Potent Dipeptide Inhibitors of the pp60^{c-src} SH2 Domain

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The design, synthesis, and evaluation of dipeptide analogues as ligands for the pp60^{c-src} SH2 domain are described. The critical binding interactions between Ac-Tyr-Glu-N(n-C$_5$H$_{11}$)$_2$ (**2**) and the protein are established and form the basis for our structure-based drug design efforts. The effects of changes in both the C-terminal (**11−27**) and N-terminal (**51−69**) portions of the dipeptide are explored. Analogues with reduced overall charge (**92−95**) are also investigated. We demonstrate the feasibility of pairing structurally diverse subunits in a modest dipeptide framework with the goal of increasing the druglike attributes without sacrificing binding affinity.

Protein tyrosine kinases play a role in signal transduction through phosphorylation of substrate proteins as well as corresponding recognition and binding of other phosphoproteins.[2,3] The prototypical nonreceptor tyrosine kinase pp60^{c-src} contains a catalytic kinase region, the SH1 (src homology 1) domain, which is highly conserved among members of the src family, an SH2 (src homology 2) domain[4,5] that binds phosphotyrosine-containing proteins, and an SH3 (src homology 3) domain[4,5] that recognizes proline-rich sequences.[6] Overexpression or hyperactivation of pp60^{c-src} has been implicated in the development of human colon and breast carcinomas;[2] thus, entities that modulate pp60^{c-src}-regulated signal transduction pathways offer potential value as antiproliferative agents.[7−11]

The src SH2 domain has been the subject of numerous investigations. This domain is a region of approximately 100 amino acids that shares sequence similarity with other members of the src family of proteins, as well as other nonreceptor kinases (abl, lck, fyn, etc.),[12] and preferentially binds tyrosine-phosphorylated (Y*) proteins. The SH2 domain may participate in the transmission of signals through the formation of complexes with specific phosphoproteins such as epidermal growth factor receptor (EGFR),[13] platelet-derived growth factor receptor (PDGFR),[14] and focal adhesion kinase (FAK).[15] Alternatively, SH2 domains may act as adapters between phosphorylated receptors and other signaling proteins[16,17] or by regulating the activity of the kinase domain.[18] Thus, an entity that specifically disrupts or inhibits protein−protein interactions involving the src SH2 domain might interrupt signal transduction processes perhaps making such inhibitors useful chemotherapeutic agents.

The preferred binding sequence for the src SH2 domain has been determined,[19] and structural studies have defined the conformation of ligands complexed to the src SH2 domain.[20−23] The subsequent crystalliza-tion[13] of a high-affinity (IC$_{50}$ ≈ 1 μM) pentapeptide, AcY*EEIE (**1**), bound to the src SH2 domain has prompted the search for alternative peptide and/or peptidomimetic ligands.[24−27] These results reinforced both the feasibility of a structure-based design approach and our desire to identify lower-molecular-weight inhibitors using X-ray crystallographic studies to guide target selection. A dipeptide, Ac-Tyr-Glu-N(n-C$_5$H$_{11}$)$_2$ (**2**), emerged as our first success in this strategy and served as the lead molecule for subsequent studies. This result confirmed the viability of an approach to reduce the size and complexity of SH2 ligands as part of a drug discovery program.
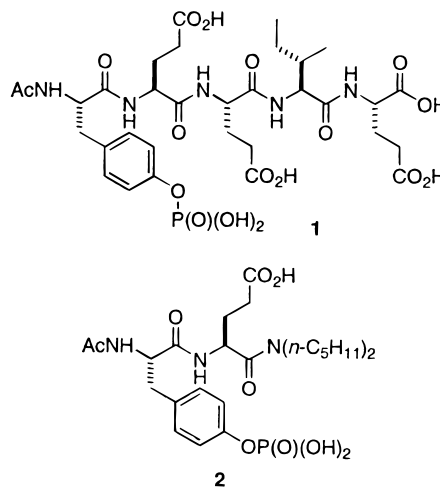
The emergence of information on the role of pp60^{c-src} in signal transduction and our ability to rapidly characterize the interaction of ligands with the src SH2 domain at a molecular level prompted us to target SH2 inhibitors as novel cancer chemotherapeutics. The results of our investigations including the successful identification of dipeptide ligands are described herein.
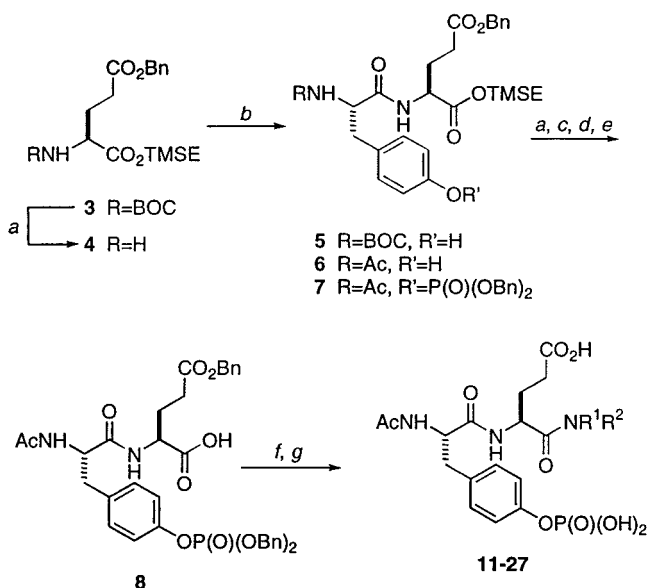
Chemistry

A synthetic route was developed to introduce diverse C-terminal substitutions onto the dipeptide framework
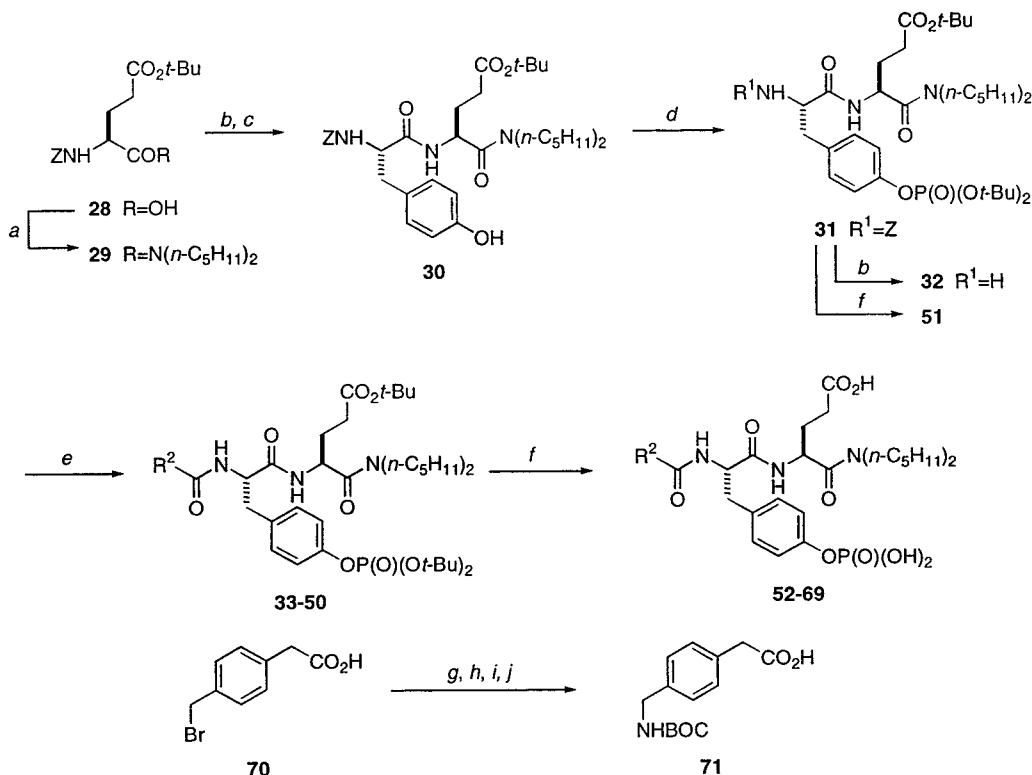
Scheme 1[a]

^{a} Reagents: (a) HCl/dioxane; (b) BOC-Tyr-OH, BOP, HOBT, Et$_3$N; (c) AcOSuc; (d) [(BnO)$_2$P(O)]$_2$O; (e) HF–pyridine; (f) NMM, ClCO$_2$i-Bu, HNR^{1}R^2; (g) H$_2$, Pd–C.

in the penultimate step (Scheme 1). Toward that end, the key coupling intermediate **8** was synthesized. Removal of the carbamate protecting group in **3** and subsequent coupling of H-Glu(OBn)-OTMSE (**4**) with BOC-Tyr-OH delivered dipeptide **5**. Installation of the N-terminal acetyl group yielded **6** which was phosphorylated with tetrabenzyl pyrophosphate.[28] Subsequent fluoride-catalyzed cleavage[29] of the silyl group in **7** set the stage for preparation of a series of C-terminal analogues. Since such a strategy introduces the poten-
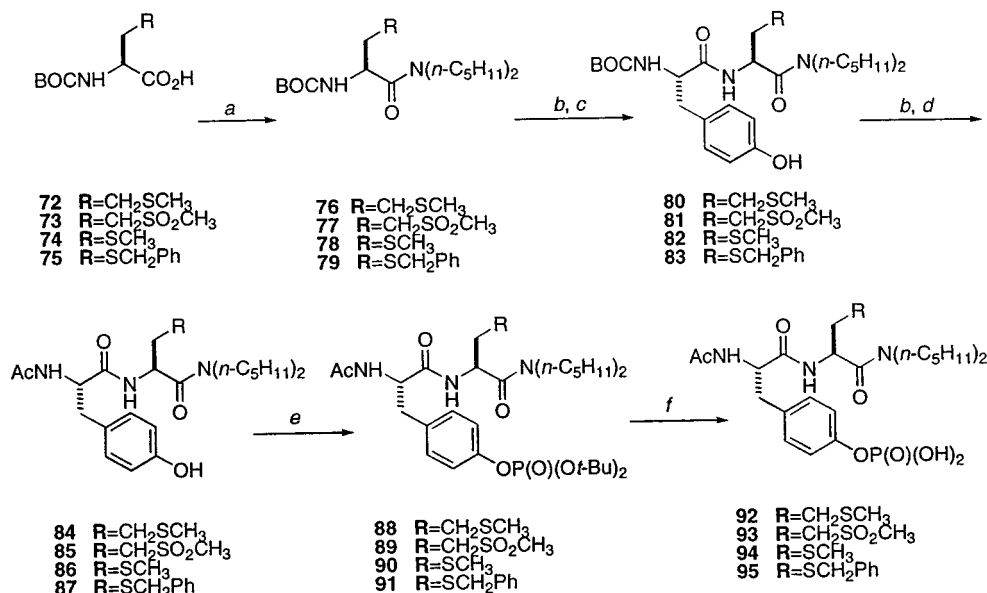
tial for epimerization at the carbon atom adjacent to the reacting center,[30] a number of methods were surveyed to identify a procedure amenable to the synthesis of these analogues. Ultimately, a protocol involving low-temperature formation of the mixed anhydride (−78 °C, 4-methylmorpholine, isobutyl chloroformate, THF) followed by the addition of the desired amine generated a diverse set of amides with <5% of the epimeric adduct, as determined by ^{1}H NMR and reverse-phase HPLC analyses.[31] Removal of the phosphate-protecting groups and concomitant unmasking of the glutamic acid delivered compounds **11–27**.

The preparation of dipeptide analogues **51–69**, containing variations in the N-terminus of the dipeptide, is depicted in Scheme 2. Conversion of amino acid **28** to the corresponding amide **29** was accomplished using the mixed anhydride protocol.[32] Removal of the nitrogen-protecting group and subsequent coupling (DCC, HOBT)[33] with Z-Tyr-OH delivered **30**. The phosphate moiety was introduced as the diester [NaH, ClP(O)(Ot-Bu)$_2$][34] via alkylation of the phenolic oxygen to afford dipeptide **31**. Attachment of the N-terminal capping group was achieved by removing the Z group by hydrogenolysis, and the primary amine **32** was reacted with electrophiles to produce **33–50**. Finally, acid-catalyzed deprotection of the phosphate produced the targets **51–69**; for **69** this treatment with acid also cleaved the BOC group introduced during the synthesis of fragment **71**.

The synthesis of analogues **92–95**, with variations in the Y* + 1 position of the dipeptide framework, is shown in Scheme 3. Conversion of the BOC-protected amino acids **72–75** to the corresponding amides **76–79** was accomplished using the mixed anhydride protocol.[32]

Scheme 2[a]

^{a} Reagents: (a) NMM, ClCO$_2$i-Bu, HN(n-C$_5$H$_{11}$)$_2$; (b) H$_2$, Pd–C; (c) Z-Tyr-OH, DCC, HOBT, Et$_3$N; (d) NaH, ClP(O)(Ot-Bu)$_2$; (e) R^{2}CO$_2$H, (R^{2}CO)$_2$O, R^{2}COCl, or R^{2}NCO; (f) TFA; (g) SOCl$_2$, DMF, pyridine; (h) PhCH$_2$OH, Et$_3$N, DMAP; (i) NaN$_3$; (j) BOC$_2$O, H$_2$, Pd–C.

Scheme 3[a]

72 R=CH$_2$SCH$_3$	**76** R=CH$_2$SCH$_3$	**80** R=CH$_2$SCH$_3$
73 R=CH$_2$SO$_2$CH$_3$	**77** R=CH$_2$SO$_2$CH$_3$	**81** R=CH$_2$SO$_2$CH$_3$
74 R=SCH$_3$	**78** R=SCH$_3$	**82** R=SCH$_3$
75 R=SCH$_2$Ph	**79** R=SCH$_2$Ph	**83** R=SCH$_2$Ph

84 R=CH$_2$SCH$_3$	**88** R=CH$_2$SCH$_3$	**92** R=CH$_2$SCH$_3$
85 R=CH$_2$SO$_2$CH$_3$	**89** R=CH$_2$SO$_2$CH$_3$	**93** R=CH$_2$SO$_2$CH$_3$
86 R=SCH$_3$	**90** R=SCH$_3$	**94** R=SCH$_3$
87 R=SCH$_2$Ph	**91** R=SCH$_2$Ph	**95** R=SCH$_2$Ph

[a] Reagents: (a) NMM, ClCO$_2$$i$-Bu, HN($n$-C$_5H_{11}$)$_2$; (b) HCl/dioxane; (c) BOC-Tyr-OH, DCC, HOBT, Et$_3$N; (d) AcOH, DCC, HOBT, Et$_3$N; (e) NaH, ClP(O)(Ot-Bu)$_2$; (f) HCl/Et$_2$O.

Removal of the nitrogen-protecting group and subsequent coupling (DCC, HOBT)[33] with BOC-Tyr-OH delivered the dipeptides **80−83**; repetition of this sequence using acetic acid in the final coupling procedure delivered **84−87**. The phosphate moiety was introduced as before followed by deprotection of the phosphate to produce the targets **92−95**.

Results and Discussion

The X-ray crystallographic analysis of the pentapeptide **1** complexed to the src SH2 domain clearly established the presence of two distinct binding regions on the surface of the protein: (1) a pocket in which the phosphotyrosine residue is bound and (2) a lipophilic pocket in which the Ile residue at the Y* + 3 position is bound. We reasoned that a dipeptide with appropriate substitution at its carboxyl terminus might bind similarly to the src SH2 domain if the phosphotyrosine-containing subunit remained intact. Using an in vitro binding assay,[13] dipeptides were evaluated as inhibitors of src SH3−SH2:phosphoprotein interactions using **1** as the standard; for the purpose of comparison and to normalize data from different experiments, results for individual peptides are presented as a ratio, IC$_{50}$(test)/IC$_{50}$(standard). A survey of hydrophobic amines produced Ac-Y*-E-N(n-C$_5$H$_{11}$)$_2$ (**2**)[23] which binds with high affinity (ratio ≈ 8) to the src SH2 domain. The key interactions between the lipophilic cleft of the src SH2 domain and the di-n-pentyl amide of **2** observed in the crystal structure (Figure 1) marked the successful replacement of the three C-terminal residues in **1** with a fragment that would access the hydrophobic pocket, occupied by Ile at the Y* + 3 position in **1**, without compromising interactions within the Y* binding pocket. In fact, the X-ray structure mirrored that obtained with the pentapeptide: (1) the dipeptide binding in an extended conformation with a hydrogen bond between its Tyr-NH and His204; (2) placement of a single n-pentyl chain into the Y* + 3 hydrophobic pocket framed by Ile217, Gly239, and Leu240; (3) interaction between the Y* ring and Arg158 as well as hydrophobic packing against the side-chain atoms of Lys206; and (4) alignment of the phosphate with Ser180, Thr182, Arg158, and Arg178.

Encouraged by the high-affinity binding obtained with **2**, we prepared a series of dipeptide inhibitors varied at the carboxyl terminus. A diverse set of amines were chosen to help elucidate the gross binding requirements of the lipophilic binding site. Compounds **9−14** (Table 1) were designed to determine the relative importance of the n-pentyl chains. The unfunctionalized primary amide **10** proved to bind weakly (ratio = 200) when compared with the pentapeptide **1**. A dramatic improvement in binding affinity was observed with the single C-terminal n-pentyl substituent present in **11**. A further, but comparatively smaller, increase in binding affinity was realized with the N-methyl, N-pentyl substitution of **12** presumably due to an increase in conformational rigidity[35] of this tertiary amide. The length of the alkyl chain was also varied, compounds **13** and **14**, to discern the depth of this pocket. While there were changes in binding affinity across the range of alkyl groups studied, the optimum substitution of the dipeptide was achieved using the di-n-pentyl amide as in **2**.

In the X-ray crystal structure of the dipeptide **2**, one of the n-pentyl chains faces the predominantly lipophilic cavity, occupied by Ile in the case of **1**, while the other alkyl chain appears to be directed away from the protein surface toward solvent. Compounds **15** and **16** were designed to probe the use of differentially substituted amides, that is, possessing one lipophilic fragment and one more hydrophilic subunit, in an effort to accommodate both of the binding relationships mentioned above simultaneously. This approach offered the potential to generate a better overall solvation/desolvation profile for the ligand during its association with the protein. Unfortunately, no improvement in binding versus **2** was observed with either **15** or **16**.
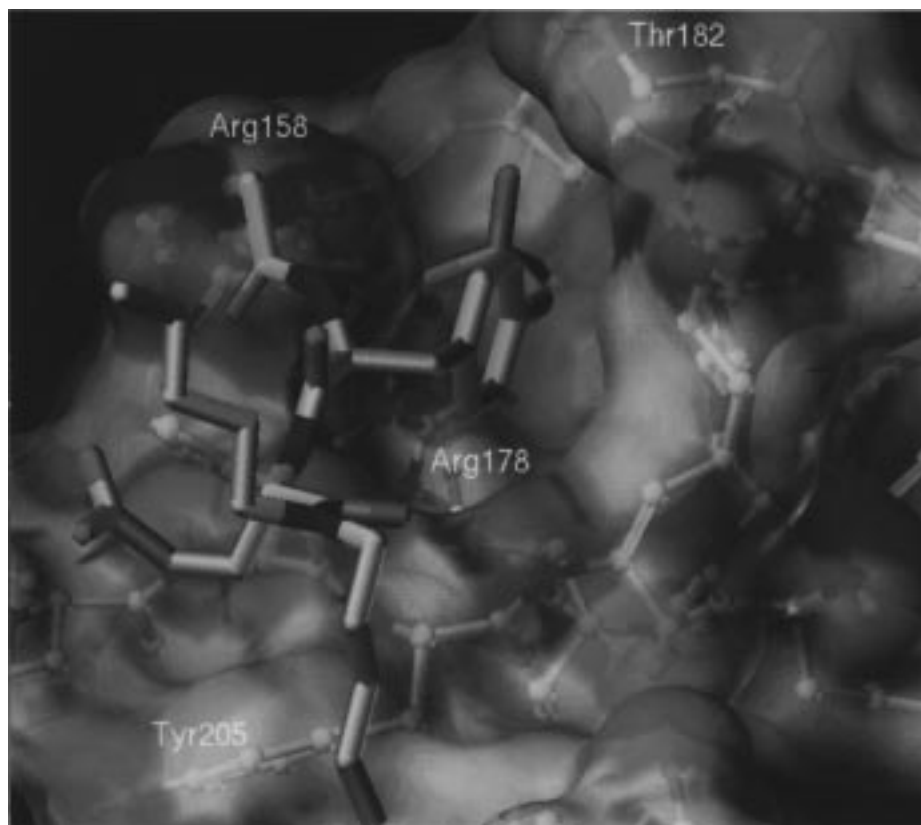
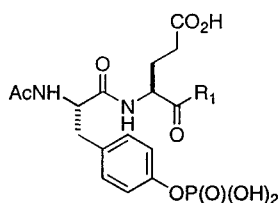**Figure 1.** X-ray structure of **2** bound to the pp60c−src SH2 domain.

An alternative series of compounds were designed to incorporate functionality in the amide that would fill the Y* + 3 binding pocket and/or potentially form a hydrogen-bonding interaction with amino acid residues lining this pocket. To explore this idea of increasing the size of the amide, variously substituted indoles were prepared, and evaluation of this series revealed a surprising tolerance for diversity in terms of binding within the Y* + 3 pocket. For example, the indole **22** and both of the related, substituted indoles (**23**, **24**) were equipotent when linked through a two-carbon chain; that is, no increase in binding affinity was observed with the inclusion of a potential hydrogen bond partner substituted on the indole ring. Interestingly, a 3-fold improvement in binding affinity was observed with a three-carbon tether (**20**, **21**).

Significant improvements in binding affinity were observed using modifications which optimized a hydrogen-bonding interaction between the constituents of the C-terminal amide and a side-chain oxygen or backbone carbonyl of residues lining the Y* + 3 binding pocket. Initial studies showed that the simple carboxamide **18** bound weakly, when compared to the corresponding acid **19**, suggesting a preference for ionizable functionality deep within this pocket. More dramatic results were observed when an alkyl chain terminating in a hydroxyl group was utilized. For example, the hexanol **25** was equipotent with **2**; however, homologation with one and two methylene units delivered heptanol **26** and octanol **27** with improved the binding affinity by 2- and 3-fold, respectively, when compared with **2**. While the X-ray crystal analysis of **25** (Figure 2) confirmed a hydrogen-bonding interaction between the alkanol and the hydroxyl group of Thr218, in practice this compound displayed a propensity toward both internal lactoniza-

tion and dimerization with the Glu carboxylic acid. While these compounds offered desirable solutions for increasing binding potency from a structural perspective, their physical properties made them less interesting for further investigations.

Our earlier work had established the binding properties of several dipeptides capped as the N-terminal acetamide. Subsequent analysis of X-ray structures reinforced several features about interactions between this portion of these dipeptides and the protein in the complexes: (1) the phosphotyrosine pocket and adjacent space appeared sufficiently large to accommodate functional groups other than acetyl to evaluate the relative importance of steric and electronic variations on binding affinity; (2) the array of hydrogen bonds in the Y* pocket of the protein:ligand complex was highly conserved; (3) the phosphotyrosine pocket was inadequately protected from exposure to solvent.

Several N-terminal analogues of **2** were prepared in an attempt to determine the impact of the above-mentioned features on binding affinity (Table 2). Incorporation of more sterically demanding aryl- and heteroaryl-containing amides delivered dipeptides with binding affinities comparable to that observed for **2**. These results suggested that binding relied on the maintenance of a hydrogen-bonding interaction between the acetamide carbonyl of the dipeptide and Arg158 of the protein rather than the ability to fill the phosphotyrosine pocket. To attenuate this hydrogen bond and determine the impact on binding affinity, a series of electronically different N-terminal analogues, including substituted benzamides and ureas, were synthesized and evaluated as ligands for the src SH2 domain. The affinities of these analogues on the whole were not demonstrably different from the aforementioned amides;

Table 1. C-Terminal Analogues of **2**

Cmpd	R	Ratio[a]	Cmpd	R	Ratio[a]
1	EIE	1	18	(structure)	115
9	OH	606	19	(structure)	36.7
10	NH_2	200	20	(structure)	9.3
11	(structure) HN	16.6	21	(structure)	11.6
12	(structure) CH_3 N	12.0	22	(structure) CH_3	31.8
2	(structure) N	7.9	23	(structure) OH	31.5
13	(structure) N	24.0	24	(structure) OCH_3	29.7
14	(structure) N	327	25	(structure) CH_3	6.4
15	(structure) OH	16.7	26	(structure) CH_3	3.6
16	(structure) CO_2H	48.0	27	(structure) CH_3	2.2
17	(structure) CO_2Et	26.0			

[a] Ratio = $IC_{50}(test)/IC_{50}(AcY^*EEIE)$.

however, variations exist within each structural class. The benzamide-based analogues **62**−**65** showed a modest (3−5-fold) preference for electron-rich systems; the anomalous result with **64** may be attributed to increased solvation of the nitro group. In the urea series, a more pronounced preference (6−13-fold) exists for the electron-rich derivatives **66** and **67**, as compared to analogue **68**, supporting the hypothesis that overall binding affinity is influenced by the hydrogen bond strength of this N-terminal carbonyl.

A network of hydrogen bonds anchors a phenyl phosphate in the phosphotyrosine binding pocket of the src SH2 domain which suggests the possibility for increasing binding affinity by strengthening such interactions between the ligand and the protein. Intramolecular association of the N-terminal components of the ligand might further improve its binding by capping the pocket and minimizing the need for solvation of the Y* binding site. Compound **69** was designed and prepared using this rationale as well as to explore the potential for preorganization in this segment of the ligand. This N-terminal aromatic system effectively shields the pocket from solvent through a series of hydrogen bonds; however, there is a decrease in binding affinity for this compound. Closer examination of the X-ray structure (Figure 3) shows that the distal oxygen of the phosphate contacts the pendant amino group as well as Thr182 and Ser180; the phosphate has rotated from its normal position to accommodate this new array. Also, the tyrosine-based phenyl ring of the peptide has rotated to allow formation of an edge-to-face interaction between the two aromatic rings of the ligand. Presumably this combination of interactions has distorted the binding trajectory of the phosphotyrosine resulting in decreased affinity.

The work to date had relied exclusively on a Y* + 1 glutamate for binding to the src SH2 domain, and we were aware of potential difficulties, such as poor cell penetration properties, associated with such highly charged compounds. To address these issues, we considered replacement of glutamic acid to reduce overall charge while retaining, or improving, binding affinity. Studies began with an analysis of the **2**:SH2 complex which showed the glutamic acid side chain packed
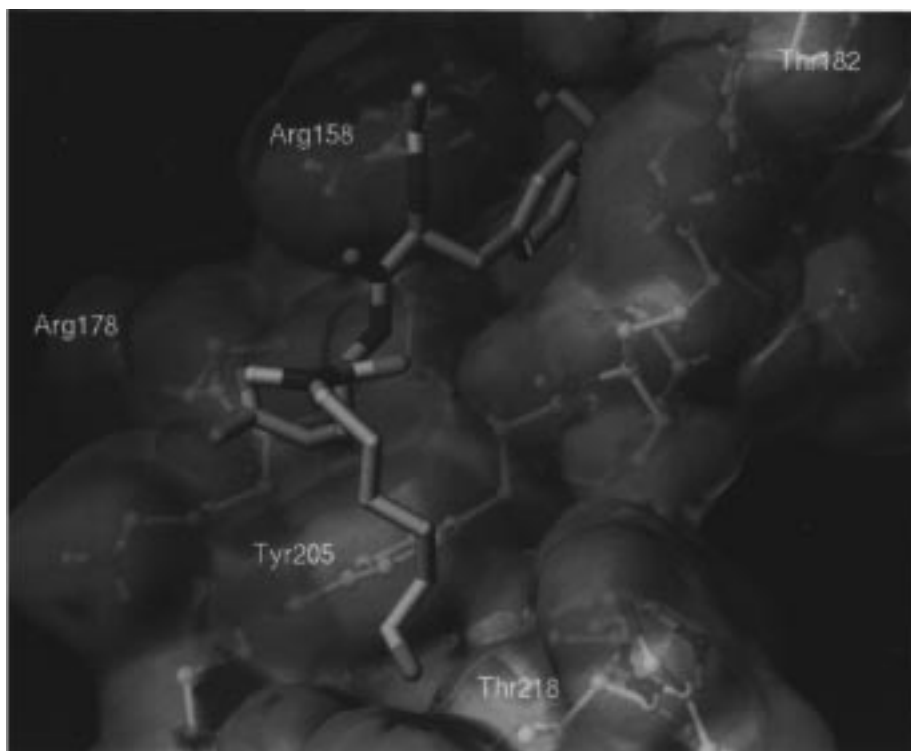
Figure 2. X-ray structure of **25** bound to the pp60^{c-src} SH2 domain.

against the phenyl ring portion of Tyr205 and its carboxyl terminus being canted toward an electrostatic interaction with His204 to offset negative charge. This proximity to Tyr205 and the reported ability of sulfur to interact with aromatic systems[36,37] prompted us to install an amino acid with a sulfur-containing side chain. Thus, a series of sulfur-based dipeptides (Table 3) were screened, with the methionine-containing dipeptide **92** having the highest affinity for the src SH2 domain. The X-ray analysis (Figure 4) of the complex revealed improved hydrophobic stacking between the methionine side chain and Tyr205 when compared to the corresponding atoms of the glutamate in the parent complex. Interestingly, the sulfur atom did not align with the aromatic ring but rather was positioned to interact with the phenolic hydroxyl group suggesting that a shorter tether might orient the sulfur atom near the π cloud. Unfortunately, a decrease in binding affinity was realized in parallel studies with the *S*-methylcysteine analogue **94** where the position of the sulfur atom forced the terminal methyl group into the π cloud. Noteworthy is the fact that a high-affinity dipeptide with a neutral Y* + 1 residue emerged from this study.

Conclusions

A strategy to identify dipeptide ligands for the pp60^{c-src} SH2 domain using structure-based design has yielded compounds with binding affinities comparable to that of pentapeptide **1**. The dipeptide **2** utilized a simple *N,N*-dialkyl amide to replace the amino acid residues C-terminal to the phosphotyrosine in **1** and served as a template for further modifications of the dipeptide framework. Subsequent variations in both the C- and N-terminal regions of **2** have produced analogues with satisfactory binding affinities and have helped us

elucidate the structural requirements and preferences of the protein. Confirmation of the ability to incorporate diverse structural motifs into a modest dipeptide which retains binding affinity remains the principal advantage of this approach.

The inability of highly charged compounds of the type described herein to penetrate cells would affect their chemotherapeutic utility for intracellular targets. Therefore, reduction of the overall charge of these dipeptides has been an important focus of this program. The strategy to use an uncharged residue adjacent to the phosphotyrosine together with the information obtained in the aforementioned studies has enabled the production of potent inhibitors with reduced charge relative to **2**. Efforts to extend the scope of these results will be the subject of further investigations.

Experimental Section

General Experimental. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR data were obtained using a Varian Unity Plus 300 or Unity Plus 400 spectrometer; chemical shifts are reported in parts per million (ppm) and referenced to solvent or internal TMS. Mass spectral data were obtained using either a Perkin-Elmer Sciex API III or JEOL JMS mass spectrometer. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Unless noted otherwise, anhydrous solvents and reagents were purchased from commercial suppliers and used as received. Flash chromatography was carried out using EM Science silica gel 60, 230−400 mesh. Isolated yields of purified materials are reported.

BOC-Glu(OBn)-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (3). BOC-Glu(OBn)-OH (5.00 g, 14.8 mmol) was dissolved in CH$_3$CN (50 mL) at room temperature. 2-(Trimethylsilyl)ethanol (4.20 mL, 29.3 mmol), 1,3-dicyclohexylcarbodiimide (DCC; 3.1 g, 15.0 mmol), and pyridine (3.60 mL, 44.5 mmol) were added, and the reaction solution was stirred for 18 h. A white precipitate formed and was removed from the reaction by suction filtration. The filtrate was diluted with CH$_2$Cl$_2$ (150 mL) and

Table 2. N-Terminal Analogues of **2**
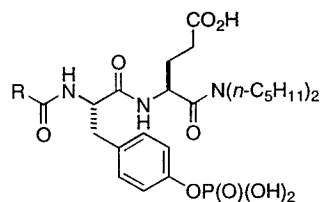
Cmpd	R	Ratio[a]	Cmpd	R	Ratio[a]
2	Me	7.9	**60**	PhCH$_2$SCH$_2$	18.9
51	PhCH$_2$O	79.7	**61**	F$_3$C	57.2
52	Ph	15.3	**62**	(4-Cl)Ph	39.0
53	PhCH$_2$	16.5	**63**	(4-F$_3$C)Ph	25.4
54	PhCH$_2$CH$_2$	10.8	**64**	(4-O$_2$N)Ph	5.7
55	PhSCH$_2$	17.7	**65**	(4-MeO)Ph	7.2
56	(4-HO)PhCH$_2$CH$_2$	26.7	**66**		26.9
57		17.7	**67**		13.5
58	PhCH$_2$CH$_2$CH$_2$	27.2	**68**		184.4
59	PhCH$_2$OCH$_2$	32.7	**69**		92.1

[a] Ratio = IC$_{50}$(test)/IC$_{50}$(AcY*EEIE).

Table 3. Y* + 1 Analogues of **2**

Cmpd	R	Ratio[a]
2	CH$_2$CO$_2$H	7.9
92	CH$_2$SMe	16.5
93	CH$_2$S(O$_2$)Me	75.3
94	SMe	33.1
95	SCH$_2$Ph	155.4

[a] Ratio = IC$_{50}$(test)/IC$_{50}$(AcY*EEIE).

washed (1 × 100 mL) with each of the following aqueous solutions: 1 M aqueous HCl, saturated NaHCO$_3$, and water. The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure to provide 6.00 g (93%) of **3** as a clear, colorless, viscous oil.

HCl·H-Glu(OBn)-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (4). BOC-Glu(OBn)-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (6.00 g, 13.7 mmol) was treated with HCl/dioxane (12.5 mL of a 4 M solution, 50.0 mmol) until **3** was no longer detectable by TLC. The volatiles were removed in vacuo, and the residue was solidified by trituration with Et$_2$O to provide 5.10 g (98%) of **4** as an amorphous, light-yellow solid.

BOC-Tyr-Glu(OBn)-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (5). BOC-Tyr-OH (3.39 g, 12.1 mmol) and **4** (4.05 g, 10.8 mmol) were combined and dissolved in DMF (15 mL). To this solution was added (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (4.83 g, 10.9 mmol) followed by *i*-PrNEt$_2$ (4.55 mL, 26.1 mmol). The reaction mixture was stirred for 6 h and then diluted with EtOAc (100 mL) and saturated

aqueous NaHCO$_3$ (100 mL). The layers were separated, and the organic phase was washed with 0.10 M aqueous HCl (1 × 100 mL) and brine (1 × 100 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with 2:3 EtOAc−hexane, to afford 5.30 g (81%) of **5** as a white foam.

Ac-Tyr-Glu(OBn)-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (6). A solution of **5** (1.55 g, 2.58 mmol) in HCl/dioxane (2.50 mL of a 4 M solution, 10.0 mmol) was stirred for 2 h. The volatiles were removed in vacuo to afford a brown-yellow foam that was taken up in CH$_2$Cl$_2$ (20 mL), treated with acetic acid *N*-hydroxysuccinimide ester (AcOSuc) (570 mg, 3.61 mmol) followed by *i*-PrNEt$_2$ (0.90 mL, 5.16 mmol), and stirred for 24 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with 100 mL each of the following aqueous solutions: saturated NaHCO$_3$, 1 M HCl, and brine. The organic phase was concentrated under reduced pressure, and the residue was purified by flash chromatography, elution with 75:25 EtOAc−hexane, to afford 1.20 g (86%) of **6** as an amorphous cream-colored solid.

Ac-Tyr[P(O)(OBn)$_2$]-Glu(OBn)-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (7). A solution of **6** (610 mg, 1.12 mmol) in CH$_2$Cl$_2$ (8 mL) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.35 mL, 2.34 mmol) and tetrabenzyl pyrophosphate (995 mg, 1.85 mmol). The reaction mixture was stirred for 3 h, diluted with EtOAc (100 mL), and washed with 100 mL each of the following aqueous solutions: saturated NaHCO$_3$, 0.50 M HCl, and brine. The organic phase was concentrated under reduced pressure, and the residue was purified by flash chromatogra-
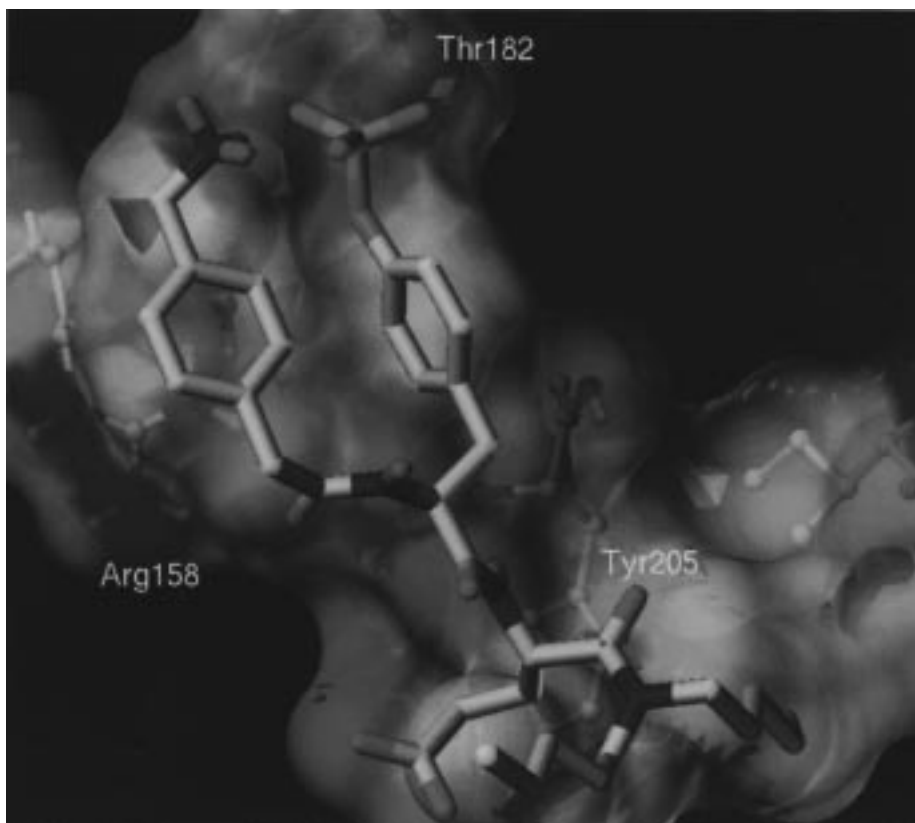
Figure 3. X-ray structure of **69** bound to the pp60[c−src] SH2 domain.

phy, eluting with 3:2 EtOAc−hexane, to afford 790 mg (88%) of **7** as a viscous oil.

Ac-Tyr[P(O)(OBn)₂]-Glu(OBn)-OH (8). To a solution of **7** (400 mg, 0.50 mmol) in CH_2Cl_2 (5 mL), in a plastic reaction vessel, at 0 °C was added HF·pyridine (0.40 mL), and the reaction mixture was stirred for 2 h while being followed closely by TLC; *significant dephosphorylation occurred with longer reaction times*. The reaction mixture was diluted with CH_2Cl_2 (250 mL) and H_2O (200 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (1 × 150 mL), and the combined organic layers were treated with KF (10 g) followed by Na_2SO_4 and then filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography, elution with 95:4:1 CH_2-Cl_2−MeOH−AcOH, to provide 150 mg (44%) of **8** as a white foam.

Compounds **11**−**27** were synthesized using the following method described for **14**.

Ac-Tyr[P(O)(OH)₂]-Glu-N(n-C₇H₁₅)₂ (14). To a solution of **8** (46 mg, 0.07 mmol) in THF (1 mL) at −70 °C were added 4-methylmorpholine (21 μL, 0.19 mmol) and isobutyl chloroformate (9 μL, 0.069 mmol). This solution was stirred for 5 min prior to the addition of HN(n-C₇H₁₅)₂ (53 μL, 0.20 mmol). The reaction mixture was warmed to −50 °C and stirred for 2.5 h. At the low temperature, the reaction mixture was diluted with EtOAc (25 mL) and saturated aqueous NaHCO₃ (25 mL). The biphasic solution was warmed to room temperature, and the layers were separated. The organic layer was washed with H_2O (1 × 30 mL) and brine (1 × 30 mL), dried (Na₂SO₄), and concentrated under reduced pressure. The residue was purified by column chromatography, elution with 4:1 EtOAc−hexane, to provide a clear viscous oil. This material was dissolved in EtOH (1.5 mL), treated with 10% Pd−C, and stirred under H_2 (1 atm) for 6.5 h. The reaction mixture was purged with N_2 and filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to provide 23 mg (57%) of **14** as an amorphous white solid: MS (ESI) m/z 628 (M + H). Anal. (C₃₀H₅₀N₃O₉P·1.5H₂O) C, H, N.

Ac-Tyr[P(O)(OH)₂]-Glu-N(n-C₆H₁₃)₂ (13): ¹H NMR (300 MHz, CD₃OD) δ 0.92 (m, 6H), 1.32 (m, 12H), 1.50 (m, 2H), 1.63 (m, 2H), 1.79 (m, 1H), 1.90 (s, 3H), 1.97 (m, 1H), 2.40 (m, 2H), 2.84 (m, 1H), 3.12 (m, 2H), 3.22 (m, 1H), 3.48 (m, 2H), 4.59 (m, 1), 4.82 (m, 1H), 7.10 (d, 2H, J = 8.3 Hz), 7.22 (d, 2H, J = 8.3 Hz); MS (ESI) m/z 598 (M − H). Anal. (C₂₈H₄₆N₃O₉-P·1.5H₂O) C, H, N.

Ac-Tyr[P(O)(OH)₂]-Glu-N[(n-C₅H₁₁)(CH₂CH₂OH)] (15): ¹H NMR (300 MHz, CD₃OD) δ 0.98 (m, 3H), 1.39 (m, 4H), 1.6 (m, 1H), 1.70 (m, 1H), 1.95 (m, 4H), 2.06 (m, 1H), 2.41 (m, 1H), 2.54 (m, 1H), 2.87 (m, 1H), 3.14 (m, 1H), 3.38 (m, 3H), 3.61 (m, 4H), 4.62 (m, 1H), 7.17 (d, 2H), 7.24 (d, 2H); MS (FAB) m/z 544 (M − H). Anal. (C₂₃H₃₆N₃O₁₀P) C, H, N.

Ac-Tyr[P(O)(OH)₂]-Glu-N[(n-C₅H₁₁)(CH₂CH₂CO₂H)] (16): ¹H NMR (300 MHz, CD₃OD) δ 0.95 (m, 3H), 1.31 (m, 6H), 1.60 (m, 2H), 1.81 (m, 1H), 1.89 (s, 3H), 1.91 (m, 1H), 2.38 (m, 2H), 2.47 (m, 1H), 2.70 (m, 1H), 2.82 (m, 1H), 3.04 (m, 1H), 3.37 (m, 2H), 3.42 (m, 1H), 3.57 (m, 1H), 3.65 (m, 1H), 4.55 (m, 1H), 4.85 (1H), 7.12 (d, 2H, J = 8.4 Hz), 7.16 (d, 2H, J = 8.4 Hz); MS (ESI) m/z 574 (MH⁺). Anal. (C₂₄H₃₆N₃O₁₁P·1.5H₂O) C, H, N.

Ac-Tyr[P(O)(OH)₂]-Glu-N[(n-C₅H₁₁)(CH₂CH₂CO₂Et)] (17): ¹H NMR (300 MHz, CD₃OD) δ 0.95 (m, 3H), 1.32 (m, 9H), 1.66 (m, 2H), 1.97 (s, 3H), 2.40 (m, 2H), 2.57 (m, 1H), 2.71 (m, 1H), 2.84 (m, 1H), 3.10 (m, 2H), 3.39 (m, 1H), 3.60 (m, 2H), 4.13 (m, 2H), 4.59 (m, 1H), 5.86 (m, 1H), 7.13 (d, 2H, J = 8.4 Hz), 7.22 (d, 2H, J = 8.4 Hz); MS (FAB) m/z 602 (MH⁺), 624 (M + Na). Anal. (C₂₆H₄₀N₃O₁₁P·1.0H₂O) C, H, N.

Ac-Tyr[P(O)(OH)₂]-Glu-NH[(CH₂)₆CONH₂] (18): ¹H NMR (300 MHz, CD₃OD) δ 1.35 (m, 4H), 1.48 (m, 2H), 1.89 (m, 1H), 1.92 (s, 3H), 2.04 (m, 1H), 2.20 (m, 2H), 2.34 (m, 2H), 2.84 (m, 1H), 3.15 (m, 3H), 4.28 (m, 1H), 4.53 (m, 1H), 7.11 (d, 2H), 7.23 (d, 2H); MS (FAB) m/z 559 (MH⁺); HRFAB-MS calcd for C₂₃H₃₆N₄O₁₀P (MH⁺) 559.216 12, found 559.216 12 (MH⁺). Anal. (C₂₃H₃₅N₄O₁₀P·1.5H₂O·1i-PrOH) C, H, N.

Ac-Tyr[P(O)(OH)₂]-Glu-NH[(CH₂)₆CO₂H] (19): ¹H NMR (300 MHz, CD₃OD) δ 1.31 (m, 6H), 1.48 (m, 2H), 1.59 (m, 2H), 1.84 (m, 1H), 1.89 (s, 3H), 2.05 (m, 1H), 2.33 (m, 2H), 2.38 (m, 2H), 2.85 (dd, 1H), 3.16 (m, 3H), 4.30 (m, 1H), 4.58 (m, 1H),
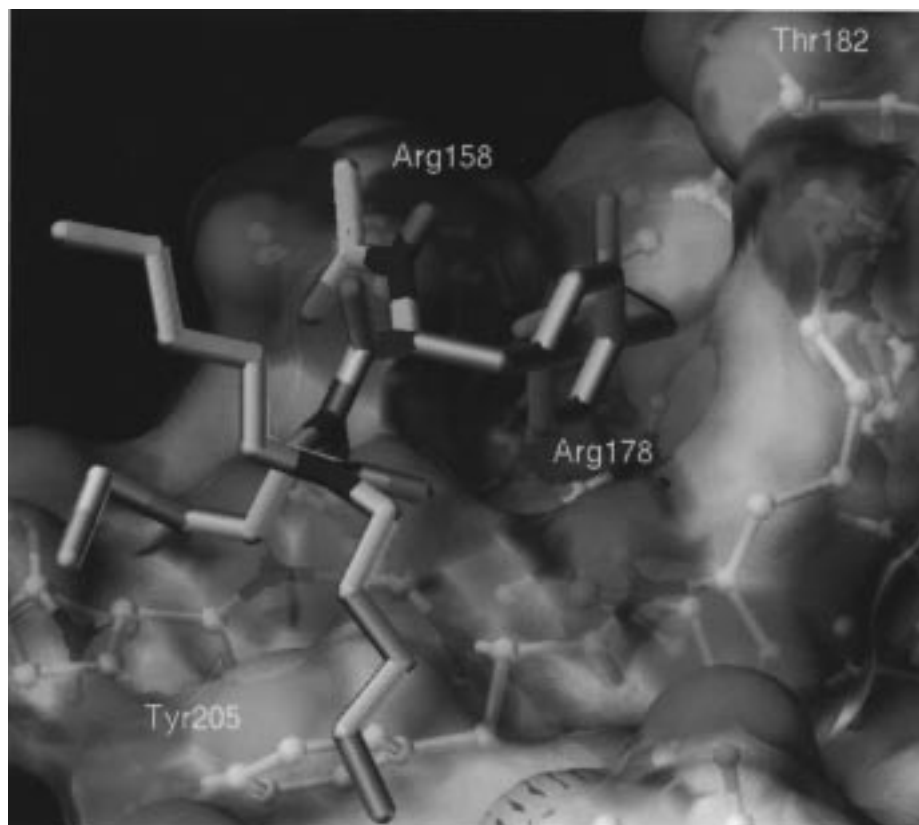
Figure 4. X-ray structure of **92** bound to the pp60^{c-src} SH2 domain.

7.14 (d, 2H), 7.24 (d, 2H), 7.80 (t, 1H, exchangeable), 8.19 (d, 1H, exchangeable); MS (ESI) m/z 572 (M − H). Anal. (C$_{24}$H$_{36}$N$_3$O$_{11}$P·1H$_2$O·0.5i-PrOH) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-N[(CH$_3$)[3-(3-propyl-2-carboxamidoindole)]] (20): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (m, 1H), 1.80 (s, 3H), 1.95 (m, 3H), 2.25 (m, 2H), 2.69 (m, 2H), 2.80 (m, 1H), 3.00 (m, 4H), 3.51 (m, 2H), 4.51 (m, 1H), 4.80 (m, 1H), 6.99 (br s, 2H), 7.04 (d, 2H), 7.14 (d, 2H), 7.22 (m, 2H), 7.59 (m, 4H), 8.22 (d, 1H). MS (FAB) m/z 646 (MH$^+$). Anal. (C$_{29}$H$_{36}$N$_5$O$_{10}$P·2H$_2$O) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-N[(CH$_3$)[3-(3-propylindole)]] (21): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (m, 1H), 1.80 (s, 3H), 1.92 (m, 2H), 2.23 (m, 2H), 2.70 (m, 2H), 2.96 (m, 4H), 3.42 (m, 2H), 4.50 (m, 1H), 4.80 (m, 1H), 6.95 (m, 1H), 7.04 (m, 4H), 7.14 (d, 2H), 7.32 (d, 1H), 7.50 (d, 1H), 7.60 (m, 2H), 10.38 (br s, 1H); MS (FAB) m/z 603 (MH$^+$). Anal. (C$_{28}$H$_{35}$N$_4$O$_9$P·1H$_2$O·0.5i-PrOH) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-N[(CH$_3$)[2-(3-ethylindole)]] (22): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.77 (m, 2H), 1.81 (s, 3H), 2.21 (m, 2H), 2.79 (m, 2H), 2.97 (m, 5H), 3.61 (m, 2H), 4.51 (m, 1H), 4.77 (m, 1H), 7.06 (m, 7H), 7.34 (m, 1H), 7.58 (m, 2H), 10.45 (br s, 1H); MS (FAB) m/z 589 (MH$^+$). Anal. (C$_{27}$H$_{33}$N$_4$O$_9$P·1H$_2$O·0.5i-PrOH) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-NH[2-(3-ethyl-5-hydroxyindole)] (23): ^{1}H NMR (300 MHz, CD$_3$OD) δ 1.87 (m, 1H), 1.91 (s, 3H), 2.20 (m, 1H), 2.3 (m, 2H), 2.85 (m, 3H), 3.06 (m, 1H), 3.48 (m, 2H), 4.30 (m, 1H), 4.56 (m, 1H), 6.63 (d, 1H), 6.93 (s, 1H), 7.02 (s, 1H), 7.12 (m, 3H), 7.20 (d, 2H), 7.80 (t, 1H, exchangeable), 8.19 (d, 1H, exchangeable), 10.40 (br s, 1H); MS (FAB) m/z 591 (MH$^+$). Anal. (C$_{26}$H$_{31}$N$_4$O$_{10}$P·0.5H$_2$O·1i-PrOH) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-NH[2-(3-ethyl-5-methoxyindole)] (24): ^{1}H NMR (300 MHz, CD$_3$OD) δ 1.86 (m, 1H), 1.90 (s, 3H), 2.30 (m, 2H), 2.81 (m, 1H), 2.90 (m, 2H), 3.04 (dd, 1H), 3.48 (m, 2H), 3.82 (s, 3H), 4.29 (m, 1H), 4.53 (m, 1H), 6.72 (d, 1H), 7.06 (s, 2H), 7.12 (d, 2H), 7.19 (m, 3H), 7.81 (t, 1H, exchangeable), 8.17 (d, 1H, exchangeable), 10.35 (br s, 1H); MS (ESI) m/z 603 (M − H). Anal. (C$_{27}$H$_{33}$N$_4$O$_{10}$P·2.25H$_2$O) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-N(CH$_3$)[(CH$_2$)$_6$OH] (25): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (m, 4H), 1.46 (m, 4H), 1.73 (m, 1H), 1.80 (s, 3H), 1.91 (m, 1H), 2.22 (m, 2H), 2.79 (dd, 1H), 2.91 (m, 3H), 3.00 (dd, 1H), 3.31 (m, 2H), 3.42 (m, 2H), 4.48 (m, 1H), 4.78 (m, 1H), 7.03 (d, 2H), 7.17 (d, 2H), 7.59 (d, 2H, exchangeable); MS (FAB) m/z 546 (MH$^+$). Anal. (C$_{26}$H$_{31}$N$_4$O$_{10}$P·0.5i-PrOH) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-N(CH$_3$)[(CH$_2$)$_7$OH] (26): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (m, 6H), 1.53 (m, 4H), 1.78 (m, 1H), 1.80 (s, 3H), 1.98 (m, 1H), 2.30 (m, 2H), 2.92 (m, 5H), 3.39 (m, 4H), 4.52 (m, 1H), 4.80 (m, 1H), 7.08 (d, 2H), 7.19 (d, 2H), 7.62 (br s, 2H, exchangeable); MS (FAB) m/z 560 (MH$^+$). Anal. (C$_{24}$H$_{38}$N$_3$O$_{10}$P·0.5H$_2$O) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Glu-N(CH$_3$)[(CH$_2$)$_8$OH] (27): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (m, 8H), 1.48 (m, 4H), 1.77 (m, 1H), 1.80 (s, 3H), 1.90 (m, 1H), 2.23 (m, 2H), 2.80 (m, 1H), 2.93 (m, 4H), 3.37 (m, 4H), 4.50 (m, 1H), 4.78 (m, 1H), 7.03 (d, 2H), 7.14 (d, 2H), 7.60 (br d, 2H, exchangeable); MS (ESI) m/z 574 (MH$^+$). Anal. (C$_{25}$H$_{40}$N$_3$O$_{10}$P·1H$_2$O) C, H, N.

Z-Glu(Ot-Bu)-N(n-C$_5$H$_{11}$)$_2$ (29). Isobutyl chloroformate (4.25 mL, 32.8 mmol) was added to a solution of **28** (10.1 g, 29.8 mmol) and 4-methylmorpholine (9.82 mL, 89.5 mmol) in THF (150 mL) at −20 °C. The mixture was stirred for 5 min, treated with HN(n-C$_5$H$_{11}$)$_2$ (6.03 mL, 29.8 mmol), and allowed to warm to room temperature over 14 h. The mixture was diluted with saturated aqueous NaHCO$_3$ (ca. 50 mL) and concentrated under reduced pressure. The residue was extracted with EtOAc (2 × 150 mL). The combined organic extracts were washed with saturated aqueous NaHCO$_3$ (1 × 50 mL), H$_2$O (1 × 50 mL), and saturated aqueous NaCl (1 × 50 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (4:1), to provide 11.3 g (80%) of **29** as an oil.

Z-Tyr-Glu(Ot-Bu)-N(n-C$_5$H$_{11}$)$_2$ (30). A mixture of **29** (11.3 g, 23.7 mmol) and 10% Pd−C (200 mg) in MeOH (120 mL) was stirred under H$_2$ (1 atm) for 4 h. The reaction mixture was purged with N$_2$ and filtered through a pad of Celite with additional MeOH (ca. 100 mL), and the filtrate was concen-

trated under reduced pressure to provide 8.15 g (90% yield) of H-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ as an oil. A solution of H-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (8.10 g, 23.7 mmol) in CH$_2$Cl$_2$ (20 mL) was added to a solution of Z-Tyr-OH (7.46 g, 23.7 mmol), DCC (5.37 g, 26.1 mmol), and HOBT (3.52 g, 26.1 mmol) in CH$_2$Cl$_2$ (80 mL) and DMF (20 mL) at 0 °C. Et$_3$N (4.00 mL, 28.4 mmol) was added, and the mixture was allowed to warm to room temperature over 16 h. The reaction mixture was filtered through a pad of Celite using CH$_2$Cl$_2$ (ca. 100 mL), and the filtrate was concentrated under reduced pressure. The residue was diluted with H$_2$O and extracted with EtOAc (2 × 150 mL). The combined organic extracts were washed with H$_2$O (3 × 50 mL) and saturated aqueous NaCl (1 × 50 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (2:1), to provide 11.8 g (78%) of **30** as a foam.

Z-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (31). A solution of **30** (1.00 g, 1.56 mmol) in THF (8 mL) was added to a suspension of NaH (78 mg of 60% oil dispersion, 1.96 mmol) in THF (6 mL) at 0 °C, and the mixture was stirred for 30 min. A solution of di-*tert*-butyl phosphorochloridate (429 mg, 1.88 mmol) in THF (2 mL) was added, and the mixture was allowed to warm to room temperature over 4 h. The mixture was treated with saturated aqueous NaHCO$_3$ (ca. 20 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with H$_2$O (2 × 25 mL) and saturated aqueous NaCl (1 × 25 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (3:2), to provide 730 mg (56%) of **31** as an oil.

H-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (32). A mixture of **31** (1.13 g, 1.36 mmol) and 10% Pd−C (100 mg) in MeOH (14 mL) was stirred under H$_2$ (1 atm) for 3 h. The reaction mixture was purged with N$_2$ and filtered through a pad of Celite with additional MeOH (ca. 20 mL), and the filtrate was concentrated under reduced pressure to provide 935 mg (98%) of **32** as an oil.

PhCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (33). A solution of **32** (436 mg, 0.63 mmol) and benzoic acid (77 mg, 0.63 mmol) in CH$_2$Cl$_2$ (2.75 mL) and DMF (1.25 mL) was treated with DCC (142 mg, 0.69 mmol) and HOBT (93 mg, 0.69 mmol) at 0 °C. Et$_3$N (0.11 mL, 0.79 mmol) was added, and the mixture was allowed to warm to room temperature overnight. The reaction mixture was filtered through a pad of Celite using CH$_2$Cl$_2$ (ca. 10 mL), and the filtrate was concentrated under reduced pressure. The residue was diluted with H$_2$O and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with saturated aqueous NaHCO$_3$ (1 × 10 mL), H$_2$O (2 × 10 mL), and saturated aqueous NaCl (1 × 10 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (2:1), to provide 441 mg (88%) of **33** as a foam.

PhCH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (34): prepared in this fashion from **32** and phenylacetic acid; 320 mg (60%) as a white foam.

PhCH$_2$CH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (35): prepared in this fashion from **32** and 3-phenyl-propionic acid; 272 mg (57%) as a white foam.

PhSCH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (36): prepared in this fashion from **32** and (phenylthio)-acetic acid; 290 mg (63%) as a white foam.

(4-OH)PhCH$_2$CH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (37): prepared in this fashion from **32** and 3-(4-hydroxyphenyl)propionic acid; 375 mg (52%) as a white foam.

(3-Pyridyl)CH$_2$CH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (38): prepared in this fashion from **32** and 3-(3-pyridyl)propionic acid; 685 mg (66%).

PhCH$_2$CH$_2$CH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (39): prepared in this fashion from **32** and 4-phenyl-butyric acid; 316 mg (65%) as a colorless oil.

PhCH$_2$SCH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (41): prepared in this fashion from **32** and (benzylthio)-acetic acid; 307 mg (62%) as a colorless oil.

(4-Cl)PhCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (43): prepared in this fashion from **32** and 4-chlorobenzoic acid; 470 mg (90%) as a white foam.

(4-CF$_3$)PhCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (44): prepared in this fashion from **32** and 4-(trifluoromethyl)benzoic acid; 476 mg (88%) as a foam.

(4-NO$_2$)PhCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (45): prepared in this fashion from **32** and 4-nitrobenzoic acid; 319 mg (69%) as a white foam.

(4-MeO)PhCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (46): prepared in this fashion from **32** and 4-methoxy-benzoic acid; 301 mg (66%) as a white foam.

(4-BOCNHCH$_2$)PhCH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (50): prepared in this fashion from **32** and **71**; 301 mg (60%) as a yellow foam.

PhCH$_2$OCH$_2$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (40). Benzyloxyacetyl chloride (0.13 mL, 0.79 mmol) was added to a suspension of **32** (530 mg, 0.76 mmol) and NaH (36 mg of 60% oil dispersion, 0.90 mmol), in THF (15 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C, treated with saturated aqueous NaHCO$_3$ (ca. 5 mL), concentrated under reduced pressure, and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with saturated aqueous NaHCO$_3$ (1 × 5 mL), H$_2$O (1 × 5 mL), and saturated aqueous NaCl (1 × 5 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (gradient, 3:2−1:5), to provide 336 mg (53%) of **40** as a white foam.

CF$_3$CO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (42). Trifluoroacetic anhydride (0.11 mL, 0.79 mmol) was added to a mixture of **32** (530 mg, 0.76 mmol) and NaH (36 mg of 60% oil dispersion, 0.90 mmol) in THF (15 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C, treated with saturated aqueous NaHCO$_3$ (ca. 5 mL), concentrated under reduced pressure, and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with saturated aqueous NaHCO$_3$ (1 × 5 mL), H$_2$O (1 × 5 mL), and saturated aqueous NaCl (1 × 5 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (gradient, 3:2−1:5), to provide 261 mg (44%) of **42** as a white foam.

(4-NO$_2$)PhNHCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (49). A catalytic amount of DMAP was added to a solution of **32** (590 mg, 0.85 mmol) and 4-nitrophenyl isocyanate (154 mg, 0.94 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C, and the mixture was allowed to warm to room temperature overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography, elution with hexanes−EtOAc (gradient, 3:2−1:5), to provide 491 mg (67%) of **49** as a foam.

(4-MeO)PhNHCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (48): prepared in this fashion from **32** and 4-methoxy-phenyl isocyanate; 266 mg (34%) as a foam.

(4-NH$_2$)PhNHCO-Tyr[P(O)(O*t*-Bu)$_2$]-Glu(O*t*-Bu)-N(*n*-C$_5$H$_{11}$)$_2$ (47). A mixture of **49** (229 mg, 0.27 mmol) and 10% Pd−C (44 mg) in MeOH (2.6 mL) was stirred under H$_2$ (1 atm) for 4 h. The reaction mixture was purged with N$_2$ and filtered through a pad of Celite with additional MeOH (ca. 100 mL), and the filtrate was concentrated under reduced pressure. The oily residue was purified by flash chromatography, elution with MeOH−CH$_2$Cl$_2$ (gradient, 1:19−1:4), to provide 204 mg (93%) of **47** as a white foam.

Z-Tyr[P(O)(OH)$_2$]-Glu-N(*n*-C$_5$H$_{11}$)$_2$ (51). TFA (0.40 mL, 5.19 mmol) was added to a solution of **31** (200 mg, 0.24 mmol) in CH$_2$Cl$_2$ (2 mL). The mixture was stirred for 16 h and concentrated under reduced pressure to give a foam. This foam was washed with hexane (3 × 5 mL) and Et$_2$O (2 × 5 mL), and excess solvent was removed in vacuo to provide 138 mg (86%) of **51** as a white foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, $J = 8.4$ Hz, 1H), 7.47 (d, $J = 8.6$ Hz, 1H), 7.27 (comp, 7H), 7.05 (d, $J = 8.0$ Hz, 2H), 4.95 (comp, 2H), 4.73 (ddd, $J = 8.8, 8.8, 4.5$ Hz, 1H), 4.26 (m, 1H), 3.37 (comp, 3H), 3.27 (m, 1H), 3.19 (dt, $J = 13.2, 7.0$ Hz, 1H), 2.92 (dd, $J = 13.8, 3.3$ Hz, 1H), 2.68 (m, 1H), 2.28 (dd, $J = 7.4, 7.0$ Hz, 2H),

1.85 (m, 1H), 1.68 (m, 1H), 1.56 (m, 2H), 1.45 (m, 2H), 1.39–1.15 (comp, 8H), 0.89 (t, J = 7.1 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); MS (ESI) m/z 662 (M – H, 100); HRFAB-MS calcd for $C_{32}H_{46}N_3O_{10}P$ (MH$^+$) 664.2999, found 664.3005 (MH$^+$). Anal. ($C_{32}H_{46}N_3O_{10}P\cdot0.5H_2O$) C, H, N.

PhCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (52): prepared in this fashion from **33**; 255 mg (75%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, J = 8.3 Hz, 1H), 8.26 (d, J = 8.5 Hz, 1H), 7.76 (d, J = 7.3 Hz, 1H), 7.50 (dd, J = 7.3, 7.3 Hz, 1H), 7.43 (dd, J = 7.7, 7.3 Hz, 2H), 7.30 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.2 Hz, 2H), 4.73 (ddd, J = 13.1, 8.8, 4.4 Hz, 1H), 4.67 (dd, J = 8.3, 3.5 Hz, 1H), 3.34 (m, 2H), 3.26 (m, 1H), 3.09 (m, 1H), 3.02 (dd, J = 13.1, 3.4 Hz, 1H), 2.92 (m, 1H), 2.28 (dd, J = 7.2, 7.0 Hz, 2H), 1.84 (m, 1H), 1.69 (m, 1H), 1.55 (m, 2H), 1.42 (m, 2H), 1.34–1.15 (comp, 8H), 0.85 (t, J = 7.0 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); MS (FAB) m/z 634 (M + H, 68), 105 (100); HRFAB-MS calcd for $C_{31}H_{44}N_3O_9P$ (MH$^+$) 634.2893, found 634.2894 (MH$^+$). Anal. ($C_{31}H_{44}N_3O_9P\cdot0.25H_2O$) C, H, N.

PhCH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (53): prepared in this fashion from **34**; 218 mg (91%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.3 Hz, 1H), 7.21 (dd, J = 7.2, 7.0 Hz, 2H), 7.16 (d, J = 7.2 Hz, 1H), 7.13 (d, J = 8.3 Hz, 2H), 7.07 (d, J = 7.0 Hz, 2H), 6.98 (d, J = 8.2 Hz, 2H), 4.71 (ddd, J = 9.0, 8.8, 4.2 Hz, 1H), 4.55 (ddd, J = 9.0, 8.8, 3.8 Hz, 1H), 3.36 (comp, 4H), 3.24 (m, 1H), 3.12 (dt, J = 13.3, 7.0, 6.8 Hz, 1H), 2.92 (dd, J = 13.8, 4.2 Hz, 1H), 2.69 (dd, J = 13.8, 8.8 Hz, 1H), 2.23 (dd, J = 7.7, 7.6 Hz, 2H), 1.80 (m, 1H), 1.73–1.59 (comp, 6H), 1.29–1.14 (comp, 8H), 0.88 (t, J = 7.1 Hz, 3H), 0.84 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 648 (M + H, 40), 158 (100); HRFAB-MS calcd for $C_{32}H_{46}N_3O_9P$ (MH$^+$) 648.3050, found 642.3047 (MH$^+$). Anal. ($C_{32}H_{46}N_3O_9P$) C, H, N.

PhCH₂CH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (54): prepared in this fashion from **35**; 84 mg (41%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.22 (dd, J = 7.4, 7.3 Hz, 2H), 7.13 (comp, 5H), 7.00 (d, J = 8.4 Hz, 2H), 4.69 (ddd, J = 8.8, 8.8, 4.5 Hz, 1H), 4.52 (ddd, J = 9.0, 9.0, 4.0 Hz, 1H), 3.34 (m, 2H), 3.25 (m, 1H), 3.09 (m, 1H), 2.89 (dd, J = 13.8, 4.0 Hz, 1H), 2.69 (t, J = 7.7 Hz, 2H), 2.64 (m, 1H), 2.33 (t, J = 7.7 Hz, 2H), 2.24 (dd, J = 7.5, 7.5 Hz, 2H), 1.84 (m, 1H), 1.66 (m, 1H), 1.54 (m, 2H), 1.43 (m, 2H), 1.34–1.15 (comp, 8H), 0.85 (t, J = 7.1 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); MS (FAB) m/z 662 (M + H, 25), 158 (100); HRFAB-MS calcd for $C_{33}H_{48}N_3O_9P$ (MH$^+$) 662.3206, found 662.3194 (MH$^+$). Anal. ($C_{33}H_{48}N_3O_9P\cdot0.5H_2O$) C, H, N.

PhSCH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (55): prepared in this fashion from **36**; 90 mg (94%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 8.3 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 7.24 (comp, 4H), 7.14 (m, 1H), 7.09 (d, J = 8.3 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 4.68 (dd, J = 13.2, 8.9 Hz, 1H), 4.55 (ddd, J = 8.3, 7.3, 4.0 Hz, 1H), 3.64 (s, 2H), 3.34 (m, 2H), 3.23 (m, 1H), 3.10 (m, 1H), 2.89 (dd, J = 14.0, 4.0 Hz, 1H), 2.70 (dd, J = 14.0, 9.2 Hz, 1H), 2.23 (m, 2H), 1.83 (m, 1H), 1.66 (m, 1H), 1.55 (m, 2H), 1.44 (m, 2H), 1.34–1.11 (comp, 8H), 0.84 (t, J = 7.0 Hz, 3H), 0.86 (t, J = 7.2 Hz, 3H); MS (FAB) m/z 680 (M + H, 55), 158 (100); HRFAB-MS calcd for $C_{32}H_{46}N_3O_9PS$ (MH$^+$) 680.2771, found 680.2778 (MH$^+$). Anal. ($C_{32}H_{46}N_3O_9PS\cdot0.5H_2O$) C, H, N.

(4-OH)PhCH₂CH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (56): prepared in this fashion from **37**; 75 mg (93%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (br s, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.3 Hz, 1H), 7.13 (d, J = 8.3 Hz, 2H), 7.01 (d, J = 8.2 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 6.61 (d, J = 8.3 Hz, 2H), 4.69 (ddd, J = 8.8, 8.8, 4.4 Hz, 1H), 4.51 (ddd, J = 8.9, 8.9, 4.1 Hz, 1H), 3.34 (m, 2H), 3.23 (m, 1H), 3.08 (m, 1H), 2.89 (dd, J = 13.9, 4.1 Hz, 1H), 2.65 (dd, J = 13.9, 9.7 Hz, 1H), 2.56 (m, 2H), 2.27 (comp, 4H), 1.84 (m, 1H), 1.66 (m, 1H), 1.53 (m, 2H), 1.43 (m, 2H), 1.34–1.15 (comp, 8H), 0.86 (t, J = 7.0 Hz, 3H), 0.83 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 678 (M + H, 27), 158 (100); HRFAB-MS calcd for $C_{33}H_{48}N_3O_{10}P$ (MH$^+$) 678.3156, found 678.3154 (MH$^+$). Anal. ($C_{33}H_{48}N_3O_{10}P\cdot0.5H_2O$) C, H, N.

(3-Pyridyl)CH₂CH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (57): prepared in this fashion from **38**; 55 mg (33%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 5.5 Hz, 1H), 8.23 (s, 1H), 8.22 (d, J = 8.5 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.55 (dd, J = 8.0, 5.5 Hz, 1H), 7.11 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 8.2 Hz, 2H), 4.68 (ddd, J = 8.8, 8.8, 4.5 Hz, 1H), 4.51 (ddd, J = 9.4, 9.0, 3.8 Hz, 1H), 3.34 (m, 2H), 3.25 (m, 1H), 3.09 (m, 1H), 2.88 (m, 1H), 2.79 (m, 2H), 2.59 (dd, J = 13.8, 10.7 Hz, 1H), 2.42 (m, 2H), 2.23 (m, 2H), 1.83 (m, 1H), 1.66 (m, 1H), 1.54 (m, 2H), 1.43 (m, 2H), 1.34–1.15 (comp, 8H), 0.86 (t, J = 7.0 Hz, 3H), 0.83 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 663 (M + H, 100); HRFAB-MS calcd for $C_{32}H_{47}N_4O_9P$ (MH$^+$) 663.3159, found 663.3157 (MH$^+$). Anal. ($C_{32}H_{47}N_4O_9P\cdot0.5TFA$) C, H, N.

PhCH₂CH₂CH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (58): prepared in this fashion from **39**; 97 mg (39%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.24 (dd, J = 7.5, 7.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 7.3 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 4.76 (ddd, J = 8.8, 8.8, 4.5 Hz, 1H), 4.51 (ddd, J = 9.2, 9.2, 3.8 Hz, 1H), 3.37 (m, 2H), 3.24 (m, 1H), 3.07 (m, 1H), 2.92 (dd, J = 13.7, 3.8 Hz, 1H), 2.67 (dd, J = 13.7, 10.4 Hz, 1H), 2.44 (t, J = 7.5 Hz, 2H), 2.24 (t, J = 6.3 Hz, 2H), 2.03 (m, 2H), 1.82 (m, 1H), 1.67 (t, J = 7.5 Hz, 2H), 1.62 (m, 1H), 1.53 (m, 2H), 1.41 (m, 2H), 1.34–1.15 (comp, 8H), 0.86 (t, J = 7.1 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); MS (FAB) m/z 676 (M + H, 45), 158 (100); HRFAB-MS calcd for $C_{34}H_{50}N_3O_9P$ (MH$^+$) 676.3363, found 676.3360 (MH$^+$). Anal. ($C_{34}H_{50}N_3O_9P\cdot0.5H_2O$) C, H, N.

PhCH₂OCH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (59): prepared in this fashion from **40**; 74 mg (28%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.33 (comp, 5H), 7.14 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.2 Hz, 2H), 4.71 (ddd, J = 8.8, 8.8, 4.3 Hz, 1H), 4.60 (ddd, J = 8.6, 8.6, 4.3 Hz, 1H), 4.44 (s, 2H), 3.83 (ABq, J_{AB} = 15.0 Hz, $\Delta\nu_{AB}$ = 15.0 Hz, 2H), 3.36 (m, 2H), 3.25 (m, 1H), 3.10 (m, 1H), 2.97 (dd, J = 13.7, 4.0 Hz, 1H), 2.83 (dd, J = 13.8, 9.0 Hz, 1H), 2.25 (m, 2H), 1.85 (m, 1H), 1.69 (m, 1H), 1.54 (m, 2H), 1.45 (m, 2H), 1.34–1.11 (comp, 8H), 0.85 (t, J = 7.0 Hz, 3H), 0.84 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 678 (M + H, 62), 158 (100); HRFAB-MS calcd for $C_{33}H_{48}N_3O_{10}P$ (MH$^+$) 678.3156, found 678.3160 (MH$^+$). Anal. ($C_{33}H_{48}N_3O_{10}P\cdot0.5TFA$) C, H, N.

PhCH₂SCH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (60): prepared in this fashion from **41**; 107 mg (44%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 7.4, 7.3 Hz, 2H), 7.22 (comp, 3H), 7.18 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 4.71 (ddd, J = 8.8, 8.8, 4.4 Hz, 1H), 4.56 (ddd, J = 8.8, 8.8, 4.0 Hz, 1H), 3.64 (ABq, J_{AB} = 13.0 Hz, $\Delta\nu_{AB}$ = 13.3 Hz, 2H), 3.35 (m, 2H), 3.26 (m, 1H), 3.08 (m, 1H), 3.00 (s, 2H), 2.95 (dd, J = 13.9, 4.0 Hz, 1H), 2.73 (dd, J = 13.9, 9.5 Hz, 1H), 2.26 (m, 2H), 1.84 (m, 1H), 1.68 (m, 1H), 1.55 (m, 2H), 1.44 (m, 2H), 1.34–1.15 (comp, 8H), 0.85 (t, J = 7.2 Hz, 3H), 0.83 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 694 (M + H, 22), 158 (100); HRFAB-MS calcd for $C_{33}H_{48}N_3O_9PS$ (MH$^+$) 694.2927, found 694.2921 (MH$^+$). Anal. ($C_{33}H_{48}N_3O_9PS\cdot0.5H_2O$) C, H, N.

CF₃CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (61): prepared in this fashion from **42**; 95 mg (46%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (d, J = 8.5 Hz, 1H), 8.49 (d, J = 8.3 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 4.71 (ddd, J = 8.8, 8.8, 4.6 Hz, 1H), 4.58 (dd, J = 8.0, 3.5 Hz, 1H), 3.35 (m, 2H), 3.28 (m, 1H), 3.09 (m, 1H), 2.99 (dd, J = 13.8, 3.5 Hz, 1H), 2.83 (dd, J = 13.7, 5.7 Hz, 1H), 2.26 (m, 2H), 1.84 (m, 1H), 1.69 (m, 1H), 1.56 (m, 2H), 1.43 (m, 2H), 1.34–1.11 (comp, 8H), 0.87 (t, J = 7.0 Hz, 3H), 0.83 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 626 (M + H, 65), 158 (100); HRFAB-MS calcd for $C_{26}H_{39}F_3N_3O_9P$ (MH$^+$) 626.2454, found 626.2460 (MH$^+$). Anal. ($C_{26}H_{39}F_3N_3O_9P\cdot0.5H_2O$) C, H, N.

(4-Cl)PhCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (62): prepared in this fashion from **43**; 131 mg (49%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 8.3 Hz, 1H), 8.27 (d, J = 8.3 Hz, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.49 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 4.67

(m, 2H), 3.32 (m, 2H), 3.24 (m, 1H), 3.07 (m, 1H), 2.99 (dd, J = 13.7, 3.3 Hz, 1H), 2.87 (dd, J = 13.7, 11.3 Hz, 1H), 2.25 (dd, J = 7.3, 7.0 Hz, 2H), 1.84 (m, 1H), 1.67 (m, 1H), 1.51 (m, 2H), 1.40 (m, 2H), 1.34−1.11 (comp, 8H), 0.83 (t, J = 7.0 Hz, 3H), 0.80 (t, J = 7.2 Hz, 3H); MS (FAB) m/z 668 (M + H, 30), 158 (100); HRFAB-MS calcd for $C_{31}H_{43}ClN_3O_9P$ (MH$^+$) 668.2504, found 668.2498 (MH$^+$). Anal. ($C_{31}H_{43}ClN_3O_9P\cdot0.5H_2O$) C, H, N.

(4-CF₃)PhCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (63): prepared in this fashion from **44**; 48 mg (21%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 4.73 (comp, 2H), 3.34 (m, 2H), 3.28 (m, 1H), 3.10 (m, 1H), 3.03 (dd, J = 13.9, 3.7 Hz, 1H), 2.91 (m, 1H), 2.28 (dd, J = 7.2, 7.0 Hz, 2H), 1.86 (m, 1H), 1.70 (m, 1H), 1.55 (m, 2H), 1.42 (m, 2H), 1.34−1.15 (comp, 8H), 0.85 (t, J = 7.0 Hz, 3H), 0.82 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 702 (M + H, 45), 173 (100); HRFAB-MS calcd for $C_{32}H_{43}F_3N_3O_9P$ (MH$^+$) 702.2767, found 702.2759 (MH$^+$). Anal. ($C_{32}H_{43}F_3N_3O_9P\cdot0.75H_2O$) C, H, N.

(4-NO₂)PhCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (64): prepared in this fashion from **45**; 111 mg (93%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (d, J = 8.4 Hz, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.28 (d, J = 8.7 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 4.73 (comp, 2H), 3.33 (m, 2H), 3.27 (m, 1H), 3.10 (m, 1H), 3.04 (m, 1H), 2.90 (dd, J = 12.5, 12.5 Hz, 1H), 2.28 (dd, J = 7.1, 7.0 Hz, 2H), 1.86 (m, 1H), 1.70 (m, 1H), 1.55 (m, 2H), 1.42 (m, 2H), 1.34−1.11 (comp, 8H), 0.85 (t, J = 6.9 Hz, 3H), 0.82 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 679 (M + H, 35), 158 (100); HRFAB-MS calcd for $C_{31}H_{44}N_4O_{11}P$ (MH$^+$) 679.2744, found 679.2751 (MH$^+$). Anal. ($C_{31}H_{44}N_4O_{11}P\cdot0.25H_2O$) C, H, N.

(4-MeO)PhCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (65): prepared in this fashion from **46**; 126 mg (53%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, J = 8.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 4.72 (ddd, J = 8.8, 7.0, 4.6 Hz, 1H), 4.65 (ddd, J = 9.6, 9.6, 3.8 Hz, 1H), 3.78 (s, 3H), 3.35 (m, 2H), 3.25 (m, 1H), 3.07 (m, 1H), 3.01 (dd, J = 13.8, 3.8 Hz, 1H), 2.91 (dd, J = 12.3, 11.4 Hz, 1H), 2.27 (dd, J = 7.0, 7.0 Hz, 2H), 1.84 (m, 1H), 1.69 (m, 1H), 1.55 (m, 2H), 1.42 (m, 2H), 1.34−1.11 (comp, 8H), 0.85 (t, J = 7.0 Hz, 3H), 0.82 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 664 (M + H, 30), 135 (100); HRFAB-MS calcd for $C_{32}H_{46}N_3O_{10}P$ (MH$^+$) 664.2999, found 664.3014 (MH$^+$). Anal. ($C_{32}H_{46}N_3O_{10}P\cdot0.5TFA$) C, H, N.

(4-NH₂)PhNHCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (66): prepared in this fashion from **47**; 78 mg (49%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (s, 1H), 8.33 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 8.7 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.22 (d, J = 8.0 Hz, 1H), 4.72 (ddd, J = 8.9, 8.9, 4.3 Hz, 1H), 4.49 (dd, J = 12.4, 7.8 Hz, 1H), 3.36 (m, 2H), 3.26 (m, 1H), 3.12 (m, 1H), 2.94 (dd, J = 14.0, 4.3 Hz, 1H), 2.76 (dd, J = 14.0, 7.8 Hz, 1H), 2.25 (m, 2H), 1.85 (m, 1H), 1.68 (m, 1H), 1.53 (m, 2H), 1.45 (m, 2H), 1.34−1.11 (comp, 8H), 0.85 (t, J = 6.8 Hz, 3H), 0.84 (t, J = 7.0 Hz, 3H); MS (ESI) m/z 662 (M − H, 100); HRFAB-MS calcd for $C_{31}H_{46}N_5O_9P$ (MH$^+$) 664.3111, found 664.3107 (MH$^+$). Anal. ($C_{31}H_{46}N_5O_9P\cdot0.67TFA$) C, H, N.

(4-MeO)PhNHCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (67): prepared in this fashion from **48**; 107 mg (55%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.32 (d, J = 8.3 Hz, 1H), 7.22 (d, J = 8.9 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 9.0 Hz, 2H), 6.12 (d, J = 8.0 Hz, 1H), 4.72 (ddd, J = 8.8, 8.8, 4.4 Hz, 1H), 4.50 (dd, J = 13.6, 7.8 Hz, 1H), 3.67 (s, 3H), 3.34 (m, 2H), 3.26 (m, 1H), 3.13 (m, 1H), 2.94 (dd, J = 12.6, 4.4 Hz, 1H), 2.75 (dd, J = 13.6, 8.0 Hz, 1H), 2.25 (m, 2H), 1.82 (m, 1H), 1.68 (m, 1H), 1.53 (m, 2H), 1.45 (comp, 2H), 1.34−1.18 (comp, 8H), 0.86 (t, J = 6.8 Hz, 3H), 0.84 (t, J = 7.0 Hz, 3H); MS (FAB) m/z 679 (M + H, 43), 158 (100); HRFAB-MS calcd for $C_{32}H_{47}N_4O_{10}P$ (MH$^+$) 679.3108, found 664.3102 (MH$^+$). Anal. ($C_{32}H_{47}N_4O_{10}P\cdot0.25H_2O$) C, H, N.

(4-NO₂)PhNHCO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅H₁₁)₂ (68): prepared in this fashion from **49**; 137 mg (65%) as a foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.45 (d, J = 8.6 Hz, 1H), 8.11 (d, J = 9.2 Hz, 2H), 7.56 (d, J = 9.3 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 7.01 (J = 8.2 Hz, 2H), 6.54 (d, J = 8.0 Hz, 1H), 4.73 (ddd, J = 8.9, 8.9, 4.3 Hz, 1H), 4.55 (dd, J = 12.2, 7.6 Hz, 1H), 3.35 (m, 2H), 3.26 (M, 1H), 3.13 (m, 1H), 2.98 (dd, J = 13.8, 4.3 Hz, 1H), 2.80 (dd, J = 13.8, 7.6 Hz, 1H), 2.28 (m, 2H), 1.85 (m, 1H), 1.69 (m, 1H), 1.55 (m, 2H), 1.45 (m, 2H), 1.34−1.15 (comp, 8H), 0.85 (J = 7.0 Hz, 3H), 0.83 (t, J = 7.1 Hz, 3H); MS (FAB) m/z 694 (M + H, 37), 158 (100); HRFAB-MS calcd for $C_{31}H_{44}N_5O_{11}P$ (MH$^+$) 694.2853, found 679.2850 (MH$^+$). Anal. ($C_{31}H_{44}N_5O_{11}P\cdot0.5H_2O$) C, H, N.

(4-H₂NCH₂)PhCH₂CO-Tyr[P(O)(OH)₂]-Glu-N(*n*-C₅-H₁₁)₂ (69): prepared in this fashion from **50**; 157 mg (73%) as a white foam; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (br s, 2H), 8.31 (d, J = 8.5 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8.0 Hz, 2H), 4.71 (dd, J = 13.2, 8.8 Hz, 1H), 4.48 (ddd, J = 10.0, 10.0, 3.2 Hz, 1H), 3.87 (br s, 2H), 3.50 (d, J = 13.2 Hz, 1H), 3.36 (m, 2H), 3.30 (m, 1H), 3.17 (d, J = 13.2 Hz, 1H), 3.10 (m, 1H), 2.92 (d, J = 11.2 Hz, 1H), 2.63 (m, 1H), 2.25 (m, 2H), 1.83 (m, 1H), 1.66 (m, 1H), 1.55 (m, 2H), 1.44 (m, 2H), 1.36−1.15 (comp, 8H), 0.87 (t, J = 6.9 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); MS (FAB) m/z 677 (M + H, 100); HRFAB-MS calcd for $C_{33}H_{50}N_4O_9P$ (MH$^+$) 677.3315, found 677.3323 (MH$^+$). Anal. ($C_{33}H_{49}N_4O_9P\cdot0.6TFA$) C, H, N.

4-[[(*tert*-Butoxycarbonyl)amino]methyl]phenylacetic Acid (71). Thionyl chloride (2.25 mL, 30.8 mmol) was added to a suspension of 4-(bromomethyl)phenylacetic acid (**70**) in PhCH₃ (200 mL), DMF (10 mL), and pyridine (2.70 mL, 33.4 mmol) at 0 °C. The reaction mixture was stirred for 5 min, then warmed to room temperature, stirred for an additional 75 min, and concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (200 mL), cooled to 0 °C, treated with benzyl alcohol (3.20 mL, 30.9 mmol), Et₃N (3.10 mL, 22.1 mmol), and a catalytic amount of DMAP, and stirred at ambient temperature overnight. The reaction mixture was washed with half-saturated aqueous NaHCO₃ (1 × 50 mL), H₂O (1 × 50 mL), 2 N aqueous NaOH (1 × 50 mL), and saturated aqueous NaCl (1 × 50 mL), dried (Na₂SO₄), and concentrated under reduced pressure. The residue was passed through a plug of silica gel (230−400 mesh), elution with hexanes−EtOAc (gradient, 5−10%), to provide 1.51 g (22%) of the benzyl ester as a brown amorphous solid that was dissolved in DMF (50 mL) and treated with sodium azide (605 mg, 9.30 mmol). The reaction mixture was stirred at room temperature overnight, diluted with H₂O (ca. 50 mL), and extracted with EtOAc (2 × 175 mL). The combined organic extracts were washed with H₂O (4 × 100 mL), half-saturated aqueous NaHCO₃ (1 × 100 mL), and saturated aqueous NaCl (1 × 150 mL), dried (Na₂SO₄), and concentrated under reduced pressure to give 1.28 g (98%) of the azide as a yellow oil. The azide (653 mg, 2.32 mmol) was dissolved in MeOH (25 mL), treated with BOC₂O (767 mg, 3.51 mmol) and 10% Pd−C (36 mg), and then stirred under H₂ (1 atm) overnight. The reaction mixture was flushed with N₂ and filtered through a pad of Celite with MeOH (ca. 25 mL), and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (25 mL), treated with 10% aqueous KHSO₄, and stirred vigorously for 5 min. The layers were separated, and the aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with H₂O (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried (Na₂SO₄), and concentrated under reduced pressure to give an oil that was triturated with Et₂O and heptane to provide 141 mg (23%) of **71** as an amorphous white solid.

BOC-Cys(Me)-OH (74). BOC₂O (4.36 g, 20.0 mmol) was added portionwise to a solution of H-Cys(Me)-OH (2.70 g, 20.0 mmol) and NaOH (1.20 g, 30.0 mmol) in H₂O (20 mL) and 1,4-dioxane (20 mL). The reaction mixture was stirred for 3 h and acidified (pH 4) with 10% aqueous KHSO₄, and the aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with saturated aque-

ous NaCl (1 × 50 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure to give 4.55 g (97%) of **74** as an oil that solidified on standing.

BOC-Met-N(*n*-C$_5$H$_{11}$)$_2$ (76). Isobutyl chloroformate (0.46 mL, 3.53 mmol) was added to a solution of **72** (800 mg, 3.20 mmol) and 4-methylmorpholine (1.10 mL, 9.60 mmol) in THF (32 mL) at −20 °C. The mixture was stirred for 5 min, treated with HN(*n*-C$_5$H$_{11}$)$_2$ (0.65 mL, 3.20 mmol), and allowed to warm to room temperature over 3.5 h. The mixture was added to EtOAc (ca. 100 mL), washed with saturated aqueous NaHCO$_3$ (1 × 50 mL), H$_2$O (1 × 50 mL), and saturated aqueous NaCl (1 × 50 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (5:1), to provide 1.17 g (94%) of **76** as an oil.

BOC-Met(O$_2$)-N(*n*-C$_5$H$_{11}$)$_2$ (77): prepared in this fashion from **73**; 960 mg (96%) as a solid.

BOC-Cys(Me)-N(*n*-C$_5$H$_{11}$)$_2$ (78): prepared in this fashion from **74**; 1.35 g (70%) as a colorless oil.

BOC-Cys(Bn)-N(*n*-C$_5$H$_{11}$)$_2$ (79): prepared in this fashion from **75**; 1.67 g (95%) as a colorless oil.

BOC-Tyr-Met-N(*n*-C$_5$H$_{11}$)$_2$ (80). HCl (3.0 mL of a 4 M solution in dioxane, 11.9 mmol) was added to a solution of **76** (1.15 g, 2.96 mmol) in CH$_2$Cl$_2$ (6 mL). The mixture was stirred for 14 h and concentrated under reduced pressure, and the residue was washed with Et$_2$O (3 × 5 mL). Excess solvent was removed in vacuo to provide 950 mg (98%) of the hydrochloride as a foam. A solution of HCl-H-Met-N(*n*-C$_5$H$_{11}$)$_2$ (1.02 g, 3.14 mmol) in CH$_2$Cl$_2$ (10 mL) was added to a solution of BOC-Tyr-OH (883 mg, 3.14 mmol), DCC (712 mg, 3.46 mmol), and HOBT (467 mg, 3.46 mmol) in CH$_2$Cl$_2$ (15 mL) and DMF (7 mL) at 0 °C. Et$_3$N (0.53 mL, 3.77 mmol) was added, and the mixture was allowed to warm to room temperature over 16 h. The reaction mixture was filtered through a pad of Celite using EtOAc (ca. 75 mL), and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (ca. 150 mL), washed with H$_2$O (3 × 25 mL) and saturated aqueous NaCl (1 × 25 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with hexanes−EtOAc (2:1), to provide 1.40 g (81%) of **80** as a foam.

BOC-Tyr-Met(O$_2$)-N(*n*-C$_5$H$_{11}$)$_2$ (81): prepared in this fashion from **77**; 935 mg (75%) as a white foam.

BOC-Tyr-Cys(Me)-N(*n*-C$_5$H$_{11}$)$_2$ (82): prepared in this fashion from **78**; 1.56 g (90%) as a foam.

BOC-Tyr-Cys(Bn)-N(*n*-C$_5$H$_{11}$)$_2$ (83): prepared in this fashion from **79**; 1.72 g (80%) as a white foam.

Ac-Tyr-Met-N(*n*-C$_5$H$_{11}$)$_2$ (84). HCl (2.52 mL of a 4 M solution in dioxane, 10.0 mmol) was added to a solution of **80** (1.39 g, 2.52 mmol) in CH$_2$Cl$_2$ (5 mL). The mixture was stirred for 3 h and concentrated under reduced pressure to provide 1.19 g (97%) of the hydrochloride as a foam. A solution of HCl-H-Tyr-Met-N(*n*-C$_5$H$_{11}$)$_2$ in CH$_2$Cl$_2$ (2 mL) was added to a solution of AcOH (31 mg, 0.51 mmol), DCC (116 mg, 0.56 mmol), and HOBT (76 mg, 0.56 mmol) in CH$_2$Cl$_2$ (2 mL) and DMF (1 mL) at 0 °C. Et$_3$N (0.11 mL, 0.79 mmol) was added, and the reaction mixture was allowed to warm to room temperature over 18 h. The mixture was filtered through a pad of Celite using EtOAc (ca. 50 mL), and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (ca. 75 mL), washed with H$_2$O (3 × 20 mL) and saturated aqueous NaCl (1 × 20 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with EtOAc, to provide 208 mg (82%) of **84** as a foam.

Ac-Tyr-Met(O$_2$)-N(*n*-C$_5$H$_{11}$)$_2$ (85): prepared in this fashion from **81**; 210 mg (46%) as a white foam.

Ac-Tyr-Cys(Me)-N(*n*-C$_5$H$_{11}$)$_2$ (86): prepared in this fashion from **82**; 520 mg (88%) as a white foam.

Ac-Tyr-Cys(Bn)-N(*n*-C$_5$H$_{11}$)$_2$ (87): prepared in this fashion from **83**; 535 mg (88%) as a white foam.

Ac-Tyr[P(O)(O*t*-Bu)$_2$]-Met-N(*n*-C$_5$H$_{11}$)$_2$ (88). A solution of **84** (108 mg, 0.22 mmol) in THF (1 mL) was added to a suspension of NaH (313 mg of 60% oil dispersion, 0.77 mmol)

at 0 °C in THF (0.7 mL), and the mixture was stirred for 30 min. A solution of di-*tert*-butyl phosphorochloridate (75 mg, 0.33 mmol) in THF (0.5 mL) was added, and the mixture was allowed to warm to room temperature over 16 h, then treated with saturated aqueous NaHCO$_3$ (ca. 5 mL), and poured into EtOAc (ca. 50 mL). The layers were separated, and the organic phase was washed with saturated aqueous NaHCO$_3$ (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, elution with 50:1 EtOAc−MeOH, to provide 85 mg (57%) of **88** as a foam.

Ac-Tyr[P(O)(O*t*-Bu)$_2$]-Met(O$_2$)-N(*n*-C$_5$H$_{11}$)$_2$ (89): prepared in this fashion from **85**; 81 mg (54%) as a colorless oil.

Ac-Tyr[P(O)(O*t*-Bu)$_2$]-Cys(Me)-N(*n*-C$_5$H$_{11}$)$_2$ (90): prepared in this fashion from **86**; 236 mg (59%) as a white foam.

Ac-Tyr[P(O)(O*t*-Bu)$_2$]-Cys(Bn)-N(*n*-C$_5$H$_{11}$)$_2$ (91): prepared in this fashion from **87**; 270 mg (66%) as an oil.

Ac-Tyr[P(O)(OH)$_2$]-Met-N(*n*-C$_5$H$_{11}$)$_2$ (92). HCl (0.51 mL of a 1 M solution in Et$_2$O, 0.51 mmol) was added to a solution of **88** (70 mg, 0.10 mmol) in CH$_2$Cl$_2$ (1.0 mL). The mixture was stirred for 3 h and concentrated under reduced pressure. Excess solvent was removed in vacuo to provide 58 mg (98%) of **92** as a foam: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.75 (br s, 1H), 7.06 (comp, 5H), 5.05 (m, 1H), 4.75 (m, 1H), 3.51 (m, 1H), 3.36 (m, 2H), 3.10 (m, 2H), 2.85 (m, 1H), 2.48 (m, 2H), 2.07 (s, 3H), 1.92 (m, 2H), 1.88 (s, 3H), 1.63 (m, 2H), 1.52 (m, 2H), 1.43−1.18 (comp, 8H), 0.92 (t, J = 6.6 Hz, 3H), 0.87 (t, J = 6.9 Hz, 3H); MS (FAB) m/z 574 (M + H, 43), 158 (100); HRFAB-MS calcd for C$_{26}$H$_{44}$N$_3$O$_7$PS (MH$^+$) 574.2716, found 574.2713 (MH$^+$). Anal. (C$_{26}$H$_{44}$N$_3$O$_7$PS·0.5H$_2$O) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Met(O$_2$)-N(*n*-C$_5$H$_{11}$)$_2$ (93): prepared in this fashion from **89**; 51 mg (91%) as an off-white foam; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.03 (br s, 1H), 7.43 (br s, 1H), 7.04 (comp, 4H), 5.05 (m, 1H), 4.73 (m, 1H), 3.48 (m, 1H), 3.33 (m, 2H), 3.10 (comp, 4H), 2.91 (s, 3H), 2.82 (m, 1H), 2.19 (m, 2H), 1.82 (s, 3H), 1.63 (m, 2H), 1.49 (m, 2H), 1.41−1.13 (comp, 8H), 0.90 (t, J = 6.8 Hz, 3H), 0.86 (t, J = 7.0 Hz, 3H); MS (FAB) m/z 606 (M + H, 85), 136 (100); HRFAB-MS calcd for C$_{26}$H$_{44}$N$_3$O$_9$PS (MH$^+$) 606.2614, found 606.2615 (MH$^+$). Anal. (C$_{26}$H$_{44}$N$_3$O$_9$PS·0.5H$_2$O) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Cys(Me)-N(*n*-C$_5$H$_{11}$)$_2$ (94): prepared in this fashion from **90**; 176 mg (96%) as a white foam; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.87 (br s, 1H), 7.30 (br s, 1H), 7.08 (comp, 4H), 5.04 (dd, J = 14.9, 7.7 Hz, 1H), 4.76 (dd, J = 7.7, 6.6 Hz, 1H), 3.49 (m, 1H), 3.38 (ddd, J = 14.9, 14.9, 7.7 Hz, 2H), 3.14 (m, 1H), 3.03 (dd, J = 13.8, 5.5 Hz, 1H), 2.87 (comp, 2H), 2.72 (dd, J = 13.8, 5.5 Hz, 1H), 2.11 (s, 3H), 1.89 (s, 3H), 1.65 (comp, 2H), 1.53 (comp, 2H), 1.41−1.20 (comp, 8H), 0.92 (t, J = 7.0 Hz, 3H), 0.87 (t, J = 7.0 Hz, 3H); MS (FAB) m/z 560 (M + H, 55), 158 (100); HRFAB-MS calcd for C$_{25}$H$_{42}$N$_3$O$_7$PS (MH$^+$) 560.2559, found 560.2549 (MH$^+$). Anal. (C$_{25}$H$_{42}$N$_3$O$_7$PS) C, H, N.

Ac-Tyr[P(O)(OH)$_2$]-Cys(Bn)-N(*n*-C$_5$H$_{11}$)$_2$ (95): prepared in this fashion from **91**; 220 mg (98%) as a white foam; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (br s, 1H), 7.27 (comp, 5H), 7.05 (comp, 4H), 6.84 (br s, 1H), 5.00 (dd, J = 14.2, 8.0 Hz, 1H), 4.78 (dd, J = 13.8, 8.0 Hz, 1H), 3.71 (s, 2H), 3.52 (ddd, J = 13.8, 8.0, 7.5 Hz, 1H), 3.23 (m, 2H), 3.09 (m, 1H), 2.99 (dd, J = 13.8, 5.5 Hz, 1H), 2.88 (dd, J = 14.2, 5.5 Hz, 1H), 2.79 (dd, J = 13.6, 8.0 Hz, 1H), 2.63 (dd, J = 13.6, 6.0 Hz, 1H), 1.89 (s, 3H), 1.61−1.46 (comp, 4H), 1.39−1.19 (comp, 8H), 0.92 (t, J = 7.2 Hz, 3H), 0.85 (t, J = 7.0 Hz, 3H); MS (FAB) m/z 636 (M + H, 78), 158 (100); HRFAB-MS calcd for C$_{31}$H$_{46}$N$_3$O$_7$PS (MH$^+$) 636.2872, found 636.2858 (MH$^+$). Anal. (C$_{31}$H$_{46}$N$_3$O$_7$PS) C, H, N.

SH2 ELISA. The evaluation of peptides using ELISA, including the expression and purification of recombinant proteins, was conducted as described previously.[13] Briefly, autophosphorylated epidermal growth factor receptor tyrosine kinase (EGFR-TK) in buffer, in the presence of 1 mM Na$_3$-VO$_4$, was diluted to 0.66 μM, and a 50-μL aliquot was placed in each well of 96-well Nunc Maxisorp plates (USA/Scientific Plastics, Ocala, FL). After incubation, the plates were washed, and the wells were filled with blocking buffer and incubated

overnight. Finally, the plates were washed, wrapped in aluminum foil, and stored at −20 °C for up to 8 weeks.

Binding of the glutathione *S*-transferase (GST) src SH3−SH2 fusion protein to autophosphorylated EGFR was quantitated by enzyme-linked immunosorbent assay (ELISA). The fusion protein was diluted in blocking buffer containing 1 mM Na_3VO_4 and 5 mM dithiothreitol (DTT), and 50 μL of each dilution was added in triplicate to wells in EGFR-TK-coated plates. After incubation, the plates were washed, and the presence of bound src fusion protein was detected by affinity-purified anti-src antibody. After incubation, the plates were washed, and anti-src antibody was detected by alkaline phosphatase-linked goat anti-mouse IgG, F_c-specific (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA); the plates were incubated and washed as before. Following a final wash with alkaline phosphatase (AP) buffer, 50 μL of 1 mg/mL *p*-nitrophenyl phosphate in AP buffer was placed in each well and incubated. The plates were read on a Molecular Devices (Menlo Park, CA) UVmax plate reader at 405 nm.

For the competitive ELISA, aqueous peptide stock solutions, pH 7.0, were serially diluted in buffer containing 1 mM Na_3VO_4 and 5 mM DTT and incubated with the GST src SH3−SH2 fusion protein. Each peptide solution (50 μL) was added to wells of EGFR-TK-coated plates in triplicate before processing as described above. The activity is expressed as a percentage of the activity shown by the control: $y = 100 \times (OD_{obs} - OD_{blank})/(OD_{max} - OD_{blank})$. Data available for nonlinear curve fitting were evaluated to determine the IC_{50} values. If a nonlinear fit could not be obtained, the IC_{50} values were obtained by linear interpolation.

Using this competition assay and **1** as the standard, peptides were tested as inhibitors of src SH3−SH2:phosphoprotein interactions. The IC_{50} of **1** was 0.67 ± 0.04 μM. For the purpose of comparison and to normalize data from different experiments, results for individual peptides are presented as a ratio: $IC_{50}(test)/IC_{50}(standard)$.

Crystallography. Crystallographic data of peptides bound to the human pp60^(c−src) SH2 domain were obtained as reported previously.[23] Briefly, human pp60^(c−src) SH2:dipeptide crystals were grown by the hanging drop vapor diffusion method where protein was mixed with an equimolar amount of peptide. Data were collected on a Rigaku RAXIS IIC area detector mounted on a Siemens rotating anode X-ray generator. Using the structure of the SH2:**1** complex[13] as a starting model, additional structures were solved. Structures were manually rebuilt using FRODO,[39] refined with X-PLOR,[40] and evaluated using PROCHECK.[41]

Supporting Information Available: Atomic coordinates are available from the Protein Data Bank,[38] Brookhaven National Laboratory for (compound, PDB file code): **1**, 1SHD; **2**, 1A1B. Coordinates for compounds **25**, **69**, and **92** will be submitted. Spectral data for compounds **3**−**8**, **28**−**50**, **71**, **74**, and **76**−**91** (12 pages). Ordering information is given on any current masthead page.

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