Syntheses of Novel 25-Hydroxyvitamin D_3 Haptens having Chemical Bridges at the C-11α Position[1]

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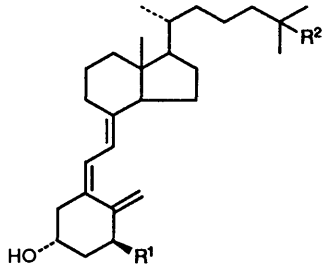
The serum or plasma levels of 25-hydroxyvitamin D_3 **1a** is useful for the evaluation of vitamin D status in various clinical or nutritional disorders. To obtain antibodies to compound **1a** which are highly specific and useful for development of immunoassays, two novel haptenic derivatives, 11α-(3-carboxypropionyloxy)-25-hydroxyvitamin D_3 **2a** and 11α-(4-carboxybutyryloxy)-25-hydroxyvitamin D_3 **2b** were synthesized each in 21 steps from 11α-hydroxydehydroepiandrosterone **3**.

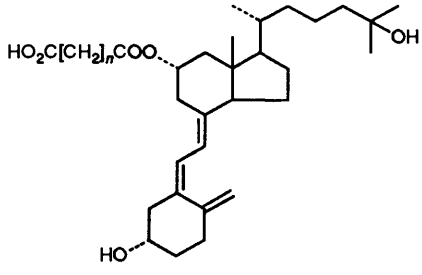
The serum or plasma levels of 25-hydroxyvitamin D_3 [25(OH)D_3, **1a**] and 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2$-D_3, **1b**], which are a major circulating metabolite and the most potent form of vitamin D_3 (D_3, **1c**), respectively, are useful for the evaluation of vitamin D status in various clinical or nutritional disorders.[2,3] The 25(OH)D_3 and 1,25$(OH)_2D_3$ levels are now measured usually by competitive radioassays using serum vitamin D binding protein and intestine vitamin D receptor, respectively.[4] However, both methods require tedious and time-consuming pretreatment of biological fluids to remove interfering substances.

Immunoassays using highly specific antibodies are therefore expected as an alternative methodology which is more simple and feasible for routine use. In recent years, a number of antibodies have been raised against the haptens linked to carrier proteins through C-3 or a position on the side chain,[4] including those prepared in our laboratory.[5] However, almost all the antibodies lacked sufficient specificity to omit or exceedingly simplify the pretreatment of the specimens in clinical application. It was anticipated that the use of the hapten–carrier conjugates exposing both the A-ring and side chain would provide antibodies having much higher specificity, the C-11α position of the metabolites being an attractive coupling site with the carrier protein.

From these points of view we have undertaken the syntheses of the haptenic derivatives of the D_3 metabolites having chemical bridges at their C-11α position. The present paper reports the syntheses of two novel haptenic derivatives of 25(OH)D_3, namely 11α-(3-carboxypropionyloxy)-25(OH)D_3 **2a** and 11α-(4-carboxybutyryloxy)-25(OH)D_3 **2b**. The properties of the resulting antibodies raised against compound **2b** are also described briefly.



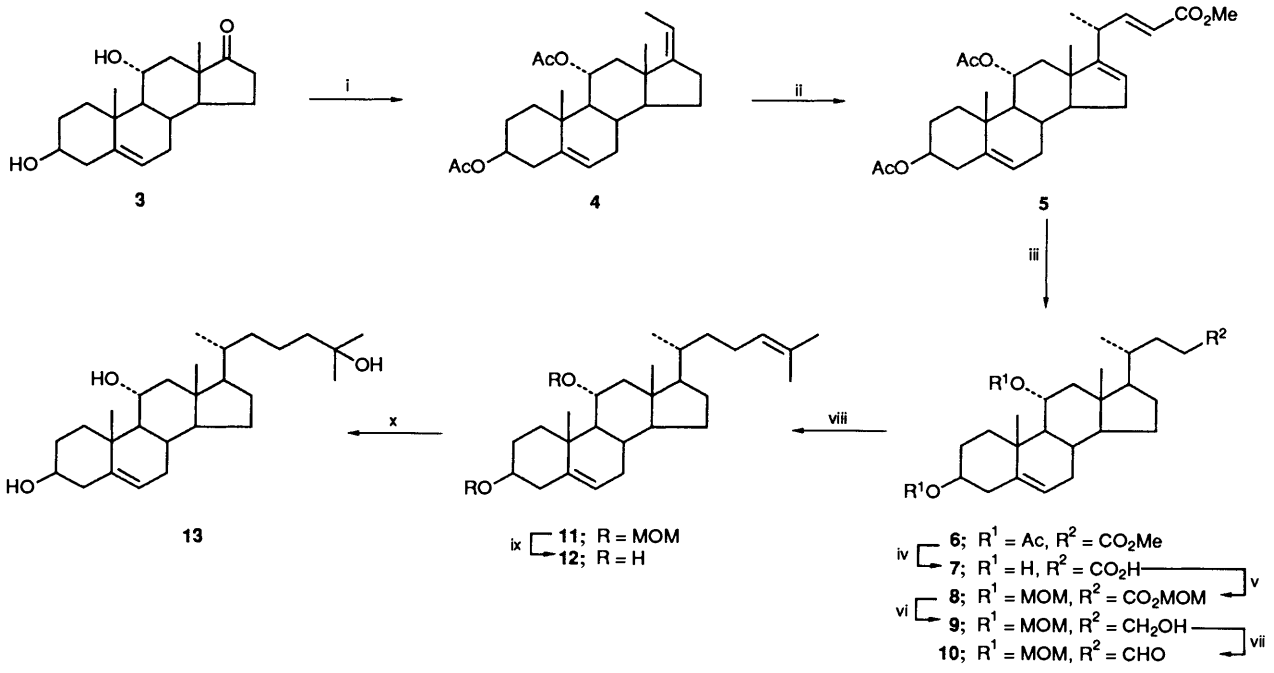
1a; R^1 = H, R^2 = OH
1b; R^1 = R^2 = OH
1c; R^1 = R^2 = H



2a; n = 2
2b; n = 3

Results

11α-Hydroxydehydroepiandrosterone **3**, obtained from dehydroepiandrosterone by microbial hydroxylation,[6] was chosen as a suitable starting material. Initially, the 25-hydroxy side chain having the necessary absolute configuration was stereoselectively constructed (Scheme 1). The Wittig reaction of ketone **3** with ethylidenetriphenylphosphorane followed by one-pot acetylation[7] afforded the diene diacetate **4** in 96% yield. A ^{1}H NMR difference nuclear Overhauser effect (NOE) experiment on compound **4** indicated the proximity between 12β-H and 21-H_3, from which the [17(20)*Z*]-configuration of the compound was confirmed. The ene reaction of compound **4** with methyl propiolate and ethylaluminium dichloride[7a] gave the (20*R*)-ester **5** in 91% yield. The formation of the corresponding (20*S*)-ester was not observed by HPLC or ^{1}H NMR spectroscopy. Subsequent catalytic hydrogenation of compound **5** with Pt/C[7] proceeded at the C-16 and C-22 double bonds selectively from the less hindered α-face, and thus the 5-ene ester **6** having the desired configuration (17β, 20*R*; the assignment is described below) could be obtained in 96% yield. Saponification of triester **6** gave the acid **7**, which was then converted into the aldehyde **10** by a sequence of reactions: the usual methoxymethylation of acid **7** to give the fully protected compound **8**, reduction of ester **8** with lithium aluminium hydride ($LiAlH_4$) to afford the alcohol **9**, and oxidation of compound **9** with pyridinium chlorochromate (PCC) to provide aldehyde **10** in 74% overall yield from triester **6**. Wittig reaction of compound **10** with isopropylidenetriphenylphosphorane[8] gave the diene **11**, whose methoxymethyl groups were subsequently removed under acidic conditions to give the diene diol **12** in 87% yield from aldehyde **10**. The introduction of the C-25 hydroxy group to compound **12** was effected by oxymercuriation and demercuriation[9] to give the intermediate **13** having the required sidechain structure in satisfactory overall yield from the starting substance (49% from compound **3**).



Scheme 1 *Reagents:* i, $EtPh_3PBr$, Bu^tOK, THF; then Ac_2O, DMAP, pyridine; ii, $HC{\equiv}CCO_2Me$, $EtAlCl_2$, CH_2Cl_2; iii, H_2, Pt/C, AcOEt; iv, KOH, MeOH–THF; v, $MeOCH_2Cl$, Pr^i_2NEt, DMF–THF; vi, $LiAlH_4$, THF; vii, PCC, CH_2Cl_2; viii, Pr^iPh_3PI, PhLi, THF; ix, HCl, THF; x, $Hg(OAc)_2$, aq. THF; then $NaBH_4$, NaOH:MOM = CH_2OMe.

In the next sequence of reactions, triol **13** was transformed into the 5,7-diene triol derivative **18** in which the 3β-hydroxy group was selectively protected (Scheme 2). Compound **13** was subjected to selective silylation using a limited amount of *tert*-butyldimethylsilyl chloride (TBSCl) (1.2 mol equiv.), and the 3-monosilyl ether **14** thus obtained in 91% yield was converted into its 11-acetate **15** quantitatively by the usual acetylation. Allylic bromination of compound **15** with *N*-bromosuccinimide (NBS) and a catalytic amount of 2,2′-azoisobutyronitrile (AIBN) followed by dehydrobromination with 2,4,6-collidine (2,4,6-trimethylpyridine) provided a mixture of several components containing the 5,7-diene derivative of acetate **15**, together with the 4,6-diene isomer. Since the separation of the dienes was not achieved by usual silica gel chromatography, the mixture was treated with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)[10] to convert the 5,7-diene selectively into the Diels–Alder adduct **16**, which was easily isolated by flash column chromatography, using silica gel, in 51% yield. Although the acetyl and PTAD groups in adduct **16** could be removed simultaneously by reaction with $LiAlH_4$ in boiling tetrahydrofuran (THF),[1,10] the desired compound **18** was obtained in only poor yield (34%). On the other hand, a two-step procedure, that is, deacetylation of compound **16** with potassium hydroxide followed by the removal of PTAD group from the diol **17** by refluxing in 1,1,3,3-tetramethylguanidine[11] gave compound **18** in improved yield (85% from **16**).

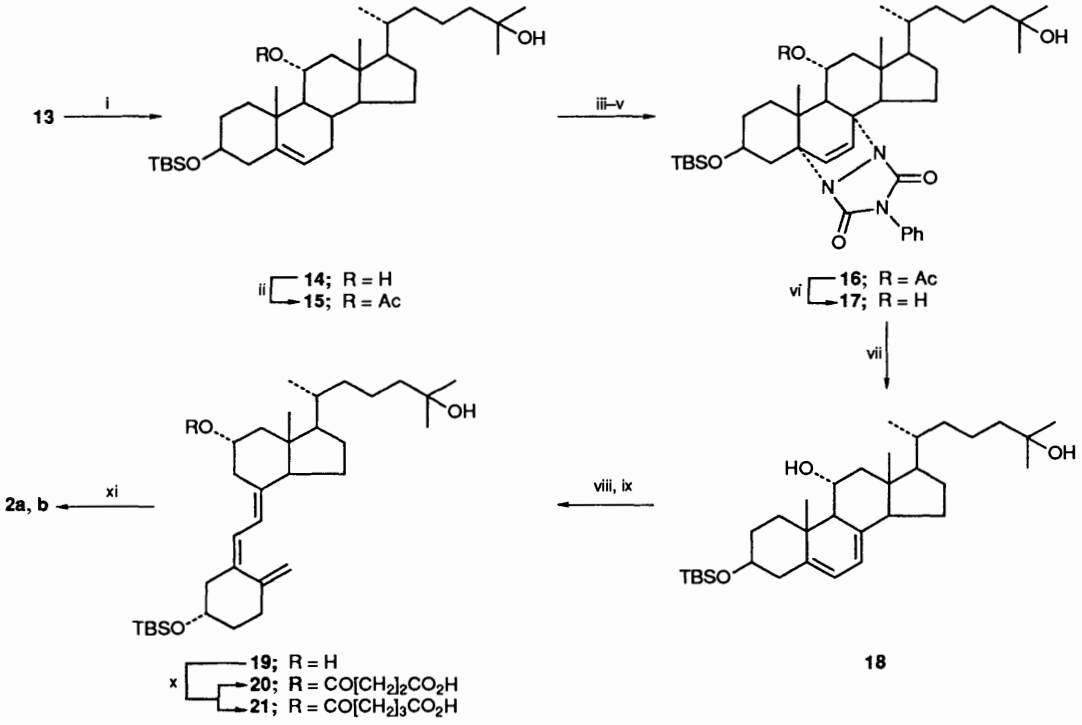
Irradiation of diene **18** with a high-pressure mercury lamp (400 W) through a Vycor filter and subsequent thermal isomerization at room temperature afforded a reaction mixture from which D_3 derivative **19** was separated in 31% yield by preparative TLC (PLC). Treatment of compound **19** with succinic or glutaric anhydride gave the hemisuccinate **20** or the hemiglutarate **21**, both of which were then subjected to desilylation with tetrabutylammonium fluoride (TBAF) to provide the desired haptens **2a**, **b** in 32 and 58% yield respectively, from compound **19**.

The stereochemistry of the introduced side chain was determined by transformation of the silyl ether **14** into 25-hydroxycholesterol (Scheme 3). Hence, compound **14** was converted into the imidazolylthiocarbonyl derivative **22**, which was then treated with tributyltin hydride to give the 11-deoxygenated compound **23**.[12] Desilylation of compound **23** gave the diol **24**, whose m.p. and ^1H NMR data including the chemical shift of the C-21 methyl group [δ 0.93; demonstrating its (20*R*)-configuration] were in good agreement with those of 25-hydroxycholesterol.[13] These results led us to conclude that the haptens **2a**, **b** as well as the compounds **6**–**21** all possess the side chain with the natural (17β,20*R*)-configuration.

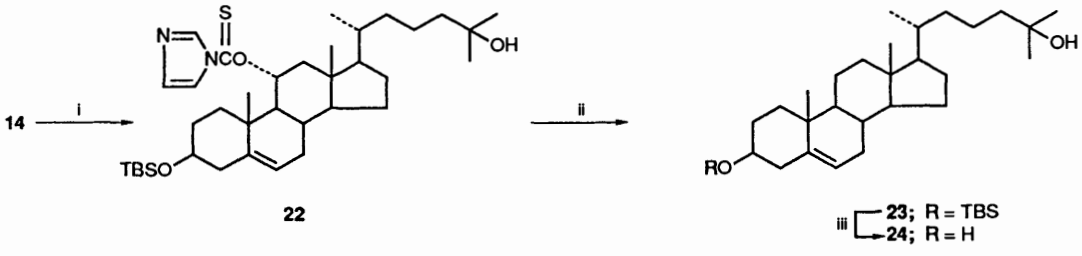
All the novel compounds (**2a**, **b** and **4**–**22**) exhibited satisfactory spectral data. It should be noted that, in the ^1H NMR spectra of the D_3 analogues (**2a**, **b** and **19**–**21**), we assigned the twin singlet-like signals due to the exocyclic methylene protons at C-19, characterizing the vitamin D structure, as follows: the lower-field resonance to 19(*E*)-H and the higher-field one to 19(*Z*)-H, that is, in the reverse order to the conventional assignment for D_3 and D_2 derivatives.[14] This was based on the results of ^1H NMR difference NOE experiments performed on compounds **19** and **2b**: irradiation of the highfield twin signal enhanced the 7-H signal as well as that of the other 19-H signal, while no NOE was observed between the downfield one and 7-H (Fig. 1).

Discussion

We have succeeded in the syntheses of the novel haptens **2a**, **b**, each in 21 steps, and in 1.9 and 3.4% overall yield, respectively, from compound **3**. As far as we are aware, this is the first report of haptenic derivatives of vitamin D metabolites having the chemical bridge at a position other than at C-3 or on the side chain.[4] The hapten **2b** has already been coupled with bovine serum albumin by the active-ester method to give the hapten–carrier conjugate, for which antibodies showing satisfactorily high titer (>1:40 000), high affinity to **1a** (K_a 0.96–2.6 × 10^9 dm^3 mol^{-1}), and suitable specificity in a radioimmunoassay.[15] In the development of enzyme immunoassay (EIA), the use of an enzyme-labelled antigen having a bridge shorter than that used for antibody production (*i.e.*, for linkage of hapten to carrier) has been shown to be advantageous in increasing the assay



Scheme 2 *Reagents and conditions:* i, TBSCl, imidazole, DMF; ii, Ac_2O, pyridine; iii, NBS, AIBN, hexane; iv, 2,4,6-collidine, xylene (mixed isomers); v, PTAD, CH_2Cl_2; vi, KOH, MeOH–THF; vii, 1,1,3,3-tetramethylguanidine; viii, *hv*, Et_2O; ix, room temp., hexane–THF; x, succinic **20** or glutaric **21** anhydride; xi, TBAF, THF:TBS = Bu^tMe_2Si.



Scheme 3 *Reagents:* i, TCDI, 1,2-dichloroethane; ii, Bu^n_3SnH, toluene; iii, TBAF, THF

sensitivity.[16] Therefore, a sensitive 'bridge heterologous' EIA could be established by the combination of the above mentioned antibody and the enzyme-labelled antigen prepared with the hapten **2a**. Details of these results will be reported subsequently.

Experimental

M.p.s were recorded with a Yanagimoto micro melting point apparatus, and are uncorrected. Optical rotations were measured on a JASCO DIP-181 digital polarimeter, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. UV spectra were taken on a Union Giken SM-401 spectrophotometer for solutions in ethanol. The low- and high-resolution MS spectra [electron impact (EI) or fast-atom bombardment (FAB) ionization] were determined with a Hitachi M-80 and a JEOL JMS-DX-303 spectrometer, respectively. ^1H NMR spectra were obtained with a JEOL JNM-FX-100 (100 MHz), JNM-EX-270 (270 MHz) or JNM-GX-400 (400 MHz) spectrometer. $CDCl_3$ was used as the solvent with tetramethylsilane as internal standard unless stated otherwise. *J*-Values are given in Hz. Column and flash column chromatography were carried out with Merck silica gel 60 (70–230 mesh) and Wakogel FC-40 (20–40 μm), respectively. PLC was carried out with Merck silica gel 60 F_{254} (0.5 mm). All air-sensitive reactions were carried out under argon or nitrogen. The phrase 'dried and evaporated' indicates drying with Na_2SO_4 followed by evaporation of the solvents under reduced pressure.

[17(20)Z]-*Pregna*-5,17(20)-*diene*-3β,11α-*diyl Diacetate** **4**.—Ethyltriphenylphosphonium bromide (14.7 g, 39.6 mmol) was added portionwise to a stirred suspension of Bu^tOK (4.87 g, 43.4 mmol) in THF (50 cm^3) at room temperature. The resulting mixture was further stirred at 55 °C (bath temperature) for 1 h. After addition of a solution of 11α-hydroxydehydroepiandrosterone **3** (2.00 g, 6.58 mmol) in THF (25 cm^3), the mixture was refluxed for 1 h and then cooled to room temperature. Pyridine (24 cm^3), Ac_2O (12 cm^3) and 4-(dimethylamino)pyridine (DMAP) (80 mg) were added to the resulting solution, and the mixture was stirred at room temperature for 1 h. The resulting mixture was extracted with Et_2O, and the organic layer was washed (water; 5% aq. HCl; water; 5% aq. $NaHCO_3$; and brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 7:1) to give *compound* **4** (2.52 g, 96%) as a solid, δ(400 MHz) 0.96 (3 H, s, 18-H_3), 1.11 (3 H, s, 19-H_3), 1.63 (3 H, d, *J* 7.0, 21-H_3), 2.03 (6 H, s, 2 × OAc), 2.63 (1H, dd, *J* 11.9 and 5.5, 12β-H), 3.52 (1 H, m, 3α-H) and 4.92–5.48 (3 H, m, 6-, 11β- and 20-H); *m/z* (EI) 340 (M^+ − AcOH, 93.5%), 280 (340 − AcOH, 65.7), 265 (59.3), 160 (73.5) and 145 (100).

* Nomenclature according to ref. 17.

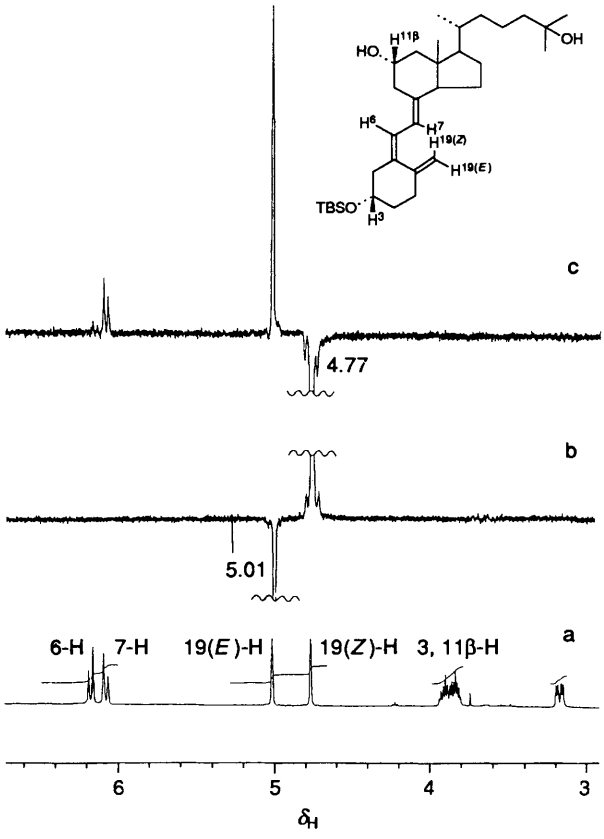


Fig. 1 ^{1}H NMR (normal and difference NOE) spectra of the vitamin D_3 derivative **19**: a, normal spectrum; b and c, difference NOE spectra on irradiation at δ 5.01 and 4.77, respectively

Methyl (22E)-3β,11α-*Diacetoxychola*-5,16,22-*trien*-24-*oate* **5**.—Ethylaluminium dichloride (1 mol dm^{-3} solution in hexane; 28.7 cm^3) was added dropwise to a solution of compound **4** (2.30 g, 5.74 mmol) and methyl propiolate (0.95 cm^3, 11.4 mmol) in CH_2Cl_2 (25 cm^3), and the mixture was stirred at room temperature for 2.5 h. The resulting solution was poured into chilled 5% aq. $NaHCO_3$, and the mixture was extracted with Et_2O. The organic layer was washed (brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 5:1) to give *compound* **5** (2.53 g, 91%) as a solid, δ(100 MHz) 0.84 (3 H, s, 18-H_3), 1.15 (3 H, s, 19-H_3), 2.02 and 2.03 (each 3 H, s, OAc), 3.73 (3 H, s, CO_2Me), 4.59 (1 H, m, 3α-H), 5.16–5.60 (3 H, m, 6-, 11β- and 16-H), 5.77 (1 H, dd, *J* 16 and 1, 23-H) and 6.93 (1 H, dd, *J* 16 and 8, 22-H); *m/z* (EI) 484 (M^+, 0.06%), 424 (M^+ − AcOH, 91.6), 349 (44.8), 251 (27.4) and 145 (100).

Methyl 3β,11α-*Diacetoxychol*-5-*en*-24-*oate* **6**.—A solution of compound **5** (2.53 g, 5.22 mmol) in AcOEt (250 cm^3) was stirred with 5% Pt/C (510 mg) at room temperature under hydrogen for 50 min. After removal of the catalyst by filtration, the solvent was evaporated off. The crude product obtained was purified by flash column chromatography (hexane–AcOEt, 8:1) to give *compound* **6** (2.46 g, 96%) as needles, m.p. 139–142 °C (from MeOH); $[\alpha]_D^{19}$ −57.8 (*c* 1.03, $CHCl_3$) (Found: C, 71.15; H, 9.2. $C_{29}H_{44}O_6$ requires C, 71.28; H, 9.08%); δ(100 MHz) 0.75 (3 H, s, 18-H_3), 0.90 (3 H, d, *J* 5, 21-H_3), 1.01 (3 H, s, 19-H_3), 2.01 and 2.03 (each 3 H, s, OAc), 3.66 (3 H, s, CO_2Me), 4.54 (1 H, m, 3α-H), 5.24 (1 H, m, 11β-H) and 5.42 (1 H, br d, 6-H).

3β,11α-*Dihydroxychol*-5-*en*-24-*oic Acid* **7**.—A solution of triester **6** (2.46 g, 5.03 mmol) in THF (50 cm^3), MeOH (50 cm^3) and 30% KOH (100 cm^3) was refluxed for 2.5 h. After removal of the organic solvent, the remaining aqueous solution was acidified with 10% aq. HCl. The resulting precipitate was collected, and washed with water to give crude *compound* **7** (1.95 g) as a solid, which was used without further purification. Recrystallization from MeOH gave analytically pure acid **7** as needles, m.p. 253.5–255 °C; $[\alpha]_D^{15}$ −33.2 [*c* 0.20, in $CHCl_3$–MeOH (1:1)] (Found: C, 73.6; H, 10.0. $C_{24}H_{38}O_4$ requires C, 73.80; H, 9.81%); δ[100 MHz; $(CD_3)_2SO$] 0.64 (3 H, s, 18-H_3), 0.90 (3 H, d, *J* 5, 21-H_3), 1.05 (3 H, s, 19-H_3) and 5.25 (1 H, br d, 6-H).

Methoxymethyl 3β,11α-*Bis*(*methoxymethoxy*)*chol*-5-*en*-24-*oate* **8**.—Pr^i_2NEt (5.0 cm^3, 29.4 mmol) was added to a solution of crude acid **7** (1.95 g) in *N*,*N*-dimethylformamide (DMF; 15 cm^3) and THF (27 cm^3) at 0 °C, and the mixture was stirred at room temperature for 30 min. Chloromethyl methyl ether (1.9 cm^3, 25.3 mmol) was then added to the solution, and the mixture was stirred at 60 °C (bath temperature) for 4.5 h. The resulting solution was poured into water, and the mixture was neutralized with 5% aq. $NaHCO_3$ and then extracted with AcOEt. The organic layer was washed (brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 3:1) to give *compound* **8** (2.38 g, 90% from triester **6**) as a solid, δ(100 MHz) 0.69 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* 6, 21-H_3), 1.13 (3 H, s, 19-H_3), 3.37 (6 H, s, 2 × OCH_2OMe), 3.46 (3 H, s, CO_2CH_2OMe), 3.88 (1 H, m, 11β-H), 4.68 (4 H, s, 2 × OCH_2OMe), 5.22 (2 H, s, CO_2CH_2OMe) and 5.36 (1 H, br d, 6-H).

3β,11α-*Bis*(*methoxymethoxy*)*chol*-5-*en*-24-*ol* **9**.—$LiAlH_4$ (863 mg, 22.7 mmol) was added to a solution of ester **8** (2.38 g, 4.55 mmol) in THF (50 cm^3) at 0 °C. The resulting suspension was stirred at room temperature for 15 min, and quenched with 1 mol dm^{-3} NaOH. The mixture was extracted with AcOEt, and the organic layer was washed (brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 3:2) to give *compound* **9** (1.92 g, 91%) as needles, m.p. 77.5–79 °C (from hexane–Et_2O); $[\alpha]_D^{19}$ −30.5 (*c* 0.84, $CHCl_3$) (Found: C, 72.2; H, 10.9. $C_{28}H_{48}O_5$ requires C, 72.37; H, 10.41%); δ(100 MHz) 0.69 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* 6, 21-H_3), 1.13 (3 H, s, 19-H_3), 3.37 (6 H, s, 2 × OMe), 3.90 (1 H, m, 11β-H), 4.68 (4 H, s, 2 × OCH_2O) and 5.38 (1 H, br d, 6-H).

3β,11α-*Bis*(*methoxymethoxy*)*chol*-5-*en*-24-*al* **10**.—A solution of the alcohol **9** (1.91 g, 4.11 mmol) in CH_2Cl_2 (20 cm^3) was added to a suspension of PCC (1.33 g, 6.17 mmol) in CH_2Cl_2 (60 cm^3), and the mixture was stirred at room temperature for 5 h. After dilution with Et_2O, the resulting mixture was placed on a short column of silica gel 60 (~8 g) and eluted with Et_2O. The crude product thus obtained was purified by flash column chromatography (hexane–AcOEt, 3:1) to give *compound* **10** (1.71 g, 90%) as needles, m.p. 89–91 °C (from hexane); $[\alpha]_D^{19}$ −27.3 (*c* 0.10, $CHCl_3$) (Found: C, 72.5; H, 10.3. $C_{28}H_{46}O_5$ requires C, 72.69; H, 10.02%); δ(100 MHz) 0.69 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 6, 21-H_3), 1.13 (3 H, s, 19-H_3), 3.37 (6 H, s, 2 × OMe), 3.88 (1 H, m, 11β-H), 4.68 (4 H, s, 2 × OCH_2O), 5.41 (1 H, br d, 6-H) and 9.77 (1 H, t, *J* 2, CHO).

3β,11α-*Bis*(*methoxymethoxy*)*cholesta*-5,24-*diene* **11**.—PhLi [2 mol dm^{-3} solution in cyclohexane–Et_2O (7:3); 8.50 cm^3] was added to a suspension of isopropyltriphenylphosphonium iodide (7.17 g, 16.6 mmol) in THF (100 cm^3), and the mixture was stirred at room temperature for 30 min. A solution of aldehyde **10** (1.71 g, 3.70 mmol) in THF (30 cm^3) was added to the suspension, and the resulting mixture was stirred at room temperature for 30 min, and was then quenched by addition of

water. The mixture was extracted with Et_2O and the organic layer was washed (brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 8:1) to give *compound* **11** (1.75 g, 97%) as needles, m.p. 76–77 °C (from hexane); $[\alpha]_D^{20}$ −29.3 (*c* 0.10, $CHCl_3$) (Found: C, 75.9; H, 11.0. $C_{31}H_{52}O_4$ requires C, 76.18; H, 10.72%); δ(100 MHz) 0.69 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* 6, 21-H_3) 1.13 (3 H, s, 19-H_3), 1.60 and 1.68 (each 3 H, s, 26- and 27-H_3), 3.37 (6 H, s, 2 × OMe), 3.88 (1 H, m, 11β-H), 4.07 and 4.68 (each 2 H, s, together OCH_2O), 5.08 (1 H, m, 24-H) and 5.36 (1 H, br d, 6-H).

Cholesta-5,24-diene-3β,11α-diol **12**.—A solution of diene **11** (1.75 g, 3.58 mmol) in THF (200 cm^3) and 6 mol dm^{-3} HCl (40 cm^3) was stirred at room temperature for 31 h. After neutralization with $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed (brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 2:3) to give *compound* **12** (1.29 g, 90%) as needles, m.p. 167–169 °C (from AcOEt); $[\alpha]_D^{20}$ −41.7 (*c* 0.10, $CHCl_3$) (Found: C, 80.4; H, 11.4. $C_{27}H_{44}O_2 \cdot 1/6\ H_2O$ requires C, 80.34; H, 11.07%); δ(100 MHz) 0.70 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 5, 21-H_3), 1.17 (3 H, s, 19-H_3), 1.60 and 1.68 (each 3 H, s, 26- and 27-H_3), 3.54 (1 H, m, 3α-H), 4.02 (1 H, m, 11β-H), 5.04 (1 H, br t, 24-H) and 5.40 (1 H, br d, 6-H).

Cholest-5-ene-3β,11α,25-triol **13**.—An aqueous solution of $Hg(OAc)_2$ (2.35 g, 7.37 mmol in 10 cm^3) was added to a solution of diol **12** (1.18 g, 2.95 mmol) in THF (30 cm^3) and the mixture was stirred at room temperature for 5 h. Then, 3 mol dm^{-3} NaOH (25 cm^3) and $NaBH_4$ (0.5 mol dm^{-3} solution in 3 mol dm^{-3} NaOH; 25 cm^3) were added to the resulting mixture, which was then stirred further at room temperature for 15 min. After addition of NaCl, the THF layer was separated and the aqueous layer was extracted with AcOEt. The AcOEt layer was washed (brine), combined with the THF layer, and the solvents were evaporated off. The crude product was purified by flash column chromatography ($CHCl_3$–MeOH, 20:1) to give *compound* **13** (1.12 g, 91%) as needles, m.p. 194–195 °C (from aq. MeOH); $[\alpha]_D^{20}$ −28.0 [*c* 0.10, $CHCl_3$–MeOH (1:1)] (Found: C, 75.6; H, 11.2. $C_{27}H_{46}O_3 \cdot 1/2H_2O$ requires C, 75.83; H, 11.08%); δ(270 MHz); [$^2H_5$]pyridine + D_2O) 0.72 (3 H, s, 18-H_3), 0.97 (3 H, d, *J* 6.6, 21-H_3), 1.39 (3 H, s, 19-H_3), 1.45 (6 H, s, 26- and 27-H_3), 3.92 (1 H, m, 3α-H), 4.30 (1 H, m, 11β-H) and 5.51 (1 H, br d, 6-H).

3β-(tert-*Butyldimethylsiloxy*)*cholest-5-ene-11α,25-diol* **14**.—A mixture of triol **13** (1.01 g, 2.41 mmol), TBSCl (436 mg, 2.89 mmol) and imidazole (410 mg, 6.02 mmol) in DMF (15 cm^3) was stirred at room temperature for 45 min. The mixture was diluted with AcOEt, washed (water), dried and evaporated. The crude product was purified by column chromatography (hexane–AcOEt, 2:1) to give *compound* **14** (1.17 g, 91%) as plates, m.p. 200–202 °C (from MeOH); $[\alpha]_D^{15}$ − 23.5 (*c* 0.20, $CHCl_3$) (Found: C, 73.8; H, 11.6. $C_{33}H_{60}O_3Si \cdot 1/4H_2O$ requires C, 73.75; H, 11.35%); δ(100 MHz) 0.06 (6 H, s. $SiMe_2$), 0.70 (3 H, s, 18-H_3), 0.89 (9 H, s, SiBu^t), 1.16 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.48 (1 H, m, 3α-H), 4.02 (1 H, m, 11β-H) and 5.33 (1 H, br d, 6-H).

3β-(tert-*Butyldimethylsiloxy*)-25-*hydroxycholest-5-en-11α-yl Acetate* **15**.—A solution of diol **14** (1.07 g, 2.01 mmol) in pyridine (14 cm^3)–Ac_2O (7 cm^3) was stirred at room temperature for 5 h. A small amount of water was added to the resulting solution, which was stirred for a further 30 min. The mixture was then extracted with Et_2O, and the organic layer was washed (water; chilled 5% aq. HCl; water, 5% aq. $NaHCO_3$; and brine), dried and evaporated. The crude product was purified by column chromatography (hexane–AcOEt, 4:1) to give *compound* **15** (1.12 g, 97%) as prisms, m.p. 149–151 °C (from MeOH); $[\alpha]_D^{26}$ −36.9 (*c* 0.32, $CHCl_3$) (Found: C, 72.9; H, 10.9. $C_{35}H_{62}O_4Si$ requires C, 73.11; H, 10.87%); δ(100 MHz) 0.05 (6 H, s, $SiMe_2$), 0.75 (3 H, s, 18-H_3), 0.88 (9 H, s, SiBu^t), 1.08 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 2.01 (3 H, s, OAc), 3.42 (1 H, m, 3 α-H) and 5.04–5.42 (2 H, m, 6- and 11β-H).

11α-*Acetoxy*-3β-(tert-*butyldimethylsiloxy*)-25-*hydroxy*-4′-*phenyl*-5,8-[1,2]*epi*[1,2,4]*triazolo*-5α,8α-*cholest*-6-*ene*-3′,5′-*dione* **16**.—A mixture of the 5-ene **15** (1.00 g, 1.74 mmol), NBS (402 mg, 2.26 mmol) and AIBN (20 mg) in hexane (80 cm^3) was refluxed for 30 min. After the mixture had cooled to room temperature, the resulting precipitate was filtered off. The filtrate was concentrated under reduced pressure, and the residue thus obtained was dissolved in xylene (mixed isomers) (40 cm^3). After addition of 2,4,6-collidine (5.0 cm^3), the mixture was refluxed for 1 h. The resulting solution was diluted with AcOEt, washed (water; chilled 5% aq. HCl; water; 5% aq. $NaHCO_3$; and brine), dried and evaporated. The residue was dissolved in CH_2Cl_2 (20 cm^3), and a solution of PTAD (0.2 mol dm^{-3} in CH_2Cl_2) was added dropwise to the solution until a faint red colour due to PTAD persisted. After additon of the PTAD, the mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the crude product thus obtained was purified by flash column chromatography (hexane–AcOEt, 2:1) to give *compound* **16** (665 mg, 51%) as needles, m.p. 224–226 °C (from MeOH); $[\alpha]_D^{15}$ −63.5 (*c* 0.10, $CHCl_3$) (Found: C, 68.9; H, 9.1; N, 5.7. $C_{43}H_{65}N_3O_6Si$ requires C, 69.04; H, 8.76; N, 5.62%); δ(100 MHz) 0.08 and 0.10 (each 3 H, s, SiMe), 0.88 (9 H, s, SiBu^t), 1.20 (6 H, s, 26- and 27-H_3), 2.04 (3 H, s, OAc), 3.12 (1H, dd, *J* 14 and 6, 9α-H), 4.32 (1 H, m, 3α-H), 4.86 (1 H, m, 11β-H), 6.26 (2 H, ABq, 6- and 7-H) and 7.12–7.48 (5 H, m, Ph).

3β-(tert-*Butyldimethylsiloxy*)-11α,25-*dihydroxy*-4′-*phenyl*-5,8-[1,2]*epi*[1,2,4]*triazolo*-5α,8α-*cholest*-6-*ene*-3′,5′-*dione* **17**.—A solution of compound **16** (468 mg, 0.626 mmol) in a mixture of MeOH (20 cm^3), THF (20 cm^3) and 10% aq. KOH (10 cm^3) was stirred at room temperature for 1.5 h. The resulting mixture was extracted with AcOEt, and the organic layer was washed (water; then brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 3:2) to give *compound* **17** (423 mg, 96%) as a foam, δ(100 MHz) 0.08 and 0.10 (each 3 H, s, SiMe), 0.81 (3 H, s, 18-H_3), 0.89 (9 H, s, SiBu^t), 1.16 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.06 (1 H, dd, *J* 14 and 6, 9α-H), 3.76 (1 H, m, 3α-H), 4.35 (1 H, m, 11β-H), 6.27 (2 H, ABq, 6- and 7-H) and 7.26–7.50 (5 H, m, Ph).

3β-(tert-*Butyldimethylsiloxy*)*cholesta-5,7-diene-11α,25-diol* **18**.—A solution of adduct **17** (373 mg, 0.528 mmol) in 1,1,3,3-tetramethylguanidine (10 cm^3) was refluxed for 2 h. The resulting solution was diluted with AcOEt, washed (water; chilled 5% aq. HCl; water; 5% aq. $NaHCO_3$; and brine), dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 2:1) to give *compound* **18** (249 mg, 89%) as a pale yellow solid [Found: M^+(EI), 530.4159. $C_{33}H_{58}O_3Si$ requires *M*, 530.4152]; δ(100 MHz) 0.06 (6 H, s, $SiMe_2$), 0.62 (3 H, s, 18-H_3), 0.89 (9 H, s, SiBu^t), 0.96 (3 H, d, *J* 6, 21-H_3), 1.10 (3 H, s, 19-H_3), 1.22 (6 H, s, 26- and 27-H_3), 3.54 (1 H, m, 3α-H), 4.16 (1 H, m, 11β-H) and 5.20–5.60 (2 H, m, 6- and 7-H); *m/z* (EI) 530 (M^+, 50.0%), 455 (M^+ − Bu^t − H_2O, 23.2), 380 (75.8), 365 (81.9), 362 (63.7), 251 (M^+ − side chain − Bu^tMe_2SiOH − H_2O, 47.7) and 225 (100).

(5Z,7E)-(3S)-3-(tert-*Butyldimethylsiloxy*)-9,10-*secocholesta-5,7,10(19)-triene-11α,25-diol* **19**.—A solution of diene **18** (87.0 mg, 0.164 mmol) in Et_2O (400 cm^3) was irradiated

intermittently (for 30 s, 60 s and 30 s), with a 400 W high-pressure mercury lamp through a Vycor filter, at 0 °C whilst under argon bubbling. After removal of the solvent under reduced pressure, the residue was dissolved in a mixture of hexane (40 cm^3) and THF (8 cm^3) and stored in the dark at room temperature under argon for 7 days. The solvent was evaporated off and the crude product thus obtained was purified by PLC (hexane–AcOEt, 3:1, developed three times) to give *compound* **19** (27.0 mg, 31%) as a foam [Found: M^+ (EI), 530.4280. $C_{33}H_{58}O_3Si$ requires *M*, 530.4152]; λ_{max} 265 nm; λ_{min} 230 nm; δ(400 MHz) 0.06 and 0.07 (each 3 H, s, SiMe), 0.57 (3 H, s, 18-H_3), 0.89 (9 H, s, SiBu^t), 0.97 (3 H, d, *J* 6.1, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.79–3.95 (2 H, m, 3- and 11β-H), 4.77 [1 H, br s, 19(*Z*)-H], 5.01 [1 H, br s, 19 (*E*)-H], 6.07 (1.H, d, *J* 11.1, 7-H) and 6.17 (1 H, d, *J* 11.1, 6-H); *m/z* (EI) 530 (M^+, 7.79%), 513 (55.1), 495 (35.1), 251 (M^+ − side chain − Bu^tMe_2SiOH − H_2O, 21.4), 193 (100) and 118 (54.6).

(5Z,7E)-(3S)-3-(tert-*Butyldimethylsiloxy*)-25-*hydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl* 3-*Carboxypropionate* **20**.—A mixture of compound **19** (8.5 mg, 16.0 μmol) and succinic anhydride (255 mg, 2.55 mmol) in pyridine (0.2 cm^3) was stirred at room temperature for 4 days. A small amount of water was added to the mixture, which was then stirred for a further 1 h. The mixture was then extracted with AcOEt, and the organic layer was washed (water), dried and evaporated. The crude product was purified by PLC ($CHCl_3$–MeOH, 15:1) to give *compound* **20** (4.7 mg, 47%) as a pale yellow foam, λ_{max} 265 nm; λ_{min} 231 nm; δ(400 MHz) 0.07 and 0.08 (each 3 H, s, SiMe), 0.60 (3 H, s, 18-H_3), 0.88 (9 H, s, SiBu^t), 0.94 (3 H, d, *J* 5.9, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 2.64 (4 H, br s, $CO[CH_2]_2CO$), 3.83 (1 H, m, 3-H), 4.74 [1 H, br s, 19 (*Z*)-H], 4.94–5.07 [2 H, m + br s, 11β- and 19 (*E*)-H] and 6.10 (2 H, ABq, 6- and 7-H).

(5Z,7E)-(3S)-(tert-*Butyldimethylsiloxy*)-25-*hydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl* 4-*Carboxybutyrate* **21**.—A mixture of compound **19** (27.0 mg, 50.8 μmol) and glutaric anhydride (870 mg, 7.62 mmol) in pyridine (0.5 cm^3) was stirred at room temperature for 4 days. The mixture was worked up as described for the homologue **20**, and purified by PLC (hexane–AcOEt, 1:1, developed twice) to give *compound* **21** (26.0 mg, 79%) as a foam, λ_{max} 265 nm; λ_{min} 232 nm; δ(400 MHz) 0.07 and 0.08 (each 3 H, s, SiMe), 0.61 (3 H, s, 18-H_3), 0.88 (9 H, s, SiBu^t), 0.94 (3 H, d, *J* 5.9, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.83 (1 H, m, 3-H), 4.75 [1 H, br s, 19 (*Z*)-H], 4.96–5.05 [2 H, m + br s, 11β- and 19(*E*)-H] and 6.11 (2 H, ABq, 6- and 7-H).

(5Z,7E)-(3S)-3,25-*Dihydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl* 3-*Carboxypropionate* **2a**.—A solution of compound **20** (4.7 mg, 7.46 μmol) and TBAF (0.22 mmol) in THF (0.42 cm^3) was stirred at room temperature for 3 h. The resulting solution was diluted with AcOEt, washed (water; then brine), dried and evaporated. The crude product was purified by PLC (toluene–EtOH, 8:1, developed three times) to give *compound* **2a** (2.6 mg, 68%) as an oil [Found: $(M - H)^-$ (FAB), 515.3380. $C_{31}H_{47}O_6$ requires *M*, 515.3373]; λ_{max} 265 nm; λ_{min} 230 nm; δ(400 MHz) 0.60 (3 H, s, 18-H_3), 0.94 (3 H, d, *J* 5.9, 21-H_3), 1.22 (6 H, s, 26- and 27-H_3), 3.96 (1 H, m, 3-H), 4.79 [1 H, d, *J* 2.4, 19 (*Z*)-H], 5.00 (1 H, m, 11β-H), 5.04 [1 H, br s, 19(*E*)-H], 6.11 (1 H, d, *J* 11.2, 7-H) and 6.19 (1 H, d, *J* 11.2, 6-H).

(5Z,7E)-(3S)-3,25-*Dihydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl* 4-*Carboxybutyrate* **2b**.—A solution of compound **21** (25.0 mg, 38.7 μmol) and TBAF (1.2 mmol) in THF (2.2 cm^3) was stirred at room temperature for 30 min. The mixture was worked up as described for compound **2a**, and was then purified with PLC (AcOEt–MeOH, 20:1, developed twice) to give *compound* **2b** (15.0 mg, 73%) as a foam [Found: $(M - H)^-$ (FAB) 529.3506. $C_{32}H_{49}O_6$ requires *M*, 529.3529]; λ_{max} 265 nm; λ_{min} 230 nm; δ(400 MHz) 0.60 (3 H, s, 18-H_3), 0.94 (3 H, d, *J* 5.4, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.94 (1 H, m, 3-H), 4.78 [1 H, d, *J* 2.0, 19(*Z*)-H], 4.97 (1 H, m, 11β-H), 5.04 [1 H, br s, 19 (*E*)-H], 6.10 (1 H, d, *J* 11.2, 7-H) and 6.20 (1 H, d, *J* 11.2, 6-H).

O-[3β-(tert-*Butyldimethylsiloxy*)-25-*hydroxycholest*-5-*en*-11α-*yl*] *Imidazole*-1-*carbothioate* **22**.—A solution of diol **14** (18.5 mg, 34.7 μmol) and 1,1′-thiocarbonyldiimidazole (TCDI) (25.0 mg, 0.140 mmol) in 1,2-dichloroethane (1 cm^3) was refluxed for 2 h, and was then stirred at room temperature for 12 h. After further addition of TCDI (25.0 mg), the resulting mixture was refluxed again for 1 h. Removal of the solvent gave a crude product, which was purified by flash column chromatography (toluene–AcOEt, 2:1) to give *compound* **22** (17.1 mg, 77%) as a solid, δ(100 MHz) 0.04 (6 H, s, $SiMe_2$), 0.80 (3 H, s, 18-H_3), 0.87 (9 H, s, SiBu^t), 1.16 (3 H, s, 19-H_3), 1.20 (6 H, s, 26- and 27-H_3), 3.41 (1 H, m, 3α-H), 5.37 (1 H, br d, 6-H), 6.00 (1 H, m, 11β-H) and 7.04, 7.64 and 8.31 (each 1 H, m, imidazolyl H).

3β-(tert-*Butyldimethylsiloxy*)*cholest*-5-*en*-25-*ol* **23**.—A solution of ester **22** (17.1 mg, 27.8 μmol) in toluene (1 cm^3) was added dropwise during 20 min to a refluxing solution of Bu_3SnH (0.128 cm^3, 0.460 mmol) in toluene (1 cm^3). The resulting mixture was then refluxed for 1 h. Removal of the solvent gave a crude product, which was purified by flash column chromatography (hexane–AcOEt, 8:1) to give the title *compound* **23** (11.3 mg, 82%) as a solid, δ(100 MHz) 0.06 (6 H, s, $SiMe_2$), 0.68 (3 H, s, 18-H_3), 0.89 (9 H, s, SiBu^t), 1.00 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.44 (1 H, m, 3α-H) and 5.24 (1 H, br d, 6-H).

Cholest-5-*ene*-3β,25-*diol* **24**.—A solution of compound **23** (10.5 mg, 20.3 μmol) and TBAF (0.2 mmol) in THF (0.4 cm^3) was stirred at room temperature for 5 h. The resulting solution was diluted with AcOEt, washed (water; then brine), dried and evaporated. The crude product was purified by PLC (hexane–AcOEt, 2:1) to give the diol **24** (6.1 mg, 75%) as needles, m.p. 181.5–183.5 °C (from MeOH) (lit.,[13a] 179–181 °C); δ(100 MHz) 0.68 (3 H, s, 18-H_3), 0.93 (3 H, d, *J* 6, 21-H_3), 1.01 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.48 (1 H, m, 3α-H) and 5.30 (1 H, br d, 6-H).

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