STEROID AND BILE ACIDS AMIDE CONJUGATES WITH D-GLUCOSAMINE

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Received October 27, 2010
Accepted December 6, 2010
Published online January 5, 2011

New type of amide conjugates of steroid and bile acids with D-glucosamine **1** and **2** were prepared. Title compounds are prepared *via* acid chloride or using *N*-[({[(1*E*)-1-cyano-2-ethoxy-2-oxoethylidene]amino}oxy)(dimethylamino)methylidene]-*N*-methylmethanaminium tetrafluoroborate as condensation agent. They were examined for gelation properties with negative results. Per-*O*-acetylated D-glucosamine hydrochloride was prepared in one step procedure from D-glucosamine hydrochloride by acetylation in a mixture of acetyl chloride and acetic acid.

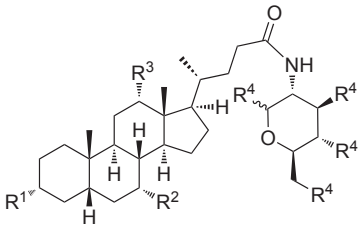
Keywords: Steroids; Carbohydrates; Amides; Bile acids.

In the search of carbohydrate conjugated steroids and their supramolecular properties we found interesting new properties[1] as well as in the group of steroids conjugated through amide function[2]. Some of the amide conjugates them are well known for their gelation properties[3,4]. The aim of preparation of the new type of the title compounds was to enable their utilization in supramolecular structures[5]. Similar types of compounds, derived from 3β,7β-dihydroxy-5-cholen-24-oic acid and 3β-hydroxy-7-oxo-5-cholen-24-oic acid were prepared as reference compounds for the study of abnormalities of bile acid synthesis and of the metabolism of NP-C1 patients[6]. As gelators were synthesized amino acid conjugates of bile acids. The reaction was carried out in the presence of diethyl phosphoryl cyanide as condensation agent[7]. Similarly, as an organogelator conjugate of cholic acid and 5-amino-1,10-phenanthroline was prepared[2]. As surface active compound it was synthesized conjugate of dehydrocholic acid[8], using *N*-mercaptothiazoline active intermediate. The compounds were prepared as surfactants, solubilizers of linoleic acid without denaturing soybean lipoxygenase to have potential applications in membranology, enzymology and extraction of proteins from biological systems. Such types of amide

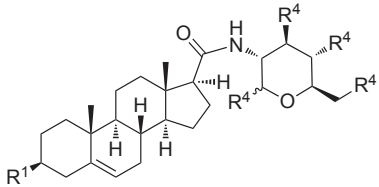
doi:10.1135/cccc2010105

conjugates are i.a. suitable for construction of synthetic immunomodulators[9], as liposome formation modulators, or as components of constructs and products exhibiting transport and complexing properties to medicinal substances. In some aspects they could be also considered as a part of the group of compounds with a heterocycle linked to the side chain of steroid, cf. e.g. ref.[10].

In this study, presented preliminarily on a Czech conference[11], we prepared and studied two main types of sugar-steroid amide conjugates, one derived from bile acids as cholic, lithocholic, deoxycholic (type A) and second, derived from etienic acid (type B).



type A



type B

Ia, $R^1 = R^2 = R^3 = R^4 = OH$
Ib, $R^1 = R^4 = OH$; $R^2 = R^3 = H$
Ic, $R^1 = R^2 = R^3 = O=$; $R^4 = OH$
If, $R^1 = R^3 = R^4 = OH$; $R^2 = H$
IIa, $R^1 = R^2 = R^3 = OCOH$; $R^4 = OAc$
IIb, $R^1 = OCOH$; $R^4 = OAc$; $R^2 = R^3 = H$
IIc, $R^1 = R^2 = R^3 = O=$; $R^4 = OAc$
IIf, $R^1 = R^3 = OCOH$; $R^2 = H$; $R^4 = OAc$
IIg, $R^1 = R^3 = R^4 = OCOH$; $R^2 = H$

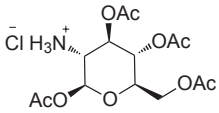
Id, $R^1 = R^4 = OH$
IId, $R^1 = OCOH$; $R^4 = OAc$
IIe, $R^1 = ONO_2$; $R^4 = OAc$
IIh, $R^1 = OCOH$; $R^4 = OH$
IIi, $R^1 = ONO_2$; $R^4 = OH$

RESULTS AND DISCUSSION

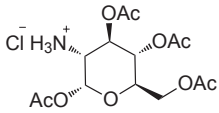
Method of preparation of the amide conjugates consists of the reaction of protected steroid acid chloride with protected D-glucosamine or of the reaction of protected steroid acid with protected or unprotected D-glucosamine with condensation agent TOTU (*N*-[({[(1*E*)-1-cyano-2-ethoxy-2-oxoethylidene]amino}oxy)(dimethylamino)methylidene]-*N*-methylmethanaminium tetrafluoroborate), both with reasonable results.

For all syntheses there was developed, however, one step preparation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride (**3a**) and a mixture of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α/β-D-gluco-

pyranose hydrochlorides **3a** and **3b** by acetylation in a mixture of acetyl chloride and acetic acid[12]. For the synthesis of steroid amide conjugates protected glucosamine **3a** was used. After deprotection we did not analyze the anomeric purity of the conjugates as it was not the prime aim of the study, the lipophility of both being almost identical.



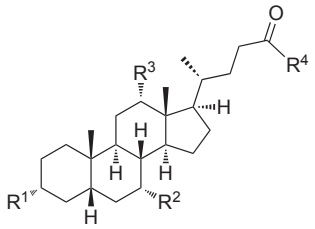
3a



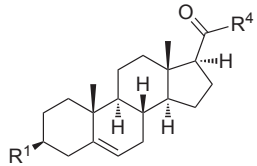
3b

The reaction procedure developed gives reasonable yield of the β-anomer and enables simple work-up. The procedure given in Experimental gives the best yields, change of the reaction conditions give either lower yield or contaminant α-anomer. Both were distinguished by the NMR spectra, where for α-anomer we used as distinguishing marker peat at 6.45 d (1 H, $J_{1,2}$ = 3.81 Hz, H-1) and for β-anomer similarly at 5.85 d (1 H, $J_{1,2}$ = 8.79 Hz, H-1).

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride and its anomeric partner are usually prepared by multistep reaction[13] or with special catalysts[14].



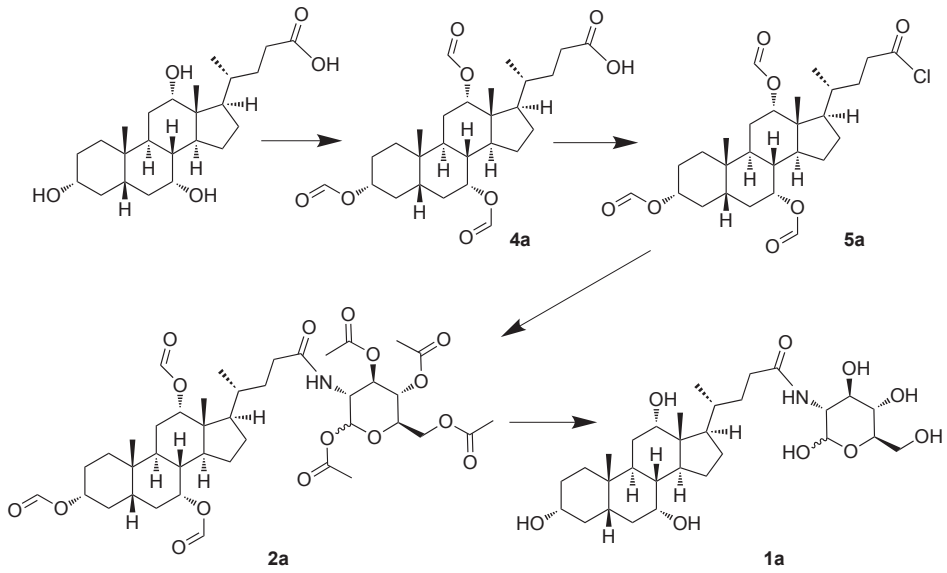
type A



type B

IVa, $R^1 = R^2 = R^3$ = OCOH; R^4 = OH
IVb, R^1 = OCOH; R^4 = OH; $R^2 = R^3$ = H
IVf, $R^1 = R^3$ = OCOH; R^2 = H; R^4 = OH
Va, $R^1 = R^2 = R^3$ = OCOH; R^4 = Cl
Vb, R^1 = OCOH; R^4 = Cl; $R^2 = R^3$ = H
Vc, $R^1 = R^2 = R^3$ = O=, R^4 = Cl
Vf, $R^1 = R^3$ = OCOH; R^2 = H; R^4 = Cl

IVd, R^1 = OCOH; R^4 = OH
Vd, R^1 = OCOH; R^4 = Cl



SCHEME 1
Example of synthetic pathway

Starting protected steroids were formylated by formic acid[2], or nitrated by a mixture of nitric acid–acetic acid. Steroid acid chlorides were prepared by the reaction with oxalyl chloride. Deprotection of acyl protecting groups on sugar and steroid part was done by alkaline hydrolysis. Cleavage of nitrates was done by the usage of zinc in protic media[15] (Scheme 1).

Conjugates synthesized were examined (for concentrations 5 and 10 mg ml^{-1}) for their gelation properties in methanol, chloroform, dichloromethane, acetone, toluene, ethanol, water and also their mixtures (1:1) at room and lowered temperatures (+4 °C). There was also tried a method from literature[16] where the addition of SDS (sodium dodecyl sulfate) was used to induce gelation. Different changes of temperature were tested for the induction of gelation but in all cases we observed negative results. Further properties are being investigated. These new types of steroidal derivatives could be further simply derived to control their polarity as well as noncovalent bonding (H-bond) capabilities.

EXPERIMENTAL

TLC was performed on HF_{254} plates (Merck), detection by UV light or by spraying with a solution of 5 g of $Ce(SO_4)_2(H_2O)_4$ in 500 ml of 10% H_2SO_4 and subsequent heating. Flash column chromatography was performed on silica gel (Merck, 100–160 μm) in solvents, dis-

tilled prior to use. Optical rotations were measured in chloroform solutions on a Rudolph Research Autopol IV polarimeter at 25 °C and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} with concentration in 10 g l^{-1}. Routine UV spectra were recorded on a Varian Cary 50 UV-VIS spectrometer; wavelengths are given in nm, molar absorptivity ε in m^2 mol^{-1} (in parentheses). IR spectra (wavenumbers in cm^{-1}) were recorded on a Perkin–Elmer PE 580 spectrometer in $CHCl_3$ solutions at 23 °C, unless stated otherwise. ^{1}H and ^{13}C NMR spectra were taken in deuterochloroform (Aldrich, 99.8% D) on a Bruker AVANCE 500 (500.1 MHz for ^{1}H) FT NMR spectrometer at 300 K if not stated otherwise. As standard, the internal signal of tetramethyl silane (δ 0.0) for ^{1}H and central line of solvent (δ 77.0) for ^{13}C spectra were used. Chemical shifts are presented in ppm (δ-scale), coupling constants (*J*) in Hz. Mass spectra were taken on a Q TOF micromass spectrometer with direct inlet (ESI) or on a ZAB-EQ (VG Analytical) instrument (FAB) with Xe ionization, accelerating voltage 8 kV. Microanalysis was performed on an elemental analyzer Perkin–Elmer 2400 Series II CHNS/O.

All solvents used were of highest degree of purity and used as received. Steroids were purchased from Steraloids, Inc. USA.

2-Deoxy-2-{[3α,7α,12α-trihydroxy-24-oxo-5α-cholan-24-yl]amino}-D-glucopyranose (**1a**)

Method A. To cholic acid triformate[17] **4a** (1.7 g, 3.6 mmol) benzene (30 ml) and oxalyl chloride (5.5 ml, 30 mmol) were added at room temperature. The reaction mixture was stirred for 1 h, and after that evaporated to dryness. The obtained chloride of cholic acid triformate[2] **5a** was mixed with protected glucosamine **3a** (1.4 g, 3.6 mmol), pyridine (50 ml) and dichloromethane (50 ml), and the reaction mixture was stirred at room temperature for 5 h. Then, methanol (50 ml) was added and the mixture was stirred for another 1 h, and after that evaporated to dryness and chromatographed on a column of silica (200 g) in toluene–acetone. The obtained protected conjugate **2a** (541 mg, 0.66 mmol, 18.5%) was a yellowish amorphous powder of $[\alpha]_D^{20}$ +65.2 (*c* 0.5, $CHCl_3$). The conjugate **2a** was dissolved in methanol (30 ml), and 0.05 M MeONa (12 ml, methanolic solution) was added. The mixture was stirred for 20 h. After all **2a** was gone, the mixture was neutralized by Dowex 50. Dowex 50 was filtered off and the filtrate evaporated to 416 mg yellowish solid which was further purified on a column of silica (25 g) in chloroform–methanol. Obtained **1a** (230 mg, 0.4 mmol, 61.3%) was a yellowish solid of $[\alpha]_D^{20}$ +36.4 (*c* 0.25, CH_3OH). ^{1}H NMR: 0.38 s (3 H, H-21); 0.55 s (3 H, H-18); 0.65 s (3 H, H-19); 0.70–2.10 m (23 H, H-steroid bundle); 2.95–4.8 (characteristic protons of saccharide). IR: ν(OH) + ν(H_2O): 3401 vs, vbr; ν(C–OH): 1077 s, 1042 s; amide I: 1671 s, br; amide II: 1537 m, br; amide III: 1301 m, br; δ_s (CH_3): 1377; β_s (CH_2) α to C=O: 1416 m. For $C_{30}H_{51}NO_9$ (569.73) calculated: monoisotopic mass 569.3564, MS-ESI found: [M + Na$^+$] 592.44; calculated: 63.24% C, 9.02% H, 2.46% N, found: 62.32% C, 8.86% H, 2.20% N.

Method B. Hydrochloride **3a** (583 mg, 1.5 mmol), cholic acid triformate[14] **4a** (536 mg, 1.09 mmol) and *N*-[({[(1*E*)-1-cyano-2-ethoxy-2-oxoethylidene]amino}oxy)(dimethylamino)-methylidene]-*N*-methylmethanaminium tetrafluoroborate (433 mg, 1.32 mmol) were dissolved in dichloromethane (20 ml) and *N*,*N*′-dimethylformamide (5 ml), and *N*-ethyldiisopropyl amine (0.8 ml, 5.8 mmol) was added to the reaction mixture. Then the mixture was stirred at room temperature for 30 h and finally distributed between chloroform and water (50 ml each). The protected conjugate **2a** was separated in organic phase, evaporated and further purified on a column of silica (60 g) in chloroform–methanol. The obtained **2a** (560 mg, 0.68 mmol, 45%) was a yellowish substance. It was dissolved in methanol (30 ml)

and treated with 0.05 M MeONa (12 ml, methanolic solution) for 20 h under stirring at room temperature. The reaction mixture was neutralized by Dowex 50, filtered and evaporated. The obtained yellowish material (416 mg) was purified on a column of silica (25 g) in chloroform–methanol. It was obtained **1a** (230 mg, 0.4 mmol, 61.3%) as a yellowish amorphous material of the same properties as under *A*).

2-Deoxy-2-{[3α-hydroxy-24-oxo-5β-cholan-24-yl]amino}-D-glucopyranose (**1b**)

Method A. Lithocholic acid formate[18] **4b** (482 mg, 1.2 mmol) was mixed with benzene (20 ml) and oxalyl chloride (1.5 ml, 17.2 mmol). The reaction was carried out during 1 h at room temperature under stirring. Then it was evaporated to dryness and the obtained chloride **5b** immediately mixed with pyridine (20 ml), dichloromethane (20 ml), carbohydrate **3a** (435 mg, 1.1 mmol). The resulted mixture was stirred at room temperature for 5 h. Then it was diluted with methanol (15 ml) and further stirred for 1 h, evaporated and chromatographed on a column of silica (60 g) in toluene–acetone. It was obtained **2b** (250 mg, 0.32 mmol, 28%) as a white amorphous solid of $[\alpha]_D^{20}$ +48 (*c* 1, $CHCl_3$). ^{1}H NMR: 0.60 s (3 H, H-21); 0.86 s (3 H, H-18); 0.92 s (3 H, H-19); 1.0–2.0 m (20 H, H-steroidal bundle); 2.02 s (3 H, Ac-$C_{2'}$); 2.08 s (3 H, Ac-$C_{4'}$); 2.18 s (3 H, Ac-$C_{3'}$); 2.30 s (3 H, Ac-$C_{1'}$); 4.0–6.2 (characteristic protons of carbohydrate); 4.82 s (1 H, NH). For $C_{39}H_{59}NO_{12}$ (733.89) calculated: 63.83% C, 8.10% H, 1.91% N; found: 63.55% C, 8.30% H, 1.70% N.

Conjugate **2b** (342 mg, 0.44 mmol) was dissolved in methanol (30 ml) and treated with 0.05 M MeONa (10 ml, methanolic solution) for 20 h under stirring at room temperature. The reaction mixture was neutralized by Dowex 50, filtered and evaporated. The obtained yellowish material was purified on a column of silica (25 g) in toluene–acetone. It was obtained **1b** (103 mg, 0.18 mmol, 41%) of $[\alpha]_D^{20}$ + 41.2 (*c* 0.5, CH_3OH). ^{1}H NMR: 0.70 s (3 H, H-21); 0.93 s (3 H, H-18); 0.95 s (3 H, H-19); 1.0–2.4 m (20 H, H-steroid); 3.2–5.1 (characteristic range of carbohydrate protons). IR: ν(OH) + ν(H_2O): 3421 vs, br; ν(C–OH): 1068 s, 1039 s; amide I: 1653 s, br; amide II: 1541 m, br; amide III: 1304 w, br; $\delta_s(CH_3)$: 1376 m; β_s (CH_2) α to C=O: 1419 m. For $C_{30}H_{51}NO_7$ (537.73) calculated: monoisotopic mass 537.3666, MS-ESI found: [M + Na^+] 560.40; calculated: 67.01% C, 9.56% H, 2.60% N, found: 62.35% C, 9.37% H, 2.18% N.

Method B. Carbohydrate **3a** (583 mg, 1.5 mmol), lithochoic acid formate[15] **4b** (485 mg, 1.2 mmol) and *N*-[({[(1*E*)-1-cyano-2-ethoxy-2-oxoethylidene]amino}oxy)(dimethylamino)-methylidene]-*N*-methylmethanaminium tetrafluoroborate (492 mg) were dissolved in dichloromethane (20 ml) and *N*,*N*′-dimethyl formamide (5 ml) and *N*-ethyldiisopropyl amine (0.8 ml, 5.8 mmol) and the resulting mixture was stirred at room temperature for 30 h. The mixture was distributed between chloroform and water. The protected conjugate was taken into organic phase which was evaporated and further purified on a column of silica (50 g) in toluene–acetone with the yield of white amorphous **2b** (400 mg (45%) of the same properties as under *A*).

2-Deoxy-2-{[3,7,12,24-tetraoxo-5β-cholan-24-yl]amino}-D-glucopyranose (**1c**)

Method A. Carbohydrate **3a** (540 mg, 1.4 mmol), dehydrocholic acid (456 mg, 1.13 mmol) and *N*-[({[(1*E*)-1-cyano-2-ethoxy-2-oxoethylidene]amino}oxy)(dimethylamino)methylidene]-*N*-methylmethanaminium tetrafluoroborate (526 mg) were mixed in dichloromethane (20 ml) and *N*-ethyldiisopropyl amine (0.8 ml, 5.8 mmol), and the mixture was stirred at room temperature for 30 h. Then it was evaporated and purified on a column of silica (100 g)

in chloroform–methanol. There was obtained conjugate **2c** as a white amorphous solid (306 mg, 0.54 mmol, 38.3%) that was dissolved in methanol (20 ml) and treated with 0.05 M MeONa (8 ml, methanolic solution) for 2 h under stirring at room temperature. The reaction mixture was neutralized by Dowex 50, filtered and evaporated. The obtained yellowish material was purified on a column of silica (25 g) in chloroform–methanol to yield yellowish amorphous conjugate **1c** (68 mg, 0.12 mmol, 25%) of $[\alpha]_D^{20}$ +24.8 (*c* 0.25, CH_3OH). ^{1}H NMR: 0.85 s (3 H, H-21); 1.10 s (3 H, H-18); 1.40 s (3 H, H-19); 1.5–2.55 m (20 H, characteristic steroid bundle); 2.8–5.5 (characteristic saccharide protons). IR: ν(OH) + ν(H_2O): 3418 s, vbr; ν(C–OH): 1053 s; ν(C=O): 1710 vs; amide I: 1655 m, br; amide II: 1530 m, br – bonded, 1511 m – free; δ_s (CH_3): 1382 m, 1365 w; β_s (CH_2) α to C=O: 1434 m. For $C_{38}H_{45}NO_9$ (563.68) calculated: monoisotopic mass 563.3094, MS-ESI found: [M] 564.3; calculated: 63.92% C, 8.05% H, 2.42% N, found: 63.60% C, 7.95% H, 2.13% N.

Method B. Dehydrocholic acid (806 mg, 2 mmol) was dissolved in benzene (20 ml) and oxalyl chloride (3 ml, 34.4 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. It was evaporated and chloride **5c** immediately mixed with pyridine (20 ml) and carbohydrate **3a** (300 mg, 0.78 mmol). The mixture was stirred at room temperature for 24 h. Then it was mixed with methanol (15 ml), stirred for 1 h and evaporated. The residue was purified on a silica gel column (100 g) in toluene–acetone. There was obtained **1c** (195 mg, 34%) as a white amorphous material of identical properties as under *A*).

2-Deoxy-2-{[3α,12α-dihydroxy-24-oxo-5β-cholan-24-yl]amino}-D-glucopyranose (**1f**)

Method A. Steroid acid formate[15] **4f** (538 mg, 1.2 mmol) was mixed with benzene (20 ml) and oxalyl chloride (1.5 ml, 17.2 mmol). The reaction was carried out during 1 h at room temperature under stirring. Then it was evaporated to dryness and the obtained chloride **5f** was immediately mixed with pyridine (50 ml) and carbohydrate **3a** (400 mg, 1.04 mmol), and stirred at room temperature for 5 h. Then the mixture was mixed with methanol (15 ml), stirred for further 1 h and evaporated. The residue was purified on a silica column (150 g) in toluene–acetone. It was obtained white amorphous **2f** (517 mg, 64.9%) of $[\alpha]_D^{20}$ 65.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR: 0.72 s (3 H, H-18); 0.82 s (3 H, H-21); 0.92 s (3 H, H-19); 1.0–2.0 m (20 H, steroidal bundle); 2.02 s (3 H, Ac-$C_{6'}$); 2.08 s (3 H, Ac-$C_{4'}$); 2.19 s (3 H, Ac-$C_{3'}$); 2.35 s (3 H, Ac-$C_{1'}$); 4.5 m (1 H, H-2′); 4.84 m (1 H, H-4′); 5.6 m (1 H, H-3′); 5.7 m (1 H, H-3); 6.15 m (1 H, H-2′); 7.05 s (1 H, NH); 8.02 s (1 H, CHO-C_{12}); 8.12 s (1 H, CHO-C_3). For $C_{40}H_{59}NO_{14}$ (777.89) calculated: monoisotopic mass 777.3936, MS-ESI found: [M + Na^+] 800.3429.

Amide **2f** (100 mg, 0.13 mmol) was dissolved in methanolic 0.03 M CH_3ONa (60 ml). After 1 h, the reaction was neutralized by adding Dowex H^+ and filtered. After evaporation the residues were purified on a silica gel column (40 g) in chloroform. It was obtained white amorphous **1f** (50 mg, 70%) of $[\alpha]_D^{20}$ + 36.4 (*c* 0.25, CH_3OH). ^{1}H NMR: 0.38 s (3 H, H-21); 0.55 s (3 H, H-18); 0.65 s (3 H, H-19); 0.70–2.10 m (23 H, H-steroid); 2.95–4.8 (saccharide part). For $C_{30}H_{51}NO_8$ (553.72) calculated: monoisotopic mass 553.3615, MS-ESI found: [M + Na^+] 592.44; calculated: 65.07% C, 9.28% H, 2.53% N, found: 58.32% C, 8.56% H, 2.00% N.

Method B. Deoxycholic acid formate **4f** (449 mg, 1 mmol), carbohydrate **3a** (461 mg, 1.2 mmol) and *O*-[(ethoxycarbonyl)cyanomethylenamino]-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (394 mg, 1.2 mmol) were mixed with dichloromethane (20 ml), DMF (8 ml) and *N*-ethyldiisopropylamine (0.6 ml) was added. The reaction was stirred under argon for 24 h. The reaction mixture was extracted three times between $CHCl_3$ and water.

Organic phases were dried by $MgSO_4$ and purified on a silica gel column (50 g) in toluene–acetone. There was obtained white amorphous **2f** (290 mg, 37%) of identical properties as under *A*).

2-Deoxy-2-({[3α-(hydroxy)androst-5-en-17β-yl]carbonyl}amino)-D-glucopyranose (**1d**)

Method A. To etienic acid formate **4d** (346 mg, 1 mmol, prepared by the known procedure[15] from etienic acid[19]), benzene (30 ml) and oxalyl chloride (1 ml, 5.4 mmol) were added and the mixture was stirred at room temperature for 1 h and after that evaporated to dryness. Chloride **5d** was taken into dichloromethane (10 ml) and added dropwise to the solution of carbohydrate **3a** (210 mg, 0.4 mmol) in pyridine (30 ml). The mixture was stirred at room temperature for 3 h. Then the reaction was quenched by addition of MeOH (15 ml) and the mixture was evaporated to dryness. The residue was chromatographed on silica (150 ml) in dichloromethane–methanol with the yield of conjugate **2d** (113 mg, 46.2%) in the form of yellowish amorphous solid. ^{1}H NMR: 0.74 s (3 H, 3 × H-18); 1.02 s (3 H, 3 × H-19); 2.02 s (3 H, 3 × H-acetyl); 2.08 s (3 H, 3 × H-acetyl); 2.16 s (3 H, 3 × H-acetyl); 2.36 m (3 H, H-4, H-16, H-17); 4.02 m (2 H, 2 × H-6′); 4.25 m (1 H, H-3); 5.38 m (1 H, H-6); 8.02 s (1 H, H-formyl). Conjugate **2d** was dissolved in methanol (20 ml) and treated with 0.05 M MeONa (8 ml, methanolic solution) for 20 h under stirring at room temperature. The reaction mixture was neutralized by Dowex 50, filtered and evaporated. After evaporation, the residues were purified on a silica gel column (25 g) in chloroform–methanol. It was obtained white amorphous **1d** (88 mg, 93%) of $[\alpha]_D^{20}$ + 0.7 (*c* 0.5, CH_3OH). ^{1}H NMR: 0.7 m (3 × H-18); 1.02 s (3 × H-19); 1.05–2.36 m (22 × H-steroid); 3.73 m (6 × H, $C_{2'}$-$C_{6'}$); 5.14 m (1 H-1′), 5.35 m (1 × H-6). For $C_{26}H_{41}NO_7$ (479.62) calculated: monoisotopic mass 479.2883, MS-ESI found: [M + H$^+$] 480.3.

Method B. Etienic acid formate **4d** (346 mg, 1 mmol), nonprotected glucosamine hydrochloride (258 mg, 1.2 mmol) and *O*-[(ethoxycarbonyl)cyanomethylenamino]-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (394 mg, 1.2 mmol) were mixed with DMF (20 ml) and *N*-ethyldiisopropyl amine (0.5 ml) was added. The reaction mixture was stirred under argon for 24 h. Then it was extracted three times between ether and water. Organic phases were dried by $MgSO_4$ and purified on a silica gel column (40 g) in chloroform–methanol. There was obtained white amorphous **2h** (220 mg, 43%). For $C_{27}H_{41}NO_8$ (507.63) calculated: monoisotopic mass 507.283217, MS-ESI found: [M + Na$^+$] 508.2. This product was immediately deprotected as given for amide **2d** with the yield of **1d** (88 mg, 93%) identical as under *A*).

2-Deoxy-2-({[3α-(hydroxy)androst-5-en-17β-yl]carbonyl}amino)-D-glucopyranose (**1d**)

Method A. Etienic acid nitrate[20] **4e** (365 mg, 1 mmol) was dissolved in benzene (30 ml) and oxalyl chloride (2 ml, 10.8 mmol) was added. The mixture was stirred at room temperature for 1 h. Then the reaction mixture was evaporated to dryness. Crude **5e** was dissolved in dichloromethane (10 ml). 1,3,4,6-Tetra-*O*-acetyl-D-glucosamine hydrochloride (210 mg, 0.4 mmol) was dissolved in pyridine (30 ml) and to this solution the above chloride in dichloromethane was added. The mixture was stirred at room temperature for 3 h. Then methanol (15 ml) was added, and the reaction mixture was evaporated to dryness and twice co-evaporated with toluene. The residue was chromatographed on a silica gel column (50 g) in toluene–ethanol. The chromatography afforded **2e** (154 mg, 61%) as a yellowish amorphous solid. ^{1}H NMR: 0.98 s (3 H, 3 × H-18); 1.02 s (3 H, 3 × H-19); 1.04–1.95 m (20 H, 20 ×

H-steroid); 1.98 m (3 H, 3 × H-acetyl); 2.01 m (3 H, 3 × H-acetyl); 4.21 m (2 H, 2 × H-6′); 4.90 m (1 H, H-3); 5.53 m (1 H, H-6); 5.91 m (1 H, H-1′). For $C_{34}H_{48}N_2O_{13}$ (692.77) calculated: monoisotopic mass 692.3156, MS-ESI found: 693.5 [M + H^+]. Amide **2e** (350 mg, 0.5 mmol) was dissolved in THF (13.7 ml), acetic acid (3.4 ml) and distilled water (0.7 ml). Zinc powder (511 mg, 7.8 mmol) was added in small portions during 3 h. The reaction mixture was filtered and washed with chloroform. The filtrate was extracted successively with water, $NaHCO_3$ and water again. Organic phases were evaporated and dissolved in 0.1 M MeONa (10 ml, methanolic solution) for 1 h under stirring at room temperature. Reaction mixture was neutralized by Dowex 50, filtered and evaporated. After evaporation the residues were purified on a silica gel column (25 g) in chloroform–methanol. It was obtained white amorphous **1d** (188 mg, 57%) identical as under *A*).

Merthod B. Etienic acid nitrate **4e** (182 mg, 0.5 mmol), nonprotected glucosamine hydrochloride (130 mg, 0.6 mmol) and *O*-[(ethoxycarbonyl)cyanomethylenamino]-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (197 mg, 0.6 mmol) were mixed with DMF (10 ml) and *N*-ethyldiisopropyl amine (0.3 ml) was added. The reaction was stirred under argon for 24 h. The reaction mixture was extracted three times between ether and water. Organic phases were dried by $MgSO_4$ and purified on a silica gel column (35 g) in dichloromethane–methanol. There was obtained white amorphous compound **2i** (170 mg, 67%). For $C_{26}H_{40}N_2O_9$ (524.62) calculated: monoisotopic mass 524.27338, MS-ESI found: 525.2 [M + H^+]. This product was immediately deprotected as given for amide **2e** with the yield of **1d** (100 mg, 65%) identical as under *A*).

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-b-D-glucopyranose hydrochloride (**3a**)

D-Glucosamine hydrochloride (5.2 g, 24.1 mmol) was mixed with acetic acid (47 ml) and acetyl chloride (24 ml) and the mixture was stirred under argon at room temperature. After 3 h, chloroform (24 ml) was added and the mixture was further stirred for 24 h. Then it was evaporated to dryness, the residue was treated with boiling ethanol (25 ml) and filtered. The filtrate was cooled to yield **3a** (3.53 g, 43%). Properties of compound prepared were identical with those from literature[21]. The α- and β-anomers were distinguished by the NMR spectra, where for α-anomer we used as distinguishing marker peak at 6.45 d (1 H, $J_{1,2}$ = 3.81 Hz, H-1) and for β-anomer similarly at 5.85 d (1 H, $J_{1,2}$ = 8.79 Hz, H-1).

This work was supported by the NATO grant CBP.EAP.CLG.982972 (PD), by the Ministry of Education, Youth and Sports of the Czech Republic with project No. MSM6046137305 (LŠ, JT), and 2B06024-SUPRAFYT of the National Research Program II (ZN). Elemental analyses were done by the Analytical Department of the IOCB AS CR Prague.

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