Design and Synthesis of New Bile Acid-Based Macrocycles

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A strategy for the synthesis of new macrocycles built on 7-deoxycholic acid is described.

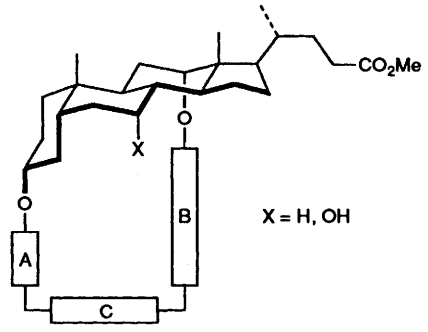
During the past two decades, research on the design, synthesis and evaluation of various types of artificial receptors has reached a new dimension.[1] A variety of synthetic receptors of diverse shapes and sizes has been constructed and evaluated for their ability to bind both molecular and ionic guests. Hydrogen bonding, cation-π interaction, π-stacking as well as hydrophobic interactions have been made use of to form a host–guest pair.[2] A variety of chiral receptors has also been synthesized, some of which show exceptionally high degree of enantioselectivity when binding chiral guest molecules,[3] and in many of these chiral receptors, natural products have frequently been used as the source of chirality.[4]

In recent years, bile acids have attracted the attention of several groups,[5,6] including ours,[7] since they are readily available in the chiral form and also possess a unique disposition of hydroxyl groups on one surface of the molecule. Davis and co-workers have contributed significantly to the development of novel macrocyclic structures built on bile acids.[5]

We have been interested in using cholic and 7-deoxycholic acids as a scaffold to create new macrocyclic structures. Our primary goal has been the construction of macrocyclic units between the 3- and the 12-hydroxyl groups of these bile acids. These two hydroxyl groups are *ca.* 6 Å apart,† hence building a macrocycle attached to these two groups should produce a large enough cavity for the complexation of small molecules. The availability of the 7-hydroxy group in cholic acid provides the opportunity to have additional functionality in conjunction with a macrocycle attached to the other two hydroxyl groups.

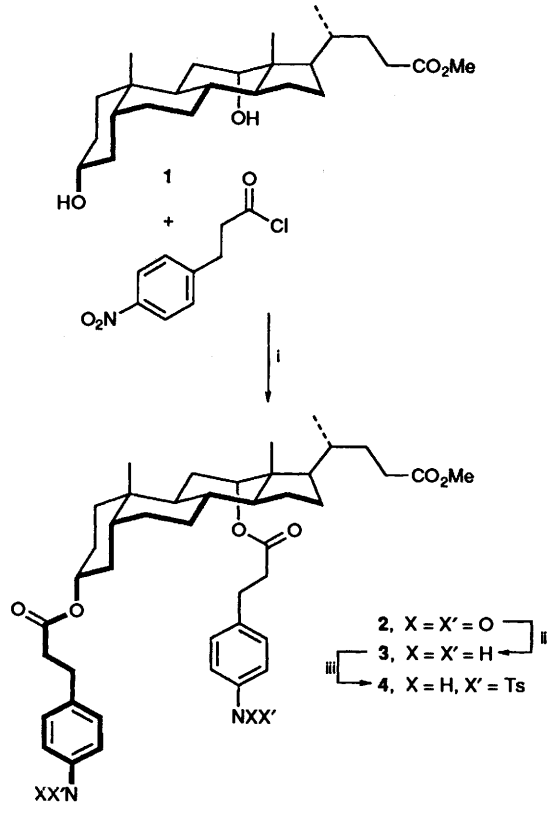
We chose a modular approach as depicted in Scheme 1, for the design of the macrocycles joined to the bile acid moiety. In this scheme, the two 'arms' A and B are attached to the two hydroxyl groups and subsequently joined through the linker C. Since a variety of modules (A, B and C) can be envisaged, we felt that this approach would allow one to create a multitude of new molecular structures, and in this paper, we report our efforts towards the synthesis of such species.

Our initial studies were based on 7-deoxycholic acid **1**, which possesses two hydroxyl 'handles'. Even though these two hydroxyl groups exhibit very different chemical reactivities, it was first decided to attach identical groups at these two hydroxyls (*i.e.*, A = B) in order to simplify the synthesis. Molecular modelling, using the DTMM‡ program, showed that compounds having two *p*-substituted 3-phenylpropanoic acid fragments attached to the 3 and the 12 positions of the bile acid would have enough room between them to encapsulate



Scheme 1 Modular approach towards the construction of steroidal macrocycles

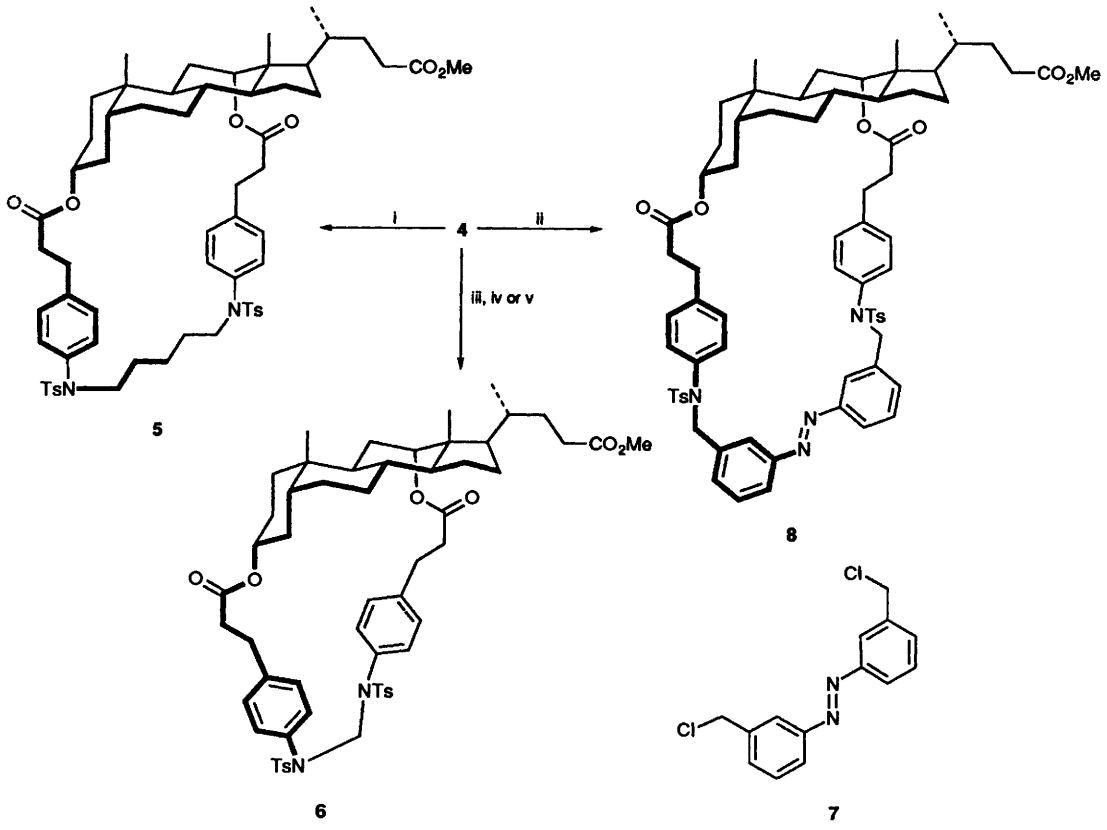
monocyclic aromatic guest molecules. Accordingly, 3-(4-nitrophenyl)propanoates were attached to methyl 7-deoxycholan-24-oate to yield compound **2** in 85% yield. The nitro groups were then reduced and the resulting diamine **3** was converted into the bis 4-methylbenzenesulfonamide derivative **4** (55%) using 4-methylbenzenesulfonyl chloride (Scheme 2). We felt that compound **4** would be a common intermediate since one could imagine that the two nitrogen atoms could be bridged by



Scheme 2 *Reagents and conditions:* i, CaH_2, $BnEt_3N^+Cl^-$, PhMe, reflux; ii, $SnCl_2 \cdot 2H_2O$, EtOAc; iii, TsCl–pyridine–DMAP

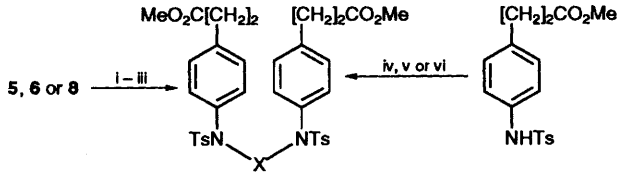
† The distance between the oxygen atoms at the 3 and the 12 positions was calculated from the X-ray structural data.[10] The two C–O vectors are not exactly parallel and diverge away from the steroid.

‡ The Desk Top Molecular Modeller program was used to construct the molecules from the X-ray structure of cholic acid and PCMODEL minimized structures of the aromatic fragments. We thank the Bioinformatic Centre of this Institute for providing computational facilities.



Scheme 3 *Reagents and conditions:* i, $Br[CH_2]_5Br$, K_2CO_3–MeCN, 78 °C; ii, **7**, K_2CO_3–MeCN; iii, $TEGTs_2$, K_2CO_3–DMF; iv, K_2CO_3–DMF; v, CH_2I_2, K_2CO_3–MeCN

an α,ω-bisalkylating agent. Once again, molecular modelling suggested that a pentamethylene chain could be easily 'inserted' between the two nitrogen atoms. Indeed, coupling of compound **4** with 1,5-dibromopentane in the presence of K_2CO_3 in acetonitrile gave the macrocyclic compound **5** in 70% yield (Scheme 3). Interestingly, when the cyclization was performed with other bisalkylating agents [*e.g.* 1,7-dibromoheptane or ($TEGTs_2$), the bis(4-methylbenzenesulfonate) derived from triethylene glycol], either poor yield or no product formation was observed. This observation, in conjunction with molecular modelling studies, clearly delineated the requirement of an optimum spacer length for efficient cyclization. The time duration for the addition, as well as high dilution conditions, played crucial roles. At higher concentrations, both the sulfonamide nitrogens were alkylated by 5-bromopentyl groups. The structure of compound **5** was elucidated by a combination of spectroscopic methods, including COSY and NOESY. In addition, the non-bile part of the macrocycle was removed from the steroid by alkaline hydrolysis and then methylated, and this methyl ester was found to be identical to the authentic material **9** synthesized independently, as shown in Scheme 4. It is interesting to note that the two nitrogen atoms of compound **4** may also be bridged by as small a group as a single methylene unit. This methylene-bridged compound **6** was produced during an attempted cyclization of compound **4** with $TEGTs_2$ in DMF. We subsequently discovered that product **6** was also formed (19%) in DMF in the absence of any alkylating agent. It is noteworthy that even though there is literature precedence[8] for a methylene transfer from DMF, harsher conditions (K-metal or KH) were necessary. Another interesting aspect of this reaction is that in our case it leads to strained macrocycle **6** although it could give a 2:2 adduct with lesser steric congestion. The cyclization forces the two aromatic rings from the side arms to lie at close proximity and, as a result,



9 X = $[CH_2]_5$
10 X = $[CH_2]$
11 X = H_2C–C_6H_4–N=N–C_6H_4–CH_2 (3, 3′)

Scheme 4 *Reagents and conditions:* i, KOH–MeOH–dioxane; ii, H_3O^+; iii, CH_2N_2; iv, $Br[CH_2]_5Br$, K_2CO_3–MeCN; v, CH_2I_2, K_2CO_3–MeCN; vi, **7**, K_2CO_3–MeCN

the aromatic signals appear *ca.* 0.6 ppm upfield of the corresponding resonances for compound **4**. Molecular modelling studies carried out by INSIGHT II also revealed the close proximity of the aromatic rings (Fig. 1). The structure was confirmed by spectroscopic and chemical methods (conversion of compound **6** into compound **10**, shown in Scheme 4). On a large scale, compound **6** may be prepared by treating the sulfonamide **4** with CH_2I_2 in acetonitrile in the presence of anhydrous K_2CO_3 (48%). The proximity of the two aromatic rings in compound **6** makes it an attractive species for further physical studies. Attempts to grow crystals of compound **6** suitable for X-ray analysis have not been successful so far.

We have also bridged the two nitrogen atoms of compound **4** with a longer spacer containing a visible chromophore. A bisalkylating agent **7** derived from diphenyldiazene was chosen for this purpose.[9] The macrocycle **8** was constructed from compound **4** and the diazene **7** using the above-mentioned conditions in 48% yield. Visible absorption at $\lambda_{max}(CHCl_3)$ 448 nm and the cleavage studies (conversion of compound **8** into

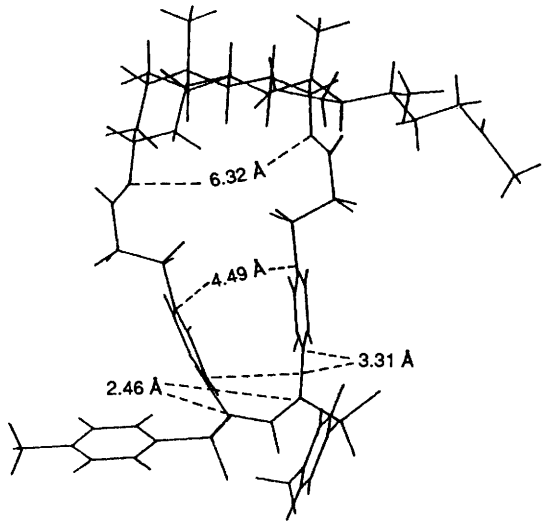


Fig. 1 INSIGHT II (DISCOVER) minimized structure of compound **6**

compound **11**, Scheme 4) as used for compound **5** support the structure shown for this macrocycle **8**.

In conclusion, we believe that the methodology described here provides a versatile and straightforward route to synthesize chiral macrocycles on a bile acid backbone. A similar strategy using cholic acid will allow us to place additional functionality at C-7 of the steroid. Evaluation of compounds **5** and **8** as receptors and work towards the construction of steroidal macrocycles with higher degree of preorganization is in progress and will be reported in due course. The mechanism involved in the formation of compound **6** is also under study.

Experimental

M.p.s were recorded in open capillaries and are uncorrected. ^{1}H NMR were recorded at 90 (JEOL FX-90Q), 270 (Bruker WH-270) or 400 (Bruker AMX 400) MHz. ^{13}C NMR were recorded either at 22.5 (JEOL FX-90Q) or 100.6 (Bruker AMX 400) MHz. IR spectra were recorded on Perkin-Elmer model 781 or Hitachi model 270-50 spectrophotometers. Mass spectra were recorded on a JEOL MSD-300 mass spectrometer. Optical rotations were obtained on a JASCO DIP-370 Digital polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. UV spectra were recorded on a Shimadzu UV2100 spectrometer. All solvents were dried and distilled before use. Tetrahydrofuran and toluene were distilled from sodium–benzophenone; dichloromethane and acetonitrile were distilled from P_2O_5; dry DMF was obtained after azeotropic removal of water with benzene and distilling from anhydrous BaO; pyridine was partially dried by storing over KOH and then distilled from CaH_2; and methanol was distilled from magnesium methoxide. Anhydrous potassium carbonate was dried at 500 °C in a furnace. All reactions were carried out with the use of standard procedures for the exclusion of moisture, unless otherwise noted.

Methyl 3α,12α-*Bis*[3-(4-*nitrophenyl*)*propanoyloxy*]-5β-*cholan*-24-*oate* **2**.—3-(4-Nitrophenyl)propanoic acid (2.03 g, 10.4 mmol) was converted into the acid chloride by reaction with thionyl chloride (3 cm^3, 41.3 mmol). Then, to a solution of the crude acid chloride (2.22 g, 10.4 mmol) in dry toluene (13 cm^3), methyl 3α,12α-dihydroxy-5β-cholan-24-oate (1.62 g, 3.9 mmol), CaH_2 (2.11 g, 50.2 mmol) and benzyltriethylammonium chloride (58 mg, 0.2 mmol) were added successively. The mixture was heated (bath temp. 90 °C) with efficient stirring for 20 h, then diluted with ethyl acetate (25 cm^3) and filtered through a bed of Celite. The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield the crude product, which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (2%) to yield the title compound **2** (2.43 g, 80%) as a pale-yellow, fluffy solid, m.p. 120–122 °C (from CH_2Cl_2–EtOH); $[\alpha]_D^{23}$ +59.5 (*c* 0.17 in $CHCl_3$); ν_{max}(thin film)/cm^{-1} 2932vs, 1737vs, 1606s and 1344s; δ_H(90 MHz; $CDCl_3$) 0.71 (3 H, s, 18-H), 0.91 (3 H, s, 19-H), 1.02–1.54 (br m, steroidal CH and CH_2), 2.74 (4 H, m, Ar-CH_2), 3.02 (4 H, m, ArCH_2CH_2), 3.68 (3 H, s, CO_2Me), 4.67 (1 H, br s, 3-H), 5.07 (1 H, s, 12-H), 7.44 (4 H, m, Ar-H) and 8.12 (4 H, d, *J* 9, Ar-H); δ_C(22.5 MHz; $CDCl_3$) 174.5, 171.2, 171, 129, 123.7, 75.6, 74, 51.4, 47, 45, 41.5, 35.4, 35, 34.8, 34, 32, 31, 27, 26.5, 25.5, 23, 22.5, 17.5 and 12; *m*/*z* 760 (M^+, 100%), 713 (17), 194 (54), 176 (52), 146 (20), 106 (17), 76 (17) and 45 (20).

Methyl 3α,12α-*Bis*[3-(4-N-*Tosylaminophenyl*)*propanoyloxy*]-5β-*cholan*-24-*oate* **4**.—A mixture of the dinitro compound **2**, $SnCl_2$·$2H_2O$ and EtOAc (6 cm^3) was heated (bath temp. 60 °C) with stirring under N_2 atmosphere for 2 h. The mixture was then poured into $NaHCO_3$ solution (10%, 20 cm^3) and extracted with ethyl acetate (15 cm^3 × 2). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the diamino compound **3** (0.146 g, 85.4%) which was used directly for tosylation.

To a solution of methyl 3α,12α-bis[3-(4-aminophenyl)-propanoyloxy]-5β-cholan-24-oate **2** (0.197 g, 0.27 mmol) in pyridine (0.5 cm^3), tosyl chloride (0.155 g, 0.81 mmol) in pyridine (0.5 cm^3) was added, followed by 4-(dimethylamino)pyridine (0.084 g, 0.68 mmol). The mixture was heated (bath temp. 120 °C) with vigorous stirring for 24 h and then poured into water (25 cm^3). The crude product was extracted with ethyl acetate (15 cm^3 × 2) and washed with dilute HCl (2 mol dm^{-3}, 15 cm^3 × 3), water, brine, 7% $NaHCO_3$ solution (15 cm^3 × 3), water, brine and finally dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the crude ditosylated product, which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (10%) to yield compound **4** (0.153 g, 55%) as an amorphous solid; $[\alpha]_D^{22}$ +29.1 (*c* 0.48 in $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3244s, 2926vs, 1728vs, 1602w, 1338s and 1158s; δ_H(270 MHz; $CDCl_3$) 0.55 (3 H, d, *J* 6.1, 21-H), 0.65 (3 H, s, 18-H), 0.86 (3 H, s, 19-H), 0.95–1.79 (br m), 2.34 (3 H, s, Ts-Me), 2.35 (3 H, s, Ts-Me), 2.47 (2 H, t, *J* 7.3, Ar-CH_2), 2.61 (2 H, t, *J* 7.6, Ar-CH_2), 2.77–2.91 (4 H, m, ArCH_2-CH_2), 3.68 (3 H, s, CO_2Me), 4.66 (1 H, br m, 3-H), 5.04 (1 H, s, 12-H), 6.90–7.07 (8 H, m, Ar-H), 7.16–7.21 (4 H, m, Ts-H) and 7.60–7.65 (4 H, m, Ts-H); δ_C(22.5 MHz; $CDCl_3$) 174.9, 172.2, 171.8, 143.4, 137.1, 136.2, 135.0, 129.3, 128.8, 127, 121.8, 121.6, 74.1, 51.5, 49.2, 47.3, 44.9, 41.6, 35.8, 34.6, 33.7, 30.9, 30.1, 26.5, 25.4, 22.8, 21.3, 17.1 and 12.1; *m*/*z* 853 (M^+ − Ts, 6.5%), 573 (70), 534 (40), 371 (26), 319 (46), 255 (79), 164 (80), 118 (84), 91 (100) and 44 (38).

Methyl 3α,12α[*Pentane*-1,5-*diylbis*(N-*tosylimino*-p-*phenyleneethylenecarbonyloxy*)-5β-*cholan*-24-*oate*.—To a 25 cm^3 two-necked, round-bottomed flask, fitted with a reflux condenser and a rubber septum, K_2CO_3 (0.235 g, 1.7 mmol) and acetonitrile (1.9 cm^3) were added. To this vigorously stirred mixture, a solution of compound **4** (0.103 g, 0.102 mmol) and 1,5-dibromopentane (0.025 g, 0.10 mmol) in acetonitrile (1.9 cm^3) was added *via* syringe over a period of 8 h at 78 °C. Heating was then continued for 16 h, and after cooling to room temperature, the mixture was diluted with ethyl acetate (25 cm^3) and filtered to remove the K_2CO_3. The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the crude product,

which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (1%) to yield compound **5** (0.070 g, 70%) as an amorphous solid [Found $(M + H)^+$ 1077.533 27. $C_{62}H_{18}N_2O_{10}S_2$ requires $(M + H)^+$ 1077.5332]; $[\alpha]_D^{24}$ +24.4 (*c* 0.98 in $CHCl_3$); $\lambda_{max}(CHCl_3)$/nm 246.3 (ε/dm^3 mol^{-1} cm^{-1} 14 140); ν_{max}(thin film)/cm^{-1} 2926vs, 2860s, 1731vs, 1602s, 1452s, 1380s, 1347vs, 1290s, 1257s and 1161vs; δ_H(400 MHz; $CDCl_3$) 0.73 (3 H, s, 18-H), 0.81 (3 H, d, *J* 6.3, 21-H), 0.91 (3 H, s, 19-H), 0.97–2.37 (br m), 2.42 (6 H, s, Ts-Me), 2.47–2.62 (4 H, m), 2.90 (4 H, m), 3.37–3.48 (4 H, m, N-CH_2), 3.64 (3 H, s, CO_2-Me), 4.76 (1 H, br m, 3-H), 5.13 (1 H, s, 12-H), 6.81 (2 H, d, *J* 8.3, Ar-H), 6.90 (2 H, d, *J* 8.3, Ar-H), 7.08 (4 H, m, Ar-H), 7.25 (4 H, m, Ts-H) and 7.46 (4 H, m, Ts-H); δ_C(22.5 MHz; $CDCl_3$) 174.5, 171.7, 143.4, 140.5, 139.9, 138.8, 137.3, 135.5, 135.1, 129.4, 128.9, 128.6, 127.6, 78.5, 77.2, 75.9, 73.6, 51.5, 50.5, 49.3, 47.7, 45.0, 41.2, 37.0, 36.0, 35.4, 34.7, 34.1, 31.8, 31.0, 30.2, 29.6, 27.6, 27.3, 26.6, 26.5, 25.1, 23.4, 22.9, 21.4, 17.4 and 12.3; *m*/*z* 1077.4 [$(M + H^+$, 12%)], 922 (55), 767 (22), 708 (15), 552 (27), 371 (84), 232 (100) and 178 (53).

Cleavage of the Macrocycle **5** *to yield Dimethyl Pentane*-1,5-*diylbis*(N-*tosylimino*-p-*phenyleneethylenecarboxylate*) **9**.—A mixture of compound **5** (0.020 g, 0.018 mmol), 10% methanolic KOH (0.5 cm^3) and dioxane (0.5 cm^3) was heated (bath temp. 65 °C) for 5 h and, after acidification with 2 mol dm^{-3} HCl to pH 3, the products were extracted with ethyl acetate (15 cm^3). The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was then removed under reduced pressure to yield the crude products. The residue was dissolved in diethyl ether (10 cm^3) and treated with an excess of diazomethane. After 30 min, the excess diazomethane and the solvent were removed by bubbling through N_2 and then the product was dried *in vacuo*. The crude product was column chromatographed on silica gel with ethyl acetate–$CHCl_3$ (5%) as the eluent to yield compound **9** (0.010 g, 73%); ν_{max}(thin film)/cm^{-1} 3023vs, 2926vs, 1738vs, 1600s, 1512s, 1440w, 1345s, 1290w and 1089s. δ_H(270 MHz; $CDCl_3$) 1.33 (6 H, s, C_5H_{10}-*H*), 2.44 (6 H, s, Ts-*Me*), 2.65 (4 H, m, Ar-CH_2), 2.96 (4 H, s, CH_2CO_2), 3.46 (4 H, br s, CH_2-N), 3.69 (6 H, s, CO_2Me) and 7.32 (16 H, m, Ar-H).

Authentic Dimethyl Pentane-1,5-*diylbis*(-N-*tosylimino*-p-*phenyleneethylenecarboxylate*) **9**.—To a vigorously stirred and heated (bath temp. 78 °C) mixture of methyl 3-(4-*N*-tosylaminophenyl)propanoate (0.176 g, 0.5 mmol) and K_2CO_3 (0.91 g, 6.6 mmol) in acetonitrile (1 cm^3), a solution of 1,5-dibromopentane (0.076 g, 0.05 cm^3, 0.33 mmol) in acetonitrile (0.5 cm^3) was added *via* syringe over a period of 6 h. Heating was continued for 24 h and, after cooling to room temperature, the mixture was diluted with ethyl acetate (25 cm^3) and filtered to remove the K_2CO_3. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the crude product, which was purified by column chromatography on silica with ethyl acetate–$CHCl_3$ (5%) to yield compound **9** (0.142 g, 73%) as a glass; ν_{max}(thin film)/cm^{-1} 3022vs, 2926vs, 1737vs, 1599s, 1512s, 1440w, 1344s, 1290w and 1089s; δ_H(90 MHz; $CDCl_3$) 1.32 (6 H, br s, C_5H_{10}-H), 2.41 (6 H, s, Ts-Me), 2.61 (4 H, m, Ar-CH_2), 2.92 (4 H, m, CH_2-CO_2), 3.42 (4 H, br m, N-CH_2), 3.68 (6 H, s, CO_2Me), 6.85–7.32 (16 H, m, Ar-H); δ_C(22.5 MHz; $CDCl_3$) 173, 143.3, 140.2, 137.2, 135.2, 129.3, 128.7, 127.6, 51.5, 50.2, 35.2, 30.4, 29.6, 27.5, 23.2, 21.4 and 11; *m*/*z* 735 (M^+, 40%), 579 (81), 346 (43), 246 (100), 218 (43), 192 (51), 155 (62), 144 (27), 132 (39), 118 (88), 106 (28) and 91 (100).

Reaction of Compound **4** *with* K_2CO_3 *and DMF to form Methyl*3α,12α[*Methylenebis*(N-*tosylimino*-p-*phenyleneethylenecarbonyloxy*)-5β-*cholen*-24-*oate*] **6**.—To a 25 cm^3 two-necked, round-bottomed flask fitted with a reflux condenser and a rubber septum, were added K_2CO_3 (0.330 g, 2.38 mmol), DMF (4.0 cm^3) and methyl 3α,12α-bis[3-(4-*N*-tosylaminophenyl)propanoyloxy]-5β-cholan-24-oate **4** (0.115 g, 0.11 mmol). The mixture was then heated (bath temp. 125 °C) with vigorous stirring for 48 h, cooled to room temperature, diluted with ethyl acetate (25 cm^3) and filtered to remove the K_2CO_3. The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4 and filtered. Volatiles were removed under reduced pressure to yield the crude product, which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (2%) to yield compound **6** (0.022 g, 19%) as a colourless solid; ν_{max}(thin film)/cm^{-1} 2940vs, 1720vs (CO), 1600w, 1510s, 1455s, 1360vs, 1180vs, 1040s, 920s, 740s and 690s; δ_H(270 MHz; $CDCl_3$) 0.68 (3 H, s, 18-H), 0.77 (3 H, d, *J* 6, 21-H), 0.86 (3 H, s, 19-H), 1.00–1.83 (br m), 2.38 (3 H, s, Ts-*Me*), 2.42 (3 H, s, Ts-*Me*), 2.27–2.80 (8 H, m, Ar-CH_2), 3.61 (3 H, s, CO_2Me), 4.73 (1 H, br m, 3-H), 5.08 (1 H, s, 12-H), 5.32 (1 H, d, *J* 13.5, N-C*H*H), 5.62 (1 H, d, *J* 13.5, N-CH*H*), 6.27 (2 H, d, *J* 8.2, Ar-H), 6.44 (2 H, d, *J* 8.2, Ar-H), 6.76–6.81 (4 H, m, Ar-H), 7.25 (4 H, m, Ts-H) and 7.58–7.70 (4 H, m, Ts-H).

Direct Synthesis of Methyl 3α,12α-[*Methylenebis*(N-*tosylimino*-p-*phenyleneethylenecarbonyloxy*)-5β-*cholan*-24-*oate*] **6**.—To a 25 cm^3 two-necked, round-bottomed flask fitted with a reflux condenser and a rubber septum, K_2CO_3 (0.676 g, 4.89 mmol) and acetonitrile (10.0 cm^3) were added. To this vigorously stirred mixture, a solution of compound **4** (0.471 g, 0.466 mmol) and diiodomethane (0.137 g, 0.041 cm^3, 0.511 mmol) in acetonitrile (10.0 cm^3) was then added *via* syringe over a period of 8 h at 78 °C. Heating was then continued for 16 h and, after cooling to room temperature, the mixture was diluted with ethyl acetate (25 cm^3) and filtered to remove the K_2CO_3. The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the crude product, which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (3%) to yield compound **6** (0.025 g, 52%); $[\alpha]_D^{22}$ +34.6 (*c* 0.55 in $CHCl_3$); $\lambda_{max}(CHCl_3)$/nm 243 (ε/dm^3 mol^{-1} cm^{-1} 18 180); ν_{max}(thin film)/cm^{-1} 2920vs, 1725vs, 1599w, 1353s, 1161s, 1086w, 849w and 750s; δ_H(270 MHz; $CDCl_3$) 0.70 (3 H, s, 18-H), 0.78 (3 H, d, *J* 6.1, 21-H), 0.88 (3 H, s, 19-H), 0.90–1.90 (br m), 2.41 (3 H, s, Ts-*Me*), 2.44 (3 H, s, Ts-*Me*), 2.09–2.83 (8 H, m, Ar-CH_2), 3.63 (3 H, s, CO_2Me), 4.75 (1 H, br m, 3-H), 5.10 (1 H, s, 12-H), 5.34 (1 H, d, *J* 13.5, N-C*H*H), 5.65 (1 H, d, *J* 13.5, N-CHH), 6.30 (2 H, d, *J* 8.3, Ar-H), 6.47 (2 H, d, *J* 8.3, Ar-H), 6.79–6.83 (4 H, m, Ar-H), 7.25 (4 H, m, Ts-H) and 7.61–7.65 (4 H, m, Ts-H); δ_C(100.61 MHz; $CDCl_3$) 174.52, 171.70, 171.55, 143.79, 143.72, 141.47, 139.97, 136.77, 136.43, 136.06, 129.77, 129.56, 128.34, 128.18, 127.95, 127.91, 76.19, 73.27, 67.08, 51.49, 49.90, 47.64, 45.10, 41.38, 41.21, 37.4, 36.10, 35.74, 30.99, 30.89, 30.73, 30.54, 27.34, 26.42, 25.91, 25.13, 23.36, 22.67, 22.56, 21.61, 17.66, 14.13, 12.43 and 11.44; *m*/*z* (FAB) 1021 (M^+, 72%), 879 (20), 866 (46), 850 (18), 709 (67), 546 (22), 371 (78), 332 (38), 255 (23), 176 (35), 155 (41), 132 (46), 118 (87), 91 (100), 83 (59), 69 (39), 55 (59) and 41 (41).

Cleavage of the Macrocycle **6** *to yield Dimethyl Methylenebis*(N-*tosylimino*-p-*phenyleneethylenecarboxylate*) **10**.—A mixture of compound **6** (0.0785 g, 0.074 mmol), 10% methanolic KOH (0.5 cm^3) and dioxane (0.5 cm^3) was heated (bath temp. 65 °C) for 5 h with vigorous stirring and, after acidification with 2 mol dm^{-3} HCl to pH 3, the products were extracted with ethyl acetate (15 cm^3). The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the crude products. The residue

was dissolved in diethyl ether (10 cm^3) and treated with an excess of diazomethane at 0 °C. After 30 min, the excess diazomethane and the solvent were removed by bubbling through with N_2 and the products were dried *in vacuo*. The crude product was column chromatographed on silica with EtOAc–$CHCl_3$ (5%) to yield compound **10** (0.042 g, 83%), m.p. 99 °C (from EtOH); ν_{max}(thin film)/cm^{-1} 2902vs, 1737vs, 1596s, 1458s, 1035w and 849w; δ_H(270 MHz; $CDCl_3$) 2.39 (6 H, s, Ts-*Me*), 2.64 (4 H, t, $ArCH_2CH_2$), 2.96 (4 H, t, Ar-CH_2), 3.68 (6 H, s, CO_2Me), 5.49 (2 H, s, N-CH_2-N) and 6.84–7.23 (16 H, m, Ar-H).

Authentic Dimethyl Methylenebis(N-*tosylimino*-p-*phenyleneethylenecarboxylate*) **10**.—A mixture of methyl 3-(4-*N*-tosylaminophenyl)propanoate (0.147 g, 0.446 mmol), K_2CO_3 (0.709 g, 5.13 mmol), acetonitrile (3 cm^3) and diiodomethane (0.06 g, 0.018 cm^3, 0.224 mmol) was vigorously stirred with heating (bath temp. 75 °C) for 24 h. The cooled reaction mixture was diluted with ethyl acetate (15 cm^3) and filtered to remove K_2CO_3. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield crude product which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (2%) to yield compound **10** (0.093 g, 62%), m.p. 99 °C (from EtOH); ν_{max}(thin film)/cm^{-1} 2902vs, 1737vs, 1596s, 1458w and 849w; δ_H(270 MHz; $CDCl_3$) 2.38 (6 H, s, Ts-H), 2.64 (4 H, t, $ArCH_2CH_2$), 2.95 (4 H, t, Ar-CH_2), 3.67 (6 H, s, CO_2Me), 5.50 (2 H, s, N-CH-N) and 6.84–7.23 (16 H, m, Ar-H); δ_C(100.61 MHz; $CDCl_3$) 173.10, 143.62, 140.99, 136.12, 135.55, 129.84, 129.41, 128.92, 127.51, 127.25, 121.96, 62.92, 51.68, 35.37, 30.52 and 21.54; *m/z* 678 (M^+), 536 (25), 523 (33), 487 (26), 463 (71), 422 (27), 347 (100), 314 (22), 181 (98), 155 (100), 131 (39), 118 (100), 106 (38) and 91 (100).

Methyl 3α,12α-[*Diazenebis*(m-*phenylenemethylene*-N-*tosylimino*-p-*phenyleneethylenecarbonyloxy*)]-5β-*cholan*-24-*oate* **8**.—To a 25 cm^3 two-necked round-bottomed flask fitted with a reflux condenser and a rubber septum, K_2CO_3 (0.339 g, 2.45 mmol) and acetonitrile (4.9 cm^3) were added. To this vigorously stirred mixture, a solution of compound **4** (0.215 g, 0.212 mmol) and bis[3-(chloromethyl)phenyl)]diazene **7** (0.062 g, 0.222 mmol) in acetonitrile (4.9 cm^3) was added *via* syringe over a period of 8 h at 75 °C. Heating was continued for a further 16 h and, after cooling to room temperature, the mixture was diluted with ethyl acetate (25 cm^3) and filtered to remove K_2CO_3. The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4 and then filtered. The solvent was removed under reduced pressure to yield the crude product, which was purified by column chromatography on silica with EtOAc–$CHCl_3$ (2%) to yield compound **8** (0.124 g, 48%) as an orange foam; $[\alpha]_D^{22}$ +22.5 (*c* 0.58 in $CHCl_3$); λ_{max}($CHCl_3$)/nm 243 (ε/dm^3 mol^{-1} cm^{-1} 28 690), 324.6 (18 430) and 443.4 (470); ν_{max}(thin film)/cm^{-1} 2920vs, 1765vs (CO), 1670s, 1590s, 1500s, 1340s, 1160s, 905s and 720s; δ_H(270 MHz; $CDCl_3$) 0.70 (3 H, s, 18-H), 0.79 (3 H, d, *J* 5.9, 21-H), 0.88 (3 H, s, 19-H), 1.01–1.85 (br m), 2.44 (6 H, s, Ts-*Me*), 2.17–2.54 (br m), 2.76–2.85 (4 H, m, $ArCH_2$-CH_2), 3.65 (3 H, s, CO_2Me), 4.64–4.85 (5 H, m, Ar-CH_2-N and 3-H), 5.07 (1 H, s, 12-H) and 6.90–7.90 (24 H, m, Ar-H); δ_C(100.61 MHz; $CDCl_3$) 174.6, 172.4, 172.11, 152.45, 152.17, 143.74, 143.69, 140.22, 140.02, 137.24, 137.07, 136.94, 136.58, 135.34, 135.18, 131.34, 130.75, 129.66, 129.62, 129.36, 129.13, 128.77, 128.71, 128.66, 127.84, 127.81, 123.31, 122.78, 122.05, 76.19, 74.32, 54.10, 51.57, 49.28, 47.70, 45.22, 41.94, 35.83, 35.76, 34.82, 34.58, 34.21, 32.50, 31.13, 30.92, 30.51, 27.43, 27.03, 26.96, 26.80, 25.95, 25.76, 23.50, 23.22, 21.64, 17.66, 14.32 and 12.43; *m/z* (FAB) 1214 (M^+, 76%), 1075 (61), 861 (72), 845 (91), 827 (27), 706 (46), 690 (68), 671 (41), 542 (41), 371 (76), 154 (100), 136 (72), 107 (40), 91 (42), 77 (40) and 55 (22).

Cleavage of the Macrocycle **8** *to yield Dimethyl Diazenebis*(m-*phenylenemethylene*-N-*tosylimino*-p-*phenyleneethylenecarboxylate*) **11**.—A mixture of compound **8** (0.104 g, 0.086 mmol), 10% methanolic KOH (0.5 cm^3) and dioxane (0.5 cm^3) was heated (bath temp. 65 °C) for 5 h with vigorous stirring and, after acidification with 2 mol dm^{-3} HCl to pH 3, the products were extracted with ethyl acetate (15 cm^3). The organic layer was washed with 7% $NaHCO_3$ solution, water, brine and finally dried over anhydrous Na_2SO_4 and then filtered. The solvent was removed under reduced pressure to yield the crude products. The residue was dissolved in diethyl ether (10 cm^3) and treated with an excess of diazomethane at 0 °C. After 30 min, the excess diazomethane and solvent were removed by evaporation with a current of N_2 and then the products were dried *in vacuo*. The crude product (911 mg) was column chromatographed on silica with EtOAc–$CHCl_3$ (2%) to yield compound **11** (400 mg, 82%); ν_{max}(thin film)/cm^{-1} 2908vs, 1719vs, 1617s, 1515s, 1170s, 1014w and 753w; δ_H(90 MHz; $CDCl_3$) 2.46 (6 H, s, Ts-H), 2.51–2.99 (8 H, m, Ar-CH_2CH_2), 3.60 (6 H, s, CO_2Me), 4.77 (4 H, s, Ar-CH_2-N) and 6.74–7.83 (24 H, m, Ar-H).

Authentic Dimethyl Diazenebis(m-*phenylenemethylene*-N-*tosylimino*-p-*phenyleneethylenecarboxylate*) **11**.—A mixture of methyl 3-(4-*N*-tosylaminophenyl)propanoate (0.262 g, 0.792 mmol), K_2CO_3 (0.940 g, 6.8 mmol), acetonitrile (2.5 cm^3) and bis[3-(chloromethyl)phenyl]diazene (0.122 g, 0.393 mmol) was vigorously stirred with heating (bath temp. 78 °C) for 24 h and then cooled to room temperature. The mixture was diluted with ethyl acetate (15 cm^3) and filtered to remove the K_2CO_3. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and then filtered. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica with EtOAc–light petroleum (40–60 °C, 30%) to yield compound **11** (100 mg, 44%) as an orange solid; ν_{max}(thin film)/cm^{-1} 2920s, 1731vs, 1599s, 1437w, 1347s, 1155s, 1089s and 750w; δ_H(90 MHz; $CDCl_3$) 2.442 (6 H, s, Ts-H) 2.50–2.964 (8 H, m, Ar-CH_2CH_2), 3.61 (6 H, s, CO_2Me), 4.771 (4 H, s, Ar-CH_2-N) and 6.80–7.78 (24 H, m, Ar-H); δ_C(50 MHz; $CDCl_3$) 175.85, 152.27, 143.52, 143.37, 140.10, 137.13, 136.84, 135.25, 131.35, 130.75, 129.34, 128.64, 127.56, 125.54, 124.32, 122.65, 121.38, 54.25, 54.62, 51.39, 35.03, 30.15 and 21.38; *m/z* 872 (M^+), 717, 561, 528, 438, 333, 283, 260, 209, 163, 106, 65 and 44.

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