Synthesis of Novel 4α-Substituted Sterols

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A series of novel 4α-substituted sterols (**9**, **10**, **11**, **14**, **15**, and **19**) has been synthesized as potential inhibitors of sterol 4-demethylation. The key intermediate was 4α-carbomethoxy-5α-cholestan-3-one (**1**), which was synthesized by regiocontrolled alkylation with methyl cyanoformate. Epoxidation of 4α-vinyl-5α-cholestan-3β-ol (**9**) gave the diasteroisomeric epoxides (**20** and **21**) whose stereochemistry was deduced from NMR analysis and deuterium labeling.

During the conversion of lanosterol to cholesterol by rat liver microsomes, the two methyl groups at C-4 of lanosterol are removed oxidatively. This demethylation process involves O_2–NADPH linked oxidation by the methyl sterol oxidase system, followed by an oxidative decarboxylation sequence.[1]

These processes are of mechanistic and pharmacological interest, and we have been engaged in the development of specific inhibitors of the enzymes involved. An earlier communication from this laboratory described[2] studies on a mechanism-based inhibitor of 4-methylsterol oxidase. We report here the synthesis of a series of novel 4α-substituted sterols, which were designed as inhibitors of the sterol 4-demethylase system. Our synthetic strategy centered on the key intermediate **1**, which could readily be modified to yield the desired 4α-substituted compounds. The difficulties associated with the synthesis of the known[3] β-keto ester **1** stem from the preferred enolization of 3-keto-5α steroids toward C-2. This problem has been previously negotiated by the use of such procedures as methyl magnesium carbonate carboxylation at C-4 of the steroidal 5α 1-en-3-one system[3] or the Stork and d'Angelo reductive alkylation process.[4]

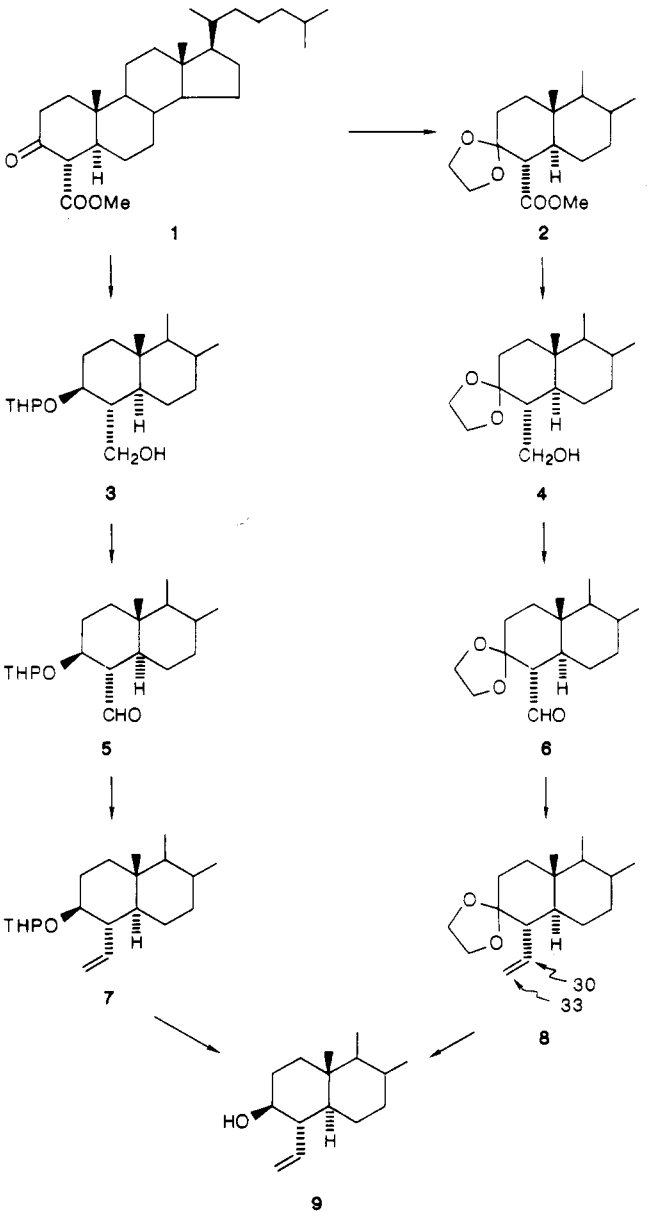
In this work we have used the potent C-acylating agent methyl cyanoformate[5] to generate 4α-carbomethoxy-5α-cholest-1-en-3-one from 5α-cholest-1-en-3-one, which was first deprotonated with lithium diisopropylamide (LDA). Hydrogenation of the 1,2-double bond in this 4α-carbomethoxy compound using 5% palladium–charcoal then gave the desired product **1** in 94% overall yield from 5α-cholest-1-en-3-one. We believe this conversion is superior in yield and practicality to the available literature procedures.

We were now ready to use compound **1** to insert vinyl, allenyl and ethynyl groups at the C-4 position. These transformations required either the protection of the 3-carbonyl group as the ethylene ketal or the reduction of the 3-ketone to a 3β-hydroxy group with subsequent conversion to the 3β-tetrahydropyranyl ether (Scheme I).

In our first synthetic experiments the known[3] 3-ethylene ketal **4** was used, but we subsequently prepared and used 4α-(hydroxymethyl)-5α-cholestan-3β-ol THP ether (**3**).[6]

Compound **3** and the 3-ethylenedioxy analogue **4** (obtained by lithium aluminum hydride reduction of the 4α-carbomethoxy-3-ethylene ketal **2** gave the 4α-formyl compounds **5**[7] and **6**, respectively, upon oxidation with Collins reagent.[8]

Scheme I



These 4α-formyl intermediates were then homologated to the desired 4α-vinyl, 4α-dihalovinyl, ethynyl, cyanoethynyl, and allenyl sterols. The Zn–$TiCl_4$–CH_2Br_2

(1) Gaylor, J. L. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 502–507.
(2) Bartlett, D. L.; Robinson, C. H. *J. Am. Chem. Soc.* **1982**, *104*, 4729–4730.
(3) Czarny, M. R.; Nelson, J. A.; Spencer, T. A. *J. Org. Chem.* **1977**, *42*, 2941–2944 and references cited therein.
(4) Bartlett, D. L. Ph.D. Dissertation, The Johns Hopkins University, 1982.
(5) Mander, L. N.; Sethi, P. *Tetrahedron Lett.* **1983**, *24*, 5425–5428.
(6) Ekhato, I. V.; Silverton, J. V.; Robinson, C. H. *J. Org. Chem.* **1988**, *53*, 2180–2183.
(7) Ekhato, I. V.; Robinson, C. H. *J. Chem. Soc., Perkin Trans. 1*, in press.
(8) Collins, J. C.; Hess, W. W. *Org. React.* **1972**, *54*, 5–10.

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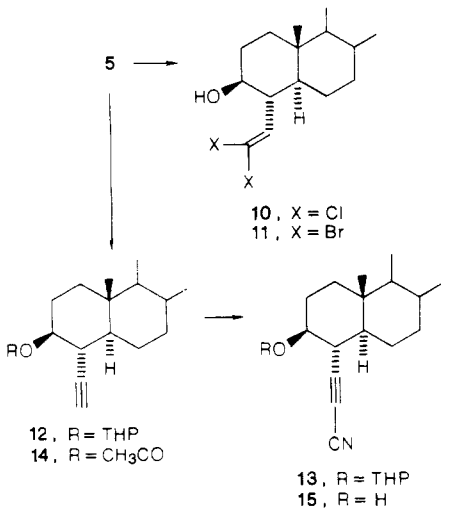
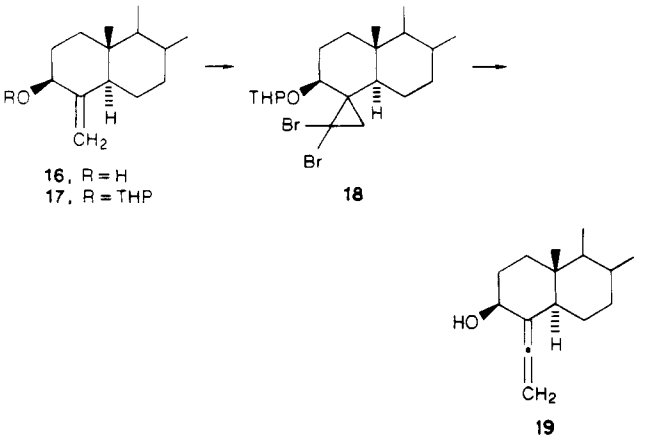
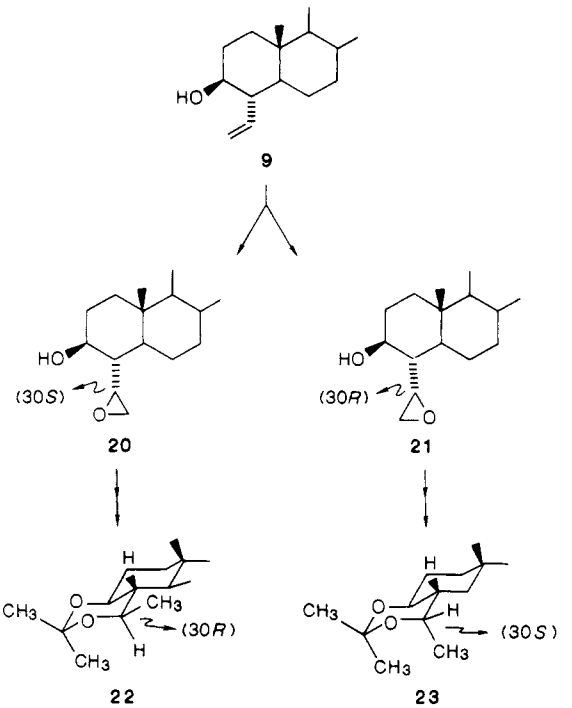
Scheme II



Scheme III



Scheme IV



methylenation methodology was adopted for the synthesis of the 4α-vinyl compound **9**, and the complex was prepared according to the Lombardo procedure.[9] With this reagent, the 4α-formyl compound **5** could be homologated and then deprotected in very good yield (90%) to give 4α-vinyl-5α-cholestan-3β-ol (**9**). The 4α-formyl-3-ethylenedioxy compound **6** was similarly homologated and then deketalized and reduced at C-3 with sodium borohydride to give the same 4α-vinyl product **9**.

The same procedure, when applied to the synthesis of 4α-(33-dichloro)vinyl-5α-cholestan-3β-ol (**10**) and the 4α-(33-dibromo)vinyl analogue (**11**) was unsuccessful, apparently due to the instability of the methylenation complex derived from bromoform or carbon tetrabromide or carbon tetrachloride. Modified Wittig procedures[10] eventually furnished the dichloro- and dibromovinyl compounds (**10** and **11**) from **5** although in low yields (Scheme II). The ethynyl compounds **14** and **15** were derived from an oily 4α-monochlorovinyl compound made in turn by the condensation of the aldehyde **5** with (chloromethylene)-triphenylphosphorane. Dehydrohalogenation of this chlorovinyl intermediate to 4α-ethynyl-5α-cholestan-3β-ol tetrahydropyranyl ether (**12**) was accomplished with *n*-butyllithium. The cyanoethynyl compound **13** was prepared via compound **12**, which was converted to the lithium salt followed by quenching of the reaction mixture with phenyl cyanate.[11] In the final steps, the tetrahydropyranyl protection at C-3 was removed in each case with aqueous HCl in warm tetrahydrofuran solution to give **14** and **15**. Attempts to make 4α-(33-difluoro)vinyl-5α-cholestan-3β-ol using literature procedures[12,13] were unsuccessful.

The rearrangement of gem-dihalogen-substituted cyclopropanes with methyllithium to give allenic compounds is a well-established procedure.[14] For our purpose, this represented the most convenient route to the desired 4-allenyl-5α-cholestan-3β-ol (**23**) from the known[3] olefin precursor **16**. Accordingly the 3-tetrahydropyranyl ether[6] of compound **16** was converted to 4-spiro(33-dibromo)-cyclopropane-5α-cholestan-3β-ol tetrahydropyranyl ether (**18**) by using bromoform and potassium *tert*-butoxide in hexane, although only in 14% yield. Optimization of yield was not explored. From the spirodibromocyclopropane (**18**) the allenic sterol (**19**) was obtained in quantitative yield via methyllithium-promoted rearrangement followed by deprotection with aqueous acid in tetrahydrofuran (Scheme III).

Finally, the diastereoisomeric epoxides **20** and **21**, which we needed as potential affinity labels for the enzyme system, were obtained by oxidation of 4α-vinyl-5α-cholestan-3β-ol (**9**) with *m*-chloroperoxybenzoic acid, followed by column chromatographic separation (Scheme IV).

Spectroscopic Properties and Stereochemistry. It was possible to deduce the configuration at the C-4 position of the above 5α-sterol derivatives by proton NMR analysis. The C-4 proton, by correlation with the known 3β-hydroxy and 5α-configurations of all the compounds, was determined to be of the β-configuration in each case. For example, in compounds **10**, **11**, **14**, and **15**, two diaxial cou-

(9) Lombardo, L. *Tetrahedron Lett.* **1982**, 4293–4296.
(10) (a) Chapleur, Y. *J. Chem. Soc., Chem. Commun.* **1984**, 449–450. (b) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769–3772.
(11) Zweifel, G.; Murray, R. E. *Synthesis* **1980**, 150–151.
(12) Fuqma, S. A.; Damian, W. G.; Silverstein, R. M. *J. Org. Chem.* **1965**, *30*, 2543–2545. Mase, D. G.; Barton, D. J. *Synth. Commun.* **1983**, 297–300. Barton, D. J.; Greenlimb, P. E. *J. Org. Chem.* **1975**, *40*, 2796–2801. Barton, D. J.; Wheaton, G. A. *J. Org. Chem.* **1983**, *48*, 917–927.
(13) Vinson, W. A.; Prickett, K. S.; Shahic, B.; Ortiz de Montellano, P. R. *J. Org. Chem.* **1983**, *48*, 4661–4668.
(14) Rutledge, F. T. In *Acetylenes and Allenes, Addition Cyclization and Polymerization Reactions*; Reinhold: New York, 1969; p 13–14.

plings in the range of 9.2–10.6 Hz were observed for the C-4 proton. Other spectroscopic data (IR, MS, NMR) and combustion analyses were consistent with the assigned structures.

The stereochemistry at the C-30 position in the diastereoisomeric epoxides **20** (30*S*) and **21** (30*R*) was established by a combination of NMR analysis and deuterium labeling. Compounds **20** and **21** were each reduced with lithium aluminum hydride, and the resulting 3β,30-diol in each case was derivatized as the acetonide, via acid-catalyzed reaction with 2,2-dimethoxypropane (Scheme IV). It was expected that these acetonides, which do not permit rotation about the C-4–C-30 bond, would permit assignment of stereochemistry at C-30 on the basis of C-4H–C-3OH coupling constants. The proton NMR spectrum of the acetonide **23** obtained from the chromatographically more polar epoxide **21** showed a multiplet at 4.06 ppm and a doublet at 1.34 ppm. These chemical shifts are assigned respectively to the C-30 methine proton and the C-30 methyl substituent. In the epimeric acetonide **22**, corresponding signals appeared at 3.71 and 1.25 ppm.

A proton decoupling experiment was performed on acetonide **23** in which the C-30 methyl was irradiated and gave a J value of 5.4 Hz for the resulting doublet at 4.06 ppm shown by the C-30 methine proton, consistent with a synclinal relation between this proton and the 4β-proton, and therefore the 30*S* configuration. When the acetonide **22** was submitted to a similar experiment, the C-30 methine proton unexpectedly appeared as a singlet. A standard ^{1}H–2D correlation experiment (COSY) was then performed to obtain cross-peaks between protons with coupling greater than 2.4 Hz. The experiments indicated that whereas in compound **23** the C-4 proton and C-30 methyl chemical shifts are separated by about 0.5 ppm, the corresponding signals in compound **22** overlapped. Evidence that this overlap accounted for the unexpected result in the decoupling experiment with acetonide **22** was then obtained via the synthesis of the deuterium-labeled C-30 [$^2H_3$]methyl (**22a**) analogue of compound **22**. This deuterated analogue **22a** was made from 4α-formyl-5β-cholestan-3β-ol 3-tetrahydropyranyl ether by methylenation with Zn–$TiCl_4$–$C^2H_2Br_2$, followed by the removal of the tetrahydropyranyl ether, epoxidation with *m*-chloroperoxybenzoic acid, lithium aluminum deuteride reduction of the 4α-(30*S*)-[33-$^2H_2$]oxirane and derivatization of the resulting 4α-(30*R*)-[33-$^2H_3$]hydroxyethyl compound as the acetonide. The highfield NMR spectrum of **22a** showed a doublet due to the C-30 methine proton at 3.71 ppm, J = 9.5 Hz, consistent with antiperiplanar geometry between it and the C-4β proton, and therefore the 30*R* configuration in compound **22**.

In summary, we have synthesized a series of potential inhibitors of steroid demethylation. These new sterols all possess an α-oriented two-carbon chain at C-4 and are derived from 4-formyl sterol intermediates.

Experimental Section

General Methods. All reactions involving the use of organolithium compounds were carried out in flame-dried flasks and under an atmosphere of argon. Melting points were determined on a Kofler hot stage and are uncorrected. IR spectra were recorded on a Perkin-Elmer 462 spectrometer, in $CHCl_3$ unless otherwise noted. UV spectra were obtained on a Perkin-Elmer Lambda 3 instrument. ^{1}H NMR spectra were recorded in $CDCl_3$ with either an IBM FT (80 MHz) spectrometer or a Varian XL-200 MHz spectrometer. Mass spectra were obtained on an LKB 9000 spectrometer or a VG70S instrument. High-pressure liquid chromatographic separations were performed on a Waters Associates Model 6000 instrument; a Whatman semipreparative column was used for normal-phase separations. Column chromatography was performed on silica gel according to Still's method.[15]

4α-Carbomethoxy-5α-cholestan-3-one (1). To a solution of diisopropylamine (2.62 mL, 18.74 mmol) in dry THF (39 mL) at −20 °C under argon was added *n*-butyllithium (12.49 mL, 1.55 M in hexane). After 30 min, the temperature was lowered to −78 °C, and a solution of 5α-cholest-1-en-3-one (6.0 g, 15.6 mmol) in dry tetrahydrofuran (THF) (15.6 mL) was added. The reaction was stirred at 0 °C for 1 h and cooled to −78 °C. Hexamethylphosphoramide (1.59, 15.6 mmol) and methyl cyanoformate (1.54 g, 18.74 mmol) [CAUTION: Hazardous if inhaled, swallowed, or absorbed through the skin (see Aldrich Material Safety Data Sheet)] were added, and the mixture was stirred for 10 min, poured onto ice–water (500 mL), and extracted with methylene chloride (2 × 300 mL). The organic extract was washed with brine, dried (Na_2SO_4), and evaporated in vacuo to afford 4α-carbomethoxy-5α-cholest-1-en-3-one:[3] NMR (80 MHz) δ 7.20 (d, 1, J = 10.7 Hz, vinyl H), 5.90 (d, 1, J = 10.3 Hz, vinyl H), 3.77 (s, 3, $COOCH_3$), 3.37 (d, 1, J = 12.3 Hz, 4β-H); UV (hexane) λ_{max} 223 nm (ε 10000).

The total crude product from the above reaction was dissolved in cyclohexane–THF (4:1, 250 mL) and was hydrogenated at atmospheric pressure over 5% Pd/C at room temperature for 3 h. After filtration through a layer of Celite, the solvent was removed in vacuo to afford 4α-carbomethoxy-5α-cholestan-3-one (6.25 g, 93%): mp 173–174 °C (from methanol) [lit.[3] mp 171–172 °C]; IR 1735 and 1710 cm^{-1}; NMR (80 mHz) δ 3.75 (s, 3, $COOCH_3$), 3.25 (d, 1, J = 12 Hz, 4β-H).

4α-(Hydroxymethyl)-5α-cholestan-3-one 3-Ethylene Ketal (4). To a stirred solution of 4α-carbomethoxy-5α-cholestan-3-one 3-ethylene ketal[4] (**2**, 3.4 g) in anhydrous ether (150 mL, freshly distilled from lithium aluminum hydride) was added lithium aluminum hydride (600 mg), and the mixture was stirred at room temperature overnight. Excess lithium aluminum hydride was destroyed by dropwise addition of ethyl acetate, the mixture was placed in an ice bath, and a saturated solution of sodium sulfate was added dropwise until coagulation occurred. Dichloromethane and then anhydrous Na_2SO_4 were added, and the suspension was filtered and evaporated to dryness in vacuo. The solid product was crystallized from acetone to afford **4** (3.0 g, 93%): mp 184–185 °C (lit.[3] mp 182–184 °C); IR 3600 cm^{-1}; NMR δ 4.00–3.80 (b s and m, OCH_2CH_2O and 4α-OCH_2).

4α-Vinyl-5α-cholestan-3-one 3-Ethylene Ketal (8). To a suspension of Collins reagent (20.0 g) in CH_2Cl_2 (200 mL) was added 4α-(hydroxymethyl)-5α-cholestan-3-one ethylene ketal (**4**, 4.5 g), and the mixture was stirred at room temperature for 15 min, filtered through a layer of Celite, and passed through a short column of Florisil. The eluent was washed with 10% aqueous HCl, water, and brine. After drying (Na_2SO_4), the solvent was removed in vacuo to yield the desired 4α-formyl compound. A solution of the crude aldehyde in dry CH_2Cl_2 (50 mL) was treated with pregenerated Zn–$TiCl_4$–CH_2Br_2 complex added in portions over 1 h until the reaction was complete (TLC). [The Zn–$TiCl_4$–CH_2Br_2 complex was generated by the addition of CH_2Br_2 (4.04 mL) to a stirred suspension of zinc dust (11.5 g) in dry THF (100 mL, freshly distilled from lithium aluminum hydride), keeping the temperature between −40 °C and −50 °C. To the stirred mixture at the same temperature was then added titanium tetrachloride (4.6 mL) over 10 min. The mixture was allowed to warm to −5 °C and stirred for 3 days before use.] The reaction mixture was diluted with ether (400 mL) and, while stirring in an ice bath, was treated with 5% aqueous $NaHCO_3$ solution. The resulting slurry was treated with Celite and filtered, and the organic phase was separated and washed with water and brine. After drying (Na_2SO_4) the solvent was removed in vacuo, and the crude product was purified by column chromatography (5% ethyl acetate in hexane). The resulting 4α-vinyl-5α-cholestan-3-one ethylene ketal, (**8**, 3.8 g, 85%) had mp 150–152 °C (from acetone); IR (KBr) 1645 cm^{-1}; NMR δ 5.80–4.87 (m, 3, $CH{=}CH_2$), 3.87 (m, 4, OCH_2CH_2O). Anal. Calcd for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.34; H, 11.26.

4α-Vinyl-5α-cholestan-3β-ol (9). (a) 4α-Vinyl-5α-cholestan-3-one 3-ethylene ketal (**8**, 3.5 g) was heated in refluxing 80% aqueous acetic acid (100 mL) for 1 h, and the solution was cooled

(15) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

to room temperature, diluted with water, and extracted with ether/CH_2Cl_2 (3:1, 300 mL). The organic extract was washed successively with saturated $NaHCO_3$, water, and brine, dried (Na_2SO_4), and evaporated in vacuo to afford the crude 3-oxo product. The latter was dissolved in benzene/methanol (1:3, 150 mL) and treated at 0 °C with 2 molar equiv of sodium borohydride for 5 min. After dropwise addition of dilute HCl the reaction mixture was evaporated in vacuo. The residue was taken up in ether, and the ether extract was washed with water and then brine and dried (Na_2SO_4). The product obtained after removal of the solvent was purified by silica gel chromatography (15% ethyl acetate in hexane) to give 4α-vinyl-5α-cholestan-3β-ol (**9**, 2.4 g, 76%): mp 124–125 °C (from acetone); IR (KBr) 3580, 3550, 3300, 1640 cm^{-1}; MS m/z 414 (M^+), 399, 397, 382; NMR δ 5.43 (m, 1, J = 9.8 Hz, 30-H), 5.13 (dd, 2, J = 2.1 and 17 Hz, 33-CH_2), 3.18 (m, 1, 3α-H). Anal. Calcd for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 84.18; H, 12.19.

(b) Alternative Preparation of Compound 9. To a solution of 4α-formyl-5α-cholestan-3β-ol tetrahydropyranyl ether[7] (**5**, 3.2 g, 6.4 mmol) in dry CH_2Cl_2 (50 mL) was added Zn–$TiCl_4$–CH_2Br_2, prepared as above, in portions over 1 h, while the reaction was monitored by TLC. At the completion of the reaction, anhydrous ether (300 mL) was added, and then the reaction mixture was cooled in an ice bath, 5% $NaHCO_3$ solution was added cautiously, and the resulting slurry was made granular by the addition of Celite and filtered. The organic phase was separated, and the aqueous portion was extracted with ether (2 × 200 mL); the combined organic extracts were washed with brine and dried (Na_2SO_4). The crude product was deprotected in boiling THF (200 mL) containing 10% aqueous HCl (20 mL) for 45 min and was purified by column chromatography and crystallized from acetone to give 4α-vinyl-5α-cholestan-3β-ol (**9**, 2.4 g, 90%), with melting point and spectroscopic data as reported above.

4α-(33-Dichloro)vinyl-5α-cholestan-3β-ol (10). 4α-Formyl-5α-cholestan-3β-ol tetrahydropyranyl ether (**5**, 400 mg, 0.8 mmol) in dry THF (10 mL) was added to a stirred suspension of hexamethylphosphorus triamide (0.319 mL, 1.76 mmol) and carbon tetrachloride (0.32 mL, 3.3 mmol) in dry THF (20 mL) under nitrogen at −28 °C. After being stirred for 1 h at −28 °C and then for 48 h at 25 °C, the mixture was diluted with ether (100 mL), washed with water (3 × 100 mL) and then with brine, and dried (Na_2SO_4). Evaporation in vacuo gave an oily product, which was deprotected by treatment with 10% HCl (10 mL) in THF (100 mL) for 45 min at 80 °C. The solvent was removed in vacuo, and the crude product was dissolved in ether (100 mL), which was washed with water (3 × 100 mL) and brine and dried (Na_2SO_4) The product obtained was crystallized from petroleum ether (35–60 °C) to give 4α-(33-dichloro)vinyl-5α-cholestan-3β-ol (**10**, 300 mg, 78%): mp 145–146 °C; IR (KBr) 3600–3200, 1620 cm^{-1}; MS m/z 482 (M^+) and other ions at m/z 462, 449, 425, 373, 355, 327, and 311; NMR (80 MHz) δ 5.50 (d, 1, J = 10 Hz, 30-H), 3.19 (m, 1, 3α-H) and 2.21 (dd, 1, J = 9.2 Hz, 4β-H). Anal. Calcd for $C_{29}H_{48}OCl_2$: C, 72.03; H, 10.00; Cl, 14.67. Found: C, 72.06; H, 9.92; Cl, 14.90.

4α-(33-Dibromo)vinyl-5α-cholestan-3β-ol (11). 4α-Formyl-5α-cholestan-3β-ol tetrahydropyranyl ether (**5**, 1.40 g), in CH_2Cl_2 (20 mL), was added in one portion to prepared ylid [from zinc dust (1.14 g) in CH_2Cl_2 (200 mL) + Ph_3P (4.58 g) and CBr_4 (5.79 g) stirred under nitrogen for 24 h at 25 °C]. The reaction mixture was stirred at 25 °C and was monitored by TLC. After 15 h, it was diluted with pentane (400 mL) and filtered. The residual tar was dissolved in CH_2Cl_2 (200 mL), and Celite was added. The slurry was filtered, and the solid cake was washed with pentane. Solvent was removed in vacuo to afford an oily crude product. The tetrahydropyranyl ether function was removed by treatment in tetrahydrofuran (100 mL) with 10% HCl (10 mL) at 80 °C for 45 min. Flash chromatography on silica gel (10% ethyl acetate in hexane) gave after crystallization from petroleum ether (35–60 °C) 4α-(33-dibromo)vinyl-5α-cholestan-3β-ol (**11**, 936 mg, 58%): mp 168–169 °C; MS m/z 572 (M^+) and other ions at m/z 554, 539, 515, 475, 417, 399, 373, and 353; NMR (80 MHz) δ 6.10 (d, 1, J = 9.9 Hz, 30-H), 3.33 (m, 1, 3α-H), 2.33 (dd, 1, J = 10.6 Hz, 4β-H). Anal. Calcd for $C_{29}H_{48}OBr_2$: C, 60.84; H, 8.45; Br, 27.91. Found: C, 60.81; H, 8.37; Br, 28.09.

4α-Ethynyl-5α-cholestan-3β-ol Tetrahydropyranyl Ether (12) and 3β-Acetoxy Analogue (14). To a stirred suspension of (chloromethyl)triphenylphosphonium chloride (6.3 g), in dry THF (140 mL), was added n-butyllithium (8.0 mL, 1.6 M in hexane) at −78 °C. The mixture was stirred at 25 °C for 30 min under argon, and 4α-formyl-5α-cholestan-3β-ol tetrahydropyranyl ether (**5**, 1.8 g) in anhydrous THF (20 mL) was added. The mixture was then refluxed for 1 h. After the mixture was cooled to 25 °C, saturated NH_4Cl (30 mL) was added, and the mixture was extracted with chloroform (3 × 100 mL). The combined organic extracts were washed with brine and dried (Na_2SO_4). The crude product was flash chromatographed on silica gel (10% ethyl acetate in hexane) to afford the crude oily vinyl chloride. This crude product in anhydrous THF (100 mL) at −78 °C under nitrogen was treated with n-butyllithium (12 mL, 1.6 M in hexane) added dropwise with stirring. After 1 h the temperature was raised to 28 °C, and stirring was continued for 1 h. The reaction was quenched with saturated NH_4Cl (30 mL) and concentrated in vacuo. The residue was extracted with ether (3 × 100 mL), and the combined ethereal extracts were washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The product was crystallized from hexane to afford 4α-ethynyl-5α-cholestan-3β-ol tetrahydropyranyl ether, (**12**, 1.68 g, 94%). A portion was deprotected with 10% HCl in THF (as described above) and characterized by acetylation (pyridine–acetic anhydride) to 4α-ethynyl-5α-cholestan-3β-ol-3β-acetate (**14**): mp 130–132 °C (acetone); IR ($CHCl_3$) 3300 and 1720 cm^{-1}; MS m/z 454 (M^+) and others at m/z 439, 412, 394, 379, 354, 299, 239, and 225; NMR (80 MHz) δ 4.76 (m, 1, 3α-H), 2.40 (dd, 1, J = 9.4 Hz, 4β-H), 2.07 (s, 3, 3β-$OCOCH_3$); ^{13}C NMR δ 170.42, 84.32, 75.90, 70.48, 56.31, 56.22, 54.13, and 48.96. Anal. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 82.11; H, 11.12.

4α-(33-Cyano)ethynyl-5α-cholestan-3β-ol (15). n-Butyllithium (1.06 mL, 1.6 M in hexane) was added dropwise to a solution of 4α-ethynyl-5α-cholestan-3β-ol tetrahydropyranyl ether (**12**, 800 mg) in dry ether (20 mL) maintained at −78 °C under argon. Phenyl cyanate[11] [CAUTION] (0.20 mL) was added dropwise, keeping the temperature below −60 °C. The mixture was stirred for 30 min at −78 °C and then allowed to warm to −40 °C. After the mixture was stirred for an additional 30 min, it was allowed to warm to 25 °C, diluted with ether, poured into a 6 M solution of NaOH (30 mL), and then shaken vigorously, and the aqueous layer was separated. The ether extract was washed with saturated NaCl solution and dried (Na_2SO_4) to give crude **13**. The tetrahydropyranyl protecting group was removed with 10% HCl (5 mL) in THF (50 mL) at 60 °C for 45 min to give a yellow solid, which was purified by flash chromatography on silica gel (20% ethyl acetate in hexane). Crystallization from acetone yielded 4α-(33-cyano)ethynyl-5α-cholestan-3β-ol (**15**, 680 mg, 96%): mp 168–170 °C; IR ($CHCl_3$) 3600 and 2280 cm^{-1}; UV (hexane) λ_{max} 273.6 nm (ε 2300); MS m/z 437 (M^+) and others at m/z 422, 380, 352, 324, 297, 282, and 268; NMR (80 Hz) δ 3.60 (m, 1, 3α-H), 2.43 (t, 1, J = 10.2 Hz, 4β-H); ^{13}C NMR δ 105.16, 89.07, 73.38, 57.62, 54.09, 48.23. Anal. Calcd for $C_{30}H_{47}NO$: C, 82.32; H, 10.82; N, 3.20. Found: C, 82.60; H, 10.77; N, 3.07.

4-Allenyl-5α-cholestan-3β-ol (19). (a) To an ice-cooled mixture of 4-methylene-5α-cholestan-3β-ol tetrahydropyranyl ether[7] (**17**, 500 mg, 1.03 mmol) and potassium *tert*-butoxide (579 mg, 5.15 mmol) in dry hexane (30 mL) was added bromoform (0.459 mL, 5.15 mmol) dropwise. The resulting suspension was stirred overnight at 25 °C and poured into a mixture of ether (100 mL) and water (100 mL). The aqueous phase was separated and extracted with ether (2 × 100 mL). The combined organic extract was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The tetrahydropyranyl grouping was cleaved [10% HCl (2 mL) in THF (20 mL) at 80 °C for 45 min], and column chromatography on silica gel (15%, ethyl acetate in hexane) yielded the 4-spiro(33-dibromo)cyclopropane product (**18**, 80 mg, 14%). Further elution gave 4-methylene-5α-cholestan-3β-ol (**16**, 320 mg, 78%).

(b) A solution of the above 4-spiro(33-dibromo)cyclopropanyl-5α-cholestan-3β-ol tetrahydropyranyl ether (**18**, 92 mg, 0.14 mmol) in anhydrous ether (10 mL) was treated with methyllithium (0.1 mL, 1.55 M in ether) added dropwise over 3 min at 0 °C. The mixture was stirred overnight at 25 °C, and poured into ether (20 mL) and water (20 mL). The aqueous layer was separated, extracted with ether (2 × 20 mL) dried (Na_2SO_4) and concentrated in vacuo. The product was deprotected [10% HCl

(1.0 mL) in THF (10 mL) at 80 °C for 45 min] and crystallized from acetone to afford 4-allenyl-5α-cholestan-3β-ol (**19**, 56 mg, 97%): mp 108–110 °C; IR ($CHCl_3$) 3560 and 1955 cm^{-1}; MS m/z 412 (M^+) 412 and other ions at m/z 397, 394, 379, 369, and 257; NMR (80 MHz) δ 5.03 (app t, 2, J = 3.8 Hz, 33-CH_2), 4.00 (m, 1, 3α-H). Anal. Calcd for $C_{29}H_{48}O$: C, 84.4; H, 11.72. Found: C, 84.29; H, 11.84.

3β-Hydroxy-5α-cholestane-4α-(30*S* and 30*R*)-oxiranes (20 and 21). To a solution of 4α-vinyl-5α-cholestan-3β-ol (**9**, 500 mg, 1.2 mmol) in CH_2Cl_2 (30 mL) at −79 °C was added a solution of 80% *m*-chloroperoxybenzoic acid (260 mg, 1.2 mmol). The solution was allowed to warm to 25 °C and was stirred for 30 h before dilution with CH_2Cl_2. The mixture was washed three times with 5% $NaHCO_3$ and then with water and saturated NaCl. After drying (Na_2SO_4) the solvent was removed in vacuo, and the oily product was purified by flash column chromatography on silica gel (15% ethyl acetate in hexane) to afford (a) unchanged 4α-vinyl-5α-cholestan-3β-ol (**9**, 50 mg), (b) (30*S*)-3β-hydroxy-5α-cholestane-4α-(30,33)-oxirane (less polar isomer **20**, 375 mg, 80%) [mp 144–146 °C (acetone); IR (KBr) 3580, 3600–3300 cm^{-1}; MS m/z 430 (M^+), 415, 412, 397, 355; NMR δ 3.70 (m, 1, J = 4.5, 11.0 Hz, 3α-H); 2.80 (m) and 2.4 (tr) (30-H and 33-CH_2). Anal. Calcd for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 81.07; H, 11.76], and (c) (30*R*)-3β-hydroxy-5α-cholestane-4α-(30,33)-oxirane (more polar isomer **21**, 45 mg, 10%) [mp 180–182 °C (MeOH); IR (KBr) 3450 cm^{-1}; MS m/z 430 (M^+), 415, 412, 397, 355; NMR δ 3.35 (m, 1, 3α-H), 2.94 (m) and 2.79 (d) (30-H and 33-CH_2). Anal. Calcd for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.53; H, 11.47].

(30*S*)-4α-(Hydroxyethyl)-5α-cholestan-3β-ol 3,30-Acetonide (23). A solution of (30*R*)-4α-oxirane (**21**, 120 mg) in ether (20 mL) at 25 °C was reduced with lithium aluminum hydride (50 mg) added in one portion. After the mixture was stirred for 30 min, excess lithium aluminum hydride was destroyed with ethyl acetate added dropwise, followed by coagulation of the aluminate with saturated Na_2SO_4. The mixture was dried (anhydrous Na_2SO_4), filtered, and evaporated in vacuo. Without further purification, the crude diol was dissolved in 2,2-dimethoxypropane (2.0 mL) containing *p*-toluenesulfonic acid (1 mg). After 19 h at 25 °C the solvent was removed completely in vacuo, and the residue was taken up in ether, washed with saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated in vacuo. Crystallization from methanol gave (30*S*)-4α-(hydroxyethyl)-5α-cholestan-3β-ol 3,30-acetonide (**23**, 96 mg, 72%): mp 158–160 °C; MS m/z 472 (M^+) and other ions at m/z 457, 415, 397, 370, 355, 245; NMR δ 4.06 (m, 1, J = 5.4 and 7 Hz), 3.68 (m, 1), 1.35 (d, 6, J = 5.4 Hz). Anal. Calcd for $C_{32}H_{56}O_2$: C, 81.29; H, 11.94. Found: C, 81.12; H, 11.80.

(30*R*)-4α-(Hydroxyethyl)-5α-cholestan-3β-ol 3,30-Acetonide (22). The (30*S*)-4α-oxirane (**20**, 60 mg) was similarly reduced with lithium aluminum hydride in ether, followed by treatment with 2,2-dimethoxypropane to afford (30*R*)-4α-(hydroxyethyl)-5α-cholestan-3β-ol 3,30-acetonide (**22**, 62 mg, 94%): mp 104–106 °C; MS m/z 472 (M^+) and other ions at 457, 397, 370, 355; NMR δ 3.71 (dd, 1, J = 5.6, 9.2 Hz, 30-H), 3.50 (m, 1, 3α-H), 1.25 (d, J = 5.6 Hz, 30-CH_3). Anal. Calcd for $C_{32}H_{56}O_2$: C, 81.29; H, 11.94. Found: C, 81.21; H, 11.86.

(30*R*)-4α-[33-$^2H_3$](Hydroxyethyl)-5α-cholestan-3β-ol 3,30-Acetonide (22a). To a solution of 4α-formyl-5α-cholestan-3β-ol 3-tetrahydropyranyl ether (**5**, 500 g) in dry CH_2Cl_2 (30 mL) was added Zn–$TiCl_4$–$C^2H_2Br_2$ complex [from zinc dust (5.8 g), titanium tetrachloride (2.3 mL), and $C^2H_2Br_2$ (5.0 g)] prepared previously as in the preparation of compound **9** above. Workup exactly as for that reaction gave the [33-$^2H_2$] analogue of compound **9**, namely 4α-[33-$^2H_2$]vinyl-5α-cholestan-3β-ol (360 mg, 86%): MS m/z 416 (M^+); NMR δ 5.43 (d, 1, J = 10.0 Hz, 30-H), 3.18 (m, 1, 3α-H).

The above 4α-[33-^{2}H]vinyl compound (250 mg) was then epoxidized in dry CH_2Cl_2 (20 mL) with *m*-chloroperoxybenzoic acid (80%, 200 mg) at 25 °C for 18 h. Workup exactly as for the preparation of the oxiranes **20** and **21** gave, after chromatography in silica gel, [33-$^2H_2$]-(30*S*)-3β-hydroxy-5α-cholestane-4α-(30,33)-oxirane (212 mg, 82%): NMR δ 3.70 (m, 1, 3α-H), 2.80 (d, 1, J = 9.2 Hz, 30-H). This material was now reduced with lithium aluminum deuteride (120 mg) in anhydrous ether (20 mL) at 25 °C for 24 h. The resulting 4α-[33-$^2H_3$]-(30*R*)-(hydroxyethyl)-5α-cholestan-3β-ol was, without purification, treated with 2,2-dimethoxypropane (4 mL) containing *p*-toluenesulfonic acid (1 mg) for 24 h at 25 °C. Workup as for the preparation of compound **22** gave the desired 4α-[33-$^2H_3$]-(30*R*)-(hydroxyethyl)-5α-cholestan-3β-ol 3,30-acetonide (**22a**, 180 mg, 77%): mp 102–104 °C; MS m/z 475 (M^+), 460, 400; NMR δ 3.71 (d, 1, J = 9.5 Hz, 30-H), 3.50 (m, 1, 3α-H). Isotopic composition (MS) (mol %): d_0 = 0.12, d_1 = 0.20, d_2 = 1.65, d_3 = 96.89, d_4 = 1.51.

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