

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

Hongwu Gao and Jerry Ray Dias*

Department of Chemistry, University of Missouri – Kansas City,
 Kansas City, MO 64110-2499, USA
 E-mail: diasgp@cctr.umkc.edu

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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 tailor-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 bufalitin 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 7 α -OH and 12 α -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 NaBH₄ without disrupting the 24-carboxylic ester linkages. It is well known that NaBH₄ reduction of the 7-oxo gives 7 α -OH^{[5][6]} and reduction of the 12-oxo frequently gives both 12 α - and 12 β -OH.^{[7][8]} The effect of cholic acid macrocyclization on NaBH₄ reduction of these oxo groups has been delineated by this study.

[◇] Part II: Ref.^[18].

Results and Discussion

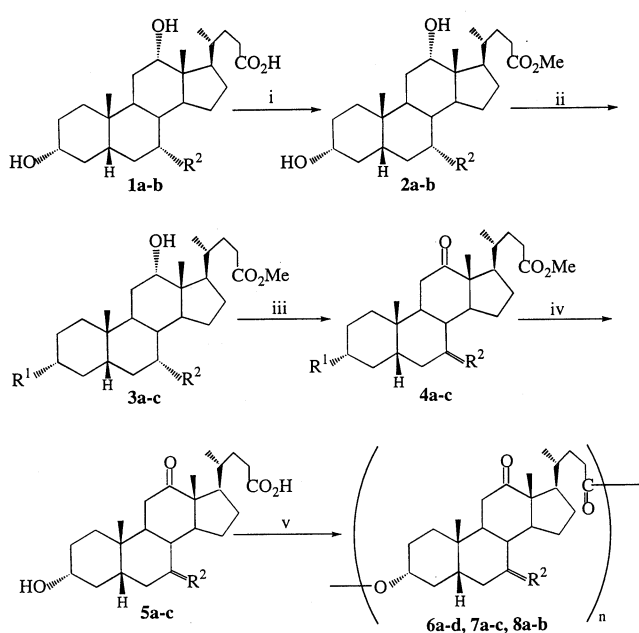
Preparation of Monomers for Dimerization and Cyclization

Cholic acid and deoxycholic acid (**1a**, **b**, Scheme 1) were methylated in methanol previously treated with acetyl chloride to give **2a**, **b**. The 3 α -hydroxy groups of **2a**, **b** were selectively reacted with excess ethyl chlorocarbonate in pyridine per the method of Fieser et al.^{[9][10]} affording the 3-carbethoxy derivatives **3a**, **b** in good yield. A simple one-pot selective 3 α -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 3 α - and 7 α -position^{[9][10]} gives the 3 α ,7 α -diacetoxy-12 α -hydroxy compound **3c**. Oxidation of this product with potassium chromate^{[9][10]} afforded methyl 3 α ,7 α -diacetoxy-12-oxo-5 β -cholan-24-oate (**4c**). The same oxidation on **3a** gave a mixture of 7,12-dioxo (**4a**) and 7-oxo-12 α -hydroxy product. Jones' oxidation gave more satisfactory results. The 3 α -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 3 α -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 3 α ,7 α -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-

Scheme 1



Reagents and Results: (i) acetyl chloride, MeOH, rt., 2h, **2a**, **2b**; (ii) ethyl chloroformate, pyridine, 0°C, 1h, **3a**, **3b**; Ac₂O, pyridine, benzene, rt, 2h, **3c**; (iii) Jones' reagent, acetone, **4a**, **4b**; K₂CrO₄, AcOH, rt, 15h, **4c**; (iv) NaOH (3M), THF, MeOH, reflux, 10h, **5a**, **5b**; Na₂CO₃, MeOH, THF, 5h, **5c**; (v) 2,6-dichlorobenzoyl chloride, DMAP, toluene, reflux, 48h, **6a-d**, **7a-c**; **8a**, **8b**.

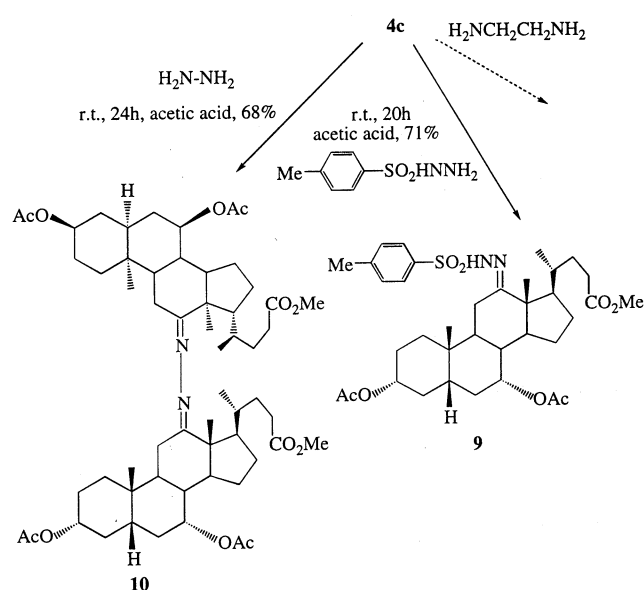
Cmpds.	R ¹	R ²	n	yield (%)
1a–2a	—	OH	—	—, 96
1b–2b	—	H	—	—, 94
3a	EtOCO ₂	OH	—	80
3b	EtOCO ₂	H	—	86
3c–4c	OAc	H, OAc	—	86, 94
4a	EtOCO ₂	=O	—	81
4b	EtOCO ₂	H, H	—	85
5a	—	=O	—	92
5b	—	H, H	—	94
5c	—	H, OAc	—	95
6a–6d	—	=O	2, 3, 4, 5	5, 38, 8, 3
7a–7c	—	H, H	2, 3, 4	18, 32, 10
8a–8b	—	H, OAc	2, 3	9, 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 pre-

Scheme 2

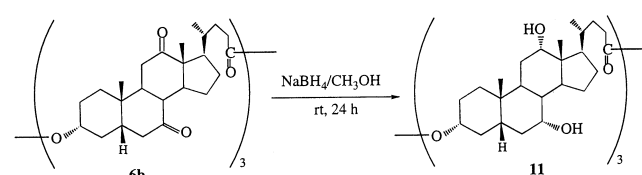


viously observed in the cyclizations of 7-deoxycholic acid derivatives.^{[12][19]} The synthesis of the cholaphane cyclodimer of cholic acid with aromatic spacer groups is reported by Bonar-Law and Davis.^[20] The low synthesis yield of tetramer(**6c**: 8%, **7c**: 10%) is consistent with our cyclization results of lithocholic acid.^[21] Large scale cyclization of **5a** also permitted isolation of some cyclopentamer **6d**.

NaBH₄ Reduction

In 1958, Wheeler and Mateos reported the reactivity of ketones at different positions in the steroid nucleus towards NaBH₄.^{[22][23]} They found the following order of reactivities: Δ⁵-3-oxo > Δ⁸⁽¹⁴⁾-3-oxo > 3-oxo(A,*B-cis*) > 3-oxo(A,*B-trans*) > 6-oxo > 7-oxo > Δ⁴-3-oxo > 12-oxo > 17-oxo > 20-oxo > 11-oxo. In the 5β-H series reduction of 7-ketochocholic 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 NaBH₄, 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 ¹H NMR chemical shifts. With 7α- and 12α-hydroxy groups, 7β- and 12β-H have chemical shifts around δ =

Scheme 3



3.96. With 7 β - and 12 β -hydroxy groups, 7 α - and 12 α -H would have chemical shifts around $\delta = 3.40$.^[8] The preference for production the tris-7 α ,12 α -dihydroxy isomer results because the NaBH₄ 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]

¹H-NMR Spectra

The C²⁰-methyl group shows a doublet because of spin-spin coupling with a hydrogen at 20-carbon. After oxidation of 12 α -OH to the 12-oxo group, C¹⁸-H and C¹⁹-H became deshielded from $\delta = 0.68$ to 1.03 and $\delta = 0.90$ to 1.03, respectively; the C²¹-H shifts upfield from $\delta = 0.97$ 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 $\delta = 1.03$ to 0.80 and $\delta = 1.03$ to 0.95, respectively, and the 21-methyl becomes further shielded ($\delta = 0.85$ 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 C¹¹ α -carbonyl protons are coupled to the C⁹ axial proton and appear as a doublet of doublet near $\delta = 2.5$. After regeneration of 12 α -OH, C¹⁸-H, and C¹⁹-H are shielded from $\delta = 1.08$ to 0.70 and $\delta = 1.08$ to 0.85, respectively. Upon hydrolysis of the 3 α -carbethoxy, the geminal 3 β -hydrogen becomes shifted upfield from $\delta = 4.53$ –4.56 to 3.61–3.69. After hydrolysis of 3 α -OAc, the geminal 3 β -hydrogen shifts upfield from $\delta = 4.58$ 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.

¹³C-NMR Spectra

Within a steroid family, a given substituent produces similar effects on the ¹³C-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 ¹³C-NMR chemical shifts for C-20 of most cholanes were least variable and occurred around $\delta = 35 \pm 1$.^[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 ¹³C-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 ¹³C 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 ¹³C-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 $\delta = 45.1$ 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 $\delta = 25.6$ to 37.6 or by 12 ppm. Similarly, C-17 shifted from $\delta = 47.2$ to 57.1, and we determined that C-9 shifted from $\delta = 28.9$ to 35.5. Comparison of the ¹³C-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 ¹³C-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 ¹³C-NMR assignments, the NMR spectra of **7b** agree with previously published ones.^[24]

Table 1. ¹³C NMR (CDCl₃) of 3 α -hydroxy-7 α -acetoxy-12-oxo-5 β -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 ₂ H ₅ CO (eq.)	170.19	169.94	—	—
C ₂ H 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 3 α -functional groups and the 17-sidechain. For all the intermediates, the 17-sidechain can be lost before or after the 3 α -functional group is lost; while the 3 α -OH is always lost after 7 α -OH, the 3 α -carbethoxy can be lost before or after the 7 α -OH

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

Assignment	4b	7a	7b	7c
C-18	11.71	11.39	11.51	11.68
$\text{CH}_3\text{CH}_2\text{O}$	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	—	—	—
$\text{CH}_3\text{CH}_2\text{O}$	63.62	—	—	—
C-3	76.66	74.35	73.36	73.36
EtOCO	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 3 α -hydroxy-7,12-dioxo-5 β -cholan-24-oic acid derivatives

Assignment	4a	6a	6b	6c	6d
C-18	11.80	11.41	11.62	11.74	11.83
$\text{CH}_3\text{CH}_2\text{O}$	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	—	—	—	—
$\text{CH}_3\text{CH}_2\text{O}$	63.78	—	—	—	—
C-3	75.97	71.75	72.04	72.10	72.18
EtOCO	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 12 α -OH. Three kinds of FAB mass spectral peaks $[\text{MNa} + \text{Na}]^+$, $[\text{MNa}]^+$, $[\text{M} + \text{H}]^+$ 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 7 α ,12 α -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-7 α ,12 α -dihydroxy-5 β -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-H₃); 0.88 (s, 3 H, 19-H₃); 0.99 (d, 3 H, 21-H₃); 3.43 (broad, 1 H, 3 β -H); 3.66 (s, 3 H, COOCH_3); 3.82 (s, 1 H, 7 β -H); 3.94 (s, 1 H, 12 β -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-H₃); 0.90 (s, 3 H, 19-H₃); 0.97 (d, 3 H, 21-H₃); 3.66 (s, 3 H, COOCH_3); 3.85 (s, 1 H, 7 β -H); 3.98 (s, 1 H, 12 β -H); 4.14 (q, 2 H, 3-EtOCO); 4.41 (broad, 1 H, 3 β -H). — MS (EI): 494.3 $[\text{M}]^+$ (2), 476.3 $[\text{M} - \text{H}_2\text{O}]^+$ (3), 458.3 $[\text{M} - 2\text{H}_2\text{O}]^+$, 404.3 $[\text{M} - \text{C}_3\text{H}_6\text{O}_3]^+$ (5), 386.2 $[\text{M} - \text{H}_2\text{O} - \text{C}_3\text{H}_6\text{O}_3]^+$ (29), 368.2 $[\text{M} - 2\text{H}_2\text{O} - \text{C}_3\text{H}_6\text{O}_3]^+$ (42), 253 $[\text{M} - 2\text{H}_2\text{O} - \text{C}_9\text{H}_{17}\text{O}_5]^+$ (100).

Methyl 3 α ,12 α -Dihydroxy-5 β -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, 3 β -H); 3.66 (s, 3 H, COOCH_3); 3.98 (s, 1 H, 12 β -H).

Methyl 12 α -Hydroxy-3 α -carbethoxy-5 β -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-H₃); 0.90 (s, 3 H, 19-H₃); 0.98 (d, 3 H, 21-H₃); 3.66 (s, 3 H, COOCH_3); 3.99 (s, 1 H, 12 β -H); 4.16 (q, 2 H, 3-EtOCO); 4.43 (broad, 1 H, 3 β -H). — MS (EI): 478.3 $[\text{M}]^+$ (2), 460 $[\text{M} - \text{H}_2\text{O}]^+$ (5), 388 $[\text{M} - \text{C}_3\text{H}_5\text{O}_3]^+$ (5), 370 $[\text{M} - \text{H}_2\text{O} - \text{C}_3\text{H}_6\text{O}_3]^+$ (31), 255 $[\text{M} - \text{H}_2\text{O} - \text{C}_9\text{H}_{17}\text{O}_5]^+$ (100).

Methyl 3 α ,7 α -Diacetoxy-12 α -hydroxy-5 β -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-H₃); 0.92 (s, 3 H, 19-H₃); 0.97 (d, 3 H, 21-H₃); 2.02 (s, 3 H, 3-OAc); 2.03 (s, 3 H, 7-OAc); 2.3 (m, 2 H, 23-H₂); 3.66 (s, 3 H, COOCH_3); 3.99 (s, 1 H, 12 β -H); 4.58 (broad, 1 H, 3 β -H); 4.89 (s, 1 H, 7 β -H). — MS (EI): 506 $[\text{M}]^+$ (1), 446 $[\text{M} - \text{HOAc}]^+$ (3), 428 $[\text{M} - \text{HOAc} - \text{H}_2\text{O}]^+$ (11), 386 $[\text{M} - 2\text{HOAc}]^+$ (10), 368 $[\text{M} - 2\text{HOAc} - \text{H}_2\text{O}]^+$ (38), 253 $[\text{M} - 2\text{HOAc} - \text{H}_2\text{O} - \text{C}_6\text{H}_{11}\text{O}_2]^+$ (100).

Methyl 3 α -Carbethoxy-7,12-dioxo-5 β -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 NaHCO₃ and NaCl solutions, dried with Na₂SO₄, and concentrated under reduced pressure to afford **4a** (20 g, 80.6%): m.p. 135–137°C (ref.^[10] m.p. 137–138°C). – ¹H NMR (CDCl₃): 0.83 (d, 3 H, 21-H₃); 1.03 (s, 6 H, 18-H₃; 19-H₃); 2.36 (m, 2 H, 11-H₂); 2.71 (dd, 2 H, 6-H₂); 2.90 (m, 1 H, 8-H); 3.66 (s, 3 H, COOCH₃); 4.14 (q, 2 H, 3-EtOCO); 4.53 (broad, 1 H, 3 β -H). – MS (EI): 490.3 [M]⁺ (19), 472.3 [M – H₂O]⁺ (19), 400.3 [M – C₃H₆O₃]⁺ (15), 359.3 (25), 269.2 (78), 245 (100).

Methyl 3 α -Carbethoxy-12-oxo-5 β -cholan-24-oate (4b) was prepared in similar fashion, m.p. 152–154°C (ref.^[10] m.p. 157–158°C) (85%). – ¹H NMR (CDCl₃): 0.84 (d, 3 H, 21-H₃); 1.02 (s, 6 H, 18-H₃; 19-H₃); 2.40 (m, 2 H, 11-H₂); 3.66 (s, 3 H, COOCH₃); 4.16 (q, 2 H, 3-EtOCO); 4.56 (broad, 1 H, 3 β -H). – MS (EI): 476.3 [M]⁺ (33), 458.3 [M – H₂O]⁺ (3), 386.3 [M – C₃H₅O₃]⁺ (69), 231.2 (100).

Methyl 3 α ,7 α -Diacetoxy-12-oxo-5 β -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). – ¹H NMR (CDCl₃): 0.85 (d, 3 H, 21-H₃); 1.03 (s, 6 H, 18-H₃; 19-H₃); 2.02 (s, 3 H, 3-OAc); 2.03 (s, 3 H, 7-OAc); 2.32 (m, 2 H, 23-H₂); 2.5 (dd, 2 H, 11-H₂); 3.66 (s, 3 H, COOCH₃); 4.58 (broad, 1 H, 3 β -H); 4.99 (s, 1 H, 7 β -H). – MS (EI): 504 [M]⁺ (4), 444 [M – HOAc]⁺ (56), 384 [M – 2 HOAc]⁺ (22), 269 [M – 2 HOAc – C₆H₁₁O₂]⁺ (76), 229 (100).

3 α -Hydroxy-7,12-dioxo-5 β -cholan-24-oic Acid (5a): To a solution of **4a** (10 g, 20.4 mmol) in CH₃OH (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 Na₂SO₄ 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). – ¹H NMR (CDCl₃): 0.85 (d, 3 H, 21-H₃); 1.03 (s, 6 H, 18-H₃; 19-H₃); 2.35 (m, 2 H, 11-H₂); 2.75 (dd, 2 H, 6-H₂); 2.88 (m, 1 H, 8-H); 3.61 (m, 1 H, 3 β -H). – FAB/MS (3-NBA): 405.2 [M + 1]⁺, 387.2 [M + 1 – H₂O]⁺, 369.2 [M + 1 – 2 H₂O]⁺, 353.2, 307.

3 α -Hydroxy-12-oxo-5 β -cholan-24-oic Acid (5b): was prepared in similar fashion, m.p. 163–165°C (94%). – ¹H NMR (CDCl₃): 0.91 (d, 3 H, 21-H₃); 1.07 (s, 6 H, 18-H₃; 19-H₃); 2.48 (m, 2 H, 11-H₂); 3.69 (s, 1 H, 3 β -H). – MS (EI): 390.3 [M]⁺ (100), 372.3 [M – H₂O]⁺ (39.10), 354.3 [M – 2 H₂O]⁺ (3), 289.3 [M – C₅H₉O₂]⁺ (12.65), 249.2 (43.23), 231.2 (70.45).

3 α -Hydroxy-7 α -acetoxy-12-oxo-5 β -cholan-24-oic Acid (5c): The acid **5c** was prepared by saponification of **4c** with satd Na₂CO₃^[12]. Yield: 95%, m.p. 240–242°C. – ¹H NMR (CDCl₃): 0.85 (d, 3 H, 21-H₃); 1.03 (s, 6 H, 18-H₃; 19-H₃); 2.03 (s, 3 H, 7-OAc); 2.3 (m, 2 H, 23-H₂); 2.49 (dd, 2 H, 11-H₂); 3.48 (broad, 1 H, 3 β -H); 4.7 (hump, OHs); 4.98 (s, 1 H, 7 β -H). – MS (EI): 448 [M]⁺ (6), 388 [M – HOAc]⁺ (9), 370 [M – HOAc – H₂O]⁺ (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%. – ¹H NMR (CDCl₃): 0.87 (d, 6 H, 21-H₃, 21'-H₃); 1.01 (s, 12 H, 18-H₃, 18'-H₃; 19-H₃, 19'-H₃); 2.33 (m, 4 H, 11-H₂, 11'-H₂); 2.74 (dd, 4 H, 6-H₂, 6'-H₂); 2.88 (m, 2 H, 8-H, 8'-H); 4.80 (m, 2 H, 3 β -H, 3' β -H). – FAB/MS (3-NBA): 773.3 [M + 1]⁺, 755.4 [M + 1 – H₂O]⁺, 739.3, 723.4.

Cyclotrimer 6b: m.p. 320–322°C, yield: 38%. ¹H NMR (CDCl₃): 0.84 (d, 9 H, 21-H₃, 21'-H₃, 21''-H₃); 1.03 (s, 18 H, 18-H₃, 18'-H₃, 18''-H₃; 19-H₃, 19'-H₃, 19''-H₃); 2.30 (m, 6 H, 11-H₂, 11'-H₂, 11''-H₂); 2.75 (dd, 6 H, 6-H₂, 6'-H₂, 6''-H₂); 2.84 (m, 3 H, 8-H, 8'-H, 8''-H); 4.70 (m, 3 H, 3 β -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%. ¹H NMR (CDCl₃): 0.84 (d, 12 H, 21-H₃, 21'-H₃, 21''-H₃, 21'''-H₃); 1.03 (s, 24 H, 18-H₃, 18'-H₃, 18''-H₃, 18'''-H₃; 19-H₃, 19'-H₃, 19''-H₃, 19'''-H₃); 2.28 (m, 8 H, 11-H₂, 11'-H₂, 11''-H₂, 11'''-H₂); 2.75 (dd, 8 H, 6-H₂, 6'-H₂, 6''-H₂, 6'''-H₂); 2.85 (m, 4 H, 8-H, 8'-H, 8''-H, 8'''-H); 4.70 (m, 4 H, 3 β -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%. – ¹H NMR (CDCl₃): 0.84 (d, 15 H, 21-H₃, 21'-H₃, 21''-H₃, 21'''-H₃, 21''''-H₃); 1.03 (s, 30 H, 18-H₃, 18'-H₃, 18''-H₃, 18'''-H₃, 18''''-H₃; 19-H₃, 19'-H₃, 19''-H₃, 19'''-H₃, 19''''-H₃); 2.28 (m, 10 H, 11-H₂, 11'-H₂, 11''-H₂, 11'''-H₂, 11''''-H₂); 2.75 (dd, 10 H, 6-H₂, 6'-H₂, 6''-H₂, 6'''-H₂, 6''''-H₂); 2.85 (m, 5 H, 8-H, 8'-H, 8''-H, 8'''-H, 8''''-H); 4.70 (m, 5 H, 3 β -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%. – ¹H NMR (CDCl₃): 0.93 (d, 6 H, 21-H₃, 21'-H₃); 1.05 (s, 12 H, 18-H₃, 18'-H₃; 19-H₃, 19'-H₃); 2.49 (m, 4 H, 11-H₂, 11'-H₂); 4.72 (m, 2 H, 3 β -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%. – ¹H NMR (CDCl₃): 0.92 (d, 9 H, 21-H₃, 21'-H₃, 21''-H₃); 1.08 (s, 18 H, 18-H₃, 18'-H₃, 18''-H₃; 19-H₃, 19'-H₃, 19''-H₃); 2.49 (m, 6 H, 11-H₂, 11'-H₂, 11''-H₂); 4.78 (m, 3 H, 3 β -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%. – ¹H NMR (CDCl₃): 0.92 (d, 12 H, 21-H₃, 21'-H₃, 21''-H₃, 21'''-H₃); 1.08 (s, 24 H, 18-H₃, 18'-H₃, 18''-H₃, 18'''-H₃; 19-H₃, 19'-H₃, 19''-H₃, 19'''-H₃); 2.49 (m, 8 H, 11-H₂, 11'-H₂, 11''-H₂, 11'''-H₂); 4.77 (m, 4 H, 3 β -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%. – ¹H NMR (CDCl₃): 0.81 (d, 6 H, 21-H₃, 21'-H₃); 0.97 (s, 12 H, 18-H₃, 18'-H₃; 19-H₃, 19'-H₃); 2.10 (s, 6 H, 7-OAc, 7'-OAc); 2.24 (m, 4 H, 23-H₂, 23'-H₂); 2.4 (dd, 4 H, 11-H₂, 11'-H₂); 4.60 (m, 2 H, 3 β -H, 3' β -H); 5.03 (s, 2 H, 7 β -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. – C₅₂H₇₆O₁₀ (860): Calcd: C 72.56, H 8.84. Found: C 72.26, H 9.36.

Cyclotrimer 8b: m.p. 310–312°C, yield: 35%. – ¹H NMR (CDCl₃): 0.86 (d, 9 H, 21-H₃, 21'-H₃, 21''-H₃); 1.03 (s, 18 H, 18-H₃, 18'-H₃, 18''-H₃; 19-H₃, 19'-H₃, 19''-H₃); 1.98 (s, 9 H, 7-OAc, 7'-OAc, 7''-OAc); 2.34 (m, 6 H, 23-H₂, 23'-H₂, 23''-H₂); 2.5 (dd, 6 H, 11-H₂, 11'-H₂, 11''-H₂); 4.60 (m, 3 H, 3 β -H, 3' β -H, 3'' β -H); 4.97 (broad s, 3 H, 7 β -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 – H₂O]⁺. – C₇₈H₁₁₄O₁₅ (1290): Calcd: C 72.56, H 8.84. Found: C 72.59, H 9.01.

Methyl 3 α ,7 α -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). – ¹H NMR (CDCl₃): 0.62 (d, 3 H, 21-H₃); 0.80 (s, 3 H, 18-H₃); 0.95 (s, 3 H, 19-H₃); 2.03 (s, 3 H, 3-OAc); 2.04 (s, 3 H, 7-OAc); 2.45 (s, 3 H, Ar-CH₃); 3.67 (s, 3 H, COOCH₃); 4.53 (broad, 1 H, 3 β -H); 4.90 (s, 1 H, 7 β -H); 6.0 (broad, 1 H, -SO₂NHN=); 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 Na₂CO₃ soln., NaCl soln., dried with Na₂SO₄, and evaporated to afford a residue from which **10** (0.68 g, 68%) was obtained by chromatography (*n*-hexane/ethyl acetate, 1:10): m.p. 125–127°C. – ¹H NMR (CDCl₃): 0.96 (d, 6 H, 21-H₃, 21'-H₃); 0.97 (s, 6 H, 18-H₃, 18'-H₃); 1.04 (s, 6 H, 19-H₃, 19'-H₃); 2.00 (s, 6 H, 3-OAc, 3'-OAc); 2.04 (s, 6 H, 7-OAc, 7'-OAc); 3.66 (s, 6 H, COOCH₃, COOCH₃); 4.62 (m, 2 H, 3 β -H, 3' β -H); 4.99 (broad s, 2 H, 7 β -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]⁺. – C₅₈H₈₈N₂O₁₂ (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 CH₃OH (20 ml) was added NaBH₄ (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 CHCl₃ 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. – ¹H NMR (CDCl₃): 0.70 (s, 9 H, 18-H₃, 18'-H₃, 18''-H₃); 0.85 (s, 9 H, 19-H₃, 19'-H₃, 19''-H₃); 0.90 (d, 9 H, 21-H₃, 21'-H₃, 21''-H₃);

3.85 (s, 3 H, 7 β -H, 7' β -H, 7'' β -H); 3.96 (s, 3 H, 12 β -H, 12' β -H, 12'' β -H); 4.70 (m, 3 H, 3 β -H, 3' β -H, 3'' β -H). – ¹³C NMR (CDCl₃): 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 + Na]⁺, 1193.9 [MNa]⁺, 326.0, 176.1.

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