Expedited Articles

Utilization of a Peptide Lead for the Discovery of a Novel PTP1B-Binding Motif†

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Examination of the PTP1B inhibitory potency of an extensive series of phosphotyrosyl (pTyr) mimetics (**Xxx**) expressed in the EGFr-derived hexapeptide platform Ac-Asp-Ala-Asp-**Xxx**-Leu-amide previously led to the finding of high inhibitory potency when **Xxx** = 4-(phosphono-difluoromethyl)phenylalanyl (F_2Pmp) (K_i = 0.2 μM) and when **Xxx** = 3-carboxy-4-carboxy-methyloxyphenylalanyl (K_i = 3.6 μM). In the first instance, further work led from the F_2Pmp-containing peptide to monomeric inhibitor, 6-(phosphonodifluoromethyl)-2-naphthoic acid (K_i = 22 μM), and to the pseudo-dipeptide mimetic, *N*-[6-(phosphonodifluoromethyl)-2-naphthoyl]-glutamic acid (K_i = 12 μM). In the current study, a similar approach was applied to the 3-carboxy-4-carboxymethyloxyphenylalanyl-containing peptide, which led to the preparation of monomeric 5-carboxy-6-carboxymethyloxy-2-naphthoic acid (K_i = 900 μM). However, contrary to expectations based on the aforementioned F_2Pmp work, incorporation of this putative pTyr mimetic into the pseudo-dipeptide, *N*-[5-carboxy-6-carboxymethyloxy-2-naphthoyl]-glutamic acid, resulted in a substantial loss of binding affinity. A reevaluation of binding orientation for 5-carboxy-6-carboxymethyloxy-2-naphthoic acid was therefore undertaken, which indicated a 180° reversal of the binding orientation within the PTP1B catalytic site. In the new orientation, the naphthyl 2-carboxyl group, and not the *o*-carboxy carboxymethyloxy groups, mimics a phosphoryl group. Indeed, when 5-carboxy-2-naphthoic acid itself was examined at neutral pH for inhibitory potency, it was found to have K_i = 31 ± 7 μM, which is lower than parent 5-carboxy-6-carboxymethyloxy-2-naphthoic acid. In this fashion, 5-carboxy-2-naphthoic acid (or more appropriately, 6-carboxy-1-naphthoic acid) has been identified as a novel PTP1B binding motif.

Introduction

Development of small molecule protein-tyrosine phosphatase (PTP) inhibitors has important implications for the treatment of a variety of diseases including certain cancers and diabetese.[2,3] Among PTPs, the human nonreceptor PTP1B enzyme is important both because it was the first PTP to have its X-ray crystal structure reported[4] and because it has been shown to potentially contribute to some forms of diabetes.[5] For these reasons, it provides an ideal model system in which to explore PTP inhibitor development. Binding of peptidyl phosphotyrosyl (pTyr)-containing substrate to PTPs involves two components: (1) critical recognition of the pTyr "phenyl phosphate" moiety in a highly conserved (H/V)CX_5R(S/T) signature motif within the catalytic pocket and (2) secondary interactions of amino acids in the substrate neighboring the pTyr residue, with features outside the catalytic pocket.[6] A principle focus of considerable research to date has centered on interactions within the pTyr-binding pocket[7] and a number of small molecule, non-phosphorus-containing structures which bind to PTP1B with from reasonable to high affinity have been discovered using either screening or computer-assisted pharmacore-based methods.[3]

One approach toward inhibitor development is the utilization of peptides as platforms for small molecule lead discovery. Exemplary of this, display of pTyr mimicking amino acid residues (**Xxx**) incorporated into the epidermal growth factor receptor ($EGFr_{988-993}$)-derived sequence "Ac-Asp-Ala-Asp-Glu-**Xxx**-Leu-amide", which is a high affinity PTP1B substrate when **Xxx** = pTyr (K_m = 3.2 μM),[8] have been utilized to define phenyl phosphate mimicking structures that replicate binding interactions of pTyr itself.[9,10] As a case in point, the high PTP1B affinity of peptide **1a** (Figure 1), when **Xxx** = phosphono(difluoromethyl)phenylalanine (F_2Pmp),[11] provided initial evidence that aryldifluoromethylphosphonates such as (difluoro(2-naphthyl)methyl)phosphonic acids are extremely good PTP-directed phenyl phosphate mimetics.[12] More recently, a similar "peptide-

† For preliminary disclosures of this work, see ref 1.

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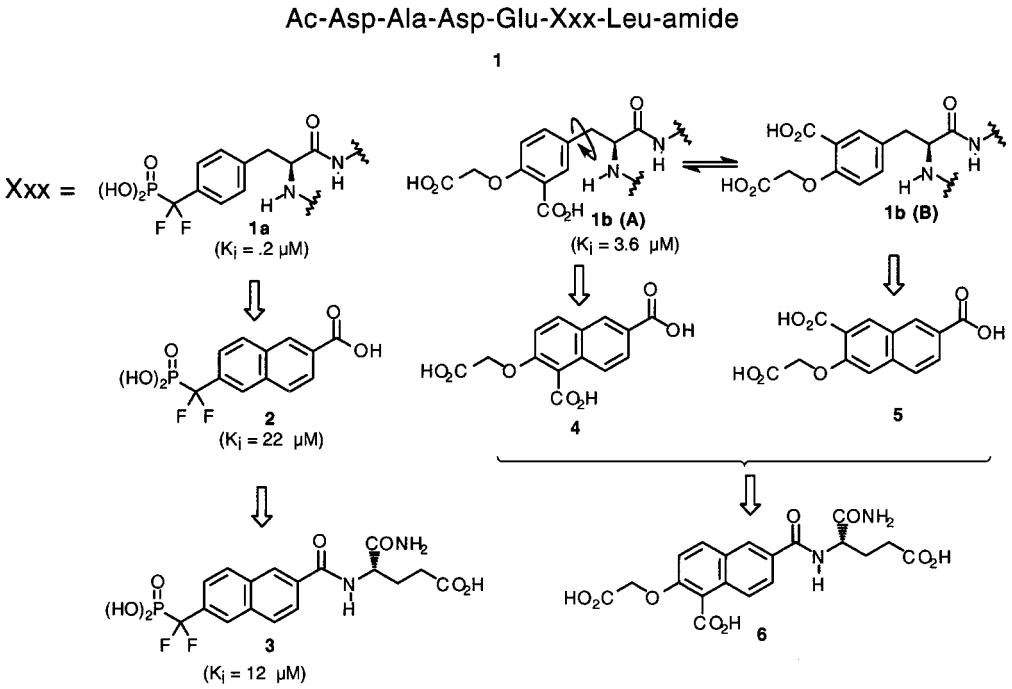
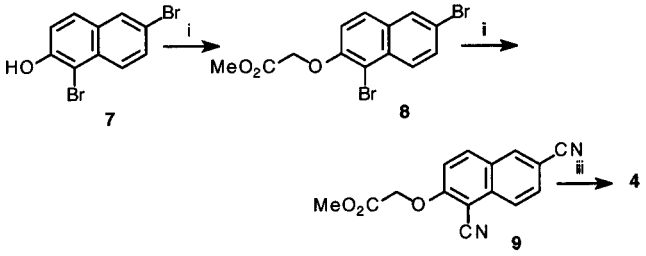


Figure 1. Rationale for the design of monomeric pTyr mimetics **4** and **5** and dipeptide mimetic **6** mimetics using peptide **1b** as a lead, based on a similar approach in going from **1a** to **2** to **3**.[25,26]

Scheme 1[a]



[a] Reagents and conditions: (i) methyl bromoacetate, K_2CO_3, DMF, 100%; (ii) CuCN, DMF, 46%; (iii) a. NaOH, H_2O; b. HCl, 100%.

based" approach has been applied to the discovery of high affinity nonphosphonate-based pTyr mimetics.[13] Reported herein is the utilization of one of these lead pTyr mimetics, 3-carboxy-4-carboxymethyloxyphenylalanyl residue **1b**,[14] as a starting point for the discovery of a new naphthyl-based motif which displays good PTP1B affinity.

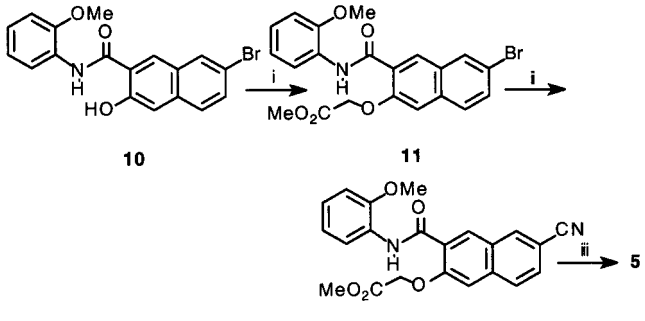
Synthesis

The synthesis of conformationally constrained analogue **4** (Figure 1) from commercially available 1,6-dibromo-2-naphthol (**7**) was readily achieved by initial O-alkylation (methyl bromoacetate) to provide **8**,[15] followed by transformation to the dicyano compound **9** using CuCN as a coupling reagent,[16] and finally hydrolysis of ester and cyano functionality to provide the target compound **4** (Scheme 1).[17]

Treatment of commercially available (Aldrich) naphthol AS BI ((7-bromo-3-hydroxy(2-naphthyl))-*N*-benzamide, **10**) in a similar fashion provided isomeric analogue **5** (Scheme 2).

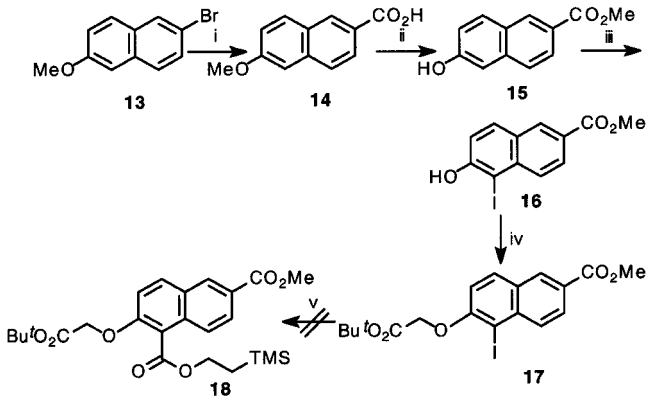
By analogy to the synthesis of peptide **1b**,[14] compound **18** was initially sought as a variant of constrained analogue **4** bearing carboxyl protection at positions 5 and 6 which rendered it compatible with peptide coupling of the free 2-carboxyl to ultimately provide pseudo-

Scheme 2[a]



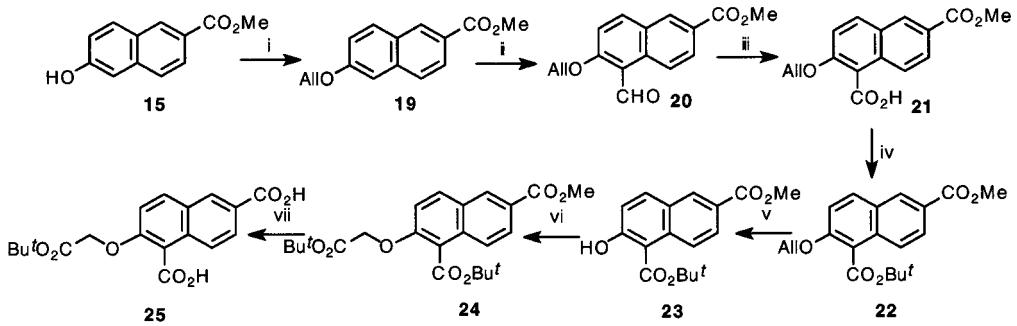
[a] Reagents and conditions: (i) methyl bromoacetate, K_2CO_3, DMF, 88%; (ii) CuCN, DMF, 56%; (iii) a. NaOH, H_2O; b. HCl, 100%.

Scheme 3[a]



[a] Reagents and conditions: (i) a. CuCN, DMF; b. NaOH, EtOH−H_2O; c. HCl, 98%; (ii) a. BBr_3, CH_2Cl_2; b. MeOH, 99%; (iii) NaI, chloramine-T, 99%; (iv) *tert*-butyl bromoacetate, K_2CO_3, DMF, 91%: (v) TMS−CH_2CH_2-OH, $Pd(OAc)_2$, CO, $Ph_2PCH_2CH_2PPh_2$.

Scheme 4[a]



[a] Reagents and conditions: (i) allyl bromide, NaH, DMSO, 91%; (ii) Cl_2CHOCH_3, $TiCl_4$, CH_2Cl_2, 77%; (iii) sulfamic acid, NaClO, H_2O–acetone, 100%; (iv) *tert*-butyl 2,2,2-trichloroacetimide, $BF_3{\cdot}OEt_2$, 58%; (v) $NaBH_4$, $Pd(PPh_3)_4$, THF; (vi) *tert*-butyl bromoacetate, K_2CO_3, 63% for two steps; (vii) LiOH, THF–H_2O, 100%.

dipeptide **6** (Figure 1). The approach to **18** (Scheme 3) began with carbonylation of commercially available 2-bromo-6-methoxynaphthol (**13**) to yield 6-methoxy-2-naphthoic acid (**15**) which was O-demethylated with BBr_3[18] and esterified in a one-pot sequence to yield methyl 6-hydroxy-2-naphthoate (**15**). Introduction of iodine at the 5-position using NaI and chloramine T[19] provided **16**, which upon O-alkylation (*tert*-butyl bromoacetate) gave key intermediate **17**. With **17** in hand, several conditions were examined to effect carboxylation to desired **18** without success.

Failure to prepare **18** was potentially attributable to steric hindrance at the crowded 5-position. The high degree of steric crowding at the 5-position suggested that carboxyl functionality situated at this position could exhibit reduced reactivity under conditions envisioned for eventual coupling of the 2-carboxyl, thereby eliminating the formal need for carboxyl protection at the 5-position. Therefore, target **25** was devised, bearing a free 5-carboxyl group. Synthesis of **25** started with intermediate **15**, which was initially protected as its allyl ether **19**,[20] then the carboxyl group was introduced in a two-step fashion[21] via aldehyde **20** which was then oxidized[22] to acid **21** (Scheme 4). Temporary protection[23] of the newly generated 5-carboxy as its *tert*-butyl ester (**22**), followed by removal of the 6-*O*-allyl protection,[24] gave **23**, which was alkylated to **24** in 63% yield for two steps. Treatment with LiOH selectively hydrolyzed both the 2- and 5-esters in the presence of the 6-ester, providing desired target **25** suitably protected for coupling of the 2-carboxyl group.

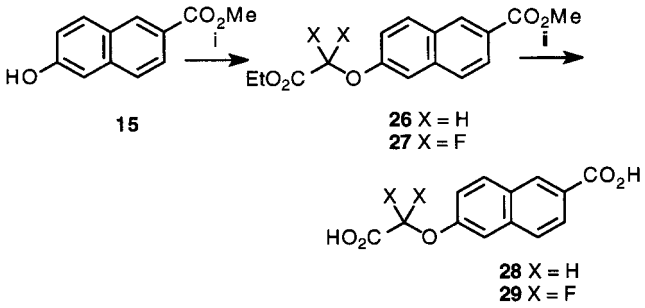
Analogues **28** and **29**, which lacked carboxyl functionality ortho to the 6-position, were prepared in straightforward fashion from common intermediate **15** as shown in Scheme 5.

To prepare **33** and **34** (Scheme 6), methyl 6-methoxy-2-naphthoate was subjected to the two-step approach of Scheme 4, whereby carboxyl functionality was first introduced at the aldehyde level (compound **31**) followed by oxidation (compound **32**).

Results and Discussion

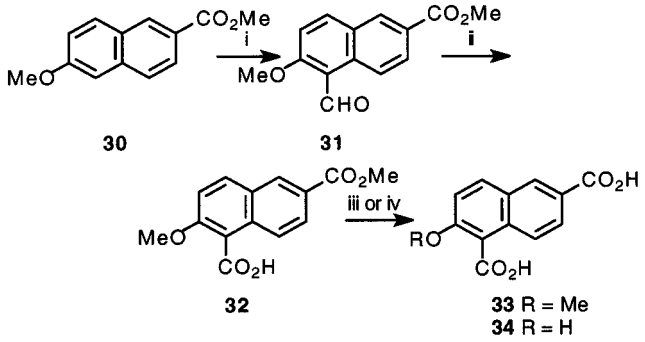
Rationale. The current study was intended as a peptide-lead approach to non-phosphonate-containing small molecule PTP1B inhibitor discovery. Its rationale was predicated on the use of pTyr mimicking amino acid residues (**Xxx**), displayed in the sequence "Ac-Asp-Ala-Asp-Glu-**Xxx**-Leu-amide" (**1**, Figure 1), to define phenyl

Scheme 5[a]



[a] Reagents and conditions: (i) ethyl bromoacetate, K_2CO_3, 68% or CF_2BrCO_2Et, K_2CO_3, 40%; (ii) LiOH, 97% (for **26**), 95% (for **27**).

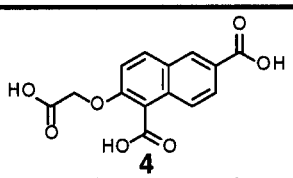
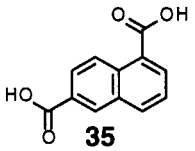
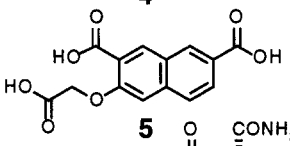
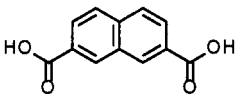
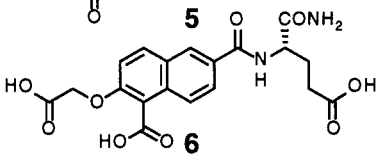
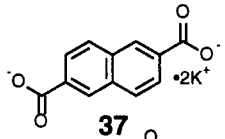
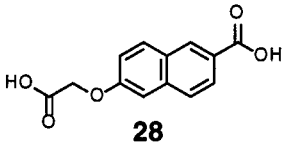
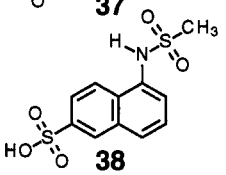
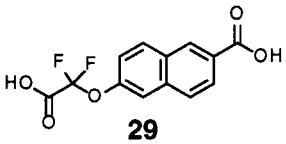
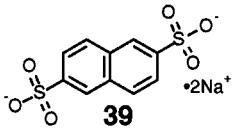
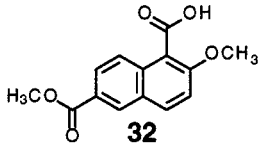
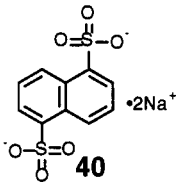
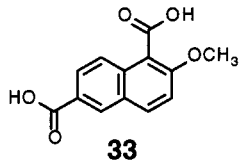
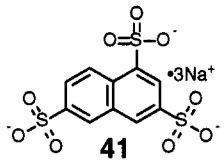
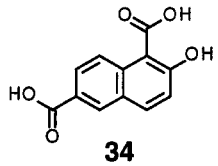
Scheme 6[a]



[a] Reagents and conditions: (i) Cl_2CHOCH_3, $TiCl_4$, CH_2Cl_2, 54%; (ii) sulfamic acid, NaClO, H_2O-acetone, 100%; (iii) NaOH, EtOH–H_2O; (iv) BBr_3, CH_2Cl_2, 100%.

phosphate-mimicking motifs which can be recognized with high affinity in the PTP1B catalytic site. This approach is conceptually similar to our previous study, where the initial lead[11] provided by the F_2Pmp-containing peptide (**1a**, Figure 1), resulted in the development of 6-(phosphonodifluoromethyl)-2-naphthoic acid (**2**) as a moderately potent small molecule PTP1B inhibitor which functioned as a monomeric pTyr mimetic.[25,26] The starting point for the current study was the previously reported 3-carboxy-4-carboxymethyloxyphenylalanyl residue (**1b**), which was among the highest affinity non-phosphonate-containing pTyr mimetics against PTP1B in a peptide context.[14] On the basis of the above-mentioned transformation of **1a** to **2** in the F_2Pmp study, it was envisioned that **1b** could be translated to isomeric 5-carboxy- and 7-carboxy 6-(carboxymethyloxy)-2-naphthoic acids **4** and **5**, respectively. Two iso-

Table 1. Inhibition of PTP1B by Naphthyl-Based Inhibitors

Structure	IC_{50} (μM)	Structure	IC_{50} (μM)
 **4**	K_i = 900	 **35**	K_i = 31 +/- 7
 **5**	K_i = 250	 **36**	>>3,200
 **6**	3800	 **37**	>>4000
 **28**	9400	 **38**	29000
 **29**	9400	 **39**	>>4000
 **32**	2800	 **40**	28000
 **33**	20000	 **41**	>>4000
 **34**	12000		

mers were required, because unlike F_2Pmp, which is symmetrical about its phenyl axis of rotation and can therefore be represented by a single 6-substituted 2-naphthoic acid (**2**), *m*-carboxy residue **1a** can exhibit conformational isomerism about its phenyl axis of rotation (**A** and **B**, Figure 1). Accordingly, 5-carboxy- and 7-carboxy 2-naphthoic acids (**4** and **5**, respectively) were designed as conformationally constrained representations of these rotamers.

Initial Results. Analogues **28** and **29** (Table 1) represent naphthyl-based mimetics of 4-carboxymethyloxyphenylalanine and 4-carboxy(difluoromethyl)oxyphenylalanine, respectively.[27] The low inhibitory potency is consistent with the reported poor PTP1B affinity of the former pTyr mimetic when expressed in the model peptide **1**.[13] Compounds **4** and **5** exhibited enhanced potencies, with the 5-carboxy analogue **4** (K_i = 900 μM) showing approximately 10-fold higher affinity than **28**. This indicated the importance of the added 5-carboxyl group. The position of the carboxyl also affected affinity, since the isomeric 7-carboxy isomer **5** (K_i = 250 μM) was approximately 3-fold more potent than **4**.

Examination of Dipeptide Mimetic 6, and Re-evaluation of Binding Mode. From preliminary results, it appeared that either compound **4** or compound **5** could be utilized as a naphthyl-based mimetic of **1b** in a manner similar to the previous use of **2** as a mimetic of F_2Pmp-containing **1a** (Figure 1).[25] In that previous study, coupling of **2** to a Glu residue resulted in pseudo-dipeptide **3**, whose approximate 2-fold increase in affinity was attributable to interaction of the

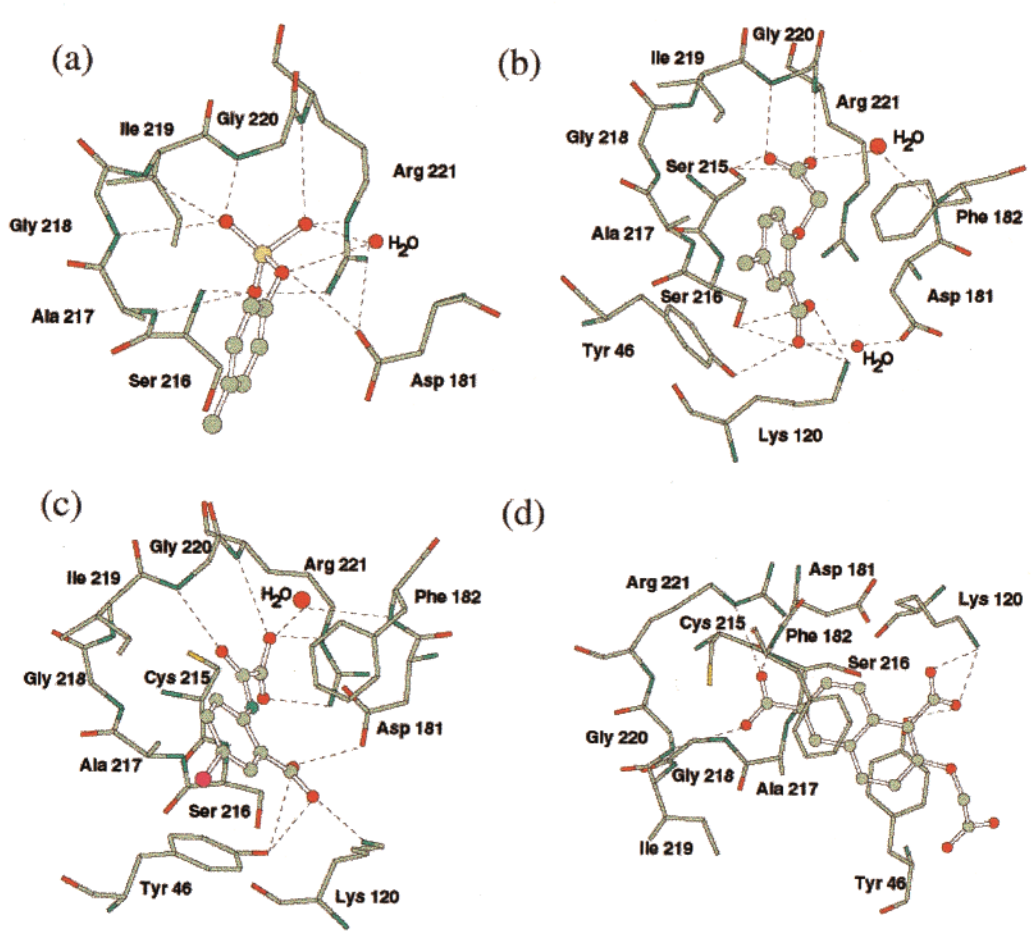


Figure 2. Binding interactions within the PTP1B signature motif. (a) Binding of a pTyr phenyl phosphate as indicated by X-ray crystallography.[6] (b) Molecular modeling prediction of 3-carboxy-4-carboxymethyloxyphenyl binding as reported in ref 14. (c) Binding of a 2-(oxalylamino)-benzoic acid moiety as indicated by X-ray crystallography.[28] (d) Preferred docking mode of compound **4** utilizing unbiased techniques as indicated in the Experimental Section.

Glu γ-carboxyl with residues outside the pTyr binding pocket. By analogy, pseudo-dipeptide **6** was designed as a further elaboration of **4**. Surprisingly, however, rather than exhibiting enhanced potency, compound **6** (IC_{50} = 3800 μM, Table 1) displayed a significant decrease in affinity relative to **4**. This necessitated a reevaluation of the originally hypothesized binding mode of **4**.

Comparison of the suggested mode of binding for peptide **1b** (Figure 2b)[14] with the binding of pTyr, as indicated by X-ray crystallography (Figure 2a),[6] shows that the carboxymethyl group of **1b** interacts with the signature motif loop in a manner similar to several of the interactions displayed by the phosphate group in pTyr; however, its 3-carboxyl group participates in additional hydrogen bonding to residues Tyr 46, Lys 120, Ser 216, and Asp 181 (through a bridging H_2O molecule), which does not occur in the case of pTyr. A recently published crystal structure of a related 2-(oxalylamino)-benzoic acid PTP1B inhibitor also shows the benzoyl carboxy group interacting with Tyr 46, Lys 120, and directly with Asp 181 (Figure 2c),[28] supporting this suggested mode of binding for **1b**. The same mode of binding was assumed initially for compound **4**; however, the extremely deleterious effect of incorporating **4** into dipeptide mimetic **6** prompted a docking study using three different methods: Flexidock,[29] the low mode conformational search in Macromodel 6.5 according to Kolossavary,[30] and QMD-docking[31] (see Experimental Section).[32] In all cases, the protein atoms were kept fixed. Consistently, all methods gave a binding mode reversed by 180° to that which had been expected based in Figure 2b. In other words, the 6-carboxymethyloxy group was oriented away from the catalytic cleft, with the 2-carboxyl occupying a "phosphate-mimicking" position within the catalytic cleft (Figure 2d). It was also observed in this model that the 5-carboxyl group made key binding interactions as well.

The 2,5-Dicarboxynaphthyl Moiety as a New Phenyl Phosphate Mimetic. To further explore this unexpected binding mode, the inhibitory potencies in a series of 12 mono-, di-, and tricarboxy/amido/sulfono-substituted naphthyls were determined (compounds **32**−**41**, Table 1). Of these, only 2,5-dicarboxy-substituted analogue **35** (K_i = 31 ± 7 μM, Table 1) exhibited good affinity.[33] The poor affinities of isomeric 2,6- and 2,7-dicarboxy analogues (compounds **37** and **36**) indicate the importance of the "2,5-dicarboxynaphthyl" motif. Additionally, loss of affinity upon introduction of func-

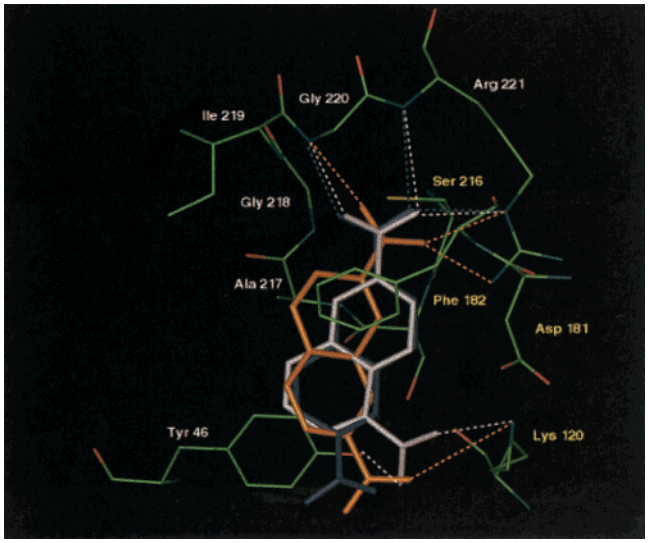


Figure 3. Docking of bis-carboxynaphthyls into the PTP1B signature motif as described in the Experimental Section: white, 2,5-dicarboxy (**35**); blue, 2,6-dicarboxy (**37**); and red, 2,7-dicarboxy (**36**).

tionality into the 6-position (compounds **33** and **34**) further supports the sensitivity of the environment surrounding the 5-carboxyl group.

To gain a clearer understanding of the effect of the naphthyl-substitution pattern on binding affinity, all 12 ligands were docked by the QMD-dock method allowing flexibility for selected residues within the catalytic site. Comparing the binding modes of **35** with **36** and **37** (Figure 3), it was evident that, while maintaining interactions of the 2-carboxyl within the phosphate-binding loop, for **36** and **37** the positions of the 7- and 6-carboxyl groups respectively, are less favorable for interactions with Tyr 46, Lys 120, and Ser 216 (Figure 3). Additionally, it was observed for compound **34**, which contains an added hydroxyl at the 6-position, that the hydroxyl group does not undergo hydrophilic interactions, and the same was true for the third sulfonate group of **41** (not shown). Therefore, although **34** and **41** include the parent "2,5-diacidic" arrangement of **35**, the desolvation energy expended on binding is not compensated for by additional hydrogen bonds.

Conclusions

We have previously examined the PTP1B inhibitory potencies of a series of pTyr mimetics expressed in a hexapeptide platform, with the intent of defining phenyl phosphate-mimicking functionality which could be utilized in non-peptide, small molecule PTP1B inhibitors. Although the initial direction of this work resulted in aryl difluorophosphonate-containing inhibitors, it was of considerable interest to define nonphosphonate-containing, carboxy-based phenyl phosphate mimetics for use in small molecule inhibitor design. In the present study, we have taken as a starting point the *o*-carboxy-carboxymethyloxyphenyl motif, which was one of the most potent phenyl phosphate mimetics in the peptide platform. On the basis of our approach with difluorophosphonomethyl inhibitors, two isomeric 2-naphthoic acid analogues (**4** and **5**) were examined as conformationally constrained variants of the parent 3-carboxy-4-carboxymethyloxyphenylalanyl residue, in which the *o*-carboxy-carboxymethyloxyphenyl portion was intended to replicate the interactions of phenyl phosphate. When incorporation of one of these monomeric inhibitors (**4**) into dipeptide **6** resulted in significant loss of PTP1B affinity, a reevaluation of binding orientation for **4** was undertaken. This indicated that the naphthyl 2-carboxyl group, and not the *o*-carboxy carboxymethyloxy groups, was mimicking a phosphoryl group. A reevaluation of binding orientation for 5-carboxy-6-carboxymethyloxy-2-naphthoic acid was therefore undertaken, which indicated a 180° reversal of the binding orientation within the PTP1B catalytic site. In the new orientation, the naphthyl 2-carboxyl group and not the *o*-carboxy carboxymethyloxy groups mimics a phosphoryl group. Indeed, when 5-carboxy-2-naphthoic acid itself was examined for inhibitory potency, it was found to have a K_i value at neutral pH of 31 ± 7 μM, which is lower than parent **4**. This therefore identifies 5-carboxy-2-naphthoic acid (or more appropriately, 6-carboxy-1-naphthoic acid) as a new binding motif for PTP1B inhibitors, wherein one acidic group accommodates the position of pTyr phosphoryl oxygen atoms and the other interacts with residues Tyr 46, Lys 120, and Ser 216. The enzyme−ligand interactions displayed by this binding mode are similar to the originally proposed binding mode of 2-(carboxymethyloxy)-benzoic acid type PTP1B inhibitors (**1b**),[14] which formed the conceptual basis of the study. The proposed binding is also quite similar to the actual binding of 2-(oxalylamino)-benzoic acid, as evidenced by a recently reported X-ray structure,[28] although the binding affinity of 5-carboxy-2-naphthoic acid is greater than this latter monomeric phenyl phosphate mimetic under physiologically relevant conditions [2-(oxalylamino)-benzoic acid $K_i = 200$ μM at pH 7.0].[28] It should be noted, however, that in translating a flexible 3-carboxy-4-carboxymethyloxyphenylalanyl residue into the rigid planar naphthyl system the spatial distribution of carboxyl groups about the naphthyl ring was radically different than anticipated. The 2,5-dicarboxy naphthalene motif reported herein provides a new approach toward phenyl phosphate mimicking functionality, which may be of use in the design of carboxy-based PTP1B inhibitors.

Experimental Section

Recombinant Human PTP1B. The cDNA encoding the catalytic domain of human PTP1B (amino acids 1 to 321) was obtained using the polymerase chain reaction (PCR) from a human fetal brain cDNA library (Stratagene). The PCR primers used were 5′-AGCTGGATCCATATGG AGATGGAAAAGGAGTT (encoding both a *BamH*I and a *Nde*I site) and 3′-ACGCGAATTCTTAATTGTGTGGCTCCAGGATTCG (encoding an *Eco*RI site). The PCR product was digested with *BamH*I and *Eco*RI and subcloned into a pUC118 vector. The PTP1B coding sequence was confirmed by DNA sequencing. The coding region for PTP1B was then cut from pUC118-PTP1B with *BamH*I and *Eco*RI and ligated to the corresponding sites of plasmid pT7-7. The PTP1B coding sequence was placed in frame downstream of the phage T7 RNA polymerase promoter at the *Nde*I site of pT7-7 to provide the translational initiation at Met 1 of PTP1B. The resulting plasmid pT7-7/PTP1B was used to transform *Escherichia coli* BL21(DE3). An overnight culture of BL2(DE3) cells with the pT7-7 expression vector containing the mutant PTP1B was diluted 1:100 into 1 L of 2 × YT medium containing 100 μg/mL ampicillin. The culture was grown at 37 °C until absorbance at 600 nm reached 0.6, at which point the cells were induced with 0.4 mM isopropyl β-D-thiogalatoside for 6 h. The cells were

harvested by centrifugation and stored at −20 °C. The frozen cell pellet was thawed at room temperature and resuspended in 30 mL of ice-cold buffer A (100 mM 2-(4-morpholino)-ethane sulfonic acid, pH 6.5, 1 mM DTT) and lysed by two passes through a French press at 1300 psi. All of the following steps were then carried out at 4 °C. The lysate was centrifuged at 15 000 rpm (Dupont SS-34 rotor) for 30 min. The supernatant was incubated with 50 mL of CM-Sephadex C50 equilibrated with buffer A and shaken gently for 40 min. The resin was washed three times with the same volume of buffer A, loaded onto a column, and washed again with 10 bed volumes of buffer A. The protein was then eluted from the column by a linear gradient from 0 to 0.5 M NaCl in 200 mL of buffer A. The positions of the peak fractions were assessed by Coomassie Blue staining of SDS−PAGE. Peak fractions which contain homogeneous PTP1B were combined into a final pool of protein, which was then concentrated to 30 mg/mL.

Phosphatase Assay. The PTP1B phosphatase activity was assayed at 30 °C in a reaction mixture (0.2 mL) containing appropriate concentrations of *p*-nitrophenyl phosphate (*p*NPP) as substrate. The buffer used was pH 7.0, 50 mM 3,3-dimethylglutarate, 1 mM EDTA. The ionic strength of the solution was kept at 0.15 M using NaCl. The reaction was initiated by addition of enzyme and quenched after 2−3 min by addition of 1 mL of 1 N NaOH. The nonenzymatic hydrolysis of the substrate was corrected by measuring the control without the addition of enzyme. The amount of product *p*-nitrophenol was determined from the absorbance at 405 nm using a molar extinction coefficient of 18 000 M^{-1} cm^{-1}. Steady state kinetic parameters were evaluated by fitting directly the v vs [S] data to the Michaelis−Menten equation using KINETASYST (IntelliKinetics, State College, PA).

Inhibition Constant Determination. Inhibition constants for the small PTP inhibitors were determined for PTP1B in the following manner. The initial rate at eight different *p*NPP concentrations (0.2 K_m to 5 K_m) was measured at three different fixed inhibitor concentrations.[34] The inhibition constant and inhibition pattern were evaluated using a direct curve-fitting program KINETASYST (IntelliKinetics, State College, PA).

Molecular Modeling. All force field simulations were performed with the Insight II 98.0/Discover 3.0 modeling package[31] using the cff91 force field (running on SGI computers). A dielectric constant of 1.00 and the cell multipole method with fine accuracy were used for all nonbonding interactions except for the high-temperature quenched molecular dynamics (QMD) simulations. The crystal structure (PDB code 1BZJ)[26] of [1,1-difluoro-1-(6′-carboxy-naphth-2′-yl)]methylphosphonate bound to PTP1B was used as the starting geometry for PTP1B. For ligand **4**, 10 quenched molecular dynamics (QMD) cycles were performed with different seeds for the random number generator for each cycle. Each QMD cycle consisted of 60 ps MD at 2000 K in an NVT ensemble, keeping all protein atoms fixed except the hydrogen atoms of Tyr46, Lys120, and Ser216. A distance constraint between the C-4 atom of the ligand naphthyl ring and the nitrogen atom of Ser222 of PTP1B was applied using a flat bottom potential with a force constant of 0.0 for the distance range of 0−12 Å and 1000.0 for longer distances. The vdW interactions were scaled to 2%, and the Coulombic interactions to 20% during the high-temperature MD simulations. The coordinates were saved every 200 fs and subsequently minimized by 300 steps of the Polak-Ribiere conjugated gradient algorithm (CG-PR). For each of the 10 runs, the lowest energy conformation out of the 300 minimized structures was stored. The frame with lowest energy among the 10 stored structures is depicted in Figure 2d. The QMD-docking for ligands **35**, **36**, and **37** was performed in a similar way which only differed in allowing side chain flexibility for Tyr46, Arg47, Asp48, Lys120, Asp181, Phe182, Ser215, Ser216, Ala217, Ile219, and Arg221, and minimizing the lowest energy complex of the 10 QMD runs by 1000 steps of CG-PR using the esff-force field. The results are depicted in Figure 3.

Synthesis. General Synthetic Methods. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA, and fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. Where indicated, FABMS matrixes used were glycerol (Gly) or nitrobenzoic acid (NBA). ^{1}H NMR data are reported in ppm relative to TMS and referenced to the solvent in which they were run. Solvent was removed by rotary evaporation under reduced pressure, and silica gel chromatography was performed using Merck silica gel 60 with a particle size of 40−63 μm. Anhydrous solvents were obtained commercially and used without further drying. HPLCs were conducted using a Waters Prep LC4000 system having photodiode array detection and binary solvent systems as indicated where A = 0.1% aqueous TFA and B = 0.1% TFA in acetonitrile and either Vydac C_{18} (10 μ) Peptide & Protein or Advantage C_{18} (5 μm) columns (preparative size, 20 mm dia. × 250 mm long with a flow rate of 10 mL/min; semi-preparative size, 10 mm dia. × 250 mm long, with a flow rate of 2 mL/min.).

Methyl 2-(1,6-Dibromo-2-naphthyloxy)acetate (8). To a solution of 1,6-dibromo-2-naphthol (**7**) (3 g, 10 mmol) in anhydrous DMF (100 mL) was added methyl bromoacetate (5.8 g, 38 mmol) and potassium carbonate (3.3 g, 25 mmol), and the mixture was heated to 60∼70 °C and stirred (7 h). Solvent was removed under vacuum, H_2O (20 mL) was added to quench the reaction, and the mixture was subjected to an extractive workup, dried (Na_2SO_4), and concentrated. Purification by silica gel flash chromatography afforded **8** (3.78 g, 100% yield): mp 138−140 °C; H NMR ($CDCl_3$) δ 8.13 (1H, d, J = 9.3 Hz), 7.96 (1H, d, J = 1.7 Hz), 7.70 (1H, J = 9.0 Hz), 7.64 (1H, dd, J = 2.0, 9.3 Hz), 7.18 (1H, d, J = 9.0 Hz), 4.85 (2H, s), 3.83 (3H, s). FABMS ($^+$VE) *m/z* 372 $(M + H)^+$.

Methyl 2-(1,6-Dicyano-2-naphthyloxy)acetate (9). To a solution of **8** (0.74 g, 2.0 mmol) in anhydrous DMF (10 mL) was added copper(I) cyanide (430 mg, 4.8 mmol). The mixture was refluxed (4 h), then cooled to room temperature, and poured into a solution of $FeCl_3 \cdot 6H_2O$ (800 mg) and HCl (37%, 2 mL) in H_2O (12 mL). After being maintained at 60∼70 °C (10 min), the mixture was subjected to an extractive workup, dried (Na_2SO_4), and concentrated. Purification by silica gel flash chromatography afforded **9** (247 mg, 46%): mp 193−195 °C; H NMR ($CDCl_3$) δ 8.25∼8.21 (2H, m), 8.10 (1H, d, J = 9.3 Hz), 7.81 (1H, dd, J = 1.5, 8.8 Hz), 7.25 (1H, d, J = 9.3 Hz), 4.10 (2H, s), 3.84 (3H, s). CIMS ($^+$VE, NH_3) *m/z* 284 $(M + NH_4^+)$.

2-Carboxymethoxy)naphthalene-1,6-dicarboxylic Acid (4). A solution of bis-nitrile **9** (120 mg, 0.45 mmol) in EtOH−H_2O (1:1; 5 mL) containing sodium hydroxide (200 mg; 5 mmol) was refluxed (12 h) and then cooled to room temperature. Ethanol was removed by evaporation, and the resulting aqueous solution was acidified with 1.0 M aqueous HCl, subjected to an extractive workup, dried (Na_2SO_4), and taken to dryness to provide crude product (143 mg). Purification by HPLC gave final product **4** as a white solid: mp 266 °C (dec); H NMR (DMSO-d_6) δ 8.56 (1H, s), 8.1 (1H, d, J = 9.3 Hz), 7.97 (1H, dd, J = 1.5, 8.8 Hz), 7.87 (1H, d, J = 9.0 Hz), 7.64 (1H, d, J = 9.3 Hz), 4.95 (2H, s). Anal. ($C_{14}H_{10}O_7 \cdot 1.29H_2O$) C, H.

Methyl 2-{6-Bromo-3-[*N*-(2-methoxyphenyl)carbamoyl]-2-naphthyloxy}acetate (11). To a solution of Naphthol AS BI (**10**) (1.28 g, 3.4 mmol) in anhydrous DMF (20 mL) was added methyl bromoacetate (2.0 g, 1.31 mmol) and potassium carbonate (1.1 g, 8.6 mmol), and the mixture was heated to 60∼70 °C with stirring (5 h). Solvent was removed under vacuum, H_2O (20 mL) was added to quench the reaction, and the mixture was subjected to an extractive workup, dried (Na_2SO_4), and concentrated. Purification by silica gel flash chromatography afforded **11** (1.34 g, 88% yield): mp 150−152 °C; H NMR ($CDCl_3$) δ 10.30 (1H, s), 8.73 (1H, s), 8.59 (1H, dd, J = 2.0, 7.6 Hz), 8.08 (1H, s), 7.62 (2H, s), 7.14 (1H, s), 7.12∼7.01 (2H, m), 6.94 (1H, dd, J = 1.7, 7.8 Hz), 5.00 (2H, s), 3.95 (3H, s), 3.85 (3H, s). Anal. ($C_{21}H_{18}BrNO_5$) C, H, N.

Methyl 2-{6-Cyano-3-[*N*-(2-methoxyphenyl)carbamoyl]-2-naphthyloxy}acetate (12). To a solution of **11** (889 mg, 2.0 mmol) in anhydrous DMF (10 mL) was added copper(I)

cyanide (214 mg, 2.4 mmol). The mixture was refluxed (4 h), then cooled to room temperature, and poured into a solution of $FeCl_3 \cdot 6H_2O$ (800 mg) and HCl (37%, 2 mL) in H_2O (12 mL). After being maintained at 60∼70 °C (10 min), the mixture was subjected to an extractive workup, dried (Na_2SO_4), and concentrated. Purification by silica gel flash chromatography afforded **12** (450 mg, 56% yield): mp 163−165 °C; H NMR ($CDCl_3$) δ 10.23 (1H, s), 8.87 (1H, s), 8.58 (1H, dd, J = 1.7, 7.6 Hz), 8.31 (1H, s), 7.83 (1H, d, J = 8.8 Hz), 7.66 (1H, dd, J = 1.7, 8.6 Hz), 7.20 (2H, s), 7.13∼7.05 (2H, m) 6.95 (1H, dd, J = 1.7, 8.0 Hz), 5.02 (2H, s), 3.92 (3H, s), 3.85 (3H, s). FABMS ($^+$VE) m/z 391.2 $(M + H)^+$. Anal. ($C_{22}H_{18}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

3-Carboxymethoxy)naphthalene-2,7-dicarboxylic Acid (5). A solution of nitrile **12** (100 mg, 0.26 mmol) in EtOH−H_2O (1:1; 5 mL) containing sodium hydroxide (200 mg; 5 mmol) was refluxed (12 h), and then cooled to room temperature. Ethanol was removed by evaporation, and the resulting aqueous solution was acidified with 1.0 M aqueous HCl, subjected to an extractive workup, dried (Na_2SO_4), and taken to dryness to provide crude product (75 mg). Purification by HPLC gave final product **5** as white solid: mp 278 °C (dec); H NMR ($CD_3OD + D_2O$) δ 7.44 (1H, s), 7.90 (1H, dt, J = 9.0, 1.7 Hz), 8.07 (1H, dt, J = 8.5, 0.5 Hz), 8.48 (1H, d, J = 0.7 Hz), 8.62 (1H, s). FABMS ($^-$VE) m/z 289 $(M - H)^-$. Anal. ($C_{14}H_{10}O_7 \cdot 0.15CF_3CO_2H \cdot H_2O$) C, H.

Dipeptide Mimetic 6. A total of 1.0 g (0.42 mmol) of *N*-Fmoc Rink amide resin (Bachem, Lot 513049) was swollen with NMP (3 × 5 mL), then deblocked with 20% piperidine in NMP (5 mL wash then 5 mL for 20 min), and washed with NMP (10 × 5 mL). An HOBt active ester, prepared by reacting Fmoc-L-Glu (Ot-Bu) (893 mg, 2.1 mmol), HOBt (283 mg, 2.1 mmol), and DIPCDI (265 mg, 2.1 mmol) in NMP (5 mL) (10 min), was added to the washed and deblocked resin, and the mixture was rocked at room temperature (10 h). The resin was washed with NMP (10 × 5 mL) and CH_2Cl_2 (10 × 5 mL), and then dried overnight under high vacuum to provide Fmoc-L-Glu (Ot-Bu)-Rink amide resin (1.21 g, 97% yield). A portion of the resin (250 mg, 0.10 mmol) was swollen with NMP (3 × 1.5 mL), deblocked with 20% piperidine in NMP (1.5 mL wash then 1.5 mL, 20 min), and washed with NMP (10 × 1.5 mL). An HOBt active ester, prepared by reacting 2-*tert*-butoxycarbonylmethoxy-naphthalene-1,6-dicarboxylic acid (**25**) (87 mg, 0.25 mmol), HOBt (34 mg, 0.25 mmol), and DIPCDI (40 mL, 0.25 mmol) in NMP (1.5 mL) (10 min), was added, and the resin was rocked at room temperature (overnight). The resin was washed with NMP (10 × 1.5 mL) and CH_2Cl_2 (10 × 1.5 mL), and then cleaved by treatment with a solution of TFA (1.85 mL):H_2O (0.1 mL):triethylsilane (50 μL) (1 h). Supernatant was collected by filtration, and the resin was washed with TFA (2 × 2 mL) and CH_2Cl_2 (2 × 2 mL). The combined filtrate was evaporated, 5 mL of H_2O was added, and the mixture was taken to dryness. This procedure was repeated two times. Residue was purified by HPLC to provide **6** (14 mg, 33% yield). H NMR (DMSO-d_6) δ 8.60 (1H, s), 8.23 (1H, d, J = 9.3 Hz), 8.09 (1H, d, J = 8.1 Hz), 8.02 (1H, dd, J = 1.7, 8.8 Hz), 7.87 (1H, d, J = 8.8 Hz), 7.52 (1H, d, J = 9.0 Hz), 4.86 (2H, s), 4.27 (1H, m), 2.20 (2H, t, J = 7.81 Hz), 1.98 (1H, m), 1.77 (1H, m). FABMS ($^-$VE) m/z 417 $(M - H)^-$. HR-FABMS calcd for $C_{19}H_{18}N_2O_9$: 417.0934. Found: 417.0947.

6-Methoxy-2-naphthoic Acid (14). To a solution of 2-bromo-6-methoxynaphthalene (**13**) (10.0 g, 42.2 mmol) in anhydrous DMF (120 mL) was added copper(I) cyanide (4.53 g, 50.4 mmol, 1.2 equiv). The mixture was refluxed (4 h), then cooled to room temperature, and poured into a solution of $FeCl_3 \cdot 6H_2O$ (15.8 g) and HCl (37%, 40 mL) in H_2O (200 mL). After being maintained at ∼70 °C (20 min), the mixture was subjected to an extractive workup, dried (Na_2SO_4), and concentrated. Concentration under vacuum afforded crude 2-cyano-6-methoxynaphthalene (7.78 g; 100% yield). The nitrile was dissolved in EtOH−H_2O (1:1; 100 mL) containing (8.0 g, 0.2 mol) of sodium hydroxide; the resulting solution was refluxed overnight. After cooled to room temperature, ethanol was evaporated, and the residue was acidified with concentrated HCl and subjected to an extractive workup, dried (Na_2SO_4), and taken to dryness to provide crude **14**, which was recrystallized from ethyl acetate to give 6.00 g of pure product, with an additional 2.4 g being recovered from the filtrate (98% combined yield): mp 107−108 °C; H NMR (DMSO-d_6) δ 8.56 (1H, s), 8.18 (1H, dd, J = 1.7, 8.5 Hz), 7.91 (1H, d, J = 8.8 Hz), 7.72 (1H, d, J = 8.6 Hz), 7.19∼7.10 (2H, m), 3.90 (3H, s). FABMS ($^-$VE) m/z 201.1 $(M - H)^-$. Anal. ($C_{12}H_{10}O_3$) C, H.

Methyl 6-Hydroxy-2-naphthoate (15). To 6-methoxy-2-naphthoic acid (**14**) (4.044 g, 20.0 mmol) in anhydrous CH_2Cl_2 (100 mL) was added dropwise 1.0 M BBr_3 in CH_2Cl_2 (40 mL, 40 mmol) at −78 °C under argon, and the mixture was stirred at −78 °C (1 h), then raised to room temperature and stirred overnight. To the mixture at −78 °C was added anhydrous MeOH (10 mL), and then the mixture was warmed to room temperature and stirred (30 min). Solvent was evaporated to dryness, additional MeOH (10 mL) was added, and the mixture taken to dryness and purified (silica gel flash chromatography) to give **15** (3.91 g, 99% yield): mp 168−170 °C; H NMR (DMSO-d_6) δ 8.50 (1H, d, J = 1.7 Hz), 7.98 (1H, d, J = 8.6 Hz), 7.87 (1H, dd, J = 1.7, 8.8 Hz), 7.77 (1H, d, J = 8.6 Hz), 7.19∼7.17 (2H, m), 3.88 (3H, s). FABMS ($^+$VE) m/z 202.1 (M^+). Anal. ($C_{12}H_{10}O_3 \cdot 0.1H_2O$) C, H.

Methyl 6-Hydroxy-5-iodo-2-naphthoate (16). Sodium iodide (2.23 g, 14.9 mmol) was dissolved in boiling acetic acid (19 mL), and added carefully to a solution of chloramine-T trihydrate (4.20 g, 14.9 mmol) in acetic acid (3.75 mL) with stirring and cooling. To a solution of methyl 6-hydroxy-2-naphthoate (**15**) (2.87 g, 14.2 mmol) in acetic acid (7.5 mL) at 80 °C was added rapidly with stirring the in situ generated solution of ICl, and the solution was maintained at 80 °C (1 h), then poured into crushed ice, subjected to an extractive workup, and dried (Na_2SO_4). Concentration and purification by silica gel flash chromatography afforded **16** (4.61 g, 99% yield): mp 165−166 °C; H NMR (CD_3OD) δ 8.47 (1H, s), 8.11 (1H, d, J = 8.8 Hz), 8.02 (1H, dd, J = 1.7, 9.0 Hz), 7.87 (1H, d, J = 8.79 Hz), 7.22 (1H, J = 8.8 Hz), 3.95 (3H, s). FABMS ($^+$VE) m/z 328 (M^+). HR-FABMS calcd for $C_{12}H_{10}O_3I$ (MH^+): 328.9675. Found: 328.9676.

***tert*-Butyl 2-[1-Iodo-6-(methoxycarbonyl)-2-naphthyloxy]acetate (17).** To a solution of **16** (2.624 g, 6.9 mmol) in anhydrous DMF (48 mL) were added *tert*-butyl bromoacetate (5.10 g, 26.2 mmol) and potassium carbonate (2.27 g, 17.2 mmol), and the mixture was heated to 60∼70 °C and stirred overnight. The reaction mixture was cooled to room temperature, acetone (50 mL) was added, and precipitated solid was filtered off. The resulting solution was evaporated to dryness, and the residue was extracted with ethyl acetate, washed with water and brine, then dried (Na_2SO_4) and concentrated. Purification by silica gel flash chromatography afforded **17** (3.22 g, 91% yield). H NMR ($CDCl_3$) δ 8.51 (1H, d, J = 1.71 Hz), 8.21 (1H, d, J = 8.79 Hz), 8.10 (1H, dd, J = 1.7, 9.0 Hz), 7.92 (1H, d, J = 8.79 Hz), 7.10 (1H, d, J = 9.0 Hz), 4.76 (2H, s), 3.99 (3H, s), 1.49 (9H, s).

Methyl 6-Allyloxy-2-naphthoate (19). To a solution of sodium hydride (851 mg, 21.3 mmol) in DMSO (10 mL) was added a solution of methyl 6-hydroxy-2-naphthoate (**15**) (3.91 g, 19.5 mmol) in DMSO (10 mL) at 0 °C under argon. The solution was stirred at 0 °C (20 min), then a solution of allyl bromide (2.57 g, 21.3 mmol) in DMSO (5 mL) was added, and the mixture was stirred at ambient temperature overnight. The reaction mixture was poured into ice-cold H_2O (50 mL) and extracted with ethyl acetate, washed with water and brine, then dried (Na_2SO_4) and concentrated. Purification by silica gel flash chromatography afforded **19** (4.30 g, 91% yield): mp 80−82 °C; H NMR ($CDCl_3$) δ 8.61 (1H, s), 8.03 (1H, dd, J = 1.7, 8.8 Hz), 7.86 (1H, d, J = 9.0 Hz), 7.75 (1H, d, J = 8.8 Hz), 7.24 (1H, dd, J = 2.4, 9.0 Hz), 7.17 (1H, d, J = 2.4 Hz), 6.21∼6.06 (1H, m), 5.54∼5.33 (2H, m), 4.70∼4.67 (2H, m), 3.97 (3H, s). FABMS ($^+$VE) m/z 242.2 (M^+). Anal. ($C_{13}H_{14}O_3$) C, H.

Methyl 6-Allyloxy-5-formyl-2-naphthoate (20). To a solution of $TiCl_4$ (2.07 g, 10.9 mmol) and α,α-dichloromethyl methyl ether (645 mg, 5.6 mmol) in anhydrous CH_2Cl_2 (8 mL) at 0 °C was added a solution of methyl 6-allyloxy-2-naphthoate (**19**) (1.21 g, 5.0 mmol) in anhydrous CH_2Cl_2 (8 mL), and the

solution was stirred at 0 °C (1 h), then raised to room temperature and stirred overnight. The reaction was quenched by addition of dilute aqueous HCl, then subjected to an extractive workup, dried (Na_2SO_4), and concentrated. Purification by silica gel flash chromatography afforded **20** (1.045 g, 77.3% yield: mp 108−109.5 °C) with 107 mg of starting material being recovered. H NMR ($CDCl_3$) δ 10.95 (1H, s), 9.32 (1H, d, J = 9.28 Hz), 8.52 (1H, d, J = 1.7 Hz), 8.18 (1H, dd, J = 1.7, 9.0 Hz), 8.14 (1H, d, J = 9.0 Hz), 7.34 (1H, d, J = 9.3 Hz), 6.20∼6.04 (1H, m), 5.54∼5.36 (2H, m), 4.86∼4.83 (2H, m), 3.98 (3H, s). FABMS ($^+$VE) m/z 271.1. $(M + H)^+$. Anal. ($C_{16}H_{14}O_4$) C, H.

2-Allyloxy-6-(methoxycarbonyl)naphthoic Acid (21). Sulfamic acid (870 mg, 8.95 mmol) was added at 0 °C over 20 min to a solution of methyl ester **20** (1.045 g, 4.05 mmol) in acetone:H_2O (2:1, 33 mL). Sodium chlorite (80%, 524 mg, 4.63 mmol) was then added, and the solution was stirred at 0 °C (30 min), then concentrated, extracted with CH_2Cl_2, washed with brine, and dried over $MgSO_4$. Solvent was evaporated under vacuum to afford **21** (1.12 g, 97%). H NMR ($CDCl_3$) δ 8.56 (1H, d, J = 1.5 Hz), 8.31 (1H, d, J = 9.0 Hz), 8.13 (1H, dd, J = 1.7, 9.0 Hz), 8.04 (1H, d, J = 9.3 Hz), 7.35 (1H, d, J = 9.0 Hz), 6.19∼6.04 (1H, m), 5.56∼5.34 (2H, m), 4.86∼4.83 (2H, m), 3.99 (3H, s).

***tert*-Butyl 2-Allyloxy-6-(methoxycarbonyl)naphthoate (22).** To a solution of methyl ester **21** (1.43 g, 5.0 mmol) in anhydrous CH_2Cl_2 (8 mL) were added *tert*-butyl 2,2,2-trichloroacetimide (1.79 mL, 10.0 mmol) in cyclohexane (17 mL) at 0 °C followed by BF_3·etherate (100 μL), and the reaction mixture was stirred at room temperature (16 h). Solid $NaHCO_3$ was added, solid precipitate was filtered off, and the filtrate was taken to dryness. Purification by silica gel flash chromatography provided **22** (1.04 g, 58% yield): mp 105−106 °C; H NMR ($CDCl_3$) δ 8.55 (1H, d, J = 1.5 Hz), 8.07 (1H, dd, J = 1.7, 9.0 Hz), 7.95 (1H, d, J = 9.0 Hz), 7.83 (1H, d, J = 8.8 Hz), 7.03 (1H, d, J = 9.0 Hz), 6.15∼6.00 (1H, m), 5.52∼5.43 (1H, m), 5.34∼5.28 (1H, m), 4.76∼4.72 (2H, m), 3.98 (3H, s), 1.68 (9H, s). FABMS ($^+$VE) m/z 343 $(M + H)^+$. Anal. ($C_{20}H_{22}O_5$) C, H.

***tert*-Butyl 2-{1-[(*tert*-Butyl)oxycarbonyl]-6-(methoxycarbonyl)-2-naphthyloxy}acetate (24).** To a solution of methyl ester **22** (342 mg, 1.00 mmol) in anhydrous THF (8 mL) was added $Pd(PPh_3)_4$ (23 mg, 0.02 mmol), and the solution was stirred at room temperature (5 min). A solution of $NaBH_4$ (58 mg, 1.5 mmol) in THF (1.5 mL) was added dropwise at 0 °C with monitoring by TLC. When all starting material had disappeared, unreacted $NaBH_4$ was destroyed by addition of acetone (10 mL), then solvent was evaporated, and residue placed under high vacuum to yield compound **23** as a solid (mp 106−107 °C) which was sufficiently pure for further use. [H NMR ($CDCl_3$) δ 10.83 (1H, s), 8.55 (1H, s), 8.10 (1H, d, J = 8.8 Hz), 7.99 (1H, dd, J = 1.7, 9.0 Hz), 7.85 (1H, d, J = 8.8 Hz), 7.28 (1H, dd, J = 1.7, 9.0 Hz), 3.89 (3H, s), 1.61 (9H, s). FABMS ($^+$VE) m/z 303.2 $(M + H)^+$.Anal. ($C_{17}H_{18}O_5$) C, H.] Crude **23** was dissolved in DMF (10 mL), and to this solution were added K_2CO_3 (346 mg, 2.5 mmol) and *tert*-butyl bromoacetate (742 mg, 3.8 mmol), and the reaction mixture was stirred at room temperature overnight. To the mixture was added acetone (30 mL), and precipitated solid was filtered off. The filtrate was evaporated, and the resulting residue was purified by silica gel flash chromatography to yield **24** as a white solid (271 mg, 63% yield): mp 67−68 °C; H NMR ($CDCl_3$) δ 8.64 (1H, d, J = 1.7 Hz), 8.24 (1H, d, J = 8.8 Hz), 8.03 (1H, dd, J = 1.7, 8.8 Hz), 7.74 (1H, d, J = 8.8 Hz), 7.47 (1H, d, J = 9.0 Hz), 4.89 (2H, s), 3.91 (3H, s), 1.60 (9H, s), 1.43 (9H, s). FABMS ($^+$VE) m/z 416.2 $(M + H)^+$. Anal. ($C_{23}H_{28}O_7$) C, H.

2-{[(*tert*-Butyl)oxycarbonyl]methoxy}naphthalene-1,6-dicarboxylic Acid (25). To an ice-cold solution of **24** (238 mg, 0.55 mmol) in THF (14 mL) was added aqueous LiOH·H_2O (0.2 M, 8.25 mL, 1.65 mmol), and the mixture was allowed to warm to room temperature and stirred (15 h). The solution was diluted with EtOAc (35 mL) and washed with ice-cold 0.2 N HCl brine (35 mL), and the aqueous phase subjected to an extractive workup, dried (Na_2SO_4), and concentrated to afford **25** (203 mg, 100% yield): H NMR (DMSO-d_6) δ 8.65 (1H, d, J = 1.5 Hz), 8.26 (1H, d, J = 9.3 Hz), 8.07 (1H, dd, J = 1.7, 8.8 Hz), 7,77 (1H, d, J = 8.8 Hz), 7.53 (1H, d, J = 9.3 Hz), 4.96 (2H, s), 1.65 (9H, s).

Ethyl 2-[6-(Methoxycarbonyl)(2-naphthyloxy)]acetate (26). To a solution of methyl 6-hydroxy-2-naphthoate (**15**) (600 mg, 3.0 mmol) in anhydrous DMF (20 mL) was added K_2CO_3 (1.0 g, 7.5 mmol) followed by ethyl bromoacetate (1.8 mL, 11.4 mmol), and the mixture was stirred with heating (60−70 °C, overnight). After cooling to room temperature, the mixture was diluted with EtOAc (300 mL), the resulting white precipitate was filtered off, and the residual solution was washed with brine and dried (Na_2SO_4). Evaporation to dryness and purification by silica gel chromatography (EtOAc:hexane) provided **26** as a white solid (584 mg, 68% yield): mp 76−79 °C; H NMR ($CDCl_3$) δ 8.52 (1H, s), 8.01 (1H, s, J = 8.6 Hz), 7.85 (1H, d, J = 8.9 Hz), 7.72 (1H, d, J = 8.6 Hz), 7.29−7.24 (1H, m), 7.07 (1H, s), 4.74 (2H, s), 4.28 (2H, t, J = 7.1 Hz), 3.94 (3H, s), 1.29 (3H, q). FABMS ($^+$VE) m/z 288.1 $(M + H)^+$. Anal. ($C_{16}H_{16}O_5 \cdot {}^3/_4H_2O$) C, H.

Ethyl 2,2-Difluoro-2-[6-(methoxycarbonyl)(2-naphthyloxy)]acetate (27). Reaction of methyl 6-hydroxy-2-naphthoate (**15**) as described for the synthesis of **26**, with the use of ethyl bromodifluoroacetate instead of ethyl bromoacetate, provided **27** as a white solid in 40% yield: mp 216.5−217.5 °C; H NMR ($CDCl_3$) δ 8.58 (1H, s), 8.08(1H, dd, J = 8.6, 1.6 Hz), 7.95 (1H, d, J = 8.9 Hz), 7.84 (1H, d, J = 8.6 Hz), 7.68 (1H, s), 7.4 (1H, dd, J = 8.9, 2.2 Hz), 4.4 (2H, s), 3.97 (3H, s), 1.35 (3H, q). FABMS ($^+$VE) m/z 324 $(M + H)^+$. Anal. ($C_{16}H_{14}O_5F_2 \cdot {}^5/_4H_2O$) C, H.

6-(Carboxymethoxy)naphthalene-2-carboxylic Acid (28). A mixture of **26** (115 mg, 0.4 mmol) and LiOH·H_2O (80 mg, 2.0 mmol) in H_2O (15 mL) was stirred at room temperature (overnight). The mixture was adjusted with 3 N HCl to ∼pH 2, then MeOH was removed under reduced pressure, and residue was subjected to an extractive workup (EtOAc). Evaporation of solvent provided **28** as a white solid (95 mg, 97% yield): mp 306−309 °C; H NMR (DMSO-d_6) δ 12.95 (1H, br s), 8.52 (1H, s), 8.04 (1H, d, J = 8.9 Hz), 7.90−7.87 (2H, m), 7.35−7.26 (m, 2H), 4.84 (2H, s). FABMS ($^-$VE) m/z 245 $(M - H)^-$. Anal. ($C_{13}H_{10}O_5 \cdot {}^1/_4H_2O$) C, H.

6-(Carboxydifluoromethoxy)naphthalene-2-carboxylic Acid (29). Treatment of **27** as described above for the preparation of **28** provided product **29** as a white solid in 95% yield: mp > 360 °C; H NMR (CD_3OD) δ 6.95 (1H, s), 6.51 (1H, d, J = 8.6 Hz), 6.38 (1H, d, J = 9.0 Hz), 6.24 (1H, d, J = 8.6 Hz), 6.13 (1H, s), 5.84 (1H, dd, J = 8.9, 1.8 Hz). FABMS ($^-$VE) m/z 281 $(M - H^-)$. HR-FABMS calcd for $C_{13}H_8O_5F_2$: 281.0273 $(M - H)$. Found: 281.0262.

Methyl 5-Formyl-6-methoxy-2-naphthoate (31). Formylation of methyl 6-methoxy-2-naphthoate (**30**) by a procedure similar to that employed for the conversion of compound **19** to **20** provided product **31** in 54% yield. H NMR ($CDCl_3$) δ 10.90 (1H, s), 9.23 (1H, d, J = 9.0 Hz), 8.64 (1H, d, J = 1.7 Hz), 8.19 (2H, d, J = 9.0 Hz), 7.39 (1H, d, J = 9.3 Hz), 4.11 (3H, s), 3.99 (3H, s). FABMS ($^+$VE) m/z 245 $(M + H)^+$.

2-Methoxy-6-(methoxycarbonyl)naphthalenecarboxylic Acid (32). Treatment of **31** in a manner similar to that used to prepare compound **21** from **20** yielded product **32** in quantitative yield. H NMR ($CDCl_3$) δ 8.58 (1H, d, J = 1.7 Hz), 8.44 (1H, d, J = 9.0 Hz), 8.14 (1H, dd, J = 2.0, 9.0 Hz), 8.11 (1H, d, J = 9.3 Hz), 7.49 (1H, d, J = 9.3 Hz), 4.12 (3H, s), 3.99 (3H, s). FABMS ($^-$VE) m/z 259 $(M - H)^-$. HR-FABMS calcd for $C_{14}H_{11}O_5$ $(M - H)$: 259.0606. Found: 259.0605.

2-Methoxynaphthalene-1,6-dicarboxylic Acid (33). Treatment of a solution of **32** in in EtOH:H_2O (1:1) with excess sodium hydroxide, followed by evaporation of EtOH and acidification with aqueous HCl (1.0 N), provided **33** as a white precipiate in quantitative yield. H NMR (DMSO-d_6) δ 13.11 (2H, s, br), 8.61 (1H, d, J = 1.5 Hz), 8.25 (1H, d, J = 9.0 Hz), 8.00 (1H, dd, J = 1.7, 8.8 Hz), 7.75 (1H, d, J = 8.8 Hz), 7.60 (1H, d, J = 9.3 Hz). 3.96 (3H, s). FABMS ($^-$VE) m/z 245 $(M - H)^-$. HR-FABMS calcd for $C_{13}H_9O_5$ $(M - H)$: 245.0450. Found: 245.0469.

2-Hydroxynaphthalene-1,6-dicarboxylic Acid (34). Treatment of a suspension of **33** in anhydrous CH_2Cl_2 with a solution of BBr_3 in CH_2Cl_2 (1.0 M) at −78 °C and stirring at room temperature (overnight), followed by pouring into ice-cold H_2O, provided a precipitated solid which was collected by filtration. Crystallization from acetonitrile−H_2O gave **34** in quantitative yield. H NMR (DMSO-d_6) δ 8.53 (1H, d, J = 1.5 Hz), 8.42 (1H, d, J = 9.0 Hz), 8.17 (1H, d, J = 9.0 Hz), 8.01 (1H, dd, J = 1.7, 9.0 Hz), 7.27 (1H, d, J = 9.0 Hz). FABMS ($^-$VE) m/z 231 $(M - H)^-$. HR-FABMS calcd for $C_{12}H_7O_5$ (M − H): 231.0293. Found: 231.0316.

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