Supporting Information

Experimental Section

General. The ^{1}H and ^{13}C NMR spectra were generated at 400 and 100 MHz, utilizing 7.28 and 77.0 ($CDCl_3$) ppm as the references respectively. Elemental analyses were performed at Oneida Research Services, Inc., Whitesboro, NY. Fast Atom Bombardment mass spectral analysis was carried out at M-Scan Inc., West Chester, PA. The starting material, 3-keto-23,24-bisnorchol-4-ene-22-ol (**1**), was purchased from Pharmacia & Upjohn, Kalamazoo, MI 49001.

(7-α-)-3-Ketobisnorchol-4-ene-7,22-diol (2). *Diplodia gossypina* (ATCC 20576) was grown on potato dextrose agar for 48 h until good growth was observed. This culture was used to inoculate a medium composed of NZ amine HD (20 g/L, Quest International), corn steep liquor (3 g/L) and dextrose (50 g/L), which had been sterilized by autoclaving for 20 min. The culture was incubated on a rotary shaker at 250 rpm at 23 ^{0}C for 48 hours. Two, two-liter capacity Biostat B fermentors (B. Braun, Allentown, PA) were sterilized empty for one hour before one liter of sterile medium was aseptically added to each fermentor. Media, which consisted of distilled water (1 L), glucose (20 g), sodium chloride (5 g), potassium hydrogenphosphate (5 g), soybean flour (5 g), yeast extract (5 g), and SAG 471 antifoam (1 mL), was sterilized for 20 min. For incubation, pH and dissolved oxygen (DO) probes were calibrated, fermentor agitation and temperature were set to 800 rpm and $24^{0}C$, respectively. During incubation, the aeration rate was set 1 L/minute without DO control. Each fermentor was inoculated with a 48 h old, 100 mL inoculum culture. At 24 h after inoculation, dextrose (10 g) and of 3-keto-23,24-bisnorchol-4-ene-22-ol (**1**) (3.0 g, 9.1 mmol) dissolved in dimethylformamide (12 mL) was added to each fermentor. The incubation was continued while the pH of the culture was controlled at 5.0 by automatic addition of 2 N HCl or 2 N NaOH. During the course of the biotransformation, the dissolved oxygen level was measured at approximately 45%. Samples were taken at 40 h and 48 h and 64 h, when the culture was harvested. The samples were analyzed for the substrate **1** and the 7α-hydroxy product **2** by HPLC (Phenomenex Kromasil 5 μ C4 100 A^{0} 250 x 4.6 mm column, flow rate of 1 mL/min, gradient of water/acetonitrile (90:10 to 10:90 in 20 minutes)). The estimated yield of **2** at the peak concentration during fermentation was 800 mg/L (25%) by this method (based on 50% recovery). To recover product, the cultures (500 mL of each) were combined, and extracted with 9:1 chloroform:acetonitrile (4 x 1L) and 9:1 ethyl acetate:acetonitrile (4 x 1L). The solvent was concentrated to 20 mL on a rotary evaporator before being dried completely using a vacuum concentrator (Savant Instruments). The residue (1.27 g) was dissolved in ethyl acetate and chloroform (10 mL total) and loaded on a silica gel column (35 g). Flash chromatography (gradient with 0 to 100% ethyl acetate in hexane) afforded recovered **1** (155 mg) and product **2** (155 mg, mp 185-187 ^{o}C, lit. mp 197.5-200 ^{o}C[4a], MW 346.52, FW 348.32), ^{1}H NMR ($CDCl_3$): δ 5.83 (s, 1H), 3.99 (br s, 1H), 3.65 (d of d, J = 10.5 and 3.1 Hz, 1H), 3.38 (d of d, J = 10.5 and 6.9 Hz, 1H), 2.65-2.61 (m, 1H), 2.46-2.38 (m, 3H), 2.07-1.23 (m,

15H), 1.21 (s, 3H), 1.07 (d, J = 6.7 Hz, 3H), 0.75 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 198.7, 167.5, 126.8, 68.4, 67.8, 52.3, 50.2, 45.2, 42.5, 40.9, 39.8, 39.0, 38.7, 38.4, 35.4, 33.9, 27.6, 23.6, 20.8, 17.0, 16.7, 11.8; Anal. Calcd for $C_{22}H_{34}O_3$-0.1H_2O: C, 75.87; H, 9.90. Found: C, 75.76; H, 9.68.

(5-α-, 7-α-)-3-Ketobisnorcholan-7,22-diol (3). Liquid ammonia (125 mL) was treated with tetrahydrofuran (15 mL) and lithium (300 mg, 43 mmol) and stirred for 30 min. Then a solution of **2**[4a] (352 mg, 1.20 mmol) in tetrahydrofuran (20 mL) and ethanol (0.4 mL) was added. The reaction mixture was stirred for 40 min and then 20 g of ammonium chloride was added. The solvent was evaporated under nitrogen and the residue was treated with water (200 mL) and extracted with ethyl acetate (3 x 75 mL). The organic phase was washed with brine, dried over sodium sulfate, filtered, and evaporated. Purification of the resulting solid by flash chromatography on silica gel (hexane-ethyl acetate- methanol 10:10:1) afforded pure **3** (251 mg, 71%, mp 221-223 ^{o}C, MW 348.53); ^{1}H NMR ($CDCl_3$): δ 3.86 (br s, 1H), 3.65-3.62 (m, 1H), 3.39-3.36 (m, 1H), 2.34-1.18 (m, 23H), 1.05 (d, J = 6.6 Hz, 3H), 1.01 (s, 3H), 0.71 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 67.9, 67.4, 52.4, 50.2, 45.2, 44.1, 42.7, 39.5, 39.2, 39.0, 38.7, 38.1, 36.5, 35.6, 27.7, 23.7, 21.2, 16.7, 11.9, 10.4; MS (+FAB): 349 ($[M+1]^+$, 100), 331 (52); Anal. Calcd for $C_{22}H_{36}O_3$: C, 75.82; H, 10.41. Found: C, 75.71; H, 10.19.

(5-α-, 7-α-)-3-Dioxolane Bisnorcholan-7,22-diol (4). To a mixture of steroid **3** (101 g, 0.290 mol) and anhydrous ethylene glycol (800 mL) was added chlorotrimethylsilane (200 mL, 1.58 mol) over 60 min at rt under nitrogen. The reaction mixture was stirred at rt for 19 h. The mixture was poured slowly into saturated sodium bicarbonate solution (1 L) and extracted with dichloromethane (3 x 500 mL). The organic layer was washed with brine (3 x 150 mL) and dried over sodium sulfate (20 g). After filtration and evaporation, the product was recrystallized from ethyl acetate in hexane (800 mL). The solid was filtered and washed with hexane (150 mL) to afford **4** (96.14 g, 84%, mp 173-175 ^{o}C, MW 392.58); ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 4H), 3.83 (br s, 1H), 3.65 (d of d, J = 10.4 and 3.1 Hz, 1H), 3.36 (d of d, J = 10.4 and 7.1 Hz, 1H), 2.0-1.8 (m, 3H), 2.7-1.1 (m, 21H), 1.05 (d, J = 6.6 Hz, 3H), 0.82 (s, 3H), 0.69 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 109.2, 67.8, 64.1, 52.4, 50.3, 45.6, 42.7, 39.5, 39.3, 38.8, 37.4, 36.2, 36.1, 35.7, 35.5, 31.2, 27.7, 23.7, 20.9, 16.7, 11.9, 10.3; MS (+FAB): 394 ($[M+1]^+$, 100); Anal. Calcd for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.15; H, 10.15.

(5-α-, 7-α-)-3-Dioxolane-7-hydroxy Bisnorcholan-22-al (5). To a solution of **4** (100 g, 255 mmol) in methylene chloride (1,200 mL) was added potassium bromide (3.19 g, 26.8 mmol) and sodium bicarbonate (10.97 g, 130 mmol) dissolved in water (120 mL). The cooled (0 ^{o}C) reaction mixture was treated with TEMPO (1.20 g, 7.7 mmol) and 10-13% sodium hypochlorite (170 mL, 275-358 mmol). After stirring (magnetic) for 2 h at 0 ^{o}C, the reaction mixture was treated with sodium thiosulfate (20 g, 126 mmol) in water (220 mL). The organic phase was separated, washed with brine (3 x 70 mL), dried over sodium sulfate (30 g), filtered, and concentrated *in vacuo* for 18 h at rt to afford **5**

(99.5 g, 98%, MW 390.57, FW 397.77); ^{1}H NMR ($CDCl_3$): δ 9.57 (d, J = 3.4 Hz, 1H), 3.95 (s, 4H), 3.83 (br s, 1H), 3.76 (m, 1H), 2.35 (m, 1H), 2.0-1.2 (m, 21 H), 1.13 (d, J = 6.8 Hz, 3H), 0.83 (s, 3H), 0.72 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 204.9, 109.0, 67.6, 64.0, 50.8, 49.7, 49.3, 45.4, 43.0, 39.3, 39.0, 37.3, 36.2, 35.9, 35.6, 35.4, 31.0, 26.8, 23.8, 20.7, 13.3, 12.1, 10.2; MS (+FAB): 391 ($[M+1]^+$, 100); Anal. Calcd for $C_{24}H_{38}O_4$-0.4H_2O: C, 72.47; H, 9.83. Found: C, 72.49; H, 9.77.

(5-α-, 7-α-)-3-Dioxolane-7-hydroxy Cholest-23-en-24-one (7). A mixture of 97% sodium *t*-butoxide (37 g, 373 mmol) and anhydrous tetrahydrofuran (400 mL) was stirred for 10 min under nitrogen and then a solution of **6**[10](94 g, 423 mmol) in tetrahydrofuran (150 mL) was added in one portion. The mixture initially warmed to 41 ^{o}C, but returned to 24 ^{o}C while stirring (45 min). Then a solution of **5** (99.48 g, 250 mmol) in tetrahydrofuran (400 mL) was added over 60 min. The reaction mixture was stirred overnight at rt (18 h) and then water was added (30 mL). The reaction mixture was concentrated *in vacuo* and treated with cyclohexane (1200 mL), toluene (600 mL) and water (160 mL). The organic layer was separated, washed with brine (3 x 100 mL) and water (160 mL), dried over sodium sulfate (30 g), filtered, and evaporated to yield a solid. The crude solid was recrystallized from ethyl acetate in cyclohexane and dried *in vacuo* at 50 ^{o}C for 5 h to yield **7** (94.64 g, 82%, mp 177-178 ^{o}C, MW 458.69); ^{1}H NMR ($CDCl_3$): δ 6.72 (d of d, J = 15.7 and 9.0 Hz, 1H), 6.07 (d, J = 15.7 Hz, 1H), 3.94 (s, 4H), 3.83 (br s, 1H), 2.85 (hept, J = 6.9 Hz, 1H), 2.29 (m, 1H), 2.0- 1.1 (m, 22 H), 1.11 (m, 9H), 0.83 (s, 3H), 0.71 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 204.5, 152.4, 126.2, 109.1, 67.8, 64.1, 54.9, 50.4, 45.6, 43.0, 40.0, 39.5, 39.3, 38.1, 37.4, 36.3, 36.1, 35.7, 31.2, 28.1, 23.6, 20.9, 19.3, 18.6, 18.4, 12.1, 10.3; MS (+FAB): 459 ($[M+1]^+$, 92), 99 (100); Anal. Calcd for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.57; H, 9.87.

(5-α-, 7-α-, 24*S*-)-7, 24-Dihydroxy-3-dioxolane Cholest-23-ene (8). A dried and nitrogen blanketed reactor was charged with 1 M (*R*)-MeCBS reagent in toluene (20 mL, 20 mmol) and 1 M borane-tetrahydrofuran complex in tetrahydrofuran (25 mL, 25 mmol) and stirred for 2 h at rt. The reaction mixture was cooled (-15 to -28 ^{o}C), treated with steroid **7** (9.16 g, 20 mmol) in tetrahydrofuran (150 mL), and stirred for 2 hr (-20 to –28 ^{o}C). The reaction mixture was treated with methanol (25 mL) with stirring for 18 hr at rt, and then repeatedly evaporated by distillation and treated with methanol (4 x 30 mL) to exchange solvents. Finally methanol (70 mL) was added and the reaction mixture was brought to reflux, cooled in the freezer (no crystals formed), and concentrated *in vacuo*. Recrystallization from acetonitrile (100 mL), filtration, and evaporation at 50-60 ^{o}C for 7 hr afforded crystals of **8** (7.43 g, 80%, mp 121-125 ^{o}C, MW 460.70, FW 464.31); ^{1}H NMR ($CDCl_3$): δ 5.5-5.3 (m, 2H), 3.94 (s, 4H), 3.82 (br s, 1H), 3.75 (m, 1H), 2.2-1.1 (m, 25H), 1.05 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H), 0.83 (s, 3H), 0.70 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 139.5, 128.6, 109.2, 78.5, 67.8, 64.1, 55.5, 50.6, 45.6, 42.6, 40.0, 39.5,39.4, 37.5, 36.2, 36.1, 35.7, 35.6, 33.9, 31.2,

28.7, 23.6, 20.9, 20.4, 18.3, 18.1, 12.0, 10.3 ; MS (+FAB): 462 ($[M+1]^+$, 100); Anal. Calcd for $C_{29}H_{48}O_4$-0.2H_2O: C, 75.02; H, 10.51. Found: C, 75.00; H, 10.48.

(5-α-, 7-α-, 24*R*-)-7, 24-Dihydroxy-3-dioxolane Cholestane (9). Steroid **8** (10.0 g, 21.5 mmol), toluene (170 mL), triethylamine (1 mL), and 10% platinum on carbon (0.5 g) were combined under 50 psi of hydrogen in a Parr apparatus (19 h). The reaction mixture was filtered through Celite® (10 g), washed with chloroform and ethyl acetate (10 mL total), and concentrated *in vacuo* to afford a solid, which was recrystallized from ethyl acetate in hexane (180 mL). The solid was filtered and concentrated at 50-60 ^oC under vacuum for 7 h to afford pure **9** (9.24 g, 92%, mp 161-163 ^oC, MW 462.72, FW 466.32); ^1H NMR ($CDCl_3$): δ 3.95 (s, 4H), 3.84 (br s, 1H), 3.33 (br s, 1H), 2.0-1.1 (m, 29H), 0.93 (m, 9H), 0.83 (s, 3H), 0.67 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 109.2, 77.0, 67.8, 64.1, 55.9, 50.5, 45.5, 42.6, 39.5, 37.4, 36.2, 36.1, 35.7, 35.5, 33.5, 32.0, 31.2, 30.5, 28.2, 23.6, 20.9, 18.8, 18.6, 17.2, 11.8, 10.3; MS (+FAB): 463 ($[M+1]^+$, 100); Anal. Calcd for $C_{29}H_{50}O_4$-0.2H_2O: C, 74.70; H, 10.89. Found: C, 74.48; H, 10.49.

(5-α-, 7-α-, 24*R*-)-7, 24-Dihydroxy-3-ketocholestane (10). Steroid **9** (2.03 g, 4.35 mmol), *p*-toluenesulfonic acid (200 mg), water (1 mL), and acetone (100 mL) were combined with stirring for 4 h. The reaction mixture was concentrated *in vacuo* and treated with dichloromethane (100 mL) and saturated sodium bicarbonate solution (50 mL). The organic layer was removed, washed with brine (3 x 25 mL), dried over sodium sulfate (10 g), filtered, and evaporated at 50-60 ^oC. The solid was recrystallized from ethyl acetate in hexane (50 mL), filtered, washed with hexane, and dried *in vacuo* at 50-60 ^oC for 7 hr to afford **10** (1.63 g, 89%, mp 151-153 ^oC, MW 418.67); ^1H NMR ($CDCl_3$): δ 3.88 (br s, 1H), 3.33 (br s, 1H), 2.5-1.1 (m, 29H), 1.02 (s, 3H), 0.94 (m, 9H), 0.71 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 212.0, 76.9, 67.3, 56.1, 50.3, 45.1, 44.1, 42.6, 39.4, 39.0, 38.1, 38.0, 36.6, 35.8, 35.6, 33.6, 32.1, 30.6, 28.2, 23.6, 21.1, 18.9, 18.6, 17.3, 11.8, 10.4; MS (+FAB): 419 ($[M+1]^+$, 100); Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07. Found: C, 77.25; H, 11.04.

Potassium (5-α-, 7-α-, 24*R*-)-7-Hydroxy-3-keto-cholestan-24-yl Sulfate (11). A dried and nitrogen blanketed flask was treated with compound **10** (2.09 g, 5.0 mmol) dissolved in anhydrous pyridine (30 mL). Sulfur trioxide pyridine complex (836 mg, 5.25 mmol, 1.05 equiv.) dissolved in pyridine (20 mL) was added to the reaction mixture, which was stirred for 4 h at rt. Water was added (10 mL) and the pyridine was removed by concentration *in vacuo* at 40 ^oC. The residue was treated with ethyl acetate (50 mL) and potassium chloride (1.12 g, 15 mmol) dissolved in water with stirring for 1.5 h. The potassium salt of **11** was collected on Celite® (3 g) by filtration, washed with ethyl acetate (50 mL) and water (10 mL), and dissolved in 1 N potassium hydroxide in methanol (10 mL, 10 mmol) and methanol (100 mL). The methanol was removed *in vacuo* to dryness and the solid was washed with water (30 mL), filtered, and dried *in vacuo* at rt for 20 hr to afford **11** (2.10 g, 77%, MW 536.82, FW

544); ^{1}H and ^{13}C NMR were identical to published spectra. HPLC analysis by the method described previously[2] indicated a diastereomeric excess of 95 % which is comparable to what was achieved previously (94%).