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

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Supporting Information

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 glycosidic linkages.5 These motions can be interrogated by molecular dynamics (MD) and related simulations.2 NMR and other experimental methods, and computationally by flexibilities (time-dependent motions).2 The latter analysis leads to potential synergies in the conformations of side-chain conformation depends on molecular context, with flanking groups playing a dominant role in determining the properties of J in solution. To quantify these effects, ensembles of J-couplings containing four values were used to determine the precision and accuracy of several 2-parameter statistical models of rotamer distributions across J 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 J 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.

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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,6–10 less attention has been paid to O-acetyl side-chains, such as those installed in vivo in the biologically important monosaccharides, N-acetyl-neuraminic acid and N-acetyl-α-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 1α/1β, 2-O-acetyl-D-glucopyranoses 2α/2β, and 6-O-acetyl-D-glucopyranoses 3α/3β (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 13C-Labeled Compounds 1–3. Chemicals.** All chemicals except 13C-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-glucopyranose with Acetyl Chloride.** To a cold solution of 1,2,5,6-di-O-isopropylidene-α-D-glucopyranose (6.6 g, 25.4 mmol) in 40 mL of anhydrous pyridine was added [1-13C] or [2-13C] 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 CH₂Cl₂, and sequentially extracted with 1N aqueous HCl, sat. aqueous NaHCO₃, and distilled water. The organic phase was dried over Na₂SO₄ 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-α-D-glucofuranose, 4 (3.8 g, 12.5 mmol; quantitatively based on labeled acetyl chloride).

Deacetonation of 3-O-[13C]Acetyl-1,2;5,6-di-O-isopropylidene-α-D-glucopyranoside 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 × 60 cm). Elution with ethyl acetate afforded a mixture of monoacetylated D-glucoses (generated by acyl group migration during deacetonation). The major component of this mixture, identified by 1H and 13C NMR, was 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 JCH and JCC values.

NMR Spectroscopy. 1H and 13C{1H} NMR spectra were recorded on a 600-MHz spectrometer in D₂O solvent at 25 °C (5 mm tubes) and with digital resolutions of <0.05 Hz/point.1 JCH and JCC values were measured directly from signal multiplicities observed in 1D 1H and 13C{1H} NMR spectra, respectively, of 13C-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 C₂₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋_-
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 GLYCAM06\textsuperscript{10} (version j) force field was employed for all simulations. Structures were solvated with TIP3P\textsuperscript{33} water using a 12 Å buffer in a cubic box, using the LEaP module in the AMBER14 software package.\textsuperscript{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 thermostat\textsuperscript{33} for temperature control. All covalent bonds involving hydrogen atoms were constrained using the SHAKE algorithm,\textsuperscript{34} allowing a simulation time step of 2 fs throughout the simulations. After the equilibration, production simulations were carried out with the GPU implementation\textsuperscript{35} of PMEMD.CUDA module and trajectory frames were collected every 1 ps for a total of 1 μs. 1–4 nonbonded interactions were not scaled\textsuperscript{36} 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 \( \theta \), in crystal structures similar to 1–3 were extracted from the Cambridge Structural Database via ConQuest using the queries shown in Scheme 6.\textsuperscript{37,38} Structure 14 queried for 3, structure 15 for 1α/β and 2β, and structure 16 for 2α. Mean positions and CSDs of \( \theta \), for each data set were calculated using Mercury.\textsuperscript{39}

RESULTS AND DISCUSSION

Spin-Coupling Constants Sensitive to \( \theta \), in 1–3. Two bonds define O-acetyl side-chain conformation in 1β (Scheme 7): \( \theta_1 \) defines rotation about the C3–O3 bond, and \( \sigma_1 \) defines rotation about the C1′–O3 bond. In principle, \( \theta_1 \) is free to rotate through 360° in solution, whereas \( \sigma_1 \) determines cis/trans configuration of the ester and is likely to be more constrained than \( \theta_1 \) (e.g., C2′–C1′–O3–C3 torsions of either 0° (cis) or 180° (trans)). Inspection of 1β reveals ten spin-couplings that are potentially sensitive to \( \theta_1 \), and one that is potentially sensitive to \( \sigma_1 \) (Scheme 7). DFT calculations on model structure 5 were conducted to determine the sensitivities of these spin-couplings to rotation about \( \theta_1 \). The geminal \( J_{C1,C2} \) and the vicinal \( J_{C1,C4} \), \( J_{C1,C2} \) and \( J_{C1,C4} \) were treated quantitatively for use in conformational modeling of \( \theta_1 \) in 1–3 (see below). The general behaviors of the remaining six spin-couplings (Figure 1) are discussed here.

Calculated overall changes in \( J_{C1,C3} \), \( J_{C2,C2} \), \( J_{C3,C3} \), \( J_{C2,C4} \), and \( J_{C2,C4} \) as a function of \( \theta_1 \) are as follows: \( J_{C1,C3} \) (2.6 Hz); \( J_{C2,C2} \) (6.7 Hz); \( J_{C3,C3} \) (6.5 Hz); \( J_{C2,C4} \) (4.6 Hz); \( J_{C3,C3} \) (4.5 Hz); and \( J_{C2,C4} \) (1.4 Hz) (Figure 1). Considering only staggered conformers (\( \theta_1 \) = 60°, 180°, −60°), \( J_{C2,C4} \) is large when \( \theta_1 \) = 60° and −60°, that is, when one of the O3 lone-pair orbitals is anti to the C3–H3 bond. Calculated values of \( r_{C2H} \) are as follows: 1.0956 Å (60°); 1.0951 Å (180°); and 1.0958 Å (−60°). Larger values of \( J_{C2H} \) at \( \theta_1 \) = 60° and −60° correspond to larger \( r_{C2H} \) contrary to expectation,\textsuperscript{40} indicating that factors other than bond length affect \( J_{C2H} \) in these systems. In contrast, when the O-acetyl side-chain is replaced by an OH group, \( r_{C2H} \) is larger in the two rotamers having one of the oxygen lone pairs anti to the CH bond, and smaller \( J_{C2H} \) values are associated with these rotamers.

Plots of \( J_{C2,C2} \) and \( J_{C3,C3} \) (Figure 1) are essentially mirror images and show nearly identical dynamic range (≈6.5 Hz). As found for \( J_{C2H} \), \( J_{C3,H3} \), and \( J_{C3,C4} \) values increase with increasing \( r_{C2H} \), contrary to the behavior of the corresponding hydroxyl compound.\textsuperscript{40} Plots of \( J_{C2H} \) and \( J_{C3,H3} \) (Figure 1) are also essentially mirror images, with more positive values (\( J_{C2H} \) at \( \theta_1 \) = 60°; \( J_{C3,H3} \) at \( \theta_1 \) = −60°) 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.\textsuperscript{40} \( J_{C2,C4} \) shows a weak dependence on \( \theta_1 \) (Figure 1) that deviates from that observed in the corresponding hydroxyl compound in which \( \theta_1 \) = 180° (O2 lone pairs anti to both C–C bonds in the pathway) gives a more positive coupling than found in the remaining two rotamers.\textsuperscript{40}

These results show that the behaviors of \( J_{C2H} \), \( J_{C3,C3} \), \( J_{C2,C4} \), and \( J_{C3,C3} \) 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).

Scheme 7. NMR J-Couplings Sensitive to \( \theta_1 \) and \( \sigma_1 \) in 1β

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
Energetics of Rotation about θX. Plots of total energy determined by DFT for 5 and 6 (Figure 2A) are nearly symmetric about θX = 180°, with the global energy minimum located at θX = 0° and a local minimum located at θX = 180°. 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 θX 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 θX = 0° 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 θX = ∼160°. This displacement is presumably caused by steric interactions between the equatorial 2-O-acetyl side-chain and the adjacent axial O1 that occur when θX assumes values of ∼180°−360°. The energy difference between the two minima in 7 is ∼20 kJ/mol. These findings show that the rotational behavior of θX 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 σx in 1−3. DFT calculations on 5−9 were conducted at fixed values of σx, 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 3JC2′,C3 in 5 and 1β, respectively. A plot of calculated 3JC2′,C3 as a function of θX in 5 is shown in Figure 3. The experimental 3JC2′,C3 in 1β of 1.3 ± 0.1 Hz is very similar to the calculated value of 1.4 Hz at θX = 0 (Figure 3). The calculated value of 3JC2′,C3 at θX = 180° 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 θX in 1−3. Experimental J-couplings involving O-acetyl side-chain atoms were measured by introducing 13C isotopes into the side-chain (Scheme 2). This approach limited J-coupling measurements to four values (e.g., 2JC1′,C3, 3JC1′,H3, 3JC1′,C2, and 3JC1′,C4 in 1β). The three vicinal J-couplings, which display Karplus dependencies on θX (see below), were used in conformational analyses of θX of 1−3. Geminal 2JC1′,C3 values

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Figure 1. Calculated 1J and 2J values in 5 as a function of θX (A) 1JC3,H3, (B) 1JC2,C3, (C) 1JC3,C4, (D) 2JC2,H3, (E) 2JC4,H3, and (F) 2JC2,C4. Red points correspond to the three perfectly staggered rotamers of θX. Overall changes in each J-value are shown in green.
were also used in these analyses because they show a systematic dependence on $\theta_x$ with a dynamic range of 2.2 Hz (Figure 4A). $J$ values are commonly affected by the bond angle subtended by the coupled nuclei, and linear relationships between the $C_1'-O_X-C_X$ bond angles and $2J_{C_1',CX}$ values in 5–8 are observed (Figures 4B and S1), with $2J_{C_1',CX}$ values becoming more negative as the bond angle increases. This bond angle effect underlies the dependence of $2J_{C_1',CX}$ on $\theta_X$.

Vicinal $^{13}C-^1H$ Spin-Coupling Constants. $J_{C_1',C_X}$ values were calculated in model structures 5–9 as a function of $\theta_X$, where $x = 2, 3,$ or 6 (Figure 5). No appreciable differences were observed between the $C_1'-O_X-C_X-H_X$ 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, and calculated $J_{C_1',C_X}$ values are $\sim 5$ Hz at $\theta_X = 0^\circ$ and $\sim 9.5$ Hz at $\theta_X = 180^\circ$. A Karplus curve reported previously for the same coupling pathway is also shown in Figure 5. 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 $J_{C1,Hx}$ values except for $J_{C1,H6}$ in 9, for which a second equation was parametrized to account for the phase shift.

$$J_{C1,Hx} = 3.92 - 2.11 \cos(\theta) + 0.05 \sin(\theta) + 3.34 \cos(2\theta) + 0.07$$

$$\sin(2\theta) - 0.04 \cos(3\theta) + 0.05 \sin(3\theta) \quad \text{rms 0.22 Hz} \quad (2)$$

$$J_{C1,H6} = 4.06 + 0.80 \cos(\theta) + 1.63 \sin(\theta) - 1.99 \cos(2\theta) + 2.91$$

$$\sin(2\theta) - 0.15 \cos(3\theta) - 0.20 \sin(3\theta) \quad \text{rms 0.12 Hz} \quad (3)$$

**Vicinal $^{13}C$–$^{13}C$ Spin-Coupling Constants.**

Vicinal $^{13}C$–$^{13}C$ spin-coupling constants calculated in 5–9 fall into two groups when plotted against $\theta_x = C1'–O_x–C_x–H_x$ (Figure 6A,B). A torsion angle of 0° between the coupled carbons (i.e., when $\theta_x = 120^\circ$) gives a calculated $J_{CCOC}$ of ~2 Hz, whereas a torsion angle of 180° ($\theta_x = 300^\circ$) gives a calculated $J_{CCOC}$ of 4–5 Hz. Curves obtained for $C1'–O_x–C_x–C_y$ ($y = x \pm 1$) coupling pathways in which one of the coupled carbons ($C_y$) is C1 (an anomic carbon) (e.g., $J_{C1,CL}$ in 7 and 8) (Figure 6B) differ from the curves for pathways involving nonanomic carbons, especially for C–O–C–C torsion angles near 180° ($\theta_x$ near 60°). At $\theta_x$ near 60°, $J_{C1,CL}$ 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-anomic (5 and 6), and eq 6 for the case where one of the coupled carbons is anomic (7 and 8).

$$J'_{C1,CL} = 1.56 + 0.36 \cos(\theta) - 0.73 \sin(\theta) - 0.75 \cos(2\theta) - 1.22$$

$$\sin(2\theta) - 0.14 \cos(3\theta) + 0.02 \sin(3\theta) \quad \text{rms 0.13 Hz} \quad (4)$$

$$J''_{C1,CL} = 1.44 + 0.33 \cos(\theta) - 0.74 \sin(\theta) - 0.68 \cos(2\theta) - 1.21$$

$$\sin(2\theta) - 0.14 \cos(3\theta) + 0.16 \sin(3\theta) \quad \text{rms 0.04 Hz} \quad (5)$$

$$J'_{C1,CL} = 1.86 + 0.51 \cos(\theta) - 1.08 \sin(\theta) - 0.94 \cos(2\theta) + 1.46$$

$$\sin(2\theta) - 0.25 \cos(3\theta) - 0.17 \sin(3\theta) \quad \text{rms 0.16 Hz} \quad (6)$$

**Geminal $^{13}C$–$^{13}C$ Spin-Coupling Constants.**

Geminal $^{13}C$–$^{13}C$ spin-couplings in 5–9 involving C1′ as a coupled nucleus depend on $\theta_x$ (Figure 7). This dependency is a manifestation of the effect of $\theta_x$ on the C1′–Ox–Cx bond angle, which in turn influences $J_{C1',CL}$ (Figure 4; Figure S1). The curves for $J_{C1,CL}$ 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 $\theta_x$ (Figure S1). The curves for 5 and 6, in which the C–O–C–C–H4 bond angle is rectangular (the minimum at $\theta_x = \sim 240^\circ$ is absent), is attributed to a lack of steric hindrance that allows smaller C–O–C bond angles at these values of $\theta_x$ (Figure S1).

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

$$J'_{C1,CL} \quad (\text{for } 5/6) = -3.81 + 0.64 \cos(\theta) - 0.14 \sin(\theta) + 0.84 \cos(2\theta)$$

$$+ 0.05 \sin(2\theta) - 0.29 \cos(3\theta) - 0.03 \sin(3\theta) \quad \text{rms 0.10 Hz} \quad (7)$$

$$J''_{C1,CL} \quad (\text{for } 7) = -3.79 + 0.95 \cos(\theta) - 0.45 \sin(\theta) + 0.42 \cos(2\theta)$$

$$- 0.14 \sin(2\theta) - 0.28 \cos(3\theta) + 0.36 \sin(3\theta) \quad \text{rms 0.13 Hz} \quad (8)$$
Statistical Modeling of $\theta_3$ in 1–3. Experimental spin-couplings in 1$\alpha$ and 1$\beta$ (Scheme 2; Table 1) are essentially identical, indicating qualitatively that the effect of anomeric configuration on the conformational behavior of $\theta_3$ for an equatorial 3-O-acetyl side-chain is small. The same conclusion pertains to the 6-O-acetyl side-chains in 3$\alpha$ and 3$\beta$. In contrast, experimental spin-couplings in 2$\alpha$ and 2$\beta$ 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 $\theta_3$ (Figure 8, Table 2). The MD results exhibit multimodality for $\theta_x$ 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 $\theta_x$ 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 $\theta_x$ 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

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

<table>
<thead>
<tr>
<th>$J$-coupling</th>
<th>1$\alpha$</th>
<th>1$\beta$</th>
<th>2$\alpha$</th>
<th>2$\beta$</th>
<th>3$\alpha$</th>
<th>3$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{C1',C3}$</td>
<td>± 2.8</td>
<td>± 2.8 (−2.7)</td>
<td>± 2.8 (−2.7)</td>
<td>± 2.8 (−2.7)</td>
<td>± 2.8 (−2.7)</td>
<td>± 2.8 (−2.7)</td>
</tr>
<tr>
<td>$J_{C1',H3}$</td>
<td>3.9</td>
<td>3.9 (4.6)</td>
<td>3.9 (4.6)</td>
<td>3.9 (4.6)</td>
<td>3.9 (4.6)</td>
<td>3.9 (4.6)</td>
</tr>
<tr>
<td>$J_{C1',C2}$</td>
<td>1.0</td>
<td>1.0 (1.2)</td>
<td>1.0 (1.2)</td>
<td>1.0 (1.2)</td>
<td>1.0 (1.2)</td>
<td>1.0 (1.2)</td>
</tr>
<tr>
<td>$J_{C1',C4}$</td>
<td>1.0</td>
<td>1.0 (1.1)</td>
<td>1.0 (1.1)</td>
<td>1.0 (1.1)</td>
<td>1.0 (1.1)</td>
<td>1.0 (1.1)</td>
</tr>
<tr>
<td>$J_{C1',C5}$</td>
<td>1.1</td>
<td>1.3 (1.5)</td>
<td>1.3 (1.5)</td>
<td>1.3 (1.5)</td>
<td>1.3 (1.5)</td>
<td>1.3 (1.5)</td>
</tr>
<tr>
<td>$J_{C1',C6}$</td>
<td>± 2.6 (−2.7)</td>
<td>± 2.6 (−2.7)</td>
<td>± 2.6 (−2.7)</td>
<td>± 2.6 (−2.7)</td>
<td>± 2.6 (−2.7)</td>
<td>± 2.6 (−2.7)</td>
</tr>
<tr>
<td>$J_{C1',C2}$</td>
<td>3.6 (3.7)</td>
<td>3.6 (3.7)</td>
<td>3.6 (3.7)</td>
<td>3.6 (3.7)</td>
<td>3.6 (3.7)</td>
<td>3.6 (3.7)</td>
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<tr>
<td>$J_{C1',C3}$</td>
<td>0.7 (1.1)</td>
<td>0.7 (1.1)</td>
<td>0.7 (1.1)</td>
<td>0.7 (1.1)</td>
<td>0.7 (1.1)</td>
<td>0.7 (1.1)</td>
</tr>
<tr>
<td>$J_{C1',C4}$</td>
<td>2.0 (2.1)</td>
<td>2.0 (2.1)</td>
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<td>$J_{C1',C5}$</td>
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<td>1.1 (1.4)</td>
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<td>2.4 (3.0)</td>
<td>2.4 (3.0)</td>
<td>2.4 (3.0)</td>
<td>2.4 (3.0)</td>
<td>2.4 (3.0)</td>
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<tr>
<td>$J_{C1',H6}$</td>
<td>2.8</td>
<td>2.8 (3.1)</td>
<td>2.8 (3.1)</td>
<td>2.8 (3.1)</td>
<td>2.8 (3.1)</td>
<td>2.8 (3.1)</td>
</tr>
<tr>
<td>$J_{C1',H6}'$</td>
<td>2.5</td>
<td>2.5 (2.3)</td>
<td>2.5 (2.3)</td>
<td>2.5 (2.3)</td>
<td>2.5 (2.3)</td>
<td>2.5 (2.3)</td>
</tr>
</tbody>
</table>

$^a$In Hz ± 0.1 Hz, in $^2$H$_2$O at 25 °C. Signs of the experimental $^2J$ values were not determined. $^b$Values shown in parentheses were calculated from the MD histograms shown in Figure 8; 10 for 1$\beta$, 11 for 2$\alpha$, 12 for 2$\beta$, and 13 for 3$\beta$. $^c$The more shielded H6 proton in 3 is defined as H6'.

Figure 8. Histograms showing the distributions of $\theta_x$ in 11 (A), 12 (B), 10 (C), and 13 (D) obtained from 1-μs aqueous molecular dynamics (MD) simulations.
minima in plots of calculated total energy as a function of $\theta_X$ (Figure 2) for 5, 7, and 8, which model $1/\beta$, $2/\alpha$, and $2/\beta$, 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 $\theta_X$ in 1–3 differ (Figure 9), with 1 and 2/3 giving values close to the idealized value of $0^\circ$ in which the C–O bond of the ester eclipses the C–H bond of the alcoholic carbon. This eclipsing is reduced in 2/2 where the mean position of $\theta_x$ is $\sim -20^\circ$. The O-acetyl side-chains in 3/3 and 3/3 deviate significantly from an eclipsed geometry, giving mean positions of $\theta_x$ near $-60^\circ$. 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 3/3 is 472 the least sterically constrained and gives the largest CSD. Compounds 1/3/3 and 2/3/3 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 2/3, one of the flanking hydroxyl groups is in an axial orientation, allowing more rotational freedom about $\theta_2$ and a CSD intermediate between the other three models. These results indicate that the statistical method presented herein can 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 $\theta_x$, 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 Lorentz.$^{24}$ (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

Table 2. Mean Positions, Circular Standard Deviations (CSDs), and RMS Errors Calculated Using Different Models to Analyze Redundant $J$-Couplings in 1–3

<table>
<thead>
<tr>
<th>cmpd</th>
<th>X-ray$^a$</th>
<th>MD$^b$</th>
<th>raised cosine</th>
<th>uniform</th>
<th>power of cosine</th>
<th>von Mises</th>
<th>Lorentz</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\alpha$ (2-OAc)</td>
<td>$-9.4$</td>
<td>$-16.4$</td>
<td>$-21.9$ (13)</td>
<td>$-21.9$ (12)</td>
<td>$-22.3$ (13)</td>
<td>$-22.5$ (13)</td>
<td>$-25.8$ (13)</td>
</tr>
<tr>
<td>$2\beta$ (2-OAc)</td>
<td>$-0.2$</td>
<td>$-2.8$</td>
<td>$-1.0$ (10)</td>
<td>$-1.0$ (10)</td>
<td>$-1.1$ (10)</td>
<td>$-1.1$ (10)</td>
<td>$-1.3$ (12)</td>
</tr>
<tr>
<td>$1$ (3-OAc)</td>
<td>$-0.2$</td>
<td>$-0.1$</td>
<td>$0.6$ (12)</td>
<td>$0.6$ (12)</td>
<td>$0.7$ (12)</td>
<td>$0.6$ (12)</td>
<td>$0.7$ (14)</td>
</tr>
<tr>
<td>$3$ (6-OAc)</td>
<td>$-55.8$</td>
<td>$-63.0$</td>
<td>$-66.5$ (30)</td>
<td>$-66.1$ (31)</td>
<td>$-66.7$ (29)</td>
<td>$-66.4$ (26)</td>
<td>$-66.6$ (19)</td>
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</tbody>
</table>

RMS errors (Hz)

<table>
<thead>
<tr>
<th>cmpd</th>
<th>X-ray$^a$</th>
<th>MD$^b$</th>
<th>raised cosine</th>
<th>uniform</th>
<th>power of cosine</th>
<th>von Mises</th>
<th>Lorentz</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\alpha$ (2-OAc)</td>
<td>$28.9$</td>
<td>$29.4$</td>
<td>$28.5$ (11)</td>
<td>$28.1$ (12)</td>
<td>$29.3$ (9)</td>
<td>$29.9$ (16)</td>
<td>$40.2$ (19)</td>
</tr>
<tr>
<td>$2\beta$ (2-OAc)</td>
<td>$16.5$</td>
<td>$23.0$</td>
<td>$23.8$ (9)</td>
<td>$23.1$ (10)</td>
<td>$24.8$ (8)</td>
<td>$25.3$ (13)</td>
<td>$39.8$ (15)</td>
</tr>
<tr>
<td>$1$ (3-OAc)</td>
<td>$16.5$</td>
<td>$24.2$</td>
<td>$22.6$ (9)</td>
<td>$22.1$ (10)</td>
<td>$23.4$ (10)</td>
<td>$23.8$ (13)</td>
<td>$37.0$ (15)</td>
</tr>
<tr>
<td>$3$ (6-OAc)</td>
<td>$45.1$</td>
<td>$51.9$</td>
<td>$40.1$ (8)</td>
<td>$37.3$ (8)</td>
<td>$40.4$ (9)</td>
<td>$42.5$ (12)</td>
<td>$52.1$ (11)</td>
</tr>
</tbody>
</table>

$^a$See Statistical Analysis of Crystallographic Data Section for details. $^b$See Molecular Dynamics (MD) Simulations Section for details.
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 2α 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, raise 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 values far from the mean position.

Since the models for 1, 2α, 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 $J_{C1,C0}$ 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, 2α, 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 $\theta_1$ (for $J_{C1,C0}$, $J_{C3,C0}$, $J_{C2,HH}$, $J_{C3,HH}$, $J_{C1,CM}$, $J_{C1,CH}$, and $J_{C1,CA}$). Because of experimental limitations, only four $J$-couplings were parametrized in this work and used to evaluate $\theta_1$ in six 13C-labeled compounds: $1\alpha$, $1\beta$, $2\alpha$, $2\beta$, $3\alpha$, and $3\beta$ (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 $J$-couplings showed that anomeric configuration does not affect $\theta_1$ in $1\alpha$, $1\beta$, $3\alpha$, and $3\beta$, but the effect was significant in $2\alpha$ and $2\beta$ 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. 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 Dunitz and by González-Outeiriño et al. These prior studies determined the conformational preferences of $\theta$ in secondary alcohols based on a survey of structures in the Cambridge Structural Database, and derived a Karplus equation for $J_{C1,3\alpha}$ from $J$-couplings in conformers with $\theta = 0^\circ$, 60°, and 180°. The present study extends this prior work by reparametrizing the $J_{C1,3\alpha}$ Karplus equation, parametrizing three new spin-couplings sensitive to θ (for example, $J_{C1,CD}$, $J_{C1,CL}$, and $J_{C1,CL}$ in 1), and treating ensembles of four redundant J-couplings to give rotameric populations about $\theta_1$ 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 $\theta_1$ 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 ($\phi$) and psi ($\psi$) 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. 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. 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.

## ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b10028.

Torsional constraints used in DFT calculations of S–9; NMR J-couplings calculated by DFT in S–9; definitions of θ rotamers in 1–9; plots showing correlations between 3J_C1,H6 and C1′–O–C1  bond angle in S–8 (x = 2 or 3); plot of calculated 3J_C1,H6 in 9 after shifting the latter curve shown in Figure 5 by 120°; plots of calculated 3J_C1,C2 as a function of θ, in S–9, where x = 2, 3, or 6 and y = x ± 1; parameter space for uniform models of 1, 2α, and 2β; partial 13C{1H} NMR spectrum (150 MHz) of 15α and 17β; partial 13C{1H} NMR spectrum (150 MHz) showing signals arising from C3 of 15′α and 15′β, and C2 of 2β; partial 13C{1H} NMR spectrum (150 MHz) showing signals arising from C2 of 15′β, C2 of 2α, C3 of 2β, and C5 of 3β; partial 13C{1H} NMR spectrum (150 MHz) showing signals arising from C3 of 15′α, C2 of 15′α, C5 of 3α, and C4 of 15′α/β; partial 1H NMR spectrum (600 MHz) showing signals arising from H3 of 15′α and 15′β; partial 1H NMR spectrum (600 MHz) showing signals arising from H6 of 15′α and 15′β; partial 1H NMR spectrum (600 MHz) showing signals arising from H2 of 2α and 2β; Cartesian coordinates for B3LYP optimized conformers of S; complete ref 16; and complete ref 32 (PDF)

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Notes

The authors declare no competing financial interest.

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