The difluoromethylenephosphonothioic acid group as a phosphate surrogate for obtaining PTP-1B inhibitors. Transformation of difluoromethylenephosphonic acids into difluoromethylenephosphonothioic acids

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Dedicated to Professor Keiichiro Fukumoto on his 70th birthday
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Abstract
Aryldifluoromethylphosphonic acids were transformed into the corresponding phosphonothioic acids *via* their bis-(2-cyanoethyl) esters for the evaluation of their biological effects on protein tyrosine phosphatase **1B**. Difluoro(2-naphthyl)-methylphosphonothioic acid 5d showed better inhibitory potency and higher hydrophobicity than the corresponding difluoromethylene-phosphonic acid analogue **1d**.

Keywords: Phosphonothioic acids, bis-(2-cyanoethyl) esters, enzyme inhibitors, protein tyrosine phosphatases

Introduction

Naturally-occurring phosphate derivatives play pivotal roles in various cellular processes including signal transduction.[1] Non-hydrolyzable phosphate mimetics have been studied for the design of potential enzyme inhibitors and probes for the elucidation of biochemical processes. Extensive studies have been devoted to the synthesis and biological evaluation of (α,α-difluoromethylene)phosphonic acid (DFMPA) derivatives 1, as non-hydrolyzable mimetics of phosphates.[2] As a result, many enzyme inhibitors with significant activity have been identified.[3] Although the DFMPA group has proved to be useful for obtaining inhibitors against a variety of enzymes, highly polar and hydrophilic DFMPA-derivatives often exhibit poor cellular uptake.[4] Consequently, recent efforts in this research field are focused on developing new-generation

mimetics of a phosphate ester.[5] Among them, (α,α-difluoromethylene)phosphonothioic acid (DFMPTA) derivatives, 2, featuring a less-polar phosphorus–sulfur bond,[6] are proposed as a class of non-hydrolyzable phosphate mimetics possessing unique and useful properties.[7] However, the physical and biological properties of DFMPTA compounds have not been studied extensively, owing to the lack of efficient synthetic methods.

1 (DFMPA) **2** (DFMPTA)

Figure 1

In the search for DFMPA-based inhibitors of protein tyrosine phosphatases (PTPs), which are key regulatory enzymes in many cellular processes such as cell proliferation and differentiation, we and others have identified the novel DFMPA derivatives **1b, 1d** and **1e**, which acted as small molecular inhibitors for protein tyrosine phosphatase 1B (PTP-1B).[4,5,8] As a part of our program to create small molecular inhibitors of PTP-1B with a better inhibition profile, we became interested in the synthesis and biological evaluation of their DFMPTA analogues. In this paper, we describe an efficient method for the transformation of DFMPAs into DFMPTAs. We also report the results of preliminary studies on the inhibitory activity of the DFMPTA analogues against PTP-1B.

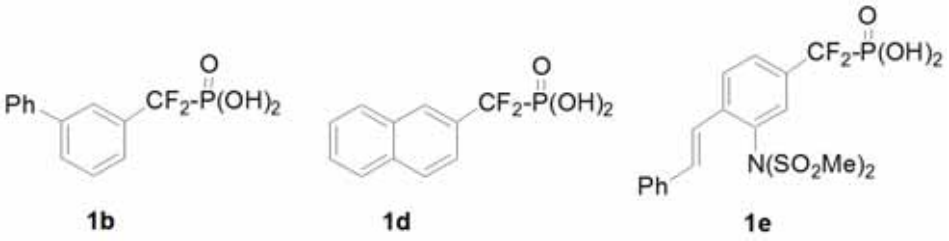


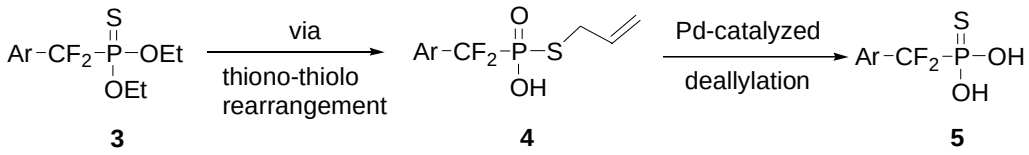
Figure 2

Results and Discussion

Synthesis of aryldifluoromethylphosphonothioic acids

Although *O*,*O*-diethyl aryldifluoromethylphosphonothioates **3** could be synthesized readily,[9] the deprotection of **3** to the free acids **5** using trimethylsilyl bromide (TMSBr), a conventional deprotecting reagent for diethyl phosphonates, was problematic.[7,9,10] Then, we recently devised a new method for converting **3** to **5** through a sodium iodide-assisted thiono–thiolo rearrangement

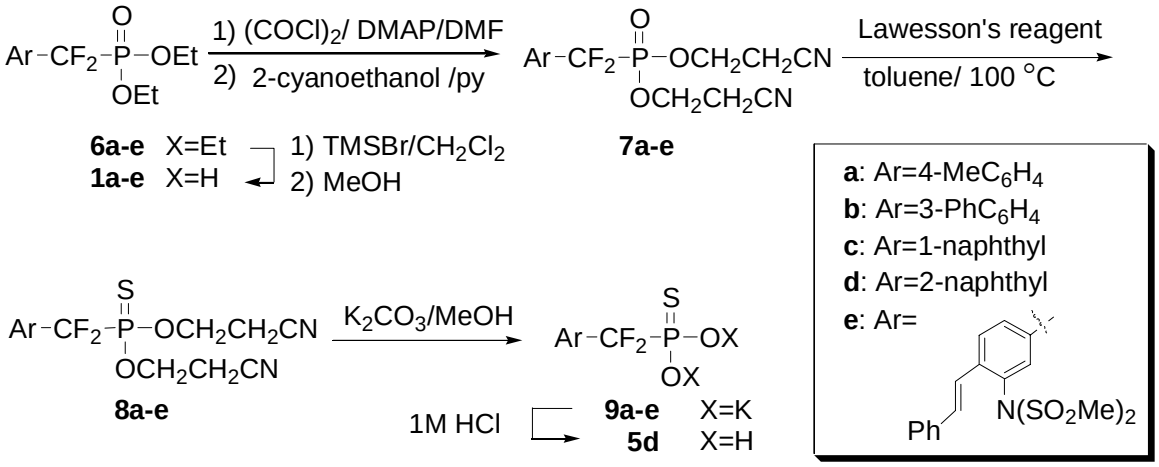
and the subsequent Pd-catalyzed de-allylation reaction, as shown in Scheme 1.[9] However, it was rather difficult to obtain reproducible yields in the de-allylation step in this sequence.



3 **4** **5**

Scheme 1

Therefore, in the present studies, we examined an alternative method for the synthesis of **5**, in which diethyl phosphonates **6a–e** were first transesterified with 2-cyanoethanol,[11] followed by sequential thionylation and deprotection of the 2-cyanoethyl group as shown in Scheme 2. The starting diethyl phosphonates **6a–e** were prepared according to the method described previously, and transformed into the corresponding free acids **1a–e** by TMSBr-mediated deprotection.[8,9,12] Treatment of **1a–e** with oxalyl chloride in boiling CH_2Cl_2 in the presence of *N*,*N*-dimethylaminopyridine (DMAP) and DMF for 10 h gave the corresponding dichlorides, which were subsequently treated with 2-cyanoethanol in pyridine to give the bis-(2-cyanoethyl) esters **7a–e** in 72–91% yield. Thionylation of **7a–e** with Lawesson's reagent [2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide] in toluene at 100 °C gave **8a–e** in 47–91% yield. Deprotection of the 2-cyanoethyl group was achieved with K_2CO_3 in MeOH to give the potassium salts **9a–e** in 47–76% yield. Acidification of **9d** with 1*M* HCl, followed by extraction with ether, gave the free acid **5d** in 83% yield.



Scheme 2

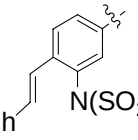
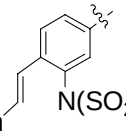
Physical properties and biological effects

To examine the features of DFMPTAs in their physical properties and biological effects, we first determined the octanol/water distribution coefficient (*D*) of **5d** by a shake-flask method.[13] The *D*

value of the phosphonothioic acid **5d** was estimated to be 0.89 at 25 °C, and is approximately 5-times larger than that (0.18) of the corresponding phosphonic acid analogue **1d**. Consequently, **5d** is sufficiently lipophilic to be extracted with organic solvents such as ether, whereas **1d** could not be extracted with ether from the aqueous solution. The second dissociation constants (pKa_2) of **5d** and **1d** were also determined to be 4.5 and 5.4, respectively, by a standard titration method. These results reveal that **5d** would exist mainly in the dianionic form at cellular pH (6.5) as in the case of **1d**, but might possess the property of enhancing cellular penetration, owing to its relatively high hydrophobicity.[14,15]

With these observations in mind, we next examined the inhibitory activity of **5d** against PTP-1B in comparison with that of **1d**. The results are shown in Table 1. The IC_{50} of **5d** was determined to be 418.5 μ*M*, which shows that **5d** possesses a slightly higher inhibitory potency than **1d**. While the inhibitor **5d** is slightly labile at room temperature, the corresponding potassium salt **9d** was found to be stable and shows approximately the same inhibitory potency as **1d**. Therefore, the inhibitory potencies for the DFMPTA analogues of **1b** and **1e** were evaluated by using their potassium salts **9b** and **9e**. However, **9b** and **9e** were 5- and 11-times, respectively, poorer inhibitors than their DFMPA analogues **1b** and **1e**.

Table 1. IC_{50} values of aryldifluoromethylphosphonic acids and aryldifluoromethyl-phosphonothioic acid derivatives against PTP-1B

$Ar\text{-}CF_2P(O)(OH)_2$	IC_{50} (μM)	$Ar\text{-}CF_2P(S)(OX)_2$	IC_{50} (μM)
1d (Ar=2-naphthyl)	718.1[a), b)]	**5d** (Ar=2-naphthyl, X=H)	418.5
		9d (Ar=2-naphthyl, X=K)	858.1
1b (Ar=3-PhC_6H_4)	252.2[b)]	**9b** (Ar=3-PhC_6H_4, X=K)	1204.3
1e (Ar= )	54.1[a)]	**9e** (Ar=  , X=K)	625.0

[a)] The value was obtained from ref 8. [b)] The IC_{50} values of **1d** and **1b** were determined by Taylor to be 35 μ*M* and 15 μ*M*, respectively, using different assay systems from ours (ref. 5a).

In summary, we have developed an efficient method for the preparation of novel (α,α-difluoromethylene)phosphonothioic acids from (α,α-difluoromethylene)phosphonic acids through thionylation and deprotection of their bis-(2-cyanoethyl) esters. The method allowed us to synthesize DFMPTA-derived aromatic compounds that possess moderate inhibitory potency against PTP-1B.

Experimental Section

General Procedures. All melting points are uncorrected. All reactions were carried out under a nitrogen atmosphere. NMR data were obtained on a Bruker DPX 400 for a solution in the indicated solvent, unless otherwise specified. ^{13}C-NMR (100 MHz) and ^{31}P-NMR (162 MHz) were taken with broadband ^{1}H decoupling. The chemical shifts of ^{31}P are recorded relative to external 85% H_3PO_4. ^{19}F-NMR spectra (376 MHz) were measured using benzotrifluoride (BTF) as an internal reference. PTP-1B was purchased from Upstate Biotech. Inc. "RT" denotes room temperature.

Typical example of preparation of aryldifluoromethylphosphonic acids. Difluoro-(4-methyl-phenyl)methylphosphonic acid (1a)

To a stirred solution of **6a**[12] (3.14 g, 11.3 mmol) in CH_2Cl_2 (23 mL) was added TMSBr (4.47 mL, 33.9 mmol). The mixture was stirred at RT for 20 h. The volatile component of the mixture was evaporated and the residue was treated with MeOH (23 mL) at 25 °C for 2 h. The solvent was removed *in vacuo* to leave a semi-solid. A crystalline material was collected, and washed with cold $CHCl_3$ to give **1a** (2.48 g, 98%). Decomp. 89–90 °C. ^{1}H NMR (CD_3OD) δ 7.47 (2H, d, J = 8.1 Hz), 7.26 (2H, d, J = 8.1 Hz), 2.36 (3H, s). ^{13}C-NMR (CD_3OD) δ 141.8, 132.1 (dt, J_{CP} = 13.4 Hz, J_{CF} = 22.3 Hz), 129.9, 127.2 (t, J_{CF} = 6.0 Hz), 120.0 (dt, J_{CP} = 213.8 Hz, J_{CF} = 260.2 Hz), 21.3. ^{19}F-NMR (CD_3OD) δ –51.3 (d, J_{PF} = 121.9 Hz). ^{31}P NMR (CD_3OD) δ 4.34 (t, J_{PF} = 121.9 Hz). IR (KBr) 1602, 1021, 901, 824 cm^{-1}. FAB-MS *m/z* 223 (MH^+). Anal. Calcd for $C_8H_9O_3F_2P.2/3H_2O$: C, 41.04; H, 4.45. Found: C, 40.86; H, 4.46.

[1,1'-Biphenyl]-3-yl(difluoro)methylphosphonic acid (1b). Prepared from **6b**[9] in 90% yield in a manner analogous to that for the preparation of **1a**. Decomp. 141 °C. ^{1}H NMR (CD_3OD) δ 7.83 (1H, s), 7.74 (1H, d, J = 7.6 Hz), 7.63 (2H, d, J = 7.6 Hz), 7.58 (1H, d, J = 7.6 Hz), 7.54 (1H, d, J = 7.6 Hz), 7.45 (2H, d, J = 7.6 Hz), 7.36 (1H, t, J = 7.6 Hz). ^{13}C NMR (CD_3OD) δ 142.6, 141.5, 135.6 (dt, J_{CP} = 13.4 Hz, J_{CF} = 22.1 Hz), 130.0 (2xC), 129.9, 128.8, 128.0, 126.1 (t, J_{CF} = 6.7 Hz), 125.9 (t, J_{CF} = 6.0 Hz), 119.8 (dt, J_{CP} = 212.1 Hz, J_{CF} = 260.9 Hz). ^{19}F-NMR (CD_3OD) δ –46.6 (d, J_{PF} = 113.3 Hz). ^{31}P-NMR (CD_3OD) δ 4.06 (t, J_{PF} = 113.3 Hz). IR (KBr) 3034, 1226, 1048, 928, 755, 701 cm^{-1}. ESI-MS *m/z* 285 (MH^+). Anal. Calcd for $C_{13}H_{11}O_3F_2P$: C, 54.94; H, 3.90. Found: C, 54.47; H, 4.06.

Difluoro(1-naphthyl)methylphosphonic acid (1c). Prepared from **6c**[12](5.43 g, 17.3 mmol) in 91% yield in a manner analogous to that for the preparation of **1a**. Mp 55–56 °C. ^{1}H-NMR (CD_3OD) δ 8.44 (1H, d, J = 8.0 Hz), 7.99 (1H, d, J = 8.2 Hz), 7.94–7.87 (1H, m), 7.81 (1H, d, J = 7.4 Hz), 7.57–7.46 (3H, m). ^{13}C-NMR (CD_3OD) δ 135.6, 132.7, 131.4, 130.8 (dt, J_{CP} = 13.1 Hz, J_{CF} = 20.2 Hz), 129.6, 127.7–127.3 (2xC), 127.0, 125.4, 121.8 (dt, J_{CP} = 211.2 Hz, J_{CF} = 261.4 Hz). ^{19}F-NMR (CD_3OD) δ –39.6 (d, J_{PF} = 112.3 Hz). ^{31}P-NMR (CD_3OD) δ 4.46 (t, J_{PF} = 112.3 Hz). IR (KBr) 1120, 1046, 797, 772 cm^{-1}. FAB-MS *m/z* 259 (MH^+). Anal. Calcd for $C_{11}H_9O_3F_2P.H_2O$: C, 47.84; H, 4.01. Found: C, 47.63; H, 4.44.

{3-[Bis-(methylsulfonyl)amino]-4-[(*E*)-2-phenylethenyl]phenyl}(difluoro)methylphosphonic acid (1e). Prepared from **6e**[8] (540 mg, 1 mmol) in 96% yield in a manner analogous to that for the preparation of **1a**. Mp 92–94 °C. ^{1}H NMR (CD_3OD) δ 7.99 (1H, d, J = 8.4 Hz), 7.74 (1H, d,

J = 8.4 Hz), 7.62 (1H, s), 7.57 (2H, d, *J* = 7.4 Hz), 7.47 (1H, d, *J* = 16.2 Hz), 7.38 (2H, t, *J* = 7.4 Hz), 7.33 (1H, d, *J* = 16.2 Hz), 7.30 (1H, t, *J* = 7.4 Hz), 3.45 (6H, s). ^{13}C NMR (CD_3OD) δ 142.1, 137.6, 135.4, 135.0 (dt, J_{CP} = 13.5 Hz, J_{CF} = 23.1 Hz), 133.1, 130.9, 130.0, 129.8, 129.4, 127.9, 123.6, 119.0 (dt, J_{CP} = 211.6 Hz, J_{CF} = 261.4 Hz), 43.4. ^{19}F NMR (CD_3OD) δ –47.1 (d, J_{PF} = 111.0 Hz). ^{31}P-NMR (CD_3OD) δ 5.43 (t, J_{PF} = 111.0 Hz). IR (KBr) 3040, 1369, 1159, 978, 764 cm^{-1}. FAB-MS *m/z* 482 (MH^+). Anal. Calcd for $C_{17}H_{18}NO_7F_2PS_2.2H_2O$: C, 39.46; H, 4.29; N, 2.71. Found: C, 39.25; H, 4.42; N, 2.69.

Typical example of preparation of bis-(2-cyanoethyl) phosphonates. bis-(2-Cyanoethyl) difluoro(4-methylphenyl)methylphosphonate (7a)

To a suspension of **1a** (10 mmol, 2.22 g) in CH_2Cl_2 (20 mL) containing DMF (78 mg, 1 mmol) and DMAP (122 mg, 1 mmol) was added oxalyl chloride (3.1 mL, 35 mmol) over a period of several minutes. The mixture was heated under reflux for 10 h, then concentrated by rotary evaporation to give the crude phosphonic acid dichloride. To a solution of the crude phosphonic acid dichloride in THF (5 mL) was added a solution of 2-cyanoethanol (1.7 mL, 25 mmol) and pyridine (2.0 mL, 25 mmol) in THF (5 mL) at 0 °C. After being stirred for 30 min at 0 °C, then 10 h at RT, the mixture was poured into saturated aq. NH_4Cl and extracted with chloroform. The extracts were washed with brine, dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (hexane:EtOAc=1:1) to give **11a** (2.39 g, 73%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.52 (2H, d, *J* = 7.8 Hz), 7.30 (2H, d, *J* = 7.8 Hz), 4.45–4.33 (2H, m), 4.32–4.21 (2H, m), 2.76–2.63 (4H, m), 2.41 (3H, s). ^{13}C NMR ($CDCl_3$) δ 141.7, 129.3, 128.0 (dt, J_{CP} = 14.2 Hz, J_{CF} = 22.1 Hz), 125.9 (dt, J_{CP} = 1.9 Hz, J_{CF} = 6.3 Hz), 117.7 (dt, J_{CP} = 223.3 Hz, J_{CF} = 263.3 Hz), 116.1, 62.7 (d, J_{CP} = 6.4 Hz), 21.2, 19.7 (d, J_{CP} = 5.7 Hz). ^{19}F-NMR ($CDCl_3$) δ –44.9 (d, J_{PF} = 124.9 Hz). ^{31}P-NMR ($CDCl_3$) δ 7.53 (t, J_{PF} = 124.9 Hz). IR (film) 2256, 1281, 1264, 1054, 1007, 820 cm^{-1}. EI-MS *m/z* 328 (M^+). HR-MS (EI) calcd for $C_{14}H_{15}N_2O_3F_2P$: 328.0788. Found: 328.0789.

Bis-(2-cyanoethyl) [1,1´-biphenyl]-3-yl-(difluoro)methylphosphonate (7b). Prepared from **1b** (5.48 g, 19.3 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=1:1) gave **7b** (4.82 g, 71%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, s), 7.77 (1H, d, *J* = 7.6 Hz), 7.65–7.61 (3H, m), 7.58 (1H, t, *J* = 7.6 Hz), 7.47 (2H, t, *J* = 7.4 Hz), 7.39 (1H, t, *J* = 7.4 Hz), 4.49–4.39 (2H, m), 4.34–4.24 (2H, m), 2.83–2.78 (4H, m). ^{13}C NMR ($CDCl_3$) δ 141.6, 139.3, 131.6 (dt, J_{CP} = 14.1 Hz, J_{CF} = 21.7 Hz), 129.8, 129.1, 128.7, 127.8, 126.9, 124.7 (t, J_{CF} = 5.4 Hz), 124.4 (t, J_{CF} = 6.0 Hz), 117.5 (dt, J_{CP} = 222.0 Hz, J_{CF} = 263.7 Hz), 116.1, 62.7 (d, J_{CP} = 6.4 Hz), 19.5 (d, J_{CP} = 5.6 Hz). ^{19}F-NMR ($CDCl_3$) δ –45.2 (d, J_{PF} = 122.4 Hz). ^{31}P NMR ($CDCl_3$) δ 7.33 (t, J_{PF} = 122.4 Hz). IR (film) 2256, 1281, 1226, 1048, 1006, 760 cm^{-1}. EI-MS *m/z* 390 (M^+). Anal. Calcd for $C_{19}H_{17}N_2O_3F_2P$: C, 58.47; H, 4.39; N, 7.18. Found: C, 58.75; H, 4.41; N, 7.01.

Bis-(2-cyanoethyl) difluoro(1-naphthyl)methylphosphonate (7c). Prepared from **1c** (5.31 g, 20.6 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=1:1) gave **7c** (6.4 g, 85%) as an oil. ^{1}H NMR ($CDCl_3$) δ 8.38 (1H, d, *J* = 8.7 Hz), 8.02 (1H, d, *J* = 8.3 Hz), 7.91 (1H, d, *J* = 8.1 Hz), 7.85 (1H, d, *J* = 7.4 Hz), 7.63–7.46 (3H, m), 4.47–4.32 (2H, m), 4.28–

4.15 (2H, m), 2.78–2.58 (4H, m). ^{13}C NMR ($CDCl_3$) δ 133.7, 132.4, 129.2, 128.6, 127.0, 126.6 (t, J_{CP} = 13.9 Hz, J_{CF} = 19.9 Hz), 126.3 (dt, J_{CP} = 3.6 Hz, J_{CF} = 9.8 Hz), 126.2, 125.0 (t, J_{CF} = 4.7 Hz), 124.3, 119.2 (dt, J_{CP} = 220.7 Hz, J_{CF} = 264.6 Hz), 116.1, 62.6 (d, J_{CP} = 6.5 Hz), 19.4 (d, J_{CP} = 5.9 Hz). ^{19}F-NMR ($CDCl_3$) δ –39.0 (d, J_{PF} = 120.9 Hz). ^{31}P NMR ($CDCl_3$) δ 7.50 (t, J_{PF} = 120.9 Hz). IR (film) 2256, 1282, 1127, 1081, 1039, 1005, 776 cm^{-1}. EI-MS *m/z* 364 (M^+). Anal. Calcd for $C_{17}H_{15}N_2O_3F_2P$: C, 56.05; H, 4.15; N, 7.69. Found: C, 55.77; H, 4.25; N, 7.81.

Bis-(2-cyanoethyl)difluoro(2-naphthyl)methylphosphonate (7d). Prepared from **1d**[12] (6.23 g, 24.1 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=1:1) gave **7d** (5.8 g, 72%) as colorless crystals (mp 82–84 °C). ^{1}H NMR ($CDCl_3$) δ 8.18 (1H, s), 8.02–7.94 (2H, m), 7.90 (1H, d, *J* = 8.8 Hz), 7.68 (1H, d, *J* = 8.8 Hz), 7.65–7.55 (2H, m), 4.50–4.22 (4H, m), 2.82–2.65 (4H, m). ^{13}C-NMR ($CDCl_3$) δ 134.2, 132.2, 128.8, 128.3 (dt, J_{CP} = 14.0 Hz, J_{CF} = 21.7 Hz), 127.9, 127.7, 127.0, 126.7 (dt, J_{CP} = 3.3 Hz, J_{CF} = 7.3 Hz), 122.1 (t, J_{CF} = 5.4 Hz), 117.8 (dt, J_{CP} = 222.8 Hz, J_{CF} = 263.7 Hz), 116.1, 62.8 (d, J_{CP} = 6.5 Hz), 19.7 (d, J_{CP} = 5.7 Hz). ^{19}F-NMR (376 MHz, $CDCl_3$) δ –44.9 (d, J_{PF} = 123.5 Hz). ^{31}P NMR ($CDCl_3$) δ 7.45 (t, J_{PF} = 123.5 Hz). IR (KBr) 2253, 1278, 1133, 1049, 745 cm^{-1}. EI-MS *m/z* 364 (M^+). Anal. Calcd for $C_{17}H_{15}N_2O_3F_2P$: C, 56.05; H, 4.15; N, 7.69. Found: C, 55.75; H, 4.28; N, 7.66.

Bis-(2-cyanoethyl) {3-[bis-(methylsulfonyl)amino]-4-[(*E*)-2-phenylethenyl]phenyl} (difluoro)-methylphosphonate (7e). Prepared from **1e** (4.53 g, 9.4 mmol). Column chromatography on silica gel (hexane:EtOAc=1:1) gave **7e** (3.91 g, 71%) as colorless crystals (mp 150–151 °C). ^{1}H-NMR (CD_3COCD_3) δ 8.14 (1H, d, *J* = 8.3 Hz), 7.81 (1H, d, *J* = 8.3 Hz), 7.77 (1H, s), 7.65 (2H, d, *J* = 7.5 Hz), 7.55 (1H, d, *J* = 16.5 Hz), 7.48 (1H, d, *J* = 16.5 Hz), 7.43 (2H, t, *J* = 8.3 Hz), 7.35 (1H, t, *J* = 8.3 Hz), 4.55–4.34 (4H, m), 3.56 (6H, s), 2.99–2.89 (4H, m). ^{13}C-NMR CD_3COCD_3) δ 142.8, 137.4, 135.7, 133.5, 132.7 (dt, J_{CP} = 14.0 Hz, J_{CF} = 22.7 Hz), 130.8 (t, J_{CF} = 5.7 Hz), 129.9, 129.8, 129.2 (t, J_{CF} = 5.6 Hz), 128.2, 127.9, 123.3, 118.1 (dt, J_{CP} = 221.6 Hz, J_{CF} = 263.8 Hz), 117.7, 64.6 (d, J_{CP} = 6.5 Hz), 43.8, 20.2 (d, J_{CP} = 6.0 Hz). ^{19}F NMR (CD_3COCD_3) δ –45.9 (d, J_{PF} = 117.5 Hz). ^{31}P NMR (CD_3COCD_3) δ 3.81 (t, J_{PF} = 117.5 Hz). IR (KBr) 3012, 2974, 2953, 2253, 1607, 1365, 1164, 1038 cm^{-1}. ESI-MS *m/z* 610 (MNa^+), 588 (MH^+). HR-MS (ESI) calcd for $C_{23}H_{25}N_3O_7F_2PS_2$ (MH^+): 588.0840. Found: 588.0853. Anal. Calcd for $C_{23}H_{24}N_3O_7F_2PS_2.3/2H_2O$: C, 44.95; H, 4.43; N, 6.84. Found: C, 45.02; H, 4.46; N, 6.88.

Typical example of thionylation of bis-(2-cyanoethyl) phosphonates with Lawesson's reagent. *O,O*-Bis-(2-cyanoethyl) difluoro(4-methylphenyl)methylphosphonothioate (8a). A solution of **7a** (2.41 g, 7.34 mmol) and Lawesson's reagent (1.78 g, 4.4 mmol) in toluene (7 mL) was heated at 100 °C for 30 min. After cooling to RT, the resulting precipitates were filtered off. The filtrates were evaporated and the residue was chromatographed on silica gel (hexane:EtOAc=4:1) to give **8a** (1.3 g, 52%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.48 (2H, d, *J* = 6.9 Hz), 7.27 (2H, d, *J* = 6.9 Hz), 4.44–4.32 (2H, m), 4.31–4.16 (2H, m), 2.82–2.60 (4H, m), 2.39 (3H, s). ^{13}C NMR ($CDCl_3$) δ 141.5, 129.0, 127.9 (dt, J_{CP} = 15.3 Hz, J_{CF} = 22.5 Hz), 126.6 (t, J_{CF} = 3.1 Hz), 119.0 (dt, J_{CP} = 185.3 Hz, J_{CF} = 268.5 Hz), 116.1, 62.5 (d, J_{CP} = 6.0 Hz), 21.3, 19.6 (d,

J_{CP} = 6.6 Hz). ^{19}F NMR ($CDCl_3$) δ –44.5 (d, J_{PF} = 130.9 Hz); ^{31}P NMR ($CDCl_3$) δ 78.3 (t, J_{PF} = 130.9 Hz). IR (film) 2254, 1263, 1101, 1028, 1004, 801 cm^{-1}. EI-MS *m/z* 344 (M^+). HR-MS (EI) calcd for $C_{14}H_{15}N_2O_2F_2PS$: 344.0560. Found: 344.0553.

***O,O*-Bis-(2-cyanoethyl) [1,1'-biphenyl]-3-yl(difluoro)methylphosphonothioate (8b).** Prepared from **7b** (4.62 g, 11.8 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=6:1) gave **8b** (2.3 g, 47%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.94 (1H, s), 7.85 (1H, d, *J* = 8.9 Hz), 7.73–7.57 (4H, m), 7.52 (2H, t, *J* = 9.0 Hz), 7.42 (1H, t, *J* = 9.0 Hz), 3.93–3.76 (2H, m), 3.72–3.57 (2H, m), 1.76 (4H, t, *J* = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 141.4, 139.8, 131.5 (dt, J_{CP} = 15.1 Hz, J_{CF} = 22.0 Hz), 129.9, 128.9, 127.9, 127.1, 125.5, 125.4, 118.9 (dt, J_{CP} = 183.5 Hz, J_{CF} = 268.8 Hz), 116.1, 62.6 (d, J_{CP} = 6.0 Hz), 19.6 (d, J_{CP} = 6.5 Hz). ^{19}F-NMR ($CDCl_3$) δ –44.9 (d, J_{PF} = 127.5 Hz). ^{31}P-NMR ($CDCl_3$) δ 78.2 (t, J_{PF} = 127.5Hz). IR (film) 2255, 1226, 1101, 1048, 1003, 802, 758, 702 cm^{-1}. ESI-MS *m/z* 407 (MH^+). HR-MS (ESI) calcd for $C_{19}H_{18}N_2O_2F_2PS$ (MH^+): 407.0795. Found: 407.0803.

***O,O*-Bis-(2-cyanoethyl) difluoro(1-naphthyl)methylphosphonothioate (8c).** Prepared from **7c** (2.2 g, 6.0 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=3:1) gave **8c** (1.0 g, 49%) as an oil. ^{1}H NMR ($CDCl_3$) δ 8.38 (1H, d, *J* = 8.6 Hz), 8.00 (1H, d, *J* = 8.2 Hz), 7.90 (1H, d, *J* = 7.2 Hz), 7.81 (1H, d, *J* = 7.2 Hz), 7.64–7.52 (3H, m), 4.44–4.28 (2H, m), 4.22–4.12 (2H, m), 2.63 (4H, t, *J* = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ 133.7, 132.3, 129.5, 128.7, 127.7 (dt, J_{CP} = 3.3 Hz, J_{CF} = 9.7 Hz), 126.8, 126.4 (dt, J_{CP} = 14.7 Hz, J_{CF} = 19.9 Hz), 126.0, 125.4 (t, J_{CF} = 6.0 Hz), 124.3, 120.6 (dt, J_{CP} = 183.0 Hz, J_{CF} = 270.1 Hz), 116.1, 62.4 (d, J_{CP} = 6.0 Hz), 19.3 (d, J_{CP} = 6.6 Hz). ^{19}F-NMR ($CDCl_3$) δ –37.5 (d, J_{PF} = 128.1 Hz). ^{31}P-NMR ($CDCl_3$) δ 78.3 (t, J_{PF} = 128.1 Hz). IR (film) 1594, 1261, 1115, 1027 cm^{-1}. EI-MS *m/z* 380 (M^+). HR-MS (EI) calcd for $C_{17}H_{15}N_2O_2F_2PS$: 380.0560. Found: 380.0566.

***O,O*-Bis-(2-cyanoethyl) difluoro(2-naphthyl)methylphosphonothioate (8d).** Prepared from **7d** (1.67 g, 4.58 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=5:1) gave **8d** (1.59 g, 91%) as colorless crystal (mp 54–55 °C). ^{1}H-NMR ($CDCl_3$) δ 8.15 (1H, s), 8.00–7.86 (3H, m), 7.66 (1H, d, *J* = 8.6 Hz), 7.63–7.55 (2H, m), 4.45–4.33 (2H, m), 4.31–4.19 (2H, m), 2.70 (4H, t, *J* = 6.1 Hz). ^{13}C NMR ($CDCl_3$) δ 134.2, 132.1, 128.8, 128.3, 128.2 (dt, J_{CP} = 18.6 Hz, J_{CF} = 25.6 Hz), 127.8, 127.7, 127.3 (dt, J_{CP} = 3.3 Hz, J_{CF} = 6.7 Hz), 126.9, 122.9 (t, J_{CF} = 5.6 Hz), 119.0 (dt, J_{CP} = 185.1 Hz, J_{CF} = 268.8 Hz), 116.2, 62.6 (d, J_{CP} = 6.0 Hz), 19.6 (d, J_{CP} = 6.5 Hz). ^{19}F NMR ($CDCl_3$) δ –44.4 (d, J_{PF} = 129.6 Hz). ^{31}P-NMR ($CDCl_3$) δ 78.0 (t, J_{PF} = 129.6 Hz). IR (film) 2254, 1419, 1262, 1071, 1029, 1002, 795 cm^{-1}. EI-MS *m/z* 380 (M^+). Anal. Calcd for $C_{17}H_{15}N_2O_2F_2PS.1/2H_2O$: C, 52.44; H, 4.14; N, 7.19. Found: C, 52.08; H, 4.08; N, 6.96.

***O,O*-Bis-(2-cyanoethyl) {3-[bis-(methylsulfonyl)amino]-4-[(*E*)-2-phenylethenyl]phenyl}-(difluoro)-methylphosphonothioate (8e).** Prepared from **7e** (2.92 g, 4.97 mmol). Purification by column chromatography on silica gel (hexane:EtOAc=1:1) gave **8e** (2.22 g, 74%) as crystals (mp 148–149 °C). ^{1}H NMR ($CDCl_3$) δ 7.90 (1H, d, *J* = 8.3 Hz), 7.75 (1H, d, *J* = 8.3 Hz), 7.56 (1H, s), 7.55 (2H, d, *J* = 7.3 Hz), 7.46 (1H, d, *J* = 16.2 Hz), 7.41 (2H, t, *J* = 7.3 Hz), 7.34 (1H, t,

J = 7.3 Hz), 7.22 (1H, d, *J* = 16.2 Hz), 4.46–4.33 (2H, m), 4.32–4.20 (2H, m), 3.45 (6H, s), 2.78–2.66 (4H, m). ^{13}C NMR ($CDCl_3$) δ 141.7, 135.8, 135.3, 131.7, 131.2 (dt, J_{CP} = 15.1 Hz, J_{CF} = 23.1 Hz), 129.9, 129.2, 129.1, 129.0, 127.2, 127.0, 122.2, 117.9 (dt, J_{CP} = 185.8 Hz, J_{CF} = 269.6 Hz), 116.3, 62.9 (d, J_{CP} = 5.9 Hz), 43.4, 19.6 (d, J_{CP} = 6.9 Hz). ^{19}F NMR ($CDCl_3$) δ –45.9 (d, J_{PF} = 126.6 Hz). ^{31}P-NMR ($CDCl_3$) δ 76.7 (t, J_{PF} = 126.6 Hz). IR (KBr) 3020, 2937, 2256, 1605, 1363, 1167, 1029 cm^{-1}. ESI-MS *m/z* 604 (MH^+). HR-MS (ESI) calcd for $C_{23}H_{25}N_3O_6F_2PS_3$ (MH^+): 604.0611. Found: 604.0615.

Typical deprotection procedure for the bis-(2-cyanoethyl) phosphonothioates

Potassium difluoro(4-methylphenyl)methylphosphonothioate (9a). To a stirred solution of **8a** (1.15g, 3.34 mmol) in MeOH (15 mL) was added K_2CO_3 (1.38 g, 10.1 mmol). After being stirred at RT for 2.5 h, the mixture was diluted with water and extracted with ether (3x10 mL). The volatile component of the aqueous extracts was removed *in vacuo* and the resulting precipitates were purified by reversed-phase column chromatography on ODS. Elution with water gave **9a** (798 mg, 76%) as colorless crystals (mp>250 °C). The spectroscopic data of **9a** were identical to those of the authentic sample prepared previously.[9]

Potassium [1,1´-biphenyl]-3-yl(difluoro)methylphosphonothioate (9b). Prepared from **8b** (2.06 g, 5.07 mmol). Purification by column chromatography on ODS (water) gave **9b** (1.38 g, 72%) as colorless crystals (mp>250 °C). The spectroscopic data of **9b** were identical to those of the authentic sample prepared previously.[9]

Potassium difluoro(1-naphthyl)methylphosphonothioate (9c). Prepared from **8c** (910 mg, 2.39 mmol). Purification by column chromatography on ODS (water) gave **9c** (400 mg, 47%) as colorless crystals (mp>250 °C). The spectroscopic data of **9c** were identical to those of the authentic sample prepared previously.[9]

Potassium difluoro(2-naphthyl)methylphosphonothioate (9d). Prepared from **8d** (1.22 g, 3.21 mmol). Purification by column chromatography on ODS (water) gave **9d** (843 mg, 75%) as colorless crystals (mp>250 °C). The spectroscopic data of **9d** were identical to those of the authentic sample prepared previously.[9]

Potassium {3-[bis-(methylsulfonyl)amino]-4[(*E*)-2-phenylethenyl]phenyl}(difluoro)methyl phos-phonothioate (9e). Prepared from **8e** (1.24 g, 2.05 mmol). Purification by column chromatography on ODS (water) gave **9e** (563 mg, 48%) as colorless crystals (decomp. 197–198 °C). ^{1}H NMR (D_2O) δ 7.81 (1H, d, *J* = 8.2 Hz), 7.70 (1H, d, *J* = 8.2 Hz), 7.65 (1H, s), 7.48 (2H, d, *J* = 6.9 Hz), 7.29 (2H, t, *J* = 6.9 Hz), 7.26–7.17 (3H, m), 3.44 (6H, s). ^{13}C-NMR (D_2O) δ 139.6, 137.3 (dt, J_{CP} = 12.8 Hz, J_{CF} = 23.4 Hz), 136.5, 134.4, 130.4, 129.4, 129.3, 129.2, 127.2, 126.9, 122.3, 121.7 (dt, J_{CP} = 136.8 Hz, J_{CF} = 273.2 Hz), 43.3. ^{19}F NMR (D_2O) δ –42.3 (d, J_{PF} = 94.7 Hz). ^{31}P-NMR (D_2O) δ 45.7 (t, J_{PF} = 94.7 Hz). IR (KBr) 3388, 1633, 1366, 1161, 1009, 763, 523 cm^{-1}. ESI-MS *m/z* 574 (MH^+). HR-MS (ESI) calcd for $C_{17}H_{17}NO_6F_2K_2PS_3$ (MH^+): 573.9198. Found: 573.9211.

Difluoro(2-naphthyl)methylphosphonothioic acid (5d). The potassium salts **9d** (600 mg, 1.58 mmol) were dissolved in water, and the solution was acidified with 1*M* HCl. The mixture

was extracted with ether (5x10 mL), and the extracts dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on ODS. Elution with water gave **5d** (360 mg, 83%) as colorless crystals (decomp. 81–83 °C). ^{1}H NMR (D_2O) δ 8.03 (1H, s), 7.82–7.69 (3H, m), 7.59 (1H, d, J = 8.6 Hz), 7.43–7.36 (2H, m). ^{13}C-NMR (D_2O) δ 136.4, 134.8, 133.7 (dt, J_{CP} = 13.4 Hz, J_{CF} = 22.3 Hz), 131.3, 130.7, 130.5, 130.3, 129.6, 129.4 (dt, J_{CP} = 2.1 Hz, J_{CF} = 7.2 Hz), 126.2 (t, J_{CF} = 5.8 Hz), 123.6 (dt, J_{CP} = 157.4 Hz, J_{CF} = 265.8 Hz). ^{19}F NMR (D_2O) δ –43.1 (d, J_{PF} = 110.5 Hz). ^{31}P-NMR (D_2O) δ 56.4 (t, J_{PF} = 110.5 Hz). IR (KBr) 3637, 3059, 1687, 1508, 1191, 948, 861, 814, 748 cm^{-1}. FAB-MS *m/z* 275 (MH^+). HR-MS (FAB) calcd for $C_{11}H_{10}O_2F_2PS$ (MH^+): 275.0107. Found: 275.0117.

Assay and inhibition of PTP-1B. PTP-1B activity was assessed according to the manufacturer's instructions. Briefly, the activity of PTP-1B was assessed at 25 °C in 96-well plates with *p*-nitrophenyl phosphate (*p*NPP) as substrate. The assay mixture contained 5 μL of 40 mM $NiCl_2$ in water, 5 mM of a bovine serum albumin solution (5 mg/mL in water), 5 μL of PTP-1B-agaroses (0.05 units) and 65 μL of 50 m*M* Tris-HCl buffer (pH 7.0)/0.1 m*M* $CaCl_2$ that, if indicated, contained various concentrations of inhibitor, and pre-incubation followed for 15 min. The enzyme reaction was started by the addition of 120 μL of a *p*NPP solution (1.5 mg/mL in 50 m*M* Tris-HCl buffer). After incubation for 30 min, the reaction was stopped by adding 20 mL of a 13% (w/v) K_2HPO_4 solution, and the absorbance at 405 nm was measured. The non-enzymatic hydrolysis of *p*NPP was corrected by measuring the control without the addition of enzyme. IC_{50} values were determined as the concentration of compounds that give 50% of the control enzyme activity. The experiments were carried out in triplicate at 5 or 8 different inhibitor concentrations. The inhibitor concentrations were plotted as the y-axis and the remaining activity (%) as the x-axis, and the concentration of inhibitor that give a 50% inhibition was calculated, using the curve-fit equation, CA-Cricket Graph III.

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