Solid-State NMR Measurements of $^{13}$C-$^{13}$C Spin-Couplings in Carbohydrates: Validation of the Effects of Conformation on Coupling Magnitudes

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Abstract
Carbohydrates are flexible molecules that play pivotal roles in many biological processes. Identifying the factors that influence their structures is vital to understanding the effects of glycosylation on the functions of glycoproteins and glycolipids. Although NMR provides unique structural information in solution, measured NMR parameters are time-averaged by molecular motion, making it difficult to correlate them directly to structure. The latter complication can be eliminated in some cases by combining isotopic labeling, solid-state NMR (ssNMR), x-ray crystallography, and density functional theory (DFT) calculations. To illustrate this application, methyl β-D-[1,3,13C3]glucopyranoside 1 was prepared and $^{2}J_{C1,C3}$ values of +4.6 ± 0.1 Hz and +6.5 ± 0.3 Hz were measured in solution and in the crystalline state, respectively. The origin of the ~2 Hz difference was identified by conducting DFT calculations on 1. $^{2}J_{C1,C3}$ Values were calculated as a function of the C1-C2-O2-H torsion angle, and the results yielded a value of ~6.0 Hz for a C1-C2-O2-H torsion angle of 90°, the value of 6.0 Hz correlates directly with a fixed C1-C2-O2-H torsion angle of 90°. This integrated approach, which exploits measurements of J-couplings by ssNMR, provides valuable new experimental data to validate the results of DFT calculations, and has potential applicability in structural studies of crystalline saccharide-receptor complexes.

A. Background
Experimental J-couplings measured in solution are averaged by the molecular motions of the molecule. For example, rotations of the two C-O bonds $\theta_1$ and $\theta_2$ in glucopyranosyl ring 1 affect the value of $^{1}J_{C1,C2}$. Rotation of the C5-C6 bond $\omega$ in 1 affects $^{2}J_{C1,C3}$ because of the changing disposition of O6 with respect to the C1-O5-C5-C6 coupling pathway, while rotation of the C2-O2 bond $\theta_2$ in 1 exerts a significant affect on $^{2}J_{C1,C3}$ (rotating $\theta_1$ does not). Complications arising from time-averaging can be addressed by combining $^{13}$C isotopic labeling, solid-state NMR (ssNMR), x-ray crystallography, and density functional theory (DFT) calculations.

C. Synthesis of doubly $^{13}$C-labeled samples
Cyanohydrin reduction using K$_{13}$CN and D-[2-$^{13}$C]arabinose yielded, after purification and Fischer glycosidation, glycoside 2 in >98% chemical purity. Compound 3 was prepared by K$_{13}$CN and D-[1-$^{13}$C]glucose, and K$_{13}$CN via 1,2,3-tri-O-propanoyl-α-D-[1-$^{13}$C]xylo-pentodialdo-1,4-furanose. Compounds 2 and 3 were crystallized from water/methanol solvent.

D. $J_{CC}$ Values in aqueous solution
$^{2}J_{C1,C3}$ and $^{1}J_{C1,C6}$ values were measured in 100 % $^2$H$_2$O at 150 MHz for $^{13}$C.

$^{2}J_{C1,C3} = (+)4.6$ Hz; $^{1}J_{C1,C6} = (+)4.3$ Hz (signs determined by experiment)

E. $J_{CC}$ Values in the solid state
Application of the pulse sequence developed by Thureau et al. (JMR 2013, 231, 90) enabled selective measurement of long-range homonuclear $^{13}$C values in solid (crystalline) samples of 2 and 3.

$^{2}J_{C1,C3} = 6.5$ Hz; $^{1}J_{C1,C6} = 4.0$ Hz.

Summary
$^{2}J_{C1,C3}$ and $^{1}J_{C1,C6}$ values were measured on doubly $^{13}$C-labeled methyl β-D-glucopyranosides in solution and in the solid state.

The C1-C2-O2-H torsion angle obtained from the x-ray structure corresponds to a $^{2}J_{C1,C3}$ value of ~6.1 Hz based on DFT calculations. This value is in good agreement with the $^{2}J_{C1,C3}$ value obtained by ssNMR, which is ~2 Hz larger than that obtained in solution. For $^{1}J_{C1,C6}$, there is very good agreement between the DFT and ssNMR results.

Solid-state NMR measurements of $J_{CC}$ values allow direct correlations between coupling magnitude and molecular conformation since the effects of molecular averaging are eliminated.

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