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AN EFFICIENT SYNTHESIS OF A 4′-PHOSPHONODIFLUOROMETHYL-3′-FORMYL-PHENYLALANINE CONTAINING SRC SH2 LIGAND

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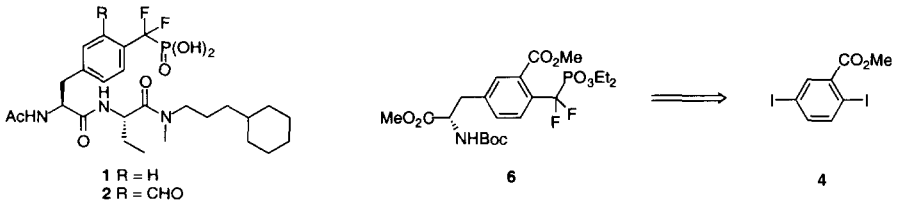
Abstract: A CuBr-mediated, regioselective cross-coupling between methyl 2,5-diiodobenzoate (**4**) and [(diethoxyphosphinyl)difluoromethyl]zinc bromide is reported. Palladium-catalyzed incorporation of an amino acid side chain, followed by subsequent modifications resulted in the rapid construction of **2**. Compound **2** was designed to engage Cys188 of the Src SH2 domain, however, this was not observed spectroscopically.

The SH2 domain of Src, a non-receptor protein tyrosine kinase (PTK), plays a critical role in signaling cascades through the recognition of phosphotyrosine-containing peptides. Unique to this member of the Src family is the presence of a cysteine (Cys188) in the phosphotyrosine binding pocket. We and others[1–3] became intrigued by the presence of this thiol, and its location relative to the 3′-position of the pTyr side chain. Modelling predicted a 3.9 Å gap into which a small electrophile, appropriately positioned, might engage the thiol resulting in improved affinity and selectivity. Precedent for this type of interaction comes from the previously reported design and synthesis of reversible, high affinity ligands for cysteine proteases in which an active site contains a reactive thiol.[4–7] Although it is unlikely that the thiol of Cys188 is fully ionized at neutral pH, the concentration of positively-charged residues in the pocket is likely to decrease the pK_a. In fact, in a very elegant study Singer and Forman-Kay[8] determined the pK_as of His57 and pTyr, in a PLC-γ_1 C-terminal SH2 domain-pY1021 complex, to be significantly reduced due to the proximity of these groups to the highly charged pTyr binding pocket. Based on these observations, and due to the hydrolytic instability of phosphates, we chose as one of our initial targets, a 4′-phosphonodifluoromethyl-phenylalanine (F_2Pmp) derivative containing a 3′-formyl substituent. Although there are now several reported syntheses of F_2Pmp moieties,[9–11] the relatively recent disclosure by Shibuya et al.[12] describing a CuBr-mediated cross-coupling between [(diethoxyphosphinyl)difluoromethyl]zinc bromide and aryl iodides seemed attractive. In fact, we envisioned

Figure 1



1 R = H
2 R = CHO

6

4

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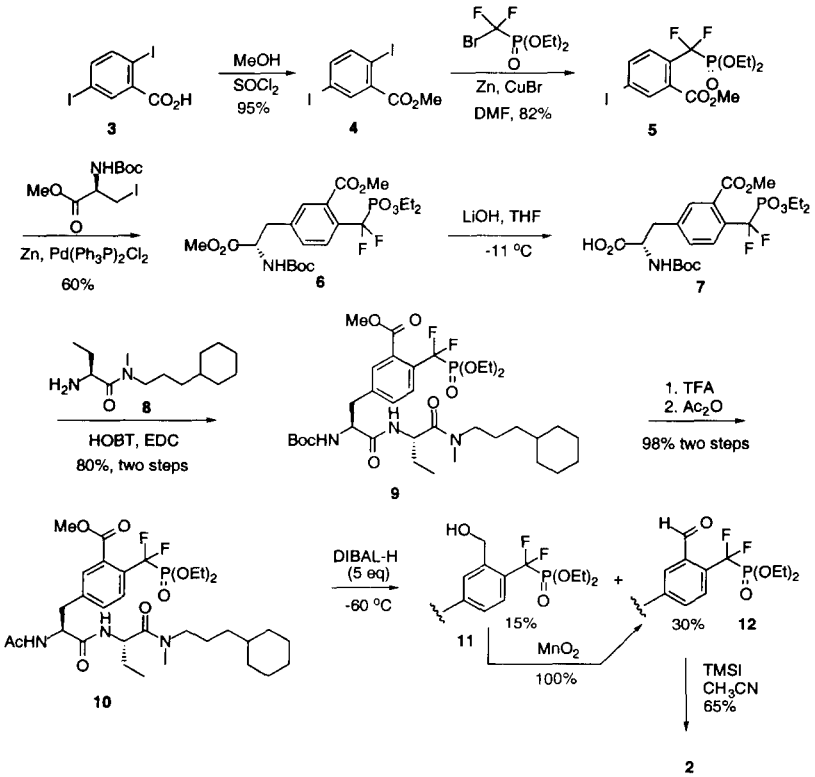
(Figure 1) the central core of **2** arising from the regioselective cross-coupling of methyl 2,5-diiodobenzoate (**4**). This strategy would provide rapid access to the tri-substituted core **6**, necessary for the construction of **2**, as well as other 3′-substituted F_2Pmp derivatives which might be of interest.

The synthesis of **2** started from commercially available 2,5-diiodobenzoic acid (**3**, Scheme 1). Esterification with methanol and catalytic $SOCl_2$ yielded the requisite ester **4**. The key cross-coupling experiment was carried out according to the literature[12] and resulted in the regioselective formation of **5** in 82% yield. Analysis of the crude mixture by NMR indicated the presence < 2% of the alternate regioisomer. While there are many examples in the literature that describe successful cross-couplings using symmetrical bis-haloarenes,[13] there are relatively few examples that demonstrate such remarkable regioselectivity when coupled to a tri-substituted, dissymmetric core.[14–17] Presumably, the regiochemistry derives from the capacity of the benzoate ester to chelate and direct delivery of the organometallic species as well as the *ortho* directing effect of the ester on the incoming nucleophile. Continuing from **5**, installation of the amino acid side chain was achieved following the procedure of Burke and Smyth[18] to give **6**. Chemoselective saponification followed by coupling with amine **8**[19] yielded **9** in 80% yield. Removal of the Boc group and acylation of the resulting amine furnished acetamide **10**. Reduction of ester **10** with DIBAL-H at -60 °C resulted in the formation of aldehyde **12** along with a small amount of the over-reduced alcohol **11**. Alcohol **11** was transformed quantitatively to **12** with MnO_2. Finally, deprotection of **12** with TMSI yielded the desired aldehyde **2**.

Scheme 1



Binding data for compounds **1**[20] and **2** were 20 and 22 μM, respectively, using a standard BIAcore assay.[21] Interestingly, incorporation of the aldehyde moiety resulted in no increase in affinity for the Src SH2 domain. Based on our own work,[22] and that of others,[1,2] incorporation of an aldehyde functionality onto phosphate containing inhibitors resulted in a two-to six-fold increase in affinity that could be attributed to the formation of a reversible, covalent bond (thiohemiacetal) with Cys188. To determine whether **2** was covalently modifying the protein we titrated **2** with doubly-labeled SH2 domain. In the case of phosphate containing aldehydes which engage Cys188, Cβ of Cys188 shifts downfield about 6 ppm.[22] The spectrum of **2**, fully titrated with protein, was identical to that for the complex formed with **1**, indicating that **2** does not engage Cys188. Additionally, a resonance attributable to the aldehyde was present in both spectra.

In an attempt to understand why this compound does not engage Cys188, especially when the 3′-formyl pTyr analog clearly does, we examined compound **2** in our Src SH2 model. We have developed a computer model of Src SH2 based on the high resolution crystal structure of pYEEI bound to Lck SH2,[23] which has been able to distinguish aldehyde containing compounds that do and do not engage Cys188. In the model, Ser188 is "mutated" to a cysteine. To examine compounds containing the aldehyde moiety, the thiohemiacetal of both stereoisomers covalently bound to Cys188 was constructed. These complexes were then submitted to intensive conformational searching and energy minimization using the FLO99 molecular modelling program.[24] When compound **2** was tested in our model no low energy conformation was found that was similar to that of pYEEI (see note 25 for more details); there are several possible explanations for this. First, the bond lengths of both the C-CF_2 and CF_2-P are longer than the C-O and O-P bonds found in phosphates. Furthermore, the phosphorus atom in both cases occupies the same position in the binding site (the phosphate "hole"), and the pTyr+1 hydrogen bond to the protein is maintained. Therefore, the positions of the intervening atoms adjust slightly in the case of F_2Pmp (to accommodate the longer bonds) relative to pTyr, moving the aromatic ring and thus precluding the aldehyde in **2** from engaging the nucleophile. Secondly, Charifson et al. have calculated that a CF_2 group is approximately 33% larger by volume than an oxygen atom.[1] Therefore, the formation of the thiohemiacetal may be more sterically unfavorable adjacent to the sp^3-hybridized CF_2 group in **2** relative to its oxygen counterpart in the phosphate. Finally, in the case of a phosphate-containing ligands there is a well-defined water-mediated hydrogen-bonding network normally observed between the pTyr oxygen (C-O-P) and Lys βD6 and the βC loop. Formation of a thiohemiacetal disrupts this network resulting in alterations in side chain and βC loop positions.[22] Introduction of the CF_2 group may further modify such hydrogen-bonding networks, and result in disruption of energetically favorable interactions. The result, therefore, is that instead of engaging the cysteine, the phenyl ring of **2** presumably rotates 180° projecting the aldehyde out of the pocket away from the cysteine residue. Modelling indicates that in this conformation, the aldehyde does not interact with the protein, providing a plausible explanation for the similar IC_{50}s of both **1** and **2**.

The facile synthesis of a 3′-formyl F_2Pmp as a new pTyr mimetic has been achieved. The rapid construction of highly functionalized intermediate **6** was achieved by the regioselective cross-coupling of readily available diiodide **4** using methods previously described. Interestingly, compound **2** did not engage Cys188; however, structural analysis (NMR) and molecular modeling studies provide some potential explanations for this data.

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25. The 25 lowest energy conformations are retained and compared to the conformation pYEEI adopts in the crystal structure of pYEEI bound to Lck SH2. Compounds that engage Cys188 adopt a conformation very similar to that of pYEEI without significant van der Waals repulsion with binding site atoms while retaining a reasonable ligand strain energy (e.g., less than 40 kJ). Compounds that do not engage Cys188 display significant shifts in the position of the tyrosine ring or can only adopt the pYEEI conformation at the expense of high internal energy (i.e., large ligand strain energy).