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A new highly stereoselective synthesis of cerebrosterol, an agonist of the nuclear receptor LXRs

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Abstract—The title compound, cerebrosterol **1**, was synthesized stereoselectively (97% d.e.) in 41% overall yield from methyl hyodeoxycholanate **2** in 10 steps with desmosterol acetate **8** as the key intermediate and the modified Sharpless asymmetric dihydroxylation as the key step.

1. Introduction

Cerebrosterol (24*S*-hydroxycholesterol, **1**) is present in small amounts in human and animal brain,[1] adrenals,[2] and liver.[3] It was reported that the liver X receptors, LXRα[4] and LXRβ,[5] are the specific biological sensors controlling the metabolism and reverse transportation of cholesterol in the body. This operates by means of formation of a permissive heterodimer with the 9-*cis*-retinoic acid receptor RXR,[6] which regulates the transcriptional expression of their target genes, such as CYP7A,[7] CETP,[7,8] ABC-1,[9] and ApoE,[10] etc. Subsequently, LXRs have become a promising targets for the development of drugs for the treatment for atherosclerosis.[10,11] Thus, as one of the most potent agonists of LXRs,[7a,12] cerebrosterol can be a potential drug for treatment for atherosclerosis. Moreover, cerebrosterol is involved in cholesterol homeostasis and in some diseases of the brain such as Alzheimer's.[13] Hence it might also be used as an early biochemical marker for diagnosis of Alzheimer's and vascular demented diseases.[13f]

The early syntheses of cerebrosterol were performed by benzoylation of a mixture of 24*R*- and 24*S*-hydroxycholesterol, followed by either resolution via recrystallization or chromatography and hydrolysis.[14] Recently several stereoselective methods have been reported for introducing a 24*S*-hydroxy group into the sidechain. One of them is the coupling of a C_{22}-sulfone derived from stigmasterol with a chiral C_5 epoxide or a chiral C_5 iodide to give a 24*S*-hydroxysteroid.[15] A second method is the reduction of a cholest-25-en-24-one system with 3 molar equivalents of Noyori's (*S*)-(−)-2,2′-dihydroxy-1,1′-binaphthyl lithium aluminum hydride reagent, which gave the 24*S*-alcohol with 94% d.e.[16] Furthermore, addition of diisopropylzinc to steroidal 24-aldehyde with a chiral β-amino alcohol as ligand also gave 24*S*-hydroxycholesterol in good yield with high diastereoselectivity (d.e. 87%).[17] Corey and Grogan[18] disclosed a successful Sharpless dihydroxylation of desmosterol (**8**, C_3–OH) in 82% yield with 92% d.e. However, this method still required a long reaction time. This led us to develop an efficient and highly stereoselective method using readily available starting materials, such as methyl hyodeoxycholanate (Me-HDCA, **2**), which is also a ligand of LXRα.[19] Here we report a new method for highly stereoselective synthesis of the target molecule **1** using Me-HDCA **2** as the starting material.

2. Results and discussion

Me-HDCA, which is very readily available in China, is selected as the starting material because the 3α and 6α-dihydroxy group can be easily converted to the Δ^5-3β-ol moiety[20]. The AB-ring of Me-HDCA itself is essential for subtype selective ligation of LXRα.[19] The 24-carboxylic acid sidechain can also be transformed to other desired ones,[21] such as that in desmosterol, which is a common precursor to many LXR ligands, including 24*S*,25-epoxycholesterol; all these will expand the scope of the ligands of LXRs.

Our synthesis of cerebrosterol is divided into two stages. In the first stage, the desmosterol acetate **8**, an important intermediate of cerebrosterol, was synthesized as depicted in Scheme 1. Thus, the 3α,6α-dihydroxy groups in **2** were protected with dimethoxymethane to afford the 3α,6α-bismethoxy methyl ether **3** in 91% yield. Then, **3** was subjected to reduction with lithium aluminum hydride in dry tetrahydrofuran to afford 24-alcohol **4** in high yield

Keywords: cerebrosterol; Sharpless AD reaction; stereoselective.

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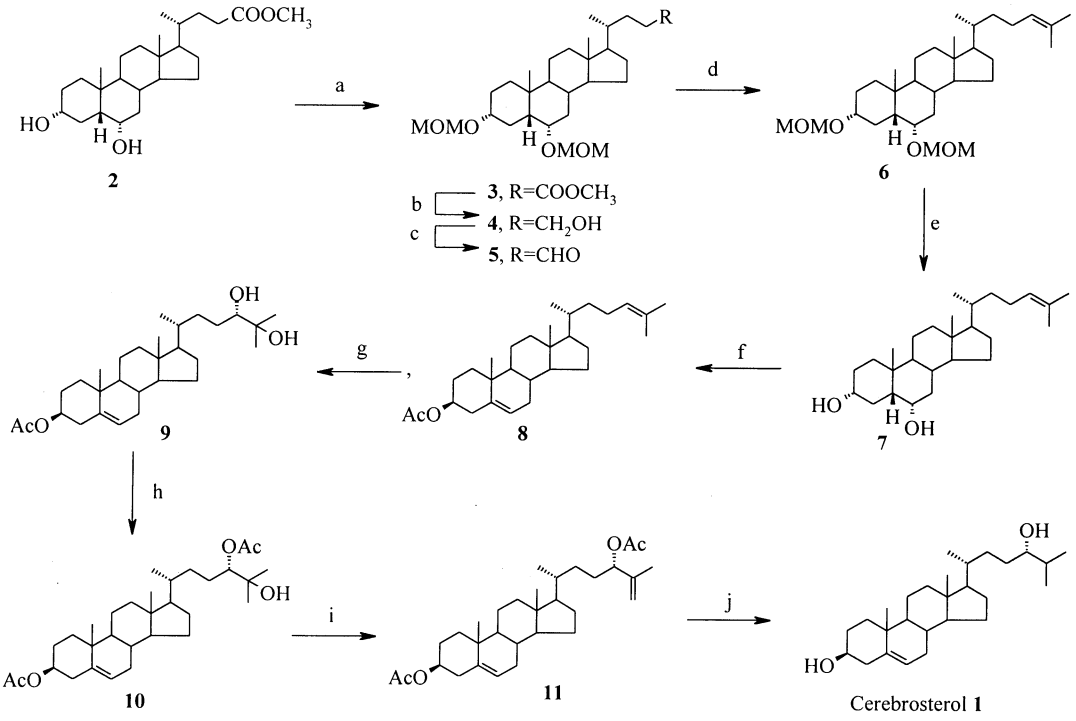

Scheme 1. *Reagents and conditions*: (a) $CH_3OCH_2OCH_3$, P_2O_5, $CHCl_3$, rt 91%; (b) $LiAlH_4$, THF, rt 94%; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C, 100%; (d) BuLi, $Ph_3P^+CH(CH_3)_2I^-$, THF, rt, 97%; (e) PPTS, *t*-Butanol, reflux, 89%; (f) TsCl, Py, 0°C, then KOAc, DMF–H_2O, 105°C, then Ac_2O, Py, rt, 87%; (g) $(DHQ)_2PHAL$, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, *t*-Butanol–H_2O–Methyl Butyl Ether (2.5:2.5:3), rt 98.6% d.e., 83%; (h) Ac_2O, Py, rt, 95%; (i) CH_3SO_2Cl, DMAP, Et_3N, CH_2Cl_2, 0°C rt 91%; (j) NH_2OH, EtOAc, DMF, 95°C, then KOH, MeOH, rt, 90%.

(94%). Swern oxidation of **4** provided the 24-aldehyde **5** in quantitative yield, which was treated with the THF solution of *n*-butyl lithium and isopropyltriphenylphosphonium iodide at rt to give Δ^{24}-compound **6** in 97% yield. Removal of 3α,6α-dihydroxy protective groups by reflux with PPTS in *t*-butanol gave Δ^{24}-5β-3α,6α-dihydroxycholestene **7** in 89% yield. Ditosylation of **7** with *p*-toluenesulfonyl chloride in pyridine at 0°C, followed by treatment of the resulting crude mixture with potassium acetate in DMF–water at 105°C[20] and acetylation with acetic anhydride and pyridine[22] gave desmosterol acetate **8** in 87% yield. Thus the important intermediate was synthesized from Me-HDCA **2** in 6 steps in 64% overall yield on a multigram scale. The desmosterol could also be used for syntheses of another oxysterol 24*S*,25-epoxycholesterol[18,23] and some other cytotoxic oxydesmosterols.[24]

With the key intermediate in hand, our attention turned to the second stage of stereoselective synthesis of cerebrosterol from desmosterol acetate. In this stage, the key step is the stereoselective introduction of 24*S*,25-dihydroxy groups into the sidechain via Sharpless catalytic asymmetric dihydroxylation.[25] In the course of the application of Sharpless catalytic asymmetric dihydroxylation on the unsaturated steroidal sidechain, we found that the low solubility of steroids in *t*-butanol–water solvent system led to the lower value of %d.e and the longer reaction time.[18,23] Fortunately, we found that addition of methyl *t*-butyl ether as an auxiliary solvent to the Sharpless dihydroxylation solvent system increased the solubility of the steroid, and consequently gave better results.[26] Thus, using an improved Sharpless catalytic asymmetric dihydroxylation, **8** was dihydroxylated with $(DHQ)_2PHAL$ and $K_2OsO_2(OH)_4$ in *t*-BuOH–H_2O–methyl *t*-butyl ether (2.5:2.5:3) within 20 h to give 3β-acetoxy-24*S*,25-dihydroxycholesterol **9** in 98.6% d.e. and 83% yield. The result is much better than the usual AD-reaction procedure in terms of d.e. value and reaction time.[18,23] Selective acetylation of the 24*S*-hydroxy of **9** with acetic anhydride and pyridine gave the diacetate **10** in 95% yield. Dehydration of **10** with methanesulfonyl chloride, triethylamine and DMAP provided $\Delta^{5,25}$-3β,24*S*-dihydroxycholestadiene–diacetate **11** in 91% yield.[27] Finally, diimide reduction[28] of **11** with high regioselectivity followed by hydrolysis with potassium hydroxide in methanol gave the target molecule, cerebrosterol **1** in 90% yield with 97% d.e.

3. Conclusion

The main advantages of the present synthesis of cerebrosterol include the easy availability of starting material, a high yield in the preparation of the key intermediate desmosterol acetate, high stereoselectivity for introduction of the 24*S*-hydroxy group and high regioselectivity for the diimide reduction of the double bonds. Furthermore, this method could be used to prepare various analogs of cerebrosterol. Extension of the improved Sharpless AD reaction to the unsaturated sidechain of steroid is in progress.

4. Experimental

4.1. General

All melting points are uncorrected. IR spectra were recorded

with FT-IR apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded at 300 and 75 MHz in $CDCl_3$. Chemical shifts are reported in ppm relative to TMS as internal standard. Mass spectra were recorded by EI methods. Flash column chromatography was carried out with silica gel (300–400 mesh). Solvent THF was distilled over sodium, with dichloromethane being distilled over CaH_2. The d.e. value was determined by HPLC analysis on Inersil ODS-3 column with CH_3CN–H_2O as an eluent.

4.1.1. Methyl 3,6-dimethoxymethyl-3α,6α-dihydroxy-5β-cholanate (3). To a solution of methyl hyodeoxycholanate **2** (5.00 g, 12.3 mmol) in dry chloroform (75 mL) and dimethoxymethane (16.30 mL, 184 mmol) was added phosphorus pentoxide (5.5 g, 36.9 mmol) with stirring at room temperature. After being stirred for 8 h, the mixture was filtered through a pad of silica gel, washed with chloroform (3×30 mL). Removal of the solvent in vacuo afforded a light yellow oil (7.565 g), which was purified by flash chromatography (Pet ether/ethyl acetate 25:l) to afford pure **3** (5.562 g, 91%). Recrystallization from methanol/water gave colorless needles: mp 78–79°C; $[\alpha]_D^{16}$=+11.9 (*c*, 0.3, $CHCl_3$) (lit.[29] mp 79–79.5°C; $[\alpha]_D^{25}$=+14.2 (*c*, 0.72, $CHCl_3$)); MS-EI(*m*/*z*): 426 (M^+−CH_3OH), 430 (M^+−2CH_3OH), 370 (M^+−2$HOCH_2OCH_3$); IR (cm^{-1}): 1743 ($COOCH_3$); ^{1}H NMR δ 0.62 (3H, s,18-CH_3), 0.90 (3H, s, 21-CH_3), 0.89 (3H, s, 18-CH_3), 2.22 (2H, m, 23-CH_2), 3.35 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.50 (1H, m, 3β-H), 3.65 (3H, s, $COOCH_3$), 3.89 (1H, m, 6β-H), 4.62 and 4.64 (2H, each d, J_1=6.8 Hz, J_2=6.5 Hz, $-OCH_2O-$), 4.65 and 4.70 (2H, each d, J_1=6.6 Hz, J_2=6.7 Hz, OCH_2O)

4.1.2. 3,6-Dimethoxymethyl-3α,6α,24-trihydroxy-5β-cholane (4). A solution of **3** (5.038 g, 10.20 mmol) in dry tetrahydrofuran (50 mL) was added to a suspension of lithium aluminum hydride (572 mg, 15.0 mmol) in dry tetrahydrofuran (50 mL) under argon over 30 min. The reaction mixture was stirred for another hour before being quenched with $Na_2SO_4{\cdot}10H_2O$, filtered through a pad of Celite, and washed with ethyl acetate. Removal of the solvent in vacuo and purification by flash chromatography (Pet. ether/ethyl acetate 10:1) afforded pure **4** (4.483 g, 94%): mp 74.5–75°C; $[\alpha]_D^{23}$=+20.6 (*c*, 0.5, $CHCl_3$); MS-EI (*m*/*z*): 404 (M^+−$HOCH_2OCH_3$), 401 (M^+−2$HOCH_3$–H_2O), 371 (M^+−2$HOCH_3$−HCHO), 342 (M^+−2$HOCH_2OCH_3$); IR (cm^{-1}): 3486 (OH); ^{1}H NMR δ 0.62 (3H, s, 18-CH_3), 0.91 (3H, d, *J*=8.2 Hz, 21-CH_3), 0.97 (3H, s, 19-CH_3), 3.35 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.50 (1H, m, 3β-H), 3.60 (2H, t, *J*=6.3 Hz, 24-CH_2), 3.90 (1H, m, 6β-H), 4.6 and 4.63 (2H, each d, J_1=6.4 Hz, J_2=6.8 Hz, $-OCH_2O$), 6.66 and 6.70 (2H, each d, J_1=6.6 Hz, J_2=6.7 Hz, $-OCH_2O$); Anal. Calcd for $C_{28}H_{50}O_5$: C, 72.04%; H, 10.80%; Found. C, 72.19%; H, 10.88%.

4.1.3. 3,6-Dimethoxymethyl-3α,6α-dihydroxy-5β-cholane-24-al (5). A solution of dimethyl sulfoxide (3.25 mL, 45.85 mmol) in dry dichloromethane (10 mL) was added to a solution of oxalyl chloride (2 mL, 22.93 mmol) in dry dichloromethane (50 mL) stirred in a dry ice–acetone bath under argon over 10 min. After stirring for another 10 min, a solution of 4 (9.437 g, 20.33 mmol) in dichloromethane (35 mL) was added dropwise over 15 min. The stirring was continued for another 35 min. Triethylamine (17 mL) was added dropwise to the reaction mixture and a large amount of white solid appeared. After stirring for 10 min, the reaction mixture was allowed to warm to room temperature and washed in turn with sat. NH_4Cl (3×50 mL), sat. $NaHCO_3$(3×50 mL), brine (3×50 mL) and dried over sodium sulfate. Removal of the solvent in vacuo and purification by flash chromatography (Pet. Ether/ethyl acetate 20:1) afforded pure compound **5** (colorless oil 9.427 g, 100%). $[\alpha]_D^{20}$=+10.6 (*c*, 0.34, $CHCl_3$); MS-EI(*m*/*z*): 464 (M^+), 400 (M^+−2MeOH), 340 (M^+−2CH_3OCH_2OH); IR (cm^{-1}): 1726 (–CHO); ^{1}H NMR δ: 0.64 (3H, s, 18-CH_3), 0.91 (3H, s, 21-CH_3), 0.92 (3H, s, 19-CH_3), 3.36 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), 3.50 (1H, m, 3β-H), 3.91 (1H, m, 6β-H), 4.63 and 4.65 (2H, each d, J_1=6.7 Hz, J_2=6.1 Hz, $-OCH_2O-$), 4.67 and 4.71 (2H, each d, J_1=6.80 Hz, J_2=6.85 Hz, $-OCH_2O-$), 9.77 (1H, t, *J*=1.6 Hz, 24-CHO); Anal. Calcd for $C_{28}H_{48}O_5{\cdot}1/3H_2O$: C, 71.45%; H, 10.42%; Found. C, 71.52%; H, 10.58%.

4.1.4. Δ^{24}-3,6-Dimethoxymethyl-3α,6α-dihydroxy-5β-cholestene (6). *n*-Butyl lithium (2.6 M, 0.70 mL) was added to a suspension of isopropyltriphenylphosphonium iodide (684 mg, 1.58 mmol) in dry tetrahydrofuran, giving a deep red solution. Then **5** (367 mg, 0.79 mmol) in dry tetrahydrofuran (8 mL) was added. After stirring for 4.5 h, TLC showed disappearance of starting material. Sat. NH_4Cl (2 mL) was added to the reaction mixture, and a large amount of white solid appeared. The mixture was filtered through a pad of Celite and washed with ethyl acetate (2×20 mL). The filtrate was then washed with 5% HCl (3×10 mL), sat. $NaHCO_3$ (3×10 mL) and brine (3×10 mL) and dried over sodium sulfate. Removal of the solvent in vacuo and purification by flash chromatography (Pet. Ether/acetone 30:1) afforded **6** as a colorless oil (375 mg, 97%): $[\alpha]_D^{22}$=+14.4 (*c*, 0.17, $CHCl_3$); MS-EI (*m*/*z*): 491 (M^++1), 430 (M^++1−OCH_2OCH_3), 414 (M^++1−OCH_2OCH_3–Me), 397 (M^++1−2$HOCH_3$–2Me), 381 (M^++1−2$HOCH_3$–MeO–Me), 367 (M^++1−2$HOCH_2OCH_3$), 351 (M^+−2$HOCH_2OCH_3$–Me); ^{1}H NMR δ: 0.62 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 0.92 (3H, d, *J*=6.8 Hz, 21-CH_3), 1.59 (3H, s, 26-CH_3), 1.63 (3H, s, 27-CH_3), 3.35 and 3.36 (3H, s, OCH_3), 3.49 (1H, m, 3β-H), 3.89 (1H, m, 6β-H), 4.62 (2H, s, $-OCH_2O$), 4.66 and 4.70 (2H, each d, *J*=7.1 Hz, $-OCH_2O$), 5.08 (1H, t, *J*=7.1 Hz, 24-H); Anal. Calcd for $C_{31}H_{54}O_4{\cdot}1/4\,H_2O$: C, 75.17%; H, 11.09%; Found: C, 74.99%; H, 11.25%.

4.1.5. Δ^{24}-3α,6α-Dihydroxy-5β-cholestene (7). A mixture of **6** (150 mg, 0.306 mmol) and PPTS (335 mg, 1.34 mmol) in *t*-butanol (15 mL) was refluxed for 10 h. After removal of the solvent in vacuo, the residue was dissolved in ethyl acetate (50 mL) and washed with 10% HCl (3×10 mL), sat. $NaHCO_3$ (3×10 mL) and brine (3×5 mL) and dried over sodium sulfate. Removal of the solvent in vacuo and purification by flash chromatography (Pet. ether/acetone 4:1) afforded pure **7** (granular crystal, 140 mg, 89%): mp 168–169°C; $[\alpha]_D^{20}$=+10.6 (*c*, 0.6, $CHCl_3$); MS-EI (*m*/*z*): 402 (M^+), 384 (M^+−H_2O), 369 (M^+−H_2O–Me), 351 (M^+−2H_2O–Me); IR (cm^{-1}): 3369 (OH); ^{1}H NMR δ 0.64 (3H, s, 18-CH_3), 0.89 (3H, s, 19-CH_3), 0.92 (3H, d, *J*=7.5 Hz, 21-CH_3), 3.61 (1H, m,

3β-H), 4.05 (1H, m, 6β-H), 5.09 (1H, t, J=6.6 Hz, 24-H); ^{13}C NMR δ 130.9, 125.2, 71.6, 68.1, 56.3, 56.2, 48.5, 42.9, 40.0, 39.9, 36.1, 36.0, 35.6, 35.0, 34.9, 30.2, 29.3, 28.2, 25.7, 24.8, 24.3, 23.5, 20.8, 18.6, 17.6, 12.0; Anal. Calcd for $C_{27}H_{46}O_2 \cdot 1.5H_2O$: C, 75.47%; H, 11.49%; Found. C, 75.29%; H, 11.33%.

4.1.6. $\Delta^{5,24}$-3β-Hydroxy-cholestadiene-acetate (desmosterol acetate) (8). A solution of **7** (4.020 g, 10 mmol) in pyridine (10 mL) was cooled to 0°C and *p*-toluenesulfonyl chloride (4.736 g, 25 mmol) was added. The resulting mixture was stirred for two days. The reaction mixture was poured into a ice-cooled dilute HCl solution (150 mL) and a large amount of precipitate appeared. After stirring for 2 h, the mixture was extracted with ethyl acetate (3×50 mL). The combined organic layer was washed with sat. $NaHCO_3$ (3×150 mL), brine (3×150 mL), dried over sodium sulfate and concentrated. The resulting residue was dissolved in a mixture of DMF (40 mL) and water (4.3 mL). Potassium acetate (10.03 g) was added. The mixture was heated to 105°C and stirred for 5 h. The reaction mixture was cooled to room temperature and poured into ice-cooled dilute HCl solution. A large amount of precipitate was produced, which was filtered off and washed with a large amount of water until neutral, and dried to give a light yellow solid residue (4.08 g). TLC showed it contained the 3β-OH compound. The residue was dissolved in a mixture of acetic anhydride (40 mL) and pyridine (40 mL) and stirred at room temperature for 25 h. The reaction mixture was taken up in ethyl acetate, washed with water (5×100 mL), 10% HCl (3×100 mL), sat. $NaHCO_3$ (3×100 mL), brine (3×100 mL) and dried over sodium sulfate. Removal of the solvent in vacuo and purification by flash chromatography (Pet. ether/acetate 9:1) afforded pure **8** (3.705 g, 87%): mp 90.5–91°C (lit.[23] mp 91–93°C); $[\alpha]_D^{22}$=−40.8 (*c*, 0.3, $CHCl_3$); MS-EI (*m/z*): 411 (M^+−Me), 366 (M^+−HOAc), 351 (M^+−Me−HOAc); IR (cm^{-1}): 1732 (COO); ^{1}H NMR δ 0.71 (3H, s, 18-CH_3), 0.94 (3H, d, J=6.5 Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 1.61 (3H, s, 26-CH_3), 1.69 (3H, s, 27-CH_3), 2.03 (3H, s, CH_3CO), 2.32 (1H, m, 7-H), 4.61 (1H, m, 3α-H), 5.10 (1H, m, 24-H), 5.38 (1H, d, J=4.7 Hz, 6-H); ^{13}C NMR δ 170.6, 139.7, 130.9, 125.2, 122.6, 74.0, 56.7, 56.1, 50.1, 50.0, 42.4, 39.7, 38.1, 37.0, 36.6, 36.1, 35.6, 31.9, 31.8, 28.2, 27.8, 25.7, 24.7, 24.3, 21.4, 21.0, 19.3, 18.6, 11.9; Anal. Calcd for $C_{29}H_{46}O_2$: C, 81.63%; H, 10.87%; Found: C, 81.64%; H, 11.16%.

4.1.7. Δ^5-3β,24*S*,25-Trihydroxycholestene-3-acetate (9). A solution of $K_3Fe(CN)_6$ (3.257 g, 9.87 mmol), K_2CO_3 (1.362 g, 9.87 mmol), $CH_3SO_2NH_2$ (313 mg, 3.29 mmol), $(DHQ)_2PHAL$ (128 mg, 5 mol%), and $K_2OsO_2(OH)_4$ (12 mg, 1 mol%) in *t*-butanol–water (1:1, 33 mL) was cooled to 0°C. A solution of **8** (1.400 g, 3.29 mmol) in methyl *t*-butyl ether (20 mL) was added dropwise. The resulting mixture was stirred vigorously for 20 h. The reaction was quenched at 0°C with sodium sulfite (4.930 g). Stirring was continued for another hour. The aqueous layer was extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with 2 M KOH (3×25 mL), 10% HCl (3×10 mL), sat. $NaHCO_3$ (3×10 mL), brine (3×10 mL), dried over sodium sulfate and evaporated to give the crude product, which was purified by flash chromatography (Pet. ether/acetone 4:1) to afford pure **9** (1.258 g, 98.6% d.e., 83%): mp 135–136°C, $[\alpha]_D^{23}$=−27.2 (*c*, 0.9, $CHCl_3$); MS-EI (*m/z*): 400 (M^+−HOAc), 382 (M^+−HOAc−H_2O); IR (cm^{-1}): 3445 (OH), 1735 (CH_3CO_2); ^{1}H NMR δ 0.68 (3H, s, 18-CH_3), 0.94 (3H, d, J=6.5 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.18 (3H, s, 26-CH_3), 1.22 (3H, s, 27-CH_3), 2.04 (3H, s, CH_3CO_2), 2.32 (2H, d, J=8.2 Hz, 7-H), 3.28 (1H, m, 24β-H), 4.61 (1H, m, 3α-H), 5.38 (1H, d, J=4.7 Hz, 6-H); ^{13}C NMR δ 170.6, 139.7, 122.6, 79.6, 74.0, 73.2, 56.7, 56.0, 50.0, 42.4, 39.7, 38.1, 37.0, 36.6, 36.0, 33.3, 31.9, 28.3, 28.2, 27.8, 26.5, 24.3, 23.2, 21.4, 21.0, 19.3, 18.8, 11.9; Anal. Calcd for $C_{29}H_{48}O_4 \cdot 1/4\ H_2O$: C, 74.88%; H, 10.51%; Found: C, 75.08%; H, 10.70%.

4.1.8. Δ^5-3β,24*S*,25-Trihydroxycholestene-3,24-diacetate (10). A solution of **9** (336 mg, 0.730 mmol) in acetic anhydride (4 mL) and pyridine (4 mL) was stirred at room temperature for 9 h. The reaction mixture was partitioned between ethyl acetate (50 mL) and water. The organic layer was washed with 5% HCl (3×10 mL), sat. $NaHCO_3$ (3×10 mL), brine (3×10 mL), dried over sodium sulfate, evaporated and purified by flash chromatography (Pet. ether/ acetone 4:1) to afford pure compound **10** (378 mg, 95%): mp 175–176°C; $[\alpha]_D^{22}$=−31.1 (*c*, 0.5, $CHCl_3$); MS-EI (*m/z*): 485 (M^++1−H_2O), 442 (M^+−CH_3COOH), 425 (M^++1−CH_3COOH−H_2O); IR (cm^{-1}): 3470 (OH), 1734 (CH_3CO_2), 1713 (CH_3CO_2); ^{1}H NMR δ 0.67 (3H, s, 18-CH_3), 0.93 (3H, d, J=6.5 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.20 (3H, s, 26-CH_3), 1.20 (3H, s, 27-CH_3), 2.03 (3H, s, 3β-CH_3CO_2), 2.11 (3H, s, 24α-CH_3CO_2), 4.60 (1H, m, 3α-H), 4.72 and 4.76 (1H, d, d, J_1=2.4 Hz, J_2=10.1 Hz, 24β-H), 5.37 (1H, d, J=4.8 Hz, 6-H); ^{13}C NMR δ 171.3, 170.5, 139.7, 122.6, 80.8, 74.0, 72.5, 56.6, 55.7, 50.0, 42.3, 38.1, 37.0, 36.6, 35.8, 32.5, 31.9, 28.1, 27.8, 26.8, 26.0, 25.0, 24.2, 21.4, 21.1, 21.0, 19.3, 18.8, 11.9; Anal. Calcd for $C_{31}H_{50}O_5$: C, 74.06%; H, 10.02%; Found. C, 74.14%; H, 10.06%.

4.1.9. $\Delta^{5,25}$-3β,24*S*-Dihydroxycholestadiene-diacetate (11). To a solution of **10** (110 mg, 0.219 mmol), DMAP (1.075 mg, 5 mol%) and triethylamine (0.09 mL, 0.65 mmol) in dichloromethane (2 mL) stirred at 0°C under argon was added dropwise methanesulfonyl chloride (0.0256 mL, 0.33 mmol). After 2 h, the reaction mixture was diluted with ethyl acetate (20 mL), washed with brine (3×20 mL), dried over sodium sulfate, concentrated and purified by flash chromatography (Pet. Ether/acetone 4:1) to afford pure **11** (64 mg, 91%, recovered **10** 37 mg): mp 95–96°C; $[\alpha]_D^{24}$=−43.3 (*c*, 0.13, $CHCl_3$); MS-EI (*m/z*): 424 (M^+−CH_3COOH), 364 (M^+−2CH_3COOH), 349 (M^++1−2CH_3COOH−H_2O); IR (cm^{-1}): 3080 (C=CH_2), 1733 (CH_3CO_2), 1654 (C=CH_2), 904 (C=CH_2); ^{1}H NMR δ 0.67 (3H, s, 18-CH_3), 0.93 (3H, d, J=6.5 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.72 (3H, s, 27-CH_3), 2.03 (3H, s, 3β-CH_3CO_2), 2.06 (3H, s, 24β-CH_3CO_2), 4.60 (1H, m, 3α-H), 4.89 (1H, t, J=1.4 Hz, 26-H), 4.94 (1H, s, 26-H), 5.12 (1H, t, J=6.8 Hz, 24β-H), 5.38 (1H, d, J=4.9 Hz, 6-H); Anal. Calcd for $C_{31}H_{48}O_4$: C, 76.82%; H, 9.98%; Found: C, 76.74%; H, 10.01%.

4.1.10. Cerebrosterol (1). To a suspension of $NH_2OH \cdot HCl$ (3.59 g, 51.7 mmol) and DMF (10 mL) stirred at 0°C was

added KOH (85%, 3.41 g, 51.7 mmol). After being stirred for 30 min, the mixture was filtered and the solid was washed with DMF (2–3 mL). The combined filtrate and washings were cooled to 0°C, and then ethyl acetate (2.22 mL, 22.6 mmol) was added dropwise. After stirring for another 30 min, the solution was added dropwise to another flask containing **11** (35 mg, 0.064 mmol) stirred at 90–95°C. After stirring for 5 h, the mixture was cooled to room temperature, water (15 mL) was added. The mixture was then extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with water (3×50 mL), 5% HCl (3×50 mL), sat. $NaHCO_3$ (3×50 mL), brine (3×50 mL), dried over sodium sulfate and evaporated to afford the crude product which was dissolved in methanol (5 mL) containing water (0.3 mL) and KOH (30 mg) and refluxed for 5 h. After removal of solvents in vacuo, the residue was dissolved in ethyl acetate (50 mL), washed with water (3×10 mL), brine (3×10 mL), dried over sodium sulfate, evaporated and purified by flash chromatography (Pet. Ether/ethyl acetate 6:1) to afford pure cerebrosterol **1** (26 mg, 90%, 97% d.e.): mp 181–182°C (lit.[14a] 175–176°C; lit.[30] mp 181–182.5°C); $[\alpha]_D^{24}=-49.5$ (*c*, 0.028, $CHCl_3$) (lit.[30] $[\alpha]_D=-48.3$, $CHCl_3$); MS-EI (*m/z*): 402 (M^+), 384 (M^+-H_2O), 369 (M^+-H_2O–Me), 351 (M^+ $2H_2O$–Me); IR (cm^{-1}): 3369 (OH); ^1H NMR δ 0.68 (3H, S, 18-CH_3), 0.89 (3H, d, J=6.8 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 3.30 (1H, m, 24-H), 3.53 (1H, m, 3α-H), 5.35 (1H, d,J=5.1 Hz, 6-H); ^{13}C NMR δ_{ppm}: 140.8 (5-C), 121.8 (6-C), 77.5 (24S-C), 77.3 (24R-C), 71.9 (3-C), 56.8 (14-C), 56.0 (17-C), 50.2 (9-C), 42.4 (13-C), 42.4 (4-C), 39.9 (12-C), 37.3 (1-C), 36.6 (10-C), 36.0 (20-C), 33.2 (25-C), 32.3 (22-C), 32.1 (2-C), 32.0 (8-C), 31.8 (7-C), 30.8 (23-C), 28.3 (16-C), 24.4 (15-C), 21.2 (11-C), 19.5 (19-C), 19.2 (21-C), 18.9 (27-C), 16.8 (26-C), 12.0 (18-C); Anal. Calcd for $C_{27}H_{46}O_2\cdot 1/2\ H_2O$: C, 78.78%, H, 11.51%; Found: C, 78.58%, H, 11.67%.

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