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Calorimetric Measurement of the CH/π Interaction Involved in the Molecular Recognition of Saccharides by Aromatic Compounds

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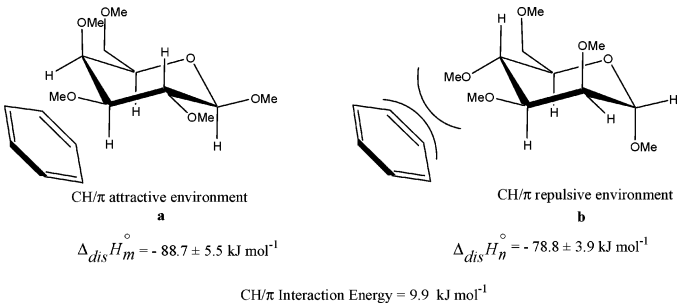
Received September 2, 2007



Can a benzene molecule differentiate between two isomeric carbohydrates? It is generally accepted that two factors govern molecular recognition: complementarity and preorganization. Preorganization requires the presence of cavities for positioning the host's groups of complementary nature to those of the guest. This study shows that, in fact, groups should be complementary to recognize each other (for the case presented here, it is controlled by the CH/π interaction) but preorganization is not essential. Since weak interactions have their origin in dispersion forces, they also have impact on the enthalpic term of the free energy, so it was considered that their participation can be demonstrated by measuring the energy involved. For recognition to happen, two conditions must be satisfied: specificity and associated stabilizing energy. In this study we evaluated the heat of dissolution of different carbohydrates such as methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside using different aromatic solvents. The solvation enthalpies in benzene were -78.8 ± 3.9 and -88.7 ± 5.5 kJ mol^{-1} for each carbohydrate, respectively; and these values yielded a CH/π energy of interaction of 9.9 kJ mol^{-1}. In addition, NMR studies of the effect of the addition of benzene to chloroform solutions of the two carbohydrates showed that benzene specifically interacts with the hydrogen atoms of the pyranose ring at positions 3, 4, and 5 located on the α face of the methyl-β-galactoside, so it is, in fact, able to recognize it. Thus, the interactions between carbohydrates and the aromatic residues of proteins occur in the absence of the confinement generated by the protein structure. By experimentally measuring the energy associated with this interaction and comparing it to theoretical calculations, it was also possible to unequivocally determine the existence of CH/π interactions between carbohydrates and proteins.

Introduction

The so-called weak interactions such as the hydrogen bonds or the interactions originating from dispersion forces are the heart of Supramolecular Chemistry and have their origin in electrostatic interactions such as dipole−dipole, dipole−quadrupole, and quadrupole−quadrupole among others. This is the origin of some noncovalent interactions that allow a specific association between a host and its guest.[1] This phenomenon is known as molecular recognition and it is generally accepted that the existence of host cavities in molecules of biological

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(1) Anslyn, E. V.; Dougherty, D. A. *Modern Physical Organic Chemistry*; University Science Books: Sausalito, CA, 2006; p 207.

10.1021/jo701926r CCC: $40.75
Published on Web 01/04/2008

origin such as enzymes and antigens is essential for recognition.[2] This phenomenon is known as preorganization. Emil Fischer[3−5] was the first scientist to explain the selectivity that is characteristic of enzymatic reactions using the rigid lock and key model.[6] In 1958, Daniel Koshland[7,8] proposed that a protein can show some flexibility that would allow an adaptation to the substrate. This led to the establishment of the induced fit model. The two models imply that preorganization plays a central role. On the other hand, the term complementarity, where the structure of the host is complementary to that of the guest, is the other factor that influences molecular recognition. Apparently, both factors are essential for recognition.

Sugar−protein interactions play an important role in a wide range of fundamental biological processes that include metabolic regulation, growth, embriogenesis, and apoptosis among many others.[9−11] The determination of the mechanisms by which carbohydrates recognize proteins (lectins, antibodies, and enzymes among others) remains a fundamental question in biochemistry. This information will be useful in the future to control and manipulate the interactions and design structural mimics of oligosaccharides of pharmaceutical interest.[12] For this, it is important to understand the structural, thermodynamic, and kinetic phenomena that control the manner in which a carbohydrate is attached to its receptor by the participation of an aromatic molecule.

Interestingly it is usual to find aromatic amino acid residues (tryptophan, tyrosine, and phenylalanine) in the protein active sites that recognize and bond carbohydrates.[13,14] Carbohydrate−protein complexes that have been studied by X-ray diffraction show that the carbohydrate is positioned in such a way that at least three hydrogen atoms of the hydrophobic region (that includes the C−H bonds of the tetrahydropyran ring) are oriented toward the amino acid aromatic nucleus (Figure 1).

For recognition to exist, two conditions should be satisfied: specificity and stabilizing energy. Since the energy associated with the recognition process of the system is important, the evaluation of the energy was considered an extremely important factor for the molecular recognition process. Thus, the evaluation of the thermodynamic properties of the carbohydrate−aromatic compound system is most relevant. Stabilizing weak interactions play an important role on the enthalpic term of the free Gibbs energy, so it was considered that if this could be measured, it would be a clear sign of their existence and relevance.

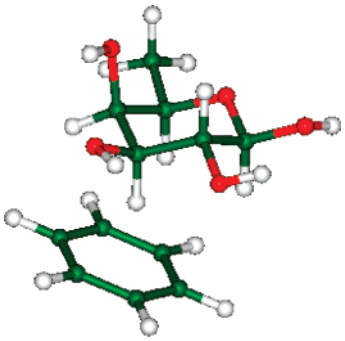


FIGURE 1. Supramolecular structure of the fucose and benzene complex determined at MP2/6-31G(d,p) including BSSE during the optimization process.[15]

Recent studies demonstrated theoretically and experimentally the existence of stabilizing interactions between fucose (a model of galactose free of conformational implications due to the rotation of the CH_2−OH segment) and benzene (Figure 1). The determination of the stabilization energy was performed using ab initio methods at the MP2/6-31G(d,p) level of theory considering a counterpoise correction during optimization of the supramolecule. This energy was on the order of 12.5 kJ mol^{-1}, approximately 4.16 kJ mol^{-1} for each C−H bond involved in the interaction. On the other hand, the structure of the complex was very sensitive to the inclusion of the correction for the basis set superposition error (BSSE) during the optimization. It was, thus, demonstrated that the use of density functionals that lack terms that adequately describe the long-distance interaction is not good for evaluating the energy of the system since it has its origin in the dispersion forces.[15,16] In addition, the authors established that the interaction produced by benzene (or phenol) on the hydrogen atoms of the α face of the methyl-β-galactoside is the most important, because the specific ^{1}H NMR resonances undergo upfield shifting upon addition of phenol. This behavior has been taken as direct proof of the existence of CH/π interaction.[15] The fact that this interaction does not require a well-defined mold or a structured rigid frame created by the protein where the conditions for interaction are generated is notable.

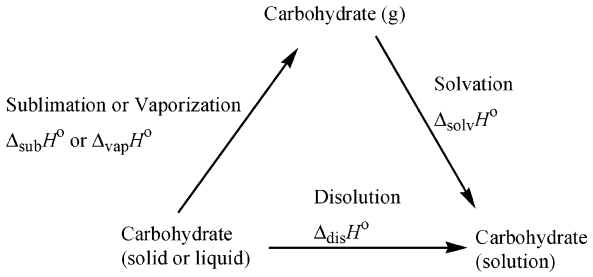
A study published recently exposed experimental evidence of the CH/π interaction through the use of near-IR vibrational spectra of individual carbohydrate conformers isolated under molecular beam conditions in the gas phase.[17] Since the analysis of weak interactions is controversial[18] and it is important to establish the mechanisms by which these interactions occur, the experimental determination of the interaction energy between an aromatic compound and a carbohydrate where the energy

(2) Smith, M. B.; March, J. *March's Advanced Organic Chemistry. Reactions, Mechanisms and Structure;* Wiley: New York, 2007.
(3) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 2985.
(4) Lichtenthaler, F. W. *Angew. Chem., Int. Ed.* ***1994****, 33, 2364.*
(5) Lemieux, R.; Spohr, U. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 1.
(6) Lichtenthaler, F. W. *Eur. J. Org. Chem.* **2002**, 4083.
(7) Koshland D. E. *Proc. Natl. Acad. Sci.* **1958**, *44, 98.*
(8) Koshland, D. E., Jr. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2375.
(9) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C. H. *Chem. Rev.* **1998**, *98*, 833.
(10) Wies, W. L.; Drickamer, K. *Ann. Rev.* **1996**, *65*, 441.
(11) Vyas, N. K. *Curr. Opp. Struct. Biol.* **1991**, *1*, 732.
(12) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637.
(13) Sauter, N. K.; Hanson, J. E.; Glick, G. D.; Brown, J. H.; Crowther, R. L.; Park, S. J.; Skehel, J. J.; Wiley, D. C. *Biochemistry* **1992**, *31*, 9609. Emsley, P.; Fotinov, C.; Black, I.; Fairweather, N. F.; Charles, I. G.; Watts, C.; Hewitt, E.; Isaacs, N. W. *J. Biol. Chem.* **2000**, *275*, 8889. Pascal, J. M.; Day, P. J.; Monzingo, A. F.; Ernst, S. R.; Robertus, J. D.; Iglesias, R.; Pérez, Y.; Ferreras, J. M.; Citores, L.; Girbes, T. *Proteins*: *Struct. Funct. Genet.* **2001**, *43*, 319−326.
(14) Fernández-Alonso, M. C. Interacciones proteína-carbohidrato: Estudio Teórico de los requisitos energéticos para la distorsión de anillos de piranosa y origen de las interacciones carbohidrato-anillo aromático. Ph.D. Thesis, Universidad de Vigo-Centro de Investigaciones Biológicas, Vigo, Spain, 2006.
(15) Fernández-Alonso, C. M.; Cañada, F. J.; Jiménez-Barbero, J.; Cuevas, G. *J. Am. Chem. Soc.* **2005**, *127*, 7379.
(16) Jiménez-Barbero, J.; Cañada, F.; Cuevas, G.; Asensio, J. L.; Aboitiz, N.; Canales, A.; Chávez, M. I.; Fernández-Alonso M. C.; García-Herrero, A.; Mari, S.; Vidal, P. In Protein-Carbohydrate Interactions: A Combined Theoretical and NMR Experimental Approach on Carbohydrate-Aromatic Interactions and on Pyranose Ring Distortion; Vliegenthart, J. F. G., Woods, R. J., Eds.; NMR Spectroscopy and Computer Modeling of Carbohydrates. Recent Advances. *ACS Symp. Ser.* **2006**, *930*, 60.
(17) Screen, J.; Stanca-Kaposta, C.; Gamblin, D. P.; Liu, B.; Macleod, N. A.; Snoek, L.; Davis, B. G.; Simons, J. P. *Angew. Chem., Int. Ed.* **2007**, *46*, 3644. Stanca-Kaposta, E. C.; Gamblin, D. P.; Screen, J.; Lui, B.; Snoek, L. C.; Davies, B. G.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2007**, *8*, 4444.
(18) Dunitz, J. D.; Gavezzotti, A. *Angew. Chem., Int. Ed.* ***2005****, 44, 1766.*

associated with the CH/π interaction is evaluated is fundamental and is the topic of the present study. Trying to determine the contribution of the enthalpic and entropic terms to the free energy associated with the donor−acceptor-complex equilibria may prove challenging. This is because the first term includes the effects of the covalent bonds, the noncovalent interactions of electrostatic origin, the stereoelectronic effects, and hydrogen bonds among others, while the second term encompasses the evaluation of the change of degrees of freedom experienced by the compounds that participate in the chemical equilibrium under study. These include the changes in degrees of freedom of the solvent, the host, and the guest as well as the product of the interaction, the supramolecule. The contribution of the enthalpic term to the free conformational energy has allowed the understanding of conformational processes[19,20] as well as molecular recognition.[21] For the latter, it has been recently demonstrated that the interaction between *Amarantus caudatus* Antimicrobial Peptide 2 (AcAMP2)-like peptides and the trimer of *N*-acetylglucosamine $(GluNAc)_3$ is enthalpically driven and that both hydrogen bonds and van del Waals forces contribute to the stability of the complexes in aqueous solution.[22] This is in sharp contrast to the previous idea that suggested that the liberation of the molecules from the solvents present at the binding site would make entropy the dominating term.[23] In this sense, it would be important to establish the role of the enthalpic term in the interaction and evaluate its magnitude in order to obtain fundamental information. This information would be quite useful for the understanding of the selectivity of receptors toward certain carbohydrates.[24−29] This information can be used to rationally manipulate the carbohydrate−protein interaction to improve the selectivity of compounds of pharmaceutical importance.[30]

There are two approaches to determine the interaction energy of interest. The first one is based on the evaluation of the solvation energy between the carbohydrate and the aromatic substrate. The term solvation process refers to the energetic and structural changes occurring in a system during the process of transferring molecules from the gas phase into the liquid solvent. Those changes are not only accompanied by disruption of intermolecular bonds in the solvent but also involve the formation of new interactions between the molecules of solute and solvent.[31] So, for both carbohydrates, the difference in the enthalpy of solvation must be proportional to the magnitude of the interaction of each isomer with the solvent molecules. Scheme 1 shows the terms required to determine this thermodynamic property.

SCHEME 1. Thermodynamic Determination of the Heat of Solvation



The enthalpy of phase change as well as the dissolution enthalpy can be determined by direct measurement in a calorimeter.[32] However, these numbers can be useful to directly quantify the interaction energy for solute and solvents through a second approximation that can be established as:

$$\Delta E_{dis} = Z[E_{1,2} - {}^1/_2(E_{1,1} + E_{2,2})] \quad (1)$$

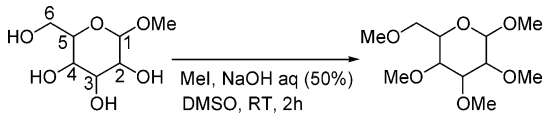
where ΔE_{dis} is the dissolution enthalpy, Z is the number of molecules in the carbohydrate solvation sphere, $E_{1,2}$ is associated with the molecular interaction between the carbohydrate molecules and the solvent, $E_{1,1}$ is the energy associated with the intermolecular interaction of the carbohydrate in the condensed phase and is directly proportional to the sublimation enthalpy, and finally $E_{2,2}$ is the cohesive energy of the liquid molecules that is proportional to the vaporization enthalpy. The direct interaction energy between the carbohydrate molecules and the aromatic solvent can be calculated through eq 1 and can be easily quantified through the enthalpies of solution and of change of phases applied to the solvation model.

Results and Discussion

Two carbohydrate derivatives were used for this study. The first, permethylated galactose,[33] is a derivative of fucose that, according to theoretical calculations and experimental results, has a CH/π interaction when combined with an aromatic compound.[15] The second, a mannose derivative,[34] lacks the interaction and was used as a reference. Since carbohydrates have a polar region (hydroxyl groups), they are insoluble in aromatic substrates that lack the groups that can form strong hydrogen bonds. The energy associated with the CH/π interaction is less than the energy associated with the hydrogen bonds. Therefore, the use of aromatic compounds capable of forming hydrogen bonds to determine the CH/π interaction is inadequate because it is possible to mask the interaction of interest. Thus, aromatic solvents such as benzene are useful to determine the interaction energy of interest. To use benzene and its derivatives as solvents, it was necessary to modify the carbohydrates through permethylation using iodomethane (Scheme 2). This reaction changes the amphiphilic character of the carbohydrate

(19) Torres, L. A.; Rojas, A.; Cuevas, G.; Juaristi, E. *J. Phys. Org. Chem.* **1994**, *7*, 561.
(20) Juaristi, E.; Cuevas, G. *Tetrahedron*, **1999**, *55*, 359.
(21) Kronis, K. A.; Carver, J. P. *Biochemistry* **1985**, *24*, 834.
(22) Chavez, M. I.; Andreu, C.; Vidal, P.; Aboitiz, N.; Freire, F.; Groves, P.; Asensio, J. L.; Asensio, G.; Muraki, M.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur. J.* **2005**, *11*, 7060.
(23) Quiocho, F. A. *Pure Appl. Chem.* **1989**, *61*, 1293.
(24) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2978. Davies, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1998**, *37*, 2270.
(25) Vacca, A.; Nativi, C.; Cacciarini, M.; Pergoli, R.; Roelens, S. *J. Am. Chem. Soc.* **2004**, *126*, 16456.
(26) Nativi, C.; Cacciarini, M.; Francesconi, O.; Vacca, A.; Moneti, G.; Ienco, A.; Roelens, S. *J. Am. Chem. Soc.* **2007**, *129*, 4377.
(27) Francesconi, O.; Ienco, A.; Moneti, G.; Nativi, C.; Roelens, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 6693.
(28) Calderone, V.; Fragai, M.; Luchinat, C.; Nativi, C.; Richichi, B.; Roelens, S. *ChemMedChem.* **2006**, *1*, 598.
(29) Cacciarini, M.; Cordiano, E.; Nativi, C.; Roelens, S. *J. Org. Chem.* **2007**, *72*, 3933.
(30) Bernardi, A.; Arosio, D.; Potenza, D.; Sánchez-Medina, I.; Mari, S.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur. J.* **2004**, *10*, 4395.
(31) Krestov, G. A. *Thermodynamics of solvation*; Ellis Horwood: New York, 1991.
(32) Berry, R. S.; Rice, S. A.; Ross, J. *Physical Chemistry*; John Wiley and Sons: New York, 1980.
(33) Asres, D. D.; Perreault, H. *Can. J. Chem.* **1999**, *77*, 319. Rathbone, E. B.; Stephen, A. M.; Pachler, K. G. R. *Carbohydr. Res.* ***1971***, *20*, *141*.
(34) Harada, M.; Montgomery, R. *Carbohydr. Res.* **1969**, *11*, 467.

SCHEME 2. Synthesis of the Modified Carbohydrates of Interest



by modifying the hydroxyl groups.[35,36] The use of the methyl groups was considered adequate because it does not present strapping problems due to the steric volume. The modification was done using standard methods starting with the corresponding methyl pyranosides. The theoretical calculations and the recently published NMR experiments[15] were useful to find the stabilization of methyl-β-galactoside through a CH/π interaction. However, this interaction was not found for methyl-α-mannoside. During this study permethylation of the two carbohydrates rendered methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside[33] (**1**) and methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside (**2**)[34] with purity greater than 99.8% determined through the use of gas chromatography.

Since the specific ^{1}H NMR signals of a group of protons attached to the pyran ring that undergo upfield shifting upon addition of an aromatic compound has been taken as direct evidence of the existence of CH/π interactions, the modified carbohydrates were used for the NMR study.[37] Thus, the effect of benzene on the chemical shift of the hydrogen atoms of the molecules of interest was evaluated. Tables S1 and S2 (see the Supporting Information) show the effect that consecutive additions of the aromatic substrate have on the chemical shifts of the different hydrogen atoms. Except for the hydrogen atom at position H2, all other hydrogen atoms of the methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside (**2**) show a shielding effect from the solvent. This effect can be evaluated through the difference $\Delta\delta = \delta(CDCl_3) - \delta(C_6D_6)$, since the chemical shift in the initial chloroform solution is slowly modified as the proportion of benzene increases. A positive value in the shift of the hydrogen atoms of molecule **2** shows a net shielding. Thus, the H3 hydrogen atom goes from 3.15 to 2.97 ppm, H4 goes from 3.65 to 3.45 ppm, and H5 goes from 3.57 to 3.33 ppm. This behavior is in sharp contrast to the effect that the same solvent has over compound **1**, where an opposed effect is observed on the same hydrogen atoms, i.e., H3 goes from 3.49 to 3.68 ppm, H4 goes from 3.42 to 3.75 ppm, and H5 goes from 3.55 to 3.82 ppm.

It is interesting to point out that a protective effect of benzene over the protons at position 6 of carbohydrate **2** is observed. However, the effect is not protective for carbohydrate **1**. All methyl groups are exposed to the solvent and in all cases show a protective effect that is increased as the benzene concentration is increased in the solution. These results support previously reported observations[15] and unequivocally confirm the presence of a CH/π interaction between benzene and the carbohydrate only for compound **2** that substitutes chloroform from the first solvation sphere.

Two important conclusions can be drawn from these results. The first one is the fact that even when the solvent−methyl group interactions are approximately similar for both compounds, the difference in the solvent−solute interaction energies, if existent, can be attributed to the CH/π interaction. The second is that the solvation of compound **2** is not random, even when there is no cavity or a preorganized site. In fact, the benzene ring protects the region of positions 3−6 of the carbohydrate as shown in Figure 2b. The benzene molecule is oriented due to the presence of the CH bonds at positions 3, 4, and 5 as has been determined by theoretical calculations,[15] as a consequence of molecular recognition. This supramolecular arrangement does not occur in compound **1** (Figure 2a), which experiences solvation but not recognition. This can be observed through the fact that the most protected proton at position 6 is the *pro*-R that tends to orient itself toward the zone where benzene is located.[16] The presence of the equatorial methoxyl group at position 4 of molecule **1** generates a repulsive environment for benzene. The equatorial methoxy group at C4 prevents the arrangement of the three C−H bond pattern that is a condition for the existence of the CH/π interaction.[16]

Figure 3 shows the evolution of the chemical shift of the carbohydrate's ring protons when benzene is added. These results provide support to the model where a benzene molecule approaches selectively to the α face of compound **2**. The negative slopes imply the shielding that is characteristic of compound **2** while the positive slopes show the unshielding of compound **1**.

With these results, a calorimetric study of the dissolution process of carbohydrates in aromatic solvents was designed to determine the energy associated with the interaction. Table 1 shows the solvation enthalpy data of compounds **1** and **2** obtained through the solvation model (Scheme 1). The solvents used for this study were 1-methoxy-4-methylbenzene (**3**), *o*-xylene (**4**), *m*-xylene (**5**), *p*-xylene (**6**), 1,2-dimethoxybenzene (**7**), 1,3-dimethoxybenzene (**8**), and benzene (**9**). In all cases, the substituents make the aromatic compound a better donor in comparison to benzene. It would be expected that if the electronic richness of benzene is increased, in the Hammett sense,[38] the interaction would be stronger if it had a hydrogen bond character. The solvents used to modify the term $\Delta_{dis}H^o_m$ can be observed in Table 1. The data used to estimate the enthalpies of dissolution for every solvent are described in the Supporting Information.

The term $\Delta_{dis}H^o_m$ is a measure of the amount of heat needed to break the molecular interactions of compound **1** that has a liquid nature and the crystalline network of compound **2**. This explains the magnitude of these values that is considerably larger for the solid than for the liquid. A positive value of this quantity is useful to establish that the compound is more stable in pure form than in solution because breaking the crystalline structure requires energy. An exothermic dissolution can be observed for only two of the cases and both belong to solutions of 1,3-dimethoxybenzene and benzene.

On the xylene series (**4**−**6**) the dissolution is markedly endothermic while the compounds that have oxygen (**3**, **7**, **8**) make it less endothermic and even exothermic. This trend is also observed in the dissolution heat of compound **2** even when, for these results, all observed cases are endothermic.

The solvation process is global, as described in the introduction of this paper. In this case, once the crystalline network is broken, the solvent interacts with the solute and it is possible to evaluate the energy exchanged in the process. Since both compounds under study vary in the configuration of the

(35) Quiocho, F. A. *Biochem. Soc. Trans.* **1993**, *21*, 442.
(36) Elgavish, S.; Sanan, B. *J. Mol. Biol.* **1998**, *277*, 917.
(37) Terraneo, G.; Potenza, D.; Canales, A.; Jiménez-Barbero, J.; Baldridge, K. K.; Bernardi, A. *J. Am. Chem. Soc.* **2007**, *129*, 2890.
(38) Johnson, C. D. *The Hammett Equation;* Cambridge University Press: Cambridge, England, 1973.

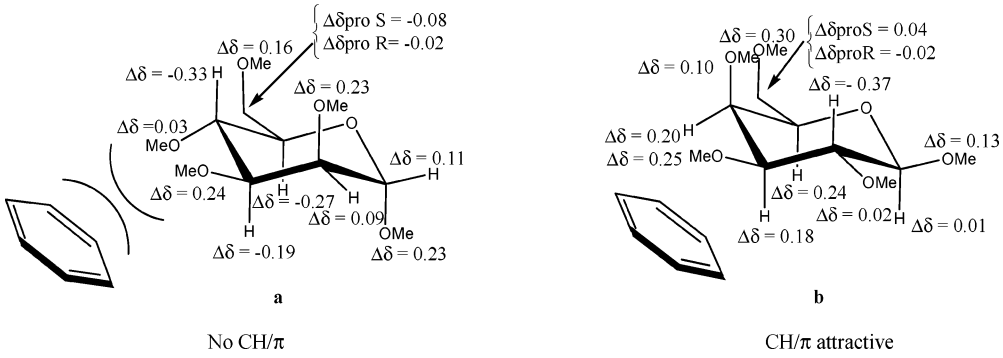


FIGURE 2. Effect of the CH/π interaction in the chemical shift in ppm of carbohydrates studied here measured by $\Delta\delta = \delta(CDCl_3) - \delta(C_6D_6)$. (a) benzene-mannoside solution and (b) benzene-galactoside supramolecule.

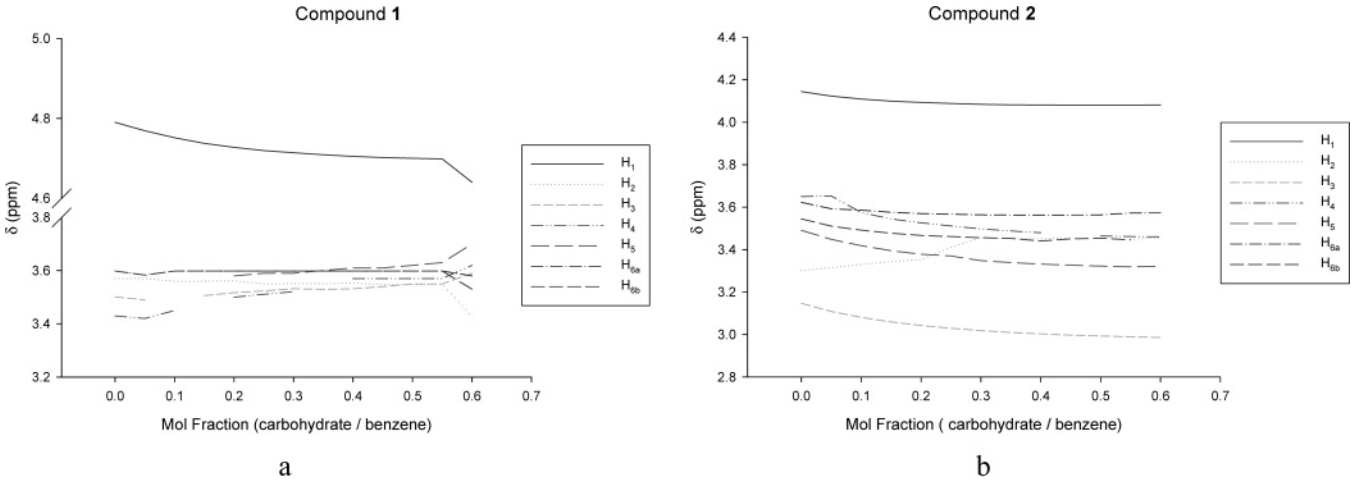


FIGURE 3. Dependence of the ring protons chemical shift of compounds **1** (a) and **2** (b) with respect to the addition of benzene in solutions of $CDCl_3$.

TABLE 1. Enthalpies of Solvation (in kJ mol^{-1}) of Methyl 2,3,4,6-Tetra-*O*-methyl-α-D-mannopyranoside (1) and Methyl 2,3,4,6-Tetra-*O*-methyl-*β*-D-galactopyranoside (2) in Different Aromatic Solvents (1:10, mol:mol) Determined by Calvet Microcalorimetry at 303.15 K[a]

	1			**2**		
solvent	$\Delta_{dis}H^o_m$	Δ_{solv}	$H^o_m\sigma_{tot}$ (±)[b]	$\Delta_{dis}H^o_m$	δ_{solv}	$H^o_m\sigma_{tot}$ (±)[b]
3	0.938	−74.239	3.898	17.391	−87.854	5.655
4	2.623	−72.554	3.899	18.914	−86.331	5.536
5	2.996	−72.181	3.897	19.386	−85.859	5.547
6	2.625	−72.552	3.897	19.249	−85.996	5.564
7	1.055	−74.122	3.897	17.970	−87.275	5.582
8	−1.069	−76.246	3.899	16.175	−89.070	5.545
9	−3.649	−78.826	3.906	16.537	−88.708	5.536

[a] The value of $\Delta^g_l H^o_m$ for **1** is constant: 75.177 kJ mol^{-1}. $\Delta^g_s H^o_m = 105.245$ for **2**. [b] σ_{tot} is the overall uncertainty, calculated from the uncertainties on the enthalpies of dissolution and phase change (see the Supporting Information).

stereogenic center at position 4, the region that shows the most changes in the NMR study, compound **1** cannot have the CH/π interaction, while compound **2** does have the interaction (Figure 2).

The solvation energy $\Delta_{solv}H^o_m$ of compound **1** establishes a more stable system for benzene with a value of -78.8 ± 3.9 kJ mol^{-1} in relation to the other aromatic solvents. This is followed in terms of stability by solvation processes where oxygenated solvents are present with the least stable systems being those formed with xylene isomers. Of the xylene isomers, the more stable interactions are those where *o*- and *p*-xylene are present. The energy difference between the least and the most stable systems is of 6.6 kJ mol^{-1}. As can be expected the $\Delta_{solv}H^o_m$ values are considerably higher for compound **2** (-88.7 ± 5.5 kJ mol^{-1}). For this solute, benzene does not generate the most stable system. In fact, the most stable system is generated by 1,3-dimethoxybenzene (Table 1). Xylenes contribute to a lesser extent to stability, but *m*-xylene contributes the least stabilization energy. The energetic difference between the extremes is 3.2 kJ mol^{-1}.

It is generally accepted that the energy difference between compounds **1** and **2** is due mainly to the CH/π energy. This can be estimated through eq 2.

$$\text{CH}/\pi \text{ energy} = \Delta_{solv}H^o_{m(1)} - \Delta_{solv}H^o_{m(2)} \qquad (2)$$

It is possible to take into account that the $\Delta_{solv}H^o_{m(1)}$ term includes only the interactions between the methyl groups and the solvent, while the $\Delta_{solv}H^o_{m(2)}$ term also includes the CH/π interaction (due to recognition); thus, the interaction between the carbohydrates and benzene gives a CH/π energy of 9.9 kJ mol^{-1}. This is in agreement with the value calculated at the MP2/6-31G(d,p) level when the base superposition correction is included (12.5 kJ mol^{-1}),[15] and with previous results showing that enthalpy is a relevant contribution for the binding process.[22]

Recently, the protein−carbohydrate interaction was evaluated using a simple model that was useful to establish that the energy of interaction is approximately 20.9 kJ mol^{-1}.[37] This was done using the B3LYP functional and the DZV(2d,p) base for the optimization of the molecular geometry followed by a single point calculation using the MP2 method with the same basis. This was done taking advantage of an intramolecular interaction that is free of BSSE. The calculation of the energy was done using an isodesmic reaction where the energy difference between the elements of the reaction is attributed only to the CH/π interaction. Since the nature of the exo-anomeric effect present in both molecular systems is different, it is possible that the final value of the energy obtained at the MP2/DZV(2d,p)//B3LYP/DZV(2d,p) level has some of this contribution. In addition, the original geometries were calculated at the B3LYP/DZV(2d,p) level. However, it is well-known that this level does not describe long-distance interactions adequately, thus the geometry reported using this erroneous calculation is far from the best. The carbohydrate−aromatic compound interactions have been widely studied using computational methods and the energy values oscillate between 10.5 and 20.9 kJ mol^{-1} when the MP2 method is used. Calculations using the Hartree−Fock method and hybrid functionals describe small and even repulsive interactions.[39−41] Therefore, the different levels of theory can be used to somewhat justify the repulsive interactions as well as different magnitudes of attractive interactions. So, it is very important to have the experimental reference value of 9.9 kJ mol^{-1} to estimate the quality of the theoretical values available.

The presence of the substituents increases the interaction energy because it increases the ring's donating capacity. However, xylenes produce similar stabilizing interactions (13.8 kJ mol^{-1} for **4**, 13.4 for **6**, and 13.7 for **5**) with respect to ethers (13.2 kJ mol^{-1} for **7** and 12.8 kJ mol^{-1} for **8**). Ether **3** generates a CH/π energy of 13.6 kJ mol^{-1} similar to that of *m*-xylene **5**. This shows that benzene derivatives with strong donating groups do not necessarily produce the more stabilizing interactions, because the stronger methoxy group produces a weaker enthalpic effect with respect to the methyl group, in contrast with the previous suggestion.[22]

These results show that the enthalpic term is a relevant contribution in the carbohydrate−aromatic compound recognition process. At 303 K with benzene being the molecule of interaction, the entropic term would be the same as the enthalpic term when it reaches 32.67 J K mol^{-1}, which is possible because high ΔS^o values have been reported,[42] over 32.67 J K mol^{-1}. The final interaction value would be the balance between both terms and where the enthalpic term is present and is relevant as is demonstrated here, and this has been described already.[22] Nevertheless, entropic contributions evaluated by NMR techniques must be carefully interpreted, for example, in small molecules their contribution to the conformational equilibrium is very high and this cannot be easily explained.[20] However, the elevated values have not been questioned in other processes associated with proteins where the same techniques have been used. Thus, the elevated entropies may be due to issues directly related to the determination and would not really represent the physical phenomenon.

The model proposed by Berry, Rice, and Ross was used to compare the interaction energy values obtained through the solvation energy.[32] The results for both compounds are shown in Table 2. The determination of the $\Delta^g_l H^o_m$ values is described on the Experimental Section. The vaporization enthalpy values for aromatic solvents were taken from the literature.[43,44] Since this energy has not been determined for 1-methoxy-4-methylbenzene and 1,3-dimethoxybenzene, values of the isomers 1-methoxy-3-methylbenzene and 1,2-dimethoxybenzene were used, so the values derived from either one of these compounds are only approximations.

The values described are useful to confirm the participation of the CH/π interaction in the stabilization of compound **2** since $E_{1,2}$ is always higher for compound **2** in relation to compound **1**. In this case, the dispersion of values is small, and different from the previous case, the benzene yields the most stabilized system. This fact can be justified if the steric size of the aromatic compounds is considered.

The use of the 1 to 10 ratio of the carbohydrate in relation to the aromatic solvent guaranteed the saturation of the solvation sphere. It can be accepted that, in accordance with Figure 1 and Tables S1 and S2 (Supporting Information), this sphere is saturated with three benzene molecules for each carbohydrate molecule. At this point, most protons attached to the pyrane ring stop suffering an important effect on the chemical shift (Figure 3). This implies that the effect remains approximately constant. Nevertheless, the methyl groups exposed to the solvent suffer continuous shielding. The calculation of the interaction energy considering, in an extreme case, only three molecules of solvent for each one of the solutes is increased to practically 4.0 kJ mol^{-1} when compared to 10 molecules for the case of molecule **2**. However, it remains practically constant for molecule **1** (Table 2). This can be attributed to the fact that the interaction energy is distributed between a smaller number of neighboring molecules. In consequence, the difference in the interaction energy between compounds **1** and **2** is also increased to 4 kJ mol^{-1} making it more favorable by 20.5 kJ mol^{-1} for compound **2**. This is in comparison with the 16.6 kJ mol^{-1} obtained using 10 molecules in the solvation sphere. This for benzene, for example, makes the energy go from 17.1 to 21.7 kJ mol^{-1}. These results are in line with the fact that compound **2** experiences a stabilizing interaction in accordance with molecular recognition. This is followed by solvation while molecule **1** only experiences solvation. This implies the interaction of a benzene molecule with one carbohydrate molecule[15] because both the energy observed experimentally and the calculated energy where the carbohydrate interacts with only one molecule of benzene are comparable. It is important to

(39) Spiwok, V.; Lipovova, P.; Skalova, T.; Vondrackova, E.-; Dohnalek, J.; Hasek, J.; Kralova, B. *J. Comput-Aided Mol. Des.* **2005**, *19*, 887.
(40) Sujatha, M. S.; Sasidhar, Y. U.; Balaji, P. V. *Biochemistry* **2005**, *44*, 8554.
(41) Sujatha, M. S.; Sasidhar, Y. U.; Balaji, P. V. *Protein Sci.* **2004**, *13*, 2502.
(42) Kronis, K. A.; Carver, J. P. *Biochemistry* **1985**, *24*, 834.
(43) Majer, J.; Svoboda, V.; Lencka, M. *J. Chem. Thermodyn.* **1985**, *17*, 365.
(44) Cox, J. D.; Pilcher, G. *Thermochemistry of Organic and Organometallic Compounds*; Academic Press: New York, 1970.

TABLE 2. Enthalpies of Interaction of Methyl 2,3,4,6-Tetra-*O*-methyl-α-D-mannopyranoside (1) and Methyl 2,3,4,6-Tetra-*O*-methyl-β-D-galactopyranoside (2) in Different Aromatic Solvents (1:3 and 1:10, mol:mol). Determined from Calvet Microcalorimetry at 303.15 K (in kJ mol^{-1})

	1				2					
solvent	$\Delta_l^g H_m^o$	$E_{12}{}^a$	$E_{12}{}^b$	σ_{tot} (±)	$\Delta_l^g H_m^o$	$E_{12}{}^a$	$E_{12}{}^b$	σ_{tot} (±)	$\Delta E_{1,2}{}^a$	$\Delta E_{1,2}{}^b$
3	51.500[c]	63.432	63.651	2.121	51.500[c]	80.112	84.170	2.893	16.680	20.518
4	43.450	59.576	60.188	1.949	43.450	76.239	80.652	2.767	16.663	20.464
5	42.680	59.228	59.927	1.959	42.680	75.901	80.425	2.774	16.673	20.497
6	40.000	57.851	58.463	1.959	40.000	74.547	79.039	2.775	16.696	20.575
7	66.900[d]	71.144	71.390	2.212	66.900[d]	87.869	92.063	2.959	16.725	20.672
8	66.900[d]	70.931	70.682	2.212	66.900[d]	87.690	91.464	2.958	16.759	20.782
9	33.920	54.183	53.332	1.959	33.920	71.236	75.095	2.774	17.053	21.763

[a] Value for 10 molecules. [b] Value for 3 molecules. [c] The value is the value reported for the isomer 1-methoxy-3-methylbenzene.[43−44] [d] The value is from 1,2-dimethoxybenzene.[43−44]

TABLE 3. Enthalpies of Dissolution and Solvation (in kJ mol^{-1}) of Methyl Acetate in Different Aromatic Solvents (1:10, mol:mol), Determined by Calvet Microcalorimetry at 303.15 K[a]

solvent	Q_{dis} (J)	$\Delta_{dis}H_m^o$	$\Delta_{solv}H_m^o$
3	1.72	0.642	−28.700 ± 0.026
4	4.68	1.75	−27.570 ± 0.018
5	4.8	1.83	−27.490 ± 0.049
6	4.42	1.725	−27.600 ± 0.009
7	3.44	1.35	−27.970 ± 0.035
9	4.21	1.073	−28.250 ± 0.007

[a] The value of $\Delta_l^g H_m^o$ is constant: ΔH_{vap} (303.15) = 29.32 kJ mol^{-1}.

differentiate between the recognition and the solvation processes. The former implies only one benzene molecule associated with a specific region of the carbohydrate through weak interactions. The latter includes several solvent molecules randomly associated with the carbohydrate.

In summary, the results are not affected importantly when three or ten molecules are considered within the solvation sphere because, in fact, regardless of the excess of solvent, the number of molecules involved in the first solvation sphere of the carbohydrate is three. Only one molecule is responsable for the recognition process and, as a consequence, its main energetic contribution.

During the NMR experiment, when benzene is added, the methyl groups of both carbohydrates show a shift to the lower field as was stated before. This led us to several questions: What is the effect in energetic terms of the contribution of methyl groups once it is solvated? Could this mask the CH/π interaction of interest? In order to answer these questions, a study on the effect of benzene over a methyl acetate in chloroform solution was conducted (Table S18, Supporting Information) and the heat of solvation of methyl acetate in different aromatic compounds was also determined. The results are shown in Table 3.

The NMR study was useful to establish that both methyl groups, of different chemical nature due to the acidic nature of the hydrogens of the acetyl group, suffer equally the protective effect of benzene. So, the methyl acetate is solvated by the benzene and it orients itself toward the hydrogen atoms. This behavior must be similar to that expected experimentally on the five methoxy groups of the carbohydrates.

It can be observed that the $\Delta_{solv}H_m^o$ value presented in Table 3 is slightly exothermic and has negligible variation with the nature of the aromatic compound in this study. This is also usually the case for the carbohydrates used in the study. Since both methyl groups can be solvated in the same manner, for the case of benzene, it can be considered that each methyl group contributes with approximately 14.1 kJ mol^{-1}, so it can be concluded that the energy associated with the pyranoside **1** corresponds to five times this value, so the additivity principle is preserved and undoubtedly, the value associated with compound **2** has its origin on the stabilization that goes with the CH/π interaction.[45]

Since saccharides may be considered as amphipathic substances,[46] the substitution of the carbohydrate's hydroxyl groups with methyl groups increases the hydrophobic properties and facilitates the access of the aromatic compound to the proximity of the pyrane ring. Solvation in the absence of specific interactions would be random. However, a pattern originated by the specific and stabilizing CH/π interaction would make the system orderly. NOE experiments were performed to detect the possibility of close proximity between the carbohydrate and benzene. For both carbohydrates the signal of a mixture of benzene-d_6 and benzene (1:1) was inverted with a 180 selective pulse, and the corresponding NOEs were monitored after mixing periods of 1300 ms. The different behavior for both compounds is notorious. For **2** small but detectable NOEs (Figure 4b) were observed for all the annular protons. The larger NOEs (integrated in relation to the solvent) are H1 (0.28), H2 (0.15), H3 (0.56), H4 (0.16), H5 (0.32), H6a (0.15), and H6b (0.14), which confirm a structured complex. For compound **1** the determined NOEs of the ring protons were very small, close to zero or negative: H3 (0.14), H4 (0.08), H5 (0.06), H6a (0.14), and H6b (0.07), but 0.67 for H1 (Figure 4a). Interestingly, the H1 proton of compound **1** is the only one that suffers shielding by adding benzene (Figure 3a), a fact that is relevant to build the first solvation sphere. As is expected, all the methyl groups showed significant NOEs because of the direct interaction with benzene.

Conclusions

When the calorimetric and NMR results are combined with the previously reported computational calculations,[15] it can be demonstrated that the CH/π interaction is stabilizing in a magnitude that ranges from 9 to 13 kJ mol^{-1}, depending on the aromatic substrate in use, and is of enthalpic nature. These values were determined using the solvation energy method that is useful to obtain an energy value that can be compared with that calculated using the MP2/6-31G(d,p) level of theory including the BSSE during optimization. The calculation of the interaction energy confirmed the existence of a carbohydrate−

(45) For example, see: Kopp, H. as reported in: Glasstone, S. *Textbook of Physical Chemistry*, 2nd ed.; Van Nostrand: New York, 1946; p 525. Rossini, F. D. *J. Res. Natl. Bur. Stand.* **1931**, *6*, 37:7, 329. Rossini, F. D. *J. Res. Natl. Bur. Stand.* **1934**, *13*, 21. Prosen, E. J.; Johnson, W. H.; Rossini, F. D. *J. Res. Natl. Bur. Stand.* **1946**, *37*, 51.

(46) Quiocho, F. A. *Curr. Top. Microbiol. Inmunol.* **1988**, *139*, 135.

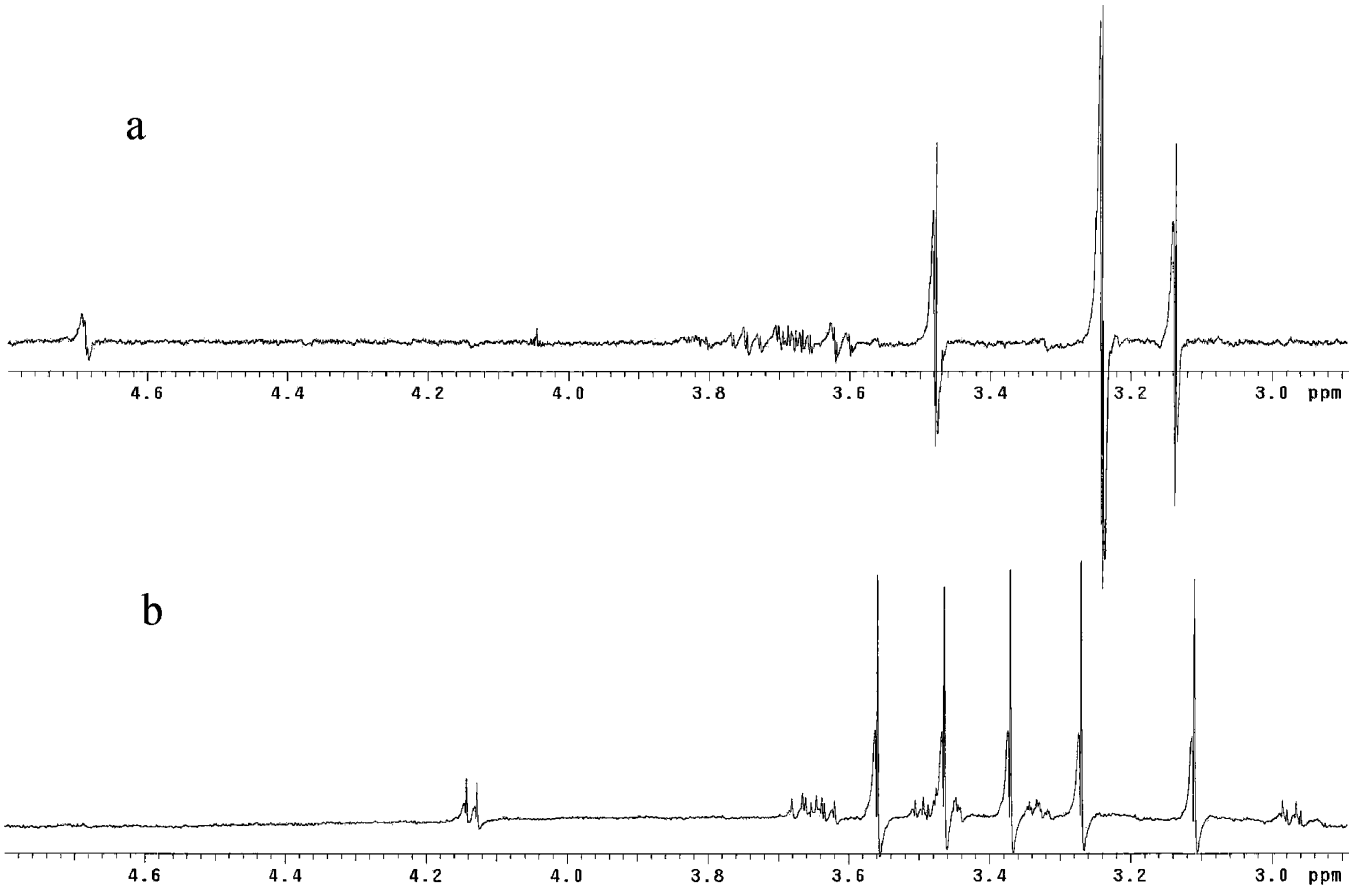


FIGURE 4. Comparative NOE effect of benzene-permethylated mannoside (a) and benzene-permethylated galactoside (b) at 298 K.

aromatic compound interaction that is stabilizing in energy and allowed to establish that at least three molecules of benzene are associated with the solute. These values can be used to establish that the HF and B3LYP theoretical methods are not useful to approach this problem because the dispersion terms are not included and should not be used for this purpose in the future. The value of the magnitude of the CH/π interaction does not vary with the electronic nature of the benzene substituent in the Hammett sense. Interestingly, the carbohydrate−aromatic compound interaction does not require the existence of a specific cavity or an environment generated by protein residues (confinement, preorganization) to occur and has a relevant enthalpic contribution. By experimentally measuring the energy associated with this interaction and comparing it to theoretical calculations it was possible to unequivocally determine the existence of CH/π interactions between carbohydrates and proteins.

It is well-known that solvents in general have properties that make them a macroscopic continuum characterized by physical properties such as density, the dielectric constant, the refraction index, etc.;[47] but as a discontinuum, it has individual molecules that interact among themselves through specific interactions. We show that these specific interactions are the origin of molecular recognition and these interactions in turn have their origin, at least partially, in electrostatic interactions.

Experimental Section

General Procedure To Carry Out the Permethylation of Carbohydrates. Methyl-D-pyranoside (1 g, 5.15 mmol, 1.0 equiv) was dissolved in dimethyl sulfoxide (18 mL). NaOH aq disolution (50%, w/w) (1.8 mL, 8.75 mmol, 1.7 equiv) was added slowly. The mixture was stirred to form a gel-suspension and CH_3I (1.93 mL, 30.99 mmol, 6.00 equiv) was added dropwise. The reaction mixture was stirred for 2 h. Water (100 mL) was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3×100 mL).[48] The combined organic phases were washed with sat. aq $Na_2S_2O_3$ disolution (200 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The reaction mixture was dissolved in EtOAc (30 mL), activated charcoal was added (20%, w/w), then the black suspension was stirred into a water-bath for 20 min. The mixture was filtered over celite after 8 h. Finally the product methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside was recrystallized from hexane (≥90%) and the methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside was treated with activated charcoal three more times, to obtain 99.8% purity for each compound. NMR signal assignments were done using HSQC, HMBC, NOESY, and COSY. 2D experiments are included in the Supporting Information.

Methyl 2,3,4,6-Tetra-*O*-methyl-α-D-mannopyranoside (1).[34] Yield: 3.475 g (90%). Yellow oil. IR (film): 2980, 2910, 2829, 1451, 1377, 1325, 1291, 1191, 1114, 1065, 997, 972, 925, 883, 844, 796, 663, 633 cm^{-1}. ^1H NMR (500 MHz, C_6D_6): δ 3.14 (s, 3H), 3.24 (s, 3H,), 3.24 (s, 3H), 3.24(s, 3H), 3.47 (m, 1H), 3.48 (s, 3H), 3.62 (dd, $J = 2.0, 10.5$, 1H), 3.67 (dd, $J = 5.5, 10.5$, 1H), 3.68 (dd, $J = 3.0, 9.0$, 1H), 3.75 (t, $J = 9.5$, 1H), 3.82 (ddd, $J = 2.0, 5.5, 9.5$, 1H), 4.68 (d, $J = 2.0$, 1H). ^{13}C NMR (125 MHz, C_6D_6): δ 54.4, 57.2, 58.9, 59.0, 60.5, 72.5, 72.6, 77.1, 77.4, 82.6, 99.0. CI-MS: 251 ($[M + H]^+$, $[C_{11}H_{22}O_6]^{H+}$).

(47) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 2nd ed.; VCH: Weinheim, Germany, 1990.

(48) Wang, H.; Sun, L.; Glazebnik, S.; Zhao, K. *Tetrahedron Lett.* **1995**, *36*, 2953. Majer, J.; Svoboda, V.; Lencka, M. *J. Chem. Thermodyn.* **1985**, *17*, 365.

Methyl 2,3,4,6-Tetra-*O*-methyl-*β*-D-galactopyranoside (2).[33] Yield: 735 mg (73%). White solid. mp 43.34 °C. IR (KBr): 2932, 2824, 1551, 1388, 1256, 1185, 1124, 1079, 1050, 1000, 965, 754, 692 cm^{-1}. ^1H NMR (500 MHz, C_6D_6): δ 2.97 (dd, J = 3.0, 9.5 1H), 3.10 (s, 3H), 3.27 (s, 3H), 3.33 (ddd, J = 1.5, 6.5, 13.0, 1H), 3.37 (s, 3H), 3.45 (dd, J = 1.0, 3.0, 1H), 3.46 (s, 3H), 3.50 (dd, J = 5.5, 9.0, 1H), 3.56 (s, 3H), 3.64 (dd, J = 7.5, 9.5, 1H), 3.68, (dd, J = 7.5, 10.0, 1H), 4.14 (d, J = 7.5, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.6, 58.2, 59.1, 60.6, 61.1, 70.8, 73.1, 74.9, 80.6, 84.1, 104.5. CI-MS: 251 ($[M + H]^+$, $[C_{11}H_{22}O_6]^{H+}$).

General Procedure To Measure the Enthalpies of Disolution by Heat Flux Calorimetry. Dissolution experiments were performed by heat flux calorimetry, using a differential Setaram C80 Calvet calorimeter working in isothermal mode. The sensors of the calorimeter are two fluxmeters with a calorimetric resolution of 0.12 μW and a detection limit in power of 2 to 5 μW, which are assembled inside of a calorimetric block with a temperature control of at least ±0.001 K. An adequate temperature control in the environmental conditions of our laboratory is attained maintaining the calorimetric block at 303.15 K, so all the dissolution experiments were performed at this temperature. For this set of experiments, stainless steel mixing with membrane vessels was employed, utilizing masses around 50 mg for each pyranoside and an amount of 350 to 55 mg of aromatic solvent, which were the largest masses able to be located in each of the containers of the vessel, in order to generate a maximal thermal signal. The resulting molar relation of the carbohydrate:solvent, after the dissolution process, was 1:10. The masses of the substances involved in a dissolution experiment with this technique were measured in a MC210 P Sartorius balance sensitive to 10 μg.

Once the disolution vessels are loaded in the fluxmeters of the Calvet Calorimeter, temperature and heat flux are stabilized by around 60 min then data acquisition begins. Five minutes is enough to get a good initial baseline, then the Teflon membrane of the mixing vessel is broken and reversing of the C80 is performed several times to promote the disolution process.

Analysis of the amplified dissolution curves, generated by the data treatment software of the C80 calorimeter, showed that for the quantity of energy of 7.0 J involved in some of the dissolution experiments, the transfer of heat finished before 120 min. At the end of the experiment of dissolution, the difference between the initial and final baseline is not more than 0.01 mW, which introduces a maximal uncertainty in the measurement of the area under the measured curve of ±0.069 J. The C80 calorimeter works at constant pressure, consequently the integration of the curve of heat flux as a function of time releases directly the enthalpy of dissolution of each carbohydrate in the respective aromatic solvent. In Table 1 the uncertainty associated with the average value of enthalpy of dissolution represents the standard deviation. The tables including all the experimental data involved in the dissolution experiments are provided in the Supporting Information.

Enthalpies of Sublimation and Vaporization by Differential Scanning Calorimetry. The calorimetric measurements of enthalpies of sublimation or vaporization of the carbohydrates were performed using the isothermal mode operation of a modified DSC7 calorimeter.[49,50]

The sensitive element of this device is a Perkin-Elmer DSC7 calorimetric holder assembly within a vacuum chamber connected to the DSC7 control device by an electrical feed. The vaporization system is evacuated with a rotary vacuum pump and residual pressure inside of the chamber is monitored by a pressure gauge, which is relayed to a Pirani gauge control. The Perkin-Elmer commercial aluminum open standard pans were utilized as sublimation cells. A working temperature of 333.15 K was established as the most recommendable from preliminary experiments.

In each experiment of sublimation, samples of around 10.0 mg of the solid carbohydrates were placed inside the sublimation pan and were weighed on a Sartorius 4503 microbalance sensitive to 1.0 μg.

Once the prepared sample pans are loaded in the calorimetric sensor, temperature and heat flux are stabilized and data acquisition begins. Five minutes is enough to get a good initial baseline, then a valve connected to the vacuum pump is opened, and the pressure inside the vacuum chamber is downloaded quickly to promote the sublimation process.

During loading, thermal stabilization and pressure change due to initial evacuation and a small fraction of the substance sublimes or vaporizes, therefore, the calculation of mass lost is necessary and was performed by independent experiments as has been previously described.[49,50]

Data acquisition and integration of the area under the change of phase curve were performed using the Pyris software of the DSC7 calorimeter. Knowing the area under the sublimation curve, the initial mass, the loss of mass during loading, and thermal stabilization, the enthalpy of sublimation was obtained using the relation:

$$\Delta H_{\text{sub or vap}} = \text{change phase area (W·s)}/\{\text{initial mass (g)} - \text{lost mass (g)}\} \quad (3)$$

The tables providing detailed experimental data of the change of phase experiments are supplied in the Supporting Information. There the uncertainty associated to the average value of enthalpy of sublimation or vaporization of the pyranosides represents the standard deviation.

Currently the calorimetric holder assembly associated with the vacuum system and that of the commercial DCS7 are calibrated for energy and temperature using high-purity samples of indium and zinc.

NMR Experiments. Modified methyl pyranoside (108 mg, 0.43 mmol) was dissolved in $CDCl_3$ (0.5 mL) and the NMR spectrum was measured in a 500 MHz spectrometer. After that C_6D_6 (19.4 μL, 0.42 mmol) was added several times starting from 1:0.5 (carbohydrate/benzene) to get a carbohydrate saturated dissolution with benzene (see Tables S1 and S2, Supporting Information) and in each case the spectrum was recorded. NOE difference were done using the cycling technique[51] (cyclenOe) with mixing times of 1300 ms and internal subtraction of data acquired by on-resonance and off-resonance selective excitation on alternate scans.

Acknowledgment. L.B.I. acknowledges Conacyt for financial support. We also thank Francisco Ginez Carbajal for his technical assistance for the disolution experiments. We are grateful to the reviewers for their useful comments that led to the improvement of this paper. This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) for financial support via grants 49921-Q, P47679-Q, and DGAPA grant IN-209606. We are grateful to Rebeca López-García for the revision of the English version of this manuscript.

Supporting Information Available: ^1H NMR chemical shifts (ppm) of compounds **1** and **2** (Tables S1−S2, pp S4 and S5), enthalpies of solution of compounds **1** and **2** in solvents **3**−**9** (Tables S3−S15, pp S6−S18), and enthalpies of Vaporization of compounds **1** and **2** (Tables S16−S17, pp S19−S20), ^1H NMR chemical shifts (ppm) of methyl acetate and mixtures of benzene and chloroform (Table S18, p S21), ^1H NMR chemical shifts (ppm) of compounds **1** and **2** of benzene-d_6 and chloroform-*d* (Table S18, p S22), Sidgwick's temperature correction (p S23), and ^1H, ^{13}C, NOESY, COSY, HMBC, and HSQC 500 MHz NMR spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO701926R

(49) Rojas-Aguilar, A.; Orozco-Guareño, E.; Martínez-Herrera, M. *J. Chem. Thermodyn.* **2001**, *33* 1405.

(50) Rojas, A.; Orozco, E. *Thermochim. Acta* **2003**, *405*, 93.

(51) Kinns, M.; Sanders, J. K. M. *J. Magn. Reson.* **1984**, *56*, 518.