Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3275–3280

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL PHOSPHATE MIMETICS FOR THE DESIGN OF NON-PEPTIDYL INHIBITORS OF PROTEIN TYROSINE PHOSPHATASES

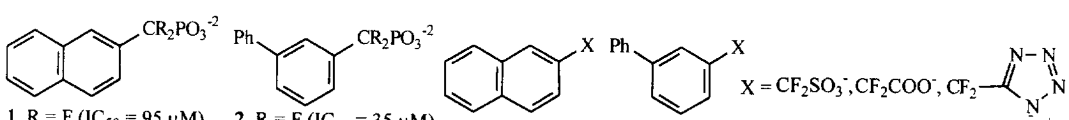
Christopher C. Kotoris, Mei-Jin Chen, and Scott D. Taylor*

Department of Chemistry, University of Toronto, Mississauga Campus, 3359 Mississauga Road North, Mississauga, Ontario, Canada, L5L 1C6

Received 18 August 1998; accepted 6 October 1998

Abstract: Benzylic α,α-difluorosulfonates, α,α-difluorotetrazoles, and α,α-difluorocarboxylates of type **5** and **6** were synthesized and examined as potential phosphate biosteres for PTP1B inhibition. The α,α-difluorosulfonates and α,α-difluorotetrazoles were found to be more effective inhibitors than the analogous compounds bearing the fluoromalonyl group, a phosphate biostere currently being used for PTP inhibition.

The phosphorylation and dephosphorylation of tyrosine residues in proteins by protein tyrosine kinases (PTK's) and protein tyrosine phosphatases (PTP's) is an important cellular regulatory mechanism.[1] Recent studies have found that overexpression of certain PTP's occurs in a number of disease states.[1] Consequently, there has been considerable interest in developing inhibitors of these enzymes.[2] Among the most effective of the PTP inhibitors reported to date are peptidyl inhibitors bearing the nonhydrolyzable phosphotyrosine mimetic, difluoromethylenephosphonyl phenylalanine (F_2Pmp).[3a,b] It has also been shown that even certain simple aromatics bearing the difluoromethylenephosphonic acid (DFMP) group, such as **1** and **2**, are relatively good competitive inhibitors of PTP's, while their non-fluorinated counterparts **3** and **4** are very poor inhibitors.[3c,d,e] X-ray crystallographic and kinetic studies suggest that the enhanced binding of DFMP-bearing inhibitors is most likely the result of a strong H-bond between the fluorines and residues in the active site and is not due to pKa effects.[3a,d,f]



1, R = F (IC_{50} = 95 μM) **3**, R = H **2**, R = F (IC_{50} = 35 μM) **4**, R = H **5** **6**

Although the DFMP group has proven to be an effective phosphate mimetic for generating PTP inhibitors, inhibitors bearing this group are not cell permeable due to the highly polar nature of the dianionic DFMP group.[3c] The dianionic malonyl or fluoromalonyl (CF-malonyl) groups have been employed as phosphate surrogates for PTP inhibition with the CF-malonyl group being the more effective of the two.[4a,b,c] Although compounds bearing these phosphate mimics are considerably less effective PTP inhibitors than their

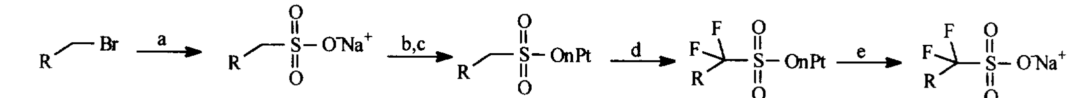
PII: S0960-894X(98)00598-8

DFMP-bearing analogues,[4a,b] they are more readily converted into enzyme-labile diesters for efficient delivery across cell membranes.

As part of our program to create small molecule, nonpeptidyl inhibitors of PTP's,[3d,e] we became interested in developing phosphate surrogates that are as or more effective than the CF-malonyl group yet would not require further chemical modification for cellular studies. Anticipating that monoanionic functionalities may be more amenable to cellular studies than dianionic species, we decided to determine if monoanionic groups such as the α,α-difluorotetrazole (CF_2-tetrazole), α,α-difluorosulfonate (CF_2-sulfonate), or α,α-difluorocarboxylate (CF_2-carboxylate) moieties could act as effective phosphate biosteres for PTP inhibition. Our approach was to construct compounds of type **5** and **6** and compare their inhibitory potency to the analogous compounds bearing the DFMP and CF-malonyl groups. Here we report the synthesis of this class of compounds and their evaluation as inhibitors of PTP1B.

Syntheses

α,α-Difluorosulfonates of type **5** and **6** were constructed as shown in Scheme 1. β-bromomethylnaphthalene **7** and *m*-(phenyl)benzyl bromide **8** were converted into the sulfonate salts **9** and **10** by reaction with sodium sulfite in acetone/water. Reaction of the sulfonate salts with $POCl_3$ [5] gave the



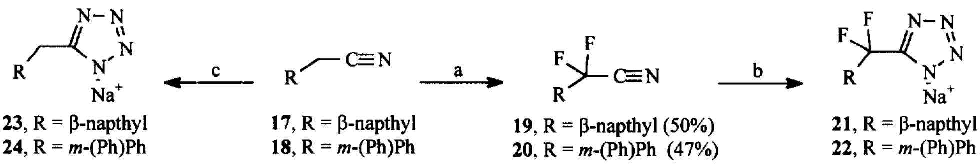
7, R = β-napthyl; **8**, R = *m*-(Ph)Ph — **9**, R = β-napthyl (73%); **10**, R = *m*-(Ph)Ph (85%) — **11**, R = β-napthyl (67%); **12**, R = *m*-(Ph)Ph (95%) — **13**, R = β-napthyl (86%); **14**, R = *m*-(Ph)Ph (59%) — **15**, R = β-napthyl (92%); **16**, R = *m*-(Ph)Ph (88%)

(a) 1.1 equiv Na_2SO_3, acetone/water (5/3), reflux; (b) 5 equiv $POCl_3$, sulfolane/CH_3CN, 55 - 65 °C; (c) 1.1 equiv neopentyl alcohol, 1.15 equiv 2,6-lutidine, THF; (d) 1.1 equiv *t*-BuLi, THF, -78 °C, 1 h followed by 1.2 equiv NFSi, THF, -78 °C, 1 h (repeat); (e) 1.1 equiv LiBr, butanone, reflux 48 h followed by Na^+ ion exchange column.

Scheme 1

sulfonyl chlorides which were reacted with neopentyl alcohol to give esters **11** and **12**. As with benzylic α,α,-difluoromethylenephosphonate esters,[6] we have also found that benzylic α,α-difluorosulfonate neopentyl esters can also be prepared by electrophilic fluorination using N-fluorobenzenesulfonimide (NFSi).[7] Thus, **11** and **12** were treated with 1.1 equiv *t*-BuLi at -78 °C followed by the addition of 1.2 equiv NFSi and this process was repeated to give fluorinated esters **13** and **14** in good to excellent yields. Reaction of **13** and **14** with LiBr in refluxing butanone gave the CF_2-sulfonates **15** and **16** in excellent yield.

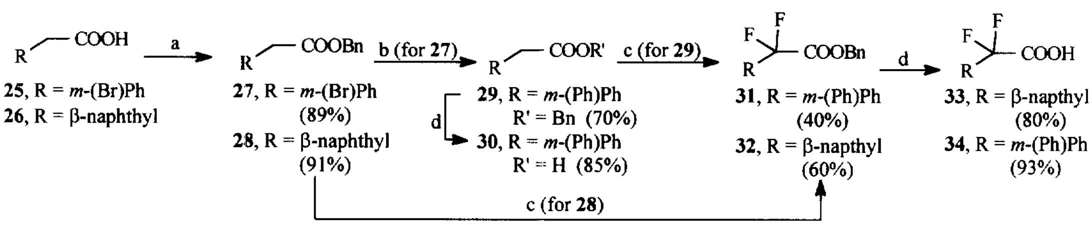
Synthesis of the CF_2-tetrazoles is outlined in Scheme 2. We have found that these compounds were readily obtained by subjecting β-naphthylacetonitrile **17** and *m*-(phenyl)phenylacetonitrile **18** to electrophilic fluorination with *t*-BuLi/NFSi[7] to give the α,α,-difluorinated nitriles **19** and **20** followed by reaction with sodium azide in DMF (quantitative) to give fluorinated tetrazoles **21** and **22** as their sodium salts. The nonfluorinated analogues, **23** and **24**, were obtained from **17** and **18** by reaction with NaN_3/NH_4Cl.



(a) 2.2 equiv *t*-BuLi, THF, -78 °C, 1 h followed by 2.5 equiv NFSi, THF, -78 °C, 3 h; (b) NaN_3, DMF 65 °C, 3 h; (c) NaN_3, NH_4Cl, DMF 80 °C, 24 h.

Scheme 2

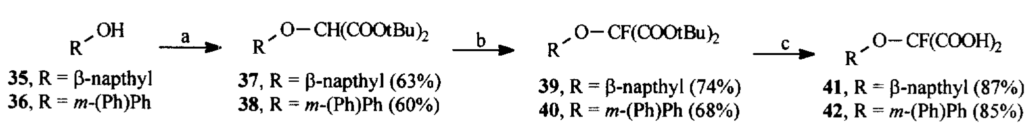
For the preparation of the CF_2-carboxylates, we again chose electrophilic fluorination[8] as the method for introducing fluorine (Scheme 3). Thus, acids **25** and **26** were converted to benzyl esters **27** and **28** in excellent yields using Cs_2CO_3 and benzyl bromide. A Suzuki reaction with ester **27** and phenylboronic acid yielded the biphenyl ester **29**. Esters **28** and **29** were treated with 1.1 equiv LDA at -78 °C followed by the addition of 1.2 equiv NFSi and this process was repeated to give difluoro esters **31** and **32** in modest to good yield. Hydrogenolysis of **31** and **32** yielded the CF_2-carboxylates **33** and **34** in good yields.



(a) Cs_2CO_3, 1.1 equiv benzyl bromide, $MeOH/H_2O$ (5/2); (b) 1.2 equiv $PhB(OH)_2$, 1.5 equiv Na_2CO_3, 5 mol% $Pd(OAc)_2$, rt, 24 h; (c) 1.1 equiv LDA, THF, -78 °C, 1 h followed by 1.2 equiv NFSi, THF, -78 °C, 3 h, (repeat); (d) 1 atm H_2, 5% Pd/C, EtOAc, 12 h.

Scheme 3

Fluoromalonyl analogues (Scheme 4) were prepared using the procedure recently developed by Burke and coworkers for the synthesis of fluoromalonyl derivatives.[4a,b,9] Thus, phenol derivatives **35** and **36** were



(a) Rh(II)acetate (4.4 mol%), 1.2 equiv di-*tert*-butyl-α-diazomalonate, dry benzene, reflux 18 h; (b) 1.1 equiv NaHMDS, THF, -78 °C, 1 h followed by 1.1 equiv NFSi, THF, -78 °C 3 h; (c) 90% TFA/CH_2Cl_2, rt, 1 h.

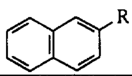
Scheme 4

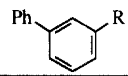
refluxed in benzene in the presence of di-*tert*-butyl α-diazomalonate[10] and rhodium diacetate to give *t*-butyl malonate esters **37** and **38**. Fluorinated derivatives **41** and **42** were prepared in good yields via electrophilic fluorination[4b] of **37** and **38** followed by hydrolysis with TFA/CH_2Cl_2.

Inhibition Studies

We initiated the inhibition studies by first examining compounds **9**, **10**, **23**, **24**, **26**, and **30** to determine if these nonfluorinated species were effective PTP inhibitors. These were examined for inhibition using 500 μM of the compounds, fluorescein diphosphate as substrate at K_m concentration (20 μM) and PTP1B[11] as a model PTP as previously described[3e] except the assay mixture contained 10% DMSO. The results are given in Table 1. All of these compounds are poor inhibitors of PTP1B. In general, their nonfluorinated phosphate counterparts **3** and **4** appear to be slightly better inhibitors with the exception of the naphthyl tetrazole derivative **23**.

Table 1. Percent Inhibition of PTP1B with 500 μM Nonfluorinated Compounds.



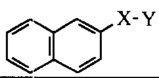
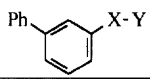


Compound	R	Percent Inhibition[a]	Compound	R	Percent Inhibition[a]
9	$CH_2SO_3^-$	5 ± 2	**10**	$CH_2SO_3^-$	10 ± 2
23	CH_2-tetrazole	12 ± 2	**24**	CH_2-tetrazole	15 ± 2
26	CH_2COO^-	4 ± 2	**30**	CH_2COO^-	10 ± 2
3	$CH_2PO_3^{-2}$	9 ± 2	**4**	$CH_2PO_3^{-2}$	17 ± 2

[a]Errors are reported as ± the standard deviation of two determinations.

IC_{50}'s were determined for the fluorinated compounds **15**, **16**, **21**, **22**, **33**, **34**, **41**, and **42**. The results are given in Table 2. These compounds were better inhibitors than their nonfluorinated counterparts although none were as effective as their DFMP analogues. The CF_2-carboxylates **33** and **34** exhibited the highest IC_{50}'s and were 18 and 29 times poorer inhibitors than their DFMP-bearing analogues, respectively. The CF_2-sulfonate group was the most effective phosphate biostere with compounds **15** and **16** being only 5 and 7.6 times less effective inhibitors than **1** and **2**. Although it has been shown that sulfate-bearing compounds are inhibitors of PTP's,[12] the well-known lability of phenolic sulfates[13] limits their use as potential therapeutics. In contrast, we have found that the CF_2-sulfonates described here are stable compounds. Sulfonates, whether bearing α-fluorines or not, are highly acidic and so **9**, **10**, **15**, and **16** should be completely ionized at the pH under which these studies were performed (pH 6.5). Thus, the enhanced inhibitory effect of the CF_2-sulfonates compared to their non-fluorinated counterparts is most likely a result of a direct interaction of the fluorines with residues in the enzyme active site and is not due to pK_a effects. The CF_2-tetrazole derivatives **21** and **22** were 6.5- and 13-fold less effective inhibitors than **1** and **2**. The pK_a's of the conjugate acids of **21** and **23** were determined by potentiometric titration in 10% DMSO/H_2O to be 3.9 and 5.3, respectively, and so both exist mainly in the anionic form at pH 6.5. This suggests that the enhanced inhibition found with **21** and **22** as compared to **23** and **24** is most likely a result of a direct interaction of the fluorines with residues in the enzyme active site. Compounds **16** and **22** were examined in more detail.[14] Both were found to be competitive inhibitors with K_i's of 49 ± 7 μM (for **16**) and 98 ± 9 μM (for **22**). Although the CF_2-sulfonate and CF_2-tetrazole compounds were not as effective inhibitors as their DFMP analogues, it is important to note that these compounds were better inhibitors than the CF-malonyl compounds **41** and **42**. Thus, it appears that the CF_2-sulfonate and CF_2-tetrazole groups are more effective phosphate biosteres than the CF-malonate group. Although it is possible that the CF_2-sulfonate compounds, as is the case with the CF-malonyl derivatives, may require caging for cellular studies, it is very possible that this will not be the case for the CF_2-tetrazole compounds since tetrazole-bearing compounds usually exhibit good cellular penetration.[15]

Table 2. IC_{50}'s Values for Fluorinated Compounds.

							
Compound	**X**	**Y**	**IC_{50} (μM)[a]**	**Compound**	**X**	**Y**	**IC_{50} (μM)[a]**
15	CF_2	SO_3^-	175 ± 10	**16**	CF_2	SO_3^-	115 ± 9
21	CF_2	tetrazole	230 ± 12	**22**	CF_2	tetrazole	195 ± 10
33	CF_2	COO^-	640 ± 18	**34**	CF_2	COO^-	435 ± 12
41	OCF	$(COO^-)_2$	320 ± 11	**42**	OCF	$(COO^-)_2$	250 ± 10
1	CF_2	PO_4^{-2}	35 ± 5[b]	**2**	CF_2	PO_4^{-2}	15 ± 3[b]

[a]IC_{50}'s were determined using eight different inhibitor concentrations. Errors are reported as ± the standard deviation of two determinations. [b]The presence of 10% DMSO in the assay mixture results in lower IC_{50}'s than previously reported values (see ref 3d) which were obtained in 100% aqueous solution.

In summary, the CF_2-tetrazole, CF_2-sulfonate and CF_2-carboxylate groups were examined as potential replacements for the DFMP group for obtaining nonpeptidyl PTP inhibitors. Although not as effective as the DFMP group, the CF_2-sulfonate and CF_2-tetrazole groups appear to be more effective phosphate biosteres than the CF-malonyl group, a phosphate surrogate currently being used for PTP inhibition. Examination of these compounds with other PTP's and studies to determine if the CF_2-tetrazole and CF_2-sulfonate compounds are suitable for cellular studies are in progress and will be reported in due course. It should also be noted that some of the procedures described in this study have the potential to provide novel classes of compounds with applications beyond the scope of PTP inhibitors. The CF_2-sulfonate and CF_2-tetrazole groups may also be useful in the development of inhibitors of other therapeutically important proteins that recognize pTyr (such as SH2 domains). The benzyl tetrazole group has been used extensively as a biostere for acidic residues in medicinal chemistry[15] and the α-difluorination of this moiety will provide a potential route for increasing the bioactivity of these compounds. Finally, CF_2-sulfonates may also find use in the development of inhibitors of enzymes such as steroid sulfatases, aryl sulfatases and proteins that bind tyrosylsulfates.

Acknowledgements.

We thank the Natural Sciences and Engineering Council of Canada and Merck Frosst Canada Inc. for financial support. We also thank Drs. Zheng Huang, Chidambaram Rhamachandran, Qingping Wang and Yves Leblanc for helpful discussions

References and Notes

1. For a recent review on PTP's see: Zhang, Z-Y. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 1.
2. Burke, T. R. *Drugs of the Future* **1992**, *17*, 119.
3. (a) Burke, T. R.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129. (b) Chen, L.; Wu, L.; Otaka, A.; Smyth, M. S.; Roller, P. R.; Burke, T. R.; den Hertog, J.; Zhang, Z-Y. *Biochem. Biophys. Res. Comm.* **1995**, *216*, 976. (c) Kole, K. H.; Smyth, M. S.; Russ, P. L.; Burke, T. R. *Biochem. J* **1995**, 311, 1025. (d) Taylor, S. D.; Kotoris, C. C.; Dinaut, A. N.; Wang, Q.; Ramachandran, C.; Huang, H. *Bioorg. Med. Chem.* in press. (e) Wang, Q.; Huang, Z.; Ramachandran, C.; Dinaut, A. N. and Taylor, S. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 345. (f) Burke, T. R.; Ye. B.; Yan, X.; Wang, S.; Jia, Z., Chen, L.; Zhang, Z-Y.; Barford, D. *Biochemistry* **1996**, *35*, 15898.
4. (a) Kole, H. K.; Akamatsu, M.; Ye, B.; Yan, X.; Barford, D.; Roller, P. P.; Burke, T. R. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 817. (b) Burke, T. R.; Ye, B.; Akamatsu, M.; Ford, H.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. R. *J. Med. Chem.* **1996**, *39*, 1021. (c) Akamatsu, M.; Roller, P. R.; Chen, L.; Zhang, Z-Y.; Ye, B.; Burke, T. R. *Bioorg. Med. Chem.* **1997**, *5*, 157.
5. Abdellaoui, H.; Depreux, P.; Lesieur, D.; Pfeiffer, B.; Bontempelli, P. *Synthetic Commun.* **1995**, 1303.
6. (a) Taylor, S. D.; Kotoris, C. C.; Dinaut, A. N.; Chen, M-J. *Tetrahedron* **1998**, *54*, 1691. (b) Taylor, S. D.; Dinaut, A. N.; Thadani, A. T.; Huang, Z. *Tetrahedron Lett.* **1996**, *37*, 8089.
7. A detailed analysis and the scope of this reaction is in progress and will be reported in due course.
8. NFSi has previously been used for preparing α,α,-difluorocarboxylic esters. For example see: Differding, E.; Ruegg, G. M.; Lang, R. W. *Tetrahedron Lett.* **1991**, *32*, 1779.
9. Ye, B.; Burke, T. R. *Tetrahedron Lett.* **1995**, *36*, 4733.
10. Ledon, H. *Synthesis* **1974**, 347.
11. PTP1B is an intracellular PTP consisting of a single catalytic domain. There is now considerable evidence that this enzyme may be involved in the down-regulation of insulin signaling by dephosphorylating specific phosphotyrosine residues on the insulin receptor kinase. Thus, PTP1B is now being considered as a potential therapeutic target to help combat certain forms of diabetes. See: Bandyopadhyay, D.; Kusari, A.; Kenner, K. A.; Liu, F.; Chernoff, J.; Gustafson, T. A.; Kusari, J. *J. Biol. Chem.* **1997**, *272*, 1639 and references therein.
12. (a) Liotta, A. S.; Kole, H. K.; Fales, H. M.; Roth, J.; Bernier, M. *J. Biol. Chem.* **1994**, *269*, 22996. (b) Kole, H. K.; Garant, M. J.; Fales, H. M.; Roth, J.; Bernier, M. *J. Biol. Chem.* **1996**, *271*, 14302. (c) Desmarais, S.; Jia, Z.; Ramachandran, R. *Arch. Biochem. Biophys.* **1998**, *354*, 225.
13. Bettlelheim, F. R. *J. Am. Chem. Soc.* **1954**, *76*, 2838.
14. K_i's were determined using the procedure outlined in reference 3d
15. Burger, A. *Prog. Drug Res.* **1991**, *37*, 287.