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Synthesis and spectroscopic data analyses of 5β-cholane derivatives

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Facile synthesis and spectroscopic data analyses of three 5β-cholane derivatives, 3α-tosyloxy-5β-cholan-24-ol (**3**), 5β-cholan-24-ol (**4**) and 5β-cholan-24-yl tosylate (**5**), are described.

Keywords: steroids, lithocholic acid, reduction, tosylation, NMR

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Steroids are a group of pharmacologically active molecules widely found in both plant and animal kingdoms. The steroid structure is based on four carbocyclic rings arranged as in cyclopenta[*a*]phenanthrene, which is usually fully or partially reduced. Small changes in the functionalities attached to the steroid skeleton can render significant changes in their biological activities and pharmaceutically important physicochemical properties (1, 2). However, highly specific reactions are required to produce functionalised steroids with therapeutic use and commercial value (3). Lithocholic acid (**1**), a secondary bile acid biosynthesised from cholesterol, has one functional group at each end of the molecule, and could easily be modified to its biologically and pharmaceutically important derivatives. As part of our continuous studies on the synthesis of various steroidal monomers and dimers (4–8), we now report on the synthesis and comprehensive spectroscopic data analyses of three lithocholic acid derivatives, 3α-tosyloxy-5β--cholan-24-ol (**3**), 5β-cholan-24-ol (**4**) and 5β-cholan-24-yl tosylate (**5**).

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EXPERIMENTAL

General procedures

All chemicals and solvents were used without further purification. The reactions were monitored and the purity of the products was assessed by thin-layer chromatography (TLC) performed on aluminium sheets silica gel 60 F_{254} (0.25 mm thickness) pre-coated TLC plates (Merck, Germany) and visualised under UV illumination and/or by I_2 vapour. Melting points of the products were determined on a Gallenkamp melting point apparatus (Gallenkamp, UK). Chemical Ionisation Mass Spectrometry (CIMS) analyses were performed in the EPSRC Central Mass Spectroscopy Facility in Swansea, UK, on a Micromass Quattro II triple quadrupole instrument (Waters, UK) in chemical desorption mode using ammonia as CI gas. Mass accuracy was within 0.4 Da. CI source temperature was 170 °C and electron energy was 59 eV. Infrared spectra (wave numbers ν_{max} in cm^{-1}) were recorded on an ATI Mattson Genesis FTIR spectrophotometer (Mattson, Germany). Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity INOVA 400 MHz (400 MHz for ^1H and 100 MHz for ^{13}C nucleus) NMR spectrometer (Varian, USA). Chemical shifts δ were in ppm downfield from TMS, using the middle resonance of $CDCl_3$ (7.25 ppm for ^1H and 77.23 ppm for ^{13}C NMR) as an internal standard, and the coupling constant J in Hz. Spectra were recorded with a probe temperature of 25 °C.

Syntheses

The steroid starting material (methyl litocholate, **1**) was synthesised and identified previously in our labs (4).

*3α-Tosyloxy-5β-cholan-24-oic acid methyl ester (**2**).* – Tosylation of methyl lithocholate (**1**, 1.0 g, 2.56 mmol) was performed following the procedure described in the literature (9). The title compound **2** was found as a white solid: 1.17 g, 84%; m.p. 107–108 °C (m.p. ref. 8: 107–109 °C); IR (9), ^1H NMR (9) and ^{13}C NMR (10); CIMS *m/z*: 562 $[M+NH_4]^+$.

*3α-Tosyloxy-5β-cholan-24-ol (**3**).* – To a stirred solution of lithium aluminum hydride (LAH, 3.67 mmol) in dry tetrahydrofuran (THF, 15 mL), a solution of 3α-tosylate-5β-cholan-24-oic acid methyl ester (**2**, 500 mg, 0.92 mmol) in dry THF (10 mL) was added dropwise under N_2. After 18 h, the mixture was treated dropwise with a saturated Na_2SO_4 solution until white precipitation formed. The solid was filtered off. The filtrate was concentrated, the residue was taken up in ether, washed with H_2O, dried by addition of anhydrous magnesium sulphate and evaporated to dryness to obtain the title compound **3** as a white amorphous solid: 371 mg, 78%; m.p. 124–126 °C (m.p. ref. 11: 127.5–128.5 °C); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3349br (alcoholic OH), 2929s (CH), 2864s (CH), 1599m (PhC=C), 1449m, 1358m, 1261w, 1175vs (OSO_2), 1098m, 1050m, 926s, 867m, 813m, 757m and 668m; ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.58 (s, 3H, 18-Me), 0.84 (s, 3H, 19-Me), 0.87 (d, J = 6.5 Hz, 3H, 21-Me), 2.40 (s, 3H, Ph-**Me**), 3.56 (m, 2H, 24-OCH_2), 4.41 (m, 1H, 3β-OCH), 3α-OTs: 7.75 (d, J = 8.5 Hz, 2H, 2 x Ph-**H**), 7.28 (d, J = 7.9 Hz, 2H, 2 x Ph-**H**); ^{13}C NMR data are in Table I; CIMS *m/z*: 534 $[M + NH_4]^+$.

*Table I. ^{13}C NMR data of compounds **3–5***

Carbon no.	Chemical shifts (δ, ppm)[a]		
	3	**4**	**5**
1	35.0	37.6	37.6
2	26.2	21.4	21.3
3	83.3	27.1	27.0
4	33.1	27.3	27.2
5	42.1	43.8	43.7
6	26.8	26.6	26.6
7	27.5	27.6	27.5
8	35.7	36.0	35.9
9	40.3	40.6	40.5
10	34.3	35.6	35.4
11	20.8	20.9	20.8
12	40.1	40.4	40.3
13	42.7	42.8	42.7
14	56.4	56.7	56.6
15	24.2	24.3	24.2
16	28.3	28.4	28.2
17	56.1	56.3	56.1
18	12.0	12.1	12.0
19	23.1	24.3	24.3
20	35.6	35.4	35.2
21	18.6	18.7	18.4
22	31.8	31.9	25.6
23	29.4	29.5	31.4
24	63.6	63.7	71.3
3-OTs			
1	144.6	–	–
2, 6	129.8	–	–
3, 5	127.9	–	–
4	133.4	–	–
4-Me	21.6	–	–
24-OTs			
1	–	–	144.6
2, 6	–	–	129.8
3, 5	–	–	127.9
4	–	–	133.4
4-Me	–	–	21.6

[a] Spectra obtained in $CDCl_3$ (100 MHz).

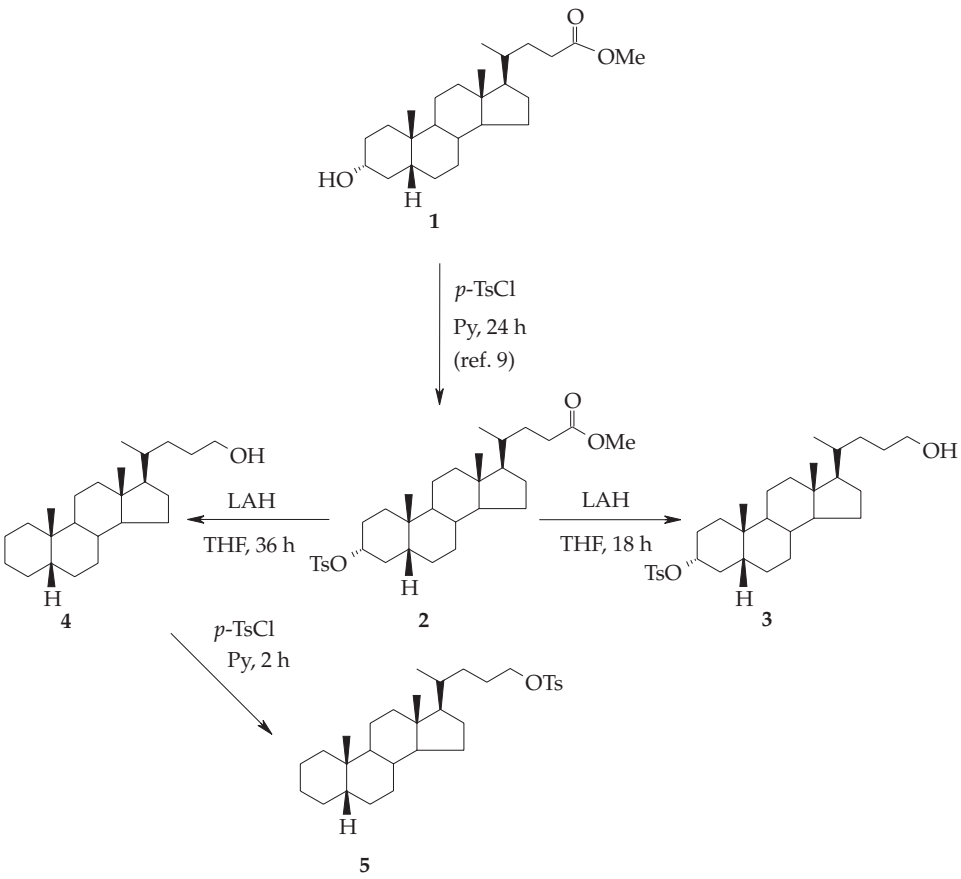
*5β-Cholan-24-ol (**4**).* – 3α-Tosyloxy-5β-cholan-24-oic acid methyl ester (**2**, 500 mg, 0.92 mmol) in dry THF (10 mL) was reduced using excess LAH (3.67 mmol) in dry THF (15 mL) under N_2. After 36 h, the mixture was treated dropwise with a saturated Na_2SO_4 solution until white precipitation was formed. After purification, the title compound **4** was obtained as a colourless solid: 239 mg, 75%; m.p. 124–125 °C (m.p. ref. 12: 127–129 °C); IR and ^{1}H NMR data were identical with published data (13); ^{13}C NMR data are in Table I; CIMS *m/z*: 364 $[M+NH_4]^+$.

*5β-Cholan-24-yl tosylate (**5**).* – Tosylation of **3** (100 mg, 0.29 mmol) was carried out using 1.2 molar excess of *p*-TsCl (66 mg, 0.35 mmol) in dry pyridine (2 mL) at 0–4 °C for 2 h. Ice and H_2O were added to the reaction mixture and extracted with diethylether, washed with dil. HCl and H_2O. The organic layer was separated, dried by addition of anhydrous magnesium sulphate and evaporated to dryness. The residue was subjected to preparative thin layer chromatography (PTLC, eluted with 10% EtOAc in petroleum-ether) to obtain the title compound **5** as a white solid: 61 mg, 42%; m.p. 98 °C (m.p. ref. 11: 99–100.5 °C); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3029w (aromatic CH), 2926s (CH), 2865s (CH), 1599m (aromatic C=C), 1449m, 1362m, 1261w, 1176vs (OSO_2), 1096m, 1019m, 927s, 813m, 758m and 664m; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.56 (s, 3H, 18-Me), 0.81 (d, J = 6.5 Hz, 3H, 21-Me), 0.87 (s, 3H, 19-Me), 3.96 (m, 2H, 24-OCH_2), 24-OTs: 7.75 (d, J = 8.2 Hz, 2H, 2 x Ph-**H**), 7.30 (d, J = 8.2 Hz, 2H, 2 x Ph-**H**), 2.41 (s, 3H, Ph-**Me**); ^{13}C NMR data are in Table I; CIMS *m/z*: 501 $[M+H]^+$, 518 $[M+NH_4]^+$.

RESULTS AND DISCUSSION

Tosylates are important functional groups in organic synthesis. An advantage of converting a hydroxyl group, a poor leaving group, to a tosylate group, an excellent leaving group, is that it can be easily displaced by nucleophilic substitution to produce a variety of organic compounds. Treatment of methyl lithocholate (**1**) with excess *p*-TsCl in dry pyridine provided 3α-tosyloxy ester **2** (9, 10) (Scheme 1). Reduction of **2** with excess LAH in dry THF over 18 h provided 3α-tosylate-5β-cholan-24-ol (**3**). It was observed that the 3α-tosyloxy was difficult to displace by hydride at ambient temperature over 18 h. The IR absorption band at 3349 cm^{-1} indicated the presence of an alcoholic OH in **3** and the CIMS spectrum displayed the $[M+NH_4]^+$ ion at *m/z* 534. The ^{1}H NMR of **3** showed signals similar to those of **2**, with the exception that, instead of a carbomethoxy, a signal for the C-24 oxymethylene (δ 3.56, 2H) was present. The identity of **3** was confirmed by comparing its mp, IR and ^{1}H NMR data with the published data (11). The ^{13}C NMR confirmed further the presence of the C-24 oxymethylene in **3** by displaying a signal at δ 63.6. When the reduction was carried out for 36 h, both 3α-tosyloxy and 24-ester functionalities were reduced to give 5β-cholan-24-ol (**4**) (Scheme 1). The identity of **4** was established by comparing its m.p., IR and ^{1}H NMR data with the published data (12, 13). The structure was confirmed further by ^{13}C NMR and CIMS analyses. The comprehensive ^{13}C NMR data for **3** and **4** are presented here for the first time.

The 24-hydroxy **4** was treated with 1.2 molar equivalent of *p*-TsCl in dry pyridine to produce 24-yl tosylate **5** (Scheme 1). The primary alcohol was highly reactive towards *p*-TsCl and the reaction was completed within 2 h at 4 °C. The 24-yl tosylate **5** was ob-



Scheme 1

tained as a semi-solid after purification by PTLC and the mp of **5** was comparable to its published data (11). The molecular mass was confirmed from the observed $[M+H]^+$ and $[M+NH_4]^+$ ions, respectively, at *m/z* 501 and 518 in its CIMS spectrum. In the ^1H NMR spectrum, in addition to the signals associated with the protons of the parent steroid nucleus (**5**), 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 3.96) of the resonance for the C-24 methylene protons of **5** (compared to that of **4** at δ 3.60) confirmed the attachment of the tosyl unit at C-24. This fact was further corroborated by its ^{13}C NMR data where the C-24 carbon resonance was observed at a further deshielded position (δ 71.3) compared to that of **4** (δ 63.7). All spectroscopic data for **5**, including IR, ^1H NMR, ^{13}C NMR and MS, are presented here for the first time.

CONCLUSIONS

The importance of tosylation in the conversion and functionalisation of steroid molecules has been demonstrated. Comprehensive spectroscopic analyses of the synthesised 5β-cholane derivatives (**3**–**5**) provided complete ^{13}C NMR assignment for these compounds for the first time.

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S A Ž E T A K

Sinteza i spektroskopska analiza derivata 5β-kolana

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Opisana je jednostavna sinteza i spektroskopska analiza tri derivata 5β-kolana, 3β-tosiloksi-5β-kolan-24-ol (**3**), 5β-kolan-24-ol (**4**) i 5β-kolan-24-il tosilat (**5**).

Ključne riječi: steroidi, litokolna kiselina, redukcija, tosilacija, NMR

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