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α,α-Difluoro-β-ketophosphonates as potent inhibitors of protein tyrosine phosphatase 1B

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Abstract—A novel series of inhibitors that contain an aryl α,α-difluoro-β-ketophosphonate group has been synthesized and evaluated against protein tyrosine phosphatase 1B. These compounds exhibit strong inhibitory activity, the best of which has a K_i value of 0.17 μM. These results demonstrate that aryl α,α-difluoro-β-ketophosphonates are powerful phosphotyrosine mimetics for the development of potent PTP inhibitors.

1. Introduction

Protein tyrosine phosphatases (PTPs) are signal transduction enzymes, which catalyze the hydrolysis of phosphotyrosine residues (*p*Tyr). These enzymes play a critical role in regulating cell proliferation, differentiation and metabolism. It has been shown that PTPs are implicated in a number of human diseases such as diabetes, cancer, inflammation, and immune dysfunctions.[1] In particular, PTP1B is a negative regulator of insulin signaling and acts by dephosphorylation of the insulin receptor.[2] Recent knock-out studies in mice have demonstrated that PTP1B is an attractive therapeutic target for the treatment of Type II diabetes and obesity.[3] Therefore, it would be highly desirable to develop PTP inhibitors as therapeutic agents as well as valuable probes for studying cellular signal transduction.

A common strategy in the development of PTP inhibitors has been the incorporation of bioisosteric replacements of the labile *p*Tyr in appropriate peptide substrates or small molecule scaffolds.[4] These nonhydrolyzable *p*Tyr mimetics serve as warheads to direct the molecule to the catalytic phosphate-binding site of phosphatases. A wide variety of *p*Tyr mimetics have been reported including α,α-difluoromethylphosphonates,[5a–c] sulfotyrosines,[5d] fluoro-*O*-malonyltyrosines,[5e] cinnamic acids,[5f] oxa-acetic acids,[5g] *O*-carboxymethyl salicylic acids,[5h] 2-(oxalylamino)-benzoic acids,[5i] and diaryloxamic acids.[5j] However, many of these *p*Tyr mimetics are not quite effective or have very limited cell permeability. Therefore, the discovery of new *p*Tyr mimetics is needed and will greatly facilitate the development of potent and selective PTP inhibitors in the field.

As part of our efforts to identify novel PTP inhibitors, we have synthesized and evaluated aryl α,α-difluoro-β-ketophosphonates as a new type of *p*Tyr mimetics, and explored their use in the development of small molecule inhibitors for PTP1B. To the best of our knowledge, inhibitory activity of this class against phosphatases has not been reported in the literature. In an early study Kluger has shown that acetonylphosphonate, a nonfluorinated β-ketophosphonate, is a competitive inhibitor of acetoacetate decarboxylase.[6] Recently, other compounds containing β-ketophosphonate moiety have been identified as inhibitors of serine protease cathepsin G[7] and glyceraldehyde-3-phosphate dehydrogenase.[8] On the other hand, it has been shown that fluorinated *p*Tyr mimetics like α,α-difluoromethylphosphonates[5a–c] and fluoro-*O*-malonyltyrosines[5e] are much more potent PTP inhibitors than the corresponding nonfluorinated analogues due to direct interactions of the fluorines with the enzyme active site residues. Thus, aryl α,α-difluoro-β-ketophosphonates (Fig. 1) are designed to stimulate a variety of specific interactions with residues in the active site of the phosphatase. While the difluoromethylphosphonate moiety targets the phosphate-binding

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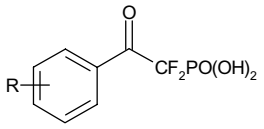


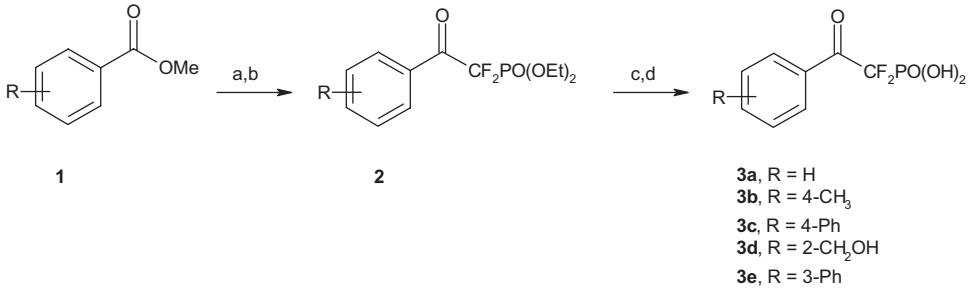
Figure 1. Aryl α,α-difluoro-β-ketophosphonates.

site through electrostatic interactions, the carbonyl group adjacent to the aromatic would be able to form additional hydrogen bonds with residues within the *p*Tyr binding pocket. Our approach to identify PTP inhibitors was to begin with simple aryl α,α-difluoro-β-ketophosphonates and then build up a series of analogues based on small molecule scaffolds.

2. Results and discussion

Our first goal was to make simple aryl α,α-difluoro-β-ketophosphonates and evaluate their biological activity. Scheme 1 shows the synthesis of target compounds **3a**–**e**. Although there are several reports on the preparation of α,α-difluoro-β-ketophosphonate diesters,[9] the method by Blades et al. describing a cerium-mediated reaction of benzoyl esters with lithium difluoromethylphosphonate provides the most direct and reproducible approach.[9d] The key intermediate esters **2** were prepared by the reaction of benzoyl methyl esters **1** with lithium difluoromethylphosphonate, generated by the addition of diethyl difluoromethylphosphonate to LDA containing cerium(III) chloride in THF. Subsequently, an aqueous work-up with 1 N hydrochloric acid gave phosphonate diethyl esters **2** in about 60% yields. Deprotection of **2** was achieved by treatment with excess TMSBr,[10] followed by hydrolysis, to give the desired phosphonic acids **3** in quantitative yields.

The inhibitory activity against PTP1B was measured using *O*-methyl fluorescein monophosphate (OMFP) as substrate,[11] and IC_{50} values are shown in Table 1. For representative compounds, inhibition kinetics were also determined,[11] and K_i values are given in Table 1. The



Scheme 1. Reagents and conditions: (a) $HF_2PO(OEt)_2$, LDA, $CeCl_3$, THF, −78 °C; (b) HCl, H_2O; (c) TMSBr, DCM; (d) H_2O.

Table 1. Inhibition of PTP1B by aryl α,α-difluoro-β-ketophosphonates[a]

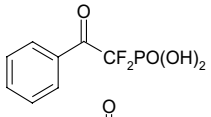
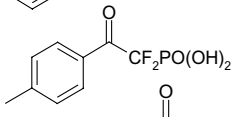
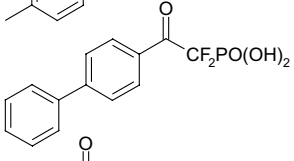
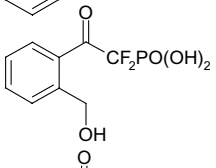
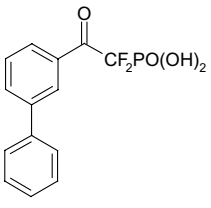
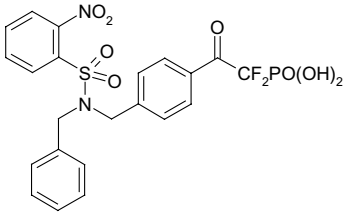
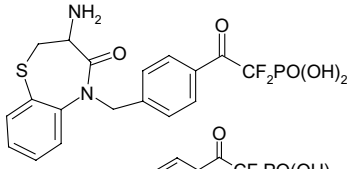
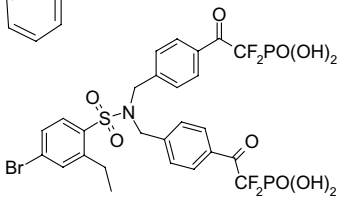
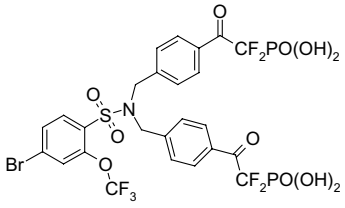
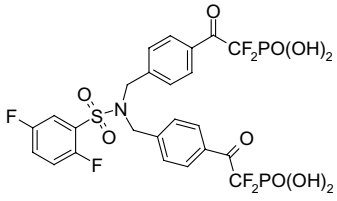
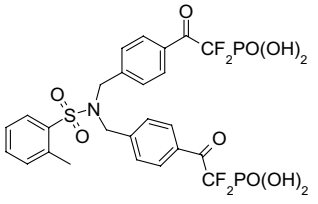
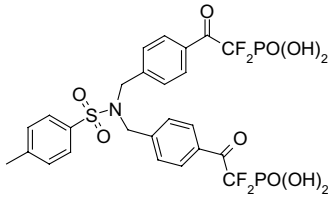
Compound	Structure	IC_{50} (μM)	K_i (μM)
3a		76	129
3b		62	71
3c		50	ND[b]
3d		31	ND
3e		180	ND

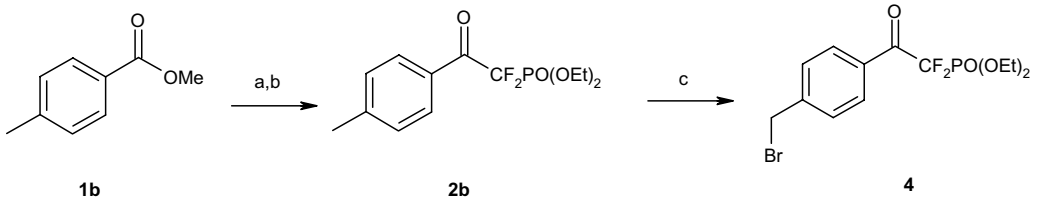
Table 1 (*continued*)

Compound	Structure	IC_{50} (μM)	K_i (μM)
7		12	12
8		8.8	ND
10a		1.3	0.57
10b		0.5	0.28
10c		0.7	ND
10d		0.6	0.17
10e		1.1	ND

[a]Values are means of duplicate experiments, errors are usually within ±10%.
[b]Not determined.

simple phenyl α,α-difluoro-β-ketophosphonate **3a** shows competitive inhibition with an IC_{50} of 76 μM and a K_i of 129 μM, respectively. Introduction of a methyl group (**3b**) or a phenyl group (**3c**) at the *para* position slightly improved inhibitory potency. Compound **3d** with a hydroxymethyl group at the *ortho* position also exhibit good inhibitor activity. Interestingly, compound **3c** ($IC_{50} = 50$ μM) with a phenyl group at the *para* position is three-times more potent than **3e** ($IC_{50} = 180$ μM) where the phenyl group is at the *meta* position, suggesting that the interaction from the *para*-substituted phenyl group with the enzyme is more favorable.

Encouraged by these results, we then explored the method to incorporate *para*-substituted phenyl α,α-difluoro-β-ketophosphonate in small molecule scaffolds.



Scheme 2. Reagents and conditions: (a) $HF_2PO(OEt)_2$, LDA, $CeCl_3$, THF, −78 °C; (b) HCl, H_2O; (c) NBS/CCl_4, benzoyl peroxide, reflux.

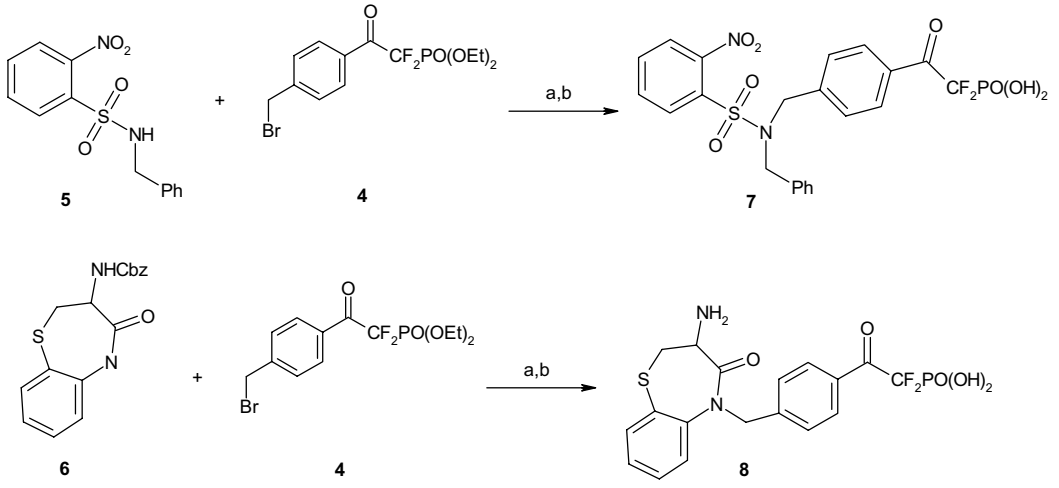
Accordingly, we developed a building block approach, which utilized 4-bromomethyl diethyl benzoyldifluorophosphonate **4** as a useful intermediate (Scheme 2). The reaction of 4-methyl benzoyl methyl ester **1b** with lithium difluoromethylphosphonate under the above conditions gave the corresponding phosphonate diester **2b**. Diester **2b** was allowed to reflux with NBS in carbon tetrachloride in the presence of benzoyl peroxide to produce the desired benzyl bromide **4** in 87% yield.

With bromide **4** in hand, we explored the reaction with several molecular scaffolds such as carboxyamide or sulfonamide, since they are known to function as a good hydrogen bond acceptor in many biological systems. As shown in Scheme 3, the reaction of **4** with 1 equiv of *N*-benzyl-2-nitro-benzenesulfonamide **5** at 70 °C in the presence of K_2CO_3 gave the corresponding phosphonate diester. After TMSBr treatment and hydrolysis, phosphonic acid **7** was isolated by preparative HPLC. Under similar conditions, the reaction of **4** with 3-Cbz-amino-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one **6**[12] followed by TMSBr treatment resulted in phosphonic acid **8**.

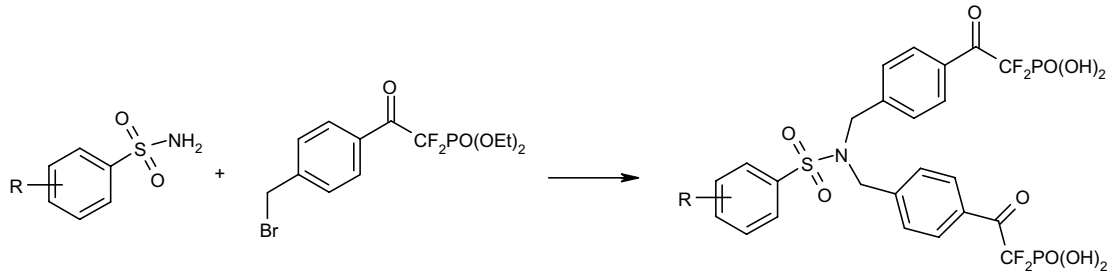
As shown in Table 1, sulfonamide **7** inhibited PTP1B in a competitive manner with an IC_{50} of 12 μM and K_i of 12 μM, respectively. Compared to simple aryl derivative **3a** or **3b**, compound **7** exhibits 5–6-fold improvement in potency. Compound **8** containing a benzothiazepinone scaffold was also 7-fold more potent than **3b**, inhibiting PTP1B with an IC_{50} of 8.8 μM. These results suggest that additional recognition elements can lead to better inhibitory activity, as more favorable interactions of the molecule with the active site residues are possible.[13] More importantly, both carboxamides and benzothiazepinones represent potential drug scaffolds for the development of potent PTP1B inhibitors. One can imagine designing more potent analogues by introducing various substituents around these scaffolds that achieve favorable interactions within the enzyme active site.

The recent discovery that PTP1B has a second *p*Tyr binding site adjacent to the catalytic pocket has led to the development of bifunctional *p*Tyr analogues against PTP1B.[14a,b] Based on our results with the new *p*Tyr mimetics and sulfonamide scaffold, we synthesized several bis(aryl α,α-difluoro-β-ketophosphonates) **10** according to Scheme 4. The reaction of sulfonamides **9** with 2 equiv of compound **4** in the presence of K_2CO_3 gave the corresponding bifunctional phosphonate esters. After TMSBr treatment and hydrolysis, bis-phosphonic acids **10** were isolated by preparative HPLC.

These bis(aryl α,α-difluoro-β-ketophosphonates) are very potent inhibitors of PTP1B with IC_{50} values in the range of 0.5–1.3 μM, as shown in Table 1. They are reversible and competitive inhibitors, and kinetic experiments show no evidence of time-dependent inhibition. The best compound from this class, **10d**, has an IC_{50} of 0.6 μM and a K_i of 0.17 μM. Compared to **3a** or **3b**, compounds **10** exhibit a significant increase in potency by 2 orders of magnitude. Presumably introduction of the second α,α-difluoro-β-ketophosphonate



Scheme 3. Reagents and conditions: (a) K_2CO_3, ACN, 70 °C; (b) TMSBr, DCM, then H_2O.



9 **4**

10a, R = 2-Et, 4-Br
10b, R = 2-OCF_3, 4-Br
10c, R = 2-F, 5-F
10d, R = 2-Me
10e, R = 4-Me

Scheme 4. Reagents and conditions: (a) K_2CO_3, ACN, 70 °C; (b) TMSBr, DCM, then H_2O.

group provides additional binding interactions with the second phosphate-binding site or nearby region in PTP1B. The results also demonstrate that aryl α,α-difluoro-β-ketophosphonates are powerful *p*Tyr mimetic for developing potent PTP inhibitors based on small molecule scaffolds.

One of the most effective *p*Tyr mimetics that have been developed to date is α,α-difluoromethylphosphonates.[5a–c] Our compounds, α,α-difluoro-β-ketophosphonates, exhibit comparable or even better inhibitory activity against PTP1B than α,α-difluoromethylphosphonates. For example, the simple phenyl difluoromethylphosphonate has been reported to inhibit PTP1B with an IC_{50} of 610 μM[5c] and a K_i of 2500 μM,[5b] while the corresponding **3a** is more active ($IC_{50} = 76$ μM, $K_i = 129$ μM). 4-Biphenyl difluorometh-ylphosphonate has an IC_{50} of 210 μM,[5c] and the corresponding **3c** has better activity with an IC_{50} of 50 μM against PTP1B. There results indicate that the carbonyl group adjacent to the aromatic can form effective interactions with the active site residues similar to the phosphate oxygen in *p*Tyr or the difluoromethyl group in difluoromethylphosphonates. Since the carbonyl group in these compounds is highly activated and electrophilic, and they can easily form hydrates in aqueous solution, it is also possible that the hydrated form can effectively mimic the water molecule in the active site[15] and therefore exhibit high binding ability to the enzyme. In addition, there is a potential for these compounds to react with the active site Cys or nitrogen nucleophiles of PTP1B in a reversible co-valent fashion to form hemithioketals or enamines. Further biochemical studies are required to elucidate detailed mechanism of inhibition of this class of inhibitors.

Compared to difluoromethylphosphonates, α,α-difluoro-β-ketophosphonates have one extra atom spacing between the aromatic and the phosphonate group. The strong inhibitory activity of our compounds against PTP1B suggests that the additional spacer derived from the carbonyl group between the aromatic and phosphonate has no adverse effects on the ability of the phosphonate group to bind to the enzyme active site. The other known examples of *p*Tyr mimetics with the same atom spacing as α,α-difluoro-β-ketophosphonates are aryloxymethylphosphonates,[16] which have been reported as relatively weak inhibitors against PTP1B. It will be of interest to replace the carbonyl group of α,α-difluoro-β-ketophosphonate with other bioisosteric atoms or functional groups and explore the spatial and structural requirements that are important for high affinity binding to the active site of PTP1B.

In conclusion, we have synthesized and evaluated a new class of PTP1B inhibitors, α,α-difluoro-β-ketophosphonates. The approach described here represents an example of utilizing these *p*Tyr mimetics to identify potent inhibitors based on small molecules scaffolds, which provide lead structures for further design and development of novel PTP1B inhibitors as potential therapeutic agents. Further studies with this class of compounds should gain additional mechanistic and structural insights into molecular recognition in signal transduction processes.

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References and notes

1. (a) For recent reviews see: Zhang, Z.-Y. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 1; (b) Evans, J. L.; Jallal, B. *Exp. Opin. Invest. Drugs* **1999**, *8*, 139.
2. (a) Neel, B. G.; Tonks, N. K. *Curr. Opin. Cell Biol.* **1997**, *9*, 93; (b) Byon, J. C. H.; Kusari, A. B.; Kusari, J. *Mol. Cell. Biochem.* **1998**, *182*, 101.

3. Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C.-C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544.
4. For recent reviews see: (a) Burke, T. R., Jr.; Yao, Z.-J.; Liu, D.-G.; Voigt, J.; Gao, Y. *Biopolymers* **2001**, *60*, 32; (b) Zhang, Z.-Y. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 209; (c) Blaskovich, M. A.; Kim, H.-O. *Exp. Opin. Ther. Patents* **2002**, *12*, 871; (d) Taylor, S. D. *Curr. Top. Med. Chem.* **2003**, *3*, 759.
5. (a) Burke, T. R., Jr.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129; (b) Yao, Z.-J.; Ye, B.; Wu, X.-W.; Wang, S.; Wu, L.; Zhang, Z.-Y.; Burke, T. R., Jr. *Bioorg. Med. Chem.* **1998**, *6*, 1799; (c) Taylor, S. D.; Kotoris, C. C.; Dinaut, A. N.; Wang, Q.; Ramachandran, C.; Huang, Z. *Bioorg. Med. Chem.* **1998**, *6*, 1457; (d) Desmarais, S.; Jia, Z.; Ramachandran, C. *Arch. Biochem. Biophys.* **1998**, *354*, 225; (e) Burke, T. R., Jr.; Ye, B.; Akamatsu, M.; Ford, H., Jr.; Yan, X.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. P. *J. Med. Chem.* **1996**, *39*, 1021; (f) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787; (g) Malamas, M. S.; Sredy, J.; Moxham, C.; Katz, A.; Xu, W.; McDevitt, R.; Adebayo, F. O.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Taylor, J. R. *J. Med. Chem.* **2000**, *43*, 1293; (h) Larsen, S. D.; Barf, T.; Liljebris, C.; May, P. D.; Ogg, D.; O'Sullivan, T. J.; Palazuk, B. J.; Schostarez, H. J.; Stevens, F. C.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 598; (i) Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Moller, K. B. *J. Biol. Chem.* **2000**, *275*, 7101; (j) Szczepankiewicz, B. G.; Liu, G.; Hajduk, P. J.; Abad-Zapatero, C.; Pei, Z.; Xin, Z.; Lubben, T. H.; Trevillyan, J. M.; Stashko, M. A.; Ballaron, S. J.; Liang, H.; Huang, F.; Hutchins, C. W.; Fesik, S. W.; Jirousek, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 4087.
6. Kluger, R.; Nakaoka, K. *Biochemistry* **1974**, *13*, 910.
7. Greco, M. N.; Hawkins, M. J.; Powell, E. T.; Almond, H. R., Jr.; Corcoran, T. W.; Garavilla, L. D.; Kauffman, J. A.; Recacha, R.; Chattopadhyay, D.; Andrade-Gordon, P.; Maryanoff, B. E. *J. Am. Chem. Soc.* **2002**, *124*, 3810.
8. Ladame, S.; Bardet, M.; Perie, J.; Willson, M. *Bioorg. Med. Chem.* **2001**, *9*, 773.
9. (a) Obayashi, M.; Ito, E.; Matsui, K.; Kondo, K. *Tetrahedron Lett.* **1982**, *23*, 2323; (b) Burton, D. J.; Ishihara, T.; Maruta, M. *Chem. Lett.* **1982**, 755; (c) Ladame, S.; Willson, M.; Perie, J. *Eur. J. Org. Chem.* **2002**, 2640; (d) Blades, K.; Lequeux, T. P.; Percy, J. M. *Tetrahedron* **1997**, *53*, 10623.
10. Salomon, C. J.; Breuer, E. *Tetrahedron Lett.* **1995**, *36*, 6759.
11. The PTP1B assay was carried out at ambient temperature using OMFP as a substrate in 62.5 mM MOPS buffer, pH 7.0, containing 62.5 mM NaCl, 0.125 mg/mL BSA, 0.0125% Brij35, 1.25 mM EDTA and 1.25 mM DTT. The reactions were initiated by the addition of enzyme and quenched by the addition of stop solution (0.25 N NaOH). Fluorescence emissions from the product *o*-methyl fluorescein were measured using an appropriate plate reader and converted to units of product formation. IC_{50} values were calculated by nonlinear regression using the dose-response equation in GraphPad Prism. For inhibitor kinetics, best curve fit for Lineweaver–Burk plots and K_i values was determined using the curve-fitting programs. 3-Phenyl-α,α-difluorobenzylphosphonic acid (compd **12** from Ref. 5c) and a peptidic 2-carboxymethoxybenzoic acid (compd **VI** from Ref. 15b) were routinely used as reference compounds, which gave IC_{50} and K_i values that were consistent with reported values. In some experiments, PTP1B activity was also measured as the rate of hydrolysis of *p*-nitrophenyl phosphate.
12. See, for example: Schwarz, M. K.; Tumelty, D.; Gallop, M. A. *J. Org. Chem.* **1999**, *64*, 2219.
13. From a different class of compounds, we found that α,α-difluoro-β-ketophosphonates based on tetrahydro-β-carboline scaffold showed poorer activity against PTP1B compared to **3b**, presumably due to unfavorable interactions of the scaffold with the enzyme.
14. (a) Puius, Y. A.; Zhao, Y.; Sullivan, M.; Lawrence, D. S.; Almo, S. C.; Zhang, Z.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13420; (b) Jia, Z.; Ye, Q.; Dinaut, A. N.; Wang, Q.; Waddleton, D.; Payette, P.; Ramachandran, C.; Kennedy, B.; Hum, G.; Taylor, S. D. *J. Med. Chem.* **2001**, *44*, 4584.
15. For the active site water molecule in X-ray structures see: (a) Burke, T. R., Jr.; Ye, B.; Yan, X. J.; Wang, S. M.; Jia, Z. C.; Chen, L.; Zhang, Z. Y.; Barford, D. *Biochemistry* **1996**, *35*, 15989; (b) Bleasdale, J. E.; Ogg, D.; Palazuk, B. J.; Jacob, C. S.; Swanson, M. L.; Wang, X. Y.; Thompson, D. P.; Conradi, R. A.; Mathews, W. R.; Laborde, A. L.; Stuchly, C. W.; Heijbel, A.; Bergdahl, K.; Bannow, C. A.; Smith, C. W.; Svensson, C.; Liljebris, C.; Schostarez, H. J.; May, P. D.; Stevens, F. C.; Larsen, S. D. *Biochemistry* **2001**, *40*, 5642.
16. Ibrahimi, O. A.; Wu, L.; Zhao, K.; Zhang, Z.-Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 457.