J|A|C|S
ARTICLES
Published on Web 02/27/2002

Using Equilibrium Isotope Effects To Detect Intramolecular OH/OH Hydrogen Bonds: Structural and Solvent Effects

Thomas E. Vasquez, Jr.,[†] Jon M. Bergset,[†] Matthew B. Fierman,[†] Alshakim Nelson,[†] Joshua Roth,[†] Saeed I. Khan,[‡] and Daniel J. O'Leary*,[†]

Contribution from the Department of Chemistry, Pomona College, 645 North College Avenue, Claremont, California 91711, and Department of Chemistry and Biochemistry, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, California 90024

Received August 17, 2001

Abstract: A comparative ^{1}H NMR study of partially deuterated 1,3- and 1,4-diols has demonstrated that intramolecular hydrogen bonds of different geometry can give rise to equilibrium isotope shifts of opposite sign in hydrogen-bond-accepting solvents such as DMSO-d_6, acetone-d_6, and THF-d_8. The sign inversion is interpreted in terms of the ability of solvent molecules to form competitive intermolecular hydrogen bonds with the diol and in terms of the limiting chemical shifts for the interior and exterior hydroxyl groups. Deuterium is shown to prefer the intermolecular solvent hydrogen bond by 10.9 ± 0.5 cal/mol for 1,4-diol **3** dissolved in DMSO-d_6 at room temperature. Pyridine-d_5 is shown to be capable of amplifying positive (downfield) isotope shifts measured in DMSO-d_6, in some cases by as much as a factor of 3. Its use is demonstrated for the assignment of the syn or anti relative configuration of 2,4-pentanediol and for the amplification of isotope shifts used to detect intramolecular hydrogen bonds in α- and β-cyclodextrin. Studies in apolar solvents such as CD_2Cl_2 and benzene-d_6 reveal that the isotope shift is negative (upfield) for all hydrogen bond geometries studied. Larger isotope shifts are measured in benzene-d_6, and a rationale for this amplification is presented. The use of apolar solvents is particularly useful for assigning the syn or anti configuration of 2,4-pentanediol.

Introduction

The use of hydroxyl groups in solution-phase NMR structural studies presents experimental challenges, largely a consequence of rapid chemical exchange among hydroxyl groups and, in some cases, protic solvents. Hydroxyl exchange rates can be slowed by dissolving in DMSO-d_6 or acetone-d_6,[1−5] by supercooling[6] aqueous solutions, or by using organic cosolvents.[7,8] Recent work from our laboratory has demonstrated the feasibility of using OH/OH scalar coupling as a method for detecting spatially proximal hydroxyl groups.[9] Intramolecular OH/OH hydrogen bonds in carbohydrates can also be detected with isotope effects[10] manifest in the ^{1}H or ^{13}C NMR spectra of partially deuterated compounds,[11] a method referred to as SIMPLE (secondary *i*sotope *m*ultiplets of *p*artially *l*abeled *e*ntities) NMR. This technique has been applied as a qualitative test for spatially proximal OH groups.

One of the first systems studied with the SIMPLE method was the cyclodextrins (Figure 1). Results obtained from the cyclodextrins are reviewed here for the purpose of introducing how SIMPLE is used for hydrogen bond detection. When α-cyclodextrin is dissolved in DMSO-d_6, sharp hydroxyl resonances are observed for OH-2, OH-3, and OH-6. When the hydroxyl groups are partially deuterated, either by prior exchange or by addition of an exchangeable deuterium source, new resonances are observed for OH-2 and OH-3 but not OH-6. The intensity of the new isotopically shifted resonances was found to increase as the deuterium content within the sample increased. Furthermore, the OH-2 and OH-3 isotope shifts were found to be of opposite sign. In α-cyclodextrin, for example,

* To whom correspondence should be addressed. E-mail: doleary@pomona.edu.
[†] Pomona College.
[‡] University of California, Los Angeles.

(1) Corio, P. L.; Rutledge, R. L.; Zimmerman, J. R. *J. Am. Chem. Soc.* **1958**, *80*, 3163−3164.
(2) Kivelson, D.; Kivelson, M. G. *J. Mol. Spectrosc.* **1958**, *2*, 518−523.
(3) McGreer, D. E.; Mocek, M. M. *J. Chem. Educ.* **1963**, *40*, 358−361.
(4) Chapman, O. L.; King, R. W. *J. Am. Chem. Soc.* **1964**, *86*, 1256−1258.
(5) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* **1966**, *22*, 3061−3083.
(6) Poppe, L.; Van Halbeek, H. *Nat. Struct. Biol.* **1994**, *1*, 215−216.
(7) Adams, B.; Lerner, L. *J. Am. Chem. Soc.* **1992**, *114*, 4827−4829.
(8) Adams, B.; Lerner, L. *J. Magn. Reson.* **1992**, *96*, 604−607.
(9) Fierman, M.; Nelson, A.; Khan, S. I.; Barfield, M.; O'Leary, D. J. *Org. Lett.* **2000**, *2*, 2077−2080. A theoretical treatment of these scalar couplings has been completed: Barfield, M.; Bergset, J. M.; O'Leary, D. J. *Magn. Res. Chem.* **2001**, *39*, S115−S125.
(10) For a recent review, see: Bolvig, S.; Hansen, P. E. *Curr. Org. Chem.* **2000**, *4*, 19−54.
(11) For representative ^{1}H applications, see: (a) Lemieux, R. U.; Bock, K. *Jpn. J. Antibiot.* **1979**, *32*, S163−S177. (b) Christofides, J. C.; Davies, D. B. *J. Chem. Soc., Chem. Commun.* **1982**, 560−562. (c) Christofides, J. C.; Davies, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 5099−5105. (d) Christofides, J. C.; Davies, D. B. *J. Chem. Soc., Chem. Commun.* **1985**, 1533−1534. (e) Christofides, J. C.; Davies, D. B.; Martin, J. A.; Rathbone, E. B. *J. Am. Chem. Soc.* **1986**, *108*, 5738−5743. (f) Davies, D. B.; Christofides, J. C. *Carbohydr. Res.* **1987**, *163*, 269−274. (g) Everett, J. R. *J. Chem. Soc., Chem. Commun.* **1987**, 1878−1880. (h) Uhlmann, P.; Vasella, A. *Helv. Chem. Acta* **1992**, *75*, 1979−1994. (i) Hansen, P. E.; Christofferson, M.; Bolvig, S. *Magn. Reson. Chem.* **1993**, *31*, 893−902. (j) Angyal, S. J.; Christofides, J. C. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1485−1491. (k) Dabrowski, J.; Grosskurth, H.; Baust, C.; Nifant'ev, N. E. *J. Biomol. NMR* **1998**, *12*, 161−172.

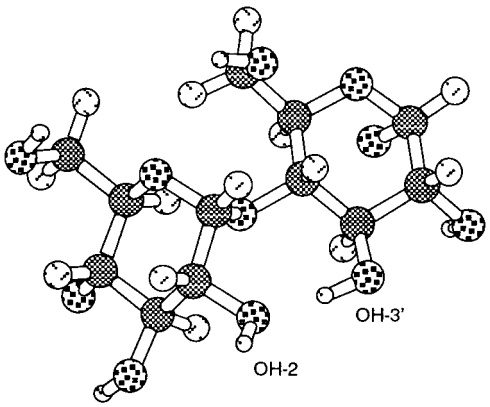
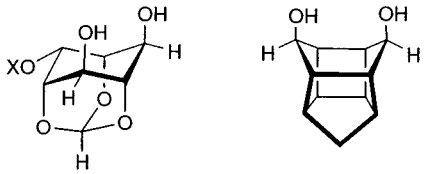


Figure 1. Representative 1,4-linked α-D-glucose disaccharide structural element from α-cyclodextrin (cyclohexaamylose) neutron diffraction structure, showing an intramolecular hydrogen bond between OH-2 and OH-3′. The O-2/O-3′ distance is 2.894 Å (ref 12).

a +10 ppb downfield shift was observed for OH-2, and an upfield shift of similar magnitude was reported for OH-3.[11b] The interpretation of these results was linked to static features found in cyclodextrin crystal structures[12] and to a NMR study of hydroxyl chemical shift temperature coefficients in DMSO-d_6, which found that OH-3 was prone to serving as a hydrogen bond donor in an interresidue OH/OH hydrogen bond.[13] In solid-state cyclodextrin structures (Figure 1), OH-3′ is usually found as a hydrogen bond donor in an interresidue hydrogen bond involving OH-2 as an acceptor; this arrangement was postulated to persist in DMSO solution. Downfield isotope shifts were assigned to acceptor hydroxyl groups, and upfield isotope shifts were indicative of donor hydroxyl groups. The data suggested that the sign of the isotope shift could be used to assign the donor/acceptor role of hydroxyl groups.[14] The magnitude of the OH-2 isotope shift was also found to decrease slightly across the series (α, +10 ppb; β, +9 ppb; γ, +8 ppb); this effect was ascribed to the decreasing OH-3′/OH-2 oxygen−oxygen distance found in the cyclodextrin X-ray structures (α, 3.00 Å; β, 2.86 Å; γ, 2.80 Å).

In a series of papers that examined deuterium isotope effects on ^{13}C chemical shifts of diols and carbohydrates, Reuben found that certain hydrogen bond-mediated isotope shifts arise as a consequence of an isotopic perturbation of intramolecular hydrogen bond equilibria.[15,16] We have proposed[17] that the difference in the sign of the isotope shifts in apolar and polar solvents arises as a consequence of the equilibrium isotope effect. A qualitative description of the NMR isotope effect, which operates under the condition of slow intermolecular chemical exchange but rapid intramolecular site exchange (OH_{in} vs OH_{out}), is shown in Figure 2. In a study of rigid 1,3-diols, deuterium was found to prefer the intramolecular bond in partially deuterated (OH/OD) substrates (e.g., **1**) dissolved in solvents such as benzene-d_6 and $CDCl_3$.[17] This conclusion was made after observing upfield isotope shifts for 1,3-diols dissolved in apolar solvents, which suggests that deuterium prefers the bridging position. As a result of deuterium's preference for the bridging position, the partner OH group averages slightly to the exterior position. With the assumption that the inner hydroxyl chemical shift is deshielded relative to the outer hydroxyl group, i.e., $\delta OH_{in} > \delta OH_{out}$, the hydroxyl signal from the OH/OD isotopomer shifts to high field relative to the OH/OH isotopomer. The NMR results observed in apolar solvents are in agreement with experimental[18] and theoretical[19,20] studies of intermolecular hydrogen bonds in the water dimer, where deuterium has been shown to have a preference for the bridging position by some 200 cal/mol. On the other hand, deuterium was proposed to prefer the intermolecular hydrogen bond when inositol **1** was dissolved in hydrogen bond acceptor solvents such as DMSO-d_6 and acetone-d_6. This conclusion was made on the basis of **1** exhibiting a positive isotope shift, with the assumption that the limiting chemical shifts remain $\delta OH_{in} > \delta OH_{out}$ for diols dissolved in a polar solvent such as DMSO-d_6 (Figure 2B). By employing compounds containing OH/OH pairs sharing a plane of symmetry, this work lent further support for the idea that SIMPLE NMR effects arise as a consequence of equilibrium isotope effects and also established that correlating the sign of an isotope effect with the donor/acceptor role of a hydroxyl group is prone to inconsistencies.



1 X = Si*t*-Bu(CH$_3$)$_2$
2 X = H

3

Results and Discussion

The Isotope Shift in Hydrogen-Bond-Accepting Solvents. To study the NMR isotope shift as a function of hydrogen bond geometry, we synthesized a series of 1,4-diols to compare with our earlier results obtained with 1,3-diols. To our surprise, 1,4-diol **3**[21] yielded markedly different isotope shifts when compared with **1** in a number of solvents. Namely, upfield isotope shifts were recorded for partially deuterated **3** dissolved in DMSO-d_6, acetone-d_6, and THF-d_8, whereas downfield shifts were observed for **1** in the same solvents (Table 1).

The change in sign of the isotope shift for **1** vs **3** in DMSO-d_6 was considered as arising from the difference in hydrogen bond geometry. Whereas an X-ray structure for inositol **2** has been reported,[11h] no such data were available for cage diol **3**. We were able to secure X-ray quality crystals of **3** by slow evaporation of a water/acetone solution. The diffraction analysis[22] is shown in Figure 3. Relevant structural parameters,

(12) Klar, B.; Hingerty, B.; Saenger, W. *Acta Crystallogr., Sect. B* **1980**, *36*, 1154−1165.
(13) St-Jacques, M.; Sundararajan, P. R.; Taylor, K.; Marchessault, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 4386−4391.
(14) This suggestion was also made by Lemieux and Bock in their pioneering study of partially deuterated sucrose in DMSO-d_6. See ref 11a.
(15) Reuben, J. *J. Am. Chem. Soc.* **1985**, *107*, 1756−1756 and references cited therein.
(16) Saunders: M.; Jaffe, M. H.; Vogel, P. *J. Am. Chem. Soc.* **1971**, *93*, 2558−2559.
(17) Craig, B. N.; Janssen, M. U.; Wickersham, B. M.; Rabb, D. M.; Chang, P. S.; O'Leary, D. J. *J. Org. Chem.* **1996**, *61*, 9610−9613.
(18) (a) Tursi, A. J.; Nixon, E. R. *J. Chem. Phys.* **1970**, *52*, 1521−1528. (b) Engdal, A.; Nelander, B. *J. Chem. Phys.* **1987**, *86*, 1819−1823.
(19) Buckingham, A. D.; Fan-Chen, L. *Int. Rev. Phys. Chem.* **1981**, *1*, 253−269.
(20) Scheiner, S.; Cuma, M. *J. Am. Chem. Soc.* **1996**, *118*, 1511−1521.
(21) Marchand, A. P.; LaRoe, W. D.; Sharma, G. V. M.; Suri, S. C.; Reddy, D. S. *J. Org. Chem.* **1986**, *51*, 1622−1625.
(22) The structure was solved by standard methods. The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre.

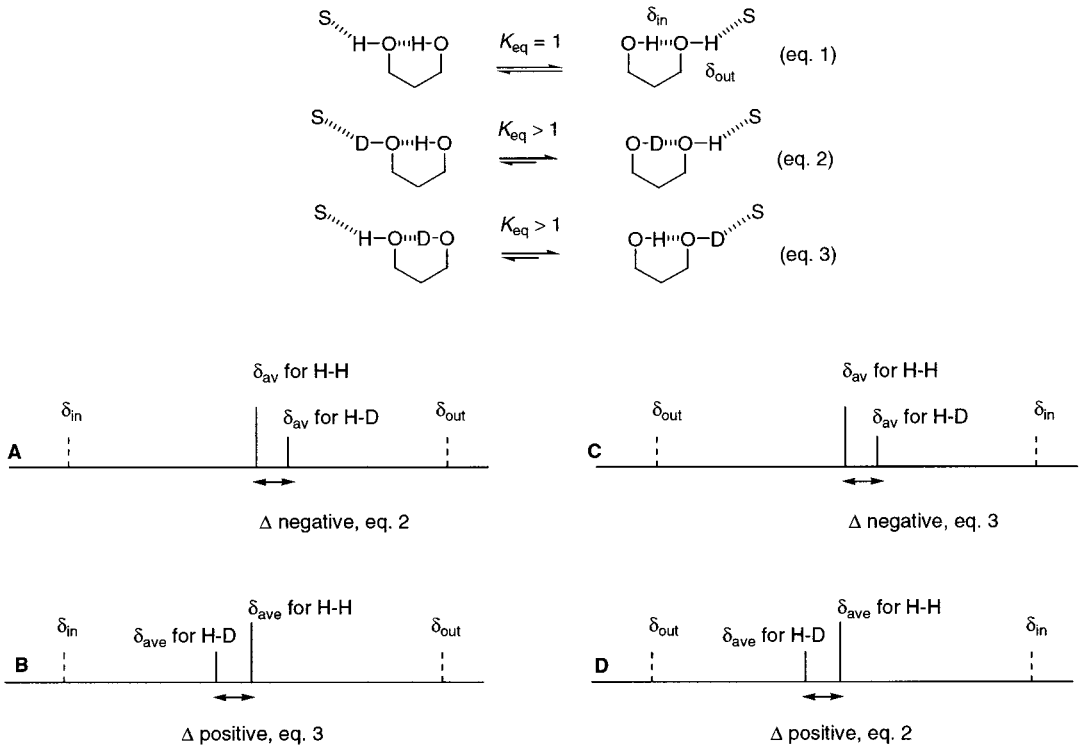


Figure 2. Hypothetical ^{1}H NMR spectra for hydroxyl groups in a symmetrical diol (eq 1) and unsymmetrical deuterated isotopomers (eqs 2 and 3). (A) A model consistent with upfield (negative) isotope shift associated with deuterium having a preference for the intramolecular hydrogen bond for $\delta_{in} > \delta_{out}$(eq 2). (B) A model consistent with downfield (positive) isotope shift associated with deuterium preferring the intermolecular hydrogen bond for $\delta_{in} > \delta_{out}$ (eq 3). (C) A model consistent with upfield (negative) isotope shift associated with deuterium having a preference for the intermolecular hydrogen bond for $\delta_{out} > \delta_{in}$ (eq 3). (D) A model consistent with downfield (positive) isotope shift associated with deuterium preferring the intramolecular hydrogen bond for $\delta_{out} > \delta_{in}$ (eq 2).

Table 1. Equilibrium Isotope Effects (ppb) in Diols **1** and **3**

solvent	Δ, **1**	Δ, **3**
DMSO-d_6	+29.9	−12.0
acetone-d_6	+14.0	−11.0
THF-d_8	+15.4	−12.3
CD_2Cl_2	−8.4	−46.0

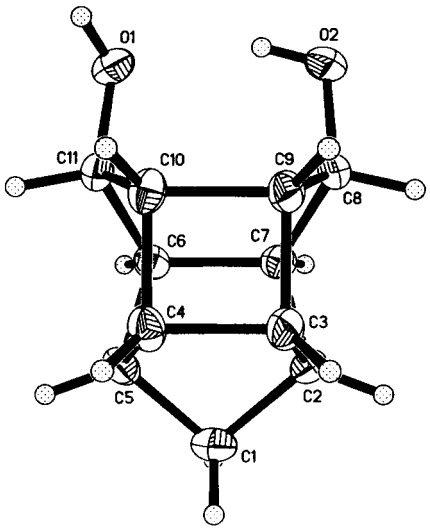
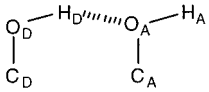


Figure 3. X-ray structure of cage diol **3**.

together with X-ray structural data for **2**, are listed in Table 2. Based upon the diffraction data, the hydrogen bond is shorter and more linear in **3** ($\angle O_A-O_D-H_D = 11.2°$, $r_{O-O} = 2.60$ Å) than in **2** ($\angle O_A-O_D-H_D = 23.9°$, $r_{O-O} = 2.78$ Å). Given the uncertainty in determining the position of hydrogen atoms via X-ray analysis, as well as unknown crystal packing effects, we also compared the geometries of **2** and **3** using ab initio calculations. Geometries were computed with the HF/6-31G** and B3LYP/6-31G** basis set as implemented in Gaussian 94.[23] The relevant structural parameters are listed in Table 2. Not surprisingly, the calculations also suggest a shorter and more linear hydrogen bond in **3** (B3LYP/6-31G**: $\angle O_A-O_D-$

Table 2. Comparison of Computed and Experimental Hydrogen Bond Geometries in Diols **2**, **3**, **8**, and **10**



compd	parameter[a]	HF/6-31G**	B3LYP/6-31G**	X-ray
2[11h]	$\angle(O_A-O_D-H_D)$, deg	28.4	25.1	23.9
	$r(O_A-O_D)$, Å	2.784	2.761	2.768
3	$\angle(O_A-O_D-H_D)$, deg	19.2	15.8	11.2
	$r(O_A-O_D)$, Å	2.705	2.690	2.602
8	$\angle(O_A-O_D-H_D)$, deg		13.5	
	$r(O_A-O_D)$, Å		2.63	
10	$\angle(O_A-O_D-H_D)$, deg		13.9	
	$r(O_A-O_D)$, Å		2.62	

[a] D, donor; A, acceptor.

(23) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, Revision E.2; Gaussian, Inc.: Pittsburgh, PA, 1995.

$H_D = 15.7°$, $r_{O-O} = 2.687$ Å) than in **2** (B3LYP/6-31G**: $\angle O_A-O_D-H_D = 25.1°$, $r_{O-O} = 2.762$ Å).

The isotope effect sign inversion for 1,3- vs 1,4-diols could arise from several factors. For instance, the relative strengths of intra- vs intermolecular hydrogen bonds could dictate the preference of the heavy isotope for the inside vs outside positions. Alternatively, the sign inversion could be due to a reversal of the limiting chemical shifts (see, for example, Figure 2A vs 2D). As mentioned earlier, the $\delta OH_{in} > \delta OH_{out}$ shift assignment should be valid for studies conducted in apolar, non-hydrogen-bonding solvents such as dichloromethane and benzene. In contrast, Vasella and Bernet have recently shown that OH resonances for hydrogen bond donors are actually shifted upfield relative to OH resonances for hydrogen bond acceptors for certain diols dissolved in DMSO-d_6.[24] For a unidirectional hydrogen bond involving an unambiguous donor/acceptor arrangement, such as that found in the gingkolides, the acceptor OH group resonates downfield of the donor OH group. In an inositol system the data were less compelling. The diaxial OH chemical shift in **2** (5.45 ppm, the average of the inside and outside positions) is shielded relative to a fully solvated axial OH group (5.71 ppm) in des-hydroxy **4**. Provided that **2** and **4** are considered suitably related compounds, this suggests that the outside position is deshielded relative to the inside position when these compounds are dissolved in DMSO-d_6. On the other hand, when one of the diaxial OH groups in **2** is protected as the benzyl ether (i.e., **5**), the axial OH resonance was shielded relative to that in **2**. Based upon an analysis of the vicinal H−C−O−H coupling constant, it was suggested that the OH group in **5** is externally solvated to the extent of ca. 80% in DMSO-d_6. These data, in contrast with **2/4** comparison, suggest the inside chemical shift is actually deshielded relative to the outside shift for an inositol-derived 1,3-diol. If this is the case, then the downfield isotope shift could arise from deuterium having a slight preference for the intermolecular hydrogen bond, as shown in Figure 2B.

The experimental evidence for the limiting hydroxyl chemical shift assignments in cage diol **3** is as follows. The OH groups in diol **3** have a chemical shift of 6.30 ppm in DMSO-d_6, whereas the isolated and presumably fully solvated axial OH group in the axial/equatorial cage diol **6**[25] is shielded (3.93 ppm) by comparison (Figure 4). This is opposite to what was observed in the inositol series, and using this datum alone it would be logical to propose that $\delta OH_{in} > \delta OH_{out}$. In contrast with the inositol example, the hydroxyl group in monobenzyl ether **7**[26] appears to remain exclusively in the intramolecular hydrogen bond configuration, as evidenced by the 12.0 Hz J(H−C−O−H) coupling constant. This value is in excellent agreement with that predicted for a 180° H−C−O−H dihedral angle by the parametrized Karplus equation.[27] If the OH group is "locked" in the intramolecular hydrogen bond, then its chemical shift should closely approximate the limiting value for δOH_{in}. As the OH group in **7** is shielded (5.65 ppm) relative to OH groups of diol **3** (6.30 ppm, average of δOH_{in} and δOH_{out}), it would also be logical to propose that $\delta OH_{out} > \delta OH_{in}$. The observation of an upfield isotope shift in diol **3**, together with the chemical shift assignment, is consistent with deuterium again shifting its preference to the intermolecular hydrogen bond, as depicted in Figure 2C.

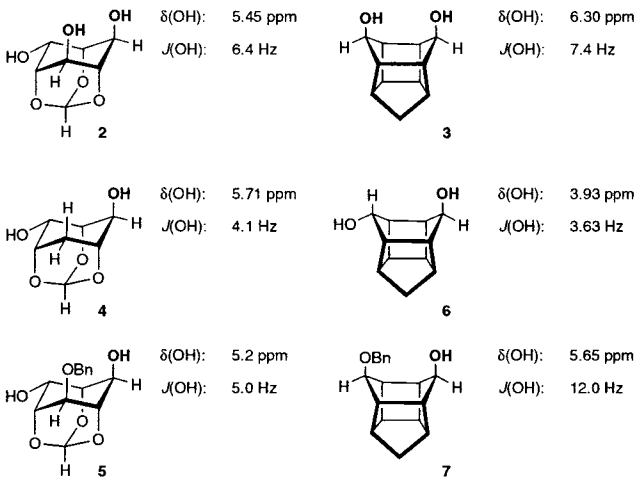


Figure 4. Hydroxyl chemical shifts and coupling constants for inositols **2**, **4**, and **5** (data taken from ref 24) and cage diols **3**, **6**, and **7** dissolved in DMSO-d_6.

Having access to a reasonable approximation of the limiting OH chemical shifts for symmetric diol **3**, coupled with the observed isotope shift, provides the opportunity to calculate the perturbation of the equilibrium constant and resulting free energy difference associated with the preference of deuterium for the intermolecular hydrogen bond. Using Saunders's expression[16] $K = (\Delta\omega - D)/(\Delta\omega + D)$, where $\Delta\omega$ (520.2 ± 0.1 Hz) is the chemical shift difference between the external and bridging OH groups and D is the isotope shift (4.8 ± 0.1 Hz), we calculate $K = 1.018 \pm 0.0008$ and $\Delta G° = 10.9 \pm 0.5$ cal/mol at room temperature. To the best of our knowledge, this is the first experimental estimate for the energetic preference of deuterium in a SIMPLE measurement. As far as conformational equilibrium isotope effects are concerned, a value of 11 cal/mol is quite small and comparable with the "*A* value" for deuterium in cyclohexane (deuterium prefers the equatorial site by some 6−8 cal/mol).[28]

We wanted next to explore the isotope shift in DMSO-d_6 for a series of 1,3- and 1,4-diols with different hydrogen bond geometries (Figure 5). Accordingly, we synthesized symmetric cage diols **8** and **9** according to an established procedure.[29] Diols **10** and **11** were prepared using an adaptation of this procedure.[26] The diethynyl diol **8** exhibited an isotope shift (−3 ppb) that was smaller than the parent cage diol **3** (−12 ppb). The isotope shifts for the divinyl, dimethyl, and diallyl cage diols (**9**, **10**, and **11**) were all found to be on the order of −38 ppb; this value remains the largest upfield shift that we have observed for any diol dissolved in DMSO-d_6.

It was anticipated that cage structures having tertiary OH groups would provide diols with shorter O−O distances due to

(24) Bernet, B.; Vasella, A. *Helv. Chem. Acta* **2000**, *83*, 995−1021.
(25) Cookson, R. C.; Crundwell, E.; Hill, R. R.; Hudec, J. *J. Chem. Soc.* **1964**, 3062−3075.
(26) Refer to the Supporting Information for a full description of synthetic procedures and compound characterization.
(27) The relevant equation is $^3J(H-C-O-H) = 10.4 \cos^2\theta - 1.5 \cos\theta + 0.2$. See: Fraser, R. I.; Kaufman, M.; Morand, G.; Govil, G. *Can. J. Chem.* **1969**, *47*, 403−409.
(28) (a) Anet, F. A. L.; Kopelevich, M. *J. Am. Chem. Soc.* **1986**, *108*, 1355−1356. (b) Anet, F. A. L.; O'Leary, D. J. *Tetrahedron Lett.* **1989**, *30*, 1059−1062.
(29) (a) For the synthesis of diol **8**, see: Bott, S. G.; Marchand, A. P.; Alihodzic, S.; Kumar, K. A. *J. Chem. Cryst.* **1998**, *28*, 251−258. (b) For the synthesis of diol **9**, see: Marchand, A. P.; Kumar, K. A.; McKim, A. S.; Mlinaric-Majerski, K.; Kragol, G. *Tetrahedron* **1997**, *53*, 3467−3474.

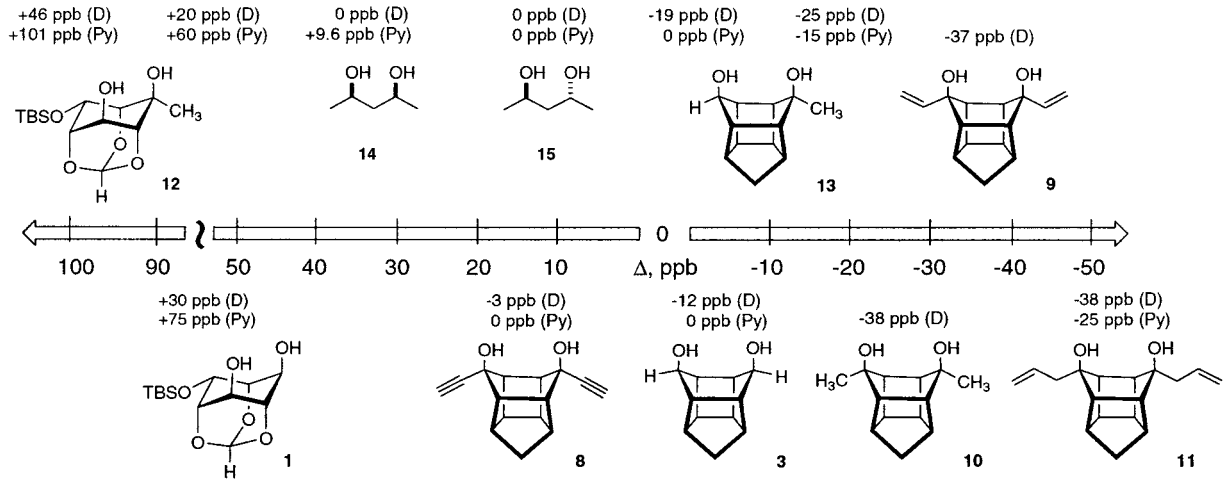


Figure 5. Summary of isotope shifts measured for compounds in DMSO-d_6 (D) and pyridine-d_5 (Py).

a steric buttressing effect.[30] A comparison of the B3LYP/6-31G** optimized geometries of diols **3**, **8**, and **10** is shown in Table 2. The computations reveal that the hydrogen bond geometry is nearly identical for the dimethyl diol **10** and diethynyl diol **8**, with both compounds having shorter and marginally more linear hydrogen bonds relative to the parent diol **3**. It is interesting, then, that the isotope shift for diethynyl diol **8** (−3 ppb) is much smaller than the dimethyl diol **10** (−38 ppb). The diethynyl isotope shift is also smaller than that of the parent diol **3**, despite the former compound having a more favorable bond geometry. A factor that can influence the isotope shifts is the degree to which solvent associates with the hydroxyl groups. For example, Bernet and Vasella have shown that the OH groups in inositols have enhanced association with DMSO due to their enhanced acidity caused by flanking antiperplanar C−O bonds.[24] Enhanced acidity could be a factor in comparing the isotope shifts for propargylic (**8**, −3 ppb) and homoallylic or methyl groups (**10** or **11**, −38 ppb), although the large shift observed for the allylic diol **9** (−37 ppb) does not support this conclusion. The limiting chemical shifts for the interior and exterior positions will also help determine the sign and magnitude of the isotope shift, and it is worth noting that the magnetic anisotropy associated with the acetylenic function in **8** could provide a strong shielding effect for the exterior OH group, thus lessening the OH_{in}/OH_{out} chemical shift difference. This comparison serves to underscore the difficulties associated with using the SIMPLE technique to reveal quantitative information about the geometry of an intramolecular hydrogen bond.

As was shown in our earlier study, the unsymmetrical inositol diol **12** exhibited shifts of different magnitude for the secondary and tertiary hydroxyl groups in DMSO-d_6, with a +46 ppb isotope shift observed for the secondary hydroxyl group and a +20 ppb isotope shift for the tertiary hydroxyl group. The isotope shifts in the unsymmetrical methyl cage diol **13**[26] were found to be intermediate in value (secondary OH, −19 ppb; tertiary OH, −25 ppb) when compared with those in the parent cage diol **3** and the dimethyl cage diol **10**.

The isotope shifts observed in model compounds dissolved in DMSO-d_6 have thus far revealed that the isotope shifts can range from +50 to −40 ppb. Given that a zero crossing exists for the SIMPLE method in DMSO-d_6, these data also suggest that a small or zero isotope shift does not necessarily imply the absence of an intramolecular hydrogen bond. It could be that a reasonably favorable intramolecular OH/OH hydrogen bond geometry would not exhibit a measurable isotope shift in DMSO-d_6. Factors such as the relative strengths of the intra- vs the intermolecular hydrogen bond, as well as the limiting chemical shifts, have to be taken into consideration.

It is worth comparing these data in light of the early observation of isotope shifts of different sign for OH-2 and OH-3 in the cyclodextrin series. Lemieux, who observed similar sign inversions for different sucrose hydroxyl groups, explained these effects as possibly arising from a donor/acceptor identity of a particular hydroxyl group in a fixed orientation. This remains an intriguing proposal, although Lemieux based his analysis on a discussion of intrinsic, rather than equilibrium, isotope effects. Arguing against this idea is our observation that upfield and downfield isotope shifts are possible in compounds containing a plane of symmetry, i.e., with hydroxyl groups exchanging their acceptor/donor roles. Additionally, Reuben has argued that SIMPLE isotope effects should be maximal when the equilibrium governing donor/acceptor interchange is near unity (eq 1, Figure 2).[15] Using our data in a qualitative sense, the downfield isotope shift for the cyclodextrin OH-2 in DMSO-d_6 might be associated with a weaker intramolecular hydrogen bond, while the upfield OH-3 isotope shift may be a signature for a shorter and stronger hydrogen bond.

Pyridine Can Amplify Certain Isotope Shifts. If solvents such as DMSO-d_6 are capable of forming intermolecular hydrogen bonds that can compete with intramolecular hydrogen bonds with respect to the limiting chemical shifts, then it seemed reasonable that a more Lewis basic solvent could be used to amplify these isotope effects.[31] To test this hypothesis, we first measured the isotope shifts for inositol derivative **12** in pyridine-d_5 (Figure 6). The isotope shifts in this compound were found to be 2−3 times larger in pyridine when compared to corresponding values in DMSO-d_6. For example, the secondary

(30) All structures were optimized with the external −OH group syn to the cyclobutane ring. Computations were not performed on diols **9** or **11** due to the multiple conformations associated with the vinyl and allyl substituents.

(31) We thank Professor Charles Perrin of the University of California at San Diego for making this suggestion.

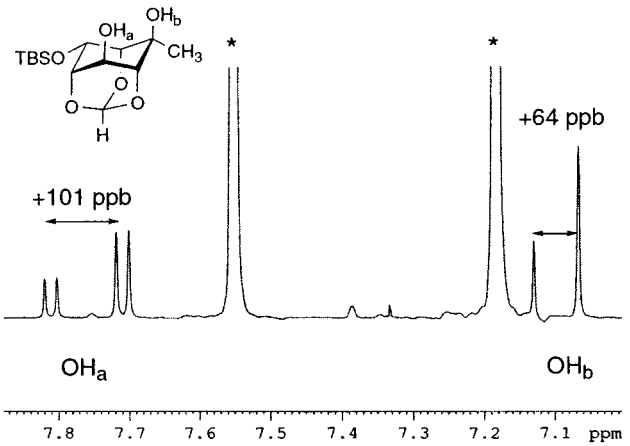


Figure 6. 400 MHz ^{1}H data for the hydroxyl region of partially deuterated diol **12** dissolved in pyridine-d_5. Downfield isotope shifts arising from the OH/OD isotopomers are observed for both secondary (OH_a, +101 ppb) and tertiary (OH_b, +64 ppb). Asterisks denote residual ^{1}H solvent signals.

hydroxyl group in diol **12** exhibits an isotope shift of +46 ppb in DMSO-d_6 and +101 ppb in pyridine-d_5. An even greater amplification was observed for the tertiary hydroxyl group: +20 ppb in DMSO-d_6 vs 64 ppb in pyridine-d_5. The isotope shift for inositol diol **1** in pyridine-d_5 was found to be +75 ppb (+29 ppb in DMSO-d_6).

A number of the cage diols were also examined in pyridine-d_5. In every case, the isotope shift in pyridine-d_5 was found to be smaller in magnitude relative to that in DMSO-d_6. For example, in pyridine-d_5 the diallyl diol **11** produced an isotope shift of −25 ppb (−38 ppb in DMSO-d_6). The same behavior was observed in the unsymmetrical cage diol **13**, with the tertiary hydroxyl group isotope shift decreasing to −15 ppb (−25 ppb in DMSO-d_6) and the secondary hydroxyl group decreasing to 0 ppb (−19 ppb in DMSO-d_6). Isotope shifts were not observed in pyridine-d_5 for cage diols **3** (−12 ppb in DMSO-d_6) and **8** (−3 ppb in DMSO-d_6). What emerges from this comparison with the DMSO-d_6 data is that isotope effects in pyridine-d_5 are generally shifted in the same direction, i.e., toward more positive values. As a result, pyridine-d_5 may be used to amplify positive isotope shifts observed in DMSO-d_6, or in certain cases it can be used to render a negative isotope shift positive (see below).

The utility of pyridine for amplifying certain isotope effects was demonstrated by using two additional systems. The first was a mixture[32] of *syn*-2,4-pentanediol (**14**) and *anti*-2,4-pentanediol (**15**) (Figure 5), an interesting test case due to the prevalence of acyclic 1,3-diols in natural products. In DMSO-d_6, neither isomer produced measurable isotope shifts upon partial deuteration. When pyridine-d_5 was employed, however, an isotope shift of +9.6 ppb was measured for the syn isomer, while no isotope shift was detected for the anti isomer. The syn isomer is predisposed to forming a stable intramolecular hydrogen bond, resulting in a six-membered ring with equatorially positioned methyl groups. The anti isomer can also form an intramolecular hydrogen bond, though an extended non-hydrogen-bonded conformation is also energetically favorable and perhaps preferred in a hydrogen-bond-accepting solvent.

(32) A 1:1 mixture of *syn*- and *anti*-2,4-pentanediol was used for these studies. The peaks arising from the anti isomer were identified by comparison with an authentic sample.

Table 3. Isotope Shifts Measured in DMSO-d_6 and Pyridine-d_5 for α- and β-Cyclodextrin

cyclodextrin	solvent	temp (°C)	Δ, OH-2 (ppb)	Δ, OH-3′ (ppb)
α	DMSO-d_6	22	+10.0	−10.4
β	DMSO-d_6	22	+8.7	−10.5
α	pyridine-d_5	−20	+41.1	+10.2
β	pyridine-d_5	−20	+41.3	+18.2

On the basis of these results, it appears feasible to use pyridine-d_5 to diagnose the syn relationship of 1,3-diols when isotopic perturbation is not measurable in DMSO-d_6.

α- and β-cyclodextrin were selected as representative carbohydrates previously studied[11b] using the isotopic perturbation method in DMSO-d_6 (Table 3). As mentioned earlier, downfield isotope shifts were observed for the cyclodextrin OH-2, and upfield shifts of unspecified magnitude were reported for OH-3′. We elected to remeasure the isotope shifts for α- and β-cyclodextrin in DMSO-d_6 for purposes of clarifying the magnitude of the OH-3′ isotope shifts. The results are shown in Table 3. The OH-2 isotope shifts were found to be in agreement with the previous study, and the OH-3′ isotope shifts were found to be −10 ppb for both α- and β-cyclodextrins.

The measurements in pyridine-d_5 were taken at moderately low temperature (−20 °C) in order to shift the hydroxyl resonances away from the pyridine-d_5 solvent resonances. In pyridine-d_5 at −20 °C, the OH-2 isotope shift was found to be +41 ppb for both cyclodextrins. At room temperature this same signal exhibited an isotope shift of +31 ppb. The lower freezing point of pyridine-d_5 (−46 °C) relative to that of DMSO-d_6 (+18 °C) makes pyridine-d_5 attractive from the standpoint of being able to lower the sample temperature in order to solve peak overlap problems. Lowering the sample temperature can also provide larger isotope shifts in some cases.[33] Perhaps more interesting is that the OH-3′ isotope shift is rendered positive in pyridine-d_5 (α, +10.2 ppb; β, +18.2 ppb). This is the one case where we have observed pyridine's ability to reverse the sign of an isotope shift measured in DMSO-d_6. Like that observed in DMSO-d_6, the OH group thought to participate in the persistent intramolecular hydrogen bond (OH-3′) shows a smaller, less negative isotope shift relative to the OH group thought to be more exposed to solvent (OH-2). The ability of pyridine to promote large isotope shifts identifies this solvent as an additional medium for studies of intramolecular hydrogen bonding in carbohydrates, natural products, and synthetic intermediates.

The Isotope Shift for Diols in Apolar Solvents. Isotope shifts measured in CD_2Cl_2 and benzene-d_6 provide a measure of the isotope effect in the absence of any strong intermolecular interactions with solvent. The isotope shifts for a number of substrates are shown in Figure 7. In contrast with the DMSO-d_6 data, all of the isotope shifts in CD_2Cl_2 and benzene-d_6 were found to be negative (upfield). This observation is consistent with deuterium preferring the bridging intramolecular hydrogen bond and with $\delta OH_{in} > \delta OH_{out}$, which is a reasonable assumption in solvents such as these.

As described in our earlier paper, benzene-d_6 can be used to amplify isotope shifts for diols exhibiting intramolecular

(33) NMR equilibrium isotope shifts generally get larger with decreasing temperature. We have found this to be the case for diols exhibiting positive isotope shifts. In contrast, negative isotope shifts in systems such as **3** dissolved in DMSO-d_6 appear to get larger with increasing temperature. The origin of this temperature dependence is not yet understood.

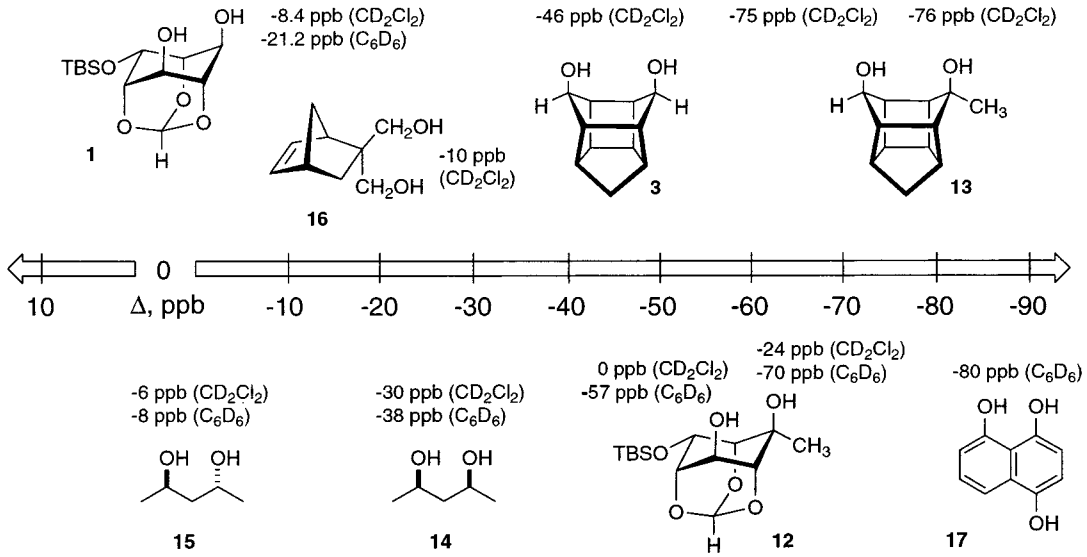


Figure 7. Summary of isotope shifts (Δ) measured for compounds dissolved in apolar solvents (CD_2Cl_2 and C_6D_6).

hydrogen bonds.[17] For some rigid 1,3-diols (e.g., **1** and **12**), this amplification can be as large as a factor of 3. The origin of this amplification is probably due to an interaction between the "outside" hydroxyl group and the π-face of the aromatic solvent. Such an interaction has been suggested to constitute a very weak hydrogen bond.[34] If the outside hydroxyl group is preferentially associated with the π-face of benzene, then its chemical shift would be expected to be more shielded relative to its value in a solvent such as CD_2Cl_2.[35] A greater (OH_{in}/OH_{out}) chemical shift difference would be expected to provide a larger isotope shift, all other things being equal.

Isotope shifts were also observed for the primary hydroxyl groups in 5-norbornene-2,2-dimethanol[36] (**16**, −10 ppb, CD_2Cl_2). It is noteworthy that diol **16** did not produce measurable isotope shifts in DMSO-d_6. 1,4,8-Trihydroxynaphthalene (**17**)[37] was found to exhibit isotope shifts of −80 ppb in benzene-d_6. The 1,8-naphthalenediol structural motif is found in certain natural products; it appears that the application of isotope shift measurements in apolar solvents can be used to detect this arrangement of OH groups.

We were unable to measure isotope shifts for several of the cage structures (**8**, **10**, and **11**) in CD_2Cl_2 or benzene-d_6 due to complications arising from what we believe to be association effects. For the two cases where we were able to measure isotope shifts (cage diols **3** and **13**), sharp hydroxyl resonances (required for observing small isotope shifts) were observed only in highly dilute CD_2Cl_2 solution (<0.1 mg/mL). Similar experiments did not produce sharp hydroxyl peaks for diols **8**, **10**, or **11**.[38]

Apolar solvents were found to be particularly useful for assigning the configuration of *syn*- and *anti*-2,4-pentanediol. In CD_2Cl_2 there is a markedly larger isotope shift for *syn*-1,3-pentanediol (**14**, −30 ppb) compared with the anti isomer (**15**, −6 ppb). As discussed earlier, this is consistent with the syn isomer being able to form a more stable intramolecular hydrogen bond relative to the anti isomer. Benzene-d_6 was found to promote a modest amplification of these shifts (**14**, −38 ppb; **15**, −8 ppb).[39] Assigning the syn or anti configuration of acyclic 1,3-diols (such as those found in acetate- and propionate-derived natural products) by means of isotope shifts represents a potential alternative to traditional methods using NMR analysis of derivatized (e.g., acetonide) diols and polyols. These chemical derivatization methods have, however, proven utility for cases involving contiguous arrays of 1,3-diols, such as those found in polyhydroxylated natural products.[40,41] The application of isotopic perturbation to extended arrays of 1,3-diols is an area of active research in our laboratory, and the results of these preliminary investigations are encouraging.

Conclusions

OH/OH hydrogen bonds are often dynamic species and exhibit a range of bond angles, atomic distances, and strengths. As illustrated in Figure 8, the compounds employed in the present study have hydrogen bond geometries that span the range typically found in naturally occurring compounds.[42] As such, results obtained from these compounds should define the range of values derived from NMR methods for intramolecular hydrogen bond detection.

Although the detailed origins of the SIMPLE NMR method remain unclear, it has been employed in numerous studies of intramolecular OH/OH hydrogen bonding. The work presented here has provided evidence for a range of positive, negative, and zero isotope shifts for a series of structurally well-defined diols dissolved in hydrogen-bond-accepting solvents such as DMSO-d_6. The sign inversion is interpreted in terms of the

(34) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: New York, 1991.
(35) Pople, J. A.; Schneider, W. G.; Bernstein, H. J. *High-Resolution Nuclear Magnetic Resonance*; McGraw-Hill: New York, 1959.
(36) This compound was purchased from Aldrich Chemical Co.
(37) This compound was prepared by sodium dithionite reduction of juglone using a textbook procedure for the preparation of 2-methyl-1,4-naphthohydroquinone: Fieser, L. F.; Williamson, K. L. *Organic Experiments*, 6th ed.; D. C. Heath and Co.: Lexington, 1987.
(38) Certain 1,3-diols are known to dimerize, see: Lopez de la Paz, M.; Jimenez-Barbero, J.; Vicent, C. *J. Chem. Soc., Chem. Commun.* **1998**, 465−466.
(39) In conducting these experiments, it was found that the diol concentration was required to be on the order of 0.5 mg/mL; at higher concentrations positive isotope shifts were measured for the anti isomer, presumably due to association.
(40) Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9−17.
(41) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, *31*, 7099−7100.
(42) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford: New York, 1997.

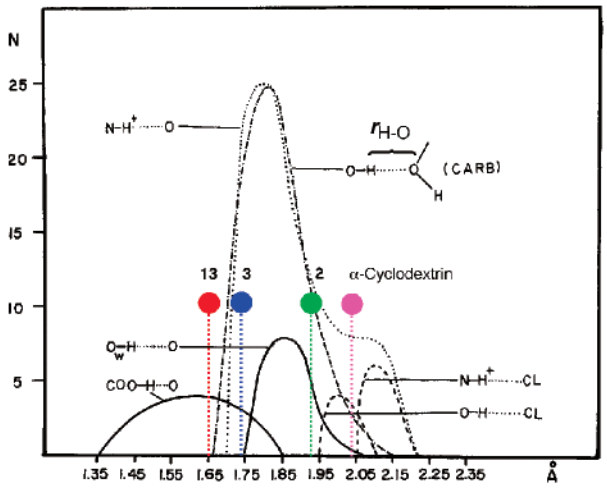


Figure 8. Distribution of hydrogen bond lengths observed in 32 amino acid and 24 carbohydrate neutron diffraction structures. $r(H\cdots O_A)$ distances as obtained from X-ray diffraction data: **13** = 1.65 Å,[9] **3** = 1.74 Å, **2** = 1.93 Å.[11h] Value for α-cyclodextrin (2.04 Å) taken from neutron diffraction data in ref 12 (figure adapted, with permission, from ref 42).

ability of solvent molecules to compete for intermolecular hydrogen bonds in the presence of the intramolecular bond and the limiting chemical shifts for the interior and exterior hydroxyl groups. The use of pyridine-d_5 was shown to amplify the positive isotope effects in the 1,3-diols. On the other hand, pyridine-d_5 diminished the negative isotope shifts in the 1,4-diols. This same trend was observed in the cyclodextrin series, where the positive isotope shift for OH-2 is magnified and the isotope shift sign for OH-3′ is reversed from negative (DMSO-d_6) to positive (pyridine-d_5). A detailed understanding of this behavior has not yet been realized. In connection with these observations, it will be of interest to measure the isotopic fractionation factors[43] to see if these values correlate with the observed sign inversions. In apolar solvents such as CD_2Cl_2 and benzene-d_6, both the 1,3- and 1,4-diols exhibited negative isotope shifts; benzene-d_6 magnified the shifts observed in CD_2Cl_2. We have found that, by applying these solvent effects, it is possible to reliably assign the relative configuration of *syn*- or *anti*-2,4-pentanediol. Further work, especially from the theoretical standpoint, needs to be done in order to clarify the origins of these isotope shifts. Work along these lines has been initiated in our laboratory, and results will be communicated in due course.

(43) Jarret, R. M.; Saunders, M. *J. Am. Chem. Soc.* **1985**, *107*, 2648−2654.

Experimental Section

NMR Measurements. NMR spectra were recorded at room temperature (22 °C, unless specified otherwise) on a Bruker Avance 400 MHz NMR spectrometer. Acquisition parameters: 16 scans, 4195 Hz sweep width, 32K file size, 0.128 Hz/pt digital resolution. Processing parameters: in certain cases, Gaussian resolution enhancement was applied in order to resolve very small (<2 ppb) isotope shifts. Isotope shifts were obtained via the resident spectrometer software peak-picking algorithm, which takes the observed maximum point and fits a parabola through it and its two nearest neighbors. Using the acquisition parameters described above, we estimate the uncertainty in any given measurement to be ±0.1 ppb or ±0.04 Hz at 400 MHz. This estimation was obtained by performing a statistical analysis of the peak-to-peak separation within the five-line ^{1}H multiplet arising from the trace amount of DMSO-d_5.

Isotopic perturbation studies were performed by dissolving the appropriate compound (ca. 1 mg) in a deuterated solvent (800 μL) with partial deuteration of hydroxyl groups accomplished by careful addition, to the NMR tube, of D_2O or CD_3OD via a microliter syringe. In some cases a stock solution (1% v/v) of D_2O or CD_3OD in the NMR solvent (e.g., DMSO-d_6) was used to facilitate small additions of exchangeable deuterium to the NMR tube. A trace amount of neutral aluminum oxide (Al_2O_3, J. T. Baker) was added to samples dissolved in pyridine-d_5, CD_2Cl_2, C_6D_6, and DMSO-d_6. This measure was taken to scavenge any soluble acidic catalysts that can serve to broaden OH resonances by chemical exchange.

Acknowledgment. This work was supported in part by the National Science Foundation, the Research Corporation, and the Camille and Henry Dreyfus Foundation. T.E.V. is an ACS Scholar. We thank the California Alliance for Minority Participation in the Sciences (CAMP-NSF) for a summer research fellowship for A.N., and Dr. Charles G. Wade of the IBM Almaden Research Center for providing a summer research fellowship for J.R. under the auspices of the San Jose State University NSF Goali Grant. Mass spectral data were provided by the UCLA Mass Spectrometry Facility.

Supporting Information Available: Synthetic procedures used for the preparation of alcohol **7** and diols **10**, **11**, and **13** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA016879F