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Effect of Hydrogen Bonds on pK_a Values: Importance of Networking

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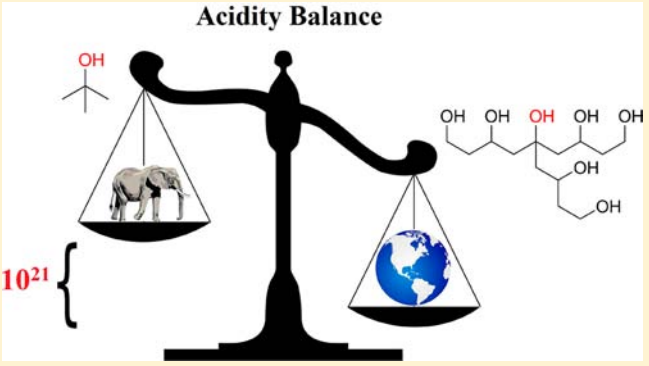
Supporting Information

ABSTRACT: The pK_a of an acyclic aliphatic heptaol ($(HOCH_2CH_2CH(OH)CH_2)_3COH$) was measured in DMSO, and its gas-phase acidity is reported as well. This tertiary alcohol was found to be 10^{21} times more acidic than *tert*-butyl alcohol in DMSO and an order of magnitude more acidic than acetic acid (i.e., pK_a = 11.4 vs 12.3). This can be attributed to a 21.9 kcal mol^{-1} stabilization of the charged oxygen center in the conjugate base by three hydrogen bonds and another 6.3 kcal mol^{-1} stabilization resulting from an additional three hydrogen bonds between the uncharged primary and secondary hydroxyl groups. Charge delocalization by both the first and second solvation shells may be used to facilitate enzymatic reactions. Acidity constants of a series of polyols were also computed, and the combination of hydrogen-bonding and electron-withdrawing substituents was found to afford acids that are predicted to be extremely acidic in DMSO (i.e., pK_a < 0). These hydrogen bond enhanced acids represent an attractive class of Brønsted acid catalysts.



INTRODUCTION

Enzymes commonly make use of general-acid and -base catalysis to accelerate a wide range of chemical transformations, some of which require transition-state stabilizations of 20 kcal mol^{-1} or more to account for the observed rates of reaction.[1] The bulk of this stabilization energy is typically provided by hydrogen bonds, which also serve as templates for proton transfer processes. Measured hydrogen bond strengths are less than 10 kcal mol^{-1} in solution,[2] however, and this difference (<10 vs ≥20 kcal mol^{-1}) led Cleland, Gerlt, and Gassman, Kreevoy, and Frey to propose stronger low-barrier hydrogen bonds (LBHBs) as a means by which enzymes can accelerate reactions.[3] These short strong hydrogen bonds were invoked to account for the enzymatic reactions of chymotrypsin, serine protease, citrate synthase, and triose phosphate isomerase, among others. In this paper we propose a second stabilizing feature that may be employed by enzymes to enhance catalysis through outer-sphere solvating hydrogen-bonding networks. This principle is demonstrated using a small molecule model system (i.e., $(HOCH_2CH_2CH(OH)CH_2CH_2)_3COH$ (**1**)).

LBHBs are characterized by short A–B distances in A···H···B complexes, downfield chemical shifts in ^{1}H NMR spectra, low isotope fractionation factors, and broad vibrational stretching bands at reduced frequencies in infrared spectra. These physical characteristics are noncontroversial, but whether they arise in systems with unusually strong hydrogen bonds is contentious.[4,5] An alternative to the LBHB proposal is to use multiple but ordinary hydrogen bonds in the first or inner solvation shell. For example, Herschlag et al. measured the pK_a values of *ortho*-substituted benzoic acids with one or two hydrogen-bond-donating groups in dimethyl sulfoxide (DMSO), since this solvent has a smaller dielectric constant than water (i.e., 46.8 vs 78.4) and can serve as a better medium for modeling the active site of an enzyme.[6] The formation of two direct hydrogen bonds to the carboxylate anion center in the conjugate base was found to increase the acidity of the benzoic acid by up to 8.0 pK_a units (i.e., 10.9 kcal mol^{-1}). Subsequently, two and even three inner shell hydrogen bonds to a singly charged site were found to dramatically increase the gas-phase acidity of acyclic aliphatic alcohols.[7] For example, the tertiary tetraol $(HOCH_2CH_2)_3COH$ was found to be as acidic as HCl, and its deprotonation enthalpy is 43.2 ± 2.4 kcal mol^{-1} more favorable than that for *tert*-butyl alcohol.[8] This acidifying effect is due to the stronger hydrogen bonds in $(HOCH_2CH_2)_3CO^-$ (Figure 1) compared to its conjugate acid. The magnitude of this enhancement not surprisingly is reduced in condensed media, but the tetraol is still 16.1 pK_a units (i.e., 21.8 kcal mol^{-1}) more acidic than *tert*-butyl alcohol in DMSO; it is also a stronger acid than phenol by 2 pK_a units (i.e., 2.7 kcal mol^{-1}) in this solvent.[9]

Intramolecular hydrogen bonds in folded proteins are known to be more stable than the corresponding hydrogen bonds to water in the proteins' unfolded states.[10] There are no good available models, however, for addressing any stabilization beyond the first internal hydrogen bond shell in an enzyme's active site. As a result, it is difficult to predict or even account for many enzyme mutation studies.[11,12] Double mutant cycles

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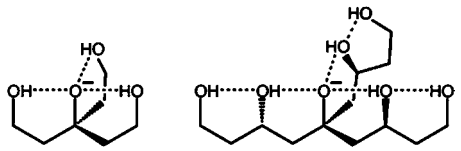

Figure 1. Most favorable hydrogen-bonding arrangements for $(HOCH_2CH_2)_3CO^-$ and $(HOCH_2CH_2CH(OH)CH_2)_3CO^-$.

offer a means to address this problem,[13] but to begin to investigate the hydrogen bond arrays employed by enzymes, we decided to measure the DMSO acidity of a flexible polyol (i.e., **1**) whose conjugate base can be stabilized by two different types of hydrogen bonds (Figure 1). That is, an array where three hydrogen bonds can form to a tertiary alkoxide anion center and three additional interactions between hydroxyl groups can be produced. The latter type of hydrogen bond is often thought to be energetically unimportant since it is present in the acid and conjugate base (or analogously in an enzyme–substrate bound complex and the corresponding reaction transition state), but this is not the case as will be shown. Computations are also reported on **1** and related compounds to further probe the consequences of hydrogen bond networks and the influence electron-withdrawing groups can have on these species.

EXPERIMENTAL SECTION

General Procedures. Glassware, syringes, NMR tubes, and needles were dried in ovens and stored in a desiccator containing rubber septa and phosphorus pentoxide. DMSO and DMSO-d_6 were dried under reflux at 1.7 Torr over CaH_2 for several hours at 60–65 °C. Both solvents were also distilled under these conditions and stored on occasion for up to several days in dark vials that contained activated 3 Å molecular sieves; activation of the sieves was carried out by heating in a furnace to 320 °C for 1 d. Pentane was dried and distilled over phosphorus pentoxide and used to triply rinse the mineral oil away from a 30% suspension of potassium hydride before the KH was used to make dimsyl anion. Dimsyl potassium was freshly prepared daily by reacting KH with DMSO or DMSO-d_6 at room temperature over a 30 min period. All of the dry solvents were degassed immediately before use by bubbling dry argon through them for approximately 20 min.

pK_a Determinations. Heptaol **1** was synthesized and purified by medium-pressure liquid chromatography (MPLC) as previously described.[14] Each sample of the *RRR*/*SSS* diastereomer was dried overnight under high vacuum (~10^{-5} Torr) and either used right away or stored in a desiccator for up to a few days. Millimolar concentrations of the heptaol were used to measure its pK_a by ^{1}H NMR spectroscopy as previously described.[7,15] Six determinations were carried out with 9-(carboxymethyl)fluorene serving as the reference compound (i.e., indicator) since it has a well-established pK_a value of 10.35.[9,16]

Gas-Phase Measurements. Electrospray ionization of a methanol/water solution (3:1, v/v) of **1** afforded the M − 1 ion at m/z 295 in a 3 T Ion Spec FTMS instrument. This ion was isolated and cooled with a pulse of argon up to a pressure of ~10^{-5} Torr before being allowed to react with HBr and 2,4-dinitrophenol. In separate experiments, Br^- and 2,4-dinitrophenoxide where generated by electron ionization in the analyzer cell of a Finnegan dual-cell FTMS instrument that was controlled with an Ion Spec data system. These ions were transferred to the source cell, cooled with a pulse of argon, isolated, and then allowed to interact with heptaol **1** which was introduced into the instrument via the solid probe inlet.

Computations. Spartan '08 was used to carry out Monte Carlo and systematic conformational searches with the MMFF force field (molecular mechanics) and the AM1 Hamiltonian (semiempirical calculations) on a variety of alcohols and their conjugate bases.[17] B3LYP/6-311+G(d,p)[18] and M06-2X/maug-cc-pVT(+d)Z)[19,20] density functional theory single-point energies were then obtained with the Gaussian 09 suite of programs[21] on all of the conformers that were found to be within 3–5 kcal mol^{-1} of the most favorable structures that had been located. The lowest energy species that resulted were fully optimized, and harmonic vibrational frequencies were computed using the same two methods as employed for the single-point energy calculations. Gas-phase acidities (ΔG°_{acid}) were subsequently calculated at 298 K using unscaled vibrational frequencies, but in carrying out the temperature correction, all weak modes ≤260 cm^{-1} were replaced by $^1/_2RT$. The entropies of the alcohols were also corrected for an entropy of mixing term as reported by Gutherie[22] and previously assessed for these compounds.[7]

Liquid-phase pK_a values in DMSO were computed using the conductor-like polarizable continuum model.[23] Both B3LYP/6-311+G(d,p) and M06-2X/maug-cc-pVT(+d)Z single-point energies were obtained using 70 surface elements (tesserae) and an area of 0.2 Å for each sphere. The "iterative" keyword was used for solving the polarized continuum model electrostatic problem and calculating the polarization charges to a convergence threshold of 10^{-12} in a maximum number of steps set to 1000. Although it has previously been reported that solvent effects on the differences between gas- and liquid-phase geometries are negligible,[24] we decided to optimize the condensed-phase structures and recompute the vibrational frequencies to compare the differences for the compounds studied herein. In accord with the literature, the resulting geometries and energies only led to small differences. In this work, relative pK_a values were computed using ethanol as the reference compound, and they were converted to absolute values by using the experimental acidity of ethanol (pK_a = 29.8).[9] For the fluorinated polyols, B3LYP/6-31+G(d) computations were carried out on previously located structures to obtain their pK_a values.[7,25] In both cases noncorrected entropies were used.

RESULT AND DISCUSSION

Proton transfer processes play a key role in most enzymatic reactions, and thus, the acidities of different functional groups in enzymatic active sites are critically important for delineating detailed mechanistic pathways.[1,26] Changes in the dielectric constant of the local environment, which typically is hydrophobic, will alter the aqueous pK_a values. This is well recognized, but there are limited pK_a data available for compounds bound to an enzyme.[27] Enzymes are also replete with hydrogen bond donors and acceptors, and consequently, they have elaborate networks of hydrogen bonds. It is less well recognized that these hydrogen bond arrays can affect acidities, and very few investigations have been carried out in this regard.[6,7] To probe the consequences of hydrogen bonding at noncharged sites in an acid and its conjugate base, the acidity of heptaol **1** was measured and computed in DMSO.

The acidity of heptaol **1** was determined by a previously reported ^{1}H NMR method relative to 9-(carbomethoxy)-fluorene,[7,15] since the latter compound is a suitable indicator and its pK_a value of 10.35 previously had been established.[9] Six determinations of the equilibrium constant revealed that it is independent of the heptaol concentration when this polyol is in the low millimolar range, and pK_a(**1**) = 11.4 ± 0.2. This is a remarkable finding in that **1** is an order of magnitude more acidic than acetic acid (pK_a = 12.3),[9] and it is the most acidic saturated alcohol of its kind that has been measured to date (i.e., an alcohol containing only C, H, and O atoms). Three different ionization sites can be envisioned for this polyol, but computations indicate that the tertiary alkoxide is significantly more stable than the secondary or primary anions both in the gas phase and in solution. That is, the relative M06-2X/maug-cc-pVT(+d)Z free energies for the tertiary, secondary, and primary ions are 0.0, 4.3, and 12.3 kcal mol^{-1} in the gas phase

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and 0.0, 3.7, and 10.9 kcal mol^{-1} in DMSO as predicted using the polarized continuum model (PCM).[23,28] The experimental pK_a is also in excellent accord with previous predictions of 10.5 and 11.7 based upon linear correlations between the computed B3LYP gas-phase deprotonation enthalpies and free energies, respectively, at the tertiary hydroxyl group with the measured DMSO pK_a values of three polyols (i.e., $(HOCH_2CH_2)_3COH$) (**2**), $(HOCH_2CH_2)_2CHOH$ (**3**), and $HOCH_2CH_2CH_2OH$ (**4**)).[7] It is well reproduced too by the PCM calculations that follow.

In recent years advances in electronic structure theory have made it possible to reliably calculate pK_a values in different solvents.[29] This has been accomplished most commonly by computing gas-phase acidities and then using a polarized continuum model to obtain the energies of the acid and its conjugate base in a bulk dielectric environment consistent with the solvent of interest. In this study, B3LYP and the newer and often superior M06-2X density functional were employed along with the 6-311+G(d,p) and maug-cc-pVT(+d)Z basis sets, respectively. Gas-phase deprotonation free energies for methanol, ethanol, *tert*-butyl alcohol, polyols **1**−**4**, phenol, and acetic acid are given in Table 1.[7,8,30] As expected, both methods do very well in reproducing the experimental values, but the M06-2X functional has the smaller average unsigned error (0.5 vs 1.5 kcal mol^{-1}) and the smaller maximum outlier (i.e., 1.4 vs 2.6 kcal mol^{-1}).

Table 1. Experimental and Theoretical Gas-Phase Acidities ($\Delta G°_{acid}$)[a]

compd	B3LYP/6-311+G(d,p)	M06-2X/maug-cc-pVT(+d)Z	exptl[b]
CH_3OH	372.9	374.9	375.5 ± 0.6
CH_3CH_2OH	369.8	372.1	372.3 ± 0.8
$(CH_3)_3COH$	366.9	368.6	369.2 ± 0.7
$HOCH_2CH_2CH_2OH$ (**4**)	355.4	355.8	355.8 ± 2.0
$(HOCH_2CH_2)_2CHOH$ (**3**)	343.5	343.8	342.4 ± 1.2
$(HOCH_2CH_2)_3COH$ (**2**)	334.5	335.0	334.4 ± 1.7
$(HOCH_2CH_2CH(OH)CH_2)_3COH$ (**1**)	319.9[c]	320.2[c]	313.5 ± 5.0
PhO*H*	339.2	341.0	341.5 ± 1.0[d]
CH_3CO_2H	339.3	340.2	339.9 ± 1.7[d]
av unsigned error	1.5	0.5	

[a]All values are in kilocalories per mole. [b]References 7 and 8. [c]The same entropy correction for **2** given in ref 7 was used. This point was not used in assessing errors because the experimental value has a large uncertainty. [d]See ref 30.

The gas-phase acidity of **1** has not been previously reported, but it is predicted to be about as acidic as HBr ($\Delta G°_{acid}$ = 318.3 ± 0.1 kcal mol^{-1}),[8] and the adiabatic electron binding energy of its conjugate base was experimentally determined to be the same as that for dihydrogen phosphate (i.e., 4.60 ± 0.1 vs 4.57 ± 0.01 eV, respectively).[14,31] To assess the computational prediction, preliminary acidity measurements were carried out on the heptaol. Electrospray ionization of **1** afforded its conjugate base, which was protonated by 2,4-dinitrophenol but not HBr ($\Delta G°_{acid}$ = 308.6 ± 2.0[32] and 318.3 ± 0.1 kcal mol^{-1}, respectively) even after a long reaction time (i.e., 5 min). In the reverse direction, bromide anion was found to deprotonate the heptaol, but 2,4-dinitrophenoxide did not. These results indicate that the acidity of **1** is between the values for HBr and 2,4-dinitrophenol. That is, $\Delta G°_{acid}$(**1**) = 313.5 ± 5.0 kcal mol^{-1}, which is in reasonable accord with our predictions, but suggests that when a more precise value is determined, it will be on the high end of the experimental range.

PCM computations were carried out using eq 1 to provide predictions of DMSO pK_a values, where [$\Delta G°_{rxn}$ (kcal mol^{-1})]/1.364 = pK_a(HX) − pK_a(CH_3CH_2OH) or pK_a(HX) = 29.8 + [$\Delta G°_{rxn}$ (kcal mol^{-1})]/1.364 (Table 2). This approach eliminates the difficulty in dealing with the solvation energy of the proton and takes advantage of the greater accuracy in computing relative energies compared to absolute values. Both the B3LYP and M06-2X predictions are in good accord with the experimental values and have average unsigned errors of 1.4 pK_a units. The maximum absolute deviation from experiment (3.2 vs 3.1 pK_a units, respectively) is also virtually the same for the two methods, and the overall results are similar in accuracy to those of a larger study of 105 organic acids previously reported.[29b]

$$HX + CH_3CH_2O^- \rightarrow X^- + CH_3CH_2OH \quad (1)$$

Table 2. Experimental and Theoretical DMSO pK_a Values[a]

compd	B3LYP/6-311+G(d,p)[b]	M06-2X/maug-cc-pVT(+d)Z[b]	exptl
CH_3OH	32.2(−3.2)	30.0(−1.0)	29.0[c]
$(CH_3)_3COH$	30.8(1.4)	29.7(2.5)	32.2[c]
$HOCH_2CH_2CH_2OH$ (**4**)	23.9(1.5)	22.3(3.1)	25.4 ± 0.3[d]
$(HOCH_2CH_2)_2CHOH$ (**3**)	20.1(−0.4)	18.3(1.4)	19.7 ± 0.2[d]
$(HOCH_2CH_2)_3COH$ (**2**)	16.4(−0.3)	14.8(1.3)	16.1 ± 0.2[d]
$(HOCH_2CH_2CH(OH)CH_2)_3COH$ (**1**)	13.6(−2.2)	11.7(−0.3)	11.4 ± 0.2
$(HOCF_2CF_2)_2CFOH$ (**3F**)	−3.6[e]		
$(HOCF_2CF_2)_3COH$ (**2F**)	−4.4[e]		
$(HOCF_2CF_2CF(OH)CF_2)_3COH$ (**1F**)	−17.3[e]		
PhO*H*	18.2(−0.8)	19.4(−1.4)	18.0[c]
CH_3CO_2H	13.7(−1.4)	12.8(−0.5)	12.3[c]
av unsigned error	1.4	1.4	

[a]Ethanol was used as a reference compound, and its experimental pK_a (29.8) was employed as indicated in the text. [b]Parenthetical values correspond to the error in pK_a units (i.e., pK_a(exptl) − pK_a(calcd)). [c]See ref 9. [d]See ref 7. [e]These pK_a values were computed at the B3LYP/6-31+G(d) level.

Tetraol **2** has two kinds of hydroxyl groups, three primary OH substituents and one tertiary site. The latter position is predicted to be 4.7 (B3LYP/6-311+G(d,p)) and 4.2 (M06-2X/maug-cc-pVT(+d)Z) pK_a units more acidic than the primary alcohol sites, and consequently, it is expected that the conjugate base is a tertiary alkoxide anion. On the basis of the experimental pK_a values, the tetraol is found to be 16.1 pK_a units more acidic than *tert*-butyl alcohol. This difference is mainly due to the three additional hydroxyl groups in **2** and can be largely attributed to the stabilization of the conjugate base brought about by three intramolecular hydrogen bonds (Figure 1). On average this corresponds to an acidity enhancement of 5.4 pK_a units or 7.3 kcal mol^{-1} per hydrogen bond. Heptaol **1** is ~10^5 times more acidic than tetraol **2** (and a stronger acid than *tert*-butyl alcohol by a factor of 10^{21}!), indicating that the

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stabilization of the charge in the deprotonated anion goes beyond the first internal hydrogen bond shell. As a result of the primary interactions between the alkoxide ion center and the three secondary hydroxyl groups, some of the excess electron density (charge) is delocalized onto the secondary OH substituents. This makes them better hydrogen bond acceptors than they would be otherwise. These outer or second solvation shell interactions between the uncharged primary and secondary hydroxyl groups are stronger in the conjugate base of **1** than in the acid, resulting in an average stabilization of 1.6 pK_a units (i.e., 2.1 kcal mol^{-1}) per hydrogen bond. This corresponds to ∼1/3 of the energy of the inner hydrogen bond shell, but more of these interactions can arise, and they maybe used for transition-state stabilization in enzyme-catalyzed reactions.[33] Both types of solvation shells could also alter the acidities and basicities of common functional groups when they are in a biological environment.

The hydrogen bond stabilization energies of the deprotonated aliphatic polyols **1**−**4** are not as large as they could be, in part, because the spacer length between the hydroxyl groups was not optimized. By incorporating two methylenes between the OH substituents, intramolecular six-membered rings are formed in the conjugate bases. These ring structures are too small to accommodate linear hydrogen bonds. For example, in the heptaol anion the M06-2X/maug-cc-pVT(+d)Z O−H···O bond angles span from 151° to 153° in the first solvation shell (i.e., in the O$^-$···H−O hydrogen bonds) and from 142° to 147° in the second (outer) solvation shell; similar values are observed in the B3LYP structures. Entropy also works against these acyclic anions, particularly when compared to an enzyme-bound substrate. If one corrects the experimental ΔpK_a(tetraol **2** − *tert*-butyl alcohol) energy difference to account for the entropies by using the computed ΔS values, the average stabilization energy increases to 10 kcal mol^{-1} per hydrogen bond. In a lower dielectric constant (ε) medium than DMSO (ε = 46.8)[21] the hydrogen bond strength should be stronger.[34] On the basis of the results of Chen et al. and Pan and McAllister, if ε were ∼5, then the hydrogen bond strength would increase by 50%. These results suggest that strong hydrogen bonds can be formed in solution even though they have not been measured to date.

Brønsted acids are commonly employed as catalysts in many chemical transformations, including nonbiological processes.[35] Hydrogen-bond-enhanced acids such as **1** are interesting in this regard, particularly since they can be chiral and used, in principle, to carry out enantioselective protonations. To assess whether the conjugate bases of polyols can be further stabilized by incorporating electron-withdrawing groups, the pK_a values of perfluorinated **1**−**3** (i.e., **1F**−**3F**) were computed (Table 2).[36] All three of these compounds are predicted to have negative pK_a values in DMSO, which would make them more acidic than HCl (pK_a = 1.8), HBr (pK_a = 0.9), and CF_3SO_3H (pK_a = 0.3).[9] These Brønsted acids, consequently, represent a tunable system which can be exploited. The preparation, characterization, and catalytic ability of such species will be reported in a subsequent paper.

CONCLUSIONS

The pK_a of the heptaol **1** ($(HOCH_2CH_2CH(OH)CH_2)_3COH$) was measured in DMSO, and this saturated aliphatic tertiary alcohol was found to be 10^{21} times more acidic than *tert*-butyl alcohol and an order of magnitude more acidic than acetic acid. This remarkable acidity enhancement is largely attributable to the hydrogen bond network in the conjugate base of **1**. Three hydrogen bonds between the tertiary alkoxide center and the secondary hydroxyl groups result in a 22 kcal mol^{-1} stabilization. The oxygen atoms of the hydrogen bond donors are also better hydrogen bond acceptors than in the neutral acid because some of the excess charge is delocalized onto them. These secondary interactions (or second solvation shell) lead to an additional 6.4 kcal mol^{-1} stabilization, and as a result **1** is 10^5 times more acidic than $(HOCH_2CH_2)_3COH$ (**2**). Neutral−neutral hydrogen bonds such as this typically are ignored when accounting for enzyme catalysis because they are considered to be weak and are present in the substrate-bound enzyme as well as the reaction transition state. However, our results indicate that the energetic consequences of hydrogen bonding in a charged species are not short-range in nature and that the movement of a charged center may lead to transition-state stabilization in an enzyme-catalyzed process. The importance of hydrogen bond networks can be tested experimentally via double mutant cycles,[13] the incorporation of unnatural amino acids,[37] and the analyses of the molecular structures in the protein data bank. Computationalists and enzyme designers may also wish to look beyond the active site to tune hydrogen bond interactions.[38]

Application of the polarized continuum model provides predicted DMSO pK_a values that are in good accord with the experimental values (i.e., ±∼2 pK_a units). Electron-withdrawing groups are found to increase the acidities of the polyols that were examined such that they are predicted to be stronger acids than HCl. As a result, it appears that the combination of hydrogen-bonding and electron-withdrawing substituents can lead to potent Brønsted acids with adjustable acidities in nonprotic media. The characterization and utility of such species warrants further investigation, and our initial results will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

Computed geometries and energies along with the complete citation to refs 21 and 38. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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