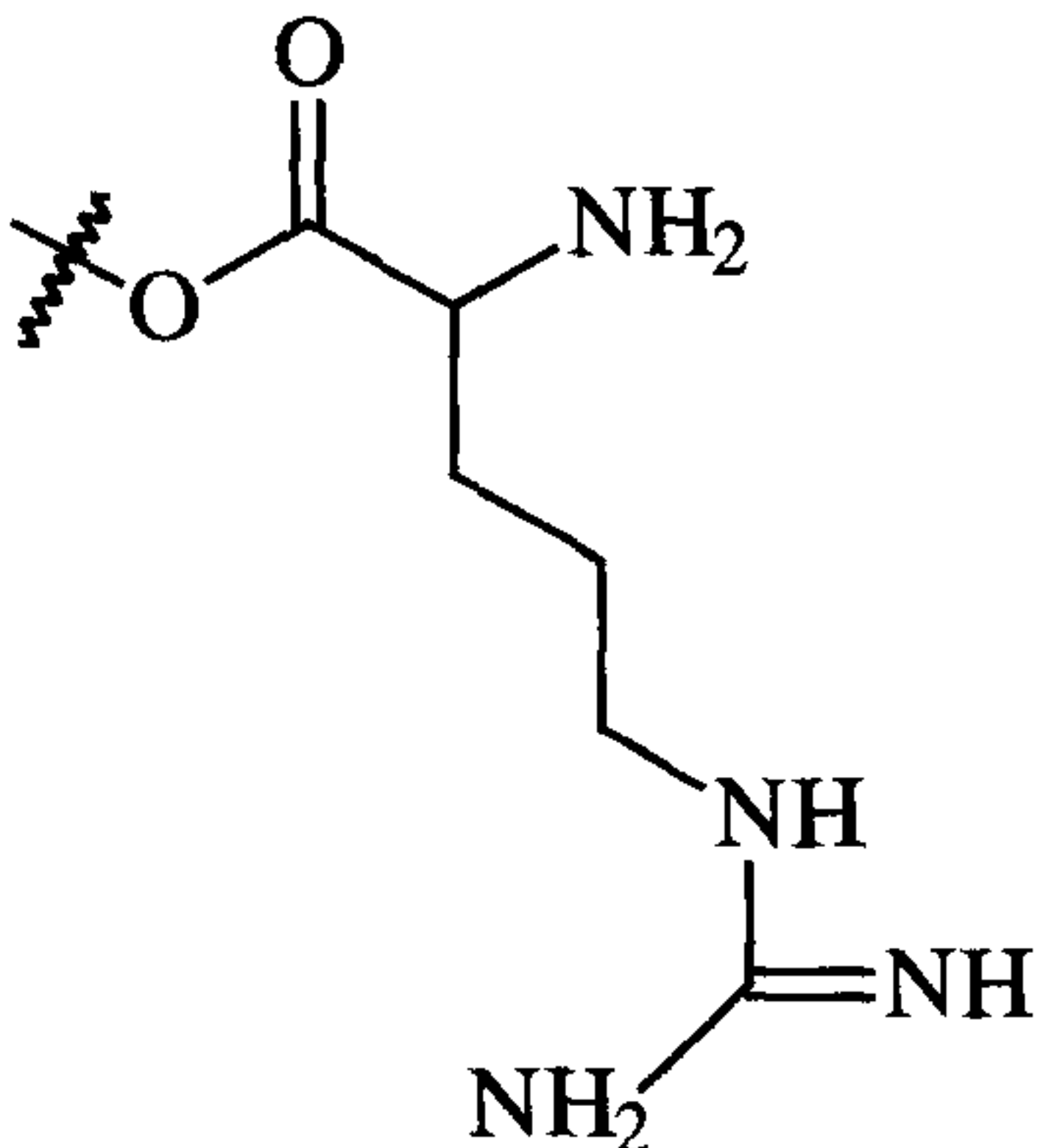
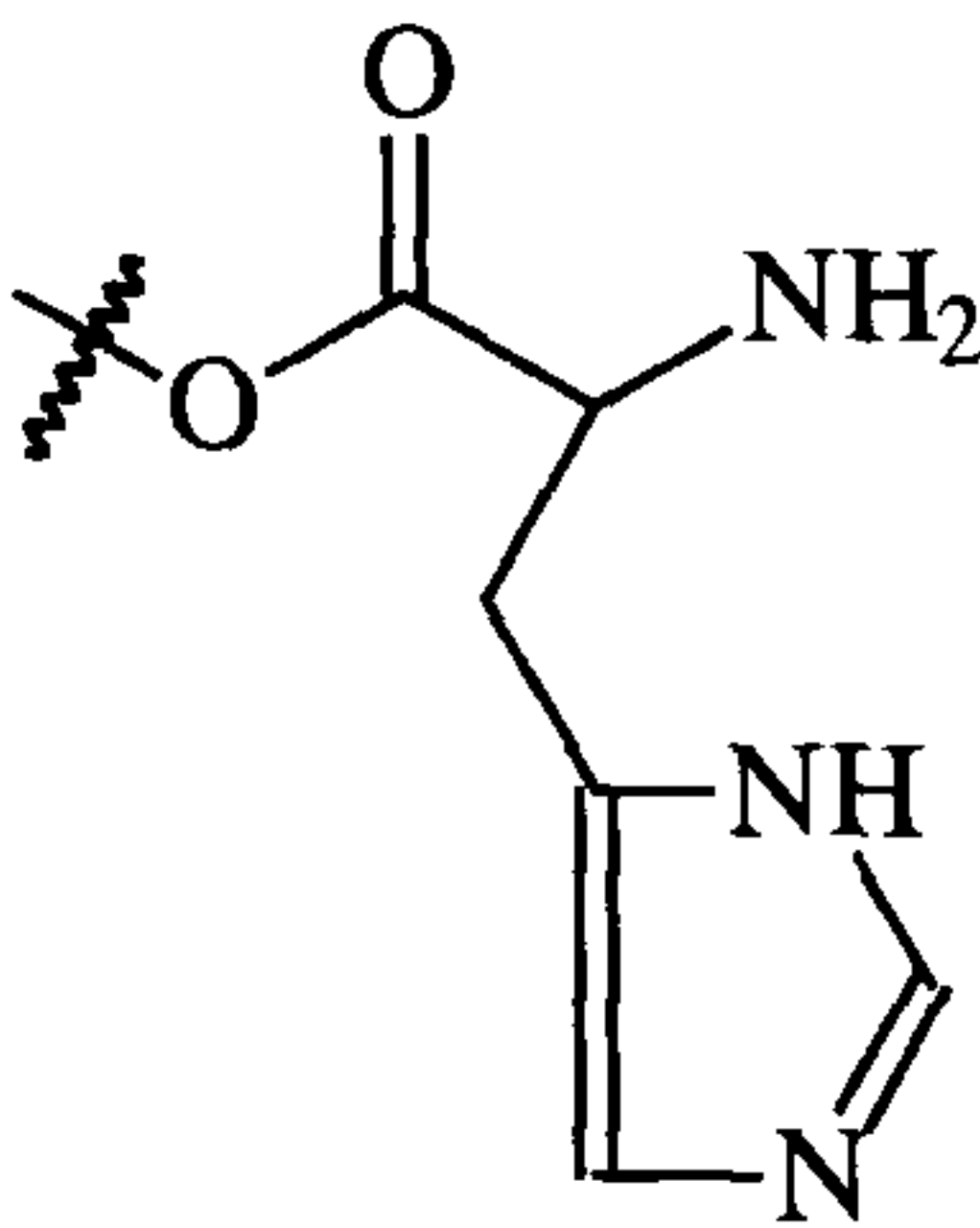
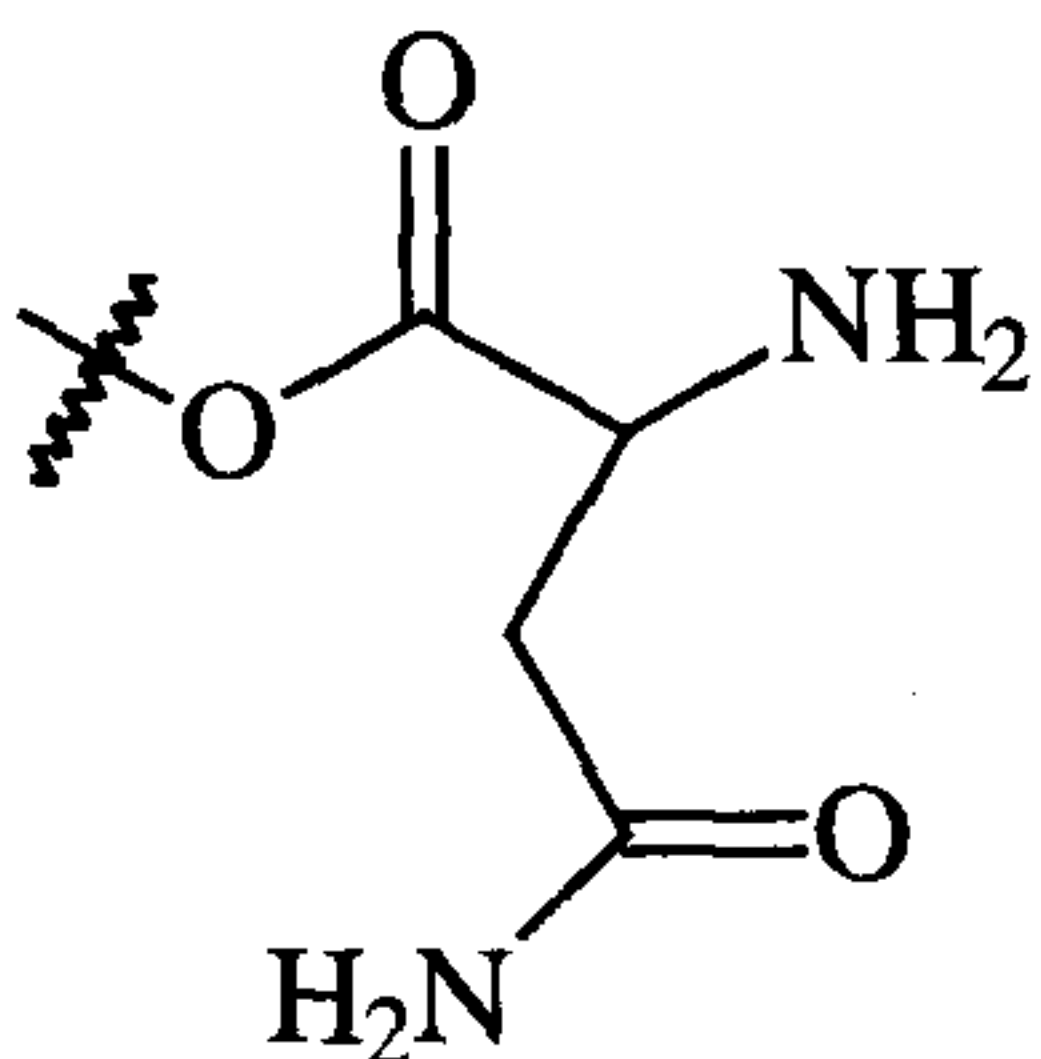
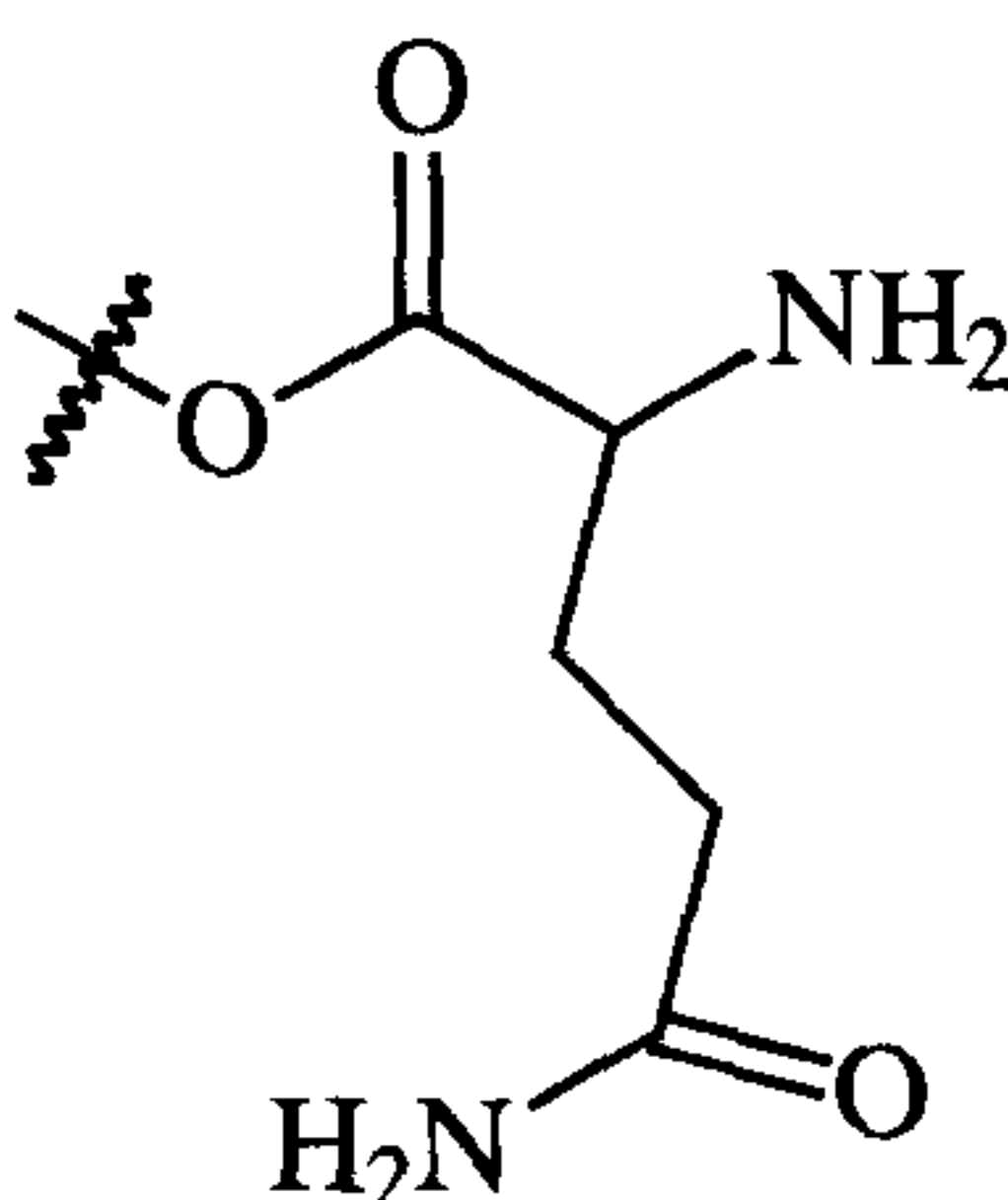
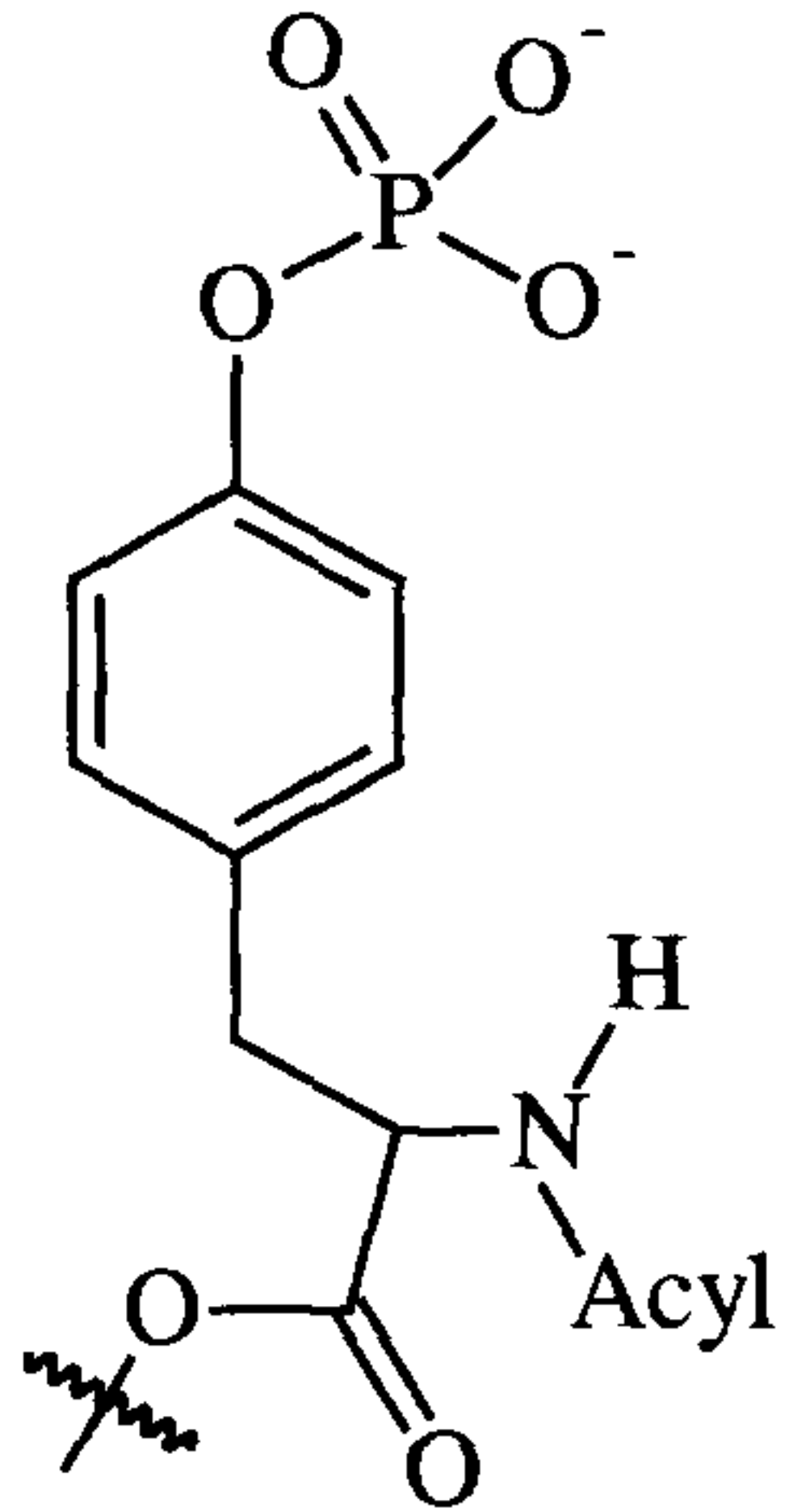
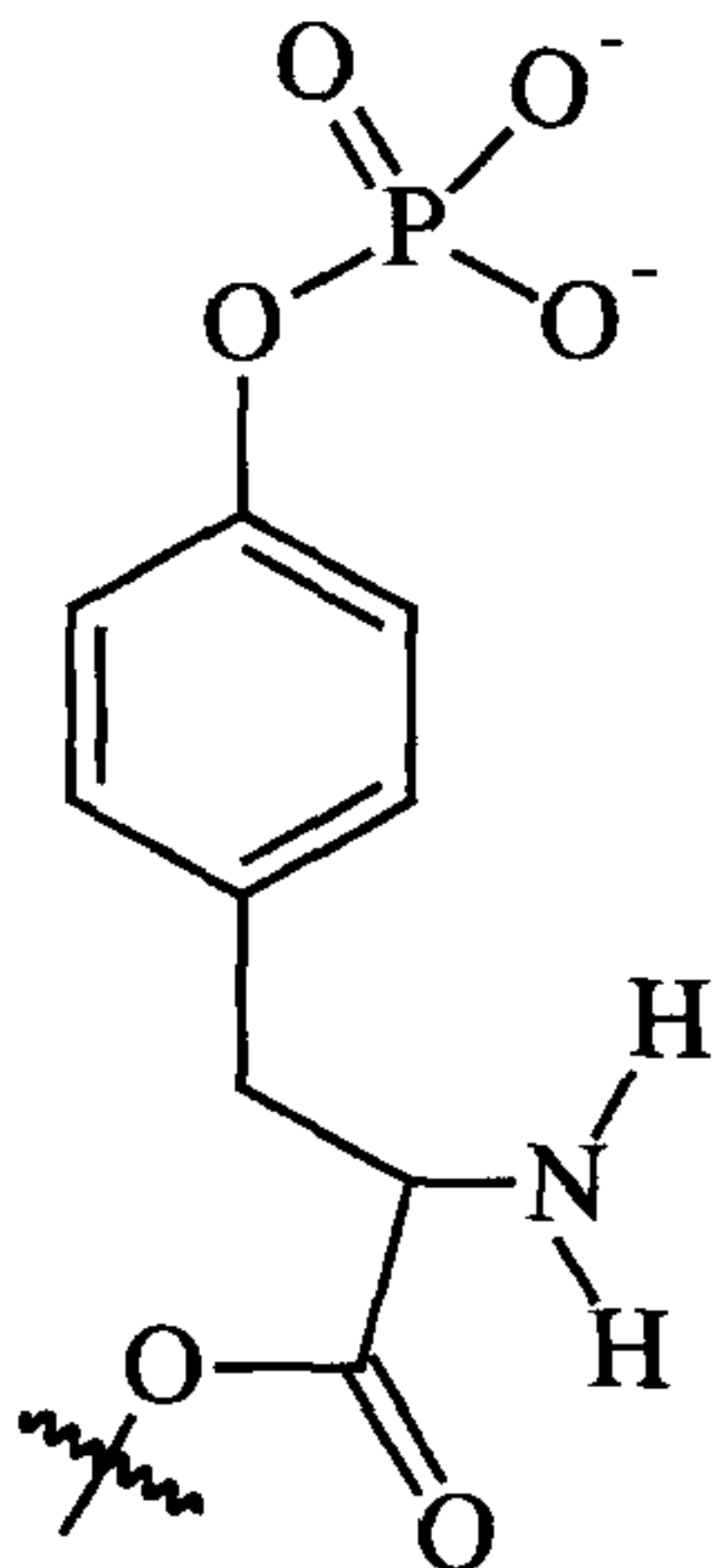
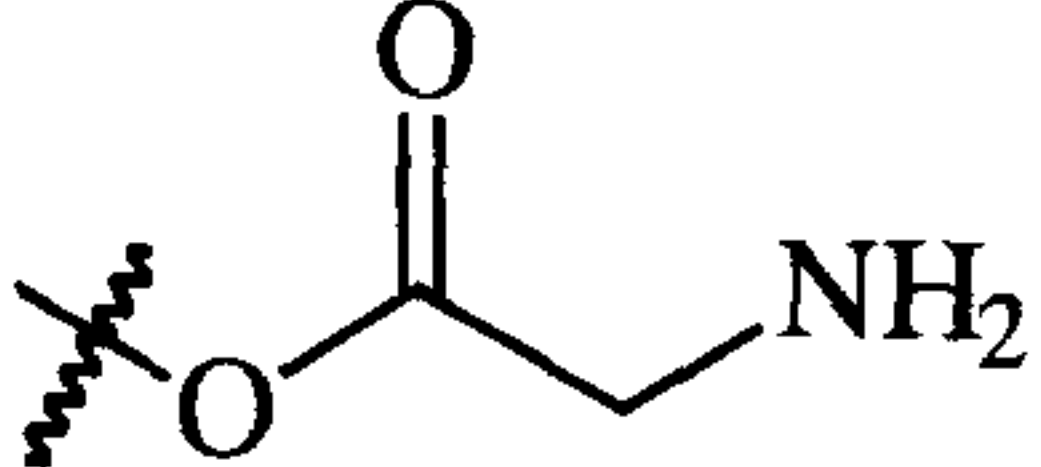
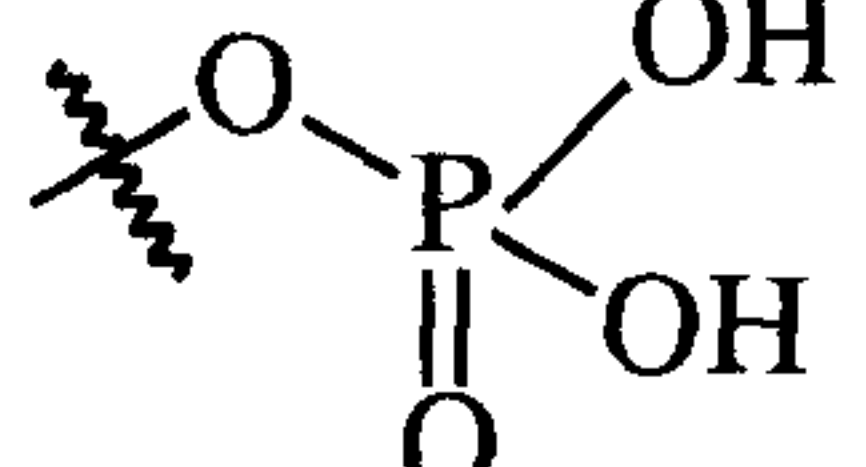
	
	
	
	

TABLE IV - ESTER PRODRUG MOIETIES

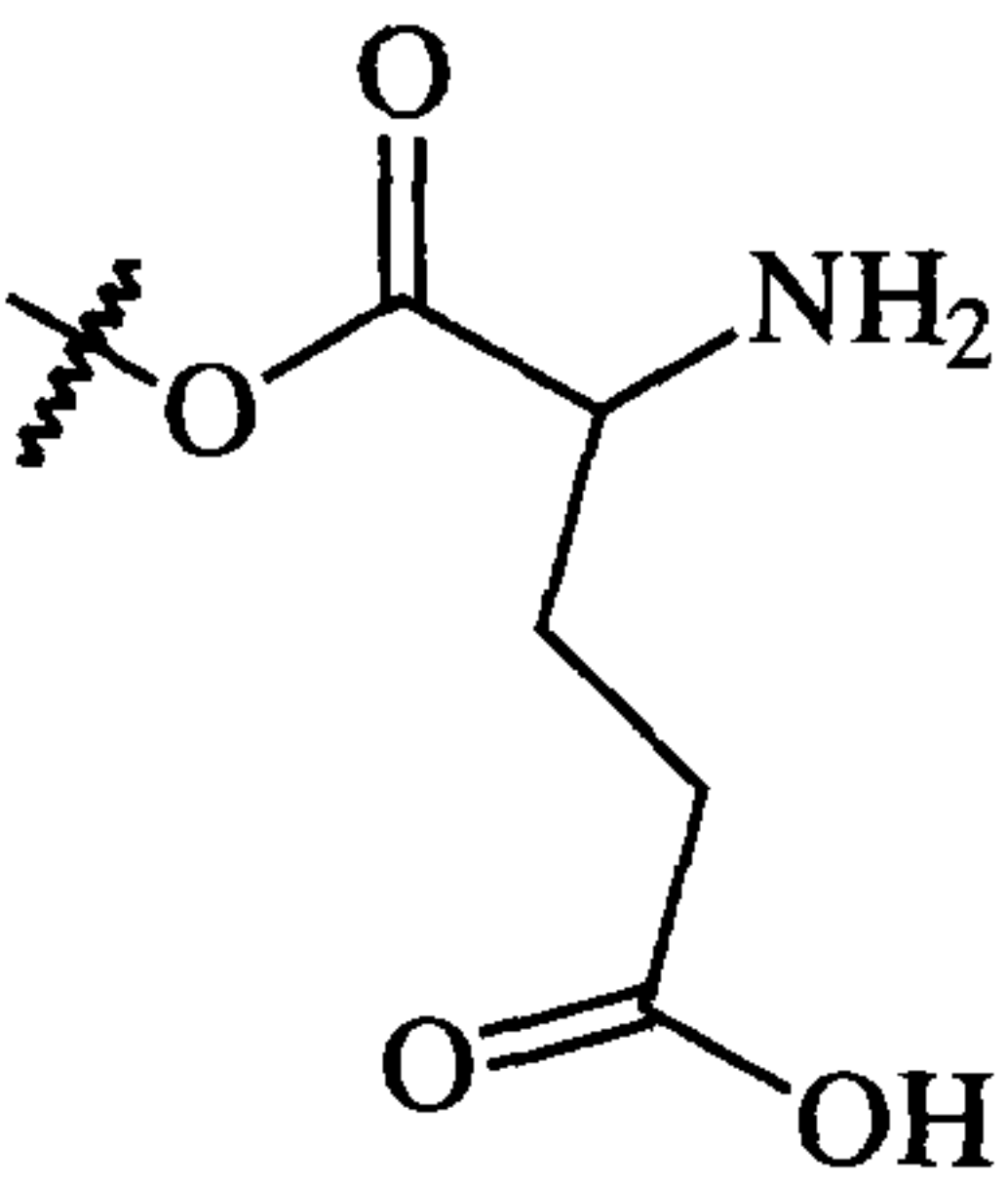
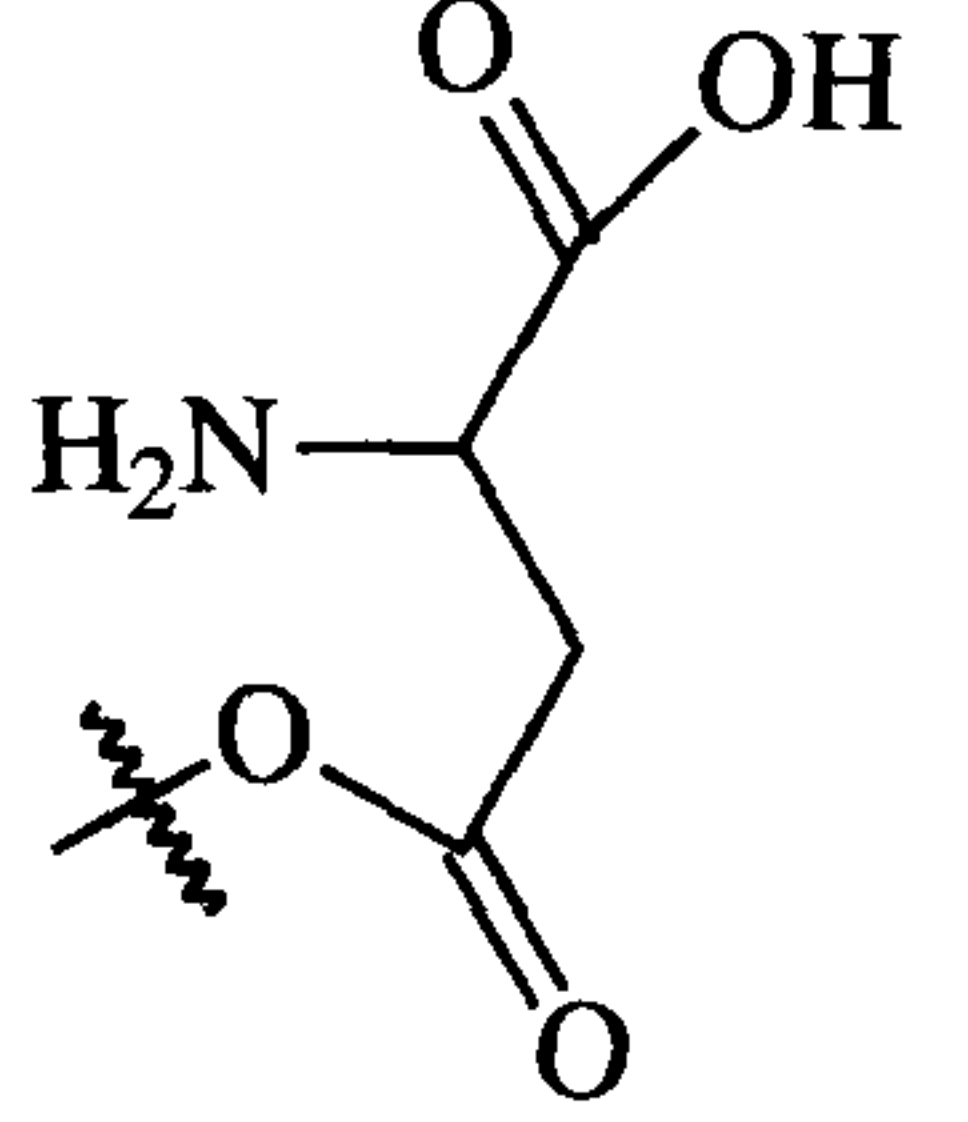
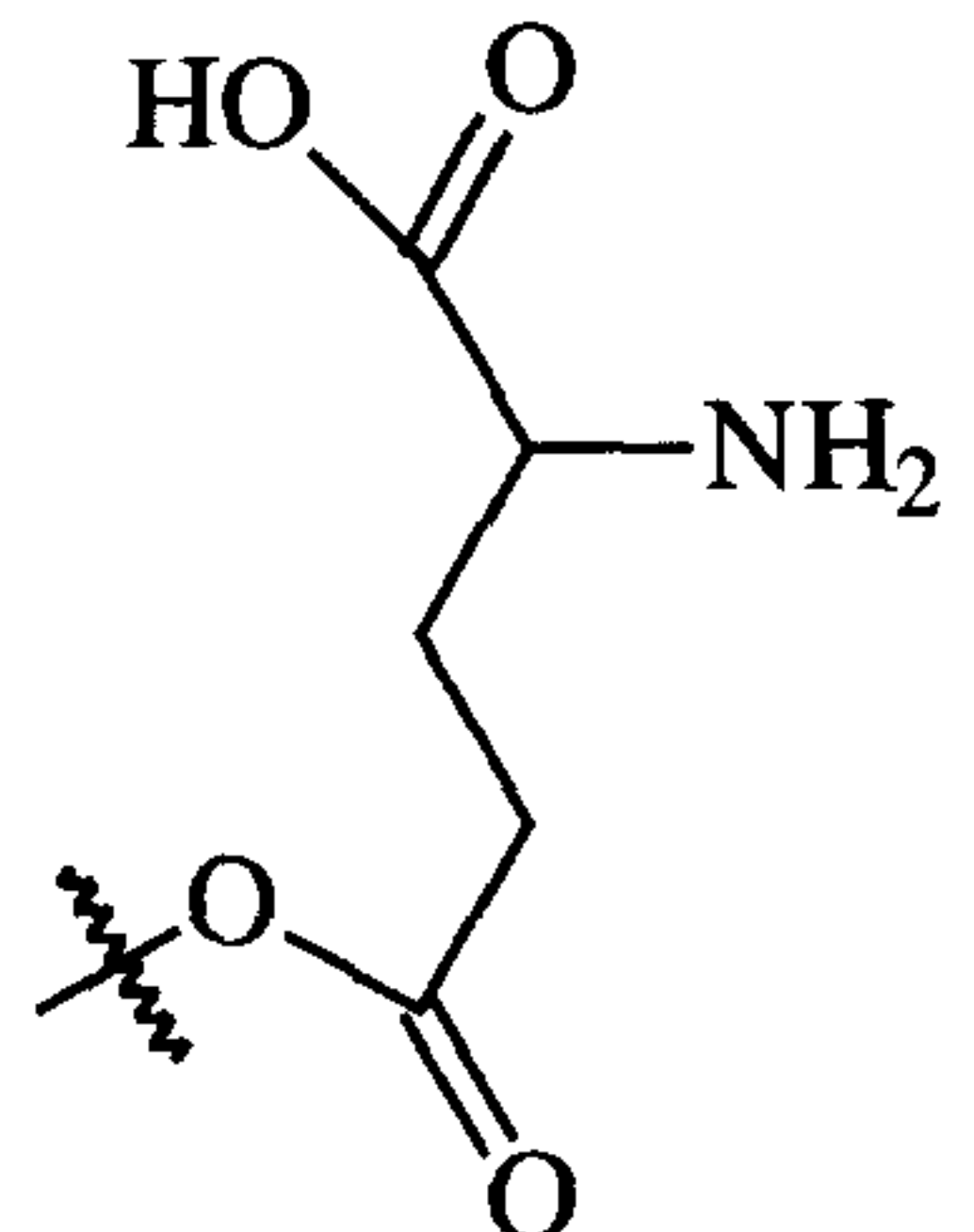
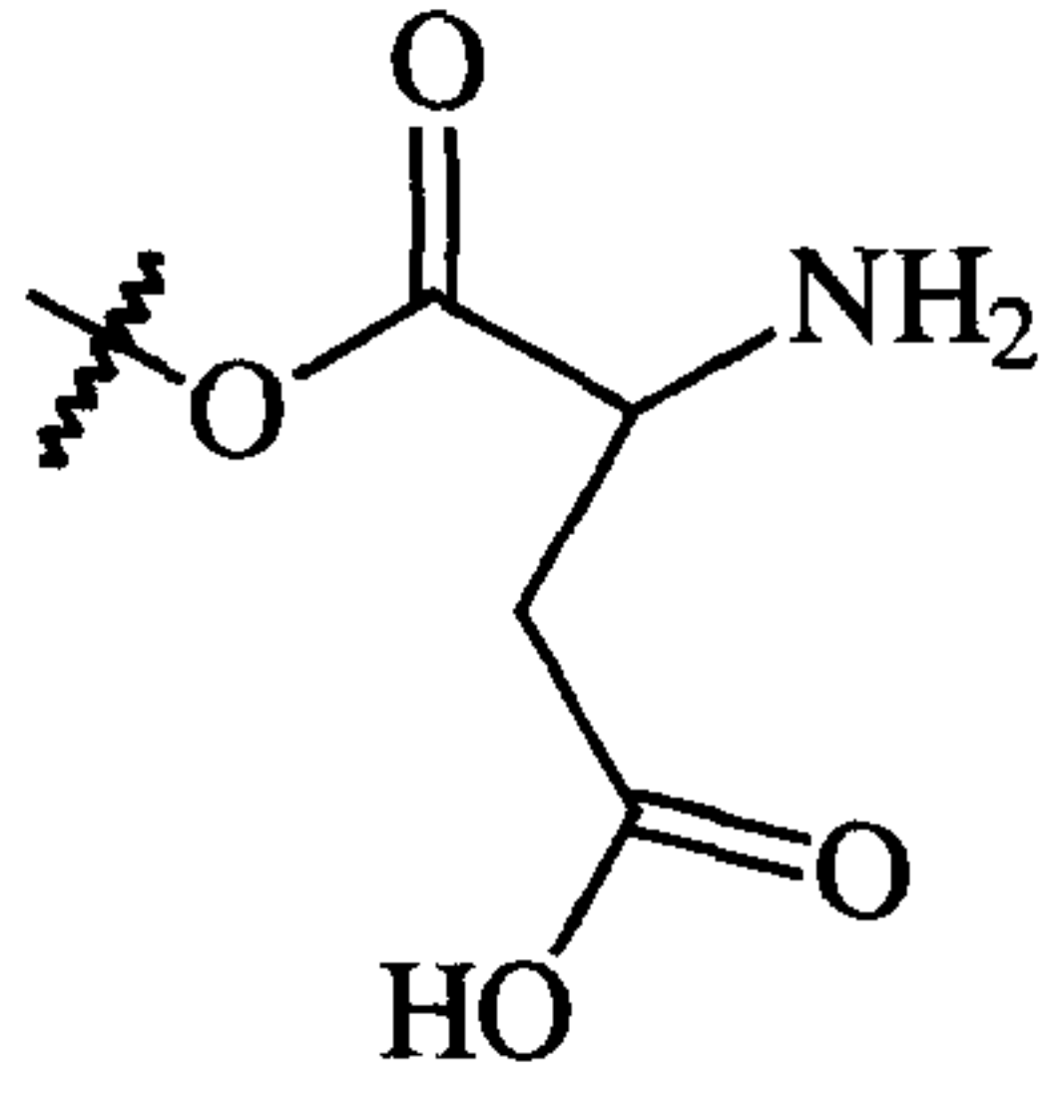
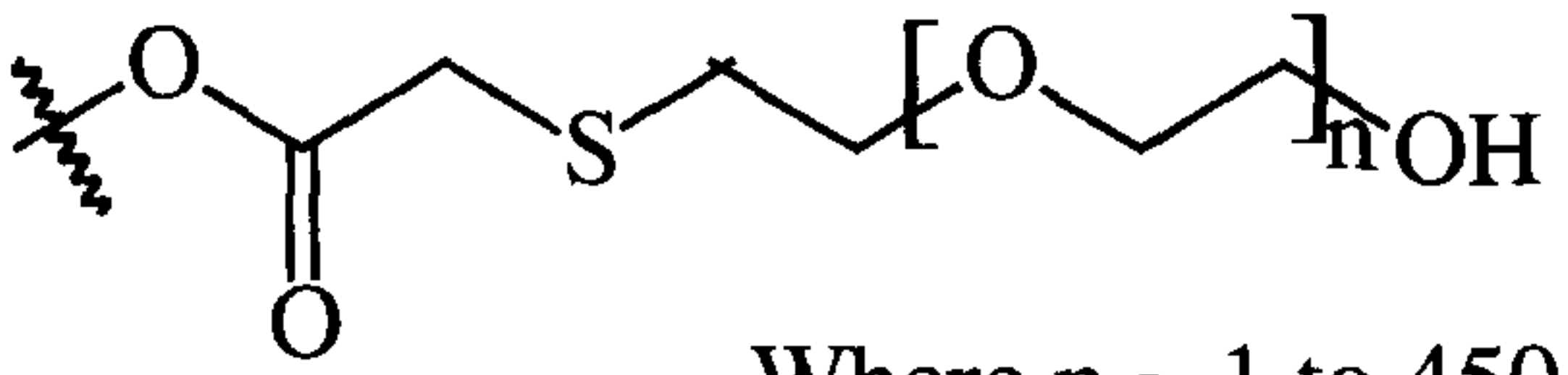
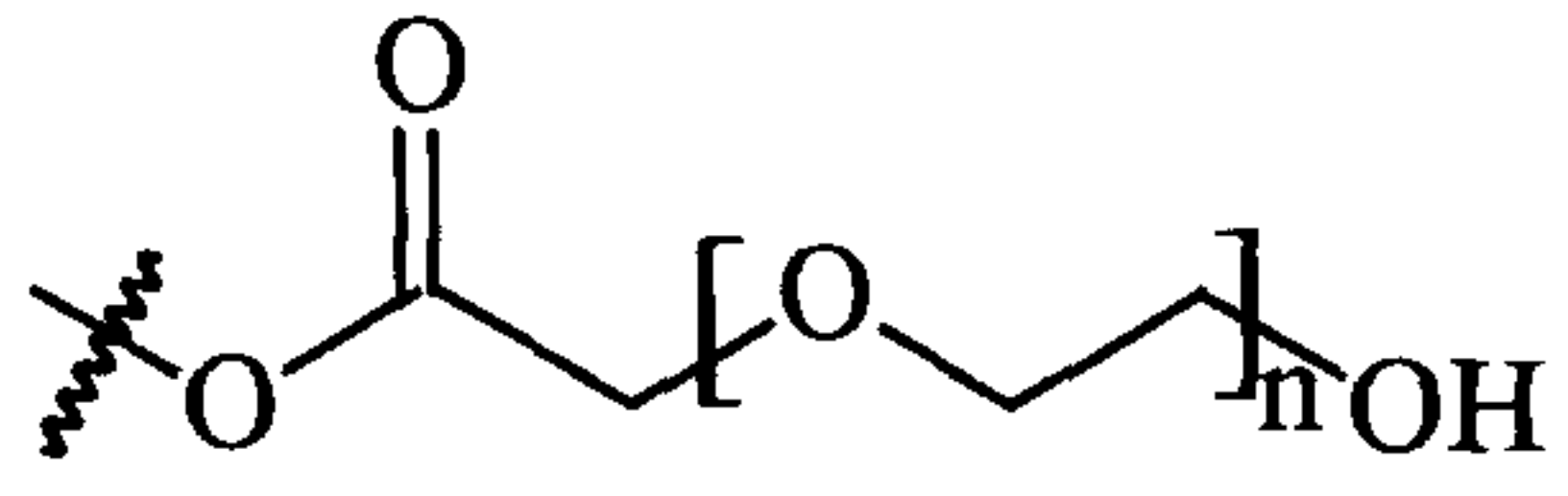
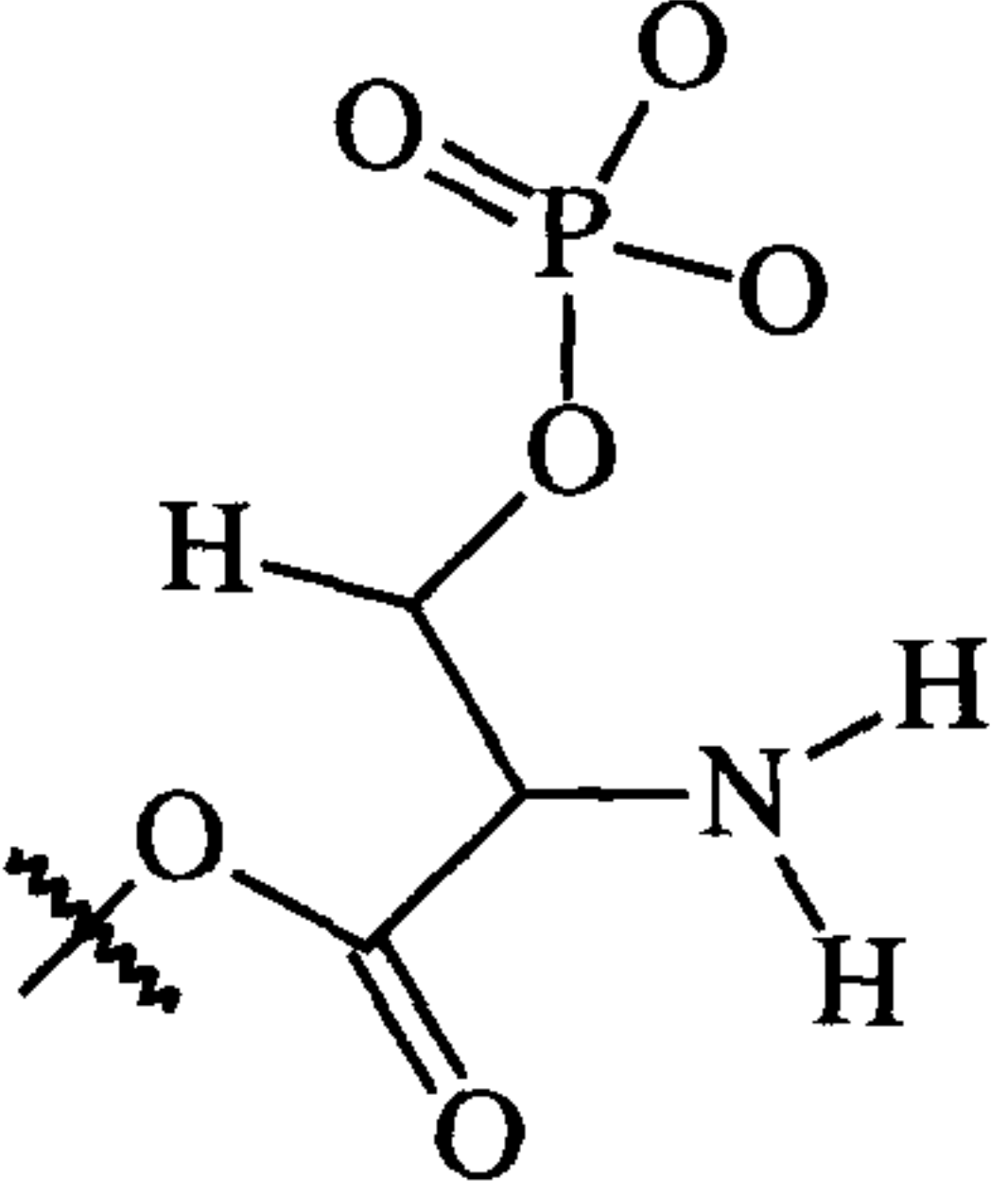
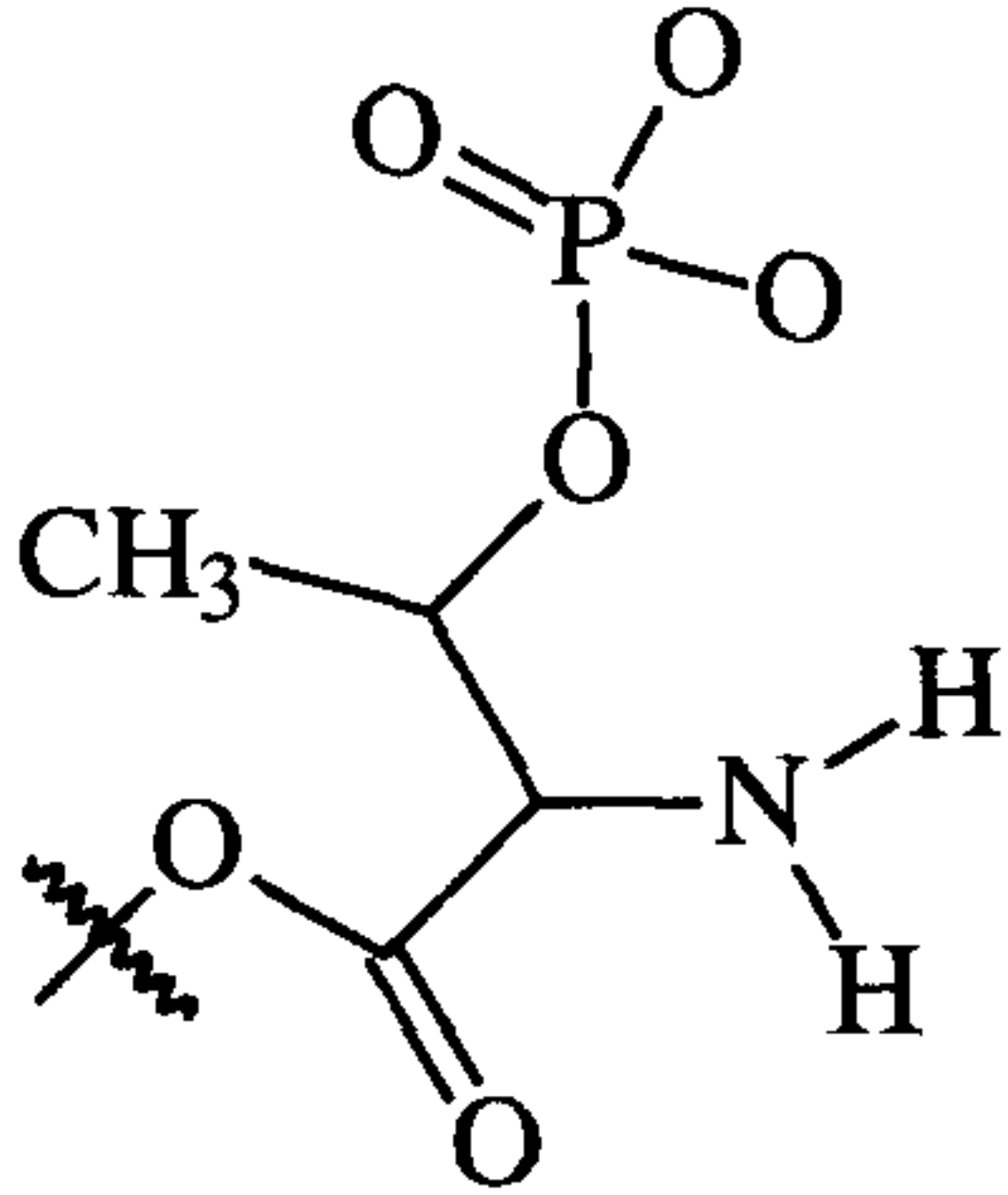
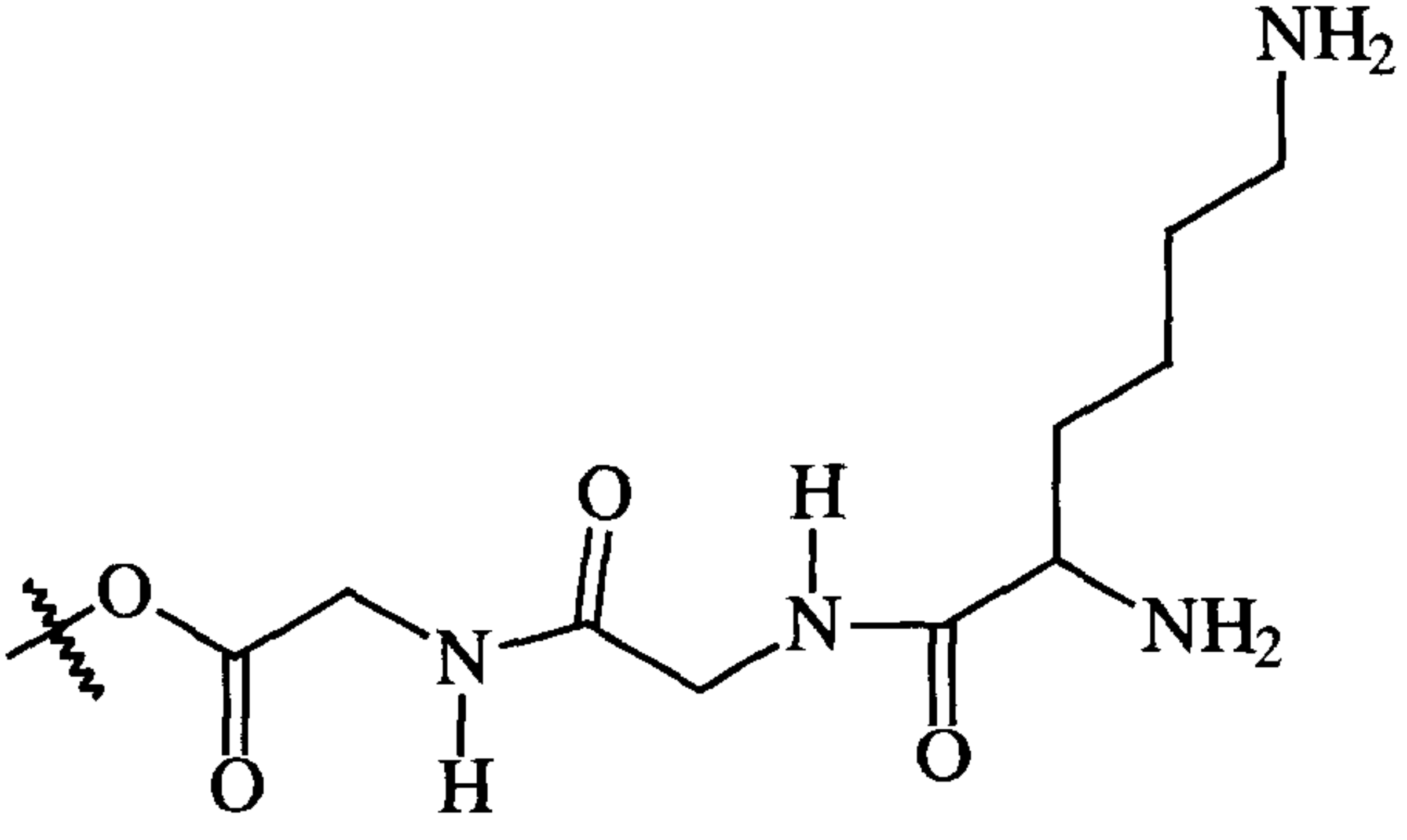
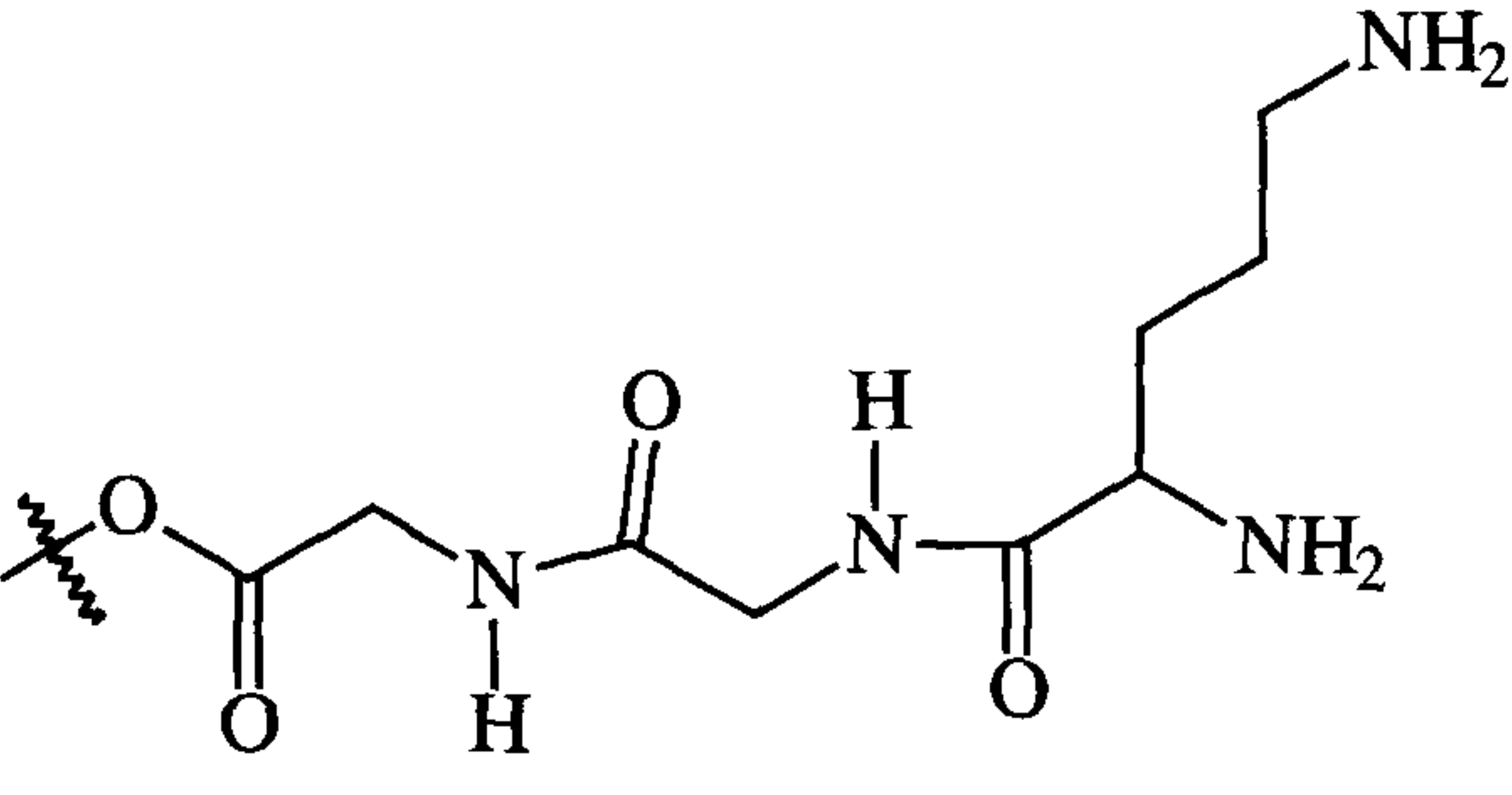
	
	
 Where n = 1 to 450	 Where n = 1 to 450
	
	

TABLE IV - ESTER PRODRUG MOIETIES

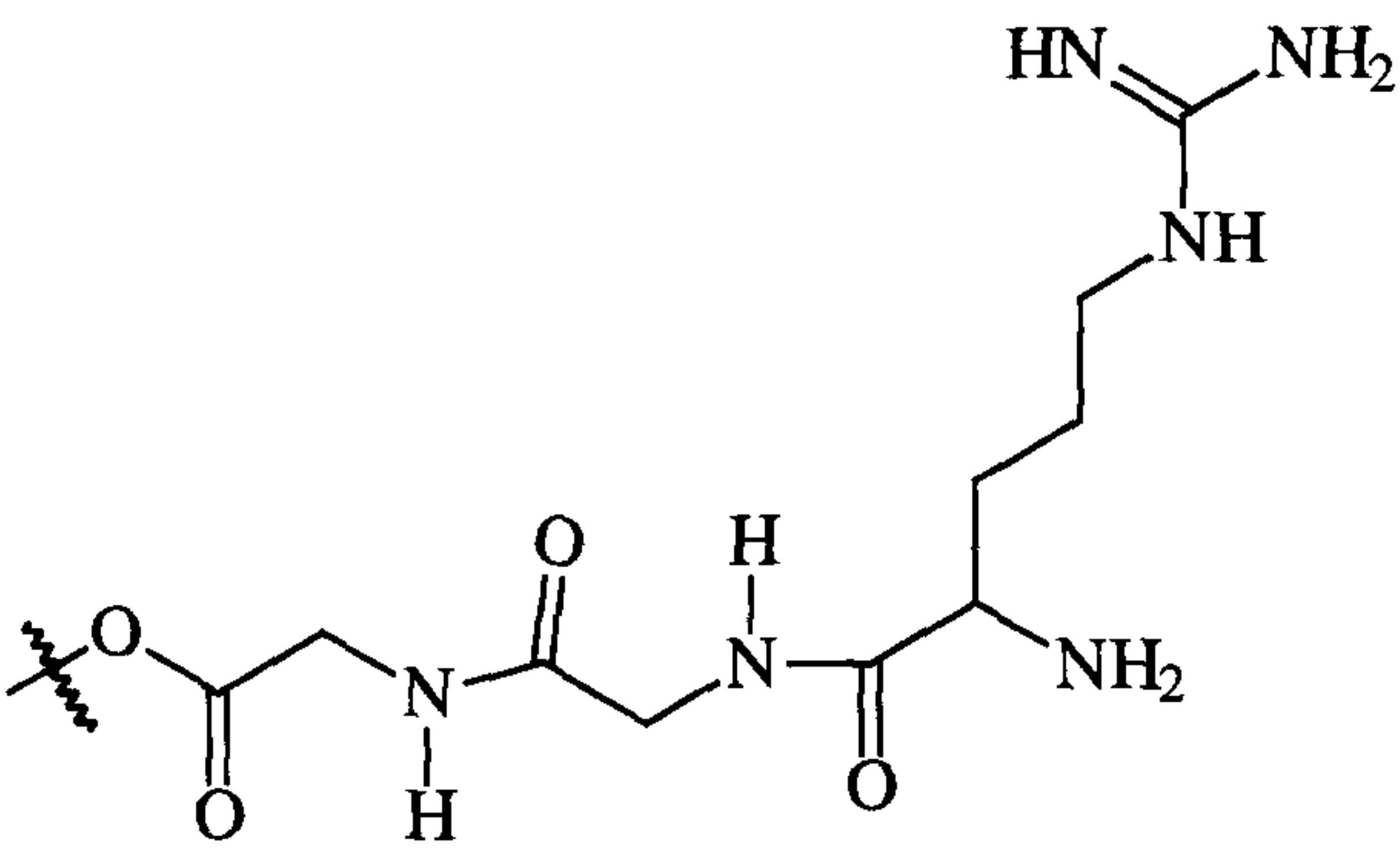
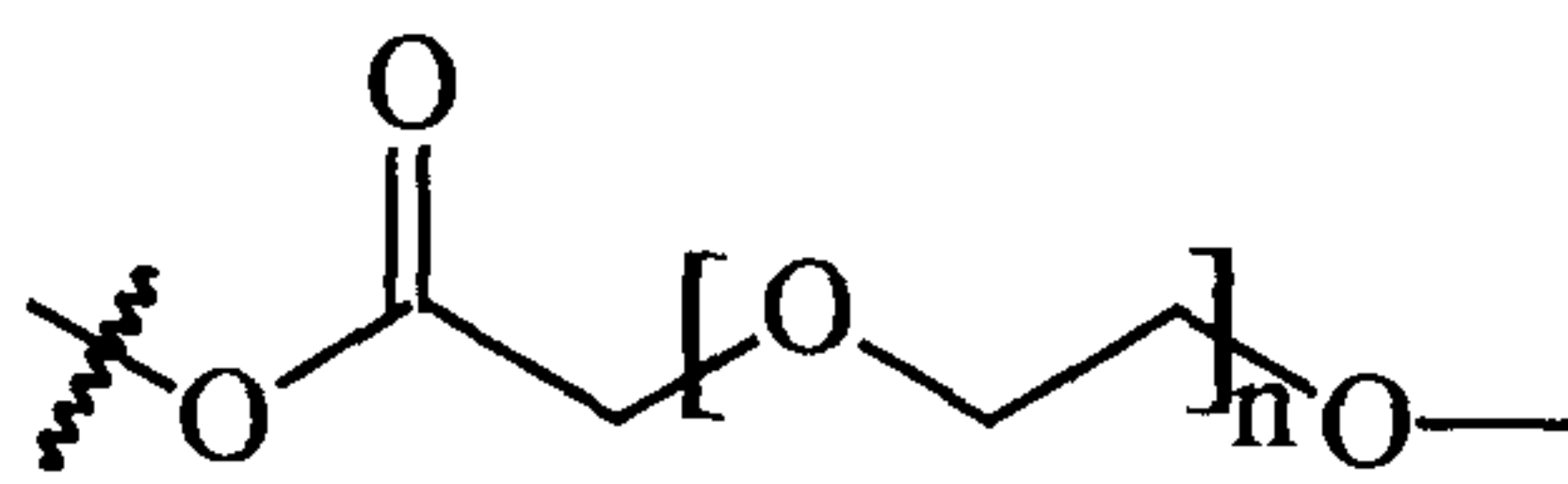
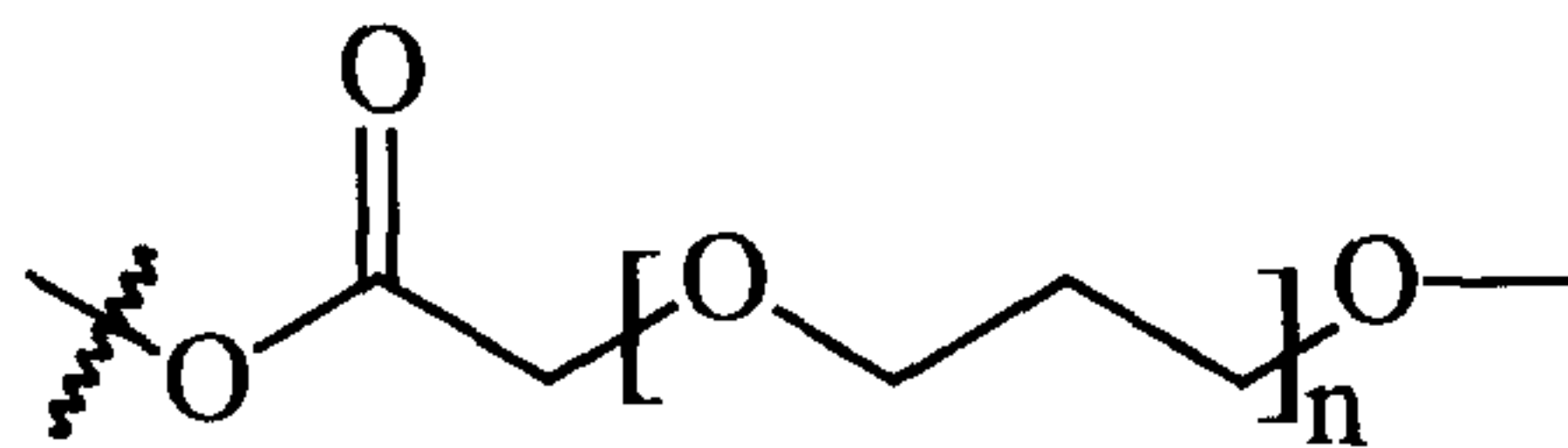
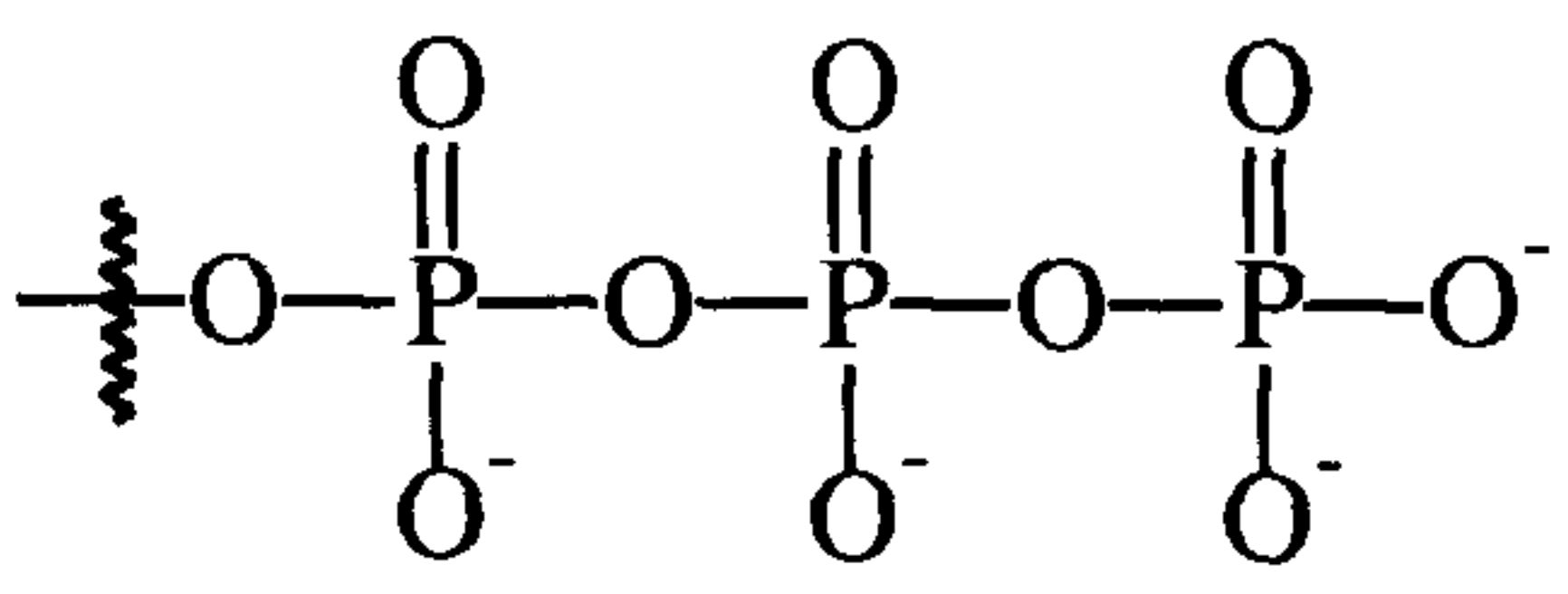
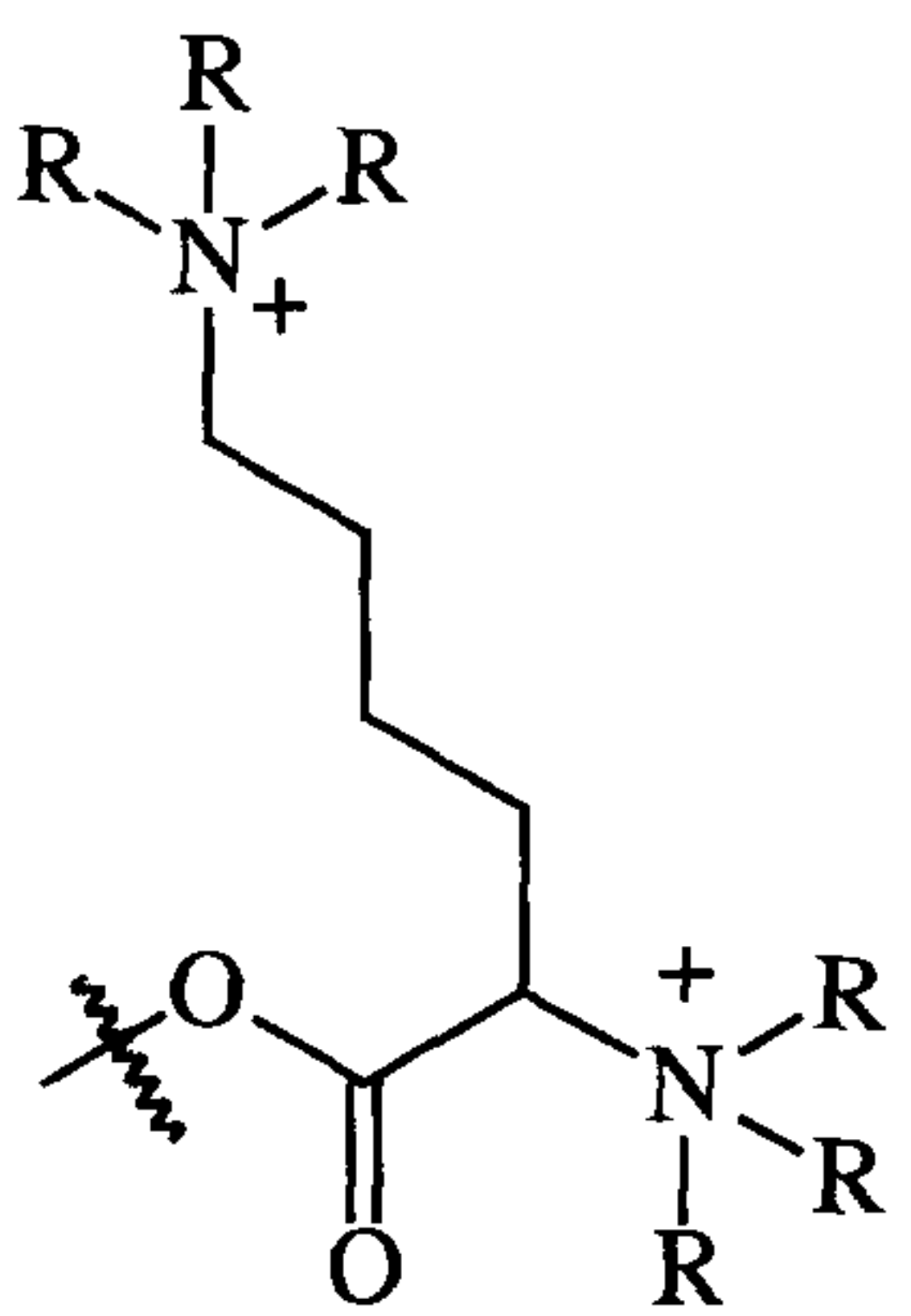
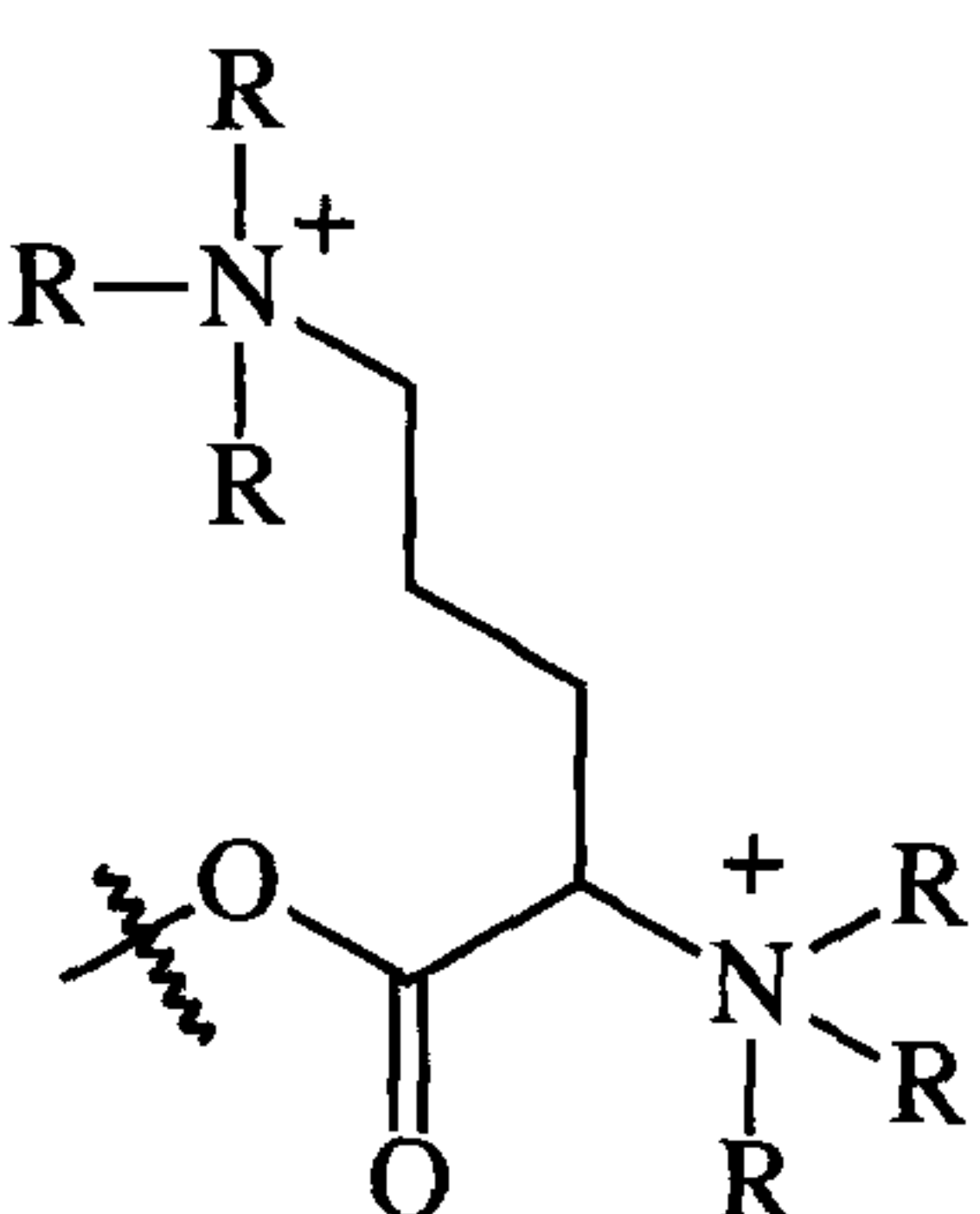
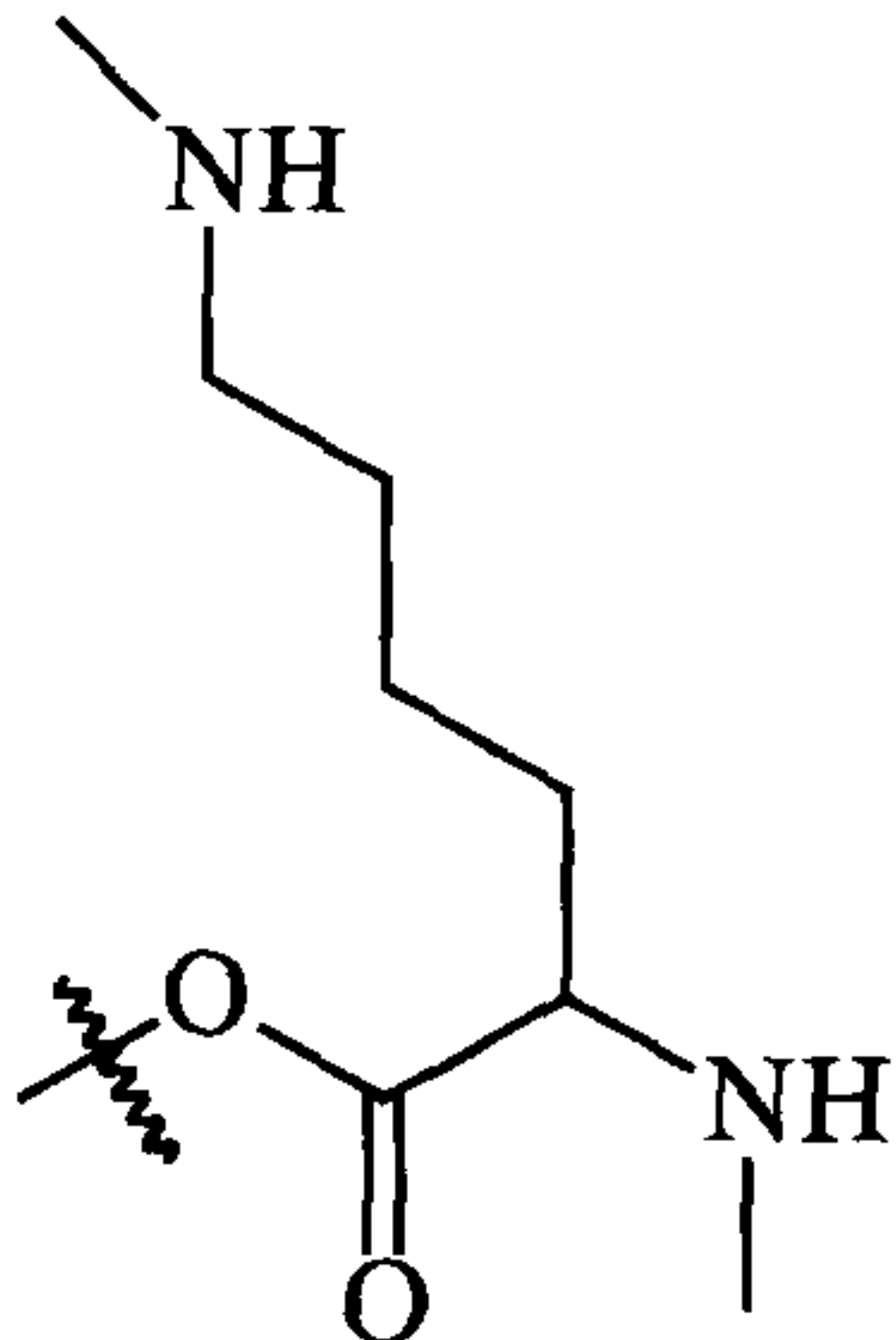
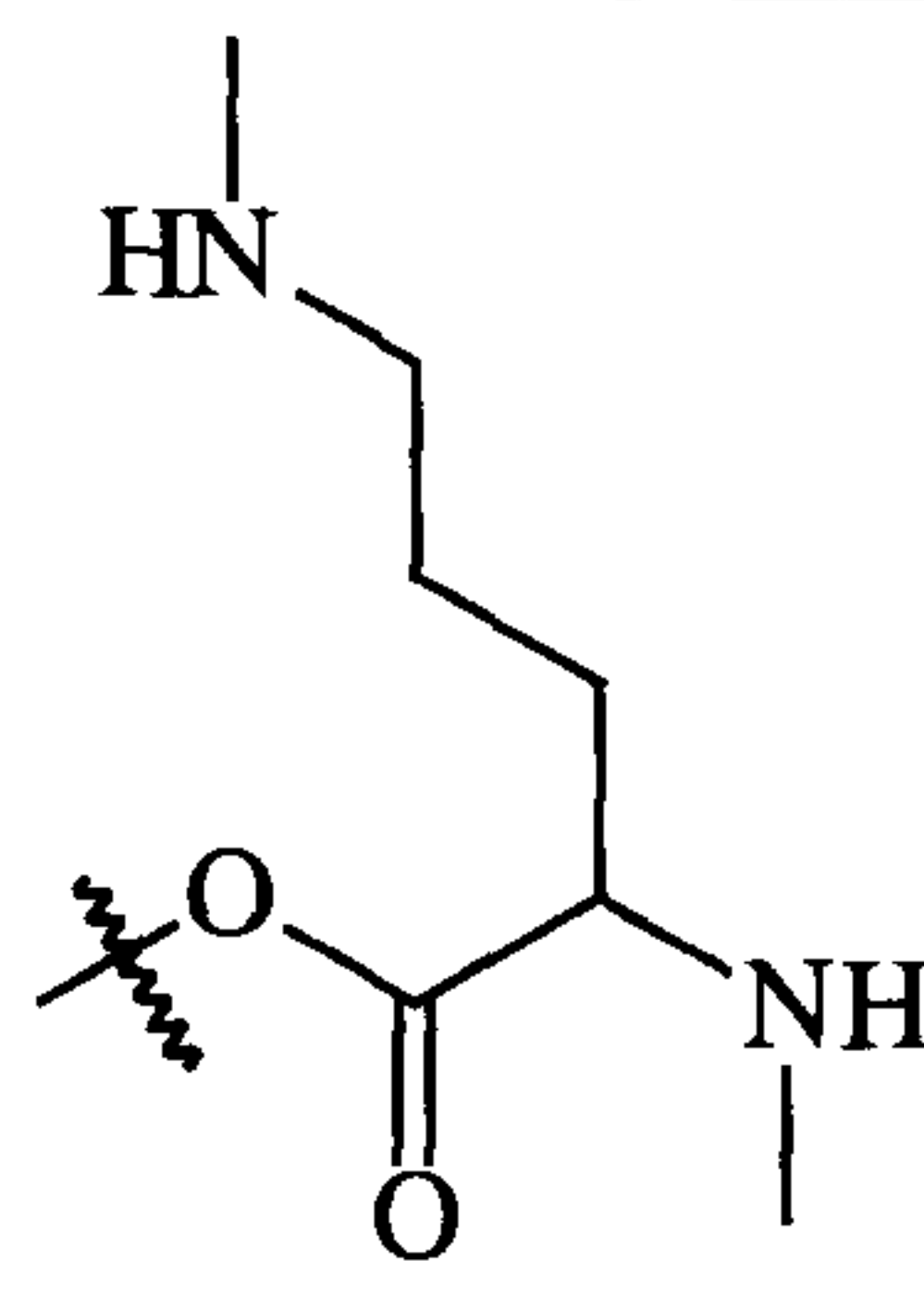
	 Where n = 1 to 450
 Where n = 1 to 450	
 Wherein each R is independent H or C₁ to C₁₀ alkyl	 Wherein each R is independent H or C₁ to C₁₀ alkyl
	

TABLE IV – ESTER PRODRUG MOIETIES

X₅ may comprise (a) a solubilizing moiety selected from the group consisting of: a moiety having one or more ionic entities at physiological pH; a moiety having multiple hydrogen bonding functionalities, such as –OH or amide; a monophosphate; a diphosphate; a triphosphate; a monosaccharide; an oligosaccharide; a polysaccharide; an oligopeptide; a polypeptide; an amino acid; an alpha amino acid a polyether and a combination thereof; and (b) a linking moiety selected from the group consisting of: -O- ; -O-C(=O)-Z-; -NH-C(=O)-Z-; -CH₂OC(=O)-; -C(=O)O-; -C(=O)HN-; where Z is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or is substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR', -NR'₂, NO₂, -CO₂H, -CO₂R', and epoxide and individual carbon atoms may be replaced by S, O, N, NR', or NR'₂ atoms; and each R', is independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or substituted with one or more of: OH, =O, SH, F, Br, Cl, I, NH₂, -NHR'₁'', -N(R'₂'')₂, NO₂ and -CO₂H where R'₁'' and R'₂'' are independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group.

Compounds of the invention are often made by preparing or purchasing pelorol, a pelorol precursor or a pelorol analog and preparing the desired compound. Details regarding the synthesis of compounds of the invention are described below.

Synthesis of Compounds and Assays for Activity

Pelorol may be obtained from natural sources as taught in the prior art. Solvent fractionation and/or chromatography may be employed. Examples of such derivatization steps as applied to different compounds of Formulas 1 and/or 2 are shown in more detail below.

The presence of SHIP 1 modulating compounds in a preparation may be determined by use of a variety of assays, including by biological assays which may be readily adapted from known procedures, including cell or animal based assays which monitor changes in: nitric oxide production from activated macrophages; IgE induced mast cell degranulation; LPS induced macrophage activation; TNF- α expression or activity. In addition, standard assays for agents which mediate inflammatory activity in living subjects may be employed. Adaptation of these assays is facilitated by the availability of SHIP 1^{-/-} and SHIP 1^{+/-} mice and bone marrow derived macrophages. In addition, the availability of anti-SHIP 1 antibodies facilitates use of immunoassay formats. Such assays may also be used to assess activity of compounds prepared by total synthesis, as described herein.

Total Synthesis of Compounds

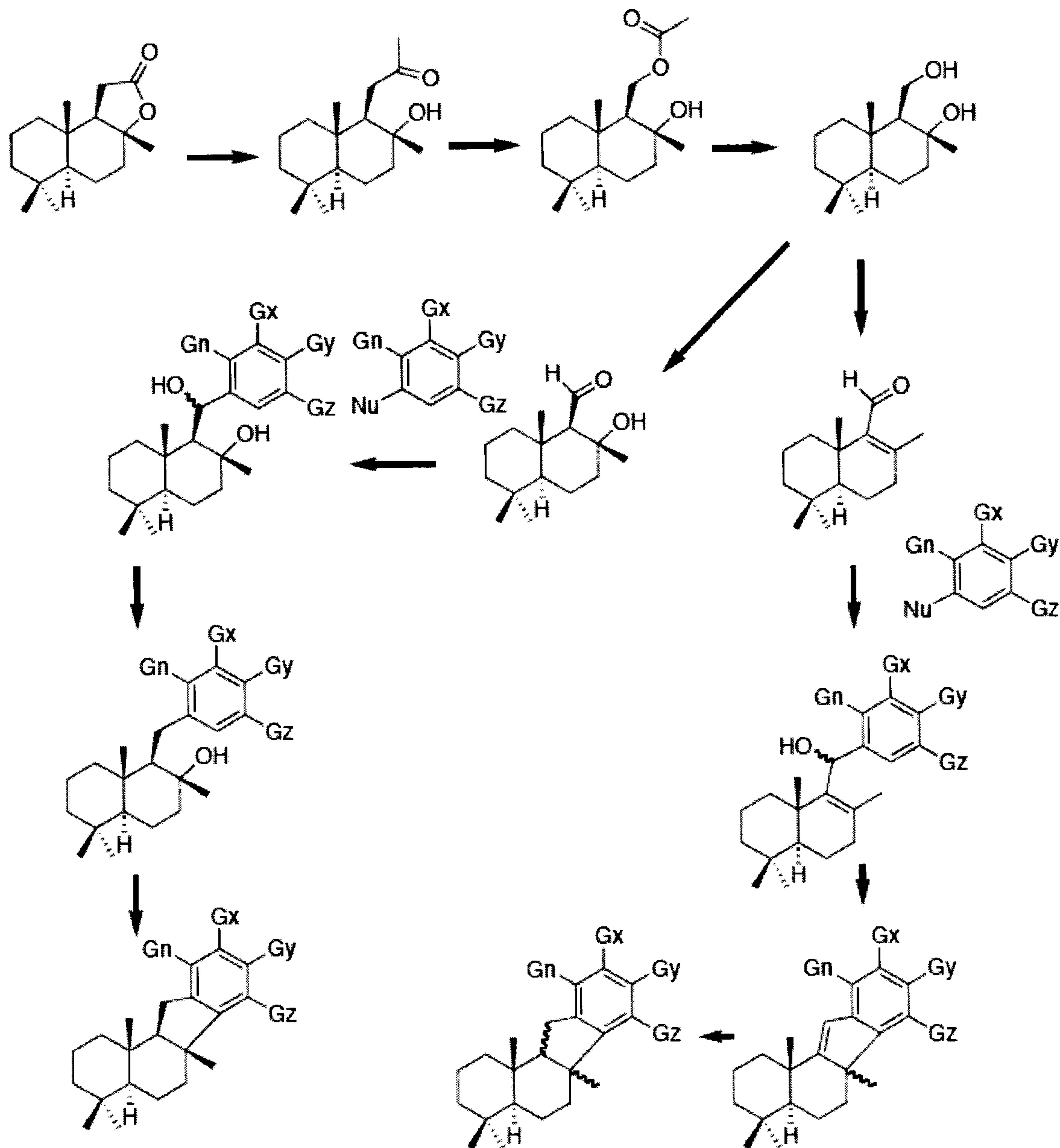
A synthetic scheme for making embodiments of the invention and intermediates and precursors of embodiments of the invention is provided herein. Table 7 provides examples of embodiments, intermediates and precursors of embodiments of such a synthesis with examples of different compounds of the invention which may be prepared. In the synthesis methods shown in Table 7, compounds of the invention and intermediates of compounds of the invention shown therein may be conveniently based on sclareolide as a starting material.

Appropriate derivatives of sclareolide providing desired Gⁿ, G^x, G^y and/or G^z substituents may be employed. Nu is a nucleophile, often lithium, and Gⁿ, G^x, G^y and/or G^z are often an activating group such as -OMe or -NHAc when carbons 15, 14, 13 and/or 12 respectively, are intended for modification. In circumstances where substituents Gⁿ, G^x, G^y and/or G^z are not

intended for modification each substituent may remain as found in the starting material or be appropriately altered to provide the desired substituents for the end product. Protecting groups may be employed on G^n , G^x , G^y and/or G^z .

- 5 The synthesis of a pelorol analog, are described in the prior art (see for example international publication number WO 2004/035601, which is incorporated herein by reference). More specific and detailed examples of syntheses of compounds of the invention may be found in the examples.

TABLE 7
Synthesis of SHIP 1 Modulating Compounds and Prodrugs



5 Pharmaceutical Compositions, Dosages, Administration and Indications

Compounds for use in this invention may be formulated into pharmaceutical compositions in any number of ways, which would be known to a person of skill in the art. The person of skill in the art may be expected to select appropriate pharmaceutically acceptable salts as well as appropriate pharmaceutically acceptable excipients, diluents, and carriers.

10 Compounds according to the invention can be provided in therapeutically- or prophylactically-acceptable amounts, in any pharmaceutically acceptable carrier. Methods

well known in the art for making such pharmaceutical formulations are found in, for example, “Remington: The Science and Practice of Pharmacy” (21st edition), ed. A. Gennaro, 2005, Mack Publishing Company, Easton, PA, incorporated by reference herein. Pharmaceutical formulations according to the present invention may, for example, contain excipients, sterile
5 water, or saline, ethanol, methanol, dimethyl sulfoxide, polyalkylene glycols such as polyethylene glycol, propylene glycol, or other synthetic solvents, oils of vegetable origin, or hydrogenated naphthalenes.

Compounds according to the invention may include hydrophobic compounds, for example, compounds that are substantially insoluble in water, but are freely soluble in solvents
10 such as, for example, ethanol, methanol, dimethyl sulfoxide, or chloroform, or combinations thereof. Formulations containing such hydrophobic compounds may be provided using, for example, micelles, which are formed by amphiphilic compounds under certain conditions. In aqueous solutions, micelles are capable of incorporating hydrophobic compounds in their hydrocarbon cores, or within the micelle walls. Hydrophobic compounds may also be
15 provided by solubilization in triglycerides (oils), for example, a digestible vegetable oil. The solubilized hydrophobic compound in the oil phase may be dispersed in an aqueous solution and stabilized using emulsifying agents, if desired. Alternatively, the hydrophobic compound may be provided in oil and delivered, for example, to the gastrointestinal system where bile salts may function as *in vivo* emulsifiers. Hydrophobic compounds may also be provided as
20 microemulsions which, like emulsions, are liquid dispersions of oil and water, but have smaller particles with an oil phase in a micelle-like “core.” Hydrophobic compounds according to the invention may also be provided together with a polymeric carrier, for example, a carbohydrate such as starch, cellulose, dextran, cyclodextrin, methylcellulose, or hyaluronic acid, or a polypeptide, such as albumin, collagen, or gelatin. Other modes of formulation of hydrophobic
25 compounds may include liposomes, natural and synthetic phospholipids, or solvents, for example, dimethyl sulfoxide or alcohols.

The pharmaceutical compositions of the invention may be formulated so as to provide controlled release of the active compound(s) over a period of time. Thus, the formulations could contain, for example, an amount of the compound that would be toxic if administered as
30 a single dose, but whose controlled release does not exceed toxic levels. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers, for example, may be used to control the release of the compounds. Other potentially useful delivery systems for modulatory

compounds according to the present invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

A "therapeutically effective amount" of a compound is an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result using a compound according to the invention. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects.

A "prophylactically effective amount" of a compound refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount.

Amounts considered sufficient will vary according to the specific compound used, the mode of administration, the stage and severity of the disease, the age, sex, weight, and health of the individual being treated, and concurrent treatments.

A range for therapeutically or prophylactically effective amounts of the compounds of the invention may be 0.1nM-0.1M, 0.1nM-0.05M, 0.05nM-15 μ M, 0.01nM-10 μ M, 0.1 μ M-1 μ M, 0.1 μ M-0.6 μ M or 0.3 μ M-0.6 μ M. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions.

Dosage ranges set forth herein are exemplary only and do not limit the dosage ranges that may be selected by medical practitioners. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

In general, compounds of the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population). In some circumstances however, such as in severe disease conditions, it may be necessary to administer substantial excesses of the compositions.

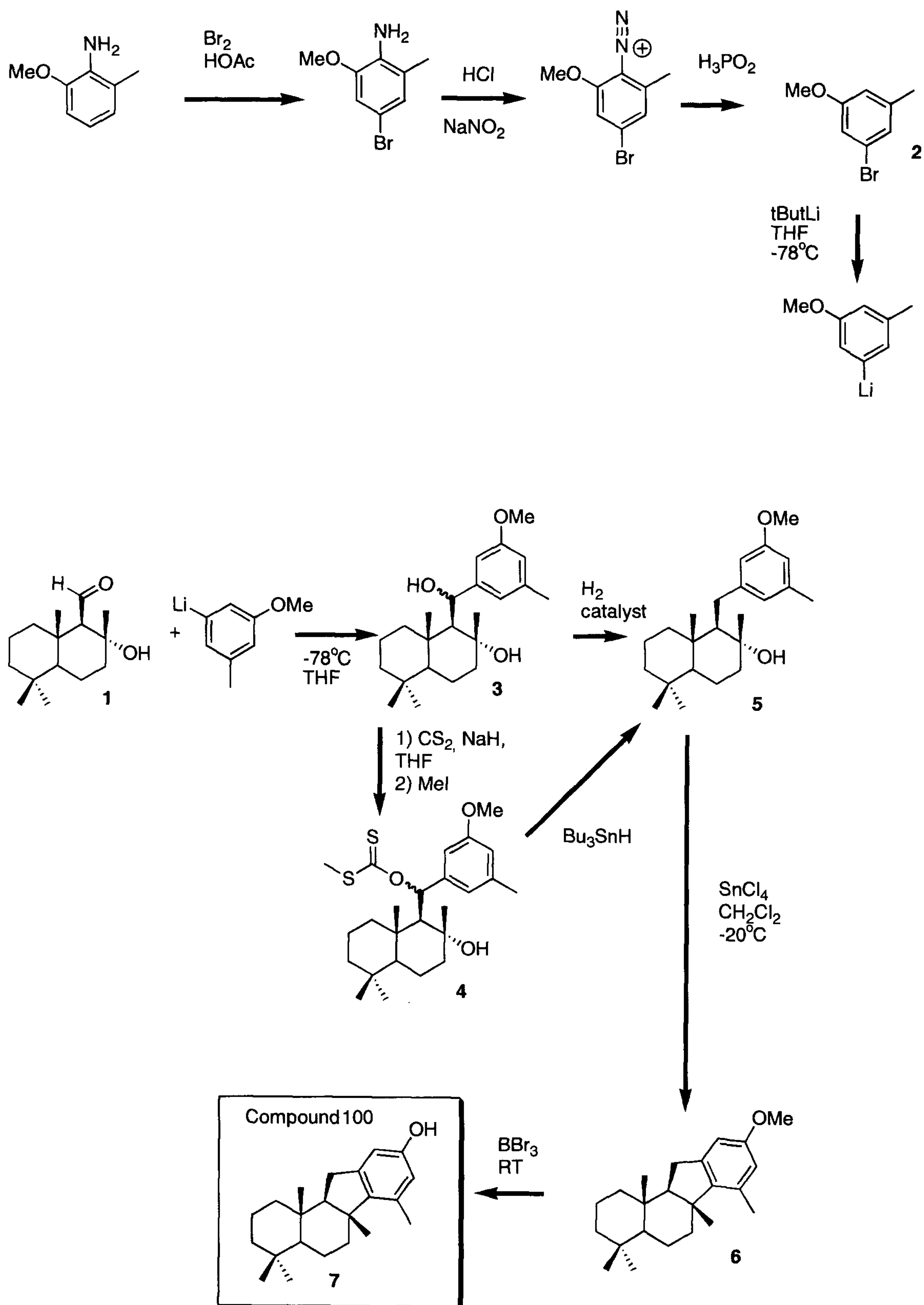
Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients, depending on the

therapeutic or prophylactic objectives. Any appropriate route of administration may be employed, for example, systemic, parenteral, intravenous, subcutaneous, transdermal, transmucosal, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, topical, surgical, or oral administration. The formulations used may vary according to the chosen route of administration. Thus, for oral administration, the formulations may be in the form of tablets or capsules; for inhalants, the formulations may be in the form of powders, nasal drops, or aerosols; for transmucosal administration, the formulations may be nasal sprays or suppositories; for transdermal administration, the formulations may be creams, ointments, salves, or gels; etc.

Therapeutically effective or prophylactically effective amounts of SHIP 1 modulators and pharmaceutical compositions of this invention may be administered to patients in need of treatment or prophylaxis for cancer (neoplastic diseases), other cell proliferative disorders, inflammatory diseases and immune diseases. Neoplastic diseases include but are not limited to: leukemias, carcinomas, sarcoma, melanomas, neuroblastoma, capillary leak syndrome and hematological malignancies. Diseases with an inflammatory component include, but are not limited to: rheumatoid arthritis, multiple sclerosis, Guillan-Barre syndrome, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, psoriasis, graft versus host disease, host versus graft, lupus erythematosus, Alzheimer's disease and insulin-dependent diabetes mellitus. Diseases related to inappropriate activation of macrophage-related cells of the reticuloendothelial lineage include osteoporosis.

Pelorol and other compounds having the structure of Formula 1 exhibit SHIP 1 agonist activity. By activating SHIP 1, such agonists are particularly useful in the treatment of inflammatory diseases such as sepsis/septic shock, colitis, inflammatory bowel syndrome, and those involving macrophage proliferation or activation; neoplastic diseases such as myeloid and lymphoid leukemias; as an immunosuppressive agent such as in transplant rejection; hematopoietic disorders; and for affecting mast cell degeneration such as in the treatment or prophylaxis of allergies.

In the following Examples and Figures, the terms "Compound #", "MN#" "AQXMN#" and "AQX-MN#" are all equivalent. For example, "Compound 100" is the same as "MN100" and is the same as "AQXMN100" and is the same as "AQX-MN100".

Example 1 - Synthesis of Compound 100:

Drimane-8 α ,11-diol was prepared according to Kuchkova et al; *Synthesis*, 1997, 1045

Bromomethoxytoluene (2) was prepared according to Chan et al; *J. Med. Chem.* (2001), 44, 1866

5

Preparation of Aldehyde (1)

Drimane-8 α ,11-diol (17.5g, 72.8mmol) was dissolved in 1L CH₂Cl₂. Diisopropylethylamine (50.7mL, 291.2mmol) was added and the solution was cooled to -15°C. A solution of Pyr-SO₃ (46.3g, 291.2mmol) in DMSO (250mL) was added dropwise over a period of 20min, then the
10 reaction was stirred cold for an additional 5 min. To the cold reaction was added 1M HCl (500mL), and the organic layer was partitioned. The aqueous layer was washed with an additional 200mL CH₂Cl₂. The pooled organic layers were then washed with saturated NaHCO₃, dried over Na₂SO₄ and concentrated. The crude product was purified by column chromatography (Hex: EtOAc) to yield 10.5g aldehyde (1) (44.1mmol, 60.1% yield) as a white
15 semisolid.

Preparation of diol (3)

Bromomethoxytoluene (2) (3.64g, 18.29mmol) was dissolved in 35mL dry THF under an argon atmosphere. This solution was cooled to -78°C, and tBuLi (21.5mL, 36.6mmol) was
20 added dropwise via syringe. The solution was stirred for 10 min at -78°C, then warmed to RT for 20 min. The solution was re-cooled to -78°C, and a solution of aldehyde (1) (1.45g, 6.09mmol) in 6mL dry THF was added via syringe. The solution was stirred at -78°C 2h, after which the reaction was quenched with the addition of 1M HCl. EtOAc (100mL) was added, and the organic phase was washed with 1M HCl, followed by saturated NaHCO₃. The organic
25 phase was dried over MgSO₄, filtered and concentrated. The crude reaction mixture was purified by column chromatography (Hex:EtOAc) to yield diol (3) (1.94g, 88.5% yield).

¹H NMR (CDCl₃) δ 0.34 (td, J= 13.3, 3.6 Hz, 1H), 0.77 (s, 3H), 0.82 (s, 3H), 0.90 (m, 1H), 0.97 (td, 13.5, 3.6 Hz, 1H), 1.02 (s, 3H), 1.13 (m, 1H). 1.16 (m, 1H), 1.23 (m, 1H) 1.33 (m,
30 1H), 1.40 (m, 1H), 1.54 (s, 3H), 1.56 (m, 1H), 1.63 (m, 1H), 1.84 (dt, 12.2, 3.3Hz, 1H), 2.12 (d,

8.1Hz, 1H), 2.33 (s, 3H), 3.79 (s, 3H), 4.79 (d, 8.1Hz, 1H), 6.61 (s, 1H), 6.78 (s, 1H), 6.85 (s, 1H).

¹³C NMR (CDCl₃) δ 15.9, 18.3, 19.8, 21.50, 21.53, 26.1, 33.2, 33.5, 38.6, 40.8, 41.3, 44.0, 55.1, 55.8, 62.84, 62.85, 76.0, 110.5, 113.6, 120.7, 139.8, 149.0, 159.7

5 HRESIMS calcd for C₃₂H₃₆O₃Na 383.2562, found 383.2563

Preparation of xanthate (4)

Diol (3) (1.94g, 5.39mmol) was dissolved in 20mL dry THF under an argon atmosphere. To this solution was added NaH (237mg, 60% in oil, 5.93mmol). The reaction was then heated to
10 50°C until the solution was clear orange. The reaction was cooled to 0°C, and CS₂ (1mL, 16.6mmol) was added. The solution was stirred for 20 min at 0°C, then warmed to RT for an additional 20 minutes, after which MeI (1mL, 16.6mmol) was added. The reaction was stirred at RT for 1hour, then concentrated to dryness. The crude mixture was dissolved in EtOAc, and washed with 3x H₂O. The organic solution was dried over MgSO₄, filtered and concentrated to
15 yield a mixture of xanthate (4) and fragmentation product, ketone (approx 4:1). This product mixture was used in the next step without further purification.

¹H NMR (CDCl₃) δ 0.56 (td, 12.9, 3.5Hz, 1H), 0.77 (s, 3H), 0.80 (s, 3H), 0.87 (dd, 12.2, 2.4 Hz, 1H), 0.99 (dt 13.6, 3.8Hz, 1H), 1.02 (s, 3H), 1.28 (m, 1H), 1.31 (m, 1H), 1.34 (m, 1H),
20 1.45 (m, 1H), 1.50 (s, 3H), 1.55 (m, 1H), 1.65 (m, 1H), 1.75 (m, 1H), 1.78 (m, 1H), 1.81 (m, 1H), 2.18 (d, 5.2 Hz 1H), 2.28 (s, 3H), 2.38 (s, 3H), 3.75 (s, 3H), 5.18 (d, 5.2Hz, 1H), 6.5 (s, 1H), 6.7 (s, 1H), 6.8 (s, 1H)

¹³C NMR (CDCl₃) δ 13.0, 15.9, 18.3, 20.2, 21.3, 21.6, 26.3, 33.26, 33.30, 40.2, 41.0, 41.3, 46.0, 46.8, 55.0, 55.9, 65.1, 74.2, 110.9, 112.3, 120.9, 139.5, 149.9, 159.4, 189.7

25 HRESIMS calcd for C₂₅H₃₈O₃S₂Na 473.2160, found 473.2159

Preparation of alcohol (5)

Xanthate (4) and ketone were dissolved as a crude mixture in 50mL toluene, and placed under an argon atmosphere. Bu₃SnH (2.9mL, 10.78mmol) was added, and the solution was heated.
30 Once at reflux, a catalytic amount of VAZO (1,1'-Azobis(cyclohexanecarbonitrile)) (approx 50mg) was added through the top of the condensor. The solution was refluxed for 1hour, then an additional amount of VAZO was added (approx 50mg). The solution was refluxed for

another 45 min, after which TLC analysis (20% EtOAc:Hex) indicated the reaction to be complete. The reaction was cooled, then concentrated to dryness. Flash chromatography of the crude product yielded alcohol (5) (1.12g, 3.23mmol, 60% yield, 2 steps) as a white foam.

5 ^1H NMR (CDCl_3) δ 0.78 (s, 3H), 0.85 (s, 3H), 0.87 (s, 3H), 0.90 (m, 1H), 0.93 (m, 1H), 0.96 (m, 1H), 1.09 (td, 13.3, 3.9Hz, 1H), 1.25 (s, 3H), 1.31 (m, 1H), 1.35 (m, 1H), 1.39 (m, 1H), 1.43 (m, 1H), 1.54 (m, 1H), 1.64 (m, 1H), 1.70 (m, 1H), 1.84 (dt, 12.4, 3.1Hz, 1H), 2.27 (s, 3H), 2.60 (dd, 14.7, 4.5Hz, 1H), 2.70 (dd, 14.7, 5.9Hz, 1H), 3.75 (s, 3H), 6.49 (s, 1H), 6.63 (s, 1H), 6.68 (s, 1H)

10 ^{13}C NMR (CDCl_3) δ 15.4, 18.4, 20.2, 21.4, 21.5, 24.5, 31.2, 33.2, 33.3, 39.1, 40.3, 41.7, 44.0, 55.0, 56.0, 63.0, 74.1, 111.3, 111.9, 122.1, 139.2, 145.9, 159.5

HRESIMS calcd for $\text{C}_{23}\text{H}_{36}\text{O}_2\text{Na}$ 367.2613, found 367.2615

Preparation of tetracycle (6)

15 Alcohol (5) (1.12g, 3.23mmol) was dissolved in 10 mL CH_2Cl_2 and cooled to 0°C . To this solution was added SnCl_4 (1mL) neat. The orange solution was then stirred for 1 hour at 0°C , followed by quenching with MeOH. The reaction was extracted into EtOAc, and washed with 2x satd NaHCO_3 . The organic phase was dried over MgSO_4 , filtered and concentrated to yield tetracycle (6) (1.05g, 3.20mmol, 99% yield). This compound was used without further
20 purification.

^1H NMR (CDCl_3) δ 0.86 (s, 6H), 0.98 (m, 1H), 1.02 (s, 3H), 1.06 (s, 3H), 1.17 (td, 13.5, 4.2Hz, 1H), 1.24 (m, 1H), 1.40 (m, 2H), 1.54 (m, 2H), 1.71 (m, 4H), 2.27 (s, 3H), 2.34 (m, 1H), 2.49 (dd, 14.5, 6.2Hz, 1H), 2.60 (m, 1H), 3.74 (s, 3H), 6.41 (s, 1H), 6.62 (s, 1H)

25 ^{13}C NMR (CDCl_3) δ 15.7, 17.9, 18.6, 19.1, 19.9, 20.7, 28.6, 32.6, 32.9, 36.5, 37.9, 38.5, 39.7, 42.1, 54.8, 56.7, 64.2, 107.9, 113.4, 117.9, 132.5, 143.8, 157.3

HRESIMS calcd for $\text{C}_{23}\text{H}_{35}\text{O}$ $[\text{M}+\text{H}]^+$ 327.2688, found 327.2685

Preparation of Compound 100 (7)

30 Tetracycle (6) (1.05g, 3.20mmol) was dissolved in 15 mL DCM. To this solution was added a solution of BBr_3 (1.0M in DCM) (3.20mL, 3.20mmol). The solution was stirred at RT for 2 hours, then concentrated to dryness. The brown residue was dissolved in EtOAc, then washed

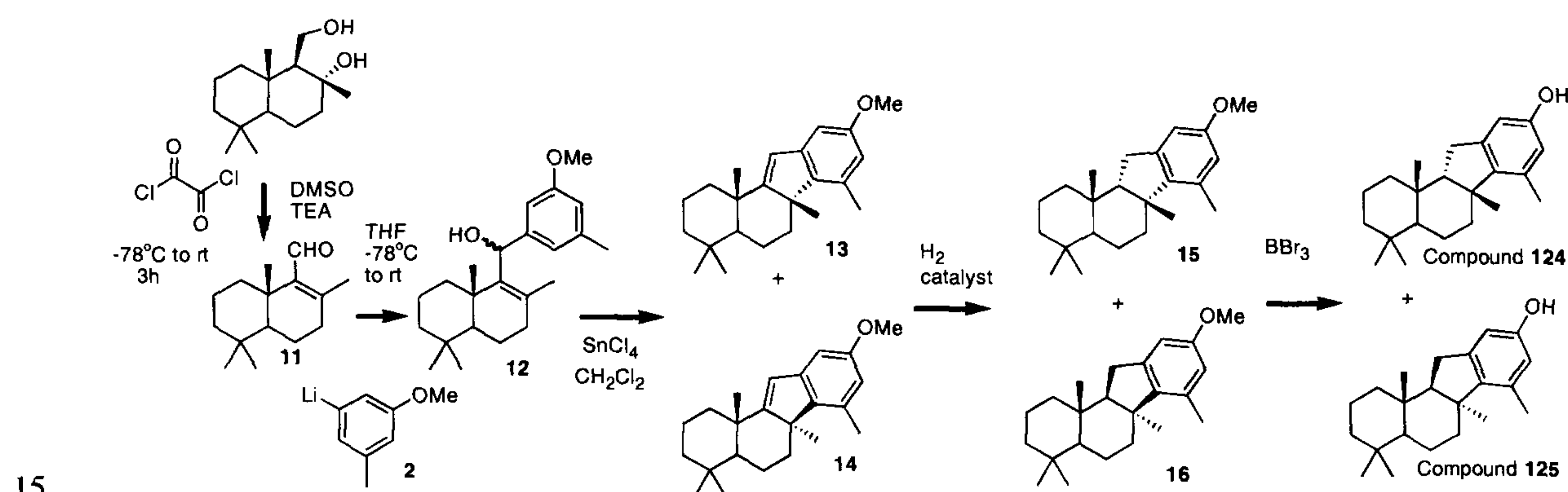
with H₂O until the pH of the aqueous layer was neutral. The crude product was purified by flash chromatography to yield Compound 100 (7) (931mg, 2.98mmol, 93% yield) as a white solid.

¹H NMR (CDCl₃) δ 0.88 (s, 6H), 0.97 (m, 1H), 1.00 (m, 1H), 1.04 (s, 3H), 1.07 (s, 3H), 1.18 (td, 13.2, 4.2Hz, 1H), 1.42 (m, 1H), 1.43 (m, 1H), 1.53 (m, 1H), 1.58 (m, 1H), 1.71 (m, 1H), 1.73 (m, 1H), 1.74 (m, 1H), 1.75 (m, 1H), 2.26 (s, 3H), 2.35 (dt, 11.7, 3.0Hz, 1H), 2.48 (dd, 14.35, 6.44Hz, 1H), 2.59 (m, 1H), 6.36 (d, 1.9Hz, 1H), 6.55 (d, 1.9Hz, 1H)

¹³C NMR (CDCl₃) δ 16.1, 18.3, 18.8, 19.6, 20.4, 21.1, 29.0, 33.1, 33.4, 37.0, 39.0, 40.1, 42.6, 47.1, 57.1, 64.5, 109.9, 115.1, 133.1, 144.2, 144.7, 153.5

HRESIMS calcd for C₂₂H₃₃O [M+H]⁺ 313.2531, found 313.2526

Example 2 - Synthesis of Compound 124 and Compound 125



Experimental for preparation of Compound 124 and Compound 125

Preparation of 12

Bromide 2 (1.41g, 7.09mmol) was dissolved in 30ml dry THF under an argon atmosphere and cooled to -78°C. tBuLi (8.3ml, 1.7M in pentane, 14.2mmol) was added over a period of 10min and the solution was warmed to rt. After 15 min, the solution was recooled to -78°C and stirred for an additional 30 min. A solution of enal 11 (521mg, 2.36mmol) in 8mL dry THF was then added to the cold solution and the reaction was stirred at -78°C for 30 min. 1M HCl was then added and the reaction was warmed to rt. The crude product was extracted into EtOAc and washed with satd. NaHCO₃. The organic phase was dried over MgSO₄, filtered

and concentrated. The crude compound was purified by flash chromatography to yield alcohol 12 (451mg, 1.32mmol, 56% yield).

^1H NMR (CDCl_3) δ 0.88 (s, 3H), 0.91 (s, 3H), 1.12 (s, 3H), 1.17 (m, 1H), 1.20 (m, 1H), 1.27 (s, 3H), 1.34 (td, 12.9, 3.5 Hz, 1H), 1.41-1.75 (m, 6H), 2.01 (m, 2H), 2.31 (s, 3H), 3.77 (s, 3H), 5.33 (s, 1H), 6.55 (s, 1H), 6.75 (s, 1H), 6.83 (s, 1H)

^{13}C NMR (CDCl_3) δ 18.9, 19.1, 20.4, 21.5, 21.7, 21.8, 33.3, 33.4, 34.8, 37.1, 38.9, 41.5, 52.5, 55.1, 69.6, 108.4, 111.7, 118.5, 133.3, 138.9, 143.4, 147.6, 159.6

HRESIMS calcd for $\text{C}_{23}\text{H}_{34}\text{O}_2\text{Na}$ 365.2457, found 365.2458

Preparation of 13 and 14

Alcohol 12 (450mg, 1.32mmol) was dissolved in 10mL CH_2Cl_2 under an argon atmosphere and cooled to -78°C . SnCl_4 (1mL) was added and the resulting yellow solution was stirred for 15min. 1M HCl was added to the cold solution and the mixture was allowed to warm to rt.

The layers were separated and the organic phase was washed with 2x H_2O , dried over MgSO_4 , filtered and concentrated. The crude product was purified by flash chromatography to yield 13 and 14 (284mg, 0.88mmol, 67% yield) as a 1:1 mixture.

Preparation of 15 and 16

A 1:1 mixture of epimers 15 and 16 (84mg) was dissolved in 5mL 1:1 MeOH:DMF. 10% Pd/C (32mg) was added, and the slurry was saturated with H_2 . The solution was stirred for 16h under a balloon of H_2 , after which the solid catalyst was filtered off and washed with EtOAc. The organic phase was washed with 3x H_2O , dried over MgSO_4 , filtered and concentrated to yield 15 and 16 (80mg, 95% yield) as a 1:1 mixture.

Preparation of Compound 124 and Compound 125

A 1:1 mixture of 15 and 16 (80mg, 0.24mmol) was dissolved in 0.5mL CH_2Cl_2 . BBr_3 (2mL, 1M in CH_2Cl_2 , 2.0mmol) was added and the solution was stirred at rt for 15 min. The reaction was quenched with slow addition of MeOH, and the crude reaction mixture was concentrated under vacuum. The crude product was purified by flash chromatography to yield a 1:1 mixture of Compound 124 and Compound 125. Compound 124 was fractionally crystallized from the mixture by cooling from toluene.

¹H NMR (CDCl₃) δ 0.87 (s, 3H), 0.89 (s, 3H), 1.20 (m, 2H), 1.25 (s, 3H), 1.30-1.45 (m, 7H), 1.62 (s, 3H), 1.70 (m, 1H), 1.85 (dd, 12.0, 8.4Hz, 1H), 2.01 (m, 1H), 2.33 (s, 3H), 2.73 (dd, 15.5, 8.3Hz, 1H), 2.78 (m, 1H), 4.51 (s, 1H), 6.39 (s, 1H), 6.50 (s, 1H)

¹³C NMR (CDCl₃) δ 17.9, 19.7, 21.5, 24.1, 25.8, 32.5, 33.1, 33.5, 36.1, 36.2, 37.9, 41.9, 46.4,
5 47.5, 61.9, 108.4, 115.8, 133.9, 142.4, 143.3, 153.2

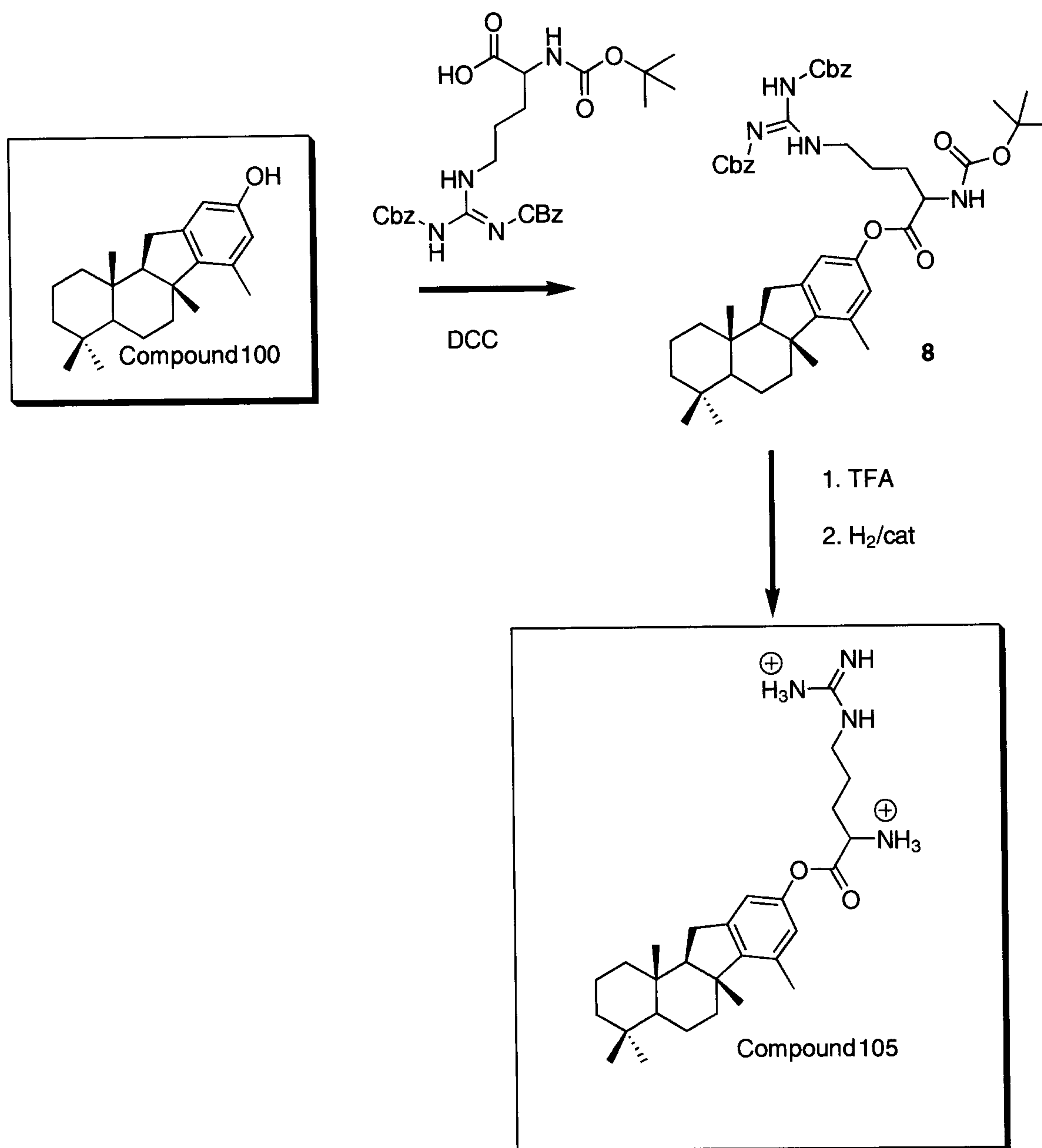
HRESIMS calcd for C₂₂H₃₃O [M+H]⁺ 313.2531, found 313.2533

Compound 125 was fractionally crystallized from the enriched remainder from CH₃CN.

¹H NMR (CDCl₃) δ 0.47 (s, 3H), 0.80 (s, 3H), 0.89 (s, 3H), 0.90 (m, 1H), 1.00 (dd, 11.3, 4.4
10 Hz, 1H), 1.17 (m, 1H), 1.18 (s, 3H), 1.29 (m, 1H), 1.40 (m, 2H), 1.52 (m, 1H), 1.62 (m, 1H), 1.70 (m, 2H), 2.33 (s, 3H), 2.52 (dt, 14.4, 5.5Hz, 1H), 2.62 (d, 16.9Hz, 1H), 2.97 (dd, 16.9, 8.0Hz, 1H), 4.52 (s, 1H), 6.35 (s, 1H), 6.47 (s, 1H)

¹³C NMR (CDCl₃) δ 15.2, 18.0, 19.1, 19.5, 21.4, 30.5, 31.7, 32.8, 32.9, 34.3, 36.9, 40.7, 41.7,
47.7, 52.0, 62.1, 108.3, 115.3, 133.2, 140.7, 145.6, 153.4

15 HRESIMS calcd for C₂₂H₃₃O [M+H]⁺ 313.2531, found 313.2533

Example 3 - Synthesis of Compound 105:

Experimental for preparation of Compound 105:

- 5 Compound 100 (7), (60.4mg, 0.193mmol), $N\alpha$ -Boc- $N\delta,N\omega$ -di-Z-L-Arg-OH (157.3mg, 0.290mmol) and DMAP (~2mg) were combined in 3mL CH_2Cl_2 . DIPC was added, and the solution was stirred for 2h at RT. The reaction was concentrated, and purified by flash chromatography to yield 8 as a white foam.

10 ^1H NMR ($CDCl_3$) δ 0.86 (s, 6H), 0.96 (s, 2H), 1.01 (s, 3H), 1.05 (s, 3H), 1.17 (m, 1H), 1.39 (m, 1H), 1.43 (s, 9H), 1.50 (m, 1H), 1.58 (m, 1H), 1.70 (m, 2H), 1.73 (m, 2H), 1.78 (m, 2H), 1.92 (m, 1H), 2.25 (s, 3H), 2.32 (m, 1H), 2.47 (dd, 14.6, 6.1Hz, 1H), 2.58 (m, 1H), 4.04 (m,

2H), 4.47 (s, br, 1H), 5.12 (s, 2H), 5.22 (2H), 6.52 (s, 1H), 6.70 (s, 1H), 7.27 (m, 3H), 7.35 (m, 7H), 9.24 (s, br, 1H), 9.45 (s, br, 1H)

^{13}C NMR (CDCl_3) δ 14.1, 16.0, 18.2, 18.7, 19.4, 20.0, 20.9, 21.0, 24.9, 28.2 (3C), 28.8, 29.3, 32.9, 33.2, 36.9, 38.5, 40.0, 42.4, 44.1, 47.3, 53.4, 56.9, 60.2, 64.2, 66.9, 68.8, 79.7, 115.5, 120.8, 127.60, 127.62, 128.2, 128.3 (2C), 128.7 (2C), 132.9, 134.6, 136.8, 144.3, 148.1, 149.3, 155.3, 155.7, 160.4, 163.7, 171.5

HRESIMS calcd for $\text{C}_{49}\text{H}_{65}\text{N}_4\text{O}_8$ $[\text{M}+\text{H}]^+$ 837.4802, found 837.4805

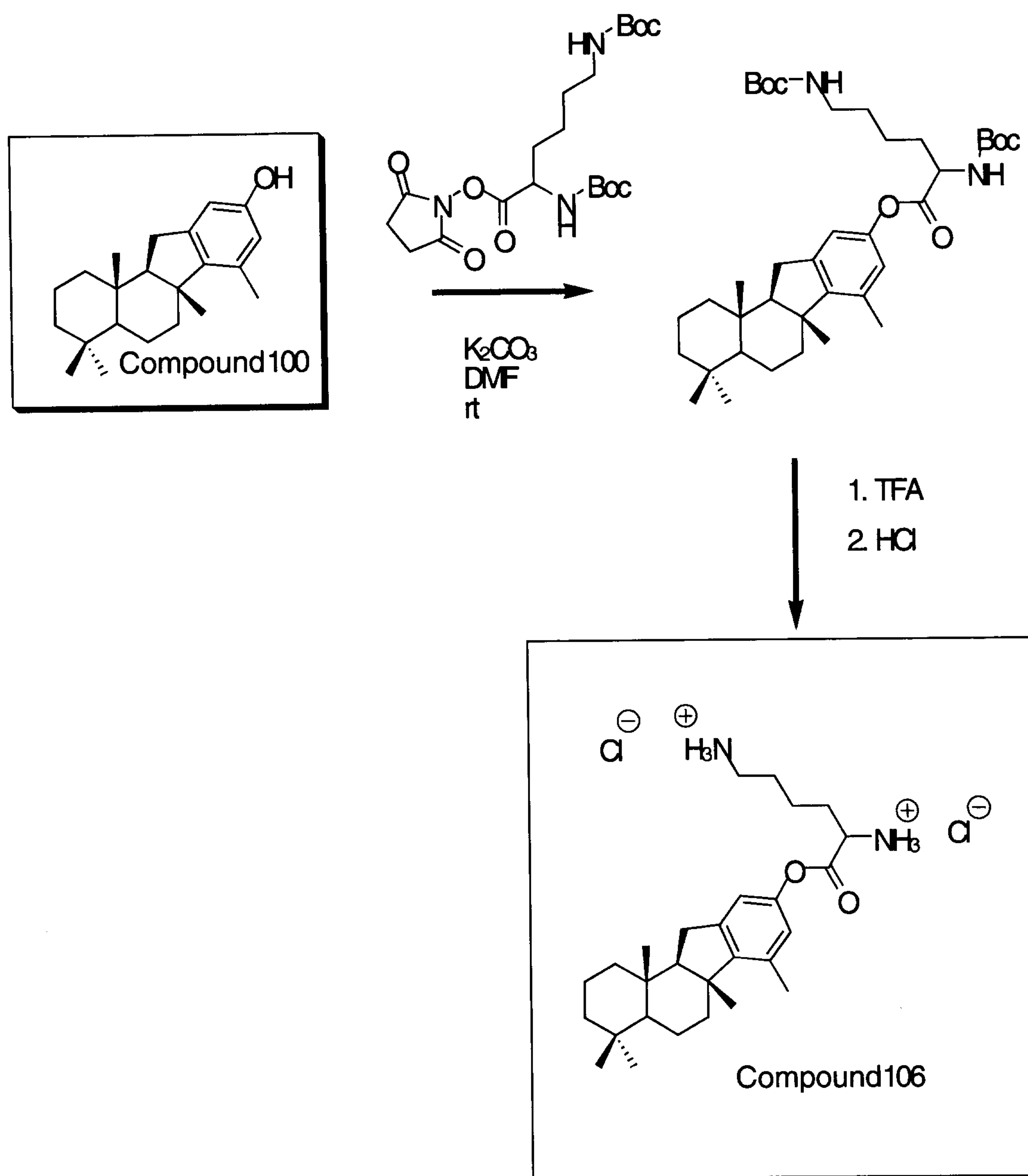
Preparation of Compound 105

Compound 8 was dissolved in 3mL 70%TFA/ CH_2Cl_2 and stirred for 1h. The solvents were then evaporated, and the resulting residue was redissolved in toluene and concentrated to dryness. The resulting solid was then dissolved in 15mL MeOH and 100mg Pd/C (10% wt) was added. The solution was saturated with H_2 and stirred overnight under a hydrogen balloon. The Pd/C was filtered off and the solution was concentrated to dryness. The resulting solid was dissolved in 10mL H_2O and 50uL 1M HCl was added. After stirring 5 min, the solution was lyophilized to yield Compound 105 as a white powder.

^1H NMR (CD_3OD) δ 0.86 (s, 3H), 0.87 (s, 3H), 1.01 (m, 2H), 1.05 (s, 3H), 1.08 (s, 3H), 1.19 (td, 13.9, 4.2Hz, 1H), 1.41 (m, 2H), 1.52 (m, 1H), 1.66 (m, 2H), 1.72 (m, 4H), 1.85 (m, 2H), 2.13 (m, 2H), 2.28 (s, 3H), 2.39 (m, 1H), 2.51 (dd, 14.6, 6.1Hz, 1H), 2.64 (m, 1H), 4.33 (t, 6.3Hz, 1H), 6.64 (s, 1H), 6.83 (s, 1H)

^{13}C NMR (CD_3OD) δ 16.7, 19.1, 19.3, 20.5, 20.6, 21.5, 25.7, 28.7, 29.8, 33.9, 34.0, 38.2, 40.0, 41.3, 41.6, 43.7, 48.7, 53.7, 58.3, 66.0, 116.5, 121.9, 134.5, 145.8, 149.2, 151.3, 158.8, 169.4

HRESIMS calcd for $\text{C}_{28}\text{H}_{45}\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$ 469.3543, found 469.3540

Example 4 - Synthesis of Compound 106:

Experimental for preparation of Compound 106:

- 5 Compound 100 (7) (41.7mg, 0.133mmol) was dissolved in 4mL DMF. K_2CO_3 (37mg, 0.266mmol) was added, and the solution was stirred for 10 min. Boc-Lys(Boc)-OSu (115.3mg, 0.266mmol) was added, and the solution was stirred for 18h at RT. The reaction was extracted into EtOAc, and washed with 3 x H_2O . The organic phase was dried, filtered and concentrated. The crude product was purified by flash chromatography to yield (23) (80.6mg, 0.126mmol,
- 10 95% yield) as a white foam.

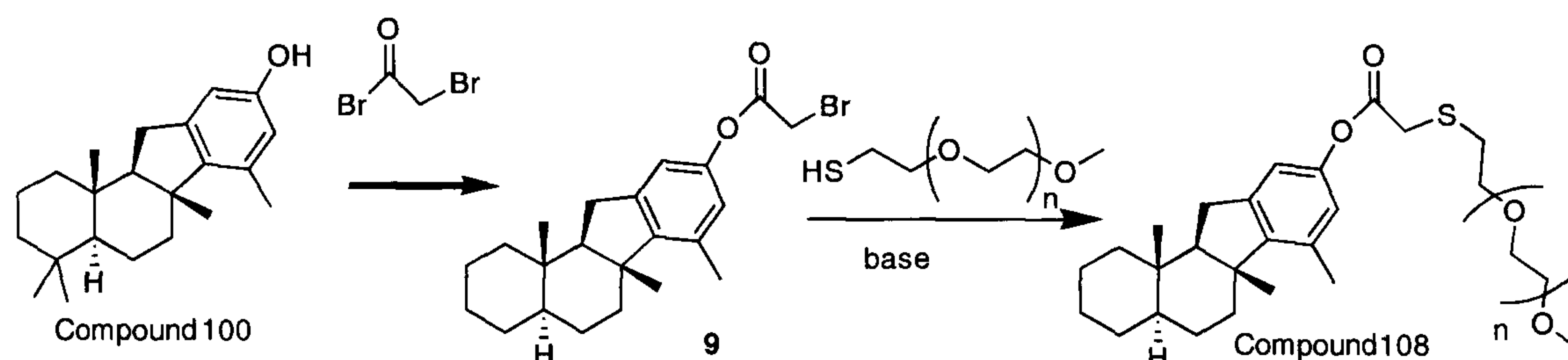
This foam was dissolved in 2mL CH₂Cl₂ and TFA (2mL) was added. The solution was stirred at RT for 2h, then concentrated to dryness. Toluene (3mL) was added, and the solution was concentrated to dryness again. The resulting residue was dissolved in 5mL H₂O and 100μL 1M HCl was added. The aqueous solution was then filtered through a 0.22μm syringe filter, and lyophilized to yield Compound 106-2HCl as a white powder.

¹H NMR (CD₃OD) δ 0.82 (s, 3H), 0.83 (s, 3H), 0.96 (m, 2H), 1.01 (s, 3H), 1.04 (s, 3H), 1.16 (td, 13.5, 4.5Hz, 1H), 1.36 (m, 2H), 1.50 (m, 1H), 1.59 (m, 3H), 1.69 (m, 5H), 2.04 (m, 2H), 2.24 (s, 3H), 2.36 (m, 1H), 2.48 (dd, 14.7, 6.2Hz, 1H), 2.60 (m, 1H), 2.92 (m, 2H), 3.23 (m, 1H), 4.23 (m, 1H), 6.58 (s, 1H), 6.76 (s, 1H)

¹³C NMR (CD₃OD) δ 16.7, 19.0, 19.4, 20.5, 20.7, 21.5, 23.3, 28.1, 29.8, 31.1, 33.8, 34.0, 38.3, 40.1, 40.3, 41.4, 43.7, 53.9, 58.4, 66.1, 116.5, 121.9, 134.5, 145.9, 149.3, 151.4, 169.4

HRESIMS calcd for C₂₈H₄₅N₂O₂ [M+H]⁺ 441.3481, found 441.3484

Example 5 - Synthesis of Compound 108:



Experimental for preparation of Compound 108:

Compound 100 (7: 12.1mg, 0.039mmol) was dissolved in 1mL CH₂Cl₂. DMAP (~1mg) was added, followed by Bromoacetyl bromide (5.1μL, 0.059mmol), and the reaction was stirred overnight. Concentration of the reaction, followed by flash chromatography yielded bromide (9) (12.9mg, 0.030mmol, 77% yield).

¹H NMR (CDCl₃) δ 0.86 (s, 6H), 0.97 (m, 1H), 1.02 (s, 3H), 1.07 (s, 3H), 1.18 (td, 13.5, 4.5Hz, 1H), 1.25 (s, 1H), 1.41 (m, 2H), 1.52 (m, 1H), 1.60 (m, 1H), 1.71 (m, 2H), 1.77 (m, 2H), 2.29

(s, 3H), 2.35 (m, 1H), 2.52 (dd, 14.6, 6.1Hz, 1H), 2.62 (m, 1H), 4.00 (s, 2H), 6.59 (s, 1H), 6.78 (s, 1H)

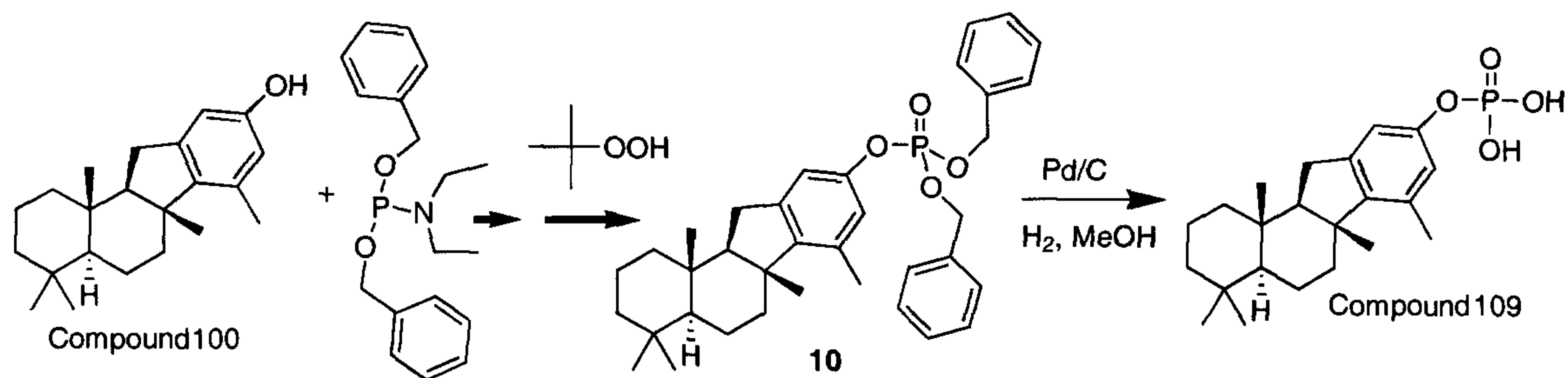
^{13}C NMR (CDCl_3) δ 16.1, 18.3, 18.9, 19.5, 20.1, 21.1, 25.7, 28.9, 33.1, 33.3, 37.0, 38.6, 40.1, 42.5, 47.4 57.0, 64.3, 115.3, 120.6, 133.2, 144.6, 148.1, 149.8, 166.2

5 HRESIMS calcd for $\text{C}_{24}\text{H}_{33}\text{O}_2$ $^{79}\text{BrNa}$ 455.1562, found 455.1550

HRESIMS calcd for $\text{C}_{24}\text{H}_{33}\text{O}_2$ $^{81}\text{BrNa}$ 457.1541, found 457.1522

Bromide 9 (6.06 g, 11 mmol) was added portionwise over a period of 30 min. to a solution of HS-PEG (35 g, MW 6000) and *N,N*-diisopropylethylamine (2.7 mL) in acetonitrile (90mL) under nitrogen at 0°C. After addition, the ice bath was removed and the mixture was allowed to warm to room temperature. After 3-4 hours, 2-propanol (1200 mL) was added over 30 min. After an addition 1.5 h, the resulting solid was collected on a Buchner funnel and washed with 2 X 150 mL of 2-propanol. The wet cake was then dissolved in acetonitrile (80 mL) containing 0.5% $i\text{Pr}_2\text{NEt}$ at 0-5°C and precipitated by addition of 2-propanol (1000mL). The resulting solid was collected and washed with 2-propanol and dried in vacuo to give Compound 108.

Example 6 - Synthesis of Compound 109:



20 Experimental for the synthesis of Compound 109:

General Procedure: Steinberg, G.M. *J. Org. Chem.* (1950), 15, 637.

Specific tyrosine phosphorylation; Gibson, B.W et al. *J. Am. Chem. Soc.* (1987), 109, 5343.

25 Compound 100 (7) (250mg, 0.80mmol) was slurried in tetrazole/MeCN solution (18mL, 8.1mmol). THF was added until the solution was clear (~10mL). To this solution was added dibenzyl N,N-diethylphosphoramidite (1.0g, 85%, 2.7mmol), and the reaction was stirred at RT for 1h. To the reaction was then added 10mL t-butylhydroperoxide (70% in H_2O) and the

solution was stirred vigorously for an additional 30 min. The reaction mixture was extracted into EtOAc, washed with 1x Na₂S₂O₅, 1x 1M HCl, then with satd NaHCO₃. The organic layer was dried over MgSO₄, filtered and concentrated. The crude product was purified by flash chromatography to yield 10 (280mg, 0.49mmol, 61% yield).

5

¹H NMR (CDCl₃) δ 0.88 (s, 6H), 0.96 (m, 1H), 0.99 (m, 1H), 1.03 (s, 3H), 1.06 (s, 3H), 1.19 (m, 1H), 1.26 (m, 1H), 1.43 (m, 2H), 1.53 (m, 1H), 1.60 (m, 1H), 1.72 (m, 3H), 2.24 (s, 3H), 2.33 (m, 1H), 2.47 (dd, 14.6, 6.3Hz, 1H), 2.58 (m, 1H), 5.11 (s, 2H), 5.13 (s, 2H), 6.64 (s, 1H), 6.84 (s, 1H), 7.33 (s, 10H)

10 ¹³C NMR (CDCl₃) δ 16.0, 18.2, 18.7, 19.4, 20.1, 21.0, 28.8, 32.9, 33.3, 36.9, 38.6, 40.0, 42.4, 47.2, 56.9, 64.3, 69.6, 69.7, 114.3, 114.4, 119.7, 119.8, 127.9, 128.4, 133.1, 135.5, 135.6, 144.5, 148.0, 148.1, 148.5

HRESIMS calcd for C₃₆H₄₆O₄P [M+H]⁺ 573.3134, found 573.3117

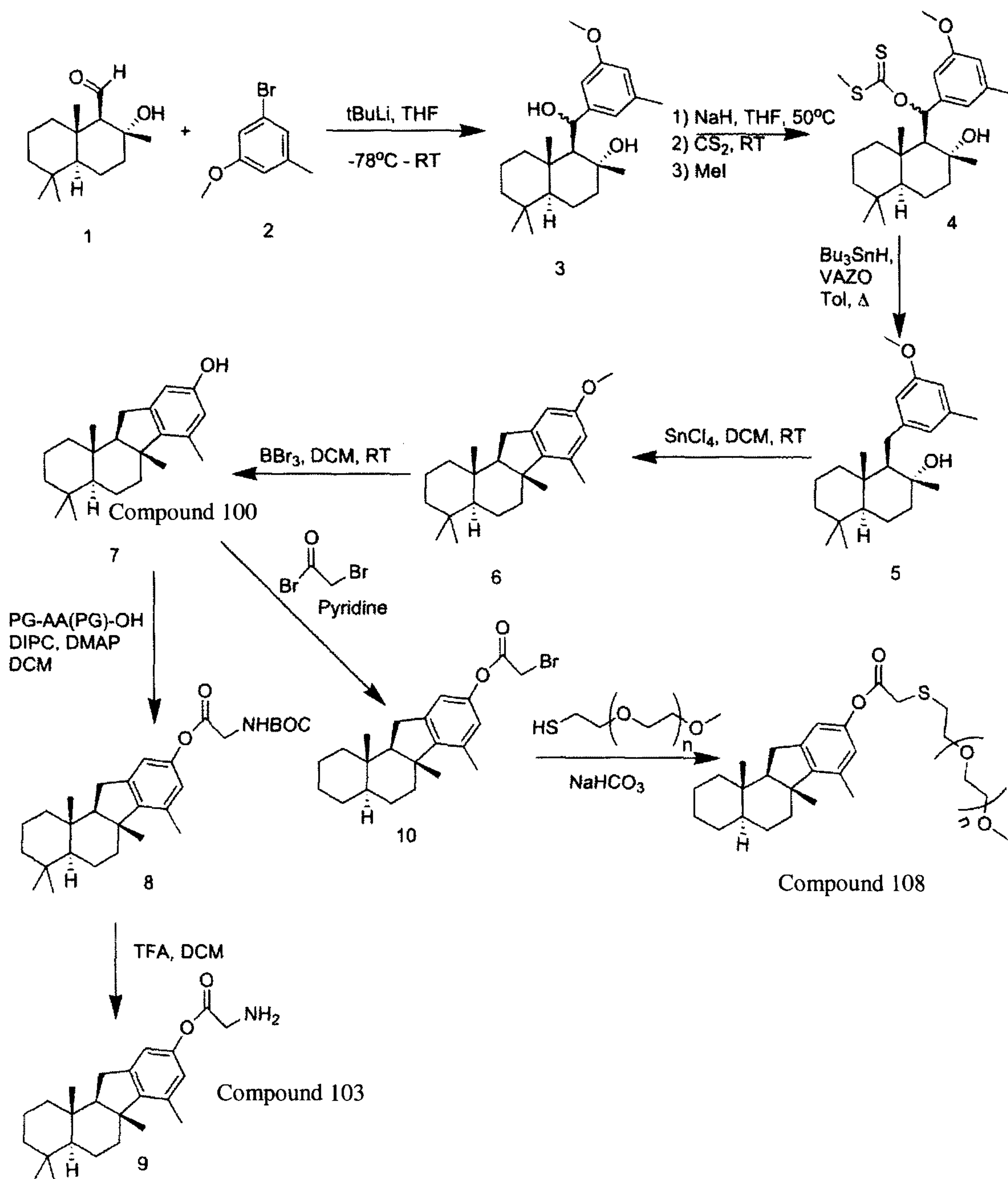
15 **Preparation of Compound 109**

Compound 10 (280mg, 0.49mmol) was dissolved in MeOH (8mL), and 10% Pd/C was added (30mg). The solution was saturated with H₂ and stirred for 18h at RT. The reaction was then filtered through a 0.45μm membrane and concentrated to dryness to yield Compound 109 (150mg, 0.38mmol, 78% yield) as a white powder.

20 ¹H NMR (CD₃OD) δ 0.83 (s, 6H), 0.93 (m, 2H), 1.00 (s, 6H), 1.14 (m, 1H), 1.35 (m, 1H), 1.38 (m, 1H), 1.48 (m, 1H), 1.65 (m, 5H), 2.21 (s, 3H), 2.31 (m, 1H), 2.41 (dd, 14.5, 6.0Hz, 1H), 2.55 (m, 1H), 6.64 (s, 1H), 6.82 (s, 1H)

¹³C NMR (CD₃OD) δ 16.7, 19.2, 19.4, 20.7, 21.6, 29.8, 33.9, 34.0, 38.2, 40.1, 41.3, 43.7, 48.5, 49.8, 58.4, 66.0, 115.8, 121.3, 134.1, 145.4, 149.1, 150.4

25 HRESIMS calcd for C₂₂H₃₃O₄NaP 415.2014, found 415.2028

Example 7 - Synthesis of Compound 103 and Compound 108

5 Drimane-8 α ,11-diol was prepared according to Kuchkova et al; *Synthesis*, 1997, 1045

Bromomethoxytoluene (2) was prepared according to Chan et al; *J. Med. Chem.* 44, 1866

Preparation of Aldehyde (1)

Drimane-8 α ,11-diol (17.5g, 72.8mmol) was dissolved in 1L CH₂Cl₂. Diisopropylethylamine (50.7mL, 291.2mmol) was added and the solution was cooled to -15°C. A solution of Pyr-SO₃ (46.3g, 291.2mmol) in DMSO (250mL) was added dropwise over a period of 20min, then the
5 reaction was stirred cold for an additional 5 min. To the cold reaction was added 1M HCl (500mL), and the organic layer was partitioned. The aqueous layer was washed with an additional 200mL CH₂Cl₂. The pooled organic layers were then washed with saturated NaHCO₃, dried over Na₂SO₄ and concentrated. The crude product was purified by column chromatography (Hex: EtOAc) to yield 10.5g aldehyde (1) (44.1mmol, 60.1% yield) as a white
10 semisolid.

Preparation of diol (3)

Bromomethoxytoluene (2) (3.64g, 18.29mmol) was dissolved in 35mL dry THF under an argon atmosphere. This solution was cooled to -78°C, and tBuLi (21.5mL, 36.6mmol) was
15 added dropwise via syringe. The solution was stirred for 10 min at -78°C, then warmed to RT for 20 min. The solution was re-cooled to -78°C, and a solution of aldehyde (1) (1.45g, 6.09mmol) in 6mL dry THF was added via syringe. The solution was stirred at -78°C 2h, after which the reaction was quenched with the addition of 1M HCl. EtOAc (100mL) was added, and the organic phase was washed with 1M HCl, followed by saturated NaHCO₃. The organic
20 phase was dried over MgSO₄, filtered and concentrated. The crude reaction mixture was purified by column chromatography (Hex:EtOAc) to yield diol (3) (1.94g, 88.5% yield) as a diastereomeric mixture.

Preparation of xanthate (4)

Diol (3) (1.94g, 5.39mmol) was dissolved in 20mL dry THF under an argon atmosphere. To this solution was added NaH (237mg, 60% in oil, 5.93mmol). The reaction was then heated to 50°C until the solution was clear orange. The reaction was cooled to 0°C, and CS₂ (1mL, 16.6mmol) was added. The solution was stirred for 20 min at 0°C, then warmed to RT for an additional 20 minutes, after which MeI (1mL, 16.6mmol) was added. The reaction was stirred
30 at RT for 1hour, then concentrated to dryness. The crude mixture was dissolved in EtOAc, and washed with 3x H₂O. The organic solution was dried over MgSO₄, filtered and concentrated to

yield a mixture of xanthate (4) and fragmentation product, ketone (12) (approx 4:1). This product mixture was used in the next step without further purification.

Preparation of alcohol (5)

5 Xanthate (4) and ketone (12) were dissolved as a crude mixture in 50mL toluene, and placed under an argon atmosphere. Bu_3SnH (2.9mL, 10.78mmol) was added, and the solution was heated. Once at reflux, a catalytic amount of VAZO (1,1'-Azobis(cyclohexanecarbonitrile)) (approx 50mg) was added through the top of the condensor. The solution was refluxed for 1hour, then an additional amount of VAZO was added (approx 50mg). The solution was
10 refluxed for another 45 min, after which TLC analysis (20% EtOAc:Hex) indicated the reaction to be complete. The reaction was cooled, then concentrated to dryness. Flash chromatography of the crude product yielded alcohol (5) (1.12g, 3.23mmol, 60% yield, 2 steps) as a white foam.

Preparation of tetracycle (6)

15 Alcohol (5) (1.12g, 3,23mmol) was dissolved in 10 mL CH_2Cl_2 and cooled to 0°C . To this solution was added SnCl_4 (1mL) neat. The orange solution was then stirred for 1 hour at 0°C , followed by quenching with MeOH. The reaction was extracted into EtOAc, and washed with 2x satd NaHCO_3 . The organic phase was dried over MgSO_4 , filtered and concentrated to yield tetracycle (6) (1.05g, 3.20mmol, 99% yield). This compound was used without further
20 purification.

Preparation of Compound 100 (7)

Tetracycle (6) (1.05g, 3.20mmol) was dissolved in 15 mL DCM. To this solution was added a solution of BBr_3 (1.0M in DCM) (3.20mL, 3.20mmol). The solution was stirred at RT for 2
25 hours, then concentrated to dryness. The brown residue was dissolved in EtOAc, then washed with H_2O until the pH of the aqueous layer was neutral. The crude product was purified by flash chromatography to yield Compound 100 (7) (931mg, 2.98mmol, 93% yield) as a white solid.

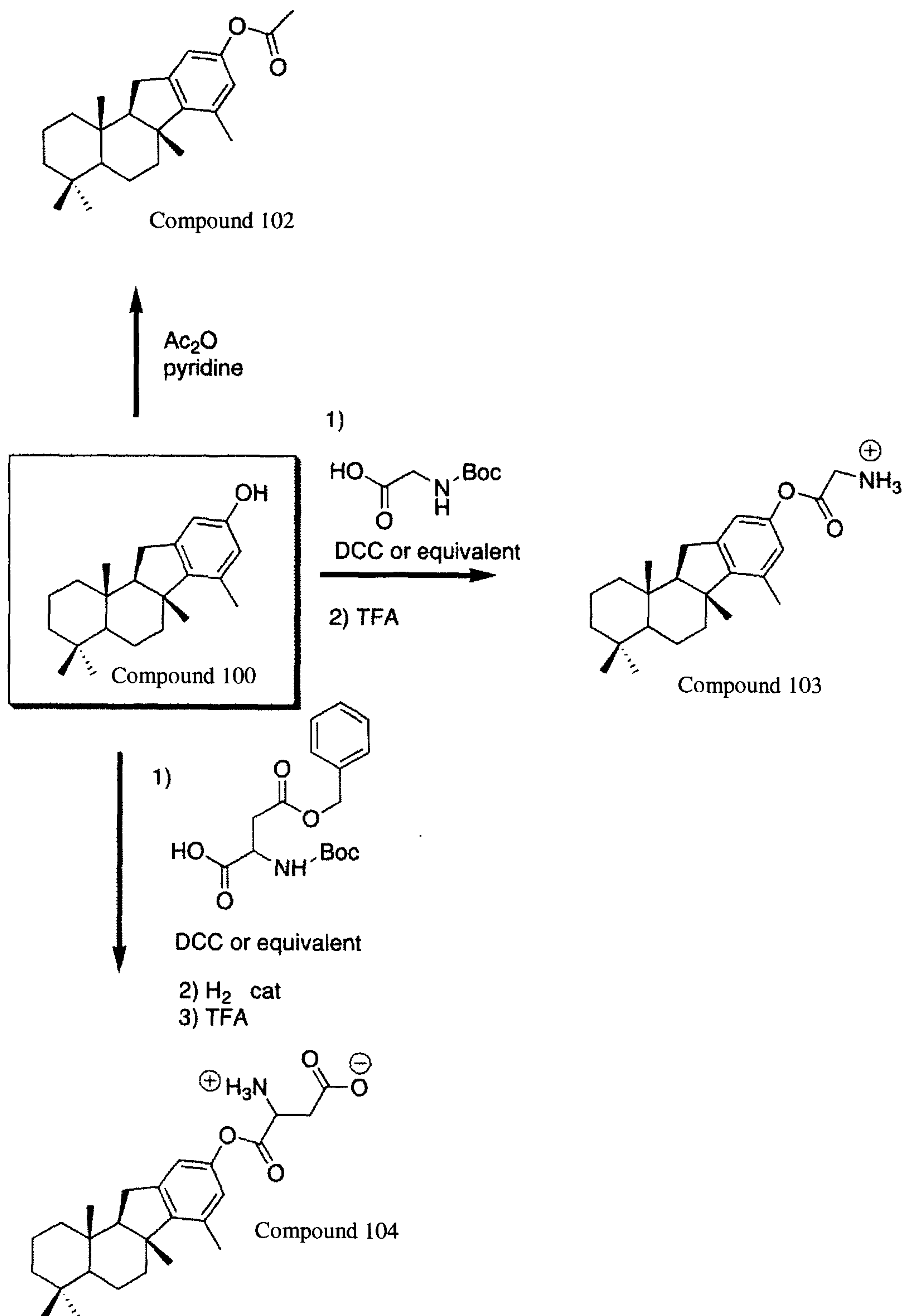
30 Preparation of glycine prodrug (9)

Compound 100 (7) (36.1mg, 0.116mmol), Boc-Gly-OH (30.5mg, 0.174mmol), DMAP (~2mg) were combined in 1mL CH_2Cl_2 . 1,3-diisopropylcarbodiimide (27uL, 0.174mmol) was added,

and the solution was stirred at RT for 2 hours. The reaction was then directly submitted to flash chromatography to yield a white foam (9). This compound was dissolved in 50% TFA/CH₂Cl₂ for 1 hour. The solution was concentrated, redissolved in toluene, and concentrated to dryness. Et₂O (15mL) was added, and the compound was triturated until the precipitate
5 appeared as a uniform solid. Centrifugation of the mixture, followed by washing of the solid with Et₂O yielded prodrug (9) (44.5mg, 0.092mmol, 80% yield) as the TFA salt.

Preparation of Pegylated prodrug (Compound 108)

To a solution of (7) (43.0g, 100mmol) in acetonitrile at 0°C were added α -bromoacetic acid
10 (19.46 g, 140mmol) and DMAP (610mg, 5 mmol). The reaction mixture was then treated with a solution of DCC (29.87 g, 145 mmol) in acetonitrile (200 mL) dropwise over 30 min, then stirred at 0°C for 2.5h. The white solid formed was removed by filtration and washed with acetonitrile (2 X 100 mL). The combined acetonitrile washes were then added to H₂O
(4000mL) over 15 min. After stirring for another 15 min, the resulting solid was collected and
15 washed with H₂O (2 X 250ml) and IPA (2 X 200mL) and then dried in vacuo. The collected white solid (6.06 g, 11 mmol) was added portionwise over a period of 30 min. to a solution of HS-PEG (35 g, MW 6000) and *N,N*-diisopropylethylamine (2.7 mL) in acetonitrile (90mL) under nitrogen at 0°C. After addition, the ice bath was removed and the mixture was allowed to warm to room temperature. After 3-4 hours, 2-propanol (1200 mL) was added over 30 min.
20 After an addition 1.5 h, the resulting solid was collected on a Buchner funnel and washed with 2 X 150 mL of 2-propanol. The wet cake was then dissolved in acetonitrile (80 mL) containing 0.5% ⁱPr₂NEt at 0-5°C and precipitated by addition of 2-propanol (1000mL). The resulting solid was collected and washed with 2-propanol and dried in vacuo to give Compound 108.

Example 8 – Synthesis of Compound 102, Compound 103 and Compound 104

Example 9 - Compound 103 inhibits TNF alpha production better than Compound 100

An assay to determine the relative inhibition of TNF α production by Compound 103 compared to Compound 100 was conducted as follows.

5 J774.1 macrophage cells were plated at 2×10^5 cells/well in 24 well plates. The next day the media was changed and Compound 100, Compound 103 or cyclodextrin carrier were added to the wells at the indicated concentrations for 30 min prior to stimulation of the cells with 2 ng/mL lipopolysaccharide (LPS). LPS activation of macrophages leads to production of TNF alpha which can be detected in the culture supernatant and quantified by ELISA. The results
10 are depicted in a graph in Figure 1.

Example 10 - Compound 106 inhibits macrophage TNF α production

An assay to determine the inhibition of macrophage TNF α by varying concentrations of Compound 106 was carried out as follows.

15 J2M macrophage cells were plated at 2×10^5 cells/well in 24 well plates. The next day the media was changed and Compound 106 or PBS carrier were added to the wells at the indicated concentrations for 30 min prior to stimulation of the cells with 2 ng/mL lipopolysaccharide (LPS). LPS activation of macrophages leads to production of TNF α which can be detected in
20 the culture supernatant and quantified by ELISA. The results are depicted in a graph in Figure 2.

Example 11 - Compound 106 inhibits calcium influx in mast cells

An assay to determine the inhibition of calcium influx in mast cells by Compound 106 was
25 carried out as follows.

The activation of mast cells by IgE receptor crosslinking leads to an influx of calcium into the cells, followed by degranulation and secretion of pro-inflammatory mediator. Bone marrow derived mast cells were loaded with the fluorescent calcium indicator dye Fura-2 prior to
30 treatment for 1 hr with Compound 106 or PBS carrier. Cells were then stimulated or not with an anti IgE antibody to cross-link the IgE receptors. Calcium influx was then monitored by fluorimetry. The results are depicted in a graph in Figure 3.

Example 12 - Compound 108 inhibits TNF α production in Wild Type (WT) but not Knock-out (KO) macrophages

Peritoneal macrophages isolated from wild-type (WT) or SHIP knock-out (KO) mice were in 24 well plates in CSF-1 containing media. The next day the media was changed and

5 Compound 108 or PBS carrier were added to the wells at the indicated concentrations for 60 min prior to stimulation of the cells with 2 ng/mL lipopolysaccharide (LPS). LPS activation of macrophages leads to production of TNF alpha which can be detected in the culture supernatant and quantified by ELISA. The results are depicted in a graph in Figure 4.

10 **Example 13 – Assay Screening of SHIP Modulators and Prodrugs thereof**

Various different SHIP modulating compounds and prodrugs thereof were tested in various different assays.

Assay 1) *In vitro* testing in a SHIP enzyme assay. Test compounds were dissolved in a
15 suitable solvent (e.g. EtOH, DMSO and others) and diluted into aqueous buffer (20 mM Tris HCl, pH 7.5 and 10 mM MgCl₂). SHIP enzyme assays were performed in 96-well microtitre plates with 10 ng of enzyme/well in a total volume of 25 μ L of 20 mM Tris HCl, pH 7.5 and 10 mM MgCl₂. SHIP enzyme was incubated with test extracts (provided in solvent) or vehicle for
15 min at 23°C before the addition of 100 μ M inositol-1,3,4,5-tetrakisphosphate (Echelon
20 Biosciences Inc, Salt Lake City, Utah). After 20 min at 37°C and the amount of inorganic phosphate released assessed by the addition of Malachite Green reagent and absorbance measurement at 650 nm.

Assay 2) Macrophage TNF- α production. J774.1a macrophage cells were treated with
25 10 μ g/mL of test compound dissolved in solvent (e.g. cyclodextran) for 40 minutes prior to the addition of 100ng/mL LPS. Culture supernatants were collected after 2 hr and 5 hr for TNF- α determination by ELISA.

Assay 3) Macrophage TNF- α NO assay. J774.1a macrophage cells were treated with
30 10 μ g/ml of test compound dissolved in solvent for 40 minutes prior to the addition of LPS. Culture supernatants were collected after 24 hr. for determination of NO concentration using the Griess reagent.

Assay 4) Stimulation of mast cells by Fc ϵ RI crosslinking. Mast cells were pre-loaded overnight in BMMC medium lacking IL-3 with 0.1 μ g/ml anti-DNP IgE (SPE-7, Sigma, Oakville, Ont). For calcium flux measurements, cells were incubated with 2 μ M fura
5 2-acetoxymethyl ester (Molecular Probes, Eugene, OR) in Tyrode's buffer at 23°C for 45 min. Cells were then washed and incubated in the presence of the test compound 30 min prior to stimulation with the indicated concentration of DNP-human serum albumin (DNP-HSA). Calcium influx was monitored by spectrofluorometry as described previously. For analysis of
intracellular signaling, cells were pre-loaded with anti-DNP IgE as above, pre-treated with the
10 test compound for 30 min at 37°C and stimulated with 20 ng/ml DNP-HSA for 5 min. Total cell lysates were then prepared and analyzed for phospho-PKB, phospho-p38^{MAPK}, phospho-MAPK, Grb-2 (Cell Signalling, Mississauga, Ont) and SHIP⁶ by immunoblot analysis.

Assay 5) Mouse acute cutaneous anaphylaxis model. 6-8 week old CD1 mice (University of British Columbia Animal Facility, Vancouver, BC) were sensitized to the hapten DNP by cutaneous application of 25 μ L of 0.5% dinitrofluorobenzene (DNFB) (Sigma, Oakville, Ont) in acetone to the shaved abdomen of mice for two consecutive days. 24 hrs
later, test substances (dissolved in 10 μ L of 1:2 DMSO:MeOH) were painted on the right ear
20 while the left ear received vehicle control. 30 min after drug application, DNFB was applied to both ears to induce mast cell degranulation. A 6 mm punch was taken from the ear and immediately frozen on dry ice for subsequent determination of neutrophil myeloperoxidase (MPO) activity.

Assay 6) Mouse endotoxemia model. 6-8 week old C57Bl6 mice (VCHRI Mammalian Model of Human Disease Core Facility, Vancouver, BC) were orally administered the test compound 30 min prior to an IP injection of 2 mg/kg of LPS (*E. Coli* serotype 0111:B4, Sigma, Oakville, Ont). Blood was drawn 2 hrs later for determination of plasma TNF α by ELISA.

Assay 7) *In vitro* Multiple Myeloma (MM) assay. The ability of SHIP activators to reduce tumor cell survival was assessed in MM cell lines treated with the test compound. The lines OPM1, OPM2, MM.1S and RPMI 8226 were plated at a density of 1 x 10⁵ cells/mL in

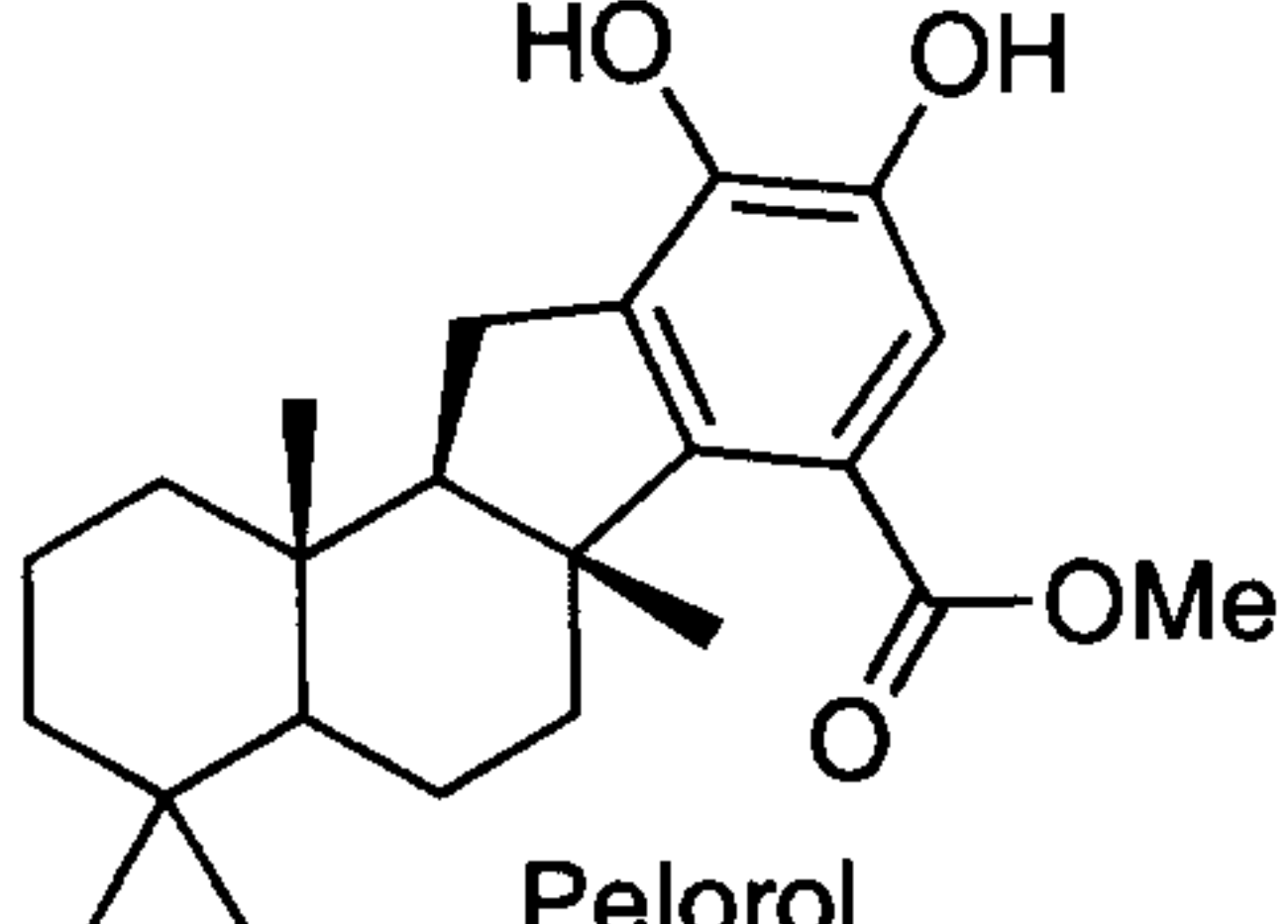
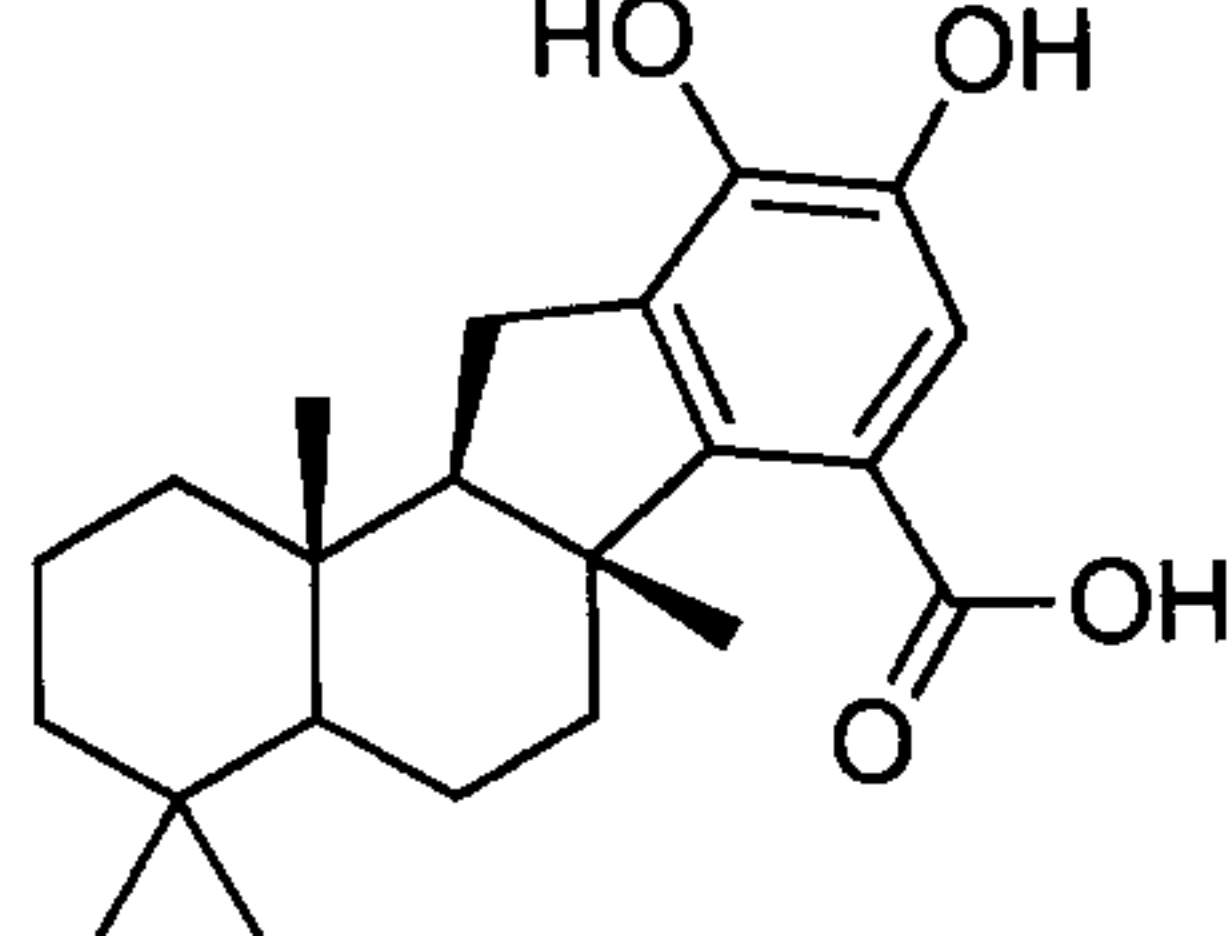
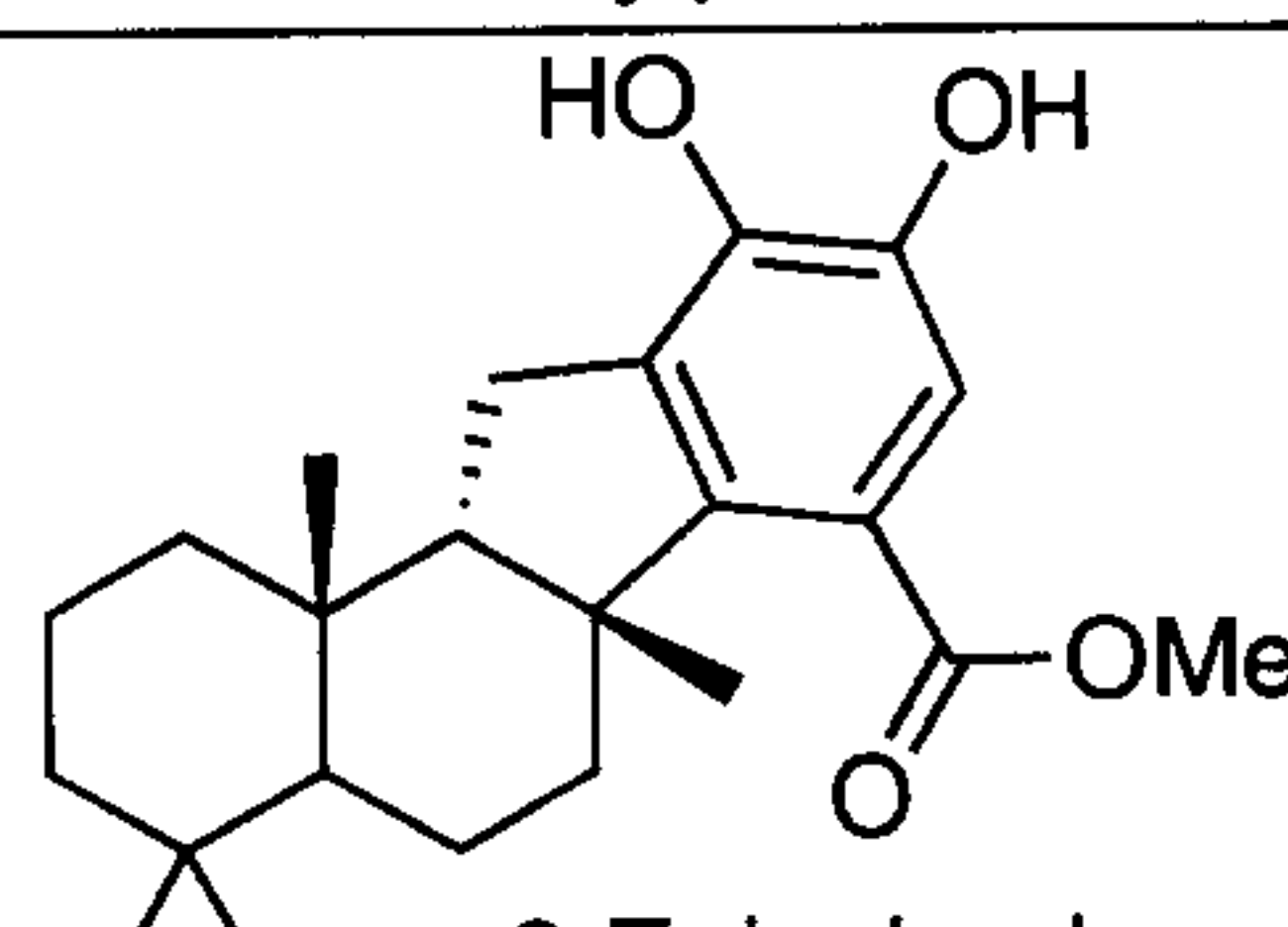
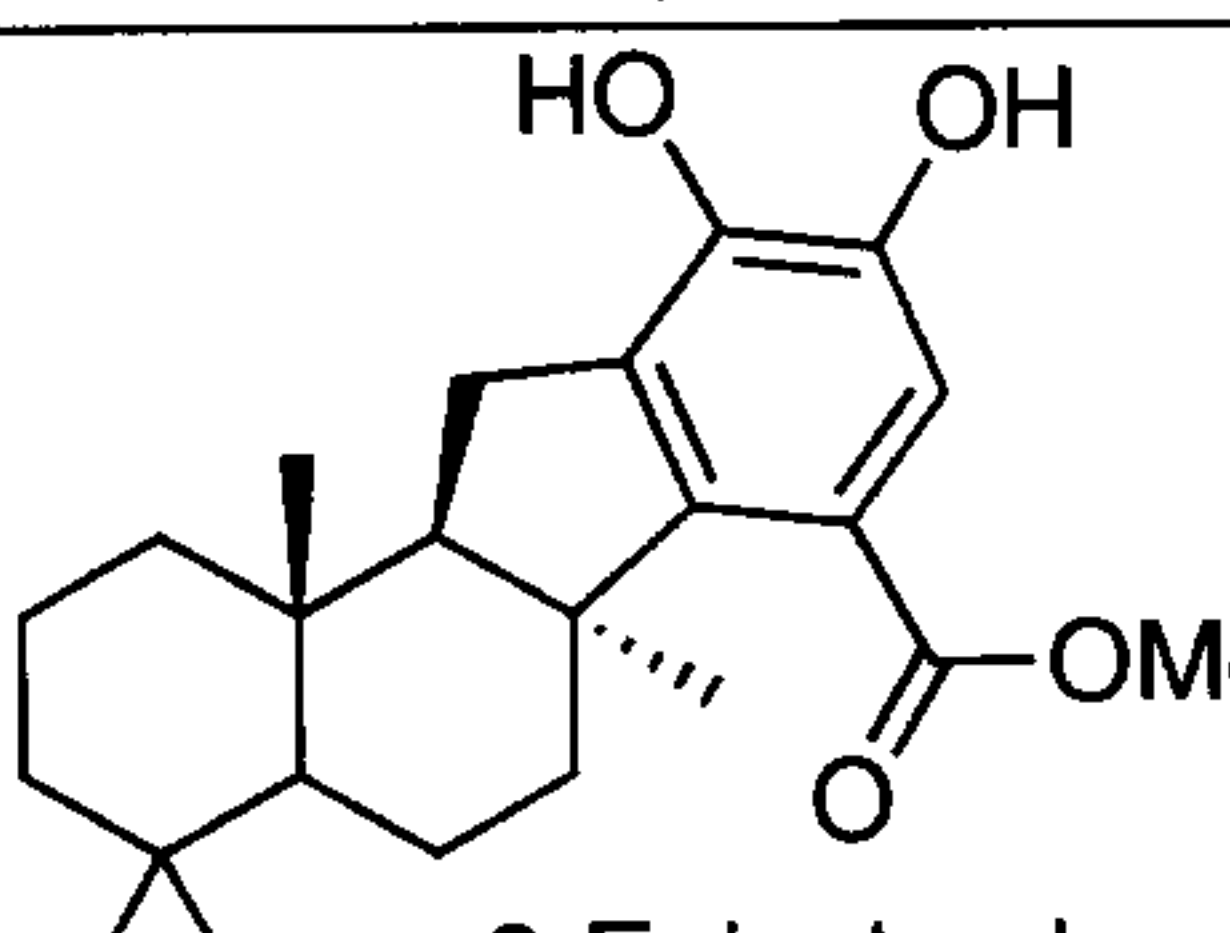
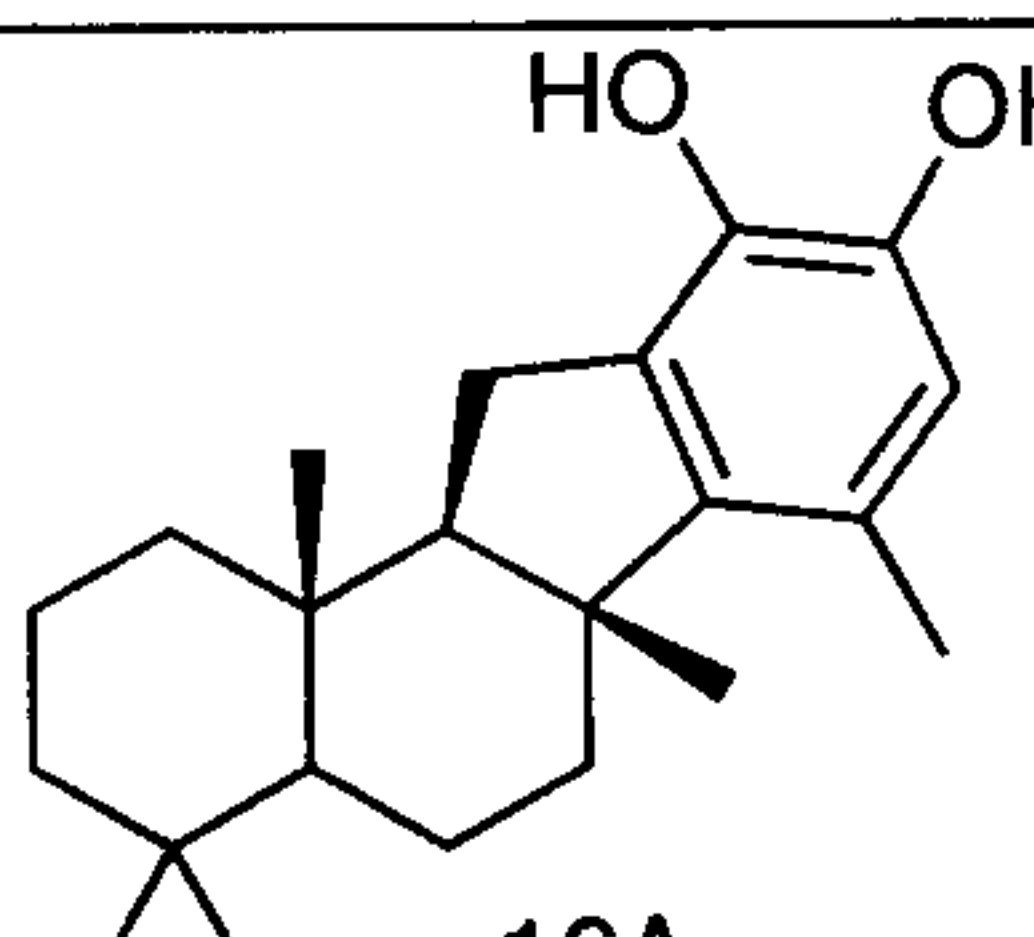
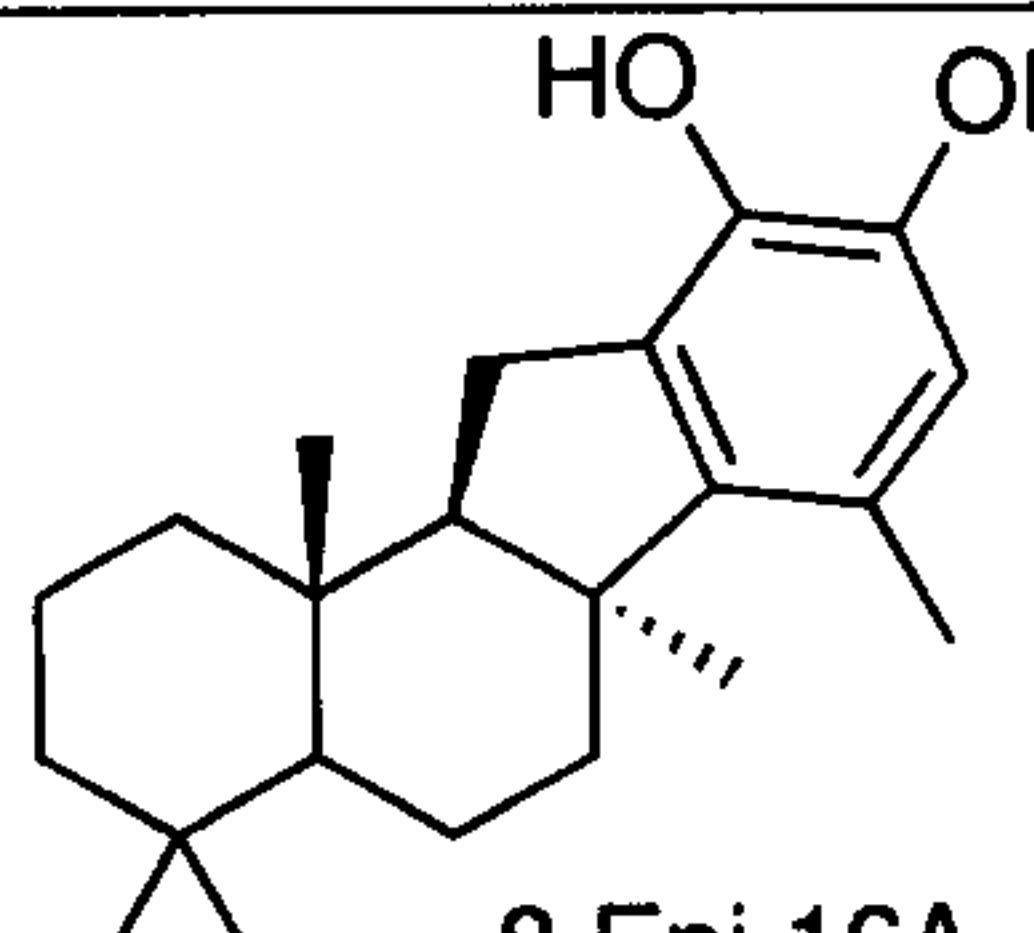
200 μ L of medium with various concentrations of the test compound, and viable cell numbers were determined on day 3 and day 5 by trypan blue exclusion. The lines RPMI 8226 and U266 were plated at a density of 1×10^6 cells/mL in 250 μ L of medium with various concentrations of the test compound. At day 4, the medium of each culture was replaced by fresh medium
5 containing the same concentration of test compound. At day 7, the viable cell number of each culture was determined by trypan blue exclusion.

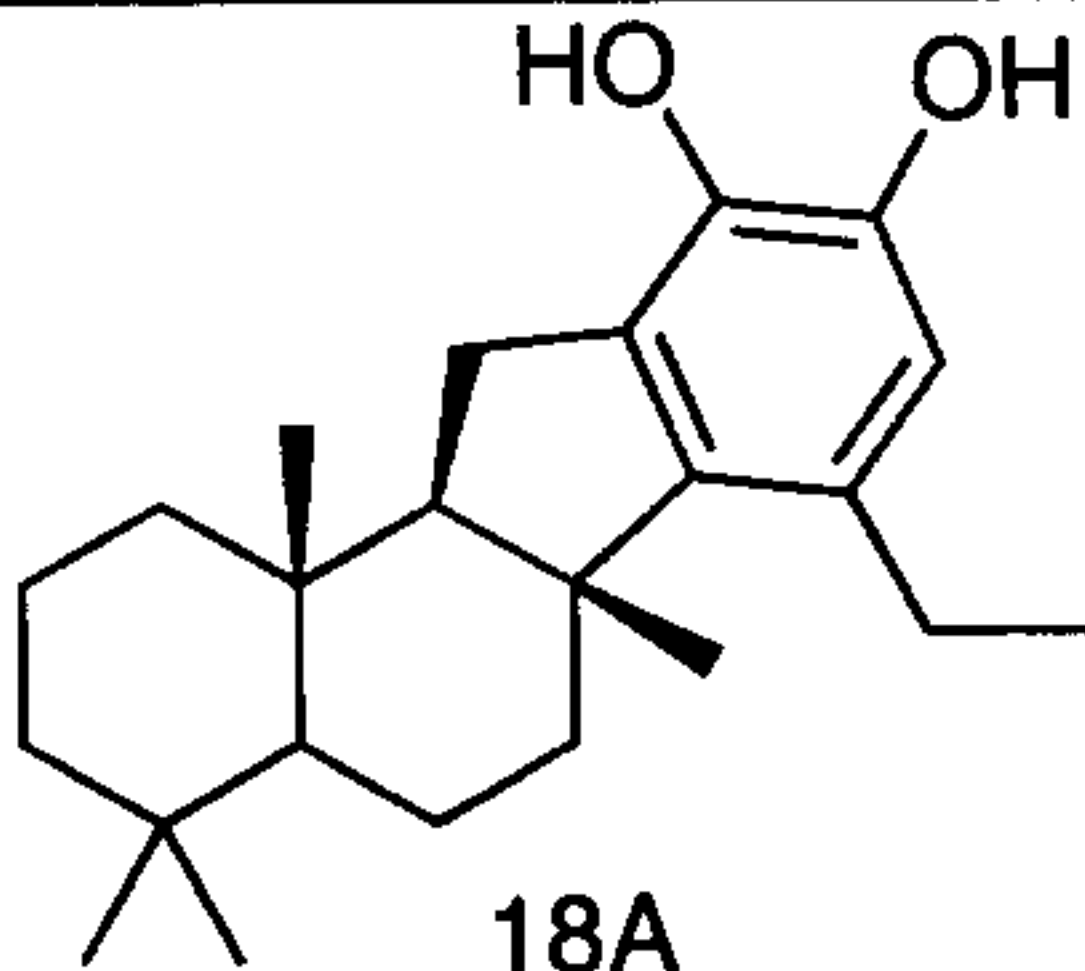
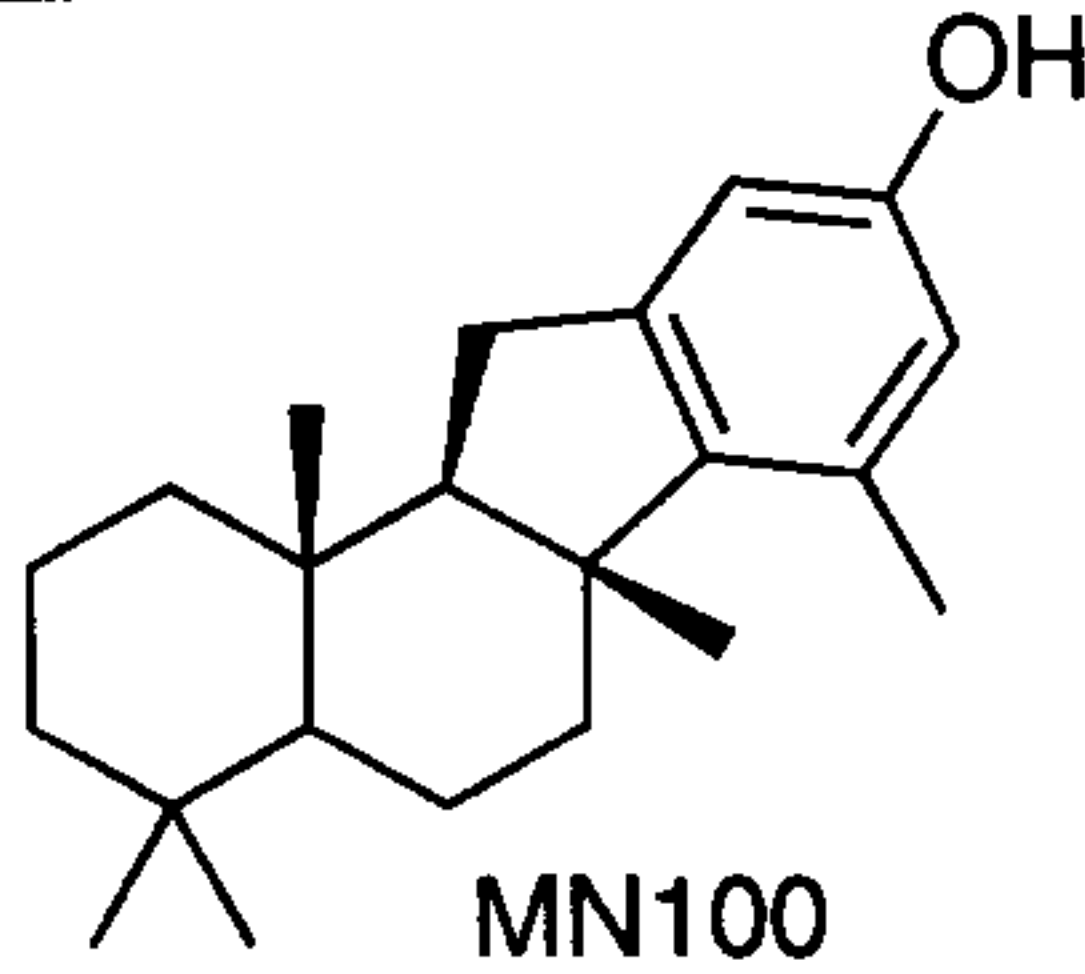
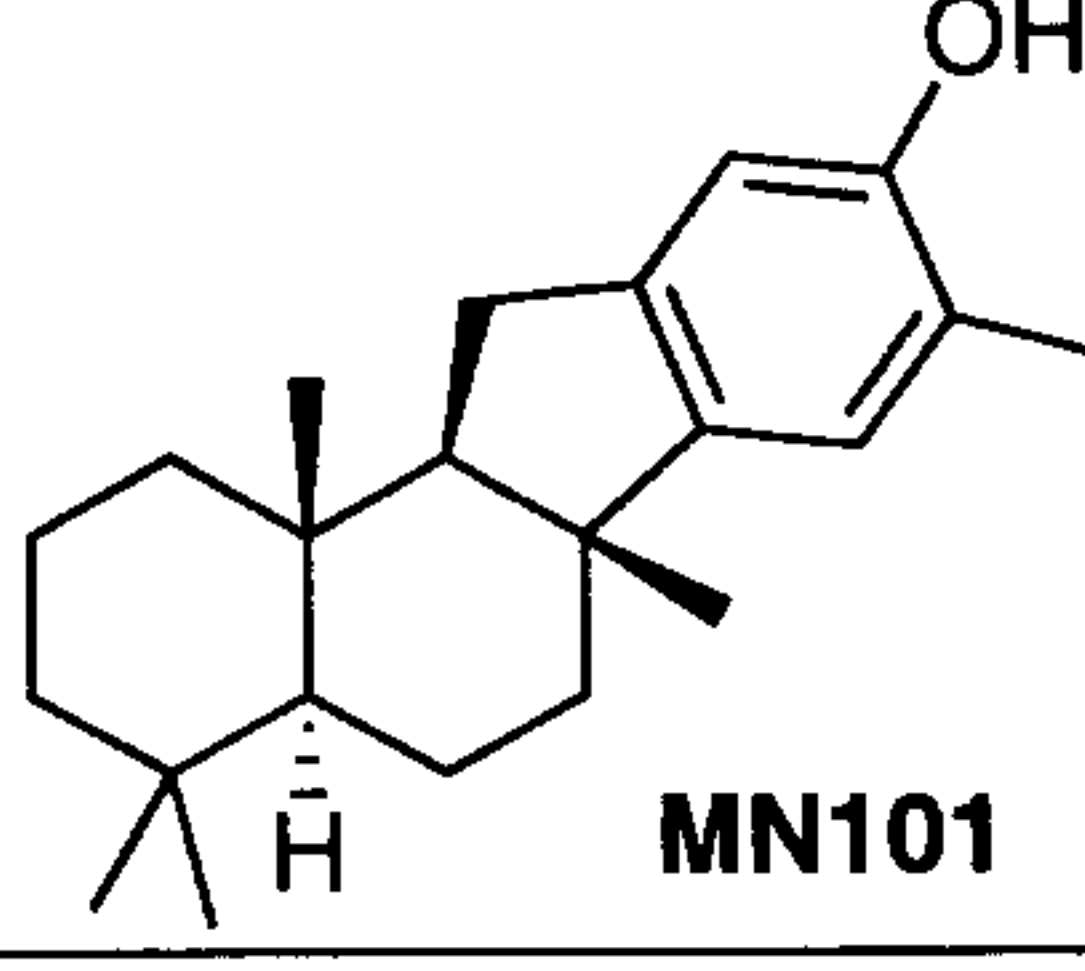
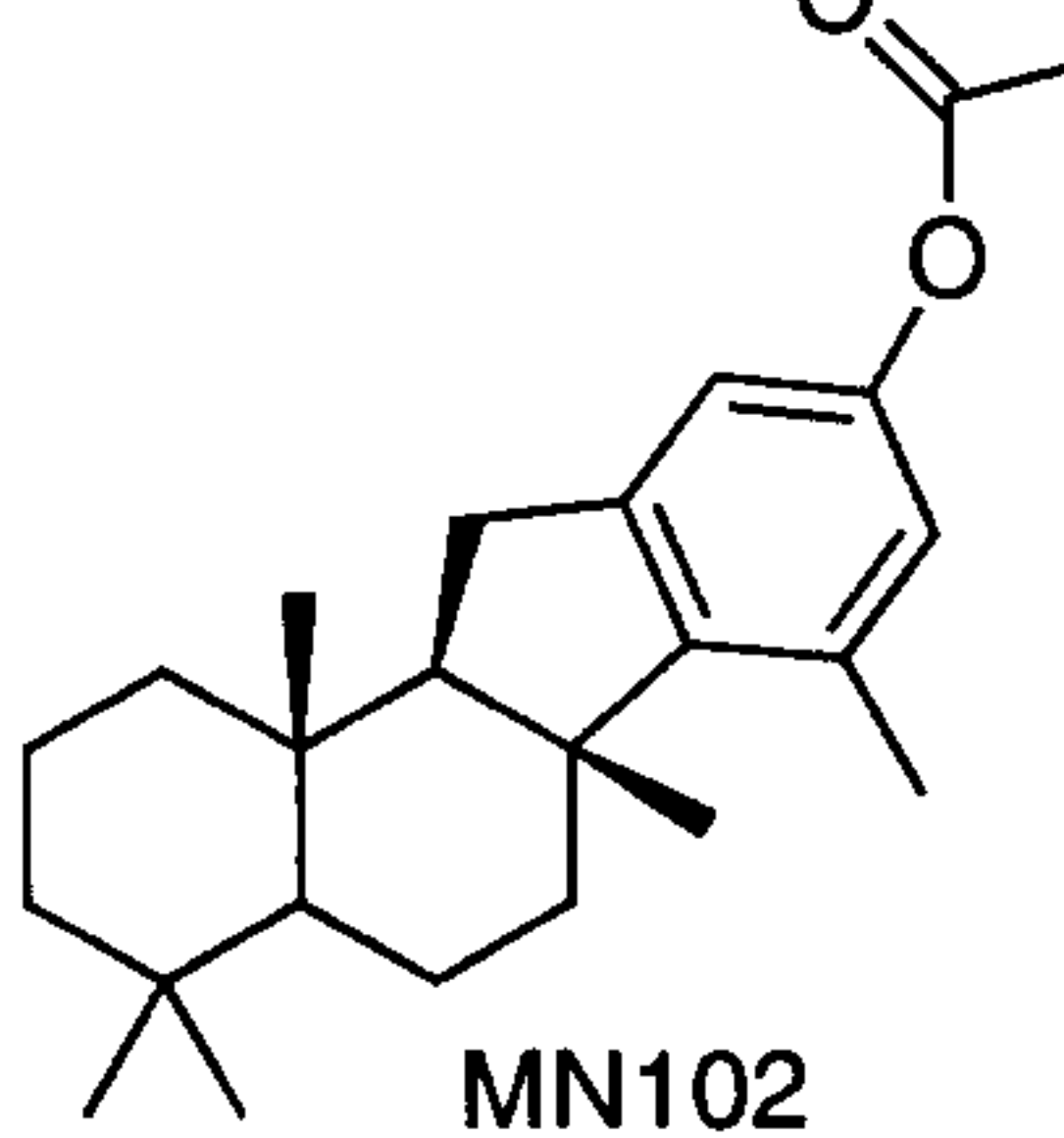
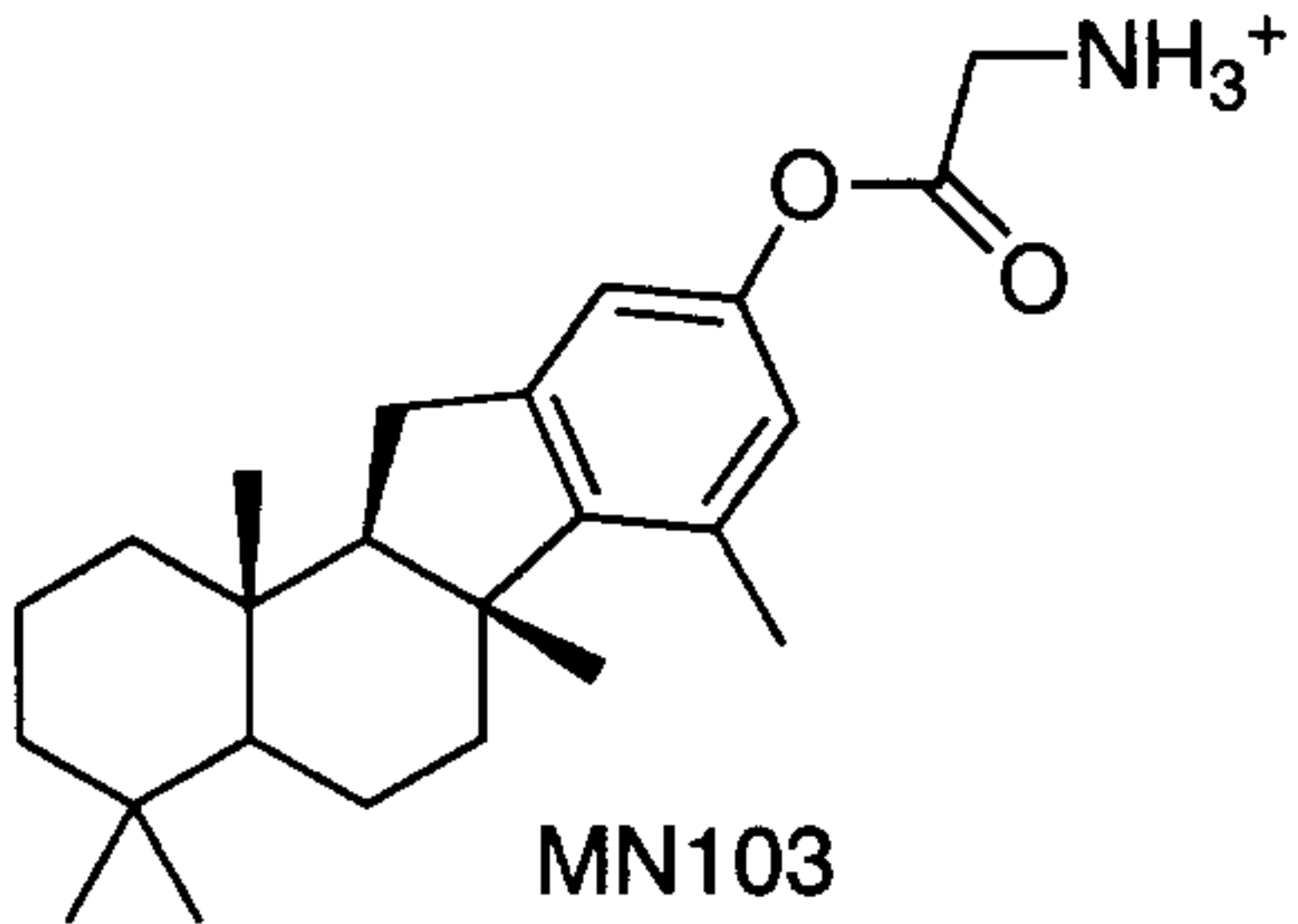
MM cell lines were cultured in 96 well plates seeded with 3×10^4 cells suspended in 200 μ L of medium along with various concentrations of test compound (and associated cyclodextran
10 vehicle control), with LY294002 serving as a positive control in the experiments. After 24-48 hrs of culture, 1 Ci of [3H]-thymidine (GE Healthcare, Baie D'Urfe, Canada) being added for the final 8 hours. Cells were harvested and DNA associated radioactivity was measured via liquid scintillation counting using a Wallac Microbeta counter (Perkin-Elmer; Boston, MA).

15 **Assay 8) *In vivo* Multiple Myeloma (MM) assay.** Mice were inoculated with at two sites each with 3×10^6 luciferase expressing OPM2 cells suspended in 50 μ L of growth medium and 50 μ L of Matrigel basement membrane matrix (Becton Dickenson; Bedford, MA). Tumors were injected subcutaneously in the upper and lower flanks of the mice and allowed to establish for 2 weeks. After 2 weeks, a test compound or control vehicle was administered in a
20 subcutaneous oil depot at a dose of 50 mg/kg every 3 days.

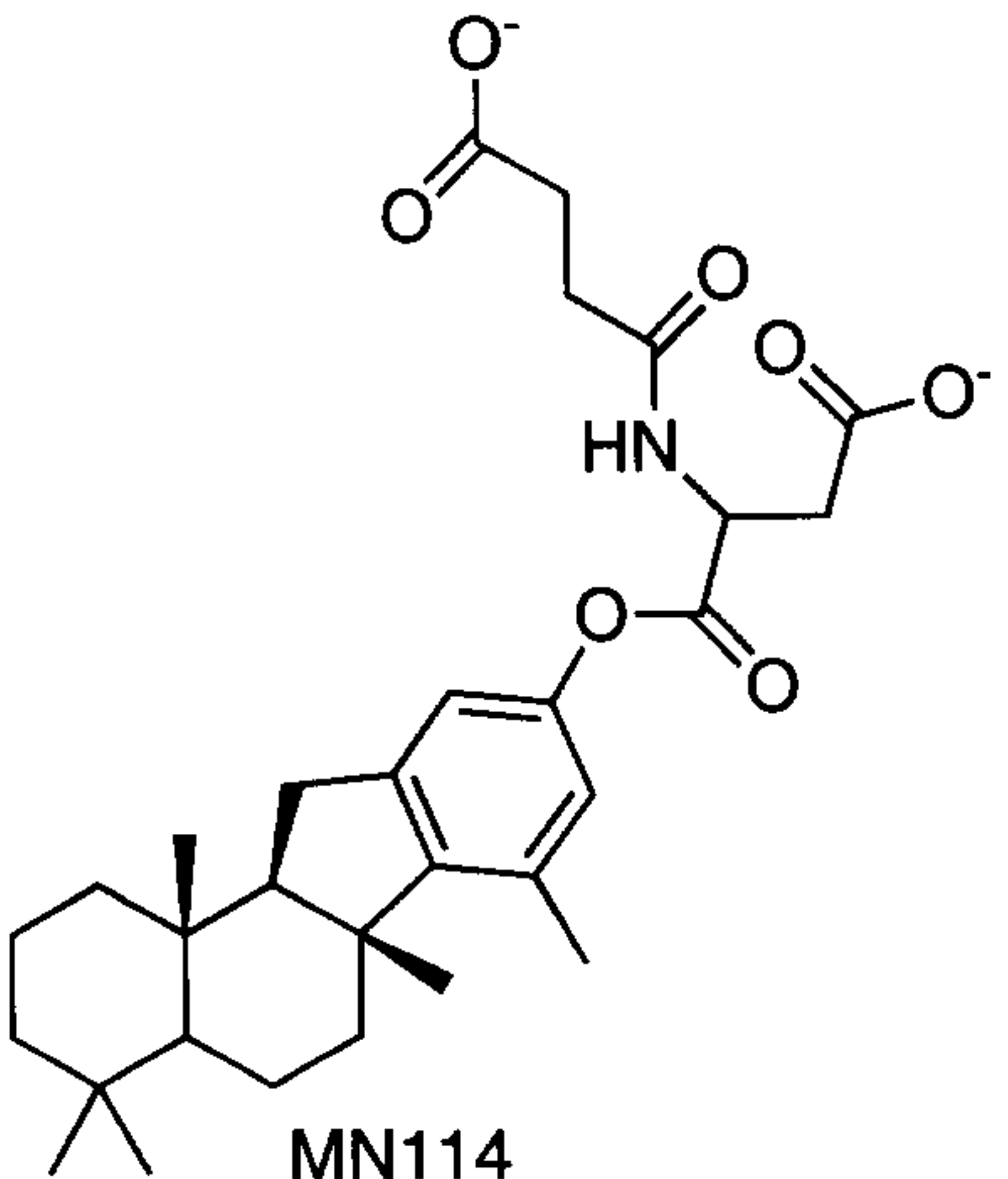
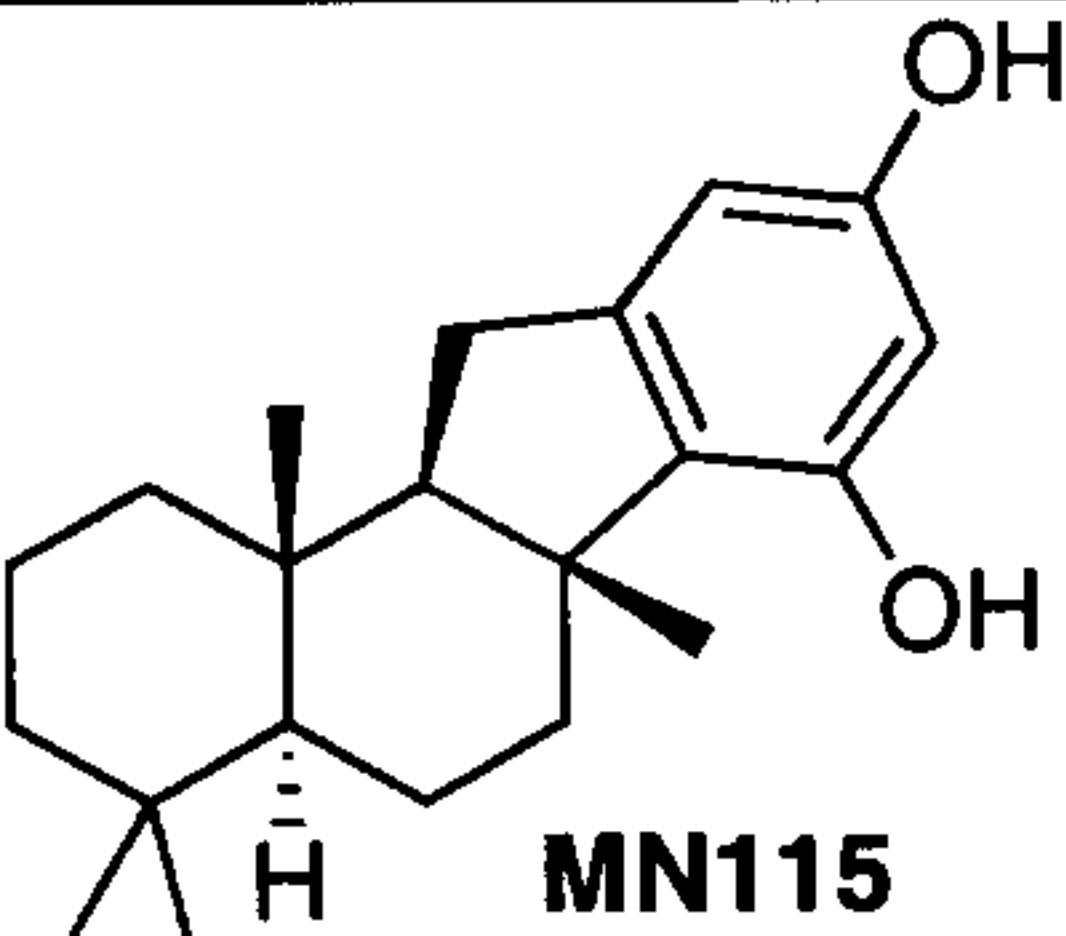
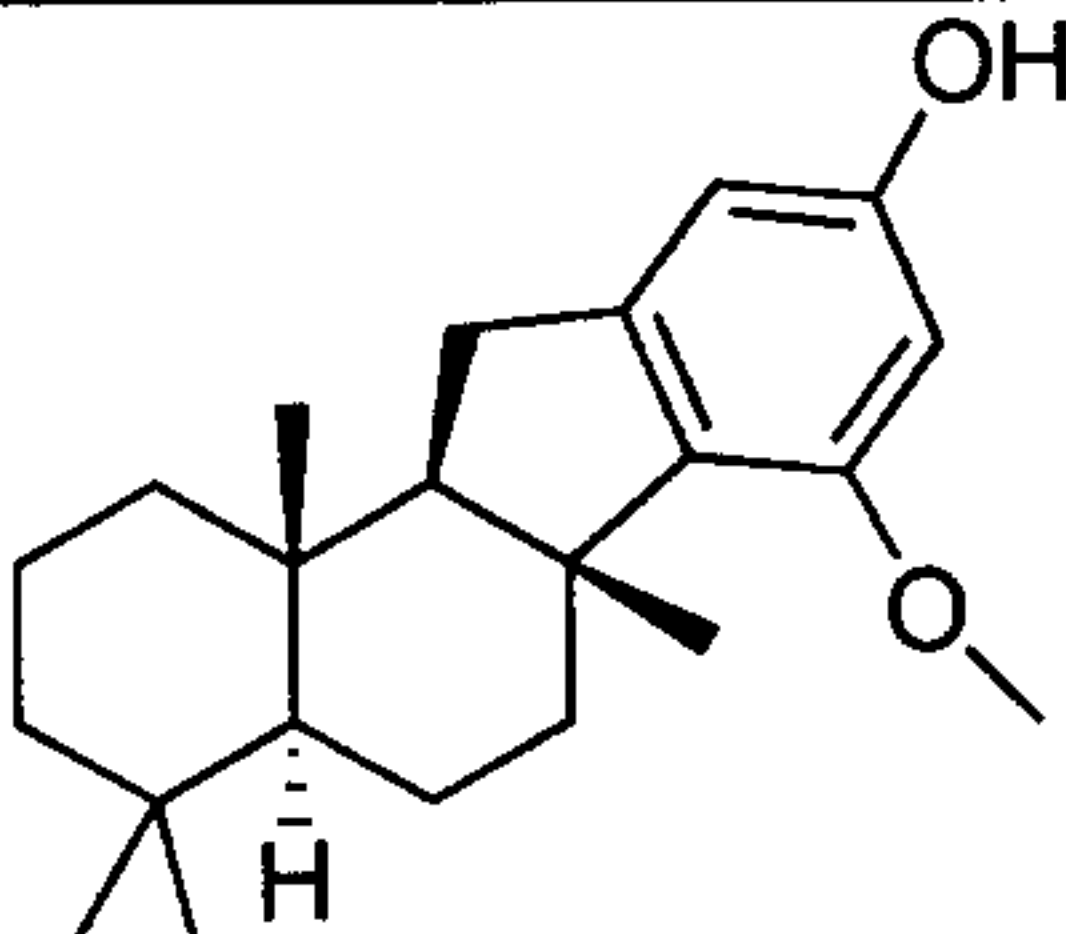
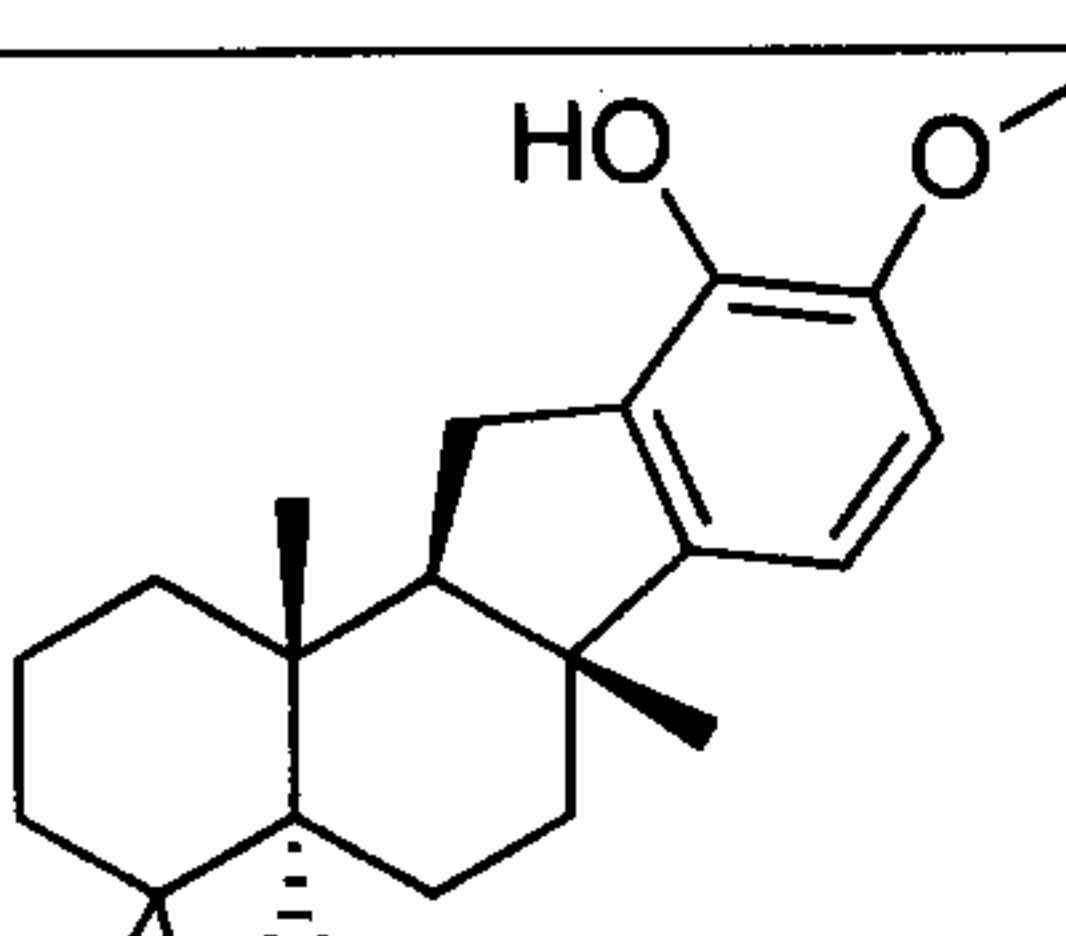
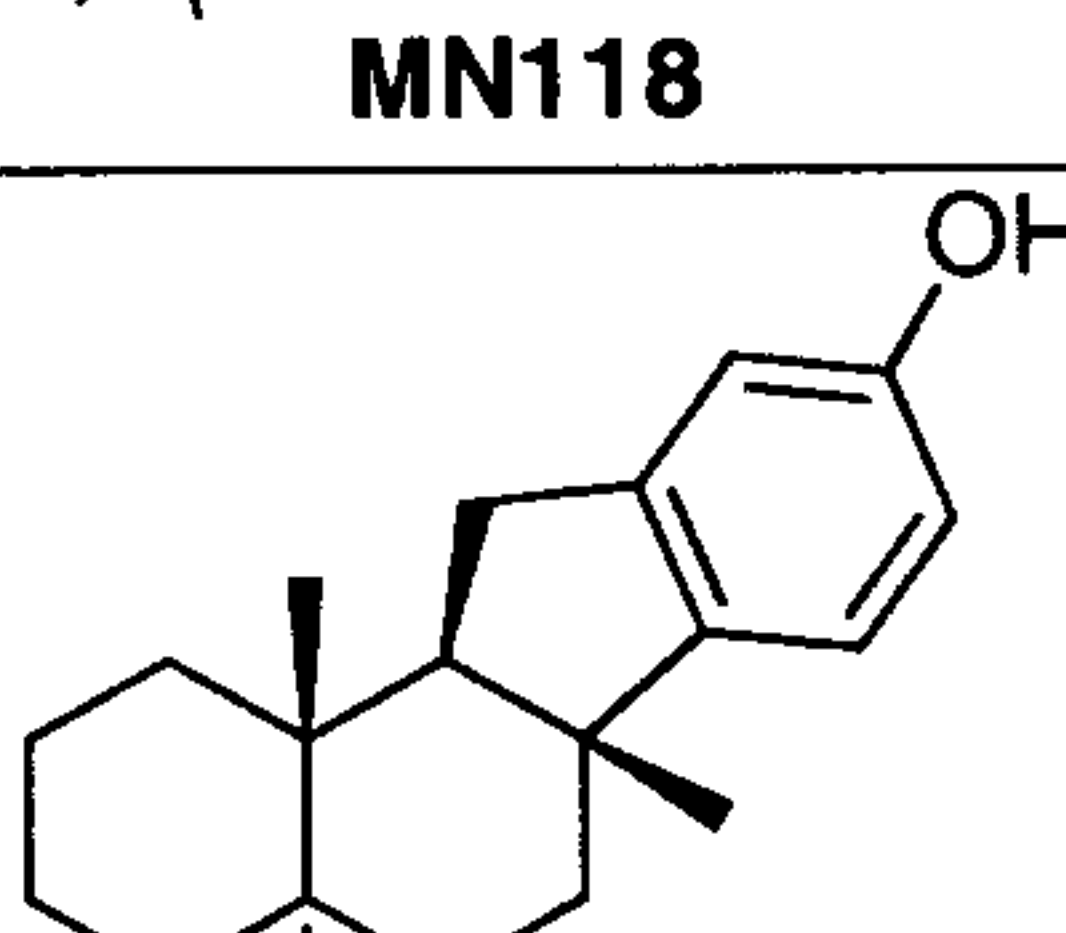
Tumors were measured using bioluminescence imaging on the Xenogen IVIS 200. Mice received intra-peritoneal injections of 200 μ L of D-luciferin at 3.75 mg/mL in sterile PBS. Mice were then anesthetized with isofluorane and imaged 15 minutes post-injection of
25 luciferin. Quantification of tumor size was performed using the Living Image™ software.

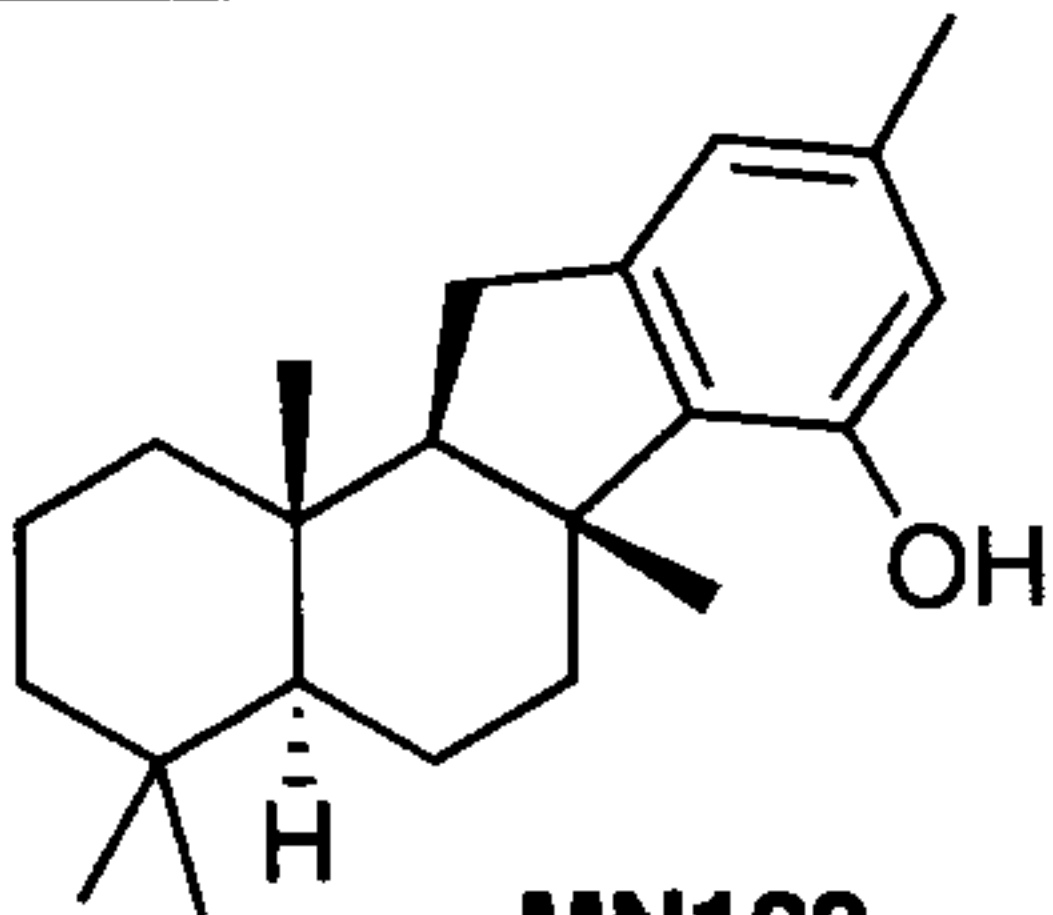
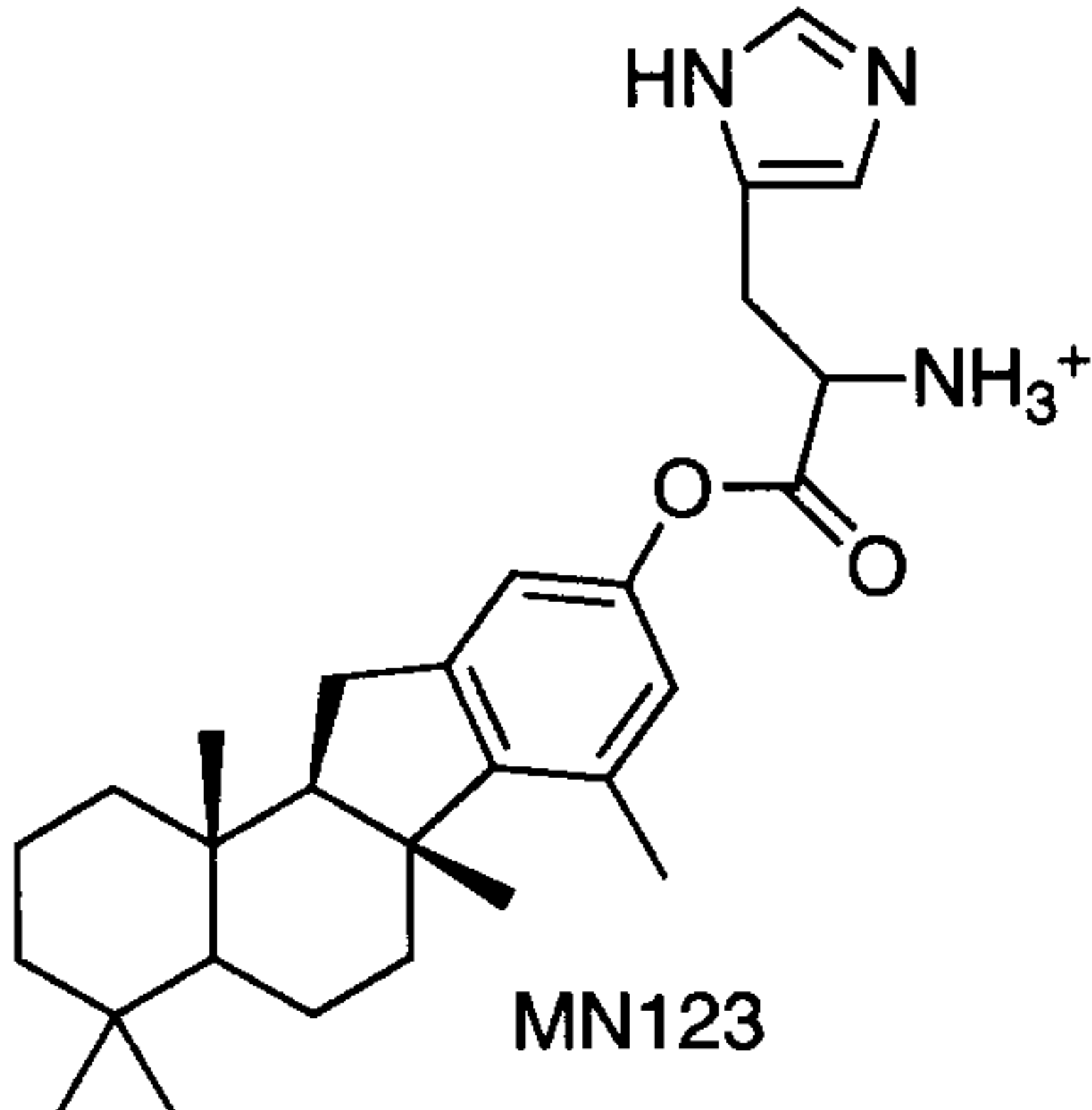
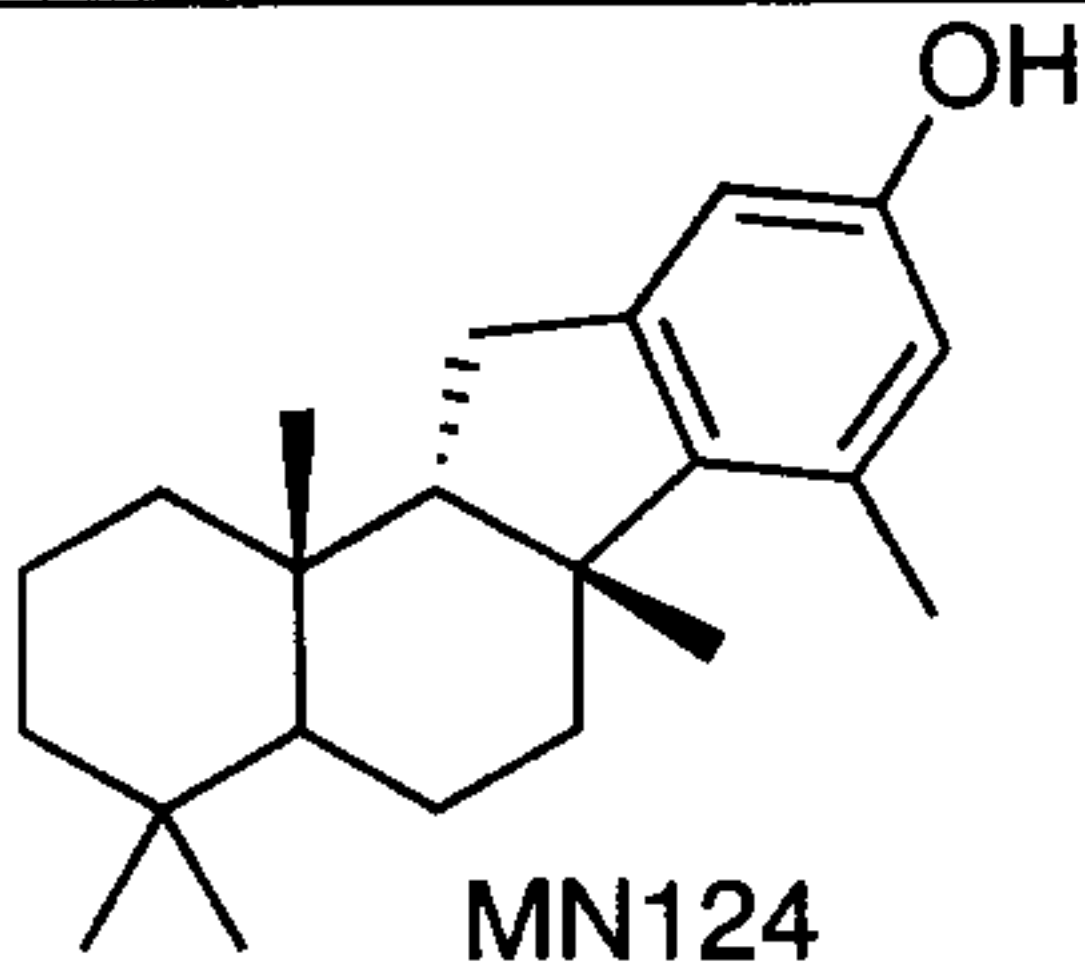
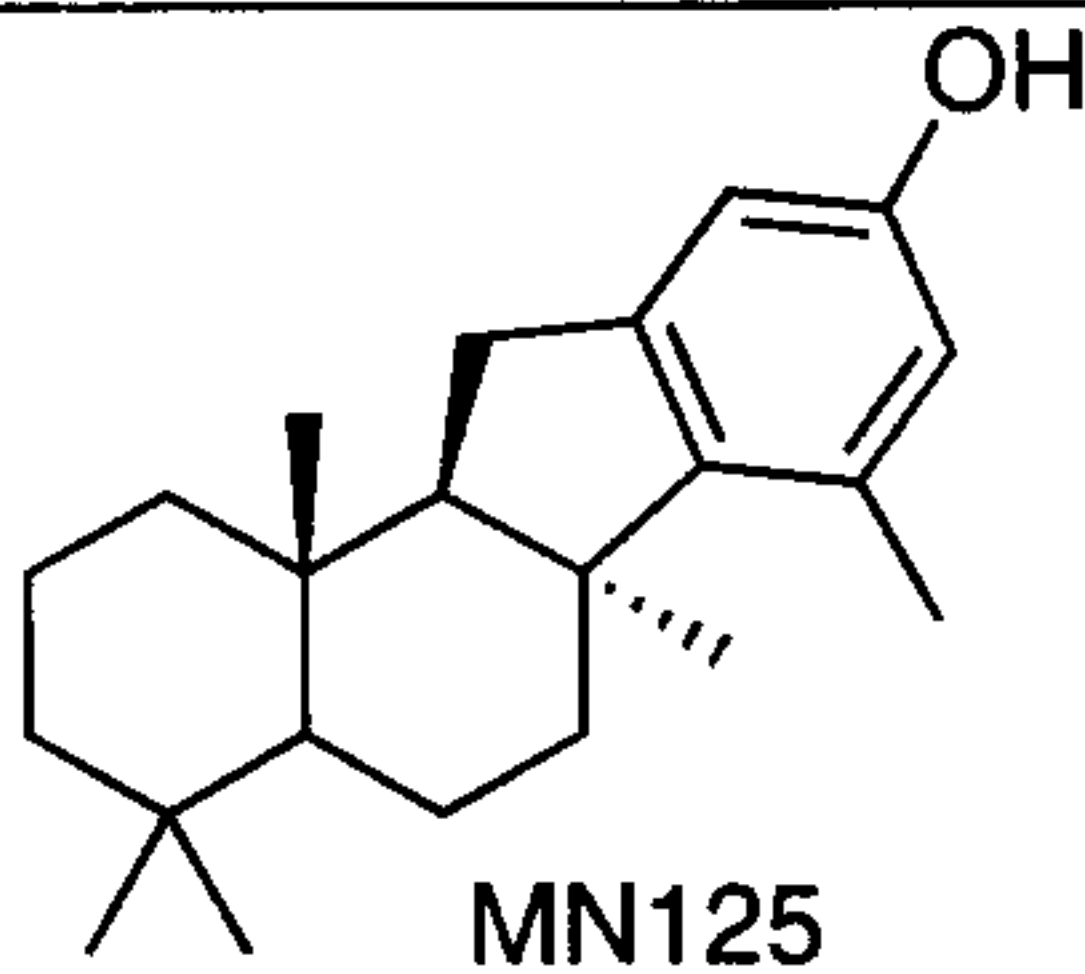
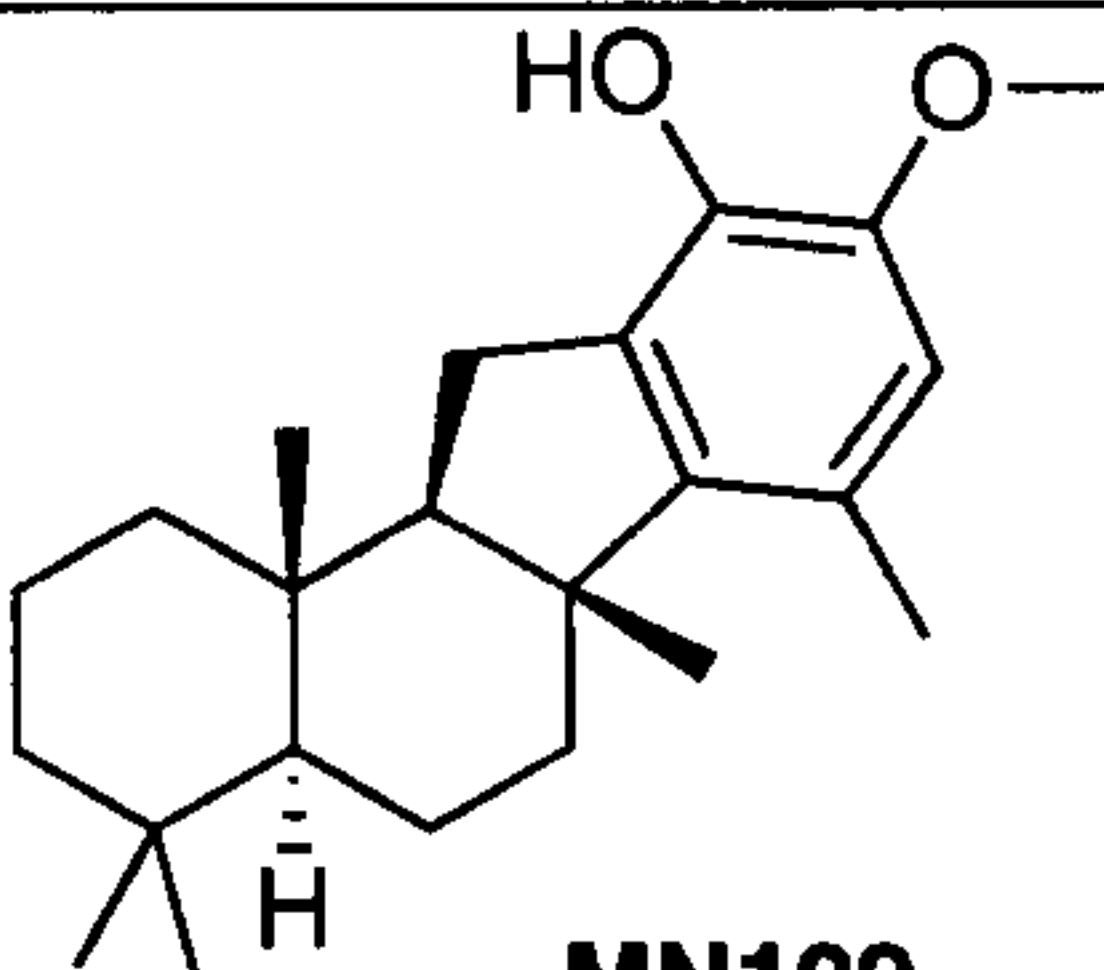
Various different SHIP modulating compounds and prodrugs thereof were tested in assays as described above and the results are shown quantitatively in the table below, where a '+' indicates a positive result for desired activity, a '-' indicates a negative result for desired
30 activity, and a 'NT' indicates no testing.

Assay Number →		1	2	3	4	5	6	7	8
Compound Structure ↓	Cmpd No.								
 Pelorol	N/A	+	+	+	NT	NT	NT	NT	NT
 Desmethypelorol	N/A	+	+	+	NT	NT	NT	NT	NT
 9-Epipelorol	N/A	+	+	+	NT	NT	NT	NT	NT
 8-Epipelorol	N/A	+	+	+	NT	NT	NT	NT	NT
 16A	AQX-016A	+	+	+	NT	+	+	+	+
 8 Epi 16A	N/A	+	NT	NT	NT	NT	NT	NT	NT

Assay Number →		1	2	3	4	5	6	7	8
Compound Structure ↓	Cmpd No.								
 18A	18A	+	+	+	NT	NT	NT	NT	NT
 MN100	100	+	+	+	+	+	+	+	+
 MN101	101	NT	+	+	NT	NT	NT	+	NT
 MN102	102	NT	+	+	NT	NT	NT	NT	NT
 MN103	103	NT	+	+	NT	NT	NT	NT	NT

75

Assay Number →		1	2	3	4	5	6	7	8
Compound Structure ↓	Cmpd No.								
 MN114	114	NT	+	+	NT	NT	+	NT	NT
 MN115	115	NT	NT	NT	NT	NT	NT	+	NT
 MN117	117	NT	+	+	NT	NT	NT	+	NT
 MN118	118	NT	-	+	NT	NT	NT	+	NT
 MN121	121	NT	+	+	NT	NT	NT	+	NT

Assay Number →		1	2	3	4	5	6	7	8
Compound Structure ↓	Cmpd No.								
 MN122	122	NT	-	+	NT	NT	NT	+	NT
 MN123	123	NT	+	+	NT	NT	NT	NT	NT
 MN124	124	NT	+	+	+	NT	NT	+	NT
 MN125	125	NT	+	+	+	NT	NT	+	NT
 MN129	129	NT	-	+	NT	NT	NT	NT	NT

Example 14

The ability of SHIP activators to reduce tumor cell survival was assessed in multiple myeloma (MM) cell lines treated with Compound 100 or AQX-016A. The lines OPM1, OPM2, MM.1S

5 and RPMI 8226 were plated at a density of 1×10^5 cells/mL in 200 μ L of medium with various

concentrations of Compound 100, and viable cell numbers were determined on day 3 and day 5 by trypan blue exclusion. The lines RPMI 8226 and U266 were plated at a density of 1×10^6 cells/mL in 250 μ L of medium with various concentrations of AQX-016A. At day 4, the medium of each culture was replaced by fresh medium containing the same concentration of AQX-016A. At day 7, the viable cell number of each culture was determined by trypan blue exclusion. Experiments were performed in triplicate. Compound 100 inhibits MM proliferation at lower concentrations than AQX-016A and the results are illustrated graphically in Figures 5A, 5B and 5C.

Example 15

Proliferation (DNA synthesis) assays. Proliferation was measured by measuring incorporation of [^{3} H]-thymidine into cells. MM cell lines were cultured in 96 well plates seeded with 3×10^4 cells suspended in 200 μ L of medium along with various concentrations of Compound 100 or AQX-016A (and associated cyclodextran vehicle control), with LY294002 serving as a positive control in the indicated experiments. After 24-48 hrs of culture, 1 μ Ci of [^{3} H]-thymidine (GE Healthcare, Baie D'Urfe, Canada) being added for the final 8 hours. Plates were frozen, which also aided in cell lysis, to terminate the experiments. Cells were then harvested onto glass fibre filters using an automatic cell harvester (TomTech; Orange, CT) and DNA associated radioactivity was measured via liquid scintillation counting using a Wallac Microbeta counter (Perkin-Elmer; Boston, MA). Wells were set up in triplicate and data is expressed as mean $\pm$ SEM. The results are illustrated graphically in Figures 6A, 6B, 6C, 6D and 6E.

Example 16

Formulation of compounds

For *in vitro* testing in the SHIP enzyme assay, AQX-016A and Compound 100 were dissolved in EtOH and diluted into aqueous buffer (20 mM Tris HCl, pH 7.5 and 10 mM MgCl_2). The actual concentration of drug in solution was determined by optical density measurement at 280 nm (λ_{max} for both compounds) after high speed centrifugation at 14 000 X g for 30 min to remove precipitated drug. For testing on cells, compounds were formulated in the carrier cyclodextrin (Cyclodex Technologies, High Springs, FL) at 6 mM (2 mg/mL). For oral administration to animals, compounds were dissolved in 100% cremophore EL (Sigma-Aldrich

Canada, Oakville, Ontario) at 150 mM (50 mg/mL) prior to dilution to 6 mM in phosphate buffer saline. However, while these compounds caged in cyclodextrin or formulated in cremophore EL micelles were very soluble in aqueous solution, they could not be used in the SHIP enzyme assays because of interference from both cyclodextrin and cremophore EL.

5

Production of recombinant SHIP enzyme and SHIP C2 domain

N-terminal His₆ tagged SHIP enzyme was expressed in mammalian 293T cells by transient transfection with pME18S-His-SHIP plasmid and purified to >95% homogeneity by Ni-chelating bead chromatography (Qiagen, Mississauga, Ontario). Recombinant SHIP C2 domain (amino acid residues 725 to 863) was expressed in *E. coli* transformed with a pET28C expression vector constructed as described below. Recombinant protein purified from the cell lysates by Nichelating bead chromatography was >95% pure.

***In vitro* SHIP enzyme assay.** SHIP enzyme assays were performed in 96-well microtitre plates with 10 ng of enzyme/well in a total volume of 25 µL of 20 mM Tris HCl, pH 7.5 and 10 Mm MgCl₂. SHIP enzyme was incubated with test extracts (provided in DMSO) or vehicle for 15 min at 23°C before the addition of 100 µM inositol-1,3,4,5-tetrakisphosphate (Echelon Biosciences Inc, Salt Lake City, Utah). After 20 min at 37°C and the amount of inorganic phosphate released assessed by the addition of Malachite Green reagent and absorbance measurement at 650 nm. SHIP2 enzyme was purchased from Echelon Biosciences (Salt Lake City, Utah) and an equivalent amount of inositol phosphatase activity was used in the *in vitro* enzyme assay. Enzyme data are expressed as the mean of triplicates +/- SEM. Experiments were performed at least 3 times. (Figures 7A and 7B).

Compound 100 is as biologically active as AQX-016A at lower concentratons

AQX-016A was substantially more active on SHIP^{+/+} than SHIP^{-/-} cells indicates that AQX-016A specifically targets SHIP. However, the presence of a catechol moiety within AQX-016A (Figure 7A) was potentially problematic since catechols can exhibit activities independent of their specific protein pocket binding interaction. For example, catechols can bind metals or be oxidized to an ortho-quinone which can lead to covalent modification of proteins through redox reactions. A non-catechol version of AQX-016A designated Compound 100 (Nodwell M. and Andersen RJ, *manuscript in preparation*). Analogous to AQX-016A, Compound 100 enhanced SHIP enzyme activity *in vitro* (Figure 7A and 7B).

Like AQX-016A, Compound 100 also selectively inhibited TNF α production from SHIP^{+/+} but not SHIP^{-/-} macrophages (Figure 7C). The EC₅₀ for this inhibition was 0.3 – 0.6 μ M. Oral administration of Compound 100 also efficiently inhibited the LPS-induced elevation of plasma TNF α levels in the mouse endotoxemia model (Figure 7D).

5

Example 17

Production of SHIP^{+/+} and SHIP^{-/-} Bone Marrow Derived Macrophages and Mast cells.

Bone marrow cells were aspirated from 4 to 8 week old C57Bl6 x 129Sv mixed background mice and SHIP^{+/+} and SHIP^{-/-} mast cells prepared as described previously. Bone marrow

10 derived macrophages from SHIP^{+/+} and SHIP^{-/-} mice were obtained and maintained in IMDM supplemented with 10% FCS, 150 μ M MTG, 2% C127 cell conditioned medium as a source of macrophage colony stimulating factor (M-CSF) (macrophage medium)

LPS stimulation of macrophages. For the analysis of LPS-stimulated TNF α production, 2
15 $\times 10^5$ cells were plated the night before in 24 well plates in macrophage medium. The next day, the medium was changed and AQX-016A or carrier was added to cells at the indicated concentrations for 30 min prior to the addition of 10 ng/mL LPS. Supernatants were collected for TNF α determination by ELISA (BD Biosciences, Mississauga, ON, Canada). For analysis of intracellular signaling, 2 $\times 10^6$ cells were plated the night before in 6 cm tissue culture plates.
20 The next day, the cells were cultured in macrophage medium without M-CSF for 1 hr at 37°C and then pretreated with AQX-016A or carrier for 30 min prior to the addition of 10 ng/mL LPS for 15 min. Cells were washed with 4°C PBS and resuspended in lysis buffer (50 mM Hepes, 2 mM EDTA, 1mM NaVO₄, 100 mM NaF, 50 mM NaPP_i and 1%NP40) supplemented with Complete Protease Inhibitor Cocktail (Roche, Montreal, Canada). Lysates were rocked at
25 4°C for 30 min and clarified by centrifuging 20 min at 12000 x g. Lysates were then made 1 x in Laemmli's buffer, boiled 2 min and loaded onto 7.5% SDS polyacrylamide cells. Immunoblot analysis for phospho PKB (Cell Signalling, Mississauga, Ont), SHIP and actin (Santa Cruz, Santa Cruz, CA) were carried out as described previously.

30 **Stimulation of mast cells by Fc ϵ RI crosslinking.** Mast cells were pre-loaded overnight in BMMC medium lacking IL-3 with 0.1 μ g/ml anti-DNP IgE (SPE-7, Sigma, Oakville, Ont). For calcium flux measurements, cells were incubated with 2 μ M fura 2-acetoxymethyl ester

(Molecular Probes, Eugene, OR) in Tyrode's buffer at 23°C for 45 min. Cells were then washed and incubated in the presence of vehicle control, LY294002 or AQX-016A 30 min prior to stimulation with the indicated concentration of DNP-human serum albumin (DNP-HSA). Calcium influx was monitored by spectrofluorometry. For analysis of intracellular signaling, cells were pre-loaded with anti-DNP IgE as above, pre-treated with AQX-016A or buffer control for 30 min at 37°C and stimulated with 20 ng/ml DNP-HSA for 5 min. Total cell lysates were then prepared and analyzed for phospho-PKB, phospho-p38^{MAPK}, phospho-MAPK, Grb-2 (Cell Signalling, Mississauga, Ont) and SHIP⁶ by immunoblot analysis.

AQX-016A inhibits macrophage and mast cell activation

The target specificity and biological efficacy of AQX-016A were assessed by comparing AQX-016A's effects on PI3K-regulated processes in primary SHIP^{+/+} vs SHIP^{-/-} macrophages and mast cells. Both LPS-induced macrophage and IgE-induced mast cell activation involve activation of PI3K-dependent pathways which have previously been shown to be negatively regulated by SHIP. LPS stimulation of macrophages is associated with a PIP3-dependent release of pro-inflammatory mediators such as TNF α . The action of AQX-016A on SHIP^{+/+} vs SHIP^{-/-} bone marrow derived macrophages was examined. Cells were pretreated for 30 min with AQX-016A prior to stimulation with 10 ng/mL of LPS for 2 h. AQX-016A was able to suppress TNF α production in SHIP^{+/+} cells by 30% at 3 μ M and 50 % at 15 μ M (Figure 8A). In contrast, SHIP^{-/-} cells, TNF α production was indistinguishable from non-AQX-016A treated cells 13 at 3 μ M and was suppressed 15% at 15 μ M. For comparison, the PI3K inhibitor LY294002 inhibited both SHIP^{+/+} and SHIP^{-/-} macrophages to the same extent (up to ~40% at 15 μ M). Activation of mast cells via IgE + antigen crosslinking of their IgE receptors results in elevation of intracellular calcium levels. As shown in Figure 8B, AQX-016A selectively inhibited IgE + antigen-induced calcium entry to a substantially greater degree in SHIP^{+/+} than in SHIP^{-/-} bone marrow derived mast cells whereas LY294002 inhibited both SHIP^{+/+} and SHIP^{-/-} mast cells to the same extent. These data were consistent with AQX-016A inhibiting PI3K-dependent macrophage and mast cell responses in a SHIP-dependent manner.

The ability of AQX-016A to inhibit activation of PIP₃-dependent downstream signalling proteins in SHIP^{+/+} vs SHIP^{-/-} cells was assessed. LPS stimulation of macrophages results in PKB phosphorylation. AQX-016A preferentially inhibited, in a dose dependent manner, LPS-stimulated PKB phosphorylation in SHIP^{+/+} but not in SHIP^{-/-} macrophages. Similarly, AQX-016A inhibited the phosphorylation of PKB, p38^{MAPK} and ERK in SHIP^{+/+} but not in SHIP^{-/-} mast cells. Similar protein loading was confirmed by reblotting with either antibodies to PKB or Grb2. We also examined the ability of AQX-016A to inhibit PKB activation in non-hematopoietic, prostate epithelial LNCaP cells, which do not express SHIP. The human prostate cancer cell line LNCaP exhibits constitutive activation of PKB due to the loss of PTEN expression. LY294002 efficiently suppressed PKB phosphorylation whereas AQX-016A had no effect at doses up to 60 μM. Thus, AQX-016A inhibits PIP₃-regulated intracellular signal transduction events in SHIP expressing hematopoietic cells, but not in SHIP-deficient hematopoietic or non-hematopoietic cells.

15 Example 18

Mouse endotoxemia model. 6-8 week old C57Bl6 mice (VCHRI Mammalian Model of Human Disease Core Facility, Vancouver, BC) were orally administered the indicated dose of AQX-016A, Compound 100 or dexamethasone or carrier 30 min prior to an IP injection of 2 mg/kg of LPS (*E. Coli* serotype 0111:B4, Sigma, Oakville, Ont). Blood was drawn 2 hrs later for determination of plasma TNFα by ELISA. Results are representative of 3 independent experiments. (Figures 7D and 9)

AQX-016A inhibits inflammation *in vivo*

AQX-016A's ability to provide protection by inhibiting inflammatory reactions *in vivo* was assessed in mouse models. The mouse model of endotoxic shock involves intraperitoneal (IP) injection of bacterial LPS and measurement of serum TNFα levels 2 hrs later. We orally administered AQX-016A or the steroidal drug dexamethasone to mice 30 min prior to the LPS challenge. AQX-016A reduced the level of serum TNFα and did so to the same extent as dexamethasone (Figure 9).

30 Example 19

Mouse acute cutaneous anaphylaxis model. 6-8 week old CD1 mice (University of British Columbia Animal Facility, Vancouver, BC) were sensitized to the hapten DNP by cutaneous

application of 25 μ L of 0.5% dinitroflourobenzene (DNFB) (Sigma, Oakville, Ont) in acetone to the shaved abdomen of mice for two consecutive days. 24 hrs later, test substances (dissolved in 10 μ L of 1:2 DMSO:MeOH) were painted on the right ear while the left ear received vehicle control. 30 min after drug application, DNFB was applied to both ears to induce mast cell degranulation. A 6 mm punch was taken from the ear and immediately frozen on dry ice for subsequent determination of neutrophil myeloperoxidase (MPO) activity. Compound 100's ability to inhibit cutaneous anaphylaxis was assessed.

Anaphylactic or allergic responses are mediated by allergen-induced degranulation of pre-sensitized mast cells. The mouse ear edema/cutaneous anaphylaxis model involves pre-sensitization of mice with the haptenizing agent dinitrofluorobenzene (DNFB). One week later the allergic reaction is elicited by painting DNFB onto the ears of the mice. The efficacy of potential anti-inflammatory compounds is tested by topical application of the test substance to one ear and comparing the resulting ear edema or inflammation of the two ears. As shown in Figure 10A, topically applied Compound 100 dramatically inhibited allergen-induced inflammation compared to the vehicle control-treated ear. AQX-016A was also able to inhibit DNFB-induced inflammation in this model.

AQX-016A inhibited mast cell degranulation in CD1 mice sensitized to hapten DNP by cutaneous application of 25 μ L of 0.5% (DNFB) in acetone to the shaved abdomen of mice for two consecutive days was also shown (Figure 10B). 20 μ Ci of tritiated thymidine ($[^3\text{H}]$ -Tdr (GE Healthcare, Piscataway, NJ) was injected IP one week after the first DNFB application. $[^3\text{H}]$ -Tdr labels rapidly dividing cells of the mouse, including neutrophils (30). 24 hrs later, test substances (dissolved in 10 μ L of 1:2 DMSO:MeOH) were painted on the right ear while the left ear received vehicle control. 30 min after drug application, DNFB was applied to both ears to induce mast cell degranulation. The resulting inflammatory cell infiltration was quantified by taking a 6mm diameter punch from the ear 1 hr later for dissolution in Solvable (Perkin Elmer-Packard, Woodbridge, Ont) and liquid scintillation counting as described. The ability of test substances to inhibit mast cell degranulation was then determined by calculating the ratio of $[^3\text{H}]$ -Tdr in the test (right) ear vs the control (left) ear as described (30). One group of mice had DNFB applied only to the left ear leaving the right ear noninflamed, in order to control for basal $[^3\text{H}]$ -Tdr incorporation into ear parenchymal cells.

Example 20**Construction of the SHIP Δ C2 mutant and isolated C2 domain**

A His6 tagged SHIP Δ C2 domain deletion mutant (deleting residues 725 to 863) in the mammalian expression vector pME18S was generated by a standard PCR-based methodology.

- 5 An N-terminal His6 C2 domain construct was also generated by PCR inserted into the pET28C bacterial expression vector using EcoRI and NdeI restriction sites.

Protein Lipid Overlay Assays

- Protein lipid overlay (PLO) assays were performed essentially as described with minor
10 modifications. Lyophilized phosphatidylinositol-3,4-bisphosphate diC16 (PIP₂, Echelon Biosciences, Salt Lake City, UT) was reconstituted in a 2:1.8 solution of methanol and water. PVDF membranes (Millipore, Mississauga, Ont) were initially wetted in methanol for 1 minute, and washed 3 X 5 min with water, and gently agitated in TBST buffer (20 mM Tris pH 7.5, 0.15 M NaCl (TBS) with 0.05% Tween 20) at 23°C overnight. Treated membranes were
15 air-dried and dilutions of reconstituted lipids were spotted in 1 μ l aliquots to give the indicated amount of PIP₂ per membrane spot. Membranes were dried completely and blocked with blocking buffer (3% BSA in TBS with 0.05% NaN₃) for 1 h at 23°C. Purified, recombinant C2 domain was diluted into blocking buffer (5 μ M final) and treated with 4 μ M Compound 100 or EtOH control for 30 min at 23°C prior to overnight incubation with the PIP₂ spotted
20 membranes. Membranes were washed 10 times over 50 min in TBST buffer at 23°C and incubated with anti-His₆ mouse IgG (Qiagen, Mississauga, Ont) for 1 h at 23°C. Membranes were washed as above and incubated with Alexa Fluor 660 anti-mouse goat anti-mouse IgG (Invitrogen, Burlington, Ont) for 1 h at 23°C. After washing, bound proteins were detected and quantified on a Li-Cor Odyssey scanner (Lincoln, NE).

25

SHIP is an allosterically activated enzyme

- The allosteric regulation of enzymes has remained under-appreciated primarily because allosteric effectors are not easy to find. The molecular mechanism by which Compound 100 activated SHIP was investigated, first by performing classical enzyme kinetic analysis of its
30 phosphatase activity. Activity measurements were performed with substrate concentrations from 10 – 100 μ M. Plots of the initial reaction velocity at each substrate concentration is predicted to exhibit a hyperbolic profile if SHIP obeys conventional Michaelis-Menten kinetics. However, SHIP displayed sigmoidal reaction kinetics suggesting allosteric activation

by its end-product (Figure 11A). Addition of the SHIP product PI-3,4-P₂ to the enzyme reaction activated wild-type SHIP enzyme to a similar extent as Compound 100 (Figure 11B).

The SHIP protein contains a C2 domain located at the carboxyterminal end of its phosphatase domain. C2 domains were first described in the protein kinase C family where it serves to bind Ca²⁺, but C2 domains have since been identified in other proteins where they have been shown to bind to a variety of ligands including lipids. SHIP lacking its the C2 domain (Δ C2 SHIP) was prepared. As shown in Figure 11B, although Δ C2 SHIP was as active as the wild-type SHIP, its activity could not be enhanced by the addition of either PI-3,4-P₂ or Compound 100. This suggests that the C2 domain may be required for the allosteric activation of SHIP activity and that it may be the binding site for its allosteric activators such as PI-3,4-P₂ and Compound 100.

Example 21

Scintillation Proximity Assays

Compound 100 was radiolabelled with tritium by GE Healthcare (Piscataway, NJ) to a specific activity of 42 Ci/mmol. Copper chelate (His-Tag) YSi SPA Scintillation Beads (GE healthcare, Piscataway, NJ) were diluted in 0.25% BSA/TBS to 1.5 mg/mL and recombinant, His₆-tagged protein added at the indicated concentrations: wild-type (1 pM), Δ C2 SHIP enzyme (1 pM) or C2 domain (10 nM). Protein was allowed to bind 1 h at 23°C, and 250 μ g of beads were aliquoted per well of a 96-well plate. 5 μ Ci of [³H]-Compound 100 was added per well, the plate gently agitated for 30 min and the amount of bead associated radioactivity quantified by counting in a Wallac BetaPlate plate scintillation counter.

Isolated recombinant, His₆-tagged C2 domain was expressed and its PI-3,4-P₂ binding ability was determined using protein lipid overlay assays. Purified C2 domain was incubated with membrane strips spotted with PI-3,4-P₂ and bound protein detected using an anti-His6 antibody. As shown in Figure 11C the C2 domain bound PI-3,4-P₂ and this binding was inhibited by Compound 100, consistent with both Compound 100 and PI-3,4-P₂ interacting with the C2 domain at a common binding site. Compound 100 was verified to directly bind the C2 domain using scintillation proximity assays (SPAs) in which SPA beads were coated with

either the C2 domain or control protein (BSA) prior to incubation with [³H]-Compound 100. As shown in Figure 11D, the C2 domain did interact with [³H]-Compound 100. In complementary studies, [³H]-Compound 100 bound to wild-type SHIP but not to SHIP lacking its C2 domain (Figure 11E). Together, these data are consistent with Compound 100 directly binding to SHIP's C2 domain, resulting in allosteric activation of the enzyme.

Example 22

A novel paradigm for inhibiting PI3K signaling through activation of the phosphatases that negatively regulate this pathway is provided. SHIP is a particularly good target for immune/hematopoietic disorders because of its restricted expression to hematopoietic cells. Because the relative activity of phosphatases present in a cell will influence the efficacy of kinase inhibitors, as discussed by Knight and Shokat, SHIP agonists may also be used to potentiate the activation of PI3K inhibitors and promote tissue targeting of PI3K inhibitors to the hematopoietic/immune cell compartment. Initial toxicology studies suggest both AQX-016A and Compound 100 are well tolerated and do not significantly affect peripheral blood cell counts or bone marrow progenitor numbers (data not shown).

Compound 100 exhibits efficacy at a submicromolar EC₅₀ (Figure 7C) and this suggests that it possesses a low likelihood of off-target effects based on calculations by Knight and Shokat.

Compound 100 had minimal off-target effects on a screen of 100 other kinases and phosphatases (Figures 12A and 12B). Compound profiling activity was undertaken using 100 protein kinase and phosphatase targets by SignalChem (Richmond, BC, Canada. www.signalchem.com) against compound Compound 100 (2 μM final concentration). Protein kinase assays were performed in the presence of 50 μM ATP at 30°C for 15 min. Protein phosphatase activities were determined using pNPP as substrate and were also performed at 37°C for 15 min. The activity of the enzymes in the presence of Compound 100 was compared to that in the vehicle control and expressed as a % change in activity relative to that observed in the vehicle control. Changes in activity of <25% were not considered significant. Enzymes affected by Compound 100 are plotted in an expanded graph in Figure 12B.

Example 23

MM Xenograft murine model. Mice were inoculated with at two sites each with 3 x 10⁶ luciferase expressing OPM2 cells suspended in 50 μL of growth medium and 50 μL of

Matrigel basement membrane matrix (Becton Dickenson; Bedford, MA). Tumors were injected subcutaneously in the upper and lower flanks of the mice and allowed to establish for 2 weeks. After 2 weeks, Compound 100 or control vehicle was administered in a subcutaneous oil depot at a dose of 50 mg/kg every 3 days.

5

Tumors were measured using bioluminescence imaging on the Xenogen IVIS 200. Mice received intra-peritoneal injections of 200 μ L of D-luciferin at 3.75 mg/mL in sterile PBS. Mice were then anesthetized with isofluorane and imaged 15 minutes post-injection of luciferin. Quantification of tumor size was performed using the Living Image™ software. The results are illustrated graphically in Figures 13 and 14.

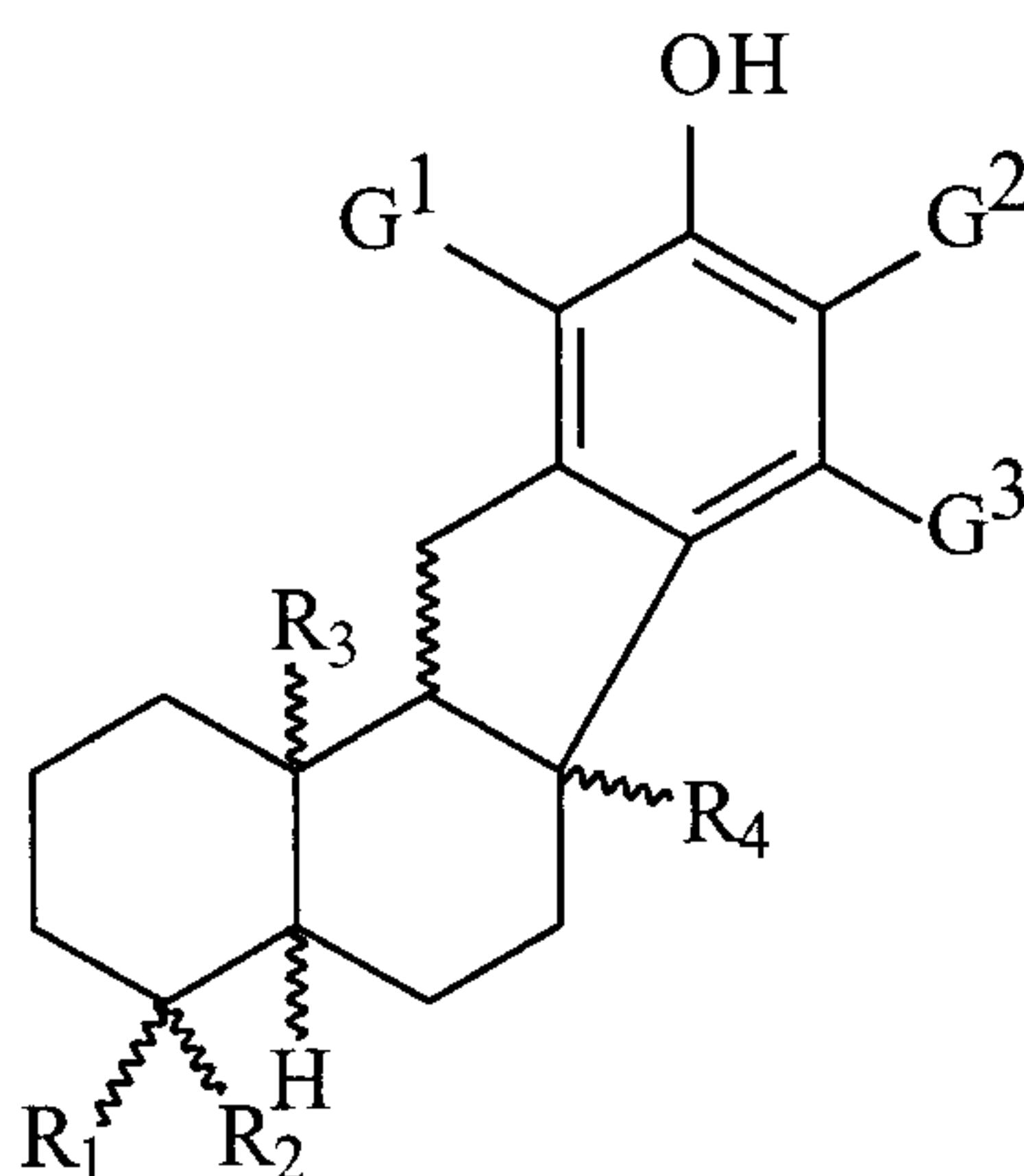
10

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of the teachings of this invention that changes and modification may be made thereto without departing from the spirit or scope of the appended claims. All patents, patent applications and publications referred to herein are hereby incorporated by reference.

15

What is claimed is:

1. A compound of Formula 1 and salts thereof:



Formula 1

wherein;

R_1 and R_2 are independently selected from the group consisting of: $-CH_3$, $-CH_2CH_3$, $-CH_2OH$, $-CH_2OR_1'$, $-CHO$, $-CO_2H$, and $-CO_2R_2'$;

R_3 and R_4 are independently selected from the group consisting of: H , $-CH_3$, $-CH_2CH_3$, $-CH_2OH$, $-CH_2OR_3'$, $-CHO$, $-CO_2H$, and $-CO_2R_4'$;

R_1' , R_2' , R_3' , and R_4' , are independently a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group that is unsubstituted or substituted with one or more of: OH , $=O$, SH , F , Br , Cl , I , NH_2 , $-NHR_1''$, $-N(R_2'')_2$, NO_2 and $-CO_2H$ where R_1'' and R_2'' is a linear, branched, or cyclic, saturated or unsaturated one to ten carbon alkyl group;

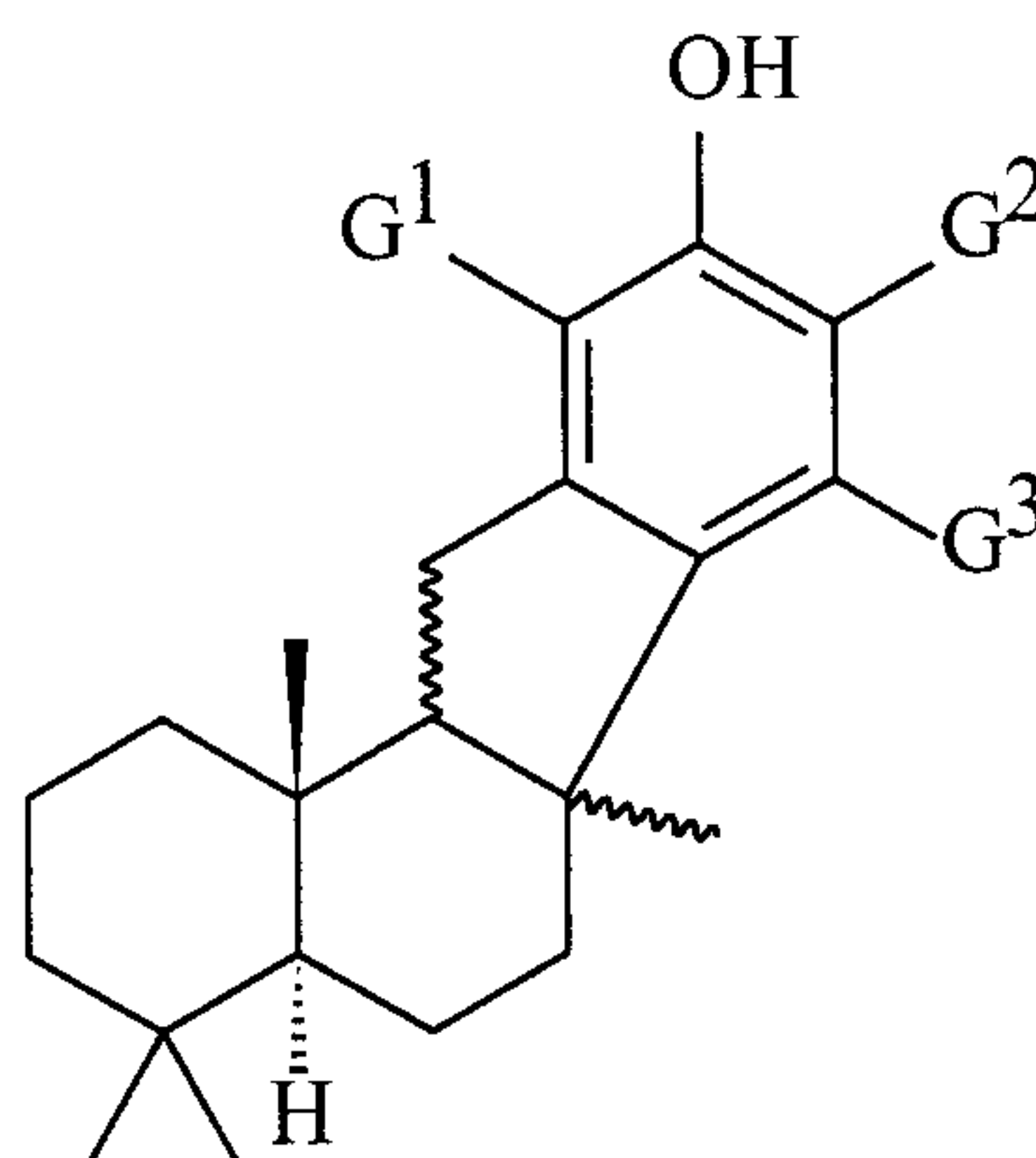
G_1 is selected from the group consisting of: $O-(C_1-C_{10} \text{ alkyl})$ and H ;

G_2 is H or $C_1-C_{10} \text{ alkyl}$; and

G_3 is selected from the group consisting of: H , $-OH$, $C_1-C_{10} \text{ alkyl}$ and $O-(C_1-C_{10} \text{ alkyl})$.

2. The compound of claim 1 or a salt thereof wherein G^1 and G^2 are H .
3. The compound of claim 2 or a salt thereof wherein one or both of R_1 and R_2 are selected from the group consisting of: methyl, ethyl, $-CH_2OH$, $-CH_2OR_1'$, or $-CH_2OR_2'$.

4. The compound of any claim 3 or a salt thereof wherein R_1' , R_2' , R_3' , and/or R_4' in R_1 and R_2 are selected from the group consisting of: methyl, ethyl, propyl or butyl.
5. The compound of claim 2 or a salt thereof wherein R_1 is methyl or ethyl.
6. The compound of claim 2 or a salt thereof wherein R_2 is methyl or ethyl.
7. The compound of claim 2 or a salt thereof wherein R_1 and R_2 are both methyl.
8. The compound of claim 2 having Formula 2 and salts thereof:



Formula 2

wherein;

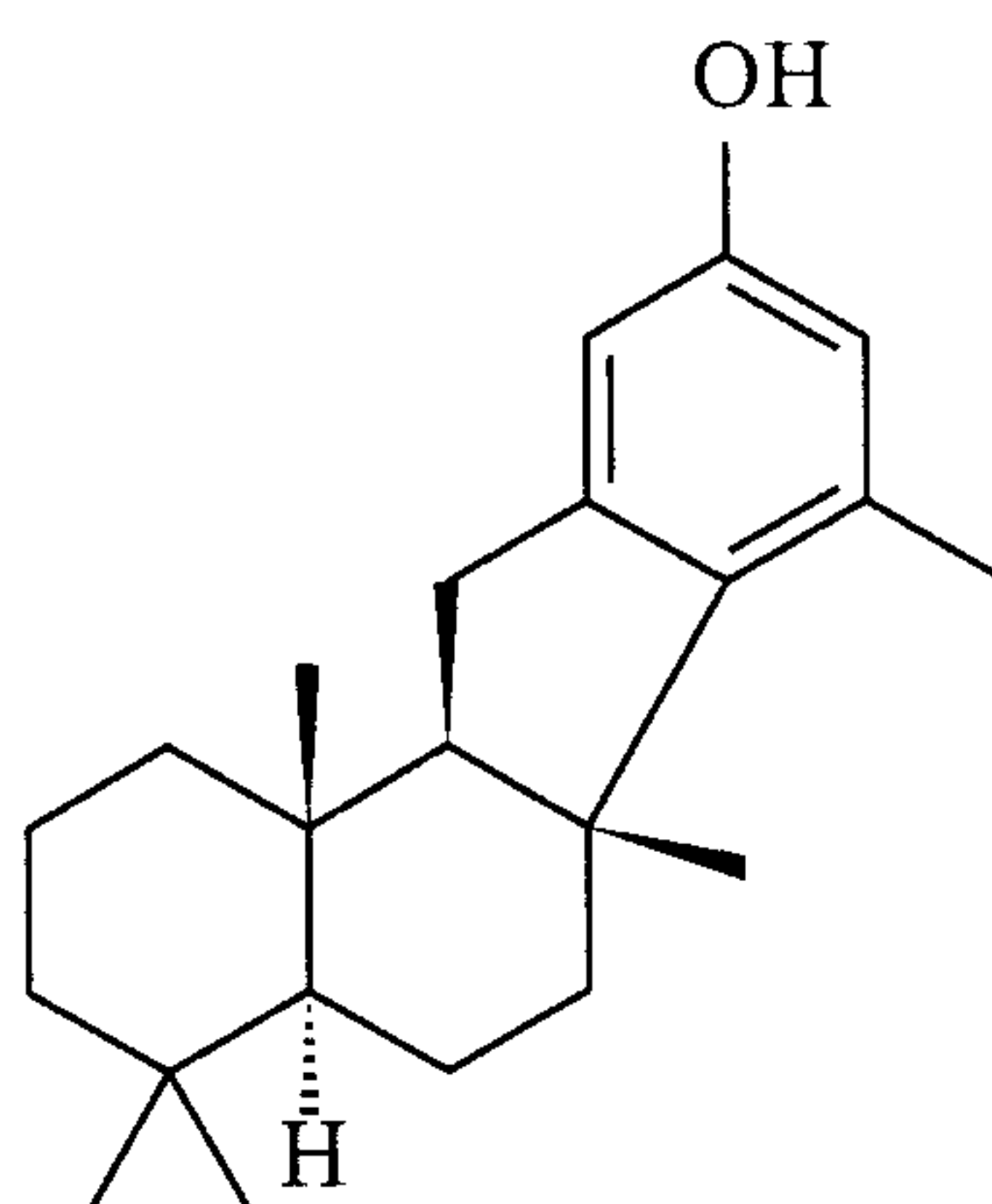
G_1 is H;

G_2 is H; and

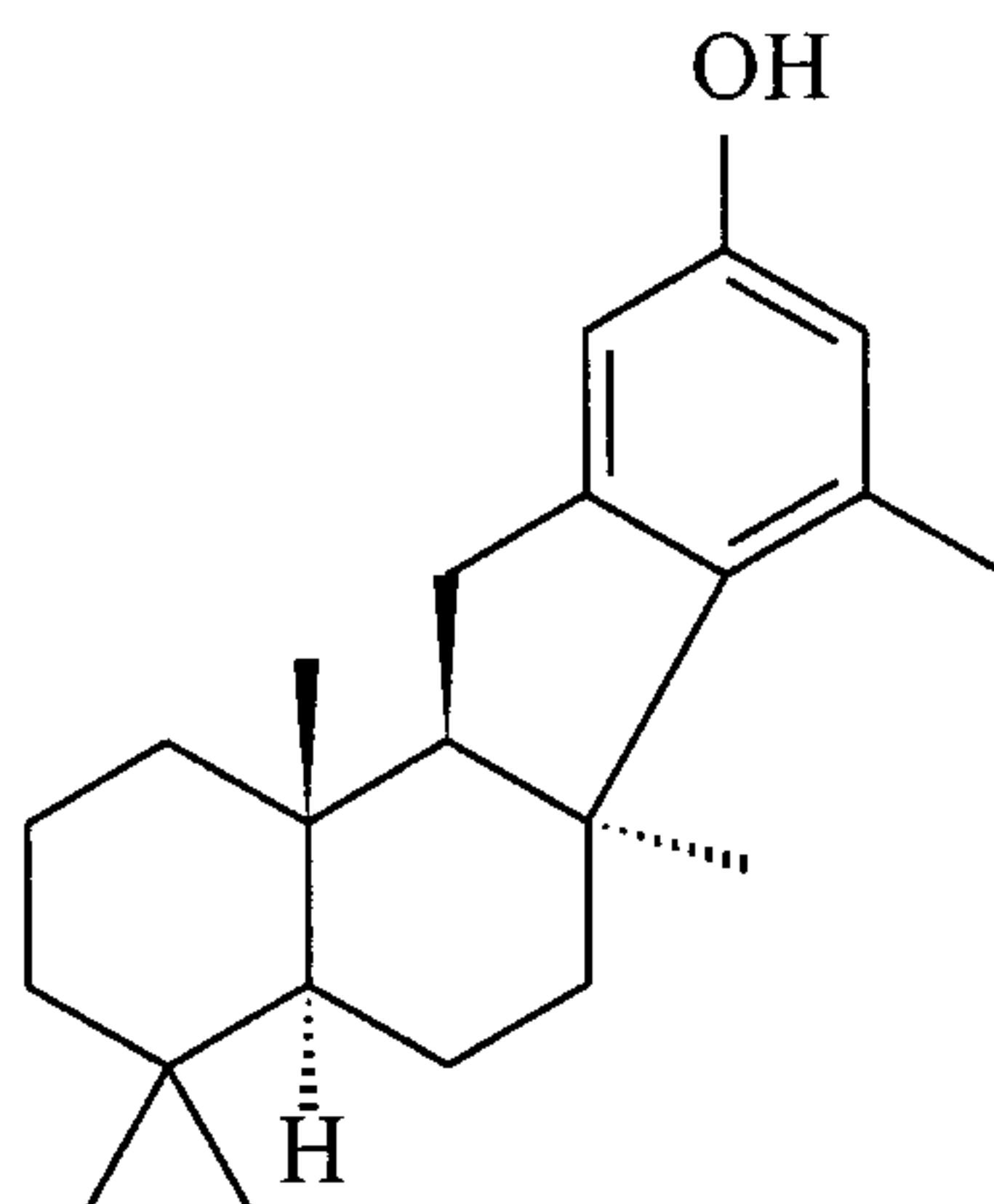
G_3 is selected from the group consisting of: H, -OH, C_1 - C_{10} alkyl and O-(C_1 - C_{10} alkyl).

9. The compound of claim 8 or a salt thereof wherein G_3 is selected from the group consisting of: H, methyl and O-methyl.
10. The compound of claim 8 or a salt thereof wherein G_3 is -O-methyl.
11. The compound of claim 8 or a salt thereof wherein G_3 is H.
12. The compound of claim 8 or a salt thereof wherein G_3 is methyl.

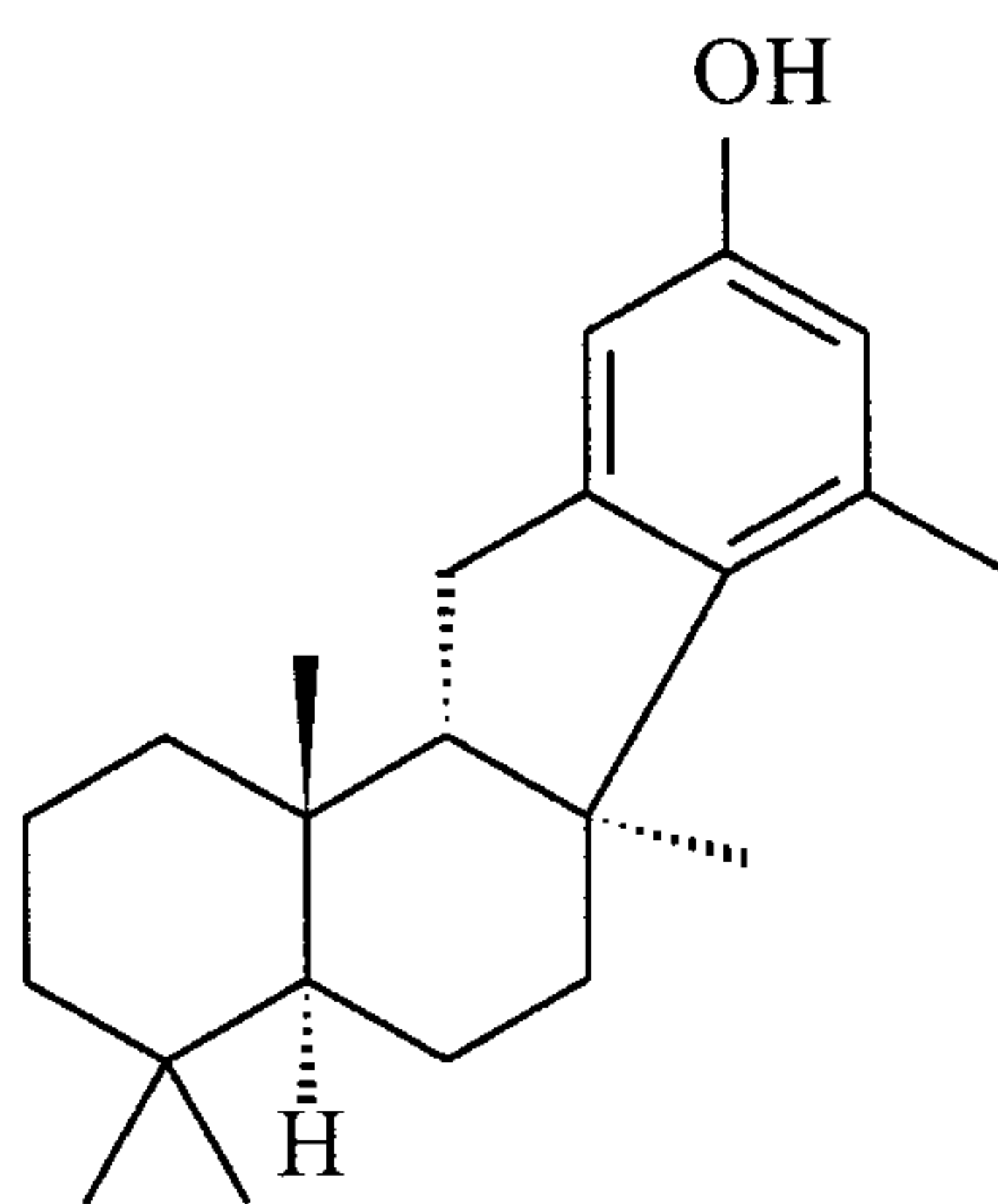
13. The compound of claim 2 having the structure:



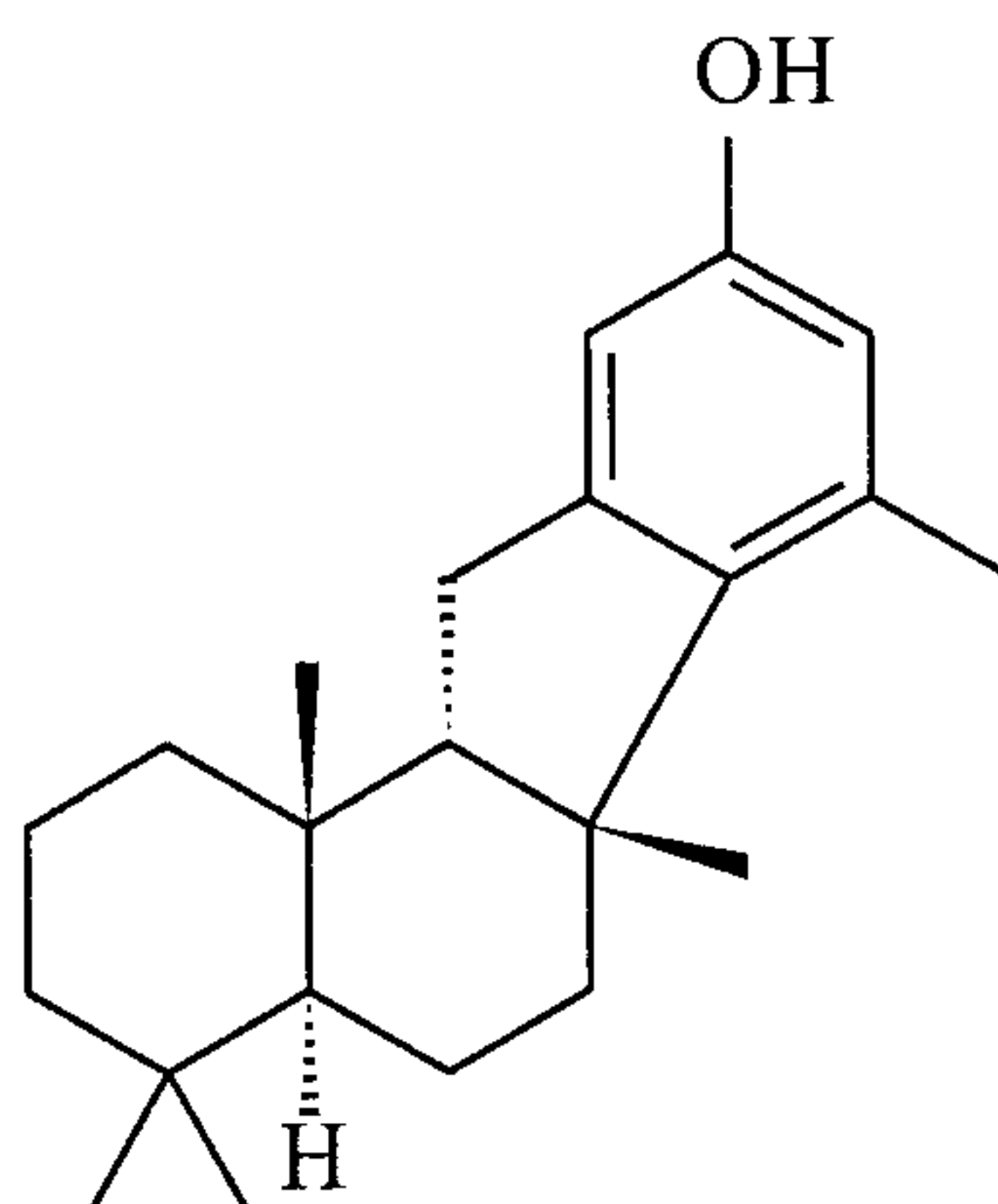
14. The compound of claim 2 having the structure:



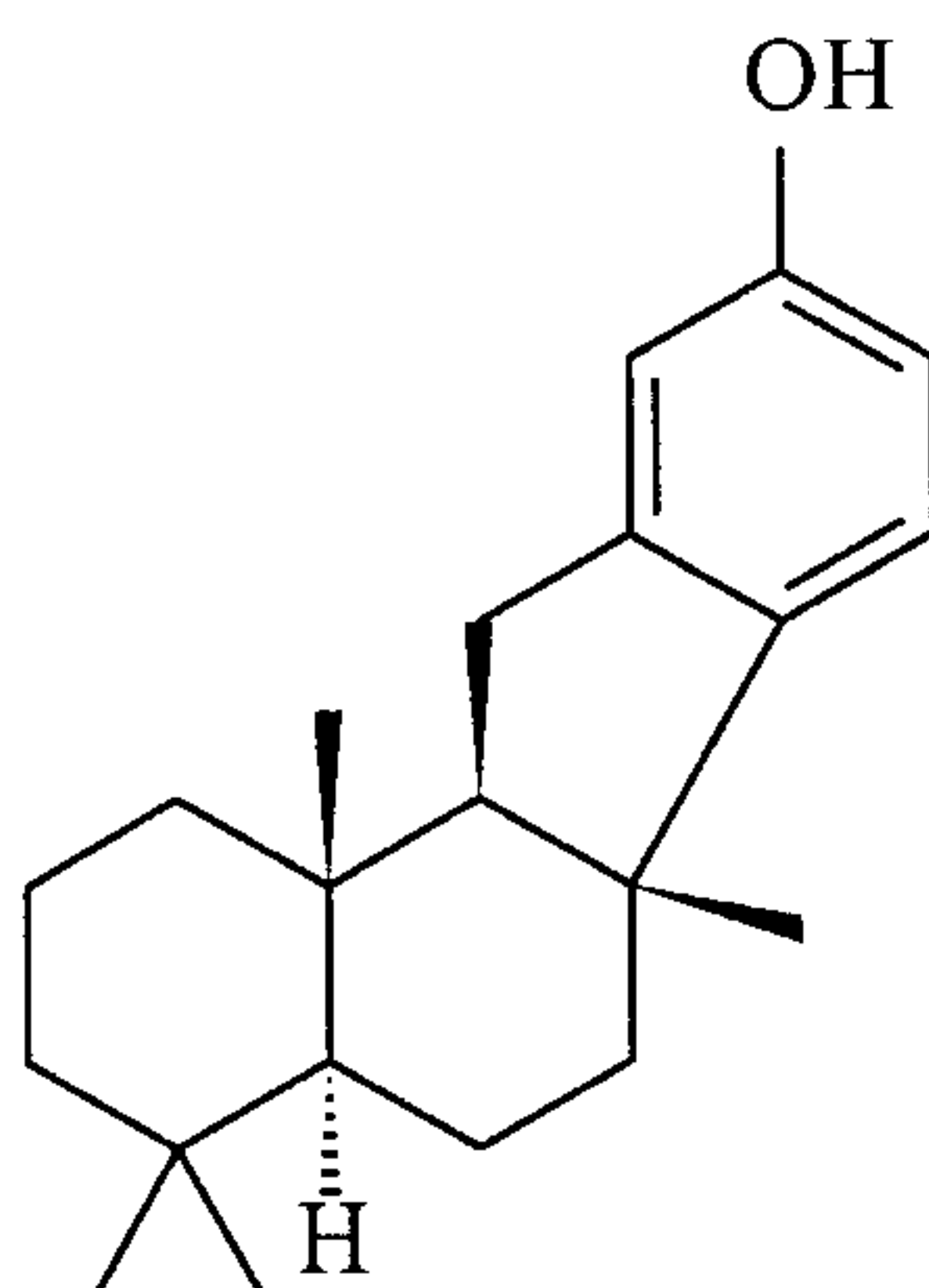
15. The compound of claim 2 having the structure:



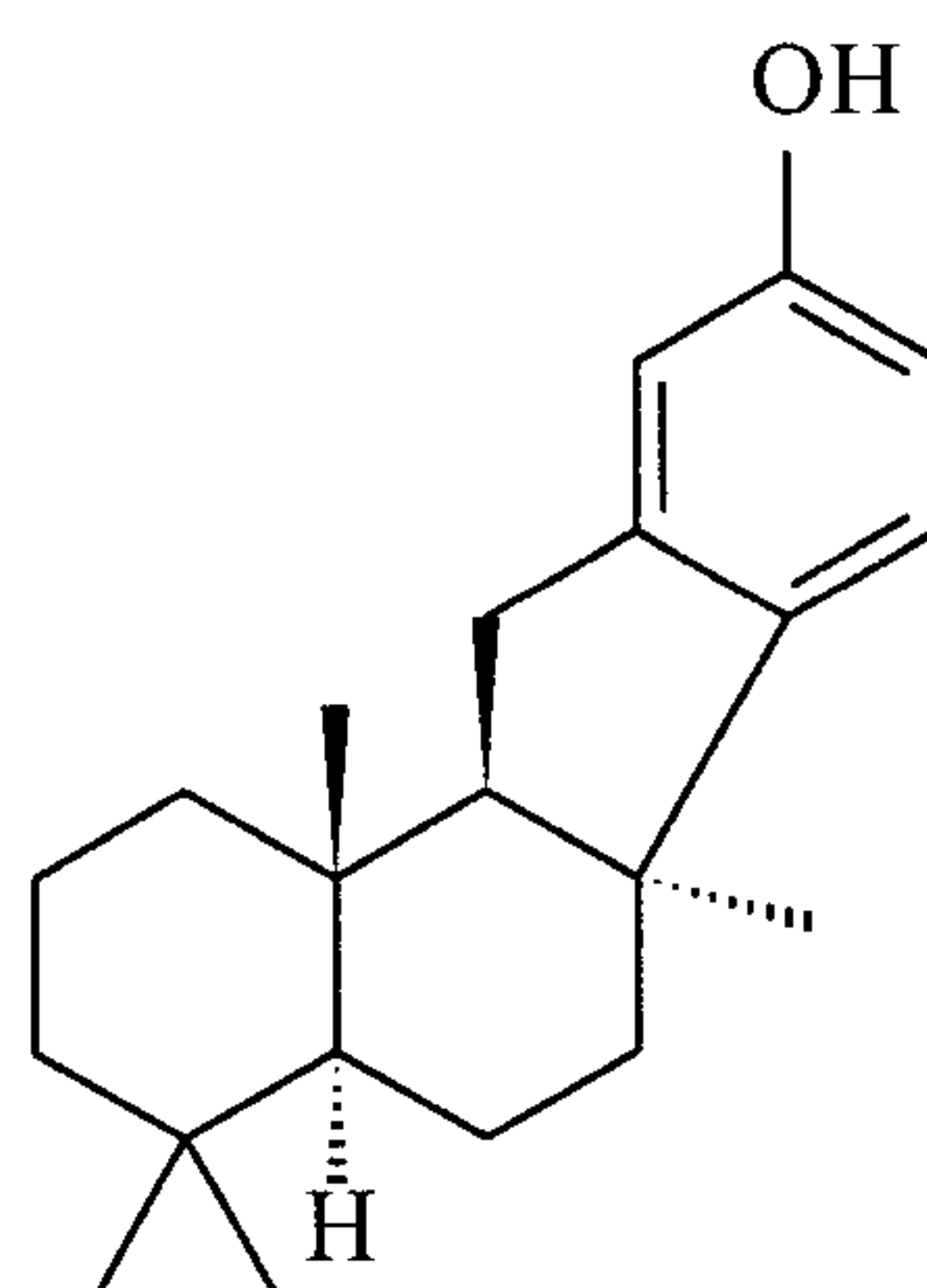
16. The compound of claim 2 having the structure:



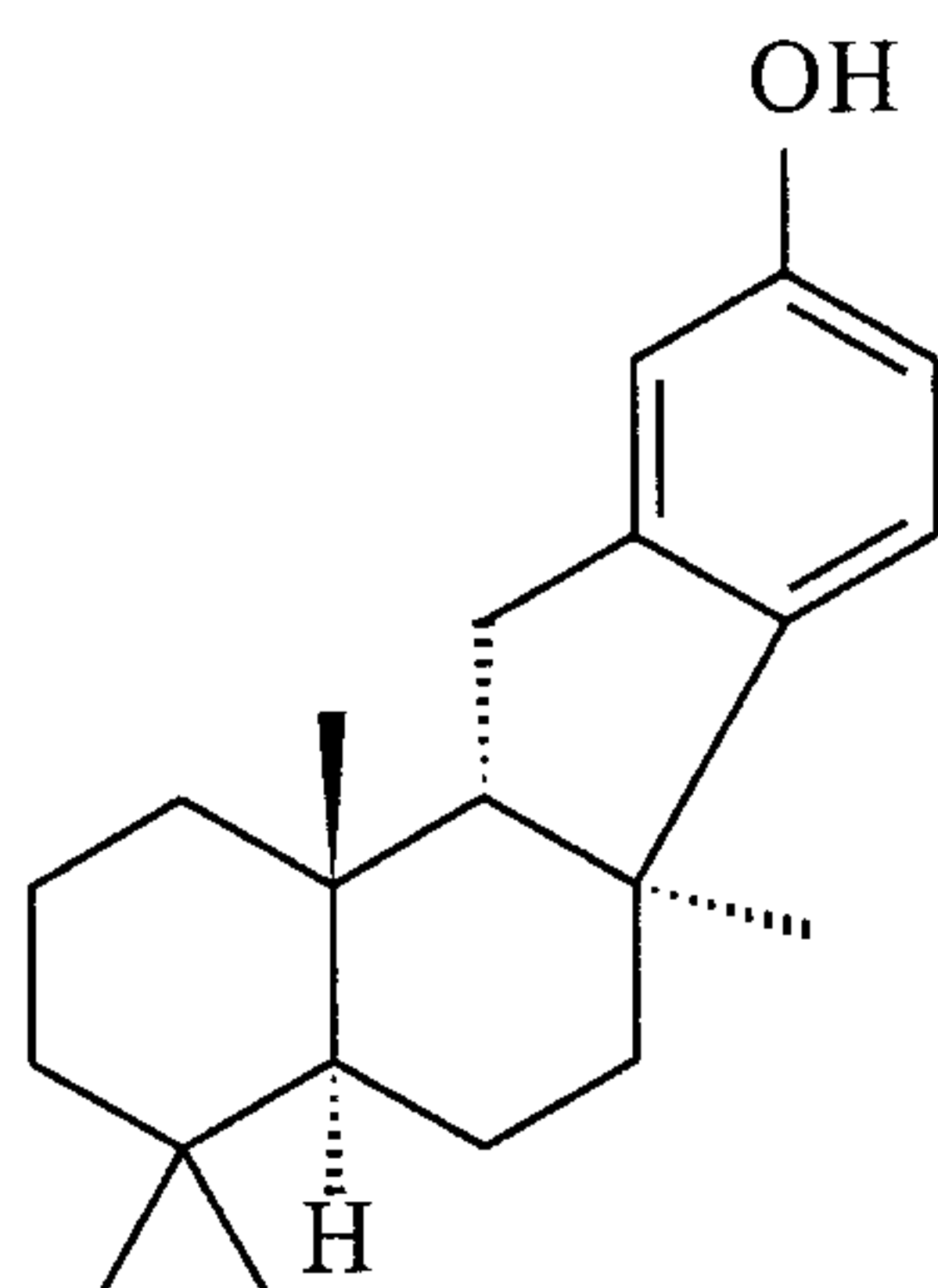
17. The compound of claim 2 having the structure:



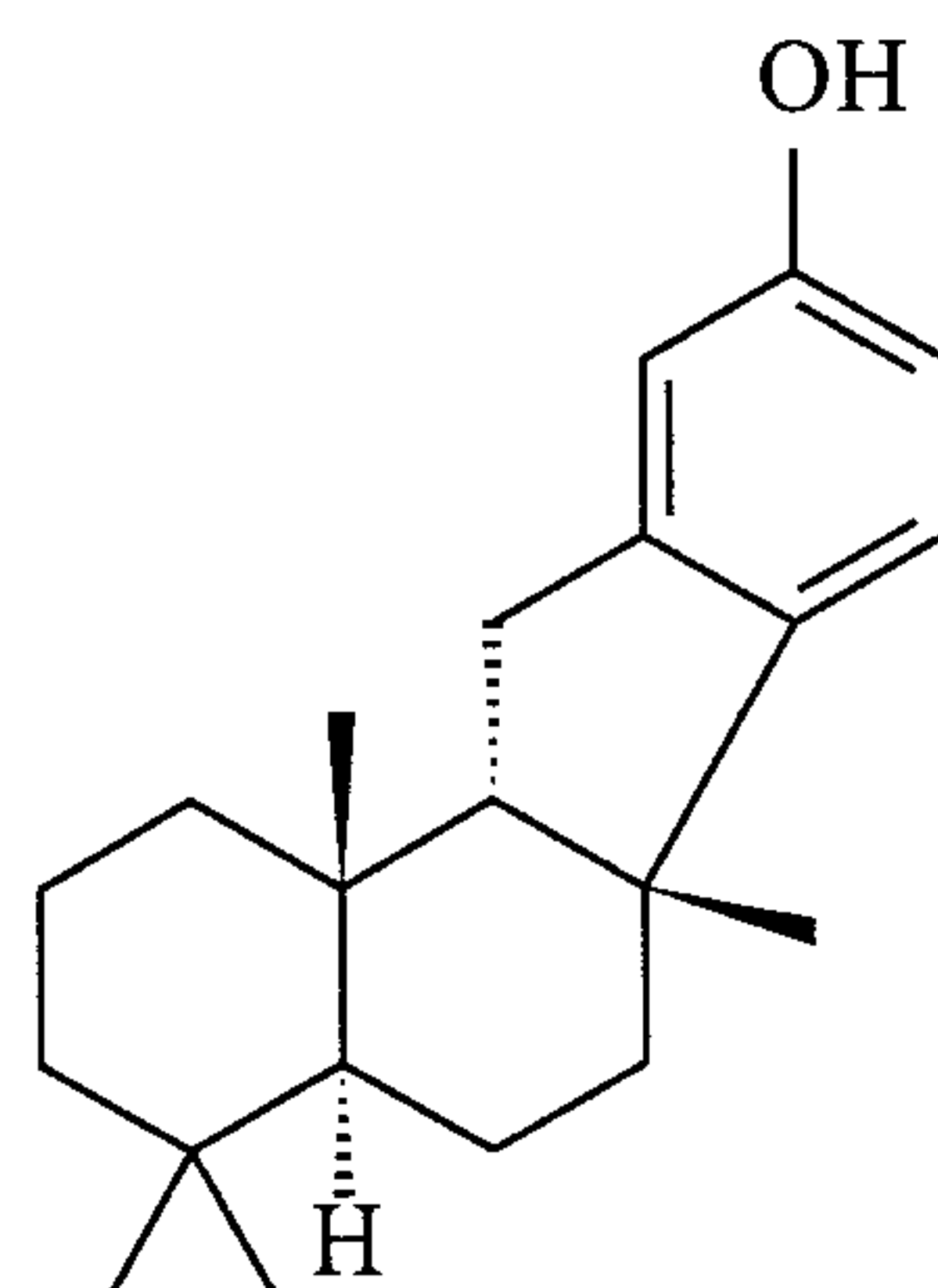
18. The compound of claim 2 having the structure:



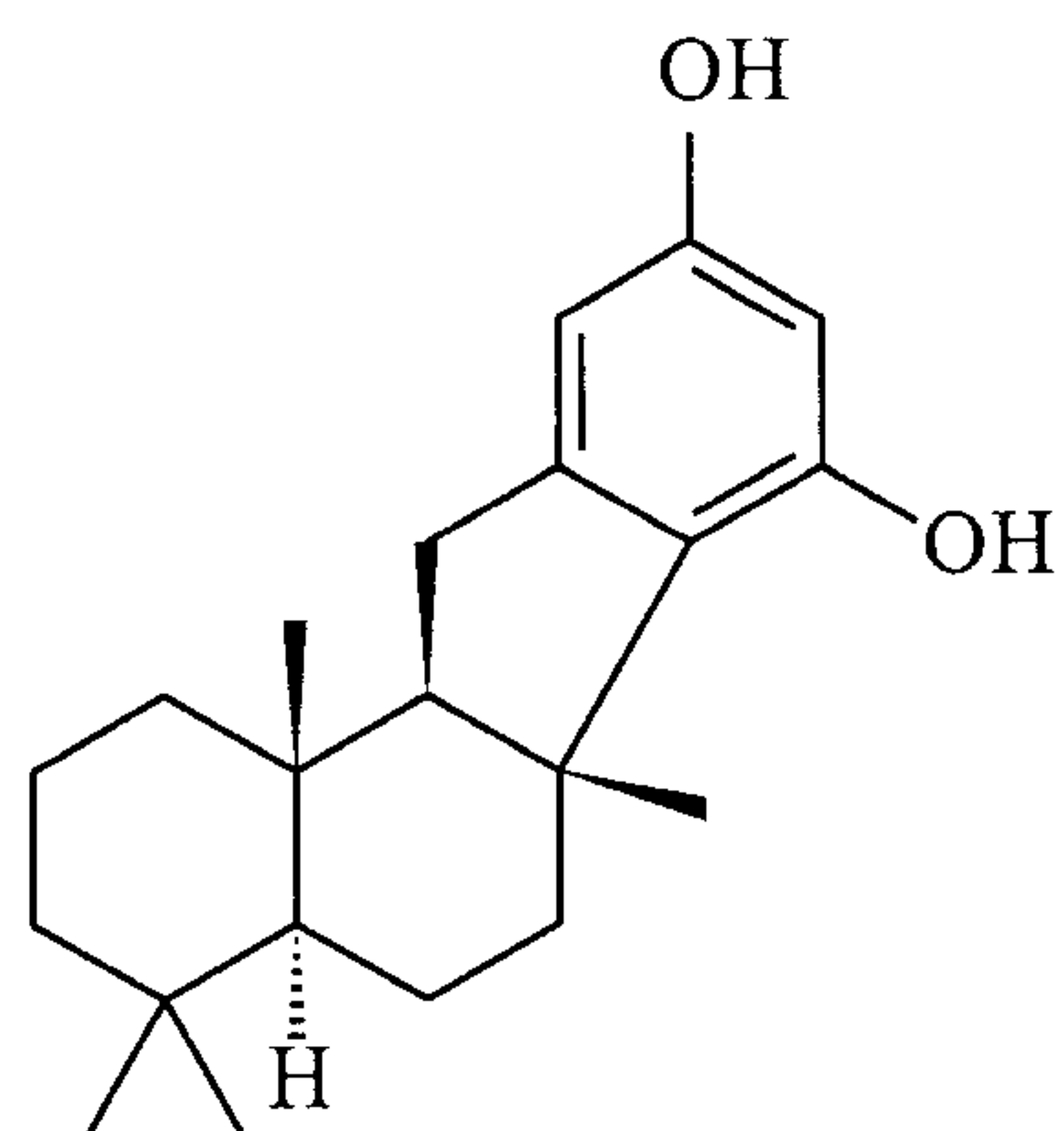
19. The compound of claim 2 having the structure:



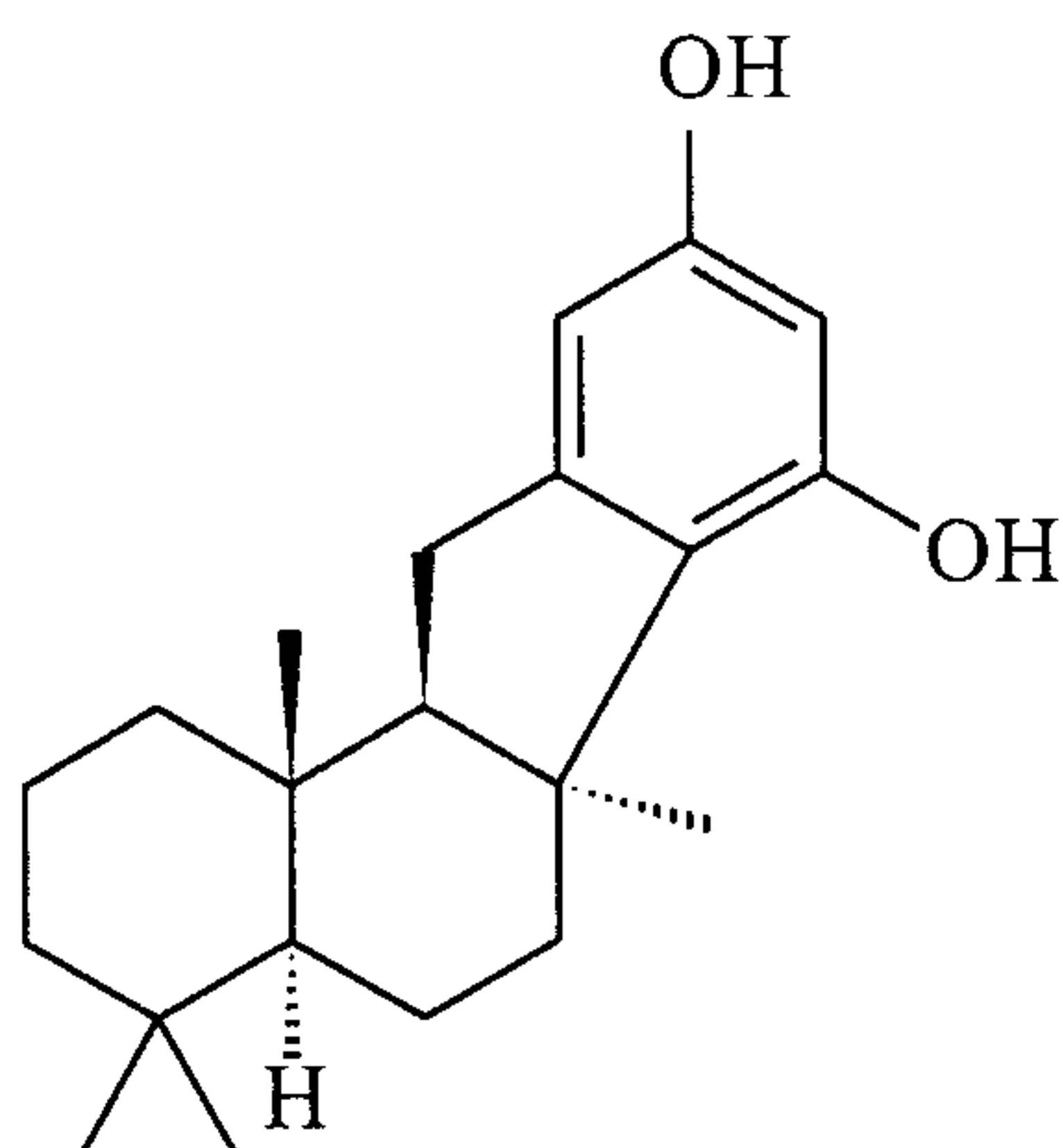
20. The compound of claim 2 having the structure:



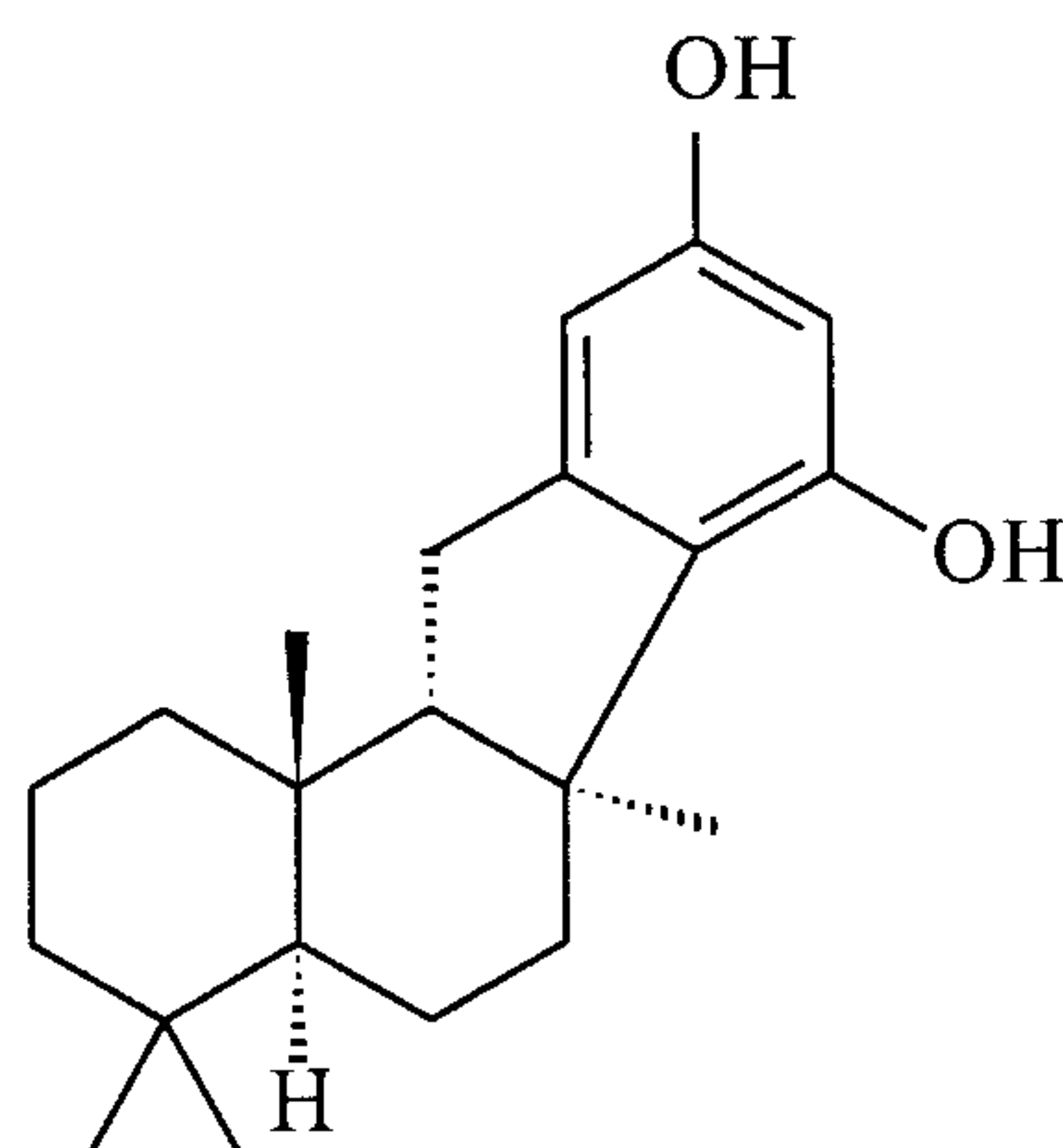
21. The compound of claim 2 having the structure:



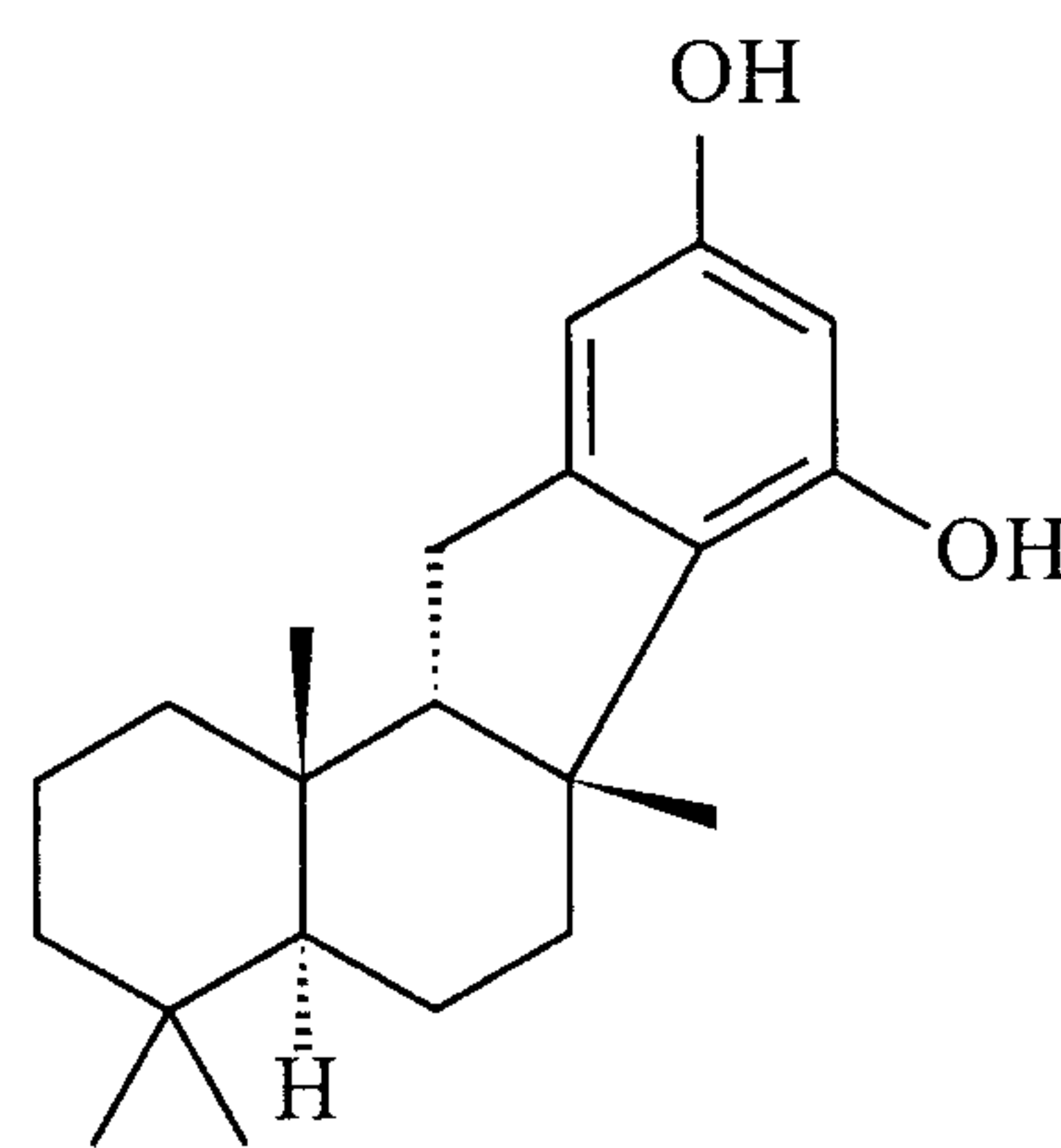
22. The compound of claim 2 having the structure:



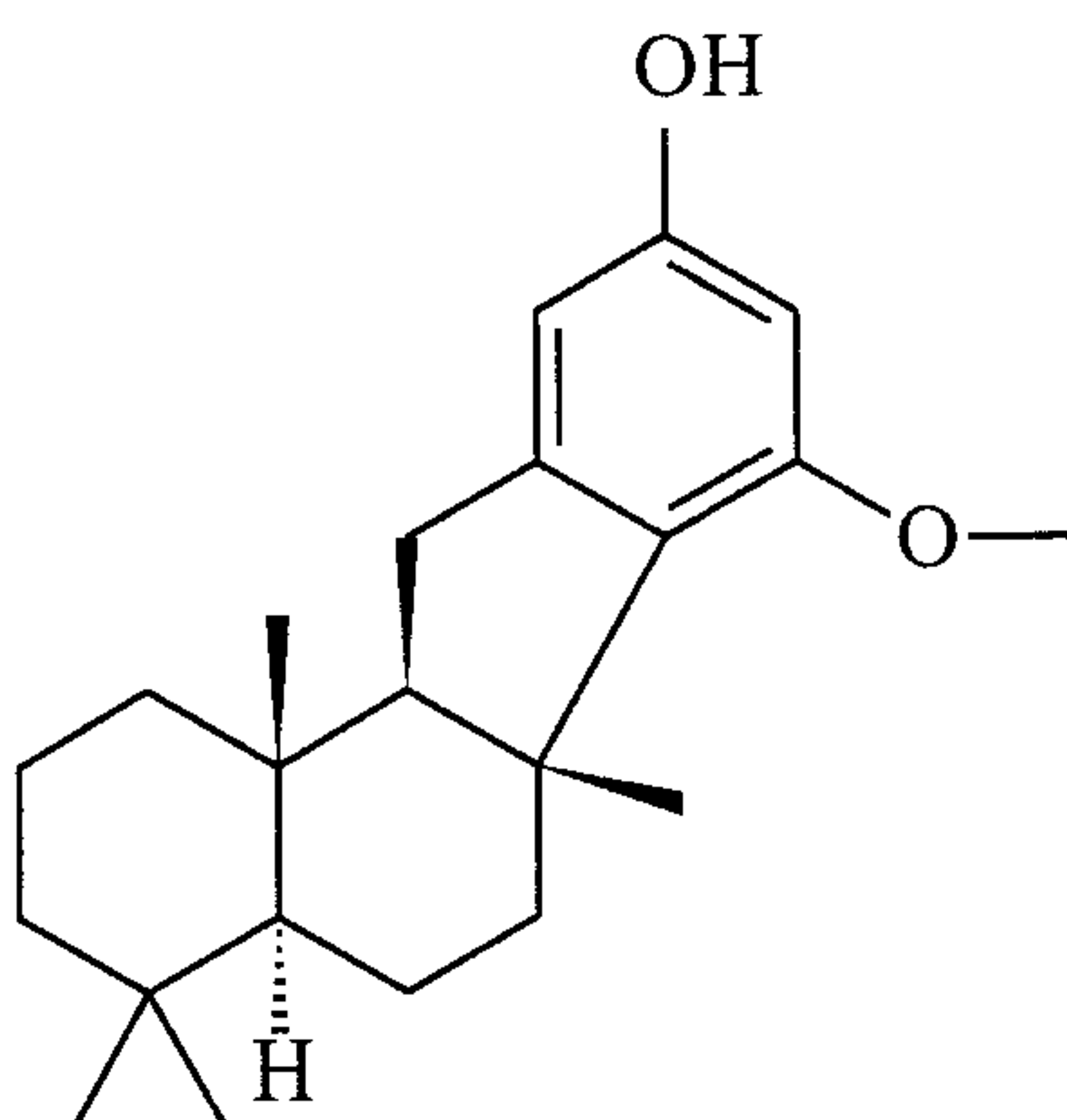
23. The compound of claim 2 having the structure:



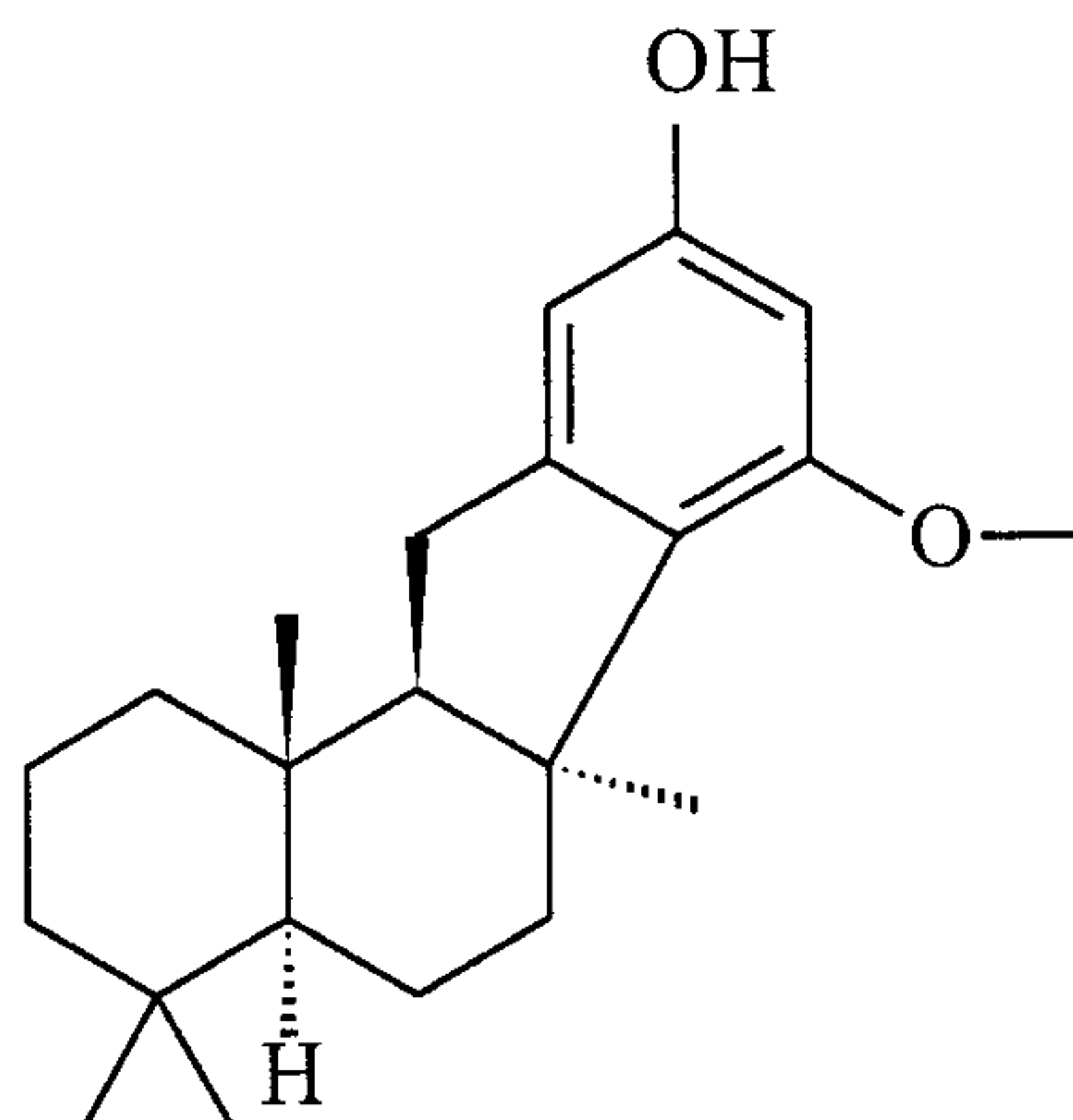
24. The compound of claim 2 having the structure:



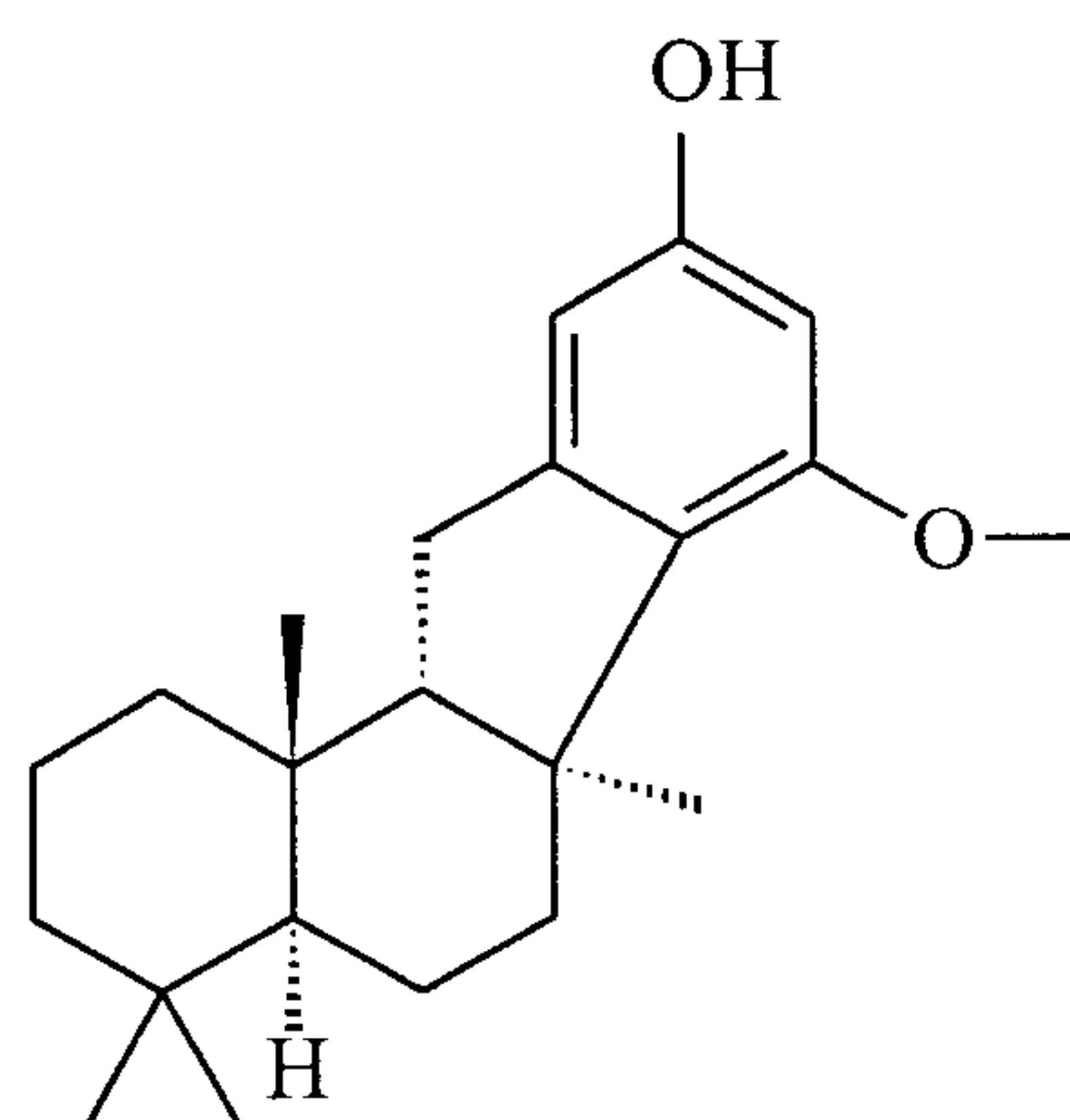
25. The compound of claim 2 having the structure:



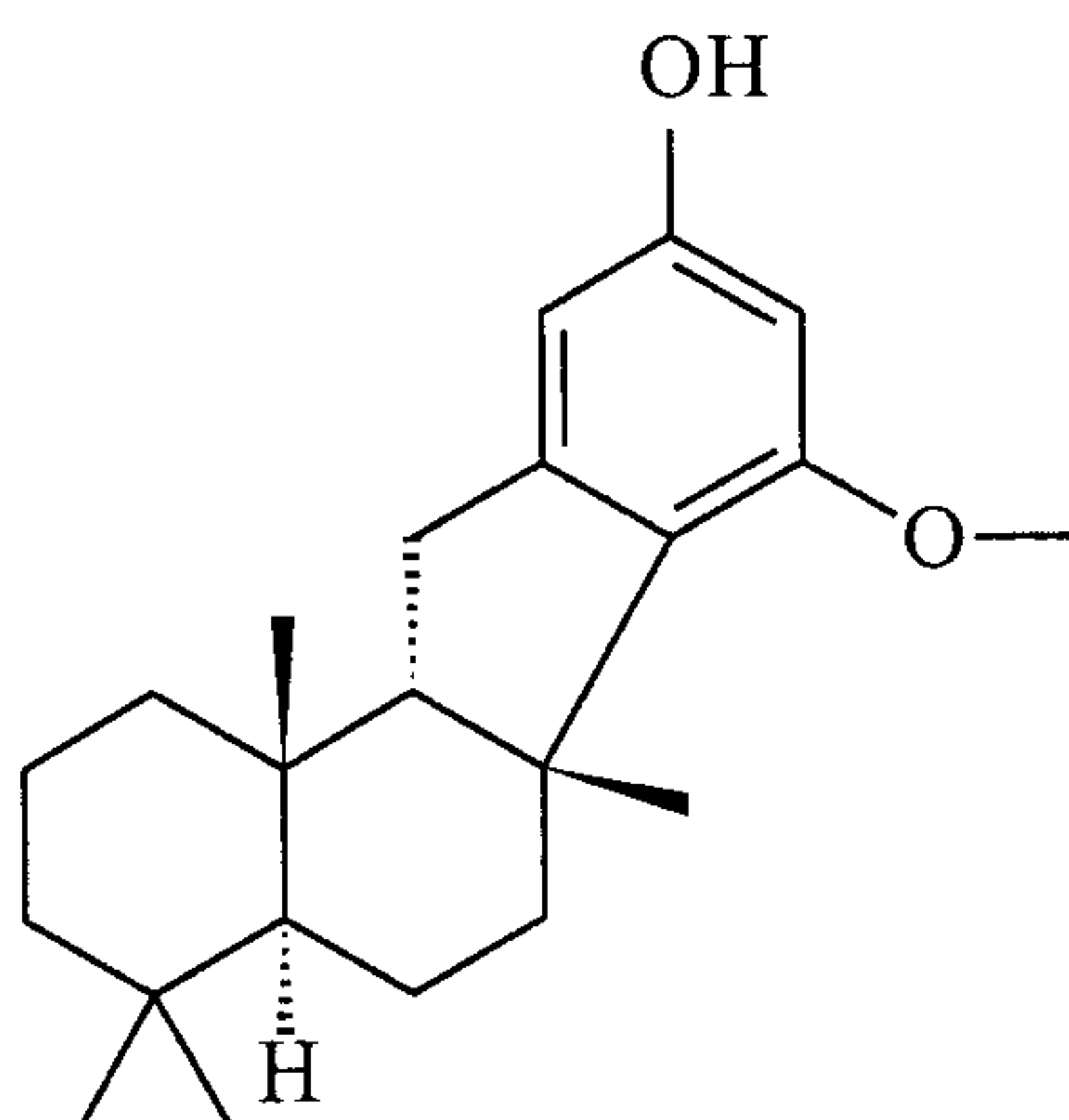
26. The compound of claim 2 having the structure:



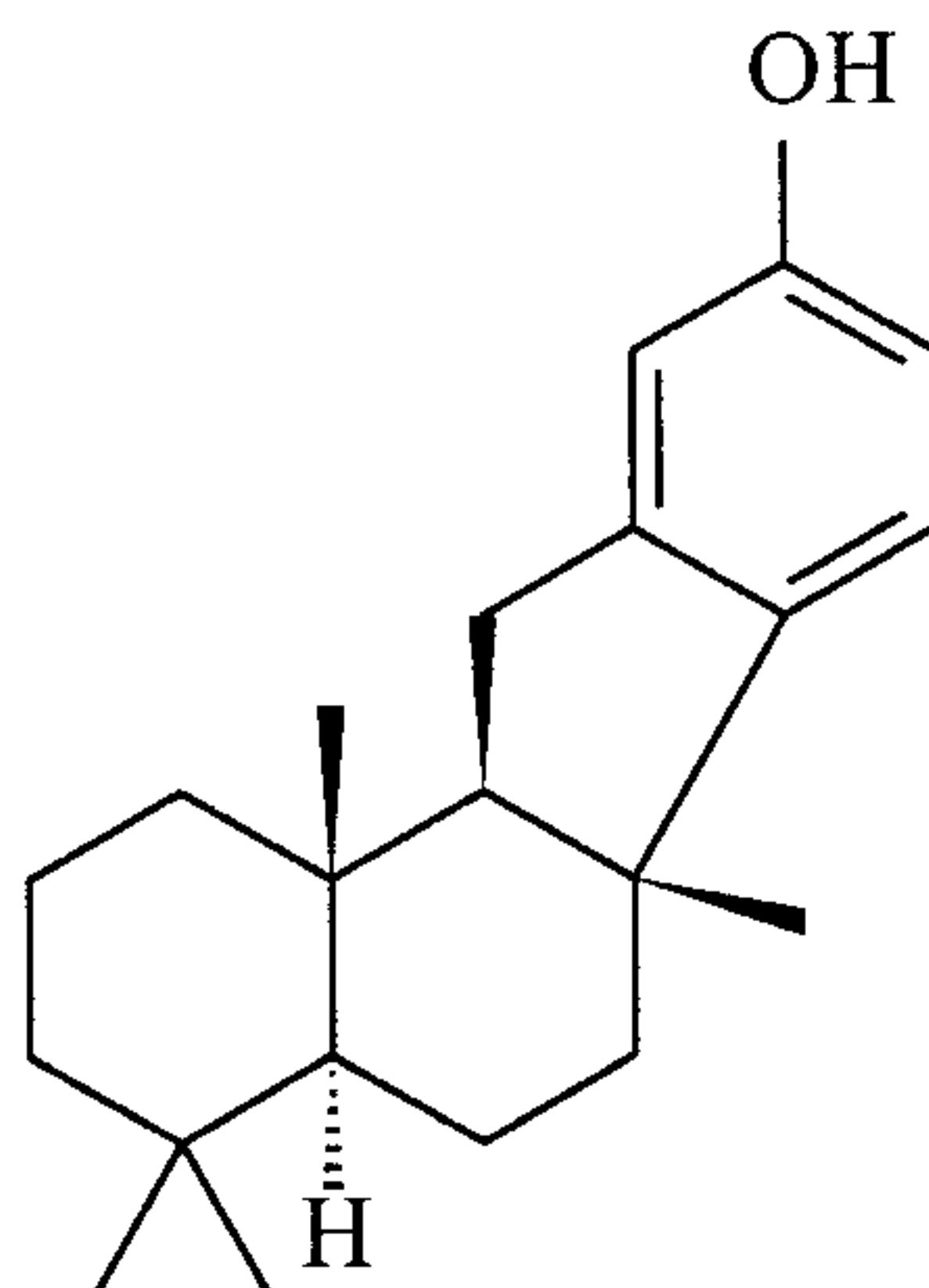
27. The compound of claim 2 having the structure:



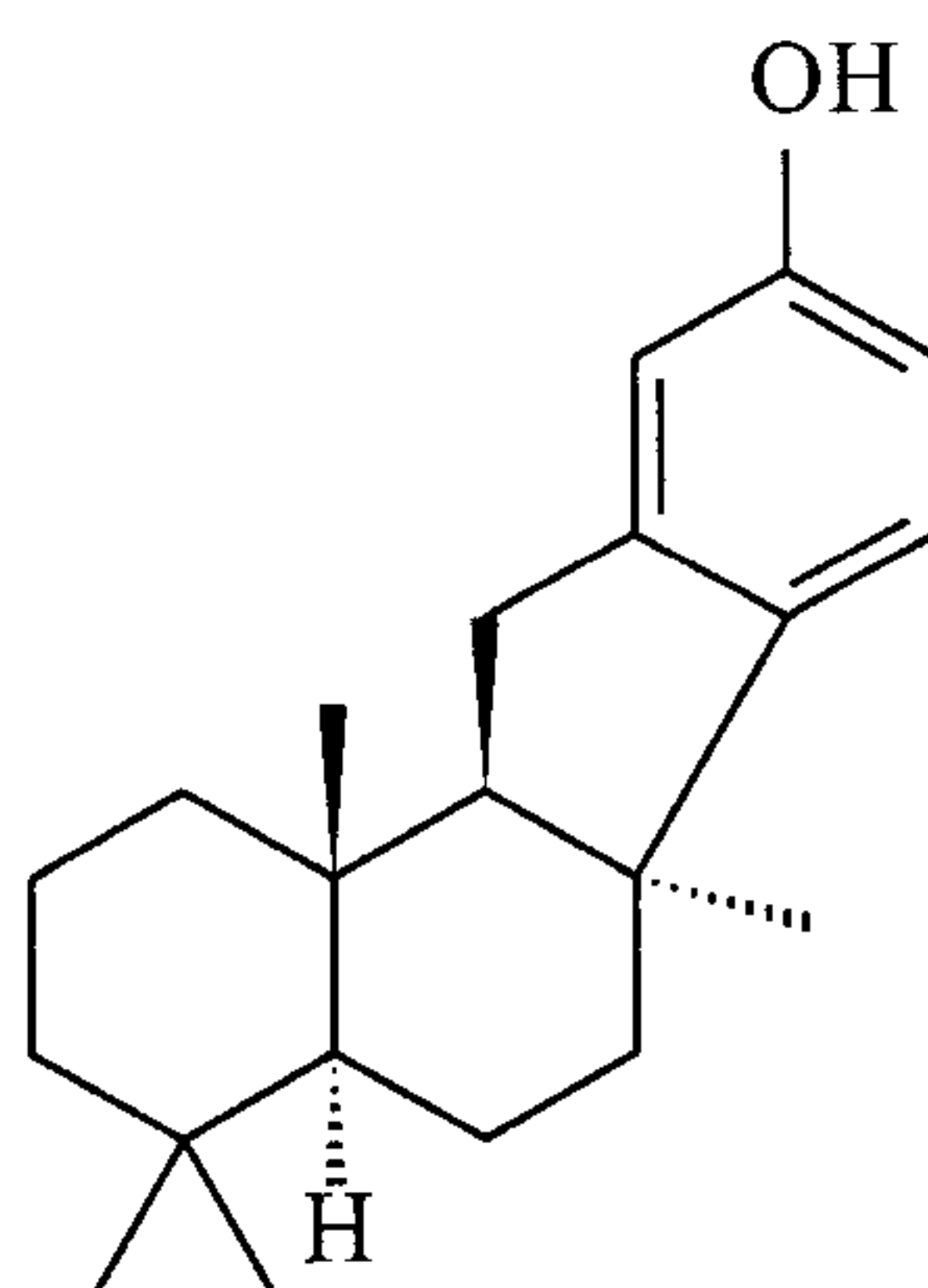
28. The compound of claim 2 having the structure:



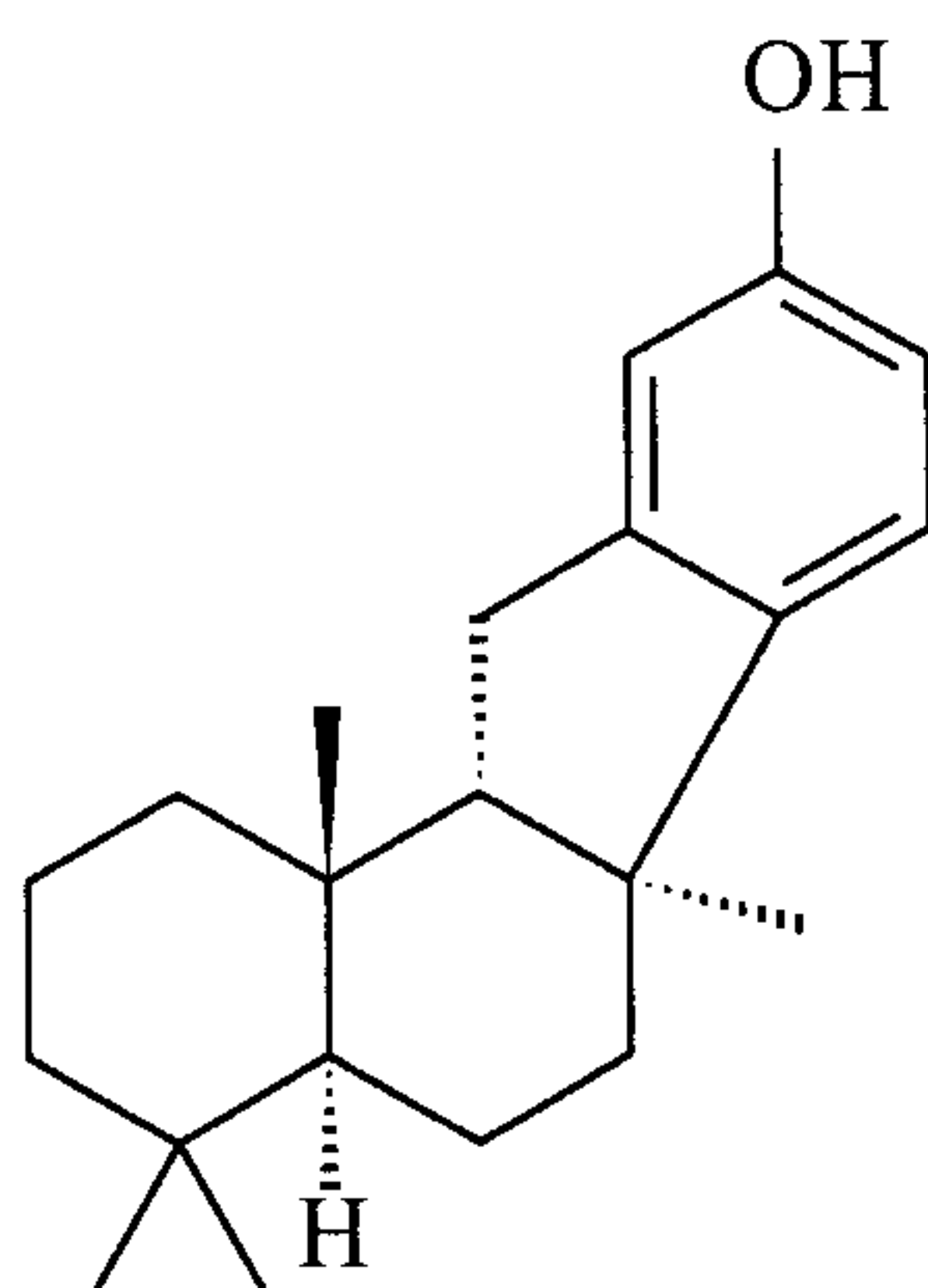
29. The compound of claim 2 having the structure:



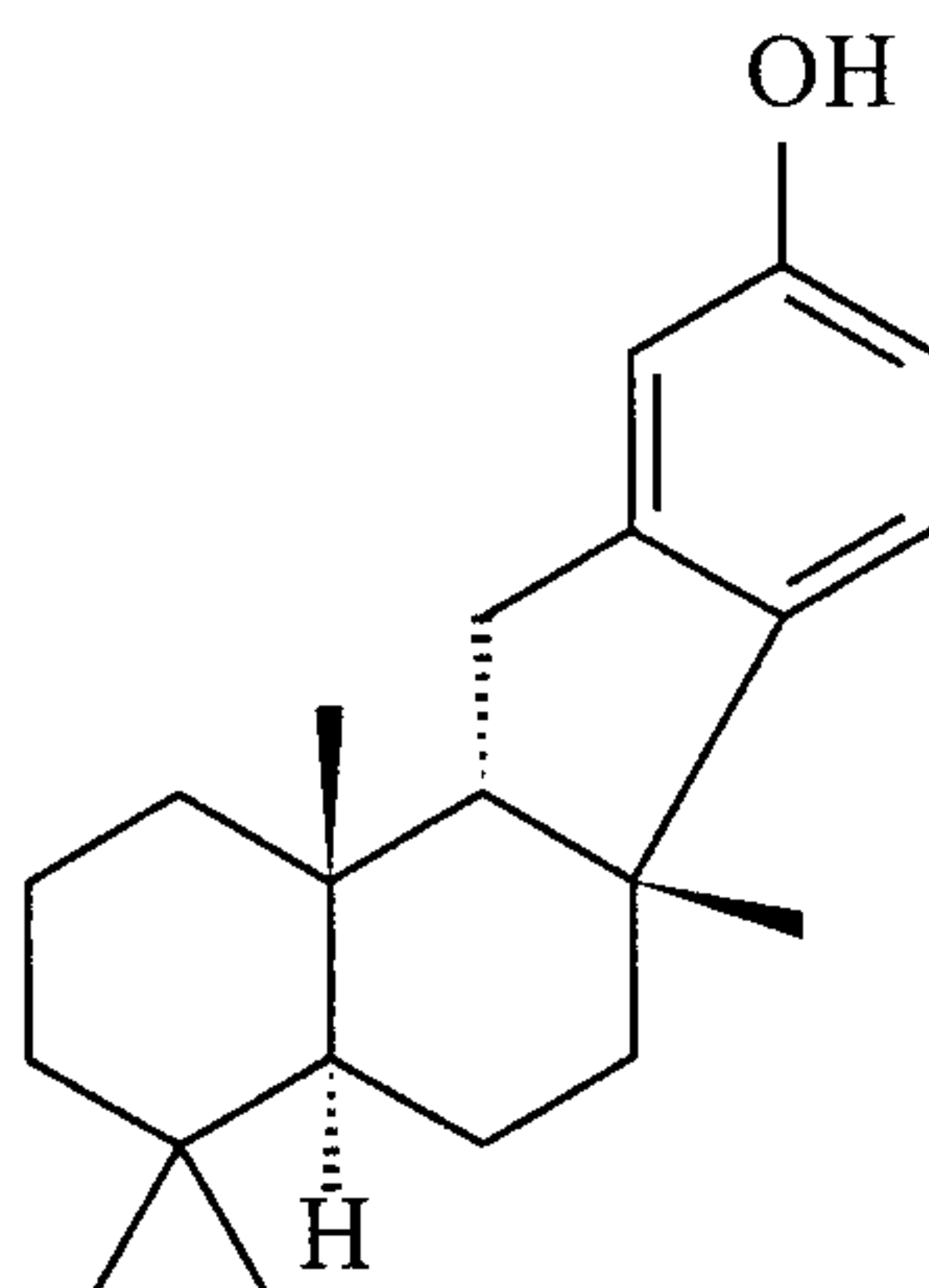
30. The compound of claim 2 having the structure:



31. The compound of claim 2 having the structure:



32. The compound of claim 2 having the structure:



33. A pharmaceutical composition comprising a compound of any one of claims 2 to 32 or salt thereof and a pharmaceutically acceptable excipient.

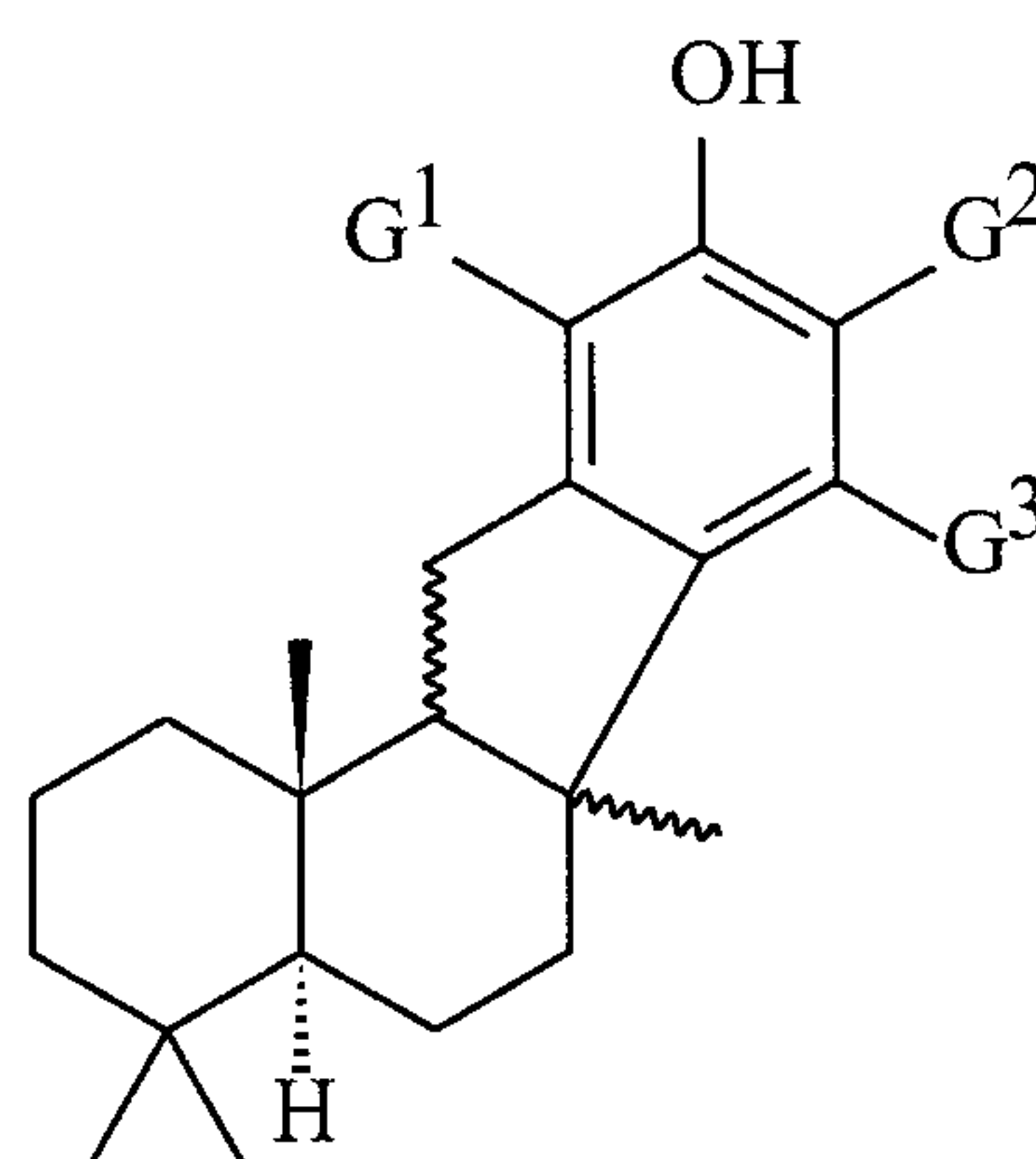
34. A compound of any one of claims 2 to 32 or salt thereof for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.

35. The compound or composition of claim 34 wherein the neoplastic disorder or condition is a blood cancer, multiple myeloma, chronic myeloid leukemia or acute myelogenous leukemia.

36. The compound or composition of claim 34 wherein the disorder or condition is an autoimmune disorder.

37. The compound or composition of claim 34 wherein the disorder or condition is an inflammatory disorder or condition.
38. The compound or composition of claim 37 wherein the inflammatory disorder or condition is rheumatoid arthritis or inflammatory bowel syndrome.
39. The compound or composition of claim 38 wherein the inflammatory bowel syndrome is Crohn's disease or ulcerative colitis.
40. Use of a compound of any one of claims 2 to 32 or salt thereof for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.
41. Use of a compound of any one of claims 2 to 32 or salt thereof for the preparation of a medicament for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.
42. The use of claim 40 or 41 wherein the neoplastic condition is a blood cancer, multiple myeloma, chronic myeloid leukemia or acute myelogenous leukemia.
43. The use of claim 40 or 41 wherein the immune disorder or condition is an autoimmune disorder.
44. The use of claim 40 or 41 wherein the disorder or condition is an inflammatory disorder or condition.
45. The use of claim 44 wherein the inflammatory disorder or condition is rheumatoid arthritis or inflammatory bowel syndrome.
46. The use of claim 45 wherein the inflammatory bowel syndrome is Crohn's disease or ulcerative colitis.
47. The compound of claim 1 or a salt thereof wherein G¹ is O-(C₁-C₁₀ alkyl).

48. The compound of claim 47 or a salt thereof wherein one or both of R_1 and R_2 are selected from the group consisting of: methyl, ethyl, $-\text{CH}_2\text{OH}$, $-\text{CH}_2\text{OR}_1'$, or $-\text{CH}_2\text{OR}_2'$.
49. The compound of claim 48 or a salt thereof wherein R_1' , R_2' , R_3' , and/or R_4' , in R_1 are selected from the group consisting of: methyl, ethyl, propyl or butyl.
50. The compound of claim 47 or a salt thereof wherein R_1 is methyl or ethyl.
51. The compound of claim 47 or a salt thereof wherein R_2 is methyl or ethyl.
52. The compound of claim 47 or a salt thereof wherein R_1 and R_2 are both methyl.
53. The compound of claim 47 having Formula 2 and salts thereof:



Formula 2

wherein;

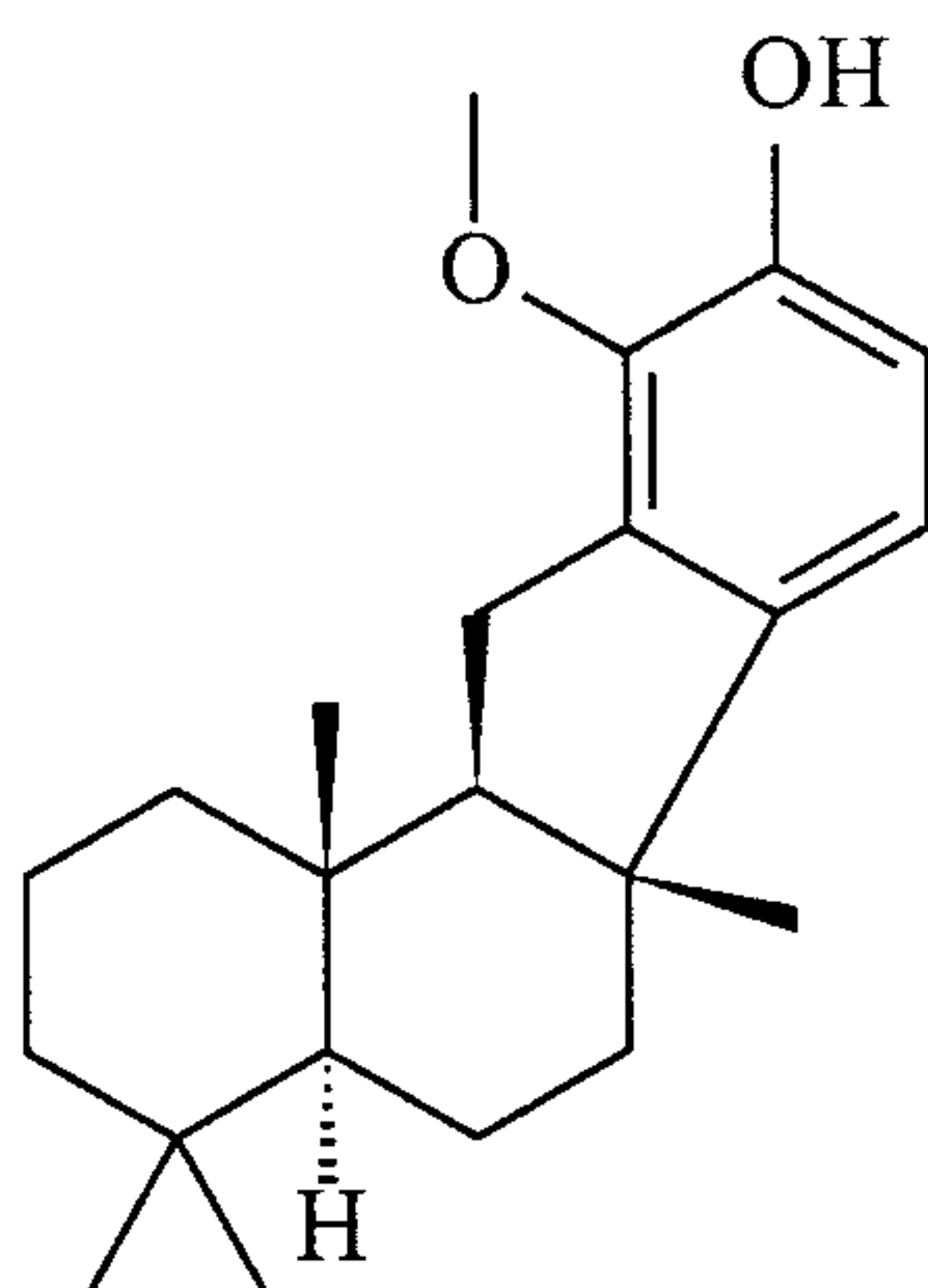
G_1 is $\text{O}-(\text{C}_1\text{-C}_{10} \text{ alkyl})$;

G_2 is H or $\text{C}_1\text{-C}_{10} \text{ alkyl}$; and

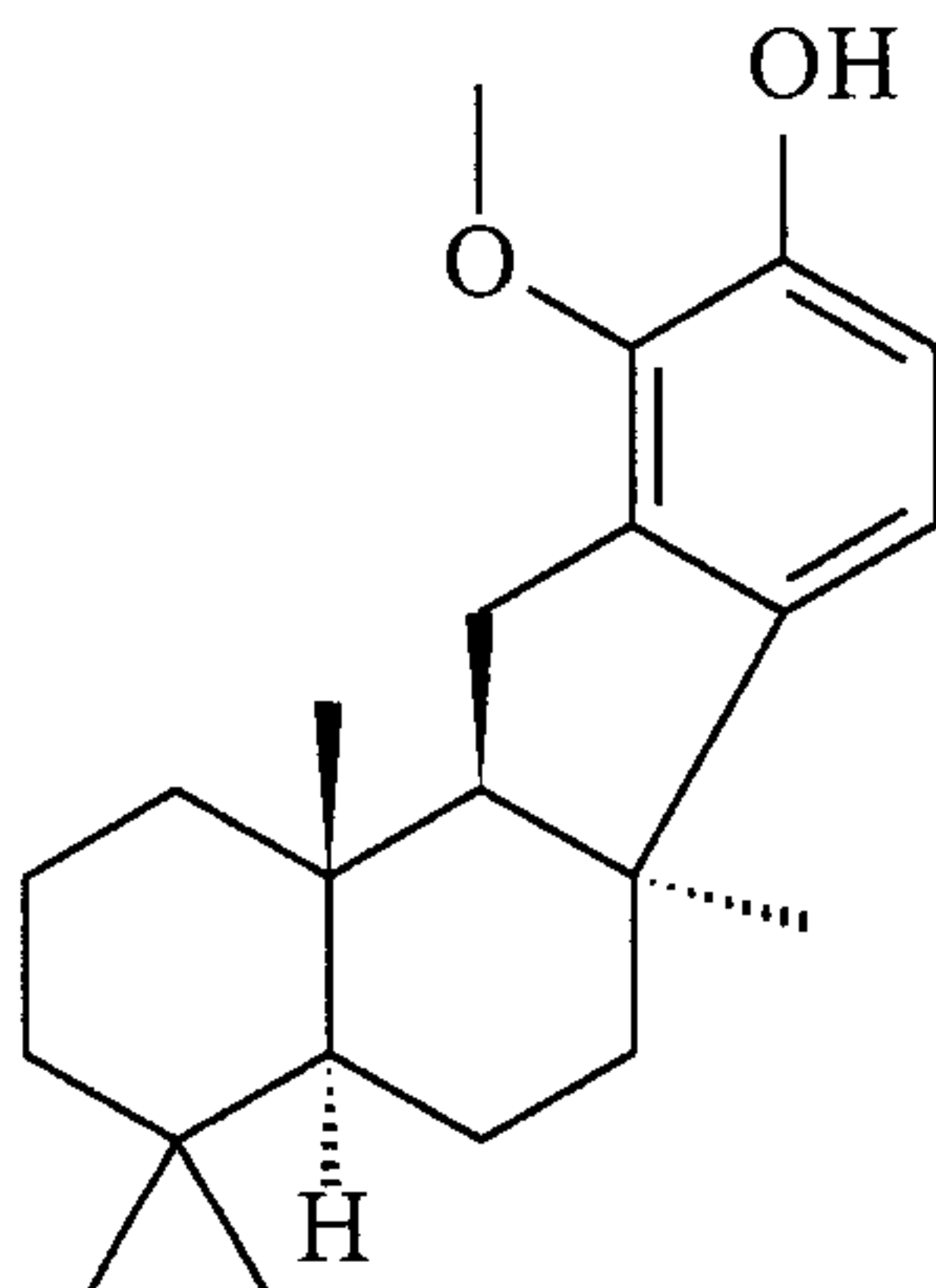
G_3 is selected from the group consisting of: H, $-\text{OH}$, $\text{C}_1\text{-C}_{10} \text{ alkyl}$ and $\text{O}-(\text{C}_1\text{-C}_{10} \text{ alkyl})$.

54. The compound of claim 53 or a salt thereof wherein G_2 is H or methyl; and G_3 is selected from the group consisting of: H, methyl and O-methyl.
55. The compound of claim 53 or a salt thereof wherein only one of G_2 and G_3 is -O-methyl.

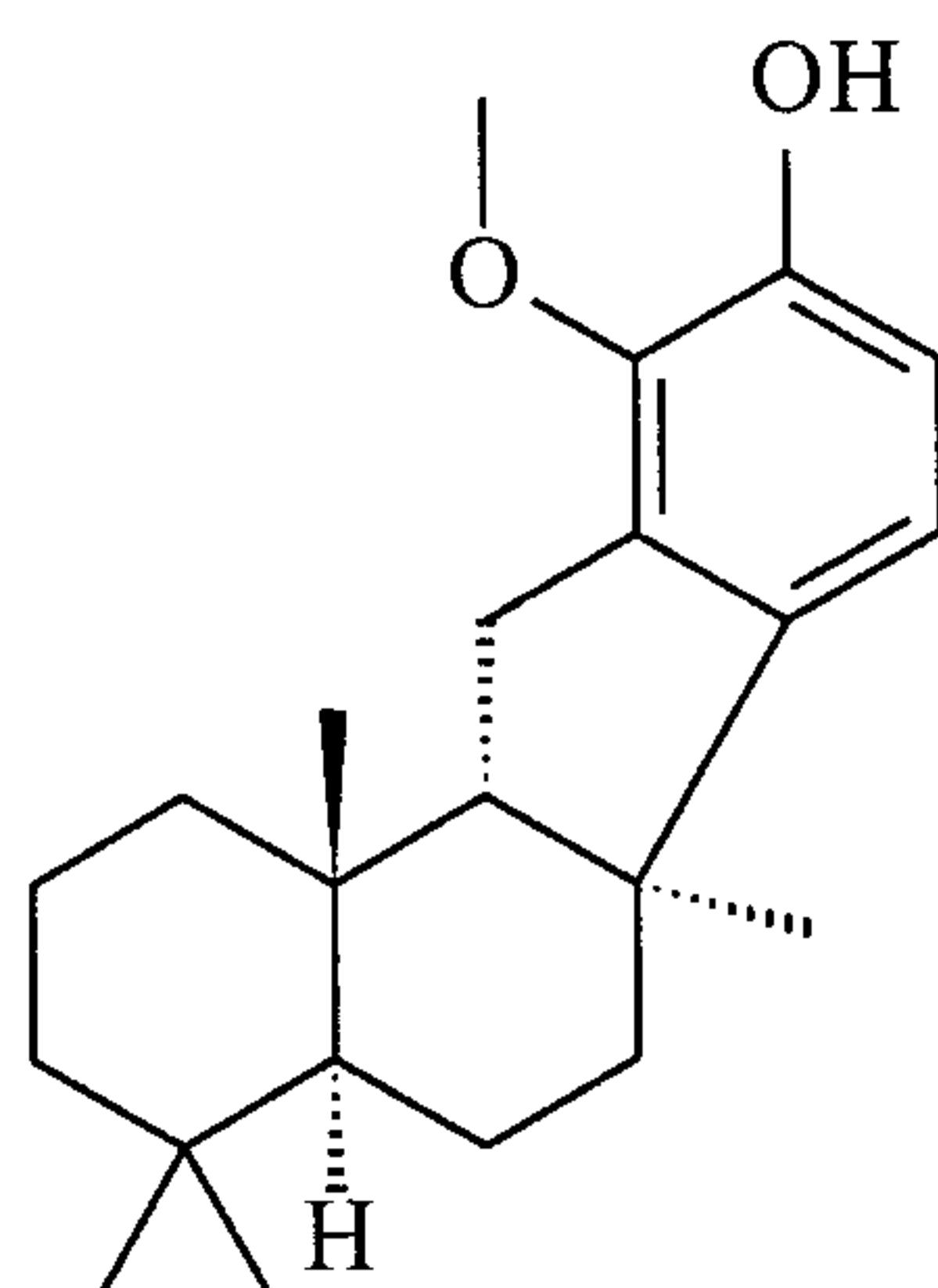
56. The compound of claim 53 or a salt thereof wherein at least one of G_2 and G_3 is H.
57. The compound of claim 53 or a salt thereof wherein G_3 is methyl.
58. The compound of claim 53 or a salt thereof wherein G_2 and G_3 are H.
59. The compound of claim 47 having the structure:



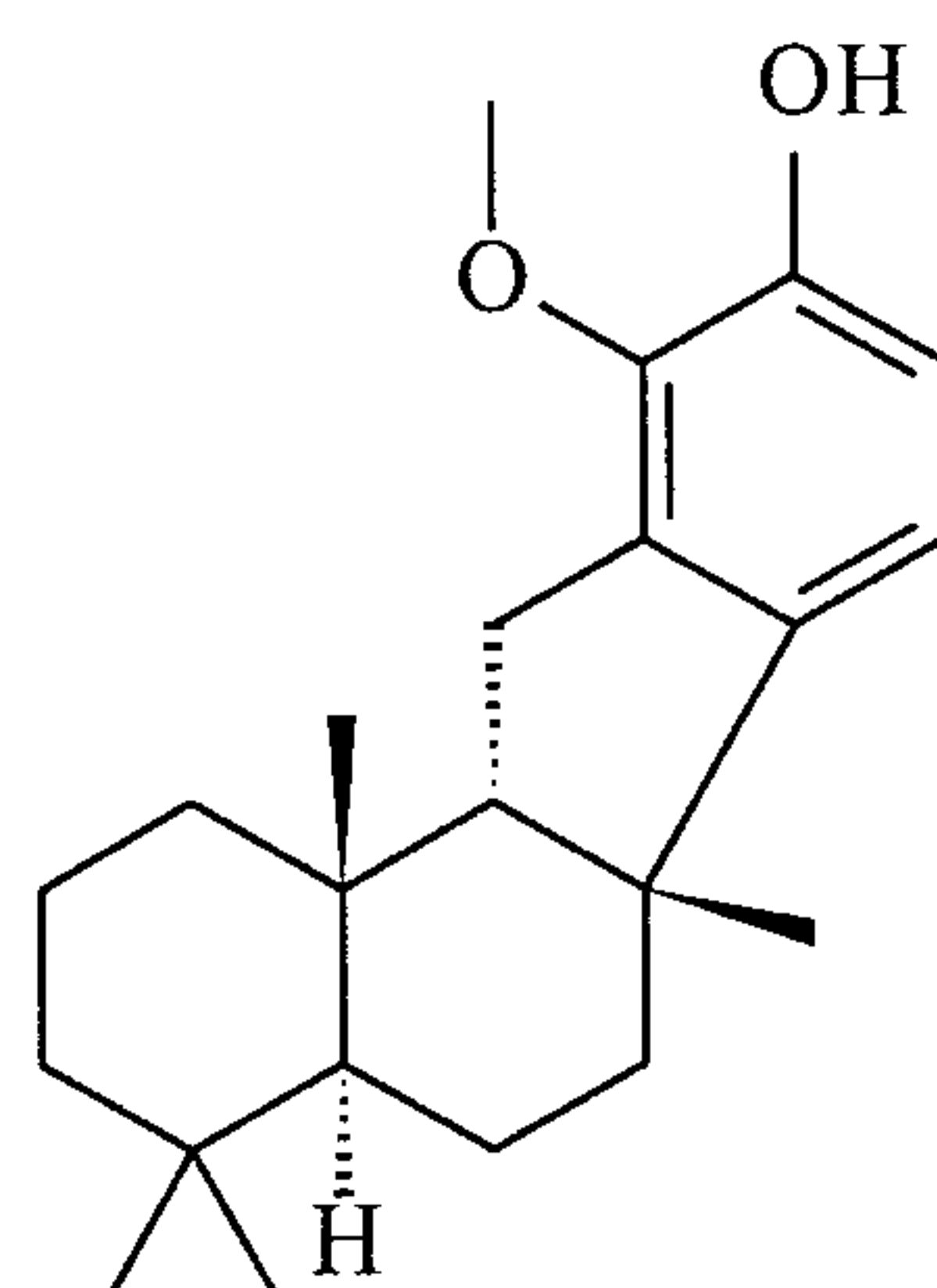
60. The compound of claim 47 having the structure:



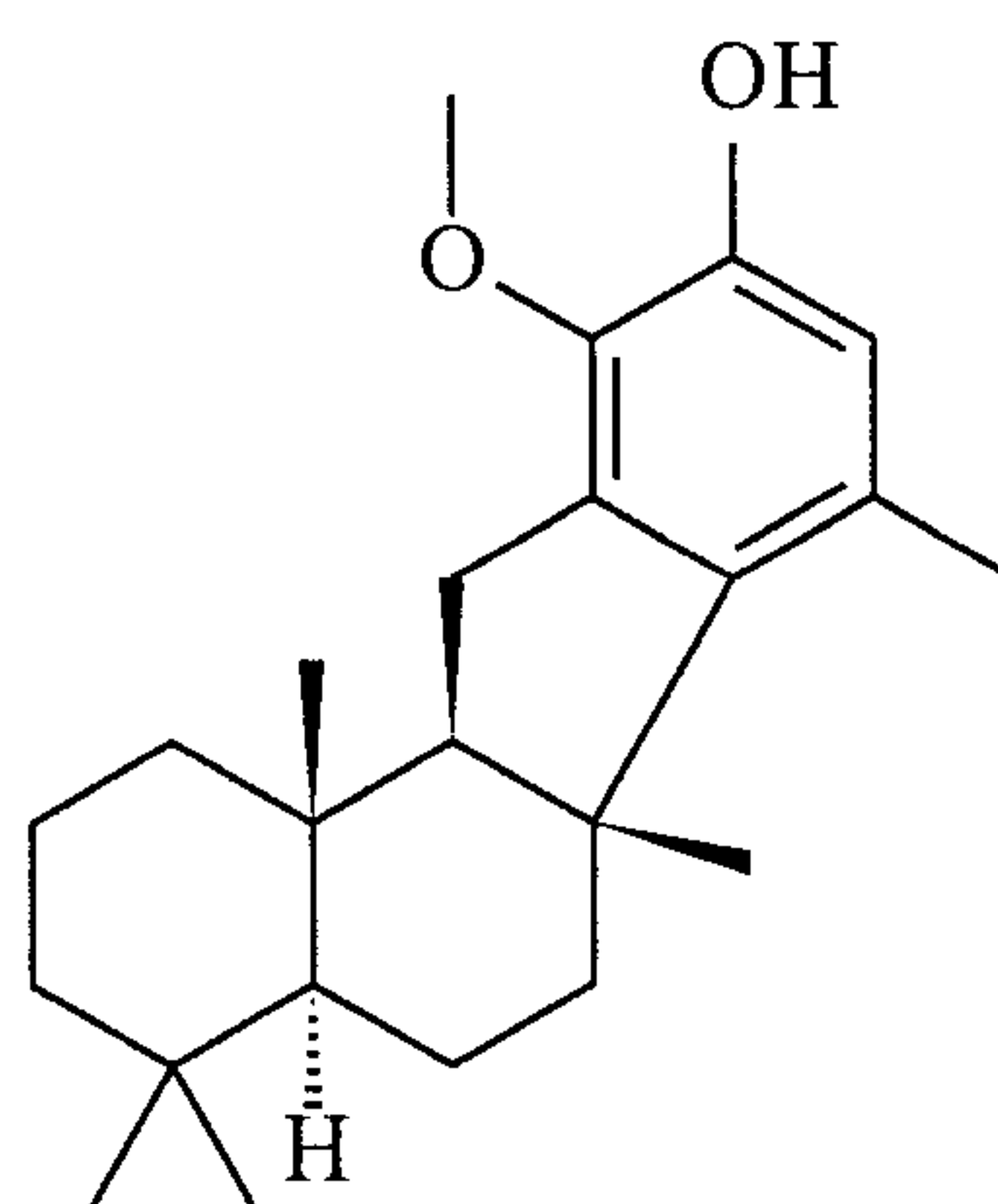
61. The compound of claim 47 having the structure:



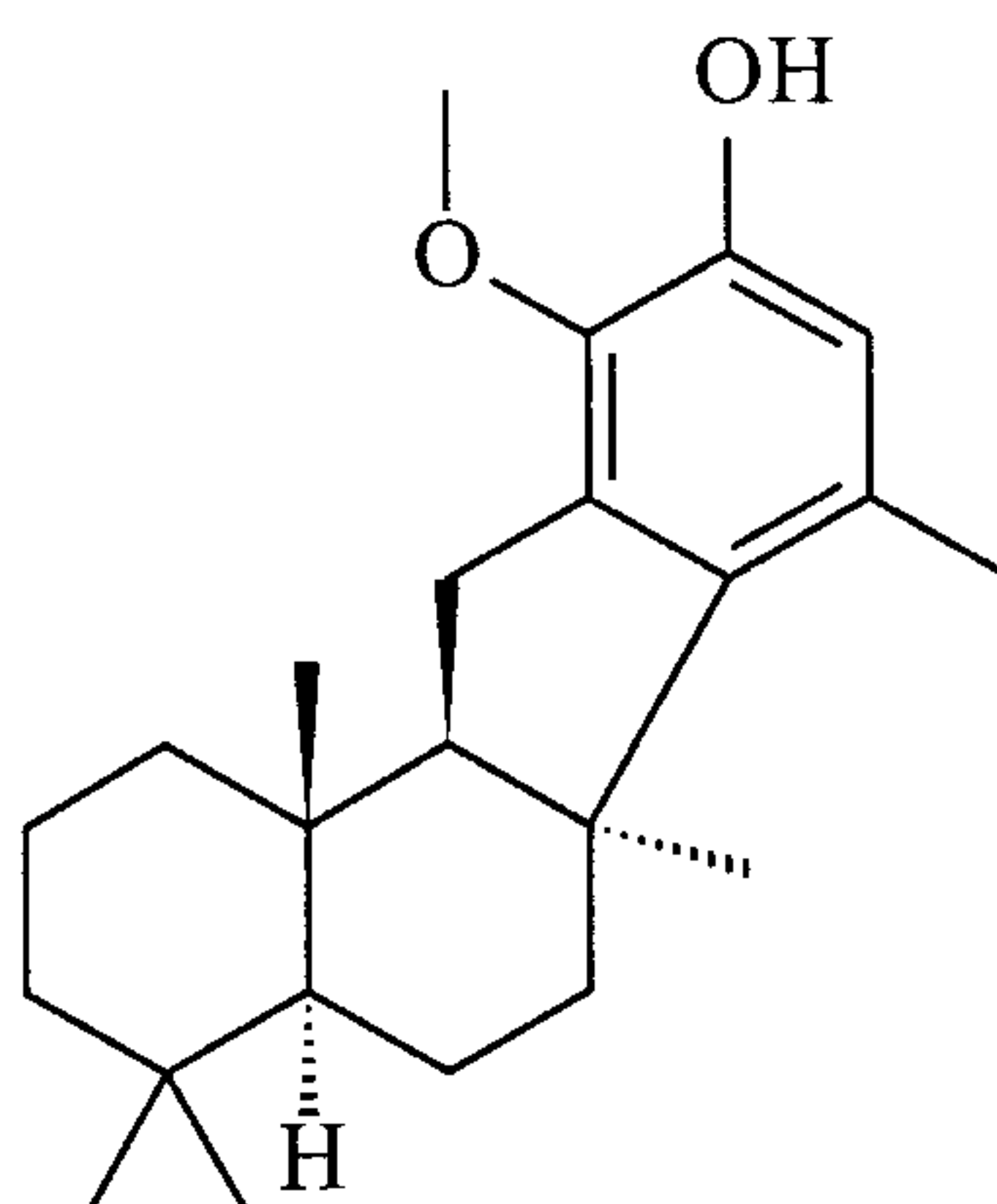
62. The compound of claim 47 having the structure:



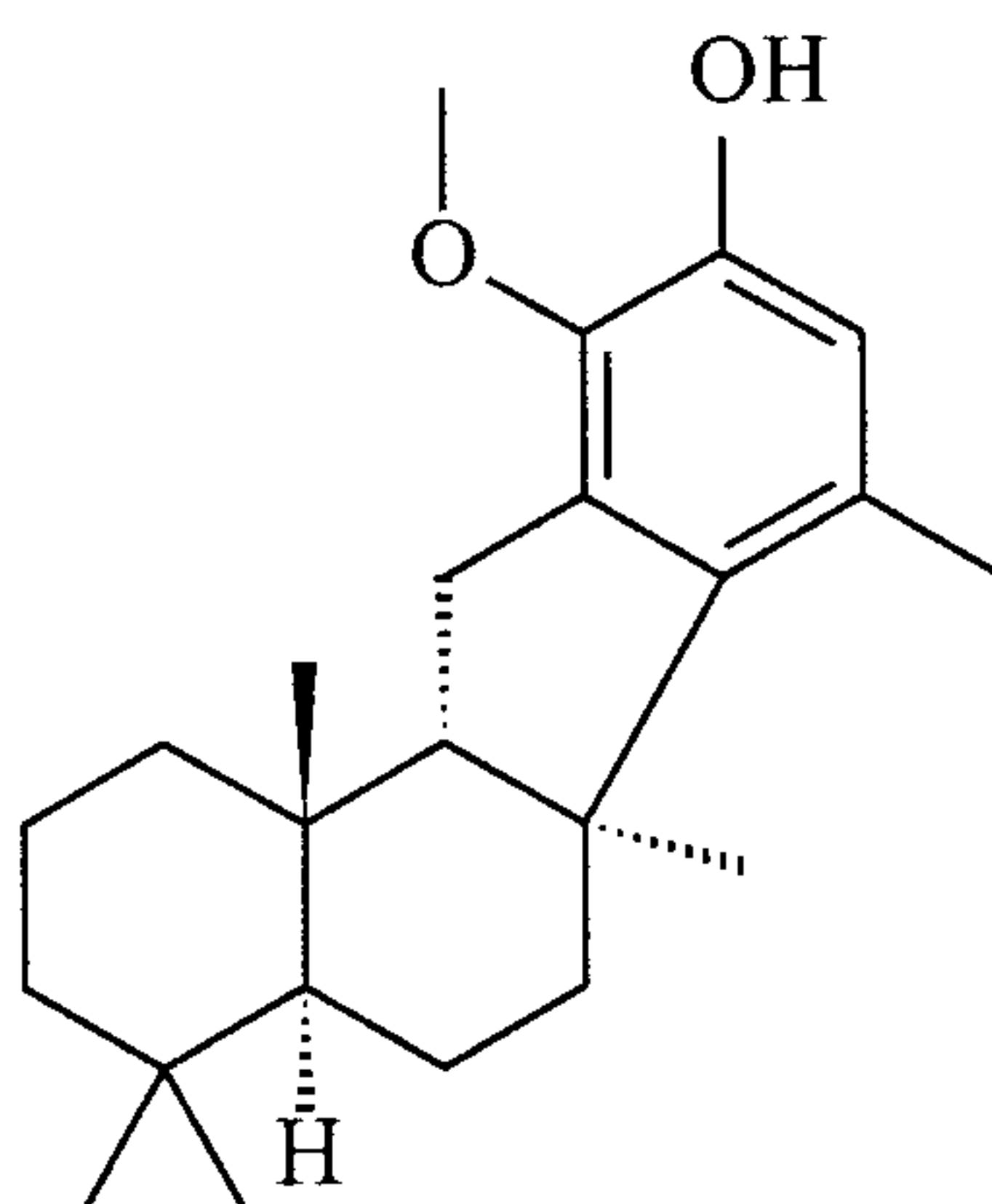
63. The compound of claim 47 having the structure:



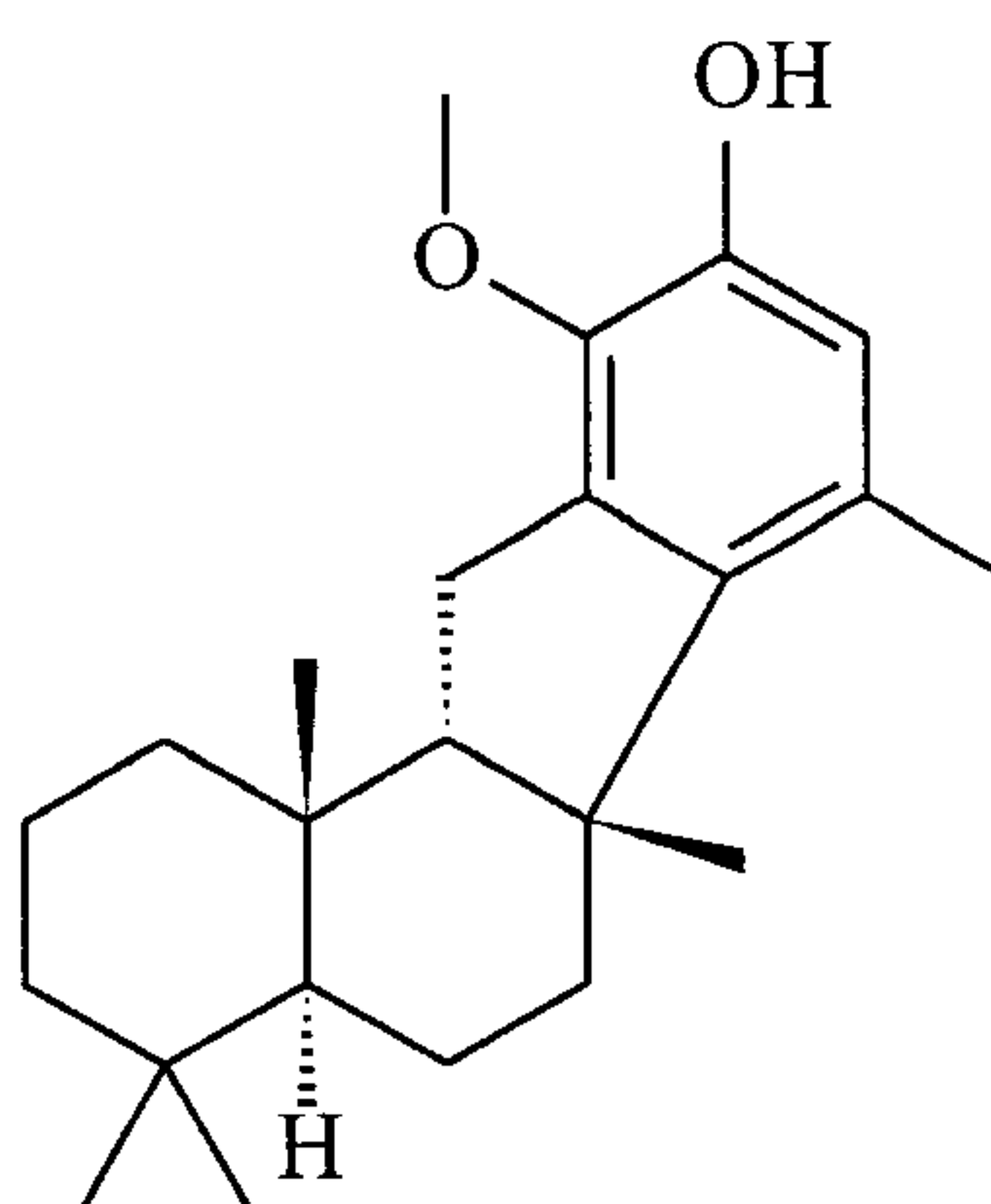
64. The compound of claim 47 having the structure:



65. The compound of claim 47 having the structure:



66. The compound of claim 47 having the structure:



67. A pharmaceutical composition comprising a compound of any one of claims 47 to 66 or salt thereof and a pharmaceutically acceptable excipient.
68. A compound of any one of claims 47 to 66 or salt thereof for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.
69. The compound of claim 68 wherein the neoplastic disorder or condition is a blood cancer, multiple myeloma, chronic myeloid leukemia or acute myelogenous leukemia.
70. The compound of claim 68 wherein the disorder or condition is an autoimmune disorder.
71. The compound of claim 68 wherein the disorder or condition is an inflammatory disorder or condition.
72. The compound of claim 71 wherein the inflammatory disorder or condition is rheumatoid arthritis or inflammatory bowel syndrome.
73. The compound of claim 72 wherein the inflammatory bowel syndrome is Crohn's disease or ulcerative colitis.
74. Use of a compound of any one of claims 47 to 66 or salt thereof for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.
75. Use of a compound of any one of claims 47 to 66 or salt thereof for the preparation of a medicament for the treatment or prophylaxis of an inflammatory, neoplastic, hematopoietic or immune disorder or condition.
76. The use of claim 74 or 75 wherein the neoplastic disorder or condition is a blood cancer, multiple myeloma, chronic myeloid leukemia or acute myelogenous leukemia.
77. The use of claim 74 or 75 wherein the disorder or condition is an autoimmune disorder.

78. The use of claim 74 or 75 wherein the disorder or condition is an inflammatory disorder or condition.

79. The use of claim 78 wherein the inflammatory disorder or condition is rheumatoid arthritis or irritable bowel syndrome.

80. The use of claim 79 wherein the inflammatory bowel syndrome is Crohn's disease or ulcerative colitis.

81. A method of prophylaxis or treatment of an immune, hematopoietic, inflammatory or neoplastic disorder or condition comprising administering to a patient in need of said prophylaxis or treatment, an effective amount of a pharmaceutical composition according to claim 33 or 67.

82. The method of claim 81 wherein the neoplastic condition is a blood cancer, multiple myeloma, chronic myeloid leukemia or acute myelogenous leukemia.

83. The method of claim 81 wherein the disorder or condition is an autoimmune disorder.

84. The method of claim 81 wherein the disorder or condition is an inflammatory disorder or condition.

85. The method of claim 84 wherein the inflammatory disorder or condition is rheumatoid arthritis or inflammatory bowel syndrome.

86. The method of claim 85 wherein the inflammatory bowel syndrome is Crohn's disease or ulcerative colitis.

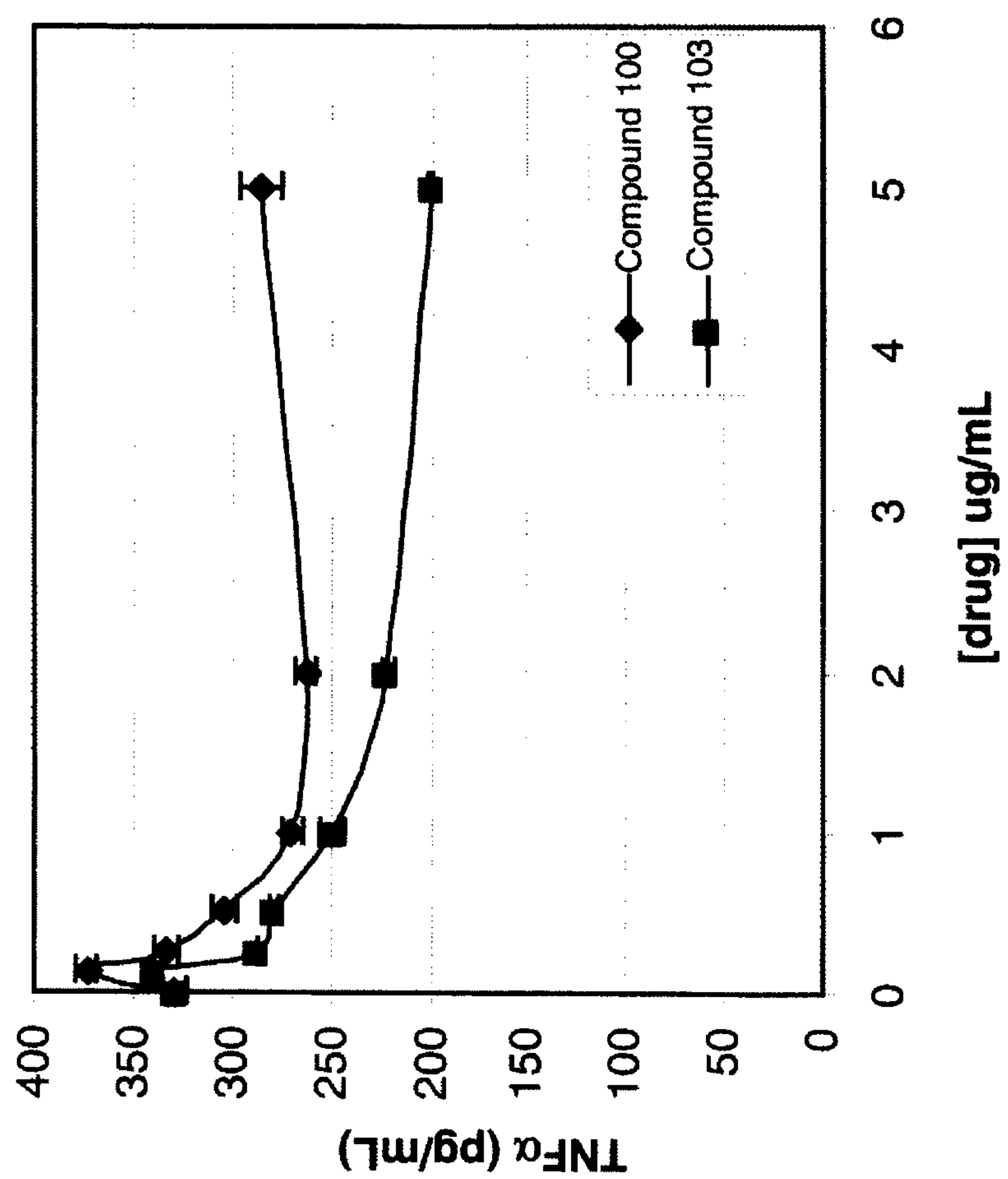
Fig. 1

Fig. 2

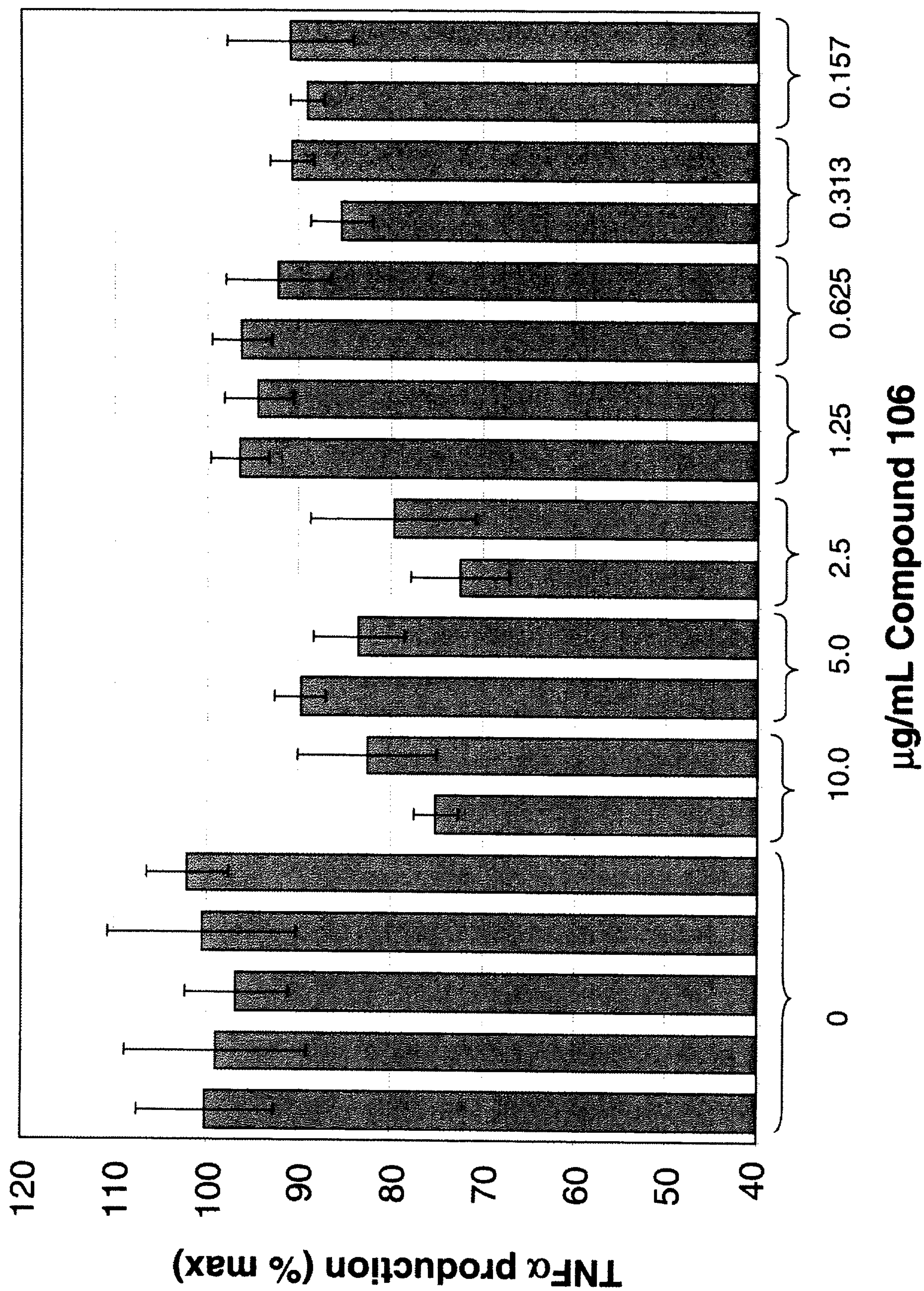


Fig. 3

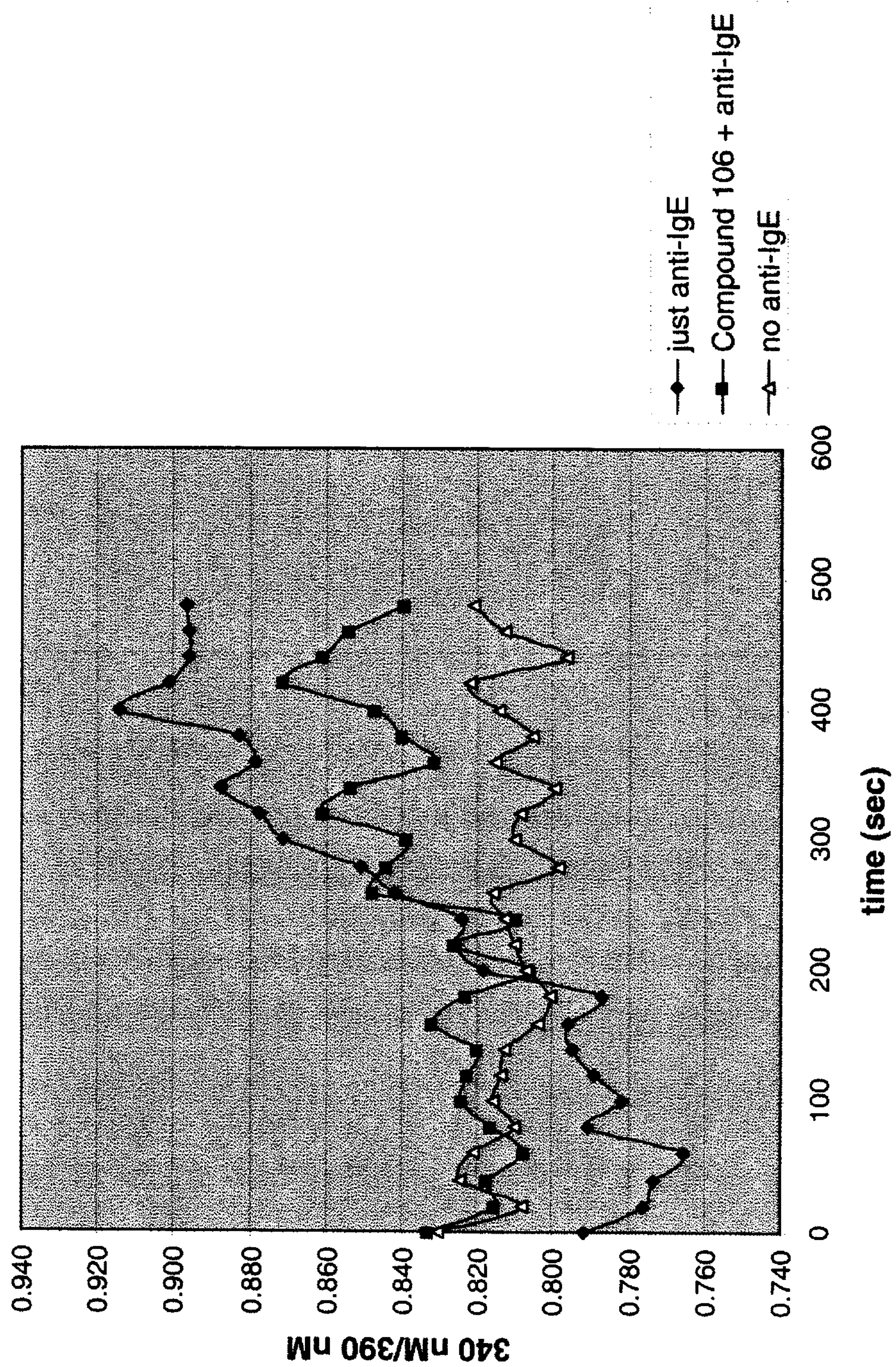


Fig. 4

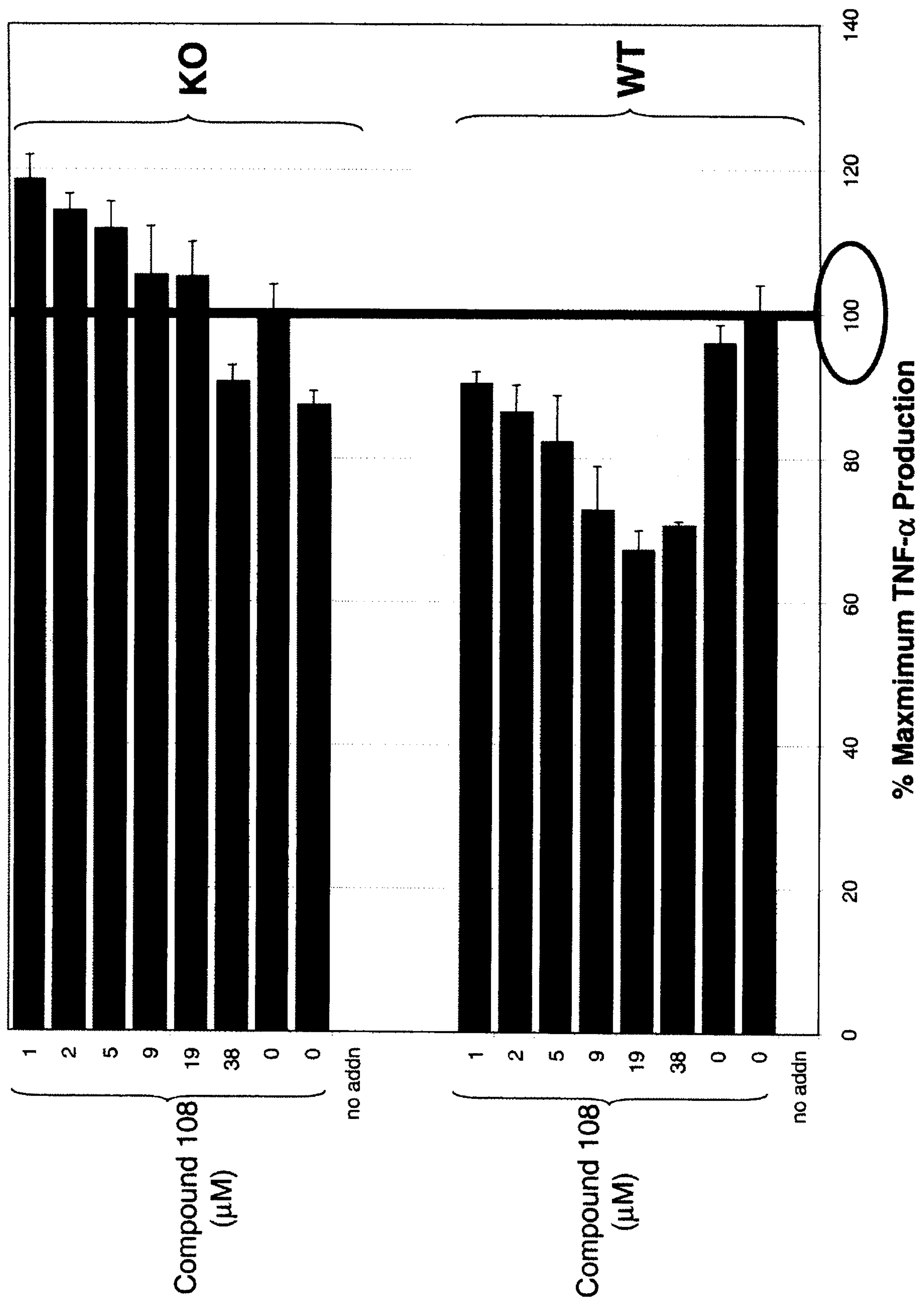


Fig. 5

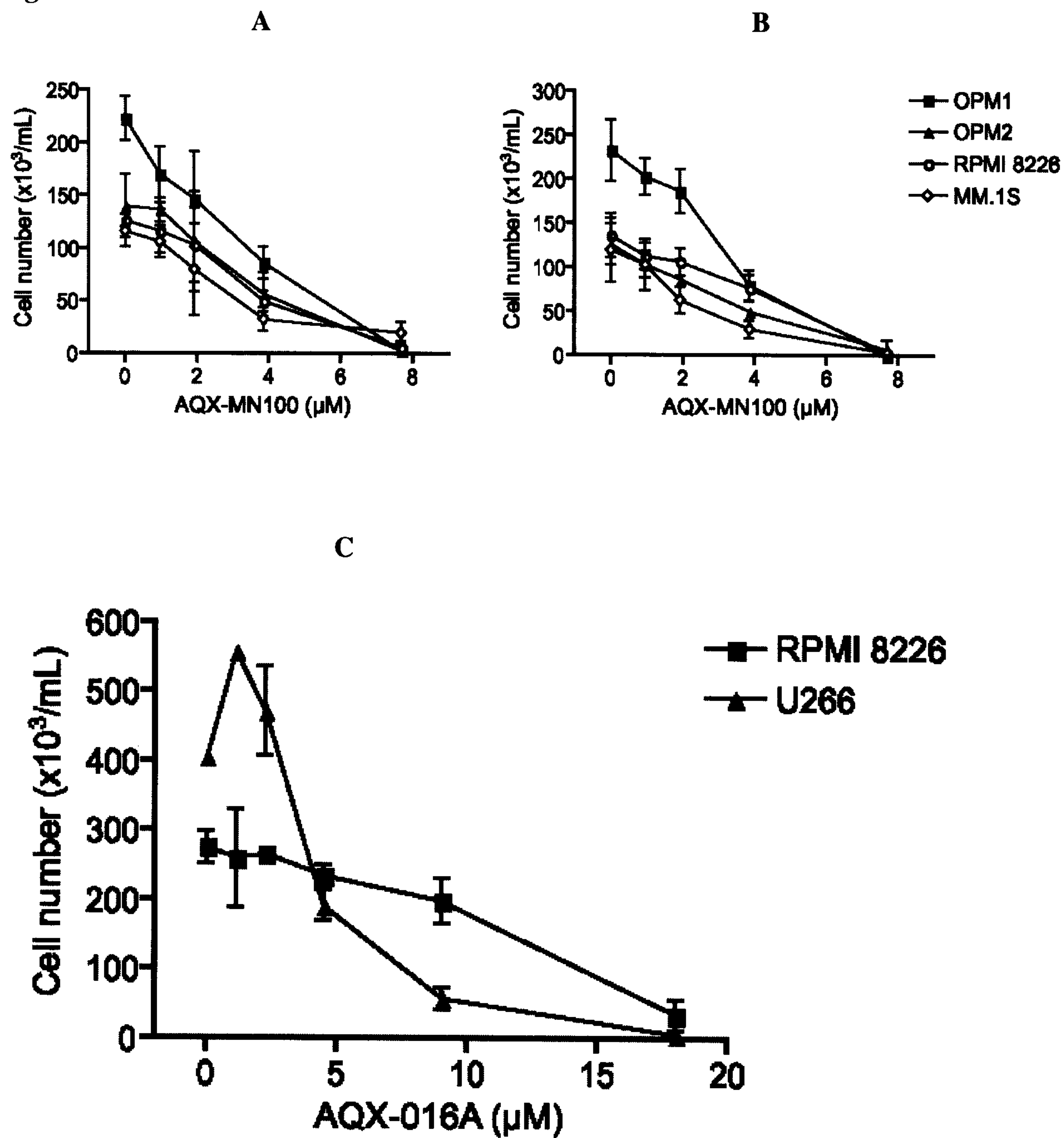


Fig. 6

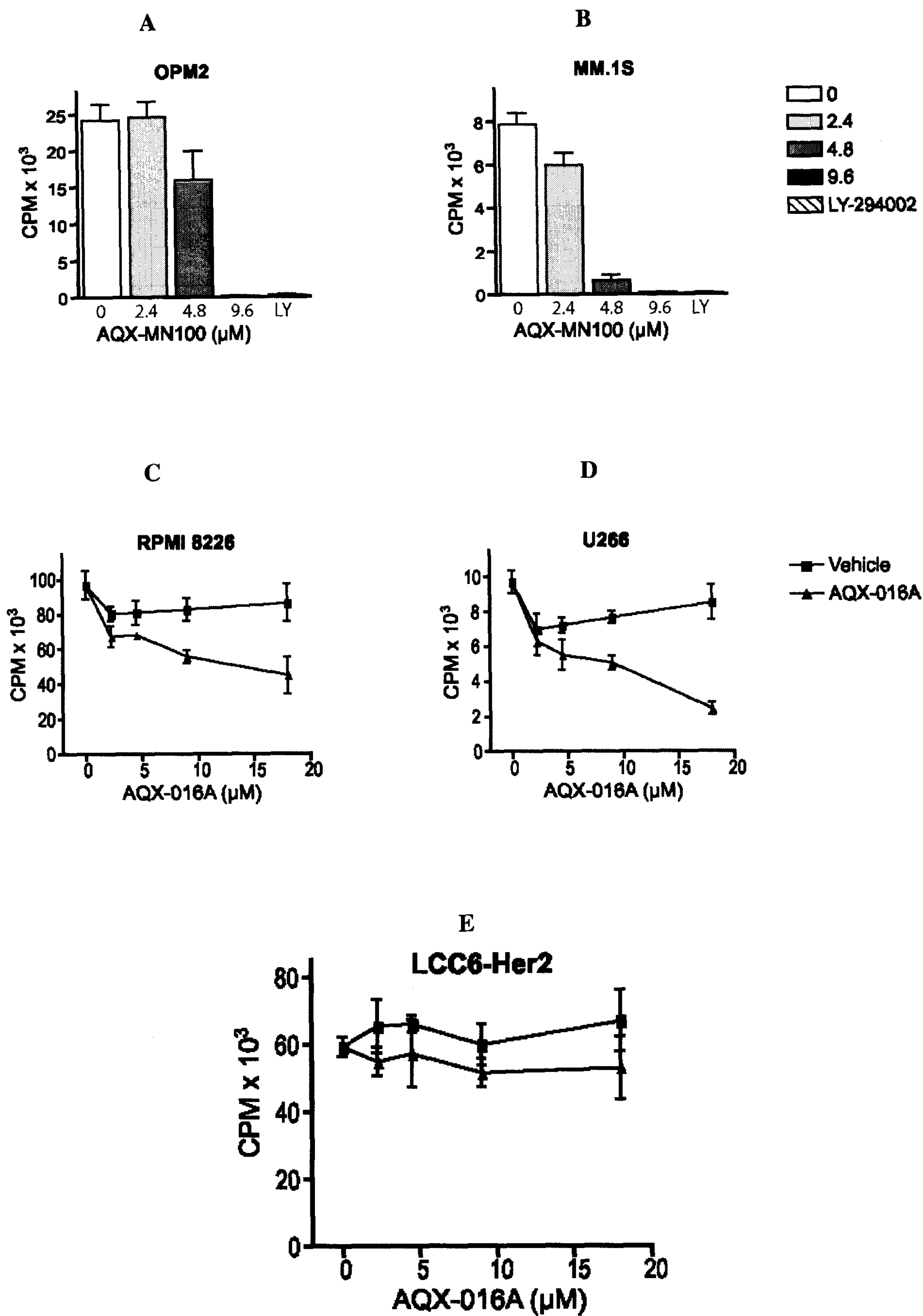


Fig. 7

A

AQX-MN100 Activates SHIP

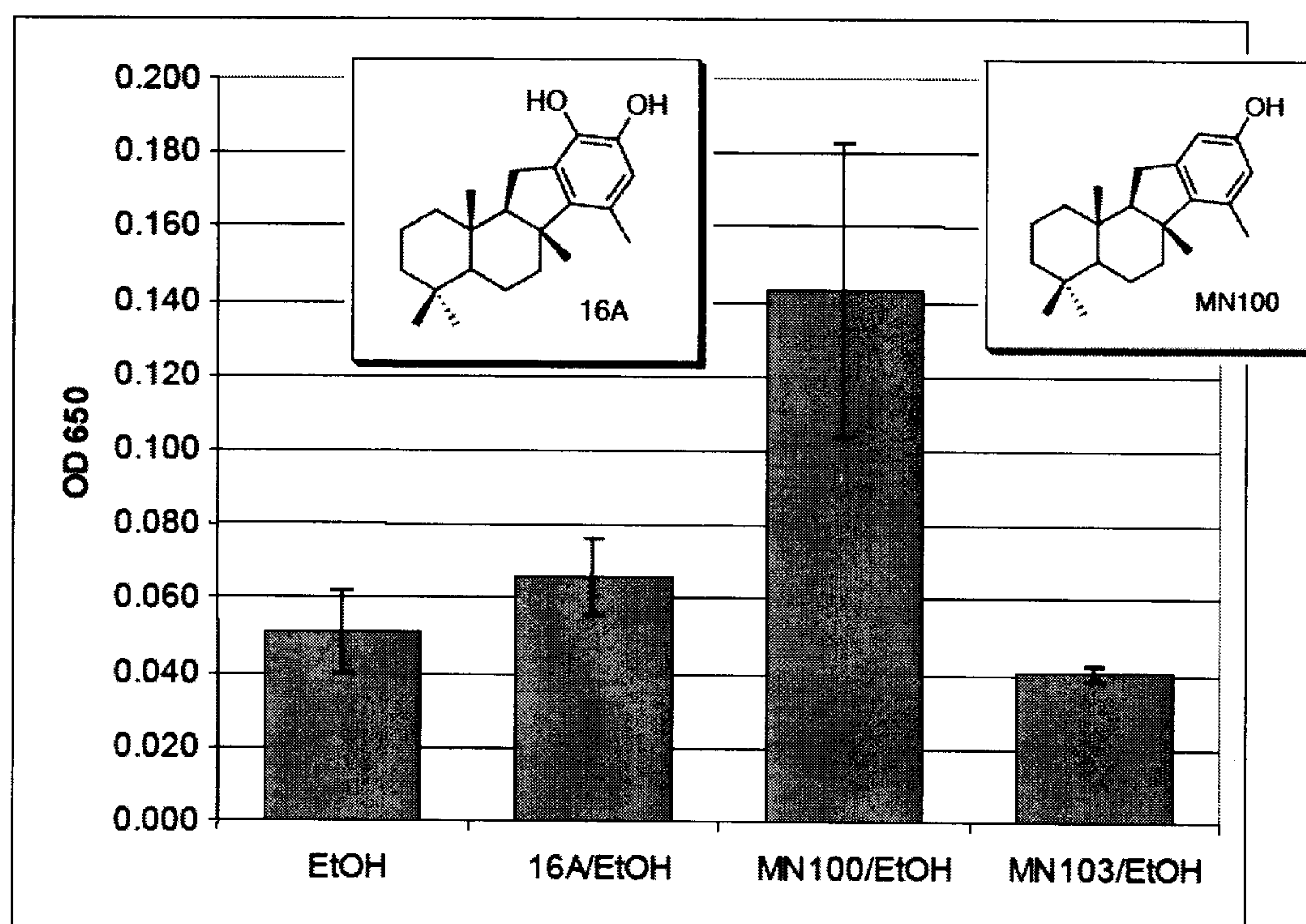


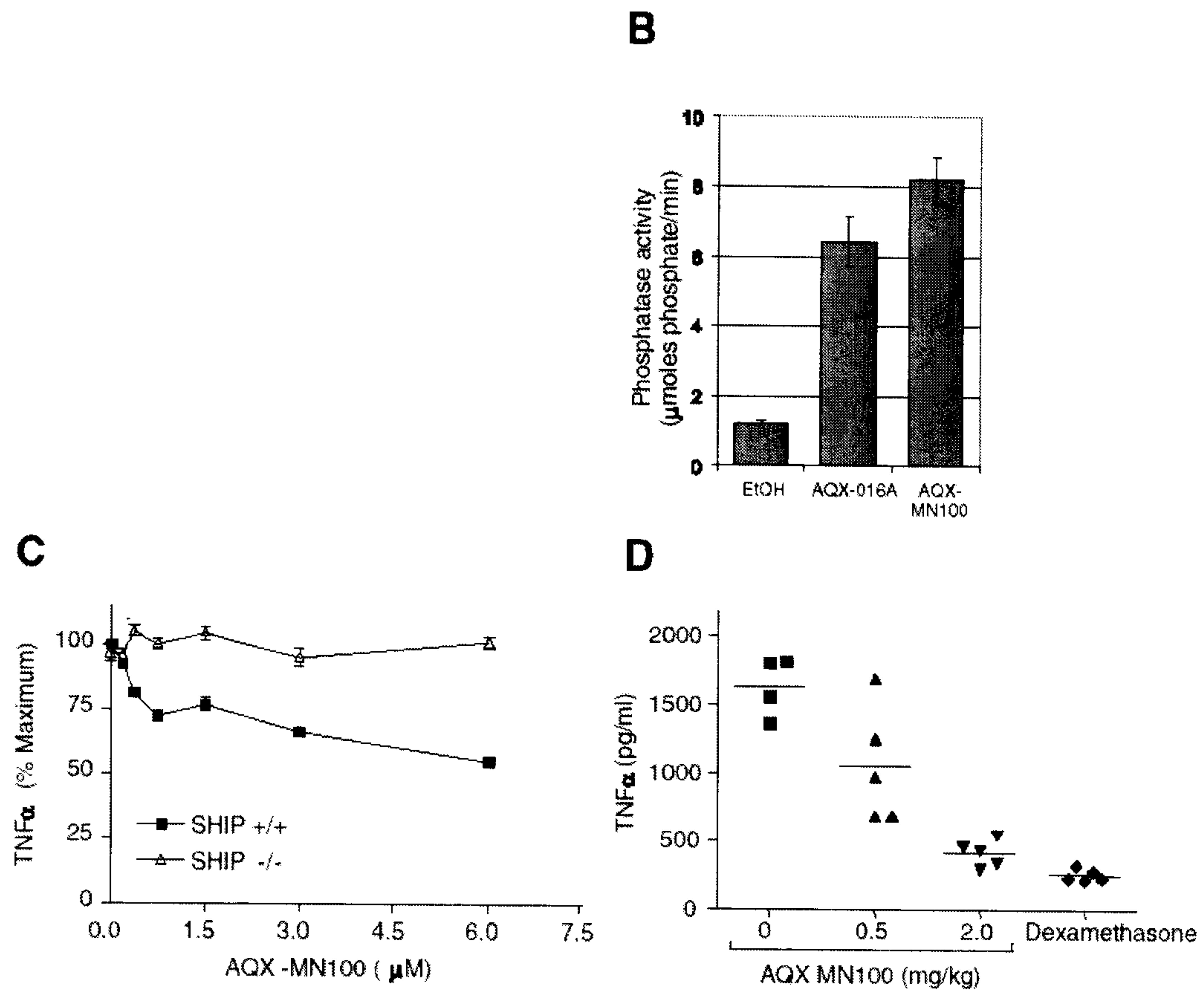
Fig. 7 (cont)

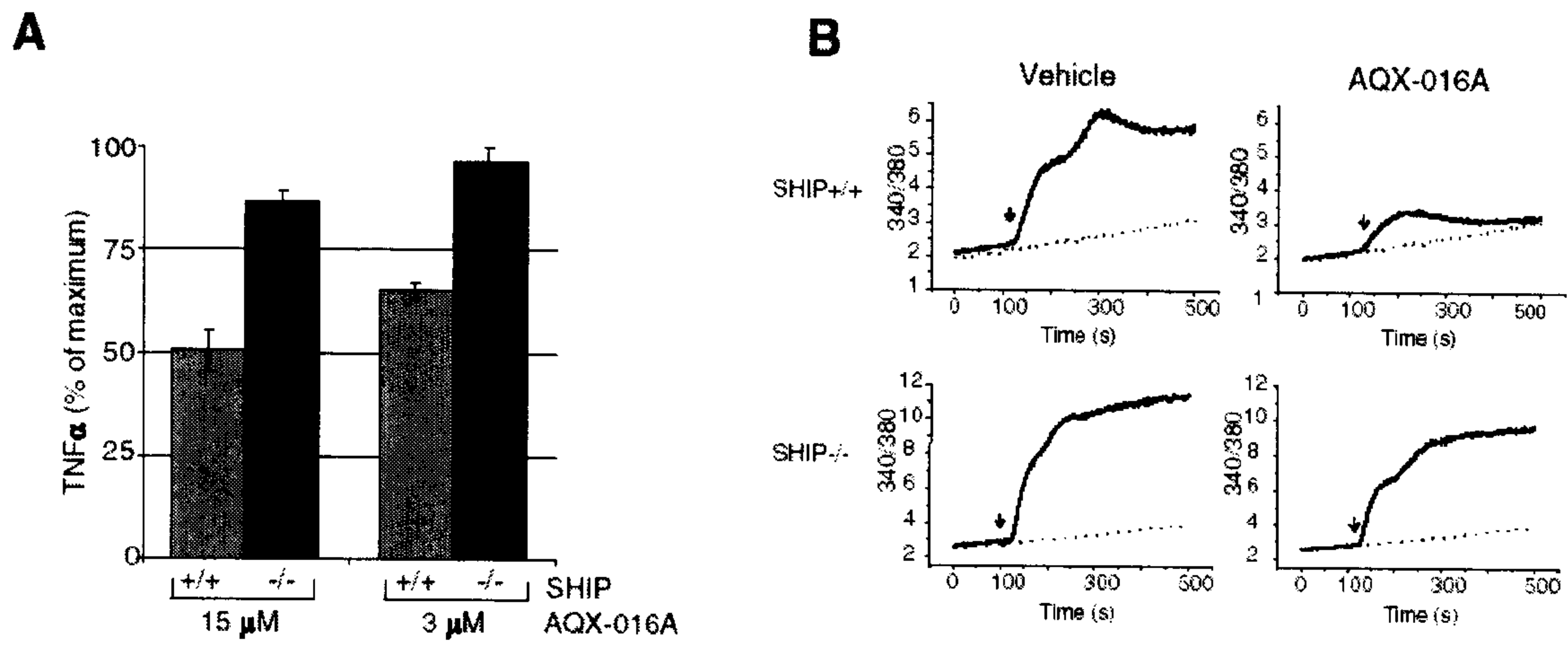
Fig. 8

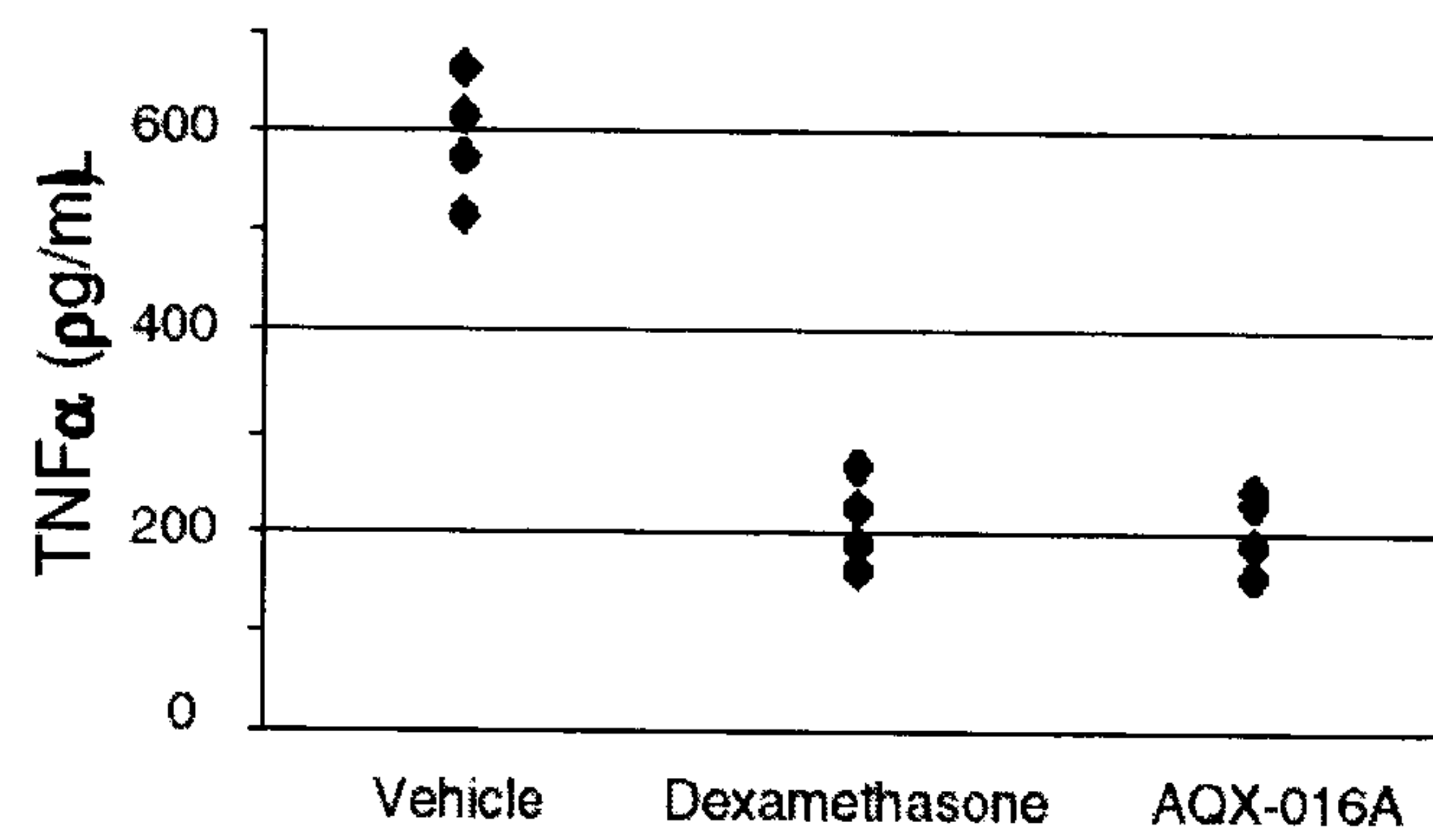
Fig. 9

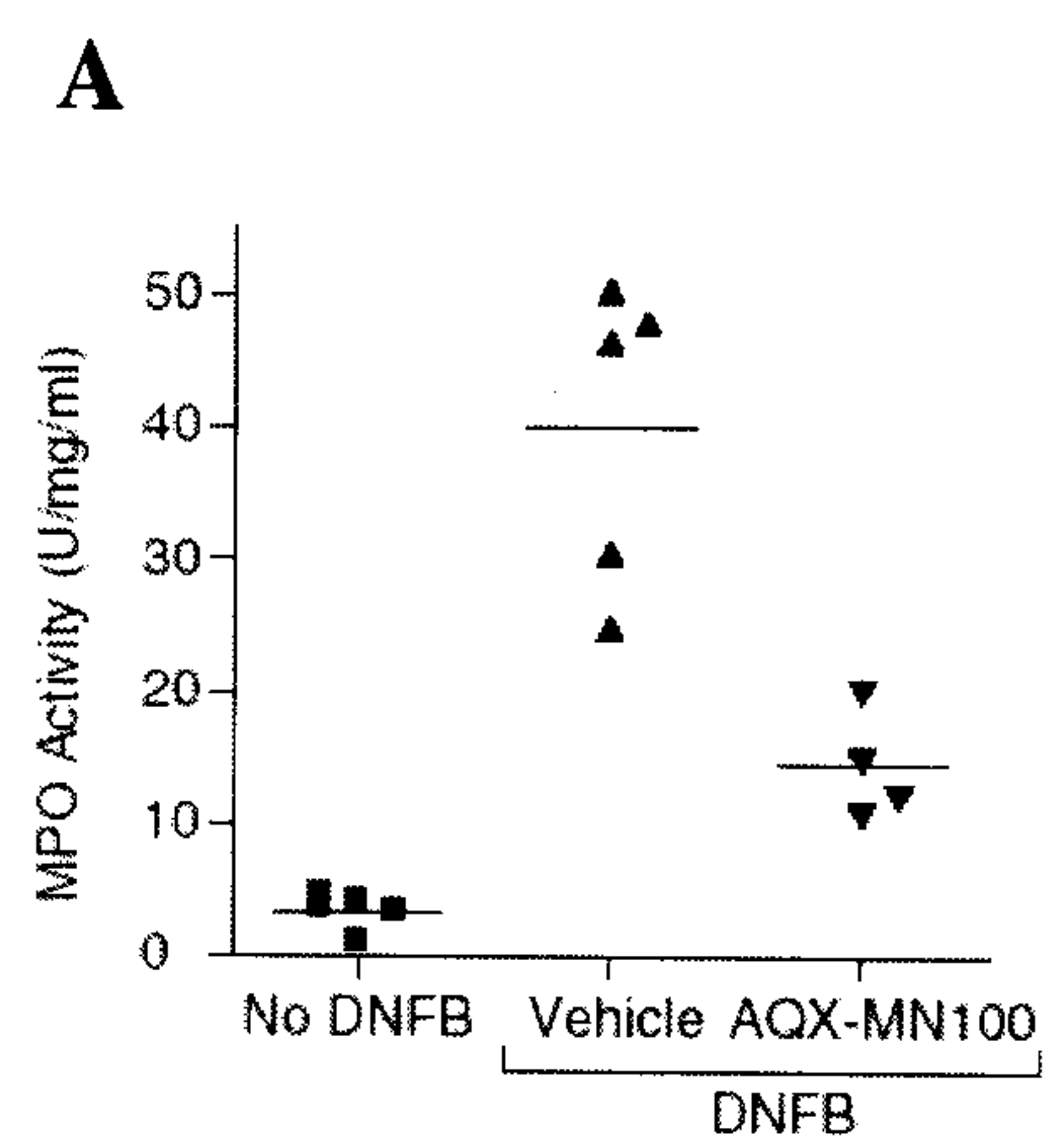
Fig. 10

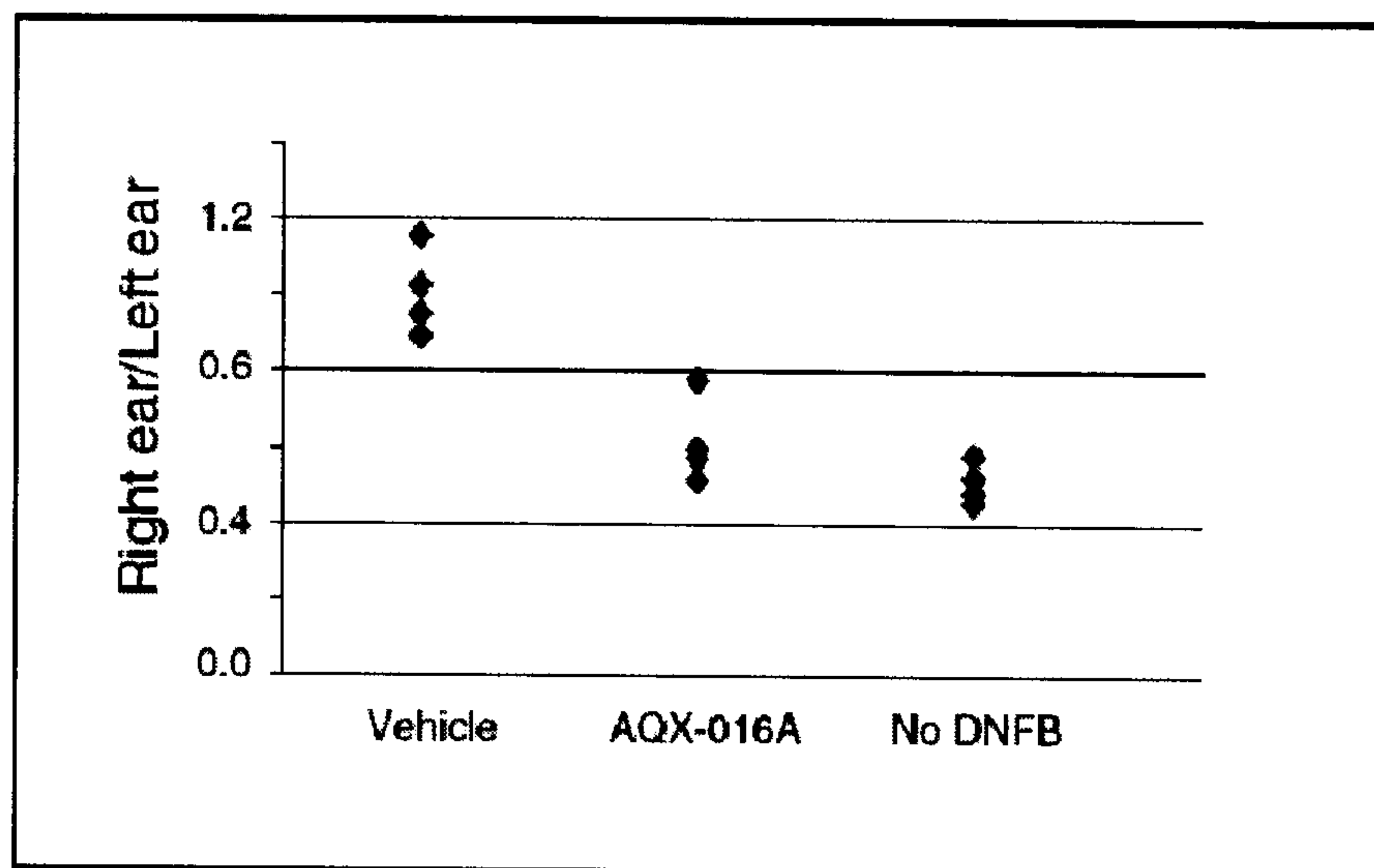
Fig. 10 (cont)**B**

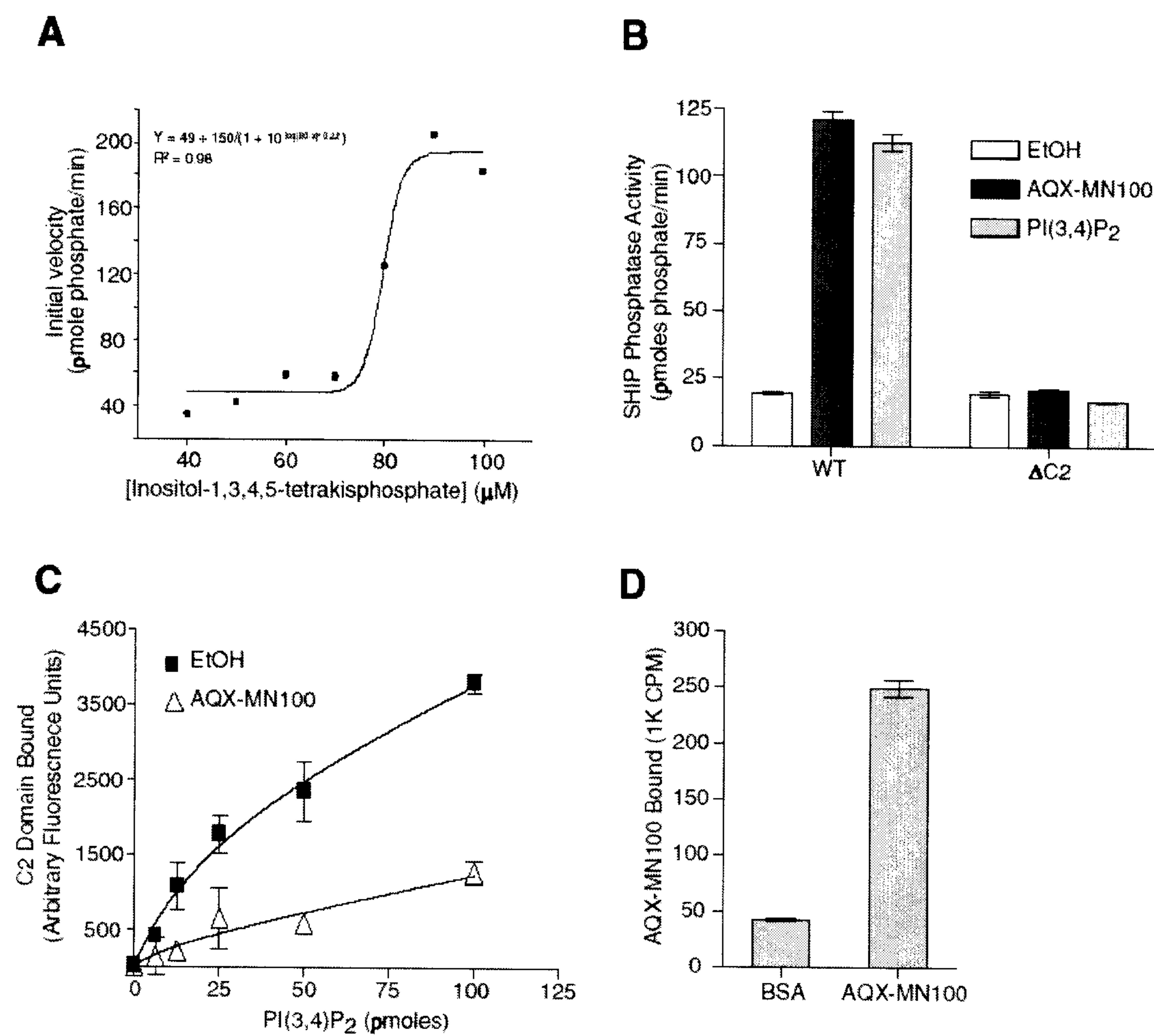
Fig. 11

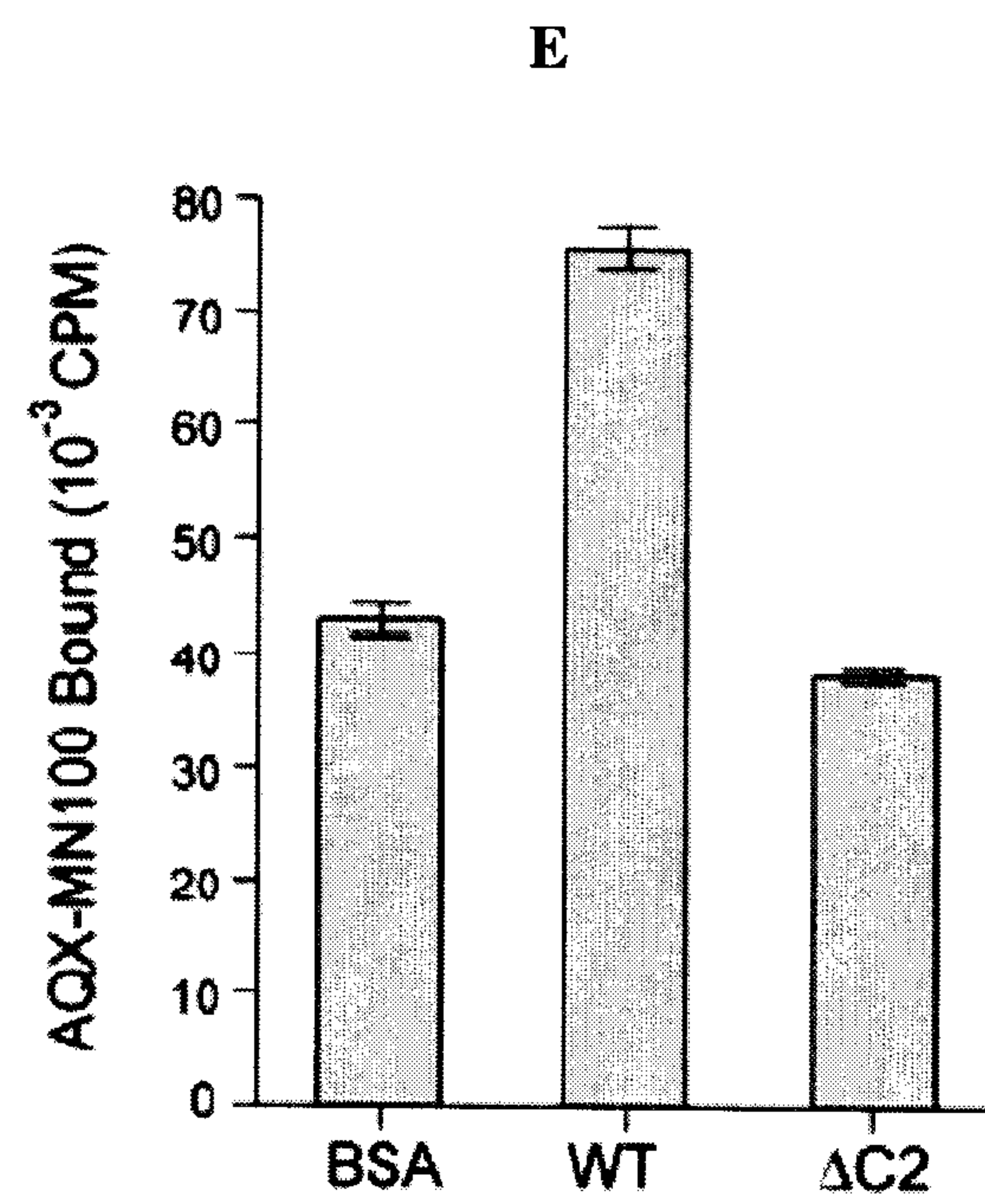
Fig. 11 (cont)

Fig. 12

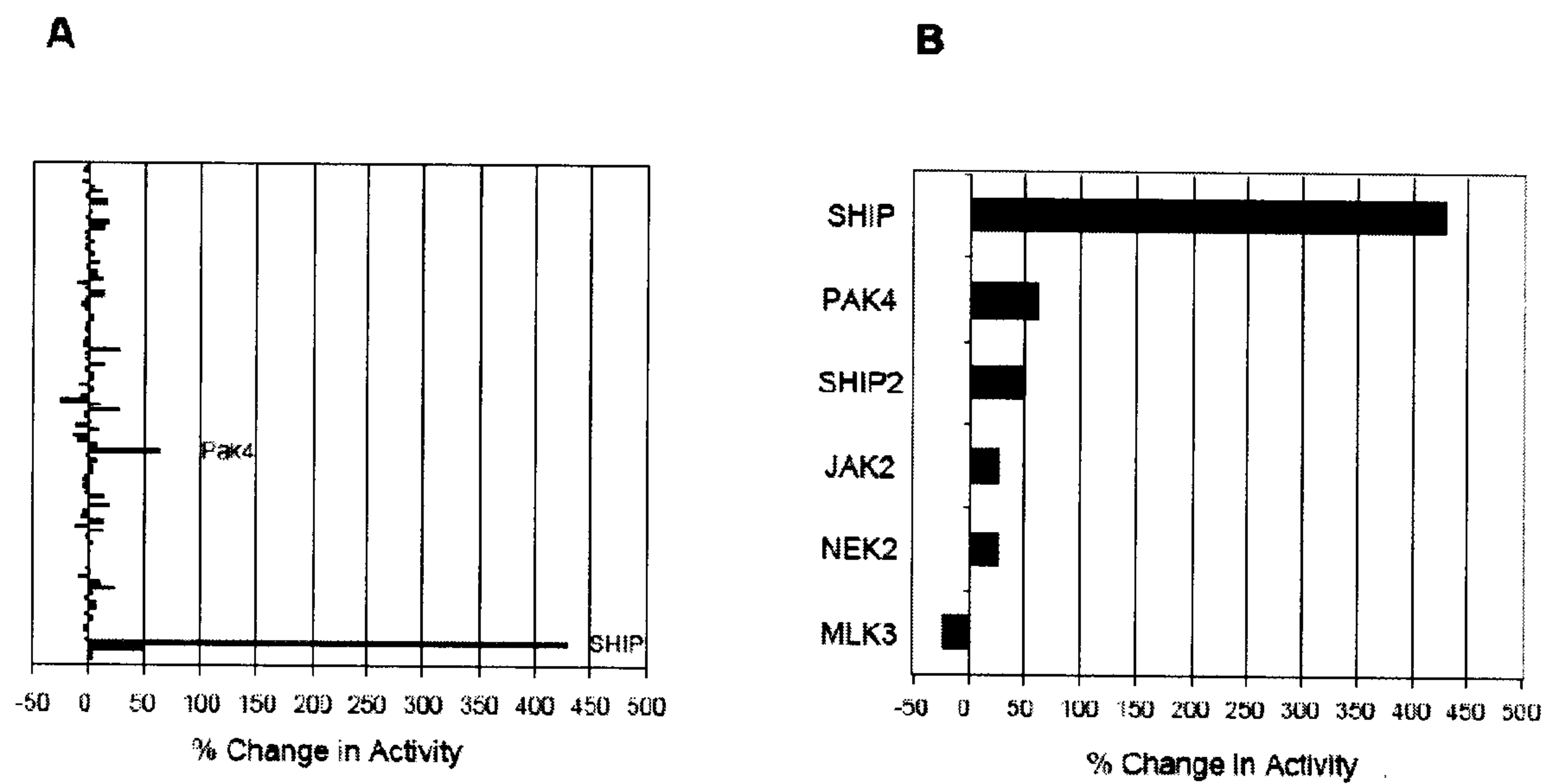


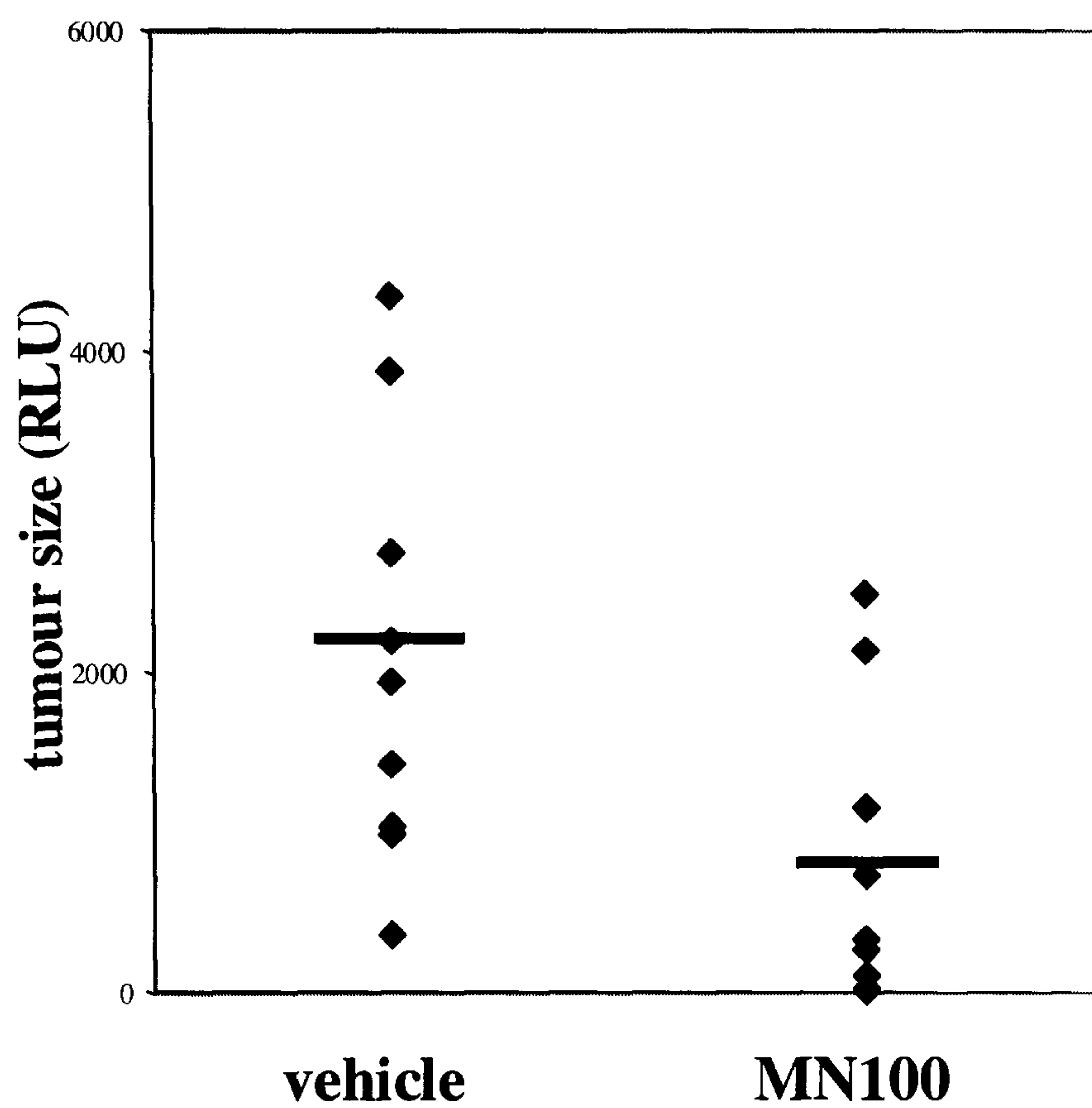
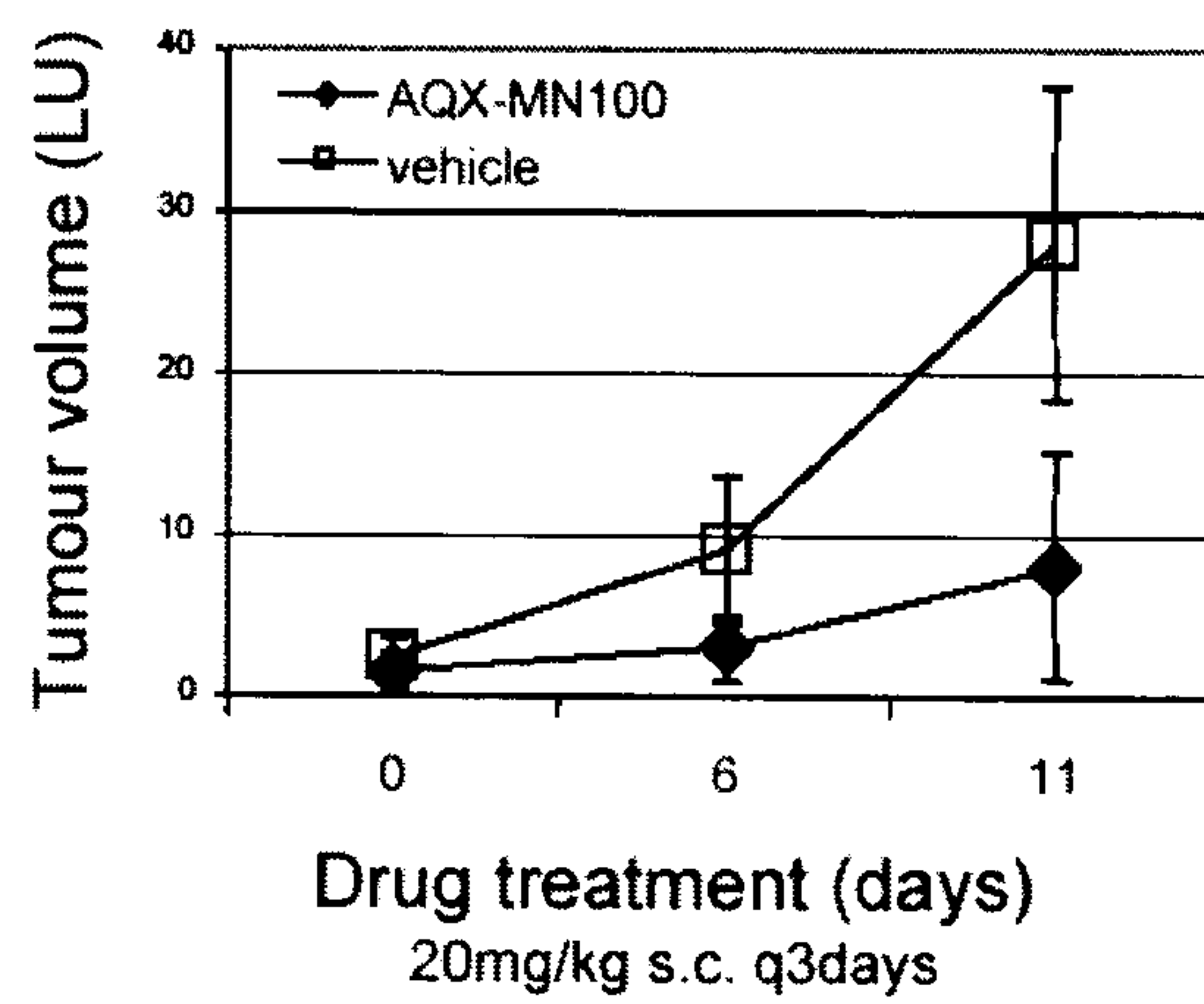
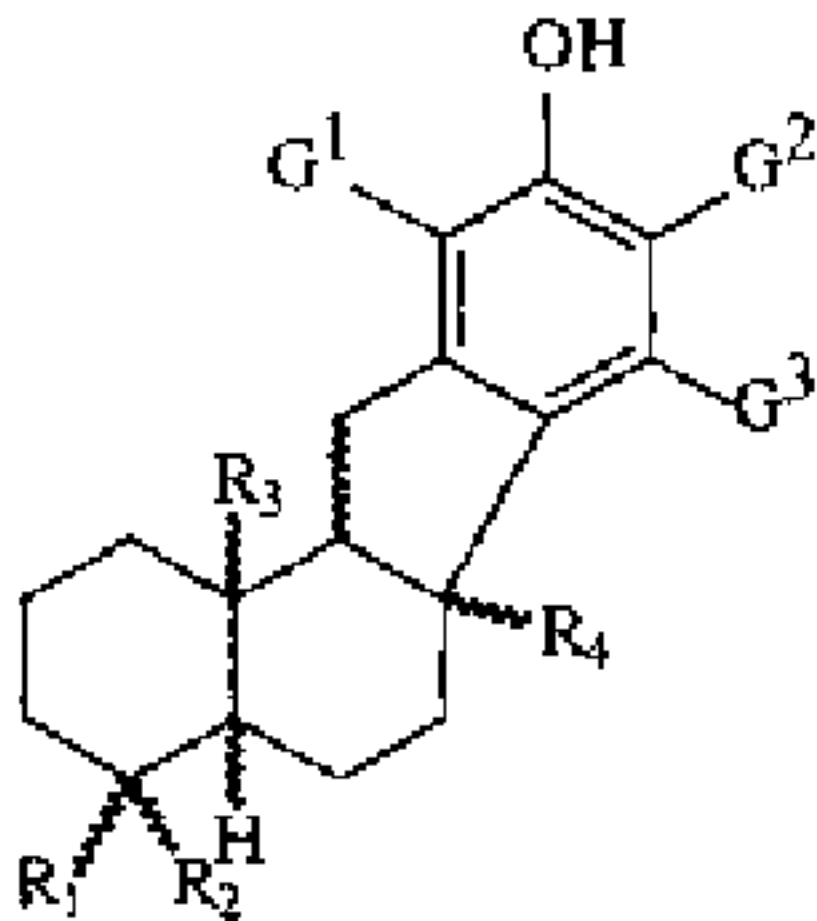
Fig. 13

Fig. 14



(I)