^{1}H NMR chemical shifts of hydroxy protons in conformational analysis of disaccharides in aqueous solution

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In a continuing study on the use of hydroxy protons in conformational analysis of saccharides in aqueous solution by NMR spectroscopy, a number of disaccharides with 1,3-, 1,4- and 1,6-linkages have been investigated. The ^{1}H-NMR chemical shifts, vicinal coupling constants ($^3J_{CH,OH}$), temperature coefficients and rates of exchange with the solvent have been measured for the hydroxy protons. Most of the hydroxy proton signals in the disaccharides have chemical shifts similar ($|\Delta\delta| \leq 0.3$ ppm) to those in the corresponding methyl monosaccharides. Three hydroxy protons, however, O(2)H in β-L-Fuc*p*-(1→3)-α-D-Glc*p*-OMe, O(3)H in β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe and O(6)H in β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe show a large upfield shift ($\Delta\delta \leq -0.3$ ppm) attributed to the proximity of the hydroxy proton to the ring oxygen of the neighbouring sugar. In β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe, the chemical shift, $^3J_{CH,OH}$ and temperature coefficient of the O(3)H signal indicate that a weak hydrogen bond interaction exists between O(3)H and O(5′). In β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe, the chemical shift and the NOEs involving the O(6)H signal suggest a preference for the *trans-gauche* conformation around the C5–C6 bond.

Introduction

We have previously reported[1,2] NMR studies on the use of hydroxy protons for the conformational analysis of a series of methyl α-D-galactopyranosides 3,4-di-substituted with D-glucose and L-fucose. We have shown that both weak and persistent hydrogen bond interactions can be determined from the NMR data (chemical shifts, coupling constants, temperature coefficients and rates of exchange with water) obtained from hydroxy protons. To our knowledge, it was the first time that strong and persistent hydrogen bond interactions were shown to exist in aqueous solution. It was also shown that the chemical shifts of hydroxy protons are very sensitive to the proximity and orientation of electron lone pairs of non-protonated oxygens.

However, only a few data on the chemical shifts of hydroxy protons in water are available, and it is necessary to investigate several different systems to assess the reliability of the measurements and determine the limitations of using hydroxy protons as conformational probes. Additionally, since the branched trisaccharides previously investigated[1,2] are relatively constrained molecules, it was necessary to determine if similar information could be obtained from hydroxy protons in more flexible systems such as disaccharides. For this reason, we have investigated a series of 1,3-, 1,4- and 1,6-linked disaccharides (Scheme 1). The conformational analysis of these compounds using ^{1}H- and ^{13}C-NMR chemical shifts and Hard Sphere Exo Anomeric effect (HSEA) and Geometry of Saccharides (GSEA) calculations has been reported.[3–6] In this paper, we show how the NMR data obtained from hydroxy protons can be used to monitor different conformational features of the disaccharides. The final goal of these investigations is to show that hydroxy protons can be used as additional conformational probes in structural studies, and also that the chemical shifts of hydroxy protons can be important in conformational studies.

α-L-Fuc*p*-(1→3)-α-D-Gal*p*-OMe	**1**	β-L-Fuc*p*-(1→3)-α-D-Gal*p*-OMe	**2**
α-D-Glc*p*-(1→3)-α-D-Gal*p*-OMe	**3**	β-D-Glc*p*-(1→3)-α-D-Gal*p*-OMe	**4**
α-L-Fuc*p*-(1→3)-α-D-Glc*p*-OMe	**5**	β-L-Fuc*p*-(1→3)-α-D-Glc*p*-OMe	**6**
α-D-Fuc*p*-(1→3)-α-D-Glc*p*-OMe	**7**	α-L-Fuc*p*-(1→3)-α-D-Man*p*-OMe	**8**
α-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe	**9**	β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe	**10**
β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe	**11**	α-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe	**12**
α-D-Glc*p*-(1→6)-α-D-Glc*p*-OMe	**13**	β-D-Glc*p*-(1→6)-β-D-Glc*p*-OMe	**14**
β-D-Glc*p*-(1→6)-β-D-Gal*p*-OMe	**15**	β-L-Fuc*p*-(1→6)-α-D-Gal*p*-OMe	**16**

Scheme 1 Disaccharides investigated.

Results and discussion

^{1}H-NMR chemical shifts

After careful preparation of the NMR samples (see Experimental section), the rate of exchange of the hydroxy protons with the solvent is slow enough so that they can be observed as relatively narrow lines ($\nu_{1/2}$ <10 Hz). It is then possible to make their assignment on the basis of scalar connectivities to the aliphatic protons from DQF-COSY and TOCSY experiments. The ^{1}H-NMR chemical shifts (δ, ppm) and chemical shift differences $\Delta\delta$ (chemical shifts of the hydroxy proton signals in the disaccharides minus those in the corresponding monosaccharide methyl glycoside) for the hydroxy protons of disaccharides **1**–**16** (Scheme 1) obtained at −8 °C are listed in Table 1. The chemical shifts and coupling constants for the methyl glycosides of the monosaccharides have been reported previously,[1] with the exception of those of α-D-Man*p*-OMe which are listed in Table 1.

Inspection of Table 1 and Fig. 1 shows that: (i) Most of the hydroxy protons show only minor effects by the glycosylation and the NMR signals have $|\Delta\delta| \leq 0.30$ ppm. (ii) One 1,3-linked disaccharide, β-L-Fuc*p*-(1→3)-α-D-Glc*p*-OMe **6**, and two 1,4-linked disaccharides, β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe **10** and β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe **11**, have each one hydroxy proton signal with a large negative $\Delta\delta$. That is O(2)H in **6**, O(3)H in **10** and O(6)H in **11**. Our previous studies[1,2] have shown that large upfield shifts ($\Delta\delta$ −0.3 to −1.4 ppm) are observed for hydroxy protons which are close in space to a non-protonated oxygen, either a ring oxygen or a neighbouring glycosidic linkage oxygen. Thus, in the disaccharides **6**, **10** and **11**, the upfield shifts observed for the hydroxy proton signals are attributed to their proximity to a non-protonated oxygen atom. The 3-D representations of one minimum energy conformation (Fig. 2) of **6**, **10** and **11** obtained from energy minimization using MM2

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Table 1 ^{1}H-NMR chemical shifts[a] (δ) and chemical shift differences ($\Delta\delta$ = chemical shift in the disaccharide glycoside minus chemical shift in the corresponding monosaccharide methyl glycoside) at −8 °C in 85% H_2O–15% $(CD_3)_2CO$. A positive difference indicates a downfield shift. The hydroxy protons with large $|\Delta\delta|$ (≥0.3 ppm) are indicated in bold

Cpd		O(2′)H	O(3′)H	O(4′)H	O(6′)H	O(2)H	O(3)H	O(4)H	O(6)H
1	δ	6.01	5.99	5.99		6.41		6.15	6.056
	$\Delta\delta$	−0.10	0.07	0.03		0.25		0.21	0.03
2	δ	6.49	6.05	5.95		6.00		5.93	6.05
	$\Delta\delta$	0.03	0.04	0.09		−0.15		0.01	0.03
3	δ	6.34	6.39	6.38	5.88	6.19		6.00	6.06
	$\Delta\delta$	0.03	0.04	0.01	−0.11	0.04		0.06	0.04
4	δ	**6.66**	6.53	6.41	6.04	**6.48**		5.82	6.05
	$\Delta\delta$	**0.34**	0.01	−0.04	0.02	**0.33**		−0.12	0.03
5	δ	5.92	5.96	5.95		6.19		6.43	6.01
	$\Delta\delta$	−0.20	0.05	−0.01		−0.13		0.06	0.06
6	δ	6.42	6.10	6.00		**6.00**		6.49	6.03
	$\Delta\delta$	−0.03	0.08	0.13		**−0.31**		0.12	0.07
7	δ	5.91	5.94	5.95		6.200		6.38	5.99
	$\Delta\delta$	−0.20	0.03	−0.01		−0.12		0.01	0.04
8	δ	6.14	5.95	5.94		6.38		6.40	5.97
	$\Delta\delta$	0.03	0.03	−0.02		0.13		0.07	−0.05
9	δ	**6.48**	5.95	5.99		6.30	6.14		6.09
	$\Delta\delta$	**0.36**	0.04	0.03		0.14	0.17		0.06
10	δ	6.49	6.09	5.99		6.25	**5.33**		6.06
	$\Delta\delta$	0.04	0.08	0.13		0.10	**−0.64**		0.03
11	δ	6.37	6.06	5.92		6.41	6.38		**5.48**
	$\Delta\delta$	−0.08	0.05	0.05		0.10	0.03		**−0.48**
12	δ	6.04	5.94	5.94		6.36	6.22		5.91
	$\Delta\delta$	−0.08	0.03	−0.02		0.04	−0.13		−0.05
13	δ	6.27	6.39	6.43	5.90	6.38	6.37	6.41	
	$\Delta\delta$	−0.04	0.04	0.06	−0.06	0.06	0.02	0.04	
14	δ	6.57	6.40	6.35	5.89	6.54	6.43	6.47	
	$\Delta\delta$	−0.08	−0.11	−0.11	0.13	−0.11	−0.08	0.02	
15	δ	6.54	6.44	6.37	5.91	6.44	6.04	5.86	
	$\Delta\delta$	−0.11	−0.07	−0.08	−0.10	−0.04	−0.03	0.01	
16	δ	6.45	6.04	5.91		6.19	6.01	6.05	
	$\Delta\delta$	−0.01	0.03	0.05		0.04	0.04	0.11	
17[b]	δ	6.25	6.12	6.33	6.02				

[a] δ ± 0.02 ppm. [b] α-D-Man*p*-OMe.

show that O(2)H in **6**, O(3)H in **10** and O(6)H in **11** are close to the O(5′) oxygen. Fig. 2 also shows that the three hydroxy protons are pointing (located) between the two lone pairs of the O(5′) oxygen. It is of interest to note that the $\Delta\delta$ of the primary hydroxy proton, O(6)H in **11**, is larger than the $\Delta\delta$ of the secondary hydroxy proton O(2)H in **6**. The $\Delta\delta$ of O(6)H in **11** is also larger than that measured for O(6)H close to ring oxygens in branched trisaccharides ($\Delta\delta$ of ~−0.3 ppm). In the branched trisaccharides,[2] the $\Delta\delta$ of primary hydroxy protons close to a ring oxygen was always smaller than the $\Delta\delta$ of secondary hydroxy protons in a similar position. One could expect smaller $\Delta\delta$ for primary hydroxy protons than for secondary hydroxy protons since changes in the conformational equilibrium for the hydroxymethyl groups will change the distance and orientation of the OH proton relative to the oxygen lone pairs, and thereby contribute to large changes in hydroxy proton chemical shifts. It is possible that in **11**, one of the three rotamers is strongly preferred due to inter-residual stabilization. These chemical shifts should however be interpreted only qualitatively, since disaccharides are flexible molecules and the measured chemical shift represents an average for all conformations existing in solution. Thus, even very small variations in distances to oxygen atoms and lone pair orientations could have a pronounced effect on the chemical shifts. The possibility of hydrogen bond interactions should also be considered. It is well accepted that protons involved in hydrogen bonds are deshielded. Thus, for hydrogen bonded hydroxy protons the deshielding due to hydrogen bonding will counteract the shielding due to stereoelectronic and proximity effects, and the chemical shift measured will be a result of the two opposite contributions.

(iii) Two disaccharides have hydroxy protons experiencing a large downfield shift. These are O(2)H and O(2′)H in **4** and O(2′)H in **9**. Downfield shifts were also measured[2] in branched trisaccharides, and are not due, as shown by the average values of $^3J_{H,OH}$, k_{ex}, and dδ/dT (*vide infra*), to hydrogen bonding. The effect of glycosylation on the ring protons is usually a deshielding of the protons across the glycosidic bond as well as of the protons at the two neighbouring sites of the aglycon.[7] The magnitude of the deshielding depends on the type of monosaccharide, anomeric linkage, and conformation around the glycosidic bond. The main causes for this deshielding are the steric repulsion between hydrogens and the fixation of oxygen lone pairs close in space to the hydrogens in question.[8] It is possible that hydroxy protons are also subject to the same effect, and that the downfield shift of the O(2)H and O(2′)H signals in **4** and the O(2′)H signal in **9** is due to the proximity of the protons to the glycosidic linkage oxygen with more directed lone pairs. In **9**, the O(3)H is also deshielded but to a smaller extent (0.14 ppm).

(iv) The $|\Delta\delta|$ differences are smallest (≤0.13 ppm) in 1,6-linked disaccharides. These small $\Delta\delta$ indicate that the hydroxy protons do not have any close contact to a non-protonated oxygen atom, at least not for a significant amount of time, and the data support the higher degree of conformational flexibility in 1,6-linked disaccharides. Thus, small $\Delta\delta$ can be good indicators of conformational flexibility, and/or of absence of close contacts with non-protonated oxygen atoms.

Conformational analysis of 1–16 using NMR data from hydroxy protons

The coupling constants, temperature coefficients, NOEs and chemical exchange interactions have been determined for the hydroxy protons in all of the disaccharides studied. The chemical exchanges observed in the ROESY spectra between hydroxy protons are represented in Fig. 1. A chemical exchange

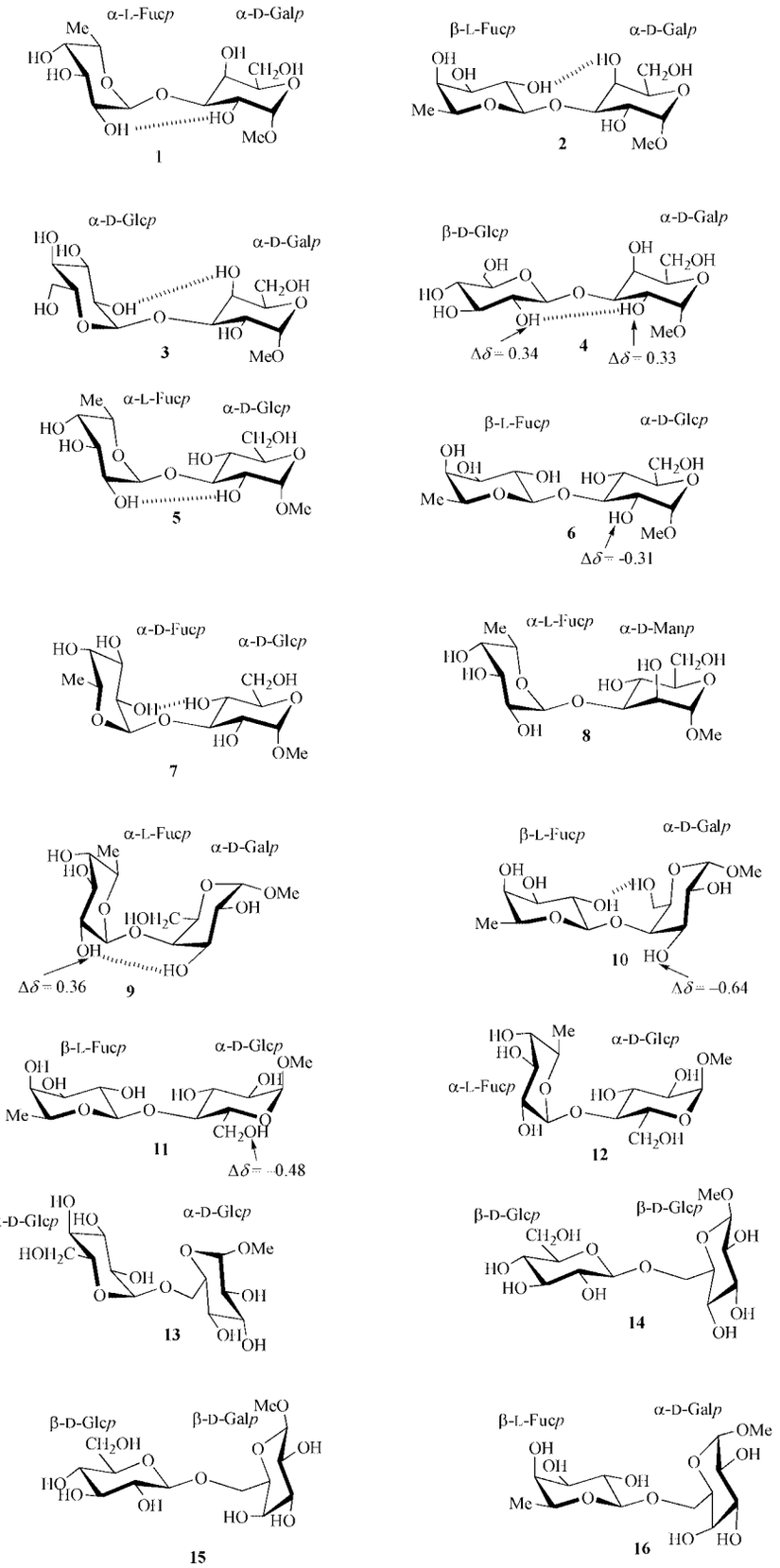
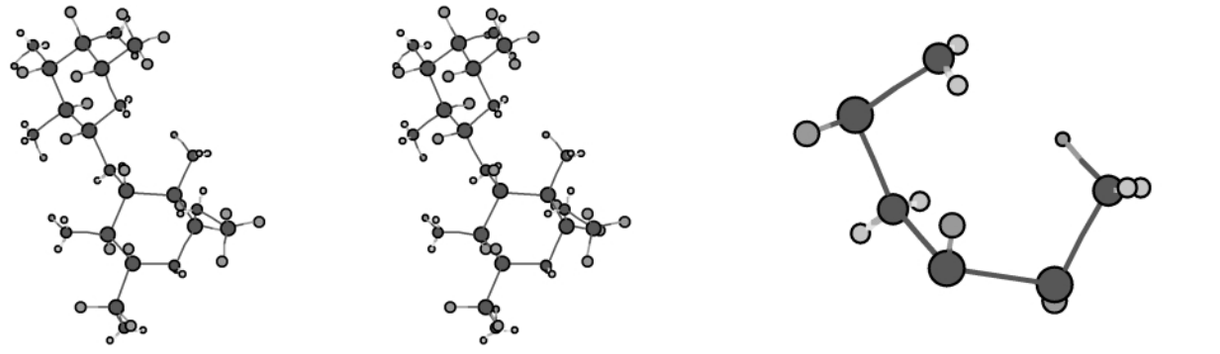
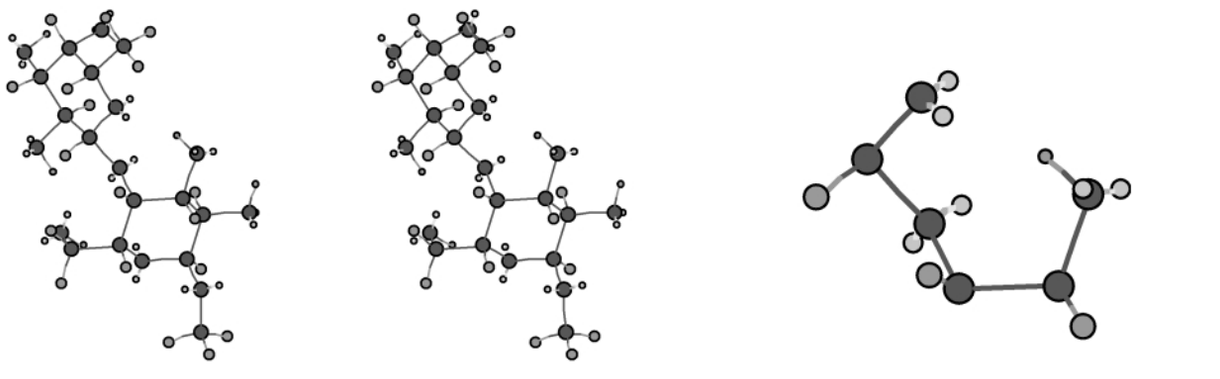


Fig. 1 2-D representation of disaccharides **1**–**16**. The hydroxy protons with large $\Delta\delta$ are indicated. The inter-residue chemical exchange interactions between hydroxy protons are drawn as dashed lines.

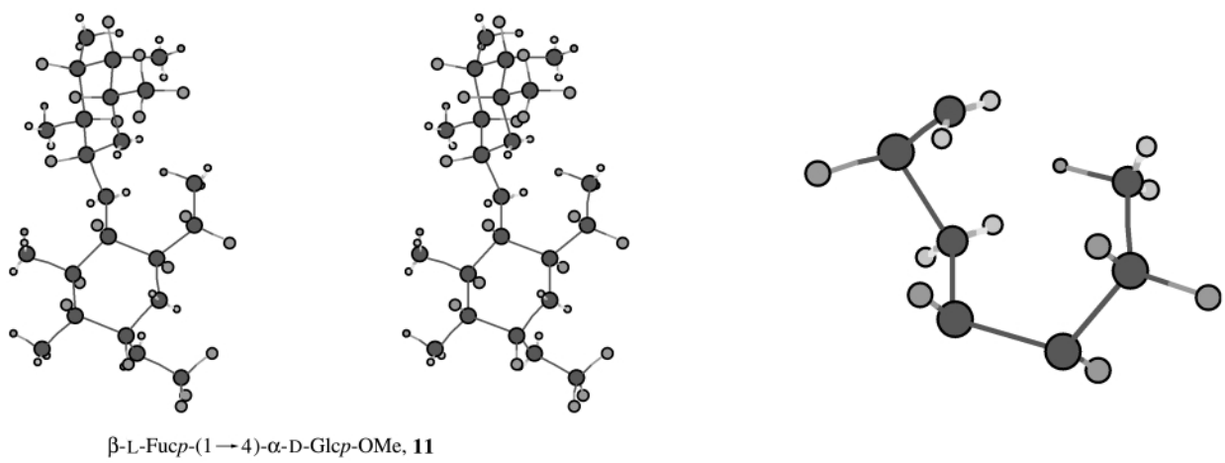
cross-peak is found between the O(2′)H signal and the O(2)H signal in **1**, **4**, **5**, and between O(2′)H and O(4)H in **2**, **3** and **7**. Thus, the same interactions are observed for disaccharides having similar stereochemistry and conformation around the glycosidic bond. These data support previous studies[3–6] which have shown that α-D/β-L-sugars on one hand, and β-D/α-L-sugars on the other have similar spatial arrangement around the glycosidic bond. The existence of hydrogen bonds is most easily detected from the NMR data of hydroxy protons. Hydroxy protons involved in hydrogen bonding are expected to have relatively smaller temperature coefficients, coupling constants which do not represent conformational averaging but which instead indicate a restricted rotation around the H–C–O–H bond, and a slower rate of exchange with water. Thus, if for



β-L-Fuc*p*-(1→3)-α-D-Glc*p*-OMe, **6**



β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe, **10**



β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe, **11**

Fig. 2 3-D models of disaccharides **6**, **10** and **11**. Note that the electron lone pairs are shown as small "atoms".

any of the hydroxy protons, one of these parameters has a value which differs from that of the other hydroxy protons, it could be an indication of involvement in a hydrogen bond. Since only hydroxy protons in **6**, **10** and **11** have NMR parameters which are different from average, only the δ, $\Delta\delta$, $^3J_{H,OH}$, k_{ex} and $d\delta/dT$ data for these are listed in Table 2. The NMR data for the hydroxy protons of the other disaccharides are available upon request from the authors. As mentioned earlier in (ii), one hydroxy proton signal in each compound, namely O(2)H in **6**, O(3)H in **10**, and O(6)H in **11**, has a large negative $\Delta\delta$ indicating its proximity to the ring oxygen of the neighbouring sugar. According to the Karplus equation derived for hydroxy protons,[9] vicinal coupling constants of the order of 5.5 ± 0.5 Hz indicate a free rotation of the hydroxy group around the C–O bond. A hydrogen bond which enforces some particular dihedral angle could be reflected in a deviation of the coupling constant for that hydroxy proton from the rotationally averaged value. Thus, in β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe, **10**, the large ^3J-value (8.7 Hz compared to 5.1 Hz in the monosaccharide methyl glycoside) measured for O(3)H indicates a restricted rotation with a preference for the *trans* orientation. This large *J*-value together with its lower temperature coefficient (7.3 ppb °C^{-1} compared to ~10.4–13.0 ppb °C^{-1} for the other hydroxy protons) suggest the involvement of O(3)H in a hydrogen bond. Due to the proximity of its NMR signal to the water resonance, the rate of exchange of O(3)H with water could not be calculated. The large upfield shift measured for the O(3)H signal together with MM2 calculations suggest that the hydrogen bond partner of O(3)H is O(5′). In addition, the *trans* orientation of the O(3)H proton relative to H(3) places O(3)H at a distance and orientation favorable for hydrogen bonding with O(5′). This hydrogen bond is similar to the one observed between O(3)H and O(5′) in cellobiose (β-D-Glc*p*-(1→4)-β-D-Glc*p*-OMe) in the crystal[10] and in DMSO solution,[11] and for

Table 2 ^{1}H-NMR chemical shifts[a] (δ), chemical shift differences ($\Delta\delta$), temperature coefficients ($d\delta/dT$, ppb °C^{-1}), $^3J_{HO,CH}$ coupling constants (J, Hz) and exchange rates (k_{ex}, s^{-1}) for the hydroxy protons of disaccharides **6**, **10** and **11** measured at −8 °C in 85% H_2O–15% $(CD_3)_2CO$

		O(2′)H	O(3′)H	O(4′)H	O(2)H	O(3)H	O(4)H	O(6)H
6	δ	6.42	6.10	6.00	6.00		6.49	6.03
	$\Delta\delta$	−0.03	0.08	0.13	−0.31		0.12	0.07
	J	3.8	5.1	5.3	2.5		5.1	6.1
	$d\delta/dT$	11.0	11.0	11.1	11.1		11.1	12.7
	k_{ex}	23	32	[b]	[b]		25	[b]
10	δ	6.49	6.09	5.99	6.25	5.33		6.06
	$\Delta\delta$	0.04	0.08	0.13	0.10	−0.64		0.03
	J	5.0	5.3	5.7	5.3	8.1		5.3
	$d\delta/dT$	10.4	11.2	11.4	12.9	7.3		13.0
	k_{ex}	15	19	11	13	[c]		14
11	δ	6.37	6.06	5.92	6.41	6.38		5.48
	$\Delta\delta$	−0.08	0.05	0.05	0.10	0.03		−0.48
	J	5.9	4.9	5.1	5.1	4.2		5.5
	$d\delta/dT$	10.2	11.9	12.7	12.1	10.0		10.2
	k_{ex}	[b]	25	63	[b]	[b]		[c]

[a] $\delta \pm 0.02$ ppm. [b] Not measured owing to spectral overlap. [c] Could not be measured owing to the proximity of the signal to the water peak.

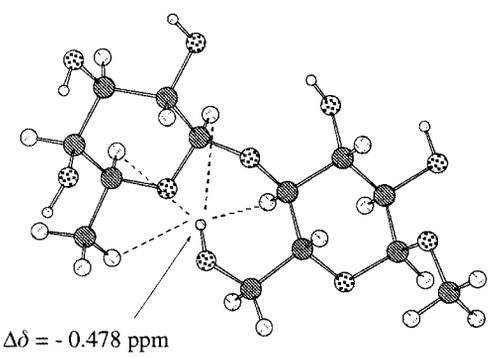


Fig. 3 Schematic representation of disaccharide **11**, showing the NOE connectivities from O(6)H which support, together with the upfield shift of O(6)H, a *tg* conformation about the C5–C6 bond.

galabioside (α-D-Gal*p*-(1→4)-β-D-Gal*p*-OMe) in the crystal.[12] The hydrogen bond in galabioside is however replaced by a weak O(6)H–O(2′)H hydrogen bond in water solution.[13] NMR studies[14] on the conformation of lactose in water have also shown that O(3)H of the Glc*p* residue is hydrogen bonded to O(5′) of the Gal*p* moiety. The O(3)H proton of Glc*p* in lactose is also shielded by 0.45 ppm as compared to that of the monosaccharide methyl glycoside, but the possible origin of this upfield shift was not mentioned. These disaccharides are all 1,4-linked sugars with similar stereochemistry around the glycosidic bond, and the fact that in some cases the hydrogen bond interaction does not persist or is different in water solution might be due to different hydration and solvation properties.

In β-L-Fuc*p*-(1→4)-α-D-Glc*p*-OMe, **11**, the temperature coefficient and exchange rate for O(6)H are similar to those of the other hydroxy protons. The values of 5.5 Hz measured for the $^3J_{H,OH}$ indicate a free rotation of the hydroxy group around the H–C–O–H bond. These NMR data do not suggest the involvment of O(6)H in a hydrogen bond interaction. The large upfield shift of the O(6)H signal together with the NOEs between O(6)H and C(1′)H and between O(6)H and C(4)H, which indicate a *trans-gauche* orientation around the C5–C6 bond agree with a close distance between O(6)H and O(5′) (Fig. 3). The NOEs observed between O(6)H–C(5′)H and O(6)H–C(6)H confirm that O(6)H is pointing toward the ring oxygen O(5′), at least for a significant population of the conformers. The hydroxymethyl groups in carbohydrates usually exist in three staggered orientations (*gauche-gauche*, *gg*; *trans-gauche*, *tg*; and *gauche-trans*, *gt*) that correspond to local minima. In solution, the amount of each rotamer is usually determined from the $^3J_{H5,H6}$-values of the coupling constants, using a generalized Karplus equation. However, in **11**, the two C(6)H signals have the same chemical shift making the measurement of the $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$-values difficult. For glucopyranoses in solution, roughly equimolar proportions of *gg* and *gt* conformers are usually found, while only minor amounts of *tg* conformer are present. Calculations in vacuum[15–17] have predicted that the *tg* conformation has the lowest energy due to an O(6)H–O(4) intramolecular hydrogen bond. Inclusion of solvent[15–17] shows a stabilization of the *gauche* (*gg*, *gt*) conformation over the *tg* conformation due to better solvation of the OH groups. Thus, the preference for the *tg* conformation found for **11** might be due to the fact that the stabilization through the intermolecular hydrogen bond between O(5) and O(6)H is more important than stabilization through hydrogen bonding to water. The different behaviour of the hydroxymethyl group in **11** is also seen from the chemical shifts of the C(6)H protons. α-D-Glc*p* is a constituent of the disaccharides **3**, **5**, **6**, **7**, **11**, **12** and **13**. In all these compounds, the two C(6)H signals have different chemical shifts[3–6] with the exception of **11**, for which the two C(6)H signals have the same chemical shift. In β-L-Fuc*p*-(1→3)-α-D-Glc*p*-OMe, **6**, the small J-value of 2.5 Hz measured for O(2)H indicates little rotational freedom around the C(2)–O(2) bond and an orientation of O(2)H favourable for hydrogen bonding with O(5′). This small value of J-coupling should also be compared to that of 6 Hz measured for methyl α-D-glucopyranoside.[1] Since the temperature coefficient is high (11.1 ppb °C^{-1}) and similar to that of the other hydroxy protons, the hydrogen bond interaction, if present, must be considered as weak. As a result of spectral overlap, the rate of exchange with water could not be measured.

Conclusion

The hydroxy protons can be used as additional probes for the conformational analysis of disaccharides by NMR spectroscopy, and three types of structural information can be obtained. (i) Hydroxy protons experiencing an upfield shift relative to those of the corresponding monosaccharide methyl glycoside indicate that they are in spatial proximity to the ring oxygen of the neighbouring sugar. Since the upfield shift of the hydroxy proton is also often accompanied by a slower rate of exchange with water, it is possible that steric interference by the ring oxygen with solvation causes the upfield shift of the resonance for the hydroxy proton. (ii) Coupling constants deviating from average values, low temperature coefficients, and slow rates of exchange with water indicate hydrogen bonding interaction. (iii) The observation of inter-residue NOEs and/or chemical exchanges involving hydroxy protons can be used to better characterize the conformation around the glycosidic linkage. The additional structural information obtained from

hydroxy protons could, together with the other data obtained from ^{1}H and ^{13}C NMR and from molecular mechanics and dynamics calculations, allow the conformations of disaccharides in solution to be better defined. In this work and in previous studies,[1,2,13] we found that the hydroxy protons involved in hydrogen bonding with a ring oxygen O(5) still experience an upfield shift. Since protons involved in hydrogen bond interactions should be deshielded, it is possible that (if compared, for example, to proteins or nucleic acids) the hydrogen bond interaction is weak due to the strong hydration of sugars and thereby the influence on the chemical shift is small. Since the question of the effect of using low temperature and 15% of $(CD_3)_2CO$ on the conformation can arise, it is important to note that the structural information obtained in the present study is in good agreement with what has been previously reported for D_2O solutions and from HSEA/GSEA calculations.[3–6]

Experimental

Sample preparation

The disaccharides **1**–**16** were available from previous studies.[3–6] The methyl glycosides of monomers were obtained from Sigma. The NMR sample tubes were soaked for a minimum of 1 h in a 50 mM solution of phosphate buffer, pH 7, to minimise adsorption of impurities from glass.[18] All compounds were purified on an Amberlite MB-3 mixed ion-exchange resin before use.

NMR spectroscopy

All NMR experiments were performed on a Bruker DRX-600 spectrometer operating at 600.13 MHz for proton observation. Compounds **1**–**16** were dissolved in a mixture of 85% H_2O–15% $(CD_3)_2CO$ to give a sample concentration of *ca.* 50 mM. The addition of acetone to the samples allowed the sample temperature to be lowered to −15 °C without freezing. The NMR spectra were recorded at −5 or −8 °C except for the temperature coefficients, which were measured by variation of the temperature from −15 to 20 °C in steps of 5 °C. The ^{1}H-NMR spectra were referenced by setting the residual acetone-d_5 signal to $\delta_H = 2.204$. The WATERGATE scheme[19] was used for water suppression in both one and two-dimensional ^{1}H-NMR experiments. The 2-D NMR spectra (DQF-COSY, TOCSY, NOESY and ROESY) were recorded in the phase-sensitive mode using the TPPI method.[20] The rates of exchange of the hydroxy protons with water were calculated from 2-D phase-sensitive chemical exchange experiments.[21] Mixing times of 3 to 24 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).

MM2 calculations

Chem3D plus version 3.5 for Macintosh was used. Minimization was performed with the "MM2" force field. The default convergence criterion was used (rms [root mean square] force 0.1 kcal mol^{-1} Å^{-1}). The starting structures were the published minimum energy conformations calculated using the HSEA and GSEA methods.

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