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Stereospecific Synthesis of 24-Propylcholesterol Isolated from the Texas Brown Tide

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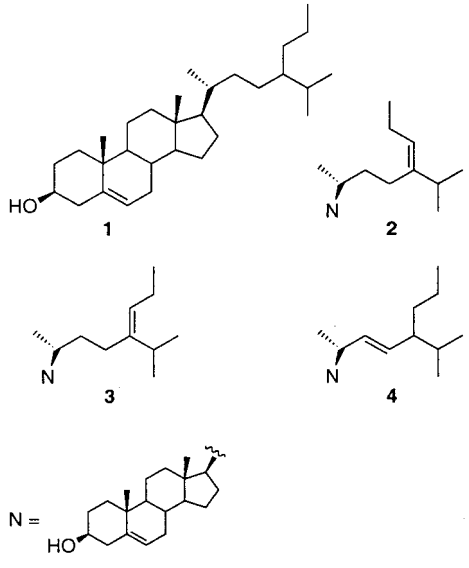
Abstract—The alga that causes the 'Texas brown tide', *Aureoumbra lagunensis*, contains 24-propylcholesterol, a potentially useful biomarker for this organism. The stereochemical configuration at C-24 was determined through synthesis using the Johnson orthoester Claisen rearrangement. Both (24*R*)- and (24*S*)-24-propylcholesterol, as well as (24*R*)- and (24*S*)-Δ^{22} 24-propylcholesterol, were synthesized and characterized. The naturally occurring isomer was found to be (24*R*)-24-propylcholesterol.

Introduction

During our analysis of the sterol composition of the causative organism of the 'Texas brown tide', *Aureoumbra lagunensis*, we isolated a rare marine sterol, 24-propylcholesterol (**1**). Sterols with *n*-propylidene or *n*-propyl subtitution at C-24 appear to be limited to the Pelagophyceae, a small class of marine chromophyte algae.[1] These algae feature (24*E*)-24-propylidenecholesterol (**2**) as their dominant sterol, the biosynthesis of which follows an unusual mechanism.[2] In recent years, members of this group of algae have been implicated in the 'brown tide' of the northeastern United States as well as the 'Texas brown tide' along the Gulf of Mexico.[3,4] We recently isolated the (24*Z*)-isomer of 24-propylidenecholesterol (**3**) from the 'brown tide' alga *Aureococcus anophagefferens*, where it may represent a unique biomarker.[1] 24-Propylcholesterol (**1**) may similarly be useful as a biomarker for the 'Texas brown tide' alga, *Aureoumbra lagunensis*.



24-Propylcholesterol (**1**) was first proposed on the basis of a molecular ion of 428 amu as a constituent of the ocean quahog *Arctica islandica*.[5] It was subsequently isolated as 2% of the total sterols of a marine alga of chrysophyte affinities and characterized by ^{1}H NMR spectroscopy.[6] The alga (*Pulvinaria sp.*) from which it was isolated is member of the Sarcinochrysidales, a group which has been recently assigned to the Pelagophyceae.[3] Another member of this class of algae, *Nematochrysopsis roscoffensis*, was also found to contain a sterol that was tentatively assigned as 24-propylcholesterol (**1**) by GC-MS.[7] Recently 24-propylcholesterol (**1**) has been detected by GC-MS in marine sediments, where it is found together with small amounts of what is believed to be its Δ^{22}-derivative (**4**).[8] Although 24-propylcholesterol (**1**) was isolated from *Aureoumbra lagunensis* and *Pulvinaria sp.* as a single isomer by ^{1}H NMR, a survey of the literature showed that its stereochemical configuration at C-24 was unknown.

In order to determine the stereochemical configuration at C-24 of **1**, we undertook the stereocontrolled synthesis of both isomers of this compound. In addition, both C-24 stereoisomers of Δ^{22}-24-propylcholesterol (**4**) were synthesized.

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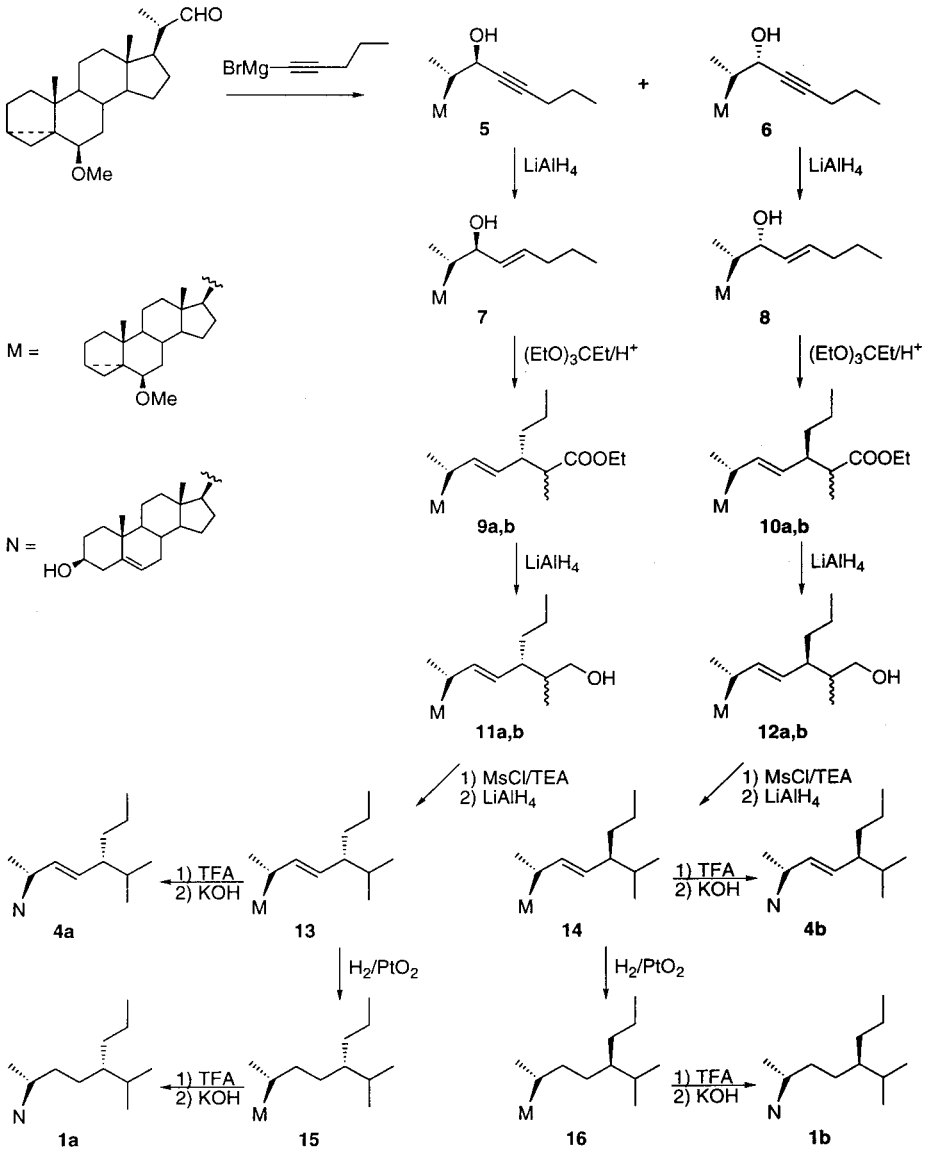


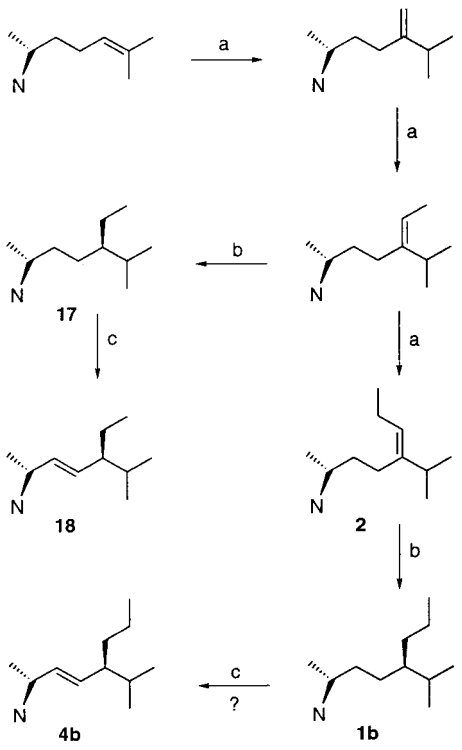
Scheme 1. Synthesis of (24*R*)- and (24*S*)-24-propylcholesterols.

Results and Discussion

The synthesis of each C-24 stereoisomer of 24-propylcholesterol (**1**) was achieved using the Claisen rearrangement of allylic alcohols **7** and **8** (Scheme 1). This approach was first used by Sucrow and co-workers for the stereospecific synthesis of sterols bearing 24-ethyl substitution.[9,10] We employed the Johnson orthoester procedure to effect the rearrangement,[11] using a modification of the procedures of Djerassi and co-workers in their synthesis of the oogoniol side chain.[12]

Our synthesis followed the procedure employed to synthesize 24-ethyl sterols, with the substitution of 1-pentyne for 1-butyne. The stereochemical assignment of the C_{28} allylic alcohols **7** and **8** was made by comparison with the literature data reported for the analogous C_{27} alcohols arising from 1-butyne. Thus, the polarity on silica gel of 22α-alcohols **5** and **7** was less than that of their 22β-epimers **6** and **8**, consistent with the reported relative polarities of the analogous C_{27} alcohols.[12,13] A close match was observed in the NMR data of the allylic alcohols **7** and **8** with the data reported for the analogous allylic alcohols obtained in the 1-butyne sequence.[13] Thus, the data for the protons of the C-21 methyl and the C-22 alcohol methine of **7** corresponded closely to those reported for the C_{27} analog: 0.89 (d, J=6.3 Hz) vs. 0.90 (d, J=6.5 Hz) and 4.21 (d, J=4.7, 1 H) vs. 4.22 (d, J=5 Hz), respectively, as did the data for the C-23 and C-24 olefinic protons: 5.49 (dd, J=15.5, 5.0 Hz) and 5.61 (dt, J=15.5, 6.6 Hz) vs. 5.50 and 5.66 (AB part of $ABMX_2$, J=15.5, 6, and 5 Hz).[13] Likewise, the NMR data for **8** were very similar to those reported for its C_{27} analog: 0.94 (d, J=6.7 Hz, H-21) vs. 0.95 (d, J=7 Hz, H-21), 4.13 (dd, J=7.4, 3.3 Hz, H-22) vs. 4.13 (dd, J=7.5, 3.5 Hz, H-22), 5.46 (ddt, J=15.4, 7.4, 1.2 Hz, 1H, H-23) vs. 5.46 (ddt, J=15.5, 7.5, 1.4 Hz, H-23), 5.65 (dt, J=15.4, 6.6 Hz, 1H, H-24) vs. 5.70 (dt, J=15.5, 6 Hz, H-24).[13]

Claisen rearrangement of **7** and **8** was effected under the conditions of the Johnson orthoester reaction (Scheme 1). Complete stereocontrol was achieved at C-24, but a 1:1 mixture of C-25 epimers was isolated for each of the



Scheme 2. Proposed biosynthesis of 24-propylcholesterol. (a) SAM-sterol methyltransferase; (b) $\Delta^{24(28)}$-sterol reductase; (c) Δ^{22}-sterol desaturase.

products (**9** and **10**). This was of no consequence, since the ester groups were ultimately reduced to methyl groups with concomitant loss of chirality at C-25. However, in order to characterize each isomer of the γ,δ-unsaturated esters **9** and **10**, they were separated by reverse phase HPLC. The alcohols (**11** and **12**) obtained upon reduction of esters **9** and **10** also were characterized separately, but were pooled prior to deoxygenation via LAH reduction of their mesylates. Hydrogenation of the Δ^{22} double bonds of **13** and **14**, followed by deprotection of the nucleus led to each of the two isomers of 24-propylcholesterol (**1a**,**b**). Omission of the hydrogenation step led to the two isomers of Δ^{22}-24-propylcholesterol (**4a**,**b**).

Comparison of the ^{1}H NMR spectrum of the specimen of 24-propylcholesterol (**1**) isolated from *Aureoumbra lagunensis* with the synthetic sterols showed this to be identical with the 24*R*-isomer (**1b**). The NMR data reported for the 24-propylcholesterol (1) isolated from *Pulvinaria sp.* also match the 24*R*-isomer (**1b**).[6] Thus, it appears that in the Pelagophyceae formation of 24-propylcholesterol (**1**) occurs in a stereochemically analogous process to the formation of sitosterol (**17**) in higher plants (Scheme 2). It is noteworthy that both sitosterol (**17**) and its Δ^{22} derivative stigmasterol (**18**) occur in the Pelagophyceae.[6,7] Based on these relationships, we predict that Δ^{22}-propylcholesterol (**4**), if it occurs in pelagophyte algae, will have the 24*S*-configuration (**4b**).

Conclusion

We have stereospecifically synthesized both C-24 stereoisomers for 24-propylcholesterol (**1a**,**b**) and for Δ^{22}-propylcholesterol (**4a**,**b**). Comparison of the ^{1}H NMR spectra shows that (24*R*)-24-propylcholesterol (**1b**) is the stereoisomer found in the 'Texas brown tide' alga *Aureoumbra lagunensis*.

Experimental

General procedures

NMR spectra were acquired using Bruker Avance-300 and Bruker Avance-600 instruments using $CDCl_3$ as the solvent and referenced to residual $CHCl_3$ signals (^{1}H: 7.262 ppm). GC-MS data was obtained using a Hewlett-Packard 5890 series II gas chromatograph with a Hewlett-Packard 5989B mass spectrometer. Optical rotations were measured using a JASCO DIP-1000 polarimeter. TLC was performed on aluminum backed plates coated with a 0.25 mm layer of Si gel 60 F254. HPLC was carried out using a Waters 6000A pump, Waters 410 differential refractometer, and two Altex Ultrasphere ODS 5-mm 10×250 mm columns in series, at a flow rate of 3 ml/min MeOH.

26-Methyl-6β-methoxy-3α,5-cyclo-5α-27-norcholest-23-yn-22-ols (5,6). To a stirred solution of 1-pentyne (0.3 ml, 3.0 mmol) in 5 ml of THF at −15°C, a 1.0 ml of a 2.5 M solution of EtMgBr in Et_2O (2.5 mmol) was added dropwise. The mixture was stirred for 30 min at −15°C, then for an additional hour at rt. A portion of this solution (4 ml) was added dropwise to a stirred solution of (20*S*)-6β-methoxy-3α,5-cyclo-5α-pregnane-20-carboxaldehyde[12] (421 mg, 1.32 mmol) in 3 ml of THF at 0°C. After 30 min, the reaction was quenched by the addition of brine and dil. HCl, and extracted with hexane/ethyl acetate (4:1). Silica gel chromatography (eluent: hexane/ethyl acetate, 39:1, 29:1, 19:1) gave 179 mg (37%) of the (22*R*)-isomer (**5**) and 153 mg (31%) of (22*S*)-isomer (**6**). **5**: TLC R_f=0.78 (hexane/ethyl acetate, 4:1); ^{1}H NMR (300 MHz) 4.46 (m, 1H, H-22), 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 2.19 (td, *J*=7.0, 1.8 Hz, 2H, H-25), 1.01 (d, *J*=6.6 Hz, 3H, H-21), 1.02 (s, 3H, H-19), 0.98 (t, *J*=7.2 Hz, 3H, H-27), 0.73 (s, 3H, H-18). **6**: TLC R_f=0.63 (hexane/ethyl acetate 4:1); ^{1}H NMR (300 MHz) 4.41 (m, 1H, H-22), 3.30 (s, 3H, OMe), 2.75 (m, 1H, H-6), 2.17 (td, *J*=7.0, 1.8 Hz, 2H, H-25), 1.02 (d, *J*=6.6 Hz, 3H, H-21), 1.00 (s, 3H, H-19), 0.98 (t, *J*=7.2 Hz, 3H, H-27), 0.72 (s, 3H, H-18); mass spectrum, *m/z* (relative intensity) 412 (M^+, $C_{28}H_{44}O_2$, 29), 397 (27), 380 (37), 357 (48), 283 (100), 257 (13), 215 (18), 213 (20), 159 (35), 145 (33), 105 (49), 81 (41), 55 (34); HRMS *m/z* 412.3335 (calcd for $C_{28}H_{44}O_2$, 412.3341).

(22*S*,23*E*)-26-Methyl-6β-methoxy-3α,5-cyclo-5α-27-norcholest-23-en-22-ol (7). A solution of **5** (155 mg) in 10 ml of ether/THF (1:1) was treated with 165 mg of $LiAlH_4$ at reflux under N_2. After 17 h the reaction was quenched by addition of brine and dilute HCl, and extracted with hexane and then Et_2O. Silica gel chromotography (eluent: hexane/EtOAc, 39:1) gave 91 mg (60%) of **7**: TLC R_f=0.45 (hexane/ethyl acetate, 4:1); ^{1}H NMR (300 MHz) 5.61 (dt, *J*=15.5, 6.6 Hz, 1H, H-24), 5.49 (dd, *J*=15.5, 5.0 Hz, 1H, H-23), 4.21 (d, *J*=4.7 Hz, 1H, H-22), 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.02 (s, 3H, H-19), 0.90 (t, *J*=7.3 Hz, 3H, H-27), 0.89 (d, *J*=6.3 Hz, 3H, H-21), 0.72 (s, 3H, H-18).

(22*R*,23*E*)-26-Methyl-6β-methoxy-3α,5-cyclo-5α-27-norcholest-23-en-22-ol (8). Treatment of 124 mg of **6** as described above gave 70 mg (56%) **8**: TLC R_f=0.52 (benzene/ethyl acetate, 9:1); ^{1}H NMR (300 MHz) 5.65 (dt, *J*=15.4, 6.6 Hz, 1H, H-24), 5.46 (ddt, *J*=15.4, 7.4, 1.2 Hz, 1H, H-23), 4.13 (dd, *J*=7.4, 3.3 Hz, 1H, H-22), 3.32 (s, 3H, OMe), 2.76 (m, 1H, H-6), 1.02 (s, 3H, H-19), 0.94 (d, *J*=6.7 Hz, 3H, H-21), 0.91 (t, *J*=7.3 Hz, 3H, H-27), 0.74 (s, 3H, H-18); mass spectrum, *m*/*z* (relative intensity) 414 (M^+, $C_{28}H_{46}O_2$, 4), 399 (5), 382 (6), 364 (7), 359 (8), 316 (24), 284 (100), 253 (26), 213 (35), 173 (16), 159 (29), 121 (42), 99 (32), 81 (31), 57 (23), 55 (22); HRMS *m*/*z* 414.3488 (calcd for $C_{28}H_{46}O_2$, 414.3498).

Ethyl (22*E*,24*S*)-24-propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-oate (9). Allylic alcohol **7** (153 mg) was heated with 3.0 ml triethyl orthopropionate and 4 drops of propionic acid in 60 ml of toluene at 140°C for 45 min with slow distillative removal of ethanol. Evaporation under reduced pressure gave 153 mg (83%) of **9**. Purification by HPLC gave isomer 1 (**9a**) and isomer 2 (**9b**) in a 1:1 ratio. **9a**: TLC R_f=0.71 (hexane/ethyl acetate, 9:1), HPLC t_R 47.7 min; ^{1}H NMR (600 MHz) 5.22 (dd, *J*=15.1, 8.8 Hz, 1H, H-22 or 23), 5.09 (dd, *J*=15.2, 9.2 Hz, 1H, H-22 or 23), 4.08 (m, 2H, OCH_2CH$_3$), 3.32 (s, 3H, OMe), 2.77 (t, *J*=2.7 Hz, 1H, H-6), 2.36 (quint, *J*=7.0 Hz, 1H, H-25), 1.25 (t, *J*=7.1 Hz, 3H, OCH$_2$CH_3), 1.10 (d, *J*=7.0 Hz, 3H, H-26 or H-21), 1.02 (s, 3H, H-19), 0.98 (d, *J*=6.6 Hz, 3H, H-26 or H-21), 0.86 (t, *J*=7.1 Hz, 3H, H-30), 0.72 (s, 3H, H-18). **9b**: TLC R_f=0.71 (hexane/ethyl acetate, 9:1), HPLC t_R 49.6 min; ^{1}H NMR (600 MHz) 5.24 (dd, *J*=15.2, 8.7 Hz, 1H, H-22 or 23), 4.96 (dd, *J*=15.2, 9.3 Hz, 1H, H-22 or 23), 4.12 (m, 2H, OCH_2CH$_3$), 3.33 (s, 3H, OMe), 2.77 (t, *J*=2.8 Hz, 1H, H-6), 2.29 (quint, *J*=7.0 Hz, 1H, H-25), 1.26 (t, *J*=7.1 Hz, 3H, OCH$_2$CH_3), 1.05 (d, *J*=7.0 Hz, 3H, H-26 or H-21), 1.03 (s, 3H, H-19), 1.00 (d, *J*=6.6 Hz, 3H, H-26 or H-21), 0.86 (t, *J*=7.1 Hz, 3H, H-30), 0.73 (s, 3H, H-18).

Ethyl (22*E*,24*R*)-24-propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-oate (10). Treatment of 70 mg **8** as described above gave 84 mg (100%) of **10**. Purification by HPLC gave isomer 1 (**10a**) and isomer 2 (**10b**) in a 1:1 ratio. **10a**: TLC R_f=0.43 (hexane/ethyl acetate, 9:1), HPLC t_R 46.6 min; ^{1}H NMR (300 MHz) 5.25 (dd, *J*=15.2, 8.4 Hz, 1H, H-22 or 23), 5.09 (dd, *J*=15.2, 8.9 Hz, 1H, H-22 or 23), 4.09 (q, *J*=7.1 Hz, 2H, OCH_2CH$_3$), 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 2.38 (quint, *J*=6.9 Hz, 1H, H-25), 1.24 (t, *J*=7.1 Hz, 3H, OCH$_2$CH_3), 1.09 (d, *J*=7.0 Hz, 3H, H-26 or H-21), 1.02 (s, 3H, H-19), 0.99 (d, *J*=6.6 Hz, 3H, H-26 or H-21), 0.86 (t, *J*=6.9 Hz, 3H, H-30), 0.72 (s, 3H, H-18). 10b: TLC R_f=0.43 (hexane/ethyl acetate, 9:1), HPLC t_R 47.6 min; ^{1}H NMR (300 MHz) 5.25 (dd, *J*=15.2, 8.6 Hz, 1H, H-22 or 23), 4.93 (dd, *J*=15.2, 9.1 Hz, 1H, H-22 or 23), 4.12 (q, *J*=7.1 Hz, 2H, OCH_2CH$_3$), 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 2.27 (quint, *J*=6.8 Hz, 1H, H-25), 1.26 (t, *J*=7.1 Hz, 3H, OCH$_2$CH_3), 1.06 (d, *J*=6.8 Hz, 3H, H-26 or H-21), 1.02 (s, 3H, H-19), 1.01 (d, *J*=6.8 Hz, 3H, H-26 or H-21), 0.85 (t, *J*=6.9 Hz, 3H, H-30), 0.72 (s, 3H, H-18); mass spectrum, *m*/*z* (relative intensity) 498 (M^+, $C_{33}H_{54}O_3$, 19), 483 (25), 466 (100), 451 (22), 443 (28), 365 (9), 313 (17), 283 (22), 253 (99), 211 (75), 159 (55), 137 (77), 109 (79), 93 (55), 81 (66), 55 (58); HRMS *m*/*z* 498.4070 (calcd for $C_{33}H_{54}O_3$, 498.4073).

(22*E*,24*S*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-ol isomer 1 (11a). Reduction of **9a** with $LiAlH_4$ in ether at rt gave, after the usual workup, **11a** in 100% yield. TLC R_f=0.55 (hexane/ethyl acetate, 4:1); ^{1}H NMR (300 MHz) 5.24 (dd, *J*=15.2, 8.3 Hz, 1H, H-22 or 23), 5.10 (dd, *J*=15.3, 9.1 Hz, 1H, H-22 or 23), 3.60 (dd, *J*=10.7, 5.0 Hz, 1H, H-26), 3.43 (dd, *J*=10.8, 6.4 Hz, 1H, H-26), 3.33 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.02 (s, 3H, H-19), 1.01 (d, *J*=6.1 Hz, 3H, H-26 or H-21), 0.93 (d, *J*=6.8 Hz, 3H, H-26 or H-21), 0.86 (t, *J*=6.7 Hz, 3H, H-30), 0.73 (s, 3H, H-18).

(22*E*,24*S*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-ol isomer 2 (11b). Reduction of **9b** as above gave **11b**. TLC R_f=0.55 (hexane/ethyl acetate, 4:1); ^{1}H NMR (300 MHz) 5.24 (dd, *J*=15.1, 8.4 Hz, 1H, H-22 or 23), 5.09 (dd, *J*=15.3, 9.3 Hz, 1H, H-22 or 23), 3.52 (dd, *J*=10.7, 6.6 Hz, 1H, H-26), 3.44 (dd, *J*=10.7, 6.0 Hz, 1H, H-26), 3.33 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.02 (s, 3H, H-19), 1.01 (d, *J*=6.9 Hz, 3H, H-26 or H-21), 0.87 (t, *J*=7.0 Hz, 3H, H-30), 0.83 (d, *J*=6.9 Hz, 3H, H-26 or H-21), 0.73 (s, 3H, H-18).

(22*E*,24*R*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-ol isomer 1 (12a). Reduction of **10a** as above gave **12a**. TLC R_f=0.47 (hexane/ethyl acetate, 4:1); ^{1}H NMR (300 MHz) 5.22 (dd, *J*=15.2, 8.5 Hz, 1H, H-22 or 23), 5.08 (dd, *J*=15.2, 9.1 Hz, 1H, H-22 or 23), 3.61 (dd, *J*=10.7, 4.9 Hz, 1H, H-26), 3.43 (dd, *J*=10.7, 6.6 Hz, 1H, H-26), 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.02 (s, 3H, H-19), 1.01 (d, *J*=6.3 Hz, 3H, H-26 or H-21), 0.94 (d, *J*=6.8 Hz, 3H, H-26 or H-21), 0.86 (t, *J*=6.9 Hz, 3H, H-30), 0.73 (s, 3H, H-18).

(22*E*,24*R*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-ol isomer 2 (12b). Reduction of **10b** as above gave **12b**. TLC R_f=0.47 (hexane/ethyl acetate, 4:1); ^{1}H NMR (300 MHz) 5.22 (dd, *J*=15.2, 8.5 Hz, 1H, H-22 or 23), 5.08 (dd, *J*=15.2, 9.2 Hz, 1H, H-22 or 23), 3.51 (dd, *J*=10.7, 6.9 Hz, 1H, H-26), 3.43 (dd, *J*=10.6, 6.4 Hz, 1H, H-26), 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.02 (s, 3H, H-19), 1.01 (d, *J*=7.2 Hz, 3H, H-26 or H-21), 0.87 (t, *J*=6.9 Hz, 3H, H-30), 0.83 (d, *J*=6.9 Hz, 3H, H-26 or H-21), 0.73 (s, 3H, H-18); mass spectrum, *m*/*z* (relative intensity) 456 (M^+, $C_{31}H_{52}O_2$, 26), 441 (29), 424 (77), 401 (36), 365 (13), 313 (28), 255 (82), 253 (91), 227 (53), 213 (44), 199 (26), 159 (64), 109 (75), 95 (100), 81 (96), 69 (71), 55 (94); HRMS *m*/*z* 456.3965 (calcd for $C_{31}H_{52}O_2$, 456.3967).

(22*E*,24*R*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-ene (13). A mixture of **11a** and **11b** (82 mg) was converted to their mesylates by dissolving in 1 ml of CH_2Cl_2 containing 40 μl of triethylamine and treating with 15 ml of methylsulfonyl chloride at 0°C under nitrogen. After 15 min, an additonal 20 ml of triethylamine and 8 ml of methylsulfonyl chloride were added to the mixture to ensure complete reaction. After an additional 15 min, the reaction was quenched with 1 ml saturated $NaHCO_3$ solution and extracted with hexane/ethyl acetate (4:1). The

organic layer was extracted with dil. HCl and brine. After evaporation, 80 mg (89%) was obtained. Reduction of the mesylates with $LiAlH_4$ in Et_2O at rt gave, after the usual workup, **13** in 100% yield. ^1H NMR (300 MHz) 5.15 (dd, J=15.1, 8.2 Hz, 1H, H-22 or 23), 5.02 (dd, J=15.2, 8.8 Hz, 1H, H-22 or 23), 3.33 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.03 (s, 3H, H-19), 1.02 (d, J=6.6 Hz, 3H, H-21), 0.86 (t, J=7.0 Hz, 3H, H-30), 0.84 (d, J=6.7 Hz, 3H, H-26 or H-27), 0.79 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.73 (s, 3H, H-18).

(22*E*,24*S*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholest-22-ene (14). Treatment of the alcohols **12a** and **12b** (82 mg) as described above gave the mesylates in 97% yield. Likewise **14** was obtained by $LiAlH_4$ reduction of the mesylates in 90% yield. ^1H NMR (300 MHz) 5.14 (dd, J=15.2, 8.3 Hz, 1H, H-22 or 23), 5.02 (dd, J=15.2, 8.8 Hz, 1H, H-22 or 23), 3.33 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.03 (s, 3H, H-19), 1.01 (d, J=6.7 Hz, 3H, H-21), 0.86 (t, J=6.8 Hz, 3H, H-30), 0.85 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.80 (d, J=6.7 Hz, 3H, H-26 or H-27), 0.73 (s, 3H, H-18).

(24*S*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholestane (15). Catalytic hydrogenation of **13** (17 mg) over PtO_2 under ambient conditions in ethyl acetate for 2.5 h gave, after filtration through silica gel and evaporation, **15** in 100% yield. ^1H NMR (300 MHz) 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.03 (s, 3H, H-19), 0.92 (d, J=6.7 Hz, 3H, H-21), 0.88 (t, J=6.9 Hz, 3H, H-30), 0.83 (d, J=6.9 Hz, 3H, H-26 or H-27), 0.81 (d, J=7.1 Hz, 3H, H-26 or H-27), 0.72 (s, 3H, H-18).

(24*R*)-24-Propyl-6β-methoxy-3α,5-cyclo-5α-cholestane (16). Hydrogenation of **14** in the same way gave **16** in 100% yield. ^1H NMR (300 MHz) 3.32 (s, 3H, OMe), 2.77 (m, 1H, H-6), 1.02 (s, 3H, H-19), 0.92 (d, J=6.4 Hz, 3H, H-21), 0.88 (t, J=6.9 Hz, 3H, H-30), 0.83 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.81 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.72 (s, 3H, H-18).

(22*E*,24*S*)-24-Propylcholest-5-en-3β-ol (1a). Deprotection of **15** (18 mg) was accomplished by treatment with 1.5 ml of 5% TFA in toluene at rt. After 6 min, 1.5 ml of 10% KOH/MeOH was added and the reaction mixture was stirred for another 2 min. The mixture was diluted with brine and extracted with hexane/ ethyl acetate (2:1). Evaporation gave 15.6 mg (88%) of **1a**. $[\alpha]_D^{21}=-29.5°$ (c 0.40, CH_2Cl_2); ^1H NMR (300 MHz) 5.35 (m, 1H, H-6), 3.52 (m, 1H, H-3), 1.01 (s, 3H, H-19), 0.92 (d, J=6.6 Hz, 3H, H-21), 0.88 (t, J=7.1 Hz, 3H, H-30), 0.83 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.81 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.68 (s, 3H, H-18); mass spectrum, m/z (relative intensity) 428 (M^+, $C_{30}H_{52}O$, 100), 413 (23), 410 (38), 395 (18), 343 (28), 317 (18), 273 (10), 255 (10), 231 (7), 213 (11), 159 (13), 145 (18), 107 (22), 105 (22), 95 (21), 93 (18), 91 (19), 81 (21), 79 (16), 57 (37), 55 (26); HRMS m/z 428.4017 (calcd for $C_{30}H_{52}O$, 428.4018).

(22*E*,24*R*)-24-Propylcholest-5-en-3β-ol (1b). Through the above procedure, **1b** was obtained in 93% yield from **16**. $[\alpha]_D^{21}=-31.4°$ (c 0.50, CH_2Cl_2); ^1H NMR (300 MHz) 5.35 (m, 1H, H-6), 3.51 (m, 1H, H-3), 1.01 (s, 3H, H-19), 0.93 (d, J=6.6 Hz, 3H, H-21), 0.88 (t, J=7.0 Hz, 3H, H-30), 0.83 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.81 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.68 (s, 3H, H-18); mass spectrum, m/z (relative intensity) 428 (M^+, $C_{30}H_{52}O$, 100), 413 (22), 410 (38), 395 (18), 343 (28), 317 (17), 273 (11), 255 (10), 231 (7), 213 (10), 159 (13), 145 (18), 107 (23), 105 (23), 95 (21), 93 (18), 91 (19), 81 (23), 79 (18), 57 (42), 55 (27); HRMS m/z 428.4021 (calcd for $C_{30}H_{52}O$, 428.4018).

(22*E*,24*R*)-24-Propylcholest-5,22-dien-3β-ol (4a). Through the above procedure, **4a** was obtained in 99% yield from **13**. $[\alpha]_D^{21}=-40.3°$ (c 0.55, CH_2Cl_2); ^1H NMR (300 MHz) 5.35 (m, 1H, H-6), 5.16 (dd, J=15.2, 8.2 Hz, 1H, H-22 or 23), 5.03 (dd, J=15.2, 8.8 Hz, 1H, H-22 or 23), 3.51 (m, 1H, H-3), 1.02 (d, J=6.3 Hz, 3H, H-21), 1.01 (s, 3H, H-19), 0.86 (t, J=6.8 Hz, 3H, H-30), 0.85 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.79 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.70 (s, 3H, H-18); mass spectrum, m/z (relative intensity) 426 (M^+, $C_{30}H_{50}O$, 100), 411 (7), 408 (8), 393 (8), 383 (18), 365 (26), 314 (10), 300 (23), 271 (26), 255 (22), 159 (22), 151 (13), 145 (21), 133 (25), 105 (27), 97 (35), 95 (29), 93 (26), 91 (28), 81 (43), 79 (28), 69 (54), 67 (25), 55 (64); HRMS m/z 426.3862 (calcd for $C_{30}H_{52}O$, 426.3862).

(22*E*,24*S*)-24-Propylcholest-5,22-dien-3β-ol (4b). Through the above procedure, **4b** was obtained in 99% yield from **14**. $[\alpha]_D^{21}=-40.5°$ (c 0.52, CH_2Cl_2); ^1H NMR (300 MHz) 5.35 (m, 1H, H-6), 5.14 (dd, J=15.2, 8.3 Hz, 1H, H-22 or 23), 5.02 (dd, J=15.2, 8.8 Hz, 1H, H-22 or 23), 3.52 (m, 1H, H-3), 1.02 (d, J=6.5 Hz, 3H, H-21), 1.01 (s, 3H, H-19), 0.86 (t, J=7.0 Hz, 3H, H-30), 0.85 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.80 (d, J=6.8 Hz, 3H, H-26 or H-27), 0.70 (s, 3H, H-18); mass spectrum, m/z (relative intensity) 426 (M^+, $C_{30}H_{50}O$, 100), 411 (7), 408 (8), 393 (8), 383 (17), 365 (27), 314 (10), 300 (23), 271 (26), 255 (22), 213 (9), 152 (14), 145 (22), 133 (27), 109 (31), 97 (36), 95 (33), 93 (29), 91 (31), 81 (46), 79 (31), 69 (58), 67 (29), 55 (72); HRMS m/z 426.3864 (calcd for $C_{30}H_{52}O$, 426.3862).

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