Efficient, Stereoselective Synthesis of 24(*S*),25-Epoxycholesterol

Nicholas C. O. Tomkinson and Timothy M. Willson

Department of Medicinal Chemistry, Glaxo Wellcome Research and Development, Research Triangle Park, North Carolina 27709

Jonathon S. Russel and Thomas A. Spencer*

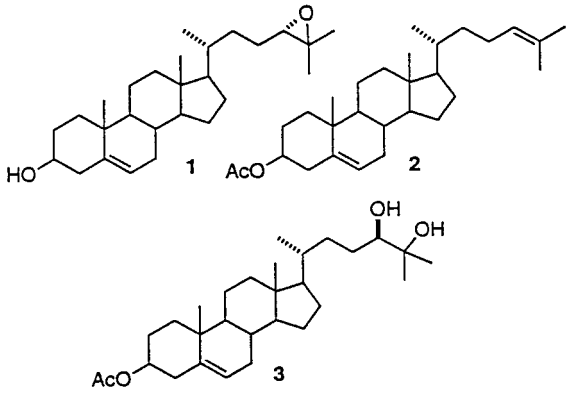
Department of Chemistry, Dartmouth College, Hanover, New Hampshire 03755

Received August 26, 1998

Efficient, stereoselective syntheses of 24(*S*),25-epoxycholesterol (**1**) have been developed starting from cholenic acid (**4**) or stigmasterol (**8**), both featuring as the key step Sharpless asymmetric dihydroxylation of desmosterol acetate (**2**). This work permits preparation of gram quantities of **1** for further evaluation as a natural regulator of cholesterol metabolism, specifically, e.g., as a ligand for the LXRα nuclear receptor.

Since 24(*S*),25-epoxycholesterol (**1**) was shown to be formed enzymatically from squalene 2,3(*S*);22(*S*),23-dioxide[1] and identified as a mammalian natural product,[2] evidence has slowly accumulated consistent with participation of that oxysterol in the natural regulation of cholesterol metabolism.[3−6] An important recent development in this regard was the discovery that the previously orphan nuclear LXR receptors are activated by oxysterols,[7,8] including **1** at physiologic concentration. In response to **1**, the LXRα receptor subtype regulates transcription of the gene encoding cholesterol 7α-hydroxylase,[8] the enzyme that catalyzes the rate-limiting step in cholesterol degradation. The fact that 24(*S*),25-epoxycholesterol has a unique biosynthetic origin among putatively regulatory oxysterols, being formed via squalene dioxide rather than by oxidation of cholesterol,[5] has been used to explain the effectiveness of inhibitors of oxidosqualene cyclase as cholesterol-lowering agents, since cyclase inhibition leads to accumulation of squalene dioxide and, consequently, **1**.[9−11]

In view of this mounting evidence of the biochemical importance of **1**, it was desired to have access to substantial quantities of this epoxide, free of the C24 epimer (*epi*-**1**), which is not known to be naturally occurring. To date, only a few milligrams of **1** have ever been prepared at one time, usually by painstaking HPLC separation of the mixture of C24 epimeric epoxides formed by peracid treatment of desmosterol.[12] Development of an efficient, highly stereoselective, and reasonably economical synthesis of **1** is described herein.



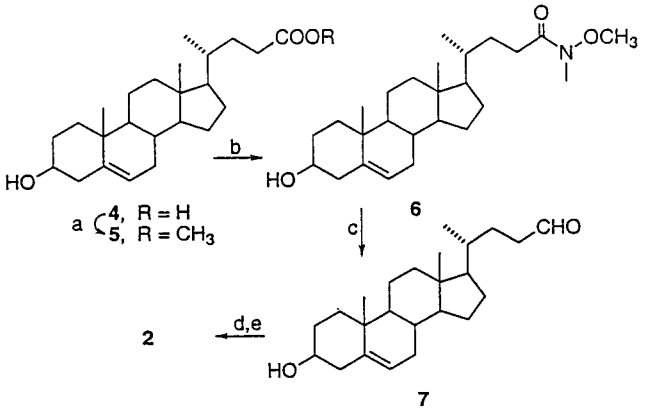
The fundamental strategy adopted for steroselective generation of the 24(*S*),25-epoxide function was to apply Sharpless asymmetric dihydroxylation[13] to an appropriate derivative of desmosterol, such as acetate **2**, to produce 24(*R*),25-diol **3**. Mesylation of the secondary hydroxyl group of **3**, followed by treatment with base, would then lead to **1**.

However, desmosterol is extremely expensive and available only in limited supply, so it was also essential to develop an efficient and economical synthesis of key prospective intermediate **2**. A number of syntheses of desmosterol from a variety of steroidal precursors have been reported;[14] we decided to attempt to seek improvement of two of these previous approaches. The first approach utilized as starting material cholenic acid (**4**), which has been converted, as different derivatives of the 3β-hydroxyl group, to C24 aldehydes,[15,16] which in turn

(1) Nelson, J. A.; Steckbeck, S. R.; Spencer, T. A. *J. Biol. Chem.* **1981**, *256*, 1067−1068.
(2) Nelson, J. A.; Steckbeck, S. R.; Spencer, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 6974−6975.
(3) Spencer, T. A.; Gayen, A. K.; Phirwa, S.; Nelson, J. A.; Taylor, F. R.; Kandutsch, A. A.; Erickson, S. K. *J. Biol. Chem.* **1985**, *260*, 13391−13394.
(4) Saucier, S. E.; Kandutsch, A. A.; Taylor, F. R.; Spencer, T. A.; Phirwa, S.; Gayen, A. K. *J. Biol. Chem.* **1985**, *260*, 14571−14579.
(5) Spencer, T. A. *Acc. Chem. Res.* **1994**, *27*, 83−90.
(6) Sinensky, M.; Logel, J.; Phirwa, S.; Gayen, A. K.; Spencer, T. A. *Exp. Cell Res.* **1987**, *171*, 492−497.
(7) Janowski, B. A.; Willy, P. J.; Rama Devi, T.; Falck, J. R.; Mangelsdorf, D. A. *Nature* **1996**, *383*, 728−731.
(8) (a) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J.-L.; Sundseth, S. S.; Winegar, D. A.; Blanchard, D. E.; Spencer, T. A.; Willson, T. M. *J. Biol. Chem.* **1997**, *272*, 3137−3140. (b) Peet, D. J.; Turley, S. D.; Ma, W.; Janowski, B. A.; Lobaccaro, J. A.; Hammer, R. E.; Mangelsdorf, D. J. *Cell* **1998**, *93*, 693−704.
(9) Mark, M.; Müller, P.; Maier, R.; Eisele, B. *J. Lipid Res.* **1996**, *37*, 148−158.
(10) Morand, O. H.; Aebi, J. D.; Dehmlow, H.; Ji, Y.-H.; Gains, N.; Lengsfeld, H.; Himber, J. *J. Lipid Res.* **1997**, *38*, 373−390.
(11) Eisele, B.; Budzinski, R.; Müller, P.; Maier, R.; Mark, M. *J. Lipid Res.* **1997**, *38*, 564−575.
(12) Saucier, S. E.; Kandutsch, A. A.; Gayen, A. K.; Nelson, J. A.; Spencer, T. A. *J. Lipid Res.* **1990**, *31*, 2179−2185.
(13) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.
(14) Kircher, H. W.; Rosentein, F. U. *J. Org. Chem.* **1987**, *52*, 2586−2588. References to other syntheses of desmosterol are provided in this paper.

10.1021/jo981753v CCC: $15.00
Published on Web 12/05/1998

Scheme 1[a]

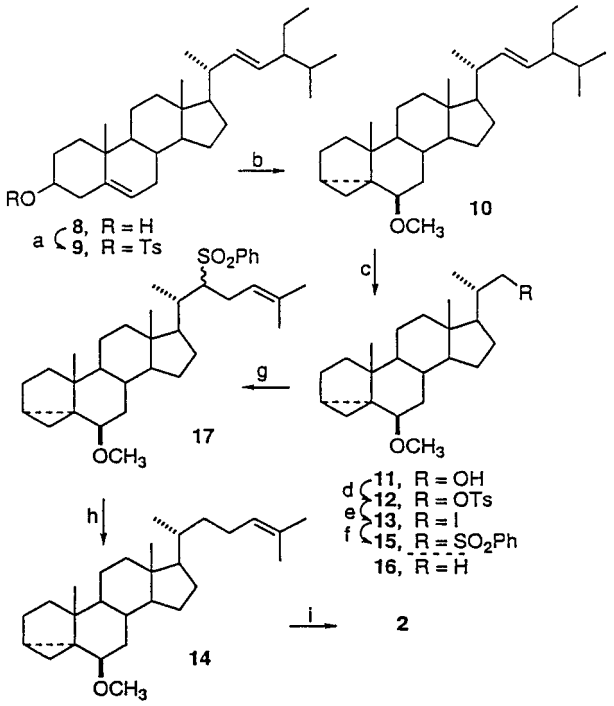


[a] Reagents: (a) CH_2N_2; (b) $(CH_3)_2AlCl$, $CH_3O(CH_3)NH_2Cl$; (c) $(iBu)_2AlH$; (d) AcCl, py; (e) $(CH_3)_2CPPh_3$.

can be transformed to the desmosterol skeleton by Wittig isopropylidenation.[3,17] Our variation on this theme proceeded, as outlined in Scheme 1, via esterification of **4** to **5** (98%), conversion to Weinreb amide **6** by the Nakata procedure (94%),[18] and reduction to aldehyde **7** (89%), followed by acetylation and isopropylidenation to afford **2** (82%).

The second approach utilized the less costly stigmasterol (**8**) as starting material and, as shown in Scheme 2, followed the route developed by Partridge et al.,[19] through tosylation to **9**, methanolysis to *i*-steroid **10**, reductive ozonolysis to **11**, and conversion, via tosylate **12**,[20] to iodide **13** in 42% overall yield, compared to the originally reported 41%.[19] Coupling of **13** with π-(dimethylallyl)nickel bromide in 65% yield has been used in a previous synthesis of desmosterol,[21] but we wished to avoid large-scale use of $Ni(CO)_4$. Apfel[22] effected direct alkylation of a C22 bromide analogous to **13** with prenyllithium in 39% yield, and we hoped to be able to improve this type of coupling by, for example, use of organocuprate methodology. However, extensive efforts, with or without involvement of a variety of cuprates, to couple **13** or tosylate **12** with prenyl organometallic reagents,[23] or to couple metalated **13** with prenyl bromide,[24] all resulted in either very low yield or in a high proportion of product of coupling at the undesired, more

Scheme 2[a]



[a] Reagents: (a) TsCl, py; (b) CH_3OH, Δ; (c) (1) O_3, CH_2Cl_2, (2) $NaBH_4$; (d) TsCl, py; (e) KI, acetone, Δ; (f) $PhSO_2Na$, DMF, Δ; (g) (1) BuLi, (2) 4-bromo-2-methyl-2-butene; (h) Li, NH_3; (i) HOAc, Δ.

substituted, terminus of the allylic system.[25] The best yield of desired alkylation product **14** was obtained by reaction of **13** with prenyllithium by the procedure of Apfel,[22] which afforded a mixture judged by ^1H NMR to contain 51% **14**, 7% of the alternate alkylation isomer, and 17% of reduction product **16**.[26]

Attention was then turned to known C22 sulfone **15**, which was formed in 92% yield from **13** upon treatment with sodium phenyl sulfinate in DMF at a temperature intermediate between those employed by Ourisson[27] and Moriarty.[28] Alkylation of **15** with prenyl bromide gave 81% of **17** as a separable mixture of diastereomers. The phenylsulfonyl group was removed with Li/NH_3 to produce 74% of **14**, which was heated in acetic acid[19] to afford 87% of **2**.

With adequate quantities of key intermediate **2** available by either route, its asymmetric dihydroxylation by the Sharpless procedure[13] was explored. Gratifyingly, dihydroxylation was found to give the desired 24(*R*),25-diol **3** in 92% yield using in situ generated AD-mix-β reagent with addition of $CH_3SO_2NH_2$ to improve catalyst turnover[13] (Scheme 3). This reaction was regio- and

(15) Kris Prasad, V. V.; Ponticorvo, L.; Lieberman, S. *J. Steroid Biochem.* **1984**, *21*, 733−736.

(16) Okamoto, M.; Tabe, M.; Fujii, T.; Tanaka, T. *Tetrahedron: Asymmetry* **1995**, *6*, 767−778.

(17) Fagerlund, U. H. M.; Idler, D. R. *J. Am. Chem. Soc.* **1957**, *79*, 6473−6475.

(18) Shimizu, T.; Osaka, K.; Nakata, T. *Tetrahedron Lett.* **1997**, *38*, 2685−2688.

(19) Partridge, J. J.; Faber, S.; Uskokovic, M. R. *Helv. Chim. Acta* **1974**, *57*, 764−770.

(20) Direct conversion of **11** to **13**, as reported by Schmittberger and Uguen (Schmittberger, T.; Uguen, D. *Tetrahedron Lett.* **1996**, *37*, 29−32) gave poor results in our hands.

(21) Dasgupta, S. K.; Crump, D. R.; Gut, M. *J. Org. Chem.* **1974**, *39*, 1658−1661.

(22) Apfel, M. A. *J. Org. Chem.* **1978**, *43*, 2284−2285.

(23) Prenyl Grignard reagent (PGR) + **12** or **13**: (a) Lipshutz, B. H.; Hackmann, C. *J. Org. Chem.* **1994**, *59*, 7437−7444. PGR + **12** + various copper reagents (Cu): (b) Tamura, M.; Kochi, J. *Synthesis* **1971**, 303−305. (c) Fouquet, G.; Schlosser, M. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 82−83. (d) Schlosser, M. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 701−706. (e) Reference 25. Prenyllithium (PL): (f) Seyferth, D.; Weiner, M. A. *J. Org. Chem.* **1961**, *26*, 4797−4800. PL + **12** or **13**: (g) Reference 22. PL + **12** or **13** + Cu: See ref 23b−d. (h) Lipshutz, B. H.; Elworthy, T. R. *J. Org. Chem.* **1990**, *55*, 1695−1696. (i) Lipshutz, B. H.; Ellsworth, E. L.; Dimock, S. H.; Smith, R. A. J. *J. Am. Chem. Soc.* **1990**, *112*, 4404−4410.

(24) Lithiated **13** + prenyl bromide + Cu: Gorlier, J.-P.; Hamon, L.; Levisalles, J.; Wagnon, J. *J. Chem. Soc. Chem. Commun.* **1973**, 88.

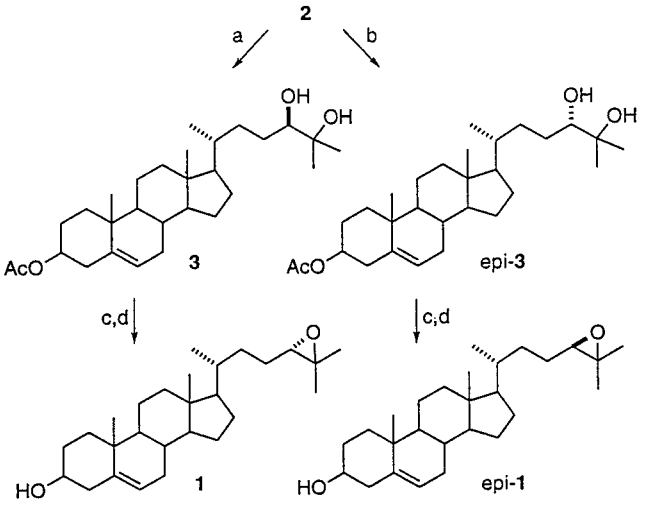
(25) Morisaki, M.; Shibata, M.; Duque, C.; Imamura, N.; Ikekawa, N. *Chem. Pharm. Bull.* **1980**, *28*, 606−611. This work describes an analogous "backwards" coupling product from the same type of reaction of a C22 tosylate.

(26) Dasgupta, S. K.; Gut, M. *J. Org. Chem.* **1975**, *40*, 1475−1477. This paper reports that **12** gives only **16** or dimeric product upon reaction with prenyl Grignard reagent. Ikekawa, ref 25, also reports a reduction product analogous to **16**.

(27) Koch, P.; Nakatani, Y.; Luu, B.; Ourisson, G. *Bull. Soc. Chim. Fr.* **1983**, 189−194.

(28) Moriarty, R. M.; Enache, L. A.; Kinney, W. A.; Allen, C. S.; Canary, J. W.; Tuladhar, S. M.; Guo, L. *Tetrahedron Lett.* **1995**, *36*, 5139−5142.

Scheme 3[a]



[a] Reagents: (a) AD-mix-β, $CH_3SO_2NH_2$; (b) AD-mix-α, $CH_3SO_2NH_2$; (c) CH_3SO_2Cl, py; (d) K_2CO_3, CH_3OH, H_2O.

stereospecific; no dihydroxylation of the $\Delta^{5,6}$ double bond or formation of 24(*S*)-diol (*epi*-**3**) could be detected by ^{13}C NMR. Diol **3** was converted to its C24 mesylate, which was treated with K_2CO_3 in methanol to generate the epoxide function and, more slowly, to free the C3 hydroxyl group. Dilution of the reaction mixture with H_2O provided the hemihydrate of 24(*S*),25-epoxycholesterol (**1**) as a white powder in 87% yield from **3** (80% from **2**, with one purified intermediate). 24(*R*),25-Epoxycholesterol (*epi*-**1**) was also prepared from intermediate **2** by a parallel route by use of the AD-mix-α reagent[13] in the asymmetric dihydroxylation step. The C24 configuration of **1** was initially confirmed by HPLC comparison with samples of **1** and *epi*-**1** generated by HPLC separation of the diastereomeric mixture.[12] To provide final confirmation of the relative configurations, single-crystal X-ray analysis was performed on both **1** and *epi*-**1**. The resulting structures showed unequivocally that the assignment of C24 stereochemistry to **1** and *epi*-**1** was correct.[29]

The synthesis of **1** from cholenic acid (**4**) requires 8 steps and affords 54% overall yield. From stigmasterol (**8**), which costs approximately 1% as much as **4**, preparation of **1** requires 12 steps and affords 16% overall yield. All steps in either synthesis can readily be conducted on a multigram scale.

Experimental Section

NMR spectra were taken in $CDCl_3$ on either a 300 or 400 MHz spectrophotometer. The ^1H and ^{13}C chemical shifts are reported in units of δ from TMS. Tetrahydrofuran (THF) and ether were distilled from sodium/benzophenone. Acetone was distilled from calcium carbonate onto 3 Å molecular sieves. Pyridine was distilled from calcium hydride onto 4 Å molecular sieves. Methanol was distilled from magnesium and iodine onto 3 Å molecular sieves. Acetic acid was distilled from acetic anhydride. All reactions were magnetically stirred. Melting points are uncorrected.

Flash column chromatography was carried out on EM Reagent silica gel 60 (230−400 mesh). Thin-layer chromatography (TLC) was conducted on EM plastic sheets precoated with silica gel 60 F-245. Visualization was obtained by exposure to 5% phosphomolybdic acid in ethanol. $MgSO_4$ was used to dry all solvents and organic layers from reaction mixtures. Cholenic acid was purchased from Steraloids Inc., Wilton, NH. All other reagents, unless noted, were obtained from Aldrich Chemical Co., Milwaukee, WI.

5-Cholenic Acid-3β-ol *N*-Methoxy-*N*-methylamide (6). To a stirred suspension of 2.54 g (26 mmol) of CH_3ONHCH_3·HCl in 100 mL of CH_2Cl_2 under N_2 at 0 °C was added 26 mL (26 mmol, 1 M in hexane) of $(CH_3)_2AlCl$ over 5 min, and the mixture was stirred for 1 h while the temperature rose to rt. Then a solution of 2.0 g (5.15 mmol) of **5**, prepared in 98% yield by diazomethane esterification of **4**, in 80 mL of CH_2Cl_2 was added dropwise via cannula, stirring was continued at rt for 4 h, and 80 mL of phosphate buffer (pH 8.0) was added. The resulting mixture was stirred for 20 min, diluted with 200 mL of $CHCl_3$, and filtered through Celite, which was washed with 200 mL of $CHCl_3$. The aqueous layer was extracted with 3 × 100 mL of $CHCl_3$, and the combined organic layers were washed with brine, dried, and evaporated to give 2.02 g (94%) of **6**, which was recrystallized from EtOAc/hexanes to give **6**: mp 138−139 °C; $[\alpha]_D$ −7 (*c* 0.03, $CHCl_3$); ^1H NMR 5.28 (t, J = 2.6 Hz, 1 H), 3.62 (s, 3 H), 3.46 (m, 1 H), 3.11 (s, 3 H), 2.38−2.16 (m, 4 H), 1.95−0.82 (m, 26 H), 0.94, (s, 3 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.62 (s, 3 H); ^{13}C NMR 140.8, 121.7, 71.8, 61.2, 56.7, 55.9, 50.1, 42.4, 42.3, 39.7, 37.2, 36.5, 35.6, 31.9, 31.6, 30.7, 28.9, 28.1, 24.3, 21.1, 19.4, 18.5, 11.9; MS *m*/*z* 418.3 (MH^+). Anal. Calcd for $C_{26}H_{43}N_1O_3$: C, 74.77; H, 10.38; N, 3.35. Found: C, 74.72; H, 10.50; N, 3.41.

3β-Hydroxycholest-5-en-24-al (7). To a stirred solution of 2.0 g (4.54 mmol) of **6** in 70 mL of CH_2Cl_2 under N_2 at −78 °C was added a solution of 9.08 mL (1.0 M in toluene, 9.08 mmol) of DIBAH in 10 mL of CH_2Cl_2 over 1 h, and stirring was continued at −78 °C for 30 min. This mixture was added via cannula to a cooled (0 °C) solution of 12 g of sodium potassium tartrate in 40 mL of H_2O, and the resulting mixture was stirred for 3 h during which time the biphasic mixture became clear. The aqueous phase was extracted with 3 × 50 mL of ether, and the combined organic layers were washed with brine, dried, and evaporated to give crude **7**, which was purified by chromatography (3:17 EtOAc/hexanes) to give 1.53 g (89%) of **7**, which was recrystallized from CH_2Cl_2/CH_3OH to give **7**: mp 132−133 °C; $[\alpha]_D$ −14 (*c* 0.03, $CHCl_3$); ^1H NMR 9.7 (t, J = 1.9 Hz, 1 H), 5.28 (t, J = 2.5 Hz, 1 H), 3.49 (m, 1 H), 2.49−0.82 (m, 26 H), 0.94 (s, 3 H), 0.86 (d, J = 6.5 Hz, 3 H), 0.61 (s, 3 H); ^{13}C NMR 203.3, 140.8, 121.7, 121.6, 71.8, 56.7, 55.8, 50.9, 50.0, 42.4, 42.3, 40.9, 39.7, 37.2, 36.5, 35.3, 31.9, 31.6, 28.2, 27.9, 24.2, 21.0, 19.4, 18.4, 11.9; MS *m*/*z* 381.2 (M + Na). Anal. Calcd for $C_{24}H_{38}O$·H_2O: C, 76.55; H, 10.71. Found: C, 76.62; H, 10.65.

Desmosterol Acetate (2) from 7. A solution of 2.0 g (5.6 mmol) of **7**, 10 mL of CH_2Cl_2, and 5 mL of pyridine was cooled to 0 °C and treated with 0.794 mL (11.2 mmol) of acetyl chloride. After 30 min, 5 mL of H_2O was added, and the reaction mixture was diluted with 100 mL of EtOAc. The organic layer was washed with 3 × 50 mL of 2 M hydrochloric acid, 50 mL of H_2O, and 50 mL of brine, dried, and evaporated to give the acetate of **7**, mp 145−147 °C (lit.[15] mp 146−148 °C), which was dissolved in 30 mL of THF. A suspension of 4.82 g (11.2 mmol) of isopropyltriphenylphosphonium iodide in 50 mL of THF was cooled to 0 °C and treated with 4.46 mL (11.2 mmol, 2.5 M in hexanes) of *n*-BuLi, and stirring was continued at 0 °C for 30 min, after which time the THF solution of acetate was added via cannula. The reaction mixture was allowed to warm to rt, stirred for 18 h, and quenched with 50 mL of saturated NH_4Cl solution, and the aqueous phase was extracted with 3 × 50 mL of EtOAc. The combined organic layers were washed with 150 mL of H_2O and 150 mL of brine, dried, and evaporated to give crude **2**, which was chromatographed (1:19 EtOAc/hexanes) to give 1.95 g (82%) of **2**: mp 91−92 °C (lit.[14] mp 94−95 °C): ^1H NMR 5.38−5.33 (m, 1H), 5.07 (t, J = 9 Hz, 1H), 4.65−4.53 (m, 1H), 2.33−2.27 (m, 2H), 2.01 (s, 3H), 1.66 (s, 3H), 1.58 (s, 3H), 1.01 (s, 3H), 0.93−0.91 (d, J = 6 Hz, 3H), 0.66 (s, 3H); ^{13}C NMR 170.8, 139.8, 131.1, 125.4, 122.8, 74.2, 56.9, 56.2, 50.2, 42.5, 39.9, 38.3,

(29) Atomic coordinates, bond lengths and angles, and thermal parameters for **1** and *epi*-**1** have been deposited at the Cambridge Crystallographic Data Centre, with registration nos. 102766 for **1** and 102765 for *epi*-**1**.

37.2, 36.8, 36.3, 35.8, 32.1, 32.0, 28.4, 28.0, 26.0, 24.9, 24.5, 21.7, 21.2, 19.5, 18.8, 17.9, 12.1.

3α,5-Cyclo-22-iodo-5α-23,24-bisnorcholan-6*β*-ol 6-Methyl Ether (13). The sequence of Partridge et al.[19] was used to convert stigmasterol (**8**) to **13** with the following results and modifications in procedure, if any, for each step. Tosylate **9**, mp 146−147.5 °C (lit.[19] mp 148−149 °C), 95% from **8**, was converted to **10** in 71% yield, but consumption of **8** required 12 h at reflux. Similar results were obtained with the procedure of Steele and Mosettig.[30] Ozonolysis of **10** was conducted as described by Salmond and Sobala,[31] followed by $NaBH_4$ reduction and workup as described by Boger and Coleman,[32] to afford **11** (81%), which was carried on to tosylate **12** (88%), mp 144−146 °C (lit.[19] mp 144−145 °C), using a workup described by Gut et al.,[21] and then to iodide **13** (87%), mp 103.5−104.5 °C (lit.[19] mp 103−104 °C).

3α,5-Cyclo-22-phenylsulfonyl-5α-23,24-bisnorcholan-6*β*-ol 6-Methyl Ether (15). According to a modification of a procedure by Ourisson,[27] a mixture of 500 mg (1.1 mmol) of **13** and 542 mg (3.3 mmol) of sodium benzenesulfinate in 10 mL of DMF was heated (oil bath temp 95−100 °C) for 3 h with stirring under argon. The reaction mixture was treated with 10 mL of water and extracted with 3 × 10 mL of ether. The combined ether layers were washed with 3 × 10 mL of water and 2 × 10 mL of brine, dried, filtered, and evaporated to afford 570 mg of colorless residue that was recrystallized from ether to yield 475 mg (92%) of colorless **15**: mp 142−143 °C (lit.[27] mp 143−144 °C); ^{1}H NMR 7.91−7.87 (m, 2H), 7.65−7.52 (m, 3H), 3.29 (s, 3H), 3.15−3.10 (dd, J = 3, 12 Hz, 1H), 2.87−2.79 (dd, J = 12, 15 Hz, 1H), 2.73 (t, J = 3 Hz, 1H), 1.18−1.16 (d, J = 6 Hz, 3H), 0.98 (s, 3H), 0.66 (s, 3H).

3α,5-Cyclo-5α-cholest-24-en-22-phenylsulfonyl-6*β*-ol 6-Methyl Ether (17). To a solution of 854 mg (1.82 mmol) of **15** in 10 mL of THF was added dropwise 1.1 mL (2.00 mmol) of 1.8 M butyllithium in pentane at −78 °C with stirring under argon. After being stirred for 15 min, the yellow solution was treated dropwise with 1.36 mg (9.10 mmol) of neat 4-bromo-2-methyl-2-butene. The mixture was allowed to warm to room temperature over 2 h, and stirring was continued for 12 h. The solvent was evaporated, and the residue was dissolved in 15 mL of EtOAc and washed with 15 mL of saturated NH_4Cl solution. The aqueous layer was extracted with 2 × 15 mL of EtOAc, and the combined organic layers were washed with 15 mL of 5% $NaHCO_3$ solution and 15 mL of brine, dried, filtered, and evaporated to afford 827 mg of residue that was recrystallized twice from acetone to yield 793 mg (81%) of colorless **17** (mp 163−167 °C). Chromatography (1:9 EtOAc/hexanes) of 205 mg of crude **17** afforded 25 mg of a less polar C22 epimer and 64 mg of a more polar C22 epimer. Less polar isomer: mp 166−168 °C; ^{1}H NMR 7.88−7.87 (m, 2H), 7.85−7.84 (m, 3H), 4.76 (t, J = 6 Hz, 1H), 3.31 (s, 3H), 3.11−3.03 (m, 1H), 2.76 (t, J = 3 Hz, 1H), 2.47−2.33 (m, 1H), 2.24−2.11 (m, 2H), 1.59 (s, 3H), 1.38 (s, 3H), 1.33−1.30 (d, J = 9 Hz, 3H), 1.01 (s, 3H), 0.63 (s, 3H), 0.63 (s, 3H); ^{13}C NMR 141.1, 135.8, 133.4, 129.2, 128.4, 119.8, 82.6, 67.1, 56.8, 56.5, 53.2, 48.1, 43.6, 43.2, 40.2, 37.6, 35.4, 35.2, 33.5, 30.8, 28.9, 27.0, 25.9, 25.2, 24.3, 23.0, 21.7, 19.5, 17.9, 14.7, 13.3, 11.8; FAB-HRMS (MH^+) calcd for $C_{34}H_{51}O_3S$ 539.3553, found 539.3559. More polar isomer: mp 163−165 °C; ^{1}H NMR 7.83−7.80 (m, 2H), 7.61−7.48 (m, 3H), 4.79 (t, J = 6 Hz, 1H), 3.29 (s, 3H), 3.07−3.02 (m, 1H), 2.73 (t, J = 3 Hz, 1H), 2.58−2.50 (m, 1H), 2.43−2.34 (m, 2H), 1.51 (s, 3H), 1.49 (s, 3H), 1.11−1.08 (d, J = 9 Hz, 3H), 0.98 (s, 3H), 0.64 (s, 3H); ^{13}C NMR 140.1, 133.6, 133.4, 129.1, 128.7, 121.4, 82.5, 67.3, 56.8, 54.2, 48.1, 43.6, 43.3, 40.5, 35.4, 35.2, 34.5, 33.5, 30.7, 28.5, 25.8, 25.1, 24.2, 22.9, 22.8, 21.6, 19.5, 18.0, 15.0, 13.3, 12.2. Anal. Calcd for $C_{34}H_{50}O_3S$: C, 75.79; H,9.36. Found: C, 75.77; H. 9.30.

3α,5-Cyclo-5α-cholest-24-en-6*β*-ol 6-Methyl Ether (14). A solution of 152 mg (22.0 mmol) of lithium in 8 mL of 3:1 ammonia/THF at −78 °C was treated dropwise with 296 mg (0.55 mmol) of **17** dissolved in 3 mL of THF and 0.5 mL of absolute ethanol. The blue solution was stirred for 30 min at −78 °C, quenched by dropwise addition of acetone until the solution became colorless, and kept at rt for 2 h to allow the ammonia to evaporate. The residue was dissolved in 10 mL of EtOAc and washed with 10 mL of water. The aqueous layer was washed with 2 × 10 mL of EtOAc, and the combined organic layers were washed with 25 mL of saturated NH_4Cl solution and 25 mL of brine, dried, filtered, and evaporated to afford 418 mg of residue that was chromatographed on $AgNO_3$-impregnated silica gel[33] (2:98 EtOAc/hexanes) to yield 162 mg (74%) of colorless oily **14**: mp 60−62 °C after crystallization from methanol (lit.[21] mp 64−66 °C); ^{1}H NMR 5.06 (t, J = 9 Hz, 1H), 3.30 (s, 3H), 2.74, (t, J = 3 Hz, 1H), 1.66 (s, 3H), 1.58 (s, 3H), 1.01 (s, 3H), 0.93−0.91 (d, J = 6 Hz, 3H), 0.66 (s, 3H); ^{13}C NMR 131.1, 125.5, 82.6, 56.8, 56.7, 56.5, 48.2, 43.6, 43.0, 40.5, 36.3, 35.8, 35.5, 35.3, 33.6, 30.7, 28.5, 26.0, 25.2, 25.0, 24.4, 23.0, 21.7, 19.5, 18.8, 17.8, 13.3, 12.5.

Desmosterol Acetate (2) from 14. According to a modification of a procedure by Partridge,[19] a solution of 335 mg (0.84 mmol) of **14** in 5 mL of glacial HOAc was heated (oil bath temp 70 °C) with stirring for 6 h. The mixture was treated with 5 mL of water and extracted with 3 × 5 mL of EtOAc. The organic layers were washed with 3 × 5 mL of water and 3 × 5 mL of saturated $NaHCO_3$ solution, dried, filtered, and evaporated to afford 351 mg of residue that was chromatographed (2:98 EtOAc/hexanes) to yield 313 mg (87%) of **2:** mp 91−93 °C.

24(*R*),25-Dihydroxycholesteryl-3*β*-acetate (3). To a mixture of 10 mg (0.023 mmol) of $K_2Os_2(OH)_4$, 73 mg (0.094 mmol) of $(DHQD)_2PHAL$, 970 mg (7.03 mmol) of K_2CO_3, and 2.32 g (7.03 mmol) of potassium ferricyanate was added 20 mL of H_2O and 20 mL of *t*-BuOH. The resulting mixture was stirred vigorously at rt until it was clear, and then 223 mg (2.34 mmol) of $CH_3SO_2NH_2$ was added. After 15 min, 1.0 g (2.3 mmol) of **2** was added in one portion, and stirring was continued for 4 d. The reaction mixture was cooled to 0 °C, treated with 4 g of Na_2SO_3, and stirred at 0 °C for 1 h. The aqueous layer was extracted with 4 × 50 mL of ether, and the combined organic layers were washed with 2 × 100 mL of 1 N potassium hydroxide solution and 100 mL of brine, dried, and evaporated to give crude **3**, which was chromatographed (3:7 EtOAc/hexanes) to give 0.993 g (92%) of **3**, which was recrystallized from methanol to give **3**: mp 158−159 °C (lit.[34] mp 164−165 °C); $[\alpha]_D$ −36 (*c* 0.01, CHC_{l3}); ^{1}H NMR 5.31 (d, J = 4.8 Hz, 1 H), 4.54 (m, 1 H), 3.27 (dd, J = 6.2, 6.0 Hz, 1 H), 2.25 (m, 2 H), 1.97 (s, 3 H), 1.93−0.89 (m, 25 H), 1.15 (s, 3 H), 1.09 (s, 3 H), 0.95 (s, 3 H), 0.87 (d, J = 6.5 Hz, 3 H), 0.62 (s, 3 H); ^{13}C NMR 170.6, 139.7, 122.6, 78.8, 77.2, 74.0, 73.2, 56.6, 56.0, 50.0, 42.3, 39.7, 38.1, 37.0, 36.6, 35.6, 32.8, 31.9, 28.3, 28.1, 27.8, 26.6, 24.3, 23.2, 21.5, 21.0, 19.3, 18.6, 11.9; FAB-HRMS (MH^+) calcd for $C_{29}H_{49}O_4$ 461.3628, found 461.3628. Anal. Calcd for $C_{29}H_{48}O_4$: C, 75.61; H, 10.50. Found: C, 75.09; H, 10.48.

24(*S*),25-Epoxycholesterol (1). To a stirred solution of 1.0 g (2.17 mmol) of **3** in 20 mL of CH_2Cl_2 was added 1 mL of pyridine. The resulting solution was cooled to 0 °C, and 0.34 mL (4.34 mmol) of CH_3SO_2Cl was added. The mixture was allowed to warm to rt, stirred overnight, diluted with 100 mL of EtOAc, washed with 3 × 50 mL of 2 N hydrochloric acid and 50 mL of brine, dried, and evaporated to give a crude mesylate, which was dissolved in 20 mL of CH_3OH and 2 mL of H_2O and treated with 1.5 g (11 mmol) of K_2CO_3. The mixture was stirred at room temperature for 18 h and diluted with 50 mL of H_2O. The resulting precipitate was filtered, washed with water, and dried under vacuum to give 0.756 g (87%) of a hemihydrate of **1** as a white powder: mp 156−157 °C;[35] $[\alpha]_D$

(30) Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571−572 used KOAc in place of pyridine in the methanolysis of **9** for 3 h at reflux in the conversion of a tosylate analogous to **9** to an *i*-steroid methyl ether. For the use of KOAc in CH_3OH at reflux for 24 h, see: Catalan, C. A. N.; Kokke, W. C. M. C.; Duque, C.; Djerassi, C. *J. Org. Chem.* **1983**, *48*, 5207−5214.

(31) Salmond, W. G.; Sobala, M. C. *Tetrahedron Lett.* **1977**, 1695−1698.

(32) Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 4796−4807.

(33) Gupta, A. S.; Dev, S. *J. Chromatogr.* **1963**, *12*, 189−195.

(34) Partridge, J. J.; Uskokovic, M. R. U. S. Patent 4,038,272, 1977.

−44 (*c* 0.01, $CHCl_3$); ^{1}H NMR 5.35 (d, *J* = 5.3 Hz, 1 H), 3.51 (m, 1 H), 2.68 (m, 1 H), 2.31 (m, 2 H), 2.28−1.95 (m, 5 H), 1.86−0.89 (m, 26 H), 1.3 (s, 3 H), 1.26 (s, 3 H), 1.00 (s, 3 H), 0.94 (d, *J* = 6.5 Hz, 3 H), 0.68 (s, 3 H); ^{13}C NMR 140.8, 121.7, 77.2, 71.8, 65.0, 58.2, 56.7, 56.0, 50.1, 42.3, 42.3, 39.8, 37.3, 36.5, 35.7, 32.5, 31.9, 31.7, 28.2, 25.7, 25.0, 24.3, 21.1, 19.4, 18.7, 18.6, 11.9; MS *m*/*z* 423.3 (M + Na). Anal. Calcd for $C_{27}H_{44}O_2 \cdot 0.5\ H_2O$: C, 79.16; H, 11.07. Found: C, 79.07; H, 10.92.

Comparison of the HPLC retention time of this **1** with that of **1** and *epi*-**1** previously prepared[12] was conducted under conditions previously described for analysis of 24(*S*)- vs 24(*R*)-hydroxycholesterol,[36] except that 98:2 hexane/2-propanol was used as solvent at a flow rate of 1 mL/min. Typical retention times were as follows: **1**, 14.92 min; *epi*-**1**, 15.34 min. X-ray analysis[29] was performed on a crystal grown from EtOAc.

(35) Anhydrous **1**, mp 160−162 °C, has previously been fully characterized: Reference 2. See also: Steckbeck, S. R. PhD Dissertation, Dartmouth College, 1981.

(36) Saucier, S. E.; Kandutsch, A. A.; Gayen, A. K.; Swahn, D. K.; Spencer, T. A. *J. Biol. Chem*. **1989**, *264*, 6863−6869.

24(*R*),25-Epoxycholesterol (*epi*-1). 24(*R*),25-Epoxycholesterol (*epi*-**1**) was synthesized by the procedure described above for **1** except that $(DHQ)_2PHAL$ was used as the chiral ligand in the asymmetric dihydroxylation protocol. Thus, 47 mg (0.11 mmol) of **2** yielded 31 mg (63%) of *epi*-**1** as a white solid: mp 154−155 °C (lit.[2] mp 166.5−168 °C); $[\alpha]_D$ −26 (*c* 0.4, $CHCl_3$); ^{1}H NMR 5.29 (d, *J* = 5.3 Hz, 1 H), 3.46 (m, 1 H), 2.62 (t, *J* = 6.1 Hz, 1 H), 2.25−2.16 (m, 2 H), 1.98−1.75 (m, 5 H), 1.56−0.83 (m, 19 H), 1.24 (s, 3 H), 1.19 (s, 3 H), 0.94 (s, 3 H), 0.87 (d, *J* = 6.5 Hz, 3 H), 0.62 (s, 3 H); ^{13}C NMR 140.8, 121.7, 71.8, 64.8, 58.4, 56.7, 55.8, 50.1, 42.3, 42.3, 39.7, 37.2, 36.5, 35.6, 32.4, 31.9, 31.6, 28.2, 25.4, 24.9, 24.3, 21.0, 19.4, 18.7, 18.6,. 11.8 ppm; MS *m*/*z* 423.3 (M + Na). X-ray analysis[29] was performed on a crystal grown from Et_2O.

Acknowledgment. The research at Dartmouth was supported by NIH grant HL52069. The HPLC analyses of **1** and *epi*-**1** were kindly performed by Dansu Li. The X-ray structures of **1** and *epi*-**1** were determined by Peter White, University of North Carolina, Chapel Hill.

JO981753V