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Synthesis of the α and β anomer of an *N*-triglycosyl dipeptide*

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ABSTRACT

O-α-D-Glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-1-*N*-[L-aspart-1-oyl-(L-proline)-4-oyl]-α- and -β-D-glucopyranosylamine have been prepared, as models for a derivative possibly present in the glomerular basement membrane of rats, by condensation of the corresponding D-glucosyl-dipeptide derivatives with 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-isomaltopyranosyl bromide in the presence of mercuric cyanide, followed by deprotection of the trisaccharide-dipeptide derivatives.

INTRODUCTION

Shibata *et al.*[2] isolated and purified from the glomerular basement membrane of rats a new glycopeptide (nephritogenoside) that was active for the induction of glomerulonephritis in homologous animals[3]. This glycopeptide is composed of three D-glucose units, α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-D-Glc, and 21 amino acids [^{1}Asn-Pro-Leu-Phe-Gly-Ile-Ala-Gly-Glu-Asp-Gly-Pro-Thr-Gly-Pro-Ser-Gly-Ile-Val-Gly-^{21}Gln], and the (potentially) reducing α-D-glucose unit is linked *N*-glucosylically to an *N*-terminal asparagine unit[4]. The synthesis of model glycoproteins and glycopeptides is important because these compounds may have many biological properties. In our previous papers[5], we reported the syntheses of an *N*-glycosyl linkage between the trisaccharide glucosylamine, *O*-α-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosylamine and L-aspartic acid or L-glutamic acid (or both), of the glycopeptide and of the neoglycoprotein, as models of corresponding derivatives possibly present in the glomerular basement membrane of rats.

The protected glycopeptide, *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-di-*O*-benzyl-1-*N*-[N-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-proline methyl ester)-4-oyl]-α-D-glucopyranosylamine (**7α**) was obtained by coupling the respective monosaccharide derivative, 2,3-di-*O*-benzyl-1-*N*-[L-aspart-1-oyl-(L-proline methyl ester)-4-oyl]-α-D-glucopyranosylamine

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(**6α**) and 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-isomaltosyl bromide. Removal of the *tert*-butoxycarbonyl group and deacylation of compound **7α**, followed by debenzylation gave **10α**, which is a suitable key compound for completion of the nephritogenoside synthesis. The method described is generally applicable to the attachment of oligopeptides for elongation of the peptide residue, and the β anomer (**10β**) of the trisaccharide-dipeptide was also prepared.

RESULTS AND DISCUSSION

The present synthesis of the glycopeptide differs from those previously reported[5] in that the 2,3-di-*O*-benzylated synthon was used to avoid acetyl protecting groups. This benzyl protecting group was chosen so that the u.v. absorption of the resulting product would give an indication of its identity. 2,3-Di-*O*-benzyl-α-*4,6-O*-isopropylidene,β-D-glucopyranosylamine (**4α,4β**) was obtained by hydrogenation, in the presence of Lindlar's catalyst of 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-α-D-glucopyranosyl azide (**3**), which had been prepared from α-D-glucopyranosyl azide[6] (**1**) by isopropylidenation and benzylation. The mixture of **4α,4β** was coupled with the dipeptide, *N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-L-proline methyl ester (**12**), in the presence of *O,O*-diethylcyanophosphonate (Et_2PC) to give 2,3-di-*O*-benzyl-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-proline methyl ester)-4-oyl]-4,6-*O*-isopropylidene-α,β-D-glucopyranosylamine (**5α,5β**). The dipeptide (**12**) was obtained by coupling 4-benzyl *N*-(*tert*-butoxycarbonyl)-L-aspartate with L-proline methyl ester, followed by debenzylation. The amines **4α** and **4β** have the same R_F value and could not be isolated by chromatography. In the ^{13}C-n.m.r. spectrum of the mixture, the C-1 signal of the α-D-glycosylamine was at δ 78.7, and δ 86.6 for the β-D anomer. Similarly, the ^1H-n.m.r. spectrum of the α-D anomer was characterized by prominent, well resolved doublets in the range for H-1α at δ 4.93 (*J* 4.95 Hz) and at δ 4.16 (*J* 8.61 Hz) for H-1β of the sugar component.

Several conditions of azide reduction were examined (Table I). In each case, the β-D anomer was obtained in preponderant proportion. Use of Lindlar's catalyst in 1:1

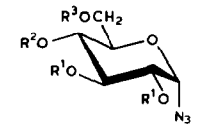
TABLE I

Yield and ratio of anomers obtained under various conditions of reduction of azide **3**

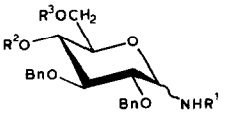
Reaction conditions	*Solvent*	*Yield(%)*[a]	*Ratio of α-to-β anomer*[a]
Reduction agent			
Lindlar's, Et_3N (1.25 eqs.)	Oxolan	95	1:6
Lindlar's, Et_3N (1.25 eqs.)	1:1 Oxolan–methanol	95	2:11
Lindlar's, Et_3N (12.5 eqs.)	1:1 Oxolan–methanol	95	10:31
H_2S gas, Et_3N	4:1 Pyridine-water	65	[b]
$NaBH_4$	2-Propanol	80	1:6
PtO_2, Et_3N	Methanol	95	5:29

[a] Yields and α-to-β ratios were calculated from integration of H-1 signal of the ^1H-n.m.r. spectra. [b] Only β anomer.

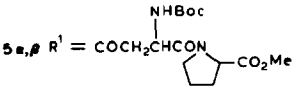
oxolane–methanol in the presence of a large amount of triethylamine, **5α** and **5β** in the ratio of 1:3. They were separated by silica gel column chromatography. The presence of a doublet at δ 5.75 (J 5.5 Hz) in the ^{1}H-n.m.r. spectrum of **5α** established the α-D configuration of the newly coupled residue. The β-D-linked anomer showed a signal at δ 6.06 (J 7.7 Hz). The isopropylidene group of both compounds was successively split off to give **6α** and **6β**. Condensation of **6α** with 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-isomaltosyl bromide in the presence of mercuric cyanide in nitromethane afforded the desired *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-di-*O*-benzyl-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-proline methylester)-4-oyl]-α-D-glucopyranosylamine (**7α**) in 62% yield. No other α-D-glycoside was observed in this reaction and the structure of **7α** was unambiguously ascertained by ^{1}H-n.m.r. spectroscopy. The signals for the carbomethoxy methyl and *tert*-butoxy methyl groups appeared at δ 3.67 and 1.42, respectively, and that for the acetyl methyl groups at δ 2.00–2.11. In the ^{13}C-n.m.r. spectrum, the signal for C-1′ was at δ 100.9, consistent with the newly introduced β-D-glycosyl linkage. The other ^{1}H-n.m.r. and ^{13}C-n.m.r. data were in accordance with the proposed structure. Removal of the *tert*-butoxycarbonyl and methyl ester groups with 85% formic acid gave **8α**, the acetyl group of which was removed to give **9α**, and hydrogenation afforded the target



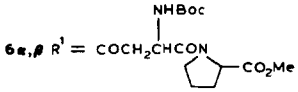
1 $R^1 = R^2 = R^3 = H$
2 $R^1 = H; R^2, R^3 = CMe_2$
3 $R^1 = Bn; R^2, R^3 = CMe_2$



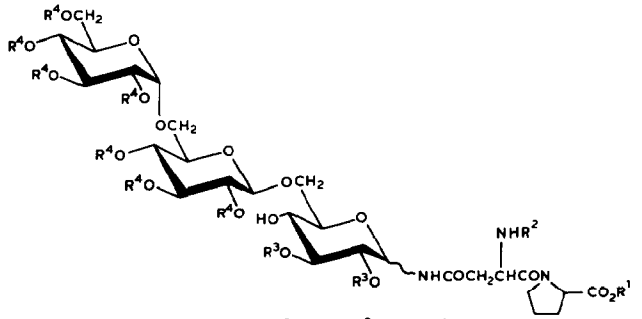
4α,β $R^1 = H; R^2, R^3 = CMe_2$



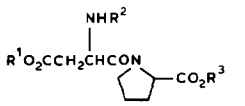
5α,β $R^1 =$; $R^2, R^3 = CMe_2$



6α,β $R^1 =$; $R^2 = R^3 = H$



7α,β $R^1 = Me, R^2 = Boc, R^3 = Bn, R^4 = Ac$
8α,β $R^1 = R^2 = H, R^3 = Bn, R^4 = Ac$
9α,β $R^1 = R^2 = R^4 = H, R^3 = Bn$
10α,β $R^1 = R^2 = R^3 = R^4 = H$



11 $R^1 = Bn, R^2 = Boc, R^3 = Me$
12 $R^1 = H, R^2 = Boc, R^3 = Me$
13 $R^1 = R^2 = R^3 = H$
Boc = Me_3CCO

TABLE II

^{13}C-N.m.r. data (δ) for glycopeptide derivatives **5**–**10** and **13**[a]

Carbon atom	*Compound*													
	5α	**6α**	**7α**	**8α**	**9α**	**10α**	**5β**	**6β**	**7β**	**8β**	**9β**	**10β**	**13**	**13**[b]
C-1	74.6	74.7	77.3	77.2	79.0	79.5	79.7	79.1	79.3	78.9	80.3	82.1		
2	77.3	77.5	77.7	77.7	82.8	72.3[c]	80.7	81.1	80.7	80.5	82.0	74.4		
3	78.8	80.2	80.7	80.4	83.1	74.4	82.6	85.4	85.2	85.1	86.9	79.2		
4	64.0	70.9	69.5[c]	69.8	71.6	72.2[c]	68.8	69.9	68.4	68.2	71.7	72.2[c]		
5	72.8	74.7	71.5	72.5[c]	77.9	74.8	74.5	76.8	76.2	76.7	78.8	79.5		
6	62.4	62.1	67.4	67.5	71.3	71.3	62.2	61.4	67.2	67.3	71.3	72.1[c]		
C-1′			100.9	100.4	104.3	105.4			100.5	99.9	104.8	105.7		
2′			71.2	71.3	76.4	75.8[e]			72.6[c]	72.6	76.6[c]	74.7		
3′			72.8	72.7[c]	77.4	78.8			72.8[c]	72.6	77.9	78.7		
4′			69.3[c]	69.3	73.6	72.5[d]			69.3	69.3	73.5	72.1[c]		
5′			72.3	72.6[c]	76.2	77.2			71.4	71.4	76.4[c]	77.2		
6′			66.5	66.6	69.6	68.9			66.4	66.6	69.9	68.3		
C-1″			95.9	95.8	99.7	100.8			95.6	95.4	99.8	100.7		
2″			70.1	70.1	73.8	74.7			70.3	70.1[c]	73.7[d]	74.7		
3″			70.6[d]	70.7	75.2	76.1			70.8	70.7	75.2	75.9		
4″			68.5	68.5	73.6	72.6[d]			68.7	68.4	73.5[d]	72.3[e]		
5″			70.5[d]	70.7	75.1	76.0[e]			70.1	70.0[c]	75.2	75.9		
6″			61.9	61.9	62.5	63.6			61.9	62.1	62.5	63.3		
Asp-α[f]	49.5	49.7	49.5	52.7	55.6(c)	56.8(c)	49.2	48.9	49.6	52.3	55.3(c)	56.7(c)	54.2(c)	54.3
					53.0(t)	54.9(t)					53.4(t)	54.7(t)	53.9(t)	
β	36.5	38.9	39.6	36.6	39.7(c)	41.4(c)	39.9	39.3	40.5	36.2	40.2(c)	41.5(c)	38.0(t)	38.1
					36.3(t)	38.3(t)					35.9(t)	38.2(t)	36.4(c)	
Pro-α[f]	58.8	59.0	59.0	59.2	60.3(t)	61.9(t)	59.3	59.3	59.2	59.0	60.3(t)	61.3(t)	61.0(c)	60.8
					59.6(c)	61.1(c)					59.6(c)	61.2(c)	60.8(t)	
β	28.9	28.9	28.9	29.7	30.7(c)	31.0(c)	28.9	29.0	29.0	28.3	30.7(c)	31.1(c)	29.7(t)	29.5
					29.9(t)	30.5(t)					29.9(t)	30.7(t)	28.9(c)	
γ	24.8	24.8	24.9	22.6	23.5(t)	24.6(t)	24.8	24.7	25.0	22.5	23.4(t)	24.8(t)	25.8(c)	23.6
					22.8(c)	24.1(c)					22.8(c)	24.2(c)	23.8(t)	
δ	46.9	47.0	47.0	45.8	47.3(c)	48.5(c)	47.0	47.2	46.9	45.5	47.7(c)	48.7(c)	48.6(t)	47.2
					46.5(t)	48.4(t)					46.8(t)	48.5(t)	46.6(c)	

[a] For solutions of **5α**, **5β**,**6α**,**6β**,**7α**,**7β**,**8α**, and **8β** in $CDCl_3$; for solutions of **9α** and **9β** in CD_3OD, and for solutions of **10α**,**10β**, and **13** in D_2O. [b] Recorded at 80°. [c,d,e] The values in each column may be interchanged. [f] (c) *cis* and (t) *trans*.

compound, *O*-(α-D-glucopyranosyl)-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-1-*N*-[L-aspart-1-oyl-(L-proline)-4-oyl]-α-D-glucopyranosylamine (**10α**) in 93% yield. The configuration of **10α** was confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy; signals for H-1, H-1′, and H-1″ were observed at δ 5.55 (*J* 5.3), 4.49 (*J* 7.9), and 4.93 (*J* 3.5 Hz), and signals for C-1, C-1′, and C-1″ at δ 79.5, 105.4, and 100.8 with $^1J_{C,H}$ 165.9, 162.3, and 169.7 Hz, respectively (see also Table II).

Condensation of **6β** with 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-isomaltosyl bromide in the presence of silver triflate or mercuric cyanide afforded **7β** in 40 and 15% yield, respectively. The β-D anomer of the trisaccharide-dipeptide (**7β**) was also prepared according to the method described for the α-D anomer.

The ^{13}C-n.m.r. spectra of **9α**,**9β**,**10α**, **10β**, and **13**, showed a doubling of the peptide peak which was anticipated because of the *cis–trans* isomerism present in these compounds. ^{13}C-N.m.r. evidence of *cis–trans* isomerism of the amide bond involving the nitrogen atom of the proline residue was reported by Thomas and Williams[7], and Dorman and Bovey[8]. The assignments of the *cis*- and *trans*-forms were based on analogy with the *N*-acetyl group of proline which prefers the *trans*-form in all solvents. In the case of peptides, the chemical-shift difference between the α-carbon atoms in the two forms of the proline residue is remarkably small, as is the corresponding shift between the carbon atoms, yet the signals for the β-and γ-carbon atoms in the two forms are well separated. In our synthetic glycopeptide, however, no such chemical-shift difference between the two forms of the proline residue could be observed, but the signals of the α- and β-carbon atoms of the asparagine residue were well separated.

EXPERIMENTAL

General methods. — Optical rotations were measured with a JASCO DIP-4 digital polarimeter. ^{1}H-N.m.r. spectra were recorded with a FX-100 spectrometer, and ^{13}C-n.m.r. spectra with a GSX-400 instrument at room temperature; tetramethylsilane was the internal standard for solutions in $CDCl_3$ and CD_3OD, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solutions in D_2O. Thin-layer chromatography was conducted on precoated silica gel plates (Merck GF-254), and the compounds were detected by quenching of u.v. fluorescence and by spraying with 10% H_2SO_4 or a 5% methanolic ninhydrin solution. Column chromatography was carried out on silica gel (Merck Kieselgel 60).

α-D-*Glucopyranosyl azide* (**1**). — This compound was obtained by the procedure described in a previous paper[6].

4,6-O-*Isopropylidene*-α-D-*glucopyranosyl azide* (**2**). — A mixture of **1** (12 g, 0.06 mol) and 2,2-dimethoxypropane (60 mL) in *N*,*N*-dimethylformamide (40 mL) was stirred for 12 h at room temperature in the presence of 4-toluenesulfonic acid (0.3 g). The solvent was evaporated *in vacuo* to give a syrup (13.82 g, 96.6%), $[\alpha]_D^{25}$ + 184° (*c* 0.36, chloroform); ^{1}H-n.m.r. ($CDCl_3$); δ 5.42 (d, 1 H, *J* 3.8 Hz, H-1), 1.44, and 1.50 (each s, 3 H, CH_3).

Anal. Calc. for $C_9H_{15}N_3O_5$; C, 44.08; H, 6.16; N, 17.13. Found: C, 43.98; H, 6.19; N, 17.09.

2,3-Di-O-benzyl-4,6-O-isopropylidene-α-D-glucopyranosyl azide (**3**). — To a solution of **2** (7.14 g, 0.03 mol) in *N,N*-dimethylformamide (48 mL) was added NaH (9.6 g), and the mixture was stirred for 1 h. After cooling at 0°, benzyl bromide (12 mL) was added dropwise, and then the mixture was stirred for 4.5 h at 20°. Excess NaH was decomposed by addition of methanol and the mixture was partitioned between water and ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4), and concentrated *in vacuo*. The syrup was purified by column chromatography to give **3** (5.04 g, 40.5%), m.p. 52° (from methanol), $[\alpha]_D^{24}$ +88° (*c* 0.40, chloroform); ^{1}H-n.m.r. ($CDCl_3$); δ 7.30–7.26 (*m*, 10 H, arom.), 5.12 (*d*, 1 H, *J* 4.0 Hz, H-1), 1.41, and 1.44 (each s, 3 H, CH_3).

Anal. Calc. for $C_{23}H_{27}N_3O_5$; C, 64.93; H, 6.40; N, 9.88. Found: C, 64.64; H, 6.52; N, 9.67.

2,3-Di-O-benzyl-4,6-O-isopropylidene-α,β-D-glucopyranosylamine (**4α, 4β**). — A solution of **3** (5.04 g, 0.01 mol) in 1:1 oxolan–methanol (135 mL) was hydrogenolyzed under atmospheric pressure in the presence of Lindlar's catalyst (3.5 g) and triethylamine (32 mL) for 24 h at room temperature. The catalyst was filtered off and the filtrate was evaporated to dryness to give **4α, 4β** (4.66 g, 95%); ^{1}H-n.m.r. ($CDCl_3$): δ 4.93 (d, *J* 4.95 Hz, H-1α) and 4.16 (d, *J* 8.61 Hz, H-1β).

Other conditions of reduction are shown in Table I. When H_2S was used, it was bubbled through a solution of **3** (20 mg) in pyridine (2 mL) and water (0.4 mL) in the presence of triethylamine for 48 h at room temperature while the solution was stirred.

[4-Benzyl N-*(*tert*-butoxycarbonyl)*-L*-aspart-1-oyl]*-L*-proline methyl ester* (**11**). — To a solution of L-proline methyl ester hydrochloride (17.8 g, 0.11 mol), triethylamine (18 mL), and 4-benzyl *N*-(*tert*-butoxycarbonyl)-L-aspartate (34.9 g, 0.11 mol) in dichloromethane–*N,N*-dimethylformamide 10:1 (v/v) (165 mL) was added diethyl cyanophosphonate (21 mL) and, after 12 h at room temperature, the mixture was extracted with ethyl acetate (250 mL). This solution was washed successively with saturated $NaHCO_3$ (2 × 50 mL), 10% citric acid (2 × 50 mL), and water, and dried (Na_2SO_4). After evaporation of the solvent *in vacuo*, the residue was chromatographed on silica gel with 10:1 benzene–acetone as the eluent. The fractions containing a material having R_F 0.78 (2:1 benzene–acetone) were pooled and evaporated (35.0 g, 75%), $[\alpha]_D^{24}$ −60° (*c* 2.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35 (m, 5 H, arom.), 5.12 (s, 2 H, PhCH_2), 3.63 (s, 3 H, OMe), and 1.42 (s, 9 H, CMe_3).

Anal. Calc. for $C_{22}H_{30}N_2O_7$; C, 60.82; H, 6.96; N, 6.45. Found: C, 60.72; H, 6.85; N, 6.63.

N-*(*tert*-Butoxycarbonyl)*-L*-aspartyl-L*-proline methyl ester* (**12**). — A solution of **11** (6.44 g, 15 mmol) in ethanol (40 mL) was hydrogenated in the presence of 10% Pd–C (500 mg) under atmospheric pressure for 12 h at room temperature. The catalyst was filtered off and the filtrate was evaporated to dryness to give **12** (4.5 g, 88%), syrup, $[\alpha]_D^{24}$ −45° (*c* 1.6, chloroform), t.l.c. (2:1 benzene–acetone) R_F 0.06; ^{1}H-n.m.r. ($CDCl_3$): δ 3.68 (s, 3 H, OMe) and 1.42 (s, 9 H, CMe_3).

Anal. Calc. for $C_{15}H_{24}N_2O_7$; C, 52.32; H, 7.02; N, 8.13. Found; C, 52.54; H, 7.12; N, 8.25.

L-*Aspartyl*-L-*proline* (**13**). — A solution of **12** (927 mg, 2.69 mmol) in 85% formic acid (5 mL) was stirred for 3 h at room temperature, and then evaporated *in vacuo*. The residue was chromatographed on silica gel in 13:6:1 (v/v, lower phase) chloroform–methanol–water as an eluent to give **13**, 71% yield, (440 mg), $[\alpha]_D^{23}$ −86.5° (*c* 0.27, methanol).

Anal. Calc. for $C_9H_{14}N_2O_5$; C, 46.95; H, 6.13; N, 12.17. Found: C, 46.73; H, 6.25; N, 12.46.

2,3-Di-O-*benzyl-1*-N-*(*N*[*tert*-*butoxycarbonyl)*-* L-*aspart-1-oyl-(*L-*proline methyl ester)*-4-*oyl*]-α-4,6-O-*isopropylidene*- (**5α**) *and* -β-D-*glucopyranosylamine* (**5β**). — To a solution of **4α, β** (1.06 g, 2.7 mmol) in oxolan (15 mL) were added dipeptide **12** (0.92 g, 2.7 mmol), diethyl cyanophosphonate (0.54 mL), and triethylamine (1.04 mL). The mixture was stirred for 8 h at room temperature, diluted with ethyl acetate (30 mL), and washed with water, 10% citric acid solution, and water, dried, and concentrated to give a syrup which was chromatographed on silica gel with 4:1 and then 3:1 (v/v) benzene–acetone. The first eluate was evaporated to dryness to give **5α** (255 mg, 13.2%), $[\alpha]_D^{24}$ −4.0° (*c* 5.9, chloroform), t.l.c. (2:1 benzene–acetone) R_F 0.72; ^{1}H-n.m.r. ($CDCl_3$): δ 5.75 (d, 1 H, *J* 5.5 Hz, H-1), 3.63 (s, 3 H, OMe), 1.42 (s, 9 H, CMe_3), 1.37, and 1.48 (each s, 3 H, Me). The latter eluate gave **5β** (789 mg, 41.0%), $[\alpha]_D^{24}$ +3.1° (*c* 0.9, chloroform). t.l.c. (2:1 benzene–acetone) R_F 0.65; ^{1}H-n.m.r. ($CDCl_3$): δ 6.06 (d, 1 H, *J* 7.7 Hz, H-1), 3.64 (s, 3 H, OMe), 1.42, 1.49 (each s, 3 H, Me), and 1.38 (s, 9 H, CMe_3).

Anal. Calc. for $C_{38}H_{51}N_3O_{11}$: C, 62.88; H, 7.08; N, 5.79. Found for **5α**: C, 62.72; H, 7.03; N, 5.84. Found for **5β**: C, 62.68; H, 7.02; N, 5.88.

2,3-Di-O-*benzyl-1*-N-*[N*-(tert-*butoxycarbonyl*)-L-*aspart-1-oyl-(N*-(tert-*butoxycarbonyl*)L-*proline methyl ester)-4-oyl]*-α-D-*glucopyranosylamine* (**6α**). — Compound **5α** (450 mg, 0.63 mmol) was treated with 80% acetic acid (5 mL) at 40° for 3 h. The solution was concentrated to give a syrup (325 mg, 75%), $[\alpha]_D^{24}$ +1.4° (*c* 3.4, chloroform); t.l.c. (10:1 chloroform–methanol) R_F 0.40; ^{1}H-n.m.r. ($CDCl_3$): δ 3.63 (s, 3 H, OMe) and 1.40 (s, 9 H, CMe_3).

Anal. Calc. for $C_{35}H_{47}N_3O_{11}$: C. 61.30; H, 6.91; N, 6.13. Found: C, 60.83; H, 6.61; N, 6.00.

β-D *Anomer* (**6β**). — This compound was prepared as described for **6α**, yield 394 mg (91%), m.p. 92°, $[\alpha]_D^{24}$ −5.3° (*c* 0.4, chloroform), t.l.c. (10:1 chloroform–methanol) R_F 0.36; ^{1}H-n.m.r. ($CDCl_3$): δ 5.87 (d, *J* 8.4 Hz, H-1), 3.62 (s, 3 H, OMe), and 1.39 (s, 9 H, CMe_3).

Anal. Calc. for $C_{35}H_{47}N_3O_{11}$: C, 61.30; H, 6.91; N, 6.13. Found. C, 59.83; H, 6.75; N, 5.92.

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-di*-O-*benzyl-1*-N-*[*N-(tert-*butoxycarbonyl*)-L-*aspart-1-oyl-(*L-*proline methyl ester)-4-oyl]*-α-D-*glucopyranosylamine* (**7α**). — To a solution of **6α** (240 mg, 0.36 mmol) in nitromethane (8 mL) were added 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-isomaltosyl bromide (734 mg, 1.06 mmol), $Hg(CN)_2$ (400 mg), and molecular sieves 4A (320 mg), and the suspension was stirred for 6 h at 55° under Ar gas. The solids were filtered off, the filtrate was poured into water, extracted with chloroform, washed with water, and concentrated, and the residual crude product was chromatographed on silica

gel with 1:4 (v/v) hexane–ethyl acetate. The fractions containing the trisaccharide-dipeptide having R_F 0.30 (1:3 hexane–ethyl acetate) were pooled and concentrated (284 mg, 62%) $[\alpha]_D^{24}$ +26° (*c* 0.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$); δ 7.36–7.24 (m, 10 H, arom), 3.67 (s, 3 H, OMe), 2.11–2.00 (each s, 21 H, 7 OAc), and 1.42 (s, 9 H, CMe_3).

Anal. Calc. for $C_{61}H_{81}N_3O_{28}$: C, 56.17; H, 6.26; N, 3.22. Found. C, 55.73; H, 6.10; N, 3.04.

*β-*D *Anomer* (**7β**). — To a solution of **6β** (547 mg, 0.8 mmol) in nitromethane (8 mL) were added 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-isomaltosyl bromide (1.05 g, 1.5 mmol), silver triflate (205 mg), tetramethylurea (0.07 mL), and molecular sieves 4A (160 mg). The suspension was stirred for 2 h at −14° and then kept at room temperature for 6 h. The solids were filtered off, the filtrate was poured into water, extracted with chloroform, washed with water, and concentrated, and the residue was purified by column chromatography in 1:5 (v/v) hexane–ethyl acetate as an eluent. The fractions containing the trisaccharide-dipeptide having R_F 0.23 were concentrated to give **7β** (418 mg, 40%); when $Hg(CN)_2$ was used, the yield was low (15%); $[\alpha]_D^{24}$ +22° (*c* 2.7, chloroform), t.l.c. (1:3 hexane–ethyl acetate) R_F 0.23; ^{1}H-n.m.r. ($CDCl_3$): δ 7.36–7.24 (m, 10 H, arom.), 3.72 (s, 3 H, OMe), 2.09–2.00 (each s, 21 H, 7 OAc), and 1.40 (s, 9 H, CMe_3).

Anal. Calc. for $C_{61}H_{81}N_3O_{28}\cdot 2H_2O$; C, 54.66; H, 6.39; N, 3.14. Found. C, 54.52; H, 6.51; N, 3.26.

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-di-*O*-benzyl-1-*N*-[*L*-aspart-1-oyl-(*L*-proline)-4-oyl]-α-*D*-glucopyranosylamine* (**8α**). — A solution of **7α** (103.2 mg) in 85% formic acid (12 mL) was stirred for 5 h at room temperature, and then extracted with chloroform. The organic layer was concentrated to dryness to give **8α** (yield 66.9 mg, 71%), $[\alpha]_D^{24}$ +34° (*c* 0.5, chloroform), t.l.c. (12:1 chlororform–methanol) R_F 0.66; ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.25 (m, 10 H, arom.) and 2.11–2.01 (each s, 21 H, 7 OAc).

Anal. Calc. for $C_{55}H_{71}N_3O_{26}$; C, 55.51; H, 6.01; N, 3.53. Found. C, 55.26; H, 5.72; N, 3.38.

*β-*D *Anomer* (**8β**). — This compound was prepared as described for **8α**, yield 84.9 mg (90%), $[\alpha]_D^{24}$ +1.2° (*c* 1.3, chloroform), t.l.c. (12:1 chloroform–methanol) R_F 0.49; ^{1}H-n.m.r. ($CDCl_3$): δ 7.36–7.24 (m, 10 H, arom.) and 2.10–2.00 (each s, 21 H, 7 OAc).

Anal. Calc. for $C_{55}H_{71}N_3O_{26}$; C, 55.51; H, 6.01; N, 3.53. Found. C, 55.72; H, 6.13; N, 3.40.

O-α-D-*Glucopyranosyl-(1→6)-*O*-β-*D*-glucopyranosyl-(1→6)-2,3-di-*O*-benzyl-1-*N*-[*L*-aspart-1-oyl-(*L*-proline)-4-oyl]-α-*D*-glucopyranosylamine* (**9α**). — To a solution of **8α** (48.9 mg, 42 μmol) in methanol (6 mL) was added sodium methoxide (90 mg). After 8 h, when deacetylation was complete [t.l.c. (chloroform–methanol–water 5:4:1) R_F 0.73], the solution was de-ionized with Amberlite IRC-50 (H^+) cation-exchange resin and filtered from the resin. The filtrate was concentrated to give a syrup which was chromatographed on a column of Sephadex LH-20 with methanol to give **9α** (36.6 mg, 99%), $[\alpha]_D^{23}$ + 44.5° (*c* 1.2, methanol); ^{1}H-n.m.r. (CD_3OD): δ 7.38–7.23 (m, 10 H, arom.).

Anal. Calc. for $C_{41}H_{57}N_3O_{19}\cdot 0.5H_2O$; C, 54.42, H, 6.46; N, 4.64. Found. C, 54.35; H, 6.17; N, 4.45.

β-D *Anomer* (**9β**). — This compound was prepared as described for **9α**, yield 32.8 mg (89%); ^{1}H-n.m.r. (CD_3OD): δ 7.38–7.10 (m, 10 H, arom.).

Anal. Calc. for $C_{41}H_{57}N_3O_{19}\cdot 0.5H_2O$; C, 54.42; H, 6.46; N, 4.64. Found. C, 54.29; H, 6.23; N, 4.72.

O-α-D-*Glucopyranosyl-(1→6)*-O-*β*-D-*glucopuranosyl-(1→6)-1*-N-*[*L-*aspart-1-oyl-(*L-*proline)-4-oyl]*-α-D-*glucopyranosylamine* (**10α**). — To a solution of **9α** (24.4 mg, 28 μmol) in 1:1 ethanol–water (4 mL) was added 10% Pd–C (40 mg). The suspension was stirred for 24 h under H_2, and then filtered and concentrated to dryness. The residue was chromatographed on Sephadex G-10. The water eluate was lyophilized to give a white powder (18.2 mg, 93%), $[\alpha]_D^{23}$ +97° (*c* 0.3, water), t.l.c. (1:1:1:1 butanol–acetic acid–ethyl acetate–water) R_F 0.19; ^{1}H-n.m.r. (D_2O): δ 5.55 (d, *J* 5.3 Hz, H-1), 4.93 (d, *J* 3.5 Hz, H-1″), and 4.49 (d, *J* 7.9 Hz, H-1′).

Anal. Calc. for $C_{27}H_{45}N_3O_{19}\cdot 2.5H_2O$: C, 42.63; H, 6.62; N, 5.52. Found: C, 42.42; H, 6.25; N, 5.18.

β-L *Anomer* (**10β**). — This compound was prepared as described for **10α**, yield 16.0 mg (82%), $[\alpha]_D^{23}$ +34° (*c* 0.6, water), t.l.c. (1:1:1:1 butanol–acetic acid–ethyl acetate–water) R_F 0.19; ^{1}H-n.m.r. (D_2O): δ 4.973 (d, *J* 9.5 Hz, H-1), 4.970 (*d*, *J* 3.5 Hz, H-1″), and 4.522 (d, *J* 7.9, H-1′).

Anal. Calc. for $C_{27}H_{45}N_3O_{19}$: C, 45.31; H, 6.34; N, 5.87. Found: C, 45.06; H, 6.55; N, 5.62.

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