Stereospecific Syntheses of the Four Epimers of 7,22-Dihydroxycholesterol

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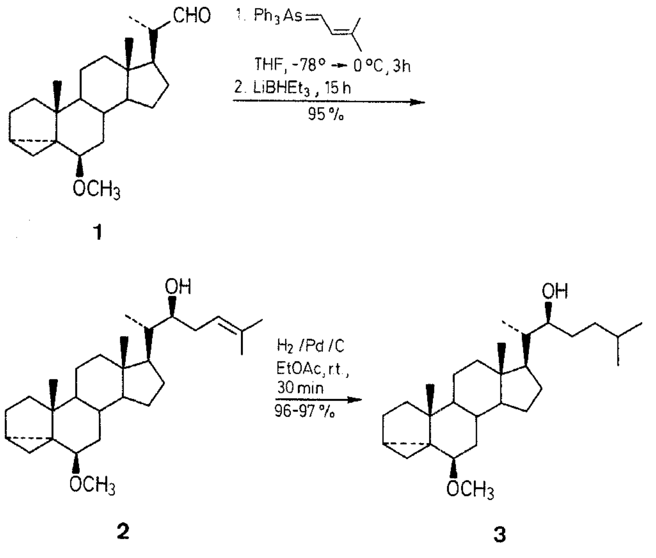
Four stereospecific pathways are presented yielding each of the four epimers of 7,22-dihydroxycholesterol from aldehyde **1**, in over 50% yield.

Sterols and triterpenes bearing a hydroxyl group in either position 7 or position 22 have been shown to be selectively cytotoxic towards tumor cells cultured *in vitro*.[1,3] Preliminary studies have also shown immunosuppressive properties for these compounds.[3,4] We have recently described the syntheses of 7α- and 7β-hydroxycholesterols[5] and the stereospecific preparation of 22-hydroxylated triterpenes and steroids.[6] We now propose four stereospecific pathways yielding each of the four epimers of 7,22-dihydroxycholesterol from aldehyde **1** in overall yields of over 50%. Two of the compounds (22*R*-epimers) have been tested recently for their antitumor activity.[7] The four of them are now being evaluated in our laboratory, both for their antitumor activity and for their immunosuppressive properties.

22*S* Epimers

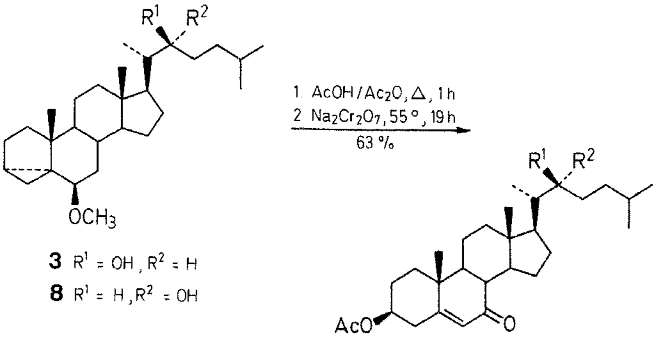
The starting material, aldehyde **1**, is obtained from stigmasterol by the classical procedure.[8] As described in our previous paper,[6] the best method to build a side-chain hydroxylated in position 22*S* is the condensation of the aldehyde with an arsenic ylide, which occurs with a high degree of asymmetric induction, followed by the reduction of the intermediate epoxide: (3-methyl-2-butenyl)triphenylarsonium tetrafluoroborate is reacted with butyllithium in tetrahydrofuran to form the ylide. Condensation with aldehyde **1** yields the α,β-unsaturated epoxide, which is reduced *in situ* by lithium triethylborohydride. Alcohol **2** is obtained in 95% yield and over 96% enantiomeric excess (Scheme **A**). Alcohol **2** is hydrogenated into alcohol **3** over palladium in ethyl acetate. This hydrogenation being almost quantitative, a direct reduction of the α, β-unsaturated epoxide to the saturated alcohol[9] has not been investigated.



Scheme **A**

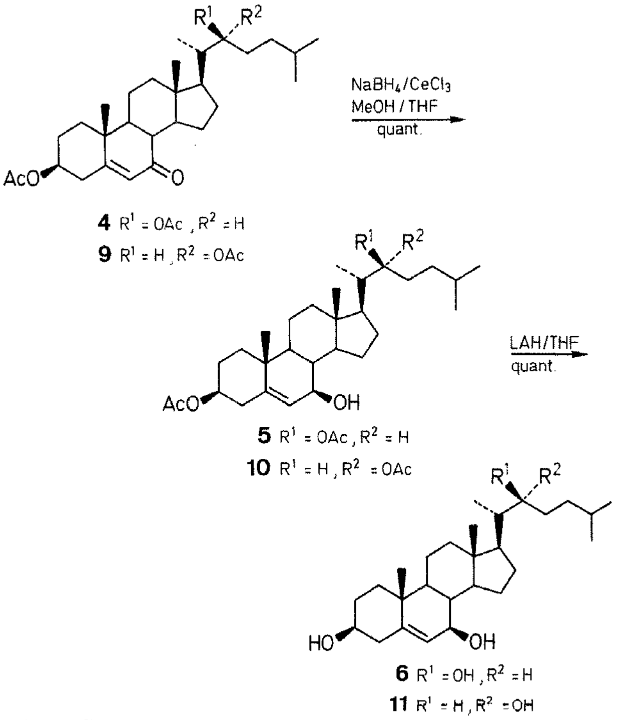
The three subsequent steps, deprotection of the i-ether, acetylation of the 22-hydroxyl and allylic oxidation, are achieved in one pot: alcohol **3** is refluxed in a mixture of acetic acid and acetic anhydride. The temperature is allowed to decrease to 55° and sodium chromate is added to achieve oxidation at C-7. Ketone **4** is isolated in 63% yield. (Scheme **B**).

Among the oxidation procedures tested – chromium trioxide/pyridine in dichloromethane, *tert*-butylhydroperoxide/chromium hexacarbonyl in acetonitrile, sodium chromate or dichromate in acetic acid - the latter two reagents gave the best and the most reproducible yields.



Scheme **B**

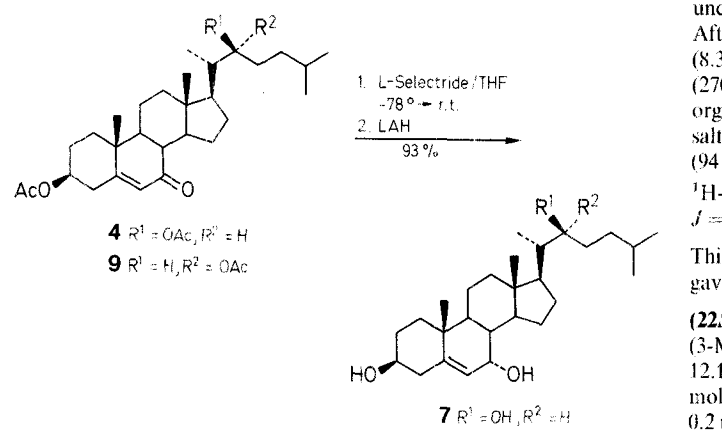
Reduction of 7-ketocholesterol derivatives into the corresponding 7β-hydroxlated compounds is achieved with sodium borohydride in the presence of cerium trichloride and meth-



Scheme **C**

anol:[5] ketone **4** and cerium trichloride heptahydrate are dissolved in tetrahydrofuran/methanol, 2:1. Sodium borohydride is slowly added while stirring. Quenching with aqueous hydrochloric acid and extraction with dichloromethane yields diacetate **5** quantitatively. The acetates are reduced with lithium aluminum hydride in tetrahydrofuran. 7*β*,22*S*-Dihydroxycholesterol **6** is isolated quantitatively.

Reduction of 7-ketocholesterol derivatives into the corresponding 7*α*-hydroxylated compounds is achieved with L-Selectride:[5] ketone **4** is dissolved in tetrahydrofuran and the solution is stirred at −78° under argon. L-Selectride is added, and the mixture is allowed to reach room temperature. Even if an excess of L-Selectride is used, the 22-monocetate remains untouched, and this monoacetylated product can be isolated. At this stage, we prefer to add lithium aluminium hydride directly to the solution and to stir a few minutes at room temperature. The 22-acetate is reduced, and 7*α*,22*S*-dihydroxycholesterol **7** is isolated in 93% yield (Scheme **D**).



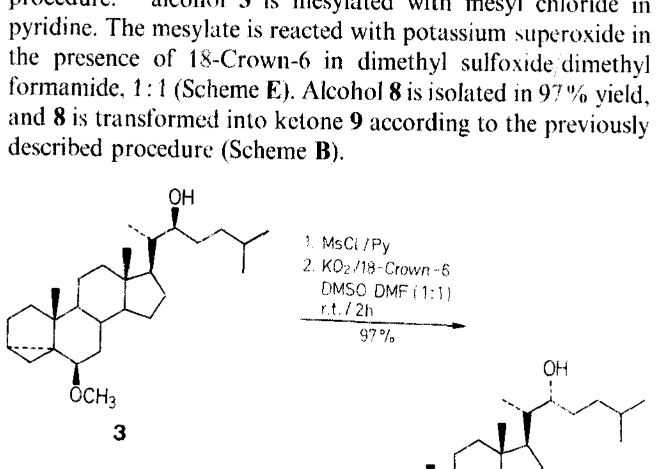
4 R^1 = OAc, R^2 = H
9 R^1 = H, R^2 = OAc

7 R^1 = OH, R^2 = H
12 R^1 = H, R^2 = OH

Scheme **D**

22*R* Epimers

Alcohol **3** is inverted into alcohol **8** according to Corey's procedure:[10] alcohol **3** is mesylated with mesyl chloride in pyridine. The mesylate is reacted with potassium superoxide in the presence of 18-Crown-6 in dimethyl sulfoxide/dimethyl formamide, 1:1 (Scheme **E**). Alcohol **8** is isolated in 97% yield, and **8** is transformed into ketone **9** according to the previously described procedure (Scheme **B**).



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Scheme **E**

Ketone **9** is reduced as described previously to yield diacetate **10** quantitatively. Reduction of the acetates yields 7*β*,22*R*-dihydroxycholesterol **11** quantitatively (Scheme **C**).

Ketone **9** is reduced successively with L-Selectride and with lithium aluminum hydride to yield 7*α*,22*R*-dihydroxycholesterol **12** in 93% yield (Scheme **D**).

The four epimers of 7,22-dihydroxycholesterol are thus obtained stereospecifically from aldehyde **1** with an overall yield of 55 ± 3%.

Melting points were measured on a Reichert hot-stage microscope and are uncorrected.

$[\alpha]_D^{20}$ were measured on a Perkin-Elmer 141 polarimeter. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyses of the CNRS. ^{1}H-NMR spectra were run on a 200 MHz Bruker spectrometer. Mass spectra were recorded on a Thomson-Houston THN 208 or an LKB 9000 spectrometer. TLC were run on precoated plates of silica gel 60F254 (Merck) and silica gel (40–63 μ, Merck) was used for column chromatography.

(3-Methyl-2-butenyl)triphenylarsonium Tetrafluoroborate:
Triphenylarsine (6 g, 19.5 mmol) is dissolved in CH_3CN (80 mL) and 1-bromo-3-methyl-2-butene (4 mL, 90% pure, ~1.5 equiv) is added under argon. The mixture is protected from light with aluminum foil. After 15 h, the solution is evaporated to dryness. The crude bromide (8.3 g) is added to a solution of $NaBF_4$ (200 g, ~100 equiv) in water (270 mL) and extracted with CH_2Cl_2/hexane, 2:1 (3 × 100 mL). The organic phase is dried (Na_2SO_4) and evaporated to dryness. The crude salt is triturated in Et_2O (100 mL). White crystals form; yield: 8.45 g (94%); m.p. 149–151 °C.

^{1}H-NMR ($CDCl_3$): δ = 1.38, 1.69 (2 s, 2 × 3 H, CH_3); 4.65 (d, 2 H, J = 8 Hz, CH_2); 5.3 (t, 1 H, J = 8 Hz, CH); 7.8 (m, 15 H_{arom}).

This salt is more soluble in THF than the corresponding bromide and gave better yields in the coupling reaction with aldehydes.

(22*S*)-22-Hydroxy-6*β*-methoxy-3*α*,5-cyclo-5*α*-cholest-24-ene (2):
(3-Methyl-2-butenyl)triphenylarsonium tetrafluoroborate (5.647 g, 12.19 mmol) is stirred at −78 °C in THF (70 mL). A solution of 1.56 molar BuLi in THF is added dropwise until a slight color persists (ca. 0.2 mL), and then BuLi (7.5 mL, 11.7 mmol) is added at once. After 15 min. stirring, aldehyde **1** (3.45 g, 10 mmol) in THF (20 mL) is added dropwise to the red solution. The solution remains slightly colored. The temperature is allowed to reach +10° in 2 h 40 min. The solution is then cooled to −78° and a 1 M solution of $LiBHEt_3$ in THF (40 mL, 40 mmol) is added. The solution is allowed to reach slowly room temperature, and after 15 h, it is evaporated to dryness. The residue is dissolved in hexane (200 mL), washed with water (3 × 100 mL) and dried ($MgSO_4$). Evaporation of this solution yields almost quantitatively a mixture of alcohol **2** and triphenylarsine (7.75 g). The two compounds are separated by chromatography on silica using hexane/EtOAc, 95:5, as eluent. Alcohol **2** is obtained as a colorless oil; yield: 3.93 g (95%).

^{1}H-NMR ($CDCl_3$): δ = 0.44 (dd, 1 H, J = 5 Hz, 8 Hz, H-3); 0.75 (s, 3 H, CH_3-18); 0.97 (d, 3 H, J = 6.7 Hz, CH_3-21); 1.03 (s, 3 H, CH_3-19); 1.66, 1.75 (2 s, 2 × 3 H, CH_3-26 and CH_3-27); 2.78 (t, 1 H, J = 2.7 Hz, H-6); 3.33 (s, 3 H, CH_3O); 3.65 (m, 1 H, H-22); 5.17 (m, 1 H, H-24).

MS (EI, 70 eV): *m/e* (%) = 414 (M^+, 1); 399 (1), 388 (1), 382 (5), 344 (35), 329 (24), 313 (100).

(22*S*)-22-Hydroxy-6*β*-methoxy-3*α*,5-cyclo-5*α*-cholestane (3):
The unsaturated alcohol **2** (2.74 g, 6.61 mmol) is dissolved in EtOAc (250 mL). Palladium (5%) on carbon is added (350 mg), and the solution is stirred 80 min in an H_2 atmosphere. Filtration of the catalyst and evaporation of the solvent give the saturated alcohol **3**; yield: 2.66 g (97%); m.p. 40–45° (Lit.[11] oil).

$C_{28}H_{48}O_2$	calc.	C 80.71	H 11.61
(416.7)	found	80.72	11.55

^{1}H-NMR ($CDCl_3$): δ = 0.44 (dd, 1 H, J = 5 Hz, 8.2 Hz, H-3); 0.74 (s, 3 H, CH_3-18); 0.90 (d, 9 H, J = 6.5 Hz, CH_3-21 + CH_3-26 + CH_3-27); 1.03 (s, 3 H, CH_3-19); 2.78 (t, 1 H, J = 2.7 Hz, H-6); 3.34 (s, 3 H, CH_3O), 3.63 (dd, 1 H, J = 4.4 Hz, 7.5 Hz, H-22).

MS (EI, 70 eV): *m/e* (%) = 416 (M^+, 100), 401 (41), 384 (90), 361 (63), 358 (28).

(22*S*)-22-Hydroxy-7-oxocholesterol Diacetate (4):
The i-ether **3** (1.07 g, 2.57 mmol) is refluxed one hour in HOAc (7.5 mL); Ac_2O (4.5 mL) is added and reflux is maintained for 45 min.

The temperature is allowed to decrease to 55° and $Na_2Cr_2O_7$ (2 g) is added slowly. The temperature is maintained 16 h. Water (30 mL) is added and the solution is extracted with CH_2Cl_2 (3 × 20 mL). The organic phase is washed with water (3 × 20 mL) and evaporated. Chromatography using hexane/EtOAc (85:15 to 70:30) as eluent affords the pure compound; yield: 810 mg (63%); no m.p. below 230 °C; subl. > 185 °C (EtOH/hexane); $[\alpha]_D^{20}$ −81° ($CHCl_3$, $c = 6.6$).

$C_{31}H_{48}O_5$	calc.	C 74.36	H 9.66
(500.7)	found	74.58	9.87

^{1}H-NMR ($CDCl_3$): δ = 0.69 (s, 3 H, CH_3-18); 0.88 (d, 6 H, J = 6.6 Hz, CH_3-26, CH_3-27); 0.98 (d, 3 H, J = 6.7 Hz, CH_3-21); 1.21 (s, 3 H, CH_3-19); 2.04, 2.06 (2 s, 2 × 3 H, CH_3CO_2-3, CH_3CO_2-22); 4.73 (m, 1 H, H-3); 4.94 (~ t, 1 H, J = 7 Hz, 7.4 Hz, H-22); 5.71 (s, 1 H, H-6).

MS (EI, 70 eV): m/e (%) = 500 (M^+, < 1), 440 (100), 380 (22), 365 (< 1).

(22*S*)-7*β*,22-Dihydroxycholesterol 3,22-Diacetate (5):
Ketone **4** (207 mg, 0.413 mmol) and cerium trichloride heptahydrate (154 mg, 0.413 mmol) are dissolved in THF/CH_3OH, 2:1 (3 mL). Sodium borohydride (~ 30 mg, < 1 mmol) is added slowly with stirring. After 5 min, the reaction is quenched with 5% HCl (1 mL), and the solution is extracted with CH_2Cl_2 (2 × 5 mL). The organic phase is washed with water (5 mL), dried ($MgSO_4$), and evaporated to give alcohol **5**; yield: 209 mg (100%); m.p. 192–194 °C (needles from EtOH/hexane); $[\alpha]_D^{20}$ −24° ($CHCl_3$, $c = 2.4$).

$C_{31}H_{50}O_5$	calc.	C 74.06	H 10.02
(502.7)	found	73.90	9.99

^{1}H-NMR ($CDCl_3$): δ = 0.71 (s, 3 H, CH_3-18); 0.88 (d, 6 H, J = 6.6 Hz, CH_3-26, CH_3-27); 0.98 (d, 3 H, J = 6.7 Hz, CH_3-21); 1.07 (s, 3 H, CH_3-19); 2.05 (2 s, 2 × 3 H, CH_3CO_2-3, CH_3CO_2-22); 3.85 (d, 1 H, J = 7.7 Hz, H-7); 4.63 (m, 1 H, H-3); 4.95 (~ t, 1 H, J = 7 Hz, 7.4 Hz, H-22); 5.32 (s, 1 H, H-6).

MS (EI, 70 eV): m/e (%) = 502 (M^+), 484, 442 (100), 424.

(22*S*)-7*β*,22-Dihydroxycholesterol (6):
Diacetate **5** (199 mg, 0.396 mmol) in THF (10 mL) is reacted with LAH (~ 30 mg, ~ 0.8 mmol) for 15 min at room temperature; $Na_2SO_4 \cdot 10\,H_2O$ is added, allowed to react for 10 min and filtered. Evaporation of the solvent yields the triol **6**; yield: quantitative; m.p. 112–115 °C (acetone/cyclohexane); $[\alpha]_D^{20}$ −9.5° ($CHCl_3$, $c = 1.6$).

$C_{27}H_{46}O_3 \cdot H_2O$	calc.	C 74.26	H 11.08
(436.7)	found	74.55	11.32

^{1}H-NMR ($CDCl_3$): δ = 0.72 (s, 3 H, CH_3-18); 0.90 (d, 6 H, J = 6.5 Hz, CH_3-26, CH_3-27); 0.92 (d, 3 H, J = 6.5 Hz, CH_3-21); 1.06 (s, 3 H, CH_3-19); 3.6 (m, 2 H, H-3 and H-22); 3.85 (d, 1 H, J ~ 7.6 Hz, H-7); 5.30 (s, 1 H, H-6); H_2O cryst.: δ = 1.43.

MS (EI, 70 eV): m/e (%) = 418 (M^+, 55), 400 (100), 382 (13), 300 (25).

(22*S*)-7*α*,22-Dihydroxycholesterol (7):
Ketone **4** (160 mg, 0.320 mmol) in THF (6 mL) is stirred under argon at −78° with a 1 M solution of L-Selectride in THF (1 mL). After 15 min, LAH (30 mg) is added, and the solution is allowed to reach room temperature. After 15 min, acetone (0.5 mL) is added, and stirring is continued until all the LAH has reacted. The solution is evaporated, and the residue is treated with 5% HCl (5 mL) and CH_2Cl_2 (10 mL). The organic phase is washed with water (3 × 5 mL), dried (Na_2SO_4), and evaporated. Chromatography using CH_2Cl_2/EtOH, 95:5, as eluent gives pure triol **7**; yield: 125 mg (93%); m.p. 179–181 °C (acetone/cyclohexane); $[\alpha]_D^{20}$ −54° ($CHCl_3$, $c = 1.6$).

$C_{27}H_{46}O_3 \cdot 1/4\,H_2O$	calc.	C 76.64	H 11.08
(423.2)	found	76.45	11.10

^{1}H-NMR ($CDCl_3$): δ = 0.71 (s, 3 H, CH_3-18); 0.90 (d, 6 H, J = 6.5 Hz, CH_3-26, CH_3-27); 0.925 (d, 3 H, J = 6.5 Hz, CH_3-21); 1.01 (s, 3 H, CH_3-21); 3.6 (m, 2 H, H-3, H-22); 3.86 (br s, 1 H, H-7); 5.61 (d, 1 H, J = 5.2 Hz, H-6); H_2O cryst.: δ = 1.43.

MS (EI, 70 eV): m/e (%) = 418 (M^+, 23), 400 (100), 382 (6), 300 (16).

(22*R*)-22-Hydroxy-6*β*-methoxy-3*α*,5-cyclo-5*α*-cholestane 8:
Alcohol **3** (165 mg, 0.396 mmol) in pyridine (2 mL) is stirred under argon with mesyl chloride (0.5 mL) 0.5 h at 0° and 4.5 h at room temperature. Ice is added and the solution is extracted with CH_2Cl_2 (3 × 5 mL). The organic phase is washed with water until the pH is neutral. The solution is then dried (Na_2SO_4) and evaporated. The crude mesylate (215 mg in DMSO/DMF, 5:7 (12 mL) is reacted with 18-Crown-6 (1.1 g) and freshly powdered potassium superoxide (95 mg, 1.34 mmol) for 2 h under argon. Water (30 mL) and a few drops of 1 N HCl are added. The solution is extracted with Et_2O (3 × 30 mL). After washing with water (2 × 10 mL), drying (Na_2SO_4), and evaporation of the ether phase, the crude alcohol obtained is chromatographed on silica using hexane/EtOAc, 90:10, as eluent to afford pure **8**; yield: 160 mg (97%); m.p. 57–62 °C (EtOH/H_2O) (Lit.[11] oil).

$C_{28}H_{48}O_2$	calc.	C 80.71	H 11.61
(416.7)	found	80.74	11.79

^{1}H-NMR ($CDCl_3$): δ = 0.44 (dd, 1 H, J = 5 Hz, 8.1 Hz, H-3); 0.74 (s, 3 H, CH_3-18); 0.91 (dd, 6 H, J = 2.1 Hz, 6.4 Hz, CH_3-26, CH_3-27); 0.92 (d, 3 H, J = 6.7 Hz, CH_3-21); 1.03 (s, 3 H, CH_3-19); 2.78 (t, 1 H, J = 2.7 Hz, H-6); 3.33 (s, 3 H, CH_3O); 3.62 (bd, 1 H, J = 9.7 Hz, H-22).

MS (EI, 70 eV): m/e (%) = 416 (M^+, 100), 401 (38), 384 (90), 361 (64), 284.

(22*R*)-22-Hydroxy-7-oxocholesterol Diacetate (9):
Preparation from **8** according to the method described for the preparation of **4**; yield: 63%; m.p. 204–205 °C; $[\alpha]_D^{20}$ −84° ($CHCl_3$, $c = 0.42$).

$C_{31}H_{48}O_5$	calc.	C 74.36	H 9.66
(500.7)	found	74.61	9.70

^{1}H-NMR ($CDCl_3$): δ = 0.69 (s, 3 H, CH_3-18); 0.88 (dd, 6 H, J = 10 Hz, 6.6 Hz, CH_3-26, CH_3-27); 0.93 (d, 3 H, J = 6.8 Hz, CH_3-21); 1.22 (s, 3 H, CH_3-19), 2.05, 2.06 (s, 2 × 3 H, CH_3CO_2-3, CH_3CO_2-22); 4.72 (m, 1 H, H-3); 4.88 (m, 1 H, H-22); 5.72 (s, 1 H, H-6).

MS (EI, 70 eV): m/e (%) = 500 (M^+ 3), 440 (100), 380 (37).

(22*R*)-7*β*,22-Dihydroxycholesterol 3,22-Diacetate (10):
Preparation from **9** according to the previously described procedure for the preparation of **5**; yield: quantitative; m.p. 71–75 °C (acetone/water/MeOH); $[\alpha]_D^{20}$ −2° ($CHCl_3$, $c = 4.3$).

$C_{31}H_{50}O_5$	calc.	C 74.06	H 10.02
(502.7)	found	74.11	10.24

^{1}H-NMR ($CDCl_3$): δ = 0.70 (s, 3 H, CH_3-18); 0.89 (dd, 6 H, J = 1.2 Hz, 6.6 Hz, CH_3-26, CH_3-27); 0.94 (d, 3 H, J = 6.8 Hz, CH_3-21); 1.07 (s, 3 H, CH_3-19); 2.05 (2 s, 2 × 3 H, CH_3CO_2-3, CH_3CO_2-22); 3.85 (d, 1 H, J = 7.9 Hz, H-7); 4.63 (m, 1 H, H-3); 4.87 (m, 1 H, H-22). 5.32 (s, 1 H, H-6).

MS (EI, 70 eV): m/e (%) = 502 (M^+, 4), 484 (1), 442 (100), 424 (15), 382 (5).

(22*R*)-7*β*,22-Dihydroxycholesterol (11):
Preparation from **10** according to the procedure described for the preparation of **6**; yield: quantitative; m.p. 182–184 °C (EtOH/cyclohexane); $[\alpha]_D^{20}$ +2° ($CHCl_3$, $c = 1.1$).

^{1}H-NMR ($CDCl_3$): δ = 0.72 (s, 3 H, CH_3-18); 0.91 (dd, 6 H, J = 2.3 Hz, 6.5 Hz, CH_3-26, CH_3-27); 0.94 (d, 3 H, J = 7 Hz, CH_3-21); 1.06 (s, 3 H, CH_3-19); 3.6 (m, 2 H, H-7, H-22); 3.85 (d, 1 H, J = 7.5 Hz, H-7); 5.30 (s, 1 H, H-6).

MS (EI, 70 eV): m/e (%) = 418 (M^+, 16), 400 (100), 382 (4), 300 (9).

(22*R*)-7*α*,22-Dihydroxycholesterol (12):
Preparation from **9** according to the procedure described for the preparation of **7**. The purification is achieved by preparative TLC (elution with EtOAc); yield: 93%; Subl. > 185 °C (THF/H_2O); $[\alpha]_D^{20}$ −60° ($CHCl_3$, $c = 1.6$).

^{1}H-NMR ($CDCl_3$): δ = 0.71 (s, 3 H, CH_3-18); 0.90 (dd, 6 H, J = 1.7 Hz, 6.2 Hz, CH_3-26, CH_3-27); 0.94 (d, 3 H, J = 6.6 Hz, CH_3-21); 1.00 (s, 3 H, CH_3-19); 3.6 (m, 2 H, H-3, H-22); 3.86 (br s, 1 H, H-7); 5.63 (d, 1 H, J = 5.2 Hz, H-6).

MS (EI, 70 eV): m/e (%) = 418 (M^+, 67), 400 (100), 382 (20), 300 (40).

Triols **11** and **12** appear to cristallize with solvents or gases (H_2CO_3) since satisfactory microanalyses could not be obtained.

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