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Comparative ^{1}H NMR study of hydroxy protons in galabioside and its *S*-linked 4-thiodisaccharide analogue in aqueous solution

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Abstract

The NMR data obtained from hydroxy protons have been used to investigate the presence and absence of intramolecular hydrogen bonding in aqueous solutions of 2-(trimethylsilyl)ethyl galabioside (α-D-Gal*p*-(1→4)-β-D-Gal*p*-O$(CH_2)_2SiMe_3$) and the *S*-linked 4-thiodisaccharide analogue. The data show that there is a weak hydrogen bond interaction between O-6H and O-2′H in galabioside, but not in the thio-analogue. The results are in good agreement with those reported for the substances in a Me_2SO-d_6 solution. It is also shown that the existence of a hydrogen bond can be quite easily monitored by comparing the NMR data of the hydroxy protons. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Conformation analysis; NMR; Galabioside; Thioglycoside; Hydrogen bonds; Hydroxy protons

1. Introduction

In previous studies, it was shown that 2-(trimethylsilyl)ethyl galabioside (α-D-Gal*p*-(1→4)-β-D-Gal*p*-O$(CH_2)_2SiMe_3$) (**1**) has, in Me_2SO-d_6 solution, a hydrogen bond between O-2′H and O-6H [1–3]. In the *S*-linked 4-thiodisaccharide analogue **2**, this hydrogen bond does not exist [3]. Since the analogue is also a much less efficient inhibitor of the bacterial pilus adhesin $PapG_{J96}$, it has been suggested [3] that this hydrogen bond is necessary for strong binding between galabioside and the adhesin.

The presence and absence of hydrogen bonding in **1** and **2** was deduced from NMR data obtained in Me_2SO-d_6 solution and from distance measurements in energy-minimised conformations [3]. Since hydrogen bonds which exist in Me_2SO-d_6 solution do not always persist in aqueous solution [4,5], and since other hydrogen bonds can be formed [1], an intriguing question was whether the O-2′H–O-6H hydrogen bond exists in water. The most direct way of investigating hydrogen bonding is to observe the hydroxy protons by NMR spectroscopy [4–18]. Hydroxy protons involved in hydrogen bonding are expected to have relatively smaller temperature coefficients, a slower rate of exchange with water, and coupling constants which do not represent conformational averaging but instead indicate a restricted rotation around the H–C–O–H bond. Other factors may influence these parameters, but if for any of the hydroxy

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protons, a value differs from that of the other OH protons, it could be an indication of involvement in a hydrogen bond. For this reason, we have measured the ^{1}H NMR chemical shifts, temperature coefficients, coupling constants, exchange rates with the solvent and NOEs for the hydroxy protons in **1** and **2**. We expected that if O-2′H and O-6H are hydrogen bonded in **1**, they should have at least one NMR parameter that differs from that of the other hydroxy protons. The lack of hydrogen bonding in **2** should be directly reflected by similar NMR data for all hydroxy protons.

2. Results and discussion

Assignment of hydroxy proton resonances.—The hydroxy proton resonances in **1** and **2** were assigned on the basis of scalar connectivities to the ring protons from DQF-COSY and TOCSY spectra recorded at −5 °C. The chemical shifts, chemical shift differences $\Delta\delta$ (chemical shifts of the hydroxy proton signals in the disaccharide minus that in the corresponding monosaccharide), temperature coefficients, coupling constants and exchange rates for the hydroxy protons in **1** and **2** are reported in Table 1. The OH signals for **1** and **2** are shown in Fig. 1. The chemical shifts and coupling constants for the hydroxy protons in the monosaccharide methyl glycosides α-D-Gal*p*-OMe (**3**) and β-D-Gal*p*-OMe (**4**) have been reported previously [17] and are given in Table 2 for comparison. To take into account the effect of a sulfur atom on the chemical shifts and coupling constants of neighbouring hydroxy protons in **2**, we have also measured these parameters in the corresponding monosaccharides **5** and **6**, in which the O-1 or O-4 atom, respectively, is replaced by a sulfur atom (Scheme 1, Table 2).

Effect of sulfur substitution on chemical shifts and coupling constants of neighbouring hydroxy protons.—A comparison of NMR data for α-D-Gal*p*-OMe (**3**) and α-D-Gal*p*-S$(CH_2)_2SiMe_3$ (**5**) (Table 2) shows that the chemical shifts and coupling constants of the O-3H, O-4H and O-6H signals are not affected by the substitution with a sulfur at the C-1 position. Only the chemical shift of O-2H is affected, and substitution by a sulfur atom leads to the downfield shift of the O-2H signal from 6.123 ppm in **3** to 6.301 ppm in **5**. A comparison of data for β-D-Gal*p*-OMe (**4**) and 4-thio-β-D-Gal*p*-O$(CH_2)_2SiMe_3$ (**6**) (Table 2) shows that the chemical shifts and coupling constants of the O-2H and O-6H signals are not affected by substitution at the C-4 position. The O-3H-proton on the other hand is deshielded by 0.457 ppm in **6** in comparison with **4**. The C-3H proton is also deshielded by 0.38 ppm, while the C-4H proton is shielded by 0.31 ppm (data not shown).

The chemical shifts of the hydroxy proton signals in **1** are similar to those in the monosaccharides **3** and **4**. Also, the chemical shifts of the hydroxy proton signals in **2** are

Table 1
^{1}H NMR chemical shifts (δ), chemical shift differences ($\Delta\delta$), $^3J_{HO,CH}$ coupling constants (J), temperature coefficients ($d\delta/dT$), and exchange rates (k_{ex}) measured at −5 °C in 85% H_2O–15% $(CD_3)_2CO$, for the hydroxy protons of disaccharides **1** and **2**

	1					**2**				
	δ (ppm)	$\Delta\delta$ [a] (ppm)	J (Hz)	$d\delta/dT$ (ppb/deg)	k_{ex} (s^{-1})	δ (ppm)	$\Delta\delta$ (ppm)	J (Hz)	$d\delta/dT$ (ppb/deg)	k_{ex} (s^{-1})
O-2′H	6.205	0.082	9.1	−12.3	11	6.501	0.200	5.3	−14.2	34
O-3′H	6.039	0.094	5.7	−12.7	19	6.049	0.104	5.7	−12.2	31 [b]
O-4′H	5.910	0.002	5.3	−13.4	13	5.997	0.044	5.5	−14.7	20
O-6′H	5.796	−0.199	5.8	−12.5	40	5.880	−0.073	5.6	−14.3	>100 [c]
O-2H	6.309	−0.168	4.9	−13.7	14	6.398	−0.046	4.4	−14.2	20
O-3H	5.765	−0.307	5.1	−12.6	10	5.858	−0.671	6.1	−13.6	13
O-6H	6.078	0.006	5.3	−14.0	17	6.049	−0.048	6.0	−14.6	31 [b]

[a] Chemical shifts of the hydroxy proton signal in the disaccharide minus that of the corresponding monosaccharide **3**–**6**. A positive difference indicates a downfield shift.
[b] Overlap.
[c] The exchange process was very rapid as seen from the disappearance of the diagonal peaks at mixing times >12 ms.

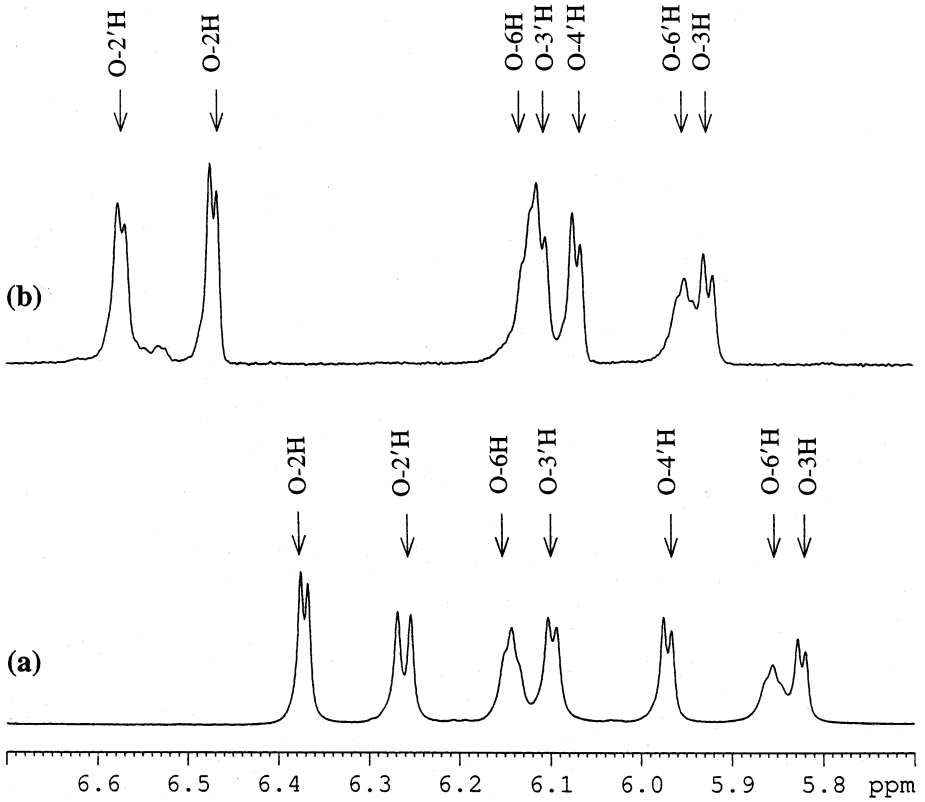


Fig. 1. One-dimensional ^{1}H NMR spectra of hydroxy protons of (a) **1** and (b) **2**, obtained at -10 °C in 85% H_2O–15% $(CD_3)_2CO$ (because of a better quality of NMR spectra, the OH signals are shown at -10 °C instead of -5 °C).

Table 2
^{1}H NMR chemical shifts (δ) and $^3J_{HO,CH}$ coupling constants (J), measured at -5 °C in 85% H_2O–15% $(CD_3)_2CO$, for the hydroxy protons of monosaccharides **3**–**6**

	3		**4**		**5**		**6**	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
O-2H	6.123	4.8	6.477	4.0	6.301	4.8	6.444	4.4
O-3H	5.945	5.1	6.072	5.6	5.945	5.8	6.529	4.9
O-4H	5.909	5.7	5.852	5.6	5.953	5.5	[a]	[a]
O-6H	5.995	5.0	6.072	5.0	5.953	5.1	6.097	5.2

[a] The NMR signal of the SH proton was not observed.

similar to those in the monosaccharides **5** and **6**. Thus, the differences in chemical shifts observed between the OH protons in **1** and **2** are mainly due to the substitution by sulfur. An exception is found for the O-3H signal in **2**, where the large $\Delta\delta$ of -0.671 ppm suggests conformational effects.

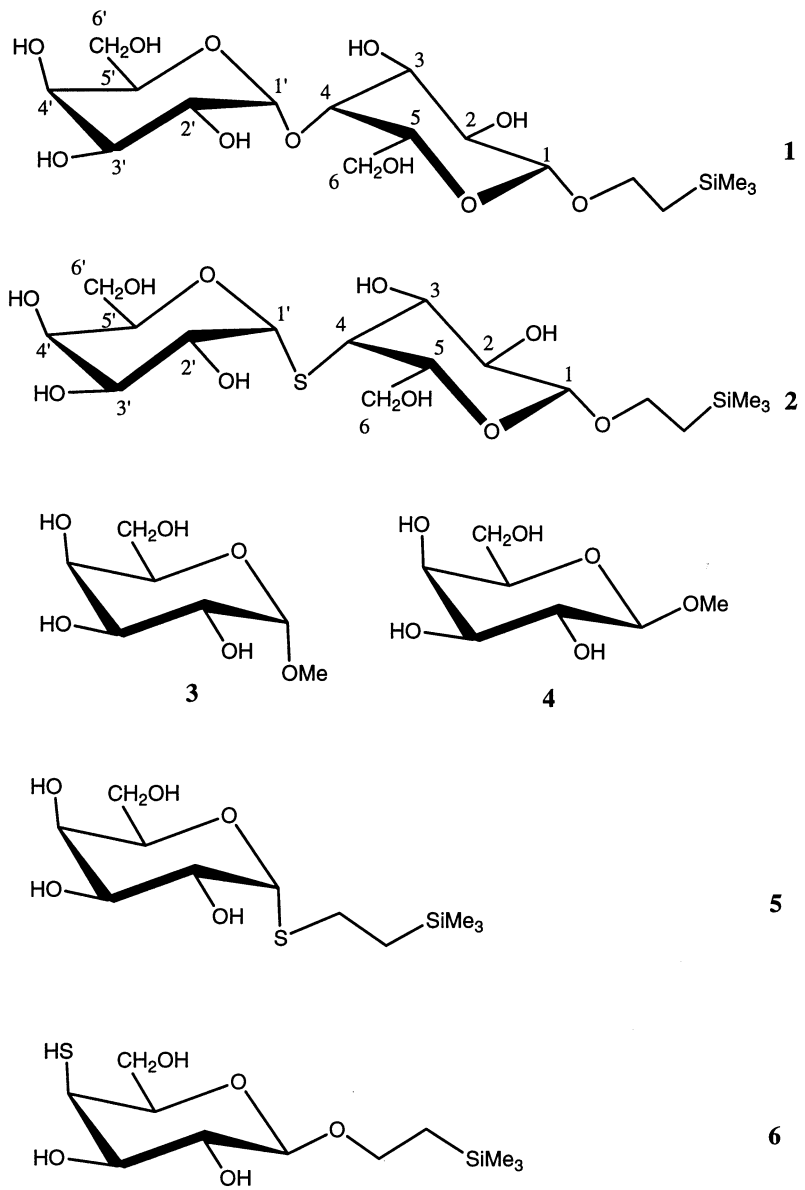
*Chemical shifts and temperature coefficients of hydroxy protons in **1** and **2**.*—Most of the hydroxy proton signals in **1** and **2** have chemical shifts similar to those in the corresponding monosaccharide glycosides ($\Delta\delta \leq |0.20|$ ppm). Exception is found for O-3H, which is shielded by 0.307 and 0.671 ppm in disaccharides **1** and **2** in comparison with monosaccharides **4** and **6**, respectively. It should also be noted that in **1** and **2**, the C-5′H signal is strongly deshielded due to its van der Waals contact with O-3, which requires that the C-5′H–O-3 distance is less than 3 Å [3].

The chemical shifts of hydroxy proton signals are subject to several effects that are difficult to predict. In previous studies on branched trisaccharides [17,18], it was shown

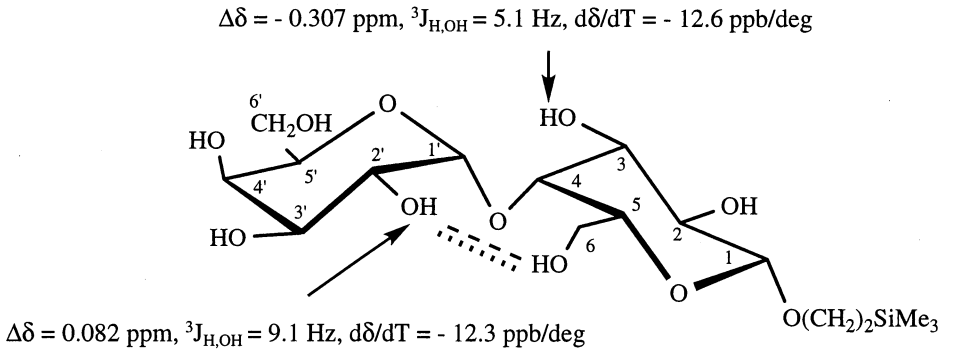
that the chemical shifts of hydroxy proton signals could be correlated to the proximity of the hydroxy protons to other oxygen atoms. Thus, upfield shifts were observed for signals of hydroxy protons close to non-protonated oxygens with the largest shifts observed for hydroxy protons close to O-5 oxygens. Distance measurements on the energy-minimised conformations reported previously [3] showed a short distance between O-3H and O-5′ in both **1** and **2**. In accordance with previous studies [17,18], we anticipate that this spatial proximity might lead to the upfield shift observed for the O-3H signal in both **1** and **2**.

The hydroxy protons for a series of disaccharides are currently under investigation [19]. One of them, β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe (**7**), has a spatial arrangement around the glycosidic bond which is similar [20] to that of **1**. The NMR data, including temperature coefficients, vicinal coupling constants and exchange rates, have shown that in **7**, O-3H is shielded by 0.64 ppm and that there is a strong hydrogen bond between O-3H and O-5′ [19] (Fig. 2).

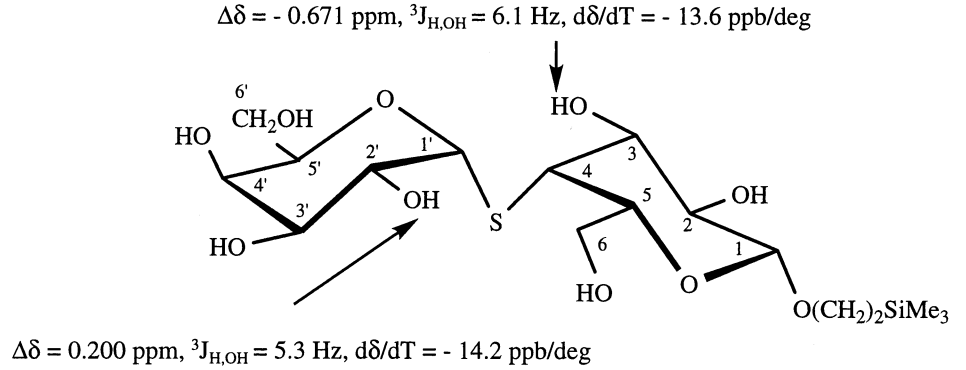
Since hydrogen bonded protons are expected to be deshielded, the measured chemical shift for O-3H must be a balance between



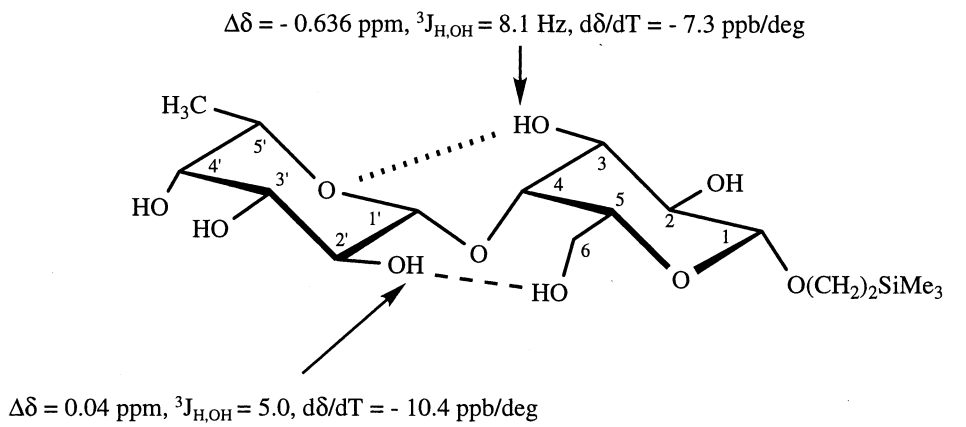
Scheme 1. Structures of disaccharides **1** and **2** and monosaccharides **3**–**6**.



1



2



7

Fig. 2. Schematic representation of disaccharides **1**, **2** and **7** (β-L-Fuc*p*-(1→4)-α-D-Gal*p*-OMe), showing with dotted lines the inter-residue hydrogen bonds and with dashed lines the inter-residue NOEs. The data for **7** are taken from Ref. [19].

the deshielding effect of polarization of the O–H bond, and the shielding effect of near electron clouds. Thus, the larger upfield shift observed for the O-3H signal in **7** in comparison with that in **1** might be due to different hydrogen bond configurations. The larger upfield shift experienced by O-3H in **2** relative to **1** (Fig. 2) could also be due to a closer contact and a different orientation of O-3H relative to O-5′. Ab initio calculations are now

in progress to determine the possible origin(s) of the upfield shifts measured for some hydroxy protons.

The temperature coefficients calculated for the hydroxy protons in **1** and **2** are in the range −12.5 to −14.0 ppb/deg in **1**, and −12.2 to −14.7 ppb/deg in **2** (Table 1). These values are much larger than the temperature coefficients reported for hydroxy protons involved in strong hydrogen bonding in aqueous solution [18,19]. All hydroxy protons also have similar temperature coefficients in both **1** and **2**, with the exception of O-2′H and O-6H, which have slightly higher $d\delta/dT$ values in **2** than in **1** (−14.2 and −12.3 ppb/deg, respectively, for O-2′H, −14.3 and −12.5 ppb/deg, respectively, for O-6H).

Vicinal coupling constants for hydroxy protons ($^3J_{HO,CH}$).—According to the Karplus equation derived for hydroxy protons [21], 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 angle could be reflected in a deviation of the coupling constant for that hydroxy proton from the rotationally averaged value. $^3J_{HO,CH}$ values around 5.5 ± 1 Hz are measured for most of the hydroxy protons, implying a free rotation around the C–O axis. Exception is found in **1** for O-2′H, which has a $^3J_{HO,CH}$ value of 9.1 Hz. This large value indicates a relatively restricted rotation with a preference for the trans orientation. In **2**, the $^3J_{HO,CH}$ value of O-2′H is 5.3 Hz (4.8 Hz in the monosaccharide **5**), suggesting a more free rotation of the hydroxy group around the C-2′–O-2′ axis.

Rate of exchange with water.—Hydroxy protons which are to some degree protected from contact with the solvent should have slower exchange rates. Since exchange rates are very sensitive to pH, solvent composition and to catalysis by small traces of impurities, a comparison of k_{ex} should only usually be carried out for hydroxy protons within one compound. Table 1 and Fig. 3 show that in **1**, O-2′H and O-3H have the lowest exchange rate, while O-6′H is exchanging notably faster than the other hydroxy protons. The reason for this is that it is a primary hydroxy group, more accessible to solvent. O-6H, on the other hand, which is also a primary hydroxy group, has a k_{ex} of the same order of magnitude as the secondary hydroxy protons. In **2**, O-2′H has an exchange rate similar to that of the other secondary hydroxy protons, while O-3H still has a relatively smaller k_{ex}. The rate of exchange of O-6H and O-3′H could not be measured due to spectral overlap at all temperatures investigated, and the rate of exchange of O-6′H with water was too fast at −5 °C (>100 s^{-1}) to be accurately measured. The differences between exchange rates are too small to allow strong conclusions, but the slightly smaller k_{ex} measured for O-2′H, O-3H and O-6H in **1** might indicate that they are protected from exchange with water, maybe through hydrogen bond interactions. In the thioglycoside **2**, O-2′H has become more accessible for exchange with the solvent, while O-3H is still protected. It should be noted that while the $^3J_{HO,CH}$ value of 6.1 Hz for O-3H might indicate conformational averaging, it is also in agreement with a C-3H–C-3–O-3–O-3H torsion angle which allows hydrogen bonding between O-5′H and O-3H. It is also possible that the lower k_{ex} measured for O-3H is caused by a limited accessibility of the water [5], and not by hydrogen bonding.

NOEs and chemical exchange.—Several cross-peaks involving the hydroxy protons were observed in the NOESY spectra of disaccharides **1** and **2** (Table 3). ROESY spectra were also recorded to discriminate between cross-peaks due to dipolar relaxation and chemical exchange. From Table 3, it can be seen that in **1** there is a chemical exchange cross-peak between O-2′H and O-6H. This cross-peak does not exist in the spectrum of **2**. Exchange cross-peaks can be diagnostic of spatial proximity and also of hydrogen bond interaction [22,23], but the presence of hydrogen bonds should be confirmed by other techniques. Thus, in **1**, the large $^3J_{HO,CH}$ value measured for O-2′H, together with its slightly lower k_{ex}, support the involvement of this hydroxy group in a hydrogen bond interaction. The lower k_{ex} of O-6H in comparison with k_{ex} of O-6′H together with the O-6H–C-1′H and O-6H–C-2′H NOEs (Table 3) indicate the involvement of O-6H in hydrogen bonding in **1** and might suggest an O6-H–O-2′ hydrogen bond direction. It is also of interest to note that in **1** there is a chemical exchange

cross-peak between O-2H and O-3H. This cross-peak is weaker in **2**. The O-2H–O-3H interaction might be stabilized by the interaction between O-3H and O-5′ and between O-2′H and O-6H. For example, ^{1}H NMR isotope shifts measured [24] for methyl maltoside in Me_2SO-d_6 solution have revealed the presence of a O-2′H–O-3′H hydrogen bonding stabilized by a stronger O-2′H–O-3H interresidue hydrogen bond.

The NOEs involving O-6H allow the determination of the favoured conformation around the C5–C6 bond. In **1**, NOEs are found from O-6H to C-1′H and C-4H. The NOE between O-6H and C-1′H indicates that both the gauche–gauche (gg) and trans–gauche (tg) conformations are possible, but not the gauche–trans (gt) conformation. The O-6H–C-4H NOE and O-6H–O-2′H chemical exchange show that the gg conformation is

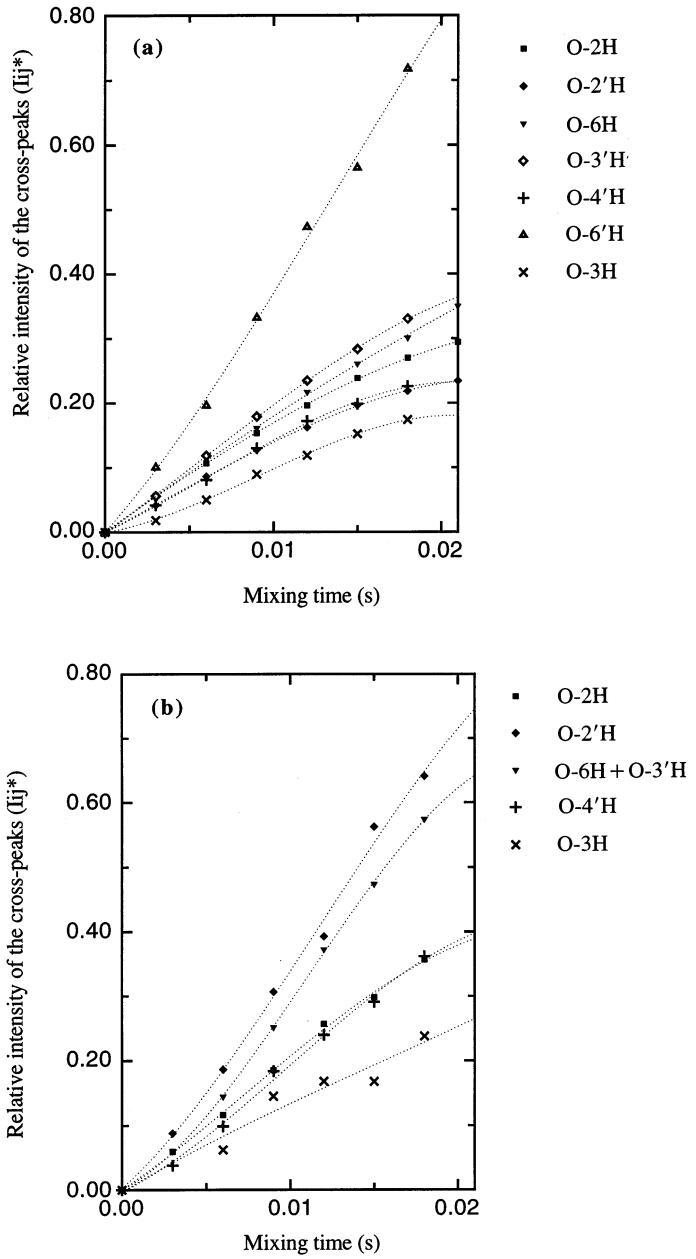


Fig. 3. Build-up curves for the chemical exchange between hydroxy protons and water in (a) disaccharide **1** and (b) disaccharide **2**.

Table 3
NOE [a] and chemical exchange [b] connectivities involving hydroxy protons observed in the NOESY spectra (−5 °C, 85% H_2O–15% $(CD_3)_2CO$, mixing time 100 ms) of **1** and **2**

1		**2**	
O-2′H–O-6H	strong		
O-2′H–C-1′H	medium	O-2′H–C-1′H	weak
O-2′H–C-2′H	strong	O-2′H–C-2′H	strong
O-2′H–C-3′H	strong	O2′-H–C-3′H	medium
O-3′H–C-2′H	strong	O-3′H–C-2′H	strong
O-3′H–C-3′H	strong	O-3′H–C-3′H	strong
O-3′H–C-4′H	strong	O-3′H–C-4′H	medium
O-4′H–C-2′H	medium	O-4′H–C-2′H	medium
O-4′-H–C-3′H	weak		
O-4′H–C-4′H	strong	O-4′H–C-4′H	strong
O-4′H–C-6′H	medium	O-4′H–C-6′H	medium
O-6′H–C-4′H	medium		
O-6′H–C-5′H	medium		
O-6′H–C-6′H	strong	O-6′H–C-6′H	strong
O-2H–O-3H	medium	O-2H–O-3H	weak
O-2H–C-1H	medium	O-2H–C-1H	medium
O-2H–C-2H	strong	O-2H–C-2H	strong
O-2H–C-3H	strong	O-2H–C-3H	weak
O-3H–C-2H	medium	O-3H–C-2H	medium
O-3H–C-3H	strong	O-3H–C-3H	strong
O-3H–C-4H	strong	O-3H–C-4H	strong
O-3H–C-1′H	medium	O-3H–C-1′H	weak
O-3H–C-6′H	strong	O-3H–C-6′H	medium
O-3H–C-5′H	strong	O-3H–C-5′H	strong
O-6H–C-4H	weak		
O-6H–C-5H	weak	O-6H–C-5H	weak
O-6H–C-6H	strong	O-6H–C-6H	strong
O-6H–C-1′H	medium	O-6H–C-1′H	weak
O-6H–C-2′H	weak		

[a] Between hydroxy and ring protons.
[b] Between hydroxy protons.

preferred. In the tg orientation, O-2′H and O-6H are too far away to give an exchange cross-peak. The NOEs between O-6H and C-1′H and between O-6H and C-4H, together with the chemical exchange between O-6H and O-2′H, indicate a preference for the gg conformation. Thus, the gg conformation, which is usually predicted to be the least populated in galactopyranosides, can be stabilised through an inter-residue hydrogen bond. It should be noted that the values of the $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ ($\approx 5-7$ Hz) measured in water and in Me_2SO-d_6 [3] suggest that all three conformations are possible. These values of *J*-coupling constants together with the values of the temperature coefficients of O-2′H and O-6H indicate that the O-6H–O-2′ hydrogen bond and the gg conformation around the C5–C6 bond occur transiently. In **2**, no O-2′H–O-6H and O-6H–C-4H cross-peaks were observed, and the O-6H–C-1′H cross-peak was very weak. The O-3H–C-5′H NOE observed in the NOESY spectra of both **1** and **2** goes hand in hand with the chemical shift measured [3] for C-5′H in **1** (4.36 ppm) and **2** (4.41 ppm). This deshielding of C-5′H is due to its van der Waals contact with O-3 [3], and it requires that the C-5′H–O-3 distance is less than 3 Å.

All these data are in good agreement with previous results [3] obtained from molecular mechanics calculations. From these data, it was shown that only the gg conformation in **1** combines the required short distance (<2.8 Å) between C-5′H and O-3 with a distance between O-2′ and O-6 that allows hydrogen bonding (<3 Å). On the other hand, no conformations of **2** could display both O-2′–O-6 and C-5′H–O-3 distances of less than 3 Å. Comparison of the gg conformation in **1** with the gg conformation in **2**, and the tg in **1** with tg in **2**, showed that in the conformations that display a reasonable C-5′H–O-3 distance (as required by the ^{1}H NMR data), the O-2′–O-6 distance in **2** is more than 0.8 Å larger than in **1**. This indicates that an O-2′–O-6H hydrogen bond could only be formed in the natural galabiosides and not in the thio analogue.

3. Conclusions

It has been suggested that while the temperature coefficients of hydroxy protons may reveal relatively strong intramolecular hydrogen bonds, chemical exchange may be an important parameter in probing weaker, transient hydrogen bonds in aqueous solution. Thus, the chemical exchange between O-6H and O-2′H, together with the large $^3J_{HO,CH}$ value (9.1 Hz) measured for O-2′H, suggest the existence of a weak hydrogen bond interaction between O-6H and O-2′H in galabioside **1**. In the *S*-linked 4-thiodisaccharide analogue **2**, the $^3J_{HO,CH}$ value of 5.3 Hz measured for O-2′H and the absence of an O-2′H–O-6H chemical exchange cross-peak suggest that there is no hydrogen bond interaction between O-2′H and O-6H. These data are in good agreement with those obtained from NMR in Me_2SO-d_6 solution, and from

computational methods. This investigation also shows that the lack of hydrogen bonding can be quite easily monitored by comparing the NMR data obtained for the hydroxy protons in the different compounds.

4. Experimental

General procedure.—The synthesis of disaccharides **1** and **2** has been described [3]. The ^{1}H NMR spectra of compounds **9** and **10** were recorded in $CDCl_3$ solution with a Bruker DRX 400 spectrometer. Tetramethylsilane was used as an internal standard and the chemical shifts are reported in ppm (δ scale). Optical rotations were measured with a Perkin–Elmer 241 polarimeter. Reactions were monitored by TLC on Silica Gel FG_{254} (E. Merck, Darmstadt, Germany).

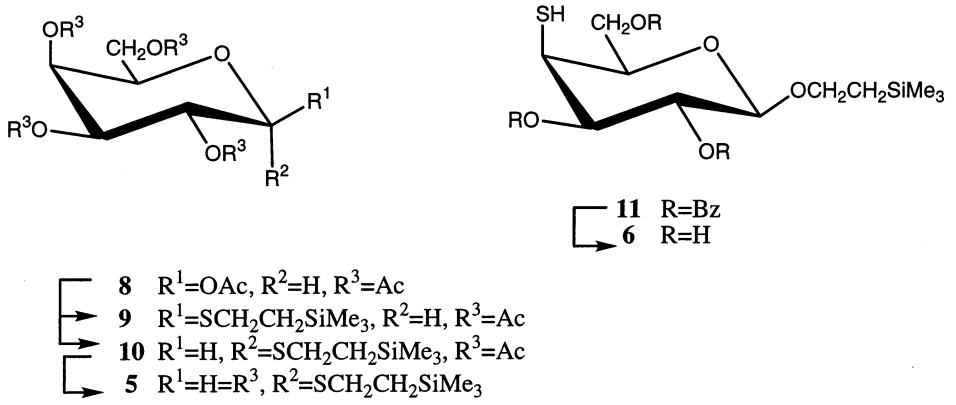
Synthesis of compounds **5**, **6** *and* **8**–**11**

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (**9**) *and 2-(trimethylsilyl)ethyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-galactopyranoside* (**10**). To β-D-galactopyranose pentaacetate (**8**) (741.5 mg, 1.90 mmol) in dry CH_2Cl_2 (5.7 mL) under argon, 2-(trimethylsilyl)ethanethiol (282 mL, 3.80 mmol) and $BF_3{\cdot}Et_2O$ (286 mL, 2.27 mmol) were added. Saturated aq $NaHCO_3$ and CH_2Cl_2 were added after 80 min, the CH_2Cl_2 was dried (Na_2SO_4) and then concentrated. Column chromatography (SiO_2, 4:1 heptane–EtOAc) gave **10** (71.3 mg, 8%), followed by **9** (581.9 mg, 66%). Compound **9** had $[\alpha]_D^{25}$ −18° (*c* 0.65, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.43 (dd, 1 H, J_{34} 3.2 Hz, J_{45} 1.1 Hz, H-4), 5.23 (dd, 1 H, J_{12} 10.0 Hz, J_{23} 10.9 Hz, H-2), 5.04 (dd, 1 H, H-3), 4.50 (d, 1 H, H-1), 4.16 (dd, 1 H, J_{56a} 6.9 Hz, J_{6a6b} 11.3 Hz, H-6a), 4.10 (dd, 1 H, J_{56b} 6.5 Hz, H-6b), 3.92 (m, 1 H, H-5), 2.74 (t, 2 H, *J* 8.8 Hz, SCH_2–), 2.16, 2.07, 2.06, 1.98 (4 s, 3 H each, –OAc), 0.86 (t, 2 H, –CH_2Si–), 0.00 (s, 9 H, –$SiMe_3$). Anal. Calcd for $C_{19}H_{32}O_9SSi$: C; 49.1, H; 6.9. Found: C; 49.0, H; 6.8. Compound **10** had $[\alpha]_D^{25}$ +168° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.73 (d, 1 H, J_{12} 5.4 Hz, H-1), 5.42 (dd, 1 H, J_{34} 3.2 Hz, J_{45} 1.1 Hz, H-4), 5.25 (dd, 1 H, J_{23} 10.9 Hz, H-2), 5.19 (dd, 1 H, H-3), 4.55 (m, 1 H, H-5), 4.10 (dd, 1 H, J_{56a} 5.8 Hz, J_{6a6b} 11.2 Hz, H-6a), 4.06 (dd, 1 H, J_{56b} 7.0 Hz, H-6b), 2.54 (t, 2 H, *J* 8.8 Hz, –SCH_2–), 2.12, 2.05, 2.02, 1.96 (4 s, 3 H each, –OAc), 0.85 (t, 2 H, –CH_2Si–), 0.00 (s, 9 H, –$SiMe_3$). HRMS Calcd for $C_{19}H_{32}O_9SSiNa$ [M + Na]: 487.1434. Found; 487.1432.

2-(Trimethylsilyl)ethyl 1-thio-α-D-galactopyranoside (**5**). To compound **10** (33.2 mg, 71 mmol) in MeOH (3 mL) under argon, 1 M NaOMe in MeOH (30 mL) was added. The mixture was neutralized with Duolite C436 (H^+) after 18 h, filtered and concentrated. Column chromatography (SiO_2, 9:1 CH_2Cl_2–MeOH) gave **5** (15.6 mg, 74%), $[\alpha]_D^{25}$ +121° (*c* 0.13, MeOH). ^{1}H NMR (85% H_2O–15% $(CD_3)_2CO$): δ 5.52 (d, 1 H, J_{12} 5.9 Hz, H-1), 4.28 (ddd, 1 H, J_{45} 1.2 Hz, J_{56a} 5.14 Hz, J_{56b} 7.7 Hz, H-5), 4.11 (dd, 1 H, J_{23} 10.3 Hz, H-2), 3.97 (dd, 1 H, J_{34} 3.5 Hz, H-4), 3.75 (m, 2 H, H-6), 3.73 (dd, 1 H, H-3), 2.68 (m, 2 H, –SCH_2–), 0.97 (m, 1 H, –CH_2Si), 0.84 (m, 1 H, –CH_2Si–), 0.02 (s, 9 H, –$SiMe_3$). HRMS Calcd for $C_{11}H_{24}O_5SSiNa$ (M + Na): 319.1011. Found; 319.1004.

2-(Trimethylsilyl)ethyl 4-thio-β-D-galactopyranoside (**6**). To 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-thio-β-D-galactopyranoside (**11**) [3] (11.4 mg, 19 mmol) in MeOH (1 mL) under argon, 1 M NaOMe in MeOH (10 mL) was added. $NaBH_4$ (10 mg) was added after 19 h to reduce traces of disulfide formed. The mixture was neutralized with Duolite C436 (H^+) after 20 h, filtered and concentrated. The residue was dissolved in water (2 mL) and applied onto a Waters SepPac Plus C18 cartridge. The cartridge was washed with water (5 mL) followed by elution with 60% MeOH (5 mL) to give **11** (5.0 mg, 83%), $[\alpha]_D^{25}$ +49° (*c* 0.4, MeOH). ^{1}H NMR (85% H_2O–15% $(CD_3)_2CO$): δ 4.39 (d, 1 H, J_{12} 7.9 Hz, H-1), 4.03 (m, 1 H, –OCH_2CH_2–), 3.97 (dd, 1 H, J_{23} 9.9 Hz, J_{34} 4.2 Hz, H-3), 3.95 (m, 2 H, H-6), 3.93 (m, 1 H, H-5), 3.72 (m, 1 H, –OCH_2CH_2–), 3.49 (d, 1 H, H-4), 3.29 (dd, 1 H, H-2), 1.06 (m, 1 H, –CH_2Si–), 0.95 (m, 1 H, –CH_2Si–), 0.02 (s, 9 H, –$SiMe_3$). HRMS Calcd for $C_{11}H_{24}O_5SSiNa$ [M + Na]: 319.1011. Found; 319.0996 (Scheme 2).

Sample preparation for NMR studies.—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 impu-



Scheme 2. Structures of monosaccharides **8**–**11** involved in the synthesis of **5** and **6**.

rities from glass [4]. The compounds were purified on an Amberlite Mβ-3 mixed ion-exchange resin prior to NMR experiments.

NMR spectroscopy.—The NMR experiments on samples in H_2O solutions were performed on a Bruker DRX 600 spectrometer operating at 600.13 MHz for proton observation. Compounds **1**–**6** were dissolved in a mixture of 85% H_2O–15% $(CD_3)_2CO$ to give a sample concentration of ca. 100 mM. The addition of acetone to the samples allowed lowering of the sample temperature to −15 °C without freezing. All spectra unless specified were recorded at −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 $\delta_H = 2.204$ ppm. One- and two-dimensional ^{1}H NMR spectra were acquired using the WATERGATE pulse sequence [25] for water suppression. The 2D NMR spectra were recorded in the phase-sensitive mode using the TPPI method [26]. NOESY [27] and ROESY [28] spectra were recorded with mixing times (t_m) of 50 and 100 ms with 256 spectra of 2K data points. For each FID, 16 scans were recorded using a repetition delay of 2 s. The data were zero-filled to 2 × 1K before applying a π/2 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 [29]. Mixing times of 3–18 ms in steps of 3 ms were used. A total of 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 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** and **2** were visualised using the Chem3D plus version 3.5 for Macintosh. The starting structures were the published [3] minimum-energy conformations calculated using MM3(92).

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