Synthesis of Cyclocholates and Derivatives, III[◇]

Cyclocholates with 12-Oxo and 7,12-Oxo Groups

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Syntheses of bile acid cyclooligomers with 12- and 7,12-oxo groups (**6a−d**, **7a−c**, **8a−b**) by the Yamaguchi method are described. Cyclotrimerization is the principal reaction route for these cholic acid systems. Conversion of 7- and 12-hydroxy groups in cholic acid (**1a−b**) to oxo groups (**4a−c**, **5a−c**), followed by macrocyclization (**6a−d**, **7a−c**, **8a−b**) and selective reduction of the oxo groups back to hydroxy ones without cleaving the 24-carboxylic ester linkages (**11**) constitutes a new strategy in the synthesis of cyclocholates having unprotected hydroxy groups.

Introduction

For more than a century, synthetic chemists have demonstrated their ability to construct molecules capable of fulfilling a huge variety of requirements. Recently, the synthesis of taylor-made dimeric and oligomeric steroids has become of interest because of their possible application as biochemical/cellular membrane models and as components in molecular engineering.[1][2] Initially, the synthesis of dimeric steroids occurred as accidental byproducts and then were produced by design.[2] Of the dimeric steroids with various inter-steroid linkages, ring A−ring A connections are observed to be the most prevalent whereas ring C−ring C connections are least. For example, Pettit and co-workers[3] isolated a ring A−ring A steroid dimer byproduct during their total synthesis of bufalitoxin and bufotoxin, and more recently Gouin and Zhu[4] synthesized several ring A−ring A steroid dimers by design. The synthesis of oligomeric steroids, in general, poses many undefined parameters requiring investigation. Toward defining these parameters, our recent syntheses directed toward evaluating the effect of the 12- and 7,12-oxo groups on macrocyclization reactions of cholic acid derivatives are described in this paper. Our previous macrocyclization studies used acetate esters to protect the 7α-OH and 12α-OH groups. Here we show an alternative strategy which involves converting these hydroxy groups in cholic acid monomers to oxo groups and after macrocyclization these oxo groups are reduced back to hydroxy groups with $NaBH_4$ without disrupting the 24-carboxylic ester linkages. It is well known that $NaBH_4$ reduction of the 7-oxo gives 7α-OH[5][6] and reduction of the 12-oxo frequently gives both 12α- and 12β-OH.[7][8] The effect of cholic acid macrocyclization on $NaBH_4$ reduction of these oxo groups has been delineated by this study.

[◇] Part II: Ref.[18].

Results and Discussion

Preparation of Monomers for Dimerization and Cyclization

Cholic acid and deoxycholic acid (**1a**, **b**, Scheme 1) were methylated in methanol previously treated with acetyl chloride to give **2a**, **b**. The 3α-hydroxy groups of **2a**, **b** was selectively reacted with excess ethyl chlorocarbonate in pyridine per the method of Fieser etal.[9][10] affording the 3-carbethoxy derivatives **3a**, **b** in good yield. A simple one-pot selective 3α-acetylation of cholic and deoxycholic acid by transesterification with acetate esters (ethyl, propyl, or butyl) in the presence of catalytic amount of *p*-TsOH and water was recently reported by Kuhajda and coworkers.[11] The selective diacetylation of compound **3b** at the 3α- and 7α-position[9][10] gives the 3α,7α-diacetoxy-12α-hydroxy compound **3c**. Oxidation of this product with potassium chromate[9][10] afforded methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (**4c**). The same oxidation on **3a** gave a mixture of 7,12-dioxo (**4a**) and 7-oxo-12α-hydroxy product. Jones′ oxidation gave more satisfactory results. The 3α-carbethoxy group and methyl ester were easily removed by refluxing **4a**, **b** with alkali (3 M NaOH) under formation of **5a**, **b**. The selective hydrolysis of 3α-OAc and methyl ester of **4c** to **5c** was achieved by refluxing with saturated sodium carbonate in methanol and THF for five hours as described by our early reports on similar compounds.[12]

Dimerization

The dimerization of **4c** with hydrazine and ethylenediamine was first attempted with Bortolini′s method without success.[13] In 1982, Chang and Iida reported a procedure to convert compound **4c** into 3α,7α-diacetoxy-12-oxocholanoate tosylhydrazone **9** (Scheme 2).[14] Employing equivalent reaction conditions, we obtained the bishydrazone dimer **10** with hydrazine but no monomeric hydrazone prod-

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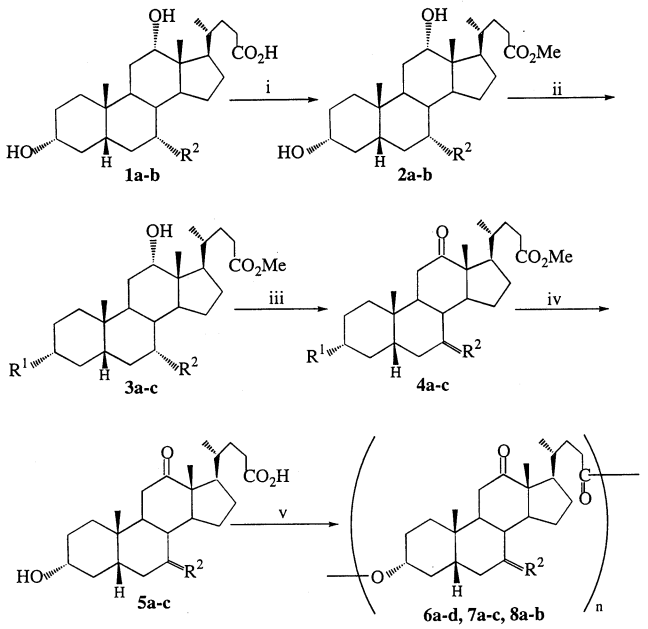
Scheme 1



Reagents and Results: (i) acetyl chloride, MeOH, rt., 2h, **2a, 2b**;
(ii) ethyl chloroformate, pyridine, 0^0C, 1h, **3a, 3b**; Ac_2O, pyridine, benzene, rt, 2h, **3c**;
(iii) Jones' reagent, acetone, **4a, 4b**; K_2CrO_4, AcOH, rt, 15h, **4c**;
(iv) NaOH (3M), THF, MeOH, reflux, 10h, **5a, 5b**; Na_2CO_3, MeOH, THF, 5h, **5c**;
(v) 2,6-dichlorobenzoyl chloride, DMAP, toluene, reflux, 48h, **6a-6d**; **7a-7c**; **8a**, **8b**.

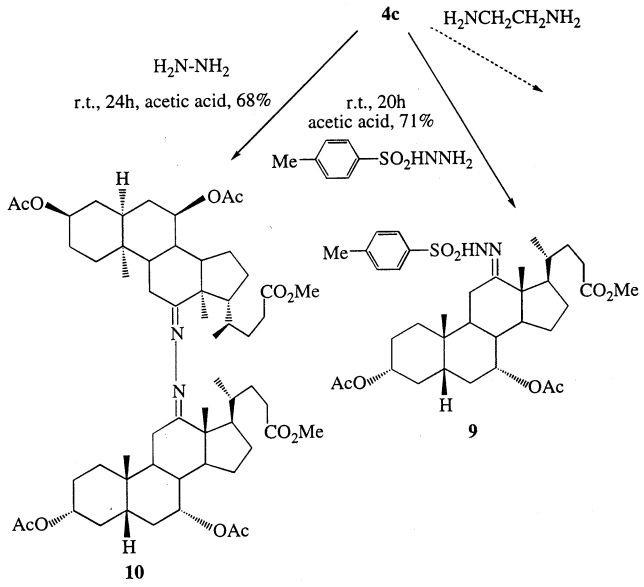
Cmpds.	R^1	R^2	*n*	yield (%)
1a−2a	−	OH	−	−, 96
1b−2b	−	H	−	−, 94
3a	$EtOCO_2$	OH	−	80
3b	$EtOCO_2$	H	−	86
3c−4c	OAc	H, OAc	−	86, 94
4a	$EtOCO_2$	=O	−	81
4b	$EtOCO_2$	H, H	−	85
5a	−	=O	−	92
5b	−	H, H	−	94
5c	−	H, OAc	−	95
6a−6d	−	=O	2, 3, 4, 5	5, 38, 8, 3
7a−7c	−	H, H	2, 3, 4	18, 32, 10
8a−8b	−	H, OAc	2, 3	9, 35

uct. Examination of Dreiding models shows that while both the *s-trans* and *s-cis* conformation have maximum N-N orbital overlap, the *s-trans* has a minimal steric interaction, whereas *s-cis* conformation has greater steric repulsion. Although high energy barriers may limit the rotation of N−N bond, it is possible that the (*Z'*-*Z*), (*Z'*-*E*) and (*E'*-*E*) isomers are interchangeable through an in-plane conversion via pseudorotation.[15]

Cyclization

The cyclooligomers were prepared from appropriately prepared monomeric hydroxy acids **5a−c** by Yamaguchi macrolactonization[16][17][18] using 2,6-dichlorobenzoyl chloride as the coupling agent (Scheme 1). Cyclotrimerization of **5a−c** is preferred over cyclodimerization and cyclotetramerization. Because the 7-oxo and 12-oxo groups exhibit reduced steric effects, cyclodimers (**6a**: 5%, **7a**: 18%, **8a**: 9%) were also obtained. The cyclodimers were previously observed in the cyclizations of 7-deoxycholic acid derivatives.[12][19] The synthesis of the cholaphane cyclodimer of cholic acid with aromatic spacer groups is reported by Bonar-Law and Davis.[20] The low synthesis yield of tetramer(**6c**: 8%, **7c**: 10%) is consistent with our cyclization results of lithocholic acid.[21] Large scale cyclization of **5a** also permitted isolation of some cyclopentamer **6d**.

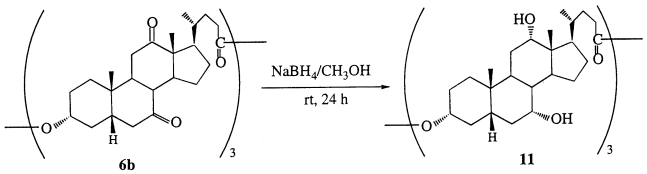
Scheme 2



$NaBH_4$ Reduction

In 1958, Wheeler and Mateos reported the reactivity of ketones at different positions in the steroid nucleus towards $NaBH_4$.[22][23] They found the following order of reactivities: Δ^5-3-oxo > $\Delta^{8(14)}$-3-oxo > 3-oxo(A,B-*cis*) > 3-oxo(A,B-*trans*) > 6-oxo > 7-oxo > Δ^4-3-oxo > 12-oxo > 17-oxo > 20-oxo > 11-oxo. In the 5β-H series reduction of 7-ketocholanic acid gives a 75−85% of 7α-hydroxy-cholanic acid with only traces of the 7β-ol.[5][6] When 12-oxocholanoic acids were reduced by $NaBH_4$, a 12-hydroxy epimeric mixture in which the 12β/12α ratio of ca. 0.55 was obtained.[7][8] The *tert*-butylamineborane reagent reduced 12-oxocholanic acids to an epimer mixture having a 12β/12α ratio of ca. 3.7. In this work, the reduction of 7,12-dioxo group in cyclotrimer **6b** gave mainly cyclotrimer **11** (69% yield) having 7α,12α-dihydroxy groups on each mer (Scheme 3). The configurations of 7,12-dihydroxy groups were established by the ^{1}H NMR chemical shifts. With 7α- and 12α-hydroxy groups, 7β- and 12β-H have chemical shifts around δ =

Scheme 3



3.96. With 7β- and 12β-hydroxy groups, 7α- and 12α-H would have chemical shifts around δ = 3.40.[8] The preference for production the tris-7α,12α-dihydroxy isomer results because the $NaBH_4$ attack occurs from the outer perimeter of the cyclotrimer for steric reasons. These results should be compared to the 3% yield of **11** obtained by direct macrocyclization of cholic acid.[16]

^{1}H-NMR Spectra

The C^{20}-methyl group shows a doublet because of spin-spin coupling with a hydrogen at 20-carbon. After oxidation of 12α-OH to the 12-oxo group, C^{18}-H and C^{19}-H became deshielded from δ = 0.68 to 1.03 and δ = 0.90 to 1.03, respectively; the C^{21}-H shifts upfield from δ = 0.97 to 0.85 because the conformation of the 17-sidechain places these protons within the shielding zone of the 12-carbonyl group. After the 12-oxo in **5c** is transformed to a 12-hydrazone moiety in **9**, the 18-methyl and 19-methyl groups undergo an upfield shift from δ = 1.03 to 0.80 and δ = 1.03 to 0.95, respectively, and the 21-methyl becomes further shielded (δ = 0.85 shifts to 0.62) because of the added effect of diamagnetic anisotropy of the aromatic ring-current. This ring-current effect is absent in **10**. The C^{11} α-carbonyl protons are coupled to the C^9 axial proton and appear as a doublet of doublet near δ = 2.5. After regeneration of 12α-OH, C^{18}-H, and C^{19}-H are shielded from δ = 1.08 to 0.70 and δ = 1.08 to 0.85, respectively. Upon hydrolysis of the 3α-carbethoxy, the geminal 3β-hydrogen becomes shifted upfield from δ = 4.53−4.56 to 3.61−3.69. After hydrolysis of 3α-OAc, the geminal 3β-hydrogen shifts upfield from δ = 4.58 to 3.48. Generally, the chemical shifts of the same functional groups on two different monomers overlap. This can be demonstrated by integrations. Compared with cholic acid system, the same functional groups of deoxycholic acid system shift to the lower field.

^{13}C-NMR Spectra

Within a steroid family, a given substituent produces similar effects on the ^{13}C-NMR chemical shifts of carbons at and near the site of substitution. Thus, a very useful approach in spectral assignments is through comparison of closely related compounds. Previously we have noted that the ^{13}C-NMR chemical shifts for C-20 of most cholanes were least variable and occurred around δ = 35±1.[21] Additionally, since the 17-sidechain of cyclocholates undergoes a major change in flexibility and molecular tension as the cycle size goes from cyclodimer to cyclotetramer, the largest ^{13}C-NMR chemical shift differences with changes in cycle size occur in the C-17 and C-20 to C-24 carbons.[21] In comparison of the ^{13}C NMR, significant deshielding of the C-20 and C-21 carbons occurred in the cyclodimer **8a** relative to the cyclotrimer **8b** and **10**. The 12-carbonyl has a strong deshielding effect on C-9, C-11, C-12, C-13, and C-17. Because the high field chemical shifts for C-18, C-21, and C-19 and the chemical shifts for C-14, C-13, and C-17 are well separated from the other ^{13}C-NMR peaks, their peak assignment is more easily done and allows one to use this information to gauge the influence of steroid skeleton substituents on the remaining proximate carbon positions. For example, in going from a 12-OAc to a 12-oxo group, C-13 shifted from δ = 45.1 to 53.1. Thus, one should expect C-11 to be shifted by a similar value, namely, approximately 8 ppm; in actuality it was shifted from δ = 25.6 to 37.6 or by 12 ppm. Similarly, C-17 shifted from δ = 47.2 to 57.1, and we determined that C-9 shifted from δ = 28.9 to 35.5. Comparison of the ^{13}C-NMR spectra of 7,12-diacetyl-[20] and 7-acetyl-12-oxo-cyclotricholate (**8b**) showed that all the respective chemical shift differences, by-and-large, were <1 ppm except for the following: C-11, 25.8 to 37.8; C-12, 75.7 to 213.4; C-13, 45.0 to 53.3; C-14, 44.1 to 45.7; C-17, 46.1 to 57.0; C-18, 12.3 to 11.3; C-21, 17.3 to 18.4. Comparison of the ^{13}C-NMR spectra of 7-acetyl-12-oxo-cyclotricholate (**8b**) and 7,12-dioxo-cyclotricholate (**6b**) showed that all the respective chemical shift differences, by-and-large, were <1 ppm except for the following: C-15, 23.71 to 25.06; C-9, 37.59 to 44.37; C-6, 31.83 to 44.92; C-8, 38.03 to 45.17; C-5, 40.29 to 45.37; C-14, 45.71 to 48.92; C-7, 70.72 to 209.09. Except for minor variation in ^{13}C-NMR assignments, the NMR spectra of **7b** agree with previously published ones.[24]

Table 1. ^{13}C NMR ($CDCl_3$) of 3α-hydroxy-7α-acetoxy-12-oxo-5β-cholan-24-oic acid derivatives

Assignment	**4c**	**10**	**8a**	**8b**
C-18	11.52	12.44	11.28	11.34
C-21	18.56	19.96	20.81	18.44
*CH_3*CO (ax.)	21.41	21.42	21.81	21.29
*CH_3*CO (eq.)	21.41	21.67	–	–
C-19	22.14	22.38	23.47	22.03
C-15	23.78	23.90	23.47	23.71
C-2	26.57	27.29	25.58	26.38
C-16	27.39	27.60	26.87	27.71
C-23	30.50	30.87	29.89	30.69
C-22	31.24	31.56	31.35	31.19
C-6	31.36	31.99	31.35	31.83
C-10	34.57	34.98	33.84	34.49
C-1	34.92	34.98	34.81	34.82
C-4	34.92	35.61	35.54	34.82
C-20	35.54	35.85	37.42	35.84
C-9	35.54	36.21	37.42	37.59
C-11	37.60	36.21	37.89	37.76
C-8	37.89	38.09	38.04	38.03
C-5	40.52	40.94	40.26	40.29
C-14	46.39	47.32	43.12	45.71
C-13	53.13	51.67	52.67	53.31
C-17	57.13	53.78	56.35	56.99
OCH_3	51.49	51.59	–	–
C-7	70.54	70.96	71.06	70.72
C-3	73.57	73.99	72.47	72.92
C_2H_5*CO* (eq.)	170.19	169.94	–	–
C_2H_5*CO* (ax.)	170.67	170.37	170.36	169.83
C-24	174.60	174.49	174.01	173.70
C-12	213.98	184.56	211.55	213.38

Mass Spectra

All these spectra have the peaks for loss of 3α-functional groups and the 17-sidechain. For all the intermediates, the 17-sidechain can be lost before or after the 3α-functional group is lost; while the 3α-OH is always lost after 7α-OH, the 3α-carbethoxy can be lost before or after the 7α-OH

Table 2. ^{13}C NMR ($CDCl_3$) of 3α-hydroxy-12-oxo-5β-cholan-24-oic acid derivatives

Assignment	**4b**	**7a**	**7b**	**7c**
C-18	11.71	11.39	11.51	11.68
*C*H$_3$CH$_2$O	14.31	–	–	–
C-21	18.62	19.57	18.53	18.54
C-19	22.75	22.78	22.72	22.72
C-15	24.38	24.10	24.28	24.39
C-16	26.02	26.01	25.99	26.01
C-7	26.38	26.53	26.36	26.32
C-2	27.00	27.21	26.91	26.92
C-6	27.54	27.81	27.69	27.75
C-23	30.57	28.36	30.17	30.89
C-22	31.31	30.18	30.85	(30.89)
C-4	32.15	32.00	32.19	32.17
C-10	34.89	35.15	34.98	34.95
C-8	35.36	35.52	35.33	35.34
C-1	35.68	35.94	(35.33)	35.68
C-20	(35.68)	(35.94)	35.64	(35.68)
C-11	38.09	37.95	38.06	38.07
C-9	41.42	41.10	41.33	41.22
C-5	44.03	43.40	43.95	44.07
C-13	46.52	43.89	45.22	45.22
C-17	57.50	57.17	57.40	57.48
C-14	58.60	58.69	58.68	58.87
OCH$_3$	51.43	–	–	–
CH$_3$*C*H$_2$O	63.62	–	–	–
C-3	76.66	74.35	73.36	73.36
EtO*CO*	154.63	–	–	–
C-24	174.60	176.47	173.98	173.56
C-12	214.31	214.38	214.19	214.25

Table 3. ^{13}C NMR ($CDCl_3$) of 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acid derivatives

Assignment	**4a**	**6a**	**6b**	**6c**	**6d**
C-18	11.80	11.41	11.62	11.74	11.83
*C*H$_3$CH$_2$O	14.23	–	–	–	–
C-21	18.59	20.87	18.53	18.60	18.68
C-19	22.36	22.26	22.41	22.42	22.46
C-15	25.14	25.05	25.06	25.08	25.12
C-2	25.75	25.10	25.77	25.81	25.86
C-16	27.63	27.16	27.93	27.82	27.60
C-23	30.47	29.72	30.12	30.66	30.61
C-22	31.27	30.14	31.01	31.64	31.85
C-10	33.05	32.94	33.10	33.21	33.21
C-1	33.64	33.90	33.80	33.81	33.79
C-4	35.52	34.94	35.40	35.46	35.36
C-20	35.75	35.67	35.70	35.76	35.85
C-11	38.30	38.09	38.34	38.36	38.35
C-9	44.86	42.21	44.37	44.92	44.96
C-6	45.15	44.99	44.92	45.04	45.25
C-8	45.39	(44.99)	45.17	45.22	45.44
C-5	45.58	45.37	45.37	45.34	(45.44)
C-14	48.86	49.07	48.92	48.92	48.95
C-13	51.77	51.13	51.78	51.77	51.79
C-17	56.81	56.41	56.74	56.80	56.89
OCH$_3$	51.41	–	–	–	–
CH$_3$*C*H$_2$O	63.78	–	–	–	–
C-3	75.97	71.75	72.04	72.10	72.18
EtO*CO*	155.17	–	–	–	–
C-24	174.46	174.18	173.67	173.56	173.59
C-7	208.92	208.46	209.09	208.88	209.13
C-12	212.19	210.69	211.89	212.13	212.34

and 12α-OH. Three kinds of FAB mass spectral peaks $[MNa + NaI]^+$, $[MNa]^+$, $[M + H]^+$ were present in all spectra.

Conclusion

Nine bile acid cyclooligomer analogs with 12-oxo and 7,12-dioxo have been synthesized by Yamaguchi Method and characterized by ^1H NMR, ^{13}C NMR, MS spectroscopy. Under Yamaguchi reaction conditions, the 12-oxo and 7,12-dioxo derivatives of cholic acid give mainly cyclic trimer products. A new synthetic strategy for the production of cyclotricholates with unprotected 7α,12α-dihydroxy groups has been demonstrated.

We gratefully thank the *Nebraska Center* for determining Mass Spectrometry.

Experimental Section

Column chromatography was carried out using Grade 62 (60−200 mesh) silica gel and eluted by hexane/ethyl acetate solvent system. Melting points were determined on a Fisher-Johns melting-point apparatus and are uncorrected. − ^1H-NMR and ^{13}C-NMR spectra were measured at 250 MHz and 63 MHz (Bruker) in $CDCl_3$ as solvent and TMS as internal standard. − Mass spectra were recorded on a VG20-253 or VGZAB-HS Spectrometer. − All new products were homogeneous by TLC, NMR, and mass spectroscopy.

Methyl 3-Carbethoxy-7α,12α-dihydroxy-5β-cholan-24-oate (**3a**): Methyl cholate **2a** was prepared by published method[10]. Yield: 96%, m.p. 153−155°C (ref.[10] m.p. 156−157°C). − ^1H NMR ($CDCl_3$): 0.67 (s, 3 H, 18-H$_3$); 0.88 (s, 3 H, 19-H$_3$); 0.99 (d, 3 H, 21-H$_3$); 3.43 (broad, 1 H, 3β-H); 3.66 (s, 3 H, COOCH$_3$); 3.82 (s, 1 H, 7β-H); 3.94 (s, 1 H, 12β-H).

Compound **3a** was prepared by esterification of **2a** with ethyl chloroformate in pyridine[10]. Yield: 80.4%, m.p. 175−177°C (ref.[10] m.p. 176−177°C). − ^1H NMR ($CDCl_3$): 0.68 (s, 3 H, 18-H$_3$); 0.90 (s, 3 H, 19-H$_3$); 0.97 (d, 3 H, 21-H$_3$); 3.66 (s, 3 H, CO-OCH$_3$); 3.85 (s, 1 H, 7β-H); 3.98 (s, 1 H, 12β-H); 4.14 (q, 2 H, 3-EtOCOO); 4.41 (broad, 1 H, 3β-H). − MS (EI): 494.3 $[M]^{+\cdot}$ (2), 476.3 $[M - H_2O]^{+\cdot}$ (3), 458.3 $[M - 2\ H_2O]^{+\cdot}$, 404.3 $[M - C_3H_6O_3]^{+\cdot}$ (5), 386.2 $[M - H_2O - C_3H_6O_3]^{+\cdot}$ (29), 368.2 $[M - 2\ H_2O - C_3H_6O_3]^{+\cdot}$ (42), 253 $[M - 2\ H_2O - C_9H_{17}O_5]^+$ (100).

Methyl 3α,12α-Dihydroxy-5β-cholan-24-oate (**2b**) was prepared in similar fashion, m.p. 90−92°C (ref.[10] m.p. 75−86°C) (93.9%). − ^1H NMR ($CDCl_3$): 0.67 (s, 3 H, C-18); 0.90 (s, 3 H, C-19); 0.96 (d, 3 H, C-21); 3.60 (broad, 1 H, 3β-H); 3.66 (s, 3 H, COOCH$_3$); 3.98 (s, 1 H, 12β-H).

Methyl 12α-Hydroxy-3α-carbethoxy-5β-cholan-24-oate (**3b**) was prepared in similar fashion, m.p. 135−137°C (ref.[10] m.p. 137−140°C) (86%). − ^1H NMR ($CDCl_3$): 0.67 (s, 3 H, 18-H$_3$); 0.90 (s, 3 H, 19-H$_3$); 0.98 (d, 3 H, 21-H$_3$); 3.66 (s, 3 H, COOCH$_3$); 3.99 (s, 1 H, 12β-H); 4.16 (q, 2 H, 3-EtOCOO); 4.43 (broad, 1 H, 3β-H). − MS (EI): 478.3 $[M]^{+\cdot}$ (2), 460 $[M - H_2O]^{+\cdot}$ (5), 388 $[M - C_3H_5O_3]^{+\cdot}$ (5), 370 $[M - H_2O - C_3H_6O_3]^{+\cdot}$ (31), 255 $[M - H_2O - C_9H_{17}O_5]^+$ (100).

Methyl 3α,7α-Diacetoxy-12α-hydroxy-5β-cholan-24-oate (**3c**): Compound **3c** was prepared by esterification of **2a** with acetic anhydride[10]. Yield: 86%, m.p. 183−185°C (ref.[10] m.p. 187−188°C). − ^1H NMR ($CDCl_3$): 0.68 (s, 3 H, 18-H$_3$); 0.92 (s, 3 H, 19-H$_3$); 0.97 (d, 3 H, 21-H$_3$); 2.02 (s, 3 H, 3-OAc); 2.03 (s, 3 H, 7-OAc); 2.3 (m, 2 H, 23-H$_2$); 3.66 (s, 3 H, COOCH$_3$); 3.99 (s, 1 H, 12β-H); 4.58 (broad, 1 H, 3β-H); 4.89 (s, 1 H, 7β-H). − MS (EI): 506 $[M]^{+\cdot}$ (1), 446 $[M - HOAc]^{+\cdot}$ (3), 428 $[M - HOAc - H_2O]^{+\cdot}$ (11), 386 $[M - 2\ HOAc]^{+\cdot}$ (10), 368 $[M - 2\ HOAc - H_2O]^{+\cdot}$ (38), 253 $[M - 2\ HOAc - H_2O - C_6H_{11}O_2]^+$ (100).

Methyl 3α-Carbethoxy-7,12-dioxo-5β-cholan-24-oate (**4a**): A solution of **3a** (25 g, 50.6 mmol) in acetone (300 ml) was treated with Jones′ reagent (27 ml) as it was being magnetically stirred and chilled on an ice bath. After complete addition of the Jones′ reagent, the ice bath was removed and stirring continued at rt for 10 min and then it was quenched with 2-propanol (30 ml). The solution was concentrated and diluted with water and extracted with ethyl acetate. The ethyl acetate was washed with $NaHCO_3$ and NaCl solutions, dried with Na_2SO_4, and concentrated under reduced pressure to afford **4a** (20 g, 80.6%): m.p. 135−137°C (ref.[10] m.p. 137−138°C). − ^{1}H NMR ($CDCl_3$): 0.83 (d, 3 H, 21-H_3); 1.03 (s, 6 H, 18-H_3; 19-H_3); 2.36 (m, 2 H, 11-H_2); 2.71 (dd, 2 H, 6-H_2); 2.90 (m, 1 H, 8-H); 3.66 (s, 3 H, $COOCH_3$); 4.14 (q, 2 H, 3-EtOCOO); 4.53 (broad, 1 H, 3β-H). − MS (EI): 490.3 $[M]^{+\cdot}$ (19), 472.3 $[M - H_2O]^{+\cdot}$ (19), 400.3 $[M - C_3H_6O_3]^{+\cdot}$ (15), 359.3 (25), 269.2 (78), 245 (100).

Methyl 3α-Carbethoxy-12-oxo-5β-cholan-24-oate (**4b**) was prepared in similar fashion, m.p. 152−154°C (ref.[10] m.p. 157−158°C) (85%). − ^{1}H NMR ($CDCl_3$): 0.84 (d, 3 H, 21-H_3); 1.02 (s, 6 H, 18-H_3; 19-H_3); 2.40 (m, 2 H, 11-H_2); 3.66 (s, 3 H, $COOCH_3$); 4.16 (q, 2 H, 3-EtOCOO); 4.56 (broad, 1 H, 3β-H). − MS (EI): 476.3 $[M]^{+\cdot}$ (33), 458.3 $[M - H_2O]^{+\cdot}$ (3), 386.3 $[M - C_3H_5O_3]^{+\cdot}$ (69), 231.2 (100).

Methyl 3α,7α-Diacetoxy-12-oxo-5β-cholan-24-oate (**4c**): Compound **4c** was prepared by oxidation of **3c** with potassium chromate[10]. Yield: 94%, m.p. 179−181°C (ref.[10] m.p. 177−179°C). − ^{1}H NMR ($CDCl_3$): 0.85 (d, 3 H, 21-H_3); 1.03 (s, 6 H, 18-H_3; 19-H_3); 2.02 (s, 3 H, 3-OAc); 2.03 (s, 3 H, 7-OAc); 2.32 (m, 2 H, 23-H_2); 2.5 (dd, 2 H, 11-H_2); 3.66 (s, 3 H, $COOCH_3$); 4.58 (broad, 1 H, 3β-H); 4.99 (s, 1 H, 7β-H). − MS (EI): 504 $[M]^{+\cdot}$ (4), 444 $[M - HOAc]^{+\cdot}$ (56), 384 $[M - 2\ HOAc]^{+\cdot}$ (22), 269 $[M - 2\ HOAc - C_6H_{11}O_2]^+$ (76), 229 (100).

3α-Hydroxy-7,12-dioxo-5β-cholan-24-oic Acid (**5a**): To a solution of **4a** (10 g, 20.4 mmol) in CH_3OH (120 ml) and THF (120 ml), NaOH (3 M, 180 ml) was added. The solution was refluxed for 10 h. The extra base was neutralized by conc HCl, extracted with ethyl acetate. The organic layer was washed by NaCl soln., dried with Na_2SO_4 and concentrated under reduced pressure to afford **5a** (7.6 g, 92%): m.p. 185−187°C (ref.[25] m.p. 192−193.5°C). − ^{1}H NMR ($CDCl_3$): 0.85 (d, 3 H, 21-H_3); 1.03 (s, 6 H, 18-H_3; 19-H_3); 2.35 (m, 2 H, 11-H_2); 2.75 (dd, 2 H, 6-H_2); 2.88 (m, 1 H, 8-H); 3.61 (m, 1 H, 3β-H). − FAB/MS (3-NBA): 405.2 $[M + 1]^+$, 387.2 $[M + 1 - H_2O]^+$, 369.2 $[M + 1 - 2\ H_2O]^+$, 353.2, 307.

3α-Hydroxy-12-oxo-5β-cholan-24-oic Acid (**5b**): was prepared in similar fashion, m.p. 163−165°C (94%). − ^{1}H NMR ($CDCl_3$): 0.91 (d, 3 H, 21-H_3); 1.07 (s, 6 H, 18-H_3; 19-H_3); 2.48 (m, 2 H, 11-H_2); 3.69 (s, 1 H, 3β-H). − MS (EI): 390.3 $[M]^{+\cdot}$ (100), 372.3 $[M - H_2O]^{+\cdot}$ (39.10), 354.3 $[M - 2\ H_2O]^{+\cdot}$ (3), 289.3 $[M - C_5H_9O_2]^{+\cdot}$ (12.65), 249.2 (43.23), 231.2 (70.45).

3α-Hydroxy-7α-acetoxy-12-oxo-5β-cholan-24-oic Acid (**5c**): The acid **5c** was prepared by saponification of **4c** with satd Na_2CO_3[12]. Yield: 95%, m.p. 240−242°C. − ^{1}H NMR ($CDCl_3$): 0.85 (d, 3 H, 21-H_3); 1.03 (s, 6 H, 18-H_3; 19-H_3); 2.03 (s, 3 H, 7-OAc); 2.3 (m, 2 H, 23-H_2); 2.49 (dd, 2 H, 11-H_2); 3.48 (broad, 1 H, 3β-H); 4.7 (hump, OHs); 4.98 (s, 1 H, 7β-H). − MS (EI): 448 $[M]^{+\cdot}$ (6), 388 $[M - HOAc]^{+\cdot}$ (9), 370 $[M - HOAc - H_2O]^{+\cdot}$ (41), 205 (100).

General Procedure of Cyclization: A mixture of *monomer* (3.4 mmol), 2,6-dichlorobenzoyl chloride (0.53 ml, 3.7 mmol), DMAP (1.6 g, 13 mmol) and sodium-dried toluene (800 ml) were refluxed for 48 h. The solvent was concentrated under reduced pressure, the residue was flash chromatographed on a silica gel column and crystallized to afford *cyclooligomers*.

Cyclodimer **6a**: m.p. 305−307°C, yield: 5%. − ^{1}H NMR ($CDCl_3$): 0.87 (d, 6 H, 21-H_3, 21′-H_3); 1.01 (s, 12 H, 18-H_3, 18′-H_3; 19-H_3, 19′-H_3); 2.33 (m, 4 H, 11-H_2, 11′-H_2); 2.74 (dd, 4 H, 6-H_2, 6′-H_2); 2.88 (m, 2 H, 8-H, 8′-H); 4.80 (m, 2 H, 3β-H, 3′β-H). − FAB/MS (3-NBA): 773.3 $[M+1]^+$, 755.4 $[M + 1 - H_2O]^+$, 739.3, 723.4.

Cyclotrimer **6b**: m.p. 320−322°C, yield: 38%. ^{1}H NMR ($CDCl_3$): 0.84 (d, 9 H, 21-H_3, 21′-H_3, 21″-H_3); 1.03 (s, 18 H, 18-H_3, 18′-H_3, 18″-H_3; 19-H_3, 19′-H_3, 19″-H_3); 2.30 (m, 6 H, 11-H_2, 11′-H_2, 11″-H_2); 2.75 (dd, 6 H, 6-H_2, 6′-H_2, 6″-H_2); 2.84 (m, 3 H, 8-H, 8′-H, 8″-H); 4.70 (m, 3 H, 3β-H, 3′β-H, 3″β-H). FAB/MS (3-NBA + NaI): 1331.3 $[MNa + NaI]^+$, 1181.6 $[MNa]^+$, 1042.5, 846.7, 469.3.

Cyclotetramer **6c**: m.p. 315−317°C, yield: 8%. ^{1}H NMR ($CDCl_3$): 0.84 (d, 12 H, 21-H_3, 21′-H_3, 21″-H_3, 21‴-H_3); 1.03 (s, 24 H, 18-H_3, 18′-H_3, 18″-H_3, 18‴-H_3; 19-H_3, 19′-H_3, 19″-H_3, 19‴-H_3); 2.28 (m, 8 H, 11-H_2, 11′-H_2, 11″-H_2, 11‴-H_2); 2.75 (dd, 8 H, 6-H_2, 6′-H_2, 6″-H_2, 6‴-H_2); 2.85 (m, 4 H, 8-H, 8′-H, 8″-H, 8‴-H); 4.70 (m, 4 H, 3β-H, 3′β-H, 3″β-H, 3‴β-H). FAB/MS (3-NBA + NaI): 1718.4 $[MNa + NaI]^+$, 1568.6 $[MNa]^+$, 1425.9, 1181.7, 1039.7, 873.3, 619.1, 469.3, 325.9.

Cyclopentamer **6d**: m.p. 260−262°C, yield: 3%. − ^{1}H NMR ($CDCl_3$): 0.84 (d, 15 H, 21-H_3, 21′-H_3, 21″-H_3, 21‴-H_3, 21⁗-H_3); 1.03 (s, 30 H, 18-H_3, 18′-H_3, 18″-H_3, 18‴-H_3, 18⁗-H_3; 19-H_3, 19′-H_3, 19″-H_3, 19‴-H_3, 19⁗-H_3); 2.28 (m, 10 H, 11-H_2, 11′-H_2, 11″-H_2, 11‴-H_2, 11⁗-H_2); 2.75 (dd, 10 H, 6-H_2, 6′-H_2, 6″-H_2, 6‴-H_2, 6⁗-H_2); 2.85 (m, 5 H, 8-H, 8′-H, 8″-H, 8‴-H, 8⁗-H); 4.70 (m, 5 H, 3β-H, 3′β-H, 3″β-H, 3‴β-H, 3⁗β-H). − FAB/MS (3-NBA): 1932.9 $[M + 1]^+$, 1142.9, 755.6, 613.3, 550.7, 419.4, 307.1.

Cyclodimer **7a**: m.p. 145−147°C, yield: 18%. − ^{1}H NMR ($CDCl_3$): 0.93 (d, 6 H, 21-H_3, 21′-H_3); 1.05 (s, 12 H, 18-H_3, 18′-H_3; 19-H_3, 19′-H_3); 2.49 (m, 4 H, 11-H_2, 11′-H_2); 4.72 (m, 2 H, 3β-H, 3′β-H). − FAB/MS (3-NBA): 745.5 $[M + 1]^+$, 447.3, 355.3, 307.1.

Cyclotrimer **7b**: m.p. 350−352°C, yield: 32%. − ^{1}H NMR ($CDCl_3$): 0.92 (d, 9 H, 21-H_3, 21′-H_3, 21″-H_3); 1.08 (s, 18 H, 18-H_3, 18′-H_3, 18″-H_3; 19-H_3, 19′-H_3, 19″-H_3); 2.49 (m, 6 H, 11-H_2, 11′-H_2, 11″-H_2); 4.78 (m, 3 H, 3β-H, 3′β-H, 3″β-H).[24] − FAB/MS (3-NBA): 1117.7 $[M + 1]^+$, 745.5 $[2/3M + 1]^+$, 447.3, 355.3, 289.1.

Cyclotetramer **7c**: m.p. 245−247°C, yield: 10%. − ^{1}H NMR ($CDCl_3$): 0.92 (d, 12 H, 21-H_3, 21′-H_3, 21″-H_3, 21‴-H_3); 1.08 (s, 24 H, 18-H_3, 18′-H_3, 18″-H_3, 18‴-H_3; 19-H_3, 19′-H_3, 19″-H_3, 19‴-H_3); 2.49 (m, 8 H, 11-H_2, 11′-H_2, 11″-H_2, 11‴-H_2); 4.77 (m, 4 H, 3β-H, 3′β-H, 3″β-H, 3‴β-H). − FAB/MS (3-NBA): 1490 $[M + 1]^+$, 1117.8 $[3/4M + 1]^+$, 822.7, 745.5 $[1/2M + 1]^+$, 682.6.

Cyclodimer **8a**: m.p. 220−222°C, yield: 9%. − ^{1}H NMR ($CDCl_3$): 0.81 (d, 6 H, 21-H_3, 21′-H_3); 0.97 (s, 12 H, 18-H_3, 18′-H_3; 19-H_3, 19′-H_3); 2.10 (s, 6 H, 7-OAc, 7′-OAc); 2.24 (m, 4 H, 23-H_2, 23′-H_2); 2.4 (dd, 4 H, 11-H_2, 11′-H_2); 4.60 (m, 2 H, 3β-H, 3′β-H); 5.03 (s, 2 H, 7β-H, 7′β-H). − FAB/MS (3-NBA): 861.4 $[M + 1]^+$, 800.4 $[M - HOAc]^+$, 447.3, 369.2 $[1/2M - HOAc]^+$, 353.2, 307, 273. − $C_{52}H_{76}O_{10}$ (860): Calcd: C 72.56, H 8.84. Found: C 72.26, H 9.36.

Cyclotrimer **8b**: m.p. 310−312°C, yield: 35%. − ^{1}H NMR ($CDCl_3$): 0.86 (d, 9 H, 21-H_3, 21′-H_3, 21″-H_3); 1.03 (s, 18 H, 18-H_3, 18′-H_3, 18″-H_3; 19-H_3, 19′-H_3, 19″-H_3); 1.98 (s, 9 H, 7-OAc, 7′-OAc, 7″-OAc); 2.34 (m, 6 H, 23-H_2, 23′-H_2, 23″-H_2); 2.5 (dd, 6 H, 11-H_2, 11′-H_2, 11″-H_2); 4.60 (m, 3 H, 3β-H, 3′β-H, 3″β-H); 4.97 (broad s, 3 H, 7β-H, 7′β-H, 7″β-H). − FAB/MS (3-NBA):

1291.3 $[M + 1]^+$, 1231.5 $[M + 1 - HOAc]^+$, 1171.6 $[M + 1 - 2 HOAc]^+$, 1111.7 $[M + 1 - 3 HOAc]^+$, 1093.7 $[M + 1 - 3 HOAc - H_2O]^{+\cdot}$. – $C_{78}H_{114}O_{15}$ (1290): Calcd: C 72.56, H 8.84. Found: C 72.59, H 9.01.

Methyl 3α,7α-Diacetoxy-12-oxocholanate Tosylhydrazone (**9**): Compound **9** was prepared by published method[14]. Yield: 71%, m.p. 147–149°C (ref.[14] m.p. 146–147°C). – ^{1}H NMR ($CDCl_3$): 0.62 (d, 3 H, 21-H_3); 0.80 (s, 3 H, 18-H_3); 0.95 (s, 3 H, 19-H_3); 2.03 (s, 3 H, 3-OAc); 2.04 (s, 3 H, 7-OAc); 2.45 (s, 3 H, Ar-CH_3); 3.67 (s, 3 H, $COOCH_3$); 4.53 (broad, 1 H, 3β-H); 4.90 (s, 1 H, 7β-H); 6.0 (broad, 1 H, -SO_2NHN=); 7.33 (dd, 2 H, Ar); 7.85 (dd, 2 H, Ar).

Dimerization of **4c** *to Bishydrazone* **10**: To a stirred solution of **4c** (1 g, 2 mmol) in acetic acid (15 ml) was added slowly anhydrous hydrazine (0.065 ml, 2.1 mmol) while chilling in an ice bath. After stirring at rt for 24 h, the solution was poured in water (50 ml) and extracted with ethyl acetate. The combined ethyl acetate was washed with satd Na_2CO_3 soln., NaCl soln., dried with Na_2SO_4, and evaporated to afforded a residue from which **10** (0.68 g, 68%) was obtained by chromatography (*n*-hexane/ethyl acetate, 1:10): m.p. 125–127°C. – ^{1}H NMR ($CDCl_3$): 0.96 (d, 6 H, 21-H_3, 21′-H_3); 0.97 (s, 6 H, 18-H_3, 18′-H_3); 1.04 (s, 6 H, 19-H_3, 19′-H_3); 2.00 (s, 6 H, 3-OAc, 3′-OAc); 2.04 (s, 6 H, 7-OAc, 7′-OAc); 3.66 (s, 6 H, $COOCH_3$, $COOCH_3$′); 4.62 (m, 2 H, 3β-H, 3′β-H); 4.99 (broad s, 2 H, 7β-H, 7′β-H). – FAB/MS (3-NBA): 1005.6 $[M + 1]^+$, 945.5 $[M + 1 - HOAc]^+$, 885.4 $[M + 1 - 2 HOAc]^+$, 849.4, 789.4, 502.2 $[M/2]^+$, 442 $[M/2 - HOAc]^+$, 382.2 $[M/2 - 2 HOAc]^{+\cdot}$. – $C_{58}H_{88}N_2O_{12}$ (1004): Calcd: C 69.32, H 8.76, N 2.79. Found: C 70.03, H 9.20, N 2.59.

Preparation of **11** *by Reduction Cyclotrimer* **6b**: To a stirred solution of compound **6b** (0.1 g, 0.086 mmol) in CH_3OH (20 ml) was added $NaBH_4$ (0.06 g, 1.59 mmol) at 0°C. After the solution was stirred for 24 h at 0°C, dilute HCl were added to acidify the solution, the solvent was removed. The residue was dissolved in $CHCl_3$ and washed with water, brine, dried and finally evaporated to dryness. The crude product was purified by column chromatography to yield **11** (0.07 g, 69%) as a colorless solid: m.p. 258–260°C. – ^{1}H NMR ($CDCl_3$): 0.70 (s, 9 H, 18-H_3, 18′-H_3, 18′′-H_3); 0.85 (s, 9 H, 19-H_3, 19′-H_3, 19′′-H_3); 0.90 (d, 9 H, 21-H_3, 21′-H_3, 21′′-H_3); 3.85 (s, 3 H, 7β-H, 7′β-H, 7′′β-H); 3.96 (s, 3 H, 12β-H, 12′β-H, 12′′β-H); 4.70 (m, 3 H, 3β-H, 3′β-H, 3′′β-H). – ^{13}C NMR ($CDCl_3$): 12.46 (C-18), 17.34 (C-21), 22.58 (C-19), 23.09 (C-15), 26.63 (C-9), 26.87 (C-16), 27.47 (C-11), 28.45 (C-2), 30.20 (C-22), 30.45 (C-23), 34.14 (C-6), 34.46 (C-10), 34.63 (C-1), 34.92 (C-20), 35.21 (C-4), 39.65 (C-8), 41.13 (C-14), 42.22 (C-5), 45.77 (C-13), 46.46 (C-17), 68.23 (C-7), 72.78 (C-3), 73.81 (C-12), 173.90 (C-24). – FAB/MS (3-NBA + NaI): 1344.0 $[MNa + NaI]^+$, 1193.9 $[MNa]^+$, 326.0, 176.1.

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