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The Nephritogenic Glycopeptide from Rat Glomerular Basement Membrane. II.¹⁾

Synthesis of O-(α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-N-(L- β -aspartyl)- α -D-glucopyranosylamine (α -D-Glc-(1 $\rightarrow$ 6)- β -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc-(1 $\rightarrow$ Asn))

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O-(α -D-Glucopyranosyl)-(1 $\rightarrow$ 6)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-N-(L- β -aspartyl)- α -D-glucopyranosylamine has been prepared, as a model of a derivative possibly present in the glomerular basement membrane of rats, by condensation of the corresponding trisaccharide α -amine with α -ethyl benzyloxycarbonyl-L-aspartate in the presence of diethylphosphorocyanidate. This was followed by hydrogenolysis, de-O-acetylation, and deethoxylation of the resulting trisaccharide-amino acid linked derivative to remove the protecting groups. The ¹³C-nuclear magnetic resonance spectra of this product and related glycosylamine derivatives are analyzed and discussed.

Keywords—glomerulonephritis; glycopeptide; nephritogenoside; condensation; catalytic reduction; glucopyranosylamine

Shibata *et al.*²⁾ isolated and purified from the glomerular basement membrane of rats a new glycopeptide that has activity for the induction of glomerulonephritis in homologous animals.³⁾ From methylation analysis data,⁴⁾ carbon-13 nuclear magnetic resonance (CMR) data, the results of a concanavalin A test,⁵⁾ and a consideration of CMR data in comparison with those of related synthetic glycosylamine derivatives,¹⁾ Shibata *et al.*⁶⁾ proposed **1** as the structure of the nephritogenoside (Chart 1). Thus, **1** contains a new type of carbohydrate-peptide linkage with α -D-configuration.

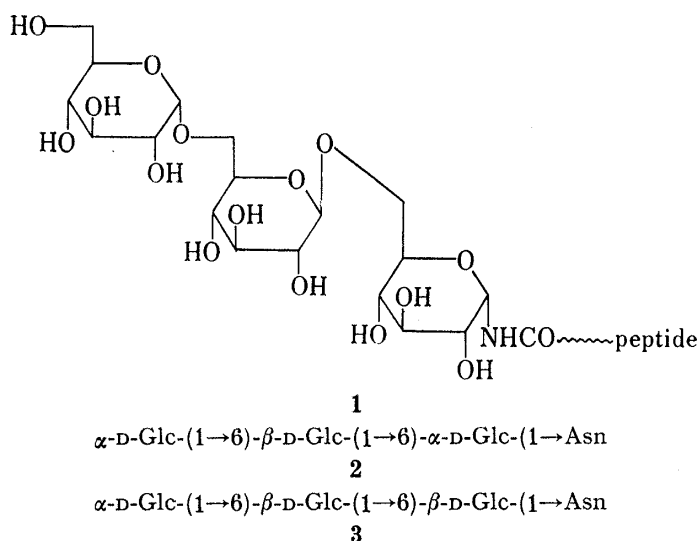


Chart 1

We report here the synthesis of α -D-Glc-(1 $\rightarrow$ 6)- β -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc-(1 $\rightarrow$ Asn (**2**) and its β -isomer of the reducing end group, α -D-Glc-(1 $\rightarrow$ 6)- β -D-Glc-(1 $\rightarrow$ 6)- β -D-Glc-(1 $\rightarrow$ Asn (**3**).

2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl azide (**4**) was obtained by the procedure of the previous paper.¹⁾ De-O-acetylation of **4** with triethylamine in 50% methanolic solution gave an 89.5% yield of **5** (mp 166–167°C). Tritylation of **5** followed by acetylation afforded a 2,3,4-tri-O-acetyl-6-O-trityl- α -D-glucopyranosyl azide (**6**) ($[\alpha]_D +148.1^\circ$). Detritylation was attempted by a slight modification of Randazzo's method.⁷⁾ A benzene solution of **6** was boiled under reflux for 24 h in the presence of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ to give a 2,3,4-tri-O-acetyl- α -D-glucopyranosyl azide (**7**) ($[\alpha]_D +163.6^\circ$). This selective removal of the trityl group under the above conditions avoided hydrolysis or migration of other groups. When anhydrous CuSO_4 was used, migration of the acetyl group occurred. Compound **7** was also prepared according to the method of Ogawa *et al.*⁸⁾; *i.e.*, by treatment with 4:1 AcOH– H_2O for 6 h at 55°C. The compounds obtained *via* both routes had the same physical constants and spectral data.

The synthesis of compound **2** corresponding to **1** was started by condensation of 2,3,4,2',3',4',6'-hepta-O-acetyl- α -D-isomaltosyl bromide (**11**) [which was prepared by the condensation of 2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl chloride⁹⁾ with 1,2,3,4-tetra-O-acetyl- β -D-glucopyranose¹⁰⁾ in the presence of mercuric cyanide, followed by hydrogenolysis and acetylation¹¹⁾ and finally bromination] with compound **7** in the presence of mercuric cyanide. This gave the fully protected trisaccharide O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- α -D-glucopyranosyl azide (**12**), in 20.3% yield. The formation of a (1 $\rightarrow$ 6)- β -glycosidic linkage in **12** without acetyl migration was confirmed by its ^{13}C -nuclear magnetic resonance (^{13}C -NMR) spectrum, which showed the deshielded signal for C-6 at δ 66.8 and the signal for C-1' at δ 100.2. The proton magnetic resonance (^1H -NMR) spectrum showed the axial H-1 of the glucopyranosyl residue as a one-proton doublet at δ 4.56 ($J=8$ Hz), the equatorial C-1 proton of the α -D-glucopyranosyl azide residue as a doublet at δ 5.62 ($J=4$ Hz) and also the equatorial H-1 of the non-reducing end glucopyranosyl residue as a doublet at δ 4.98 ($J=4$ Hz). The same trisaccharide **12** was also synthesized *via* another route. 2,3,4,2',3',4',6'-Hepta-O-acetyl- α -D-isomaltosyl bromide (**11**) was treated with 1,2,3,4-tetra-O-acetyl- β -D-glucopyranose in the presence of mercuric cyanide to give O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-1,2,3,4-tetra-O-acetyl- β -D-glucopyranose (**13**). Trisaccharide azide (**12**) was prepared by treating O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- β -D-glucopyranosyl chloride, prepared from **13** according to Mills *et al.*,¹²⁾ with sodium azide in hexamethylphosphoric triamide (HMPT). Samples of compound **12** obtained by the two routes were identical. Catalytic reduction of **12** in tetrahydrofuran (THF) over Lindlar's catalyst gave a mixture of O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- α -D-glucopyranosylamine (**14**) ($[\alpha]_D +92.1^\circ$) and, together with α -D-glucosylamine, its β -isomer at C-1 (**15**) ($[\alpha]_D +14.6^\circ$) in the ratio of 2:1. Condensation of α -D-glucosylamine **14** with α -ethyl benzyloxycarbonyl-L-aspartate¹³⁾ gave O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-N-(O-ethyl-N-benzyloxycarbonyl-L- β -aspartyl)- α -D-glucopyranosylamine (**16**) in 90% yield using diethylphosphorocyanidate (DEPC).¹⁴⁾ The ^1H -NMR spectrum of the product showed a benzyloxy group signal at δ 7.36, a 10-acetyl-group signal at δ 2.05–1.94 and an ethoxyl methyl signal at δ 1.26. The ^{13}C -NMR data indicated three anomeric carbon atoms at 99.8 (C-1'), 96.0 (C-1''), and 73.9 (C-1), and two α and β carbons of the aspartic acid residue at δ 50.4 and 36.9, respectively. Removal of the benzyloxycarbonyl group of **16** by catalytic hydrogenation afforded O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-N-(O-ethyl-L- β -aspartyl)- α -D-glucopyranosylamine (**18**). Subsequently, **18** was de-O-acetylated and deethoxylated with triethylamine in 50% methanolic solution at room temperature to give the target compound, O-(α -

D-glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl-(1→6)-N-(L-β-aspartyl)-α-D-glucopyranosylamine (2) ($[\alpha]_D + 72.4^\circ$). The $^1\text{H-NMR}$ data indicated three anomeric protons at δ 5.56 ($J=4$ Hz), 4.95 ($J=3.5$ Hz), and 4.50 ($J=7.5$ Hz), and the $^{13}\text{C-NMR}$ data showed three anomeric carbon atoms at δ 103.7 (C-1'), 99.0 (C-1''), and 77.6 (C-1).

In a parallel route, the trisaccharide β-amine **15** was condensed with α-ethyl benzyloxycarbonyl-L-aspartate as described above to give the corresponding β-linked carbohydrate-amino acid compound, O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-N-(O-ethyl-N-benzyloxycarbonyl-L-β-aspartyl)-β-D-glucopyranosylamine (**17**) in 91.3% yield. The $^1\text{H-NMR}$ spectrum of the product showed a benzyloxy group signal at δ 7.36, a 10-acetyl-group signal at δ 2.06–1.94, and an ethoxyl methyl signal at δ 1.22. The $^{13}\text{C-NMR}$ data indicated three anomeric carbon atoms at δ 97.9 (C-1'), 95.3 (C-1''), and 77.8 (C-1), thus, indicating the β-configuration of the N-glycosyl linkage. Decarbobenzyloxylation, de-O-acetylation, and deethoxylation gave O-(α-D-glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl-(1→6)-N-(L-β-aspartyl)-β-D-glucopyranosylamine (**3**). The $^1\text{H-NMR}$ data indicated three anomeric protons at δ 4.98 ($J=8$ Hz), 4.94 ($J=4$ Hz), and 4.50 ($J=8$ Hz), and the $^{13}\text{C-NMR}$ data three anomeric carbon atoms at δ 103.9 (C-1'), 99.0 (C-1''), and 80.4 (C-1).

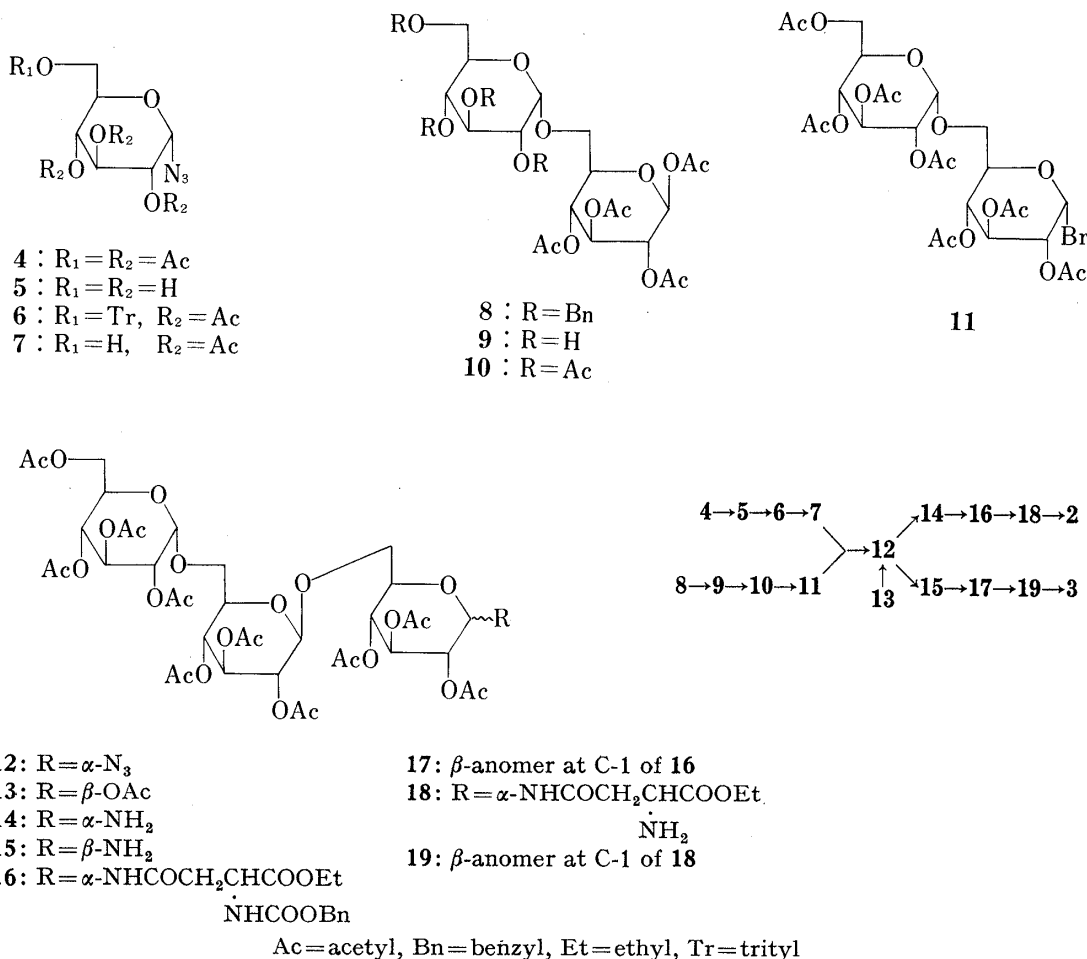


Chart 2

The synthetic route is illustrated in Chart 2. The ^{13}C shifts of the glycosylamine derivatives and related compounds are listed in Table I. The $^{13}\text{C-NMR}$ spectra indicate that the chemical shifts of the sugar moieties of compounds **1** and **2** are very similar, and are clearly different from those of the β-isomer (**3**).

TABLE I. ^{13}C Chemical Shifts examined in CDCl_3^a (δ)

Carbon atom	Compound														
	5	6	7	8	9	10	12	13	14	16	2	1	15	17	3
C-1	90.1	86.3	86.3	91.6	91.9	91.5	86.0	91.6	76.3	73.9	77.6	77.6	84.9	78.7	80.4
C-2	71.6	70.1	69.7	70.3	72.0	70.7	69.6	70.5	69.1	69.1	70.4	70.8	72.1	70.3	72.7
C-3	73.6	70.4	70.4	73.0	73.5	73.3	69.7	73.9	69.9	70.2	72.7	72.4	73.4	75.1	77.5
C-4	70.1	68.4	68.5	68.9	68.5	68.8	67.9	68.3	68.0	67.5	70.4	70.8	69.1	68.8	70.4
C-5	74.7	71.0	72.1	72.9	73.0	72.8	70.5	72.8	70.6	70.8	72.9	74.1	73.8	75.9	77.7
C-6	61.4	61.6	60.6	65.8	65.8	67.5	67.4	67.1	67.4	67.3	69.5	70.4	67.6	67.7	70.0
C-1'				96.9	98.8	95.9	100.2	100.2	99.6	99.8	103.7	104.1	100.3	100.6	103.9
C-2'				79.9	70.3	68.5	70.9	70.7	71.3	71.3	74.1	74.8	71.1	72.9	73.0
C-3'				81.5	74.0	70.4	72.6	71.0	72.7	72.9	77.0	77.2	72.8	74.5	77.0
C-4'				77.6	69.7	66.2	69.0	69.1	68.4	68.6	70.5	71.2	69.0	69.9	70.5
C-5'				70.3	72.0	70.0	72.8	72.8	72.7	72.9	75.4	77.2	72.8	73.8	75.5
C-6'				68.5	61.4	61.8	66.8	68.3	67.2	67.3	66.7	69.6	67.5	67.2	66.7
C-1''							95.7	95.8	95.9	96.0	99.0	100.2	95.9	96.8	99.0
C-2''							68.3	68.5	68.4	69.1	72.9	72.4	68.5	71.6	72.9
C-3''							70.5	70.3	70.6	70.8	74.3	75.5	70.7	72.1	74.3
C-4''							66.4	67.5	66.8	66.7	70.6	71.6	66.5	69.5	70.7
C-5''							69.9	70.0	69.9	69.9	74.3	74.1	70.0	71.8	74.3
C-6''							61.7	61.9	61.8	61.9	61.6	62.0	61.8	62.6	61.6
α										50.4	51.2			51.6	52.2
β (aspartyl residue)										36.9	37.6			38.4	36.3

a) Compounds **1**, **2**, **3** and **5** were measured in D_2O and **17** was measured in CD_3OD .

Experimental

Melting points were determined with a Yanagimoto microapparatus and are uncorrected. The ^1H -NMR spectra were recorded on a JNM MH-100 spectrometer, and the ^{13}C -NMR spectra were obtained at 25.0 MHz in the pulsed Fourier transform mode on JEOL FX-100 instruments. Optical rotations were recorded with a Union Giken PM-201 automatic digital polarimeter. Thin-layer chromatography (TLC) was conducted on precoated silica gel plates (Merck GF-254), and the detection of compounds was achieved by quenching of UV fluorescence and with 10% sulfuric acid solution. Column chromatography was carried out using silica gel (Merck Kieselgel 60).

Materials—2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl azide was obtained by the procedure of the previous paper.¹⁾ 2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl chloride was synthesized by well established procedures.⁹⁾ 1,2,3,4-Tetra-O-acetyl- β -D-glucopyranose was prepared according to the method of Whistler *et al.*¹⁰⁾

α -D-Glucopyranosyl Azide (5)—2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl azide (6 g) was O-deacetylated with triethylamine (4 ml) in 50% methanolic solution (100 ml). The reaction solution was evaporated to dryness and the residue was crystallized from methanol to give **5** (2.95 g, 89.5%). mp 166–167°C. $[\alpha]_D^{25} + 268^\circ$ ($c=1.0$, MeOH). ^1H -NMR (D_2O) δ : 5.52 (1H, d, $J=4$). ^{13}C -NMR data are given in Table I. *Anal.* Calcd for $\text{C}_8\text{H}_{11}\text{N}_3\text{O}_5$: C, 35.13; H, 5.40; N, 20.48. Found: C, 35.24; H, 5.43; N, 20.59.

2,3,4-Tri-O-acetyl-6-O-trityl- α -D-glucopyranosyl Azide (6)—A mixture of **5** (2.97 g) and triphenylmethyl chloride (3.8 g) was stirred in pyridine (14 ml) at 40°C for 18 h, then cooled. Next, 8.4 ml of acetic anhydride was added, and the solution was stirred for 3 h. The mixture was poured into ice-water, and extracted with CHCl_3 . The CHCl_3 extracts were washed with water, dried and concentrated under reduced pressure to give a syrup which contained (TLC) a major (R_f 0.47) and a minor (R_f 0.21) component. The syrup was chromatographed on silica gel with the same solvent system as on TLC (benzene–acetone 6: 1). The eluate containing the major component was evaporated to dryness and the residue was crystallized from ethanol to give pure **6** (7.55 g, 96.9%). mp 62.0–62.5°C. $[\alpha]_D^{25} + 148.1^\circ$ ($c=1.0$, CHCl_3). ^1H -NMR (CDCl_3) δ : 5.71 (1H, d, $J=4$), 7.70–6.90 (15H, m, arom.).

2,3,4-Tri-O-acetyl- α -D-glucopyranosyl Azide (7)—a) A solution of **6** (1.2 g) in 400 ml of benzene was boiled in the presence of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (31 g) under reflux for 24 h. The reaction mixture was filtered, and the filtrate was concentrated to give a syrup.

b) A solution of **6** (2 g) in 50 ml of acetic acid–water (4: 1) was stirred at 55°C for 6 h. The reaction solution was poured into ice-water. After the removal of the precipitate by filtration, the filtrate was ex-

tracted with CHCl_3 . The CHCl_3 layer was washed with water, dried and concentrated to give a syrup (1 g, 86.5%). $[\alpha]_D^{25} + 163.6^\circ$ ($c=0.55$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 5.59 (1H, d, $J=4$), 5.41 (1H, t, $J=9$), 5.04 (1H, t, $J=9$), 4.90 (1H, d.d, $J=4, 9$), 4.02 (1H, m), 3.62 (1H, s, OH), 2.05, 2.01, 1.98 (each s, 3H, OAc).

O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-1,2,3,4-tetra-O-acetyl- β -D-glucopyranose (8)—A solution of 2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl chloride (2 g) [prepared from 2,3,4,6-tetra-O-benzyl- α -D-glucopyranose according to the method of Austin *et al.*¹⁰] in nitromethane (30 ml) was added to a mixture of 1,2,3,4-tetra-O-acetyl- β -D-glucopyranose (2 g), mercuric cyanide (4 g), and molecular sieve 4 (1 g) in the same solvent (10 ml). After being stirred for 21 h at 40°C , the mixture was filtered and the filtrate was extracted with CHCl_3 . The CHCl_3 extracts were washed with water, dried and concentrated to a syrup, which was chromatographed on a column of silica gel with 4:1 (v/v) benzene-acetone as an eluent. The disaccharide fraction was collected and evaporated to dryness to give pure **8** (2.05 g, 41%). $[\alpha]_D^{25} + 47.2^\circ$ ($c=0.45$, CHCl_3).

O-(α -D-Glucopyranosyl)-(1 $\rightarrow$ 6)-O-1,2,3,4-tetra-O-acetyl- β -D-glucopyranose (9)—Catalytic hydrogenolysis of **8** (600 mg) over 10% Pd-C (60 mg) in acetic acid (40 ml) gave the compound **9** (334 mg, 95%). $^1\text{H-NMR}$ (CDCl_3) δ : 5.81 (1H, d, $J=7.8$), 2.07 (3H, s, OAc), 2.02 (9H, s, $3 \times \text{OAc}$).

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-1,2,3,4-tetra-O-acetyl- β -D-glucopyranose = β -Isomaltose Octaacetate¹¹) (10)—A solution of **9** (5 g) in 30 ml of pyridine and 25 ml of acetic anhydride was stirred at room temperature for 11 h. The solution was poured into ice-water, and the resulting crystalline mass was collected and crystallized from ethanol (7.75 g, 78.2%). mp $145\text{--}146^\circ\text{C}$. $[\alpha]_D^{25} + 95.0^\circ$ ($c=1.1$, CHCl_3). These physical data were coincident with those in the literature.¹²

2,3,4,2',3',4',6'-Hepta-O-acetyl- α -D-isomaltosyl Bromide (11)—A 25% solution of hydrogen bromide in acetic acid (37 ml) was added to a solution of **10** (7.75 g) in chloroform (37 ml) under cooling and the whole was stirred for 9 h at 0°C . The reaction solution was poured into ice-water and extracted with CHCl_3 . The CHCl_3 extracts were washed with water, dried and concentrated *in vacuo* to yield a crystalline mass (7.12 g, 89.4%). $[\alpha]_D^{25} + 178.2^\circ$ ($c=1.2$, CHCl_3).

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- α -D-glucopyranosyl Azide (12)—A solution of **11** (4.6 g) in nitromethane (48 ml) was added to a mixture of **7** (2.4 g) and mercuric cyanide (8.7 g) in the same solvent (20 ml). After being stirred for 46 h at 60°C , the resulting solution was filtered and the filtrate was extracted with CHCl_3 . The CHCl_3 layer was washed with water and evaporated to a syrup, which was chromatographed on silica gel using 4:1 (v/v) chloroform-acetone as an eluent. The eluate containing the trisaccharide fraction was evaporated to dryness and the residue was crystallized from ethanol to give pure **12** (1.4 g, 20.3%). mp $57\text{--}59^\circ\text{C}$. $[\alpha]_D^{25} + 104.5^\circ$ ($c=0.5$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 5.62 (d, $J=4$, H-1), 4.98 (d, $J=4$, H-1'), 4.56 (d, $J=8$, H-1'). *Anal.* Calcd for $\text{C}_{38}\text{H}_{51}\text{N}_3\text{O}_{25}$: C, 48.05; H, 5.41; N, 4.42. Found: C, 48.37; H, 5.45; N, 4.13.

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-1,2,3,4-tetra-O-acetyl- β -D-glucopyranose (13)—A solution of **11** (1 g) in nitromethane was added to a nitromethane solution (20 ml) of 1,2,3,4-tetra-O-acetyl- β -D-glucopyranose (0.7 g), mercuric cyanide (0.6 g), and Drierite (0.5 g) over a period of 0.5 h. The solution was stirred for 24 h at 40°C , then cooled to room temperature. The CHCl_3 extracts were washed successively with saturated aqueous sodium hydrogen carbonate, saturated sodium chloride, and water, then dried with sodium sulfate, and concentrated. Chromatography of the residue on silica gel gave a major fraction (1.47 g, 75%). *Anal.* Calcd for $\text{C}_{40}\text{H}_{54}\text{O}_{27}$: C, 49.69; H, 5.63. Found: C, 49.73; H, 5.69.

An Alternative Route to O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- α -D-glucopyranosyl Azide (12)—Crushed anhydrous aluminium chloride (200 mg) was added to a solution of trisaccharide undecaacetate (**13**) (300 mg) in chloroform (2 ml) and the mixture was stirred for 2 h at room temperature. After usual work-up, the organic layer was concentrated to give a syrupy trisaccharide β -chloride, which was immediately treated with sodium azide in HMPT to give a 65% yield of compound **12**.

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- α -D-glucopyranosylamine (14)—A solution of **12** (447 mg) in 4 ml of THF was hydrogenated in the presence of Lindlar's catalyst (100 mg) and triethylamine (0.3 ml) at room temperature and atmospheric pressure. The filtrate was concentrated to a syrup, which contained (TLC) three components (R_f 0.19, R_f 0.36, and R_f 0.54; benzene-acetone 3:1 (v/v)). In this system the α -amine showed R_f 0.19. The syrup was chromatographed on silica gel using 3:1 (v/v) benzene-acetone to provide pure **14** (198.2 mg, 45.6%). mp $119\text{--}120^\circ\text{C}$. $[\alpha]_D^{25} + 92.1^\circ$ ($c=4.2$, CHCl_3). *Anal.* Calcd for $\text{C}_{38}\text{H}_{53}\text{NO}_{25}$: C, 49.41; H, 5.78; N, 1.52. Found: C, 49.63; H, 5.88; N, 1.47, and its β -isomer at C-1, O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- β -D-glucopyranosylamine (**15**) (101.7 mg, 23.4%). mp $95\text{--}97^\circ\text{C}$. R_f 0.36. $[\alpha]_D^{25} + 14.6^\circ$ ($c=2.2$, CHCl_3). *Anal.* Calcd for $\text{C}_{38}\text{H}_{53}\text{NO}_{25}$: C, 49.41; H, 5.78; N, 1.52. Found: C, 49.58; H, 5.84; N, 1.54.

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-N-(O-ethyl-N-benzoyloxycarbonyl-L- β -aspartyl)- α -D-glucopyranosylamine (16)— α -Ethyl benzoyloxycarbonyl-L-aspartate (26.7 mg), DEPC (75 mg), and triethylamine (0.05 ml) were added to a solution of **14** (83 mg) in THF (1.85 ml) and the whole was stirred for 40 h at 0°C . The reaction mixture

was diluted with AcOEt (50 ml) then washed successively with 5% HCl, water, saturated sodium hydrogen carbonate, and saturated sodium chloride. Drying followed by concentration gave a syrup, which was chromatographed on silica gel with 3:2 (v/v) benzene-acetone. The eluate was evaporated to dryness to give **16** (97.1 mg, 90%). $[\alpha]_D^{25} + 57.1^\circ$ ($c=0.8$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 7.36 (5H, s, arom), 2.05 (3H, s, OAc), 2.01 (12H, s, 4 $\times$ OAc), 2.00 (3H, s, OAc), 1.97 (6H, s, 2 $\times$ OAc), 1.94 (6H, s, 2 $\times$ OAc), 1.26 (3H, t, $J=8$). *Anal.* Calcd for $\text{C}_{52}\text{H}_{68}\text{N}_2\text{O}_{30}$: C, 52.00; H, 5.71; N, 2.33. Found: C, 52.10; H, 5.78; N, 2.40.

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-N-(O-ethyl-L- β -aspartyl)- α -D-glucopyranosylamine (18)—A solution of **16** (33.9 mg) in THF (6 ml) was hydrogenated in the presence of PtO_2 (22 mg) for 3 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated to dryness to give **18** (28.5 mg, 94.6%).

O-(α -D-Glucopyranosyl)-(1 $\rightarrow$ 6)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-N-(L- β -aspartyl)- α -D-glucopyranosylamine (2)—A solution of **18** (20.4 mg) in 50% methanolic solution (2.1 ml) was treated with 0.72 ml of triethylamine and the mixture was kept overnight at room temperature. The solution was concentrated *in vacuo* to provide a powder (11.1 mg, 93.9%). mp 146–147°C. $[\alpha]_D^{25} + 72.4^\circ$ ($c=0.33$, H_2O). $^1\text{H-NMR}$ (D_2O) δ : 5.56 (d, $J=4$, H-1), 4.95 (d, $J=3.5$, H-1'), 4.50 (d, $J=7.5$, H-1'). *Anal.* Calcd for $\text{C}_{22}\text{H}_{38}\text{N}_2\text{O}_{18}$: C, 42.72; H, 6.19; N, 4.53. Found: C, 42.83; H, 6.21; N, 4.62.

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-N-(O-ethyl-N-benzyloxycarbonyl-L- β -aspartyl)- β -D-glucopyranosylamine (17)— α -Ethyl benzyloxycarbonyl-L-aspartate (20.5 mg), DEPC (21.3 mg), and triethylamine (0.03 ml) were added to a solution of **15** (64.6 mg) in THF (1.1 ml) and the whole was stirred for 40 h at 0°C. After the usual processing, column chromatography on silica gel with 3:2 (v/v) benzene-acetone as the solvent gave **17** (76.7 mg, 91.3%). mp 100–102°C, $[\alpha]_D^{25} + 46.4^\circ$ ($c=3.8$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 7.36 (5H, s, arom), 2.06 (12H, s, 4 $\times$ OAc), 2.04 (12H, s, 4 $\times$ OAc), 1.96, 1.94 (3H, each s, OAc), 1.22 (3H, t, $J=8$). *Anal.* Calcd for $\text{C}_{52}\text{H}_{68}\text{N}_2\text{O}_{30}$: C, 52.00; H, 5.71; N, 2.33. Found: C, 52.13; H, 5.78; N, 2.43.

O-(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-N-(O-ethyl-L- β -aspartyl)- β -D-glucopyranosylamine (19)—A solution of **17** (25 mg) in THF (2 ml) was hydrogenated in the presence of PtO_2 (20 mg) for 3 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated to dryness to give **19** (20 mg, 90%). $[\alpha]_D^{25} + 30.4^\circ$ ($c=1.25$, CHCl_3). *Anal.* Calcd for $\text{C}_{44}\text{H}_{62}\text{N}_2\text{O}_{28}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.64; H, 5.92; N, 2.68.

O-(α -D-Glucopyranosyl)-(1 $\rightarrow$ 6)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-N-(L- β -aspartyl)- β -D-glucopyranosylamine (3)—A solution of **19** (20 mg) in 50% methanolic solution (2 ml) was treated with 0.7 ml of triethylamine and the mixture was kept overnight. The solution was concentrated *in vacuo* to provide a powder (11.0 mg, 94.9%). mp 142–143°C. $[\alpha]_D^{25} + 14.2^\circ$ ($c=0.85$, H_2O). $^1\text{H-NMR}$ (D_2O) δ : 4.98 (d, $J=8$, H-1), 4.94 (d, $J=4$, H-1'), 4.50 (d, $J=8$, H-1'). *Anal.* Calcd for $\text{C}_{22}\text{H}_{38}\text{N}_2\text{O}_{18}$: C, 42.72; H, 6.19; N, 4.53. Found: C, 42.85; H, 6.23; N, 4.63.

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