Carbohydrate Hydrogen-Bonding Cooperativity − Intramolecular Hydrogen Bonds and Their Cooperative Effect on Intermolecular Processes − Binding to a Hydrogen-Bond Acceptor Molecule

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The high hydroxy (OH) group content in carbohydrates makes the study of carbohydrate OH···XH and OH···X H-bond energetics fundamental to understanding of carbohydrate recognition. There is, however, a relative lack of knowledge concerning the factors that allow a carbohydrate to participate in recognition events stabilised by intermolecular H bonds. We therefore present here a systematic study on the factors that determine the formation of a well-defined intramolecular H-bonding network between carbohydrate hydroxy groups, and its cooperative or anti-cooperative influence on selected intermolecular processes mediated by H bonds. With this in mind, we first determined the H-bonding networks of a series of carbohydrate derivatives – monoalcohols, 1,2- and 1,3-diols and amidoalcohols – by ^{1}H NMR and FT-IR spectroscopy. The hydroxy groups of these compounds showed different abilities to form intramolecular H bonds, depending on their relative positions and configurations on the pyranose ring, and on the nature of the adjacent functional groups. It has also been shown that both the directionality and strength of the intramolecular H-bonding network of a carbohydrate govern the formation of cooperative or anti-cooperative H-bond centres, with consequent repercussions on the thermodynamics of the intermolecular H-bonding interactions of the carbohydrate in question. From this study, some general rules for the prediction of the intramolecular H-bonding network characteristics of a given carbohydrate and its influence on the energetics of intended intermolecular recognition processes have been inferred. The results presented here give a new perspective over understanding of the role of the H-bonding interactions in carbohydrate recognition and have fundamental implications for the rational design of glycoconjugates incorporating H-bonding motifs with geometrical and electronic complementarity to given receptor molecules.

Introduction

Oligosaccharides mediate biological processes such as cell-cell recognition, including the infection of cells by bacteria and viruses, and the behaviour of enzymes and other proteins. They also fulfil various functions in immune response.[1,2]

The intermolecular forces believed responsible for carbohydrate recognition are hydrophobic interactions, hydrogen bonding (H bonding) and cation binding.[3−5] Their relative contribution depends on the topology of the sugar − that is, the particular orientation of the exposed carbohydrate hydrophilic and hydrophobic residues − and on the medium in which the process is taking part.[6−9]

There is, however, a relative lack of knowledge on basic aspects of the noncovalent interactions responsible for carbohydrate recognition, and this is an important drawback for the design of interaction processes involving even the simplest monosaccharides.[10] So far, for example, there are no rules for the noncovalent selective binding of glucose vs. galactose. In this respect, carbohydrates can be regarded as "unknown biomolecules".

The high hydroxy (OH) group content in carbohydrates makes the study of carbohydrate OH···XH and OH···X H-bond energetics fundamental to the understanding of carbohydrate recognition. However, the experimental study of such interactions is a difficult task. Spectroscopic techniques, such as NMR and FT-IR, are very convenient for the study of OH···H bonds in aprotic solvents. In aqueous media, however, the observation of OH groups by NMR is complicated because of the fast rate of exchange between OH and water, compared to, for example, protein and nucleic acid NH resonances. In vibrational spectroscopy, the broad hydroxy stretching vibrations of water typically

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swamp the sugar OH bands in dilute solution. These experimental limitations have made it difficult to obtain both structural and thermodynamic data on H bonds involving hydroxy groups. In comparison with amide H bonds, there is an additional difficulty for the study of hydroxy H bonds. Whilst H-bond donor and acceptor centres are well defined in amide H bonds, OH groups have dual donor-acceptor character, which impedes a priori identification of the H-bond *directionality*. The orientation of the major H-bond conformer conditions the formation of the rest of the H-bond network in a particular direction (defined by the positions of the donor and acceptor). We define this orientation of a specific H bond or H-bond network as its *directionality*. Consequently, it is much more complicated to make this prediction in highly hydroxylated compounds such as carbohydrates.

A general characteristic of molecular interactions is cooperativity.[11−14] Cooperativity between H bonds[15] implies that an H bond, A−H···B, between a proton donor group A−H and a proton acceptor group or molecule B, will become stronger when a second H bond is formed between a further A*−H group and the A−H group already involved in the first H bond. For instance, A*−H···A−H···B H bonds show this property in all biological structures.

Even though there are many theoretical studies on H-bonding cooperativity,[16−21] experimental evidence for this property in solution is scarce.[22−27]

Evidence for intramolecular σ-cooperativity (mutual enhancement of intramolecular H bonds) has been reported for carbohydrates, both in solid state and in solution.[28−32]

Intermolecular σ-cooperativity (mutual enhancement of intra- and intermolecular H bonds) in carbohydrates is well documented in the solid state.[9,33] In contrast, evidence of carbohydrate intermolecular σ-cooperativity in solution is limited.[34−38] Our results have demonstrated that, at least in nonpolar media, intramolecular OH···OH H bonds significantly influence the effectiveness of intermolecular processes stabilised by H bonds and that carbohydrate OH groups do not act as independent H-bonding interaction centres, but as H-bond arrays.

At this stage, more information about the influence of the directionality and strength of the intramolecular H-bond network on the ability of a carbohydrate to participate in intermolecular recognition processes in solution is required. Work related to the factors underlying the intramolecular H-bond strength in hydroxylated compounds simpler than carbohydrates,[39−41] as well as in hydroxy ethers,[42] has been reported. These studies examined how intramolecular OH···OH H bonds affect the formation of intermolecular H bonds in simple aliphatic diols.[43,44] Vasella and coworkers have carried out several detailed investigations on the effect of the strength and directionality of the intramolecular H bond network on the yield, regioselectivity and stereoselectivity of the glycosidation reactions between protected sugar vicinal diols and 1-azasugars.[45,46] To the best of our knowledge, however, within the field of molecular recognition, there are no experimental reports available in the literature that contemplate the geometrical requirements of the H-bonding network that would allow a carbohydrate to participate in those recognition events stabilised by intermolecular H bonds. This lack of basic information, together with the importance of this topic for carbohydrate molecular recognition, prompted us to initiate a systematic study on the factors that determine the formation of a well-defined intramolecular H-bonding network between carbohydrate hydroxy groups, as well as on its cooperative or anti-cooperative influence. For this purpose, some selected intermolecular processes mediated by H bonds have been selected.

We report now on the study of the intramolecular H-bonding networks of a series of carbohydrate derivatives − monoalcohols, 1,2- and 1,3-diols and amidoalcohols (compounds **1−16**; Figure 1) − by ^{1}H NMR and FT-IR spectroscopy. The structural theme central to the design of compounds **1−16** was the ability of the carbohydrate OH groups to form intramolecular H bonds depending on their relative position and configuration on the pyranose ring, and on the nature of the adjacent functional groups. It was further intended to show that both the directionality and strength of the intramolecular H-bonding network of a given carbohydrate determines the formation of cooperative or anti-cooperative H-bond centres. Cooperative H-bond centres will be denominated as either "good donors" or "good acceptors". To verify the relevance of the generation of these cooperative H-bonding centres on carbohydrate recognition energetics, we have chosen two intermolecular recognition processes: carbohydrate self-assembly (manuscript in preparation), and complexation of a H-bond acceptor molecule (see below). The NMR spectroscopic data here were usually recorded in $CDCl_3$, while the FT-IR spectra were measured in CH_2Cl_2. This kind of approach to the study of H bonds in nonpolar solvents is frequently found in the literature. In fact, only small differences regarding H-bonding competition have been found between these particular two solvents, and for the type of analysis described below they can be considered as similar, noncompetitive solvents. Indeed, IR spectra have also in some cases been recorded in $CDCl_3$, providing analogous results.

^{1}H NMR titration experiments (in $CDCl_3$) of compounds **1−16** with pyridine (Py), as a model H-bond acceptor molecule have, as shown below, enabled correlations to be established between relative OH configuration and stability of the intermolecular process. Analysis of the IR spectra of these compounds in CH_2Cl_2/Py has allowed evaluation of the changes in the O−H stretching region upon interaction with Py. These data have been used to evaluate the strengthening of the intramolecular H bonds as a consequence of the intermolecular OH···Py association (evaluation of intermolecular H-bonding cooperativity).

From these results, it has been possible to establish several rules with which to predict the intramolecular H-bonding network characteristics of a given carbohydrate and its influence on the energetics of intended intermolecular recognition processes. These give a new perspective to the understanding of the role of the H-bonding interactions in

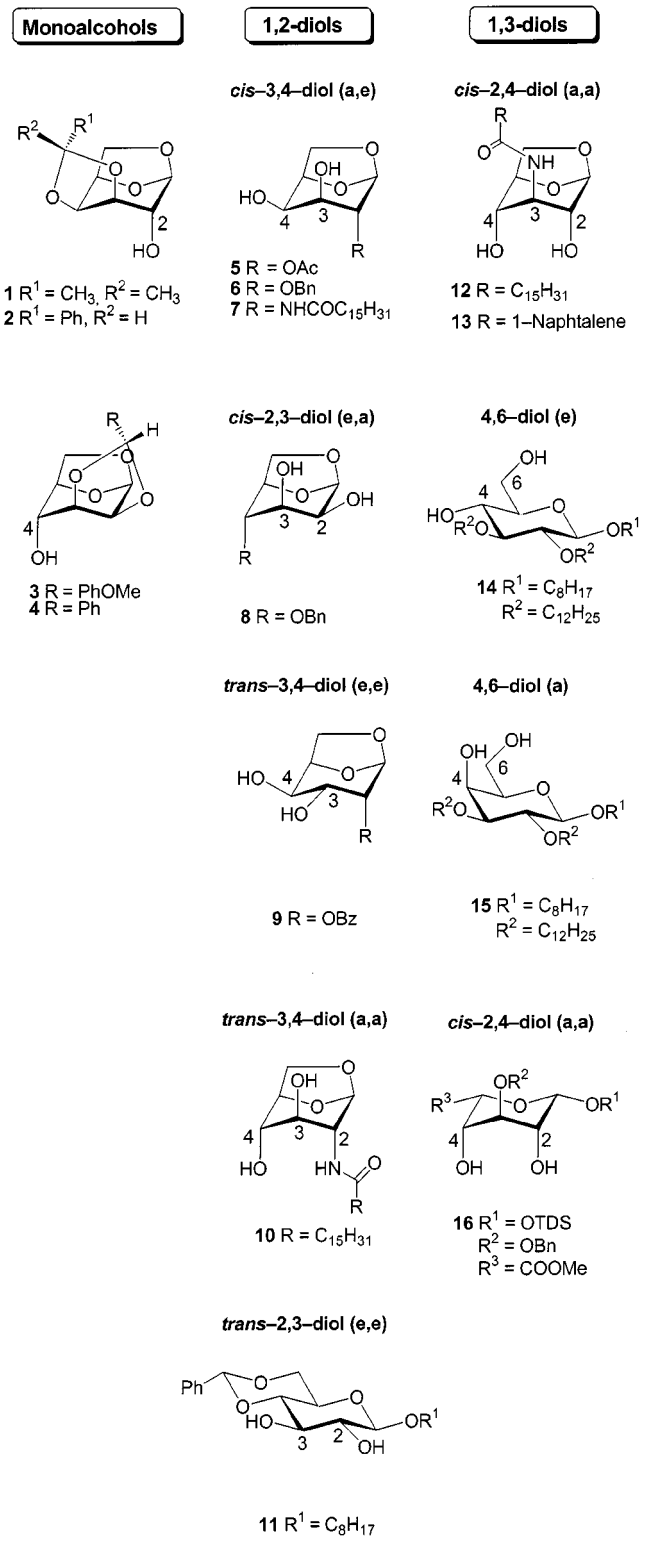

Figure 1. Sugar diols and amido alcohols (**1**−**16**) studied

carbohydrate recognition and have fundamental implications for the rational design of glycoconjugates containing H-bonding motifs with geometrical and electronic complementarity to given receptor molecules.

Results and Discussion

1. Elucidation of the Intramolecular H-Bonding Network

1.1 ^{1}H NMR and FT-IR Data

The ^{1}H NMR spectra of dilute solutions (5×10^{-4} M of compounds **1**−**16** in $CDCl_3$ showed all hydroxy resonances to be well resolved, and they were easily assigned by their coupling patterns ($^3J_{CH,OH}$). Intermolecular exchange of hydroxy protons destroys the coupling information. Such exchange can easily be controlled and manipulated simply by dilution and careful solvent handling (passing $CDCl_3$ through dry basic alumina to remove traces of water and acid).[47] However, because of overlapping of the signals of 3-H and 4-H, the OH resonances of compound **9** could not be unambiguously assigned, either by analysis of $^3J_{CH,OH}$, or by irradiation of the 3-H or 4-H resonance signals. The relative configurations and separations of the carbohydrate H-bonding groups (hydroxy and amide groups) depend on the pyranose ring conformation. A conformational analysis based on coupling constant values indicated that all 1,6-anhydro-β-D-pyranose derivatives except compound **9** have $^1C_4$-chair conformations. The $J_{2,3}$ and $J_{3,4}$ values (≈ 4.2 Hz) revealed that compound **9** exists in a 1:1 $^1C_4$ chair/$B_{O,3}$ boat conformational equilibrium.[48] Coupling constant analysis of **11**, **14** and **15** supported a $^4C_1$-chair ring conformation for these compounds.

Table 1. NMR parameters for sugar derivatives **1**−**16** in $CDCl_3$

	$J_{OH,H}$ [Hz][a]	δ [ppm][a]	$\Delta\delta/\Delta T$[b] [ppb/K]
1	8.9 (2-OH)	1.93	−2.3
3	9.2 (4-OH)	2.31	−2.3
5	9.0 (3-OH)	2.96	−2.5
	7.2 (4-OH)	2.58	−2.2
6	7.8 (3-OH)	2.45	−1.9
	8.4 (4-OH)	2.88	−2.6
7	8.5 (3-OH)	3.10	−3.1
	6.0 (4-OH)	2.68	−8.7
	9.0 (2-NH)	5.60	−2.3
8	5.1 (2-OH)	2.65	−1.1
	8.4 (3-OH)	2.40	−2.5
9[c]	2.5 (3-OH)	2.35	−2.3
	6.0 (4-OH)	2.00	−1.4
10	6.1 (3-OH)	2.57	−2.1
	4.3 (4-OH)	1.96	−1.6
	9.0 (2-NH)	6.07	−1.5
11	2.6 (2-OH)	2.32	−2.7
	2.5 (3-OH)	2.50	−2.2
12	9.7 (2-OH)	2.83	−2.4
	5.4 (4-OH)	2.72	−4.3
	7.8 (3-NH)	6.07	−1.2
14	2.4 (4-OH)	2.27	−2.0
	5.7, 6.6 (6-OH)	1.89	−1.9
15	ca. 0 (4-OH)	2.57	−1.8
	3.9, 9.3 (6-OH)	2.36	−3.3
16	0 (2-OH)	2.79	−1.3
	11.4 (4-OH)	3.76	−2.5

[a] $T = 299$ K. [b] $c = 5 \times 10^{-4}$ M. Temperature coefficients obtained between 297 and 218 K. [c] The assignments may be inverted.

Tables 1 and 2 summarise some experimental and calculated ^{1}H NMR parameters (δ, $^3J_{CH,OH}$, $\Delta\delta/\Delta T$) of the OH and NH resonances of compounds **1−16** in dilute chloroform solutions. These parameters have all been used as a diagnosis for intramolecular H bonding. Further structural analysis of the data shown in this table was carried out by considering that:

(1) Large $^3J_{CH,OH}$ values (9−10 Hz) indicate dihedral angles of 150−170°, and small values (1.5−2.5 Hz) angles of 90° ($^3J_{CH,OH} = 10.4\cos^2\Phi - 1.5\cos\Phi + 0.2$).[49] Both large and small $^3J_{CH,OH}$ values indicate the participation of OH groups in H bonds. Intermediate coupling constants are usually observed for a freely rotating OH group,[50] but can also be associated with a conformational equilibrium between H-bonded isomers.[45]

(2) The hydroxy chemical shifts contain useful information on H bonding. H-bonded hydroxy groups usually show resonances that are more deshielded than those of free OH groups.[47,51]

(3) The temperature dependence of the OH[52,53] and NH[54−56] resonances permits H bonds to be detected and H-bond energies evaluated.[57] In dilute samples in nonpolar organic solvents, the value of $\Delta\delta/\Delta T$ reflects the perturbation of possible H bonds with temperature, and weak interactions with the solvent (weak H bonds in $CDCl_3$ or van der Waals interactions in CCl_4 or CH_2Cl_2). In these solvents, small or intermediate $\Delta\delta/\Delta T$ values are expected for solvent-exposed OH and NH groups, and small values for intramolecular H-bonded OH and NH groups.[58]

Dilute solution IR spectroscopy, in which the timescale permits the direct observation of the free [$(OH)_{free}$] and intramolecular H-bonded [$(OH)_{intra}$] hydroxy stretching vibrations, is commonly used to confirm the presence of an intramolecular H bond. Carefully applied, the technique may also be used to determine the extent and relative energy of intramolecular H bonds.[42][59] FT-IR spectra were recorded for compounds **1−16** in dry CH_2Cl_2 at concentrations at which the intermolecular association was reduced to a minimum, taking into account the dissolution limits of the compounds. The frequencies of the O−H bond stretching vibrations of the free [$\nu(OH)_{free}$] and intramolecular H-bonded [$\nu(OH)_{intra}$] hydroxy groups are given in Table 3. The FT-IR spectra of these sugar derivatives exhibited $\nu(OH)_{free}$ bands, in which ν(OH) is represented by the peak maximum, at values between 3610−3588 cm^{-1}. The bands observed between 3580 and 3500 cm^{-1} are attributable to intramolecular H-bonded OH stretching vibrations [$\nu(OH)_{intra}$]. In compounds with an amide moiety, N−H stretching vibrations [ν(NH)] can be observed between 3435−3425 cm^{-1}. Other broad bands, which often appear as shoulders, can be identified between 3500 and 3370 cm^{-1} and are attributable to intermolecular association. Features

Table 2. Torsion angle values for the possible rotamers around the C−O bonds of the hydroxy groups; the expected vicinal coupling constants according to $10.4\cos^2\Phi - 1.5\cos\Phi + 0.2$ and their comparison with the experimental values are also given; $\Phi - 1.5\cos\Phi + 0$ is the H−C−O−H torsion angle for the hydroxy group attached to the carbon atom shown as subscript; superscripts a and b stand for the H-bonding isomer according to the figures in the text; the best agreement is given in **bold**; the lowest energy minimum according to MM3* calculation is given in *italics*

	Rotamer Φ_2 [a]/J_2 [a] [Hz]	Rotamer Φ_3 [a]/J_3 [a] [Hz]	Rotamer Φ_4 [a]/J_4 [a] [Hz]	Rotamer Φ_2 [b]/J_2 [b] [Hz]	Rotamer Φ_3 [b]/J_3 [b] [Hz]	Rotamer Φ_4 [b]/J_4 [b] [Hz]
$1_{theor.}$				**143/7.8**		
$1_{exp.}$	8.9	−	−	8.9	−	−
$3_{theor.}$						**153/9.7**
$3_{exp.}$	−	−	9.2	−	−	9.2
$5_{theor.}$	−	***−56/2.8***	***42/5.0***	−	**145/8.4**	**153/9.7**
$5_{exp.}$	−	9.0	7.2	−	9.0	7.2
$6_{theor.}$	−	*−60/2.1*	*39/5.2*	−	**146/8.5**	**153/9.7**
$6_{exp.}$	−	7.8	8.4	−	7.8	8.4
$7_{theor.}$	−	*−61/2.0*	***−47/3.7***	−	**146/8.5**	**154/9.8**
$7_{exp.}$	9.0	8.5	6.0	9.0	8.5	6.0
$8_{theor.}$	***48/3.8***	***64/1.9***	−	**180/12.1**	**168/11.6**	−
$8_{exp.}$	5.1	8.4	−	5.1	8.4	−
$9_{theor.}$				−	−166/11.2	55/2.8
$9_{exp.}$	−	2.5	6.0	−	2.5	6.0
$10_{theor.}$	**143/7.8**	157/10.4	**−39/5.2**			
$10_{exp.}$	9.0	6.1	4.3	9.0	6.1	4.3
$11_{theor.}$	***−52/3.2***	***53/3.0***	−	***51/3.4−***	***−53/3.2***	−
$11_{exp.}$	2.6	2.5	−	2.6	2.5	−
$12_{theor.}$	***−178/12.0***	***138/7.2***	***41/5.0***	−45/4.3	141/7.5	178/12.0
$12_{exp.}$	9.7	7.8	2.4	9.7	7.8	2.4
$14^*_{theor.}$			**−53/3.2**			
$14^*_{exp.}$			2.4	−	−	
$15^*_{theor.}$			***59/2.2***			
$15^*_{exp.}$			< 1	−	−	< 1
$16_{theor.}$	−176/12.0	−	35/5.9	***−59/2.2***	−	***179/12.1***
$16_{exp.}$	< 1	−	11.4	< 1	−	11.4

between 3687−3682 cm^{-1} arise from trace water. Further analysis of these IR data were carried out taking the following considerations into account:

1) The initial interpretation of the FT-IR spectra is based upon the assumption that the free and intramolecularly associated H bonds can be observed at sufficiently distinct frequencies, and that the variation in the bond strengths within each of these OH bond types is sufficiently small as to allow effective consideration of two unique band maxima, which we will denominate ν_f for the representative frequency of $\nu(OH)_{free}$ and ν_i for the representative frequency of $\nu(OH)_{intra}$. This assumption, although simplistic, allows us to create an overall view of the hydrogen-bonding characteristics of the systems described here. However, it is clear that the distribution of H-bond strengths between intramolecularly hydrogen-bonded conformers is significant in some cases, which is reflected in the large bandwidths observed.

2) The magnitude of the difference in frequency between the absorptions of the free (ν_f) and of the bonded (ν_i) maxima is customarily taken as a measure of the strength of the H bond[60][61] and is denominated as $\Delta\nu_{OH}$. The value of $\Delta\nu_{OH}$ depends on a number of factors. Higher values of $\Delta\nu_{OH}$ − i.e., stronger H bonds − are favoured by acidity of the donor OH, basicity of the acceptor site, and approach of the three-atom bridge to linearity. It is a very useful parameter for the correlation of molecular geometries and for drawing comparisons between H-bonded structures containing the same donor and acceptor function.

3) According to the literature,[60][62] the free/bonded intensity ratios (I_f/I_i) should be related to the proportion of molecules engaged in intramolecular H bonding for a given system. Assuming that this is indeed the case in 1,2-diols, similar values of $(OH)_{free}$ and $(OH)_{intra}$ absorption intensities would indicate the presence of one free and one bonded OH group, whereas a value of $I_f/I_i = 0.4$ would suggest that both hydroxy groups are simultaneously H-bonded.[62] In this respect, several comments should be made. It is essential to consider the relative absorbance, A (areas), of the bands and not the peak intensities (I) as representative of the HB distribution. Three general cases to compare the relative intensities, A_f/A_i, in a semiquantitative manner can be described.[62][63] When $A_f/A_i \approx 1$, this suggests that an equilibrium between free and associated OH groups that approaches an equimolar distribution exists; when $A_f/A_i >> 1$, this suggests that conformations that do not favour H-bond formation are prevalent; and when $A_f/A_i << 1$, this suggests that conformations that do favour H bond formation are prevalent, and the lower the value of A_f/A_i, the greater the tendency to form intramolecular associations.

4) The nature of the intramolecular H bonds can be characterised by the frequency range in which ν_i is observed. In CH_2Cl_2 (the solvent used for all the FT-IR experiments presented here) absorptions between 3600−3585 cm^{-1} have been shown to be characteristic of H-bonded diequatorial 1,2-*trans*-diols (five-membered ring *trans*), bands between 3580 and 3560 cm^{-1} of H-bonded equatorial OH groups in a 1,2-*cis*-diol (five-membered ring *cis*), and those between 3550 and 3500 cm^{-1} of an H-bonded OH group in a six-membered ring [1,3-*cis*-diol (a,a)], or of intermolecular H bonds.[45,64]

Table 3. Observed IR frequencies for sugars **1**−**16** between 3650−3400 cm^{-1}

	$c \times 10^{-2}$ [M]	ν_f [a] [cm^{-1}]	ν_i [a] [cm^{-1}]	ν_{other} [cm^{-1}]
1	5	3605	3571	3480[b] [c]
3	5	3603	3572	3487[b] [c]
5	1	3602	3546	3480[b] [c]
6	5	3605	3543	3489[b] [c]
7	1	3600	3540	3430[a] [d]
8	5	3605	3555	3389[b] [c] [d]
9	3	−	3592, 3557[c]	3476[b] [c]
10	0.1	3597	−	3471[b] [c]
11	5	−	3590, 3552[c]	3425[a] [d]
12	0.05	3591	3551	3475[b]
14	5	3593	3532[c]	3434[a] [d]
15	5	3606[c]	3562, 3530[c]	3476[b] [c]
16	3.3	3589[c]	3554, 3507	3471[b] [c], 3460[b] [c]

[a] Absorptions independent of concentration. [b] Broad, concentration-dependent absorptions (intermolecular associations). [c] Shoulder (approximate frequency given). [d] ν_{NH} absorptions.

Structures corresponding to the possible hydrogen-bonding networks involving the hydroxy groups (type a and b in the figures of the text) were used as starting geometries and submitted to exhaustive energy minimisation. In all cases, the geometries obtained from molecular mechanics calculations with the MM3* program and the GB/SA solvent model for chloroform were used for analysis. In particular, torsion angles and interatomic distances were extracted from the MM3*-optimised geometries. Special attention was paid to the geometrical aspects (angles and distances), independently of the relative steric energies provided by the program, since the energy values may be rather approximate. Therefore, the discussions below are based on comparison of the experimental NMR (Table 1) and IR (Table 3) parameters with those expected for the different hydrogen-bonding isomers, and not on the energy values (Table 2). It also should be mentioned that, in those cases for which conformational equilibria have been shown, no attempt to give accurate figures for the different populations of conformers has been made, and only a semiquantitative conclusion is given. In fact, both the torsion angles and the estimated coupling constants derived from these values may be subject to errors and so the percentages of conformers could be misleading.

1.2 Intramolecular H-Bonding Network of Monoalcohols 1 and 3

Compounds **1** and **3** were designed as axial monoalcohols with OH moieties located at different positions with respect to the anomeric centre (**1**, OH on position 2, and **3**, OH on position 4). Additionally, they were used as models to study how the H-bond properties of one OH group are influenced by the presence of a second OH group in a 1,3-diaxial relative configuration to the first, by comparing the

data from these compounds with those from 1,3-diols **12** and **16**.

The $J_{OH,H}$ values (8.9 Hz for **1** and 9.2 Hz for **3**) and $\Delta\delta/\Delta T$ values (−2.3 ppb/K for both compounds) indicated that both hydroxy groups are probably intramolecularly H-bonded to 5-O. Distances obtained from MM3* calculations in chloroform supported these H bonds [d(O2···O5) = 2.96 Å for **1** and d(O2···O5) = 2.94 Å for **3**, with H−O−C−H torsion angles higher than 150° in absolute value]. In the infrared spectra of **1** and **3**, the intramolecular association bands are observed at the same frequency, at around 3571 cm^{-1}, which further implies the involvement of the hydroxy group in the same type of H bond in both compounds. This result is in good agreement with the 2-OH and 4-OH → 5-OH bond inferred from NMR spectroscopic data analysis. The isomer ratio present in solution, calculated from the peak areas, presents a value close to unity for **1**, suggesting a 1:1 equilibrium between H-bonded and free OH isomers. The value of A_f/A_i appears to be lower in **3** than in **1**, which suggests that the formation of the intramolecular H bond is more favoured in this compound (Figure 2).

1.3 Intramolecular H-Bonding Network of 1,2-Diols 5−11

The 1,2-diols **5−11** are carbohydrate derivatives designed to study the influence of the relative configurations of the hydroxy groups (*cis* or *trans*) on the intramolecular H-bonding network of a given carbohydrate. They differ in the position of the hydroxy groups on the pyranose ring with respect to the anomeric centre, and on the nature of their neighbouring groups (acyl or *O*-alkyl). They can be classified into two groups (Figure 1): (I) 1,2-*cis*-diols (**5−8**) and (II) 1,2-*trans*-diols (**9−11**). Group (I) comprises *cis*-diols with relative *galacto* [axial, equatorial OH groups; (*a*,*e*)] (**5−7**) and *manno* (*e*,*a*) (**8**) configurations, and diols with adjacent acyl (**5** and **7**) and *O*-alkyl (**6** and **8**) groups. Group (II) comprises *trans* diequatorial (*e*,*e*) (**9** and **10**) and *trans* diaxial (*a*,*a*) (**10**) diols.

The ^1H NMR parameters for the OH resonances of the group (I) galactose derivatives **5** and **7** with an *O*- or an *N*-acyl group in position 2, respectively, follow the same trend. The $^3J_{CH,OH}$ values for 3-OH are 9.0 and 8.5 for **5** and **7**, and 7.2 and 6.0 Hz, respectively, for the 4-OH resonances. The dependence of the chemical shift on temperature ($\Delta\delta/\Delta T$) for both 3-OH resonances is similar in each compound (−2.5 and −3.1 ppb/K), while for 4-OH the temperature coefficient is higher in **7** (−8.7 ppb/K) than in **5** (−2.2 ppb/K) and, in addition, $\delta_{OH3} > \delta_{OH4}$ for both compounds. In principle, two five-membered ring hydrogen-bonding networks may be envisaged, with *3-O−H → 4-O−H* (isomer a) or *4-O−H → 3-O−H* (isomer b) directions (Figure 3). The coupling values (Table 2) indicate the existence of an equilibrium between the two forms, with a major contribution from the *4-O−H → 3-O−H* b isomer, especially for compound **5**. Nevertheless, the data presented here (J and $\Delta\delta/\Delta T$) also indicate the presence in the equilibrium of the H-bonded isomer in which the five-membered ring intramolecular H bond (see Figure 3) with the *3-O−H → 4-O−H* H bond is present (mainly in **7**). In this case, the polarisation of the H-bonding network in the *3-O−H → 4-O−H* direction makes 4-OH a "good donor" in intermolecular processes. The analysis of the FT-IR spectra of **5** and **7** is also in accordance with the existence of both forms (Table 3). The frequencies of ν_i observed between 3546−3540 cm^{-1}, and the very similar values of A_f/A_i found for the two compounds (between 0.4−0.5 for **5** and **7**, respectively) imply that the involvement of 3-OH and 4-OH in intramolecular hydrogen bonds is similar for both compounds. The not complete agreement between NMR spectroscopic data and IR could be a reflection of the change of solvent from $CDCl_3$ to CH_2Cl_2.The 2-NH and 5-O moieties in **7** are at H-bonding distance (see Figure 3). In accordance with the presence of this intramolecular H bond (*N−H → 5-O*) in solution, the NH resonance has a ^3J value of 9.0 Hz (antiperiplanar conformation of the N−H and C−H bonds) and a very low $\Delta\delta/\Delta T$ value (−2.3 ppb/K). This H bond was confirmed by IR data. The NH stretching frequency of methylacetylamide, used as a model for the free N−H bond,[56] is at 3460 cm^{-1}. The difference in frequency between this value and that observed for the sugar amide **7** at 3430 cm^{-1} ($\Delta\nu_{NH}$ = 30 cm^{-1}) is consistent with the involvement of the NH bond of **7** in an intramolecular H bond.[34]

Comparison of **6** and **8**, both 1,2-*cis*-diols with one axial and one equatorial hydroxy group (*a*,*e*) and one adjacent *O*-alkyl group, gives some indication of the effect of the different positions of the diols with respect to the anomeric centre. Compounds **6** and **8** (see Table 1) show similar ^1H NMR parameters for both OH resonances. The similar values of $\Delta\delta/\Delta T$(OH) (−1.9 and −2.6 ppb/K for **6** and −1.1 and −2.5 ppb/K for **8**) indicate that both hydroxy groups are involved in intramolecular H bonds. However, the ratio of A_f/A_i varies between **6** (0.25−0.31) and **8** (0.32−0.59), indicating a lower average population of free OH groups in **6,** as can be seen from Figure 3. Furthermore, the value of ν_i is more than 10 cm^{-1} lower for **6** than for **8**, which clearly implies a higher average intramolecular hydrogen-bond strength in **6**, as would be expected for a cooperative array

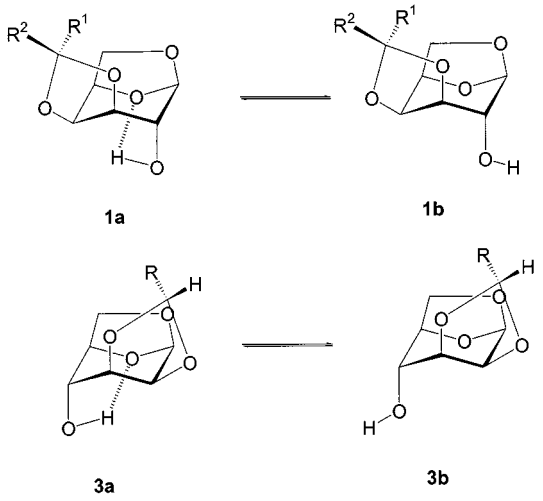

Figure 2. Intramolecular H bonds in monoalcohols **1** and **3**

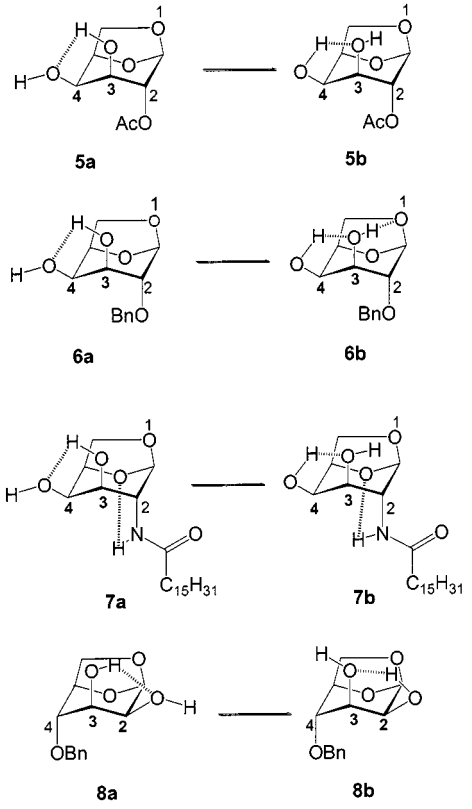

Figure 3. Intramolecular H bonds in 1,2-*cis*-diols **5**−**8**

(*4-O*−*H* → *3-O*−*H* → *1-O*). In these two derivatives, the natural tendency found in simple alcohols of axial hydroxy groups to be H-bond donors to equatorial hydroxy groups[41] is compensated, and an equilibrium of isomers is found. This observation is in agreement with Vasella's descriptions[46] for sugar diols. In the case of **6**, both H-bonding arrays are also taking place (**6a** and **6b**), as may be deduced by the $^3J_{CH,OH}$ values (8.4 and 7.8 Hz), but from the expected calculated *J* values, **6a** is less populated than the corresponding **5a** and **7a** rotamers. In the case of **8**, a similar H-bond isomer, equilibria may also be deduced from both the experimental NMR and IR data. In this case, and unlike that of **6**, the higher acidity of 2-OH may also contribute to the generation of the *2-O*−*H* → *3-O*−*H* H-bonding isomer, in which the equatorial 2-OH acts as a donor to the axial 3-OH (Figure 3). In this case, this observation is in complete agreement with the IR data.

The effect on the carbohydrate intramolecular H-bond network of the nature of the groups adjacent to the hydroxy group can be evaluated by comparing the H-bonding features found for compounds **5** and **7**, with adjacent *O*- and *N*-acyl groups, respectively, and **6**, with adjacent *O*-alkyl groups (Figure 3). In all cases, an equilibrium between both a- and b-type H-bonding isomers may be found. However, the relative abundance of both forms is different. This fact is probably due to the different natures of the acyl and the benzyl groups, which should modify the availability of the hydroxy groups to act either as better hydrogen-bonding donors or as acceptors.

Group (II) is composed of diequatorial (*e*,*e*) (**9** and **10**) and diaxial (*a*,*a*) (**10**) diols (Figure 4). As previously commented, the conformational analysis of the pyranose ring of the *3*,*4-trans* diequatorial (*e*,*e*) *diol* **9** indicated that, in chloroform solution, it adopts a major $^4C_1 \leftrightarrows B_{O,3}$ equilibrium. The OH resonances could not be unequivocally assigned, but they do present ^3J values of 2.4 Hz (small) and 6.6 Hz (intermediate), while the values of $\Delta\delta/\Delta T$ (−2.3 and −1.4 ppb/K) are small for both resonances. These NMR parameters can be explained on the basis of the equilibrium presented in Figure 4. At a first glance, the interpretation of the FT-IR spectrum of **9** in CH_2Cl_2 appears to be contradictory. However, the data is in accordance with the proposed equilibrium if the two bands that can be distinguished are attributed to intramolecular H-bonded hydroxy groups, with the free OH band being too weak to be observed. Thus, the intense band at 3592 cm^{-1} may be characteristic of a five-membered ring *trans* H bond (present in **9b** and **9c**), and the weaker band, which appears as a shoulder at around 3552 cm^{-1}, could be due to a six-membered ring H bond (in isomers **9a** and **9b**). In this case, the presence of the aforementioned H-bond isomer equilibrium in solution does not generate a particularly "good donor" H-bond centre in the molecule.

The 3,4-*trans* diaxial diol (**10**) features a neighbouring amide group in a 1,3-diaxial configuration with respect to 4-OH (see Figure 4). This particular configuration leaves the amide 2-NH at a H-bonding distance from 4-OH (six-membered ring *cis* H bond). This H bond was confirmed by the value of the NMR parameters of 2-NH ($^3J_{NH,H\text{-}2}$ = 9.0 Hz and $\Delta\delta/\Delta T$ = −1.5 ppb/K). The OH resonances showed medium-sized $^3J_{CH,OH}$ values, 6.1 Hz (3-OH) and 4.3 Hz (2-OH), but small $\Delta\delta/\Delta T$ values, namely −2.1 (3-OH) and −1.6 ppb/K (2-OH). Thus, the ^{1}H NMR spectroscopic data for **10** are consistent with 2-NH acting as a donor to 4-OH and/or 5-O, with neither OH group strongly involved in intramolecular hydrogen bonds. Indeed, their involvement in hydrogen bonds should give much higher $^3J_{CH,OH}$ values (see Table 2). Moreover, the FT-IR spectrum shows a free OH band at 3597 cm^{-1}, in agreement with the NMR spectroscopic data. As mentioned previously, the difference between ν(NH) of **10** (3425 cm^{-1}) with that for the model of a free amide (*N*-methylacetamide, $\Delta\nu_{NH}$ = 35 cm^{-1}) confirms the presence of an intramolecular H bond, *2-N*−*H* → *4-O*−*H* in **10** (see Figure 4). In this case, 4-*OH* is a "good donor" (cooperative donor), able to participate efficiently in intermolecular processes.

The 4,6-benzylidene-β-D-glucopyranoside **11**, a *2*,*3-trans* diequatorial diol (*ee*), showed very small and similar $J_{CH,OH}$ and $\Delta\delta/\Delta T$ values for OH resonances ($^3J_{CH,OH}$ = 2.5 Hz and $\Delta\delta/\Delta T$ = −2.7 and −2.2 ppb/K). The presence of a vicinal acceptor group (OR) in a 1,2-*trans* configuration with respect to both OH groups (O−R groups in positions 1 and 4 of the pyranose ring) permits an equilibrium of H-bonding isomers in solution (Figure 4). The expected couplings for both OH resonances in a-, b- and c-type hydrogen-bond isomers are analogous. In all putative isomers of **11**, both hydroxy groups are involved in a five-membered ring

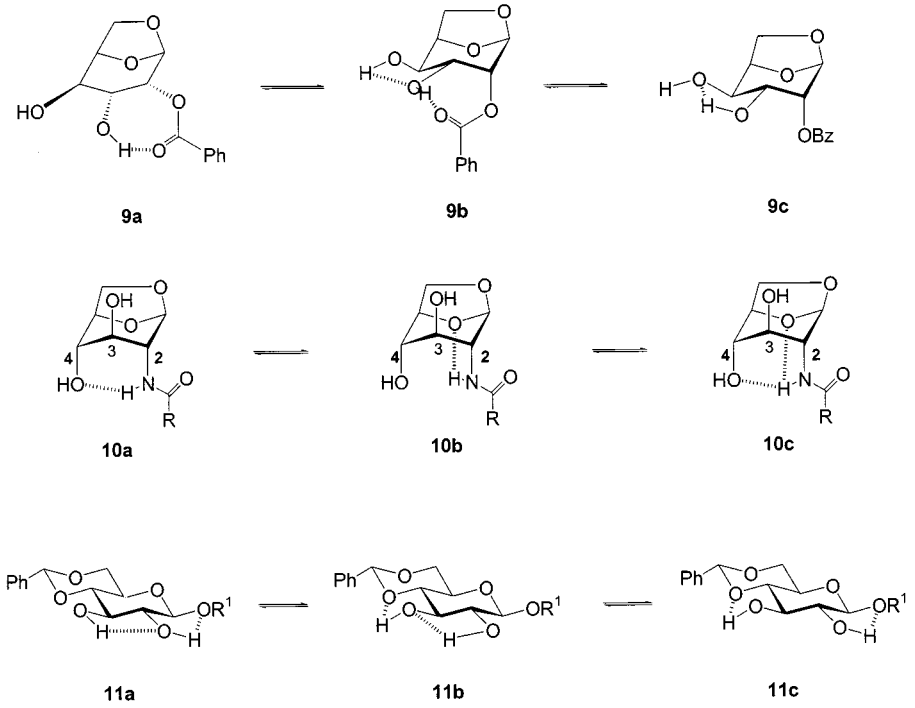

Figure 4. Intramolecular H bonds in 1,2-*trans*-diols **9**−**11**

trans H bond in which the hydroxy groups are H-bond donors to the ether oxygen atom of the adjacent 1-OR and 4-OR hydrogen atoms. The FT-IR spectra of **11** exhibits a unique OH stretching band at 3590 cm^{-1}, which is characteristic of a five-membered ring *trans* H bond. It appears that both 2-OH and 3-OH are involved in the same type of intramolecular H bond, which is only feasible if **11c** is viewed as the major isomer. Thus, both OH groups are H-bond donors in intramolecular H bonds, which determines that the H-bond network does not generate any "good donors" (cooperative donor), and terminates within the molecule.

1.4 Intramolecular Hydrogen Bonds in the 1,3-Diols 12−16

Intramolecular hydrogen bonds within 1,3-diols are known to be stronger than those existing within 1,2-diols.[42,60,65] A series of 1,3-diols (**12**−**16**) was chosen in order to evaluate the influence of conformational flexibility on the H-bonding network (see Figure 1). By this criterion, these compounds can be classified into two groups. Group (I) could be described as conformationally rigid, since the compounds present a 1,3-*cis* diaxial diol configuration due to the position of both hydroxy groups in a six-membered ring of a $^1C_4$ chair (**12**, **13** and **16**). Group (II) is made up of conformationally flexible diols, since the H-bond centres involve positions 4 and 6 of a pyranose ring; and their flexibility is derived from the existence of rotational freedom about the C5−C6 bond of the hydroxymethyl group.

The rigid 1,3-diols derived from 1,6-anhydro-β-D-glucopyranoside 2,4-*cis* diaxial diols (*a*,*a*) **12**−**13** and glucuronic acid **16** [2,4-*cis* diaxial diol (*a*,*a*)] differ in the relative configurations of the anomeric residue (axial **12**/equatorial **16**) (see Figure 5). Both geometries allow the formation of a six-membered ring *cis* H bond between the two hydroxy groups in a rigid framework: the $^1C_4$ chair of the pyranose ring (see Figure 5). The flexible 1,3-diols (**14** and **15**) involve positions 4 and 6 of a pyranose ring and differ in the relative configuration of the hydroxy group in position 4, axial in galactose (**15**) and equatorial in glucose (**14**).

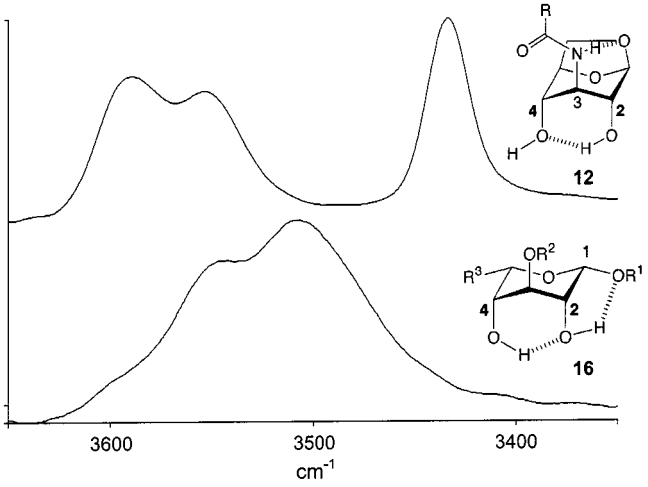

Figure 5. Intramolecular H bonds and FT-IR spectra of rigid 1,3-*cis* diaxial diols **12** and **16**

The hydroxy resonances (2-OH and 4-OH) of diols **12** and **13** showed clear differences in their ^1H NMR parameters (see Tables 1 and 2). The $^3J_{CH,OH2}$ value of ca. 10 Hz is consistent with a fixed conformation of the H−C−O−H angle at about 170°, and with participation in an H bond. The value of $\Delta\delta/\Delta T$ of the 2-OH resonance of **12** (−2.5

ppb/K) is also indicative of its involvement in an intramolecular H bond. Molecular modelling suggests that it could be H-bonded to 4-OH and/or 5-O. On the other hand, the 4-OH resonance has a medium-sized $^3J_{CH,OH}$ value of 5.4 Hz and a $\Delta\delta/\Delta T$ value of −4.3 ppb/K, both consistent with the 4-OH proton being exposed to the solvent. The amide resonance of **12** has ^{1}H NMR parameters $^3J_{NH,CH}$ = 8 Hz and $\Delta\delta/\Delta T$ = −1.2 ppb/K, indicating that the NH proton participates in an intramolecular H bond to the closest H-bond acceptor, the 1,6-anhydro bridging oxygen atom 1-O (see Figure 5). The direction of the hydrogen bond from 2-OH to 4-OH in **12** was further supported by partial deuteration experiments. The spectra of a partially deuterated sample (60%) of **12** in $CDCl_3$ at low concentration showed a negative isotopic effect (−16.5 ppb) for the 2-OH resonance, which revealed that 2-OH behaves as H-bond donor to 4-OH.[66] The IR data obtained for **12** complements the ^{1}H NMR spectroscopic data; two bands of similar intensity are observed in the IR spectrum at ν_f = 3591 cm^{-1} and ν_i = 3551 cm^{-1} (see Table 3). The relative intensities of these bands, $A_f/A_i \approx 1$, could be regarded as additional evidence of an equimolar distribution of free and intramolecularly bound OH groups, with the bound OH frequency consistent with that of a six-membered ring intramolecular H bond. No clear indication of the presence of an absorption band at around 3572 cm^{-1}, which would be characteristic of a five-membered ring intramolecular H bond involving *2-O−H → 5-O*, can be observed. These results support the intramolecular H-bond array shown in Figure 5 for **12** and **13**. Thus, the H bond between the two diaxial hydroxy groups, *2-O−H → 4-O−H* in **12** and **13**, is highly directional, and so generates a "good H-bond donor" centre (4-OH) and a "good H bond acceptor" centre (2-OH). This polarisation is probably due to the higher acidity of 2-OH compared to 4-OH, due to its proximity to the anomeric centre.

The two OH groups of the rigid 1,3-*cis*-diol (*a*,*a*) **16**[67] are in the same position with respect to the anomeric centre and present the same relative configuration as those in **12**, but the anomeric substituent is equatorial instead of axial (see Figure 5). It is important to point out that the ^{1}H NMR parameters of the OH resonances of **16** are very different from those previously mentioned for **12**. The *J* values ($^3J_{CH,OH2}$ = < 1 Hz and $^3J_{CH,OH4}$= 11.4 Hz) and $\Delta\delta/\Delta T$ values (−2.5 and −1.3 ppb/K for 4-OH and 2-OH, respectively) of the OH resonances of **16** are indicative of the participation of the OH protons in intramolecular H bonds and, to lend further support to this interpretation, the OH protons resonate at very low field (δ = 3.76 and 2.79) compared to the rest of the diols studied. The IR spectrum of **16** shows two clearly defined OH absorption bands (Table 3) corresponding to a five-membered ring *cis* intra H bond (at 3554 cm^{-1}), and a six-membered ring *cis* H bond (at 3507 cm^{-1}), agreeing with the NMR spectroscopic data and confirming the presence of two intramolecular H bonds.[67] The $\nu(OH)_{free}$ appears as a weak shoulder with ν_f estimated at around 3589 cm^{-1}. The low wavenumber values of the most intense absorption bands for diol **16** are indicative of strong intramolecular H bonds. These experimental IR data are evidence for the cooperative strengthening of both intramolecular H bonds (*4-O−H → 2-O−H → 1-OR*) (see Figure 5).

Thus, the different relative configuration of the neighbouring acceptor group 1-OR, which is 1,2-*cis* in **16** and 1,2-*trans* in **12**, is at the origin of the inversion of the directionality of the H-bonding network in these molecules. The 2-OH of **16** can be an H-bond donor to 1-O (five-membered ring *cis* H bond), which gives rise to a drastic change in the directionality of the H-bonding networks in both molecules, thus modifying the "cooperative nature" of the H-bonding centres. Whilst 4-OH in **12** is a "good cooperative donor", ready to become involved in an intermolecular process, this hydroxy group in **16** is involved in a strong intramolecular H bond, thus acting as a "good cooperative acceptor".

We also compared the effect of conformational rigidity on the formation of a 1,3-intramolecular H bond by choosing two flexible diols, the glucose form (4eq, 6-OH) **14** and the galactose analogue (4ax, 6-OH) **15**. The two derivatives, from their NMR parameters (see Table 1), show the hydroxy group at position 4 involved in intramolecular H bonds ($^3J_{CH,OH4}$ = 2.4−2.8 Hz, $\Delta\delta/\Delta T$ = −1.8/−2.0 ppb/K) either with 6-OH or with 3-OR. The 6-OH resonance of the *gluco* configuration derivative showed medium-sized *J* values (5.7/6.6 Hz for **14**), indicating that 6-OH in the major H-bonding isomer in solution is probably "a free OH". For the galactose derivative **15**, in contrast, this 6-OH resonance showed *J* values that suggest that the rotational freedom of the OH group is restricted by one intramolecular H bond ($^3J_{CH,OH6}$ = 3.9 and 9.3 Hz and $\Delta\delta/\Delta T$(6-OH) = −3.3 ppb/K). We thus decided to estimate the rotamer population around C-5−C-6 for **14** and **15**, from the ^3J values ($^3J_{H5,H6}$ and $^3J_{H5,H6'}$), with the intention of correlating the structural information implicit in *J* values with the presence of a particular population of H-bonding isomers in solution. In these cases, 6-OH could be H-bonded to either 4-OH or 5-O, for each diol. This information, together with the $^3J_{CH,OH}$ values, allowed us to identify the major H-bonding isomer present in solution for each diol. Since direct *J* measurement was impossible, due to significant spectral overlap, selective inversion of the 1-H resonance experiments was required in order to assign 5-H and to measure the $^3J_{H5,H6}$ and $^3J_{H5,H6'}$ values. Assignment of the 6-H *proS* and *proR* resonances was not possible. For this reason, the population of each rotamer was calculated by considering both possible assignments of 6-H protons. The calculation was performed by assuming the existence of three possible alternated conformations: *gg*, *gt* and *tg*. The populations for each rotamer can be calculated by using both *J* values, $J_{5,6S}$ and $J_{5,6R}$, and taking into account the obvious fact that the total population for the three conformers has to be 1.[68,69] Table 4 shows the obtained population distribution estimated for rotamers around C-5−C-6 for **14** and **15**. The *gg* rotamer was the most populated for **14** in each possibility (A and B), in agreement with the expectations for glucose derivatives. In this rotamer, 6-OH is never at H-bond-

Table 4. Distribution of rotamer populations around C-5−C-6 for diols **14** and **15** in chloroform

[a]	*J* [Hz]	*gg*	*gt*	*tg*
14 (A)	$J_{5,6s}$ = 4.6 $J_{5,6R}$ = 4.4	53%	17%	30%
14 (B)	$J_{5,6s}$ = 4.4 $J_{5,6R}$ = 4.6	52%	21%	27%
15 (A)	$J_{5,6s}$ = 6.0 $J_{5,6R}$ = 5.5	35%	23%	42%
15 (B)	$J_{5,6s}$ = 5.5 $J_{5,6R}$ = 6.0	34%	30%	36%

[a] A and B are the two possible *J* value assignments.

ing distance (*r*) either from 4-OH or from 5-O (see Figure 6, a). This is in agreement with the intermediate ^3J values measured for 6-OH (free OH). For the galactose derivative **15**, it should be mentioned that form (A) has previously been found[68] for similar derivatives in chloroform solution. In **15**A, the three rotamers will allow 6-OH to be H-bonded either to 4-OH or to 5-O (Figure 6, b) The existence of a hydrogen bond for 6-OH is also in agreement with the $^3J_{OH,H6}$ and $^3J_{OH,H6'}$ values. The IR spectra of these diols are not conclusive, and it is impossible to assign the bands clearly, since a considerable degree of overlapping can be observed in both compounds. However, it is clear from the spectra that equilibrium of H-bonding isomers exists, and that the population of H-bonded species is higher in **15** than in **14**. In any case, the small expected values of $^3J_{OH,H4}$ in both cases (Table 2), seem to indicate that they adopt particular orientations, probably involved in intramolecular hydrogen bonding. Nevertheless, although the expected couplings in both possibilities (hydrogen-bonded either to 3-O or to 6-O) are always small and these small values are in agreement with the experimental data, the geometries provided by the MM3* calculations indicate that the 4-OH moieties in both **14** and **15** are most probably hydrogen-bonded to 3-O.

To conclude, our results indicate that the conformational differences between the rigid (**12** and **16**) and flexible (**14** and **15**) 1,3-diols are responsible for their differential H-bonding behaviour and result in different degrees of effectiveness of the six-membered ring H bond. The fixed positions of the hydroxy groups in **12** and **16** favour the existence of highly populated H-bonded isomers with stable six-membered ring H bonds. The directionality of the H bond is dependent on the nature and orientation of the neighbouring groups. However, the rotational freedom about C-5−C-6 in diols **14** and **15** results in conformational equilibria in which the six-membered ring H-bonded isomers are not heavily populated, as in **12** and **16**. Therefore, conformational flexibility is also a key point to take into ac-

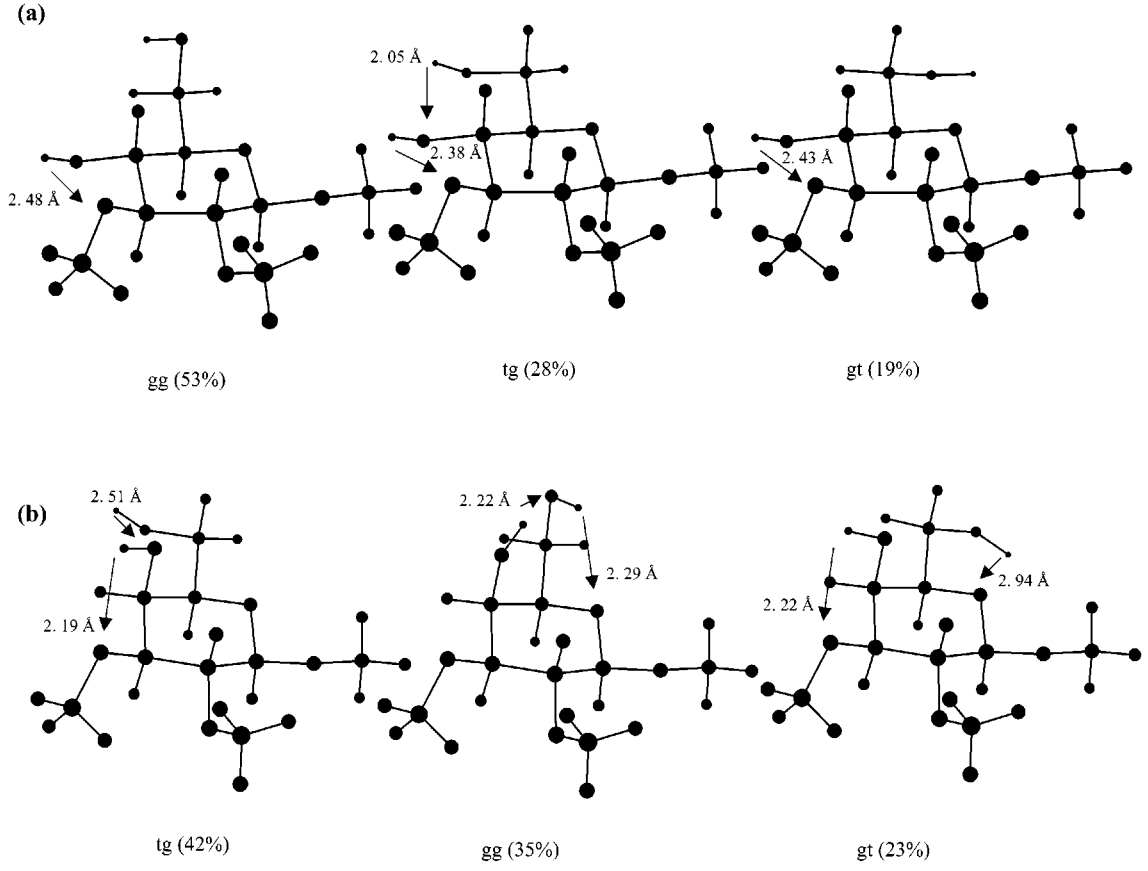

Figure 6. Intramolecular H bonds for 1,3-flexible diols **14** and **15**; distribution of rotamer population (C-5−C-6) for diol **14** (a) and **15** (b) in chloroform

count in the design of carbohydrate moieties with effective cooperative H bonds.

2. Complexes with Pyridine − Strengthening of the Intramolecular H Bond

The complexing properties of the diols with an H-bond acceptor − pyridine (Py)[70] − in nonpolar media were also explored. The aim was to investigate the influence and relevance of the intramolecular H-bond networks, as key features by which to identify diols that favour this intermolecular process. Furthermore, this study also provided experimental evidence on the directionality of the intramolecular H-bond network, locating the specific hydroxy group that, thanks to intramolecular H-bond formation, acts in the diols as a "good H-bond donor centre".

The binding constants of the complexes formed by the diols **1**−**16** and pyridine were determined by ^{1}H NMR titration in $CDCl_3$. Chemical-induced shifts of the OH resonances upon addition of small amounts of Py (see Table 5) were followed. The 1,2-*cis*-diol (*a*,*e*) **5**, 1,3-*cis*-diol (*a*,*a*) **12** and 1,3-*cis*-amidoalcohol (*a*,*a*) **10** exhibited the strongest interaction with pyridine: between −1.4 and −1.3 kcal mol^{-1}. From the chemical-induced shifts of the sugar OH resonances on coordination to Py, complexation was inferred to occur at 4-OH; for all these sugar−Py complexes, the 4-OH resonance shifted 1.4 ppm further downfield than the 3-OH resonance. No analysis of the *J* parameters for the hydroxy groups was performed, since for the Py/sugar molar ratio employed and the weak binding constants, the percentage of bound state was rather small, the *J* parameters not appreciably changing from their free-state values. From the results described above, the intramolecular H-bond network may generate a "good H-bond donor" (4-OH), and so the intramolecular H-bond network seems to be playing a role in this intermolecular process. Notably, the most relevant result comes from comparison of the different Py complexing abilities shown by the 1,3-rigid *cis*-diols (*a*,*a*) **12** and **16** (see Table 5). Whilst **12** formed a very stable complex with Py (−1.3 kcal mol^{-1}), **16** did not interact at all under the same conditions. This result, once again, reflects a difference in the intramolecular H bonding between **12** and **16**. As mentioned previously, **12** seems to present a "cooperative H-bond donor OH" (4-OH), whilst **16** does not, since both 2-OH and 4-OH groups are involved in strong intramolecular H bonds. The complexation of an H-bond acceptor, based on the intramolecular H-bond network of **16**, is anti-cooperative.[71] Thus, anti-cooperativity is playing an active role in not allowing the interaction of **16** with Py.

The flexible 1,3-diols **14** and **15**, involving positions 4 and 6 of glucose and galactose, also show very different behaviour towards Py, once more consistent with the proposed intramolecular H-bonding network (Figure 6). The galactose derivative **15** does not interact with Py, whilst the glu-

Table 5. Binding constants of pyridine complexes formed by **1**−**16** in $CDCl_3$

	Signal	$\Delta\delta_{exp.}$ [a] [ppm]	Saturation[b] [%]	K_a (error)[c] [M^{-1}]	$\Delta G°$ [kcal mol^{-1}]	K_a [d] [M^{-1}]
1	2-OH	+1.873	43	5.3 (2.0%)	−0.9	−
3	4-OH	+1.679	32	3.5 (0.8%)	−0.7	−
5	3-OH	+1.123	57	9.6 (1.1%)	−1.3	**10.5 ± 0.9**
	4-OH	+2.480	61	11.0 (1.4%)	−1.4	
	6-H*endo*	+0.076	59	10.3 (1.4%)	−1.4	
6	3-OH	+1.246	44	5.4 (0.6%)	−1.0	5.4 ± 0.1
	4-OH	+1.897	44	5.4 (0.7%)	−1.0	
7[e]	3-OH	+0.669	45	5.8 (1.2%)	−1.0	6.6 ± 0.9
	4-OH	+2.045	49	6.9 (1.5%)	−1.1	
8	2-OH	+1.123	55	8.6 (1.6%)	−1.3	8.1 ± 0.7
	3-OH	+2.480	53	7.7 (2.3%)	−1.2	
	6-H*endo*	+0.076	56	8.8 (1.6%)	−1.3	
9	3-OH	+1.712	51	7.2 (1.2%)	−1.2	6.8 ± 0.9
	4-OH	+1.292	48	6.3 (1.2%)	−1.1	
10[e]	2-NH	+0.235	58	10.5 (1.6%)	−1.4	**11.1 ± 0.4**
	4-OH	+2.502	59	11.2 (2.2%)	−1.4	
11	2-OH	+1.148	39	4.4 (1.1%)	−0.9	4.4 ± 0.0
	3-OH	+1.051	39	4.4 (1.1%)	−0.9	
12[e]	2-OH	+1.145	54	8.1 (0.3%)	−1.2	**8.7 ± 0.9**
	4-OH	+2.053	56	9.1 (1.2%)	−1.3	
14	4-OH	+0.738	22	2.0 (1.8%)	−0.4	1.9 ± 0.2
	6-OH	+0.439	20	1.8 (1.8%)	−0.3	
15	4-OH	+0.164	7	0.5 (1.4%)	+0.4	−
	6-OH	+0.637	11	0.9 (1.0%)	+0.1	
16	2-OH	+0.157	14	0.3 (2.6%)	+0.7	−
	4-OH	+0.073	16	1.1 (1.4%)	−0.2	

[a] $\Delta\delta_{experimental} = \delta_{free} - \delta_{bound}$. [b] % of complex. [c] Deviation of the experimental data from calculated. [d] Mean value considered. [e] The NH resonance was not taken into account for the obtained average K_a media because the measured value differs from the rest by more than 0.3 kcal mol^{-1}.

cose derivative **14** binds to it weakly, presumably through the 6-OH group. This 6-OH is a "free hydroxy" group (independent), and thus less effective as an H-bond acceptor than a "good H-bond acceptor", such as 4-OH in **10** and **12**.

In summary, two main conclusions can be drawn from the pyridine binding experiments:

i. When there is a "good (cooperative) donor H-bond centre" in the sugar, the intermolecular interaction with an acceptor is more effective than in those sugars that only present "free OH" groups. This conclusion arises from comparison of the ΔG^{o} values of the complexes of monoalcohols (**1** and **3**), and 1,3-diols with 6-OH free (**14**), with the ΔG^{o} values of the complexes formed by 1,2-*cis*-diols (**5−8**), and 1,3-*cis*-diol (*a*,*a*) and amidoalcohol (**12** and **10**).

ii. The intramolecular H-bond networks in **15** and **16** terminate within the molecule (intramolecularly) and these molecules therefore do not present a donor OH group capable of coordinating pyridine. Thus, anti-cooperativity is also very effective and diols **15** and **16**, which have stable intramolecular H bonds, do not interact with Py.

When H-bond cooperativity influences an intermolecular process between an intramolecularly H-bonded substrate and an H-bond acceptor, the intramolecular H bond that is at the origin of the cooperative effect is also strengthened.[22,23,44,72] As a similar example, Lemieux observed that addition of an equimolar quantity of [D_6]DMSO (H-bond acceptor) to a monosaccharide solution induced a conformational change, which he associated with the strengthening of an intramolecular H bond.[73]

FT-IR spectra of dilute solutions of the sugar diols **1−16** were also recorded in dry CH_2Cl_2/Py (7%). The aim of these experiments was to use OH frequency shifts as a measure of the perturbation of the O−H stretching vibration upon interaction with the H-bonding acceptor molecule. In principle, it should be expected that, in the presence of a given H-bond acceptor, the more acidic the alcohol, the stronger the H bond formed. In any case, different levels of perturbation should be expected, depending on the relative configuration of OH groups.

The IR spectra of the monoalcohols **1** and **3** showed that the absolute intensity of both νOH_{free} and νOH_{intra} bands decreased in the same proportion upon addition of Py. A single fairly symmetrical intermolecular association band appeared with a maximum at around 3190 cm^{-1}. In contrast, the IR spectra obtained from the Py binding experiments of the diols were rather more complicated combination spectra. Indeed, they included contributions both from complexed and from noncomplexed sugar molecules, each species with its own particular conformational differences. In an attempt to simplify the spectroscopic data, we adopted the differential method suggested by Kleeberg et al. as a reasonable criterion for subtracting the spectral contributions from the noncomplexed molecules. Thus, the extinction coefficient, ε, was adjusted at the observed free OH frequency, ν_f to $\varepsilon(\nu_f)\cdot\approx 0$, by interactive subtraction of the sugar/CH_2Cl_2 solution from the sugar−CH_2Cl_2/Py solution. According to Kleeberg's protocol,[44] analysis of the difference spectra [(1,4-butanediol + Py) − 1,4-butanediol] indicated that the addition of bases to a diol solution should result in the appearance of two new bands at the expense of the OH_{free} and OH_{intra} bands. In the complexes $OH_A\cdots OH_B\cdots$Py, the band corresponding to $\nu(OH_A)$ appeared at a higher frequency than that corresponding to $\nu(OH_B)$. In our systems, however, considerable degrees of overlapping of these bands were observed and they turned to be much broader than the OH_{free} and OH_{intra} bands. In order to obtain a proper and reliable differentiation between the inter- and intramolecular associations, the study of such systems by low-temperature matrix-isolation methods would be desirable.[23] Nevertheless, a preliminary analysis of our spectra allowed us to gain some insight into the nature of the intermolecular association region of the difference spectra. In most of the compounds studied, the complexed systems showed at least two new bands in this region when compared with the sugar solutions alone. One of them appeared at a lower frequency, and may be associated with the OH group involved in the intermolecular OH···Py hydrogen bond, which we have defined as OH_B. The second one could correspond to the OH group involved in a perturbed or cooperatively strengthened intramolecular OH···OH bond, which we have defined as OH_A. The observation of the latter band at the high frequency side of the OH_B observed for the monoalcohols is in agreement with the observations of Kleeberg et al.[44] The precise frequencies of these bands were very difficult to measure, even after spectral subtraction of the noncomplexed sugar by a combination of spectroscopic techniques such as curve-resolving and derivative spectra. However, estimations were obtained for the complexes of **5**, **6**, **7**, **8** and **12**. The 1,2-*cis*-diols **5−8** exhibited approximated νOH_A values oscillating between 3330 and 3270 cm^{-1} and νOH_B values between 3170 and 3130 cm^{-1}. The 1,3-*cis* diaxial diol **12** also showed evidence for at least two broad, overlapping absorptions, associated with νOH_A at 3306 cm^{-1} and νOH_B at 3153 cm^{-1}.

In order to compare the relative strengthening of the intramolecular OH···OH hydrogen bonds in the Py-complexed systems, a cooperativity factor based on the work of Maes et al.[23] was also estimated. In our case, the cooperativity factor *A* was defined as a numerate value representing the relative cooperative effect that the intermolecular interaction $OH_B\cdots$Py has on the intramolecular hydrogen bond $OH_A\cdots OH_B$: $A = \Delta\nu_A/\Delta\nu_i$, where the $\Delta\nu$ values are calculated with respect to the free OH bond frequency for each compound, such that $\Delta\nu_A = \nu_f - \nu_A$, and $\Delta\nu_i = \nu_f - \nu_i$.

Cooperativity factors calculated in this manner are numbers with values > 1, which represent the strengthening of the intramolecularly associated OH_A bond with respect to the unperturbed OH_{intra} bond. Several options for estimating $\Delta\nu_A$ and $\Delta\nu_i$ have been reported.[60] In order to calculate *A*, we employed the observed values for the free OH stretch, ν_f. However, we did not calculate the cooperativity factor *B*, due to the lack of a suitable monoalcohol with a fully free OH group as a model for the $OH_f\cdots$Py interaction. The

Table 6. Specific IR frequencies for sugars in CH_2Cl_2 and in the difference spectra, and calculated cooperativity factors, *A*, for the strengthening of the intramolecular H bond $OH_A\cdots OH$ due to the formation of the intermolecular H bond, $OH_B\cdots Py$

IR[a]	Sugar in CH_2Cl_2		Difference spectra[a] of sugar in CH_2Cl_2/Py		*A*[b]	
	ν_f [cm^{-1}]	ν_i [cm^{-1}]	νOH_A [c] [d] [cm^{-1}]	νOH_B [c] [cm^{-1}]	ν_{other} [e] [cm^{-1}]	
5	3602	3546	3330	3133	3515	4.9
6	3605	3543	3320	3141	3520	4.6
7	3600	3540	3280	3165	3493	5.3
8	3605	3555	3270	3167	3525	6.7
12	3591	3551	3306	3153	3525	7.7

[a] See text for explanation. [b] Calculated as described in text. [c] Approximate bandhead frequency for broad overlapping bands which appear between 3350 and 3100 cm^{-1} according to previous work; ref.[44] [d] Shoulder. [e] Bands observed in the intramolecular association region.

observed frequencies in the complexed and noncomplexed sugars and the calculated values of the cooperativity factor *A* obtained for compounds **5**−**8** and **12**, given in Table 6, showed that, in all cases, the intramolecular hydrogen bonds are significantly strengthened upon formation of the intermolecular OH_B ··Py H bond. The most significant cooperative effect was found for the highly stable six-membered intramolecular H bond in **12** with respect to the 1,2-diols.

Conclusions

The directionalities and strengths of the intramolecular H-bonding networks of compounds **1**−**16** in nonpolar media have been determined from ^1H NMR and FT-IR parameters. In the pyranose cyclic form of naturally occurring monosaccharides, both rigid and flexible 1,3-diol and 1,2-diol motifs can, in general, be found. Our results have shown that, in terms of geometry, carbohydrate OH···OH H bonds share some features with those of simpler hydroxylated compounds, such as cyclohexanediols. Intramolecular OH···OH hydrogen bonds are more stable in 1,3-diols than in 1,2-diols, and more stable in 1,2-*cis*-diols than 1,2-*trans*-diols. However, what differentiates the carbohydrates is the presence of functional groups adjacent to these hydroxy groups, such as OR, NHCOR (amido), the anomeric centre, and 5-O, which play an active role in the carbohydrate H-bonding network. Their participation implies a polarisation of the H bonds between OH groups, which modulates the final directionality and strength of the intramolecular OH···OH H bonds of a given carbohydrate. Their H-bonding character (H-bond donor or acceptor groups) and location (position and relative configuration) with respect to the diol motif determines whether the intramolecular H-bond network terminates within the molecule, leaving only acceptor H-bond centres in the molecule. This fact is extremely important for the definition of the nature of the molecular recognition processes in which a carbohydrate is able to participate, because it determines whether or not "good H-bond acceptors and/or donors" are formed.

In carbohydrate derivatives containing a 1,2-*cis* (*a*,*e*) diol motif, the natural tendency of the axial OH to be an H-bond donor to the equatorial OH, as seen in simpler diols, may be modified, depending on the situation of the equatorial OH with respect to the anomeric centre, and the presence of neighbouring groups, as also observed by Vasella and co-workers. The equatorial OH can also be an H-bond donor, which gives rise to an H-bond isomer mixture that can be detected in solution, and the tendency can even be reversed.

Some basic rules for rational design of carbohydrate derivatives with OH···OH hydrogen-bonding motifs useful for molecular interactions in apolar media can be devised from our results. One of them is that neighbouring groups can be strategically located to polarise intramolecular H bonds with an intended directionality. This will enhance the donor or acceptor character of a particular OH, making it a cooperative H-bond centre. An electron-withdrawing neighbouring group makes the closest OH more acidic, polarising the intramolecular H-bonding network in the direction in which that OH acts as H-bond donor and, thereby generating a major H-bond isomer. The hydroxy group at position 2 of the pyranose ring is more acidic than the others, due to its proximity to the anomeric centre. This makes it a better H-bond donor.

A group with a unique H-bond donor or acceptor character, located at H-bonding distance to a particular OH, can be used to initiate the H-bond network and polarise it in the desired direction.

Intramolecular H bonds are more favourable than intermolecular ones. Therefore, if an intramolecular H-bond network can terminate within the sugar molecule, it does. Neighbouring H-bond acceptor groups in a *cis* configuration relative to the last OH involved in the intramolecular H bond make the H-bond network terminate intramolecularly. Thus, only "good acceptors" (cooperative acceptors) are generated in the molecule. Therefore, if a "good donor" (cooperative) OH is required for intermolecular interactions, the sugar derivative should lack a 1,2- or 1,3-*cis*-related acceptor group at an H-bonding distance; otherwise the H bond will be formed intramolecularly.

The effectiveness of 4-OH as an H-bond donor in **12** is due to the high stability and suitable directionality of the intramolecular H bond involving 2-OH···4-OH. The 1,3-*cis* diaxial diol located in a rigid framework generates the most

stable intramolecular H bond of all the carbohydrate derivatives studied here. This rigid diol contains all the features mentioned above as needed to be an effective and cooperative H-bond donor centre. The amide in position 3 makes both OH groups more acidic; however, 2-OH has its H-bond donor character additionally enhanced due to its proximity to the anomeric centre. This factor is essential to polarise the intramolecular H bond in the direction 2-O−H → 4-O−H. The lack of an H-bond acceptor group at an H-bonding distance to 4-OH (6-OH is involved in the 1,6-anhydro bridge) avoids the intramolecular termination of the H-bond network. 4-OH is an ideal cooperative H-bond donor ready to participate in intermolecular processes.

The pyridine complexing experiments have shown that the interaction mediated by a hydroxy group involved in an intramolecular H bond as an acceptor ("good donor", or a cooperative donor) is more effective (**10** and **12**) than that involving a "free OH" (i.e., **14**). Additionally, the results indicate that the existence of a stable intramolecular H-bond network is not enough to enhance the intermolecular processes by cooperativity. It is also necessary to have adequate directionality. This means that the anti-cooperative effect is also effective in counteracting the intermolecular process in nonpolar media. The best evidence for this is the significant difference between **12** and **16** on complexation with Py. The former gives a very stable complex (presents a "good donor" H-bonding centre), whilst the latter does not interact.

The cooperative strengthening of the intramolecular *OH* → *OH* H bonds by intermolecular complexation of Py was also measured by IR spectroscopy. The results show that all intramolecular H bonds are strengthened upon complexation with Py. The intramolecular H bond that shows the largest degree of strengthening is the most stable (present in **12**). Although this has been predicted by theory, little experimental evidence for this observation has appeared in the literature to date.

In order to allow carbohydrates to use their OH groups in cooperative intermolecular processes, a stable H bond with correct directionality is needed. Molecules and functional groups strategically placed to interact within the H-bond network can be used as "additives" to polarise the network suitably and hence to obtain the energetic advantage from H-bonding cooperativity.

Once a well-defined H bond is formed between the OH and amido groups of a sugar pyranose ring, these H-bonding groups no longer act as independent H-bonding centres, but as H-bonding arrays. This fact introduces a new perspective on carbohydrate OH groups and, as such, it is extremely important for de novo molecular recognition design purposes, at least in nonpolar media.

We are at present extending this study on the basic features of the H-bonding behaviour of carbohydrate OH groups in nonpolar solvents to aqueous media. We believe that at least the strongest H bonds in noncompetitive media may have some relevance for the formation of cooperative intermolecular H-bond arrays in water solution. Moreover, the relevance of a cooperative H-bonding network in the molecular recognition of a carbohydrate by a protein in aqueous solution has already been demonstrated by NMR.[74]

Experimental Section

Compounds and Solvents: Carbohydrate derivatives **1**−**16** were synthesized (see synthesis section in the Supporting Information), and dried under high vacuum and heated overnight at 40 °C in the presence of P_2O_5 prior to the ^{1}H NMR and FT-IR experiments. All ^{1}H NMR studies were performed with freshly prepared solutions in $CDCl_3$, which was always passed through basic alumina and collected over 4 Å molecular sieves. The alumina and molecular sieves employed were freshly activated by heating at 600 °C under high vacuum. All FT-IR experiments were carried out with freshly prepared solutions in CH_2Cl_2. Pyridine used in binding studies and CH_2Cl_2 for IR experiments were distilled and dried according to conventional methods and stored over 4 Å molecular sieves and under Ar.

^{1}H NMR Experiments: The binding abilities of compounds **1**−**16** to pyridine were investigated by ^{1}H NMR titration experiments. Carbohydrate derivative solutions were obtained by diluting a known volume of a stock solution, previously prepared by dissolving a weighed amount of sugar in $CDCl_3$, to 2.5 mL. Then, 0.5 mL of the resulting solution was introduced into the NMR tube and 1.9 mL was used to obtain the titrant sugar/pyridine (0.19 M) solution. The carbohydrate concentrations ranged between 8×10^{-5} M and 1×10^{-3} M, depending on the auto-association ability of the particular carbohydrate. A minimum of twelve additions of the sugar/pyridine solution were made to the sugar solution, and one 1D NMR spectrum (299 K) was recorded after each addition. The experiments were repeated twice. The binding constants were extracted from a least-squares fitting, using a well-established procedure.[75] In order to measure the $^3J_{5,6}$ and $^3J_{5,6'}$ coupling constants of 14 and 15, 1*D*-DPFGSE-NOESY experiments were performed on these compounds (5×10^{-2} M) with the sequence of Shaka et al.[76] In both compounds, the resonance of 1-H was selectively inverted. The mixing time used was 600 ms. To determine the temperature coefficients ($\Delta\delta/\Delta \cong T$) of the exchangeable resonances of compounds **1**−**16**, ^{1}H NMR variable-temperature experiments were carried out at concentrations ranging from 8×10^{-5} M to 1×10^{-3} M, depending on the auto-association ability of the investigated carbohydrate. Six spectra were recorded at distinct temperatures in the 297−318 K range. All measurements of $\Delta\delta/\Delta \cong T$ were carried out at least twice. $\Delta\delta/\Delta \cong T$ values were obtained from a linear fit. Differences smaller than 0.3 ppb/k were observed between values of $\Delta\delta/\Delta \cong T$ of a given resonance determined from different experiments.

FT-IR Experiments: Carbohydrate solutions were prepared by dissolving a weighed amount of compound in dry CH_2Cl_2 or 7% pyridine/CH_2Cl_2. Spectra were recorded at concentrations at which intermolecular H bonding was negligible, with a Nicolet 520B spectrometer with a liquid cell (KBr windows) of 0.1 mm pathlength. Background spectra were recorded with the solvent or solvent mixture in the cell. This inevitably produces saturation in several regions of the spectra, but a clear spectral window down to around 3100 cm^{-1} was available. The spectroscopic data were analysed by GRAMS (Galactic Inc.) and Spectrum (Perkin−Elmer) software. In the estimation of peak frequencies, several methods were employed to confirm the validity of the values obtained, including simple peak-picking (estimated bandhead maximum), derivative spectra and curve resolving. In the latter method, three well-ac-

cepted band-shape methods were employed and cross-referenced: Gaussian, mixed Gaussian-Lorentzian, and Pearson VII. The number of peaks used was based solely on the visual observations, and on the residuals observed in the fitted band profile. The correlation coefficient was not considered to be the most important factor, since this invariably introduces more bands in the fit to obtain higher R^2 values, irrespective of the possible band assignments. In most cases, there was a good correspondence between each method for specific bands. In the case of the Py complexing experiments, initial visual estimation of the intermolecularly associated band positions, based on the work of Kleeberg,[44] was refined by curve-fitting. The frequencies given are the mean values between the upper and lower limits obtained from a series of fitted spectra. Although the errors in the measurement of the band positions, which vary from sugar to sugar, are important, generating standard deviation values in the range of 0.9−1.5 in the calculated value of A (except in 8, for which the standard deviation is 2.4), the trend of strengthening of the intramolecular hydrogen bond is observed in all cases.

Molecular Modelling: Molecular mechanics calculations were carried out by using the general-purpose MM3* force field with the GB/SA solvent model for chloroform. The different possible orientations of hydroxy groups giving rise to hydrogen bonding in both clockwise and counterclockwise orientations were used as starting geometries and submitted to exhaustive energy minimisation. Torsion angles and interatomic distances were derived from the output geometries. The corresponding final structures are given in the figure of the Supporting Information.

Syntheses: Compounds **1**−**6**, **8** and **9** were synthesised according to published procedures. The amine precursors of amides **7** and **10** were synthesised from D-galactal and D-glucal according to the literature[77] (Supporting Information is available; see also footnote on the first page of this article).

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