The nephritogenic glycopeptide from rat glomerular basement membrane: synthesis of α-D-glucopyranosylamine derivatives

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This paper is dedicated to Prof. Raymond U. Lemieux on the occasion of his 60th birthday

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N-(L-β-aspartyl)-α-D-glucopyranosylamine has been prepared, as a model of a corresponding derivative possibly present in glomerular basement membrane of rats, by the condensation of α-ethyl benzyloxycarbonyl-L-aspartate with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylamine in the presence of diethylphosphorocyanidate. This was followed by hydrogenolysis, de-*O*-acetylation, and deethoxylation of the resulting 2,3,4,6-tetra-*O*-acetyl-*N*-(α-ethyl benzyloxycarbonyl-L-β-aspartyl)-α-D-glucopyranosylamine to remove the protecting groups. The ^{13}C nmr spectra of the corresponding glycosylamine derivatives are analyzed and discussed.

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La *N*-(L-β-aspartyl) α-D-glucopyrannosylamine, un modèle d'un dérivé correspondant qui est probablement présent dans la sous-membrane glomérulaire du rat, est obtenue par condensation du benzyloxycarbonyl L-α-aspartate d'éthyle sur la tétra-*O*-acétyl-2,3,4,6 α-D-glucopyrannosylamine en présence du phosphocyanidate diéthylique. Cette étape est suivie de l'élimination des groupes protecteurs par une hydrogénolyse, une dé-*O*-acétylation et une dééthoxylation de la tétra-*O*-acétyl-2,3,4,6 *N*(α-éthyl benzyloxycarbonyl L-β-aspartyl) α-D-glucopyrannosylamine qui en résulte. On a analysé les spectres rmn du ^{13}C des dérivés correspondants de la glycosylamine.

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Introduction

Shibata *et al.* (1) isolated from the glomerular basement membrane of rats, and purified, a new glycopeptide that has an activity consisting of induction of glomerulonephritis in homologous animals (nephritogenic activity) (2). The methylation analyses (3, 4), the concanavalin A test, and ^{13}C nmr spectral analyses (5) indicate that the sugar moiety of this glycopeptide has the Glc α-(1→6)Glc β-(1 → 6)Glc structure shown in Fig. 1. This is the first example of a new type of carbohydrate–protein linkage among natural compounds, of a direct, *N*-glycosyl linkage between a glycosyl and an amino acid residue. This paper reports the synthesis of *N*-acylated glycosylamines and studies on the ^{13}C nmr spectra of the above-mentioned glycopeptide and model compounds.

Results and Discussion

Marks and Neuberger (6) have described the synthesis and some properties of *N*-(L-β-aspartyl)-β-D-glucopyranosylamine. The preparation of this compound by a different procedure has also been described by Coutsogeorgopoulos and Zervas (7). Garg and Jeanloz (8) have also described the synthesis of *N*-(L-β- and α-aspartyl)-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamines. All these syntheses produced β-D-glucopyranosylamine derivatives.

We now report the synthesis of the *N*-acetyl, *N*-(L-β-aspartyl)-α,β-D-glucopyranosylamine derivatives which were needed for ^{13}C nmr studies. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (**1**) was obtained by the procedure of Bertho (9). The azide was hydrogenated in tetrahydrofuran over Adam's catalyst to give the amino compound (**2**). Compound **2** was acetylated and also condensed with α-ethyl benzyloxycarbonyl-L-aspartate to give **3** and **5**, respectively. These compounds were deblocked in the usual manner to give *N*-acetyl-, *N*-(L-β-aspartyl)-β-D-glucopyranosylamine **4** (mp 255°C) and **7** (mp 253°C), respectively. The basic physical and chemical properties are coincident with those reported earlier (7).

For synthesis of α-D-glucopyranosylamine derivatives, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide (**9**) was prepared by treating 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride (**8**) with sodium azide in hexamethylphospholic triamide (HMPT). This S_N2 reaction gave the inverted product in a yield of 65% and neighbouring group participation with retention of configuration is not observed (10). Hydrogenation of **9** in methanol gave the desired key intermediate glycosylamine derivative (**10**).

The α-ethyl benzyloxycarbonyl-L-aspartate was

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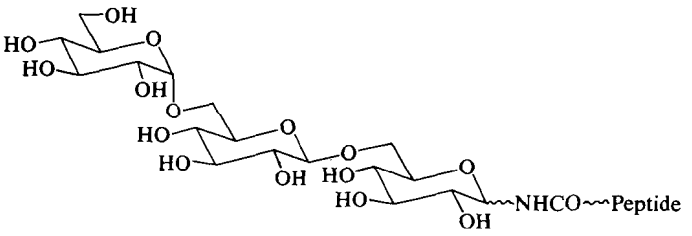
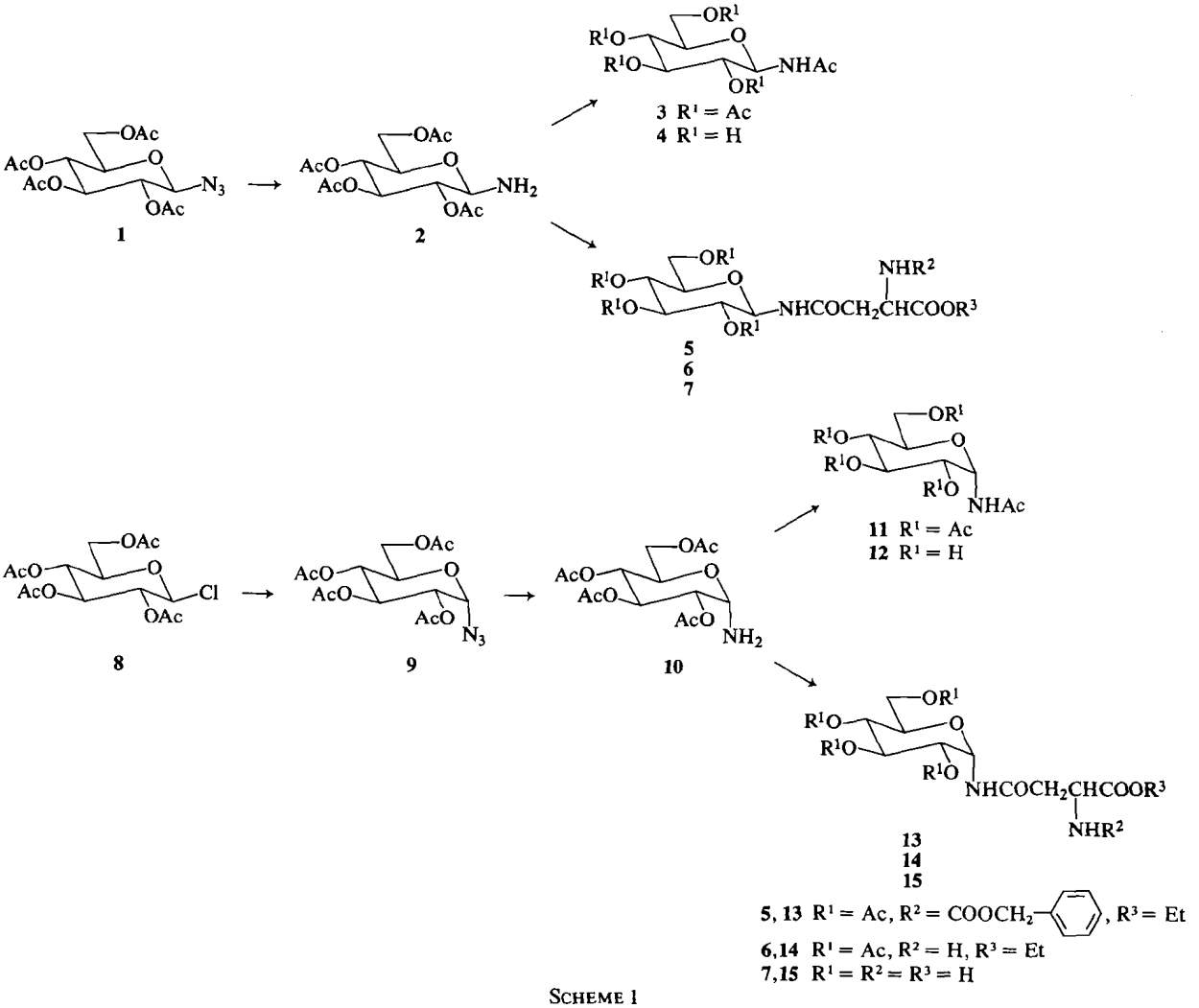


FIG. 1

readily prepared in high yield by the procedure of Wünsch and Zwiek (11) from benzyloxycarbonyl-L-aspartic acid.

The next step involved the condensation of α-ethyl benzyloxycarbonyl-L-aspartate with **10**, which gave the α-aspartyl-glucopyranosylamine derivative (**13**) in 90% yield using the DEPC method of Shioiri *et al.* (12). Removal of the benzyloxycarbonyl group by catalytic hydrogenation afforded the required tetra-*O*-acetyl-*N*-(L-β-aspartyl)-α-D-glucopyranosylamine (**14**). Thus, **14** was deacetylated and deethoxylated with triethylamine in 50% methanolic solution at room temperature to give a *N*-(L-β-aspartyl)-α-D-glucopyranosylamine (**15**) ($[\alpha]_D$ +34.1°). The *N*-acetylation of compound **10** followed by de-*O*-acetylation of the *N*-acetate af-



SCHEME 1

forded a *N*-acetyl-α-D-glucopyranosylamine (**12**) ($[\alpha]_D$ +15.0°). The synthetic route is illustrated in Scheme 1. The ^{13}C shifts of the glucosylamine derivatives are listed in Table 1.

The assignment of the α-D-configuration to the azide compound (**9**) was made by comparing its spectrum with that of β-D-azide compound (13). The signal of the anomeric carbon atom is always found at lowest field and normally C-1 of the β-anomer is at lower field than that of the α-anomer. The signals of C-4 and C-6 remain almost constant throughout the series of compounds described here and they are therefore readily assigned in most cases. The assignments of the closely spaced signals of C-3 and C-5 were made by the selective proton decoupling of H-3 and H-5, respectively. The ^{13}C nmr spectra indicate that the chemical shifts of the sugar moiety of compounds **12** and **15** are very similar to each other, and are obviously different from their respective β-isomers (**4**, **7**). A complete similarity of the *N*-glycosyl moieties of the glycopeptide and compound **15** was observed.

Consequently, a glycopeptide that has a nephritogenic activity has its *N*-glycosyl linkage in the α-D-configuration.

Experimental

General Methods

Melting points were determined with a Yanagimoto microapparatus and are uncorrected. The ^1H nmr spectra were recorded on a JNM MH-100 spectrometer with tetramethylsilane as the internal standard; abbreviations used: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; and m, multiplet. The ^{13}C nmr spectra were obtained at 25.0 MHz in the pulsed Fourier transform mode on JEOL FX-100 instruments. Thin-layer chromatography was conducted on precoated silica gel plates (Merck GF-254) and column chromatography on silica gel (Merck Kieselgel 60).

TABLE 1. ^{13}C Chemical shifts of glucosylamine derivatives examined in $CDCl_3$

Compound	δ ppm							
	C-1	C-2	C-3	C-4	C-5	C-6	β (Aspartyl residue)	α (Aspartyl residue)
1	87.9	70.8	72.7	68.0	74.1	61.7		
9	86.1	69.7	69.8	68.1	70.1	61.7		
2	84.9	72.2	72.7	69.0	73.3	62.4		
10	76.7	68.5	68.5	68.1	73.3	62.1		
3	78.2	70.7	73.0	68.3	73.6	61.8		
11	74.5	68.7	68.7	68.2	70.4	62.0		
4	80.1	72.6	77.3	70.1	78.3	61.4		
12	77.4	70.2	73.5	70.2	73.9	61.4		
5	78.0	70.6	72.7	68.1	73.7	61.8	37.8	50.5
13	74.2	68.3	69.1	68.0	70.2	62.3	36.8	50.4
7[a]	80.0	72.7	77.3	70.1	78.4	61.4	36.1	51.9
15[a]	77.4	70.2	73.5	70.2	73.9	61.4	37.3	53.1
Glycopeptide[a] (*N*-glycosyl residue)	77.6	70.8	72.4	70.8	74.1	70.4		

[a]Compounds **7**, **15**, and glycopeptide were measured in D_2O (internal 1,4-dioxane (67.4 ppm)).

Materials

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine were synthesized by well established procedures (9). α-Ethyl benzyloxycarbonyl-L-aspartate was prepared from benzyloxycarbonyl-L-aspartic acid according to the method of Wünsch and Zwiek (11).

*2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl Azide (9)*

A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride (**8**) (7.2 g), prepared from penta-*O*-acetyl-β-D-glucopyranose according to Mills *et al.* (14), in 75 mL of HMPT was added to 15 g of sodium azide. The mixture was stirred for 3.5 h, then it was filtered and the filtrate concentrated to dryness. The residue was crystallized, and recrystallization from methanol gave 4.77 g (65%), mp 91–92°C; $[\alpha]_D^{24}$ +156.6° (*c* 1.06, $CHCl_3$); R_f 0.77 (benzene–acetone 4:1); ^1H nmr ($CDCl_3$) δ: 1.98, 2.00, 2.04, 2.06 (each s, 3H, CH_3CO), 4.06–4.30 (m, 3H, H-5, 6, 6'), 4.92 (q, J = 4, 8 Hz, 1H, H-2), 5.00 (t, J = 8 Hz, 1H, H-4), 5.33 (t, J = 8 Hz, 1H, H-3), 5.62 (d, J = 4 Hz, 1H, H-1). *Anal.* calcd. for $C_{14}H_{19}O_9N_3$: C 45.04, H 5.13, N 11.26; found: C 45.30, H 5.06, N 10.99.

*2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosylamine (10)*

A solution of 3.36 g of azide (**9**) in 15 mL of methanol was hydrogenated at room temperature and atmospheric pressure for 13 h in the presence of Adams' catalyst (0.45 g). After removal of the catalyst by filtration, the filtrate was concentrated to a syrup, which contained (tlc) three components (R_f 0.17, R_f 0.29, R_f 0.4). In this system the α-amine possessed R_f 0.17. The syrup was chromatographed on silica gel using benzene–acetone (4:1) to provide pure **10** (0.63 g, 21% yield), mp 62.5–63°C, $[\alpha]_D^{24}$ +108.7° (*c* 7.5, $CHCl_3$). *Anal.* calcd. for $C_{14}H_{21}O_9N$: C 48.41, H 6.09, N 4.03; found: C 48.50, H 6.19, N 3.83.

*N-Acetyl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylamine (11)*

A solution of 100 mg of **10** in 2 mL of pyridine and 2 mL of acetic anhydride was allowed to stand at room temperature for 8 h. The solution was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with water, dried, and concentrated to give a syrup; $[\alpha]_D^{20}$ +14.65° (*c* 4.0, $CHCl_3$). *Anal.* calcd. for $C_{16}H_{23}O_{10}N$: C 50.52, H 6.09, N 3.68; found: C 50.48, H 6.12, N 3.59.

N-Acetyl-α-D-glucopyranosylamine (12)

Compound **11** (100 mg) was *O*-deacetylated with triethylamine in 50% MeOH solution. The reaction solution was evaporated to give a syrup; $[\alpha]_D^{20}$ + 15.02° (*c* 0.9, H_2O). *Anal.* calcd. for $C_8H_{15}O_6N$: C 43.44, H 6.83, N 6.33; found: C 43.56, H 6.90, N 6.29.

*2,3,4,6-Tetra-*O*-acetyl-N-(α-ethyl Benzyloxycarbonyl-L-aspartyl)-α-D-glucopyranosylamine (13)*

To a solution of **10** (114 mg) in THF (5 mL) α-ethyl benzyloxycarbonyl-L-aspartate (96.3 mg), DEPC (diethyl phosphorocyanidate) (100 mg), and 0.15 mL of triethylamine were added. The mixture was stirred for 12 h at 0°C. The reaction mixture was diluted with AcOEt (50 mL), and successively washed with 5% HCl, water, saturated $NaHCO_3$, and saturated NaCl. Drying followed by evaporation gave a syrup which contained (tlc) a major (R_f 0.56) and a minor (R_f 0.76) component and some slower-moving components. The syrup was chromatographed on silica gel with the same solvent system as

on tlc (chloroform–methanol 4:1). The eluate of the major component was evaporated and crystallized from ethanol to give pure **13** (185 mg, 90%); $[\alpha]_D^{20}$ +64.6° (*c* 3.2, $CHCl_3$); ^1H nmr ($CDCl_3$) δ: 1.25 (t, 3H, $—CH_2CH_3$), 2.02, 2.03, 2.06, 2.08 (each s, 3H, $COCH_3$), 2.90 (q, 2H, $—CH_2CH_3$), 5.15 (s, 2H, $—CH_2—$⟨⟩), 5.65–5.90 (br, 1H, NH), 7.38 (m, 5H, arom. H). *Anal.* calcd. for $C_{28}H_{36}O_{14}N_2 \cdot 2H_2O$: C 50.91, H 6.10, N 4.24; found: C 50.46, H 5.93, N 4.27.

2,3,4,6-Tetra-O-*acetyl*-N-(α-*ethyl*-L-β-*aspartyl*)-α-D-*glucopyranosylamine* (*14*)

A solution of **13** (98 mg) in THF (8 mL) was hydrogenated in the presence of PtO_2 (30 mg) for 12 h at room temperature. The catalyst was filtered off, and the filtrate evaporated to dryness to give **14** (75.4 mg, 98%); $[\alpha]_D^{23}$ +54.1° (*c* 0.8, $CHCl_3$). *Anal.* calcd. for $C_{20}H_{30}O_{12}N_2$: C 48.98, H 6.17, N 5.71; found: C 49.06, H 6.22, N 5.82.

N-(L-β-*Aspartyl*)-α-D-*glucopyranosylamine* (*15*)

To a solution of **14** (18.9 mg) in 50% methanol (2 mL) 0.18 mL of triethylamine was added and kept overnight at room temperature. The solution was concentrated *in vacuo*. A syrup was obtained, yielding 16 mg (99%); $[\alpha]_D^{24}$ +34.1° (*c* 0.9, H_2O). *Anal.* calcd. for $C_{10}H_{18}O_8N_2$: C 40.82, H 6.17, N 9.53; found: C 40.74, H 6.03, N 9.64.

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