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Preliminary communication

A new simple synthesis of amino sugar β-D-glycosylamines

LEONID M. LIKHOSHERSTOV, OLGA S. NOVIKOVA, VARVARA A. DEREVITSKAJA, and NIKOLAY K. KOCHETKOV

N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow B-334 (U.S.S.R.)

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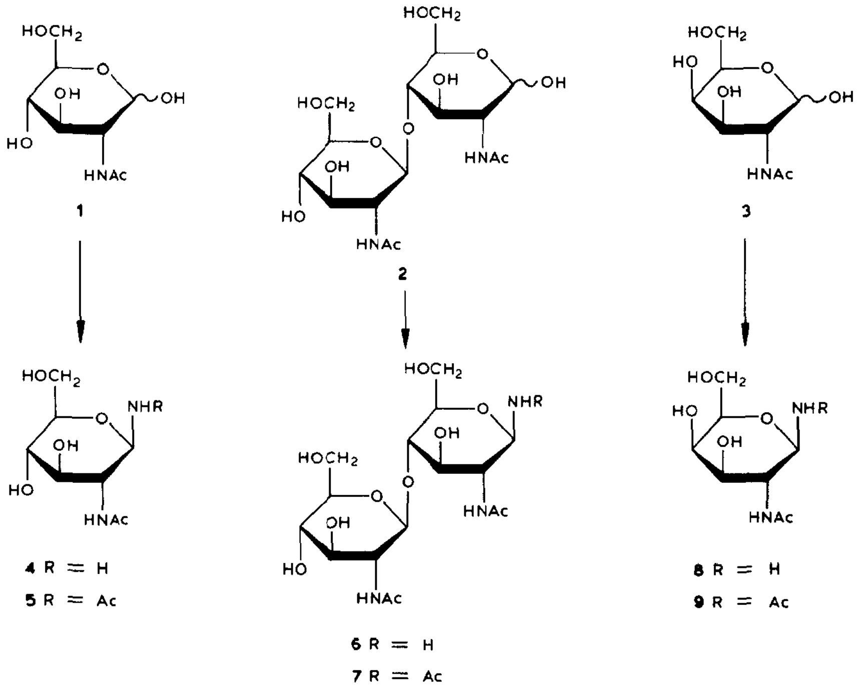
2-Acetamido-2-deoxy-β-D-glucopyranosylamine[1,2] is used mainly for the synthesis of various 1-*N*-acyl derivatives which model the *N*-glycosylamine linkage of glycoproteins[3]. The synthesis of amino sugar β-glycosylamines *via* glycosyl azides requires several steps[2,4,5].

We report herein a convenient, one-step synthesis of amino sugar β-glycosylamines by direct condensation of 2-acetamido-2-deoxy-D-glucose (**1**), 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (**2**), and 2-acetamido-2-deoxy-D-galactose (**3**) with ammonium hydrogencarbonate to give, in 50–80% yield, glycosyl-



amines **4**, **6**, and **8**. The reaction of **1** and **2** with saturated, aqueous ammonium hydrogencarbonate was monitored by p.c. (Whatman 1 paper; 4:8:5 2-methyl-1-propanol–1-propanol–water with detection by silver nitrate–potassium hydroxide, ninhydrine, and chlorine–potassium iodide–starch reagents). Major products having R_F 0.32 and 0.23 (**4** and **6**) were detected along with the starting amino sugars and minor products (R_F 0.22 and 0.1, respectively). The latter compounds are possibly the bis(glycosylamines), the formation of which (preferably in methanol) was reported earlier[6]. These products were practically absent when the concentration of **1** and **2** was < 0.2M. The yield of **4** reached 80% when the reaction of **1** with ammonium hydrogencarbonate was carried out at 20° (45 days) and 60% at 30° (6 days). In a sealed tube, this reaction gave **4** in a yield of not >30%.

In a typical experiment, the reaction mixture was diluted with equal volumes of water, and ammonium hydrogencarbonate was removed by concentration *in vacuo* (bath temperature 27–30°) to the original volume. This procedure was repeated 6 to 7 times. Owing to the lability of the glycosylamines, subsequent operations were carried out in the cold (0–5°). The resulting solution was made neutral (pH 6–6.5) with Amberlist 15 (H^+) ion-exchange resin, and the resin was filtered off, and washed with water and methanol to give in the effluent the starting sugar. The glycosylamines were eluted with methanolic 0.5M ammonia, and the eluate was concentrated to ~5% of its original volume, whereupon **6** was obtained in crystalline form. Addition of ether gave amorphous **4** and **8**.

The purity of the glycosylamines **4**, **6**, and **8** was determined by t.l.c. (Merck Silica gel 60, glass plates) in 1:1 chloroform–methanol with detection by conc. sulfuric acid and ninhydrine. They were free from the respective C-2 epimers, as determined by quantitative determination (amino acid analyzer) of 2-amino-2-deoxyglucose from **4** and **6**, and 2-amino-2-deoxygalactose (2-amino-2-deoxytalose was absent) from **8** after hydrolysis with hydrochloric acid for 16 h at 100°.

2-Acetamido-2-deoxy-β-D-glucopyranosylamine (**4**) was obtained in 60% yield (30°, 6 days), as amorphous solid, sint. 70°, dec. 103–112°, $[\alpha]_D^{20}$ −4.7° (*c* 1.7, water); t.l.c. R_F 0.23; lit.[7] m.p. 140–143°, $[\alpha]_D^{19}$ −9.9° (*c* 1.9, water); lit.[6] sint. 70°, dec. 104–110°.

2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (**6**) was obtained in 65% yield (30°, 7 days), m.p. 221–222° (dec., from methanol), $[\alpha]_D^{20}$ −11.0° (*c* 1.1, water); t.l.c. R_F 0.12.

Anal. Calc. for $C_{16}H_{29}N_3O_{10}$: C, 45.39; H, 6.90; N, 9.92. Found: C, 44.97; H, 6.82; N, 9.59.

2-Acetamido-2-deoxy-β-D-galactopyranosylamine (**8**) was obtained in 50% yield (30°, 5 days), amorphous, $[\alpha]_D^{23}$ +35.0° (*c* 1.2, water); t.l.c. R_F 0.22.

Anal. Calc. for $C_8H_{16}N_2O_5$: C, 43.58; H, 7.32; N, 12.72. Found: C, 43.12; H, 7.67; N, 13.15.

The structures and β-D configurations of the *N*-glycosyl linkages of **4**, **6**, and **8** were confirmed by ^{13}C-n.m.r. spectroscopy (Table I). The ^{13}C-n.m.r. data of **4** were

TABLE I

^{13}C-N.M.R. DATA FOR COMPOUNDS 4, 6, AND 8

Compound	*Chemical shifts* (δ)[a]							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*CH_3*
4	84.9	57.1	75.3	70.8	77.5	61.6	175.4	23.0
6[b]	85.2	56.9[c]	74.2	81.0	76.4	61.4	175.6	23.3[c]
6[d]	102.5	56.7[c]	74.6	70.9	77.0	61.7	175.6	23.2[c]
8	85.4	54.1	72.5	68.9	76.8	62.0	175.7	23.2

[a]For a solution in D_2O, from signal of internal methanol. [b]Reducing end. [c]Assignments may be reversed. [d]Nonreducing end.

identical with those reported previously[6]. The signals for **6** and **8** were assigned on the basis of the published data[6] for **4**, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose[8], and 2-acetamido-2-deoxy-D-galactose[9].

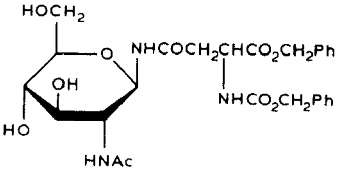
N-Acetylation of **4**, **6**, and **8** with acetic anhydride in aqueous methanol at 20° afforded the 1-*N*-acetylglycosylamines **5**, **7**, and **9**, respectively. The physical constants for **5** were very similar to those reported previously[10]. The ^{1}H-n.m.r. data confirmed the structures of the triacetate **7** and diacetate **9**. 2-Acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine (**5**, 80%), m.p. 264–265° (from ethanol), $[\alpha]_D^{20}$ +25.0° (*c* 1.0, water); lit.[10] m.p. 265–266°, $[\alpha]_D^{20}$ +26.4° (*c* 1.0, water). 2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine (**7**, 85%), m.p. 303–304° (dec., from methanol), $[\alpha]_D^{20}$ +2.5° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O, 250 MHz): δ 5.08 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.65 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 2.10 (s, 3 H, Ac), and 2.04 (s, 6 H, Ac).

Anal. Calc. for $C_{18}H_{31}N_3O_{11}$: C, 46.44; H, 6.71; N, 9.02. Found: C, 46.21; H, 6.82; N, 8.85.

2-Acetamido-1-*N*-acetyl-2-deoxy-β-D-galactopyranosylamine (**9**, 80%), m.p. 290–292° (dec., from aqueous methanol), $[\alpha]_D^{23}$ +77.5° (*c* 1.0, water); ^{1}H-n.m.r.: δ 4.98 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 2.00 (s, 3 H, Ac), and 2.01 (s, 3 H, Ac).

Anal. Calc. for $C_{10}H_{18}N_2O_6$: C. 45.79; H, 6.91; N, 10.68. Found: C, 45.35; H, 6.80; N, 10.42.

Glycosylamine **4** was the starting compound for the synthesis of model glycopeptides that can be used for studying the cleavage of *N*-glycosyl bond in glycoproteins by alkali–lithium borohydride treatment[11]. Condensation of 1-benzyl *N*-benzyloxycarbonyl-4-(*N*-succinimido)-L-aspartate, prepared by the procedure of Anderson *et al.*[12], with **4** in 80% aqueous *N,N*-dimethylformamide (20°, 16 h) gave 2-acetamido-1-*N*-(1-benzyloxy-*N*-benzyloxycarbonyl-4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**10**, 50%), amorphous solid, $[\alpha]_D^{21}$ +9.8° (*c* 1.0, methanol).



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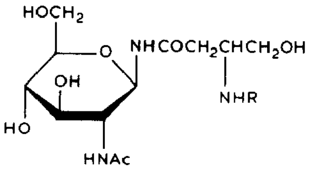

11 R = CO_2CH_2Ph

12 R = $H_2^+Cl^-$

13 R = Ac

Anal. Calc. for $C_{27}H_{33}N_3O_{10}$: C, 57.95; H, 5.94; N, 7.50. Found: C, 57.58; H, 6.01; N, 7.43.

The structure of **10** was confirmed by the quantitative determination (amino acid analyzer) of 2-amino-2-deoxyglucose and aspartic acid (1:1) after hydrolysis with 4M HCl (100°, 16 h). Treatment of **10** with $LiBH_4$ in 70% aqueous *tert*-butyl alcohol (0°, 2 h) reduced the ester group to yield **11**, the structure of which was confirmed by comparison of the i.r. spectra of **10** and **11** (the latter compound had ν 1730 cm^{-1} absent) and by detection of only 2-amino-2-deoxyglucose (aspartic acid was absent) in a hydrolyzate of **11**. Crude **11** was hydrogenolyzed in the presence of Pd–C in 50% aqueous methanol to give 2-acetamido-1-*N*-(3-amino-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine, which was isolated (40%) as the hydrochloride **12** and *N*-acetyl derivative **13**. 2-Acetamido-1-*N*-(3-amino-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine hydrochloride (**12**), m.p. 235–236° (dec., from methanol–ether), $[\alpha]_D^{21}$ +15.0° (*c* 1.4, water).

Anal. Calc. for $C_{12}H_{24}ClN_3O_7$: C, 40.28; H, 6.76; N, 11.74. Found: C, 39.96; H, 6.87; N, 11.25.

2-Acetamido-1-*N*-(3-acetamido-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine (**13**), m.p. 281–283° (dec., from aqueous ethanol), $[\alpha]_D^{21}$ +24.0° (*c* 1.2, water).

Anal. Calc. for $C_{14}H_{25}N_3O_8$: C, 46.27; H, 6.93; N, 11.56. Found: C, 45.84; H, 7.03; N, 11.41.

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