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Note

The α-D configuration of the glycosyl–N linkage in the nephritogenic glycopeptide isolated from rat glomerular, basement membrane

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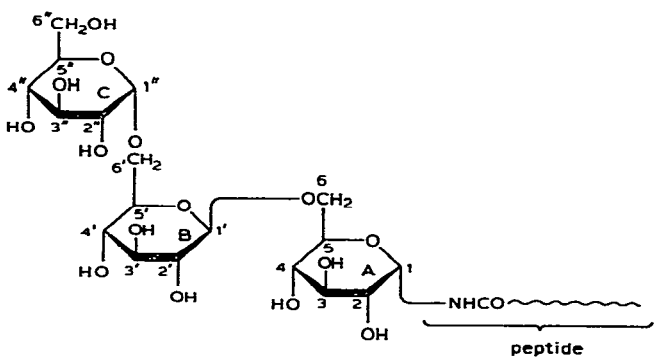
Shibata *et al.*[1] isolated and purified a new glycopeptide (**1**) from the glomerular, basement membrane (GBM) of rats. Compound **1** has biological activity, namely, induction of proliferative glomerulonephritis in homologous animals[2,3]. It is important to elucidate the chemical structure of **1**, in order to clarify the relationship between the chemical structure and this nephritogenic activity at the molecular level. It has recently been made clear[4] that the structure of the trisaccharide moiety in **1** is α-D-glucosyl-(1→6)-β-D-glucosyl-(1→6)-D-glucose, and that the D-glucosyl residue at the potentially reducing terminus is directly bonded to the peptide moiety at C-1 in an N–glycosyl linkage.

We now report the anomeric configuration of the N–glycosyl linkage in **1**, ascertained by detailed study of the ^{13}C-nuclear magnetic resonance (n.m.r.) spectra of **1**, analogous (but smaller) glycopeptides, and related sugar compounds.



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EXPERIMENTAL

The ^{13}C-n.m.r. spectra were recorded with a Varian XL-100-15A spectrometer operating at 25.16 MHz for a solution of each of compounds **2–9**, **12**, and **13** (5–20 mg) in deuterium oxide (0.7 mL) in a Pyrex tube (10 mm o.d.). The Fourier-transform mode was used, with 8192 data points. The general, spectral parameters for compounds **2–9**, **12**, and **13** were: spectral width, 5 kHz; acquisition time, 0.8 s; pulse width, 20 μs (corresponding to a pulse of ~45°); pulse delay, 5 s; total transients, 10,000 (~15 h); and sensitivity enhancement, 0.20. The chemical shifts were read from the signal of the methyl carbon atoms of *tert*-butyl alcohol, and were tentatively corrected as the chemical shifts from tetramethylsilane [δ (from Me_4Si) = δ (from *tert*-BuOH) + 32.2 p.p.m.]. The temperature of the sample was ~30°.

RESULTS AND DISCUSSION

In order to determine the anomeric configuration (α or β) of the linkage between the sugar and peptide moieties of **1**, the ^{13}C-n.m.r. spectra of *N*-acetyl-α- (**2**) and -β-D-glucopyranosylamine* (**3**) were measured under almost the same conditions as for **1** (see Fig. 1). The results are shown in Table I.

As these are new types of compounds, the ^{13}C-n.m.r. spectra of α-D-glucopyranose (**4**), β-D-glucopyranose (**5**), 2-amino-2-deoxy-α-D-glucopyranose (**6**), 2-amino-2-deoxy-β-D-glucopyranose (**7**), 2-acetamido-2-deoxy-α-D-glucopyranose (**8**), and 2-acetamido-2-deoxy-β-D-glucopyranose (**9**) were measured, to aid assignment of the peaks in the ^{13}C-n.m.r. spectra of **2** and **3**. The assignments of the carbon signals of compounds **4–9** were made from the reference data[5–12].

On comparing the chemical shifts of the D-glucopyranoses (**4** and **5**) with those

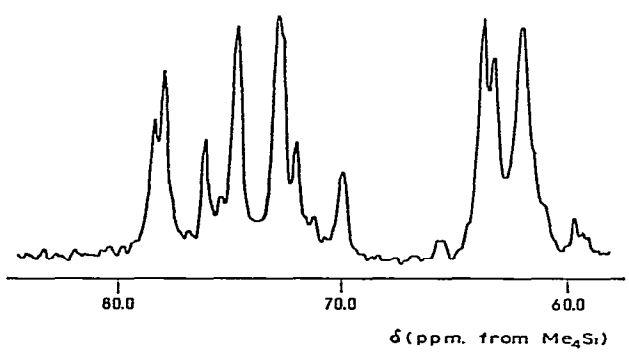


Fig. 1. ^{13}C-N.m.r. spectrum of the sugar moiety in glycopeptide **1**. [The sample of solution of **1** (39 mg) in deuterium oxide (0.4 mL) was measured in a tube (5 mm o.d.) at 30°. Spectral parameters: data points, 4096; spectral width, 5 kHz; pulse width, 41 μs (90° pulse); pulse delay, 0.0 s; acquisition time, 0.40 s; transients, 180,000 (20 h); sensitivity enhancement, 0.2. A little aggregation occurred.]

*The compounds were synthesized by Dr. T. Takeda and his co-workers.

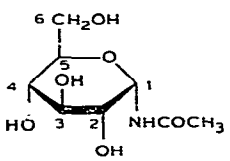
TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS FOR THE SUGAR MOIETY IN THE GLYCOPEPTIDES AND ANALOGOUS SUGAR COMPOUNDS

Compound	*δ (p.p.m. from Me_4Si)*										
1	77.7 77.3	72.1 74.2	75.5 74.0	72.1 72.5	74.2[a] 71.7	69.6	63.6	63.2[b]			
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CH_3CO*	*CONH*	*α-CH*	*β-CH_2*	*CO_2H*
2	79.1	71.9	75.6	71.9	75.2	63.1	24.6	178.6			
3	81.8	74.3	80.0	71.8	79.0	63.1	24.6	178.0			
4	94.3	73.7	74.8	71.8	73.7	63.0					
5	98.2	76.1	77.8	71.8	77.8	63.0					
6	94.7	57.6	75.7	72.3	74.2	63.6					
7	98.9	60.0	78.5	72.2	78.0	63.6					
8	93.4	56.7	74.1	72.7	73.3	63.2	24.5	177.1			
9	97.5	59.3	78.5	72.4	76.5	63.5	24.8	177.3			
12	79.1	71.9	75.6	71.9	75.2	63.1		178.5	49.2	38.4	178.5
13	81.7	74.4	80.1	71.8	79.0	63.1		175.7	53.6	37.7	175.5

[a]The signals assigned to the carbon atoms in the potentially reducing, terminal D-glucosyl residue. [b]The signals assigned to the D-glucosyl group or to the other D-glucosyl residue, or to the amino acid residues.

of the 2-amino-2-deoxy-D-glucopyranoses (**6** and **7**), it was found that the signal of C-2 (involved in $>CHNH_2$) in **6** and **7** is at considerably higher field than that of C-2 ($>CHOH$) in **4** and **5** [the chemical-shift difference ($\Delta\delta$) = + 16.1 p.p.m. for the α and β anomers], whereas those of C-1 and C-3–C-6 in **6** and **7** are at slightly lower field than those in **4** and **5** ($\Delta\delta$ = −0.2 to −0.9 p.p.m.). From these data, the chemical shifts of C-1 of α- (**10**) and β-D-glucopyranosylamine (**11**) may, on the basis



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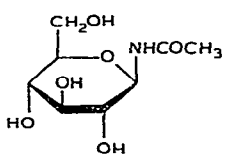

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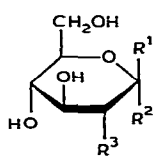
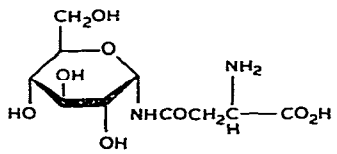


4 R^1 = H, R^2 = R^3 = OH
5 R^1 = OH, R^2 = H, R^3 = OH
6 R^1 = H, R^2 = OH, R^3 = NH_2
7 R^1 = OH, R^2 = H, R^3 = NH_2
8 R^1 = H, R^2 = OH, R^3 = $NHCOCH_3$
9 R^1 = OH, R^2 = H, R^3 = $NHCOCH_3$
10 R^1 = H, R^2 = NH_2, R^3 = OH
11 R^1 = NH_2, R^2 = H, R^3 = OH

of the chemical shifts of α- and β-D-glucopyranose, be roughly calculated as follows; C-1 of **10**: 94.3 — 16.1 = 78.2 p.p.m., and C-1 of **11**: 98.2 — 16.1 = 82.1 p.p.m.

Then, on comparing the chemical shifts of C-2 of **6** and **7** with those of their acetamido analogs (**8** and **9**), it was revealed that the chemical shift of C-2 bonded to an $NHCOCH_3$ group is at higher field than that of C-2 bonded to an NH_2 group ($\Delta\delta$ = —0.9 p.p.m. for the α-, and —0.7 p.p.m. for the β-, anomer). Therefore, using these values, the expected chemical shifts of C-1 in **2** and **3** are as follows: C-1 of **2**: 78.2 — 0.9 = 77.3 p.p.m., and C-1 of **3**: 82.1 — 0.7 = 81.4 p.p.m.

These calculated values of chemical shift are very close to the values experimentally obtained (79.1 p.p.m. for **2** and 81.8 p.p.m. for **3**). The chemical-shift difference between the C-1 atoms bonded to the $NHCOCH_3$ group in **2** and **3** ($\Delta\delta$ = 2.7 p.p.m.) is almost the same as that between the C-2 atoms in **8** and **9** (2.6 p.p.m.). This result strongly indicates the validity of the assignments described; thus, those for the C-1 atoms in **2** and **3** are now obvious. The assignments for atoms C-2–C-6 in **2** and **3** were made by using the same procedures. The results are shown in Table I.



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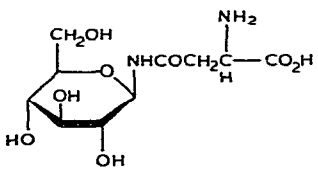

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As it had been reported[4] that the trisaccharide moiety of **1** is bonded to an amino acid of the peptide moiety (and not to the amido group of acetamide), the next step was to investigate the difference between the chemical shifts of the C-1 atoms in *N*-acetyl-D-glucopyranosylamine and **1**. As the model compounds, we chose *N*-(L-aspart-4-oyl)-α- and -β-D-glucopyranosylamine (**12** and **13***), because there are a number of examples[13,14] of glycoproteins in which a 2-acetamido-2-deoxy-D-glucopyranosylamine residue is bonded to an L-aspartic acid residue at C-1. The chemical shifts of **12** and **13** are also shown in Table I. It is obvious that **2** and **12** have almost the same chemical shifts of C-1–C-6, and that **3** and **13** show almost the same chemical shifts. This indicates that the effect on the chemical shift of C-1 on changing the acetamido group to an amino acid group (C-1-$NHCOCH_3$ → C-1-$NHCOCH_2R$) is very small in the glycopeptide system studied, suggesting the validity of the analogy between the chemical shifts of **1** and those of **2** and **3**.

The chemical shifts of **1** are also included in Table I. Among the signals for **1**, the signals at 77.3, 74.2, 74.0, 72.5, 71.7, 69.6, 63.6, and 63.2 p.p.m. are assigned to the D-glucosyl residue and the D-glucosyl group (B and C rings). The signal at 77.7 p.p.m., which is assigned to C-1 in **1**, is close to that (79.1 p.p.m.) for C-1 in **2**, and the discrepancy between 77.7 p.p.m. for **1** and 81.8 p.p.m. for **3** is too large.

*This compound was synthesized by Dr. I. Yamashina and co-worker.

In addition, the spectrum of **1** shows no signals corresponding to those for C-3 and C-5 in **3** (80.0 and 79.0 p.p.m., respectively), whereas those for C-2–C-5 of **2** fit reasonably well to those for **1**, indicating that **1** and **2** have the same configuration at the anomeric carbon atom in the D-glucosyl residue (A) or group attached to the nitrogen atom. Thus, it may be concluded that **1** has an α-glycosyl–N linkage between the D-glucosyl residue A and the peptide residues.

This is a remarkable new finding, because, until now, there has been no example reporting an N–α-glycosyl linkage in D-glucose glyco-peptides (-proteins) among natural compounds. Many investigators have reported[13,14] the β-glycosyl–N linkage in 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine systems. Thus, **1** contains a new type of carbohydrate–protein linkage.

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