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STRUCTURE AND REARRANGEMENT REACTIONS OF SOME DIGLYCOSYLAMINES*†

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ABSTRACT

Di-β-D-glucopyranosylamine (**2**) was prepared by transglycosylation from β-D-glucopyranosylamine. *O*-Acetylation of **2** led to partial anomerization and the formation of the expected octa-*O*-acetyl β,β derivative and its α,β anomer in the ratio 1:2.4. Similarly, transglycosylation of β-D-xylopyranosylamine afforded di-β-D-xylopyranosylamine (**7**). As with **2**, the *O*-acetylation of **7** led to partial anomerization and formation of the hexa-*O*-acetyl α,β anomer of **7**, as well as the expected β,β *O*-acetylated derivative. *O*-Deacetylation with ammonia in methanol of (2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)amine (**7**) led to recovery of the β,β-linked starting material. These rearrangement reactions are interpreted in terms of unfavorable nonbonded interactions which modify the thermodynamic equilibrium of the diglycosylamines.

INTRODUCTION

Glycosylamines are compounds of interest for the enzymology of carbohydrates, since they are considered as active-site-directed, reversible inhibitors of glycosidases[1,2]. They usually bind more tightly with enzymes than their oxygen counterparts, provided that the basic character of their nitrogen atom is not diminished[2]. They have been used to study the specificity of β-D-glucosidase[3], and several *pseudo* glycosylamines, such as acarbose, are known as potent inhibitors of intestinal α-D-glucosidase. Some of these compounds have been proposed as oral antidiabetic agents[4]. The main drawback of glycosylamines, however, is their propensity to rearrange in solution *via* immonium-ion intermediates, which leads to

*Dedicated to Burckhardt Helferich in commemoration of the hundredth anniversary of his birth.
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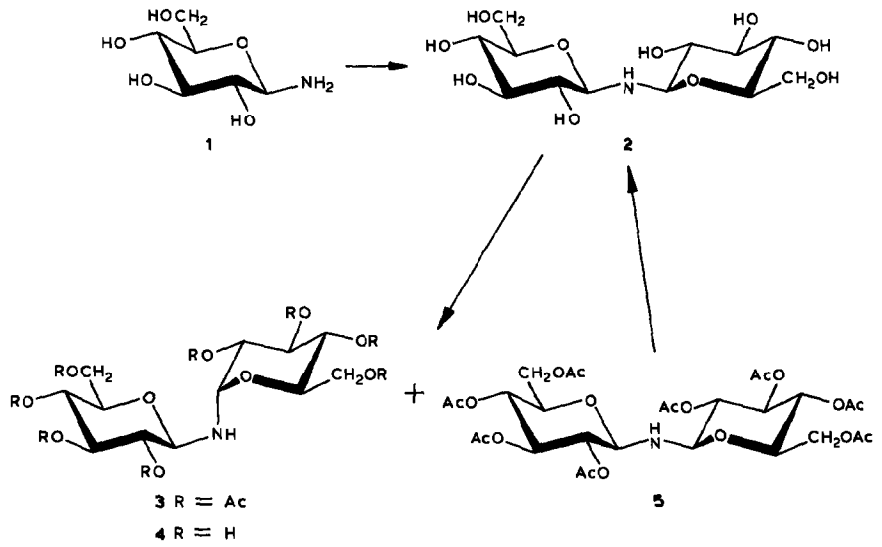
mutarotation and possible hydrolysis[5]. Diglycosylamines may be expected to be more stable than monoglycosylamines, since the combined electron-withdrawing effect of the two ring-oxygen atoms may decrease the basicity of the glycosylamine nitrogen. Nevertheless several reports in the literature mention unusual rearrangement reactions of these compounds. This paper deals with a reinvestigation of the reactivity of diglucosyl- and dixylosyl-amines in view of their possible use as inhibitors of glycosidases.

Aldopentoses and aldohexoses form glycosylamines when treated with concentrated alcoholic solutions of ammonia in the cold[5]. The structure of glycosylamines was originally thought to be acyclic[6]; however, Helferich assumed[7] that these compounds should have a cyclic structure, and this point is now well established[5]. When an unsubstituted glycosylamine is heated in a suitable solvent, two molecules condense to form the corresponding diglycosylamine. This reaction is called the dimerisation[8] or transglycosylation[9] of glycosylamines. In most cases, however, the anomeric configurations of the diglycosylamines have not yet been unambiguously assigned. Brigl and Keppler[10] isolated an "α-diglucosylamin-octacetat" from the reaction of D-glucopyranosylamine **1** with D-glucose in pyridine, followed by the addition of acetic anhydride. Deacetylation with methanolic ammonia led to a crystalline diglucosylamine to which they assigned the α,α-D-anomeric configuration on the basis of its highly positive optical rotation. The same octa-*O*-acetyl derivative was prepared by Hodge and Moy[11], who confirmed the α,α-D-anomeric configuration. However, Tóth and coworkers[12] proved by ^{1}H- and ^{13}C-n.m.r. spectroscopy that this compound was (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine.

"Di-D-xylosylamine"[9] was prepared by the transglycosylation of xylosylamine **6** without establishing the configuration of the *N*-glycosyl bond. Two crystalline per-*O*-acetyl derivatives were prepared by *O*-acetylation of "di-D-xylosylamine". One of them (m.p. 218–219°) was called "hexa-*O*-acetyl-di-D-xylosylamine" whereas the other one has not yet been identified[9].

RESULTS AND DISCUSSION

In fact, the main product of the transglycosylation of β-D-glucopyranosylamine (**1**) is di-β-D-glucopyranosylamine (**2**). The course of the transglycosylation in boiling methanol was monitored by measurements of the optical rotation as well as by nitrogen determinations. The reaction, under the given conditions, was complete within 2 h. The chromatographically pure compound **2** was isolated by silica gel column chromatography, and its structure was proved by mass, ^{1}H- and ^{13}C-n.m.r. spectroscopy. The f.a.b. mass spectrum of **2** showed an intense protonated molecular ion at m/z 342 in agreement with a dimeric structure. Furthermore the ^{13}C-n.m.r. spectrum of **2** exhibited 6 different carbon signals, pointing to a symmetrical molecule. The value of the chemical shift of C-1 (88.17 p.p.m., Table I) indicated a β,β configuration, since the values for the α,β anomer **4** were 90.25 (C-1β) and 84.90



p.p.m. (C-1α). This point was further confirmed by the value of the anomeric coupling constant in the ^{1}H-n.m.r. ($J_{1,2}$ 8.5 Hz).

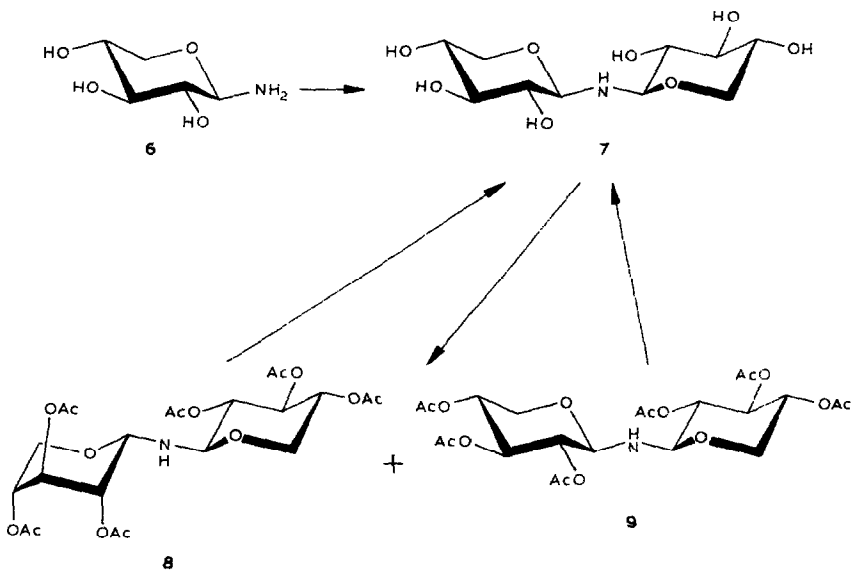
A mixture of (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (**3**) and bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (**5**) was formed by *O*-acetylation of diglucosylamine **2**. The pure, crystalline octa-*O*-acetyl derivatives **3** and **5** were obtained by fractional crystallization of this mixture[13] in the approximate ratio 2.4:1. The revision of the structure of the octa-*O*-acetyl derivative **3**, previously known as "α-diglucosylamin-octacetat"[10,11], has already been described[12].

The proof of the structure of compound **5** was provided by ^{1}H- and ^{13}C-n.m.r. spectroscopy as well as by mass spectrometry. As expected, the c.i. mass spectra of the octa-*O*-acetyl derivatives **3** and **5** were identical, showing M + H^+ ions at *m/z* 678. The pyranoid structures of both rings were proved by ions of the A series at *m/z* 331, 271, 229, 169, 109, and 81 (ref. 14). In the ^{13}C-n.m.r. spectrum of **5**, only 6 carbon signals appeared, just as in the spectrum of diglucosylamine **2**. From the ^{1}H-n.m.r. spectrum, the coupling constant $J_{1,2}$ was shown to be 8.8 Hz (Table II), indicating a β,β configuration. The formation of two per-*O*-acetyl derivatives by the *O*-acetylation of diglucosylamine was previously observed[9]. *N*-Acetylation of the octa-*O*-acetyl derivative **3** is furthermore known to yield *N*-acetyl-bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine[12].

(α-D-Glucopyranosyl)(β-D-glucopyranosyl)amine (**4**) was prepared by the *O*-deacetylation of the per-*O*-acetyl derivative **3**. This compound was previously described as di-α-D-glucopyranosylamine[10,11]. However, its ^{13}C-n.m.r. spectrum exhibits 12 carbon signals, indicating a non-symmetrical molecule with the α- and β-D configurations (C-1β 90.25 p.p.m. and C-1α 84.90 p.p.m.). The values of the anomeric coupling constants in the ^{1}H-n.m.r. spectrum (Table II) confirmed this

structure and a $^4C_1$ conformation for both heterocycles. The *O*-deacetylation of bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (**5**) yielded the β,β-diglucopyranosylamine (**2**) previously prepared by the transglycosylation of glucosylamine **1**.

The transglycosylation of β-D-xylopyranosylamine (**6**) afforded di-β-D-xylopyranosylamine (**7**). The presence of 5 signals in the ^{13}C-n.m.r. spectrum and the value of the coupling constant $J_{1,2}$ (7.6 Hz) gave evidence for a symmetrical molecule having the β configuration at both anomeric sites.



O-Acetylation of dixylosylamine **7** afforded a mixture of both (2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)amine (**8**) and bis (2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-amine (**9**). The pure, crystalline hexa-*O*-acetyl derivatives **8** and **9** were isolated by fractional crystallization of this mixture[15]. The c.i. mass spectra of compounds **8** and **9** were identical. Intense M + H^+ ions at *m/z* 534 and ions at *m/z* 259, 199, 157, 139, 115, 97, and 69, indicative of a pyranose structure for both rings, were found in these spectra. The n.m.r.-signals of the protons of the hexa-*O*-acetyl derivative **9** were the same for both xylosyl units. The value of the coupling constant $J_{1,2}$ (8.6 Hz), as well as other values (Tables I and II), suggest that this symmetrical per-*O*-acetylated dixylosylamine has the β,β configuration. The chemical shift of C-1 (87.80 p.p.m.) of compound **9** and of diglycosylamines and their per-*O*-acetyl derivatives in general is at somewhat higher magnetic field than that of C-1 in glycosyl glycosides, indicating the weaker deshielding effect of the NH group. *O*-Deacetylation of the per-*O*-acetyl derivative **9** gave di-β-D-xylopyranosylamine **7**.

The structure of hexa-*O*-acetyl derivative **8** was proved by ^{1}H-n.m.r. spectroscopy. The configurations at C-1 and C-1′, respectively, were confirmed by the

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS FOR DIGLYCOSYLAMINES **2,4,5,** AND **7–9**

Compound	*Chemical shifts*[a] *(δ)*											
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*
2	88.17	73.99	77.97	71.06	77.97	62.16						
4	90.25	73.90	77.36	70.98	77.75	61.87	84.90	71.54	73.00	70.98	71.28	61.63
5	87.42	71.04	72.92	68.70	72.60	68.18						
7	88.60	73.60	77.93	70.59	67.28							
8	88.90	72.15	73.24	69.83	64.61		83.11	68.25	69.15	70.25	61.58	
9	87.80	71.82	73.03	69.98	64.18							

[a]The primed numbers refer to the α-D-linked glycosyl unit.

TABLE II

^{1}H-N.M.R. SPECTRAL DATA FOR DIGLYCOSYLAMINES **2,4,5,** AND **7–9**

Compound	*Chemical shifts (δ)*								*Coupling constants (Hz)*							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*	*H-6'* *H-6'*	*NH*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{4,5'}$	$J_{5,5'}$	$J_{5,6}$ $J_{5,6'}$ $J_{6,6'}$	$J_{1,NH}$
2	4.26d	3.22t	3.47t	3.32–3.43m			3.87q 3.68q	—	8.5	9.0	9.0	—			2.5 5.5 12.3	—
4 α	4.95d	3.73q	3.65t	3.60–3.72m					4.9	9.5	9.8					—
β	4.14d	3.65t	3.52t	3.35–3.45m			3.82q, 3.75q		8.7	9.3	9.3				2.1–6.0	
5	4.25t	4.83t	5.28t	5.04t	3.69		4.29q 4.12q	3.08t	8.8	9.0	9.8	9.5			3.0 8.5	8.0
7	3.86	2.88	3.10	3.20	3.63	2.97		3.17t	7.60	8.90	8.90	5.40	10.80	11.4		8.0
8 α	4.76	5.19	4.71m		3.94q	3.77q		3.04q	5.5	5.45	—	6.28	3.85	12.3		9.4
β	4.14t	4.83t	5.21t	4.95s	4.05q	3.29t			8.5	9.21	9.21	5.38	10.8	11.03		8.5
9	4.17t	4.74t	5.21t	4.91s	4.01q	3.26q		2.99t	8.59	9.28	9.28	5.46	10.57	11.50		8.51

coupling constants $J_{1,2}$ (8.50 Hz, β anomer) and $J_{1',2'}$ (5.50 Hz, α anomer). The Karplus type of dependence can also be used to infer the dihedral angles of the N–H and C-1–H bonds[16]. The values of the coupling constants $J_{1,NH}$ (8.5 Hz, β anomer and 9.4 Hz, α anomer) are not considered as averaged values, because at 20°, hindered rotation around NΘC-1 is not probably because of the steric arrangement. The values suggest that both pyranose rings are disposed in such a way that the anomeric protons are transoid to the NH proton. Furthermore, the differences between the values of the coupling constants of the protons on the individual pyranose rings suggest that the conformations of the rings are different. The values of $J_{4,5a}$ (5.38 Hz) and $J_{4,5b}$ (10.80 Hz) for the β-linked residues are typical for a $^4C_1$ conformation; by contrast the values for $J_{4,5a}$ (6.28 Hz) and $J_{4,5b}$ (3.85 Hz) of the α-linked moiety reveal that this part of the molecule has adopted the $^1C_4$ conformation. In such an arrangement of the structure **8** the nonbonded interactions between NH and the *O*-acetyl groups at C-2 and C-2′ of the respective pyranose rings are minimal, and this may explain the unusual conformation of this molecule. The anomeric configurations at both sites in compound **8** were confirmed by the ^{13}C chemical shifts (C-1β, 88.90 p.p.m.); C-1α, 83.11 p.p.m., and by a measurement of the heteronuclear anomeric coupling constants ($J_{C\text{-}1,H\beta}$ 151.5 Hz; $J_{C\text{-}1,H\alpha}$ 157.8 Hz).

Partial anomerization of the *N*-glycosyl bond takes place during the *O*-acetylation of di-β-D-xylopyranosylamine (**7**), as in the *O*-acetylation of di-β-D-glucopyranosylamine (**2**), and this may be due to unfavorable nonbonded interactions, in the β,β configuration, of one equatorial 2-*O*-acetyl group with the equatorial glycosylamine group at C-1. *O*-Deacetylation of compound **8** with ammonia in methanol gave dixylosylamine **7**, indicating that the *N*-glycosyl bond was anomerized under these conditions by a process already discussed by Isbell and Frush[9].

As previously described[17], hexa-*O*-acetyl derivative **8** could be prepared from xylosylamine **6** directly without isolation of dixylosylamine **7**; in this case, the symmetrical hexa-*O*-acetyl derivative **9** did not crystallize.

The values of the optical rotations of the anomeric pairs of glycosylamines **2** and **4** and **3** and **5**, as well as **8** and **9**, are in agreement with Hudson's rule.

Both diglucosylamines **2** and **4** were strong competitive inhibitors of β-D-glucosidase from *Aspergillus phoenicis* and *Aspergillus niger*. Di-β-D-xylopyranosylamine **7** caused a mixed type inhibition of the β-D-xylosidase from *Aspergillus niger*[18].

EXPERIMENTAL

General. — Melting points were determined on a Kofler micro hot-stage. Solutions were evaporated under diminished pressure at 30–40°. Compounds **1**, **6** and **7** were prepared according to ref. 9, as well as by the methods listed below. Thin-layer chromagraphy was conducted on plates on silica gel (Silufol, Kavalier)

with (*a*) 5:1:1 methanol–1,4-dioxane–heptane or (*b*) 1:1 benzene–ethyl acetate. On thin-layer plates, the components were located by spraying with 5% sulfuric acid in ethanol and heating. Preparative chromatography was performed on columns (60 × 3 cm) of silica gel (0.04–0.1 mm) eluted with solvent *a*. Mass spectra were recorded, either in the chemical-ionization mode (ammonia–isobutane, 100 eV, accelerating potential 2 kV, emission current 520 μA, source temp. 150°, on an AEI-Kratos MS-30 instrument, using the direct inlet technique, or in the f.a.b.-ionization mode (Xe, glycerol matrix, accelerating potential 7–8 keV) on an AEI-Kratos MS-50 mass spectrometer fitted with an f.a.b. 11 NV, Ion Tech atom-gun and a Mat SS-200 Finnigan (DEC-PDP 11-34) computer. N.m.r. spectra were recorded at 298 K on a Bruker AM-300 FT spectrometer, with chemical shifts referenced to tetramethylsilane. Per-*O*-acetyl derivatives of diglycosylamines were examined in $CDCl_3$ solutions containing Me_4Si. Unsubstituted diglycosylamines were examined in D_2O solutions, using methanol as the internal standard (δ_C 50.15). The instrumental parameters were: for ^1H spectra 300.13 MHz, spectral width 3.5 kHz, 16 K data points; for ^{13}C spectra 75.46 MHz, spectral width 17 kHz, 32 K data points. The signals of the protons were assigned using 2D COSY 45 homocorrelated spectra, and those of the carbon atoms by selective decoupling.

β-D-Glucopyranosylamine (**1**). — Prepared according to ref. 11, **1** had 7.8% N (calc. 7.8) and $[\alpha]_D^{20}$ +20.8° (water); lit.[9] $[\alpha]_D^{20}$ +20.8° (water); lit.[11] $[\alpha]_D^{25}$ +20° (*c* 2, water). The f.a.b. mass spectrum showed a signal at *m/z* 180 (100, $[M+H]^+$) together with signals at *m/z* 342 (32), 521 (4) and 681 indicating the presence of a substantial proportion of a diglucosylamine.

Analytical control of the transglycosylation of glucosylamine **1**. — Compound **1** (4 g) in dry methanol (200 mL) was boiled under reflux for 5 h with the exclusion of external moisture. Individual samples were taken at intervals, evaporated, dried to constant weight over P_2O_5, and analyzed. The values for N content (%) and $[\alpha]_D$ (deg., *c* 2.0, water) were: initial, 7.70, +21.9°; 30 min, 4.96, +21.2°; 60 min, 4.37, +20.2°; 2 h, 4.13, +18.4°; 5 h, 4.15, +15.0°.

Di-β-D-glucopyranosylamine (**2**). — *a*. β-D-Glucopyranosylamine (**1**) (5 g) in dry methanol (250 mL) was heated for 5 h at reflux temperature with the exclusion of external moisture. The solution was cooled to 0°, ether was added (100 mL) until a precipitate formed, and after 24 h at 0° this was filtered off. The crude product was purified on a silica gel column, using solvent *a* as eluent. The fractions containing chromatographically homogeneous substance were evaporated and the resulting colorless solid was dried to constant weight over P_2O_5 to give diglucosylamine **2** as a foam (3.1 g; 65%), $[\alpha]_D^{20}$ + +6.0° (*c* 2.0, water, 2 min); lit.[9] for "β-di-D-glucosylamine dihydrate" $[\alpha]_D^{20}$ −21.1° (water); lit.[10] for "β-diglucosylamin" $[\alpha]_D$ −20.9° (water, 10 min); f.a.b.-m.s.: *m/z* 683 (4, $[2M + H]^+$), 521 (3, $[M + GlcNH_2]^+$), 342 (100, $[M + H]^+$), and 180 (50 $[GlcNH_2]^+$).

Anal. Calc. for $C_{12}H_{23}NO_{10}$: C, 42.2; H, 6.8; N, 4.1. Found: C, 42.1; H, 6.9; N, 4.2.

b. Octa-*O*-acetyl derivative **5** (4.7 g) was kept in methanol–ammonia

solution[11] (150 mL) for 48 h at 0°. The solution was evaporated, and to the syrupy residue dry methanol (30 mL) and acetone (10 mL) were added until a precipitate formed. After standing for 24 h at 0° the solid was filtered off. The crude product (t.l.c., solvent *a*) was chromatographed on a silica gel column using solvent *a* as eluent to give pure **2** (1.5 g; 63%), $[\alpha]_D^{20}$ +5.0° (*c* 2.0, water, 2 min). The ^{13}C-n.m.r. spectrum was identical with that of diglucosylamine prepared by method *a*.

*(2,3,4,6-Tetra-*O*-acetyl-α-*D*-glucopyranosyl)(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)amine* (**3**) *and bis(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)amine* (**5**). — β-D-Glucopyranosylamine **1** (18 g) in dry methanol (900 mL) was boiled under reflux (2 h) with the exclusion of external moisture. Methanol was removed by evaporation, a mixture of acetic anhydride (90 mL) and pyridine (180 mL) was added to the dry residue and the solution was stirred at 20°. The course of *O*-acetylation was checked by t.l.c. using solvent *b*. After 24 h the reaction mixture was then poured into icewater (2 L) whereupon during occasional shaking the mixture of octa-*O*-acetyl derivatives **3** and **5** crystallized. The crystalline mixture was filtered off, dried, and dissolved in ethanol (2 L) at 70°. The acetyl derivative **3** (11.6 g; 34%) crystallized on cooling the solution to 0°. The pure product was obtained by recrystallization from ethanol (230 mL) and acetone (130 mL), m.p. 212–213°, $[\alpha]_D^{20}$ +88.0° (*c* 2.0, chloroform); lit.[10] for "α-diglucosylamin-octacetat", m.p. 216–217°, $[\alpha]_D$ +87° (chloroform); lit.[11] m.p. 211–212°, $[\alpha]_D^{25}$ +89.4° (*c* 2.5, chloroform); c.i.-m.s.: *m/z* 678 (100, $[M + H]^+$), 618 (30, $MH^+ - 60$), 558 (10), 431 (10), 376 (10), 331 (48), 169 (40, A_3), 109 (20, A_4), and 81 (30).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.6; H, 5.8; N, 2.1. Found: C, 49.5; H, 5.9; N, 2.0.

The filtrate from the separation of compound **3** was concentrated (70 mL), heated (50°), and cooled (20°) to produce crystalline per-*O*-acetyl derivative **5** (4.7 g; 14%). After recrystallization from ethanol the product had m.p. 154°, $[\alpha]_D^{20}$ +16.9° (*c* 2.0, chloroform); lit.[10] for "β-diglucosylamin-octacetat", m.p. 135–140 → 190–192°, $[\alpha]_D$ +7.6° (chloroform); c.i.-m.s.: *m/z* 678 (60, $[M + H]^+$), 618 (10, $MH^+ - 60$), 390 (100), 366 (75), 348 (10), 331 (90, A_1), 271 (15, $A_1 - 60$), 229 (10), 186 (10), 169 (30, A_3), 109 (15, A_4), and 81 (20).

Anal. Found: C, 49.6; H, 5.8; N, 2.0.

α-D-Glucopyranosyl-β-D-glucopyranosylamine (**4**). — The *O*-deacetylation of octa-*O*-acetyl derivative **3** gave **4**, m.p. 170–171°, $[\alpha]_D^{20}$ +88.5° (*c* 2.0, water, 2 min); lit.[10] for "α-diglucosylamin", m.p. 167–168°, $[\alpha]_D$ +85.1°; lit.[11] for "di-α-D-glucosylamine", m.p. 165–166°, $[\alpha]_D^{25}$ +90.0° (*c* 2.0, water, 3–5 min); f.a.b.-m.s.: *m/z* 683 (2,$[M + H]^+$), 342 (100, $[M + H]^+$).

Anal. Calc. for $C_{12}H_{23}NO_{10}$: C, 42.2; H, 6.8; N, 4.1. Found: C, 42.0; H, 6.8; N, 4.3.

β-D-Xylopyranosylamine (**6**). — Prepared according to ref. 9, compound **6** gave an f.a.b. mass spectrum showing a signal at *m/z* 150 (100, $[M + H]^+$), together with signals at *m/z* 282 (41), 431 (3), and 563, indicating the presence of a substantial proportion of a dixylosylamine.

*(2,3,4-tri-*O*-acetyl-α-*D*-xylopyranosyl)(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl) amine* (**8**) *and bis(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)amine* (**9**). — A mixture of acetic anhydride (75 mL) and pyridine (150 mL) was added to di-D-xylosylamine[9] **7** (15 g), and the solution was stirred for 24 h at 20°. The reaction mixture was then poured into ice-water (1 L), causing the mixture of hexa-*O*-acetyl derivatives **8** and **9** to crystallize. The crystalline mixture was filtered off, dried, and dissolved in ethanol (1 L). The hexa-*O*-acetyl-α,β derivative **8** crystallized on cooling the solution to 0° (10.3 g; 36%). The crude product was purified by recrystallization from ethanol, m.p. 224–225°, $[\alpha]_D^{20}$ +15.1°, (*c* 2.0, chloroform); lit.[9] m.p. 218–219°, $[\alpha]_D^{20}$ +16.8° (*c* 2.0, chloroform); c.i.-m.s.: *m/z* 534 (100, $[M + H]^+$), 474 (25, $MH^+ - 60$), 414 (10, $MH^+ - 2 \times 60$), 359 (8), 304 (15), 259 (70, A_1), 199 (45, $A_1 - 60$), 157 (22, C_1), 139 (38, A_2), 128 (10, B_2), 115 (10, C_2), 97 (30, A_3), and 69 (20).

Anal. Calc. for $C_{22}H_{31}NO_{14}$: C, 49.5; H, 5.9; N, 2.6. Found: C, 49.3; H, 5.9; N, 2.5.

The filtrate from the recovery of hexa-*O*-acetyl derivative **8** was concentrated (100 mL), heated (50°), and cooled (20°) to give crystalline hexa-*O*-acetyl derivative **9** (5.1 g; 18%). The pure product was obtained by recrystallization from ethanol, m.p. 151–152°, $[\alpha]_D^{20}$ −28.0° (*c* 2.0, chloroform); c.i.-m.s.: *m/z* 678 (100, $[M + H]^+$), 618 (30, $MH^+ - 60$), 558 (10), 431 (10), 376 (10), 331 (48), 169 (40, A_3), and 109 (20, A_4).

Anal. Found: C, 49.5; H, 5.9; N, 2.4.

*Di-β-*D*-xylopyranosylamine* (**7**). — *a. From hexa-*O*-acetate* **8**. Compound **8** (3.3 g) was held in methanol–ammonia solution (120 mL)[11] for 48 h at 0°. The solution was evaporated, acetone (50 mL) was added, and on cooling the mixture to 0°, dixylosylamine **7** crystallized (1.3 g; 75%). For recrystallization, the compound was dissolved in a minimum volume of water, methanol (10 mL) was added, and then sufficient acetone to produce turbidity. After 24 h at 0° crystalline **7** was separated, m.p. 164°, $[\alpha]_D^{20}$ −45.4° (*c* 2.0, water, 2 min); lit.[9] m.p. 154–155°, $[\alpha]_D^{20}$ −44.3° (*c* 1.4, water, 10 min); f.a.b.-m.s.: *m/z* 563 (5, $[2M + H]^+$) and 282 (100, $[M + H]^+$).

Anal. Calc. for $C_{10}H_{19}NO_8$: C, 42.7; H, 6,8; N, 5.0. Found: C, 42.6; H, 6.8; N, 4.9.

*b. From hexa-*O*-acetate* **9**. Per-*O*-acetyl derivative **9** was *O*-deacetylated by the same method as for compound **8**. The product was dixylosylamine **7** having m.p. 164°, $[\alpha]_D^{20}$ −45.2° (*c* 2.0, water, 2 min), and a ^{13}C-n.m.r. spectrum identical with that of the product from **8**.

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