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Notable chenodeoxycholic acid oligomers—synthesis, characterization, and 7α-OR steric hindrance evaluation

H. R. Ferhat Karabulut,[a,b,*] Suad A. Rashdan[b] and Jerry Ray Dias[b,*]

[a]*Department of Chemistry, Trakya University, Edirne 22030, Turkey*
[b]*Department of Chemistry, University of Missouri, Kansas City, MO 64110-2499, USA*

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Abstract—The steric hindrance of the 7α-OR group in bile acid derivatives is very different from the 12α-OR group. The effect of this difference in steric hindrance on the synthesis of cyclocholates has been evaluated with support from AM1 and MM2 calculations. The ^{13}C NMR parameters for cheneodeoxycholic acid-based cyclocholates have been determined.

1. Introduction

Recent attention has focused on the synthesis of bile acid-based dendritic oligomers. There are two types of bile acid dendrons—those involving multiple attachments of the bile acid 24-carboxyls to a non-bile acid polyol or to the 3-, 7-, and 12-hydroxyl groups of cholic acid.[1] Another important synthesis using bile acids is their cyclization into macrocyclic systems. There are three alternative models for dimerization of the bile acid unit—head to head, tail to tail, or head to tail—where the 3α-OH is the tail end of the bile acid and the C-24 carboxyl group is the head. The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are converted to secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), by respective dehydroxylation by anaerobic bacteria during passage through the small intestines and colon. CDCA has been implicated as playing a special role in the farnesoid X receptor (FXR) regulation in the homeostasis and metabolism of cholesterol to bile acids and is a constituent in traditional Chinese medicine, which is commonly used for the treatment of cardiovascular diseases.[2,3] Thus far, the majority of the literature on bile acid oligomerization has focused on CA, DCA, and LCA.[4] Very few macrocyclization reactions using CDCA have been reported.[5] Herein, we present some major examples using CDCA.

In a series of experiments Blickenstaff and co-workers established that the order of increasing steric hindrance for the hydroxyl groups on bile acids is 3<12<7, i.e., the 3α-OH is the least sterically hindered and the 7α-OH is most sterically hindered.[6] Consistent with the Blickenstaff and co-workers' results is the MM2 optimized structures of the trifluoroacetoxy esters of the cyclotricholates of DCA and CDCA. The trifluoroacetoxy ester groups in the former were essentially symmetrically distributed with an average distance between the carbons of the three trifluoromethyl groups being 7 Å. In the latter cyclocholate, the trifluoroacetoxy ester groups were not symmetrically distributed but had distances of 6.5, 12, and 12 Å.[7] As a consequence, this order of steric hindrance suggests that we should be able to perform macrocyclization of CDCA without having to perform protection and deprotection of the 7α-OH group in much the same way as lithocholate is cyclized.[8] In this study, we synthesized the cyclotri(chenodeoxycholate) [cyclotriCDCA] esters in order to be used for synthesis of cholatubes and related nanocontainers and at the same time evaluated the consequence of this steric hindrance.[9] These compounds have potential use as the models for ion channel transportation.[10] Also, because FXR regulates the biosynthesis of bile acids by a feedback repression of genes encoding for cholesterol 7α-hydroxlase and 12α-hydroxylase and CDCA has been identified as the endogenous ligand for FXR, these derivatives may have potential use as drugs for cholesterol reduction therapies.[2]

2. Results and discussion

2.1. Synthesis

Dimer **2** (Chart 1) was easily synthesized by previously proven methods.[8,12] Dimer **2** is new and provided us with the needed spectral data for comparison with the spectral data of the cyclocholates. The cyclocholate **3a** was synthesized by both the DCC/DMAP method and Yamaguchi macrocyclization method,[8,12] which the latter proved to give

* Corresponding authors. Tel.: +1 816 235 2284; fax: +1 816 235 5502; e-mail addresses: hrfk@yahoo.com; diasj@umkc.edu

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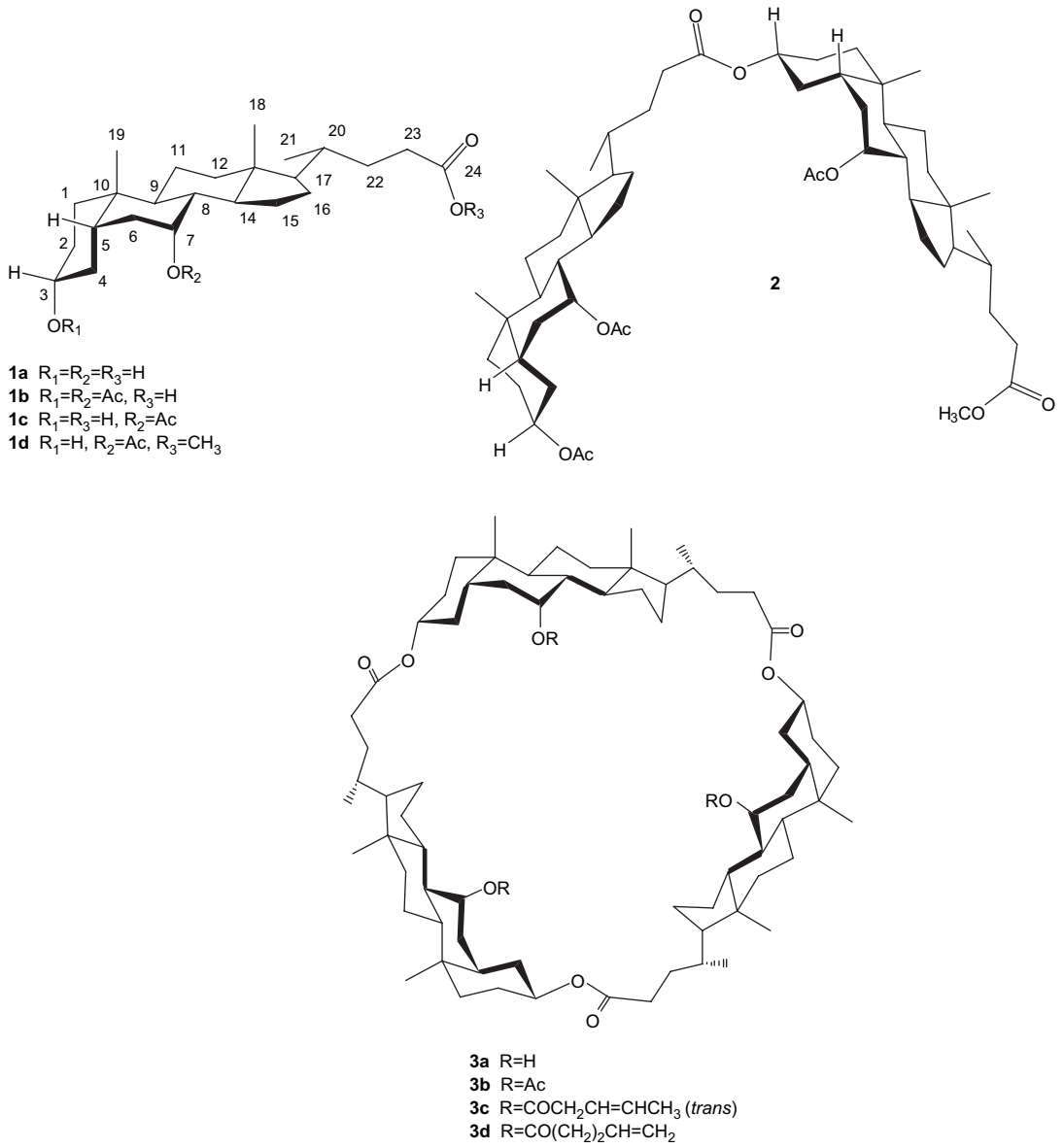


Chart 1.

approximately 10% better yields. The removal of *N*,*N*′-dicyclohexylurea co-product in the DCC method was laborious. Overall the yields of the cyclocholate derivatives starting from CDCA appear to be lower by approximately 20% than those starting from DCA,[12] which we attribute to greater steric hindrance of the 7α-ester moiety compared to the 12α-ester moiety. While the major products were trimeric cyclocholates (**3a**–**3d**), lesser amounts of dimeric and tetrameric cyclocholates were also generated.

2.2. NMR Spectra

In cyclization of CDCA, the change of ^{1}H NMR chemical shifts of the 3β-H from 3.5 ppm for the 3α-OH to 4.6 ppm for the 3α-ester was diagnostic. The 3β-H and 7β-H have distinctive chemical shifts and peak shapes in the ^{1}H NMR, the former is both more shielded and broader. The assignments of all the chemical shifts were made by careful comparison of closely related compounds published previously.[11] The ^{13}C NMR spectrum of dimer **2** conforms with our previous analysis of related dimers.[12] Most of the chemical shifts for the two 5β-cholanoate steroid moieties in dimer **2** coincided with notable exceptions occurring for the carbons having major different chemical environments, i.e., the C3 and C3′ (δ 73.9 and 74.2) and C24 and C24′ (δ 173.8 and 174.7) carbons.

The average of chemical shifts of the steroid skeletal carbons of the cyclotrimers **3a**–**3d** are 34.8 (C1), 28.0 (C2), 73.9 (C3), 34.3 (C4), 40.9 (C5), 32.3 (C6), 71.1 and 68.4 (C7, for RCO and OH, respectively), 38.3 (C8), 34.2 (C9), 34.8 (C10), 20.6 (C11), 39.7 (C12), 42.5 (C13), 50.7 (C14), 23.6 (C15), 26.7 (C16), 53.8 (C17), 11.7 (C18), 22.6 (C19), 35.2 (C20), 18.3 (C21), 31.1 (C22), 30.2 (C23), and 173.9 (C24). Some guiding generalizations for these chemical shift assignments can be made.[11] In going from the 3α-OH to the 3α-ester group (as in the cyclization reactions), a ^{13}C shielding effect of approximately 4 ppm at C2

(31–27 ppm) and C4 (39–34 ppm) occurs and a ^{1}H deshielding effect of approximately 1.1 ppm is observed for the 3α-H (3.5–4.6 ppm). While the assignments of C1, C4, C9, and C10 can be rearranged, the current order we believe gives the best fit.[11] In going from the 7α-OH to the 7α-ester group, a ^{1}H deshielding of approximately 1.0 ppm for the 7β-H (3.9 −4.9 ppm) is observed.[11,12]

2.3. Steric features of CDCA derivatives

Our previous X-ray crystallographic results for the cyclotrimer of the acetate ester of DCA and the cyclotetramer of the acetate ester of 24-norCA made it apparent that the 12α-OAc groups were thrusted into the cyclocholate cavity while the 7α-OAc groups were oriented in a direction away from the cyclocholate cavity.[13] These results are interpreted as being consistent with the larger steric interaction associated with the 7α-OR group compared to the 12α-OR group. The larger steric effect associated with the 7α-OR group derives from the A-ring being folded toward the α-side of the bile acid 5β-skeleton.

The AM1 optimized ground state structure of the tricyclocholate of CDCA $(CDCA)_3$ has been found to possess approximately C_3 symmetry and a cavity diameter of 10.3 Å. The global minimum was found using molecular dynamics simulation, which gave a chemically intuitive conformation similar to that obtained by our previous results for the triacetate ester of cyclotriDCA.[13] Assuming the enthalpy of formation is an additive quantity and using the AM1 enthalpies of formation of the secondary alcohol *i*-PrOH as the minimum strain case, the enthalpy of formation of each ester group formed between a secondary alcohol and carboxylic acid is estimated via Eq. 1 to be equal to 7.1 kcal/mol.

$$i\text{-PrOH(g)} + \text{HOAc(g)} \rightarrow i\text{-PrOAc(g)} + \text{H}_2\text{O(g)} \quad (1)$$

$$\Delta H_f\ (\text{kcal/mol}) = -106.2 - 59.2 - (-69.5 - 103.0) = 7.1$$

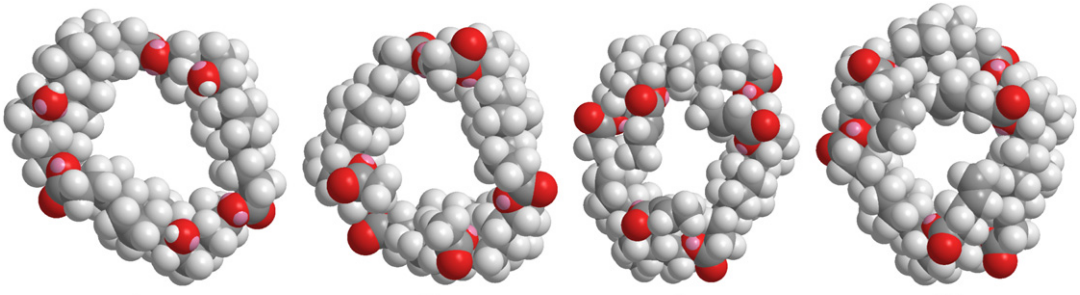
Three ester groups would require 21.3 kcal/mol (3×7.1 kcal/mol).

$$3\text{CDCA(g)} \rightarrow (\text{CDCA})_3\text{(g)} + 3\text{H}_2\text{O(g)} \quad (2)$$

$$\Delta H_f\ (\text{kcal/mol}) = -605.1 + 3(-59.2) - 3(-270.1) = 27.6$$

From Eq. 2, it is calculated that formation of $(CDCA)_3$ requires 27.6 kcal/mol. Subtraction of 21.3 kcal/mol for the enthalpy of formation of three ester groups from the enthalpy of formation of $(CDCA)_3$ (27.6 kcal/mol) results in 6.3 kcal/mol of strain energy. In comparison to the same calculation done previously for $(12\alpha\text{-OAc–DCA})_3$ (−2.8 kcal/mol),[13] the strain energy of $(CDCA)_3$ is higher by 9.1 kcal/mol [6.3−(−2.8) kcal/mol]. This higher strain energy compares the smaller 7α-OH groups in $(CDCA)_3$ versus the larger 12α-OAc groups in $(12\alpha\text{-AcO–DCA})_3$. This is again consistent with the 7α-OR group being more sterically congested than the 12α-OR group.

The space-filling depictions of **3a**–**3d** shown below were generated by MM2 energy minimization. These depictions all have approximately C_3 symmetry and show that the 7α-OR moieties are oriented in the same direction away from the cyclotricholate cavity in agreement with our prior X-ray crystallographic results of analogs.[13] The approximately equilateral triangle formed within the cyclotrimer cavity by drawing a line from one 7α-O moiety (cf. red capped with white and pink hemispheres on **3a**) to another has an average side length of 11.3 Å. The average distance of the 7α-hydroxyl hydrogen (white hemisphere on red sphere) in **3a** from the nearest steroid skeletal hydrogen is 2.2 Å, and the average distance of an acetyl hydrogen in **3b** from the nearest steroid skeletal hydrogen is 2.8 Å. From the depictions of **3a**–**3d**, it is apparent that as the acyl groups get larger from **3b** to **3d** (left to right), the cavity entrance from the side shown becomes more obstructed.



3. Conclusion

This is the first extensive study of cyclocholates built of CDCA in which the relative steric hindrance of the 7-OR group and the ^{13}C NMR parameters have been evaluated. In this work, we obtained approximately 20% lower yields of the head-to-tail cyclotrimers from CDCA than from DCA, which is consistent with the greater steric hindrance of the 7α-OR group versus the 12α-OR group.[12] Increasing crowding occurs on one side of the cyclotriCDCA skeleton as the 7α-acyl moiety gets larger. Of these prodigious cyclic systems, we conjecture that hydroxyl **3a** and acetoxy **3b** have the potential of occurring naturally in biological systems.

4. Experimental section

4.1. General

Proton magnetic resonance (^{1}H NMR) and carbon magnetic resonance (^{13}C NMR) spectra were recorded at 250 MHz and 62.9 MHz (Bruker Ac 250 upgraded with a tecmag computer interface), respectively, in chloroform-*d* (7.27 and 77.2, 77.7 and 78.2 ppm, respectively) with 1% TMS as internal standard. Chemical shifts are reported in parts per million on δ scale.[14] Flash chromatography (FC) was carried out using silica gel 230–400 mesh. Thin layer chromatography (TLC) was carried out on silica gel precoated glass plates. Spots were visualized by spraying cerium(IV) sulfate/sulfuric acid developing solution and charring with heat. The FAB mass spectra were determined by the Nebraska Center for Mass Spectrometry.[14]

4.1.1. 3α,7α-Diacetoxy-5β-cholanoic acid (1b). To CDCA (2.00 g, 5.11 mmol), acetic anhydride (4.0 mL, 42 mmol) and Py (6.0 mL, 47 mmol) were added. After reacting for 24 h at rt, the reaction mixture was poured into ice (20 g) containing 5 mL of concd HCl and diacetate **1b** (3α,7α-diacetoxy-5β-cholanic acid) formed as a solid (2.40 g, 99%) was collected by filtration. Mp 215–218 °C; ^{1}H NMR δ 0.64 (s, 3H, 18-CH_3), 0.93 (d, 3H, 21-CH_3), 0.95 (s, 3H, 19-CH_3), 2.03 (s, 3H, OAc), 2.05 (s, 3H, Ac), 2.4 (m, 2H, 23-CH_2), 4.59 (m, H, 3β-H), 4.89 (s, H, 7β-H); ^{13}C NMR δ 11.7 (C18), 18.2 (C21), 20.6 (C11), 21.5 (7-*C*H_3CO), 21.6 (3-*C*H_3CO), 22.7 (C19), 23.5 (C15), 26.8 (C2), 28.0 (C16), 30.7 (C23), 30.9 (C22), 31.3 (C6), 34.0 (C9), 34.6 (C4), 34.9 (C10), 35.1 (C20), 35.2 (C1), 37.9 (C8), 39.5 (C12), 40.9 (C5), 42.7 (C13), 50.4 (C14), 55.7 (C17), 71.3 (C7), 74.2 (C3), 170.6 (3-CH_3*C*O), 170.8 (7-CH_3*C*O), 180.0 (C24). MS (EI, 70 eV) (*m*/*z*): 476.5 [M]$^+$, 416.4 [M−HOAc]$^+$, 356.4 [M−2HOAc]$^+$, 341.3 [M−2HOAc–CH_3]$^+$, 255.3. Anal. Calcd for $C_{28}H_{44}O_6$: C, 70.56; H, 9.30. Found: C, 70.24; H, 9.41.

4.1.2. Methyl 3α-hydroxy-7α-acetoxy-5β-cholanoate (1d). To an ice-cooled soln made by reacting acetyl chloride (1 mL) with CH_3OH (10 mL), diacetate **1b** was added. After allowing to stand at rt for 3 h, the soln was diluted with hexanes (1 mL) and the methyl acetate **1d** was formed as a crystalline solid. Mp 105–110 °C; ^{1}H NMR δ 0.64 (s, 3H, 18-CH_3), 0.91 (d, 3H, 21-CH_3), 0.92 (s, 3H, 19-CH_3), 2.05 (s, 3H, OAc), 2.4 (m, 2H, 23-CH_2), 3.48 (m, H, 3β-H), 3.66 (s, 3H, OCH_3), 4.88 (s, H, 7β-H); ^{13}C NMR δ 11.9 (C18), 18.4 (C21), 20.8 (C11), 21.8 (7-*C*H_3CO), 22.9 (C19), 23.7 (C15), 28.2 (C16), 30.7 (C2), 31.0 (C23), 31.1 (C22), 31.6 (C6), 34.3 (C9), 34.9 (C10), 35.3 (C20), 35.4 (C1), 38.1 (C8), 39.0 (C4), 39.7 (C12), 41.3 (C5), 42.8 (C13), 50.5 (C14), 51.7 (OCH_3), 55.8 (C17), 71.5 (C7), 71.9 (C3), 170.9 (7-CH_3*C*O), 174.9 (C24). MS (EI, 70 eV) (*m*/*z*): 448.4 [M]$^+$, 388.4 [M−HOAc]$^+$, 370.3 [M−HOAc–H_2O]$^+$, 341.3 [M−HOAc–H_2O–CH_3]$^+$, 255.3. Anal. Calcd for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.51; H, 9.99.

4.1.3. 3α-Hydroxy-7α-acetoxy-5β-cholanoic acid (1c). To a soln of diacetate **1b** (1.0 g, 2.1 mmol) in CH_3OH (14 mL) and THF (14 mL), satd Na_2CO_3 (22 mL) was added. This soln was heated at reflux for 4 h. The excess base was neutralized with concd HCl and extracted with EtOAc. The organic layer was washed by satd NaCl soln, dried with anhyd Na_2SO_4, filtered, and concentrated in vacuo to afford monoacetate **1c** (497 mg, 55%). Mp 175–180 °C; ^{1}H NMR δ 0.64 (s, 3H, 18-CH_3), 0.91 (d, 3H, 21-CH_3), 0.92 (s, 3H, 19-CH_3), 2.05 (s, 3H, OAc), 2.3 (m, 2H, 23-CH_2), 3.49 (m, H, 3β-H), 4.87 (s, H, 7β-H); ^{13}C NMR δ 11.9 (C18), 18.6 (C21), 20.9 (C11), 21.8 (7-*C*H_3CO), 22.9 (C19), 23.7 (C15), 28.2 (C16), 30.7 (C23), 31.0 (C22), 31.5 (C6), 31.6 (C2), 34.3 (C9), 34.9 (C10), 35.4 (C20), 35.4 (C1), 38.1 (C8), 38.9 (C4), 39.8 (C12), 41.3 (C5), 42.8 (C13), 50.6 (C14), 51.7 (OCH_3), 55.9 (C17), 71.6 (C7), 71.8 (C3), 170.9 (7-CH_3*C*O), 180.0 (C24). MS (EI, 70 eV) (*m*/*z*): 416.5 [M−H_2O]$^+$, 356.5 [M−H_2O–HOAc]$^+$, 341.3 [M−HOAc–H_2O–CH_3]$^+$, 255.3. Anal. Calcd for $C_{26}H_{42}O_5$: C, 71.85; H, 9.74. Found: C, 72.06; H, 9.82.

4.1.4. Dimer 2. A mixture of diacetate **1b** (143 mg, 0.3 mmol), 2,6-dichlorobenzoyl chloride (63 mg, 0.3 mmol), Et_3N (30 mg, 0.3 mmol), and THF (5 mL) was heated at reflux for 2 h. Methyl acetate **1d** (134 mg, 0.3 mmol), DMAP (150 mg, 1.23 mmol), and benzene (10 mL) were added to the reaction mixture, which was refluxed for another 24 h. The solvent was concentrated in vacuo, and the residue was chromatographed on a silica gel column using 1:3 hexanes/EtOAc to afford dimer **2** (249 mg, 0.275 mmol, 92%). Mp 195–200 °C; ^{1}H NMR δ 0.67 (s, 6H, 18-CH_3's), 0.93 (d, 6H, 21-CH_3's), 0.95 (s, 6H, 19-CH_3's), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.07 (s, 3H, Ac), 2.3 (m, 4H, C23's), 3.69 (s, 3H, OCH_3), 4.63 (m, 2H, 3β-H's), 4.90 (br s, 2H, 7β-H's); ^{13}C NMR δ 11.7 (C18, C18′), 18.3 (C21, C21′), 20.6 (C11, C11′), 21.5 (7-*C*H_3CO, 7-*C*H_3CO′), 21.6 (3-*C*H_3CO), 22.7 (C19, C19′), 23.5 (C15, C15′), 26.8 (C16, C16′), 28.0 (C2, C2′), 31.0 (C23, C23′), 31.3 (C22, C22′), 31.6 (C6, C6′), 34.0 (C9, C9′), 34.6 (C4, C4′), 34.8 (C10, C10′), 34.9 (C20, C20′), 35.3 (C1, C1′), 37.9 (C8, C8′), 39.5 (C12, C12′), 40.9 (C5, C5′), 42.7 (C13, C13′), 50.4 (C14, C14′), 51.5 (OCH_3), 55.8 (C17, C17′), 71.2 (C7, C7′), 73.90 (C3), 74.16 (C3′), 170.41 (7-CH_3*C*O, 7-CH_3*C*O′), 170.65 (3-CH_3*C*O), 173.8 (C24), 174.7 (C24′). MS (FAB, 3-NBA+NaI) (*m*/*z*): 929.6 [dimer+Na]$^+$, 1079.5 [dimer+Na_2I]$^+$; calcd for $C_{55}H_{86}O_{10}$+Na$^+$ 929.62.

4.1.5. Cyclochenodeoxycholates (cyclodimer, cyclotrimer 3a, cyclotetramer, cyclopentamer). DCC/DMAP method: To stirred soln of CDCA (1.56 g, 4.0 mmol) in anhyd CH_2Cl_2 (125 mL), DCC (2.74 g, 13.2 mmol) and DMAP (1.73 g, 14 mmol) were added. This reaction mixture was stirred for 48 h at rt. The mixture was filtered, the filtrate was washed with 1 N HCl, satd $NaHCO_3$, satd NaCl soln, and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography on silica gel with 3:1 hexanes/EtOAc to give an overall mixture of 340 mg (23%).

Yamaguchi method: A mixture CDCA (235 mg, 0.6 mmol), 2,6-dichlorobenzoyl chloride (126 mg, 0.6 mmol), DMAP (300 mg, 2.46 mmol), and toluene (20 mL) were refluxed for 24 h. The soln was extracted with ether and dried (Na_2SO_4). The solvent was concentrated in vacuo, and the residue was chromatographed on silica gel with 3:1 hexanes/EtOAc to give an overall mixture of 122 mg (54%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.64 (s, 3H, 19-CH_3), 0.91 (s, 3H, 19-CH_3), 0.92 (d, 3H, 21-CH_3), 3.88 (s, 1H, 7α-H), 4.62

(m, 1H, 3α-H). MS (FAB, 3-NBA+NaI) (*m/z*): 771.5 [cyclodimer+Na]$^+$, 921.5 [cyclodimer+Na_2I]$^+$, 1145.7 [cyclotrimer+Na]$^+$, 1295.7 [cyclotrimer+Na_2I]$^+$, 1519.9 [cyclotetramer+Na]$^+$, 1669.9 [cyclotetramer+Na_2I]$^+$, 1894.3 [cyclopentamer+Na]$^+$. Careful chromatography over silica gel with gradient elution with hexanes/EtOAc led to separation of the cyclotrimer **3a**. ^{1}H NMR δ 0.66 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.93 (d, 3H, 21-CH_3), 3.87 (s, 1H, 7-H), 4.63 (m, 1H, 3-H); ^{13}C NMR δ 35.0 (C1), 28.1 (C2), 73.9 (C3), 34.6 (C4), 41.1 (C5), 32.9 (C6), 68.4 (C7), 39.3 (C8), 34.2 (C9), 35.0 (C10), 20.6 (C11), 39.6 (C12), 42.6 (C13), 50.5 (C14), 23.6 (C15), 26.7 (C16), 54.4 (C17), 11.6 (C18), 22.7 (C19), 35.2 (C20), 18.3 (C21), 30.4 (C22), 30.0 (C23), 173.9 (C24). MS (LR-FAB, 3-NBA+LiI) (*m/z*): 1129.7 [cyclotrimer+Li]$^+$, 1263.6 [cyclotrimer+Li_2I]$^+$; HRMS (FAB, 3-NBA+NaI) calcd for $C_{72}H_{114}O_9$+Na^+ 1145.8361, found 1145.8375 (1.3 ppm).

4.1.6. Cyclodi- and cyclotri(chenodeoxycholate) acetates. DCC/DMAP method: To stirred soln of CDCA (1.56 g, 4.0 mmol) in anhyd CH_2Cl_2 (125 mL), DCC (2.74 g, 13.2 mmol) and DMAP (1.73 g, 14 mmol) were added. This reaction mixture was stirred for 48 h at rt. Then acetic anhydride (0.22 mL, 4.0 mmol) was added to the mixture and stirred for 24 h. The mixture was filtered, the filtrate was washed with 1 N HCl, satd $NaHCO_3$, brine, and H_2O, dried ($MgSO_4$), and evaporated to dryness. The crude product was purified by column chromatography with hexanes/EtOAc (polarity was increased slowly per 9:1, 8:2, 7:3, 6:4, and 5:5) to give 52 mg (3.1%) of cyclodimer and 73.6 mg (4.4%) of **3b**.

Yamaguchi method: To a stirred soln of CDCA (1.34 g, 1.2 mmol) in anhyd CH_2Cl_2, 2,6-dichlorobenzoyl chloride (0.6 mL, 0.886 g, 4.23 mmol) was added. After 1 h, DMAP (0.43 g, 3.6 mmol) was added. After being stirred for 24 h at rt in a sealed flask, acetic anhydride (0.34 mL, 0.36 g, 3.6 mmol) was added to the reaction mixture, and this reaction mixture was stirred for another 24 h at rt. This mixture was washed with 1 N HCl, satd $NaHCO_3$, brine, and H_2O, dried ($MgSO_4$), and the CH_2Cl_2 solvent was evaporated. The crude product was purified by column chromatography with hexanes/EtOAc (the solvent ratio was slowly changed per 9:1, 8:2, 7:3, 6:4, and 5:5) to give 170 mg (11.3%) of **3b**.

4.1.7. Cyclodi(chenodeoxycholate) diacetate. R_f=0.39 (hexanes/EtOAc, 2:1); ^{1}H NMR δ 0.66 (s, 3H, C18), 0.95 (br s, 6H, C19, C21), 2.06 (s, 7α-CH_3CO), 4.62 (m, 1H, 3β-H), 4.84 (s, 1H, 7β-H); ^{13}C NMR δ 34.6 (C1), 27.8 (C2), 74.2 (C3), 34.5 (C4), 40.9 (C5), 32.9 (C6), 71.1 (C7), 38.0 (C8), 34.5 (C9), 34.6 (C10), 20.5 (C11), 39.7 (C12), 42.3 (C13), 51.3 (C14), 23.6 (C15), 26.7 (C16), 53.1 (C17), 11.7 (C18), 22.6 (C19), 34.9 (C20), 17.5 (C21), 31.4 (C22), 28.8 (C23), 174.2 (C24), 170.6 (7α-CH_3CO_2), 21.5 (7α-CH_3CO_2). MS (LR-FAB, 3-NBA+LiI) (*m/z*): 839.8 [cyclodimer+Li]$^+$, 973.6 [cyclodimer+Li_2I]$^+$.

4.1.8. Cyclotri(chenodeoxycholate) triacetate (3b). R_f=0.40 (hexanes/EtOAc, 2:1); ^{1}H NMR δ 0.66 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 0.96 (s, 3H, 21-CH_3), 2.06 (s, 3H, 7α-CH_3CO), 4.62 (m, 1H, 3β-H), 4.84 (s, 1H, 7β-H); ^{13}C NMR δ 34.6 (C1), 27.8 (C2), 74.3 (C3), 34.3 (C4), 40.9 (C5), 33.0 (C6), 71.1 (C7), 37.9 (C8), 34.3 (C9), 34.7 (C10), 20.7 (C11), 39.8 (C12), 42.3 (C13), 51.3 (C14), 23.6 (C15), 26.7 (C16), 53.1 (C17), 11.7 (C18), 22.6 (C19), 34.9 (C20), 18.5 (C21), 31.5 (C22), 28.9 (C23), 174.4 (C24), 170.7 (7α-CH_3CO_2), 21.6 (7α-CH_3CO_2). MS (LR-FAB, 3-NBA+LiI) (*m/z*): 1256.1 [cyclotrimer+Li]$^+$, 1390.0 [cyclotrimer+Li_2I^+]; HRMS (FAB) calcd for $C_{78}H_{120}O_{12}$+Na 1271.8677, found 1271.8665 (1.0 ppm).

4.1.9. Cyclotri(chenodeoxycholate) tricrotonoate (3c). To a stirred soln of CDCA (2.35 g, 6.0 mmol) in anhyd toluene (200 mL), 2,6-dichlorobenzoyl chloride (1.01 mL, 7.05 mmol) was added. After 1 h, DMAP (3.0 g, 24.6 mmol) was added. After stirring for 15 h at reflux temp, toluene was evaporated. The mixture was diluted with $CHCl_3$, filtered, and the solvent was evaporated to dryness. The cyclocholate **3a** was dissolved in dry CH_2Cl_2 and a mixture of 2,6-dichlorobenzoyl chloride (1.212 mL, 1.772 g, 8.46 mmol), crotonic anhydride (1.07 mL, 1.11 g, 7.2 mmol), and DMAP (0.87 g, 7.2 mmol) was added. The reaction mixture was then stirred for 48 h at rt and CH_2Cl_2 was evaporated to dryness. The residue was crystallized from $CHCl_3$/hexane and ethyl acetate/hexane mixture to afford 0.745 g (0.56 mmol, 28%) **3c** as colorless crystals. R_f=0.31 (hexane/EtOAc, 2:1); ^{1}H NMR δ 0.65 (s, 3H, 18-CH_3), 0.94 (br s, 6H, 19-CH_3, 21-CH_3), 4.62 (m, 1H, 3β-H), 4.92 (s, 1H, 7β-H), 5.80 (d, 1H, 7α-CH_3CH=$CHCO_2$), 6.90 (m, 1H, 7α-CH_3CH=$CHCO_2$); ^{13}C NMR δ 34.9 (C1), 28.1 (C2), 73.6 (C3), 34.4 (C4), 40.7 (C5), 31.5 (C6), 70.9 (C7), 37.9 (C8), 34.2 (C9), 34.7 (C10), 20.6 (C11), 39.7 (C12), 42.6 (C13), 50.7 (C14), 23.5 (C15), 26.7 (C16), 55.7 (C17), 11.6 (C18), 22.6 (C19), 35.2 (C20), 18.1 (C21), 31.2 (C22), 30.8 (C23), 173.5 (C24), 165.2 (7α-CH_3CH=$CHCO_2$), 17.9 (7α-CH_3CH=$CHCO_2$), 143.7 (7α-CH_3CH=$CHCO_2$), 123.4 (7α-CH_3CH=$CHCO_2$). MS (LR-FAB, 3-NBA+LiI) (*m/z*): 1327.7 [cyclotrimer+H]$^+$, 1333.7 [cyclotrimer+Li]$^+$; HRMS (FAB, 3-NBA) calcd for $C_{84}H_{126}O_{12}$+H^+ 1327.9327, found 1327.9312 (1.2 ppm).

4.1.10. Cyclotri(chenodeoxycholate) tripentenoate (3d). To a stirred soln of CDCA (2.35 g, 6.0 mmol) in anhyd toluene (200 mL), 2,6-dichlorobenzoyl chloride (1.01 mL, 7.05 mmol) was added. After 1 h, DMAP (3 g, 24.6 mmol) was added to the stirring mixture. After stirring for 15 h at reflux temp, toluene was evaporated. The mixture was diluted with $CHCl_3$, filtered, and $CHCl_3$ was evaporated to dryness. The cyclocholate **3a** was dissolved in dry CH_2Cl_2 and a mixture of 2,6-dichlorobenzoyl chloride (3.97 mL, 5.8 g, 27 mmol), 4-pentenoic acid (2.5 mL, 2.45 g, 24.5 mmol), and triethylamine (0.92 mL, 668 mg, 6.6 mmol) was added. The reaction mixture was then stirred for 30 h at rt, the CH_2Cl_2 was evaporated, and the residue was crystallized from $CHCl_3$/hexane mixture to afford 600 mg (0.44 mmol) 21.9% **3d** as a white solid. R_f=0.37 (hexanes/EtOAc, 2:1); ^{1}H NMR δ 0.65 (s, 3H, 18-CH_3), 0.94 (br s, 6H, 19-CH_3, 21-CH_3), 2.40 (br s, 4H, 7α-CH_2=$CHCH_2CH_2CO_2$), 4.60 (m, 1H, 3β-H), 4.92 (s, 1H, 7β-H), 5.05 (crude t, 7α-CH_2=$CHCH_2CH_2CO_2$), 5.83 (m, 7α-CH_2=$CHCH_2CH_2CO_2$); ^{13}C NMR δ 34.8 (C1), 28.0 (C2), 73.9 (C3), 34.0 (C4), 40.8 (C5), 31.6 (C6), 71.3 (C7), 37.9 (C8), 33.7 (C9), 34.6 (C10), 20.4 (C11), 39.5 (C12), 42.7 (C13), 50.4 (C14), 23.6 (C15), 26.6 (C16), 55.9 (C17), 11.7 (C18), 22.6 (C19), 35.4 (C20), 18.3 (C21), 31.3 (C22), 30.9 (C23), 173.9 (C24), 172.3

(7α-CH_2=$CHCH_2CH_2CO_2$), 136.9 (7α-CH_2=$CHCH_2$-CCH_2O_2), 115.6 (7α-CH_2=$CHCH_2CH_2CO_2$), 28.8 (7α-CH_2=$CHCH_2CH_2CO_2$), 34.0 (7α-CH_2=$CHCH_2CH_2CO_2$). MS (LR-FAB, 3-NBA+LiI) (*m/z*): 1375.9 [cyclotrimer+Li]$^+$; HRMS (FAB) calcd for $C_{87}H_{132}O_{12}$+Li^+ 1375.9879, found 1375.9842 (2.7 ppm).

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14. Supporting spectra for compounds **2** and **3a**–**3d** can be obtained from the last author upon request.