

Hydrogen bonding and cooperativity effects on the assembly of carbohydrates

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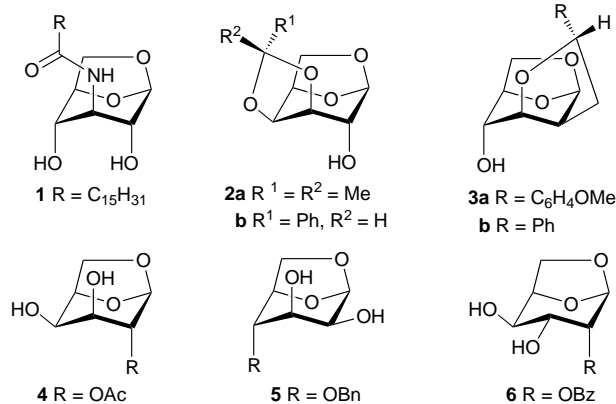
The effect of intramolecular hydrogen bonds on the cooperative assembly of carbohydrate derivatives has been evaluated; the 1,3-diaxial OH...OH intramolecular hydrogen bond is at the origin of the dimerization of diol **1**; and one intermolecular OH...OH bond accounts for 2.5 kcal mol⁻¹ in CDCl₃ and 3 kcal mol⁻¹ in CDCl₃-CCl₄.

Hydrophobic interactions, hydrogen bonding and cation binding are at the origin of the recognition processes in which carbohydrates are involved. The study of carbohydrate OH...OH hydrogen bond energetics is of fundamental interest for understanding these recognition processes.

One particular characteristic of hydrogen bonding is cooperativity, considered as the enhancement of the first hydrogen bond (HB) between a donor and an acceptor when a second HB is formed between one of these two species and a third partner.¹ A quantitative treatment of HB cooperativity was described initially by Huyskens.² Theoretical³ and experimental⁴ methods have also allowed the quantification of this effect. Evidence of intramolecular σ -cooperativity in carbohydrates has arisen from neutron diffraction data of crystalline structures of mono- and di-saccharides.⁵ In solution, intramolecular cooperativity has been studied by NMR spectroscopy in polar solvents⁶ and by FTIR.⁷ In contrast, evidence of intermolecular cooperativity in carbohydrates has only come from neutron diffraction data of carbohydrates⁸ and protein-carbohydrate complexes.⁹

This report represents a contribution within a project to design self-assembled structures based on carbohydrate intermolecular OH...OH hydrogen bonds.¹⁰ Here we have evaluated the energetic advantage of establishing cooperative intermolecular HBs for assembling simple carbohydrates. In addition, we have also studied the relative stereochemistry of OH groups that favour this process.

The possibility of self-assembly of diols of 1,6-anhydro- β -D-glycopyranosides of different relative configuration and position with respect to the anomeric centre [2,4 (*a,a*) *cis*-diol **1**, 3,4 (*a,e*) *cis*-diol **4**, 2,3 (*e,a*) *cis*-diol **5** and 3,4 (*e,e*) *trans*-diol **6**] has been explored.[‡] The only diol that showed significant aggregation behaviour in CHCl₃ was the glucose 1,3-diaxial diol **1**.



A detailed study of the intramolecular HB network for the monomer of **1** was carried out in dilute CDCl₃ solution by ¹H NMR spectroscopy. Additionally, the axial monoalcohols,

1,6-anhydro- β -D-glycopyranosides **2a** and **3a**, were used as models to study the influence of a second hydroxy group with a 1,3-diaxial orientation, as present in diol **1**, on intermolecular cooperativity.

Monoalcohols **2a** and **3a** in CDCl₃ at low concentration show high ³J_{CH,OH} values (9 Hz), consistent with a fixed conformation of the CHOH angle (larger than 150°), which can be attributed to an hydrogen bonded OH.^{6c} For the 1,3-diaxial diol **1**, the OH(2) resonance follows the same trend [as expected for hydrogen bonding to OH(4) or O(5)] but, in contrast, the OH(4) resonance now has a medium size ³J_{CH,OH} value, indicating that it is not hydrogen bonded. Neither of the OH(2) or OH(4) resonances achieve exchange decoupling at any accessible concentration, a characteristic feature of fixed OHs.^{6c}

Partial deuteration of **1** in CDCl₃ at low concentration shows that the OH(2) resonance has a negative isotopic effect (-0.0165 ppm), consistent with OH(2) being a donor.^{6b} Therefore, these results show that both monoalcohols **2a** and **3a** have their hydroxy groups intramolecularly fixed by a HB to O(5). In contrast, for **1** the 1,3-diaxial orientation of both hydroxy groups favour OH(2) to be hydrogen bonded, as a donor, to OH(4). These HBs must affect the self-assembly characteristics of the different compounds.

The characterization of the aggregates in solution was performed using different methods. ¹H NMR dilution experiments in CDCl₃ at 299 K of **1-6** allowed us to calculate the stability constants of the dimerization process.[§] Neither of the monoalcohols **2a** or **3a** dimerize.[¶] In contrast, diol **1** [2,4(*a,a*)] presents a dimerization constant of 70 M⁻¹ at 299 K ($\Delta G^\circ = -2.5 \pm 0.1$ kcal mol⁻¹).

Vapour pressure osmometry measurements (VPO)^{||} in CHCl₃ suggested that, for a concentration range between 0.05–0.01 M, the monoalcohols **2a** and **3a** are monomers. On the other hand, **1** presents a molecular weight which corresponds to 1.6 times that of the monomer. This value is in agreement with the percentage of dimer which is present in solution according to the NMR-derived stability constant (80%).

Chemical shifts, ³J values and temperature coefficients also indicate that the assembly is mediated by OH...OH hydrogen bonds. Table 1 shows that the coefficients of OH(2) for **2a** and OH(4) for **3a** are not concentration dependent, in contrast with the observations made for **1**.¹² The ³J_{CH,OH} values of **1-3** at high concentration indicate their involvement in HBs, with the exception of OH(4) of **1** which shows a J value of 5.4 Hz at all concentrations. The NH of **1** does not show any concentration dependence for the NMR parameters. This experimental evidence is consistent with the amide not being involved in the self-association process.^{||}

The clear difference in the solution self-assembly behaviour of **1** with respect to monoalcohols **2a** and **3a** indicates that the addition of the extra OH in a pyranoid ring having a 1,3-*syn* diaxial orientation accounts for an extra stabilization of the dimer of 2.5 kcal mol⁻¹ in CDCl₃ compared to the monoalcohols. As a test for a non-intramolecular hydrogen bonded OH, ethanol under the same conditions measured for **1-6** did not show any measurable dimerization constant.

Thermodynamic parameters for the dimerization of **1** in CDCl₃-CCl₄ (1–1.3) were obtained from a Van't Hoff plot (from 296–318 K). Values of $\Delta H^\circ = -6.5$ kcal mol⁻¹ and ΔS°

Table 1 ¹H NMR chemical shifts coupling constants and temperature coefficients of OH resonances of **1**, **2a** and **3a** at two different concentrations

Compound	Concentration/ mM	δ (ppm)	OH(2) ³ J ^a /Hz	$\Delta\delta_{\text{OH}(2)}/\Delta T^b$ (ppm K ⁻¹)	δ (ppm)	OH(4) ³ J ^a /Hz	$\Delta\delta_{\text{OH}(1)}/\Delta T^b$ (ppm K ⁻¹)
1	110	4.00	9.0	-1.0×10^{-2}	4.68	5.4	-1.6×10^{-2}
	0.05	2.82	10.2	-2.3×10^{-3}	2.73	5.4	-4.0×10^{-3}
2a	110	2.21	8.4	-5.5×10^{-3}	—	—	—
	0.05	1.95	8.7	-2.2×10^{-3}	—	—	—
3a	110	—	—	—	2.534	8.7	-5.2×10^{-3}
	0.05	—	—	—	2.304	9.3	-2.4×10^{-3}

^a Data at 298 K. ^b Measured between 297 and 313 K.

= +11.8 kcal mol⁻¹ K⁻¹ were estimated. The stability constant of the dimer at 299 K was 150 M⁻¹ ($\Delta G^\circ = -3.0 \pm 0.1$ kcal mol⁻¹). Ethanol under the same conditions showed a dimerization constant of 0.1 M⁻¹.

To quantify the effect of cooperativity on the dimerization process, it is important to know the structure of the dimer. In principle, the structure of the dimer present in solution could be of two types: an open dimer with one cooperative intermolecular HB stabilizing the dimer [Fig. 1(a)], and a closed dimer with two intermolecular HBs established in a cyclic and cooperative way [Fig. 1(b)]. The implication of O(5) in the network cannot be excluded.

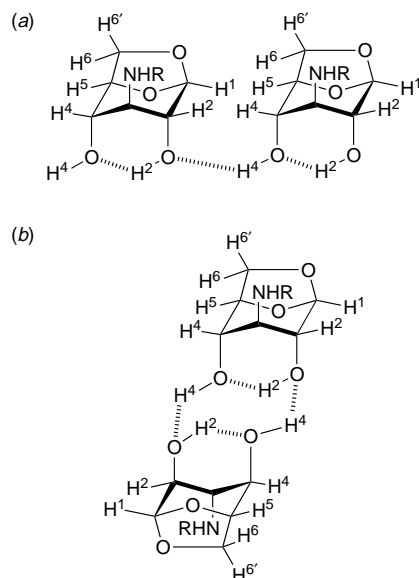


Fig. 1 Schematic representation of two possible dimeric structures for **1**: (a) open dimer and (b) closed dimer

NOESY experiments of a concentrated solution of **1** (0.1 M) were performed in order to obtain structural information about the dimer. Since two free OH groups are present in the molecule, regular experiments as well as MINSY-type spectra¹³ were recorded in order to exclude chemical exchange-mediated cross peaks. Thus, besides the regular NOESY spectrum, additional experiments saturating OH(2) and OH(4) hydroxy groups were recorded. The obtained results unambiguously indicate the presence of intermolecular NOEs. In particular, H(1)/H(6_{exo}), H(1)/H(6_{endo}), H(1)/H(5), H(2)/H(4), H(5)/H(2) and H(1)/H(4) cross-peaks were detected. These NOEs are not seen when the experiments are carried out at low concentration and are only compatible with the existence of an open dimer structure. This open structure is also supported by the measured ³J_{CH,OH} values. MM2* calculations** of this structure show that it is stable and account for the observed NOEs. This structure shows that the carbonyl group cannot be hydrogen bonded to any donor moiety.

Thus, a single cooperative intermolecular OH...OH bond accounts for 2.5 kcal mol⁻¹ in CDCl₃ and 3 kcal mol⁻¹ in CDCl₃-CCl₄.

The difference in the self-assembly behaviour of **1** with respect to diols **4**, **5** and **6** has to be related to the difference in strength and directionality of the intramolecular HBs.^{7c,14}

We are now extending this study to diols involving other positions in the pyranoid ring in order to evaluate the energetic advantage of intermolecular HB cooperativity.

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Notes and References

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‡ All new compounds gave satisfactory elemental analyses and spectroscopic data. ³J analysis shows that all compounds adopt a chair conformation in CDCl₃ solution.

§ All dilution experiments were carried out three times and the ΔG° values were reproducible within ± 0.1 kcal mol⁻¹. Dilution data were fitted to a dimerization process using an infinite non-cooperative model. We thank Dr C. A. Hunter (University of Sheffield) for kindly providing the fitting program.

¶ Diols **4** and **5** showed small dimerization constants (1.8 and 1.4 M⁻¹, respectively).

|| Additionally, a concentration dependent FTIR experiment shows no evidence for intermolecular hydrogen bonded species involving the NH or C=O of the amide (ν_{NH} 4335 and ν_{CO} 1772 cm⁻¹ at high and low concentration).

** Molecular Mechanics Calculations were carried out using MM2* with the GB/SA solvent model for CHCl₃.

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