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SYNTHESIS OF 2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE AND DIMER FORMATION

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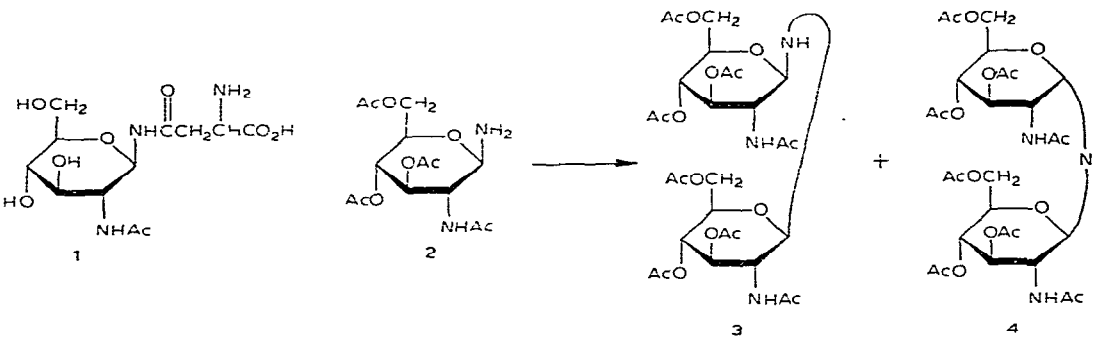
ABSTRACT

The synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (**2**), the key intermediate in glycopeptide synthesis, has been improved. The dimerization of **2** has been studied as a model for its activity in biological systems. The formation of β,β and α,β dimers from **2** and their interconversions could be readily followed by ^{13}C-n.m.r. spectroscopy, and a probable mechanism of their formation involving an acyclic immonium ion intermediate has been proposed.

INTRODUCTION

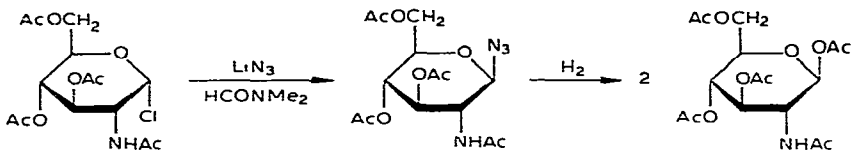
In the course of our studies of the synthesis and biological activities of analogs of 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**1**), we have utilized 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (**2**) as a key intermediate. Unexpectedly, **2** was found to have significant growth-inhibitory



activity against mouse mammary adenocarcinoma (TA3) cells in culture, as well as to inhibit the incorporation of L-leucine and D-glucosamine in leukemic cells (P288) likewise in culture[1]. On storage at room temperature, **2** was found to be unstable, readily forming 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β- and -α-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (**3** and **4**, respectively).

This facile self-condensation reaction of **2** may serve as a model for the reaction of this compound with other nucleophilic reagents in a biological system, particularly those in the active sites of enzymes, such as the ε-amino group of lysine and the SH group of cysteine. Hence, it could be expected that an investigation of this reaction, with respect to reaction conditions, stereochemistry of the products, and mechanism, may lead to clarification of the biological activity of this compound and of glycosylamines in general.

The synthesis of **2** has presented some problems in relation to yields, convenience, and safety. Therefore, this investigation was preceded by an examination of procedures already reported, and by the development of improvements. Starting with either 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**5**) or bromide, the halogen atom is displaced by the azido group to give **6**, which is catalytically reduced to **2**. We have found the procedure by Horton[2] for the synthesis



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of **5** to be preferable to other methods[3,4]. Treatment of **5** with silver azide in chloroform gave the expected azido derivative **6** in good yield, but the reaction mixture exploded in one instance (an explosion has also been reported earlier by Marks *et al.*[5]). This led us to explore the preparation of the azido intermediate with alkali azides under various conditions. When **5** was treated with sodium azide in aqueous acetone[6], **6** was obtained in 59% yield along with 15% of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose as a by-product. Yamamoto *et al.*[7], using sodium azide in formamide at 85°, have reported a high yield of **6** from **5**, but Bolton *et al.*[8] and Cowley *et al.*[9], using the same solvent and reagent but the glycosyl bromide instead of the chloride **5**, obtained **6** in only 52–56% yield. Finally, an excellent yield (75–80%) of **6** from **5** was obtained with finely powdered lithium azide in dry *N*,*N*-dimethylformamide at 75–80°.

Hydrogenation of **6** in an ethanol or ethyl acetate solution gave the desired key intermediate **2** in good yield. The main by-products were the dimers **3** and **4**. Formation of these dimers was first reported by Yamamoto *et al.*[7], and was later studied in more detail by Bolton *et al.*[8]. We observed that this dimerization also takes place on storage of the solid compound at room temperature; thus, a considerable variation in physical constants for **2** have been reported[5,7,10,11].

Facile formation of dimers **3** and **4** from **2** occurred at room temperature during silica gel chromatography with methanol–chloroform as eluent, or methanol or ethanol solution boiling under reflux. The dimers were also formed during an attempted synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-

guanidine, which involved heating **2** with *S*-methylisothiourea hydrogensulfate[12] or 1-amidino-3,5-dimethylpyrazole nitrate[13] in ethanol. The two dimers **3** and **4** could be readily separated by t.l.c. in 1:9 methanol–chloroform and isolated by chromatography on a silica gel column. Initially, the partial conversion of **3** into **4** in chloroform solution was studied by ^{1}H-n.m.r. spectroscopy at 100 MHz (see Fig. 1), but

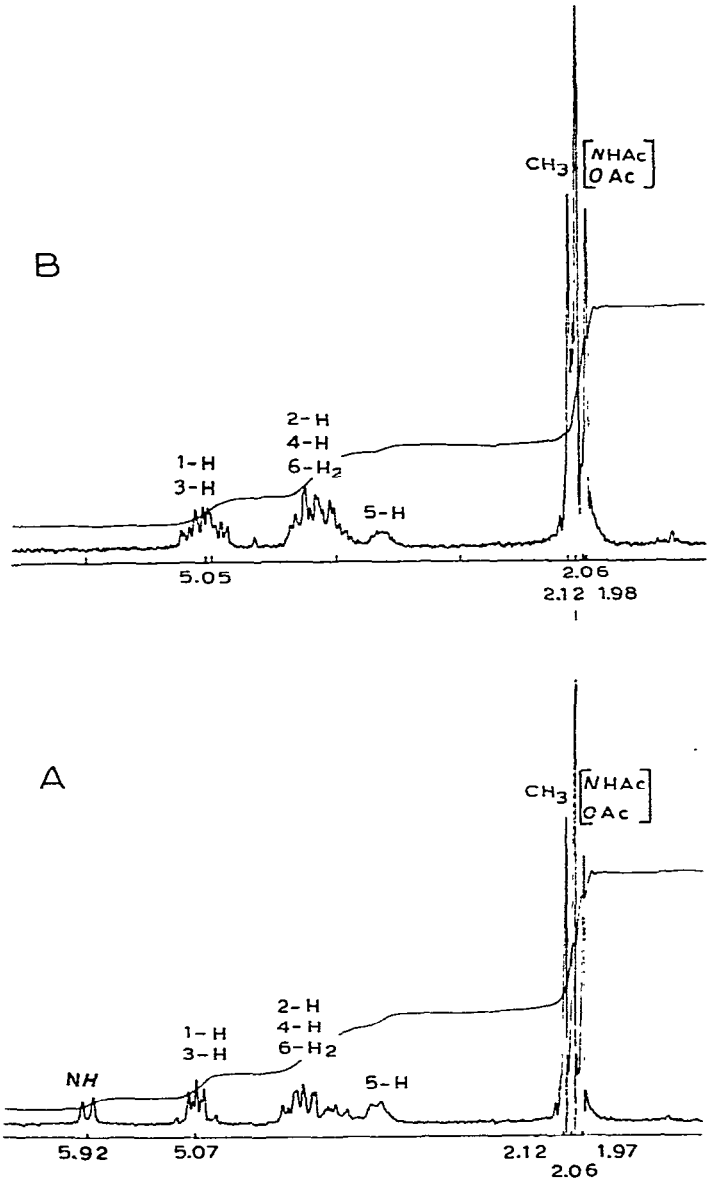


Fig. 1. A. ^{1}H-N.m.r. spectrum (100 MHz) of **3** in $CDCl_3$, showing an NH doublet at δ 5.92 and a relatively simple pattern in the anomeric region at δ 5.07. B. Same as A, after 24 h. The anomeric region around δ 5.05 shows a complex pattern, even after being exchanged with deuterium.

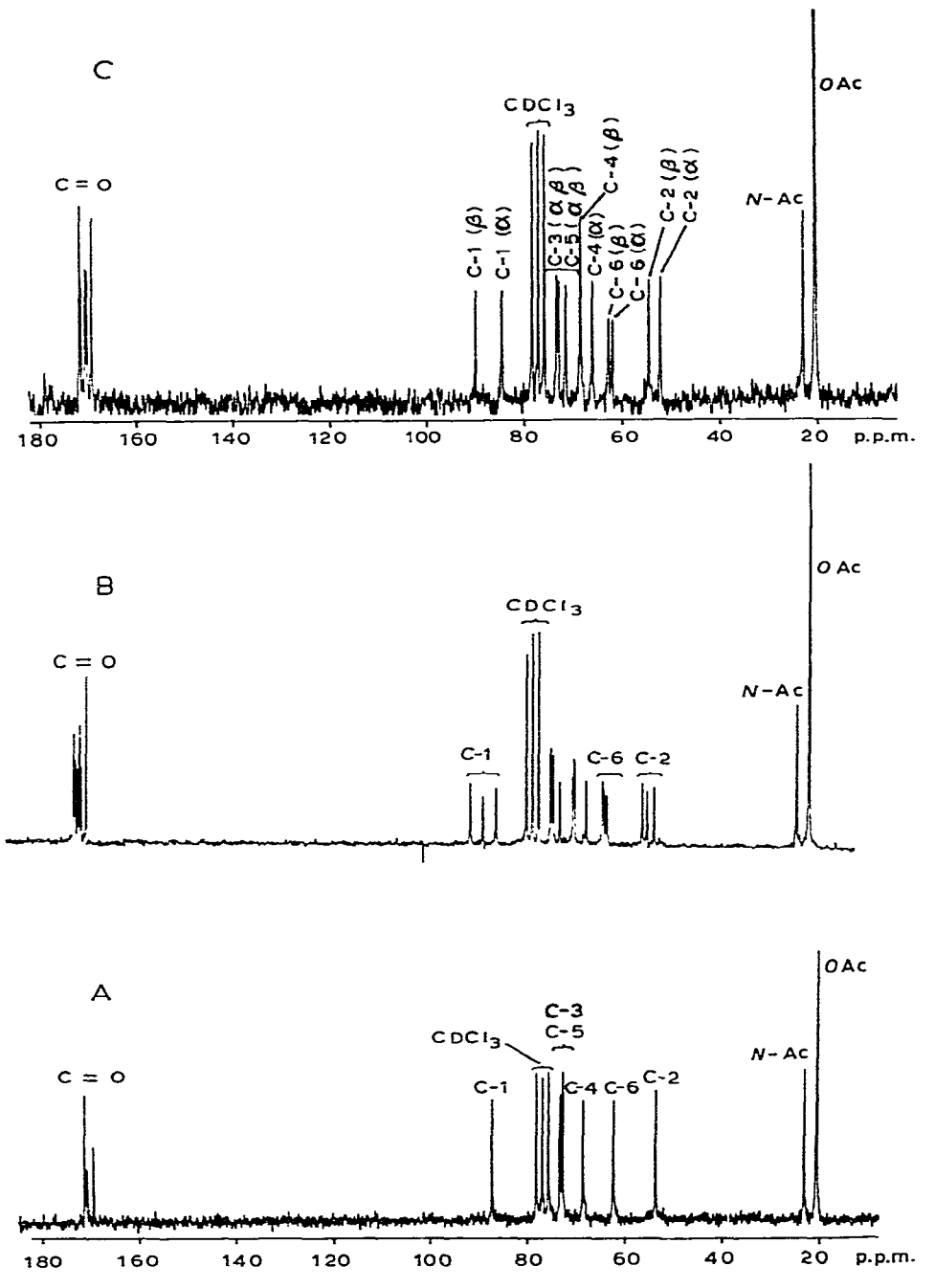


Fig. 2. A. ^{13}C-N.m.r. spectrum (25.4 MHz) of 3 in $CDCl_3$. The simplicity of the spectrum indicates symmetry. B. Same as in A, but after being kept for 3 weeks. Note the complexity of the pattern, indicating a mixture of 3 and 4. C. ^{13}C-N.m.r. spectrum of 4, showing "doubling" of peaks of each monomer because of asymmetry.

this change could be more clearly observed by ^{13}C-n.m.r. spectroscopy. A fresh solution of **3** in chloroform-*d* (Fig. 2A) showed a ^{13}C-n.m.r. spectrum very similar to that of **2**, indicating that the dimer (β,β isomer) is symmetrical. A similar simplicity of the ^{13}C-n.m.r. spectra due to symmetry has also been observed for α,α- and β,β-trehalose[14]. On the other hand, the ^{13}C-n.m.r. spectrum of the α,β isomer (Fig. 2C) is more complex, showing two peaks due to the anomeric carbon atoms, as well as other carbon atoms, corresponding to the two parts of the molecule. This feature is also analogous to the ^{13}C-n.m.r. spectrum of α,β-trehalose[14]. Fig. 2B shows a much more complex ^{13}C-n.m.r. spectral pattern for **3**, after the chloroform-*d* solution had been kept at room temperature for 3 weeks. C-1, C-2, and C-6 each distinctly shows three separate peaks, one peak due to **3** (Fig. 2A), and two peaks due to **4** (Fig. 2C), suggesting that the partial conversion of **3** to **4** took place as was indicated also by optical rotation and t.l.c. studies (see Experimental). The interconversion of **3** into **4** was accelerated by the presence of traces of hydrogen chloride in chloroform-*d*. In absolute chloroform-*d*, this interconversion was found to be very sluggish.

The ^{13}C shifts of the acetylated derivatives **2**, **3**, **4**, **6**, and **7** are reported in Table I. The C-2 resonances are readily recognized by their considerable upfield displacement and are in the range of 52.9–54.8 p.p.m. The assignments to 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (**7**) were made by comparing its

TABLE I

^{13}C-N.M.R. SPECTRAL DATA OF **2**, **3**, **4**, **6**, AND **7**

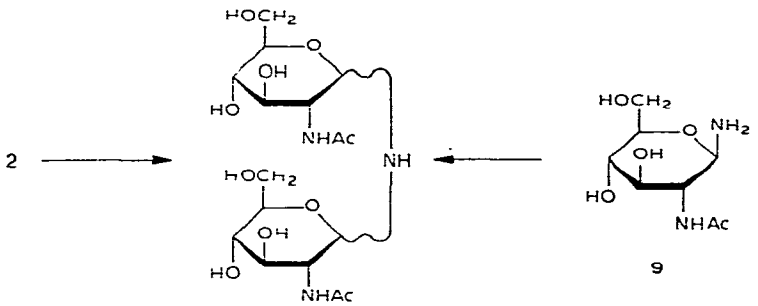
Compd.	*Chemical shifts*							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CH_3(NAc)*	*CH_3(OAc)*
2	86.1	54.8	73.5	69.1	72.6	62.6	23.1	20.6
3	87.2	53.7	73.3	68.6	72.4	62.3	23.2	20.6
4[a]	89.9	54.4	[b]	68.3	[b]	62.6	23.2	20.7
4[c]	84.5	52.1	[d]	66.0	[d]	61.9		
6	88.2	54.0	72.2	68.5	73.8	62.0	23.1	20.6
7	92.5	52.9	72.8	68.3	72.8	61.9	23.1	20.6, 20.8

[a]Resonance of β-D-glucosyl group. [b]Either two of the following: 73.3, 72.9, and 71.4. [c]Resonance of α-D-glucosyl group. [d]Either two of the following: 68.3, 73.3, 72.9, and 71.4.

spectrum with that of its α anomer[15]; in the β anomer, both C-3 and C-5 resonances are coincident. Assignments of ^{13}C resonances to the 1-azido compound **6** were based on ^{1}H–^{13}C off-resonance decoupling experiments. The original assignments of ^{1}H resonances[16] were confirmed at 100 MHz by a series of homonuclear decoupling experiments. Since H-3 and H-5 resonances are well separated in the ^{1}H-n.m.r. spectrum, the ^{13}C resonances could be unequivocally assigned to peaks at 72.2 and 73.8 p.p.m., respectively. This method, however, was not unambiguous for the C-1 and C-4 resonances, as they were insufficiently separated in the ^{1}H-n.m.r. spectrum. Recent studies of the effects of the azido group on the ^{13}C spectra of steroids have

shown that the azido group has a deshielding effect that may be compared to that of the hydroxyl group[17]. Thus, C-1, which bears the azido group in **6**, is the most deshielded ring carbon-atom at 88.2 p.p.m., whereas the C-4 resonance assumes the usual upfield position at 68.5 p.p.m. The ^{13}C assignments to the 1-amino derivative **2** for C-3 and C-5 have been determined by a series of off-resonance experiments, as for **6**, and their positions were found to be reversed. The resonances of the β,β dimer **3** follow very closely that of the monomer. The same can be said of the β-linked residue of the α,β dimer. In the α-linked residue, however, the anomeric carbon was moved upfield by 5.4 p.p.m., and this trend has been maintained also for C-2 and C-4 resonances, indicating steric effects as is evident from molecular models of the α,β-dimer.

The optical rotation of a solution of **2** in methanol changed from $[\alpha]_D^{24}$ -5.2 to $+16.3°$ (*c* 1.2, methanol) at room temperature over a period of 8 weeks. This could not be ascribed to the conversion to the α anomer of **2** or possibly to acetyl migration, as the ^1H-n.m.r. spectrum showed no methyl peaks due to the *O*-acetyl groups. A similar change in optical rotation was also observed for the de-*O*-acetylated derivative **9** in methanol solution. T.l.c., and n.m.r. and i.r. spectral data of the product indicate that it consisted of the de-*O*-acetylated isomeric dimers **8**. It was found to be identical



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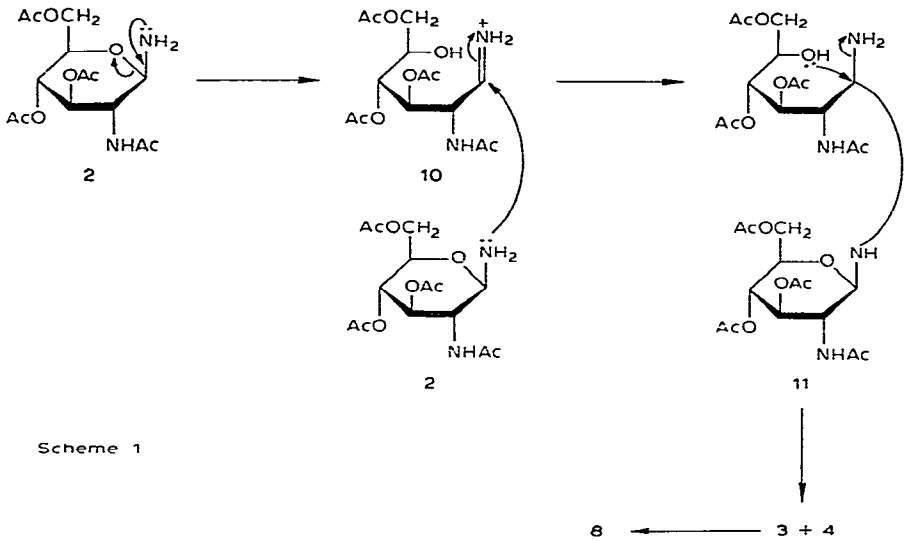
with the product obtained by de-*O*-acetylation of **3** and **4** by treatment with 10% triethylamine in aqueous methanol at room temperature. This finding was further confirmed by the acetylation of **8** with acetic anhydride in pyridine, to give a mixture of **3** and **4**, It should be noted that the secondary amino group at C-1 could not be acetylated. This finding is in accord with the observation made earlier by Bolton *et al.*[8], and may be attributed to the decreased basicity of the amino function due to the combined electron-withdrawing effects of the two ring oxygen atoms as well as to steric hindrance.

A probable mechanism of dimer formation is shown in Scheme 1. Initially, **2** in methanol is converted[18] into the acyclic immonium ion intermediate **10**, which then reacts with a second molecule of **2** to give the intermediate **11**. The latter undergoes ring closure, with elimination of the amine group at the anomeric carbon atom as ammonia, giving **3** and **4**. The ammonia liberated in this process hydrolyzes the *O*-

acetyl group, giving de-*O*-acetylated dimers **8**. It should be pointed out that a similar mechanism could be in operation on the surface of a biological receptor, once **2** becomes attached to it, giving rise to the covalently-bound sugar. In this case, the other reaction partner could be the ε-amino group of lysine or any other nucleophilic group.



Scheme 1

Formation of both **3** and **4** during the reaction could be readily visualized, as the cyclic immonium ion intermediate could be attacked by NH_2-1 of **2**, giving both isomers. The formation of **4** from **3** in chloroform solution is probably catalyzed by the formation of hydrogen chloride with time. This suggests that the ring-opening is preceded by protonation of the ring oxygen atom. Addition of one drop of M deuterium chloride to the chloroform-*d* solution of **3** converted the solute rapidly into a mixture of **3** and **4** in a 1:2 ratio, as determined by integration of the ^{13}C resonances of C-1 atoms (Fig. 2B). On keeping the acidified solution for several days, there was only a very slight increase in the relative amount of **4**. Although formation of the α,α isomer could not be excluded, we could not detect it by ^{13}C-n.m.r. The greater thermodynamic stability of **4**, as compared with **3**, is dependent on factors that are as yet not understood.

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined by the capillary method. I.r. spectra were recorded with a Perkin–Elmer 457 spectrophotometer, and n.m.r. spectra with Varian A-60A and Varian XL-100 instruments. ^1H-N.m.r. spectra were recorded in the continuous wave (CW) mode and ^{13}C-n.m.r. spectra in the Fourier transform (FT) mode, with the positions of the peaks expressed in δ from the tetramethylsilane or 1,4-dioxane signals. Optical rotations were

measured with a Perkin–Elmer 141 polarimeter. Thin-layer chromatograms were run on Merck HF-254 silica gel plates, and spots on chromatograms were detected with iodine vapor, by u.v. absorption, or by spraying with a ninhydrin solution.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**6**). — *Method A* (*with silver azide*). A suspension of silver azide in chloroform was prepared by mixing aqueous solutions of sodium azide (2.5 g) and silver nitrate (6.3 g), and washing the precipitate by decantation with water, followed by ethanol, ether, and chloroform. While the silver azide–chloroform suspension was heated under reflux, water droplets appeared in the condenser; these were removed by gentle distillation. To the water-free chloroform suspension (50 ml), **5** (4.5 g) was added, and the reaction mixture was heated to gentle reflux (at 80° bath temperature) with stirring for 3 h. It was cooled and filtered (care being taken not to dry the precipitate, as unreacted silver azide may explode), and the filtrate was evaporated *in vacuo*. The residue was crystallized from ethyl acetate and ether (yield 3.7 g, 72%), m.p. 170–171° (lit.[7] m.p. 159–160°; lit.[19] m.p. 160–161°; and lit.[9] m.p. 169–170°); $[\alpha]_D^{25}$ −46.3° (*c* 1.1, chloroform); ν_{max}^{KBr} 3350 (NH), 2942, 2888 (CH), 2110 (N_3), 1745 (C=O, acetoxy) 1664, 1520 (C=O, amide), and 1235 cm^{-1} (acetate): ^1H n.m.r. ($CDCl_3$): δ 2.02 (s, 3 H, NHCOCH_3), 2.07, 2.12 (2s, 9 H, OCOCH_3), 4.23 (2 H, H_2-6), 6.62 (d, 1 H, *J* 9 Hz, H-1), 5.10 (q, 1 H, *J* 9 Hz, H-4), 5.40 (q, 1 H, *J* 9 Hz, H-3), and 6.87 (d, 1 H, *J* 9 Hz, N*H*).

Anal. Calc. for $C_{14}H_{20}N_4O_8$: C, 45.15; H, 5.42; N, 15.05. Found: C, 45.32; H, 5.59; N, 14.79.

Method B (*with lithium azide*). A mixture of **5** (3.75 g) and lithium azide (0.75 g, dried and finely powdered) in *N,N* dimethylformamide (10 ml, dried over calcium hydride and distilled) was heated at 70–80° with stirring for 3 h. The reaction mixture was cooled to room temperature and then evaporated *in vacuo* at 40°. The residue was dissolved in chloroform (30 ml), and the solution was washed with cold water (5 ml). The water wash was back extracted with chloroform (15 ml × 2). The combined chloroform extracts were dried (Drierite) and evaporated, and the residue was crystallized from ethyl acetate–ether solution, m.p. 170–171°; yield 2.9 g (76%). The product was identical with the product obtained earlier by method A on the basis of mixed m.p., i.r., and t.l.c. When anhydrous acetone was used as the solvent, the yield of **6** was only 65.5%.

Reaction of **5** *with sodium azide in aqueous acetone. Formation of* **6** *and of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranose.* — Sodium azide (0.35 g, finely powdered) was added to a solution of **5** (1.83 g) in acetone (27 ml) and water (10 ml). The reaction mixture was heated at 60° with stirring for 1 h, kept at room temperature for 2 days, and then evaporated *in vacuo* at room temperature. The residue was extracted with chloroform (50 ml), which was washed with cold water (5 ml). The water wash was back-extracted with chloroform (10 ml × 2), and the combined chloroform extracts were evaporated *in vacuo*. T.l.c. of the residue in 1:9 (v/v) methanol–chloroform showed two spots (R_F 0.41 and 0.51). The spot of higher R_F corresponds to that of **6**. The residue was chromatographed on silica gel

(Bio-Sil A, 100–200 mesh; 1.9 × 56 cm column), with 1:9 (v/v) methanol–chloroform as eluent. The fractions corresponding to the spot of higher R_F were pooled and evaporated, and the residue was crystallized from a mixture of ethyl acetate and ether (yield 1.1 g, 59%), m.p. 170–171°; t.l.c. and i.r. spectrum were identical with those of the product obtained by method A, and no depression of the mixed melting point was observed.

The fractions corresponding to the spot of lower R_F were pooled and evaporated *in vacuo*. The mixture was crystallized from ether–petroleum ether (yield 0.25 g, 14.5%), m.p. 90° (lit.[3] m.p. 65–75°); optical rotation, i.r., and n.m.r. correspond to those found for 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose. The compound is identical with the product obtained by hydrogenolysis of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside.

*Formation of bis(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyranosyl)-amines* **3** *and* **4** *as by-products during catalytic reduction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl azide* (**6**). — A solution of **6** (4.0 g) in ethyl acetate (200 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of Adam's platinum oxide catalyst (0.3 g) for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to a small volume, when crystalline material separated out. This was filtered off, washed with ether, and dried. The main product (**2**) was recrystallized twice from ethanol (yield 2.6 g, 70%); the compound shrank and turned light brown at 145–146° and decomposed at 235–240°, $[\alpha]_D^{24}$ −22.9° (*c* 1.15, chloroform); lit.[10] m.p. 225–230°, $[\alpha]_D^{25}$ −5.2° (*c* 1.27, chloroform); lit.[5] m.p. 152–153° $[\alpha]_D$ −13° (*c* 1.0, chloroform); lit.[11] m.p. 233–234° (dec.) $[\alpha]_D$ −19.6 to −23.3° (*c* 1.5, chloroform); lit.[7] m.p. 147–149°, $[\alpha]_D^{30}$ −14° (*c* 1.0, chloroform); ν_{max}^{KBr} 3400 (NH), 2965, 2888 (CH), 1750, 1732 (C=O, acetoxyl), 1661, 1535 (C=O, amide), and 1235 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 2.00 (s, 3 H, $NHCOCH_3$), 2.05, 2.12 (2 s, 9 H, $OCOH_3$), 2.20 (s, 2 H, NH_2-1), 3.72 (broad, 1 H, H-5), 4.17 (m, 2 H, H_2-6), 5.09 (d, 1 H, CH-1 anomeric), and 6.45 (d, 1 H, *J* 9 Hz, NH). On storage at room temperature, **2** turned into a black mass, and t.l.c. indicated the formation of dimers along with unidentified products.

The combined mother liquors were evaporated *in vacuo* to dryness, and the residue was crystallized from ethyl acetate and petroleum ether to give 0.35 g of material, m.p. 223–225° (dec.); t.l.c. (1:9, v/v, methanol–chloroform) of the product gave two major spots (R_F 0.20 and 0.33). The product was chromatographed on silica gel (Bio-Sil A; 100–200 mesh) with methanolic chloroform (5–20%) as eluent. The compound corresponding to spot R_F 0.20 was isolated, yielding 0.17 g (4%) of **3**, identified on the basis of its optical rotation and ^{13}C-*n.m.r. spectrum*, m.p. 267–268° (after two crystallizations from ethanol), $[\alpha]_D^{21}$ −28.5° (*c* 1.2, chloroform) changed to +19° after 3 weeks; t.l.c. in 1:9 (v/v) methanol–chloroform of the chloroform solution (used in the optical rotation study) showed two spots (R_F 0.20 and 0.33) corresponding to the β,β (**3**) and α,β (**4**) isomers, respectively; ν_{max}^{KBr} 3330 (NH), 2980 (CH), 2881, 1745 (C=O, acetoxyl), 1658, 1540 (C=O, amide), and 1240 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 6 H, $NHCOCH_3$), 2.06, 2.12, (2s, 18 H, $COCH_3$), 3.59 (br, 2 H,

H-5), 4.12 (m, 8 H, H-2, H_2-6, H-4), 5.07 (m, 4 H, H-1, H-3), and 5.92 (d, 2 H, *J* 9 Hz, N*H*COCH$_3$).

Anal. Calc. for $C_{28}H_{41}N_3O_{16}$: C, 49.77; H, 6.13; N, 6.22. Found: C, 49.95; H, 6.35; N, 6.40.

The fractions corresponding to the upper spot (R_F 0.33) were pooled and evaporated, and the residue was crystallized from ethanol, yielding 0.11 g (3%), m.p. 253–254°, $[\alpha]_D^{20}$ +38.8° (*c* 1.2, chloroform); ν_{max}^{KBr} 3340 (NH) 2977 (CH), 2945, 1735 (C=O, acetoxyl), 1652, 1515 (C=O, amide), and 1235 cm^{-1} (acetate): ^{1}H-n.m.r. ($CDCl_3$): δ 1.96 (s, 6 H, NHCOC*H*$_3$), 2.03, 2.10 (2 s, 18 H, OCOCH$_3$), 3.64 (br., 2 H, H-5), 4.20 (m, 7 H, H-2, H_2-6, H-4), 5.04 (m, 5 H, H-1, H-3, H-4), 5.92 (d, 1 H, *J* 8 Hz, NH-1), and 6.21 (d, 2 H, *J* 8 Hz, N*H*COCH$_3$).

Formation of **3** *and* **4** *from* **2** *in boiling methanol.* — A solution of **2** (0.6 g) in methanol (25 ml) was heated under reflux on a steam bath for 72 h, when the color of the solution turned light brown. T.l.c. (1:9, v/v, methanol–chloroform) of the methanol solution showed the presence of very little starting material and the formation of **3** and **4** along with other products. The solution was evaporated *in vacuo* to dryness, and the residue was chromatographed on a silica gel (Bio-Sil A, 100–200 mesh) column in methanolic chloroform and increasing the proportion of methanol from 5 to 20%. The amount of **3** isolated was 62 mg (11%), and that of **4**, 52 mg (8.8%). The identity of these products with authentic samples was shown by mixed m.p., and i.r. and n.m.r. spectroscopy.

*Formation of the de-*O*-acetylated dimer* **8** *from a methanolic solution of* **2** *at room temperature.* — A solution of **2** (0.5 g) in methanol (50 ml) was kept for 89 days at room temp., after which t.l.c. (ethanol) of the reaction mixture gave one spot, which differed from that of the starting material. The solution was concentrated *in vacuo* to a small volume (~5 ml). Ethyl acetate was added until turbidity was reached, when a white precipitate was obtained. After filtration and washing with ether, it was recrystallized from a mixture of methanol and ethyl acetate, yielding 0.21 g (68%) of **8**, m.p. 174–175°, $[\alpha]_D^{21}$ +30.1° (*c* 1.04, methanol): ν_{max}^{KBr} 3510–3190 (NH, OH), 2930, 2890 (CH), 1650, 1560 (C=O, amide): ^{1}H-n.m.r. (D_2O): δ 1.99 (s, 6 H, NHCOC*H*$_3$) and 4.28 (d, 2 H, *J* 9 Hz, H-1).

Anal. Calc. for $C_{16}H_{29}N_3O_{10}\cdot H_2O$: C, 43.53; H, 7.09; N, 9.52. Found: C, 43.81; H, 7.07; N, 9.28.

*De-*O*-acetylation of dimer* **3**. — A solution of **3** (0.4 g) in aqueous methanol (40 ml) containing 10% triethylamine was stirred for 5 h at room temp. The solution was evaporated to remove traces of triethylamine and methyl acetate. The residue was crystallized from a mixture of methanol and ethyl acetate (yield 0.21 g, 83%), m.p. 174–175° (shrinks at 151°); t.l.c. (1:9, v/v, methanol–chloroform) of the product had an R_F value identical with that of the product obtained in the preceding experiment.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl azide.* — A solution of **6** (2 g) in 50% aqueous methanol (40 ml) containing 10% triethylamine was stirred for 16 h at room temperature and evaporated *in vacuo*. Water (10 ml) was added to the residue and evaporated to remove traces of triethylamine and methyl acetate. The residue was

dissolved in water (20 ml), the solution washed with ethyl acetate, and evaporated, and the residue crystallized form 2-propanol–ether (yield 0.95 g, 72%), m.p. 147–148° (frothing); lit.[16] m.p. 142° (dec.); lit.[20] m.p. 140–145°; $[\alpha]_D^{25}$ −25.9° (*c* 1.01, water); ν_{max}^{KBr} 3480–3160 (OH, NH), 2858 (CH), 2120 (N_3), 1660, and 1548 (C=O, amide); ^{1}H-n.m.r. (D_2O): δ 2.00 (s, 3 H, NHCOCH_3), anomeric proton partially obscured by HDO peak at 4.75 (d, 1 H, *J* 9 Hz, H-1).

Anal. Calc. for $C_8H_{14}N_4O_5$: N, 22.75. Found: N, 22.54.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosylamine*[16] (**9**). — A solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide (0.7 g) in methanol (60 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of Adam's platinum oxide catalyst (0.05 g) for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated to a small volume (~5 ml) *in vacuo*. Ether was added, when a white precipitate separated out. The ether was decanted off, and the white precipitate was dried *in vacuo*. The compound was very hygroscopic; it shrank at 70° and became foamy at 104–110° [lit.[16] m.p. 140–143° (dec.)]; t.l.c. (1:1, v/v, methanol–chloroform) showed one ninhydrin-positive spot (R_f 0.26); ν_{max}^{KBr} 3530–3160 (NH, NH_2, OH), 2940, 2885 (CH), 1650, and 1555 cm^{-1} (C=O, amide); ^{1}H-n.m.r. (D_2O): δ 2.00 (s, 3 H, NHCOCH_3), the anomeric proton was obscured by the HDO peak at 4.91; ^{13}C-n.m.r. (D_2O): δ 175.9 (C=O), 85.4 (C-1), 78.1 (C-5), 75.8 (C-3), 75.8 (C-3), 71.3 (C-4), 62.1 (C-6), 57.6 (C-2), and 23.5 (CH_3 of NHAc).

Anal. Calc. for $C_8H_{16}N_2O_5 \cdot H_2O$: C, 40.32; H, 7.63. Found: C, 40.98; H, 7.61.

Formation of **8** *from* **9**. — A solution of **9** (100 mg) in methanol (50 ml) was kept for 2 months at room temperature, when optical rotation was positive. The solution was evaporated *in vacuo* to a small volume (~5 ml). Ethyl acetate was added until turbidity, when a white precipitate separated out. The mixture was filtered, and the residue was washed with ethyl acetate and dried, m.p. 173–175° (shrinks at 152°); t.l.c. and i.r. indicated that the compound was identical with that obtained earlier by the de-*O*-acetylation of **3**.

Formation of **3** *and* **4** *during attempted synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylguanidine by treating* **2** *with 1-amidino-3,5-dimethylpyrazole nitrate.* — A mixture of **2** (50 mg) and 1-amidino-3,5-dimethylpyrazole nitrate (32 mg) in absolute alcohol (5 ml) was heated for 5 h in a steam bath, and was then kept for 48 h at room temperature, when needle-shaped crystalline material separated out. The crystals were filtered off, washed with ether, and dried, m.p. 243–246° (dec); t.l.c. and i.r. indicated that the product was a mixture of dimers **3** and **4**.

Formation of **3** *and* **4** *during attempted synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylguanidine by treating* **2** *with S-methylisothiourea hydrogensulfate.* — A mixture of **2** (50 mg) and *S*-methylisothiourea hydrogensulfate (32 mg) in absolute alcohol (5 ml) was heated for 4 h on a steam bath, and then kept for 16 h at room temperature. No thiol odor in the reaction mixture resulting from the liberation of methanethiol was observed, indicating that no reaction had taken place. The solution was evaporated *in vacuo*, and the residue was dissolved in dry *N*,*N*-dimethylformamide (5 ml) and heated to 100–110° with stirring for 4 h,

when the solution turned light brown. (Some precipitated *S*-methylisothiourea hydrogensulfate, however, was left with the solutes.) The suspension was filtered, the filtrate was evaporated *in vacuo*, and the residue was crystallized from alcohol, m.p. 236–240°; t.l.c. (1:9, v/v, methanol–chloroform) and i.r. data indicate that the product is a mixture of **3** and **4**, as was found in the preceding experiment.

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