Synthesis of 3α-chlorinated methyl lithocholate derivatives

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Methyl lithocholate (**1**), a bile acid derivative which contains a rigid steroid part as well as a flexible side chain, has been used as the starting material for the convenient synthesis of 3α-chlorinated methyl lithocholate, 3α-chloro-5β-cholan-24-oic acid methyl ester (**2**), and three other chlorinated derivatives, 3α-chloro-5β-cholan-24-ol (**3**), 3α-chloro-5β-cholan-24-yl tosylate (**4**) and ethyl (3α-chloro-5β-cholan)-24-yl oxalate (**5**). The identity of all these synthesised steroidal molecules (**2**–**5**) has been determined by spectroscopic analyses.

Keywords: steroid, methyl lithocholate, oxalyl chloride, ethyl oxalate, NMR

Bile acids are naturally occurring amphiphilic compounds. Methyl lithocholate (**1**), a bile acid derivative, is a structurally well-known compound containing a rigid steroid part as well as a flexible side chain with an ester functional group at the end of the chain and can easily be modified to its derivatives. As part of our on-going studies on the synthesis of various new C-24 steroidal derivatives,[1,2] we now report on the convenient synthesis and spectroscopic analyses of four new 3α-chlorinated methyl lithocholate derivatives, 3α-chloro-5β-cholan-24-oic acid methyl ester (**2**), 3α-chloro-5β-cholan-24-ol (**3**), 3α-chloro-5β-cholan-24-yl tosylate (**4**) and ethyl (3α-chloro-5β-cholan)-24-yl oxalate (**5**).

Alcohols react with a variety of reagents to yield alkyl halides. The most commonly used reagents are hydrogen halides, PBr_3, $SOCl_2$ and $POCl_3$. All of these reactions result in cleavage at the C–O bond of the alcohol. Secondary and tertiary alcohols can react by a mechanism that involves the formation of a carbocation, which then reacts with a nucleophile in an S_N1 reaction to give alkyl halides. Thus, the synthesis of 3α-chloro-5β-cholan-24-oic acid methyl ester (**2**) from methyl lithocholate (**1**) in pyridine using $POCl_3$ resulted in the retention of stereochemistry.

Four new 3α-chlorinated methyl lithocholate derivatives (**2**–**5**) were synthesised starting from methyl lithocholate (**1**) (Scheme 1). Since the 3α-hydroxy in **1** is equatorial, it reacted readily with $POCl_3$ in presence of pyridine to give 3α-chloro ester **2**. In the ^{13}C NMR (Table 1) the chemical shift for C-3 (δ 61.6) was in the range for an α orientation of the chlorine at C-3.[3]

The relative stereochemistry at C-3 was further confirmed from a ^1H–^1H NOESY experiment (Fig. 1). The ESIMS spectrum of **2** displayed the $[M + H]^+$ ion at *m/z* 409 (~75%) and 411 (~25%) due to the presence of two isotopes of chlorine, and confirmed the molecular formula $C_{25}H_{41}ClO_2$ for this compound.

Esters can easily be reduced to primary alcohols by treatment with a very reactive reducing agent lithum aluminium hydride.

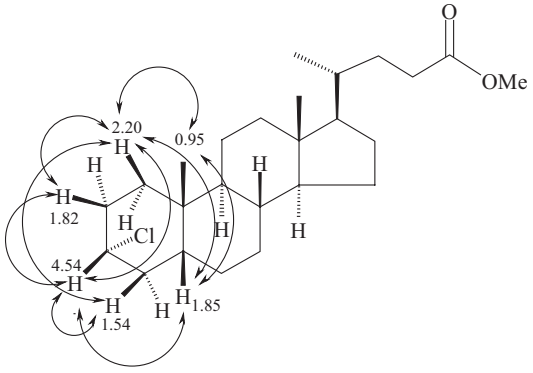
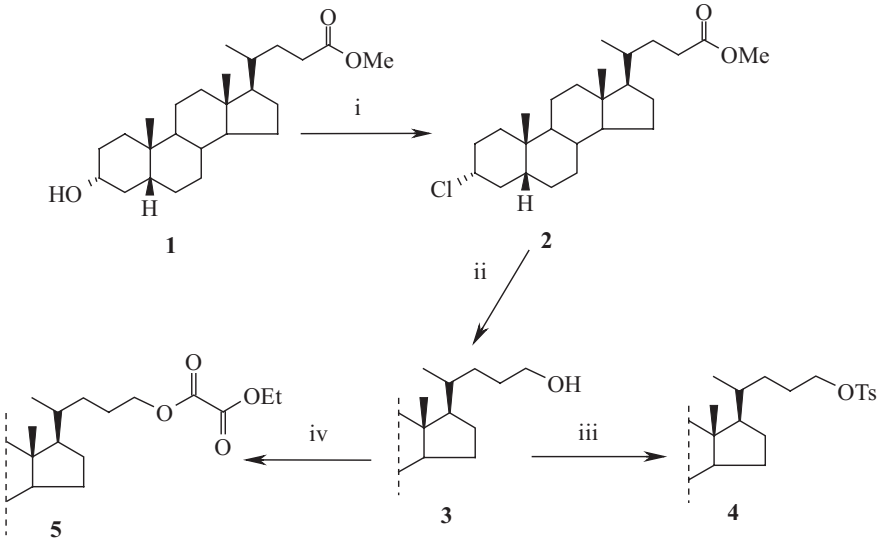


Fig. 1 Key nOe correlations observed in the ^1H–^1H NOESY spectrum of **2**.



Scheme 1 (i) $POCl_3$, dry pyridine, r.t., 18 h; (ii) LAH, THF, r.t., 18 h; (iii) *p*-TsCl, dry pyridine, 4°C, 2 h (iv) $(COCl)_2$, DCM, r.t., 18 h, then EtOH was added and evaporated at 40°C, 1 h.

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Table 1 ^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **3**–**5**

Carbon no.	Chemical shifts (δ) in ppm			
	2	**3**	**4**	**5**
1	34.5	34.5	34.5	34.5
2	28.9	28.9	28.9	28.9
3	61.6	61.6	61.6	61.6
4	30.0	30.0	30.3	30.0
5	36.6	36.6	36.8	36.6
6	26.5	26.5	27.2	26.5
7	26.4	26.4	26.4	26.4
8	35.7	35.7	35.9	35.7
9	40.4	40.4	40.4	40.4
10	35.2	35.2	35.2	35.2
11	21.0	21.0	20.8	21.0
12	40.2	40.2	40.2	40.2
13	42.7	42.7	42.7	42.7
14	56.6	56.6	56.6	56.6
15	24.2	24.2	24.2	24.2
16	28.2	28.3	28.2	28.2
17	56.0	56.2	56.1	56.0
18	12.0	12.1	12.1	12.1
19	23.7	23.7	23.7	23.7
20	35.4	35.6	35.4	35.3
21	18.3	18.6	18.5	18.5
22	31.1	31.8	31.4	24.9
23	31.0	29.4	25.6	31.7
24–OCH_2	–	63.6	71.2	63.1
24–CO–Me	174.7	–	–	–
24–OMe	51.5	–	–	–
24–OTs				
1–	–	144.6	–	
2, 6	–	–	129.8	
–				
3, 5	–	–	127.9	–
4–	–	133.3	–	
4–Me	–	–	21.6	–
24–OCH_2**C**H_3	–	–	–	14.0
24–O**C**H_2CH_3	–	–	–	67.7
24–**C**O–CO–OEt	–	–	–	157.9
24–CO–**C**O–OEt	–	–	–	158.0

The 3α-chloro ester **2** was reduced to 3α-chloro alcohol **3** by excess LAH in dry THF (Scheme 1). In the ^1H NMR and ^{13}C NMR spectra (Table 1) of **3**, instead of the signals for the carbomethoxy group, a signal for the C-24 oxymethylene (δ_H 3.57 and δ_C 63.6) was observed.

Tosylation of primary alcohol is rapid and takes only a few hours at 4°C. 3α-Chloro-24-tosylate **4** (Scheme 1) was obtained from the 3α-chloro-alcohol **3** in 2 h. The presence of the tosyl group was confirmed from ^1H and ^{13}C NMR analyses. In its ^1H NMR spectrum, in addition to the signals associated with the protons of the parent steroid nucleus (**3**), signals at δ 7.75 (2H, J = 8.2 Hz) and δ 7.30 (2H, J = 8.2 Hz) for the *p*-di-substituted benzene ring system and at δ 2.41 for the methyl group of the tosyl moiety were observed. The downfield shift (δ 3.97) of the resonance for the C-24 methylene protons of **4** (compared to that of **3** at δ 3.57) confirmed the attachment of the tosyl unit at C-24. In its ^{13}C NMR spectrum (Table 1), the C-24 carbon resonance was observed at a further deshielded position (δ 71.2) compared to that of 3 (δ 63.6).

Ethyl (3α-chloro-5β-cholan)-24-yl oxalate (**5**) was synthesised from alcohol **3** using excess oxalyl chloride (Scheme 1) in DCM. The reaction mixture was quenched with EtOH and the solvent evaporated at 40°C. The ^1H and ^{13}C NMR (Table 1) of **5**, in addition to the signals assignable to the starting material, exhibited signals for an ethyl oxalate moiety. The deshielded ^1H and ^{13}C NMR chemical shifts (compared to the starting material **3**) of C-24 oxymethylene (δ_H 4.23 and δ_C 67.7) confirmed the attachment of the ethyl oxalate group at C-24.

Experimental

The steroid starting material (methyl lithocholate, **1**) was synthesised and identified previously in our lab.[4] Oxalyl chloride $(COCl)_2$, phosphorous oxychloride ($POCl_3$), *p*-TsCl and LAH were purchased from Aldrich. All chemicals and solvents were used throughout without further purification. The reactions were monitored and the purity of the products was assessed by thin-layer chromatography (TLC) performed on silica gel (Merck type 60) and visualised under UV illumination and/or by I_2 vapour. Melting points of the products were determined on a Gallenkamp melting point apparatus. IR spectra (wavenumbers in cm^{-1}) were recorded on an ATI Mattson Genesis FTIR spectrophotometer as KBr pellets. NMR spectra were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) using residual solvent peak as internal standard. Chemical shifts (δ) are reported in ppm downfield from TMS, using the middle resonance of $CDCl_3$ (7.25 ppm for ^1H and 77.23 ppm for ^{13}C) as an internal standard and coupling constants (J) in Hz. MS analyses were performed on a Finnigan MAT95 spectrometer and high-resolution mass spectroscopic analyses were performed at the EPSRC Mass Spectrometry Service at Swansea.

3α-Chloro-5β-cholan-24-oic acid methyl ester **(2)**: A solution of methyl lithocholate (**1**, 100 mg, 0.26 mmol) in dry pyridine (3 ml) was treated with phosphorous oxychloride (0.2 ml) and allowed to stand at r.t. for 18 h (Scheme 1). The reaction mixture was quenched with ice and water and the mixture was extracted with DCM (3 × 15 ml), the organic extracts were separated and dried ($MgSO_4$) to yield a yellow gummy product. The crude product was purified by PTLC (step gradient elution 10% EtOAc in pet-ether, 15% EtOAc in pet-ether). The title compound **2** was obtained as a white solid (69 mg, 65%), m.p. 84–85°C. IR ($CHCl_3$): ν_{max} cm^{-1} 2949vs (C–H), 2867 s (C–H), 1739vs (methyl ester C=O), 1445 s, 1379 m, 1278 m, 1253 m, 1168 m (ester C–O), 1016w, 859w, 712 m (C–Cl) and 616 m. ^1H NMR (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, 18-Me), 0.87 (d, J = 6.5 Hz, 3H, 21-Me), 0.95 (s, 3H, 19-Me), 3.62 (s, 3H, 24-OMe), 4.54 (m, 1H, 3β-CHCl) and ^{13}C NMR (100 MHz, $CDCl_3$). Table 1.

ESIMS: *m/z* 409/411 $[M + H]^+$. HR-ESIMS: Found: $[M + H]^+$, 409.2875/411.2846. $C_{25}H_{41}ClO_2+H^+$ requires 409.2873/411.2844.

3α-Chloro-5β-cholan-24-ol **(3)**: Reduction of **2** (60 mg, 0.15 mmol) was performed using LAH (23 mg, 4 molar equivalent) in dry THF (15 ml) for 18 h at r.t. The crude product was purified by PTLC (step gradient elution 10% EtOAc in pet-ether, 15% EtOAc in pet-ether, 18% EtOAc in pet-ether). The title compound **3** was obtained as a slowly solidifying gummy product (42 mg, 74%), m.p. 109–110°C. IR ($CHCl_3$): ν_{max} cm^{-1} 3340br (O–H), 2934 s (C–H), 2863 s (C–H), 1445 m, 1377 m, 1279 m, 1056 m (alcoholic C–O), 758 m, 712 m (C–Cl) and 667 m. ^1H NMR (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, 18-Me), 0.88 (d, J = 6.8 Hz, 3H, 21-Me), 0.95 (s, 3H, 19-Me), 3.57 (m, 2H, 24-OCH_2), 4.54 (m, 1H, 3β-CHCl) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. ESIMS: *m/z* 381/383 $[M + H]^+$. HR-ESIMS: Found: $[M + H]^+$, 381.2927/383.2898. $C_{24}H_{41}ClO+H^+$requires 381.2924/383.2895.

3α-Chloro-5β-cholan-24-yl tosylate **(4)**: Tosylation of **3** (40 mg, 0.11 mmol) was carried out using *p*-TsCl (0.13 mmol, 1.2 molar equivalent) in dry pyridine (2 ml) at 4°C for 2 h. The crude oil was purified by PTLC (step gradient elution 10% EtOAc in pet-ether, 15% EtOAc in pet-ether, 20% EtOAc in pet-ether). The title compound **4** was obtained as a thick oil (28 mg, 48%). IR ($CHCl_3$): ν_{max} cm^{-1} 3031w (aromatic C–H), 2932vs (C–H), 2862 (C–H), 1599 m (Ph C =C), 1450 m, 1360 m, 1176vs (O–SO_2), 1099 m, 927 m, 867 m, 814 m, 755 m, 714 s (C–Cl) and 667 m. ^1H NMR (400 MHz, $CDCl_3$): δ 0.58 (s, 3H, 18-Me), 0.85 (s, 3H, 19-Me), 0.87 (d, J = 6.2 Hz, 3H, 21-Me), 4.54 (m, 1H, 3β-CHCl), 3.97 (m, 2H, 24-OCH_2), 24-OTs: 7.75 (d, J = 8.2 Hz, 2H, 2 × Ph-**H**), 7.30 (d, J = 8.2 Hz, 2H, 2 × Ph-**H**), 2.41 (s, 3H, Ph-**Me**) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. ESIMS: *m/z* 535/537 $[M + H]^+$, 557/559 $[M + Na]^+$. HR-ESIMS: Found: $[M + H]^+$, 535.30127/537.2983. $C_{31}H_{47}ClO_3S+H^+$ requires 535.30125/537.29835.

Ethyl (3α-chloro-5β-cholan-24-yl) oxalate **(5)**: A stirred solution of alcohol **3** (50 mg, 0.13 mmol) in DCM (3 ml) was treated dropwise with oxalyl chloride (85 mg, 4 molar equiv.) at r.t. After 18 h, the resulting mixture was quenched cautiously with EtOH (5 ml) and rotary evaporated for 1 h at 40°C to dryness. The crude oily substance was purified by PTLC (step gradient elution 10% EtOAc in pet-ether, 20% EtOAc in pet-ether, 30% EtOAc in pet-ether). The title compound **5** was obtained as an oil (43 mg, 69%). IR ($CHCl_3$): ν_{max} cm^{-1} 2946 s (C–H), 2923 s (C–H), 2863 s (C–H), 1765 s (oxalate C=O), 1743 s (ethyl and oxalate C=O), 1446 m, 1377 m, 1308 m, 1184 s (ester C-O), 1016 m, 983 m, 860 m, 758 m and 711 m. ^1H NMR (400 MHz): δ 0.62 (s, 3H, 18-Me), 0.90 (s, J = 6.8 Hz, 3H, 21-Me), 0.96 (s, 3H, 19-Me), 1.36 (t, J = 7.2 Hz, 3H, -OCH_2CH_3), 4.23 (br s, 2H, 24-CH_2O), 4.31 (q, J = 7.2 Hz, 2H, -OCH_2CH_3), 4.86 (m, 1H, 3β-CH-O); ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. FABMS *m/z*: 481/483 $[M + H]^+$, 503/505 $[M + Na]^+$. HR-FABMS: Found: $[M + NH_4]^+$, 498.3352/500.3323. $C_{28}H_{45}ClO_4+NH_4^+$requires 498.3350/500.3321.

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