Interactions of Hydroxy Compounds and Sugars with Anions[1]

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Complexations of aliphatic monohydroxy compounds, of *trans*-1,2 cyclohexanediol, and of several glucose and galactose derivatives with two to four free hydroxy groups are measured in chloroform with peralkylammonium salts containing different anions. It is shown that NMR titrations with up to four different OH signals as well as with some CH signals allow accurate and consistent calculation of equilibrium constants *K* and complexation induced shifts (CIS). The anions used generally show an increasing affinity in the order *iodide* < *benzenesulfonate* < *bromide* < *diphenyl phosphate* < *chloride* < *benzenecarboxylate* (benzoate). The *K* values increase from secondary to primary alcohols, and again substantially to vicinal diols, culminating at up to $K = 10^3$ M^{-1} for 1-dodecyl glucose or galactose compounds. The observed CIS and *K* values agree with the formation of 3 different 1:1 complexes of similar stability for the phosphate receptor, with binding one anion between the 2-, 3-, 4-, and 6-OH groups of the glucoside, or only one 1:1 complex in the interaction of halides with sugars. Vicinal $^3J_{HOCH}$ coupling constants are analyzed before and after complexation and provide insight into the hydrogen bond network of the sugar derivatives.

Introduction

The selective binding of carbohydrates by noncovalent interactions is of enormous biological importance.[2] At the same time, it is a topic which only recently has begun to be explored by synthetic supramolecular chemistry. This is due to the rather weak intermolecular forces, characterized by small hydrogen bonding donor and acceptor numbers for hydroxyl groups, which are placed at the lower ends of corresponding acidity and basicity scale.[3] Aoyama et al.[4a] have shown, using phenolic macrocycles, predominant interactions with hydroxy groups in 1,4-position of pyranoses. Very recently, Bonar−Law and Sanders[5] have described a particularly effective steroid-capped porphyrin receptor for sugars. Aoyama et al.[4b] have presented evidence that lipophilic interactions of sugar C−H bonds with aromatic receptor moieties can provide weak additional stabilizations. Other receptors for sugars have been reported by Davis,[6] Gellman,[7] Still,[8] Anslyn[9] et al. and, using reverse micelles, Greenspoon et al.[10] Recently, weak complexes of some sugars with cyclodextrins have also been described[11] even with water as competing solvent.

Nature has overcome the problem of inherently weak hydrogen bonds mostly by providing in suitable protein structures strong acceptors and donors in the form of acidic and basic amino acids, respectively.[2a] Synthetic hosts containing phosphonates as anionic acceptors were recently described by Hamilton et al.[12] while our work was still in progress. We wanted to explore systematically different anionic functions for the complexation of aliphatic hydroxy compounds including carbohydrates. The anions studied comprise carboxylates as the entities used by nature, as well as phosphates, sulfonates, and halides, the latter being of interest mostly as they can interfere with other hydrogen bond acceptor sites also in biological matrices. As will be shown, the binding capacities of these anions differ substantially in spite of their equal charges. Surprisingly, there are almost no numbers characterizing the acceptor qualities of the anions even in the vast tabulations of acceptor and donor molecules published by Raevksy or by Abraham et al.[3] Another aim of the present study was therefore to provide such data, particularly in combination with hydroxy groups as hydrogen bond donors.

The major problem associated with the weak hydrogen bonds is the need to maintain usually an aprotic environment, which is materialized inside globular proteins.[2a] This is the reason why in the present work, as in other studies,[5,6,8,9,12] lipophilic solvents such as chloroform are used. Aoyama et al.[4c] have recently shown that, even using strongly anionic macrocyclic hosts, only weak complexes with carbohydrates are formed in water. Our strategy was based on the use of tetraalkylammonium ions as counterions in order to allow solubility of the salts in lipophilic solvents. The alkyl chains were planned to be long and/or bulky so that the anionic charges could be exposed to the OH-groups of the substrates or not. The question to which degree the alkyl chains would shield the anion from contact with the OH groups, and whether formation of the extremely tight ion pairs in such media would interfere too much, was investigated by using also hexadecyltrimethylammonium (CTA) besides tetradecylammonium (TDA) derivatives (Chart 1).

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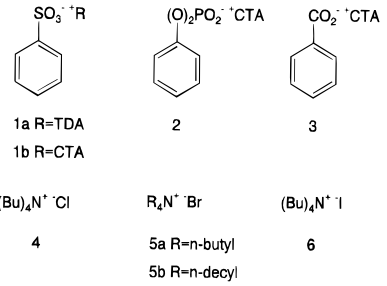
0022-3263/96/1961-1429$12.00/0

Chart 1. Acceptors and Alcohols Used in This Work. TDA = Tetradecyl ammonium. CTA = Cetyltrimethylammonium



The substrates (Chart 2) were so chosen that binding contributions of single hydroxy and of vicinal dihydroxy groups in different positions could be evaluated; the latter may be expected to show higher basicities and acidities as the result of known[13] intramolecular hydrogen bonds, which was studied by NMR spectroscopy. The use of partially protected sugars and of configurational isomers allowed evaluation of specificities, as well as the accumulative effects of the up to four OH groups present in the *n*-dodecyl β-D-glycopyranosides used.

Methods. The Use of OH NMR Signals for Complexation Studies. One major obstacle in the study of carbohydrate complexes is the lack of large NMR shielding differences between free and complexed material, which is the basis of most investigations with supramolecular systems. Another problem which in fact blurs many investigations is the extreme effect of water and acid traces.[14] Thus, in a 2.0 mM solution of *n*-dodecyl β-D-galactopyranoside (**18**) in chloroform with 3 mM water (see Experimental Section), the OH resonances of the sugar were clearly observable; after addition of 1% (only 2.0×10^{-5} M) *p*-toluenesulfonic acid they became broad, and with 2% acid the OH signals disappeared completely. It is likely for this reason that NMR was until now barely used for the study of carbohydrate associations, except where CH signals happened to show enough differences.[6,8,9,12] NH signals in amides, nucleobases, etc., are less sensitive to random medium effects and are therefore routinely used for studying corresponding equilibria. We show for the first time that, under suitable precautions, almost all OH signals in sugars can be used routinely for the evaluation of equilibrium constants *K* and complexation induced shifts (CIS values) by NMR titrations.

As documented, for instance, with Figure 1, not only the different OH signals in a sugar where observable, but also CH signals in typical titration experiment show satisfactory agreement with computer simulated least square fit curves[15] and usually yield constants *K* within a rather narrow range (Tables 2−5). The model used for all associations was based on a 1:1 complex formation. As the fitting showed no systematic deviation, and the *K* values for one system generally showed no significant disagreement between evaluation of different NMR signals, there was no justification to use other models, such as different 1:1 complexes which certainly are present with the sugars (see below). Self-association of the substrates was considered to be negligible in view of experimental dilution shifts of the OH signal; with >0.02 ppm these were much smaller than the CIS values with the other acceptors (see below and Tables 1−5).

Assignment of the signals was achieved by COSY45 experiments at different concentrations where necessary (see Experimental Section), allowing the unambiguous assignment of all sugar OH signals in chloroform (Figure 2). The *CIS* values obtained from the least square fit were found to be rather uniformly in the range of 2.5 to 3 ppm deshielding; their scatter may be partially due to undetectable traces of acids. Thus, addition of 1% *p*-toluenesulfonic acid with respect to tetraalkylammonium diphenyl phosphate to a solution containing 60% complexed 1,2-*trans*-cyclohexanediol (**11**) with **2** led to an additional deshielding of 0.54 ppm. Addition of 3% of the acid to the same solution produced 1.3 ppm downfield shift of the OH signal; with 10% acid the OH signal disappeared. There is no significant correlation between the *CIS* and the *K* values, suggesting a fairly constant charge depletion at the protons involved in hydrogen bonding to the different anions.

Results and Discussion

In spite of tight ion pairing in chloroform, steric shielding of the anion by the peralkylammonium cation does not prevent formation of hydrogen bond complexes with stabilities reaching values beyond $K = 10^3$ M (Table 5). The acceptor compounds **1a** and **1b** differ substantially in the alkyl chains of the cation, yet interact with the same energy with donor **11**. The same is observed in the case of **5a** and **5b** (Table 2).

A. Monohydroxy Derivatives. These provided data for the weak donor capacities of single OH groups compared to the vicinal diols or polyols, as well as for the difference between primary and secondary OH groups (Table 1). The number of anions tested with the simple alcohols was restricted to a few cases in view of the small constants *K*, and the therefore smaller degrees of complexation which could be obtained in the titrations, with the consequence of larger errors involved here. Nevertheless, the results show, as the diols discussed below, a stronger binding to chloride than to bromide and a 10 times higher equilibrium constant with primary compared to secondary alcohols.

B. *trans*-1,2-Cyclohexanediol. Diol **11**, containing gauche vicinal OH groups as the most frequent structural motif in carbohydrates, was used to compare the ability of many different anions to act as acceptors (Table 2). Noticeably, halides were found to be as efficient as oxygen-containing anions, in the usual activity sequence Cl > Br > I, which is in line with the p*K* differences of the corresponding hydrogen halides in aqueous systems. A similar order has been observed in studies of anion complexation with different hydrogen bonding host structures.[16] Fluoride in the form of tetraalkylammonium salts, unfortunately, was too hygroscopic for these mea-

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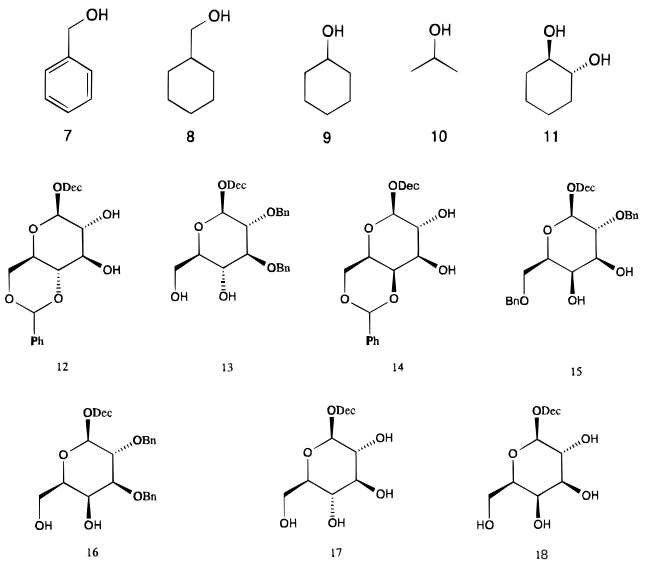
Chart 2



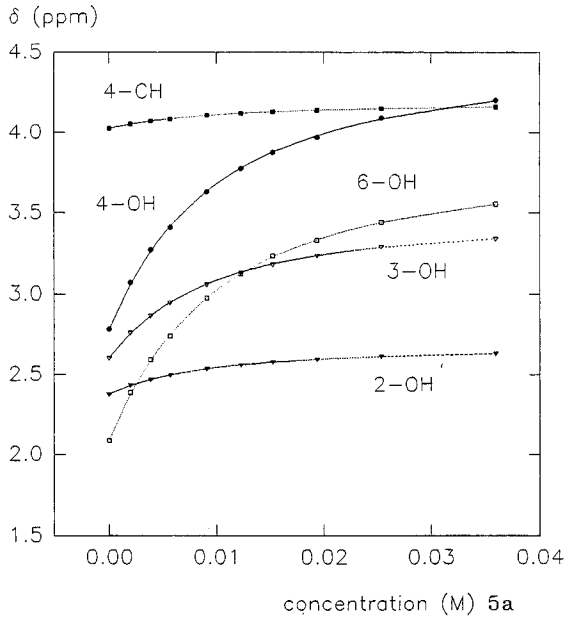


Figure 1. Experimental points and calculated curves for the binding of **18** and **5a**. All OH signals and that of 4-CH for compound **18** are shown.

surements, but is expected to compete very effectively with organic anions such as carboxylates. For the organic anions, aryl derivatives were chosen with the hope to observe NMR shielding effects by ring current anisotropies. The donor strength of the organic anions decreases substantially in spite of the same total charge, with the following order: carboxylate > phosphate ≫ sulfonate, which is interesting in view of the role of acidic amino acids in carbohydrates binding proteins. Again, the ΔG_{cplx} differences qualitatively follow the order of pK values in aqueous solution, reflecting the decreasing stabilities of the corresponding anions. This order qualitatively agrees with the one found by Wilcox et al.[16c] in the interaction of these anions with an urea.

The reason for the almost 20 times larger association found for the diol in comparison to the monohydroxy derivatives, which also is substantiated with the protected sugars (see below), may be seen in the acidity of a proton which is already involved in the well known intramolecular hydrogen bond. Recent data accumulated

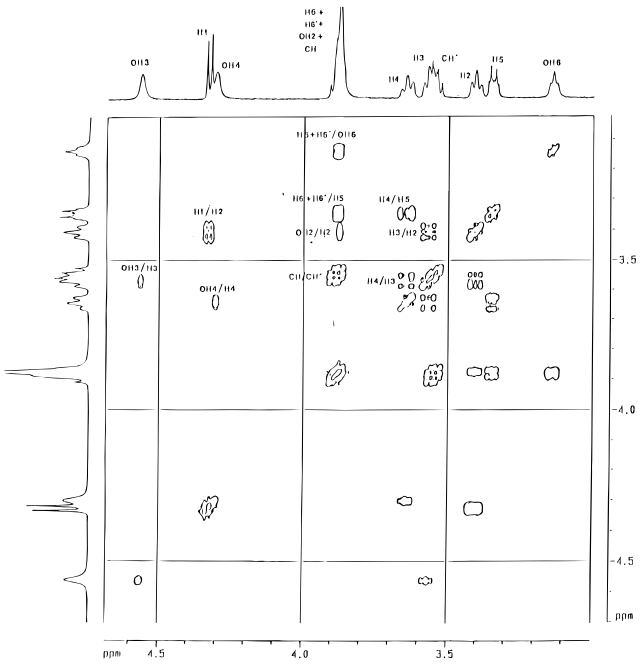


Figure 2. COSY45 for a 13 mM solution of **17** in $CDCl_3$.

Table 1. Association with Monohydroxy Compounds[a]

alcohol	acceptor	proton	K (M^{-1})	ΔG (kcal mol^{-1})	CIS (ppm)
7	**4**	OH	17	1.68	4.60
7	**5a**	OH	3.6	0.76	4.40
8	**2**	OH	10.7	1.41	2.43
9[b]	**2**	α-CH	0.9[c]	0.08	−0.34
		β-CH2	0.9[c]	0.08	−0.19
		β-CH2′	0.9[c]	0.08	−0.18
10[b]	**2**	α-CH	1.2	0.10	−0.24
		β-CH3	1.3	0.14	−0.18

[a] In $CDCl_3$ at 298 ± 1 K. Association constants K (error, standard deviation, <10% unless noted otherwise); free energies of complexation, ΔG; complexation induced shifts, *CIS*, positive values = deshielding, negative values = shielding. [b] The OH signal dissapeared after the first addition and could not be used. [c] Estimated error in K ± 25%.

Table 2. Association with 1,2-*trans*-Cyclohexanediol (11)[a]

anion	proton	$K_{ass.}$ (M^{-1})	ΔG (kcal mol^{-1})	CIS (ppm)
1a	OH	5.4	1.00	2.93
	β-CH	6.8	1.12	−0.11
1b	OH	5.3	0.99	6.48
	β-CH	5.8	1.05	−0.17
2[b]	OH	19.4	1.76	5.62
	β-CH	13.0	1.53	−0.13
	β-CH′	15.6	1.63	−0.17
3[b]	OH	31.3	2.05	2.87
	β-CH	32.9	2.08	−0.10
	β-CH′	47.0	2.27	−0.09
4	OH	34	2.10	2.44
5a	OH	11	1.43	2.45
5b	OH	9.3	1.33	2.67
6	OH	3.6	0.76	1.83

[a] See footnote a to Table 1. [b] Error, standard deviation, in K < 30%.

by Abraham[17] suggest acidities of vicinal diols to be much higher (with $\Sigma\alpha = 0.58$) than those of mono- ($\Sigma\alpha = 0.37$), or other dihydroxy derivatives ($\Sigma\alpha_{av} = 0.37$ if only one hydrogen bond complex per OH group is taken into account). That one proton can participate in several hydrogen bonds, in this case one being intra- and the

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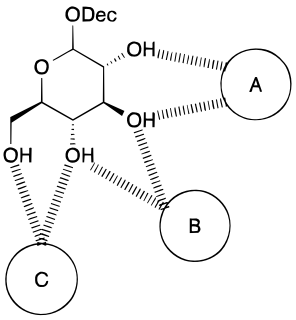

Figure 3. Diol type binding sites in compound **17**.

Table 3. Association with Partially Protected Sugars 12−16[a]

alcohol	proton	$K_{ass.}$ (M^{-1})	ΔG (kcal mol^{-1})	CIS (ppm)
11	OH	11	1.43	2.45
12	OH2	6.8	1.14	2.54
	OH3	6.8	1.14	2.67
13	OH4	9.6	1.34	2.90
	OH6	9.5	1.34	2.45
	OH2	3.4	0.73	1.81
	OH3	3.6	0.76	2.58
15	OH3	1.8	0.35	2.82
	OH4	1.9	0.38	2.39
16	OH4	7.3	1.18	1.42
	OH6	7.4	1.19	2.64
	CH4	7.5	1.20	0.48

[a] See footnote a to Table 1.

other intermolecular, is in line with structural findings in the solid state,[18] as well as with current views and calculational processing of hydrogen bonds based essentially on electrostatic interactions. Alternative explanations can be based on the formation of two instead of one hydrogen bonds to an anion with the diols. Intramolecular hydrogen bonding in similar partially protected sugar derivatives have been shown to be not very strong in chloroform.[19] Changes in coupling constants observed with galactose derivative **14** actually do speak for breaking of intramolecular hydrogen bond by complexation.

C. Binding to Partially Protected Sugars. The sugars **17** and **18** have three different binding sites (Figure 3). Compound **17** has two of the 1,2-*trans*-cyclohexanediol type and one of a 1,3-diol type binding sites, while **18** has one 1,2-*trans*-cyclohexanediol, one 1,2-*cis*-cyclohexanediol, and one 1,3-diol type binding sites. In order to check the binding capacities of the different binding sites, we decided to prepare the partially protected sugars **12**−**16** and to study their interactions with the ammonium bromide **5a**. The results are listed in Table 3.

Binding of bromide to partially protected sugars **12** to **16** largely reflects the affinities and *CIS* values observed with the simple model compounds. Thus, **12** and **13** show similar values as the diol **11**, indicating the same binding mode and donor acidity. The situation is different in the case of galactose derivatives: **16** shows the same affinity as **11**−**13**, but compounds **14** and **15** show an energetic disadvantage compared to the other derivatives. A similar difference has been observed before by Anslyn et al.[9] and is believed to be due to the difference of intramolecular hydrogen bond strength between *cis*- and *trans*-1,2-cyclohexanediol. The glucose derivative with free 3-OH and 4-OH was not prepared because its synthesis is not trivial, and its binding capacity is expected to be close to compound **11**.

Table 4. Association with *n*-Dodecyl *β*-D-Glucopyranoside (17)[a]

		1a	**2**	**4**	**5**	**6**
OH2	K_{ass}	*b*	470	615	153	26
	ΔG	*b*	3.66	3.82	2.99	1.94
	CIS	*b*	1.48	0.55	0.42	0.24
OH3	K_{ass}	*b*	902	686	166	*d*
	ΔG	*b*	4.04	3.88	3.04	*d*
	CIS	*b*	3.15	2.38	1.75	*d*
OH4	K_{ass}	*b*	889	695	165	*d*
	ΔG	*b*	4.04	3.89	3.04	*d*
	CIS	*b*	3.43	2.96	2.28	*d*
OH6	K_{ass}	*b*	589	603	151	25
	ΔG	*b*	3.79	3.81	2.98	1.91
	CIS	*b*	1.92	1.39	1.14	0.85
CH1	K_{ass}	33.5	*c*	*c*	*c*	*c*
	ΔG	2.09	*c*	*c*	*c*	*c*
	CIS	−0.03	*c*	*c*	*c*	*c*
	ΔG_{av}	2.09	3.88	3.85	3.01	1.92

[a] See footnote a to Table 1. [b] Proton not observable in the titration. [c] Proton not shifted with the interaction. [d] Proton not distinguishable at some points of the titration due to signal overlapping.

D. Binding to *n*-Dodecyl *β*-D-Glucopyranoside (17). Compound **17**, in which only one alkyl chain was introduced for the sake of chloroform solubility, showed free enegies of complexation with the anions much higher than the ones observed with the diols (Table 4). These sugar derivatives offer four OH groups for binding. The increase of the effective concentration of binding sites within one donor molecule is reflected in the high constants *K* which nevertheless could be derived without systematical deviations from fitting to a 1:1 model. In the anion concentration range used, the formation of 1:2 or 1:3 complexes, containing more than one anion, is disfavored. In contrast, formation of three different 1:1 complexes A, B, and C (Figure 3) is suggested by the fact that the sugar derivatives **12** and **13** with vicinal diols as well as the diol **11** show similar complex stabilities.

The formation of three different 1:1 complexes A, B, C (Figure 3) is reflected to a different degree also in the observed *CIS* and *K* values for the interaction between compound **17** and the phosphate **2**. In this case, all 4 OH signals show a good fitting to a 1:1 complexation mode; however, the 3-OH and 4-OH signals yield *CIS* and *K* values roughly twice as large as obtained with the 2-OH and 6-OH signals. This indicates that the central OH groups 3 and 4 are involved twice in different complexes, namely either A and B, or B and C, respectively, whereas the 2-OH and 6-OH groups are involved only once (in A or C). A Job plot[20] for the binding of **17** and **2** (Figure 4) showed a maximum between $x = 0.5$ and $x = 0.6$, indicating only a preference for 1:1 complexes over 2:1 complexes, which obviously coexist in solution to a minor degree.

The situation is different in the complexation of **17** by halides. In this case, the *K* values are similar for the 4 OHs and a Job plot showed a maximum at $x = 0.5$; the *CIS* values follow the order 4-OH > 3-OH > 6-OH > 2-OH. This can be due to the formation of only one 1:1 complex between the halide and the 3- and 4-OH groups

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(20) Job, A. *Ann. Chim. (10th Series)* **1928**, *9*, 113−204.

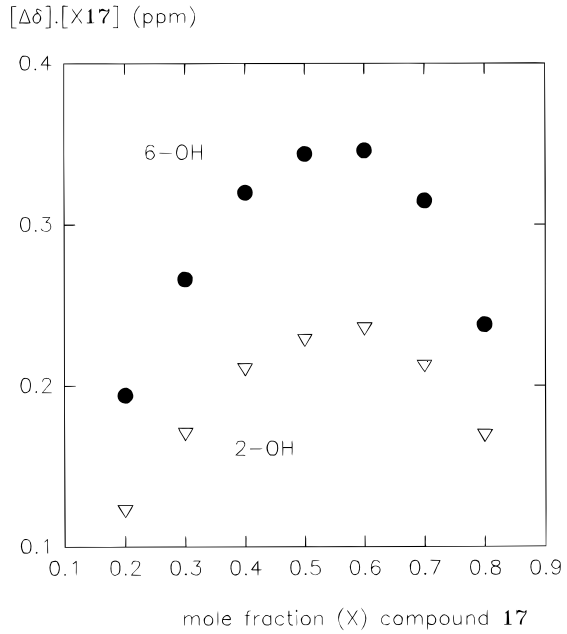

Figure 4. Job plot for the binding of **17** and **2**. Concentration of **17** + **2** was held constant at 2×10^{-3} M.

Table 5. Association with *n*-Dodecyl β-D-Galactopyranoside (18)[a]

		1a	2	3	4	5
OH2	K_{ass}	*b*	210	*b*	597	136
	ΔG	*b*	3.18	*b*	3.80	2.92
	CIS	*b*	1.85	*b*	0.42	0.33
OH3	K_{ass}	*b*	471	*b*	530	134
	ΔG	*b*	3.66	*b*	3.73	2.91
	CIS	*b*	2.94	*b*	1.10	0.89
OH4	K_{ass}	*b*	396	*b*	567	121
	ΔG	*b*	3.56	*b*	3.77	2.85
	CIS	*b*	2.72	*b*	2.15	1.78
OH6	K_{ass}	*b*	331	*b*	551	120
	ΔG	*b*	3.45	*b*	3.75	2.85
	CIS	*b*	2.86	*b*	2.51	1.86
CH1	K_{ass}	38.7	360	*c*	*c*	*c*
	ΔG	2.17	3.50	*c*	*c*	*c*
	CIS	−0.05	−0.07	*c*	*c*	*c*
CH4	K_{ass}	52.0	*c*	1067	−	126
	ΔG	2.35	*c*	4.15	−	2.88
	CIS	−0.06	*c*	0.182	−	0.171
	ΔG_{av}	2.26	3.47	4.15	3.76	2.88

[a] See footnote a to Table 1. [b] Proton not observable. [c] Proton not shifted with complexation.

which show the highest *CIS* values; 2-OH and 6-OH would still be involved in intramolecular hydrogen bonds. Upon complex formation the oxygens 3 and 4 become more basic, in other words, better acceptors and the intramolecular hydrogen bonds OH(2)−O(3) and OH(6)−O(4) would be stronger than before complex formation. The larger *CIS* value for 6-OH compared to 2-OH might be due to the higher flexibility of the CH_2OH group to form intramolecular hydrogen bonds.

E. Binding to *n*-Dodecyl β-D-Galactopyranoside (18) (Table 5). The *CIS* values observed for the interaction of compound **18** with phosphate **2** suggest that the interaction takes place with 3-OH, 4-OH, and 6-OH, all of them showing comparable, but higher values than the one observed for 2-OH. In this case, a 1:1 complex including *three* hydrogen bonds is possible between acceptor **2** and **18**, as galactose provides an *axial* OH directed toward the acceptor. This is suggested by molecular modeling[21] (Figure 5), indicating the OH groups in position 3, 4, and 6 as donors. A Job plot

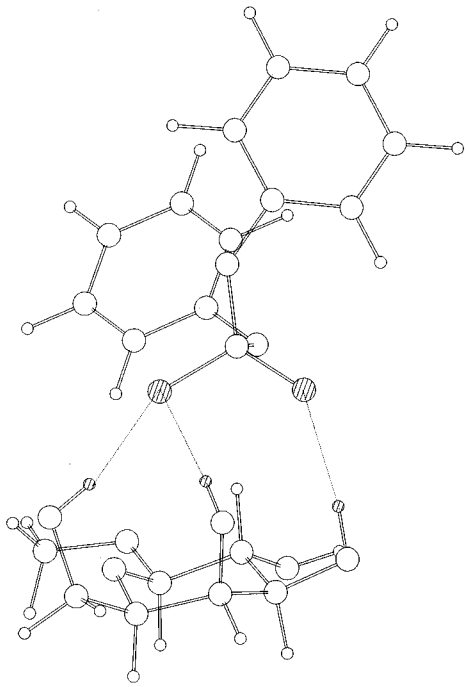

Figure 5. Structure with three hydrogen bonds obtained for the complex [**18** + **2**] using CHARMm 4.0.[21]

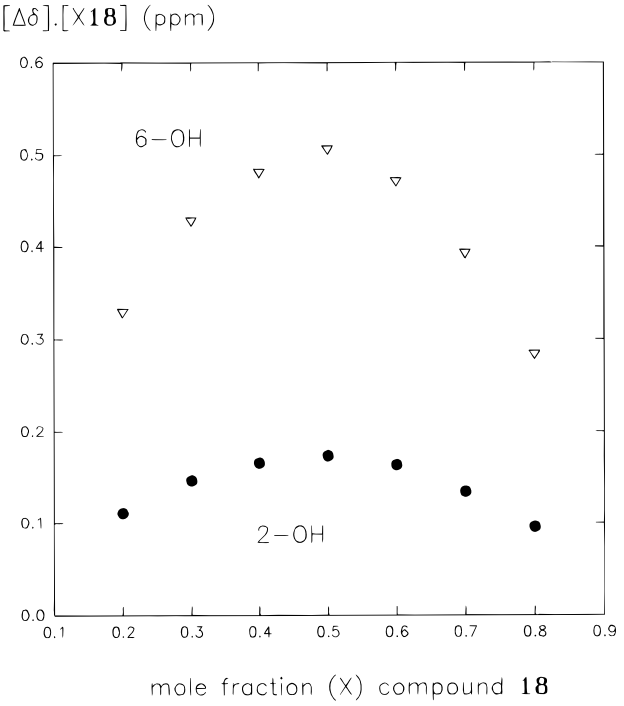

Figure 6. Job plot for the binding of **18** and **2**. Concentration of **18** + **2** was held constant at 2×10^{-3} M.

(Figure 6) for **18** and **2** gave a maximum at $x = 0.5$ indicating a 1:1 complexation mode. A three-fold hydrogen bond is not possible for acceptor **5a** which can interact only with two OH groups at the same time. The results in Table 3 clearly indicate that, for the complex **18** + **5a**, interaction with binding site A (2-OH and 3-OH, as in compound **14**) or B (3-OH and 4-OH, as in compound **15**) is less favorable than interaction with binding site C (4-OH and 6-OH, as in compound **16**). Consequently, the complex is formed with 4-OH and 6-OH as suggested by the *CIS* values obtained for this interaction. A Job plot for this interaction gave a

Table 6. Coupling Constants for Compounds 12−18 before and after Complexation with 5a[a]

sugar	hydroxyl	δ_0 (ppm)	J_0[b]	J_{exp} (%compl.)	ΔJ
β-D-glu **17**	OH2	2.39	2.2 (5.0)	*d*	–
	OH3	2.64	1.9 (4.7)	*d*	–
	OH4	2.52	2.4 (5.0)	*d*	–
	OH6	1.99	6.5[c] (5.8,6.0)	*d*	–
4,6-Bd-glu **12**	OH2	2.45	2.4	3.0 (58)	1.0
	OH3	2.63	2.2	3.0 (58)	1.0
2,3-di-Bn-glu **13**	OH4	2.18	2.3	4.2 (64)	3.0
	OH6	1.99	6.7[c]	6.8[c] (47)	0.2
β-D-gal **18**	OH2	2.37	1.8	*d*	–
	OH3	2.60	4.6	6.7 (72)	3.0
	OH4	2.78	2.9	4.0 (71)	1.6
	OH6	2.08	5.2/7.8	6.9[c] (63)	–
4,6-Bd-gal **14**	OH2	2.41	1.6	2.5 (48)	1.9
	OH3	2.46	8.8	6.5 (55)	4.2
2,6-di-Bn-gal **15**	OH3	2.43	4.0	5.1 (26)	4.2
	OH4	2.49	3.2	3.3 (27)	0.4
2,3-di-Bn-gal **16**	OH4	2.57	2.8	3.0 (45)	0.4
	OH6	2.06	3.9/8.9	5.1[c] (37)	–
4,6-Bd-α-gal **19**	OH2	2.29	9.1	–	–
	OH3	2.04	8.0	–	–
4,6-Bd-β-gal **20**	OH2	2.63	1.6	–	–
	OH3	2.60	8.9	–	–

[a] In $CDCl_3$ at 298 ± 1 K. Compounds **12**−**18** are *n*-dodecyl β-D-derivatives. Compounds **19** and **20** are methyl glycosides. Values given for compounds **19** and **20** have been taken from reference 18. [b] Numbers in brackets correspond to $^3J_{HOCH}$ in DMSO and have been taken from reference 22. [c] Unresolved triplet. [d] Coupling constant not distinguishible.

maximum at $x = 0.5$. If only one 1:1 complex is formed between the acceptor and 4- and 6-OH, the observed *CIS* values for 2- and 3-OH are due to the strengthening of the intramolecular hydrogen bonding network in the sugar after the complex has been formed. The difference in the *CIS* value between 2- and 3-OH reflects then that 3-OH is closer to the main interaction than 2-OH and, therefore, exhibits a higher *CIS* value.

F. Complexation and HOCH NMR Coupling Constants. As shown recently by Vasella et al.,[19] H−O−C−H coupling constants can provide valuable insight into the geometries of intramolecular hydrogen bond network in carbohydrates. Bonar−Law and Sanders[5] have described the HOCH coupling constants of α- and β-alkyl glycosides of glucose, galactose, and mannose in chloroform. We observed changes of these coupling upon complexation which can be used to derive information on the underlying geometries. Table 6 contains both the observed maximal value $^3J_{max}$ as well as the one expected for 100% complexation ($^3J_{cplx}$); these were obtained by dividing $^3J_{max}$ over the complexation degree calculated from the evaluated *K* values at the known concentrations of host and guest. For the analysis of ^3J we used a Karplus type equation[22] with empirically derived coefficients describing the variation of ^3J with the torsional angle Φ in the system HOCH with the following form:

$$^3J_{HOCH}\,(\mathrm{Hz}) = 10.4\cos^2\Phi - 1.5\cos\Phi + 0.2 \quad \text{(eq 1)}$$

Vasella et al.[19] have studied the HOCH coupling constants of some partially protected methyl glycosides in chloroform, observing that an equatorial OH shows characteristic $^3J_{HOCH}$ values of ca. 2, 4, or 9 Hz when it has in the vicinity an equatorial OH, an unprotected axial OH, or a protected axial OH, respectively.

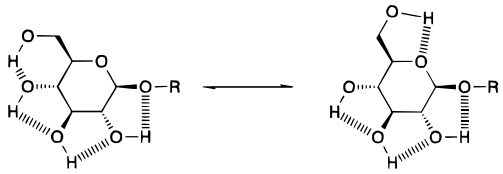

Figure 7. Most stable conformers obtained for **17** using CHARMm 4.0 showing the most probable intramolecular hydrogen bonding net for each conformer.

Molecular modeling[21] was used to calculate the torsional angles in the different conformers with intramolecular hydrogen bonds in order to compare the experimental values with the calculated ones. Compound **17** can be assumed to exist as two hydrogen bound species as shown in Figure 7. In both conformers, 2-OH, 3-OH, and 4-OH have torsional angles around 70°, with a small coupling constant around 1 Hz according to eq 1. This is in line with the experimental values around 2 Hz. The 6-OH signal appears as a triplet with very close *J* value for both 6-HOCHa (6a-OH) and 6-HOCHb (6b-OH). In conformer A, the calculated Φ values are 160° for 6a-OH and 70° for 6b-OH corresponding to *J* values of 11 and 1 Hz, respectively, while, in conformer B, these values are −80° for 6a-OH and −171° for 6b-OH. The average experimental value (6 Hz) suggests similar stabilities for both conformers. The same situation is observed in the partially protected sugars **12** and **13** before complexation with small coupling constants for 2-OH, 3-OH and 4-OH close to 2 Hz. The coupling constant value increases slightly in these protons when the complex is formed, suggesting a change in the torsional angle due to complexation.

The galactose derivative **18** can be described by three different conformers of very similar energy as shown in Figure 8. The 2-OH group shows $^3J_{HOCH}$ very close to the ones observed for 2-OH, 3-OH, and 4-OH in compound **17**, the reason being that 2-OH has only equatorial vicinal oxygens and, consequently, torsional angles of 70° in all conformers. 3-OH has one equatorial (2-OH) and one axial (4-OH) neighbor; when 3-OH acts as a donor to 2-OH the expected Φ is 70° corresponding to a *J* value of 2 Hz, but when it acts as donor to 4-OH this value is around 170° corresponding to a *J* value of 12 Hz. The observed *J* (4.6 Hz) is close to the average value of the three conformers. In the case of 4-OH, *J* is slightly larger than in the case of 2-OH, likely due to an intramolecular hydrogen bond with the pyran oxygen (conformer not shown). In this conformer, the torsional angle of 4-OH is close to −170° corresponding to a ^3J of 12 Hz, and the presence of a small concentration of this conformer would explain the increase of ^3J. 6-OH reflects two different coupling constants because the average of ^3J in the three conformers is different for 6-HOCHa and 6-HOCHb. The coupling constants of the hydroxyls change after complexation, indicating that their orientation is different in the complex. The most remarkable change occurs in the coupling constant observed for 3-OH. As it has been shown before, the main interaction must take place between the bromide and the hydroxyls 4 and 6 because they show the largest *CIS* values, and, when the complex is formed, ^3J for 3-OH increases to a value close to the one observed for compound **14**, behaving as if 4-OH were protected. On the other hand, when compound **14** interacts with the bromide **5a**, the 3-OH coupling con-

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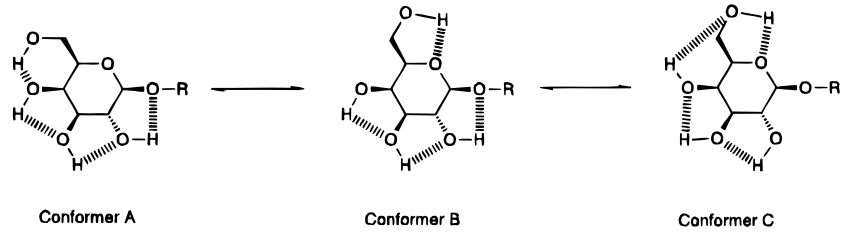

Figure 8. Most stable conformers obtained for **18** using CHARMm 4.0 showing the most probable intramolecular hydrogen bonding net for each conformer.

stant decreases from 8.8 to 4.2 Hz, indicating the partial breakage of the intramolecular hydrogen bond OH(3)−O(4) while forming the complex.

Experimental Section

^{1}H-NMR Spectra were obtained using a Bruker AM-400 (all titrations) or a Bruker DRX-500 (2D experiments) spectrometer. Molecular mechanics calculations[21] were performed on a Silicon Graphics Indy workstation using the QUANTA program with the CHARMm force field.

Binding Studies. $CDCl_3$ was deacidified with anhydrous K_2CO_3 and dried with 4 Å molecular sieves. The water content was checked by integration of the ^{1}H-NMR peak at 1.6 ppm, using DMSO as internal standard, and it was found to be 2.5−3.0 mM. Self-association of 1,2-*trans*-cyclohexanediol and dodecyl sugars was checked with dilution experiments and was found to be negligible at or below the following concentrations: 1,2-*trans*-cyclohexanediol, 10^{-2} M, *n*-dodecyl β-D-glucopyranoside, 1.7 mM, and *n*-dodecyl β-D-galactopyranoside, 2.0 mM. The dilution experiments could not be fitted to a 1:1 self-association mode, suggesting the presence of aggregates different from a 1:1 stoichiometry in the self-association.

Water Effects. A 1.7 mM solution of *n*-dodecyl β-D-glucopyranoside in $CDCl_3$ was titrated with wet chloroform; it was found that the OH resonances were observable when the water concentration was below 6.0 mM. In order to check the influence of water on the *K* values, two titrations were carried out with different water content (16 and 2.5 mM) for the interaction of 1,2-*trans*-cyclohexanediol (**11**) with TBABr (**5a**), and the *same* association constant within the error was obtained in both cases (10.7 and 11.3 M^{-1}, respectively).

The assignment of the 3- and 4-OH signals in the case of compound **17** was not clear by COSY45 when the concentration of the sample was 1.7 mM, but it was possible to assign the signals in a 2D experiment when the concentration was 13 mM (Figure 2). A dilution experiment showed that the relative positions of both protons did not change in the range 20−1.5 mM, permitting the assignment of both signals.

Titrations/Solvent Effect of Acetonitrile. Titrations were carried out as described before.[15] All titrations were done at least twice in order to ensure reproducibility, usually with deviations below 10% in *K* and *CIS* values. A titration of the interaction of compound **16** and bromide **5a** in deuterated acetonitrile gave $K = 17 \pm 1$ M^{-1}, representing an energetic advantage of 0.6 kcal/mol in respect to the same interaction measured in chloroform ($K = 7.5$ M^{-1}). This result reflects that acetonitrile is a weak acceptor while chloroform is a weak donor; as the OHs are acting as donors, the *K* in chloroform is smaller than the one in acetonitrile because chloroform competes with the sugar for the acceptor.

Solvents were refluxed and distilled using the following drying agents: for dichloromethane, CaH_2; for dimethylformamide, CaO; for acetone, K_2CO_3.

Synthesis. *n*-Dodecyl 4,6-*O*-Benzylidene-*n*-β-D-Glucopyranoside (12). *n*-Dodecyl β-D-glucopyranoside (434 mg, 1.25 mmol) and α,α'-dimethoxytoluene (0.46 mL, 3 mmol) were mixed in Cl_2CH_2 (70 mL). *p*-Toluenesulfonic acid (cat.) was added, and the starting suspension became a clear solution after 15 min. The mixture was stirred for 4 h. Water (70 mL) was added, and the organic phase was extracted with 5% $NaHCO_3$ and then with water, dried over $MgSO_4$, and concentrated. The residue was stirred with hexane for 20 min and a gel was formed. The gel was filtrated and washed with hexane (5 × 5 mL), and the final product was obtained (396 mg, 73%). ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.51-7.28$ (m, 5H, aromatic), 5.54 (s, 1H, benzylidene), 4.40 (d, $^3J(H,H) = 7.7$ Hz, 1H; CH1), 4.35 (dd, $^3J(H,H) = 4.9$ Hz, $^3J'(H,H) = 10.5$ Hz, 1H), 3.92−3.77 (m, 3H), 3.60−3.44 (m, 4H), 2.62 (d, $^3J(H,H) = 2.2$ Hz, 1H; OH3), 2.44 (d, $^3J(H,H) = 2.4$ Hz, 1H, OH2), 1.67−1.60 (m, 2H, $CH_2\beta$), 1.30−1.24 (m, 18H), 0.88 (t, $^3J(H,H) = {}^3J'(H,H) = 6.6$ Hz, CH_3). $C_{25}H_{40}O_6$ (436.6): calcd C 68.78, H 9.23; found: C 68.85, H 9.10.

***n*-Dodecyl 4,6-*O*-Benzylidene-2,3-di-*O*-benzyl-B-D-glucopyranoside.** *n*-Dodecyl 4,6-*O*-benzylidene-β-D-glucopyranoside (800 mg, 1.83 mmol) was dissolved in a suspension of NaH (338 mg, 14.7 mmol) in DMF (25 mL), and the mixture was stirred for 1 h and then cooled to 0 °C. Benzyl bromide (0.85 mL, 7,34 mmol) was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. MeOH (20 mL) was carefully added and, after all the NaH was quenched, water (20 mL) was added. A white precipitate was obtained, filtered, washed with methanol (5 × 5 mL), and dried. This product was used in the next step without further purification.

***n*-Dodecyl 2,3-Di-*O*-benzyl-β-D-glucopyranoside (13).** The precipitate obtained in the last step was dissolved in $CHCl_3$−MeOH 1:1 (150 mL), and *p*-toluenesulfonic acid (20 mg) was added. The mixture was stirred for 10 h at room temperature. Water (150 mL) was added, and the organic phase was washed with saturated $NaHCO_3$ (2 × 25 mL) and then with water (2 × 25 mL). The organic phase was dried over $MgSO_4$, and the solvents were evaporated. Column chromatography of the residue gave the final product as a white solid (702 mg, 73% in two steps). ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.38-7.29$ (m, 10H, aromatic), 4.98 (d, $^3J(H,H) = 11.6$ Hz, 1H, benzylic), 4.97 (d, $^3J(H,H) = 10.8$ Hz, 1H, benzylic) 4.72 (d, $^3J(H,H) = 11.0$ Hz, 1H, benzylic), 4.66 (d, $^3J(H,H) = 11.6$ Hz, 1H, benzylic), 4.45 (d, $^3J(H,H) = 7.3$ Hz, 1H; H1), 3.95−3.85 (m, 2H), 3.77−3.70 (m, 1H), 3.58−3.31 (m, 5H), 2.19 (d, $^3J(H,H) = 2.3$ Hz, 1H, OH4), 1.99 (t, $^3J(H,H) = {}^3J'(H,H) = 6.7$ Hz, 1H; OH6); 1.70−1.63 (m, 2H; $CH_2\beta$); 1.45−1.20 (m, 18H); 0.88 (t, 1H, $^3J(H,H) = {}^3J'(H,H) = 6.4$ Hz, 1H; CH_3). $C_{32}H_{48}O_6$ (528.7): calcd C 72.69, H 9.15; found: C 72.71, H 9.08.

Preparation of the corresponding galactose derivatives will be reported elsewhere.[24] All other substrates were commercially available products and were used without further purification.

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