Synthesis of cyclocholates and derivatives. Part II.† Selective synthesis of cyclotetracholates from linear dimers

Hongwu Gao and Jerry Ray Dias*

Department of Chemistry, University of Missouri-Kansas City, Kansas City, MO 64110-2499, USA

Selective synthesis of cyclic tetramers **3** and hexamers **4** of cholic acid was achieved from the cycloesterification of dimeric cholic acids **2** by using 2,6-dichlorobenzoyl chloride and 4-dimethylaminopyridine (DMAP). The results suggest that the cyclotetramer is the preferred even oligomeric cycle size for both cholic acid and the 24-norcholic acid system, consistent with our previous results for lithocholic acid. The structures of all dimeric hydroxy acids and cyclooligomers were determined by ^{1}H NMR, ^{13}C NMR and mass spectrometries.

Cyclic bile acids (cyclocholates) provide unique molecular architectures that can be exploited in molecular engineering and recognition.[1,2] Cyclocholate mixtures composed principally of dimers to tetramers can be prepared by Yamaguchi macrolactonization[3] of monomeric hydroxy acids using 2,6-dichlorobenzoyl chloride as the coupling reagent[4] in a one-pot procedure, followed by chromatographic separation.[5–8] Another strategy synthesized the cyclocholates from monomers by transesterification under thermodynamic, equilibrating conditions using a potassium methoxide–crown ether complex.[9] In both methods, cyclotrimers were the main products for the cholic acid system and cyclotetramers (1–13%) were minor products. For the 24-norcholic acid system, cyclotetramerization was the principal reaction route.[7] In 1993, Bonar-Law and coworkers reported a more efficient method on a large scale to combine two differentially protected monomers and then to cyclize the resulting linear dimer.[10,11] A similar synthetic strategy was used for the multistep synthesis of tetrameric forms of lithocholic acid derivatives.[8] In this method, the carboxyl group was protected as the corresponding benzyl ester *via* reaction with benzyl alcohol by using DCC and DMAP; the 3-hydroxyl group was protected with *tert*-butyldimethylsilyl chloride. After the dimerization, the TBDMS and benzyl protecting groups were cleaved by aqueous HF and catalytic hydrogenation, respectively. It took seven steps to synthesize cyclotetramers with 25% yield (45% in the last step).[8] Here we report a two-step method with 50% yield to synthesize selectively cyclotetramers of cholic acid derivatives. This method utilizes our previously synthesized linear dimers consisting of all combinations of cholic acid and 24-norcholic acid.[12] This work is directed toward building a combinatorial library containing all combinations and oligomeric sizes of linear and cyclic cholates and describes the selective synthesis of three out of six possible cyclotetracholates and two out of 13 possible cyclohexacholates made from cholic acid and 24-norcholic acid monomers.

Results and Discussion

Synthesis

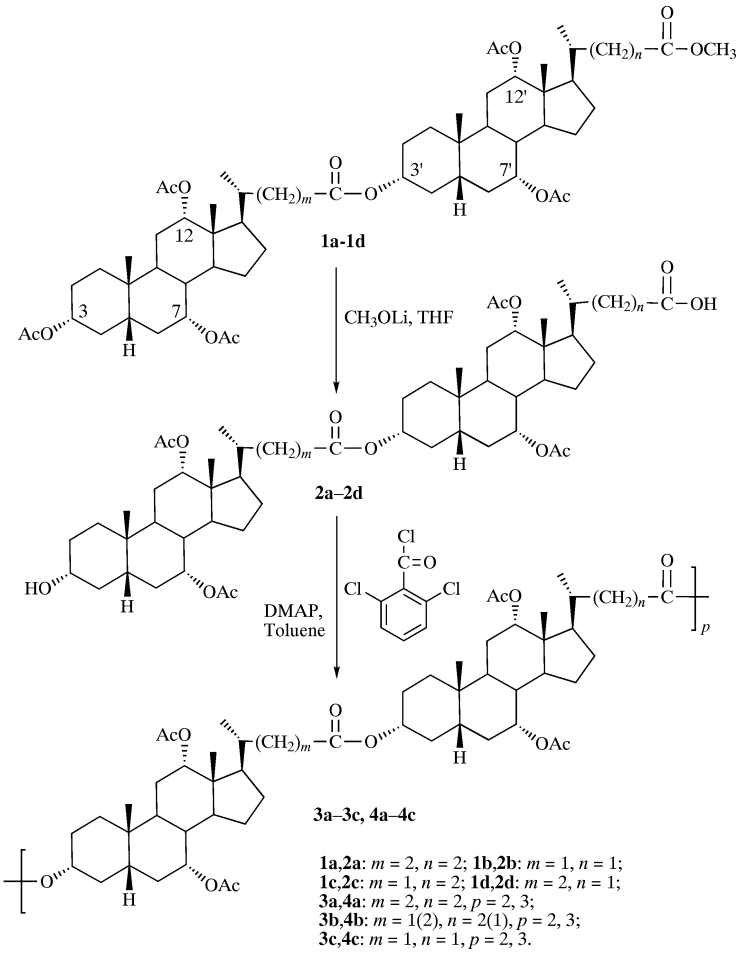
The starting dimers **1** were prepared by our previous methods.[12] The regioselective hydrolysis of the methyl ester protecting groups on the ring A-ring A cholic acid dimers by using aqueous lithium hydroxide with tetrahydrofuran was first reported by Gouin and Zhu.[13] We used lithium methoxide instead of lithium hydroxide to hydrolyze selectively the 3-OAc and methyl ester simultaneously (Scheme 1). The ester bonds between two monomers and 7α-OAc, 12α-OAc were kept intact because of a steric effect. Compared with 24-methyl ester, 24-normethyl ester requires a longer reaction time for saponification because the shorter 17-side chain gave a larger steric effect. The following cyclization gave cyclotetramers (56–67%) as major products and cyclohexamers (3–12%) as minor products. Compared with the previous results for the lithocholic and 12-acetyldeoxycholic acid systems,[7,8] the cyclodimer was not detected, presumably because of the added steric effect from 7α-OAc.[5,9] The cyclization of **2c** and **2d** gave the same mixed cyclooligomers with different yields (**3b**: 62, 56%; **4b**: 4.3, 12%). The somewhat lower yield of cyclotetramer **3b** and corresponding higher yield of cyclohexamer **4b** in the reaction of **2d** in comparison to **2c** seems to be the result of more steric interaction associated with the shorter 17-side chain of the free carboxylic acid belonging to the 24-norcholic acidmer. Because of the small amount of reactant **2d**, no cyclohexamer **4c** was isolated.

Spectroscopic characterization

^{1}H NMR spectra. All the ^{1}H NMR spectra are summarized in Table 1. The 20-methyl groups show a doublet because of coupling with the hydrogen at C-20. After the regioselective hydrolysis of 3α-OAc and 24-methyl ester, the 3β-H shifts upfield from 4.57 to 3.50 ppm. On the other hand, the chemical shifts of 3′β-H, 7β-H, 7′β-H, 12β-H and 12′β-H did not shift. The 18-methyl, 18′-methyl; 19-methyl, 19′-methyl; 21-methyl, 21′-methyl; 7-OAc, 7′-OAc and 12-OAc, 12′-OAc in mixed dimer **2c** showed different chemical shifts. The 24-nor carboxylic acids **2b** and **2d** gave broad peaks around 6.5 ppm. After cyclization, 3β-H shifts back downfield from 3.50 to 4.58 ppm. Compared with the homogeneous oligomers, the mixed cyclotetramer **3b** gave two kinds of 3β-H ($\delta_{24} = 4.58$ ppm $<$ $\delta_{24\text{-nor}} = 4.68$ ppm). On the other hand, the mixed cyclohexamer **4b** did not show this difference.

^{13}C NMR spectra. All the ^{13}C NMR spectra assignments in Tables 2 and 3 were done empirically. For the four acetates in the dimers **2**, the eight acetates in the cyclotetramers **3** and the twelve acetates in cyclohexamers **4**, the 7-OAc can easily be distinguished from 12-OAc by the different chemical shifts of the methyl carbon and carbonyl carbon. In dimers **2**, the six

† For Part I see ref. 7.



Scheme 1

kinds of carbons connected with oxygen gave different chemical shifts from 70.73 to 76.66 ppm. After the 3-OAc hydrolyzed to 3-OH, C-3 shifts from 73.90–73.94 to 71.57–71.73 ppm. In going from dimer **2a** to dimer **2b**, the 18-methyl groups shifts downfield from 17.59 to 18.63 ppm. This is due to the 18-methyl group in **2b** being closer to the carboxyl group. In the mixed dimers (**2c** and **2d**) and mixed cyclooligomers (**3c** and **3d**), C-20, C-21 and C-22 show the same chemical shift pattern. In all the dimers (**2a**–**d**), C-3 with an ester group is more deshielded than C-3 with a hydroxy group. For the linear dimers **1**–**2**, we regard the 3α-OH group as the tail end and the C-24(C-23) carboxyl group as the head of the cholic acid. Because of symmetry, the ^{13}C signals for the same position in each mer of the cyclooligomers **3a** and **4a** coincide (Table 3), whereas **3b** and **4b** give two sets of ^{13}C signals (Table 2).

Mass spectra. The mass spectra of the oligomers are summarized in Table 4. The mass spectra of the dimers **2a**–**d** revealed the minor presence of some monomer and exhibited peaks associated with two metal atoms $[(M + Na) - H + Na]^+$ not present in the spectra of the cyclocholates; this is because the free —COOH forms —COONa.

Combinations and isomers of cyclocholates

If a one-to-one mixture of 7,12-diacetylcholic acid and 7,12-diacetyl-24-norcholic acid are reacted per Yamaguchic macrolactonization conditions, one should expect to obtain all possible combinations and permutation isomers of cyclodimers to cyclohexamers. With these monomers there are 3, 4, 6, 8 and 13 possible cyclodimeric, cyclotrimeric, cyclotetrameric, cyclo-

Table 1 Partial ^1H NMR spectra

Compound	C-18	C-21	C-19	7-OAc	12-OAc	3β-H	3′β-H	7β-H	12β-H
2a	0.73(s)	0.81(d)	0.91(s)	2.08(s)	2.13(s)	3.50(m)	4.57(m)	4.90(s)	5.08(s)
2b	0.77(s)	0.87(d)	0.91(s)	2.08(s)	2.13(s)	3.51(m)	4.58(m)	4.91(s)	5.08(s)
2c	0.73(s)	0.82(d)	0.91(s)	2.05–2.11(m)		3.52(m)	4.58(m)	4.91(s)	5.08(s)
	0.76(s)	0.87(s)							
2d	0.73(s)	0.82(d)	0.91(s)	2.08(s)	2.12(s)	3.50(m)	4.57(m)	4.90(s)	5.08(s)
3a	0.74(s)	0.83(d)	0.93(s)	2.07(s)	2.13(s)	4.58(m)	—	4.92(s)	5.08(s)
4a	0.73(s)	0.83(d)	0.93(s)	2.07(s)	2.13(s)	4.58(m)	—	4.91(s)	5.09(s)
3b	0.75(s)	0.84(m)	0.92(s)	2.00–2.17(m)		4.58(m)	4.68(m)	4.91(s)	5.08(s)
4b	0.73(s)	0.82(m)	0.92(s)	2.05–2.17(m)		4.58(m)	4.58(m)	4.91(s)	5.09(s)
3c	0.77(s)	0.84(d)	0.90(s)	2.07(s)	2.11(s)	4.67(m)	—	4.92(s)	5.09(s)

Table 2 ^{13}C NMR spectra of **2a–d**, **3b** and **4b**

	Tail/head[a]				norCA/CA[a]	
Assignment	**2a**	**2b**	**2c**	**2d**	**3b**	**4b**
C-18, 18′	12.27	12.21	12.25	12.26	12.20	12.26
C-21, 21′	17.59	18.63	18.68	17.53	18.44	18.69
			17.58	18.75	17.46	17.55
CH_3CO(C-7, 7′)	21.47	21.51	21.48	21.42	21.29	21.43
CH_3CO(C-12, 12′)	21.65	21.65	21.70	21.58	21.46	21.59
C-19, 19′	22.58	22.56	22.56	22.56	22.52	22.56
C-15, 15′	22.82	22.78	22.80	22.78	22.82	22.80
C-11, 11′	25.60	25.55	25.59	25.54	25.60	25.60
					25.89	
C-2, 2′	27.08	27.03	27.11	27.14	26.87	26.94
					26.64	
C-16, 16′	27.16	27.31	27.29	27.14	27.52	27.29
					27.00	
C-9, 9′	28.93	28.93	28.97	28.89	29.30	29.11
					28.87	28.88
C-23, 23′	30.81	172.68	178.87	30.43	173.59	173.59
	30.48	177.89	30.45	180.36	30.02	30.88
C-6, 6′	31.37	30.38	31.35	31.37	31.70	31.23
		31.29	31.24		31.01	
C-22, 22′	31.37	32.96	32.99	30.43	32.75	33.06
			30.68	32.97	29.48	29.11
C-10, 10′	34.34	34.30	34.33	34.28	34.12	33.17
					33.90	
C-1, 1′	34.43	34.64	34.52	34.58	34.49	33.91
C-4, 4′	34.64	34.80	34.63	34.87	34.63	34.31
C-20, 20′	34.88	40.99	40.98	34.87	40.52	40.77
			34.81	38.63	34.86	34.63
C-8, 8′	37.79	37.76	38.45	37.78	37.54	37.77
	38.69	38.46	37.78			
C-5, 5′	41.05	41.61	41.61	41.01	40.92	40.93
					40.75	
C-14, 14′	43.42	43.47	43.49	43.37	43.72	43.42
C-13, 13′	45.12	45.12	45.07	45.07	45.39	45.04
					45.06	
C-17, 17′	47.50	47.15	47.43	47.43	47.37	47.60
		47.43	47.17			
C-7, 7′	70.73	70.74	70.83	70.84	70.67	70.76
	70.85	70.83				
C-3, 3′	71.68	71.63	71.73	71.57	73.76	73.92
	73.94	73.93	73.93	73.90	73.50	73.69
C-12, 12′	75.44	75.30	75.33	75.41	76.60	75.47
	76.54	76.60	76.54	76.66	75.62	
CH_3CO(C-7, 7′)	170.20	170.38	170.37	170.25	169.88	170.02
					169.72	
CH_3CO(C-12, 12′)	170.44	170.55	170.59	170.55	170.18	170.27
	170.55				170.01	170.14
C-24, 24′	173.58	—	—	173.55	—	—
			172.67	—	173.22	173.40

[a] One value is given if the same for both.

pentameric and cyclohexameric combinations and ring permutation isomers, respectively. For example, Fig. 1 lists all possible cyclotetracholates composed of cholic acid (CA) and 24-norcholic acid (norCA). In Fig. 1, we adapt a notation

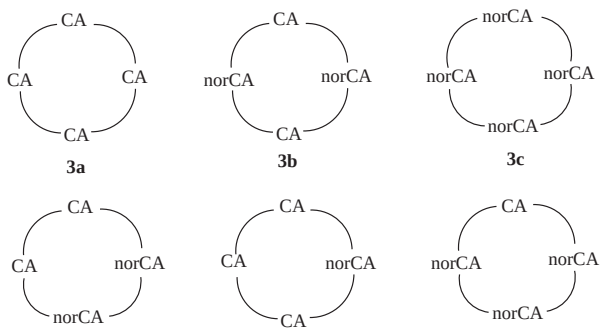


Fig. 1 All possible cyclotetracholates made from cholic and 24-norcholic acid monomers

similar to that employed for dimers and oligomers of amino acids, *i.e.*, peptides and proteins. The cyclotetramers made up of two cholic and 24-norcholic acid monomers exist in two permutation ring isomers—one with adjacent cholic mers and one with nonadjacent cholic mers (**3b**). Similarly, cyclohexamer **4b** is only one of three possible permutation ring isomers.

Conclusion

Select cyclotetramers and cyclohexamers of 7,12-diacetylcholic acid have been synthesized from linear dimers by the Yamaguchi method. Unlike the linear dimer of lithocholic acid, which is devoid of steric hindrance associated with the presence of 7α-acetoxy and 12α-acetoxy groups, no cyclodimers were obtained in this work. The success of this synthesis was predicated by conditions that are not conducive to transesterification, which would have given cyclotrimers.

Table 3 ^{13}C NMR spectra of 3α-hydroxy-7α,12α-diacetoxy-5β-cholan-24-oic acid derivatives

Assignment	$C_{31}H_{48}O_8$ [a]	$C_{112}H_{168}O_{24}$ [b]	$C_{168}H_{252}O_{36}$ [c]
C-18	12.25	12.25	12.27
C-21	17.53	17.65	17.59
CH_3CO(C-7)	21.45	21.45	21.42
CH_3CO(C-12)	21.65	21.58	21.59
C-19	22.57	22.56	22.56
C-15	22.85	22.77	22.81
C-11	25.59	25.56	25.59
C-2	26.93	26.95	26.95
C-16	27.23	26.95	27.09
C-9	28.93	28.83	28.91
C-23	30.81	30.51	31.01
C-22	30.93	31.18	31.25
C-6	31.29	31.29	31.25
C-10	34.39	33.90	34.39
C-1	34.64	34.39	34.39
C-4	34.64	34.65	34.65
C-20	34.73	34.87	34.65
C-8	37.79	37.80	37.79
C-5	41.0	40.94	40.95
C-14	43.43	43.31	43.42
C-13	45.12	45.00	45.06
C-17	47.42	46.58	47.25
OCH_3	51.55	—	—
C-7	70.72	70.78	70.77
C-3	74.12	73.98	73.93
C-12	75.44	75.48	75.45
CH_3CO(C-7)	170.3	170.06	170.19
CH_3CO(C-12)	170.54	170.25	170.37
C-24	174.56	173.58	173.52

[a] Methyl-3α,7α,12α-triacetoxy-5β-cholan-24-oate.[7] [b] Cyclotetramer of 3α-hydroxy-7α-diacetoxy-5β-cholan-24-oic acid (**3a**). [c] Cyclohexamer of 3α-hydroxy-7α,12α-diacetoxy-5β-cholan-24-oic acid (**4a**).

Experimental

Column chromatography was carried out using Grade 62 (60–200 mesh) silica gel and eluted by a *n*-hexane–ethyl acetate solvent system. Reactions and chromatography fractions were analyzed using Fisher 250 micron silica gel G (5 × 20 cm) TLC plates. All TLCs were developed with 1 : 1 *n*-hexane–ethyl acetate. Visualization was done by either spraying the plates with a $Ce(SO_4)_2$ plus H_2SO_4 solution and briefly heating or keeping the plates in an iodine bottle. Toluene was dried with sodium. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR (proton decoupled) spectra were measured at 250 and 63 MHz (Bruker), respectively, in $CDCl_3$ as solvent and with TMS as internal standard. Mass spectra were recorded on a VG20-253 or VGZAB-HS spectrometer.

Regioselective hydrolysis of the 3α-dimers of bile acids

To a solution of 3α-dimer (2 mmol) in THF (40 mL) at room temperature was added aqueous lithium methoxide (40 mL, 0.2 N). The resulting suspension was stirred at room temperature for 10 h (for the 24-methyl ester, **1a** and **1c**) or 20 h (for the 24-nor methyl ester, **1b** and **1d**). The solvent (THF) was then removed *in vacuo* and the solution was acidified with 10% hydrochloric acid to neutral pH. The precipitate was extracted by ethyl acetate (3 × 30 mL) and the collected organic layers were washed with brine, dried with Na_2SO_4 and concentrated *in vacuo* to afford the dimer.

Dimer 2a. Mp 208–210 °C, yield: 84.6%.

Dimer 2b. Mp 164–166 °C, yield: 84.8%.

Dimer 2c. Mp 148–150 °C, yield: 79.4%.

Dimer 2d. Mp 198–200 °C, yield: 80.5%.

General cyclization procedure

A mixture of dimer (1.7 mmol), 2,6-dichlorobenzoyl chloride (0.27 mL, 1.8 mmol), DMAP (0.8 g, 7 mmol) and toluene (800 mL) was refluxed for 48 h. The solvent was concentrated under reduced pressure, the residue was flash chromatographed on a silica-gel column (eluent: *n*-hexane–EtOAc) and crystallized to afford cyclooligomers.

Cyclotetramer 3a. Mp 218–220 °C, yield: 65.5%.

Cyclohexamer 4a. Mp 215–217 °C, yield: 2.9%.

Cyclotetramer 3b. Mp 195–197 °C, yield: 62% (**2c**), 56% (**2d**).

Cyclohexamer 4b. Mp 238–240 °C, yield: 4.3% (**2c**), 12% (**2d**).

Cyclotetramer 3c. Mp 288–290 °C (287–289 °C[7]), yield: 67%.

Acknowledgements

The FAB mass spectra were determined by the Nebraska Center for Mass Spectrometry.

Table 4 Partial FAB mass spectra taken in 3-NBA plus NaI

Compound	$[(M + Na) - H + Na]^+$	$[M + Na]^+$	Miscellaneous peaks
2a	1011.7	989.7	628.9, 393.3, 325.9
2b	983.7	961.7	501.3, 393.3, 325.9
2c	977.7	975.7	325.9, 176.1
2d	997.7	975.7	628.9, 475.9, 325.9
3a	—	1921.6	685.6, 469.5, 176.1
3b	—	1893.3	1419.8 $[M + Na - C_{28}H_{42}O_6]^+$ 1031.6, 325.9, 176.0
3c	—	1865.3	341.2, 176.1
4a	—	2869.8	469.3, 176.0
4b	—	2827.7	2367.7 $[M + Na - C_{27}H_{40}O_6]^+$ 1893.5 $[M + Na - C_{27}H_{40}O_6 - C_{28}H_{42}O_6]^+$, 1507.2, 798.8, 648.8, 469.5, 176.1

References

1 Y. Li and J. R. Dias, *Chem. Rev.*, 1997, **97**, 283.
2 P. Wallimann, T. Marti, A. Furer and F. Diederich, *Chem. Rev.*, 1997, **97**, 1567.
3 J. Inanaga, K. Hirata, H. Saeki, T. Katsuki and M. Yamaguchi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1989.
4 P. P. Wanders, L. Thijs and B. Zwanenburg, *Tetrahedron Lett.*, 1987, **28**, 2409.
5 R. P. Bonar-Law and J. K. M. Sanders, *Tetrahedron Lett.*, 1992, **33**, 2071.
6 R. P. Bonar-Law and J. K. M. Sanders, *Tetrahedron Lett.*, 1993, **34**, 1677.
7 H. W. Gao and J. R. Dias, *Synth. Commun.*, 1997, **27**, 757.
8 Y. X. Li and J. R. Dias, *Synthesis*, 1997, 425.
9 P. A. Brady, R. P. Bonar-Law, S. J. Rowan, C. J. Suckling and J. K. M. Sanders, *Chem. Commun.*, 1996, 319.
10 H. L. Anderson, R. P. Bonar-Law, L. G. Mackay, M. S. Nicholson and J. K. M. Sanders in *Supramolecular Chemistry*, ed. V. Balzani and L. De Cola, Kluwer, Dordrecht, 1992, p. 359.
11 R. P. Bonar-Law, L. G. Mackay and J. K. M. Sanders, *J. Chem. Soc., Chem. Commun.*, 1993, 456.
12 H. W. Gao and J. R. Dias, *J. Prakt. Chem.*, 1997, **339**, 187.
13 S. Gouin and X. X. Zhu, *Steroids*, 1996, **61**, 664.

Received in New Haven, CT, USA, 24th July 1997;
Paper 7/09178H