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Note

Synthesis of *N*-(L-glutam-5-oyl)-α-D-glucopyranosylamine and *O*-α-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-*N*-(L-glutam-5-oyl)-α-D-glucopyranosylamine*

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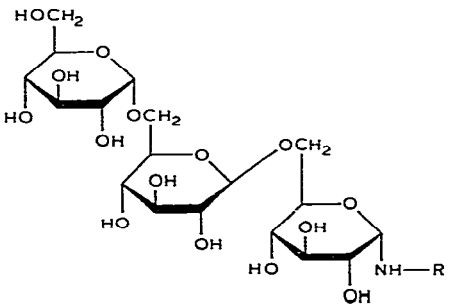
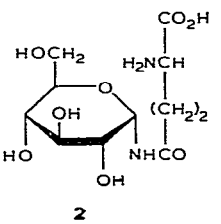
Shibata *et al.*[1] isolated and purified from the glomerular basement membrane of rats a new glycopeptide that has activity for the induction of glomerulonephritis in homologous animals[2]. From a consideration of ^{13}C-n.m.r. data in comparison with those of related synthetic glycosylamine derivatives[3], Shibata and Nakanishi[4] proposed **1** as the structure of the nephritogenoside. This is the first example of an *N*-α-D-glycosyl linkage in a natural D-glucose-containing glycopeptide (or glycoprotein)[5].

In a previous paper[6], we reported the synthesis of an *N*-glycosyl linkage between the trisaccharide glycosylamine, *O*-α-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyrano-



1 R = Peptide

3 R = $CO(CH_2)_2\underset{\displaystyle NH_2}{\underset{|}{C}}H—CO_2H$



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*The nephritogenic glycopeptide from rat glomerular-basement membrane. Part 3.

syl-(1→6)-*O*-α-D-glucopyranosylamine and L-aspartic acid. As it is not clear whether the amino acid linked to the glycosylamine residue by an *N*-glycosyl linkage is an L-aspartic acid or L-glutamic acid residue, we synthesized *N*-(L-glutam-5-oyl)-α-D-glucopyranosylamine (**2**) and *O*-α-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-*N*-(L-glutam-5-oyl)-α-D-glucopyranosylamine (**3**) as models of corresponding derivatives possibly present in the glomerular basement-membrane of rats, and studied the ^{13}C-n.m.r. spectra of these model compounds.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosylamine (**4**) was obtained by the procedure described in a previous paper[3]. 1-Ethyl *N*-(benzyloxycarbonyl)-L-glutamate, readily prepared in high yield by the procedure of Wünsch and Zwiek[7] from *N*-(benzyloxycarbonyl)-L-glutamic acid, was condensed with **4**, by use of the diethyl phosphorocyanidate method of Shioiri *et al.*[8], to give, in 82% yield, the *N*-(L-glutam-5-oyl)-D-glucopyranosylamine derivative **5**. Removal of the benzyloxycarbonyl group by catalytic hydrogenation afforded 2,3,4,6-tetra-*O*-acetyl-*N*-(1-ethyl-L-glutam-5-oyl)-α-D-glucopyranosylamine (**6**), which was *O*-deacetylated and

TABLE I

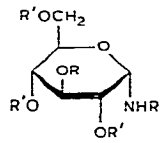
^{13}C-N.M.R. DATA (δ) FOR COMPOUNDS **1–3**, **5**, **6**, **9**, AND **10**[a]

Carbon atom	*Compound*						
	1	**2**	**3**	**5**	**6**	**9**	**10**
Sugar residues							
C-1	77.6	77.4	77.7	73.9	73.3	73.5	73.4
C-2	70.8	70.3	70.5	68.5	68.3	69.1	68.9
C-3	72.4	73.5	72.7	69.2	68.3	70.0	69.5
C-4	70.8	70.3	70.5	68.2	68.3	67.4	67.8
C-5	74.1	73.9	73.0	70.3	68.3	70.7	70.4
C-6	70.4	61.4	69.5	61.9	62.0	67.4	67.2
C-1′	104.1		103.8			99.9	100.1
C-2′	74.8		74.2			71.3	71.8
C-3′	77.2		77.0			72.8	73.1
C-4′	71.2		70.6			68.6	68.9
C-5′	77.2		75.4			72.8	73.0
C-6′	69.6		66.7			67.4	67.4
C-1″	100.2		99.0			96.1	96.3
C-2″	72.4		73.0			69.1	68.9
C-3″	75.5		74.2			70.7	71.0
C-4″	71.6		70.7			66.5	67.2
C-5″	74.1		74.2			69.8	70.4
C-6″	62.6		61.7			61.9	62.2
Amino acid residue							
C-2		59.2	59.5	52.5	55.6	53.3	55.5
C-3		26.2	26.5	27.3	24.8	27.4	24.9
C-4		30.6	30.9	30.0	29.3	29.9	29.1

[a]For solutions of **1–3** in D_2O, and for solutions of **5**, **6**, **9**, and **10** in chloroform-*d*.

deethylated with triethylamine in 50% methanolic solution at room temperature to give *N*-(L-glutam-5-oyl)-α-D-glucopyranosylamine (**2**).

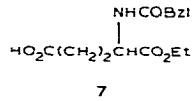
Similarly, condensation of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranosylamine[6] (**8**) with 1-ethyl *N*-(benzyloxycarbonyl)-L-glutamate gave, in 47% yield, *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-*N*-(1-ethyl *N*-benzyloxycarbonyl-L-glutam-5-oyl)-α-D-glucopyranosylamine (**9**), the ^{1}H-n.m.r. spectrum of which showed signals for a benzyloxy group, 10 acetyl groups, and an ethoxylmethyl group. The ^{13}C-n.m.r. data showed signals for three anomeric carbon atoms, and three signals for C-2, -3, and -4 of the glutamic acid residue (see Table I). Removal of the benzyloxycarbonyl group of **9** 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*-(1-ethyl L-glutam-5-oyl)-α-D-glucopyranosylamine (**10**). Subsequently, **10** was *O*-deacetylated and deethylated 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-glutam-5-oyl)-α-D-glucopyranosylamine (**3**), the ^{1}H-n.m.r. data of which show signals for three anomeric protons.



4 R = H, R′ = Ac

5 R = CO(CH$_2$)$_2$CH(NHCO$_2$BzI)CO$_2$Et, R′ = Ac

6 R = CO(CH$_2$)$_2$CH(NH$_2$)CO$_2$Et, R′ = Ac



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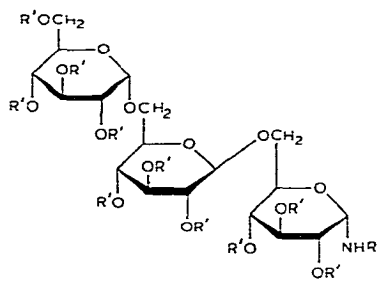

8 R = H, R′ = Ac

9 R = CO(CH$_2$)$_2$CH(NHCO$_2$BzI)CO$_2$Et, R′ = Ac

10 R = CO(CH$_2$)$_2$CH(NH$_2$)CO$_2$Et, R′ = Ac

All the compounds described gave elemental analyses in agreement with assigned structures, and no anomeric impurities were observed by ^{1}H- and ^{13}C-n.m.r. spectrometry. The ^{13}C shifts of the glycosylamine derivatives and related compounds are listed in Table I. The data for **3**, **9**, and **10** are essentially the same as those for the corresponding aspartic acid derivatives[6], except for the amino acid residue. The ^{13}C-n.m.r. spectra indicated that the chemical shifts of the sugar residues of **1** and **3** are very similar.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto microapparatus and are uncorrected. ^{1}H-N.m.r. spectra were recorded with a JNM MH-100 spectrometer, and ^{13}C-n.m.r. spectra with a FX-100 instrument, tetramethylsilane being the internal standard in both cases. Optical rotations were recorded with a Union Giken PM-201 automatic digital polarimeter. T.l.c. was conducted on precoated silica gel plates (Merck GF-254), and column chromatography on silica gel (Merck Kieselgel 60).

*2,3,4,6-Tetra-*O*-acetyl-*N*-(1-ethyl* N*-benzyloxycarbonyl-*L*-glutam-5-oyl)-α-*D*-glucopyranosylamine* (**5**). — To a solution of **4** (ref. 3, 532 mg) in oxolane (19 mL) were added 1-ethyl *N*-(benzyloxycarbonyl)-L-glutamate (455 mg), diethyl phosphorocyanidate (524 mg), and triethylamine (0.54 mL). The mixture was stirred for 12 h at 0°, and then diluted with ethyl acetate (100 mL), and successively washed with 5% hydrogen chloride, water, saturated sodium hydrogencarbonate, and saturated sodium chloride. Drying, followed by evaporation, gave a syrup, which was chromatographed on silica gel with 4:1 (v/v) chloroform–methanol. Evaporation of the eluate gave a pure syrup (800 mg, 82%), $[\alpha]_D^{20}$ +42.4° (*c* 5.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.38 (m, 5 H, arom), 2.02–2.00 (12 H, 4 OAc), and 1.32 (t, 3 H, CH_2CH_3).

Anal. Calc. for $C_{29}H_{38}N_2O_{14}$: C, 54.54; H, 6.00; N, 4.39. Found: C, 54.49; H, 5.98; N, 4.25.

*2,3,4,6-Tetra-*O*-acetyl-*N*-(1-ethyl* L*-glutam-5-oyl)-α-*D*-glucopyranosylamine* (**6**). — A solution of **5** (90 mg) in oxolane (8 mL) was hydrogenated in the presence of platinum dioxide (30 mg) for 12 h at room temperature. The catalyst was filtered off, and the filtrate evaporated to dryness to give **6** (66.1 mg, 93%) syrup $[\alpha]_D^{22}$ +64.1° (*c* 0.77, chloroform).

Anal. Calc. for $C_{21}H_{32}N_2O_{12}$: C, 50.00; H, 6.39; N, 5.55. Found: C, 50.12; H, 6.33; N, 5.50.

N-(L-*Glutam-5-oyl*)-α-D-*glucopyranosylamine* (**2**). — To a solution of **6** (25 mg) in 50% methanol (1 mL) was added triethylamine (0.24 mL). The mixture was kept overnight at room temperature, and then evaporated *in vacuo* to give a syrup (15 mg, 98%), $[\alpha]_D^{22}$ +53.1° (*c* 0.64, water); ^{1}H-n.m.r. (D_2O): δ 5.52 (d, 1 H, *J* 4 Hz, H-1).

Anal. Calc. for $C_{11}H_{20}N_2O_8$: C, 42.86; H, 6.54; N, 9.09. Found: C, 42.72; H, 6.49; N, 9.13.

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)-2,3,4-tri-*O*-acetyl-*N*-(1-ethyl* N*-benzyloxycarbonyl-*L*-glutam-5-oyl)-α-*D*-glucopyranosylamine* (**9**). — To a solution of **8** (ref. 6, 204 mg) in oxolane (6 mL) were added 1-ethyl *N*-(benzyloxycarbonyl)-L-glutamate (90 mg), diethyl phosphorocyanidate (66 mg), and triethylamine (0.18 mL). The mixture was stirred for 40 h at 0°, and then diluted with ethyl acetate (100 mL), and successively washed with 5% hydrogen chloride, water, saturated sodium hydrogencarbonate, and saturated sodium chloride. Drying followed by evaporation gave a syrup, which was chromatographed on silica gel with 3:2 (v/v) benzene–acetone. The eluate was

evaporated to dryness to give **9** (126 mg, 47%), $[\alpha]_D^{20}$ +63.8° (*c* 7.26, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.36 (5 H, arom). 2.08–2.00 (30 H, 10 OAc), and 1.26 (t, 3 H, CH_2CH_3).

Anal. Calc. for $C_{53}H_{70}N_2O_{30}$: C, 52.39; H, 5.81; N, 2.31. Found: C, 52.44; H, 5.89; N, 2.36.

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-(*1-ethyl* L-*glutam-5-oyl*)-α-D-*glucopyranosylamine* (**10**). — A solution of **9** (50 mg) in oxolane (5 mL) was hydrogenated in the presence of platinum dioxide (50 mg) for 3 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated to dryness to give **10** (42 mg, 94.5%), $[\alpha]_D^{22}$ +74.0° (*c* 0.5, chloroform); t.l.c. (10:1, v/v, chloroform–methanol): R_F 0.51.

O-α-D-*Glucopyranosyl*-(*1*→*6*)-O-β-D-*glucopyranosyl*-(*1*→*6*)-N-(L-*glutam-5-oyl*)-α-D-*glucopyranosylamine* (**3**). — To a solution of **10** (38 mg) in 50% methanolic solution (2 mL) was added triethylamine (0.18 mL). The mixture was kept overnight at room temperature, and then evaporated *in vacuo* to give a powder (20 mg, 89.9%), $[\alpha]_D^{22}$ +47.6° (*c* 1.68, water); t.l.c. (2:4:1, v/v, 1-butanol–acetic acid–water): R_F 0.32; ^{1}H-n.m.r. (D_2O): δ 5.56 (d, *J* 4 Hz, H-1), 4.95 (d, *J* 3.5 Hz, H-1″), and 4.50 (d, *J* 8 Hz, H-1′).

Anal. Calc. for $C_{23}H_{40}N_2O_{18}$: C, 43.67; H, 6.37; N, 4.43. Found: C, 43.72; H, 6.42; N, 4.49.

ACKNOWLEDGMENT

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