

SYNTHESIS OF 5α -CHOLESTANE TYPE GLYCOSIDE SULFATES***

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Syntheses of steroid glycoside sulfates sulfated both on the steroid skeleton and in sugar part are presented. Sodium salts of 5α -cholestane- $3\beta,6\alpha$ -diol 6- β -D-glucoside 3-sulfate *V* and 3- β -D-glucoside 6-sulfate *XI* were prepared from 3β -(2-tetrahydropyranyl)- 5α -cholestane- 6α -ol (*I*) using acetyl and methoxymethyl groups for temporary protection. Sulfation in the sugar part was at first checked in pregnane series and triethylammonium salts of 3β -(β -D-glucopyranosyloxy)pregn-5-en-20-one 6'-sulfate (*XXVI*) and 4'-sulfate 2',3'-diacetate *XXVII* were prepared. Application of this method on cholestan derivatives gave triethylammonium salt of 5α -cholestane- 3β -yl β -D-glucopyranoside-6-sulfate (*XXXI*).

In our preceding papers we have described preparation of the steroid hydroxy sulfates^{1,2} with structure derived from the substances isolated from marine invertebrates. Now, we report syntheses of other naturally occurring derivatives – steroid glycoside sulfates. We prepared model derivatives of both principal groups of this type, differing in location of a sulfate group either on the steroid skeleton or on a sugar moiety.

Naturally occurring steroid glycoside sulfates sulfated on the skeleton are often^{3,4} of a 5α -cholestane type with the sugar moiety or the sulfate group on hydroxyls situated mainly in positions 3β , 6α , and 24. The sugar part is frequently constituted from D-glucose or D-xylose. In the first part of this paper we describe syntheses of both β -D-glucopyranoside sulfates of 5α -cholestane- $3\beta,6\alpha$ -diol.

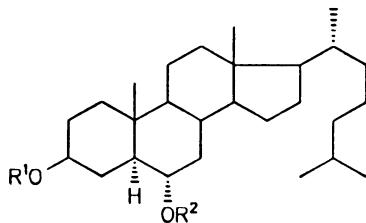
From the 3 -(2-tetrahydropyranyl)-protected derivative *I* (ref.²) and tetra-O-acetyl- α -D-glucopyranosyl bromide under insoluble silver salts catalysis we obtained β -D-glucopyranoside *II*. Mild deprotection of THP-group with tetrahydrofuran-acetic acid-2 M

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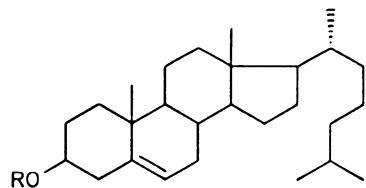
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hydrochloric acid mixture in the next step yielded derivative *III* without remarkable deacetylation in sugar part. Sulfation was then readily accomplished by trimethylamine-sulfur trioxide complex in pyridine and resulting sulfate *IV* was without puri-



<i>I</i> ,	$R^1 = \text{THP}; R^2 = \text{H}$	<i>IX</i> ,	$R^1 = \text{Ac}_4\text{Glc}; R^2 = \text{H}$
<i>II</i> ,	$R^1 = \text{THP}; R^2 = \text{Ac}_4\text{Glc}$	<i>X</i> ,	$R^1 = \text{Ac}_4\text{Glc}; R^2 = \text{SO}_3\text{NHMe}_3$
<i>III</i> ,	$R^1 = \text{H}; R^2 = \text{Ac}_4\text{Glc}$	<i>XI</i> ,	$R^1 = \text{Glc}; R^2 = \text{SO}_3\text{Na}$
<i>IV</i> ,	$R^1 = \text{SO}_3\text{NHMe}_3; R^2 = \text{Ac}_4\text{Glc}$	<i>XIII</i> ,	$R^1 = \text{TxDMS}; R^2 = \text{H}$
<i>V</i> ,	$R^1 = \text{SO}_3\text{Na}; R^2 = \text{Glc}$	<i>XIV</i> ,	$R^1 = \text{TxDMS}; R^2 = \text{THP}$
<i>VI</i> ,	$R^1 = \text{THP}; R^2 = \text{MOM}$	<i>XV</i> ,	$R^1 = \text{H}; R^2 = \text{THP}$
<i>VII</i> ,	$R^1 = \text{H}; R^2 = \text{MOM}$	<i>XVII</i> ,	$R^1 = \text{Piv}; R^2 = \text{H}$
<i>VIII</i> ,	$R^1 = \text{Ac}_4\text{Glc}; R^2 = \text{MOM}$	<i>XVIII</i> ,	$R^1 = \text{Piv}; R^2 = \text{THP}$



<i>XII</i> ,	$R = \text{TxDMS}$
<i>XVI</i> ,	$R = \text{Piv}$

$\text{Ac}_4\text{Glc} = 2,3,4,6\text{-tetra-}O\text{-acetyl-}\beta\text{-D-glucopyranosyl}$

$\text{Glc} = \beta\text{-D-glucopyranosyl}$

$\text{THP} = 2\text{-tetrahydropyranyl}$

$\text{MOM} = \text{methoxymethyl}$

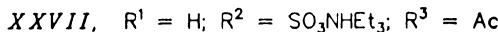
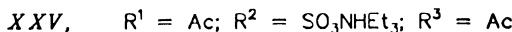
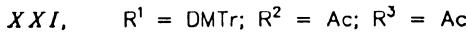
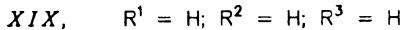
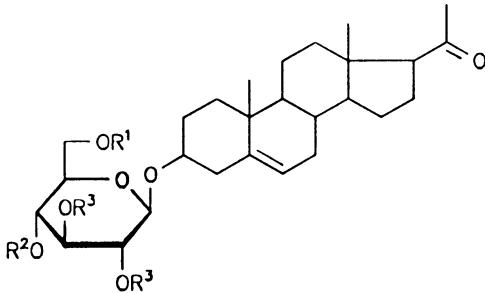
$\text{TxDMS} = \text{thexyldimethylsilyl}$

$\text{Piv} = \text{pivaloyl}$

fication deacetylated by sodium hydroxide in methanol–water mixture. The desired glycoside sulfate was isolated as sodium salt *V* by crystallization.

Similar 3-glycoside 6-sulfate *XI* was prepared from methoxymethyl derivative *VII* (available via *VI* from *I*, ref.²) in the same way: *VII* → *VIII* → *IX* → *X* → *XI*. In this case the deprotection of methoxymethyl-protected derivative *VIII* requires temperature of 50 °C and at this conditions partial saponification of acetoxy groups takes part. For the alternative synthesis 6-(2-tetrahydropyranyl)-protected derivative *XV* was needed. We developed two approaches to this derivative based on temporary thexyldimethylsilyl or pivaloyl-protection (*XII* → *XIII* → *XIV* → *XV* or *XVI* → *XVII* → *XVIII* → *XV*). However, the overall yields of *XV* in both sequences were not better than the yield of *VII* in original procedure (cf. ref.²) with methoxymethyl-protection and this approach was not longer corroborated.

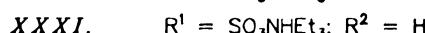
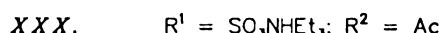
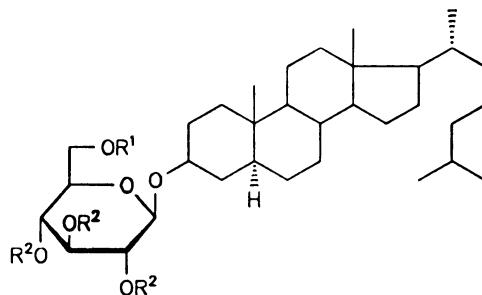
Also natural derivatives sulfated on a sugar moiety are often of the 5α-cholestane type with hydroxyl groups in 3β, 6α and 15α positions^{3,4}. Sulfate groups are located either in positions 4 or 6 of sugar. The synthesis of derivatives of this type was at first



developed on the more available pregnane model and then applied on 5α -cholestane derivatives.

We started with synthesis of partially protected derivatives of 3β -(β -D-glucopyranosyloxy)pregn-5-en-20-one (*XIX*). Using 4,4'-dimethoxytrityl group⁵ for selective protection in position 6 of sugar moiety we prepared partially acetylated glucoside *XXII* (*XIX* \rightarrow *XX* \rightarrow *XI* \rightarrow *XXII*). The classical trityl group⁶ is far less reactive in this case and requires more severe conditions for removal. The isomeric glucoside *XXIII* with free 4'-hydroxyl was then accessible from *XXII* by base-catalyzed acetyl migration⁷.

For sulfation of both partially protected glucosides (*XXII* and *XXIII*) the pyridine-sulfur trioxide complex was used. This complex reacts more slowly than the corresponding trimethylamine complex but its low solubility in the reaction medium enables easy work-up after reaction. Nevertheless, the products of sulfation were converted prior deacetylation into triethylammonium salts (*XXIV* and *XXV*), in order to modify their solubility and stability⁶. In the case of the derivative *XXIV* acetyl protecting groups were successfully removed by triethylamine-methanol-water mixture and afforded sulfate *XXVI*. Deacetylation of the derivative *XXV* failed with any modification of the conditions we have tested and yielded only derivative *XXVII*, in which acetyl groups in the 2' and 3' positions remained intact. This could be caused by the occupation of the position 4' by a sulfate as this position plays an important role in the deacetylation process⁸.



DMTr = 4,4'-dimethoxytrityl

Ac = acetyl

In the same manner as derivative *XXVI* cholestane derivative *XXXI* was prepared (*XXVIII* → *XXIX* → *XXX* → *XXXI*).

The prepared glycoside sulfate salts (*V*, *XI*, *XXVI*, and *XXXI*) were tested for anti-HIV activity. None of them showed any activity of this type. Derivative *XXVI* is highly cytotoxic on the MT4 cell culture ($IC_{50} = 4 \cdot 10^{-5}$ mol l⁻¹).

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius. Optical rotations were measured on a Perkin-Elmer 141 MC polarimeter at 25 °C. IR spectra were taken on a Perkin-Elmer PE 580 or Bruker IFS 88 spectrometer (wavenumbers in cm⁻¹). ¹H NMR spectra were obtained on Tesla BS-497 (100 MHz), Varian XL-200 (200 MHz), or on Varian UNITY-500 (500 MHz) instruments (all in FT mode) at 23 °C in deuteriochloroform with tetramethylsilane as internal standard, unless stated otherwise. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. All parameters were obtained by the first-order analysis. Column chromatography was performed on silica gel (60 – 120 μm) or on neutral alumina (Reanal, activity II), thin-layer chromatography on silica gel G according to Stahl (ICN Biochemicals). For reverse phase chromatography column (12.6 × 500 mm) packed with Separon Si C18 (Tessek, Praha; 10 μm) was used. Solutions in organic solvents were dried over anhydrous sodium sulfate and the solvents were evaporated in vacuo (about 2 kPa). Analytical samples were dried over phosphorus pentoxide at 40 °C/26 Pa for 12 h. The identity of samples prepared by different routes was checked by comparison of their IR and ¹H NMR spectra, thin-layer chromatography and mixture melting point determination.

3β-(2-Tetrahydropyranoxy)-5α-cholestane-6α-yl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside (*II*)

A dry mixture of hydroxy derivative² *I* (345 mg, 0.71 mmol), silver silicate on silica gel⁹ (700 mg) and ground molecular sieve 4A (350 mg) was stirred in vacuo (10 Pa) for 2 h. The flask was then filled with argon under slight overpressure (about 5 kPa) and dry 1,2-dichloroethane (3 ml) was injected through a septum. The mixture was stirred at room temperature for 30 min and then a solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (600 mg, 1.46 mmol) in dry 1,2-dichloroethane (2 ml) was added through the septum. After stirring at room temperature for 20 h, the solid material was filtered off on a column of Celite. The column was washed with chloroform, and the combined organic phases were washed with 5% aqueous potassium hydrogen carbonate solution and water. The solvents were evaporated, the residue was subjected to chromatography (silica gel pretreated with ammonia vapour for 24 h, 30 g; benzene – ether 1 : 1) yielding 413 mg (71%) of derivative *II*, m.p. 232 – 242 °C, $[\alpha]_D + 20^\circ$ (c 0.34, acetone). IR spectrum (chloroform): 1 750 (C=O); 1 040, 1 026 (C–O). ¹H NMR spectrum (100 MHz): 5.28 – 4.80 m, 2H (H-3', H-4'); 4.68 m, 2H (H-2', H-2 of THP group); 4.40 d, 1H (H-1', *J* = 8.0); 3.60 m, 3H (H-3, H-5', H-6 of THP group); 2.10 s, 2.07 s, 2.01 s, 1.98 s, 4 × 3H (4 × CH₃CO); 0.89 bd, 3H (3 × H-21, *J* = 6.0); 0.86 bd, 6H (3 × H-26, 3 × H-27, *J* = 6.0); 0.78 s, 3H (3 × H-19); 0.60 s, 3H (3 × H-18). For C₄₆H₇₄O₁₂ (819.1) calculated: 67.45% C, 9.11% H; found: 68.00% C, 9.70% H.

3β-Hydroxy-5α-cholestane-6α-yl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside (*III*)

The solution of derivative *II* (400 mg, 0.49 mmol) in tetrahydrofuran (25 ml), acetic acid (10 ml) and 2 M hydrochloric acid (10 ml) was stirred at room temperature for 2 h. The mixture was then diluted with ether (150 ml) and washed successively with water, saturated aqueous solution of potassium hydrogen carbonate and water. After drying the solvent was evaporated and the solid residue was chromatographed on a column of silica gel (40 g) in benzene-ether (8 : 2). Yield of derivative *III* was 184 mg (51%), m.p. 150 – 153 °C, $[\alpha]_D + 14^\circ$ (c 0.3, methanol). IR spectrum (chloroform): 3 608, 3 460 (O–H); 1 742 (C=O); 1 042 (C–O). ¹H

NMR spectrum (200 MHz): 5.18 t, 1II (II-3', J = 9.6); 5.06 t, 1II (II-4', J = 9.6); 4.96 dd, 1II (II-2', J = 7.8 and 9.7); 4.63 d, 1II (II-1', J = 7.8); 4.23 dd, 1II (II-6'a, J = 11.5 and 4.9); 4.11 dd, 1II (II-6'b, J = 11.5 and 2.5); 3.60 m, 1II (II-3); 3.50 m, 1II (II-5'); 2.08 s, 2.04 s, 2.02 s, 1.99 s, 4 \times 3II (CH_3CO); 0.88 d, 3II (3 \times II-21, J = 6.0); 0.86 bd, 6II (3 \times II-26, 3 \times II-27, J = 6.0); 0.78 s, 3II (3 \times II-19); 0.60 s, 3II (3 \times II-18). For $\text{C}_{41}\text{H}_{66}\text{O}_{11}$ (735.0) calculated: 67.00% C, 9.05% H; found: 66.80% C, 10.01% H.

6 α -(β -D-Glucopyranosyloxy)-5 α -cholestane-3 β -yl Sulfate, Sodium Salt (V)

To the solution of derivative *III* (81 mg, 0.11 mmol) in pyridine (5 ml) trimethylamine-sulfur trioxide complex (42 mg, 0.3 mmol) was added. The mixture was stirred for 24 h, then the solvent was evaporated. Crude derivative *IV* was dissolved in 5% solution of sodium hydroxide in 50% aqueous methanol (5 ml) and stirred for 2 h. The mixture was then neutralized by solid carbon dioxide and the solvent was evaporated. Crude product was crystallized from chloroform-methanol mixture to yield 60 mg (81%) of derivative *V*; m.p. 146 – 148 °C, $[\alpha]_D + 12^\circ$ (c 0.25, methanol). IR spectrum (KBr pellet): 3 425 (O-II); 1 082, 1 060, 1 037 (C-O); 1 250, 1 230 (SO_2). ^1H NMR spectrum (CD_3SOCD_3 , 100 MHz): 4.60 d, 1II (II-1', J = 8.0); 4.40 m, 1II (II-3); 0.78 s, 3II (3 \times II-19); 0.64 s, 3II (3 \times II-18). For $\text{C}_{33}\text{H}_{57}\text{NaO}_{10}\text{S}$ (668.9) calculated: 59.26% C, 8.59% H, 4.79% S; found: 59.57% C, 8.63% H, 4.51% S.

6 α -Methoxymethyl-5 α -cholestane-3 β -yl 2,3,4,6-Tetra-O-acetyl- β -D-glucopyranoside (VII)

Glycosylation of derivative² *VII* (200 mg, 0.45 mmol) in the manner used for preparation of *II* and subsequent chromatography (silica gel 20 g, light petroleum-ether 7 : 3) gave 129 mg (37%) of the glucoside *VIII*, m.p. 149 – 151 °C, $[\alpha]_D + 23^\circ$ (c 0.31, chloroform). IR spectrum (chloroform): 1 755 (C=O); 1 144, 1 096, 1 039 (C-O). ^1H NMR spectrum (100 MHz): 5.13 – 4.90 m, 3II (II-3', II-4', II-2'); 4.70 – 4.50 m, 3II (II-1' and OCH_2O of the MOM group); 4.14 m, 1II (II-6'); 3.56 m, 1II (II-3); 3.36 m, 4II (CH_3O and II-6); 2.10 s, 2.06 s, 2.04 s, 2.00 s, 4 \times 3II (4 \times CH_3CO); 0.90 bd, 3II (3 \times II-21, J = 6.0); 0.86 bd, 6II (3 \times II-26, 3 \times II-27, J = 6.2); 0.80 s, 3II (3 \times II-19); 0.63 s, 3II (3 \times II-18). For $\text{C}_{43}\text{H}_{70}\text{O}_{12}$ (779.0) calculated: 66.30% C, 9.06% H; found: 66.70%, 9.74% H.

6 α -Hydroxy-5 α -cholestane-3 β -yl 2,3,4,6-Tetra-O-acetyl- β -D-glucopyranoside (IX)

The solution of derivative *VIII* (100 mg, 0.13 mmol) in tetrahydrofuran (5 ml), acetic acid (5 ml) and 2 M hydrochloric acid (5 ml) was stirred at 50 °C for 80 min. The mixture was then cooled, diluted with ether (100 ml) and washed with water, saturated aqueous solution of potassium hydrogen carbonate and water again. After drying the solvent was removed and solid residue was chromatographed on a column of silica gel (10 g) in acetone-ether (1 : 1). Yield of derivative *IX* was 30 mg (32%), m.p. 165 – 168 °C, $[\alpha]_D + 10^\circ$ (c 0.25, chloroform). IR spectrum (chloroform): 3 619 (O-II); 1 755 (C=O); 1 232, 1 029 (C-O). ^1H NMR spectrum (500 MHz): 5.18 t, 1II (II-3', J = 9.5); 5.07 t, 1II (II-4', J = 9.7); 4.96 dd, 1II (II-2', J = 8.1 and 9.6); 4.65 d, 1II (II-1', J = 8.1); 4.25 dd, 1II (II-6'a; J = 11.7 and 5.3), 4.12 dd, 1II (II-6'b, J = 11.7 and 2.4); 3.67 m, 1II (II-3); 3.41 ddd, 1II (II-5', J = 2.8, 5.4 and 10.2); 2.12 s, 2.08 s, 2.03 s, 1.99 s, 4 \times 3H (4 \times CH_3CO); 0.89 d, 3II (3 \times II-21, J = 5.5); 0.87 bd, 6II (3 \times II-26, 3 \times II-27, J = 6.0); 0.79 s, 3II (3 \times II-19); 0.60 s, 3II (3 \times II-18). For $\text{C}_{41}\text{H}_{66}\text{O}_{11}$ (735.0) calculated: 67.00% C, 9.05% H; found: 67.02% C, 9.58% H.

3 β -(β -D-Glucopyranosyloxy)-5 α -cholestane-6 α -yl Sulfate, Sodium Salt (XI)

Derivative *IX* (40 mg, 54 μmol) was sulfated and deacetylated analogously as described for the preparation of derivative *V*. After crystallization from methanol-light petroleum 34 mg (93%) of derivative *XI* was obtained. M.p. 190 – 192 °C, $[\alpha]_D + 2^\circ$ (c 0.2, acetone). IR spectrum (KBr pellet): 3 417 (O-II); 1 054, 1 078 (C-O); 1 250, 1 230 (SO_2). ^1H NMR spectrum (CD_3OD , 200 MHz, referenced to δ 3.30 signal of methanol):

4.48 d, 1H (H-1', $J = 7.5$); 0.82 s, 3H (3 \times H-19); 0.65 s, 3H (3 \times H-18). For $C_{33}H_{57}NaO_{10}S$ (668.9) calculated: 59.26% C, 8.59% H, 4.79% S; found: 59.51% C, 8.67% H, 4.52% S.

3 β -Thexyldimethylsilyloxycholest-5-ene (XII)

To the solution of cholesterol (5.8 g, 15 mmol) in dichloromethane (60 ml), triethylamine (3 ml) and dimethylaminopyridine (70 mg, 0.57 mmol) thexyldimethylsilyl chloride (5.9 ml, 30 mmol) was added. The mixture was stirred at room temperature for 78 h, then diluted with ether and washed with water, 5% solution of citric acid, saturated solution of potassium hydrogen carbonate and water. After drying the solvent was removed and the solid residue was chromatographed on a column of alumina (230 g) in benzene–light petroleum–ether (49 : 50 : 1). After crystallization from benzene–acetone–methanol 4.0 g (50%) of derivative XII were obtained, m.p. 152–154 °C. IR spectrum (chloroform): 1 257, 847, 830 (Si–C); 1 100, 1 083 (C–O). 1H NMR spectrum (100 MHz): 5.32 d, 1H (H-6, $J = 4.0$); 3.47 m, 1H (H-3); 0.94 bd, 3H (3 \times H-21, $J = 6.0$); 0.88 bd, 6H ($CH(CH_3)_2$, $J = 7.8$); 0.85–0.83 m, 15H ($C(CH_3)_2$, 3 \times H-26, 3 \times H-27, 3 \times H-19); 0.67 s, 3H (3 \times H-18); 0.08 s, 6H ($Si(CH_3)_2$). For $C_{35}H_{64}OSi$ (529.0) calculated: 79.47% C, 12.20% H; found: 79.50% C, 12.56% H.

3 β -Thexyldimethylsilyloxy-5 α -cholestane-6 α -ol (XIII)

To a suspension of sodium borohydride (250 mg, 6.61 mmol) in tetrahydrofuran (20 ml) a solution of boron trifluoride etherate (0.5 ml, 4.1 mmol) in tetrahydrofuran (10 ml) was added via syringe through septum under argon at 0 °C. The resulting mixture was stirred for 30 min. Then, the solution of derivative XII (1.0 g, 1.89 mmol) in tetrahydrofuran (20 ml) was added and the mixture was stirred at 0 °C for 9 h. The mixture was then treated with aqueous solution of potassium hydroxide (0.5 g in 5 ml of water) and hydrogen peroxide (30%, 3 ml) and stirred again for 80 min at 0 °C. The product was extracted with ether (300 ml) and the ethereal phase was washed with water (4 \times 250 ml). After evaporation of the solvent, the crude product was purified by column chromatography (silica gel, 100 g) in light petroleum–ether (95 : 5). Yield of the product XIII was 720 mg (70%), m. p. 137–139 °C. IR spectrum (chloroform): 3 620, 3 600 sh, 3 450 (O–H); 1 100, 1 083 (C–O); 1 252 (Si–C). 1H NMR spectrum (100 MHz): 3.42 m, 2H (H-3, H-6); 0.89 bd, 3H (3 \times H-21, $J = 6.0$); 0.87 d, 6H ($CH(CH_3)_2$, $J = 7.8$); 0.83–0.80 m, 15H ($C(CH_3)_2$, 3 \times H-26, 3 \times H-27, 3 \times H-19); 0.64 s, 3H (3 \times H-18); 0.07 s, 6H ($Si(CH_3)_2$; after addition of TAI: 8.26 s, 1H ($CCl_3CONHCOO$); 4.70 m, 1H (H-6); 3.54 m, 1H (H-3); 0.90 bd, 3H (H-21, $J = 6.0$); 0.87 d, 6H ($CH(CH_3)_2$, $J = 7.8$); 0.83–0.80 m, 15H ($C(CH_3)_2$, 3 \times H-26, 3 \times H-27, 3 \times H-19); 0.64 s, 3H (3 \times H-18); 0.07 s, 6H ($Si(CH_3)_2$). For $C_{35}H_{66}O_2Si$ (547.0) calculated: 76.85% C, 12.16% H, 5.13% Si; found: 77.12% C, 12.60% H, 5.25% Si.

6 α -(2-Tetrahydropyranoyloxy)-3 β -thexyldimethylsilyloxy-5 α -cholestane (XIV)

3,4-Dihydro-2H-pyran (0.2 ml, 2.2 mmol) and *p*-toluenesulfonic acid monohydrate (2.8 mg, 15 μ mol) were added to a solution of derivative XIII (510 mg, 0.93 mmol) in benzene (6 ml) at 10 °C. After stirring at room temperature for 6 h the reaction mixture was diluted with ether (250 ml) and washed successively with water, saturated solution of potassium hydrogen carbonate and water. Organic layer was dried and the solvent was removed. The solid residue was chromatographed on a column of alumina (40 g) in light petroleum–ether (97 : 3). Yield of the oily product XIV was 411 mg (70%). IR spectrum (chloroform): 1 251, 868, 831 (Si–C); 1 075, 1 054, 1 023 (C–O). 1H NMR spectrum (200 MHz): 4.70 bs, 1H (H-2 of the tetrahydropyranoyloxy group); 3.50 m, 2H (H-3, H-6); 0.90 bd, 3H (H-21, $J = 6.0$); 0.88 bd, 6H ($CH(CH_3)_2$, $J = 7.7$); 0.86 bd, 6H (3 \times H-26, 3 \times H-27, $J = 6.0$); 0.84 s, 6H ($C(CH_3)_2$); 0.83 s, 3H (3 \times H-19); 0.64 s, 3H (3 \times H-18); 0.09 s, 6H ($Si(CH_3)_2$). For $C_{40}H_{74}O_3Si$ (631.1) calculated: 76.13% C, 11.82% H; found: 76.28% C, 12.00% H.

6 α -(2-Tetrahydropyranyloxy)-5 α -cholestane-3 β -ol (XV)

A) To the solution of derivative *XIV* (417 mg, 0.66 mmol) in tetrahydrofuran (20 ml) tetrabutylammonium fluoride (2 ml, 0.1 M solution in tetrahydrofuran) was added. The solution was stirred 15 h at 50 °C. Then the mixture was cooled, diluted with ether (300 ml) and washed with water (3 x). The organic layer was dried and the solvent removed. The solid residue was chromatographed on a column of alumina (30 g) in light petroleum-ether (6 : 4). Yield of derivative *XV* was 229 mg (71%), m.p. 94 – 96 °C. IR spectrum (chloroform): 3 609, 3 441 (O-H); 1 032, 1 022 (C=O). 1 H NMR spectrum (100 MHz): 4.78 bs, 1H (H-2 of the tetrahydropyranyloxy group); 3.52 m, 2H (H-3 and H-6); 0.90 bd, 3H (3 x H-21, J = 6.0); 0.86 bd, 6H (3 x H-26, 3 x H-27, J = 6.0); 0.83 s, 3H (3 x H-19); 0.64 s, 3H (3 x H-18). For $C_{32}H_{56}O_3$ (488.8) calculated: 78.63% C, 11.55% H; found: 77.78% C, 11.35% H.

B) Derivative *XVII* (150 mg, 0.26 mmol) was dissolved in benzene (15 ml), excess of the 1 M solution of potassium hydroxide in ethanol (to pH 10 – 12) was added and the mixture was refluxed for 24 h. The mixture was then cooled and diluted with ether (100 ml). The ethereal solution was washed with water to the neutral reaction, dried and evaporated. The solid residue was chromatographed on a column of alumina (15 g) in light petroleum-ether (8 : 2 to 6 : 4). Yield of derivative *XV* was 69 mg (54%).

Cholest-5-en-3 β -yl Pivalate (XVI)

To a solution of cholesterol (0.5 g, 1.29 mmol) and dimethylaminopyridine (158 mg, 1.3 mmol) in pyridine (5 ml) pivaloyl chloride (0.8 ml, 6.5 mmol) was added. After 5 h of stirring at room temperature the mixture was poured into ice, the precipitate was collected and crystallized from acetone. Yield of derivative *XVI* was 350 mg (58%), m.p. 162 – 163 °C. 1 H NMR spectrum (100 MHz): 5.31 bd, 1H (H-6, J = 4.5); 4.40 m, 1H (H-3); 1.17 bs, 9H ((CH₃)₃C); 0.90 d, 3H (3 x H-21, J = 6.0); 0.86 bd, 6H (3 x H-26, 3 x H-27, J = 6.0); 0.84 s, 3H (3 x H-19); 0.65 s, 3H (3 x H-18). For $C_{32}H_{54}O_2$ (470.8) calculated: 81.64% C, 11.56% H; found: 81.74% C, 11.50% H.

6 α -Hydroxy-5 α -cholestane-3 β -yl Pivalate (XVII)

This derivative was prepared from olefine *XVI* (300 mg, 0.64 mmol) by hydroboration analogously to derivative *XIII*. The product was purified by chromatography on a column of alumina (20 g) in light petroleum-ether (7 : 3). Yield of derivative *XVII* was 200 mg (64%), m.p. 194 – 196 °C, $[\alpha]_D + 36^\circ$ (c 0.28, methanol). 1 H NMR spectrum (200 MHz): 4.62 m, 1H (H-3); 3.40 m, 1H (H-6); 1.17 bs, 9H ((CH₃)₃C); 0.90 d, 3H (3 x H-21, J = 6.0); 0.86 bd, 6H (3 x H-26, 3 x H-27, J = 6.0); 0.84 s, 3H (3 x H-19); 0.65 s, 3H (3 x H-18). For $C_{32}H_{56}O_3$ (488.8) calculated: 78.63% C, 11.56% H; found: 79.00% C, 11.62% H.

6 α -(2-Tetrahydropyranyloxy)-5 α -cholestane-3 β -yl Pivalate (XVIII)

Derivative *XVII* (150 mg, 0.31 mmol) was reacted with 3,4-dihydro-2H-pyran and *p*-toluenesulfonic acid monohydrate in benzene analogously as described in the preparation of derivative *XIV*. After chromatography (alumina, 20 g) in light petroleum-ether (8 : 2) the yield of derivative *XVIII* was 157 mg (89%). 1 H NMR spectrum (200 MHz): 4.68 bs, 1H (H-2 of the tetrahydropyranyloxy group), 4.64 m, 1H (H-3); 1.17 bs, 9H ((CH₃)₃C); 0.90 d, 3H (3 x H-21, J = 6.0); 0.86 bd, 6H (3 x H-26, 3 x H-27, J = 6.0); 0.84 s, 3H (3 x H-19); 0.65 s, 3H (3 x H-18). For $C_{37}H_{64}O_4$ (572.9) calculated: 77.57% C, 11.26% H; found: 77.85% C, 11.13% H.

3 β -(2,3,4-Tri-O-acetyl- β -D-glucopyranosyloxy)-pregn-5-en-20-one (XXII)

To a stirred solution of glucoside¹⁰ *XIX* (1.5 g, 3.13 mmol) in pyridine (20 ml) cooled to 0 °C 4,4'-dimethoxytrityl chloride (2.0 g, 5.9 mmol) in pyridine (5 ml) was added. The stirring was continued at

0 °C for 2 h, then acetic anhydride (2.5 ml, 26.5 mmol) was added and the mixture was left aside at room temperature for 12 h. The solvent was evaporated (bath temperature 40 °C) and the residue coevaporated with benzene-triethylamine (100 : 1) mixture (2 × 20 ml). To a resulting white foam of derivative *XXI* 80% acetic acid (50 ml) was added and the solution was stirred at room temperature for 1 h. After evaporation of solvent and coevaporation with benzene (2 × 20 ml) crystallization from ethanol yielded 1.5 g (79%) of glucoside *XXII*, m.p. 138 – 142 °C (decomp.). IR spectrum (chloroform): 3 604 (O-II); 1 756, 1 702 (C=O); 1 040 (C-O). ¹H NMR spectrum (500 MHz): 5.36 dt, 1H (II-6, *J* = 5.4 and 2.2); 5.25 t, 1H (II-3', *J* = 10.0 and 9.5); 5.01 t, 1H (II-4', *J* = 9.9 and 9.5); 4.94 dd, 1H (II-2', *J* = 8.0 and 10.0); 4.63 d, 1H (II-1', *J* = 8.0); 3.72 dd, 1H (II-6'a, *J* = 12.5 and 2.5); 3.61 dd, 1H (II-6'b, *J* = 12.5 and 5.0); 3.52 ddd, 1H (II-5', *J* = 2.5, 5.0 and 9.9); 3.50 m, 1H (II-3); 2.53 t, 1H (II-17, *J* = 9.0); 2.13 s, 3H (II-21); 2.05 s, 2.02 s, 2 × 3H (2 × CH₃CO); 0.99 s, 3H (3 × II-19); 0.63 s, 3H (3 × II-18). For C₃₃H₄₈O₁₀ (604.7) calculated: 65.54% C, 8.00% H; found: 65.58% C, 8.02% H.

3β-(2,3,6-Tri-O-acetyl-β-D-glucopyranosyloxy)-pregn-5-en-20-one (*XXIII*)

The glycoside *XXII* (300 mg, 0.50 mmol) was dissolved in pyridine (5 ml) and water (5 ml) was added. The mixture was stirred for 48 h at room temperature, then the solvent was evaporated and the residue coevaporated with benzene (2 × 20 ml). Yield after crystallization from ethanol was 150 mg (50%), m.p. 198 – 203 °C. IR spectrum (chloroform): 3 479 (O-II); 1 752, 1 702 (C=O); 1 234, 1 086, 1 047, 1 018 (C-O). ¹H NMR spectrum (200 MHz): 5.36 bd, 1H (II-6, *J* = 5.2); 5.04 dd, 1H (II-3', *J* = 8.4 and 9.7); 4.90 dd, 1H (II-2', *J* = 7.8 and 9.6); 4.56 d, 1H (II-1', *J* = 7.7); 4.49 dd, 1H (II-6'a, *J* = 12.2 and 3.9); 4.29 dd, 1H (II-6'b, *J* = 12.2 and 2.2); 3.53 m, 3H (II-3, II-4', II-5'); 2.12 s, 6H (3 × II-21, CH₃CO); 2.09 s, 2.06 s, 2 × 3H (2 × CH₃CO); 0.99 s, 3H (3 × II-19); 0.63 s, 3H (3 × II-18). For C₃₃H₄₈O₁₀ (604.7) calculated: 65.54% C, 8.00% H; found: 65.49% C, 8.01% H.

20-Oxopregn-5-en-3β-yl 2,3,4-Tri-O-acetyl-β-D-glucopyranoside-6-sulfate, Triethylammonium Salt (*XXIV*)

Derivative *XXII* (156 mg, 0.26 mmol) was dissolved in chloroform (2 ml) and to this solution pyridine-sulfur trioxide complex (81 mg, 0.5 mmol) was added. The mixture was stirred under argon for 7 d and then 0.35 ml of triethylamine was added. After 60 min the solvent was removed and the solid residue was chromatographed on a column of silica gel (15 g) in benzene-ethanol-triethylamine (80 : 19 : 1). The oily product (96 mg, 47%) crystallized upon addition of few drops of ether; m.p. 154 – 156 °C. IR spectrum (chloroform): 2 651, 2 470 (N-II); 1 756, 1 700 (C=O); 1 250 (SO₂ overlap with C-O); 1 039, 1 005 (C-O). ¹H NMR spectrum (200 MHz): 9.57 m, 1H (NII); 5.36 bd, 1H (II-6, *J* = 5.3); 5.19 t, 1H (II-3', *J* = 9.5); 5.03 t, 1H (II-4', *J* = 9.6); 4.91 dd, 1H (II-2', *J* = 7.9 and 9.6); 4.58 d, 1H (II-1', *J* = 7.9); 4.14 d, 2H (II-6', *J* = 4.0); 3.78 m, 1H (II-5); 3.50 m, 1H (II-3); 3.15 m, 6H ((CH₃CH₂)₃NH); 2.13 s, 3H (3 × II-21); 2.05, 2.04, 1.99 s, 3 × 3H (3 × CH₃CO); 1.38 t, 9H ((CH₃CH₂)₃NH, *J* = 6.5); 0.98 s, 3H (3 × II-19); 0.63 s, 3H (3 × II-18). For C₃₉H₆₃NO₁₃S (786.0) calculated: 59.60% C, 8.08% H, 1.78% N, 4.08% S; found: 59.92% C, 7.89% H, 1.61% N, 3.98% S.

20-Oxopregn-5-en-3β-yl 2,3,6-Tri-O-acetyl-β-D-glucopyranoside-4-sulfate, Triethylammonium Salt (*XXV*)

Derivative *XXIII* (143 mg, 0.24 mmol) was sulfated analogously to the procedure described for derivative *XXIV*. The solid crude derivative *XXV* was chromatographed on silica gel (20 g) in benzene-ethanol-triethylamine (90 : 9 : 1) and 150 mg (81%) of oil was obtained. The oil crystallized upon addition of a few drops of ether after two days, m.p. 167 – 169 °C. IR spectrum (chloroform): 2 701 (N-II); 1 751, 1 699 (C=O); 1 239 (SO₂); 1 057, 1 040 (C-O). ¹H NMR spectrum (500 MHz): 9.41 m, 1H (NII); 5.36 bd, 1H

(II-6, $J = 5.0$); 5.26 t, 1II (II-3', $J = 10.0$); 4.95 dd, 1II (II-2', $J = 8.1$ and 10.0); 4.56 d, 1II (II-1', $J = 8.1$); 4.47 t, 1II (II-4', $J = 10.0$); 4.46 dd, 1II (II-6'a, $J = 12.0$ and 2.2); 4.29 dd, 1II (II-6'b, $J = 12.0$ and 6.1); 3.62 ddd, 1II (II-5', $J = 10.0$, 2.2 and 6.1); 3.48 tt, 1II (II-3, $J = 11.4$ and 4.9); 3.15 dq, 6H ((CH₃CH₂)₃NH, $J = 7.2$ and 4.9); 2.53 t, 1II (II-17, $J = 9.0$); 2.12 s, 3II (3 \times II-21); 2.08 s, 2.07 s and 2.04 s, 3 \times 3II (3 \times CH₃CO); 1.36 t, 9II ((CH₃CH₂)₃NH, $J = 7.2$); 0.98 s, 3II (3 \times II-19); 0.63 s, 3II (3 \times II-18). For C₃₉H₆₃NO₁₃S (786.0) calculated: 59.60% C, 8.08% H, 1.78% N, 4.08% S; found: 59.87% C, 7.92% H, 1.65% N, 3.99% S.

**20-Oxopregn-5-en-3 β -yl β -D-Glucopyranoside-6-sulfate,
Triethylammonium Salt (XXVI)**

Derivative **XXIV** (40 mg, 51 μ mol) was dissolved in the solution of triethylamine-methanol-water (1 : 1 : 0.5, 5 ml) and heated to 50 °C for 8 h. The solvent was then removed and the solid residue was chromatographed on reversed phase in methanol-water (1 : 1). The main fraction gave after evaporation of solvent 30 mg (89%) of oil, which crystallized upon addition of a few drops of acetone-ether (1 : 1) mixture; m.p. 132 – 134°C. IR spectrum (chloroform): 3 417 (O-II); 1 700 (C=O); 1 254, 1 197 (SO₂); 1 005 (C-O). For C₃₃H₅₇NO₁₀S (659.9) calculated: 60.07% C, 8.71% H, 2.12% N, 4.86% S; found: 59.89% C, 8.95% H, 2.08% N, 4.57% S.

**20-Oxopregn-5-en-3 β -yl 2,3-Di-O-acetyl- β -D-glucopyranoside-4-sulfate,
Triethylammonium Salt (XXVII)**

Derivative **XXV** (70 mg, 89 μ mol) was heated in methanol-water-triethylamine (1 : 1 : 0.5, 7 ml) mixture to 50 °C for 7 days. The solvent was then removed and solid residue was chromatographed on a column of silica gel (10 g) in benzene-ethanol-triethylamine (90 : 9 : 1) and (80 : 19 : 1). Yield of the oily derivative **XXVII** was 45 mg (68%). IR spectrum (chloroform): 3 452 (O-II); 2 701 (N-II); 1 734, 1 700, 1 664, 1 630 (C=O); 1 239 (SO₂); 1 056, 1 040 (C-O). ¹H NMR spectrum (200 MHz): 5.35 bd, 1H (II-6, $J = 4.5$); 5.23 t, 1II (II-3', $J = 9.8$); 4.93 dd, 1II (II-2', $J = 8.6$ and 10.0); 4.51 d, 1II (II-1', $J = 7.8$); 4.46 t, 1II (II-4', $J = 9.8$); 3.90 m, 2II (II-6'); 3.55 – 3.35 m, 2II (II-5' and II-3'); 3.13 m, 6H ((CH₃CH₂)₃NH); 2.12 s, 3II (3 \times II-21); 2.06 s, 2.04 s, 2 \times 3II (2 \times CH₃CO); 1.37 t, 9II ((CH₃CH₂)₃NH, $J = 7.0$); 0.98 s, 3II (3 \times II-19); 0.63 s, 3II (3 \times II-18). For C₃₇H₆₁NO₁₂S (744.0) calculated: 59.74% C, 8.26% H, 1.88% N, 4.31% S; found: 60.01% C, 8.20% H, 1.61% N, 4.03% S.

5 α -Cholestan-3 β -yl 2,3,4-Tri-O-acetyl- β -D-glucopyranoside (XXIX)

From glucoside **XXVIII** (337 mg, 0.61 mmol) as in preparation of **XXII** 146 mg (35%) of derivative **XXIX** was obtained, m.p. 180 – 185 °C (ethanol), $[\alpha]_D + 4.7^\circ$ (c 0.2, chloroform). IR spectrum (chloroform): 3 600 (O-II); 1 756 (C=O); 1 252, 1 040 (C-O). ¹H NMR spectrum (500 MHz): 5.24 t, 1II (II-3', $J = 9.8$ and 9.3); 5.01 t, 1II (II-4', $J = 10.0$ and 9.3); 4.92 dd, 1II (II-2', $J = 8.0$ and 9.8); 4.63 d, 1II (II-1', $J = 8.0$); 3.72 dd, 1II (II-6'a, $J = 12.2$ and 5.3); 3.60 ddd, 1II (II-6'b, $J = 12.2$, 5.0 and 2.7); 3.56 tt, 1II (II-3, $J = 11.2$ and 4.8); 3.52 ddd, 1II (II-5', $J = 2.7$, 5.3 and 10.0); 2.05 s, 2.04 s, 2.01 s, 3 \times 3II (3 \times CH₃CO); 0.89 d, 3II (3 \times II-21, $J = 6.5$); 0.86 d, 0.85 d, 2 \times 3II (3 \times II-26, 3 \times II-27, $J = 6.7$); 0.78 s, 3II (3 \times II-19); 0.64 s, 3II (3 \times II-18). For C₃₉H₆₄O₉ (676.9) calculated: 69.20% C, 9.53% H; found: 69.01% C, 9.56% H.

**5 α -Cholestan-3 β -yl 2,3,4-Tri-O-acetyl- β -D-glucopyranoside-6-sulfate,
Triethylammonium Salt (XXX)**

Derivative **XXIX** (130 mg, 0.19 mmol) was sulfated analogously to the procedure described for derivative **XXIV**. After 7 days the solvent was removed, resulted oil (130 mg, 79%) spontaneously crystallized; m.p.

173 – 175 °C. IR spectrum (chloroform): 2 651, 2 473 (N-II); 1 756 (C=O); 1 250 (SO₂ overlap with C=O), 1 038 (C=O). ¹H NMR spectrum (200 MHz): 9.60 m, 1H (NII); 5.19 t, 1H (H-3', *J* = 10.0 and 11.0); 5.00 t, 1H (H-4', *J* = 10.0); 4.89 bdd, 1H (H-2', *J* = 8.0 and 10.0); 4.58 d, 1H (H-1', *J* = 8.0); 4.12 bd, 2H (H-6', *J* = 4.0); 3.55 m, 1H (H-3); 3.38 m (1H, H-5'); 3.18 m, 6H ((CH₃CH₂)₃NH); 2.04 s, 2.03 s, 1.98 s, 3 × 3H (3 × CH₃CO); 1.37 t, 9H ((CH₃CH₂)₃NH, *J* = 6.5); 0.89 d, 3H (3 × H-21, *J* = 6.0); 0.86 bd, 6H (3 × H-26, 3 × H-27, *J* = 6.0); 0.78 s, 3H (3 × H-19); 0.64 s, 3H (3 × H-18). For C₄₅H₇₉NO₁₂S (858.2) calculated: 62.98% C, 9.28% H, 1.63% N, 3.74% S; found: 63.23% C, 8.99% H, 1.49% N, 3.48% S.

5α-Cholestan-3β-yl β-D-Glucopyranoside-6-sulfate, Triethylammonium Salt (XXXI)

Derivative XXX (100 mg, 0.12 mmol) was deacetylated analogously to the procedure described for derivative XXVI. After chromatography (reverse phase) in methanol–water (7 : 3 to 9 : 1) 70 mg (82%) of derivative XXXI was obtained; m.p. 119 – 122 °C. IR spectrum (chloroform): 3 417 (O-II); 2 650, 2 473 (N-II); 1 254, 1 197 (SO₂); 1 005 (C=O). For C₃₉H₇₃NO₉S (732.1) calculated: 63.99% C, 10.05% H, 1.91% N, 4.38% S; found: 64.20% C, 9.88% H, 1.73% N, 4.11% S.

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