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Enantioselective Synthesis of N-Boc and N-Fmoc Protected Diethyl 4-Phosphono(difluoromethyl)-L-Phenylalanine; Agents Suitable for the Solid-Phase Synthesis of Peptides Containing Nonhydrolyzable Analogues of *O*-Phosphotyrosine

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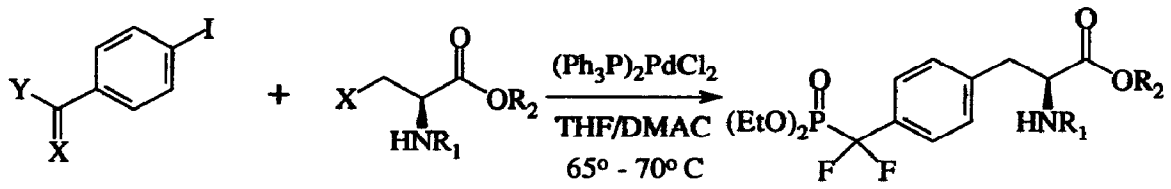
Abstract: Enantioselective convergent syntheses of N-Boc and N-Fmoc protected diethyl 4-phosphono(difluoromethyl)-L-phenylalanine are reported.

O-Phosphotyrosyl (pTyr) residues play critical roles in protein-tyrosine kinase (PTK) cellular signalling cascades, in part because they form a key recognition motif for protein-protein associations mediated by *src*-homology 2 (SH2) domains. Nonhydrolyzable pTyr mimetics are potentially valuable tools for studying these signalling phenomena, and we[1,2] and others[3,4] have previously described the synthesis of phosphonomethyl phenylalanine (Pmp) derivatives as enzymatically and hydrolytically stable analogues of *O*-phosphotyrosine, suitably protected for incorporation into peptides using solid-phase techniques. We found however, that Pmp-containing peptides were less potent than the parent pTyr-containing peptides in binding to SH2 domains.[5] We rationalized that this decreased affinity may be partially due to the higher pKa2 of the Pmp phosphonate relative to the parent phosphate,[5] as well as to the loss of hydrogen bonding interactions normally present between the phosphate ester oxygen and the SH2 domain.[6] We further postulated that fluorine substitution at the α-methylene could result in Pmp analogues which more closely approximate pTyr by lowering the pKa2 and by providing hydrogen bonding to the α-fluoromethylene similar to that displayed by the parent phosphate.[6-8] We therefore developed the technology necessary for the preparation of benzylic α,α-difluorophosphonates,[9] and applied these techniques to the synthesis of fully protected racemic α-mono- and α,α-difluoro Pmp analogues (FPmp and F_2Pmp, respectively) and incorporated them into peptides.[6-8] Use of the racemic F_2Pmp analogues results in diastereomeric D- and L-F_2Pmp containing peptides which, in most cases are easily separated by hplc.[6,8] It is however, desirable to have available starting F_2Pmp analogues in their enantiomerically pure L-forms. The recently reported synthesis of a protected L-Pmp derivative using Pd^{+2}-mediated coupling of an organozinc reagent to an aryl iodide[10] offered an attractive route to the desired L-F_2Pmp compounds. We herein report the application of this approach to the synthesis of N-Boc and N-Fmoc protected diethyl 4-phosphono(difluoromethyl)-L-phenylalanines [N-Boc L-F_2Pmp$(OEt)_2$-OH (**3d**) and N-Fmoc L-F_2Pmp$(OEt)_2$-OH (**3e**) respectively].

Scheme 1



1

a X=O ; Y=Cl
b X=O ; Y=P(O)(OEt)$_2$
c X=F$_2$; Y=P(O)(OEt)$_2$

2

a X=OH ; R$_1$=Boc ; R$_2$=Bn
b X=OTs; R$_1$=Boc ; R$_2$=Bn
c X=I ; R$_1$=Boc ; R$_2$=Bn
d X=IZn ; R$_1$=Boc ; R$_2$=Bn
e X=H ; R$_1$=Boc ; R$_2$=Bn
f X=I ; R$_1$=Boc ; R$_2$=Me
g X=I ; R$_1$=Fmoc; R$_2$=Me

3

a R$_1$=Boc ; R$_2$=Bn
b R$_1$=Boc ; R$_2$=Me
c R$_1$=Fmoc ; R$_2$=Me
d R$_1$=Boc ; R$_2$=H
e R$_1$=Fmoc ; R$_2$=H

The synthesis was begun by the Arbuzov reaction of triethyl phosphite with commercially available 4-iodobenzoyl chloride (**1a**) to give crude keto-phosphonate **1b** as a clear yellow liquid. Addition of DAST (neat, 5 eq.)[9] at -78° C to **1b** followed by stirring at 0° C (2 h), then aqueous workup ($NaHCO_3$) and chromatographic purification, yielded difluorophosphonate **1c** (64% from the acid chloride). Preparation of the requisite organozinc reagent **2d** required the synthesis of the previously reported iodo alanine derivative **2c**,[10,11] which was obtained in two high yielding steps from commercially available N-Boc L-serine benzyl ester (**2a**) by initial tosylation (TsCl, pyridine, -5° C) followed by nucleophilic displacement of the resulting tosylate in **2b** with iodide (NaI, acetone, rt). Treatment of a 0.5 M solution of **2c** in THF-N,N-dimethylacetamide (DMAC) (1:1) with acid-washed zinc dust (1 eq.) at 65° C (1 h) then provided the necessary organozinc compound **2d**.[10,11] When THF alone was used as solvent, no formation of **2d** was detected by TLC analysis even after several hours.[12] To the suspension of freshly prepared **2d** was then added a mixture of difluorophosphonate **1c**, 1 M in THF-DMAC, and $(Ph_3P)_2PdCl_2$ (5 mol %) and the reaction stirred at 65° C for 4-5 h.[13] Extractive workup (NH_4Cl/EtOAc), followed by chromatographic purification, afforded coupled product **3a** in 71% as a colorless syrup.[14] Optimized coupling was achieved utilizing a 2:1 molar ratio of **2c** to **1c**. The only detected side products were the dehalogenated N-Boc L-alanine benzyl ester (**2e**) and the bis aryl compound resulting from homo-coupling of the aryl iodide (15-20%). Both of these were readily separated from the desired product **3a** during chromatographic purification. Our success in utilizing $(Ph_3P)_2PdCl_2$ as a catalyst was in contrast to Jackson's previously reported ineffective results using this catalyst for similar coupling reactions.[11,15] The presence of the electron withdrawing phosphono(difluoromethyl) moiety in the *para*-position may be partially responsible for our success. It should also be noted that the coupling reaction failed when an aryl bromide was used.

Treatment of **3a** with 0.2 N LiOH (2 eq.) in THF at 0° C[6] gave crude final product **3d** as a colorless syrup, which was triturated with anhydrous Et_2O, then cooled to -78° C and the supernatant removed from an insoluble side product. Evaporation of solvent provided pure N-Boc L-F_2Pmp$(OEt)_2$-OH (**3d**) as a white foam in 66% yield {$[\alpha]_D^{24}$ =+8.06° (c 1.08, MeOH); lit.[14] $[\alpha]_D^{25}$ =+7.96° (c 1.08, MeOH)}.

To demonstrate the wider applicability of this method, we prepared the F_2Pmp$(OEt)_2$ methyl esters **3b** and **3c** which bear N-Boc or N-Fmoc protection, respectively. The corresponding intermediate iodo-alanine derivatives **2f** and **2g** were both synthesized from commercially available L-serine methyl ester hydrochloride by initial nitrogen protection, followed by tosylation and iodination similar to that already described for the preparation of **2c**.[11] Coupling of N-Boc protected iodo-alanine derivative **2f** to aryl iodide **1c** as mentioned above gave **3b** in 56% yield{$[\alpha]_D^{24}$ = +35.6° (c 1.05, $CHCl_3$)}. The LiOH induced hydrolysis of **3b** to final product **3d** was readily accomplished in 72% yield. Likewise, coupling of the N-Fmoc protected iodo-alanine derivative **2g** to **1c** provided 3c in 32% yield as a viscous yellow syrup.[16] Methyl ester hydrolysis of **3c** afforded final N-Fmoc L-F_2Pmp$(OEt)_2$-OH (**3e**) in 89% yield as an hygroscopic yellow foam {$[\alpha]_D^{24}$ = +41.6° (c 1.10, $CHCl_3$)}. The yields of compounds **3b** and **3c** reflect unoptimized reaction conditions. Of note is the stability of the base-labile Fmoc group toward the mildly alkaline conditions of both the coupling reaction as well as the LiOH hydrolysis.

Finally, the synthesis of intermediate N-Boc L-F_2Pmp$(OEt)_2$-OBn (**3a**) presented here compares favorably with the recently reported preparation of the same compound.[14] The reduced amount of DAST (5 eq. vs. 15 eq.) as well as a convergent route which employs fewer steps than the previously reported linear protocol may favor the present route for large scale preparations.

We have previously demonstrated the utility of racemic N-Boc F_2Pmp$(OEt)_2$-OH and N-Fmoc F_2Pmp$(OEt)_2$-OH in the solid phase synthesis of F_2Pmp-containing peptides.[6,17] These studies relied upon the hplc separation of diastereomeric D-F_2Pmp and L-F_2Pmp containing peptides, and the subsequent assignment of F_2Pmp configuration based on enzymatic digestion. Peptides prepared with enantiomerically pure F_2Pmp provide confirmation of the enzyme-based configurational assignments. Evaluation of F_2Pmp-containing peptides in SH2 assays is currently in progress. Preliminary results indicate that F_2Pmp is superior to Pmp as a pTyr mimetic in these systems.[18]

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16. Compound 3c: $[\alpha]_D^{24}$= +21.4° (c 1.05, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ: 7.75 (d, 2H, J = 7.41Hz, fluorenyl $H_{1'}$ & $H_{8'}$), 7.54 (overlapping d's, 4H, fluorenyl $H_{4'}$ & $H_{5'}$, aromatic), 7.39 (t, 2H, J = 7.13Hz, fluorenyl $H_{3'}$ & $H_{6'}$), 7.31 (q, 2H, J = 7.34 Hz, fluorenyl $H_{2'}$ & $H_{7'}$), 7.16 (d, 2H, J = 7.87 Hz, aromatic), 5.25 (d, 1H, J = 8.20 Hz, NH), 4.66 (q, 1H, J = 7.86, 5.93 Hz, H_α), 4.40 (m, 2H, fluorenyl $H_{9'}$, NCO_2CH), 4.15 (m, 5H, $POCH_2$, NCO_2CH), 3.70 (s, 3H, CO_2CH_3), 3.14 (m, 2H, H_β), 1.28 (t, 6H, J = 7.11 Hz, CH_3).
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