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The Nephritogenic Glycopeptide from Rat Glomerular Basement Membrane. II.[1] Synthesis of O-(α-D-glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl-(1→6)-N-(L-β-aspartyl)-α-D-glucopyranosylamine (α-D-Glc-(1→6)-β-D-Glc-(1→6)-α-D-Glc-(1→Asn))

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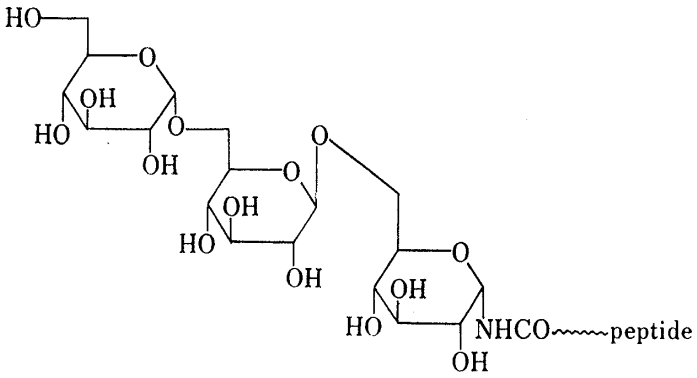
O-(α-D-Glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl-(1→6)-N-(L-β-aspartyl)-α-D-glucopyranosylamine has been prepared, as a model of a derivative possibly present in the glomerular basement membrane of rats, by condensation of the corresponding trisaccharide α-amine with α-ethyl benzyloxycarbonyl-L-aspartate 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 ^{13}C-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.



1

α-D-Glc-(1→6)-β-D-Glc-(1→6)-α-D-Glc-(1→Asn

2

α-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→Asn

3

Chart 1

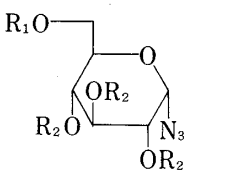
We report here the synthesis of α-D-Glc-(1→6)-β-D-Glc-(1→6)-α-D-Glc-(1→Asn (**2**) and its β-isomer of the reducing end group, α-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→Asn (**3**).

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°). 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 $CuSO_4 \cdot 5\,H_2O$ to give a 2,3,4-tri-O-acetyl-α-D-glucopyranosyl azide (**7**) ($[\alpha]_D$ +163.6°). 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.

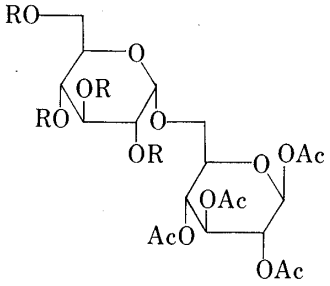
The synthesis of compound **2** corresponding to **1** was started by condensation of **2,3,4,-2',3',4',6'**-hepta-O-acetyl-α-D-isomaltosyl bromide (**11**) [which was prepared by the condensation of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl chloride[9] with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose[10] in the presence of mercuric cyanide, followed by hydrogenolysis and acetylation[11] 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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-α-D-glucopyranosyl azide (**12**), in 20.3% yield. The formation of a (1→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 (^{1}H-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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-α-D-glucopyranosylamine (**14**) ($[\alpha]_D$ +92.1°) and, together with α-D-glucosylamine, its β-isomer at C-1 (15) ($[\alpha]_D$ +14.6°) in the ratio of 2:1. Condensation of α-D-glycosylamine **14** with α-ethyl benzyloxycarbonyl-L-aspartate[13] gave 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)-α-D-glucopyranosylamine (**16**) in 90% yield using diethylphosphorocyanidate (DEPC).[14] The ^{1}H-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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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**) ([α]$_D$ +72.4°). The ^{1}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}C-NMR data showed three anomeric carbon atoms at δ 103.7 (C-1′), 99.0 (C-1″), and 77.6 (C-1).

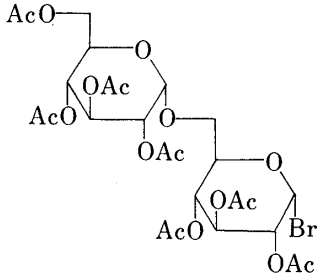
In a parallel route, the trisaccharide β-amine **15** was condensed with α-ethyl benzyloxycarbonyl-L-aspartate as described above to give the corresponding β-linked carbohydrate-amino acid compound, O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-N-(O-ethyl-N-benzyloxycarbonyl-L-β-aspartyl)-β-D-glucopyranosylamine (**17**) in 91.3% yield. The ^{1}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}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}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}C-NMR data three anomeric carbon atoms at δ 103.9 (C-1′), 99.0 (C-1″), and 80.4 (C-1).



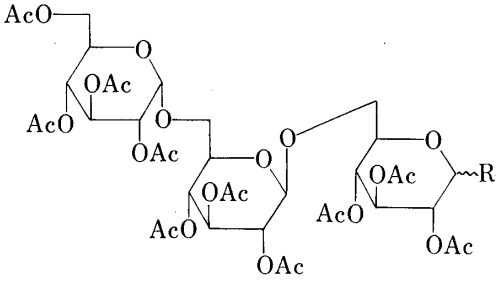
4 : R_1=R_2=Ac
5 : R_1=R_2=H
6 : R_1=Tr, R_2=Ac
7 : R_1=H, R_2=Ac



8 : R=Bn
9 : R=H
10 : R=Ac

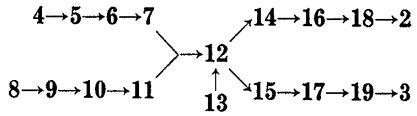


11



12: R=α-N_3
13: R=β-OAc
14: R=α-NH_2
15: R=β-NH_2
16: R=α-NHCOCH$_2$CHCOOEt
NHCOOBn

17: β-anomer at C-1 of **16**
18: R=α-NHCOCH$_2$CHCOOEt
NH_2
19: β-anomer at C-1 of **18**



Ac=acetyl, Bn=benzyl, Et=ethyl, Tr=trityl

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}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
$^{\alpha}_{\beta}$ (aspartyl residue)										50.4 36.9	51.2 37.6			51.6 38.4	52.2 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. $[\alpha]_D^{21}$ +268° (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 $C_6H_{11}N_3O_5$: 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 (*Rf* 0.47) and a minor (*Rf* 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. $[\alpha]_D^{21}$ +148.1° (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 $CuSO_4 \cdot 5H_2O$ (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%). $[\alpha]_D^{23}$ +163.6° (c=0.55, $CHCl_3$). ^{1}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→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 Å (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%). $[\alpha]_D^{23}$ +47.2° (c=0.45, $CHCl_3$).

O-(α-D-Glucopyranosyl)-(1→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}H-NMR ($CDCl_3$) δ: 5.81 (1H, d, J=7.8), 2.07 (3H, s, OAc), 2.02 (9H, s, 3×OAc).

O-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)-(1→6)-O-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose=β-Isomaltose Octaacetate[11] (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—146°C. $[\alpha]_D^{22}$ +95.0° (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%). $[\alpha]_D^{23}$ +178.2° (c=1.2, $CHCl_3$).

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-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—59°C. $[\alpha]_D^{22}$ +104.5° (c=0.5, $CHCl_3$). ^{1}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 $C_{38}H_{51}N_3O_{25}$: 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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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 $C_{40}H_{54}O_{27}$: 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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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 (*Rf* 0.19, *Rf* 0.36, and *Rf* 0.54; benzene–acetone 3: 1 (v/v)). In this system the α-amine showed *Rf* 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—120°C. $[\alpha]_D^{21}$ +92.1° (c=4.2, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{53}NO_{25}$: 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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranosylamine (**15**) (101.7 mg, 23.4%). mp 95—97°C. *Rf* 0.36. $[\alpha]_D^{21}$ +14.6° (c=2.2, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{53}NO_{25}$: 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→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)-α-D-glucopyranosylamine (16)——α-Ethyl benzyloxycarbonyl-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%). $[\alpha]_D^{22}$ +57.1° (c=0.8, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36 (5H, s, arom), 2.05 (3H, s, OAc), 2.01 (12H, s, 4×OAc), 2.00 (3H, s, OAc), 1.97 (6H, s, 2×OAc), 1.94 (6H, s, 2×OAc), 1.26 (3H, t, J=8). *Anal.* Calcd for $C_{52}H_{68}N_2O_{30}$: 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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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→6)-O-β-D-glucopyranosyl-(1→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 triethyl amine 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. $[\alpha]_D^{22}$ +72.4° (c=0.33, H_2O). ^{1}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 $C_{22}H_{38}N_2O_{18}$: 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→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)——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, $[\alpha]_D^{23}$ +46.4° (c=3.8, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36 (5H, s, arom), 2.06 (12H, s, 4×OAc), 2.04 (12H, s, 4×OAc), 1.96, 1.94 (3H, each s, OAc), 1.22 (3H, t, J=8). *Anal.* Calcd for $C_{52}H_{68}N_2O_{30}$: 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→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→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%). $[\alpha]_D^{23}$ +30.4° (c=1.25, $CHCl_3$). *Anal.* Calcd for $C_{44}H_{62}N_2O_{28}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.64; H, 5.92; N, 2.68.

O-(α-D-Glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl)-(1→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. $[\alpha]_D^{22}$ +14.2° (c=0.85, H_2O). ^{1}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 $C_{22}H_{38}N_2O_{18}$: C, 42.72; H, 6.19; N, 4.53. Found: C, 42.85; H, 6.23; N, 4.63.

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References and Notes

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