Journal of Medicinal Chemistry

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Volume 43, Number 21 October 19, 2000

Communications to the Editor

Structure-Based Design of Novel Bicyclic Nonpeptide Inhibitors for the Src SH2 Domain

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Received August 2, 2000

Introduction. The enzymes responsible for the selective phosphorylation of tyrosine residues, protein tyrosine kinases (PTKs),[1] and the corresponding dephosphorylation, protein tyrosine phosphatases (PTPs),[2] have been implicated in mediating a wide array of intracellular events including cell proliferation, migration, differentiation, metabolism, and immune response. These proteins selectively bind their targets and initiate a cascade of signaling events through a series of cytosolic modular domains that control protein−protein interactions.[3] One such domain, the Src homology 2 (SH2) domain, has been determined to play a critical role in many signaling cascades by recognizing phosphotyrosine (pTyr) sequences of cognate proteins.[4] The SH2 domain of the nonreceptor PTK Src, for example, has been shown to interact with FAK, p130^{cas}, p85, PI3-K, and p68^{sam}.[5−8] Small molecules designed to inhibit SH2-mediated protein−protein interactions provide potential molecular probes to study cellular signaling pathways and ultimately act as therapeutic agents modulating such pathways demonstrated to be involved in the pathogenesis of certain diseases. In the case of Src, elevated levels of kinase activity have been associated with several different cancers including breast[9] and colon[10] cancer. Additionally, genetic knockout studies have implicated Src in bone resorption processes.[11] Thus we were compelled to advance our efforts on the structure-based design of inhibitors of the Src SH2 domain.

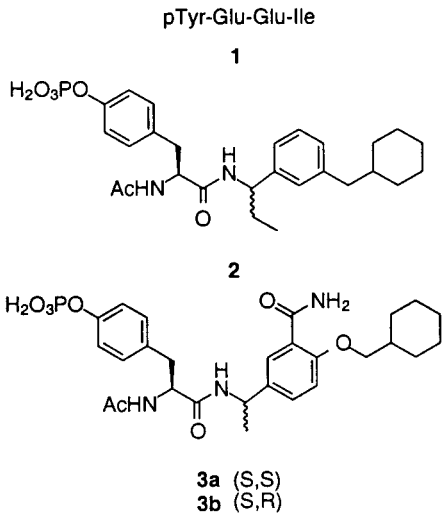

Figure 1. Chemical structures of **1**−**3**.

SH2 domains are relatively small protein modules of approximately 100 amino acids that recognize pTyr-containing sequences, thereby facilitating phosphorylation-dependent, protein−protein interactions. The secondary structure of the SH2 domain features a large central antiparallel β-sheet and two flanking α-helices. The pTyr-binding cleft is formed by three strands of the β-sheet (βB, βC, and βD), the loop between the βB and βC sheets, and Arg αA2 of the A helix.[12−14] The first structure of a ligated SH2 domain was solved using a low-affinity phosphopeptide.[15] Subsequently, a higher-affinity ligated structure was determined for Src SH2 bound to an 11-residue phosphopeptide[12,16] containing the Src family-binding motif pTyr-Glu-Glu-Ile (**1**, Figure 1). These four residues make extensive contacts with the protein. In fact, the majority of interactions with **1** involve the phosphotyrosine (in the basic pTyr pocket) and pY+3 residue Ile. The pY+1 Glu(NH) forms a hydrogen bond with His180(CO), and the Cβ and Cχ lie on the protein surface in van der Waals contact with

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10.1021/jm0003337 CCC: $19.00 © 2000 American Chemical Society
Published on Web 09/28/2000

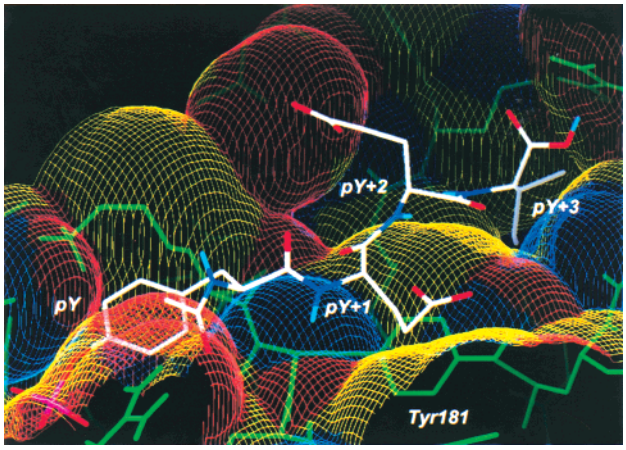


Figure 2. X-ray crystal structure of pTyr-Glu-Glu-Ile (**1**) bound to the SH2 domain of Lck illustrating the solvent-accessible surface detailed in terms of both hydrophobic and hydrogen-bonding interactions. Yellow colors refer to hydrophobic surfaces, blue colors refer to hydrogen-bond-accepting surfaces, and red colors refer to hydrogen-bond-donating surfaces.

Tyr181 and Val178, respectively (Figure 2). No additional hydrogen bonds are directly formed between the backbone of pTyr-Glu-Glu-Ile and the protein. In fact, between the pY+1 carbonyl and the pY+3 residue, the backbone lies above the solvent-accessible surface. The only interaction the pY+2 residue makes is an electrostatic one between the side-chain acid and Arg184.

Structure-Based Design. The SH2 binding site consists of two binding pockets: the pTyr and the pY+3 pocket. These pockets are separated by a solvent-accessible β-strand (βD), with no significant indentations. However, when this region was carefully visualized using an accessible surface colored to display both hydrophobic and hydrogen-bonding sites,[17] a large hydrophobic region with a slight "dimple" in the middle became apparent (Figure 2). This convex hydrophobic surface is created by the phenyl ring of Tyr181.

The stacking of aromatic rings, either parallel or perpendicular, is a well-known phenomenon observed in protein structures[18,19] and between ligands and proteins in complexes. Therefore, we sought to capitalize on the presence of the Tyr181 phenyl ring by designing molecules which would position appropriately an aromatic ring to form energetically favorable stacking interactions. For the initial design strategy, we chose not to replace the phosphate group of pTyr, although, ultimately, a suitable bioisosteric replacement would be desired.

Compound **2** (Figure 1) was the first compound advanced using an interactive structure-based design strategy.[20] To determine the likely binding of this molecule to Src SH2, we docked the molecule into a model of the binding site of Lck SH2 based on a high-resolution (1.0 A) crystal structure of Lck SH2 complexed with pTyr-Glu-Glu-Ile.[21,22] The FLO97[23] molecular modeling program was used to simulate the interactions compound **2** may form with binding site atoms. Our docking studies predicted that compound **2** would position its phenyl ring above Tyr181; however, the model showed that the cyclohexane ring was not ideal in that it did not penetrate deeply into the pY+3 pocket (Figure 3). Also, other opportunities for hydrophobic and hydrogen-bonding interactions around the Tyr181 were not utilized. Nonetheless, this compound represented a useful starting point. Compound **2** was synthesized and found to have an IC_{50} of 300 μM as a mixture of diastereomers.

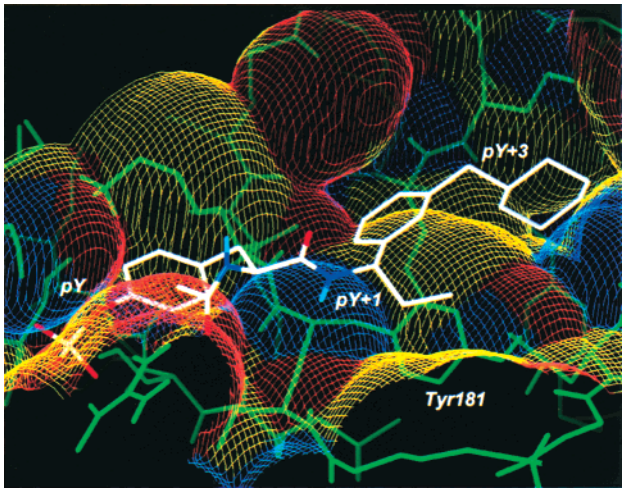


Figure 3. Model of **2** complexed with Lck SH2.

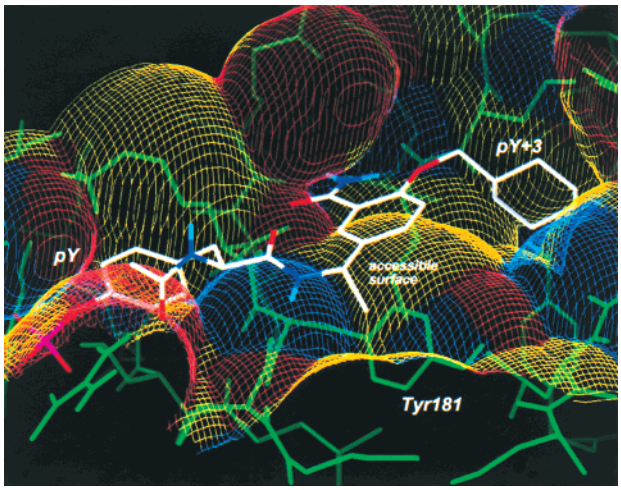
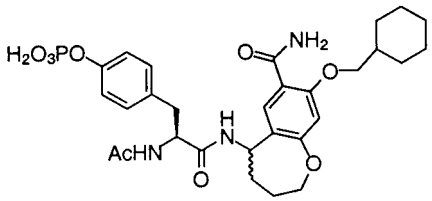
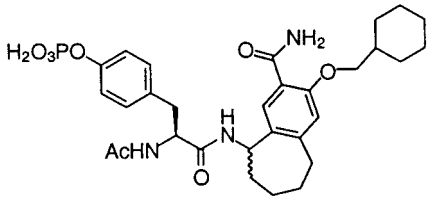


Figure 4. Model of **3a** (*S,S*) complexed with Lck SH2 indicating the hydrophobic-accessible surface.

After this initial investigation, a novel series of de novo designed nonpeptide ligands was reported, as exemplified by the construction of compound **3 (3a**, IC_{50} = 2 μM in our assay; Figure 1).[24,25] Docking studies of **3a** in our model showed numerous favorable protein−ligand interactions (Figure 4): (1) the phenyl ring of **3a** stacks perpendicular to the phenyl ring of Tyr181; (2) the primary amide of **3a** forms two hydrogen bonds with Lys182 (NH and CO) which displaces two water molecules and pulls the cyclohexane ring closer toward the protein; and (3) the cyclohexane ring of **3a** fits well into the pY+3 pocket making five hydrophobic contacts. However, analysis of the hydrophobic-accessible surface adjacent to the central benzamide ring of **3a** indicated that the phenyl ring did not extend beyond Tyr181, thereby not fully exploiting the complete hydrophobic surface of the Src SH2 domain. We, therefore, focused our search for ways to design compounds that would more fully complement the complete convex architecture created by Tyr181 while maintaining the other intermolecular contacts inherent in ligand **3a**. Our search resulted in compounds **4** and **5** (Figure 5), both which



4a (S,S)
4b (S,R)



5a (S,S)
5b (S,R)

Figure 5. Chemical structures of **4** and **5**.

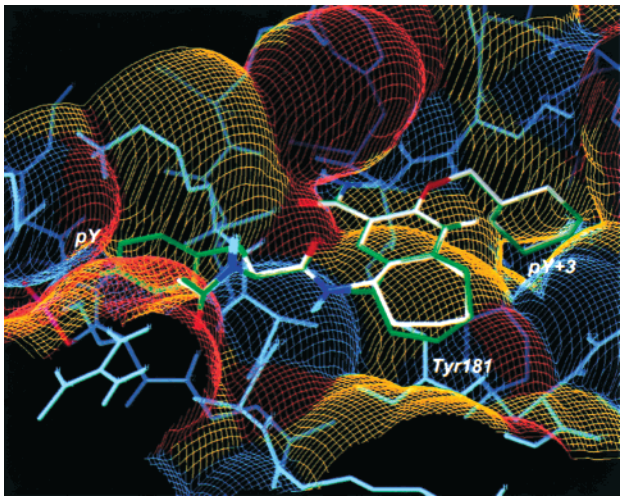
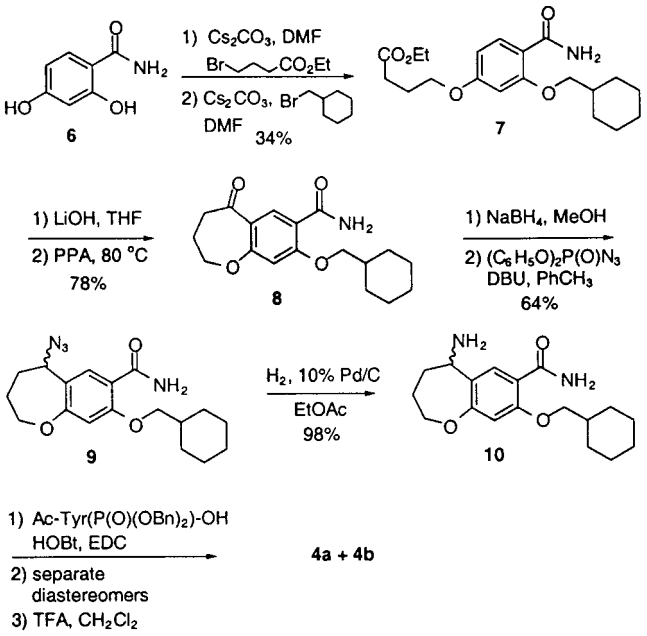


Figure 6. Model of **4a** (white) and **5a** (green) complexed with the Lck SH2.

position a seven-membered ring over the complete hydrophobic surface of Tyr181 (Figure 6). In addition to the predicted enthalpic contributions, we also believed that the increased rigidity of **4** and **5** should be entropically more favorable. In conclusion, our modeling studies predicted that compounds **4a** and **5a** (the two correct diastereomers) should bind to the SH2 domain of Src with higher affinity than does **3a**. Moreover, because compound **4a** positions an oxygen adjacent to the hydrophobic surface, we expected it would exhibit slightly weaker affinity than the related carbocycle **5a.**

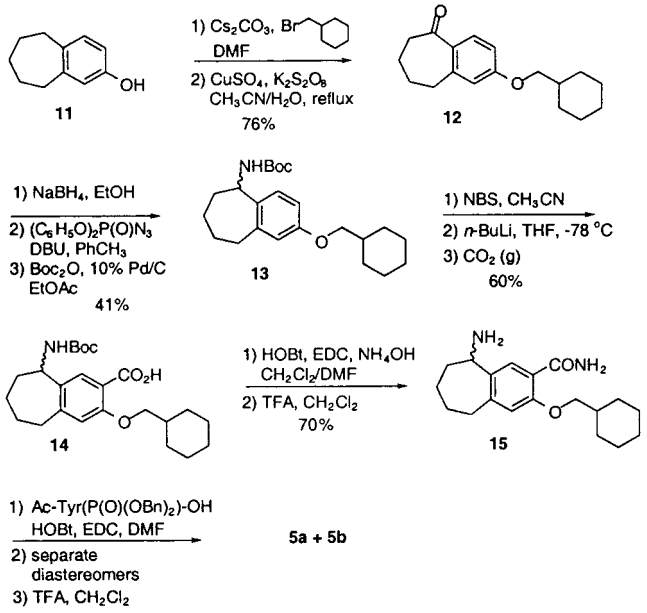
Chemistry. Preparation of the requisite amine **10** for the construction of **4** began with commercially available 2,4-dihydroxybenzamide **(6)** (Scheme 1). Sequential alkylation at the 4- and 2-positions resulted in the formation of ester **7**. Hydrolysis of **7**, followed by a PPA cyclization, afforded ketone **8.** Reduction of **8** with $NaBH_4$ gave the corresponding alcohol, which was smoothly transformed to the azide **9** with diphenyl phosphorazidate.[26] Reduction of **9** over Pd/C afforded the desired amine **10**. Amine **10** was then coupled with an appropriately protected pTyr derivative; the resulting diastereomers were separated by RP-HPLC and the benzyl groups removed with TFA to yield **4a** and **4b**.

Scheme 1. Chemical Synthesis of **4**



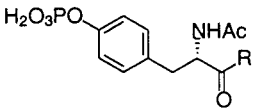
Formation of the carbobicylic amine **15** began from the bicyclic phenol **11** (Scheme 2).[27] Alkylation of phenol

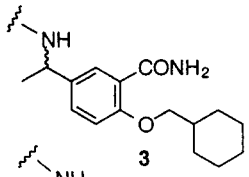
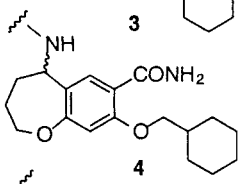
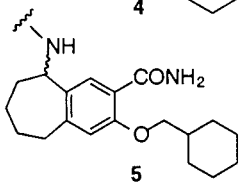
Scheme 2. Chemical Synthesis of **5**



11, followed by a regioselective oxidation with copper(II) sulfate and potassium peroxydisulfate,[28] afforded ketone **12** in 72% yield. This method of oxidation was remarkably clean relative to the more conventional reagents such as DDQ and PDC, both which gave lower yields and more complex mixtures. Reduction of **12** with $NaBH_4$, followed by installation of the azido functionality and reduction over palladium in the presence of Boc_2O, furnished the Boc-protected amine **13**. Regioselective bromination,[29] followed by lithiation and carboxylation, yielded the carboxylic acid **14**. Amide formation and subsequent deprotection with TFA afforded the requisite amine **15**. Coupling as before, followed by deprotection, furnished the desired compounds **5a** and **5b**.

Table 1. Comparative Src SH2 Binding Affinities (IC_{50}'s) for Compounds **3**−**5** Using a Competitive Fluorescence Polarization Assay



R =	IC_{50} (μM)*	
	isomer a (S,S)	isomer b (S,R)
 **3**	2	32
 **4**	0.2	1.6
 **5**	0.1	8.1

*pTyr-Glu-Glu-Ile had an IC_{50} = 5.6 μM in this assay.

Results. Src SH2 binding data for compounds **3**−**5** were determined using a fluoresence polarization assay[30] and are shown in Table 1. Incorporation of the seven-membered ring systems resulted in a 10−20-fold increased (relative to **3a**) affinity for compounds **4a** and **5a**. The carbocyclic analogue **5a** is a 2-fold better binder than the oxepin **4a.** This is consistent with modeling which predicted a slightly higher contact energy (hydrophobic interactions) for **5a** (−34 vs −32.3 for **4a**) and our own observations that when these ring systems are attached to pTyr mimics, the carbocyclic-containing compounds were uniformly more active than those which contained oxygen (data not shown). Consistent with the previously reported binding data for diastereomer **3b**,[24,25] both **4b** and **5b** were less active.

To confirm our hypothesis regarding the mode of binding, the structure of **4a** complexed with Lck SH2 (S164C) was determined by X-ray crystallography at a resolution of 1.95 Å (Figure 7). As predicted, the seven-membered ring of **4a** adopts a concave conformation which complements exquisitely the convex nature of the hydrophobic surface created by Tyr181. The benzamide carbonyl forms a hydrogen bond with the backbone NH of Lys182 (for Src, Lys206) displacing one of the two water molecules observed in the phosphopeptide complex. The second water molecule is not displaced and is hydrogen-bonded to the benzamide NH group of **4a** and the backbone carbonyl of Ile193 (for Src, Ile217). The cyclohexyl group, although predicted to extend more deeply into the hydrophobic pY+3 pocket, was not fully extended and was observed in a higher-energy conformation.

Using X-ray data available from both peptide and nonpeptide inhibitors as well as the FLO97 molecular modeling program, we have designed a series of second-generation inhibitors of the Src SH2 domain. To our knowledge, **4** and **5** represent some of the tightest binding inhibitors known for this target SH2, and such results demonstrate the effectiveness of exploiting accessible surface contacts using bicyclic templates. Further modification of this series by non-phosphate-containing pTyr isosteres holds the promise for future inhibitors with increased cell penetration and in vivo activity.[31,32]

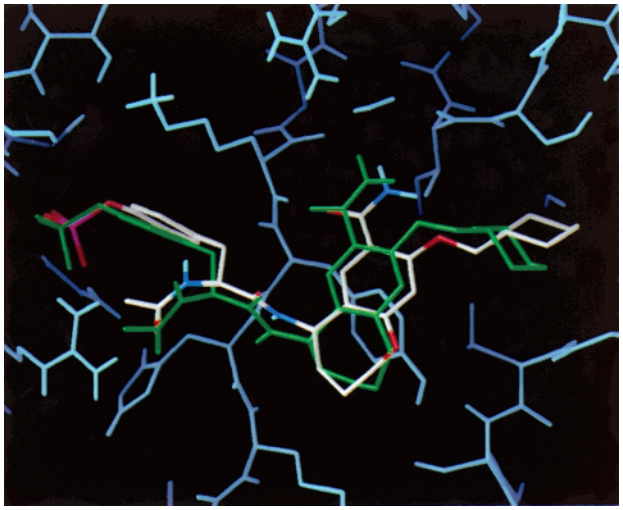


Figure 7. Superposition of the model of **4a** (white) and the X-ray crystal structure of the **4a** Lck SH2 (S164C) complex (green). The water-mediated hydrogen bonds (determined crystallographically) between the carboxamide of **4a** and the protein were not predicted as no water molecules were included in our model.

Acknowledgment. The authors thank Bonnie Marmor and Ruth Yuan for analytical data, Andy Tyler (Harvard University) for high-resolution MS, and the financial support provided by Hoechst Marion Roussel (Aventis).

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