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ON THE PROTECTION OF 3α-HYDROXY GROUP OF A/B *cis* STEROIDS

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ABSTRACT: The difficulties encountered in the protection of 3α-hydroxy group of lithocholic acid **1** with three different types of protective groups are described. Only benzyl ether derivative was found to be suitable for synthetic transformations. The methodology for the synthesis of benzyl ethers of A/B *cis* steroids is reported for the first time.

In connection with a project aimed at development of new spin labelled probes for biomembranes, we needed to protect the 3α-hydroxy group of lithocholic acid (**1**). The protection of hydroxy groups is a well studied chemical transformation, as revealed by the plethora of groups available for the purpose.[1] Although esters and carbonates and their derivatives form important class of protective groups, the envisaged strategy required a Grignard addition reaction, ruling out their utility for our purpose. The tetrahydropyranyl group[2] is readily cleaved by mild reagents and hence

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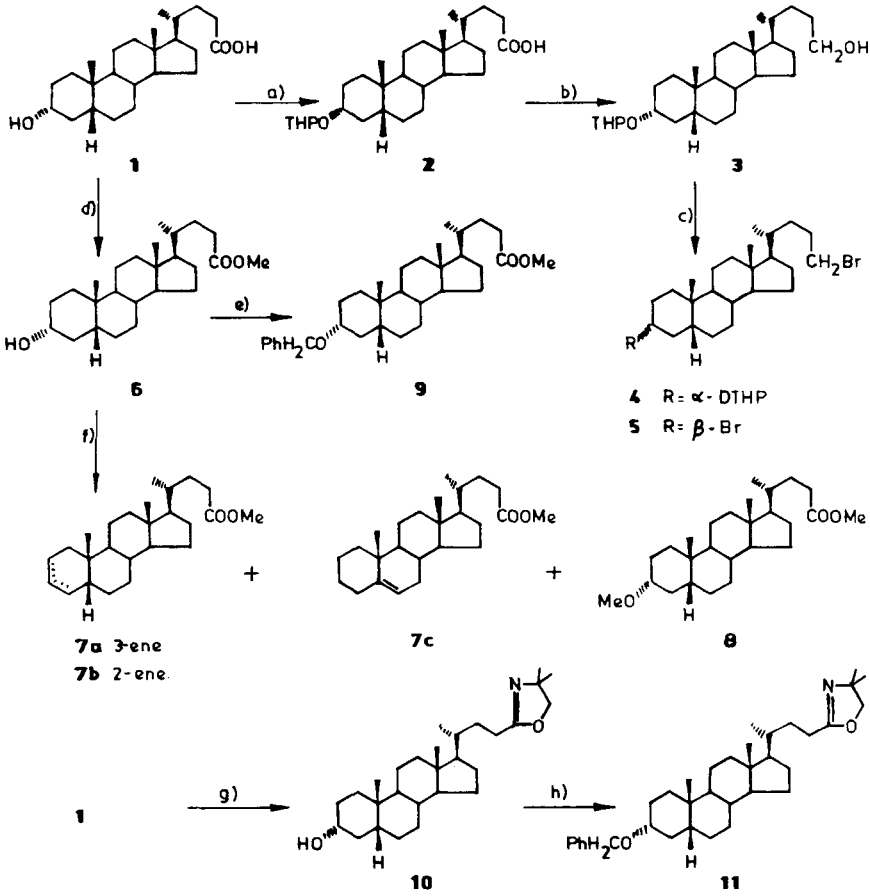
was a method of choice. Usual conditions using benzene-THF cosolvent system[3] to solubilize the acid **1** (Chart 1) provided the protected acid **2** in 80% yields. The free acid was reduced with lithium aluminium hydride to the expected[4] primary alcohol **3**. On treatment of the alcohol **3** with PPh_3-Br_2 mixture,[5] two compounds could be isolated. The desired product **4** was obtained in very low yields (26%). In addition, the reaction provided a compound in major amounts (70%) which showed characteristic molecular ion peaks at m/z 486, 488, and 490 with relative intensity ratios 1:2:1 in mass spectrum. The FT-IR showed two strong bands at 607.9 and 648.7 cm^{-1} assignable to C-Br stretchings. This, coupled with the absence of fragment corresponding to the THP group (m/z 85), indicated the formation of dibromo derivative **5**. On prolonging the reaction time, **5** was obtained as the exclusive product. The ^1H NMR spectrum of **5** shows a deshielded 19-H_3 signal at 1.00 δ indicating β-Br substitution. The displacement of the OTHP group under the reaction conditions used could be rationalized by the nucleophilic attack of $PPh_3Br^+Br^-$ species formed in the reaction.[6]

The isolation of the desired monobromo compound **4** in low yields led us to seek an alternative protective group for our purpose. Herz and Montalvo[7] have reported the synthesis of methyl ether of methyl 3β-hydroxy chol-5-en-24-oate using trimethyl orthoformate and 70% perchloric acid in quan-



CONDITIONS:

a) DHP, PTSA (catalytic amount), PhH-THF, rt, 2h.
b) LAH, THF, reflux, 3h
c) PPh_3/Br_2, pyridine, CH_2Cl_2, rt, 3h
d) MeOH / H_2SO_4 (catalytic amount), reflux, 2h
e) NaH / THF, TBAI, $PhCH_2Br$, reflux, 3·5h
f) $HC(OMe)_3$, 70 % $HClO_4$, rt, 2h
g) 2-amino-2-methyl-propan-1-ol, H_3BO_3, xylene, reflux, 48h
h) NaH / THF, TBAI, $PhCH_2Br$, reflux, 3h

Chart-1

titative yields. Thus it was thought pertinent to use similar reaction conditions for the present case. Attempts to protect the 3α-hydroxy group of **6**, however provided two compounds in yields of 55% and 15%. The product isolated in minor amount was the desired methyl ether **8** as identified by its ^{1}H NMR spectrum which showed signals at 3.35 δ due to 3-OCH_3 and 3.28 δ due to 3β-H. The major product was suspected to be of olefinic nature and was found to be a mixture of three compounds on silver nitrate impregnated tlc. The unsaturated mixture was separated by $AgNO_3$-impregnated column chromatography[8] into three compounds in the ratio of 4:2:1. The ^{1}H NMR spectrum of the major isomer showed the olefinic protons at 5.34 (dd) and 5.67-5.63 δ (m) integrating for a proton each; while these were observed at 5.48-5.56 (m) and 5.64-5.59 δ (m) in the second-major compound. Based upon these spectral features, structures **7a** and **7b** were assigned to the two isomers respectively. The third product was characterised as 5-ene **7c** isomer on the basis of single olefinic proton at 5.31-5.28 δ (m) together with the resonance of 19-H_3 at 1.01 δ.[9] The elimination reaction to provide **7** in the presence of acid, in contrast to methyl ether formation of 3β-hydroxy group of 5-ene steroid,[7] indicates the significant difference in reactivity of 3α-hydroxy group of A/B *cis* steroids.

Although the methyl ether derivative **8** was possible to prepare, the low yield of the product was a major stumb-

ling block in the multistep synthesis. The stability of the benzyl ethers to withstand various synthetic transformations, coupled with their facile deprotection, is well established. However, no report exists on the benzyl ether formation of 3α-hydroxy A/B *cis* steroids to our knowledge. Two of the most often utilized reaction conditions viz NaH/DMSO[10] and NaH/THF[11] failed to provide the benzyl ether derivative **9**. In order to solubilize the alkoxide anion, HMPA/THF cosolvent system at -5°C was used; but to no avail. The method of Golding and Ioannou[12] involving the use of solid-liquid phase transfer catalyst for the synthesis of benzyl ethers of sn-glycerol, also proved to be unsuccessful yielding the starting material back in quantitative yield. Use of Ag_2O[13] also did not help. Czrnecki et al [14] suggest the use of tetrabutyl ammonium iodide (TBAI) as a catalyst for benzylation of hindered hydroxy groups in monosaccharides. On treatment of sodium alkoxide of methyl ester **6** with benzyl bromide and TBAI in THF under reflux, the required compound **9** was obtained in a yield of 72%. Thus it was found that the benzyl ether of 3α-hydroxy group of A/B *cis* steroids formed only under the catalytic conditions of tetrabutyl ammonium iodide accompanied by elevated temperature.

The method was also applied to substrate **10**, prepared according to Barton et al ,[15] to provide the desired protected derivative **11** in good yields. The benzyl ether derivative

11 was then used for the synthesis of new spin labelled probes and this aspect of work will be the subject matter of another article.

Experimental

Melting points are reported uncorrected. Laboratory solvents were predried before use according to standard procedures. Lithocholic acid was purchased from Aldrich Chemical Co and used as such. IR spectra were recorded on Perkin Elmer 688 spectrometer. FT-IR spectrum was obtained on Nicolet 5DXB spectrometer. ^1H NMR spectra were recorded on Varian VXR 300S spectrometer as solutions in $CDCl_3$ at ambient temperature with TMS as internal standard. Mass spectra were obtained on Shimadzu QP1000 spectrometer. Elemental analyses were performed on CEST MOD.110 analyser.

3α-Tetrahydropyranyloxy-chol-24-ol (3)

A mixture of lithocholic acid (**1**) (4.0 g, 0.01 mol), freshly distilled dihydropyran (3.17 mL, 37 mmol) and catalytic amount of p-toluenesulphonic acid was stirred under nitrogen atmosphere in benzene-THF mixture (160 mL, 1:3) at RT for two hours. LAH (0.4 g, 10 mmol) was then added and the reaction mixture set up for reflux. After three hours of reflux, moist ether (10 mL) was added carefully. This was followed by further addition of water (3 mL). The reaction mixture was filtered and precipitate washed with four portions

of ethyl acetate (25 mL). The combined filtrates were dried over anhydrous $MgSO_4$, evaporated under reduced pressure to give crude **3**. Further purification by column chromatography on silica gel using 8% ethyl acetate in petroleum ether (bp 60-80°C) as eluent, provided a colourless crystalline solid (3.84 g, 80%).

IR ($CHCl_3$) $\bar{\nu}$ = 3420 cm^{-1} (broad, OH) ; ^1H NMR ($CDCl_3$) δ = 4.73-4.71 (m, 1H, OCHO), 3.96-3.89 (m, 1H, OCH'H"), 3.67-3.59 (m, 3H, OCH'H" and 24-H_2), 3.54-3.45 (m, 1H, 3β-H), 0.92 (d, J = 6.56 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.64 (s, 3H, 18-H_3) ; MS m/z = 446 (M^+).

Bromination of substrate 3

To a solution of PPh_3 (2.40 g, 9 mmol) in anhydrous CH_2Cl_2 (23 mL) was added dropwise a solution of bromine in CCl_4 (5.5 M, 1.57 mL, 86 mmol) at 0-5°C. The resultant thick white suspension was stirred for 15 min at 0°C, after which a solution of **3** (3.52 g, 78 mmol) and anhydrous pyridine (0.74 mL, 9 mmol) in CH_2Cl_2 (15 mL) was added dropwise. The reaction was kept stirring at RT for 3 hours. The clear solution was then evaporated under reduced pressure to give white semisolid residue. The residue was extracted with four portions of ether (25 mL) and filtered through a pad of neutral alumina. The resultant filtrate was dried over anhydrous Na_2SO_4, concentrated under vacuum *and further*

purified by column chromatography on neutral alumina using pentane as eluent. Compound **4** was obtained as a thick viscous liquid (1.04 g, 25.9%) while compound **5** (2.69 g, 70%) was obtained as white crystalline solid.

3α-Tetrahydropyranyloxy-24-bromo-cholane (4)

^{1}H NMR ($CDCl_3$) δ = 4.72 (m, 1H, OCHO), 3.94-3.88 (m, 1H, OCH'H") 3.70-3.62 (m, 1H, OCH'H"), 3.50 (m, 1H, 3β-H), 3.46-3.31 (m, 2H, 24-H_2), 0.92 (d, J = 6.26 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.65 (s, 3H, 18-H_3) ; MS m/z = 508, 510 (M^+).

3β,24-Dibromo-cholane (5)

mp = 82°C ; IR (KBr) $\bar{\nu}$ = 607.9 cm^{-1} (C-Br), 648.7 (C-Br) ; ^{1}H NMR ($CDCl_3$) δ = 4.80 (t, J = 2.94 Hz, 1H, 3α-H), 3.45-3.31 (m, 2H, 24-H_2), 1.00 (s, 3H, 19-H_3), 0.92 (d, J = 6.56Hz, 3H, 21-H_3), 0.65 (s, 3H, 18-H_3) ; MS m/z = 486, 487, 488, 489, 490 (M^+).

Elemental Analysis	Calculated	C	59.02	H	8.25
	Found	C	59.68	H	8.59 %

Methyl ether formation of substrate 6

Methyl lithocholate (**6**) (980 mg, 2.5 mmol) was dissolved in trimethyl orthoformate (10 mL) and perchloric acid (70%, 0.6 mL) added to it. After stirring for 2 hrs at RT, the solution was neutralized with a solution of $NaHCO_3$

in water (5%). The mixture was extracted with four portions of ether (25 mL), and the organic layer washed sequentially with $NaHCO_3$, water, brine, followed by drying over anhydrous Na_2SO_4. On concentration under reduced pressure, a viscous liquid was obtained which was purified on silica gel column using 2.5% ethyl acetate-petroleum ether (bp 60-80°C) eluent to provide compounds **7** (510.5 mg, 55%) and **8** (160 mg, 15.5%). The crude mixture **7** was separated by means of silver nitrate impregnated column chromatography using a gradient elution of 0 to 1% ethyl acetate in petroleum ether (bp 60-80°C).

Unsaturated isomeric mixture 7

IR($CHCl_3$) $\bar{\nu}$ = 1730 cm^{-1} (COOR), 690 (=CH); MS m/z = 372(M^+).

Methyl chol-3-ene-24-oate (7a)

^1H NMR ($CDCl_3$) δ = 5.67-5.63 (m, 1H, 3-H), 5.34 (dd, J = 9.9 Hz, 1.83 Hz, 1H, 4-H), 3.66 (s, 3H, $COO\underline{CH_3}$), 0.94 (s, 3-H, 19-H_3), 0.90 (d, J = 6.41 Hz, 3H, 21-H_3), 0.66 (s, 3H, 18-H_3). MS m/z = 372 (M^+).

Methyl chol-2-ene-24-oate (7b)

^1H NMR ($CDCl_3$) δ = 5.59-5.64 (m, 1H, 2-H), 5.48-5.56 (m, 1H, 3-H), 3.66 (s, 3H, $COO\underline{CH_3}$), 0.96 (s, 3H, 19-H_3), 0.90 (d, J = 6.41Hz, 3-H, 21-H_3), 0.65 (s, 3H, 18-H_3).

MS m/z = 372 (M^+).

Methyl chol-5-ene-24-oate (7c)

^1H NMR ($CDCl_3$) δ = 5.31-5.28 (m, 1H, 4-H), 3.66 (s, 3H,

$COOCH_3$), 1.01 (s, 3H, 19-H_3), 0.91 (d, J = 6.25 Hz, 3H, 21-H_3), 0.68 (s, 3H, 18-H_3).

MS m/z = 372 (M^+).

Methyl 3α-methoxy-chol-24-oate (8)

IR ($CHCl_3$) $\bar{\nu}$ = 1730 cm^{-1} (COOR), 1030 (C-O) ; ^1H NMR ($CDCl_3$) δ = 3.66 (s, 3H, $COOCH_3$), 3.35 (s, 3H, OCH_3), 3.32-3.12 (m, 1H, 3β-H), 0.93 (d, J = 6.26 Hz, 3H, 21-H_3), 0.92 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3).

MS m/z = 404 (M^+).

Methyl 3α-benzyloxy-chol-24-oate (9)

To a suspension of NaH (55% in petroleum wax, 22 mg, 0.5 mmol) in anhydrous THF (10 mL) was added dropwise under nitrogen a solution of **6** (200 mg, 0.5 mmol) in anhydrous THF (15 mL). After stirring for another 15 min freshly distilled benzyl bromide (0.06 mL, 0.5 mmol) and catalytic amount of TBAI (18.4 mg, 0.05 mmol) was added and the mixture refluxed for 3.5 hrs. After cooling, saturated NH_4Cl was added till effervescence ceases and the reaction mixture extracted with four portions of CH_2Cl_2 (25 mL). The organic layer worked up as usual and the crude product purified by column chromatography on silica gel to yield white crystalline solid (0.18 g, 72%).

mp = 70°C ; IR ($CHCl_3$) $\bar{\nu}$ = 3100, 3080, 3030 cm^{-1} (Ar-H), 1730 (COOR), 1040 (C-O) ; ^1H NMR ($CDCl_3$) δ = 7.36-7.25 (m, 5H, Ar-H), 4.56 (s, 2H, OCH_2Ph), 3.66 (s, 3H, $COOCH_3$),

0.91 (d, J = 6.25 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3) ; MS m/z = 480 (M^+).

Elemental Analysis Calculated C 79.95 H 10.06

Found C 79.83 H 10.27%

2-(3α-Hydroxy-24-norcholan-23-yl)-4,4-dimethyl-4,5-dihydro-oxazole (10)

A mixture of lithocholic acid (1) (5.0 g, 13 mmol), 2-methyl-2-amino-propan-1-ol (1.72 mL, 18 mmol) and boric acid (296 mg, 4 mmol) was dissolved in anhydrous xylene (96 mL) and the solution refluxed with azeotropic removal of water for 48 hrs. The solvent was removed by vacuum distillation and the residue dissolved in hot methanol (10 mL). To this solution, 5% aqueous K_2CO_3 (105 mL) was added and the mixture was boiled for 1 hr, cooled and extracted with four portions of ether (25 mL). The organic layer was worked up as usual and the residue so obtained was purified by column chromatography on silica gel using 5% methanol in benzene as eluent to give a white solid (5.46 g, 96%).

mp = 152°C; IR (nujol) $\bar{\nu}$ = 3230 cm^{-1} (broad, OH), 1670 (C=N), 1375, 1395 ($C(CH_3)_2$) ; ^1H NMR ($CDCl_3$) δ = 3.89 (s, 2H, $O\underline{\underline{CH}}_2$), 3.66-3.57 (m, 1H, 3β-H), 1.63 (broad, D_2O exchangeable, OH), 1.25 (s, 6H, $C(CH_3)_2$), 0.93 (d, J = 6.41 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3) ; MS m/z = 430 ($M+1^+$).

Elemental Analysis Calculated C 78.27 H 11.03 N 3.26

Found C 78.04 H 10.98 N 3.10%

2-(3α-Benzyloxy-24-norcholan-23-yl)-4,4-dimethyl-4,5-dihydro oxazole (11)

To a suspension of NaH (55% in petroleum wax, 0.21 g, 4.4 mmol) in anhydrous THF (100 mL) was added dropwise a solution of **10** (1.90 g, 4 mmol) under nitrogen. After stirring for 15 min, benzyl bromide (0.52 mL, 4 mmol) was added dropwise, followed by catalytic amount of TBAI (14.7 mg, 0.04 mmol). The resultant solution was refluxed for 4 hrs. On cooling, saturated aqueous NH_4Cl solution was added dropwise and the reaction mixture extracted with four portions of CH_2Cl_2 (25 mL). The organic layer worked up as usual and the residue so obtained was purified by column chromatography to provide a thick viscous liquid (1.54 g, 67.2%). IR ($CHCl_3$) $\bar{\nu}$ = 3100 cm^{-1}, 3070 (aromatic), 1670 (C=N), 1360, 1370 ($C(CH_3)_2$) ; ^1H NMR ($CDCl_3$) δ = 7.37-7.26 (m, 5H, Ar-H), 4.55 (s, 2H, $O\underline{CH_2}Ph$), 3.89 (s, 2H, $O\underline{CH_2}$), 3.38-3.33 (m, 1H, 3β-H), 1.25 (s, 6H, $C(CH_3)_2$), 0.93 (d, J = 6.25 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3); MS m/z = 519 (M^+).

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