CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

Structure Activity Relationships in Cholapod Anion Carriers: Enhanced Transmembrane Chloride Transport through Substituent Tuning.

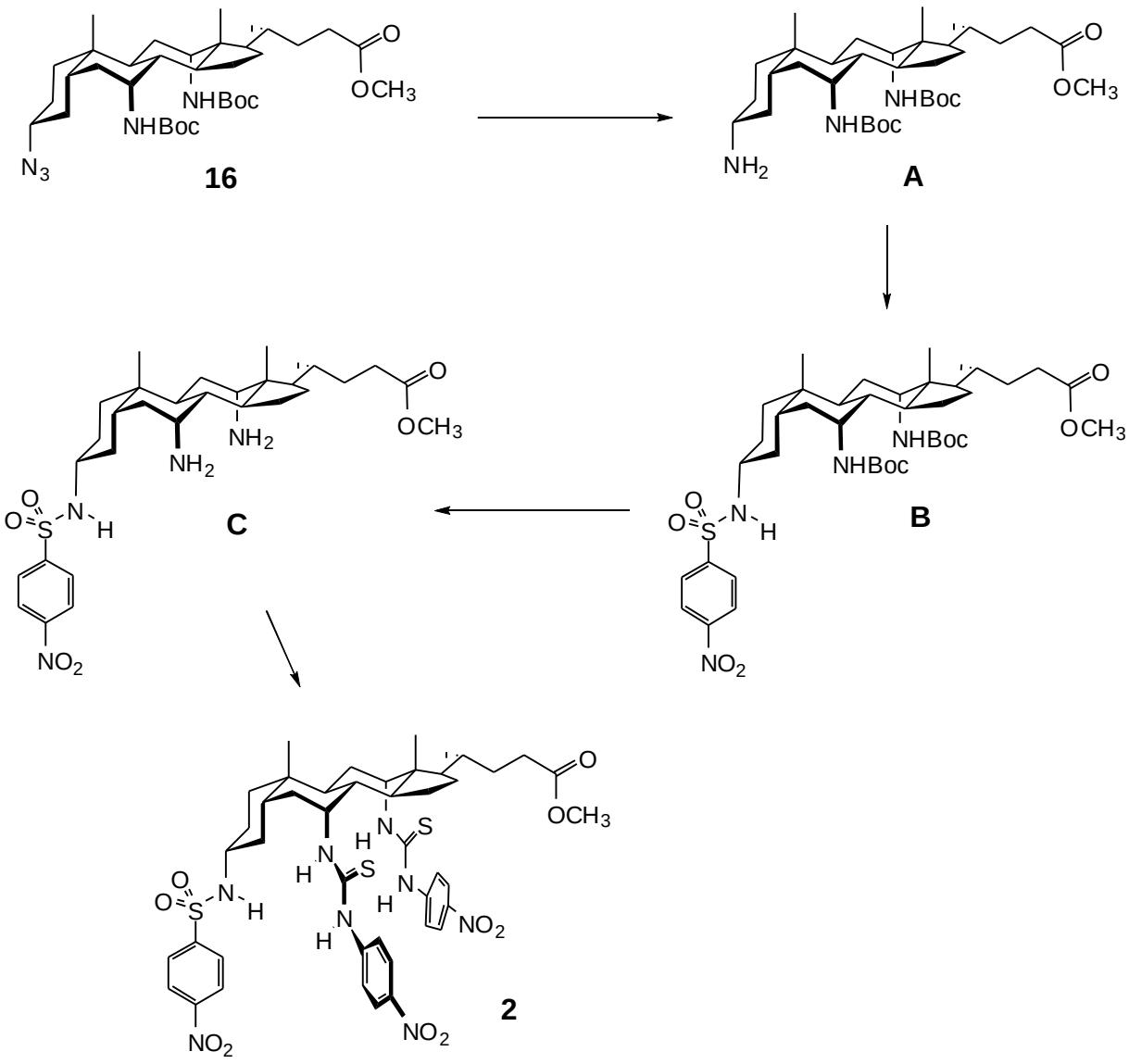
Beth A. McNally,[b] Atanas V. Koulov,[b] Timothy N. Lambert,[b] Bradley D. Smith,*[b] Jean-Baptiste Joos,[a] Adam L. Sisson,[a] John P. Clare,[a] Valentina Sgarlata,[a] Luke W. Judd,[a] Germinal Magro[a] and Anthony P. Davis*[a]

[a] *School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS (UK).*

[b] *Dept. of Chemistry and Biochemistry and Walther Cancer Research Center, University of Notre Dame, Notre Dame, Indiana 46556 (USA).*

Supporting Information

Synthesis of cholapods 2, 3, 5, 8, 9, 10, 11 and 14.



Methyl 3a-amino-7a,12a-di-[*N*-(*t*-butoxycarbonyl)amino]-5b-cholan-24-oate (A): To a solution of azide **16**[1] (232 mg, 0.36 mmol) in dry tetrahydrofuran (3.6 mL) was added a solution of trimethylphosphine in tetrahydrofuran (1 M, 0.72 mL, 0.72 mmol). The reaction was stirred overnight followed by the addition of water (130 µL, 7.2 mmol). The reaction was stirred for another 16 hours before the solvent was removed under reduced pressure. A white foam was obtained. The latter was purified by flash chromatography (gradient dichloromethane/methanol 96:4 to 8:2) yielding amine **A** as a white solid (224 mg, 100%). R_f = 0.37 (dichloromethane/methanol 85:15); ^{1}H NMR (400 MHz; $CDCl_3$, 25 °C): *d* = 0.78 (s, 3H; 18-CH_3), 0.88 (d, ^3J(H,H) = 6.4 Hz, 3H; 21-CH_3), 0.92 (s, 3H; 19-CH_3), 1.45 (s, 18H; $(CH_3)_3C$), 2.64 (m, 1H; 3β-H), 3.66 (s, 3H; $COOCH_3$), 3.67 (m, 1H; 7β-H), 3.93 (m, 1H; 12β-H), 4.80 (br s, 1H; NH), 4.87 (br s, 1H; NH); ^{13}C

NMR (100 MHz; $CDCl_3$, 25 °C): *d* = 13.74 (CH_3), 17.27 (CH_3), 23.06 (CH_2), 23.15 (CH_3), 26.82 (CH_2), 27.12 (CH_2), 28.44 (CH_3), 29.17 (CH), 30.75 (CH_2), 30.86 (CH_2), 31.27 (CH_2), 32.20 (CH_2), 34.69 (C), 34.93 (CH_2), 35.57 (CH_2), 37.01 (CH), 40.08 (CH_2), 41.83 (CH), 44.76 (C), 45.04 (CH), 47.13 (CH), 48.46 (CH), 51.57 (CH), 51.71 (CH_3), 53.11 (CH), 79.00 (C), 79.03 (C), 155.24 (C), 155.40 (C), 174.77 (C); IR (Neat): n_{max} = 3371, 2916, 2859, 1706, 1506, 1363, 1241, 1166 cm^{-1}; MS (FAB+): *m/z* (%): 621 (100) $[M+H]^+$, 642 (70) $[M+Na]^+$.

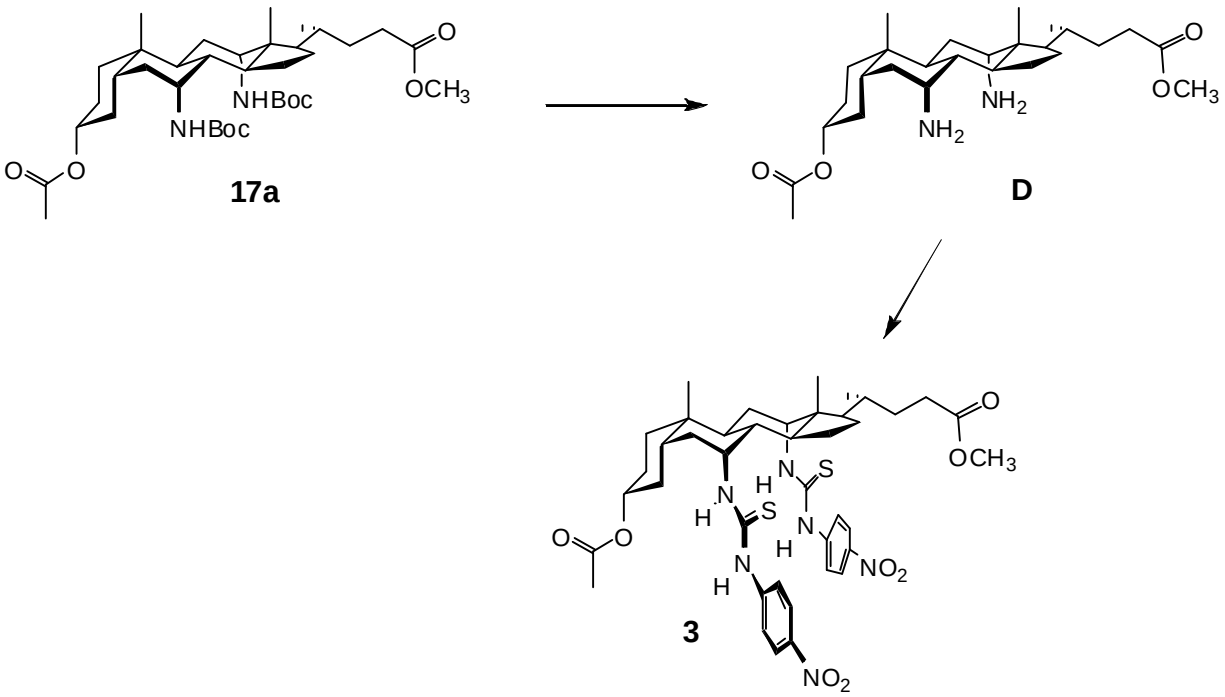
Methyl 3a-[(4-nitrobenzenesulfonyl)amino]-7a,12a-di-[*N*-(*t*-butoxycarbonyl)amino]-5b-cholan-24-oate (B): To a solution of amine **A** (1.01 g, 1.63 mmol) in dry dichloromethane (16 mL) was added triethylamine (409 mg, 564 µl, 4.04 mmol) followed by 4-nitrobenzenesulfonyl chloride (1.08 g, 4.89 mmol). The slightly orange reaction mixture was stirred for 4 hours at room temperature. The reaction mixture was diluted with dichloromethane and washed with a saturated sodium bicarbonate solution. The aqueous phase was extracted with dichloromethane and the combined organic phases were dried over magnesium sulfate. After evaporation of the solvent under reduced pressure, the crude mixture was purified by flash chromatography (dichloromethane/methanol 98:2) to give **B** as a white solid (1.03 g, 78 %). R_f = 0.28 (dichloromethane/methanol 98:2); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): *d* = 0.80 (s, 3H; 18-CH_3), 0.89 (d, ^3J(H,H) = 6.4 Hz, 3H; 21-CH_3) 0.91 (s, 3H; 19-CH_3), 1.40 (s, 18H; $C(CH_3)_3$), 2.92 (m, 1H; 3β-H), 3.59 (s, 3H, $COOCH_3$), 3.65 (m, 1H; 7β-H), 4.00 (m, 1H; 12β-H), 5.94 (br s, 1H; carbamate NH), 6.06 (br s, 1H; carbamate NH), 6.54 (br s, 1H; SO_2NH), 8.13 (d, ^3J(H,H) = 8.8 Hz, 2H; aryl H), 8.43 (d, ^3J(H,H) = 8.8 Hz, 2H; aryl H); ^{13}C NMR (100 MHz; $CDCl_3$, 25 °C): *d* = 13.67 (CH_3), 17.28 (CH_3), 22.88 (CH_3), 22.96 (CH_2), 27.13 (CH_2), 28.40 (CH_3 + CH), 30.75 (CH_2), 31.16 (CH_2), 31.87 (CH_2), 34.49 (C), 34.89 (CH), 35.52 (CH_2), 36.81 (CH), 37.46 (CH_2), 42.01 (CH), 44.75 (C + CH), 47.02 (CH), 48.45 (CH), 51.45 (CH_3), 53.12 (CH), 54.71 (CH), 79.07 (C), 124.26 (CH), 128.14 (CH), 147.60 (C), 149.92 (C), 155.47 (C), 174.54 (C); IR (Neat): n_{max} = 3396, 3350, 3260, 2948, 2867, 1703, 1533, 1498, 1450, 1431, 1364, 1348, 1242, 1164, 1067, 1045, 1013, 739 cm^{-1}; m.p. 222-224 °C (crystallised from ethyl acetate/hexane); MS (ES+): *m/z* (%): 827.6 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{41}H_{64}N_4O_{10}S$ (805.03): C 61.17, H 8.01, N 6.96; found: C 61.19, H 7.82, N 6.80.

Methyl 3a-[(4-nitrobenzenesulfonyl)amino]-7a,12a-diamino-5b-cholan-24-oate (C): Dicarbamate **B** (205 mg, 0.255 mmol) was stirred at 0 °C in a solution of trifluoroacetic acid in dry dichloromethane (30%, 7.5 mL). After 1 hour the ice bath was removed and the reaction was

allowed to stir for another 2.5 hours at room temperature. The solvent was removed under reduced pressure, redissolved in dichloromethane and washed with a saturated sodium bicarbonate solution. After extraction of the aqueous phase with dichloromethane the combined organic phases were dried over magnesium sulfate. Evaporation of the solvent yielded the pure diamino compound **C** (150 mg, 97 %) as a slightly yellow solid. R_f = 0.28 (DCM/methanolic ammonia[2] 94:6); ^{1}H NMR (400 MHz; $CDCl_3$, 25 °C): *d* = 0.70 (s, 3H; 18-CH_3), 0.87 (s, 3H; 19-CH_3), 0.96 (d, ^3J(H,H) = 6.4 Hz, 3H; 21-CH_3), 2.4-3.00 (br s; amine NH), 3.08 (m, 1H; 3β-H), 3.14 (m, 1H), 3.22 (m, 1H), 3.66 (s, 3H; $COOCH_3$), 8.07 (d, ^3J(H,H) = 8.8 Hz, 2H; aryl H), 8.34 (d, ^3J(H,H) = 8.8 Hz, 2H; aryl H); ^{13}C NMR (100 MHz; $CDCl_3$, 25 °C): *d* = 13.44 (CH_3), 17.27 (CH_3), 22.61 (CH_3), 23.56 (CH_2), 25.81 (CH), 27.58 (CH_2), 27.91 (CH_2), 29.12 (CH_2), 30.92 (CH_2), 31.12 (CH_2), 34.53 (CH_2), 34.78 (C), 35.11 (CH), 35.93 (CH_2), 38.26 (CH_2), 39.44 (CH), 41.70 (CH), 42.49 (CH), 46.09 (C), 47.54 (CH), 47.97 (CH), 51.45 (CH_3), 53.89 (CH), 54.42 (CH), 124.18 (CH), 127.98 (CH), 148.25 (C), 149.75 (C), 174.43 (C); IR (Neat): n_{max} = 3103, 2933, 2866, 1733, 1527, 1446, 1348, 1307, 1158, 1092, 852, 734, 685 cm^{-1}; MS (ES+): *m/z* (%): 605.3 (100) $[M+H]^+$; m.p. 106-108 °C (crystallised from dichloromethane/hexane); elemental analysis calcd (%) for $C_{31}H_{48}N_4O_6S.3/2\ H_2O$ (604.80): C 58.93, H 8.14, N 8.87; found: C 59.15, H 8.25, N 8.85.

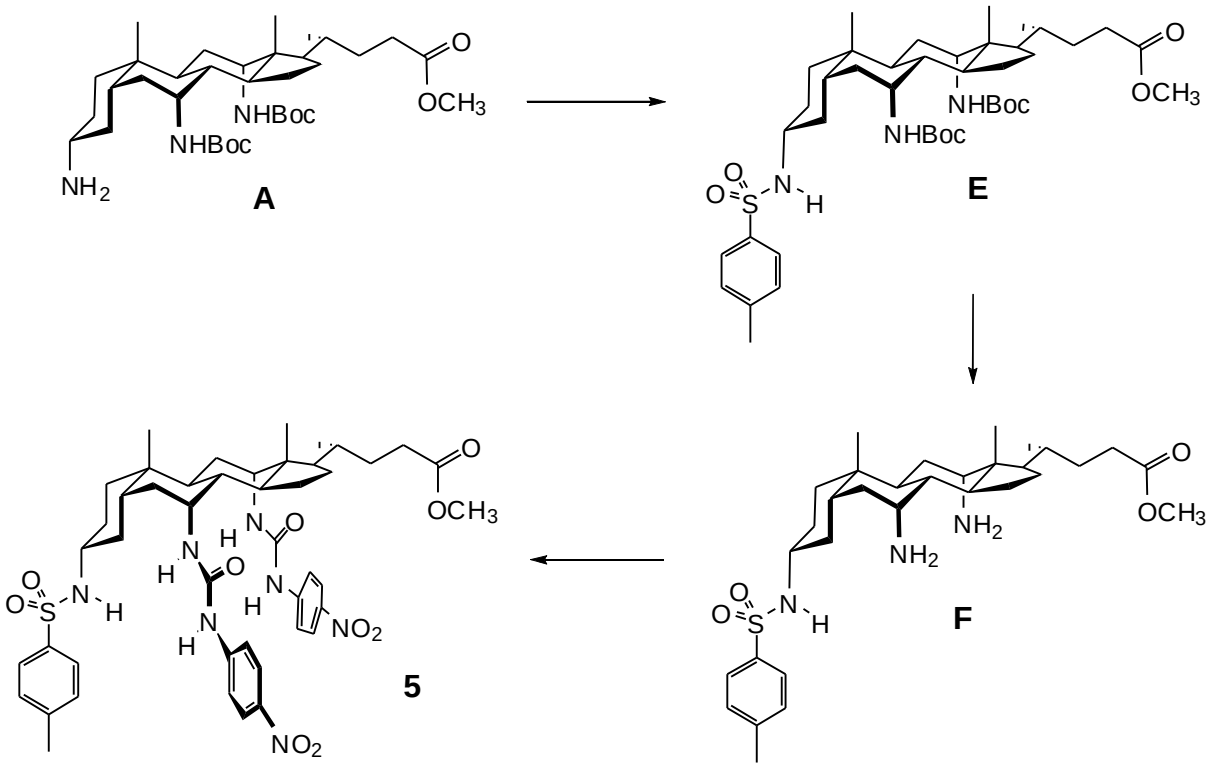
Methyl 3a-[(4-nitrobenzenesulfonyl)amino]-7a,12a-di-[(4-nitrobenzeneaminothio-carbonyl)amino]-5b-cholan-24-oate (2): A solution of 7a,12a-diamine **C** (141 mg, 0.233 mmol) and 4-nitrophenyl isothiocyanate (86 mg, 0.478 mmol) in dry dichloromethane (11 mL) was stirred overnight. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (gradient dichloromethane/methanol 98:2 to 8:2). The title compound **2** was obtained as a yellow solid (158 mg, 70%). The product was precipitated from dichloromethane by slow diffusion of diethyl ether. R_f = 0.23 (dichloromethane/methanol 9:1); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): *d* = 0.88 (d, ^3J(H,H) = 6.11 Hz, 3H; 21-CH_3), 0.94 (s, 3H; 18-CH_3), 1.05 (s, 3H; 19-CH_3), 2.86 (br s, 1H; 3β-H), 3.57 (s, 3H; $COOCH_3$), 4.67-4.82 (m, 2H, 7β- + 12β-H), 6.43 (br s, 1H; NH), 7.52 (br s, 2H; NH), 8.00-8.13 (m, 6H; aryl H), 8.28 (d, ^3J(H,H) = 9.0 Hz, 2H; aryl H), 8.29 (d, ^3J(H,H) = 8.8 Hz, 2H; aryl H), 8.38 (d, ^3J(H,H) = 8.0 Hz, 2H; aryl H), 9.50 (br s, 1H; NH), 9.78 (br s, 1H; NH); ^{13}C NMR (100 MHz; $(CD_3)_2CO$, 25 °C): *d* = 13.92 (CH_3), 17.79 (CH_3), 23.30 (CH_3), 24.16 (CH_2), 25.73 (CH_2), 27.85 (CH_2), 28.92 (CH_2), 30.60 (CH), 31.26 (CH_2), 31.53 (CH_2), 31.77 (CH_2), 35.37 (CH), 35.75 (C), 35.88 (CH_2), 37.49 (CH_2), 37.84 (CH), 42.22 (CH), 45.81 (C), 46.30 (CH), 49.34 (CH), 51.33 (CH), 51.54 (CH_3, $COOCH_3$), 55.29 (CH), 57.85 (CH), 120.88 (CH), 121.11 (CH), 121.41 (CH), 121.50 (CH), 125.27 (CH), 129.27 (CH), 143.52 (C),

143.67 (C), 147.10 (C), 147.46 (C), 150.95 (C), 174.44 (C), 180.17 (C), 180.73 (C); IR (Neat): n_{max} = 3338, 2943, 2865, 1727, 1712, 1596, 1496, 1327, 1294, 1254, 1203, 1158, 1110, 850, 735 cm^{-1};[3] MS (FAB+): *m/z* (%): 965 (100) $[M+H]^+$, 987 (90) $[M+Na]^+$; elemental analysis calcd (%) for $C_{45}H_{56}N_8O_{10}S_3.H_2O$ (964.33): C 54.97, H 5.95, N 11.40; found: C 55.02, H 5.93, N 11.27.



Methyl 3a-Acetoxy-7a,12a-bis[(4-nitrophenylaminothiocarbonyl)amino]-5b-cholan-24-oate (3): Bis-carbamate **17a** was converted into diamine **D** by treatment with TFA/CH_2Cl_2 followed by $NaHCO_3$ aq., as described previously.[4] A solution of diamine **D** (0.138 g, 0.299 mmol), 4-nitrophenyl isothiocyanate (0.134 g, 0.746 mmol) and DMAP (0.08 g, 0.65 mmol) in $CHCl_3$ (15 mL, stabilised with amylenes) was stirred at rt under N_2 for 2 d. The mixture was diluted with CH_2Cl_2, washed with aq. 4% $NaHCO_3$, dried (Na_2SO_4), and the solvent was removed *in vacuo* to give the crude product (0.387 g). Flash chromatography with 5-50% ethyl acetate/dichloromethane gradient elution gave the product **3** (0.290 g, 57%): R_f = 0.50 (dichloromethane/methanol 9:1); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): *d* = 0.88 (d, ^3J(H,H) = 5.9 Hz, 3H; 21-CH_3), 0.95 (s, 3H; 18-CH_3), 1.12 (s, 3H; 19-CH_3), 1.92 (s, 3H, CH_3CO_2), 3.57 (s, 3H; $COOCH_3$), 4.54 (br s, 1H; 3β-H), 4.73-4.80 (m, 2H, 7β- + 12β-H), 7.48 (br s, 1H; NH), 7.58 (br s, 1H; NH), 8.00-8.08 (m, 4H; aryl H), 8.19 (d, ^3J(H,H) = 3.1 Hz, 2H; aryl H), 8.21 (d, ^3J(H,H) = 3.0 Hz, 2H; aryl H), 9.44 (br s, 1H; NH), 9.78 (br s, 1H; NH). ^{13}C NMR (100 MHz; $(CD_3)_2CO$, 25 °C): *d* = 14.01 (CH_3), 17.86 (CH_3), 21.41 (CH_3),

23.20 (CH_3), 24.38 (CH_2), 25.67 (CH_2), 27.64 (CH_2), 27.96 (CH_2), 31.31 (CH_2), 31.61 (CH_2), 32.05 (C), 35.44 (CH), 35.57 (CH_2), 35.83 (C), 36.00 (C), 37.98 (CH), 41.98 (CH), 45.86 (C), 46.46 (C), 49.36 (CH), 51.42 (CH), 51.61 (CH_3, COO*C*H_3), 57.98 (CH), 74.50 (CH), 79.28 (C), 121.15 (CH), 121.60 (CH), 125.25 (CH), 125.33 (CH), 143.56 (C), 143.69 (C), 147.58 (2C), 170.27 (C), 174.51 (C), 180.45 (C), 180.77 (C); IR (Neat): n_{max} = 3337, 2946, 2870, 1731, 1596, 1496, 1325,1296, 1247, 1203, 1175, 1111, 1024, 881, 848, 750 cm^{-1};[3] MS (ES+): *m/z* (%): 845 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{41}H_{54}N_6O_8S_2.2H_2O$ (859.06): C 57.32, H 6.81, N 9.78, S 7.47; found: C 57.21, H 6.78, N 9.82, S 7.50.

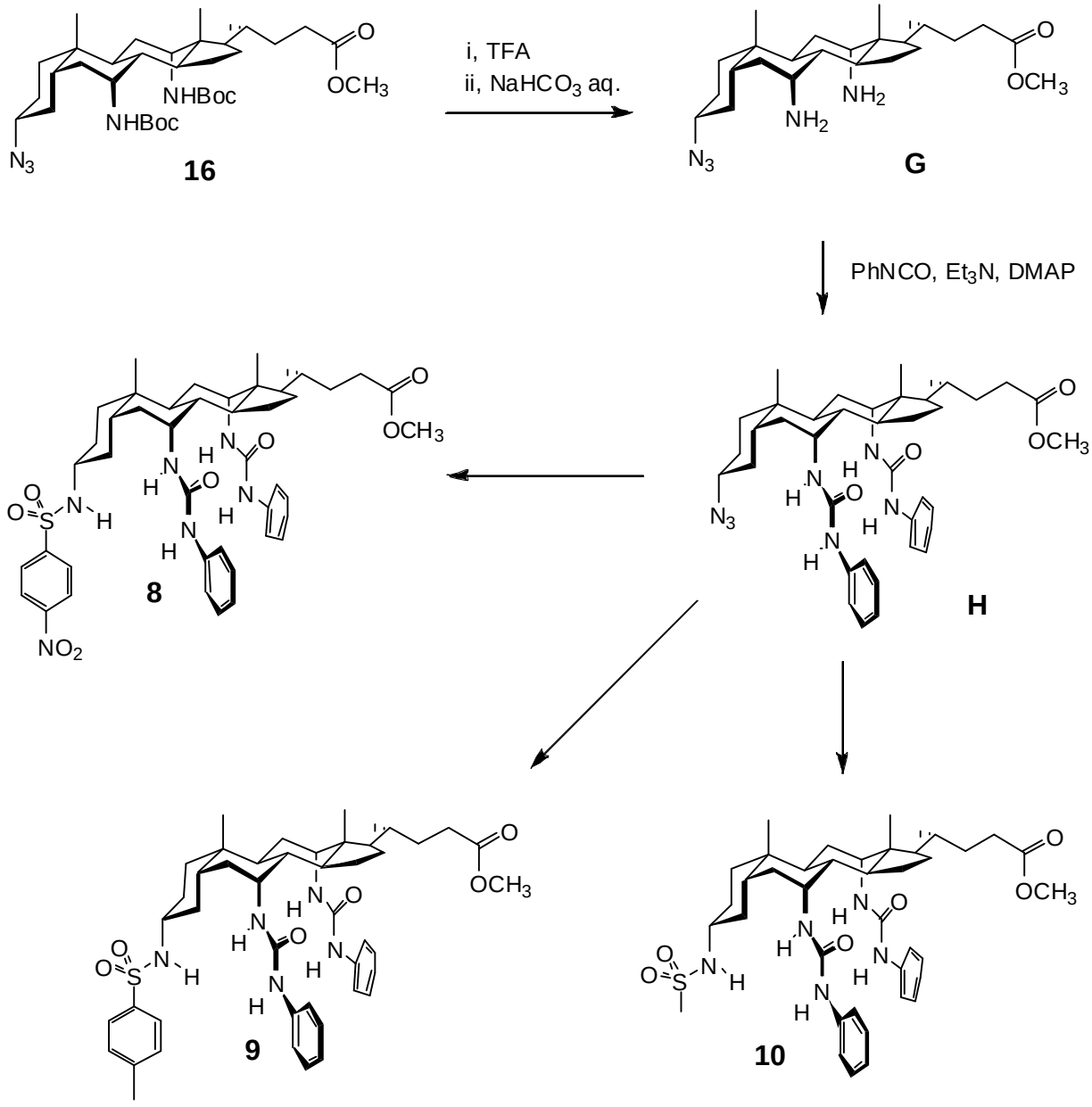


Methyl 3a-[(*p*-toluenesulfonyl)amino]-7a,12a-di-[*N*-(*t*-butoxycarbonyl)amino]-5b-cholan-24-oate (E): To a solution of amine **A** (150 mg, 0.242 mmol) and triethylamine (54 mg, 0.532 mmol, 74 µl) in dry dichloromethane (2.5 mL) was added *p*-toluenesulfonyl chloride (51 mg, 0.266 mmol). The reaction was stirred overnight at room temperature. Next the reaction mixture was diluted with dichloromethane and washed with a 0.2 M hydrochloric acid solution. The aqueous phase was extracted twice with dichloromethane. The combined organic phases were washed with water and dried over anhydrous magnesium sulfate. The crude mixture was purified by flash chromatography (dichloromethane/methanol 98/2) to give toluenesulfonamide **E** as a white solid (147 mg, 78%). R_f = 0.28 (dichloromethane/methanol 97:3); m.p. 240-242 °C (crystallised from dichloromethane/hexane);

^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): *d* = 0.82 (s, 3H; 18-CH_3), 0.92 (m, 6H; 19-CH_3 + 21-CH_3), 1.41 (s, 18H; $C(CH_3)_3$), 2.40 (s, 3H; tosylamido CH_3), 2.77 (m, 1H; 3ß-H), 3.60 (s, 3H; $COOCH_3$), 3.65 (m, 1H; 7β-H), 4.01 (m, 1H; 12β-H), 5.95-6.29 (m, 3H; NH), 7.37 (d, ^{3}J(H,H) = 7.8 Hz, 2H; aryl H), 7.73 (d, ^{3}J(H,H) = 7.8 Hz, 2H; aryl H); ^{13}C NMR (100 MHz; $(CD_3)_2CO$, 25 °C): *d* = 13.77 (CH_3), 17.34 (CH_3), 21.53 (CH_3), 22.93 (CH_3, CH_3), 23.08 (CH_2), 27.05 (CH_2), 27.25 (CH_2), 28.50 ($C(CH_3)_3$), 29.20 (CH_2), 30.80 (CH_2), 31.30 (CH_2), 31.93 (CH_2), 34.54 (C), 34.99 (CH), 35.48 (CH_2), 36.87 (CH), 37.45 (CH_2), 42.06 (CH), 44.82 (C), 45.00 (CH), 47.12 (CH), 48.46 (CH), 51.65 (CH_3), 53.11 (CH), 54.34 (CH), 79.08 (C), 127.03 (CH), 129.65 (CH), 143.15 (C), 155.5 (C), 174.93 (C); IR (Neat): n_{max} = 3369, 3291, 2958, 2928, 2861, 1702, 1517, 1500, 1362, 1322, 1242, 1155, 1069, 1043, 872, 816, 666 cm^{-1}; MS (ES+): *m/z* (%): 774.6 (30) $[M+H]^+$, 791.6 (100) $[M+NH_4]^+$, 796.6 (40) $[M+Na]^+$; elemental analysis calcd (%) for $C_{42}H_{67}N_3O_8S.1/2H_2O$ (774.06): C 64.42, H 8.75, N 5.37; found: C 64.56, H 9.11, N 5.40.

Methyl 3a-[(*p*-toluenesulfonyl)amino]-7a,12a-di-[(4-nitrobenzeneaminocarbonyl)-amino]-5b**-cholan-24-oate 5:** A solution of dicarbamate **E** (51 mg, 0.066 mmol) in dry dichloromethane (0.5 mL) was cooled in an ice bath. Trifluoroacetic acid was added (250 μL) and the reaction was stirred at 0 °C. After 20 minutes the ice bath was removed and the reaction was allowed to stir for another hour at room temperature. The solution was evaporated under reduced pressure and the residue was redissolved in dichloromethane and washed with a saturated sodium bicarbonate solution. The aqueous phase was extracted with dichloromethane (3 portions) and the combined organic phases were dried over sodium sulfate. Filtration, evaporation and drying under high vacuum gave the diamine **F** (38 mg, 0.067 mmol). This material was dissolved in dry tetrahydrofuran (2 mL) and 4-nitrophenyl isocyanate (33 mg, 0.2 mmol) was added. After stirring for 22 hours at room temperature the solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography through a short silica plug (gradient dichloromethane/ethyl acetate 95:5 to 8:2). The bisurea **5** (56 mg, 93%) was obtained as a yellow solid. An analytically pure sample was obtained by crystallisation from acetone. R_f = 0.28 (dichloromethane/methanol 9:1); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): *d* = 0.86 (d, ^{3}J(H,H) = 6.6 Hz, 3H; 21-CH_3), 0.89 (s, 3H; 18-CH_3), 0.98 (s, 3H; 19-CH_3), 2.34 (s, 3H; tosylamido CH_3), 2.74 (m, 1H; 3β-H), 3.53 (s, 3H; $COOCH_3$), 4.03 (br s, 1H; 7β-H), 4.15 (m, 1H; 12β-H), 6.05 (br d, ^{3}J(H,H) = 7.9 Hz, 1H; NH), 6.10 (br d, ^{3}J(H,H) = 3.4 Hz, 1H; NH), 6.15 (br d, ^{3}J(H,H) = 7.5 Hz, 1H; NH), 7.32 (d, ^{3}J(H,H) = 8.3 Hz, 2H; Aryl H), 7.66-7.72 (m, 6H; aryl H), 8.15 (d, ^{3}J(H,H) = 9.2 Hz, 2H; aryl H), 8.16 (d, ^{3}J(H,H) = 9.3 Hz, 2H; aryl H), 8.62 (br s, 1H, NH), 8.74 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): *d* = 13.96

(CH_3), 17.30 (CH_3), 21.74 (CH_3), 22.81 (CH_2), 23.08 (CH_3) 27.12 (CH_2), 27.83 (CH_2), 29.92 (CH_2), 30.02 (CH), 30.66 (CH_2), 31.02 (CH_2), 31.45 (CH_2), 34.04 (C), 34.59 (CH_2), 34.88 (CH), 35.56 (CH_2), 36.48 (CH), 41.94 (CH), 45.02 (C), 46.17 (CH), 48.55 (CH), 51.45 (CH_3), 52.02 (CH), 55.14 (CH), 116.32 (CH), 117.16 (CH), 124.82 (CH), 125.24 (CH), 130.74 (CH), 134.75 (CH), 140.28 (C), 141.78 (C), 144.45 (C), 146.03 (C), 146.74 (C), 153.24 (C), 153.52 (C), 174.61 (C); IR (Neat): n_{max} = 3360, 2947, 1703, 1597, 1498, 1323, 1202, 1176, 1110, 851, 812, 751, 664 cm^{-1}; MS (ES+): 902.7 (30) $[M+H]^+$, 924.7 (100) $[M+Na]^+$; m.p. 196 °C (crystallised from acetone); elemental analysis calcd (%) for $C_{46}H_{59}N_7O_{10}S$ (902.07): C 61.25, H 6.59, N 10.87; found: C 61.18, H 6.63, N 10.51.



Methyl 3a-azido-7a,12a-bis[(phenylaminocarbonyl)amino]-5b-cholan-24-oate (H): Azido-bis-carbamate **16**[1] was converted into bis-urea **H** as described previously.[5]

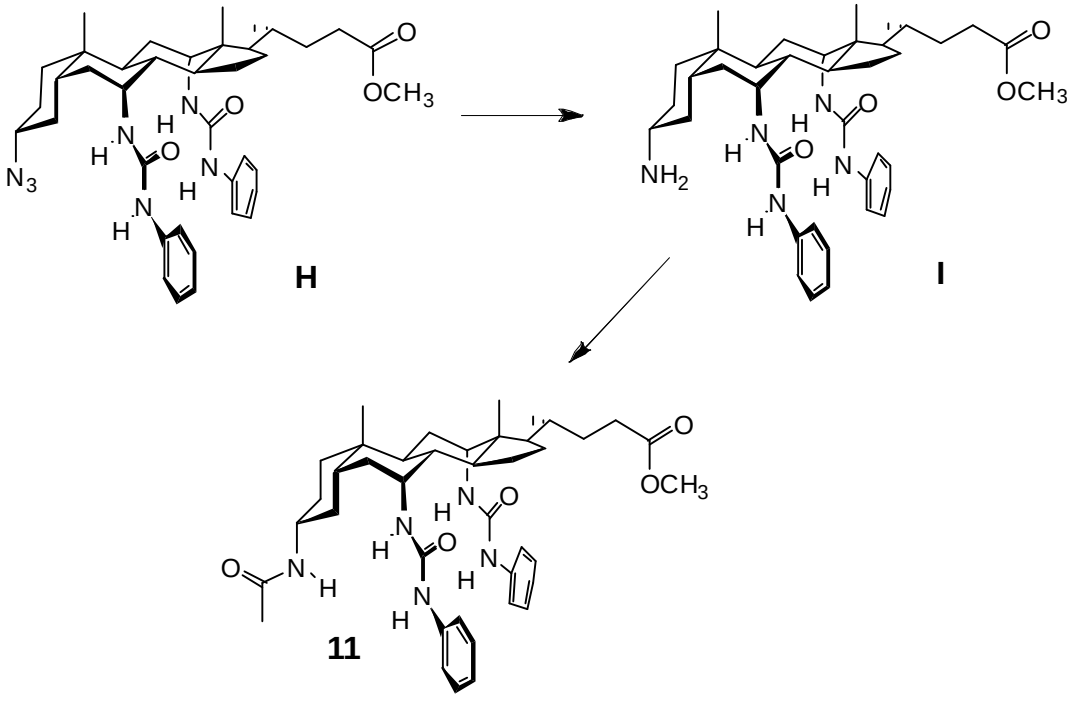
Methyl 3a-*p*-nitrobenzenesulfonamido-7a,12a-bis[(phenylaminocarbonyl)amino]-5b-cholan-24-oate (8): To a solution of azide **H** (0.3 g, 0.44 mmol) in dry THF (4.4 mL) was added trimethylphosphine (0.88 mL as a 1 M solution in THF, 0.88 mmol). The solution was stirred for 5 h before water (80 µL, 0.08 g, 4.4 mmol) was added and the solution was stirred for a further 24 h. The reaction solution was evaporated under reduced pressure and dried azeotropically by addition of toluene and re-evaporation. The dried residue and *p*-nitrobenzenesulfonylchloride (0.107 g, 0.48 mmol) were dissolved in dry DCM (9 mL) and to the stirred solution was added triethylamine (67 µL, 0.049 g, 0.48 mmol). After 24 h the solvent was removed under reduced pressure, the crude product was dissolved in dichloromethane, washed with 1 N $HCl_{(aq.)}$, dried ($MgSO_4$) and evaporated under reduced pressure. Purification of the resultant residue by flash column chromatography (eluent DCM/methanolic ammonia[2] 97:3 to 96:4) gave product sulfonamide **8** (0.100 g, 27 %) as a pale yellow solid. R_f = 0.30 (DCM/methanolic ammonia[2] 95:5); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 ^oC): δ = 0.86-0.88 (m, 3 H; 21-H_3), 0.87 (s, 3 H; 18-H_3), 0.98 (s, 3 H; 19-H_3), 2.10-2.20 (m, 1 H), 2.20-2.30 (m, 1 H), 2.80-2.90 (m, 1 H; 3β-H), 3.55 (s, 3 H; $COCH_3$), 3.97-4.04 (m, 1 H; 7β-H), 4.10-4.16 (m, 1 H; 12β–H), 5.77-5.87 (m, 2 H; 2 x CH-N**H**CO), 6.51 (s, 1 H; Ar-SO_2NH), 6.87-6.97 (m, 2 H), 7.18-7.28 (m, 4 H), 7.46-7.54 (m, 4 H), 7.90 (s, 1 H; Ar-NHCO), 8.04-8.11 (m, 3 H; 2 x Ar-CH + Ar-NHCO), 8.33-8.39 (m, 2 H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, 25 ^oC): δ = 14.1 (18-CH_3), 17.4 (21-CH_3), 23.5 (19-CH_3), 24.1 (CH_2), 27.6 (CH_2), 28.0 (CH_2), 29.1 (CH_2), 29.5 (CH), 31.3 (CH_2), 31.7 (CH_2), 33.5 (CH_2), 35.6 (CH), 35.7 (10-C), 36.2 (CH_2), 37.9 (CH_2), 38.3 (CH), 42.8 (CH), 45.5 (CH), 45.7 (13-C), 46.9 (CH), 49.1 (7-CH), 51.5 (COO**C**H_3), 53.6 (12-CH), 55.6 (3-CH), 118.6 (2 x Ar-CH), 118.7 (2 x Ar-CH), 122.1 (Ar-CH), 122.2 (Ar-CH), 125.2 (2 x Ar-CH), 129.3 (2 x Ar-CH), 129.6 (2 x Ar-CH), 129.6 (2 x Ar-CH), 141.8 (Ar-C), 141.9 (Ar-C), 147.6 (Ar-C), 151.0 (Ar-C), 155.2 (NHCONH), 155.4 (NHCONH), 174.4 (**C**$OOCH_3$); IR (Neat): ν_{max} = 3386, 2941, 1528, 1348, 1155, 747, 735, 691 cm^{-1}; MS (FAB): *m/z* (%): 843 (24) $[M+H]^+$, 865 (66) $[M+Na]^+$, 911 (100) $[M+H+NaNO_2]^+$, 933 (44) $[M+Na+NaNO_2]^+$; elemental analysis calculated (%) for $C_{45}H_{58}N_6O_8S/2.5\ H_2O$: C 63.14, H 7.42, N 9.82; found C 63.34, H 7.18, N 9.76.

Methyl 3a-*p*-toluenesulfonamido-7a,12a-bis[(phenylaminocarbonyl)amino]-5b-cholan-24-oate (9): To a solution of azido compound **H** (0.179 g, 0.260 mmol) in dry THF (2.6 mL) was added trimethylphosphine (0.52 mL as a 1 M solution in THF, 0.520 mmol). The reaction solution

was stirred for 6 h before water (47 μL, 0.047 g, 2.6 mmol) was added and the solution was stirred for a further 24 h. The reaction solution was evaporated under reduced pressure and dried azeotropically by addition of toluene and re-evaporation. The dried residue and *p*-toluenesulfonylchloride (0.055 g, 0.286 mmol) were dissolved in dry DCM (5 mL) and to the stirred solution was added triethylamine (40 μL, 0.029 g, 0.286 mmol). After 4 h the solvent was removed under reduced pressure, the crude product was dissolved in dichloromethane, washed with 1 N $HCl_{(aq.)}$, dried ($MgSO_4$) and evaporated under reduced pressure. Purification of the resultant residue by flash column chromatography (DCM/methanolic ammonia[2] 98:2) gave product sulfonamide **9** (0.106 g, 50 %) as a pale yellow solid. R_f = 0.30 (DCM/methanolic ammonia[2] 97.5:2.5); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{o}C): δ = 0.77 (s, 3 H; 18-H_3), 0.81 (s, 3 H; 19-H_3), 0.95 (d, ^3J(H,H) = 6.4 Hz, 3 H; 21-H_3), 2.15-2.26 (m, 1 H), 2.31 (bs, 3 H; Ar-CH), 2.87 (m, 1 H; 3β-H), 3.65 (s, 3 H; $COOCH_3$), 3.78 (m, 1 H; 7β-H), 4.15 (m, 1 H; 12β-H), 5.24 (bs, 1 H, CH-N**H**CO), 5.69 (bs, 1 H, CH-N**H**CO), 6.65-6.75 (m, 1 H), 6.85-6.95 (m, 1 H), 6.95-7.20 (m, 6 H), 7.23-7.31 (m, 3 H), 7.37-7.50 (m, 2 H), 7.90-7.97 (m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 ^{o}C): δ = 14.0 (18-CH_3), 17.3 (21-CH_3), 21.5 (CH), 22.9 (Ar-CH_3), 22.9 (19-CH_3), 23.0 (CH_2), 27.1 (CH_2), 27.5 (CH_2), 29.4 (CH_2), 30.7 (CH_2), 31.0 (CH_2), 31.0 (CH_2), 34.9 (CH), 34.9 (10-C), 35.0 (CH_2), 36.8 (CH_2), 36.8 (CH), 41.7 (7-CH), 44.9 (13-C), 46.0 (CH), 47.3 (CH), 48.4 (12-CH), 48.4 (CH), 51.5 (COO**C**H_3), 53.4 (3-CH), 117.8 (Ar-CH), 117.8 (Ar-CH), 117.8 (Ar-CH), 117.9 (Ar-CH), 121.7 (2 x Ar-CH), 127.7 (2 x Ar-CH), 128.7 (2 x Ar-CH), 128.9 (2 x Ar-CH), 130.5 (2 x Ar-CH), 135.3 (Ar-C0, 138.9 (Ar-C), 139.8 (Ar-C), 145.0 (Ar-C), 154.5 (NHCONH), 154.7 (NHCONH), 174.5 (**C**$OOCH_3$); IR (Neat): ν_{max} = 3417, 2945, 1696, 1531, 1375, 1148, 749, 691 cm^{-1}; MS (FAB): *m/z* (%): 812 (38) $[M+H]^+$, 834 (100) $[M+Na]^+$; elemental analysis calculated (%) for $C_{46}H_{61}N_5O_6S/0.5\ H_2O$: C 67.30, H 7.61, N 8.53; found C 67.30, H 7.60, N 8.44.

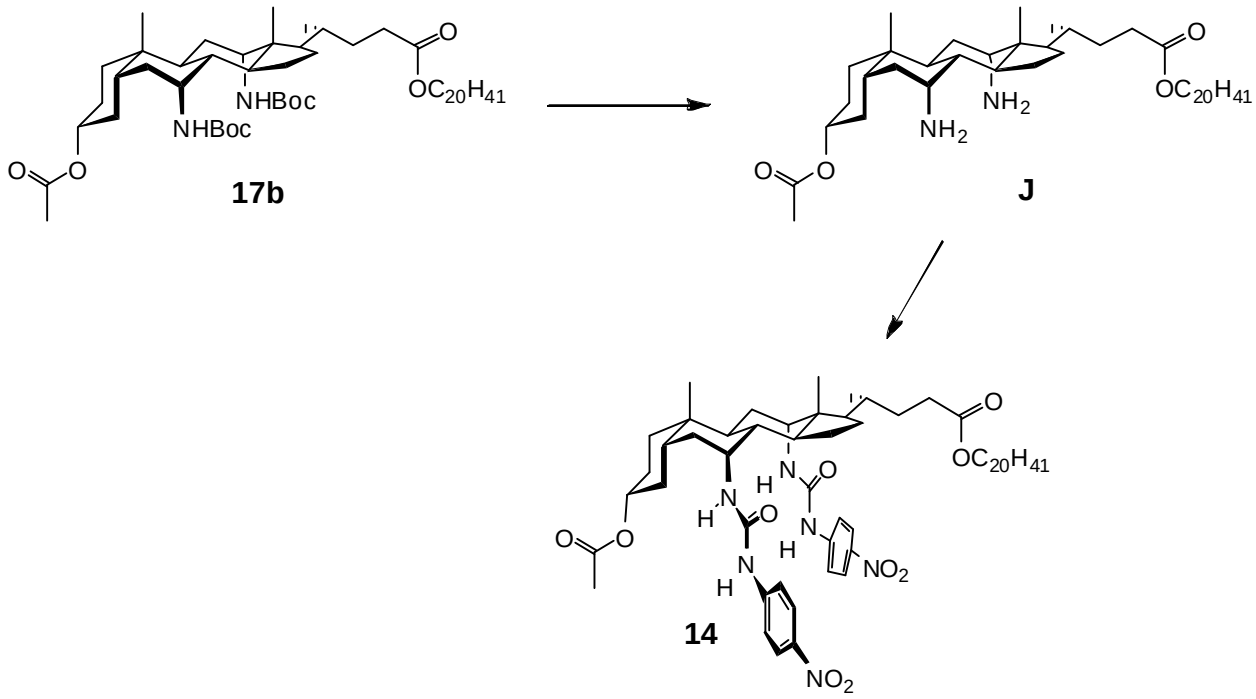
Methyl 3a-methanesulfonamido-7a,12a-bis[(phenylaminocarbonyl)amino]-5b-cholan-24-oate (10): To a solution of azido compound **H** (0.200 g, 0.292 mmol) in dry THF (2.9 mL) was added trimethylphosphine (0.58 mL as a 1 M solution in THF, 0.58 mmol). The reaction solution was stirred for 5 h before water (53 μL, 0.053 g, 2.92 mmol) was added and the solution was stirred for a further 24 h. The reaction solution was evaporated under reduced pressure and dried azeotropically by addition of toluene and re-evaporation. The dried residue and methanesulfonylchloride (34 μL, 0.050 g, 0.438 mmol) were dissolved in dry DCM (5.8 mL) and to the stirred solution was added triethylamine (61 μL, 0.044 g, 0.438 mmol). After 24 h the solvent was removed under reduced pressure, the crude product was dissolved in dichloromethane, washed with 1 N $HCl_{(aq.)}$, dried

($MgSO_4$) and evaporated under reduced pressure. Purification of the resultant residue by flash column chromatography (DCM/methanolic ammonia[2] 97:3 to 96:4) gave product sulfonamide **10** (0.100 g, 47 %) as a pale yellow solid: R_f = 0.55 (DCM/methanolic ammonia[2] 97:3); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 0.88 (s, 3 H; 18-H_3), 0.87-0.89 (m, 3 H; 21-H_3), 0.98 (s, 3 H; 19-H_3), 2.10-2.21 (m, 1 H), 2.21-2.32 (m, 1 H), 2.92 (s, 3 H; CH_3SO_2N), 3.09-3.20 (m, 1 H; 3β-H), 3.55 (s, 3 H; $COOCH_3$), 4.00 (bs, 1 H; 7β-H), 4.14-4.20 (m, 1 H; 12β-H), 5.70-5.82 (m, 3 H; 2 x CH-N**H**CO + CH_3SO_2N**H**), 6.87-6.94 (m, 2 H), 7.18-7.26 (m, 4 H), 7.45-7.59 (m, 4 H), 7.81 (s, 1 H; Ar-NHCO), 7.98 (s, 1 H; Ar-NHCO); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, 25 °C): δ = 14.2 (18-CH_3), 17.7 (21-CH_3), 23.5 (19-CH_3), 24.0 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 29.7 (CH_2), 30.0 (CH), 31.4 (CH_2), 31.7 (CH_2), 33.5 (CH_2), 35.5 (10-C), 35.7 (CH), 36.2 (CH_2), 38.1 (CH_2), 38.1 (CH), 40.5 (CH_3SO_2NH), 42.9 (CH), 45.8 (13-C), 45.8 (2 x CH), 49.1 (7-CH), 51.5 (COO**C**H_3), 53.4 (12-CH), 55.6 (3-CH), 118.6 (2 x Ar-CH), 119.4 (Ar-CH), 119.9 (Ar-CH), 122.0 (Ar-CH), 122.1 (Ar-CH), 129.5 (4 x Ar-CH), 141.8 (Ar-C), 142.0 (Ar-C), 155.3 (NHCONH), 155.4 (NHCONH), 174.4 (**C**$OOCH_3$); IR (Neat): ν_{max} = 3381, 2941, 2869, 1679, 1534, 750, 692 cm^{-1}; MS (FAB): *m/z* (%):736 (64) $[M+H]^+$, 758 (100) $[M+Na]^+$; elemental analysis calculated (%) for $C_{40}H_{57}N_5O_6S$: C 65.28, H 7.81, N 9.52; found C 65.17, H 8.19, N 9.08.



Methyl 3a-acetamido-7a,12a-bis[(phenylaminocarbonyl)amino]-5b-cholan-24-oate 11: Azide **H** (0.212 g, 0.31 mmol) and triphenylphosphine (0.105 g, 0.40 mmol) were dissolved in dry THF

(1.55 mL) and the resultant solution stirred overnight. Water (56 μL, 0.056 g, 3.1 mmol) was added and the solution refluxed overnight before being allowed to cool to rt. Solvents were removed by evaporation, addition of toluene, re-evaporation, addition of dichloromethane and further re-evaporation. Purification by chromatography through a plug of silica (eluent DCM/methanolic ammonia[2] 97:3 to 9:1) gave amine **I**[6] (0.147 g, 72 %) which was dissolved in dry DCM (4 mL). To the resultant solution was added acetic anhydride (23 μL, 0.0245 g, 0.24 mmol) followed by diisopropylethylamine (42 μL, 0.031 g, 0.24 mmol) and the reaction mixture was stirred overnight. Evaporation of the solvent under reduced pressure gave crude product which was dissolved in dichloromethane, washed with 1 N HCl, dried ($MgSO_4$) and evaporated under reduced pressure. Purification of the resultant residue by flash column chromatography (eluent DCM/methanolic ammonia[2] 96:4) gave the acetamide **11** (0.131 g, 61 % over two steps) as a white solid: R_f = 0.40 (DCM/methanolic ammonia[2] 95:5); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 ^oC): δ = 0.70 (s, 3 H; 18-H_3), 0.84 (s, 3 H; 19-H_3), 0.87 (d, $^3J(H,H)$ = 6.6 Hz, 3 H; 21-H_3), 1.98 (s, 3 H; CH_3CO), 2.12-2.20 (m, 1 H), 2.20-2.29 (m, 1 H), 3.25-3.36 (m, 1 H; 3β-H), 3.54 (s, 3 H; $COOCH_3$), 4.00-4.10 (m, 2 H; 7β-H +12β-H), 5.77 (d, $^3J(H,H)$ = 9.5 Hz, 1 H; CH-NHCO), 6.00 (d, $^3J(H,H)$ = 7.0 Hz, 1 H; CH-NHCO), 6.85-6.94 (m, 2 H), 7.17-7.26 (m, 5 H; 4 x Ar-CH + CH_3CONH), 7.49-7.59 (m, 4 H), 8.37 (s, 1 H; Ar-NHCO), 8.69 (s, 1 H; Ar-NHCO); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, 25 ^oC): δ = 14.3 (18-CH_3), 17.4 (21-CH_3), 23.4 (19-CH_3), 23.8 (CH_3CONH), 24.0 (CH_2), 27.4 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 29.8 (CH), 31.2 (CH_2), 31.7 (CH_2), 34.4 (CH_2), 35.5 (CH), 35.6 (10-C), 36.5 (CH_2), 37.2 (CH_2), 38.2 (CH), 41.9 (CH), 45.6 (CH), 45.7 (13-C), 46.0 (CH), 48.9 (7-CH), 51.5 (COO**C**H_3), 52.8 (3-CH), 54.0 (12-CH), 118.4 (Ar-CH), 118.4 (Ar-CH), 118.5 (Ar-CH), 118.5 (Ar-CH), 121.7 (Ar-CH), 121.9 (Ar-CH), 129.5 (2 x Ar-CH), 129.5 (2 x Ar-CH), 142.1 (Ar-C), 142.4 (Ar-C), 155.2 (NHCONH), 155.4 (NHCONH), 172.2 (CH_3**C**ONH), 174.4 (**C**$OOCH_3$); IR (Neat): ν_{max} = 3326, 2943, 1539, 1203, 750, 693 cm^{-1}; MS (FAB): *m/z* (%):701 (50) $[M+H]^+$, 723 (100) $[M+Na]^+$; HRMS (ES+): *m/z* calculated for $[M+Na]^+$ = 722.4252, found 722.4226.



Eicosyl 3a-Acetoxy-7a,12a-bis[(4-nitrophenylaminocarbonyl)amino]-5b-cholan-24-oate (14): The preparation of **17b** and its conversion to **J** has been described previously.[4] A solution of bis-amine **J** (0.082 g, 0.113 mmol) and 4-nitrophenyl isocyanate (0.0420 g, 0.338 mmol) in $CHCl_3$ (6 mL) under N_2 was stirred at rt for 16 h. The reaction was diluted with $CHCl_3$ (75 mL), washed with aq. 0.5 N HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL), dried (Na_2SO_4), and the solvent removed *in vacuo* to give the crude product. Radial chromatography (SiO_2, 1 mm) with 5-50% ethyl acetate/hexanes gradient elution gave pure **14** as a yellow solid (0.084 g, 65%): m.p. 225-228 °C; ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ = 0.84-0.92 (m, 9 H), 1.07-2.29 (m, 66 H), 3.97 (t, *J* = 6.6 Hz, 2 H CO_2CH_2), 4.10 (br s, 1 H), 4.20 (br d, 1 H), 4.52 (m, 1 H, 3ß-*H*), 6.17 (br s, 2 H, 6a-N*H*, 12a-N*H*), 7.75 (d, *J* = 9 Hz, 4 H, Ar-*H*), 8.14 (d, *J* = 9 Hz ,4 H, Ar-*H*), 8.63 (br s, 1 H, 12a-N*H*-Ar), 8.81 (br s, 1 H, 7a-N*H*-Ar); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ = 14.6 (18-CH_3), 14.9 ($(CH_2)_{18}CH_3$), 17.9 (21-CH_3), 21.8 (19-CH_3), 23.7, 23.8, 27.1, 27.7, 28.1, 28.4, 29.5, 29.8, 30.1, 30.2, 30.3, 30.4, 30.6, 30.7, 30.8, 30.9, 31.0, 31.1, 31.9, 32.1, 33.9, 35.9, 36.0, 36.3, 38.6, 42.6, 46.1, 47.5, 49.7, 54.4, 65.0 (CO_2CH_2), 75.0 (3-*C*H), 118.1 (Ar-*C*H), 126.4 (Ar-*C*H), 142.6 (Ar-*C*H), 148.4 (NH*C*ONH), 148.5 (NH*C*ONH), 154.8 (Ar-*C*H), 170.6 (3a-O_2CCH_3), 174.4 ($CO_2CH_2(CH_2)_{18}CH_3$); HRMS(FAB$^+$) m/z calc'd for $C_{60}H_{92}N_6O_{10}$ $[M+H]^+$: 1057.6875, Found 1057.6875.

Calculation of the Number of Lipid Molecules per Vesicle.

The vesicles used in this study possessed a diameter of 200 nm (2000 Å).
Surface area of these vesicles = $4\pi r^2 = 12.56 \times 10^6$ Å^2.

The surface areas occupied by phospholipid and cholesterol molecules have been estimated by Engelman[7] to be as follows:

Surface area per phospholipid = 71 Å^2
Surface area per cholesterol = 46 Å^2

For, phospholipid:cholesterol 7:3 mixture, the average surface area per lipid molecule is therefore 63.5 Å^2

It follows that the number of lipids in one membrane leaflet of a 200 nm vesicle is = $12.56 \times 10^6/63.5$ = 198000, and that the total number of lipid molecules per vesicle is roughly double this number, i.e. *396000 molecules/vesicle.*

References and Notes

[1]. V. del Amo, L. Siracusa, T. Markidis, B. Baragana, K. M. Bhattarai, M. Galobardes, G. Naredo, M. N. Pérez-Payán, A. P. Davis, *Org. Biomol. Chem.* **2004**, *2*, 3320.

[2] Methanolic ammonia was produced by bubbling ammonia gas through methanol at room temperature, until a saturated solution had been formed.

[3] No characteristic S-H band (-SH: 2600-2550(w)) was observed, supporting the thiourea structure as opposed to the iminothiol tautomer.

[4] T. N. Lambert, J. M. Boon, B. D. Smith, M. N. Pérez-Payán, A. P. Davis, *J. Am. Chem. Soc.* **2002**, *124*, 5276.

[5]. A. L. Sisson, V. del Amo Sanchez, G. Magro, A. M. E. Griffin, S. Shah, J. P. H. Charmant, A. P. Davis, *Angew. Chem. Int. Ed.* **2005**, *44*, 6878.

[6] For characterisation see ref [5].

[7] D. M. Engelman, *Nature* **1969**, *223*, 1279. See also S. Pikula, J. B. Hayden, S. Awasthi, Y. C. Awasthi, P. Zimniak, *J. Biol. Chem.* **1994**, *269*, 27574.