Syntheses of 11α-(3-Carboxypropanoyloxy)-1α,25-dihydroxyvitamin D_3 and 11α-(4-Carboxybutanoyloxy)-1α,25-dihydroxyvitamin D_3: Novel Haptenic Derivatives for Production of Highly Specific Antibodies to 1α,25-Dihydroxyvitamin D_3 [1]

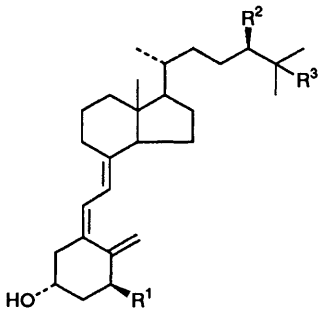
Norihiro Kobayashi, Junichi Kitahori, Hidetoshi Mano and Kazutake Shimada*
Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920, Japan

The measurement of serum/plasma levels of 1α,25-dihydroxyvitamin D_3 **1a** is important for the diagnosis of diseases influencing vitamin D metabolism. To obtain antibodies to the metabolite **1a** which are highly specific and useful for development of immunoassays, two novel haptenic derivatives, 11α-(3-carboxypropanoyloxy)-1α,25-dihydroxyvitamin D_3 **2a** and 11α-(4-carboxybutanoyloxy)-1α,25-dihydroxyvitamin D_3 **2b** were synthesized each in 19 steps from a suitably protected derivative (**3**) of 11α,25-dihydroxycholesterol.

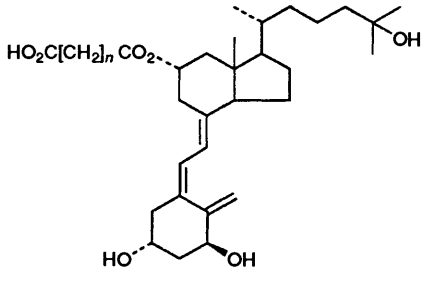
1α,25-Dihydroxyvitamin D_3 **1a** is the hormonally active form of vitamin D_3 **1b**, playing an important role in regulating calcium and phosphorus metabolism and bone resorption. The measurement of this metabolite **1a** in biological fluids is of great value for diagnosis of diseases influencing vitamin D metabolism, which is now performed usually by radioreceptor assays (RRA) using chicken intestine vitamin D receptor (VDR).[2] Although RRA exhibit excellent sensitivity, careful and time-consuming pretreatment of serum/plasma samples is necessary to remove some cross-reactive metabolites and interfering substances.

Immunoassays are therefore expected to be a novel and promising methodology for 1α,25-dihydroxyvitamin D_3 determination because of the possibility that highly specific antibodies may be producible by the immunization using well designed haptenic derivatives and, in addition, because of the excellent stability of antibodies allowing more feasible handling and a longer storage time compared with VDR. In recent years, some antibodies have been raised to the haptens linked to carrier proteins through the C-3 or a position on the side chain.[2] However, almost all these antibodies lacked the specificity for application to biological fluids without complicated pretreatments.

It is anticipated that the use of the hapten-carrier conjugates exposing both the A-ring and the side chain of a vitamin D metabolite would result in the antibodies having much higher specificity, and thus the 11α-position of the metabolite seems attractive as a coupling site for the carrier protein. For this reason, we have synthesized the haptenic derivatives of 25-hydroxyvitamin D_3 **1c** (biosynthetic precursor of 1α,25-dihydroxyvitamin D_3)[3] and (24*R*)-24,25-dihydroxyvitamin D_3 **1d** (one of the major metabolites of vitamin D_3)[4] having C-11 α bridges, from which some antibodies having useful properties have been obtained. We report here the syntheses of novel 1α,25-dihydroxyvitamin D_3 haptens, 11α-(3-carboxypropanoyloxy)-1α,25-dihydroxyvitamin D_3 **2a** and 11α-(4-carboxybutanoyloxy)-1α,25-dihydroxyvitamin D_3 **2b**. The properties of the resulting antibodies raised against compound **2b** are also described briefly.



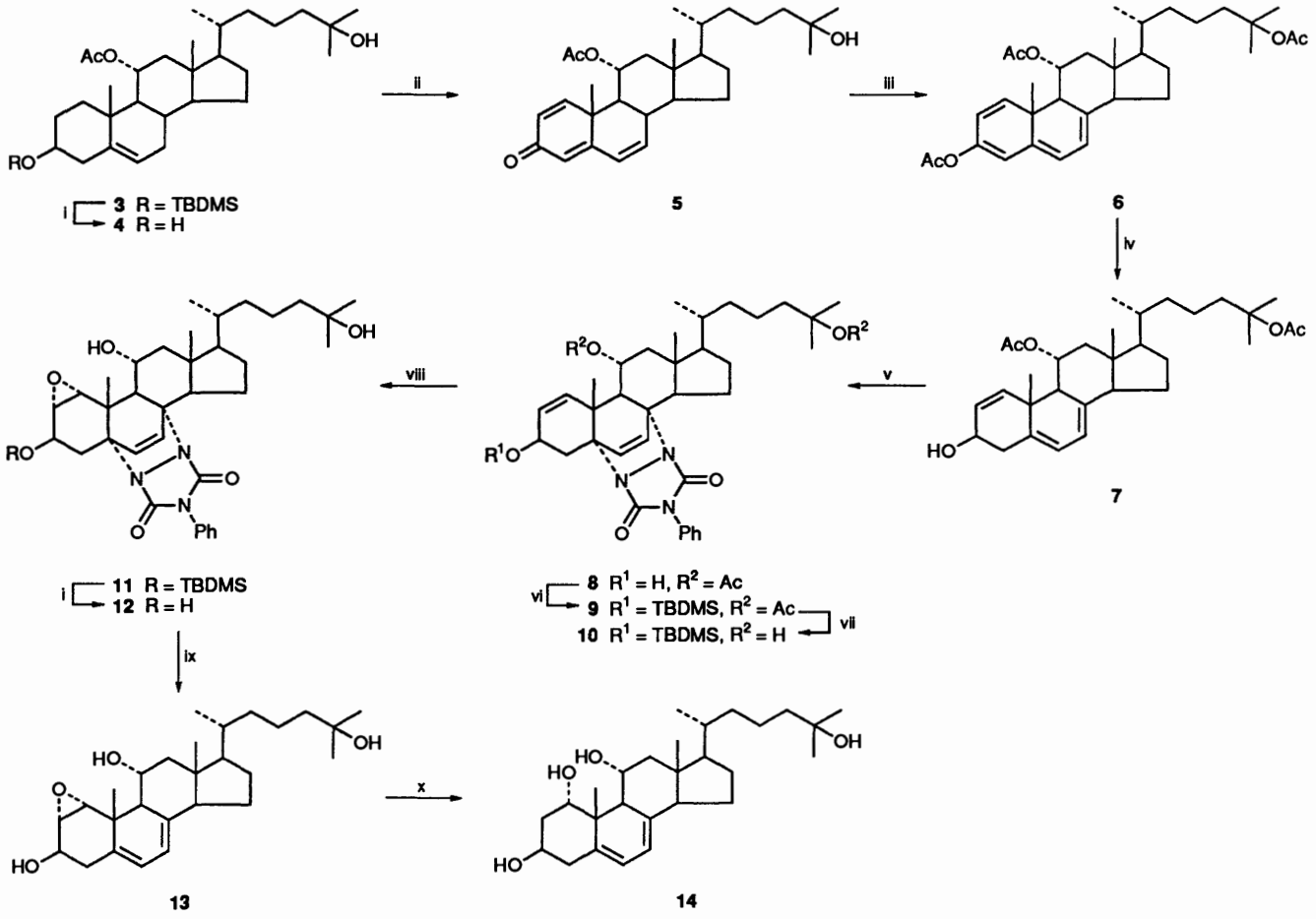
1a; $R^1 = R^3 = OH$, $R^2 = H$
1b; $R^1 = R^2 = R^3 = H$
1c; $R^1 = R^2 = H$, $R^3 = OH$
1d; $R^1 = H$, $R^2 = R^3 = OH$



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

Results and Discussion

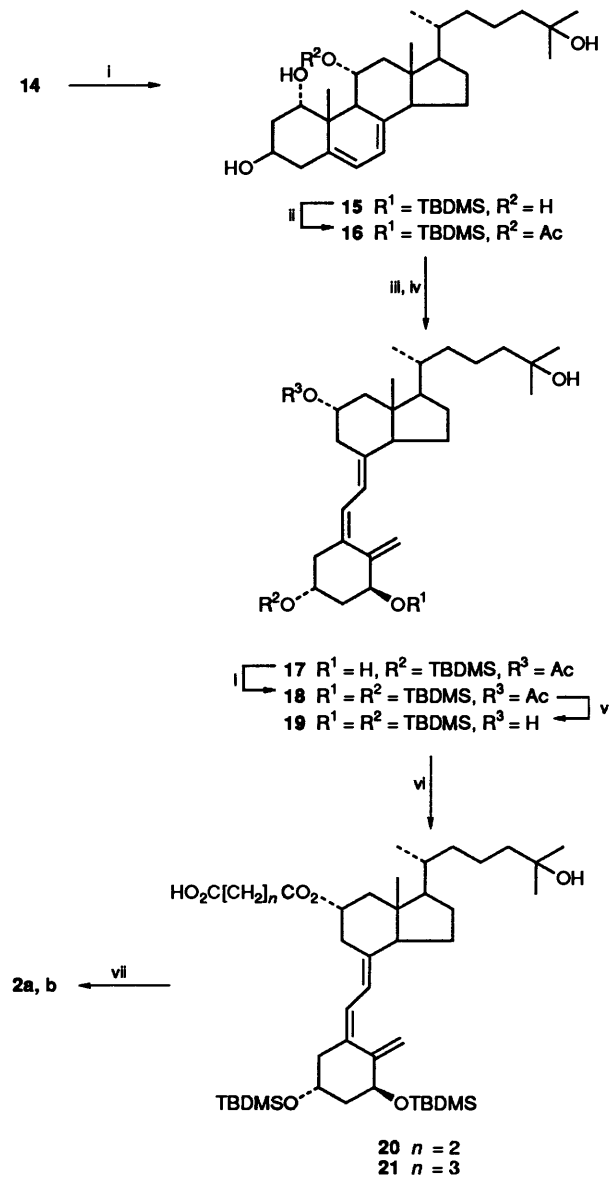
11α-Acetoxy-25-hydroxycholesterol 3-*tert*-butyldimethylsilyl (TBDMS) ether **3**, previously synthesized from 11α-hydroxydehydroepiandrosterone[5] in our laboratory,[3] was chosen as a suitable starting material for the desired haptens. A major problem in the syntheses is the regio- and stereo-selective introduction of a 1α-hydroxy group into the steroid precursor **3** leading to the key intermediate **14**. Treatment of the 1α,2α-epoxide of the 1,4,6-trien-3-one **5** with lithium metal and ammonium chloride in liquid ammonia[6] might be the most efficient procedure for this purpose. However, we chose another synthetic route based on reduction of the enol acetate **6**, because of the ease of handling of the reagents required (Scheme 1). Desilylation of the ether **3** with tetrabutylammonium fluoride (TABF) gave the diol **4** quantitatively, which was subsequently converted into the 1,4,6-trien-3-one **5** by refluxing with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 1,4-dioxane[7] (61% yield). Compound **5** was then subjected to enol acetylation with isopropenyl acetate and toluene-*p*-sulfonic acid (TsOH),[8] and the 1,3,5,7-tetraenyl



Scheme 1 *Reagents:* i, TBAF, THF; ii, DDQ, 1,4-dioxane; iii, isopropenyl acetate, TsOH, AcOBu; iv, $Ca(BH_4)_2$, MeOH–EtOH; v, PTAD, CH_2Cl_2; vi, TBDMSCl, imidazole, DMF; vii, KOH, MeOH–THF; viii, MCPBA, $CHCl_3$; ix, 1,1,3,3-tetramethylguanidine; x, $NaBH_4$, bis(2-methoxyethyl)ether: TBDMS = Bu^tMe_2Si

acetate **6** thus obtained was transformed into the 1,5,7-trien-3β-ol **7** by reduction with calcium boranuide [$Ca(BH_4)_2$] at low temperature[8–10] (40% yield through the two steps). The acetyl groups at the 11α- and 25-position were kept intact under the conditions of this reduction. After the 5,7-diene structure of compound **7** had been protected with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)[9] (88% yield), the obtained Diels–Alder adduct **8** was converted into the 3-silyl ether **9** quantitatively by treatment with *tert*-butyldimethylsilyl chloride (TBDMSCl) to allow us to perform subsequent stereoselective α-epoxidation of the double bond at C-1.[10] Saponification of compound **9** gave the diol **10** quantitatively, oxidation of which with *m*-chloroperbenzoic acid (MCPBA) proceeded smoothly and afforded the 1α,2α-epoxide **11** in 75% yield. Stereochemistry of this epoxy group was supported by a ^1H NMR difference nuclear Overhauser effect (NOE) experiment on the triol **12**, which was obtained by desilylation of compound **11**, indicating the proximity between the 1β-H and the 19-H_3. Although cleavage of the epoxy group can be performed simultaneously with removal of the PTAD group by treatment with lithium aluminium hydride ($LiAlH_4$) in boiling tetrahydrofuran (THF),[9,10] the desired 1α-hydroxy-5,7-dienes are not always obtained in satisfactory yield. Therefore, we converted the adduct **12** into the tetraol **14** by a two-step procedure: removal of the PTAD group from compound **12** by heating it in 1,1,3,3-tetramethylguanidine[11] produced the 5,7-diene **13**, which was then treated with a large excess of sodium boranuide (~ 14 mol equiv.) in bis(2-methoxyethyl)ether[12] to give the desired intermediate **14** in 57% yield from compound **11** (including a desilylation step). The presence of a hydroxy group at the 1α-position was clearly demonstrated by the ^1H NMR spectra of compound **14** obtained in [$^2H_5$]pyridine where the 1β-proton appeared at δ 4.58 as a broad singlet.

In the next reaction sequence, the 3β- and 11α-hydroxy groups of the tetraol **14** were protected differentially to enable the following regioselective introduction of the bridge structures to the 11α-position to be made (Scheme 2). Silylation of compound **14** with a limited amount of TBDMSCl (1.3 mol equiv.) gave the 3-monosilyl ether **15** in 91% yield. The usual acetylation of compound **15** with acetic anhydride at room temperature proceeded selectively at the 11α-hydroxy group to provide the 11-monoacetate **16** in 81% yield. These results are ascribable to the difference in the reactivity of the three secondary hydroxy groups on the steroid nucleus: 3β-(equatorial) > 11α-[equatorial, but is hindered by the 1β-hydrogen and the C(1)–C(10) bond] > 1α-hydroxy group (axial). Irradiation of compound **16** with a high-pressure mercury lamp (400 W, Vycor filter) followed by thermal isomerization gave a mixture from which the vitamin D_3 derivative **17** was separated by preparative TLC (PLC) (26% yield). Silylation of compound **17** with TBDMSCl afforded the 1,3-bis-TBDMS ether **18**, which was then deacetylated with potassium hydroxide to give the suitably protected compound **19** in 82% yield from compound **17**. 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 TBAF to provide the desired haptens **2a**, **b** in 41 and 80% yield, respectively, from compound **19**. All the novel compounds (**2a**, **b** and **4**–**21**) exhibited satisfactory spectral data and/or elemental analyses. In the ^1H NMR spectra of vitamin D_3 derivatives **17**–



Scheme 2 *Reagents and conditions:* i, TBDMSCl, imidazole, DMF; ii, Ac_2O, pyridine; iii, *hv*, Et_2O; iv, room temp., hexane–THF; v, KOH, MeOH; vi, succinic (**20**) or glutaric (**21**) anhydride, pyridine; vii, TBAF, THF

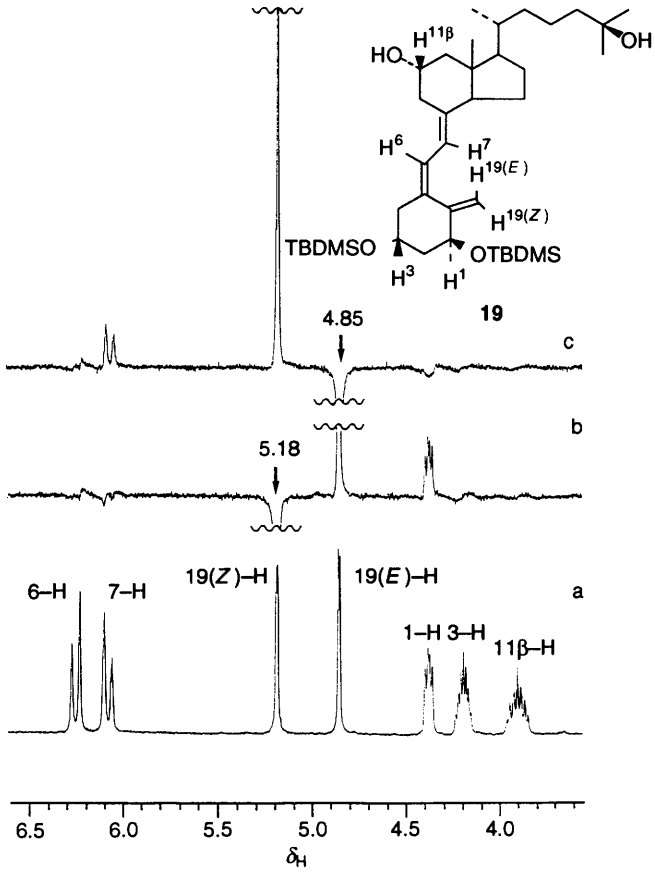
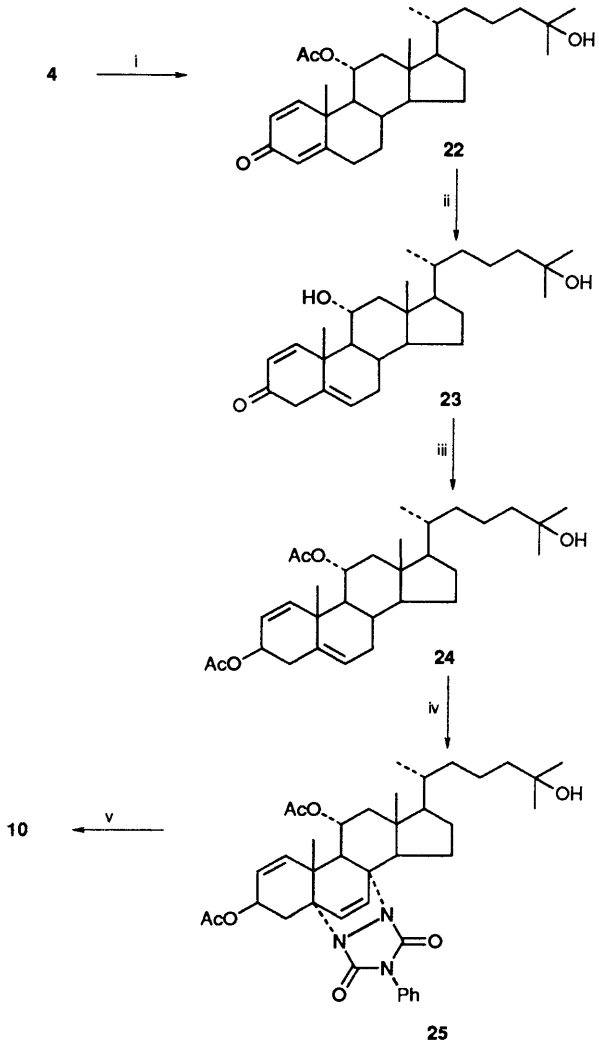


Fig. 1 ^1H NMR (normal and difference NOE) spectra of the intermediate **19**: a, normal spectrum; b and c, difference NOE spectra on irradiation at δ 5.18 and 4.85, respectively

21 and **2a, b**, we assigned the twin singlet-like signals due to the exocyclic methylene protons again in the reverse order to the conventional assignment of vitamin D_3 and D_2 derivatives as in previous reports for the 11α-derivatives of 25-hydroxyvitamin D_3[3] and 24,25-dihydroxyvitamin D_3:[4] the lower-field resonance to 19(*Z*)-H, and the higher-field one to 19(*E*)-H, where each 19-proton which is *cis* and *trans* to the hydroxylated C-1 is 'correctly' defined as 19(*Z*) and 19(*E*), respectively.[13] This assignment was based on the results of ^1H NMR difference NOE experiments performed on compound **19** in which irradiation of the high field twin signal enhanced the 7-H signal together with the other 19-H signal, while on irradiation of the downfield one, NOE was observed on the 1-H but not on the 7-H (Fig. 1). This observation may be a common feature, at least of the vitamin D_3 derivatives having an 11α-functional group.

Thus, we have succeeded in the syntheses of the novel haptens **2a**, **b**, each in 19 steps and in 0.56 and 1.1% overall yield, respectively, from compound **3**. It should be noted here that another synthetic route, involving a deconjugation[14] of the 1,4-dien-3-one **22**, converting it into the 1,5-dien-3-one **23** (Scheme 3), was unsuccessful, mainly due to a low yield in this key reaction (29%). Compound **23** was then converted into the intermediate **10** *via* the acetate **24** and the adduct **25**, as shown in Scheme 3. However, the yield of the common intermediate **10** from the diol **4** was about fourfold lower than that by the above mentioned synthetic route (Scheme 1). Hapten–carrier conjugates linked through the 11α-position seem promising for the generation of highly specific anti-1α,25-dihydroxyvitamin D_3 antibodies. However, no attempt to produce antibodies against such immunogens has been made so far, though some derivatives of the metabolite **1a** functionalized at the C-11 have recently been synthesized.[15–17] The hapten **2b** has already been coupled with bovine serum albumin by the *N*-succinimidyl ester method to give the hapten–carrier conjugate, with which rabbit antibodies showing satisfactorily high titre (optimum final dilution in a radioimmunoassay system, 1:1300–1:220 000) and high affinity for the metabolite **1a** (K_a 0.34–3.3 × 10^{10} dm^3 mol^{-1}) have been produced. Cross-reactivities with various related secosterols demonstrated that the antibodies easily recognize both the A-ring and the sidechain structures of the metabolite **1a** and are highly specific compared with the conventional antibodies.[1,18] In the development of enzyme immunoassay (EIA), the use of an enzyme-labelled antigen linked through a bridge shorter than that used for antibody production (*i.e.*, for conjugation of a hapten with a carrier) has been shown to be advantageous in increasing the assay sensitivity.[19] We expect that a sensitive and practical 'bridge heterologous' EIA of the metabolite **1a** could be developed by the combination of the above mentioned antibodies and the enzyme-labelled antigen prepared using the hapten **2a**.



Scheme 3 *Reagents* (*yield*): i, $(Pr^iO)_3Al$, cyclohexanone, toluene; then DDQ, benzene (60%); ii, NaOEt, Me_2SO; then aq. AcOH (29%; after recycling the reaction several times using recovered deacetylated derivative of compound **22**); iii, $NaBH_4$, MeOH; then Ac_2O, pyridine (87%); iv, *N*-bromosuccinimide, 2,2'-azoisobutyronitrile, CCl_4; tetrabutylammonium bromide, THF; TBAF, THF; then PTAD, CH_2Cl_2 (66%); v, KOH, MeOH; then TBDMSCl, imidazole, DMF (56%)

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 for solutions in $CHCl_3$, 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-EX-270 (270 MHz) spectrometer. $CDCl_3$ was used as the solvent, unless otherwise specified, with Me_4Si as internal standard. *J*-Values are given in Hz. High-performance liquid chromatography (HPLC) was carried out on a JASCO TRI ROTAR chromatograph equipped with a JASCO UVIDEC-100-II UV detector (265 nm). A Develosil ODS-5 column (5 μm; 15 × 0.4 cm i.d.) was used at a flow rate of 1 cm^3 min^{-1} under ambient temperature. Column and flash column chromatography were carried out with Merck silica gel 60 (60–200 mesh) and Wakogel FC-40 (20–40 μm), respectively. PLC was performed with Merck silica gel 60 F_{254} (0.5 mm). 3β-(*tert*-Butyldimethylsiloxy)-25-hydroxycholest-5-en-11α-yl acetate (the starting substance **3**) was synthesized from 11α-hydroxydehydroepiandrosterone[5] as described in the previous reports.[3] 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.

3β,25-*Dihydroxycholest*-5-*en*-11α-*yl Acetate** **4**.—A solution of 5-ene **3** (4.19 g, 7.29 mmol) and TBAF (50 mmol) in THF (150 cm^3) was stirred at room temperature for 6.5 h. The resulting solution was diluted with AcOEt, washed (brine) and then dried and evaporated. The crude product was purified by flash column chromatography (CH_2Cl_2–MeOH, 50:1) to give compound **4** (3.29 g, 98%) as a foam, δ 0.75 (3 H, s, 18-H_3), 0.91 (3 H, d, *J* 6.6, 21-H_3), 1.09 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 2.02 (3 H, s, OAc), 3.51 (1 H, m, 3α-H), 5.28 (1 H, m, 11β-H) and 5.42 (1 H, br d, 6-H); *m*/*z* (EI) 460 (M^+, 10%), 400 (M^+ − AcOH, 100), 382 (400 − H_2O, 85) and 271 (400 − side chain, 52).

25-*Hydroxy*-3-*oxocholesta*-1,4,6-*trien*-11α-*yl Acetate* **5**.—A solution of diol **4** (1.92 g, 4.17 mmol) and DDQ (3.12 g, 13.7 mmol) in 1,4-dioxane (40 cm^3) was refluxed for 18 h. After the mixture had cooled to room temperature, the resulting precipitate was filtered off. The filtrate was submitted to a short column of silica gel 60 and eluted with hexane–AcOEt (1:2). The crude product thus obtained was purified by flash column chromatography ($CHCl_3$–MeOH, 50:1) to give compound **5** (1.15 g, 61%) as a yellow brown foam, δ 0.84 (3 H, s, 18-H_3), 0.92 (3 H, d, *J* 6.3, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 1.27 (3 H, s, 19-H_3), 2.04 (3 H, s, OAc), 5.28 (1 H, m, 11β-H), 5.99–6.26 (4 H, m, 2-, 4-, 6- and 7-H) and 6.73 (1 H, d, *J* 10.2, 1-H); *m*/*z* (FAB; positive ions) 455 [$(M + H)^+$, 86%], 437 (455 − H_2O, 3.9), 377 (437 − AcOH, 14) and 171 (100); *m*/*z* (FAB; negative ions) 453 [$(M - H)^-$, 1.6%] and 153 (100).

Cholesta-1,3,5,7-*tetraene*-3,11α,25-*triyl Triacetate* **6**.—Isopropenyl acetate (36 cm^3, 331 mmol) and TsOH (1.65 g, 8.67 mmol) were added to a solution of enone **5** (3.71 g, 8.16 mmol) in AcOBu (41 cm^3), and the mixture was refluxed for 7 h. The resulting solution was diluted with AcOEt, washed (5% aq. $NaHCO_3$ and then brine) and then was dried and evaporated. Flash column chromatography (hexane–AcOEt, 4:1) of the crude product gave compound **6** (1.68 g, 38%) and 25-acetate of the enone **5** (2.15 g, 53%). This reaction was repeated twice more using the recovered acetate, and compound **6** (total 2.81 g, 64%) was finally obtained as a yellow powder, m.p. 95.5–96.5 °C (from MeOH); $[\alpha]_D^{15}$ −296.8 (*c* 0.10); δ 0.71 (3 H, s, 18-H_3), 0.88 (3 H, s, 19-H_3), 0.95 (3 H, d, *J* 6.3, 21-H_3), 1.42 (6 H, s, 26- and 27-H_3), 1.97, 2.10 and 2.19 (each 3 H, s, OAc), 5.45 (1 H, m, 11β-H) and 5.70–6.21 (5 H, m, 1-, 2-, 4-, 6- and 7-H); *m*/*z* (EI) 538 (M^+, 0.46%), 478 (M^+ − AcOH, 7.8), 418 (478 − AcOH, 2.4) and 249 (100).

3β-*Hydroxycholesta*-1,5,7-*triene*-11α,25-*diyl Diacetate* **7**.—A suspension of $NaBH_4$ (1.13 g, 29.9 mmol) in EtOH (22 cm^3) was added to a solution of $CaCl_2$ (2.26 g, 20.4 mmol) in MeOH (19 cm^3) at −10 °C, and the mixture was stirred at −10 °C for 1.5 h. A solution of tetraene **6** (580 mg, 1.08 mmol) in Et_2O (12 cm^3) was added dropwise to the resulting solution, which was stirred at 0 °C for 4 h and then at 4 °C overnight. After addition of 50% aq. AcOH, the mixture was extracted with Et_2O. The organic layer was washed (saturated aq. $NaHCO_3$

* Nomenclature according to ref. 20.

and then brine) and then was dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 2:1) to give compound **7** (333 mg, 62%) as a pale yellow foam, δ 0.69 (3 H, s, 18-H_3), 0.94 (3 H, d, J 6.3, 21-H_3), 1.07 (3 H, s, 19-H_3), 1.42 (6 H, s, 26- and 27-H_3), 1.97 and 2.05 (each 3 H, s, OAc), 4.30 (1 H, m, 3α-H) and 5.38–5.74 (5 H, m, 1-, 2-, 6-, 7- and 11β-H); *m/z* (EI) 498 (M^+, 0.30%), 438 (M^+ – AcOH, 43), 420 (438 – H_2O, 53), 378 (438 – AcOH, 37), 360 (378 – H_2O, 60) and 209 (100).

3β-*Hydroxy*-3′,5′-*dioxo*-4′-*phenyl*-3′,5′-*dihydro*-5,8-[1,2]*epi*-[1,2,4]*triazolo*-5α,8α-*cholesta*-1,6-*diene*-11α,25-*diyl Diacetate* **8**.—A solution of PTAD (5% in CH_2Cl_2) was added dropwise to a solution of triene **7** (297 mg, 595 μmol) in CH_2Cl_2 (6.0 cm^3) until a faint red colour due to PTAD persisted. The resulting mixture was stirred at room temperature for 1 h and then was concentrated under reduced pressure. The crude product thus obtained was purified by column chromatography (hexane–AcOEt, 1:2) to give *compound* **8** (351 mg, 88%) as needles, m.p. 198–199 °C (from MeOH); $[\alpha]_D^{16}$ –24.0 (*c* 0.10) (Found: C, 69.7; H, 7.7; N, 6.3. $C_{39}H_{51}N_3O_7$ requires C, 69.51; H, 7.63; N, 6.24%); δ 0.89 (3 H, s, 18-H_3), 0.94 (3 H, d, J 5.9, 21-H_3), 1.05 (3 H, s, 19-H_3), 1.41 (6 H, s, 26- and 27-H_3), 1.96 and 2.07 (each 3 H, s, OAc), 3.40 (1 H, dd, J 14.8 and 7.6, 9α-H), 4.91 and 5.04 (each 1 H, m, 3α- and 11β-H), 5.75 (1 H, dd, J 10.0 and 3.5, 2-H), 5.89 (1 H, d, J 10.0, 1-H), 6.32 and 6.44 (each 1 H, d, J 8.3, 6- and 7-H) and 7.30–7.42 (5 H, m, Ph).

3β-(tert-*Butyldimethylsiloxy*)-3′,5′-*dioxo*-4′-*phenyl*-3′,5′-*dihydro*-5,8-[1,2]*epi*[1,2,4]*triazolo*-5α,8α-*cholesta*-1,6-*diene*-11α,25-*diyl Diacetate* **9**.—A solution of adduct **8** (1.17 g, 1.74 mmol), TBDMSCl (378 mg, 2.51 mmol) and imidazole (305 mg, 4.48 mmol) in *N*,*N*-dimethylformamide (DMF; 11 cm^3) was stirred at room temperature for 1 h. The mixture was diluted with AcOEt, washed (brine) and then was dried and evaporated. The crude product was purified by column chromatography (hexane–AcOEt, 2:1) to give compound **9** (1.37 g, 100%) as a foam, δ 0.09 and 0.11 (each 3 H, s, SiMe), 0.89 (3 H, s, 18-H_3), 0.90 (9 H, s, SiBu^t), 0.93 (3 H, d, J 6.3, 21-H_3), 1.05 (3 H, s, 19-H_3), 1.42 (6 H, s, 26- and 27-H_3), 1.96 and 2.07 (each 3 H, s, OAc), 3.32 (1 H, dd, J 14.8 and 7.6, 9α-H), 4.82–5.02 (2 H, m, 3α- and 11β-H), 5.65 (1 H, dd, J 10.2 and 3.6, 2-H), 5.81 (1 H, d, J 10.2, 1-H), 6.30 and 6.43 (each 1 H, d, J 8.3, 6- and 7-H) and 7.32–7.42 (5 H, m, Ph); *m/z* (FAB; positive ions) 788 [$(M + H)^+$, 0.98%] and 73 (100); *m/z* (FAB; negative ions) 786 [$(M - H)^-$, 2.4%], 175 and 153 (100).

3β-(tert-*Butyldimethylsiloxy*)-11α,25-*dihydroxy*-4′-*phenyl*-3′,5′-*dihydro*-5,8-[1,2]*epi*[1,2,4]*triazolo*-5α,8α-*cholesta*-1,6-*diene*-3′,5′-*dione* **10**.—A solution of KOH (30% in MeOH; 20 cm^3) was added to a solution of diacetate **9** (1.36 g, 1.73 mmol) in MeOH (11 cm^3)–THF (9 cm^3), which was stirred at room temperature for 2 h. After neutralization with 50% aq. AcOH, the mixture was extracted with AcOEt. The organic layer was washed (5% aq. $NaHCO_3$ and then brine) and then was dried and evaporated. The crude product was purified by column chromatography (hexane–AcOEt, 1:2) to give *compound* **10** (1.19 g, 98%) as needles, m.p. 166.5–168 °C (from hexane–CH_2Cl_2); $[\alpha]_D^{15}$ –17.1 (*c* 0.10) (Found: C, 69.7; H, 8.8; N, 5.9. $C_{41}H_{61}N_3O_5Si$ requires C, 69.95; H, 8.73; N, 5.97%); δ 0.09 and 0.11 (each 3 H, s, SiMe), 0.82 (3 H, s, 18-H_3), 0.90 (9 H, s, SiBu^t), 0.98 (3 H, d, J 5.9, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 3.31 (1 H, dd, J 14.7 and 7.8, 9α-H), 3.78 (1 H, m, 11β-H), 4.97 (1 H, m, 3α-H), 5.62 (1 H, dd, J 10.0 and 3.5, 2-H), 5.90 (1 H, d, J 10.0, 1-H), 6.28 and 6.38 (2 H, ABq, J 8.4, 6- and 7-H) and 7.29–7.41 (5 H, m, Ph).

3β-(tert-*Butyldimethylsiloxy*)-1α,2α-*epoxy*-11α,25-*dihydroxy*-4′-*phenyl*-3′,5′-*dihydro*-5,8-[1,2]*epi*[1,2,4]*triazolo*-5α,8α-*cholest*-6-*ene*-3′,5′-*dione* **11**.—A solution of 1-ene **10** (702 mg, 997 μmol) and MCPBA (1.27 g, 7.36 mmol) in $CHCl_3$ (21 cm^3) was stirred at room temperature for 5 h. The resulting solution was diluted with $CHCl_3$, washed (10% aq. Na_2CO_3 and then brine) and then was dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 1:1) to give compound **11** (536 mg, 75%) as a powder, m.p. 148.5–149.5 °C (from hexane–CH_2Cl_2); $[\alpha]_D^{14}$ –56.1 (*c* 0.10); δ 0.12 (6 H, s, $SiMe_2$), 0.85 (3 H, s, 18-H_3), 0.92 (9 H, s, SiBu^t), 0.97 (3 H, d, J 5.6, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 1.29 (3 H, s, 19-H_3), 3.11 and 3.52 (each 1 H, d, J 3.6, 1β- and 2β-H), 3.20 (1 H, dd, J 15.0 and 8.4, 9α-H), 3.77 (1 H, m, 11β-H), 4.90 (1 H, br t, 3α-H), 6.22 and 6.34 (each 1 H, d, J 8.3, 6- and 7-H) and 7.30–7.43 (5 H, m, Ph); *m/z* (FAB; positive ions) 720 [$(M + H)^+$, 20%] and 73 (100); *m/z* (FAB; negative ions) 718 [$(M - H)^-$, 2.6%] and 175 (100).

1α,2α-*Epoxy*-3β,11α,25-*trihydroxy*-4′-*phenyl*-3′,5′-*dihydro*-5,8-[1,2]*epi*[1,2,4]*triazolo*-5α,8α-*cholest*-6-*ene*-3′,5′-*dione* **12**.—A solution of the ether **11** (535 mg, 743 μmol) and TBAF (5.3 mmol) in THF (17 cm^3) was stirred at room temperature for 80 min. The mixture was diluted with $CHCl_3$, washed (water and then brine) and then was dried and evaporated. The residue thus obtained was submitted to flash column chromatography ($CHCl_3$–MeOH, 10:1) to give compound **12** (477 mg) containing a trace amount of impurity (derived from TBAF) as a solid, which was used without further purification, δ 0.82 (3 H, s, 18-H_3), 0.96 (3 H, d, J 5.3, 21-H_3), 1.21 (6 H, s, 26- and 27-H_3), 1.26 (3 H, s, 19-H_3), 3.18 (2 H, m, 2β- and 9α-H), 3.54 (1 H, d, J 3.6, 1β-H), 3.75 (1 H, m, 11β-H), 4.99 (1 H, br t, 3α-H), 6.21 and 6.33 (each 1 H, d, J 8.3, 6- and 7-H) and 7.29–7.45 (5 H, m, Ph); *m/z* (FAB; positive ions) 606 [$(M + H)^+$, 33%] and 85 (100); *m/z* (FAB; negative ions) 604 [$(M - H)^-$, 2.8%], 153 and 33 (100).

1α,2α-*Epoxycholesta*-5,7-*diene*-3β,11α,25-*triol* **13**.—A solution of adduct **12** (crude, 390 mg) in 1,1,3,3-tetramethylguanidine (15 cm^3) was stirred at 170 °C (bath temperature) for 50 min. The mixture was diluted with AcOEt, washed (water; chilled 5% aq. HCl; water; 5% aq. $NaHCO_3$ and then brine) and then was dried and evaporated. The crude product was purified by flash column chromatography ($CHCl_3$–MeOH, 10:1) to give compound **13** (212 mg, 81% from compound **11**) as a solid, δ 0.64 (3 H, s, 18-H_3), 0.99 (3 H, d, J 6.3, 21-H_3), 1.19 (3 H, s, 19-H_3), 1.22 (6 H, s, 26- and 27-H_3), 3.34 and 3.54 (each 1 H, d, J 3.6, 1β- and 2β-H), 3.90 (1 H, dd, J 10.7 and 6.1, 3α-H), 4.27 (1 H, m, 11β-H), 5.40 (1 H, m, 7-H) and 5.71 (1 H, br d, 6-H); *m/z* (EI) 430 (M^+, 45%), 412 (M^+ – H_2O, 64), 394 (412 – H_2O, 22) and 257 (100).

Cholesta-5,7-*diene*-1α,3β,11α,25-*tetraol* **14**.—A suspension of $NaBH_4$ (80.3 mg, 2.12 mmol) in bis(2-methoxyethyl) ether (0.60 cm^3) was added dropwise to a solution of epoxide **13** (66.4 mg, 154 μmol) in bis(2-methoxyethyl) ether (1.0 cm^3) at 0 °C and then the mixture was stirred at 80 °C (bath temperature) for 1 h. After addition of a small amount of acetone, the mixture was diluted with $CHCl_3$, washed (brine) and then was dried and evaporated. The crude product was purified by flash column chromatography ($CHCl_3$ – MeOH, 10:1) to give *compound* **14** (46.6 mg, 70%) as a solid [Found: $(M + Na)^+$ (FAB), 455.3147. $C_{27}H_{44}NaO_4$ requires *m/z*, 455.3137]; λ_{max}/nm 272, 282 and 294; δ([$^2H_5$]pyridine + D_2O) 0.67 (3 H, s, 18-H_3), 0.98 (3 H, d, J 5.9, 21-H_3), 1.24 (3 H, s, 19-H_3), 1.45 and 1.46 (each 3 H, s, 26- and 27-H_3), 4.50 (1 H, m, 11β-H), 4.58 (1 H, br s, 1β-H), 4.81 (1 H, m, 3α-H) and 5.85 (1 H, m, 6-H); δ([$^2H_6$]acetone + D_2O) 0.64 (3 H, s, 18-H_3), 0.98 (3 H, s, 19-H_3), 1.01 (3 H, d, J 6.3, 21-H_3), 1.16 (6 H, s, 26- and 27-H_3), 3.91–4.06 (2 H, m, 1β- and 3α-H), 4.18 (1 H, m, 11β-H), 5.40 (1 H, m, 7-H) and 5.62 (1 H, m, 6-H); *m/z* (EI) 432

(M^+, 0.19%), 414 ($M^+ - H_2O$, 6.6), 396 (414 − H_2O, 100) and 267 (396 − side chain, 15); *m/z* (FAB; positive ions) 455 [(M + Na)$^+$, 8.2%] and 69 (100); *m/z* (FAB; negative ions) 580 [(M + triethanolamine − H)$^-$, 5.2%], 297 and 148 (100).

3β-(tert-*Butyldimethylsiloxy*)*cholesta*-5,7-*diene*-1α,11α,25-*triol* **15**.—A solution of tetraol **14** (46.5 mg, 107 μmol), TBDMSCl (21.0 mg, 139 μmol) and imidazole (19.2 mg, 282 μmol) in DMF (1.0 cm^3) was stirred at room temperature for 1.5 h. The mixture was diluted with AcOEt, washed (brine) and then was dried and evaporated. The crude product was purified by flash column chromatography ($CHCl_3$–MeOH, 20:1) to give *compound* **15** (53.3 mg, 91%) as needles, m.p. 155–155.5 °C (from hexane–CH_2Cl_2); $[\alpha]_D^{13}$ −53.0 (*c* 0.10) (Found: C, 71.7; H, 10.7. $C_{33}H_{58}O_4Si \cdot \frac{1}{4}H_2O$ requires C, 71.88; H, 10.70%); δ 0.09 (6 H, s, $SiMe_2$), 0.61 (3 H, s, 18-H_3), 0.90 (9 H, s, $SiBu^t$), 0.97 (3 H, s, 19-H_3), 1.22 (6 H, s, 26- and 27-H_3), 3.97 (1 H, br t, 1β-H), 4.07 (1 H, m, 3α-H), 4.19 (1 H, m, 11β-H), 5.43 (1 H, m, 7-H) and 5.69 (1 H, m, 6-H).

3β-(tert-*Butyldimethylsiloxy*)-1α,25-*dihydroxycholesta*-5,7-*dien*-11α-*yl Acetate* **16**.—A solution of triol **15** (98.8 mg, 181 μmol) in pyridine (1.6 cm^3)–Ac_2O (0.80 cm^3) was stirred at room temperature for 3.5 h. A small amount of water was added to the resulting solution, which was stirred for a further 10 min. The mixture was then extracted with AcOEt, and the organic layer was washed (water; chilled 5% aq. HCl; brine; 5% aq. $NaHCO_3$ and then brine) and then was dried and evaporated. The crude product was purified by flash column chromatography (hexane–AcOEt, 2:1) to give compound **16** (86.7 mg, 81%) as a solid, λ_{max}/nm 272, 282 and 294; δ 0.08 (6 H, s, $SiMe_2$), 0.68 (3 H, s, 18-H_3), 0.90 (9 H, s, $SiBu^t$), 0.94 (3 H, d, *J* 6.3, 21-H_3), 0.99 (3 H, s, 19-H_3), 1.21 (6 H, s, 26- and 27-H_3), 2.06 (3 H, s, OAc), 3.56 (1 H, br s, 1β-H), 4.04 (1 H, m, 3α-H), 5.38 (1 H, m, 11β-H), 5.45 (1 H, m, 7-H) and 5.70 (1 H, m, 6-H); *m/z* (EI) 588 (M^+, 0.58%), 510 ($M^+ - H_2O$ − AcOH, 100), 396 (M^+ − AcOH − TBDMSOH, 100) and 378 (396 − H_2O, 71). The purity of compound **16** was also confirmed by HPLC (mobile phase MeCN; t_R 10.6 min).

(5Z,7E)-(1S,3R)-3-(tert-*Butyldimethylsiloxy*)-1,25-*dihydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl Acetate* **17**.—A solution of diene **16** (86.7 mg, 147 μmol) in Et_2O (400 cm^3) was irradiated intermittently (for 10 s, 30 s, 30s, 20s and 20s) with a 400 W high-pressure mercury lamp through a Vycor filter at 0 °C with argon bubbling through the solution. After removal of the solvent under reduced pressure, the residue was dissolved in a mixture of hexane (20 cm^3) and THF (5.0 cm^3) and the solution was stored in the dark at room temperature for 14 days. The solvent was evaporated off and the crude product was purified by PLC (CH_2Cl_2–MeOH, 50:1; developed three times) to give *compound* **17** (22.3 mg, 26%) as a pale yellow oil [Found: (M + Na)$^+$ (FAB), 611.4086. $C_{35}H_{60}NaO_5Si$ requires *m/z*, 611.4108]; λ_{max}/nm 263; λ_{min}/nm 229; δ 0.07 (6 H, s, $SiMe_2$), 0.61 (3 H, s, 18-H_3), 0.87 (9 H, s, $SiBu^t$), 1.21 (6 H, s, 26- and 27-H_3), 2.06 (3 H, s, OAc), 4.20 (1 H, m, 3-H), 4.42 (1 H, br t, 1-H), 4.93 [1 H, br s, 19(*E*)-H], 4.98 (1 H, m, 11β-H), 5.27 [1 H, br s, 19(*Z*)-H], 6.08 (1 H, d, *J* 11.2, 7-H) and 6.27 (1 H, d, *J* 11.2, 6-H); *m/z* (EI) 588 (M^+, 0.44%), 510 ($M^+ - H_2O$ − AcOH, 44) and 134 (100); *m/z* (FAB) 611 [(M + Na)$^+$, 12%] and 23 (100). The purity of compound **17** was also confirmed by HPLC [mobile phase MeCN–water (19:1); t_R 7.8 min].

(5Z,7E)-(1S,3R)-1,3-*Bis*(tert-*butyldimethylsiloxy*)-25-*hydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl Acetate* **18**.—A solution of triene **17** (12.9 mg, 21.9 μmol), TBDMSCl (16.5 mg, 109 μmol) and imidazole (17.1 mg, 251 μmol) in DMF (0.30 cm^3) was stirred at room temperature for 1 h. The mixture was diluted with Et_2O, washed (water) and then was dried and evaporated. The crude product was purified by PLC (hexane–AcOEt, 2:1) to give compound **18** (13.7 mg, 89%) as a foam, λ_{max}/nm 263; λ_{min}/nm 229; δ 0.05, 0.07 and 0.10 (3 H, 6 H and 3 H, each s, 4 × SiMe), 0.59 (3 H, s, 18-H_3), 0.87 and 0.88 (each 9 H, s, $SiBu^t$), 0.94 (3 H, d, *J* 5.9, 21-H_3), 1.22 (6 H, s, 26- and 27-H_3), 2.06 (3 H, s, OAc), 4.19 (1 H, m, 3-H), 4.37 (1 H, m, 1-H), 4.83 [1 H, d, *J* 2.3, 19(*E*)-H], 4.99 (1 H, m, 11β-H), 5.17 [1 H, br s, 19(*Z*)-H], 6.09 and 6.21 (2 H, ABq, *J* 11.2, 7- and 6-H); *m/z* (EI) 702 (M^+, 0.35%), 513 (M^+ − AcOH − side chain, 8.8) and 75 (100).

(5Z,7E)-(1S,3R)-1,3-*Bis*(tert-*butyldimethylsiloxy*)-9,10-*secocholesta*-5,7,10(19)-*triene*-11α,25-*diol* **19**.—A solution of acetate **18** (17.7 mg, 25.2 μmol) and KOH (60 mg, 1.07 mmol) in MeOH (1.2 cm^3) was stirred at 0 °C for 50 min. After neutralization with 50% aq. AcOH, the mixture was extracted with AcOEt. The organic layer was washed (brine) and then was dried and evaporated. The crude product was purified by PLC (hexane–AcOEt, 1:1) to give *compound* **19** (15.3 mg, 92%) as an oil [Found: (M + Na)$^+$ (FAB), 683.4910. $C_{39}H_{72}NaO_4Si_2$ requires *m/z*, 683.4866]; λ_{max}/nm 265; λ_{min}/nm 229; δ 0.05 and 0.06 (each 6 H, s, $SiMe_2$), 0.55 (3 H, s, 18-H_3), 0.87 and 0.88 (each 9 H, s, $SiBu^t$), 0.97 (3 H, d, *J* 5.9, 21-H_3), 1.22 (6 H, s, 26- and 27-H_3), 3.90 (1 H, m, 11β-H), 4.19 (1 H, m, 3-H), 4.37 (1 H, m, 1-H), 4.85 [1 H, d, *J* 2.3, 19(*E*)-H], 5.18 [1 H, br s, 19(*Z*)-H], 6.07 (1 H, d, *J* 11.2, 7-H) and 6.24 (1 H, d, *J* 11.2, 6-H); *m/z* (EI) 660 (M^+, 3.4%), 642 ($M^+ - H_2O$, 7.7), 510 (642 − TBDMSOH, 15) and 249 (510 − TBDMSOH − side chain, 100); *m/z* (FAB) 683 [(M + Na)$^+$, 4.2%] and 73 (100).

(5Z,7E)-(1S,3R)-3-{[1,3-*Bis*(tert-*butyldimethylsiloxy*)-25-*hydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl*]*oxycarbonyl*}*propanoic Acid* **20**.—A mixture of diol **19** (10.2 mg, 15.4 μmol) and succinic anhydride (158 mg, 1.58 mmol) in pyridine (0.40 cm^3) was stirred at room temperature for 3 days. A small amount of water was added to the mixture, which was then stirred for a further 30 min. The mixture was then extracted with AcOEt, and the organic layer was washed (water) and then was dried and evaporated. The crude product was purified by PLC ($CHCl_3$–MeOH, 15:1) to give compound **20** (6.8 mg, 58%) as an oil, λ_{max}/nm 257; λ_{min}/nm 226; δ 0.04, 0.06 and 0.07 (3 H, 3 H and 6 H, each s, 4 × SiMe), 0.59 (3 H, s, 18-H_3), 0.87 and 0.88 (each 9 H, s, $SiBu^t$), 1.22 (6 H, s, 26- and 27-H_3), 4.20 (1 H, m, 3-H), 4.36 (1 H, m, 1-H), 4.83 [1 H, d, *J* 2.3, 19(*E*)-H], 5.01 (1 H, m, 11β-H), 5.17 [1 H, d, *J* 2.0. 19(*Z*)-H], 6.09 and 6.20 (2 H, ABq, *J* 11.2, 7- and 6-H).

(5Z,7E)-(1S,3R)-4-{[1,3-*Bis*(tert-*butyldimethylsiloxy*)-25-*hydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl*]*oxycarbonyl*}*butanoic Acid* **21**.—A mixture of diol **19** (15.3 mg, 23.1 μmol) and glutaric anhydride (398 mg, 3.49 mmol) in pyridine (0.40 cm^3) was stirred at room temperature for 5 days. The mixture was worked up and purified as described for the homologue **20** to give compound **21** (16.0 mg, 89%) as an oil, λ_{max}/nm 263; λ_{min}/nm 230; δ 0.05, 0.06 and 0.07 (3 H, 3 H and 6 H, each s, 4 × SiMe), 0.59 (3 H, s, 18-H_3), 0.87 and 0.88 (each 9 H, s, $SiBu^t$), 0.94 (3 H, d, *J* 5.9, 21-H_3), 1.22 (6 H, s, 26- and 27-H_3), 4.20 (1 H, m, 3-H), 4.37 (1 H, m, 1-H), 4.83 [1 H, d, *J* 2.3, 19(*E*)-H], 5.00 (1 H, m, 11β-H), 5.17 [1 H, br s, 19(*Z*)-H], 6.09 and 6.21 (2 H, ABq, *J* 11.2, 7- and 6-H).

(5Z,7E)-(1S,3R)-3-{[1,3,25-*Trihydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl*]*oxycarbonyl*}*propanoic Acid* **2a**.—A solution of hemisuccinate **20** (6.8 mg, 8.9 μmol) and TBAF (90.0 μmol) in THF (0.50 cm^3) was stirred at room temperature for 7 h. The mixture was diluted with AcOEt, washed (water and then brine) and then was dried and evaporated. The crude product was purified by PLC ($CHCl_3$–MeOH, 5:1) to give

compound **2a** (3.3 mg, 70%) as an oil [Found: $(M + Na)^+$ (FAB), 555.3297. $C_{31}H_{48}NaO_7$ requires *m/z*, 555.3298]; λ_{max}/nm 262; λ_{min}/nm 226; δ([$^2H_6$]acetone) 0.64 (3 H, s, 18-H_3), 0.97 (3 H, d, *J* 5.6, 21-H_3), 1.15 (6 H, s, 26- and 27-H_3), 4.18 (1 H, m, 3-H), 4.40 (1 H, m, 1-H), 4.87 [1 H, m, 19(*E*)-H], 4.94 (1 H, m, 11β-H), 5.33 [1 H, m, 19(*Z*)-H], 6.18 and 6.28 (2 H, ABq, *J* 11.1, 7- and 6-H); *m/z* (FAB; positive ions) 555 [$(M + Na)^+$, 2.9%] and 154 (100); *m/z* (FAB; negative ions) 531 [$(M - H)^-$, 12%] and 153 (100).

(5Z,7E)-(1S,3R)-4-{[1,3,25-*Trihydroxy*-9,10-*secocholesta*-5,7,10(19)-*trien*-11α-*yl*]*oxycarbonyl*}*butanoic Acid* **2b**.—A solution of hemiglutarate **21** (16.0 mg, 20.6 μmol) and TBAF (1.5 mmol) in THF (2.5 cm^3) was stirred at room temperature for 8 h. The mixture was worked up and purified as described for the homologue **2a** to give *compound* **2b** (10.2 mg, 90%) as an oil [Found: $(M + Na)^+$ (FAB), 569.3450. $C_{32}H_{50}NaO_7$ requires *m/z*, 569.3455]; λ_{max}/nm 264; λ_{min}/nm 229; δ([$^2H_6$]acetone + D_2O) 0.64 (3 H, s, 18-H_3), 0.97 (3 H, d, *J* 5.3, 21-H_3), 1.16 (6 H, s, 26- and 27-H_3), 4.17 (1 H, m, 3-H), 4.39 (1 H, m, 1-H), 4.87 [1 H, m, 19(*E*)-H], 4.95 (1 H, m, 11β-H), 5.33 [1 H, m, 19(*Z*)-H], 6.19 and 6.30 (2 H, ABq, *J* 11.2, 7- and 6-H); *m/z* (FAB; positive ions) 569 [$(M + Na)^+$, 7.1%] and 154 (100); *m/z* (FAB; negative ions) 545 [$(M - H)^-$, 14%], 153 and 46 (100).

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