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THE SYNTHESIS OF OLIGOSACCHARIDE–L-ASPARAGINE COMPOUNDS. PART VI. DI-*N*-ACETYLISOCHITOBIOSE–L-ASPARAGINE, 2-ACETAMIDO-6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-1-*N*-(L-ASPART-4-OYL)-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE*

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ABSTRACT

The crystalline intermediate 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**5**), obtained by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl bromide with either 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide or its 6-*O*-triphenylmethyl derivative, was reduced in the presence of Adams' catalyst to give a disaccharide amine. Condensation with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate afforded crystalline 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-*O*-acetyl-1-*N*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**9**). Catalytic hydrogenation in the presence of palladium-on-charcoal was followed by saponification to give 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**11**) in crystalline form. From the mother liquors of the reduction of **5**, a further crystalline product was isolated, to which was assigned a bisglycosylamine structure (**12**).

INTRODUCTION

As part of our program of synthesis of oligosaccharide–L-asparagine compounds, 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**11**) was synthesized. Previous work[2] has shown that di-*N*-acetylchitobiose is a more potent inhibitor of wheat-germ agglutinin

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than is 2-acetamido-2-deoxy-D-glucose, and preliminary experiments[3] indicated that 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (di-*N*-acetylchitobiose–L-asparagine)[4] was even more inhibitory than was the free disaccharide; for this reason, synthesis of the (1→6) analog (**11**) of di-*N*-acetylchitobiose–L-asparagine is of interest for a study of the inhibition of wheat-germ and other plant agglutinins in relation to the structure of the receptor sites at the surface of cancer and virus-transformed cells[5].

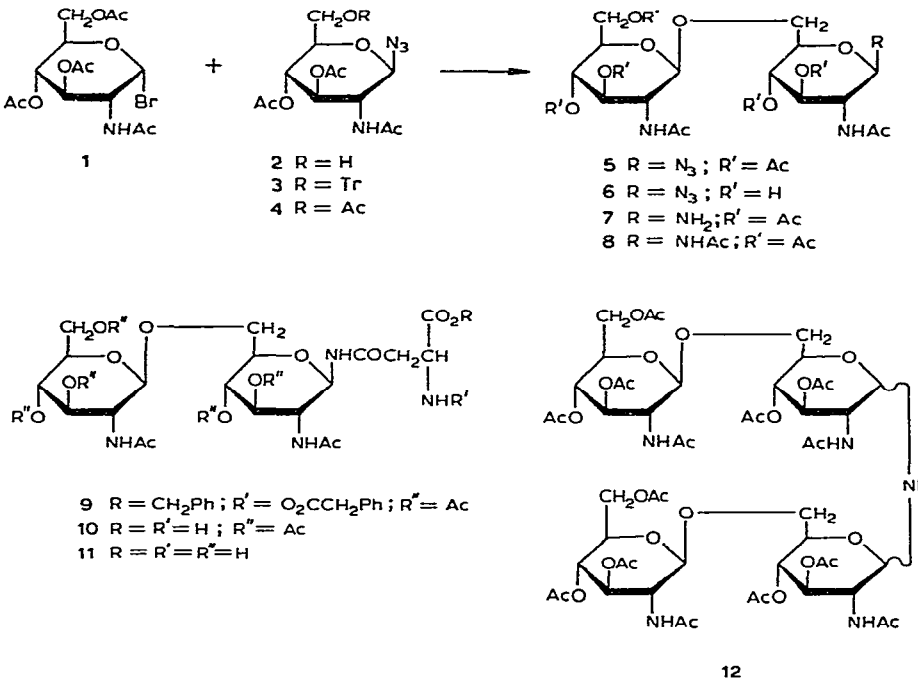
DISCUSSION

The (1→6) analog of di-*N*-acetylchitobiose cannot be obtained from natural sources and, if obtained by synthesis[6], the (1→6) glycosidic linkage is probably not resistant to the conditions of preparation of a glycosyl halide. Consequently, the present synthesis was based on the preparation of a disaccharide azide, as described previously for the preparation of 2-acetamido-2-deoxy-3- and 6-*O*-D-mannopyranosyl-β-D-glucopyranosyl azides[7,8], and subsequent reduction of the azide to the amine and condensation with a protected aspartic acid derivative.

In the condensation of 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**2**) with 2-acetamido-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide[9] (**1**) in the presence of mercuric cyanide, the crystalline disaccharide azide **5** was obtained in 10% yield; this yield is in the same range as that (5.3%) obtained after the Koenigs–Knorr condensation of **1** with 2-acetamido-1,3,4-tri-*O*-acetyl-β-D-glucopyranose in the presence of silver oxide[6]. However, the yield of the condensation could be improved by coupling **1** with the known 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-β-D-glucopyranosyl azide[10] (**3**) under the conditions described by Bredereck *et al.*[11], and pure crystalline **5** was obtained in 15% yield. Samples of compound **5**, obtained by the two routes, were shown to be identical by comparison of their melting points, optical rotations, i.r. and n.m.r. spectra, and their behavior in t.l.c.

The n.m.r. spectra of **2** and of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[12] (**4**) were recorded at 60 MHz under the same conditions. Both spectra revealed a one-proton doublet at δ 5.35 with a coupling constant J of 9.5 Hz, which is characteristic[13] of 1,2-*trans* diaxial protons ($^4C_1$ conformation); these results are in agreement with the data reported by Bolton, Hough, and Khan[14], who first proved the β-D configuration of **4** on the basis of n.m.r. studies (see also Kiyozumi *et al.*[15], and Austen and Marshall[16]). The β-D configuration of the azido group of compound **2** was thus ascertained.

The β-D configuration of the glycosidic linkage of the disaccharide azide **5** was strongly suggested by the mode of synthesis and by comparison of the molecular rotation of **5** (−19,800°) with the sum of the molecular rotations of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[9] and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[14] (−24,700°), as compared to the sum of the molecular rotations of the methyl α-D-glucopyranoside derivative[17] and of the β-D-glucopyranosyl azide (+20,100°). As additivity of molecular rotations is not a



rigorous proof, the β-D-configuration was further confirmed by study of the 100-MHz n.m.r. spectrum of **5**. The low solubility of **5** in chloroform led to poorly defined signals, and the n.m.r. spectrum was recorded in pyridine-d_5. In order to facilitate the interpretation, the spectra of both **4** and 2-acetamido-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose were recorded under the same conditions. The data obtained for the latter compound were in good agreement with those reported on chloroform solutions by Horton *et al.*[18], and for **4** by Bolton *et al.*[14] and Kiyozumi *et al.*[15], although some differences in the chemical shifts, due to the different solvent used, were observed. The pentaacetate showed the H-1 doublet at δ 6.45 ($J_{1,2}$ 3.5 Hz) and the azide **4** at δ 5.30 ($J_{1,2}$ 9.5 Hz), as compared to 6.18 ($J_{1,2}$ 3.5 Hz) (Ref. 18) and 4.88 ($J_{1,2}$ 9 Hz) (Ref. 15) in chloroform-*d*, respectively. Compound **5** showed a one-proton doublet at δ 5.21 (J 9 Hz), which was attributed to the *trans*-axial proton H-1 of the 2-acetamido-2-deoxy-β-D-glucopyranosyl azide residue, as this doublet appears in the spectrum of **4** at δ 5.30. The other anomeric signal of **5** was a one-proton doublet at δ 5.15 (J 9 Hz), attributed to the *trans*-axial H-1 proton of the interglycosidic linkage. This attribution is based on the hypothesis that an interglycosidic α-D linkage would give rise to a narrow doublet at much lower field, as shown by the spectrum of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose. The attribution of the anomeric signals observed in the spectrum of **5** is supported by the recent work of Schmitt and Sinaÿ[19], who assigned, in a derivative of 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose, the signal at δ 5.25 to the anomeric proton of the reducing residue and the signal at δ 5.10 to H-1 of the nonreducing residue. As

subsequent reactions did not involve the configurations at C-1 of both residues[10], the β-D configuration was established for compounds **5–11**.

When compound **5** was treated with sodium methoxide and the solution deionized by passage through a column of Dowex-50 (H^+) resin, no *O*-deacetylated disaccharide azide (**6**) was obtained. Instead, 2-acetamido-2-deoxy-α-D-glucopyranose was isolated as the only crystalline product, and the mother liquors contained 2-acetamido-2-deoxy-β-D-glucopyranosyl azide. The sensitivity to acid of the β-D-(1→6) linkage of **5** was also indicated by the observation that treatment of compound **12**, as just described for **5**, gave crystalline 2-acetamido-2-deoxy-α-D-glucopyranose as the major product, the mother liquors containing the known 2-acetamido-1-*N*-(2-acetamido-2-deoxy-D-glucopyranosyl)-2-deoxy-D-glucopyranosylamine[14] in impure form. A more extensive study, on a semiquantitative basis, of the hydrolysis of **6** with 50 μM sulfuric acid, 50% (v/v) aqueous acetic acid, and Dowex-50 (H^+) for various lengths of time, at room temperature and 100°, was undertaken. In all cases, the glycosidic bond was ruptured after a few min at room temperature with the formation of 2-acetamido-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranosyl azide, as shown by t.l.c. and g.l.c. of the reaction mixture. The extent of hydrolysis was considerably decreased when the solution was rapidly deionized at 0°, but treatment with a carboxylic cation-exchanger (such as Amberlite CG-50, H^+) at room temperature still caused some hydrolysis of the glycosidic bond. Although the β-D-(1→6) linkage in oligosaccharides is considered to be the least susceptible to acid[20], extensive hydrolysis of 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose under mildly acidic conditions has been previously reported[21]. Although it has been shown that methyl 2-acetamido-2-deoxy-β-D-glucopyranoside is hydrolyzed about three times as rapidly as methyl β-D-glucopyranoside[22], the great lability of the (1→6)-2-acetamido-2-deoxy-β-D-hexopyranosyl linkage in disaccharides in the presence of dilute acetic acid is nevertheless exceptional.

Hydrogenolysis of **5** gave, in addition to the disaccharide amine **7** characterized by the tri-*N*-acetyl derivative (**8**), a by-product to which the structure of a disaccharide bisglycosylamine (**12**) was assigned on the basis of earlier studies[14,23] of a similar compound formed by the monosaccharide analog of **7**. An α,α-D configuration is suggested by the isorotation rules previously used for the monosaccharide bisglycosylamine[14], after taking into consideration a contribution of $[M]_D$ +2550° or −7230° for the β-D-(1→6) linkage; the latter figures are based on a comparison between the molecular rotation of the disaccharide azide **5** with those of the monosaccharide azides **4** and **2**, respectively.

The synthesis of the fully protected intermediates **9** and **10**, as well as of the final compound **11**, followed the route described earlier[4] for the (1→4) analog. Compound **11**, obtained as the crystalline monohydrate, showed a chromatographic behavior identical with that of the di-*N*-acetylchitobiose–asparagine derivative[4].

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting points". Optical rotations were determined in 1-dm semimicro tubes, with a Perkin–Elmer Model 141 polarimeter; the chloroform used was analytical-reagent grade and contained *ca.* 0.75% of ethanol. I.r. spectra were recorded with a Perkin–Elmer Model 237 spectrophotometer, and n.m.r. spectra with Varian A-60 and HA-100 n.m.r. spectrometers for solutions in pyridine-d_5, with tetramethylsilane as the internal standard. G.l.c. of the per(trimethylsilyl) ethers was performed with a Perkin–Elmer Model 900 gas chromatograph equipped with a flame-ionization detector on 30.5 × 0.3 cm and 152.5 × 0.3 cm columns of glass beads (GLC 110, 120–140 mesh) coated with 0.1% OV-17 (Supelco Inc., Bellefonte, Pa. 16823), programmed for a rise of 10° per min from 80° to 275°, with hydrogen as carrier gas; octa-*O*-(trimethylsilyl)sucrose was used as an internal standard. Column chromatography was performed on Silica Gel Davison (60–200 mesh, grade 950, Davison Chemical, Baltimore, Md. 21226) used without pretreatment. The proportion of weight of substance to weight of silica gel was 1:60 to 1:100. The ratio of diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 2–3 ml per g of substance to be chromatographed. T.l.c. was performed on precoated Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany) and, for polar compounds, on precoated cellulose plates (layer thickness 0.10 mm; E. Merck, Darmstadt, Germany); the solvent travel-distance was *ca.* 5.5 cm. The zones were detected by spraying the chromatogram with (*A*) 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol, (*B*) 1:10 conc. sulfuric acid–ethanol, (*C*) 1% (w/v) ninhydrin in 1:1 (v/v) ethanol–acetone, and (*D*) aniline hydrogen phthalate solution[24], followed by heating on a hot plate for a few min. Evaporations were conducted *in vacuo*, with a bath temperature below 45°, unless stated otherwise. Solutions (<5 ml) in volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. M. Manser, Zürich, Switzerland.

2-Acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**2**). — A suspension of 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-β-D-glucopyranosyl azide[10] (2.25 g) in 60% acetic acid (25 ml) was heated for 20 min at 100° with occasional shaking. During this time the reaction was monitored by t.l.c. Water (25 ml) was added and the solvent was then evaporated under diminished pressure; the last traces of acetic acid were removed by repeated addition and distillation of water and finally methanol, and the syrupy residue was dried *in vacuo* at room temperature over phosphorus pentaoxide and chromatographed on a column of silica gel with 9:1 (v/v) chloroform–ethanol. Triphenylmethanol was eluted first, and then a small amount of unreacted starting-material, and finally pure **2**. Crystallization from aqueous ethanol gave 851 mg (65%) of clusters of small needles, m.p. 171–174° (with dec.), $[\alpha]_D^{20}$ −38° (*c* 1.0, methanol); i.r. data: ν_{max}^{KBr} 3460 (OH), 3305, 3260 (NH), 2120 (N_3), 1745 (OAc), 1655 (Amide I), 1555 cm^{-1} (Amide II); n.m.r. data (60 MHz): δ 5.35 (one-proton doublet, $J_{1,2}$ 9.5 Hz, *trans*-axial H-1); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*) R_F 0.3.

Anal. Calc. for $C_{12}H_{18}N_4O_7$: C, 43.64; H, 5.49; N, 16.96; O, 33.91. Found: C, 43.56; H, 5.47; N, 16.96; O, 33.73.

2-Acetamido-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**5**). — *A. From* **2**. A mixture of dry **2** (735 mg) and finely powdered mercuric cyanide (800 mg) in dry 1:1 (v/v) benzene–nitromethane (150 ml) was concentrated to 100 ml at atmospheric pressure, and then cooled to room temperature. To this mixture was added a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide[9] (**1**) in dry dichloromethane (100 ml) prepared *in situ* from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (1.0 g). The mixture was stirred for 3.5 days at room temperature, the reaction being monitored by t.l.c. Additional amounts of bromide **1**, prepared from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (1.0 g) in dry dichloromethane (100 ml), and mercuric cyanide (800 mg) were added, and the mixture was stirred for a further 4.5 days at room temperature. It was then rapidly washed with ice-cold water (3 × 75 ml), a cold, saturated, potassium hydrogen carbonate solution (3 × 75 ml), and ice-cold water (2 × 75 ml), dried (sodium sulfate), and evaporated to a residue that was dried *in vacuo* overnight. The yellow syrup (920 mg) thus obtained was chromatographed on a column of silica gel with 9:1 (v/v) chloroform–ethanol; the fractions having R_F 0.4 on t.l.c. in the same solvent mixture were combined and evaporated to give a partly crystalline residue (160 mg) consisting of a mixture of the disaccharide azide **5**, of some starting material **2**, and of a trace of a faster-moving component. This residue was rechromatographed on a column of silica gel with the same solvent mixture, to give 48 mg of pure **5**, which crystallized and was recrystallized from methanol–ether as small needles, m.p. 200–201° (with dec.), $[\alpha]_D^{20}$ −30° (*c* 0.4, chloroform); i.r. data: ν_{max}^{KBr} 3320 (NH), 2120 (N_3), 1745 (OAc), 1655 (Amide I), 1545 cm^{-1} (Amide II); n.m.r. data (100 MHz): δ 1.92–2.08 (21 H, 5 OAc and 2 NHAc), 5.15 (one-proton doublet, $J_{1,2}$ 9.0 Hz, axial H-1 of the interglycosidic linkage), 5.21 (one-proton doublet, $J_{1,2}$ 9.0 Hz, axial H-1 of the β-D-glucopyranosyl azide residue); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*) R_F 0.4.

Anal. Calc. for $C_{26}H_{37}N_5O_{15}$: C, 47.35; H, 5.65; N, 10.62; O, 36.38. Found: C, 47.44; H, 5.65; N, 10.50; O, 36.41.

After column chromatography of the less-pure fractions from the two columns and of the mother liquors, a further 100 mg of pure **5** was obtained, to afford a total of 148 mg (10%).

B. From **3**. Silver perchlorate (1.9 g) was dissolved in dry nitromethane (40 ml) under mild heating and stirring. Drierite (2.3 g) was added and the mixture was kept in the dark for *ca.* 10 min. After slow addition of 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-β-D-glucopyranosyl azide[10] (**3**, 4.7 g), the mixture was cooled to 0°, and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide[9] (**1**) (prepared *in situ* from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose, 3.65 g) in dry, alcohol-free chloroform (275 ml), was added and the mixture was treated as described by Bredereck *et al.*[11]. The partly crystalline residue (3.30 g) was chromatographed on a column of silica gel with 9:1 (v/v) chloroform–ethanol. The

fractions having R_F 0.4 on t.l.c. in the same solvent-mixture were combined and evaporated. The crystalline residue (850 mg) was recrystallized from methanol–ether to give 830 mg (15%) of **5** as needles, m.p. and mixed m.p. with the compound prepared from **2** 200–201° (with dec.), $[\alpha]_D^{20}$ −30° (*c* 0.4, chloroform); the i.r. and n.m.r. spectra, and R_F value on t.l.c., were identical with those of the compound just described.

*2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylamine* (**7**). — Compound **5** (125 mg) was dissolved in abs. ethanol (30 ml) and hydrogenated at atmospheric pressure in the presence of platinum oxide (20 mg) for 2 h at room temperature. The catalyst was filtered off, the clear solution was evaporated under diminished pressure with the bath temperature maintained below 30°, and the syrupy residue was dried *in vacuo* at room temperature to give the rather unstable amine **7** (90 mg; 75%). It showed R_F 0.4 on t.l.c. in 4:1 (v/v) chloroform–ethanol with a very minor, faster-moving contaminant, and it was immediately used for the preparation of **9** without further purification*. On one occasion, the crude compound **7** was purified by column chromatography on silica gel in 4:1 (v/v) chloroform–ethanol; the fractions containing the ninhydrin-positive material were combined and the solvent was evaporated under diminished pressure below 30° (bath temperature). The residue was crystallized and recrystallized from cold methanol to give **7** (~30%) as prisms containing one mole of methanol, m.p. 228–230° (with dec.), $[\alpha]_D^{18}$ −14° [*c* 0.1, 9:1 (v/v) chloroform–methanol]; i.r. data: ν_{max}^{KBr} 3375 (OH), 3300 (NH), 1740 (OAc), 1655 (Amide I), 1550 cm^{-1} (Amide II); t.l.c. in 4:1 (v/v) chloroform–ethanol (*A*, *C*) R_F 0.4.

Anal. Calc. for $C_{26}H_{39}N_3O_{15}\cdot CH_3OH$: C, 48.72; H, 6.51; N, 6.31. Found: C, 48.60; H, 6.07; N, 5.97.

*2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-1-*N*-acetyl-3,4-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylamine* (**8**). — Compound **7** (15 mg) was dissolved in methanol (8 ml) and acetic anhydride (1 ml) was added; the solution was kept overnight at room temperature, and then evaporated under diminished pressure. Evaporation of toluene several times, and methanol twice, from the residue gave a colorless residue that was dried *in vacuo*. It was not possible to purify this compound by recrystallization, and it was, therefore, chromatographed on a column of silica gel in 9:1 (v/v) chloroform–ethanol. The fractions having R_F 0.3 on t.l.c. in the same solvent mixture were combined and evaporated. The residue (12 mg) of pure **8** crystallized and was recrystallized from abs. ethanol to give a semi-crystalline product (9 mg, 56%), changing into long needles at 267–268°, m.p. 298–299° (with dec.), $[\alpha]_D^{20}$ −10° (*c* 0.2, chloroform); i.r. data: ν_{max}^{KBr} 3300 (NH), 1740 (OAc), 1655 (Amide I), 1540 cm^{-1} (Amide II); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*) R_F 0.3.

Anal. Calc. for $C_{28}H_{41}N_3O_{16}$: C, 49.77; H, 6.12; N, 6.22; O, 37.90. Found: C, 49.73; H, 6.10; N, 6.06; O, 37.62.

*2-Acetamido-6-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-β-*D*-gluco-*

*To prevent the formation of higher molecular-weight by-products[7,8,14,23].

pyranosyl azide (**6**). — A solution of dry **5** (44 mg) in ammonia-saturated abs. methanol (5 ml) was kept for 24 h in the cold (*ca.* 4°), the reaction being monitored by t.l.c. The solvent was evaporated off and the crystalline residue was recrystallized from ethanol to give pure **6** as clusters of small needles (26 mg, 87%) m.p. (puffing) 207–208° (with dec.), $[\alpha]_D^{18}$ −36° [*c* 0.33, 9:1 (v/v) methanol–water]; i.r. data: ν_{max}^{KBr} 3450, 3350 (OH), 3250 (shoulder, NH), 2120 (N_3), 1670, 1635 (Amide I), 1540 cm^{-1} (Amide II); t.l.c. on silica gel in 60:25:4 (v/v) chloroform–methanol–water (*A*) R_F 0.1; on cellulose in 4:1:5 (v/v) butanol–ethanol–water, organic phase (*D*) R_F 0.2.

Anal. Calc. for $C_{16}H_{27}N_5O_{10}$: C, 42.76; H, 6.06; N, 15.58; O, 35.60. Found: C, 42.62; H, 6.14; N, 15.38; O, 35.42.

*2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4-di-*O*-acetyl-1-*N*-[1-benzyl* N*-(benzyloxycarbonyl)-*L*-aspart-4-oyl]-2-deoxy-β-*D*-glucopyranosylamine* (**9**). — Compound **7** (100 mg) was dissolved in dry dichloromethane (10 ml), and 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[25] (60 mg) and *N,N'*-dicyclohexylcarbodiimide (50 mg) were added. The mixture was stirred overnight at room temperature. The *N,N'*-dicyclohexylurea that had precipitated was filtered off, and the filtrate was evaporated. The residue (197 mg) was chromatographed on a column of silica gel, which was first eluted with chloroform to remove the non-carbohydrate compounds, and then with 9:1 (v/v) chloroform–ethanol to give pure **9**. Crystallization from methanol gave 90 mg (59%) of small needles, m.p. 233–236° (with dec.), $[\alpha]_D^{20}$ −10° (*c* 0.5, chloroform); i.r. data: ν_{max}^{KBr} 3300 (NH), 1740 (C=O ester), 1700 (C=O of $-NHCO_2CH_2Ph$), 1660 (Amide I), 1550 cm^{-1} (Amide II); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*, *B*) R_F 0.6; in 19:1 (v/v) chloroform–ethanol (*A*, *B*) R_F 0.2.

Anal. Calc. for $C_{45}H_{56}N_4O_{20}$: C, 55.55; H, 5.80; N, 5.76; O, 32.88. Found: C, 55.49; H, 5.79; N, 5.83; O, 32.87.

*2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4-di-*O*-acetyl-1-*N*-(*L*-aspart-4-oyl)-2-deoxy-β-*D*-glycopyranosylamine* (**10**). — A solution of **9** (65 mg) in 90% acetic acid (10 ml) was hydrogenated in the presence of palladium-on-charcoal (15 mg) at atmospheric pressure for 2 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated to dryness; the last traces of acetic acid were removed by coevaporation of water from the residue. The residue was crystallized from water–ethanol to give 36 mg (72%) of **10**, as needles, m.p. 237–240° (dec.), $[\alpha]_D^{20}$ −13° (*c* 0.5, water); i.r. data: ν_{max}^{KBr} 3340 (broad, NH, OH), 1740 (broad, OAc and CO_2H), 1660, 1650–1640 (Amide I and Amino acid I), 1545–1525 cm^{-1} (Amide II and Amino acid II); t.l.c. on silica gel in 2:4:1 (v/v) butanol–acetic acid–water (*A*) R_F 0.4; in 12:3:5 (v/v) butanol–acetic acid–water (*A*) R_F 0.3.

Anal. Calc. for $C_{30}H_{44}N_4O_{18}$: C, 48.12; H, 5.92; N, 7.48; O, 38.47. Found: C, 48.17; H, 5.89; N, 7.57; O, 38.52.

*2-Acetamido-6-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-1-*N*-(*L*-aspart-4-oyl)-2-deoxy-β-*D*-glucopyranosylamine* (**11**). — Compound **10** (25 mg) was treated with a 0.12M aqueous solution of lithium hydroxide (2.5 ml), and kept for 1 h at room temperature. The solution was passed through a column of Amberlite CG-50 resin

(H^+, 0.6 × 12 cm) in the cold (*ca.* 4°), and the column was washed with water (10 ml). The eluate and washings were concentrated to *ca.* 0.5 ml under diminished pressure. Compound **11** was crystallized as the monohydrate by addition of ethanol, and was recrystallized from water–ethanol to give 12 mg (65%) of small prisms, m.p. 219–220° (dec.), $[\alpha]_D^{20}$ −7.5° (*c* 0.4, water); i.r. data: ν_{max}^{KBr} 3400–3300 (broad, OH and NH), 1640 (broad, Amide I and Amino acid I), 1555 cm^{-1} (broad, Amide II and Amino acid II); t.l.c. on silica gel in 2:4:1 (v/v) butanol–acetic acid–water (*A*, *C*), R_F 0.1, staining blue with ninhydrin; t.l.c. on cellulose in the same solvent system (*C*) R_F 0.3*; in 5:5:1:3 (v/v) pyridine–ethyl acetate–acetic acid–formic acid R_F *ca.* 0.4, staining blue with ninhydrin. The compound contained one molecule of water of crystallization, and extensive drying caused decomposition.

Anal. Calc. for $C_{20}H_{34}N_4O_{13}\cdot H_2O$: C, 43.16; H, 6.52; N, 10.07; O, 40.25. Found: C, 43.16; H, 6.64; N, 9.98; O, 40.04.

*Bis-[2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4-di-*O*-acetyl-2-deoxy-*D*-glucopyranosyl amine* (**12**). — From the mother liquors of **7** as well as from the first fractions eluted from the silica gel column, a second compound, giving a negative ninhydrin reaction, was isolated; it had R_F *ca.* 0.6 in 4:1 (v/v) chloroform–ethanol (*A*) but showed two distinct spots with R_F 0.4 and 0.3 in 9:1 (v/v) chloroform–ethanol (*A*), and R_F 0.25 and 0.2 in 9:1 (v/v) benzene–methanol (*A*). This material (200 mg) was chromatographed on a column of silica gel in benzene, followed by gradient mixtures of solvents of increasing polarity (benzene–ether and ether–ethyl acetate); 2:1 (v/v) ether–ethyl acetate eluted a major fraction corresponding to the faster-moving component on t.l.c. The solvent was partially evaporated under diminished pressure, and the remaining mixture was filtered through charcoal–Celite. The filtrate was evaporated to dryness and the amorphous residue (170 mg) dried *in vacuo* over phosphorus pentaoxide. It was crystallized from ether–hexane to give the disaccharide bisglycosylamine **12** as very hygroscopic, irregular prisms decomposing at 245°, $[\alpha]_D^{20}$ +50° (*c* 0.5, chloroform); i.r. data: ν_{max}^{KBr} 3360 (NH), 1740 (OAc), 1660 (Amide I), 1545–1535 cm^{-1} (Amide II); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*) R_F 0.4. The slower-moving component, possibly an isomer of **12**, was not further investigated.

Anal. Calc. for $C_{52}H_{75}N_5O_{30}\cdot 2H_2O$: C, 48.56; H, 6.19; N, 5.43; O, 39.81. Found: C, 48.51; H, 6.16; N, 5.04; O, 39.69.

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*T.l.c. of 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-1-*N*-(*L*-aspart-4-oyl)-2-deoxy-β-*D*-glucopyranosylamine on cellulose in 2:4:1 (v/v) butanol–acetic acid–water (*C*) showed R_F 0.3 (in Ref. 4, the chromatographic medium was not reported).

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