

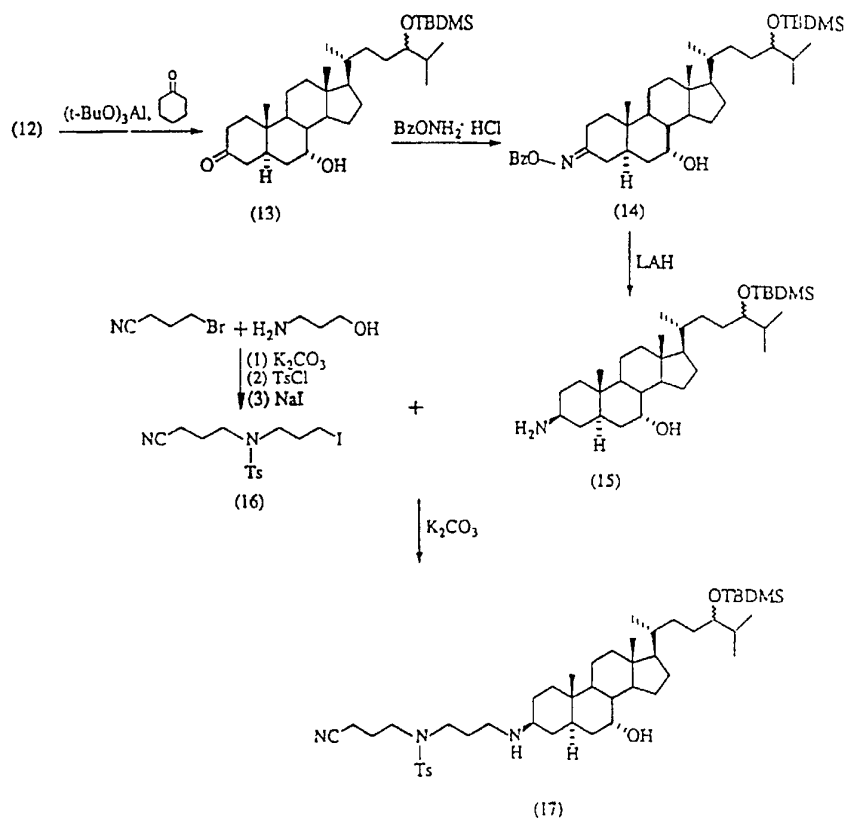


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(54) Title: CHEMICAL SYNTHESIS OF SQUALAMINE**(57) Abstract**

Methods for the chemical preparation or synthesis of the sterol antibiotic squalamine. Preferably, the preparation is by modifying the 3-position of a 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane with a spermidino moiety to form a 3 β -spermidino-7 α -hydroxy-24 ζ -ether protected group -5 α -cholestane; deprotecting the 24-position of the 3 β -spermidino-7 α -hydroxy-24 ζ -ether protected group-5 α -cholestane to the free hydroxyl; and sulfating the 24-position of the 3 β -spermidino-7 α , 24-dihydroxy-5 α -cholestane.



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Description**CHEMICAL SYNTHESIS OF SQUALAMINE**Background of the Invention

Squalamine is a novel aminosterol recently isolated from the dogfish shark, *Squalus acanthias* (K. Moore, et al., Proc. Nat. Acad. Sci. USA 90:1354-1358, 1993). This water soluble steroid exhibits potent bactericidal activity against both Gram-positive and Gram-negative bacteria. In addition, squalamine is fungicidal and exhibits lytic activity against protozoa. The molecule was initially recovered as a natural product through extraction of several tissues of the Dogfish shark, including stomach, liver, gallbladder, and spleen. Its structure was determined by fast atom bombardment mass spectroscopy and NMR. The chemical structure of squalamine is that of a cationic steroid characterized by a condensation of an anionic bile salt intermediate with spermidine. A review of the chemical database revealed that squalamine represented a new chemical entity, and a member of a novel class of antibiotics.

Summary of the Invention

The present invention provides a method of preparing squalamine which comprises alkylating the 3-position of a 3-oxo-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane with a spermidino moiety to form a 3 β -spermidino-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane; deprotecting the 24 ζ -position of the 3 β -spermidino-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane to the free hydroxyl; and sulfating the 24 ζ -position of the 3 β -spermidino-7 α , 24 ζ -dihydroxy-5 α -cholestane. The method can optionally include protecting the 7 α -hydroxy group and the spermidino amino groups during sulfating of the 24-position.

Preferably, formation of the 3 β -spermidino-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane comprises the steps of forming a 3 β -amino-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane from the 3-oxo-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane; alkylating the 3 β -amino-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane with a spermidino precursor moiety at the 3-position; and reducing the spermidino precursor moiety to form a 3 β -spermidino-7 α -hydroxy-24 ζ -protecting group ether-5 α -cholestane.

Brief Description of the Drawings

Figure 1a - 1f illustrate the pathway used for the chemical synthesis of squalamine described in Example 1.

Figure 2 illustrates the synthesis of 3-oxo-7 α -hydroxy-24-tert-butyldimethylsiloxy-5 α -cholestane (compound 13) from chenodeoxycholic acid described in Example 2.

Figure 3 illustrates the synthesis of compound 13 from chenodeoxycholic acid described in Example 3.

Figures 4a and 4b illustrate the synthesis of compound 13 from fucosterol acetate described in Example 4 and the synthesis of squalamine.

Figure 5 illustrates the synthesis of compound 13 from dehydroepiandrosterone acetate described in Example 5.

Figure 6 illustrates the synthesis of compound 13 through microbial conversion of 3, 24-diketocholestane-24-ethylenedioxyketal to the 7 α -hydroxy derivative described in Example 6.

Figure 7 illustrates the synthesis of compound 13 from pregnenolone acetate described in Example 7.

Detailed Description of Preferred Embodiments

A principal aspect of the invention provides a method for synthesizing squalamine which comprises:

modifying the 3-position of a 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane with a spermidino moiety to form a 3 β -spermidino-7 α -hydroxy-protecting group ether-5 α -cholestane;

deprotecting the 24 ζ -position of the 3 β -spermidino-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane to the free hydroxyl; and

sulfating the 24 ζ -position of the 3 β -spermidino-7 α , 24 ζ -dihydroxy-5 α -cholestane. This can further comprise the step of protecting the 7 α -hydroxy group and the spermidino amino groups during sulfation of the 24-position and deprotecting such positions after sulfation.

In a preferred embodiment of this aspect, formation of the 3 β -spermidino-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane comprises the steps of:

forming a 3 β -amino-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane from the 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane;

alkylating the 3 β -amino-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane with a spermidino precursor moiety at the 3-position; and

reducing the spermidino precursor moiety to form a 3 β -spermidino-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane.

The 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane can be formed from 3 β -hydroxy

-5-

5-cholenic acid. Preferably, this comprises the steps of protecting the hydroxyl moieties of 3 β -hydroxy-5-cholenic acid to form a 3 β -protecting group ether-24 ζ -protecting group ester of-5-cholenic acid;

homologating the 24 ζ -protected position to the 3 β ,24 ζ -diprotected cholesterol;

allylically oxidizing the 7-position of the diprotected cholesterol;

reducing the cholestene and C₇ carbonyl groups;

deprotecting the 3-position to form 3 β ,7 α -24-trihydroxy cholestane 24 ζ -ether protected alcohol; and

oxidizing to the 3-keto form.

In another preferred embodiment of this aspect, forming the 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group -5 α -cholestane comprises the steps of:

oxidizing 3-acetoxychenodeoxycholic acid into the 1,4-dienone;

reducing to 3-keto-7 α -hydroxy-5 α -cholanolic acid;

protecting the C₃ keto and 7-hydroxy functionalities;

side-chain homologating; and

deprotecting yielding 3-keto-7 α ,24-dihydroxy-5 α -cholestane 24-ether protected alcohol.

Alternatively, this can be achieved by oxidizing chenodeoxycholic acid to the 3-one-4-ene and reducing the double bond to 3-keto-7 α -hydroxy-5 α -cholanolic acid.

The following detailed description identifies compounds named in the examples by their reference numbers and should be read in conjunction with the drawings.

In accordance with the principal aspect of the invention, squalamine (25) is prepared from its cognate sodium sulfate salt (24) or other suitable hydrolyzable alkali metal salts by acid hydrolysis in the presence of a C₁-C₄ alkyl alcohol or other water soluble organic solvent. This final step consists primarily in neutralization of the salt form of the title compound.

The cognate alkali metal salt is prepared from the pyridinium sulfate (23) by mixture with a C₁-C₄ alkyl alcohol and a strong alkali metal base such as an alkali metal hydroxide, e.g., NaOH, under conditions where the pyridine moiety is cleaved to form the sulfate salt with the metal ion of the alkali metal base and pyridinium halide by-products are formed. Appropriate solvents for this step are also the C₁-C₄ alcohols.

The pyridinium intermediate (23) is formed by sulfur trioxide pyridine sulfation of compound (22) with anhydrous pyridine. Other sulfating agents that could be used include chlorosulfonic acid, trimethylsilyl chylchlorosulfonic acid, sulfur trioxide trimethylamine complex and sulfur trioxide triethylamine complex.

Compound (22) is formed by removing the carbobenzoxy (CBZ) protective groups used to protect the amine groups on the spermidine side chain. The CBZ protective groups are removed by catalytic hydrogenation using 10% palladium-charcoal and hydrogen gas. Also in this deprotection step, the tertiary butyldimethylsilyl (TBDMS)

is removed by acid cleavage using, for example, hydrochloric acid in ethanol. Other strong acids in suitable water soluble alcohol solvents can also be used. Compound (21) is prepared by acetylation of the hydroxyl at the C₇ of the steroid ring nucleus. Acetylation of this site protects it from subsequent reactions in the synthesis. Other known acylating agents can also be used to provide the derived alkyl or aryl esters. Thus, the C₇ hydroxyl is protected from sulfation at the time that the C₂₄ carbon is sulfated during preparation of compound (23).

Compound (20) is prepared by protection with CBZ. Other protecting groups which could be used for protection of these amino sites on the side chain are CBZ derivatives or sulfonylating agents, e.g., p-toluenesulfonyl chloride or p-bromophenylsulfonyl chloride. Compound (19) is prepared by treating compound (18) with lithium aluminum hydride (LAH) in diethyl ether, which reduces the terminal cyano (C N) group to a primary amine. Other chemical reducing agents which could be used include, for example, alkali metal lithiohydride derivatives, as well as catalytic hydrogenation methods, such as use of rhodium plated on alumina. Compound (18) is prepared from compound (17) by cleavage of the tosyl protective group from the tosylamide to yield the free secondary amine. Other protecting groups which could be used in this step are N-carbobenzoxy and p-bromosulfonyl.

Preparation of compound (17) from compounds (16) and (15) constitutes attachment of the spermidine side chain precursor by displacement of the iodine from N-3 (cyanopropyl)N-3-propyl iodide. The spermidine side chain precursor is attached, once the iodine has been displaced,

to the C₃β-amino group of the steroid nucleus using K₂CO₃ or any related alkali metal base.

Compound (16) is formed by a three-step reaction. The bromine of 3-cyanopropyl bromide is displaced by the amino group of 3-hydroxypropylamine, the thus formed hydrobromic acid being neutralized by K₂CO₃, to form the spermidine side chain precursor. Then, this spermidine side chain precursor is tosylated at the secondary amine and the hydroxyl moiety, such that the free secondary amine becomes a tosyl protected group and the hydroxyl becomes a tosyl ester. This protected intermediate is then reacted with sodium iodide or other suitable alkali metal halide to provide compound (16).

Compound (15) is formed from compound (14) by reductive formation of the 3β-amino group from the benzyloxyimino compound (14) using LAH as one of the appropriate reducing agents, others of which can be used are sodium borohydride and catalytic hydrogenation.

Compound (14) can also be obtained from compound (13) using alkoxyamines or other oximine groups as well as the oxime itself.

Compound (13) is prepared by an Oppenauer selective oxidation of compound (12) using aluminum tri-tert-butoxide. Other aluminum alkoxides that could be used include aluminum tri-isopropoxide. Another ketone which can be used is acetone.

Compound (12) is prepared from compound (11) by the cleavage of the C₃ acetoxy group using sodium cyanide in methanol. Other methods which could be used in this step

include sodium carbonate in methanol and lithium hydroxide in methanol.

Compound (11) is formed by reduction of the corresponding 7-one, compound (10), such as with K-selectride (potassium tri-sec-butylborohydride, Aldrich Chemical Co., Milwaukee, WI), which reduces the carbonyl stereoselectively and does not cleave the acetyl group at position 3 of compound 10. Compound 10 is formed by saturation of the 5-cholestene (compound 9) stereoselectively to the corresponding $\alpha\beta$ -trans cholestane.

Compound (9) is made by allylic oxidation of the 5-cholestene (compound 8) at the 7-position using chromium hexacarbonyl and tert-butyl hydroperoxide. Other known oxidative methods for this transformation are: tert-butyl chromate; Jones reagent; N-bromosuccinimide, H_2O , MnO_2 .

Compound (8) is formed by acetylation of compound (7). Compound (7) is made by deprotection of the C_3 tetrahydropyranol ether using pyridinium paratoluene sulfonate, or other mild acids which do not cleave the C_{24} protective group. Compound (6) is formed by protecting compound (5) at the C_{24} with tert-butyl-dimethylsilyl chloride (TBDMS) to yield the derived C_{24} TBDMS ether (6). Other trialkyl such as trimethyl and triethyl or diaryl alkyl, such as diphenylmethyl silyl groups.

Compound (5) is a Grignard addition of isopropyl magnesium halide, e.g., bromide, to the C_{24} aldehyde (4). Compound (4) is formed by Swern oxidation of the C_{24} hydroxy (3) to the derived aldehyde. Compound (3) is formed by reduction of the C_{24} tetrahydropyranyl ester (2), e.g., using lithium aluminum hydride. Compound (2) is formed by

reaction of 5-cholenic acid-3 β -ol (Steroids, Ltd., Chicago, IL) with 2, 3-dihydropyrane with pyridinium para-toluene sulfonate as a catalyst. Another protecting group that can be used for protection of the C₃ and C₂₄ groups is the tetrahydrofuranyl moiety.

Example 1Chemical Synthesis of Squalamine

The title compound, 3 β -N-1-[N(3-[4-aminobutyl])-1,3-diaminopropane]-7 α , 24 β -dihydroxy-5 α -cholestane-24-sulfate, which has been given the common name squalamine, has now been chemically synthesized. The preferred method of chemical synthesis of the title compound is described in this example with reference to the synthesis pathway and numbered compounds illustrated in Figures 1a-1f as follows.

3 β -(2-Tetrahydropyranoxy)-5-cholenic acid tetrahydropyranyl ester (2)

To a stirred solution of 3 β -hydroxy-5-cholenic acid (5-cholenic acid-3 β -ol) (1) (100 g, 270 mmole) in anhydrous CH₂Cl₂ (600 ml) was added pyridinium p-toluenesulfonate (PPTS) (3.40 g, 10 mmole) and 2,3-dihydropyran (67.3 g, 800 mmole) at room temperature. The reaction mixture was stirred for 8 hours, then it was diluted with CH₂Cl₂ (600 ml), washed with saturated NaHCO₃ solution (2 x 50 ml), brine (1 x 50 ml), dried (MgSO₄), filtered and concentrated in vacuo to get compound (2) (117.0 g, 80%) as a viscous oil. The crude compound was used in the step without further purification. ¹H NMR (400 MHz, CDCl₃) δ : 0.68 (3H, s, 18-CH), 0.94 (3H, d, 21-CH₃), 1.01 (3H, s, 19-CH₃), 2.36 (2H, m, CH₂CO₂), 3.50 (2H, m, OCH₂), 3.68 (1H, m, 3 α -H), 3.90 (2H, m, OCH₂), 4.72 (1H, t, OCHCH₂), 5.35 (1H, t, C=CH), and 5.96 (1H, t, OCHCH₂).

3 β -(2-Tetrahydropyranoxy)-5-cholen-24-ol (3)

To a stirred suspension of lithium aluminium hydride (8.20 g, 216 mmole) in anhydrous tetrahydrofuran (500 ml) was added dropwise a solution of compound (2) (117 g, 216 mmole) in anhydrous tetrahydrofuran (200 ml) at 0°C (ice bath). Then the reaction mixture was stirred at room temperature for 2 hours and decomposed by pouring onto the crushed ice and dilute hydrochloric acid. The aqueous solution was extracted with ether (3 x 500 ml). The combined ether extracts were washed with brine (1 x 70 ml), dried (MgSO₄), filtered and concentrated in vacuo to get almost pure compound (3) (81.6 g, 85%). ¹H NMR (400 MHz, CDCl₃) δ : 0.69 (3H, s, 18-CH₃), 0.96 (3H, d, 21-CH₃), 1.01 (3H, merged s, 19-CH₃), 3.50 (2H, m, OCH₂), 3.61 (2H, merged t, 24-CH₂), 3.93 (1H, m, 3 α -H), 4.72 (1H, m, OCHO), 5.35 (1H, t, C=CH-CH₂). The compound was used in the next reaction step.

3 β -(2-Tetrahydropyranoxy)-5-cholen-24-al (4)

To a stirred solution of anhydrous dimethylsulfoxide (DMSO) (51 ml, 720 mmole) in anhydrous CH₂Cl₂ (400 ml) was added dropwise a solution of oxalyl chloride (31.4 ml, 360 mmole) in anhydrous CH₂Cl₂ (200 ml) at -78°C under argon. After stirring the mixture for 20 minutes at the same temperature, a solution of compound (3) (81.6 g, 183 mmole) in anhydrous CH₂Cl₂ (200 ml) was added dropwise. The reaction mixture was stirred for 1 hour at -78°C, and then anhydrous triethylamine (100 ml, 717 mmole) was added slowly. The mixture was allowed to warm to room temperature, and quenched with saturated NH₄Cl solution. The organic phase was separated from the aqueous, and aqueous phase was extracted with CH₂Cl₂ (2 x 500 ml).

The combined organic extracts were washed with saturated NaHCO_3 solution (2 x 75 ml), brine (1 x 70 ml), dried (MgSO_4), filtered and concentrated in vacuo to get crude compound (4) (40.1 g, 49%). The compound was used in the next step without further purification. ^1H NMR (200 MHz, CDCl_3) δ : 0.68 (3H, s, 18- CH_3), 0.91 (3H, d, 21- CH_3), 1.00 (3H, s, 19- CH_3), 2.36 (2H, m, 23- CH_2), 3.49 (2H, m, OCH_2), 3.88 (1H, m, 3 α -H), 4.70 (1H, m, OCHCH_2), 5.32 (1H, m, $\text{C}=\text{CH}$), 9.75 (1H, t, 24-CHO). CIMS (m/e): 443 ($\text{M}^+ + 1$, 1%), 425 (1.2%), 383 (1.1%), 375 (3%), 357 (11%), 341 (100%), 323 (10%), 273 (4%), 255 (4%).

3 β -(2-Tetrahydropyranoxy)-5-cholestene-24 -ol (5)

To a stirred solution of compound (4) (40.0 g, 90 mmole) in anhydrous tetrahydrofuran (500 ml) was added a solution of isopropylmagnesium bromide in tetrahydrofuran (2M in THF, 135 ml, 270 mmole) dropwise at 0°C (ice bath). After complete addition, the reaction mixture was stirred at room temperature for 4 hours and then the reaction was quenched by slow addition of 2N HCl solution. The aqueous solution was extracted with ether (3 x 400 ml). The combined ether extracts were washed with water (2 x 100 ml), brine (1 x 70 ml), dried (MgSO_4), filtered and concentrated in vacuo to get crude product which was purified by flash chromatography on silica gel to get pure compound (4) (37.2 g, 85%). ^1H NMR (400 MHz, CDCl_3) δ : 0.69 (3H, s, 18- CH_3), 1.02 (3H, s, 19- CH_3), 3.32 (1H, m, 24 δ -H), 3.51 (2H, m, OCH_2), 3.92 (1H, m, 3 β -H), 4.72 (1H, t, OCHCH_2), and 5.35 (1H, t, $\text{C}=\text{CH}-\text{CH}_2$).

3 β -(2-Tetrahydropyranoxy)-24 ζ -tert-butyldimethylsiloxy-5-cholestene (6)

A solution of compound (5) (37.2 g, 76.5 mmole), tert-butyldimethylsilyl chloride (17.3 g, 114.8 mmole) and imidazole (39.0 g, 572.8 mmole) in anhydrous CH₂Cl₂ (500 ml) was stirred at room temperature for 14 hours. The reaction mixture was diluted with CH₂Cl₂ (500 ml), washed with water (1 x 250 ml), 2N HCl (2 x 100 ml), brine (1 x 100 ml), dried (MgSO₄), filtered and concentrated in vacuo to get crude compound (6) (41.4 g, 90%). The crude compound was used in the next step without purification. ¹H NMR (400 MHz, CDCl₃) δ : 0.00 & 0.01 (2 x 3H, two s, Si(CH₃)₂), 0.68 (3H, s, 18-CH₃), 0.91 (9H merged s, SiC(CH₃)₃), 1.01 (3H, s, 19-CH₃), 3.36 (1H, M, 24 δ -H), 3.52 (2H, m, OCH₂), 3.83 (1H, m, 3(α -H), 4.72 (1H, t, OCHCH₂), and 5.36 (1H, t, C=CH-CH₂).

3 β -Hydroxy-24 ζ -tert-butyldimethylsiloxy-5-cholestene (7)

A solution of compound (6) (41.4 g, 68.8 mmole) and pyridinium p-toluenesulfonate (PPTS) (0.86 g, 3.42 mmole) in methanol (600 ml) was refluxed for 4 hours. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in ether (800 ml), washed with 2N HCl (2 x 100 ml), brine (1 x 100 ml), dried (MgSO₄), filtered and concentrated in vacuo to get compound (7) (30.2 g, 85%). The crude compound was used directly in the next step.

3 β -Acetoxy-24 ζ -tert-butyldimethylsiloxy-5-cholestene (8)

A solution of compound (7) (30.0 g, 58 mmole), dry pyridine (200 ml, 2473 mmole) and acetic anhydride (30 ml, 318 mmole) was heated at 80°C (oil bath temperature) for 1 hour. Then the cold reaction mixture was poured into a crushed ice and saturated NaHCO₃ solution. The aqueous solution was extracted with ether (3 x 300 ml). The combined ether extracts were washed with saturated NaHCO₃ solution (2 x 100 ml), water (2 x 150 ml), 2NHCl (3 x 75 ml), brine (1 x 100 ml), dried (MgSO₄), filtered and concentrated in vacuo to afford the crude product which was purified by flash chromatography on silica gel to give pure compound (8) (28.6 g, 88%). ¹H NMR (400 MHz, CDCl₃) δ : 0.01 (6H, br s, Si(CH₃)₂), 0.68 (3H, s, 18-CH₃), 0.90 (9H, merged s, SiC(CH₃)₃), 1.02 (3H, s, 19-CH₃), 3.37 (1H, m, 24 ζ -H), 4.60 (1H, m, 3(α -H), and 5.38 (1H, m, C=CH-CH₂).

3 β -Acetoxy-24 -tert-butyl dimethylsiloxy-5-cholestene-7-one
(9)

A solution of compound (8) (28.0 g, 50.1 mmole), chromium hexacarbonyl (11.6 g, 52.7 mmole) and tert-butyl hydroperoxide (100 ml, 94 g, 1043 mmole) in anhydrous acetonitrile (500 ml) was refluxed under argon for 12 hours. Acetonitrile was removed in vacuo, and the residue was dissolved in ether (500 ml). The ether extract was washed with saturated NaHCO₃ (3 x 150 ml), brine (1 x 100 ml), dried (MgSO₄), filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel to give pure compound (9) (13.2 g, 46%). IR (neat): 1674 (C=CHCO), 1736 (OCOCH₃) cm⁻¹; ¹H NMR (400 MHz, CDCl₃), δ : 0.00 & 0.01 (2 x 3H, two s, Si(CH₃)₂), 0.68 (3H, s, 18-CH₃), 0.90 (9H, merged s, SiC(CH₃)₃), 1.20 (3H, s, 19-CH₃), 2.04 (3H, s, OCOCH₃), 3.38 (1H, m, 24³-H), 4.72 (1H, m, 3 -H), and 5.69 (1H, s, C=CH).

3 β -Acetoxy-24 -tert-butyldimethylsiloxy-5 α -cholestane-7-one
(10)

To a solution of compound (9) (13.0 g, 22.7 mmole) in dry ether (50 ml) was distilled in liquid ammonia (300 ml) at -78°C. Lithium (0.5 g, 72.1 mmole) was added in small portions until a blue coloration persisted for 10 minutes, after which the solution was quenched with solid NH_4Cl (50 g). Ammonia was evaporated, and the resulting residue was partitioned between water (500 ml) and ether (300 ml). The aqueous solution was extracted further with ether (3 x 200 ml). The combined ether extracts were washed with brine (1 x 100 ml), dried (MgSO_4), filtered and concentrated in vacuo to get the crude product. Flash chromatography on silica gel of the crude product gave pure compound (10) (10.6 g, 81%). IR (neat): 1711 (CO), 1736 (OCOCH_3) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 0.01 (6H, s, $\text{Si}(\text{CH}_3)_3$), 0.61 (3H, s, 18- CH_3), 0.82 (3H, merged s, 19- CH_3), 0.89 (9H, merged s, $\text{SiC}(\text{CH}_3)_3$), 2.00 (3H, s, OCOCH_3), 3.35 (1H, m, 24 β -H), and 4.66 (1H, m, 3 α -H).

3 β -Acetoxy-7 α -hydroxy-24 β -tert-butyldimethylsiloxy-5 α -cholestan-3-ol (11)

To a stirred solution of compound (10) (10.6 g, 18.4 mmole) in anhydrous tetrahydrofuran (300 ml) was added dropwise a solution of K-selectride (1.0 M in THF, 75.6 ml, 75.6 mmole) (Aldrich Chemical Co., Milwaukee, WI) at -50°C. The reaction mixture was stirred at that temperature for 5 hours, and then quenched with 30% hydrogen peroxide solution (20 ml) and saturated NH₄Cl. The aqueous solution was extracted with ether (3 x 100 ml). The combined ether extracts were washed with saturated NaHCO₃ (2 x 70 ml), water (2 x 100 ml), brine (1 x 70 ml), dried (MgSO₄), filtered and concentrated in vacuo to get the crude product. The product was purified by flash chromatography on silica gel to give pure compound (11) (8.5 g, 80%). IR (neat): 1732 (OCOCH₃), 3456 (OH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 0.01, 0.02 (6H, two s, 2 x SiCH₃), 0.63 (3H, s, 18-CH₃), 0.80 (3H, s, 19-CH₃), 0.88 (9H, s, SiC(CH₃)₃), 2.01 (3H, s, COCH₃), 3.36 (1H, m, 24 β -H), 3.82 (1H, m, 7 β -H) and 4.70 (1H, m, 3 α -H).

3 β ,7 α -Dihydroxy-24^f-tert-butyldimethylsiloxy-5 α -cholestane
(12)

A solution of compound (11) (2.20 g, 3.81 mmole) and sodium cyanide (0.20 g, 4.08 mmole) in methanol (70 ml) was refluxed for 8 hours (monitored by tlc). After completion of reaction, methanol was evaporated in vacuo, and the residue was extracted with CH₂Cl₂ (3 x 30 ml). The combined CH₂Cl₂ extracts were concentrated in vacuo to get a pure white solid compound (12) (1.85 g, 88%). ¹H NMR (400 MHz, CDCl₃) δ : 0.00, 0.01 (2 x 3H, two s, Si(CH₃)₃), 0.66 (3H, s, 18-CH₃), 0.80 (3H, s, 19-CH₃), 0.85 (1H, d, 21-CH₃), 0.91 (9H, merged s, SiC(CH₃)₃), 3.36 (1H, m, 24^f-H), 3.64 (1H, m, 3 α -H), and 3.84 (1H, m, 7 β -H). CIMS (m/e): 534 [(M⁺+1)-1, 3.7%], 533 (8.6%), 499 (16.5%), 473 (20%), 385 (97%), 367 (100%), 301 (17%), and 273 (16%).

3-Oxo-7 α -hydroxy-24 β -tert-butyldimethylsiloxy-5 α -cholestane (13)

A solution of compound (12) (1.27 g, 2.37 mmole), aluminum tri-tert-butoxide (4.75 g, 19.28 mmole) and cyclohexanone (30 ml) in anhydrous toluene (90 ml) was stirred and heated to 120°C (oil bath) for 72 hours. The reaction mixture was then cooled to room temperature, diluted with benzene (100 ml) and acidified with 2N HCl (50 ml). The organic layer was then separated from the aqueous layer. The aqueous layer was extracted with benzene (3 x 50 ml). The combined organic extracts were washed with water (1 x 50 ml), saturated NaHCO₃ (2 x 50 ml), water (1 x 50 ml), brine (1 x 50 ml), dried (MgSO₄), filtered and evaporated in vacuo to get the crude product along with cyclohexanone. Flash chromatography of the crude product using benzene followed by a gradient of EtOAc/hexane (5, 10, 20 and 40%) solvent system gave a pure white solid compound (13) (0.743 g, 59%). ¹H NMR (400 MHz, CDCl₃) δ : 0.00, 0.01 (2 x 3H, two s, Si(CH₃)₂), 0.66 (3H, s, 18-CH₃), 0.82 (3H, merged d, 21CH₃), 0.89 (9H, merged s, SiC(CH₃)₃), 0.99 (3H, s, 19-CH₃), 2.31 (4H, m, CH₂COCH₂), 3.36 (1H, m, 24 β -H), and 3.85 (1H, m, 7 β -H). CIMS (m/e): 533 (M⁺+I, 61%), 401 (34%), 383 (100%), 327 (3%), 299 (11%).

3-Benzoyloxyimino-7 α -hydroxy-24 β -tert-butyldimethylsiloxy-5 α -cholestane (14)

A solution of compound (13) (0.74 g, 1.39 mmole), benzyloxyamine hydrochloride (0.49 g, 3.07 mmole) and anhydrous pyridine (20 ml) in anhydrous ethanol (40 ml) was gently refluxed for 16 hours. Excess pyridine and ethanol was removed in vacuo, and the residue was dissolved in EtOAc (100 ml) and water (100 ml). The aqueous layer was

separated, and the EtOAc layer was washed with 2N HCl (3 x 20 ml), water (2 x 20 ml), brine (1 x 20 ml), dried (MgSO₄), filtered and evaporated in vacuo to get a white solid compound (14) (0.86g, 97%). ¹H NMR (400 MHz, CDCl₃) δ: 0.00, 0.01 (2 x 3H, two s, Si(CH₃)₂), 0.69 (3H, s, 18-CH₃), 0.89 (9H, merged s, SiC(CH₃)₃), 2.98, 3.27 (m, due to syn and anti oxime next to α-CH₂ Of A-ring), 3.37 (1H, m, 24 -H), 3.86 (1H, m, 7β-H), 5.06 (2H, br s, OCH₂C₆H₅), and 7.26-7.42 (5H, m, C₆H₅): CIMS (m/e): 638 (M⁺+I, 100%), 512 (3.4%), 488 (13%), 380 (21%).

3 β -Amino-7 α -hydroxy-24 β -tert-butylidimethylsiloxy-5 α -chole-
stane (15)

To a well stirred suspension of lithium aluminum hydride (LAH) (0.252 g, 6.66 mmole) in dry ether (100 ml) was added dropwise a solution of compound (14) (0.85 g, 1.33 mmole) in dry ether (50 ml) at room temperature. After complete addition, the reaction mixture was refluxed gently for 16 hours. The reaction mixture was cooled to 0°C (ice bath), then a solution of 2N NaOH was added dropwise until white solid granulates were formed. Ether was decanted and the residue further extracted with benzene (3 x 50 ml), and the combined organic extracts were dried (K₂CO₃), filtered and evaporated in vacuo to give compound (15) along with traces of benzyl alcohol as by-product (0.89 g total, quantitative yield). The crude product was used as such in the next step. ¹H NMR (400 MHz, CDCl₃) δ : 0.01, 0.02 (2 x 3H, two s, Si(CH₃)₂), 0.66 (3H, s, 18-CH₃), 0.79 (3H, s, 19-CH₃), 0.91 (9H, merged s, SiC(CH₃)₃), 2.69 (1H, m, 3 α -H), 3.39 (1H, m, 24 β -H), and 3.84 (1H, m, 7 β -H). CIMS (m/e): 534 (M⁺+1, 60%), 518 (14%), 476 (7%), 384 (100%), 367 (6.3%), 300 (5%).

N-(3-Cyanopropyl)-N-(3-iodopropyl)p-toluenesulfonylamine
(16)

To the stirred and refluxed mixture of 3-amino-1-propanol (3.0 g, 39.94 mmole), anhydrous potassium carbonate (6.10 g, 44.16 mmole) and anhydrous sodium iodide (0.60 g, 4.00 mmole) in anhydrous acetonitrile (150 ml) was added a solution of 3-cyanopropyl bromide (4-bromobutyronitrile) (5.91 g, 39.67 mmole) in anhydrous acetonitrile (50 ml) dropwise over a period of 6 hours. After complete addition, reflux of the reaction mixture was continued for 20 hours. The cold reaction mixture was concentrated in vacuo, then the residue was extracted with CHCl_3 (3 x 60 ml). Evaporation of CHCl_3 afforded almost pure colorless oil N-(3-cyanopropyl)-N-(3-hydroxypropyl)amine (5.65 g, 100%). ^1H NMR (400 MHz, CDCl_3) δ : 1.71 (2H, m, CH_2), 1.83 (2H, m, CH_2), 2.44 (2H, t, CH_2CN), 2.58 (1H, m, NH), 2.77 (2H, t, CH_2N), 2.89 (2H, t, CH_2N), 3.80 (2H, t, CH_2O). This compound (5.60 g, 39.38 mmole) was N,O-bis-tosylated by treating with p-toluenesulfonyl chloride (18.4 g, 96.51 mmole), anhydrous triethylamine (19.6 g, 193.71 mmole) and N,N-dimethylaminopyridine (DMAP) (2.35 g, 19.23 mmole) in dry CH_2Cl_2 at 0°C for 2 hours followed by 0°C to room temperature for 16 hours. After complete reaction, CH_2Cl_2 was removed in vacuo and then the residue was dissolved in EtOAc (150 ml), washed with water (2 x 50 ml), 2N HCl (3 x 50 ml), water (1 x 50 ml), saturated NaHCO_3 (2 x 50 ml), brine (1 x 50 ml), dried (MgSO_4), filtered and evaporated in vacuo to afford almost pure solid N-(3-cyanopropyl)-N-(3-toluenesulfonyl-oxypropyl)p-toluenesulfonylamine (14.55 g, 82%). ^1H NMR (400 MHz, CDCl_3) δ : 1.87-2.01 (4H, m, 2 x CH_2), 2.42 (2H, t, CH_2CN), 2.46 (3H, s, CH_3Ar), 2.48 (3H, s, CH_3Ar), 3.15 (4H, m, 2 x CH_2N), 4.08 (2H, t, CH_2O), 7.33, 7.38, 7.67, 7.79 (4 x 2H, four d, 2 x - C_6H_4 -). Without

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further purification, the crude N,O-bis-tosylated compound (14.50 g, 32.18 mmole) was treated with anhydrous sodium iodide (9.65 g, 64.38 mmole) in refluxing anhydrous acetone (250 ml) for 16 hours. Acetone was removed by evaporating in vacuo, and the residue was extracted with CHCl_3 (3 x 70 ml). The combined CHCl_3 extracts were evaporated in vacuo to afford a pale yellow solid compound (12.95 g, 99%). Flash chromatography on silica gel of the crude compound using EtOAc/hexane (4:6) as eluant gave pure N-(3-cyanopropyl)-N-(3-iodopropyl)p-toluenesulfonylamine (16) (11.62 g, 89%) as crystalline solid. IR (neat) 1338, 1454, (SO_2), 1597 (aromatic), 2245 (CN) cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ : 1.95 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.43 (3H, merged s, CH_3Ar), 2.44 (2H, merged t, CH_2CN), 3.13-3.20 (6H, m, 2 x CH_2N x CH_2I), 7.32, 7.68 (2 x 2H, two d, $-\text{C}_6\text{H}_4-$). ^{13}C NMR (100 MHz, CDCl_3) δ : 1.88, 14.52, 21.52, 25.11, 32.52, 47.96, 49.90, 118.82, 127.15, 129.87, 135.59, and 143.79. CIMS (m/e): 407 ($\text{M}^+ + \text{I}$, 100%), 315 (5.7%), 279 (7.6%), 251 (7.3%), 135 (11%).

3 β -N-1-[N(3-Cyanopropyl)-N(p-toluenesulfonyl)-1,3-diaminopropane]-7 α -hydroxy-24 S -tert-butyldimethylsiloxy-5 α -cholestane (17)

To a stirred and refluxed mixture of compound (15) (0.70 g, 1.31 mmole) and anhydrous potassium carbonate (0.20 g, 1.45 mmole) in anhydrous acetonitrile (200 ml) was added a solution of N-(3-cyanopropyl)-N-(3-iodopropyl)p-toluenesulfonylamine (16) (0.54 g, 1.33 mmole) in anhydrous acetonitrile (50 ml) dropwise over a period of 6 hours. After complete addition, the reaction mixture was continued to reflux for 16 hours. The cold reaction mixture was concentrated in vacuo, and the residue was extracted with CHCl_3 (3 x 60 ml). The combined CHCl_3 extracts were evaporated in vacuo to get almost pure compound (17) (1.06

g, 100%) as viscous oil. ^1H NMR (400 MHz, CDCl_3) δ : 0.02, 0.03 (2 x 3H, two s, $\text{Si}(\text{CH}_3)_2$), 0.66 (3H, s, 18- CH_3), 0.78 (3H, s, 19- CH_3), 0.90 (9H, merged s, $\text{SiC}(\text{CH}_3)_3$), 1.96 (4H, merged m, 2 x CH_2), 2.42 (4H, merged m, CH_2N x CH_2CN), 2.43 (3H, merged s, CH_3Ph), 2.62 (1H, m, 3 α -H), 3.18 (4H, m, 2 x CH_2N), 3.38 (1H, m, 24 -H), 3.84 (1H, m, 7 β -H), 7.32, 7.19 (2 x 2H, two d, ArH). CIMS (m/e): 813 ($\text{M}^+\text{+I}$, 44%), 754 (32%), 656 (52%), 575 (48%), 547 (54%), 418 (43%), 384 (26%), 334 (35%), 296 (55%), 251 (60%), 239 (87%), 155 (100%).

3 β -N-1-[N(3-Cyanopropyl)-1,3-diaminopropane]-7 α -hydroxy-24 β -tertbutyldimethylsiloxy-5 α -cholestane (18)

To a solution of compound (17) (1.0 g, 1.23 mmole) in dry tetrahydrofuran (THF) (20 ml) was distilled liquid ammonia (80 ml) at -78°C . Sodium (1.5g, 65.25 mmole) was added in small portions until a blue coloration persisted for 1 hour, and the reaction was allowed to warm to room temperature overnight. After 16 hours, the reaction mixture was quenched with ethanol. THF and ethanol from the reaction mixture were removed by evaporation in vacuo, and the residue was extracted with benzene (3 x 50 ml), dried (K_2CO_3), filtered and evaporated in vacuo to get compound (18) (0.74 g, 91%) as viscous oil. ^1H NMR (400 MHz, CDCl_3) δ : 0.01, 0.02 (2 x 3H, two s, $\text{Si}(\text{CH}_3)_2$), 0.66 (3H, s, 18- CH_3), 0.79 (3H, s, 19- CH_3), 0.91 (9H, merged s, $\text{SiC}(\text{CH}_3)_3$), 2.41-2.74 (9H, m, CH_2CN , 3 x CH_2N & 3 α H), 3.37 (1H, m, 24 β -H) and 3.83 (1H, m, 7 β -H). This crude product was used in the next step without further purification.

3 β -N-1-{N[3-(4-Aminobutyl)]-1,3-diaminopropane}-
7 α -hydroxy-24^P-tertbutyldimethylsiloxy-5 α -cholestane (19)

To a stirred suspension of lithium aluminium hydride (1.0 g, 26.35 mmole) in anhydrous ether (150 ml) was added a solution of compound (18) (0.72 g, 1.09 mmole) in anhydrous ether (50 ml) dropwise at room temperature over a period of 1 hour. After completion of the addition, the reaction mixture was refluxed for 5 hours. Then at 0°C (ice-bath) the reaction mixture was treated with a solution of 2N NaOH until white solid granulates were formed. Ether was decanted and the residue further extracted with benzene (3 x 60 ml), and the combined organic extracts were dried (KOH and K₂CO₃), filtered and evaporated in vacuo to get compound (19) (0.67 g, 93%) as colorless viscous oil. ¹H NMR (400 MHz, CDCl₃) δ : 0.00, 0.01 (2 x 3H, two s, Si(CH₃)₃), 0.66 (3H, s, 18-CH₃), 0.81 (3H, merged s, 19-CH₃), 0.91 (9H, merged s, SiC(CH₃)₃), 2.38-2.78 (9H, m, 4 x CH₂N & 3 α -H), 3.38 (1H, m, 24 -H), and 3.84 (1H, m, 7 β -H). CIMS (m/e): 662 (M⁺+I, 31%), 604 (16%), 560 (13%), 548 (100%), 530 (31%), 432 (25%), 410 (28%), 384 (12%), 321 (8%), 188 (10%), 144 (29%). Compound (19) was used in the next step without further purification.

3 β -N-1-{N[3-(4-Benzoyloxycarbonyl)aminobutyl)]-1,3-
(dibenzoyloxycarbonyl)diaminopropane}
-7 α -hydroxy-24^P-tert-butyl dimethylsiloxy-5 α -cholestane (20)

To a cold (5°C) stirred solution of compound (19) (0.67 g, 1.01 mmole) in tetrahydrofuran (20 ml) was added separately but simultaneously a solution of benzyloxycarbonyl chloride (benzyl chloroformate) (CBZ Cl) (1.31 g, 7.70 mmole) in THF (30 ml) and 2N NaOH in 30 minutes. After complete addition, the reaction mixture was allowed to warm to room

temperature, and stirred overnight. After 16 hours, THF was removed in vacuo from the reaction mixture, and the aqueous layer was extracted with benzene (3 x 50 ml). The combined benzene extracts were dried (K_2CO_3), filtered and evaporated in vacuo to get compound (20) (1.06 g, 99%) as a colorless viscous oil. 1H NMR (400 MHz, $CDCl_3$) δ : 0.01 (6H, merged two s, $Si(CH_3)_3$), 0.68 (3H, s, 18- CH_3), 0.87 (3H, merged s, 19- CH_3), 0.91 (9H, merged s, $SiC(CH_3)_3$), 2.94-3.58 (9H, m, 4 x CH_2N & 3 α -H), 3.48 (1H, merged m, 24 β -H), 3.81 (1H, m, 7 β -H), 5.10 (6H, br s, 3 x OCH_2Ph), and 7.20-7.60 (15H, m, 3 x C_6H_5). CIMS (m/e): 1064 ($M^+ + I$, 0.1%), 1017 (0.3%), 926 (2%), 518 (10%), 367 (13%), 225 (11%), 181 (23%), 163 (100%). This compound (20) was used in next step without further purification.

3 β -N-{N-[3-(4-Benzoyloxycarbonyl)aminobutyl]-1,3-(dibenzoyloxycarbonyl)diaminopropane}-7 α -acetoxy-24 β -tert-butyl-5 α -cholestane (21)

To a stirred solution of compound (20) (1.0 g, 0.94 mmole) and 4-dimethylaminopyridine (DMAP) (1.20 g, 9.82 mmole) in anhydrous CH₂Cl₂ (10 ml) was added acetic anhydride (Ac₂O) (0.78 g, 7.61 mmole) at room temperature. The reaction mixture was monitored by tlc. After 4 hours, methanol was added to the reaction mixture, then the organic solvents were evaporated in vacuo to get oily residue. The residue was dissolved in EtOAc (100 ml), washed with 2NHCl (3 x 25 ml), water (1 x 50 ml), saturated NaHCO₃ (3 x 25 ml), brine (1 x 25 ml), dried (MgSO₄), filtered and evaporated in vacuo to give compound (21) (0.89 g, 86%) as a viscous oil. ¹H NMR (400 MHz, CDCl₃) δ : 0.02, 0.03 (2 x 3H, two s, Si(CH₃)₂), 0.66 (3H, s, 18-CH₃), 0.87 (3H, merged s, 19-CH₃), 0.90 (9H, merged s, SiC(CH₃)₃), 2.11 (3H, merged s, OCOCH₃), 2.92-3.58 (9H, m, 4 x CH₂N & 3 α -H), 3.38 (1H, merged m, 24 -H), 4.88 (1H, m, 7 β -H), 5.11 (6H, br s, 3 x CH₂Ph), 7.21-7.44 (15H, m, 3 x C₆H₅). CIMS (m/e): 1107 (M⁺+1, 0.2%), 1059 (0.2%), 984 (0.5%), 968 (2%), 650 (5%), 519 (7%), 410 (8%), 223 (44%), 163 (100%).

3 β -N-1-{N-[3-(4-Aminobutyl)]-1,3-diaminopropane-7 α -acetoxy-24 β -hydroxy-5 α -cholestane trihydrochloride (22)

A mixture of compound (21) (0.46 g, 0.42 mmole), 10% palladium-charcoal (0.2g) and anhydrous ethanol saturated with HCl gas (3.0 ml) in anhydrous ethanol (30 ml) was stirred in hydrogen atmosphere (filled in a balloon) at room temperature for 20 hours. The reaction mixture was filtered through celite using a sintered glass funnel and the residue was washed with ethanol (3 x 40 ml). Combined filtrates were evaporated in vacuo to get a hygroscopic solid compound (22) (0.26 g, 90%). ^1H NMR (400 MHz, MeOH- d_4) δ : 0 (3H, s, 18-CH $_3$), 1.96 (3H, merged s, COCH $_3$), 2.95-3.85 (m, spermidine chain), 3.31 (1H, merged s, 24 β -H), and 4.90 (1H, m, 7 β -H). This compound was used in the next step without further purification.

3 β -N-1-{N[3-(4-Aminobutyl)]-1,3-diaminopropane}-7 α -acetoxy-24 β -hydroxy-5 α -cholestane 24-pyridium sulfate trihydrochloride (23)

A solution of compound (22) (0.137 g, 0.196 mmole) and sulfur trioxide pyridine complex (0.187 g, 1.175 mmole) in anhydrous pyridine (2 ml) was heated at 60-80°C (oil bath temperature) for 2 hours. Then pyridine was removed in vacuo to get a crude 24-sulfated compound (23) along with an excess of sulfur trioxide pyridine complex. The crude product was used directly without isolation in the next step.

3 β -N-1-{N[3-(4-Aminobutyl)]-1,3-diaminopropane}-7 α , 24 β -di-hydroxy-5 α -cholestane 24-sodium sulfate (24).

The crude compound (23) from the previous reaction was dissolved in methanol (15 ml) and to that solution was added 2N NaOH solution (10 ml). Then the reaction mixture was heated at 80°C (oil bath temperature) for 20 hours. The cold reaction mixture which contained compound (24) was taken in the next step without isolation.

3 β -N-1-{N[3-(4-Aminobutyl)]-1,3-diaminopropane}-7 α , 24 β -dihydroxy-5 α -cholestane 24 β -sulfate trihydrochloride (Squalamine) (25).

The above reaction mixture containing compound (24) was acidified to pH 1 with cold 2NHCl (20 ml) and then water removed in vacuo. The light grey solid compound obtained was dried in a vacuum desiccator at room temperature to provide crude squalamine hydrochloride (25) which was purified by reversed phase liquid chromatography. ^1H NMR (400 MHz, MeOH- d_4) δ : 0.60 (3H, s, 18-CH₃), 0.80 (3H, merged s, 19-CH₃), 2.88-3.57 (m, spermidine chain), 3.72 (1H, m, 7 β -H), 4.08 (1H, m, 24 -H). ^1H NMR (400 MHz, DMSO- d_6) δ : 0.51 (3H, s, 18-CH₃), 0.68 (3H, merged s, 19-CH₃), 2.61-3.25 (m, spermidine chain), 3.55 (1H, m, 7 β -H) and 3.80 (1H, m, 24 β -H). This material was identical with an authentic sample of squalamine.

Considerable flexibility appears to exist in the selection of the starting steroidal compound onto which the sulfated side chain and the spermidine moiety must be condensed. The critical intermediate in the pathway, Compound 13, characterized by the 3-keto group, trans AB ring structure, and 7 α -OH group, can be reached by several

routes. To accommodate the different starting steroids, additional variations in the choice of the chemical blocking groups required along the route are also introduced.

Example 2Synthesis of Compound 13 from Chenodeoxycholic Acid

The following description is illustrated by Figure 2. In this synthesis chenodeoxycholic acid (26) is diacetylated at the two secondary hydroxyl groups to yield diacetate (27) which is selectively hydrolyzed to (28) which in turn is oxidized to dienone (29) following the procedure of K. Ochi, I. Matsunga, M. Shindo, and C. Kaneko, J.Chem.Soc., Perkin Trans., 1979, 161. Lithium in ammonia reduction of (29) according to the procedure of A. Kallner, Acta Chem.Scand., 1967, 21:322 yields (30) which is protected at the C₇-hydroxyl group using methoxymethyl chloride to yield (31). Further reactions are formation of the C₃ ketal (32), elongation of the side chain (COOH -CH(OH)-C(CH₃)₂) as was done in Example 1 and finally deprotection at C₇ and protection at C₂₄ to yield (13).

Example 3
Alternate Synthesis of Compound 13
from Chenodeoxycholic Acid

Compound (33) formed by Oppenauer oxidation of chenodeoxycholic acid (26) is oxidized using selenium dioxide to the conjugate ketone (34) which is reduced using lithium in liquid ammonia to the saturated ketone (35). Selective oxidation of (35) using isopropyl alcohol, $(\text{CH}_3)_3\text{PO}$ and Ir(IV)Cl_4 yields (36) following the procedure of A. Kallner, Acta Chem.Scand, 1967, 21:322. The compound referenced as (35) with respect to this example is the same molecule as (30) in Example 2. It is converted to (13) using the same steps as those of Example 2.

Example 4Synthesis of Compound 13 from Fucosterol Acetate
and the Conversion of Fucosterol Acetate to Squalmine

In Example 4 the naturally occurring marine sterol fucosterol is acetylated at C₃ to yield (37) which is converted to keto compound (38) by ozonolysis following the procedure of Takeshita et al., U.S. Patent 4,022,891. Protection of the C₂₄ keto group by ketalization yields (39) which is allylically oxidized as done in Example 1 to yield the conjugated ketone (40). Lithium in liquid ammonia reduction yields (41) which is stereoselectively reduced using K-selectride as done in Example 1 to yield the C₇ alcohol (42) which is converted to (13) by deprotection at C₂₄ using acid-catalyzed hydrolysis, followed by calcium borohydride reduction of the C₂₄ keto group to the alcohol and protection with TBDMS-Cl. Cleavage of the C₃ acetate and Collin's reagent oxidation yields (13).

Compound (42) is converted to squalamine by cleavage of the acetate and Collin's reagent oxidation to yield (43) followed by benzylation of the C₇ alcohol using benzylchloride and sodium iodide in dimethylformamide to yield (44). The spermidine chain is introduced using a diprotected precursor, namely NH₂(CH₂)₃N(BOC)(CH₂)₄NHBOC. The C₇ O-benzyl derivative of the (43), namely (44), is condensed with the diprotected precursor of the spermidine side-chain followed by reduction with sodium cyanoborohydride to yield (45). Deprotection of the C₂₄ ketal yields the C₂₄ ketone (46) which is reduced with sodium borohydride to the C₂₄ alcohol (47). Sulfation of (47) with Py/SO₃ yields (48). Catalytic reduction of (48) leads to deprotection of the two BOC groups on the nitrogen

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atoms (49) as well as the C₇-O-benzyl group (50) to yield squalamine (51).

Example 5Alternative Route to Compound 13 Starting
From Dehydroepiandrosterone to Yield 24-Oxo-
Cholesteryl Acetate

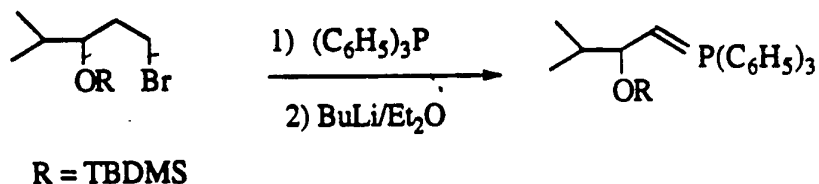
This route proceeds from dehydroepiandrosterone (52) which is converted to the C₁₇ ethylidene derivative using the Wittig reaction to yield (53) which in turn is treated with (CH₃)₂ AlCl and isopropyl vinyl ketone followed by catalytic hydrogenation to yield 24-oxocholesteryl acetate following the known procedure (B.S. Snider and E.A. Deutsch, J. Org.Chem., 1982, 47, 745). 24-Oxo cholesteryl acetate (38) can be used as in Example 4.

Example 6A Microbiological Route to Compound 13

Compound (39) is reduced via catalytic hydrogenation to (55). Chromic acid oxidation of alcohol (56) obtained by saponification of (55) is subjected to C₇ hydroxylation using *Neurospora* sp (M714), vegetable inoculum of *penicillium* sp ATCC 11598, *Phycomyces blakesleanus*, *Peziza* species, *Cephalosporium* species (Lederle Culture 2164), *Diplodia Anatalensis* ATCC 9055, or *Helminthosporium* culture to yield (43) of Example 4.

Example 7Alternate Route to Compound 13 Starting From
Pregnenolone Acetate

Starting with pregnenolone acetate (3 β -acetoxy-5-pregnen-20-one) (58), ketalization at C₂₀ yields (59) followed by allylic oxidation, lithium in liquid ammonia reduction, K-selectride reduction as done in Example 1, followed by formation of the methoxymethyl protected C₇-alcohol product (62) and selective deprotection of the C₂₀ ketal to yield (63). Wittig coupling of the protected side-chain yields (64) which upon catalytic reduction yields (65). Deprotection at C₇ yields (13). The side-chain is formed as follows:



What is Claimed is:

1. A method of preparing squalamine which comprises:
modifying the 3-position of a
3-oxo-7 α -hydroxy-24-protecting group ether-5 α -cholestane
with a spermidino moiety to form a
3 β -spermidino-7 α -hydroxy-24 ζ -ether protected group
ether-5 α -cholestane;
deprotecting the 24 ζ -position of the
3 β -spermidino-7 α -hydroxy-24 ζ -protecting group
ether-5 α -cholestane to the free hydroxyl; and
sulfating the 24-position of the 3 β -spermidin 7 α ,
24-dihydroxy-5 α -cholestane.
2. The method of claim 1 which further comprise:
protecting the 7 α -hydroxy group and the spermidino
amino groups during sulfating of the 24-position and
deprotecting such positions after sulfation.
3. The method of claim 1 wherein formation of the
3 β -spermidino-7 α -hydroxy-24 ζ -ether protected alcohol
-5 α -cholestane comprises the steps of
forming a 3 β -amino-7 α -hydroxy-24 ζ -ether protected
alcohol group -5 α -cholestane from the 3-oxo-7 α -hydroxy-24 ζ -
ether protected alcohol group-5 α -cholestane;
alkylating the 3 β -amino-7 α -hydroxy-24 ζ -ether
protected alcohol group-5 α -cholestane with a spermidino
precursor moiety at the 3-position; and
reducing the spermidino precursor moiety to form a
3 β -spermidino-7 α -hydroxy-24 ζ -ether protected alcohol group
-5 α -cholestane.
4. The method of claim 1 which further comprises:

forming the 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group -5 α -cholestane from 3 β -hydroxy 5-cholenic acid.

5. The method of claim 4 which comprises the steps of protecting the hydroxyl moieties of 3 β -hydroxy-5-cholenic acid to form a 3 β -protecting group ether-24 ζ -protecting group ester of -5-cholenic acid; homologating the 24 ζ -protected position to the 3 β , 24 ζ -diprotected cholesterol; allylically oxidizing the 7-position of the diprotected cholesterol and reducing the cholestene and C₃-carbonyl groups and deprotecting the 3-position to form 3 β , 7 α -24-trihydroxy cholestane 24 ζ -ether protected alcohol; oxidizing to the 3-keto form.

6. The method of claim 1 which further comprises: forming the 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane from chenodeoxycholic acid.

7. The method of claim 6 wherein forming the 3-oxo-7 α -hydroxy-24 ζ -ether protected alcohol group-5 α -cholestane comprises: oxidizing 3-acetoxychenodeoxycholic acid into the 1,4-dienone; reducing to 3-keto-7 α -hydroxy-5 α -cholanic acid; protecting the C₃ keto and 7-hydroxy functionalities; side-chain homologating and deprotecting yielding 3-keto-7 α ,24 ζ -dihydroxy-5 α -cholestane 24-ether protected alcohol.

8. The method of claim 6 wherein forming the 3-oxo-7 α -hydroxy-24 β -ether protected alcohol group-5 α -cholestane comprises:

- oxidizing chenodeoxycholic acid to the 3-one-4ene;
- reducing the double bond to 3-keto-7 α -hydroxy-5 α -cholanic acid; and
- converting to 3-keto-7 α ,24 β -dihydroxy-5 α -cholanic acid.

9. A method of preparing squalamine which comprises:

- ozonizing fucosterol to 24-oxocholesteryl acetate;
- protecting the 24-keto function;
- allylically oxidizing and reducing the thus formed eneone to 3 β ,7 α -dihydroxy-5 α -cholestane-24-one 24-protected ketone;

- protecting the C₃ alcohol and converting to 7 α ,24-dihydroxy-3-keto-5 α -cholestane-24-ether of the hydroxy group;

- benzylating the C₇-alcohol;
- oximating the C₃ keto group;
- reductively condensing a diprotected spermidine side-chain;

- deprotecting the C₂₄ ketal;
- reducing, sulfating and reductively deprotecting the diprotected spermidine and C₇ benzyl ether.

10. A method of preparing squalamine which comprises:

11. A method of claim 1 which further comprises forming the 3-oxo-7 α -hydroxy-24 β -ether protected alcohol group-5 α -cholestane from dehydroepiandrosterone.

12. The method of claim 13 which comprises the steps of:

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adding an ethylidene unit to
dehydroepioandrosterone acetate;
converting to 24-ketocholesteryl acetate by
treatment with isopropyl vinyl ketone and dimethyl aluminum
chloride; and
converting to 3-keto-7 α -24-dihydroxycholestane
24-ether protected alcohol.

13. The method of claim 1 which further comprises:
forming the 3-oxo-7 α -hydroxy-24 β -ether protected
group -5 α -cholestane by a microbial hydroxylation.

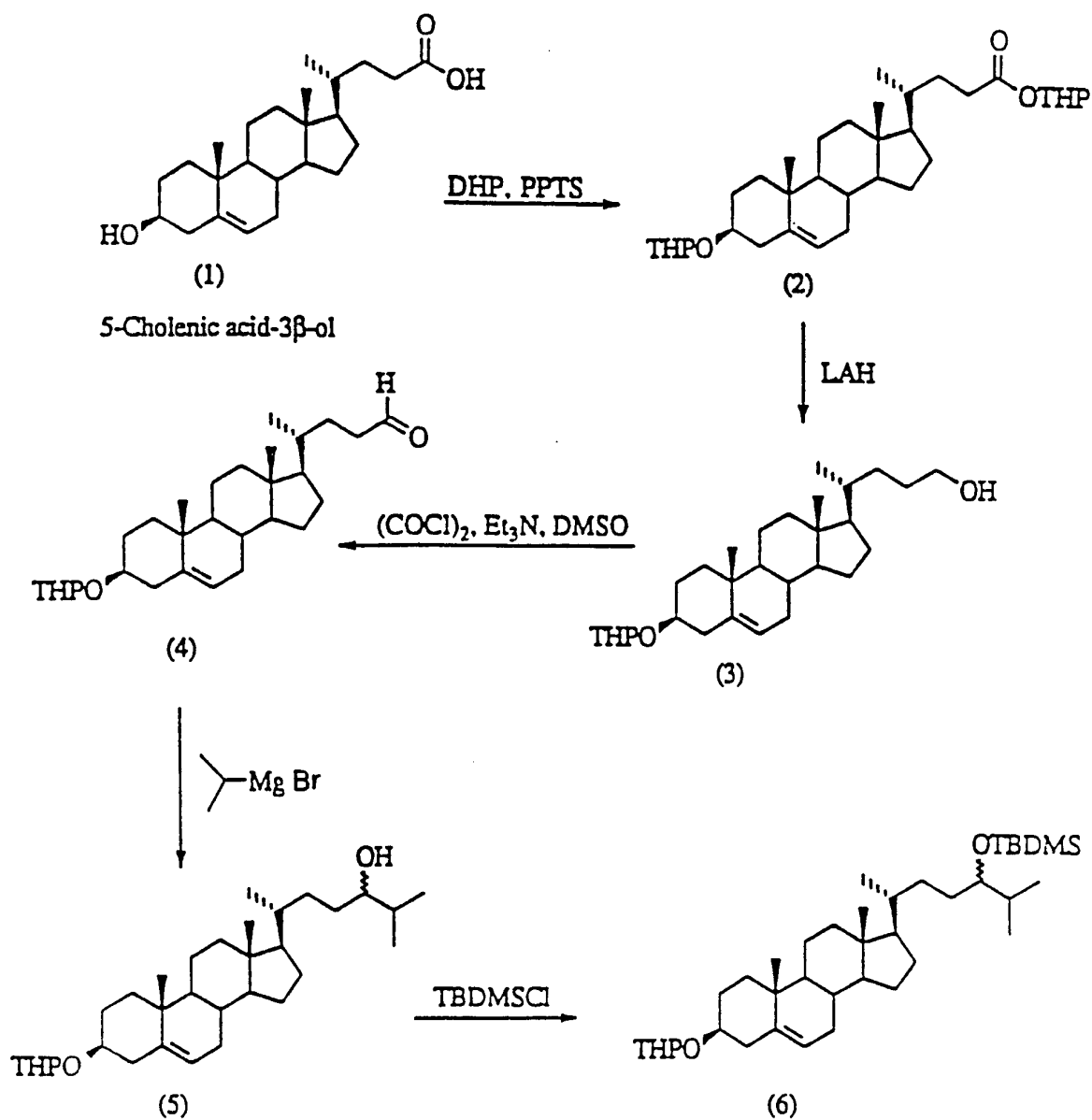
14. The method of claim 1 which further comprises
forming the 3-oxo-7 α -hydroxy-24 β -ether protected alcohol
group-5 α -cholestane from pregnenolone acetate by the
following steps:

protecting the C₂₀ keto group of pregnenolone
acetate;

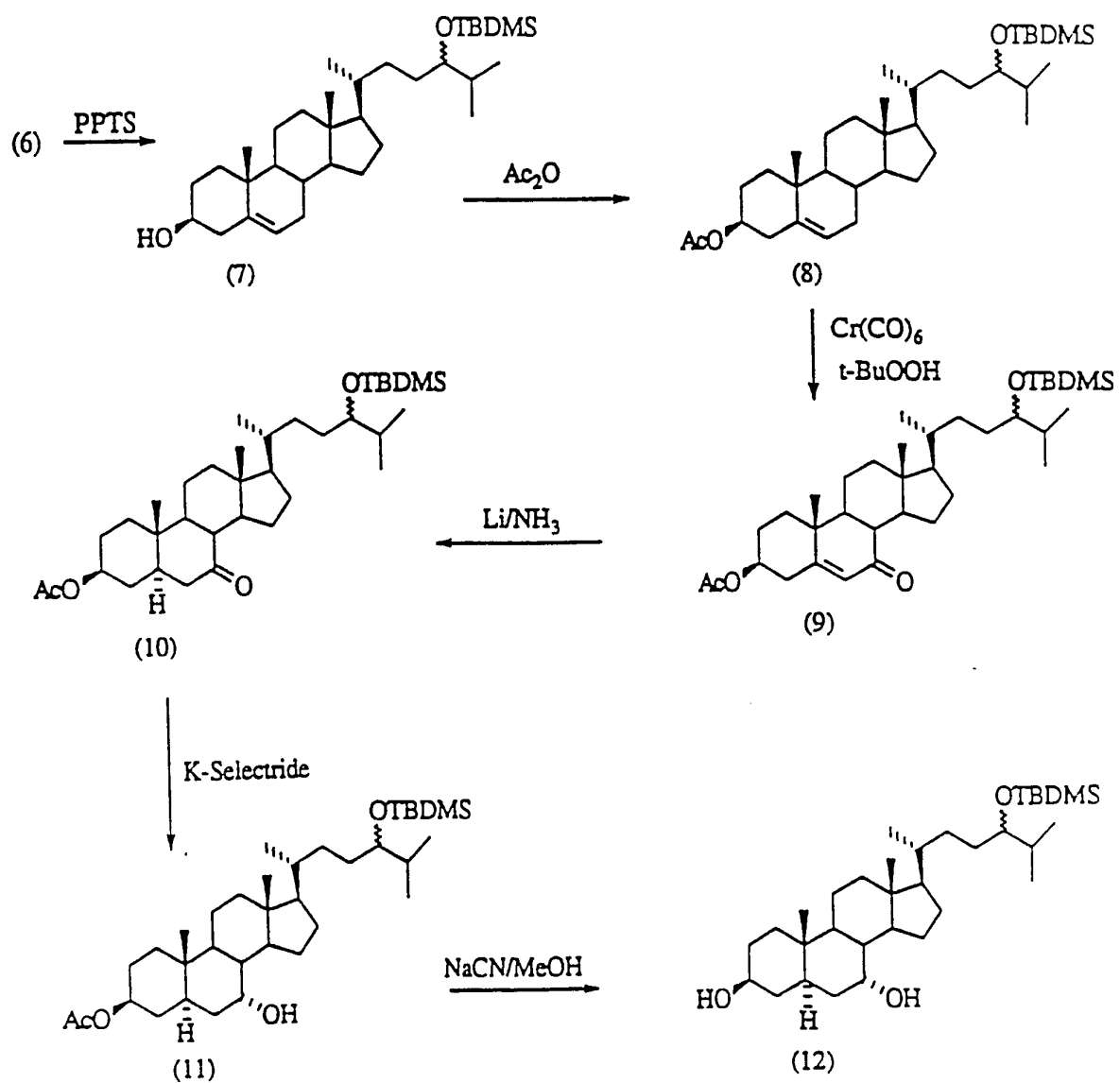
allylicallyoxidizing, reducing, and protecting the
C₇ alcohol; and

adding the side-chain comprising carbon atoms C₂₂,
C₂₃, C₂₄, C₂₅, C₂₆, C₂₇ with the C₂₄-protected hydroxyl
group.

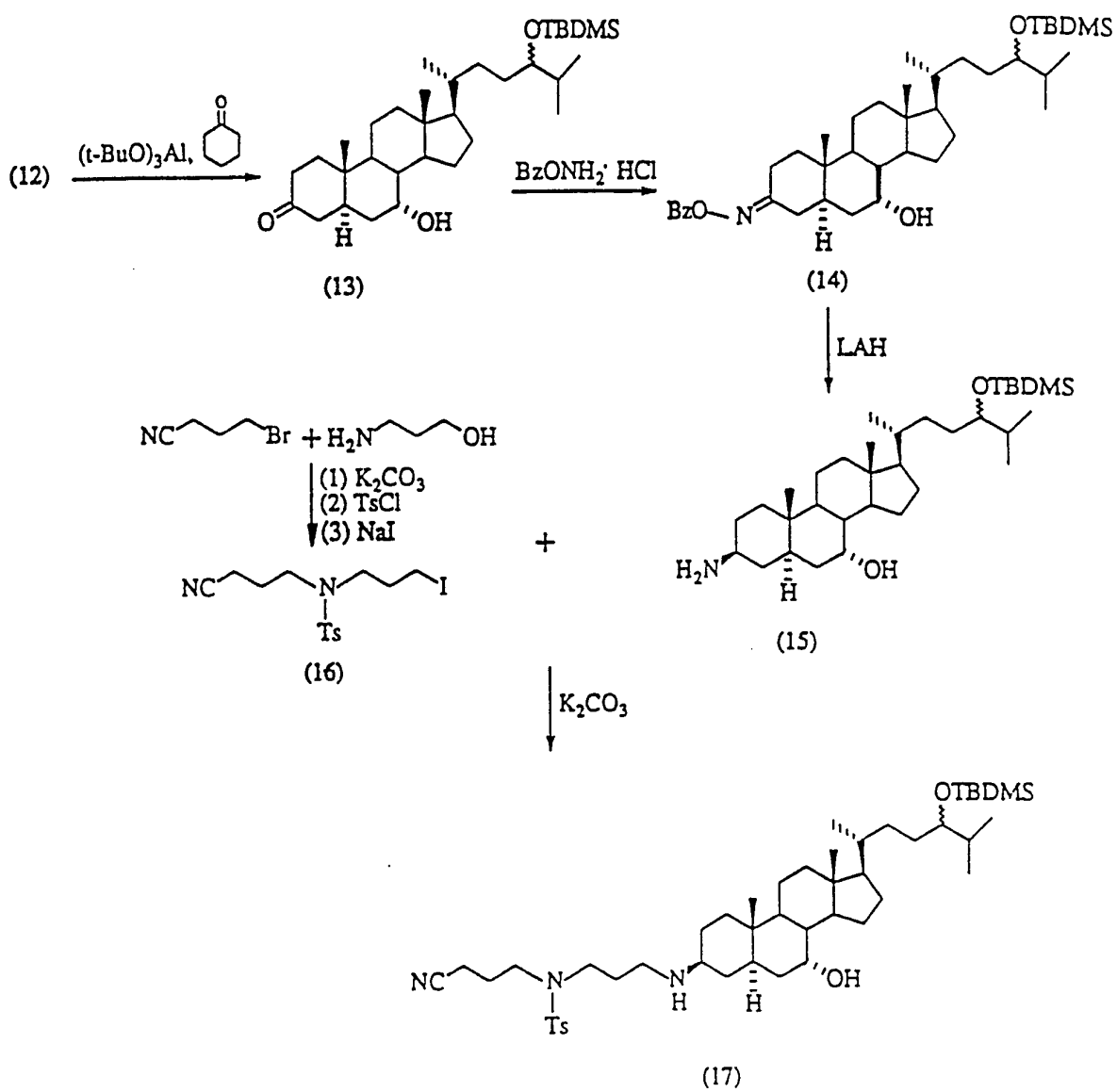
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FIG. 1a**SYNTHESIS OF SQUALAMINE**

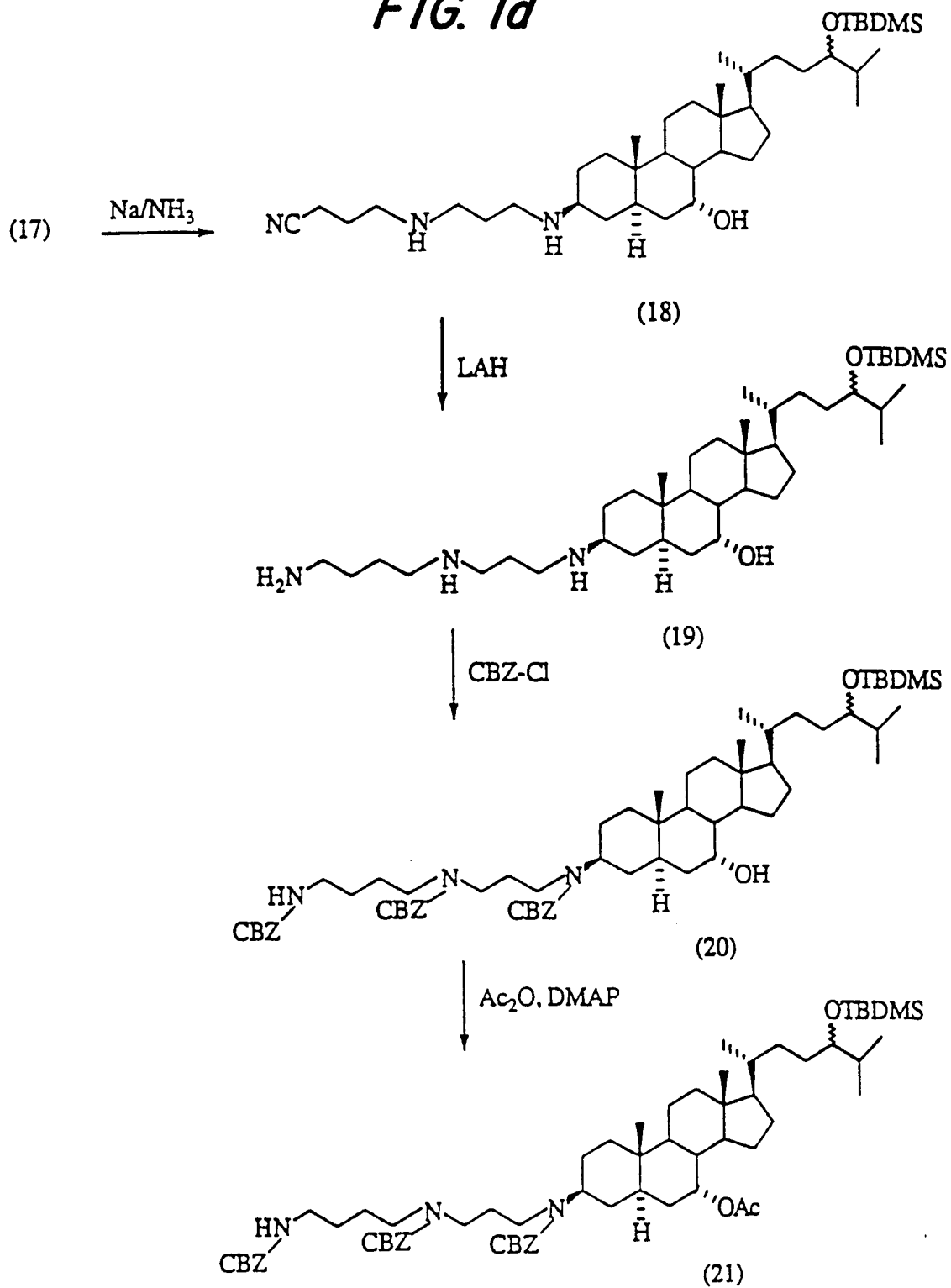
2/13

FIG. 1b

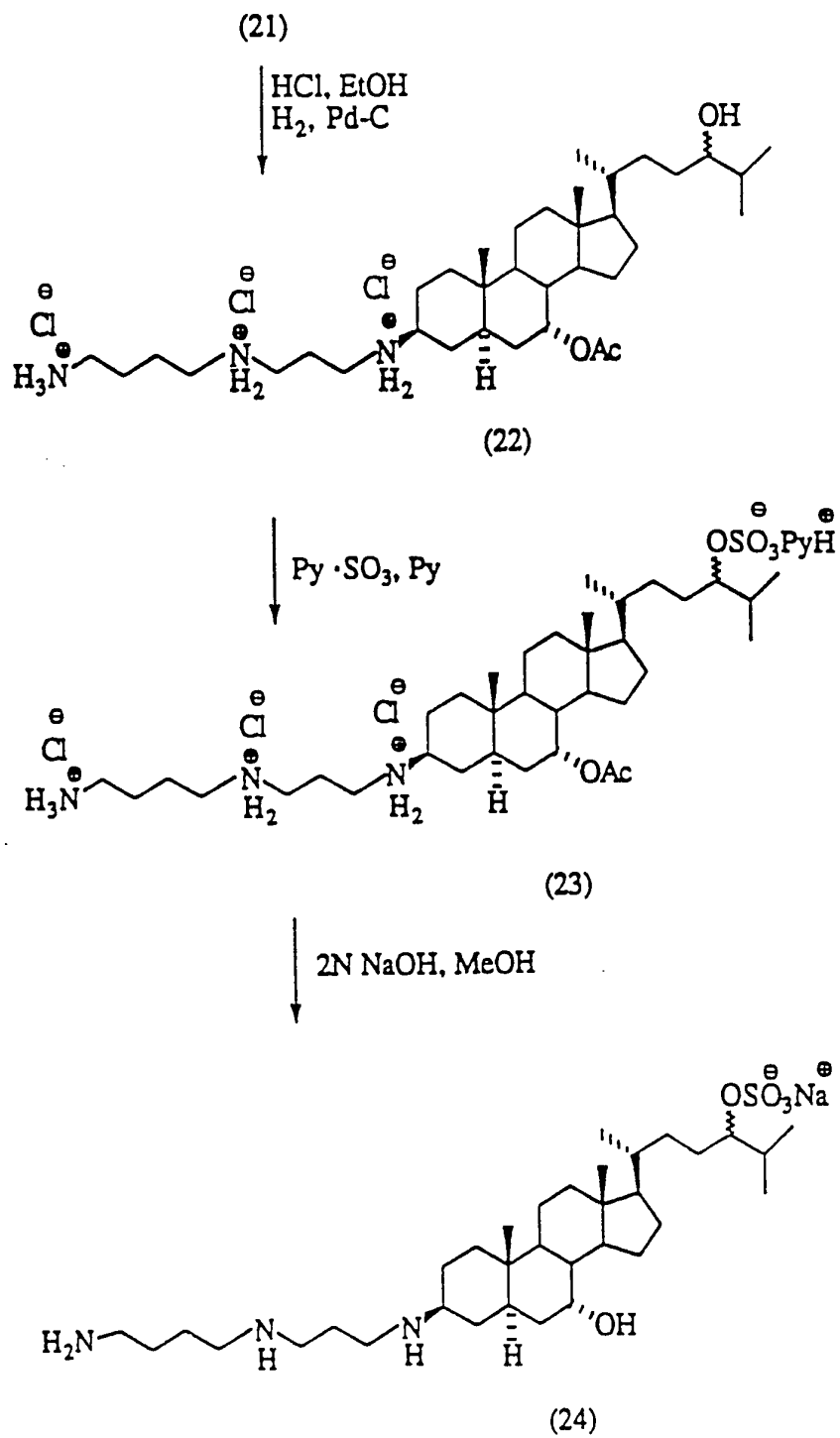
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FIG. 1c

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FIG. 1d

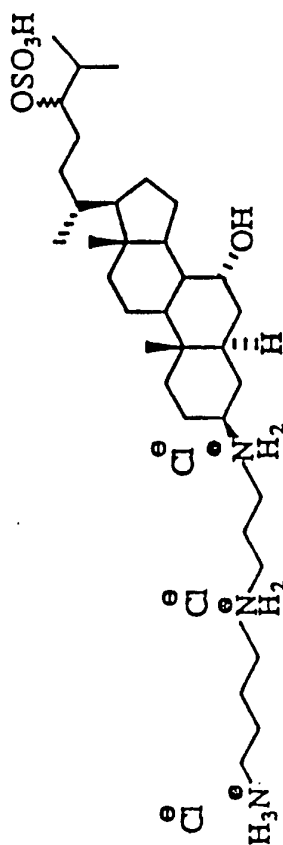
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FIG. 1e

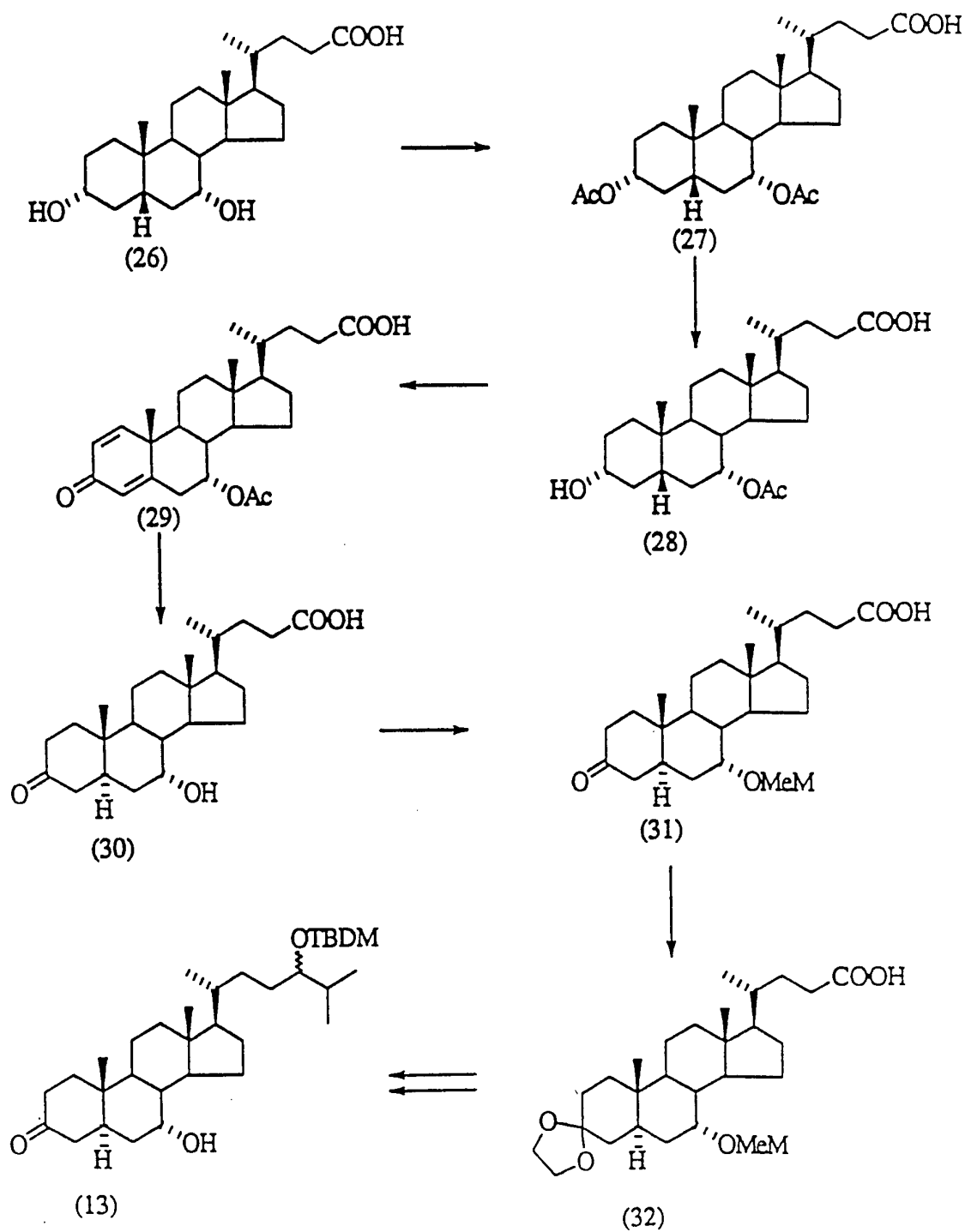
6/13

FIG. 1f

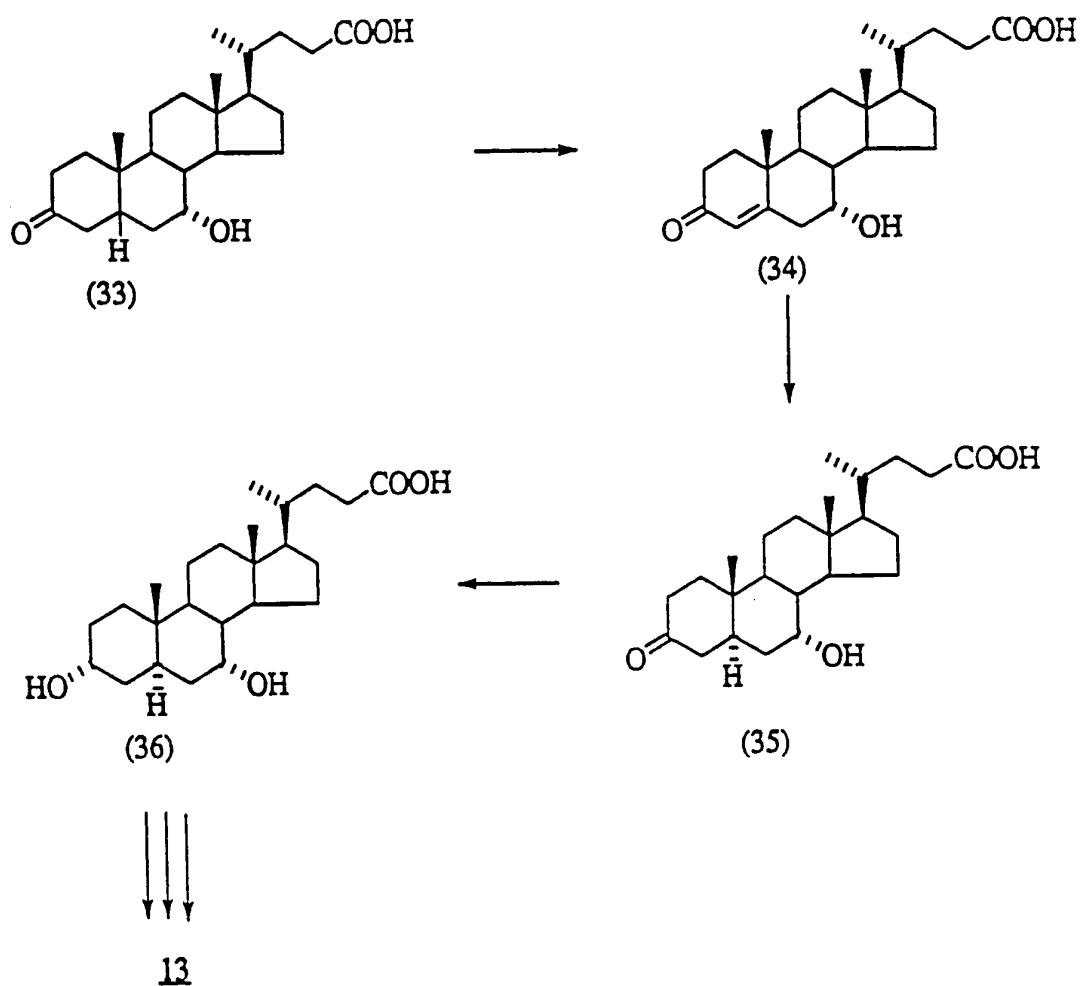
(24)

**SQUALAMINE**

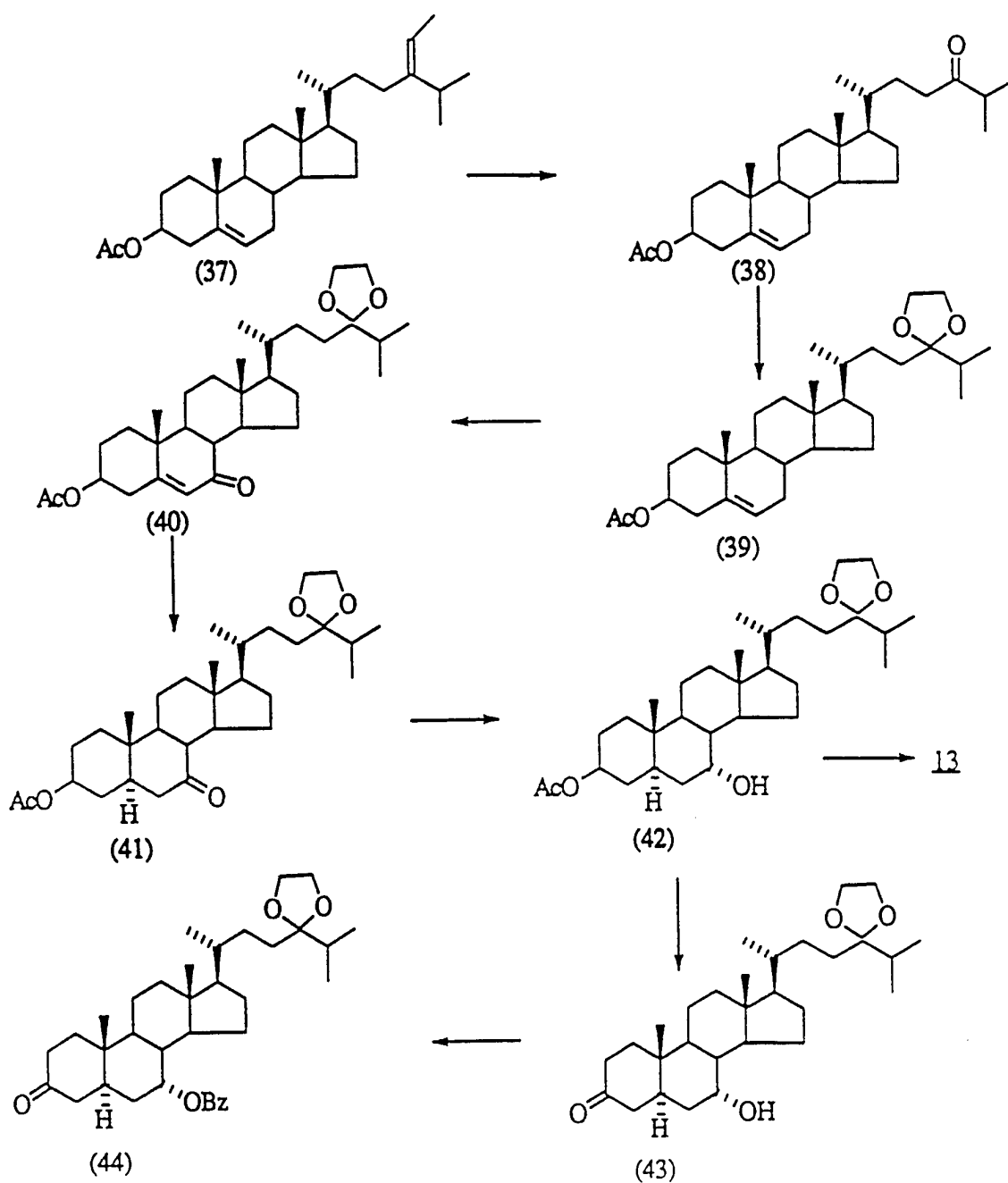
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FIG. 2

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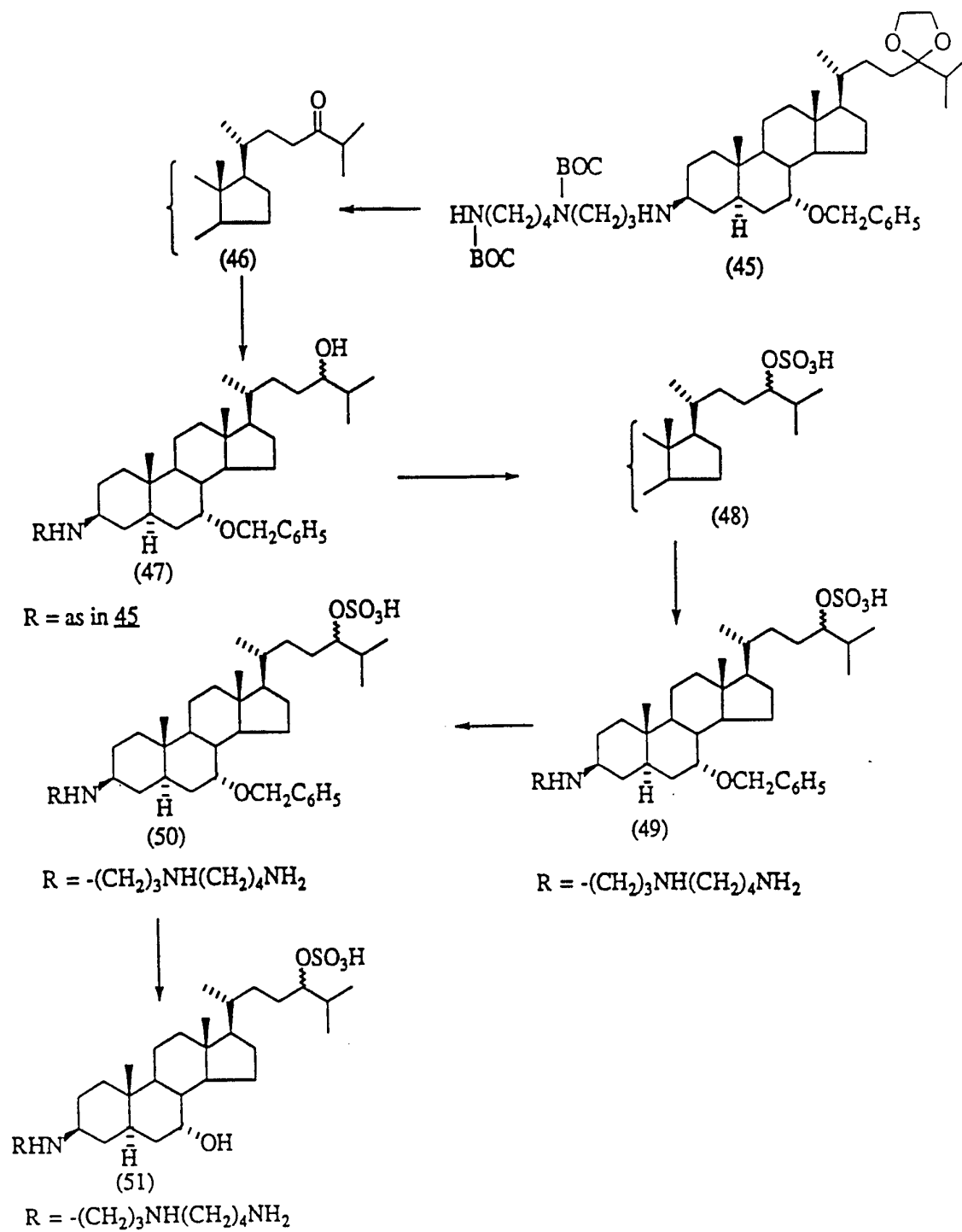
FIG. 3

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FIG. 4a

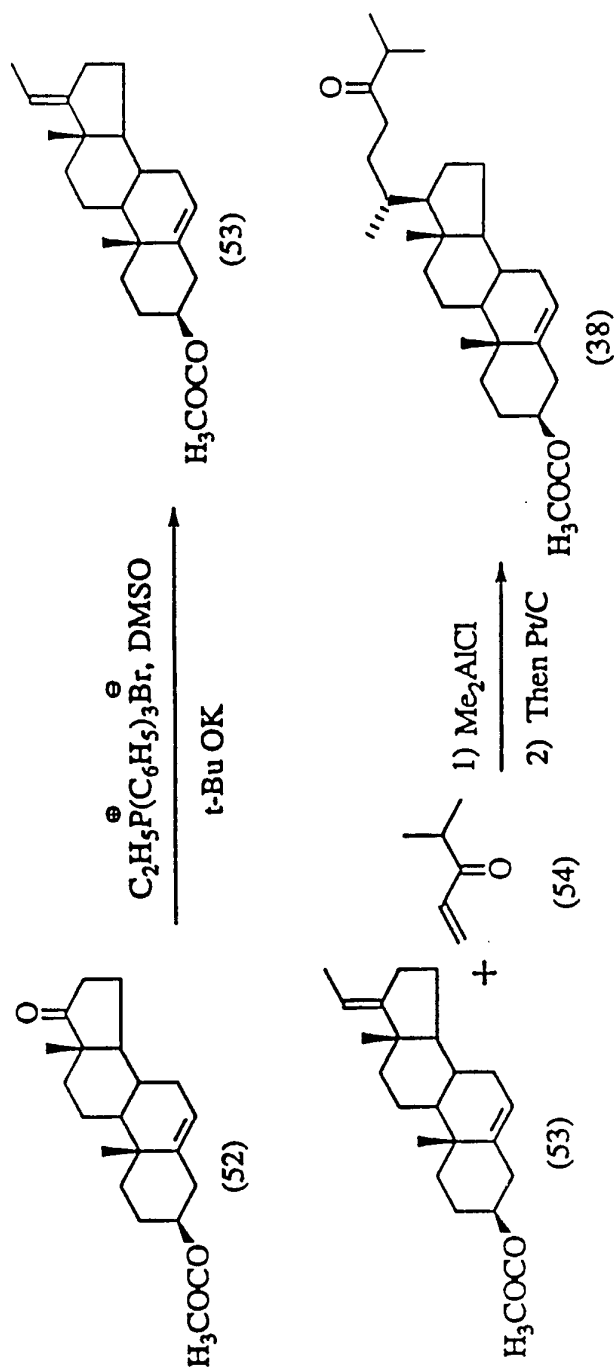
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FIG. 4b

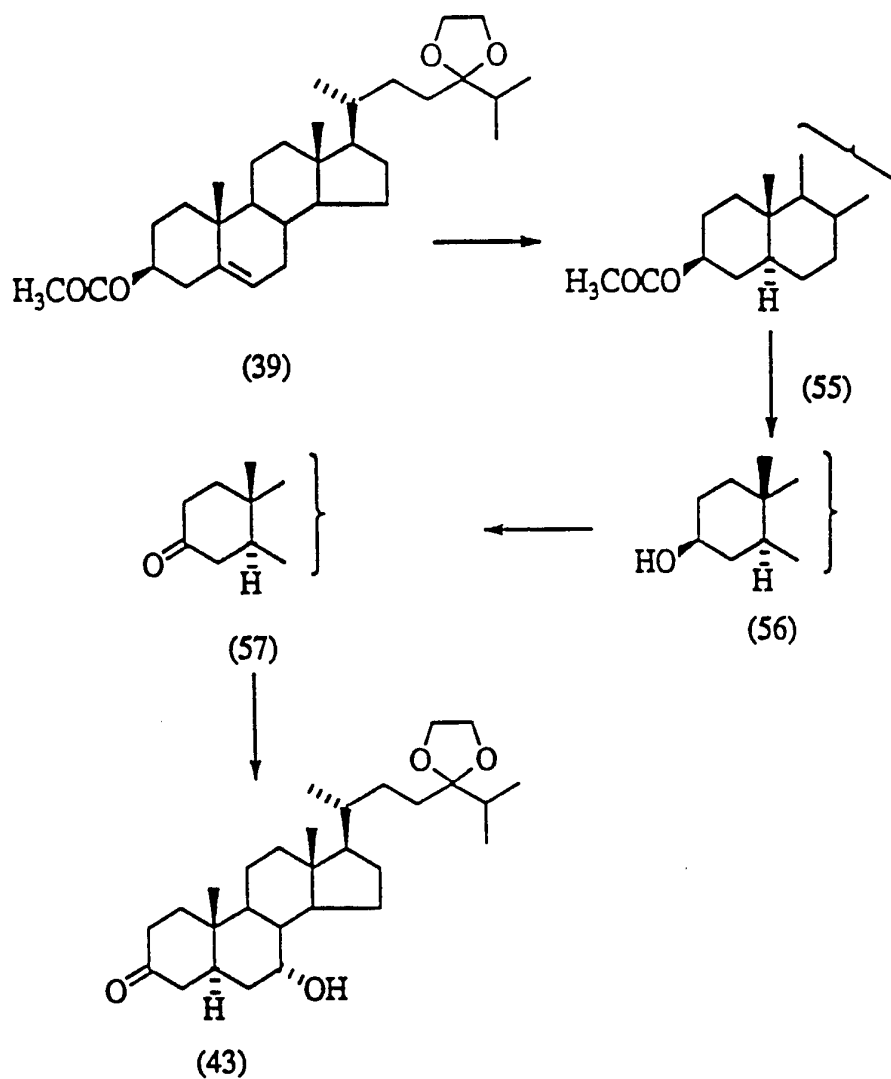


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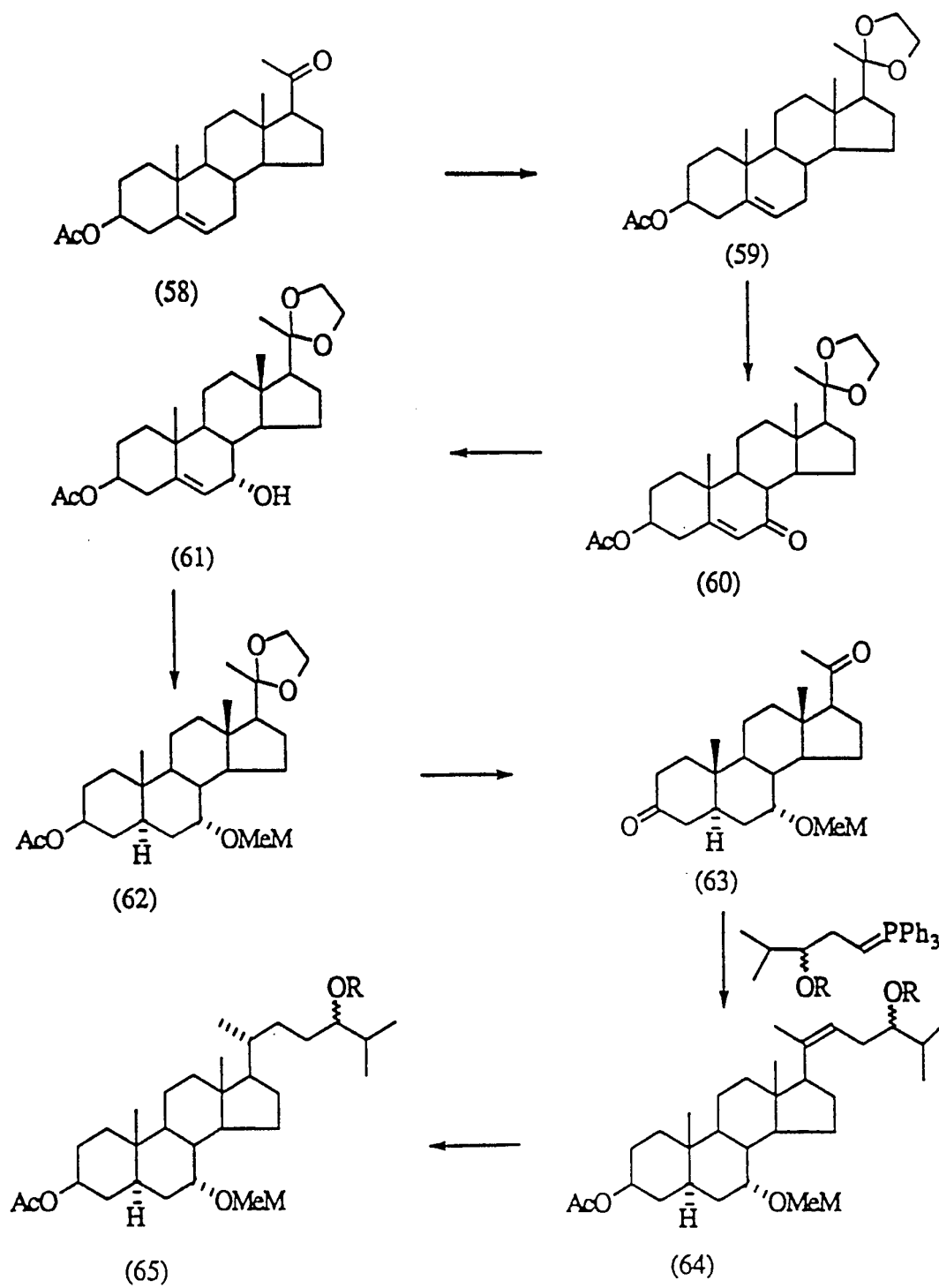
FIG. 5



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FIG. 6

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FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/01822

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07J41/00 A61K31/56 //C07J51/00, C07J17/00, C07J9/00,
C07J21/00, C07J13/00, C07J9/00, C07J7/00

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)

IPC 5 C07J A61K

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF THE CHEMICAL SOCIETY, no.11, 1967, LETCHWORTH GB pages 1045 - 1052 M. DAVIS ET AL 'Steroid amines. Part IV. 3,17-Diaminoandrostane derivatives' see page 1046 - page 1047 ---	1,9
Y	CANADIAN JOURNAL OF CHEMISTRY, vol.64, no.12, December 1986, OTTAWA CA pages 2456 - 2469 J. P. GUTHRIE 'A water soluble dimeric steroid with catalytic properties. Rate enhancements from hydrophobic binding' see page 2457 --- -/--	1,9

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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 "&" document member of the same patent family

Date of the actual completion of the international search

13 July 1994

Date of mailing of the international search report

26. 07. 94

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Moreno, C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/01822

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, vol.90, no.4, 15 February 1993, WASHINGTON DC, USA pages 1354 - 1358 K. S. MOORE ET AL 'Squalamine: an aminosterol antibiotic from the shark' see the whole document ---	1,9
A,P	WO,A,93 18773 (THE CHILDREN'S HOSPITAL OF PHILADELPHIA) 30 September 1993 see the whole document -----	1,9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PC7/US 94/01822

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU-B- 3661993	21-10-93
