4362 *J. Am. Chem. Soc.* **2001,** *123,* 4362−4363

# A Tethered Disaccharide Trapped As Its *anti* Conformer Calibrates the Karplus Relationship for $^3J_{C,H}$ Coupling Constants

Shirley A. Wacowich-Sgarbi,[†] Chang Chun Ling,
Albin Otter, and David R. Bundle*

*Department of Chemistry, The University of Alberta*
*Edmonton, Alberta, T6G 2G2, Canada*

*Received December 19, 2000*

We show here that a tethered disaccharide trapped in the *anti* conformation with a $\psi$ glycosidic torsional angle of $\sim$160° provides an ideal model compound to calibrate the Karplus type relationship that describes the torsional dependence of three bond heteronuclear coupling constants for this class of molecules. The observed value of $^3J_{C,H}$ exceeds that calculated by empirically derived Karplus relationships[1] but is less than those predicted by a recent theoretical treatment.[2]

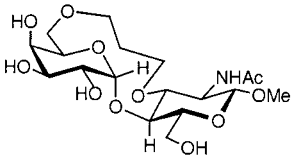
Interest in these coupling constants arises because a paucity of distance and torsional angle constraints renders the experimental elucidation of the solution conformations of oligosaccharides underdetermined.[3] This complex situation is compounded by motional averaging between numerous conformational microstates.[4] While NOE-derived distance constraints have been the most frequently used parameter to infer solution conformations of oligosaccharides, torsional angles across the glycosidic heteroatom have been recognized for some time as a valuable parameter.[5] Difficulties that once limited the measurement of this parameter have been overcome through increased use of $^{13}C$ labeling[6] and by the introduction of a variety of NMR experiments.[7] While model compounds have been used to derive experimental Karplus relationships,[1] those that closely mimic actual glycosidic bonds of oligosaccharides are restricted in number and could potentially yield anomalous couplings due to the presence of structural strain. This is especially true for the region of the Karplus type curve that corresponds to the higher-energy *anti* conformer $\psi = 180°$, a conformation whose population has been the focus of intense interest.[6a,c,8] Since the NMR pulse sequence “EXSIDE” employed to measure this coupling constant can be scaled in the F1 dimension, this parameter can be obtained with some precision.[7d,9]

**Table 1.** Selected *inter*-Residue Proton Distances in Å of **1** and **2**[a]

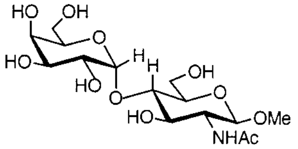
| | **1** *anti* | | | **2** *syn* | | |
|---|---|---|---|---|---|---|
| | H1′−H3 | H1′−H4 | H1′−H5 | H1′−H3 | H1′−H4 | H1′−H5 |
| exptl NOE | 2.4 | 3.6 | 2.3 | 3.6 | 2.2 | 3.9 |
| 5 ns MD sim | 2.3 | 3.7 | 2.4 | 3.7 | 2.4 | 4.4 |

[a] NOE data obtained from 600 MHz TROESY experiments, mixing time 400 ms, in pyridine-$d_5$ at 27.0 °C; distance calculations based on 2.44 Å α-Gal-H1:H2 reference distance.

The synthetic tethered disaccharide **1**[10] provides a rare example of a glycosidic linkage trapped in the high energy *anti* conformation and is the first such example for an unprotected oligosaccharide. In solution the unrestrained disaccharide **2** predominantly populates the *syn* conformation. The $^1H$ and $^{13}C$ resonances and coupling constants of **1** and **2** were assigned for solutions in $D_2O$, methanol-$d_4$, and pyridine-$d_5$ (Supporting Information). The homonuclear $^3J_{1,2}$ coupling constant of 3.8 Hz and single bond heteronuclear $^1J_{C,H}$ coupling constants unambiguously define the α configuration of the Gal-GlcNAc glycoside linkage for both disaccharides **1** and **2**.[11] The homonuclear $^3J$ coupling constants observed for the pyranose residues in each disaccharide indicate that both monosaccharide residues exist as chair conformers. However, the pattern of *inter*-residue NOEs for the two disaccharides differs dramatically. While **2** exhibited the expected NOEs for a predominant *syn* conformation with a strong NOE for the H1′/H4 pair and very weak NOEs between H1′/H3 and H1′/H5, disaccharide **1** exhibited a weak NOE between H1′/H4 and strong NOEs for the pairs H1′/H3 and H1′/H5 (Table 1). These NOEs are only consistent with the *anti* conformation. Molecular dynamics calculations performed for disaccharide **1** with and without the NOE constraints clearly indicated that the *anti* conformation is adopted and, furthermore, predicts a $\psi$ torsional angle of 162°.



**1**



**2**

Measurement of three-bond heteronuclear coupling constants across the glycosidic linkage provide time-averaged values of the two torsional angles $\phi$ and $\psi$ but due to their often small numerical value, passive coupling pathways and intrinsically low sensitivity, extraction of the parameter is often difficult, especially for compounds that are not isotopically enriched.[7c] We recently used

---

[†] The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037.

(1) (a) Mulloy, B.; Frenkiel, T. A.; Davies, D. B. *Carbohydr. Res.* **1988**, *184*, 39−46. (b) Tvaroska, I.; Hricovini, M.; Petrakova, E. *Carbohydr. Res.* **1989**, *189*, 359−362. (c) Tvaroska, I.; Taravel, F. *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 15−61.

(2) Cloran, F.; Carmichael, I.; and Serianni, A. S. *J. Am. Chem. Soc.* **1999**, *121*, 9843−9851.

(3) (a) Dabrowski, J.; Poppe, L. *J. Am. Chem. Soc.* **1989**, *111*, 1510−1511. (b) van Halbeek, H. *Curr. Opin. Struct. Biol.* **1994**, *4*, 697−709. (c) Poppe, L.; Stuike-Prill, R.; Meyer, B.; van Halbeek, H. *J. Biomol. NMR.* **1992**, *2*, 109−136. (d) Poppe, L.; van Halbeek, H. *J. Am. Chem. Soc.* **1992**, *114*, 1092−1094. (e) Bock, K.; Duss, J. *J. Carbohydr. Chem.* **1994**, *13*, 513−543.

(4) (a) Cumming, D. A.; Carver, J. P. *Biochemistry* **1987**, *26*, 6664−6676. (b) Carver, J. P. *Curr. Opin. Struct. Biol.* **1991**, *1*, 716−720. (c) Bush C. A. *Curr. Opin. Struct. Biol.* **1992**, *2*, 655−660. (d) Rutherford, T. J.; Partridge, J.; Weller, C. T.; Homans, S. W. *Biochemistry* **1993**, *32*, 12715−12724. (e) Homans, S. W. *Glycobiology* **1993**, *3*, 551−555.

(5) (a) Lemieux, R. U. *Ann. N.Y. Acad. Sci.* **1973**, *222*, 915−934. (b) Delbaere, L. T. J.; James, M. N. G.; Lemieux, R. U. *J. Am. Chem. Soc.* **1973**, *95*, 7866−7868. (c) Hamer, G. K.; Balza, F.; Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1978**, *56*, 3109−3116.

(6) (a) Milton, M. J.; Harris, R.; Probert, M. A.; Field, R. A.; Homans, S. W. *Glycobiology* **1998**, *8*, 147−153. (b) Richardson, J. M.; Evans, P. D.; Homans, S. W.; & DonohueRolfe, A. *Nat. Struct. Biol.* **1997**, *4*, 190−193. (c) Dabrowski, J.; Kozár, T.; Grosskurth, H.; Nifantev, N. E. *J. Am. Chem. Soc.* **1995**, *117*, 5534−5539.

(7) (a) Hricovini, M.; Tvaroska, I.;Hirsch, J. *Carbohydr. Res.* **1990**, *198*, 193−203. (b) Uhrin, D.; Mele, A.; Boyd, J.; Wormald, M. R.; Dwek, R. A. *J. Magn Reson.* **1992**, *97*, 411−418. (c) Poppe, L.; van Halbeek, H. *J. Magn Reson.* **1991**, *92*, 636−641. (d) Otter, A.; Lemieux, R. U.; Ball, R. G.; Venot, A. P.; Hindsgaul, O.; Bundle, D. R. *Eur. J. Biochem.* **1999**, *259*, 295−303.

(8) (a) Lipkind, G. M.; Shaskov, A. S.; Nikolaev, A. V.; Kochetkov, N. K. *Carbohydr. Res.* **1985**, *141*, 191−197. (b) Landersjo, C.; Shaskov, A. S.; Nikolaev. A. V.; Mamyan, S. S.; Kochetkov, N. K. *Bioorg. Khim.* **1987**, *13*, 1081−1093. (c) Poppe, L.; von der Leith, C.-W.; Dabrowski. J. *J. Am. Chem. Soc.* **1990**, *112*, 7762−7771. (d) Bock, K., Duus, J. O., Refin, S. *Carbohydr. Res.* **1994**, *253*, 51−67. (e) Espinosa, J. F.; Canada, F. J.; Asensio, J. L.; Martin-Pastor, M.; Dietrich, H.; Martin-Lomas, M.; Schmidt, R. R.; Jimenez-Barbero, J. *J. Am. Chem. Soc.* **1996**, *118*, 10862−10871. (f) Espinosa, J. F.; Montero, E.; Vian, A.; Garcia, J. L.; Dietrich, H.; Schmidt, R. R.; Martin-Lomas, M.; Imberty. A.; Canada, F. J.; Jimenez-Barbero, J. *J. Am. Chem. Soc.* **1998**, *120*, 1309−1318. (g) Geyer, A.; Müller, M.; Schmidt, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 6312−6313. (h) Masoud, H.; Perry, M. B.; Brisson, J.-R.; Uhrin, D.; Richards, J. C. *Can. J. Chem.* **1994**, *72*, 1466−1477. (i) Milton, M. J.; Bundle D. R. *J. Am. Chem. Soc.*, **1998**, *120*, 10547−10548.

(9) (a) Krishnamurthy, V. V. *J. Magn. Reson., Ser. A* **1996**, *121*, 33−41. (b) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, 67, 565−569.

(10) Wacowich-Sgarbi, S. A.; Bundle, D. R. Manuscript in preparation.

(11) Bock, K.; Pedersen, C. J. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293−297.

10.1021/ja005901m CCC: \$20.00

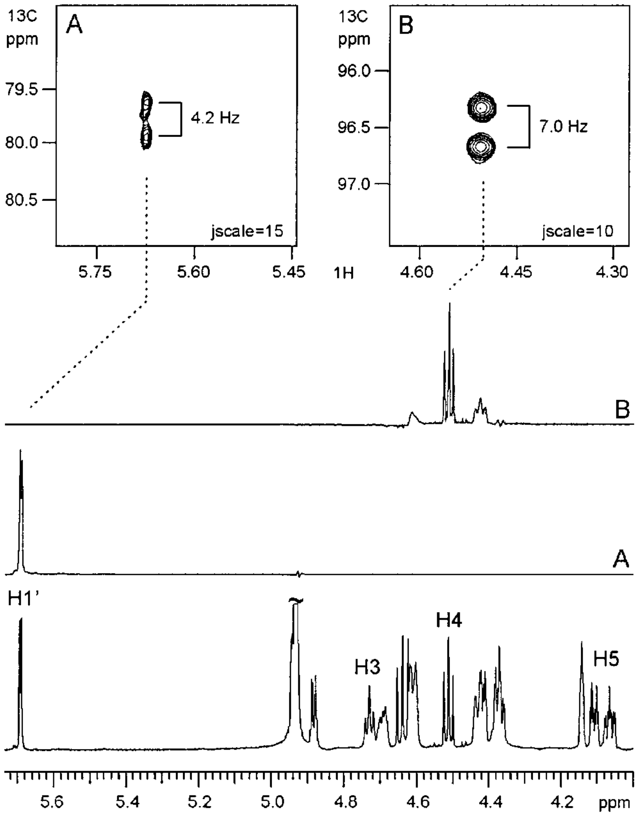


**Figure 1.** EXSIDE spectra of **1** at 800 MHz and selective excitation profiles for the H1′ and H4 protons.

the pulse sequence “EXSIDE” to measure these coupling constants for the blood group B-trisaccharide.[7d] The experiment requires selective excitation at the resonance frequency of the proton that is part of the H−C−O−C fragment. The shaped pulses must excite only the proton resonance of interest and must not perturb any protons that are coupled to this hydrogen. Here the fragments that define the $\phi$ and $\psi$ torsional angles are H1′−C1′−O1′−C4 and C1′−O1′−C4−H4. The determination of each coupling constant requires its own experiment.

For the EXSIDE experiment to provide the $^{3}J_{\mathrm{C,H}}$ coupling constant (fragment C1′−O1′−C4−H4; $\psi$ torsional angle) the resonance for H4 must be well separated from the $^{3}J$ coupled hydrogen atoms H3 and H5. To measure the coupling related to the $\phi$ torsional angle only requires that H1′ be well separated from H2′, a condition that is fulfilled for virtually all hexopyranose residues. The former condition is frequently not satisfied and for disaccharides **1** and **2** the resonances of H3, H4, and H5 were either too close or overlapped for solutions in $D_2O$, DMSO-$d_6$, and methanol-$d_4$. We resorted to the use of an aromatic solvent, pyridine-$d_5$ to disperse the resonances so that at 800 MHz field strength the three resonances were separated by at least 150 Hz. The EXSIDE experiment then provided correlation peaks (Figure 1) from which the heteronuclear coupling constants could be accurately measured (Table 2).

Although solutions in different solvents ($D_2O$, methanol-$d_4$ and pyridine-$d_5$) might be expected to experience solvent induced conformational shifts, the NOE data collected for solutions in each solvent are similar as are the coupling constants for the fragment H1′−C1′−O1′−C4. This suggests that the conformational equilibria are not significantly different in the three solvents, especially for **1**. Conclusions of this type were reported for blood group oligosaccharides that were shown by Bush et al. to exhibit solvent independent conformational preference.[12] In pyridine and methanol, the coupling constants (4.2 and 4.5 Hz) for the fragment H1′−C1′−O1′−C4 of disaccharides **1** and **2** are consistent with similar $\phi$ torsional angles. While disaccharide **2** is able to sample an unrestricted range of $\phi$ and $\psi$ torsional angles, its $^{3}J_{\mathrm{C,H}}$ values of 4.5 and 4.9 Hz are consistent with modeling data (Table 2) that suggests a narrow range of $\phi$ values with averaging of the $\psi$ torsional angle over two principal families of conformers that correspond to the *syn* and *anti* conformers. Literature data for disaccharides in DMSO or $D_2O$ solution are consistent with the sampling of the *anti* conformation.[6,9] Tethered disaccharide **1** exhibits a $^{3}J_{\mathrm{C,H}}$ value of 7 Hz that is consistent, according to the empirically derived Karplus type relationship,[1] with a $\psi$ torsional angle close to 180°. In fact the 7.0 Hz coupling constant is higher than the maximum predicted by the modified Karplus-type curve proposed by Tvaroska et al.[1b,c] or Mulloy et al.[1a]

**Table 2.** $^{3}J_{\mathrm{CH}}$ Coupling Constants in Hz and Torsional Angles of **1** and **2**[a]

| | **1** *anti* | | **2** *syn* | |
|---|---|---|---|---|
| | $\phi$ | $\psi$ | $\phi$ | $\psi$ |
| EXSIDE | 4.2 −**31°** | 7.0 −**180°** | 4.5 −**27°** | 4.9 −**21°** |
| 5 ns MD sim | 4.2 −**31°** | 6.2 −**160°** | 4.7 −**24°** | 4.7 −**24°** |

[a] Based on EXSIDE experiments at 800 (**1**) and 600 MHz (**2**) in pyridine-$d_5$ at 27.0 °C and application of the empirical Karplus relationship.[7a]

While combined NOE and molecular modeling data are consistent with a $\psi$ torsional angle close to 160°, the empirical Karplus relationship[1] would suggest a perfect *anti* conformation at $\psi = 180°$ and if $\psi = 160°$, a coupling constant of 6.1 Hz would be expected. Since the modeling and NOE data are based on three separate NOE constraints it seems more probable that the Karplus curve is under-calibrated and that a torsional angle of ∼160−170° yields a 7 Hz heteronuclear coupling constant. This implies that the numerical values for $^{3}J_{\mathrm{C,H}}$ across oligosaccharide glycosidic bonds for torsional angles close to 180° are too low by 10−20%. In support of this contention, a highly branched and sterically crowded bacterial polysaccharide was shown to possess a cellobiose disaccharide element that adopted the *anti* conformation.[8h] In this case the coupling constant was 7.4 Hz, a value also higher than the maximum predicted by the empirical relationship.

The $^{3}J_{\mathrm{C,H}}$ coupling constant for the *anti* conformation of **1**, while definitely larger than coupling constants predicted by empirical treatments,[1a−c] is smaller than predictions of the theoretical treatment.[2] More examples are therefore needed to calibrate the proportionality constants.

**Acknowledgment.** The research was supported by Natural Science and Engineering Research Council of Canada (NSERC) funding to D. R. Bundle. NMR spectra (800 MHz) were obtained at the Canadian National High Field NMR Centre (NANUC) funded by the Canadian Institutes of Health Research, NSERC, Alberta Heritage Foundation for Medical Research and the University of Alberta.

**Supporting Information Available:** Chemical shift assignments, molecular modeling parameters with minimum energy conformations of compounds **1** and **2**. This material is available free of charge via the Internet at *http://pubs.acs.org*. See any current masthead page for ordering information and Web access instructions.

JA005901M

---

(12) (a) Bush, C. A.; Yan, Z.-Y.; Rao, B. N. N. *J. Am. Chem. Soc.* **1986**, *108*, 6168−6173. (b) Mukhopadhyay, C.; Bush, C. A. *Biopolymers* **1991**, *31*, 1737−1746. (c) Yan, Z.-Y.; Rao, B. N. N.; Bush, C. A. *Carbohydr. Res.* **1988**, *180*, 111−128.