Carbohydrate Polymers 79 (2010) 241–249

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Relation between the Δ2 effect and the solution conformational entropy of aldohexoses and select methyl glycosides

Taylor D. Buley, André M. Striegel *

Department of Chemistry & Biochemistry, Florida State University, Tallahassee, FL 32306-4390, USA

ARTICLE INFO

Article history:
Received 2 July 2009
Received in revised form 27 July 2009
Accepted 29 July 2009
Available online 3 August 2009

Keywords:
Solution conformational entropy
Size-exclusion chromatography
Δ2 effect
Aldohexoses
Methyl glycosides

ABSTRACT

Configurational differences between monosaccharides directly impact properties such as cryoprotectant ability and binding selectivity. Here, we examine how diastereomeric differences among select aldohexoses and methyl glycosides affect the flexibility of the sugars in solution. Our analysis takes advantage of the entropic nature of the separation in size-exclusion chromatography (SEC). Particular attention is given to the influence of the Δ2 effect, the greater destabilization of the pyranose ring conformation when the hydroxyl group at carbon 2 (C_2) is in the axial position, on solution conformational entropy (ΔS) in aqueous solvent as well as in *N,N*-dimethyl acetamide (DMAc) and in DMAc/LiCl. For example, the rankings of the ΔS values of mannose and talose in water, relative to the other aldohexoses examined, can be directly attributed to the influence of the Δ2 effect. As part of this study, we have also investigated how the pyranose:furanose and α:β ratios affect the relative flexibilities of the sugars in solution. Several differences, not all of which we are currently able to explain, are noted between the behavior of the sugars in aqueous versus organic solvent.

1. Introduction

Aldohexose sugars are abundant in biological systems and are used by cells to fuel life functions, for signaling and recognition, and in cell–cell adhesion (Garrett & Grisham, 2005). At the dimeric and higher levels, differences in molecular flexibility imparted by anomeric configuration or glycosidic linkage are responsible for differences in binding and docking of enzymes and bacterial toxins (Lindberg et al., 1987; Rockey, Laederach, & Reilly, 2000), in "site-directed presentation" (the initiation of infection by bacteria and parasites, through interaction with glycosylation sites on the surface of the proteins of the host organism) (Carver, 1993), and for aptameric binding specificity (Yang, Goldstein, Mei, & Engelke, 1998). At the monomeric level, anomeric differences provide for the biding selectivity of the tryptophan-labeled *Escherichia coli* sugar transport protein GalP toward α-glucose over its β counterpart (Patching, Henderson, Herbert, & Middleton, 2008), while diastereomeric configurational differences between the glucose, galactose, mannose, and talose carbohydrate moiety in C-linked antifreeze glycoprotein analogues have been shown to be extremely important in modulating recrystallization-inhibition activity (Czechura, Tam, Dimitrijevic, Murphy, & Ben, 2008).

During the last several years, our group has pioneered the application of size-exclusion chromatography (SEC) (Striegel, 2005a, 2005b, 2008; Striegel, Yau, Kirkland, & Bly, 2009), an entropically-driven technique, to determining the solution conformational entropy of oligosaccharides. Our studies have quantitated the influence of anomeric configuration, glycosidic linkage, linearity versus cyclicity, and intramolecular hydrogen bonding on the flexibility of select di- and oligosaccharides in aqueous and polar aprotic organic solvents (Boone, Nymeyer, & Striegel, 2008; Boone & Striegel, 2006; Striegel, 2003a). With few exceptions (Striegel, 2003a), monosaccharides have remained unexamined by this method.

Here, we apply the SEC method to the study of various aldohexoses (Fig. 1, where only the β anomers are shown) and several of their methyl glycosides (Fig. 2). Our studies were performed both in water, at "quasi-physiological" conditions of temperature and pH, as well as in the organic solvents *N,N*-dimethyl acetamide (DMAc) and DMAc/LiCl, the latter being a preferred solvent for such difficult-to-dissolve polysaccharides as cellulose and chitin (Striegel, 1997; Striegel & Timpa, 1995). Of particular interest was investigating how the Δ2 effect, i.e., the greater destabilization of the pyranose ring conformation when the hydroxyl group at carbon 2 (C_2) is in the axial position (Izydorczyk, 2005; Juaristi & Cuevas, 1995; Reeves, 1950; Shallenberger, 1982; Stoddart, 1971), manifests itself with respect to limiting the flexibility of monosaccharides in solution, which is given quantitative meaning through the solution conformational entropy ΔS. It is hoped that a more quantitative understanding of how the Δ2 effect and the pyranose:furanose and α:β ratios influence the flexibility of aldohexoses in solution will aid in understanding features such as the differences in cryoprotectant

* Corresponding author. Tel.: +1 850 645 3211; fax: +1 850 644 8281.
E-mail address: striegel@chem.fsu.edu (A.M. Striegel).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.07.053

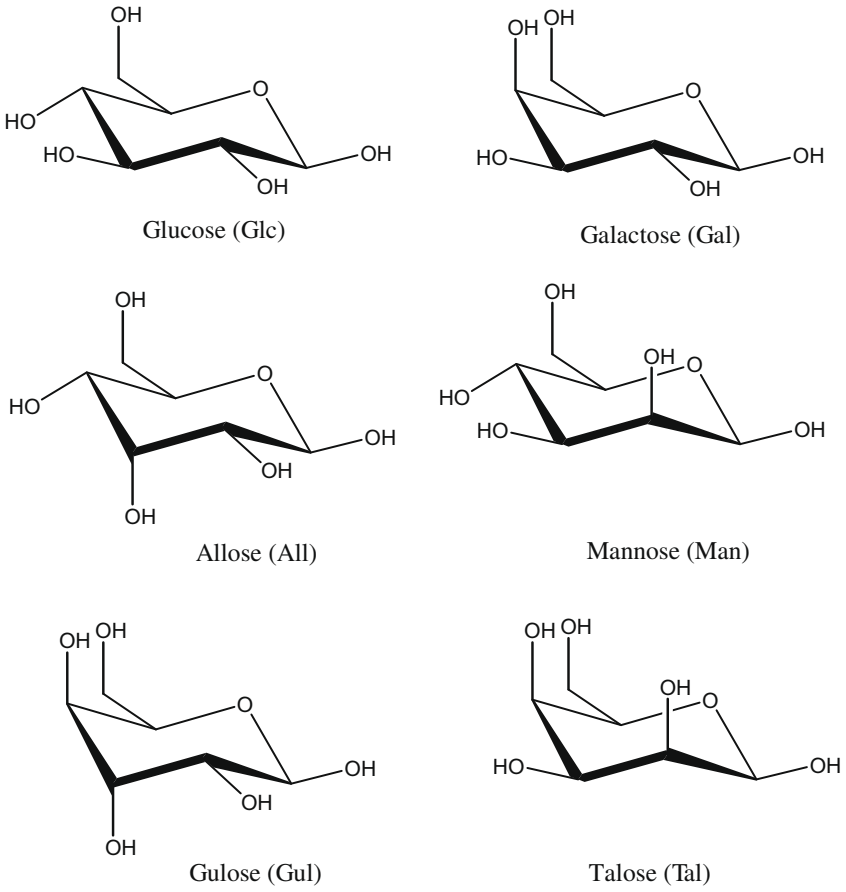

Fig. 1. Structure of the aldohexoses studied. In order to highlight the Δ2 effect in mannose and talose, the β anomers of the aldohexoses are shown.

properties of monosaccharides, mentioned above, as well as in *a priori* design of aptamers, glycovaccines, etc.

2. Experimental

2.1. Materials

All sugars were from Sigma–Aldrich (St. Louis, MO), except for gulose, which was also obtained from V-Labs (Covington, LA); all sugars were sold to at least 98% purity. DMAc, LiCl, NaOH, and NaN_3 were from VWR (West Chester, PA). LiCl was dried in a vacuum oven at 165 °C for at least 18 h and then stored in a desiccator. Preparation of DMAc/0.5% LiCl, as well as its application in the analysis of a variety of polysaccharides, is given in detail in reference (Striegel & Timpa, 1995).

2.2. Size-exclusion chromatography

Unfiltered sample solutions (100 μL of 2.5 mg/mL solutions, briefly heated to ~60 °C to ensure full dissolution) were injected into a system consisting of a Waters 2695 Separations Module (Waters Corp., Milford, MA) and either a Waters 410 (Waters) or an Optilab rEX (Wyatt Technology Corp., Santa Barbara, CA) differential refractive index detector (the untimely demise of the Waters 410 partway through the experiments led us to replace it with the Optilab rEX; results from the latter were checked for consistency with those from the former, and no inconsistencies were found between data sets). Solvent and mobile phase were either aqueous, consisting of H_2O with 0.02% NaN_3, adjusted to pH 7.4 with NaOH, or organic, consisting of either neat DMAc or DMAc/0.5% LiCl. Temperatures of the detector and the injector, sample, and column compartments were maintained at 37 °C (to confirm the predominantly entropic nature of the separation, aqueous data were also collected at 25 °C, while organic data were also collected at 50 °C. See Section 2.3 *Calculation of* $-\Delta S$ below). Flow rate when using aqueous eluent was 1.000 mL/min; when using organic eluents, it was 0.500 mL/min. The interdetector tubing between the columns and the detector was wrapped with insulating tape to prevent heat loss during transfer. For all chromatographic determinations, results are averages of at least six injections, three each from two separate sample dissolutions. Minor flow rate fluctuations were corrected by comparing the retention time of an acetone (5 μL of acetone were added to each sample solution for aqueous analysis) or toluene (5 μL of toluene were added to the each sample solution for organic analysis) marker peak in each injection (including individual glucose injections) to the average value of the marker peak for all glucose injections. For aqueous analysis, separation was performed using a set of four Ultrahydrogel 6 μm particle size, 120 Å pore size SEC columns (Waters). For organic analysis, a set of four PLgel 5 μm particle size, 50 Å pore size columns (Varian/Polymer Laboratories, Amherst, MA) was used.

2.3. Calculation of the solution conformational entropy $-\Delta S$

Calculation of the standard conformational entropy difference between the mobile and stationary phases for the monosaccharides in solution was based on the retention times of the peak maxima (V_R), as measured by SEC, as well as on the solute distribution coefficient (K_{SEC}). These two parameters are related via via (Boone & Striegel, 2006; Boone et al., 2008; Striegel, 2003a; Striegel, 2004; Striegel et al., 2009).

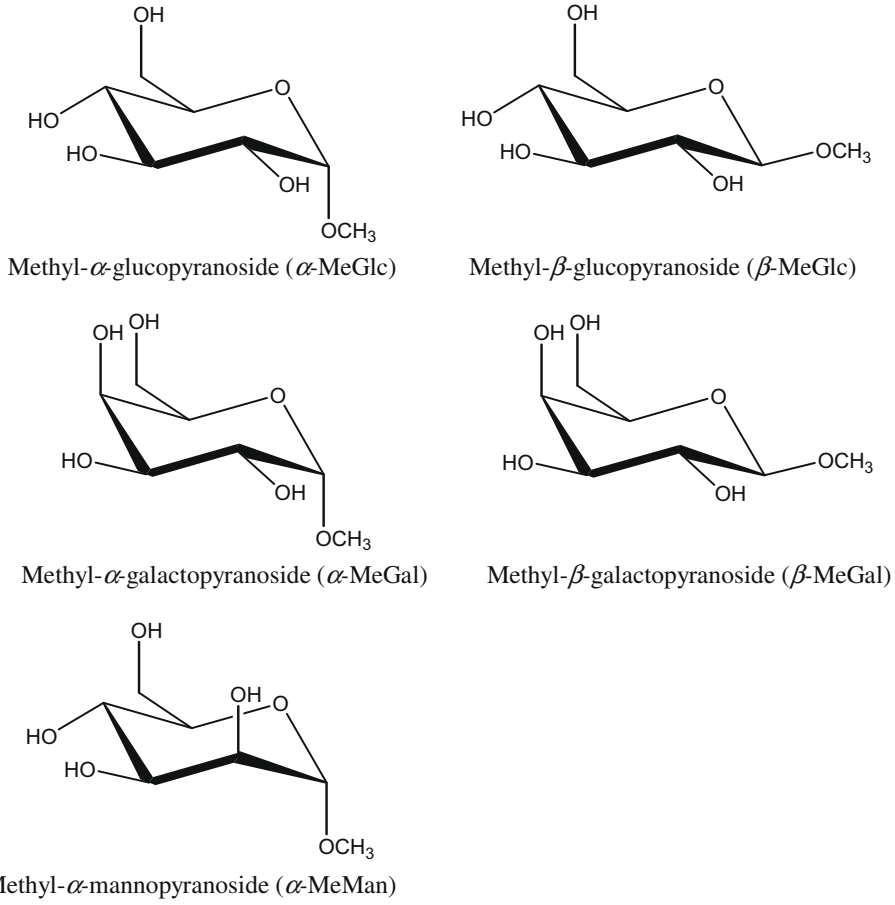

Fig. 2. Structure of the methyl glycosides studied.

$$K_{SEC} = \frac{V_R - V_0}{V_i - V_0}$$

where V_0 is the void volume of the columns and V_i is the total column volume. The aqueous SEC columns are quoted by the manufacturer as having an exclusion limit of approximately 5000 g/mol, based on analysis of poly(ethylene oxide) and poly(ethylene glycol) standards in water at room temperature. For these columns, we measured V_0 using narrow polydispersity ($M_w/M_n \leqslant 1.07$) pullulan standards of molar mass 22,800 and a 112,000 g/mol (Varian/Polymer Laboratories), and measured V_i using acetone. The columns used with the organic eluents have a nominal exclusion limit of 2000 g/mol, based on polystyrene standards in tetrahydrofuran at room temperature. Here, we measured the V_0 using a 20,000 g/mol narrow polydispersity (M_w/M_n = 1.02) linear polystyrene standard (Varian/Polymer Laboratories) and measured V_i using toluene. As the system temperature was changed from 37 to 25 °C in aqueous eluent, and from 37 to 50 °C in the organic eluents, K_{SEC} changed by no more than ~5% for any analyte, and usually substantially less than this. This strongly supports the conclusion that separation of the monosaccharides is predominantly entropic in nature (characteristic of "near-ideal" SEC behavior), as enthalpic interactions with the column packing material would lead to highly temperature-dependent values of the distribution coefficient. Consequently, we can write

$$\Delta S = R \ln K_{SEC}$$

Here, we have used R = 8.31451 J mol^{-1} K^{-1}. The standard entropy difference, ΔS, denotes the difference between the conformational entropy of the oligosaccharides in the flowing mobile phase outside the pores of the column packing versus the entropy of the oligosaccharides in the stagnant mobile phase inside the pores. The use of the negative sign, i.e., of $-\Delta S$, results from solute permeation in SEC being associated with a decrease in conformational entropy, due to the decrease in the degrees of freedom of the analyte when inside the pores of the column packing material, as compared to when the analyte is in the flowing mobile phase (i.e., in the interstitial volume outside the pores) (Striegel et al., 2009).

3. Results and discussion

Results from our experiments are given in Tables 1 and 2, for both the aldohexoses and methyl glycosides examined, in both aqueous and organic media. In what follows, we will attempt to ex-

Table 1
SEC-determined solution conformational entropy of aldohexoses.

Monosaccharide	$-\Delta S$ (J mol^{-1} K^{-1})[a]		
	H_2O	DMAc	DMAc/0.5% LiCl
Glucose	2.351 ± 0.001 (38, <1)[b]	11.97 ± 0.01	16.14 ± 0.01
Galactose	2.486 ± 0.001 (31, 6)	11.88 ± 0.01	16.05 ± 0.01
Allose	2.147 ± 0.002 (15, 8)	11.57 ± 0.01	16.01 ± 0.01
Mannose	2.103 ± 0.001 (66, <1)	11.66 ± 0.02	15.88 ± 0.01
Gulose	1.886 ± 0.001 (12, 4)	12.05 ± 0.03	16.79 ± 0.02
Talose	1.682 ± 0.001 (42, 29)	11.29 ± 0.01	15.62 ± 0.01

[a] Data constitute averages from at least six determinations, at 37 °C in H_2O and at 50 °C in DMAc and DMAc/LiCl. See Section 2 for details.
[b] First number in parenthesis corresponds to the percentage of α pyranose anomer, second number to the combined percentages of α and β furanose anomers, both data in aqueous solution at 30 °C. For all sugars, percentage of acyclics in aqueous solution at 30 °C is <0.1% (Zhu et al., 2001).

Table 2
SEC-determined solution conformational entropy of methyl glycosides.

Methyl glycoside	$-\Delta S$ (J mol^{-1} K^{-1})[a]		
	H_2O	DMAc	DMAc/LiCl
Methyl-α-glucopyranoside	2.953 ± 0.001	11.00 ± 0.01	15.12 ± 0.01
Methyl-β-glucopyranoside	3.168 ± 0.001	11.26 ± 0.01	15.32 ± 0.01
Methyl-α-galactopyranoside	3.212 ± 0.001	10.70 ± 0.01	14.86 ± 0.01
Methyl-β-galactopyranoside	3.246 ± 0.001	11.10 ± 0.01	15.15 ± 0.01
Methyl-α-mannopyranoside	2.402 ± 0.001	10.62 ± 0.01	14.66 ± 0.01

[a] Data constitute averages from at least six determinations, at 37 °C in H_2O and at 50 °C in DMAc and DMAc/LiCl. See Section 2 for details.

plain the observed differences and rankings in the $-\Delta S$ data of the methylated and unmethylated monosaccharides and the differences between the $-\Delta S$ values in water versus those in DMAc and in DMAc/LiCl, while paying particular attention to how the Δ2 effect influences the relative $-\Delta S$ rankings. (At this point, it should be noted that minor differences between some $-\Delta S$ values given in this paper and the values for the same mono- or disaccharides given elsewhere (Boone et al., 2008; Boone & Striegel, 2006; Striegel, 2003a) are almost certainly due to small differences in the average pore size, as well as the pore size distribution, of the SEC columns employed in the respective studies).

As a reminder, the Δ2 effect occurs when the C_2–O bond bisects the torsional angle between the two C_1–O bonds (Izydorczyk, 2005; Jeffrey & Yates, 1981; Juaristi & Cuevas, 1995; Kaliannan, Vishveshwara, & Rao, 1986; Lii, Chen, & Allinger, 2003; Reeves, 1950; Shallenberger, 1982; Stoddart, 1971). This is shown in Fig. 3, with the Newman projection viewed along the axis leading from C_2 to C_1 ($C_2 \rightarrow C_1$), i.e., the circle in Fig. 3 represents carbon 2 obscuring carbon 1. For the monosaccharides depicted in Fig. 1, this arrangement occurs in both β-mannose and β-talose. The Δ2 effect appears to be due to unfavorable dipolar interactions between the C_1–O_1 and C_2–O_2 dipoles in the O_1–C_1–C_2–O_2 arrangement (or, in Fig. 3, the R′O–C_1–C_2–OR arrangement) in the β anomer, when the hydroxyl group at C_2 (the OR group in Fig. 3) is axially oriented (Kaliannan et al., 1986; Reeves, 1950). In the MM4 hydrocarbon force field, the Δ2 effect has been represented by a torsion–torsion interaction between the two O_1–C_1–C_2–O_2 torsions (Lii et al., 2003).

3.1. Solution conformational entropies in aqueous solvent

The $-\Delta S$ values of the aldohexoses and methyl glycosides examined are given in the "H_2O" column of Tables 1 and 2. The experimental conditions are termed "quasi-physiological," as the aqueous solvent was maintained at pH 7.4 and at 37 °C, conditions resembling those within the human body (Boone et al., 2008). As all the analytes are charge-neutral, no electrolyte was added to the solution. Again, the negative sign on the entropy is meant to represent the loss of conformational freedom experienced upon an analyte permeating from the flowing mobile phase outside the pores into the stagnant mobile phase inside a pore of the column packing material. Because equatorial bonds are more extended than axial bonds, upon entering SEC pores of the same size a monosaccharide with a higher number of equatorially-bonded hydroxyl (or other) groups should experience a greater loss in degrees of freedom than a similar monosaccharide with less of the same equatorially-bonded groups. This corresponds to a larger $-\Delta S$ for the monosaccharide with the higher number of equatorially-bonded hydroxyls.

According to the data in Table 1, galactose has the largest $-\Delta S$ of the aldohexoses examined, and talose has the lowest, with the $-\Delta S$ ranking being Gal > Glc > All > Man > Gul > Tal. The $\Delta\Delta S$ between galactose and talose in aqueous solution is 0.804 J mol^{-1} K^{-1}. As seen in Fig. 4, for a ~1:1 mixture of the two monosaccharides, galactose and talose can be separated from each other by SEC with near-baseline resolution. The virtual lack of enthalpic contribution to the separation is evidenced by the small change in the solute distribution coefficient K_{SEC} of talose and galactose when the temperature of the experiment was varied from 37 to 25 °C: For talose, ΔK_{SEC} was 5.63%, while for galactose ΔK_{SEC} was 3.71%. It should be noted that, for enthalpically-controlled liquid chromatographic separations (e.g., in reversed-phase liquid chromatography), this 12 °C change in temperature is generally expected to correspond to a change in solute distribution coefficient of at least 10–20% (Cole & Dorsey, 1992). What is shown in Fig. 4 is thus a baseline resolution separation of monosaccharide diastereomers by a near-ideal size-exclusion mechanism, virtually devoid of enthalpic interactions. As seen by the peak integral overlaid upon the chromatograms, peak areas differ by only ~10%. This is likely due to small differences in the concentration of the solutions, as well as to small differences in the refractive indices of the monosaccharides themselves (which would affect the response of the differential refractive index detectors used) (Shallenberger, 1982).

A surprise in the $-\Delta S$ rankings of the aldohexoses in water is the larger conformational entropy of galactose compared to that of glucose, given the fact that all hydroxyl groups in glucose are equatorial, whereas in galactose the OH at C_4 is axial (Fig. 1). We do not yet have a complete explanation for this result. The conformational entropy values, which are highly reproducible (note the small standard deviations in Table 1), can only be partially explained by mutarotation and the fact that, at equilibrium in water at room temperature, solutions of glucose contain approximately 7% more α pyranose anomer than do solutions of galactose (first parenthetical datum in Table 1), though solutions of galactose contain 5–6% more furanose isomers than do solutions of glucose (second parenthetical datum in Table 1) (Zhu, Zajicek, & Serianni, 2001). Because of the OH group axially-bonded to C_1 in α anomers, these are expected to have lower $-\Delta S$ values in solution as compared to their β counterparts. Experimental evidence for this is seen when comparing the $-\Delta S$ values of α- versus β-methyl glucoside and of α-versus β-methyl galactoside (Table 1 and Fig. 2): for

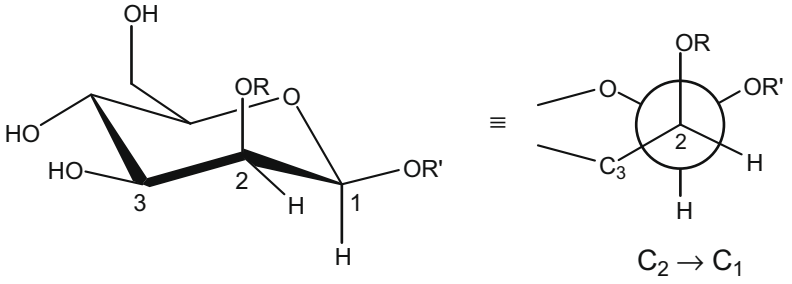

Fig. 3. The Δ2 effect. Newman projection represents view along the axis from C_2 to C_1.

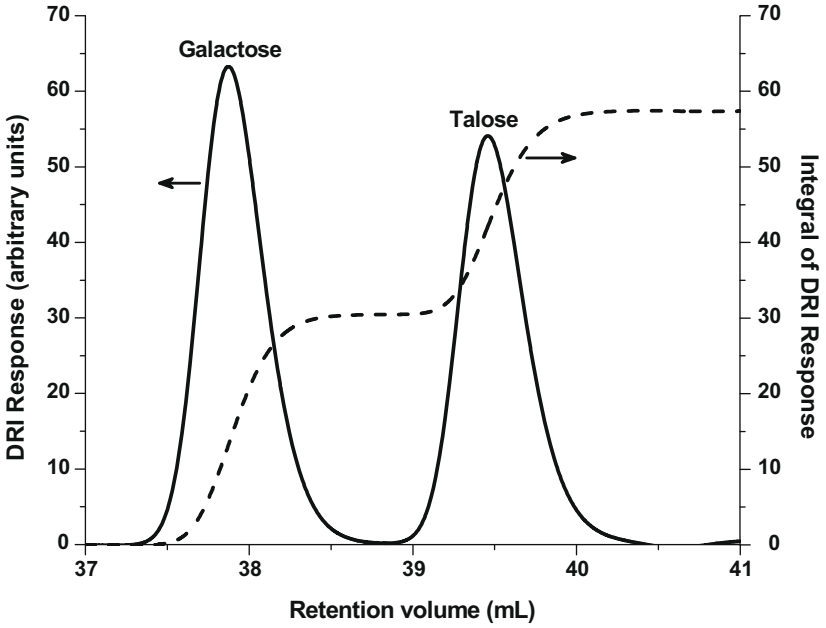

Fig. 4. Size-exclusion chromatogram of a ~1:1 mixture of galactose and talose. Left ordinate corresponds to the response from a differential refractive index (DRI) detector (solid line), right ordinate to the integral of the DRI response (dashed line). Experimental conditions: H_2O, 37 °C, pH 7.4 (see Section 2 for details). Note that both sugars elute by a near-ideal size-exclusion mechanism, as described in the text.

the non-mutarotating methyl glycosides, the solution conformational entropies of the α anomers are smaller than those of the corresponding β anomers. However, in water the $-\Delta S$ values of the methyl galactosides are larger than those of the methyl glucosides. As such, while some of the difference in the $-\Delta S$ values of glucose versus galactose may be due to the equilibrium distribution of pyranose anomers, this does not fully account for $-\Delta S_{Gal} > -\Delta S_{Glc}$.

The results for glucose versus galactose in water agree with those for lactose versus cellobiose and for isomaltose versus melibiose (Fig. 5). Lactose and cellobiose are both β-(1 → 4)-linked disaccharides, the former being a galactopyranosyl-glucopyranose (β-(1 → 4)Gal-Glc, $-\Delta S = 3.913 + 0.001$ J mol^{-1} K^{-1}) and the latter a glucopyranosyl-glucopyranose (β-(1 → 4)Glc-Glc, $-\Delta S = 3.778 \pm 0.001$ J mol^{-1} K^{-1}). The structure of isomaltose is α-(1 → 6)Glc-Glc and its $-\Delta S = 4.088 \pm 0.001$ J mol^{-1} K^{-1}, while the structure of melibiose is α-(1 → 6)Gal-Glc and its $-\Delta S = 4.266 \pm 0.001$ J mol^{-1} K^{-1}. When comparing the disaccharides in each pair to each other, the $-\Delta S$ value of the Gal-Glc disaccharides (lactose and melibiose) are lower than the $-\Delta S$ values of the Glc-Glc disaccharides with identical glycosidic linkage and anomeric configuration (cellobiose and isomaltose, respectively). While the various sets of results are internally self-consistent, the larger conformational entropy of galactose over glucose in aqueous solvent, expressed in the aldohexoses, methyl glycosides, and disaccharides, remains to be fully explained.

We next note that, with the exception of galactose, the $-\Delta S$ rankings of the aldohexoses are inversely proportional to the number of axial hydroxyls, i.e., the sugars with two axial OH groups (gulose and talose) have lower solution conformational entropies than do the sugars with one OH group (allose and mannose) which, in turn, have lower $-\Delta S$ values than does glucose, with all equatorial OHs. Within these rankings we find manifestations of the Δ2 effect: mannose, with its axial OH on C_2, has a lower $-\Delta S$ value than does allose, in which the axial OH is located on C_3. Similarly talose, with axial OHs on C_2 and C_4, has a lower $-\Delta S$ than does gulose, with axial OHs on C_3 and C_4.

The $-\Delta S$ value of talose is substantially lower than that of gulose, due to the high percentage of α pyranose anomer (42%) of talose at equilibrium in water; this is a consequence of the Δ2 effect, which is also responsible for the high percentage of α-mannose (66%) in water. In both cases, talose and mannose, the Δ2 effect provides for an increased anomeric effect (Angyal, 1968; Juaristi & Cuevas, 1995). In talose, however, the $-\Delta S$ value is additionally lowered by the high percentage (29%) of furanose anomers present at equilibrium in water.

An axial OH group on C_3, such as found in allose and gulose, has been observed to reduce the magnitude of the anomeric effect (Angyal, 1968; Juaristi & Cuevas, 1995). (Note the large percentages of β pyranose anomer for both gulose (84%) and allose (77%) in water) (Zhu et al., 2001). Unfortunately, we were unable to obtain a sample of altrose, which has axial OH groups on both C_2 and C_3, an arrangement where the Δ2 effect should be offset by the O:OH stereoelectronic interaction of the axial hydroxyl group on C_3 (Angyal, 1968; Stoddart, 1971).

Regarding the methyl glycosides examined (Fig. 2), the relative rankings of the galactosides and glucosides have already been discussed. The $-\Delta S$ values of the methyl glycosides, given in Table 2, are greater than the values of the corresponding unmethylated aldohexoses. This is due to the extra degree of freedom contributed by the O–CH_3 bond of the glycosides. The fact that the $-\Delta S$ values of the glycosides are significantly greater than the values of their unmethylated counterparts, while the former still retain the same rank order as the latter with respect to $-\Delta S$, we interpret as meaning that the entropy contributed by the bond added through the arrangement [anomeric oxygen]:[methyl group carbon]:[methyl group hydrogen] is greater than the entropy lost due to prevention of mutarotation in the non-mutarotating glycosides. Because prevention of mutarotation by methylation should not significantly decrease entropy, it is likely that mutarotation does not confer a large amount of flexibility on the unmethylated aldohexoses in aqueous solution. The parenthetical data in Table 1 give the percentage of α pyranose anomers, and the combined percentages of α and β furanose anomers, at equilibrium in water for the unmethylated aldohexoses (Zhu et al., 2001).

The much lower $-\Delta S$ value of methyl α-mannopyranoside (with an axial hydroxyl group at C_2), as compared to the values

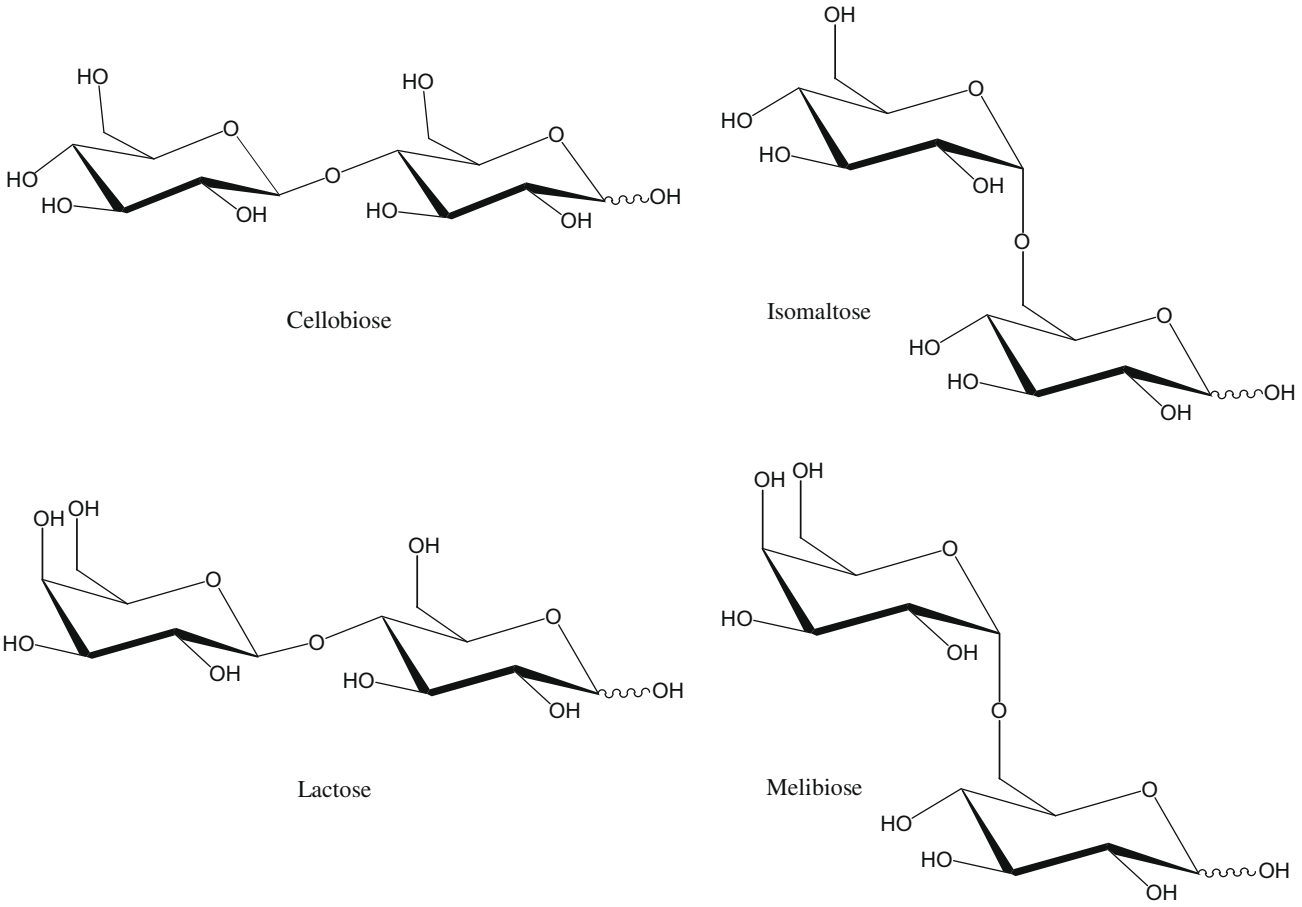

Fig. 5. Structure of the disaccharides examined.

for the various methyl galacto- and glucopyranosides, is once again attributed to the Δ2 effect in the mannopyranoside, similar to the same effect in its unmethylated counterpart mannose.

3.2. Solution conformational entropies in DMAc and DMAc/LiCl

Due to its high polarity and dielectric constant, water is good at solvating other polar or charged species. Because water is also a good hydrogen bond donor and acceptor, the hydroxyl groups of sugars will interact favorably with water, constraining the sugar molecules and limiting their flexibility. Hydrogen bonds are dynamic interactions that break and reform on a picosecond timescale (Garrett & Grisham, 2005). Hydrogen bonding between solvent and solute will not eliminate all analyte flexibility, but it will limit the extent to which the OH groups are free to rotate in sugars (Woods, Fraser-Reid, Dwek, & Edge, 1994).

Both the $-\Delta S$ values of the aldohexoses and their rankings relative to each other are different in DMAc and in DMAc/LiCl than they are in water. The $-\Delta S$ values of all the sugars examined are higher in the organic solvents used than in water. For DMAc, this observation can be attributed mostly to the diminished (relative to water) intermolecular hydrogen bonding of the sugars with the solvent. Without these relatively strong intermolecular H-bonds, the sugar molecules adopt a more extended, less constricted conformation in solution and, therefore, have a larger apparent size in DMAc than they do in water. While the aprotic nature of DMAc may lead to more intramolecular H-bonding in the sugars in this solvent than in water, leading to a less flexible, more compact structure in DMAc, the role of intramolecular H-bonding is likely to be relatively minor as compared to solute–solvent interactions (Angyal, 1968).

Another factor contributing to the larger $-\Delta S$ values in DMAc and DMAc/LiCl, as compared to the same values in water, is that the solvating organic molecule or macrocation are both substantially larger than are water molecules (Fig. 6). It should be remembered that $-\Delta S$ is the *solution* conformational entropy, i.e., the conformational entropy of the *solvated* species (Striegel et al., 2009). Due to the relative sizes of the solvents, the solvated complex of sugar with DMAc is much larger than the solvated complex of the same sugar with water. For DMAc/LiCl, the solvating species is the macrocation $[DMAc_n + Li]^+$ (where $n = 1, 2, 3$ and the chloride counterion serves as H-bond acceptor; see Fig. 6) (Striegel, 1997; Striegel, 2003b; Striegel, Piotrowiak, Boué, & Cole, 1999), a significantly larger species than neat DMAc molecules and which accounts for the much higher $-\Delta S$ values in DMAc/LiCl as compared to the values in neat DMAc.

The $-\Delta S$ rankings in DMAc follow the order Gul > Glc > Gal > Man > All > Tal, while in DMAc/LiCl the order is Gul > Glc > Gal > All > Man > Tal. It should first be noted that we are currently unable to explain the higher $-\Delta S$ of gulose in both DMAc and DMAc/LiCl, as compared to the conformational entropies of the rest of the monosaccharides. The relative ranking of gulose in the aldohexose series examined clearly differs in the organic solvents as compared to water. Measurements for gulose in the organic solvents were conducted many (dozens) of times, for five different sample concentrations ranging from 0.1 to 5.0 mg/mL, and using samples from two different sources (see Section 2). For each concentration, hexuplicate determinations were performed. We also performed measurements, at a concentration of 2.5 mg/mL in

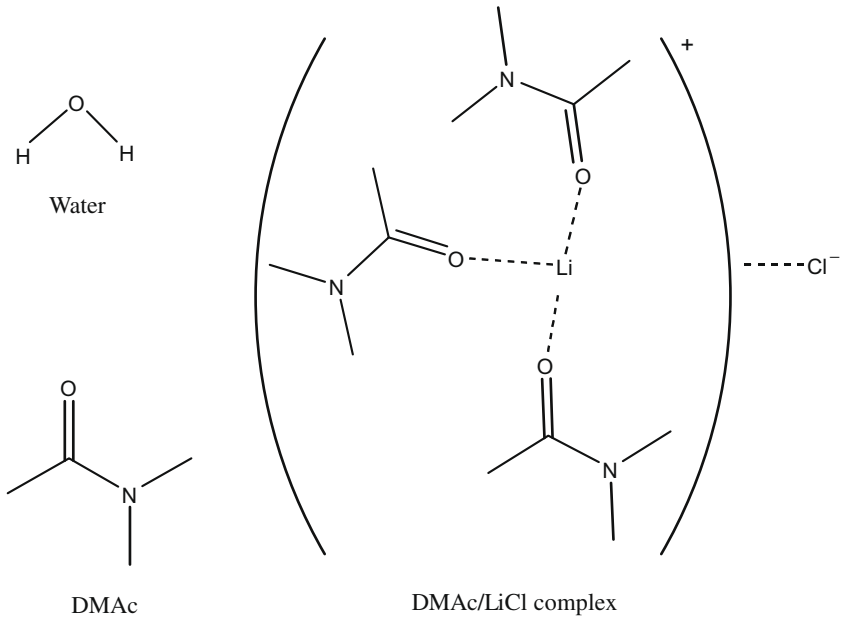

Fig. 6. Solvents used: water, *N,N*-dimethyl acetamide (DMAc), and DMAc/LiCl. For the latter, the figure depicts the structure proposed in (Striegel, 2003b).

DMAc, approximately six months apart from each other. In all cases, the $-\Delta S$ of gulose was larger than those of the other aldohexoses in the series. As noted in the previous section, the α:β ratio of gulose pyranose anomers in water is 12:84. This is a much higher percentage of β pyranose anomer than in any of the other aldohexoses examined, and suggests a highly complex H-bonding network that stabilizes the β pyranose form of the sugar. It is possible that either the β pyranose anomer (or both pyranose anomers) of gulose is stabilized even more by the polar aprotic solvents employed here than it is by water.

We also observe that once again talose, with axial OH groups at C_2 and C_4, has the lowest $-\Delta S$ value of the series in both DMAc and DMAc/LiCl., and that in both organic solvents the $-\Delta S$ value of glucose is larger than that of galactose. The latter agrees with previous result from our group for these monosaccharides, and for isomaltose and melibiose, in DMAc/LiCl (Striegel, 2003a), and with expectations based on the all-equatorial-OH arrangement in glucose versus galactose, which has an axial OH at C_4. It also agrees with the results for the α-(1 → 6)-linked glucopyranosyl-glucopyranose disaccharide isomaltose as compared to its galactopyranosyl-glucopyranoside counterpart melibiose. The relative ranking, however, is opposite the glucose versus galactose and isomaltose versus melibiose rankings in water, discussed earlier. For the monosaccharides, this difference in ranking could be due to a difference in the relative ratios of α to β pyranose anomers, and/or of pyranose to furanose structures, in DMAc or DMAc/LiCl versus the same ratios in water (Franks, 1977). For example, the percentage of glucose α pyranose anomer changes from 38% in water at room temperature to 44% in DMSO at 30 °C (Bubb, 2003; Franks, 2000); the amount of furanose anomers of galactose in water at room temperature is 6%, but rises to ~15% in DMSO (the temperature of the DMSO experiments does not appear to have been specified by Mackie and Perlin in the original publication) (Mackie & Perlin, 1966); and the amount of furanose anomers of glucose, negligible in both water and DMSO, rises to 4.5% in *N,N*-dimethyl formamide at 70 °C (Reine, Hveding, Kjølberg, & Westbye, 1974). It is also known, from the classic infrared absorption work of Tipson and Isbell, that in water the equilibrium distribution of arabino sugars such as galactose, and of ribo sugars such as talose, is higher in furanose structures than are the equilibrium distributions of xylo (glucose) and lyxo (mannose and gulose) sugars (Tipson & Isbell, 1962). As the amount of α pyranose anomer and/or of furanose structures increases, the solution conformational entropy $-\Delta S$ should become smaller. This is due to the fact that the more compact furanose and α pyranose structures should experience a smaller loss in conformational degrees of freedom (less loss of flexibility) than the corresponding pyranose and β pyranose structures, respectively, upon permeation into the pores of the SEC column packing material from the flowing interstitial mobile phase. The same reasoning can be applied to explain why, in DMAc, the $-\Delta S$ of mannose is larger than that of allose, seemingly contradicting the $\Delta 2$ effect in this solvent. It should be emphasized, however, that at present this remains a conjecture, though the amount of α pyranose anomer of mannose increases from 66% in water at room temperature to 86% in DMSO at 116 °C (Franks, 2000).

Regarding the behavior of the methyl glycosides in DMAc and DMAc/LiCl, it is quite interesting to note that the $-\Delta S$ values of the glycosides are smaller than those of their corresponding unmethylated counterparts. This is contrary to what was observed in water. In the organic solvents, the extra degree of freedom gained by replacing the anomeric hydroxyl group with a methyl group does not appear to fully compensate for the loss of entropy due to prevention of mutarotation. In DMAc and DMAc/LiCl, mutarotation of the unmethylated sugars must therefore be a significant source of flexibility for the aldohexoses examined here. The larger $-\Delta S$ values of the methyl glycosides in DMAc/LiCl, vis-à-vis in DMAc, can only be partially explained through H-bond solute–solvent interactions. If this were the case, the $-\Delta S$ values in DMAc/LiCl would be intermediate to those in DMAc and in water, as intermolecular H-bonding between the sugars and water is expected to be greater than between the same sugars and DMAc/LiCl (with the possible exception of gulose, as noted above). As with the unmethylated aldohexoses, most of the $-\Delta S$ difference for the glycosides in DMAc versus in DMAc/LiCl can be explained by the significant difference in the size of the solvating species, the neat DMAc molecule and the $[DMAc_n + Li]^+Cl^-$ complex, and by the fact that what is being measured is the conformational entropy of the solvated analytes, which will be larger in DMAc/LiCl than in neat DMAc.

We also observe for the methyl glycosides that, while in water the $-\Delta S$ values of both galactosides are larger than those of both

glucosides, in DMAc and DMAc/LiCl the $-\Delta S$ values of β-MeGlc and β-MeGal are both larger than the $-\Delta S$ values of α-MeGlc and α-MeGal (Table 2). The order in both solvents is β-MeGlc > β-MeGal > α-MeGlc > α-MeGal > α-MeMan. As was the case in aqueous solvent, the low $-\Delta S$ value of the methyl mannoside (with an axial OH at C_2) in DMAc and DMAc/LiCl is ascribed to a manifestation of the $\Delta 2$ effect.

For the glucosides and galactosides, the larger $-\Delta S$ of the β over the α anomers likely results from the more extended structure of the former as compared to the latter. This effect could also be augmented by intramolecular H-bonding between the hydrogen of the OH on C_2 and the oxygen of the aglycon in the methyl-α-glycosides. The latter conclusion is supported by proton nuclear magnetic resonance (^{1}H NMR) studies of α- and β-methyl-gluco- and galactosides in DMSO, where it was found that the coupling constant J(H,OH) and the chemical shift δ(OH) for the hydroxyl group at C_2 were larger for methyl-α-glucoside and methyl-α-galactoside than for their β counterparts (Bernet & Vasella, 2000). The same study showed that, in DMSO, the ΔJ(H,OH) and $\Delta\delta$(OH) between α- and β-methyl-galactosides (again for the OH at C_2) were both larger than the corresponding differences between α- and β-methyl-glucosides, and ranked these four glycosides in the order β-MeGlc > β-MeGal > α-MeGlc > α-MeGal with respect to chemical shift at the C_2 OH. The latter ranking is the same as the $-\Delta S$ ranking of the gluco- and galactosides studied here, in both DMAc and DMAc/LiCl, while the ΔJ and $\Delta\delta$ results agree with the larger $\Delta\Delta S$ of the galactosides, as compared to the $\Delta\Delta S$ of the glucosides, in both DMAc and DMAc/LiCl.

This leads to one last observation, which is that the $\Delta\Delta S$ between galactopyranoside anomers increases by about an order of magnitude when going from water to either DMAc or DMAc/LiCl (from 0.034 J mol^{-1} K^{-1} in water to 0.40 J mol^{-1} K^{-1} in DMAc, and to 0.29 J mol^{-1} K^{-1} in DMAc/LiCl), while the $\Delta\Delta S$ between glucopyranoside anomers increases only modestly when changing solvents (from 0.215 J mol^{-1} K^{-1} in water to 0.26 J mol^{-1} K^{-1} in DMAc, and to 0.29 J mol^{-1} K^{-1} in DMAc/LiCl). While we cannot at present explain the large difference in behavior between the glucosides and galactosides, the change in $\Delta\Delta S$ as a function of changing solvent, for both sets of glycosides, could be due to a decreased stabilization of the more polar methyl-β-glycosides as a function of decreasing solvent polarity: water is substantially more polar (and has higher dielectric constant) than is neat DMAc, with DMAc/LiCl expected to have an intermediate polarity closer to that of DMAc. From studies of the anomeric effect, it is known that the percentage of methyl-α-glycoside increases with decreasing solvent polarity (decreasing solvent dielectric constant) (Juaristi & Cuevas, 1995). More methyl-α-glycoside, which has a smaller $-\Delta S$ than its β counterpart, corresponds to a larger $\Delta\Delta S$ between glycoside anomers. Likewise, decreased stabilization of methyl-β-glycoside as a function of decreasing solvent polarity corresponds to a larger $\Delta\Delta S$ between glycoside anomers as solvent polarity decreases.

4. Conclusions

Size-exclusion chromatography, an entropically-controlled separation method, has been employed to determine the solution conformational entropy of select aldohexoses and methyl glycosides in water, DMAc, and DMAc/LiCl. Results from our studies showed the influence of the $\Delta 2$ effect on the flexibility of the monosaccharides in solution, as well as the influences of furanose:pyranose and α:β ratios. In water and DMAc/LiCl, the $\Delta 2$ effect clearly manifested itself in the relative ranking of the $-\Delta S$ values of mannose, talose, and methyl-α-mannopyranoside in the series examined, while in neat DMAc the $-\Delta S$ of allose was found to be slightly lower than that of mannose. On an absolute basis (i.e., number of J mol^{-1} K^{-1}), the $\Delta 2$ effect manifested itself more greatly in DMAc/LiCl that it did in water for mannose versus allose and for talose versus gulose. However, in terms of a relative (i.e., percentage) change in $-\Delta S$, the $\Delta 2$ effect manifested itself more greatly in the aqueous solvent for the given monosaccharide pairs. These same relationships also hold true for the behavior of methyl-α-mannopyranoside relative to the other methyl glycosides examined. Not surprisingly, the size of the solvating species greatly influenced the solution conformational entropy of the analytes.

Several of the results obtained will require further investigation, such as the seemingly abnormally-large $-\Delta S$ of gulose in DMAc and DMAc/LiCl relative to the other aldohexoses examined, and the difference in the aqueous versus organic solvent rankings of the $-\Delta S$ values of glucose versus galactose and of select galactopyranosyl-glucosyranoses versus their glucopyranosyl-glucopyranose counterparts with identical anomeric configuration and glycosidic linkage.

As noted in the Introduction, configurational differences among aldohexoses give rise to differences in the binding selectivity of sugar transport proteins and in the ability of monosaccharides to modulate recrystallization-inhibition activity. The ability of size-exclusion chromatography to quantify how isomeric and related differences influence carbohydrate flexibility in solution has great potential for glycovaccine design and for furthering our understanding of bioregulatory processes, among others. Our group continues to explore this area.

Acknowledgements

Acknowledgement is made to the Donors of the American Chemical Society Petroleum Research Fund for partial support of this research through grant 1-1312-0050. We would also like to acknowledge Mr. Dustin Richard for assistance with analysis of the disaccharides, and Dr. Marcus Boone for assistance when needed. Particular thanks are given to Sir Dai Rees, for a number of interesting and enlightening discussions during the initial stages of this project.

References

Angyal, S. J. (1968). Conformational analysis in carbohydrate chemistry. I. Conformational free energies. The conformations and α:β ratios of aldopyranoses in aqueous solution.. *Australian Journal of Chemistry, 21*, 2737–2746.

Bernet, B., & Vasella, A. (2000). ^{1}H NMR analysis of intra- and intermolecular H-bonds of alcohols in DMSO: Chemical shift of hydroxy groups and aspects of conformational analysis of selected monosaccharides, inositols, and ginkgolides. *Helvetica Chimica Acta, 83*, 995–1021.

Boone, M. A., Nymeyer, H., & Striegel, A. M. (2008). Determining the solution conformational entropy of O-linked oligosaccharides at quasi-physiological conditions: Size-exclusion chromatography and molecular dynamics. *Carbohydrate Research, 343*, 132–138.

Boone, M. A., & Striegel, A. M. (2006). Influence of anomeric configuration, degree of polymerization, hydrogen bonding, and linearity versus cyclicity on the solution conformational entropy of oligosaccharides. *Macromolecules, 39*, 4128–4131.

Bubb, W. A. (2003). NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity. *Concepts in Magnetic Resonancy Part A, 19A*, 1–19.

Carver, J. P. (1993). Oligosaccharides: How can flexible molecules act as signals? *Pure & Applied Chemistry, 65*, 763–770.

Cole, L. A., & Dorsey, J. G. (1992). Temperature dependence on retention in reversed-phase liquid chromatography. 1 Stationary-phase considerations. *Analytical Chemistry, 64*, 1317–1323.

Czechura, P., Tam, R. Y., Dimitrijevic, E., Murphy, A. V., & Ben, R. N. (2008). The importance of hydration for inhibiting ice recrystallization with C-linked antifreeze glycoproteins. *Journal of the American Chemical Society, 130*, 2928–2929.

Franks, F. (1977). Solvation and conformational effects in aqueous solutions of biopolymer analogues. *Philosophical Transactions of the Royal Society of London B, 278*, 33–57.

Franks, F. (2000). *Water – A Matrix for Life* (2nd ed.). Cambridge, UK: Royal Society of Chemistry.

Garrett, R. H., & Grisham, C. M. (2005). *Biochemistry* (3rd ed.). Belmont, CA: Brooks Cole. pp. 205–243.

Izydorczyk, M. (2005). Understanding the chemistry of food carbohydrates. In S. W. Cui (Ed.), *Food Carbohydrates – Chemistry, Physical Properties, and Applications* (pp. 1–65). Boca Raton, FL: Taylor & Francis.

Jeffrey, G. A., & Yates, J. H. (1981). Application of *ab initio* molecular-orbital calculations to the structural moieties of carbohydrates. *Carbohydrate Research, 96*, 205–213.

Juaristi, E., & Cuevas, G. (1995) *The anomeric effect*. Boca Raton, FL: CRC Press.

Kaliannan, P., Vishveshwara, S., & Rao, V. S. R. (1986). Anomeric effect in carbohydrates–an ab initio study on extended model systems. *Proceedings of the Indian Academy of Science (Chemical Science), 96*, 327–339.

Lii, J.-H., Chen, K.-H., & Allinger, N. L. (2003). Alcohols, ethers, carbohydrates, and related compounds. IV. Carbohydrates. *Journal of Computational Chemistry, 24*, 1504–1513.

Lindberg, A. A., Brown, J. E., Strömberg, N., Westling-Ryd, M., Schulz, J. E., & Karlsson, K.-A. (1987). Identification of the carbohydrate receptor for Shiga toxin produced by *Shigella dysenteriae* Type 1. *Journal of Biological Chemistry, 262*, 1779–1785.

Mackie, W., & Perlin, A. S. (1966). Pyranose–furanose and anomeric equilibria Influence of solvent and of partial methylation. *Canadian Journal of Chemistry, 44*, 2039–2049.

Patching, S. G., Henderson, P. J. F., Herbert, R. B., & Middleton, D. A. (2008). Solid-state NMR spectroscopy detects interactions between tryptophan residues of the *E. coli* sugar transporter GalP and the α-anomer of the D-glucose substrate. *Journal of the American Chemical Society, 130*, 1236–1244.

Reeves, R. E. (1950). The shape of pyranoside rings. *Journal of the American Chemical Society, 72*, 1499–1506.

Reine, A., Hveding, J. A., Kjølberg, O., & Westbye, O. (1974). The mutarotation of D-glucose and its dependence on solvent. III. The existence of α- and β-D-glucofuranose in *N*,*N*-dimethylformamide. *Acta Chemica Scandinavica B, 28*, 690–691.

Rockey, W. M., Laederach, A., & Reilly, P. J. (2000). Automated docking of α-(1 → 4)- and α-(1 → 6)-linked glucosyl trisaccharides and maltopentaose into the soybean -amylase active site. *Proteins, 40*, 299–309.

Shallenberger, R. S. (1982). *Advanced Sugar Chemistry*. Westport, CT: AVI.

Stoddart, J. F. (1971). *Stereochemistry of Carbohydrates*. New York: Wiley-Interscience.

Striegel, A. M. (1997). Theory and applications of DMAc/LiCl in the analysis of polysaccharides. *Carbohydrate Polymers, 34*, 267–274.

Striegel, A. M. (2003a). Anomeric configuration, glycosidic linkage, and the solution conformational entropy of O-linked disaccharides. *Journal of the American Chemical Society, 125*, 4146–4148. (See erratum in Striegel, A. M. (2004) *Journal of the American Chemical Society, 126*, 4740).

Striegel, A. M. (2003b). Advances in the understanding of the dissolution mechanism of cellulose in DMAc/LiCl. *Journal of the Chilean Chemical Society, 48*, 73–77.

Striegel, A. M. (2004). Thermodynamic equilibrium of the solute distribution in size-exclusion chromatography. *Journal of Chromatography A, 1033*, 241–245.

Striegel, A. M. (Ed.). (2005a) *Multiple Detection in Size-Exclusion Chromatography*. ACS Symposium Series 893. Washington, DC: American Chemical Society.

Striegel, A. M. (2005b). Multiple detection in size-exclusion chromatography of macromolecules. *Analytical Chemistry, 77*, 104A–113A.

Striegel, A. M. (2008). Size-exclusion chromatography: Smaller, faster, multi-detection, and multi-dimensions. *Analytical & Bioanalytical Chemistry, 390*, 303–305.

Striegel, A. M., Piotrowiak, P., Boué, S. M., & Cole, R. B. (1999). Inductive effect and polarizability contributions to solvent-cation binding observed in electrospray ionization mass spectrometry. *Journal of the American Society for Mass Spectrometry, 10*, 254–260.

Striegel, A. M., & Timpa, J. D. (1995). Molecular characterization of polysaccharides dissolved in Me_2NAc-LiCl by gel-permeation chromatography. *Carbohydrate Research, 267*, 271–290.

Striegel, A. M., Yau, W. W., Kirkland, J. J., & Bly, D. D. (2009). *Modern size-exclusion liquid chromatography* (2nd ed.). Wiley: New York.

Tipson, R. S., & Isbell, H. S. (1962). Infrared absorption spectra in the study of mutarotational equilibria of monosaccharides. *Journal of Research of the National Bureau of Standards, 66A*, 31–58.

Woods, R. J., Fraser-Reid, B., Dwek, R. A., & Edge, C. J. (1994). Role of nonbonded interactions in determining solution conformations of oligosaccacharides. In D. A. Smith (Ed.), *Modeling the Hydrogen Bond*. (pp. 252–268). ACS Symposium Series 569. Washington, DC: American Chemical Society.

Yang, Q., Goldstein, I. J., Mei, H.-Y., & Engelke, D. R. (1998). DNA ligands that bind tightly and selectively to cellobiose. *Proceedings of the National Academy of Sciences USA, 95*, 5462–5467.

Zhu, Y., Zajicek, J., & Serianni, A. S. (2001). Acyclic forms of [1–^{13}C]aldohexoses in aqueous solution: Quantitation by ^{13}C NMR and deuterium isotope effects on tautomeric equilibria. *Journal of Organic Chemistry, 66*, 6244–6251.