α-Fluorinated Phosphonates as Substrate Mimics for Glucose 6-Phosphate Dehydrogenase: the CHF Stereochemistry Matters

David B. Berkowitz,* Mohua Bose, Travis J. Pfannenstiel, and Tzanko Doukov

Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588-0304

dbb@unlserve.unl.edu

Received February 16, 2000

Reported is a systematic study of the "fitness" (in terms of k_{cat}/K_m) of a series of phosphonate mimics of glucose 6-phosphate (G6P) as unnatural substrates for G6P dehydrogenase from *Leuconostoc mesenteroides*. The four G6P analogues (**9**, **10**, **15a**, and **15b**) differ only in the degree of fluorination at the "bridging" phosphonate carbon. All have been synthesized from benzyl 6-*O*-trifluoromethanesulfonyl-2,3,4-tri-*O*-benzyl β-D-glucopyranoside (**6**). The phosphonates with bridging CH_2 (**9**) and CF_2 (**10**) groups are cleanly obtained by direct displacements with the appropriate $LiX_2CP(O)(OEt)_2$ reagents (X = H, F) in 15 min at −78 °C. For the (α-monofluoro)alkylphosphonates (**15a**/**b**), homologation of **6** is achieved via lithiodithiane-mediated triflate displacement, followed by aldehyde unmasking [$CaCO_3$, $Hg(ClO_4)_2$, H_2O]. Addition of diethyl phosphite anion produces diastereomeric, (α-hydroxy)phosphonates **13a**/**b** (1.4:1 ratio) which may be readily separated by chromatography. The stereochemistry of the minor diastereomer was established as 7(*S*) via X-ray crystallographic structure determination of its *p*-bromobenzoate derivative, **16b**. Treatment of the major 7(*R*) diastereomer with DAST produces α-fluorinated phosphonate **14a**, in modest yield, with inversion of configuration, as established, again, by X-ray crystallography. To our knowledge, this is first example of DAST-mediated fluorination of a (nonbenzylic, nonpropargylic) secondary (α-hydroxy)-phosphonate and thus establishes the stereochemical course of this transformation. α-Deprotonation/kinetic quenching of **14a** provides access to the 7(*R*)-epimer (**14b**). For all four protected phosphonates (**7**, **8**, **14a**, and **14b**), diethyl phosphonate ester deprotection was carried out with TMSBr, followed by global hydrogenolytic debenzylation to produce the free phosphonates, as α/β anomeric mixtures. Titrations of G6P itself and the free phosphonic acids provides second pK_a values of 6.5 (**1**, bridging-O), 5.4 (**10**, bridging-CF_2), 6.2 (**14a**, bridging-CHF), and 7.6 (**9**, bridging-CH_2). *Leuconostoc mesenteroides* G6PDH-mediated oxidation and Lineweaver−Burk analysis yields normalized k_{cat}/K_m values of 0.043 (**14b**, bridging-7(*R*)-CHF), 0.11 (**10**, bridging-CF_2), 0.23 (**14b**, bridging-CH_2), and 0.46 (**14a**, bridging-7(*S*)-CHF) relative to G6P itself, largely reflecting differences in K_m. The fact that k_{cat}/K_m increases by more than an order of magnitude in going from the 7(*R*)-α-monofluoroalkyl phosphonate (worst substrate) to the 7(*S*)-diastereomer (best substrate) is especially notable and is discussed in the context of the known phosphate binding pocket of this enzyme as revealed by X-ray crystallography (Adams, M. J. et al. *Structure* **1994**, *2*, 1073−1087).

Introduction

Phosphate esters and anhydrides are among the most common functional groups found in Nature's repertoire, particularly as displayed in her metabolic intermediates.[1] Among other features, the phosphate ester provides a convenient handle for substrate binding that has presumably led to the evolution of the many well-defined phosphate binding pockets. An important challenge for the bioorganic/medicinal chemist remains, however, to develop phosphate mimics of natural metabolites that retain high affinity for targeted enzymatic phosphate binding pockets, but are themselves resistant to phosphatase-mediated cleavage. As part of a project directed toward this goal, we report here the synthesis and enzymatic characterization of a series of (fluorinated) phosphonate analogues of D-glucose 6-phosphate.

Simple phosphonates, in which the labile P−O−C bond is replaced by a stable P−CH_2−C bond, have been explored as phosphate surrogates for some time now.[2] Yet, new members of this parent phosphonate class continue to appear on a regular basis and often emerge as useful bioorganic tools.[3] A more recent effort, inspired by Blackburn,[4] has been in the area of α-halogenated phosphonates, as potentially better "isosteric and isopolar" mimics of the phosphates themselves. Particular attention has been given to the α,α-difluorinated phosphonates, in recent years.[4−6] Among the parameters that potentially favor α-fluorinated phosphonates[7] over their nonfluorinated congeners are (a) reduced pK_{a2}, (b) increased C−CF_2−P dihedral angle, (c) increased polarity of the bridging group, and perhaps even (d) the possibility for C−F···H−X hydrogen bonding.[8,9] Sterics, on the other hand, tend to favor the simple phosphonate as literally

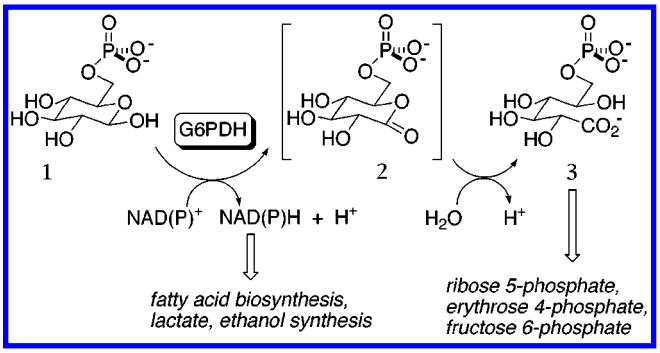
(1) For a thoughtful discussion on "Why Nature chose phosphates?" see: Westheimer, F. H. *Science* **1987**, *235*, 1173−1178.

(2) For reviews on the synthesis and study of phosphonates, see: (a) Wiemer, D. F. *Tetrahedron* **1997**, *53*, 16609−16644. (b) Engel, R. *Chem. Rev.* **1977**, *77*, 349−367.

10.1021/jo000220v CCC: $19.00
Published on Web 07/01/2000

the best isostere. (In α-fluorinated phosphonates, the C−F bond is typically 1.4−1.5 Å, 30−50% longer than the corresponding C−H bond.) Indeed, several instances have now been documented in which α,α-difluorinated phosphonates behave as excellent pyrophosphate[10] or phosphate ester mimics[11] in target enzyme active sites.

Although a fair number of (α-monofluoro)alkylphosphonates have been reported,[12−14] the biological activity of this class of phosphate mimics remains much less explored than that of either their simple phosphonate or α,α-difluorinated congeners. This is surprising in light of their iso-acidity: the second pK_a of the CHF phosphonate is often nearly identical to that of the phosphate group (pK_a ≈ 6.4).[4] We are aware of only a handful of studies in which phosphonates have been compared, as enzyme substrates or inhibitors, across the entire range of α-fluorination {$(CH_2)P\rightarrow(CHF)P\rightarrow(CF_2)P$}.[13] In none of these studies were individual (CHF)P stereoisomers studied, although O'Hagan reports differential reactivity (based upon changes in the ^{19}F NMR spectrum of the mixture) of his diastereomeric (though unassigned) glycerol 3PDH substrates.[13b]



G6PDH occupies an important place in metabolism and in human health. The enzyme catalyzes the committing step to the pentose phosphate shunt, through which ribose 5-phosphate and NADPH reducing equivalents are produced. The former is involved in nucleic acid biosynthesis and the latter in the production of reduced energy stores, particularly fatty acids. But, perhaps most importantly, in cells lacking mitochondria, such as red blood cells, G6PDH deficiency or modification (∼400 variants are known in humans) alters the cellular redox balance and can lead to anemia,[15a,b] osteoarthritis,[15c] vulnerability to oxidative stress,[15d] and even teratogenesis.[15e] Males are particularly susceptible as the G6PDH gene is carried on the X-chromosome.

As our model, we chose to study G6PDH from *Leuconostoc mesenteroides*, as this enzyme has been well-

(3) For recent examples, see: (a) Vidil, C.; Morère, A.; Garcia, M.; Barragan, V.; Hamdaoui, B.; Rochefort, H.; Montero, J.-L. *Eur. J. Org. Chem.* **1999**, 447−450 (mannose 6P analogues as ligands for the M6P/IGF II receptor). (b) Viera de Almeida, M.; Cleophax, J.; Gateau-Olesker, A.; Prestat, G.; Dubreuil, D.; Gero, S. D. *Tetrahedron* **1999**, *55*, 12997−13010 (inositol 1,4,5-triphosphate analogues). (c) Billault, I.; Vasella, A. *Helv. Chim. Acta* **1999**, *82*, 1137−1149 (glucosyl transferase inhibitors). (d) Kakinuma, H.; Shimazaki, K.; Takahashi, N.; Takahashi, K.; Niihata, S.; Aoki, Y.; Hamada, K.; Matsushita, H.; Nishi, Y. *Tetrahedron* **1999**, *55*, 25559−2572 (phosphonate transition state analogues for esterolytic, catalytic Ab development). (e) Sachpatzidis, A.; Dealwis, C.; Lubetsky, J. B.; Liang, P.-H.; Anderson, K. S.; Lolis, E. *Biochemistry* **1999**, *38*, 12665−12674 (transition state analogues for the trp synthase α-reaction/X-ray studies). (f) Arni, R. K.; Watanabe, L.; Ward, R. J.; Kreitman, R. J.; Kumar, K.; Walz, F. G., Jr. *Biochemistry* **1999**, *38*, 2452−2461 (RNAse T1 substrate analogue/X-ray studies). (g) Bedard, J.; May, S.; Lis, M.; Tryphonas, L.; Drach, J.; Huffman, J.; Sidwell, R.; Chan, L.; Bowlin, T.; Rando, R. *Antimicrob. Agents Chemotherapy* **1999**, *43*, 557−567 (anti-HCMV agents).

(4) Blackburn, G. M. *Chem. Ind. (London)* **1981**, 134−138. (b) Blackburn, G. M.; Kent, D. E.; Kolkmann, F. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1119−1125.

(5) For reviews that include discussions of the α-fluorinated phosphonates, see: (a) Tozer, M. J.; Herpin, T. F. *Tetrahedron* **1996**, *52*, 8619−8683. (b) Burton, D. J.; Yang, Z.-Y.; Qiu, W. *Chem. Rev.* **1996**, *96*, 1641−1715. (c) Welch, J. T. *Fluorine in Bioorganic Chemistry*; Wiley: New York, 1991.

(6) For recent leading references, see: Berkowitz, D. B.; Bhuniya, D.; Peris, G. *Tetrahedron Lett.* **1999**, *40*, 1869−1872.

(7) For discussions on the possible effects of fluorination α to phosphorus, see: (a) O'Hagan, D.; Rzepa, H. S. *J. C. S. Chem. Commun.* **1997**, 645−652. (b) Chen, Li.; Wu, Li.; Otaka, A.; Smyth, M. S.; Roller, P. P.; Burke, T. R., Jr.; den Hertog, J.; Zhang, Z.-Y. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 976−984. (c) Thatcher, G. R. J.; Campbell, A. S. *J. Org. Chem.* **1993**, *58*, 2272−2281.

(8) For examples of C−F···H−X hydrogen bonds based upon X-ray crystal structures, see: (a) Brady, K.; Wei, D.; Ringe, D.; Abeles, R. H. *Biochemistry* **1990**, *29*, 7600−7607. (b) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 3368−3374. (c) Murray-Rust, P.; Stallings, W. C.; Monti, C. T.; Preston R. K.; Glusker, J. P. *J. Am. Chem. Soc.* **1983**, *105*, 3206−3214.

(9) For recent discussions of the possibility of C−F···H−X hydrogen bonds in conjunction with surveys of crystallographic databases, see: (a) Dunitz, J. D.; Taylor, R. *Chem. Eur. J.* **1997**, *3*, 89−98. (b) Plenio, H.; Diodone, R. *Chem. Ber./Recueil* **1997**, *130*, 633−640. (c) Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613−12622.

(10) (a) Arabshahi, L.; Khan, N. N.; Butler, M.; Noonan, T.; Brown, N. C.; Wright, G. E. *Biochemistry* **1990**, *29*, 8620−8626 [β,γ-CF_2-dGTP as a substrate for *Bacillus subtilis* DNA pol III; $K_m < K_m$(dGTP); (b) Stremler, K. E.; Poulter, C. D. *J. Am. Chem. Soc.* **1987**, *109*, 5542−5544 (geranyl diphosphate analogues as FDP synthase pseudo-substrates).

(11) (a) Halazy, S.; Ehrhard, A.; Eggenspiller, A.; Berges-Gross, V.; Danzin, C. *Tetrahedron* **1996**, *51*, 177−184 (PNP inhibitors). (b) Burke, T. R., Jr.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129−134. (c) Kole, H. K.; Smyth, M. S.; Russ, P. L.; Burke, T. R., Jr. *Biochem. J.* **1995**, *311*, 1025−1031 (PTP inhibitors). (d) Phillion, D. P.; Cleary, D. G. *J. Org. Chem.* **1992**, *57*, 2763−2764 (EPSP synthase inactivator). (e) Hebel, D.; Kirk, K. L.; Kinjo, J.; Kovacs, T.; Lesiak, K.; Balzarini, J.; De Clercq, E.; Torrence, P. F. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 357−360 (RT inhibitors).

(12) For examples of (monofluoro)alkylphosphonate analogues of biological phosphates, see: (a) Wnuk; S. F.; Robins, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2519−2520 (UMP). (b) Chen, W.; Flavin, M. T.; Filler, R.; Xu, Z.-Q. *Tetrahedron Lett.* **1996**, *37*, 8975−8978 (acyclic phosphonate nucleosides). (c) Yokomatsu, T.; Yamagishi, T.; Matsumoto, K.; Shibuya, S. *Tetrahedron* **1996**, *52*, 11725−11738 (pTyr). (d) Burke, T. R., Jr.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Roller, P. P. *J. Org. Chem.* **1993**, *58*, 1336−1340 (pTyr). (e) Blackburn, G. M.; Langston, S. P. *Tetrahedron Lett.* **1991**, *32*, 6425−6428 (dTTP, dATP.; α−β bridge). (f) Campbell, A. S.; Thatcher, G. R. J. *Tetrahedron Lett.* **1991**, *32*, 2207−2210 (inositol phosphate). (g) Blackburn, G. M.; Guo, M.-J.; Langston, S. P.; Taylor, G. E. *Tetrahedron Lett.* **1990**, *31*, 5637−5640 (Ap_3A). (h) Blackburn, G. M.; Rashid, A. *J. C. S. Chem. Commun.* **1988**, 317−319 (2-phosphoglycerate). (i) Blackburn, G. M.; Perrée, T. D.; Rashid; A.; Bisbal; C.; Lebleu, B. *Chem. Scripta* **1986**, *26*, 21−24 (glyceraldehyde 3-phosphate; 3-phosphoglycerate, TMP). (j) Blackburn, G. M.; Eckstein, F.; Kent, D. E.; Perrée, T. D. *Nucleosides Nucleotides* **1985**, *4*, 165−167 (ATP; β−γ bridge). (k) McKenna, C. E.; Shen, P. *J. Org. Chem.* **1981**, *46*, 4573−4576 (pyrophosphate). (l) Blackburn, G. M.; England, D. A.; Kolkmann, F. *J. Chem. Soc. Chem. Commun.* **1981**, 930−932 (pyrophosphate).

(13) In related work, Blackburn has recently introduced the novel tris(phosphonomethyl) group as a supercharged, surrogate for the pyrophosphate group: (a) Liu, X.; Zhang, X.-R.; Blackburn, G. M. *J. Chem. Soc. Chem. Commun.* **1997**, 87−88 (ATP). (b) Liu, X.; Adams, H.; Blackburn, G. M. *J. C. S. Chem. Commun.* **1998**, 2619−2620 (pyrophosphate; *includes the α-fluorinated analogue*).

(14) (a) Caplan, N. A.; Pogson, C. I.; Hayes, D. J.; Blackburn, G. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 515−520 [(1, 3-bis-phosphonomethyl)arene inhibitors of phosphoglycerate kinase]. (b) Nieschalk, J.; Batsanov, A. S.; O'Hagan, D.; Howard, J. A. K. *Tetrahedron* **1996**, *52*, 165−176 (glycerol 3-phosphate). (c) Burke, T. R., Jr.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Nomizu, M.; Roller, P. P.; Wolf, G.; Case, R.; Shoelson, S. E. *Biochemistry* **1994**, *33*, 6490−6494 (pTyr). (d) For an example in the PCXYP series (pyrophosphate analogues), see: Guranowski, A.; Starzynska, E.; Gzik, L.; Langston, S. P.; Brown, P.; Blackburn, G. M. *Nucleosides Nucleotides* **1995**, *14*, 731−734 (Ap_3A analogues as substrates/inhibitors of enzymatic hydrolysis or phosphorolysis).

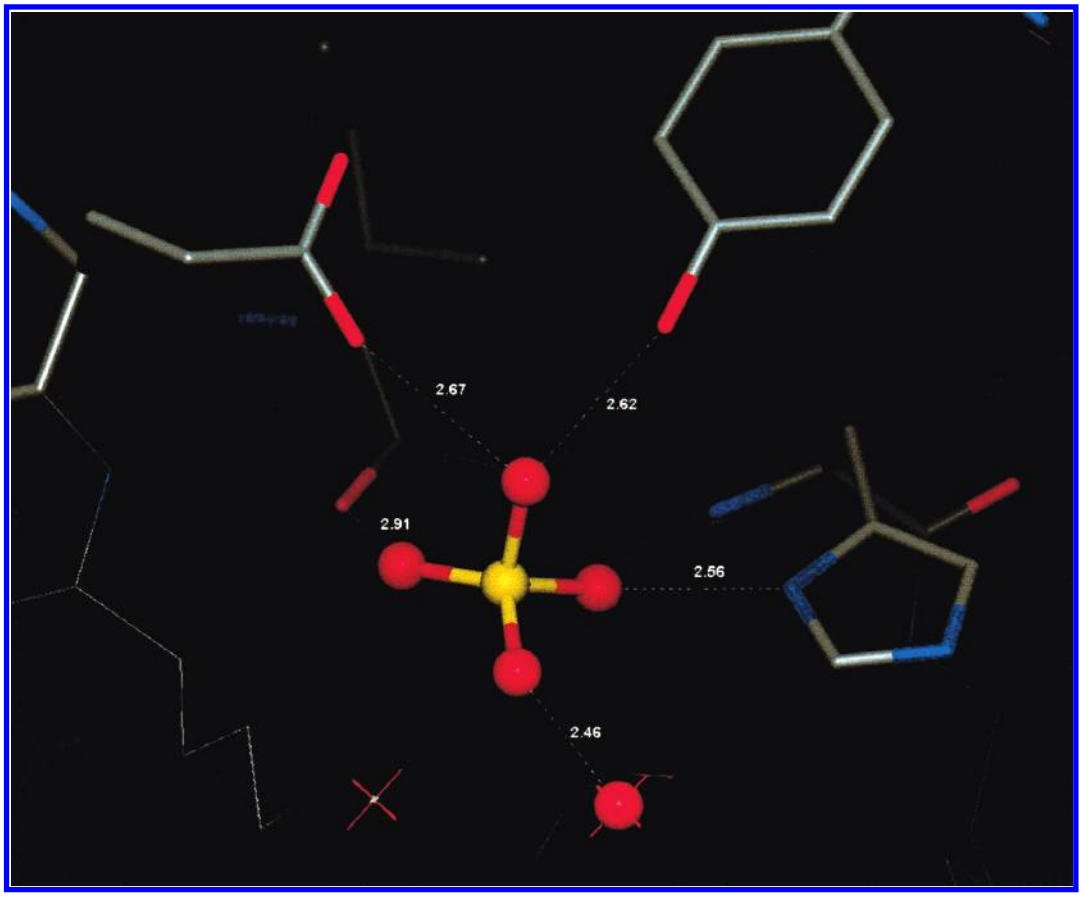


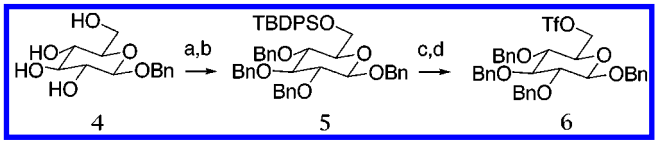
Figure 1. Active site of *Leuconostoc mesenteroides* G6PDH (PDB: 1DPG; subunit A).

studied[16] and a crystal structure is now available.[17] Although no cocrystal structure with G6P (or any other carbohydrate substrate mimic) is yet available, G6PDH readily incorporates inorganic phosphate into its active site under the conditions of enzyme crystallization. As can be seen in Figure 1, the Adams structure[17a] reveals a very well defined phosphate binding pocket surrounding this conserved phosphate. Specific phosphate contacts include a likely electrostatic interaction with His-178 and presumed H-bonds with Tyr-415 and Glu-147, as well as with the backbone carbonyl of Ile-176. There is also a water of crystallization occupying a portion of the probable sugar binding pocket.

Results and Discussion

This clearly defined phosphate binding pocket seemed a most appropriate model receptor with which to systematically evaluate phosphonate binding affinity, across the entire range of α-fluorination. Our synthetic routes to all four target phosphonates (bridging CH_2,[18] (*R*)-CHF, (*S*)-CHF, and CF_2) pass through glucopyranose triflate **6**, taking advantage of our triflate displacement methodology.[19] This triflate is readily prepared in high yield from benzyl β-D-glucopyranoside[20] (Scheme 1). The TBDPS group figures prominently in this sequence as it very selectively protects the lone primary hydroxyl group,[21] among four total, in **4**.

Scheme 1. Synthesis of Pivotal Triflate 6[a]



[a]Reagents: (a) TBDPSCl, imidazole (98%); (b) NaH, BnBr (87%); (c) TBAF, THF (88%); (d) Tf_2O, DTBMP (99%).

Construction of the C_6-C_7 bond is the key step in convergent syntheses of both the nonfluorinated phosphonate and its α,α-difluorinated congener (Scheme 2). Thus, displacement of triflate **6** with either (diethylphosphonomethyl)lithium (*n*-BuLi as base) or [diethylphosphono(difluoromethyl)]lithium (LDA as base) proceeds cleanly in 74−83% yield within 15 min at −78 °C.

(15) (a) Tian, W.-N.; Braunstein, L. D.; Apse, K.; Pang, J.; Rose, M.; Tian, X.; Stanton, R. C. *Am. J. Physiol.* **1999**, *276*, C1121−C1131. (b) Venkataram, M. N.; Al-Suwaid, A. R. *Int. J. Dermatol.* **1999**, *38*, 730−734. (c) Fioravanti, A.; Marcolongo, R.; Carcassi, A. *J. Rheumatol.* **1999**, *26*, 1425. (d) Roos, D.; van Zwieten, R.; Wijnen, J. T.; Gómez-Gallego, F.; de Boer, M.; Stevens, D.; Pronk-Admiraal, de Rijk, T.; van Noorden, C. J. F.; Weening, R. S.; Vulliamy, T. J.; Ploem, J. E.; Mason, P. J.; Bautista, J. M.; Khan, P. M.; Beutler, E. *Blood* **1999**, *94*, 2955−2962. (e) Nicol, C. J.; Zielinski, J.; Tsui, L.-C.; Wells, P. G. *FASEB J.* **2000**, *14*, 111−127.

(16) (a) Levy, H. R. *Biochem. Soc. Trans.* **1989**, *17*, 313−315. (b) Viola, R. E. *Arch. Biochem. Biophys.* **1984**, *228*, 415−424. (c) Levy, H. R.; Christoff, M.; Ingulli, J.; Ho, E. M. L. *Arch. Biochem. Biophys.* **1983**, *222*, 473−488. (d) Hermes, J. D.; Roeske, C. A.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1982**, *21*, 5106−5114.

(17) (a) Rowland, P.; Basak, A. K.; Gover, S.; Levy, H. R.; Adams, M. J. *Structure* **1994**, *2*, 1073−1087. (b) Cosgrove, M. S.; Naylor, C.; Paludan, S.; Adams, M. J.; Levy, H. R. *Biochemistry* **1998**, *37*, 2257−2267.

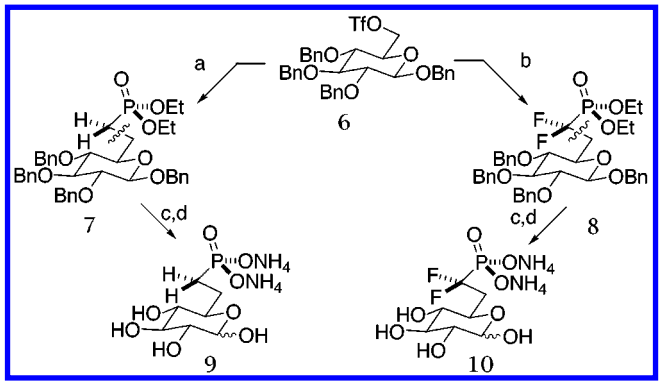
(18) For a previous synthesis and characterization of the simple, nonfluorinated analogue of G6P, see: Adams, P. R.; Harrison, R.; Inch, T. D.; Rich, P. *Biochem. J.* **1976**, *155*, 1−4 {(K_m(G6P) = 46 μM; K_m-(CH_2-phosphonate) = 192 μM; V_{rel} = 0.46 at pH 8 with yeast G6PDH}.

(19) Berkowitz, D. B., Eggen, M.; Shen, Q.; Sloss, D. G. *J. Org. Chem.* **1993**, *58*, 6174−6176.

(20) Slotta, K. H.; Heller, H. *Chem. Ber.* **1930**, *63*, 1024−1028.

(21) Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975−2977.

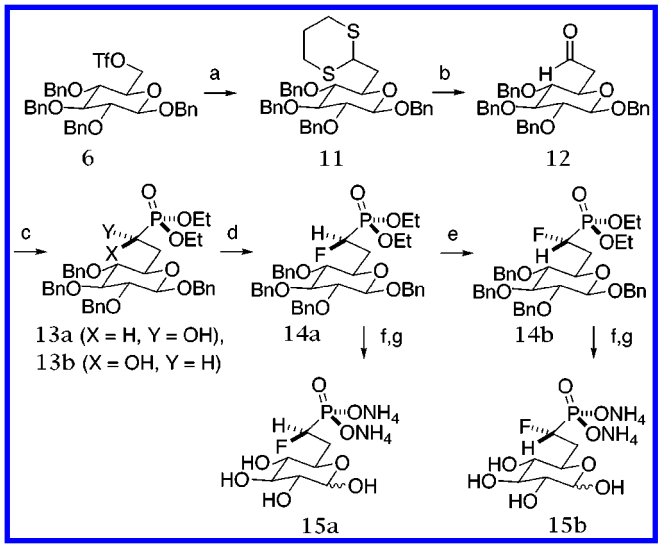
Scheme 2. Triflate Displacement Entry to Phosphono-Sugars[a]



[a] Reagents: (a) $MeP(O)(OEt)_2$, BuLi (74%); (b) $HF_2CP(O)(OEt)_2$, LDA (83%); (c) TMSBr, CH_2Cl_2; then (d) H_2, $Pd(OH)_2/C$ (69% for 9; 91% for 10; 2 steps).

Our approach to the diastereomeric, α-monofluorinated phosphonates involves initial terminal sugar homologation through coupling of triflate **6** with lithiodithiane[22] (Scheme 3). Aldehyde unmasking proceeds smoothly, via selective *S*,*S*-acetal cleavage in the presence of the anomeric *O*,*O*-acetal, to provide **12**. The Pudovik reaction[23] is then employed to fashion the C_7-P bond. Diethyl phosphite anion adds into aldehyde **12** at −78 °C to produce a pair of chromatographically separable, (α-hydroxy)phosphonates, **13a/b** in excellent overall yield. A slight preference (1.4:1) in favor of the 7(*R*)-diastereomer is observed.

Scheme 3. Installation of the α-Fluorophosphono Functionality[a]



[a] Reagents: (a) dithiane, BuLi (94%); (b) $Hg(ClO_4)_2$, $CaCO_3$, THF, H_2O (88%); (c) $HP(O)(OEt)_2$, LiHMDS; THF (93%; **13a**:**13b** = 1.4:1); (d) DAST, CH_2Cl_2 (32−50%; **14a**:**14b** = 10:1); (e) LDA, HOAc quench (98%; **14a**:**14b** = 1:1); (f) TMSBr, CH_2Cl_2; (g) H_2, $Pd(OH)_2/C$ (60% for **15a**; 70% for **15b**; 2 steps).

Initially, the diastereomeric mixture **13a/b** was treated directly with DAST, which reproducibly gave predominantly one diastereomer (10:1 selectivity) of the corresponding (α-monofluoro)phosphonate (**14a**) in modest yield. The crystal structure of **14a** establishes the (*S*) stereochemistry at the center α to phosphorus.[24] Subsequently, the DAST reaction was repeated under exactly the same conditions with the individual diastereomers **13a** and **13b**. While **13a** gives **14a** in yields comparable to those observed with the diastereomeric mixture, **13b** decomposes under the reaction conditions.

To investigate the stereochemical course of this DAST-mediated transformation, we were able to establish the stereochemistry of the starting alcohols **13a/b**, by obtaining an X-ray crystal structure of **16b**, the *p*-bromobenzoate ester of (α-hydroxy)phosphonate **13b** (the minor diastereomer).[25] The structure reveals that **13b** possesses the 7(*S*) stereochemistry. This means that **13a** must have the 7(*R*) stereochemistry, and therefore its transformation to **14a** proceeds with *inversion of configuration*, albeit in modest yield.

(22) Seebach, D.; Corey, E. J. *J. Org. Chem.* **1975**, *40*, 231−237.
(23) Pudovik,, A. N.; Konovalova, I. V. *Synthesis* **1979**, 81−96.

(24) Crystals of **14a** are at −75 ± 2 °C, triclinic, space group $P1-C^1_1$ (No. 1) with a = 8.5887(7) Å, b = 14.5270(11) Å, c = 16.9171(13) Å, α = 109.512(2)°, β = 97.058(2)°, γ = 107.051(2)°, V = 1844.1(2) Å^3 and Z = 2 molecules {d_{calcd} = 1.248 g cm^{-3}; μ_a(Mo K$_\alpha$) = 0.130 $\mu\mu^{-1}$}. A full hemisphere of diffracted intensities (omega scan width of 0.20°) was measured for a two-domain nonmerohedrally twinned specimen using graphite-monochromated Mo Kα radiation on a Bruker SMART CCD Single-Crystal Diffraction System. The two domains were related by a 180° rotation about the *a* axis and the major domain accounted for 74.6(2)% of the total sample volume. X-rays were provided by a normal-focus sealed X-ray tube operated at 50 kV and 40 mA. The lattice constants reported herein were determined with the Bruker SAINT software package using peak centers for 1659 reflections of the major domain. Similar lattice constants were obtained for the minor domain. Data frames were integrated with the Bruker program SAINT using the orientation matrixes for both domains and the resulting 19526 reflections (∼9760 with 2Θ(Mo Kα) < 50.28° for each domain) were then merged with the Bruker program TWHKL to eliminate 4439 reflections which were only partially overlapped for both both domains. A total of 9584 reflections from both domains were totally overlapped, and 5500 were not overlapped. These reflections were then combined to give a final data set which contained 10292 independent reflections; 4792 of these were totally overlapped pairs of reflections and 5500 were nonoverlapped reflections from both domains. This data set was eventually used to simultaneously refine the common structure for both domains which is reported herein. The Siemens/Bruker SHELXTL-PC software package was used to solve the structure using direct methods techniques with the data set obtained from frame integration for the major domain. This data set was discarded after using it to solve the structure and perform several cycles of preliminary least-squares structure refinement. All stages of subsequent weighted full-matrix least-squares refinement were conducted using F_o^2 data with the SHELXTL-PC Version 5 software package for nonoverlapping reflections of individual domains or totally overlapping and nonoverlapping reflections of both domains. Final agreement factors at convergence for refinement of both domains simultaneously are: R_1 (unweighted, based on F) = 0.080 for 4918 independent reflections having 2Θ (Mo Kα) < 50.28° and $I > 2\sigma(I)$; wR_2 (weighted, based on F^2) = 0.235 for 8523 independent reflections having 2Θ(Mo Kα) < 50.28° and $I > 0$. Final agreement factors for all of the data are R_1 (unweighted, based on F) = 0.203 and wR_2 (weighted, based on F^2) = 0.312 for all 10 292 independent reflections having 2Θ (Mo Kα) < 50.28°. Parallel refinements with ∼2750 nonoverlapping reflections for each individual domain gave similar (although less precise) structural parameters. The structural model incorporated isotropic thermal parameters for all hydrogen atoms as well as carbon atom C_{14} which could not be refined satisfactorily as an unrestrained anisotropic atom. The remaining nonhydrogen atoms were incorporated into the structural model with anisotropic thermal parameters. Hydrogen atoms were located from difference Fourier syntheses and included in the structural model as fixed isotropic atoms (using idealized sp^2- or sp^3-hybridized geometry and C−H bond lengths of 0.95−1.00 Å) riding on their respective carbon atoms. Methyl groups were given idealized staggered orientations. The isotropic thermal parameters of all hydrogen atoms were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameter of the carbon atom to which they are covalently bonded. The phosphonate ethoxy groups for the second crystallographically independent molecule are disordered and one of these did not refine satisfactorily when all of its atoms were allowed to vary as independent atoms. The $O_{18}-C_{48}$, $C_{48}-C_{49}$ ethoxy bond lengths and the $O_{18}\cdots C_{49}$ separation were therefore restrained to values which were 0.940, 1.000, and 1.633 times a common C−C distance that was included as a free variable in the least-squares refinement cycles. This free variable refined to a final value of 1.31(2) Å.

The reaction of DAST with secondary alcohols to produce secondary alkyl fluorides is well-known[26] and proceeds with inversion of configuration, in most cases.[26b−e] This stereochemical course has been attributed to an S_N2-like mechanism wherein fluoride displaces an intermediate DAST-derived S^{IV}-ester.[26b] However, overall retention has sometimes been observed, particularly in certain steroidal systems, presumably due either to neighboring group participation, or to an S_N1-like pathway, wherein one face of the carbocationic intermediate is well shielded.[27a,b] For other secondary alcohols in functionalized environments, the DAST reaction is known to be problematic resulting in rearranged products[27c] or elimination.[27d]

To our knowledge, this is the first reported example of use of DAST to convert a simple, unactivated secondary (α-hydroxy)phosphonate to the corresponding (α-fluoro)-phosphonate. For propargylic[28] and benzylic[12c,d,29] (α-hydroxy)phosphonates this transformation is known. However, allylic systems give rearrangement (formally an S_N2' displacement or a [3,3]-sigmatropic rearrangment).[29] As far as we are aware, only for benzylic systems, has the stereochemical course of the DAST-mediated transformation of (α-hydroxy)phosphonates to (α-fluoro)phosphonates been investigated. Initial reports suggested that this reaction goes with inversion of configuration.[12c] However, this has been corrected through a careful study by Shibuya which reveals that, in general, benzylic (α-hydroxy)phosphonates give scrambling of the α-stereochemistry upon treatment with DAST.[12d]

This is suggestive of an S_N1-like mechanism, and indeed, there has been speculation that, in general, phosphoryl groups may stabilize adjacent carbocations through resonance structures of the $C^+-{}^-P{\equiv}O^+$ variety.[29b] The observation of inversion of configuration in the transformation of **13a** to **14a** here is suggestive of an S_N2-like pathway. However, given the failure of **13b** to react via this manifold and the limited efficiency of the transformation, further studies are clearly needed to establish generality for this synthetic operation. Such studies are certainly warranted given the rapidly expanding literature on the preparation of enantiomerically enriched (α-hydroxy)phosphonates.[30]

For our purposes here, the failure of **13b** to undergo α-fluorination with DAST meant that another route to the 7(*R*) α-monofluorophosphonate analogue of G6P (**14b**) would be needed. Low-temperature deprotonation (LDA) of **14a**, followed by a kinetic quench (HOAc) provides for facile epimerization at the fluorine-bearing center (Scheme 3). Though an equimolar mixture of **14a** and **14b** is obtained, the two diastereomers are easily separated by flash chromatography, and, importantly, no decomposition occurs under the conditions of the epimerization.

All four protected phosphonate analogues, **7**, **8**, **14a**, and **14b** may be deprotected smoothly by a two-step procedure involving phosphonate ester cleavage under the Rabinowitz−McKenna−Jung conditions (TMSBr, CH_2Cl_2),[31] followed by hydrogenolysis (Pearlman's catalyst) of all benzyl ether protecting groups (Schemes 2 and 3). After considerable experimentation, the best conditions for phosphonate purification were found to involve silica gel chromatography with a mixed organic (MeCN/*i*-PrOH), aqueous (ammonium bicarbonate buffer) eluent similar to one reported by Poulter and co-workers.[32] One obtains the free phosphonates as their ammonium salts (α/β anomeric mixtures). If the free acids are desired, they may be obtained by a couple of cycles of lyophilization (see Experimental Section, Titrations).

Enzymatic Studies. Pleasingly, all four phosphonate analogues of G6P are accepted as substrates for G6PDH from *L. mesenteroides*. This enzyme utilizes either NAD^+ or $NADP^+$ as cofactor. We chose to employ the latter, and performed a Michaelis−Menten steady-state kinetic analysis under conditions of cofactor saturation. When one superimposes the kinetic data for G6P itself and each of the phosphonate analogues, in double-reciprocal form (Lineweaver−Burk plots, Figure 2), one is immediately struck by the near intersection of all lines at the *y*-intercept ($1/V_{max}$). In other words, as measured by k_{cat}, we observe only very small differences in turnover efficiency across the entire range of phosphate mimics studied. Indeed, as can be seen from Table 1, the highest values of k_{cat} (CF_2 analogue and G6P) are only a factor of 1.3 greater than the lowest k_{cat} (7(*R*)-CHF analogue).

One reason for the similarity in k_{cat} values observed, is surely rooted in Viola's finding that below pH 8.7, product dissociation is partially rate-limiting for this enzyme.[16b] Nonetheless, careful kinetic isotope effect studies carried out by O'Leary and Cleland and co-

(25) Preliminary results {R_1 (unweighted, based on F) = 0.166 for 2761 independent absorption-corrected reflections having 2Θ (Mo Kα) < 41.6° and $I > 2\sigma(I)$ and wR_2 (weighted, based on F^2) = 0.492 for all 4962 independent absorption-corrected reflections having 2Θ (Mo Kα) < 41.6°} from a low-temperature (−80 ± 2 °C) X-ray structure determination for small needle-shaped single crystals {a = 30.447(2), b = 11.077(1), and c = 14.636(1) Å, β = 97.583(2)° and Z = 4} of **16b** are shown in the Supporting Information. Since the small size and poor quality of the present crystals prevented a high precision structure determination, attempts are underway to grow larger and higher quality crystals of this and closely related compounds for an eventual high-precision X-ray structural study.

(26) (a) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574−578. (b) Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090−1092. (c) Lowe, G.; Potter, B. V. L. *J. Chem. Soc. Perkin Trans. 1* **1980**, 2029−2032. (d) Focella, A.; Bizzarro, F.; Exon, C. *Synth. Commun.* **1991**, *21*, 2165−2170. (e) Scharer, O. D.; Verdine, G. L. *J. Am. Chem. Soc.* **1995**, *117*, 10781−10782.

(27) (a) Bird, T. G. C.; Fredericks, P. M.; Jones, E. R. H.; Meakins, G. D. *J. C. S. Chem. Commun.* **1979**, 65−66. (b) Rozen, S.; Faust, Y.; Ben-Yakov, H. *Tetrahedron Lett.* **1979**, *20*, 1823−1826. (c) Albert, R.; Dax, K.; Katzenbeisser, U.; Sterk, H.; Stütz, A. E. *J. Carbohydrate Chem.* **1985**, *4*, 521−528. (d) Pansare, S. V.; Vederas, J. C. *J. Org. Chem.* **1987**, *52*, 4804−4810.

(28) (a) Benayoud, F.; de Mendonca D. J.; Digits, C. A.; Moniz, G. A.; Sanders, T. C.; Hammond. G. B. *J. Org. Chem.* **1996**, *61*, 5159−5164. (b) Sanders, T. C.; Hammond, G. B. *J. Org. Chem.* **1993**, *58*, 5598−5599.

(29) (a) Blackburn, G. M.; Kent D. E. *J. Chem. Soc., Chem. Commun.* **1981**, 511−513. (b) Blackburn, G. M.; Kent D. E. *J. Chem. Soc., Perkin Trans. 1* **1986**, 913−917.

(30) For recent asymmetric approaches to α-hydroxyphosphonates, see: (a) Sharpless, K. B.; Thomas, A. A. *J. Org. Chem.* **1999**, *64*, 8379−8385. (b) Yamagishi, T.; Yokomatsu, T.; Suemune, K.; Shibuya, S. *Tetrahedron* **1999**, *55*, 12125-12136. (c) Duxbury, J. P.; Cawley, A.; Thorton-Pett, M.; Wantz, L.; Warne, J. N. D.; Greatrex, R.; Brown, D.; Kee, T. P. *Tetrahedron Lett.* **1999**, *40*, 4403−4406. (d) Groaning, M. D.; Rowe, B. J.; Spilling, C. D. *Phosphorus, Sulfur Silicon* **1999**, *147*, 1217. (e) Groaning, M. D.; Rowe, B. J.; Spilling, C. D. *Tetrahedron Lett.* **1998**, *39*, 5485−5488. (f) Cravaotto, G.; Giovenzana, G. B.; Pagliarin R.; Sisti, M. *Tetrahedron Asymm.* **1998**, *9*, 745−748. (g) Pogatchnik, D. M.; Wiemer, D. F. *Tetrahedron Lett.* **1997**, *38*, 3495−3498. (h) Sasai, H.; Bougauchi, M.; Arai, T.; Shibasaki, M. *Tetrahedron Lett.* **1997**, *38*, 2717−2720. (i) Yokomatsu, T.; Yamagishi, T.; Shibuya S. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1527−1533. (j) Drescher, M.; Hammerschmidt, F.; Kahlig, H. *Synthesis* **1995**, 1267−1272.

(31) (a) Rabinowitz, R. *J. Org. Chem.* **1963**, *28*, 2975−2978. (b) Jung, M. E.; Lyster, M. A. *J. Am. Chem. Soc.* **1977**, *99*, 968−969. (c) McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M.-C. *Tetrahedron Lett.* **1977**, *18*, 155−158.

(32) Woodside, A. G.; Huang, Z.; Poulter, C. D. *Org. Synth.* **1987**, *66*, 211−219.

Table 1. Phosphonates as G6PDH Pseudo-Substrates

substrate	bridging group	K_m (mM)[a]	k_{cat} (s^{-1})[a]	k_{cat}/K_m ($mM^{-1}\ s^{-1}$)	$(k_{cat}/K_m)_{rel}$	
glucose 6-phosphate (**1**)	O	0.12 ± 0.01	241 ± 14	2.01 ± 0.17 × 10^3	1.00	
glucose-CH_2-phosphonate (**9**)	CH_2	0.49 ± 0.04	224 ± 14	4.57 ± 0.27 × 10^2	0.23	
glucose-CHF-phosphonate (**15a**) {(7*S*)-diastereomer}	(*S*)-CHF	0.23 ± 0.02	212 ± 6	9.22 ± 0.80 × 10^2	0.46	factor of 11
glucose-CHF-phosphonate (**15b**) {(7*R*)-diastereomer}	(*R*)-CHF	2.26 + 0.26	195 ± 14	8.60 ± 1.0 × 10^1	0.043	
glucose-CF_2-phosphonate (**10**)	CF_2	1.35 ± 0.06	248 ± 16	1.84 ± 0.12 × 10^2	0.11	

[a] All assays were at pH 7.8 as described in the Experimental Section. K_m and k_{cat} values were obtained by least-squares fitting of the $1/v_0$ vs $1/[S]$ data to a line, wherein substrate concentrations have been determined by UV (observation of the NADPH formed upon complete G6PDH-mediated oxidation to the corresponding lactone) or ^{19}F NMR (trifluoroethanol standard) in the case of **15b**. Experimental uncertainties were determined by comparing the kinetic constants obtained from several independent runs (2−3 data sets) on the same substrate.

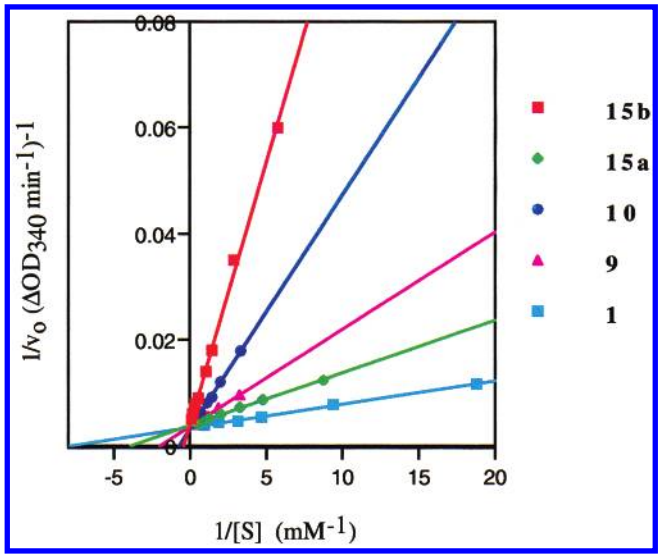


Figure 2. Superimposed Lineweaver−Burk plots for G6P (**1**) and its phosphonate analogues **9**, **10**, **15a**, and **15b** as substrates for G6PDH from *L. mesenteroides*.

workers on G6PDH from *L. mesenteroides* revealed that $k_H/k_D(V_{max}/K_m) = 3.0$ and $k_H/k_D(V_{max}) = 1.8$ at pH 8.[16c] All of our kinetic experiments have been performed at pH 7.8. So, it is clear that k_{cat} does at least partially reflect the rate of hydride transfer here. As such, our results indicate that alterations in the bridging group (from O to CX_2, X = H, F) do not have a dramatic effect on *L. mesenteroides* G6PDH-mediated hydride transfer. This is interesting as the perturbations (at least in terms of group electronegativity) are rather significant and not particularly distant from the site of enzymatic oxidation. After all, only a relatively short three-atom bridge (−C_6−C_5−O−) separates the bridging group from C_1.

On the other hand, there are notable differences in K_m. Indeed, the most striking observation is that the phosphate mimics with the lowest K_m (7(*S*)-CHF, 0.23 mM) and the highest K_m (7(*R*)-CHF, 2.3 mM) are both members of the (α-monofluoro)phosphonate family. These results clearly indicate that close attention must be paid to stereochemistry when examining this class of phosphonates. *In other words, our data show that introduction of a fluorine atom* α *to a phosphoryl group in a biological phosphate mimic must be regarded as a vectorial operation rather than a scalar one.* Furthermore, in contrast to common practice heretofore,[14] these results underscore the advantage of examining single diastereomers when deriving enzyme kinetic parameters for phosphate mimics of this variety.

Table 2. pK_a Values vs Degree of α-Fluorination

substrate	bridging group	pK_{a2}	% dianion at pH 7.8
1	O	6.5	95
9	CH_2	7.6	62
15a	(*S*)-CHF	6.2	98
10	CF_2	5.4	>99

The second pK_a values for G6P and for each type of phosphonate analogue were determined by titration. As expected, α-monofluorination produces a phosphonate functionality that is most nearly "iso-ionic" (pK_{a2} = 6.2) with the actual phosphate ester (pK_{a2} = 6.5). As can be seen in Table 2, all of the phosphonates are predominantly in their dianionic forms at the pH of the assay. Furthermore, there is really no correlation between pK_{a2} and K_m here. Clearly factors other than phosphonate ionization state are contributing to the differential binding to this phosphate binding pocket at this pH.

With an eye toward stimulating discussion on possible origins of the striking effect of CHF stereochemistry upon enzyme binding, we elected to superimpose the crystallographic coordinates of our best G6P mimic upon those of G6PDH itself, as revealed from Adams' original crystal structure.[17a,33] Accordingly, **15a** [as approximated by the coordinates of **14a** sans benzyl ether and ethyl ester protecting groups] was placed in the active site of G6PDH (PDB: 1DPG, subunit A).[34] The phosphorus atom and all three oxygen atoms of the phosphonate were superimposed upon the corresponding atoms of the conserved phosphate (HPO_4-2000). Care was taken to locate the β-C1−O(H) at an appropriate distance (3.4 Å, see Figure 3) from the ϵ-nitrogen of His^{240}, the presumed catalytic base.[17b] Note that the δ-nitrogen of this same histidine donates a hydrogen bond to Asp^{177} as part of the catalytic diad that is postulated to operate for G6PDH. To accommodate the substrate, two waters of crystallization (H_2O-1079 and H_2O-1244) were deleted. Also, the second (nonconserved) phosphate of crystallization (HPO_4-2002) has been removed for clarity.

In this way, the three phosphonate oxygens retain favorable interactions with His^{178} (δN), Tyr^{415} (OH), Ile^{176}

(33) This original *L. mesenteroides* G6PDH crystal structure contains inorganic phosphate bound to the sugar phosphate site, but no nicotinamide cofactor.[17a] A more recent structure of the same enzyme does include a bound $NADP^+$.[17b] However, in that structure, the nicotinamide ring of the cofactor is apparently so significantly disordered that its coordinates could not be defined.

(34) The coordinates of **14a** and of 1DPG were modeled in XtalView. This software was designed by Duncan McRee and is free for nonprofit use. See: http://www.sdsc.edu/CCMS/Packages/XTALVIEW/xtalview.html. For visualization purposes, the Insight II package (version 98.0, Molecular Simulations, Inc., San Diego, CA) was employed.

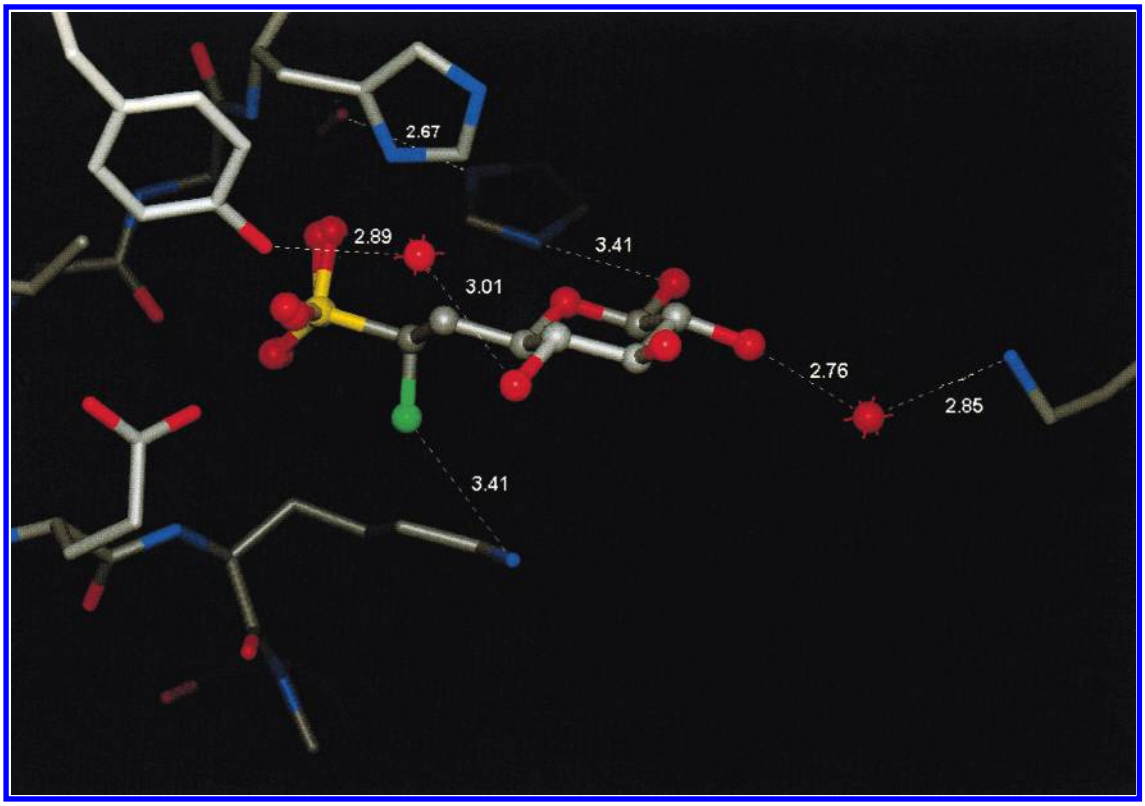


Figure 3. Compound **15a** (β anomer, as derived from the X-ray coordinates of **14a**) placed in the active site of G6PDH (PDB: 1DPG; subunit A).

(backbone carbonyl) and Glu[147] (CO_2H). In this hypothetical view of **15a** bound at the G6PDH active site, the C−F bond of this 7(*S*)-diastereomer could engage in a favorable ion−dipole interaction with the ϵ-ammonium nitrogen of Lys[148] (N−F distance = 3.4 Å, as modeled). This is particularly attractive in light of recent model studies with fluorinated piperidines in which the surprising predominance of conformers with opposing axially disposed fluorine substituents is ascribed to such ion−dipole interactions.[35,36] However, it is important to note here that Lys[148] is apparently quite mobile.[37] So, a more precise positioning of this lysine, relative to sugar phosph(on)ate, must await the crystallographic solution of either a well-ordered binary complex (E-$NADP^+$ *or* E-sugar phosphate), or perhaps, of an abortive ternary complex [e.g., E-NADPH-sugar phosph(on)ate or E-$NADP^+$-phosphogluconolactone], should such be forthcoming.

Conclusions

This report details the first study of enzyme binding affinity/turnover, as a function of both degree of fluorination and C−F stereochemistry, across a complete set of fluorinated phosphonate mimics of G6P. The syntheses of all four phosphonates diverge from glucopyranose 6-triflate (**6**) and highlight the utility of the triflate displacement approach for sugar homologation and functionalization. In the course of the synthesis of **14a**, it was established that DAST-mediated conversion of a non-benzylic, secondary (α-hydroxy)phosphonate to the (α-monofluoro)phosphonate proceeds with inversion of configuration. Steady-state enzyme kinetic analysis with *L. mesenteroides* G6PDH yields k_{cat}/K_m values of 0.043 (**15b**, bridging-7(*R*)-CHF), 0.11 (**10**, bridging-CF_2), 0.23 (**14b**, bridging-CH_2), and 0.46 (**15a**, bridging-7(*S*)-CHF) relative to G6P itself, largely reflecting differences in K_m. Though the best phosphate mimic, **15a**, (K_m = 0.23 mM vs 0.12 mM for G6P) is of the iso-acidic (pK_{a2} = 6.2 vs 6.5 for G6P) (α-monofluoro)phosphonate class, so also is the worst phosphate mimic, **15b** (K_m = 2.3 mM). These results suggest that the vectorial disposition of a C−F bond in an enzymatic phosphate binding pocket can contribute up to an order of magnitude in binding affinity in (α-monofluoro)phosphonates. Clearly further such studies are warranted to establish the generality, range, and origin of the effects of C−F stereochemistry upon receptor binding in this iso-acidic class of hydrolytically stable phosphate mimics.

Experimental Section

General. All reactions were conducted under argon atmosphere using oven-dried glassware. Methylene chloride and diisopropylamine were distilled from CaH_2. THF and Et_2O were distilled from sodium benzophenone ketyl. HMPA was distilled from Na under reduced pressure. Methanol was distilled from Mg. *n*-Butyllithium in hexanes (nominally 1.6 M) was purchased from Aldrich and titrated before each use.

(35) Snyder, J. P.; Chandrakumar, N. S.; Sato, H.; Lankin, D. C. *J. Am. Chem. Soc.* **2000**, *122*, 544−545.

(36) Several authors have recently critically examined both the CSD (Cambridge Structural Database)[9a−c] and the PDB (Protein Databank)[9a] and concluded that bona fide C−F···H−N hydrogen bonds are extremely rare. Dunitz[9a] has pointed out, however, that systems bearing a negative charge in the vicinity of the C−F bond (such as $-SerO^-$ addition complexes to α-fluorinated ketonic inhibitors) are more likely to serve as H-bond acceptors at fluorine. If indeed **15a** binds to G6PDH in its dianionic form, this factor could make such a hydrogen bond (to Lys[338], say) more favorable here.

(37) For example, in going from subunit A to subunit B of 1DPG, the ϵ-nitrogen of Lys148 moves 2.9 Å. This movement is associated with a change from a *trans*-Lys[148]-Pro[149] amide bond (subunit A) to a cis-amide bond (subunit B).

Other reagents were obtained from commercial sources and used without further purification. ^{31}P and ^{19}F NMR spectral lines are with respect to the internal (capillary) standards triphenylphosphine (−5.80 ppm) and trifluoroacetic acid (−76.5 ppm), respectively. Mass spectra were acquired at the Nebraska Center for Mass Spectrometry (University of Nebraska−Lincoln). Elemental analyses were carried out by MHW Labs (Phoenix, AZ).

Benzyl 6-*O*-*tert*-Butyldiphenylsilyl-*β*-D-glucopyranoside (17). To a solution of benzyl *β*-D-glucopyranoside **4**[20] (10.0 g, 39 mmol) and imidazole (5.27 g, 77.4 mmol) in DMF (100 mL) at −30 °C was added dropwise, via cannula, a solution of *tert*-butyldiphenylsilyl chloride (10.1 mL, 39 mmol) in DMF (50 mL). After 3.5 h the reaction mixture was poured into $NaHCO_3$ (aq.) and Et_2O. The aqueous layer was further extracted with Et_2O. The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (5% MeOH−CH_2Cl_2) provided **17** (20.0 g, 98%) as a foam: mp 51−52 °C; $[\alpha]^{22}_D$ +41 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, ($CDCl_3$) *δ* 1.06 (s, 9 H), 2.78 (br s, 3 H), 3.41 (m, 2 H), 3.54 (t, *J* = 9 Hz, 1 H), 3.61 (app t, *J* = 8, 9 Hz, 1 H), 3.91 (dd, *J* = 5, 11 Hz, 1 H), 3.94 (dd, *J* = 5, 11 Hz, 1 H), 4.34 (d, *J* = 8 Hz, 1 H), 4.55 (d, *J* = 12 Hz, 1 H), 4.86 (d, *J* = 12 Hz, 1 H), 7.38 (m, 11 H), 7.70 (d, *J* = 8 Hz, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 19.2, 26.8 (3 C), 64.6, 70.8, 71.9, 73.6, 75.0, 76.3, 101.2, 127.78, 127.80, 128.2, 128.5, 129.9, 132.9, 133.1, 135.6, 135.7, 136.9; IR (film) 3380 (br), 2930, 2860 cm^{-1}; HRMS (FAB, 3-NOBA/LiI) calcd for $C_{29}H_{36}SiO_6Li$ ($M + Li^+$) 515.2442, obsd 515.2443. Anal. Calcd for $C_{29}H_{36}SiO_6$: C, 68.47; H, 7.13. Found: C, 68.24; H, 7.21.

Benzyl 6-*O*-*tert*-Butyldiphenylsilyl-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (5). To a solution of triol **16** (8.11 g, 16 mmol) in DMF (47 mL) at 0 °C was added NaH (2.23 g of a 60% dispersion in mineral oil, 55.9 mmol). The reaction mixture was allowed to reach rt over a period of 20 min. Benzyl bromide (6.26 mL, 52.7 mmol) then was added dropwise, via syringe. After 7 h, the reaction mixture was poured into $NaHCO_3$ (aq.) and EtOAc. The aqueous layer was further extracted with EtOAc, and the combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (10% Et_2O−hexane) gave **5** (10.8 g, 87%) as a white solid: mp 74−76 °C; $[\alpha]^{22}_D$ − 4.98 (*c* 1.00, $CDCl_3$); ^1H NMR (500 MHz, ($CDCl_3$) *δ* 1.14 (s, 9 H), 3.42 (dt, *J* = 3, 9 Hz, 1 H), 3.62 (app t, *J* = 8, 9 Hz, 1 H), 3.72 (t, *J* = 9 Hz, 1 H), 3.83 (t, *J* = 9 Hz, 1 H), 4.02 (s, 2 H), 4.58 (d, *J* = 8 Hz, 1 H), 4.73 (d, *J* = 12 Hz, 1 H), 4.75 (d, *J* = 11 Hz, 1 H), 4.81 (d, *J* = 11 Hz, 1 H), 4.87 (d, *J* = 11 Hz, 1 H), 4.95 (d, *J* = 11 Hz, 1 H), 4.98 (s, *J* = 11 Hz, 1 H), 5.04 (d, *J* = 12 Hz, 1 H), 5.06 (d, *J* = 11 Hz, 1 H), 7.36 (m, 26 H), 7.77 (d, *J* = 9 Hz, 2 H), 7.83 (d, *J* = 8 Hz, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 19.3, 26.8 (3 C), 62.8, 70.6, 74.9, 75.1, 75.8 (2 C), 77.8, 82.6, 84.8, 102.3, 127.60, 127.61, 127.7, 127.74, 127.8, 127.87, 127.93, 127.98, 128.1, 128.3, 128.37 (2 C), 128.42, 133.2, 133.7, 134.8, 135.6, 135.9, 137.6, 138.3, 138.61, 138.64; IR (film) 3030, 2855, 2930 cm^{-1}; HRMS (FAB, 3-NOBA/LiI) calcd for $C_{50}H_{54}SiO_6Li$ ($M + Li^+$) 785.3850, obsd 785.3857. Anal. Calcd for $C_{50}H_{54}SiO_6$: C, 77.09; H, 6.99. Found: C, 77.23; H, 7.01.

Benzyl 2,3,4-Tri-*O*-benzyl-*β*-D-glucopyranoside (18). To a solution of silyl ether **5** (523 mg, 0.67 mmol) in THF (5.5 mL) at 0 °C was added, dropwise, a solution of tetrabutylammonium fluoride (0.739 mmol) in THF (0.739 mL). After being stirred 9 h at room temperature, the reaction was quenched with $NaHCO_3$ (aq.) and EtOAc. The aqueous layer was further extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (25% EtOAc−hexane) gave **18** (321 mg, 88%) as a white solid. [Note: Scale-up does not compromise yield. Thus, 3.90 g of **5** gives 2.33 g of **18** (87%)] mp 102−104 °C; $[\alpha]^{22}_D$ −5.63 (*c* 1.00, $CDCl_3$); ^1H NMR (300 MHz, ($CDCl_3$) *δ* 1.79 (dd, *J* = 6, 7 Hz, 1 H), 3.33 (m, 1 H), 3.45 (app t, *J* = 9 Hz, 1 H), 3.53 (app t, *J* = 9 Hz, 1 H), 3.63 (app t, *J* = 9 Hz, 1 H), 3.67 (m, 1 H), 3.83 (ddd, *J* = 3, 6, 12 Hz, 1 H), 4.53 (d, *J* = 8 Hz, 1 H), 4.60 (d, *J* = 11 Hz, 1 H), 4.66 (d, *J* = 12 Hz, 1H), 4.69 (d, *J* = 11 Hz, 1H), 4.77 (d, *J* = 11 Hz, 1H), 4.82 (d, *J* = 11 Hz 1H), 4.88 (d, *J* = 12 Hz, 1 H), 4.89 (d, *J* = 10 Hz, 1 H), 4.91 (d, *J* = 10 Hz, 1 H), 7.28 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 62.1, 71.6 74.9, 75.0, 75.1, 75.6, 77.6, 82.3, 84.6, 102.8, 127.58, 127.62, 127.82, 127.86, 127.89, 127.9, 128.0, 128.1, 128.3 (2 C), 128.5 (2 C), 137.3, 138.0, 138.3, 138.5; IR (film) 3360 (br), 3030, 2910 cm^{-1}; HRMS (FAB, 3-NOBA/Na_2CO_3) calcd for $C_{34}H_{36}O_6Na$ ($M + Na^+$) 563.2410, obsd 563.2416. Anal. Calcd for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.76; H, 6.71.

Benzyl 2,3,4-Tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-*β*-D-glucopyranoside (6). To a solution of alcohol **17** (1.00 g, 1.9 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (513 mg, 2.50 mmol) in CH_2Cl_2 (10 mL) at −40 °C was added triflic anhydride (0.39 mL, 2.3 mmol), dropwise, via syringe. After being stirred for 1.5 h at −40 °C, the volatiles were evaporated, and the residue was immediately subjected to flash chromatography (30% EtOAc−hexane) to give **6** (1.23 g, 99%) as a white solid: mp 71−72 °C; $[\alpha]^{22}_D$ +7.42 (*c* 1.00, $CDCl_3$); ^1H NMR (500 MHz, ($CDCl_3$) *δ* 3.45 (dd, *J* = 9, 10 Hz, 1 H), 3.52 (dd, *J* = 8, 9 Hz, 1 H), 3.57 (ddd, *J* = 2, 6, 10 Hz, 1 H), 3.67 (app t, *J* = 9 Hz, 1 H), 4.30 (dd, *J* = 6, 10.5 Hz, 1 H), 4.52 (d, *J* = 8 Hz, 1 H), 4.55 (d, *J* = 11 Hz, 2 H), 4.58 (dd, *J* = 2, 10.5 Hz, 1 H), 4.65 (d, *J* = 12 Hz, 1 H), 4.70 (d, *J* = 11 Hz, 1 H), 4.77 (d, *J* = 11 Hz, 1 H), 4.89 (d, *J* = 12 Hz, 1 H), 4.94 (d, *J* = 11 Hz, 1 H), 4.95 (d, *J* = 11 Hz, 1 H), 7.29 (m, 20 H); ^{13}C NMR (125 MHz, ($CDCl_3$) *δ* 71.1, 72.3, 74.7, 74.9, 75.0, 75.7, 76.4, 82.0, 84.5, 102.1, 127.7, 127.8, 128.0, 128.1, 128.17, 128.20 (2 C), 128.3, 128.38, 128.42, 128.5, 128.6, 136.9, 137.3, 138.19, 138.22; IR (ATR) 3038, 2875 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{35}H_{35}SO_8F_3Na$ 695.1902, obsd 695.1903.

Benzyl 6-Deoxy-6-(diethylphoshonomethyl)-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (7). All solutions were deoxygenated by freezing (liquid nitrogen) and subjecting to five cycles of evacuation and purging with Ar. To a solution of diethyl methylphosphonate (0.32 mL, 2.22 mmol) and HMPA (0.39 mL, 2.22 mmol) in THF (2.8 mL) at −78 °C was added *n*-butyllithium (1.59 mL of a 1.4 M solution in hexane, 2.22 mmol). The resulting solution was allowed to stir for 30 min at −78 °C. To this solution was then added, via cannula, a precooled (−78 °C) solution of the triflate **6** (50 mg, 0.74 mmol) in THF (4.2 mL). After 10 min at −78 °C, the reaction was quenched by adding NH_4Cl (aq.) and Et_2O. The aqueous layer was further extracted with Et_2O, and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (30% EtOAc−hexane) gave **7** (369 mg, 74%) as a white solid: mp 56−58 °C; $[\alpha]^{22}_D$ +2.44 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ* 1.32 (app dt, *J* = 3.5, 7 Hz, 6 H), 1.78 (m, 2 H), 1.98 (m, 1 H), 2.19 (m, 1 H), 3.28 (m, 2 H), 3.49 (dd, *J* = 8, 9 Hz, 1 H), 3.63 (app t, *J* = 9 Hz, 1 H), 4.09 (m, 4 H), 4.49 (d, *J* = 8 Hz, 1 H), 4.62 (d, *J* = 11 Hz, 1 H), 4.67 (d, *J* = 12 Hz, 1 H), 4.73 (d, *J* = 11 Hz, 1 H), 4.78 (d, *J* = 11 Hz, 1 H), 4.88 (d, *J* = 11 Hz, 1 H), 4.92 (d, *J* = 12 Hz, 1 H), 4.93 (d, *J* = 11 Hz, 1 Hz),4.96 (d, *J* = 1 Hz, 1 H),7.32 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 16.5 (br, 2 C), 21.7(d, *J* = 144 Hz), 24.9 (br), 61.5 (br, 2 C), 71.3, 74.3 (d, *J* = 17 Hz), 74.8,75.2,-75.6, 81.4, 82.5, 84.6, 102.6, 127.58, 127.61, 127.79, 127.83, 127.9, 128.0, 128.1, 128.29, 128.33, 128.37, 128.4, 137.3, 138.0, 138.4, 138.5; ^{31}P NMR (202 MHz, $CDCl_3$) *δ* 31.07; IR (ATR) 2972, 2904 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{39}H_{47}O_8PNa$ ($M + Na^+$) 697.2906, obsd 697.2914.

Benzyl 6-Deoxy-6-[(diethylphosphono)difluoromethyl]-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (8). All solutions were deoxygenated as in the synthesis of **7**. To a solution of diisopropylamine (83 *μ*L, 0.63 mmol) in THF (0.5 mL) at −78 °C was added *n*-butyllithium (490 *μ*L of 1.3 M solution in hexane, 0.63 mmol). The resulting solution was allowed to stir for 30 min at 0 °C and then cooled to −78 °C. To this solution of LDA at −78 °C were added, dropwise via cannula, a precooled (−78 °C) solution of diethyl α,α-difluoromethylphosphonate [99 *μ*L, 0.63 mmol (*d* = 1.198)] in THF (0.25 mL) and, 2 min later, a solution of triflate **6** (85 mg, 0.13 mmol) in THF (0.5 mL). After being stirred 15 min at −78 °C, the reaction was quenched by addition of NH_4Cl (aq.) and Et_2O. The aqueous layer was further extracted with Et_2O. The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (50% EtOAc−hexane) gave **8** (73.6 mg, 83%) as a white solid: mp 75−76 °C; $[\alpha]^{22}_D$ +1.6 (*c* 1.25, $CHCl_3$); ^1H

NMR (500 MHz, ($CDCl_3$) δ 1.34 (app t, J = 7 Hz, 3 H), 1.35 (app t, J = 7 Hz, 3 H), 2.17 (m, 1 H), 2.56 (m, 1 H), 3.27 (app t, J = 9 Hz, 1 H), 3.51 (app t, J = 9 Hz, 1 H), 3.65 (app t, J = 9 Hz, 1 H), 3.75 (app t, J = 9 Hz, 1 H), 4.24 (m, 4 H), 4.50 (d, J = 8 Hz, 1 H), 4.59 (d, J = 11 Hz, 1 H), 4.66 (d, J = 12 Hz, 1 H), 4.70 (d, J = 11 Hz, 1 H), 4.75 (d, J = 11 Hz, 1 H), 4.92 (m, 3 H), 4.96 (d, J = 11 Hz, 1 H), 7.31 (m, 20 H); ^{13}C NMR (125 MHz, ($CDCl_3$) δ 16.3, 16.4, 35.4−35.8 (m), 64.38, 64.42, 65.7, 68.9, 70.7, 74.7, 74.9, 80.4, 82.3, 84.7, 101.8, 116.9−122.7 (app dt, J = 126, 261 Hz), 127.57, 127.6, 127.7, 127.8, 127.86, 127.92, 128.1, 128.2, 128.3, 128.35 (2C), 128.39, 137.3, 137.8, 138.43 (2 C); ^{19}F NMR (188 MHz; ($CDCl_3$) δ −113.7 to −111.6 (dd, J = 108, 303 Hz, 1 F), −110.6 to −108.3 (ddd, J = 22, 108, 303 Hz, 1 F); ^{31}P NMR (81 MHz, ($CDCl_3$) δ 6.28 (app t, $J_{F,P}$ = 108 Hz); IR (film) 2910 cm^{-1}; HRMS (FAB, 3-NOBA/Na_2CO_3) calcd for $C_{39}H_{45}PF_2O_8Na$ (M + Na^+) 733.2718, obsd 733.2726. Anal. Calcd for $C_{39}H_{45}PF_2O_8$: C, 65.91; H, 6.38. Found: C, 66.13; H, 6.49.

Benzyl 6-Deoxy-6-(2′-dithianyl)-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (11). All solutions were deoxygenated as in the preparation of **7**. To a solution of dithiane (556 mg, 4.62 mmol) and HMPA (0.80 mL, 4.6 mmol) in THF (9 mL) at −78 °C was added *n*-BuLi (3.8 mL of a 1.22 M solution in hexane, 4.6 mmol). The resulting solution was allowed to stir for 5 min at −78 °C. To this solution was then added, via cannula, a precooled (−78 °C) solution of the triflate **6** (889 mg, 1.32 mmol) in THF (9 mL). After 10 min at −78 °C, the reaction was quenched by adding NH_4Cl (aq.) and Et_2O. The aqueous layer was further extracted with Et_2O, and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (20% EtOAc−hexane) gave **11** (800 mg, 94%) as a white solid: mp 110−111 °C; $[\alpha]^{22}_D$ +2.67 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.86 (ddd, J = 4.5, 10, 14 Hz, 1 H), 1.92 (m, 1 H), 2.08 (m, 1 H), 2.32 (ddd, J = 2.4,-10,14 Hz, 1 H), 2.75 (ddd, J = 3, 10, 14 Hz, 1 H), 2.84 (m, 3 H), 3.29 (t, J = 9 Hz, 1 H),3.5 (dd, J = 4, 10 Hz, 1 H), 3.58 (dt, J = 2, 10 Hz, 1 H), 3.64 (app t, J = 9 Hz, 1 H), 4.18 (dd, J = 4, 10 Hz, 1 H), 4.49 (d, J = 8 Hz, 1 H), 4.62 (d, J = 11 Hz, 1 H), 4.72 (d,J = 11 Hz, 2 H), 4.77 (d, J = 11 Hz, 1 H), 4.88 (d, J = 11 Hz, 1 H), 4.92 (d, J = 11 Hz, 2 H), 4.96 (d, J = 11 Hz, 1 H),7.34 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.0, 29.2, 29.7, 37.4, 43.3, 71.2, 74.8, 75.2, 75.7, 81.6, 82.5, 84.8, 102.4, 127.6 (2 C), 127.8, 127.9, 128.0 (2 C), 128.09, 128.1, 128.29, 128.34, 128.4 (2 C), 137.5, 138.0, 138.5, 138.6; IR (ATR) 2901, 2891 cm^{-1}. Anal. Calcd for $C_{38}H_{42}O_5S_2$: C, 70.99; H, 6.58. Found: C, 70.86; H, 6.68.

Benzyl 6-Deoxy-6-formyl-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (12). To a solution of the dithiane derivative **11** (964 mg, 1.50 mmol) and $CaCO_3$ (2.7 g, 27 mmol) in THF (70 mL) and water (15 mL) was added dropwise a 2 M aqueous solution of $Hg(ClO_4)_2$ (1.88 mL, 3.75 mmol). After 5 h or stirring at room temperature, the reaction mixture was diluted with Et_2O (50 mL) and filtered through a plug of neutral alumina. The filtrate was washed with water (45 mL) and extracted with Et_2O (3 × 30 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (30% EtOAc−hexane) gave **12** (724 mg, 88%) as a white solid: mp 108−109 °C; $[\alpha]^{22}_D$ +2.30 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.82 (ddd, J = 2, 9, 15 Hz, 1 H), 2.74 (ddd, J = 2, 4, 12 Hz, 1 H), 3.33 (app t, J = 9 Hz, 1 H), 3.51 (dd, J = 8, 9 Hz, 1 H), 3.66 (app t, J = 9 Hz, 1 H), 3.83 (dt, J = 4,9 Hz, 1 H), 4.54 (d, J = 8 Hz, 1 H), 4.57 (d, J = 11 Hz, 1 H),4.63 (d, J = 12 Hz, 1 H), 4.71 (d, J = 11 Hz, 1 H) 4.77 (d, J = 11 Hz, 1 H),4.85 (d, J = 12 Hz, 1 H), 4.88 (d, J = 11 Hz, 1 H), 4.94 (d, J = 11 Hz, 1 H), 4.95 (d, J = 11 Hz, 1 H), 7.3 (m, 20 H), 9.72 (t, J = 2 Hz, 1 H); ^{13}C NMR δ 45.8, 70.0, 71.2, 74.9, 75.0, 75.6, 80.6, 82.4, 84.6, 102.4, 127.7 (2 C), 127.86, 127.91, 128.0, 128.07, 128.1 (2 C), 128.35, 128.4, 128.45, 128.51, 137.1, 137.7, 138.3, 138.4, 199.6; IR (ATR) 3028, 2905, 1722 cm^{-1}. Anal. Calcd for $C_{35}H_{36}O_6$: C, 76.04; H, 6.56. Found: C, 75.90; H, 6.82.

Benzyl 6-Deoxy-6-[diethylphosphono(1′-hydroxy)-methyl]-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (13a & 13b). To a solution of diethyl phosphite (0.16 mL, 1.23 mmol) in THF (0.7 mL) at −78 °C was added LiHMDS (1.23 mL of a 1 M solution in THF, 1.23 mmol). The resulting solution was stirred for 5 min at −78 °C, followed by the addition, via cannula, of a solution of aldehyde **12** (618 mg, 1.12 mmol) in THF (5 mL) at −78 °C. After 10 min, the reaction was quenched with NH_4Cl (aq.) and Et_2O. The aqueous layer was further extracted with Et_2O. The combined organics were dried ($MgSO_4$), filtered, and evaporated. One obtains **13a/b** (650 mg, 85% total yield) as a mixture of diastereomers (1.4:1 ratio) separable by flash chromatography (50% EtOAc−hexane). On a smaller scale (93 mg of **12**), the same procedure gives **13a/b** in 93% (108 mg) total yield. Major diastereomer **13a** (first-eluting): mp 122−123 °C; $[\alpha]^{22}_D$ +2.16 (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (t, J = 7 Hz, 6 H), 1.94 (ddd, J = 9, 14.5, 21 Hz, 1 H), 2.41 (m, 1 H), 3.18 (dd, J = 3, 16 Hz, 1 H), 3.38 (app t, J = 9 Hz, 1 H), 3.5 (dd, J = 8,9 Hz, 1 H), 3.58 (dt, J = 3, 9 Hz, 1 H), 3.62 (app t, J = 9 Hz, 1 H), 4.18 (m, 5 H), 4.55 (d, J = 8 Hz, 1 H), 4.63 (d, J = 12 Hz, 1 H), 4.65 (d, J = 11 Hz, 1 H), 4.72 (d, J = 11 Hz, 1 H), 4.78 (d, J = 11 Hz, 1 H), 4.88 (d, J = 12 Hz, 1 H), 4.89 (d, J = 11 Hz, 1 H), 4.93 (d, J = 11 Hz, 1 H), 4.94 (d, J = 11 Hz, 1 H), 7.3 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.5 (br, 2 C), 33.3, 62.6 (br, 2 C), 67.2 (d, J = 165 Hz), 71.5, 74.8 (d, J = 15 Hz), 74.9, 75.2, 75.7, 81.5, 82.2, 84.5, 102.7, 127.6, 127.7, 127.8 (2 C), 127.89, 127.93, 128.0, 128.1, 128.3, 128.36, 128.4, 128.5, 136.9, 137.9, 138.3, 138.4; ^{31}P NMR (202 MHz, $CDCl_3$) δ 22.93; IR (ATR) 3251−3283 (br), 3030, 2863, 1218, 1061, 1020 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{39}H_{47}O_9PNa$ (M + Na^+) 713.2855, obsd 713.2877. Minor diastereomer **13b** (second-eluting): mp 120−122 °C; $[\alpha]^{22}_D$ +5.09 (*c* 0.60, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (app dt, J = 7, 4 Hz, 6 H), 1.83 (m, 1 H), 2.24 (m, 1 H), 2.95 (br s, 1H), 3.3 (app t, J = 9, 10 Hz, 1 H), 3.49 (app t, J = 9 Hz, 1 H), 3.65 (app t, J = 9 Hz, 1 H), 3.69 (dt, J = 2, 10 Hz, 1 H), 4.15 (dq, J = 3, 7 Hz, 5 H), 4.54 (d, J = 8 Hz, 1 H), 4.60 (d, J = 11 Hz, 1 H), 4.69 (d, J = 12 Hz, 1 H), 4.71 (d, J = 11 Hz, 1 H), 4.76 (d, J = 11 Hz, 1 H), 4.87 (d, J = 11 Hz, 1 H), 4.88 (d, J = 12 Hz, 1 H), 4.93 (app t, J = 11 Hz, 2 H), 7.32 (m, 20 H); ^{13}C NMR (125 MHz, ($CDCl_3$) 16.5 (br, 2 C), 33.1, 62.6 (2 C), 64.6 (d, J = 163 Hz), 71 (d, J = 15 Hz), 71.6, 74.9, 75.1, 75.7, 81.3, 82.5, 84.7, 102.8, 127.6, 127.7, 127.8, 127.9, 127.96, 128.0, 128.1, 128.32, 128.37 (2 C), 128.43, 137.4, 138.0, 138.4, 138.5; ^{31}P NMR (202 MHz, $CDCl_3$) δ 24.23; IR (ATR) 3293 (br), 3030, 2905 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{39}H_{47}O_9PNa$ 713.2855, obsd 713.2832.

Benzyl 6-Deoxy-6-[(diethylphosphono)-(*S*)-fluoro-methyl]-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (14a). To a solution of DAST (0.08 mL, 0.58 mmol) in CH_2Cl_2 (0.24 mL) at −78 °C was added rapidly down the sides of the flask, via cannula, a precooled (−78 °C) solution of the diastereomeric mixture (1.4: 1) *o*f the (α-hydroxy)phosphonates **13a** and **13b** (270 mg, 0.4 mmol) in CH_2Cl_2 (2.5 mL). The mixture was stirred at −78 °C for 3−4 min and then raised immediately to room temperature. After 50−60 min at room temperature, the reaction was quenched with $NaHCO_3$ (aq.) followed by extraction with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and evaporated. ^{19}F NMR of the crude showed that it contained a 10:1 mixture of diastereomers. Flash chromatography (50% EtOAc−hexane) gave **14a** [88 mg, 32% (40% based on recovered starting material)] and **14b** (6 mg; 2%). The same procedure, when repeated on a sample of pure **13a** (36 mg, 0.05 mmol), gave **14a** [14 mg, 40% (50% based on recovered starting material)]. Crystals suitable for X-ray diffraction could be obtained by recrystallization from Et_2O/hexane: mp 75−76 °C; $[\alpha]^{22}_D$ +12.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (app dt, J = 7, 5 Hz, 6H), 1.79 (br app dt, J = 13, 44 Hz, 1 H), 2.47 (m, 1H), 3.28 (t, J = 9 Hz, 1H), 3.5 (dd, J = 8, 9 Hz, 2 H), 3.65 (t, J = 9 Hz, 1 H), 4.18 (app quintet, J = 7.5 Hz, 4 H), 4.52 (d, J = 8 Hz, 1 H), 4.61 (d, J = 11 Hz, 1 H), 4.69 (d, J = 12 Hz, 1 H), 4.72 (d, J = 11 Hz, 1 H), 4.77 (d, J = 11 Hz, 1 H), 4.88 (d, J = 11 Hz, 1 H), 4.89 (d, J = 12 Hz, 1 H), 4.92 (d, J = 11 Hz, 1 H), 4.94 (app ddt, J = 2, 13, 47 Hz, 1 H), 4.95 (d, J = 11 Hz, 1 H), 7.31 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.45 (br, 2 C), 32.6 (br), 63.0 (br, 2 C), 69.4 (d, J = 11 Hz), 71.6, 74.9, 75.1, 75.7, 81.5, 82.4, 84.7, 102.6, 127.6 (2 C), 127.8, 127.89, 127.92, 127.98, 128.0, 128.1, 128.3, 128.36, 128.41, 128.5, 137.2, 137.9, 138.4, 138.5; ^{19}F NMR δ −214

(dddd, J = 12, 44, 46, 73 Hz); ^{31}P NMR (202 MHz, $CDCl_3$) δ 17.47 (d, $J_{F,P}$ =73 Hz); IR (ATR) 2905 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{39}H_{46}O_8PFNa$ (M + Na$^+$) 715.2812, obsd 715.2816.

Benzyl 6-deoxy-6-[(diethylphosphono)-(*R*)-fluoromethyl]-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (14b). To a solution of diisopropylamine (150 μL, 1.13 mmol) in THF (3 mL) at −78 °C was added *n*-butyllithium (1 mL of 1.11 M solution in hexanes, 1.13 mmol). The resulting solution was allowed to stir for 30 min at 0 °C and then cooled to −78 °C. To this solution of LDA at −78 °C was added dropwise via cannula a precooled (−78 °C) solution of **14a** (154 mg, 0.23 mmol) in THF (1.2 mL). After 1 h, a solution of HOAc (0.13 mL, 2.25 mmol) in THF (0.52 mL) cooled to −78 °C was cannulated into the reaction mixture. Upon an additional 10 min of stirring at that temperature, the reaction was quenched with $NaHCO_3$ (aq.) and Et_2O. The aqueous layer was further extracted with Et_2O. The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (50% EtOAc−hexane) gave a white solid **14b** [73 mg, 47% (98% based on recovered starting material)]: $[\alpha]^{22}_D$ −6.82 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (app dt, J = 7, 8 Hz, 6 H), 2.39 (m, 2 H), 3.47 (t, J = 9 Hz, 1 H), 3.56 (m, 2 H), 3.65 (t, J = 9 Hz, 1 H), 4.21 (m, 4 H), 4.56 (d, J = 8 Hz, 1 H), 4.66 (d, J = 11 Hz, 1 H), 4.70 (d, J = 12 Hz, 1 H), 4.75 (d, J = 11 Hz, 1 H), 4.72 (d, J = 11 Hz, 1 H), 4.77 (d, J = 11 Hz, 1 H), 4.88 (d, J = 11 Hz, 1 H), 4.89 (d, J = 12 Hz, 1 H), 4.80 (d, J = 11 Hz, 1 H), 4.93 (d, J = 11 Hz, 1 H), 4.95 (d, J = 12 Hz, 1 H), 4.96 (d, J = 11 Hz, 1 H), 4.99 (d, J = 11 Hz, 1 H), 5.12 (dddd, J = 3, 5, 9, 47 Hz), 7.34 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.4 (br, 2 C), 32.1 (d, J = 18 Hz), 63.0 (dd, J = 7, 31 Hz, 2 C),71.2, 71.4 (dd, J = 4, 11 Hz), 74.8, 75.0, 75.6, 81.2, 82.4, 84.7, 86.1 (dd, J = 171, 180 Hz), 102.5, 127.6 (2 C), 127.7, 127.74, 127.80, 127.9 (2 C), 128.0, 128.3, 128.32 (2 C), 128.4, 137.3, 138.1, 138.4, 138.5; ^{19}F NMR (470 MHz, $CDCl_3$) δ −205.5 (dddd, J = 18, 32, 47, 73 Hz); ^{31}P NMR (202 MHz, $CDCl_3$) δ 17.22 (d, $J_{F,P}$ = 73 Hz); HRMS (FAB, 3-NOBA/LiI) calcd for $C_{39}H_{46}O_8PFLi$ (M + Li$^+$) 699.3062, obsd 699.3058.

6-Deoxy-6-(phosphonomethyl)-D-glucopyranoside, Bis-Ammonium Salt (9). To a stirred solution of **7** (300 mg, 0.45 mmol) in CH_2Cl_2 (5.2 mL) at room temperature was added TMSBr (0.18 mL, 1.39 mmol), dropwise via syringe. The reaction mixture was stirred at room temperature for the required period of time (^{1}H NMR monitoring, 2 days in this case) and then concentrated by rotary evaporation. [Note: It is advantageous to use an inert cap for this reaction vessel (plastic Capplugs or glass) as common laboratory septa are unstable to TMSBr.] Several cycles of washing (MeOH) and evaporation then gave the free phosphonic acid. A suspension of this crude acid and 20% $Pd(OH)_2$/C (55 mg) in EtOAc (2.5 mL) and MeOH (2.5 mL) was shaken (Parr Hydrogenator) under hydrogen pressure (50 psi) for 1 day. Filtration, evaporation of the volatiles, and flash chromatography (50% *i*-PrOH/25% CH_3CN/25% 50 mM NH_4HCO_3) gave **9** (90 mg, 69%) as a mixture of anomers: ^{1}H (300 MHz, D_2O) β:α ≈ 3:1 as determined by integration of the anomeric H signals, δ 4.67 (d, J = 8 Hz, 1 H; β anomer), 5.24 (d, J = 4 Hz, 1 H; α anomer) (see Supporting Information for a copy of the entire NMR spectrum); ^{13}C NMR (75.5 MHz, D_2O, characteristic signals) δ 24.1 (d, J = 135 Hz; β anomer), 24.2 (d, J = 135 Hz, α anomer), 92.6 (α anomer), 96.6 (β anomer); ^{31}P NMR (202 MHz, D_2O) δ 24.97 (β anomer), 25.03 (α anomer); HRMS (FAB, TEA) calcd for $C_7H_{15}O_8P$ [(M − H)$^-$] 257.0426, obsd 257.0418.

6-Deoxy-6-(difluorophosphonomethyl)-D-glucopyranoside, Bis-Ammonium Salt (10). From **8** (44 mg, 0.06 mmol), following the deprotection procedure for **7**, was obtained **10** (18 mg, 91%; white solid) as a mixture of anomers: ^{1}H NMR (500 MHz, D_2O) β:α ≈ 3:1 as determined by integration of anomeric H signals, δ 4.71 (d, J = 8 Hz, 1 H; β anomer), 5.23 (d, J = 4 Hz, 1 H; α anomer) (see Supporting Information for a copy of the entire NMR spectrum); ^{13}C NMR (125 MHz, D_2O) δ 92.7 (α anomer), 96.6 (β anomer); ^{31}P NMR (202 MHz, D_2O) δ 4.73 (t, J = 86 Hz; β anomer), 4.81 (t, J = 86 Hz; α anomer); ^{19}F NMR (470 MHz, D_2O) δ −110.52 (dddd, J = 5, 35, 87, 281 Hz; β anomer), −110.75 (dddd, J = 5, 35, 87, 281 Hz; α anomer), 112.85 (dddd, J = 10, 30, 87, 281 Hz; β anomer), 113.69 (dddd, J = 10, 30, 87, 281 Hz; α anomer); HRMS (FAB, TEA) calcd for $C_7H_{13}O_8F_2P$ [(M−H)$^-$] 293.0238, obsd 293.0241.

6-Deoxy-6-[(*S*)-monofluorophosphonomethyl]-D-glucopyranoside, Bis-Ammonium Salt (15a). From **14a** (186 mg, 0.27 mmol) following the deprotection procedure for **7**, was obtained **15a** (50 mg, 60%; white solid) as a mixture of anomers: ^{1}H NMR (300 MHz, D_2O) β:α ≈ 2:1 as determined by integration of anomeric H signals, δ 4.65 (d, J = 8 Hz, β anomer), 5.21 (d, J = 4 Hz, α anomer) (see Supporting Information for a copy of the entire NMR spectrum); ^{13}C NMR (75.5 MHz, D_2O) δ 92.7 (α anomer), 96.6 (β anomer); ^{31}P NMR (121 MHz, D_2O); 12.4 (d, J = 62 Hz; β anomer), 12.9 (d, J = 62 Hz; α anomer); ^{19}F NMR (470 MHz, D_2O) δ −205.01 to −205.77 (overlapping app ddt, J = 12, 46, 66 Hz, 1 F from each anomer); HRMS (FAB, TEA): calcd for $C_7H_{14}O_8PF$ (M − H)$^-$ 275.0332, obsd 275.0336.

6-Deoxy-6-[(*R*)-monofluorophosphonomethyl]-D-glucopyranoside, Bis-Ammonium Salt (15b). From **14b** (90 mg, 0.13 mmol), following the deprotection procedure for **7**, was obtained **15b** (28 mg, 70%; white solid) as a mixture of anomers: ^{1}H NMR (300 MHz, D_2O) β:α ≈ 2:1 as determined by integration of anomeric H signals, δ 4.66 (d, J = 8 Hz, β anomer), 5.25 (d, J = 4 Hz, α anomer) (see Supporting Information for a copy of the entire NMR spectrum); ^{13}C NMR (75.5 MHz, D_2O) δ 92.8 (β anomer), 96.9 (α anomer); ^{31}P NMR (121 MHz, D_2O) 12.7 (d, J = 67 Hz, 1F; both anomers); ^{19}F NMR (470 MHz, D_2O) δ −199.7 to −200.4 (m, 1 F from each anomer); HRMS (FAB, TEA) calcd for $C_7H_{14}O_8PF$ [(M − H)$^-$] 275.0332, obsd 275.0338.

Benzyl 6-Deoxy-6-[diethylphosphono(1′-(4″-bromo)-benzoyloxy)methyl]-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (16a and 16b). To a solution of the diastereomeric mixture of (α-hydroxy)phosphonates **13a** and **13b** (114 mg, 0.17 mmol) in THF (1.65 mL) at −78 °C was added *n*-BuLi (0.14 mL of a 1.15 M solution in hexane, 0.17 mmol). After 5 min, a precooled solution of *p*-bromobenzoyl chloride (54.4 mg, 0.25 mmol) in THF (1 mL) was added, via cannula, and the resulting reaction mixture was stirred at −78 °C for 30 min. The reaction was quenched by addition of NH_4Cl (aq., 10 mL) and Et_2O (10 mL). The aqueous layer was further extracted with Et_2O. The combined organics were dried ($MgSO_4$), filtered, and evaporated. One obtains **16a/b** (126 mg, 88% total yield, white solid) as a mixture (1.5:1) of diastereomers separable by flash chromatography (30%→50% EtOAc−hexane). Major diastereomer **16a** (first-eluting, 76 mg): $[\alpha]^{22}_D$ −22.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 (dt, J = 3, 7 Hz, 6 H), 2.07 (m, 1H), 2.65 (dddd, J = 2, 5, 11, 15 Hz, 1 H), 3.27 (t, J = 9 Hz, 1 H), 3.46 (dd, J = 8, 9 Hz, 1 H), 3.62 (app t, J = 9 Hz, 1 H), 3.64 (app t, J = 9 Hz, 1 H), 4.14 (m, 4 H), 4.41 (d, J = 12 Hz, 1 H), 4.48 (d, J = 8 Hz, 1 H), 4.56 (d, J = 11 Hz, 1 H), 4.64 (d, J = 11 Hz, 1 H), 4.67 (d, J = 11 Hz, 1 H), 4.77 (d, J = 11 Hz, 1 H), 4.91 (t, J = 11 Hz, 3 H), 5.76 (dt, J = 5,9 Hz, 1 H), 7.11 (m, 2 H), 7.31 (m, 18 H), 7.54 (d, J = 9 Hz, 2 H), 7.93 (d, J = 9 Hz, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.4 (d, J = 5 Hz), 16.5 (d, J = 5 Hz), 32.0, 62.8 (d, J = 20 Hz), 62.9 (d, J = 18 Hz), 67.5 (d, J = 167 Hz), 71.1, 73.8 (d, J = 13 Hz), 74.7, 75.1, 75.6, 81.4, 82.3, 84.5, 102.7, 127.5, 127.6 (2 C), 127.7, 127.8, 127.9, 128.0, 128.17, 128.24 (2 C), 128.3, 128.4, 128.6, 131.3, 131.9, 137.3, 137.9, 138.4, 138.5, 164.6; ^{31}P NMR (202 MHz, ($CDCl_3$) δ 19.33 (s); IR (ATR) 3031, 2907, 1724 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{46}H_{50}O_{10}BrPNa$ (M+Na$^+$) 895.2222, obsd 895.2244; Minor diastereomer **16b** (second-eluting, 50 mg): mp 78°-79 °C; $[\alpha]^{22}_D$ −7.59 (*c* 0.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.29 (dt, J = 7, 13 Hz, 6 H), 2.00 (m, 1 H), 2.56 (m, 1 H), 3.21 (app t, J = 9,10 Hz, 1 H), 3.31 (t, J = 9 Hz, 1 H), 3.46 (app t, J = 8,9 Hz, 1 H), 3.54 (t, J = 9 Hz, 1 H), 4.20 (m, 4 H), 4.30 (d, J = 8 Hz, 1H), 4.63 (d, J = 11 Hz, 2 H), 4.67 (d, J = 11 Hz, 1 H), 4.71 (d, J = 11 Hz, 1 H), 4.87 (d, J = 11 Hz, 2H), 4.92 (d, J = 11 Hz, 1H), 4.95 (d, J = 12 Hz, 1 H), 5.91 (dt, J = 2,10 Hz, 1 H), 7.34 (m, 20 H), 7.58 (d, J = 10 Hz, 2 H), 7.91 (d, J = 10 Hz, 2 H); ^{13}C NMR (202 MHz, ($CDCl_3$) δ 16.5 (br, 2 C), 31.6, 62.8 (d, J = 9 Hz), 62.9 (d, J = 11 Hz), 64.5 (d, J = 170 Hz), 69.7 (d, J = 24 Hz), 71.1, 74.8, 75.3, 75.7, 81.3, 82.4, 84.6, 102.3, 127.60, 127.65, 127.69,

127.89, 127.95, 128.01 (2 C), 128.1, 128.22, 128.30, 128.40, 128.48, 128.7, 129.9, 131.4, 131.9, 137.5, 137.8, 138.3, 138.4, 164.6; ^{31}P NMR (202 MHz, $CDCl_3$) δ 20.03; IR (ATR) 2920, 2856, 1727 cm^{-1}; HRMS (FAB, 3-NOBA/NaI) calcd for $C_{46}H_{50}O_{10}BrPNa$ ($M + Na^+$) 897.2202 obsd 897.2204. To determine the relative stereochemistry at C-7, crystals of **16b** were grown from Et_2O/hexane and employed for X-ray diffraction.

Enzyme Standardization. G6PDH solutions were standardized (in terms of U/mL) on a regular basis to ensure that all kinetics experiments were carried out with a fixed amount of enzyme. G6PDH (*L. mesenteroides*, 200 U/mg protein, Calbiochem), β-$NADP^+$ (sodium salt, Sigma), and β-D-glucose-6-phosphate (monosodium salt, Aldrich) were used in the assay. All UV absorbance data were recorded on a Shimadzu model UV-2101PC scanning spectrophotometer. A standard assay contained 1 mM glucose 6-phosphate and 0.6 mM $NADP^+$ and Tris-HCl buffer (0.1 M, pH = 7.8) in a total volume of 1 mL.

Concentration Determinations, Phosphonate Solutions. Concentrations of the phosphonate substrate stock solutions (for **9**, **10**, **15a**, and G6P itself) were determined spectrophometrically, by complete oxidation with G6PDH and quantitation of the NADPH thereby formed. At pH 7.8, the reaction is functionally irreversible as the 6-phosphogluconolactone enzymatic product is presumably converted to 6-phosphogluconate, at least on the time scale of these measurements (ca. 1−2 h). Typically, 3 μL of the stock solution (10−75 mM) are diluted in buffer (100 mM Tris-HCl, pH 7.8; 1 mL total volume; 1 cm path length) containing 1 mM $NADP^+$ and G6PDH (*L. mesenteroides*, 25 mU). The total increase in optical density at 340 nm is then monitored at 25 °C, whereby additional enzyme (typically two 25 mU portions) is added over the time course of the assay until the absorbance readings level off. Division of $\Delta O.D._{340}$(total) by 6.22 mM^{-1} gives the total concentration of NADPH formed, reflecting the concentration of G6P or analogue initially present. For **15b**, the stock solution concentration was determined by ^{19}F NMR after diluting 1:1 with D_2O. A solution of trifluoroethanol in D_2O was employed as concentration standard.

Enzyme Kinetic Analysis (G6PDH). The assay cuvette (total volume 1 mL) containing Tris-HCl buffer (0.1 M, pH 7.8), $NADP^+$ (0.6 mM) and varying concentrations of glucose 6-phosphate (0.05, 0.1, 0.2, 0.3, 0.5, 0.8 and 1 mM) was incubated at 25 °C for 2 min. Each reaction was then initiated by the addition of enzyme (25 mU per cuvette). Initial velocities were determined by monitoring $\Delta O.D._{340}$ (against a buffer blank) vs time. (Note, control experiments showed no reduction of $NADP^+$ in the absence of enzyme.) Each assay was performed in duplicate. This same dehydrogenase assay was carried out in duplicate for phosphonates **9** (at 0.3, 0.5, 0.8, 1.0, 2.0, 3.0, 5.0 mM), **10** (at 0.3, 0.5, 0.7, 0.9, 1.5, 2.5, 3.5 mM), **15a** (at 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0 mM), and **15b** (at 0.2, 0.3, 0.7, 1.0, 1.9, 2.9, 4.0, 5.9, 10 mM). In each case, initial rates were estimated from the observed absorbance over the first 5 min of reaction, for which excellent linearity was observed. Linear least-squares fitting of $1/v_o$ vs $1/[S]$ provided K_m and V_{max} values.

pK_a Determinations. To obtain the free phosphonic acids from the ammonium salts of **9**, **10**, and **15a**, two cycles of dissolution (in distilled, deionized water), freezing and lyophilization were carried out. That ammonia had, in fact, been removed by lyophilization could be seen by monitoring the change in pH of an aqueous solution of these phosphonates (ca. pH 8 prior to lyophilization and ca. pH 3 thereafter). Titrations were carried out on 1 mg/mL solutions of the phosphonic acids, by adding a solution of base (14 mM NaOH) in 10 μL aliquots and monitoring pH with an Orion glass electrode (model 81−15) and a Corning pH meter (model 240). Following titration, the pH profile for each analogue was plotted vs amount of base added, from which the second pK_a was determined.

Acknowledgment. Financial support from the American Heart Association is gratefully acknowledged. D.B.B. is an Alfred P. Sloan Research Fellow. T.J.P. was a participant in the NSF REU (Research Experience for Undergraduates) program at the University of Nebraska. We thank Prof. Victor W. Day for performing the crystal structure analyses of **14a** and **16b**, and Prof. John J. Stezowski for access to his SGI workstation. MariJean Eggen and Darby G. Sloss are acknowledged for early experiments with **10**. This research was facilitated by grants for NMR and GC/MS instrumentation from the NIH (SIG 1-S10-RR06301) and the NSF (CHE-93000831), respectively.

Supporting Information Available: ^{1}H NMR spectra for compounds **5-12**, **13a**, **13b**, **14a**, **14b**, **15a**, **15b**, **16a**, **16b**, **17** and **18,** as well as perspective drawings of the solid-state structures for **14a** and **16b** and a summary of crystallographic parameters and tables of fractional coordinates, anisotropic thermal parameters, bond angles, bond lengths and modeled hydrogen atom coordinates for the X-ray crystal structure of **14a**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000220V