# Intra- and Intermolecular H-Bonds of Alcohols in DMSO

## $^1$H-NMR Analysis of Inter-Residue H-Bonds in Selected Oligosaccharides: Cellobiose, Lactose, *N,N′*-Diacetylchitobiose, Maltose, Sucrose, Agarose, and Hyaluronates

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Dedicated to Professor *Albert Eschenmoser* on the occasion of his 75th birthday

Inter-residue H-bonds of oligosaccharides in ($D_6$)DMSO have been assigned on the basis of a combined interpretation of the chemical shift ($\delta$(OH)), coupling constant ($J$(H,OH)), and temperature dependence ($\Delta\delta(\mathrm{OH})/\Delta T$) of OH signals. Cellobiose, lactose, and *N,N′*-diacetylchitobiose possess a completely persistent C(3)OH ⋯ OC(5′) H-bond. Maltose is characterised by flip-flop H-bonds between HO−C(3) and HO−C(2′), and agarose by two weakly persistent inter-residue H-bonds. Sucrose forms an equilibrium of differently H-bonded species, and hyaluronates possess four strong inter-residue H-bonds.

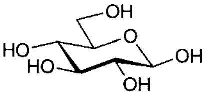
**Introduction.** – Our analysis of the $^1$H-NMR spectra of ($D_6$)DMSO solutions of selected monosaccharides, inositols, and ginkgolides has shown that an OH group involved in an intramolecular H-bond to an OR group (R = H, alkyl, or alkoxyalkyl) resonates at higher field than a similarly located OH group involved in an intermolecular H-bond to ($D_6$)DMSO [1]. Intramolecular H-bonds were conveniently detected by a combined analysis of $J$(H,OH) and $\delta$(OH) values. NOESY and SIMPLE $^1$H-NMR spectra and additional parameters such as temperature dependence and H/D exchange rate were helpful, but were required only in difficult cases. In DMSO, fully solvated equatorial OH groups are characterised by $J$(H,OH) values of 4.5 – 5.5 Hz and fully solvated axial OH groups by $J$(H,OH) values of 4.2 – 4.4 Hz. Values outside of this range indicate that the corresponding OH group acts as H-donor in an intramolecular H-bond. Formation and cleavage of H-bonds, even of strong ones, is fast in DMSO, so that $J$(H,OH) and $\delta$(OH) values reflect the conformational equilibria between differently H-bonded species.

$\delta$(OH) Values depend on constitutional, configurational, and electronic factors, and on H-bonding. We found that the $\delta$(OH) value for a fully solvated secondary OH group of monosaccharides in DMSO can be calculated on the basis of a reference value and suitable increments. The $\delta$ value of the fully solvated, equatorial HO−C(2), HO−C(3), and HO−C(4) of $\beta$-D-glucopyranose (**1**; 4.81 ppm [2]) proved a reliable reference that was used in conjunction with the following increments:

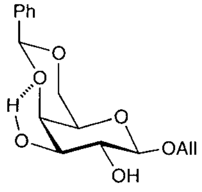
- $-0.3$ ppm for an axial OH group
- $+0.2$ to $+0.25$ ppm for replacing a vicinal OH by on OR group
- *ca.* $+0.1$ ppm for replacing another OH by an OR group
- $+0.2$ ppm for an antiperiplanar C−O bond (not applicable to equatorial OH groups that are completely involved in non-directional intermolecular H-bonds to DMSO, as in **1**)

- +0.8 to +1.3 ppm for two antiperiplanar C–O bonds
- –0.3 ppm if a vicinal OH group is (partially) H-bonded to an OR group, as, *e.g.*, for HO–C(2) of **2**.

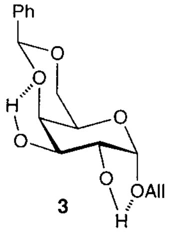
Comparison of the calculated $\delta$(OH) value to the experimental value should reveal whether the OH group in question is involved in an inter- or intramolecular H-bond. Agreement between calculated and experimental value indicates a completely solvated OH group. An upfield shift reveals that the OH group acts as H-donor in a (partial) intramolecular H-bond and a downfield shift that the OH group acts as H-acceptor.



**1**



**2**



**3**

A comparison of $J$(H,OH) and $\delta$(OH) values of oligosaccharides with those of the parent monosaccharides [2][3] should allow detection of inter-residue H-bonds. One expects that OH groups of terminal residues that do not form interresidue H-bonds are also a useful reference for fully solvated OH groups of a given oligosaccharide. We have now investigated the chemical shift of OH groups of cellobiose, maltose, sucrose, and hyaluronates in ($D_6$)DMSO solution.

**Analysis.** – 1. *Inter-Residue H-Bonds in Cellobiose, Lactose, and N,N′-Diacetylchitobiose.* Inter-residue C(3)OH $\cdots$ OC(5′) H-bonds have been observed in the solid state of celluloses [4][5], $\beta$-cellobiose [6–8], the methyl $\beta$-cellobioside · MeOH solvate [9], $\alpha$-lactose monohydrate [10–12], and $\beta$-lactose [13]. The observed dihedral angles H–C(3)–O–H are –95.2 for $\beta$-lactose, –90.0 to –70.5 for $\beta$-cellobiose, –71.1 to –63.3 for $\alpha$-lactose, and –38.7° for the methyl $\beta$-cellobioside · MeOH solvate. According to the equation of *Fraser et al.* [14], these dihedral angles correspond to $J$(3,OH) values of 0.4, 0.2–0.8, 0.8–1.6, and 5.4 Hz, respectively. Hence, for solutions, this inter-residue H-bond should be clearly evidenced by a small $J$(3,OH) value. Indeed, methyl $\beta$-cellobioside (**4**; *Fig. 1* and *Table 1*) in ($D_6$)DMSO shows a small $J$(3,OH) value of $<2$ Hz and medium $J$(H,OH) values ($>4$ Hz) for all other secondary OH groups [15–17]; similar values have been reported for methyl $\beta$-lactoside (**5**) [18]. The HO–C(3) signals show a weak temperature dependence ($\Delta\delta/\Delta T = -2.6$ and $-2.0$ ppb/K) in contrast to the other OH signals ($\Delta\delta/\Delta T = -4.5$ to $-8.0$ ppb/K). These values clearly indicate a completely persistent inter-residue H-bond in ($D_6$)DMSO solution[1]).

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[1]) In aqueous solution, however, this inter-residue H-bond persists only to *ca.* 50%, as evidenced by $J$(3,OH) of 3–3.15 Hz [17][19]. The larger $J$(3,OH) values have led to controversial conclusions. *Poppe* and *van Halbeek* [19] found a slower H/D exchange for HO–C(3) (5.6 $s^{-1}$) of **5** than for the other OH groups (8.3–21.5 $s^{-1}$), and concluded that the inter-residue C(3)OH $\cdots$ O(5′) H-bond persists completely. *Leeflang et al.* [17][20], however, insisted that the inter-residue H-bond of **4** is completely interrupted in aqueous solution, as supported by $\Delta\delta/\Delta T = -11.1$ ppb/K for HO–C(3), in the same range as the $\Delta\delta/\Delta T$ values for the other OH groups of **4** (–10.6 to –12.7 ppb/K). The criterium of the temperature dependence, however, has the disadvantage that the $\Delta\Delta\delta/\Delta T$ value is not known ($\Delta\Delta\delta/\Delta T$ is the difference between the temperature dependence of an OH group that is either intramolecularly or intermolecularly (to $H_2O$) H-bonded).

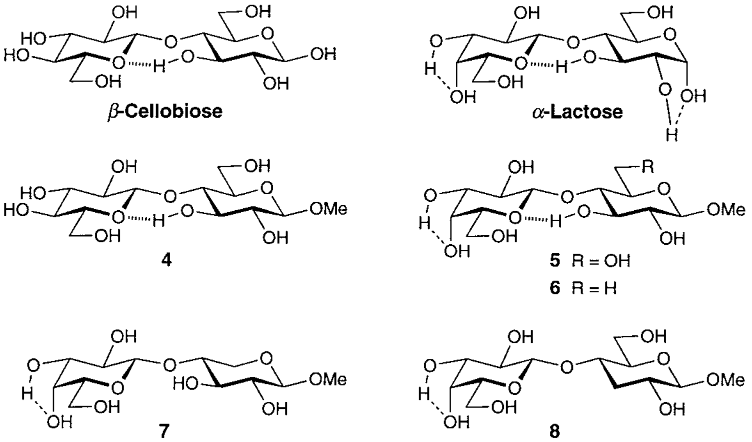

Fig. 1. *Intramolecular H-bonds in β-cellobiose, α-lactose, and their derivatives* **1**–**5** *in ($D_6$)DMSO solution* (hashed bonds indicate persistent and dashed bonds partially persistent intramolecular H-bonds)

Table 1. *Chemical Shifts* [ppm] *and Vicinal Coupling Constants* [Hz] *for OH Groups and for the Anomeric H of α/β-Lactose, the Corresponding Methyl β-Disaccharides* **4** *and* **5**, *and the Lactose Analogues* **6**–**8** *in ($D_6$)DMSO Solution at 27–30°*

| | H–C(1) (*J*(1,2)) | HO–C(1) (*J*(1,OH)) | HO–C(2) (*J*(2,OH)) | HO–C(3) (*J*(3,OH)) | HO–C(6) (*J*(6,OH)) |
|---|---|---|---|---|---|
| α-Cellobiose | 4.89 (3.2) | 6.30 (4.8) | 4.63 (6.8) | 4.465 (2.1) | 4.47 (5.8, 5.8) |
| β-Cellobiose | 4.32 (7.6) | 6.64 (6.4) | 4.95 (4.9) | 4.60 (1.6) | 4.56 (6.5, 5.5) |
| β-Cellobiose/Lactose (calc.)[a] | | 6.64 | 4.91 | 5.06 | 4.55 |
| **4** [17] | 4.12 | – | 5.18 | 4.68 (1.7) | 4.61 |
| α-Lactose [21] | 4.88 | 6.29 | 4.61 | 4.43 (*ca.* 2) | 4.42 |
| β-Lactose [21] | 4.31 | 6.61 | 4.91 | 4.58 (*ca.* 2) | 4.50 |
| **5** [18] | 4.09 (7.8) | – | 5.12 | 4.63 (< 2) | 4.53 |
| **6** [18] | 4.08 (8.1) | – | 5.11 | 4.64 (< 2) | – |
| **7** [18] | 4.03 (7.9) | – | 5.13 | 4.98 (> 4) | – |
| **8** [18] | 4.07 (8.1) | – | 4.96 | – | 4.47 |

| | H–C(1′) *J*(1′,2′)) | HO–C(2′) (*J*(2′,OH)) | HO–C(3′) (*J*(3′,OH)) | HO–C(4′) (*J*(4′,OH)) | HO–C(6′) (*J*(6′,OH)) |
|---|---|---|---|---|---|
| α-Cellobiose | 4.225 (8.0) | 5.215 (4.8) | 4.99 (5.0) | 4.97 (5.5) | 4.58 (5.8, 4.9) |
| β-Cellobiose | 4.25 (7.9) | 5.21 (4.8) | 5.00 (4.9) | 4.97 (5.5) | 4.58 (5.8, 4.9) |
| α/β-Cellobiose (calc.)[a] | | 5.02 | 4.92 | 4.89 | 4.50 |
| **4** [17] | 4.28 | 5.24 | 5.02 | 4.995 | 4.60 |
| α-Lactose [21] | 4.16 | 5.05 | 4.725 | 4.48 | 4.61 |
| β-Lactose [21] | 4.19 | 5.03 | 4.73 | 4.46 | 4.61 |
| α/β-Lactose (calc.)[a] | | 4.85 | 4.62 | 4.30 | 4.53 |
| **5** [18] | 4.19 (7.9) | 5.04 | 4.73 | 4.46 | 4.61 |
| **6** [18] | 4.18 (8.1) | 5.00 | 4.60[b] | 4.44 | 4.60[b] |
| **7** [18] | 4.20 (7.7) | 4.78 | 4.69 | 4.39 | 4.58 |
| **8** [18] | 4.15 (8.0) | 4.86 | 4.64 | 4.32 | 4.51 |

[a] Calculated from the corresponding δ(OH) values of β-D-glucopyranose, methyl β-D-glucopyranoside, and methyl β-D-galactopyranoside [2] using the increments listed in the *Introduction.* [b] Estimated value (overlapping signal).

The $^1$H-NMR spectrum of cellobiose in ($D_6$)DMSO is not known. We recorded a 500-MHz spectrum of $\alpha/\beta$-cellobiose 10 : 1 in ($D_6$)DMSO solution and assigned the OH signals with the help of a DQFCOSY.GRASP spectrum. The experimental data for the OH signals of $\alpha/\beta$-cellobiose, $\alpha/\beta$-lactose [21], their methyl $\beta$-D-glycosides **4** [17] and **5** [18], and the $\beta$-lactoside analogues **6**–**8** [18] are listed in *Table 1* together with the calculated $\delta$(OH) values for $\beta$-cellobiose and $\beta$-lactose. HO–C(2) of $\alpha$-cellobiose and $\alpha$-lactose forms a partial intramolecular H-bond to the vicinal axial HO–C(1) (depicted for $\alpha$-lactose in *Fig. 1*). This intramolecular H-bond is evidenced by $J(2,\mathrm{OH}) = 6.8$ Hz and by the upfield shift for HO–C(2) of 0.30–0.32 ppm, as related to $\delta$(HO–C(2)) of the $\beta$-anomers. Transformation of $\beta$-cellobiose and $\beta$-lactose into the methyl $\beta$-D-glycosides **4** and **5** led to a downfield shift for HO–C(2) (0.21–0.23 ppm), HO–C(3) (0.07–0.08 ppm), and HO–C(6) (0.03–0.05 ppm), as expected for an alkylation of HO–C(1). The experimental $\delta$(OH) values for HO–C(1), HO–C(2), and HO–C(6) of the reducing $\beta$-D-glucopyranosyl unit of $\beta$-cellobiose and $\beta$-lactose agree well with the calculated values ($\Delta\delta^{e/c} \leq 0.05$ ppm)[2]), while the HO–C(3) signals are strongly shifted upfield ($\Delta\delta^{e/c}$ *ca.* 0.6 ppm), evidencing that HO–C(3) is engaged in a completely persistent interresidue H-bond[3]).

A comparison of the nonreducing units of $\alpha/\beta$-cellobiose and $\alpha/\beta$-lactose, respectively, shows almost no shift difference for $\delta$(OH) and for $\delta$(H–C(1′)) ($\Delta\delta \leq$ 0.03 ppm; *Table 1*). However, these experimental $\delta$(OH) values differ from the calculated values by a downfield shift $\Delta\delta^{e/c}$ of 0.08 to 0.2 ppm. This difference between the experimental and the calculated values disappears in the 3-deoxylactoside **8** that of necessity lacks the inter-residue H-bond ($\Delta\delta^{e/c} \leq 0.02$ ppm). Thus, the inter-residue H-bond of $\alpha/\beta$-cellobiose and $\alpha/\beta$-lactose is responsible for the downfield shift for the OH signals of the nonreducing unit. The effect of this inter-residue H-bond is equivalent to a partial protonation of the ring O-atom. This protonation leads to a higher acidity of the OH groups of the nonreducing unit and, hence, to a downfield shift of these OH signals. However, it does not rationalize the observation that the downfield shift is larger for HO–C(2′) than for HO–C(4′) of $\alpha/\beta$-cellobiose ($\Delta\delta^{e/c} = 0.2$ and 0.1 ppm, resp.). Apparently, $\delta$(HO–C(2′)) is influenced by an additional factor, possibly by an interresidue H-bond between HO–C(6) and HO–C(2′). Such a H-bond has been observed in celluloses [4][5]. The slightly stronger downfield shift of HO–C(2′) agrees either with a weakly persistent unidirectional C(6)OH $\cdots$ O(2′) H-bond or a with flip-flop H-bonds. A weakly persistent inter-residue H-bond between HO–C(6) and HO–C(2′) of cellobiosides and lactosides is supported by a comparison of the $\beta$-lactoside **5** with its 6-deoxy analogue **6**[4]) where one notes a small downfield shift of 0.04 ppm for HO–C(2′) of **5** relative to **6**. In contrast to the strong C(3)OH $\cdots$ OC(5′) H-bond in cellobiosides and lactosides, there is only a weakly persistent interresidue C(3)OH $\cdots$ O(5′) H-bond in the xyloside **7**, as evidenced by $J(3,\mathrm{OH}) > 4$ Hz (the exact value is not given) [18]. The weakness of this H-bond is also evidenced by

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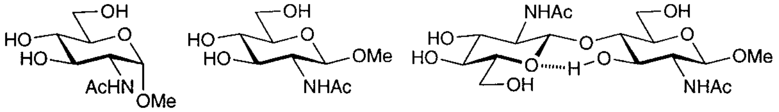
[2]) $\Delta\delta^{e/c}$ represents the difference between the experimental and the calculated chemical shift value.

[3]) It was not possible to rationalize this upfield shift [15][17] on the basis of the assumption that intramolecular H-bonds lead to a downfield shift for the H-donating OH group [22–24].

[4]) The epimeric 6-deoxy-$\beta$-cellobioside is known, but $^1$H-NMR data for its OH groups have not been published [25].

$\Delta\delta$(HO–C(3))/$\Delta T$ of $-7$ ppb/K, by $\delta$(HO–C(3)) of 4.98 ppm, and by small chemical-shift differences for the OH groups of the $\beta$-D-galactopyranosyl residue in **7** and **8**. The absence of a strong inter-residue H-bond is indeed characteristic for xylans in ($D_6$)DMSO [16], and evidences that the Me group of **6** and the $HOCH_2$ group of the cellobiosides and lactosides dramatically enhance the interresidue H-bond.

Chitin and chitin oligomers are insoluble in DMSO, with the exception of the dimeric species [16]. Several conformers obtained by rotation around the C(2)–N bond have been observed for *N*-acetyl-$\alpha$-D- and -$\beta$-D-glucosamine derivatives [26], but they have only a weak influence upon the chemical shift of HO–C(3) of the *N*-acetylglucosaminides **9** and **10** in ($D_6$)DMSO ($\Delta\delta = 0.17$ ppm; *Fig. 2*). The *J*(H,OH) and the small $\Delta\delta^{e/c}$(OH) values for **9** and **10** indicate that HO–C(3) and HO–C(4) are completely solvated. The small *J*(3,OH) value of 0.9 Hz for the *N,N′*-diacetylchitobioside **11** and the weak temperature dependence for HO–C(3) ($-3.0$ ppb/K; compare $-6.8$ ppb/K for HO–C(3′)) evidence a completely persistent C(3)OH···O(5′) interresidue H-bond which is also revealed by a large upfield shift for HO–C(3) ($\delta$(HO–C(3)) = 4.52, $\Delta\delta^{e/c} = 0.6$ ppm).



|  | **9** |  | **10** |  | **11** |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  | HO–C(4) | HO–C(3) | HO–C(4) | HO–C(3) | HO–C(4′) | HO–C(3′) | HO–C(3) |  |
| *J*: | 5.6 | 5.8 | 4.6 | 5.3 | 5.0 | 4.2 | 0.9 | Hz |
| $\delta$: | 4.98 | 4.72 | 4.98 | 4.89 | 4.92 | 4.80 | 4.52 | ppm |
| $\Delta\delta/\Delta$T: |  |  |  |  | –6.9 | –6.8 | –3.0 | ppb/K |

Fig. 2. *Chemical-shift values, temperature coefficients, and coupling constants for OH groups of the methyl glycosides* **9** *and* **10** [26] *derived from GlcNAc and of the* N,N′*-diacetylchitobioside* **11** [16] *in ($D_6$)DMSO solution at 25°* (chemical-shift values for **11** calculated from the given values at 50°) *and assignment of the inter-residue H-bond* (hashed bond)

2. *A Flip-Flop Inter-Residue H-bond in Maltose.* In the solid state, $\alpha$-maltose possesses a C(3)OH···OC(2′) H-bond [27], and $\beta$-maltose monohydrate a C(2′)OH··· OC(3) H-bond [28]; these are retained in the preferred conformations resulting from MM3* calculations (Macromodel V. 6.0 [29]). This raises the question about the preferred direction of the H-bond between HO–C(3) and HO–C(2′) in solution. Anomeric mixtures of maltose in ($D_6$)DMSO solution have been investigated, but the analysis is hampered by signal overlap. Nevertheless, *Casu et al.* had observed a downfield shift for both HO–C(3) and HO–C(2′) and postulated an 'internal H-bond', without specifying which OH acts as H-donor and which one as H-acceptor [22]. *Marchessault* and co-workers determined the temperature dependence for HO–C(3), HO–C(2′), and HO–C(6′) ($-2.8$, $-4.2$, and $-5.8$ ppb/K, resp., as deduced from the bivariate plot in [30]) and concluded that HO–C(3) is the H-donor. Finally, *Christofides* and *Davies* [31] analysed a 1:2 $\alpha/\beta$-maltose mixture by SIMPLE $^1$H-NMR spectroscopy. They postulated a co-operative intramolecular H-bond system starting in both anomers with HO–C(1) as H-donor and ending with HO–C(6′) as H-acceptor (see **M1** for $\alpha$-maltose in *Fig. 3, a*). This means that all secondary OH groups

of **M1** and its anomer should exhibit small $J$(H,OH) couplings ($\leq$2.5 Hz). This is not the case (see *Table 2*). As discussed in the preceding paper [1], such co-operative H-bonds are not formed in ($D_6$)DMSO. Recently, *Sugiyama et al.* [32] analysed maltooligosaccharides in ($D_6$)DMSO containing sufficient $D_2O$ to ensure a complete H/D exchange and concluded from interglycosidic $^3J$(H,C) couplings that maltose possesses an unfavourable conformation for an interresidue H-bond. The relevance of their results for the analysis of the conformation and H-bonding of maltose in DMSO is not clear as the question about the extent to which the conformation of maltose in ($D_6$)DMSO is modified by the addition of $D_2O$ has not been answered. One also notes that the authors have only considered the conformer possessing a $\phi$ angle of $-28°$ and ignored the conformer possessing the $\phi$ angle of $+28°$ ($\phi$ angles of $0-15°$ are ideal for an inter-residue H-bond).

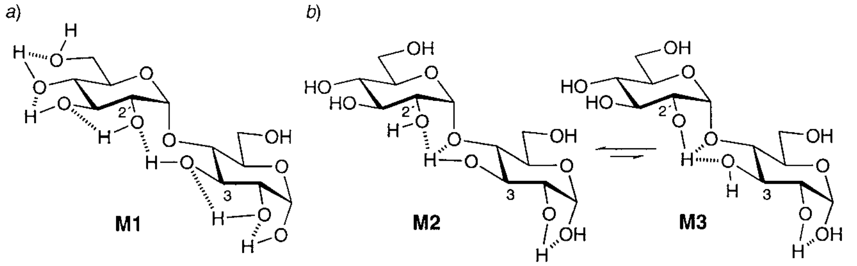


Fig. 3. *Intramolecular H-bonds in α-maltose in ($D_6$)DMSO solution* (hashed bonds), *as deduced* a) *from SIMPLE $^1$H-NMR effects* [31] *and* b) *from chemical-shift values and coupling constants for OH groups* (this work)

Table 2. *Chemical Shifts* [ppm] *and Vicinal Coupling Constants* [Hz] *for OH Groups and the Anomeric H of α- and β-Maltose in ($D_6$)DMSO at 25°*

| | H–C(1) ($J$(1,2)) | HO–C(1) ($J$(1,OH)) | HO–C(2) ($J$(2,OH)) | HO–C(3) ($J$(3,OH)) | HO–C(6) ($J$(6,OH)) |
|---|---|---|---|---|---|
| α-Maltose | 4.907 (4.0) | 6.319 (4.5) | 4.587 (6.9) | 5.270 (3.2) | 4.348 (5.8, 5.8) |
| α-Maltose (calc.)[a] | | 6.28 | 4.52 | 4.86 | 4.44 |
| β-Maltose | 4.309 (7.6) | 6.648 (6.5) | 4.937 (4.9) | 5.423 (3.2) | 4.457 (5.6, 5.6) |
| β-Maltose (calc.)[a] | | 6.64 | 4.91 | 5.06 | 4.55 |
| | H–C(1′) ($J$(1′,2′)) | HO–C(2′) ($J$(2′,OH)) | HO–C(3′) ($J$(3′,OH)) | HO–C(4′) ($J$(4′,OH)) | HO–C(6′) ($J$(6′,OH)) |
| α-Maltose | 4.976 (3.8) | 5.383 (6.2) | 4.855 (5.1) | 4.865 (5.8) | 4.474 (5.8, 5.8) |
| β-Maltose | 5.005 (3.8) | 5.396 (6.3) | 4.854 (5.0) | 4.876 (5.6) | 4.483 (5.7, 5.7) |
| α/β-Maltose (calc.)[a] | | 4.68 | 4.74 | 4.84 | 4.45 |

[a]) Calculated from the δ(OH) values of α-D-glucopyranose, β-D-glucopyranose, and methyl α-D-glucopyranoside [2] using the increments listed in the *Introduction.*

To analyse the H-bonding in α- and β-maltose, we recorded 500-MHz $^1$H-NMR spectra of *ca.* 0.085M solutions in ($D_6$)DMSO of β-maltose hydrate (α/β-ratio 1.5 : 98.5) and of a sample enriched in α-maltose (α/β ratio *ca.* 4 : 1)[5]). The $H_2O$ content of these

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[5]) We thank *Wolfgang Wenger* for this sample.

samples (1.45 and 0.35 equiv. of $H_2O$, resp.) had no influence upon the chemical shifts and the coupling constants. This was shown by drying a sample of $\beta$-maltose hydrate in ($D_6$)DMSO twice with activated 4-Å molecular sieves (see *Exper. Part*), lowering the $H_2O$ content from 2 *via* 0.45 to $<0.05$ equiv. At the same time, the $\beta/\alpha$-ratio changed from 96:4 *via* 8:2 to 6:4, but the chemical shifts of the OH signals remained constant ($\Delta\delta \leq 0.005$ ppm). The OH signals of $\alpha$- and $\beta$-maltose were assigned on the basis of a DFQCOSY.GRASP spectrum. The $\delta$(OH) and $J$(H,OH) values for corresponding OH groups of the nonreducing unit of $\alpha$- and $\beta$-maltose are nearly identical ($\Delta\delta \leq 0.015$ ppm, $\Delta J \leq 0.2$ Hz; *Table 2*), evidencing that the anomeric configuration has a negligible influence on the inter-residue H-bond. Except for $J$(3,OH), all $J$(H,OH) values of $\alpha$- and $\beta$-maltose are similar to the corresponding values of $\alpha$- and $\beta$-D-glucopyranose and methyl $\alpha$-D-glucopyranoside [2] ($\Delta J \leq 0.25$ Hz). The value of 3.2 Hz for HO−C(3) is too small for a fully solvated equatorial OH group and strongly suggests that HO−C(3) acts as H-donor in an inter-residue H-bond. The chemical-shift values for HO−C(1), HO−C(2), HO−C(6), HO−C(3′), HO−C(4′), and HO−C(6′) of $\alpha$- and $\beta$-maltose differ only slightly from the calculated values ($\Delta\delta^{e/c} \leq 0.12$ ppm), while both HO−C(3) and HO−C(2′) resonate at lower field ($\Delta\delta^{e/c}$(HO−C(3)) *ca.* 0.4 ppm, $\Delta\delta^{e/c}$(HO−C(2′)) *ca.* 0.7 ppm). This means that both HO−C(3) and HO−C(2′) act as H-bond acceptors, forming inter-residue flip-flop H-bonds (**M2** and **M3** in *Fig. 3*, *b*). The proximity of the glycosidic O-atom to the donating OH group of **M2** and **M3** suggests that these H-bonds are bifurcated. The difference between the downfield shifts for HO−C(3) and HO−C(2′) indicates a *ca.* 2:1 equilibrium of **M2** and **M3**; this is in agreement with the observed $\Delta\delta/\Delta T$ values (HO−C(3): $-2.8$ ppb/K, HO−C(2′): $-4.2$ ppb/K [30]). The determination of the conformational equilibrium from $J$(H,OH) alone is hampered by the fact that the exact $J$(H,OH) value for a H-accepting OH group is not known (compare [1]). In addition, maltose shows some flexibility around the glycosidic bonds [33], which may lead to an averaging of $J$(H,OH) for the H-donating OH group. Additional evidence for the position of the equilibrium between **M2** and **M3** may be obtained by comparing $J$(H,OH) values of the anomeric maltoses with those of methyl $\alpha$-D-glucopyranoside and the anomers of glucose, considering maltose a combination of the structural elements of the two monosaccharides. The comparison shows a larger difference between $J$(3,OH) values of the maltoses as compared to the glucoses ($\Delta J = 1.6$ Hz) than between $J$(2′,OH) values of the maltoses and methyl $\alpha$-D-glucopyranoside ($\Delta J = 0.15-0.25$ Hz). The larger difference for $J$(3,OH) suggests a preponderance of the species **M2** with HO−C(3) as H-donor to HO−C(2′).

In our hands, a SIMPLE $^1$H-NMR experiment with the maltoses led to a strong splitting of the signals for HO−C(2′) and HO−C(3) ($\beta$-maltose: $\Delta\delta$(HO−C(2′)) = +16 ppb; $\Delta\delta$(HO−C(3)): negative, exact value not determined due to signal overlap; [31] gives +10 and −10 ppb; $\alpha$-maltose: $\Delta\delta$(HO−C(2′)) = +22; $\Delta\delta$(HO−C(3)) = −7 ppb; [31] gives +10 and −10 ppb). We could not reproduce the fine splitting of the other OH signals in both anomers, observing only line broadening for HO−C(3′). Thus, SIMPLE $^1$H-NMR spectroscopy confirms the inter-residue H-bond in $\alpha$- and $\beta$-maltose. The different extent of the splitting for HO−C(2′) and HO−C(3) appears to correctly reflect the position of the conformational equilibrium, but any further interpretation of the sign and the extent of the splittings appears difficult.

3. *Weakly Persistent Inter-Residue H-Bonds in Agarose.* The repeating unit of agarose, $[(1 \rightarrow 4)$-$\alpha$-L-Gal$p$-3,6-anhydro-$(1 \rightarrow 3)$-$\beta$-D-Gal$p]_n$, consists of a 4-linked 3,6-anhydro-$\alpha$-L-galactopyranosyl and a 3-linked $\beta$-D-galactopyranosyl residue and possesses four OH groups (*Fig. 4, a*; residues indexed with A and G, resp.). In $(D_6)$DMSO solution, $HO-C(2_G)$ and $HO-C(6_G)$ of agarose show $J$(H,OH) and $\Delta\delta(OH)/\Delta T$ values [34] that are characteristic for fully solvated OH groups (*Table 3*). Significantly smaller $J$(H,OH) and slightly smaller $\Delta\delta(OH)/\Delta T$ values are observed for $HO-C(4_G)$ and $HO-C(2_A)$. This was rationalised by postulating an inter-residue $C(4_G)OH\cdots OC(5_A)$ H-bond and a conformationally biased complex, linking $HO-C(2_A)$ to $(D_6)$DMSO (as depicted in *Fig. 4, a*) [34]. The proof for such a complex (differences of $^{13}$C-NMR chemical shifts in $(D_6)$DMSO, $(D_6)DMSO/D_2O$ 1:1, and $D_2O$) is not convincing. The $HO-C(2_A)$ group is located on the *exo* side of the dioxabicyclo[3.2.1]octane skeleton and thus easily accessible to solvent. It is not clear why $HO-C(2_A)$ should form a conformationally biased complex with DMSO, and its low $J(2_A,OH)$ value of *ca.* 2 Hz must be due to another effect, most probably an intramolecular H-bond. Indeed, the $C(4_G)OH\cdots OC(5_A)$ H-bond requires a conformation where $HO-C(2_A)$ is close enough to $HO-C(2_G)$ to form an $C(2_A)OH\cdots OC(2_G)$ H-bond. Such a H-bond is suggested by MM3* modeling (gas phase; *Fig. 4, b*). Energy minimisation led to $H\cdots O$ distances of 1.73 and 1.81 Å for the $C(4_G)OH\cdots OC(5_A)$ and $C(2_A)OH\cdots OC(2_G)$ H-bonds, respectively. The calculated $H-C(4_G)-O-H$ and $H-C(2_A)-O-H$ dihedral angle of 83° corresponds to a $J$(H,OH) value of 0.2 Hz. A comparison with the experimental $J$(H,OH) values of 3.0 and 2 Hz suggests that the $C(4_G)OH\cdots OC(5_A)$ H-bond should persist to *ca.* 25% and the $C(2_A)OH\cdots OC(2_G)$ H-bond to *ca.* 50%. Since these $J$(H,OH) values are taken from a spectrum recorded at 60° (signals at 25° are broad), the faster H/H exchange expected at this temperature may contribute to reduce the $J$(H,OH) values (*cf.* Footnote 13 in [1] for such a case). This means that the persistence of the inter-residue H-bonds may be smaller than indicated by the coupling constants, as suggested by the $\Delta\delta(OH)/\Delta T$ values of $-5.8$ and $-5.2$ ppb/K. These temperature coefficients are in keeping with minor contributions of the intramolecularly H-bonded species and a slightly favoured $C(2_A)OH\cdots OC(2_G)$ H-bond. In agreement with this assignment, $HO-C(4_G)$ and $HO-C(2_A)$ show weak upfield shifts ($\Delta\delta^{e/c} = 0.1$ and 0.25 ppm), whereas $HO-C(2_G)$ shows a weak downfield shift ($\Delta\delta^{e/c} = 0.1$ ppm; *Table 3*; see *Footnote b* for the reliability of the calculated value for $HO-C(2_A)$). Thus, $HO-C(2_A)$ acts as H-donor and $HO-C(2_G)$ as H-acceptor of the inter-residue H-bond. Compared to an upfield shift for an OH group in a persistent inter-residue H-bond ($\Delta\delta^{e/c} =$ 0.6 ppm for $HO-C(3)$ of cellobiose and lactose), the upfield shift of 0.1 ppm for $HO-C(4_G)$ and of 0.25 ppm for $HO-C(2_A)$ suggests a 15% persistence of the $C(4_G)OH\cdots OC(5_A)$ and a *ca.* 40% persistence of the $C(2_A)OH\cdots OC(2_G)$ H-bond. The higher persistence of the $C(2_A)OH\cdots OC(2_G)$ H-bond is probably due to cooperativity between the intra- and the intermolecular H-bonds of $HO-C(2_G)$ (*cf.* [1]).

Unfortunately, further attempts to reveal the $C(2_A)OH\cdots OC(2_G)$ H-bond failed[6]). No split signals are found in the agarose SIMPLE $^1$H-NMR spectrum recorded at 60°.

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[6]) We thank *R. Toffanin*, *POLY-bios Research Centre,* Trieste, for measuring the SIMPLE and the NOESY $^1$H-NMR spectra.

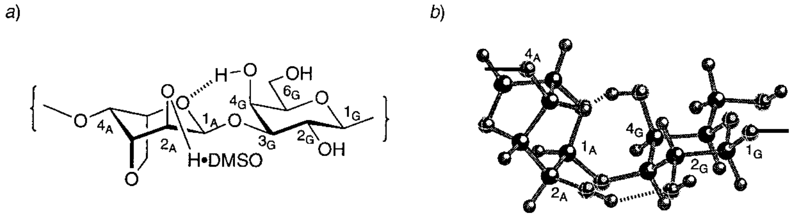

Fig. 4. *H-Bonds in the repeating unit of agarose in* $(D_6)$*DMSO solution* a) *as assigned by* [34]; b) *MM3*-modeled repeating unit possessing the interresidue* $C(4_G)OH\cdots OC(5_A)$ *and* $C(2_A)OH\cdots OC(5_G)$ *H-bonds* (hashed bonds)

Table 3. *Chemical Shifts, Vicinal Coupling Constants, and* $\Delta\delta(OH)/\Delta T$ *Values for OH Groups and the Anomeric H of Agarose in* $(D_6)$*DMSO at 25°*

| | $H-C(1_G)$ | $HO-C(2_G)$ | $HO-C(4_G)$ | $HO-C(6_G)$ | $H-C(1_A)$ | $HO-C(2_A)$ |
|---|---|---|---|---|---|---|
| $\delta$ [ppm] | 4.30 | 5.21 | 4.43 | 4.66 | 5.08 | 4.80 |
| Calc. $\delta$(OH) [ppm] | | 5.12[a]) | 4.55[a]) | 4.63[a]) | | 5.05[b]) |
| $J$(H,OH) [Hz][c]) | | 5.2 | 3.0 | 5.5, 5.5 | | *ca.* 2 |
| $\Delta\delta(OH)/\Delta T$ [ppb/K] | | −6.1 | −5.8 | −6.2 | | −5.2 |

[a]) Calculated from the $\delta$(OH) values of methyl $\beta$-D-galactopyranoside [2] using the increments listed in the *Introduction.* [b]) No $\delta$(OH) value is available for monomeric 1,3-anhydro-galactopyranoside. The best reference we have found is $\delta(HO-C(2)) = 4.80$ ppm of methyl 4,6-*O*-benzylidene-$\beta$-D-mannopyranoside [1]. The additional downfield shift for $\delta(HO-C(2_A))$ due to the antiperiplanar $C(3_A)-O$ bond is more or less compensated by the downfield shift for the $\delta(HO-C(2))$ of the mannopyranoside due to the partially persistent $C(3)OH\cdots OC(2)$ H-bond. The influence of the additional ring on $\delta(HO-C(2_A))$ is not known, but is probably negligible. A value of 5.1 ppm is obtained from $\delta$(OH) of $\beta$-D-glucopyranose and the increments listed in the *Introduction.* [c]) Not determined at 25° (broad signals). Data taken from a spectrum recorded at 60°.

A NOESY spectrum, again at 60°, showed strong saturation transfer between $H_2O$ and the OH groups, but no cross peak between $HO-C(2_A)$ and $H-C(2_G)$, or between $HO-C(2_G)$ and $H-C(2_A)$.

4. *Equilibrium of H-Bonded Species of Sucrose.* The solid state of sucrose (**12**; *Fig. 5*; glucopyranosyl and fructofuranosyl residues indexed with G and F, resp.) is characterised by the two inter-residue $C(1_F)OH\cdots O(2_G)$ and $C(6_F)OH\cdots O(5_G)$ H-bonds [35–37]. The $C(1_F)OH\cdots O(2_G)$ and $C(6_F)OH\cdots O(5_G)$ distances are 1.85 and 1.89 Å, respectively. It is still not completely clear whether and to what extent inter-residue H-bonds are present in aqueous solutions of sucrose (see [38–40] and refs. cit. therein). Solutions of sucrose in $(D_6)$DMSO have been analysed by *Bock* and *Lemieux* [41]. Based on $^1$H-NMR data (including $\Delta\delta/\Delta T$ values and H/D exchange rates) and HSEA calculations, they postulated a similar species as that found in the solid state, possessing the inter-residue $C(1_F)OH\cdots OC(2_G)$ and, perhaps, the $C(6_F)OH\cdots O(5_G)$ H-bond. Although SIMPLE $^1$H-NMR spectroscopy can not detect the inter-residue $C(6_F)OH\cdots OC(5_G)$ H-bond, *Christofides et al.* investigated sucrose in $(D_6)$DMSO solution by this method and postulated a 2 : 1 equilibrium of the species possessing the inter-residue $C(1_F)OH\cdots OC(2_G)$ and $C(3_F)OH\cdots OC(2_G)$ H-bonds [42]. *Widmalm* and co-workers performed molecular-dynamics simulation of sucrose in DMSO solution and calculated a probability of 81% for the inter-residue $C(6_F)OH\cdots OC(5_G)$

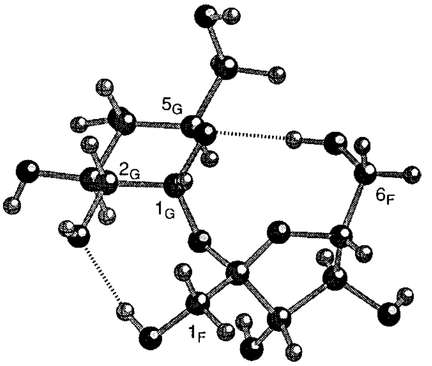

Fig. 5. *X-Ray crystal structure of sucrose* (**12**; inter-residue H-bonds marked by hashed bonds) [35]

H-bond, of 68% for the inter-residue $C(2_G)OH \cdots OC(1_F)$ H-bond (opposite H-bond direction as in the solid state), and of 30% for the intra-residue $C(1_F)OH \cdots OC(3_F)$ H-bond [39]. No probability was proposed for the intra-residue $C(1_F)OH \cdots OC(4_F)$ and $C(6_F)OH \cdots OC(3_F)$ H-bonds that have been postulated for $\beta$-D-fructofuranose in DMSO solution[7]).

These controversial interpretations prompted us to repeat the $^1$H-NMR analysis of sucrose in $(D_6)$DMSO solution. For the discussion of the data, we considered the crystal structure of sucrose (*Fig. 5*) and the species **12A**–**12C** (*Fig. 6*) that correspond roughly to those discussed in the literature, but already embody some of our interpretations.

The assignment of the OH signals of *Bock* and *Lemieux* was checked and confirmed on the basis of a DQFCOSY.GRASP spectrum (*cf.* also [45][46]). The $\Delta\delta(OH)/\Delta T$ values (*Table 4*) indicate the absence of any strongly persistent intramolecular H-bond, but are in keeping with weakly persistent unidirectional or flip-flop H-bonds, especially involving $HO-C(1_F)$, $HO-C(4_F)$, and $HO-C(6_F)$ ($\Delta\delta(OH)/\Delta T$ values from $-4.2$ to $-4.4$ ppb/K). In the absence of inter-residue H-bonds, one expects the same $\delta(OH)$ and $J(H,OH)$ values as for methyl $\alpha$-D-glucopyranoside [2] and methyl $\beta$-D-fructofuranoside[7]) [47]; *i.e.*, the calculated $\delta(OH)$ values for sucrose correspond exactly to the experimental $\delta(OH)$ values of these monosaccharides. The analysis of inter-residue H-bonds in sucrose is hampered by flip-flop H-bonds in the fructofuranosyl moiety[7]) especially when these are influenced by the inter-residue H-bonds. Nevertheless, large $\Delta\delta^{e/c}(OH)$ values should evidence inter-residue H-bonds. Such shift differences are found for $HO-C(2_G)$ that is strongly shifted downfield ($\Delta\delta^{e/c} = 0.34$ ppm), and for $HO-C(3_F)$ and $HO-C(6_F)$ that are strongly shifted upfield ($\Delta\delta^{e/c} = 0.26$ and 0.36 ppm, resp.).

We first checked whether the species in $(D_6)$DMSO solution are similar to the crystal structure. The upfield shift of $HO-C(6_F)$ may be rationalised by an inter-residue $C(6_F)OH \cdots OC(5_G)$ H-bond, as depicted in **12A** (*Fig. 6*). As for cellobiose (see

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[7]) $\beta$-D-Fructofuranose is the dominant anomer of fructose in $(D_6)$DMSO [43][44]. All OH groups resonate in the typical range for fully solvated OH groups, but show a slightly reduced temperature dependence ($\Delta\delta(OH)/\Delta T$ values between $-4.4$ and $-5.9$ ppb/K [43b]). This is rationalised by flip-flop H-bonds between HO−C(1) and HO−C(4) and between HO−C(6) and HO−C(3) (or HO−C(2)).

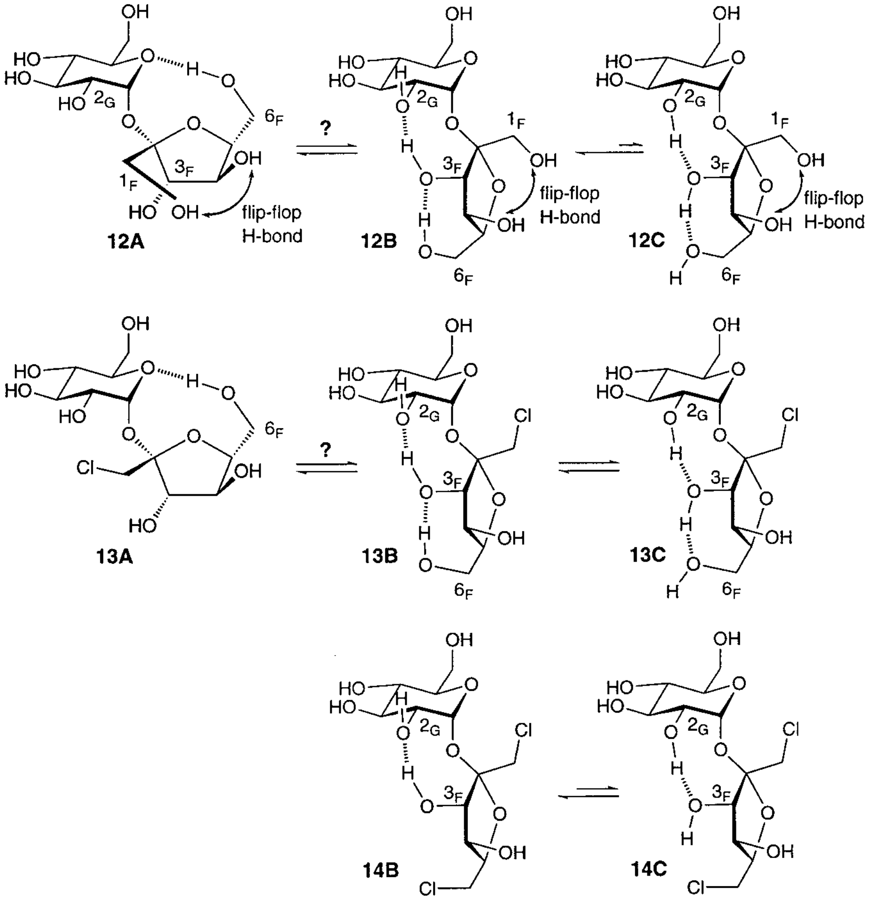

Fig. 6. *Intramolecular H-bonds of sucrose* (**12**), *1′-chloro-1′-deoxysucrose* (**13**), *and 1′,6′-dichloro-1′,6′-dideoxysucrose* (**14**) *in* ($D_6$)*DMSO solution deduced from δ(OH) and* J(*H,OH*) *values*

above), this H-bond should enhance the acidity of all OH groups of the H-bond accepting unit and induce a downfield shift. This is not the case; on the contrary, a small upfield is observed for HO–C($4_G$) and HO–C($6_G$) ($\Delta\delta^{e/c} = 0.08 - 0.09$ ppm). The inter-residue C($1_F$)OH ··· OC($2_G$) H-bond in the crystal structure is not evidenced by the solution data. The small downfield shift of HO–C($1_F$) ($\Delta\delta^{e/c} = 0.10$ ppm) indicates that HO–C($1_F$) is a slightly better H-acceptor, probably of the flip-flop H-bond, than HO–C(1) of methyl β-D-fructofuranoside, but not a (predominant) H-donor to HO–C($2_G$). Being characterised by a strong downfield shift, HO–C($2_G$) must be a H-bond acceptor and can not be a (predominant) H-donor, *e.g.*, of a C($2_G$)OH ··· OC($1_F$) or C($2_G$)OH ··· OC($1_G$) H-bond. Thus, there is no evidence that the species possess an inter-residue C($1_F$)OH ··· OC($2_G$) H-bond, which has been claimed to be preponderant in ($D_6$)DMSO [41][42]. These data imply that **12A** cannot be a dominant species in ($D_6$)DMSO.

Table 4. *$\Delta\delta(OH)/\Delta T$ Values* [ppb/K] *of Sucrose* (**12**), *$\delta(OH)$* [ppm] *and* $J(H,OH)$ [Hz] *Values of Sucrose* (**12**), *1'-Chloro-1'-deoxysucrose* (**13**) [42b], *1',6'-Dichloro-1',6'-dideoxysucrose* (**14**) [42b], *Methyl α-D-Glucopyranoside* [2], *and Methyl β-D-Fructofuranoside* [47] *in ($D_6$)DMSO Solution*

| | | HO–C($2_G$) | HO–C($3_G$) | HO–C($4_G$) | HO–C($6_G$) |
|---|---|---|---|---|---|
| Sucrose (**12**) | $\Delta\delta(OH)/\Delta T$ [a]) | – 5.0 (– 5.4) | – 6.0 (– 6.1) | – 4.6 (– 4.85) | – 5.1 (– 5.6) |
| | $\delta(OH)$ ($J$(H,OH)) | 5.02 (5.6) | 4.74 (4.9) | 4.73 (5.6) | 4.37 (5.8, 5.8) |
| Methyl α-D-glucopyranoside [b]) | $\delta(OH)$ ($J$(H,OH)) | 4.68 (6.45) | 4.74 (4.7) | 4.84 (5.5) | 4.45 (5.8, 6.2) |
| **13** | $\delta(OH)$ ($J$(H,OH)) | 4.77 (6.2) | 4.76 (5.0) | 4.77 (5.6) | 4.38 (5.2, 6.0) |
| **14** | $\delta(OH)$ ($J$(H,OH)) | 4.83 (6.0) | 4.75 (5.0) | 4.80 (5.4) | 4.49 (4.5, 6.0) |

| | | HO–C($1_F$) | HO–C($3_F$) | HO–C($4_F$) | HO–C($6_F$) |
|---|---|---|---|---|---|
| Sucrose (**12**) | $\Delta\delta(OH)/\Delta T$ | – 4.2 (– 4.6) | – 5.0 (– 5.2) | – 4.2 (– 4.7) | – 4.4 (– 4.85) |
| | $\delta(OH)$ ($J$(H,OH)) | 4.78 (6.3, 6.3) | 4.46 (8.0) | 5.16 (5.3) | 4.35 (5.6, 5.6) |
| Methyl β-D-fructofuranoside [b]) | $\delta(OH)$ ($J$(H,OH)) | 4.68 (6.7, 6.7) | 4.72 (6.9) | 5.13 (5.7) | 4.71 (6.3, 6.3) |
| **13** | $\delta(OH)$ ($J$(H,OH)) | – | 4.94 (8.0) | 5.30 (5.8) | 4.45 [c]) |
| **13** (calc.) [d]) | $\delta(OH)$ | – | 5.05 | 5.28 | 4.71 |
| **14** | $\delta(OH)$ ($J$(H,OH)) | – | 5.17 (7.7) | 5.52 (5.9) | – |
| **14** (calc.) [d]) | $\delta(OH)$ | – | 5.22 | 5.63 | – |

[a]) Values from [41] in parenthesis. [b]) $\delta$(H,OH) Values correspond to the calculated values of **12**. [c]) $J$(H,OH) not assigned. [d]) Calculated from the $\delta$(H,OH) values of methyl β-D-fructofuranoside using an increment of +0.35 ppm for a β-Cl and +0.15 ppm for a γ-Cl substituent [47].

The downfield shift of HO–C($2_G$) and the upfield shift of HO–C($3_F$) suggest a C($3_F$)OH ··· OC($2_G$) inter-residue H-bond, as depicted in **12B**. *Christofides et al.* [42] have postulated such a species as minor component of sucrose in ($D_6$)DMSO. This inter-residue H-bond may be stabilized by a co-operative intra-residue C($6_F$)OH ··· OC($3_F$) H-bond. Such a H-bond is in keeping with the upfield shift of HO–C($6_G$). Species **12C**, which possesses a homodromic H-bond system of opposite direction is, however, clearly disfavoured, as evidenced by the upfield shift of HO–C($6_F$) and by the downfield shift of HO–C($2_G$). The value of $\Delta\delta^{e/c}$(HO–C($2_G$)) (*ca.* 0.3 ppm; compare with 0.6 ppm for the completely persistent H-bond in cellobiose) agrees well with a *ca.* 1:1 equilibrium of **12B** and the corresponding solvated species, lacking the inter-residue H-bond. Minor amounts of **12A** and **12C** can not be completely excluded.

In our hands, the SIMPLE $^1$H-NMR spectrum of sucrose in ($D_6$)DMSO (500 MHz, 25°, H/D 55:45) shows a triple splitting of the original *doublet* for HO–C($2_G$) (– 7, – 5.5, and *ca.* + 2.5 ppb), a single splitting for HO–C($1_F$) (+ 3.8 ppb), and line broadening for the signal of HO–C($3_F$). The splittings, but not their sign, correspond roughly to the values published by *Christofides* and *Davies* (HO–C($2_G$): + 7, + 3.2 ppb and a weak negative value; HO–C($1_F$): – 4.3 ppb; HO–C($3_F$): + 2.2 and – 2.2 ppb [42c]). Isomerisation of the deuteriated, differently H-bonded species is slow relative to the NMR time scale, as signalled by the three isotope effects for HO–C($2_G$). According to the conventional interpretation of the sign of the splitting[8]), HO–C($2_G$) acts twice as H-donor and once as H-acceptor in H-bonds to OH groups, in

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[8]) See [1][48], for a discussion of this aspect.

contradiction to the interpretation of the chemical-shift data, and evidencing that there is an equilibrium of at least three species. The triple splitting may reflect the participation of the species due to the flip-flop equilibration of **12B** that may be accompanied by a change of the furanose ring conformation, although it is not possible to exclude that the third species is, after all, the one corresponding to the crystal structure, for which we found no other evidence.

The SIMPLE effect on HO$-$C($1_F$) prompted us to also analyse the reported $^1$H-NMR data of the 1′-chloro-1′-deoxy derivative **13** and 1′,6′-dichloride **14** [42b]. The SIMPLE $^1$H-NMR spectra of **13** and **14** [42b] show a double splitting for HO$-$C($2_G$) and HO$-$C($3_F$) (or a simple splitting and line broadening), in keeping with the hypothesis that the SIMPLE spectrum of **12** reflects an influence of HO$-$C($1_F$). The chlorides **13** and **14** lack one or two primary OH groups, and should thus allow confirmation of the correct assignment of the inter-residue H-bonds of sucrose in ($D_6$)DMSO. According to *Guthrie et al.* [47], substitution of the primary OH groups of methyl $\beta$-D-fructofuranoside by chloride leads to a downfield shift of 0.35 ppm for the $\beta$-OH and of 0.15 ppm for the $\gamma$-OH group. Using these increments, we calculated the shifts for HO$-$C($3_F$) and HO$-$C($4_F$) of **13** and **14** on the basis of methyl $\beta$-D-fructofuranoside (*Table 4*). Analysis of these values led to the following result: HO$-$C($2_G$) of **13** is a weaker H-acceptor than HO$-$C($2_G$) of **12** ($\Delta\Delta\delta^{e/c} = 0.1$ ppm), and HO$-$C($3_F$) and HO$-$C($6_F$) of **13** ($\Delta\delta^{e/c} = 0.11$ and 0.26 ppm) are weaker H-donors than HO$-$C($3_F$) and HO$-$C($2_F$) of **12**, respectively ($\Delta\delta^{e/c} = 0.26$ and 0.36 ppm), while HO$-$C($4_F$) of **13** resonates at the expected position. These values suggest an equilibrium between **13B**, the species lacking an inter-residue H-bond, and substantial amounts of **13C**; minor amounts of **13A** cannot be excluded. One expects a flip-flop equilibrium between **14B** and **14C**; this is evidenced by the chemical-shift values ($\Delta\delta^{e/c}$ values) for HO$-$C($2_G$) and HO$-$C($3_F$) (*Table 4*). HO$-$C($2_G$) of **14** resonates more strongly downfield than HO$-$C($2_G$) of **13** ($\Delta\Delta\delta^{e/c} = 0.06$ ppm), while HO$-$C($3_F$) of **14** resonates more strongly upfield than HO$-$C($3_F$) of **13** ($\Delta\Delta\delta^{e/c} =$ 0.04 ppm). While these shift differences do not allow to define the position of the equilibria between **13B** and **13C** or between **14B** and **14C**, they indicate that **14B** participates to a larger extent in the latter equilibrium than **13B** in the former. The relative decrease of the C($2_G$)OH $\cdots$ OC($3_F$) H-bonded species from **13C** *via* **14C** to **12C** is evidenced by the decrease of $J(2_G,\mathrm{OH})$ from 6.2 *via* 6.0 to 5.6 Hz.

While this analysis shows that $\delta$(OH) values are important for the assignment of intramolecular H-bonds in sucrose, it also shows that additional data are required to unambiguously define the H-bonded species.

5. *Strong Inter-Residue H-Bonds in Hyaluronates.* Hyaluronic acid is a polymer (molecular mass in the range of $10^4$ to $8 \cdot 10^6$) of a ($1 \rightarrow 4$)-$\beta$-D-Glc*p*A-($1 \rightarrow 3$)-$\beta$-D-Glc*p*NAc dimeric repeating unit (*Fig. 7, b*). Hence, one expects intramolecular H-bonds involving carboxylato and acetamido groups. Four inter-residue H-bonds have been described that are responsible for the shape of the stiffened worm-like coil [49–52]. The H-bonds are between HO$-$C(2) of Glc*p*A and the acetamido C=O group, between NH of the acetamido and the carboxylato group, between HO$-$C(4) of Glc*p*NAc and both O$-$C(5) and the carboxylato group of Glc*p*A (bifurcated H-bond), and between HO$-$C(3) of Glc*p*A and O$-$C(5) of Glc*p*NAc [49][52]. Since carboxylato and C=O groups act as H-acceptors, the chemical shift of the H-donors

is also influenced by the charge and/or by anisotropy effects, which complicate the quantification of the shift increments. As shown below, a qualitative analysis is still feasible.

The terminal units of the tetrasaccharide **15** (*Fig. 7, a*) lack some of the inter-residue H-bonds, and this aspect allows an internal correlation of the $^1$H-NMR data [52]. The carboxylato group, a strong H-acceptor, leads to a strong deshielding of the donating HX, as evidenced by the downfield shift for HO–C(4$^{IV}$), resonating at 7.09 ppm. Thus, one expects a downfield shift for the NH of an acetamido group involved in an inter-residue H-bond to a carboxylato group. Indeed, HN–C(2$^{III}$) resonates by *ca.* 1.9 ppm downfield to $\delta$(HN–C(2$^{I}$)). Its temperature dependence (–4.3 ppb/K) and its relatively large vicinal coupling (6.2 Hz)[9]) suggest a *ca.* 50% persistence of the inter-residue C(2$^{III}$)NH $\cdots$ $^-$O$_2$C H-bond. The geometry required for this H-bond favours the formation of the C(3$^{II}$)OH $\cdots$ OC(5$^{III}$) H-bond. Indeed, $\Delta\delta/\Delta T$(HO–C(3$^{II}$)) = –4.3 ppb/K and $J$(3$^{II}$,OH) = 3.5 Hz evidence a *ca.* 50% persistence also of the inter-residue C(3$^{II}$)OH $\cdots$ OC(5$^{III}$) H-bond. This interpretation is in agreement with an upfield shift for HO–C(3$^{II}$) of 0.46 ppm relative to $\delta$(HO–C(3$^{IV}$)). HO–C(4$^{I}$), HO–C(4$^{III}$), and HO–C(2$^{IV}$) are exclusively involved in inter-residue H-bonds, as revealed by small $J$(H,OH) ($\leq$ 2 Hz) and the low $\Delta\delta$(OH)/$\Delta T$ values (–1.4 to –1.0 ppb/K). HO–C(2$^{IV}$) resonates at 3.87 ppm, significantly upfield to $\delta$(HO–C(2$^{II}$)) ($\Delta\delta$ *ca.* 0.8 ppm); this is rationalised by the inter-residue H-bond and probably also by an anisotropy effect of the accepting C=O group. HO–C(4$^{I}$) and HO–C(4$^{III}$) resonate at low field (5.46 and 5.24 ppm, resp.). This means that O–C(5$^{II}$) and O–C(5$^{IV}$) cannot be the only H-acceptors of these OH groups, as this would result in upfield shifts; the observed downfield shifts must be due to the participation of the carboxylato groups in bifurcated H-bonds.

The $\delta$(OH) and $\Delta\delta$(OH)/$\Delta T$ values of tetraethylammonium hyaluronate (**16**; *Fig. 7, b*) [50] deviate only slightly from the $\delta$(OH) and $\Delta\delta$(OH)/$\Delta T$ values for the central disaccharide unit of the tetrasaccharide **15**. This evidences that HO–C(4$^{I}$) and HO–C(2$^{II}$) of tetraethylammonium hyaluronate form persistent inter-residue H-bonds, that HN–C(2$^{I}$) and HO–C(3$^{II}$) are involved to *ca.* 50% in inter-residue H-bonds, and that HO–C(6$^{I}$) is completely solvated.

The $\Delta\delta$(OH)/$\Delta T$ values of hyaluronate ethyl and benzyl esters [50] (data of ethyl ester **17** in *Fig. 7, c*) reveal a slightly weaker inter-residue H-bond of HO–C(2$^{II}$) than for HO–C(2$^{II}$) of the ammonium salt **16**. They also show that the inter-residue H-bond between HN–C(2$^{I}$) and the C=O group of the ester moiety and the one between HO–C(4$^{I}$) and O–C(5$^{II}$) are each *ca.* 50% persistent, while HO–C(6$^{I}$) and HO–C(3$^{II}$) are completely solvated. The upfield shift of HN–C(2$^{I}$) of **17** as compared to $\delta$(HN–C(2$^{I}$)) of **16** ($\Delta\delta$ = 1.9 ppm) is not due to a complete cleavage of the inter-residue H-bond, as claimed in [50], but conditioned by the different H-acceptors: charged carboxylato *vs.* uncharged EtOCO group. HO–C(4$^{I}$) of **17** is no more in a bifurcated H-bond. The absence of the carboxylato group as H-acceptor is now

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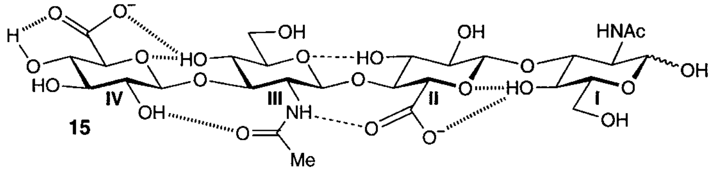
[9]) MM3* Modeling (gas phase) suggests a dihedral angle H–C(2)–N–H of *ca.* 130° for the H-bonded species, avoiding a too close contact between the carbonyl group and O–C(3) (see [26] for preferred conformations of GlcNAc derivatives). According to the equation of *Bystrov* [53], this angle corresponds to a $J$(H,NH) value of 5.0 Hz.

a)

| | HO–C(4$^{\mathrm{IV}}$) | HO–C(4$^{\mathrm{III}}$) | HO–C(6$^{\mathrm{III}}$) | HO–C(3$^{\mathrm{II}}$) | HO–C(2$^{\mathrm{II}}$) | HN–C(2$^{\mathrm{I}}$) | |
|---|---|---|---|---|---|---|---|
| *δ*: | 7.09 | 5.24 | 4.57 | 4.36 | 4.75/4.57 | 7.70/7.81 | ppm |
| *J*: | | ≤ 2 | 6.0 | 3.5 | 4.9 | 7.2/8.2 | Hz |
| *Δδ/Δ*T: | | –1.4 | –5.0 | –4.3 | –6.7 | –4.3/–2.0 | ppb/K |

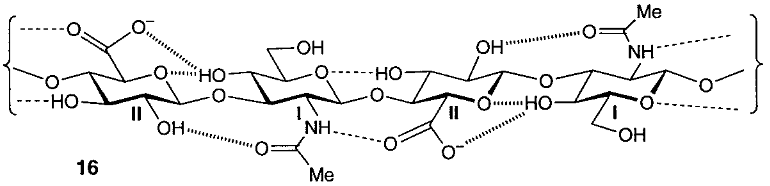


**15**

| | HO–C(3$^{\mathrm{IV}}$) | HO–C(2$^{\mathrm{IV}}$) | HN–C(2$^{\mathrm{III}}$) | HO–C(4$^{\mathrm{I}}$) | |
|---|---|---|---|---|---|
| δ: | 4.82 | 3.87 | 9.28/9.21 | 5.46 | ppm |
| *J*: | 4.9 | 1.6 | 6.2 | ≤ 1 | Hz |
| Δδ/ΔT: | –7.0 | –1.0 | –4.3 | –1.4 | ppb/K |

b)

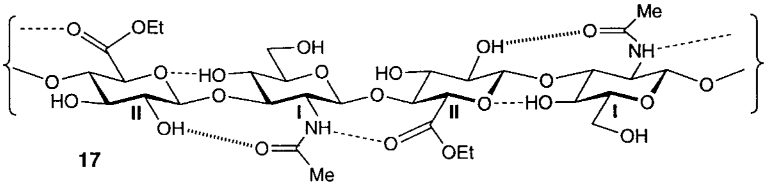
| | HO–C(6$^{\mathrm{I}}$) | HO–C(4$^{\mathrm{I}}$) | HN–C(2$^{\mathrm{I}}$) | HO–C(3$^{\mathrm{II}}$) | HO–C(2$^{\mathrm{II}}$) | |
|---|---|---|---|---|---|---|
| *δ*: | 4.60 | 5.28 | 9.38 | 4.28 | 3.95 | ppm |
| *Δδ/Δ*T: | –6.5 | +0.4 | –5.1 | –4.7 | –2.4 | ppb/K |



**16**

c)

| | HO–C(6$^{\mathrm{I}}$) | HO–C(4$^{\mathrm{I}}$) | HN–C(2$^{\mathrm{I}}$) | HO–C(3$^{\mathrm{II}}$) | HO–C(2$^{\mathrm{II}}$) | |
|---|---|---|---|---|---|---|
| *δ*: | 4.73 | 4.38 | 7.47 | 4.99 | 4.83 | ppm |
| *Δδ/Δ*T: | –6.4 | –5.2 | –4.8 | –7.1 | –3.0 | ppb/K |



**17**

Fig. 7. *δ(OH),* J*(H,OH), and Δδ(OH)/Δ*T *values of hyaluronates in* ($D_6$)*DMSO solution: assignment of completely and partially persistent inter-residue H-bonds* (hashed and dashed bonds, resp.): a) *of the Na salt of the tetrasaccharide* **15** [52], b) *of the tetraethylammonium salt* **16** *of a polymeric hyaluronate* [50], *and* c) *of the polymeric hyaluronate ethyl ester* **17** [50]

characterized by an upfield shift ($\Delta\delta = 0.9$ ppm relative to $\delta$(HO–C(4$^{\mathrm{I}}$)) of **16**), as it is expected when O–C(5$^{\mathrm{II}}$) is the only H-bond acceptor. The signals of HO–C(3$^{\mathrm{II}}$) and HO–C(2$^{\mathrm{II}}$) of **17** are shifted downfield by 0.7 and 0.9 ppm from $\delta$(HO–C(3$^{\mathrm{II}}$)) and $\delta$(HO–C(2$^{\mathrm{II}}$)) of **16**, respectively. The former shift is expected from the absence of the inter-residue H-bond. The latter shift is presumably conditioned by changes of the anisotropy of the H-acceptor, and not simply by a slightly weaker inter-residue H-bond, as indicated by the change of $J(2^{\mathrm{I}},\mathrm{NH})$ from 6.2 Hz for **16** to 8.1 Hz for **17**, corresponding to $\mathrm{H{-}C(2^{I}){-}O{-}H}$ dihedral angles of 137 and 148°, respectively.

These examples show that the combined interpretation of chemical shifts, coupling constants, and temperature dependence values is a powerful tool for the analysis and quantification of inter-residue H-bonds in oligosaccharides.

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## Experimental Part

Standard $^1$H-NMR, DFQCOSY.GRASP, and SIMPLE $^1$H-NMR spectra (only for $\alpha/\beta$-maltose and sucrose) of 0.08 – 0.09M soln. of $\alpha/\beta$-cellobiose 1:9, $\beta$-maltose monohydrate (*Fluka BioChemica MicroSelect*, $\alpha/\beta$ ratio 1.5:98.5), $\alpha/\beta$-maltose *ca.* 4:1 (prepared by heating a suspension of $\beta$-maltose monohydrate in 2-methoxyethanol to 75 – 90° [54]), and sucrose in ($D_6$)DMSO (20 – 22 mg in 0.7 ml) were measured at 298 K on a 500 MHz apparatus (*Bruker-AMX-500*). The $\Delta\delta(\mathrm{OH})/\Delta T$ values of sucrose were deduced from the 300 MHz spectra (*Varian-Gemini-300*) recorded between 298 and 368 K in 10 K intervals.

*Drying of a ($D_6$)DMSO Soln. of $\beta$-Maltose Monohydrate.* A soln. of $\beta$-maltose monohydrate (40 mg) in 2 ml of ($D_6$)DMSO was analysed by $^1$H-NMR spectroscopy (300 MHz): $\beta/\alpha$-maltose 96:4, 2 equiv. of $H_2O$. The soln. was treated with activated 4-Å molecular sieves (dried at 0.01 Torr and 140° for 24 h) for 24 h, filtered through cotton, and analysed by $^1$H-NMR spectroscopy: $\beta/\alpha$-maltose 4:1, 0.45 equiv. of $H_2O$. This procedure was repeated: $\beta/\alpha$-maltose 3:2, $<0.05$ equiv. of $H_2O$ (the signal of $H_2O$ is obscured below the signals of maltose). The $\delta$(OH) values are not influenced by the $H_2O$ content ($\Delta\delta \leq 0.005$ ppm).

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