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(54) Title: NEUROACTIVE 13,24-CYCLO-18,21-DINORCHOLANES AND STRUCTURALLY-RELATED PENTACYCLIC STEROIDS

(57) Abstract: Novel pentacyclic steroids and pentacyclic D-homosteroids comprising: (i) the tetracyclic steroid ring system or tetracyclic D-homosteroid ring system, respectively; (ii) a C(3) substituent selected from the group consisting of (a) a hydroxyl or carboxyl in the "-configuration and (b) a sulfate or other negatively charged moiety; and (iii) a fused fifth ring, the fused fifth ring comprising a hydrogen bond acceptor, and (a) in the case of the pentacyclic steroid the C(13) and C(17) carbons, or (b) in the case of the pentacyclic D-homosteroid the C(13) and C(17a) carbons, having utility as anesthetics and in the treatment of disorders relating to GABA function and activity.

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NEUROACTIVE 13,24-CYCLO-18,21-DINORCHOLANES AND STRUCTURALLY RELATED PENTACYCLIC STEROIDS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Serial No. 60/474,152, filed May 29, 2003, which is incorporated herein by reference in its entirety.

[0002] This invention was made with Government support under NIH Grant # 5 PO1 GM47969 awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] The present invention is directed to novel pentacyclic steroids and novel pentacyclic D-homosteroids that have utility as anesthetics and in the treatment of disorders relating to GABA function and activity.

[0004] Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the central nervous system. GABA activates two types of receptors, the inotropic $GABA_A$ and the metabotropic $GABA_B$ receptor. Activation of the $GABA_B$ receptor by GABA causes hyperpolarization and a resultant inhibition of neurotransmitter release. The $GABA_A$ receptor subtype regulates neuronal excitability and rapid mood changes, such as anxiety, panic, and stress response. $GABA_A$ receptors are coupled to chloride ion channels; activation of the receptor induces increased inward chloride ion flux, resulting in membrane hyperpolarization and neuronal inhibition. Drugs that stimulate $GABA_A$ receptors, such as benzodiazepines and barbiturates, have anticonvulsive effects (by reducing neuronal excitability and raising the seizure threshold) as well as anxiolytic and anesthetic effects. Recently, the effect of steroids on $GABA_A$ receptors has been demonstrated. As a result, researchers are pursuing the discovery and synthesis of neuroactive steroids that act as anesthetics and/or serve to provide treatment for disorders related to GABA function.

[0005] In addition to anesthetic properties, neuroactive steroids may be used to treat disorders related to GABA function. For example, neuroactive steroids may be used as sedative-hypnotics. Progesterone, for example, exhibits

benzodiazepine-like actions, inducing reduced sleep latency and increased non-REM sleep with only small changes in slow wave and REM sleep. Further, systemic administration of GABA-enhancing steroids has demonstrated anticonvulsant effects in animals. In addition, drugs that enhance GABA responses are often used to treat anxiety in humans. Thus, it might be expected that GABA-potentiating steroids would exhibit anxiolytic effects. In addition to uses as sedative-hypnotics, anticonvulsants, and anxiolytics, neuroactive steroids may be used to treat depression. Accumulating evidence suggests that patients with major depression have decreased levels of GABAergic neurosteroids, and that certain treatments for depression alter levels of these steroids. Although GABA is not typically thought to play a critical role in the biology of depression, there is evidence that low GABAergic activity may predispose to mood disorders. Finally, inhibition of NMDA receptors and enhancement of $GABA_A$ receptors appear to play important roles in mediating the acute effects of ethanol in the nervous system. Recent studies suggest that GABAergic neurosteroids may be involved in some of the pharmacological effects of ethanol and that neuroactive steroids may be useful in treating ethanol withdrawal.

[0006] An alternative to developing GABAergic anesthetics is to focus on steroids that inhibit NMDA receptors. However, steroids that inhibit NMDA receptors often block $GABA_A$ receptors as well, resulting in complex effects on CNS excitability. Neuroactive steroids containing a 3β-sulfate group inhibit $GABA_A$ receptors non-competitively. These agents act by enhancing $GABA_A$ receptor desensitization and display different degrees of enantioselectivity. These compounds may be useful as memory enhancers and in reversing the anesthetic effects of compounds that potentiate GABA at $GABA_A$ receptors.

SUMMARY OF THE INVENTION

[0007] Among the various aspects of the present invention, therefore, is the provision of neuroactive 13,24-cyclo-18,21-dinorcholanes, and structurally related pentacyclic steroids and pentacyclic D-homosteroids, which potentiate the effects of GABA at $GABA_A$ receptors useful for producing anesthesia or treating disorders related to GABA function such as insomnia, mood disorders, convulsive disorders,

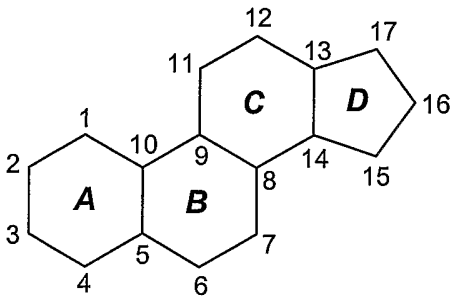
anxiety, or symptoms of ethanol withdrawal. Another aspect of the present invention is the provision of neuroactive 13,24-cyclo-18,21-dinorcholanes, and structurally related pentacyclic steroids and pentacyclic D-homosteroids that inhibit the effect of GABA at $GABA_A$ receptors useful for memory enhancement and reversing the effects of compounds that potentiate GABA at $GABA_A$ receptors.

[0008] Briefly, therefore, the present invention is directed to pentacyclic steroids and pentacyclic D-homosteroids comprising:

(i) the tetracyclic steroid ring system or tetracyclic D-homosteroid ring system, respectively;

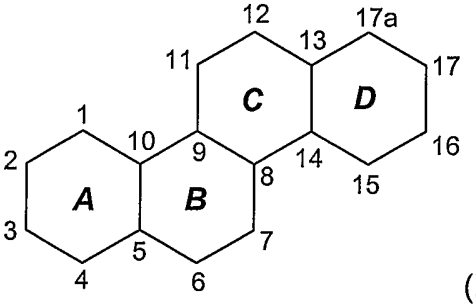
(ii) a C(3) substituent selected from the group consisting of (a) a hydroxyl or carboxyl in the α-configuration and (b) a sulfate or other negatively charged moiety; and

(iii) a fused fifth ring, the fused fifth ring comprising a hydrogen bond acceptor, and (a) in the case of the pentacyclic steroid the C(13) and C(17) carbons, or (b) in the case of the pentacyclic D-homosteroid the C(13) and C(17a) carbons, wherein the tetracyclic steroid ring system corresponds to Formula (I)



(I)

and the tetracyclic D-homosteroid ring system corresponds to Formula (II)



(II).

[0009] Other objects and features of the present invention will be in part apparent and in part pointed out hereafter.

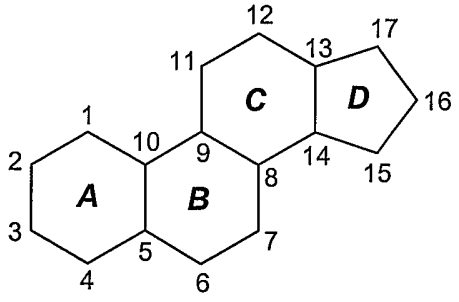
BRIEF DESCRIPTION OF THE DRAWINGS

[0010] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims and accompanying figures where:

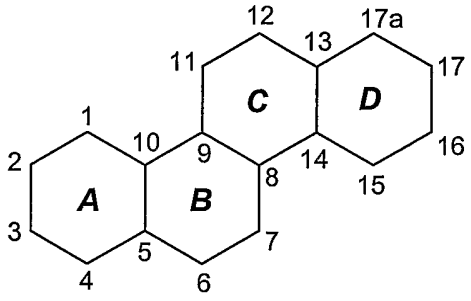
[0011] **Figure 1** shows two current traces (chloride currents) (Figure **1A** and **1B**) and two tables (Figure **1C** and **1D**) demonstrating the effect of cyclosteroids on electrophysiological responses to GABA in oocytes. In particular, Figure **1A** and Figure **1B** depict current traces for application of GABA to GABA receptors in the absence of cyclosteroids and in the presence of certain cyclosteroids; compound **35a** (Figure **1A**) and compound **35b** (Figure **1B**) and reference compound **1a** (Figure **1A**) and compound **1b** (Figure **1B**). Figure **1C** shows a table of chloride current versus specific compounds, which summarizes the effects of certain cyclosteroids tested at 500 nM against the response to 2 μM GABA alone (dotted line denotes the normalized response to GABA alone). Figure **1D** depicts a similar summary for the 5β-reduced series.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0012] In general, the present invention is directed to pentacyclic steroids and D-homosteroids. The pentacyclic steroids comprise the tetracyclic ring system of Foluma (I) and the pentacyclic D-homosteroids comprise the tetracyclic ring system of Formula (II):



(I)



(II)

In addition, the pentacyclic steroids comprise a fifth fused ring, sometimes identified herein as the "E" ring, the fifth fused ring comprising the carbon atoms at the C(13) and C(17) positions of Formula (I). Similarly, the pentacyclic D-homosteroids comprise a fifth fused ring, sometimes identified herein as the "E" ring comprising the carbon atoms at the C(13) and C(17a) positions. The pentacyclic steroids or pentacyclic D-homosteroids further comprise an E ring hydrogen bond acceptor.

[0013] In one embodiment, the compound is a pentacyclic steroid wherein each of rings A, B, C, and D of Formula (I) are saturated and the E ring is a 5- or 6-membered carbocyclic ring. Further, the E ring may be saturated. Alternatively, at least one pair of ring atoms of the E ring share a double bond. While the E ring may be fully saturated or partially unsaturated, the E ring hydrogen bond acceptor is selected from the group consisting of keto (=O), cyano (CN), acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond (that is, a carbon-carbon alkene bond between two E ring carbon atoms), and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Typically, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene ($=CH_2$), methoxy ($-OCH_3$), acetyl ($-C(O)CH_3$), =CHCN, $=CHC(O)CH_3$, and an E ring alkene bond. Additionally, the E ring may comprise substituents selected from the group consisting of halo and alkyl. Further, for any of the above variations, the hydroxyl or carboxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. For example, when the E ring is saturated and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, or $=CHC(O)CH_3$, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. Preferable linking groups include methylene, ethylene, methoxy, and ethoxy. Alternatively, when at least one pair of ring atoms of the E ring share a double bond and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, or an E ring alkene bond, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group.

[0014] In another embodiment, the compound is a pentacyclic steroid wherein at least one pair of ring atoms comprising rings A, B, C, and D of Formula (I) share a double bond and the E ring is a 5- or 6-membered carbocyclic ring. Further, the E ring may be saturated. Alternatively, at least one pair of ring atoms of the E ring share a double bond. While the E ring may be fully saturated or partially unsaturated, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Typically, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, and an E ring alkene bond. Additionally, the E ring may comprise substituents selected from the group consisting of halo and alkyl. Further, for any of the above variations, the hydroxyl or carboxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. For example, when the E ring is saturated and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, or $=CHC(O)CH_3$, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. Preferable linking groups include methylene, ethylene, methoxy, and ethoxy. Alternatively, when at least one pair of ring atoms of the E ring share a double bond and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, or an E ring alkene bond, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group.

[0015] In another embodiment, the compound is a pentacyclic D-homosteroid wherein each of rings A, B, C, and D of Formula (II) are saturated and the E ring is a 5- or 6-membered carbocyclic ring. Further, the E ring may be saturated. Alternatively, at least one pair of ring atoms of the E ring share a double bond. While the E ring may be fully saturated or partially unsaturated, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected

from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Typically, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, and an E ring alkene bond. Additionally, the E ring may comprise substituents selected from the group consisting of halo and alkyl. Further, for any of the above variations, the hydroxyl or carboxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. For example, when the E ring is saturated and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, or $=CHC(O)CH_3$, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. Preferable linking groups include methylene, ethylene, methoxy, and ethoxy. Alternatively, when at least one pair of ring atoms of the E ring share a double bond and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, or an E ring alkene bond, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group.

[0016] In yet another embodiment, the compound is a pentacyclic D-homosteroid wherein at least one pair of ring atoms comprising rings A, B, C, and D of Formula (II) share a double bond and the E ring is a 5- or 6-membered carbocyclic ring. Further, the E ring may be saturated. Alternatively, at least one pair of ring atoms of the E ring share a double bond. While the E ring may be fully saturated or partially unsaturated, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Typically, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, and an E ring alkene bond. Additionally, the E ring may comprise substituents selected from the group consisting of halo and alkyl. Further, for any of the above variations,

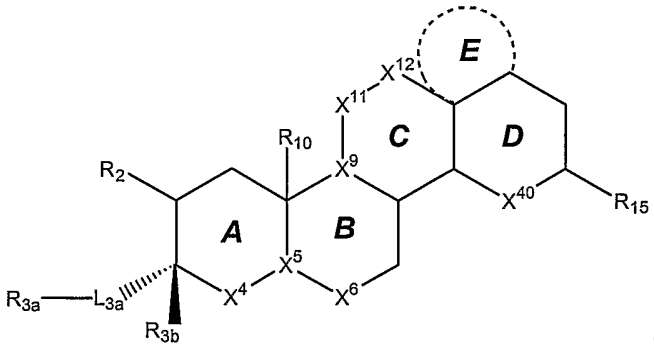
the hydroxyl or carboxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. For example, when the E ring is saturated and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, or $=CHC(O)CH_3$, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group. Preferable linking groups include methylene, ethylene, methoxy, and ethoxy. Alternatively, when at least one pair of ring atoms of the E ring share a double bond and the hydrogen bond acceptor is keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, or an E ring alkene bond, the hydroxyl in the α-configuration at C(3) may be directly bonded to the C(3) position or bonded via a linking group.

[0017] In a preferred embodiment, the compound is a pentacyclic steroid comprising the tetracyclic steroid ring system, Formula (I), wherein each of rings A, B, C, and D of Formula (I) are fully saturated, the E ring is a 6-membered carbocyclic ring, the E ring hydrogen bond acceptor is an E ring alkene bond or is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, and an epoxide; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl, and the hydroxyl or carboxyl group is directly bonded to C(3). Alternatively, the hydroxyl or carboxyl group is bonded to C(3) via a linking group as defined for any of the previous embodiments.

[0018] In another preferred embodiment, the compound is a pentacyclic steroid comprising the tetracyclic steroid ring system, Formula (I), wherein at least one pair of ring atoms comprising rings A, B, C, and D share a double bond, the E ring is a 6-membered carbocyclic ring, the E ring hydrogen bond acceptor is an E ring alkene bond or is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, and an epoxide; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl, and the hydroxyl or carboxyl group is directly bonded to C(3). Alternatively, the hydroxyl or carboxyl group is bonded to C(3) via a linking group as defined for any of the previous embodiments.

[0019] In another embodiment, the pentacyclic steroids or pentacyclic D-homosteroids comprise Formulae (I) or (II), respectively, and the steroids or D-homosteroids have the standard steroid or D-homosteroid stereochemical configuration (8β,9α,10β,13β,14α). In this embodiment, the hydrogen at C(5), if present, may be in either the α-configuration or the β-configuration.

[0020] In another embodiment, the pentacyclic steroids or pentacyclic D-homosteroids of the present invention correspond to Formula (A)



(A)

wherein

[0021] R_2 is selected from the group consisting of hydrogen, alkoxy, and substituted or unsubstituted morpholine;

[0022] R_{3a} is hydroxy or carboxyl;

[0023] R_{3b} is hydrogen, alkyl, alkenyl, or alkynyl optionally substituted with halo, hydroxy, or substituted or unsubstituted aryl;

[0024] R_5 is α- or β-hydrogen;

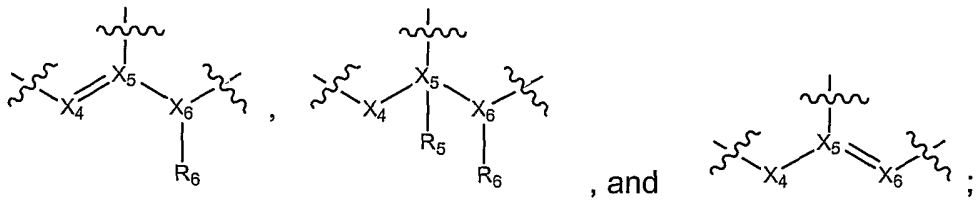
[0025] R_{10} is hydrogen or C_{1-4} alkyl;

[0026] R_{15} is hydrogen or oxo;

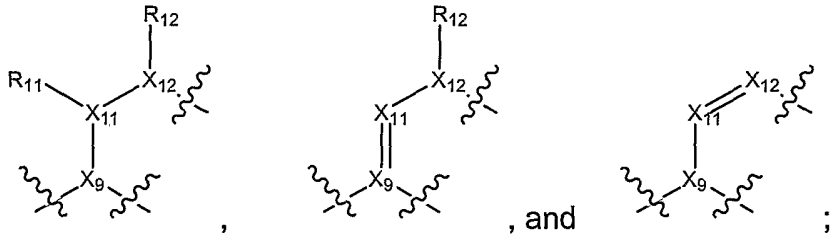
[0027] R_6, R_{11} and R_{12} are independently hydrogen or oxo;

[0028] L_{3a} is selected from the group consisting of a bond, C_{1-3} alkyl, heterosubstituted C_{1-3} alkyl, or alkoxy;

[0029] X_4-X_5-X_6 is selected from the group consisting of



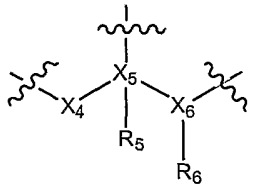
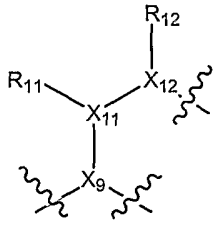
[0030] X_9-X_{11}-X_{12} is selected from the group consisting of



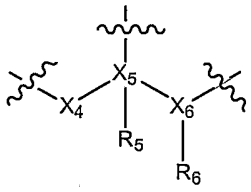
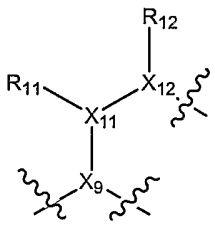
[0031] X_{40} is a bond or a carbon atom; and

[0032] E is a 5- or 6-membered carbocyclic ring, comprising a hydrogen bond acceptor.

[0033] In one embodiment where the compounds correspond to Formula (A), X_{40} is a bond. In this embodiment the carbocyclic ring, E, may be in the β-configuration in relation to the C and D rings. Further, the E ring hydrogen bond acceptor may be an E ring alkene bond or may be selected from the group consisting of keto, cyano, acyl, =CHX_1, -OX_2, -C(O)X_2, epoxide, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Preferably, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, =CHC(O)CH_3, and an E ring alkene bond. In certain preferred embodiments, the hydrogen bond acceptor is keto, cyano, or =CHCN. While the E ring may be as defined above for this embodiment, R_2 may be selected from the group consisting of hydrogen and substituted or unsubstituted morpholine. Preferably, R_2 is hydrogen. While the E ring and R_2 may be as defined above for this embodiment, L_{3a} may be a bond, methylene, ethylene, methoxy or

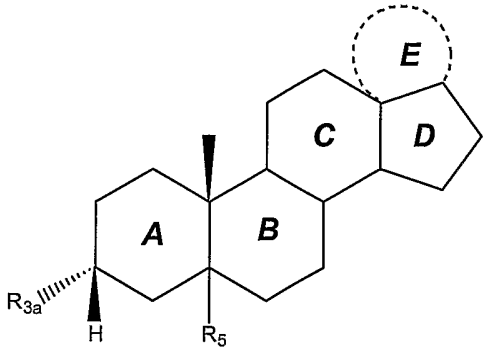
ethoxy and R_{3a} may be hydroxyl. Alternatively, L_{3a} may be a bond, methylene, ethylene, methoxy or ethoxy and R_{3a} may be carboxyl. L_{3a} is preferably a bond. In certain instances of this embodiment, X_4-X_5-X_6 may be  and X_9-X_{11}-X_{12} may be  . Further, R_{3b} may be hydrogen or ethynyl substituted with alkyl or optionally substituted aryl. R_{3b} is preferably hydrogen or ethynyl substituted with phenyl substituted with amino, dimethylamino, hydroxyl, carboxyl, or alkoxy. Still further, in this embodiment, R_{10} may be hydrogen or β-methyl.

[0034] In another embodiment where the compounds correspond to Formula (A), X_{40} is carbon. In this embodiment, the carbocyclic ring, E, may be in the *β*-configuration in relation to the C and D rings. Further, the E ring hydrogen bond acceptor may be an E ring alkene bond or may be selected from the group consisting of keto, cyano, acyl, =CHX_1, -OX_2, -$C(O)X_2$, epoxide, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Preferably, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, =$CHC(O)CH_3$, and an E ring alkene bond. In certain preferred embodiments, the hydrogen bond acceptor is keto, cyano, or =CHCN. While the E ring may be as defined above for this embodiment, R_2 may be selected from the group consisting of hydrogen and substituted or unsubstituted morpholine. Preferably, R_2 is hydrogen. While the E ring and R_2 may be as defined above for this embodiment, L_{3a} may be a bond, methylene, ethylene, methoxy or ethoxy and R_{3a} may be hydroxyl. Alternatively, L_{3a} may be a bond, methylene,

ethylene, methoxy or ethoxy and R_{3a} may be carboxyl. L_{3a} is preferably a bond. In certain instances of this embodiment, X_4-X_5-X_6 may be  and X_9-X_{11}-X_{12} may be  . Further, R_{3b} may be hydrogen or ethynyl substituted with alkyl or optionally substituted aryl. R_{3b} is preferably hydrogen or ethynyl substituted with phenyl substituted with amino, dimethylamino, hydroxyl, carboxyl, or alkoxy. Still further, in this embodiment, R_{10} may be hydrogen or β-methyl.

[0035] In a preferred embodiment, the compounds correspond to Formula (A), X_{40} is a bond or carbon, R_2 is hydrogen, R_{3b} is hydrogen, R_{10} is β-methyl, L_{3a} is a bond, and E is a 6-membered carbocyclic ring wherein the E ring hydrogen bond acceptor is an E ring alkene bond or is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. When the E ring hydrogen bond acceptor comprises an epoxide ring, the epoxide ring comprises two carbon atoms of the E ring, each in the alpha position relative to each other, and an oxygen atom.

[0036] In another embodiment of the present invention, the compounds correspond to Formula (B)



(B)

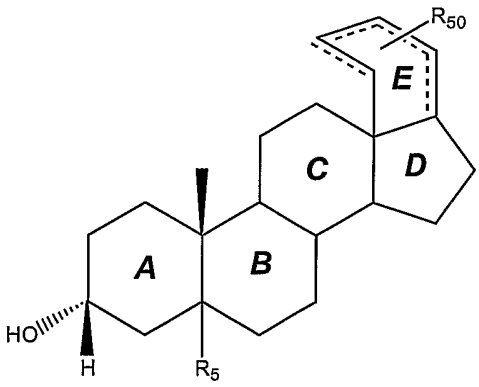
wherein

[0037] R_{3a} is hydroxyl or carboxyl;

[0038] R_5 is α- or β-hydrogen; and

[0039] E is as previously defined for any of the above embodiments.

[0040] In yet another embodiment, the compounds of the present invention correspond to Formula (C)



(C)

wherein

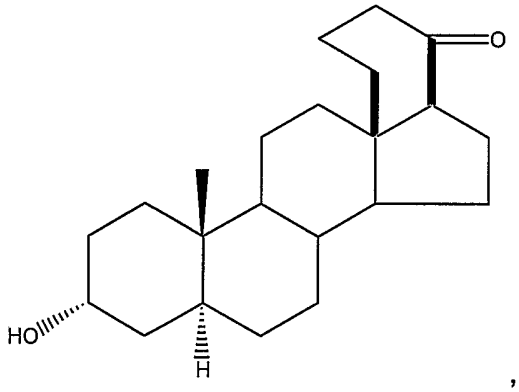
[0041] R_5 is α- or β-hydrogen;

[0042] R_{50} is selected from the group consisting of hydrogen, keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$ and an epoxide; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl, provided that when R_{50} is hydrogen, at least one pair of E ring atoms share a double bond; and

[0043] E is a 6-membered carbocyclic ring wherein the dashed lines represent optional double bonds provided E comprises no more than 2 double bonds and provided each carbon ring atom of E is sp^2 or sp^3 hybridized.

[0044] In one embodiment where the compounds correspond to Formula (C) and the E ring is in the β-configuration in relation to the C and D rings, R_5 may be α- or β-hydrogen and the E ring may be saturated or partially unsaturated. While the E ring and R_5 may be as defined above for this embodiment, R_{50} is typically selected from the group consisting of hydrogen, keto, cyano, methylene, methoxy, acetyl, =CHCN, and $=CHC(O)CH_3$. In a preferred embodiment, R_{50} is keto, cyano, or =CHCN.

[0045] In another embodiment where the compounds correspond to Formula (C), the compound is selected from the group consisting of



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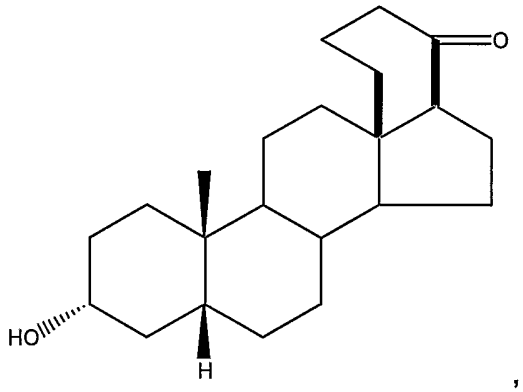

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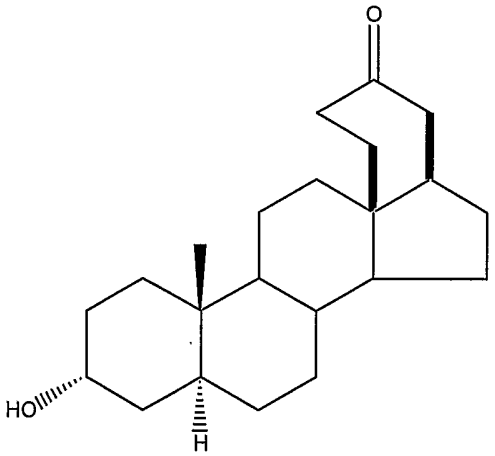

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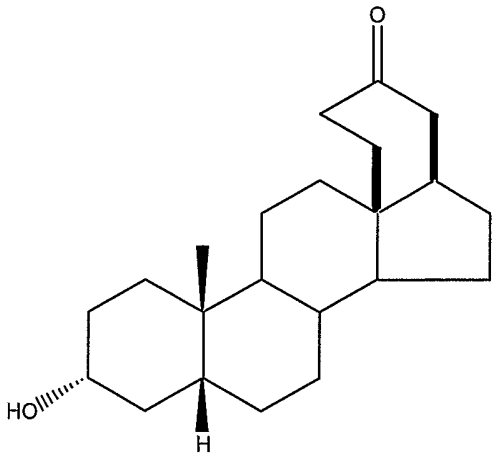

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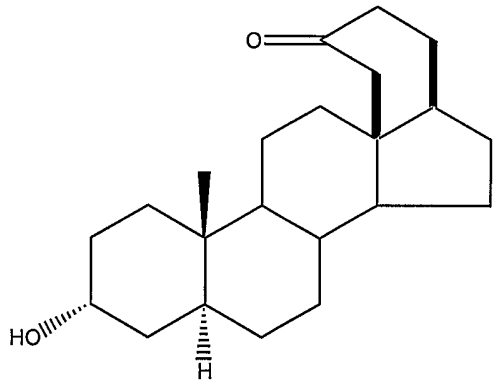
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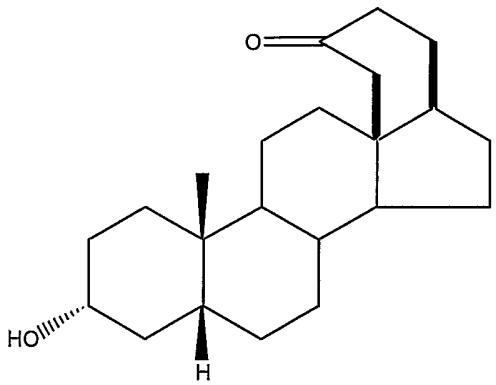
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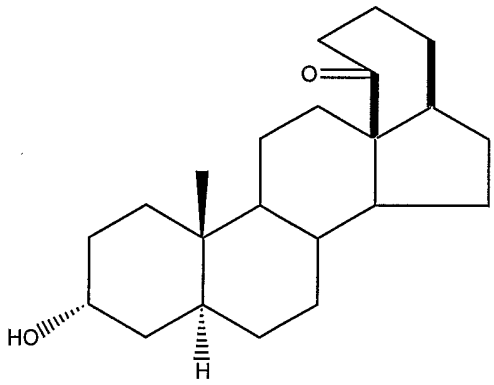
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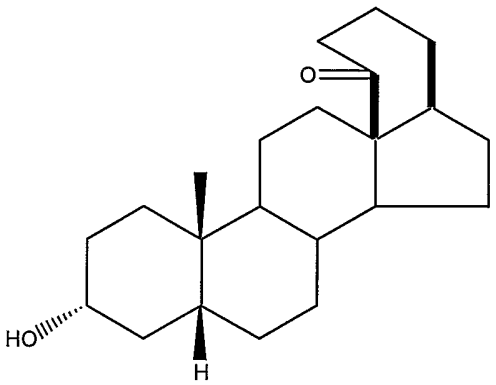
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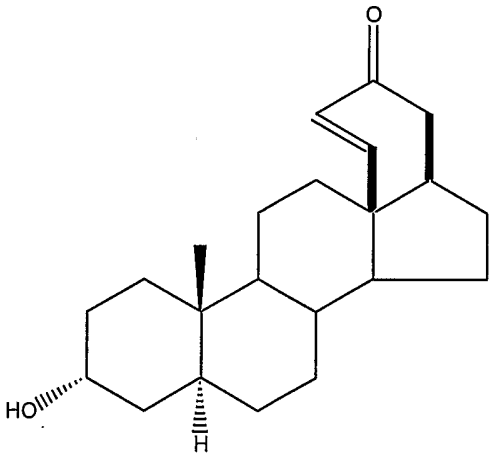
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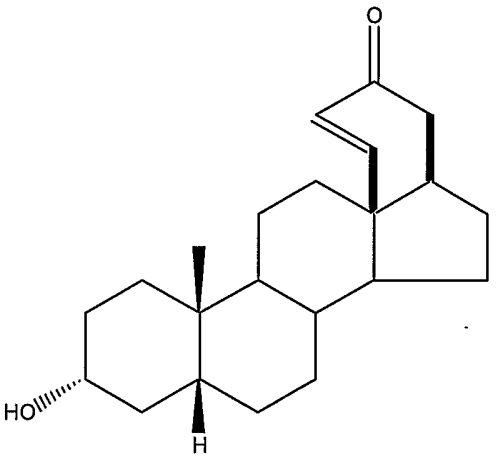
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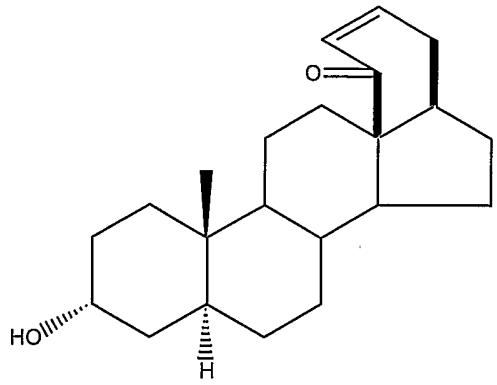
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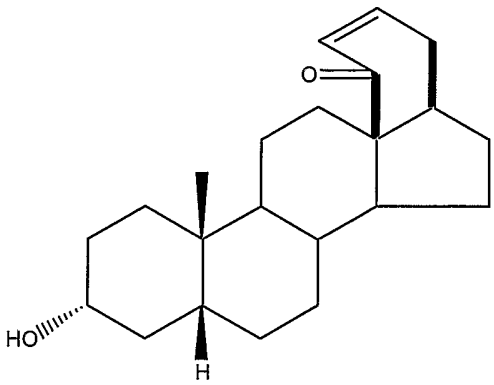
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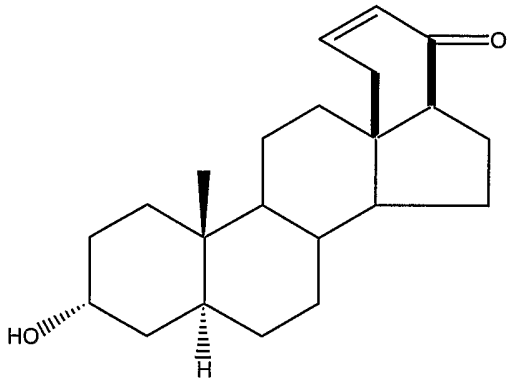
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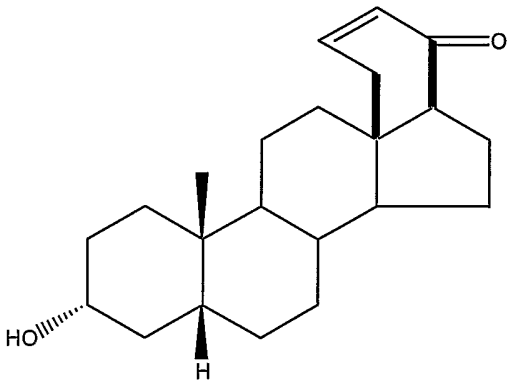
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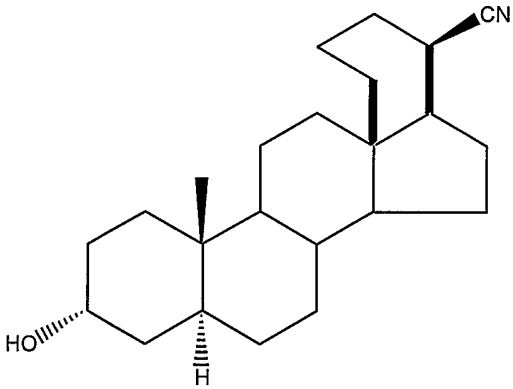
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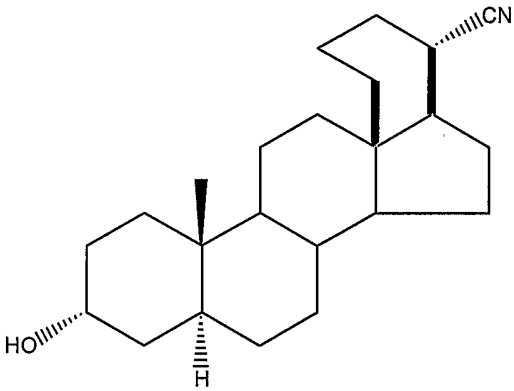
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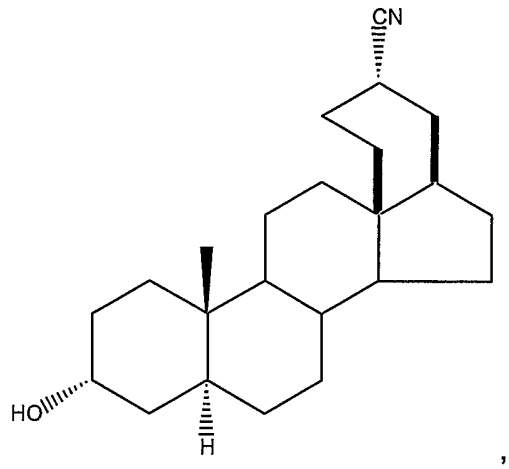
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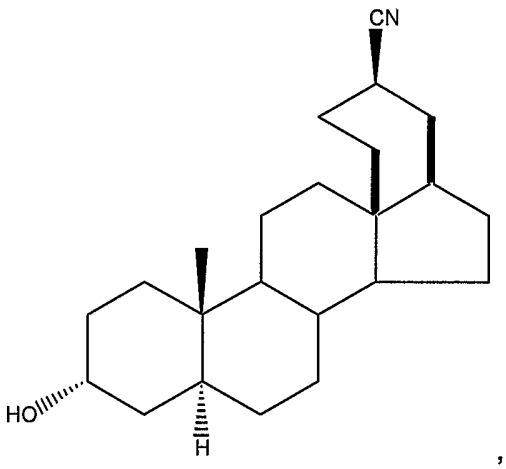
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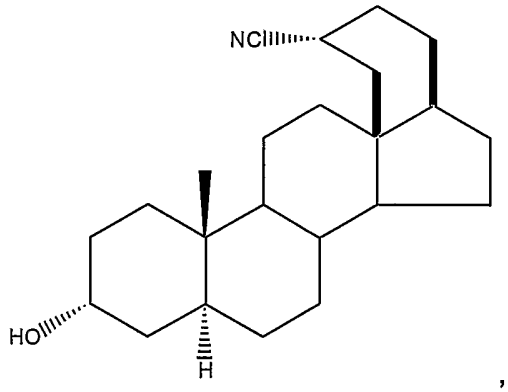
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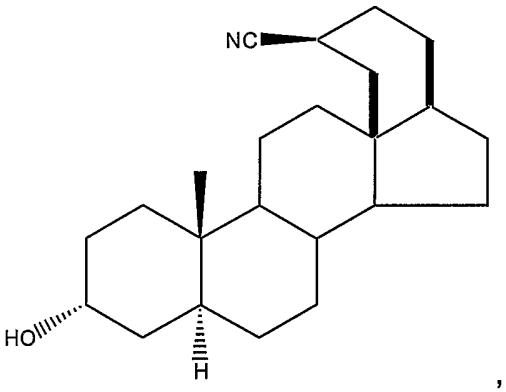
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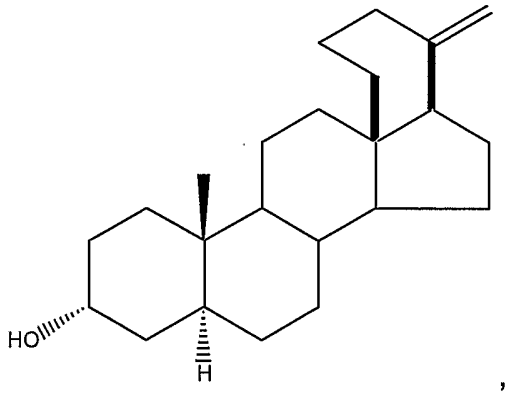
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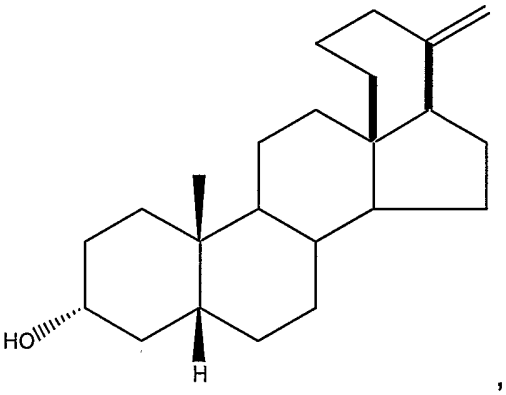
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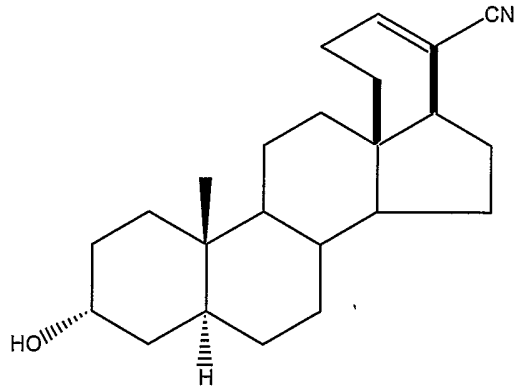
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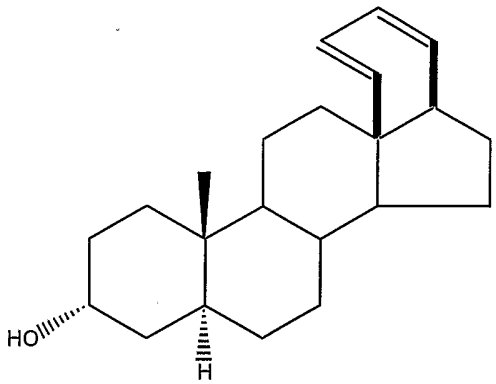
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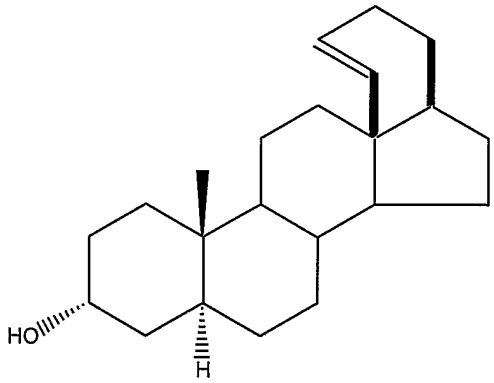
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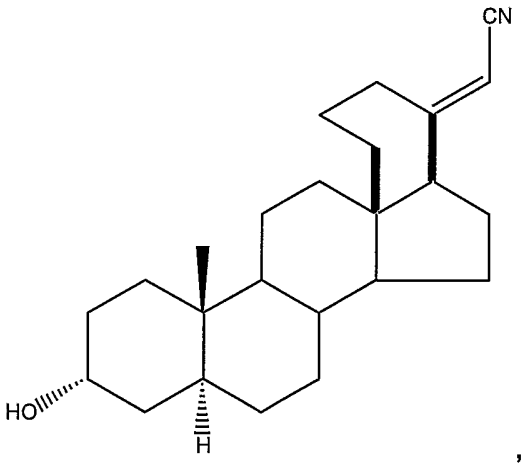
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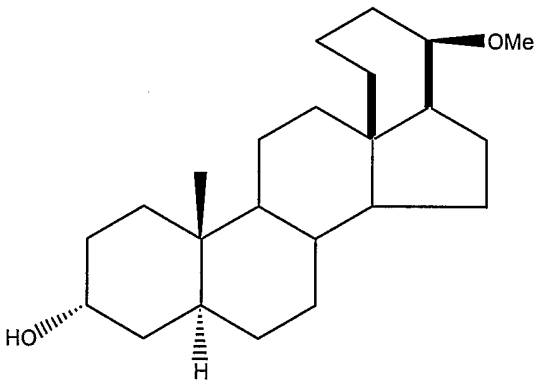
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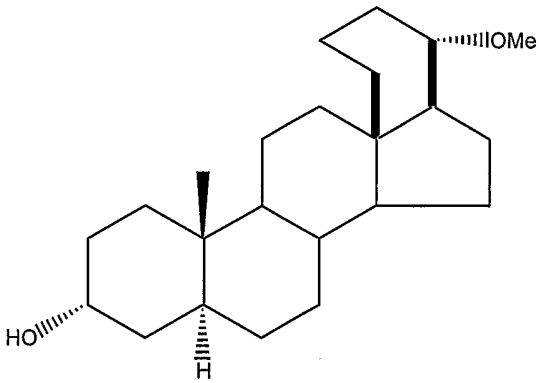
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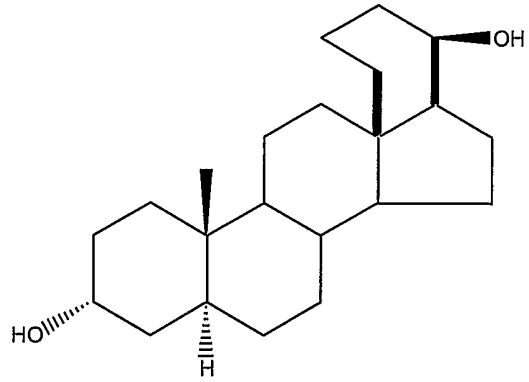
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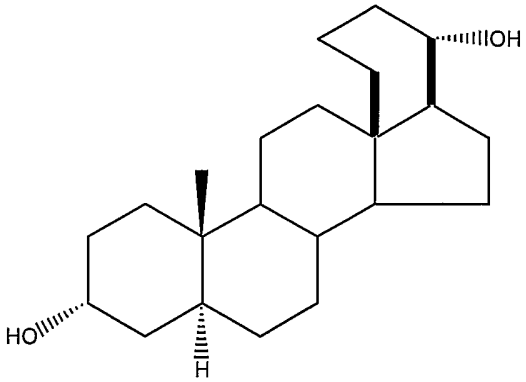
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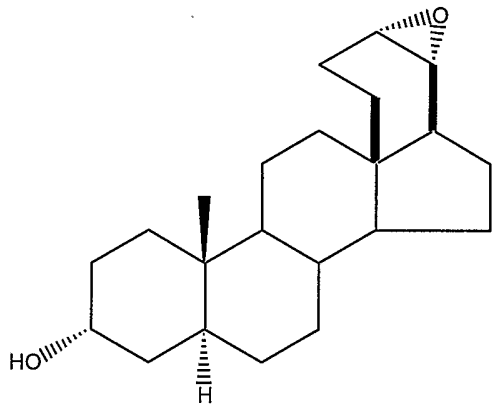
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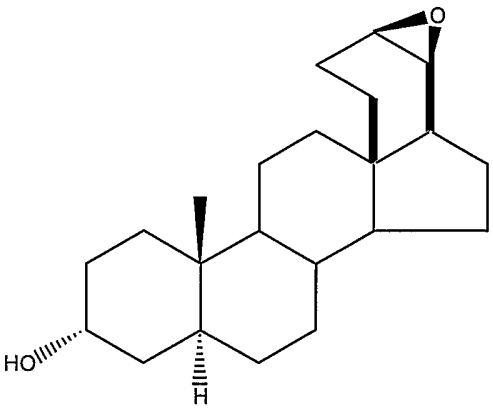
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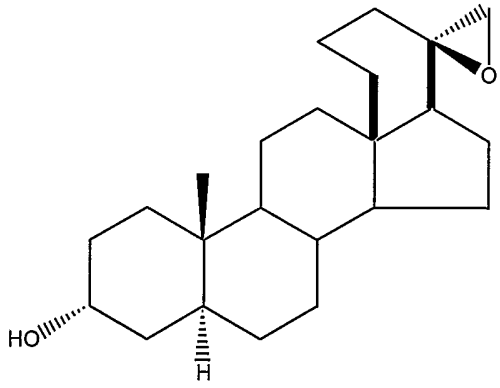
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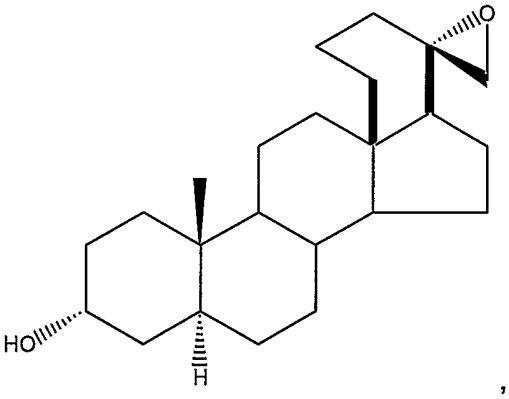
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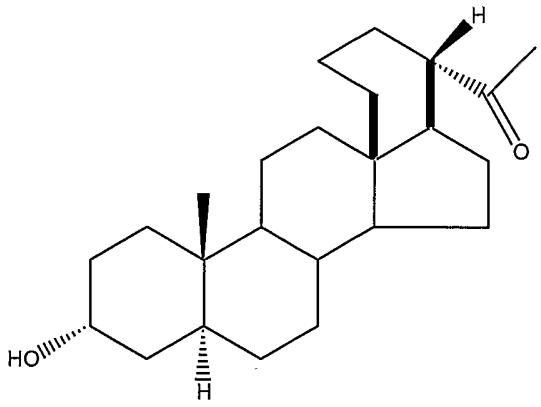

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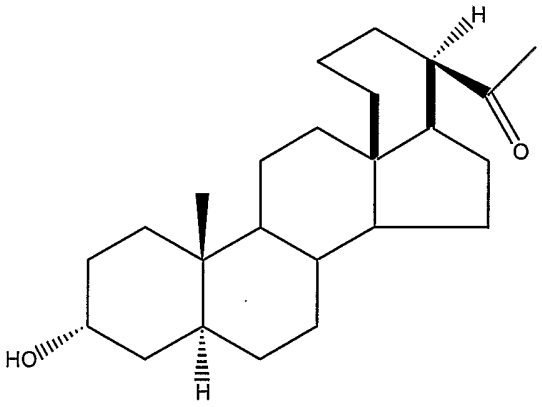

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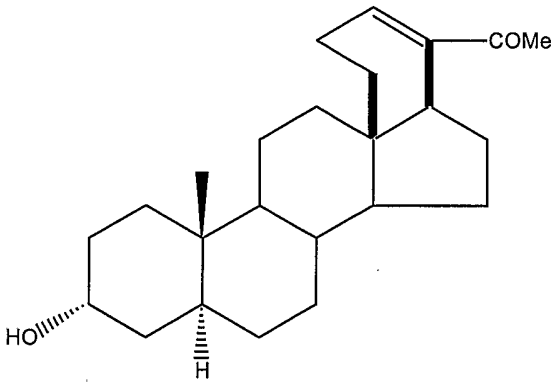

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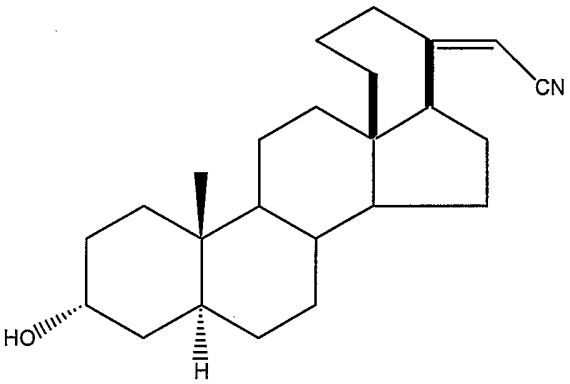

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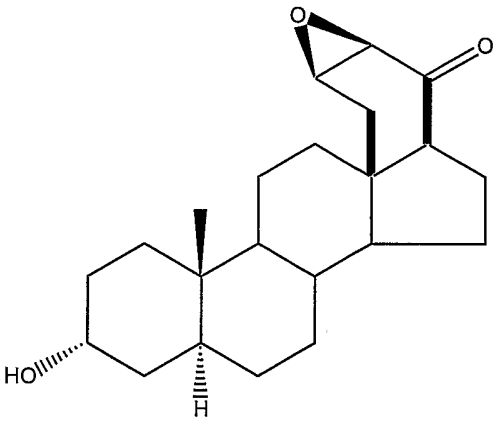

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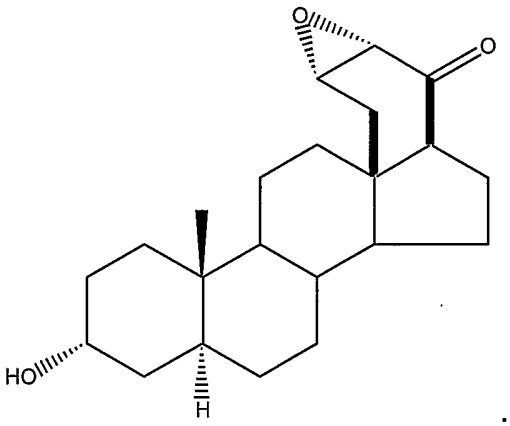

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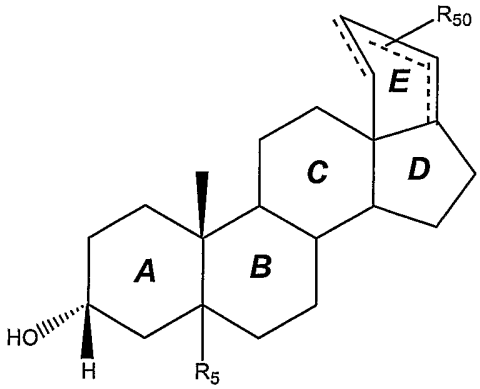
[0046] In another embodiment, the compounds of the present invention correspond to Formula (D)



(D)

wherein

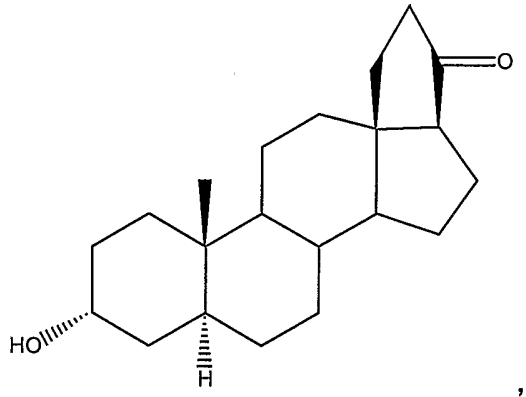
[0047] R_5 is α- or β-hydrogen;

[0048] R_{50} is selected from the group consisting of hydrogen, keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, and an epoxide; where X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl, provided that when R_{50} is hydrogen, at least one pair of E ring atoms share a double bond; and

[0049] E is a 5-membered carbocyclic ring wherein the dashed lines represent optional double bonds provided E comprises no more than 2 double bonds and each carbon ring atom of E ring is sp^2 or sp^3 hybridized.

[0050] In one embodiment where the compounds correspond to Formula (D), the E ring is in the β-configuration in relation to the C and D rings. In this embodiment, R_5 may be α- or β-hydrogen and the E ring may be saturated or partially unsaturated. While the E ring and R_5 may be as defined above for this embodiment, R_{50} is typically selected from the group consisting of hydrogen, keto, cyano, methylene, methoxy, acetyl, =CHCN, and $=CHC(O)CH_3$. In a preferred embodiment, R_{50} is =O, CN, or =CHCN.

[0051] In another embodiment where the compounds correspond to Formula (D), the compound is selected from the group consisting of



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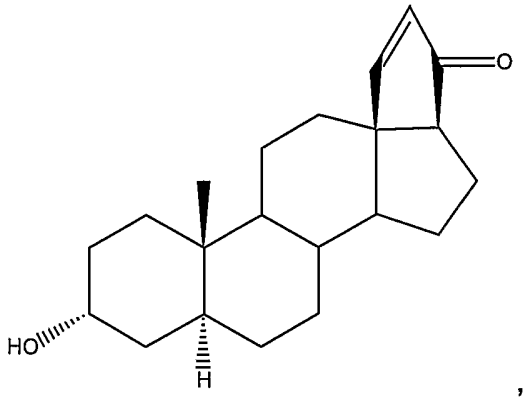

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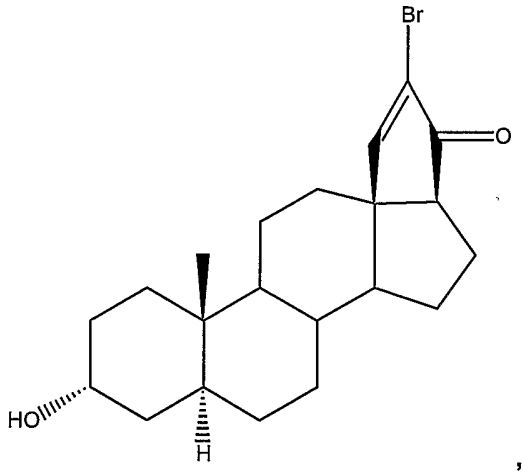

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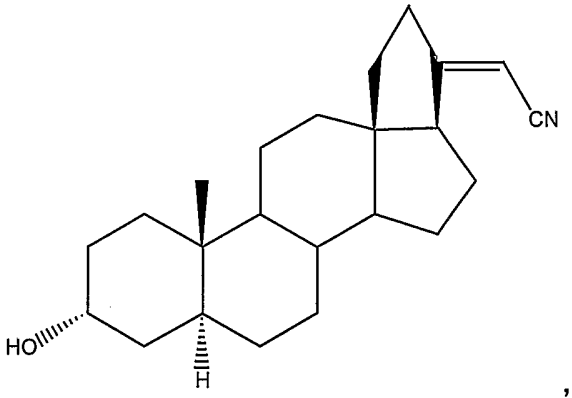

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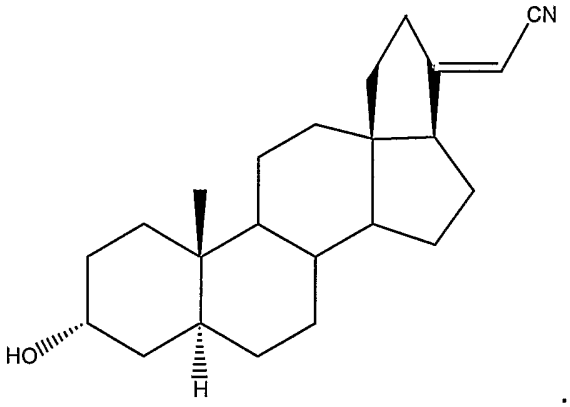
and



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[0052] In still another embodiment, the compounds of the present invention are pentacyclic steroids and pentacyclic D-homosteroids comprising:

(i) the tetracyclic steroid ring system, Formula (I) or tetracyclic D-homosteroid ring system, Formula (II), respectively;

(ii) a negatively charged substituent at physiological pH at C(3); and

(iii) a fused fifth ring, the fused fifth ring comprising a hydrogen bond acceptor, and (a) in the case of the pentacyclic steroid the C(13) and C(17) carbons, or (b) in the case of the pentacyclic D-homosteroid the C(13) and C(17a) carbons. In this embodiment, the negatively charged substituent at C(3) is typically selected from the group consisting of sulfate, carboxylate, phosphate, phosphonate, and combinations thereof, while rings A, B, C, D, and the fifth fused ring (the "E" ring) and all substituents are as previously defined for any of the above embodiments. In particular, each ring A, B, C, and D may be saturated or, alternatively, at least two of the ring atoms share a double bond. Further, the E ring may be a 5- or 6-membered carbocyclic fully saturated ring or, alternatively, at least one pair of ring atoms of the E ring share a double bond. As described previously for other embodiments, the E ring hydrogen bond acceptor may be selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; where X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. Typically, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, or an E ring alkene bond.

[0053] In a preferred embodiment, the compound is a pentacyclic steroid comprising the tetracyclic steroid ring system, Formula (I), wherein each of rings A, B, C, and D of Formula (I) are saturated or, alternatively, at least one pair of ring atoms comprising rings A, B, C, and D of Formula (I) share a double bond, the E ring is a 6-membered carbocyclic ring, the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; where X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted

hydrocarbyl, and acyl, and the negatively charged substituent at C(3) is a sulfate or carboxylate group.

[0054] Steroids having the general Formula (A) may be obtained by the methods described in the examples. The starting material for the compounds of Formula (A) may be obtained using the reaction conditions set forth in Example 1 or a similar process. Generally, cyanoketone (compound **8a** of Example 1) was prepared from the 20-ketosteroid **6a** (Example 1) in two steps (40%). The 20-keto group was then protected with ethylene glycol to give compound **9a** (83%), and this compound was reacted with DIBALH in toluene at room temperature to yield aldehyde **10a**. As described for the preparation of a different 13,24-cyclo-18,21-dinorchol-22-en-20-one, treatment of aldehyde **10a** with aqueous HCl removed the ketal protecting group and affected a Robinson annulation to yield the cyclosteroid **11a** (57% from steroid **9a**). Hydrogenation of the 22-en-20-one **11a** gave the 20-one **2a** (91%).

[0055] The compounds of Formula (A) of the present invention are useful for potentiating GABA at $GABA_A$ receptors thereby inducing anesthesia or treating disorders related to GABA function (i.e., insomnia, mood disorders, convulsive disorders, anxiety or symptoms of ethanol withdrawal) in mammals, including humans, and are preferably administered in the form of a pharmaceutical composition comprising an effective amount of a compound of the instant invention in combination with at least one pharmaceutically or pharmacologically acceptable carrier. The carrier, also known in the art as an excipient, vehicle, auxiliary, adjuvant, or diluent, is any substance that is pharmaceutically inert, confers a suitable consistency or form to the composition, and does not diminish the therapeutic efficacy of the compounds. The carrier is "pharmaceutically or pharmacologically acceptable" if it does not produce an adverse, allergic or other untoward reaction when administered to a mammal or human, as appropriate.

[0056] The pharmaceutical compositions containing the compounds of the present invention may be formulated in any conventional manner. Proper formulation is dependent upon the route of administration chosen. The compositions of the invention can be formulated for any route of administration so long as the

target tissue is available via that route. Suitable routes of administration include, but are not limited to, oral, parenteral (e.g., intravenous, intraarterial, subcutaneous, rectal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intraperitoneal, or intrasternal), topical (nasal, transdermal, intraocular), intravesical, intrathecal, enteral, pulmonary, intralymphatic, intracavital, vaginal, transurethral, intradermal, aural, intramammary, buccal, orthotopic, intratracheal, intralesional, percutaneous, endoscopical, transmucosal, sublingual and intestinal administration.

[0057] Pharmaceutically acceptable carriers for use in the compositions of the present invention are well known to those of ordinary skill in the art and are selected based upon a number of factors: the particular compound used, and its concentration, stability and intended bioavailability; the disease, disorder or condition being treated with the composition; the subject, its age, size and general condition; and the route of administration. Suitable carriers are readily determined by one of ordinary skill in the art (see, for example, J. G. Nairn, in: Remington's Pharmaceutical Science (A. Gennaro, ed.), Mack Publishing Co., Easton, Pa., (1985), pp. 1492-1517, the contents of which are incorporated herein by reference).

[0058] The compositions are preferably formulated as tablets, dispersible powders, pills, capsules, gelcaps, caplets, gels, liposomes, granules, solutions, suspensions, emulsions, syrups, elixirs, troches, dragees, lozenges, or any other dosage form that can be administered orally. Techniques and compositions for making oral dosage forms useful in the present invention are described in the following references: 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, Editors, 1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition (1976).

[0059] The compositions of the invention for oral administration comprise an effective amount of a compound of the invention in a pharmaceutically acceptable carrier. Suitable carriers for solid dosage forms include sugars, starches, and other conventional substances including lactose, talc, sucrose, gelatin, carboxymethylcellulose, agar, mannitol, sorbitol, calcium phosphate, calcium carbonate, sodium carbonate, kaolin, alginic acid, acacia, corn starch, potato starch, sodium saccharin, magnesium carbonate, tragacanth, microcrystalline cellulose,

colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, and stearic acid. Further, such solid dosage forms may be uncoated or may be coated by known techniques; e.g., to delay disintegration and absorption.

[0060] The compounds of the present invention are also preferably formulated for parenteral administration, e.g., formulated for injection via intravenous, intraarterial, subcutaneous, rectal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intraperitoneal, or intrasternal routes. The compositions of the invention for parenteral administration comprise an effective amount of the compound in a pharmaceutically acceptable carrier. Dosage forms suitable for parenteral administration include solutions, suspensions, dispersions, emulsions or any other dosage form that can be administered parenterally. Techniques and compositions for making parenteral dosage forms are known in the art.

[0061] Suitable carriers used in formulating liquid dosage forms for oral or parenteral administration include nonaqueous, pharmaceutically-acceptable polar solvents such as oils, alcohols, amides, esters, ethers, ketones, hydrocarbons and mixtures thereof, as well as water, saline solutions, dextrose solutions (e.g., DW5), electrolyte solutions, or any other aqueous, pharmaceutically acceptable liquid.

[0062] Suitable nonaqueous, pharmaceutically-acceptable polar solvents include, but are not limited to, alcohols (e.g., α-glycerol formal, β-glycerol formal, 1, 3-butyleneglycol, aliphatic or aromatic alcohols having 2-30 carbon atoms such as methanol, ethanol, propanol, isopropanol, butanol, t-butanol, hexanol, octanol, amylene hydrate, benzyl alcohol, glycerin (glycerol), glycol, hexylene glycol, tetrahydrofurfuryl alcohol, lauryl alcohol, cetyl alcohol, or stearyl alcohol, fatty acid esters of fatty alcohols such as polyalkylene glycols (e.g., polypropylene glycol, polyethylene glycol), sorbitan, sucrose and cholesterol); amides (e.g., dimethylacetamide (DMA), benzyl benzoate DMA, dimethylformamide, N-(β-hydroxyethyl)-lactamide, N, N-dimethylacetamide, 2-pyrrolidinone, 1-methyl-2-pyrrolidinone, or polyvinylpyrrolidone); esters (e.g., 1-methyl-2-pyrrolidinone, 2-pyrrolidinone, acetate esters such as monoacetin, diacetin, and triacetin, aliphatic or aromatic esters such as ethyl caprylate or octanoate, alkyl oleate, benzyl benzoate, benzyl acetate, dimethylsulfoxide (DMSO), esters of glycerin such as mono, di, or tri-

glyceryl citrates or tartrates, ethyl benzoate, ethyl acetate, ethyl carbonate, ethyl lactate, ethyl oleate, fatty acid esters of sorbitan, fatty acid derived PEG esters, glyceryl monostearate, glyceride esters such as mono, di, or tri-glycerides, fatty acid esters such as isopropyl myristrate, fatty acid derived PEG esters such as PEG-hydroxyoleate and PEG-hydroxystearate, N-methyl pyrrolidinone, pluronic 60, polyoxyethylene sorbitol oleic polyesters such as poly(ethoxylated)$_{30-60}$ sorbitol poly(oleate)$_{2-4}$, poly(oxyethylene)$_{15-20}$ monooleate, poly(oxyethylene)$_{15-20}$ mono 12-hydroxystearate, and poly(oxyethylene)$_{15-20}$ mono ricinoleate, polyoxyethylene sorbitan esters such as polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate, polyoxyethylene-sorbitan monostearate, and Polysorbate® 20, 40, 60 or 80 from ICI Americas, Wilmington, DE, polyvinylpyrrolidone, alkyleneoxy modified fatty acid esters such as polyoxyl 40 hydrogenated castor oil and polyoxyethylated castor oils (e.g., Cremophor® EL solution or Cremophor® RH 40 solution), saccharide fatty acid esters (i.e., the condensation product of a monosaccharide (e.g., pentoses such as ribose, ribulose, arabinose, xylose, lyxose and xylulose, hexoses such as glucose, fructose, galactose, mannose and sorbose, trioses, tetroses, heptoses, and octoses), disaccharide (e.g., sucrose, maltose, lactose and trehalose) or oligosaccharide or mixture thereof with a C_4-C_{22} fatty acid(s)(e.g., saturated fatty acids such as caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid and stearic acid, and unsaturated fatty acids such as palmitoleic acid, oleic acid, elaidic acid, erucic acid and linoleic acid)), or steroidal esters); alkyl, aryl, or cyclic ethers having 2-30 carbon atoms (e.g., diethyl ether, tetrahydrofuran, dimethyl isosorbide, diethylene glycol monoethyl ether); glycofurol (tetrahydrofurfuryl alcohol polyethylene glycol ether); ketones having 3-30 carbon atoms (e.g., acetone, methyl ethyl ketone, methyl isobutyl ketone); aliphatic, cycloaliphatic or aromatic hydrocarbons having 4-30 carbon atoms (e.g., benzene, cyclohexane, dichloromethane, dioxolanes, hexane, n-decane, n-dodecane, n-hexane, sulfolane, tetramethylenesulfon, tetramethylenesulfoxide, toluene, dimethylsulfoxide (DMSO), or tetramethylenesulfoxide); oils of mineral, vegetable, animal, essential or synthetic origin (e.g., mineral oils such as aliphatic or wax-based hydrocarbons, aromatic

hydrocarbons, mixed aliphatic and aromatic based hydrocarbons, and refined paraffin oil, vegetable oils such as linseed, tung, safflower, soybean, castor, cottonseed, groundnut, rapeseed, coconut, palm, olive, corn, corn germ, sesame, persic and peanut oil and glycerides such as mono-, di- or triglycerides, animal oils such as fish, marine, sperm, cod-liver, haliver, squalene, squalane, and shark liver oil, oleic oils, and polyoxyethylated castor oil); alkyl or aryl halides having 1-30 carbon atoms and optionally more than one halogen substituent; methylene chloride; monoethanolamine; petroleum benzine; trolamine; omega-3 polyunsaturated fatty acids (e.g., alpha-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, or docosahexaenoic acid); polyglycol ester of 12-hydroxystearic acid and polyethylene glycol (Solutol® HS-15, from BASF, Ludwigshafen, Germany); polyoxyethylene glycerol; sodium laurate; sodium oleate; or sorbitan monooleate.

[0063] Other pharmaceutically acceptable solvents for use in the invention are well known to those of ordinary skill in the art, and are identified in The Handbook of Pharmaceutical Excipients, (American Pharmaceutical Association, Washington, D.C., and The Pharmaceutical Society of Great Britain, London, England, 1968), Modern Pharmaceutics, (G. Banker et al., eds., 3d ed.)(Marcel Dekker, Inc., New York, New York, 1995), The Pharmacological Basis of Therapeutics, (Goodman & Gilman, McGraw Hill Publishing), Pharmaceutical Dosage Forms, (H. Lieberman et al., eds.,)(Marcel Dekker, Inc., New York, New York, 1980), Remington's Pharmaceutical Sciences (A. Gennaro, ed., 19th ed.)(Mack Publishing, Easton, PA, 1995), The United States Pharmacopeia 24, The National Formulary 19, (National Publishing, Philadelphia, PA, 2000), A.J. Spiegel et al., and Use of Nonaqueous Solvents in Parenteral Products, J. of Pharm. Sciences, Vol. 52, No. 10, pp. 917-927 (1963).

[0064] Preferred solvents include oils rich in triglycerides, for example, safflower oil, soybean oil or mixtures thereof, and alkyleneoxy modified fatty acid esters such as polyoxyl 40 hydrogenated castor oil and polyoxyethylated castor oils (e.g., Cremophor® EL solution or Cremophor® RH 40 solution). Commercially available triglycerides include Intralipid® emulsified soybean oil (Kabi-Pharmacia

Inc., Stockholm, Sweden), Nutralipid ® emulsion (McGaw, Irvine, California), Liposyn® II 20% emulsion (a 20% fat emulsion solution containing 100 mg safflower oil, 100 mg soybean oil, 12 mg egg phosphatides, and 25 mg glycerin per ml of solution; Abbott Laboratories, Chicago, Illinois), Liposyn® III 2% emulsion (a 2% fat emulsion solution containing 100 mg safflower oil, 100 mg soybean oil, 12 mg egg phosphatides, and 25 mg glycerin per ml of solution; Abbott Laboratories, Chicago, Illinois), natural or synthetic glycerol derivatives containing the docosahexaenoyl group at levels between 25% and 100% by weight based on the total fatty acid content (Dhasco® (from Martek Biosciences Corp., Columbia, MD), DHA Maguro® (from Daito Enterprises, Los Angeles, CA), Soyacal®, and Travemulsion®.

[0065] Additional minor components can be included in the compositions of the invention for a variety of purposes well known in the pharmaceutical industry. These components will for the most part impart properties which enhance retention of the compound at the site of administration, protect the stability of the composition, control the pH, facilitate processing of the compound into pharmaceutical formulations, and the like. Preferably, each of these components is individually present in less than about 15 weight % of the total composition, more preferably less than about 5 weight %, and most preferably less than about 0.5 weight % of the total composition. Some components, such as fillers or diluents, can constitute up to 90 wt.% of the total composition, as is well known in the formulation art. Such additives include cryoprotective agents for preventing reprecipitation of the steroid or D-homosteroid, surface active, wetting or emulsifying agents (e.g., lecithin, polysorbate-80, Tween® 80, pluronic 60, polyoxyethylene stearate), preservatives (e.g., ethyl-p-hydroxybenzoate), microbial preservatives (e.g., benzyl alcohol, phenol, m-cresol, chlorobutanol, sorbic acid, thimerosal and paraben), agents for adjusting pH or buffering agents (e.g., acids, bases, sodium acetate, sorbitan monolaurate), agents for adjusting osmolarity (e.g., glycerin), thickeners (e.g., aluminum monostearate, stearic acid, cetyl alcohol, stearyl alcohol, guar gum, methyl cellulose, hydroxypropylcellulose, tristearin, cetyl wax esters, polyethylene glycol), colorants, dyes, flow aids, non-volatile silicones (e.g., cyclomethicone), clays (e.g., bentonites), adhesives, bulking agents, flavorings, sweeteners, adsorbents,

fillers (e.g., sugars such as lactose, sucrose, mannitol, or sorbitol, cellulose, or calcium phosphate), diluents (e.g., water, saline, electrolyte solutions), binders (e.g., starches such as maize starch, wheat starch, rice starch, or potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropyl methylcellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidone, sugars, polymers, acacia), disintegrating agents (e.g., starches such as maize starch, wheat starch, rice starch, potato starch, or carboxymethyl starch, cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate, croscarmellose sodium or crospovidone), lubricants (e.g., silica, talc, stearic acid or salts thereof such as magnesium stearate, or polyethylene glycol), coating agents (e.g., concentrated sugar solutions including gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, or titanium dioxide), and antioxidants (e.g., sodium metabisulfite, sodium bisulfite, sodium sulfite, dextrose, phenols, and thiophenols).

[0066] Dosage from administration by these routes may be continuous or intermittent, depending, for example, upon the patient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to and assessable by a skilled practitioner.

[0067] Those with ordinary skill in administering anesthetics can readily determine dosage and regimens for the administration of the pharmaceutical compositions of the invention or titrating to an effective dosage for use in treating insomnia, mood disorders, convulsive disorders, anxiety or symptoms of ethanol withdrawal. It is understood that the dosage of the compounds will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. For any mode of administration, the actual amount of compound delivered, as well as the dosing schedule necessary to achieve the advantageous effects described herein, will also depend, in part, on such factors as the bioavailability of the compound, the disorder being treated, the desired therapeutic dose, and other factors that will be apparent to those of skill in the art. The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect the desired therapeutic response in the animal over a reasonable period of time. Preferably, an

effective amount of the compound, whether administered orally or by another route, is any amount that would result in a desired therapeutic response when administered by that route. The dosage may vary depending on the dosing schedule, which can be adjusted as necessary to achieve the desired therapeutic effect. The most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of ordinary skill in the art without undue experimentation.

[0068] In one embodiment, solutions for oral administration are prepared by dissolving the compound in any pharmaceutically acceptable solvent capable of dissolving a compound (e.g., ethanol or methylene chloride) to form a solution. An appropriate volume of a carrier which is a solution, such as Cremophor® EL solution, is added to the solution while stirring to form a pharmaceutically acceptable solution for oral administration to a patient. If desired, such solutions can be formulated to contain a minimal amount of, or to be free of, ethanol, which is known in the art to cause adverse physiological effects when administered at certain concentrations in oral formulations.

[0069] In another embodiment, powders or tablets for oral administration are prepared by dissolving a compound in any pharmaceutically acceptable solvent capable of dissolving the compound (e.g., ethanol or methylene chloride) to form a solution. The solvent can optionally be capable of evaporating when the solution is dried under vacuum. An additional carrier can be added to the solution prior to drying, such as Cremophor® EL solution. The resulting solution is dried under vacuum to form a glass. The glass is then mixed with a binder to form a powder. The powder can be mixed with fillers or other conventional tabletting agents and processed to form a tablet for oral administration to a patient. The powder can also be added to any liquid carrier as described above to form a solution, emulsion, suspension or the like for oral administration.

[0070] Emulsions for parenteral administration can be prepared by dissolving a compound in any pharmaceutically acceptable solvent capable of dissolving the compound (e.g., ethanol or methylene chloride) to form a solution. An appropriate volume of a carrier which is an emulsion, such as Liposyn® II or Liposyn® III emulsion, is added to the solution while stirring to form a pharmaceutically

acceptable emulsion for parenteral administration to a patient. If desired, such emulsions can be formulated to contain a minimal amount of, or to be free of, ethanol or Cremophor® solution, which are known in the art to cause adverse physiological effects when administered at certain concentrations in parenteral formulations.

[0071] Solutions for parenteral administration can be prepared by dissolving a compound in any pharmaceutically acceptable solvent capable of dissolving the compound (e.g., ethanol or methylene chloride) to form a solution. An appropriate volume of a carrier which is a solution, such as Cremophor® solution, is added to the solution while stirring to form a pharmaceutically acceptable solution for parenteral administration to a patient. If desired, such solutions can be formulated to contain a minimal amount of, or to be free of, ethanol or Cremophor® solution, which are known in the art to cause adverse physiological effects when administered at certain concentrations in parenteral formulations.

[0072] If desired, the emulsions or solutions described above for oral or parenteral administration can be packaged in IV bags, vials or other conventional containers in concentrated form and diluted with any pharmaceutically acceptable liquid, such as saline, to form an acceptable concentration prior to use as is known in the art.

DEFINITIONS

[0073] The term "steroid" as used herein describes an organic compound containing in its chemical nucleus the perhydrocyclopentanophenanthrene ring.

[0074] The term "D-homosteroid" as used herein describes an organic compound containing in its chemical nucleus the perhydrochrysene ring.

[0075] The term "hydrogen bond acceptor" as described herein generally describes a substance that receives hydrogen atoms from another substance, the donor. Examples of hydrogen bond acceptors include, but are not limited to the following: halogens, =O, CN, =CHCN, =CH, $=CHC(O)CH_3$, acyl, $=CHX_1$, $-OX_2$ and an epoxide, where X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl, and X_2 is selected from the group

consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl. A sp^2 hybridized carbon atom may also function as a hydrogen bond acceptor.

[0076] The term "saturated" as used herein describes the state in which all available valence bonds of an atom (especially carbon) are attached to other atoms.

[0077] The term "unsaturated" as used herein describes the state in which not all available valence bonds along the alkyl chain are satisfied; in such compounds the extra bonds usually form double or triple bonds (chiefly with carbon).

[0078] Unless otherwise indicated, the alkyl groups described herein are preferably lower alkyl containing from one to eight carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain or cyclic and include methyl, ethyl, propyl, isopropyl, butyl, hexyl and the like.

[0079] Unless otherwise indicated, the alkenyl groups described herein are preferably lower alkenyl containing from two to eight carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain or cyclic and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, hexenyl, and the like.

[0080] Unless otherwise indicated, the alkynyl groups described herein are preferably lower alkynyl containing from two to eight carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain and include ethynyl, propynyl, butynyl, isobutynyl, hexynyl, and the like.

[0081] A "carboxyl" moiety as described herein is composed of a carbonyl group and a hydroxyl group bonded to a carbon atom, commonly shown as COOH or CO_2H.

[0082] The term "acyl," as used herein alone or as part of another group, denotes the moiety formed by removal of the hydroxyl group from the -COOH group of an organic carboxylic acid, *e.g.*, RC(O)- wherein R is R_a, R_aO-, R_aS-, or R_aR_bN-, R_a and R_b are independently hydrogen, hydrocarbyl, substituted hydrocarbyl, or heterocyclo and "-" denotes the point of attachment.

[0083] The terms "hydrocarbon" and "hydrocarbyl" as used herein describe organic compounds or radicals consisting exclusively of the elements carbon and hydrogen. These moieties include alkyl, alkenyl, alkynyl, and aryl moieties. These moieties also include alkyl, alkenyl, alkynyl, and aryl moieties substituted with other

aliphatic or cyclic hydrocarbon groups, such as alkaryl, alkenaryl and alkynaryl. Unless otherwise indicated, these moieties preferably comprise 1 to 20 carbon atoms.

[0084] The "substituted hydrocarbyl" moieties described herein are hydrocarbyl moieties which are substituted with at least one atom other than carbon, including moieties in which a carbon chain atom is substituted with a hetero atom such as nitrogen, oxygen, silicon, phosphorous, boron, sulfur, or a halogen atom. These substituents include halogen, heterocyclo, alkoxy, alkenoxy, alkynoxy, aryloxy, hydroxy, protected hydroxy, keto, acyl, acyloxy, nitro, amino, amido, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[0085] The term "aryl" as used herein alone or as part of another group denote optionally substituted homocyclic aromatic groups, preferably monocyclic or bicyclic groups containing from 6 to 12 carbons in the ring portion, such as phenyl, biphenyl, naphthyl, substituted phenyl, substituted biphenyl or substituted naphthyl. Phenyl and substituted phenyl are the more preferred aryl.

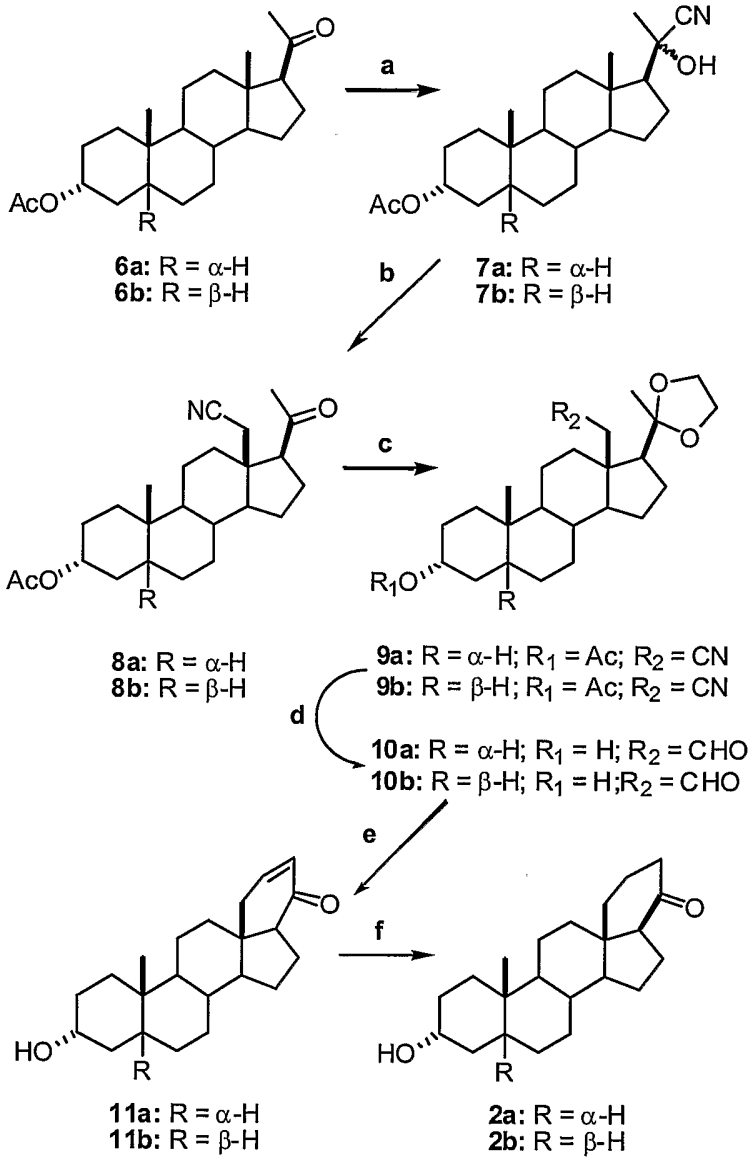
[0086] The terms "halogen" or "halo" as used herein alone or as part of another group refer to chlorine, bromine, fluorine, and iodine.

[0087] An "epoxide" moiety as described herein is an organic compound containing a reactive resulting from the union of an oxygen atom with two other atoms (usually carbon) that are joined in some other way.

[0088] As used herein, "Ac" means acetyl; "THF" means tetrahydrofuran, "NNHTs" means tosylhydrazone, "MOM" means methoxymethyl; "DMF" means dimethyl formamide, "DMSO" means dimethyl sulfoxide, "KHMDS" means potassium hexamethyldisilazane, "DBU" means 1,8-diazabicyclo[5.4.0]undec-7-ene; "DIBALH" means diisobutylaluminum hydride, and "HMPA" means hexamethylphosphoramide; "DEAD" means diethyl azodicarboxylate; "PCC" means pyridinium chlorochromate; "*m*-CPBA" means meta-chloroperbenzoic acid, and "NBS" means *N*-bromosuccinimide.

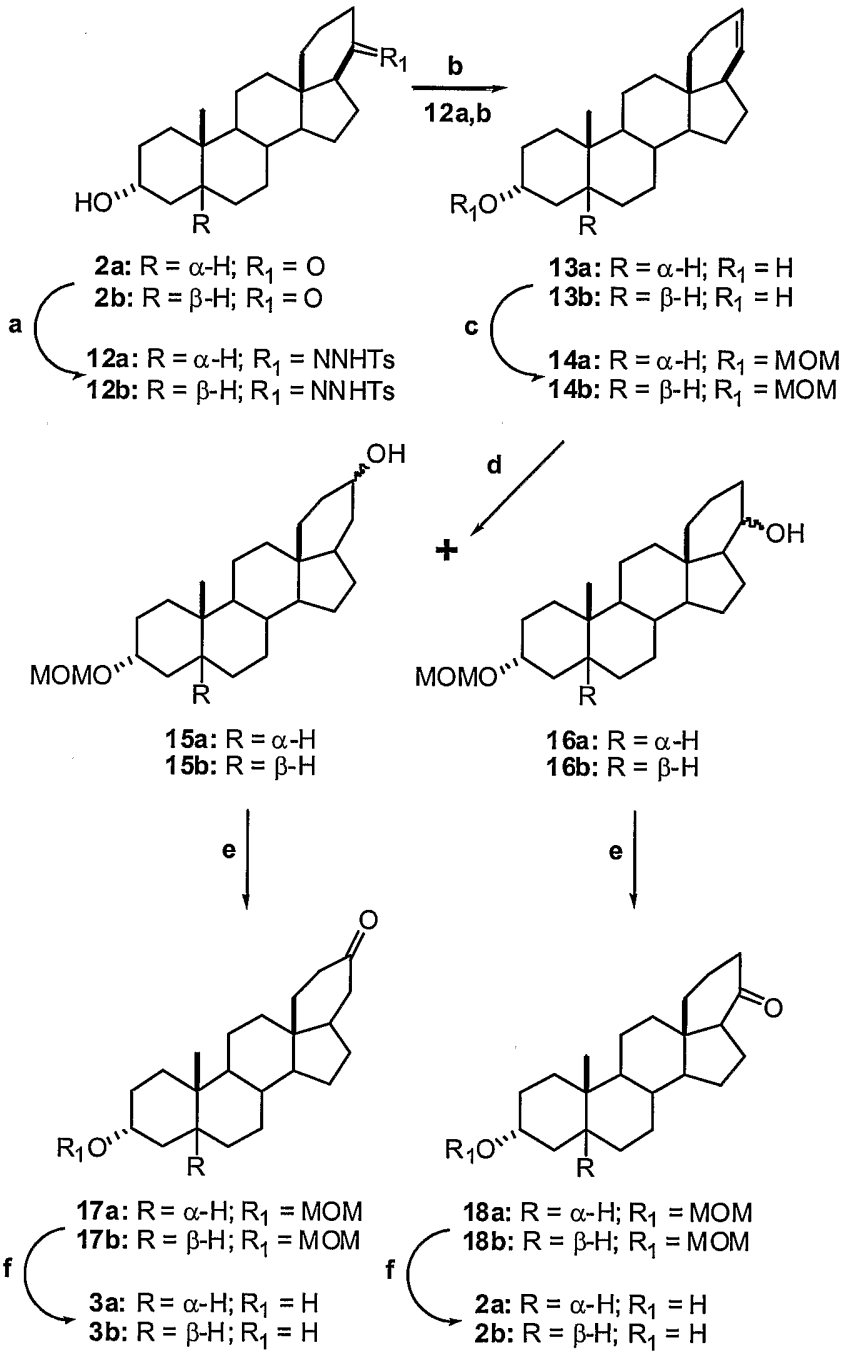
[0089] The following examples illustrate the invention.

Example 1



[0090] Reagents and conditions: (a) acetone cyanohydrin, Et_3N, 50 °C to 25 °C; (b) $Pb(OAc)_4$, $CaCO_3$, I_2, cyclohexane, reflux, hν; (c) ethylene glycol, pyridinium *p*-toluenesulfonate, toluene, reflux; (d) 1 M DIBALH in toluene, THF, -78 °C to 25 °C; (e) 4N *aq.* HCl, THF, 25 °C; (f) 5% $Pd/BaSO_4$, H_2, EtOAc, 40 psi, 25 °C.

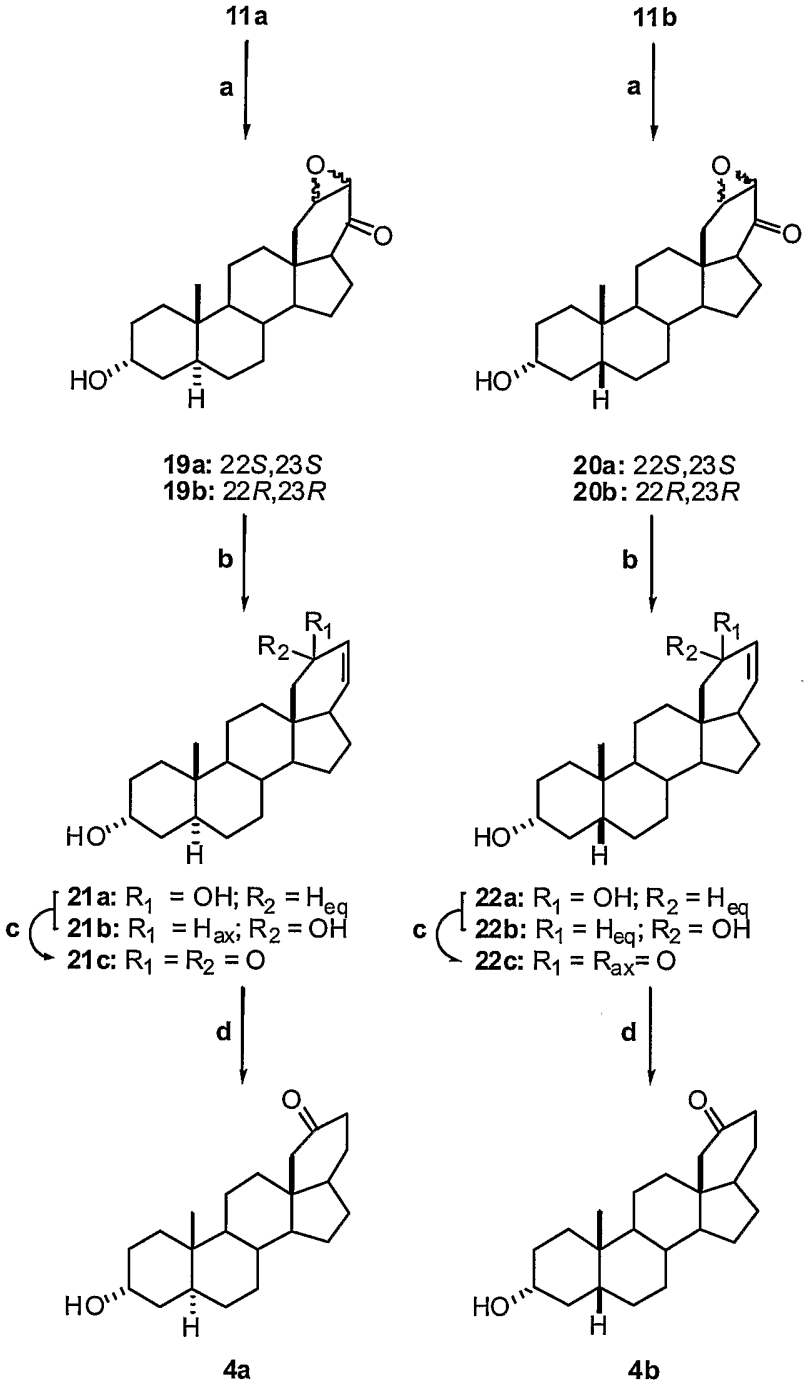
Example 2



[0091] Reagents and conditions: (a) *p*-toluenesulfonhydrazide, MeOH, conc. H_2SO_4, 25 °C; (b) 2.5 N *n*-BuLi in hexanes, THF, 0 °C to 25 °C; (c) MOMCl, (*i*-

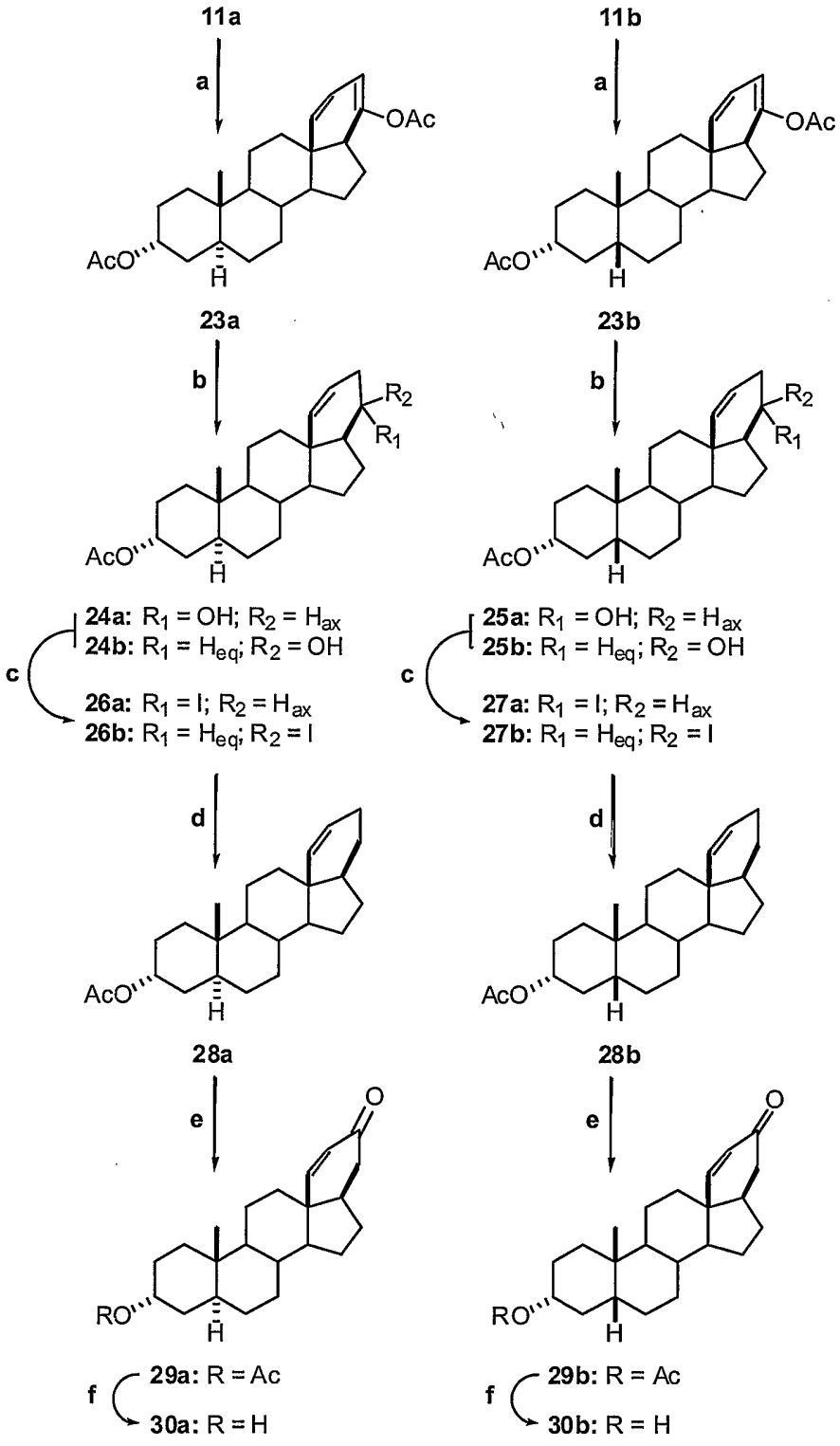
$Pr)_2NEt$, CH_2Cl_2, 25 °C; (d) i) 1 M BH_3 in THF, 0 °C to 25 °C; ii) aq. NaOH, 30% H_2O_2, 0 °C to 25 °C; (e) pyridinium chlorochromate, NaOAc, CH_2Cl_2, 25 °C; (f) 37% *aq.* HCl, MeOH, 25 °C.

Example 3



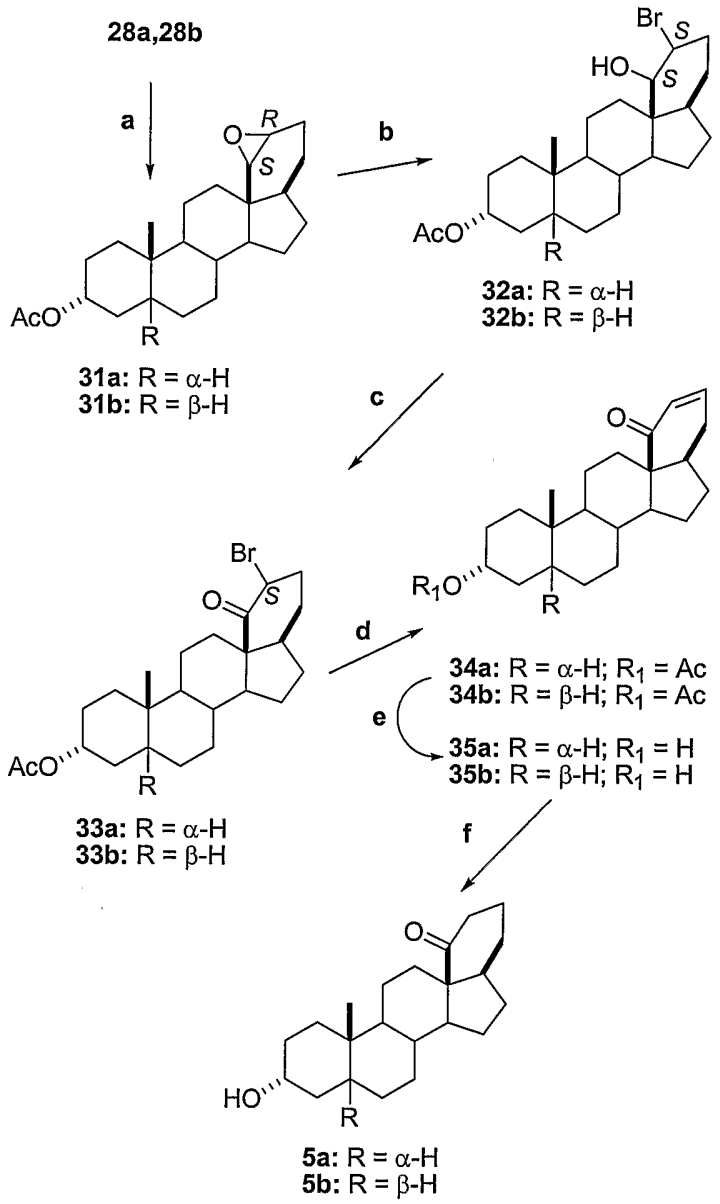
[0092] Reagents and conditions: (a) 30% H_2O_2, aq. NaOH, MeOH/1,4-dioxane, 0 °C; (b) $NH_2NH_2 \cdot xH_2O$ (x ~ 1.5), AcOH, MeOH, reflux; (c) MnO_2, $CHCl_3$, 25 °C; (d) 5% $Pd/BaSO_4$, MeOH, 40 psi, 25 °C.

Example 4


11a
a
OAc
AcO
H
11b
a
OAc
AcO
H
23a
b
R2
R1
AcO
H
23b
b
R2
R1
AcO
H
24a: R1 = OH; R2 = Hax
24b: R1 = Heq; R2 = OH
c
26a: R1 = I; R2 = Hax
26b: R1 = Heq; R2 = I
25a: R1 = OH; R2 = Hax
25b: R1 = Heq; R2 = OH
c
27a: R1 = I; R2 = Hax
27b: R1 = Heq; R2 = I
d
AcO
H
d
AcO
H
28a
e
O
RO
H
28b
e
O
RO
H
f
29a: R = Ac
30a: R = H
f
29b: R = Ac
30b: R = H

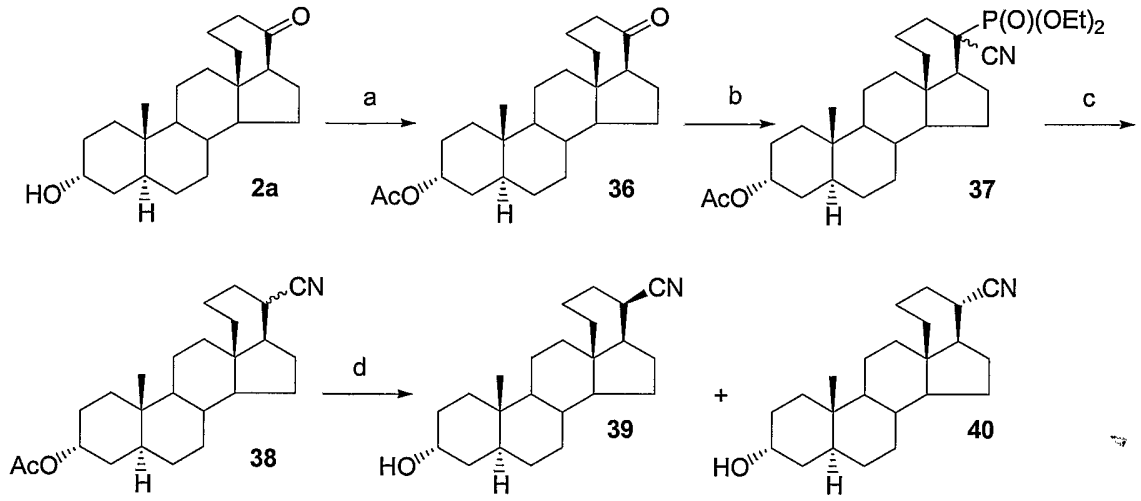
[0093] Reagents and conditions: (a) NaI, Ac_2O, Me_3SiCl, 0 °C to 25 °C; (b) $NaBH_4$, EtOH, 25 °C; (c) Ph_3P, I_2, imidazole, toluene, 95 °C; (d) SmI_2, HMPA, *i*-PrOH, THF, 25 °C; (e) CrO_3, 3,5-dimethyl pyrazole, CH_2Cl_2, 25 °C; (f) aq. NaOH, MeOH, reflux.

Example 5



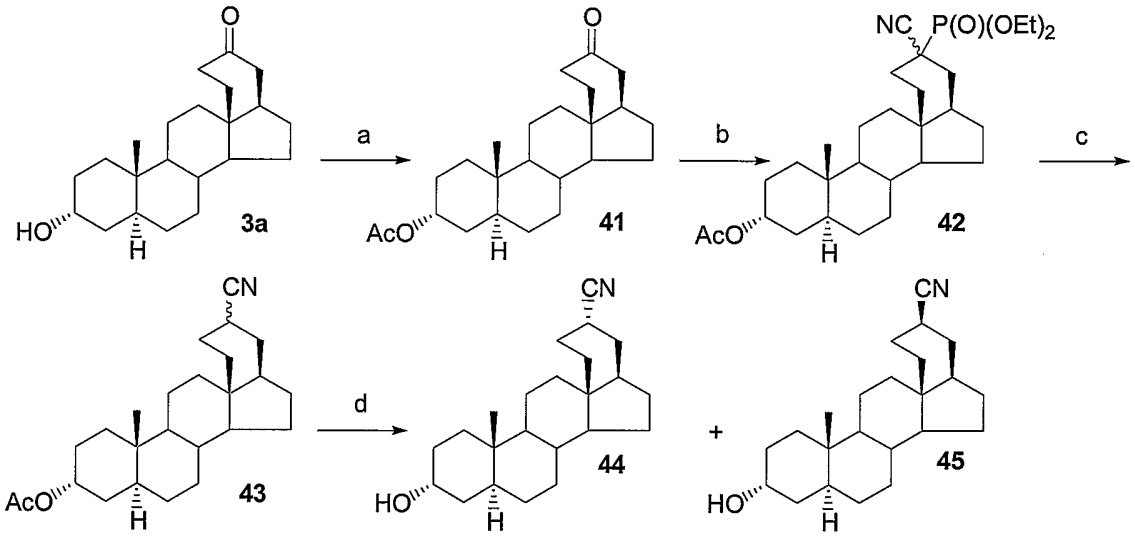
[0094] Reagents and conditions: (a) *m*-chloroperbenzoic acid, $NaHCO_3$, CH_2Cl_2, 25 °C; (b) 48% aq. HBr, MeCN, -40 °C to 25 °C; (c) Jones reagent, Me_2CO, 5 °C; (d) Li_2CO_3, LiBr, DMF, 125 °C; (e) aq. NaOH, MeOH, reflux; (f) 5% $Pd/BaSO_4$, EtOAc, 40 psi, 25 °C.

Example 6



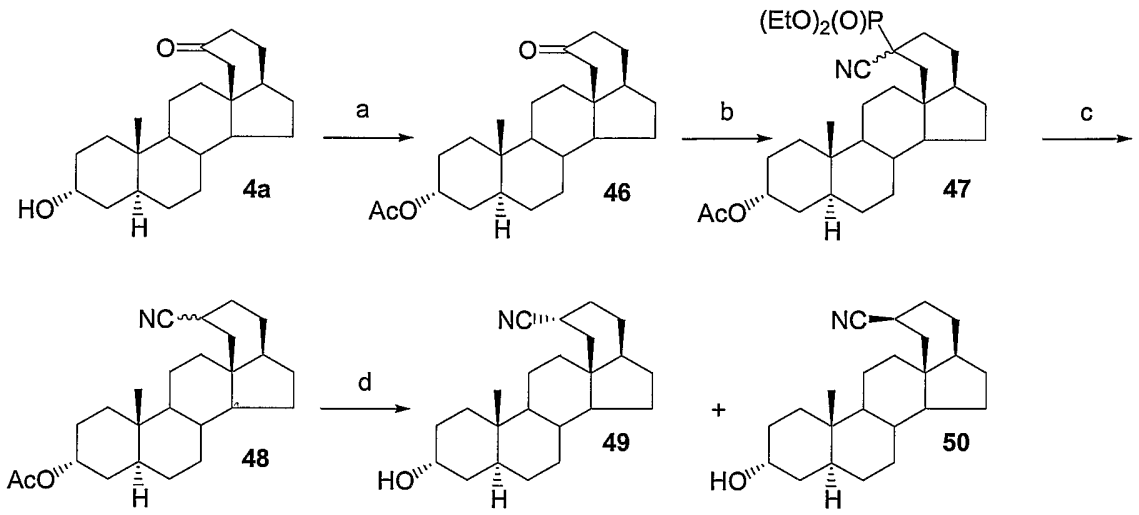
[0095] Reagents and conditions: (a) Ac_2O, pyridine, room temperature; (b) diethyl cyanophosphonate, LiCN in DMF, THF, room temperature; (c) SmI_2, MeOH, THF, room temperature; (d) NaOH, MeOH, room temperature.

Example 7



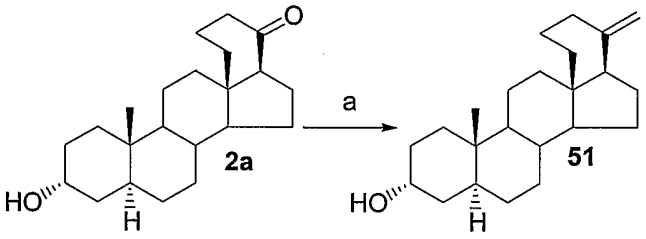
[0096] Reagents and conditions: (a) Ac_2O, pyridine, room temperature; (b) diethyl cyanophosphonate, LiCN in DMF, THF, room temperature; (c) SmI_2, MeOH, THF, room temperature; (d) NaOH, MeOH, room temperature.

Example 8



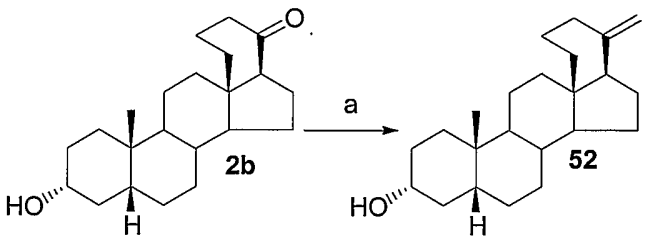
[0097] Reagents and conditions: (a) Ac_2O, pyridine, room temperature; (b) diethyl cyanophosphonate, LiCN in DMF, THF, room temperature; (c) SmI_2, MeOH, THF, room temperature; (d) NaOH, MeOH, room temperature.

Example 9



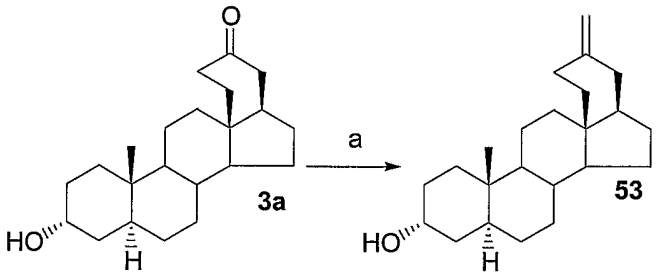
[0098] Reagents and conditions: (a) NaH, methyltriphenylphosphonium bromide, DMSO, 70 °C.

Example 10



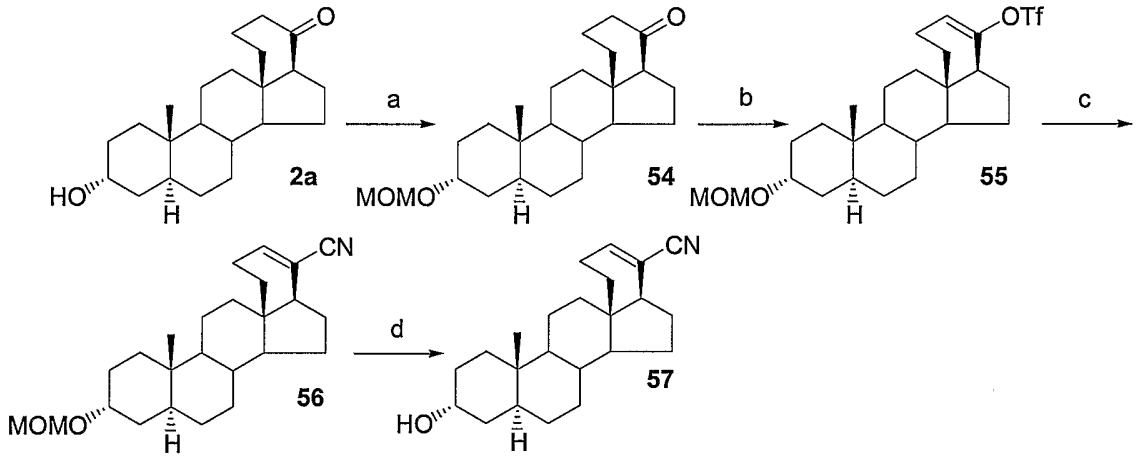
[0100] Reagents and conditions: (a) NaH, methyltriphenylphosphonium bromide, DMSO, 70 °C.

Example 11



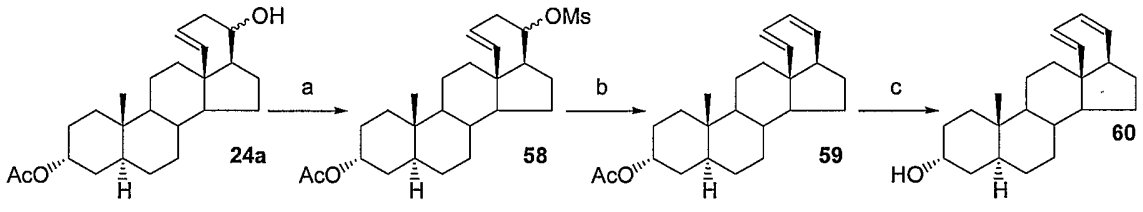
[0101] Reagents and conditions: (a) NaH, methyltriphenylphosphonium bromide, DMSO, 70 °C.

Example 12



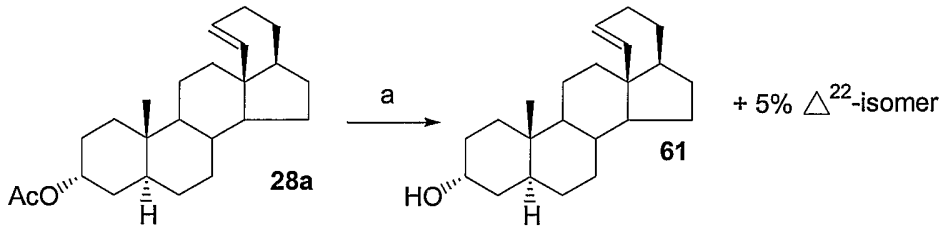
[0102] Reagents and conditions: (a) MOMCl, *N*,*N*-diisopropyl ethylamine, CH_2Cl_2, room temperature; (b) *N*-phenyltrifuoromethane sulfonamide, KHMDS, THF, -78 °C to room temperature; (c) Et_3N, Me_3SiCN, $Pd(PPh_3)_4$, benzene, reflux; (d) HCl, MeOH, CH_2Cl_2, room temperature.

Example 13



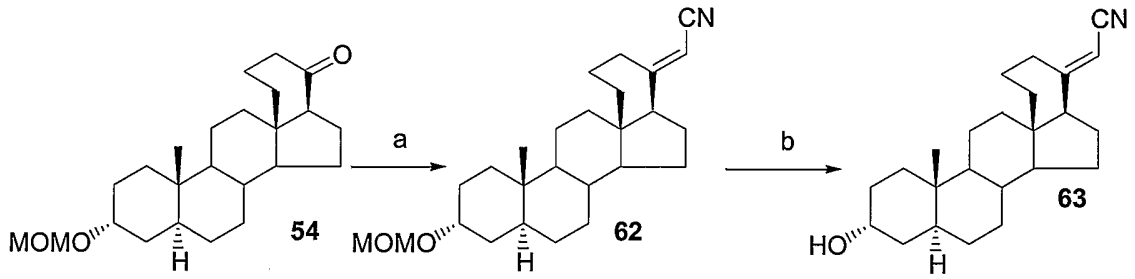
[0103] Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 0 °C room temperature; (b) DBU, toluene, reflux; (c) $LiAlH_4$, THF, room temperature.

Example 14



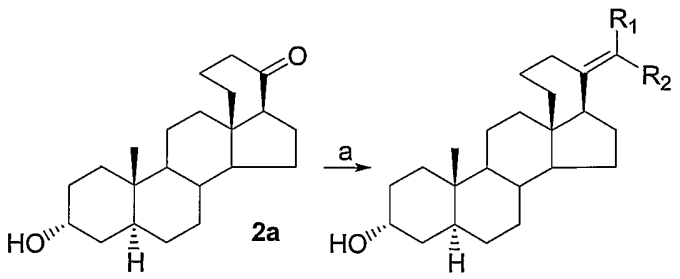
[0104] Reagents and conditions: (a) $LiAlH_4$, THF, room temperature.

Example 15



[0105] Reagents and conditions: (a) (triphenylphosphoranylidene)acetonitrile; (b) HCl, MeOH, CH_2Cl_2, room temperature.

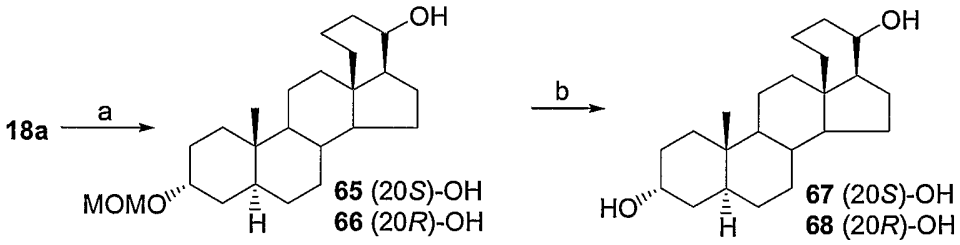
Example 16



63 R_1 = CN, R_2 = H
64 R_1 = H, R_2 = CN

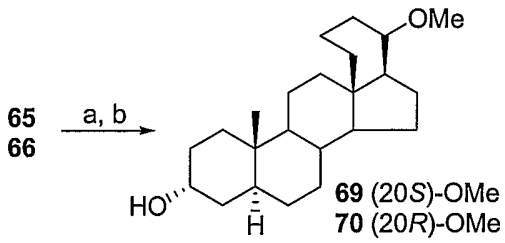
[0106] Reagents and conditions: (a) NaH, $CNCH_2P(O)(OEt)_2$, THF, reflux.

Example 17



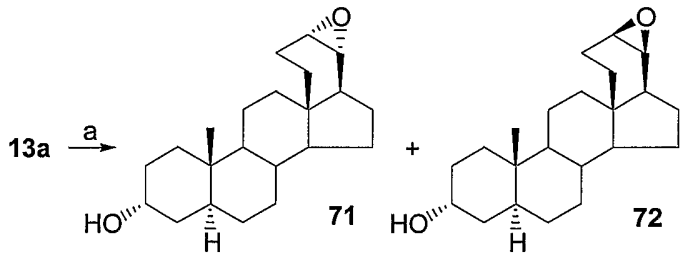
[0107] Reagents and conditions: (a) $NaBH_4$, EtOH, 25 °C; (b) HCl, MeOH-H_2O, 25 °C.

Example 18



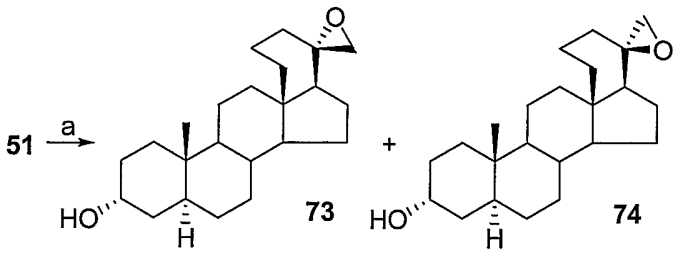
[0108] Reagents and conditions: (a) NaH, MeI, THF, reflux; (b) HCl, MeOH-H_2O, 25 °C.

Example 19



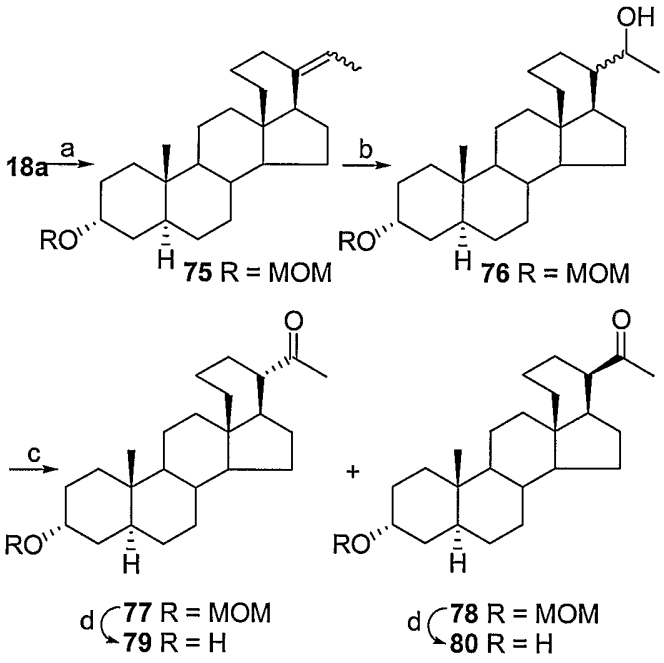
[0109] Reagents and conditions: (a) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 25 °C.

Example 20



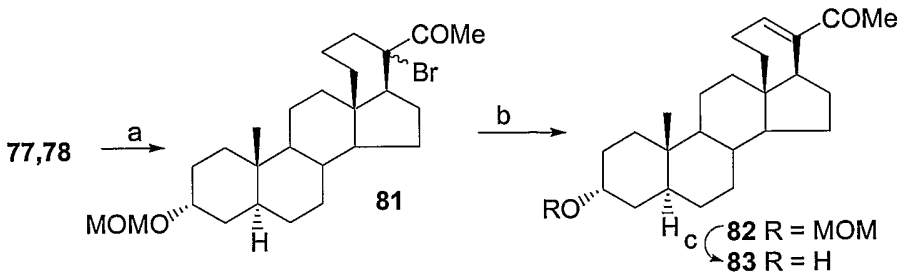
[0110] Reagents and conditions: (a) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 25 °C.

Example 21



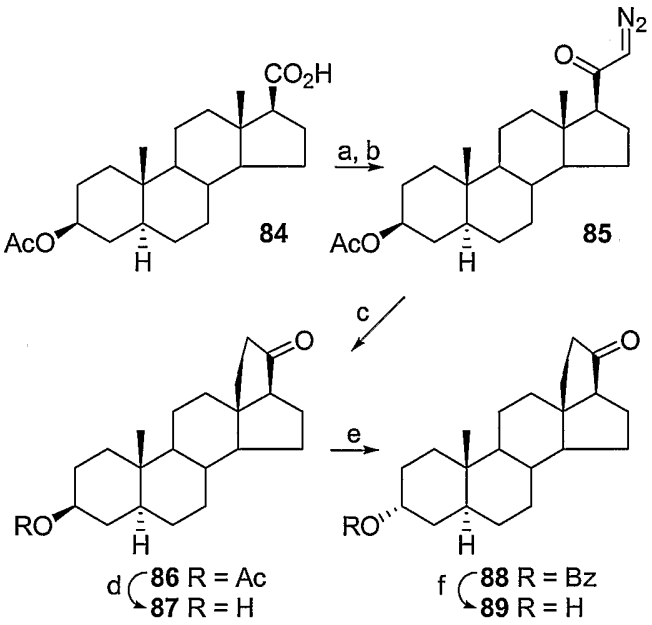
[0111] Reagents and conditions: (a) $KOBu^t$, ethyltriphenylphosphonium bromide, THF, reflux; (b) i) BH_3, THF, 0 °C to 25 °C; ii) *aq.* NaOH, 30% H_2O_2, 0 °C to 25 °C; (c) PCC, NaOAc, CH_2Cl_2, 25 °C; (d) $LiBF_4$, MeCN, H_2O, reflux.

Example 22



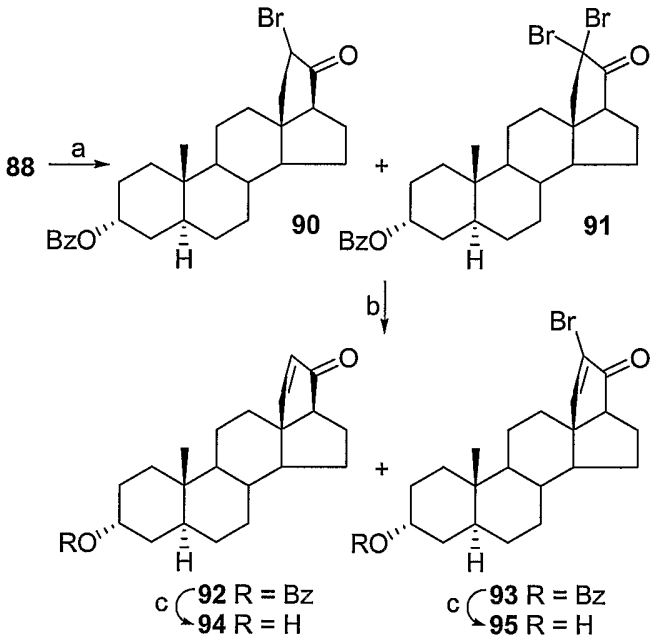
[0112] Reagents and conditions: (a) NBS, CCl_4, hν, reflux; (b) $LiCO_3$, LiBr, DMF, 130 °C; (c) HCl, MeOH-H_2O, 25 °C.

Example 23



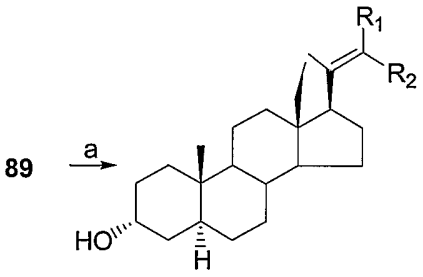
[0113] Reagents and conditions: (a) oxalyl chloride, benzene, 25 °C; (b) diazomethane, ether, 0 °C to 25 °C; (c) $Rh_2(OCOCF_3)_4$, CH_2Cl_2, 25 °C; (d) NaOH, MeOH-H_2O, reflux; (e) $PhCO_2H$, Ph_3P, DEAD, THF, 25 °C; (f) NaOH, EtOH-H_2O, reflux.

Example 24



[0114] Reagents and conditions: (a) pyridinium tribromide, THF, 25 °C; (b) Li_2CO_3, LiBr, DMF, 130 °C; (c) NaOH, EtOH-H_2O, reflux.

Example 25



96 R_1 = CN, R_2 = H
97 R_1 = H, R_2 = CN

[0115] Reagents and conditions: (a) NaH, $CNCH_2P(O)(OEt)_2$, THF, reflux.

Experimental Section for Examples 1-25

[0116] General Methods. Melting points were determined on a Kofler micro hot stage and are uncorrected. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^1H) or 75 MHz (^{13}C). IR spectra were recorded as films on a NaCl plate. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Solvents were used either as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32-63 μm) purchased from Scientific Adsorbents, Atlanta, GA.

[0117] As set forth in Example 1, compounds **11a**, **11b**, **2a**,and **2b** may be prepared by first adding a cyano group at C(20) of (3α,5α)- or (3α,5β)-(3-acetyloxy)pregnan-20-one using acetone cyanohydrin. The cyano group at C(20) migrates to C(18) through the use of a free radical initiator, such as $Pb(OA_C)_4$. The ketone at C(20) may then be converted to a ketal using any alcohol or 1,2- or 1,3-glycol. The cyano group may then be reduced to form an aldehyde group using a reducing agent, such as DIBALH. To form the carbocyclic E ring, a cyclization reaction may be carried out in acidic conditions ($pH \leq 5.5$) forming compounds **11a** and **11b**. Hydrogenation of these compounds yields compounds **2a** and **2b**, respectively.

[0118] As set forth in Example 2, compounds **3a**, **3b**, **2a**, and **2b** may be prepared by first introducing a double bond into the E ring. Depending upon the starting material, the first step may involve converting the oxo group on the E ring to NNHTs using *p*-toluenesulfonylhydrazine. The NNHTs group, if present, may then be eliminated in the presence of a strong base, such as n-butyllithium, thereby introducing a double bond into the E ring. Alternatively, the elimination may be carried out by converting the compound to the tolyslate sodium salt to eliminate hydrogen. The hydroxyl group at C(3) may then be protected using a protecting group that is stable in acid or base, such as methoxymethyl. Using, for example, diborane, a hydroxyl group may then be introduced in the E ring at positions C(20) or C(22). A mild oxidizing agent may then be used to convert the hydroxyl group to an oxo, while leaving the protecting group at C(3) intact. One example of such an

oxidizing agent is pyridinium chlorochromate. Finally, the hydroxyl at C(3) may be deprotected, using almost any acid, yielding compounds **3a**, **3b**, **2a**, and **2b**.

[0119] As set forth in Example 3, compounds **21c**, **22c**, **4a** and **4b** may be prepared using compounds **11a** and **11b** as starting materials, respectively, by first introducing an epoxide ring into the E ring using a peroxide or peracid, such as hydrogen peroxide. Hydrazine or substituted hydrazine may then be used as part of an elimination reaction to form an unsaturated E ring possessing a hydroxyl group at C(23). Oxidation of the allylic alcohol using an oxidizing agent selective for oxidation of the carbon atoms adjacent to a double bond, such as MnO_2, may then proceed yielding compounds **21c** and **22c**. These compounds may then be converted by catalytic hydrogenation to yield compounds **4a** and **4b**.

[0120] As set forth in Example 4, compounds **30a** and **30b** may be prepared starting from compounds **11a** and **11b**, respectively, using isopropenyl acetate and an acid catalyst or sodium iodide, acetic anhydride, and a trialkylsilyl halide to form the dienol acetate. A borohydride, such as sodium borohydride, may then be used to remove the acetate group from the E ring. The alcohol may then be converted to an iodide group using a nucleophilic displacement reaction. After reduction with SmI_2, the compounds may be oxidized to introduce an oxo group into the E ring. Finally, a saponification reaction or an acid catalyzed reaction yields compounds **30a** and **30b**.

[0121] As set forth in Example 5, compounds **5a** and **5b** may be prepared starting from compounds **28a** and **28b**, respectively, by first forming an epoxide ring using a peroxide, such as *m*-chloroperbenzoic acid. The epoxide ring may then be opened with HI, HCl, HBr, or HF and the compound subjected to an oxidation reaction using a chromium reagent, such as Jones reagent. The resulting compound may then be subjected to base-catalyzed elimination, acid or base hydrolysis, and hydrogenation to yield compounds **5a** and **5b**.

[0122] As set forth in Example 6, compounds **39** and **40** may be prepared by first acylating the starting compound, **2a**, with an acylating agent such as Ac_2O. Diethyl cyanophosphate may then be used, followed by SmI_2 to form the cyano group at C(20). Finally, the acetate group at C(3) may be removed to form

compounds **39** and **40**. Compounds **44** and **45** of Example 7 and compounds **49** and **50** of Example 8 may be prepared in the same manner.

[0123] As set forth in Example 9, compound **51** is prepared by converting the carbonyl of compound **2a** into a double bond using, for example, the Witting reaction. Compound **52** of Example 10 and compound **53** of Example 11 may be prepared in the same manner.

[0124] As set forth in Example 12, compound **57** may be prepared by first protecting the C(3) hydroxyl of compound **2a** with a group such as MOM or a silyl group. The carbonyl may then be modified using *N*-phenyltrifluoromethane sulfonamide. The triflate may then be converted to cyano and, finally, the C(3) hydroxyl group may be deprotected to yield compound **57**.

[0125] As set forth in Example 13, compound **60** may be prepared by converting the hydroxyl group at C(20) into a leaving group, for example, by treating with MsCl, and then performing an elimination reaction under basic conditions yielding compound **60**.

[0126] As set forth in Example 14, compound **61** may be prepared by removal of the acetate group at C(3) by hydrolysis or reduction of compound **28a**.

[0127] As set forth in Example 15, compound **63** may be prepared by subjecting compound **54** to a Wittig reaction using (triphenylphosphoranylidene)acetonitrile and then removing the protecting group at C(3).

[0128] As set forth in Example 16, compounds **63** and **64** may be prepared by subjecting compound **2a** to a Wittig reaction with (triphenylphosphoranyidene)acetonitrile.

[0129] As set forth in Example 17, compounds **67** and **68** may be prepared by reduction of the ketone group of compound **18a** using a hydride reducing agent such as sodium borohydride to obtain compounds **65** and **66** and subsequent removal of the methyoxymethyl group by acid catalyzed hydrolysis.

[0130] As set forth in Example 18, compounds **69** and **70** are prepared by methylation of the hydroxyl group of compounds **65** and **66** followed by removal of the methoxymethyl group by acid catalyzed hydrolysis.

[0131] As set forth in Example 19, compounds **71** and **72** may be prepared by epoxidation of the double bond of compound **13a** using a peracid such as meta-chloroperbenzoic acid.

[0132] As set forth in Example 20, compounds **73** and **74** may be prepared by epoxidation of the double bond of compound **51** using a peracid such as meta-chloroperbenzoic acid.

[0133] As set forth in Example 21, compounds **79** and **80** may be prepared from compound **18a** by aWittig reaction using ethyltriphenylphosphonium bromide to obtain the methyl-substituted olefin **75**, hydroboration of the double bond of compound **75** to obtain the hydroxyl group present in compound **76**, PCC oxidation of the hydroxyl group of compound **76** to give the ketone group shown in compounds **77** and **78** and removal of the methoxymethyl group by acid catalyzed hydrolysis to obtain compounds **79** and **80**.

[0134] As set forth in Example 22, compound **83** may be prepared by bromination of a mixture of compounds **77** and **78** using a reagent such as N-bromosuccinimide, elimination of the bromine to form the double bond shown in compound **82** and removal of the methoxymethyl group by acid catalyzed hydrolysis to obtain compound **83**.

[0135] As set forth in Example 23, compound **89** may be prepared from compound **84** by conversion of the carboxylic acid group to the corresponding acid chloride group and then reaction of this acid chloride with diazomethane to give the known compound **85**. Compound **85** is then cyclized using $Rh_2(OCOCF_3)_4$ to give compound **86**. The acetate group is hydrolyzed from compound **86** using base catalysis to give compound **87** and the beta configuration of the hydroxyl group in compound **87** is changed using a Mitsunobu reaction that yields compound **88** with a benzoate ester in the alpha configuration. The ester is hydrolyzed from compound **88** using base catalysis to yield compound **89**.

[0136] As set forth in Example 24, compounds **94** and **95** may be prepared by bromination of compound **88** using a brominating reagent such a pyridinium tribromide to obtain compounds **90** and **91** that are then subjected to an elimination reaction to obtain compounds **92** and **93**. Hydrolysis of the benzoate ester of

compounds **92** and **93** under conditions of basic catalysis yields compounds **94** and **95**.

[0137] As set forth in Example 25, compounds **96** and **97** may be prepared by subjecting compound **89** to a Wittig reaction with (triphenylphosphoranyidene)acetonitrile.

[0138] In addition, the hydrogen bond accepting groups on the E ring exemplified in Examples 1-25 may be converted into various other hydrogen bond accepting groups such as an epoxide, carboxy, thioester, alcohol, or ether. Generally, an epoxide ring may be formed from conversion of a ketone to the olefin intermediate, followed by treatment with peracid. A carboxy group may be formed from the hydrolysis of a cyano group. The $C{=}ONH_2$ hydrolyzes to COOH, which, if desired, can then be converted to an ester. A thioester may be formed from the accession from COOH. Ethers may be formed from the alkylation of oxygen.

(3α,5α)-3-(Acetyloxy)-20-hydroxypregnane-20-carbonitrile (7a).

[0139] Compound **7a** was prepared using a reported procedure. (*See*, Mickova, R., et al., Isolation and the Structure of the Product of Cholesterol Biodegradation by the Mutant Mycobacterium sp. CCM 3529, *Coll. Czech. Chem. Comm.* 1985, 50:1110-1113). Et_3N (0.2 mL) was added to (3α,5α)-3-(acetyloxy)pregnan-20-one (**6a**, 1.0 g, 2.77 mmol) in acetone cyanohydrin (2 mL) at 50 °C. The reaction mixture was slowly cooled to room temperature with stirring. After 3 h, water (5 mL) was added to the reaction mixture, and the white solid precipitate (mixture of compound **6a** and **7a**) was filtered and washed thoroughly with water, then dried under high vacuum at room temperature for 24 h. The precipitate was used without further purification.

[0140] An analytical sample of **7a** was purified by column chromatography (silica gel; hexanes/EtOAc, 7:1) and obtained as white crystals: mp 192–195 °C (EtOAc-hexanes); ^1H NMR δ 0.81 (s, 3H, 19-CH_3), 1.00 (s, 3H, 18-CH_3), 1.62 (s, 3H, 21-CH_3), 2.05 (s, 3H, CH_3CO_2), 5.01 (m, 1H, C*H*OAc); ^{13}C NMR δ 11.3, 13.0, 20.5, 21.5, 24.0, 25.0, 26.1, 28.2, 30.6, 31.8, 32.8 (2×C), 34.9, 35.8, 40.0, 40.2, 43.5, 54.1,

55.9, 59.1, 70.1, 71.7, 122.0 (CN), 170.7 (CO_2); IR ν_{max} 3389, 2924, 2231, 1701, 1281 cm^{-1}. Anal. ($C_{24}H_{37}NO_3$) C, H, N.

(3α,5α)-3-(Acetyloxy)-20-oxo-pregnane-18-carbonitrile (8a).

[0141] Compound **8a** was prepared using a reported procedure. (*See*, Kalvoda, J., et al., Reactions of Steroid Hypoiodites. VII. 1,4-Shift of the Nitrile Group (18-Cyanopregnanes), *Helv. Chim. Acta* 1966, 49: 424-436) I_2 (1.6 g, 6.30 mmol) was added to a refluxing mixture of $Pb(OAc)_4$ (6.0 g, 13.5 mmol) and $CaCO_3$ (2 g, 20.0 mmol) in cyclohexane (200 mL). The purple solution was refluxed for 1 h and the mixture of compound **6a** and **7a** obtained in last step [prepared from **6a** (1.0 g, 2.77 mmol)] was added. The reaction mixture was refluxed while irradiated with a 300 W tungsten lamp for 3 h and then cooled to room temperature. After filtration, the precipitate was washed thoroughly with ether. The combined filtrate was washed successively with 10% $Na_2S_2O_3$ and brine, and dried over Na_2SO_4. Removal of solvent under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 4:1) to give compound **8a** (400 mg, 40% from **6a**) and recovered compound **6a** (400 mg).

[0142] Compound **8a** was obtained as a colorless oil: ^1H NMR δ 0.80 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3CO_2), 2.29 (s, 3H, 21-CH_3), 2.70 (t, J = 9.0 Hz, 1H, C*H*$COCH_3$), 5.02 (m, 1H, C*H*OAc); ^{13}C NMR δ 11.3, 16.4, 20.6, 21.5, 23.1, 24.0, 26.0, 28.0, 31.6, 32.5, 32.7, 32.7, 35.5, 35.7, 36.0, 39.8, 46.1, 53.7, 56.5, 62.0, 69.8, 118.1 (CN), 170.5 (CO_2), 208.7 (*C*O); IR ν_{max} 2933, 2249, 1732, 1704, 1245 cm^{-1}. Anal. ($C_{24}H_{35}NO_3$) C, H, N.

(3α,5α)-3-(Acetyloxy)-20,20-[1,2-ethanediylbis(oxy)]pregnane-18-carbonitrile (9a).

[0143] A mixture of compound **8a** (1.93 g, 5.00 mmol), ethylene glycol (3.1 g, 50 mmol) and PPTS (0.64 g, 2.55 mmol, 30% W/W) in toluene (50 mL) was refluxed using a Dean-Stark apparatus under N_2 for 2 h. The reaction mixture was cooled to room temperature, washed with 10% $NaHCO_3$ and brine, and dried over Na_2SO_4.

The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 9:1) to give compound **9a** (1.78 g, 83%).

[0144] Compound **9a** was obtained as white crystals: mp 176–177 °C (EtOAc-hexanes); ^{1}H NMR δ 0.80 (s, 3H, 19-CH_3), 1.29 (s, 3H, 21-CH_3), 2.05 (s, 3H, CH_3CO_2), 2.27 (d, *J* = 16.8 Hz, 1H, CH_2CN), 2.52 (d, *J* = 16.8 Hz, 1H, CH_2CN), 4.04 (m, 4H, OCH_2CH_2O), 5.01 (m, 1H, C*H*OAc); ^{13}C NMR δ 11.2, 16.7, 20.3, 21.4, 23.0, 23.2, 23.4, 25.9, 28.0, 31.4, 32.6 (2×C), 35.0, 35.6, 36.6, 39.7, 43.7, 53.7, 56.0, 56.3, 63.0, 63.7, 69.8, 110.8 (20-C), 119.8 (CN), 170.4 (CO_2); IR ν_{max} 2937, 2241, 1730, 1237 cm^{-1}. Anal. ($C_{26}H_{39}NO_4$) C, H, N.

(3α,5α)-20,20-[1,2-Ethanediylbis(oxy)]-3-hydroxypregnane-18-carboxaldehyde (10a).

[0145] Compound **9a** (2.0 g, 4.66 mmol) in THF (120 mL) was cooled to -78°C and DIBALH (1.0 M in toluene, 23.3 mL, 23.3 mmol) was added. The colorless solution was stirred at ambient temperature for 26 h and then cooled to 0 °C. After H_2O (5 mL) was added dropwise to quench the reaction, the solvent was removed at room temperature. EtOAc (50 mL) and H_2O (20 mL) were added to the residue. Insoluble $Al(OH)_3$ was filtered through a pad of Celite 545® and washed thoroughly with EtOAc. The combined filtrate was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave aldehyde **10a** as a white solid that was partially characterized and immediately converted into cyclosteroid **11a**. Aldehyde **10a** had: ^{1}H NMR δ 0.77 (s, 3H, 19-CH_3), 1.29 (s, 3H, 21-CH_3), 3.85 (m, 4H, OCH_2CH_2O), 4.03 (m, 1H, C*H*OH), 9.82 (t, *J* = 2.4 Hz, 1H, CHO); ^{13}C NMR δ 11.1, 20.2, 22.9, 23.4, 23.5, 28.3, 28.9, 31.7, 32.0, 34.8, 35.7, 36.0, 36.3, 39.0, 40.2, 44.1, 54.1, 57.6, 57.7, 62.6, 64.0, 66.2, 111.1 (20-C), 204.9 (CHO).

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one (11a).

[0146] A solution of aldehyde **10a** in THF (100 mL) and aqueous HCl (4 N, 32 mL) was stirred under N_2 at room temperature for 16 h. THF was removed under reduced pressure. The residue was extracted with EtOAc. The combined EtOAc

extracts were washed with 10% $NaHCO_3$ and brine, and dried over Na_2SO_4. After solvent removal under reduced pressure, the residue was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 8:1) to give enone **11a** (0.86 g, 57% from **9a**).

[0147] Compound **11a** was obtained as white crystals: mp 230–232 °C (EtOAc-hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 5.96 (dd, *J* = 3.0 Hz, *J* = 9.9 Hz, 1H, CH=C*H*CO), 6.83 (m, 1H, C*H*=CHCO); ^{13}C NMR δ 11.2, 20.3, 25.8, 26.2, 27.2, 28.3, 28.9, 32.0, 32.1, 33.9, 35.3, 35.8, 36.1, 39.0, 47.5, 53.7, 56.3, 57.7, 66.3, 127.4 (CH=*C*HCO), 148.3 (*C*H=CHCO), 202.3 (CO); IR ν_{max} 3429, 2924, 1666, 1444 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (2a).

[0148] Enone **11a** (328 mg, 1.0 mmol) was dissolved in EtOAc (50 mL) and hydrogenated (40 psi, H_2, 5% Pd/$BaSO_4$, 100 mg) for 1.5 h. The reaction mixture was filtered through a pad of Celite 545® to remove catalyst and the solvent was removed under reduced pressure. The product was purified by column chromatography (silica gel; hexanes/EtOAc/CH_2Cl_2, 6:1:0.3) to give compound **2a** (300 mg, 91%) as white crystals: mp 200–202 °C (EtOAc-hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 2.50 (m, 1H, C*H*CO), 4.04 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 20.3, 22.1 (2×C), 25.1, 27.0, 28.4, 29.0, 32.1, 32.2, 33.5, 35.2, 35.8, 36.1, 37.3, 39.0, 49.6, 53.9, 56.7, 61.5, 66.4, 215.6 (CO); IR ν_{max} 3548, 2928, 1701, 1253 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α)-13,24-Cyclo-18,21-dinorchol-20(22)-en-3-ol (13a).

[0149] To the solution of compound **2a** (330 mg, 1.0 mmol) and *p*-toluenesulfonhydrazide (186 mg, 1.0 mmol) in MeOH (40 mL) was added 3 drops of 96% H_2SO_4. The reaction mixture was stirred at room temperature for 3-4 h and the MeOH was removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with 10% $NaHCO_3$ and brine, and dried over Na_2SO_4. After

solvent removal, hydrazone **12a** was obtained and used without further purification or characterization.

[0150] *n*-BuLi (2.5 M in hexanes, 1.6 mL, 4 mmol) was added dropwise to the solution of hydrazone **12a** (obtained from 1.0 mmol ketone **2a**) in anhydrous THF (10 mL) under N_2 at 0 °C. The orange solution was stirred overnight (14 h) at ambient temperature and quenched with water (0.4 mL) at 0 °C. EtOAc (50 mL) was added and the mixture was washed with saturated aqueous NH_4Cl and brine, and dried over Na_2SO_4. After solvent removal under reduced pressure, the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **13a** (201 mg, 64% from ketone **2a**).

[0151] Compound **13a** was obtained as white crystals: mp 169–170 °C (hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 4.04 (m, 1H, C*H*OH), 5.55 (m, 1H, CH=), 5.76 (m, 1H, CH=); ^{13}C NMR δ 11.2, 19.8, 20.2, 21.6, 24.7, 28.6, 29.1, 30.6, 32.3 (2 × C), 33.2, 34.7, 35.9, 36.2, 39.3, 40.9, 46.6, 54.7, 56.1, 66.6, 124.4 (CH=), 130.9 (CH=); IR ν_{max} 3307, 3019, 2927, 1430, 1002 cm^{-1}. Anal. ($C_{22}H_{34}O$) C, H.

(3α,5α)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorchol-20(22)-ene (14a).

[0152] Methoxy methylchloride (0.11 mL, 1.5 mmol) was added to compound **13a** (158 mg, 0.5 mmol) and *N,N*-diisopropyl ethylamine (0.44 mL, 5 mmol) in CH_2Cl_2 (20 mL). The resultant solution was stirred at room temperature for 24 h. The solvent was partially removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **14a** (170 mg, 95%) as white crystals: mp 77–78 °C (hexanes); ^{1}H NMR δ 0.80 (s, 3H, 19-CH_3), 3.37 (s, 3H, CH_3O), 3.83 (m, 1H, C*H*OCH_2), 4.66 (m, 2H, OCH_2O), 5.56 (m, 1H, CH=), 5.76 (m, 1H, CH=); ^{13}C NMR δ 11.4, 19.8, 20.2, 21.6, 24.7, 26.4, 28.7, 30.6, 32.3, 32.9, 33.3, 33.7, 34.7, 36.0, 39.9, 40.9, 46.6, 54.7, 55.1, 56.2, 71.7, 94.6 (OCH_2O), 124.4 (CH=), 130.9 (CH=); IR ν_{max} 3016, 2927, 1455, 1043 cm^{-1}. Anal. ($C_{24}H_{38}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-22-one (3a).

[0153] BH_3 (1.0 M in THF, 1.59 mL, 1.59 mmol) was added to compound **14a** (190 mg, 0.53 mmol) in anhydrous THF (20 mL) under N_2 at 0 °C. The resultant solution was stirred at room temperature for 3.5 h and cooled to 0 °C. Water (0.1 mL) was added to quench the reaction followed by aq. NaOH (3N, 3.0 mL) and 30% H_2O_2 (3.0 mL). The reaction mixture was stirred at ambient temperature for 1.5 h and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine until the pH was neutral and then dried over Na_2SO_4. After solvent removal under reduced pressure, the residue (a mixture of two pairs of diastereomers of **15a** and **16a**) was used without further purification or characterization.

[0154] NaOAc (130 mg, 1.59 mmol) and PCC (228 mg, 1.06 mmol) were added to the solution of compounds **15a** and **16a** in CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature for 2 h and Et_2O (50 mL) was added. The mixture was filtered through a pad of Celite 545® and washed thoroughly with ether. Solvent was removed from the combined filtrate to give a mixture of ketones **17a** and **18a** which was dissolved in CH_3OH (12 mL). Then, 37% aqueous HCl (4 mL) was added. The solution was stirred at room temperature for 1 h and MeOH was removed under reduced pressure. EtOAc (30 mL) was added to the residue and the solution was washed with water, 10% $NaHCO_3$, brine, and dried over Na_2SO_4. Solvent removal under reduced pressure gave ketones **3a** and **2a** which were separated by column chromatography (silica gel; EtOAc/CH_2Cl_2, 1:15) to give ketone **3a** (90 mg, 51% from **14a**) and recovered compound **2a** (58 mg, 33% from **14a**).

[0155] Compound **3a** was obtained as white crystals: mp 195–197 °C (EtOAc-hexanes); ^1H NMR δ 0.79 (s, 3H, 19-CH_3), 2.39 (m, 1H, CH_2CO), 2.54 (dd, J = 6.0 Hz, J = 14.4 Hz, 1H, CH_2CO), 4.05 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 20.4, 23.3, 23.6, 27.7, 28.4, 29.0, 32.0, 32.2, 32.7, 35.6, 35.8, 36.2, 36.9, 39.1, 41.2, 41.5, 48.9, 54.4, 55.6, 66.4, 213.4 (CO); IR ν_{max} 3308, 2918, 1712, 1449, 1007 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α,22*S*,23*S*)-22,23-Epoxy-3-hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (19a) and (3α,5α,22*R*,23*R*)-22,23-Epoxy-3-hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (19b).

[0156] A mixture of MeOH (5 mL), 30% H_2O_2 (1.5 mL) and aqueous NaOH (4N, 0.25 mL) was added to a solution of enone **11a** (100 mg, 0.30 mmol) in 1:1 MeOH and 1,4-dioxane (20 mL) at 0 °C. The resultant solution was stirred at 0 °C for 3-4 h. EtOAc (30 mL) was added and the organic phase was washed with water and brine, and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc/CH_2Cl_2, 4:1:0.2) to give a mixture of compound **19a** and **19b** (1.8:1) 90 mg (86%). Analytical samples were obtained by HPLC separation (silica gel; hexanes/EtOAc, 6:1).

[0157] Compound **19a** was obtained as white crystals: mp 238–240 °C (EtOAc-hexanes); ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 3.15 (d, *J* = 3.3 Hz, 1H, epoxide H-22), 3.55 (s, 1H, epoxide H-23), 4.05 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 20.1, 20.9, 24.7, 27.6, 28.3, 29.0, 32.0, 32.1, 35.1, 35.7, 36.1, 36.4, 39.0, 42.0, 51.8, 53.7, 54.8, 56.4, 57.0, 66.4, 207.1 (CO); IR ν_{max} 3551, 2925, 1691 cm^{-1}. Anal. ($C_{22}H_{32}O_3$) C, H.

[0158] Compound **19b** was obtained as white crystals: mp 194–195 °C (EtOAc-hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 3.23 (d, *J* = 3.6 Hz, 1H, epoxide H-22), 3.55 (t, *J* = 3.6 Hz, 1H, epoxide H-23), 4.05 (m, 1H, C*H*OH); ^{13}C NMR δ 11.3, 20.2, 24.2, 25.5, 26.0, 28.3, 29.0, 32.0, 32.2, 35.1, 35.8, 36.1, 36.4, 39.0, 51.5, 53.6, 54.5, 56.7, 57.9, 59.2, 66.4, 209.2 (CO); IR ν_{max} 3435, 2926, 1699 cm^{-1}. Anal. ($C_{22}H_{32}O_3$) C, H.

(3α,5α,23*S*)-13,24-Cyclo-18,21-dinorchol-20(22)-ene-3,23-diol (21a) and (3α,5α,23*R*)-13,24-Cyclo-18,21-dinorchol-20(22)-en-3,23-diol (21b).

[0159] The mixture of epoxides **19a** and **19b** (68 mg, 0.20 mmol), $NH_2NH_2{\cdot}xH_2O$ (x ~ 1.5, 136 μL, 2.4 mmol) and AcOH (2.35 μL) in MeOH (4 mL) was refluxed for 2 h. Water (20 mL) was added and the product was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with water until the pH was

neutral and then dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; CH_2Cl_2/hexanes/EtOAc, 1:1:0.2) to give compound **21a** (26 mg, 40%) and compound **21b** (14 mg, 22%).

[0160] Compound **21a** was obtained as white crystals: mp 94–96 °C (EtOAc-hexanes); ^{1}H NMR δ 0.82 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 4.22 (m, 1H, CH=CHC*H*OH), 5.72 (m, 1H, CH=C*H*CHOH), 5.97 (m, 1H, C*H*=CHCHOH); ^{13}C NMR δ 11.3, 20.4, 25.3, 28.1, 28.6, 29.1, 30.4, 32.2, 32.3, 34.8, 35.7, 35.9, 36.3, 39.3, 40.0, 46.6, 54.7, 57.0, 64.8, 66.6, 125.6 (CH=), 133.9 (CH=); IR ν_{max} 3359, 3018, 2927, 1446, 1003 cm^{-1}.

[0161] Compound **21b** was obtained as white crystals: mp 215–217 °C (EtOAc-hexanes); ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 4.28 (m, 1H, CH=CHC*H*OH), 5.57 (d, *J* = 10.2, 1H, CH=C*H*CHOH), 5.85 (m, 1H, C*H*=CHCHOH); ^{13}C NMR δ 11.3, 20.2, 24.9, 28.5, 29.0, 29.8, 31.0, 32.2, 32.3, 34.2, 34.7, 35.9, 36.2, 39.2, 44.2, 46.4, 54.6, 56.4, 65.9, 66.6, 128.4 (CH=), 132.6 (CH=); IR ν_{max} 3328, 3019, 2926, 1446, 1003 cm^{-1}.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-20(22)-en-23-one (21c).

[0162] Activated MnO_2 (420 mg, 4.83 mmol) was added to a mixture of compounds **21a** and **21b** (60 mg, 0.18 mmol) in $CHCl_3$ (20 mL). The reaction mixture was stirred at room temperature for 48 h. The MnO_2 was removed by filtration and the $CHCl_3$ was removed under reduced pressure. The residue was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 20:1) to give compound **21c** (28 mg, 47%) and a recovered mixture of compounds **21a** and **21b** (27 mg).

[0163] Compound **21c** was obtained as white crystals: mp 209–210 °C (EtOAc-hexanes); ^{1}H NMR δ 0.78 (s, 3H, 19-CH_3), 2.37 (d, *J* = 16.2 Hz, 1H, CH_2CO), 4.05 (m, 1H, C*H*OH), 5.90 (d, *J* = 9.9 Hz, 1H, C(O)C*H*=CH), 6.96 (dd, *J* = 5.4, *J* = 9.9 Hz, 1H, C(O)CH=C*H*); ^{13}C NMR δ 11.3, 19.7, 26.3, 28.3, 29.0, 29.4, 32.0, 32.2, 34.2, 34.8, 35.8, 36.2, 38.0, 39.1, 47.3, 47.4, 54.2, 56.1, 66.5, 127.2 (C(O)*C*H=CH), 152.1

(C(O)CH=*C*H), 200.3 (CO); IR ν_{max} 3467, 2926, 1664, 1451, 1004 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-23-one (4a).

[0164] Compound **21c** (28 mg, 0.085 mmol) was dissolved in MeOH (10 mL) and hydrogenated (40 psi, H_2, 5% $Pd/BaSO_4$, 9 mg) for 2 h. The reaction mixture was filtered through a pad of Celite 545® to remove catalyst and the solvent was removed under reduced pressure. The product was purified by column chromatography (silica gel; hexanes/EtOAc, 4:1) to give compound **4a** (26 mg, 94%).

[0165] Compound **4a** was obtained as white crystals: mp 185–186 °C (EtOAc-hexanes); ^1H NMR δ 0.76 (s, 3H, 19-CH_3), 4.04 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 19.8, 24.3, 24.9, 25.2, 28.3, 29.0, 31.9, 32.2, 34.2, 34.9, 35.8, 36.2, 36.5, 39.1, 40.5, 44.7, 48.8, 54.3, 56.3, 66.5, 213.7 (CO); IR ν_{max} 3435, 2921, 1704, 1005 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α)-13,24-Cyclo-18,21-dinorchola-20(22),23-diene-3,20-diol, 3,20-diacetate (23a).

[0166] A mixture of compound **11a** (200 mg, 0.61 mmol), NaI (366 mg, 2.44 mmol) and Ac_2O (9 mL) was cooled to 0 °C under N_2 and Me_3SiCl (0.31 mL, 2.44 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 24 h and then poured into 10% $NaHCO_3$ (50 mL). After the mixture was stirred for 10 min, the product was extracted using hexanes (4 × 50 mL). The combined extracts were washed with 10% $NaHCO_3$ (5 mL), 10% $Na_2S_2O_3$ (2 × 10 mL), water, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 30:1) to give compound **23a** (173 mg, 69%).

[0167] Compound **23a** was obtained as white crystals: mp 135–136 °C (hexanes); ^1H NMR δ 0.82 (s, 3H, 19-CH_3), 2.06 (s, 3H, CHOC(O)C*H*$_3$), 2.15 (s, 3H, =COC(O)C*H*$_3$), 5.02 (m, 1H, CHOAc), 5.56 (d, *J* = 5.7 Hz, 1H, CH=COAc), 5.65 (d, *J* = 9.6 Hz, 1H, C*H*=CHCH=), 5.84 (dd, *J* = 5.7 Hz, *J* = 9.6 Hz, 1H, CH=C*H*CH=); ^{13}C

NMR δ 11.4, 20.1, 21.2, 21.5, 26.1, 28.4, 29.7, 32.3 (2 × C), 32.9, 33.0, 35.9, 36.0, 37.5, 40.1, 47.9, 48.4, 54.2, 56.2, 70.1, 107.9 (*C*H=COAc), 122.4 (=*C*HCH=COAc), 127.7 (C*C*H=CH), 154.6 (=*C*OAc), 169.1 (O*C*(O)CH_3), 170.7 (O*C*(O)CH_3); IR ν_{max} 3039, 2932, 1763, 1734, 1204. Anal. ($C_{26}H_{36}O_4$) C, H.

(3α,5α,20*R*)-13,24-Cyclo-18,21-dinorchol-23-en-3,20-diol, 3-acetate (24a) and (3α,5α,20*S*)-13,24-Cyclo-18,21-dinorchol-23-en-3,20-diol, 3-acetate (24b).

[0168] Compound **23a** (170 mg, 0.41 mmol) was dissolved in EtOH (15 mL), and $NaBH_4$ (78 mg, 2.05 mmol) was added. The solution was stirred under N_2 for 6 h. Most of the EtOH was removed under reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with 5% HCl (10 mL), aqueous $NaHCO_3$ (10 mL), brine (10 mL), and dried over Na_2SO_4. The solvent was removed and the residue obtained was filtered through a short column (silica gel; hexanes/EtOAc, 8:1) to give a mixture of compounds **24a** and **24b** (140 mg, 92%). Analytical samples of compounds **24a** and **24b** were obtained by column chromatography (silica gel; hexanes/EtOAc, 20:1).

[0169] Compound **24a** was obtained as white crystals: mp 159–161 °C; ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3C(O)O), 4.10 (m, 1H, C*H*OH), 5.01 (m, 1H, CHOAc), 5.54 (m, 1H, =C*H*CH_2), 5.66 (d, 1H, *J* = 10.5 Hz, C*H*=CHCH_2); ^{13}C NMR δ 11.3, 20.6, 20.7, 21.5, 25.8, 26.0, 28.3, 30.7, 32.2, 32.8, 32.9, 35.3, 35.9, 38.6, 40.0, 47.9, 51.0, 54.5, 55.1, 66.8, 70.0, 124.7 (CH=), 129.6 (CH=), 170.6 (C(O)O); IR ν_{max} 3399, 3023, 2929, 1734, 1245. Anal. ($C_{24}H_{36}O_3$) C, H.

[0170] Compound **24b** was obtained as white crystals: mp 168–169 °C; ^{1} HNMR δ 0.83 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3C(O)O), 4.06 (m, 1H, C*H*OH), 5.02 (m, 1H, CHOAc), 5.65 (m, 1H, CH_2C*H*=), 5.86 (d, *J* = 10.5 Hz, 1H, CC*H*=); ^{13}C NMR δ 11.3, 20.7, 21.5, 25.8, 26.1 (2 × C), 28.3, 30.0, 32.2, 32.8 (2 × C), 34.9, 35.9, 39.7, 40.0, 42.8, 50.1, 54.2, 55.7, 68.4, 70.1, 124.0 (CH=), 129.7 (CH=), 170.7 (C(O)O); IR ν_{max} 3442, 3021, 2931, 1735, 1249 cm^{-1}. Anal. ($C_{24}H_{36}O_3$) C, H.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-23-ene (28a).

[0171] A mixture of compounds **24a** and **24b** (150 mg, 0.40 mmol), Ph_3P (195 mg, 0.74 mmol), imidazole (101 mg, 1.48 mmol) and I_2 (150 mg, 0.59 mmol) in toluene (15 mL) was heated at 95 °C for 1.5 h. The toluene was removed under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2) to give a mixture of compounds **26a** and **26b** in quantitative yield. This product mixture was used without further purification or characterization.

[0172] The mixture of compounds **26a** and **26b**, *i*-PrOH (0.20 mL) and HMPA (0.50 mL) in THF (1 mL) was added to a freshly made SmI_2-THF solution (0.1 M, 10 mL) under Ar. The purple solution was stirred at room temperature for 30 min and quenched with saturated aqueous NH_4Cl (1mL). The solution was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with saturated aqueous NH_4Cl, water, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 30:1) to give compound **28a** contaminated with about 20% of the 20(22),23-diene. The mixture was dissolved in acetone (15 mL) and a 2% solution of 4-phenyl-1,2,4-triazoline-3,5-dione in acetone was added dropwise at 0 °C until the pink color persisted. After all of the diene was reacted with 4-phenyl-1,2,4-triazoline-3,5-dione (monitored by TLC), the solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **28a** (94 mg, 66% from compound **24a** and **24b**) as a single spot on TLC (silica gel plate). NMR analysis showed that product **28a** contained ~10% of another inseparable and unidentified isomeric olefin product.

[0173] Compound **28a** was obtained as a white solid: mp 117–119 °C; ^1H NMR δ 0.82 (s, 3H, 19-CH_3), 2.05 (s, 3H, $CH_3C(O)O$), 5.01 (m, 1H, CHOAc), 5.67 (bs, 2H, CH=CH); ^{13}C NMR δ 11.4, 20.5, 20.9, 21.2, 21.5, 24.5, 26.1, 26.1, 28.4, 32.3, 32.9 (2 × C), 35.0, 35.9, 38.5, 40.1, 43.3, 43.8, 54.5, 55.1, 70.1, 128.0 (CH=), 129.7 (CH=), 170.7 (C(O)O); IR ν_{max} 3019, 2932, 1736, 1446 cm^{-1}. Anal. ($C_{24}H_{36}O_2$) C, H.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-23-en-22-one (29a).

[0174] A solution of CrO_3 (280 mg, 2.8 mmol) in CH_2Cl_2 (4 mL) was cooled to –15 °C and 3,5-dimethylpyrazole (274 mg, 2.85 mmol) was added in one portion. The resultant brown slurry was stirred for 30 min followed by addition of compound **28a** (40 mg, 0.112 mmol) in CH_2Cl_2 (4 mL). The mixture was stirred at –15 °C for 3 h and a 1:1 mixture of hexanes and ethyl acetate (20 mL) was added. The reaction mixture was filtered through a pad of silica gel. Solvent was removed under reduced pressure to give a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **29a** (28 mg, 67%). This product was contaminated with ~5% of an unidentified impurity which was removed after hydrolysis of the acetate group.

[0175] Partially purified compound **29a** was obtained as white solid: mp 133–138 °C; ^{1}H NMR δ 0.86 (s, 3H, 19-CH_3), 2.07 (s, 3H, $CH_3C(O)O$), 2.41 (dm, *J* = 17.4 Hz, 1H, CH_2CO), 2.62 (dd, *J* = 4.5 Hz, *J* = 17.4 Hz, 1H, CH_2CO), 5.03 (m, 1H, CHOAc), 6.01 (d, *J* = 10.5 Hz, 1H, =CHCO), 6.97 (dd, *J* = 1.5 Hz, *J* = 10.5 Hz, 1H, C*H*=CHCO); ^{13}C NMR δ 11.4, 20.7, 21.5, 26.0, 26.7, 27.5, 28.1, 32.0, 32.8, 32.9, 35.3, 35.9, 36.4, 38.2, 40.0, 43.9, 45.5, 54.2, 56.9, 69.9, 130.1 (=*C*HCO), 152.3 (*C*H=CHCO), 170.6 (C(O)O), 199.7 (CO).

(3α,5α)-3-Hydroxy-13,24-Cyclo-18,21-dinorchol-23-en-22-one (30a).

[0176] Compound **29a** (30 mg, 0.08 mmol) was dissolved in MeOH (2 mL) and 15% aq. NaOH (0.2 mL) was added. The solution was refluxed for 20 min and cooled to room temperature. EtOAc (20 mL) was added and the organic layer was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 20:1) to give compound **30a** (22 mg, 83%).

[0177] Compound **30a** was obtained as white crystals: mp 168–170 °C (Et_2O-EtOAc); ^{1}H NMR δ 0.84 (s, 3H, 19-CH_3), 2.41 (dm, *J* = 17.1 Hz, 1H, CH_2CO), 2.62 (dd, *J* = 4.5 Hz, *J* = 17.1 Hz, 1H, CH_2CO), 4.07 (m, 1H, C*H*OH), 6.00 (d, *J* = 10.2 Hz, 1H, =CHCO), 6.97 (dd, *J* = 1.8 Hz, *J* = 10.2 Hz, 1H, C*H*=CHCO); ^{13}C NMR δ 11.3,

20.7, 26.7, 27.6, 28.4, 29.0, 32.1, 32.2, 35.4, 35.8, 36.2, 36.5, 38.2, 39.1, 43.9, 45.6, 54.3, 56.9, 66.4, 130.1 (=*C*HCO), 152.5 (*C*H=CHCO), 199.9 (CO); IR ν_{max} 3320, 3028, 2926, 1679, 1445. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α,23*R*,24R)-23,24-Epoxy-13,24-cyclo-18,21-dinorcholan-3-ol, acetate (31a).

[0178] A mixture of $NaHCO_3$ (33 mg, 0.40 mmol), *m*-CPBA (103 mg, 0.60 mmol) and compound **28a** (71 mg, 0.20 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 5 hrs. It was washed successively with 5% $Na_2S_2O_3$, 10% $NaHCO_3$ and brine, and dried over Na_2SO_4. After solvent removal under reduced pressure, the residue obtained was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **31a** (67 mg, 90%).

[0179] Compound **31a** was obtained as a white solid: mp 126–128 °C [containing ~10% (23*S*,24*R*)-epoxide]; ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 2.06 (s, 3H, $CH_3C(O)O$), 2.96 (d, *J* = 3.9 Hz, 1H, epoxide 24-H), 3.16 (m, 1H, epoxide 23-H), 5.02 (m, 1H, CHOAc); ^{13}C NMR δ 11.4, 18.3, 19.4, 21.1, 21.5, 24.5, 26.0, 26.1, 28.3, 32.3, 32.8, 32.9, 34.5, 34.7, 35.9, 40.0, 40.7, 41.3, 54.3, 54.4, 54.6, 55.8, 70.0, 170.6 (C(O)O); IR ν_{max} 2930, 1735, 1235 cm^{-1}.

(3α,5α,23*S*,24R)-23-Bromo-13,24-cyclo-18,21-dinorcholan-3,24-diol, 3-acetate (32a).

[0180] Compound **31a** (71 mg, 0.19 mmol) in MeCN (10 mL) was cooled to –40 °C and hydrobromic acid (48%, 0.25 mL) was added dropwise. The solution was stirred at 0 °C for 2 h and then at room temperature for 1 h. EtOAc (50 mL) was added and the organic layer was washed with water to neutral pH, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **32a** (60 mg, 70%).

[0181] Compound **32a** was obtained as a white solid: mp 161.5–163.5 °C; ^{1}H NMR δ 0.77 (s, 3H, 19-CH_3), 2.05 (s, 3H, $CH_3C(O)O$), 3.65 (dd, *J* = 1.8 Hz, *J* = 10.5 Hz, 1H, C*H*OH), 4.32 (m, 1H, CHBr), 5.00 (m, 1H, CHOAc); ^{13}C NMR δ 11.2, 21.6,

22.6, 23.3, 24.8, 24.9, 26.1, 28.4, 31.9, 32.5, 32.9 (2 × C), 33.0, 35.3, 36.1, 40.3, 47.7, 48.2, 54.6, 58.3, 63.7, 70.3, 74.4, 170.7 (C(O)O); IR ν_{max} 3554, 2931, 1733, 1261 cm^{-1}. Anal. ($C_{24}H_{37}BrO_3$) C, H.

(3α,5α,23*S*)-3-(Acetyloxy)-23-bromo-13,24-cyclo-18,21-dinorcholan-24-one (33a).

[0182] Jones reagent was added dropwise to a solution of compound **32a** (58 mg, 0.13 mmol) in acetone (15 mL) at 5 °C until an orange color persisted. The resultant mixture was stirred at 0–5 °C for 1 h. 2-propanol was added to consume excess oxidant and the acetone was removed under reduced pressure. The residue obtained was dissolved in EtOAc (30 mL) and washed with water to neutral pH, and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 20:1) to give compound **33a** (57 mg, 98%).

[0183] Compound **33a** was obtained as a white solid: mp 207–209 °C; ^1H NMR δ 0.76 (s, 3H, 19-CH_3), 2.04 (s, 3H, $CH_3C(O)O$), 4.92 (dd, *J* = 6.0 Hz, *J* = 12.9 Hz, 1H, CHBr), 5.00 (m, 1H, CHOAc); ^{13}C NMR δ 11.5, 21.5, 21.6, 23.6, 25.2, 25.4, 26.0, 28.2, 32.3, 32.8, 32.9, 35.2 (2 × C), 35.8, 36.3, 40.0, 49.3, 54.6, 56.3, 57.0, 59.8, 70.0, 170.6 (C(O)O), 205.0 (CO); IR ν_{max} 2942, 1733, 1240 cm^{-1}. Anal. ($C_{24}H_{35}BrO_3$) C, H.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-22-en-24-one (34a).

[0184] A mixture of compound **33a** (54 mg, 0.12 mmol), Li_2CO_3 (89 mg, 1.2 mmol) and LiBr (42 mg, 0.48 mmol) in DMF was heated at 125 °C under N_2 for 24 h. After cooling to room temperature, EtOAc (30 mL) was added and the organic layer was washed with water until neutral pH, and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 45:1) to give compound **34a** (31 mg, 70%).

[0185] Compound **34a** was obtained as white crystals: mp 122–124 °C; ^1H NMR δ 0.87 (s, 3H, 19-CH_3), 2.05 (s, 3H, $CH_3C(O)O$), 5.02 (m, 1H, CHOAc), 5.80

(m, 1H, =CHCO), 6.62 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 11.5, 20.3, 21.5, 24.3, 26.1, 26.7, 27.7, 28.3, 32.7 (2 × C), 32.9, 33.1, 35.4, 36.0, 40.3, 45.1, 53.5, 54.3, 57.2, 70.2, 129.4 (=*C*HCO), 144.4 (=*C*HCH_2), 170.6 (C(O)O), 204.0 (CO); IR ν_{max} 3031, 2938, 1734, 1666, 1247 cm^{-1}. Anal. ($C_{24}H_{34}O_3$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-24-one (35a).

[0186] Using the same procedure described for the preparation of compound **30a** from compound **29a**, compound **35a** (21 mg, 80%) was prepared from compound **34a** (30 mg, 0.08 mmol).

[0187] Compound **35a** was obtained as white crystals: mp 242–243 °C; ^{1}H NMR δ 0.86 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 5.80 (m, 1H, =CHCO), 6.62 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 11.3, 20.3, 24.3, 26.8, 27.7, 28.5, 29.1, 32.4, 32.8 (2 × C), 35.5, 36.0, 36.3, 39.4, 45.1, 53.5, 54.4, 57.2, 66.6, 129.4 (=*C*HCO), 144.5 (=*C*HCH_2), 204.1 (CO); IR ν_{max} 3439, 3031, 2908, 1647 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-24-one (5a).

[0188] Using the same procedure described for the preparation of compound **2a** from compound **11a**, compound **5a** (37 mg, 92%) was prepared from compound **35a** (40 mg, 0.12 mmol).

[0189] Compound **5a** was obtained as white crystals: mp 203–204 °C (Et_2O-hexanes); ^{1}H NMR δ 0.75 (s, 3H, 19-CH_3), 4.04 (m, 1H, C*H*OH); ^{13}C NMR δ 11.3, 21.6, 23.9, 23.9, 24.0, 25.4, 28.5, 29.0, 32.3, 32.6, 35.1, 35.3, 35.9, 36.2, 39.3, 40.2, 49.7, 54.9, 56.4, 58.5, 66.6, 216.7 (CO); IR ν_{max} 3317, 2923, 1708, 1003 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorcholan-20-one (36).

[0190] Compound **2a** (100 mg, 0.303 mmol) was dissolved in pyridine (1mL), and Ac_2O (0.3 mL) was added. The mixture was stirred at room temperature for 20 h and then poured into saturated aqueous $NaHCO_3$ (10 mL). After stirring at room temperature for 10 min, the product was extracted with EtOAc. The combined

EtOAc extracts were washed with 3% HCl, 5% $NaHCO_3$ and water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **36** (105 mg, 94%).

[0191] Compound **36** was obtained as white crystals: mp 197-198 °C (hexanes); IR ν_{max} 2938, 1732, 1696, 1244 cm^{-1}; ^1H NMR δ 0.80 (s, 3H), 2.05 (s, 3H), 2.50 (m, 1H), 5.01 (m, 1H); ^{13}C NMR δ 11.4, 20.4, 21.5, 22.2 (2 × C), 25.1, 26.1, 27.0, 28.2, 32.1, 32.8 (2 × C), 33.5, 35.2, 35.9, 37.4, 40.0, 49.6, 53.9, 56.8, 61.6, 70.0, 170.7, 215.5.

(3α,5α,20*R*)-3-Hydroxy-13,24-cyclo-18,21-dinorcholane-20-carbonitrile (39) and (3α,5α,20*S*)-3-Hydroxy-13,24-cyclo-18,21-dinorcholane-20-carbonitrile (40).

[0192] To the solution of compound **36** (53 mg, 0.142 mmol) in anhydrous THF (8 mL) was added diethyl cyanophosphonate (100 mg, 0.570 mmol) and LiCN (0.5 M solution in DMF, 1.14 mL, 0.57 mmol). The mixture was stirred at room temperature for 3h and the solvent was removed under reduced pressure. The residue obtained was then purified by column chromatography (silica gel; hexanes/EtOAc, 4:1) to give compound **37** as a colorless oil that was used without further separation.

[0193] The mixture of compound **37** and MeOH (0.12 mL) in THF (3 mL) was added to a freshly made SmI_2-THF solution (0.1 M, 20 mL) under Ar. The resultant dark blue solution was stirred at room temperature overnight and then cooled to 0 °C. 3% HCl (5 mL) was added to quench the reaction. Solvent was removed under reduced pressure and the residue was dissolved in EtOAc and washed with 5% $NaHCO_3$, 5% $Na_2S_2O_3$ and brine and dried over Na_2SO_4. After solvent removal under reduced pressure, compound **38** was obtained and it was dissolved in MeOH (8 mL). Then, 2.5 N NaOH (1.2 mL) was added. The solution obtained was stirred at room temperature overnight and MeOH was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with water, 3% HCl and water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which

was purified by column chromatography (silica gel; dichloroethane) to give compound **39** (25 mg, 51% from **36**) and compound **40** (22 mg, 45% from **36**).

[0194] Compound **39** was obtained as white crystals: mp 213-214 °C (EtOAc-hexanes); IR ν_{max} 3409, 2929, 2232, 1443, 1001 cm^{-1}; ^1H NMR δ 0.76 (s, 3H), 2.75 (m, 1H), 4.04 (m, 1H); ^{13}C NMR δ 11.2, 20.0, 20.3, 21.7, 22.9, 23.5, 24.8, 27.3, 28.4, 29.0, 31.9, 32.2, 32.8, 34.6, 35.9, 36.2, 39.1, 41.7, 46.8, 54.3, 56.8, 66.5, 122.5.

[0195] Compound **40** was obtained as white crystals: mp 250-251 °C (EtOAc-hexanes); IR ν_{max} 3319, 2922, 2234, 1448, 1009 cm^{-1}; ^1H NMR δ 0.77 (s, 3H), 2.42 (m, 1H), 2.76 (m, 1H), 4.04 (m, 1H); ^{13}C NMR δ 11.2, 17.9, 20.2, 22.5, 23.6, 23.8, 25.9, 27.1, 28.4, 29.0, 32.0, 32.1, 34.1, 34.5, 35.8, 36.2, 39.1, 41.5, 46.9, 54.3, 57.1, 66.5, 123.3.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorcholan-22-one (41).

[0196] Using the same procedure described for the preparation of compound **36**, compound **41** (60 mg, 97%) was prepared from compound **3a** (55 mg, 0.167 mmol).

[0197] Compound **41** was obtained as white crystals: mp 178-180 °C (hexanes); IR ν_{max} 2936, 1733, 1237 cm^{-1}; ^1H NMR δ 0.80 (s, 3H), 2.06 (s, 3H), 2.39 (dt, J = 5.7, 14.4 Hz, 1H), 2.54 (dd, J = 5.7, 14.4 Hz, 1H), 5.02 (m, 1H); ^{13}C NMR δ 11.4, 20.4, 21.5, 23.4, 23.7, 26.1, 27.7, 28.2, 32.0, 32.7, 32.9, 32.9, 35.6, 35.9, 37.0, 40.1, 41.3, 41.6, 49.0, 54.3, 55.6, 70.0, 170.6, 213.3.

(3α,5α,22*S*)-3-Hydroxy-13,24-cyclo-18,21-dinorcholane-22-carbonitrile (44) and (3α,5α,22*R*)-3-Hydroxy-13,24-cyclo-18,21-dinorcholane-22-carbonitrile (45).

[0198] Using the same procedure described for the preparation of compound **39** and **40**, compound **44** (14 mg, 26% from **41**) and compound **45** (36 mg, 68% from **41**) were prepared from compound **41** (58 mg, 0.156 mmol). Compounds **44** and **45** were separated by HPLC (silica gel, hexanes/EtOAc, 25:1, 2 mL/min).

[0199] Compound **44** was obtained as white crystals: mp 199-200 °C (Et_2O-hexanes); IR ν_{max} 3345, 2926, 2238, 1444, 1003 cm^{-1}; ^1H NMR δ 0.76 (s, 3H), 2.02

(m, 1H), 2.58 (m, 1H), 4.04 (m, 1H); ^{13}C NMR δ 11.2, 20.1, 21.9, 23.8, 24.0, 25.0, 25.2, 28.4 (2 × C), 29.0, 32.0, 32.2, 32.9, 34.7, 35.9, 36.2, 39.2, 40.5, 44.7, 54.4, 56.7, 66.5, 123.3.

[0200] Compound **45** was obtained as white crystals: mp 244-246 °C (CH_2Cl_2-EtOAc-hexanes); IR ν_{max} 3513, 2929, 2234, 1456, 1016 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3H), 2.20 (m, 1H), 2.85 (m, 1H), 4.05 (m, 1H); ^{13}C NMR δ 11.2, 19.1, 20.1, 23.4, 23.6, 23.9, 25.8, 25.9, 28.5, 29.0, 32.0, 32.2, 33.1, 34.6, 35.9, 36.2, 39.2, 41.1, 44.5, 54.5, 56.9, 66.5, 124.2.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorcholan-23-one (46).

[0201] Using the same procedure described for the preparation of compound **36**, compound **46** (59 mg, 95%) was prepared from compound **4a** (55 mg, 0.167 mmol).

[0202] Compound **46** was obtained as white crystals: mp 183-185 °C (hexanes); IR ν_{max} 2931, 1734, 1712, 1247, 1235 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3H), 2.05 (s, 3H), 2.50 (m, 1H), 5.01 (m, 1H); ^{13}C NMR δ 11.4, 19.8, 21.5, 24.3, 24.9, 25.2, 26.0, 28.1, 31.8, 32.8, 32.8, 34.2, 34.8, 35.9, 36.4, 40.0, 40.5, 44.7, 48.7, 54.2, 56.3, 70.0, 170.5, 213.5.

(3α,5α,20*R*)-3-Hydroxy-13,24-cyclo-18,21-dinorcholane-23-carbonitrile (49) and (3α,5α,20*S*)-3-Hydroxy-13,24-cyclo-18,21-dinorcholane-23-carbonitrile (50).

[0203] Using the same procedure described for the preparation of compounds **39** and **40**, compound **49** (27 mg, 53% from **46**) and compound **50** (15 mg, 29% from **46**) were prepared from compound **46** (56 mg, 0.150 mmol). Compounds **49** and **50** were separated by HPLC (silica gel, hexanes/EtOAc, 6:1, 2 mL/min).

[0204] Compound **49** was obtained as white crystals: mp 182-183 °C (EtOAc-hexanes); IR ν_{max} 3343, 2929, 2232, 1451, 1003 cm^{-1}; ^{1}H NMR δ 0.79 (s, 3H), 2.66 (m, 1H), 2.88 (m, 1H), 4.05 (m, 1H); ^{13}C NMR δ 11.2, 20.1, 20.9, 22.9, 23.7, 24.0, 24.0, 24.7, 28.5, 29.1, 32.1, 32.1, 34.2, 34.6, 35.8, 36.2, 39.2, 41.0, 44.7, 54.5, 58.1, 66.5, 123.8.

[0205] Compound **50** was obtained as white crystals: mp 157-159 °C (Et_2O-hexanes); IR ν_{max} 3401, 2927, 2239, 1446, 1003 cm^{-1}; ^1H NMR δ 0.79 (s, 3H), 2.43 (m, 1H), 4.05 (m, 1H); ^{13}C NMR δ 11.3, 20.1, 23.4, 23.8, 24.3, 24.7, 24.8, 27.1, 28.4, 29.0, 32.0, 32.2, 33.1, 34.6, 35.9, 36.2, 39.2, 41.4, 44.3, 54.5, 57.0, 66.5, 123.5.

(3α,5α)-20-Methylene-13,24-cyclo-18,21-dinorcholan-3-ol (51).

[0206] A suspension of NaH (60% in mineral oil, 75 mg, 1.88 mmol) in anhydrous DMSO (3 mL) was heated at 75 °C for 45 min and cooled to room temperature. Methyltriphenylphosphonium bromide (690 mg, 1.93 mmol) in DMSO (2 mL) was added and stirred at room temperature for 10 min. Compound **2a** (127 mg, 0.385 mmol) in DMSO (1.5 mL) was then added. The mixture obtained was heated at 70 °C for 4 h and cooled to room temperature. Brine was added and the product was extracted with EtOAc. The combined EtOAc extracts were washed with water and brine and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 5:1) to give compound **51** (116 mg, 92%).

[0207] Compound **51** was obtained as a white solid: mp 211-213 °C (EtOAc-hexanes); IR ν_{max} 3295, 2927, 1645, 1436, 1001 cm^{-1}; ^1H NMR δ 0.77 (s, 3H), 4.04 (m, 1H), 4.61 (m, 2H); ^{13}C NMR δ 11.2, 20.4, 22.6, 23.2, 24.7, 27.4, 28.6, 29.1, 30.9, 32.3 (2 × C), 33.3, 35.0, 35.9, 36.2, 39.2, 44.9, 54.5, 55.9, 56.9, 66.6, 107.8, 150.2.

(3α,5β)-20-Methylene-13,24-cyclo-18,21-dinorcholan-3-ol (52).

[0208] Using the same procedure described for the preparation of compound **51**, compound **52** (41 mg, 82%) was prepared from compound **2b** (50 mg, 0.152 mmol).

[0209] Compound **52** was obtained as a white solid: mp 131-133 °C (hexanes); IR ν_{max} 3299, 2927, 1645, 1451, 1040 cm^{-1}; ^1H NMR δ 0.91 (s, 3H), 3.63 (m, 1H), 4.61 (m, 2H); ^{13}C NMR δ 20.4, 22.5, 23.1, 23.4, 24.7, 26.7, 27.1, 27.5, 30.5, 30.9, 33.4, 34.7, 35.3, 35.4, 36.4, 40.6, 42.1, 44.9, 55.9, 56.9, 71.8, 107.8, 150.1.

(3α,5α)-22-Methylene-13,24-cyclo-18,21-dinorcholan-3-ol (53).

[0210] Using the same procedure described for the preparation of compound **51**, compound **53** (17 mg, 43%) was prepared from compound **3a** (40 mg, 0.121 mmol).

[0211] Compound **53** was obtained as a white solid: mp 187-188 °C (hexanes); IR ν_{max} 3312, 2926, 1450 cm^{-1}; ^1H NMR δ 0.77 (s, 3H), 4.04 (m, 1H), 4.58 (s, 1H), 4.69 (s, 1H); ^{13}C NMR δ 11.2, 20.3, 23.9, 24.4, 25.5, 28.5, 29.1, 30.5, 32.2, 32.2, 33.0, 34.3, 34.9, 35.9, 36.2, 39.3, 41.3, 47.7, 54.6, 56.8, 66.6, 108.3, 147.9.

(3α,5α)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorcholan-20-one (54).

[0212] Chloromethyl methyl ether (84 mg, 1.05 mmol) was added to a solution of compound **2a** (115 mg, 0.35 mmol) and *N,N*-diisopropyl ethylamine (0.30 mL, 1.75 mmol) in CH_2Cl_2 (10 mL). The resultant solution was stirred at room temperature for 20 h. The solvent was partially removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 15:1) to give compound **54** (126 mg, 97%).

[0213] Compound **54** was obtained as white crystals: mp 138-139 °C (Hexanes); IR ν_{max} 2928, 1702, 1446, 1045 cm^{-1}; ^1H NMR δ 0.79 (s, 3H), 2.50 (m, 1H), 3.36 (s, 3H), 3.83 (m, 1H), 4.65 (m, 2H); ^{13}C NMR δ 11.3, 20.3, 22.1 (2 × C), 25.0, 26.2, 26.9, 28.4, 32.1, 32.7, 33.5, 33.5, 35.1, 35.8, 37.3, 39.6, 49.5, 53.9, 55.0, 56.7, 61.4, 71.5, 94.5, 215.3.

(3α,5α)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorchol-20(22)-en-20-ol trifluoromethanesulfonate (55).

[0214] A solution of compound **54** (128 mg, 0.342 mmol) and *N*-phenyltrifuoromethanesulfonimide (367 mg, 1.03 mmol) in anhydrous THF (20 mL) was cooled to -78 °C and KHMDS (0.5 M solution in toluene, 1.37 mL, 0.685 mmol) was added. The mixture was stirred at room temperature for 1h and saturated aqueous NH_4Cl (1 mL) was added. The product was extracted with ether and the combined ether extracts were washed with aqueous NH_4Cl and brine and dried over

Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **55** (169 mg, 97%). Compound **55** was partially characterized. It had ^1H NMR δ 0.79 (s, 3H), 3.37 (s, 3H), 3.83 (m, 1H), 4.66 (m, 2H), 5.61 (m, 1H).

(3α,5α)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorchol-20(22)-ene-20-carbonitrile (56).

[0215] A mixture of compound **55** (165 mg, 0.333 mmol), triethylamine (3 mL), trimethylsilyl cyanide (0.5 mL) and tetrakis(triphenylphosphine)palladium(0) (96.3 mg, 0.083 mmol) in benzene (10 mL) was refluxed under N_2 for 20 h. The solvent was partially removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 30:1) to give compound **56** (45 mg, 35%).

[0216] Compound **56** was obtained as white crystals: mp 136-138 °C (hexanes); IR ν_{max} 2927, 2212, 1456, 1041 cm^{-1}; ^1H NMR δ 0.79 (s, 3H), 3.37 (s, 3H), 3.83 (m, 1H), 4.66 (m, 2H), 6.50 (t, 1H, J = 3.6 Hz); ^{13}C NMR δ 11.4, 18.5, 19.9, 22.6, 24.4, 26.3, 28.5, 29.6, 32.0, 32.8, 32.8, 33.6, 34.8, 35.9, 39.7, 41.1, 47.4, 54.3, 55.1, 55.5, 71.5, 94.5, 116.5, 119.9, 142.6.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-20(22)-ene-20-carbonitrile (57).

[0217] 12 N HCl (0.6 mL) was added to a solution of compound **56** (41 mg, 0.106 mmol) in MeOH (3 mL) and CH_2Cl_2 (0.5 mL) and stirred at room temperature for 2 h. EtOAc (20 mL) was added and the organic phase was washed with water, 5% $NaHCO_3$ and brine and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 8:1) to give compound **57** (32 mg, 89%).

[0218] Compound **57** was obtained as white crystals: mp 193-195 °C (EtOAc-hexanes); IR ν_{max} 3426, 2925, 2212, 1455, 1002 cm^{-1}; ^1H NMR δ 0.79 (s, 3H), 4.04 (m, 1H), 6.50 (t, 1H, J = 3.6 Hz); ^{13}C NMR δ 11.2, 18.5, 19.9, 22.6, 24.4, 28.4, 29.0, 29.6, 32.0, 32.2, 32.7, 34.8, 35.8, 36.2, 39.1, 41.1, 47.4, 54.3, 55.5, 66.4, 116.4, 119.9, 142.6.

(3α,5α)-13,24-Cyclo-18,21-dinorchol-20(22),23-dien-3-ol acetate (59).

[0219] Mesyl chloride (20 μL, 0.258 mmol) was added to a solution of compound **24a** (24 mg, 0.065 mmol) and triethylamine (80 μL, 0.581 mmol) in CH_2Cl_2 (5 mL) at 0 °C and stirred at room temperature for 1h. The reaction mixture was washed with water and 3% HCl and water and dried over Na_2SO_4. The solvent was removed under reduced pressure to give crude mesylate **58**. It was dissolved in toluene (2 mL) and DBU (0.1 mL) was added. The mixture was refluxed for 5 h and cooled to room temperature. The reaction mixture was washed with 3% HCl and water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 20:1) to give compound **59** (19 mg, 83% from **24a**).

[0220] Compound **59** was obtained as a colorless oil; IR ν_{max} 3032, 2932, 1736, 1445, 1246 cm^{-1}; ^1H NMR δ 0.83 (s, 3H), 2.06 (s, 3H), 5.02 (m, 1H), 5.73-5.80 (m, 2H), 5.88 (m, 1H), 5.95 (m, 1H); ^{13}C NMR δ 11.4, 20.1, 21.5, 26.1, 28.4, 30.1, 32.4, 32.9, 33.0, 33.2, 35.7, 35.9, 37.4, 40.1, 44.5, 44.9, 54.3, 56.3, 70.1, 121.4, 124.1, 130.0, 131.1, 170.7.

(3α,5α)-13,24-Cyclo-18,21-dinorchol-20(22),23-dien-3-ol (60).

[0221] $LiAlH_4$ (10 mg, 0.263 mmol) was added to a solution of compound **59** (20 mg, 0.056 mmol) in anhydrous THF (5 mL) and stirred at room temperature for 20 min. The reaction mixture was cooled to 0 °C and water (0.1 mL) was added dropwise to quench the reaction. The precipitate that formed was filtered through a pad of Celite 545. The solvent was removed from the filtrate and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **60** (16 mg, 91%).

[0222] Compound **60** was obtained as a white solid: mp 136-138 °C (Hexanes); IR ν_{max} 3318, 3034, 2928, 1445, 1004 cm^{-1}; ^1H NMR δ 0.82 (s, 3H), 4.05 (m, 1H), 5.74-5.80 (m, 2H), 5.88 (m, 1H), 5.95 (m, 1H); ^{13}C NMR δ 11.2, 20.1, 28.6,

29.1, 30.1, 32.3, 32.5, 33.2, 35.7, 35.9, 36.2, 37.5, 39.2, 44.5, 44.9, 54.4, 56.3, 66.6, 121.3, 124.0, 130.1, 131.2.

(3α,5α)-13,24-Cyclo-18,21-dinorchol-23-en-3-ol (61).

[0223] Using the same procedure described for the preparation of compound **60**, compound **61** (45 mg, 85%) was prepared from compound **28a** (60 mg, 0.169 mmol).

[0224] Compound **61** was obtained as a white solid: mp 166.5–169 °C (hexanes); ^{1}H NMR δ 0.81 (s, 3H), 4.04 (m, 1H), 5.68 (bs, 2H); ^{13}C NMR δ 11.2, 20.5, 20.9, 21.3, 24.6, 26.2, 28.6, 29.1, 32.2, 32.4, 35.1, 35.9, 36.3, 38.5, 39.2, 43.4, 43.8, 54.7, 55.2, 66.6, 127.9, 129.8.

(3α,5α,20*E*)-3-Methoxymethoxy-13,24-cyclo-18-norcholan-20-ene-21-carbonitrile (62).

[0225] Compound **54** (50 mg, 0.134 mmol) and (triphenylphosphoranylidene)acetonitrile (100 mg, 0.332 mmol) was heated at 160 °C under N_2 for 15 h and then purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **62** (37 mg, 70%).

[0226] Compound **62** was obtained as white crystals: mp 159-161 °C (hexanes); IR ν_{max} 2928, 2215, 1625, 1446, 1041 cm^{-1}; ^{1}H NMR δ 0.77 (s, 3H), 2.07 (t, 1H, *J* = 9.0 Hz), 2.22 (m, 1H), 2.76 (m, 1H), 3.37 (s, 3H), 3.83 (m, 1H), 4.66 (m, 1H), 5.03 (d, 1H, *J* = 1.8 Hz); ^{13}C NMR δ 11.4, 20.3, 22.4, 22.7, 24.7, 26.3, 28.0, 28.5, 29.0, 32.1, 32.8, 33.2, 33.6, 35.1, 35.9, 40.0, 47.4, 54.2, 55.1, 56.4, 57.0, 71.6, 92.5, 94.5, 117.2, 170.1.

(3α,5α,20*E*)-3-Hydroxy-13,24-cyclo-18-norcholan-20-ene-21-carbonitrile (63).

[0227] Using the same procedure described for the preparation of compound **57**, compound **63** (34 mg, 94%) was prepared from compound **62** (41 mg, 0.103 mmol).

[0228] Compound **63** was obtained as white crystals: mp 210-212 °C (ether-hexanes); IR ν_{max} 3308, 2928, 2217, 1444 cm^{-1}; ^1H NMR δ 0.77 (s, 3H), 2.07 (t, 1H, *J* = 9.0 Hz), 2.22 (m, 1H), 2.77 (m, 1H), 4.04 (m, 1H), 5.04 (d, 1H, *J* = 1.5 Hz); ^{13}C NMR δ 11.2, 20.3, 22.4, 22.7, 24.7, 28.0, 28.4, 28.9, 29.0, 32.2 (2 × C), 33.2, 35.0, 35.8, 36.1, 39.1, 47.4, 54.2, 56.4, 56.9, 66.5, 92.5, 117.2, 170.0.

(3α,5α,20*E*)-3-Hydroxy-13,24-cyclo-18-norcholan-20-ene-21-carbonitrile (63) and (3α,5α,20*Z*)-3-Hydroxy-13,24-cyclo-18-norcholan-20-ene-21-carbonitrile (64).

[0229] Diethyl cyanomethylphosphonate (0.78 mL, 4.83 mmol) was added dropwise to the mixture of NaH (60% in mineral oil, 175 mg, 4.38 mmol) and THF (10 mL) at room temperature and refluxed for 10 min. Compound **2a** (280 mg, 0.85 mmol) in THF (20 mL) was then added and reflux was continued for 20 min. The reaction mixture was then cooled to room temperature. EtOAc (100 mL) was added and the organic phase was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue that was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 40:1) to give compound **63** (164 mg, 55%) and compound **64** (109 mg, 36%).

[0230] Compound **63** was obtained as white crystals: mp. 214-216 °C (EtOAc-hexanes); ^1H NMR δ 0.77 (s, 3H), 2.07 (t, 1H, *J* = 9.0 Hz), 2.22 (m, 1H), 2.77 (dm, 1H, *J* = 15.6 Hz), 4.04 (m, 1H), 5.04 (d, 1H, *J* = 1.5 Hz); ^{13}C NMR δ 11.1, 20.2, 22.2, 22.6, 24.6, 27.9, 28.3, 28.9 (2 × C), 32.1 (2 × C), 33.1, 34.9, 35.7, 36.0, 39.0, 47.3, 54.1, 56.3, 56.8, 66.3, 92.4, 117.2, 170.0; IR ν_{max} 3308, 2928, 2217, 1444 cm^{-1}.

[0231] Compound **64** was obtained as white crystals: mp. 201.5-203 °C (ether-hexanes); ^1H NMR δ 0.77 (s, 3H), 2.18 (m, 1H), 2.32 (m, 1H), 2.69 (t, 1H, *J* = 9.3 Hz), 4.04 (m, 1H), 5.05 (d, 1H, *J* = 1.8 Hz); ^{13}C NMR δ 11.2, 20.2, 22.3, 23.0, 24.7, 27.3, 28.4, 29.0, 31.8, 32.1, 32.1, 32.9, 35.0, 35.8, 36.1, 39.1, 47.4, 53.0, 54.1, 56.8, 66.4, 92.8, 117.1, 170.1; IR ν_{max} 3401, 2927, 2215, 1621, 1445 cm^{-1}.

(3α,5α,20*S*)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorcholan-20-ol (65) and (3α,5α,20*R*)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorcholan-20-ol (66).

[0232] The mixture of compound **18a** (180 mg, 0.48 mmol) and $NaBH_4$ (60 mg, 1.58 mmol) in EtOH (10 mL) was stirred at room temperature for 30 min and EtOH was removed under reduced pressure. EtOAc (30 mL) was added and the solution was washed with water, 5% HCl and water and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) gave compound **65** (55 mg, 30%) and compound **66** (115 mg, 64%).

[0233] Compound **65** was obtained as white solid: mp. 131-133 °C; ^{1}H NMR δ 0.78 (s, 3H), 2.29 (m, 1H), 3.37 (s, 3H), 3.82 (m, 1H), 3.91 (bs, 1H), 4.66 (m, 2H); ^{13}C NMR δ 11.4, 15.5, 20.4, 23.0, 24.1, 25.4, 26.3, 28.5, 28.8, 32.0, 32.8, 33.6, 34.4, 35.5, 35.9, 39.7, 41.1, 53.0, 54.4, 55.1, 57.3, 69.7, 71.6, 94.5; IR ν_{max} 3369, 2923, 1445, 1040 cm^{-1}.

[0234] Compound **66** was obtained as white solid: mp. 136-138 °C; ^{1}H NMR δ 0.77 (s, 3H), 1.97 (m, 1H), 3.37 (s, 3H), 3.82 (m, 1H), 3.87 (m, 1H), 4.66 (m, 2H); ^{13}C NMR δ 11.4, 20.2, 20.4, 20.6, 22.2, 23.6, 26.3, 28.5, 30.0, 32.1, 32.8, 33.6 (2 × C), 34.9, 36.0, 39.8, 44.4, 52.6, 54.6, 55.1, 57.1, 69.7, 71.6, 94.5; IR ν_{max} 3401, 2923, 1446, 1041 cm^{-1}.

(3α,5α,20*S*)-13,24-cyclo-18,21-dinorcholane-3,20-diol (67).

[0235] 37% aqueous HCl (0.6 mL) was added the solution of compound **65** (25 mg, 66 μmol) in CH_3OH (2 mL). The solution was stirred at room temperature for 1 h and EtOAc (30 mL) was added. It was washed with water, 10% $NaHCO_3$, and brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 6:1) to give compound **67** (21 mg, 95%).

[0236] Compound **67** was obtained as white crystals: mp 261-262 °C ($CHCl_3$-hexanes); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.77 (s, 3H), 2.29 (m, 1H), 3.90 (m, 1H), 4.01 (m, 1H); ^{13}C NMR δ 11.1, 15.4, 20.3, 22.8, 24.0, 25.3, 28.4, 28.5, 28.6, 32.0, 32.0,

34.3, 35.2, 35.6, 36.0, 39.0, 40.9, 52.6, 54.3, 57.2, 66.2, 69.4; IR ν_{max} 3307, 2916, 1443, 1001 cm^{-1}.

(3α,5α,20*R*)-13,24-cyclo-18,21-dinorcholane-3,20-diol (68).

[0237] Using the same procedure described for the preparation of compound **67** from compound **65**, compound **68** (20 mg, 91%) was prepared from compound **66** (25 mg, 66 μmol).

[0238] Compound **68** was obtained as white crystals: mp 269-271 °C (methanol); ^1H NMR ($CDCl_3/CD_3OD$) δ 0.78 (s, 3H), 2.00 (m, 1H), 3.85 (m, 1H), 3.98 (m, 1H); ^{13}C NMR δ 11.7, 20.9, 21.1 (2 × C), 22.8, 24.2, 29.2 (2 × C), 29.9, 32.8, 32.9, 34.3, 35.6, 36.2, 36.8, 39.8, 44.9, 53.2, 55.4, 57.8, 66.7, 69.9; IR ν_{max} 3338, 2929, 1444, 1049 cm^{-1}.

(3α,5α,20*S*)-20-Methoxy-13,24-cyclo-18,21-dinorcholan-3-ol (69).

[0239] The solution of compound **65** (30 mg, 80 μmol) in THF (3 mL) was added to the slurry of NaH (60% in mineral oil, 16 mg, 0.40 mmol) in THF (2 mL) and stirred for 10 min. MeI (27 μL, 0.44 mmol) was added and refluxed overnight. The same amount of NaH and MeI was added again and refluxed for another 1 h and cooled to room temperature. Water was added to the reaction mixture and it was extracted with EtOAc. The combined EtOAc extracts were washed with water to neutral pH and then dried over Na_2SO_4. Solvent was removed under reduced pressure and the oil obtained was dissolved in MeOH (3 mL) and 37% HCl (1 mL) was added and stirred at room temperature for 2 h. EtOAc (30 mL) was added and it was washed with water and brine to neutral pH and then dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue purified by column chromatography (silica gel; hexanes/EtOAc, 8:1) gave compound 69 (24 mg, 87%).

[0240] Compound **69** was obtained as white crystals: mp 182-183 °C (EtOAc-hexanes); ^1H NMR δ 0.76 (s, 3H), 2.25 (m, 1H), 3.28 (s, 3H), 3.32 (m, 1H), 4.03 (m, 1H); ^{13}C NMR δ 11.2, 16.1, 20.2, 23.1, 24.2, 25.4 (2 × C), 28.5, 29.0, 32.1 (2 × C),

34.5, 34.8, 35.9, 36.2, 39.2, 41.3, 48.6, 54.5, 56.0, 57.4, 66.6, 78.7; IR ν_{max} 3306, 2927, 1446, 1089 cm^{-1}.

(3α,5α,20*R*)-20-Methoxy-13,24-cyclo-18,21-dinorcholan-3-ol (70).

[0241] Using the same procedure described for the preparation of compound **69** from compound **65**, compound **70** (29 mg, 90%) was prepared from compound **66** (35 mg, 93 μmol).

[0242] Compound **70** was obtained as white crystals: mp 200-202 °C (hexanes); ^1H NMR δ 0.77 (s, 3H), 1.97 (m, 1H), 3.31 (s, 3H), 3.39 (m, 1H), 4.04 (m, 1H); ^{13}C NMR δ 11.2, 20.2, 20.4, 20.7, 22.5, 23.6, 26.6, 28.5, 29.0, 32.1, 32.2, 33.8, 34.9, 35.9, 36.2, 39.2, 44.1, 49.3, 54.6, 55.4, 57.1, 66.5, 78.5; IR ν_{max} 3293, 2921, 1445, 1103 cm^{-1}.

(3α,5α,20*R*,22*S*)-20,22-Epoxy-13,24-cyclo-18,21-dinorcholan-3-ol (71) and (3α,5α,20*S*,22*R*)-20,22-epoxy-13,24-cyclo-18,21-dinorcholan-3-ol (72).

[0243] The mixture of compound **13a** (96 mg, 0.31 mmol), $NaHCO_3$ (46 mg, 0.55 mmol) and *m*-CPBA (77% max, 106 mg, 0.47 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 4 h. The solution was washed with water, 5% $NaHCO_3$, 5% $Na_2S_2O_3$ and water and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 50:1) gave compound **71** (33 mg, 33%) and compound **72** (54 mg, 54%).

[0244] Compound **71** was obtained as white crystals: mp 206-208 °C (EtOAc-hexanes); ^1H NMR δ 0.75 (s, 3H), 3.05 (dd, 1H, *J* = 1.5, 3.9 Hz), 3.18 (dd, 1H, *J* = 3.9, 6.3 Hz), 4.03 (m, 1H); ^{13}C NMR δ 11.1, 18.8, 20.0, 20.4, 24.3, 24.6, 28.5, 29.0, 32.1 (2 × C), 34.3, 34.9, 35.8, 36.1, 39.1, 41.5, 44.7, 52.3, 54.4, 55.9, 56.7, 66.5; IR ν_{max} 3400, 2927, 1435 cm^{-1}.

[0245] Compound **72** was obtained as white crystals: mp 203.5-205 °C (acetone-hexanes); ^1H NMR δ 0.77 (s, 3H), 3.13 (m, 1H), 3.22 (m, 1H), 4.03 (m, 1H); ^{13}C NMR δ 11.1, 15.5, 19.8, 20.1, 24.3, 25.1, 28.5, 28.9, 32.2 (2 × C), 33.8, 34.8,

35.8, 36.2, 39.1, 39.8, 43.8, 53.1, 53.6, 54.4, 55.7, 66.4; IR ν_{max} 3233, 2921, 1432, 1000 cm^{-1}.

(3α,5α,20*S*)-20,21-Epoxy-13,24-cyclo-18-norcholan-3-ol (73) and (3α,5α,20*R*)-20,21-Epoxy-13,24-cyclo-18-norcholan-3-ol (74).

[0246] Using the same procedure described for the preparation of compound **71** and **72** from compound **13a**, compound **73** (30 mg, 48%) and compound **74** (26 mg, 41%) was prepared from compound **51** (60 mg, 0.18 mmol). Compounds **73** and **74** were separated by column chromatography (silica gel; CH_2Cl_2/EtOAc, 30:1).

[0247] Compound **73** was obtained as white crystals: mp. 186-192 °C (EtOAc-hexanes, decomposed); ^1H NMR δ 0.78 (s, 3H), 2.00 (m, 1H), 2.25 (m, 1H), 2.48 (d, 1H, *J* = 4.8 Hz), 2.46 (d, 1H, *J* = 4.8 Hz), 4.04 (m, 1H); ^{13}C NMR δ 11.2, 19.2, 20.2, 22.2, 24.4, 24.9, 28.5, 29.0, 29.3, 32.1, 32.2, 33.8, 34.7, 35.8, 36.2, 39.1, 43.6, 51.4, 53.4, 54.4, 56.8, 58.9, 66.5; IR ν_{max} 3307, 2927, 1445 cm^{-1}.

[0248] Compound **74** was obtained as white crystals: mp. 206-211 °C (EtOAc-hexanes, decomposed); ^1H NMR δ 0.78 (s, 3H), 2.02 (m, 2H), 2.59 (d, 1H, *J* = 5.4 Hz), 2.55 (dd, 1H, *J* = 1.5, 5.4 Hz), 4.05 (m, 1H); ^{13}C NMR δ 11.2, 20.2, 21.3, 22.2, 23.6, 24.2, 28.4, 28.9, 29.3, 32.1, 32.2, 33.5, 35.0, 35.9, 36.2, 39.1, 45.7, 53.5, 54.4, 55.7, 56.8, 59.4, 66.5; IR ν_{max} 3306, 2928, 1455 cm^{-1}.

(3α,5α)-3-Methoxymethoxy-21-methyl-13,24-cyclo-18-norchol-20-ene (75).

[0249] Ethyltriphenylphosphonium bromide (870 mg, 2.35 mmol) was added to the mixture of $KOBu^t$ (240 mg, 2.14 mmol) in THF (12 mL) at room temperature and refluxed for 30 min. Compound **18a** (165 mg, 0.44 mmol) in THF (10 mL) was then added and refluxed for another 30 min and cooled to room temperature. Water was added to the reaction mixture and it was extracted with EtOAc. The combined EtOAc extracts were washed with water and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 50:1) gave compound **75** (166 mg, 98%) as a colorless oil. It is a mixture of the two olefin isomers in the ratio >10:1.

[0250] The Main isomer has: ^{1}H NMR δ 0.78 (s, 3H), 1.55 (dd, 3H, *J* = 2.4, 6.6 Hz), 1.93 (m, 2H), 2.17 (m, 1H), 2.38 (m, 1H), 3.37 (s, 3H), 3.82 (m, 1H), 4.66 (m, 2H), 5.21 (m, 1H); ^{13}C NMR δ 11.4, 12.3, 20.3, 23.2, 23.3, 24.6, 26.1, 26.3, 28.6, 32.2, 32.6, 32.9, 33.4, 33.6, 34.9, 36.0, 39.8, 44.4, 48.3, 54.5, 55.1, 57.0, 71.6, 94.5, 116.8, 140.0; IR ν_{max} 2927, 1445, 1044 cm^{-1}.

(3α,5α,20*S*)-1-[3-Methoxymethoxy-13,24-cyclo-18,21-dinorcholan-20-yl]ethanone (77) and (3α,5α,20*R*)-1-[3-Methoxymethoxy-13,24-cyclo-18,21-dinorcholan-20-yl]ethanone (78).

[0251] BH_3 (1.0 M in THF, 1.28 mL, 1.28 mmol) was added to compound **75** (165 mg, 0.43 mmol) in anhydrous THF (15 mL) under N_2 at 0 °C. The resultant solution was stirred at room temperature for 3.5 h and cooled to 0 °C. Water (0.1 mL) was added to quench the reaction followed by aqueous NaOH (3 N, 2.6 mL) and 30% H_2O_2 (2.6 mL). The reaction mixture was stirred at ambient temperature overnight and extracted with EtOAc. The combined EtOAc extracts were washed with brine until neutral pH and then dried over Na_2SO_4. After solvent removal under reduced pressure, the residue **76** (a mixture of four diastereomers) was used without further purification or characterization.

[0252] Jones reagent was added dropwise to a solution of compound **76** in acetone (15 mL) at 0 °C until an orange color persisted. The resultant mixture was stirred at 0-5 °C for 10 min. 2-propanol was added to consume excess oxidant and the acetone was removed under reduced pressure. The residue obtained was dissolved in EtOAc (30 mL), washed with water to neutral pH, and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give a mixture of compound **77** and **78** (152 mg, 88%) in the ratio of 3:2. The compounds were further separated by column chromatography (silica gel; hexanes/EtOAc, 30:1).

[0253] Compound **77** was obtained as white solid: mp. 106-107 °C; ^{1}H NMR δ 0.75 (s, 3H), 2.16 (s, 3H), 2.42 (bs, 1H), 3.36 (s, 3H), 3.82 (m, 1H), 4.65 (m, 2H); ^{13}C NMR δ 11.3, 18.1, 20.1, 21.6, 22.6, 23.8, 26.2, 27.7, 28.1, 28.5, 32.0, 32.8, 33.6,

33.9, 34.6, 35.9, 39.7, 41.6, 46.0, 49.6, 54.3, 55.1, 57.4, 71.6, 94.5, 211.6; IR ν_{max} 3400, 2928, 1708, 1041 cm^{-1}.

[0254] Compound **78** was obtained as white solid: mp. 103-105 °C; ^1H NMR δ 0.78 (s, 3H), 2.06 (m, 1H), 2.10 (s, 3H), 2.61 (m, 1H), 3.37 (s, 3H), 3.83 (m, 1H), 4.66 (m, 2H); ^{13}C NMR δ 11.4, 20.2, 20.5, 21.6, 21.9, 22.4, 23.7, 26.3, 27.9, 28.5, 32.0, 32.9, 33.3, 33.6, 34.7, 36.0, 39.8, 42.6, 47.5, 49.4, 54.6, 55.1, 56.7, 71.6, 94.5, 211.8; IR ν_{max} 3392, 2923, 1707, 1041 cm^{-1}.

(3α,5α,20*S*)-1-[3-Hydroxy-13,24-cyclo-18,21-dinorcholan-20-yl]ethanone (79).

[0255] The mixture of compound **77** (32 mg, 80 μmol), $LiBF_4$ (64 mg, 0.68 mmol), H_2O (0.1 mL) and MeCN (3 mL) was refluxed for 3 h and cooled to room temperature. EtOAc (30 mL) was added and the solution was washed with water and brine and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 7:1) to give compound **79** (27 mg, 95%).

[0256] Compound **79** was obtained as white crystals: mp. 187-189 °C (acetone-hexanes); ^1H NMR δ 0.75 (s, 3H), 2.16 (s, 3H), 2.42 (m, 1H), 4.03 (m, 1H); ^{13}C NMR δ 11.1, 18.1, 20.1, 21.6, 22.6, 23.8, 27.7, 28.1, 28.5, 29.0, 32.0, 32.1, 33.9, 34.6, 35.8, 36.1, 39.1, 41.6, 46.0, 49.6, 54.3, 57.4, 66.5, 211.7; IR ν_{max} 3401, 2928, 1705 cm^{-1}.

(3α,5α,20*R*)-1-[3-Hydroxy-13,24-cyclo-18,21-dinorcholan-20-yl]ethanone (80).

[0257] Using the same procedure described for the preparation of compound **79** from compound **77**, compound **80** (29 mg, 93%) was prepared from compound **78** (35 mg, 87 μmol).

[0258] Compound **80** was obtained as white crystals: mp. 228-229 °C (EtOAc-hexanes); ^1H NMR δ 0.78 (s, 3H), 1.83 (m, 1H), 2.05 (m, 1H), 2.10 (s, 3H), 2.62 (m, 1H), 4.04 (m, 1H); ^{13}C NMR δ 11.2, 20.2, 20.5, 21.6, 21.9, 22.4, 23.7, 27.9, 28.5, 29.0, 32.1, 32.2, 33.3, 34.7, 35.9, 36.2, 39.2, 42.6, 47.5, 49.4, 54.6, 56.7, 66.5, 211.8; IR ν_{max} 3401, 2927, 1699 cm^{-1}.

(3α,5α)-1-[3-Methoxymethoxy-13,24-cyclo-18,21-dinorchol-20(22)-en-20-yl]ethanone (82).

[0259] The mixture of compounds **77** and **78** (100 mg, 0.25 mmol) and NBS (73 mg, 0.41 mmol) in CCl_4 (15 mL) was refluxed under N_2 while being irradiated with a 300 W tungsten lamp for 20 min and then cooled to room temperature. The solvent was partially removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 20:1) to give compound **81** (80 mg, 67%) as a mixture of two diastereomers in the ratio of 1:1.

[0260] The mixture of compound **81** (80 mg, 0.17 mmol), Li_2CO_3 (160 mg, 2.16 mmol), and LiBr (80 mg, 0.93 mmol) in DMF (3 mL) was heated at 130 °C under N_2 for 2 h and cooled to room temperature. EtOAc (30 mL) was added and the mixture was washed with water, 3% HCl and water and dried over Na_2SO_4. The solvent was removed and the residue purified by column chromatography (silica gel; hexanes/EtOAc, 20:1) gave compound **82** (42 mg, 63%).

[0261] Compound **82** was obtained as white crystals: mp. 157-159 °C (hexanes); ^{1}H NMR δ 0.80 (s, 3H), 2.27 (s, 3H), 3.37 (s, 3H), 3.83 (m, 1H), 4.66 (m, 2H), 6.78 (t, 1H, J = 3.6 Hz); ^{13}C NMR δ 11.5, 18.8, 20.0, 23.2, 24.6, 25.5, 26.3, 28.6, 30.5, 32.1, 32.8, 32.9, 33.6, 35.0, 36.0, 39.8, 41.0, 44.4, 54.5, 55.2, 55.5, 71.6, 94.5, 138.7, 143.7, 199.4; IR ν_{max} 2928, 1667, 1435, 1042 cm^{-1}.

(3α,5α)-1-[3-Hydroxy-13,24-cyclo-18,21-dinorchol-20(22)-en-20-yl]ethanone (83).

[0262] Using the same procedure described for the preparation of compound **67** from compound **65**, compound **83** (16 mg, 95%) was prepared from compound **82** (19 mg, 48 □mol).

[0263] Compound **83** was obtained as white solid: mp. 198-200 °C (ether-hexanes); ^{1}H NMR δ 0.79 (s, 3H), 2.27 (s, 3H), 4.04 (m, 1H), 6.78 (t, 1H, J = 3.9 Hz); ^{13}C NMR δ 11.2, 18.8, 20.0, 23.2, 24.6, 25.5, 28.5, 29.0, 30.5, 32.1, 32.2, 32.8, 35.0,

35.9, 36.2, 39.2, 41.0, 44.4, 54.5, 55.5, 66.5, 138.7, 143.7, 199.4; IR ν_{max} 3315, 2924, 1667, 1252 cm^{-1}.

(3β,5α)-3-Acetyloxy-21-diazopregnan-20-one (85).

[0264] Oxalyl chloride (0.12 mL, 1.38 mmol) was added to compound **84** (100 mg, 0.28 mmol) in benzene at 0 °C under N_2 and stirred at room temperature for 4 h. Removal of solvent under reduced pressure gave the acid chloride as a solid. It was used without further purification.

[0265] The acid chloride in ether (10 mL) was added to diazomethane (0.2 M in ether, 6 mL) dropwise at 0 °C and stirred at ambient temperature overnight. The solvent was removed under reduced pressure in a hood and the yellow residue obtained was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **85** (84 mg, 79%).

[0266] Compound **85** was obtained as a yellow solid: mp. 135-136 °C (133-137 °C in Pettit noted below); ^1H NMR δ 0.66 (s, 3H), 0.82 (s, 3H), 2.02 (s, 3H), 4.68 (m, 1H), 5.18 (s, 1H); ^{13}C NMR δ 12.1, 13.3, 21.0, 21.4, 22.8, 24.4, 27.3, 28.4, 31.9, 33.9, 35.4 (2 × C), 36.7, 38.6, 44.6, 44.8, 54.1, 54.7, 56.3, 61.4, 73.5, 170.6, 195.1; IR ν_{max} 2937, 2099, 1731, 1637, 1367, 1246 cm^{-1}. (See also, Pettit, G. R., et al., Steroids and Related Natural Products. LIV. Bufadienolides. 7. Synthesis of 3β-Acetoxy-5α-l4α-bufa-20,22-dienolide, *J. Org. Chem.* 1970, *35*, 1398-1404).

(3β,5α)-3-Acetyloxy-18,21-cyclopregnan-20-one (86).

[0267] Compound **85** (600 mg, 1.55 mmol) in CH_2Cl_2 (10 mL) was added to $Rh_2(OCOCF_3)_4$ in CH_2Cl_2 (50 mL) and stirred at room temperature for 10 min. Most solvent was removed under reduced pressure and the residue purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) gave compound **86** (300 mg, 54%).

[0268] Compound **86** was obtained as white crystals: mp. 146-148 °C; ^1H NMR δ 0.82 (s, 3H), 2.02 (s, 3H), 4.69 (m, 1H); ^{13}C NMR δ 12.2, 21.4, 21.6, 24.2,

26.2, 27.4, 27.7, 28.3, 32.1, 33.9, 34.3, 35.5, 35.7, 36.8, 37.0, 44.5, 51.9, 53.5, 55.5, 58.5, 73.5, 170.7, 222.6; IR ν_{max} 2933, 1733, 1244, 1027 cm^{-1}.

(3β,5α)-3-Hydroxy-18,21-cyclopregnan-20-one (87).

[0269] Compound **86** (300 mg, 0.84 mmol) was dissolved in MeOH (20 mL) and 20% aqueous NaOH (1.0 mL) was added. The solution was refluxed for 20 min and cooled to room temperature. MeOH was removed under reduced pressure and EtOAc (50 mL) was added. The organic layer was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue that was purified by column chromatography (silica gel; hexanes/EtOAc, 5:1) to give compound **87** (250 mg, 94%).

[0270] Compound **87** was obtained as white crystals: mp. 182-184 °C; ^1H NMR δ 0.80 (s, 3H), 3.59 (m, 1H); ^{13}C NMR δ 12.2, 21.6, 24.1, 26.2, 27.6, 28.4, 31.3, 32.2, 34.3, 35.5, 35.6, 36.9, 36.9, 38.0, 44.7, 51.8, 53.5, 55.5, 58.4, 71.0, 222.6; IR ν_{max} 3413, 2923, 1721, 1447, 1042 cm^{-1}.

(3α,5α)-3-Benzoyloxy-18,21-cyclopregnan-20-one (88).

[0271] DEAD (40% solution in toluene) was added dropwise to the mixture of compound **87** (250 mg, 0.79 mmol), Ph_3P (250 mg, 0.95 mmol) and $PhCO_2H$ (116 mg, 0.95 mmol) in THF (20 mL) until the yellow color persisted. The yellowish solution was stirred at room temperature for 2 h and most of THF was removed under reduced pressure. The residue obtained was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **88** (290 mg, 87%).

[0272] Compound **88** was obtained as white solid: mp. 201-204 °C; ^1H NMR δ 0.85 (s, 3H), 5.29 (m, 1H), 7.46 (t, 2H, J = 7.2 Hz), 7.57 (t, 1H, J = 7.2 Hz), 8.07 (d, 2H, J = 7.2 Hz); ^{13}C NMR δ 11.4, 21.2, 24.2, 26.2, 26.2, 27.7, 28.1, 32.1, 32.9, 33.2, 34.3, 35.7, 35.9, 37.0, 40.3, 51.9, 53.6, 55.6, 58.5, 70.5, 128.3 (2 × C), 129.5 (2 × C), 131.1, 132.7, 165.8, 222.5; IR ν_{max} 2930, 1735, 1714, 1450, 1272, 1113, 714 cm^{-1}.

(3α,5α)-3-Hydroxy-18,21-cyclopregnan-20-one (89).

[0273] Compound **88** (290 mg, 0.69 mmol) was dissolved in EtOH (20 mL) and 20% aqueous NaOH (0.8 mL) was added. The solution was refluxed for 20 min and cooled to room temperature. EtOH was removed under reduced pressure and EtOAc (50 mL) was added. The organic layer was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue that was purified by column chromatography (silica gel; hexanes/EtOAc, 5:1) to give compound **89** (210 mg, 96%).

[0274] Compound **89** was obtained as white crystals: mp. 176-178.5 °C (EtOAc-hexanes); ^{1}H NMR δ 0.78 (s, 3H), 4.05 (m, 1H); ^{13}C NMR δ 11.2, 21.2, 24.2, 26.2, 27.7, 28.3, 28.9, 32.2 (2 × C), 34.4, 35.7, 35.8, 36.1, 37.0, 39.0, 51.9, 53.6, 55.7, 58.5, 66.4, 222.7; IR ν_{max} 3307, 2923, 1736, 1448 cm^{-1}.

(3α,5α)-3-Hydroxy-18,21-cyclopregn-18(21)-en-20-one (94) and (3α,5α)-21-bromo-3-hydroxy-18,21-cyclopregn-18(21)-en-20-one (95).

[0275] The mixture of compound **88** (168 mg, 0.40 mmol) and pyridinium tribromide (180 mg, 0.56 mmol) in THF (20 mL) was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure and EtOAc (20 mL) was added. The EtOAc solution was washed with water, 5% $Na_2S_2O_3$ and water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue that was passed through a short column (silica gel; hexanes/EtOAc, 5:1) to give a mixture of 21-bromo-20-one (90) and 21,21-dibromo-20-one (**91**). The mixture of compounds was used without further separation.

[0276] The mixture of compounds **90** and **91** (160 mg, 0.32 mmol), Li_2CO_3 (300 mg), LiBr (150 mg) in DMF (5 mL) was stirred at 130 °C for 6 h and cooled to room temperature. EtOAc (50 mL) was added and the organic layer was washed with water, 5% HCl and water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the residue was separated by column chromatography (silica gel; hexanes/EtOAc, 12:1) to give partially purified compound **92** (34 mg, 25%) and compound **93** (55 mg, 41%).

[0277] Using the same procedure described for the preparation of compound **89** from compound **88**, compound **94** (22 mg, 86%) was prepared from compound **92** (34 mg, 81 μmol) and compound **95** (32 mg, 77%) was prepared from compound **93** (55 mg, 131 μmol).

[0278] Compound **94** was obtained as white crystals: mp. 139-141 °C (EtOAc-hexanes); ^{1}H NMR δ0.86 (s, 3H), 2.31 (d, 1H, *J* = 11.1 Hz), 4.07 (m, 1H), 6.19 (d, 1H, *J* = 6.0 Hz), 7.73 (d, 1H, *J* = 6.0 Hz); ^{13}C NMR δ 11.2, 21.9, 26.0, 28.3, 28.8, 29.0, 32.1, 32.3, 35.7, 35.8, 36.0, 36.3, 39.0, 54.0, 54.2, 54.6, 57.4, 66.3, 134.7, 167.3, 212.4; IR ν_{max} 3412, 2926, 1709, 1448 cm^{-1}.

[0279] Compound **95** was obtained as white crystals: mp. 220-222.5 °C (EtOAc-hexanes); ^{1}H NMR δ 0.87 (s, 3H), 2.49 (d, 1H, *J* = 11.4 Hz), 4.08 (m, 1H), 7.81 (s, 1H); ^{13}C NMR δ 11.3, 21.9, 26.0, 28.2, 29.0 (2 × C), 32.1, 32.2, 35.7, 35.7, 35.8, 36.3, 38.9, 53.2, 54.1 (2 × C), 57.0, 66.3, 125.6, 164.7, 203.7; IR ν_{max} 3538, 2919, 1717, 1446 cm^{-1}. MS (ESI) *m/z* 415 (M^++Na^+), 417 (M^++2+Na^+).

(3α,5α,20*E*)-3-Hydroxy-18,21-cyclopregnan-20-ylideneacetonitrile (96) and (3α,5α,20*Z*)-3-Hydroxy-18,21-cyclopregnan-20-ylideneacetonitrile (97).

[0280] Using the same procedure described for the preparation of compound **63** and **64** from compound **2a**, compound **96** (39 mg, 48%) and **97** (32 mg, 40%) was prepared from compound **89** (75 mg, 0.24 mmol). Compounds **96** and **97** were separated by column chromatography (silica gel; CH_2Cl_2/EtOAc, 30:1).

[0281] Compound **96** was obtained as white crystals: mp. 201-202.5 °C (acetone-hexanes); ^{1}H NMR δ 0.76 (s, 3H), 2.05 (m, 1H), 2.46 (m, 1H), 2.67 (m, 2H), 4.05 (m, 1H), 5.12 (s, 1H); ^{13}C NMR δ 11.2, 21.5, 26.1, 27.3, 28.3, 29.0, 31.8, 31.9, 32.2 (2 × C), 34.7, 35.8, 36.1, 37.0, 39.0, 53.6, 55.2, 55.3, 56.4, 66.4, 89.9, 117.5, 178.7; IR ν_{max} 3306, 2927, 2214, 1634, 1448 cm^{-1}.

[0282] Compound **97** was obtained as white crystals: mp. 226-228 °C (acetone-hexanes); ^{1}H NMR δ 0.75 (s, 3H), 2.28 (m, 1H), 2.53 (m, 2H), 2.77 (m, 1H), 4.04 (m, 1H), 5.09 (d, 1H, *J* = 1.8 Hz); ^{13}C NMR δ 11.2, 21.5, 26.0, 27.5, 28.3, 28.9,

30.9, 32.2 (2 × C), 32.8, 34.6, 35.8, 36.1, 37.0, 39.0, 53.6, 54.9, 55.2, 55.3, 66.4, 89.7, 117.7, 179.0; IR ν_{max} 3401, 2922, 2215, 1446 cm^{-1}.

Physical properties and spectroscopic data for evaluated compounds in the 5β-series

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (2b).

[0283] Compound **2b** was obtained as white crystals: mp 220–222 °C (EtOAc-hexanes); ^1H NMR δ 0.93 (s, 3H, 19-CH_3), 2.50 (m, 1H, CHCO), 3.63 (m, 1H, C*H*OH); ^{13}C NMR δ 20.3, 22.1 (2 × C), 23.3, 25.1, 26.7, 27.0, 27.1, 30.5, 33.7, 34.6, 35.4, 35.5, 36.3, 37.3, 40.2, 42.0, 49.6, 56.7, 61.5, 71.6, 215.5 (CO); IR ν_{max} 3449, 2921, 1698, 1460, 1037 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-22-one (3b).

[0284] Compound **3b** was obtained as white crystals: mp 175–177 °C (EtOAc-hexanes); ^1H NMR δ 0.93 (s, 3H, 19-CH_3), 2.38 (m, 1H, CH_2CO), 2.54 (dd, *J* = 6.0 Hz, *J* = 14.4 Hz, 1H, CH_2CO), 3.65 (m, 1H, C*H*OH); ^{13}C NMR δ 20.4, 23.3 (2 × C), 23.6, 26.4, 27.0, 27.7, 30.4, 32.8, 34.6, 35.4, 35.9, 36.3, 36.9, 40.6, 41.2, 41.5, 42.0, 49.0, 55.5, 71.6, 213.4 (CO); IR ν_{max} 3418, 2936, 1714, 1447 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-23-one (4b).

[0285] Compound **4b** was obtained as white crystals: mp 197-198 °C (Ether-hexanes); ^{1}H NMR δ 0.91 (s, 3H, 19-CH_3), 2.50 (m, 1H, CH_2CO), 3.64 (m, 1H, C*H*OH); ^{13}C NMR δ 19.8, 23.4, 24.4, 24.9, 25.2, 26.4, 26.9, 30.5, 34.4, 34.7, 35.2, 35.3, 36.3, 36.5, 40.5 (2 × C), 42.0, 44.7, 48.9, 56.3, 71.7, 213.7 (CO); IR ν_{max} 3398, 2927, 1709, 1448, 1039 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-24-one (5b).

[0286] Compound **5b** was obtained as white crystals: mp 177–178 °C (Et_2O-hexanes); ^{1}H NMR δ 0.89 (s, 3H, 19-CH_3), 3.64 (m, 1H, C*H*OH); ^{13}C NMR δ 21.7, 23.6, 23.9 (2 × C), 24.0, 25.5, 27.0, 27.1, 30.5, 34.7, 35.3, 35.4, 35.6, 36.5, 40.2, 41.1, 42.2, 49.8, 56.4, 58.5, 71.8, 216.6 (CO); IR ν_{max} 3334, 2937, 1701, 1450, 1037 cm^{-1}. Anal. ($C_{22}H_{34}O_2$).

(3α,5β)-3-(Acetyloxy)-20-hydroxypregnane-20-carbonitrile (7b).

[0287] Compound **7b** was prepared using a reported method (*See*, Slomp, G., Jr. 3,20-Dioxo-Δ^4-steriod 20-cyanohydrins. U.S. Patent 2,655,517, 1953. Chem. Abstr. 48:60638) and was obtained as a white solid: mp 168–178 °C; ^{1}H NMR δ 0.95 (s, 3H, 19-CH_3), 0.99 (s, 3H, 18-CH_3), 1.63 (s, 3H, 21-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 4.73 (m, 1H, CHOAc); ^{13}C NMR δ 13.0, 20.5, 21.4, 23.3, 24.1, 25.1, 26.2, 26.6, 26.9, 30.7, 32.2, 34.6, 35.0, 35.2, 40.3, 40.4, 41.8, 43.6, 55.9, 59.2, 71.7, 74.3, 121.9 (CN), 170.6 (C(O)O).

(3α,5β)-3-(Acetyloxy)-20-oxo-pregnane-18-carbonitrile (8b).

[0288] Compound **8b** was obtained as a colorless oil: ^{1}H NMR δ 0.94 (s, 3H, 19-CH_3), 2.04 (s, 3H, $CH_3C(O)O$), 2.29 (s, 3H, CH_3CO), 2.72 (t, *J* = 9.3 Hz, 1H, C*H*$COCH_3$), 4.73 (m, 1H, CHOAc); ^{13}C NMR δ 16.3, 20.6, 21.3, 23.1, 23.2, 23.9, 26.0, 26.5, 26.6, 32.1, 32.4, 34.4, 34.8, 35.8, 36.1, 40.1, 41.4, 46.1, 56.3, 62.0, 73.8, 118.0 (CN), 170.4 (C(O)O), 208.6 (CO).

(3α,5β)-3-(Acetyloxy)-20,20-[1,2-ethanediylbis(oxy)]pregnane-18-carbonitrile (9b).

[0289] Compound **9b** was obtained as white crystals: mp 138–140 °C; ^{1}H NMR δ 0.94 (s, 3H, 19-CH_3), 1.29 (s, 3H, 21-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 2.24 (d, *J* = 16.8 Hz, 1H, CH_2CN), 2.51 (d, *J* = 16.8 Hz, 1H, CH_2CN), 4.04 (m, 4H, OCH_2CH_2O), 4.72 (m, 1H, C*H*OAc); ^{13}C NMR δ 16.7, 20.5, 21.4, 23.2, 23.2, 23.3, 23.5, 25.9, 26.6, 26.8, 32.2, 34.5, 34.9, 35.5, 36.8, 40.3, 41.6, 43.8, 56.1, 56.5, 63.1, 63.8, 74.1, 110.8 (20-C), 119.8 (CN). 170.5 (C(O)O).

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one (11b).

[0290] Compound **11b** was obtained as white crystals: mp 253–254 °C (EtOAc); ^{1}H NMR δ 0.93 (s, 3H, 19-CH_3), 3.63 (m, 1H, C*H*OH), 5.96 (dd, *J* = 3.0 Hz, *J* = 9.9 Hz, 1H, =CHCO), 6.83 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 20.3, 23.3, 25.8, 26.3, 26.5, 26.9, 27.3, 30.5, 34.1, 34.6, 35.3, 35.6, 36.3, 40.1, 42.0, 47.6, 56.2, 57.8, 71.6, 127.4 (=*C*HCO), 148.3 (=*C*HCH_2), 202.2 (CO); IR ν_{max} 3430, 2921, 1663, 1038 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5β)-13,24-Cyclo-18,21-dinorchol-20(22)-en-3-ol (13b).

[0291] Compound **13b** was obtained as white crystals: mp 168–169 °C (hexanes); ^{1}H NMR δ 0.94 (s, 3H, 19-CH_3), 3.63 (m, 1H, C*H*OH), 5.54 (m, 1H, CH=), 5.76 (m, 1H, CH=); ^{13}C NMR δ 20.0, 20.2, 21.6, 23.4, 24.8, 26.7, 27.2, 30.6, 30.6, 33.4, 34.7, 35.1, 35.5, 36.5, 40.8, 41.0, 42.2, 46.6, 56.1, 71.8, 124.4 (CH=), 130.8 (CH=).

(3α,5β)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorchol-20(22)-ene (14b).

[0292] Compound **14b** was obtained as a colorless oil: ^{1}H NMR δ 0.93 (s, 3H, 19-CH_3), 3.37 (s, 3H, OCH_3), 3.53 (m, 1H, C*H*OCH_2O), 4.69 (s, 2H, OCH_2O), 5.54 (m, 1H, CH=), 5.75 (m, 1H, CH=); ^{13}C NMR δ 19.7, 20.1, 21.6, 23.4, 24.7, 26.7, 27.2, 27.7, 30.6, 33.3, 33.6, 34.8, 35.0, 35.4, 40.7, 40.9, 42.2, 46.6, 55.1, 56.1, 76.8, 94.5 (OCH_2O), 124.4 (CH=), 130.7 (CH=).

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-20(22)-en-23-one (22c).

[0293] Compound **22c** was obtained as white crystals: mp 209–211 °C (EtOAc-hexanes); ^{1}H NMR δ 0.92 (s, 3H, 19-CH_3), 2.35 (d, *J* = 15.9 Hz, 1H, CCH_2CO), 3.64 (m, 1H, C*H*OH), 5.90 (d, *J* = 9.6 Hz, 1H, =CHCO), 6.96 (m, 1H, =C*H*CH);^{13}C NMR δ 19.7, 23.3, 26.3, 26.4, 26.9, 29.4, 30.4, 34.4, 34.6, 35.1, 35.3, 36.3, 37.9, 40.4, 42.0, 47.3, 47.4, 56.0, 71.6, 127.1 (=*C*HCO), 152.0 (=*C*HCH), 200.2 (CO); IR ν_{max} 3446, 2932, 1677, 1451, 1042 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5β)-13,24-Cyclo-18,21-dinorchola-20(22),23-diene-3,20-diol, 3,20-diacetate (23b).

[0294] Compound **23b** was obtained as white crystals: mp 138–139 °C; ^{1}H NMR δ 0.96 (s, 3H, 19-CH_3), 2.03 (s, 3H, CHOC(O)C*H*$_3$), 2.15 (s, 3H, =CHOC(O)C*H*$_3$), 4.74 (m, 1H, CHOAc), 5.56 (d, *J* = 5.7 Hz, 1H, CH=COAc), 5.62 (d, *J* = 9.9 Hz, 1H, C*H*=CHCH=), 5.84 (dd, *J* = 5.7 Hz, *J* = 9.9 Hz, 1H, CH=C*H*CH=); ^{13}C NMR δ 20.1, 21.2, 21.4, 23.3, 26.6, 26.7, 27.0, 29.7, 32.2, 32.4, 34.7, 35.1, 36.3, 37.6, 40.5, 41.8, 47.9, 48.4, 56.1, 74.2, 107.8 (*C*H=COAc), 122.4 (=*C*HCH=COAc), 127.5 (C*C*H=CH), 154.5 (=*C*OAc), 169.0 (CHO*C*(O)CH_3), 170.5 (=CO*C*(O)CH_3).

(3α,5β,20*R*)-13,24-Cyclo-18,21-dinorchol-23-en-3,20-diol, 3-acetate (25a).

[0295] Compound **25a** was obtained as a colorless oil: ^{1}H NMR δ 0.96 (s, 3H, 19-CH_3), 2.04 (s, 3H, CH_3C(O)O), 4.11 (m, 1H, C*H*OH), 4.74 (m, 1H, CHOAc), 5.54 (m, 1H, CH_2C*H*=), 5.63 (d, *J* = 10.2 Hz, 1H, CCH=); ^{13}C NMR δ 20.6, 20.8, 21.4, 23.3, 25.9, 26.6, 26.7, 26.9, 30.7, 32.3, 34.8, 35.1, 35.7, 38.7, 40.8, 41.9, 48.0, 51.1, 55.1, 66.9, 74.3, 124.8 (CH=), 129.5 (CH=), 170.6 (C(O)O).

(3α,5β,20*S*)-13,24-Cyclo-18,21-dinorchol-23-en-3,20-diol, 3-acetate (25b).

[0296] Compound **25b** was obtained as a colorless oil: ^{1}H NMR δ 0.97 (s, 3H, 19-CH_3), 2.04 (s, 3H, CH_3C(O)O), 4.07 (m, 1H, C*H*OH), 4.74 (m, 1H, CHOAc), 5.65 (m, 1H, CH_2C*H*=), 5.84 (d, *J* = 10.8 Hz, 1H, CCH=); ^{13}C NMR δ 20.8, 21.4, 23.3,

25.9, 26.1, 26.6, 26.7, 27.0, 30.0, 32.3, 34.7, 35.0, 35.3, 39.9, 40.5, 41.8, 42.9, 50.1, 55.7, 68.4, 74.3, 124.1 (CH=), 129.6 (CH=), 170.6 (C(O)O).

(3α,5β)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-23-ene (28b).

[0297] Compound **28b** was obtained as a colorless oil: ^{1}H NMR δ 0.96 (s, 3H, 19-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 4.73 (m, 1H, CHOAc), 5.66 (bs, 2H, CH=CH); ^{13}C NMR δ 20.5, 20.8, 21.2, 21.4, 23.3, 24.6, 26.2, 26.6, 26.7, 27.0, 32.2, 34.7, 35.1, 35.4, 38.6, 40.8, 41.9, 43.4, 43.8, 55.1, 74.3, 128.0 (CH=), 129.5 (CH=), 170.5 (C(O)O).

(3α,5β)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-23-en-22-one (29b).

[0298] Compound **29b** was obtained as a white solid (contaminated with 10% unidentified impurity): ^{1}H NMR δ 1.00 (s, 3H, 19-CH_3), 2.04 (s, 3H, $CH_3C(O)O$), 2.41 (dm, *J* = 17.1 Hz, 1H, CH_2CO), 2.64 (dd, *J* = 4.2 Hz, *J* = 17.1 Hz, 1H, CH_2CO), 4.75 (m, 1H, CHOAc), 6.00 (d, *J* = 10.5 Hz, 1H, =CHCO), 6.94 (dd, *J* = 2.1 Hz, *J* = 10.5 Hz, 1H, =CHC); ^{13}C NMR δ 20.8, 21.4, 23.3, 26.4, 26.6, 26.7, 26.8, 27.6, 32.2, 34.7, 35.1, 35.7, 36.6, 38.2, 40.6, 41.8, 44.0, 45.6, 56.8, 74.1, 130.1 (=*C*HCO), 152.1 (=*C*HC), 170.5 (C(O)O), 199.7 (CO).

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-23-en-22-one (30b).

[0299] Compound **30b** was obtained as white crystals: mp 196.5–199 °C; ^{1}H NMR δ 0.99 (s, 3H, 19-CH_3), 2.41 (dm, *J* = 17.1 Hz, 1H, CH_2CO), 2.64 (dd, *J* = 4.2 Hz, *J* = 17.1 Hz, 1H, CH_2CO), 3.67 (m, 1H, C*H*OH), 6.00 (d, *J* = 10.5 Hz, 1H, =CHCO), 6.95 (dd, *J* = 2.1 Hz, *J* = 10.5 Hz, 1H, =CHC), ^{13}C NMR δ 20.8, 23.3, 26.5, 26.8, 27.0, 27.7, 30.5, 34.7, 35.4, 35.7, 36.4, 36.6, 38.2, 40.7, 42.0, 44.0, 45.6, 56.9, 71.6, 130.1 (=CHCO), 152.3 (=*C*HC), 199.8 (CO); IR ν_{max} 3412, 2933, 1677, 1038 cm^{-1}. Anal. ($C_{22}H_{32}O_2$).

(3α,5β,23*R*,24*S*)-23,24-Epoxy-13,24-cyclo-18,21-dinorcholan-3-ol, acetate (31b).

[0300] Compound **31b** was obtained as a white solid: mp 125–129 °C; ^{1}H NMR δ 0.95 (s, 3H, 19-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 2.94 (d, *J* = 3.9 Hz, 1H, epoxy 24-H), 3.16 (m, 1H, epoxy 23-H), 4.74 (m, 1H, CHOAc); ^{13}C NMR δ 18.2, 19.3, 21.2, 21.4, 23.3, 24.6, 26.2, 26.6, 26.7, 26.9, 32.2, 34.7, 34.9 (2 × C), 35.0, 40.7, 40.8, 41.4, 41.8, 54.2, 54.5, 55.8, 74.3, 170.5 (C(O)O).

(3α,5β,23*S*,24*S*)-23-Bromo-13,24-cyclo-18,21-dinorcholan-3,24-diol, 3-acetate (32b).

[0301] Compound **32b** was obtained as a white solid: mp 134–136.5 °C; ^{1}H NMR δ 0.91 (s, 3H, 19-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 3.64 (dd, *J* = 1.8 Hz, *J* = 10.5 Hz, 1H, C*H*OH), 4.32 (m, 1H, CHBr), 4.73 (m, 1H, CHOAc); ^{13}C NMR δ 21.4, 22.6, 23.3, 23.4, 24.9, 24.9, 26.7, 26.9, 27.0, 31.9, 32.3, 33.1, 34.9, 35.2, 35.7, 40.7, 42.1, 47.8, 48.3, 58.3, 63.6, 74.4, 74.5, 170.6 (C(O)O).

(3α,5β,23*S*)-3-(Acetyloxy)-23-bromo-13,24-cyclo-18,21-dinorcholan-24-one (33b).

[0302] Compound **33b** was obtained as a white solid: mp 222–225 °C; ^{1}H NMR δ 0.91 (s, 3H, 19-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 4.72 (m, 1H, CHOAc), 4.90 (dd, *J* = 6.3 Hz, *J* = 13.2 Hz, 1H, CHBr); ^{13}C NMR δ 21.4, 21.8, 23.6, 23.7, 25.2, 25.6, 26.7, 26.8, 26.9, 32.3, 34.7, 35.1, 35.4, 35.6, 36.3, 41.1, 41.9, 49.5, 56.2, 57.1, 59.9, 74.3, 170.5 (C(O)O), 204.9 (CO).

(3α,5β)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-22-en-24-one (34b).

[0303] Compound **34b** was obtained as white crystals: mp 146–148 °C; ^{1}H NMR δ 1.01 (s, 3H, 19-CH_3), 2.03 (s, 3H, $CH_3C(O)O$), 4.74 (m, 1H, CHOAc), 5.80 (dd, *J* = 2.7 Hz, *J* = 10.2 Hz, 1H, =CHCO), 6.63 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 20.3, 21.4, 23.6, 24.4, 26.7 (2 × C), 26.9, 27.0, 27.8, 32.4, 32.8, 34.8, 35.2, 35.7, 40.5, 42.1, 45.2, 53.5, 57.2, 74.5, 129.4 (=CHCO), 144.5 (=$CHCH_2$), 170.5 (C(O)O), 203.8 (CO).

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-24-one (35b).

[0304] Compound **35b** was obtained as white crystals mp 222–223 °C (EtOAc-hexanes); ^{1}H NMR δ 1.00 (s, 3H, 19-CH_3), 3.65 (m, 1H, C*H*OH), 5.80 (dd, 1H, *J* = 3.0 Hz, *J* = 10.2 Hz, =CHCO), 6.62 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 20.3, 23.6, 24.4, 26.7, 27.1 (2 × C), 27.8, 30.6, 32.9, 34.8, 35.5, 35.7, 36.5, 40.5, 42.2, 45.2, 53.5, 57.2, 71.8, 129.4 (=*C*HCO), 144.5 (=*C*HCH_2), 204.0 (CO); IR ν_{max} 3418, 2861, 1661, 1038 cm^{-1}. Anal. ($C_{22}H_{32}O_2$).

Example 26: [^{35}S]-TBPS Binding Methods.

[0305] Rat brain cortical membranes were prepared with minor modifications of the method previously reported. (Hawkinson, J. E., et al., Correlation of Neuroactive Steroid Modulation of [^{35}S]*t*-Butylbicyclophosophorothionate and [^{3}H]Flunitrazepam Binding and γ-Aminobutyric Acid$_A$ Receptor Function, *Mol. Pharmacol.*, 1994, *46*, 977–985). Briefly, frozen rat cerebral cortices (Pel-freez, Rogers, AK) were thawed and homogenized in 10 volumes of ice-cold 0.32 M sucrose using a glass/Teflon pestle. The homogenate was centrifuged at 1500 x *g* for 10 min at 4 °C. The resultant supernatant was centrifuged at 10,000 x *g* for 30 min at 4 °C. The pellet (P2) from this centrifugation was resuspended in 200 mM NaCl, 50 mM potassium phosphate buffer, pH 7.4, and centrifuged at 10,000 x *g* for 20 min at 4 °C. This washing procedure was done a total of three times, and then pellets were resuspended in buffer (~4 mL/brain) using a glass/Teflon pestle. The membrane suspension was aliquoted, frozen in liquid nitrogen, and stored at –80 °C prior to use.

[0306] [^{35}S]-TBPS Binding assays were done according to the procedure described previously (Hawkinson, J. E., et al., Correlation of Neuroactive Steroid Modulation of [^{35}S]*t*-Butylbicyclophosophorothionate and [^{3}H]Flunitrazepam Binding and γ-Aminobutyric Acid$_A$ Receptor Function, *Mol. Pharmacol.*, 1994, *46*, 977–985) with modifications. Briefly, aliquots of membrane solution (0.5 mg/mL final protein concentration in assay) were incubated with 5 µM GABA, 2 nM [^{35}S]-TBPS (45–120 Ci/mmol), and 5 µL aliquots of steroid in DMSO solution (final assay concentrations ranged from 1 nM to 10 µM), and brought to a final volume of 1 mL with 200 mM NaCl, 50 mM potassium phosphate buffer, pH 7.4. Control binding was defined as binding observed in the presence of 0.5% DMSO and the absence of steroid. Nonspecific binding was defined as binding observed in the presence of 200 µM picrotoxinin and ranged from 6.1 to 14.3% of total binding. Assay tubes were incubated for 2 hr at room temperature. A Brandel (Gaithersburg, MD) cell harvester was used for filtration of the assay tubes through Whatman/GF/C glass filter paper.

Filter paper was rinsed with 4 mL of ice-cold buffer three times. Radioactivity bound to the filters was read by liquid scintillation counter and data was fit using Sigma Plot version 3.0 to the Hill equation

i. $f = R_{max}/\{1 + ([conc]/IC_{50})^{n}\}$

[0307] Where R_{max} is the maximal effect, [conc] is steroid concentration, IC_{50} is the half-maximal inhibitor concentration and *n* is the Hill coefficient. Each data point was determined in triplicate and 2–3 full concentration-response curves were generated for each steroid.

Table 1. Displacement of [^{35}S]-TBPS binding by Cyclosteroids.

Compound	IC_{50} (nM)[a]	nHill
5α-Steroids		
1a; 20-oxo	74 ± 7	0.89 ± 0.06
5a; 24-oxo	301 ± 20	1.01 ± 0.06
4a; 23-oxo	334 ± 17	1.14 ± 0.05
2a; 20-oxo	514 ± 64	0.84 ± 0.08
3a; 22-oxo	1,440 ± 100	0.95 ± 0.06
35a; Δ^{22}-24-one	197 ± 23	1.03 ± 0.10
21c; $\Delta^{20(22)}$-23-one	243 ± 11	0.89 ± 0.03
11a; Δ^{22}-20-one	373 ± 20	1.17 ± 0.06
30a; Δ^{23}-22-one	1260 ± 100	1.00 ± 0.07
19a; (22*S*,23*S*)-epoxy-20-one	370 ± 42	0.97 ± 0.09
19b; (22*R*,23*R*)-epoxy-20-one	313 ± 18	1.10 ± 0.06
39; 20-CN (eq)	1670 ± 138	1.16 ± 0.10
40; 20-CN (ax)	173 ± 12	0.97 ± 0.06
44; 22-CN (eq)	585 ± 30	0.86 ± 0.03
45; 22-CN (ax)	44300 ± 21300	0.42 ± 0.11
49; 23-CN (ax)	574 ± 26	1.20 ± 0.06
50; 23-CN (eq)	482 ± 20	1.09 ± 0.04
13a; $\Delta^{20(22)}$-ene	569 ± 28	1.15 ± 0.05
51; 20-[*exo*-CH_2]	181 ± 9	0.96 ± 0.04
53; 22-[*exo*-CH_2]	107000 ± 29600	0.47 ± 0.06
57; $\Delta^{20(22)}$-20-CN	433 ± 30	1.05 ± 0.06
60; $\Delta^{20(22),23}$-diene	467 ± 26	1.04 ± 0.05
61; Δ^{23}-ene*	2000 ± 190	0.94 ± 0.08
63; *exo*-[CHCN]	11 ± 1.8	0.90 ± 0.08
64; *exo*-[CHCN]	1,080 ± 136	1.10 ± 0.14
67; 20-OH (ax)	613 ± 92	0.83 ± 0.09
68; 20-OH (eq)	1,540 ± 320	0.68 ± 0.09
69; 20-OMe (ax)	353 ± 38	1.07 ± 0.10
70; 20-OMe (eq)	868 ± 78	1.15 ± 0.10
71; (20*R*,22*S*)-epoxide	596 ± 71	1.06 ± 0.11
72; (20*S*,22*R*)-epoxide	323 ± 25	0.92 ± 0.05
73; *exo*-(20*S*)-epoxide	1,290 ± 83	1.66 ± 0.16
74; *exo*-(20*R*)-epoxide	64 ± 9	0.99 ± 0.11
79; 20-(acetyl) (ax)	93 ± 9	1.07 ± 0.09
80; 20-(acetyl) (eq)	2,060 ± 340	0.91 ± 0.13
83; $\Delta^{20(22)}$-20-(acetyl)	3,190 ± 398	1.64 ± 0.31
89; 20-one	204 ± 36	0.82 ± 0.10

Compound	IC_{50} (nM)[a]	nHill
94; $\Delta^{18(21)}$-20-one	745 ± 75	0.86 ± 0.07
95; $\Delta^{18(21)}$-20-one, 21-Br	94 ± 17	1.05 ± 0.16
96; *exo*-[CHCN]	168 ± 32	0.90 ± 0.13
97; *exo*-[CHCN]	17,200 ± 6,190	0.44 ± 0.10
5β-Steroids		
1b; 20-oxo	71 ± 18	0.57 ± 0.06
5b; 24-oxo	329 ± 35	0.95 ± 0.08
4b; 23-oxo	899 ± 150	0.89 ± 0.11
2b; 20-oxo	1,780 ± 180	1.36 ± 0.16
3b; 22-oxo	3,230 ± 640	0.73 ± 0.11
35b; Δ^{22}-24-one	105 ± 12	0.90 ± 0.07
22c; $\Delta^{20(22)}$-23-one	268 ± 43	0.64 ± 0.05
11b; Δ^{22}-20-one	1,570 ± 140	1.15 ± 0.10
30b; Δ^{23}-22-one	4750 ± 970	0.95 ± 0.18
13b; $\Delta^{20(22)}$-ene	484 ± 103	0.81 ± 0.11
52; 20-[*exo*-CH_2]	213 ± 21	0.92 ± 0.07

[0308][a] Results presented are from duplicate experiments performed in triplicate. Error limits are calculated as standard error of the means.

Example 27: *Xenopus* Oocyte Electrophysiological Methods.

[0309] Stage V-VI oocytes were harvested from sexually mature female *Xenopus* laevis (Xenopus One, Northland, MI) under 0.1% tricaine (3-aminobenzoic acid ethyl ester) anesthesia. Oocytes were defolliculated by shaking for 20 min at 37 °C in collagenase (2 mg/ml) dissolved in calcium-free solution containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, and 5 HEPES at pH 7.4. Capped mRNA, encoding rat $GABA_A$ receptor $\alpha 1$, $\beta 2$ and $\gamma 2L$ subunits was transcribed *in vitro* using the mMESSAGE mMachine Kit (Ambion, Austin, TX) from linearized pBluescript vectors containing receptor coding regions. Subunit transcripts were injected in equal parts (20-40 ng total RNA) 8 – 24 hours following defolliculation. Oocytes were incubated up to 5 days at 18 °C in ND96 medium containing (in mM): 96 NaCl, 1 KCl, 1 $MgCl_2$, 2 $CaCl_2$ and 5 HEPES at pH 7.4, supplemented with pyruvate (5 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and gentamycin (50 μg/ml). The cDNAs for the rat $GABA_A$-receptor subunits were originally provided by A. Tobin, University of California, Los Angels ($\alpha 1$), P. Malherbe, Hoffman-La Roche, Switzerland ($\beta 2$), C. Fraser, National Institute on Alcohol Abuse and Alcoholism ($\gamma 2L$).

[0310] Two-electrode voltage-clamp experiments were performed with a Warner OC725 amplifier 2-5 days following RNA injection. The extracellular recording solution was ND96 medium with no supplements. Intracellular recording pipettes were filled with 3 M KCl and had open tip resistances of ~ 1 MΩ. Drugs were applied from a common tip via a gravity-driven multibarrel drug-delivery system. Steroids were simultaneously co-applied with GABA. Cells were clamped at –70 mV for all experiments, and peak current during 20 s drug applications was measured. Data acquisition and analysis were performed with pCLAMP software (Axon Instruments, CA). Statistical differences were determined using a two-tailed Student's t-test.

Table 2. Modulation of rat $\alpha1\beta2\gamma2$ $GABA_A$ Receptor Function by Cyclosteroids.

Compound	Oocyte Electrophysiology[a]			
	0.1 μM	1 μM	10 μM	Gating (10 μM)
5α-Steroids				
1a; 20-oxo[b]	1.26 ± 0.14	3.89 ± 1.34	9.65 ± 3.87	0.37 ± 0.07
5a; 24-oxo	1.22 ± 0.23	3.05 ± 0.79	10.73 ± 1.87	0.39 ± 0.69
4a; 23-oxo	1.39 ± 0.38	1.64 ± 0.15	6.49 ± 0.59	0.32 ± 0.01
2a; 20-oxo	1.01 ± 0.03	1.44 ± 0.03	2.28 0.05	0 ± 0.1
3a; 22-oxo	1.30 ± 0.15	1.61 ± 0.22	2.93 ± 0.68	0.06 ± 0.06
35a; Δ^{22}-24-one	1.98 ± 0.25	8.33 ± 0.75	9.45 ± 4.19	0.07 ± 0.10
21c; $\Delta^{20(22)}$-23-one	1.59 ± 0.14	5.53 ± 0.66	16.58 ± 2.45	0.35 ± 0.12
11a; Δ^{22}-20-one	1.49 ± 0.20	5.87 ± 1.52	9.07 ± 1.86	0.72 ± 0.03
30a; Δ^{23}-22-one	1.12 ± 0.08	1.54 ± 0.30	5.07 ± 0.98	–0.04 ± 0.05
19a; (22*S*,23*S*)-epoxy-20-one	1.47 ± 0.17	1.92 ± 0.23	8.90 ± 2.10	0.23 ± 0.24
19b; (22*R*,23*R*)-epoxy-20-one	1.13 ± 0.02	2.11 ± 0.26	5.47 ± 0.62	0.21 ± 0.02
39; 20-N (eq)	1.04 ± 0.03	1.12 ± 0.08	1.70 ± 0.10	0.04 ± 0.04
40; 20-CN (ax)	1.06 ± 0.03	1.23 ± 0.02	1.27 ± 0.02	–0.01 ± 0.01
44; 22-CN (eq)	1.19 ± 0.02	1.14 ± 0.13	4.01 ± 0.42	0.03 ± 0.01
45; 22-CN (ax)	0.90 ± 0.02	0.82 ± 0.02	0.87 ± 0.01	–0.01 ± 0
49; 23-CN (ax)	—	—	1.3 ± 0.1	—
50; 23-CN (eq)	—	—	2.1 ± 0.2	—
13a; $\Delta^{20(22)}$-ene	1.53 ± 0.45	2.12 ± 0.40	2.68 ± 0.42	–0.32 ± 0.15
51; 20-[*exo*-CH_2]	0.90 ± 0.15	0.93 ± 0.08	0.95 ± 0.05	–0.06 ± 0.06
53; 22-[*exo*-CH_2]	—	—	1.05 ± 0.04	—
57; $\Delta^{20(22)}$-20-CN	0.91 ± 0.03	0.96 ± 0.04	3.18 ± 0.24	0.01 ± 0.02
60; $\Delta^{20(22),23}$-diene	—	—	1.1 ± 0.1	—
61; Δ^{23}-ene*	1.28 ± 0.08	1.47 ± 0.10	1.72 ± 0.26	0.19 ± 0.10
63; *exo*-[CHCN]	6.29 ± 1.71	12.34 ± 3.28	17.82 ± 3.75	0.01 ± 0.02
64; *exo*-[CHCN]	1.19 ± 0.04	1.27 ± 0.03	1.74 ± 0.11	0.03 ± 0.04
67; 20-OH (ax)	0.89 ± 0.03	0.94 ± 0.08	1.37 ± 0.15	-0.01 ± 0.00
68; 20-OH (eq)	0.74 ± 0.05	0.74 ± 0.05	0.79 ± 0.04	0.01 ± 0.02
69; 20-OMe (ax)	0.90 ± 0.04	1.26 ± 0.09	1.72 ± 0.28	0.00 ± 0.00
70; 20-OMe (eq)	0.74 ± 0.01	0.87 ± 0.02	1.02 ± 0.03	0.00 ± 0.00
71; (20*R*,22*S*)-epoxide	0.90 ± 0.05	1.05 ± 0.09	1.94 ± 0.23	0.00 ± 0.00
72; (20*S*,22*R*)-epoxide	0.90 ± 0.15	2.20 ± 0.25	6.39 ± 1.33	-0.01 ± 0.04
73; *exo*-(20*S*)-epoxide	0.88 ± 0.05	2.54 ± 0.40	4.84 ± 1.05	-0.02 ± 0.04

Compound	Oocyte Electrophysiology[a]			
	0.1 μM	1 μM	10 μM	Gating (10 μM)
74;*exo*-(20*R*)-epoxide	1.61 ± 0.18	5.46 ± 1.21	4.53 ± 0.75	-0.01 ± 0.01
79; 20-(acetyl) (ax)	1.00 ± 0.08	1.13 ± 0.15	1.70 ± 0.12	-0.06 ± 0.04
80; 20-(acetyl) (eq)	0.85 ± 0.02	0.77 ± 0.03	0.93 ± 0.07	0.01 ± 0.01
83;$\Delta^{20(22)}$-20 (acetyl)	0.89 ± 0.02	0.86 ± 0.04	1.15 ± 0.04	0.00 ± 0.00
89; 20-one	0.81 ± 0.02	1.02 ± 0.07	4.54 ± 0.64	0.01 ± 0.00
94; $\Delta^{18(21)}$-20-one	0.96 ± 0.02	1.41 ± 0.09	2.78 ± 0.13	-0.04 ± 0.02
95; $\Delta^{18(21)}$-20-one, 21-Br	1.25 ± 0.08	1.99 ± 0.20	8.75 ± 1.92	0.02 ± 0.02
96; *exo*-[CHCN]	1.40 ± 0.21	2.33 ± 0.30	2.90 ± 0.29	0.03 ± 0.03
97; *exo*-[CHCN]	0.88 ± 0.07	0.87 ± 0.15	1.10 ± 0.08	-0.02 ± 0.06
5β-Steroids				
1b; 20-oxo	1.20 ± 0.10	2.82 ± 0.51	9.77 ± 2.15	0.61 ± 0.03
5b; 24-oxo	1.26 ± 0.05	3.45 ± 0.29	10.42 ± 1.65	0.04 ± 0.02
4b; 23-oxo	0.97 ± 0.03	1.03 ± 0.08	3.17 ± 0.42	0.01 ± 0.01
2b; 20-oxo	0.93 ± 0.06	1.12 ± 0.14	1.51 ± 0.13	–0.25 ± 0.21
3b; 22-oxo	0.97 ± 0.01	1.11 ± 0.14	2.65 ± 0.33	–0.10 ± 0.06
35b; Δ22-24-one	1.36 ± 0.60	3.15 ± 1.19	21.38 ± 11.08	0.20 ± 0.19
22c;Δ20(22)-23-one	1.07 ± 0.10	1.83 ± 0.25	4.50 ± 0.46	–0.02 ± 0.01
11b; Δ22-20-one	1.07 ± 0.17	1.17 ± 0.11	2.34 ± 0.37	0.11 ± 0.04
30b; Δ23-22-one	1.87 ± 0.24	1.80 ± 0.25	4.44 ± 0.87	0.26 ± 0.31
1b; 20-oxo	1.20 ± 0.10	2.82 ± 0.51	9.77 ± 2.15	0.06 ± 0.03
13b; $\Delta^{20(22)}$-ene	1.44 ± 0.17	2.09 ± 0.35	3.48 ± 1.01	–0.05 ± 0.09
52; 20-[*exo*-CH_2]	1.57 ± 0.16	3.47 ± 0.50	6.22 ± 0.92	–0.02 ± 0.02

[0311][a] The GABA concentration used for the control response was 2 μM. Each compound was evaluated on at least four different oocytes at the concentrations indicated, and the results reported are the ratio of the currents measured in the presence/absence of added compound. (G) represents direct current gated by 10 μM compound in the absence of GABA, and this current is reported as the ratio of compound only current/2 μM GABA current. Error limits are calculated as standard error of the means.

[0312][b] Values reported are from Covey, D., et al., Neurosteroid Analogues. 8. Structure–Activity Studies of *N*-Acylated 17a-Aza-D-homosteroid Analogues of the

Anesthetic Steroids (3α,5α)- and (3α,5β)-3-Hydroxypregnan-20-one, *J. Med. Chem.*, 2000, *43*, 3201–3204.

[0313] Generally, **Figure 1** depicts the potentiation of electrophysiological responses to GABA by cyclosteroids using two current traces (chloride currents) (Figure **1A** and **1B**) and two tables (Figure **1C** and **1D**). In particular, Figure **1A** is a current trace (chloride current) depicting GABA currents potentiated by 5*α*-reduced cyclosteroids. The current trace depcits a two-electrode voltage-clamp recording (–70 mV) from an oocyte showing the response to 2 *μ*M GABA alone and in the presence of 500 nM test compound **35a** and in the presence of reference compound **1a**. At this concentration, effects of the steroids were readily reversible as shown by the GABA recovery panel. Figure **1B** depicts an analogous experiment in another oocyte showing effects of 5*β*-reduced compounds, **35b** and **1b**. Figure **1C** depicts a table of chloride current versus specific compounds, which summarizes the effects of certain cyclosteroids tested at 500 nM against the response to 2 *μ*M GABA alone (dotted line denotes the normalized response to GABA alone). Figure **1D** depicts a similar summary table for the 5*β*-reduced series.

Example 28: Tadpole Behavioral Methods.

[0314] Tadpole LRR was measured as described previously. (See, Wittmer, L. L., et al., Enantioselectivity of steroid-induced γ-aminobutyric acidA receptor modulation and anesthesia, *Mol. Phar.*, 1996, 50:1581-1586). Briefly, groups of 10 early prelimb-bud stage *Xenopus laevis* tadpoles (Nasco, Fort Atkinson, WI) were placed in 100 mL of oxygenated Ringer's stock solution containing various concentrations of compound. Compounds were added from a 10 mM DMSO stock (final concentration of DMSO in test solutions ≤0.1%). After equilibrating at room temperature for 3 h, tadpoles were evaluated using the LRR and LSR behavioral endpoints. LRR was defined as failure of the tadpole to right itself within 5 sec after being flipped by a smooth glass rod. LSR was defined as failure of the tadpole to swim when touched by a smooth glass rod. In general, the tadpoles regained their righting and swimming reflexes when placed in fresh oxygenated Ringer's solution. Control beakers containing up to 0.6% DMSO produced no LRR or LSR in tadpoles.

[0315] Tadpole LRR and LSR concentration-response curves were fit using Sigma Plot version 3.0 to the Hill equation

[0316] $f = R_{max}/\{1 + ([conc]/EC_{50})^{n}\}$

[0317] where R_{max} is the maximum effect, [conc] is the steroid concentration, EC_{50} is the half-maximal effective concentration, and n is the Hill coefficient.

Table 3. Cyclosteroid effects on tadpole righting and swimming reflexes.

Compounds	LRRa ED_{50} (μM)	LRR NHill	LSR[b] EC_{50} (μM)	LSR nHill
5α-Steriods				
1a; 20-one	0.42 ± 0.04	–1.83 ± 0.32	5.50 ± 0.48	–7.5 ± 1.1
5a; 24-one	0.32 ± 0.02	-1.75 ± 0.17	1.73 ± 0.03	-36.5 ± 0.07
4a; 23-one	1.12 ± 0.36	-1.75 ± 0.83	3.58 ± 1.08	-16.7 ± 28.3
2a; 20-one	1.36 ± 0.20	-2.51 ± 0.76	5.45 ± 0.07	-33.5 ± 0.1
3a; 22-one	2.37 ± 0.47	-2.02 ± 0.64	None @ 10	—
35a; Δ^{23}-24-one	1.02 ± 0.0	-17.1 ± 0.91	2.66 ± 0.0	-24.2 ± 0.0
21c;$\Delta^{20(22)}$-23-one	1.28 ± 0.38	-1.16 ± 0.26	10.0 ± 0.0	-17.0 ± 0.0
11a; Δ^{22}-20-one	3.38 ± 0.67	-2.79 ± 1.89	None @ 10	—
30a; Δ^{23}-22-one	0.73 ± 0.16	-1.57 ± 0.43	5.48 ± 0.09	-33.5 ± 0.1
19a; (22*S*,23*S*)-epoxy-20-one	1.75 ± 0.46	–1.60 ± 0.49	10.0 ± 0.0	–17.0 ± 0.04
19b; (22*R*,23*R*)-epoxy-20-one	0.96 ± 0.29	–1.14 ± 0.27	10.0 ± 0.0	–17.0
31a; (23*R*,24*R*)-epoxide**	—	—	—	—
39; 20-CN (eq)	0.49 ± 0.09	–1.21 ± 0.22	None @ 10	—
40; 20-CN (ax)	> 10	—	None @ 10	—
44; 22-CN (eq)	0.30 ± 0.03	–1.81 ± 0.31	6.28 ± 1.08	–2.98 ± 0.47
45; 22-CN (ax)	>10	—	None @ 10	—
49; 23-CN (ax)	0.35 ± 0.09	–1.49 ± 0.49	>10	—
50; 23-CN (eq)	0.35 ± 0.07	–2.11 ± 0.84	0.95 ± 0.01	–15.7 ± 0.43
13a; $\Delta^{20(22)}$-ene	>10	—	None @ 10	—
51; 20-[exo-CH_2]	>10	—	None @ 10	—
53; 22-[exo-CH_2]	—	—	—	—
57; $\Delta^{20(22)}$-20-CN	0.42	± 0.10	None @ 10	—
60; $\Delta^{20(22),23}$-diene	2.70 ± 0.72	–1.63 ± 0.49	>10	—
61; Δ^{23}-ene*	None @ 10	—	None @ 10	—
63; *exo*-[CHCN]	0.04 ± 0.00	–3.5 ± 0.54	0.08 ± 0.02	–2.33 ± 1.12
64; *exo*-[CHCN]	0.68 ± 0.15	-1.68 ± 0.48	10.2 ± 0.00	-11.4 ± 0.02
67; 20-OH (ax)	None @ 10	—	None @ 10	—
68; 20-OH (eq)	> 10	—	None @ 10	—
69; 20-OMe (ax)	None @ 10	—	None @ 10	—
70; 20-OMe (eq)	> 10	—	None @ 10	—
71; (20*R*,22*S*)-epoxide	None @ 10	—	None @ 10	—
72; (20*S*,22*R*)-epoxide	19.3 ± 30.8	-1.01 ± 0.52	None @ 10	—
73; exo-(20*S*)-epoxide	4.00 ± 1.08	-1.44 ± 0.28	None @ 10	—

Compounds	LRRa ED_{50} (μM)	LRR NHill	LSR[b] EC_{50} (μM)	LSR nHill
74; *exo*-(20*R*)-epoxide	> 10	—	None @ 10	—
79; 20-(acetyl) (ax)	None @ 10	—	None @ 10	—
80; 20-(acetyl) (eq)	None @ 10	—	None @ 10	—
83; $\Delta^{20(22)}$-20-(acetyl)	7.20 ± 6.62	-1.21 ± 0.41	None @ 10	—
89; 20-one	2.63 ± 0.07	-3.16 ± 0.35	5.48 ± 0.15	-33.4 ± 0.13
94; $\Delta^{18(21)}$-20-one	3.37 ± 0.16	-14.8 ± 6.18	9.52 ± 0.00	-18.8 ± 0.00
95; $\Delta^{18(21)}$-20-one, 21-Br	0.29 ± 0.04	-1.89 ± 0.51	0.55 ± 0.09	-33.5 ± 0.06
96; *exo*-[CHCN]	2.77 ± 1.16	-0.98 ± 0.19	None @ 10	—
97; *exo*-[CHCN]	> 10	—	None @ 10	—
5β-Steriods				
1b; 20-one	0.063 ± 0.003	-1.54 ± 0.12	0.30 ± 0.0	-6.93 ± 0.47
5b; 24-one	0.40 ± 0.12	-4.97 ± 5.29	0.89 ± 0.0	-18.2 ± 0.4
4b; 23-one	1.44 ± 0.02	-3.02 ± 0.09	3.55 ± 1.15	-17.5 ± 33.6
2b; 20-one	0.94 ± 0.55	-1.23 ± 0.64	5.48 ± 0.08	-33.5 ± 0.1
3b; 22-one	2.47 ± 0.53	-1.86 ± 0.57	9.43 ± 0.06	-18.7 ± 0.6
35b; Δ^{23}-24-one	0.40 ± 0.13	-4.97 ± 5.75	1.00 ± 0.0	-18.6 ± 0.1
22c; $\Delta^{20(22)}$-23-one	1.05 ± 0.00	-16.6 ± 0.9	2.72 ± 0.01	-22.1 ± 0.7
11b; Δ^{22}-20-one	2.56 ± 1.39	-1.59 ± 0.95	8.95 ± 0.01	-19.8 ± 0.01
30b; Δ^{23}-22-one	0.56 ± 0.9	-1.50 ± 0.27	5.48	-33.5 ± 0.1
13b; $\Delta^{20(22)}$-ene	1.28 ± 1.80	–0.64 ± 0.36	>10	—
52; 20-[*exo*-CH_2]	4.88 ± 1.12	–1.80 ± 0.55	>10	—

[0318][a] LRR = Loss of righting response. Error limits are calculated as standard error of the means.

[0319][b] LSR = Loss of swimming response. Error limits are calculated as standard error of the means.

Example 29. Elemental Analysis

2a	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37	
		Found	C, 79.79	H, 10.50	
3a	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37	
		Found	C, 80.00	H, 10.32	
4a	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37	
		Found	C, 80.13	H, 10.16	
5a	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37	
		Found	C, 79.85	H, 10.21	
7a	Anal. For $C_{24}H_{37}NO_3$	Calcd.	C, 74.38	H, 9.62	N, 3.61
		Found	C, 74.50	H, 9.50	N, 3.50
8a	Anal. For $C_{24}H_{35}NO_3$	Calcd.	C, 74.77	H, 9.15	N, 3.63
		Found	C, 74.67	H, 9.37	N, 3.44
9a	Anal. For $C_{26}H_{39}NO_4$	Calcd.	C, 72.69	H, 9.15	N, 3.26
		Found	C, 72.47	H, 8.97	N, 3.13
11a	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82	
		Found	C, 80.21	H, 9.89	
13a	Anal. For $C_{22}H_{34}O$	Calcd.	C, 84.02	H, 10.90	
		Found	C, 84.19	H, 10.92	
14a	Anal. For $C_{24}H_{38}O_2$	Calcd.	C, 80.39	H, 10.68	
		Found	C, 80.17	H, 10.59	
19a	Anal. For $C_{22}H_{32}O_3$	Calcd.	C, 76.70	H, 9.36	
		Found	C, 76.57	H, 9.50	
19b	Anal. For $C_{22}H_{32}O_3$	Calcd.	C, 76.70	H, 9.36	
		Found	C, 76.86	H, 9.24	
21a	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37	
		Found	C, 80.05	H, 10.27	
21c	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82	

		Found	C, 80.19	H, 9.63
23a	Anal. For $C_{26}H_{36}O_4$	Calcd.	C, 75.69	H, 8.80
		Found	C, 75.42	H, 8.69
24a	Anal. For $C_{24}H_{36}O_3$	Calcd.	C, 77.38	H, 9.74
		Found	C, 77.41	H, 9.83
24b	Anal. For $C_{24}H_{36}O_3$	Calcd.	C, 77.38	H, 9.74
		Found	C, 77.18	H, 9.57
28a	Anal. For $C_{24}H_{36}O_2$	Calcd.	C, 80.85	H, 10.18
		Found	C, 80.77	H, 10.12
30a	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82
		Found	C, 80.32	H, 9.83
32a	Anal. For $C_{24}H_{37}BrO_3$	Calcd.	C, 63.57	H, 8.22
		Found	C, 63.58	H, 8.36
33a	Anal. For $C_{24}H_{35}BrO_3$	Calcd.	C, 63.85	H, 7.81
		Found	C, 63.66	H, 8.00
34a	Anal. For $C_{24}H_{34}O_3$	Calcd.	C, 77.80	H, 9.25
		Found	C, 78.00	H, 9.12
35a	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82
		Found	C, 80.63	H, 9.70
2b	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37
		Found	C, 80.19	H, 10.15
3b	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37
		Found	C, 79.90	H, 10.23
4b	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37
		Found	C, 80.10	H, 10.15
5b	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37
		Found	C, 80.10	H, 10.43
11b	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82
		Found	C, 80.55	H, 9.77
22c	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82
		Found	C, 80.58	H, 9.87

30b	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82	
		Found	C, 80.60	H, 9.85	
35b	Anal. For $C_{22}H_{32}O_2$	Calcd.	C, 80.44	H, 9.82	
		Found	C, 80.56	H, 9.94	
39	Anal. For $C_{23}H_{35}NO_3$	Calcd.	C, 80.88	H, 10.33	N, 4.10
		Found	C, 81.03	H, 10.26	N, 4.15
40	Anal. For $C_{23}H_{35}NO_3$	Calcd.	C, 80.88	H, 10.33	N, 4.10
		Found	C, 80.94	H, 10.14	N, 4.14
41	Anal. For $C_{24}H_{36}O_3$	Calcd.	C, 77.38	H, 9.74	
		Found	C, 77.16	H, 9.59	
44	Anal. For $C_{23}H_{35}NO$	Calcd.	C, 80.88	H, 10.33	N, 4.10
		Found	C, 80.67	H, 10.16	N, 4.07
46	Anal. For $C_{24}H_{36}O_3$	Calcd.	C, 77.38	H, 9.74	
		Found	C, 77.44	H, 9.52	
49	Anal. For $C_{23}H_{35}NO$	Calcd.	C, 80.88	H, 10.33	N, 4.10
		Found	C, 80.65	H, 10.33	N, 4.07
50	Anal. For $C_{23}H_{35}NO$	Calcd.	C, 80.88	H, 10.33	N, 4.10
		Found	C, 80.60	H, 10.33	N, 3.80
51	Anal. For $C_{23}H_{36}O$	Calcd.	C, 84.09	H, 11.04	
		Found	C, 84.19	H, 11.00	
61	Anal. For $C_{22}H_{34}O$	Calcd.	C, 84.02	H, 10.90	
		Found	C, 84.20	H, 10.84	
63	Anal. For $C_{24}H_{35}NO$	Calcd.	C, 81.53	H, 9.98	N, 3.96
		Found	C, 81.70	H, 9.91	N, 3.95
64	Anal. For $C_{24}H_{35}NO$	Calcd.	C, 81.53	H, 9.98	N, 3.96
		Found	C, 81.48	H, 10.10	N, 3.95
65	Anal. For $C_{24}H_{40}O_3$	Calcd.	C, 76.55	H, 10.71	
		Found	C, 76.58	H, 10.83	
66	Anal. For $C_{24}H_{40}O_3$	Calcd.	C, 76.55	H, 10.71	
		Found	C, 76.38	H, 10.67	

67	Anal. For $C_{22}H_{36}O_2$	Calcd.	C, 79.46	H, 10.91
		Found	C, 79.62	H, 11.00
68	Anal. For $C_{22}H_{36}O_2$	Calcd.	C, 79.46	H, 10.91
		Found	C, 79.60	H, 10.96
69	Anal. For $C_{23}H_{38}O_2$	Calcd.	C, 79.71	H, 11.05
		Found	C, 80.04	H, 11.20
70	Anal. For $C_{23}H_{38}O_2$	Calcd.	C, 79.71	H, 11.05
		Found	C, 79.80	H, 10.89
71	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37
		Found	C, 79.88	H, 10.15
72	Anal. For $C_{22}H_{34}O_2$	Calcd.	C, 79.95	H, 10.37
		Found	C, 80.16	H, 10.18
73	Anal. For $C_{23}H_{36}O_2$	Calcd.	C, 80.18	H, 10.53
		Found	C, 80.38	H, 10.63
74	Anal. For $C_{23}H_{36}O_2$	Calcd.	C, 80.18	H, 10.53
		Found	C, 79.96	H, 10.67
77	Anal. For $C_{26}H_{42}O_3$	Calcd.	C, 77.56	H, 10.51
		Found	C, 77.70	H, 10.62
78	Anal. For $C_{26}H_{42}O_3$	Calcd.	C, 77.56	H, 10.51
		Found	C, 77.76	H, 10.70
79	Anal. For $C_{24}H_{38}O_2$	Calcd.	C, 80.39	H, 10.68
		Found	C, 80.50	H, 10.88
80	Anal. For $C_{24}H_{38}O_2$	Calcd.	C, 80.39	H, 10.68
		Found	C, 80.25	H, 10.47
82	Anal. For $C_{26}H_{40}O_3$	Calcd.	C, 77.95	H, 10.06
		Found	C, 77.84	H, 10.19
83	Anal. For $C_{24}H_{36}O_2$	Calcd.	C, 80.85	H, 10.18
		Found	C, 81.02	H, 10.37
86	Anal. For $C_{23}H_{34}O_3$	Calcd.	C, 77.05	H, 9.56
		Found	C, 77.06	H, 9.67

88	Anal. For $C_{28}H_{36}O_3$	Calcd.	C, 79.96	H, 8.63	
		Found	C, 79.98	H, 8.80	
89	Anal. For $C_{21}H_{32}O_2$	Calcd.	C, 79.70	H, 10.19	
		Found	C, 79.69	H, 9.94	
94	Anal. For $C_{21}H_{30}O_2$	Calcd.	C, 80.21	H, 9.62	
		Found	C, 80.16	H, 9.66	
95	Anal. For $C_{21}H_{29}BrO_2$	Calcd.	C, 64.12	H, 7.43	
		Found	C, 64.25	H, 7.40	
96	Anal. For $C_{23}H_{33}NO$	Calcd.	C, 81.37	H, 9.80	N, 4.13
		Found	C, 81.16	H, 9.67	N, 3.95
97	Anal. For $C_{23}H_{33}NO$	Calcd.	C, 81.37	H, 9.80	N, 4.13
		Found	C, 81.51	H, 10.00	N, 4.16

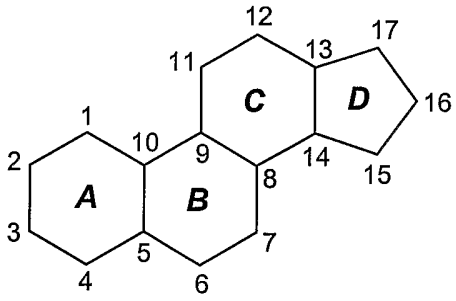
What is claimed is:

1. A pentacyclic steroid or pentacyclic D-homosteroid, the pentacyclic steroid or pentacyclic D-homosteroid comprising:

(i) the tetracyclic steroid ring system or tetracyclic D-homosteroid ring system, respectively;

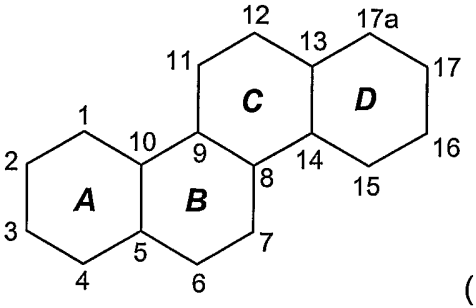
(ii) a C(3) substituent selected from the group consisting of (a) a hydroxyl or carboxyl in the α-configuration and (b) a sulfate or other negatively charged moiety; and

(iii) a fused fifth ring, E, the fused fifth ring comprising a hydrogen bond acceptor, and (a) in the case of the pentacyclic steroid the C(13) and C(17) carbons, or (b) in the case of the pentacyclic D-homosteroid the C(13) and C(17a) carbons, wherein the tetracyclic steroid ring system corresponds to Formula (I)



(I)

and the tetracyclic D-homosteroid ring system corresponds to Formula (II)



(II).

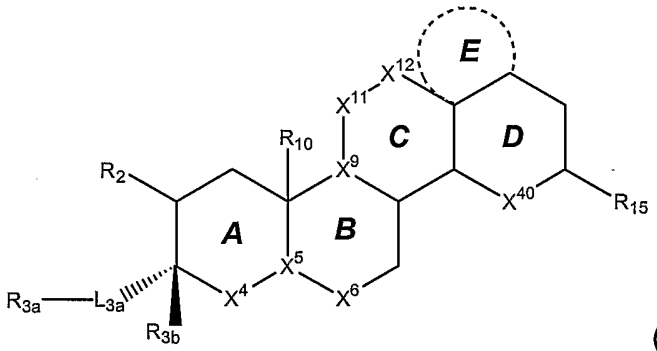
2. The steroid or D-homosteroid of claim 1 wherein each of rings A, B, C, and D are saturated.

3. The steroid or D-homosteroid of claim 1 wherein at least one pair of ring atoms comprising rings A, B, C, and D share a double bond.

4. The steroid or D-homosteroid of claim 1 wherein the fused fifth ring, E, is a 5- or 6-membered carbocyclic ring.

5. The steroid or D-homosteroid of claim 4 wherein the hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl.

6. The steroid or D-homosteroid of claim 1 having Formula (A)



(A)

wherein

R_2 is selected from the group consisting of hydrogen, alkoxy, and substituted or unsubstituted morpholine

R_{3a} is hydroxy or carboxyl;

R_{3b} is hydrogen, alkyl, alkenyl, or alkynyl optionally substituted with halo, hydroxy, or substituted or unsubstituted aryl;

R_5 is α- or β-hydrogen;

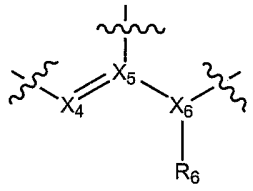
R_{10} is hydrogen or C_{1-4} alkyl;

R_{15} is hydrogen or oxo;

R_6, R_{11} and R_{12} are independently hydrogen or oxo;

L_{3a} is selected from the group consisting of a bond, C_{1-3} alkyl, heterosubstituted C_{1-3} alkyl, or alkoxy;

X_4-X_5-X_6 is selected from the group consisting of



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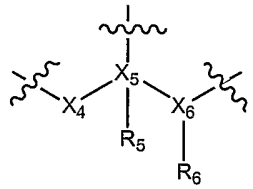

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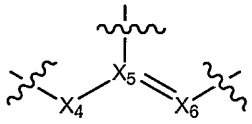
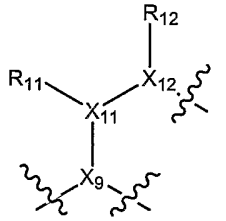


;

X_9-X_{11}-X_{12} is selected from the group consisting of



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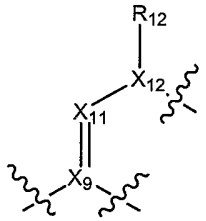

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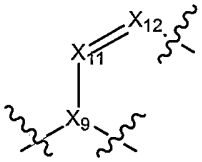


;

X_{40} is a bond or a carbon atom; and

E is a 5- or 6-membered carbocyclic ring, comprising a hydrogen bond acceptor and (i) the carbons at C(13) and C(17) when X_{40} is a bond, or (ii) the carbons at C(13) and C(17a) when X_{40} is carbon.

7. The compound of claim 6 wherein the hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl.

8. The compound of claim 7 wherein the E ring hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, epoxide, an E ring alkene bond, and combinations thereof.

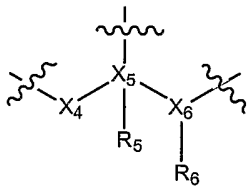
9. The compound of claim 7 wherein R_2 is hydrogen or substituted or unsubstituted morpholine.

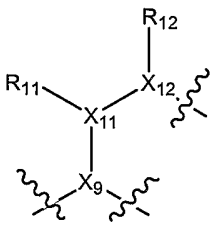
10. The compound of claim 7 wherein the carbocyclic ring, E, is in the β-configuration in relation to the C and D rings.

11. The compound of claim 7 wherein L_{3a} is selected from the group consisting of a bond, methylene, ethylene, methoxy, and ethoxy and R_3 is hydroxyl.

12. The compound of claim 11 wherein L_{3a} is a bond.

13. The compound of claim 7 wherein

X_4-X_5-X_6 is  ; and

X_9-X_{11}-X_{12} is  .

14. The compound of claim 7 wherein X_{40} is a bond.

15. The compound of claim 7 wherein R_{3b} is ethynyl substituted with alkyl or optionally substituted aryl.

16. The compound of claim 7 wherein R_{3b} is ethynyl substituted with phenyl substituted with amino, dimethylamino, hydroxyl, carboxyl, or alkoxy.

17. The compound of claim 7 wherein R_{10} is hydrogen or β-methyl.

18. The compound of claim 6 wherein

R_2 is hydrogen;

R_{3b} is hydrogen;

R_{10} is hydrogen or β-methyl;

R_{15} is hydrogen;

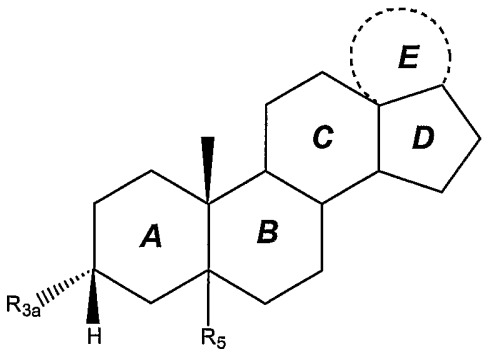
R_6, R_{11} and R_{12} are independently hydrogen or oxo;

L_{3a} is a bond; and

X_{40} is a bond.

19. The compound of claim 18 wherein the hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, epoxide, an E ring alkene bond, and combinations thereof.

20. The compound of claim 6 having Formula (B)



(B)

wherein

R_{3a} is hydroxyl or carboxyl;

R_5 is α- or β-hydrogen; and

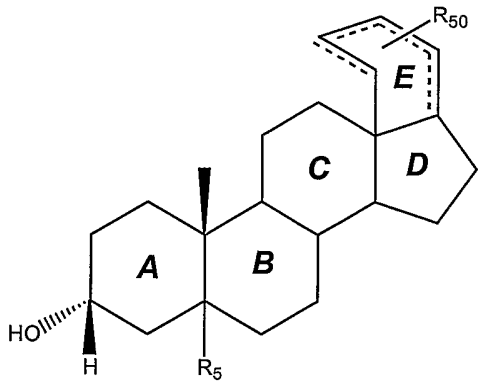
E is as defined in claim 6.

21. The compound of claim 20 wherein the E ring comprises a hydrogen bond acceptor selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl.

22. The compound of claim 21 wherein the hydrogen bond acceptor is selected from the group consisting of keto, cyano, methylene, methoxy, acetyl, $=CHCN$, $=CHC(O)CH_3$, epoxide, and combinations thereof.

23. The compound of claim 21 wherein the hydrogen bond acceptor comprises an E ring alkene bond.

24. The compound of claim 20 having Formula (C)



(C)

wherein

R_5 is α- or β-hydrogen;

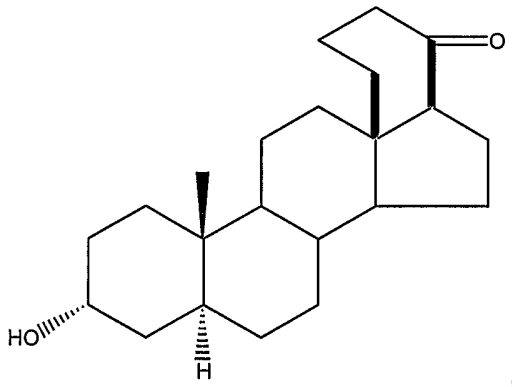
R_{50} is selected from the group consisting of hydrogen, keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted

hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl, provided that when R_{50} is hydrogen at least one pair of E ring atoms share a double bond; and

E is a 6-membered carbocyclic ring wherein the dashed lines represent optional double bonds provided E comprises no more than 2 double bonds and provided each carbon ring atom of E is sp^2 or sp^3 hybridized.

25. The compound of claim 24 wherein R_{50} is selected from the group consisting of hydrogen, keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, epoxide, and combinations thereof, provided that when R_{50} is hydrogen at least one pair of E ring atoms share a double bond.

26. The compound of claim 24 wherein the compound is selected from the group consisting of



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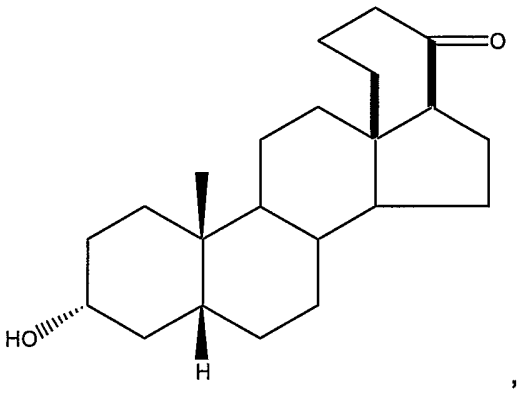

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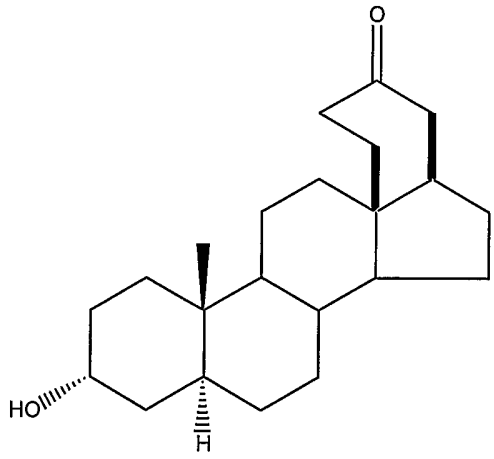
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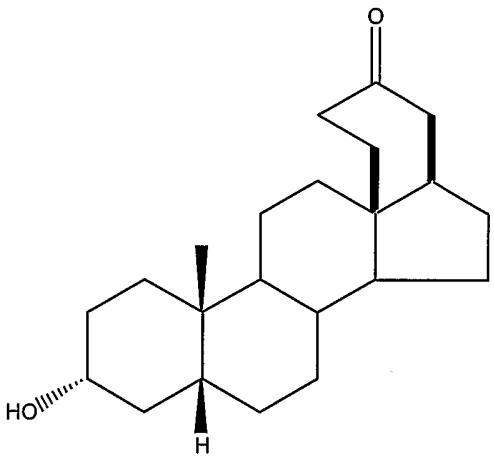
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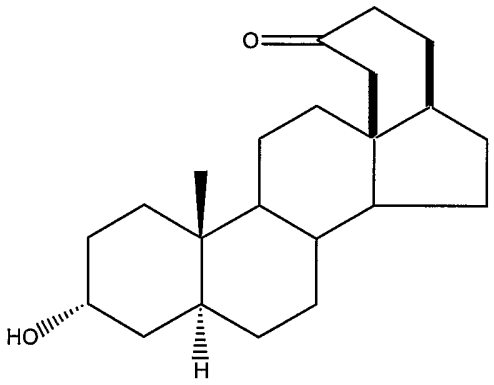
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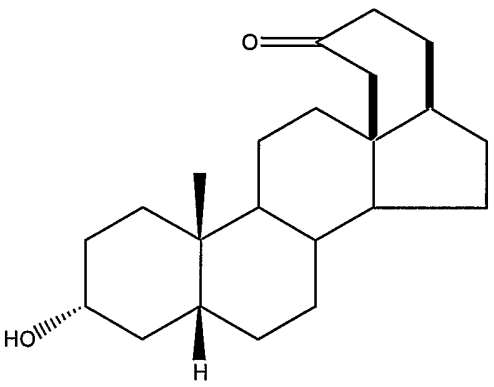
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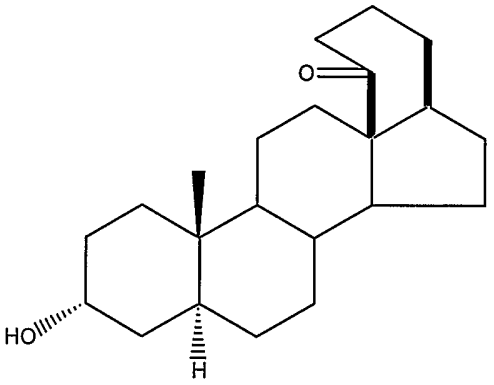
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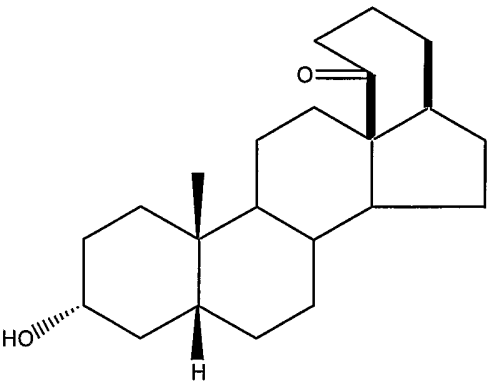
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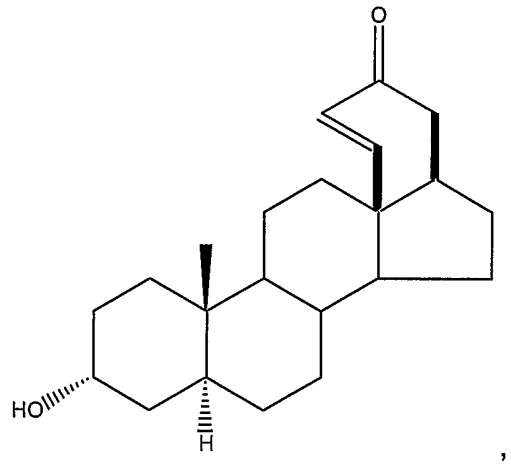
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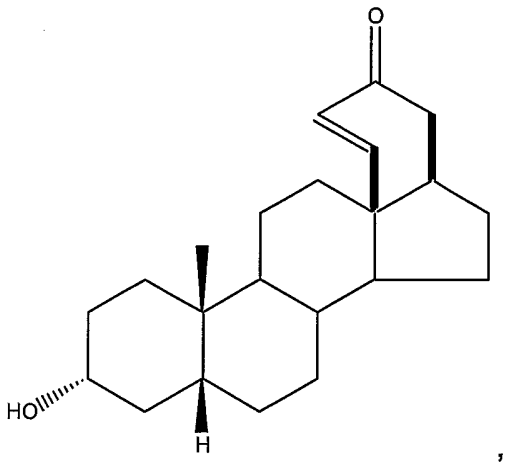
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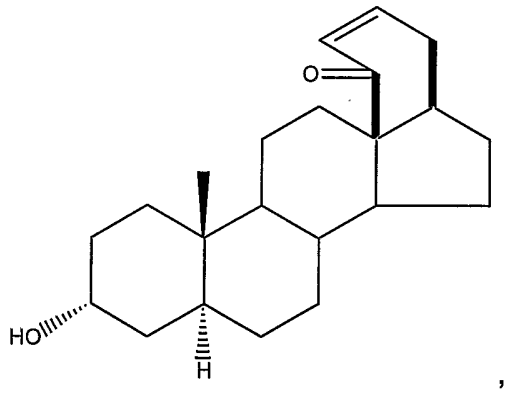
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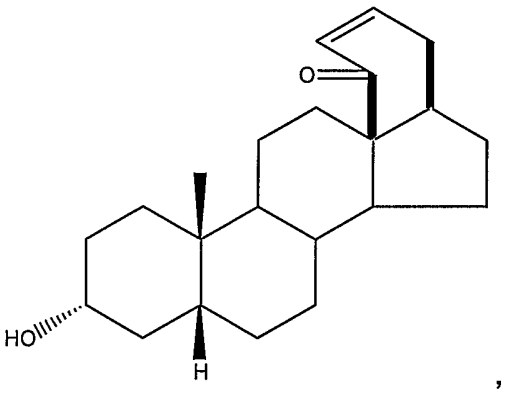
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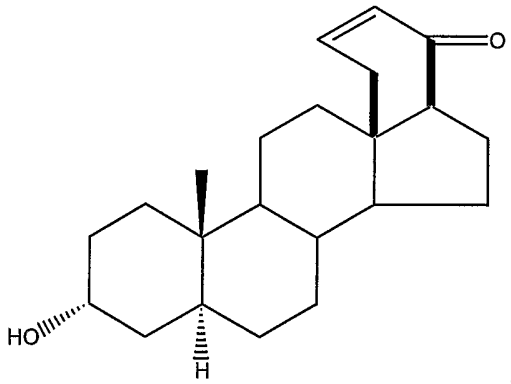
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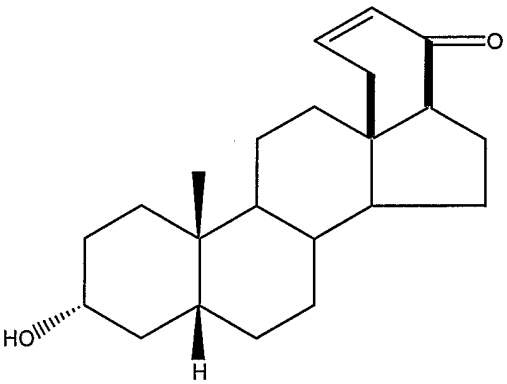
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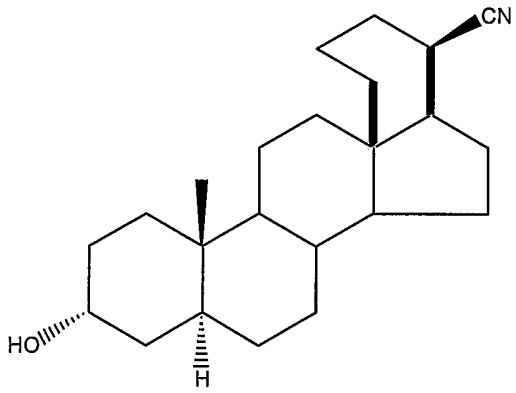
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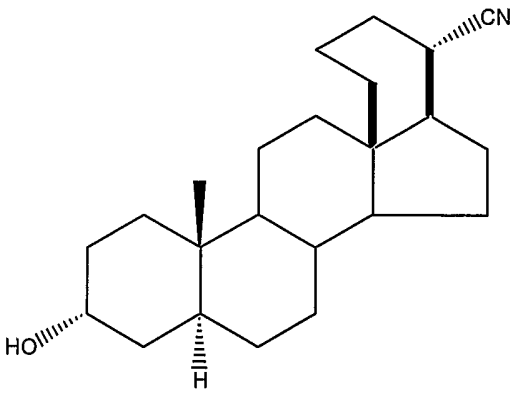
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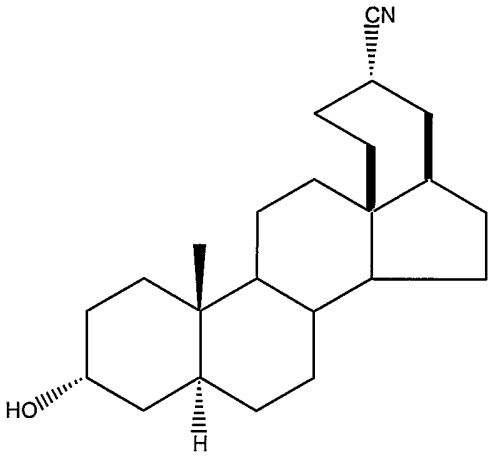
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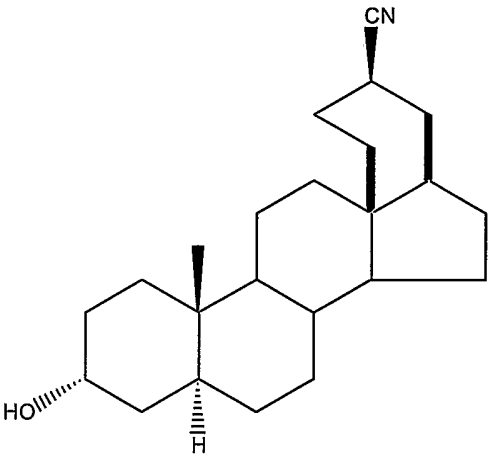
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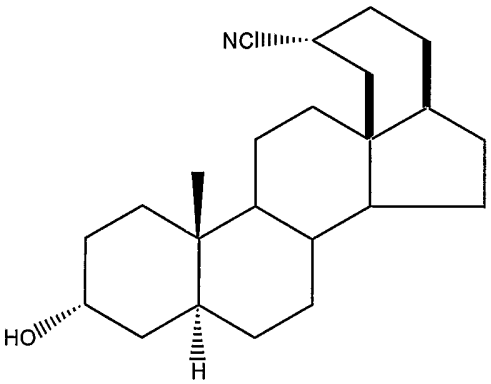
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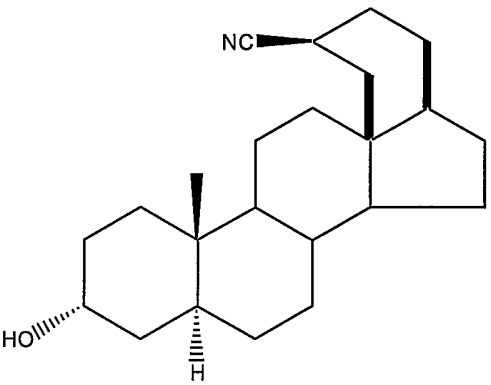
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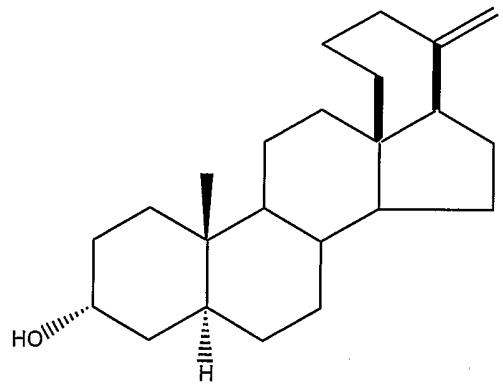
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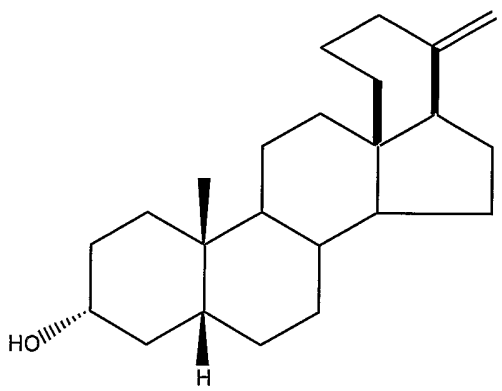
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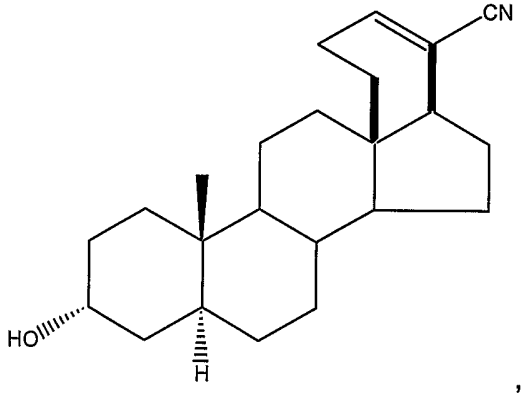
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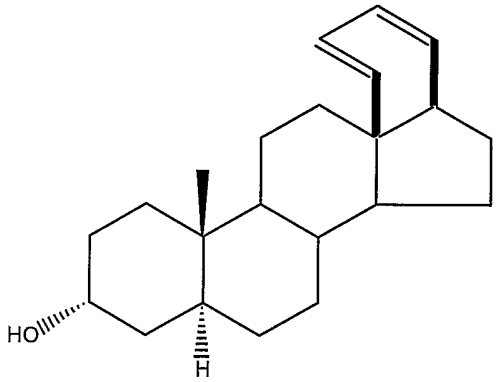
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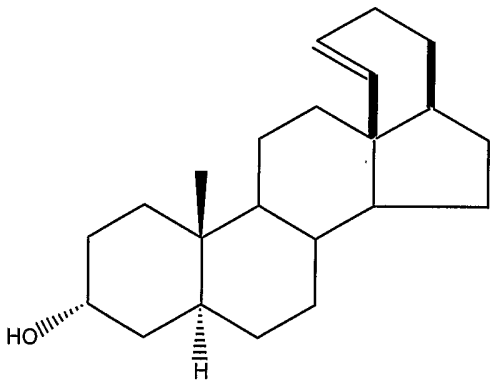
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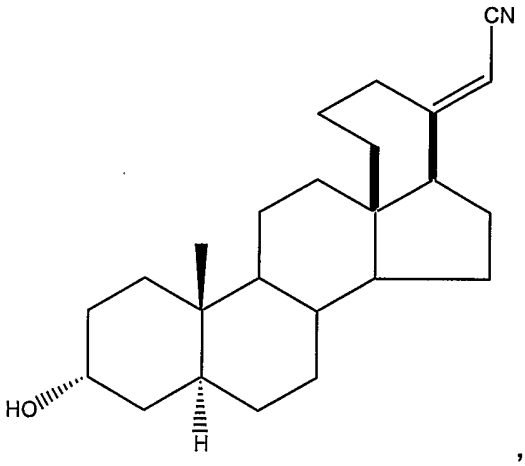
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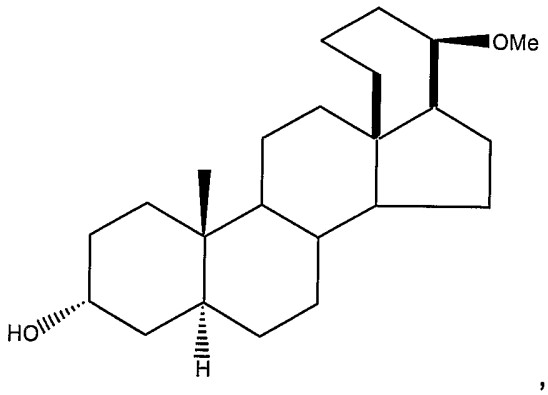
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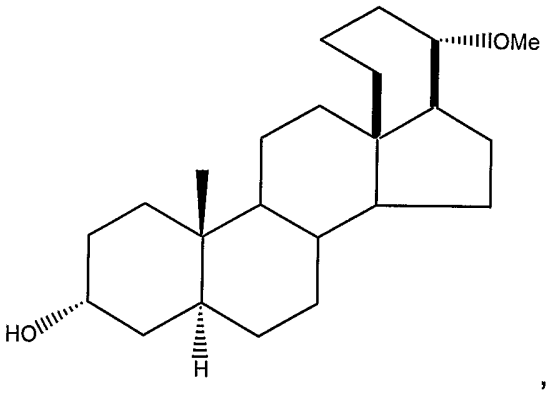
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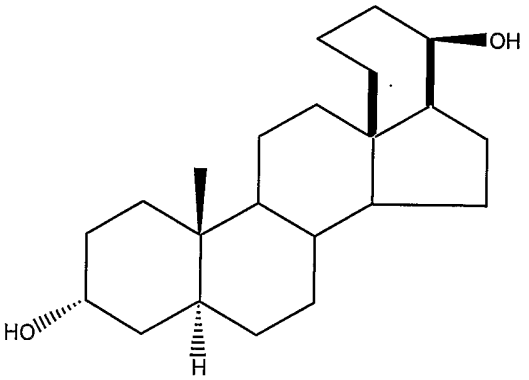
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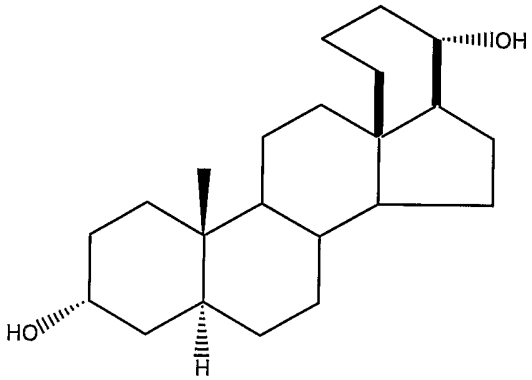
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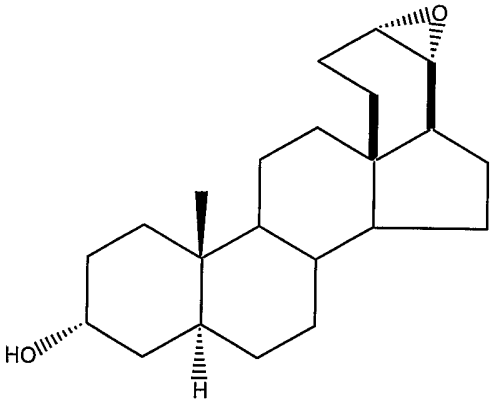
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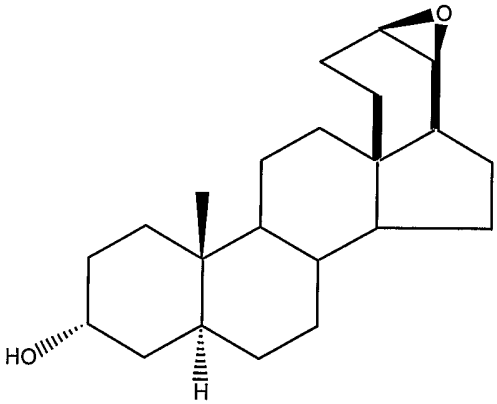
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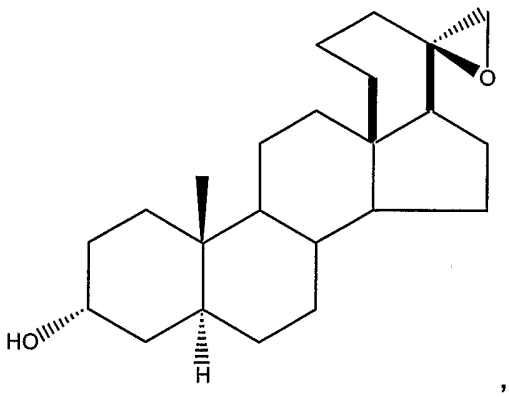
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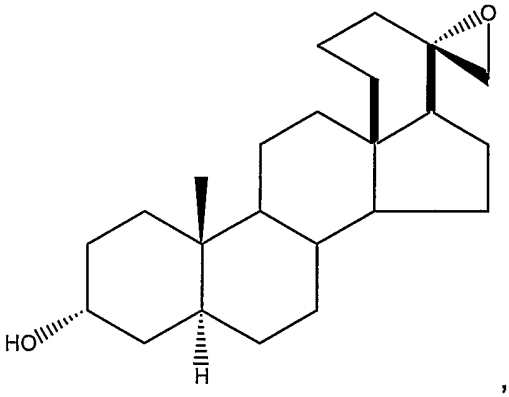
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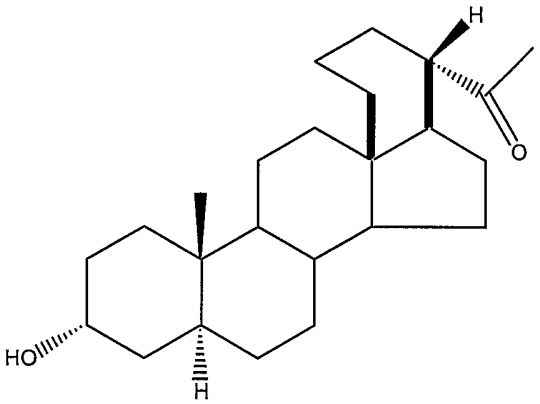
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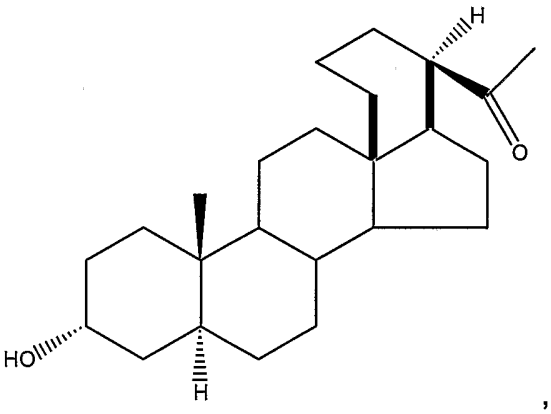
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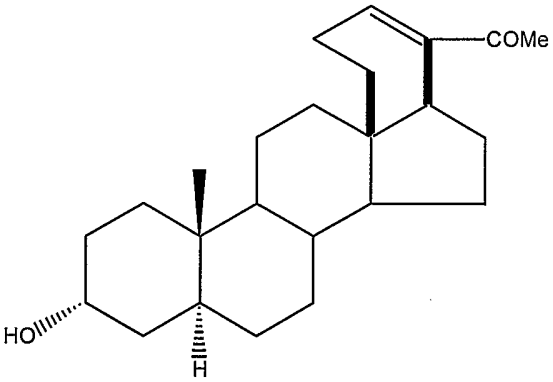
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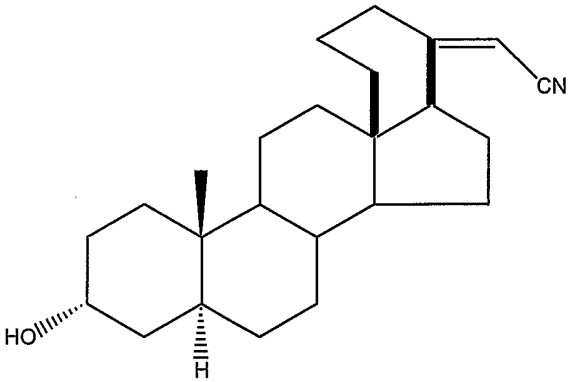
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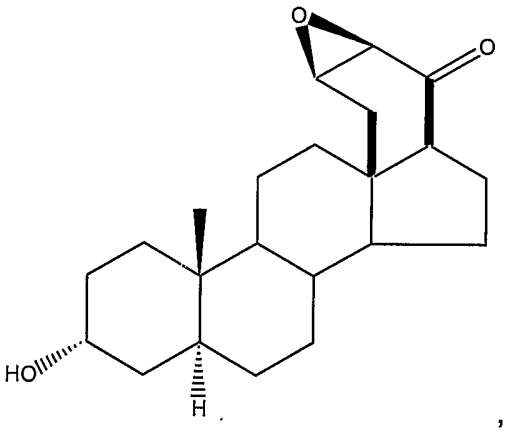

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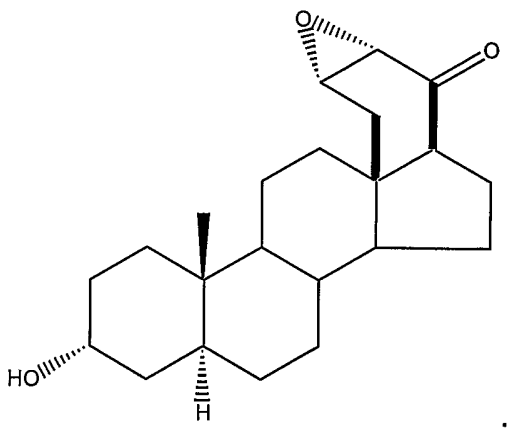

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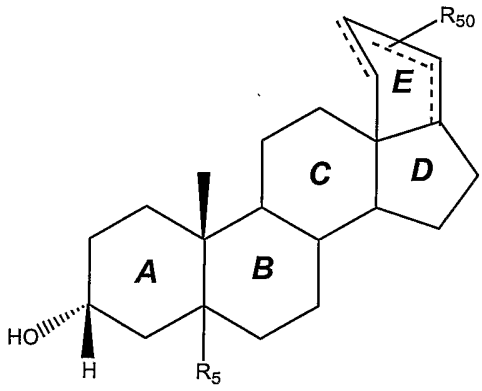
27. The compound of claim 20 having Formula (D)



(D)

wherein

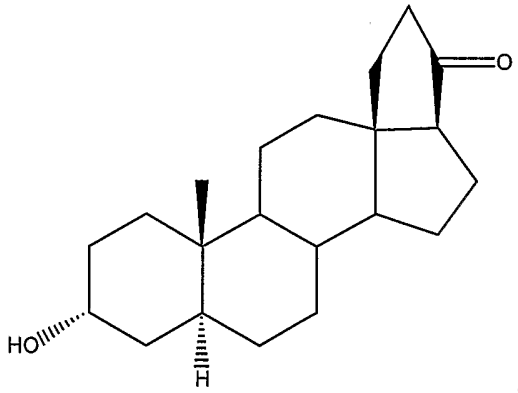
R_5 is α- or β-hydrogen;

R_{50} is selected from the group consisting of hydrogen, keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl, provided that when R_{50} is hydrogen at least one pair of E ring atoms share a double bond; and

E is a 5-membered carbocyclic ring wherein the dashed lines represent optional double bonds provided E comprises no more than 2 double bonds and provided each carbon ring atom of E is sp^2 or sp^3 hybridized.

28. The compound of claim 27 wherein the carbocyclic ring, E, is in the β-configuration in relation to the C and D rings and R_{50} is selected from the group consisting of hydrogen, keto, cyano, methylene, methoxy, acetyl, =CHCN, $=CHC(O)CH_3$, epoxide, and combinations thereof, provided that when R_{50} is hydrogen at least one pair of E ring atoms share a double bond.

29. The compound of claim 27 wherein the compound is selected from the group consisting of



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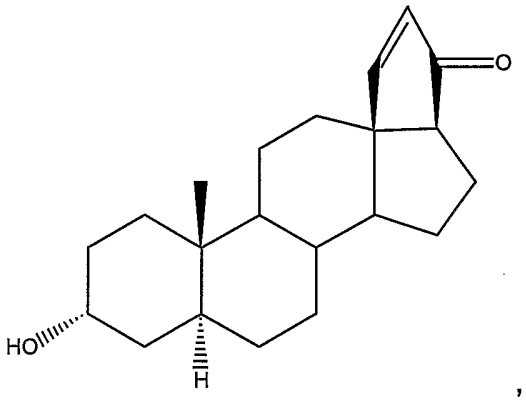

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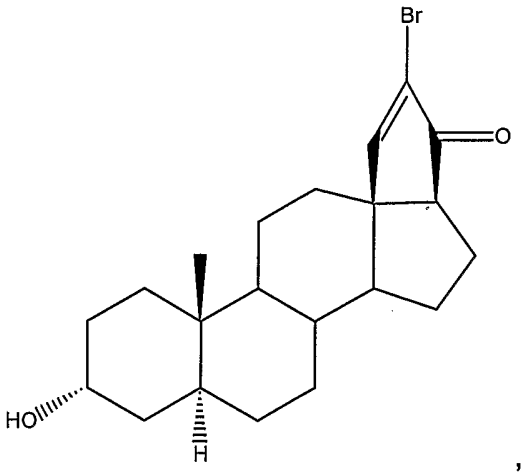

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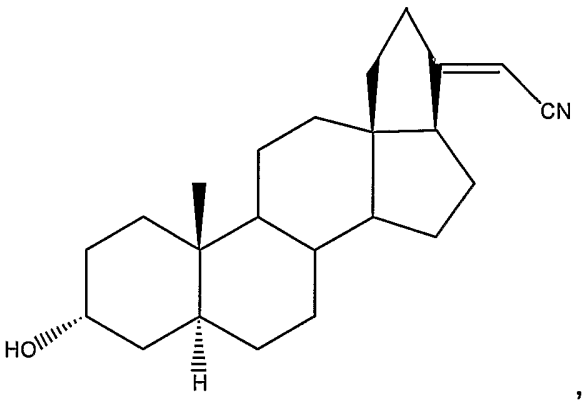

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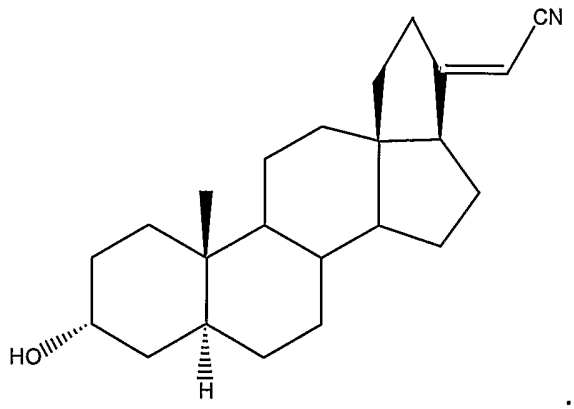


and .

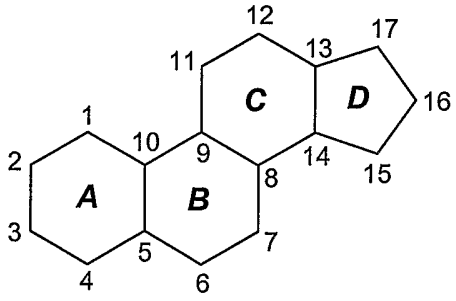
30. The steroid or D-homosteroid of claim 6 wherein the E ring is substituted by halo, alkyl, or combinations thereof.

31. A pentacyclic steroid or pentacyclic D-homosteroid, the pentacyclic steroid or pentacyclic D-homosteroid comprising:

(i) the tetracyclic steroid ring system, Formula (I), or tetracyclic D-homosteroid ring system, Formula (II), respectively;

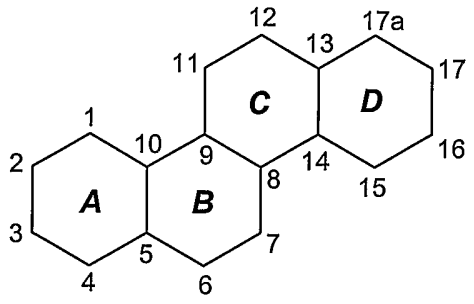
(ii) a negatively charged substituent at physiological pH at C(3); and

(iii) a fused fifth ring, E, the fused fifth ring comprising a hydrogen bond acceptor, and (a) in the case of the pentacyclic steroid the C(13) and C(17) carbons, or (b) in the case of the pentacyclic D-homosteroid the C(13) and C(17a), wherein the tetracyclic steroid and ring numbering system corresponds to Formula (I)



(I)

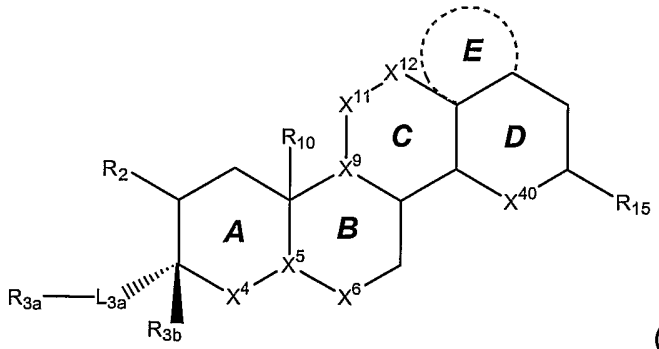
and the tetracyclic D-homosteroid and ring numbering system corresponds to Formula (II)



(II).

32. The steroid or D-homosteroid of claim 31 wherein the negatively charged substituent at C(3) is selected from the group consisting of sulfate, carboxylate, phosphate, and phosphonate.

33. The steroid or D-homosteroid of claim 31 having Formula (A)



(A)

wherein

R_2 is selected from the group consisting of hydrogen, alkoxy, and substituted or unsubstituted morpholine

R_{3a} is selected from the group consisting of sulfate, carboxylate, phosphate, and phosphonate;

R_{3b} is hydrogen, alkyl, alkenyl, or alkynyl optionally substituted with halo, hydroxy, or substituted or unsubstituted aryl;

R_5 is α- or β-hydrogen;

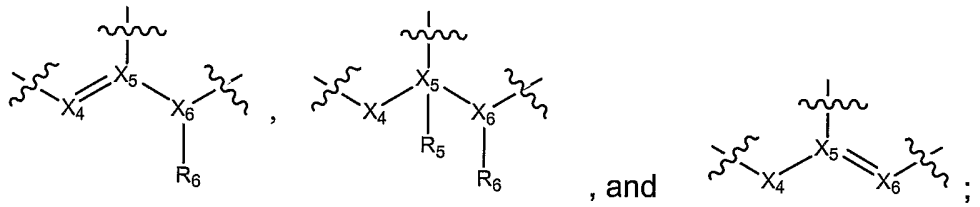
R_{10} is hydrogen or C_{1-4} alkyl;

R_{15} is hydrogen or oxo;

R_6, R_{11} and R_{12} are independently hydrogen or oxo;

L_{3a} is selected from the group consisting of a bond, C_{1-3} alkyl, heterosubstituted C_{1-3} alkyl, or alkoxy;

X_4-X_5-X_6 is selected from the group consisting of



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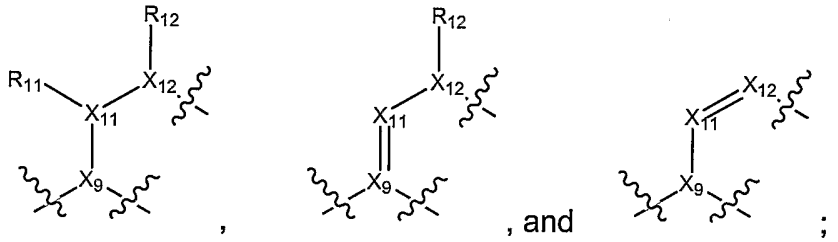
X_9-X_{11}-X_{12} is selected from the group consisting of



;

X_{40} is a bond or a carbon atom; and

E is a 5- or 6-membered carbocyclic ring, comprising a hydrogen bond acceptor and (i) the carbons at C(13) and C(17) when X_{40} is a bond, or (ii) the carbons at C(13) and C(17a) when X_{40} is carbon,

34. The compound of claim 33 wherein L_{3a} is a bond and R_{3a} is in the β-configuration.

35. The compound claim 33 wherein the hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, =CHX_1, -OX_2, -$C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl.

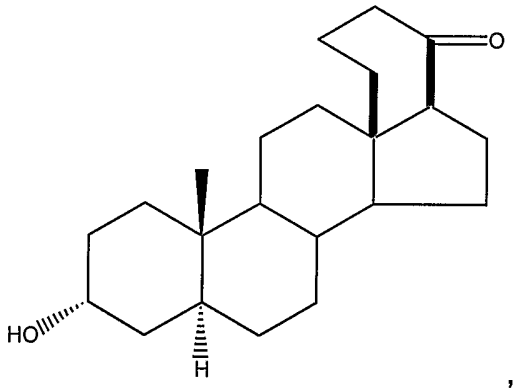
36. The compound of claim 35 wherein L_{3a} is a bond and R_{3a} is carboxyl in the α- or β-configuration.

37. A method of inducing anesthesia or treating disorders related to GABA function in a mammal, said method comprising administering a therapeutically effective amount of a pharmaceutical composition containing the steroid or D-homosteroid of claim 1 and at least one pharmaceutically acceptable carrier.

38. The method of claim 37 wherein the hydrogen bond acceptor is selected from the group consisting of keto, cyano, acyl, $=CHX_1$, $-OX_2$, $-C(O)X_2$, epoxide, an E ring alkene bond, and combinations thereof; X_1 is selected from the group consisting of hydrogen, cyano, hydrocarbyl, substituted hydrocarbyl and acyl; and X_2 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, and acyl.

39. A pharmaceutical composition comprising the pentacyclic steroid or pentacyclic D-homosteroid of claim 1 and at least one pharmaceutically acceptable carrier.

40. The composition of claim 39 where the pentacyclic steroid is selected from the group consisting of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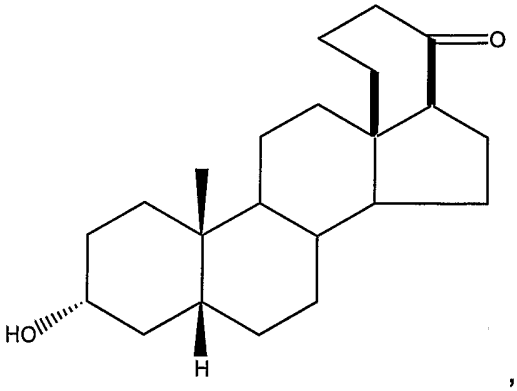
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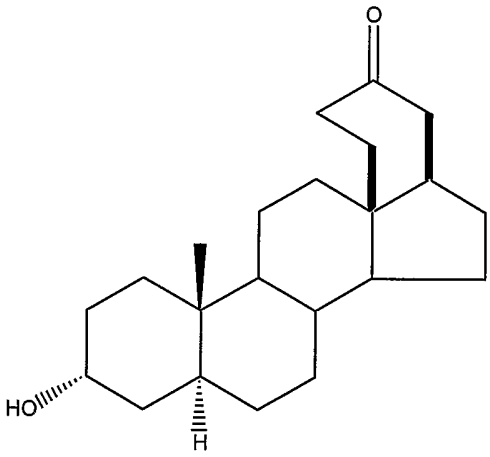
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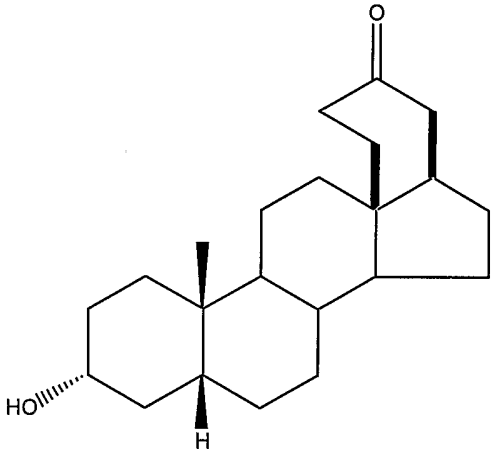
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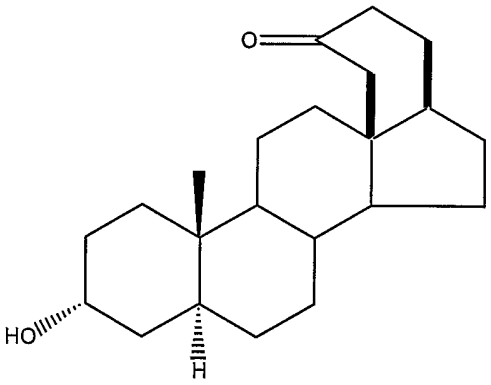
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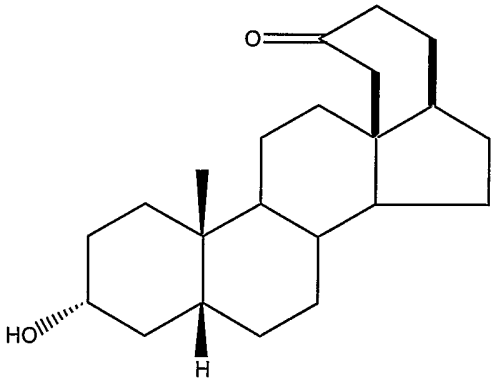
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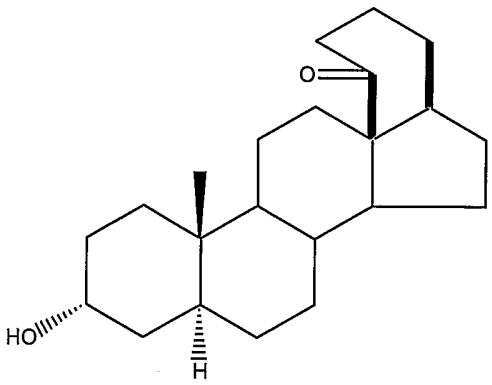
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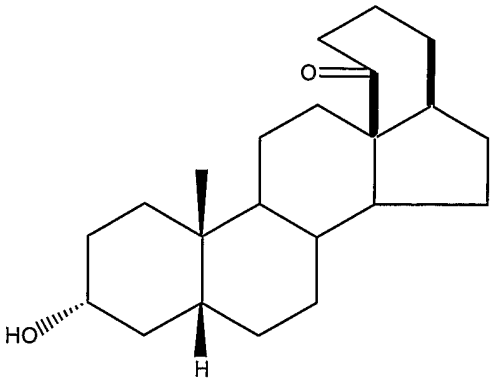
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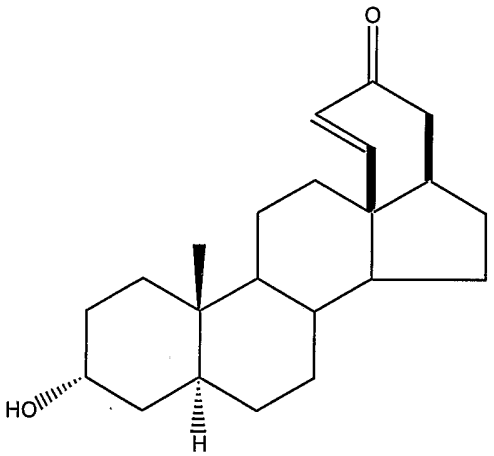
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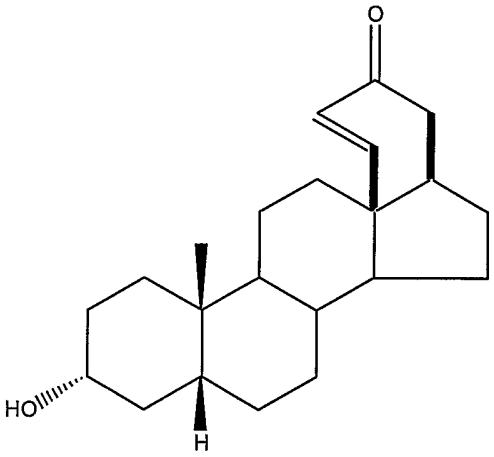
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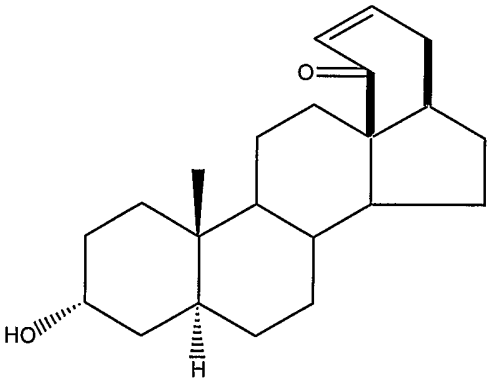
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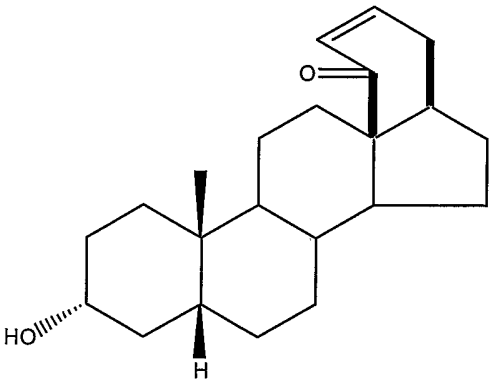
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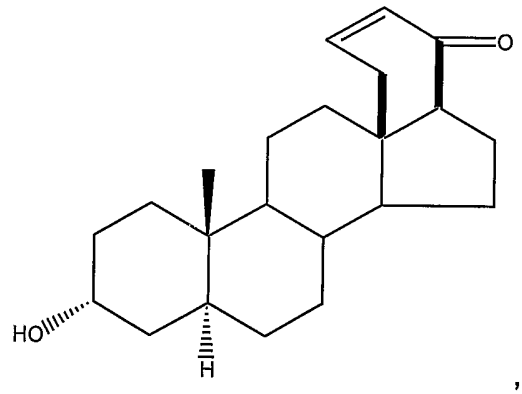
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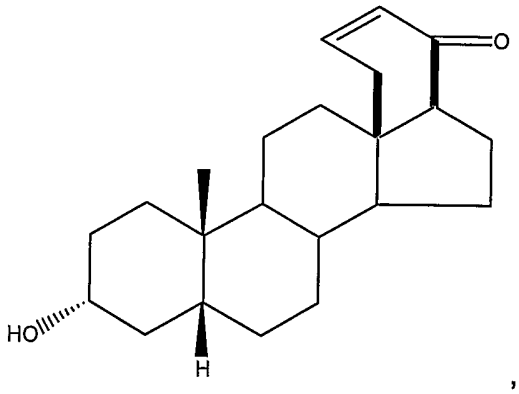
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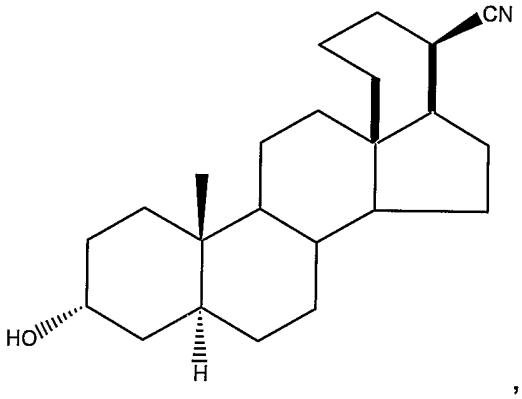
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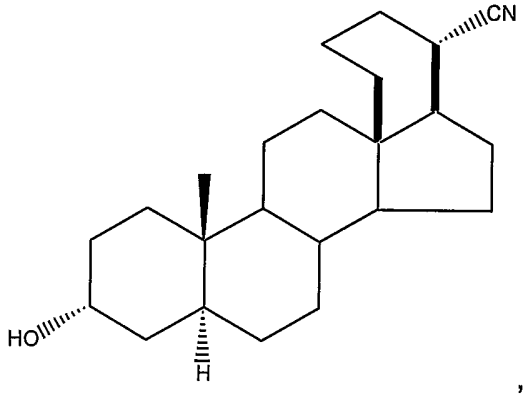
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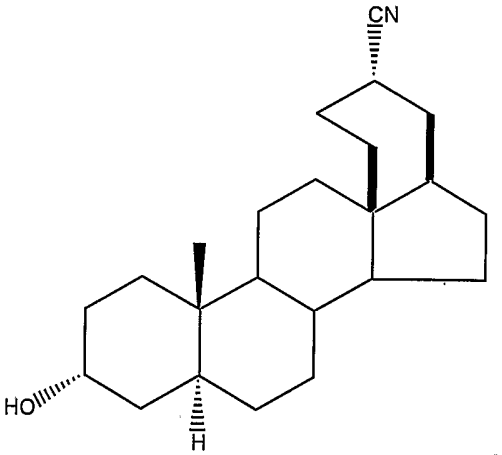
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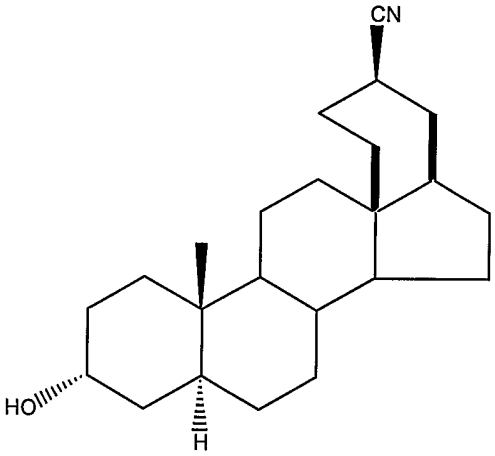
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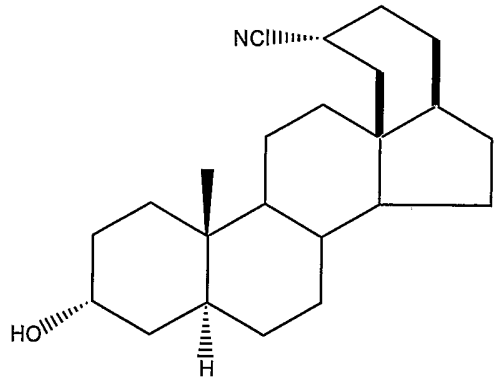
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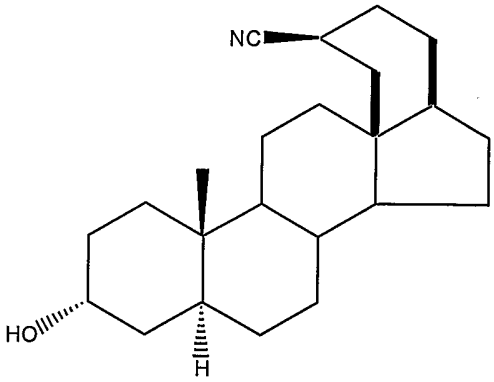
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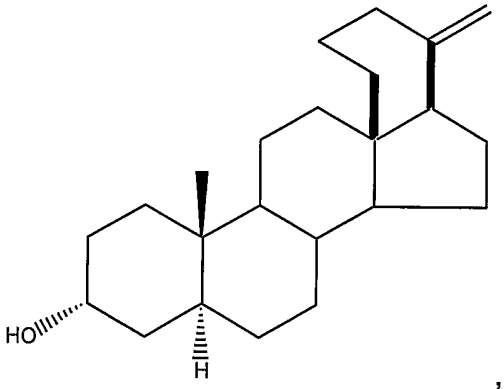
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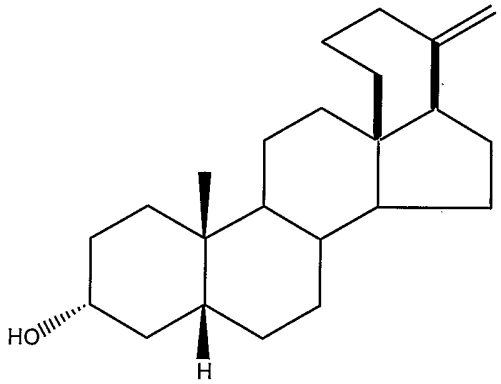
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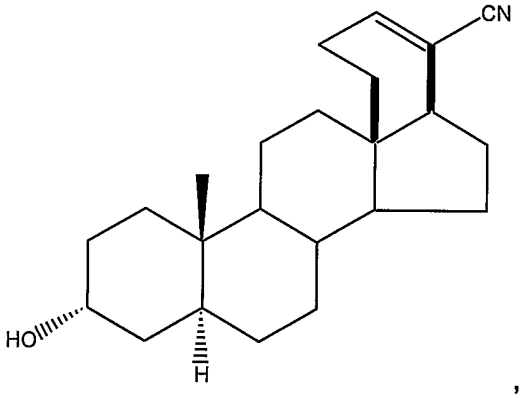
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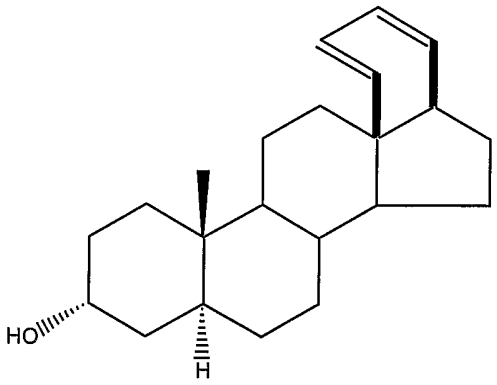
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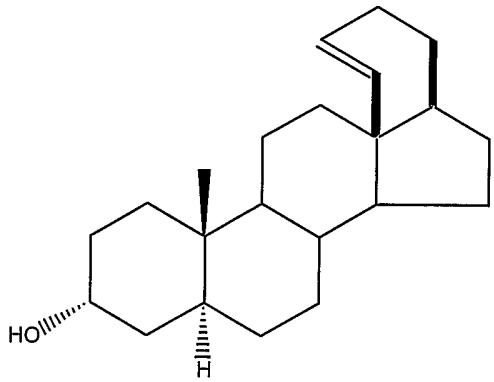
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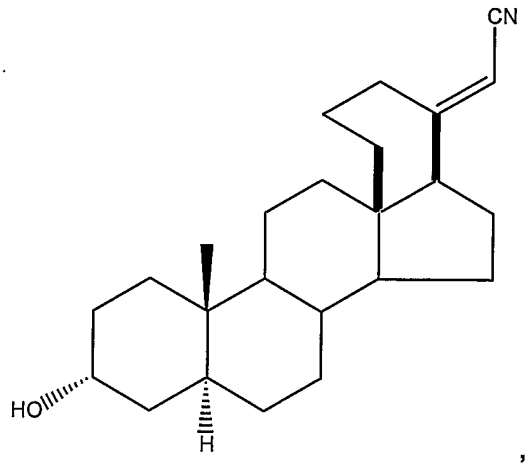
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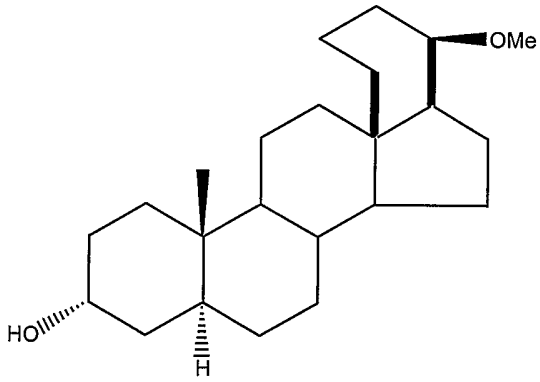
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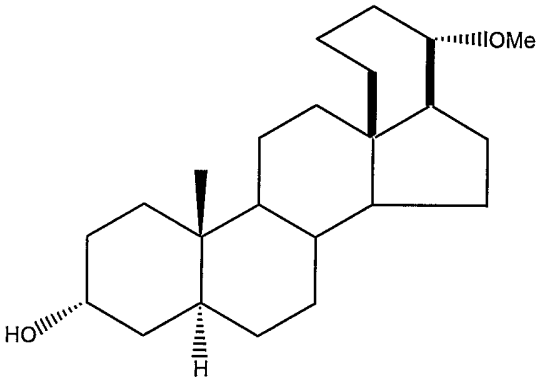
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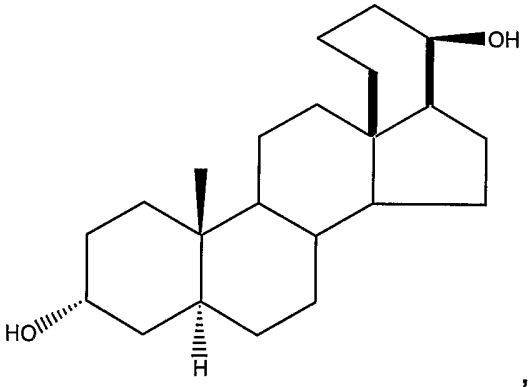
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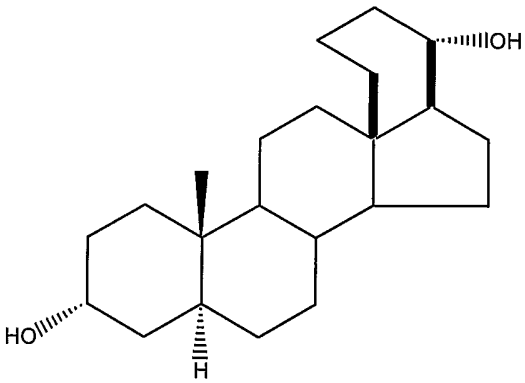
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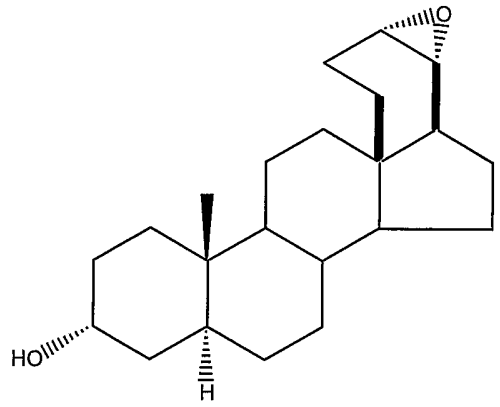
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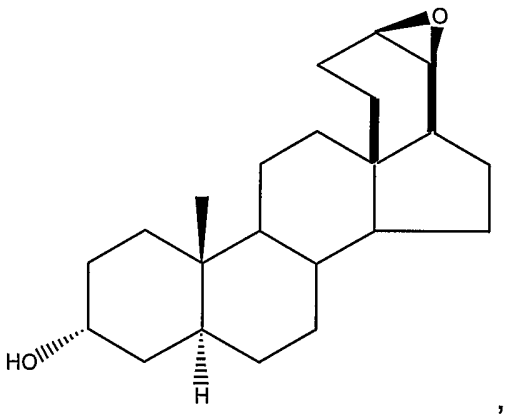
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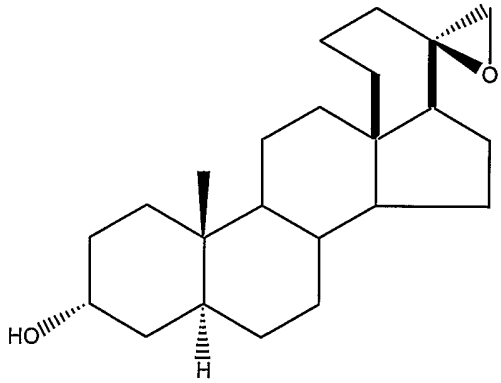
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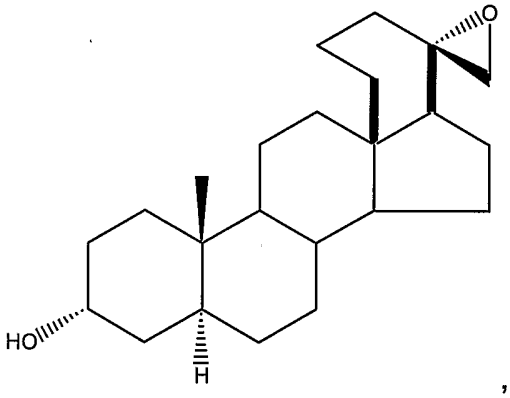
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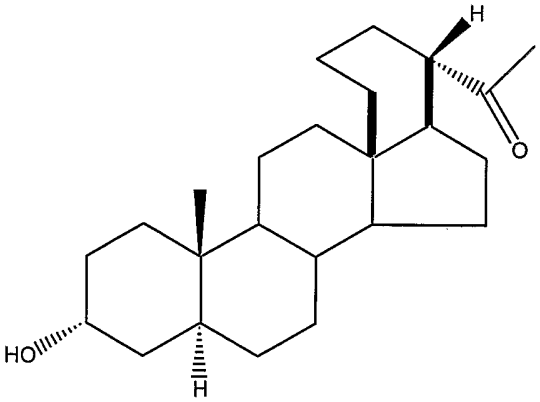
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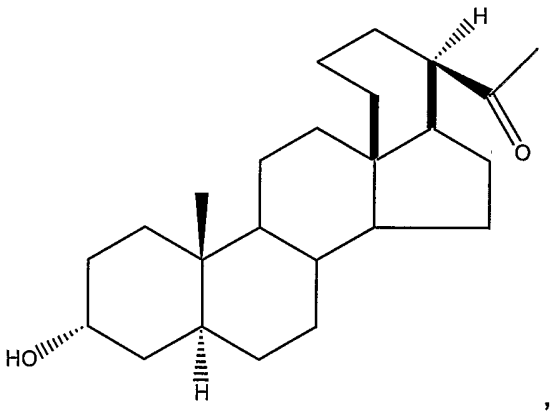
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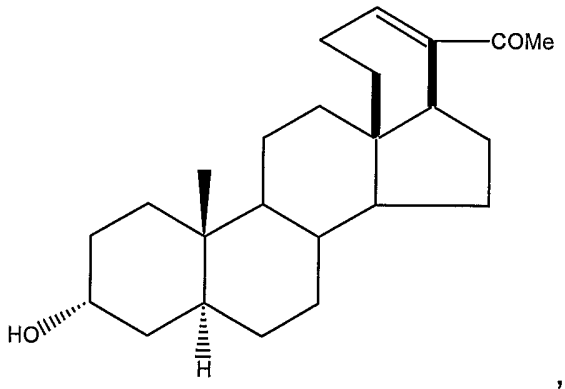
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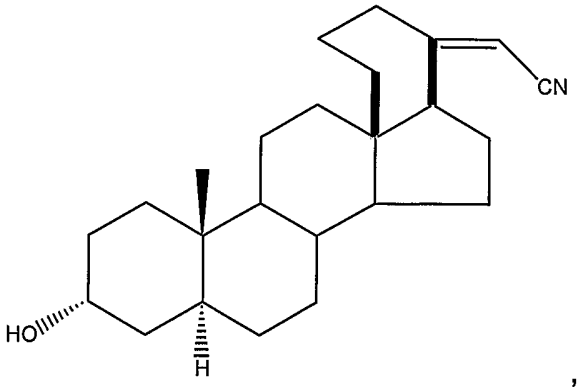
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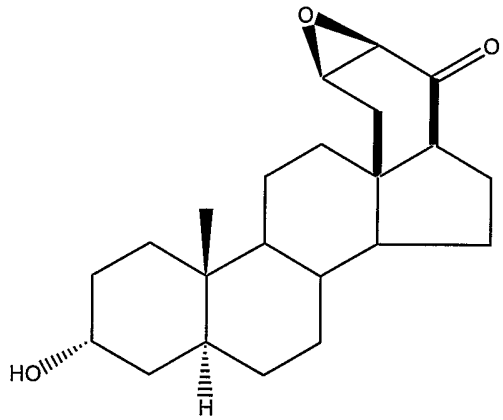
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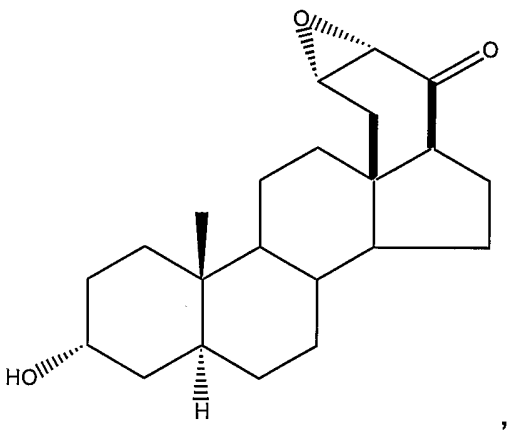
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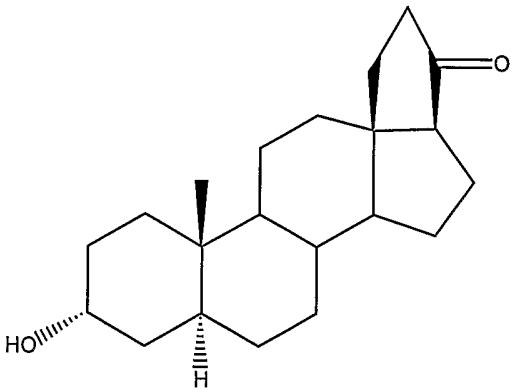
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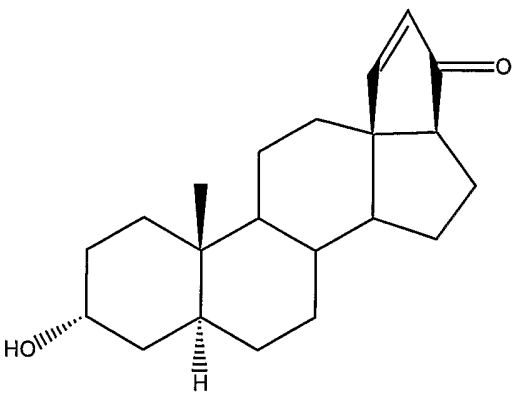
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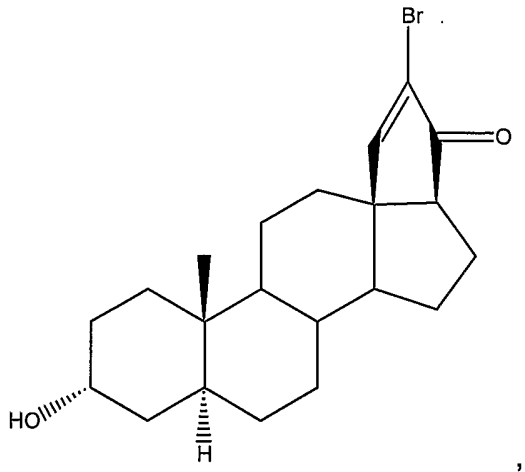

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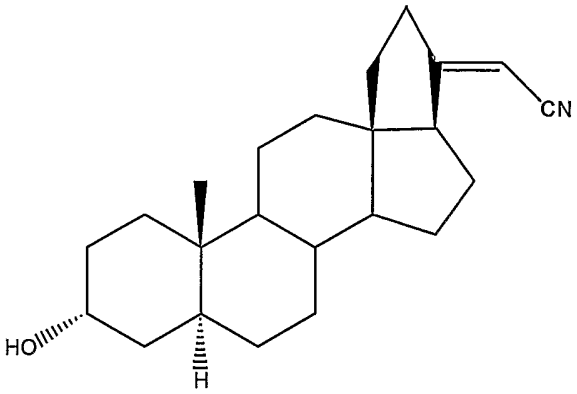
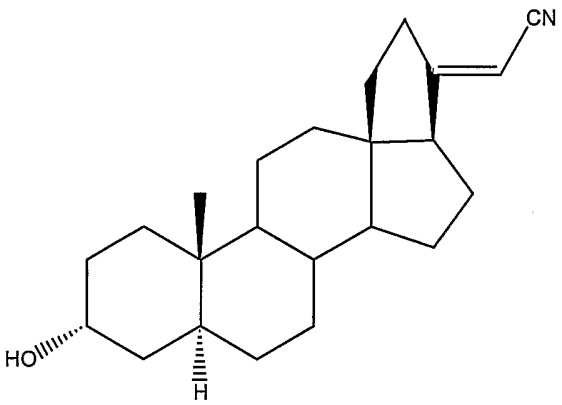




and

FIG. 1

Electrophysiological responses to GABA potentiated by cyclosteroids

FIG. 1A

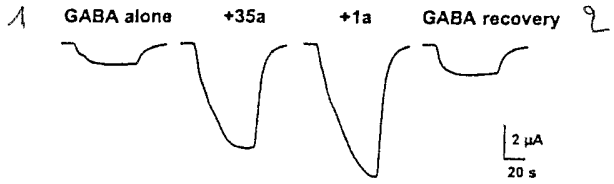


FIG. 1B

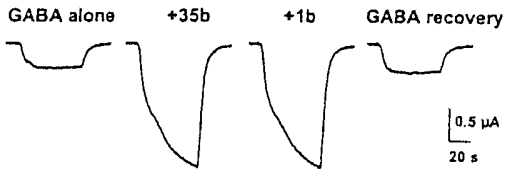


FIG. 1C

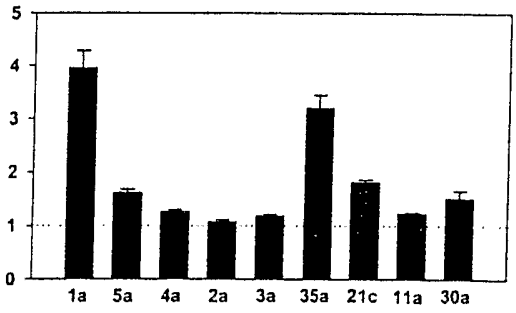
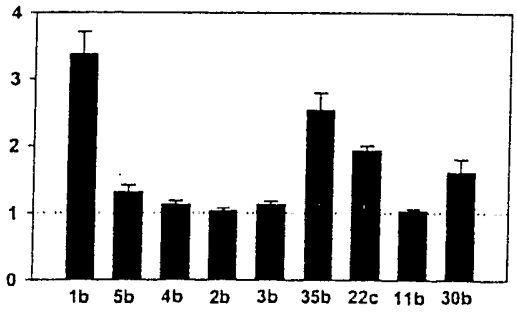


FIG. 1D



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/17055

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07J 53/00; A61K 31/56

US CL : 552/510; 514/182

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 552/510; 514/182

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

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

REGISTRY, CAPLUS, USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Bogan et al., "17,18-Cyclo Steroids." Aust. J. Chem., October 1979, Vol. 32, No. 10, pages 2323-2326, see page 2324, compounds 8a and 8b.	1, 2, 31, 32 and 39
X	Kashiwada et al., "Antitumor Agents. 180. Chemical Studies and Cytotoxic Evaluation of Cumingianosides and Cumindysoside A, Antileukemic Triterpene Glucosides with a 14, 18-Cycloapotirucallane Skeleton." J. Nat. Prod., November 1997, Vol. 60, No. 11, pages 1105-1114, especially page 1110, compounds 14 and 15.	1, 3-5, 31. 32 and 39

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

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Date of the actual completion of the international search

07 October 2004 (07.10.2004)

Date of mailing of the international search report

29 NOV 2004

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