# Synthesis and spectroscopic characterisation lithocholic acid derivatives

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The synthesis of five lithocholic acid derivatives, 5β-cholane-3α,24-diol (**2**), 3α-hydroxy-5β-cholan-24-yl tosylate (**3**), 5β-cholan-3α,24-yl ditosylate (**4**), 3α-tosyloxy-5β-cholan-24-yl chloride (**5**) and 3-oxo-5β-cholan-24-al (**6**) have been described.

**Keywords:** lithocholic acid, oxidation, reduction, tosylation, NMR

Lithocholic acid (**1**), is a secondary bile acid, with one functional group at each end of the molecule, which can be modified to useful derivatives.[1] Recently, we reported several lithocholic acid derivatives including monomers and dimers[1-4] and now we report the synthesis of five lithocholic acid derivatives, 5β-cholane-3α,24-diol (**2**), 3α-hydroxy-5β-cholan-24-yl tosylate (**3**), 5β-cholan-3α,24-yl ditosylate (**4**), 3α-tosyloxy-5β-cholan-24-yl chloride (**5**) and 3-oxo-5β-cholan-24-al (**6**) together with their full spectroscopic data.

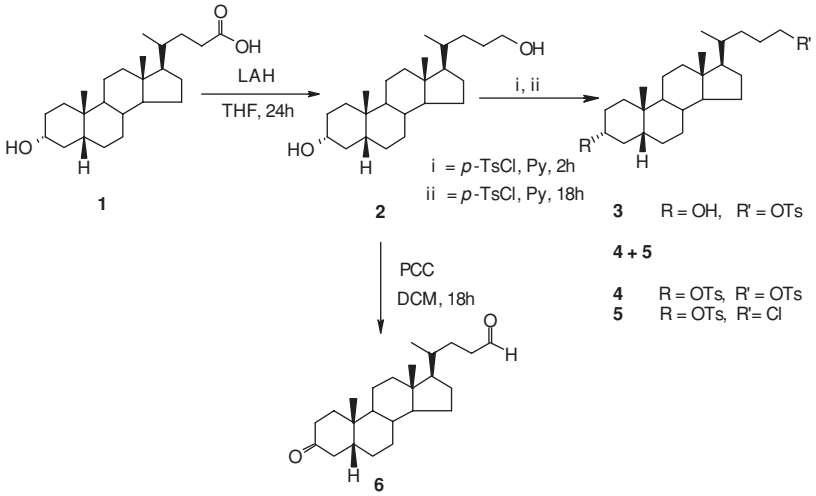
Lithocholic acid (**1**) is a natural hydroxy acid. Reduction of **1** with excess LAH produced 3α,24-diol **2** (Scheme 1) which, in its FABMS spectrum, showed $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 363 and 385. Its IR spectrum, had a broad OH absorption band at 3315 $cm^{-1}$ and no absorption band for a C=O functionality. The $^1H$ and $^{13}C$ NMR spectra (Table 1) of **2** were similar to those of **1** with the exception that ($\delta_H$ 3.60, $\delta_C$ 63.7) replaced the alcohol functionality, instead of a carboxylic acid group at C-24. Previously **2** was identified only by its m.p.[5] and a partial $^1H$ NMR[6] data. The structure was fully confirmed by the IR and $^{13}C$ NMR data analyses.

The hydroxyl groups were converted to their toluene-*p*-sulfonates which are excellent leaving groups, that can be displaced by nucleophilic substitution. The tosylates (**3**–**5**) were synthesised from diol **2**. 3α-Hydroxy-24-tosylate **3** was synthesised selectively from **2** (Scheme 1).

The ESIMS spectrum of **3** revealed the $[M+Na]^+$ ion at *m/z* 539 confirming the tosylation of **2**. In its $^1H$ NMR spectrum, the downfield shift (δ 3.97) of the resonance for the C-24 methylene protons of **3** (compared to that of **2** at δ 3.60) was due to the presence of the tosyl unit at C-24. The $^{13}C$ NMR spectrum (Table 1), the revealed deshielding of the C-24 signal (δ 71.2 as opposed to δ 63.7 for **2**). Previously this compound was also identified only on the basis of its m.p.[7] and partial $^1H$ NMR[8] data.

The diol **2** was treated with excess *p*-TsCl in dry pyridine for 18h at r.t. (Scheme 1) to obtain a mixture of **4** and **5** which were separated by PTLC (10% EtOAc in pet-ether). The FABMS spectrum of **4** exhibited the $[M+H]^+$ ion at *m/z* 671which was 308 mass unit more than that of **2**. This extra mass unit could be accounted for the presence of two tosyl units in **4**. In the $^1H$ NMR spectrum of **4**, in addition to the signals associated with the protons of the starting material **2**, signals for two *p*-di-substituted benzene ring systems [δ 7.75 (4H, *J* = 8.2 Hz), δ 7.29 (2H, *J* = 8.2 Hz), δ 7.28 (2H, *J* = 8.2 Hz)], and two deshielded methyls (δ 2.41 and 2.40) were observed confirming the presence two tosyl moieties in **4**. The downfield shift of the resonances for the oxymethine (C-3) and oxymethylene (C-24), respectively, at δ 4.41 and 3.96 for **4** (compared to that of **2** at δ 3.60 for both signals) established the attachment of the tosyl units at C-3 and C-24. In its $^{13}C$ NMR spectrum (Table 1), the C-3 and C-24 carbon signals were at further deshielded, at δ 83.2 and 71.2, as opposed to δ 71.9 and 63.7 for **2**. The HRFABMS spectrum revealed the $[M+H]^+$ ion at *m/z* 671.343985 which confirmed the molecular formula $C_{38}H_{54}O_6S_2$ for **4**.

The ESIMS spectrum of **5** displayed the $[M+Na]^+$ ion at *m/z* 557 (~75%) and 559 (~25%) for chlorine-35 and chlorine-37. In its $^1H$ NMR spectrum, in addition to the signals associated with the protons of **2**, the signals for a tosyl unit was present.




**Scheme 1** Synthesis of lithocholic acid derivatives **2–6**.

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**Table 1** $^{13}$C NMR data of compounds **2–6**

| Carbon no. | Chemical shifts (δ) in ppm | | | | |
|---|---|---|---|---|---|
| | **2** | **3** | **4** | **5** | **6** |
| 1 | 35.6 | 35.3 | 35.0 | 35.1 | 37.1 |
| 2 | 30.6 | 30.6 | 26.2 | 26.2 | 37.0 |
| 3 | 71.9 | 71.9 | 83.2 | 83.3 | 213.4 |
| 4 | 36.5 | 36.5 | 33.1 | 33.3 | 42.3 |
| 5 | 42.1 | 42.1 | 42.1 | 42.2 | 44.3 |
| 6 | 26.5 | 26.4 | 27.5 | 27.6 | 25.8 |
| 7 | 27.2 | 27.2 | 26.8 | 26.9 | 26.6 |
| 8 | 35.9 | 35.8 | 35.7 | 35.8 | 35.3 |
| 9 | 40.5 | 40.4 | 40.3 | 40.4 | 40.7 |
| 10 | 34.6 | 34.6 | 34.3 | 34.4 | 34.9 |
| 11 | 20.9 | 20.8 | 20.7 | 20.8 | 21.2 |
| 12 | 40.2 | 40.2 | 40.0 | 40.1 | 40.3 |
| 13 | 42.7 | 42.7 | 42.6 | 42.7 | 42.8 |
| 14 | 56.6 | 56.5 | 56.4 | 56.5 | 56.4 |
| 15 | 24.2 | 24.2 | 24.1 | 24.2 | 24.1 |
| 16 | 28.3 | 28.2 | 28.2 | 28.3 | 28.1 |
| 17 | 56.2 | 56.0 | 56.0 | 56.1 | 56.0 |
| 18 | 12.1 | 12.0 | 12.0 | 12.1 | 12.1 |
| 19 | 23.4 | 23.4 | 23.1 | 23.2 | 22.6 |
| 20 | 35.4 | 35.2 | 35.2 | 35.3 | 35.5 |
| 21 | 18.7 | 18.4 | 18.6 | 18.6 | 18.2 |
| 22 | 29.5 | 25.6 | 25.6 | 29.5 | 27.9 |
| 23 | 31.9 | 31.4 | 31.4 | 33.1 | 30.7 |
| 24 | 63.7 | 71.2 | 71.2 | 45.7 | 203.1 |
| *3-OTs* | | | | | |
| 1 - | - | 144.3 | 144.4 | - | |
| 2, 6 | - | - | 129.7 | 129.8 | - |
| 3, 5 | - | - | 127.6 | 127.6 | - |
| 4 - | - | 134.8 | 134.8 | - | |
| 4-Me | - | - | 21.6 | 21.7 | - |
| *24-OTs* | | | | | |
| 1 - | 144.6 | 144.6 | - | - | |
| 2, 6 | - | 129.8 | 129.9 | - | - |
| 3, 5 | - | 127.9 | 127.9 | - | - |
| 4 - | 133.3 | 133.3 | - | - | |
| 4-Me | - | 21.6 | 21.6 | - | - |

Spectra obtained in $CDCl_3$, 100 MHz

The downfield shift (δ 4.42) of the resonance for the C-3 methine proton of **5** (compared to that of **2** at δ 3.60) confirmed the attachment of the tosyl unit at C-3 and the upfield shift (δ 3.46) of the resonance for the C-24 methylene protons of **5** (compared to that of **2** at δ 3.60) confirmed the presence of the chlorine at C-24. In its $^{13}$C NMR spectrum (Table 1), the C-3 and C-24 carbon resonances were observed, at a further deshielded (δ 83.3) and shielded (δ 45.7) positions compared to those of **2** (δ 71.9 and 63.7). Previously compound **5** was identified only on the basis of its m.p. and IR data.[7] Therefore, the $^1$H and $^{13}$C NMR data for **5** are presented.

An oxidation of **2** using PCC in DCM yielded **6** (Scheme 1). The FABMS spectrum of **6** revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 359 and 381. In its $^{13}$C NMR spectrum (Table 1), among the signals for 24 carbons, the signals at δ 213.4 and 203.1 established, respectively, the presence of 3-oxo and C-24 aldehyde functionalities. The mp, IR and $^1$H NMR data were in good agreement with the published data.[9] The structure was confirmed further by $^{13}$C NMR data analysis. The $^{13}$C data are given in Table 1.

## Experimental

Lithocholic acid (**1**), LAH, PCC and *p*-TsCl were purchased from Aldrich and used as received. All chemicals and solvents were used throughout without further purification. The reactions were monitored and the purity of the products was assessed by TLC performed on silica gel (Merck type 60) and visualised under UV illumination and/or by $I_2$ vapour. Vacuum liquid chromatography (VLC) was performed on silica gel 60H. 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. Chemical shifts (δ) are reported in ppm downfield from TMS, using the middle resonance of $CDCl_3$ (7.25 ppm for $^1$H and 77.23 ppm for $^{13}$C) as an internal standard and coupling constants (*J*) in Hz. Mass spectroscopic analyses were performed at the EPSRC Mass Spectrometry Service at Swansea.

*Synthesis of 5β-cholane-3α,24-diol* (**2**): To a stirred solution of LAH (5.32 mmol, 4 equiv.) in dry THF (15 ml), a solution of lithocholic acid (**1**, 500 mg, 1.33 mmol) in dry THF (15 ml) was added dropwise under $N_2$. After 24h, the mixture was treated dropwise with a saturated $Na_2SO_4$ solution until a white precipitate formed. The solid was filtered off. The filtrate was concentrated, the residue was taken up in ether, washed with $H_2O$, dried ($MgSO_4$) and evaporated to dryness to obtain the title compound as a white amorphous solid (**2**, 452 mg, 94%), m.p. 129–130 °C (*lit.*[5] m.p. 128–130 °C). IR ($CHCl_3$): $\nu_{max}$ $cm^{-1}$ 3315br (O–H), 2926s (C–H), 2862s (C–H), 1467m, 1448m, 1376m, 1053s (alcoholic C–O), 1016m and 945w. $^1$H NMR (400 MHz, $CDCl_3$): δ 0.64 (s, 3H, 18–Me), 0.91 (s, 3H, 19–Me), 0.92 (d, *J* = 5.8 Hz, 3H, 21–Me), 3.60 (br m, 3H, 3β–OCH and 24–$OCH_2$) (*lit.*[6] $^1$H NMR: partial assignments); $^{13}$C NMR (Table 1). FABMS *m/z*: 363 $[M+H]^+$, 385 $[M+Na]^+$.

*Synthesis of 3α-hydroxy-5β-cholan-24-yl tosylate* (**3**): Tosylation of **2** (50 mg, 0.14 mmol) was carried out selectively using *p*-TsCl (0.17 mmol, 1.2 equiv.) in pyridine (5 ml) and stirred for 2h at 4 °C (ice-bath). The reaction mixture was poured into a cold $H_2O$. A white precipitate was formed which was separated by filtration and washed with $H_2O$. The residue was dissolved in ether and washed sequentially with dil HCl, $H_2O$, sat. $NaHCO_3$, separated, dried ($MgSO_4$) and reduced under pressure. The solid was purified by PTLC (10% EtOAc in pet-ether), and a colourless solid was obtained as the title compound (**3**, 31 mg, 43%), m.p. 120–121 °C (*lit.*[7] m.p. 119–120.6 °C). IR ($CHCl_3$): $\nu_{max}$ $cm^{-1}$ 3373br (O–H), 3032w (aromatic C–H), 2927s (C–H), 2864s (C–H), 1599m (Ph C=C), 1449m, 1362m, 1176s (O–$SO_2$), 1098w, 1043m, 919m, 814m and 755m. $^1$H NMR (400 MHz, $CDCl_3$): δ 0.57 (s, 3H, 18-Me), 0.81 (d, *J* = 6.8 Hz, 3H, 21-Me), 0.87 (s, 3H, 19-Me), 3.60 (m, 1H, 3β-OCH), 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**) (*lit.*[8] $^1$H NMR: partial assignments); $^{13}$C NMR (Table 1). ESIMS *m/z*: 539 $[M+Na]^+$.

*Synthesis of 5β-cholan-3α,24-yl ditosylate (4) and 3α-tosyloxy-5β-cholan-24-yl chloride* (**5**): Tosylation of **2** (100 mg, 0.28 mmol) was performed using 4 molar excess of *p*-TsCl for 18h at r.t. following the above method. After purification by PTLC (10% EtOAc in pet-ether) two compounds (**4** and **5**) were isolated.

*Compound* **4**: Colourless oil, 34 mg, 18%. IR ($CHCl_3$): $\nu_{max}$ $cm^{-1}$ 3029w (aromatic C–H), 2940vs (C–H), 2863s (C–H), 1599m (Ph C=C), 1495w, 1450m, 1360m, 1176vs (O-$SO_2$), 1098m, 925m, 814m and 755m. $^1$H NMR (400 MHz, $CDCl_3$): δ 0.54 (s, 3H, 18-Me), 0.80 (d, *J* = 6.5 Hz, 3H, 21-Me), 0.83 (s, 3H, 19-Me), 3.96 (m, 2H, 24-$OCH_2$), 4.41 (m, 1H, 3β-OCH), 3α-OTs: 7.75 (d, *J* = 8.2 Hz, 2H, 2 × Ph-**H**), 7.28 (d, *J* = 7.9 Hz, 2H, 2 × Ph-**H**), 2.40 (s, 3H, Ph–**Me**), 24-OTs: 7.75 (d, *J* = 8.2 Hz, 2H, 2 × Ph–**H**), 7.29 (d, *J* = 7.9 Hz, 2H, 2 × Ph–**H**), 2.41 (s, 3H, Ph–**Me**); $^{13}$C NMR (Table 1). FABMS *m/z*: 671 $[M+H]^+$. HRFABMS: Found: 671.343985; calc 671.343983 for $C_{38}H_{55}O_6S_2$.

*Compound* **5**: White solid, 68 mg, 45%, m.p. 109–112 °C (*lit.*[7] m.p. 109–117 °C and IR). $^1$H NMR (400 MHz, $CDCl_3$): δ 0.59 (s, 3H, 18-Me), 0.84 (s, 3H, 19-Me), 0.87 (d, *J* = 6.5 Hz, 3H, 21-Me), 3.46 (m, 2H, 24-$CH_2Cl$), 4.42 (m, 1H, 3β-OCH), 3α-OTs: 7.75 (d, *J* = 8.2 Hz, 2H, 2 × Ph–**H**), 7.28 (d, *J* = 7.9 Hz, 2H, 2 × Ph–**H**), 2.41 (s, 3H, Ph-**Me**); $^{13}$C NMR (Table 1). ESIMS *m/z*: 557/559 $[M+Na]^+$.

*Synthesis of 3-oxo-5β-cholan-24-al* (**6**): A solution of 2 (200 mg, 0.55 mmol) in dry DCM (25 ml) was treated with PCC (1.10 mmol, 2 molar equiv.), and the mixture was refluxed for 18h under $N_2$. The reaction was quenched with $H_2O$ and extracted with ether. The ethereal solution was washed with $H_2O$, $NaHCO_3$ and brine, dried over $MgSO_4$. The compound was purified by VLC (20% EtOAc in pet-ether) and identified as the title compound (6, 154 mg, 78%), m.p. 81–82 °C (lit.[9] m.p. 82–83 °C, IR and $^1$H NMR); $^{13}$C NMR (Table 1). FABMS m/z: 359 $[M+H]^+$, 381 $[M+Na]^+$.

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