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O-Acetyl Side-Chains in Monosaccharides: Redundant NMR Spin-Couplings and Statistical Models for Acetate Ester Conformational Analysis

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Abstract

α - and β -D-glucopyranose monoacetates **1–3** were prepared with selective ^{13}C enrichment in the O-acetyl side-chain, and ensembles of ^{13}C – ^1H and ^{13}C – ^{13}C NMR spin-couplings (J -couplings) were measured involving the labeled carbons. Density functional theory (DFT) was applied to a set of model structures to determine which J -couplings are sensitive to rotation of the ester bond θ . Eight J -couplings ($^1J_{\text{CC}}$, $^2J_{\text{CH}}$, $^2J_{\text{CC}}$, $^3J_{\text{CH}}$, and $^3J_{\text{CC}}$) were found to be sensitive to θ , and four equations were parametrized to allow quantitative interpretations of experimental J -values. Inspection of J -coupling ensembles in **1–3** showed that O-acetyl side-chain conformation depends on molecular context, with flanking groups playing a dominant role in determining the properties of θ in solution. To quantify these effects, ensembles of J -couplings containing four values were

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Notes

The authors declare no competing financial interest.

Supporting Information

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Torsional constraints used in DFT calculations of **5–9**; NMR J -couplings calculated by DFT in **5–9**; definitions of θ rotamers in **1–9**; plots showing correlations between $^2J_{\text{C1}'\text{CX}}$ and C1'–O_X–C_X bond angle in **5–8** ($x = 2$ or 3); plot of calculated $^3J_{\text{C1}'\text{H6R}}$ and $^3J_{\text{C1}'\text{H6S}}$ in **9** after shifting the latter curve shown in Figure 5 by 120° ; plots of calculated $^3J_{\text{C1}'\text{C}_y}$ as a function of θ_x in **5–9**, where $x = 2, 3$, or 6 and $y = x \pm 1$; parameter space for uniform models of **1**, **2a**, and **2b**; partial $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (150 MHz) of $1^{2'}\alpha$ and $1^{2'}\beta$; partial $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (150 MHz) showing signals arising from C3 of $1^{1'}\alpha$ and $1^{1'}\beta$, and C2 of **2b**; partial $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (150 MHz) showing signals arising from C2 of $1^{1'}\beta$, C2 of **2a**, C3 of **2b**, and C5 of **3b**; partial $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (150 MHz) showing signals arising from C3 of **2a**, C2 of $1^{1'}\alpha$, C5 of **3a**, and C4 of $1^{1'}\alpha/\beta$; partial ^1H NMR spectrum (600 MHz) showing signals arising from H3 of $1^{1'}\alpha$ and $1^{1'}\beta$; partial ^1H NMR spectrum (600 MHz) showing signals arising from H6 and H6' of **3a** and **3b**; partial ^1H NMR spectrum (600 MHz) showing signals arising from H2 of **2a** and **2b**; Cartesian coordinates for B3LYP optimized conformers of **5**; complete ref 16; and complete ref 32 (PDF)

used to determine the precision and accuracy of several 2-parameter statistical models of rotamer distributions across θ in **1–3**. The statistical method used to generate these models has been encoded in a newly developed program, MA'AT, which is available for public use. These models were compared to *O*-acetyl side-chain behavior observed in a representative sample of crystal structures, and in molecular dynamics (MD) simulations of *O*-acetylated model structures. While the functional form of the model had little effect on the precision of the calculated mean of θ in **1–3**, platykurtic models were found to give more precise estimates of the width of the distribution about the mean (expressed as circular standard deviations). Validation of these 2-parameter models to interpret ensembles of redundant *J*-couplings using the *O*-acetyl system as a test case enables future extension of the approach to other flexible elements in saccharides, such as glycosidic linkage conformation.

Graphical Abstract

INTRODUCTION

Carbohydrates are critical to many biological processes, such as cell–cell recognition, bacterial and viral infection, immunity, and energy metabolism.¹ The biological functions of saccharides correlate with their structures and/or their conformational flexibilities (time-dependent motions).² The latter flexibility is associated with inherent dynamical properties such as furanosyl and pyranosyl ring pseudorotation,³ side-chain conformational dynamics (e.g., hydroxymethyl⁴), and the motions of *O*-glycosidic linkages.⁵ These motions can be interrogated by NMR and other experimental methods, and computationally by molecular dynamics (MD) and related simulations.²

The multiple hydroxyl (or amino) substituents present in saccharides are often modified in biological systems to give, for example, phosphoester, sulfoester, *O*-lactoyl ether, *N*-formyl (amide), *N*-acetyl (amide), and *O*-acetyl (ester) substituents.⁶ These substitutions alter the physical properties, and thus biological functions, of saccharides by introducing net charge, new hydrogen bonding opportunities, and/or hydrophobic fragments that promote recognition and binding to cell receptors. Enzymes can regulate the presence or absence of these modifications to tune saccharide biological properties to a particular metabolic state.⁷ The opportunity for multiple substitutions on the same furanosyl or pyranosyl ring (clustering effect) leads to potential synergies in the conformations of side-chains, that is, side-chain conformations that are context dependent.

While significant efforts to characterize hydroxymethyl and *N*-acetyl side-chain behaviors in saccharides have been made,^{8–10} less attention has been paid to *O*-acetyl side-chains, such as those installed *in vivo* in the biologically important monosaccharides, *N*-acetylneuraminic acid and *N*-acetyl-D-muramic acid.^{6,11} In these systems, the presence or absence of an *O*-acetyl substituent regulates cellular migration and apoptosis, and mediates bacterial lysozyme resistance. Specific patterns of *O*-acetylation in the residues of oligosaccharides also appear to be required to elicit an antigenic response.^{12,13}

The conformations of *O*-acetyl side-chains appended to aldopyranosyl rings are, to a significant extent, determined by the chemical context in which they are found.¹⁴ In simple systems, two core structural factors pertain: (1) *O*-acetyl side-chain orientation (axial vs equatorial); and (2) the chemical identity and relative orientation of the flanking ring substituents. This situation is illustrated in Scheme 1 for an aldohexopyranosyl ring bearing an equatorial *O*-acetyl side-chain at C3. Structure **I** contains no flanking substituents. Structures **IIa/IIb** and **IIIa/IIIb** contain an OH group at one of the flanking positions, thus presenting two unique combinations of interactions with the intervening *O*-acetyl function. Structures **IV**, **V**, and **VIa/VIb** contain an OH group at both flanking positions, thus presenting three unique combinations of interactions with the intervening *O*-acetyl function. In this simple system, five unique combinations occur, leading to the possibility of five different conformational behaviors of the *O*-acetyl side-chain. A similar set of interactions pertains if the *O*-acetyl side-chain is axial. The problem is further complicated if the structures of the flanking substituents differ, and/or if they bear functionality that can interact noncovalently with the *O*-acetyl side-chain, thus influencing its conformation. Experimental methods sufficiently robust to distinguish between these different conformational scenarios are currently unavailable.

This work aims to improve current treatments of *O*-acetyl side-chain conformation in saccharides through the use of NMR *J*-coupling analysis assisted by density functional theory (DFT) calculations. J_{CH} and J_{CC} values were measured in *O*-acetyl side-chains that were ¹³C-labeled at either C1' (CO) or C2' (CH₃) in three D-glucopyranose monoacetates: 3-*O*-acetyl-D-glucopyranoses **1a/1b**, 2-*O*-acetyl-D-glucopyranoses **2a/2b**, and 6-*O*-acetyl-D-glucopyranoses **3a/3b** (Scheme 2). Using appropriate model structures, DFT was used to establish the dependencies of these *J*-couplings on *O*-acetyl side-chain conformation, and rotamer distributions about the acetate ester bond were described using several 2-parameter models. Each model yielded a mean position of the torsional distribution, and a width of this distribution, the latter describing the degree of torsional disorder. While previous studies have employed similar models to account for *O*-acetyl conformational disorder, neither the precision nor accuracy of these models have been compared to those derived from 1-parameter models that ignore disorder.¹⁵ The different steric environments of the *O*-acetyl side-chains in **1–3** were chosen with the expectation that they will influence calculated mean positions and degree of disorder in a mostly predictable manner, thus providing a test of the validity/necessity of the 2-parameter models. The experimental models were tested through comparisons to rotamer distributions determined from molecular dynamics simulations and from a large sampling of saccharide crystal structures. We show that conformational models derived exclusively from experimental constraints are comparable to those derived via MD

simulations and X-ray statistical analyses, providing validation of the redundant J -coupling approach to characterize conformationally flexible elements in saccharides.

EXPERIMENTAL SECTION

Synthesis of ^{13}C -Labeled Compounds 1–3

Chemicals—All chemicals except ^{13}C -labeled acetyl chloride (CIL) were purchased from Sigma-Aldrich Chemical Co. and used without further purification.

Acetylation of 1,2;5,6-Di-*O*-Isopropylidene- α -D-glucofuranose with Acetyl Chloride—To a cold solution of 1,2;5,6-di-*O*-isopropylidene- α -D-glucofuranose (6.6 g, 25.4 mmol) in 40 mL of anhydrous pyridine was added [1- ^{13}C]- or [2- ^{13}C]acetyl chloride (1.0 g, 12.6 mmol, 0.5 equiv). After standing at room temperature overnight, TLC (silica gel; 1:1 ethyl acetate:hexane) indicated the reaction was complete. The solution was concentrated to a minimum volume at 30 °C *in vacuo*, diluted with 100 mL of CH_2Cl_2 , and sequentially extracted with 1N aqueous HCl, sat. aqueous NaHCO_3 , and distilled water. The organic phase was dried over Na_2SO_4 and concentrated at 30 °C *in vacuo* to a minimum volume. The resulting solution was applied on a silica gel column and eluted with a hexane and ethyl acetate mixture (2:1) to give pure 3-*O*-[^{13}C]acetyl-1,2;5,6-di-*O*-isopropylidene- α -D-glucofuranose **4** (3.8 g, 12.5 mmol; quantitatively based on labeled acetyl chloride).

Deacetonation of 3-*O*-[^{13}C]Acetyl-1,2;5,6-di-*O*-isopropylidene- α -D-glucofuranose **4**—The purified **4** (3.8 g, 12.5 mmol) was hydrolyzed at 40 °C in a methanol–water solvent (3:1) containing Dowex HCR H^+ resin (~10 g) until TLC showed the complete disappearance of starting material. The mixture was then filtered to remove the resin, and the filtrate concentrated at 30 °C *in vacuo* to dryness. The solid was dissolved in a minimum volume of ethyl acetate (~5 mL) and the solution applied to a silica gel column (2.5 cm $\times$ 60 cm). Elution with ethyl acetate afforded a mixture of monoacetylated D-glucoses (generated by acyl group migration during deacetonation). The major components of this mixture, identified by ^1H and ^{13}C NMR, were 3-*O*-acetyl-D-glucose **1** (~24%), 2-*O*-acetyl-D-glucose **2** (~20%), and 6-*O*-acetyl-D-glucose **3** (~55%). This mixture was used without further purification to measure J_{CH} and J_{CC} values.

NMR Spectroscopy— ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were recorded on a 600-MHz spectrometer in D_2O solvent at 25 °C (5 mm tubes) and with digital resolutions of <0.05 Hz/point. $^1J_{\text{CH}}$ and J_{CC} values were measured directly from signal multiplicities observed in 1D ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra, respectively, of ^{13}C -labeled **1–3**. In some cases, resolution enhancement was applied to the raw FIDs prior to Fourier transformation to resolve small J -couplings (< ~0.7 Hz). Reported J -couplings are accurate to ± 0.1 Hz unless otherwise stated.

Theoretical Calculations

Geometry Optimization—Five model structures **5–9** (Scheme 3) that contain *O*-acetyl side-chains appended to C2, C3, and C6 of an aldohexopyranosyl ring were chosen for DFT calculations. Torsional constraints applied in these structures during the calculations are

shown in Scheme S1 (Supporting Information). In the 3- and 6-*O*-acetyl model structures (**5**, **6**, **9**), only one anomeric configuration (β) was studied, since configuration at C1 is not expected to influence *O*-acetyl behavior in these structures. Both anomers were studied in the 2-*O*-acetyl structures (**7**, **8**). In each model structure, the $H_X-C_X-O_X-C1'$ torsion angle in the *O*-acetyl side-chain, denoted θ_x where x denotes the ring carbon bearing the *O*-acetyl side-chain, was rotated in 15° increments through 360° , giving 24 optimized structures. In **9**, H6*R* (Scheme 4) was used as the reference atom to define θ_6 . Geometry optimizations were conducted in *Gaussian09*¹⁶ using the B3LYP functional¹⁷ and the 6-31G* basis set.¹⁸ Implicit solvation was present in all calculations using the self-consistent reaction field (SCRF) and integral equation polarizable continuum model (IEFPCM)^{19,20} parametrized for aqueous solution.

NMR Spin-Coupling Constant Calculations—The B3LYP functional¹⁷ and an extended basis set ($[5s2p1d|3s1p]$)^{8,21} were used in all *J*-coupling calculations as reported previously.^{8,9} Implicit solvation was applied in all calculations using the self-consistent reaction field (SCRF) and integral equation polarizable continuum model (IEFPCM).^{19,20} Reported *J*-couplings are unscaled, and include both Fermi and non-Fermi contact terms.

Parameterization of Spin-Coupling Equations—Equations relating the calculated *J*-couplings to θ_x were parametrized using the Fit function in *Mathematica*.²² The goodness-of-fit of each equation is reported as a root-mean-squared (RMS) deviation. When applicable, multiple data sets were parametrized as a single generalized equation.

Conformational Modeling of θ_x in 1–3—Conformational models describing rotation about θ_x in **1–3** were calculated by employing several different 2-parameter continuous, circular probability distributions in the circular package of R.^{23,24} These distributions included the von Mises, wrapped Cauchy, uniform, raised cosine, and Cartwright's power of cosine distributions.^{25,26} Monte Carlo (MC) methods were used to find the

$$J_{\text{pred}} = \int_0^{2\pi} p(\theta) J(\theta) d\theta \quad (1)$$

parameters that minimized the RMS deviation between the experimental and predicted *J*-couplings. Least-squares methods were used to ensure the model reached the global minimum of the parameter space. Each pair of parameters generated during the MC step was saved so that the parameter space of each model could be interpolated and graphed using the SciPy and Matplotlib packages in *Python*.²⁷ Predicted *J*-couplings in each model were calculated using eq 1, where $J(\theta)$ is the calculated *J*-coupling at a given value of θ_x obtained from equations derived from DFT calculations, J_{pred} is the predicted *J*-coupling, and $p(\theta)$ is the probability of a given rotamer about θ_x .²⁸

Mean positions of θ_x and circular standard deviations (CSDs) were calculated for **1a/β**, **2a/β**, and **3a/β**. Standard uncertainties in the model parameters were determined via numerically estimating the covariance matrix.²⁹ The R code used to produce these models,

hereafter referred to as MA'AT, can be found at the following website (<http://www3.nd.edu/~serlab03/DATA.html>).

Molecular Dynamics (MD) Simulations—Model structures **10–13** (Scheme 5) were built using the carbohydrate builder module at the GLYCAM website (<http://www.glycam.org>). The GLYCAM06³⁰ (version j) force field was employed for all simulations. Structures were solvated with TIP3P³¹ water using a 12 Å buffer in a cubic box, using the LEaP module in the AMBER14 software package.³² Energy minimizations were performed separately under constant volume (500 steps steepest descent, followed by 24500 steps of conjugate-gradient minimization). Each system was subsequently heated to 300 K over a period of 50 ps, followed by equilibration at 300 K for a further 0.5 ns using the nPT condition, with the Berendsen thermostat³³ for temperature control. All covalent bonds involving hydrogen atoms were constrained using the SHAKE algorithm,³⁴ allowing a simulation time step of 2 fs throughout the simulations. After the equilibration, production simulations were carried out with the GPU implementation³⁵ of PMEMD.CUDA module and trajectory frames were collected every 1 ps for a total of 1 μ s. 1–4 nonbonded interactions were not scaled³⁶ and a nonbonded cutoff of 8 Å was applied to van der Waals interactions, with long-range electrostatics treated with the particle mesh Ewald approximation. *J*-Couplings were back-calculated using one out of every 10 frames of the simulation (every 10 ps) and eqs 2–9 (see below), and the average *J*-coupling over all of these frames was taken as the predicted value obtained from the simulation.

Statistical Analysis of Crystallographic Data

Values of θ_x in crystal structures similar to **1–3** were extracted from the Cambridge Structural Database via ConQuest using the queries shown in Scheme 6.^{37,38} Structure **14** queried for **3**, structure **15** for **1 α / β** and **2 β** , and structure **16** for **2 α** . Mean positions and CSDs of θ_x for each data set were calculated using Mercury.³⁹

RESULTS AND DISCUSSION

Spin-Coupling Constants Sensitive to θ_x in **1–3**

Two bonds define *O*-acetyl side-chain conformation in **1 β** (Scheme 7): θ_3 defines rotation about the C3–O3 bond, and σ_3 defines rotation about the C1'–O3 bond. In principle, θ_3 is free to rotate through 360° in solution, whereas σ_3 determines *cis/trans* configuration of the ester and is likely to be more constrained than θ_3 (e.g., C2'–C1'–O3–C3 torsions of either 0° (*cis*) or 180° (*trans*)). Inspection of **1 β** reveals ten spin-couplings that are potentially sensitive to θ_3 , and one that is potentially sensitive to σ_3 (Scheme 7). DFT calculations on model structure **5** were conducted to determine the sensitivities of these spin-couplings to rotation about θ_3 . The geminal $^2J_{C1',C3}$, and the vicinal $^3J_{C1',H3}$, $^3J_{C1',C2}$, and $^3J_{C1',C4}$, were treated quantitatively for use in conformational modeling of θ_x in **1–3** (see below). The general behaviors of the remaining six spin-couplings (Figure 1) are discussed here.

Calculated overall changes in $^1J_{C3,H3}$, $^1J_{C2,C3}$, $^1J_{C3,C4}$, $^2J_{C2,H3}$, $^2J_{C4,H3}$, and $^2J_{C2,C4}$ as a function of θ_3 are as follows: $^1J_{C3,H3}$ (2.6 Hz); $^1J_{C2,C3}$ (6.7 Hz); $^1J_{C3,C4}$ (6.5 Hz); $^2J_{C2,H3}$ (4.6 Hz); $^2J_{C4,H3}$ (4.5 Hz); and $^2J_{C2,C4}$ (1.4 Hz) (Figure 1). Considering only staggered

conformers ($\theta_3 = 60^\circ, 180^\circ, -60^\circ$), $^1J_{C3,H3}$ is large when $\theta_3 = 60^\circ$ and -60° , that is, when one of the O3 lone-pair orbitals is anti to the C3–H3 bond. Calculated values of $r_{C3,H3}$ are as follows: 1.0956 Å (60°); 1.0951 Å (180°); and 1.0958 Å (-60°). Larger values of $^1J_{C3,H3}$ at $\theta_3 = 60^\circ$ and -60° correspond to larger r_{CH} , contrary to expectation,⁴⁰ indicating that factors other than bond length affect $^1J_{CH}$ in these systems. In contrast, when the *O*-acetyl side-chain is replaced by an OH group, r_{CH} is larger in the two rotamers having one of the oxygen lone pairs anti to the CH bond, and smaller $^1J_{CH}$ values are associated with these rotamers.

Plots of $^1J_{C2,C3}$ and $^1J_{C3,C4}$ (Figure 1) are essentially mirror images and show nearly identical dynamic range (~ 6.5 Hz). As found for $^1J_{C3,H3}$, $^1J_{C2,C3}$ and $^1J_{C3,C4}$ values increase with increasing r_{CC} , contrary to the behavior of the corresponding hydroxyl compound.⁴⁰

Plots of $^2J_{C2,H3}$ and $^2J_{C4,H3}$ (Figure 1) are also essentially mirror images, with more positive values ($^2J_{C2,H3}$ at $\theta_3 = 60^\circ$; $^2J_{C4,H3}$ at $\theta_3 = -60^\circ$) associated with rotamers having an O3 lone pair anti to the C–H and C–C bonds in the coupling pathway, contrary to observations made in hydroxyl systems.⁴⁰

$^2J_{C2,C4}$ shows a weak dependence on θ_3 (Figure 1) that deviates from that observed in the corresponding hydroxyl compound in which $\theta_3 = 180^\circ$ (O2 lone pairs anti to both C–C bonds in the pathway) gives a more positive coupling than found in the remaining two rotamers.⁴⁰

These results show that the behaviors of $^1J_{CH}$, $^1J_{CC}$, $^2J_{CCH}$, and $^2J_{CCC}$ values determined by DFT in *O*-acetylated structures differ from those of corresponding *J*-values in the hydroxyl structures. Anisotropy of the C1' carbonyl group is presumably responsible for these different properties via mechanisms that are not yet fully understood. Similar observations pertain to DFT data obtained on **6–9** (data not shown).

Energetics of Rotation about θ_x —Plots of total energy determined by DFT for **5** and **6** (Figure 2A) are nearly symmetric about $\theta_3 = 180^\circ$, with the global energy minimum located at $\theta_3 = 0^\circ$ and a local minimum located at $\theta_3 = 180^\circ$. The energy difference between the two minima is ~ 12 kJ/mol for **5** and ~ 33 kJ/mol for **6**, and activation barriers for their interconversion exceed 20 kJ/mol. DFT predicts a preferred bond torsion (global minimum) about θ_3 in **5** and **6** in which the C3–H3 and C1'–O3 bonds are eclipsed, and a local energy minimum in which the C1'–O3 bond bisects the C2–C3–C4 bond angle. Similar behavior is observed in **7** and **8** (Figure 2B), but the orientation of the adjacent OH group at C1 (axial vs equatorial) affects the energy plot substantially. When O1 is equatorial (**8**), the plot resembles that found for **5** (Figure 2A), showing an energy difference between the two minima at $\theta_2 = 0^\circ$ and 180° of ~ 11 kJ/mol. However, when O1 is axial (**7**), the right half of the plot shifts left, displacing the local energy minimum to $\theta_2 = \sim 160^\circ$. This displacement is presumably caused by steric interactions between the equatorial 2-*O*-acetyl side-chain and the adjacent axial O1 that occur when θ_2 assumes values of $\sim 180^\circ$ – 360° . The energy difference between the two minima in **7** is ~ 20 kJ/mol. These findings show that the rotational behavior of θ_x in **1–3** differs from that observed in the corresponding hydroxyl

compound, the latter obeying models involving three idealized staggered rotamers about the C3–O3 bond.⁴¹

Behavior of σ_x in 1–3—DFT calculations on **5–9** were conducted at fixed values of σ_x , namely, that having C2' anti to C3, corresponding to a *trans* geometry for the ester bond. In this study, the *trans* geometry is assumed to dominate in aqueous solution. Experimental support for this assumption derives from a comparison of calculated and experimental values of $^3J_{C2',C3}$ in **5** and **1β**, respectively. A plot of calculated $^3J_{C2',C3}$ as a function of θ_3 in **5** is shown in Figure 3. The experimental $^3J_{C2',C3}$ in **1β** of 1.3 ± 0.1 Hz is very similar to the calculated value of 1.4 Hz at $\theta_3 = 0$ (Figure 3). The calculated value of $^3J_{C2',C3}$ at $\theta_3 = 180^\circ$ is 2.1 Hz (Figure 3). These results support the assumption that the ester bond in *O*-acetyl side-chains in saccharides prefers a *trans* configuration in aqueous solution.

Experimental Spin-Coupling Constants for Conformational Analysis of θ_x in 1–3

Experimental J -couplings involving *O*-acetyl side-chain atoms were measured by introducing ^{13}C isotopes into the side-chain (Scheme 2). This approach limited J -coupling measurements to four values (e.g., $^2J_{C1',C3}$, $^3J_{C1',H3}$, $^3J_{C1',C2}$, and $^3J_{C1',C4}$ in **1β**). The three vicinal J -couplings, which display Karplus⁴³ dependencies on θ_x (see below), were used in conformational analyses of θ_x of **1–3**. Geminal $^2J_{C1',CX}$ values were also used in these analyses because they show a systematic dependence on θ_x with a dynamic range of 2.2 Hz (Figure 4A). 2J values are commonly affected by the bond angle subtended by the coupled nuclei,^{42,44} and linear relationships between the C1'–O_X–C_X bond angles and $^2J_{C1',OX,CX}$ values in **5–8** are observed (Figures 4B and S1), with $^2J_{C1',CX}$ values becoming more negative as the bond angle increases. This bond angle effect underlies the dependence of $^2J_{C1',CX}$ on θ_x .

Vicinal ^{13}C – ^1H Spin-Coupling Constants— $^3J_{C1',HX}$ values were calculated in model structures **5–9** as a function of θ_x , where $x = 2, 3$, or 6 (Figure 5). No appreciable differences were observed between the C1'–O_X–C_X–H_X coupling pathways in **5–9**, noting that one of the curves for **9** is phase-shifted by 120° (see Figure S2). These curves have Karplus character as expected;⁴³ calculated $^3J_{\text{COCH}}$ values are ~ 5 Hz at $\theta_x = 0^\circ$ and ~ 9.5 Hz at $\theta_x = 180^\circ$. A Karplus curve reported previously for the same coupling pathway is also shown in Figure 5.¹⁴ This curve has an overall shape similar to those derived using **5–9**, but a considerably reduced amplitude.

A generalized equation was parametrized to treat $^3J_{C1',HX}$ values except for $^3J_{C1',H6S}$ in **9**, for which a second equation was parametrized to account for the phase shift.

$$^3J_{C1',HX} = 3.92 - 2.11\cos(\theta) + 0.05\sin(\theta) + 3.34\cos(2\theta) + 0.07\sin(2\theta) - 0.04\cos(3\theta) + 0.05\sin(3\theta) \quad \text{rms } 0.22 \text{ Hz} \quad (2)$$

$${}^3J_{\text{C1}',\text{H6S}} = 4.06 + 0.80\cos(\theta) + 1.63\sin(\theta) - 1.99\cos(2\theta) + 2.91\sin(2\theta) - 0.15\cos(3\theta) - 0.20\sin(3\theta) \quad \text{rms } 0.12 \text{ Hz} \quad (3)$$

Vicinal ${}^{13}\text{C}$ – ${}^{13}\text{C}$ Spin-Coupling Constants—Vicinal ${}^{13}\text{C}$ – ${}^{13}\text{C}$ spin-coupling constants calculated in **5–9** fall into two groups when plotted against $\theta_x = \text{C1}'\text{--O}_x\text{--C}_x\text{--H}_x$ (Figure 6A,B). A torsion angle of 0° between the coupled carbons (i.e., when $\theta_x = 120^\circ$) gives a calculated ${}^3J_{\text{CCOC}}$ of ~ 2 Hz, whereas a torsion angle of 180° ($\theta_x = 300^\circ$) gives a calculated ${}^3J_{\text{CCOC}}$ of 4–5 Hz. Curves obtained for $\text{C1}'\text{--O}_x\text{--C}_x\text{--C}_y$ ($y = x \pm 1$) coupling pathways in which one of the coupled carbons (C_y) is C1 (an anomeric carbon) (e.g., ${}^3J_{\text{C1}',\text{C1}}$ in **7** and **8**) (Figure 6B) differ from the curves for pathways involving nonanomeric carbons, especially for C–O–C–C torsion angles near 180° (θ_x near 60°). At θ_x near 60° , ${}^3J_{\text{C1}',\text{C1}}$ values for **7** and **8** are ~ 1 Hz larger than values found for **5** and **6**. Eq 4 treats the five overlapping curves shown in Figure 6A. Two additional equations treat the two sets of curves shown in Figure 6B, eq 5 for the case where one of the coupled carbons is non-anomeric (**5** and **6**), and eq 6 for the case where one of the coupled carbons is anomeric (**7** and **8**).

$${}^3J_{\text{C1}',\text{C}_y} = 1.56 + 0.36\cos(\theta) - 0.73\sin(\theta) - 0.75\cos(2\theta) - 1.22\sin(2\theta) - 0.14\cos(3\theta) + 0.05\sin(3\theta) \quad \text{rms } 0.13 \text{ Hz} \quad (4)$$

$${}^3J_{\text{C1}',\text{C4}} = 1.44 + 0.33\cos(\theta) - 0.74\sin(\theta) - 0.68\cos(2\theta) - 1.21\sin(2\theta) - 0.14\cos(3\theta) + 0.16\sin(3\theta) \quad \text{rms } 0.04 \text{ Hz} \quad (5)$$

$${}^3J_{\text{C1}',\text{C1}} = 1.86 + 0.51\cos(\theta) + 1.08\sin(\theta) - 0.94\cos(2\theta) + 1.46\sin(2\theta) - 0.25\cos(3\theta) - 0.17\sin(3\theta) \quad \text{rms } 0.16 \text{ Hz} \quad (6)$$

Geminal ${}^{13}\text{C}$ – ${}^{13}\text{C}$ Spin-Coupling Constants—Geminal ${}^{13}\text{C}$ – ${}^{13}\text{C}$ spin-couplings in **5–9** involving C1' as a coupled nucleus depend on θ_x (Figure 7). This dependency is a manifestation of the effect of θ_x on the $\text{C1}'\text{--O}_x\text{--C}_x$ bond angle, which in turn influences ${}^2J_{\text{C1}',\text{C}_x}$ (Figure 4; Figure S1). The curves for ${}^2J_{\text{C1}',\text{C3}}$ in **5** and **6** have the same overall shape, but that for **7** (axial *O*-acetyl side-chain at C3) has a greater amplitude caused by the greater overall change in C–O–C bond angle as a function of θ_3 (Figure S1). The curves for **5** and **8**, in which the *O*-acetyl side-chain and the flanking groups are equatorial, are virtually identical. When one flanking group is axial (i.e., in **7**), the right half of the curve shifts left, analogous to behavior found for total energy (Figure 2B). The behavior of ${}^2J_{\text{C1}',\text{C6}}$ values in **9**, which deviates from a bimodal pattern (the minimum at $\theta_6 = \sim 240^\circ$ is absent), is attributed to a lack of steric hindrance that allows smaller C–O–C bond angles at these values of θ_6 (Figure S1).

Based on data in Figure 7, three parametrized equations for ${}^2J_{C1',Cx}$ were derived: eq 7 for ${}^2J_{C1',C3}$ in **5** and ${}^2J_{C1',C2}$ in **8**; eq 8 for ${}^2J_{C1',C2}$ in **7**; and eq 9 for ${}^2J_{C1',C6}$ in **9**. An equation for ${}^2J_{C1',C3}$ in **6** was also developed (see Supporting Information) but was not used in this work because experimental data in this ring system was unavailable.

$${}^2J_{C1',Cx} \text{ (for } \mathbf{5/8}) = -3.81 + 0.64\cos(\theta) - 0.14\sin(\theta) + 0.84\cos(2\theta) + 0.05\sin(2\theta) - 0.29\cos(3\theta) - 0.03\sin(3\theta) \quad \text{rms } 0.10 \text{ Hz} \quad (7)$$

$${}^2J_{C1',C2} \text{ (for } \mathbf{7}) = -3.79 + 0.95\cos(\theta) - 0.45\sin(\theta) + 0.42\cos(2\theta) - 0.14\sin(2\theta) - 0.28\cos(3\theta) + 0.36\sin(3\theta) \quad \text{rms } 0.13 \text{ Hz} \quad (8)$$

$${}^2J_{C1',C6} \text{ (for } \mathbf{9}) = -3.31 + 0.40\cos(\theta) - 0.94\sin(\theta) + 0.40\cos(2\theta) + 0.33\sin(2\theta) - 0.24\cos(3\theta) + 0.28\sin(3\theta) \quad \text{rms } 0.20 \text{ Hz} \quad (9)$$

Statistical Modeling of θ_X in 1–3—Experimental spin-couplings in **1a** and **1b** (Scheme 2; Table 1) are essentially identical, indicating qualitatively that the effect of anomeric configuration on the conformational behavior of θ_3 for an equatorial 3-*O*-acetyl side-chain is small. The same conclusion pertains to the 6-*O*-acetyl side-chains in **3a** and **3b**. In contrast, experimental spin-couplings in **2a** and **2b** differ significantly, suggesting that anomeric configuration affects the conformation of equatorial 2-*O*-acetyl side-chains. MD simulations of **11** and **12** support these conclusions, showing significantly different mean positions and circular standard deviations (CSDs) for θ_2 (Figure 8, Table 2). The MD results exhibit multimodality for θ_X in **11** and **13** (Figure 8). Spin-couplings back-calculated from the MD histograms are similar to the experimental values in most instances (Table 1), suggesting that the θ_X populations present in solution are probably similar to those predicted from the MD simulations.

Parameterized eqs 2–9 and experimental J_{CH} and J_{CC} values in **1–3** (Table 1) were used with eq 1 to calculate the mean positions of θ_X and their associated circular standard deviations (CSDs) for *O*-acetyl side-chains in **1–3** (Figure 9, Table 2). Five probability density functions were used to fit the experimental J -couplings (see below). The calculated mean positions of the resulting distributions (Figure 9) coincide with the locations of minima in plots of calculated total energy as a function of θ_X (Figure 2) for **5**, **7**, and **8**, which model **1b**, **2a**, and **2b**, respectively. The mean positions and CSDs are in good agreement with those obtained from MD simulations and from crystal structure analyses (Table 2). The mean positions of θ_X in **1–3** differ (Figure 9), with **1** and **2b** giving values close to the idealized value of 0° in which the C=O bond of the ester eclipses the C–H bond of the alcoholic carbon. This eclipsing is reduced in **2a** where the mean position of θ_2 is $\sim -20^\circ$. The *O*-acetyl side-chains in **3a** and **3b** deviate significantly from an eclipsed geometry,

giving mean positions of θ_6 near -60° . In **3**, the C=O bond has two C-H bonds to eclipse, and adopts a position between them (see Scheme S3).

The CSDs of each compound depend on the steric environment of the *O*-acetyl side-chains. Compound **3a/β** is 472 the least sterically constrained and gives the largest CSD.

Compounds **1a/β** and **2β** are more sterically constrained, with the *O*-acetyl side-chain flanked by two equatorial hydroxyl groups. They are also constrained to a very similar degree, as reflected in their similar CSDs. In **2a**, one of the flanking hydroxyl groups is in an axial orientation, allowing more rotational freedom about θ_2 and a CSD intermediate between the other three models. These results indicate that the statistical method presented herein can not only identify the preferred mean position about a given molecular torsion angle, but also reveal relative differences in disorder about similar angles in different compounds.

Mean positions of θ_x , standard errors of the mean positions, and CSDs were calculated using five different probability density functions to fit the experimental spin-couplings: raised cosine;^{45,46} uniform;^{45,46} power of cosine;⁴⁷ von Mises;²⁴ and Lorentz²⁴ (Figure 10; Table 2). These parameters were largely independent of the functional form of the model. Since each model gives a similar RMS error that reflects the goodness of the fit, estimating mean positions and CSDs accurately does not depend on the functional form of the model, with the exception of Lorentz models. However, the standard error of the mean noticeably increases for models with larger CSDs.

One parameter that depends on the functional form of the model is the standard error of the CSD, with platykurtic models (e.g., raised cosine, uniform, and power of cosine; Table 2) giving smaller standard errors than von Mises and Lorentz models. Platykurtic models are probably more sensitive to changes in the CSD because their “tails” approach a probability of zero more rapidly than do those of other models, and thus rotamers near the edge of the peak will be weighted more heavily (Figure 10). This increase in precision is of practical significance, since the predicted CSD of the von Mises models for the 2-*O*-acetyl side-chain in **2a** and the 3-*O*-acetyl side-chain in **1** cannot be easily distinguished from a 1-parameter model that does not account for molecular flexibility, while this is not the case for the uniform, raised cosine, or power of cosine models. Of the five functional forms investigated, Lorentz models consistently gave the largest CSD, standard error of the CSD, and RMS error (Table 2). This performance could be caused by the large “tails” of this function, which may assign too much weight to rotamer with θ_x values far from the mean position.

Since the models for **1**, **2a**, **2β**, and **3** consist of two parameters, visual inspection of the parameter space of each model (Figures 11 and S4) can identify model parameters that represent local minima, and determine whether these minima are significantly different from the global minimum. The parameter space of **3** displays one local minimum with an RMS error of <1 Hz (Figure 11). The relative smoothness of the parameter space for **3** could be due to the use of $^2J_{C1',C6}$, which is described essentially by a unimodal equation. This behavior contrasts with the other derived equations, which are bimodal. While local minima exist in the parameter spaces of **1**, **2a**, and **2β**, the associated RMS errors of the local

minima in each compound are significantly different than that for the global minimum (at least 0.3 Hz, Figure S4).

CONCLUSIONS

This investigation had three primary goals: (1) to identify NMR spin-couplings sensitive to *O*-acetyl conformation in *O*-acetylated saccharides; (2) to parametrize new equations relating these spin-couplings to *O*-acetyl conformation; and (3) to use redundant spin-couplings to assign *O*-acetyl conformation in aqueous solution. Of the ten *J*-couplings examined (Scheme 7), seven exhibit a sufficient dynamic range to assess θ_x (for **5**, $^1J_{C2,C3}$, $^1J_{C3,C4}$, $^2J_{C2,H3}$, $^2J_{C4,H3}$, $^2J_{C1',C3}$, $^3J_{C1',H3}$, $^3J_{C1',C2}$, and $^3J_{C1',C4}$). Because of experimental limitations, only four *J*-couplings were parametrized in this work and used to evaluate θ_x in six ^{13}C -labeled compounds: **1a**, **1b**, **2a**, **2b**, **3a**, and **3b** (Scheme 2). Only equatorial *O*-acetyl side-chains appended to an aldopyranosyl ring were considered, and the effect of flanking substituents on their conformations studied. Inspection of the ensembles of *J*-values showed that anomeric configuration does not affect θ_x in **1a**, **1b**, **3a**, and **3b**, but the effect was significant in **2a** and **2b** due to the proximity of the anomeric center to the 2-*O*-acetyl side-chain. This behavior is similar to that of *N*-acetyl side-chains in 2-acetamido sugars such as *N*-acetyl-D-glucosamine, where anomeric configuration influences the amide *cis-trans* equilibrium and the kinetics of amide *cis-trans* isomerization.⁴⁸ While axial *O*-acetyl side-chains were not investigated here, similar flanking effects on their conformations are anticipated.

Efforts to determine *O*-acetyl side-chain conformation in solution have been reported previously, notably by Schweizer and Dunitz⁴⁹ and by González-Outeiriño et al.¹⁴ These prior studies determined the conformational preferences of θ in secondary alcohols based on a survey of structures in the Cambridge Structural Database, and derived a Karplus equation for $^3J_{C1',HX}$ from *J*-couplings in conformers with $\theta = 0^\circ$, 60° , and 180° . The present study extends this prior work by reparameterizing the $^3J_{C1',HX}$ Karplus equation, parametrizing three new spin-couplings sensitive to θ (for example, $^2J_{C1',C3}$, $^3J_{C1',C2}$, and $^3J_{C1',C4}$ in **1**), and treating ensembles of four redundant *J*-couplings to give rotameric populations about θ_x for *O*-acetyl side-chains located at different sites in an aldohexopyranosyl ring. The latter treatment advances the study of *O*-acetyl conformation and other flexible elements in saccharides by providing models of rotamer populations in solution derived exclusively from experimental data. The inclusion of additional *J*-couplings sensitive to θ_x in future analyses will increase the reliability of the fit and permit more rigorous testing of alternative models. Nevertheless, the *J*-coupling analyses reported herein yield conformational models in good agreement with X-ray data and with those predicted by molecular dynamics simulations, although some of the finer details revealed by MD cannot yet be interrogated by experiment.

Although the current work focused on *O*-acetyl side-chain conformation, it validates the use of redundant *J*-couplings to determine rotameric populations about a single molecular torsion angle in solution, such as the phi (ϕ) and psi (ψ) C–O torsion angles comprising the *O*-glycosidic linkages in oligosaccharides (Scheme 8). The general conformational properties of *O*-acetyl side-chains have been documented previously, allowing a determination of the accuracy of the data analysis.¹⁴ The precision and accuracy of five

different two-parameter models for rotamer distribution across a single molecular torsion angle were tested and compared. Olsson et al.¹⁵ have shown that the flexibility of a molecular torsion angle in solution could be determined by modeling the rotamer distribution as a von Mises distribution. The present study expands this prior work by calculating the precision of this distribution in its ability to model the mean positions and CSDs of multiple rotamer distributions, comparing this precision to those obtained from the use of other statistical models, and demonstrating that it is possible to generate unique models of a given rotamer distribution. While J -couplings were the only experimental observables used in this work, eq 1 is theoretically capable of using other experimental parameters, such as nuclear Overhauser effects (NOE) and residual dipolar couplings (RDCs), as constraints provided that they can be measured reliably and suitable equations can be derived to relate them to the torsion angle of interest. The use of diverse experimental parameters in future conformational studies could be beneficial, since some of these parameters will likely have torsional dependencies that differ from the bimodal Karplus dependencies that typically describe vicinal spin-couplings. These diverse dependencies will improve the reliability of assigning unique models to rotamer distributions in solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Varki, A., Lowe, JB. Essentials of Glycobiology. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR.Hart, GW., Etzler, ME., editors. Biological Roles of Glycans. 2. Cold Spring Harbor Laboratory Press; New York: 2009. p. 75-88.
2. DeMarco ML, Woods RJ. Structural Glycobiology: A Game of Snakes and Ladders. Glycobiology. 2008; 18:426–440. [PubMed: 18390826]
3. Polacek R, Stenger J, Kaatz U. Chair-Chair Conformational Flexibility, Pseudorotation, and Exocyclic Group Isomerization of Monosaccharides in Water. J Chem Phys. 2002; 116:2973–2982.
4. Rockwell GD, Grindley TB. Effect of Solvation on the Rotation of Hydroxymethyl Groups in Carbohydrates. J Am Chem Soc. 1998; 120:10953–10963.
5. Cloran F, Carmichael I, Serianni AS. Density Functional Calculations on Disaccharide Mimics: Studies of Molecular Geometry and Trans-*O*-glycosidic $^3J_{\text{COCH}}$ and $^3J_{\text{COCC}}$ Spin-Couplings. J Am Chem Soc. 1999; 121:9843–9851.
6. Varki, A., Schauer, R. Essentials of Glycobiology. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR.Hart, GW., Etzler, ME., editors. Sialic Acids. 2. Cold Spring Harbor Laboratory Press; New York: 2009. p. 199-218.
7. Hanai N, Dohi T, Nores GA, Hakomori S. A Novel Ganglioside, De-*N*-acetyl-GM₃ (II³NeuNH₂LacCer), Acting as a Strong Promoter for Epidermal Growth Factor Receptor Kinase and as a Stimulator for Cell Growth. J Biol Chem. 1988; 263:6296–6301. [PubMed: 2834372]
8. Stenutz R, Carmichael I, Widmalm G, Serianni AS. Hydroxymethyl Group Conformation in Saccharides: Structural Dependencies of $^2J_{\text{HH}}$, $^3J_{\text{HH}}$ and $^1J_{\text{CH}}$ Spin-spin Coupling Constants. J Org Chem. 2002; 67:949–958. [PubMed: 11856043]

9. Thibaudeau C, Stenutz R, Hertz B, Klepach T, Zhao S, Wu Q, Carmichael I, Serianni AS. Correlated C-C and C-O Bond Conformations in Saccharide Hydroxymethyl Groups: Parametrization and Application of Redundant ^1H - ^1H , ^{13}C - ^1H and ^{13}C - ^{13}C NMR J -Couplings. *J Am Chem Soc.* 2004; 126:15668–15685. [PubMed: 15571389]
10. Hu X, Carmichael I, Serianni AS. N -Acetyl Side-chains in Saccharides: NMR J -Coupling Equations Sensitive to CH-NH and NH-CO Bond Conformations in 2-Acetamido-2-deoxyaldohexopyranosyl Rings. *J Org Chem.* 2010; 75:4899–4910. [PubMed: 20604546]
11. Brumfitt W, Wardlaw A, Park J. Development of Lysozyme-resistance in *Micrococcus lysodeikticus* and Its Association with an Increased O -Acetyl Content of the Cell Wall. *Nature.* 1958; 181:1783–1784. [PubMed: 13566139]
12. Verma N, Brandt J, Verma D, Lindberg A. Molecular Characterization of the O -Acetyl Transferase Gene of Converting Bacteriophage SF6 That Adds Group Antigen 6 to *Shigella flexneri*. *Mol Microbiol.* 1991; 5:71–75. [PubMed: 2014005]
13. Sweet L, Zhang W, Torres-Fewell H, Serianni A, Boggess W, Schorey J. *Mycobacterium avium* Glycopeptidolipids Require Specific Acetylation and Methylation Patterns for Signaling Through Toll-like Receptor 2. *J Biol Chem.* 2008; 283(48):33221–33231. [PubMed: 18824550]
14. González-Outeiriño J, Nasser R, Anderson JE. Conformation of Acetate Derivatives of Sugars and Other Cyclic Alcohols. Crystal Structures, NMR Studies, and Molecular Mechanics Calculations of Acetates. When Is the Exocyclic C-O Bond Eclipsed? *J Org Chem.* 2005; 70:2486–2493. [PubMed: 15787534]
15. Olsson U, Serianni AS, Stenutz R. Conformational Analysis of β -Glycosidic Linkages in ^{13}C -Labeled Glucobiosides Using Inter-residue Scalar Coupling Constants. *J Phys Chem B.* 2008; 112:4447–4453. [PubMed: 18345660]
16. Frisch, MJ., et al. Gaussian09, Revision D. Gaussian Inc; Pittsburgh, PA: 2009.
17. Becke AD. A New Mixing of Hartree-Fock and Local Density-Functional Theories. *J Chem Phys.* 1993; 98:1372–1377.
18. Hehre WJ, Ditchfield R, Pople JA. Self-Consistent Molecular Orbital Methods. XII. Further Extensions of Gaussian-Type Basis Sets for Use in Molecular Orbital Studies of Organic Molecules. *J Chem Phys.* 1972; 56:2257–2261.
19. Cancés E, Mennucci B, Tomasi J. A New Integral Equation Formalism for the Polarizable Continuum Model: Theoretical Background and Applications To Isotropic and Anisotropic Dielectrics. *J Chem Phys.* 1997; 107:3032–3041.
20. Cammi R, Mennucci B, Tomasi J. Fast Evaluation of Geometries and Properties of Excited Molecules in Solution: A Tamm-Dancoff Model with Application to 4-Dimethylaminobenzonitrile. *J Phys Chem A.* 2000; 104:5631–5637.
21. Klepach, T., Zhao, H., Hu, X., Zhang, W., Stenutz, R., Hadad, MJ., Carmichael, I., Serianni, AS. Informing Saccharide Structural NMR Studies with Density Functional Theory Calculations. In: Lütke, T., Frank, M., editors. *Glycoinformatics: Methods in Molecular Biology*. Springer; New York: 2015. p. 289-331.
22. Wolfram, S. *Mathematica: A System for Doing Mathematics by Computer*. 2. Addison-Wesley; Redwood City, CA: 1991.
23. Agostinelli, C., Lund, U. *R Package Circular: Circular Statistics (version 0.4–3)*. CA: Department of Environmental Sciences, Informatics and Statistics, Ca'Foscari University; Venice, Italy: UL: Department of Statistics, California Polytechnic State University; San Luis Obispo, California, USA: 2011.
24. Pewsey, A., Neuhausser, M., Ruxton, GD. *Circular Statistics in R*. Oxford University Press; 2013.
25. Jammalamadaka, SR., SenGupta, A. *Series on Multivariate Analysis. Vol. 5. World Scientific Publishing Co; 2001. Topics in Circular Statistics*.
26. Cartwright, DE. *Ocean Wave Spectra*. Prentice-Hall; Englewood Cliffs, NJ: 1963. p. 203-218.
27. Jones, E., Oliphant, T., Peterson, P. *SciPy: Open Source Scientific Tools for Python*. 2014.
28. Dzakula Z, Westler WM, Edison AS, Markley JL. The “CUPID” Method for Calculating the Continuous Probability Distribution of Rotamers from NMR Data. *J Am Chem Soc.* 1992; 114:6195–6199.

29. Yuen, K. Bayesian Methods for Structural Dynamics and Civil Engineering. Wiley; 2010. p. 257-262.
30. Kirschner KN, Yongye AB, Tschampel SM, Gonzalez-Outeirino J, Daniels CR, Foley BL, Woods RJ. GLYCAM06: A Generalizable Biomolecular Force Field. Carbohydrates. J Comput Chem. 2008; 29:622–655. [PubMed: 17849372]
31. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of Simple Potential Functions for Simulating Liquid Water. J Chem Phys. 1983; 79:926–935.
32. Case, DA., Babin, V., Berryman, JT., Betz, RM., Cai, Q., Cerutti, DS., Cheatham, TEI., Darden, TA., Duke, RE., Gohlke, H., et al. AMBER 14. University of California; San Francisco: 2014.
33. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR. Molecular Dynamics with Coupling to an External Bath. J Chem Phys. 1984; 81:3684–3690.
34. van Gunsteren WF, Berendsen HJC. Algorithms for Macromolecular Dynamics and Constraint Dynamics. Mol Phys. 1977; 34:1311–1327.
35. Götz AW, Williamson MJ, Xu D, Poole D, Le Grand S, Walker RC. Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 1. Generalized Born. J Chem Theory Comput. 2012; 8:1542–1555. [PubMed: 22582031]
36. Kirschner KN, Woods RJ. Solvent Interactions Determine Carbohydrate Conformation. Proc Natl Acad Sci U S A. 2001; 98:10541–10545. [PubMed: 11526221]
37. Allen FH. The Cambridge Structural Database: A Quarter of a Million Crystal Structures and Rising. Acta Crystallogr, Sect B: Struct Sci. 2002; 58:380–388.
38. Bruno IJ, Cole JC, Edgington PR, Kessler M, Macrae CF, McCabe P, Pearson J, Taylor R. New Software for Searching the Cambridge Structural Database and Visualizing Crystal Structures. Acta Crystallogr, Sect B: Struct Sci. 2002; 58:389–397.
39. Macrae CF, Edgington PR, McCabe P, Pidcock E, Shields GP, Taylor R, Towler M, van de Streek J. *Mercury*: Visualization and Analysis of Crystal Structures. J Appl Crystallogr. 2006; 39:453–457.
40. Serianni AS, Wu J, Carmichael I. One-Bond ^{13}C - ^1H Spin-Coupling Constants in Aldofuranosyl Rings: Effect of Conformation on Coupling Magnitude. J Am Chem Soc. 1995; 117:8645–8650.
41. Stolow RD, Gallo AA. Hydroxyl Proton Coupling in Cyclohexanols. Rotamer Populations of the Hydroxyl Group. Tetrahedron Lett. 1968; 9:3331–3336.
42. Cloran F, Carmichael I, Serianni AS. $^2J_{\text{COC}}$ Spin-Spin Coupling Constants Across Glycosidic Linkages Exhibit a Valence Bond-Angle Dependence. J Am Chem Soc. 2000; 122:396–397.
43. Karplus M. Contact Electron-Spin Coupling of Nuclear Magnetic Moments. J Chem Phys. 1959; 30:11–15.
44. Ando, I., Webb, GA. Theory of NMR Parameters. Academic Press; New York: 1983. p. 106
45. Hunter J. A Simple Technique for Estimating an Allowance for Uncertain Sea-Level Rise. Clim Change. 2012; 113:239–252.
46. Jammalamadaka SR, Kozubowski TJ. New Families of Wrapped Distributions for Modeling Skew Circular Data. Communications in Statistics - Theory and Methods. 2004; 33:2059–2074.
47. Cartwright, DE. Ocean Wave Spectra. Prentice-Hall; Englewood Cliffs, NJ: 1963. The Use of Directional Spectra in Studying the Output of a Wave Recorder on a Moving Ship; p. 203-218.
48. Hu X, Zhang W, Carmichael I, Serianni AS. Amide *Cis-Trans* Isomerization in Aqueous Solutions of Methyl *N*-Formyl-D-glucosaminides and Methyl *N*-Acetyl-D-glucosaminides: Chemical Equilibria and Exchange Kinetics. J Am Chem Soc. 2010; 132:4641–4652. [PubMed: 20225805]
49. Schweizer WB, Dunitz JD. Structural Characteristics of the Carboxylic Ester Group. Helv Chim Acta. 1982; 65:1547–1554.

Figure 1. Calculated 1J and 2J values in **5** as a function of θ_3 . (A) $^1J_{C3,H3}$, (B) $^1J_{C2,C3}$, (C) $^1J_{C3,C4}$, (D) $^2J_{C2,H3}$, (E) $^2J_{C4,H3}$, and (F) $^2J_{C2,C4}$. Red points correspond to the three perfectly staggered rotamers of θ_3 . Overall changes in each J -value are shown in green.

Figure 2. Total energy (kJ/mol) as a function of (A) the H3–C3–O3–C1' torsion angle (θ_3) in **5** (filled symbols) and **6** (open symbols), and (B) the H2–C2–O2–C1' torsion angle (θ_2) in **7** (open symbols) and **8** (filled symbols).

Figure 3. Calculated $^3J_{C2',C3}$ values in **5** as a function of the H3-C3-O3-C1' torsion angle θ_3 .

Figure 4.

(A) Effect of the H3-C3-O3-C1' torsion angle (θ_3) on calculated values of $^2J_{C1',C3}$ in **5**. The dynamic range is shown in red. (B) Summary of data for **5-8** showing the effect of C-O-C bond angle on calculated $^2J_{C1',CX}$, where $X = 2$ or 3 . Filled green, **5**; open green, **6**; open red, **7**; filled red, **8**. The inset shows a linear fit of the data.

Figure 5.

Calculated $^3J_{C1',HX}$ values in **5–9** as a function of θ_X , where $x = 2, 3$, and 6 . Filled green, **5**; open green, **6**; open red, **7**; filled red, **8**; filled blue, $^3J_{C1',H6R}$ and $^3J_{C1',H6S}$ in **9**; filled black, data taken from ref 14. Curves were generated by point-to-point connectivity.

Figure 6.

Calculated $^3J_{\text{C1}',\text{Cx}}$ values in **5–9** as a function of the $\text{C1}'\text{--O}_x\text{--C}_x\text{--H}_x$ torsion angle θ_x ($x = 2, 3$ or 6). (A) $^3J_{\text{C1}',\text{C3}}$ in **5** (filled blue); $^3J_{\text{C1}',\text{C4}}$ in **6** (open blue); $^3J_{\text{C1}',\text{C3}}$ in **7** (open red); $^3J_{\text{C1}',\text{C3}}$ in **8** (filled red); $^3J_{\text{C1}',\text{C5}}$ in **9** (filled black). (B) $^3J_{\text{C1}',\text{C4}}$ in **5** (filled blue); $^3J_{\text{C1}',\text{C2}}$ in **6** (open blue); $^3J_{\text{C1}',\text{C1}}$ in **7** (open red); $^3J_{\text{C1}',\text{C1}}$ in **8** (filled red).

Figure 7.

Calculated $^2J_{C1',CX}$ values in **5–9** as a function of θ_x , where $x = 2, 3$, or 6 . Filled green, $^2J_{C1',C3}$ in **5**; open green, $^2J_{C1',C3}$ in **6**; open red, $^2J_{C1',C2}$ in **7**; filled red, $^2J_{C1',C2}$ in **8**; open blue, $^2J_{C1',C6}$ in **9**.

Figure 8. Histograms showing the distributions of θ_x in **11** (A), **12** (B), **10** (C), and **13** (D) obtained from 1- μ s aqueous molecular dynamics (MD) simulations.

Figure 9.

Probability distributions across θ_x in **1**, 2α , 2β , and **3** determined from a fit of redundant experimental J -couplings (Table 1) using parametrized eqs 2–9 and a von Mises model. Red, 2α ; blue, 2β ; green, $1\alpha/\beta$; black, **3**.

Figure 10.

Different statistical models of θ_6 in **3**. Black, raised cosine; red, uniform; green, power of cosine; blue, von Mises; violet, Lorentz. Since platykurtic models approach zero more rapidly than models displaying more kurtosis, rotamers associated with θ_x values near the edge of the distribution are weighted more heavily, rendering the model more sensitive to changes in the CSD.

Figure 11.

Parameter space for the uniform model of θ_6 in **3**. Since only one minimum falls below an RMS error of 1 Hz, this minimum represents a unique solution for the rotamer distribution.

Scheme 1.
Relative Dispositions of Flanking Hydroxyl Groups in Aldohexopyranosyl Rings Bearing an *O*-Acetyl Side-Chain at C3

Scheme 2. ^{13}C -Labeled Mono-*O*-acetylated D-Glucopyranoses 1–3

Scheme 3.

Model Structures 5–9 Used in DFT Calculations, Showing Atom Numberings and Definitions of θ_x

Scheme 4.

Identification of the H6R Reference Atom Used To Define θ_6 in Structure 9

Scheme 5.

Model Structures 10–13 Used in Molecular Dynamics (MD) Simulations

Scheme 6.

Structure Queries 14–16 Used by ConQuest in the Cambridge Structural Database Search

$$\begin{array}{lll} {}^1J_{\text{C3,H3}} & {}^2J_{\text{C2,H3}} & {}^3J_{\text{C1',H3}} \\ {}^1J_{\text{C2,C3}} & {}^2J_{\text{C4,H3}} & {}^3J_{\text{C1',C2}} \\ {}^1J_{\text{C3,C4}} & {}^2J_{\text{C2,C4}} & {}^3J_{\text{C1',C4}} \\ & {}^2J_{\text{C1',C3}} & \end{array}$$

J -Couplings Sensitive to σ_3

${}^3J_{C2',C3}$

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Scheme 8.
 Conformational Elements in a $\beta(1 \rightarrow 4)$ -Linked Disaccharide

Table 1Experimental J_{CH} and J_{CC} Values^a in 1–3, and Corresponding Back-Calculated Spin-Couplings^b in 10–13 Determined from MD Histograms

J -coupling	compound				
	1 <i>a</i>	1 <i>β</i>	2 <i>a</i>	2 <i>β</i>	3 <i>a</i> 3 <i>β</i>
$^2J_{C1',C3}$	± 2.8	± 2.8 (– 2.7)			
$^3J_{C1',H3}$	3.9	3.9 (4.6)			
$^3J_{C1',C2}$	1.0	1.0 (1.2)			
$^3J_{C1',C4}$	1.0	1.0 (1.1)			
$^3J_{C2',C3}$	1.1	1.3			
$^2J_{C1',C2}$			± 2.6 (– 2.7)	± 2.7 (– 2.7)	
$^3J_{C1',H2}$			3.6 (3.7)	4.0 (4.5)	
$^3J_{C1',C1}$			0.7 (1.1)	1.3 (1.3)	
$^3J_{C1',C3}$			2.0 (2.1)	1.1 (1.4)	
$^2J_{C1',C6}$					± 2.2 ± 2.2 (– 2.7)
$^3J_{C1',H6}$					2.4 2.4 (3.0)
$^3J_{C1',H6},^c$					2.8 2.8 (3.1)
$^3J_{C1',C5}$					2.5 2.5 (2.3)

^aIn Hz ± 0.1 Hz, in 2H_2O at 25 °C. Signs of the experimental 2J values were not determined.^bValues shown in parentheses were calculated from the MD histograms shown in Figure 8; **10** for **1*****β***, **11** for **2*****a***, **12** for **2*****β***, and **13** for **3*****β***.^cThe more shielded H6 proton in **3** is defined as H6'.

Table 2

Mean Positions, Circular Standard Deviations (CSDs), and RMS Errors Calculated Using Different Models to Analyze Redundant J -Couplings in 1–3

cmpd	X-ray ^a	MD ^b	raised cosine	uniform	power of cosine	von Mises	Lorentz
mean positions (deg)							
2a (2-OAc)	-9.4	-16.4	-21.9 (13)	-21.9 (12)	-22.3 (13)	-22.5 (13)	-25.8 (13)
2β (2-OAc)	-0.2	-2.8	-1.0 (10)	-1.0 (10)	-1.1 (10)	-1.1 (10)	-1.3 (12)
1 (3-OAc)	-0.2	-0.1	0.6 (12)	0.6 (12)	0.7 (12)	0.6 (12)	0.7 (14)
3 (6-OAc)	-55.8	-63.0	-66.5 (30)	-66.1 (31)	-66.7 (29)	-66.4 (26)	-66.6 (19)
circular standard deviations (CSDs) (deg)							
2a (2-OAc)	28.9	29.4	28.5 (11)	28.1 (12)	29.3 (9)	29.9 (16)	40.2 (19)
2β (2-OAc)	16.5	23.0	23.8 (9)	23.1 (10)	24.8 (8)	25.3 (13)	39.8 (15)
1 (3-OAc)	16.5	24.2	22.6 (9)	22.1 (10)	23.4 (10)	23.8 (13)	37.0 (15)
3 (6-OAc)	45.1	51.9	40.1 (8)	37.3 (8)	40.4 (9)	42.5 (12)	52.1 (11)
RMS errors (Hz)							
2a (2-OAc)		0.22	0.22	0.21	0.23	0.23	0.29
2β (2-OAc)		0.29	0.22	0.23	0.23	0.23	0.26
1 (3-OAc)		0.37	0.19	0.20	0.20	0.20	0.24
3 (6-OAc)		0.43	0.27	0.27	0.27	0.28	0.33

^aSee Statistical Analysis of Crystallographic Data Section for details.^bSee Molecular Dynamics (MD) Simulations Section for details.