ACS OMEGA

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Cite This: ACS Omega 2018, 3, 536−543

Exploring the Role of Solvent on Carbohydrate−Aryl Interactions by Diffusion NMR-Based Studies

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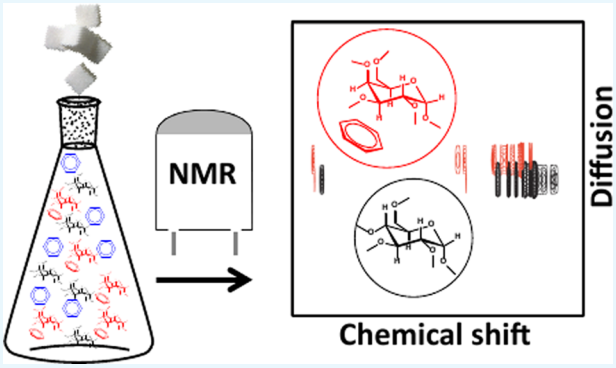
Supporting Information

ABSTRACT: Carbohydrate−protein interactions play an important role in many molecular recognition processes. An exquisite combination of multiple factors favors the interaction of the receptor with one specific type of sugar, whereas others are excluded. Stacking CH−aromatic interactions within the binding site provide a relevant contribution to the stabilization of the resulting sugar−protein complex. Being experimentally difficult to detect and analyze, the key CH−π interaction features have been very often dissected using a variety of techniques and simple model systems. In the present work, diffusion NMR spectroscopy has been employed to separate the components of sugar mixtures in different solvents on the basis of their differential ability to interact through CH−π interactions with one particular aromatic cosolute in solution. The experimental data show that the properties of the solvent did also influence the diffusion behavior of the sugars present in the mixture, inhibiting or improving their separation. Overall, the results showed that, for the considered monosaccharide derivatives, their diffusion coefficient values and, consequently, their apparent molecular sizes and/or shapes depend on the balance between solute/cosolute as well as solute/solvent interactions. Thus, in certain media and in the presence of the aromatic cosolute, the studied saccharides that are more suited to display CH−π interactions exhibited a lower diffusion coefficient than the noncomplexing sugars in the mixture. However, when dissolved in another medium, the interaction with the solvent strongly competes with that of the aromatic cosolute.



INTRODUCTION

Many biological events are initiated by molecular recognition processes that are triggered by the initial interaction between an exogenous/endogenous sugar-type ligand and membrane cell receptors.[1] Although the formation of glycan/protein complexes is driven by many factors (e.g., hydrogen bond, van der Waals forces, etc.), the role of CH−π interactions in such a process is currently receiving special attention because it may subtly tune the properties of the ligand−protein complexes. Thus, substantial efforts are being made to analyze the features that promote such interactions between carbohydrate entities and the aromatic side chains from the residues within the recognition site.[2]

Detailed X-ray crystallographic structures of sugar/protein complexes have clearly shown the stabilizing contribution of CH−π interactions in these systems,[3] whereas their detection in solution by NMR spectroscopy has also been achieved.[4] Moreover, NMR methods have been employed to directly detect intramolecular methyl−π interactions within a protein.[5]

Received: October 24, 2017
Accepted: December 28, 2017
Published: January 17, 2018

DOI: 10.1021/acsomega.7b01630
ACS Omega 2018, 3, 536−543

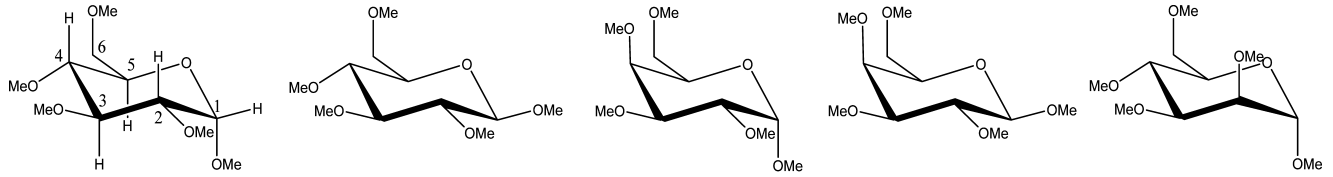


Figure 1. Compound structures, name abbreviations, and atom numbering used in the present study: α-pMeGlc(**1**) = 2,3,4,6-tetra-*O*-methyl-α-D-methylglucopyranoside, β-pMeGlc(**2**) = 2,3,4,6-tetra-*O*-methyl-β-D-methylglucopyranoside, α-pMeGal(**3**) = 2,3,4,6-tetra-*O*-methyl-α-D-methylgalactopyranoside, β-pMeGal(**4**) = 2,3,4,6-tetra-*O*-methyl-β-D-methylgalactopyranoside, and α-pMeMan(**5**) = 2,3,4,6-tetra-*O*-methyl-α-D-methylmannopyranoside.

NMR spectroscopy has also been essential for the detection of CH−π interactions in model systems using simple solutions consisting of one particular monosaccharide and one aromatic moiety in aqueous medium. Hence, the shielding of specific sugar NMR signals in the presence of the aromatic compounds,[6] as well as the detection of intermolecular nuclear Overhauser effects between these species in solution,[7] have been correlated with the formation of sugar−aromatic complexes. The role of solvent on the formation of noncovalent CH− aromatic interactions has been investigated, focusing on the analysis of certain solvent parameters that were correlated with the modulation of the observed solute−solute CH−π interactions,[8] suggesting that the interplay between solute/solvent may have a modulating effect on these interactions[9]

Because optimal sugar−aromatic interactions involve a specific spatial arrangement of CH bonds within the sugar skeleton,[6,7] some sugar moieties are more prone to establish stabilizing CH−π interactions (e.g., α/β-galactose) than others (e.g., α-mannose). Therefore, we hypothesized that different monosaccharides could be discriminated taking advantage of this recognition phenomenon. Herein, we detail our investigations to detect sugar−aromatic complexes within a sugar mixture by using diffusion-ordered spectroscopy (DOSY) NMR.

During the last decades, DOSY NMR has been proposed as a versatile tool to perform not only the virtual separation of complex mixtures but also to explore many other interesting aspects related to solute/solute and solute/solvent interactions associated with size and shape changes.[10] In this respect, remarkable examples of applications of DOSY have been reported, including folding/unfolding of proteins,[11] identification of secondary structure elements of nucleic acids,[12] detection and identification of reaction intermediates,[13] aggregation,[14] hydrogen bonding,[15] exchange,[16] metal sugar complexation,[17] host−guest complexation,[18] detection of impurities,[19] assessment of fraud in pharmaceutical formulations,[20] estimation of the molecular weight of small molecules[21] and large biomolecules,[22] among many others.

A number of strategies have been proposed to enhance the performance of DOSY NMR experiments, mostly based on subtle modifications of the medium to modify the diffusivity of the molecules. Hence, the difficult separation of a mixture of isomers with identical molecular weight has been accomplished by switching solvents.[23] The so-called "matrix-assisted" DOSY method did allow the discrimination between epimers by using a (chiral) co-adjuvant that interacts specifically with only one component of the mixture and selectively modifies its translational diffusion properties.[24] The use of chromatographic adsorbents has also been demonstrated to efficiently facilitate the separation of complex mixtures using solid-state NMR ("NMR chromatography").[25]

Herein, we provide new evidences for the intricate interplay of the solvent properties that influence the strength of CH−π interactions between sugars and aromatic compounds. The use of DOSY NMR has permitted investigating the influence of the solvent on both the separation of the different sugars (diastereomers) in mixtures as well as for the discrimination of those sugars that display intrinsic propensities to form noncovalent complexes with aromatic molecules.

RESULTS AND DISCUSSION

A series of permethylated monosaccharides known to be soluble in a variety of solvents ranging from nonpolar lipophilic cyclohexane to polar protic water were synthesized[26] (Figure 1, compounds **1**−**5**). They were considered to be suitable model systems to investigate carbohydrate/aryl interactions, as well as those solvent properties that might modulate the separation of mixtures of sugar diastereomers, as has been described for regioisomers of simple molecules.[23]

The diffusion properties of the components of equimolar binary mixtures of sugars **1**−**5**, as well as the role of the solvent properties (i.e., cyclohexane, chloroform, and water), were first investigated.

For a proper performance of DOSY NMR experiments, a scarce dispersion of the signals in the NMR spectrum of mixtures is clearly an obstacle but, in this case, the isolated signals in the studied mixtures were sufficient to unambiguously identify the diffusion coefficient values of each of their components. The one-dimensional (1D) ^{1}H NMR spectrum of a representative mixture in water is shown in Figure 2. As typically occurs with carbohydrates, most of the signals overlap within a narrow region of the spectrum, including in this case, the signals of the ring protons and also the singlets of the methyl substituents. Fortunately, for most of the analyzed mixtures, the anomeric signals and some ring proton signals from each sugar within the mixtures were clearly distinguishable.

Role of the Solvent. DOSY NMR has been already employed to separate carbohydrate mixtures that differ by molecular weight (oligo and polysaccharides),[27] branching pattern,[28] and/or shape.[29] However, to our knowledge, until now there has not been any study on the potential of diffusion NMR to separate mixtures of sugar diastereomers and on the possible influence of solvent properties to achieve this aim through specific solute/solvent interactions.

In this context, we first explored whether the separation of the components of binary mixtures of sugars **1**−**5** could be

DOI: 10.1021/acsomega.7b01630
ACS Omega 2018, 3, 536−543

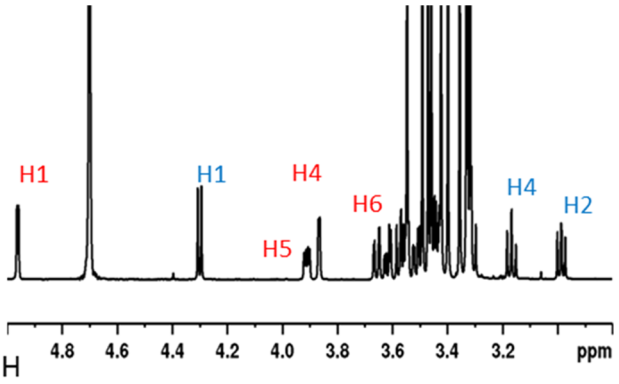


Figure 2. One-dimensional ^{1}H NMR spectrum of an equimolar mixture of β-pMeGlc(**2**)/α-pMeGal(**3**) in D_2O at 298 K (ca. 25 mM, 600 MHz). Assignments of the signals of β-pMeGlc(**2**) and α-pMeGal(**3**) are highlighted with blue (**2**) and red (**3**).

achieved exclusively by switching solvents with different properties.

In Figure 3, the DOSY spectra of the mixture α-pMeGlc(**1**)/α-pMeGal(**3**) in different solvents are shown. It is noticeable that the diffusion coefficient values of the components of the mixture, as well as the mixture separation, depend significantly on the medium properties.

The mixture in aqueous solution could not be separated as the components showed the same self-diffusion coefficient $D_{D_2O}(\mathbf{1}) = D_{D_2O}(\mathbf{3}) = 5.25 \times 10^{-10}$ m^2 s^{-1} (Figure 3c). In cyclohexane and chloroform, however, α-pMeGlc(**1**) and α-pMeGal(**3**) exhibited slight, although evident, differences in their respective diffusion coefficient values (Figure 3a,b). Interestingly, although in cyclohexane, the apparent size of α-pMeGlc(**1**) is smaller than that of α-pMeGal(**3**) ($D_{C_6D_{12}}(\mathbf{1}) = 1 \times 10^{-9}$ m^2 s^{-1} and $D_{C_6D_{12}}(\mathbf{3}) = 9.33 \times 10^{-10}$ m^2 s^{-1}), in chloroform, the opposite occurred and α-pMeGlc(**1**) had a lower self-diffusion coefficient ($D_{CDCl_3}(\mathbf{1}) = 4.78 \times 10^{-10}$ m^2 s^{-1}) than that of α-pMeGal(**3**) ($D_{CDCl_3}(\mathbf{3}) = 5.13 \times 10^{-10}$ m^2 s^{-1}).

Figure 4 shows the DOSY spectra of several binary mixtures of **1**−**5** in cyclohexane-d_{12}. Fittingly, most of the studied mixtures are separated and their components (diastereomers) showed slightly although clearly distinctive diffusion coefficient values in cyclohexane.

For instance, paying attention to the α-pMeGlc(**1**)/β-pMeGal(**4**) mixture (Figure 4a) and according to the Stokes−Einstein equation, α-pMeGlc(**1**) displays the apparent smallest hydrodynamic radius (largest diffusion coefficient, $D_{C_6D_{12}}(\mathbf{1}) = 1 \times 10^{-9}$ m^2 s^{-1}) in comparison to that of any of the galactose derivatives ($D_{C_6D_{12}}(\mathbf{3}) = D_{C_6D_{12}}(\mathbf{4}) = 9.33 \times 10^{-10}$ m^2 s^{-1}). The α/β configuration at the anomeric position of the galactose derivatives did not seem to have any influence (Figures 3a and 4a). The difference between the glucose and galactose diastereomers resides at the orientation of the methoxy group (equatorial and axial, respectively) at C4, which in turn also affects the distribution of rotamers around the C5−C6 torsions. It has been established that this distribution is also modulated by the solvent properties.[30] Thus, we initially reasoned that the axial orientation of O4 could be at the origin of the apparently larger size of the galactose derivatives when compared with the glucose analogues in cyclohexane.

The DOSY spectrum of the β-pMeGlc(**2**)/α-pMeMan(**5**) mixture (Figure 4b) showed the same trend ($D_{C_6D_{12}}(\mathbf{2}) = 1 \times 10^{-9}$ m^2 s^{-1} and $D_{C_6D_{12}}(\mathbf{5}) = 9.33 \times 10^{-10}$ m^2 s^{-1}, respectively), confirming that the configuration at the anomeric position is not critical but rather the configuration at position C2 (α-pMeMan(**5**) with axial O2) or C4 (β-pMeGal(**4**) with axial O4) is crucial for the separation. Because the distribution of the C5−C6 rotamers for mannose and glucose are analogous,[31] we concluded that the observed separation and associated size differences in the pMeGlc/pMeGal and pMeGlc/pMeMan mixtures were mostly driven by the presence of axial substituents (C2/C4) in the Gal and Man moieties. The absence of separation in the mixtures α-pMeGal(**3**)/α-pMeMan(**5**) (Figure 4c) supported this hypothesis (identical diffusion coefficient values $D_{C_6D_{12}}(\mathbf{3}) = D_{C_6D_{12}}(\mathbf{5}) = 9.33 \times 10^{-10}$ m^2 s^{-1}). Thus, the simple presence of axial substituents at C2 or C4 strongly modulates the diffusion properties and the apparent sizes of these permethylated monosaccharides (**1**−**5**) in the nonpolar cyclohexane.

As the next step, the DOSY NMR spectra of selected mixtures of **1**−**5** in aqueous solution were also analyzed. The results obtained for the mixtures in water were initially confusing because none of them could be separated by DOSY NMR and all of them exhibited the same diffusion coefficient value ($D_{D_2O}(\mathbf{1}-\mathbf{5}) = 5.25 \times 10^{-10}$ m^2 s^{-1}). We thus concluded that in contrast to the interactions in the

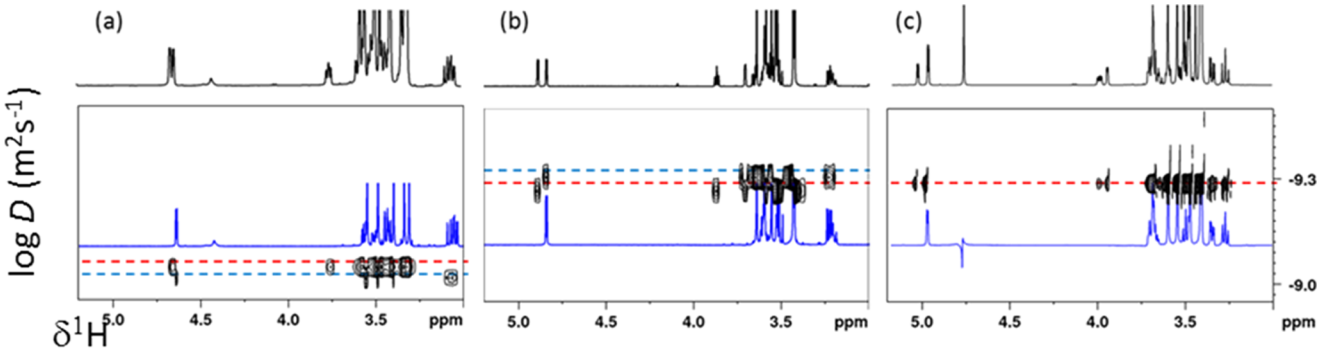


Figure 3. ^{1}H-detected two-dimensional (2D) DOSY NMR spectra of α-pMeGlc(**1**)/α-pMeGal(**3**) in (a) cyclohexane-d_{12} (ca. 40 mM), (b) $CDCl_3$ (ca. 25 mM), and (c) D_2O (ca. 25 mM). On top, the 1D ^{1}H NMR spectra of the mixtures are displayed. The 1D ^{1}H NMR spectrum of α-pMeGlc(**1**) (blue) is also shown. Dotted lines identify the diffusion coefficient of each component (blue (**1**) and red (**3**)).

DOI: 10.1021/acsomega.7b01630
ACS Omega 2018, 3, 536−543

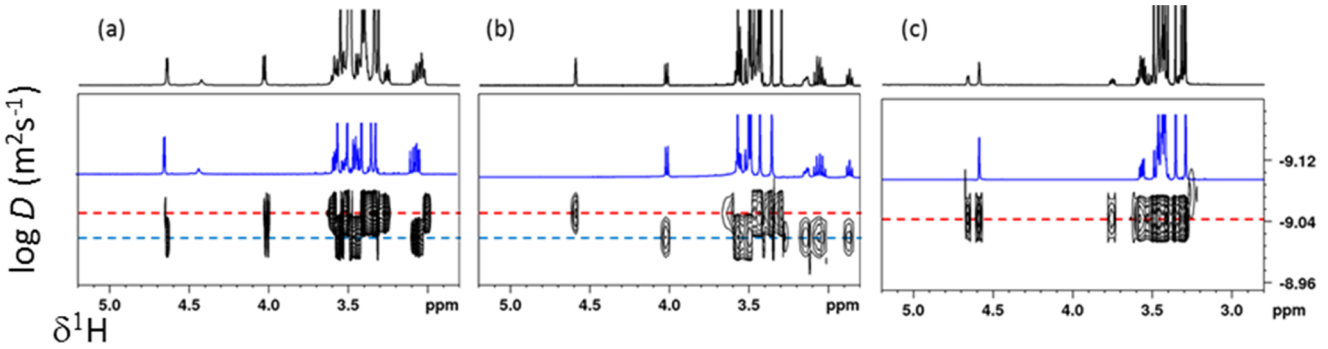


Figure 4. ^{1}H-detected 2D DOSY NMR spectra of selected binary mixtures of permethylated monosaccharides (**1**−**5**) in cyclohexane-d_{12} (ca. 40 mM): (a) α-pMeGlc(**1**)/β-pMeGal(**4**), (b) β-pMeGlc(**2**)/α-pMeMan(**5**), (c) α-pMeGal(**3**)/α-pMeMan(**5**). The 1D ^{1}H NMR spectra of (a) α-pMeGlc(**1**), (b) β-pMeGlc(**2**), and (c) α-pMeMan(**5**) are displayed in blue.

cyclohexane solutions, intermolecular hydrogen bonds in the aqueous mixtures, with water acting as either hydrogen bond donor or acceptor and compounds **1**−**5** as hydrogen bond acceptors, could make the hydrodynamic radius of the sugars rather similar, consequently yielding almost identical diffusion coefficients.

Thus, it appears that the separation of these sugar diastereomers by diffusion NMR depends on the properties of both, the solute and solvent. Hence, the apparent size of these sugars, as deduced by their corresponding coefficient diffusion values, is tuned by a complex balance between the sugar/solvent and solvent/solvent interactions.

Three's Company: Two Sugars and One Aromatic Cosolute. The previous results encouraged us to investigate whether the diffusion coefficient of the sugars, either in cyclohexane or in water, could also be altered by their specific interaction with an aromatic compound present in solution. The corroboration of this hypothesis could be taken as a novel although indirect proof of the formation of carbohydrate/aromatic complexes via CH−aromatic interactions.

First, the chemical shift perturbations (CSPs) of the NMR signals of the sugars upon addition of benzene to the mixtures in cyclohexane were detected. Interestingly, CSPs were noticeable for not only signals from those CH−π anticipated for the ring protons[6] but also for methyl groups, suggesting the existence of CH_3−π interactions (Figure 5).[5,7]

Next, the DOSY spectra of selected cyclohexane mixtures upon addition of benzene were acquired. Two representative results are presented in Figure 6. In general, the separation of most of the mixtures followed the same pattern as that in the absence of the aromatic molecules. The interval differences for the diffusion coefficients are, in general, only slightly larger, although noticeable in the presence of benzene, as exemplified in the DOSY spectrum recorded for the α-pMeGlc(**1**)/α-pMeGal(**3**)/benzene mixture in cyclohexane (Figure 6a). In contrast, the separation observed for the α-pMeGal(**3**)/α-pMeMan(**5**) mixture in the presence of benzene was remarkable (Figure 6b, $D_{C_6D_{12}}$(**3**) = 9.29 × 10^{-10} m^2 s^{-1} and $D_{C_6D_{12}}$(**5**) = 10 × 10^{-10} m^2 s^{-1}, respectively). Strikingly, these two sugars could not be separated in plain cyclohexane (Figure 4c). Besides the observed CH_3−π interactions detected as CSP, the spatial arrangement of the CH groups of the α-pMeGal(**3**) derivative fulfills the geometric requirements for establishing optimal multivalent CH−π interactions with aromatic rings (when compared with α-pMeMan(**5**)). The putative stacking interactions of this molecule with benzene may satisfactorily

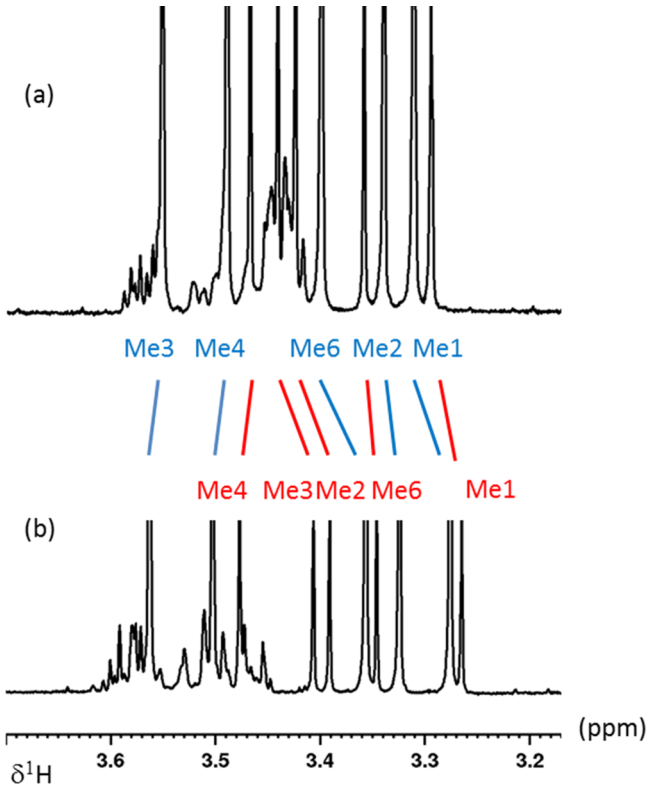


Figure 5. Expansion of the region of the 1D ^{1}H spectra of ring and methyl protons of the mixture of α-pMeGlc(**1**)/α-pMeMan(**5**) (ca. 40 mM) in cyclohexane-d_{12} in absence (a) and presence of a 10 times concentration of benzene (b). The original shift of the methyl signals are highlighted (in blue (**1**) and red (**5**)).

explain the apparent larger size of the α-pMeGal(**3**) derivative in the presence of benzene when compared to that of the α-pMeMan(**5**) analogue. Thus, for these systems in cyclohexane, the DOSY results provide an indirect proof of the existence of stabilizing CH−π and Me−π interactions between α-pMeGal-(**3**) and benzene.

Subsequently, the effect of the presence of an aromatic cosolute in aqueous mixtures was investigated. In contrast with the cyclohexane mixtures, major signal shifts were observed for the CH-ring resonances when compared to those for the O-methyl groups. The analysis of the DOSY spectra of the corresponding aqueous mixtures upon addition of phenol was, however, much less straightforward than the interpretation of the cyclohexane mixtures. In general, no clear separation was

DOI: 10.1021/acsomega.7b01630
ACS Omega 2018, 3, 536−543

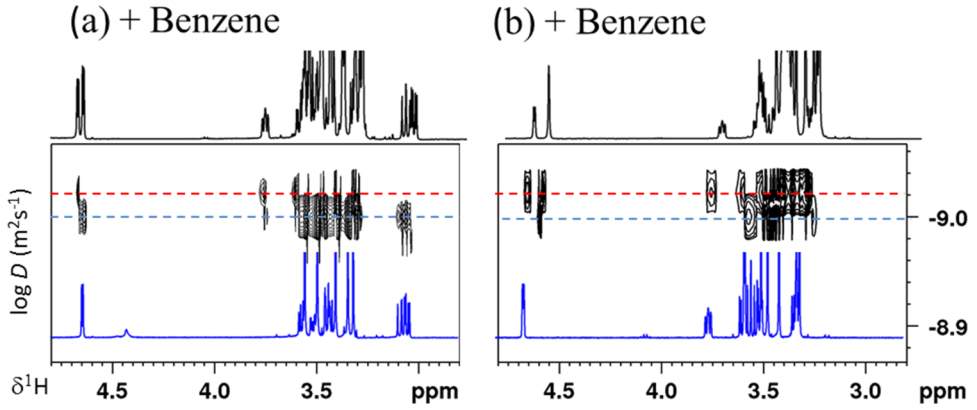


Figure 6. ^{1}H 2D DOSY spectra of the binary mixtures (a) α-pMeGlc(**1**)/α-pMeGal(**3**) and (b) α-pMeGal(**3**)/α-pMeMan(**5**) in cyclohexane-d_{12} upon addition of benzene (ca. 40 mM). One-dimensional ^{1}H NMR spectra of one of the components of the mixture are displayed in blue [(a) α-pMeGlc(**1**) and (b) α-pMeGal(**3**)].

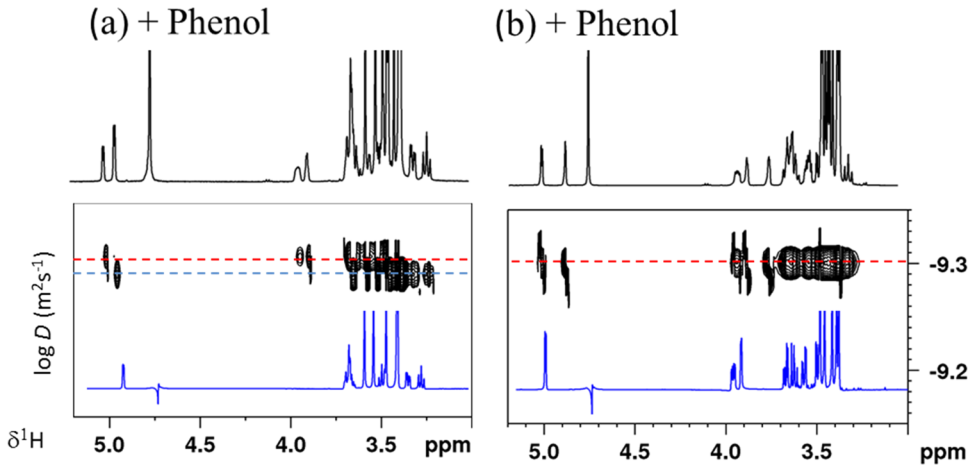


Figure 7. ^{1}H 2D DOSY spectra of the binary aqueous mixtures (a) α-pMeGlc(**1**)/α-pMeGal(**3**) and (b) α-pMeGal(**3**)/α-pMeMan(**5**) (ca. 25 mM) upon addition of phenol. One-dimensional ^{1}H spectra of one of the components of the mixture are displayed in blue [(a) (**1**) and (b) (**3**)].

achieved in the presence of phenol (Figure 7b, D_{D_2O}(**3**) = D_{D_2O}(**5**) = 4.91 × 10^{-10} m^2 s^{-1}) and only for the α-pMeGlc(**1**)/α-pMeGal(**3**)/phenol mixture, a well-defined separation of the diffusion coefficients of the sugars was observed (Figure 7a, D_{D_2O}(**1**) = 5.02 × 10^{-10} m^2 s^{-1} and D_{D_2O}(**3**) = 4.93 × 10^{-10} m^2 s^{-1}). In this case, the apparent hydrodynamic radius of α-pMeGal(**3**) increased compared to that of α-pMeGlc(**1**) when phenol was present in solution. The smaller diffusion coefficient observed for α-pMeGal(**3**) may be correlated with the tendency of α-pMeGal(**3**) to display multivalent CH−π interactions through the CH groups at positions 3, 4, and 5. However, the possible interplay of intermolecular hydrogen bonds between both solutes and the solvent hamper the analysis of the observed data.

Therefore, although the data obtained for the cyclohexane mixtures allowed for a clear-cut discrimination of the components (separation by DOSY), the lack of separation for most of the investigated mixtures in water clearly suggests that the solvent properties strongly influence the type and strength of interactions between the species in solution. The intrinsic chemical nature of each sugar diastereomer in every specific mixture (solute/solvent interactions), as well as the balance between the intermolecular interactions (i.e., hydrogen bonds and CH−π interactions), modulates the recognition process. Thus, for the CH−π contacts, solvophobic effects drive the interactions between the nonpolar groups.[32]

CONCLUSIONS

We have used diffusion NMR spectroscopy to screen the possibility of separation of the components of sugar binary mixtures in different solvents. The mixtures were composed by simple permethylated monosaccharide diastereomers. The working hypothesis relied on the existence of characteristic solvation/diffusion properties of each sugar that could be modulated by solute/solvent interactions and by the presence of aromatic rings (cosolute). Our results suggest that tiny configurational differences of the solutes (even at only one stereogenic center) drastically modify their interaction with the solvent, yielding different diffusion coefficients for the sugars in solution. Despite their obvious similarities (i.e., identical molecular weight and chemical functionalities), in nonpolar nonprotic cyclohexane, the glucose derivatives showed the smallest size and higher diffusion coefficient values compared to that of their counterparts in the mixture (galactose or mannose derivatives). Although self-diffusion coefficient values are very similar, noticeable differences were observed for the components of some of the studied mixtures. Intriguingly, the same mixtures in water (polar, protic solvent) could not be separated by DOSY, suggesting that their shapes and sizes (i.e.,

DOI: 10.1021/acsomega.7b01630
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hydrodynamic radius of the sugars) become almost identical (similar diffusion coefficient) as a consequence of the interaction with the solvent (i.e., hydrogen bond). Further experiments with specific mixtures confirmed the influence of the solvent properties to properly achieve the challenging separation by diffusion NMR.

In addition, we have probed for the first time that sugar−aromatic interactions (CH−π interactions) may modify the apparent size of monosaccharide derivatives and consequently their diffusion coefficients. This fact allows for the discrimination of the components of binary mixtures as a function of their respective ability to interact with aromatic compounds in solution. Moreover, we have shown the strong influence of the solvent properties to allow the detection of the CH−π interactions. The competition with other types of noncovalent interactions, such as hydrogen bonds, may definitively influence the attainable size and shape differences.

Overall, the presented results open up the possibility of using DOSY as an additional experimental tool to demonstrate and support the existence of intermolecular CH−π interactions during formation of specific complexes.

METHODS

NMR Samples. All permethylated monosaccharides were synthesized according to the literature description.[26] All compounds used for the synthesis, as well as phenol, benzene, and deuterated solvents (i.e., D_2O, $CDCl_3$, and cyclohexane-d_{12}), were purchased from Sigma-Aldrich.

The 600 μL NMR samples (5 mm NMR tubes) of the binary mixtures were prepared at a concentration of ca. 20−40 mM for each sugar.

Stock solutions of aromatic compounds (i.e., phenol in D_2O and benzene in cyclohexane-d_{12}) were prepared to add small volumes to the NMR samples to reach the desired sugar−aryl ratio concentrations.

The ^{1}H chemical shift scales are internally referred to trimethylsilylpropionate (D_2O), tetramethylsilane ($CDCl_3$), and to the nondeuterated solvent signal (δ 1.38 ppm) for cyclohexane-d_{12} solutions. The ^{13}C chemical shift scales are referred to acetone (δ 30.9 ppm) in D_2O and to the residual signal of chloroform (δ 77.4 ppm) and cyclohexane (δ 26.4 ppm).

NMR Experiments. All experiments were performed at room temperature (298 K) either on a Bruker Avance (500 MHz) or on a Bruker Avance II (600 MHz) spectrometer, equipped with a triple resonance high-resolution probe (TXI). The characterization of the compounds in different solvents was achieved by NMR standard experiments. NMR experimental data and assignment of **1**−**5** in different solvents are available in the Supporting Information and at NMRshiftDB[33] under the corresponding molecules identification codes (i.e., 60004073 (**1**), 60004029 (**2**), 60004074 (**3**), 60004071 (**4**), and 60004072 (**5**)). The attribution of scalar couplings was supported by ^{1}H NMR spectra simulation with SpinWorks 4.0.3 (K. Marat, University of Manitoba, 2014) using NUMRIT algorithms.[34]

For the measurement of diffusion coefficients of the components of the mixtures, data acquisition and analysis were carried out using Bruker Topspin software (Bruker BioSpin, Germany). The DOSY experiments were performed using the longitudinal eddy current delay bipolar gradient pulse sequence (ledbpgp2s) from Bruker library. The diffusion delay Δ was set at 150−200 ms, the gradient strength g (2 ms) was linearly incremented in 32−64 steps from 2 to 95% of its maximum value, longitudinal eddy currents delay of 5 ms and 8/16 scans were recorded for each experiment. Processing was achieved using 4 K points in the F2 dimension and 256 points in F1. An exponential window function with 3 Hz line broadening was applied in the F2 dimension prior to Fourier transformation. After baseline correction, the diffusion dimension was processed with the DOSY processing program (Bruker TopSpin software 3.5). A logarithmic scaling was applied to the diffusion axis, and a noise sensitivity factor of 4 and line width factor of 1 were used. The fitting of the diffusion dimension in the 2D DOSY spectra was obtained using a single exponential fit.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01630.

Assignment of the NMR (^{1}H and ^{13}C) spectra of compounds **1**−**5** in D_2O, cyclohexane-d_{12}, and $CDCl_3$; Table S1 with values of diffusion coefficients (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Kathrin König, Daniela Naumann, and Stefan Kuhn of the NMR facility of the University of Cologne for their technical support. D.D. and N.E.S. are very grateful to the University of Cologne as participants of the JobSharing project (for their support). L.J. was in part supported by the Deutsche Forschungsgemeinschaft (DFG) through the 1D NMR project (improvement of data quality in NMR, SCHL 580/3-1). The group at Spain thanks MINECO of Spain for financial support (Grants CTQ2015-64597-C2-1P and 2-P) and for the MINECO-Severo Ochoa Excellence Acreditation (SEV-2016-0644).

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