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DESIGN AND SYNTHESIS OF A PYRIDONE-BASED PHOSPHOTYROSINE MIMETIC

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Abstract: A novel pyridone-based tyrosine analog, **6**, has been designed to mimic the binding interaction of SH2 domains with phosphotyrosine (pTyr) containing peptides. Synthesis of **6** features a key Pd catalyzed coupling of β-iodoalanine with phosphonomethyl 4-pyridone triflate.

SH2 domains are phosphotyrosine-binding modules found in a variety of important signal-transducing molecules such as nonreceptor tyrosine kinases, phosphatases, and regulatory adapter proteins. Inhibitors that block SH2 domain binding have potential utility in a wide variety of therapeutic areas including metabolic diseases, cancer, inflammation and allergy.[1] Our interest lies with the high affinity IgE receptor, FcεRI, and associated tyrosine kinases and phosphatase PTP-1C.[2] Aggregation of this receptor by antigen–antibody complexes leads to the activation of *Lyn* and *Syk* with rapid phosphorylation of tyrosine residues in the β- and γ-chain cytoplasmic ITAM (immunoreceptor tyrosine-based activation motif) regions of the receptor. Association of the SH2 domain of *syk* with the phosphorylated γ-chain of FcεRI in basophils and mast cells leads to downstream activation signals and the allergic response.[3]

Structural detail provided from X-ray and NMR studies of high affinity pTyr containing peptides has guided the design of SH2-directed ligands.[4] Selective ligands for SH2 domains containing pTyr or phosphate-resistant pTyr analogs and pseudo-peptidic elements, have been developed for SH2 domains of $pp60^{c\text{-}src}$, p85 subunit of PI-3 kinase, and other proteins.[5] Ligand studies with (phosphonomethyl) phenylalanine (Pmp), wherein the phosphate ester oxygen ($>COPO_3H_2$) has been replaced by a methylene unit ($>CH_2PO_3H_2$) and Pmp analogs bearing fluorine or hydroxyl, indicate a pK_{A2} requirement (pTyr pK_{A2} = 5.7 vs. Pmp pK_{A2} = 7.1) and an H-bond to the phosphate ester oxygen.[6] It occurred to us that the inductive effect of a heterocycle on phosphonate acidity (Het-$CH_2PO_3H_2$) would result in a pK_{A2} close to that of pTyr.[7] As indicated in Figure 1, the pyridone methylphosphonate moiety was expected to maintain ionic and H-bonding interactions observed in phosphate-based ligands.[8]

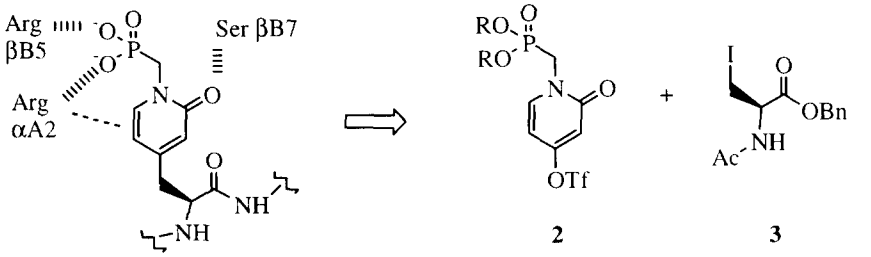

Figure 1. Modeled interactions with a SH2 domain and retrosynthesis of pyridone pTyr mimetic

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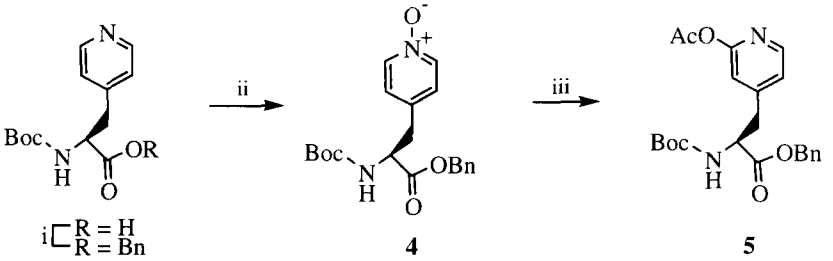
The first approach in preparing the key pyridone pTyr mimetic began with commercial (4-pyridinyl)alanine. Since pyridine to pyridone conversion has been reported for simple systems,[9] rearrangement of N^{α}-Boc-(4-pyridinyl-N-oxide)alanine benzyl ester to the corresponding (4-pyridone)alanine with acetic anhydride was investigated (Scheme 1). In the event, we established the presence of **5** in crude product by MS but the yield was low and pure material was elusive.



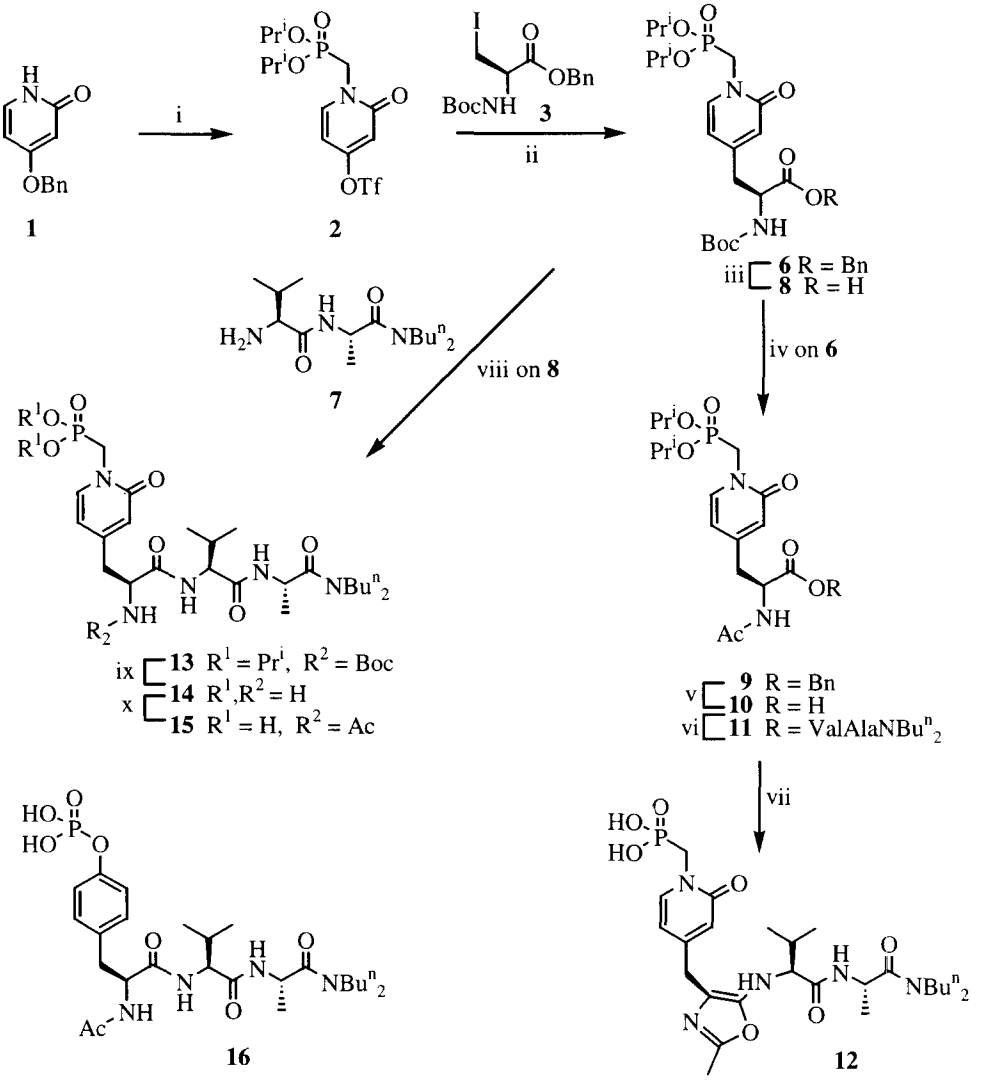
Scheme 1. (i) $CsCO_3$, DMF/H_2O, BnBr, 76% (ii) *m*-CPBA, CH_2Cl_2, 86% (iii) Ac_2O, 65°C, 2.5 h

Alternatively, the palladium catalyzed cross coupling of triflate **2**, already possessing the phosphonate moiety, and β-iodoalanine **3** appeared to be a feasible, convergent synthesis of **6**[10] (Scheme 2). Starting with commercial 4-(O-benzyl)pyridone, alkylation with $BrCH_2P(O)(O^iPr)_2$ and K_2CO_3 in acetonitrile at reflux gave N-alkylated product in 98% yield. The benzyl group of the phosphonomethylpyridone intermediate was then removed by hydrogenolysis in 96% isolated yield. The triflate moiety was introduced with triflic anhydride and triethylamine at –78 °C for 5 min in 70% isolated yield, longer reaction time led to lower yields of triflate product. Palladium catalyzed coupling of **2** with the zinc reagent of β-iodoalanine, prepared according to Jung,[11] $Pd_2(dba)_3$/o-tol_3P at 55 °C, provided the desired product **6** reproducibly in 43% yield.

Assembly of a pyridone-based ligand with recognition for SH2 domains involved the additional condensation of **6** with the peptidomimetic **7**, an entity developed for the P+1 to P+3 pockets,[12] and N^{α}-acetylation of the N-terminus. Thus, treatment of **6** with TFA and acetylation with acetic anhydride proceeded in 76% yield for the two-step transformation to give **9**. Hydrogenolysis with H_2/$Pd(OH)_2$/EtOAc gave the carboxylic acid **10** in 94% yield. Coupling of **10** with ValAla dibutyl amide **7**, afforded **11** as a single isomer revealing stereochemical integrity in the palladium coupling step. Unmasking of the phosphonate isopropyl esters with typical conditions for ethyl phosphate esters, namely iodotrimethylsilane and N,O-bis(trimethysilyl) acetamide,[13] led to the oxazole **12** in 51% isolated yield. To avoid this intramolecular cyclization and dehydration of the acetamide moiety, the N-acetyl group would need to be introduced after phosphonate ester hydrolysis. This was achieved by first coupling N-Boc acid **8** with **7** (EDCI/HOBT) to give **13** in 85% yield. Treatment of **13** with bromotrimethylsilane in acetonitrile and subsequently aqueous acetone resulted in isopropyl ester hydrolysis and Boc removal. Acetylation of the zwitterionic intermediate **14** with Ac_2O gave the desired target compound **15** as a single isomer as determined by ^1H and ^{13}C NMR analysis.[14]

The corresponding phosphate **16** (reported[12] to block the association of PDGF-β receptor with p85 C-SH2; IC_{50} = 0.077 μM) was also assembled for comparative biochemical evaluation. BIAcore analysis of **15** showed 50% inhibition of binding of the p85 N-terminal SH2 domain to a CD19 phosphopeptide at 50 μM.

By comparison, the canonical phosphopeptide **16** exhibited 98% inhibition at 20 μM. This result indicates a moderate effect by the pyridone heterocyclic on phosphonate pK_{A2}.[7] Moreover, the Arg αA2-aromatic (π-cation) interaction may be compromised in the pyridone case.[6c,8] We are continuing our studies with other SH2 domains in order to determine the potential utility of the pyridone phosphonate as a pTyr mimetic.



Scheme 2. (i) (a) K_2CO_3, CH_3CN, $BrCH_2P(O)(O^iPr)_2$, reflux, 48 h, 98%; (b) H_2/Pd/C, MeOH, rt, 2 h, 96%; (c) Et_3N, $(CF_3SO_2)_2O$, CH_2Cl_2, -78 °C, 5 min, 70% (ii) Zn dust, $Pd_2(dba)_3$/*o*-tol_3P/THF-DMA, 55 °C, 43% (iii) H_2, Pd/C, MeOH, rt, 14 h, 99% (iv) (a) TFA, CH_2Cl_2, rt, 5 min; (b) Ac_2O, NMM, CH_2Cl_2, 0 °C to rt, 76% for two steps; (v) H_2, $Pd(OH)_2$, EtOAc, 94% (vi) **7**, EDCI/HOBT, DDMF, 0 °C to rt, 85% (vii) TMSI, BSTFA, CH_2Cl_2, 0 °C to rt; TFA-H_2O-CH_3CN, rt, 1 h, 51% (viii) **7**, EDCI, HOBT, DMF, 0 °C to rt, 14 h, 85% (ix) TMSBr, CH_3CN, rt, 2 h; H_2O-acetone, rt, 14 h (x) Ac_2O, NMM, DMF, 0 °C to rt, 14 h.

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14. Compound **8:** Oil; ^1H NMR (200 MHz, $CDCl_3$) δ 1.21 (s, 3H), 1.24 (s, 3H),1.28 (s, 3H), 1.31 (s, 3H), 1.43 (s, 9H), 2.94–3.02 (m, 2H), 4.28–4.75 (m, 5H), 5.40 (br, 1H), 6.26–6.29 (d, J = 6.6 Hz, 1H), 6.56 (s, 1H), 7.43–7.46 (d, J = 6.6 Hz, ArH). MS (ES): 461 (M^+ + 1), 405 (M^+- $C(CH_3)_3$). Compound **13:** ^1H NMR (200 MHz, $CDCl_3$) δ 0.84–0.95 (m, 12H), 1.17–1.55 (m, 32H), 2.01–2.13 (m, 2H), 2.74–3.29 (m, 6H), 3.37–3.52 (m, 2H), 4.28–4.41 (m, 4H), 4.57–4.85 (m, 4H), 5.46–5.50 (d, J = 8 Hz, 1H), 6.13–6.17 (d, J = 8 Hz, 1H), 6.43 (s, 1H), 6.92–6.96 (d, J = 8Hz, 1H), 7.19–7.23 (d, J = 8 Hz, 1H), 7.38–7.42 (d, J = 8 Hz, 1H). MS (ES): 742.0 (M^++1). Compound **15:** MS (ES): 600.3 (M^+ + 1).