Acc. Chem. Res. **2003,** *36,* 426−433

Phosphotyrosyl Mimetics in the Development of Signal Transduction Inhibitors

TERRENCE R. BURKE, JR.* AND KYEONG LEE

Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, National Institutes of Health, NCI-Frederick, Frederick, Maryland

Received October 21, 2002

ABSTRACT

Phosphotyrosyl (pTyr) residues play important roles in cellular signal transduction by facilitating recognition and binding necessary for critical protein−protein interactions, and for this reason pTyr motifs represent attractive starting points in the development of signaling antagonists. Although the pTyr phosphoryl moiety is central in these phenomena, its incorporation into signaling inhibitors is contraindicated due to enzymatic lability and limited bioavailabilty associated with phosphate esters. To address these limiations, an entire field of study has arisen devoted to the design and utilization of pTyr mimetics. This Account provides a perspective on the roles of pTyr residues in signal transduction and approaches to pTyr mimetic development.

Introduction

The cytoplasmic domains of many cytokine and growth factor receptors are protein−tyrosine kinases (PTKs) that function by phosphorylating intracellular substrates in response to extracellular ligand binding (Figure 1).[1] This occurs by direct transfer of γ-phosphate from ATP to tyrosyl 4′-hydroxyls, resulting in the creation of phosphotyrosyl residues (pTyr **1**) within the substrate proteins (Figure 2A). Once formed, pTyr residues can have profound physiological effects that include alteration of enzyme activity and presentation of critical functionality needed for recognition/association by pTyr-binding modules that lead to the formation of multiprotein complexes, which results in further signal propagation. Among these latter pTyr-binding proteins are Src homology 2 (SH2) domains and phosphotyrosyl-binding (PTB) domains (Figure 1).[2−4] The third leg of this "PTK signaling triad" is served by protein−tyrosine phosphatases (PTPs) that remove pTyr phosphoryl groups and return tyrosyl residues to their nonphosphorylated states (Figure 1). Although PTPs often function as down-regulators of PTK-dependent pathways, they can serve in positive signaling roles by neutralizing inhibitory pTyr residues,[5] and their inappropriate activation can contribute to a variety of diseases, including several cancers.[6] Accordingly, development of PTK-dependent signaling antagonists has been an important area of investigation.[7−9]

Efforts to develop intracellular PTK-dependent signaling inhibitors can be categorized into three distinct categories that are predicated on the signaling triad outlined above: (1) PTK-catalytic site-directed inhibitors; (2) antagonists of pTyr-dependent binding phenomena, and (3) inhibitors of PTP activity. The central and defining roles that pTyr residues play in PTK-dependent signaling make the structure of pTyr itself a starting point for design of inhibitors directed at all three branches of the PTK signaling triad.[10−12] Since the roles served by pTyr residues are distinct for each of the three signaling branches, the importance of "pTyr motifs" and the manner in which they can be utilized for inhibitor development vary. Primarily utilizing work from our own laboratory, this Account will point out aspects of the pTyr structure that are important for its physiological actions and highlight ways in which these have been utilized for inhibitor design.

PTK Catalytic Site-Directed Agents

PTK-catalyzed transfer of phosphate is bisubstrate, with ATP serving as phosphoryl donor to Tyr-containing peptide acceptors. Accordingly, "pTyr residues" are end products and not substrates. Therefore, the utility of pTyr motifs in PTK catalytic site-directed inhibitor design is diminished relative to SH2/PTB domains and PTPs, where pTyr residues provide critical elements of ligand or substrate recognition. In fact, the most successful tyrosine kinase inhibitors have proven to be ATP-competitive in nature.[13,14] However, efforts to develop PTK catalytic site-directed inhibitors that incorporate phosphoryl elements onto tyrosyl-like structures have also been reported. As seen in Figure 2B, such inhibitors may mimic various points in the catalytic process of phosphoryl transfer. Analogues, such as **2** having formal ATP-like moieties tethered to Tyr equivalents,[15] are intended as bisubstrate inhibitors. Others containing abbreviated phosphoryl-mimicking groups, such as **3**,[16] **4**,[17] and **5**,[18] may be viewed either as transition-state analogues or as end-product inhibitors. Although some of these are highly potent in extracellular kinase assays,[16] the use of pTyr mimetics in the design PTK catalytic site-directed inhibitors has not been extensively pursued.[19,20]

Structural Features of pTyr Residues Related to Mimetic Design

In contrast, pTyr-motifs have been central in the development of antagonists directed at other components of PTK signaling, where pTyr residues afford a rich variety of features for potential inhibitor design. Importantly,

Terrence Burke was born and raised in the Pacific Northwest and received his Ph.D. in 1978 from the University of Washington, Seattle, under the direction of Professor Wendel Nelson. In 1979 he began postdoctoral studies at the National Institutes of Health, Bethesda, MD, and joined the Laboratory of Medicinal Chemistry, NCI, as a tenured principal investigator in 1989.

Kyeong Lee received her B. S. in 1993 from Ewha Womans University, Seoul, Korea, prior to obtaining her Ph.D. from the University of Georgia, Athens, under the direction of Professor Chung K. Chu. Dr. Lee is currently a Visiting Fellow in the Laboratory of Medicinal Chemistry, NCI.

10.1021/ar020127o
Published on Web 03/08/2003

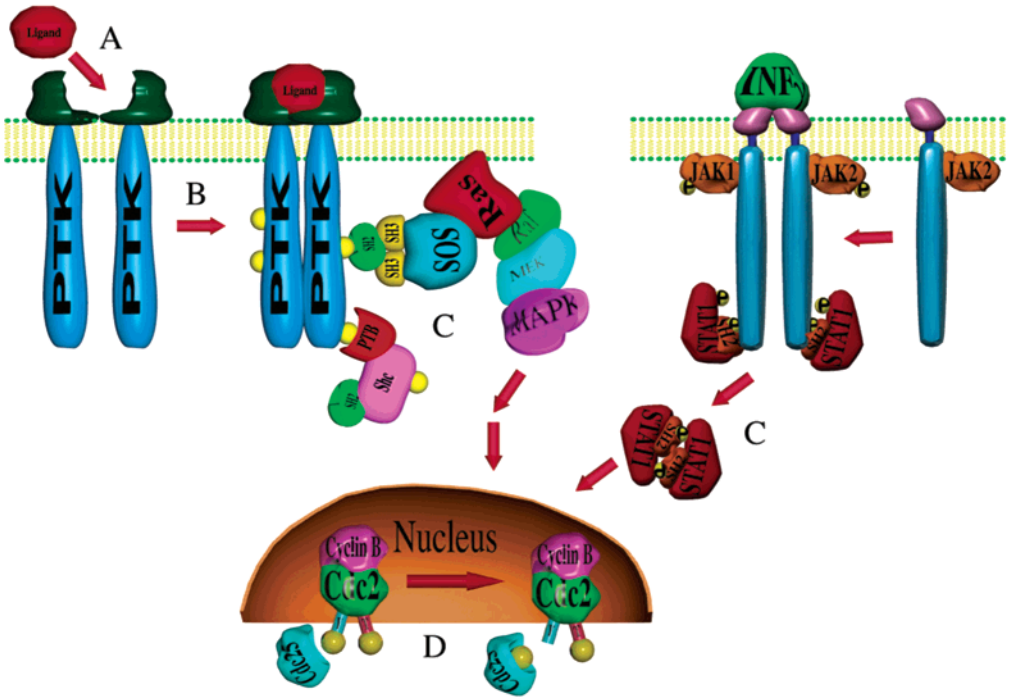


FIGURE 1. The role of pTyr residues in PTK-dependent signaling. (A) Binding of growth factors/cytokines to PTK extracellular receptors; (B) generation of intracellular pTyr residues in response to extracellular ligand binding; (C) pTyr-dependent assembly of multicomponent signaling complexes resulting in signal propagation; (D) an example of a PTP serving a positive signaling role by hydrolysis of an inhibitory tyrosyl phosphate.

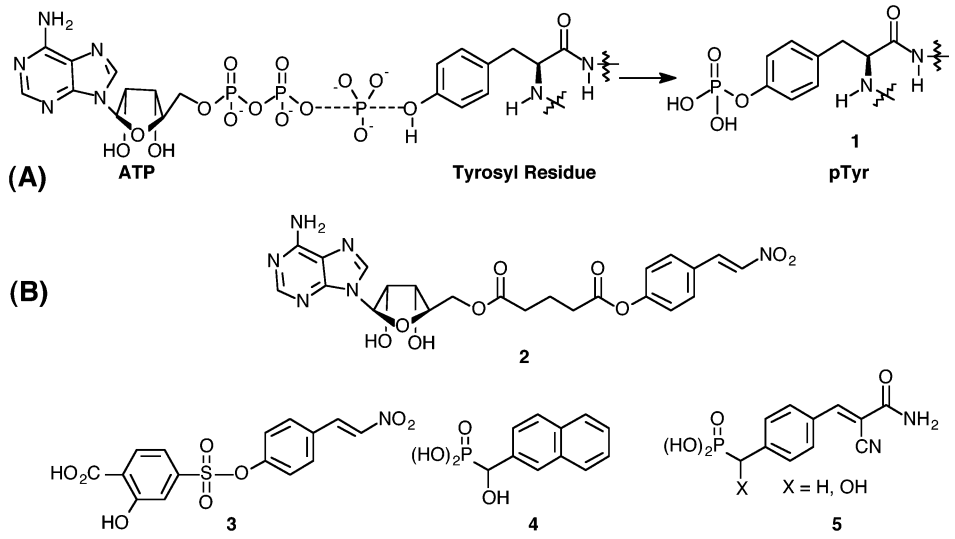


FIGURE 2. (A) PTK-mediated phosphoryl transfer between ATP and a tyrosyl residue with release of a pTyr-containing product. (B) Examples of PTK catalytic site-directed inhibitors that contain phosphate-mimicking functionality.

pTyr residues present a defining phosphoryl group that provides an array of geometrically spaced heteroatoms bearing a net (−2) charge at physiological pH (Figure 2). Binding affinity is also dependent on other features of the pTyr residue as well as amino acid residues surrounding the pTyr residue. It is the combination of a pTyr residue within the proper peptide sequence that can achieve high affinity. The availability of X-ray crystal structures of SH2 domains, PTB domains, and PTPs in complex with pTyr-bearing peptides has clarified the roles these various structural features play in binding interactions.

pTyr-SH2 Domain Interactions

SH2 domains represent a collection of modular signaling proteins that can be broadly classified into four major groups,[21] all of which share a high degree of structural homology. Subtle variations among subfamilies of SH2 domains result in differential affinity for pTyr-containing ligands, depending on the amino acid sequence proximal to the pTyr residue.[22] Although SH2 domains mediate protein−protein interactions, small pTyr-containing peptides based on the proximal amino acid sequences surrounding target pTyr residues found in full-length proteins

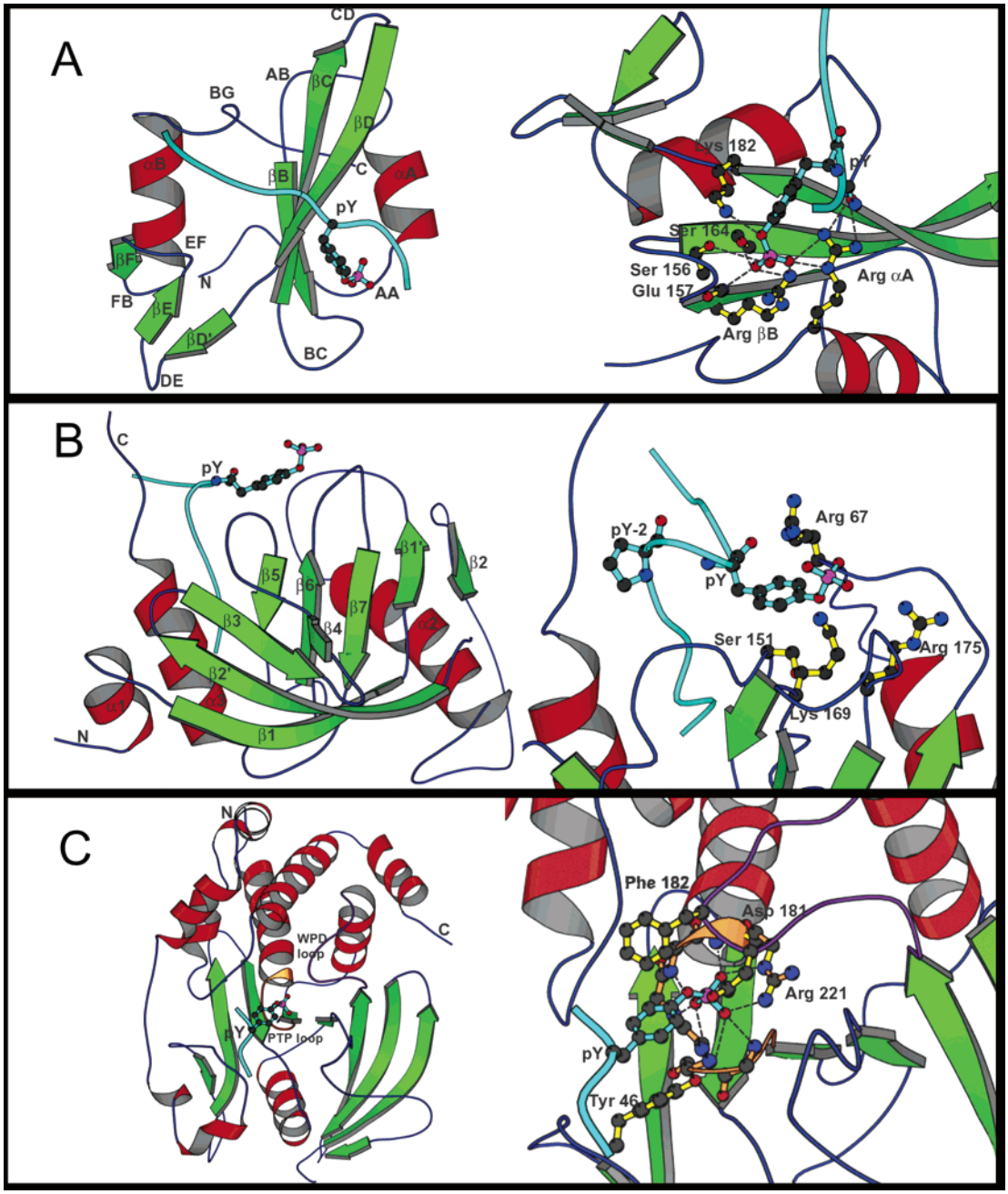


FIGURE 3. Comparison of three pTyr-binding proteins in complex with pTyr-containing ligands. (A) The p56^{lck} SH2 domain;[23] (B) the Shc PTB domain;[34] (C) a PTP-1B in its Cys215Ser form.[38] The right side of each panel highlights the pTyr binding pocket.

are able to effectively bind to SH2 domains and block their interactions with cognate pTyr-containing proteins. For this reason, short pTyr-containing peptides can serve as starting points for inhibitor development. Crystal structures of SH2 domains, including SH2 domains in complex with pTyr-containing peptides, were available early on, and a general system of nomenclature was devised to describe the common topographical features of SH2 domains (Figure 3A).[23] Binding of pTyr-containing ligands normally occurs with the pTyr residue held in a pocket bounded by the αA helix, the three antiparallel βB−βD sheets and the BC loop where the pTyr phosphoryl group interacts with two positively charged Arg residues, αA2 and βB5. The Arg αA2 residue is situated above the plane of the pTyr aryl ring where it serves a dual role by partially stabilizing the phosphoryl negative charge and undergoing aryl π-cation stabilization[24] as well as interacting with functionality originating from the pTyr α-amino group. Proximal to the pTyr-binding pocket in an area normally situated between the EF and BG loops are regions of the SH2 domain that interact with ligand residues C-terminal to the pTyr residue. (A notable exception is found with Grb2 SH2 domains.[25]) The principal function of these sites is to discriminate ligands On the basis of amino acid side chain functionality C-proximal to the pTyr residue. Over 50% of the free energy of the highest affinity interaction is derived from the pTyr residue itself, with approximately 25% of this being attributable to at least one negative

charge on the phosphoryl group.[26] However, secondary binding interactions provided by residues other than pTyr are critical, since monomeric pTyr exhibits extremely poor affinity.[27] Therefore, development of SH2 domain signaling antagonists has focused both on high-affinity pTyr mimetics as well as suitable display platforms.[28−30]

pTyr-PTB Domain Interactions

PTB domains differ from SH2 domains by recognizing ligands according to amino acid sequence on the N-terminal side of the pTyr residue, rather than the C-terminal side.[31−33] As exemplified by the NMR solution structure of the Shc PTB domain in complex with a NPQpY ligand,[34] binding of pTyr-containing ligand occurs in a β-bend conformation that is facilitated by its pY-2 Pro residue (Figure 3B). The pTyr "binding pocket" contains two Arg residues (Arg67 and Arg175) that correspond to the ArgαA and Arg βB of SH2 domains,[34] similar to what is observed for SH2 domains.[35]

pTyr-PTP Interactions

In addition to SH2 domains and PTB domains, PTPs are a third category of signaling protein that recognize and bind peptides in pTyr-dependent fashion. Although the principal interaction of most PTPs with pTyr-containing polypeptides is through catalytic domains in which they are recognized as substrates rather than ligands, PTPs are capable of binding pTyr-containing peptides as ligands. This could occur either through inactive catalytic domains such as "STYX" domains,[36] or through other special pTyr-binding domains as exemplified by the N-terminal region of the *Yersinia pestis* PTP (YopH) that interacts with pTyr-containing peptides in a manner somewhat similar to SH2 domains.[37] However, the principal approach toward PTP inhibitor development is through agents directed at the catalytic site, which is defined by the "signature motif" sequence (H/V)**C**(X5)**R**(S/T) that forms a semicircular structure about the tyrosyl phosphate group (Figure 3C).[38] This arrangement allows precise positioning and stabilization of the negatively charged phosphoryl group both by a network of hydrogen bonds and by a formal ionic bond to the signature motif Arg residue. Nucleophilic attack onto the phosphoryl moiety by the signature motif Cys thiolate anion results in formation of a thiophosphoryl intermediate and release of free tyrosyl product. Water-catalyzed hydrolysis of the thiophosphate species regenerates the active enzyme. The highly specialized manner in which pTyr residues are recognized by PTPs has rendered pTyr mimetics of particular value in development of PTP inhibitors.[12,39,40]

Phosphorus-Containing Phosphoryl Replacements

The importance of phosphoryl functionality in pTyr-dependent signaling is derived from its ability to present a system of suitably charged heteroatoms (oxygens) within a three-dimensional geometry that is appropriate for interaction with both Arg residues and hydrogen bond donor/acceptor side chains of acceptor proteins. Phosphoryl groups are potentially unsuitable for use in inhibitors due to (1) hydrolytic lability of the phosphate ester bond in the presence of cellular phosphatases and (2) hindrance of cell membrane transit caused by a (−2) charge. While the first drawback has been readily addressed by phosphonate-based species, the second area has been less satisfactorily overcome. In the current Account, presentation of phosphoryl mimetics will be categorized into phosphorus-based and carboxy-based analogues. Due to space constraints, the Account will not cover phosphoryl mimetics based on other heteroatoms; nor will it cover partial pTyr mimetics (that could more aptly be described as "phenyl phosphate" surrogates).

The most direct way to prevent phosphoryl hydrolysis is through replacement of the phosphate ester linkage by an enzymatically stable isostere. Preparation of phosphonomethyl phenylalanine (Pmp, **6**),[41] which has the bridging oxygen unit replaced by a methylene, in a form bearing bis-(*tert*-butyl) phosphonate and N-Fmoc protection (**7**),[42] allowed its incorporation into an PI-3 kinase C-terminal SH2 domain-directed peptide.[43] While being stable to phosphatases, the resulting peptide exhibited an approximate 5-fold reduction in SH2 domain-binding affinity relative to the parent pTyr-containing peptide.[44] This loss of affinity was attributed to a combination of elevated phosphonate pK_a resulting in a net reduction in formal negative charge (approximately −1.5 at pH 7) relative to the (−2) charge of the parent phosphate and loss of hydrogen bonding interactions normally afforded by the phosphoryl ester oxygen atom. It was known that incorporation of α-fluorines onto aliphatic phosphonates enhances phosphate-mimicking efficacy.[45,46] Therefore, a synthetic approach to benzylic α,α-difluorophosphonates was developed using (diethylamino)sulfur trifluoride (DAST)-mediated fluorination of ketophosphonates,[47] and this was applied to the synthesis of α,α-difluoroPmp (F_2-Pmp, **8**) in a form bearing *tert*-butyl and Fmoc protection of phosphonate and amino functionality, respectively (**9**).[48] Similarly protected analogues bearing monofluoro (FPmp, **10**) and hydroxyl (HPmp, **11**) were also prepared and incorporated in peptides directed at Src, PI-3 kinase, and Grb2 SH2 domains.[48] Against the C-terminal PI-3 kinase SH2 domain, the FPmp-containing peptide regained approximately 50% of the loss in affinity originally observed in replacing pTyr with Pmp, while the F_2Pmp-containing peptide regained all lost affinity and was equal in potency to parent pTyr.[49] These results supported the contention that pK_a values are important for SH2 domain-binding potency, since model studies using benzylic phosphonates indicated that pK_{a2} values were lowered by approximately 1 unit per α-fluorine added,[47] with the F_2Pmp being completely ionized at pH 7 in a manner similar to pTyr. It was also observed that effects of fluorination on binding varied among SH2 domains, with F_2Pmp being equipotent to pTyr against the C-terminal PI-3 kinase SH2 domain, of greater potency against the Src SH2 domain, and of reduced potency against the Grb2 SH2 domain.[49] This

demonstrated for the first time that interactions within the pTyr-binding pocket afforded by structural variations in pTyr mimetics could potentially afford a means of enhancing selectivity among SH2 domain families.

The ability of α-fluoro substituents to enhance binding potency of Pmp-containing peptides is observed for pTyr-binding proteins other than SH2 domains. Substitution of pTyr with Pmp in a Shc PTB-directed peptide sequence resulted in a 25-fold loss of binding affinity, while substitution with F_2Pmp provided a binding affinity equivalent to the parent pTyr-containing peptide.[50] Alternatively, the insulin receptor substrate-1 (IRS-1) PTB domain exhibited only poor affinity for F_2Pmp-containing peptide, which was attributed to the loss of hydrogen bonding interactions with the difluoromethylene unit that would normally be present with the pTyr phosphate ester oxygen.[50] This provides another example where alterations in the pTyr mimetic can affect binding selectivity.

In the above SH2 domain and PTB domain examples, F_2Pmp serves as a phosphatase-stable pTyr replacement that provides from 5-fold to 25-fold binding enhancement relative to Pmp. For PTPs, where pTyr residues also provide key elements of binding affinity, F_2Pmp can exhibit an approximate 1000-fold enhancement in affinity relative to Pmp.[51] The basis for the exceptional increase in PTP affinity is not fully understood, although kinetic studies have shown that it is most probably not due to phosphonate pK_a effects.[52] X-ray crystal structures of naphthyl diflouromethylphosphonic acids bound to PTP-1B have indicated a potential role for H-bonding interactions of the protein with one specific fluorine atom of the CF_2 unit.[53,54] In support of the hypothesis that binding enhancement is due preferentially to interactions of one fluorine atom, model studies with enantiopure aryl monofluoromethylphosphonic acids have indicated a 10-fold difference in affinity depending on chirality at the α-fluoromethylene center.[55] The exceptional affinity of F_2Pmp when used as a pTyr mimetic and of aryl difluoromethylphosphonic acids in general has led to their widespread use as structural motifs in PTP and SH2 domain antagonist development.[12,39] As a result, several synthesis of F_2Pmp have been reported in both its racemic[48,56] and L-forms.[57−60]

SH2 domain binding of pTyr phosphoryl groups have been shown by X-ray crystallography to involve important interactions between the two anionic acidic oxygen atoms as well as by the ester oxygen which bridges the phosphorus to the aryl ring. The ester oxygen both positions the phosphate group at a distance from the aryl ring appropriate for interaction with these Arg residues as well as engaging in hydrogen bonding interactions itself. For these reasons, considerable effort has been devoted to its suitable replacement. However, complete deletion of bridging functionality by direct attachment of the phosphorus to the aryl ring provides compound **12** (Figure 4), which maintains good SH2 domain-binding affinity.[61] In a similar fashion, removal of one anionic acidic hydroxyl to give analogues such as **13** has been achieved without significant loss of binding potency.[62] In this latter example, loss of binding interactions normally provided by the

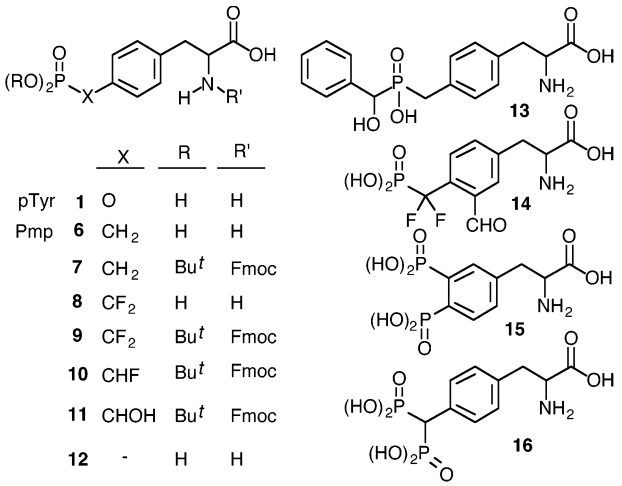
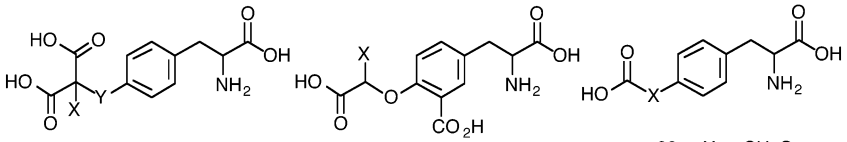


FIGURE 4. Structures of phosphorus-containing pTyr mimetics.

second acidic oxygen species is theoretically compensated for by new aromatic-cation interactions between the added phenyl ring and the αA2 Arg residue. In contrast to these examples, where removal of oxygen functionality has been examined, highly potent SH2 domain-binding ligands have also been prepared from pTyr mimetics through appendage of additional oxygen-containing groups. These include analogues **14**[63] and **15**,[64] which introduce substituents at the Phe 3-position, and analogue **16**.[65]

Carboxy-Based Phosphoryl Replacements

While phosphonic acid-based pTyr mimetics adequately address the issue of lability to phosphatases, issues of membrane transport still remain (Figure 5). Prodrug approaches to both Pmp[66] and F_2Pmp[61] have been examined as potential solutions to this latter problem. An alternative approach has been development of non-phosphorus-containing pTyr mimetics that utilize carboxylic acid groups to replicate phosphate functionality. Among early examples of this are **17** (OMT)[67,68] and **18** (FOMT)[69] that contain two geminal carboxylic acids intended to replicate the dianionic arrangement of parent pTyr. While **17** and **18** exhibit good binding to both SH2 domains and PTPs, selective enhancement of SH2 domain binding affinity relative to PTP affinity is achieved with **19**,[70] which is derived from OMT (**17**) by deletion of the ether oxygen.[71,72] Translocation of one carboxyl of OMT (**17**) from the geminal position to the aryl 3-position gave analogue **20**,[73] which exhibits potent affinity against PTP-1B[73,74] and the Grb2 SH2 domain.[75] Addition of a 3-aryl carboxyl group with maintenance of the original bis-geminal carboxyl arrangement of **19** provided tricarboxylic-based **21**, which displayed high SH2 domain-binding affinity.[76] Alternatively, reduction in the number of carboxyl groups by removing one carboxyl from OMT (**17**) to provide **22** resulted in poor affinity in both PTP[73] and SH2 domain-binding systems.[77] However, elimination of the ether oxygen from **22** gives carboxymethyl phenylalanine **23**, which shows good SH2 domain-binding affinity.[78] The enhanced SH2 domain-binding affinity of

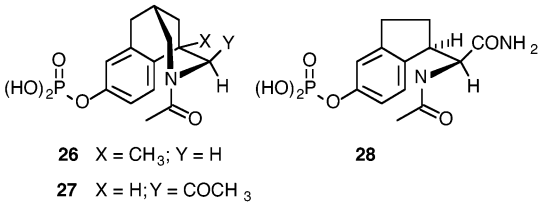


OMT **17** X = H; Y = O
FOMT **18** X = F; Y = O
19 X = H; Y = -

20 X = H
21 X = CO_2H

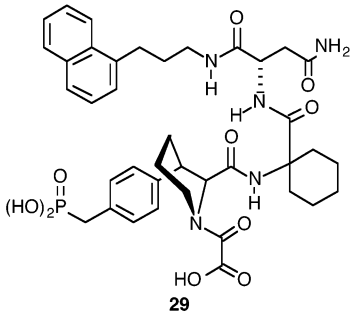
22 X = -CH_2O-
23 X = -CH_2-
24 X = -CHOH-
25 X = -CF_2-

FIGURE 5. Structures of pTyr mimetics containing carboxy-based phosphoryl replacements.

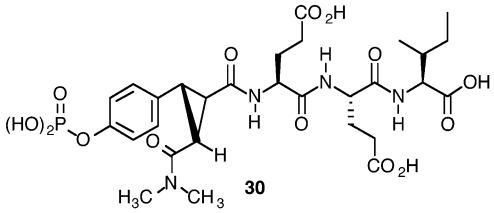


26 X = CH_3; Y = H
27 X = H; Y = $COCH_3$

28



29



30

FIGURE 6. Conformationally constrained pTyr mimetics.

23 relative to **21** can be understood based on a closer overlap in **23** of the carboxylic functionality with the pTyr phosphate oxygens than is observed with **22**,[78] where the ether oxygen extends the carboxylic group too far from the phenyl ring.[77] Addition of a hydroxyl group onto the α-methylene of **23** to provide **24** enhances SH2 domain-binding affinity;[79] however, introduction of fluorines at this center[80] (compound **25**) reduces affinity.[77] Finally, as described previously, the relative affinities of pTyr mimetics are highly dependent on the binding protein. In the case of monocarboxy pTyr mimetics where good affinity can be obtained in SH2 domain-binding systems, poor inhibition has been observed when examined against PTP-1B.[72]

Conformational Constraint in the Design of pTyr Mimetics

The importance of phosphoryl functionality to pTyr-dependent binding is reflected in its contribution to the enthalpy term in the free energy equation for binding (Figure 6).[26] This has resulted in a majority of pTyr mimetic-directed research being devoted to phosphoryl replacements. However, the free energy equation for binding also includes an adverse entropy component that arises when the highly flexible pTyr side chain goes from solution to bound conformations. One means of potentially reducing such penalties is to induce side chain conformational constraint in the pTyr residue. Monomeric ring-constrained pTyr mimetics **26**,[81] **27**,[82] and **28**,[81] as well as a Grb2 SH2 domain-directed peptide (**29**) that contains a conformationally constrained pipecolic acid-based pTyr mimetic, were ineffective at enhancing SH2 domain affinity. Th recently reported Src SH2 domain-directed peptide **30** that contains a cyclopropyl-based pTyr mimetic[83] successfully reduced entropy binding penalties relative to parent pTyr-containing peptide; however, this was matched by a reduction in enthalpy, so that net binding affinity was largely unchanged.[84] Overall, induction of conformational constraint at the level of the pTyr residue has not proven to be a significant factor in pTyr mimetic mimetic development.

Conclusions

Tyrosine kinase-depenent signal is highly dependent on the recognition and utilization of the phosphotyrosyl pharmacophore. Because of this, as outlined in this account, the structure of pTyr provides a starting point for development of diverse signaling antagonists that may lead to new generations of therapeutic agents.

References

(1) Hunter, T. Signaling − 2000 and beyond. *Cell* **2000**, *100*, 113−127.
(2) Panayotou, G.; Waterfield, M. D. The assembly of signaling complexes by receptor tyrosine kinases. *Bioessays* **1993**, *15*, 171−177.
(3) Leof, E. B. Growth factor receptor signaling: location, location, location. *Trends Cell Biol.* **2000**, *10*, 343−348.
(4) Pawson, T.; Raina, M.; Nash, P. Interaction domains: from simple binding events to complex cellular behavior. *FEBS Lett.* **2002**, *513*, 2−10.
(5) Ostman, A.; Bohmer, F. D. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. *Trends Cell Biol.* **2001**, *11*, 258−266.
(6) Blume-Jensen, P.; Hunter, T. Oncogenic kinase signaling. *Nature* **2001**, *411*, 355−365.
(7) Cohen, P. The development and therapeutic potential of protein kinase inhibitors. *Curr. Opin. Chem. Biol.* **1999**, *3*, 459−465.
(8) Levitzki, A. Protein tyrosine kinase inhibitors as therapeutic agents. *Top. Curr. Chem.* **2001**, *211*, 1−15.
(9) Shawver, L. K.; Slamon, D.; Ullrich, A. Smart drugs: Tyrosine kinase inhibitors in cancer therapy. *Cancer Cell* **2002**, *1*, 117−123.
(10) Burke, T. R., Jr.; Yao, Z.-J.; Smyth, M. S.; Ye, B. Phosphotyrosyl-based motifs in the structure-based design of protein-tyrosine kinase-dependent signal transduction inhibitors. *Curr. Pharm. Des.* **1997**, *3*, 291−304.
(11) Burke, T. R., Jr.; Gao, Y.; Yao, Z.-J. Phosphoryltyrosyl mimetics as signaling modulators and potential antitumor agents. *Biomedical Chemistry: Applying Chemical Principles to the Understanding and Treatment of Disease*, 1st ed.; John Wiley & Sons: New York, 2000; pp 189−210.

(12) Burke, T. R., Jr.; Yao, Z.-J.; Liu, D.-G.; Voigt, J.; Gao, Y. Phosphoryltyrosyl mimetics in the design of peptide-based signal transduction inhibitors. *Biopolymers* **2001**, *60*, 32−44.
(13) Morin, M. J. From oncogene to drug: Development of small molecule tyrosine kinase inhibitors as antitumor and antiangiogenic agents. *Oncogene* **2000**, *19*, 6574−6583.
(14) Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; O'Reilly, T.; Wood, J.; Zimmermann, J. Tyrosine kinase inhibitors: From rational design to clinical trials. *Med. Res. Rev.* **2001**, *21*, 499−512.
(15) Peterli, S.; Hubmann, D.; Sequin, U.; Mett, H.; Traxler, P. Nitrostyrene derivatives of adenosine 5′-glutarates modified with an alkyl spacer and their inhibitory activity on epidermal growth factor receptor protein tyrosine kinase. *Helv. Chim. Acta* **1994**, *77*, 59−69.
(16) Traxler, P. M.; Wacker, O.; Bach, H. L.; Geissler, J. F.; Kump, W.; Meyer, T.; Regenass, U.; Roesel, J. L.; Lydon, N. Sulfonylbenzoyl nitrostyrenes − potential bisubstrate type inhibitors of the EGF-receptor tyrosine protein kinase. *J. Med. Chem.* **1991**, *34*, 2328−2337.
(17) Saperstein, R.; Vicario, P. P.; Strout, H. V.; Brady, E.; Slater, E. E.; Greenlee, W. J.; Ondeyka, D. L.; Patchett, A. A.; Hangauer, D. G. Design of a selective insulin receptor tyrosine kinase inhibitor and its effect on glucose uptake and metabolism in intact cells. *Biochemistry* **1989**, *28*, 5694−5701.
(18) Burke, T. R., Jr.; Li, Z. H.; Bolen, J. B.; Marquez, V. E. Phosphonate-containing inhibitors of tyrosine-specific protein kinases. *J. Med. Chem.* **1991**, *34*, 1577−1581.
(19) Marsilje, T. H.; Milkiewicz, K. L.; Hangauer, D. G. The design, synthesis and activity of non-ATP competitive inhibitors of pp60-(C-src) tyrosine kinase. Part 1: Hydroxynaphthalene derivatives. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 477−481.
(20) Milkiewicz, K. L.; Marsilje, T. H.; Woodworth, R. P.; Bifulco, N.; Hangauer, M. J.; Hangauer, D. G. The design, synthesis and activity of non-ATP competitive inhibitors of pp60(C-src) tyrosine kinase. Part 2: Hydroxyindole derivatives. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 483−486.
(21) Songyang, Z. Recognition and regulation of primary-sequence motifs by signaling modular domains. *Prog. Biophys. Mol. Biol.* **1999**, *71*, 359−372.
(22) Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Haser, W. G.; King, F.; Roberts, T.; Ratnofsky, S.; Lechleider, R. J.; Neel, B. G.; Birge, R. B.; Fajardo, J. E.; Chou, M. M.; Hanafusa, H.; Schaffhausen, B.; Cantley, L. C. SH2 domains recognize specific phosphopeptide sequences. *Cell* **1993**, *72*, 767−778.
(23) Eck, M. J.; Shoelson, S. E.; Harrison, S. C. Recognition of a high-affinity phosphotyrosyl peptide by the Src homology-2 domain of p56(lck). *Nature* **1993**, *362*, 87−91.
(24) Umezawa, Y.; Nishio, M. CH/pi Interactions as demonstrated in the cyrstal structure of guanine-nucleotide binding proteins, Src homology 2 domains and human growth hormone in complex with their specific ligands. *Bioorg. Med. Chem.* **1998**, *6*, 493−504.
(25) Rahuel, J.; Gay, B.; Erdmann, D.; Strauss, A.; GarciaEcheverria, C.; Furet, P.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Grutter, M. G. Structural basis for specificity of Grb2-SH2 revealed by a novel ligand binding mode. *Nat. Struct. Biol.* **1996**, *3*, 586−589.
(26) Grucza, R. A.; Bradshaw, J. M.; Futterer, K.; Waksman, G. SH2 domains: From structure to energetics, a dual approach to the study of structure−function relationships. *Med. Res. Rev.* **1999**, *19*, 273−293.
(27) Lemmon, M. A.; Ladbury, J. E. Thermodynamic studies of tyrosyl-phosphopeptide binding to the SH2 domain of p56(Lck). *Biochemistry* **1994**, *33*, 5070−5076.
(28) Muller, G. Peptidomimetic SH2 domain antagonists for targeting signal transduction. *Top. Curr. Chem.* **2001**, *211*, 17−59.
(29) Garcia-Echeverria, C. Antagonists of the Src homology 2 (SH2) domains of Grb2, Src, Lck and ZAP-70. *Curr. Med. Chem.* **2001**, *8*, 1589−1604.
(30) Sawyer, T. K.; Bohacek, R. S.; Dalgarno, D. C.; Eyermann, C. J.; Kawahata, N.; Metcalf, C. A., III.; Shakespeare, W. C.; Sundaramoorthi, R.; Wang, Y.; Yang, M. G. Src homology-2 inhibitors: Peptidomimetic and nonpeptide. *Mini-Rev. Med. Chem.* **2002**, *2*, 475−489.
(31) Siegal, G. The surprisingly flexible PTB domain. *Nat. Struct. Biol.* **1999**, *6*, 7−10.
(32) Margolis, B. L. Function of PTB domain proteins. *FASEB J.* **1999**, *13*, A1422.
(33) Yan, K. S.; Kuti, M.; Zhou, M.-M. PTB or not PTB − that is the question. *FEBS Lett.* **2002**, *513*, 67−70.
(34) Zhou, M. M.; Ravichandran, K. S.; Olejniczak, E. T.; Petros, A. M.; Meadows, R. P.; Sattler, M.; Harlan, J. E.; Wade, W. S.; Burakoff, S. J.; Fesik, S. W. Structure and ligand recognition of the phosphotyrosine binding domain of Shc. *Nature* **1995**, *378*, 584−592.
(35) Bradshaw, J. M.; Mitaxov, V.; Waksman, G. Investigation of phosphotyrosine recognition by the SH2 domain of the Src kinase. *J. Mol. Biol.* **1999**, *293*, 971−985.
(36) Wishart, M. J.; Dixon, J. E. Gathering STYX: phosphatase like form predicts functions for unique protein-interaction domains. *Trends Biochem. Sci.* **1998**, *23*, 301−306.
(37) Khandelwal, P.; Keliikuli, K.; Smith, C. L.; Saper, M. A.; Zuiderweg, E. R. P. Solution structure and phosphopeptide binding to the N-terminal domain of Yersinia YopH: Comparison with a crystal structure. *Biochemistry* (in press).
(38) Barford, D.; Das, A. K.; Egloff, M. P. The structure and mechanism of protein phosphatases: Insights into catalysis and regulation. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 133−164.
(39) Burke, T. R.; Zhang, Z. Y. Protein-tyrosine phosphatases: Structure, mechanism, and inhibitor discovery. *Biopolymers* **1998**, *47*, 225−241.
(40) Zhang, Z.-Y. Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. *Annu. Rev. Pharm. Tox.* **2002**, *42*, 209−234.
(41) Marseigne, I.; Roques, B. P. Synthesis of new amino acids mimicking sulfated and phosphorylated tyrosine residues. *J. Org. Chem.* **1988**, *53*, 3621−3624.
(42) Burke, T. R., Jr.; Russ, P.; Lim, B. Preparation of 4-[bis(*tert*-butyl)-phosphonomethyl]-N-Fmoc-D,L-phenylalanine; a hydrolytically stable analogue of O-phosphotyrosine potentially suitable for peptide synthesis. *Synthesis* **1991**, *11*, 1019−1020.
(43) Shoelson, S. E.; Chatterjee, S.; Chaudhuri, M.; Burke, T. R. Solid-phase synthesis of nonhydrolyzable phosphotyrosyl peptide analogues with N(alpha)-Fmoc-(O,O-di-tert-butyl)phosphono-para-methylphenylalanine. *Tetrahedron Lett.* **1991**, *32*, 6061−6064.
(44) Domchek, S. M.; Auger, K. R.; Chatterjee, S.; Burke, T. R.; Shoelson, S. E. Inhibition of SH2 domain/phosphoprotein association by a nonhydrolyzable phosphonopeptide. *Biochemistry* **1992**, *31*, 9865−9870.
(45) Blackburn, G. M. Phosphonates as analogues of biological phosphates. *Chem. Ind. (London)* **1981**, 134−138.
(46) Blackburn, G. M.; Parratt, M. J. The synthesis of alpha-fluoroalkylphosphonates. *J. Chem. Soc., Chem. Commun.* **1983**, 886−888.
(47) Smyth, M. S.; Ford, H., Jr.; Burke, T. R., Jr. A general method for the preparation of benzylic alpha, alpha-difluorophosphonic acids; non-hydrolyzable mimetics of phosphotyrosine. *Tetrahedron Lett.* **1992**, *33*, 4137−4140.
(48) Burke, T. R.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Roller, P. P. Preparation of fluoro-4-(phosphonomethyl)-D,L-phenylalanine and hydroxy-4-(phosphonomethyl)-D,L-phenylalanine suitably protected for solid-phase synthesis of peptides containing hydrolytically stable analogues of O-phosphotyrosine. *J. Org. Chem.* **1993**, *58*, 1336−1340.
(49) Burke, T. R., Jr.; Smyth, M. S.; Otaka, A.; Nomizu, M.; Roller, P. P.; Wolf, G.; Case, R.; Shoelson, S. E. Nonhydrolyzable phosphotyrosyl mimetics for the preparation of phosphatase-resistant SH2 domain inhibitors. *Biochemistry* **1994**, *33*, 6490−6494.
(50) Giorgetti-Peraldi, S.; Ottinger, E.; Wolf, G.; Ye, B.; Burke, T. R., Jr.; Shoelson, S. E. Cellular effects of phosphotyrosine-binding domain inhibitors on insulin receptor signaling and trafficking. *Mol. Cell. Biol.* **1997**, *17*, 1180−1188.
(51) Burke, T. R.; Kole, H. K.; Roller, P. P. Potent inhibition of insulin receptor dephosphorylation by a hexamer peptide containing the phosphotyrosyl mimetic F(2)Pmp. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129−134.
(52) Chen, L.; Wu, L.; Otaka, A.; Smyth, M. S.; Roller, P. P.; Burke, T. R.; Denhertog, J.; Zhang, Z. Y. Why is phosphonodifluoromethyl phenylalanine a more potent inhibitory moiety than phosphonomethyl phenylalanine toward protein-tyrosine phosphatases? *Biochem. Biophys. Res. Commun.* **1995**, *216*, 976−984.
(53) Burke, T. R.; Ye, B.; Yan, X. J.; Wang, S. M.; Jia, Z. C.; Chen, L.; Zhang, Z. Y.; Barford, D. Small molecule interactions with protein-tyrosine phosphatase PTP1B and their use in inhibitor design. *Biochemistry* **1996**, *35*, 15989−15996.
(54) Groves, M. R.; Yao, Z. J.; Roller, P. P.; Burke, T. R.; Barford, D. Structural basis for inhibition of the protein tyrosine phosphatase 1B by phosphotyrosine peptide mimetics. *Biochemistry* **1998**, *37*, 17773−17783.
(55) Kotoris, C. C.; Wen, W.; Lough, A.; Taylor, S. D. Preparation of chiral alpha-monofluoroalkylphosphonic acids and their evaluation as inhibitors of protein tyrosine phosphatase 1B. *J. Chem. Soc., Perkin Trans. 1* **2000**, *8*, 1271−1281.

(56) Burke, T. R.; Smyth, M. S.; Otaka, A.; Roller, P. P. Synthesis of 4-phosphono(difluoromethyl)-D,L-phenylalanine and N-Boc and N-Fmoc derivatives suitably protected for solid-phase synthesis of nonhydrolyzable phosphotyrosyl peptide analogues. *Tetrahedron Lett.* **1993**, *34*, 4125−4128.
(57) Wrobel, J.; Dietrich, A. Preparation of L-(phosphonodifluoromethyl)phenylalanine derivatives as non-hydrolyzable mimetics of O− phosphotyrosine. *Tetrahedron Lett* **1993**, *34*, 3543−3546.
(58) Smyth, M. S.; Burke, T. R., Jr. Enantioselective synthesis of N−Boc and N−Fmoc protected diethyl 4-phosphonodifluoromethyl-L-phenylalanine; agents suitable for the solid-phase synthesis of peptides containing nonhydrolyzable analogues of O-phosphotyrosine. *Tetrahedron Lett.* **1994**, *35*, 551−554.
(59) Solas, D.; Hale, R. L.; Patel, D. V. An efficient synthesis of N-alpha-Fmoc-4- (phosphonodifluoromethyl)-L-phenylalanine. *J. Org. Chem.* **1996**, *61*, 1537−1539.
(60) Qabar, M. N.; Urban, J.; Kahn, M. A facile solution and solid-phase synthesis of phosphotyrosine mimetic L-4-[diethylphosphono-(difluoromethyl)]phenylalanine (F_2Pmp($EtO)_2$) derivatives. *Tetrahedron* **1997**, *53*, 11171−11178.
(61) Stankovic, C. J.; Surendran, N.; Lunney, E. A.; Plummer, M. S.; Para, K. S.; Shahripour, A.; Fergus, J. H.; Marks, J. S.; Herrera, R.; Hubbell, S. E.; Humblet, C.; Saltiel, A. R.; Stewart, B. H.; Sawyer, T. K. The role of 4-phosphonodifluoromethyl- and 4-phosphono-phenylalanine in the selectivity and cellular uptake of SH2 domain ligands. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1909−1914.
(62) Walker, C. V.; Caravatti, G.; Denholm, A. A.; Egerton, J.; Faessler, A.; Furet, P.; GarciaEcheverria, C.; Gay, B.; Irving, E.; Jones, K.; Lambert, A.; Press, N. J.; Woods, J. Structure-based design and synthesis of phosphinate isosteres of phosphotyrosine for incorporation in Grb2-SH2 domain inhibitors. Part 2. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2343−2346.
(63) Shakespeare, W. C.; Bohacek, R. S.; Narula, S. S.; Azimioara, M. D.; Yuan, R. W.; Dalgarno, D. C.; Madden, L.; Botfield, M. C.; Holt, D. A. An efficient synthesis of a 4′-phosphonodifluoromethyl-3′-formyl-phenylalanine containing Src SH2 ligand. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3109−3112.
(64) Shakespeare, W.; Yang, M.; Bohacek, R.; Cerasoli, F.; Stebbins, K.; Sundaramoorthi, R.; Azimioara, M.; Vu, C.; Selvi Pradeepan; Chester Metcalf, I.; Haraldson, C.; Merry, T.; Dalgarno, D.; Narula, S.; Hatada, M.; Lu, X.; van Schravendijk, M. R.; Adams, S.; Violette, S.; Smith, J.; Guan, W.; Bartlett, C.; Herson, J.; Iuliucci, J.; Weigele, M.; Sawyer, T. Structure-based design of an osteoclast-selective, nonpeptide Src homology 2 inhibitor with in vivo antiresorptive activity. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9373−9378.
(65) Bohacek, R. S.; Dalgarno, D. C.; Hatada, M.; Jacobsen, V. A.; Lynch, B. A.; Macek, K. J.; Merry, T.; Metcalf, C. A.; Narula, S. S.; Sawyer, T. K.; Shakespeare, W. C.; Violette, S. M.; Weigele, M. X-ray structure of citrate bound to Src SH2 leads to a high-affinity, bone-targeted Src SH2 inhibitor. *J. Med. Chem.* **2001**, *44*, 660−663.
(66) Gay, B.; Suarez, S.; Caravatti, G.; Furet, P.; Meyer, T.; Schoepfer, J. Selective Grb2 SH2 inhibitors as anti-Ras therapy. *Int. J. Cancer* **1999**, *83*, 235−241.
(67) Kole, H. K.; Ye, B.; Akamatsu, M.; Yan, X.; Barford, D.; Roller, P. P.; Burke, T. R., Jr. Protein-tyrosine phosphatase inhibition by a peptide containing the phosphotyrosyl mimetic, O-malonyltyrosine (OMT). *Biochem. Biophys. Res. Commun.* **1995**, *209*, 817−822.
(68) Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgetti-Peraldi, S.; Yan, X. J.; Roller, P. P.; Burke, T. R. L−O−(2-malonyl)-tyrosine: A new phosphotyrosyl mimetic for the preparation of Src homology 2 domain inhibitory peptides. *J. Med. Chem.* **1995**, *38*, 4270−4275.
(69) Burke, T. R.; Ye, B.; Akamatsu, M.; Ford, H.; Yan, X. J.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. P. 4′−O-[2-(2-fluoromalonyl)]-L-tyrosine: A phosphotyrosyl mimic for the preparation of signal transduction inhibitory peptides. *J. Med. Chem.* **1996**, *39*, 1021−1027.
(70) Gao, Y.; Burke, T. R., Jr. Stereoselective preparation of 4-(2′-malonyl)phenylalanine suitably protected for Fmoc-based synthesis of potent signal transduction inhibitor ligands. *Synlet* **2000**, 134−136.
(71) Gao, Y.; Luo, J.; Yao, Z.-J.; Guo, R.; Zou, H.; Kelley, J.; Voigt, J. H.; Yang, D.; Burke, T. R., Jr. Inhibition of Grb2 SH2 domain binding by nonphosphate containing ligands 2. 4-(2-Malonyl)-phenylalanine as a potent phosphotyrosyl mimetic. *J. Med. Chem.* **2000**, *43*, 911−920.
(72) Gao, Y.; Wu, L.; Luo, J. H.; Guo, R.; Yang, D.; Zhang, Z.-Y.; Burke, T. R., Jr. Examination of novel non-phosphorus-containing phosphotyrosyl mimetics against protein-tyrosine phosphatase-1B and demonstration of differential affinities toward Grb2 SH2 domains. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 923−927.
(73) Burke, T. R.; Yao, Z. J.; Zhao, H.; Milne, G. W. A.; Wu, L.; Zhang, Z. Y.; Voigt, J. H. Enantioselective synthesis of nonphosphorus-containing phosphotyrosyl mimetics and their use in the preparation of tyrosine phosphatase inhibitory peptides. *Tetrahedron* **1998**, *54*, 9981−9994.
(74) Larsen, S. D.; Barf, T.; Liljebris, C.; May, P. D.; Ogg, D.; O′Sullivan, T. J.; Palazuk, B. J.; Schostarez, H. J.; Stevens, F. C.; Bleasdale, J. E. Synthesis and biological activity of a novel class of small molecular weight peptidomimetic competitive inhibitors of protein tyrosine phosphatase 1B. *J. Med. Chem.* **2002**, *45*, 598−622.
(75) Yao, Z. J.; King, C. R.; Cao, T.; Kelley, J.; Milne, G. W. A.; Voigt, J. H.; Burke, T. R. Potent inhibition of Grb2 SH2 domain binding by non-phosphate-containing ligands. *J. Med. Chem.* **1999**, *42*, 25−35.
(76) Lange, G.; Lesuisse, D.; Deprez, P.; Schoot, B.; Loenze, P.; Benard, D.; Marquette, J.-P.; Broto, P.; Sarubbi, E.; Mandine, E. Principles governing the binding of a class of non-peptidic inhibitors to the SH2 Domain of Src studied by X-ray analysis. *J. Med. Chem.* **2002**, *45*, 2915−2922.
(77) Burke, T. R., Jr.; Luo, J.; Yao, Z.-J.; Gao, Y.; Milne, G. W. A.; Guo, R.; Voigt, J. H.; King, C. R.; Yang, D. Monocarboxylic phosphotyrosyl mimetics in the design of Grb2 SH2 domain inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 347−352.
(78) Tong, L.; Warren, T. C.; Lukas, S.; SchembriKing, J.; Betageri, R.; Proudfoot, J. R.; Jakes, S. Carboxymethyl-phenylalanine as a replacement for phosphotyrosine in SH2 domain binding. *J. Biol. Chem.* **1998**, *273*, 20238−20242.
(79) Beaulieu, P. L.; Cameron, D. R.; Ferland, J. M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; LlinasBrunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. Ligands for the tyrosine kinase p56(Lck) SH2 domain: Discovery of potent dipeptide derivatives with monocharged, nonhydrolyzable phosphate replacements. *J. Med. Chem.* **1999**, *42*, 1757−1766.
(80) Yao, Z. J.; Gao, Y.; Voigt, J. H.; Ford, H.; Burke, T. R. Synthesis of Fmoc-protected 4-carboxydifluoromethyl-L-phenylalanine: A phosphotyrosyl mimetic of potential use for signal transduction studies. *Tetrahedron* **1999**, *55*, 2865−2874.
(81) Burke, T. R., Jr.; Barchi, J. J., Jr.; George, C.; Wolf, G.; Shoelson, S. E.; Yan, X. Conformationally constrained phosphotyrosyl mimetics designed as monomeric SH2 domain inhibitors. *J. Med. Chem.* **1995**, *38*, 1386-1396.
(82) Wang, X.; Yao, Z. J.; Zhang, M.; Yang, D.; George, C.; Burke, T. R., Jr. Manuscript in preparation.
(83) Davidson, J. P.; Martin, S. F. Use of 1,2,3-trisubstituted cyclopropanes as conformationally constrained peptide mimics in SH2 antagonists. *Tetrahedron Lett.* **2000**, *41*, 9459−9464.
(84) Davidson, J. P.; Lubman, O.; Rose, T.; Waksman, G.; Martin, S. F. Calorimetric and structural studies of 1,2,3-trisubstituted cyclopropanes as conformationally constrained peptide inhibitors of Src SH2 domain binding. *J. Am. Chem. Soc.* **2002**, *124*, 205−215.

AR020127O