

## **SUPPLEMENTARY MATERIAL**

### **Synthesis and structural characteristics of lithocholate triads: steroid type channels occupied by spacer fragments**

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## 1S. Synthesis of compounds 5 and 7

**1S.1. Synthesis of bis(23-benzyloxycarbonyl-24-nor-5 $\beta$ -cholan-3 $\alpha$ -yl) terephthalate (8).** To a solution of benzyl lithocholate **3** (Yuexian & Dias, 1997) (1 g, 2.14 mmol) in anhydrous benzene (10 ml) terephthalic acid dichloride (239 mg, 1.17 mmol) and pyridine (5 ml) were added. After 4 hours, additional portion of dichloride (110 mg, 0.584 mmol) was added. The reaction was carried out overnight (total reaction time 18 hours) until all compound **3** had reacted (TLC control). Chloroform (40 ml) was added and the solution was washed twice with HCl (1N) solution, NaHCO<sub>3</sub> (5%) and brine. The organic layer was dried with MgSO<sub>4</sub> and evaporated in vacuo to give 1.14 g of the crude product, which was purified by column chromatography on silica gel with benzene as eluent. The ester **8** was obtained (838 mg) as a white solid, mp 194-195 °C (hexane-AcOEt). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.638 (s, 6H, CH<sub>3</sub>-18), 0.924 (d, 6H, J=6Hz, CH<sub>3</sub>-21), 0.967 (s, 6H, CH<sub>3</sub>-19), 4.991 (m, 2H, 3-H), 5.12 (s, 4H, O-CH<sub>2</sub>-Ph), 7.36 (m, 10H, -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>), 8.09 (s, 4H, CO-C<sub>6</sub>H<sub>4</sub>-CO). <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 11.98 (C-18), 18.22 (C-21), 20.84 (C-11), 23.31 (C-19), 24.12 (C-15), 26.30 (C-7), 26.69 (C-6), 27.01 (C-16), 28.11 (C-2), 30.94 (C-23), 31.29 (C-22), 32.29 (C-10), 34.61 (C-1\*), 35.03 (C-4\*), 35.26 (C-8\*\*), 35.73 (C-20\*\*), 40.09 (C-12), 40.47 (C-9), 41.96 (C-5), 42.71 (C-13), 55.99 (C-17), 56.43 (C-14), 66.03 (-OCH<sub>2</sub>Ph), 75.56 (C-3), 128.14, 128.20, 128.51, 129.37 134.53, 136.15, 165.39 (OOC-C<sub>6</sub>H<sub>4</sub>-COO), 170.04 (C-24). IR (KBr):  $\nu_{\max}$  = 3034, 2923, 1732, 1714, 1451, 1382, 1279, 1124, 1105, 729, 656 cm<sup>-1</sup>. MS (ES): m/z = 907 [M-2Bn+Na<sup>+</sup>]<sup>+</sup>, 994 [M-Bn+Na<sup>+</sup>]<sup>+</sup>, 882 [M-2Bn+H<sup>+</sup>]<sup>+</sup>. Anal. Calcd. for C<sub>70</sub>H<sub>94</sub>O<sub>8</sub>: C, 79.06; H, 8.91; O, 12.04. Found: C, 78.61; H, 9.10; O, 12.29.

**1S.2. Synthesis of bis(23-carboxy-24-nor-5 $\beta$ -cholan-3 $\alpha$ -yl) terephthalate (5).** To a solution of triad **8** (500 mg, 0.47 mmol) in benzene-methanol (1:1, 40 ml), Pd/C catalyst (10%, 100 mg) and ammonium formate (570 mg, 9 mmol) was added and the mixture was refluxed for 1.5 h (TLC control). The warm solution was filtered and the solid was washed with hot chloroform (twice 25 ml). The combined solutions were evaporated in vacuo. To the residue a hot mixture of chloroform-dioxane (1:1, 60 ml) was added and the solid was filtered off. The filtrate was evaporated and the residue (415 mg) was crystallized from chloroform-methanol to give compound **5** as crystals, which melt at 320°C. <sup>1</sup>H NMR (300 MHz deuterium pyridine):  $\delta$  = 0.613 (s, 6H, CH<sub>3</sub>-18), 0.912 (s, 6H, CH<sub>3</sub>-19), 1.01 (d, J=5.7Hz, 6H, CH<sub>3</sub>-21), 2.49-2.71 (m, 4H, CH<sub>2</sub>-COOH), 5.179 (m, 2H, 3-H), 8.473 (s, 4H, -C<sub>6</sub>H<sub>4</sub>-). <sup>13</sup>C NMR (300 MHz deuterium pyridine):  $\delta$  = 12.21, 18.56, 21.07, 23.36, 24.34, 26.46, 26.94, 27.24, 28.39, 31.75, 31.82, 32.53, 34.66, 35.17, 35.65, 35.81, 40.16, 40.50, 42.08, 42.80, 56.25, 75.80, 129.80, 165.11, 176.24. IR (KBr):  $\nu_{\max}$  = 3417, 2933, 2866,

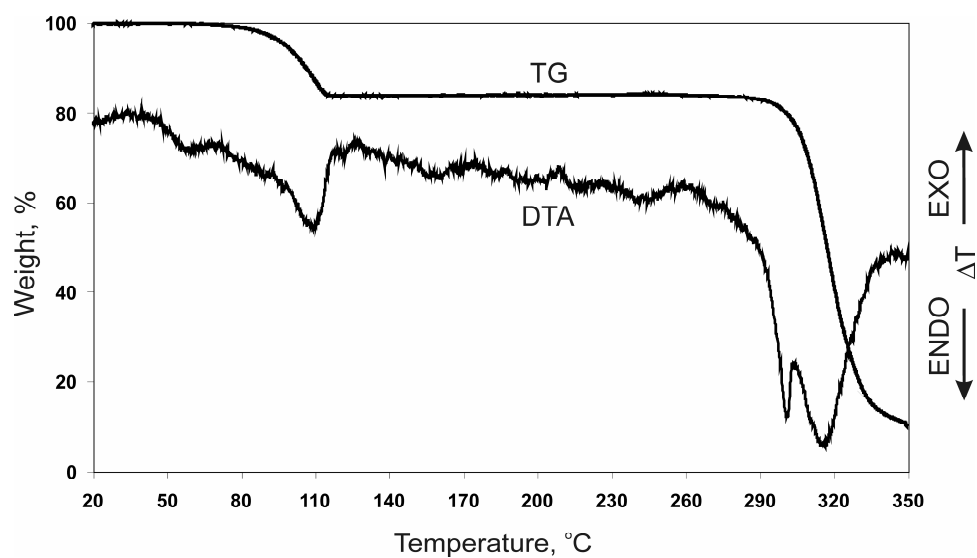
1710, 1717, 1279, 1127, 1106, 1465, 1447, 1020  $\text{cm}^{-1}$ . MS (ES):  $m/z = 882$   $[\text{M}-\text{H}]^+$ , 906  $[\text{M}+\text{Na}]^+$ . Compound **5** was crystallized from pyridine to give solvated crystals of **5+2Py**.

**1S.3. Synthesis of methyl 3 $\alpha$ -chloroformyloxy-5 $\beta$ -cholan-24-ate (4).** To a solution of methyl lithocholate **2** (Dayal, et al., 1981) (50 mg, 0.125 mmol) in anhydrous methylene chloride (2 ml) triphosgene (25 mg, 0.085 mmol) was added. To this solution kept at  $-20\text{ }^{\circ}\text{C}$  pyridine (25  $\mu\text{l}$ , 0.315 mmol) was added and the reaction mixture was stirred until all substrate reacted (TLC test). The solvent was evaporated and benzene (3 ml) was added. The organic layer was washed with HCl (1 N) and brine, dried with magnesium sulfate and evaporated. The residue was purified by column chromatography on silica gel with benzene as eluent to give compound **4** (52 mg, 90%).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ):  $\delta = 4.81$  (m, 1H, 3-H), 3.67 (s, 3H,  $-\text{COOCH}_3$ ), 2.16-2.41 (m, 2H,  $-\text{CH}_2\text{CO}_2\text{Me}$ ), 0.93 (s, 3H,  $\text{CH}_3$ -19), 0.90-0.92 (d, J = 6.3 Hz, 3H,  $\text{CH}_3$ -21), 0.64 (s, 3H,  $\text{CH}_3$ -18).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ):  $\delta = 11.97, 18.20, 20.77, 23.09, 24.10, 26.17, 26.85, 28.10, 30.93, 30.99, 31.69, 34.46, 34.74, 35.30, 35.69, 40.02, 40.37, 41.89, 42.67, 51.41, 55.93, 56.38, 83.80, 149.75, 174.71$ . IR (KBr):  $\nu_{\text{max}} = 2947, 2881, 1777, 1732, 1469, 1437, 1380, 1209, 1179, 1162, 970, 868, 825, 685\text{ cm}^{-1}$ . MS (ESI):  $m/z$ : 491  $[\text{M}+\text{K}]^+$ , 475  $[\text{M}+\text{Na}]^+$ .

**1S.4. Synthesis of bis(23-methoxycarbonyl-24-nor-5 $\beta$ -cholan-3 $\alpha$ -yl) piperazine-1,4-dicarboxylate (7).** To a solution of chloroformate **4** (300 mg, 0.66 mmol) in tetrahydrofuran (5 ml) 4-dimethylaminopyridine (80.99 mg, 0.66 mmol) and piperazine (28.5 mg, 0.33 mmol) was added and the reaction mixture was stirred overnight at room temperature. Benzene-ether (10 ml, 1:1) was added and the organic layer was washed with HCl (1 N),  $\text{NaHCO}_3$  (5%) and brine, dried with magnesium sulfate and evaporated. The crude product was purified by column chromatography on silica gel with benzene/ethyl acetate (20:1) as eluent to give compound **7** (285 mg, 94%), which crystallized from the 4:1 mixture of  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$  to give solvated crystals of **7+0.5MeOH** which melt at  $290\text{ }^{\circ}\text{C}$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ):  $\delta = 4.63$  (m, 2H, 2\*3-H), 3.66 (s, 6H,  $-\text{COOCH}_3$ ), 3.44 (s, 8H,  $-\text{N}-(\text{C}_2\text{H}_4)_2-\text{N}-$ ), 2.16-2.40 (dm, 4H, 2\* $-\text{CH}_2-\text{COOMe}$ ), 0.93 (s, 6H, 2\* $\text{CH}_3$ -19), 0.91 (d, J = 6.03 Hz, 6H, 2\* $\text{CH}_3$ -21), 0.65 (s, 6H, 2\* $\text{CH}_3$ -18).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ):  $\delta = 12.11$  (C-18), 18.34 (C-21), 20.90 (C-11), 23.39 (C-19), 24.23 (C-15), 26.39 (C-2), 27.08 (C-7), 27.13 (C-6), 28.23 (C-16), 31.04 (C-22), 31.08 (C-23), 32.78 (C-1), 34.60 (C-10), 35.09 (C-4), 35.39 (C-20), 35.80 (C-8), 40.14 (C-12), 40.47 (C-9), 41.93 (C-5), 42.75 (C-13), 43.48 (C-27), 51.51 ( $\text{OCH}_3$ ), 55.99 (C-17), 56.47 (C-14), 75.62 (C-3), 154.97 (CO-3), 174.57 (C-24). IR (KBr):  $\nu_{\text{max}} = 2971, 2945, 2937, 2863, 1748, 1728, 1695, 1466, 1435, 1376, 1359, 1309, 1233, 1158, 1105, 982, 939, 827, 756\text{ cm}^{-1}$ . MS (ESI):  $m/z$ : 942  $[\text{M}+\text{Na}]^+$ .

## 2S. Thermogravimetric analysis.

The sample of **5**+2Py was heated at a rate of 2°C/min in the He gas atmosphere, flowing at 32ml/min. TG and DTA curves shown in Fig. 1 yielded a distinct weight-loss event in the range of 79 to 115 °C, accounting for the total loss of pyridine solvent, originally present in the material (calc. 15.2%, found 15.8%), followed by a plateau from 115 to 285 °C. The wide range of the plateau indicates high thermal robustness of the solvent free framework.



**Figure 1S.**

TG-DTA diagram of the 1:2 complex of **5** with pyridine