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(α-Monofluoroalkyl)phosphonates: a class of isoacidic and ''tunable'' mimics of biological phosphates

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Abstract

In the early 1980s, Blackburn and McKenna suggested that α-fluorination might lead to phosphonates that better mimic natural phosphates. Although α-monofluorination produces phosphonates with ''matching'' second pK_a values, the α,α-difluorinated phosphonates have received more attention in the past decade or so. Recently, reported enzyme kinetic data on the α-monofluorinated phosphonates from the O'Hagan lab and from our lab suggest that the CHF stereochemistry does affect enzyme-binding, thereby providing an additional variable that may be tuned to achieve optimal binding to an active site of interest. This asymmetry also appears in structural data from the groups of Barford/Burke and Tracey on PTP1B complexes with bound α,α-difluorinated phosphonate inhibitors. In those complexes, only one of two prochiral fluorine atoms appears to interact appreciably with the enzyme. Namely, it is thought that the pro-*R* (F_{si}) fluorine is engaged in an important hydrogen bond with the Phe-182 amide NH.

Available methods for the synthesis of this class of α-monofluorinated phosphonates are reviewed. A new convergent approach, developed at Nebraska, in which the potassium anion of (α-fluoro-α-phenylsulfonylmethyl)phosphonate is used to displace primary triflates is also described. This method is particularly convenient as it allows one to perform a ''fluorinated phosphonate scan'' of an active site of interest (in what follows, we use this expression to designate the synthesis and evaluation of a complete set of the CH_2–, CF_2– and both stereoisomeric CHF-phosphonates in an active site of interest) from a single primary triflate. The properties of the title compounds in enzyme active sites are discussed, as are possible interactions of these fluorine-containing bioisosteres with active site residues.

Keywords: (α-Monofluoroalkyl)phosphonate; Phosphate mimics; Triflate displacement; Glucose 6-phosphate dehydrogenase; Glycerol 3-phosphate dehydrogenase; Protein phosphotyrosine phosphatase PTP1B

1. Introduction

Living systems have evolved an organic chemistry that prominently features fully oxygenated phosphorus(V) functionality. For example, the first step in glycogenolysis/glycolysis involves extrusion of monomeric glucose residues from the carbohydrate polymer with accompanying attachment of a phosphate ester handle (from inorganic phosphate) to each carbohydrate unit. A second phosphate monoester is installed at the phosphofructokinase step. Phosphate monoesters, being kinetically stable, but thermodynamically labile to hydrolysis, are useful on/off switches (e.g. pTyr, pSer, pThr) for enzyme regulation. The same properties make the phosphodiester a viable backbone for polymeric nucleic acids carrying genetic information (DNA, RNA). Here, kinetic stability is essential to insure fidelity in the translation of the message into phenotype, but nucleic acid digestion and recycling is also required and can be achieved by nuclease enzymes that take advantage of the favorable ester cleavage thermodynamics in water. More highly activated P(V) functionalities, including enol phosphate esters (e.g. PEP) and phosphate anhydrides (e.g. 1,3-diphosphoglycerate or ATP) are used as cellular energy currency [1].

Of course, phosphate esters/anhydrides offer several potentially favorable modes of interaction with enzymatic binding partners. These include electrostatic interactions (as the mono or dianions), hydrogen bonding (both as donors and as acceptors) or dipole–dipole interactions. The ability of phosphates to provide a binding handle for their derivatives may have contributed to their widespread evolutionary incorporation into biomolecules, from metabolites to macromolecules. Indeed, for many proteins, one can identify well-defined phosphate binding pockets. In particular, the phosphate binding pockets in glucose 6-phosphate dehydrogenase and protein phosphotyrosine phosphatase 1B (PTP1B) will be discussed in this article.

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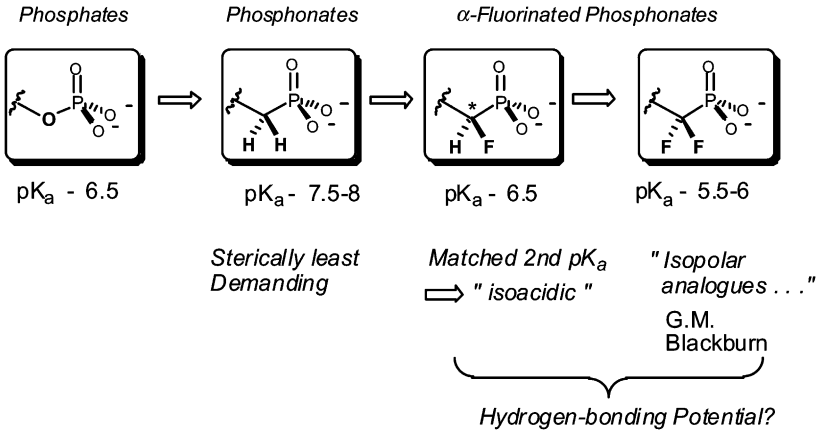


Fig. 1. (Fluorinated) phosphonates as "phosphate isosteres".

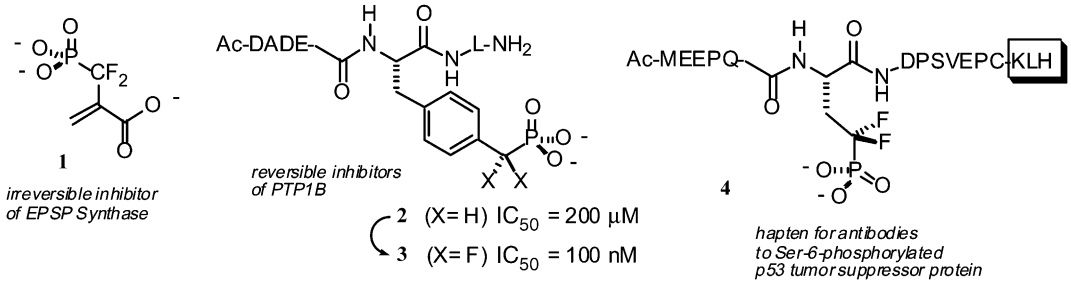


Fig. 2. α,α-Difluorinated phosphonates as bioorganic tools.

Unfortunately, the abundance of digestive phosphatases renders phosphate esters themselves impractical functional groups for drug design, with the exception of pro-drug applications (for example, Etopophos™ is a useful prodrug with enhanced water solubility [2]). For this reason, much like the peptidomimetic problem, the phosphate mimics problem has captured the attention of many in the bio-organic community. The notion that replacement of the bridging oxygen in a phosphate ester or anhydride with a CH_2 should confer inertness to phosphatase cleavage is a well established one, and simple phosphonate analogues of biological phosphates continue to be of interest [3,4]. In the 1980s, Blackburn and coworkers [5,6] and McKenna and Shen [7] suggested that superior bioisosteres might be obtained by introducing α-halogenation, and in particular, α-fluorination, into such phosphonates (see Fig. 1). Among the parameters that potentially favor α-fluorinated phosphonates[1] [8–14] over their nonfluorinated congeners are: (a) reduced pK_a (for an example in which the reduced pK_a of α,α-difluorinated phosphonates appears to correlate with enzyme (phosphoglycerate kinase) binding, see [15]), (b) increased C–CX_2–P dihedral angle, (c) increased polarity of the bridging group and (d) the possibility for C–F···H–X hydrogen bonding (for recent discussions of the possibility of C–F···H–X hydrogen bonds in conjunction with surveys of crystallographic databases, see [16–18]). Sterics, on the other hand, tend to favor the simple phosphonate as literally the best isostere (in α-fluorinated phosphonates, the C–F bond is typically 1.3–1.5 Å, 30–50% longer than the corresponding C–H bond).

Indeed, α,α-difluorinated phosphonates are known to be especially effective phosphate isosteres in a number of active sites (Fig. 2). For example, the Monsanto group found that phosphoenol pyruvate analogue **1** irreversibly inactivates EPSP synthase [19]. Burke et al. discovered that the difluorinated analogue of phosphotyrosine, when incorporated into an appropriate hexapeptide (**3**), enhances PTP1B-binding affinity 2000-fold relative to the CH_2-phosphonate-containing congener (**2**) [20]. Peptide **3** even reverses the impairment of insulin receptor function associated with the overexpression of PTP1B in some forms of diabetes [21]. Most recently, the CF_2-phosphonate analogue of phosphoserine (first synthesized in our laboratory [22,23] and later incorporated into peptide **4**), served as a useful bio-organic tool, allowing Appella and coworkers to induce otherwise unobtainable antibodies to the Ser[6]-phosphorylated form of

[1] For reviews that include discussions of the α-fluorinated phosphonates, see [8–10]. For discussions on the possible effects of fluorination α to phosphorus, see [12–14].

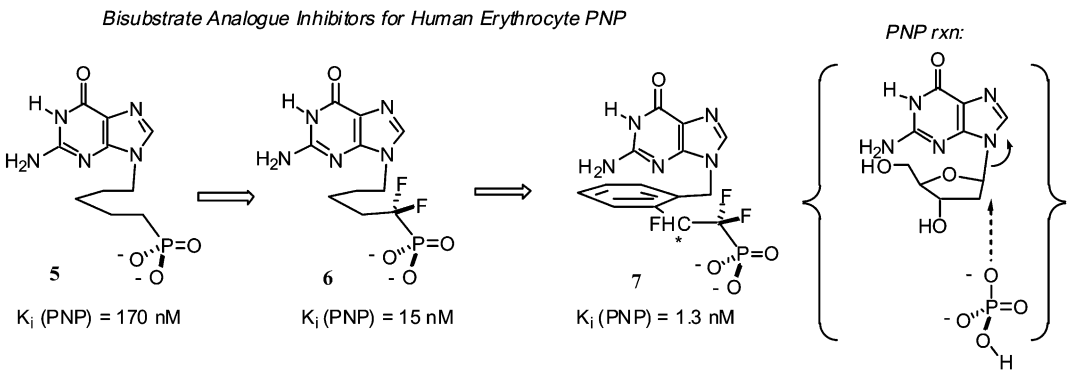


Fig. 3.

the important human tumor suppressor protein p53, for the study of its regulation [24].

Halazy and coworkers, working with a series of bisubstrate analogue inhibitors of purine nucleoside phosphorylase, took things a step further [25,26]. That is to say, they found that, in this active site, one can gain an order of magnitude in binding affinity with α-difluorination (**5** → **6**; Fig. 3). Interestingly, however, placement of an additional CHF unit in the β-position (i.e. (CHF)CF_2-phosphonate, **7**) reduces K_i by another order of magnitude.[2] The stereochemical dependence of this effect at the α-center was not examined. The results suggest that when constructing phosphonate isosteres, both flanking CHF and CF_2 units should be considered, and where possible it may even be advantageous to include both α- and β-fluorination in such phosphonates.

However, it remains the case that placement of a single CHF unit α to the phosphoryl group results in a phosphonate that is essentially iso-acidic with the phosphate itself. So, although a fair number of (α-monofluoroalkyl)phosphonates have been reported (Fig. 4; for examples, of (monofluoroalkyl)phosphonate analogues of biological phosphates, see [27–38]) somewhat surprisingly, 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. Most importantly, from the point of view of this article, the possible influence of the additional CHF stereocenter upon binding/activity has only recently begun to be examined.

Reports from O'Hagan and coworkers with glycerol 3-phosphate analogues [39,40] from our group with glucose 6-phosphate analogues [41] and from the groups of Burke and coworkers [20,42], Barford and coworkers [43], Glover and Tracey [44] on PTP1B inhibitors suggest not only that phosphonates of this class can make excellent bioisosteres, but that the CHF stereocenter may indeed serve as an additional tunable parameter within the class.[3] A detailed discussion of the findings of these groups will be presented following a discussion of synthetic methods available for accessing this class of phosphate mimics.

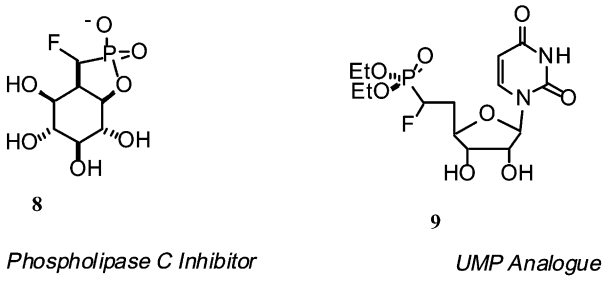


Fig. 4. Bioisoteres of the monofluorinated phosphonate class.

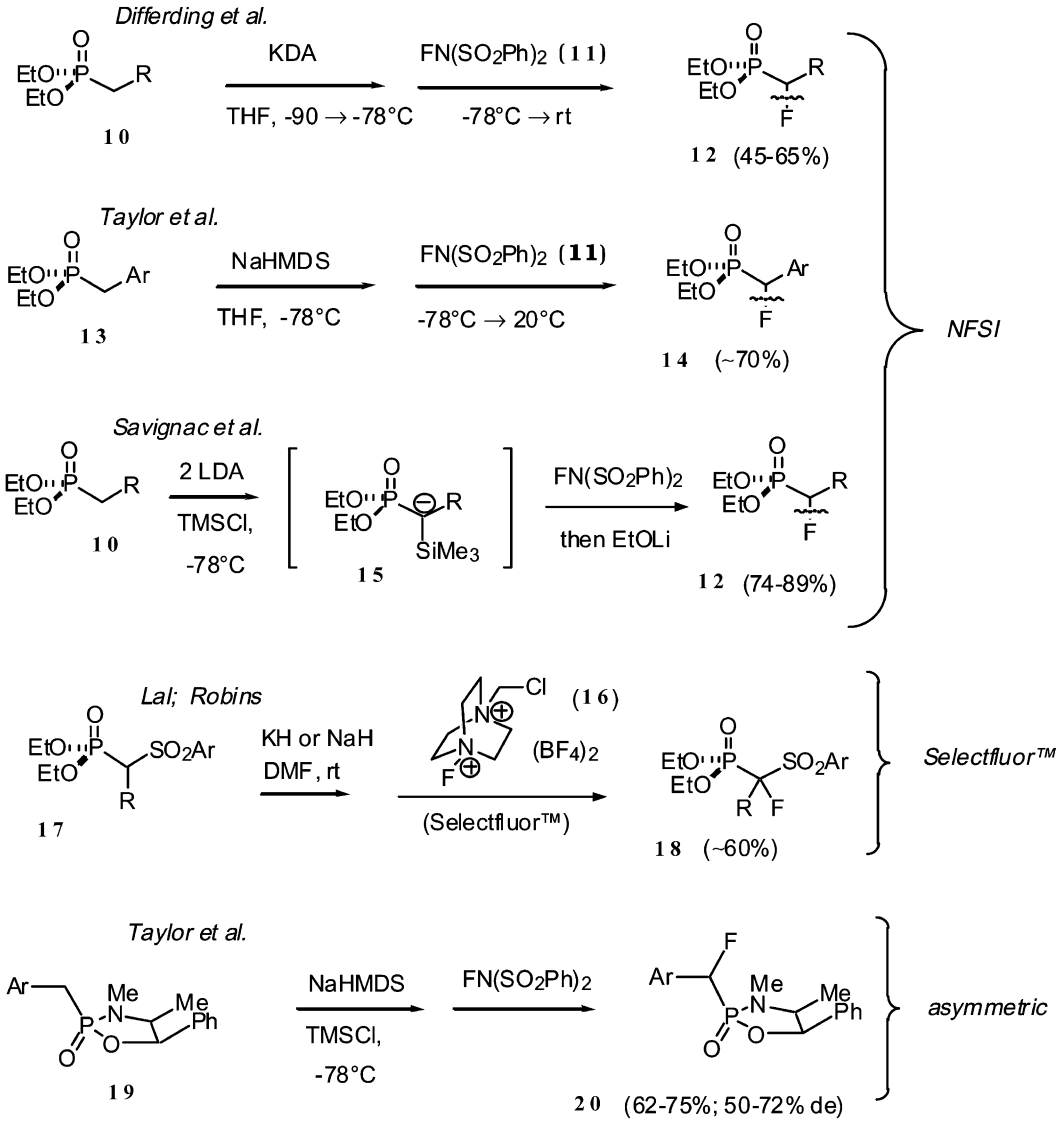
2. Synthesis of (α-monofluoroalkyl)phosphonates

2.1. Electrophilic fluorination

Among the synthetic entries into the α-monofluorinated phosphonates, two of the most common approaches involve C–X bond disconnection. The past decade has seen the

[2] The fusion of a phenyl ring to compound **6** alone results in essentially no change in binding affinity (K_i = 13 nM; [26]).

[3] There is one preliminary report on the synthesis and evaluation of (α-monofluoroalkyl)phosphonates related to the PTP1B inhibitors described by Burke and coworkers in [42,43]. However, the authors of this report begin from a thesis that appears to be the opposite of what one would predict from Burke's X-ray crystallographic data. They state "Burke's results suggest that enantiomerically pure (*R*)-(α-monofluoroalkyl)phosphonic acids may be as effective inhibitors of PTP1B as their difluoro analogues." In fact, Burke's results would suggest just the opposite; namely, that the (*S*)-(α-monofluoroalkyl)phosphonic acids would bind more tightly to PTP1B than their (*R*)-counterparts. Thus, we are hesitant to draw any conclusions from this work at this time, see [45]. [Note: S.D. Taylor has informed us that he is aware of these issues and is in the process of addressing them. One can expect a modified account of this work, in due course].

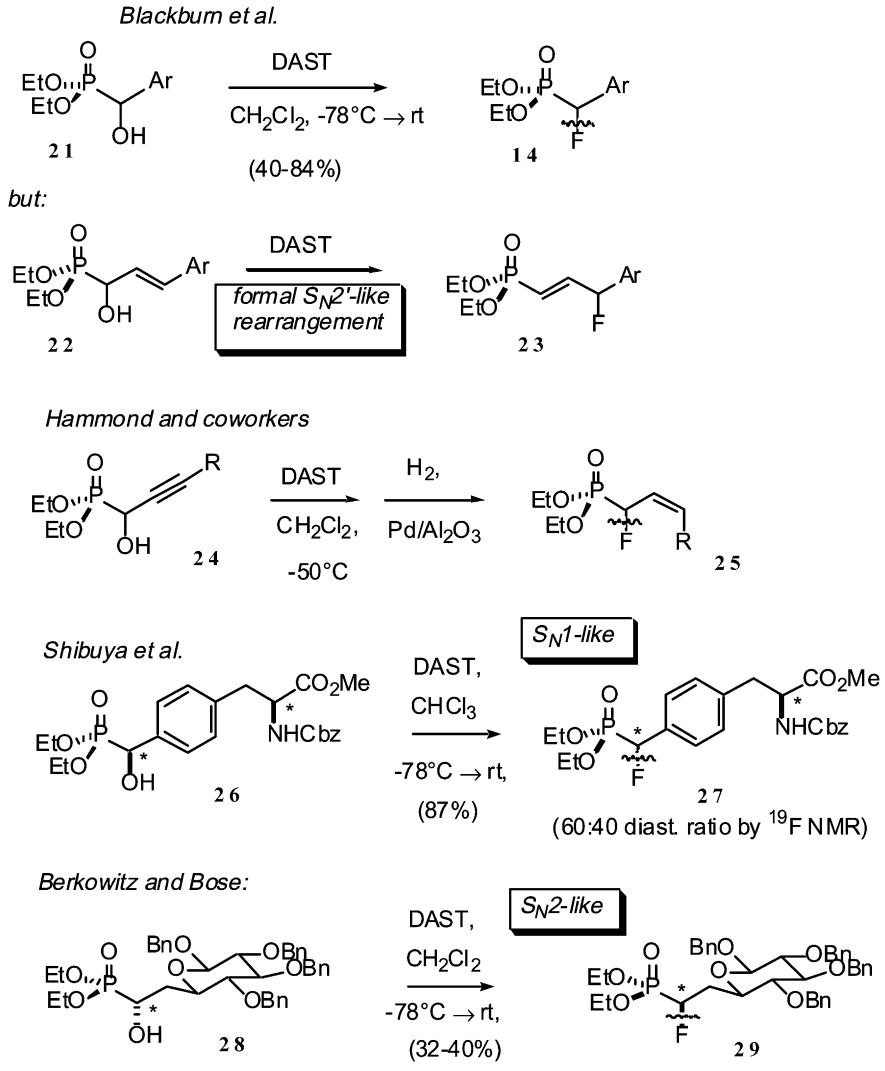


Scheme 1.

development of electrophilic fluorination as a viable approach. One generates a carbanion α to a phosphoryl group and then traps it with an X^+ equivalent. To our knowledge, Differding et al. first reported electrophilic fluorination α to phosphorus (Scheme 1; [46]). In that case, KDA was used as base to generate an "unstabilized" anion α to phosphorus. NFSI was used as F^+ equivalent and yields were modest to good. In general, as has been seen in subsequent work in other labs, the reaction proceeds more efficiently when a stabilized α-phosphoryl anion $\{(RO)_2P(O)C(Y)R^-\}$ is employed, wherein the Y group is either aryl [47], arenesulfonyl [29,50], or trialkylsilyl [48,49]. Where Y is sulfonyl, the alternative F^+ equivalent, Selectfluor™ has been successfully deployed. Savignac and coworkers have developed arguably the most general version of this chemistry, in which a trimethylsilyl group is employed as a temporary anion-stabilizing group, installed in situ, and removed under basic conditions, following α-fluorination [48,49]. In early forays into asymmetric electrophilic fluorination, Taylor has shown that fair to good ee's can be obtained through the appendage of an ephedrine ester/amide auxiliary to the phosphoryl center [45].

2.2. Nucleophilic fluorination

The reversed polarity version of this same C–F bond disconnection offers an alternative route to α-monofluorinated phosphonates. Typically, this nucleophilic fluorination route involves treatment of an alcohol with DAST (*N*,*N*-diethylaminosulfur trifluoride) and presumably passes though a S(IV)-ester intermediate. To our knowledge, the first application of this reaction to (α-hydroxyalkyl)phosphonates was by Blackburn and Kent (Scheme 2, [51,52]) The reaction was found to work well for benzylic substrates,



Scheme 2.

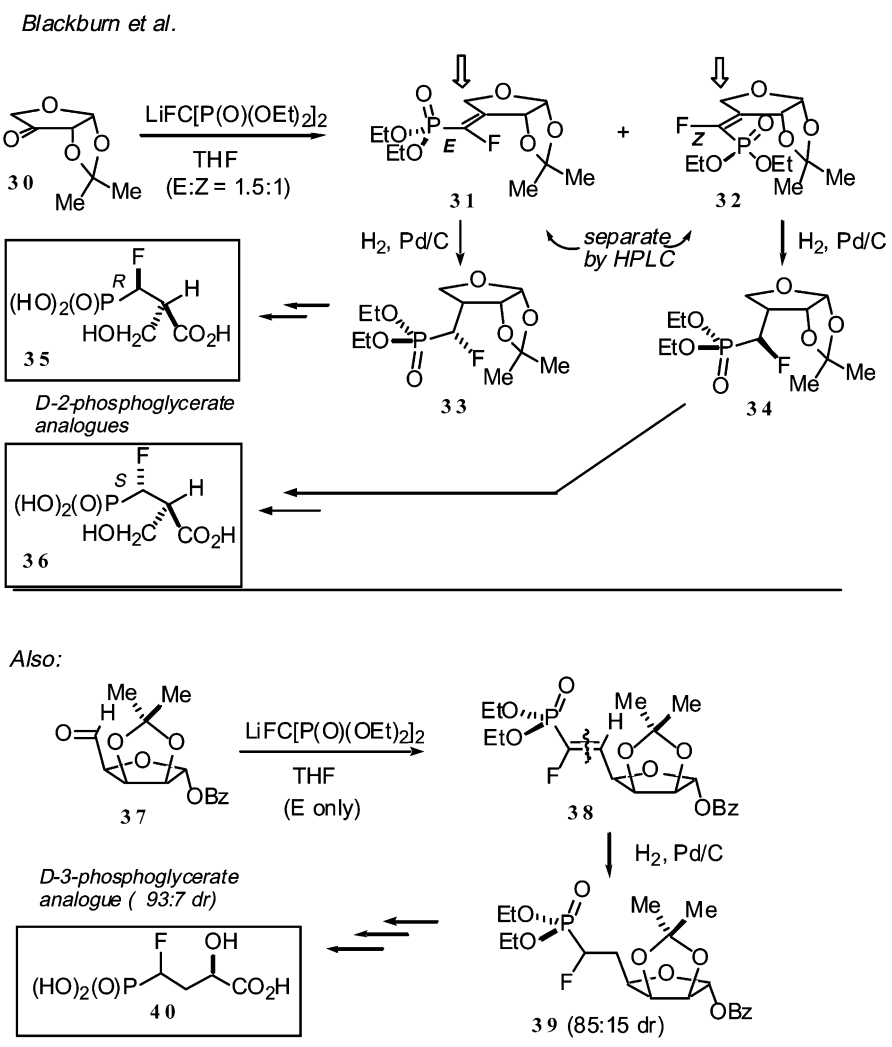
but to give rearrangement (formally an S_N2 displacement or a [3,3]-sigmatropic shift) for allyl or cinnamyl-type systems. Later, in an important advance, Hammond, found that propargylic (α-hydroxy)phosphonates do undergo clean substitution with DAST, without accompanying rearrangement [53–55]. A subsequent partial hydrogenation step leads to the elusive allylic α-fluorinated phosphonates.

There are two studies on the stereochemical course of this transformation of which we are aware. On the one hand, Shibuya showed that benzylic (α-hydroxy)phosphonates give racemization, suggestive of an S_N1-like mechanism [31]. On the other, we found recently that a secondary (α-hydroxyalkyl)phosphonate derived from glucose undergoes DAST-mediated substitution with inversion of configuration, suggestive of an S_N2-like mechanism [41]. It is important to note, however, that this transformation is quite inefficient. Blackburn and Kent have pointed out that dehydration often competes with substitution in such systems and is the predominant reaction in some cases [51].

2.3. Horner–Wadsworth–Emmons olefination approach

In the 1980s, Blackburn and Parratt reported that tetraalkyl fluoromethylenebisphosphonate anions undergo HWE-condensation with aldehydes and ketones producing protected, (α-fluoro)vinylphosphonates [56,57]. Mixtures of geometric isomers are obtained. However, with aldehydes, a pronounced preference for the *E*-isomer is generally seen. Following this initial disclosure, the Sheffield group reported the application of this approach to the synthesis of monofluorophosphonate analogues of several glycolytic intermediates, including glyceraldehyde 3-phosphate [37], 2-phosphoglycerate [36] and 3-phosphoglycerate [58].

As is illustrated in Scheme 3, in order to access individual CHF-stereoisomers in these cases, Blackburn performs the



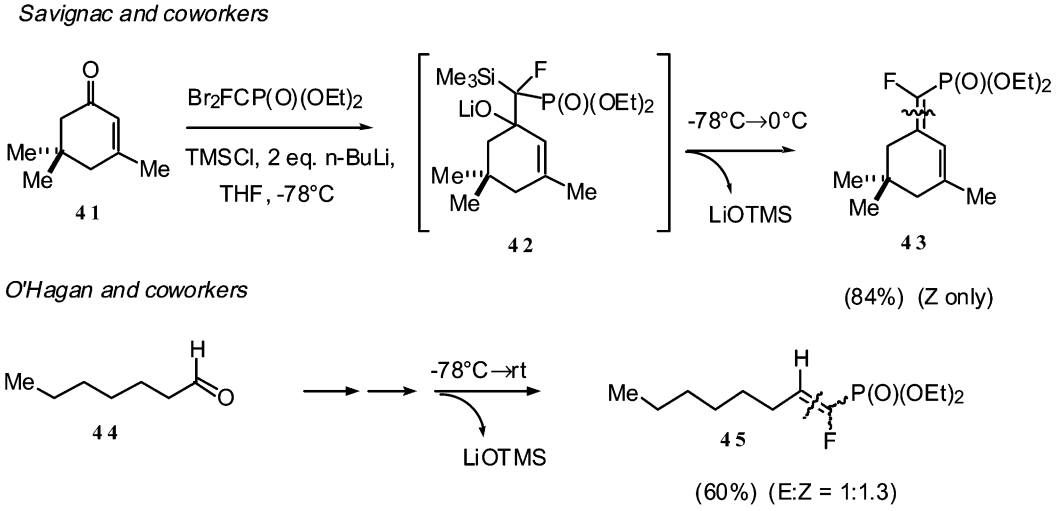
Scheme 3.

initial HWE reaction with carbonyl groups residing on relatively rigid acetonide-protected carbohydrate frameworks. The scaffolding provided by the protected sugar then imposes a significant diastereofacial bias upon a subsequent double bond hydrogenation step. Provided that geometric isomers can be separated at the (α-fluoro)vinylphosphonate stage, this approach allows one to obtain single stereoisomers of the target (α-monofluoroalkyl)phosphonates, with predictable CHF stereochemistry in some cases (i.e. hydrogenation of **31** and **32** presumably occurs from the exposed convex face). Deprotection and periodate-mediated oxidative processing then allows one to extract the desired analogues of C_3-metabolites from these carbohydrate templates of higher carbon count.

It should be noted that hydrogenolysis of the C–F bond can occur in some cases, particularly for (β-aryl-α-fluoro)-vinylphosphonates [57]. This two-step HWE condensation/hydrogenation route was more recently applied by Campbell and Thatcher to the synthesis of the racemic monofluorophosphonate analogue of myo-inositol-1,2-cyclic phosphate [34].

2.4. Peterson olefination approach

In 1996, the groups of Savignac and coworkers [59] and O'Hagan and coworkers [60] independently reported a complementary Peterson olefination entry into (α-fluoro)-vinylphosphonates (Scheme 4). In fact, Blackburn and Parratt had actually first reported one example of a related Peterson approach some time earlier [61]. In a key development leading to the maturation of this chemistry, Savignac and coworkers had developed a procedure for the in situ generation and alkylation of the lithium anion of diethyl (α-trimethylsilyl)fluoromethylphosphonate (vide infra). The condensation of this anion with carbonyl compounds then followed as a logical and important extension of that chemistry. With aldehydes, the Peterson approach generally provides a higher percentage of the *Z*-isomer than the



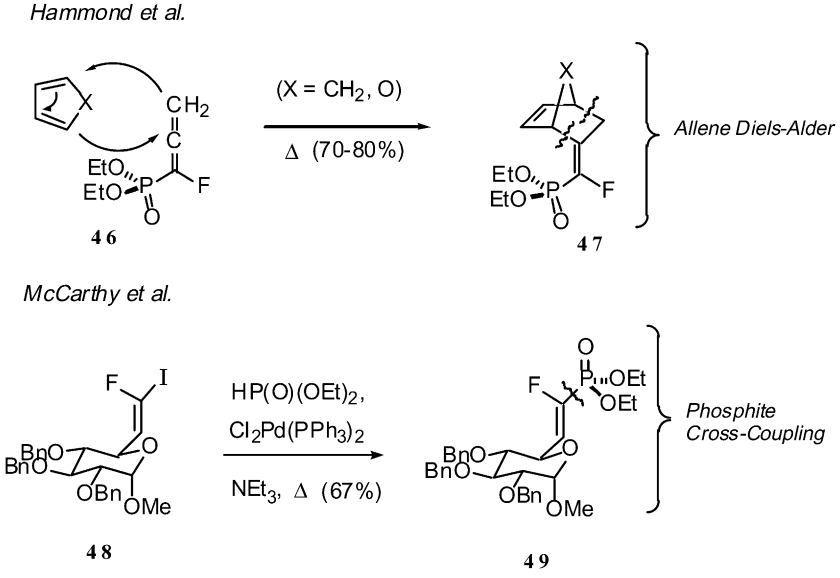
Scheme 4.

HWE approach discussed above. Nonetheless, it generally must be separated from a nearly equimolar quantity of the *E*-isomer. With cyclohexenones (e.g. **41** → **43**) or with α-substituted cyclohexanones, a marked preference for the *Z*-isomer is often seen.

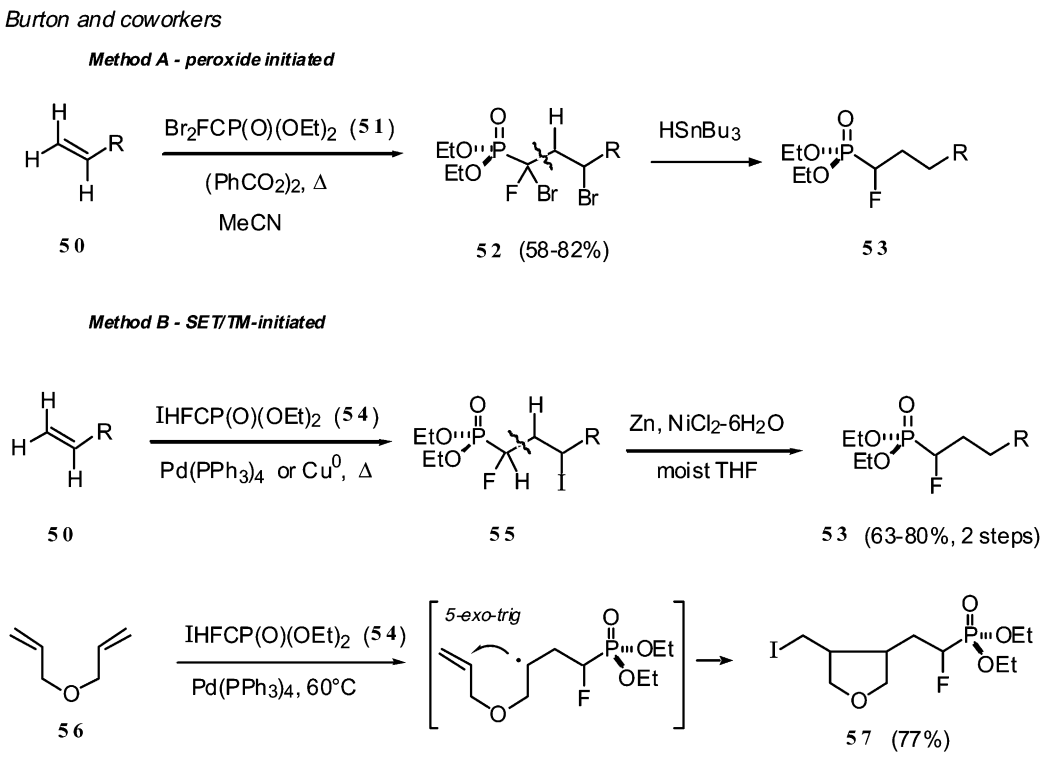
2.5. Other entries into (α-fluoro)vinylphosphonates

Given the possibility of accessing fluoroalkylphosphonates via careful hydrogenation of their fluorovinyl counterparts, a couple of alternative synthetic entries into this latter phosphonate class should be mentioned here. Quite recently, Hammond has successfully synthesized and studied the novel (α-fluoro)allenylphosphonate building block (**46**). In the presence of a range of dienes, including furan, a rather unreactive aromatic diene, **46** undergoes Diels–Alder cycloadditions, generally with good to excellent diastereoselectivity [62].

All of the aforementioned approaches that pass through (fluoro)vinylphosphonates have the advantage of being more convergent than the electrophilic and nucleophilic fluorination approaches initially discussed. While the HWE and Peterson routes involve PC_{α}–$C_{\beta}HF$ disconnection, the Hammond route is even more convergent, disconnecting simultaneously across the β- and γ-carbons (Scheme 5). So, in terms of synthetic efficiency, this chemistry holds great promise in situations where cyclic or bicyclic products are desired.



Scheme 5.



Scheme 6.

On the other hand, McCarthy reported a clever, if decidedly linear, Pd^0-mediated cross-coupling approach to (α-fluoro)vinyl phosphonates (e.g. **48** → **49**). The advantage of this strategy lies in the stereospecificity of the transformation; namely, the transition metal mediated phosphonylation of geminal fluoroiodoalkenes proceeds with retention of configuration [63]. Across a family of glucose-derived α-fluoroiodoalkenes, coupling yields were found to be quite dependent on the sugar protecting groups employed, ranging from modest to very good.

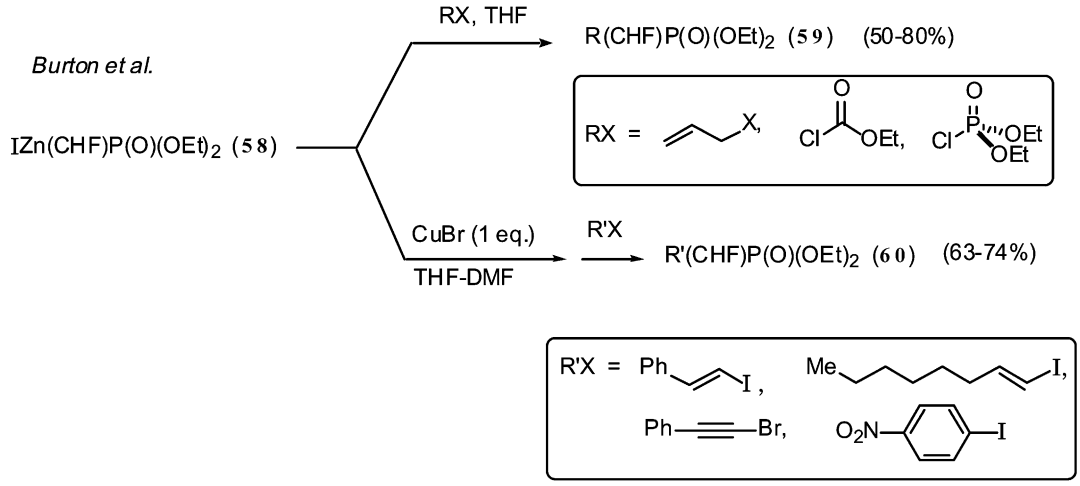
2.6. Radical-mediated P(CFH)–C bond formation

Burton and coworkers recently disclosed a pair of complementary, two step procedures for appending an α-monofluoromethylphosphonate moiety to the terminus of a monosubstituted alkene (Scheme 6). In the first of these, the alkene is heated with diethyl dibromofluoromethylphosphonate (**51**) in the presence of a peroxide initiator [64]. Debromination of the resulting α,γ-dibromo-α-fluoroalkylphosphonates is reported to be achievable through the agency of $HSnBu_3$.

The reluctance of **51** to add to functionalized alkenes prompted the Iowa group to examine the behavior of the more reactive **54** with such substrates. In this case, even alkenes bearing ethereal, silyl or alcoholic functionality in the *R* group give good yields of addition products. Here, a second step is also required to de-iodinate at the γ-position, with $Zn/NiCl_2{\cdot}6H_2O$ serving as reductant. Pleasingly, Burton notes that this two step process can be streamlined into a formal one-flask procedure, with overall yields being quite good. A radical chain mechanism is also postulated for the addition reaction with **54**, wherein the chain is initiated by SET from the zero valent transition metal present (either $Pd(PPh_3)_4$ or Cu^0) to **54**, followed by loss of iodide from the resulting radical anion. Consistent with this mechanism, diallyl ether (**56**), gives an excellent yield of the tetrahydrofuran product (**57**) that one would expect via a facile ring closure of the expected intermediate C_γ-centered radical.

2.7. TM-mediated P(CFH)–C bond formation

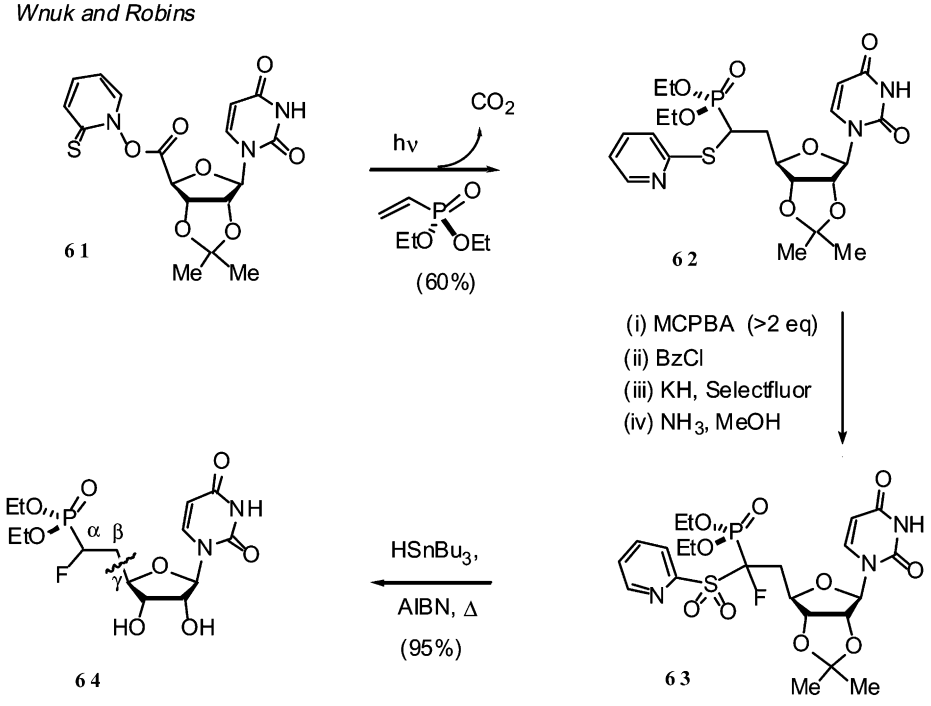
For (α-fluoroalkyl)phosphonate targets bearing β,γ- or γ,δ-unsaturation, the Iowa group has developed an even more direct synthetic approach [65]. Retrosynthetically, this chemistry also invokes a C_α,C_β-disconnection, but only a single synthetic step is required. Thus, the organozinc iodide **58** cross-couples directly with allylic halides and acid chlorides, in the absence of other late transition metals of the sort typically employed for such cross-couplings (i.e. Pd^0, Ni^0, Cu^I, Rh^I). However, if one wishes to couple directly to the sp^2 carbon of a vinyl or aryl halide, addition of one equivalent of a Cu^I salt is highly beneficial. The authors postulate that transmetallation to Cu occurs prior to coupling (Scheme 7).



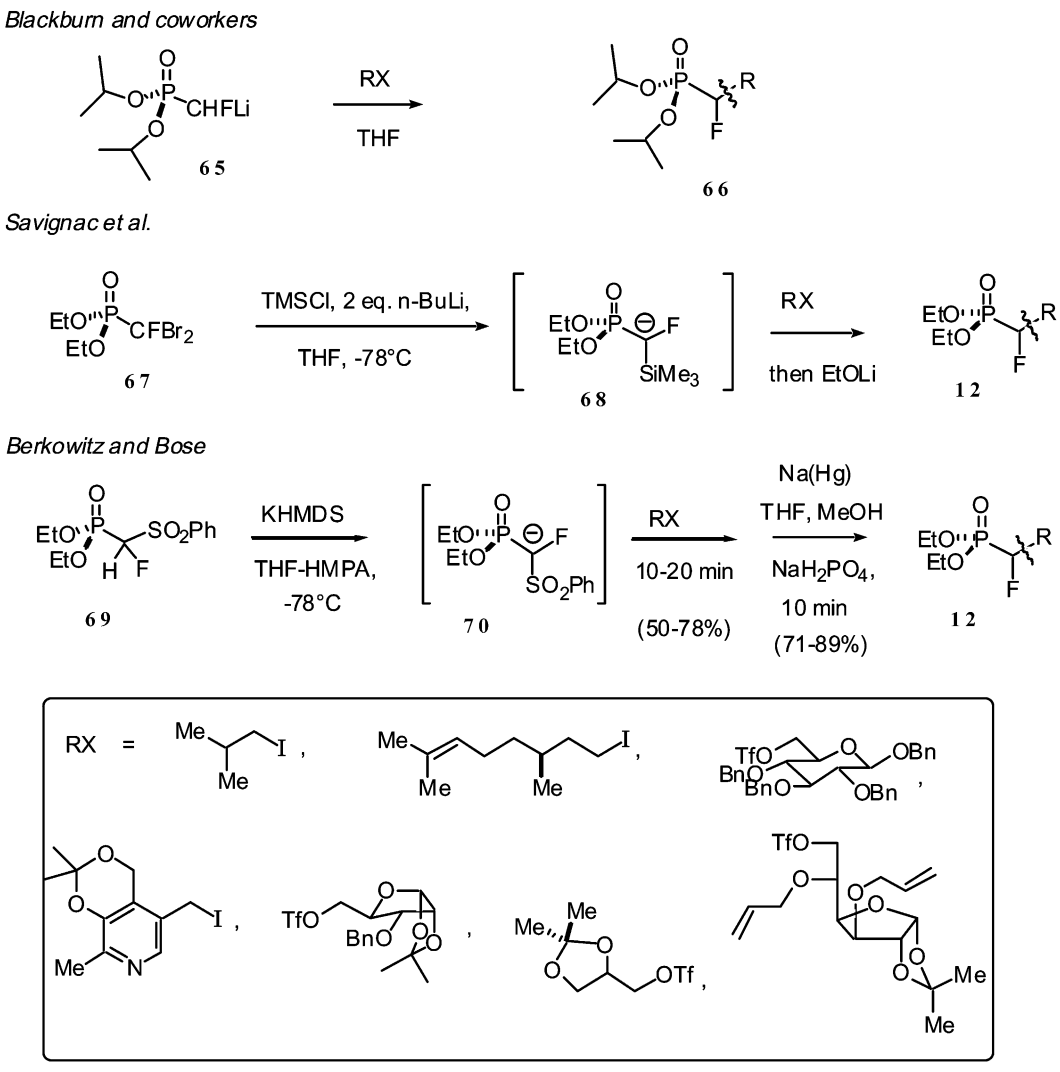
Scheme 7.

2.8. Radical-mediated P(CHSAr)CH_2–C bond formation/oxidation/electrophilic fluorination

A creative approach due to Robins involves installation of a bioisostere for the entire $CH_2OP(O)(OH)_2$ unit [29]. Retrosynthetically, this amounts to formal C_β,C_γ-disconnection. In the forward direction, one initiates this sequence with a Barton photo-induced decarboxylative chain extension (Scheme 8). The α-(2-pyridine)thioether that is obtained may be oxidized to the corresponding sulfone, thereby acidifying the α-proton and setting the stage for an electrophilic fluorination with Selectfluor. Desulfonation is accomplished by an unusual stannodesulfonation. It appears that π-deficient heterocyclic aryl alkyl sulfones are required for this reaction (i.e. the corresponding phenyl sulfone is not reduced with $HSnBu_3$). Model studies in related carbonyl systems suggest that both pyridine and pyrimidine sulfones will give this reaction. The scope of this promising new entry in α-fluorinated phosphonates remains to be explored.



Scheme 8.



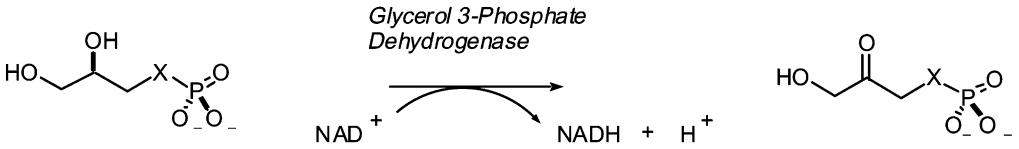
Scheme 9.

2.9. Direct displacement approaches

Among the most convergent approaches to phosphonate analogues of biological phosphates is the direct displacement of primary halides or sulfonates. For α,α-difluorinated phosphonate targets, we have found that triflate displacement with $LiF_2CP(O)(OR)_2$ is particularly advantageous, in terms of reaction time, temperature and yield, providing convenient access to analogues of sugar phosphates and phospho-amino acids [11,22,23,41,66,67].

The first direct displacement approach to (α-monofluoroalkyl)phosphonates of which we are aware is due to Blackburn, and involves the reaction of dialkyl lithio-(α-fluoromethyl)phosphonate (**65**) with alkyl halides [61,68]. Generally, however, as has been discussed recently by Hamilton and Roberts [69], such unstabilized, (α-fluoromethyl)phosphonate anions are difficult to handle, with anion decomposition often competing with the desired displacement reaction. In an elegant solution to this problem (Scheme 9), Savignac and coworkers find that in situ α-silylation leads to a much better behaved carbanion that nonetheless can be alkylated effectively with alkyl halides [70,71]. Careful treatment with lithium ethoxide, following displacement, permits α-desilylation. O'Hagan and coworkers have used this reagent to displace a functionalized primary triflate in an improved, convergent synthesis of the diastereomeric monofluorophosphonate analogues of glycerol 3-phosphate [39,40].

We have recently developed a complementary triflate displacement approach to this phosphonate class that is both convenient and applicable to the synthesis of functionalized members of the family [72]. Our method employs α-sulfonylation for carbanion stabilization. Such α-fluorinated, α-sulfonylated phosphonate anions had been well established as viable HWE reagents for the synthesis of geminal fluoroovinyl sulfones from the work of McCarthy and coworkers [73–75]. However, to our knowledge, there had been no reports of the successful use of such reagents for direct



Substrate	K_m	V_{rel}
71, X = O	0.20 mM	1.0
72, X = CH_2	0.18 mM	0.8
73a,b, X = (R,S)-CHF	0.17 mM	0.9
74, X = CF_2	0.73mM	0.8

Scheme 10.

displacement reactions in which the phosphonate group was retained and subsequently α-desulfonated without P–C cleavage [76].[4]

As is illustrated in Scheme 9, we find that deprotonation of diethyl (α-fluoro-α-phenylsulfonylmethyl)phosphonate with KHMDS at −78°C for a few minutes, followed by addition of a primary triflate and removal of the cold bath, leads to direct displacement in good to very good yields within 10–20 min. Alkylation products then undergo clean reductive desulfonation, again in a matter of minutes at RT, so long as fresh sodium amalgam is used. We find that this chemistry can be easily deployed to access a range of useful α-monofluorinated phosphonate ''bioisosteres'', including isoprenoid, carbohydrate and pyridoxyl systems. Perhaps, most importantly from a strategic point of view, this provides the ''missing link'' in our triflate displacement approach to phosphonate analogue families. Now, from a single triflate, phosphonates bearing 0, 1 and 2 α-fluorines should all be accessible in short order (vide infra) greatly facilitating ''fluorinated phosphonate scans.''

3. Evaluation of (α-monofluoro)alkylphosphonates in enzyme active sites

As has been pointed out by Nieschalk and O'Hagan [40], the enzymatic study of the α-monofluorinated phosphonates has lagged behind that of their non-fluorinated and α,α-difluorinated congeners. This is all the more surprising when one considers that, in general, pK_{a2} of the monofluoro class is most closely matched with pK_{a2} of the phosphates themselves (Fig. 1). Furthermore, at least one computational study has suggested that the electrostatic potential of this phosphonate class resembles that of the phosphate esters themselves [14]. Finally, as Blackburn noted over a decade ago [36], because of the asymmetry in this phosphonate class, there is the possibility that the placement of a single fluorine α to a phosphoryl group may functionally translate into a vectorial operation rather than simply a scalar one. This adds an interesting and potentially ''tunable'' dimension to the monofluoro class. Only recently have investigators begun to experimentally consider the directionality of α-fluorination, in addition to the degree of α-fluorination when comparing this class of phosphate mimics to others. These studies will be highlighted here.

Among the more interesting early results to be tallied was the finding by Thatcher that the monofluorophosphonate analogue (**8**) of myo-inositol-1,2-cyclic phosphate is an excellent inhibitor of PI-specific phospholipase C [34]. The stereochemical dependence of the inhibition remains an open question as racemic **8** was employed in those studies.

3.1. Glycerol 3-phosphate dehydrogenase

The first experimental evidence of which we are aware that CHF stereochemistry can influence enzyme binding and catalysis arose from O'Hagan and coworkers' investigation of a set of phosphonate analogues of glycerol 3-phosphate in the active site of G3P dehydrogenase [39,40]. This was also one of the first cases in which a complete fluorinated phosphonate scan was carried out. Namely, the O'Hagan group examined phosphonate analogues of the natural substrate across the entire range of α-fluorination and considered the α-stereochemistry for the α-monofluorinated class.

Interestingly, all four phosphonates, **72**, **73a** and **b** and **74**, are turned over by the mammalian G3PDH enzyme (Scheme 10). Moreover, under standard assay conditions, the turnover rates for all of these analogues are within 80% of the value observed for the natural substrate itself. In this active site, it appears that α-monofluorination is optimal. The differences in both K_m and V_{max} between the non-fluorinated and α-monofluorinated phosphonates are slight, but both favor the latter. And considering that the latter

[4] We are aware of only an isolated report in which McCarthy's reagent was used for nucleophilic displacement reactions. Note that these authors used only simple alkyl halides and reported being unable to reductively cleave the sulfonyl group without P–C cleavage.

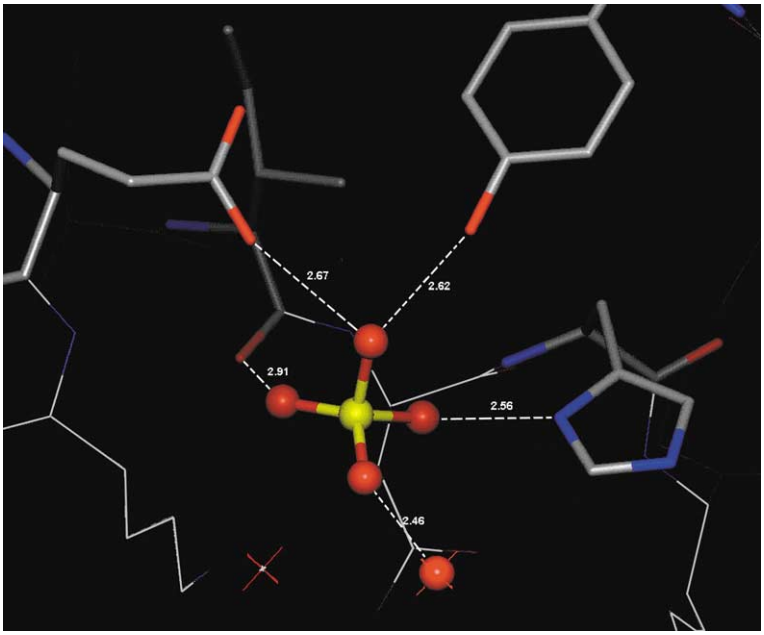


Fig. 5. The active site of glucose-phosphate dehydrogenase [78,79].

numbers are derived from a diastereomeric mixture of **73a** and **b**, the actual values for the preferred diastereomer are expected to be better than these. On the other hand, α,α-difluorination is clearly deleterious, leading to a 4–5-fold increase in K_m.

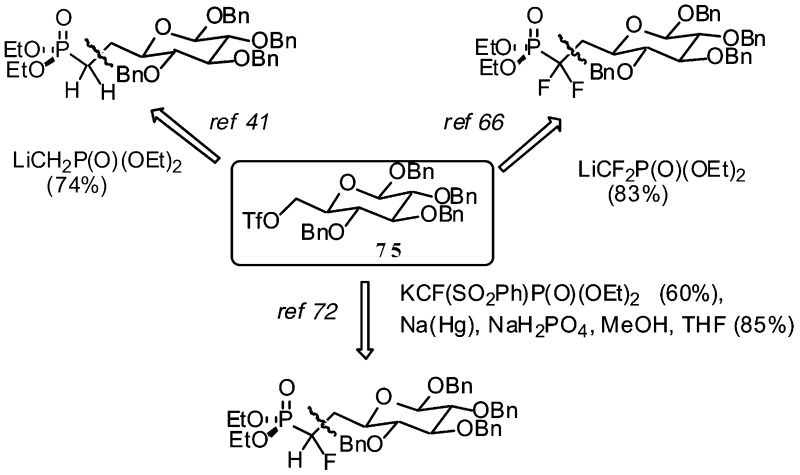
In further investigating the monofluoro analogues, O'Hagan devised a clever experiment to probe for differences in the kinetics of enzymatic processing of diastereomers **73a** and **b**. By employing proton-decoupled ^{19}F NMR, the signals for these diastereomers could be resolved. Upon incubation with rabbit muscle G3PDH, NMR revealed that the mixture became enriched in one diastereomer by 25% during the course of 1 h of reaction. A more complete understanding of the magnitude of this stereochemical preference must await the determination of kinetic constants for individual diastereomers and their stereochemical assignment. To begin to examine the origins of this preference, better structural characterization of this enzyme active site will be needed. Toward this latter end, the first X-ray crystal structure of a glycerol 3-phosphate dehydrogenase was recently solved, albeit for the trypanosomal enzyme [77].

3.2. Glucose 6-phosphate dehydrogenase

In order to address both the question of optimal degree and of optimal directionality of α-fluorination, we chose to perform a complete fluorinated phosphonate scan on the well studied enzymatic phosphate binding pocket in G6PDH. The crystal structure of the *Leuconostoc mesenteroides* G6PDH has been solved by Adams and coworkers and contains a molecule of inorganic phosphate occupying the binding pocket for the glucose 6-phosphate substrate [78,79]. As can be seen in Fig. 5, the Adams structure reveals specific phosphate contacts including 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.

All four phosphonates (bridging CH_2, (*R*)-CHF, (*S*)-CHF, and CF_2) can now be obtained from a common precursor, glucopyranose triflate **75** (Scheme 11) using triflate displacement chemistry developed in our laboratory [11,41,66,67,72]. As regards the (α-monofluoroalkyl)phosphonates, this chemistry [72] represents a significant improvement over the linear route employed in our original synthesis [41]. Note that, in this case, the individual (*R*)-CHF- and (*S*)-CHF-diastereomers can easily be separated by SiO_2 chromatography. These relative stereochemical assignments rest on the X-ray crystal structure of the (*S*)-diastereomer (**29**). This divergent, streamlined approach should be generally applicable whenever a complete set of (α-fluorinated) phosphonates is desired and whenever a primary triflate precursor can be employed.

All four phosphonate analogues of G6P are accepted as substrates for G6PDH from *L. mesenteroides*, being oxidized at C-1 with concomitant reduction of $NADP^+$ (Fig. 6). A complete Michaelis–Menten analysis was carried out. The data are analyzed here (Fig. 7) by directly least squares fitting the measured v_0 versus [*S*] values to a hyperbola (Michaelis–Menten equation). The kinetic constants that fall out of this analysis have been compared with the values obtained from the previous linear least squares fit of the double reciprocal $1/v_0$ versus 1/[*S*] data (Lineweaver–Burk



Scheme 11.

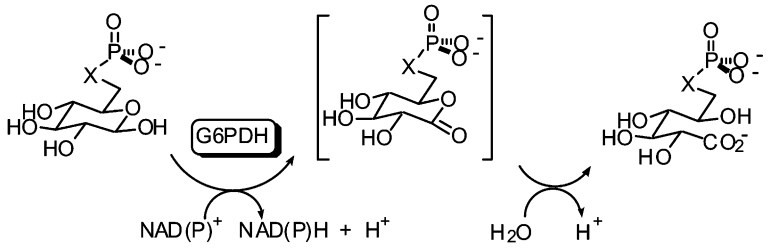


Fig. 6. Phosphonate pseudo-substrates for G6PDH.

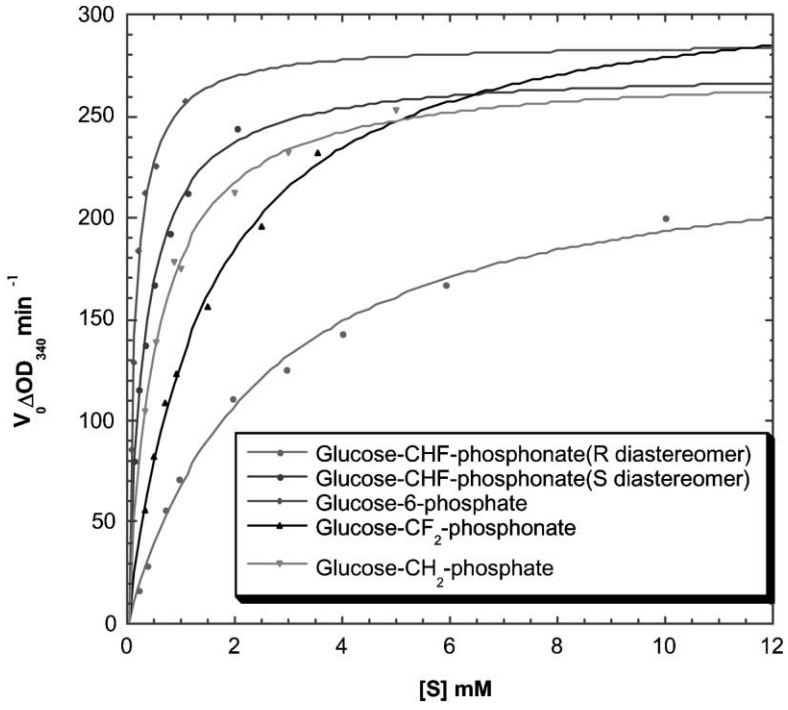


Fig. 7. Hyperbolic fit of the v_0 vs. [S] data.

Table 1
Fluorinated phosphonate scan of the G6PDH active site: *kinetic constants*

Substrate	Linear Lineweaver-Burk Fit				Hyperbolic Fit			
	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}s^{-1}$)		K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}s^{-1}$)	
Glucose-6-phosphate	0.12 ± 0.01	241 ± 14	2010 ± 170	Factor of 2.2	0.12 ± 0.01	240 ± 10	2000 ± 216	Factor of 2.3
Glucose-CH_2-phosphonate	0.49 ± 0.04	224 ± 14	457 ± 27		0.52 ± 0.03	229 ± 10	440 ± 15	
Glucose-CHF-phosphonate {(7*S*)-diastereomer}	0.23 ± 0.02	212 ± 6	922 ± 80	Factor of 10.7	0.27 ± 0.03	231 ± 7	856 ± 77	Factor of 10.7
Glucose-CHF-phosphonate {(7*R*)-diastereomer}	2.26 ± 0.26	195 ±14	86 ± 1		2.59 ± 0 .11	208 ± 3	80 ± 2	
Glucose-CF_2-phosphonate	1.35 ± 0.06	248 ± 16	184 ± 12		1.40 ± 0.05	254 ±15	181 ± 6	

plot) in Table 1. As can be seen, similar values are obtained for K_m and k_{cat} from these two treatments of the data.

On the one hand, in terms of k_{cat}, we observe only very small differences in turnover efficiency across the entire range of phosphate mimics studied. That is, the highest values of k_{cat} (CF_2-analogue and G6P) are only 1.3 times greater than the lowest k_{cat} value (7*R*-CHF analogue). Interestingly, on the other hand, there are significant differences in K_m, with members of the (α-monofluoroalkyl)-phosphonate family posting both the lowest (7*S*-CHF, 0.23–0.27 mM) and the highest K_m values (7*R*-CHF, 2.3–2.6 mM). In other words, these results show that the affinity of phosphate mimics of the α-monofluorinated phosphonate class for target enzymatic active sites may vary by an order of magnitude, depending on the α-stereochemistry. In addition to lending support to the Blackburnian notion that α-stereochemistry may provide an additional ''tunable'' variable in this phosphonate class [36], these results underscore the importance of examining single diastereomers when deriving enzyme kinetic parameters for phosphate mimics of this variety.

The second pK_a values for G6P and for each type of phosphonate analogue were determined by titration. Fig. 8 illustrates the significant effect of α-fluorination upon phosphonate acidity, with two α-fluorine atoms lowering the second pK_a by over two-orders of magnitude. On the other hand, as can be seen from Fig. 9, α-monofluorination produces a phosphonate functionality that is nearly ''isoionic'' (p$K_{a2} = 6.2$) with the actual phosphate ester (p$K_{a2} = 6.6$). Of course, all of the phosphonates are predominantly in their dianionic forms at the pH of the assay (7.8). 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.

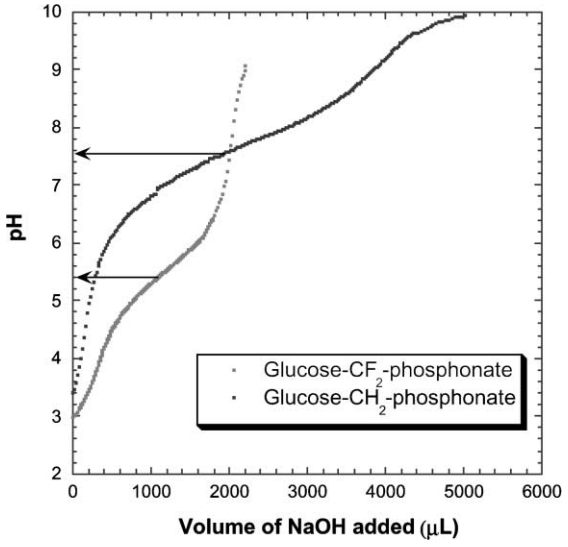


Fig. 8. Titration curves for the non-fluorinated and α,α-difluorinated G6P analogues.

With an eye toward stimulating discussion on possible origins of the striking effect of CHF stereochemistry upon enzyme binding, a manual docking experiment was carried out. The crystallographic coordinates of our best G6P mimic (**29** with the benzyl ether and ethyl ester protecting groups truncated) were placed in the active site of G6PDH, as revealed from Adams' original crystal structure (PDB: 1DPG, subunit A) [78,79].[5] The phosphorus atom and all

[5] This original *L. mesenteroides* G6PDH crystal structure contains inorganic phosphate bound to the sugar phosphate site, but no nicotinamide cofactor [78]. A more recent structure of the same enzyme does include a bound $NADP^+$ [79]. However, in that structure, the nicotinamide ring of the cofactor is apparently so significantly disordered that its coordinates could not be defined.

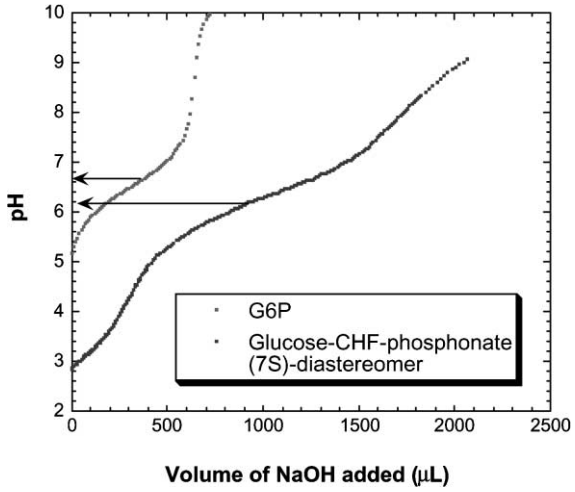


Fig. 9. Titration curves for G6P and its (7*S*)-monofluorophosphonate analogue.

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 Fig. 10) from the ε-nitrogen of His^{240}, the presumed catalytic base [79]. 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. In order to accommodate the substrate, two waters of crystallization (H_2O-1079 and H_2O-1244) were deleted. Also, the second (non-conserved) 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} (backbone carbonyl) and Glu^{147} (CO_2H). In this hypothetical view of the (7*S*)-monofluorinated G6P analogue bound at the G6PDH active site, the C–F bond may be engaged in a favorable ion-dipole interaction with the ε-ammonium nitrogen of Lys^{148} (N–F distance = 3.4 Å, as modeled). This is attractive in light of recent model studies with fluorinated piperidines in which a conformational preference for opposing axially-disposed fluorine substituents is ascribed to such ion-dipole interactions [80]. However, it is important to note here that Lys^{148} is apparently quite mobile.[6] 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.

3.3. Protein phosphotyrosine phosphatase 1B (PTP1B)

As discussed in the introduction, one of the most spectacular examples of the effect of α-fluorination upon phosphonate binding comes from the laboratory of Burke. Hexapeptide **3** containing the (α,α-difluoromethylene)phosphonate analogue of phosphotyrosine binds to PTP1B three-orders of magnitude more tightly than the otherwise identical hexapeptide that lacks α-fluorination [20]. Burke and coworkers later discovered that much simpler, α,α-difluorinated naphthylphosphonates, such as **76** [42] and **77** [43], also bind well to this active site, though K_i's here are in the mid-micromolar range (more than two-orders of magnitude higher than the K_i for **3**) (Fig. 11).

The X-ray crystal structures for each of these naphthylphosphonates bound to PTP1B have been solved by Barford and Burke and the two structures are nearly identical (0.22 rmsd). Fig. 12 displays the PTP1B-**77** complex from a viewing angle that clearly highlights the phosphate binding pocket in this enzyme. As can be seen, two electrostatic "salt bridges" are apparently formed between Arg^{221} and the phosphonate. In addition, there is an interesting array of hydrogen bonds donated by six consecutive backbone amide nitrogens from Ser^{216} through Arg^{221}.

This well-defined phosphate binding pocket is clearly quite distinct from the pocket seen in G6PDH (see Fig. 5). Recall that, in that case, a less basic *histidine* residue (rather than Arg) is engaged in the key salt bridge and the surrounding amino acid *side chains* (rather than backbone amides) donate most of the other key hydrogen bonds to the phosph(on)ate. Why then do α-difluorinated phosphonates bind especially well to the PTP1B binding pocket? For inhibitors **76** and **77**, the authors propose an unconventional F···H···N hydrogen bond between the pro-*R* fluorine (F_{si}) [81,82][7] of the inhibitor and the amide NH of Phe-182. This is illu-

[6] 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*-Lys148-Pro149 amide bond (subunit A) to a *cis*-amide bond (subunit B).

[7] For a description of the "pro-*R*/*S* system", see [81]. In this system, the assignment of prochirality designator is made by selecting one of the heterotopic ligands, L, and arbitrarily assigning it a higher priority than the other, without disturbing the priorities of the other ligands. However, in cases where these heterotopic ligands are not hydrogen, one may instead be tempted to replace one L with a hydrogen, and this can lead to the "wrong" prochirality designation. In the original report by Burke et al. describing the PTP1B-**76** crystal structure [42], the authors correctly state several times that the pro-*R* fluorine appears to be accepting a hydrogen bond from Phe-182. This fluorine is, however, mislabeled as pro-*S* once (apparently a typo) in the introduction of the 1998 follow-up paper by Barford and coworkers [43]. In any case, the X-ray crystal structures of the PTP1B-76 complex illustrated in the 1996 paper and of the PTP-77 complex (PDB = INH) reported in the 1998 paper make clear which prochiral fluorine atom is involved in this putative hydrogen bond. Nonetheless, apparent ambiguities (see [81]) in the application of this "pro-*R*/*S* system" to α,α-difluorinated phosphonates may have partially contributed to some confusion on this latter point (see [45]). Therefore, we strongly advocate the use of the F_{re}/F_{si} descriptor system [82,83] in lieu of the pro-*R*/*S* system when dealing with (α,α-difluoroalkyl)phosphonates. The re and si subscript system used here is due largely to Prelog and is arguably less ambiguous than the "pro-*R*/*S*" system. In this system, one removes one heterotopic ligand, L, from the tetrahedral prochiral center, leaving behind a trigonal face, that is either re or si. The removed ligand is labeled L_{re} or L_{si}, accordingly. This system does not require one to arbitrarily change the priority of any ligand. For discussions of the re and si subscript system, see [82,83].

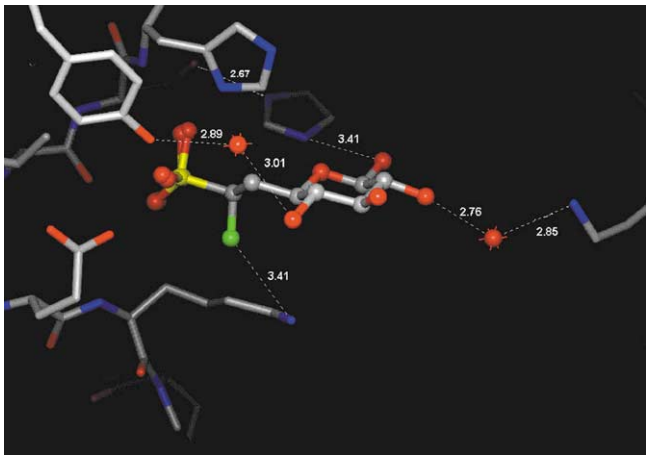


Fig. 10. A manually docked image of the (7S)-CHF-phosphonate analogue of G6P in the G6PDH active site (PDB code: 1DPG).

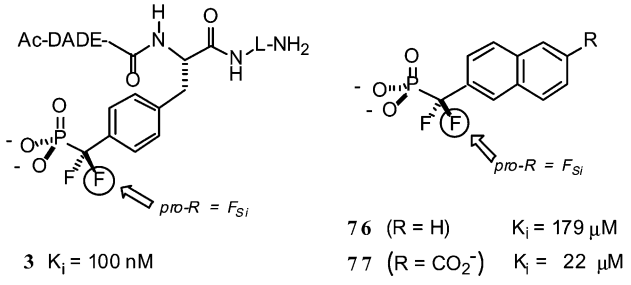


Fig. 11. Fluorinated phosphonate inhibitors of PTP1B.

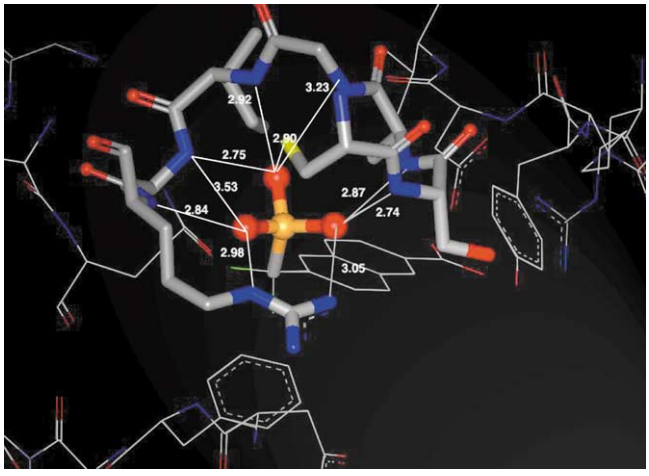


Fig. 12. X-ray crystal structure of 77 bound to PTP1B (PDB: INH; phosphonate highlighted) [43].

strated in Fig. 13 (N–F distance = 3.41 Å, N–H–F angle = 139°). Using molecular dynamics simulations, Burke et al. propose that (at least for the PTP1B-**76** structure) this hydrogen bond contributes up to −4.6 kcal/mol to the *E–I* interaction energy [42]. However, the authors also suggest that the CF_2-phosphonate may more effectively displace water molecules from the PTP1B active site than

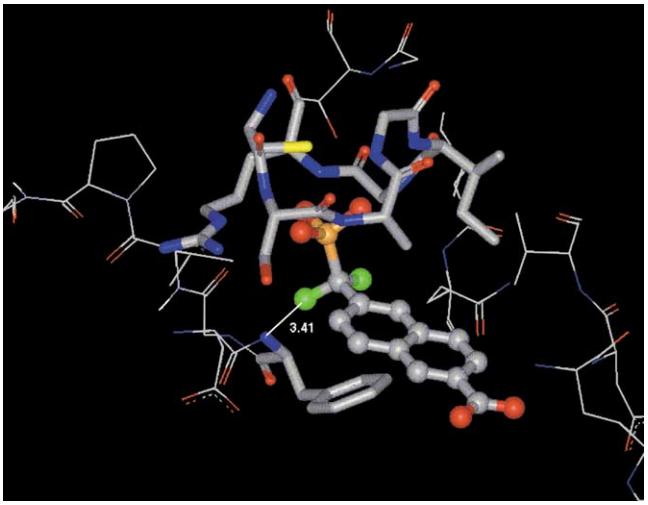


Fig. 13. Putative F···H···N hydrogen bond in the PTP1B-77 structure [43].

its nonfluorinated counterpart, resulting in a more favorable entropy of binding for the former.

On the other hand, to our knowledge, an X-ray crystal structure of the exceptionally high affinity complex, PTP1B-**3**, has not yet been determined. However, the structure of a $Cys^{215} \rightarrow Ser^{215}$ mutant with the actual DADE(pY)L peptide bound is available. Using this structure as a computational guide, Glover and Tracey performed transfer nOe experiments and restrained molecular dynamics simulations to estimate the conformation of **3** when bound to the enzyme [44]. In the end, as Burke and coworkers, Glover and Tracey postulate an important hydrogen bond between F_{si} of the inhibitor and the backbone NH of Phe-182. Indeed, the NMR structure determination points to an even shorter hydrogen bond here (∼2.0 Å F–H bond distance for PTP1B-**3**) than before (∼2.5 Å F–H bond distance for PTP1B-**77**), consistent with the high affinity of **3** for the enzyme. In light of the suggestion by both the Burke group and the Tracey group that (i) fluorine hydrogen-bonding is important in the binding of these (α,α-difluoroalkyl)phosphonates to PTP1B and that (ii) only the F_{si} atom is actually engaged in this hydrogen bond, it will be interesting to see if there is an enantiomeric preference in the corresponding (α-monofluoroalkyl)phosphonates.[3]

4. On the possibility for $^{2-}O_3P$–C–F···H–X hydrogen bonding in enzyme active sites

While recent computational studies, using both ab initio [18] and DFT [84] methods, support the notion that carbon-bound fluorine can act as a hydrogen bond acceptor, this remains an area of considerable controversy. Plausible examples of such hydrogen bonds exist, but are rare in the current crystallographic record, as has been discussed by several authors who have recently surveyed both the

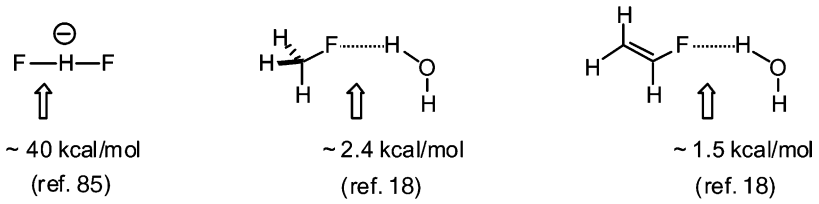


Fig. 14. Fluorine hydrogen bonding.

Cambridge Structural Database (CSD [16–18]) and the Protein Databank (PDB [16]). In contrast to anionic fluoride, which is known to be an outstanding hydrogen bond donor, as exemplified by bifluoride anion [85], O'Hagan and co-workers [18] estimate that an idealized carbon-bound fluorine hydrogen bond to water ranges from 1.4 to 2.5 kcal/mol depending upon whether the fluorine is olefinic or aliphatic (Fig. 14). The olefinic fluorine is presumed to be a poorer hydrogen bond acceptor due to delocalization of the fluorine non-bonding electrons into the π^* orbital of the adjacent alkene.

In addition to the X-ray crystal structures of enzyme bound α-fluorinated phosphonates discussed above, there are examples of perhaps related enzyme bound α-fluorinated ketones. Included among these are at least two complexes involving serine proteases in which a reasonable possibility exists for fluorine hydrogen bonding. In the first case, a β-keto-α,α-difluoroamide is tightly bound to elastase (Fig. 15). Histidine[57] appears to be engaged in a hydrogen bond with the pro-*S* fluorine (F_{si}) of the inhibitor (N–F distance = 2.75 Å, N–H–F angle = 130°).

In the second example, due to Abeles, an α,α,α-trifluorinated ketone is bound to chymotrypsin (Fig. 16). In this complex, one α-fluorine appears to be involved in either a favorable ion-dipole interaction or a hydrogen bond with His[57] (N–F distance = 3.33 Å, N–H–F angle = 125°). At the same time, another of the α-fluorines apparently accepts a hydrogen bond from the backbone NH of Gly[193] (N–F distance = 3.30 Å, N–H–F angle = 148°).

In each of these complexes, the active site serine alkoxide anion has added into the electrophilic α-fluorinated carbonyl group of the inhibitor. This produces covalent protease-inhibitor adducts in which the fluorine atom apparently acting as hydrogen bond acceptor is three bonds removed from a negatively charged oxygen atom. Dunitz has noted this pattern and suggested that systems bearing a negative charge in the vicinity of the C–F bond are more likely to serve as H-bond acceptors at fluorine [16]. We wish to point out here that the aforementioned PTP1B complexes with bound (α,α-difluoroalkyl)phosphonates (**3**, **76** or **77**) all possess this same substructural pattern. In fact, if bound in their dianionic forms, these phosphonates bear two negatively charged oxygen atoms three bonds removed from the fluorine atom thought to be acting as a hydrogen bond acceptor.

Note that in contrast to the situation with α-fluorinated ketones and α,α-difluorinated phosphonates, to our knowledge, one simply does not have any structural data yet that speak to the issue of (α-monofluoroalkyl)phosphonates forming hydrogen bonds in enzyme active sites. Such studies would clearly be of value, as the study of this class of phosphonates expands, and as closer attention is paid to stereochemistry.

However, given the exceptionally tight binding exhibited by **3** to PTP1B as compared to its nonfluorinated congener (factor of 2000 in K_i), and given the unusually high interaction energy calculated by Burke et al. for the fluorine hydrogen bond in the PTP1B-**76** complex (−4.6 kcal/mol), one is tempted to speculate that, in that the right macromolecular context, α,α-difluorinated phosphonates may be particularly good hydrogen bond acceptors, at least as carbon-bound fluorine goes. Clearly many more data points are required before one can truly make such a conjecture. Nonetheless, to stimulate discussion here, we suggest that, in some such phosphonates, a geminal fluorine anomeric effect may facilitate fluorine hydrogen bonding. This is illustrated in Fig. 17 with both the valence bond $n \rightarrow \sigma^*$ and double bond-no bond resonance pictures of such an anomeric effect.

For simple systems in the gas phase, the geminal difluoro anomeric effect is well established. Thus, for H_2CF_2, ab initio calculations from both the groups of Wolfe et al. [88] and Schleyer et al. [89] place this orbital stabilization energy in the range of 10–14 kcal/mol, in good agreement with the experimental "methyl stabilization energy" (12 ± 4 kcal/mol). Both computational and experimental evidence supports related F–C–O and F–C–N anomeric effects, though these values are lower, with calculated gas phase values generally in the 4–7 kcal/mol range, depending on the system [90]. If such an anomeric effect were operative, one would expect bond-shortening for the C–F bond involving the donating fluorine atom. One can probably put an upper limit on the magnitude of this bond-shortening at about 0.05 Å. This value is derived from the observed C–F bond-shortening in α-fluorine-stabilized carbocations as recently disclosed in an elegant low-temperature X-ray crystallographic study by Christe et al [91].

But could such an anomeric effect facilitate fluorine hydrogen bonding, if it exists in an (α,α-difluoromethylene)phosphonate, in an active site? This notion is intuitively attractive as the simple double bond–no bond resonance picture provides a mechanism by which one fluorine atom of the α,α-difluoromethylene unit would take on more fluoride character. And from the previous discussion, it stands to reason that as one traverses the spectrum from neutral,

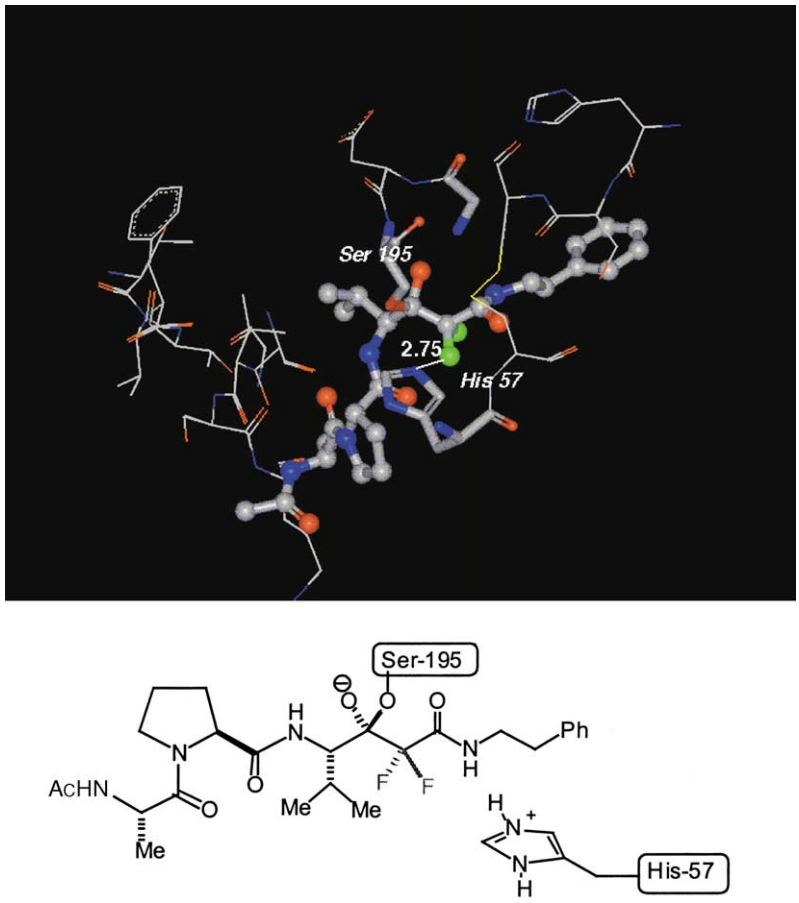


Fig. 15. A β-Keto-α,α-difluoroalkylamide inhibitor bound to elastase [86].

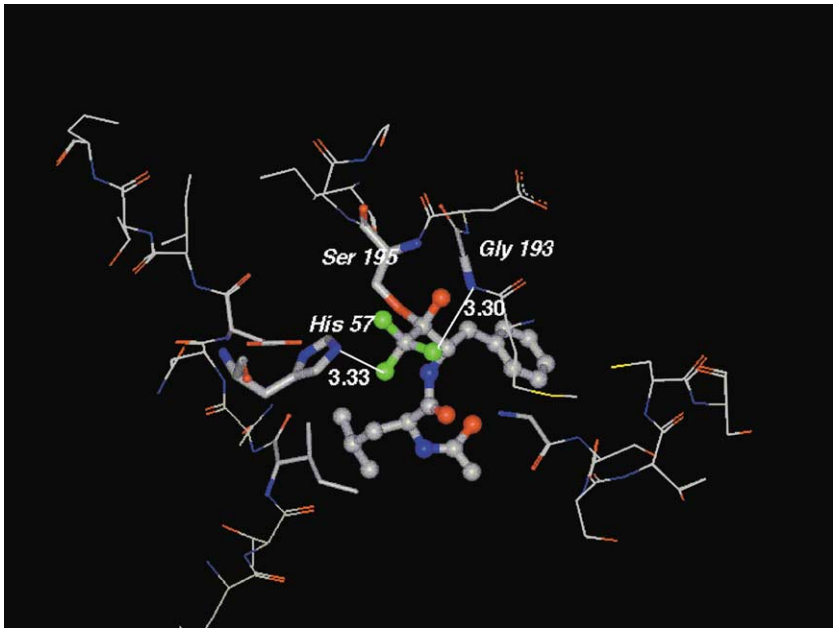


Fig. 16. An α,α,α,-trifluoromethyl ketone chymotrypsin inhibitor [87].

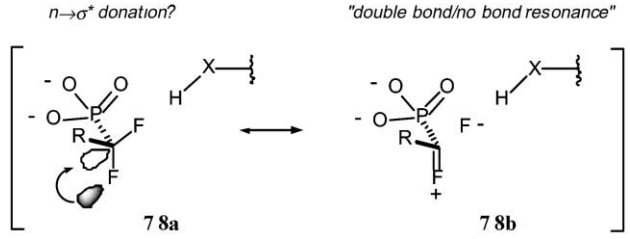


Fig. 17. Enhancing fluorine's hydrogen bond acceptor ability through a geminal fluorine anomeric effect?

carbon-bound fluorine to fluoride itself, hydrogen-bond acceptor ability should increase [18]. Moreover, one can find experimental evidence for this phenomenon, for example, in the crystal structure of the protonated aminal **79** (Fig. 18). Here, the ''all-nitrogen'' anomeric effect leads to appreciable C–N bond-shortening/lengthening in the direction expected to facilitate hydrogen bonding [92]. So, while we are aware of no evidence, at this point, for such bond length asymmetry in α,α-difluoromethylene units potentially involved in hydrogen bonds, we think that this is an idea worth considering. The difficulty in evaluating this proposition surely lies in the typical limits of precision of an enzyme-ligand co-crystal structure (typically in the range of 0.1–0.2 Å at best) which would not permit bond-shortening/lengthening of $\leq$0.05 Å to be seen. It may, however, be possible to investigate this proposition in model host–guest complexes.

In summary, when seeking to tailor a hydrolytically stable phosphate mimic for a target active site, we would advocate using a complete (fluorinated) phosphonate scan, in which a range of phosphonates (bearing from 0 to 2 α-fluorines) is evaluated. Where primary triflates can be used, the divergent triflate displacement approach permits particularly convenient access to all four such phosphonates. In this article, the potential advantage of ''iso-acidity'' and ''tunability'' in the α-monofluorinated phosphonates has been emphasized. More comparative studies on individual (*R*)- and (*S*)-stereoisomers in this family, are clearly warranted to establish just how ''tunable'' these are. In terms of enzyme binding, the CHF stereochemistry may influence such factors as unfavorable steric effects, favorable van der Waals interactions, dipole–dipole interactions, ion-dipole interactions and finally, fluorine hydrogen-bonding. This latter phenomenon remains controversial and difficult to evaluate. For example, entropic effects, such as differential displacement of bound water, may underlie differences in binding affinity for two ligands. Just the same, at least for several α,α-*difluorinated* phosphonates in the pre-organized PTP1B active site, the crystallographic data may be consistent with one such C–F_{si}···HN(Phe-182) hydrogen bond. We have further speculated that fluorine hydrogen bonds in such phosphate mimics, if borne out by future tests, may benefit from a fluorine anomeric effect that endows the accepting fluorine atom with greater fluoride-like character. Where hydrogen bonding seems to be operative (e.g. from X-ray data), a comparison of active site binding affinities of both the ''matched'' and ''mis-matched'' stereoisomers in the α-monofluoro phosphonate series with the α,α-difluorinated phosphonate should provide a qualitative test for this (presumably directionalized) fluorine hydrogen bonding. At the same time, it should provide at least circumstantial evidence for or against this geminal difluoride-enhanced hydrogen bonding model.

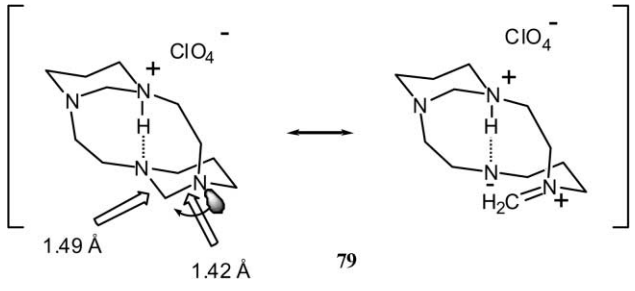


Fig. 18. C–N bond lengths from the crystal structure of protonated aminal 79.

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