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A re-examination of the difluoromethylenesulfonic acid group as a phosphotyrosine mimic for PTP1B inhibition

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ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is involved in the down-regulation of insulin signaling and is a well-validated therapeutic target for the treatment of diabetes and obesity. Key to the design of potent inhibitors of PTP1B is a moiety that effectively mimics the phosphate group of the natural phosphotyrosine substrate. Difluoromethylsulfonomethylphenylalanine (F_2Smp) is one of the best monoanionic pTyr mimics reported to date. However, the difluoromethylenesulfonic acid (DFMS) group as a phosphate mimic has not been carefully evaluated in the context of a non-peptidyl platform. Here we present a careful examination of the DFMS group as a phosphate mimic. This was achieved by first constructing an analog of a previously reported high affinity, non-peptidyl PTP1B inhibitor (compound **2**, IC_{50} = 8 nM) in which a difluoromethylenephosphonic acid group is replaced with the DFMS moiety (compound **6**). We also report the synthesis of its non-fluorinated methylenesulfonic analog (compound **7**), as well as two other derivatives in which a distal sulfonamide moiety is replaced with a difluoromethylenesulfonamide group (compounds **8** and **9**). Compounds **2** and **6**–**9** were examined as PTP1B inhibitors. Replacing the distal sulfonamide moiety with a difluoromethylenesulfonamide group had only a modest effect on inhibitor potency. However, compound **6** was approximately a 1000-fold poorer inhibitor than compound **2**. Most significantly, inhibition studies with compound **7** and a peptide bearing sulfonomethylphenylalanine revealed that the fluorines have little effect on the potency of the DFMS-bearing inhibitors. This is in contrast to a previous assumption that the fluorines in DFMS-bearing inhibitors contributed significantly to their potency. This may in part explain the large difference in potency between the DFMS and DFMP-bearing compounds. These results also demonstrate that sulfonomethylphenylalanine, a pTyr mimic that is readily constructed, is a relatively good pTyr mimic in comparison to most others that have been reported when examined in the context of the DADE-X-LNH_2 peptide platform.

1. Introduction

Protein tyrosine phosphatases (PTPs) catalyze the dephosphorylation of phosphotyrosine residues in peptides and proteins. Among the approximately 100 human tyrosine phosphatases that have been identified so far,[1] protein tyrosine phosphatase 1B (PTP1B) has garnered the most attention as far as inhibitor design is concerned.[2] PTP1B is involved in the down-regulation of insulin signaling and is considered to be a well-validated therapeutic target for the treatment of diabetes and obesity.[3] A key component in the design of PTP1B inhibitors and indeed, almost all phosphatase inhibitors, is the presence of a hydrolytically stable phosphotyrosine (pTyr) or phosphate mimic and dozens of such mimics have been reported.[4] One of the earliest, and still one of the most effective mimics, is the difluoromethylenephosphonic acid (DFMP, $-CF_2PO_3^{-2}$) group.[5] A wide variety of highly potent PTP1B inhibitors, such as peptide **1**[5a–c] (K_i = 24 nM)[6] and small molecule **2** (IC_{50} = 8 nM),[7] have been prepared bearing this mimic (Fig. 1). However, the potential problems that are associated with developing highly polar and charged phosphonate compounds into therapeutics have led to an intense search for phosphate mimics that are less highly charged yet still contribute significantly to inhibitor potency when incorporated into a small molecule platform. Several years ago we examined the difluoromethylene sulfonic acid (DFMS, $-CF_2SO_3^-$) group as a phosphate mimic for PTP1B inhibition.[8a,b] We reasoned that the DFMS group would be an effective monoanionic phosphate mimic for several reasons. First, Desmarais and coworkers examined a series of peptides bearing sulfotyrosine (sTyr or sY) as PTP1B inhibitors and found that even relatively simple peptides, such as AcDE(sY)L, are good reversible competitive inhibitors with IC_{50}'s in the low μM region which indicates that these peptides

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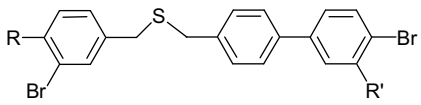
bind almost as well as the best peptide substrates.[9] These results suggested to us that the substitution of the phosphorus in pTyr for a sulfur (to sTyr) does not have a serious deleterious effect on ligand binding. Second, it has been shown that peptide **1** is almost 1000 times more effective as a pTyr mimic than the corresponding peptide bearing phosphonomethylphenylalanine (the non-fluorinated analog of peptide **1**).[5a,c] Moreover, pH and crystallographic studies suggest that the inhibitory-enhancing effect of the fluorines in DFMP-bearing inhibitors is due to H-bonding of the fluorines with residues in the active site and not due to a pK_a effect.[5c,10] Although the DFMS-bearing peptide **3** was found to be over 100-fold (IC_{50} = 10 μM) less potent a competitive PTP1B inhibitor than peptide **1**, the DFMS group is still a much more effective phosphate mimic than other monoanionic mimics that have been evaluated in this hexapeptide platform.[8b,11] This 100-fold difference in potency was in stark contrast to the only 6-fold difference in potency between naphthyl sulfonate compound **4** (IC_{50} = 175 μM) and its phosphonate analog **5** (IC_{50} = 35 μM), two relatively simple non-peptidyl competitive inhibitors.[8a] One possible reason for the 100-fold versus 6-fold difference is that the peptide scaffold may be preventing the F_2Smp residue from forming optimal interactions in the active site as suggested by Gao et al. for other monoanionic pTyr mimics.[11] However, neither compound **4** nor **5** was q highly potent inhibitor and, consequently, this simple naphthyl platform may not be the most effective platform for assessing the DFMS group as a phosphate mimic in a non-peptidyl platform. In order to make a more complete assessment of the DFMS group as a phosphate mimic for PTP1B inhibition, we wished to replace the DFMP group in a potent a non-peptidyl competitive inhibitor, such as compound **2**,[7] with a DFMS group and then compare their inhibitory potencies. In this report, we describe an effective synthesis of inhibitor **2**, its DFMS analog **6**, and its non-fluorinated methylenesulfonyl analog **7** (Figs. 1 and 2). The synthesis of two other derivatives, **8** and **9**, in which the sulfonamide moiety in compounds **2** and **6** is replaced with a difluoromethylenesulfonamide group is also described. Inhibition studies with these compounds and PTP1B reveal surprising results concerning the use of benzylicsulfonates as PTP1B inhibitors.

2. Results and discussion

2.1. Syntheses

The general approach to the synthesis of compounds **2** and **6**–**9** is outlined in Scheme 1.[12a] The protected aryl phosphonate or sulfonate portions (**10**–**12**) would be prepared bearing a thioacetate which, upon thiol deprotection, would be reacted in situ with the biphenyl sulfonamide portions, **13** and **14**, bearing a benzylic bromide moiety. Deprotection of the phosphonate and sulfonate would yield the final compounds.

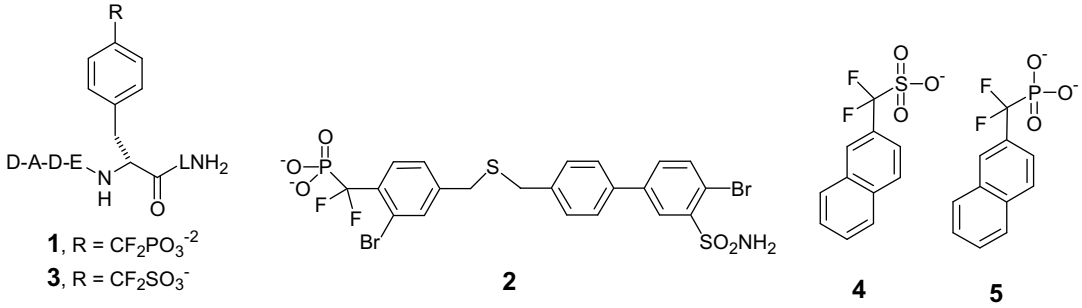


6, R = $CF_2SO_3^{-1}$, R' = SO_2NH_2
7, R = $CH_2SO_3^{-1}$, R' = SO_2NH_2
8, R = $CF_2PO_4^{-2}$, R' = $CF_2SO_2NH_2$
9, R = $CF_2SO_3^{-1}$, R' = $CF_2SO_2NH_2$

Figure 2. Structures of compounds **6**–**9**.

Although Romsicki et al. reported[7] that the synthesis of inhibitor **2** is described in a patent by Sing et al., we were unable to find any mention of **2** in this document.[12a] The preparation of the phosphonate portion of compound **2**, phosphonate **10**, was described,[12a] however, the synthesis of compound **2** itself as well as the biphenyl sulfonamide portion, compound **13**, was not. Compound **2** is just one of a series of similar compounds reported by MerckFrosst,[7,12a–c] several of which are potent PTP1B inhibitors and some of these compounds have proven to be useful as tools for elucidating the role of PTP1B in signaling pathways.[7,13a,b] The *ortho*-bromo phosphonate portion is a common feature of this series of inhibitors and so an efficient synthesis of phosphonate **10** as well as its precursors is of considerable interest. Sing et al. prepared phosphonate **10** in seven steps in an overall 17% yield starting from readily prepared ethyl 4-amino-3-bromobenzoate (Scheme 2).[12a] In this synthesis, the fluorines were introduced by subjecting α-ketophosphonate **19** to DAST, which gave the corresponding α,α-difluoro derivative **20** in a 46% yield. Although this is a viable synthesis of **10** we wished to avoid using DAST for safety reasons and also reduce the number of steps. We reasoned that a more efficient and DAST-free synthesis could be achieved by introducing the fluorines by electrophilic fluorination of a benzylic phosphonate.[14a–c] Thus, phosphonate **22** was prepared in 94% yield from benzyl bromide **21**[15] via a Michaelis–Arbuzov reaction using triethylphosphite (Scheme 3). Electrophilic fluorination of phosphonate **22** using NaHMDS and *N*-fluorobenzenesulfonimide (NFSi)[14a–c] gave fluorophosphonate **23** in 57% yield. Reduction of the ester using $NaBH_4$ gave alcohol **24** in 72% yield which was converted into bromide **20** in 83% yield using CBr_4/PPh_3. Finally, reaction of bromide **20** with potassium thioacetate gave phosphonate **10** in 92% yield. Thus, phosphonate **10** was prepared in just five steps with an overall yield of 29%, a significant improvement over the previously reported procedure.

The sulfonate analog of **10**, compound **11**, was prepared using a strategy similar to that used for phosphonate **10** in that a benzylic sulfonate was prepared and then subjected to electrophilic fluorination (Scheme 4). Reaction of benzyl bromide **21** with potassium thioacetate gave thioester **25** in 99% yield. Oxidative chlorination

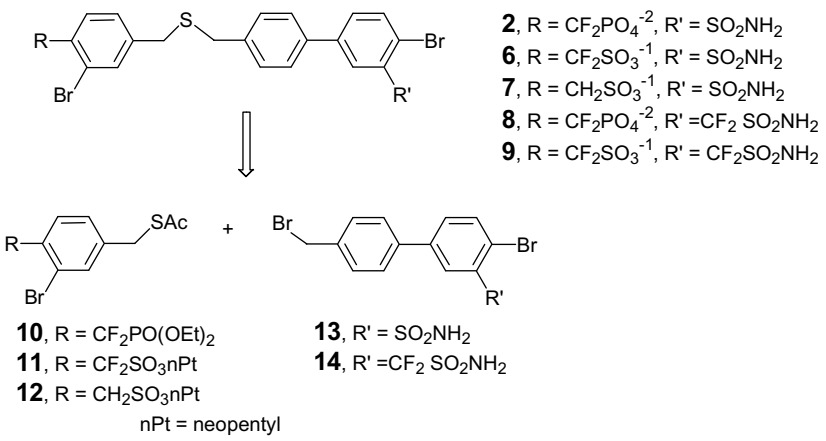


1, R = $CF_2PO_3^{-2}$
3, R = $CF_2SO_3^-$

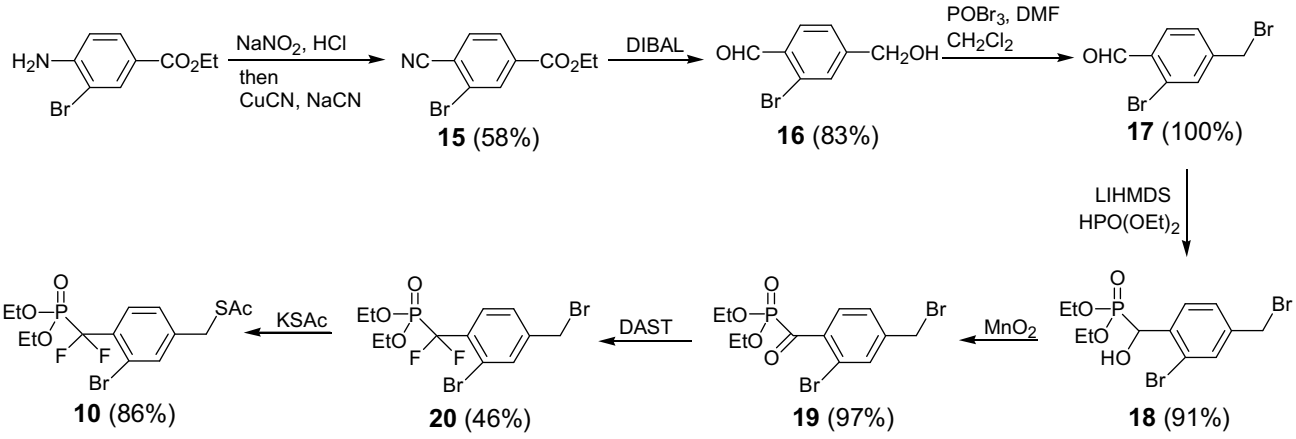
2

4 **5**

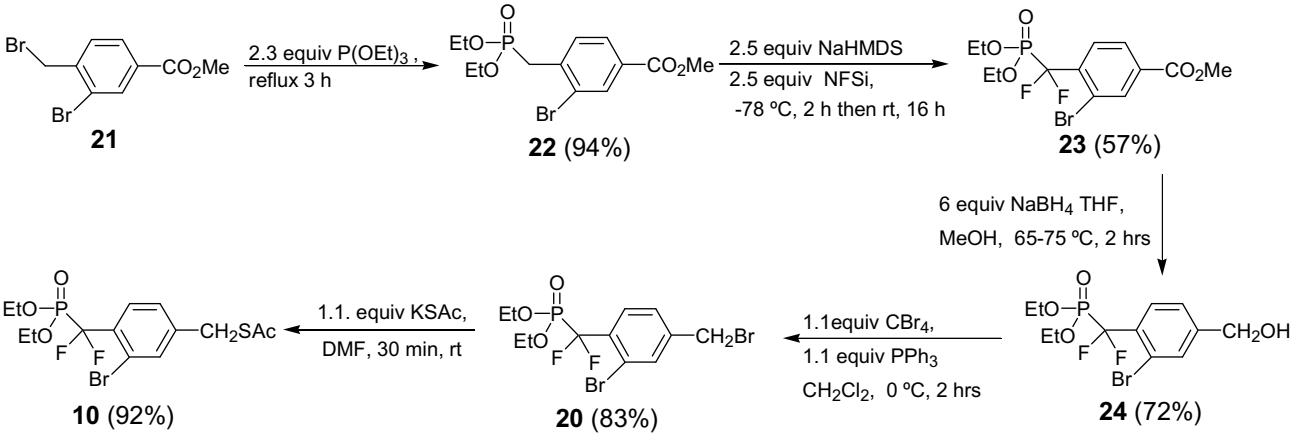
Figure 1. PTP1B inhibitors **1**–**5**.



Scheme 1.



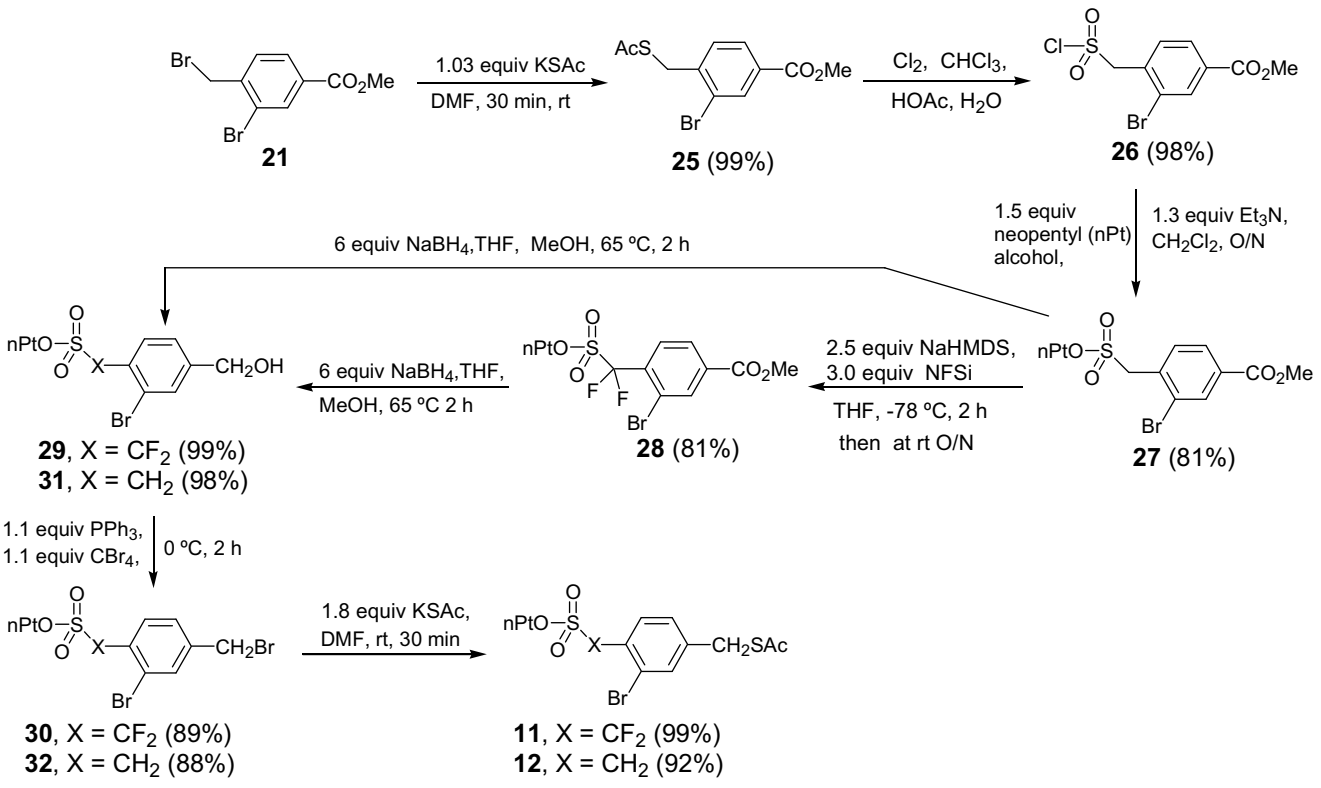
Scheme 2.



Scheme 3.

of **25** gave chlorosulfonate **26**, which was isolated in a 98% yield. Chlorosulfonate **26** was converted into neopentyl (nPt) ester **27** in 81% yield using neopentyl alcohol and triethylamine. Electrophilic fluorination[8b,16a,b] of benzylic sulfonate **27** proceeded well giving difluoromethylenesulfonate **28** in 81% yield. Reduction of the ester moiety in **28**, bromination of the resulting alcohol **29**, and conversion of benzylbromide **30** to sulfonate **11** were performed using the same procedures described above for the synthesis of **10** and all three reactions proceeded in excellent yields. The overall yield of sulfonate **11** from benzyl bromide **21** was 54%. The non-fluorinated sulfonate **12** was prepared from ester **27** using the same reduction, bromination, and thioacetylation procedure and all three of these reactions proceeded in excellent yield (Scheme 4, compounds **31** and **32**).

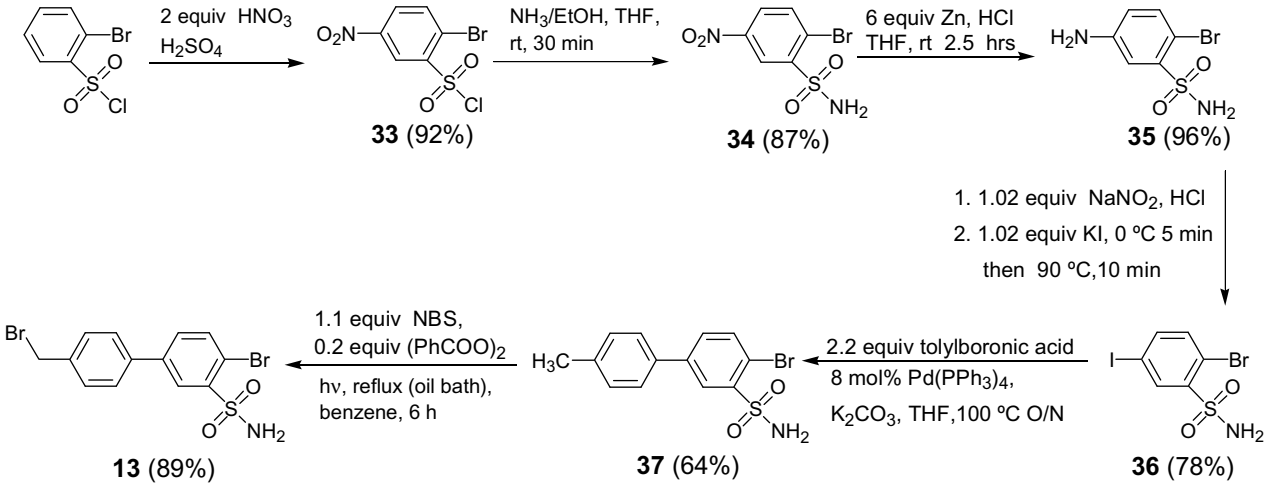
As mentioned previously, we were unable to find any description in the primary or patent literature of the biphenyl sulfonamide



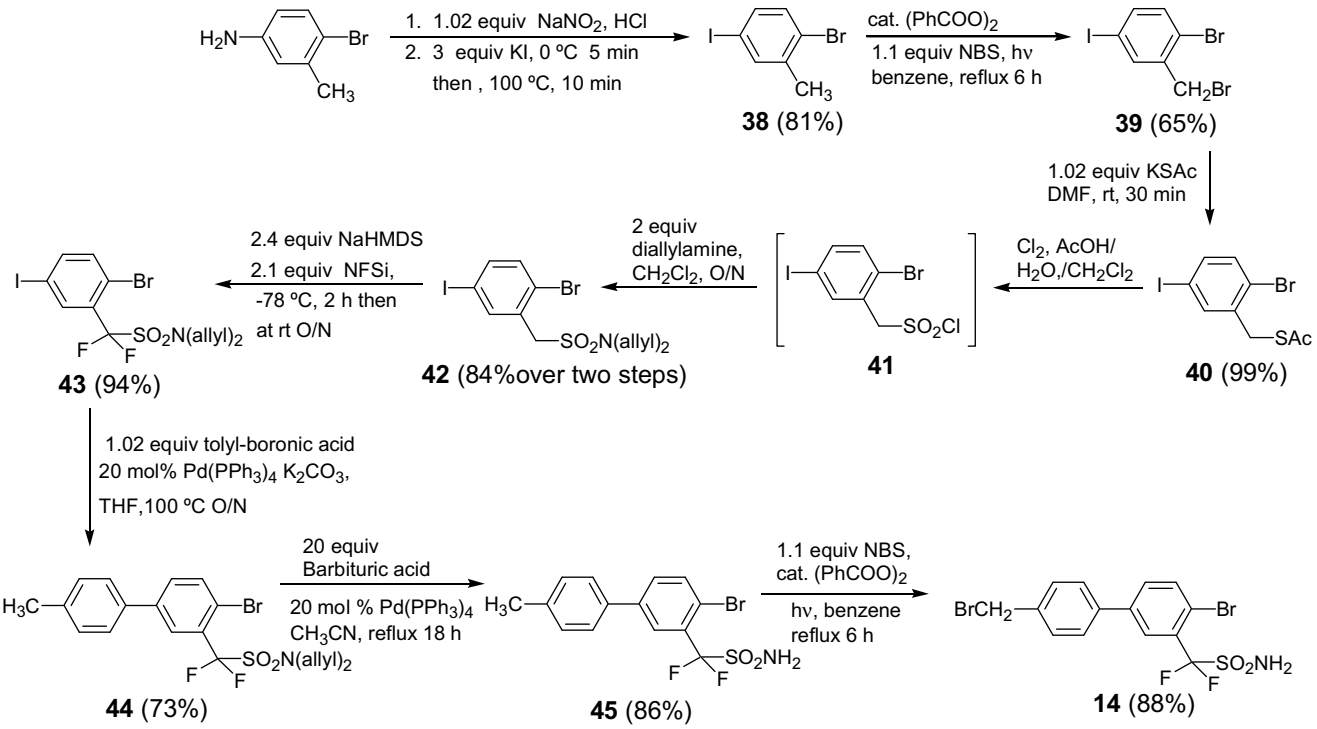
Scheme 4.

portion of inhibitor **2**, compound **13**. Our synthesis of compound **13** is shown in Scheme 5. Nitration of commercially available 2-bromobenzenesulfonyl chloride gave compound **33** in an isolated 96% yield.[17] Reaction of the sulfonyl chloride with ammonia in ethanol gave sulfonamide **34** in 87% yield.[17] The nitro group in **34** was readily reduced to the amine **35** in 96% yield using Zn/HCl. Diazotization of **35** followed by reaction of the diazonium salt with KI gave compound **36** in 78% yield. A tolyl group was installed using a Suzuki reaction which gave biphenyl derivative **37** in 64% yield. Finally, sulfonamide **13** was prepared in 89% yield by irradiating a refluxing solution (oil bath) of **37** and NBS in the presence of a catalytic amount of benzoylperoxide with a 250 W lamp. The overall yield of **13** was 36%.

Our synthesis of biphenyl α,α-difluorosulfonamide **14** is outlined in Scheme 6. 4-Bromo-3-methylaniline was converted to iodobenzene derivative **38** in 81% yield by diazotization and then reaction of the diazonium salt with KI (Scheme 6).[18] Bromination of **38** using the same procedure as for the bromination of **37** (NBS, benzoylperoxide, *hν*), where the reaction was heated to reflux using an oil bath, resulted in the formation of a significant quantity of deiodinated material. However, it was found that by heating the reaction to gentle reflux with the heat generated from the lamp (no oil bath), deiodination could be kept to a minimum and the desired benzyl bromide derivative **39** was obtained in a 65% yield after careful chromatography. Compound **39** was reacted with KSAc and the



Scheme 5.

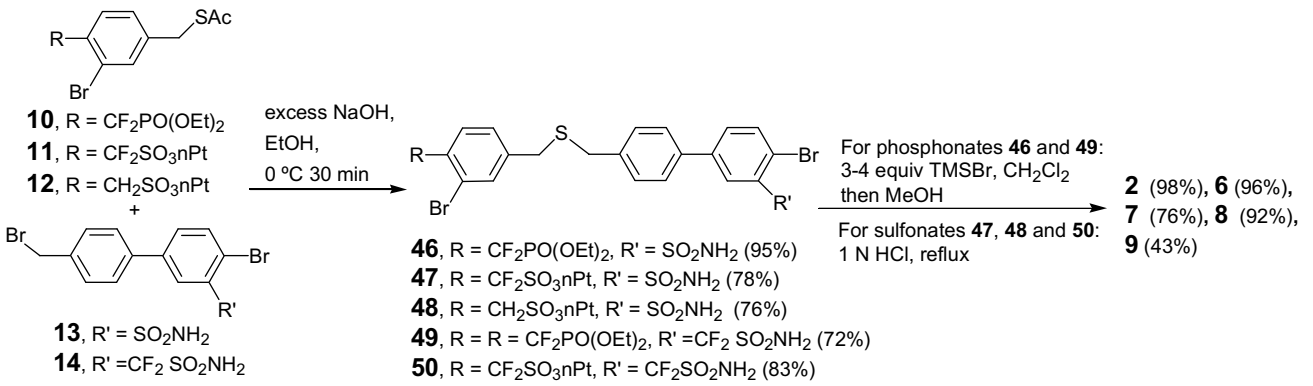


Scheme 6.

resulting thioacetate **40** (99% yield) was subjected to oxidative chlorination to give crude sulfonyl chloride **41**. We were unable to isolate **41** in pure form and so crude **41** was reacted with diallyl amine to give protected sulfonamide **42** in an 84% yield (two steps). Once again, we found that the fluorines could be readily introduced by electrophilic fluorination of benzylic sulfonamide **42** using NaHMDS/NFSi.[19a,b] This gave the desired difluoromethylenesulfonamide **43** in an outstanding 94% yield. A Suzuki reaction between **43** and tolylboronic acid gave biaryl species **44** in 73% yield. The temperature of this reaction was crucial to its success as temperatures over 100 °C resulted in a significant amount of reaction occurring at the bromine while temperatures less than 100 °C resulted in a slow and often incomplete reaction even after 24 h. The allyl groups in **44** were removed in 86% yield using 20 mol% $Pd(PPh_3)_4$ and barbituric acid in refluxing acetonitrile.[19a] Bromination of the resulting primary sulfonamide **45** using the same procedure as described for the bromination of **38** gave compound **14** in 88% yield. The overall yield of **14** was 23%.

Phosphonate **10** was coupled to sulfonamides **13** and **14** to give compounds **46** and **49** in 95% and 72% yields, respectively, by stirring a cold aqueous ethanolic solution of the sulfonamides and the phosphonate containing excess NaOH for 30 min. (Scheme 7). The phosphonate groups were deprotected by treatment with TMSBr then with methanol which gave phosphonate compounds **2** and **8** in 98% and 92% yield as their free acids which could be converted to their sodium salts using a Dowex Na^+ form ion exchange column.

Compounds **47**, **48** and **50** were prepared in good yield by coupling sulfonates **11** and **12** to sulfonamides **13** and **14** using the same procedure as that described for **46** and **49** (Scheme 7) The neopentyl groups from compounds **47**, **48**, and **50** were removed using refluxing 1 N HCl. Compounds **6** and **7** were obtained in pure form as their ammonium salts in 96% and 76% yield, respectively, by subjecting the crude material to silica gel flash chromatography using $MeOH/NH_4OH/CH_2Cl_2$ as eluent. This purification procedure was not successful for compound **9** and RP-HPLC was required to obtain pure **9** and the deprotection yield was 43%.



Scheme 7.

2.2. Inhibition studies

IC_{50} determinations were carried out under conditions similar to those reported by MerckFrosst for compound **2**[7,20] (50 mM Bis–Tris HCl buffer, pH 6.3 containing 2% glycerol, 0.1% Triton X-100, 4% DMSO, 4.5 mM DTT and 1.8 mM EDTA) with the only significant difference being the presence of 4% DMSO.[21] 6,8-Difluoro-4-methylumbelliferyl phosphate (DiFMUP) was used as substrate at K_m concentration (5 μM).[20,21] Although none of the new compounds (**6**–**9**) were as potent as the parent compound **2**, some surprising and informative results were obtained (Table 1). Compound **6** is a 1000-fold less potent inhibitor than its phosphonate analog, compound **2**. This large difference in potency prompted us to examine whether the modality of inhibition of sulfonate **6** was different from that of competitive inhibitors **2**–**5**. However, sulfonate **6** also behaved as a purely competitive inhibitor with a K_i of 10.6 μM and inhibition was reversible (Fig. 3). Surprisingly, inhibition studies with the nonfluorinated analog of inhibitor **6**, compound **7**, revealed that it has an IC_{50} that is almost the same as that of compound **6**. More detailed studies with inhibitor **7** revealed that it too is a competitive inhibitor with a K_i of 10.3 μM which is essentially the same as that of compound **6** The non-fluorinated analog of peptide **3**, peptide **51** (Fig. 4), was never examined as a PTP1B inhibitor. However, the non-fluorinated analog of inhibitor **4** exhibited only 10% inhibition at 500 μM[8a] which indicates that the fluorines in inhibitor **4** contribute significantly to the binding of compound **4** to PTP1B. Therefore, we had assumed that the fluorines in peptide **3** were also making a significant contribution to the potency of this peptide. To determine if this is indeed the case, we prepared peptide **51**[22] and examined it, as well as peptide **3**, as PTP1B inhibitors under the conditions described above. Under these conditions, peptide **3** exhibited an IC_{50} of 24 μM. Most significantly, the IC_{50} of peptide **51** was 44 μM, which is less than two-fold higher than that of peptide **3**. Thus, our previous assumption that the fluorines in peptide **3** contributed significantly to its potency was incorrect. The inability of the fluorines in inhibitors **3** and **6** to make beneficial contacts with active site residues may partly explain the very large (100- to 1000-fold) difference in potency between these compounds and their DFMP analogs.[23] On the other hand, compound **4** is much smaller than peptide **3** and compound **6**, and so compound **4** may have greater freedom of motion in the active site. If so, then this may allow the fluorines in inhibitor **4** to make some beneficial contacts with active site residues while residues in peptide **3** and moieties in compound **6** outside the active site may restrict the mobility of the arylDFMS group in the active site which could limit the ability of the fluorines to make beneficial contacts. It is worth mentioning that, with the exception of sulfonodifluoromethylphenylalanine (in peptide **3**), sulfonomethylphenylalanine (in peptide **51**) is a considerably more effective pTyr mimic than other monoanionic pTyr mimics that have been examined in this hexapeptide (DADE-X-LNH_2, X = pTyr mimic) platform.[11]

Table 1
Inhibition of PTP1B with compounds **2**, and **6**–**9**

Compound	IC_{50} (μM)	K_i (μM)
2	0.0060 ± 0.0004	ND
6	13 ± 3	10.6 ± 0.4
7	19 ± 3	10.3 ± 1.8
8	0.030 ± 0.002	0.024 ± 0.003
9	6.0 ± 0.4	3.3 ± 0.5

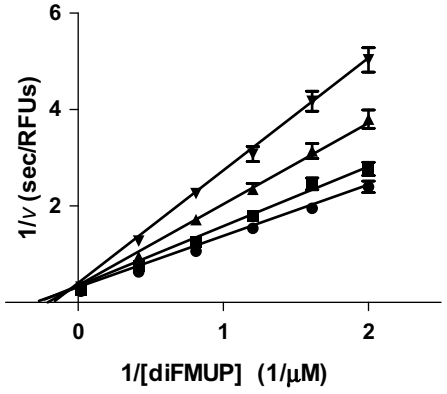
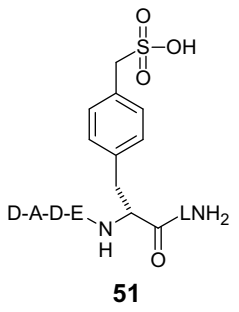


Figure 3. Lineweaver–Burk plot for the inhibition of PTP1B with inhibitor **6** in 50 mM Bis–Tris HCl, pH 6.3, 20% glycerol, 5 mM DTT, 2 mM EDTA, 0.1% Triton X-100. (●) 0 μM, (■) 5 μM, (▲) 10 μM, (▼) 20 μM.



51

Figure 4. Structure of peptide **51**.

In compounds **8** and **9** the sulfonamide moiety in compounds **2** and **6** is replaced with a difluoromethylenesulfonamide group. This modification was pursued since this allowed us to increase the hydrophobicity of these compounds while at the same time lowering the pK_a of the sulfonamide by about three orders of magnitude (from 10.5 to 7.5) by making just a minor structural change.[24a,b] Phosphonate inhibitor **8** exhibited a 5-fold increase in IC_{50} compared to compound **2** (Table 1). Further studies revealed that **8** is a competitive inhibitor with a K_i of 24 nM, still a very potent inhibitor. However, sulfonate **9** exhibited a slight decrease in IC_{50} compared to sulfonate **6** (Table 1). Moreover, inhibitor **9** exhibited reversible mixed inhibition (Fig. 5) with a K_i of 3.3 μM and an αK_i of 12.5 μM indicating that this more hydrophobic compound is probably binding somewhat differently to PTP1B compared to compound **8** and this may account for their different trends in K_i's.

Although monoanionic inhibitors **6**, **7**, and **9** are considerably less potent than their phosphonate counterparts, they compare favorably, in terms of their affinity for PTP1B, to the majority of

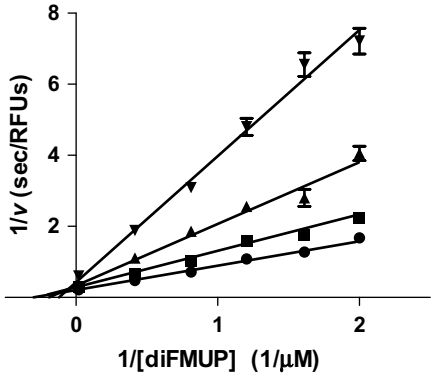


Figure 5. Lineweaver–Burk plot for the inhibition of PTP1B with inhibitor **9** in 50 mM Bis–Tris HCl, pH 6.3, 20% glycerol, 5 mM DTT, 2 mM EDTA, 0.1% Triton X-100. (●) 0 μM, (■) 2.5 μM, (▲) 5 μM, (▼) 10 μM.

the best non-peptidyl monoanionic PTP1B inhibitors that have been reported in the literature. Amarasinghe et al. reported a monoanionic sulfamide PTP1B inhibitor with an IC_{50} of 2.3 μM though the mode of inhibition was not reported.[25a] Researchers at Abbott have reported monoanionic inhibitors bearing either a 2-(hydroxyphenoxy)phenyl acetic acid moiety or a isoxazole carboxylic acid group with K_i's of 6–9 μM.[25b,c] Researchers at Sunesis have reported a monoanionic pyrazine carboxylic acid-based PTP1B competitive inhibitor with a K_i of 4 μM.[25d] However, it should be noted that neither our sulfonate inhibitors nor any of the other above mentioned monoanionic inhibitors are as potent as the monoanionic inhibitors bearing the (*S*)-isothiazolidinone phosphate mimic recently reported by workers at Incyte.[25e] Although the (*S*)-isothiazolidinone phosphate mimic has never been examined in the DADE-X-LNH_2 platform, studies with other smaller peptides as well as with non-peptidyl compounds bearing this moiety suggest that it is the most effective monoanionic phosphate reported to date and appears to be even more effective than the DFMP group.

3. Conclusions

An efficient synthesis of protected phosphonate **10**, an important biophore for obtaining potent non-peptidyl PTP1B inhibitors, as well as an effective synthesis of known DFMP-bearing inhibitor **2**, one of the most potent non-peptidyl PTP1B inhibitors reported to date, was achieved. The synthesis of the DFMS analog of compound **2** (compound **6**), and that of its non-fluorinated methylenesulfonic analog (**7**), as well as two other derivatives in which a distal sulfonamide moiety is replaced with a difluoromethylenesulfonamide group, were also described. DFMS-bearing inhibitor **6** was a much less potent PTP1B inhibitor than phosphonate **2**. Replacing the distal sulfonamide moiety with a difluoromethylenesulfonamide group in compound **2** did not yield a more potent inhibitor though this substitution in compound **6** did yield a slightly better inhibitor. Surprisingly, inhibition studies with the non-fluorinated sulfonates, compound **7** and peptide **51** bearing sulfonomethylphenylalanine, revealed that the fluorines had little effect on the potency of the DFMS-bearing inhibitors which was in contrast to our previously held assumption that the fluorines in DFMS-bearing inhibitors contributed significantly to their potency. This may in part explain the large difference in potency between the DFMS and DFMP bearing compounds. These results also show that sulfonomethylphenylalanine, a pTyr mimic that is more easily prepared than sulfonodifluoromethylphenylalanine,[16b,22] is one of the best monoanionic pTyr mimics reported so far when examined in the context of the DADE-X-LNH_2 platform.

4. Experimental

4.1. Chemistry

4.1.1. General

All starting materials and reagents were obtained commercially and used without further purification with the exception of TMSBr, which was distilled under Ar and stored under Ar in a Schlenk tube. Tetrahydrofuran (THF) was distilled from sodium metal in the presence of benzophenone under argon. CH_2Cl_2 was distilled from calcium hydride under nitrogen. DMF was distilled under aspirator pressure from CaH and stored under Ar. Methanol was dried by refluxing over magnesium metal and distilled under Ar and stored over 4 Å molecular sieves. Flash chromatography was performed using silica gel (60 Å, 230–400 mesh). For ^1H NMR in $CDCl_3$, chemical shifts (δ) are reported in ppm relative to tetramethylsilane (δ 0.0, internal standard). For ^1H NMR in CD_3OD, chemical shifts are reported in ppm relative to the solvent residual peak (δ 3.31, central peak). For ^1H NMR in D_2O, chemical shifts are reported in ppm relative to the solvent residual peak (δ 4.79). For ^1H NMR in DMSO-d_6, chemical shifts are reported in ppm relative to the solvent residual peak (δ 2.49). For ^1H NMR in acetone-d_6, chemical shifts (δ) are reported in ppm relative to the solvent residual peak (δ 2.09). For ^{13}C NMR in $CDCl_3$, chemical shifts are reported in ppm relative to $CDCl_3$ (δ 77.0 for the central peak). For ^{13}C NMR spectra run in CD_3OD, chemical shifts are reported in ppm relative to the solvent residual peak (δ 49.00, central peak). For ^{13}C NMR in DMSO-d_6, chemical shifts are reported in ppm relative to DMSO-d_6, (δ 39.5 for the central peak). For ^{13}C NMR spectra run in D_2O, chemical shifts are reported in ppm relative to CH_3OH in D_2O (δ 49.5, external standard). For ^{13}C NMR run in acetone-d_6, chemical shifts (δ) are reported in ppm relative to the solvent residual peak (δ 30.6). All ^{31}P NMR spectra chemicals shifts are reported in ppm relative to 85% H_3PO_4 (δ 0.0, external standard). All ^{19}F spectra chemical shifts are reported in ppm relative to an external $CFCl_3$ standard (δ 0.0, $CFCl_3$). CIMS were run in the positive mode using ammonia as ionizing. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected.

4.1.2. {[2-Bromo-4-(4′-bromo-3′-sulfamoylbiphenyl-4-ylmethylsulfanylmethyl)-phenyl]difluoromethyl}phosphonic acid (2)

To a solution of **46** (0.113 g, 0.159 mmol) in dry CH_2Cl_2 (10 mL) under Ar was added trimethylsilyl bromide (0.97 g, 4.0 equiv, 0.63 mmol) and the mixture was stirred at rt for 20 h. The mixture was concentrated and then the residue was dissolved in methanol and stirred for 30 min. The solution was concentrated and the residue was dissolved in methanol and concentrated again. This gave pure **2** as a colorless oil (0.103 g, 98%). For inhibition studies, compound **2** was dissolved in water and passed through a Dowex Na^+ form ion exchange column to give **2** as a white disodium salt. The spectral data given below is for the free acid. ^1H NMR (300 MHz, acetone-d_6) δ 3.73 (2H, s), 3.75 (2H, s), 7.40–7.43 (3H, d, J = 8 Hz), 7.58–7.64 (4H, m), 7.75 (1H, dd, J = 8.4 Hz), 7.86 (1H, d, J = 8.3 Hz), 8.32 (1H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ 34.2, 35.2, 118.0, 118.3 (dt, J = 264.0, 216.0 Hz), 119.7, 126.9, 127.6, 127.8, 129.9, 130.1 (t, J = 7.7 Hz), 131.2, 135.5, 135.6, 137.1, 138.8, 140.3, 143.2, 143.6, 205.5; ^{31}P NMR (121 MHz, acetone-d_6) δ 5.4 (t, J = 111.0 Hz); ^{19}F NMR (282 MHz, acetone-d_6) δ −105.0 (d, J = 112.7 Hz). LR$^-$ESIMS m/z (relative intensity) 653 ([M−H]$^-$, 100); HR$^-$ESIMS m/z calcd for $C_{21}H_{18}Br_2F_2NO_5PS_2$ ([M-H]$^-$), 653.8620, found 653.8614.

4.1.3. [2-Bromo-4-(4′-bromo-3′-sulfamoylbiphenyl-4-ylmethylsulfanyl methyl)phenyl]difluoromethanesulfonate, ammonium salt (6)

A solution of compound **47** (0.0112 g, 0.0160 mmol) in 1 N HCl (10 mL) was refluxed for 4 h. The solution was concentrated by high-vacuum rotary evaporation and the resulting residue was subjected to flash chromatography (2:0.5:10 MeOH/NH_4OH/CH_2Cl_2) which gave compound **6** as a white ammonium salt (0.010 g, 95%). ^1H NMR (500 MHz, CD_3OD) δ 3.64 (2H, s), 3.68 (2H, s), 7.32 (1H, d, J = 8.1 Hz), 7.38 (2H, d, J = 8.2 Hz), 7.55 (1H, s), 7.59 (2H, d, J = 8.2 Hz), 7.67 (1H, d, J = 8.2 Hz), 7.70 (1H, dd, J = 8.3 Hz), 7.83 (1H, d, J = 8.3 Hz), 8.33 (1H, d, J = 2.3 Hz); ^{19}F NMR (282 MHz, CD_3OD) δ −99.1; ^{13}C NMR (75 MHz, CD_3OD) δ 34.0, 34.8, 117.7, 119.7 (t, J = 279.6 Hz), 120.1, 126.5, 127.0, 127.3, 129.5, 129.9, 130.5 (t, J = 7.7 Hz), 131.0, 135.1, 135.3, 137.1, 138.5, 140.4, 142.7, 143.0; LR$^-$ESIMS m/z (relative intensity) 653 ([M−$^+NH_4$]$^-$, 100); HR$^-$ESMS m/z calcd for $C_{21}H_{17}Br_2F_2NO_5S_3$ ([M−$^+NH_4$]$^-$), 653.8525, found 653.8525.

4.1.4. [2-Bromo-4-(4′-bromo-3′-sulfamoylbiphenyl-4-ylmethylsulfanylmethyl)phenyl]-methanesulfonate, ammonium salt (7)

A suspension of compound **48** (0.160 g, 0.232 mmol) in 1 N HCl (15 mL) was refluxed for 12 h. The solution was concentrated by high-vacuum rotary evaporation and the resulting residue was subjected to flash chromatography (2:0.5:10 MeOH/NH_4OH/CH_2Cl_2) which gave pure compound **7** as a white ammonium salt (0.100 g, 76%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.63 (2H, s), 3.60 (2H, s), 4.25 (2H, s) 7.21 (1H, d, J = 7.9 Hz), 7.37 (2H, d, J = 8.2 Hz), 7.45 (1H), 7.53 (1H, d, J = 7.9 Hz), 7.58 (2H, d, J = 8.2 Hz), 7.70 (1H, dd, J = 8.3 Hz), 7.83 (1H, d, J = 8.3 Hz), 8.32 (1H, d, J = 2.2 Hz) ; ^{13}C NMR (75 MHz, CD_3OD) δ 34.3, 34.7, 55.7, 117.8, 124.9, 120.1, 126.5, 127.3, 127.6, 129.6, 130.9, 131.5, 132.0, 132.8, 135.4, 137.1, 138.7, 140.5, 142.8; LR$^-$ESIMS m/z (relative intensity) 618 ($[M-NH_4^+]^-$, 100); HR$^-$ESIMS m/z calcd for $C_{21}H_{18}Br_2NO_5S_3$ ($[M-^+NH_4]^-$), m/z 617.8714, found 617.8714.

4.1.5. ({2-Bromo-4-[4′-bromo-3′-(difluorosulfamoylmethyl) biphenyl-4-ylmethylsulfanylmethyl]phenyl}difluoromethyl) phosphonic acid (8)

Prepared in a 92% yield from compound **49** using the same procedure as that described for compound **2**. The following quantities were used: **49** (0.026 g, 0.034 mmol), dry CH_2Cl_2 (4 mL), and TMSBr (0.016 g, 2.9 equiv, 0.10 mmol). For inhibition studies, compound **8** was dissolved in water and passed through a Dowex Na^+ form ion exchange column to give **8** as a white disodium salt. The spectral data given below are for the free acid. ^{1}H NMR (300 MHz, CD_3OD) δ 3.64 (2H, s), 3.68 (2H, s) 7.32–7.38 (3H, m), 7.56 (4H, m), 7.67 (1H, d, J = 8.2 Hz), 7.79 (1H, d, J = 8.4 Hz), 7.90 (1H, s); ^{31}P NMR (121 MHz, CD_3OD) δ 5.5 (t, J = 110.5 Hz); ^{19}F NMR (282 MHz, CD_3OD) δ −99.2, −106.0 (d, J = 110.1 Hz); ^{13}C NMR (125.8 MHz, CD_3OD) δ 34.0, 38.5, 119.2 (bs), 119.7 (bs), 120.1 (t, J = 283.2 Hz), 126.6, 127.3, 128.87 (t, J = 8.0 Hz), 129.5, 129.7 (t, J = 8.1 Hz) 130.9, 131.5, 135.2, 135.8, 137.3, 138.4, 139.9, 142.9 LR$^-$ESIMS m/z (relative intensity) 703 ($[M-1]^-$, 100); HR$^-$ESIMS m/z calcd for $C_{22}H_{17}Br_2F_4NO_5PS_2$ $(M-H)^-$ m/z 703.8589, found 703.8597.

4.1.6. {2-Bromo-4-[4′-bromo-3′-(difluorosulfamoylmethyl) biphenyl-4-ylmethylsulfanylmethyl]-phenyl}difluoromethanesulfonic acid (9)

A suspension of **50**, (0.090 g, 0.116 mmol), in 1 N HCl (20 mL) was refluxed for 4 h. The solution was concentrated by high-vacuum rotary evaporation which gave 75 mg of crude **9** as a light brown oil. Silica gel flash chromatography (2:0.5:10 MeOH/NH_4OH/CH_2Cl_2) of crude **9** failed to give pure product. To obtain pure **9** the impure material from the silica flash column was subjected to reverse-phase semi-preparative HPLC (C-18 column, 55% 0.1% TFA in H_2O/45% CH_3CN to 20% 0.1% TFA in H_2O/80% CH_3CN over 60 minutes, flow rate = 8 mL/min, R_t = 39 min) which gave pure **9** as its free acid (0.035 g, white powder, 43%). ^{1}H NMR (300 MHz, acetone-d_6) δ 3.71 (2H, s), 3.74 (2H, s), 7.35 (1H, d, J = 8.0 Hz), 7.42 (2H, d, J = 8.1 Hz) 7.57–7.63 (3H, m), 7.70–7.78 (2H, s) 7.84 (1H, d, J = 8.4 Hz), 7.95 (1H, d, J = 1.8 Hz); ^{19}F NMR (285 MHz, acetone-d_6) δ −99.1, −99.0; ^{13}C NMR (75 MHz, CD_3OD) δ 34.1, 35.0, 119.2, 119.8 (t, J = 280.5 Hz), 120.1 (t, J = 284.2 Hz), 126.6, 127.2, 128.9 (t, J = 21.5 Hz), 128.9 (t, J = 7.8 Hz), 129.6, 129.9 (t, J = 22.5 Hz), 130.6 (t, J = 7.7 Hz), 131.0, 135.2, 135.9, 137.2, 138.4, 139.9, 143.1; LR$^-$ESIMS m/z (relative intensity) 704 ($[M-H]^-$, 100); HR$^-$ESIMS m/z calcd for $C_{22}H_{16}Br_2F_4NO_5S_3$ ($[M-H]^-$) 703.8493, found 703.8483.

4.1.7. Thioacetic acid *S*-{3-bromo-4-[(diethoxyphosphoryl)difluoromethyl]benzyl}ester (10)

A solution of **20** (0. 777 g, 1.79 mmol) and potassium thioacetate (0.228 g, 1.12 equiv, 1.57 mmol) in DMF (15 mL) was stirred at rt for 30 min. The DMF was removed by high-vacuum rotary evaporation and the residue dissolved in CH_2Cl_2. The resulting solution was washed with water, then dried (Na_2SO_4), and concentrated. The residue was subjected to flash chromatography (1:1 EtOAc/hexane) which gave pure **10** as a colorless oil (0.711 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (6H, t, J = 7.1 Hz), 2.21 (3H, s), 3.93 (2H, s), 4.03–4.16 (4H, m), 7.19 (1H, d, J = 8.1 Hz), 7.40 (1H, d, J = 8.1 Hz), 7.47 (1H, s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 6.51 (t, J = 114.4 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ −104.0 (d, J = 114.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.3 (d, J = 5.4), 30.1, 32.0, 64.9 (d, J = 6.9 Hz), 117.9 (dt, J = 265.9, 219.1 Hz), 120.0 (t, J_{CP} = 3.0), 127.6, 129.8 (dt, J = 8.7, 1.8 Hz), 130.5 (J = 21.3, 14.3), 135.4, 142.5 (d, J = 1.41), 194.1; LREIMS m/z (%) 430 (M^+, 23), 389 (100); HREIMS m/z calcd for $C_{14}H_{18}B_rF_2O_4PS$ (M^+) 429.9815, found 429.9819.

4.1.8. Thioacetic acid *S*{3bromo-4-[(2,2-dimethylpropoxysulfonyl) difluoromethyl]-benzyl}ester (11)

Prepared in a 96% yield from compound **30** using the same procedure as that described for compound **10**. The following quantities were used: **30** (0.100 g, 0.22 mmol), potassium thioacetate (0.047 g, 1.8 equiv, 0.40 mmol), DMF (5 mL). The residue was subjected to flash chromatography (0.5:9.5 EtOAc/hexane) which gave **11** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (9H, s), 2.32 (3H, s), 4.03 (2H, s), 4.12 (2H, s), 7.33 (1H, d, J = 8.1 Hz), 7.57 (1H, d, J = 8.2 Hz), 7.62 (1H, s); ^{19}F NMR (285 MHz, $CDCl_3$) δ −94.8; ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.7, 29.6, 30.1, 31.9, 84.6, 120.5 (t, J = 286.7 Hz), 120.9, 126.0 (t, J = 21.1 Hz), 127.8, 130.8 (t, J = 7.9 Hz), 135.8, 144.3, 194.0; LR-ESIMS m/z (relative intensity) 462 (M^+, 100); HR-ESIMS m/z calcd for $C_{15}H_{19}BrF_2O_4S_2$ (M^+) 462.0220, found 462.0224.

4.1.9. Thioacetic acid *S*-(3-bromo-4-isobutoxysulfonylmethylbenzyl) ester (12)

Prepared using the same procedure as that used for compound **10**. The following quantities were used: compound **32** (0.555 g, 1.34 mmol), potassium thioacetate (0.179 g, 1.80 equiv, 1.57 mmol), DMF (5 mL). The residue was subjected to flash chromatography (1:4 EtOAc/ hexane) which gave pure **12** as a white solid (92%). Mp 108–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (9H, s), 2.31 (3H, s), 3.76 (2H, s|), 4.02 (2H, s), 4.54 (2H, s), 7.23 (1H, d, J = 7.8 Hz), 7.43 (1H, d, J = 7.9 Hz), 7.52 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 30.3, 31.7, 32.2, 55.4, 79.5, 125.4, 127.0, 128.3, 132.6, 133.4, 140.9, 194.4; LRCIMS m/z (relative intensity) 426 ($[M+NH_4]^+$, 100), 258 (10), 214 (35); HREIMS m/z calcd for $C_{13}H_{18}BrO_3S_2$ ($[M-(CH_3CO)]^+$) 364.9886, found 364.9876.

4.1.10. 4-Bromo-4′-bromomethylbiphenyl-3-sulfonic acid amide (13)

A solution of compound **37** (0.300 g, 0.92 mmol), *N*-bromosuccinimide (0.181 g, 1.1 equiv, 1.02 mmol) and benzoylperoxide (0.048 g, 0.20 equiv, 0.18 mmol) in benzene (25 mL) was refluxed (oil bath) under Ar for 6 h during which a 250 W lamp was shone on the reaction. The reaction was quenched with water then extracted with EtOAc. The combined organics were dried (Na_2SO_4) and concentrated and the resulting residue subjected to flash chromatography (2:8 EtOAc/hexane) to give **13** as a white solid (0.333 g, 89%). Mp 151–153 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 4.68 (2H, s), 6.76 (2H, br s), 7.58 (2H, d, J = 8.3 Hz), 7.68 (2H, d, J = 8.3 Hz), 7.76 (1H, d, J = 8.2 Hz), 7.87 (1H, d, J = 8.2 Hz), 8.33 (1H, d, J = 2.3 Hz); ^{13}C NMR (75 MHz, acetone-d_6) δ 32.9, 118.3, 127.2, 127.6, 130.0, 131.3, 135.7, 138.4, 138.7, 140.0, 143.3; LREIMS m/z (relative intensity) 403 (M^+, 11), 326 (100), 165 (22); HREIMS m/z calcd for $C_{13}H_{11}Br_2NO_2S$ (M^+) 402.8877, found 402.8885.

4.1.11. (4-Bromo-4′-bromomethylbiphenyl-3-yl)difluoro-methanesulfonamide (14)

To a solution of **45** (0.100 g, 0.266 mmol) in benzene (7 mL) were added *N*-bromosuccinimide (0.052 g, 1.1 equiv, 0.29 mmol) and benzoylperoxide (0.008 g, 0.1 equiv, 0.03 mmol). The mixture was gently refluxed under Ar by placing a 250 W lamp close to the reaction vessel. After 3 h, water was added and the mixture was extracted with EtOAc. The combined organics were concentrated and the resulting residue was subjected to flash chromatography twice (3.5:6.5 EtOAc/hexane) which gave pure **14** as a white solid (0.105 g, 88%). Mp 152–154 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 4.68 (2H, s), 7.39 (2H, br s), 7.57 (2H, d, J = 7.6 Hz), 7.66 (2H, d, J = 7.5 Hz), 7.75 (1H, d, J = 10.5 Hz), 7.84 (1H, d, J = 8.0 Hz), 7.94 (1H, s); ^{19}F NMR (285 MHz, acetone-d_6) δ −98.6; ^{13}C NMR (75 MHz, acetone-d_6) δ 32.9, 119.7, 120.2 (t, J = 284.5 Hz), 127.2, 129.2 (t, J = 7.6 Hz), 130.0, 131.34, 136.2, 138.3, 138.6, 139.6, 142.5; LREIMS m/z (relative intensity) 453 (M^+, 12), 395 (100), 296 (68); HREIMS m/z calcd for $C_{14}H_{11}Br_2F_2NO_2S$ (M^+) 452.8845, found 452.8838.

4.1.12. [(2-Bromo-4-bromomethylphenyl)difluoromethyl]phosphonic acid, diethyl ester (20)

To a solution of **24** (0.80 g, 2.2 mmol) in CH_2Cl_2 (20 mL) at 0 °C were added carbon tetrabromide (0.78 g, 1.1 equiv, 2.4 mmol) and triphenylphosphine (0.62 g, 1.1 equiv, 2.4 mmol), and the mixture was stirred for 2 h. The reaction was quenched with water, extracted with CH_2Cl_2, and the combined organics were dried (Na_2SO_4) and concentrated. The resulting residue was subjected to flash chromatography (1:1 EtOAc/hexane) which gave pure **20** as a colorless oil (0.78 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (6H, t, J = 7.1 Hz), 4.06–4.19 (4H, m), 4.30 (2H, s), 7.30 (1H, d, J = 8.2 Hz), 7.47 (1H, d, J = 8.2 Hz), 7.58 (1H, s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 5.30 (t, J = 111.0 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ −104.0 (d, J = 112.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.3 (d, J = 5.5 Hz), 30.7, 65.0 (d, J = 6.9 Hz), 117.8 (dt, J = 266.2, 218.7 Hz), 120.1 (app. dt, J_{CP} = 3.1 Hz, J = 3.1 Hz), 127.7, 130.1 (dt, J = 8.7, 1.9 Hz), 131.55 (dt, J = 21.4, 14.2 Hz), 135.6, 141.9 (d, J = 1.4 Hz). LRCIMS m/z (%) 454 ([M + NH_4]$^+$, 100), 355 (11); HREIMS m/z calcd for $C_{12}H_{15}B_rF_2O_3P$ ([M − Br]$^+$) 356.9889, found 356.9881.

4.1.13. 3-Bromo-4-(diethoxyphosphorylmethyl)benzoic acid methyl ester (22)

This was prepared according to the procedure of Gilbert et al.[26] A mixture of 3-bromo-4-(bromomethyl)benzoic acid methyl ester (**21**)[15] (4.05 g, 13.3 mmol) and triethyl phosphite (5.33 g, 2.40 equiv, 32.0 mmol) was refluxed for 3h. The excess triethyl phosphite was removed by distillation and the residue was subjected to flash chromatography (1:1 EtOAc/Hexanes) which gave pure **22** as a colorless oil (4.55 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (6H, t, J = 7.1 Hz), 3.41 (2H, d, J = 22.5 Hz), 3.88 (3H, s), 3.97–4.10 (4H, m), 7.49 (1H, dd, J = 8.1 Hz), 7.89 (1H, d, J = 7.9 Hz), 8.20 (1H, s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 24.1. LREIMS m/z (%) 333 ([M−OMe]$^+$, 4) 285 (100) 229 (85); HREIMS m/z calcd for $C_{12}H_{15}BrO_4P$ ([M−OMe]$^+$), 332.9891, found 332.9884.

4.1.14. 3-Bromo-4-[(diethoxyphosphoryl)difluoromethyl]benzoic acid, methyl ester (23)

To a solution of phosphonate **22** (2.00 g, 5.47 mmol) and *N*-fluorobenzenesulfonimide (4.31 g, 2.50 equiv, 13.7 mmol) in dry THF (50 mL) at −78 °C was added NaHMDS (1.0 M in THF, 13.7 mL, 2.50 equiv, 13.7 mmol) over 10 min. The mixture was stirred for 2 h at −78 °C, then allowed to warm to rt, and stirred for 16 h. The reaction was quenched with a satd NH_4Cl solution, extracted with CH_2Cl_2, and the combined organic layers dried (Na_2SO_4) and concentrated. The residue was subjected to flash chromatography (1:1 EtOAc/hexane) which gave **23** as a white solid (1.25 g, 57%). Mp 35 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09 (6H, t, J = 7.2 Hz), 3.67 (3H, s), 3.93–4.07 (4H, m), 7.45 (1H, d, J = 8.3 Hz), 7.76 (1H, d, J = 8.2 Hz), 8.03 (1H, s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 4.9 (t, J = 111.0 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ −105.0 (d, J = 110.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.1 (d, J = 5.4), 52.34, 64.8 (d, J = 6.9 Hz,), 117.5 (dt, J = 266.6, 217.0 Hz), 119.9 (app. dt, J = 2.9 Hz, J = 2.9 Hz), 127.8, 129.7 (dt, J = 8.7, 1.7 Hz), 133.2, 135.6 (dt, J = 21.1, 14.1 Hz), 135.9, 164.4; LREIMS m/z (%) 400 (M^+, 2), 321 (100), 265 (48); HREIMS m/z calcd for $C_{13}H_{16}BrF_2O_5P$ (M^+) 399.9887, found 399.9876.

4.1.15. [(2-Bromo-4-hydroxymethylphenyl)difluoromethyl]-phosphonic acid diethyl ester (24)

A suspension of **23** (1.20 g, 3.00 mmol) and $NaBH_4$ (0.67 g, 6.0 equiv, 18 mmol) in dry THF (8 mL) under Ar was stirred at 65 °C for 15 min. MeOH (8 mL) was added dropwise over 20 min and the mixture was stirred for 2 h at 65 °C. The reaction was quenched with a satd NH_4Cl solution, water was added and the mixture was extracted several times with CH_2Cl_2. The combined organisc were dried (Na_2SO_4) and concentrated. The residue was subjected to flash chromatography (4:1 EtOAc/hexane) which gave pure **24** as a colorless oil (0.80 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (6H, t, J = 7.1 Hz), 4.02–4.21 (5H, m, OH peak merged), 4.53 (2H, s), 7.23 (1H, d, J = 8.2 Hz), 7.44 (1H, d, J = 8.2 Hz), 7.58 (1H, s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 5.5 (t, J = 114.6 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ 104.0 (d, J = 115.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.3 (d, J = 5.5), 62.9, 65.2 (d, J = 7.0 Hz), 117.5 (dt, J = 265.7, 220.7 Hz), 119.9, 125.1, 129.6 (dt, J = 8.7, 1.9 Hz), 129.8 (dt, J = 21.3, 14.3 Hz), 133.1, 146.4; LRCIMS m/z (%) 390 ([M + NH_4]$^+$, 100), 312 (20); HREIMS m/z calcd for $C_{12}H_{16}F_2O_4P$ ([M−Br]$^+$) 293.0754, found 293.0760.

4.1.16. 4-Acetylsulfanylmethyl-3-bromo-benzoic acid methyl ester (25)

A solution of compound **21** (21.3 g, 69.0 mmol) and potassium thioacetate (8.07 g, 1.03 equiv, 70.8 mmol) in DMF (60 mL) was stirred at rt for 30 min. The reaction was diluted with water and extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4), then concentrated, and the resulting solid residue recrystallized in CH_2Cl_2/hexane which gave compound **25** as a crystalline white solid (20.65 g, 99%). Mp 62–63 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 3.86 (3H, s), 4.19 (2H, s), 7.47 (1H, d, J = 8.0 Hz), 7.84 (1H, dd, J = 8.1 Hz), 8.15 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.2, 33.7, 52.4, 124.3, 128.6, 130.7, 131.0, 133.8, 142.2, 165.4, 194.4; LRCIMS m/z (relative intensity) 320 ([M+NH_4]$^+$, 100), 223 (42); HREIMS m/z calcd for $C_{10}H_8O_2BrS$ ([M−OMe]$^+$) 270.9423, found 270.9431.

4.1.17. 3-Bromo-4-chlorosulfonylmethylbenzoic acid methyl ester (26)

To a suspension of thioacetate **25** (5.03 g, 16.7 mmol) in glacial acetic acid (32 mL) and ice water (16 mL) was added $CHCl_3$ dropwise until the mixture become clear. The reaction was placed in an ice bath and Cl_2 gas was bubbled through the mixture for 2 h. Nitrogen was then bubbled through the solution to remove excess Cl_2 and then mixture was diluted with water and extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated to give pure **26** as colorless fine crystals (5.32 g, 98%). Mp 99–101 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (3H, s), 5.16 (2H, s), 7.68 (1H, d, J = 8.1 Hz), 8.04 (1H, dd, J = 8.0 Hz), 8.33 (1H, d, J = 1.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.7, 69.4, 126.2, 128.8, 130.8, 133.2, 133.3, 134.6, 164.8; LREIMS m/z (relative intensity) 325 (M^+, 6) 227 (100); HREIMS m/z calcd for $C_9H_8O_4BrClS$ (M^+) 325.9015, found 325.9020.

4.1.18. 3-Bromo-4-(2,2-dimethylpropoxysulfonylmethyl)benzoic acid methyl ester (27)

To a solution of compound **26** (5.06 g, 15.5 mmol) and neopentyl alcohol (2.04 g, 1.5 equiv, 23.3 mmol), in dry CH_2Cl_2 (50 mL) at 0 °C under Ar was added triethylamine (2.03 mL, 1.3 equiv, 20.2 mmol) dropwise over 10 min. The mixture was stirred for 16 h at rt, quenched with H_2O, and extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated and the resulting residue subjected to flash chromatography which gave pure **27** as a colorless crystalline solid (4.72 g, 81%). Mp 51–53 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (9H, s), 3.75 (2H, s), 3.84 (3H, s), 4.59 (2H, s), 7.56 (1H, d, J = 8.1 Hz), 7.90 (1H, dd, J = 7.9 Hz), 8.2 (1H, d, J = 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 31.7, 52.5, 55.5, 79.6, 125.4, 128.6, 132.1, 132.6, 132.9, 134.1, 165.0; LREIMS m/z (relative intensity) 378 (M^+, 6), 229 (100); HREIMS m/z calcd for $C_{14}H_{19}BrO_5S$ (M^+) 378.0137, found 378.0142.

4.1.19. 3-Bromo-4-(2,2-dimethylpropoxysulfonylmethyl)benzoic acid methyl ester (28)

To a solution of compound **27** (2.00 g, 5.28 mmol) and N-fluorobenzenesulfonimide (5.00 g, 3.0 equiv, 15.9 mmol) in dry THF (100 mL) at −78 °C was added NaHMDS (1.0 M in THF, 13.3 mL, 2.5 equiv, 13.3 mmol) over 30 min. The mixture was stirred for 2 h at −78 °C then allowed to warm to rt, and stirred for 16 h. The reaction was quenched with aq satd NH_4Cl solution and extracted with diethyl ether. The combined organics were dried (Na_2SO_4) and then concentrated. The resulting residue was subjected to flash chromatography which gave pure **28** as a white solid (1.78 g, 81%). Mp 61–63 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (9H, s), 3.90 (3H, s), 4.15 (2H, s), 7.74 (1H, d, J = 8.3 Hz), 8.02 (1H, d, J = 8.3 Hz), 8.31 (1H, s); ^{19}F NMR (285 MHz, $CDCl_3$) δ −95.5; ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 32.1, 52.8, 85.0, 120.3 (t, J = 287.4 Hz), 121.08 (t, J = 2.5 Hz), 128.1, 130.9 (t, J = 13.9 Hz), 131.32 (t, J = 21.0 Hz), 134.7, 136.6, 164.4; LR$^+$ESIMS m/z (relative intensity) 415 ($[M+1]^+$, 100); HR$^+$ESIMS m/z calcd for $C_{14}H_{18}BrF_2O_5S$ ($[M+1]^+$) 415.0026, found 415.0034.

4.1.20. (2-Bromo-4-hydroxymethyl-phenyl)difluoromethanesulfonic acid, 2,2-dimethyl-propyl ester (29)

A suspension of compound **28** (1.00 g, 2.41 mmol) and $NaBH_4$ (6.0 equiv, 14.5 mmol) in dry THF (8 mL) was gently refluxed under Ar for 15 min. MeOH (8 mL) was added dropwise over 20 min and the mixture was stirred under gentle reflux for a further 2 h. The reaction was quenched with satd aq NH_4Cl solution, water was then added and the mixture was extracted with diethyl ether. The combined organics were dried (Na_2SO_4) and then concentrated. The resulting residue was subjected to flash chromatography which gave pure **29** as light pinkish oil (0.92 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (9H, s), 4.12 (2H, s), 4.55 (2H, s), 7.29 (1H, d, J = 8.1 Hz), 7.58 (1H, d, J = 8.1 Hz), 7.63 (1H, s); ^{19}F NMR (285 MHz, $CDCl_3$) δ −94.7; ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 32.1, 63.0, 84.8, 120.7 (t, J = 286.8 Hz), 120.9 (t, J = 2.7 Hz), 125.3, 125.9 (t, J = 21.1 Hz), 130.7 (t, J = 7.8 Hz), 133.4, 147.4; LRCIMS m/z (relative intensity) 404 $(M+NH_4)^+$, 100).

4.1.21. (2-Bromo-4-bromomethylphenyl)difluoromethanesulfonic acid, 2,2-dimethylpropyl ester (30)

To a solution of **29** (0.900 g, 2.30 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C were added carbon tetrabromide (0.85 g, 1.10 equiv, 2.56 mmol) and triphenylphosphine (0.610 g, 1.1 equiv, 2.56 mmol) and stirred for 2 h. The mixture was concentrated at rt by rotary evaporation and the resulting residue subjected to flash chromatography which gave pure **30** as a colorless oil (0.93 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (9H, s), 4.15 (2H, s), 4.38 (2H, s), 7.43 (1H, d, J = 8.1 Hz), 7.65 (1H, d, J = 8.2 Hz), 7.73 (1H, s); ^{19}F NMR (285 MHz, $CDCl_3$) δ −95.0; ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 30.3, 32.1, 84.9, 120.4 (t, J = 286.9 Hz), 121, 127.1 (t, J = 21.2 Hz), 128.0, 131.1 (t, J = 8.8 Hz), 136.1, 143.6; LRCIMS m/z (relative intensity) 466 ($[M + NH_4]^+$, 100); HREIMS m/z calcd for $C_{13}H_{16}BrF_2O_3S$ ($[M-Br]^+$) 370.9946, found 370.9948.

4.1.22. (2-Bromo-4-hydroxymethylphenyl)methanesulfonic acid, 2,2-dimethylpropyl ester (31)

A suspension of compound **27** (0.570 g, 1.51 mmol) and $NaBH_4$ (0.335 g, 6.0 equiv, 9.05 mmol) in dry THF (8 mL) was gently refluxed under Ar for 15 min. MeOH (8 mL) was added, dropwise over 20 min and gentle refluxing was continued for an additional 2 h. The reaction was quenched with a satd aq NH_4Cl solution, H_2O was added and the mixture was extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) then concentrated, and the resulting residue subjected to flash chromatography which gave pure **31** as a colorless oil (0.525 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (9H, s), 3.05 (1H, br s), 3.77 (2H, s), 4.54 (4H, s), 7.22 (1H, d, J = 7.9), 7.43 (1H, d, J = 7.8 Hz), 7.55 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 31.7, 55.4, 63.4, 79.6, 125.5, 125.9, 126.7, 131.1, 132.5, 144.1; LREIMS m/z (relative intensity) 350 (M^+, 10), 199 (100); HREIMS m/z calcd for $C_{13}H_{19}BrO_4S$ (M^+) 350.0187, found 350.0190.

4.1.23. (2-Bromo-4-bromomethylphenyl)methanesulfonic acid, 2,2-dimethylpropyl ester (32)

To a solution of compound **31** (0.580 g, 1.65 mmol) in CH_2Cl_2 (25 mL) at 0 °C were added carbon tetrabromide (0.606 g, 1.10 equiv, 1.83 mmol) and triphenylphosphine 0.480 g, 1.10 equiv, 1.83 mmol) and the mixture was stirred for 1 h. The mixture was concentrated by rotary evaporation at rt and the resulting residue was subjected to flash chromatography which gave pure **32** as a white solid (0.593 g, 86%). Mp 81–83 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (9H, s), 3.77 (2H, s), 4.36 (2H, s), 4.55 (2H, s), 7.32 (1H, d, J = 7.9 Hz), 7.48 (1H, d, J = 7.9 Hz), 7.61 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 31.3, 31.7, 55.3, 79.6, 125.5, 128.2, 128.5, 132.9, 133.6, 140.5; LREIMS m/z (relative intensity) 411 (M^+, 4) 333 (20), 262 (100); HREIMS m/z calcd for $C_{13}H_{18}Br_2O_3S$ (M^+) 411.9343, found 411.9337.

4.1.24. 2-Bromo-5-nitrobenzenesulfonyl chloride (33)

To a suspension of 2-bromobenzenesulfonyl chloride (5.00 g, 19.6 mmol) in conc. H_2SO_4 (80 mL) was added a solution of conc. H_2SO_4 (3.6 mL)/69% HNO_3 (3.6 mL, 39.2 mmol, 2 equiv) dropwise over a period of 30 min. The mixture becomes a clear yellow solution. The mixture was stirred for 4 h, then added slowly to ice water (800 mL) with vigorous stirring. After the ice melted the suspension was extracted with $CHCl_3$. The combined organics were dried ($MgSO_4$) and concentrated to give **33** as a crude pale yellow solid. ^{1}H NMR of the crude solid revealed that it consisted of 95% of the desired product **33** and 5% of the isomer resulting from nitration at the 3-position. Re-crystallization from hexane gave pure **33** (5.40 g, 92%). Mp 91–92 °C (lit.[17] mp 92 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1H, d, J = 8.5 Hz), 8.37 (1H, d, J = 8.4 Hz), 9.00 (1H, s).

4.1.25. 2-Bromo-5-nitrobenzenesulfonamide (34)

To a solution of **33** (5.60 g, 18.8 mmol) in dry THF (80 mL) at 0 °C was added slowly a solution of NH_3 in EtOH (2 M, 80 mL). The mixture was allowed to warm to rt and then stirred for 20 min. Water was added and the mixture was extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated and the resulting residue solid was washed with cold CH_2Cl_2 which gave compound **34** as a white solid (4.6 g, 87%). Mp 197–199 °C (lit.[17] mp 204–205 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ

7.95 (2H, br s), 8.11 (1H, d, J = 8.7 Hz), 8.27 (1H, dd, J = 8.6 Hz), 8.65 (1H, d, J = 2.5 Hz).

4.1.26. 5-Amino-2-bromobenzenesulfonamide (35)

To a solution of **34** (0.140 g, 0.500 mmol) in THF/conc. HCl (6.0 mL, 1:1) was added Zn powder (0.195 g, 6.0 equiv, 3.00 mmol) portion-wise. The mixture was stirred at rt for 2.5 h and then neutralized with satd aq $NaHCO_3$. The mixture was diluted with water and extracted with EtOAc. The combined organics were concentrated and the residue subjected to flash chromatography which gave compound **35** as a white solid (0.120 g, 96%). Mp 148–150 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 5.22 (2H, br s), 6.47 (2H, br s), 6.73 (1H, dd, J = 8.5 Hz), 7.37 (1H, d, J = 8.6 Hz), 7.4 (1H, d, J = 2.7 Hz); ^{13}C NMR (300 MHz, acetone-d_6) δ 103.2, 114.9, 118;.2, 135.1, 142.6, 148.3; LREIMS m/z (relative intensity) 250 (M^+, 100) 170 (17); HREIMS m/z calcd for $C_6H_7BrN_2O_2S$ (M^+), 249.9412, found 249.9409.

4.1.27. 2-Bromo-5-iodobenzenesulfonamide (36)

To a solution of compound **35** (0.250 g, 1.00 mmol) dissolved in H_2O/conc. HCl (4 mL, 3:1) at 0 °C was added a solution of $NaNO_2$ (0.070 g, 1.02 equiv, 1.02 mmol) in H_2O (3 mL) and stirred for 5 min. To this was added a solution of KI (0.498 g, 3.0 equiv, 3.00 mmol) in H_2O (3 mL) and the mixture was heated at 90 °C for 10 min. Water was added and the mixture was extracted with EtOAc. The combined organics were washed with an aq solution of $Na_2S_2O_3$ then dried (Na_2SO_4) and concentrated. The resulting residue was subjected to flash chromatography (3:7 EtOAc/hexane) which gave **36** as a white solid (0.283 g, 78%). Mp: 179–181 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 6.86 (2H, br s), 7.60 (1H, dd, J = 8.3 Hz), 7.85 (1H, d, J = 8.2 Hz), 8.35 (1H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ 91.7, 119.2, 136.9, 137.9, 142.1, 144.2; LREIMS m/z (relative intensity) 361 (M^+, 100) 280 (19), 154 (12); HREIMS m/z calcd for $C_6H_5BrINO_2S$ $(M)^+$ 360.8269, found 360.8269.

4.1.28. 4-Bromo-4′-methylbiphenyl-3-sulfonic acid amide (37)

To a solution of **36** (0.181 g, 0.5 mmol), tetrakis(triphenylphosphine)palladium(0) (0.058 g, 8 mol%) and 4-tolylboronic acid (0.15 g, 2.2 equiv, 1.1 mmol) in dry THF (20 mL) was added a 2M solution of K_2CO_3 (2 mL) and the mixture was refluxed under Ar for 16 h. The reaction was diluted with water and extracted with EtOAc. The combined organics were dried (Na_2SO_4) and concentrated and the resulting residue subjected to flash chromatography (2:8 EtOAc/hexane) which gave **37** as a white solid (0.105 g, 64%). Mp 155–157 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.38 (3H, s), 5.27 (2H, br s), 7.24 (2H, d, J = 7.7 Hz), 7.44 (2H, d, J = 7.7 Hz), 7.55 (1H, d, J = 8.2 Hz), 7.72 (1H, d, J = 8.2 Hz), 8.30 (1H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ 20.2, 117.6, 126.6, 127.4, 129.8, 131.0, 135.5, 135.6, 138.3, 140.6, 143.1; LREIMS m/z (relative intensity) 325 (M^+, 100), 245 (71), 165 (30); HRMS m/z calcd for $C_{13}H_{12}BrNO_2S$ (M^+) 324.9772, found 324.9775.

4.1.29. 1-Bromo-4-iodo-2-methylbenzene (38)

The following procedure is based on that of Kiang et al.[18] To a solution of 4-bromo-3-methylaniline (2.06 g, 11.0 mmol) in H_2O/conc.HCl (60 mL, 3:1) at 0 °C was added a solution of $NaNO_2$ (0.774 g, 1.02 equiv, 11.2 mmol) in H_2O (8 mL) and stirred for 5 min. A solution of KI (5.48 g, 3.00 equiv, 33.0 mmol) in H_2O (8 mL) was added and the reaction was heated to 100 °C for 10 min. After cooling to rt, water was added and the mixture was extracted with EtOAc. The combined organics were washed with an aq solution of $Na_2S_2O_3$, then dried (Na_2SO_4), and concentrated. The resulting residue was subjected to flash chromatography (100% hexane) to give **38** as white solid (2.63 g, 81%). ^{1}H NMR was consistent with that reported in the literature.[18] ^{1}H NMR (300 MHz, acetone-d_6) δ 2.33 (3H, s), 7.22 (1H, dd, J = 10.0 Hz), 7.33 (1H, d, J = 8.3 Hz), 7.55 (1H, dd, J = 1.4 Hz).

4.1.30. 1-Bromo-2-bromomethyl-4-iodobenzene (39)

The following procedure is based on that of Kiang et al.[18] To a solution of **38** (1.43 g, 4.80 mmol) in benzene (20 mL) were added *N*-bromosuccinimide (0.974 g, 1.1 equiv, 5.32 mmol) and benzoylperoxide (0.234 g, 0.20 equiv, 0.964 mmol). The mixture was gently refluxed under Ar by placing a 250W lamp close to the reaction vessel. After 6 h, water was added and the mixture was extracted with CH_2Cl_2. The combined organics were washed with satd aq $NaHCO_3$, then dried (Na_2SO_4), and concentrated. The resulting residue was subjected to flash chromatography three times (hexane) which gave pure **39** as a white solid (1.18 g, 65%). The ^{1}H NMR was consistent with that reported in the literature.[18] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (2H, s), 7.27 (1H, d, J = 8.4 Hz), 7.45 (1H, dd, J = 8.4 Hz), 7.75 (1H, d, J = 2.0 Hz).

4.1.31. Thioacetic acid *S*(2-bromo-5-iodobenzyl) ester (40)

Prepared using the same procedure as that described for compound **10**. The following quantities were used: **39** (6.56 g, 17.5 mmol), potassium thioacetate (2.19 g, 1.10 equiv, 0.40 mmol), DMF (50 mL). Pure **40** (6.49 g, 99%) was obtained as a crystalline white solid after aqueous workup. Mp 70–72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 4.09 (2H, s), 7.18 (1H, d, J = 8.4 Hz), 7.35 (1H, dd, J = 8.3 Hz), 7.71 (1H, d, J = 1.8 Hz), ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.4, 33.5, 92.7, 124.4, 134.4, 137.9, 139.5, 139.7, 194.3; LREIMS m/z (relative intensity) 370 (M^+, 3), 291 (100); HREIMS m/z calcd for C_9H_8BrIOS (M^+) 369.8524, found 369.8527.

4.1.32. (2-Bromo-5-iodophenyl)methanesulfonyl chloride (41)

Chlorine gas was bubbled through a solution of **40** (0.370 g, 1.00 mmol) in acetic acid/water (16 mL, 3:1) at 0 °C for 1 h. The mixture was purged with N_2 and air to remove excessive Cl_2, water was added, and the mixture was extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated. The resulting sulfonyl chloride was unstable and so the crude product was used immediately for the next step.

4.1.33. *N*,*N*-Diallyl-(2-bromo-5-iodo-phenyl)methane sulfonamide (42)

To a solution of crude **41** in CH_2Cl_2 (15 mL) was added diallylamine (0.31 mL, 2.5 equiv, 2.5 mmol) and the mixture was stirred for 1 h. Water was added and the mixture was extracted with CH_2Cl_2 and the combined organics were dried (Na_2SO_4) and concentrated. The resulting residue was subjected to flash chromatography (0.5:9.5 EtOAc/hexane) which gave pure **42** as a white solid (0.383 g, 84% over two steps). Mp 60–62 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (4H, d, J = 6.4 Hz), 4.33 (2H, s), 5.17 (4H, d, J = 11.7 Hz), 5.65 (2H, m), 7.27 (1H, d, J = 8.4 Hz), 7.46 (1H, d, J = 8.4 Hz), 7.82 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.7, 57.3, 92.4, 119.5, 125.5, 131.5, 132.7, 134.6, 139.1, 141.1; LREIMS m/z (relative intensity) 455 (M^+, 3) 399 (12), 331 (100); HREIMS m/z calcd for $C_{13}H_{15}BrINO_2S$ (M^+) 454.9052, found 454.9055.

4.1.34. *N*,*N*-Diallyl-*C*-(2-bromo-5-iodophenyl)-*C*,*C*-difluoromethanesulfonamide (43)

To a solution of **42** (1.46 g, 3.21 mmol) and *N*-fluorobenzenesulfonimide (2.09 g, 2.10 equiv, 6.64 mmol) in dry THF (50 mL) at −78 °C was added NaHMDS (1.0 M in THF, 7.54 mL, 2.40 equiv, 7.54 mmol) over 10 min. The mixture was stirred at −78 °C for 2 h, then allowed to warm to rt, and then stirred for 16 h. The reaction was quenched with satd aq NH_4Cl and then extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4), then concentrated, and the resulting residue was subjected to flash chromatog-

raphy (0.5:9.5 EtOAc/hexane) which gave pure **43** as a white solid (1.44 g, 94%). Mp 89–91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (4H, d, J = 6.5 Hz), 5.22–5.29 (4H, m), 5.72–5.86 (2H, m), 7.37 (1H, d, J = 8.4 Hz), 7.61 (1H, d, J = 8.4 Hz), 7.92 (1H, d, J = 1.9 Hz); ^{19}F NMR (285 MHz, $CDCl_3$) δ −97.0; ^{13}C NMR (75 MHz, $CDCl_3$) δ 50.1, 91.8, 120.2, 120.6 (t, J = 285 Hz), 120.8, 130.21 (t, J = 21.7 Hz), 132.2, 137.1, 139.1 (t, J = 8.2 Hz), 141.8; LREIMS m/z (relative intensity) 491 (M^+, 3), 331 (100), 204 (10); HREIMS m/z calcd for $C_{13}H_{13}BrF_2INO_2S$ (M^+) 490.88631 found 490.8871.

4.1.35. *N,N*-Diallyl(4-bromo-4′-methyl-biphenyl-3-yl)difluoromethanesulfonamide (44)

To a solution of **43** (0.461 g, 0.940 mmol), tetrakis(triphenylphosphine)palladium(0) (0.231 g, 20 mol%) and 4-tolylboronic acid (0.150 g, 1.10 equiv, 1.02 mmol) in dry THF (25 mL) was added and a 2 M aq K_2CO_3 solution (2.0 mL). The mixture was refluxed under Ar for 16 h. After cooling to rt, water was added and the mixture was extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated and the resulting residue subjected to flash chromatography (1:4 EtOAc/hexane) which gave pure **44** as a white solid (0.313 g, 73%). Mp 80–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (3H, s), 4.05 (4H, d, J = 7.5 Hz), 5.25–5.32 (4H, m), 5.86 (2H, m), 7.25 (2H, d, J = 8.0 Hz), 7.47 (2H, d, J = 8.1 Hz) 7.52 (1H, d, J = 8.4 Hz), 7.73 (1H, d, J = 8.3 Hz), 7.89 (1H, d, J = 2.1 Hz); ^{19}F NMR (285 MHz, $CDCl_3$) δ −96.3; ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 50.2, 119.3, 120.0, 121.8 (t, J = 284 Hz), 126.9, 128.6 (t, J = 9.1 Hz), 129.2 (t, J = 8.0 Hz), 129.8, 131.1, 132.5, 135.8, 136.0, 138.2, 140.5; LREIMS m/z (relative intensity) 455 (M^+, 9), 295 (100); HREIMS m/z calcd for $C_{20}H_{20}BrF_2NO_2S$ (M^+) 455.0366, found 455.0357.

4.1.36. (4-Bromo-4′-methylbiphenyl-3-yl)difluoromethanesulfonamide (45)

A solution of **44** (0.320 g, 0.70 mmol), tetrakis(triphenylphosphine)palladium(0) (0.161 g, 20 mol%), and 1,3-dimethylbarbituric acid (2.18 g, 20.0 equiv, 14.0 mmol) in dry CH_3CN (30 mL) was refluxed vigorously for 18 h. Water was added and the mixture was extracted with EtOAc and the combined organics concentrated. The residue was dissolved in THF/6N HCl (120 mL, 3:1), stirred for 1h, diluted with H_2O, and extracted with EtOAc. The combined organics were dried (Na_2SO_4), then concentrated, and the resulting residue subjected to flash silica chromatography (3:7 EtOAc/hexane) which gave pure **45** as a white solid (0.225 g, 86%). Mp 149–151 °C; ^{1}H NMR (300 MHz, a acetone-d_6) δ 2.38 (3H, s), 5.08 (2H, br, s), 7.24 (2H, d, J = 6.4 Hz), 7.44 (2H, d, J = 7.8 Hz) 7.55 (1H, d, J = 8.4 Hz), 7.74 (1H, d, J = 8.3 Hz), 7.87 (1H, s,); ^{19}F NMR (285 MHz, acetone-d_6) δ −98.6; ^{13}C NMR (75 MHz, acetone-d_6) δ 20.2, 119.0 (t, J = 2.5 Hz), 120.3 (t, J = 283.3 Hz), 126.7, 128.4 (t, J = 16.4 Hz), 129.0 (t, J = 7.8 Hz), 129.8, 131.1, 135.6, 136.1, 138.3, 140.3; LREIMS m/z (relative intensity) 375 (M^+, 20), 295 (100); HREIMS m/z calcd for $C_{14}H_{12}BrF_2NO_2S$ (M^+) 374.9740, found 374.9737.

4.1.37. {[2-Bromo-4-(4′-bromo-3′-sulfamoylbiphenyl-4-ylmethylsulfanylmethyl)phenyl]difluoro-methyl}phosphonic acid diethyl ester (46)

To a suspension of **10** (0. 100 g, 1 equiv, 0.233 mmol) and **13** (0.094 g, 1 equiv, 0.233 mmol) in EtOH (5 mL) was added $CHCl_3$ (3 mL) to make the solution clear. The solution was cooled to 0 °C (ice bath) and an aqueous solution of NaOH (2.0 mL, 2 N, 17 equiv, 4.0 mmol) was added and the mixture stirred for 30 min. The mixture was diluted with water and extracted with EtOAc. The combined organics were dried (Na_2SO_4) and concentrated. The resulting residue was subjected to flash chromatography (3:7 EtOAc/hexane) which gave pure **46** as a semisolid (0.158 g, 96%). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.27 (6H, t, J = 7.0 Hz), 3.75 (4H, br s), 4.13–4.24 (4H, m), 6.80 (2H), 7.40–7.45 (3H), 7.57–7.63 (4H, m), 7.74 (1H, dd, J = 8.2 Hz), 7.85 (1H, d, J = 8.2 Hz), 8.30 (1H, d, J = 1.7 Hz); ^{31}P NMR (121 MHz, acetone-d_6) δ 6.2 (t, J = 110.9 Hz); ^{19}F NMR (282 MHz, acetone-d_6) δ −104.0 (d, J = −111.6 Hz); ^{13}C NMR (75 MHz, acetone-d_6) δ 16.7 (d, J = 5.2), 35.2, 36.1, 65.5 (d, J = 6.8 Hz), 118.8, 119.1 (dt, J = 265.1, 218.4 Hz), 120.3, 127.7, 128.4, 128.8, 130.7, 130.8 (t, J = 7.3 Hz), 132.0, 136.4, 136.5, 137.9, 139.5, 141.1, 144.0, 144.8; LR$^+$ESIMS m/z (relative intensity) 712 ([M+1]$^+$, 100); HR$^+$ESIMS m/z calcd for $C_{25}H_{26}Br_2F_2NO_5PS_2$ ([M+1]$^+$) 711.9403, found 711.9418.

4.1.38. [2-Bromo-4-(4′-bromo-3′-sulfamoylbiphenyl-4-ylmethylsulfanylmethyl)phenyl]-difluoromethanesulfonic acid, 2,2-dimethylpropyl ester (47)

Prepared using the same procedure as that described for **46**. The following quantities were used: compound **11** (0.033 g, 1.0 equiv, 0.074 mmol), compound **13** (0.030 g, 1.0 equiv, 0.074 mmol), EtOH (5 mL), and 2 N NaOH (2.0 mL, 54 equiv, 4.0 mmol). Flash chromatography (3:7 EtOAc/hexane) of the crude residue gave pure **47** as a semisolid (0.054 g, 78%). ^{1}H NMR (300 MHz, acetone-d_6) δ 0.98 (9H, s), 3.77 (2H, s), 3.79 (2H, s), 4.20 (s, 2H), 6.8 (2H, s), 7.42 (2H, d, J = 8.1 Hz), 7.55 (1H, d, J = 8.1 Hz), 7.63 (2H, d, J = 8.1 Hz), 7.69–7.77 (3H), 7.86 (1H, d, J = 8.3 Hz), 8.33 (1H, d, J = 2.1 Hz); ^{19}F NMR (285 MHz, acetone-d_6) δ −95.3; ^{13}C NMR (75 MHz, acetone-d_6) δ 25.1, 31.8, 34.3, 35.3, 84.6, 118.0, 120.3 (t, J = 2.6 Hz), 120.7 (t, J = 285.3 Hz), 125.5 (t, J = 21.2 Hz), 126.9, 127.6, 128.4, 129.9, 130.8 (t, J = 7.9 Hz), 131.2, 135.6, 136.1, 137.2, 138.6, 140.3, 143.2, 146.1; LR$^+$ESIMS m/z (relative intensity) 742 ([M+NH$_4$]$^+$, 100); HR$^+$ESIMS m/z calcd for $C_{26}H_{31}Br_2F_2N_2O_5S_3$ ([M+NH$_4$]$^+$) 742.9730, found 742.9739.

4.1.39. [2-Bromo-4-(4′-bromo-3′-sulfamoylbiphenyl-4-ylmethylsulfanylmethyl)phenyl]-methanesulfonic acid, 2,2-dimethylpropyl ester (48)

Prepared using the same procedure as that used for compound **46**. The following quantities were used: compound**13** (0.101 g, 1.0 equiv, 0.250 mmol), **12** (0.102 g, 1.0 equiv, 0.250 mmol), EtOH (5 mL), and 2 N NaOH (2.0 mL, 16 equiv, 4.0 mmol). The residue was subjected to flash chromatography (3:7 EtOAc/hexane) which gave pure **48** as semisolid (0.130 g, 76%). ^{1}H NMR (300 MHz, acetone-d_6) δ 0.92 (9H, s), 3.72 (2H, s), 3.74 (2H, s), 3.88 (s, 2H), 4.72 (2H), 6.81 (2H, s), 7.36–7.43 (3H, m), 7.54–7.64 (4H, m), 7.74 (1H, d, J = 8.3 Hz), 7.85 (1H, d, J = 8.3 Hz), 8.32 (1H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ 25.4, 31.4, 34.5, 35.2, 54.7, 79.3, 118.0, 125.1, 126.9, 127.3, 127.5, 128.4, 129.8, 131.2, 132.9, 133.3, 135.6, 137.0, 138.8, 140.3, 141.9, 143.1; LR$^+$ESIMS m/z (relative intensity) 707 ([M + NH$_4$]$^+$, 100); HR$^+$ESIMS m/z calcd for $C_{26}H_{33}Br_2N_2O_5S_3$ ([M+NH$_4$]$^+$) m/z 706.9918, found 706.9924.

4.1.40. ([2-Bromo-4-[4′-bromo-3′-(difluorosulfamoylmethyl) biphenyl-4-ylmethylsulfanyl-methyl)phenyl]difluoromethyl) phosphonic acid diethyl ester (49)

Prepared from compounds **10** and **14** in 72% yield using the same procedure as that described for **46**. The following quantities were used: **14** (0.030 g, 1.0 equiv, 0.066 mmol), **10** (0.029 g, 1.0 equiv, 0.066 mmol), EtOH (5 mL), and 2 N NaOH (1.0 mL, 30 equiv, 2.0 mmol). Flash chromatography (2:3 EtOAc/hexane) of the crude residue gave pure **49** as a semisolid. ^{1}H NMR (300 MHz, acetone-d_6) δ 1.28 (6H, t, J = 6.3 Hz), 3.76 (4H, br s), 4.13–4.24 (4H, m), 7.41–7.47 (5H, NH_2 peak merged), 7.57–7.65 (4H, m), 7.76 (1H, d, J = 8.2 Hz), 7.85 (1H, d, J = 8.2 Hz), 7.94 (1H, d, J = 1.7 Hz); ^{31}P NMR (121 MHz, acetone-d_6) δ 5.1 (t, J = 110.9 Hz); ^{19}F NMR (282 MHz, acetone-d_6) δ −98.6, −104.0 (d, J = 111.8 Hz); ^{13}C NMR (125.75 MHz, acetone-d_6) δ 14.3, 35.2, 36.1, 65.5 (d, J = 6.7 Hz), 119.1 (dt, J = 265.0, 218.2 Hz), 120.2, 120.4 (t, J = 3.6 Hz), 121.1 (t, J = 283.3 Hz), 127.8, 128.8, 129.8 (t, J = 22.7 Hz), 130.0 (t,

J = 7.6 Hz), 130.7, 130.9 (t, J = 7.9 Hz), 131.1 (dt, J = 21.5, 15.0 Hz), 132.1, 136.4, 137.0, 138.0, 139.6, 140.8, 144.9. LR$^+$ESIMS m/z (relative intensity) 761 ([M+1]$^+$, 100); HR$^+$ESIMS m/z calcd for $C_{26}H_{27}Br_2F_4NO_5PS_2$ (M+1)$^+$ m/z 761.9388 found 761.9371.

4.1.41. [2-Bromo-4-[4′-bromo-3′-(difluorosulfamoylmethyl) biphenyl-4-ylmethylsulfanylmethyl]-phenyl}difluoromethanesulfonic acid 2,2-dimethyl-propyl ester (50)

Prepared using the same procedure as that described for **46**. The following quantities were used: **11** (0.038 g, 1.0 equiv, 0.085 mmol), **14** (0.039 g, 1.0 equiv, 0.085 mmol), EtOH (10 mL), 2 N NaOH (1.0 mL, 23.5 equiv, 2.0 mmol). Flash chromatography (3:7 EtOAc/hexane) of the crude residue gave pure **50** as a semi-solid (0.055 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (9H, s), 3.58 (2H, s), 3.64 (2H, s), 4.16 (2H, s), 5.16 (2H, s), 7.33 (3H, s), 7.47–7.62 (5H, m), 7.76 (1H, d, J = 8.2 Hz), 7.89 (1H, s); ^{19}F NMR (285 MHz, $CDCl_3$) δ −96.9, −94.7; ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 32.2, 34.6, 35.6, 84.8, 119.7, 120.1 (t, J = 284.4 Hz), 120.7 (t, J = 286.3 Hz), 120.96, 125.9 (t, J = 21.1 Hz), 127.2, 127.7 (t, J = 21.3 Hz), 127.9, 129.5 (t, J = 7.8 Hz), 129.7, 130.8 (t, J = 7.8 Hz), 131.6, 136.1, 136.2, 137.5, 137.8, 140.2, 144.7; LR$^+$ESIMS m/z (relative intensity) 704, ([M-C_5H_{11}]$^+$, 100); HR$^+$ESIMS m/z calcd for $C_{22}H_{16}Br_2F_4NO_5S_3$ ([M−C_5H_{11}]$^+$), 703.8493, found 703.8507.

4.1.42. Synthesis of peptide 51

Fmoc-L-Phe(p-CH_2SO_3Na)OH was prepared according to the procedure of Miranda et al.[22] Solid-phase peptide synthesis was carried out manually using Fmoc strategy using the Rink amide AMS resin (0.71 mmol/g). The resin was preswollen in CH_2Cl_2 for 2 h. Coupling of Fmoc-L-LeuOH was carried out with the pentafluorophenyl ester of Fmoc-L-leucine (4.0 equiv), HOBt (4.0 equiv) in DMF (stirred 10 min at room temperature before being added to the resin) and then shaken with resin (2× 60 min). The resin was then rinsed with DMF (6× 3 min) and CH_2Cl_2 (2× 3 min). All remaining coupling reactions were carried out as follows: A solution of Fmoc-amino acid (4.0 equiv), HATU or HBTU (4.0 equiv), HABt or HOBt (4.0 equiv), DIEA (4.0 equiv), and DMF (3 mL) was stirred at room temperature for 10 min. This solution was then added to the resin and shaken for 60 min. Each coupling was carried out twice. The resin was washed after each coupling with DMF (3 mL, 6× 3 min) and CH_2Cl_2 (3 mL, 2× 3 min). The Fmoc group was removed using 20% piperidine/DMF (2× 15 min), and then the resin was washed with DMF (3 mL, 4× 3 min) 1:1 DMF:CH_2Cl_2 (3 mL, 1× 3 min) and CH_2Cl_2 (3 mL, 1× 3 min). The side chains of both the Asp and Glu were protected as *t*-butyl esters. Once the peptide was assembled the final Fmoc group was removed and the resin dried overnight. The resin was placed in a small round-bottomed flask and Reagent K (82.5% TFA, 2.5% ethanedithiol, 5% water, 5% thioanisole, and 5% phenol) was added and stirred for 2.5 hours at room temperature. The mixture was filtered and the residue evaporated. To this mixture was added cold methyl *t*-butylether (0 °C) and the suspension transferred to a 35 mL centrifuge tube. The mixture was centrifuged at 3500 rpm at 0 °C for 10 min and then the solvent was decanted off. This process was repeated two more times affording a white crude peptide. The peptide was dried overnight in the fumehood, then placed in water and cooled, and lyophilized which gave crude peptide **51** as a fluffy white powder. Pure peptide **51** was obtained by semi-preparative RP-HPLC using a C-18 column (250 × 21.2 mm, 10 micron) and was eluted using a linear gradient of 95% H_2O containing 0.1%TFA/5% CH_3CN to 60% H_2O containing 0.1%TFA/40% CH_3CN over 70 minutes at a flow rate of 8 mL/min. The retention time was 32 min and the peptide was detected using a detector set at 220 nM. Analytical RP-HPLC (C-18 column, 250 × 4.6 mm, 10 μ) revealed the peptide to be greater than 97% pure. LR$^-$ESIMS m/z (relative intensity) 800 ([M−H]$^-$, 100), HR$^-$ESMS m/z calculated for $C_{32}H_{47}N_7O_{15}S$ (M−H)$^-$ 800.2773, found 800.2764.

4.2. Inhibition studies

4.2.1. IC_{50} and K_i determinations

Stock solutions of the inhibitors were prepared in 40% DMSO/60% buffer containing 50 mM Bis–Tris HCl, pH 6.3. 10 μL of each inhibitor stock solution was added to the wells of a 96-well microtiter plate containing 90 μL of 5.5 μM diFMUP in 50 mM Bis–Tris HCl, pH 6.3, 5 mM DTT, and 2 mM EDTA. The reactions were initiated at 25 °C with 10 μL of a 30 nM solution of PTP1B[27] in a buffer containing 50 mM Bis–Tris HCl, pH 6.3, 20% glycerol, 5 mM DTT, 2 mM EDTA, and 0.1% Triton X-100. The production of fluorescent product diFMU was monitored for 10 min using a spectrofluorimeter platereader with excitation and emission at 360 nM and 460 nM, respectively. The initial rates of enzyme activity in relative fluorescence units per second (RFU/s) were used to determine the IC_{50}'s and K_i's. For IC_{50}'s, the ratio of the initial rate in the presence of inhibitor (V_i) to that in the absence of inhibitor (V_o) was calculated and plotted as a semi-log curve in Grafit, from which the IC_{50} value was calculated based on the following equation: $V_i = V_o/[1 + ([I]/IC_{50})^S] + B$, where V_i is the initial rate of reaction at an inhibitor concentration of [I]; V_o is the velocity in the absence of inhibitor; B is background and s is the slope factor equal to $V_o - B$. For K_i's, data were plotted as Lineweaver–Burk graphs and K_i values were calculated from replots of the slopes or intercepts of the Lineweaver–Burk graphs according to the equations for mixed or competitive inhibition.

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Supplementary data

Supplementary data (Lineweaver–Burk plots for compound **7** and **8**, IC_{50} plot for peptides **3** and **51** and data from reversibility experiments) associated with this article can be found in the online version at doi:10.1016/j.bmc.2008.05.062.

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