Novel Macrocyclic Bile Acid Derivatives; Selective and Easy Binding of Two Cholic Acid Moieties at the 3- and 3′-Positions

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Abstract: The synthesis of macrocyclic derivatives of bile acids (cholaphanes) is elaborated using the formation of ester or amide linkages at the 3α,3′α- and 24,24′-positions of lithocholic, deoxycholic or cholic acids. The conditions were found under which the selective diacylation at the 3α,3′α- and 24,24′-positions of two molecules of cholic acid by dichloroanhydrides of arene(hetarene)dicarboxylic acid proceeds in good yields without prior protection of the 7α- and 12α-hydroxy groups.

Key words: macrocycles, bile acids, cyclophanes, cholaphanes, cyclizations

Bile acids are naturally occurring chiral compounds with a rigid steroidal skeleton that possess one to three endo-hydroxy groups and an *iso*-pentanoic acid side chain. The chemistry of the macrocyclic derivatives of bile acids has been flourishing since the 1990s due to the possible use of such compounds as selective receptors for cations and anions[1–5] and because they are such versatile organic molecules.[6–11] Another interesting use of macrocycles derived from bile acids is ion channels modeling.[12]

Up to now, various macrocycles of this type, which contain from a single to several bile acid units, have been synthesized and investigated. The simplest molecules from the synthetic point of view are the cyclocholates, which are often obtained by the Yamaguchi macrolactonization method[1,6,13,14] or by the use of dicyclohexylcarbodiimide for condensation.[15] Similar cyclic amides of bile acids – cyclocholamides[7,8,16,17] – are also known which, in some cases, contain amino acids fragments.[18–20] The Ugi reaction has been used for the synthesis of N-substituted cyclocholamides.[21,22]

Cholaphanes are another type of steroidal macrocycles; these contain 2–4 bile acid moieties which are linked by different spacers at the 3- and 24-positions of the steroidal backbone. Such molecules can exist as 'head-to-tail' (**I**) and 'head-to-head' (**II**) isomers (Figure 1). Diamide[23,24] or ester[25] linkages are the most common with spacer **A**, while ester groups are most frequently used with spacer **B**.[23,25,26] More rare cases include ether groups as spacers **A** and **B**.[27] Syntheses of cholaphanes (**II**) often starts with spacer **A** formation. The second step is the cyclization of the dimeric intermediate through formation of the spacer **B**. The Yamaguchi method employing ester formation gives such macrocycles in poor 9–11% yield.[23,25] The reaction of steroidal 3-(bromoacetates) with dicesium terephthalate or with dicesium 3,5-pyridinedicarboxylate affords cholaphanes in high yields 70–95%.[11,24,26]

The use of ester or ether bonds in **A** spacers between the 24- and 24′-positions is not frequent. Ester spacers are synthesized by the reaction of lithocholic acid chloroanhydride with corresponding diols.[25] The yields of the intermediate acylic 24,24′-diester do not exceed 40%. Ether bridges are synthesized by the preliminary reduction of

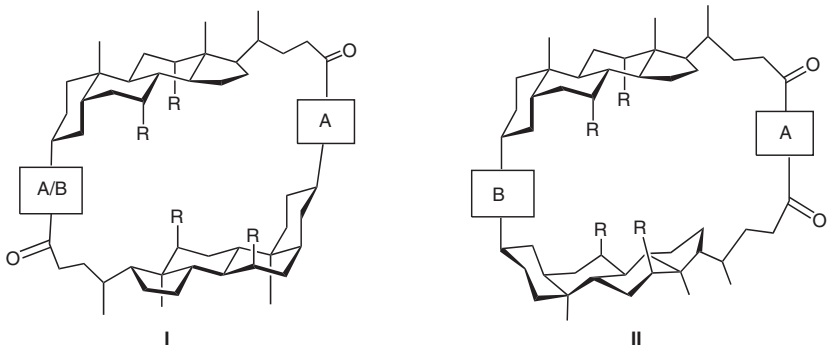

Figure 1

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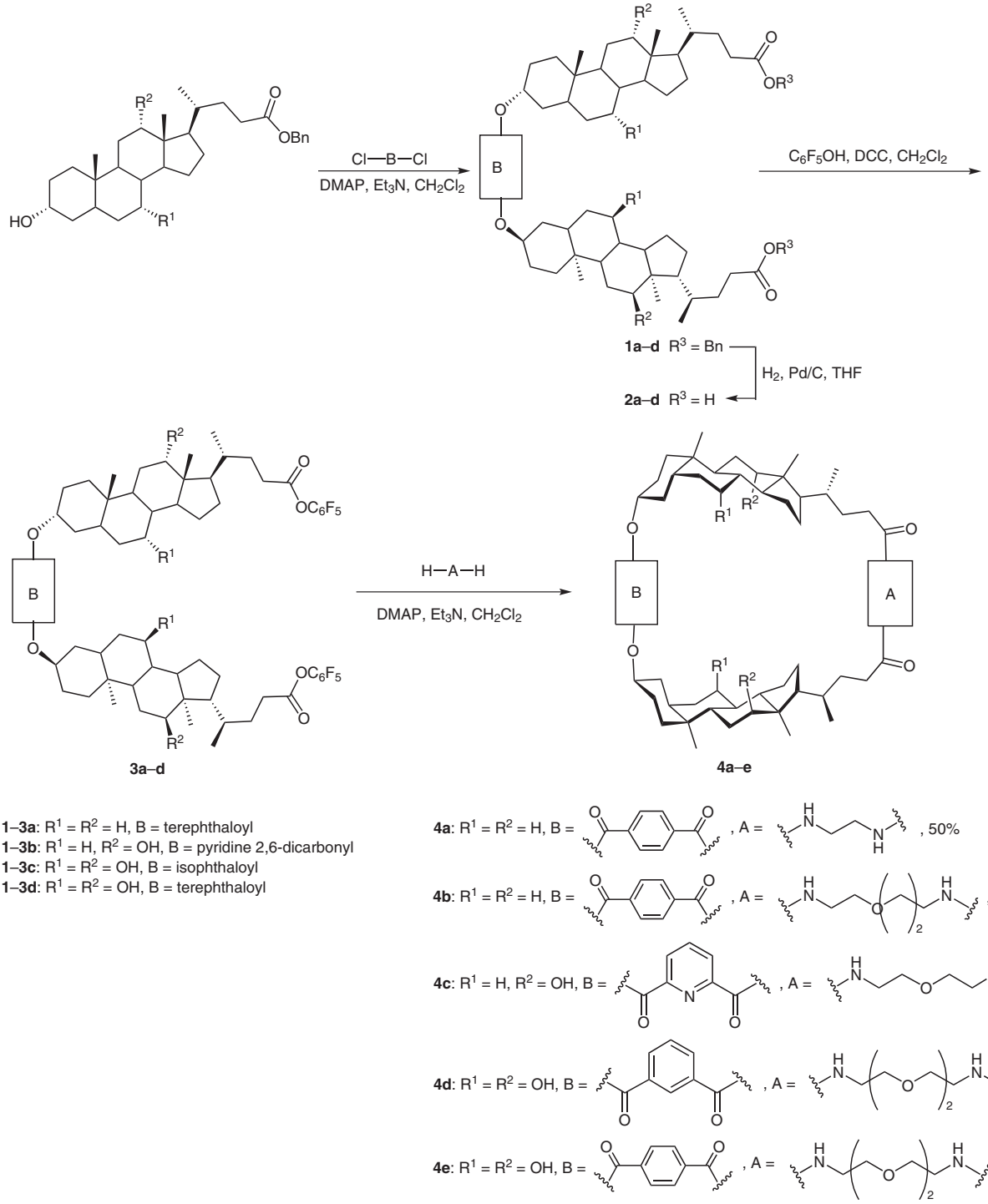
the bile acid's carboxyl group to the hydroxy group. It is to be noted that the approach using ether spacers is laborious because it demands multiple protecting and deprotecting steps.[27] Cyclic dimers in which the hydroxy groups at the 7α- and 12α-positions of the cholic acid are linked have also been described.[28]

Also widespread are macrocycles consisting of one bile acid moiety[3] connected to cholacrown ethers.[4,29] Palladium-catalyzed amination,[30,31] metathesis,[32] and copper-catalyzed 1,3-dipolar cycloaddition[33] have become important new approaches to the generation of macrocyclic structures with steroidal fragments.

Analysis of the literature data shows that the synthesis of macrocycles based on deoxycholic and cholic acids using chloroanhydrides of organic acids is often hindered by their non-selectivity towards hydroxy groups at the 3α-, 7α- and 12α-positions. Due to this fact, lithocholic acid is widely used for the synthesis of macrocycles because it possesses only one hydroxy group at position 3α. However, this acid has a hydrophobic backbone and possesses



1–3a: $R^1 = R^2 = H$, B = terephthaloyl
1–3b: $R^1 = H$, $R^2 = OH$, B = pyridine 2,6-dicarbonyl
1–3c: $R^1 = R^2 = OH$, B = isophthaloyl
1–3d: $R^1 = R^2 = OH$, B = terephthaloyl

4a: $R^1 = R^2 = H$, B = , A = , 50%

4b: $R^1 = R^2 = H$, B = , A = , 37%

4c: $R^1 = H$, $R^2 = OH$, B = , A = , 37%

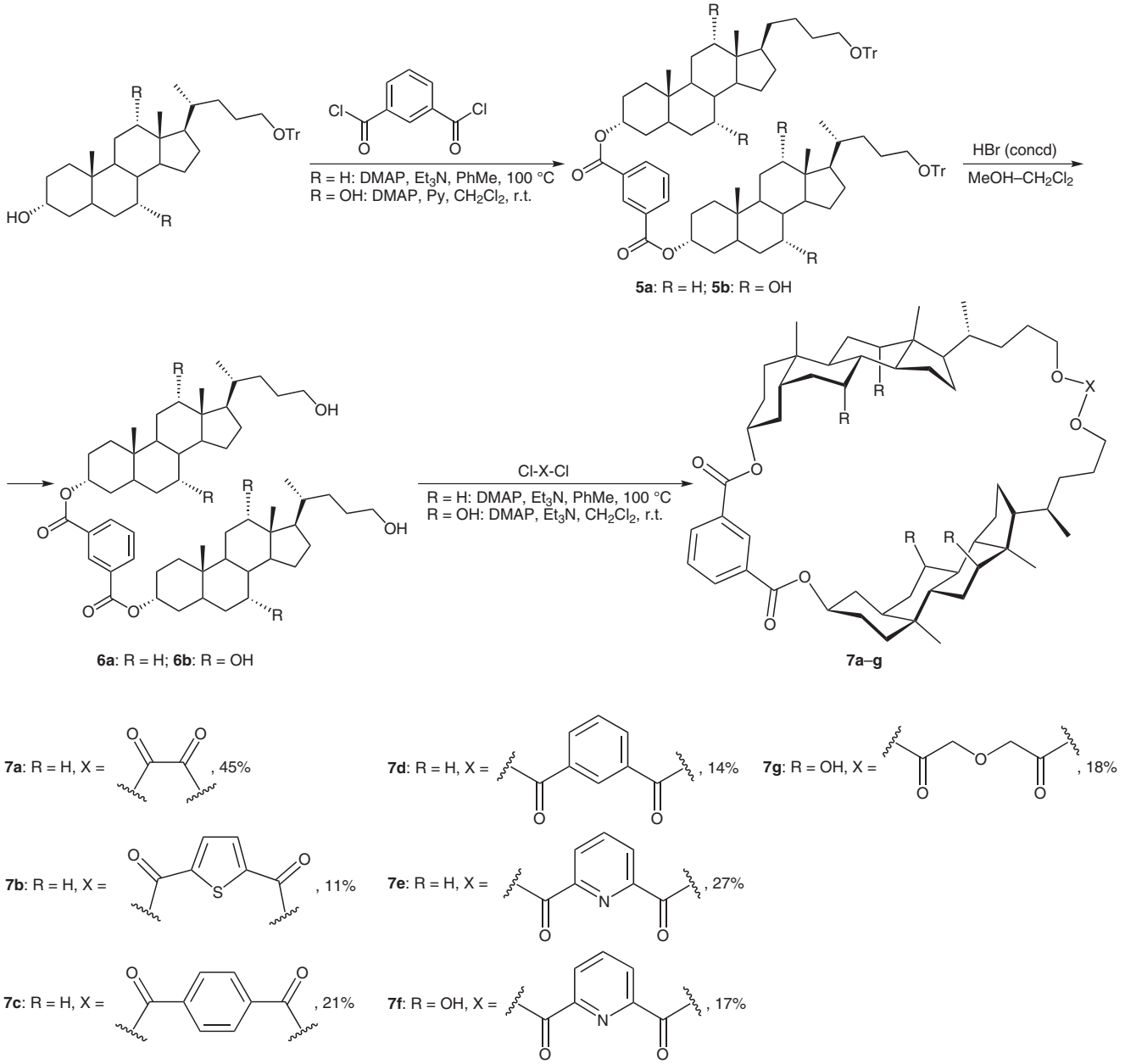
4d: $R^1 = R^2 = OH$, B = , A = , 34%

4e: $R^1 = R^2 = OH$, B = , A = , 28%

Scheme 1

less valuable coordination properties. We have also found that deoxycholic and cholic acids cannot be used in palladium-catalyzed amination reactions because of the low solubility of intermediate products.[30,31] All these facts taken together forced us to try more classical methods for the synthesis of new types of cholaphanes and to search for convenient procedures for acylation of cholic acid derivatives.

Recently, we have proposed a method for the synthesis of lithocholaphanes by the initial formation of the spacer **B**. In these molecules either both spacers **A** and **B** were equipped with ester groups, or spacer **B** contained ester groups and spacer **A** contained a (poly)oxadiamide bridge.[34] It is known that the reactivity of the equatorial 3α-hydroxy group in cholic acid in the acylation reaction is higher than that of the axial 7α- and 12α-hydroxy groups. In some cases this gives rise to the possibility of selective acylation of the 3α-hydroxy of cholates, for example by bromoacetyl bromide[11,24] or by chloroacetic anhydride.[5] Trimesoyl chloride was also proposed for selective acylation of three molecules of cholic acid in toluene and DMAP under reflux at the 3-position.[35] However, we were unable to carry out selective bis(acylation) of two molecules of methyl cholate with dichloroanhydrides of arenedicarboxylic acids under conditions described in the papers mentioned above. In this paper we demonstrate that, by modifying this approach, one can obtain cholaphanes from cholic acid without initial protection of the hydroxy groups at positions 7α and 12α. The approach uses a selective acylation of cholic acid esters at the 3α- and 3′α-positions (Scheme 1).



Scheme 2

Previously, we presented the synthesis of a diester linker binding positions 3α and 3′α of the lithocholic acid benzyl esters. This was achieved by the action of aromatic dicarboxylic acid dichloroanhydrides in toluene at 100 °C for 12 hours.[34] However, since this method proved to be useless for the synthesis of deoxycholic and cholic acid dimers similar to **1b**,**c** due to competitive acylation of the 7α- and 12α-hydroxy groups, we have found milder conditions for selective 3α,3′α-diacylation: in our new protocol, dichloromethane or tetrahydrofuran was used instead of toluene, and the reaction was run at ambient temperature. These reaction conditions allowed us to obtain compounds **1b–d** in yields up to 65% without formation of significant admixtures of products in which the hydroxy groups at the 7- and 12-positions were affected. To form the diamide spacer **A**, we proposed the reaction of diamines with pentafluorophenyl esters at positions 24 and 24′. Yields at the macrocyclization step varied from 34% to 50%, which is 1.5–2 times higher than described in the literature for analogous compounds. The yields of cholaphanes did not change significantly when changing lithocholic acid for deoxycholic or cholic acids.

Another approach was elaborated for the synthesis of cholaphanes with two diester bridges (Scheme 2). In this case, mild acylation conditions (CH_2Cl_2, 20 °C, DMAP, pyridine) afforded the cholic acid dimer **5b** in 56% yield. Deprotection according to a standard procedure gave the corresponding 24,24′-diols, which were introduced into the reaction with dicarboxylic acid dichloroanhydrides. In the case of the transformation of **6b** into **7f** and **7g**, mild acylation conditions were used again to prevent undesirable acylation at positions 7 and 12.

Cholaphanes thus obtained were characterized using ^{1}H and ^{13}C NMR as well as MALDI-TOF and ESI-TOF MS techniques. The most important feature of the ^{1}H NMR spectra of the target compounds, which supports the proposed structure of cholaphanes, is substantial deshielding of the 3β-protons (from 3.4 ppm to 4.8 ppm). A similar deshielding was observed for the lithocholic acid benzyl ester after its cyclization into the dimeric product **1a**. In the case of the cyclization using diamide spacers, a notable shielding of the protons at C23 occurred (from 2.7 ppm to 2.1 ppm) whereas in the case of the diester bridge formation in **7a–g** we observed deshielding of the protons at C24 (from 3.6 to 4.4–4.5 ppm).

In conclusion, we report here a straightforward and synthetic route to bile acid derived macrocycles which can be utilized as an aid in designing suitable receptors and hosts to be used in various supramolecular systems and in nanochemical applications.

Lithocholic acid was purchased from Sigma, deoxycholic and cholic acids were purchased from Acros Organic and used without further purification. Isophthalic and terephthalic acids and other reagents were analytical grade reagents and used as purchased. All solvents used in chromatography were also analytical grade reagents and used without further purification. Column chromatography was performed on silica gel 60 (0.040–0.063 mm) from Merck. The benzyl ester of lithocholic acid[36] and the benzyl esters of deoxycholic and cholic acids[37] were prepared according to the literature. 5β-Cholane-3α,24-diol, 24-triphenylmethoxy-5β-cholan-3α-ol, dibenzyl 3α,3′α-(terephthaloyloxy)bis(5β-cholan-24-oate) (**1a**), 3α,3′α-(terephthaloyloxy)bis(5β-cholan-24-oic acid) (**2a**), bis(pentafluorophenyl) 3α,3′α-(terephthaloyloxy)bis(5β-cholan-24-oate) (**3a**), bis(24-triphenylmethoxy)-3α,3′α-(isophthaloyloxy)bis(5β-cholane) (**5a**), 3α,3′α-(isophthaloyloxy)bis(5β-cholan-24-ol) (**6a**) were obtained according to Valkonen et al.[34] 5β-Cholane-3α,7α,12α,24-tetraol and 24-triphenylmethoxy-5β-cholane-3α,7α,12α-triol were synthesized as previously described.[38] ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker Avance 400 spectrometer; ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra were recorded on a Bruker Avance DRX 500 FT spectrometer. Methylene and methyne protons of steroid cores which are present in ^{1}H NMR spectra as complex unresolved multiplets in the 1.0–2.8 ppm region are not indicated. Only significant peaks are shown. MALDI-TOF spectra were recorded on Bruker Daltonics Ultraflex mass spectrometer using dithranol as matrix. Electrospray mass spectroscopic measurements were performed by using an LCT time of flight (TOF) mass spectrometer with electrospray ionization (ESI; Micromass LCT).

Dibenzyl 3α,3α′-[Arene(hetarene)dicarbonyloxy]bis[7*H*(OH),12α-hydroxy-5β-cholan-24-oate]s (1b–d); General Procedure

To the corresponding dichloroanhydride of arene(hetarene)dicarboxylic acid (0.225 mmol) in anhyd THF (1.5 mL), DMAP (18 mg, 0.14 mmol), Et_3N (0.26 mL) and the benzyl ester of deoxycholic acid (241 mg, 0.5 mmol) or the benzyl ester of cholic acid (249 mg, 0.5 mmol) were added consecutively. The mixture was stirred for 18 h at r.t., diluted with CH_2Cl_2 (50 mL) and washed with 5% $NaHCO_3$ (2 × 10 mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography [(CH_2Cl_2–MeOH, 99:1; R_f = 0.70) for **1b** or (CH_2Cl_2–MeOH, 20:1; R_f = 0.50) for **1c**,**d**] to give **1b–d** as white crystalline solids.

Dibenzyl 3α,3α′-(2,6-Pyridinedicarbonyloxy)bis(12α-hydroxy-5β-cholan-24-oate) (1b)

Yield: 173 mg (70%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, ^3J = 7.8 Hz, 2 H, ArH-3,5), 8.15 (t, ^3J = 7.8 Hz, 1 H, ArH-4), 7.34 (m, 10 H, Ph), 5.10 (m, 4 H, CH_2-Ph), 5.06 (m, 2 H, 3β-H, 3β′-H), 3.95 (br s, 2 H, 12β-H, 12β′-H), 2.45–2.22 (m, 4 H, 23-CH_2, 23′-CH_2), 0.94 (m, 12 H, 21-CH_3, 21′-CH_3, 19-CH_3, 19′-CH_3), 0.64 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0 [C(24)=O], 164.0 (ArC=O) 148.9 (ArC-2,6), 138.0 (ArC-4), 136.1 (PhC-1), 128.5 (PhC-2,6), 128.1 (PhC-3,4,5), 127.6 (ArC-3,5), 76.4 (3-CH), 73.0 (12-CH), 66.0 (Ph-CH_2), 48.2, 47.2, 46.4, 41.8, 35.9, 35.0, 34.9, 34.1, 33.5, 31.9, 31.2, 30.8, 28.6, 27.4, 26.9, 26.3, 26.0, 23.6 (19-CH_3), 23.0, 17.2 (21-CH_3), 12.7 (18-CH_3).

Dibenzyl 3α,3α′-(Isophthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-oate) (1c)

Yield: 109 mg (43%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.63 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.18 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.47 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 7.34 (m, 10 H, Ph), 5.10 (m, 4 H, CH_2-Ph), 4.84 (m, 2 H, 3β-H, 3β′-H), 3.97 (br s, 2 H, 12β-H, 12β′-H), 3.85 (br s, 2 H, 7β-H, 7β′-H), 2.53–2.38 (m, 4 H, 23-CH_2, 23′-CH_2), 0.97 (d, ^3J = 5.8 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.93 (s, 6 H, 19-CH_3, 19′-CH_3), 0.67 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0 [C(24)=O], 165.3 (ArC=O), 136.1 (PhC-1), 133.4 (ArC-4,6), 131.2 (ArC-1,3), 130.7 (ArC-2), 128.5 (PhC-2,6), 128.2 (PhC-3,4,5 and ArC-5), 75.2 (3-

CH), 72.9 (12-CH), 68.2 (7-CH), 66.1 (Ph-CH_2), 47.2, 46.5, 42.0, 41.2, 39.4, 35.2, 35.1, 34.8, 34.7, 34.4, 31.3, 30.8, 28.4, 27.4, 26.7, 26.6, 23.1 (19-CH_3), 22.4, 17.3 (21-CH_3), 12.5 (18-CH_3).

Dibenzyl 3α,3α′-(Terephthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-oate) (1d)
Yield: 165 mg (65%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (s, 4 H, ArH-2,3,5,6), 7.34 (m, 10 H, Ph), 5.10 (m, 4 H, CH_2-Ph), 4.83 (m, 2 H, 3β-H, 3β′-H), 3.99 (br s, 2 H, 12β-H, 12β′-H), 3.86 (br s, 2 H, 7β-H, 7β′-H), 2.54–2.38 (m, 4 H, 23-CH_2, 23′-CH_2), 0.97 (d, ^3J = 5.9 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.93 (s, 6 H, 19-CH_3, 19′-CH_3), 0.68 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0 [C(24)=O], 165.4 (ArC=O), 136.0 (PhC-1), 134.4 (ArC-1,4), 129.3 (ArC-2,3,5,6), 128.5 (PhC-2,6), 128.2 (PhC-3,4,5), 75.4 (3-CH), 72.9 (12-CH), 68.2 (7-CH), 66.1 (Ph-CH_2), 47.3, 46.5, 42.0, 41.2, 39.5, 35.2, 35.1, 34.8, 34.7, 34.4, 31.3, 30.8, 28.4, 27.4, 26.7, 26.6, 23.1 (19-CH_3), 22.5, 17.3 (21-CH_3), 12.5 (18-CH_3).

3α,3α′-[Arene(hetarene)dicarbonyloxy]bis[7*H*(OH),12α-hydroxy-5β-cholan-24-oic Acid]s (2b–d); General Procedure
To a mixture of the corresponding dibenzyl ester **1b–d** (0.64 mmol) and 10% Pd/C (20 mg), anhyd THF (10 mL) was added. The flask was purged with H_2 for 1–2 s, connected to a balloon of H_2 and stirred for 24 h at r.t. The mixture was filtered through a short plug of silica gel to remove the catalyst. The filtrate was evaporated to dryness to give **2b–d** as a white crystalline solid.

3α,3α′-(2,6-Pyridinedicarbonyloxy)bis(12α-hydroxy-5β-cholan-24-oic Acid) (2b)
Yield: 563 mg (96%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (d, ^3J = 7.8 Hz, 2 H, ArH-3,5), 7.99 (t, ^3J = 7.8 Hz, 1 H, ArH-4), 5.01 (m, 2 H, 3β-H, 3β′-H), 3.95 (br s, 2 H, 12β-H, 12β′-H), 0.94 (m, 12 H, 21-CH_3, 21′-CH_3, 19-CH_3, 19′-CH_3), 0.67 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.9 [C(24)=O], 164.1 (ArC=O), 148.7 (ArC-2,6), 138.3 (ArC-4), 127.9 (ArC-3,5), 76.9 (3-CH), 73.1 (12-CH), 48.0, 47.0, 46.5, 42.0, 35.9, 35.2, 35.0, 34.2, 33.5, 31.9, 31.2, 30.7, 28.5, 27.5, 27.0, 26.3, 26.1, 23.7 (19-CH_3), 23.0, 17.3 (21-CH_3), 12.7 (18-CH_3).

3α,3α′-(Isophthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-oic Acid) (2c)
Yield: 600 mg (99%).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.58 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.20 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.51 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.83 (m, 2 H, 3β-H, 3β′-H), 3.98 (br s, 2 H, 12β-H, 12β′-H), 3.87 (br s, 2 H, 7β-H, 7β′-H), 2.50–2.35 (m, 4 H, 23-CH_2, 23′-CH_2), 0.96 (d, ^3J = 5.8 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.94 (s, 6 H, 19-CH_3, 19′-CH_3), 0.70 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, CD_3OD): δ = 178.9 [C(24)=O], 166.6 (ArC=O), 134.6 (ArC-4,6), 132.6 (ArC-1,3), 131.1 (ArC-2), 129.9 (ArC-5), 77.2 (3-CH), 74.0 (12-CH), 69.0 (7-CH), 48.1, 47.5, 43.0, 42.9, 41.0, 36.8, 36.4, 36.0, 35.9, 35.6, 32.6, 32.5, 29.5, 28.7, 27.9, 27.7, 24.2 (19-CH_3), 23.0, 17.7 (21-CH_3), 13.0 (18-CH_3).

3α,3α′-(Terephthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-oic Acid) (2d)
Yield: 598 mg (99%).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.05 (s, 4 H, ArH-2,3,5,6), 4.83 (m, 2 H, 3β-H, 3β′-H), 4.01 (br s, 2 H, 12β-H, 12β′-H), 3.88 (br s, 2 H, 7β-H, 7β′-H), 2.55–2.38 (m, 4 H, 23-CH_2, 23′-CH_2), 0.99 (d, ^3J = 5.8 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.94 (s, 6 H, 19-CH_3, 19′-CH_3), 0.71 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, CD_3OD): δ = 180.6 [C(24)=O], 170.5 (ArC=O), 135.9 (ArC-1,4), 130.4 (ArC-2,3,5,6), 77.3 (3-CH), 74.0 (12-CH), 69.0 (7-CH), 48.1, 47.5, 43.0, 42.9, 41.0, 36.9, 36.4, 36.1, 36.0, 35.6, 32.7, 32.5, 29.5, 28.7, 27.9, 27.6, 24.2 (19-CH_3), 23.0, 17.7 (21-CH_3), 13.0 (18-CH_3).

Bis(pentafluorophenyl) 3α,3α′-[Arene(hetarene)dicarbonyloxy]bis[7*H*(OH),12α-hydroxy-5β-cholan-24-oate]s (3b–d); General Procedure
To a stirred solution of the corresponding **2b–d** (0.61 mmol) in anhyd CH_2Cl_2 (17 mL), pentafluorophenol (282 mg, 1.53 mmol) and dicyclohexylcarbodiimide (380 mg, 1.84 mmol) were added at the same time. After being stirred for 18 h at r.t., the mixture was diluted with CH_2Cl_2 (20 mL) and filtered. The filtrate was washed with 5% $NaHCO_3$ (10 mL), and H_2O (10 mL), then dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 98:2; R_f = 0.60).

Bis(pentafluorophenyl) 3α,3α′-(2,6-Pyridinedicarbonyloxy)bis(12α-hydroxy-5β-cholan-24-oate) (3b)
Yield: 457 mg (60%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.25 (d, ^3J = 7.8 Hz, 2 H, ArH-3,5), 8.01 (t, ^3J = 7.8 Hz, 1 H, ArH-4), 5.03 (m, 2 H, 3β-H, 3β′-H), 4.00 (br s, 2 H, 12β-H, 12β′-H), 2.78–2.55 (m, 4 H, 23-CH_2, 23′-CH_2), 1.03 (d, ^3J = 5.7 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.94 (s, 6 H, 19-CH_3, 19′-CH_3), 0.70 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0 [C(24)=O], 164.0 (ArC=O), 148.8 (ArC-2,6), 140.0–130.0 (m, C_6F_5), 138.2 (ArC-4), 127.7 (ArC-3,5), 76.4 (3-CH), 73.1 (12-CH), 48.3, 47.1, 46.5, 41.8, 35.9, 35.0, 34.9, 34.1, 33.6, 31.9, 30.7, 30.4, 28.7, 27.4, 26.9, 26.3, 26.0, 23.6 (19-CH_3), 23.0, 17.2 (21-CH_3), 12.7 (18-CH_3).

Bis(pentafluorophenyl) 3α,3α′-(Isophthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-oate) (3c)
Yield: 678 mg (87%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.63 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.19 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.48 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.85 (m, 2 H, 3β-H, 3β′-H), 4.02 (br s, 2 H, 12β-H, 12β′-H), 3.88 (br s, 2 H, 7β-H, 7β′-H), 2.76–2.55 (m, 4 H, 23-CH_2, 23′-CH_2), 1.05 (d, ^3J = 5.8 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.94 (s, 6 H, 19-CH_3, 19′-CH_3), 0.73 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0 [C(24)=O], 165.4 (ArC=O), 140.0–130.0 (m, C_6F_5), 133.5 (ArC-4,6), 131.2 (ArC-1,3), 130.7 (ArC-2), 128.3 (ArC-5), 75.3 (3-CH), 72.9 (12-CH), 68.3 (7-CH), 47.1, 46.5, 42.0, 41.2, 39.5, 35.2, 35.1, 34.8, 34.7, 34.5, 31.3, 30.7, 28.5, 27.4, 26.8, 26.7, 23.1 (19-CH_3), 22.4, 17.2 (21-CH_3), 12.5 (18-CH_3).

Bis(pentafluorophenyl) 3α,3α′-(Terephthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-oate) (3d)
Yield: 663 mg (85%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (s, 4 H, ArH-2,3,5,6), 4.84 (m, 2 H, 3β-H, 3β′-H), 4.03 (br s, 2 H, 12β-H, 12β′-H), 3.88 (br s, 2 H, 7β-H, 7β′-H), 2.78–2.57 (m, 4 H, 23-CH_2, 23′-CH_2), 1.06 (d, ^3J = 5.8 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.94 (s, 6 H, 19-CH_3, 19′-CH_3), 0.73 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0 [C(24)=O], 165.4 (ArC=O), 140.0–130.0 (m, C_6F_5), 134.4 (ArC-1,4), 129.3 (ArC-2,3,5,6), 75.5 (3-CH), 73.0 (12-CH), 68.3 (7-CH), 47.2, 46.6, 42.0, 41.2, 39.4, 35.2, 35.0, 34.8, 34.7, 34.5, 30.7, 30.4, 28.4, 27.4, 26.8, 26.7, 23.1 (19-CH_3), 22.5, 17.2 (21-CH_3), 12.5 (18-CH_3).

Preparation of Macrocycles 4a–e; Typical Procedure
To a stirred solution of the corresponding **3a–d** (0.06 mmol) in CH_2Cl_2 (6 mL), DMAP (22 mg, 0.18 mmol), Et_3N (25 μL, 0.18

mmol) and the corresponding diamine (0.06 mmol) was added in one portion. After stirring for 12–15 h, the mixture was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (20 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 20:1; R_f = 0.1~0.3).

Cyclic *N*,*N*′-(Ethanediyl) 3α,3′α-(Terephthaloyloxy)bis(5β-cholan-24-amide) (4a)
Yield: 27 mg (50%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.10 (s, 4 H, ArH-2,3,5,6), 6.19 (br s, 2 H, NH), 5.05 (m, 2 H, 3β-H, 3β′-H), 3.45–3.33 (m, 4 H, NH-CH_2), 2.18–2.08 (m, 4 H, 23-CH_2, 23′-CH_2), 1.06–0.95 (m, 12 H, 19-CH_3, 19′-CH_3, 21-CH_3, 21′-CH_3), 0.66 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.0 (NHC=O), 165.0 (ArC=O), 134.7 (ArC-1,4), 129.5 (ArC-2,3,5,6), 74.2 (3-CH), 57.1, 54.6, 42.7, 41.4, 40.7 (CH_2-NH), 40.6, 40.4, 35.5, 34.8, 34.7, 34.3, 31.9, 31.6, 31.5 (23-CH_2), 28.0, 26.7, 26.4, 26.1, 24.0, 23.1 (19-CH_3), 20.9, 18.5 (21-CH_3), 12.1 (18-CH_3).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{58}H_{86}N_2O_6Na$: 929.64; found: 929.67.

Cyclic *N*,*N*′-(3,6-Dioxa-1,8-octanediyl) 3α,3′α-(Terephthaloyloxy)bis(5β-cholan-24-amide) (4b)
Yield: 21 mg (37%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.09 (s, 4 H, ArH-2,3,5,6), 5.79 (t, ^3J = 5.5 Hz, 2 H, NH), 5.05 (m, 2 H, 3β-H, 3β′-H), 3.66–3.46 (m, 12 H, NH-CH_2, O-CH_2), 2.11–2.03 (m, 4 H, 23-CH_2, 23′-CH_2), 0.97 (s, 6 H, 19-CH_3, 19′-CH_3), 0.95 (d, ^3J = 6.5 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.67 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.7 (NHC=O), 165.2 (ArC=O), 134.5 (ArC-1,4), 129.4 (ArC-2,3,5,6), 75.0 (3-CH), 70.1 (O-CH_2), 69.9 (O-CH_2), 56.8, 55.1, 42.7, 41.8, 40.7, 40.3, 39.1 (CH_2-NH), 35.7, 35.0, 34.9, 34.5, 32.2, 32.2, 31.8 (23-CH_2), 28.2, 26.9, 26.7, 26.3, 24.1, 23.3 (19-CH_3), 20.9, 18.5 (21-CH_3), 12.1 (18-CH_3).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{62}H_{94}N_2O_8Na$: 1017.69; found: 1017.70.

Cyclic *N*,*N*′-(3-Oxa-1,5-pentanediyl) 3α,3α′-(2,6-Pyridinedicarbonyloxy)bis(12α-hydroxy-5β-cholan-24-amide) (4c)
Yield: 22 mg (37%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27 (d, ^3J = 7.8 Hz, 2 H, ArH-3,5), 7.97 (t, ^3J = 7.8 Hz, 1 H, ArH-4), 6.10 (m, 2 H, NH), 4.89 (m, 2 H, 3β-H, 3β′-H), 3.98 (br s, 2 H, 12β-H, 12β′-H), 3.56–3.38 (m, 8 H, $NHCH_2CH_2O$), 0.99 (d, ^3J = 6.3 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.95 (s, 6 H, 19-CH_3, 19′-CH_3), 0.66 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.7 (NHC=O), 164.7 (ArC=O), 148.7 (ArC-2,6), 138.1 (ArC-4), 127.9 (ArC-3,5), 77.0 (3-CH), 73.0 (12-CH), 70.1 (O-CH_2), 47.6, 47.6, 46.6, 42.0, 39.2, 36.1, 35.2, 35.0, 34.4, 34.3, 33.6, 32.0, 31.8, 28.7, 27.4, 27.1, 26.2, 26.1, 23.9 (19-CH_3), 23.1, 17.8 (21-CH_3), 12.8 (18-CH_3).

Cyclic *N*,*N*′-(3,6-Dioxa-1,8-octanediyl) 3α,3α′-(Isophthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-amide) (4d)
Yield: 22 mg (35%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.33 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.6 Hz, 2 H, ArH-4,6), 8.21 (br s, 1 H, ArH-2), 7.58 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 7.45 (m, 2 H, NH), 5.02 (m, 2 H, 3β-H, 3β′-H), 3.96 (br s, 2 H, 12β-H, 12β′-H), 3.91 (br s, 2 H, 7β-H, 7β′-H), 4.22–3.47 (m, 12 H, CH_2-O, CH_2-NH), 1.14 (d, ^3J = 6.7 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.96 (s, 6 H, 19-CH_3, 19′-CH_3), 0.73 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.4 (NHC=O), 165.4 (ArC=O), 134.9 (ArC-4,6), 130.8 (ArC-1,3), 128.8 (ArC-2), 127.9 (ArC-5), 75.2 (3-CH), 72.5 (12-CH), 71.0 (O-CH_2), 70.6 (O-CH_2), 68.2 (7-CH), 46.3, 44.5, 43.8, 41.2, 39.2, 39.1, 35.5, 35.4, 35.0, 34.6, 34.1, 31.2, 30.5, 28.7, 28.1, 27.5, 25.5, 22.9 (19-CH_3), 22.6, 17.6 (21-CH_3), 11.8 (18-CH_3).

MALDI-TOF: *m*/*z* [M + H]$^+$ calcd for $C_{62}H_{95}N_2O_{12}$: 1059.69; found: 1059.45.

Anal. Calcd for $C_{62}H_{94}N_2O_{12}$: C, 70.29; H, 8.94; N, 2.64. Found: C, 69.99; H, 8.77; N, 2.55.

Cyclic *N*,*N*′-(3,6-Dioxa-1,8-octanediyl) 3α,3α′-(Terephthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-amide) (4e)
Yield: 18 mg (28%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (s, 4 H, ArH-2,3,5,6), 5.86 (m, 2 H, NH), 4.90 (m, 2 H, 3β-H, 3β′-H), 3.99 (br s, 2 H, 12β-H, 12β′-H), 3.87 (br s, 2 H, 7β-H, 7β′-H), 3.64–3.42 (m, 12 H, CH_2-O, CH_2-NH), 1.01 (d, ^3J = 6.4 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.92 (s, 6 H, 19-CH_3, 19′-CH_3), 0.70 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.7 (NHC=O), 165.3 (ArC=O), 134.4 (ArC-1,4), 129.4 (ArC-2,3,5,6), 74.7 (3-CH), 72.8 (12-CH), 70.0 (O-CH_2), 69.8 (O-CH_2), 68.2 (7-CH), 46.4, 45.6, 42.6, 41.1, 39.4, 39.1, 35.1, 34.8, 34.5, 34.4, 34.3, 31.5, 31.3, 28.6, 27.3, 27.1, 26.6, 23.0 (19-CH_3), 22.5, 17.5 (21-CH_3), 12.5 (18-CH_3).

MALDI-TOF: *m*/*z* [M + H]$^+$ calcd for $C_{62}H_{95}N_2O_{12}$: 1059.69; found: 1059.92.

Anal. Calcd for solvate **4e***CH_2Cl_2 $C_{63}H_{96}Cl_2N_2O_{12}$: C, 66.12; H, 8.46; N, 2.45. Found: C, 66.30; H, 8.41; N, 2.30

Bis(24-triphenylmethoxy)-3α,3′α-(isophthaloyloxy)bis(5β-cholane-7α,12α-diol) (5b)
To isophthaloyl dichloride (57 mg, 0.28 mmol) in CH_2Cl_2 (1.5 mL), DMAP (18 mg, 0.2 mmol), pyridine (0.33 mL) and 24-triphenylmethoxy-5β-cholan-3α,7α,12α-triol (400 mg, 0.63 mmol) were added consecutively at r.t. The mixture was stirred for 24 h, diluted with CH_2Cl_2 (50 mL) and washed with 5% $NaHCO_3$ (2 × 20 mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (CH_2Cl_2–MeOH, 20:1; R_f = 0.40) to give **5b** as a white crystalline solid.

Yield: 222 mg (56%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.63 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.18 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.47 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 7.44–7.18 (m, 30 H, PhH-2,3,4,5,6), 4.84 (m, 2 H, 3β-H, 3β′-H), 4.00 (br s, 2 H, 12β-H, 12β′-H), 3.86 (br s, 2 H, 7β-H, 7β′-H), 3.02 (m, 4 H, 24-CH_2, 24′-CH_2), 0.96 (d, ^3J = 6.4 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.93 (s, 6 H, 19-CH_3, 19′-CH_3), 0.68 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3 (ArC=O), 144.5 (PhC-1), 133.4 (ArC-4,6), 131.2 (ArC-1,3), 130.7 (ArC-2), 128.6 (PhC-2,6), 128.5 (ArC-5), 127.6 (PhC-3,5), 126.7 (PhC-4), 86.2 (C-Ph_3), 75.2 (3-CH), 72.9 (12-CH), 68.2 (7-CH), 64.0 (24-CH_2), 47.5, 46.5, 42.0, 41.2, 39.5, 35.3, 35.2, 34.8, 34.6, 34.4, 32.2, 28.3, 27.5, 26.8, 26.7, 26.6, 23.2 (19-CH_3), 22.5, 17.7 (21-CH_3), 12.5 (18-CH_3).

3α,3′α-(Isophthaloyloxy)bis(5β-cholane-7α,12α,24-triol) (6b)
Compound **5b** (0.16 mmol) was dissolved in a mixture of MeOH (8 mL) and CH_2Cl_2 (2 mL). To this solution aq 48% HBr (0.3 mL) was added and the mixture was refluxed for 3 h. The product was extracted with CH_2Cl_2 (2× 70 mL) and the organic layer was washed with H_2O (20 mL), dried over $MgSO_4$ and concentrated. The resulting oil was purified by chromatography on silica gel (CH_2Cl_2–MeOH, 20:1; R_f = 0.05).

Yield: 125 mg (85%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.18 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.48 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.83 (m, 2 H, 3β-H, 3β′-H), 4.00 (br s, 2 H, 12β-H, 12β′-H), 3.86 (br s, 2 H, 7β-H, 7β′-H), 3.61 (m, 4 H, 24-CH_2, 24′-CH_2), 0.98 (d, ^3J = 6.4 Hz, 6 H, 21-CH_3), 0.93 (s, 6 H, 19-CH_3, 19′-CH_3), 0.69 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.4 (ArC=O), 133.5 (ArC-4,6), 131.3 (ArC-1,3), 130.6 (ArC-2), 128.3 (ArC-5), 75.4 (3-CH), 73.0 (12-CH), 68.3 (7-CH), 63.4 (24-CH_2), 47.4, 46.5, 42.0, 41.2, 39.5, 35.4, 35.2, 34.9, 34.7, 34.4, 31.8, 29.5, 28.3, 27.5, 26.8, 26.7, 23.2 (19-CH_3), 22.5, 17.7 (21-CH_3), 12.6 (18-CH_3).

Preparation of Macrocycles 7a–e; Typical Procedure

In a vial with a screw cap, a mixture of **6a** (68 mg, 0.08 mmol), DMAP (3 mg, 0.02 mmol), the corresponding dichloroanhydride (1 equiv) and Et_3N (0.5 mL) in toluene (8 mL) was heated on an oil bath (100 °C) until TLC analysis revealed the reaction was no longer proceeding (15–20 h). The solvent was removed under reduced pressure and the residue was subjected to column chromatography (CH_2Cl_2; R_f = 0.20~0.25) to give **7a–e** as white crystalline solids.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(5β-cholan-24-yloxy)oxalate (7a)

Yield: 33 mg (45%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.47 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.28 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.53 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.96 (m, 2 H, 3β-H, 3β′-H), 4.40–4.20 (m, 4 H, 24-CH_2, 24′-CH_2), 0.99 (s, 6 H, 19-CH_3, 19′-CH_3), 0.93 (d, ^3J = 6.5 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.68 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 165.6 (ArC=O), 158.1 (C=O), 134.4 (ArC-4,6), 131.2 (ArC-1,3), 128.9 (ArC-2), 128.6 (ArC-5), 76.3 (3-CH), 66.8 (24-CH_2), 56.5, 56.4, 42.8, 42.3, 40.6, 40.3, 35.9, 35.5, 35.2, 34.8, 32.5, 31.8, 28.8, 27.2, 26.9, 26.4, 25.6, 24.3, 23.5 (19-CH_3), 21.0, 18.7 (21-CH_3), 12.0 (18-CH_3).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{58}H_{84}O_8Na$: 931.61; found: 931.64.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(5β-cholan-24-yloxy)-2,5-thiophenedicarboxylate (7b)

Yield: 9 mg (11%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.46 (t, ^3J = 1.7 Hz, 1 H, ArH-2), 8.25 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.77 (s, 2 H, Ar′H-3,4), 7.53 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.95 (m, 2 H, 3β-H, 3β′-H), 4.40–4.20 (m, 4 H, 24-CH_2, 24′-CH_2), 0.98 (s, 6 H, 19-CH_3, 19′-CH_3), 0.93 (d, ^3J = 6.5 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.66 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 165.7 (ArC=O), 161.5 (Ar′C=O), 138.5 (Ar′C-2,5), 134.2 (ArC-4,6), 133.2 (Ar′C-3,4), 131.3 (ArC-1,3), 129.0 (ArC-2), 128.6 (ArC-5), 76.1 (3-CH), 65.2 (24-CH_2), 56.6, 56.5, 42.8, 42.2, 40.6, 40.3, 35.9, 35.1, 35.0, 34.8, 32.4, 31.7, 28.6, 27.2, 26.8, 26.4, 25.3, 24.4, 23.4 (19-CH_3), 20.9, 18.5 (21-CH_3), 12.0 (18-CH_3).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{62}H_{86}O_8SNa$: 1013.59; found: 1013.65.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(5β-cholan-24-yloxy)terephthalate (7c)

Yield: 17 mg (21%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.41 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.20 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 8.08 (s, 4 H, Ar′H-2,3,5,6), 7.50 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.86 (m, 2 H, 3β-H, 3β′-H), 4.39–4.26 (m, 4 H, 24-CH_2, 24′-CH_2), 0.97 (s, 6 H, 19-CH_3, 19′-CH_3), 0.95 (d, ^3J = 6.5 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.66 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.0 (ArC=O), 165.8 (Ar′C=O), 134.3 (Ar′C-1,4), 133.9 (ArC-4,6), 131.6 (ArC-1,3), 129.5 (Ar′C-2,3,5,6), 128.9 (ArC-2), 128.5 (ArC-5), 77.9 (3-CH), 65.5 (24-CH_2), 56.6, 55.9, 42.7, 42.2, 40.6, 40.2, 35.9, 35.6, 35.1, 34.8, 32.4, 32.0, 28.6, 27.2, 26.8, 26.5, 25.5, 24.2, 23.5 (19-CH_3), 20.9, 18.8 (21-CH_3), 12.0 (18-CH).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{64}H_{88}O_8Na$: 1007.64; found: 1007.73.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(5β-cholan-24-yloxy)isophthalate (7d)

Yield: 14 mg (14%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.59 (br s, 1 H, Ar′H-2), 8.46 (br s, 1 H, ArH-2), 8.25 (m, 4 H, ArH-4,6, Ar′H-4,6), 7.53 (m, 2 H, ArH-5, Ar′H-5), 4.96 (m, 2 H, 3β-H, 3β′-H), 4.50–4.23 (m, 4 H, 24-CH_2, 24′-CH_2), 0.97 (s, 6 H, 19-CH_3, 19′-CH_3), 0.95 (d, ^3J = 6.5 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.65 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 165.9 (Ar′C=O), 165.7 (ArC=O), 134.3 (ArC-4,6), 134.1 (Ar′C-4,6), 131.3 (ArC-1,3), 130.9 (Ar′C-1,3), 129.8 (Ar′C-2), 129.0 (ArC-2), 128.7 (Ar′C-5), 128.6 (ArC-5), 76.1 (3-CH), 65.6 (24-CH_2), 56.5, 56.3, 42.8, 42.2, 40.6, 40.3, 35.9, 35.2, 35.1, 34.7, 32.4, 32.3, 28.3, 27.2, 26.8, 26.4, 25.3, 24.4, 23.4 (19-CH_3), 20.9, 18.6 (21-CH_3), 12.0 (18-CH_3).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{64}H_{88}O_8Na$: 1007.64; found: 1007.57.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(5β-cholan-24-yloxy)-2,6-pyridinedicarboxylate (7e)

Yield: 21 mg (27%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.44 (t, ^4J = 1.7 Hz, 1 H, ArH-2), 8.27 (d, ^3J = 7.8 Hz, 2 H, Ar′H-3,5), 8.24 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.97 (t, ^3J = 7.8 Hz, 1 H, Ar′H-4), 7.52 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.93 (m, 2 H, 3β-H, 3β′-H), 4.54–4.31 (m, 4 H, 24-CH_2, 24′-CH_2), 0.97 (s, 6 H, 19-CH_3, 19′-CH_3), 0.94 (d, ^3J = 6.5 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.63 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 165.7 (ArC=O), 165.2 (Ar′C=O), 148.6 (Ar′C-2,6), 137.9 (Ar′C-4), 134.2 (ArC-4,6), 131.3 (ArC-1,3), 129.0 (ArC-2), 128.6 (ArC-5), 127.8 (Ar′C-3,5), 76.3 (3-CH), 66.5 (24-CH_2), 56.5, 56.4, 42.8, 42.2, 40.6, 40.3, 35.9, 35.2, 35.1, 34.8, 32.4, 32.2, 28.3, 27.2, 26.8, 26.4, 25.4, 24.4, 23.4 (19-CH_3), 20.9, 18.6 (21-CH_3), 12.0 (18-CH_3).

MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{63}H_{87}NO_8Na$: 1008.63; found: 1008.59.

Preparation of Macrocycles 7f–g; General Procedure

A mixture of **6b** (46 mg, 0.05 mmol), DMAP (3 mg, 0.02 mmol), the corresponding dichloroanhydride (0.05 mmol), and Et_3N (0.5 mL) in anhyd CH_2Cl_2 (8 mL) was stirred at r.t. until TLC analysis revealed the end of the reaction (24 h). The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2–MeOH, 48:2; R_f = ~0.20) to give **7f**,**g** as white crystalline solids.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-yloxy)-2,6-pyridinedicarboxylate (7f)

Yield: 9 mg (17%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.55 (t, ^4J = 1.6 Hz, 1 H, ArH-2), 8.30 (d, ^3J = 7.8 Hz, 2 H, Ar′H-3,5), 8.20 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.98 (t, ^3J = 7.8 Hz, 1 H, Ar′H-4), 7.50 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.79 (m, 2 H, 3β-H, 3β′-H), 4.56–4.31 (m, 4 H, 24-CH_2, 24′-CH_2), 4.01 (br s, 2 H, 12β-H, 12β′-H), 3.85 (br s,

2 H, 7β-H, 7β′-H), 1.01 (d, ^3J = 6.4 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.93 (s, 6 H, 19-CH_3, 19′-CH_3), 0.68 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.5 (ArC=O), 165.1 (Ar′C=O), 148.5 (Ar′C-2,6), 138.0 (Ar′C-4), 133.8 (ArC-4,6), 131.1 (ArC-1,3), 130.3 (ArC-2), 128.4 (ArC-5), 127.9 (Ar′C-3,5), 75.4 (3-CH), 73.1 (12-CH), 68.1 (7-CH), 66.5 (24-CH_2), 47.6, 46.6, 41.8, 41.3, 39.7, 35.1, 34.9, 34.8, 34.5, 32.3, 29.6, 28.4, 27.5, 26.8, 26.6, 25.7, 23.4 (19-CH_3), 22.5, 17.9 (21-CH_3), 12.6 (18-CH_3).

MS (MALDI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{63}H_{88}NO_{12}$: 1050.63; found: 1050.55.

Anal. Calcd for $C_{63}H_{87}NO_{12}$: C, 72.04; H, 8.35; N, 1.33. Found: C, 71.81; H, 8.43; N, 1.29.

Cyclic 3α,3′α-(Isophthaloyloxy)bis(7α,12α-dihydroxy-5β-cholan-24-yloxy)-3-oxaglutarate (7g)
Yield: 9 mg (18%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.54 (t, ^4J = 1.6 Hz, 1 H, ArH-2), 8.21 (dd, $^3J_1$ = 7.8 Hz, $^4J_2$ = 1.7 Hz, 2 H, ArH-4,6), 7.51 (t, ^3J = 7.8 Hz, 1 H, ArH-5), 4.79 (m, 2 H, 3β-H, 3β′-H), 4.27–4.12 (m, 8 H, 24-CH_2, 24′-CH_2, OCH_2), 4.02 (br s, 2 H, 12β-H, 12β′-H), 3.87 (br s, 2 H, 7β-H, 7β′-H), 0.98 (d, ^3J = 6.4 Hz, 6 H, 21-CH_3, 21′-CH_3), 0.95 (s, 6 H, 19-CH_3, 19′-CH_3), 0.71 (s, 6 H, 18-CH_3, 18′-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9 (ArC=O), 165.5 (C=O), 133.8 (ArC-4,6), 131.1 (ArC-1,3), 130.0 (ArC-2), 128.5 (ArC-5), 75.6 (3-CH), 73.1 (12-CH), 68.4 (7-CH), 68.1 (OCH_2), 66.3 (24-CH_2), 47.5, 46.5, 41.7, 41.3, 39.7, 35.1, 34.9, 34.8, 34.6, 32.0, 29.7, 28.5, 27.5, 26.8, 26.5, 25.5, 23.4 (19-CH_3), 22.5, 17.7 (21-CH_3), 12.7 (18-CH_3).

MS (MALDI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{60}H_{88}NaO_{13}$: 1039.61; found: 1039.53.

Anal. Calcd for $C_{60}H_{88}O_{13}$: C, 70.84; H, 8.72. Found: C, 70.57; H, 8.51.

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