Investigation of the Hydrogen Bonding Properties of a Series of Monosaccharides in Aqueous Media by ^{1}H NMR and IR Spectroscopy

Joanne Hawley,[a] Nick Bampos,[b] Nuria Aboitiz,[a] Jesus Jiménez-Barbero,[a] Manuela López de la Paz,[a] Jeremy K. M. Sanders,[b] Pedro Carmona,[c] and Cristina Vicent*[a]

Keywords: Amino alcohols / Carbohydrates / Conformation analysis / Cooperative phenomena / Hydrogen bonds

A technique, based on ^{1}H NMR and IR experiments, to characterise intramolecular hydrogen bonds in aqueous medium in a series of amino, amido and ammonium sugar derivatives has been established. Three groups of molecules, representing *amides* (**4**, **5** and **6**), *amines* (**7** and **8**) and *ammonium salts* (chlorides **9** and **10**, and phosphates **11** and **12**), with different relative configurations of their functional groups, have been investigated to assess the effect of the nature and the stereochemistry of these groups on the hydrogen-bonding features of the sugar. The deduced features in water solution are compared to those obtained previously in nonpolar solvents. The phosphate salts of amines **7** and **8** (**11** and **12**) were also prepared, in order to evaluate the influence of the OH groups on the binding of the phosphate counterion, and the possibility of establishing cooperative hydrogen bonds involving the phosphate group. The data presented here indicate that the 1,3-*cis*-diaxial-type configuration in sugar diols and amino alcohols produces an intramolecular *six-membered-ring* hydrogen bond that survives in water and, moreover, offers the possibility to establish cooperative intermolecular hydrogen bonds.

Introduction

Biological recognition of carbohydrates relies upon multiple *weak* interactions.[1−4] Although still controversial, hydrogen bonds and van der Waals forces seem to be the most important ones.[5−7] Under physiological conditions, carbohydrates are highly hydrated, and so water molecules must be displaced from their surface before a hydrogen bond to a receptor molecule may be formed.[8,9] The role that hydrogen bonding plays in carbohydrate recognition is uncertain,[10] and studies of intra- and intermolecular hydrogen bonding in carbohydrates in aqueous solution are thus essential to improve our understanding of the interactions of these molecules in nature. The high hydroxy group content in carbohydrates makes the study of carbohydrate OH···XH and OH···X hydrogen-bond energetics fundamental to the understanding of carbohydrate recognition. Moreover, the cooperative effect of many hydrogen bonds acting together is believed to play an important role in biological interactions. Hydrogen-bonding cooperativity, defined as the change in the strength of a hydrogen bond when a second hydrogen bond is formed between either the hydrogen-bond donor or acceptor (of the existing hydrogen bond) and a third hydrogen-bond group, may enhance (positive cooperativity) or weaken (negative cooperativity) a hydrogen-bond interaction.[11−14] The experimental study of hydrogen bonds involving OH groups in aqueous media, either by IR or by NMR, is a difficult task. Furthermore, the obtaining of experimental evidence of hydrogen-bonding cooperativity in aqueous solution has proved to be even more difficult.[15−17]

In principle, the most direct probe with which to study the involvement in hydrogen bonds of OH groups of sugars by ^{1}H NMR[18−20] is the hydroxy resonance, as it is sensitive to solvent changes and to their implication in hydrogen bonding. Unfortunately, observation of the OH ^{1}H NMR resonance in aqueous medium is complicated by the fast exchange of the OH proton with water protons. In favourable cases, it is possible to use the WATERGATE or similar pulse sequences to suppress the water ^{1}H NMR signal efficiently, without affecting the resonance of exchangeable protons or influencing signals close to the water signal.[21] Moreover, in acquisition of spectra at low temperature, the OH signals of aqueous sugar samples can be observed without the use of special precautions and, in some cases, coup-

[a] Dep. Química Orgánica Biológica, Instituto de Química Orgánica, CSIC,
Juan de la Cierva 3, 28806 Madrid, Spain
[b] University of Cambridge,
Lensfield Road, Cambridge, CB2 1EW, UK
[c] Instituto de Estructura de la Materia, CSIC,
Serrano 121, 28006 Madrid, Spain

ling constants of the OH peaks have been resolved. There is an additional difficulty for the study of OH hydrogen bonds. While hydrogen-bond donor and acceptor centres are well defined in other systems (NH in peptides and in nucleotides), OH groups have dual donor/acceptor character, and the prediction of the donor/acceptor character of every OH is much more difficult for highly hydroxylated compounds such as carbohydrates.[22−24]

In order to detect intramolecular hydrogen bonds in water, it is necessary to rely on the measurement of one or several of the common ^{1}H NMR resonance parameters: chemical shifts, vicinal coupling constants, temperature coefficients, exchange rates with solvent and interresidue NOEs may be extracted to infer the existence of such hydrogen bonds. In fact, interresidue hydrogen bonds have been identified in disaccharide and in linear and branched trisaccharide moieties.[25,26] To the best of our knowledge, however, no evidence for the existence of intramolecular hydrogen bonds in a simple monosaccharide in aqueous solution has yet been reported.

The efficiency of IR spectroscopy in characterisation of hydrogen bonds in nonpolar solvents is well known.[27,28] In aqueous media, however, the strong hydroxy ν(O−H) and amide ν(N−H) bands present in sugar derivatives in nonpolar media are masked by the corresponding ones generated by water, and consequently the 4000−3000 cm^{-1} spectral region cannot be used for the study of hydrogen bonding.

Nevertheless, carbohydrate hydroxy groups cause medium-intensity ν(C−O) and δ(O−H) bands, which can be used as diagnostic signals for the presence of hydrogen bonds. This is supported by the fact that in alcohols in the vapour state, in which hydrogen-bonded interactions are absent, no upshift of the ν(C−O) bands is observed upon *O*-deuteration of these hydroxy compounds.[29] In contrast, hydrogen-bonded alcohols, either in the liquid state or dissolved in nonpolar solvents, show ν(C−O) upshifts upon deuteration[30] as a result of Fermi resonance involving ν(C−O) and δ(O−D) vibrations. Consequently, this ν(C−O) shifting can be expected whenever O−H···O hydrogen bonds are present, even in aqueous medium. In connection with this, hydroxy−water and hydroxy−hydroxy hydrogen bonds can be distinguished by the magnitude of the ν(C−O) shift. In the case of ethanol in water (5% w/w), the ν(C−O) upshift upon deuteration is about 2 cm^{-1}.[31] It is obvious that stronger ν(C−O) upshifting is to be expected when carbohydrate intramolecular hydrogen bonds are present in water solution; otherwise these would be broken by water. We have used this infrared spectroscopic method to identify intramolecular hydrogen bonds in the carbohydrates studied here for the first time.

Sugar diol and amido alcohol arrangements are "common features" found in carbohydrates present in antibiotics and anticancer drugs[32,33] that interact with specific sequences of DNA in the minor groove. Their involvement in hydrogen-bonding interactions with the phosphate groups present in the groove has been postulated. Additionally, 1,2- and 1,3-amino alcohol hydrogen-bonding motifs are found in the potent amino sugar antibiotic family,[34,35] which interact specifically with RNA. Under physiological conditions these amines are protonated, and so electrostatic interactions, and presumably hydrogen bonds, are relevant to their interaction processes. Efforts have been made to determine the contribution of the interaction between the neighbouring OH and the ammonium groups on phosphate binding, depending on the different relative configurations of these particular OH groups.[36] We have found that, in nonpolar media, the glucose amido alcohol **1** presents a very efficient bidentate binding motif complementary to a phosphate salt ($\Delta G^\circ = -4.4$ kcal mol^{-1}),[37] the intramolecular hydrogen bond being the origin of the extra stabilisation by cooperativity.

In this context, and following our studies on the role of the nonadditive (cooperative) effect of hydrogen bonds in carbohydrates,[38−41] we report here on the hydrogen-bonding properties of a series of water-soluble 1,2- and 1,3-diol and amino alcohol monosaccharide derivatives (**4**−**12**), with *gluco* and *galacto* configurations in the 1,6-anhydrosugar series (see Figure 1) in aqueous solution.

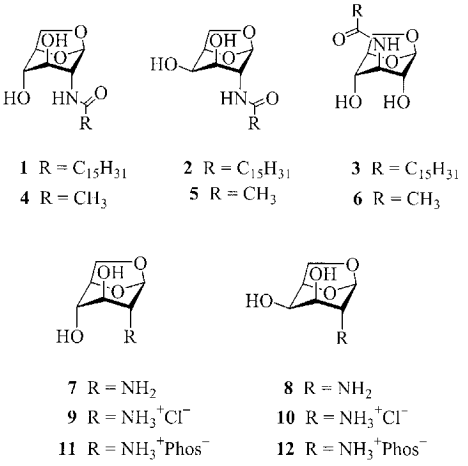


Figure 1. Carbohydrate derivatives studied in this work [Phos$^-$ = tetrabutylammonium bis(3,5-di-*tert*-butylphenyl) phosphate]

These derivatives were selected on the basis of our previous findings in nonpolar media of stable and directional intramolecular hydrogen bonds for a particular relative orientation of the hydrogen-bonding centres in the corresponding organic-soluble derivatives **1**, **2** and **3** (see Figure 2).

Thus, three groups of molecules, representing *amides* (**4**, **5** and **6**), *amines* (**7** and **8**) and *ammonium salts* (chlorides **9** and **10**, and phosphates **11** and **12**), with different relative configurations of their functional groups, were investigated both by NMR and by IR to assess the effect of the nature and the stereochemistry of these groups on the hydrogen-bond features of the sugar. The features deduced in water solution were compared to those obtained previously in nonpolar solvents.[28,38] The phosphate salts of amines **7** and **8** (**11** and **12**) were prepared in order to evaluate the influence of OH groups in the binding of the phosphate

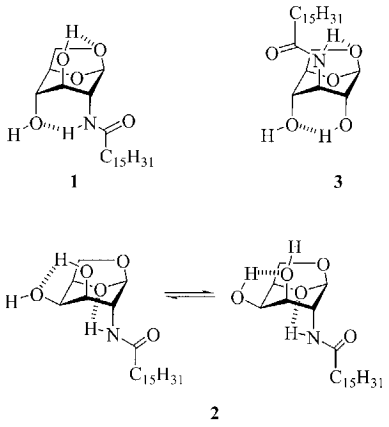


Figure 2. Hydrogen-bonding pattern of sugar derivatives **1**, **2** and **3** in chloroform

counterion, and the possibility of establishing cooperative hydrogen bonds involving the phosphate group.

Results and Discussion

1. Amides 4−6 in Water Solution and Their Analogues 1−3 in Chloroform Solution

In preliminary communications we have examined the hydrogen-bonding abilities of a series of 1,2- and 1,3-diols and amido alcohols, with either *gluco* or *galacto* configurations, in chloroform solution.[38,39] Monoalcohols were also used as models to study the influence of a second hydroxy group with 1,3-diaxial or 1,2-diequatorial orientations on cooperativity. NMR, IR and vapour-pressure osmometry experiments allowed us to demonstrate that analogue **3**, with the 1,6-anhydro-gluco configuration and a 1,3-*syn*-diaxial orientation of the hydroxy groups, forms stable dimers in nonpolar media, where the intramolecular *six-membered ring hydrogen bond* 2-OH → 4-OH is the origin of the effectiveness of the dimerization. More recently,[40] we have also shown that analogue **3** is able to interact and to form stable complexes ($\Delta G^\circ = -4.4$ kcal/mol) with the cytidine-guanosine (**CG**) base pair, through cooperativity-enhanced hydrogen bonds between the hydroxy groups of the sugar, the carbonyl group of **G** and the NH_2 group of **C**.

The results for the water-soluble sugar amides **4**−**6** can be summarised as follows. In all cases, the exchangeable proton resonances of the three compounds were sharp and well resolved in ^{1}H NMR spectra acquired at low temperatures, and the coupling constants of CH sugar ring resonances indicated that all of them displayed $^1C_4$ chair conformations under these experimental conditions. This is relevant, since the formation of intramolecular hydrogen bonds in **6** is more likely to occur when a chair conformation is adopted than when a boat conformation is present. The conformations of 1,6-anhydro-β-D-glucopyranoses are known to be solvent-dependent, and appreciable populations of boat-like conformers have been observed in DMSO.[42,43] Analogously, the hexadecanoyl and naphthaloyl amido-substituted analogue of **6** (compound **3**) has also been shown to present a chair conformation in [D]chloroform over the 297−318-K temperature range.

The ^{1}H NMR spectra of **4**−**6** were fully assigned by TOCSY and NOESY experiments at low temperature with solvent suppression (presaturation) (Table 1).

Table 1. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) of sugar amides **4**−**6** in water solution (H_2O/15% [D_6]-acetone)

Proton	**4**		**5**		**6**	
	δ	*J*	δ	*J*	δ	*J*
1-H	5.50	1.2	5.38	1.1	5.48	1.0
2-H	3.80	1.1	4.07	1.2	3.68	1.1
3-H	3.79	1.3	4.03	1.1	3.79	1.2
4-H	3.77	1.0	3.89	1.3	3.59	1.0
5-H	4.67	0.6, 5.4	4.51	0.6, 5.5	4.62	0.5, 5.5
6-H_{endo}	4.21	9.1	4.30	−8.9	4.04	−9.0
6-H_{exo}	3.69	−	3.67	−	3.77	−
3-OH/2-OH	6.30	−	6.20	−	6.97	−
4-OH	6.46	−	5.90	−	6.35	−
NH	8.04	8.7	8.34	8.8	8.19	8.2

In the NOESY spectra of **4** and **5**, NOE cross-peaks were detected between the OH signals and their respective vicinal proton signals. No other NOE cross-peaks from the OH resonances were observed. Furthermore, the amide signals of **4** and **5** exhibited NOE cross-peaks to several ring protons (1-H, 2-H, 3-H, 4-H). It was also evident that the OH protons of **4** and **5** underwent chemical exchange with water protons, but no exchange cross-peaks between amide and water protons were detected. Temperature coefficients of −10.6 and −10.3 ppb K^{-1}, and −10.5 and −11.1 ppb K^{-1} were determined for the 3-OH and 4-OH resonances of **4** and **5**, respectively. In both compounds, the difference between the temperature coefficients of the two OH protons was insufficient to be significant, since the experimental error in the determination of temperature coefficients in 15% [D_6]acetone in water was estimated to be between 0.5 and 1.0 ppb K^{-1}. In any case, the temperature coefficients of the OH resonances of **4** and **5** indicated that the corresponding OH groups were equally exposed to solvent and that, as such, participation of these OH groups in intramolecular hydrogen bonds was unlikely, or the population of the intramolecular hydrogen-bonding isomer was too small to produce changes in this parameter. Furthermore, the magnitude of the temperature coefficients of the OH resonances of **4** and **5** was consistent with the OH protons being engaged in hydrogen bonding to solvent water molecules, rather than involved in intramolecular hydrogen bonds.

Analysis of the ^{1}H NMR spectra of aqueous solutions of **6** acquired at low temperatures (< 278 K) showed sharp and well-resolved lines for the exchangeable proton signals. Moreover, the resolution of the NH and OH proton resonances improved as the proportion of [D_6]acetone in water was increased and the acquisition temperature was lowered. In 15% [D_6]acetone, for example, both sugar OH signals were resolved at 278 K, whereas in pure water only one broad OH peak was observed at 268 K.

Little variation was observed in the exchangeable proton chemical shifts of **6** over the range of aqueous compositions studied. The OH resonances were slightly displaced to lower field as the proportion of [D_6]acetone in the solvent was decreased, while the chemical shift of the NH resonance was essentially uninfluenced by small changes in the solvent composition. For example, at 263 K, on decreasing the solvent acetone content from 15 to 10%, the NH chemical shift was virtually unaffected, and the OH peaks were displaced downfield by 0.02−0.05 ppm. No change in the chemical shifts or coupling constants of the H−C protons was detected on varying the solvent composition or temperature. At 263 K in 15% acetone, the couplings of the OH signals were resolved, although the coupling pattern could not be identified properly. Under the same conditions, the amide proton was a doublet with a coupling constant of 8.2 Hz. The behaviour of the rest of the sugars in water was therefore studied using 15% [D_6]acetone in water as the aqueous medium.

For **6**, temperature coefficients of −12.9 and −9.8 ppb K^{-1} were determined for its OH resonances. In this case, the difference between these values is greater than the margin of error in determining temperature coefficients. The temperature coefficient of 4-OH was similar to the experimentally determined temperature coefficient for a non-intramolecularly hydrogen-bonded OH proton. (^{1}H NMR spectra of a monoalcohol in which there was no possibility of the formation of intramolecular hydrogen bonds were acquired in 15% [D_6]acetone in water at distinct temperatures in order to calculate the temperature coefficient of a non-intramolecularly hydrogen-bonded OH proton resonance. Unfortunately, the most suitable substrates for this purpose were not water-soluble and 2-propanol was employed. By using the WATERGATE pulse sequence, the OH resonance of 2-propanol was resolved at temperatures below approximately 283 K and the corresponding temperature coefficient was determined to be −11.3 to −12 ppb K^{-1}.) In contrast, the temperature coefficient of 2-OH ($\Delta\delta/\Delta T = -9.8$ ppb K^{-1}) was significantly smaller, although still rather large in relation to the reported values for those OH protons involved in strong intramolecular hydrogen bonds in [D_6]acetone/water.

On the other hand, the temperature coefficients of the NH resonances of **4**−**6** were too close to each other (between 7.3 and 8.0 ppb K^{-1}) to provide evidence for differential involvement of the respective N−H protons in hydrogen bonding

Although less evident and probably shorter lived, the NMR behaviour of **6** in water solution resembles that of its analogue **3** in chloroform, in which a 2-HO → 4-OH intramolecular hydrogen bond could persist for a limited amount of time. In contrast, compounds **4** and **5** did not show any NMR indication of the existence of intramolecular hydrogen bonding as previously found in chloroform for **1** and **2** (Figure 2).

As regards IR experiments, band shifts in opposite directions due to coupling (Fermi resonance) can provide information on the interactions between functional groups. This coupling can be either electromagnetic or mechanical. In the first case, coupling is generated by electromagnetic interaction of parallel or antiparallel oriented similar dipoles, while mechanical coupling is caused by electronic fluctuations of groups contacting each other through, for instance, hydrogen bonds. Generally speaking, hydrogen-bonding studies of aqueous solutions of organic compounds by infrared spectroscopy are hampered by the ν(O−H) bands of water. However, on the basis of the above band shifts and by considering ν(C−O) and δ[O−H(O−D)] bands, it is possible to obtain information regarding the hydrogen bonds between different polar groups. Additionally, δ(NH_3) was observed for the detection of hydrogen-bond formation in the ammonium salts.

We therefore performed IR experiments in water solution. The ν(C−O) and amide II bands of the three sugar amides **4**−**6** were examined for intramolecular hydrogen bonds (see Table 2).

Table 2. Infrared bands (cm^{-1}) obtained for the amide-containing sugars **4**−**6** in H_2O and D_2O solutions

Band	Solvent	**4**		**5**		**6**	
ν(C−O)	H_2O	1138	1089	1117	1060	1146	1079
	D_2O	1152	1123	1139	1091	1159	1100
Amide II	H_2O	1559		1559		1549	

The amide II bands of compounds **4** and **5** appeared at the same frequency (1559 cm^{-1}). As the two amides differ only in the relative configuration of 4-OH, the results seem to indicate that the 1,3-diaxial relative orientation of 4-OH and 2-NH in **4** does not affect the amide group. Two situations can be considered: either both of them are free or both are involved in the same type of hydrogen bonding. In nonpolar media, **1** (an analogue of **4**) represents a *six-membered ring intramolecular hydrogen bond* between the two diaxial substituents[28] (see Figure 2). Sugar **6**, which has the amide group in position 3, displayed this band at lower frequency (1549 cm^{-1}). Unfortunately, we do not have an analogue of **6** that could be used as a model for non-intramolecularly hydrogen-bonded 3-NH.

In the 1200−900-cm^{-1} spectral region, upshifts of two ν(C−O) bands in compound **5** were detected upon deuteration (see Figure 3a), with the 1117- and 1060-cm^{-1} bands shifting to 1139 and 1091 cm^{-1}, respectively. As described above, shifts of the ν(C−O) and δ(O−D) bands in opposite directions means that C−O and/or O−H groups are involved in intramolecular interactions.

Unambiguous assignment of these bands to particular C−O and O−H bonds could not be performed without isotopic C and O labelling of the molecule, and so two possible hydrogen-bonding isomers could be conceived of, involving a hydrogen bond between 3-OH and either 1-CO or 4-OH. Since two − and not three − ν(C−O) upshifts were detected upon deuteration of this compound, the double interaction, 4-OH···3-OH···1-OC, cannot be considered. This result is completely in accordance with the hydrogen-bonding isomer equilibrium we have proposed in nonpolar me-

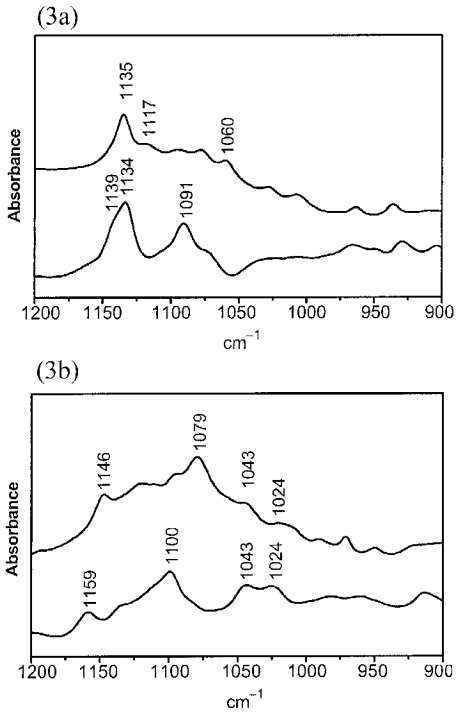


Figure 3. IR spectra (1200−900-cm^{-1} region) of compounds **5** (3a) and **6** (3b) in H_2O (upper spectra) and D_2O (lower spectra)

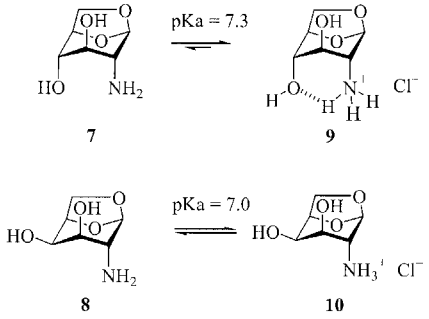
dia between the OH···OH centres of **2**, the organic-soluble analogue of **5** (see Figure 2).[28]

In the case of the sugar amide **4**, two ν(C−O) bands at 1138 and 1089 cm^{-1} shifted upon deuteration to 1152 and 1123 cm^{-1}, respectively. This is compatible with the existence of a 3-OH···1-OC hydrogen bond, as found in its organic-soluble analogue **1**. The most significant result was the finding that the 1,3-*cis*-diaxial diol **6** also showed two ν(C−O) upshifts (Figure 3b), from 1146 and 1079 cm^{-1} to 1159 and 1100 cm^{-1}, respectively. This is in agreement with the NMR spectroscopic data in water, and confirms the presence of an intramolecular hydrogen bond between the diaxial diol 2-OH and 4-OH, forming a *six-membered ring intramolecular hydrogen bond*, in accordance with the previously characterised intramolecular hydrogen bond in the corresponding analogue **3** in nonpolar solvents (Figure 2).[38] This has proved to be essential for effective binding in intermolecular processes, due to hydrogen-bonding cooperativity.[28,37−41]

2. Sugar Amines 7 and 8

Unfortunately, the exchangeable proton resonances of the amines **7** and **8** were not resolved under the experimental conditions used to acquire the ^{1}H NMR spectra of the corresponding amides **4** and **5** as described above. This situation was not surprising, as the more basic amine group would be expected to scavenge protons more readily than an amide group and cause a fast rate of proton exchange. The rest of the signals were assigned by conventional methods as described earlier. The coupling constants allowed us to deduce that the conformation of each amine was a $^1C_4$ chair.

The pK_a values have been used as a tool to characterise the existence of polar interactions in nitrogen-containing sugars.[17] The resonance signals of 2-H in **7** and **8** appeared characteristically isolated at high field (δ = 2.78 and 3.06 ppm), and so were used as probes in a pH titration (in D_2O at 299 K) in order to determine the pK_a values of the sugars. It was observed that the chemical shifts of protons 1-H and 5-H of **7** and **8** were also sensitive to the pH, being displaced downfield by δ = 0.25 and 0.13 ppm, respectively; pK_a values of 7.3 and 7.0 were determined for **7** and **8**. As the error in the measurement of the pK_a values by this procedure is < 0.1 pK_a units, these data established that **7** was protonated more readily than **8**. Since the only structural difference between the two compounds resides in the configuration at C-4, the measured difference between these pK_a values could be attributed to the appearance − or enhancement − of a nonpolar interaction, probably an intramolecular hydrogen bond,[17] between the ammonium group at C-2 and 4-OH after protonation of the *gluco* derivative **7**. Such an interaction would not be possible upon protonation of the *galacto* analogue **8**, and so a stabilisation of protonated **7** relative to protonated **8** would be provided (see Figure 4).



7 **9** **8** **10**

Figure 4. Protonation equilibrium of amine derivatives **7** and **8**, showing the possible intramolecular hydrogen-bonding patterns

3. Ammonium-Containing Sugars 9−12: Effect of the Counterion; Hydrogen-Bonding Interaction with Phosphate

The paper on 1,2- and 1,3-amino alcohol interaction motifs in the molecular recognition of aminoglycoside antibiotics has motivated efforts to determine the differences in binding to phosphate depending on the different relative configurations of OH groups close to the ammonium group.[36] In nonpolar media, we have previously found that the glucose amido alcohol **1** represents a very efficient bidentate binding motif complementary to a phosphate salt (ΔG° = −4.4 kcal mol^{-1}),[37] the intramolecular hydrogen bond being the origin of extra stabilisation by cooperativity. The water-soluble derivatives **9**−**12** were therefore studied in order to perform a comparative study in aqueous media.

The ^{1}H NMR spectra of the ammonium chloride (**9**−**10**) and ammonium phosphate sugars (**11**−**12**) in water/16% acetone revealed a clear difference from the respective amines (**7** and **8**) and amides (**4** and **5**). Generally, lower temperatures were required to observe the NH and OH proton resonances for **9**−**12** than for **4**−**8**. For **11**, in fact, the

OH resonances were not sufficiently well resolved even at the lowest temperature attainable without freezing in 15% [D_6]acetone/water. The observed data (Table 3) could not be correlated either with the type of counteranion [chloride or tetrabutylammonium bis(3,5-di-*tert*-butylphenyl) phosphate] or with the configuration of the functional groups.

Table 3. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) of sugar amides **9**−**12** in water solution (H_2O/15% [D_6]acetone)

Proton	**9**		**10**		**11**		**12**	
	δ	*J*	δ	*J*	δ	*J*	δ	*J*
1-H	5.70	1.0	5.63	1.0	5.70	1.0	5.63	1.0
2-H	3.32	1.1	3.55	1.1	3.34	1.1	3.56	1.1
3-H	3.93	1.2	4.16	1.2	3.92	1.2	4.16	1.2
4-H	3.82	1.0	4.43	1.0	3.86	1.0	4.16	1.0
5-H	4.80	0.5, 5.5	4.61	0.5, 5.5	4.76	0.5, 5.5	4.62	0.5, 5.5
6-H_{endo}	4.60	−9.0	4.16	−9.0	4.30	−9.0	4.44	−9.0
6-H_{exo}	3.83	–	3.76	–	3.86	–	3.76	
OH[a]	6.63	–	6.25	–	–	–	6.27	
OH[a]	7.01	–	6.35	–	–	–	6.36	
NH	6.70	8.2	6.90	8.2	8.30	8.2	8.42	8.2

[a] The assignment of hydroxy peaks makes no distinction between OH groups in positions 3 and 4.

The difficulty in resolving the OH resonances of **9**−**12** also hampered the determination of the corresponding temperature coefficients; where OH resonances could be observed, the temperature range over which they were resolved was generally too small to allow the temperature coefficient to be measured accurately.

Fortunately, IR experiments proved to be very useful for characterisation of intramolecular hydrogen bonds in these compounds, whereas the obtaining of NMR information presented serious experimental problems. We detected intramolecular hydrogen bonds in the two ammonium chloride derivatives **9** and **10** by measurement of the ν(C−O) frequencies (see Table 4). In the ammonium phosphate derivatives **11** and **12** we also compared their $\nu_s(PO_2^-)$ bands.

Table 4. Infrared bands (cm^{-1}) obtained for the ammonium-containing sugars **9**−**12** in H_2O and D_2O solutions

Band	Solvent	**9**	**10**	**11**	**12**
ν(C−O)	H_2O	1123 1082	1121 1065	1124 1091	1123 1067
	D_2O	1151 1113	1143 1073	1124 1089	1143 1074
$\delta_s(NH_3^+)$	H_2O	1533	1538	1555	1540
$\nu_s(PO_2^-)$	H_2O	–	–	1088	1089

By the method described above, upshifting of two ν(C−O) bands upon deuteration was observed for the two

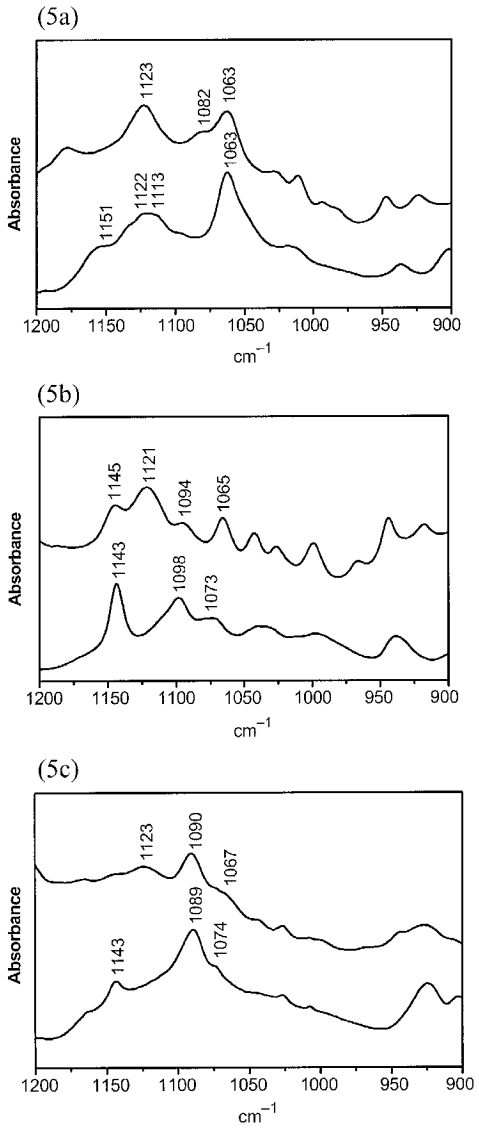


Figure 5. IR spectra (1200−900-cm^{-1} region) of compounds **9** (5a), **10** (5b) and **12** (5c) in H_2O (upper spectra) and D_2O (lower spectra)

ammonium chloride salts **9** and **10** (see Figure 5a), which means that there is an intramolecular hydrogen bond involving two C−O/O−H groups in each molecule.

As expected, these results are consistent with those obtained for the analogous sugar amide derivatives **4** and **5** in water, and compatible with those proposed for their corresponding chloroform-soluble derivatives **1** and **2** (see Figure 2).

On the other hand, comparison of the $\delta_s(NH_3^+)$ bands in **9** and **10** (1533 and 1538 cm^{-1}, respectively) yielded no evidence of an intramolecular hydrogen bond between NH_3^+ and 4-OH in **9**, in which case it would be expected to show $\delta_s(NH_3^+)$ at a higher frequency than in **10**.

With regard to the effect of the counterion on the establishment of intra- and intermolecular hydrogen bonds involving the phosphate ion, not only ν(C−O), but also $\delta_s(NH_3^+)$ and $\nu_s(PO_2^-)$ bands were observed. Phosphate salts **11** and **12**, which only differ in the orientation of 4-

OH, showed very different behaviour in the three absorptions. Additionally, they behaved differently from their corresponding chlorine salts (**9** and **10**).

Comparison of the $\delta_s(NH_3^+)$ bands in phosphate salts **11** and **12** showed differences between the two compounds. A significantly higher frequency (1555 cm^{-1}) was observed for this band in **11** than in **12** (1540 cm^{-1}). This suggests that an intramolecular hydrogen bond (absent in **12**) exists between 2-NH^+ and 4-OH in **11**, NH_3^+ being the hydrogen-bonding donor group.

Additionally, comparison of $\nu_s(PO_2^-)$ in both **11** and **12** deuterated salts (see Table 4) with the same band in a non-carbohydrate model salt [tetrabutylammonium bis(3,5-di-*tert*-butylphenyl) phosphate] (1091 cm^{-1}) showed downshifts of 2 cm^{-1} for compound **11** and 1 cm^{-1} for compound **12**. This observation implies that PO_2^- is involved in an additional stabilisation of the complex, presumably through intermolecular hydrogen bonding that is stronger in **11** than in **12**.

Upshifting of two ν(C−O) bands was observed in **12** upon deuteration (Figure 5c). In fact, the bands affected by a Fermi resonance are the same as in the chloride salt **10**, which suggests the conclusion that the hydrogen-bond model existing in compound **10** (either 3-OH···1-OC or 3-OH···4-OH) remains in the presence of phosphate anion. On the contrary, no clearly visible upshifting of ν(C−O) bands was observed for the phosphate salt **11** upon deuteration (see Table 4), which suggests that the hydrogen bonding involving the 3-OH group is different from that in the homologous chloride salt **9** (see above), in which the upshifting of two ν(C−O) bands was detected. All of these results seem to show that, in the presence of phosphate anion, 3-OH no longer interacts with 1-CO but preferentially forms a more stable intermolecular hydrogen bond with PO_2^-.

This last evidence furthermore suggests that the intermolecular interaction between the sugar and the anion PO_2^- in **11** could be explained by the formation of an intramolecular 2-NH^+···4-OH hydrogen bond stabilised by cooperativity, and in a reciprocal manner, that the interaction with the phosphate is enhanced by the same effect.

Finally, the differences found in the 1200−900-cm^{-1} spectral region between the chloride (**9**) and phosphate (**11**) salts in D_2O (Figure 6) can be attributed to interactions

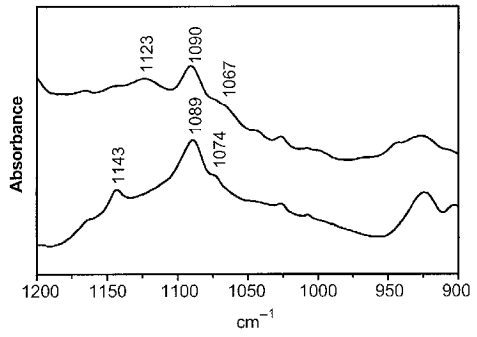


Figure 6. IR spectra (1200−900-cm^{-1} region) of compounds **9** (upper spectrum) and **11** (lower spectrum) in D_2O

with their respective anions. It seems that a strong hydrogen bond with the PO_2^- group is present in **11**, and is responsible for shifting the ν(C−O) absorption to higher frequencies (see Table 4). This is apparent for the ν(C−O) band of **9** (1151 cm^{-1}) and the homologous **11** (1164 cm^{-1}).

These are relevant results, since the interaction with the phosphate anion in water seems to be dependent on the relative stereochemistry of the carbohydrate hydrogen-bonding centres in the proximity to the ammonium group. Furthermore, they suggest that the hydrogen-bonding array present in **9** (and **11**) could be a good candidate in the design of carbohydrate RNA binders.

Conclusions

We have established a technique, based on ^1H NMR and IR experiments, with which to characterise intramolecular hydrogen bonds in aqueous medium in a series of amino, amido and ammonium sugar derivatives containing 1,2- or 1,3-diol moieties (**4**−**12**). Essentially, *six-membered ring intramolecular hydrogen bonds* between 2-OH and 4-OH in **6**, and 2-NH^+ and 4-OH in **9** and **11** have proved to be long-lived in water. Experimental evidence was provided by low-temperature NMR experiments in a 15% [D_6]acetone/water solution, in which chemical shifts, coupling constants and temperature coefficients of the sugar protons were studied. Infrared spectra of aqueous samples, particularly the detection of upshifts of ν(C−O) bands upon deuteration, has been decisive in the unambiguous characterisation of the intramolecular CO···HO−C hydrogen bonds.

Thus, both NMR and IR showed the presence of an intramolecular 2-OH···4-OH hydrogen bond (*six-membered ring cis-intra-hydrogen bond*) in compound **6**. In addition, infrared spectroscopy was able to detect hydrogen-bond interactions between 3-OH and 1-O in compound **4**, and 3-OH···4-OH or 4-OH···3-OH hydrogen bonding (*five-membered ring cis-intra-hydrogen bond*) in compound **5**, for which NMR spectroscopic data were not conclusive. These particular hydrogen bonds have been observed previously in nonpolar media for their corresponding organic soluble derivatives **1**, **2** and **3**. *The six-membered ring intramolecular hydrogen bond* in **3** allows efficient cooperative intermolecular interactions in nonpolar media.[28,37] As far as the ammonium-containing sugars **9** and **10** are concerned, they show IR spectra indicative of the same hydrogen bonds (involving the OH groups) as observed in their respective amide analogues **4** and **5**.

As regards the possible formation of an intramolecular hydrogen bond between 2-NH and 4-OH in the 1,3-*cis*-diaxial glucose derivatives **4** (amido alcohol), **7** (amino alcohol) and **9** (ammonium chloride alcohol) in water, only the pK_a difference between amines **7** and **8** can be taken as evidence of the existence of a certain percentage of the hydrogen-bonding isomer in solution. This particular hydrogen bond has been characterised in nonpolar media for the same configuration in analogue **1**. In contrast, if the chloride counterion is changed for phosphate (compound **11**),

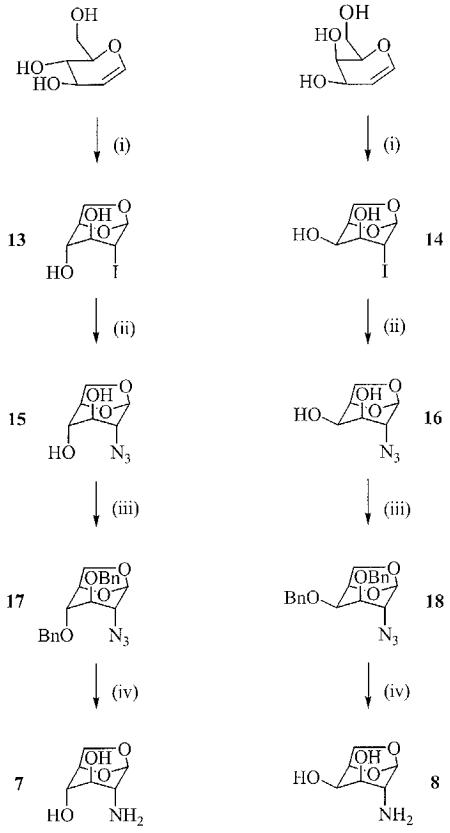
IR spectroscopy confirms not only the presence of this intramolecular hydrogen bond, but also the participation of 3-OH in the interaction with phosphate.

In conclusion, the data reported here indicate that the 1,3-*cis*-diaxial-type configuration in sugar diols and amino alcohols results in an intramolecular hydrogen bond (forming a *six-membered ring intramolecular hydrogen bond*) that survives in water and, moreover, offers the potential to establish cooperative intermolecular hydrogen bonds. This fact may have implications in the design of molecular recognition processes, especially towards DNA and RNA, where this type of sugar structures is very common.

Experimental Section

1. General Methods and Materials: All chemicals were purchased from Aldrich and Sigma. Molecular sieves were activated at 300 °C for 5 h. All reaction solvents were distilled prior to use. Chromatography was performed with Merck 7734 silica gel 60, and TLC on silica gel plates (Kieselgel 60). Compounds were viewed under UV light and/or by dipping in H_2SO_4/MeOH (1:10, v/v) followed by subsequent charring at 140 °C.

2. Synthesis: The syntheses of all the compounds prepared here were not optimised, since the major aim of this work is to examine their hydrogen-bonding properties. Amines **7** and **8** were prepared from D-glucal and D-galactal, respectively, as shown in Scheme 1. Compounds **7** and **8** were the precursors for the rest of derivatives, including amide acetates **4** and **5**, ammonium chlorides **9** and **10** and ammonium phosphates **11** and **12**. The benzylated azides **17** and **18** were prepared according to previously described procedures, with minor modifications. Since the intermediates in the syntheses of **7** and **8** had been characterised previously, **13**−**18** were not recrystallised and were used in the synthetic procedure after purification by column chromatography. Treatment of **13** and **14** with sodium azide yielded **15** and **16**, respectively. However, azides of opposite configuration were also obtained. The products were purified after benzylation of the crude mixtures to give **17** and **18**. The benzylated azides **17** and **18** were hydrogenolysed by using palladium hydroxide on carbon in the presence of trifluoroacetic acid, the trifluoroacetate salts of the corresponding ammonium-containing sugars thus being obtained. The corresponding free amines **7** and **8** were isolated after ion-exchange column chromatography of the crude products. The acetate amides **4** and **5** were prepared from **7** and **8**, respectively, by stirring with acetic anhydride. In a parallel manner, the corresponding *gluco* and *galacto* ammonium chlorides **9** and **10** were derived from **7** and **8**, respectively, by addition of an excess of hydrochloric acid. Ammonium phosphate salts **11** and **12** were prepared analogously from **7** and **8** after addition of 1 equiv. of sodium bis(3,5-di-*tert*-butylphenyl) phosphate in aqueous solution. In both cases, the solvent water was removed by lyophilisation.



Scheme 1. Synthetic route for the preparation of amines **7** and **8** (i) a. $(Bu_3Sn)_2O/CH_3CN$, b. I_2/CH_2Cl_2; (ii) $NaN_3/DMF/H_2O$; (iii) a. NaH/DMF, b. BnBr; (iv) Pd-OH/C, H_2, TFA CH_3OH/H_2O

1,6-Anhydro-2-deoxy-2-iodo-β-D-glucopyranose (14): D-Glucal (1.37 g, 9.4 mmol) and molecular sieves (3 Å) were placed in a two-necked, round-bottomed flask, equipped with a condenser. The complete apparatus was exposed to vacuum and argon (3 cycles), and dry acetonitrile (55 mL) was then introduced into the system. The resulting mixture was stirred with warming until the substrate sugar was dissolved. Bis(tributyltin) oxide (3.8 mL, 7.5 mmol) was then added, and the colourless solution was heated under reflux under argon for 3 h; the solution became opaque during the course of the reaction. After the mixture had cooled to room temperature, the solvent was removed by evaporation under reduced pressure and the residual oil was dried under high vacuum. The residue was taken up in dry dichloromethane (55 mL) under argon at room temperature and subsequently cooled in an ice/water bath. Iodine (2.85 g, 11.2 mmol) was added to the dichloromethane solution, and a colour change to dark brown was immediately observed. The mixture was allowed to stir at 0 °C for 15 min, and filtered through a Celite plug to remove tin salts and molecular sieves. The Celite was washed with dichloromethane, the filtrate and dichloromethane washings were combined, and their volume was reduced to approximately 5 mL by rotary evaporation. The concentrated dichloromethane solution thus obtained was diluted with hexane (50 mL) and stirred with aqueous sodium thiosulfate (50 mL) for 12 h, after which the aqueous and organic phases were then colourless. The aqueous phase was extracted with ethyl acetate (6 × 100 mL) and then dried with sodium sulfate, and the solvent was removed to give a white, oily solid. The crude product was dissolved in methanol, mixed with silica and concentrated to dryness in rarotary evaporator. Flash column chromatography (silica; 18 cm, hexane/acetone, 3:2) gave **14** as white solid (1.65 g, 6.1 mmol, 65%).

1,6-Anhydro-2-deoxy-2-iodo-β-D-galactopyranose (13): D-Galactal (6.9 g, 47.2 mmol) and molecular sieves (3 Å) were placed in a two-necked, round bottomed flask equipped with a condenser. The complete apparatus was exposed to vacuum and argon (3 cycles), and dry acetonitrile (55 mL) was then introduced into the system.

The resulting mixture was stirred with warming until the substrate sugar was dissolved. Bis(tributyltin) oxide (3.8 mL, 7.5 mmol) was then added, and the colourless solution was heated under reflux under argon for 3 h; the solution became opaque during the course of the reaction. After the mixture had cooled to room temperature, the solvent was removed by evaporation under reduced pressure and the residual oil was dried under high vacuum. The residue was taken up in dry dichloromethane (55 mL) under argon at room temperature and subsequently cooled in an ice/water bath. Iodine (2.85 g, 11.2 mmol) was added to the dichloromethane solution and a colour change to dark brown was immediately observed. The mixture was stirred at 0 °C for 15 min, and filtered through a Celite plug to remove tin salts and molecular sieves. The Celite was washed with dichloromethane, the filtrate and dichloromethane washings were combined, and their volume was reduced to approximately 5 mL by rotary evaporation. The concentrated dichloromethane solution thus obtained was diluted with hexane (50 mL) and stirred with aqueous sodium thiosulfate (50 mL) for 12 h, after which the aqueous and organic phases were colourless. The aqueous phase was extracted with ethyl acetate (6 × 100 mL) and then dried with sodium sulfate, and the solvent was removed to give a white, oily solid. The crude product (TLC: silica; hexane/acetone, 3:2; 2 elutions) was dissolved in methanol, mixed with silica concentrated to dryness by rotary evaporation. Flash column chromatography (silica, 18 cm; hexane/acetone, 3:2) gave **13** as white solid (65%).

1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranose (15) and 1,6-Anhydro-2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranose (17): Compound **13** (3.66 g, 13.4 mmol) was heated with sodium azide (25 mmol) in aqueous DMF overnight, until the reaction was complete. Flash column chromatography of the product (ethyl acetate/hexane, 2:1; product loaded onto silica) yielded fractions containing two species [TLC: silica; hexane/acetone, 3:2; 2 elutions: R_f(**15**) = 0.18]. The more polar fraction was an impurity. When dissolved in dichloromethane, the mixture of products produced a yellow solution; a white solid was precipitated from this solution on addition of hexane. Material (1.51 g) containing **15** (impure) was obtained after purification by column chromatography (IR: $\tilde{\nu}$ = 2070 cm^{-1}). The yield is not quoted since a mixture was obtained and used in the further step. This mixture was treated with benzyl bromide in excess. Flash column chromatography (silica; hexane/ethyl acetate, 7:1) gave **17** in an overall yield of 51% (from **13**) when the synthesis was carried out on a 13.4-mmol scale.

1,6-Anhydro-2-azido-2-deoxy-β-D-galactopyranose (16) and 1,6-Anhydro-2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-galactopyranose (18): Compound **14** (1.65 g, 6.1 mmol) was heated with sodium azide (25 mmol) in aqueous DMF overnight, until the reaction was complete. Flash column chromatography of the product (ethyl acetate/hexane, 2:1; product loaded onto silica) yielded fractions containing two species [TLC: silica; hexane/acetone, 3:2; 2 elutions: R_f(**16**) = 0.20]. The more polar fraction was an impurity. When dissolved in dichloromethane, the mixture of products produced a yellow solution; a white solid was precipitated from this solution on addition of hexane. Material (0.80 g) containing azide **16** (impure) was obtained after purification by column chromatography (IR: $\tilde{\nu}$ = 2070 cm^{-1}). The yield is not quoted since a mixture was obtained and used in the next step. This mixture was treated with benzyl bromide in excess. Flash column chromatography (silica; hexane/ethyl acetate, 7:1) gave **18** (1.0 g, in an overall yield of 45% from **14**) when the synthesis was carried out on a 6.1-mmol scale.

2-Amino-1,6-anhydro-2-deoxy-β-D-glucopyranose (7): TFA (5 equiv.) was added to a stirred solution (45 mM) of **17** (500 mg, 1.36 mmol) in methanol/THF (5:1), followed by palladium hydroxide on carbon (3 equiv.). The resulting mixture was purged with hydrogen and stirred under hydrogen for 10 h. The mixture was then filtered through Celite and washed with ethanol and ethyl acetate. The filtrate was concentrated under reduced pressure to yield a colourless oil. TLC (silica; dichloromethane/methanol, 100:5) showed complete conversion of the starting material. The product was then dissolved in milli-q water and loaded on to a column of Dowex 50 wx8-200 resin, prepared by washing with 5% aqueous HCl, followed by water until the eluent was neutral. Milli-q water (200 mL) was then eluted through the column (under gravity) to remove salts produced in the reaction. To collect the product, aqueous ammonium hydroxide solution (0.5 M) was eluted; fractions from the column were monitored by TLC (2-propanol/water/concentrated aqueous ammonia solution, 7.2:2.5:1) and those containing **7** were lyophilised to remove solvent water. (Variation in the efficiency of ion-exchange chromatography on Dowex 50 wx8-200 resin was observed; in some cases two column runs were necessary). Lyophilisation produced **7** as a white solid (194 mg, 1.2 mmol, 88%), which was recrystallised from methanol/diethyl ether to give 92 mg (0.57 mmol, 42%) of pure crystalline material. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 261 K): δ = 4.65 (d, $^3J_{5,6exo}$ = 5.1 Hz, 1 H, 5-H), 4.21 (d, $^2J_{6endo,6exo}$ = 7.7 Hz, 1 H, 6-H$_{endo}$), 3.80−3.74 (m, 3 H, 6-H$_{exo}$, 4-H, 3-H), 2.78 (s, 1 H, 2-H) ppm.

2-Amino-1,6-anhydro-2-deoxy-β-D-galactopyranose (8): TFA (5 equiv.) was added to a stirred solution (45 mM) of **18** (900 mg, 2.45 mmol) in methanol/THF (5:1), followed by palladium hydroxide on carbon (3 equiv.). The resulting mixture was purged with hydrogen and stirred under hydrogen for 10 h. The mixture was then filtered through Celite and washed with ethanol and ethyl acetate. The filtrate was concentrated under reduced pressure to yield a colourless oil. TLC (silica; dichloromethane/methanol, 100:5) showed complete conversion of the starting material. The product was then dissolved in milli-q water and loaded onto a column of Dowex 50 wx8-200 resin, prepared by washing with 5% aqueous HCl, followed by water until the eluent was neutral. Milli-q water (200 mL) was then eluted through the column (under gravity) to remove salts produced in the reaction. To collect the product, aqueous ammonium hydroxide solution (0.5 M) was eluted; fractions from the column were monitored by TLC (2-propanol/water/concentrated aqueous ammonia solution, 7.2:2.5:1) and those containing **8** were lyophilised to remove solvent water. ^{1}H NMR spectra of a monoalcohol in which there was no possibility of the formation of intramolecular hydrogen bonds were acquired in 15% [D$_6$]acetone in water at distinct temperatures in order to calculate the temperature coefficient of a non-intramolecularly hydrogen-bonded OH proton resonance. Unfortunately, the most suitable substrates for this purpose were not water-soluble and 2-propanol was employed. By using the WATERGATE pulse sequence, the OH resonance of 2-propanol was resolved at temperatures below approximately 283 K and the corresponding temperature coefficient was determined to be −11.3 to −12 ppb K^{-1}. Lyophilisation produced **8** as a white solid (345 mg, 2.14 mmol, 87%). ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 270 K): δ = 5.39 (s, 1 H, 1-H), 4.51 (dd, $^3J_{5,6exo}$ = 4.6 Hz, 1 H, 5-H), 4.37 (d, $^2J_{6endo,6exo}$ = 7.9 Hz, 1 H, 6-H$_{endo}$), 4.11 (d, J = 4.2 Hz, 1 H), 3.92 (d, J = 4.3 Hz, 1 H), 3.67 (m, 1 H), 3.06 (s, 1 H, 2-H) ppm. ES MS: calcd. for $C_6H_{11}NO_4$ 161.16; found (positive mode): m/z = 162.1 [M + H]$^+$; found (negative mode): m/z = 160.1 [M − Na]$^+$.

2-Acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (4): Acetic anhydride (5 equiv.) was added to a stirred solution of **7** (0.65 mmol)

in methanol (Merck), and the resulting mixture was stirred overnight. TLC (silica; dichloromethane/methanol, 100:20) then showed the complete consumption of the starting material. The reaction mixture was concentrated under reduced pressure to yield a yellow oil; toluene was repeatedly added to the residue, and the solvents were evaporated. Compound **4** was obtained as a white solid (52 mg, 0.26 mmol, 40%) that was recrystallised from methanol/diethyl ether. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 270 K): δ = 8.04 (d, $^3J_{NH,H2}$ = 8.7 Hz, 1 H, NH), 6.455 (s, 1 H, 4-OH), 6.30 (s, 1 H, 3-OH), 5.46 (s, 1 H, 1-H), 4.67 (d, $^3J_{5,6exo}$ = 5.5 Hz, 1 H, 5-H), 4.22 (d, $^2J_{6endo,6exo}$ = 7.8 Hz, 1 H, 6-H$_{endo}$), 3.79−3.77 (m, 4 H, 6-H$_{exo}$, 2-H, 3-H, 4-H), 2.06 (s, 3 H, CH$_3$) ppm.

2-Acetamido-1,6-anhydro-2-deoxy-β-D-galactopyranose (5): Acetic anhydride (5 equiv.) was added to a stirred solution of **8** (0.47 mmol) in methanol (Merck), and the resulting mixture was stirred overnight. TLC (silica; dichloromethane/methanol, 100:20) then showed the complete consumption of the starting material. The reaction mixture was concentrated under reduced pressure to yield a yellow oil, toluene was repeatedly added to the residue, and the solvents were evaporated. Compound **5** was obtained as a white solid (40 mg, 0.20 mmol, 42%), which was recrystallised from methanol/diethyl ether. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 270 K): δ = 8.34 (d, 1 H, $^3J_{NH,2-H}$ = 8.8 Hz, NH), 6.20 (s, 1 H, 3-OH), 5.90 (s, 1 H, 4-OH), 5.42 (s, 1 H, 1-H), 4.54 (d, $^3J_{5,6exo}$ 5.5 Hz, 1 H, 5-H), 4.42 (d, $^2J_{6endo,6exo}$ = 7.9 Hz, 1 H, 6-H$_{endo}$), 4.09 (d, $^3J_{2-H,NH}$ = 8.9 Hz, 1 H, 2-H), 4.03 (s, 1 H, 4-H), 3.91 (s, 1 H, 3-H), 3.70 (m, 1 H, 6-H$_{exo}$), 2.04 (s, 1 H, CH$_3$) ppm. ES MS: calcd. for C$_8$H$_{13}$NO 203.1; found (positive mode): *m*/*z* = 226.0 [M + Na]$^+$.

2-Ammonio-1,6-anhydro-2-deoxy-β-D-glucopyranose Chloride (9): A solution of hydrochloric acid in milli-q water (a few drops from a pipette of concentrated HCl in approximately 5 mL of milli-q water) was prepared and added dropwise to a solution of recrystallised **7** (0.12 mmol) in milli-q water, until the pH was shown to be acidic, by spotting (with a fine capillary) onto pH indicator paper. The acidic solution thus obtained was lyophilised to yield a white solid that was recrystallised from methanol/diethyl ether. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 261 K): δ = 7.01 (br. s, 1 H, OH), 6.70 (s, 3 H, NH), 6.63 (br. s, 1 H, OH), 5.70 (s, 1 H, 1-H), 4.76 (d, $^3J_{5,6exo}$ = 5.5 Hz, 1 H, 5-H), 4.30 (d, $^2J_{6endo,6exo}$ = 7.8 Hz, 1 H, 6-H$_{endo}$), 3.91 (s, 1 H, 3-H), 3.857 (s, 2 H, 4-H, 6-H$_{exo}$), 3.34 (s, 3 H, 2-H) ppm.

2-Ammonio-1,6-anhydro-2-deoxy-β-D-galactopyranose Chloride (10): A solution of hydrochloric acid in milli-q water (a few drops from a pipette of concentrated HCl in approximately 5 mL of milli-q water) was prepared and added dropwise to a solution of recrystallised **8** (0.19 mmol) in milli-q water, until the pH was shown to be acidic, by spotting (fine capillary) onto pH indicator paper. The acidic solution thus obtained was lyophilised to yield a white solid, which was recrystallised from methanol/diethyl ether. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 270 K): δ = 6.9 (v br. s, 3 H, NH), 6.35 (br. s, 1 H, OH), 6.25 (br. s, 1 H, OH), 5.63 (s, 1 H, 1-H), 4.62 (s, 1 H, 5-H), 4.44 (d, $^2J_{6endo,6exo}$ = 8.0 Hz, 1 H, 6-H$_{endo}$), 4.16 (s, 2 H, 3-H, 4-H), 3.76 (m, 1 H, 6-H$_{exo}$), 3.55 (s, 1 H, 2-H) ppm. ES MS: calcd. for C$_6$H$_{12}$NO$_4$Cl 197.6; found (positive mode): *m*/*z* = 162.1 [M − Cl]$^+$; found (negative mode): *m*/*z* = 196.1 [M − H]$^+$.

2-Ammonio-1,6-anhydro-2-deoxy-β-D-glucopyranose Bis(3,5-di-*tert*-butylphenyl) Phosphate (11): Recrystallised **7** (0.12 mmol) was dissolved in milli-q water in a vial, and a second solution containing an equimolar quantity of sodium bis(3,5-di-*tert*-butylphenyl) phosphate in milli-q water was prepared. This solution was then transferred to the vial containing the sugar by micropipette. The solvent water was removed by lyophilisation, and a white solid was obtained and recrystallised from methanol/diethyl ether. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 261 K): δ = 8.30 (br. s, 3 H, NH), 7.41 (m, 2 H, H$_{aryl}$), 7.24 (m, 3 H, H$_{aryl}$), 5.70 (s, 1 H, 1-H), 4.78 (s, 1 H, 5-H), 4.30 (d, $^2J_{6endo,6exo}$ = 7.9 Hz, 1 H, 6-H$_{endo}$), 3.92 (s, 1 H, 3-H), 3.86 (s, 2 H, 4-H, 6-H$_{exo}$), 3.34 (s, 1 H, 2-H) ppm.

2-Ammonio-1,6-anhydro-2-deoxy-β-D-galactopyranose Bis(3,5-di-*tert*-butylphenyl) Phosphate (12): Recrystallised **8** (0.19 mmol) was dissolved in milli-q water in a vial, and a second solution, containing an equimolar quantity of sodium bis(3,5-di-*tert*-butylphenyl) phosphate in milli-q water, was prepared. This solution was then transferred to the vial containing the sugar, by micropipette. The solvent water was removed by lyophilisation, and a white solid was obtained and recrystallised from methanol/diethyl ether. ^{1}H NMR (500 MHz; [D$_6$]acetone/H$_2$O, 15:85; 270 K): δ = 8.42 (br. s, 3 H, NH), 7.41 (m, 2 H, H$_{aryl}$), 7.24 (m, 3 H, H$_{aryl}$), 6.36 (s, 1 H, OH), 6.27 (s, 1 H, OH), 5.64 (s, 1 H, 1-H), 4.62 (s, 1 H, 5-H), 4.44 (d, $^2J_{6endo,6exo}$ = 8.0 Hz, 1 H, 6-H$_{endo}$), 4.16 (s, 2 H, 3-H, 4-H), 3.76 (m, 1 H, 6-H$_{exo}$), 3.56 (s, 1 H, 2-H) ppm. ES MS: calcd. for C$_{18}$H$_{22}$NO$_8$P 411.3; found (positive mode): *m*/*z* = 412.3 [M + H]$^+$.

Sodium Bis(3,5-di-*tert*-butylphenyl) Phosphate: Ion-exchange resin (WA30, Supelco) was placed in a column (gravity packing). A volume of aqueous hydrochloric acid (2 M) equal to twice the volume of resin was passed through the column under gravity. Water was then passed through until the eluent was neutral; this procedure was repeated, substituting the hydrochloric acid solution with an aqueous sodium hydroxide solution (2.5 M). The resin was then washed with methanol, drained of excess solvent and stored until required. Bis(3,5-di-*tert*-butylphenyl) phosphate (350 mg, 1.4 mmol) was stirred with a large excess of resin (volume of 2 mL) in methanol (3 mL) overnight, or until analysis by NMR spectroscopy showed that the reaction was complete. [Differences between the aromatic resonances of bis(3,5-di-*tert*-butylphenyl) phosphate and sodium bis(3,5-di-*tert*-butylphenyl) phosphate allowed the conversion from the former to the latter to be monitored. ^{1}H NMR data of bis(3,5-di-*tert*-butylphenyl) phosphate for comparison with the corresponding data of sodium bis(3,5-di-*tert*-butylphenyl) phosphate: ^{1}H NMR (300 MHz; [D$_6$]DMSO; 299 K): δ = 7.36 (m, 2 H, H$_{aryl}$), 7.17 (m, 3 H, H$_{aryl}$) ppm.] The product was isolated by removing the resin by filtration, concentrating the filtrate and recrystallising; 90 mg (0.33 mol, 24%) of the salt was collected from the first recrystallisation. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 299 K): δ = 7.20 (m, 2 H, H$_{aryl}$), 7.11 (m, 2 H, H$_{aryl}$), 6.93 (t, *J* = 7.0 Hz, 1 H, H$_{aryl}$) ppm. ES MS: calcd. for C$_{12}$H$_{10}$NaO$_4$P 272.2; found (positive mode): *m*/*z* = 295.0 [M + Na]$^+$; found (negative mode): *m*/*z* = 249.1 [M − Na]$^+$.

3. ^{1}H NMR Studies in Aqueous Solution: For all ^{1}H NMR studies of **4**−**12** in aqueous solution, the corresponding monosaccharide was dried under high vacuum and a temperature of 308 K prior to use. Sample solutions for ^{1}H NMR spectroscopy were prepared at concentrations ranging from 2 × 10^{-3} to 8 ×10^{-3} M, and freshly filtered milli-q water was always employed. Throughout this work, the pH of the NMR samples was not varied. All the spectra in water solution were recorded with solvent suppression; usually, the WATERGATE pulse sequence was employed, although some spectra were also carried out with presaturation (Cambridge). A preliminary investigation was carried out to select the proper conditions for the acquisition of spectra of the aqueous sugar solutions. Usually, as the first step, several NMR spectra were recorded for a saccharide concentration of around 2 mM, employing different

combinations of [D_6]acetone and water. In particular, the [D_6]acetone content of the solvent was varied from 5% to 15%. A number of spectra were also acquired at various temperatures in pure water using a [D_6]acetone capillary. [Where an acetone capillary was employed the ^{1}H NMR spectra were referenced to the 2-H resonance at $\delta = 3.86$ ppm. An acetone resonance was observed at $\delta \approx 3.3$ ppm and a second resonance associated with the solvent was observed at $\delta \approx 4.3$ ppm. The chemical shift and linewidth of the latter resonance varied with temperature and it is proposed that its resonance was attributable to water within the capillary tube.] Room-, high- and low-temperature ^{1}H NMR spectra of the samples were acquired with Varian UNITY 500 (Madrid) and Bruker DRX 500 (Cambridge) spectrometers. All NMR spectra were recorded with careful control of the temperature. In all cases, chemical shifts (δ) are quoted in ppm, the downfield direction being positive, and are referenced to DSS, although acetone was used as external standard (capillary). Coupling constants (J) are given in Hz and uncertainties quoted as $\pm$ 0.1 Hz.

4. Measurement of Temperature Coefficients: As a first step, ^{1}H NMR spectra were acquired for each of the sugars **4**−**12** at various temperatures in 15% [D_6]acetone/water, in order to calculate the temperature coefficients of the NH and OH resonances. These values are shown in Tables 1 and 3, along with the representative coupling constants and chemical shifts. Five spectra were acquired at distinct temperatures in the 263−278-K range to determine temperature coefficients of sugar resonances in aqueous solution (15% [D_6]acetone in water). All measurements of temperature coefficients were carried out at least twice. The temperature coefficients were taken from the experiment in which the chemical shift/temp data exhibited the closest fit to a straight-line plot. Generally, differences smaller than 0.5 ppb K^{-1} were observed between values of temperature coefficients of a given signal determined from different experiments. Differences smaller than 0.6 ppb K^{-1} were observed between temperature coefficients of a given signal determined at high and low concentrations. This variation in temperature coefficients was similar to the experimental error.

5. Titration NMR Experiments: Titration experiments to determine the pH of the amino sugars in water solution were also carried out. The amino sugar was dissolved in D_2O (8 mM) and the pH of the obtained solution was determined. The NMR spectrum was then measured and the pH was determined again. The pH of the sample solution was varied by $\approx$ 0.5 pH units by the addition of small quantities (< 5 μL) of HCl and NaOH solutions in D_2O. Repetition of the complete procedure was performed a minimum of twelve times. The sampled pH (average of the measured values prior to and after the acquisition of each spectrum) and the observed chemical shift of 2-H were fitted to a straight line to obtain the corresponding pK_a. The pH-meter readings were not corrected for samples in D_2O.

6. Infrared Spectroscopy: The infrared spectra were recorded with a Perkin−Elmer 1725X Fourier transform infrared spectrometer assisted by a personal computer for signal storage, display and processing. For each aqueous solution spectrum, 32 scans were coadded at a spectral resolution of 2 cm^{-1}. Cells with ZnSe windows and 12 μm path length were used for measurement of the spectra. To compensate for ^1H_2O and ^2H_2O absorptions, the solvents were placed in the same kind of cell. Spectral contributions from residual water vapour in the sample chamber were eliminated by use of a set of water vapour spectra measured under identical conditions. The subtraction factor was varied until the second derivative of the 2000−1700-cm^{-1} absorption region was featureless.

Acknowledgments

Financial support by the DGES (Grant BQU2000-1501-C02-01) and the TMR European project (FMRX-CT98-0231) are acknowledged. M. L. P. is grateful to the Comunidad de Madrid for a predoctoral fellowship and to J. H. for a postdoctoral TMR fellowship.

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Received December 3, 2001
[O01568]