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^{13}C Solid-state NMR chemical shift anisotropy analysis of the anomeric carbon in carbohydrates

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Abstract—^{13}C NMR solid-state structural analysis of the anomeric center in carbohydrates was performed on six monosaccharides: glucose (Glc), mannose (Man), galactose (Gal), galactosamine hydrochloride (GalN), glucosamine hydrochloride (GlcN), and *N*-acetyl-glucosamine (GlcNAc). In the 1D ^{13}C cross-polarization/magic-angle spinning (CP/MAS) spectrum, the anomeric center C-1 of these carbohydrates revealed two well resolved resonances shifted by 3–5 ppm, which were readily assigned to the anomeric α and β forms. From this experiment, we also extracted the ^{13}C chemical shift anisotropy (CSA) tensor elements of the two forms from their spinning sideband intensities, respectively. It was found out that the chemical shift tensor for the α anomer was more axially symmetrical than that of the β form. A strong linear correlation was obtained when the ratio of the axial asymmetry of the ^{13}C chemical shift tensors of the two anomeric forms was plotted in a semilogarithmic plot against the relative population of the two anomers. Finally, we applied REDOR spectroscopy to discern whether or not there were any differences in the sugar ring conformation between the anomers. Identical two-bond distances of 2.57 Å (2.48 Å) were deduced for both the α and β forms in GlcNAc (GlcN), suggesting that the two anomers have essentially identical sugar ring scaffolds in these sugars. In light of these REDOR distance measurements and the strong correlation observed between the ratio of the axial asymmetry parameters of the ^{13}C chemical shift tensors and the relative population between the two anomeric forms, we concluded that the anomeric effect arises principally from interaction of the electron charge clouds between the C-1–O-5 and the C-1–O-1 bonds in these monosaccharides.

Keywords: Anomeric effect; Solid-state NMR; Asymmetry in the electron cloud distribution; Chemical shift anisotropy

1. Introduction

The anomeric effect, the tendency of an electronegative substituent at the anomeric carbon to assume the axial rather than the equatorial conformation, has long been recognized as an important phenomenon in carbohydrates. The effect was first discovered by Edward[1] and formulated by Lemieux and Chü[2] about half a century ago [for a review, see Ref. 3 and references cited therein]. Over the years, the natural selectivity regarding O- and N-linked carbohydrates and their associated anomeric effects have attracted much attention. It has been argued that the increased stability of the axial polar groups at the anomeric carbon is due to the repulsive interaction between the ring dipole generated by the electron lone pairs and the nearly parallel polar bond in the equatorial conformer.[1] The detailed molecular structure and free-energy analyses of the equatorial and axial conformations of the monosaccharides are of interest to carbohydrate chemists since the monosaccharides serve as model systems for the more sophisticated carbohydrate molecules.

To address the origin of the anomeric effect further, we have appealed to high-resolution solid-state NMR spectroscopy. There are two advantages of solid-state NMR spectroscopy. First, solid-state NMR spectroscopy is capable of determining certain intrinsic

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anisotropic properties that can be directly correlated with the anomeric effect in carbohydrates. Secondly, unwanted solvent effects[4,5] can be ignored in the solid phase as demonstrated in this study.

Studies of six important monosaccharide compounds were carried out in this work: glucose (Glc), mannose (Man), galactose (Gal), galactosamine hydrochloride (GalN), glucosamine hydrochloride (GlcN), and *N*-acetyl-glucosamine (GlcNAc) with either ^{13}C-1 or ^{13}C-1/^{15}N isotope labeling. It was found that both the ^{13}C isotropic chemical shift and the axial asymmetry parameter in the chemical shift tensor are sensitive to the anomeric effect. The α and β anomers revealed two resonances in the ^{13}C NMR spectrum separated by 3–5 ppm. From the ^{13}C chemical shift tensors derived, we showed that the tensors for the α anomer are more anisotropic and axially symmetrical than those of the β form. In addition, a strong correlation was uncovered between the ratio of the axial asymmetry parameter of the ^{13}C chemical shift tensor (η_β/η_α) and the relative population of the two anomers (P_β/P_α). Finally, we applied REDOR spectroscopy[6,7] to compare the two-bond ^{15}N–{C-2}–^{13}C internuclear distance in the α and β forms for both GlcNAc and GlcN, in order to ascertain whether or not there exists any differences in the sugar ring conformation between the two anomers.

2. Results and discussion

2.1. ^{13}C CP/MAS intensity

It is reasonable to assume that the relative population of the α and β forms in monosaccharides is governed by different electronic distributions at the anomeric center associated with the two anomers.

In principle, it is possible to obtain the relative population of the two anomeric forms (P_β/P_α) in solids from ^{13}C cross-polarization[8]/magic-angle spinning[9] (CP/MAS) solid-state NMR experiments. The 1D ^{13}C spectra of GlcN, Glc, and Man are shown in Figure 1. The anomeric C-1 carbon exhibited two well distinct ^{13}C resonances at near 90 ppm with different intensities. These resonances are unambiguously assigned to the α and β forms, respectively, in accordance to their spectral positions in the solution phase.[10] Normally, the α intensity shifted is upfield shifted by 3–5 ppm and dominates the β intensity by a factor of 3–30, depending on the sugars.

However, two conditions must be met before the ^{13}C CP/MAS relative intensity is directly related to the population ratio P_β/P_α. First, it is essential that the CP efficiency be the same for the two anomers in the ^{13}C CP/MAS experiment. Second, the relative intensity should not be influenced by different CP/MAS experimental settings such as the CP contact time. To investigate the first

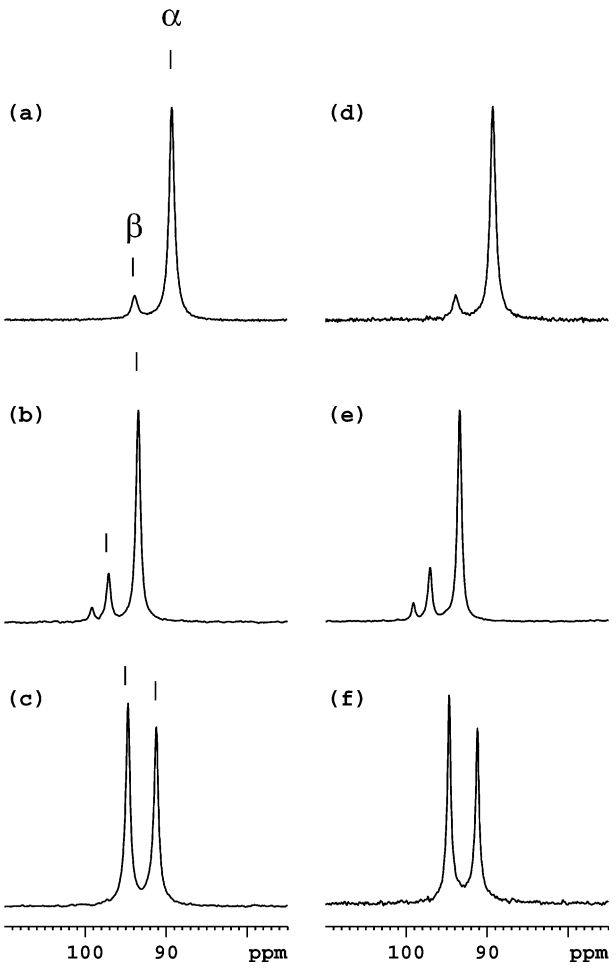


Figure 1. 1D CP/MAS ^{13}C NMR spectra of (a) GlcN, (b), Glc, and (c) Man, and one-pulse ^{13}C NMR spectra of (d) GlcN, (e), Glc, and (f) Man, respectively. As indicated in the text, the two nonequal resonances are readily assigned to the anomeric α and β forms. The dominant α form is shifted upfield by 3–5 ppm relative to the β form. The relative intensities observed between the two experiments are basically the same, suggesting that there is no magnetization-transfer bias between the two anomers. Samples were specifically ^{13}C- or ^{13}C/^{15}N-isotope labeled at the anomeric carbon and the amine (or amide) nitrogen. All spectra were acquired at room temperature and referenced to the glycine carboxyl carbon at 176.4 ppm.

issue, we conducted ^{13}C one-pulse experiments in parallel with the ^{13}C CP/MAS. The results are shown in Figure 1. Regardless of the long T_1 relaxation time, the ^{13}C spectra obtained from the one-pulse experiment were found to be identical to that of the CP/MAS experiment, implying that the CP intensities of the two anomers were equally affected. To check whether or not the observed intensities are CP contact time dependent, we monitored the ^{13}C CP/MAS intensities at different contact-times. Although the overall ^{13}C CP intensity of the α and β forms followed an 'exponential rise–exponential decay' behavior dominated by a combination of T_{CH} and $T_{1\rho}$ relaxation mechanisms,[20,21] the relative populations remained the same over a wide CP range of 0.5–20 ms

(data not shown). Thus, no MAS dependence was detected on the ^{13}C relative intensity between the α and β forms of these sugars. Based on these observations, we conclude that ^{13}C CP/MAS spectroscopy offers a reliable method to quantify the relative amounts of the anomeric α and β forms.

In this NMR study, a total of six monosaccharides were characterized by ^{13}C CP/MAS spectroscopy. The anomeric carbon resonances were simulated with two Lorentzian lines with the appropriate widths, frequencies, and intensities. The population ratios (P_β/P_α) of the anomeric α and β forms deduced from the curve-fitting analysis are given in Table 1, together with the fitting line widths. As indicated, these sugars revealed different combinations of the anomeric α and β form populations. The heterogeneity of the crystalline phase also varied from sample to sample, as indicated by the fitting line widths from 50 to 100 Hz. This inhomogeneous broadening mainly arises from molecular packing and does not affect the overall population ratios.

2.2. Chemical shift tensor

When the MAS speed was set relatively low, i.e., below the size of the chemical shift anisotropy (CSA) of the anomeric center, the 1D ^{13}C CP/MAS spectrum was flanked by a number of spinning sidebands,[11] as demonstrated in Figure 2. Following Herzfeld and Berger,[12] we extracted the chemical shift anisotropy (CSA) tensor elements from the MAS spinning sideband intensities. Since the MAS spinning sideband intensities were modulated by the sample spinning speed, for higher accuracy, we minimized the uncertainty by iteratively checking the theoretical values with the experimental data acquired at various speeds. The final chemical shift tensor elements were determined from the average of a number of measurements with standard deviation of ±0.5 ppm. The results are summarized in Table 1 for the sugars examined in this work.

It was found that the ^{13}C chemical shift tensors for the α anomer are more axially symmetrical; that is, the axial asymmetry parameter η ($= (\sigma_{22} - \sigma_{11})/(\sigma_{33} - \sigma_{iso})$) values are relatively smaller. Also all the tensor values of the α anomers are shifted upfield relative to those of the β anomers, in agreement with the analysis of a set of methyl glycoside samples in a single crystal study reported earlier by Liu et al.[13] Note that the ^{13}C chemical shift tensor elements of the β anomer in Gal and GalN were difficult to determine with reasonable accuracy because their intensities were extremely low. Assuming that the deviation of the chemical shift tensor elements from the corresponding isotropic chemical shift between the α and β forms remain the same as that in the case of methyl galactopyranoside,[13] one can estimate the chemical shift tensor elements of the β anomer from the α form for these sugars by simple calculation. These estimates are also included in Table 1. Interestingly, these predicted results nicely fitted into the overall trend

Table 1. Summary of ^{13}C chemical shifts, chemical shift tensor elements, dipolar strengths, and free-energy differences for the anomeric α and β forms in Glc, Man, Gal, GalN, GlcN, and GlcNAc[a]

	Glc		Man		Gal		GalN		GlcN		GlcNAc	
	α	β	α	β	α	β	α	β	α	β	α	β
Relative population %[b]	82 (48)	18 (48)	48 (51)	52 (48)	96 (48)	4 (33)	98 (44)	2 (49)	90 (58)	10 (76)	80 (73)	20 (98)
σ_{iso}[c]	93.4	97.0	91.2	94.7	92.4	96.7	89.7	95.1	89.3	93.9	93.0	96.8
σ_{33}	66.9	70.2	67.1	74.7	65.8	71.3[d]	61.4	68.0[d]	62.4	68.8	67.0	73.7
σ_{22}	97.3	99.2	98.7	99.8	95.2	96.0[d]	96.4	99.3[d]	92.0	95.8	97.7	99.8
σ_{11}	115.9	121.6	107.9	110.0	116.2	115.8[d]	111.4	111.7[d]	113.4	117.1	114.3	117.0
$-(\sigma_{33} - \sigma_{iso})$	26.5	26.8	24.1	20.1	26.6	25.4	28.3	27.1	26.9	25.1	26.0	23.1
η[e]	0.70	0.84	0.38	0.51	0.79	0.78	0.53	0.46	0.80	0.86	0.64	0.74
$\Delta\sigma$[f]	−39.7	−40.2	−36.2	−30.2	−39.9	−34.6	−42.5	−37.5	−40.7	−37.7	−39.0	−34.6
D (Hz)[g]							−200		−200	−200	−180	−180
r (Å)[h]							2.48		2.48	2.48	2.57	2.57
ΔE (kcal/mol)	0.90		−0.05		1.88		2. 30		1.30		0.82	

[a] Samples were specifically ^{13}C- and/or ^{15}N-labeled. For details, see Experimental. Chemical shift tensor elements were the average of measurements at various MAS speeds with standard deviation of ±0.5 ppm.

[b] Data was deduced from Lorentzian curve fitting with an uncertainty of ±3%; the half-width at half-height of the fitting curve are given in bracket in unit of Hz.

[c] Chemical shifts were referenced to the carbonyl carbon of glycine at 176.4 ppm.

[d] Data were calculated from that of the α form assuming that the deviation of chemical shift tensor elements from the isotropic chemical shift for the α and β form were the same as that for methyl α- and β-D-galactopyranoside.[13]

[e] Chemical shift tensor axial asymmetry $\eta = (\sigma_{22} - \sigma_{11})/(\sigma_{33} - \sigma_{iso})$.

[f] Chemical shift anisotropy $\Delta\sigma = \sigma_{33} - (\sigma_{11} + \sigma_{22})/2$.

[g] Dipolar strength D was determined from REDOR spectroscopy using the curve-fitting simulation algorithm[7] with an uncertainty of less than 5%.

[h] r is the internuclear distance for ^{13}C-1–{C-2}–^{15}N derived from dipolar strength D.

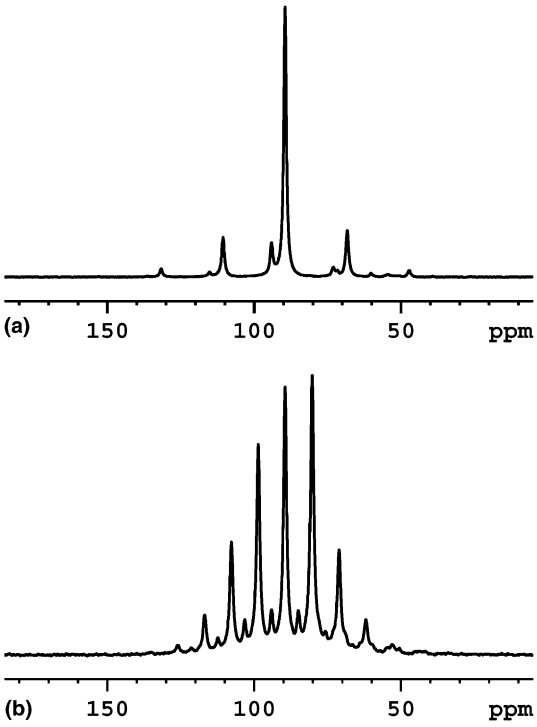
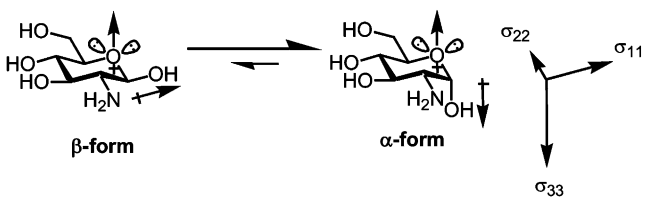


Figure 2. 1D CP/MAS ^{13}C spectra of ^{13}C-1-labeled GlcN acquired at MAS of (a) 1590 Hz, and (b) 690 Hz, respectively. The isotropic chemical shift resonance was flanked by a number of MAS spinning sideband intensities that were used to extract the ^{13}C chemical shift tensor elements for the α and β forms. The average of the chemical shift tensor values acquired at different MAS speeds was taken and summarized in Table 1. MAS speed was optimized in order to prevent any sideband overlapping.

depicted by the experimental data determined for the other monosaccharides: Glc, Man, GlcN, and GlcN (see Fig. 5). This linear correlation between η_β/η_α and P_β/P_α will be discussed in detail below.

Knowing that the chemical shift tensor orientations can be directly referred to the molecular structure in the single crystal, one can correlate the three principal tensor elements with the molecular orientation: the direction of σ_{33} approximately coincides with the C-1–O-1 bond; that of σ_{22} is close to the C-1–O-5 bond; and the direction of σ_{11} is perpendicular to the O-5–C-1–O-1 plane in the α configuration, as depicted in Scheme 1.[14] These orientation-dependent chemical shift tensor elements should provide a reliable determinant of the distribution of the electron cloud in the vicinity of the anomeric carbon center.



Scheme 1.

2.3. Solvent effect

It was pointed out by the reviewers that the relative ^{13}C intensity and the chemical shift tensor elements observed for a given sugar might depend on the experimental conditions employed in the crystallization procedures, specifically, the solvent and temperature. In an attempt to clarify this point, we dissolved ^{13}C singly labeled Glc into three different solvents, namely, water, ethanol, and *N*,*N*-dimethylformamide (DMF), respectively, and then recrystallized the Glc for solid-state NMR measurements. The details of the recrystallization procedures are described in the Experimental. Supposedly, if there is any solvent effect, one might expect to discern variations in the α and β anomeric population ratio, and possibly, dramatic changes in the ^{13}C CP/MAS spectra as suggested by solution NMR data (data not shown). For comparison, the ^{13}C spectra of the 'untreated' and recrystallized samples are shown in Figure 3. Interestingly, these ^{13}C CP/MAS spectra revealed very similar isotropic chemical shifts and almost identical CSA spinning sideband patterns. The curve-fitting simulation analysis also revealed at most a 2–4% variation in the relative population of the anomers for these samples. The same recrystallization procedures were applied to another monosaccharide GlcN, and again, the curve-fitting data of the resulting ^{13}C CP/MAS spectra revealed no significant change in the relative anomeric population (data not shown). Thus, no solvent effect was observed on the NMR in the solid state.

2.4. REDOR spectroscopy

Structurally, the α and β forms could differ slightly in their sugar ring scaffolds. To evaluate this possibility, we implemented REDOR spectroscopy,[6,7] a tool that has been successfully applied in molecular structural determinations,[15–17] to probe the two-bond internuclear distance C-1–{C-2}–N. Supposedly, a deformation in the sugar ring could induce small variations in the two-bond distances around the sugar ring. The REDOR spectroscopy exploits the dipolar interaction, which is inversely proportional to the cube of the internuclear distance, to afford accurate distance measurements. As a control, we carried out a similar internuclear distance measurement with the same technique on the one-bond ^{13}C-1/^{15}N doubly labeled glycine standard compound. An accuracy of ±0.05 Å was deduced from the fitting analysis using the dipolar dephasing simulation algorithm described elsewhere.[7] Given the high degree of accuracy, REDOR spectroscopy could be used to determine internuclear distance in the carbohydrate compounds of interest here.

Three amino sugars with specific ^{13}C-1–{C-2}–^{15}N isotope labeling were chosen for measurement of the two-bond distance: GalN, GlcN, and GlcNAc. REDOR

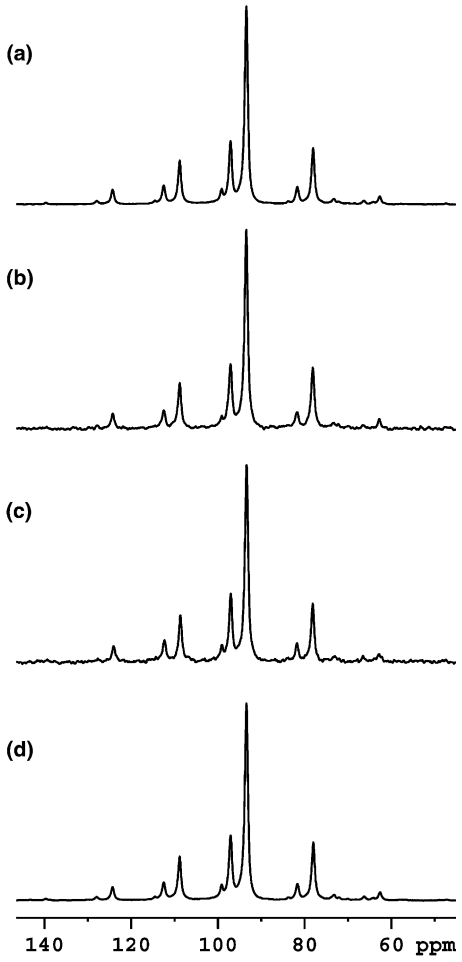


Figure 3. 1D CP/MAS ^{13}C spectra of untreated ^{13}C-1-labeled Glc (a), and the same samples after recrystallization from different solvents, water (b), ethanol (c), and DMF (d), respectively. For comparison, the ^{13}C spectra have been rescaled to the same intensity height. Note that the isotropic chemical shifts and anisotropic chemical shift spinning sidebands of the anomeric α and β forms showed no discernible difference, indicating that solvent effects are negligible. Spectra were acquired at a MAS speed of 1160 Hz.

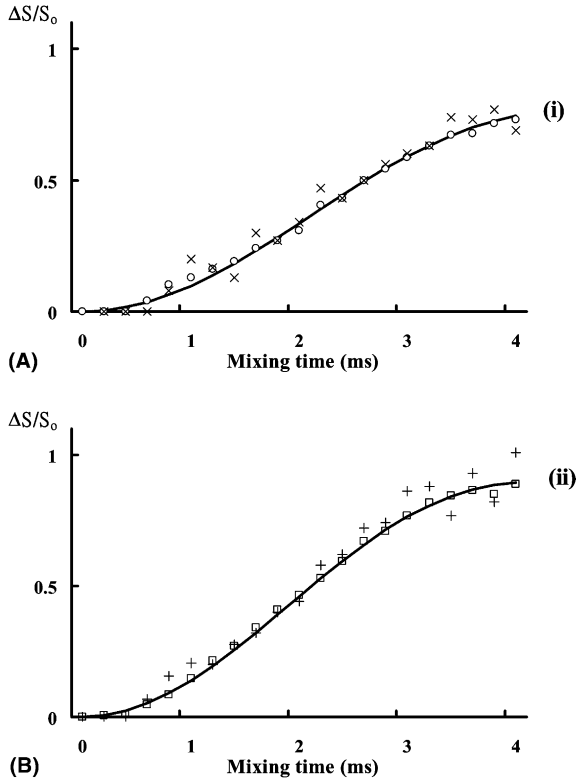


Figure 4. ^{13}C–^{15}N distance determinations for the anomeric α form and β form in (A) GlcNAc and (B) GlcN carbohydrate compounds as probed by REDOR spectroscopy. REDOR dipolar dephasing intensity ratio $\Delta S/S_o$ versus the mixing time for the α form (○) and for the β form (×) in GlcNAc and the α form (□) and for the β form (+) in GlcN. Solid lines (i) and (ii) represent best-fit curves with the following parameters: (i) dipolar strength of −180 Hz with a scaling factor of 0.74; (ii) dipolar strength of −200 Hz with a scaling factor of 0.86. Internuclear distances of 2.57 Å and 2.48 Å ± 0.08 Å were deduced from the best fits of the data for both the α and β forms in GlcNAc and GlcN, respectively.

distance analyses and the corresponding ^{13}C-1/^{15}N dipolar dephasing curves are given in Figure 4. Internuclear distances of 2.48 Å (200 Hz), 2.48 Å (200 Hz), and 2.57 Å (180 Hz) were extracted from the best fits of the dephasing curves for both the α and β forms in GalN, GlcN, and GlcNAc, respectively (see Table 1), within the uncertainty of ±0.08 Å. The results obtained indicated that the sugar ring conformations of two anomers are identical.

Computer molecular simulations confirmed these REDOR results. Energy minimization calculations showed that the sugar ring prefers a most probable *chair* conformation without any substantial difference between the α and β anomers (data not shown). Based on these REDOR measurements and energy calculations, we conclude that there is no difference in the sugar ring scaffold between the α and β anomers in any of these sugars. On the other hand, a ~0.1 Å difference in the C-1–{C-1}–N distance was observed between GlcNAc and GlcN. This result would suggest a small difference in the ring conformation between these two sugars. This is a significant result, as it demonstrates that the REDOR experiment is capable of discriminating subtle differences between ring conformations in sugars.

2.5. Correlation between η_β/η_α and P_β/P_α

As noted earlier, we have obtained a strong correlation between η_β/η_α and P_β/P_α for the anomeric carbon (C-1) for the various sugars examined in this study. In the absence of any difference in the sugar scaffold between the two anomers, the free-energy difference between the two configurations (ΔG) should reflect differences in the steric/electronic effects related to differences in the electronic distributions between the two anomers. The dimensionless axial asymmetry parameter $\eta = (\sigma_{22} - \sigma_{11})/(\sigma_{33} - \sigma_{iso})$ should provide a measure of the

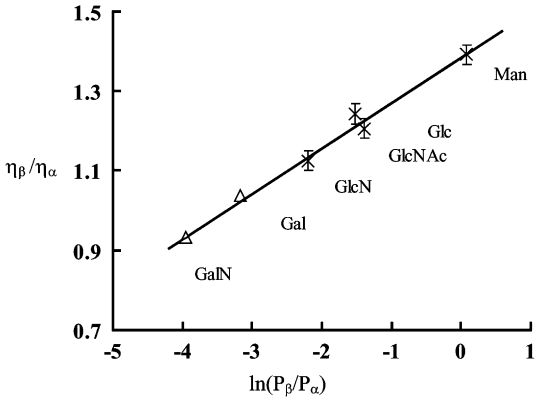


Figure 5. Correlation between the axial asymmetry η of the ^{13}C chemical shift tensor for the anomeric carbon and the anomeric population ratio P_β/P_α for the various sugars studies in this work. The experimental data (×) for η_β/η_α and P_β/P_α for Glc, Man, GlcN, and GlcNAc are taken from Table 1. In the cases of Gal and GalN, the ^{13}C CSA for the β anomer were calculated from that of the methyl α- and β-D-galactopyranoside[13] (see text for details). Consistent with the other sugars, these data for Gal and GalN (△) followed the same linear relationship represented by the solid line.

asymmetry of the electron density about the anomeric center. In light of this, a direct correlation might be expected between η_β/η_α and P_β/P_α the ratio of the axial asymmetry parameter η of the ^{13}C chemical shift tensor and the population ratio of the two anomers. As shown in Figure 5, a linear correlation is, indeed, obtained when the ratio η_β/η_α is plotted against the logarithm of P_β/P_α. That there should be such a strong correlation between these two quantities, one microscopic and the other macroscopic, argues for the same electronic factors affecting both the axial asymmetry parameter of the chemical shift tensor for the anomeric carbon as well as the free-energy difference between the α and β anomers.

2.6. Free-energy difference

For most of the sugars that we have studied here, the α anomer is the predominant form. Accordingly, $P_\beta/P_\alpha = \exp(-\Delta G/k_BT) \approx \exp(-\Delta H/k_BT) \approx \exp(-\Delta E/k_BT)$, assuming that ΔS and $\Delta V \approx 0$ between the two anomeric forms, so that $\Delta E \approx -k_BT\ln(P_\beta/P_\alpha)$. At the same time, $\eta_\beta/\eta_\alpha = [(\sigma_{22}-\sigma_{11})_\beta/(\sigma_{22}-\sigma_{11})_\alpha]\cdot[(\sigma_{33}-\sigma_{iso})_\alpha/(\sigma_{33}-\sigma_{iso})_\beta] \approx (\sigma_{22}-\sigma_{11})_\beta/(\sigma_{22}-\sigma_{11})_\alpha$. The free-energy difference that quantifies the anomeric effect is given in Table 1. The linear correlation depicted in Figure 5 suggests that ΔE is proportional to η_β/η_α, for Glc, GlcN, GlcNAc, and Man, the carbohydrates that we have studied. Since σ_{22} is the tensor component when the magnetic field is directed along the C-1–O-5 bond, and σ_{11} the tensor component when the magnetic field is directed perpendicular to the O-5–C-1–O-1 plane, $(\sigma_{22}-\sigma_{11})$ is related to the electron distribution associated with the O-5–C-1 and the C-1–O-1 bonds, including the lone pairs on the O-1 oxygen. Accordingly, the quantity $(\sigma_{22}-\sigma_{11})_\beta/(\sigma_{22}-\sigma_{11})_\alpha$ should provide some measure of the difference in the interaction of these bond electron densities when the sugar is in the β and α anomers, respectively. In light of the excellent correlation depicted between η_β/η_α and the logarithm of the population ratio P_β/P_α or $-\Delta E/k_BT$, the anomeric effect is clearly electronic in origin, related to electron repulsion between the electron distribution associated with the O-5–C-1 and the C-1–O-1 bonds when the sugar is in the β anomeric state versus the corresponding interaction in the α anomeric state.

3. Summary

We have presented the first detailed structural analysis of the anomeric effect in carbohydrate by ^{13}C NMR solid-state spectroscopy. Specifically, the orientation-dependent CSA tensor has been utilized to characterize the anomeric effect in six representative monosaccharides: Glc, Man, Gal, GalN, GlcN, and GlcNAc. It was found that, in every case, all tensor values of the α anomer were shifted upfield compared to those of the β anomer, and the CSA tensors are more axially symmetrical for the α relative to the β form. In addition, we were able to relate the free-energy difference between the α and β anomers to the degree of axial asymmetry of the chemical shift tensor in the plane containing the O-5–C-1–O-1 bonds, and showed that the anomeric effect is electronic in origin.

Thus, we have demonstrated the capability of solid-state NMR spectroscopy to characterize the anomeric effect in monosaccharides. We believe that these solid-state NMR approaches are applicable to similar structural analysis of more complex carbohydrate systems, including galactosylceramides, glycopeptides, and other biologically related amino sugars.

4. Experimental

4.1. Sample

Six ^{13}C- or $^{13}C/^{15}N$-isotopically labeled carbohydrates were used in this study. ^{13}C-1(99%)- and ^{15}N(98%)-double-labeled GlcN (D-glucosamine hydrochloride) was purchased from Isotech, Inc. (Miamisburg, OH). ^{13}C(99%)- and ^{15}N(98%)-double-labeled GalN (D-galactosamine hydrochloride), Man (D-Mannose), and Gal (D-galactose) with ^{13}C-1(99%)-labeled were purchased from Omicron, Biochemicals, Inc. (South Bend, IN). ^{13}C-1(99%)-labeled Glc (D-glucose) was from CIL (Andover, MA). These samples were used without further purification. A sample of 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine, abbreviated as GlcNAc)

was prepared via modification of the GlcN following the procedures reported elsewhere.[18] These isotopically labeled samples were mixed with unlabeled molecules (natural abundance) at a weight ratio of 1:1.

4.2. Recrystallization

^{13}C-labeled glucose (500 mg each) was dissolved into three different solvents; water, ethanol, and *N*,*N*-dimethylformamide (DMF) (2 mL each), respectively, and heated to reflux. The resulting solutions were refrigerated (−20 °C) overnight. Recrystallized white-powdered solid samples were obtained after filtering and drying under vacuum.

4.3. NMR spectroscopy

In the CP/MAS experiments, ^{1}H TPPM decoupling[19] was applied with rf amplitudes of 62.5 kHz during t_1 period. In the Hartmann–Hahn[8] polarization transfer, the contact time was 1–5 ms. The REDOR experiments were performed using triple resonance probe (^{1}H/^{13}C/^{15}N) at ambient temperature. ^{1}H 90° pulse lengths of 3.5–4.7 μs were used, with contact time of 1 ms and recycle delay of 5–6 s. In the triple resonance REDOR experiments, B_1 field strengths of 55, 40, and 62.5 kHz in ^{13}C (observed), ^{15}N (dipolar recoupling), and ^{1}H (decoupling) were used, respectively. In the ^{13}C one-pulse experiment, the ^{13}C 90° pulse length was 4.2 μs, and a long recycle delay of 600 s was used to allow for the long T_1 relaxation in the ^{13}C. During data acquisition, ^{1}H cw decoupling was applied with B_1 field strengths of 58 kHz. All NMR spectra were obtained on a Bruker Avance 300 MHz NMR spectrometer, operating at a proton/carbon frequency of 300.13/75.47 MHz at ambient temperature. Powdered samples were packed in double-bearing 4-mm zirconium oxide MAS rotors for all the solid-state measurements.

4.4. Spectral simulation of the ^{13}C resonances

The ^{13}C resonances of the anomeric center were simulated by varying the intensity and the line width of Lorentizan lineshapes in order to extract the relative population. Simulation was performed on SGI workstation using 'xedplot' module under XwinNMR software program provided by Bruker Inc. An uncertainty of less than 4% was estimated in the curve-fitting analysis.

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