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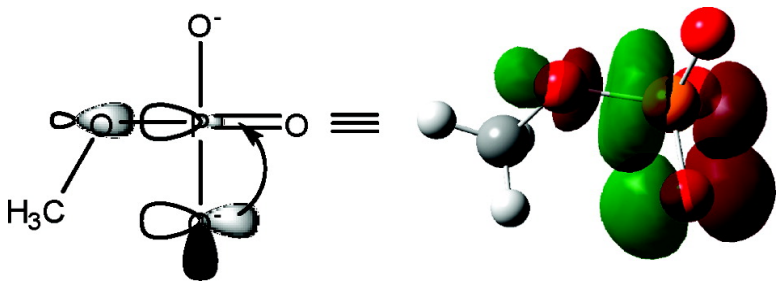
J. Am. Chem. Soc., **2008**, 130 (11), 3349-3358 • DOI: 10.1021/ja073652x



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Anomeric Effect in "High Energy" Phosphate Bonds. Selective Destabilization of the Scissile Bond and Modulation of the Exothermicity of Hydrolysis

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Abstract: A natural bonding orbital (NBO) analysis of phosphate bonding and connection to experimental phosphotransfer potential is presented. Density functional calculations with the 6-311++G(d,p) basis set carried out on 10 model phosphoryl compounds verify that the wide variability of experimental standard free energies of hydrolysis (a phosphotransfer potential benchmark) is correlated with the instability of the scissile O−P bond through computed bond lengths. NBO analysis is used to analyze all delocalization interactions contributing to O−P bond weakening. Phosphoryl bond lengths are found to correlate strongest ($R = 0.90$) with the magnitude of the ground-state n(O) → σ^*(O−P) anomeric effect. Electron-withdrawing interactions of the substituent upon the σ(O−P) bonding orbital also correlate strongly with O−P bond lengths ($R = 0.88$). However, an analysis of σ^*(O−P) and σ(O−P) populations show that the increase in σ^*(O−P) density is up to 6.5 times greater than the decrease in σ(O−P) density. Consequently, the anomeric effect is more important than other delocalization interactions in impacting O−P bond lengths. Factors reducing anomeric power by diminishing either lone pair donor ability (solvent) or antibonding acceptor ability (substituent) are shown to result in shorter O−P bond lengths. The trends shown in this work suggest that the generalized anomeric effect provides a simple explanation for relating the sensitivity of the O−P bond to diverse environmental and substituent factors. The anomeric n(O) → σ^*(O−P) interaction is also shown to correlate strongly with experimentally determined standard free energies of hydrolysis ($R = -0.93$). A causal mechanism cannot be inferred from correlation. Equally, a *P*-value of 1.2×10^{-4} from an *F*-test indicates that it is unlikely that the ground-state anomeric effect and standard free energies of hydrolysis are coincidentally related. It is found that as the exothermicity of hydroylsis increases, the energy stabilization of the ground-state anomeric effect increases with selective destabilization of the high-energy O−P bond to be broken in hydrolysis. The anomeric effect therefore partially counteracts a larger resonance stabilization of products that makes hydrolysis exothermic and needs to be considered in achieving improved agreement between calculated and empirical energies of hydrolysis. The avenues relating the thermodynamic behavior of phosphates to underlying structural factors via the anomeric effect are discussed.

Introduction

Described as the centerpiece of biochemical processes,[1] phosphoryl transfer reactions, shown in Figure 1, have long been the subject of extensive investigation.[1−25] One aspect of phosphoryl transfer that remains poorly understood are the factors governing O−P phosphoryl bond weakening and whether such factors can be connected to characteristic properties of phosphates, such as thermodynamic instability. Investigations into the weakening of the O−P bond lack consensus on whether O−P bonds change as a function of substituent and environment, how influential such changes are to thermodynamic parameters, and why they occur.

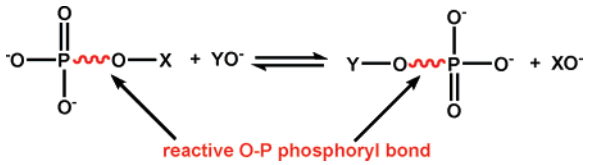

Figure 1. Phosphoryl transfer reaction scheme.

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(1) Allen, K. N.; Dunaway-Mariano, D. *Trends. Biochem. Sci.* **2004**, *29* (9), 495−503.
(2) Lipmann, F. *Adv. Enzymol. Relat. Subj. Biochem.* **1941**, *1*, 99−162.
(3) Boyer, P. D.; Stokes, B. O.; Wolcott, R. G.; Degani, C. *Fed. Proc.* **1975**, *34* (8), 1711−1717.
(4) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877−919.
(5) Westheimer, F. H. *Science* **1987**, *235*, 1173.
(6) Cleland, W.; Hengge, A. *FASEB J.* **1995**, *9* (15), 1585−1594.
(7) Diaz, N.; Field, M. J. *J. Am. Chem. Soc.* **2004**, *126* (2), 529−542.
(8) Dittrich, M.; Hayashi, S.; Schulten, K. *Biophys. J.* **2003**, *85* (4), 2253−2266.
(9) Lopez, X.; Schaefer, M.; Dejaegere, A.; Karplus, M. *J. Am. Chem. Soc.* **2002**, *124* (18), 5010−5018.

Specifically, X-ray crystal structures show almost a 0.07 Å change in O−P bond length when the pK_a of the substituent group is reduced by 8.6 units.[26−30] Meanwhile, ^{18}O isotopic shifts in ^{31}P NMR show no significant change in bond orders as a function of either substituent or medium.[30,31] Vibrational spectroscopy demonstrates clear but small (~0.04 Å when substituent pK_a values vary by 12 pK units) changes in O−P bond lengths as a function of substituent or solvent.[21] However, a much larger change in O−P bond lengths (~0.09 Å) between a model acetyl phosphate and analogous aspartyl phosphate in enzymatic environments is reported using similar techniques.[32] Theoretical calculations show distinct O−P bond lengths change as a function of charge, conformation, and environment.[13,15−17,33,34] For example, Florian and Warshel[13] report that O−P bond lengths shorten with decreasing charge of the phosphoryl group. This trend was described to reflect stronger covalent bonding in neutral species.[13] In contrast, dianionic species displayed more polar and longer O−P bonds. Loncke and Berti[35] show similar correlations between protonation effects, O−P bond lengths, and calculated bond dissociation energies. Distinct changes in axial O−P bond lengths are evident in rotations about O−P−O−X dihedrals.[36−41] Finally, a 0.04 Å O−P bond length change in GTP on binding to Ras has been predicted.[42]

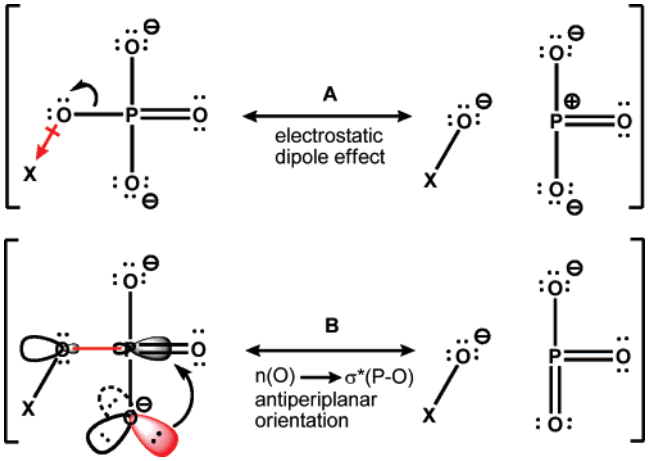


Figure 2. Charge-transfer interactions contributing to O−P bond weakness.

Even when there are clear trends between O−P bond lengths and the substituent, it is difficult to assess the contribution of O−P bond weakness to thermodynamics, in part because of the dangers of attributing a cause and effect relationship through correlations.[21] Most theoretical and experimental data have suggested small energetic changes with O−P bond lengthening and consequently are thought to have little impact upon phosphotransfer potential. Using force constants calculated from vibrational frequencies and theoretical data, Cheng and co-workers suggested a 1 kcal/mol energetic impact for a 0.1 Å change.[21,43] However, Barth and Bezlyepkina attributed a 15 kcal/mol impact for a 0.09 Å change in O−P bond length between model acetyl phosphate and aspartyl phosphate in ADP-insensitive phosphoenzyme using vibrational frequencies from infrared spectroscopy.[32]

The weakness of the O−P bond in triphosphates and diphosphates that possess adjacent oxygen atoms have been rationalized by classical electrostatic repulsion.[16,44,45] An alternative explanation for the weakness of the O−P bond involves charge-transfer effects stemming from the electronegativity of the phosphate substituent.[21,46] Charge transfer was first proposed by Kirby and co-workers for the weakness of the C−O bond in a variety of substituted aryl acetals.[27,47] It was suggested that strongly electronegative substituents (low pK_a) withdraw electron density from the C−O σ bond and result in a longer, more polar bond. This is sometimes explained as an electrostatic dipole effect, where highly electron-withdrawing substituents create a dipole moment that stabilizes longer and weaker C−O bonds.[21] This argument has been extended to the O−P bond in phosphates.[21,27] The substituent charge-transfer effect can be described by the flow of charge, as shown Figure 2a.

The other most common charge-transfer factor proposed for O−P bond weakening in phosphates[48−86] was proposed by

(10) Benkovic, S. J.; Schray, K. J. Chemical Basis of Biological Phosphoryl Transfer. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1973; Vol. 8, pp 201−238.
(11) Dzeja, P. P.; Terzic, A. *J. Exp. Biol.* **2003**, *206* (12), 2039−2047.
(12) Williams, N. H. *Biochim. Biophys. Acta* **2004**, *1697* (1−2), 279−287.
(13) Florian, J.; Warshel, A. *J. Phys. Chem. B* **1998**, *102* (4), 719−734.
(14) Florian, J.; Warshel, A. *J. Am. Chem. Soc.* **1997**, *119* (23), 5473−5474.
(15) Florian, J.; Aqvist, J.; Warshel, A. *J. Am. Chem. Soc.* **1998**, *120* (44), 11524−11525.
(16) Strajbl, M.; Shurki, A.; Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (25), 14834−14839.
(17) Klahn, M.; Rosta, E.; Warshel, A. *J. Am. Chem. Soc.* **2006**, *128* (47), 15310−15323.
(18) Aqvist, J.; Kolmodin, K.; Florian, J.; Warshel, A. *Chem. Biol.* **1999**, *6* (3), R71−R80.
(19) Admiraal, S. J.; Schneider, B.; Meyer, P.; Janin, J.; Veron, M.; Deville-Bonne, D.; Herschlag, D. *Biochemistry* **1999**, *38* (15), 4701−11.
(20) Admiraal, S. J.; Herschlag, D. *J. Am. Chem. Soc.* **2000**, *122* (10), 2145−2148.
(21) Cheng, H.; Nikolic-Hughes, I.; Wang, J. H.; Deng, H.; O'Brien, P. J.; Wu, L.; Zhang, Z.-Y.; Herschlag, D.; Callender, R. *J. Am. Chem. Soc.* **2002**, *124* (38), 11295−11306.
(22) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111* (19), 7579−7586.
(23) Hollfelder, F.; Herschlag, D. *Biochemistry* **1995**, *34* (38), 12255−12264.
(24) Maegley, K. A.; Admiraal, S. J.; Herschlag, D. *PNAS* **1996**, *93* (16), 8160−8166.
(25) O'Brien, P. J.; Herschlag, D. *Biochemistry* **2002**, *41* (9), 3207−3225.
(26) Jones, P. G.; Sheldrick, G. M.; Kirby, A. J.; K. W. Y. A. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1984**, *40* 550−552.
(27) Jones, P. G.; Sheldrick, G. M.; Kirby, A. J.; K. W. Y. A. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1984**, C40, 547−549.
(28) Caughlan, C. N.; *Inorg. Chem.* **1967**, *6*, (11), 1998−2002.
(29) Garbassi, F.; Giarda, L.; Fagherazzi, G. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Commun.* **1972**, *28*, 1665−1670.
(30) Sorensen-Stowell, K.; Hengge, A. C. *J. Org. Chem.* **2005**, *70* (12), 4805−4809.
(31) Sorensen-Stowell, K.; Hengge, A. C. *J. Org. Chem.* **2005**, *70* (21), 8303−8308.
(32) Barth, A.; Bezlyepkina, N. *J. Biol. Chem.* **2004**, *279* (50), 51888−51896.
(33) Florian, J.; Strajbl, M.; Warshel, A. *J. Am. Chem. Soc.* **1998**, *120* (31), 7959−7966.
(34) Aqvist, J.; Kolmodin, K.; Florian, J.; Warshel, A. *Chem. Biol.* **1999**, *6* (3), R71−R80.
(35) Loncke, P. G.; Berti, P. J. *J. Am. Chem. Soc.* **2006**, *128* (18), 6132−6140.
(36) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B. *J. Am. Chem. Soc.* **1977**, *99* (24), 8048−8049.
(37) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B.; Momii, R. *J. Am. Chem. Soc.* **1977**, *99* (12), 4170−4172.
(38) Gorenstein, D. G. *Chem. Rev.* **1987**, *87* (5), 1047−1077.
(39) Taira, K.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106* (25), 7825−7831.
(40) Taira, K.; Fanni, T.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106* (5), 1521−1523.
(41) Range, K.; McGrath, M. J.; Lopez, X.; York, D. M. *J. Am. Chem. Soc.* **2004**, *126* (6), 1654−1665.
(42) Klahn, M.; Schlitter, J.; Gerwert, K. *Biophys. J.* **2005**, *88* (6), 3829−3844.
(43) Cheng, H.; Sukal, S.; Deng, H.; Leyh, T. S.; Callender, R. *Biochemistry* **2001**, *40* (13), 4035−4043.
(44) Shurki, A.; Strajbl, M.; Schutz, C. N.; Warshel, A. *Methods Enzymol.* **2004**, *380*, 52−84.
(45) Voet, D.; Voet, J. G. *Biochemistry*; 2nd ed.; John Wiley: New York, 1995; pp 428−434.
(46) Jones, P. G.; Kirby, A. J. *J. Am. Chem. Soc.* **1984**, *106* (21), 6207−6212.
(47) Allen, F. H.; Kirby, A. J. *J. Am. Chem. Soc.* **1984**, *106* (21), 6197−6200.
(48) Gautier, A. *Heterocycles* **2006**, *67* (2), 823−837.
(49) Whitehead, A.; McReynolds, M. D.; Moore, J. D.; Hanson, P. R. *Org. Lett.* **2005**, *7* (15), 3375−3378.
(50) Egli, M.; Minasov, G.; Tereshko, V.; Pallan, P. S.; Teplova, M.; Inamati, G. B.; Lesnik, E. A.; Owens, S. R.; Ross, B. S.; Prakash, T. P.; Manoharan, M. *Biochemistry* **2005**, *44* (25), 9045−9057.
(51) Matsukawa, S.; Yamamoto, Y.; Akiba, K.-y. *Heterocycles* **2003**, *59* (2), 707−719.

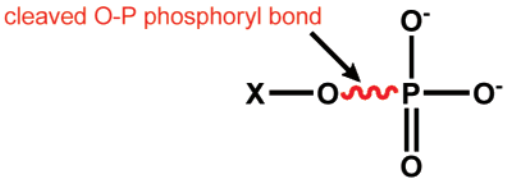
Gorenstein and co-workers in 1977 when investigating the reactions of phosphate diesters.[36−40] Calculations showed selective weakening of O−P ester bonds when oxygen atom lone pairs adopted an antiperiplanar orientation toward that bond. Further theoretical and experimental studies on the conformational preference of phosphates, such as in nucleic acids and their counterparts, pentacoordinated phosphates, phosphorothionates, and bicyclic and acyclic phosphates, also suggested O−P bond weakening due to stereoelectronic effects.[33,38−40,87−96] The flow of charge suggested by Gorenstein is shown in Figure 2b. Both types of charge-transfer effects stabilize nominally similar resonance structures. As a result, it is important to investigate O−P bond weakening in the context of different charge-transfer effects as a first step to developing a better understanding of how and why phosphates respond to different substituent and environmental factors.

The intent of this work is to explore three aspects of ground-state O−P bond weakening and possible connections to the exothermicity of hydroylsis. First, the most important charge-transfer interactions (e.g., anomeric versus substituent) responsible for scissile O−P bond weakening are investigated across a series of phosphate compounds ranging in phosphotransfer potential. Second, the structural, electronic, and external factors responsible for selective O−P bond weakening are explored. Finally, the energetic consequence of product resonance stabilization and the ground-state anomeric effect is examined to explain the observed exothermicity of hydrolysis and possible connection to phosphotransfer potentials. Electronic structure methods and natural bond orbital (NBO) analysis are used to provide the necessary atomistic and electronic detail to identify and quantify the origin of O−P bond length differences and energies. The results provide a foundation for understanding both the bonding behavior of monosubstituted phosphates and its connection to phosphotransfer potential.

Methods

All electronic structure calculations were carried out using G03.[97] Initial conformations for all structures were obtained from either prior published calculations,[98,99] crystal structures,[100,101] or potential energy scans about select dihedrals using PM3 semiempirical molecular orbital theory.[102] Minimum energy structures were geometry optimized using B3LYP density functional method[103,104] and the 6-311++G(d,p) basis set.[105,106] All calculations were performed in water using the PCM model.[107] Charges were calculated using ESP methods, namely the Merz−Kollmann method.[108] Prior calculations on the N−P bond in phosphagens[109] and tests on selected structures showed that this level of theory was sufficient to identify the changes in phosphoryl bond lengths associated with the generalized anomeric effect. More extensive basis sets and higher levels of theory did not change any general trend. Frequency calculations were performed in conjunction with all energy minimizations to

(52) Zhang, X.; Harrison, D. H. T.; Cui, Q. *J. Am. Chem. Soc.* **2002**, *124* (50), 14871−14878.
(53) Labande, A.; Ruiz, J.; Astruc, D. *J. Am. Chem. Soc.* **2002**, *124* (8), 1782−1789.
(54) Toney, M. D. *Biochemistry* **2001**, *40* (5), 1378−1384.
(55) Velikian, I.; Acharya, P.; Trifonova, A.; Foldesi, A.; Chattopadhyaya, J. *J. Phys. Org. Chem.* **2000**, *13* (5), 300−305.
(56) Alber, F.; Folkers, G.; Carloni, P. *J. Phys. Chem. B* **1999**, *103* (29), 6121−6126.
(57) Acharya, P.; Nawrot, B.; Sprinzl, M.; Thibaudeau, C.; Chattopadhyaya, J. *J. Chem. Soc., Perkin Trans.* **1999**, *2* (7), 1531−1536.
(58) Parker, E. J.; Coggins, J. R.; Abell, C. *J. Org. Chem.* **1997**, *62* (24), 8582−8585.
(59) Tole, P.; Lim, C. Do stereoelectronic effects control the structure and reactivity of trigonal-bipyramidal phosphoesters? *ACS Symp. Ser.* **1993**, *539* (Anomeric Effect and Associated Stereoelectronic Effects), 240−55.
(60) Johnson, L. N.; Hu, S. H.; Barford, D. *Faraday Discuss.* **1992**, *93* (Structure and Activity of Enzymes), 131−42.
(61) Uchimaru, T.; Tanabe, K.; Nishikawa, S.; Taira, K. *J. Am. Chem. Soc.* **1991**, *113* (11), 4351−3.
(62) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113* (10), 3980−3.
(63) Voityuk, A. A. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1990**, *11*, 2528−32.
(64) Kluger, R.; Taylor, S. D. *J. Am. Chem. Soc.* **1990**, *112* (18), 6669−71.
(65) Van Genderen, M. H. P.; Kook, L. H.; Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1989**, *108* (1), 28−35.
(66) Gorenstein, D. G.; Chang, A.; Yang, J. C. *Tetrahedron* **1987**, *43*, (3), 469−78.
(67) Gorenstein, D. G.; Schroeder, S. A.; Miyasaki, M.; Fu, J. M.; Roongta, V.; Abuaf, P.; Chang, A.; Yang, J. C. *Bioact. Mol.* **1987**, *3* (Biophosphates Their Analogues), 487−502.
(68) Fanni, T. A. *Stereoelectronic effects in phosphates, phosphorothionates, and phosphoranes*; 1985.
(69) Kluger, R.; Thatcher, G. R. J. *J. Am. Chem. Soc.* **1985**, *107* (21), 6006−11.
(70) Yakovlev, G. I.; Bocharov, A. L.; Moiseev, G. P.; Mikhailov, S. N. *Bioorg. Khim.* **1985**, *11* (2), 205−10.
(71) Yakovlev, G. I.; Bocharov, A. L.; Moiseev, G. P.; Mikhailov, S. N. *FEBS Lett.* **1985**, *179* (2), 217−20.
(72) Taira, K.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106* (25), 7825−31.
(73) Taira, K.; Fanni, T.; Gorenstein, D. G. *J. Org. Chem.* **1984**, *49* (23), 4531−6.
(74) Taira, K.; Fanni, T.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106* (5), 1521−3.
(75) Gorenstein, D. G.; Rowell, R.; Taira, K. *ACS Symp. Ser.* **1981**, 171 (Phosphorus Chem.), 69−75.
(76) Gorenstein, D. G.; Luxon, B. A.; Goldfield, E. M. *J. Am. Chem. Soc.* **1980**, *102* (5), 1757−9.
(77) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B. 2. *J. Am. Chem. Soc.* **1979**, *101* (20), 5869−75.
(78) Luxon, B. A. *Stereoelectronic effects in phosphate esters*; 1977.
(79) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B. *J. Am. Chem. Soc.* **1977**, *99* (24), 8048−9.
(80) Kar, D. Part I. Stereoelectronic effects in phosphate esters. Part II. Proton nuclear magnetic resonance study on the binding of trans-cinnamaldehyde to a-chymotrypsin; 1977.
(81) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B.; Momii, R. *J. Am. Chem. Soc.* **1977**, *99* (12), 4170−2.
(82) Lehn, J. M.; Wipff, G. *J. Chem. Soc., Chem. Commun.* **1975**, *19*, 800−2.
(83) Whitehead, A.; McReynolds Matthew, D.; Moore Joel, D.; Hanson Paul, R. *Org. Lett.* **2005**, *7* (15), 3375−8.
(84) Zhang, X.; Harrison David, H. T.; Cui, Q. *J. Am. Chem. Soc.* **2002**, *124* (50), 14871−8.
(85) Yakovlev, G. I.; Bocharov, A. L.; Moiseyev, G. P.; Mikhaylov, S. N. *FEBS Lett.* **1985**, *179* (2), 217−20.
(86) Tsai, M. D.; Weintraub, H. J.; Byrn, S. R.; Chang, C.; Floss, H. G. *Biochemistry* **1978**, *17* (16), 3183−8.
(87) Cramer, C. J.; Gustafson, S. M. *J. Am. Chem. Soc.* **1994**, *116* (2), 723−734.
(88) Cramer, C. J.; Gustafson, S. M. *J. Am. Chem. Soc.* **1993**, *115* (20), 9315−9316.
(89) Gauss, J.; Cramer, C. *Chem. Phys. Lett.* **1988**, *150*, 280.
(90) Storer, J. W.; Uchimaru, T.; Tanabe, K.; Uebayasi, M.; Nishikawa, S.; Taira, K. *J. Am. Chem. Soc.* **1991**, *113* (14), 5216−5219.
(91) Zhou, D.-M.; Taira, K. *Chem. Rev.* **1998**, *98* (3), 991−1026.
(92) Banavali, N. K.; MacKerell, A. D. *J. Am. Chem. Soc.* **2001**, *123* (28), 6747−6755.
(93) Hernandez, J.; Ramos, R.; Sastre, N.; Meza, R.; Hommer, H.; Salas, M.; Gordillo, B. *Tetrahedron* **2004**, *60* (48), 10927−10941.
(94) Cramer, C. J.; Denmark, S. E.; Miller, P. C.; Dorow, R. L.; Swiss, K. A.; Wilson, S. R. *J. Am. Chem. Soc.* **1994**, *116* (6), 2437−2447.
(95) Kirby, A. J. *Adv. Phys. Org. Chem.* **1994**, *29*, 87−184.
(96) Denehy, E.; White, J. M.; Williams, S. J. *Chem. Commun.* **2006**, *3*, 314−316.
(97) Frisch, M. J.; et al. *Gaussian 03*, revision D.01; Gaussian, Inc.: Pittsburgh, PA, 2003.
(98) Hwang, M.-J.; Chu, P.-Y.; Chen, J.-C.; Chao, I. *J. Comput. Chem.* **1999**, *20* (16), 1702−1715.
(99) Meagher, K. L.; Redman, L. T.; Carlson, H. A. *J. Comput. Chem.* **2003**, *24* (9), 1016−1025.
(100) Crowhurst, G. S.; Dalby, A. R.; Isupov, M. N.; Campbell, J. W.; Littlechild, J. A. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1999**, *55* (11), 1822−1826.
(101) Flachner, B.; Kovari, Z.; Varga, A.; Gugolya, Z.; Vonderviszt, F.; Naray-Szabo, G.; Vas, M. *Biochemistry* **2004**, *43* (12), 3436−3449.
(102) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10* (2), 221−264.
(103) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098−3100.
(104) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37* (2), 785−789.
(105) Francl, M. M.; Pietro, W. J.; Hehre, W. J.; Binkley, J. S.; Gordon, M. S.; Defrees, D. J.; Pople, J. A. *J. Chem. Phys.* **1982**, *77*, 3654−3665.
(106) Clark, T.; Chandrasekhar, J.; Spitznagel, G. W.; Schleyer, P. v. R. *J. Comput. Chem.* **1983**, *4*, 294.
(107) Miertus, S.; Scrocco, E.; Tomasi, J. *J. Chem. Phys.* **1981**, *55*, 117.
(108) Besler, B. H.; Merz, K. M.; Kollman, P. A. *J. Comput. Chem.* **1990**, *11*, (4), 431−439.
(109) Ruben, E. A.; Chapman, M. S.; Evanseck, J. D. *J. Am. Chem. Soc.* **2005**, *127* (50), 17789−17798.



Substituent		ΔG hydrolysis (kcal/mol)
X = 1:	glycerate (glycerol-3-phosphate)	-2.2
2:	methanolate (methyl monophosphate)	-2.2
3:	3,4,5,6 tetrahydroxytetrahydropyranyl methanolate (glucose-6-phosphate)	-3.3
4:	3,4,5 trihydroxy 6 tetrahydropyranolate (glucose-1-phosphate)	-5.0
5:	pyruvate enol (phosphoenol pyruvate)	-6.8
6:	methyl monophosphate (methyl diphosphate)	-8.5
7:	methyl diphosphate (methyl triphosphate)	-8.5
8:	acetate (acetyl phosphate)	-10.3
9:	3-phosphoglycerate (1,3 bisphosphoglycerate)	-11.8
10:	carbamate (carbamyl phosphate)	-12.5

Figure 3. Phosphoryl compounds modeled.

characterize all stationary points as minima. Coordinated compounds were calculated without the inclusion of basis set superposition error.

Natural bond order (NBO) analysis was performed using the NBO 3.1 program[110] interfaced into the Gaussian program. NBO transforms the nonorthogonal atomic orbitals from the HF wavefunction into natural atomic orbitals (NAO), natural hybrid orbitals (NHO), and natural bond orbitals (NBO) each of which are complete and orthonormal. This allows electron density to be treated in a more intuitive manner, i.e., localized onto bonds and atoms, leading to a better description of the molecule as a localized Lewis structure. In effect, NBO transformation provides filled orbitals that are more concentrated in terms of occupancies. This then allows delocalizing interactions to be treated as a perturbation through second-order perturbation theory. The $E(2)$ energy values from the second-order perturbation method then provide a reasonable quantitative description of the magnitude of such delocalizing interactions.[111−113] NBO deletion analysis was performed using the HF/6-311++G(d,p) method on B3LYP/6-311++G(d,p) optimized structures because of the inconsistency of deletion operations using DFT wavefunctions.

The NBO method has been cited for overestimating charge-transfer energies as compared to other methods, such as the Morukuma method[114] and other localized wavefunction methods.[115] However, it has also been reported that the trends computed by NBO are the same as that produced by other methods.[116] Since the charge-transfer energies are compared in a series of phosphates in this study, the NBO method will overestimate the charge transfer in each compound by the same amount. The most important advantage of using the NBO method is that it provides a more quantitative method of measuring relative electron delocalization (charge transfer) of individual orbital interactions.

Results and Discussion

Charge-Transfer Interactions and O−P Bond Weakening. A molecular orbital approach is used to identify the most important charge-transfer interactions in the weakening of the O−P bond. First, a series of phosphate compounds ranging in phosphotransfer potential are modeled to compare computed bond length changes against experimentally known standard free energies of hydrolysis. Delocalizations lengthening the O−P bond length involve interactions either removing electron density from σ(O−P) or donating electron density into σ^*(O−P). Both types of interactions are correlated to O−P bond lengths. Thus, the relative increase in σ^*(O−P) density versus the relative decrease in σ(O−P) is compared.

Geometric Structures. The phosphoryl compounds modeled, as shown in Figure 3, were chosen to span the wide range of free energies of hydrolysis from *high energy* (−12.5 kcal/mol) to *low energy* (−2.2 kcal/mol) systems.[45,117,118] It is well-known that standard free energies of hydrolysis of phosphate compounds are different from the free energies ultimately utilized in biological environments. Nevertheless, standard conditions are used to make relative and controlled comparisons between different phosphate compounds. Optimized structures are shown in Figure 4.

A direct correlation ($R = -0.88$) between experimental standard free energies of hydrolysis and calculated O−P phosphoryl bond lengths is found, as shown in Figure 5. This correlation suggests a connection between O−P phosphoryl bond strengths and phosphotransfer potentials, where bond weakening effects contribute to exothermic free energies of hydrolysis.

***σ**(O−P) Interactions and Correlations.** The most dominant interaction donating electron density into σ^*(O−P) was that of the generalized anomeric n(O) → σ^*(O−P) effect. NBO second-order energy, $E(2)$, estimations of these interactions were ∼25 kcal/mol. Much less dominant were $\sigma(O_{nonbridging}-P) \rightarrow \sigma^*(O-P)$, which was on the order of 2 kcal/mol and so are not included in the discussion. The sum of all generalized anomeric n(O) → σ^*(O−P) contributions is considered. Reactive phosphoryl O−P bond lengths are found to correlate ($R = 0.90$) with the n(O) → σ^*(O−P) generalized anomeric effect, as shown in Figure 6a. Importantly, the computed anomeric effect energies are found to correlate ($R = -0.93$) with the experimental standard free energies of hydrolysis, as shown in Figure

(110) Glendening, E. D.; Badenhoop, J. K.; Reed, A. E.; Carpenter, J. E. Weinhold, F. *NBO 3.1*, 1996.
(111) Alabugin, I. V.; Zeidan, T. A. *J. Am. Chem. Soc.* **2002**, *124* (12), 3175−3185.
(112) Alabugin, I. V.; Mariappan, M.; Zeidan, T. A. *J. Am. Chem. Soc.* **2003**, *125* (46), 14014−14031.
(113) Cramer, C. J.; Kelterer, A. M.; French, A. D. *J. Comput. Chem.* **2001**, *22* (11), 1194−1204.
(114) Kitaura, K.; Morokuma, K. *Int. J. Quant. Chem.* **1976**, *10* (2), 325−340.
(115) Mo, Y.; Peyerimhoff, S. D. *J. Chem. Phys.* **1998**, *109* (5), 1687−1697.
(116) Mo, Y.; Gao, J. *Acc. Chem. Res.* **2007**, *40* (2), 113−119.
(117) Garrett, R. H.; Grisham, C. M. *Biochemistry*, 2nd ed.; Saunders: Fort Worth, 1999; pp 56−80.
(118) Berg, J.; Tymoczko, J. L.; Stryer, L. *Biochemistry*, 5th ed.; W. H. Freeman & Co.: New York, 2002; pp 374−383.

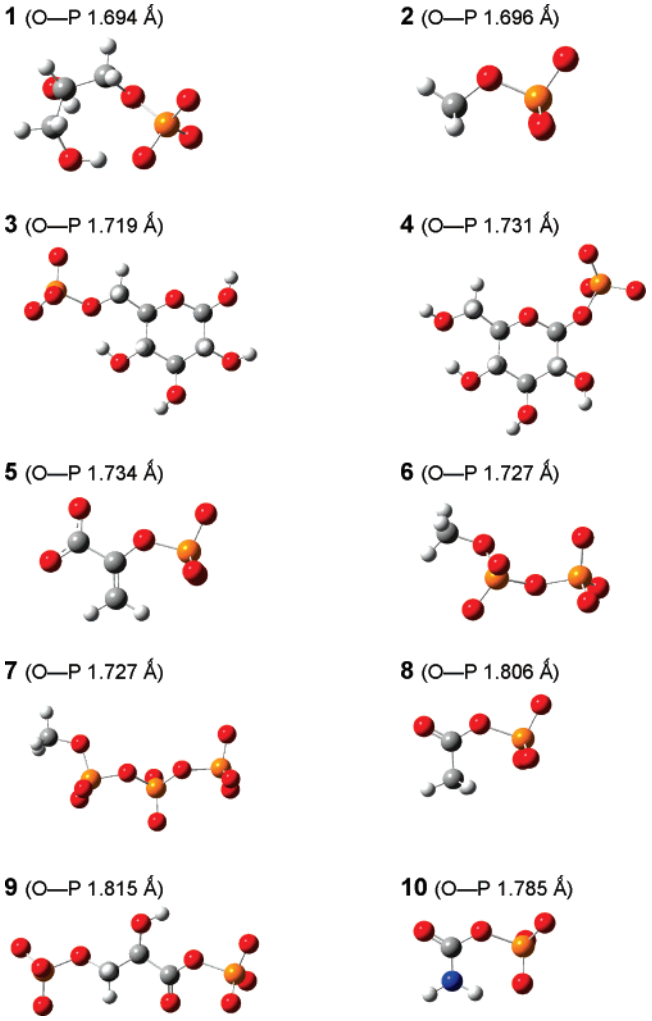


Figure 4. Optimized structures using the B3LYP/6-311++G(d,p) level of theory with energy minimized high-energy bond length given in parentheses. Phosphorus atoms are illustrated in orange, oxygen in red, carbon in gray, and nitrogen in blue.

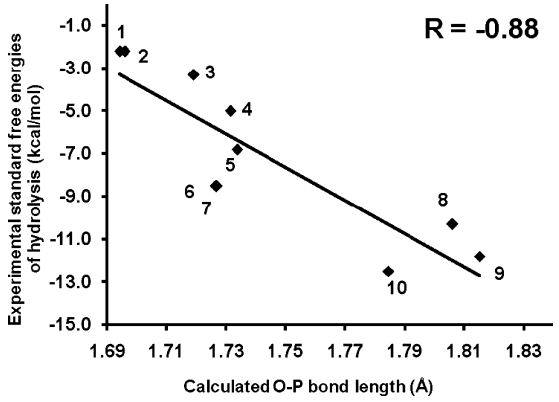


Figure 5. Correlation between standard free energies of hydrolysis and O−P bond lengths.

6b. It is known that cause cannot be inferred from correlation, but an F-test gives a *P*-value of 1.2×10^{-4}, indicating that there is little chance that the anomeric effect is correlated to phosphoryl transfer potential by coincidence.

The net anomeric effect reported is a sum of *E*(2) contributions from all three antiperiplanar phosphoryl oxygen atoms. Occasionally more than one contribution into the same orbital can cause anti-cooperative interactions,[113] which may lead to

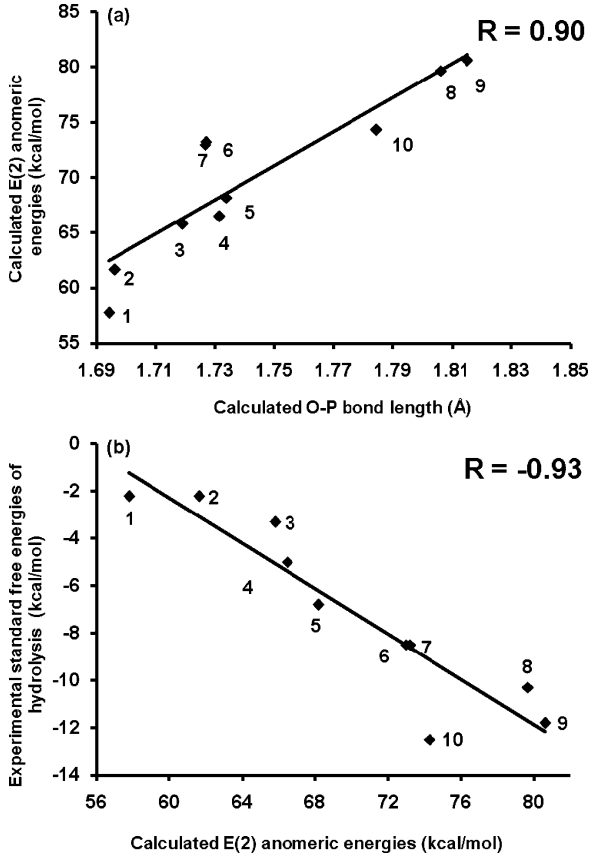


Figure 6. Correlation between the n(O) → σ^*(O−P) generalized anomeric effect (a) O−P bond lengths, and (b) experimental standard free energies of hydrolysis.

different values in overall anomeric energy and not give the correlations given in Figure 6. To check that the correlations were correct and that the interactions were not anti-cooperative, an NBO deletion analysis comparing the total *E*(2) energy values to the simultaneously deleted interactions was performed.[111,112] With all structures, no evidence of anti-cooperative effects was observed and correlations hold, as shown by Figure S1 in Supporting Information. It is noted that O−P bonds for structures **6** and **7**, corresponding to ATP and ADP, tend to agree somewhat less with experimental free energies of hydrolysis and calculated *E*(2) anomeric contributions (Figures 5 and 6), suggesting that while anomeric effects appear to be important, other factors also contribute to O−P weakness in those compounds.[16,44,45]

***σ*(O−P) Interactions and Correlations.** In keeping with intuitive expectations that σ bonds are poor donors, charge transfer from σ(O−P) interactions were found to be small in magnitude compared to σ^*(O−P) interactions. For example, acetyl phosphate possessed a σ(O−P) → σ^*(C−O) interaction of 5.6 kcal/mol. The correlation between O−P bond lengths and these interactions ($R = 0.88$), as shown in Figure 7a, yield a comparable correlation as found with the anomeric effect ($R = 0.90$). The bond weakening of σ(O−P) correlates less strongly with the experimental free energies of hydrolysis ($R = -0.71$), as shown in Figure 7b.

***σ*(O−P) vs *σ**(O−P) Density.** Given that bond lengths are influenced by the relative populations of σ and σ^* orbitals, the decrease in σ(O−P) and the increase in σ^*(O−P) density was plotted versus the O−P bond length, as shown in Figure 8a.

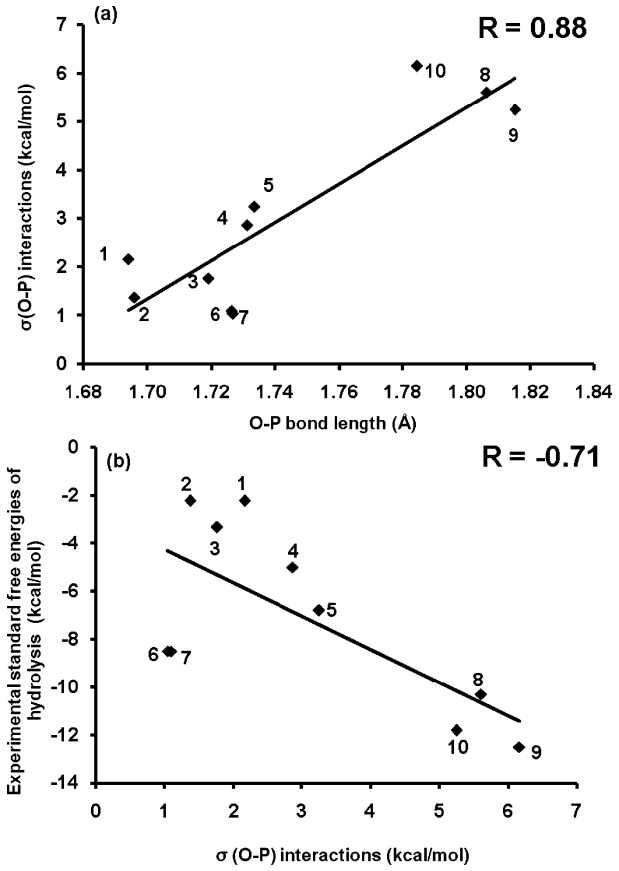


Figure 7. Correlation between σ(O−P) delocalizations into the substituent group with (a) O−P bond length, and (b) experimental standard free energies of hydrolysis.

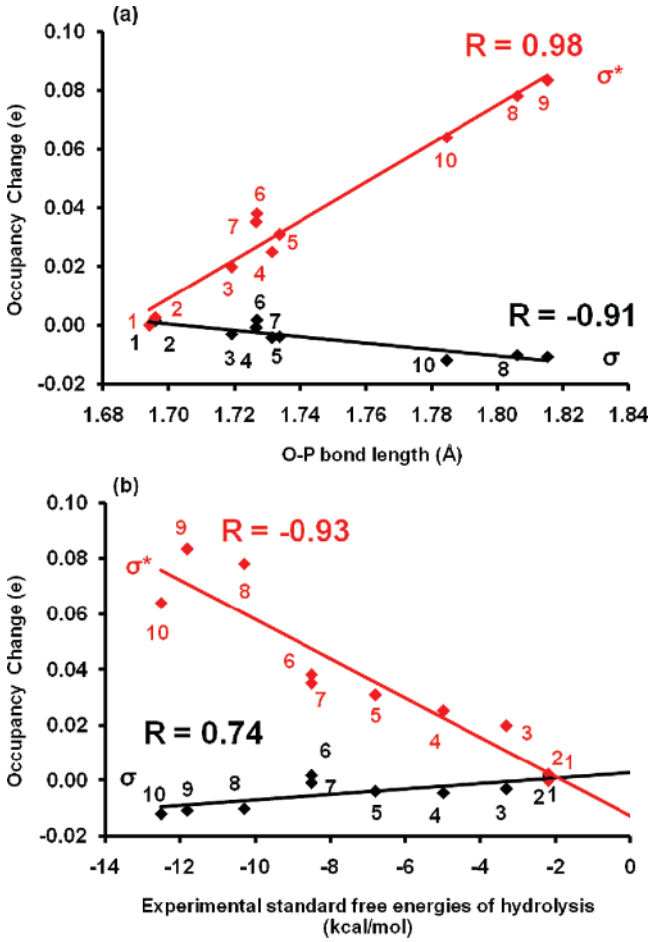


Figure 8. (a) Decrease in σ(O−P) and increase in σ*(O−P) occupancies and their correlation with O−P bond lengths. (b) Corresponding occupancy change and correlation with the experimental free energies of hydrolysis.

The difference between σ*(O−P) and σ(O−P) occupancies and their relationship to experimental free energies of hydrolysis (Figure 8b) also suggests that the generalized anomeric effect is more important in impacting experimental free energies of hydrolysis than other O−P bond weakening factors. Hence, while electron-withdrawing effects of the substituent upon the bonding orbital is shown to have some influence (Figure 7a, slope = 40 kcal/mol Å) on the weakness of the O−P bond, the anomeric effect is shown to be far more significant (Figure 6a, slope = 155 kcal/mol Å). As a check, the phosphate was removed and substituted with a proton, and the change in σ-(O−H) versus σ*(O−H) was compared. The increase in σ*-(O−H) was similar to the decrease in σ(O−H), Figure S2, underscoring the importance of the anomeric effect.

Anomeric Enhancement by Substituents. As described earlier, the increased polarity of the C−O bond, as a result of electron-withdrawing substituents, was given as a reason for longer C−O bonds by Kirby and extended to O−P bonds.[47] A greater contribution of the charge-separated resonance form leads to a greater stabilization of longer C−O bonds. However, as Kirby concedes, it is not immediately self-evident why an increasing dipole at C−O would lead to an increase in bond lengths.[47] Kirby points out that an ionic bond is weaker than a covalent bond, but as ionic character increases, greater electrostatic attraction traditionally leads to a shortening of bonds.[119] The generalized anomeric effect offers a straightforward explanation to reconcile why more polar O−P bonds increase in length.

The lowering in energy of orbitals is often due to an increase in polarity.[111] A relationship between σ* orbital energies and polarity is found, as shown in Figure 9. Using the population difference between O and P as an index of polarity (this is also equal to the difference between squares of polar coefficients) of the O−P bond, an increased polarity of the σ*(O−P) bond is found to correlate with a lower σ*(O−P) orbital energy, as shown in Figure 9a, with an enhanced anomeric effect, Figure 9b, and with experimental free energy of hydrolysis, Figure 9c.

Orbital energies of both the lone pair and O−P antibond confirm that it is the lowering in energy of the σ*(O−P) that is most responsible for reducing the average n(O) and σ*(O−P) energy gap and enhancing the anomeric effect, as shown in Figure 10. Thus, as polarity is increased, the σ*(O−P) energy is decreased, and the energy gap between n(O) and σ*(O−P) is reduced, leading to a more enhanced anomeric effect and consequently longer O−P bonds.

Environmental Effects. Ground-state stereoelectronic effects will undoubtedly be different between enzymatic and nonenzymatic conditions. As such, the computed correlations are tested by including external influences that crudely approximate enzymatic conditions/interactions. Polar solvents are known to accelerate cleavage rates of phosphates.[120] However, the effect of polar solvents on O−P bond lengths has been less well

(119) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: Ithaca, NY, 1939.

(120) Grzyska, P. K.; Czyryca, P. G.; Golightly, J.; Small, K.; Larsen, P.; Hoff, R. H.; Hengge, A. C. *J. Org. Chem.* **2002**, *67* (4), 1214−1220.

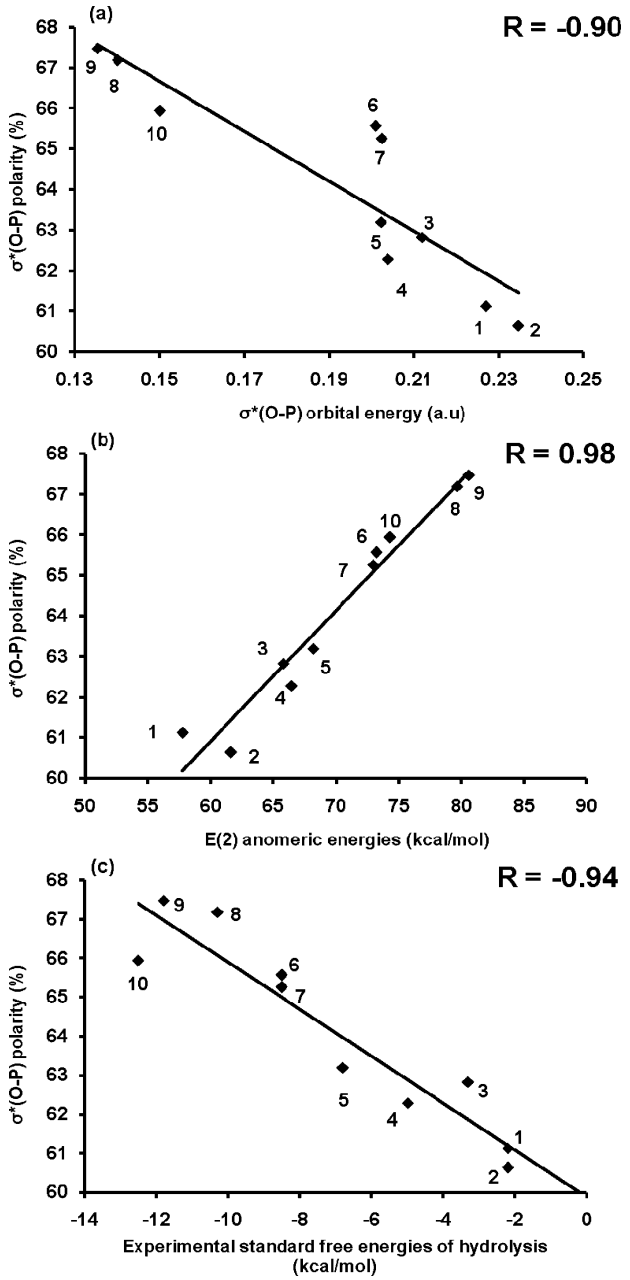


Figure 9. (a) Correlation between σ^*(O−P) polarity and orbital energies. (b) Correlation between σ^*(O−P) polarity and anomeric energies. (c) Correlation between σ^*(O−P) polarity and experimental free energies of hydrolysis.

defined. ^{18}O isotopic shifts in ^{31}P NMR show no significant change in the bond orders. But more precise IR experiments show small but distinct increases in O−P bond lengths as DMSO concentrations increase.[21] To account for this trend, it was suggested that less favorable electrostatic interactions between DMSO and nonbridging oxygen atoms preferred less charge accumulation on those atoms and provided an electrostatic incentive to shorten those bonds. Water molecules form more stabilizing electrostatic interactions with the nonbridging oxygen atoms. In doing so, lone pairs on the oxygen atoms are likely sequestered and less able to contribute to the anomeric effect. To test this idea, a methyl monophosphate complex with water was modeled using the B3LYP/6-311++G(d,p) level of theory. Specifically, methyl monophosphate coordinated with three

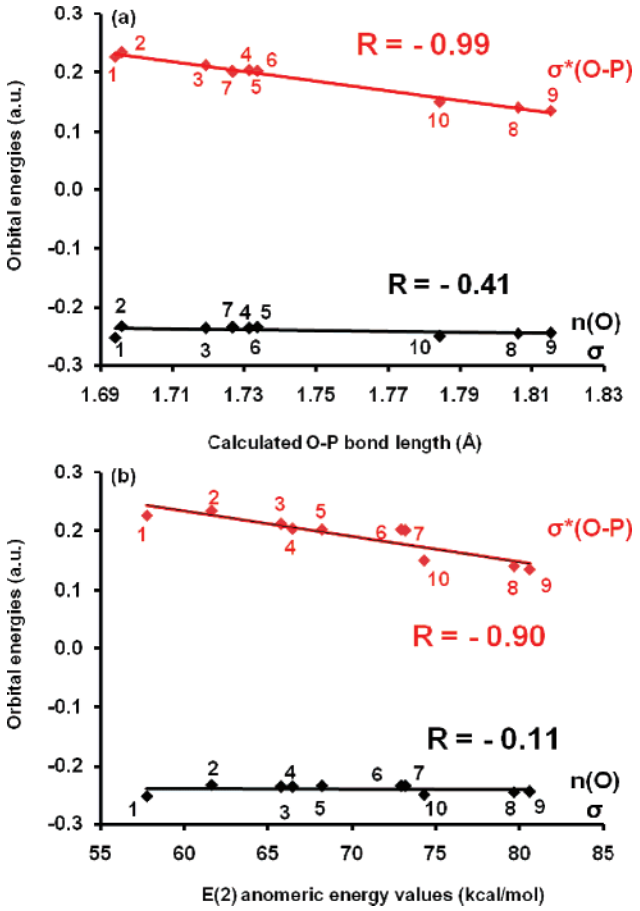


Figure 10. (a) Relationship between orbital energies and O−P bond lengths. (b) Relationship between orbital and anomeric energies.

water molecules and optimized in water using PCM[107,121] was compared to methyl monophosphate coordinated to three DMSO molecules optimized in DMSO also using PCM. It was found that the reactive O−P bond for a simple methyl monophosphate did increase ∼0.03 Å (experimental value ∼0.015 Å) in moving from water to DMSO. This increase in bond lengths was matched by an increase in $E(2)$ values for the anomeric effect by ∼11 kcal/mol showing once again the connection between O−P bond lengthening and the anomeric effect.

To further investigate how environmental factors influence the anomeric effect, five methyl triphosphate molecules (a model of ATP) were optimized in the gas phase. Subsequently, PCM single point energy evaluations were performed on the gas phase structures to model the effects of water. Three of these structures were chosen to increase the protonation state sequentially at the terminal γ-phosphoryl group: **11**, fully deprotonated, **12** protonated once, and **13** protonated twice (Figure 11). The aim of choosing three protonation states was to reduce sequentially the anomeric effect within the terminal phosphoryl and observe the effects upon the geometry of the O−P bond. Protonation reduces the anomeric effect by removing electron lone pair donation into the Os−Pγ antibonding orbital.

The remaining two structures, **14** and **15**, compare two different Mg^{2+} chelated schemes in reference to **11** in order to observe the effects of electrostatics upon Os−Pγ bond lengths. Structure **14** is coordinated with oxygen atoms on Pα, Pβ, and

(121) Miertus, S.; Tomasi, J. *J. Chem. Phys.* **1982**, *65*, 239.

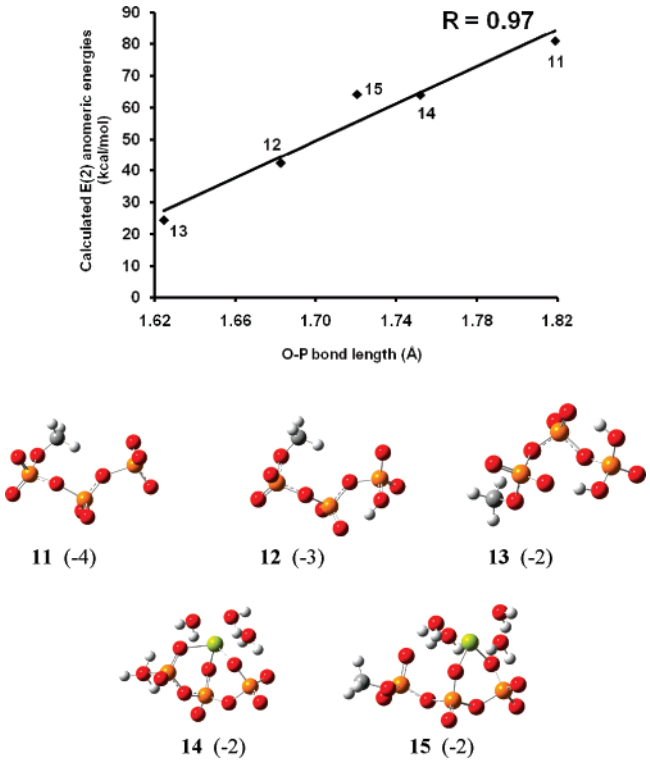


Figure 11. Correlation between terminal O−P bond lengths and the magnitude of the n(O) → σ^*(Os−P) and optimized geometries of triphosphate structures.

Pγ. This type of coordination is slightly unusual but has been reported for the transition state analogue of arginine kinase.[91] A more common coordination scheme is shown in structure **15**, where Mg^{2+} is coordinated with oxygen atoms on Pα and Pβ.[122] Three and four water molecules complete the coordination of Mg^{2+} in systems **14** and **15**, respectively.

As shown in Figure 11, the comparison between **11** and both **14** and **15** show that there is a clear geometric shortening of the terminal O−P bond with increasing protonation or coordination of Mg^{2+}. The computed results emphasize that the geometric changes within triphosphates are influenced by the anomeric effect. Charge transfer between Mg^{2+} and the lone pairs on oxygen atoms on the phosphoryl groups reduces the availability of the same lone pairs to donate into the antibonding σ^*(O−P) orbitals, thus strengthening the O−P bond. The shortening of the terminal O−P bond with increasing protonation reiterates how reducing lone pair donor ability strengthens the O−P bond.

Ground-State Anomeric Connection to Phosphotransfer Potential. The relationship between the anomeric effect and the standard free energies of hydrolysis is of great interest, but not entirely straightforward. A weaker, high-energy, phosphoryl bond should result in a less endothermic bond breaking step and thus increase the overall exothermicity of hydrolysis. However, the ground-state anomeric effect lowers the energy of the system, and so should reduce the exothermicity of the process. To understand how the anomeric effect impacts high-energy character, we investigated other well-known reasons for phosphate thermodynamics.

Apart from ATP and ADP whose high-energy nature has been attributed to electrostatic repulsion,[16,44,45,117] the high-energy nature of other phosphates has been attributed to the resonance stability of the products of hydrolysis.[16,45] A quantitative value for resonance stability can be inferred from the free energy of deprotonation. The resonance-stabilized acetate product of hydrolysis from high energy acetyl phosphate is far more stable than the methanolate product of hydrolysis of the low-energy methyl monophosphate. As the stability of the hydrolyzed phosphate ($H_2PO_4^-$) is common for both high and low-energy phosphates, the pK_a values of the substituent are a quantitative indicator of resonance stability of the products. As shown in Table 1, the predicted relative resonance stability of high- versus low-energy substituents are much greater than the relative free energies of hydrolysis of high versus low-energy phosphates. For example, the difference in standard free energies of hydrolysis of acetyl phosphate versus methyl monophosphate is 8.1 kcal/mol. However, the difference in stability of the acetate ion versus methanolate ion as calculated from pK_a values is 15.3 kcal/mol. Thus, a difference of 7.6 kcal/mol results between predicted and observed free energies. Across the board the systematic error persists, where the energetic differences of the substituent is almost double the differences in hydrolysis free energies of their phosphate counterparts, as shown in Table 1. Thus, resonance stability of products alone cannot quantitatively account for the experimental free energies of hydrolysis. There must be some other factor that offsets the $\Delta\Delta G$ of deprotonation (accounting for the deficit in the right column of Table 1).

As described by Briggs et al. with regard to acetals and glucosides, the anomeric effect preferentially stabilizes a ground-state structure closer in energy and geometry to the fragmented products.[125] It is conceivable that the enhanced anomeric effect in high-energy phosphates stabilizes ground states making them closer in energy to their hydrolysis products. This could be the factor explaining the discrepancy alluded to above (between resonance stability of the substituent and observed standard free energies of hydrolysis), as further discussed below.

Energetic Consequence of the Ground-State Anomeric Effect. In order for the anomeric effect to account for the deficit between $\Delta\Delta G$ deprotonation and $\Delta\Delta G$ hydrolysis, the anomeric effect must account for up to 7.6 kcal/mol in energy. As described by Kirby, stereoelectronic effects such as the anomeric effect are easily observed, but whose consequences are more difficult to measure energetically.[126] At the heart of the problem of measuring the anomeric effect is the difficulty in designing molecular systems free of steric, environmental, or other factors that impact energetics. Typically, orbital positioning is controlled through specific molecular conformations. Gorenstein and coworkers reported a minimum anomeric contribution of 1.4 kcal/mol for ground-state phosphites and phosphates, but suspected that it could range up to 9.9 kcal/mol.[36,38,39] To the best of our knowledge, no other theoretical treatments offer significantly different values.[33,56,127]

(122) Stryer, L.; Berg, J. M.; Tmoczko, J. L. *Biochemistry*, 5th ed.; Freeman: New York, 2001.
(123) Jencks, W. P.; Regenstein, J. *Handbook of Biochemistry and Molecular Biology*; Cleveland, 1976; pp 305−351.
(124) Popov, K. I.; Sultanova, N. M.; Ronkkomaki, H. *Russian J. Phys. Chem.* **2006**, *80* (10), 1680−1682.
(125) Briggs, A. J.; Glenn, R.; Jones, P. G.; Kirby, A. J.; Ramaswamy, P. *J. Am. Chem. Soc.* **1984**, *106* (21), 6200−6206.
(126) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: Berlin, 1983.
(127) Dejaegere, A.; Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1991**, *113* (11), 4353−4355.

Table 1.

	$\Delta G_{hydrolysis}$ (kcal/mol)	pK_a[a] substituent	$\Delta\Delta G_{hydrolysis}$[b] (kcal/mol)	$\Delta\Delta G_{deprotonation}$[c] (kcal/mol)	$\Delta\Delta G_{deprotonation} - \Delta\Delta G_{hydrolysis}$ (kcal/mol)
methyl monophosphate	−2.2	15.5	0.0	0.0	0.0
glycerol phosphate	−2.2	14.2	0.0	−1.9	−1.9
glucose-1-phosphate	−5.0	12.3	−2.8	−4.6	−1.8
methyl diphosphate	−8.5	6.3	−6.3	−13.1	−6.8
methyl triphosphate	−8.5	6.3	−6.3	−13.1	−6.8
acetyl phosphate	−10.3	4.8	−8.1	−15.3	−7.2
1,3-bisphosphoglycerate	−11.8	3.4	−9.6	−17.2	−7.6

[a] pK_a values are obtained according to Jencks,[123] except for glucose.[124] pK_a values for glucose indicated the C1 carbon as the most likely choice of protonation.[124] No values were found for C6. No values were found for carbamate (most likely because of the lability of the C−N Bond) or the enol form of pyruvate. [b] $\Delta\Delta G$ hydrolysis refers to differences in standard free energies of hydrolysis between a phosphoryl compound and methyl monophosphate, $[\Delta G_{phosphoryl\ compound} - \Delta G_{methyl\ monophosphate}]$. [c] $\Delta\Delta G$ deprotonation refers to differences in stability of the methanolate ion versus other substituents as calculated using pK_a values at 39 °C, $[\Delta\Delta G = RT \ln K_1/K_2 = RT \ln (10^{15.5} - 10^{pK_a})$.

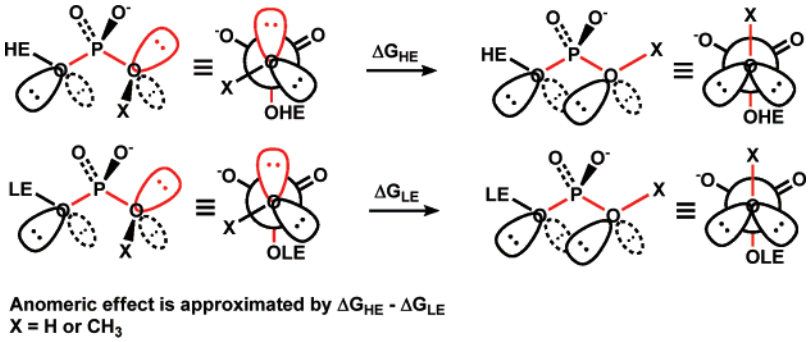


Figure 12. Calculation of the enhanced anomeric effect in high versus low-energy compounds using different conformations.

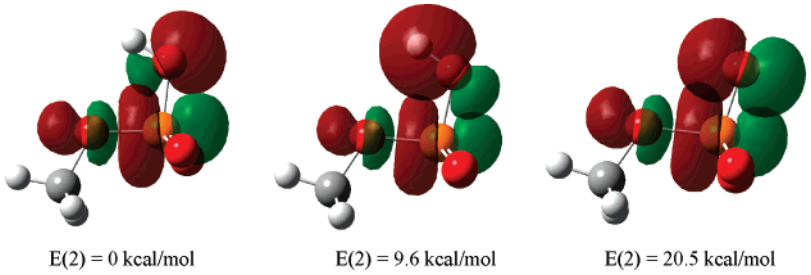


Figure 13. Distortion of antiperiplanar lone pair overlap for methyl monophosphate.

In the same spirit of controlling the positioning of lone-pair electrons to the antibonding orbital of the high-energy bond, one phosphoryl oxygen was either protonated or methylated, as previously utilized by Gorenstein (Figure 12).[38] The O−P−O−X moiety is rotated to shift the lone pairs on the oxygen away from an antiperiplanar configuration. It is debatable if steric factors do not contribute to the energetics, but such conformational differences offer a reasonable method of removing the antiperiplanar configuration of the lone pairs.[127]

Acetyl phosphate and methyl monophosphate were used as prototypes for high and low-energy phosphates. Acetyl phosphate and methyl monophosphate are small and geometrically similar enough such that steric factors should not differ significantly between the two and so cancel out. The enhanced anomeric effect in acetyl phosphate over methyl monophosphate was calculated to lower the ground-state energy by 1.8 (X = H) and 2.4 kcal/mol ($X = CH_3$). Thus, the anomeric effect appears to fall short of counterbalancing the predicted free energy from product stabilization using this particular method to account for the anomeric energy.

However, the computed anomeric effect is underestimated due to the method employed. Protonating or methylating a phosphoryl oxygen atom distorts the antiperiplanar lone pair such that overlap with the σ^*(O−P) is significantly reduced. The impact upon orbital overlap is shown in Figure 13.

Two phenomena of interest occur. First, the NBO $E(2)$ energy values show no appreciable n(O) → σ^*(O−P) interaction in such distorted antiperiplanar configuration because of hybridization changes (methyl monophosphate shown). Note that the phase shown is arbitrary in NBO because of second-order perturbation approximation for $E(2) = 0$ kcal/mol. Second, appreciable overlap is obtained, not from the lone-pair as employed in the anomeric effect but from the p-orbital participating in the σ C−H bond through a σ(C−H) → σ^*(O−P) interaction, where $E(2) = 9.6$ kcal/mol. This should not be mistaken as the specific orbital interaction under investigation. Thus, rotation about the O−P−O−X dihedral about 180° never captures the overlap or stereoelectronic impact delivered by the actual anomeric effect, where $E(2)$ is computed to be 20.5 kcal/mol. For an accurate

energetic determination of the anomeric effect, a system without hybridization changes is necessary.

As previously discussed, the NBO method has been cited for overestimating charge-transfer energies compared to other methods of decomposing ab initio intermolecular interaction energies.[114,115] Thus, the 20.5 kcal/mol is certainly an overestimate. A rough approximation of the overestimation can be achieved from the water dimer. For the water dimer, charge-transfer estimates by the Kitaura and Morokuma (KM)[114] and NBO methods are −1.8[128] and −9.3[129] kcal/mol. It has been noted that NBO analysis stresses the role of orbital interaction between filled and unfilled orbitals, whereas the KM analysis emphasizes classical electrostatics from overlapping charge distributions.[116,130] Assuming that the KM analysis is correct, then NBO overestimates the intermolecular interaction by 9.3 kcal/mol/1.8 kcal/mol = 5.2 times. Thus, applied to the current anomeric interaction, the NBO interaction of 20.5 kcal/mol divided by 5.2 results in an interaction energy of 4.0 kcal/mol. Thus, it is reasonable to suggest that the anomeric effect partly counterbalances product resonance stabilization and accounts for at least part of the 7.6 kcal/mol difference between predicted resonance-stabilized and observed free energies of hydroylsis.

It is interesting that estimations of O−P bond destabilization energies from experimental methods also do not seem to reach a consensus with values ranging from 1 kcal/mol[21] to 15 kcal/mol[32] per 0.1 Å stretch. Both values were calculated using force constants converted from vibrational stretching frequencies. The 1 kcal/mol per 0.1 Å stretch was derived from O−P bond distortion studies on *p*NPP in sequentially increasing concentrations of DMSO. The 15 kcal/mol per 0.1 Å stretch was obtained from the difference in O−P bond lengths between acetyl phosphate and its near analogue aspartyl phosphate in ADP-insensitive phosphoenzyme (E2-P). It is noted that *p*NPP has a weaker electron withdrawer as a subtituent group as compared to acetyl phosphate (pK_a of R in pnPP is 7.1 compared to 4.8 in acetyl phosphate). The trends produced in this work suggest that the anomeric effect is enhanced in acetyl phosphate. One possible explanation therefore is that the greater destabilization energy stems from the enhanced anomeric effect in acetyl phosphate as compared to *p*NPP.

Other effects cannot be discounted from playing a role in impacting the thermodynamic poise of phosphates. However, the estimation of the anomeric effect contribution presented here is significant enough to underline the importance of the correlation between free energies of hydrolysis and the anomeric effect. The correlations presented in this work provide a baseline and fundamental understanding of the connection between exothermicity and ground-state stereoelectronic effects, which may be tested in more complex models, and the target of future work.

Conclusion

Compelling evidence is presented suggesting the importance of the generalized anomeric effect in O−P bond weakening and contribution to the exothermicity of hydrolysis. Strong correlations between O−P bond lengths and the magnitude of the n(O) → σ*(O−P) anomeric effect, as calculated by NBO, has been shown. Comparisons of σ*(O−P) versus σ(O−P) occupancies show that the anomeric effect is most important in determining the strength of the O−P bond. The n(O) → σ*(O−P) anomeric effect also correlates strongly with experimental standard free energies of hydrolysis. It is suggested that the anomeric effect impacts phosphotransfer potential by stabilizing ground-state geometries that are closer in energy and geometry to the hydrolyzed products. Protonation, electrostatics, and solvent are shown to alter the magnitude of the anomeric effect and consequently O−P bond strengths. Changes in lone-pair donor ability (by protonation or coordination with Mg^{2+}) affect the magnitude of the anomeric effect and institute significant changes in O−P bond lengths. Changes in σ*(O−P) acceptor ability as a consequence of increased polarity also impact O−P bond lengths. Articulating the strength of the O−P bond as a function of both lone pair donor ability and antibond acceptor ability allows a better understanding of how and why O−P bonds are sensitive to diverse surroundings. With the comparative NBO analysis presented, it is shown that a calculated stereoelectronic anomeric effect directly destabilizes the "high energy" bonds in question and is highly correlated to the experimental free energies of hydrolysis. Destabilization of this bond is only one of several likely effects in hydrolysis, so the correlation is not perfect, and correlation itself is not enough to infer causal effect, but the observation that the correlation holds across a wide spectrum of "high energy" compounds is striking and likely to be important. It may provide a starting point for understanding the chemistry behind nature's "choices" of different phosphates as repositories of potential energy and to use in driving otherwise endothermic processes. Given that charge-transfer effects are often associated with catalysis,[17,24,42,131−133] it is suggested that a deeper understanding of ground-state anomeric effects of high-energy bonds may help in further defining it consequences in important biological reactions, such as phophoryl transfer.

Acknowledgment. This work was funded in part by an American Heart Association (Florida, Puerto Rico affiliate) predoctoral fellowship (0415212B, 0615243B) to E.A.R., and NIH GM077643 to M.S.C. In addition, J.D.E. is grateful to the NSF and DoD (CHE-0649182, CHE-0321147, CHE-0723109, CHE-0354052, AAB/PSC CHE-030008P), Department of Education (P116Z040100 and P116Z050331), and SGI and Gaussian Corporations for the support of this work. The authors thank the FSU School of Computational Science and Information Technology (CSIT) for use of the IBM p-Series. The authors thank Prof. Dan Herschlag and members of his laboratory for critical reading of the manuscript.

Supporting Information Available: Gaussian archives for all structures at B3LYP/6-311++G(d,p). Supplementary discussion and figures on cooperativity effects of the anomeric interaction. Figure S1. Full Gaussian reference. This material is available free of charge via the Internet at http://pubs.acs.org.

JA073652X

(128) Umeyama, H.; Morokuma, K. *J. Am. Chem. Soc.* **1977**, *99* (5), 1316−1332.
(129) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88* (6), 899−926.
(130) Glendening, E. D.; Streitwieser, A. *J. Chem. Phys.* **1994**, *100* (4), 2900−2909.
(131) Allin, C.; Gerwert, K. *Biochemistry* **2001**, *40* (10), 3037−3046.
(132) Grigorenko, B. L.; Nemukhin, A. V.; Shadrina, M. S.; Topol, I. A.; Burt, S. K. *Proteins: Struct., Funct., Bioinf.* **2007**, *66* (2), 456−466.
(133) Topol, I. A.; Cachau, R. E.; Nemukhin, A. V.; Grigorenko, B. L.; Burt, S. K. *Biochim. Biophys. Acta* **2004**, *1700* (1), 125−136.