Molecular Recognition of Carbohydrates Using a Synthetic Receptor. A Model System To Understand the Stereoselectivity of a Carbohydrate−Carbohydrate Interaction in Water

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Received June 26, 1995[⊗]

Abstract: We have determined by ^{1}H NMR the association constants between a synthetic receptor, glycophane **1**, and a series of 4-nitrophenyl and 2,4-dinitrophenyl glycosides (D-*gluco*, D-*galacto*, D-*manno*, D-*xylo*, L-*arabino*, and L-*fuco*) with axial and equatorial configuration at the anomeric center. The results indicate both a stabilization of the complexes due to the carbohydrate moieties and a stereoselectivity in the interaction. The equatorial glycosides are complexed with similar free energies of binding (~2.7 Kcal·mol^{-1}), while in the axial glycosides the free energy of binding changes with the stereochemistry of the sugar (from 2.6 to 3.3 Kcal·mol^{-1}). In contrast, the complexes formed between α-cyclodextrin and the 4-nitrophenyl glycosides did not show either additional stabilization nor selectivity due to the carbohydrate moieties. Differential changes in the free energy of binding in the glycophane **1**−4-nitrophenyl glycosides complexes result from changes in the chemical nature of the substituents of the guest. The ΔG also changes with the orientation of the hydroxyl groups that are in contact with the receptor but do not necessarily participate in hydrogen bonding interactions. Molecular mechanics and dynamics calculations gave different geometries for the axial and equatorial glycosides which agree with the geometries expected based on the ^{1}H NMR data. The results show that in our models lipophilic forces between carbohydrate surfaces mainly determine the stability of the association. Parallels are drawn between the forces involved in our models and those proposed for the biological associations of carbohydrates.

Molecular recognition of carbohydrates is characterized, as it is the case with other biological molecules, by the confluence of many weak polar and nonpolar intermolecular forces. However, the understanding of the contribution of these forces in carbohydrate recognition is probably more intriguing due to their molecular complexity. Whereas two identical amino acids can only build a dipeptide, two monosaccharides can form up to 11 different disaccharides. Consequently, it is to be expected that the language used by carbohydrates to express the affinity and selectivity for their receptors be much more complicated than that used by amino acids or nucleic acids.

The most important structural information about the intermolecular forces involved in carbohydrate recognition comes from the crystal structure of complexes formed between oligosaccharides and lectins or antibodies[1] and, more recently, from studies of glycolipid−glycolipid interactions.[2] From these studies hydrogen bonding and lipophilic interactions have been proposed as responsible forces for specificity. The importance of aromatic side-chain/saccharide stacking in stabilizing protein−carbohydrate interactions has also been recognized. Important questions to be answered are how polar and nonpolar groups of saccharides contribute to express affinity and selectivity and how these groups manage to strip the water molecules from between the reacting partners to make all the process energetically favorable for both the carbohydrate and the water molecules. However, to answer these questions not only structural but also energetic information is required.

Some time ago, Lemieux[3] anticipated the importance of lipophilic interactions and differential solvation effects on amphiphilic surfaces in the stability and selectivity of carbohydrate interactions. In contrast, other authors[4] have attributed the specificity and the driving energy for binding mainly to hydrogen bonding interactions. This paper attempts to address these questions using a model system to assess the role of the hydroxyl and lipophilic groups of a saccharide in the affinity and selectivity of their interactions in water. Recently, model receptors have been used in the study of the noncovalent interactions involved in carbohydrate recognition.[5] Most of these studies have been based on the potentiality of the hydroxyl groups to form hydrogen bonds in apolar solvents. However, few of these studies have been carried out in water,[5c,k] where carbohydrates are highly hydrated and their ability to form hydrogen bonds with the receptor has to compete with water. In this medium carbohydrates should be considered as am-

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⊗ Abstract published in *Advance ACS Abstracts*, November 1, 1995.

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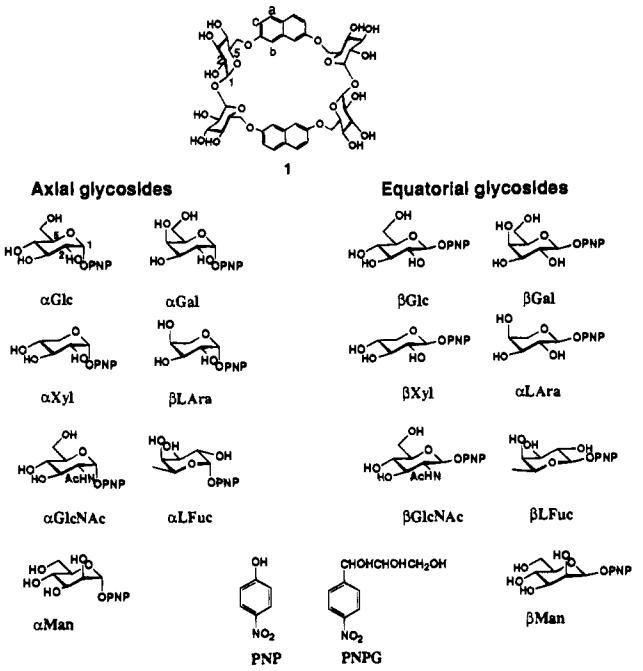
Chart 1



phiphiles with disperse hydrophilic and lipophilic areas so that forces, other than hydrogen bonds, probably play a role in the stability and selectivity of the interaction.[6]

We have recently used a model system constituted by a new type of receptors (glycophanes) and 4-nitrophenyl glycosides to study the binding of carbohydrates in water.[7] The receptor, glycophane **1**, (Chart 1) is constituted by a disaccharide, α,α-trehalose, and naphthalene molecules and may be considered as a cyclodextrin-cyclophane hybrid. Using this system we showed the existence of interactions in water between small saccharides, even though calorimetric evidences had previously suggested that this is an unfavorable process.[8] Our preliminary results with glycophane **1** and 4-nitrophenyl α- and β-gluco-, galacto-, and mannopyranosides showed evidence of both a stabilizing contribution due to the carbohydrate moieties and an α/β stereoselectivity in the interaction. These effects were not observed in a different model system constituted by the PNP-glycosides and α-cyclodextrin (αCD)[7]. We now have extended these studies in an attempt to understand the structural origin of the affinity and selectivity of this interaction and the contribution of the different polar and nonpolar groups of the carbohydrate moiety. We have used glycophane **1** and a broad range of the 4-nitrophenyl glycopyranosides (**PNP-Gly**) of D-glucose (**Glc**), D-xylose (**Xyl**), *N*-acetyl-D-glucosamine (**GlcNAc**), D-galactose (**Gal**), L-arabinose (**L-Ara**), L-fucose (**L-Fuc**), and D-mannose (**Man**) with axial and equatorial configuration at the anomeric center (Chart 1). These substrates have been chosen because of their distinct configurations which allowed us to evaluate the influence of both the nature of the substituents and the stereochemistry of the saccharides on the strength and selectivity of the interaction. Moreover, the interaction of glycophane **1** and the 2,4-dinitrophenyl (DNP) glycosides of D-glucose, D-xylose, D-galactose, D- and L-arabinose, and D- and L-fucose with equatorial configuration at the anomeric center has also been studied. Simultaneously, a comparative study between the complexes of α-cyclodextrin (αCD) and the 4-nitrophenyl glycosides and those of glycophane **1** has been carried out to assess the selective character of the interaction in the case of **1** with the PNP-glycosides. Our results indicate that the stability of the complex is related to lipophilic interactions (van der Waals and desolvation forces) and that the orientation of the hydroxyl groups in the substrate influences the free energy of these interactions, even though these groups do not seem to be involved in hydrogen bonding with the receptor.

Results and Discussion

The stability constants (K_a) of the interaction in D_2O between the glycophane **1** and the 4-nitrophenyl glycosides have been determined by ^1H NMR. In each binding experiment, the host concentration was held constant, while the concentration of the guest was increased. Upon the addition of the guest, the aromatic and the $H_{6\ endo}$ protons of the host shifted upfield (~0.3 ppm), while the H-1 and H-2 of the trehalose moiety moved downfield (~0.2 ppm). This pattern was the same in all cases, suggesting a similar binding geometry for all complexes. The induced chemical shifts by the β-glycosides were always smaller than those induced by the α-glycosides. For comparative reasons, the K_a of the aglycon (**PNP**) as well as the 1-(4-nitrophenyl) glycerol (**PNPG**), a lineal chain saccharide, were also determined.

The stability constants obtained at 30 °C and the corresponding free energy of binding ($-\Delta G$) are given in Table 1. To calculate K_a values we have used the upfield chemical shift change of the aromatic Hb proton of the host *versus* the guest concentration.[9] In all measurements the percent of saturation achieved was near 70%. All titration curves were carried out two or three times, and the values were reproducible within ±0.1 kcal·mol^{-1} in ΔG. In order to draw conclusions from the Gibbs free energies of binding, we have estimated the error associated with these measurements. The maximum percent of error in the binding constants was estimated to be 20%, and the temperature error was ±1 °C. Thus, the calculated error for ΔG is ±0.1 kcal/mol.

Binding Selectivity of Glycophane 1. The results shown in Table 1 reveal the following points: (1) All glycosides associate with the glycophane **1**. The association constants in the equatorial series are all in the same range of magnitude (~2.7 kcal·mol^{-1}), while in the axial series range from 2.6 to 3.3 kcal·mol^{-1}, depending on the configuration of the sugar. (2) In all complexes a stabilizing contribution of the carbohydrate moiety to the binding was observed. This stabilization could

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(9) In our previous measurements titrations were followed by ^1H NMR on the H1 and Hb resonances of **1**. We now know that measurements on H1 gave considerable errors, and we have used in this work only measurements on Hb for the determination of K_a. This is the origin of discrepancies between the values now given and those previously reported.

Table 1. Association Constants (K_a, M^{-1}) and Free Energy of Binding (ΔG, kcal·mol^{-1}) of the Complexes between Glycophane **1** and the *p*-Nitrophenyl Glycosides, *p*-Nitrophenol, and 1-(*p*-Nitrophenyl)glycerol in D_2O at 303 K

axial glycosides	K_a	$-\Delta G$	$-\Delta\Delta G_{PNP}$[a]	$-\Delta\Delta G_{PNPG}$[b]	equatorial glycosides	K_a	$-\Delta G$	$-\Delta\Delta G_{PNP}$[a]	$-\Delta\Delta G_{PNPG}$[b]
αGlc	111	2.8 ± 0.1	1.3	1.0	βGlc	98	2.7 ± 0.1	1.2	0.9
αXyl	155	3.0 ± 0.1	1.5	1.2	βXyl	96	2.7 ± 0.1	1.2	0.9
αGlc NAc	177	3.1 ± 0.1	1.6	1.3	βGlcNAc	96	2.7 ± 0.1	1.2	0.9
αMan	162	3.0 ± 0.1	1.5	1.2	βMan	70	2.6 ± 0.1	1.1	0.8
αGal	79	2.6 ± 0.1	1.1	0.8	βGal	91	2.7 ± 0.1	1.2	0.9
βLAra	166	3.0 ± 0.1	1.5	1.2	αLAra	81	2.6 ± 0.1	1.1	0.8
αLFuc	242	3.3 ± 0.1	1.8	1.5	βLFuc	99	2.8 ± 0.1	1.3	1.0
PNP	13	1.5 ± 0.1							
PNPG	21	1.8 ± 0.1	0.3						

[a] ΔG glycosides − ΔG *p*-nitrophenol in kcal·mol^{-1}. [b] ΔG glycosides − ΔG 1-(*p*-nitrophenyl) glycerol in kcal·mol^{-1}.

Table 2. Association Constants (K_a, M^{-1}) and Free Energy of Binding (ΔG, kcal·mol^{-1}) of the Complexes Formed between Glycophane **1** and 2,4-Dinitrophenyl Glycosides in D_2O at 303 K

DNP glycosides	K_a	$-\Delta G$	$-\Delta\Delta G_{DNP}$
βGlc	646	3.9 ± 0.1	0.3
βXyl	939	4.1 ± 0.1	0.5
βGal	525	3.8 ± 0.1	0.2
αLAra	745	4.0 ± 0.1	0.4
αDAra	1200	4.3 ± 0.1	0.7
βLFuco	787	4.0 ± 0.1	0.4
βDFuco	780	4.0 ± 0.1	0.4
DNP	420	3.6 ± 0.1	

be estimated from the comparison of the ΔG values with those of the complexes formed between **1** and *p*-nitrophenol (**PNP**) as well as with 1-(4-nitrophenyl) glycerol (**PNPG**) (Table 1). (3) The magnitude of the stabilization was higher for the axial glycosides and was influenced by the stereochemistry of the sugar (from 1.1 to 1.8 kcal·mol^{-1}). The highest free energy of binding was observed for α **LFuc** with an additional stabilization of 1.8 kcal·mol^{-1} related to **PNP** and 1.5 kcal·mol^{-1} with respect to the glycerol derivative (**PNPG**). (4) The contribution of the carbohydrate to the stability of the binding in the case of the equatorial glycosides is similar for all of them and smaller than that of the axial glycosides. These results indicate some axial/equatorial selectivity of the glycophane **1**. Finally, comparison of the chain saccharide **PNPG** complex with those of the pyranose derivatives shows the stabilizing effect of the pyranose ring related to a chain arrangement in a polyhydroxy compound.

In the case of the 2,4-dinitrophenyl glycosides (DNP-Gly), only the equatorial isomers could be measured.[10] Comparison of the ΔG values of the equatorial PNP-glycosides (Table 1) with those of the DNP-glycosides (Table 2) indicates a higher stability for the latter complexes. This increase is due to the major contribution of the aglycon, the 2,4-dinitrophenol (ΔG_{DNP} 3.6 kcal·mol^{-1}), to the stability of the complexes. However, the contribution of the carbohydrate moiety to the stability of the complex is lower than that of the PNP-glycosides. This can be attributed to the existence of a smaller number of van der Waals contacts between both host and guest carbohydrate moieties in the DNP-complexes. In fact, the hindrance of the NO_2-group in ortho-position avoids a deep inclusion of the guest in the cavity.

α-Cyclodextrin-PNP-Glycosides Binding. Even a smaller number of vdW contacts between host and guest carbohydrate moieties are to be expected in the complexes formed between αCD and the PNP-glycosides (Figure 1). A comparison of the results shown in Table 1 with those obtained for the complexes formed between αCD and the PNP-glycosides (Table 3) indicates that there is not additional stabilization due to the sugar

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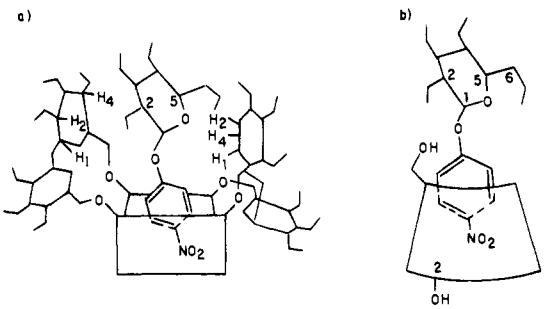

Figure 1. Schematic representation of the complexes of (a) glycophane **1**-PNP-α-D-glucopyranoside and (b) α-cyclodextrin-PNP-α-D-glucopyranoside based on CPK models.

Table 3. Association Constants (K_a, M^{-1}) and Free Energy of Binding (ΔG, kcal·mol^{-1}) of the Complexes Formed between α-CD and the *p*-Nitrophenyl Glycosides, *p*-Nitrophenol, and 1-(*p*-Nitrophenyl)glycerol in D_2O at 303 K

axial glycosides	K_a	$-\Delta G$	equatorial glycosides	K_a	$-\Delta G$
αGlc	129	2.9 ± 0.1	βGlc	83	2.6 ± 0.1
αGal	120	2.8 ± 0.1	βGal	127	2.9 ± 0.1
αMan	170	3.0 ± 0.1	βMan	122	2.8 ± 0.1
βLAra	150	3.0 ± 0.1	αLAra	111	2.8 ± 0.1
αLFuc	137	2.9 ± 0.1	βLFuc	106	2.8 ± 0.1
PNP (pH3)	192	3.11 ± 0.03[a]			
PNPG	122	2.9 ± 0.1			

[a] Binding constant calculated by calorimetry in H_2O at 298 K.

moiety in these complexes with respect to those between αCD and **PNP** as well as **PNPG**. Axial and equatorial glycosides have similar association constants, in contrast to the axial/equatorial selectivity observed for glycophane **1**. Furthermore, the stereochemistry of the sugar does not seem to influence to the same extent than in the case of **1** the free energy of binding (ΔG 2.8−3.0 kcal·mol^{-1} for all glycosides). These differences in complexing properties between glycophane **1** and αCD may be due to the different orientation of the interacting sugar surfaces. In the case of αCD, the carbohydrate moiety of the guest will remain mainly in contact with the bulk water during the interaction, while in the glycophane it will approach to the β-face of the glucose units of the trehalose molecules (Figure 1). This different approach makes van der Waals contacts between both host and guest carbohydrate faces possible in the glycophane system, thus favoring desolvation of both surfaces upon binding. Differences in van der Waals contacts due to the different relative orientation of the sugar and the aromatic ring in the axial and the equatorial PNP- glycosides may be the origin of the observed selectivity showed by glycophane **1** for these compounds.

Influence of the Nature and Orientation of the Substituents on Binding Selectivities. The deeper encapsulation of C1, C2, and C5 of the pyranose ring of the guests in the complexes with **1** should result on a major influence of the nature of the

substituents and the configuration of the sugars at these positions on the binding. Comparison of the different free energies of binding resulting by changing the substituents at these carbons will give information about the role of the hydroxyl groups at these positions.

Substitution of the hydroxymethyl group at C5 in the axial *gluco* derivative (**αGlc**) by hydrogen to give the corresponding glycoside with *xylo* configuration (**αXyl**) increases the binding in 0.2 kcal·mol^{-1} (Table 1). An increase of 0.3 kcal·mol^{-1} in the binding is observed by substitution of the OH-group at C2 by the less polar acetamide group to give the α-D-glucosamine derivative (**αGlcNAc**). A similar effect is observed by changing the equatorial by the axial orientation of the OH-2 ($\Delta\Delta G_{\alpha Man\text{-}\alpha Glc}$ 0.3 kcal·mol^{-1}). In contrast, the same changes in the equatorial series (**β Glc → β Xyl, β Glc → β GlcNAc, β Glc → β Man**) do not influence the ΔG values of binding. The same trend is observed for the *galacto* series. Comparison of the binding of **αGal** with the β-L-*arabino* derivative (**βLAra**) indicates that the CH_2OH group at C5 has a destabilizing effect of 0.4 kcal·mol^{-1}, whereas no change is observed on going from **βGal** to **αLAra**. Evidence for higher contribution to the binding by apolar substituents was also obtained from the comparison of the binding of **αGal** with the corresponding 6-deoxy-derivative with L-configuration (i.e., the α-L-*fuco* derivative; $\Delta\Delta G_{\alpha LFuc\text{-}\alpha Gal}$ = 0.7 kcal·mol^{-1}).

Clearly, these results show that the presence of a hydroxymethyl group at position 5 in the axial derivatives has a destabilizing effect. The above results also suggest that the equatorial hydroxyl group at position 2 has a similar destabilizing effect since substitution by H or acetamide groups increases the binding. This trend was not found in the equatorial glycosides, indicating a different geometry for axial and equatorial complexes.

We have tried to assess the tridimensional structure of the complexes on the basis of the intermolecular NOEs observed for the different geometries. In all complexes, significant NOEs were found between the aromatic protons of the host and those of the guests, indicating the inclusion of the aromatic ring of the guest within the cavity of the host (Figures 1−3 in supporting information.). In addition, for **αMan**, short average sugar−sugar intermolecular interproton distances were deduced from NOE and ROESY experiments (Figure 4 in supporting information. Nevertheless, given the dynamic nature of the complex and the small NOEs involved, unambiguous conclusions on the structure of the complexes could not be extracted from the NOE alone. Attempts to crystallize both complexes in order to assess the geometries also failed. At this point, molecular modeling studies were performed to complement the above results on the interacting geometries of axial and equatorial complexes.

Molecular Modeling. Studies began determining possible structures of the free host. The conformational analysis of the isolated host was performed by a Monte Carlo (MC) approach as described in the Experimental Section. Ten different conformers were found within a steric energy difference of 12 kJ·mol^{-1}. Assuming that entropy difference among these conformers is negligible, the respective population of these conformers, calculated from a Boltzmann distribution function, is indicated in Table 4 in the supporting information.

Several characteristics are common to these conformers. All glucose units adopt the $^4C_1$ chair conformations, and the glycosidic linkages can be described by torsion angles (Φ/Φ, *ca.* 70°) which are in agreement with the *exo*-anomeric effect[11]

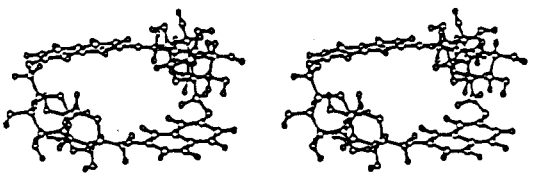

Figure 2. Stereoview of the global minimum calculated for glycophane **1**.

(Table 4 in supporting information). Independently of the MC protocol which was employed, all the hydroxymethyl groups adopt either *gauche-gauche* or *gauche-trans* conformations. Thus, no alternative *trans-gauche* conformation was found, in agreement with the expectations for glucose lateral chains. For the ten conformers, geometries with four, three, or two hydroxymethyl groups in orientation *gt* was found. The global minimum (Figure 2) shows the four hydroxymethyl groups with *gt* conformation. This minimum was also the structure most frequently found in the MC search, independently of the protocol used. It can also be observed that, although, some conformers present an appropriate cavity for complexation of an aromatic guest molecule, other local minima would not permit complexation to occur, since the cavity is blocked (Figure 5 in supporting information).

Molecular dynamics simulations performed using the global minimum as starting geometry indicated that this conformation is fairly stable (supporting information). No variation in the glycosidic linkages nor the hydroxymethyl groups was observed. Only small oscillations around the starting values were detected (ca. ±15° for the hydroxymethyl groups and ±10° for the glycosidic linkages). Larger variations were observed for the linkages involving O6−C_{Ar} and O6−C6.

Although the NMR data are averaged among the different forms present in solution, they satisfactorily agree with the computational MM3* calculations, in particular regarding the glycosidic linkage orientation and the conformational equilibrium of the hydroxymethyl groups. The ^{1}H- and ^{13}C-NMR spectra of the host indicated a fast conformational equilibrium in the NMR chemical shift time scale, since only one set of signals was observed for each kind of proton and carbon. A medium size NOE (from ROESY and NOESY experiments) between the anomeric proton of a glucose moiety and H-5 of the contiguous residue indicates the *exo*-anomeric orientation[11] of the glycosidic linkages. In addition, the average coupling constants between H-5 and H-6_{endo} and H-6_{exo} (2.3 and 5.4 Hz, respectively), confirm that there is an ~1:1 equilibrium between the *gg* and *gt* forms. Also the trivial NOEs between the aromatic protons and between these protons and H-6_{endo} were found. The only discrepancy between the NMR data and molecular dynamics simulations affects the lack of transitions between the *gg* and *gt* orientations of the glucose lateral chains during the MD simulation. Nevertheless, this lack of transitions is not unusual, since it has been already reported for sugars.[12] Much longer simulation times seem to be necessary to account for these transitions in a quantitative way.[13]

Molecular Modeling of the Complexes. The guests α- and β-PNP-gluco- and mannopyranosides were docked to the glycophane host as described in the experimental part. Although all the conformations found for the free host show two different faces, only one side of the cavity produced energetically acceptable complexes, independently of either the α,β configuration or the nature (*manno, gluco*) of the sugar. This energetically favorable host−guest orientation maximizes the

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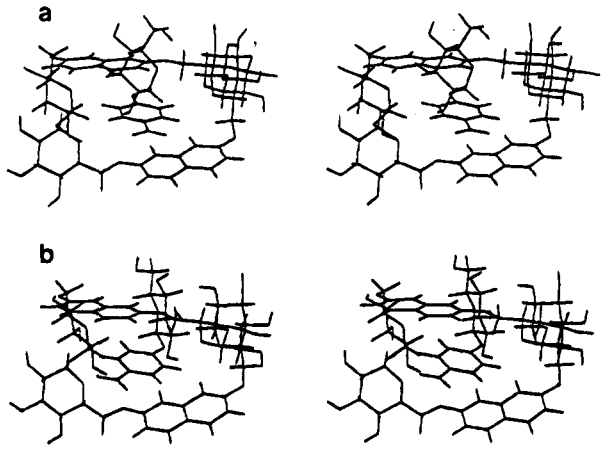

Figure 3. Stereoview of the calculated global minima structures for the complexes. (a) **1**·αGlc and b) **1**·βGlc.

interactions between both host and guest carbohydrate moieties. Two different conformations were found for every complex within a range of 12 kJ·mol^{-1}. The two major conformations differ in the orientation of one hydroxymethyl group of the receptor. Therefore, and according to the calculations, it is possible to form stable complexes having either the *gg* or *gt* conformations of the lateral chains. The change of one hydroxymethyl group from *gt* to *gg* orientation does not substantially modify the geometrical features of the complexes. A stereoview of the global minima conformers for the α- and *β-gluco* complexes is given in Figure 3. The corresponding torsion angles are similar to those of the free host.

The conformational stability of the complexes was also tested by MD simulations (Figure 6 in supporting information). The complexes are perfectly stable during the whole 500 ps simulation. Although the aromatic PNP ring is within the cavity, there are still significant fluctuations of the torsion angles, in particular, those involving the conexion of O-6 to C-6 and to the naphthalene rings (*ca.* ±25°). On the other hand, the glycosidic linkages and the lateral chains are slightly more restricted in the complexes than in the isolated host. It is interesting to note that the lateral chains of the guest present transitions between their corresponding *gg* and *gt* orientations.

The global minima structures of the **1**·αMan and **1**·βMan complexes are represented in Figure 4. The two structures explain the ^{1}H NMR observed upfield shift for the aromatic and $H6_{endo}$ protons and the downfield shift for the anomeric proton of the host upon complexation of all the guests. The model geometries show that in both α- and β- complexes the aromatic ring and the region around the anomeric centre (C1, C2, C5, and C6) are deep in the cavity originating vdW contacts between trehalose and mannose moieties. Besides the expected aromatic−aromatic interaction, vdW contacts between the naphthalene and the sugar moieties are also present.

The calculated geometries also show clearly the different orientation of the axial and equatorial glycopyranosides within the cavity. As a consequence a higher number of vdW contacts below 3.7 Å are established with the axial glycosides than with the equatorial derivatives. These differences are also observed in the calculated geometries for the α- and *β-gluco* complexes. But the most striking differences between both geometries are the orientation of the hydroxyl groups of the guest within the cavity. In the **1**·αGlc complex OH2, OH3, OH4, and OH6 of the glucose ring present vdW contacts below 3.7 Å with the hydrophobic patch CH1−CH2−CH4−CH6 of the trehalose moiety. Hydrogen bonding interactions could be also established between OH2 and OH6 of the guest and OH2 of the

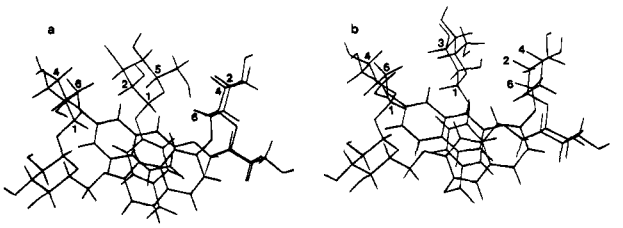

Figure 4. A view of the calculated global minima structures for the complexes (a) **1**·αMan and (b) **1**·βMan.

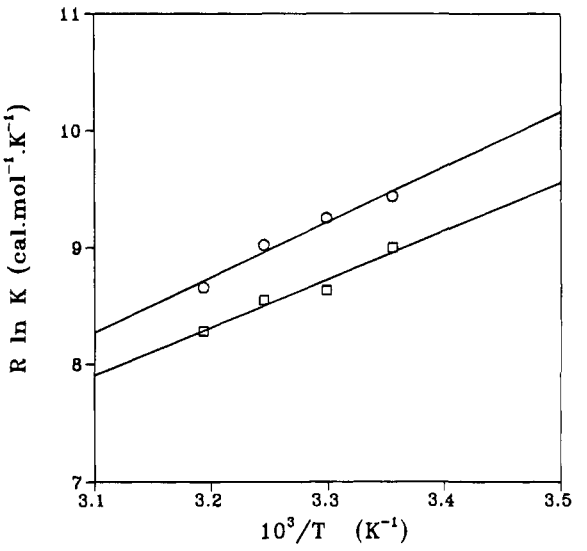

Figure 5. Van't Hoff plot for **1**·αGlc (○) and **1**·βGlc (□) complexes.

trehalose moiety, while in the **1**·βGlc complex all the hydroxyl groups are directed to the bulk water, and no contacts with the trehalose moiety are present (Figure 3). Differences in the orientation of the hydroxyl groups in the **1**·αMan and **1**·βMan complexes (Figure 4) could also explain the differences observed in their free energy of binding ($\Delta\Delta G_{\alpha Man\text{-}\beta Man}$ 0.4 kcal·mol^{-1}). In the αMan complex OH2 and OH4 are in contact with bulk water, while OH3 and OH6 present vdW contacts with the lipophilic surface of the trehalose moiety. As it was the case of the αGlc complex, an intermolecular hydrogen bond between OH6 of the guest and OH2 of the trehalose moiety could be established, while in the βMan complex only the OH2 group is directed to the hydrophobic CH1−CH2−CH4−CH6 patch of the trehalose moiety, the rest of the hydroxyl groups being bound to water. Thus, these calculated geometries are consistent with the differences in free energy of binding experimentally observed for axial and equatorial complexes.

In order to better evaluate the forces involved in this association accurate values of enthalpy, entropy, and heat capacity would be required. We are now engaged in a study by calorimetry of the complexes formed between the PNP-glycosides and αCD, but unfortunately the low solubility of glycophane **1** in water did not allow until now the calorimetric study with this system. Although the enthalpy and entropy values obtained from van't Hoff analysis may be only approximated, we have carried out this analysis for the complexes formed between glycophane **1** and 4-nitrophenyl α- and β-D-*gluco* derivatives. ^{1}H NMR spectra were recorded at 298, 303, 308, and 313 K which results in $\Delta H = -4.8$ and $T\Delta S = -1.9$ kcal·mol^{-1} for the axial glycoside and $\Delta H = -4.1$ and $T\Delta S = -1.4$ kcal·mol^{-1} for the equatorial analogue at 298 K (Figure 5). These values are similar to those found for monosaccharide associations to lectins[14] and to those reported for apolar guest associations with cyclophanes.[15] In these cases, it has been

observed that the enthalpy of binding is more negative than the free energy of binding. These values show also a higher enthalpy of binding for the axial glycosides.[16]

In conclusion, all these results show that in our models differential changes in the free energy of binding result from changes in the chemical nature (polar/apolar) of the substituents in the guest and/or in the orientation of hydroxyl groups that are in contact with the receptor but do not necessarily participate in hydrogen bonding interactions. In contrast, changes in hydroxyl groups that are farthest from the receptor do not influence the free energy of binding. These effects, similar to those found for the binding of modified oligosaccharides by lectins,[17] can arise from changes in both the vdW contacts between the OH groups and the lipophilic surfaces of the receptor and/or in the hydration forces involved in the solvation/desolvation[18] of the complementary amphiphilic surfaces of the sugars.[6a]

This paper constitutes an attempt to establish a link between the molecular structure and the free energy of a carbohydrate–carbohydrate interaction in water. It has been shown that in our glycophane–PNP-glycosides model system, lipophilic forces between carbohydrate surfaces mainly determine the stability of the association. In contrast, in the αCD-PNP-glycoside system neither additional stabilization nor selectivity due to the presence of the carbohydrate were observed. We would like to stress that these results indicate, as anticipated by Lemieux, that "the main driving force for complex formation likely occurs when the adjacent complementary nonpolar surfaces come together"[19] and also agree with the suggestion by Hakomori that "complementarity of two interacting carbohydrates could be based on hydrophobic interactions between the respective hydrophobic surfaces".[2a] It follows then that the role of the hydroxyl groups in a carbohydrate is not only significantly related to the formation of hydrogen bonding interactions but also to the strengthening of the lipophilic interactions. The orientation of the hydroxyl groups can influence this strengthening modulating the shape of the complementary amphiphilic surfaces thus favoring the vdW contacts and assisting the release of high-energy water molecules from around these surfaces. This assessment is at the heart of the long standing debate concerning the importance of hydrogen bonding interactions *versus* lipophilic and hydration effects in the association of carbohydrates in aqueous solution.[4,6a,20] In this regard, it would be of interest to mention the recent works by Kahne et al. concerning the role of the oligosaccharide in the chromomycin A_3–Mg^{2+} complex[21] and the influence of hydrogen bonding in the aggregation of glycosylated bile acids.[22] These systems have some similarities in their behavior with our model systems. In all of them an interaction is additionally stabilized by the presence of a carbohydrate moiety. In the first case[21] the stabilization of a dimer complex is attributed to the presence of a sugar-aromatic chromophore (chromomycinone) interaction. In the other case, hydrogen bonding interactions are invoked as the origin of the lower critical micelle concentration observed in glycosylated *versus* nonglycosylated bile acids.[22b] We suggest that the increase in aggregation observed in the glycosylated bile acids may be also due to not only hydrogen bond formation between the sugar moieties but also lipophilic carbohydrate–carbohydrate interactions, similar to those observed in our model systems. This observation is also supported by the crystal structure of these bile acids,[22a] where, in addition to the intermolecular hydrogen bond formation between opposing sugars, the contacts between nonpolar carbohydrate surfaces are maximized. These results, along with our results, strongly support the ideas developped by Lemieux about the forces involved in the molecular recognition of carbohydrates.

Experimental Section

General Methods. NMR spectra were recorded on Varian spectrometers. Chemical shifts in D_2O were referenced to external DMSO (2.5 ppm) in a coaxial tube. Glycophane **1** was prepared as published.[7] The *p*-nitrophenyl glycosides were obtained from commercial sources (Sigma and Aldrich) except the *p*-nitrophenyl α-D-xylopyranoside (αXyl) which was synthesized using a procedure developed in our laboratory. The 2,4-dinitrophenyl glycosides were also prepared by us.[10]

Binding Studies. A stock solution for the host (0.09 mM) was prepared by dissolving the host in D_2O. Stock solution for the guests (10–25 mM) were prepared from the stock solution of the host in order to have a constant concentration of the host during the titration. Binding constants were determined by adding in portions via microsyringe a solution of guest to a solution of host. The ^1H NMR spectrum of each solution was recorded, and the chemical shifts of the singlet of Hb of the host obtained at nine different host:guest concentration ratios was used in an iterative least-squares fitting procedure.[23] The temperature was kept constant at 303 ± 1 K.

Computation Procedure. All calculations were performed in a Silicon Graphics workstation with the MM3* force field as integrated in MACROMODEL v4.5.[24] The MM3* program differs from the regular MM3[25] in the treatment of the electrostatic term, since it employs charge–charge instead of dipole–dipole interacions. Studies began by determining possible host structures. The initial structure was obtained from an MM3* minimization using the MACROMODEL program and the GB/SA solvation model described by Still and coworkers.[26] The starting geometry was built by setting the glycosidic torsion angles of the trehalose moieties, Φ and Φ′, defined as H-1′-C-1′-O-1′-C-1, and H-1-C-1-O-1′-C-1′, close to the *exo*-anomeric position, *ca.* 60°, since this orientation has been shown to be strongly predominant for trehalose and trehalose derivatives.[27] The conformation of the lateral chains was set to *gauche-gauche*, which corresponds to

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an ω angle (H-5-C-5-C-6-O-6) *ca.* 180°. This conformation as well as that of the *gauche-trans* (60°) has been shown to be preferred for glucose hydroxymethyl groups both in solution and in the solid state.[28] The elevated number of torsions in the host (16) prompted us to choose a Monte Carlo (MC) approach for the conformational search of all possible host conformers. One of the bonds between one naphthalene moiety and the O-6 atom of its corresponding glucose moiety was chosen as the ring closure bond. The glucose moieties were left at their stable $^4C_1$ chair conformations. First, a global search using 2200 MC steps was performed: each Monte Carlo step began with the starting geometry of the previous step. A number of torsions between 2 and 13 were randomly selected for modification at each MC step. After each MC step, the resultant geometry was minimized using 5000 gradient conjugate steps. Structures were tested for duplication according to a least square criterion (0.4 Å) and to the Numbering System Rotation implemented in Macromodel for highly symmetric molecules. In addition, the torsion angles of the accepted structures were further examined visually. An energy window of 50 kJ·mol^{-1} was used as criterion to accept a given conformation. After elimination of the repeated minima, the accepted conformations were saved for subsequent analysis. Second, five different geometries, randomly chosen from the set of accepted structures, were submitted to independent MC global searches. For every starting geometry, 1000 MC steps were performed. The other criteria remained the same. Third, seven geometries representing the seven possible combinations of orientations of the hydroxymethyl groups, were taken as starting structures for different local searches. Since two conformations, *gauche-gauche* (*gg*) and *gauche-trans*, (*gt*) are preferred for the hydroxymethyl groups, and there are four of these groups in the host, one could think of $2^4 = 16$ possible combinations. However, the number of possibilities is reduced to seven, due to the symmetry of the molecule. For every starting geometry, 500 MC steps were carried out. The lateral chains were left at their initial conformation. The number of torsions randomly varied were between 2 and 9. Thus, 10 700 MC steps were performed in total. The global searches provided 412 conformers, while the seven local searches provided 621 conformers. Before comparing the structures obtained from the different pathways, a criterion of 12 kJ/mol over the global minimum was established to select the final set of conformers. Thus, 27 conformers were selected. After fitting and comparison of the structures obtained from the independent MC searches, this number was reduced to ten. These conformations are numbered from 1 to 10 starting at the global minimum in increased order of energy. The global minimum conformation was found 35 times during the protocol, while conformers 3, 6, and 7 were found 8, 7, and 20 times respectively. All the other conformers were found less than 5 times. The torsion angles for these conformations are given in the supporting information.

The global minimum was extensively minimized and, then, its conformational stability was checked using 500 ps of molecular dynamics simulations (MD) by using the MM3* force field. The Shake option[29] to fix C−H bonds was employed during the simulation, and the temperature was kept fixed at 300 K by coupling to a temperature bath.[30] Trajectory frames were saved every 0.5 ps.

Docking Studies. Docking of α- and β-p-nitrophenyl glucopyranosides and mannopyranosides to the glycophane host was performed by using the MACROMODEL v4.5 package. Initially, the four guest molecules were extensively minimized using the MM3* program, with the aromatic ring oriented as to satisfy the *exo*-anomeric effect, given by Φ angle (H-1-C-1-O-1-Ar) of *ca.* 60° for β-glycosides and −60° for α-glycosides. The ten minima found in the previous analysis for the isolated host molecule were then used to dock the different substrates, according to the following protocol: First, every substrate was docked manually into the cavity and the complex was extensively minimized. Since the cavities of all ten conformers of the host present two different faces, calculations for the two modes of substrate entry into the host cavity were performed. Second, 100 MC steps for the host−guest molecule were carried out, with random translation and rotation of the guest around the cavity. Limits between 0 and 2 Å and between 0 and 15° were used for translation and rotation, respectively. In all cases, those complexes which had a steric energy difference of 12 kJ/mol over the corresponding global minimum were not considered for subsequent analysis.

Apart from the calculations which employed the GB/SA solvent model,[26] two different dielectric constants, $\epsilon = 1$ and $\epsilon = 10$ D, were used for additional *in vacuo* calculations to test the possibility of overestimation of hydrogen bond formation. Indeed, more than twofold different conformers were generated when employing $\epsilon = 1$ D than for the other cases. The analysis of these complexes ($\epsilon = 1$) allowed us to deduce that many of them were unrealistic and showed distorted geometries. Nevertheless, all the complexes deduced when using $\epsilon = 10$ D were also present when using the GB/SA solvation model, or $\epsilon = 1$. Therefore, only the geometries obtained with $\epsilon = 10$ D were finally considered. The conformational stability of the global minima found for the four different host−guest complexes was checked by performing independent 500 ps MD simulations, as described above for the free host. No constraints were used to mantain the guest inside the cavity. The average enthalpy at 300 K was estimated from these simulations.

Acknowledgment. This work was supported by the Dirección General de Investigación Científica y Técnica (Grant PB-87-0367) and Boehringer Ingelheim España, S.A. We thank Prof. Martín-Lomas for helpful discussions and for his continuous support. S.K.S. thanks the Ministerio de Educación y Ciencia for a postdoctoral fellowship. We thank also Prof. M. Bernabé for his help with the van't Hoff analysis and J. L. Asensio for his help with the molecular modeling studies. One of the referees of this paper is also gratefully acknowledged for the helpful comments.

Supporting Information Available: ^{1}H NMR titration of glycophane **1** with 4-nitrophenyl α-D-mannopyranoside, 1D NOE and ROESY experiments of the complexes **1**·αMan and **1**·βMan, molecular mechanics and dynamics calculations of glycophane **1** and the complexes **1**·αGlc, **1**·βGlc, **1**·αMan, **1**·βMan, ^{1}H NMR titration data and simulated curve fits for **1**·αGlc complex at 25, 30, 35, and 40 °C, and table containing torsion angles, steric energies, and population of the ten low energy conformers of glycophane **1** (14 pages). This material is contained in many libraries on microfiche, inmediately follows this article in the microfilm version of the journal, can be orderd from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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