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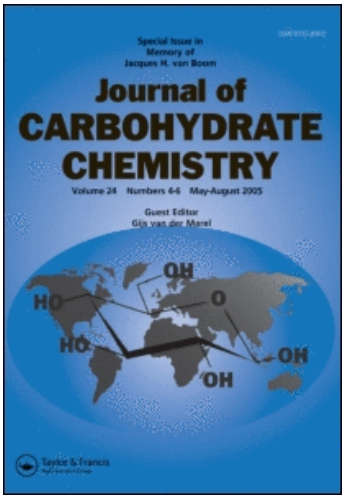

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J. CARBOHYDRATE CHEMISTRY, 19(9), 1291–1303 (2000)

^{1}H NMR STUDIES OF HYDROXY PROTONS OF ASN- AND SER-LINKED DISACCHARIDES IN AQUEOUS SOLUTION

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ABSTRACT

The hydroxy protons of β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc, β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-*N*-Asn, β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Me and of β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Ser in aqueous solution have been investigated using ^{1}H NMR spectroscopy. The chemical shifts, coupling constants, temperature coefficients, exchange rates and NOEs have been measured. The O(3)H proton of β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc and β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-*N*-Asn, and the O(2')H proton of β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc and β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Ser have values which differ significantly from the other hydroxy protons. Both these hydroxy protons are shielded when compared to those of the corresponding monosaccharide methyl glycosides. This shielding is attributed to the proximity of these protons to the O(5') oxygen and to the 2-acetamido group, respectively. In β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc and β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-*N*-Asn, the O(3)H proton has restricted conformational freedom with a preferred orientation towards the O(5') oxygen, and is protected from exchange with the bulk water through a weak hydrogen bond interaction with O(5'). In β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Me and β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Ser, the O(2')H is protected from exchange with the bulk water by the 2-acetamido group. The conformations of the disaccharides are not affected by the amino acid, and no interaction in terms of hydrogen bonding between the sugars and the amino acid residue could be observed.

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INTRODUCTION

Glycoproteins constitute an important class of biopolymers and are involved in many biological processes. Most oligosaccharide moieties of glycoproteins can be categorized into one of two major classes based on the type of linkage between the oligosaccharide and the protein. These two major linkages are: N-linked, where the oligosaccharide is linked to the side chain amide group of an asparagine residue through an N-glycosidic bond, and O-linked, where the oligosaccharide is linked to the side chain hydroxyl group of a serine or threonine residue through an O-glycosidic bond. The core structure of oligosaccharides attached to asparagine consists of the disaccharide β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc and the core structure of oligosaccharides linked to serine or threonine consists of the disaccharide β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc. Little is known about the effect of the saccharides on the conformation of the peptide moieties, or how the peptides affect the conformation of the saccharides. However, most of the biological activities of the glycoproteins appear to be generated by the cooperation of the sugar and peptide moieties, and the structural properties of the sugar-peptide linkage region are considered to be important.[1,2] Since the exchangeable amide and hydroxy protons of amino acids and saccharides often are involved in intramolecular interactions such as hydrogen bonding, it is important to be able to study these when investigating the conformations of glycopeptides. The observation of amide protons by NMR in aqueous solution is possible, but it is much more difficult to detect the hydroxy protons due to their rapid exchange with the bulk water. However, when they can be observed, important additional structural information can be obtained.[3-12] In this work, we have investigated the hydroxy protons of the disaccharide β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc (**1**) and the glycoside β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Me (**3**) in aqueous solution by NMR spectroscopy (Scheme 1). The data have been compared to those of their amino acid linked counterparts β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-*N*-Asn (**2**) and β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Ser (**4**)

Conformational studies using NMR have already been reported for disaccharides **1**[13,14] and **3**[15,16] and for glycopeptides structurally related to **2**[13,14] and **4**.[17,18] However, to our knowledge, no information on the NMR of the hydroxy protons has been reported for these compounds. The goal of this work was: (i) To determine if new structural information can be obtained from the hydroxy protons. More specifically, as part of a general study on the chemical shifts of hydroxy protons, we are interested in finding out if they can be used as additional structural probes in conformational analysis. (ii) To determine if and how the conformations of the disaccharides are changed upon linkage to an amino acid. For this, we have assigned the exchangeable hydroxy protons and amide protons, and measured several of their properties such as chemical shifts, vicinal coupling constants, exchange rates with water, temperature coefficients, NOEs and ROEs.

RESULTS AND DISCUSSION

To be able to observe signals from hydroxy protons by NMR, their rate of exchange with water was reduced by a lowering of the temperature. Acetone-d_6 was added

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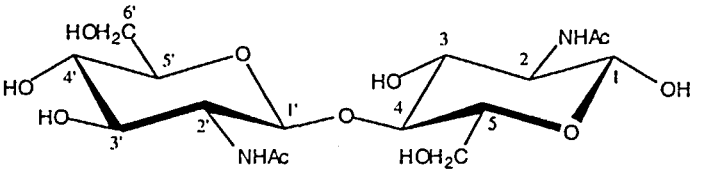

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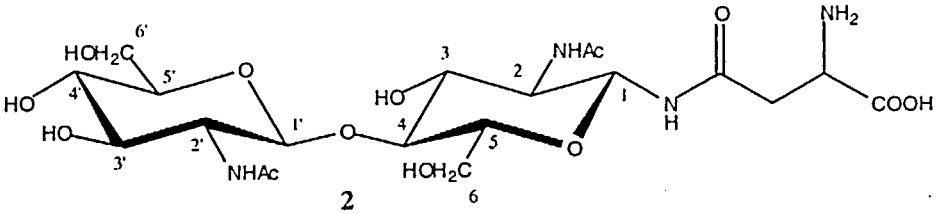

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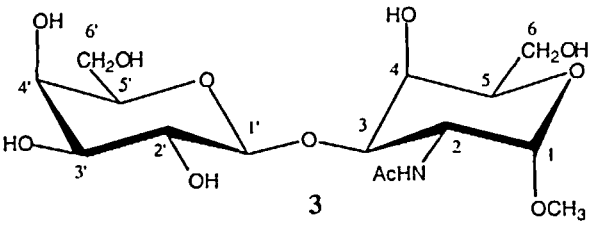

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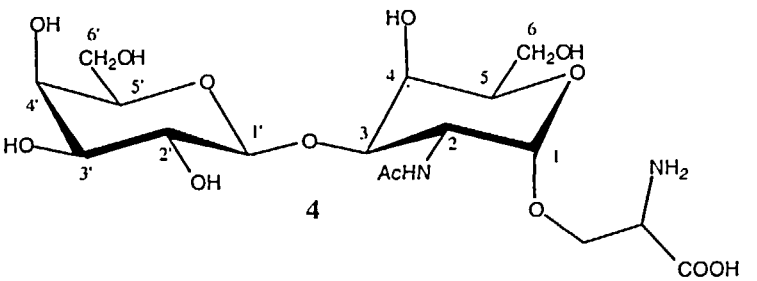

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Scheme 1

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to give 85% H_2O/15% $(CD_3)_2CO$ solutions of **1** - **4** down to -12 °C without freezing. At this temperature, the rate of exchange of the hydroxy protons was low enough so that they could be assigned from two-dimensional DQF-COSY and TOCSY spectra on the basis of scalar connectivities to the C-H protons.

β-D-GlcpNAc-(1→4)-β-D-GlcpNAc (1), β-D-GlcpNAc-(1→4)-β-D-GlcpNAc-N-Asn (2):

Figure 1a shows the NH and OH region of the ^1H NMR spectrum of β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc (**1**) where signals for both the hydroxy protons of the α and β anomers can be observed. The signal of the hydroxy proton O(1β)H of the equatorial hydroxy group is deshielded by 0.65 ppm compared to O(1α)H of the axial hydroxy group. The long range effect of the configuration at C(1) is also seen on the chemical shift of the O(3)H signal [O(3β)H is deshielded by 0.13 ppm from O(3α)H]. For comparison with compound **2** (Fig. 1b), only the β form of β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc will be discussed.

Inspection of Figure 1 and Table 1 shows that each hydroxy proton signal has similar chemical shift in **1** and **2**. Table 1 also shows that all hydroxy proton signals of **1** and **2**, with the exception of O(3)H, have chemical shifts very similar ($|\Delta\delta| \leq 0.14$ ppm) to those measured for signals from the corresponding monosaccharide methyl glycosides. The O(3)H signal, on the other hand, is shielded by 0.459 and 0.415 ppm in **1** and **2**, respectively. Previous studies[10-12] on saccharides have shown that such large upfield shifts are observed for signals of hydroxy protons close to the O(5) oxygen of a neighboring sugar. Distance measurements on energy-minimized conformations show that in both **1** and **2**, such a short distance exists between O(3)H and O(5'). It should be noted that this short O(3)H-O(5') distance is a common feature for all 1,4-linked disaccharides having the same geometry and glycosidic bond conformation.

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All hydroxy protons (Table 1) of **1** and **2**, with the exception of O(3)H, have $^3J_{HO.CH}$-values around 5 Hz, characteristic of free rotation of the hydroxy group around the C-O bond.[19] The small $^3J_{HO.CH}$-values for O(3)H of 2.2 Hz in **1** and 2.6 Hz in **2** indicate a more restricted rotation around the C(3)-O(3) bond with a preference for a *gauche* orientation of the O(3)H and C(3)H protons. The *gauche* orientation places O(3)H at a distance and orientation favorable for hydrogen bonding with O(5'). Thus, the restricted rotation might be caused by hydrogen bonding to O(5').

Hydroxy protons that are protected from contact with the solvent should have lower exchange rates than hydroxy protons in contact with the solvent. The rate of exchange for the hydroxy protons in **1** and **2** with the bulk water was measured at -12 °C from a series of eight chemical exchange experiments recorded with mixing times between 3 and 24 ms (Fig. 2a and 2b). In both **1** and **2**, O(3)H has the lowest rate of exchange with water supporting its participation in hydrogen bonding. Hydroxy protons involved in strong hydrogen bond interaction should have low temperature coefficients ($d\delta/dT \leq -3$ ppb/°C). The dependence on temperature of the chemical shifts of the exchangeable hydroxy and amide proton signals in **1** and **2** was measured from a series of one-dimensional spectra obtained at different temperatures. Table 1 shows that all protons have temperature coefficients between 8.8 and 13.5 ppb/°C in **1**, and between 7.4 and 12.1

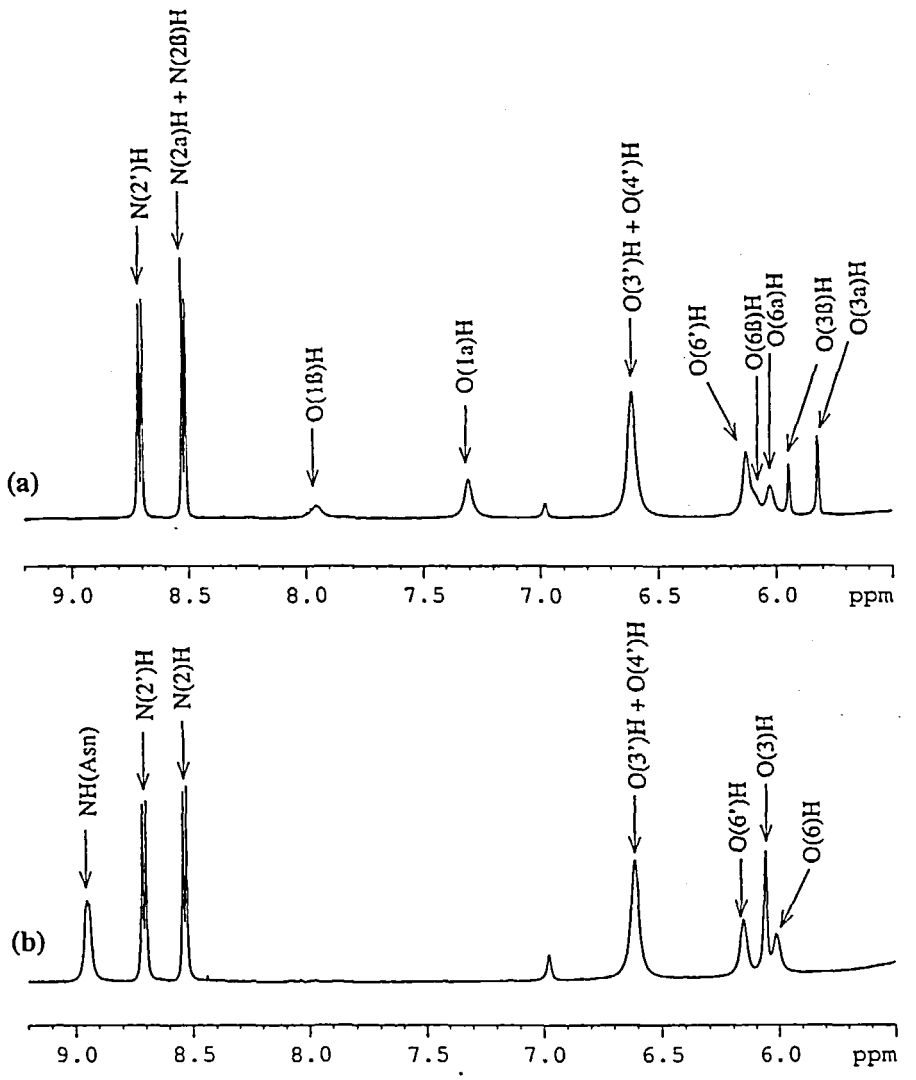

Figure 1 One-dimensional ^{1}H NMR spectra of the NH and OH region of (a) **1**, and (b) **2**, recorded at -12 °C in 85% H_2O / 15% $(CD_3)_2CO$.

ppb/°C in **2**, the amide protons having slightly lower coefficients than the hydroxy protons. The fact that O(3)H has a temperature coefficient similar to that of the other hydroxy protons suggests that the hydrogen bond interaction with O(5') is weak. The NOEs and chemical exchanges involving the exchangeable NH and OH protons are listed in Table 2. Most of the interactions are within a sugar residue. It is only O(3)H which shows inter-residue NOEs to C(1')H and C(5')H, and N(2')H to one of the C(6)H protons.

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Table 1. ^{1}H NMR chemical shifts (δ), chemical shift differences (Δδ),[a] ^3J-values (J /Hz), temperature coefficients (dδ/dT), and exchange rates (k_{ex}/s^{-1}) measured at -12 °C in 85% H_2O-15% $(CD_3)_2CO$ for the NH and OH protons of compounds **1** and **2**.

		NH(Asn)	N(2')H	N(2)H	O(3')H	O(4')H	O(6')H	O(1)H	O(3)H	O(6)H
1	δ	-	8.708	8.518 (β) 8.512 (α)	6.610	6.604	6.132	7.305 (α) 7.955 (β)	5.818 (α) 5.945 (β)	6.024 (α) 6.094(β)
	Δδ	-	0.223	0.034	0.134	0.046	0.048	0.069[h]	-0.459[b]	0.080[b]
	J	-	9.7	8.1	4.2[c]	5.7[c]	9.5[c,d]	6.4[c,e]	2.2[e]	10.0[c,d,e]
	dδ/dT	-	-9.44	-8.99	-10.1[f]	-10.1[f]	-13.5[g]	-8.79[e]	-9.10[e]	-13.5[e,g]
	k_{ex}	-	-[h]	-[h]	45[f]	45[f]	33[g]	65[e]	22[e]	33[e,g]
2	δ	8.945	8.707	8.533	6.613	6.614	6.159	-	6.061	6.013
	Δδ	-	0.222	0.048	0.137	-0.04	0.075	-	-0.415	-0.071
	J	7.8	9.7	9.4	5.3[c]	5.3[c]	10.1[c]	-	2.6	9.7[c,d]
	dδ/dT	-7.37	-9.16	-7.96	-11.6[f]	-11.6[f]	-12.1	-	-10.6	-10.1
	k_{ex}	-[h]	-[h]	-[h]	47[f]	47[f]	34	-	13	45

a. Chemical shift of the hydroxy proton signal in the disaccharide minus that of the corresponding monosaccharide methyl glycoside. A positive difference indicates a downfield shift. b. Chemical shift of the hydroxy proton signal in the disaccharide minus that of the corresponding signal in *N*-acetyl-β-D-glucosamine. c. Strong resolution enhancement applied. d. $^3J_{O(6)H,C(6)Ha} + {}^3J_{O(6)H,C(6)Hb}$. e. Measured and calculated only for the β anomer. f. The signals of O(3')H and O(4')H are overlapping. g. The signals of O(6')H and O(6)H are overlapping. h. Could not be calculated since the rate of exchange of NH protons with water at this temperature is too low.

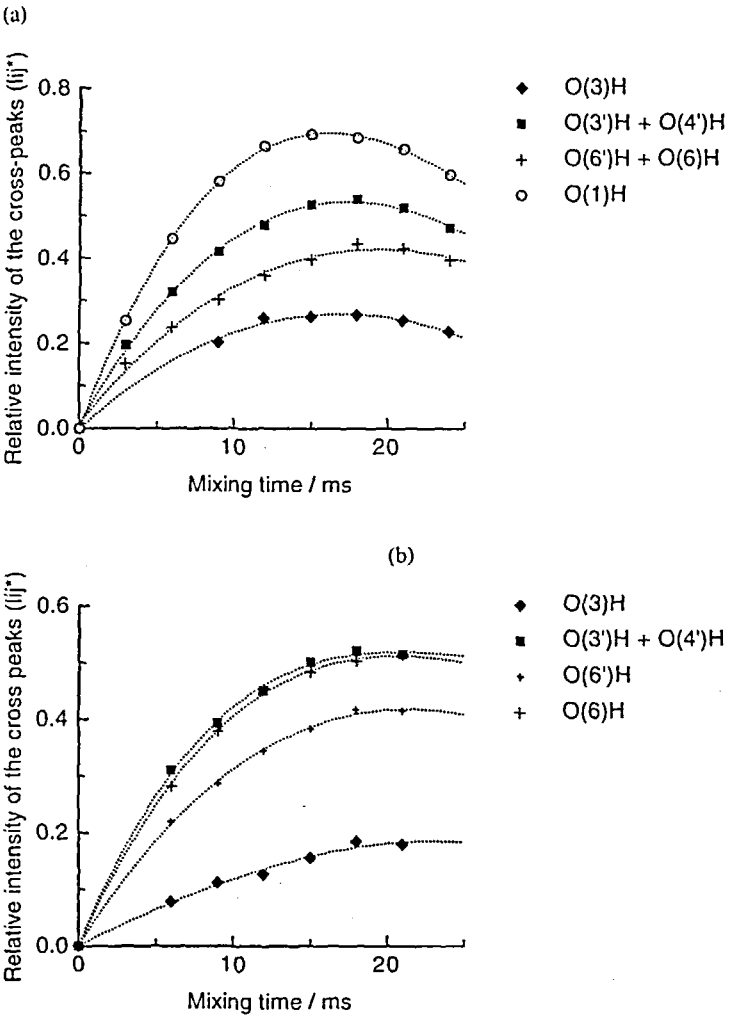

Figure 2. Build-up curves for the chemical exchange between hydroxy protons and water of (a) **1** and (b) **2**.

The preferred conformation of the 2-acetamido group was determined from the $^3J_{N(2')H,C(2')H}$- and $^3J_{N(2)H,C(2)H}$-values. The large values measured for **1** and **2** indicate a preferred *anti* conformation around the C(2')-N(2') and C(2)-N(2) bonds. For both **1** and **2**, the amide proton of GlcNAc has stronger NOEs to C(2)H and C(3)H and a weaker NOE to C(1)H, indicating that the average conformation of the acetamido group is not restricted by the introduction of Asn. NOESY experiments on **2** showed that N(1)H has a strong NOE to C(2)H and a very weak NOE to C(1)H. These NOEs and the large $^3J_{N(1)H,C(1)H}$-value of 7.8 Hz (Table 1) indicate an *anti* orientation of the C(1)H proton relative

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Table 2. NOEs and ROEs observed for the exchangeable NH and OH protons in **1**[a] and **2** at -12 °C (mixing time 100 ms for NOESY and 100 ms for ROESY).

	1		**2**	
N(1)H			C(1)H	weak
			C(2)H	stron
			HαAsn	weak
			HβAsn	strong
			Hβ'Asn	medium
N(2)H	C(1)H	weak	C(1)H	weak
	C(2)H	medium	C(2)H	medium
	C(3)H	strong	C(3)H	strong
	C(4)H	weak	C(4)H	weak
	C(5)H	weak	C(5)H	weak
	C(Ac)H	strong	C(Ac)H	strong
	O(3)H	weak	O(3)H	medium
N(2')H	C(1')H	medium	C(1')H	medium
	C(2')H	medium	C(2')H	strong
	C(3')H	strong	C(3')H	strong
	C(4')H	weak	C(4')H	weak
	C(Ac')H	strong	C(Ac')H	strong
	O(3')H	weak	O(3')H	weak
	C(6)H	weak	C(6)H	medium
O(3)H	C(2)H	medium	C(2)H	medium
	C(3)H	strong	C(3)H	strong
	C(4)H	strong	C(4)H	strong
	C(5')H	weak	C(5')H	medium
	C(1')H	weak	C(1')H	medium
O(6)H[b]	C(6)H	weak	C(6)H	medium
O(3')H[c]	C(2')H	medium	C(2')H	weak
	C(3')H	medium	C(3')H	medium
	C(4')H	medium	C(4')H	medium
	C(5')H	weak	C(5')H[d]	weak
O(6')H[b]	-	-	C(4')H	weak
	C(5')H	weak	C(5')H[d]	weak
	C(6')H	weak	C(6')H[d]	weak

a. Only the data for the β-anomer are given. b. The signals for O(6')H and O(6β)H of **1** are not well separated in the two-dimensional-NMR spectra. c. The signals for O(3')H and O(4')H of **1** and **2** are overlapping. d. The signals for C(5')H and C(6')H of **2** are overlapping.

to the N(1)H proton. The strong NOE between N(1)H and the β protons of Asn together with the absence of NOE between C(1)H and the β protons of Asn suggest that the Asn γ-amide bond which connect the disaccharide to the amino acid is in the *anti* conformation.

β-D-Galp-(1→3)-α-D-GalpNAc-O-Me (3), β-D-Galp-(1→3)-α-D-GalpNAc-O-Ser (4)

All hydroxy proton signals have $|\Delta\delta| \leq 0.150$ ppm, with the exception of the O(2')H signal, which has a large $\Delta\delta$ of -0.387 ppm in **3** and -0.420 ppm in **4** (Table 3). Inspection of the minimum energy conformations of **2** and **3** shows that O(2')H is close

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Table 3. ^{1}H NMR chemical shifts (δ), chemical shift differences (Δδ),[a] ^3J-values (J /Hz), temperature coefficients (dδ/dT) and exchange rates (k_{ex}/s^{-1}) measured at -10 °C in 85% H_2O-15% $(CD_3)_2CO$ for the NH and OH protons of compounds **3** and **4**.

		NH (Ser)	N(2)H	O(2')H	O(3')H	O(4')H	O(6')H	O(4)H	O(6)H
3	δ	-	8.495	6.198	6.140	5.985	6.260	6.059	6.155
	Δδ	-	0.141	-0.387	-0.027	0.038	0.083	-0.013	0.077
	J	-	9.9	4.9	5.9	5.3[b]	10.8[b,c]	4.0[b]	10.0[b,c]
	dδ/dT	-	-11.1	-13.4	-13.1[d]	-12.9	-16.0	-15.0	-13.1[d]
	k_{ex}	-	-[e]	7	12[d]	17	15	10	12[d]
4	δ	missing	8.408	6.151	6.052	5.915	6.216	6.052	6.216
	Δδ		0.054	-0.420	-0.105	-0.019	0.059	0.002	0.150
	J		9.9	3.7	3.9[b]	4.9[b]	10.1[b,c]	3.9[b]	10.1[b,c]
	dδ/dT		-9.85	-13.3	-14.8[f]	-12.2	-13.9[g]	-14.8[f]	-13.9[g]
	k_{ex}		-[e]	56	262[f]	222	115[g]	262[f]	115[g]

a. Chemical shift of the hydroxy proton signal in the disaccharide minus that of the corresponding monosaccharide methyl glycoside. A positive difference indicates a downfield shift. b. Extensive resolution enhancement function applied. c. $^3J_{O(6)H,C(6)Ha} + {}^3J_{O(6)H,C(6)Hb}$. d. The signals of O(3')H and O(6)H are overlapping. e. Could not be calculated since the rate of exchange of NH protons with water at this temperature is too low. f. The signals of O(3')H and O(4)H are overlapping. g. The signals of O(6')H and O(6)H are not well separated in the 2D spectra.

Table 4. Experimental NOEs and ROEs observed for the exchangeable NH and OH protons in **3** and **4** at -12 °C (mixing time 100 ms for NOESY and 200 ms for ROESY).

	3		**4**	
N(2)H	C(1)H	weak	-	-
	C(2)H	medium	C(2)H	weak
	C(3)H	medium	C(3)H	medium
	C(Ac)H	strong	C(Ac)H	strong
	C(1')H	weak	C(1')H	weak
O(4)H[a]	C(2)H	weak	C(2)H	weak
	C(4)H	medium	C(4)H	medium
	C(6)H	weak	-	-
O(6)H[b,c]	C(5)H	weak	-	-
	C(6)H	weak	C(6)H	weak
O(2')H	C(1')H	weak	C(1')H	weak
	C(2')H	medium	C(2')H	medium
	C(3')H	weak	C(3')H	weak
O(3')H[a,b]	C(2')H	medium	C(2')H	weak
	C(3')H	medium	C(3')H	weak
	C(4')H	weak	-	-
	C(6')H	weak	C(6')H	weak
O(4')H	C(2')H	weak	-	-
	C(4')H	medium	C(4')H	weak
	C(6')H	weak	-	-
O(6')H[c]	C(6')H	weak	C(6')H	weak

a. The signals for O(3')H and O(4)H of **4** are overlapping. b. The signals for O(6)H and O(3')H of **3** are not well separated in the two-dimensional NMR spectra. c. The signals for O(6')H and O(6)H of **4** are overlapping.

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to the 2-acetamido group of the neighboring sugar residue. In disaccharides of similar geometry such as **2** and **3**, but with a 2-hydroxy group instead of a 2-acetamido group, the O(2')H proton has either a very small $\Delta\delta$ or a positive $\Delta\delta$.[20] The upfield shift experienced by O(2')H is thus probably due to the influence of the 2-acetamido group. The $^3J_{OH,CH}$-values are all in the range 4 - 6 Hz indicating conformational averaging. All temperature coefficients are in the range -9.8 to -16 ppb/°C suggesting that no hydrogen bond interaction exists in the disaccharides. Table 3 shows that in **3** and **4**, the exchange rate of O(2') with the bulk water is lower than that of the other hydroxy protons. The lower rate of exchange of O(2')H with water in **3** and **4** is probably due to a limited accessibility of water because of the proximity to the 2-acetamido group of the neighboring sugar, and not to hydrogen bonding. As in **1** and **2**, the large $^3J_{N(2)H,C(2)H}$-value of 9.9 Hz measured in **3** and **4** indicates a preference for the *anti* conformation around the C(2)-N(2) bond. Only intra-residue NOEs were observed between OH and CH protons of **3** and **4** (Table 4), with the exception for the NOE between N(2)H and C(1')H.

CONCLUSION

Hydrogen bond interactions between the peptide and sugar moieties have been shown to exist in DMSO solution of small glycopeptides.[21] No such interaction is observed in the compounds investigated in the present study, and the presence of one amino acid does not affect the conformations of the disaccharides. These results are not surprising since the introduction of a single amino acid is not expected to affect significantly the conformational flexibility of the disaccharide.

In both β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc and β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-*N*-Asn, the O(3)H proton is shielded relative to that in the corresponding monosaccharide. This shielding, attributed to the proximity to O(5'), together with the small $^3J_{OH,CH}$-value, which indicates a restricted rotation around the C(3)-O(3) bond, and the lower exchange rate of O(3)H with water, suggest the presence of hydrogen bonding between O(5') and O(3)H. However, the high value of the temperature coefficient of O(3)H indicate that the hydrogen bonding is weak. MD studies have shown[13] that this hydrogen bond exists *in vacuo*, but is replaced by hydrogen bonds with the solvent water molecules in aqueous solution. The present work shows that some transient hydrogen bond interaction must, however, be present. In β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Me and β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-*O*-Ser, the O(2')H proton is shielded relative to the same proton in the corresponding monosaccharide methyl glycoside and in β-D-Gal*p*-(1→3)-α-D-Gal*p*-*O*-Me. This shielding is attributed to the proximity to the 2-acetamido group. In the two disaccharides **1** and **3**, and in their amino acid linked counterparts **2** and **4**, the hydroxy proton, which experiences an upfield shift, also shows a lower rate of exchange with water. It is possible that the steric interference caused by the oxygen ring in **1** and **2**, and by the 2-acetamido group in **3** and **4**, competes with solvation and leads to the upfield shift of the hydroxy proton resonances. This work agrees well with previous studies[10-12,20] where we have shown that when the chemical shift of a signal from a hydroxy proton in a saccharide substantially differs from that in the monosaccharide methyl glycoside, it is an indication of a particular conformational feature. It also confirms the potential use of hydroxy proton chemical shifts in structural analysis. Since the question on the value of using low temperature for conformational studies can arise, it is important to note that the conformation of the disaccharides (**1**) and (**3**) determined in this work is very similar to the conformations reported for higher temperature in aqueous solution.[13-16] This work is now being extended to larger glycopeptides where interactions between the sugars and the amino acids are more likely.

EXPERIMENTAL

Compounds **1** and **2** were purchased from Sigma and compound **3** was available in the laboratory. Compound **4** was synthesized as previously reported.[22] Additional

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purification of compound **4** was performed on a Bio-Gel P-2 gel filtration column (BIO-RAD, USA). All NMR experiments on samples **1** - **4** were performed on a BRUKER DRX-600 spectrometer operating at 600.13 MHz for proton observation. Compounds **1** - **4** were dissolved in a mixture of 85% H_2O/15% $(CD_3)_2CO$. The pH for **1** and **2** was 5.5, and the pH for **3** and **4** was 5. A microprobe was used, which allowed the use of volumes of 150 µL to give sample concentrations of 25 mM, 25 mM, 17 mM, 34 mM for **1**, **2**, **3** and **4**, respectively. $(CD_3)_2CO$ was added to the samples to allow lowering of the sample temperature to -12 °C without freezing. All spectra unless specified were recorded at -12 °C except for the temperature coefficients, which were measured by variation of the temperature from -10 °C to 25 °C in steps of 5 °C. ^{1}H NMR spectra were referenced by setting the residual acetone-d_5 signal to δ_H = 2.204 ppm. One and two-dimensional ^{1}H NMR spectra were acquired using the WATERGATE pulse sequence[23] for water suppression. The 2D TOCSY and NOESY spectra were recorded in the phase-sensitive mode using the TPPI method.[24] The 2D DQF-COSY and ROESY spectra were recorded in the phase-sensitive mode using the state-TPPI method.[25] NOESY spectra were recorded with mixing times (τ_m) of 100, 200 and 300 ms. ROESY spectra were recorded with a mixing time of 100 ms for **1** and **2**, and 100 and 200 ms for **3** and **4**. Repetition delays of 1.5 s and 256 or 512 spectra of 2K data points were used. The data were zero-filled to 2K × 1K before applying a $\pi/4$ shifted sine-square bell window function in both dimensions. The rates of exchange of the hydroxy protons with water were calculated from 2D phase-sensitive chemical exchange experiments.[26] Mixing times of 3 to 18 (or 24 ms) ms in steps of 3 ms were used. 128 FIDs of 2K data points were acquired and a recycle delay of 1.5 s was used. A polynomial baseline correction was applied in both dimensions. The volumes of the NOE cross-peaks and diagonal peaks were measured using the program AURELIA (Bruker, Germany). The initial build-up rates of the exchange cross-peak volumes were determined from the spectra, and the volumes of the hydroxyl proton diagonal peaks at zero mixing time were obtained by extrapolation from the volumes of the diagonal peaks in the spectra. The exchange rate constants were calculated as the ratio of the initial build-up rates of the exchange peaks over the volume of the diagonal peaks at zero mixing time. The 3D structures of **1** - **4** were visualized using the Chem3D plus version 3.5 for Macintosh.

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