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Enantioselective Synthesis of Protected L-4-[Sulfonamido(difluoromethyl)]phenylalanine and L-4-[Sulfonamido(methyl)]phenylalanine and an Examination of Hexa- and Tripeptide Platforms for Evaluating pTyr Mimics for PTP1B Inhibition

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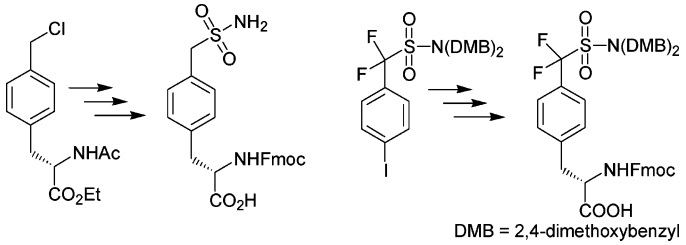

The first enantioselective syntheses of L-4-(sulfonamidomethyl)phenylalanine and L-[sulfonamido-(difluoromethyl)]phenylalanine suitably protected for peptide syntheses are described. A key step in the synthesis of L-(sulfonamidomethyl)phenylalanine was an oxidative chlorination on Ac-L-Phe(4-CH_2-$SCOCH_3$)-OEt to give crude Ac-L-Phe(4-CH_2SO_2Cl)-OEt, which could be reacted with amines to give the corresponding sulfonamides. Key to the preparation of L-[sulfonamido(difluoromethyl)]phenylalanine was a highly enantioselective reaction involving William's auxiliary and a benzylic bromide intermediate. These amino acids were incorporated into two peptide sequences, DADE-X-LNH_2 and FmocGlu(OBn)-X-LNH_2, which have previously been employed as platforms for assessing pTyr mimics for inhibition of protein tyrosine phosphatase 1B (PTP1B). Inhibition studies with these and other peptides and PTP1B revealed that good inhibition could be obtained using the tripeptide platform, although the presence of a pTyr mimic was not required for good inhibition. These results suggest that the FmocGlu(OBn)-X-LNH_2 tripeptide platform is not suitable for assessing pTyr mimics for PTP1B inhibition.

Introduction

The design and synthesis of hydrolytically stable phosphotyrosine (pTyr) mimetics has expanded in recent years to the point where it has become almost an entire field of study unto itself.[1] This is mainly due to their application as inhibitors and probes of therapeutically significant enzymes involved in protein tyrosine phosphorylation or dephosphorylation and proteins that bind to phosphotyrosine.[1] Such proteins include protein tyrosine kinases (PTKs), protein tyrosine phosphatases (PTPs), and SH2 domain binding proteins. The application of pTyr mimics to the development of inhibitors of PTPs has been a particularly active area of research, with a distinct emphasis on PTP1B, an enzyme that is involved in the down regulation of insulin signaling and a well-validated therapeutic target for the treatment of diabetes and obesity.[2,3]

pTyr mimics for PTP1B inhibition are often initially assessed by incorporating them into peptide platforms with the D-A-D-E-X-LNH_2 (X = pTyr mimic) sequence being the most common. This hexapeptide corresponds to a phosphorylated sequence (X = pTyr) in the epidermal growth factor receptor and is a good substrate for PTP1B. The most effective pTyr mimic, in the context of this hexapeptide platform and PTP1B inhibition, is difluorophosphonomethylphenylalanine (F_2Pmp,

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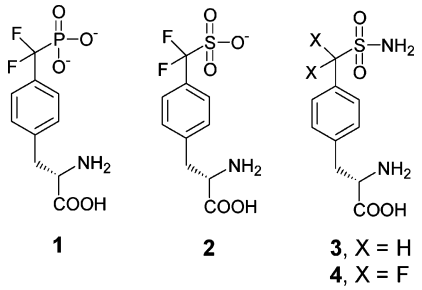

FIGURE 1. pTyr mimics **1**−**4**.

1) (Figure 1).[4] The difluoromethylenephosphonic acid (DFMP) group has also been shown to be a very effective mimic even in nonpeptidyl scaffolds.[2,5] However, it has been suggested that the dianionic nature of this mimic limits its cell permeability.[1] Consequently, a wide variety of other pTyr mimics have been synthesized and evaluated in this hexapeptide.[6] We recently demonstrated that difluorosulfonomethylphenylalanine (F_2Smp, **2**) is the most effective nonhydrolyzable monoanionic pTyr mimic for PTP1B inhibition when incorporated into this hexapepide platform.[7] Most other monoanionic pTyr mimics bear a carboxylic acid moiety, though these are not particularly effective in the hexapeptide platform.[6] Gao et al. have provided a possible explanation as to why the D-A-D-E-X-LNH_2 peptides bearing certain pTyr mimics have a poor affinity for PTP1B.[6] Although the peptide provides beneficial binding contacts with residues outside of the active site, it limits the depth of insertion and freedom of the pTyr mimic within the catalytic pocket. Hence, it was suggested that studies using this hexapeptide platform may not allow one to assess the true effectiveness of certain pTyr mimics.[6] More recently, Lee et al. demonstrated that a wide variety of monoanionic and dianionic pTyr mimics exhibiting poor affinity for PTP1B in the hexapeptide platform[6] were considerably more effective in tripeptide platforms such as FmocGlu(OBn)-X-LNH_2 though none were as potent as the F_2Pmp-bearing hexapeptide.[8,9] Similar results were also found when these tripeptides were examined as inhibitors of Yersinia PTP (YopH). On the basis of these results, it was suggested that certain monoanionic COOH-based pTyr mimics, which were considered to be of limited utility based on the hexapeptide studies, were indeed good pTyr mimics.[8]

The difference in potency between several of the monoanionic and dianionic mimics studied by Lee at al, when incorporated into the tripeptide mentioned above, was surprisingly small. Indeed, tripeptides bearing monoanionic carboxylic acid-based mimics exhibited affinities for PTP1B only 2−3-fold greater than the analogous F_2Pmp-bearing tripeptide.[8,9] These results led us to question whether a neutral pTyr mimic could also be effective in the tripeptide platform. An effective neutral pTyr mimic would be very useful in increasing the bioavailability of PTP inhibitors. Neutral pTyr mimics are rare. Researchers at Sugen have suggested, on the basis of studies with nonpeptidyl PTP1B inhibitors, that the trifluoromethylsulfonamido group is an effective neutral phosphate mimic.[10] We chose to examine the sulfonamide **3** as a potential pTyr mimic. The sulfonamide group has found widespread use as a pharmacophore in medicinal chemistry and numerous bioactive agents bear this functionality.[11] It has a geometry similar to that of the phosphate group yet does not bear a negative charge. Recently, Chen et al. reported a racemic synthesis of amino acid **3** and its incorporation into the D-A-D-E-X-LNH_2 platform.[12] Although these workers found this peptide to be a very poor inhibitor of PTP1B, this amino acid was never examined as a pTyr mimic in the FmocGlu(OBn)-X-LNH_2 platform where the potential constraints of the hexapeptide platform mentioned above may not be as severe. Burke and co-workers have shown that peptides bearing F_2Pmp can be up to 1000-fold more potent PTP1B inhibitors than their nonfluorinated analogues.[4a−c] pH studies,[4c] computational studies, and X-ray crystallographic analyses[13] of a DFMP-bearing inhibitor complexed to PTP1B strongly suggest that the effect of the fluorines is due to interaction of the fluorines with residues in the active site and is not due to pK_a effects. Consequently, we also decided to examine the fluorinated analogue of **3**, compound **4**, as a pTyr mimic. Here, we report an enantioselective synthesis of amino acids **3** and **4**, suitably protected for peptide synthesis, their incorporation into the tri- and hexapeptide platforms described above, and an evaluation of these peptides as inhibitors of PTP1B. These studies yielded some important results concerning the utility of the tripeptide scaffold for assessing pTyr mimics.

Results and Discussion

Chen et al. recently reported a racemic synthesis of Fmoc-protected **3**.[12] After incorporation into the D-A-D-E-X-LNH_2 hexapeptide, the resulting diastereomeric peptides were separated by HPLC before inhibition studies. We wished to develop an enantioselective synthesis of protected **3** to avoid having to separate diastereomeric peptides. Chen et al.'s synthesis of Fmoc-protected racemic **3** was achieved by reacting compound **5** with sodium sulfite followed by conversion of the resulting sulfonate to the sulfonyl chloride using PCl_5, which was reacted with ammonium hydroxide to give sulfonamide **6** in 31% yield from **5** (Scheme 1). Refluxing the sulfonamide in 6 N HCl followed by reaction with Fmoc-OSu gave Fmoc-protected racemic **3**. Miranda et al. have shown that crude L-Phe(4-CH_2-SO_3Na)-OH can be prepared by reacting the hydrochloride salt of L-Phe(4-CH_2Cl)-OH with sodium sulfite.[14] Although this presented a potential route to protected L-**3** starting with

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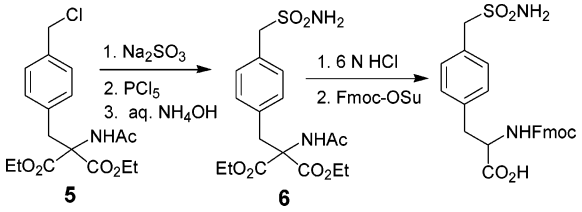
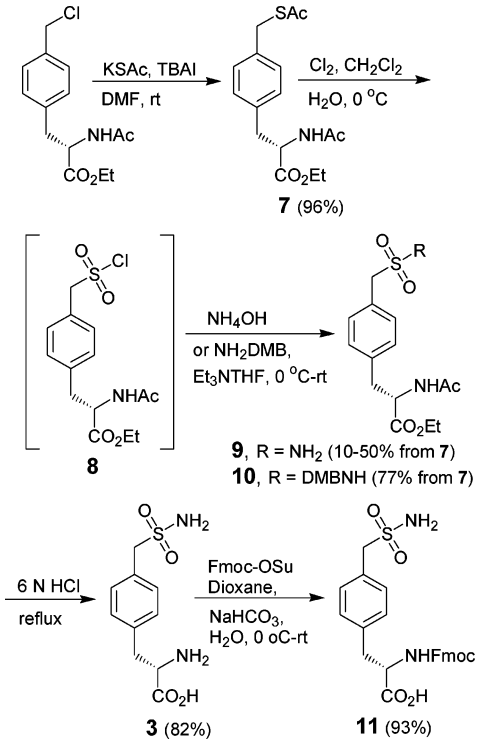
SCHEME 1. Chen et al.'s Synthesis of Fmoc-Protected Racemic 3



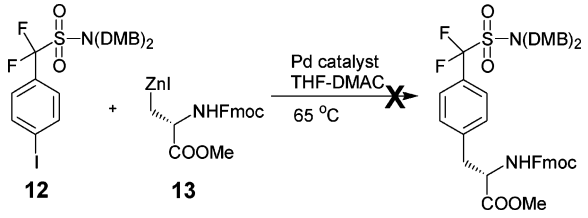
SCHEME 2. Synthesis of Fmoc-Protected 3



protected L-Phe(4-CH_2Cl)-OH and a series of reactions involving sodium sulfite, PCl_5 and finally ammonia, the modest yields of these reactions obtained by Chen et al. on compound **5** prompted us to investigate an alternative route, preferably one which did not require the synthesis and purification of polar sulfonate salts. To this end, Ac-L-Phe(4-CH_2Cl)-OEt[14,15] was reacted with potassium thioacetate to give thioacetate **7** in 96% yield (Scheme 2). Oxidative chlorination of **7** with Cl_2 in dichloromethane−water provided crude sulfonyl chloride **8**. Reaction of crude **8** with aq NH_4OH in various solvents or NH_3/MeOH gave sulfonamide **9**; however, the yields were inconsistent ranging from 10 to 50%, and purification of the product was difficult. However, reaction of crude **8** with 2,4-dimethoxybenzylamine gave secondary sulfonamide **10** in 77% yield (from **7**). Acid hydrolysis of **10** gave amino acid **3** in 82% yield. Treatment of **3** with Fmoc-OSuc yielded the desired Fmoc-protected amino acid **11** in 93% yield. HPLC analysis of two diastereomeric dipeptides, Fmoc-**3**-L-Leucine-NH_2 and Fmoc-**3**-DL-Leucine-NH_2, revealed that the ee of **11** was >97%.

Before a synthesis of protected **4** was undertaken, the issue of the protection of the sulfonamide moiety was addressed. Harrington and co-workers demonstrated that the presence of fluorines alpha to a sulfonamide leads to a linear acidity increase

SCHEME 3. Attempted Synthesis of Protected 4 by a Cross-Coupling Approach



of approximately 1.5 pK_a units per fluorine.[16] The pK_a of the sulfonamide moiety in **3** can be estimated to be approximately 10.5.[17] On the basis of Harrington's studies, the pK_a of the sulfonamide moiety in **4** would be expected to be approximately 7.5. Although the nonfluorinated amino acid **3** was readily incorporated into the hexapeptide platform mentioned above with the sulfonamide group unprotected, we were concerned that the lower pK_a of the sulfonamide protons in compound **4** could potentially result in side reactions during peptide synthesis that might not be encountered with **3**.[18,19] Therefore, we decided to incorporate amino acid **4** into peptides with the sulfonamide protected. To reduce the number of synthetic manipulations, it was deemed advantageous to use the same sulfonamide protecting group for the synthesis of protected amino acid **4** and its incorporation into peptides. We recently reported that α,α-difluorosulfonamides can be prepared by electrophilic fluorination using *N*-fluorobenzenesulfonimide (NFSi).[20] In this paper, we introduced the bis(2,4-dimethoxybenzyl) (DMB) group as a new sulfonamide protecting group and this was particularly effective for the preparation of α-fluorosulfonamides. Benzylic sulfonamides protected with this group readily underwent electrophilic fluorination and were stable to wide variety of reaction conditions yet the group could be easily removed using TFA/CH_2Cl_2. Thus, we anticipated that the DMB group would be suitable for the construction of protected **4** and for peptide synthesis using Fmoc chemistry.

We initially examined a cross-coupling route to protected **4** since it represented a relatively direct route to this compound and has been applied to the synthesis of other pTyr mimics including compounds **1** and **2** (Scheme 3).[21] However, numerous attempts to couple bis(dimethoxybenzyl)-protected sulfonamide **12**[20] to zincate **13** with a variety of catalysts and under different reaction conditions were unsuccessful. Therefore, we examined an alternative approach using William's auxiliary, a route that has also been used in the synthesis of other pTyr mimics (Scheme 4).[6] Compound **12** was treated with *n*-BuLi followed by the addition of DMF to give the aldehyde **14** in 77% yield. Conversion to the benzyl bromide **16** was achieved by reducing the aldehyde moiety of **14** to alcohol **15** with $NaBH_4$ (90%) followed by treatment of **15** with CBr_4/PPh_3 (95%). Reaction of **16** with the lithium enolate of William's lactone gave compound **17** in 80% yield. The desired protected amino acid **18** was readily obtained by hydrogenation of **17** using $PdCl_2$ as

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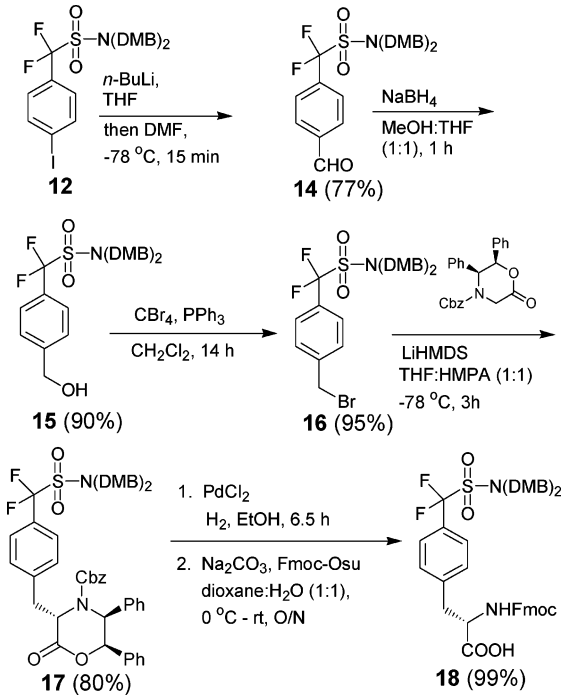
(21) See: Liu, S.; Dockendorf, C.; Taylor, S. D. *Org. Lett.* **2001**, *3*, 1571 and references therein.

SCHEME 4. Synthesis of Fluorosulfonamide 18



catalyst followed by treating the crude product with Fmoc-OSuc in dioxane−aq Na_2CO_3 (99%, two steps). The ee of the protected F_2Samp, **18**, was determined by incorporating **18** into the two dipeptides, Fmoc-**4**-L-leucine and Fmoc-**4**-DL-leucine. HPLC analysis of the dipeptides revealed that **18** was obtained in a >97% ee.[22]

Amino acids **11** and **18** were incorporated into the hexa- and tripeptide platform mentioned above using standard solid-phase peptide synthesis protocols employing Fmoc chemistry. Purification by preparative RP-HPLC gave the desired peptides DADE-**3**-LNH_2 (**19**), DADE-**4**-LNH_2 (**20**), FmocGlu(OBn)-**3**-LNH_2 (**21**), and FmocGlu(OBn)-**4**-LNH_2 (**22**).

IC_{50}'s with PTP1B were performed in bis-tris buffer at pH 6.5, containing 15 mM NaCl, 2 mM EDTA, 0.001% Triton X-100 and 7.5% DMSO using 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) as substrate at K_m concentration (20 μM).[23] The IC_{50}'s are given in Table 1. Hexapeptides **19** and **20** showed no inhibition even at 1.0 mM. These results are consistent with Chen et al.'s studies on peptide **19**, which showed that this compound was a very poor inhibitor of PTP1B.[12] However, tripeptides **21** and **22** exhibited IC_{50}'s of 3.4 and 6.4 μM, respectively. Clearly, the fluorines in peptides **20** and **22** had little or no impact on inhibitory potency. We also prepared tripeptide **23** bearing F_2Smp (**2**) and found that it exhibited an IC_{50} similar to **21** and **22** as well as hexapeptide **24**.[7] Indeed, the IC_{50} values for tripeptides **21**−**23** are, in general, not significantly different from those reported by Lee et al. for a variety of monoanionic carboxylic acid-based pTyr mimics,

(22) The ee of **18** could also be determined by ^{19}F NMR. ^{19}F NMR of the F_2Samp-DL-leucine dipeptide exhibited a singlet corresponding to one diastereomeric dipeptide as well as an ABQ doublet of doublets corresponding to the other diastereomeric dipeptide. ^{19}F NMR of the F_2Samp-L-leucine dipeptide exhibited only a single peak. We could not detect any of the ABQ doublet of doublets corresponding to the other diastereomer confirming that F_2Samp was prepared in very high (>97%) enantiomeric purity.

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TABLE 1. Inhibition of PTP1B with Peptides 19−25

peptide	IC_{50} (μM)
DADE-**3**-LNH_2 (**19**)	0% inhibition at 1 mM
DADE-**4**-LNH_2 (**20**)	0% inhibition at 1 mM
FmocGlu(OBn)-**3**-LNH_2 (**21**)	3.4 ± 0.4
FmocGlu(OBn)-**4**-LNH_2 (**22**)	6.4 ± 1.0
FmocGlu(OBn)-**2**-LNH_2 (**23**)	7.4 ± 1
DADE-**2**-LNH_2 (**24**)	10 ± 1[a]
FmocGlu(OBn)-Phe-LNH_2 (**25**)	4.1 ± 1.0

[a] From ref 7.

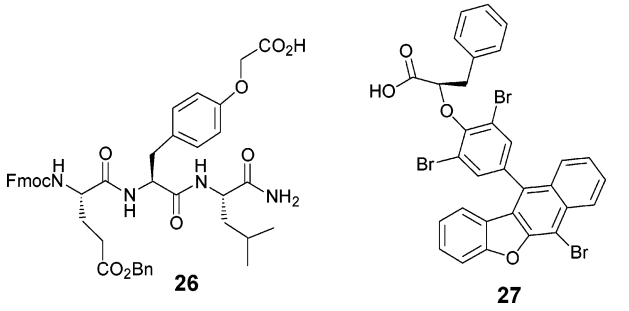

FIGURE 2. Tripeptide inhibitor (**26**) of YopH and PTP1B and a hydrophobic inhibitor (**27**) of PTP1B.

as well as F_2Pmp (**1**), in the same tripeptide platform.[8] The similarity of the IC_{50}'s of tripeptides **21** and **22** to tripeptide **23** and to those of Lee et al. prompted us to question if the pTyr mimics were contributing at all to the potency of the tripeptides. An examination of the data reported by Lee et al.[8] revealed that tripeptide FmocGlu(OBn)-Phe-LNH_2 (**25**), a control in which no phosphate mimic is present on the phenylalanine side chain, had not been evaluated as a PTP1B inhibitor. Therefore, we synthesized peptide **25** and evaluated it as a PTP1B inhibitor. This peptide exhibited an IC_{50} of 4.1 μM which is similar to the IC_{50}'s for **21**−**23** and many of the tripeptides reported by Lee et al. with PTP1B. This result raises the possibility that the phosphate "mimic" portion of the tripeptide inhibitors contributes little or not at all to their inhibitory potency. It is possible that the phosphate-mimicking portion of these tripeptides do not bind or binds very poorly in the active site or elsewhere on the protein and that the majority of the binding energy is a result of the interaction of a hydrophobic group on the inhibitor with a hydrophobic region on the protein. Lee et al. have recently shown that the presence of a hydrophobic aromatic hydrocarbon moiety at the N-terminus was important for obtaining good PTP1B inhibition with their tripeptides.[9]

Lee et al. examined in some detail the kinetics of YopH inhibition with tripeptide **26** (Figure 2) which has an IC_{50} of 1.8−2.8 μM with YopH and 2.9−4.6 μM with PTP1B.[8,9] It did not exhibit a strictly competitive mode of inhibition. It showed a steep IC_{50} curve with a slope factor of 3−5 depending upon the enzyme concentration suggesting that more than one molecule of inhibitor binds to YopH. IC_{50} values increased as the concentration of YopH increased. They also observed a time dependency in that preincubation with YopH for 5 min decreased the IC_{50} from 3.2 to 1.5 μM. Treatment of YopH with 200 μM inhibitor **26** for 30 min followed by dilution into excess substrate resulted in a 40% loss of activity. It is interesting to note that some of these phenomena were also encountered with hydrophobic inhibitor **27** (Figure 2) and PTP1B in that it too displayed time-dependent inhibition and

nonclassical inhibition patterns.[24,25] Using **23** as a model inhibitor, we have found that some of the above phenomena encountered with **26** and **27** also occur with PTP1B. A Lineweaver−Burk plot did not exhibit classical inhibition patterns and we were unable to determine a K_i from the plot. The IC_{50}'s depended upon PTP1B concentration. At 1.5 nM enzyme, the IC_{50} of **23** was 4.3 μM while at 12 nM enzyme the IC_{50} increased to 12 μM, however, the slope factors were 1.2−1.4. We did not observe a significant time dependence upon inhibition. We also examined the reversibility of inhibition by incubating 150 nM PTP1B with 50 μM of **23** for either 15 s or 60 min and then diluting the mixture 50-fold into a solution containing 1.0 mM DIFMUP and assaying enzyme activity. In each case, only 5−10% of the activity was recovered. Moreover, even after extensive dialysis, no activity was recovered. As pointed out by Lee et al. and others,[8,24] these results are consistent with the properties associated with nonspecific promiscuous inhibitors.[26−28] However, these workers also point out that some of the observed effects are not as pronounced as previously reported for promiscuous inhibitors.[8]

In summary, we have described the first enantioselective synthesis of protected L-4-[sulfonamido(methyl)]phenylalanine (**3**) and L-4-[sulfonamido(difluoromethyl)]phenylalanine (**4**) and incorporated these species into a hexapeptide that has been widely used for assessing pTyr mimics as well as the tripeptide, FmocGlu(OBn)-X-LNH_2, that has recently been used by others for evaluating pTry mimics.[8] Although the tripeptides bearing these mimics were relatively good inhibitors, our results suggest that the phosphate mimicking portion of the tripeptides may not be contributing significantly to their potency. Moreover, studies with inhibitor **23** reveals that this class of tripeptide inhibitor exhibits nonclassical inhibition patterns with PTP1B. As mentioned above, it has been suggested that the hexapeptide platform may limit the depth of insertion and freedom of the pTyr mimic within the catalytic pocket and this may result in artificially low potencies for certain pTyr mimics.[6,8] Our results do not suggest that this hypothesis is incorrect though they do raise serious questions as to the value of the FmocGlu(OBn)-X-LNH_2 peptide as a platform for assessing pTyr mimics for PTP1B inhibition. Indeed, our results emphasize an important point about assessing pTyr mimics in any platform in that it is prudent to compare the platform bearing the pTyr mimic to the analogous structure lacking the phosphate mimicking portion.

Although amino acids **3** and **4** were not good pTyr mimics for PTP1B, they may also prove to be useful in the development of inhibitors and probes of other enzymes. Chen et al. have shown that peptide **19** was a modest inhibitor of Yersinia PTP.[12] The presence of fluorines α to the sulfonamide moiety might enhance its potency with Yersinia PTP. It is also worthy of note that tyrosine sulfation has been recognized as an important post-translational modification.[29] The growing list of proteins that bind to sulfotyrosine include viral proteins that recognize sulfated receptor proteins on cell surfaces.[30,31] Hence, a need has also arisen for hydrolytically stable sulfotyrosine mimics. We have recently demonstrated that the difluoromethylene-sulfonamide group is a relatively good nonhydrolyzable replacement of the sulfate group in estrone sulfate in that this species is a relatively good reversible inhibitor of steroid sulfatase.[32] Thus, amino acid **4** may prove to be an effective sTyr mimic and useful in the development of inhibitors of protein−protein interactions.

Experimental Section

Syntheses. L-2-Acetylamino-3-(4-acetylsulfanylmethylphenyl)-propionic Acid Ethyl Ester (7). To a stirred 0 °C solution of L-(4-chloromethyl)phenylalanine[14,15] (48 g, 12.3 mmol), TBAI (452 mg, 1.23 mmol), and DMF (49 mL) was added potassium thiol acetate (1.54 g, 13.5 mmol). The reaction was gradually warmed to rt and stirred overnight. The reaction was quenched with water, extracted with EtOAc (×3), washed with brine, dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (60:40:4 hexanes/EtOAc/MeOH) yielded 3.81 g (96%) of **7** as a white solid: mp 47−48 °C; ^{1}H NMR (300 MHz) δ 7.20 (2H, d, J = 7.9 Hz), 7.03 (2H, d, J = 7.9 Hz), 5.90 (1H, bd, J = 7.5 Hz), 4.84 (1H, dd, J = 7.6, 5.8 Hz), 4.17 (2H, q, J = 7.1 Hz), 4.08 (2H, s), 3.09 (2H, d, J = 5.6 Hz), 2.35 (3H, s), 1.99 (3H, s), 1.24 (3H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz) δ 194.8 (C═O), 171.5 (C═O), 169.6 (C═O), 136.2 (C_{arom}), 134.9 (C_{arom}), 129.3 (CH_{arom}), 128.7 (CH_{arom}), 61.2 (CH_2), 53.0 (CH), 37.3 (CH_2), 32.8 (CH_2), 30.1 (CH_3), 22.8 (CH_3), 13.9 (CH_3); LR-EIMS m/z (relative intensity) 323 (M^+, 15), 264 ($M^+ - NH_2Ac$, 100); HR-EIMS m/z calcd for $C_{16}H_{21}NO_4S$ 323.1191 found 323.1194.

L-2-Acetylamino-3-{4-[(2,4-dimethoxybenzylsulfamoyl)methyl]-phenyl}propionic Acid Ethyl Ester (10). Chlorine gas was slowly bubbled through a cooled 0 °C solution of thiol acetate **7** (1.98 g, 6.12 mmol) and CH_2Cl_2/H_2O (34 mL: 7 mL). Once a yellow color appeared, the Cl_2 was stopped and argon was bubbled through to remove the excess Cl_2. The mixture was poured into EtOAc, washed with H_2O and brine, and then dried over $MgSO_4$. Removal of the solvent by rotary evaporation yielded crude sulfonyl chloride that was used in the next step without further purification. A solution of dimethoxybenzylamine (1.53 g, 9.18 mmol) and NEt_3 (929 mg, 9.18 mmol) in THF (40 mL + 10 mL) was added via cannula to a 0 °C solution of sulfonyl chloride and THF (50 mL). The reaction was allowed to warm to rt and stir overnight. The reaction was quenched with NH_4Cl, extracted with EtOAc (×3), washed with $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was removed in vacuo and purified by flash chromatography (50:50 to 80:20 EtOAc/hexanes) yielded 1.36 g (77%) of **10** as a off-white solid: mp 91−93 °C; ^{1}H NMR (300 MHz) δ 7.16 (1H, d, J = 8.0 Hz), 7.06 (4H, s), 6.47−6.44 (2H, m), 6.30 (1H, d, J = 7.8 Hz), 5.09 (1H, t, J = 6.1 Hz), 4.80 (1H, dd, J = 13.9, 6.2 Hz), 4.18−4.11 (4H, m), 4.02 (2H, s), 3.80 (3H, s), 3.76 (3H, s), 3.09 (1H, dd, J = 13.9, 6.0 Hz), 3.00 (1H, dd, J = 13.9, 6.4 Hz), 1.93 (3H, s), 1.23 (3H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz) δ 171.4 (C═O), 169.7 (C═O), 160.9 (C_{arom}), 158.4 (C_{arom}), 136.4 (C_{arom}), 130.6 (C_{arom}), 130.3 (CH_{arom}), 129.3 (CH_{arom}), 127.9 (CH_{arom}), 117.6 (C_{arom}), 103.8 (CH_{arom}), 98.5 (CH_{arom}), 61.3 (CH_2), 58.5 (CH_2), 55.2

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(CH_3), 55.1 (CH_3), 53.0 (CH), 43.2 (CH_2), 37.3 (CH_2), 22.8 (CH_3), 13.9 (CH_3); LR-EIMS *m*/*z* (relative intensity) 326 ($M^+ - C_9H_{12}O_2$, 3), 249 ($M^+ - C_9H_{12}SO_4N$, 15); LR-CIMS (NH_3) 496 ($M + NH_4^+$, 30); positive-ion HR-ESIMS calcd for $C_{23}H_{31}N_2O_7S$ ($M + H^+$) 479.1852, found 479.1842.

L-4-[Sulfonamido(methyl)]phenylalanine (3). A solution of amino acid **10** (1.00 g, 2.09 mmol) in 6 N HCl (17.4 mL) was refluxed overnight. The solution was cooled and the solvent removed in vacuo. The resulting solid was dissolved in EtOH (85 mL), and propylene oxide (2 mL) was added. The reaction was stirred overnight, which resulted in the precipitation of a white solid. The solid was filtered to yield pure amino acid 540.6 mg (82%) of amino acid **3** as a white solid. Spectral data matched literature data:[12] ^{1}H NMR (300 MHz, D_2O) δ 3.00 (1H, dd, J = 14.7, 7.7 Hz), 3.13 (1H, dd, J = 14.7, 5.4 Hz), 3.87 (1H, overlapping dd, J = 6.8 Hz), 4.34 (2H, s), 7.19 (2H, d, J = 8.0 Hz), 7.29 (2H, d, J = 8.0 Hz).

L-Fmoc-4-[sulfonamido(methyl)]phenylalanine (11). To a stirred 0 °C mixture of amino acid **3** (392 mg, 1.52 mmol), $NaHCO_3$ (687 mg, 8.49 mmol), and H_2O (15 mL) were added Fmoc-OSu (624 mg, 1.85 mmol) and dioxane (15 mL). The reaction was gradually warmed to room temperature and stirred overnight. The mixture was acidified with 1.2 N HCl, extracted with EtOAc (×4), washed (brine), dried ($MgSO_4$), filtered, and concentrated. Recrystallization of the residue in EtOAc yielded 677 mg (93%) of compound **11**. Spectral data matched literature data:[12] ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.87 (1H, dd, J = 14.1, 10.4 Hz), 3.08 (1H, dd, J = 13.7, 4.5 Hz), 4.19 (6H, m), 6.82 (2H, s), 7.31 (6H, m), 7.39 (2H, m), 7.65 (2H, dd, J = 7.2, 4.2 Hz), 7.70 (2H, d, J = 8.4 Hz), 7.87 (2H, m, J = 7.5 Hz).

Bis(2,4-dimethoxybenzyl)difluoro(4-benzaldehyde)methanesulfonamide (14). To a stirred −78 °C solution of iodo compound **12** (3.49 g, 5.50 mmol) in THF (110 mL) was added 1.98 M *n*-BuLi (3.05 mL, 6.05 mmol). After 5 min at −78 °C, DMF (1.28 mL, 16.5 mmol) was added. The reaction was stirred for 15 min and then quenched with saturated NH_4Cl. The aqueous layer was extracted with EtOAc (×3), and the combined organics were dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (15:85 to 25:75 to 40:60 EtOAc/hexanes) yielded 2.28 g of aldehyde **14** as a white solid (77%): mp 141−142 °C; ^{1}H NMR (300 MHz) δ 10.10 (1H, s), 7.98 (2H, d, J = 8.2 Hz), 7.84 (2H, d, J = 8.2 Hz), 7.17 (2H, d, J = 8.4 Hz), 6.38 (2H, dd, J = 8.4, 2.4 Hz), 6.29 (2H, d, J = 2.3 Hz), 4.51 (4H, s), 3.77 (6H, s), 3.66 (6H, s); ^{13}C NMR (75 MHz) δ 191.3 (C═O), 160.3 (C_{arom}), 158.1 (C_{arom}), 138.2 (C_{arom}), 135.0 (t, J = 22.8 Hz, C_{arom}), 130.2 (CH_{arom}), 129.4 (CH_{arom}), 127.9 (t, J = 5.9 Hz, CH_{arom}), 121.6 (t, J = 283.2 Hz, CF_2), 116.2 (C_{arom}), 103.8 (CH_{arom}), 97.6 (CH_{arom}), 55.2 (CH_3), 54.8 (CH_3), 46.7 (CH_2): ^{19}F NMR (282 MHz) δ −101.5; LR-EIMS *m*/*z* (relative intensity) 535 (M^+, 11), 151 (100), 178 (69); HR-ESIMS calculated for $C_{26}H_{27}F_2NO_7S$ ($M + Na^+$) 558.1368, found 558.1389.

Bis(2,4-dimethoxybenzyl)difluoro(4-benzyl alcohol)methanesulfonamide (15). To a stirred 0 °C solution of aldehyde **14** (1.58 g, 2.95 mmol) in THF (15 mL) and methanol (15 mL) was added $NaBH_4$ (111 mg, 2.95 mmol). After 1 h, the reaction was quenched with saturated NH_4Cl. The mixture was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2 (×3), and the combined organics were dried ($MgSO_4$), filtered, and concentrated. Flash chromatography of the residue (25:75 to 33:67 to 40:60 to 50:50 EtOAc/hexanes) gave 1.42 g of alcohol **15** as a white solid (90%): mp 123 °C; ^{1}H NMR (300 MHz) δ 7.66 (2H, d, J = 8.1 Hz), 7.47 (2H, d, J = 8.0 Hz), 7.18 (2H, d, J = 8.4 Hz), 6.37 (2H, dd, J = 8.4, 2.2 Hz), 6.27 (2H, d, J = 2.2 Hz), 4.77 (2H, s), 4.49 (4H, s), 3.77 (6H, s), 3.65 (6H, s); ^{13}C NMR (75 MHz) δ 160.2 (C_{arom}), 158.1 (C_{arom}), 144.8 (C_{arom}), 130.3 (CH_{arom}), 128.3 (t, J = 22.9 Hz, C_{arom}), 127.3 (t, J = 6.0 Hz, CH_{arom}), 126.5 (CH_{arom}), 122.3 (t, J = 282.6 Hz, CF_2), 116.6 (C_{arom}), 103.8 (CH_{arom}), 97.6 (CH_{arom}), 64.4 (CH_2), 55.3 (CH_3), 54.9 (CH_3), 46.7 (CH_2); ^{19}F NMR (282 MHz) δ −100.6; LR-ESIMS *m*/*z* (relative intensity) 560 ($M + Na^+$, 100); HR-ESIMS calcud for $C_{26}H_{29}F_2NO_7SNa$ ($M + Na^+$) 560.1525, found 560.1518.

Bis(2,4-dimethoxybenzyl)difluoro(4-benzyl bromide)methanesulfonamide (16). To a cooled 0 °C solution of benzyl alcohol **15** (1.69 g, 3.14 mmol), PPh_3 (1.23 g, 4.71 mmol), and CH_2Cl_2 (175 mL) was added CBr_4 (1.56 g, 4.71 mmol) portionwise. The ice bath was removed and the solution stirred overnight. Silica gel was added to the mixture and the solvent removed in vacuo. Flash chromatography (25:75 to 33:67 EtOAc/hexanes) afforded 1.80 g of benzyl bromide **16** as a white solid (95%): mp 113−114 °C; ^{1}H NMR (300 MHz) δ 7.64 (2H, d, J = 8.2 Hz), 7.49 (2H, d, J = 8.2 Hz), 7.17 (2H, d, J = 8.4 Hz), 6.38 (2H, dd, J = 8.4, 2.3 Hz), 6.28 (2H, d, J = 2.3 Hz), 4.50 (2H, s), 4.49 (4H, s), 3.77 (6H, s), 3.65 (6H, s); ^{13}C NMR (75 MHz) δ 160.3 (C_{arom}), 158.1 (C_{arom}), 141.3 (C_{arom}), 130.3 (CH_{arom}), 129.4 (t, J = 23.0 Hz, C_{arom}), 129.0 (CH_{arom}), 127.6 (t, J = 5.9 Hz, CH_{arom}), 122.0 (t, J = 282.7 Hz, CF_2), 116.5 (C_{arom}), 103.8 (CH_{arom}), 97.6 (CH_{arom}), 55.3 (CH_3), 54.9 (CH_3), 46.7 (CH_2), 32.0 (CH_2); ^{19}F NMR (282 MHz) δ −100.9; LR-ESIMS *m*/*z* (relative intensity) 624 ($M + Na^+$, 44), 622 ($M + Na^+$, 44), 602 ($M + H^+$, 29), 600 ($M + H^+$, 29), 301 (100); HR-ESIMS calcd for $C_{26}H_{29}BrF_2NO_7S$ ($M + H^+$) 600.0872, found 600.0869.

3-(4-{[Bis(2,4-dimethoxybenzyl)sulfamoyl]difluoromethyl}-benzyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (17). To a stirred −78 °C solution of benzyl bromide **16** (1.80 g, 3.00 mmol) and William's lactone (969 mg, 2.50 mmol) in THF/HMPA (75 mL:7.5 mL) was added 0.96 M LiHMDS (2.7 mL, 2.62 mmol) dropwise. After 3 h at −78 °C, the reaction was quenched with EtOAc. The layers were separated, and the EtOAc layer was washed with water and brine. After drying ($MgSO_4$), filtration, and concentration, the crude lactone was purified by flash chromatography (25:75 to 33:67 EtOAc/hexanes) to provide 1.82 g of lactone **17** as a foamy white solid (80%), mp 91−94 °C. Two conformers were observed at room temperature in a 1:2 ratio: ^{1}H NMR (300 MHz) δ major comformer 7.68 (2H, d, J = 8.0 Hz), 7.39−7.34 (2H, m), 7.21−7.03 (11H, m), 6.78 (2H, d, J = 7.0 Hz), 6.59 (2H, d, J = 7.3 Hz), 6.48 (2H, d, J = 7.6 Hz), 6.37 (2H, dd, J = 8.4, 2.0 Hz), 6.27 (2H, d, J = 1.9 Hz), 5.34−5.32 (1H, m), 5.07 (1H, d, J = 12.3 Hz), 4.96 (1H, d, J = 12.3 Hz), 4.85 (1H, d, J = 2.6 Hz), 4.51 (4H, s), 4.45 (1H, d, J = 2.6 Hz) 3.74 (6H, s), 3.62 (6H, s), 3.48−3.41 (2H, m); ^{1}H NMR (300 MHz, DMSO-d_6, 373K) δ 7.62−7.59 (2H, m), 7.49 (2H, bm), 7.35 (2H, s), 7.25−7.14 (7H, m), 7.10−7.04 (4H, m), 6.98−6.96 (2H, m), 6.60−6.57 (2H, m), 6.42−6.41 (4H, m), 5.89 (1H, s), 5.27−5.26 (1H, bs), 5.16−5.12 (1H, m), 5.02 (2H, s), 4.36 (4H, s), 3.73 (6H, s), 3.66 (6H, s), 3.59−3.52 (2H, m); ^{13}C NMR (75 MHz) δ 168.9 (maj, C═O), 168.6 (min, C═O), 160.2 (C_{arom}), 158.0 (C_{arom}), 154.3 (maj, C═O), 153.8 (min, C═O), 140.1 (maj, C_{arom}), 139.5 (min, C_{arom}), 135.6 (maj, C_{arom}), 135.4 (C_{arom}), 135.0 (min, C_{arom}), 133.5 (min, C_{arom}), 133.4 (maj, C_{arom}), 130.2 (CH_{arom}), 129.9 (CH_{arom}), 129.7 (CH_{arom}), 129.0 (t, J = 23.1 Hz, C_{arom}), 128.7 (CH_{arom}), 128.4 (CH_{arom}), 128.2 (CH_{arom}), 127.8 (CH_{arom}), 127.8 (CH_{arom}), 127.4 (CH_{arom}), 127.4 (CH_{arom}), 126.6 (CH_{arom}), 126.4 (CH_{arom}), 122.1 (t, J = 282.5 Hz, CF_2), 116.4 (CH_{arom}), 103.7 (CH_{arom}), 97.6 (CH_{arom}), 79.0 (min, CH), 78.7 (maj, CH), 68.3 (min, CH), 67.7 (maj, CH), 60.1 (CH_2), 58.8 (CH), 55.2 (CH_3), 54.8 (CH_3), 46.6 (CH_2), 40.1 (min, CH_2), 38.6 (maj, CH_2); ^{19}F NMR (282 MHz) δ −100.5 (ABQ, J = 260.4 Hz), −100.6 (s); LR-EIMS *m*/*z* (relative intensity) 906 (M^+, 6), 151 (100), 91 (72), 178 (60); HR-ESIMS calcd for $C_{50}H_{48}F_2N_2O_{10}SNa$ ($M + Na^+$) 929.2889, found 929.2936.

3-(4-{[Bis-(2,4-dimethoxybenzyl)sulfamoyl]-difluoromethyl}-phenyl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionic Acid Methyl Ester (18). To a stirred solution of lactone **17** (1.62 g, 1.79 mmol) and ethanol (14 mL) in dioxane (14 mL) was added $PdCl_2$ (158 mg, 0.893 mmol). This suspension was stirred under a H_2 atmosphere (balloon) for 6.5 h before the suspension was spun down via centrifuge to remove the catalyst. The solvent was removed and the crude amino acid titurated with Et_2O (×3) to yield a gummy white solid. The crude amino acid was dissolved in

dioxane (12 mL), and a solution of Na_2CO_3 (568 mg, 5.36 mmol) in water (14 mL) was added. The mixture was cooled to 0 °C, and a solution of Fmoc-OSuc (632 mg, 1.88 mmol) and dioxane (2 mL) was added dropwise. The ice bath was removed and the mixture stirred overnight. The mixture was acidified with 1 N HCl and the layer separated. The product was extracted with EtOAc (×5), and the combined organics were washed with brine, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (0 to 1 to 2 to 5 to 10% methanol in chloroform) yielded 1.4 g of Fmoc-protected amino acid **18** as a white foam (99%): mp 79−80 °C; ^{1}H NMR (300 MHz) δ 7.75−7.72 (2H, m), 7.58−7.53 (4H, m), 7.39−7.35 (2H, m), 7.30−7.21 (4H, m), 7.15 (2H, d, J = 8.4 Hz), 6.34 (2H, d, J = 8.4 Hz), 6.25 (2H, s), 5.35 (1H, d, J = 7.3 Hz), 4.69 (1H, m), 4.41 (4H, s) overlapping, 4.36 (1H, m), 4.17 (1H, m), 3.73 (6H, s), 3.62 (6H, s), 3.17 (2H, m); ^{13}C NMR (75 MHz) δ 174.9 (C═O), 160.4 (C_{arom}), 158.1 (C_{arom}), 155.9 (C═O), 143.8 (C_{arom}), 141.4 (C_{arom}), 139.9 (C_{arom}), 130.5 (CH_{arom}), 129.6 (CH_{arom}), 128.7 (CH_{arom}), 127.9 (t, J = 20.9 Hz, C_{arom}), 127.2 (CH_{arom}), 125.2 (CH_{arom}), 122.2 (t, J = 282.7 Hz, CF_2), 120.1 (CH_{arom}), 116.7 (C_{arom}), 104.0 (CH_{arom}), 97.9 (CH_{arom}), 67.2 (CH_2), 55.4 (CH_3), 55.0 (CH_3), 54.5 (CH), 47.2 (CH), 46.8 (CH_2), 37.7 (CH_2); ^{19}F NMR (282 MHz) δ −100.6; LR-ESIMS m/z (relative intensity) 839 (M + Na^+, 12), 834 (M + NH_4^+, 9), 855 (M + K^+, 6); HR-ESIMS calcd for $C_{43}H_{42}N_2O_{10}F_2SNa$ (M + Na^+) 839.2426, found 839.2422.

General Protocol for Hexapeptide and Tripeptide Synthesis. Solid-phase peptide synthesis was carried out manually using Fmoc strategy employing Rink amide AMS resin (0.71 mmol/g). The resin was preswollen in CH_2Cl_2 for 2 h (minimum), the Fmoc group was removed using 20% piperidine/DMF (3 mL, 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). All Fmoc deprotections were carried out in this manner. Coupling of the first amino acid was carried out with the pentafluorophenyl ester of Fmoc-L-leucine (4.0 equiv) and HOBt (4.0 equiv) in DMF (stirred 10 min at room temperature before being added to the resin) and then shaken with resin (2 × 30 to 90 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 30−90 min. Each coupling was carried out twice, except for unnatural Fmoc-amino acids **9** and **16** which were coupled once overnight (2.0−4.0 equiv). The resin was washed after the second coupling with DMF (3 mL, 6 × 3 min) and CH_2Cl_2 (3 mL, 2 × 3 min). The side chains of both the Asp and Glu were protected as *tert*-butyl esters for peptides **19** and **20** or benzyl ester for the Glu in the case of tripeptides **21**−**23** and **25**. Once the peptide was assembled, the final Fmoc group was removed and the resin dried overnight. The resin was placed in a small round-bottom flask, and Reagent K (82.5% TFA, 2.5% ethanedithiol, 5% water, 5% thioanisole, and 5% phenol) was added (1.5 mL per 100 mg of resin) and stirred for 2.5 h at room temperature. The mixture was filtered and the residue evaporated. **Hexapeptide Workup.** To this mixture was added cold methyl *tert*-butyl ether (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 yielding a fluffy white powder. **Tripeptide Workup.** The peptides were precipitated with the addition of diethyl ether or chloroform and filtered to give fluffy white powders. Purification was accomplished by RP-HPLC or silica gel column chromatography.

DADE-3-LNH$_2$ (19). Preparative HPLC of the precipitated peptide (Vydac 218TP1022-C18 reversed-phase column) using a linear gradient of 90/10 H_2O (0.025% TFA)−CH_3CN to 70/30 H_2O (0.025% TFA)−CH_3CN over 36 min gave pure **19** as a white powder. Analytical HPLC (Vydac 218TP54 C18 column) using a linear gradient of 90/10 H_2O (0.025% TFA)−CH_3CN to 70/30 H_2O (0.025% TFA)−CH_3CN over 36 min showed a single peak (retention time = 14.0 min): positive-ion HRESMS m/z calcd for $C_{32}H_{49}N_8O_{14}S$ (M + H)$^+$ 801.3089, found 801.3017.

DADE-4-LNH$_2$ (20). Preparative HPLC of the precipitated peptide (Vydac 218TP1022-C18 reversed-phase column) using a linear gradient of 90/10 H_2O (0.025% TFA)−CH_3CN to 70/30 H_2O (0.025% TFA)−CH_3CN over 36 min gave pure **20** as white powder. Analytical HPLC (Vydac 218TP54 C18 column) using a linear gradient of 90/10 H_2O (0.025% TFA)−CH_3CN to 70/30 H_2O (0.025% TFA)−CH_3CN over 36 min showed a single peak (retention time = 21.3 min): negative-ion HRESMS m/z calcd for $C_{32}H_{45}F_2N_8O_{14}S$ (M − H)$^-$ 835.2744, found 835.2701.

FmocGlu(OBn)-3-LNH$_2$ (21). Purification by column chromatography (0 to 1 to 2.5 to 5% MeOH/$CHCl_3$) gave pure **21** as a white powder. Analytical HPLC (Phenomenex Jupiter Proteo reversed-phase column) using H_2O (0.1% TFA)−CH_3CN as eluent (linear gradients of 60/40 to 25/75 from 0 to 30 min, 25/75 to 1/99 from 30 to 40 min, 1/99 from 40 to 55 min, retention time = 18.5 min) indicated greater than 98.6% purity: positive-ion HRESMS m/z calcd for $C_{43}H_{50}N_5O_9S$ (M + H)$^+$ 812.3298, found 812.3329.

FmocGlu(OBn)-4-LNH$_2$ (22). Purified by column chromatography (0 to 1 to 2.5 to 5% MeOH/$CHCl_3$) gave pure **22** as a white powder. Analytical HPLC (Phenomenex Jupiter Proteo reversed-phase column) using H_2O (0.1% TFA)−CH_3CN as eluent (linear gradients of 60/40 to 25/75 from 0 to 30 min, 25/75 to 1/99 from 30 to 40 min, 1/99 from 40 to 55 min, retention time = 25.7 min) indicated greater than 98.7% purity: positive-ion HRESMS m/z calcd for $C_{43}H_{48}F_2N_5O_9S$ (M + H)$^+$ 848.3126, found 848.3141.

FmocGlu(OBn)-2-LNH$_2$ (23). Rink Resin (284 mg, 0.202 mmol) and Fmoc-L-4-[neopentylsulfono(difluoromethyl)phenyl-alanine[2] (237 mg, 0.403 mmol) were used. Purification by column chromatography (0 to 1 to 2.5 to 5% MeOH/$CHCl_3$) yielded 59.4 mg (17%) of tripeptide **23** with the sulfonate moiety protected with a neopentyl group (white solid): positive-ion LR-ESIMS m/z (relative intensity) 919 (M + H^+, 100); positive-ion HR-ESIMS calcd for $C_{48}H_{57}F_2N_4O_{10}S$ (M + H^+) 919.3763, found 919.3765. The purified neopentyl tripeptide (48.9 mg, 0.0532 mmol) was dissolved in methyl ethyl ketone (4 mL) and LiBr (5 mg, 0.0585 mmol) and refluxed for 2 days. The solvent was removed in vacuo and the residue dissolved in H_2O. This solution was washed with ether (×3), and the aqueous layer was then lyophilized to yield 55.2 mg of crude tripeptide **23**. Purification by semipreparative reversed-phase HPLC (Phenomenex Jupiter Proteo reversed-phase column) using H_2O (0.1% TFA)−CH_3CN as eluent (linear gradients of 50/50 to 30/70 from 0 to 65 min,30/70 to 1/99 from 65 to 75 min, retention time = 42.7 min) gave 15 mg of pure **23**. Analytical HPLC (Phenomenex Jupiter Proteo reverse phase column) using H_2O (0.1% TFA)−CH_3CN as eluent (linear gradients of 50/50 to 20/80 from 0 to 30 min, 20/80 to 1/99 from 30 to 40 min, 1/99 from 40 to 55 min, retention time = 22.5 min) indicated greater than 98.8% purity: positive ion HRESMS calculated for $C_{43}H_{47}F_2N_4O_{10}S$ (M + H)$^+$ 849.2971, found 849.2981.

FmocGlu(OBn)-Phe-LNH$_2$ (25). Purification by silica gel column chromatography (0 to 1 to 2.5 to 5% MeOH−$CHCl_3$) gave tripeptide **25** as a white powder. Analytical HPLC (Phenomenex Jupiter Proteo reverse phase column) using H_2O (0.1% TFA)−CH_3CN as eluent (linear gradients of 60/40 to 25/75 from 0 to 30 min, 25/75 to 1/99 from 30 to 40 min, 1/99 from 40 to 55 min, retention time = 28.2 min) indicated greater than 98.5% purity: positive-ion HRESIMS calcd for $C_{42}H_{47}N_4O_7$ (M + H)$^+$ 719.3406, found 719.3445.

IC$_{50}$ Determinations. Ten microliters of a solution of a stock solution of inhibitor in DMSO−water was added to the wells of a microtiter plate containing 70 μL of 0.1 M bis-tris, pH 6.3, 2 mM EDTA, 5 mM DTT, 0.001% Triton. A control was prepared by adding 10 μL of DMSO−water instead of inhibitor. To this, 10 μL of 200 μM diFMUP (Invitrogen) stock in 2% DMSO was added

to bring the volume up to 90 μL. The assay was initiated by the addition of 10 μL of PTP1B stored in 20 mM tris, pH 7.5, 150 mM NaCl, 5 mM DTT, 0.1 mM EDTA, and 20% glycerol. A control was performed by adding 10 μL of enzyme storage buffer. The final concentration of inhibitor in the assay wells was 100, 80, 60, 40, 20, 15, 10, 5, 2.5, 1, 0.5, and 0 μM. The final concentration of DMSO was 7.5%. The final concentration of diFMUP substrate in the assay was 20 μM, the previously determined K_m value under these conditions. The final concentration of PTP1B ranged from 1.5 to 3.0 to 6.0 nM. The production of fluorescent product diFMU was monitored for 10 min at 30 °C using a spectrofluorometer platereader with excitation and emission at 360 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}. 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 semilog 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 $V_o - B$.

Assay for Time-Dependent Inhibition with Inhibitor 23. The IC_{50} of **23** was determined using the procedure described above except the inhibitor solutions were incubated with PTP1B (3 nM) for 30 min at 30 °C before initiation of the reaction with DiFMUP (20 μM final concentration).

Assay for Irreversible Inhibition with Inhibitor 23. Ten microliters of a 500 μM stock solution of **23** in DMSO−water was added to 80 μL of 0.1 M bis-tris, pH 6.3, 2 mM EDTA, 5 mM DTT, 0.001% Triton, and 10 μL of 1.5 μM PTP1B. A control was similarly made with 10 μL of DMSO−water instead of inhibitor. Upon initiation of the reaction at $t = 0$ and after 20, 40, and 60 min of incubation, a 2 μL aliquot was withdrawn at $t = 15$ s and $t = 60$ min and added to a 96-well microtiter plate containing 98 μL of 1.0 mM diFMUP (approximately 50 × K_m) in 0.1 M bis-tris, pH 6.3, 2 mM EDTA, 5 mM DTT, 0.001% Triton for a 50-fold dilution of the incubation mixture. The final concentration of the inhibitor in the assay was 1 mM, and the final concentration of enzyme was 150 nM. The production of diFMU was followed for 10 min as described above. The percent of activity remaining in the presence of inhibitor compared to that of the control was determined. After the aliquot was withdrawn from the preincubation mixture after 60 min, the remainder was transferred to a dialysis membrane (10 kDa cutoff, SpectraPor) and dialyzed against 500 mL of 0.1 M bis-tris, pH 6.3, 2 mM EDTA, 5 mM DTT, 0.001% Triton, changed twice over 24 h at 4 °C. At 24 h the amount of activity remaining in the enzyme−inhibitor mixture and its control was measured by withdrawing 2 μL into 98 μL of 1 mM diFMUP (approximately 50 × K_m) in 0.1 M bis-tris, pH 6.3, 2 mM EDTA, 5 mM DTT, 0.01% Triton as described above.

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Supporting Information Available: ^{1}H, ^{13}C, and ^{19}F NMR (when applicable) for new compounds and HPLC chromatograms for peptides **19**−**23** and **25**. Experimental procedures for the synthesis of dipeptides prepared for determining the enantiomeric excess of compounds **11** and **18** and HPLC chromatograms and ^{19}F NMR (when applicable) of the resulting dipeptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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