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N-α-Fmoc-4-Phosphono(difluoromethyl)-L-phenylalanine: A New O-Phosphotyrosine Isosteric Building Block Suitable for Direct Incorporation into Peptides

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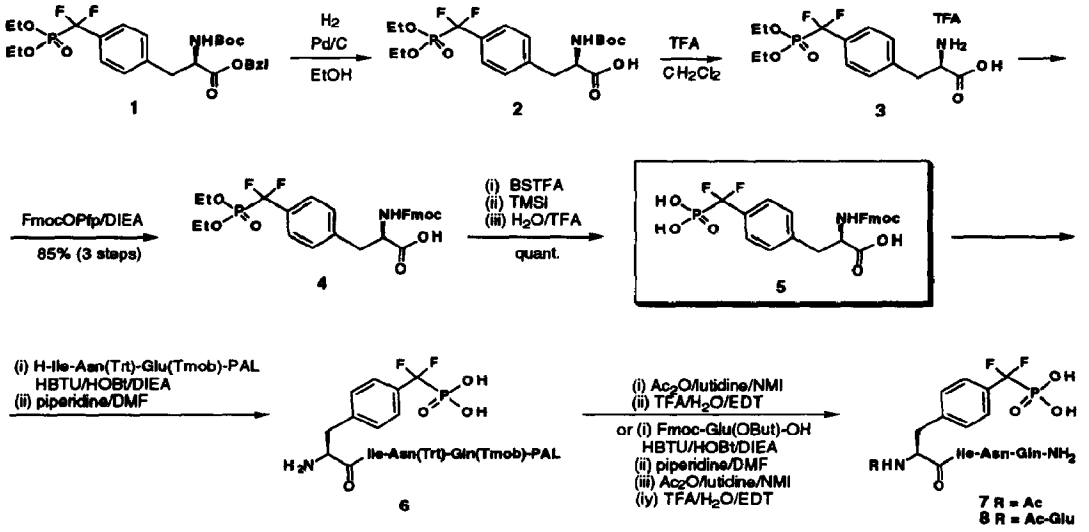
Abstract: *An efficient preparation of N-α-Fmoc-4-phosphono(difluoromethyl)-L-phenylalanine 5 is described. The synthesis of this phosphotyrosine isostere involves deprotection of the penultimate diethylphosphonate intermediate 4 with BSTFA/TMSI as the key step. Building block 5 can be utilized directly for incorporation into peptides without protection of the side chain phosphonic acid group, as illustrated by the efficient preparation of model difluorophosphonopeptides Ac-F_2Pmp-Ile-Asn-Gln-NH_2 and Ac-Glu-F_2Pmp-Ile-Asn-Gln-NH_2.*

The ability of O-phosphotyrosine containing proteins to bind to Src homology 2 (SH2) motifs in certain cytoplasmic proteins is crucial for signaling pathways of tyrosine kinase growth factor receptors.[1] Insight into the mechanistic details of these interactions might permit controlled regulation of cellular signal transduction, and lead to rational design of drugs for a number of aberrant signal expression related diseases.[2] This has prompted a growing interest in the synthesis of enzymatically and hydrolytically stable O-phosphotyrosine analogs that can be used as tools for studying signaling phenomena in cells.[2a, 3-5]

Recently, 4-phosphono(difluoromethyl)-L-phenylalanine (F_2Pmp) has received much attention as a potent and hydrolytically stable O-phosphotyrosine bioisostere for incorporation into SH2-binding peptides.[3-6] The corresponding N-protected diethylphosphonate [F_2Pmp(OEt)$_2$] precursors have been synthesized both in racemic[3] and enantiomerically pure L-forms.[4,5] The reported use of racemic Fmoc-F_2Pmp(OEt)$_2$-OH for preparation of phosphonopeptides requires deprotection of the phosphonate group after construction of peptide sequences is completed.[6] This requires addition of reagents like TMSBr or TMSOTf to standard cocktails employed for removal of peptide side chain protecting groups and cleavage of peptides from resins. In certain cases, this may result in complications impeding the synthesis and purification of target peptides. Thus, our attempts to prepare peptides Ac-F_2Pmp-Ile-Asn-Gln-NH_2 **7** and Ac-Glu-F_2Pmp-Ile-Asn-Gln-NH_2 **8** using similar protocols were problematic. The major product after deprotection/cleavage of the resin-supported peptide Ac-F_2Pmp(OEt)$_2$-Ile-Asn(Trt)-Gln(Tmob)-PAL under reported conditions[6] was the mono-deprotected peptide

Ac-F_2Pmp(OEt)-Ile-Asn-Gln-NH_2, whereas longer reaction times resulted in a formation of mixtures of products. Similar results of incomplete deprotection of Phe[p-$CH_2PO_3Et_2$] containing peptides under analogous conditions,[6] and with trimethylsilyl bromide[7] have been reported. The quest for a more efficient synthetic strategy that would eliminate problems associated with the deprotection of side chain phosphonate group during the synthesis of difluorophosphonophenylalanine peptides, was thus indicated.

Techniques of combinatorial chemistry have recently emerged as powerful tools for efficient drug discovery.[8] Among these, methods employing light-directed parallel chemical synthesis[8a] and encoded synthetic libraries of peptides (ESL)[8c-f] appear to be very promising. ESL strategy involves use of oligonucleotides to encode a combinatorial synthesis of peptides on a polymer microbeads. Significantly, the diethylphosphonate deprotection procedure[6] is potentially incompatible with ESL due to lability of oligonucleotide linkages.[8c] The problem of incomplete deprotection of the F_2Pmp$(OEt)_2$-containing peptides coupled with our desire to synthesize such peptides in a combinatorial ESL format made it necessary for us to devise a new synthetic strategy that would circumvent these problems. The successful use of side chain unprotected phosphotyrosine for preparation of phosphotyrosine peptides has been recently reported in the literature.[9] The similarity of phosphate and the difluorophosphonic acid groups prompted our studies toward the elaboration of F_2Pmp building block with unprotected phosphonate function for direct incorporation into peptides.



Our efforts commenced with the preparation of building block Fmoc-L-F_2Pmp$(OH)_2$-OH **5**. Boc-L-F_2Pmp$(OEt)_2$-OBzl **1** was prepared according to literature procedure[4] and hydrogenated over 10% Pd/C in ethanol to afford the acid **2** in quantitative yield. Subsequent deprotection of **2** with 10% TFA in CH_2Cl_2 smoothly yielded **3**.[10] This simple preparation of amino acid **3** provides for an easy access to any desirable N-protected form of the building block F_2Pmp. Indeed, **3** was efficiently converted to Fmoc-derivative **4** {$[\alpha]_D$ = 42.0° (c = 1.0, $CHCl_3$)} with Fmoc-OPfp/DIEA.[10] This route compares favourably with the alternative

procedure starting from 4-iodobenzoyl chloride and protected L-serine.[5] Unlike the reported method, the current procedure does not require alkaline hydrolysis at any step and therefore avoids posibilities of Fmoc-deprotection and/or racemization of the amino acid during synthesis. Indeed, additional diastereomers were not detected (HPLC[11] and NMR) when **4** was incorporated into peptides Ac-L-F_2Pmp(OEt)$_2$-I-N-Q-NH_2 and Ac-E-F_2Pmp(OEt)$_2$-I-N-Q-NH_2 using standard protocol employing PAL resin.[12]

During attempted conversion of **4** to **5**, a number of deprotection conditions (1M TMSOTf/2M DMS in TFA/EDT/m-cresol,[6] TMSBr in CH_2Cl_2,[7] BSTFA/TMSBr in CH_2Cl_2, BBr_3 in CH_2Cl_2, TMSI in MeCN[13] etc.) were tried but failed to give satisfactory results in our hands. The main problems were incomplete deprotection (into mono-dealkylated compound Fmoc-L-F_2Pmp(OEt)-OH) and formation of side products, such as the transesterification product Fmoc-L-F_2Pmp-OEt. After some experimentation, we found that optimal conditions for the deprotection of the diethylphosphonate group in compound **4** are sequential treatment with bis(trimethylsilyl)trifluoroacetamide (BSTFA; 11 equivalents) in CH_2Cl_2 at RT for 1 h followed by TMSI (8 equivalents) at -20 °C with gradual warming to RT over 3 h. The solvent and excess reagents were removed by concentration *in vacuo* (0.5 Torr., RT, 10 h) and the crude product was desilylated with aqueous TFA in MeCN (1:1:2 TFA/H_2O/MeCN) at RT for 1h. This procedure afforded compound **5** in nearly quantitative yield[10] {$[\alpha]_D$ = 7.0° (c = 0.18, DMSO)} and adequate purity (>90% by HPLC) to enable its direct use in subsequent reactions. The relatively low reactivity of **4** towards TMSBr as compared to other phosphonate esters[14] might in part be accounted for by the neighbouring difluoromethylene group which decreases the Lewis basicity of the phosphonate ester oxygens thus preventing their efficient reaction with TMSBr and subsequent dealkylation. A combination of the stronger Lewis acidity of TMSI with its capability to generate a strongly nucleophilic iodide anion probably makes it superior than TMSBr as a dealkylation reagent.[13,15] BSTFA serves to quench HI that may form during the course of reaction or from decomposition of the reagent, thereby ensuring mild and essentially neutral reaction conditions. Compound **5** is obtained as an yellowish glass and can be stored at -18 °C for at least 2 months without detectable decomposition (HPLC).

The applicability of building block **5** toward synthesis of F_2Pmp peptides was assessed by synthesis of model peptides **7** and **8** (Scheme). Solid phase synthesis was performed on PAL resin in standard fashion.[12] The crude peptides were obtained in nearly quantitative yields after cleavage from the resin and found to be >90% pure by HPLC.[11] These were further purified by HPLC and characterized by ^1H (COSY), ^{19}F and ^{31}P NMR and FABMS.[16] In contrast to previously reported couplings with 4-(phosphono)methylphenylalanine,[17] little or no by-products resulted from side reactions involving free P-OH groups. This is probably due to the electron withdrawing influence of the difluoromethylene group that reduces the nucleophilic reactivity of the phosphonic acid group.

In summary, an efficient preparation of Fmoc-L-F_2Pmp-OH **5** from its diethylphosphonate precursor **4** employing BSTFA/TMSI is described. The side chain unprotected **5** is a convenient building block for preparation of corresponding phosphotyrosine isostere peptides, as illustrated by its direct utility in the efficient synthesis of model difluorophosphonopeptides **7** and **8**.

Notes and References:

1. (a) Mayer, B.J.; Baltimore, D. Trends Cell. Biol., **1993**, 3, 8; (b) Pawson, T.; Gish, G.D. Cell, **1992**, 71, 359; (c) Birge, R.B.; Hanafusa, H. Science, **1993**, 262, 1522.

2. (a) Ullrich, A.; Schlessinger, J. Cell, **1990**, 61, 203; (b) Lowenstein, E.J.; Daly, R.J.; Batzer, A.G. et al. Cell, **1992**, 70, 431; (c) Waksman, G.; Shoelson, S.E.; Pant, N.; Cowburn, D.; Kutiyan, J. Cell, **1993**, 72, 779; (d) Songyang, Z.; Shoelson, S.E.; Chaudhuri, M. et al. Cell,**1993**, 72, 767.

3. Burke, T.R., Jr.; Smyth, M.S.; Otaka, A.; Roller, P.P. Tetrahedron Lett., **1993**, 34, 4125.

4. Wrobel, J.; Dietrich, A. Tetrahedron Lett., **1993**, 33, 3543.

5. When our work was completed, an alternative route to compound **4** *via* its methyl ester has been reported: Burke, T.R., Jr.; Smyth, M.S. P.P. Tetrahedron Lett., **1994**, 35, 551.

6. Otaka, A.; Burke, T.R., Jr.; Smyth, M.S.; Nomizu, M.; Roller, P.P. Tetrahedron Lett., **1993**, 34, 7039.

7. Garbay-Jaureguiberry, C.; Ficheux, D.; Roques, B.P. Int. J. Peptide Protein Res.,**1992**, 39, 523.

8. (a) Fodor, S.P.A.; Read, J.L.; Pirrung, M.C.; Stryer, L.; Lu, E.T.; Solas, D. Science, **1991**, 251, 713; (b) Baum, R.M. Chem. Eng. News, **1994**, 20; (c) Needels, M.C.; Jones, D.G.; Tate, E.H.; Heinkel, G.L.; Kochersperger, L.M.; Dower, W.J.; Barrett, R.W.; Gallop, M.A. Proc. Natl. Acad. Sci. USA, **1993**, 90, 10700; (d) Brenner, S.; Lerner, R.A. Proc. Natl. Acad. Sci. USA, **1992**, 89, 5181; (e) Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. J. Med. Chem. **1994**, 37, 1233; (f) Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. J. Med. Chem. **1994**, 37, 1385.

9. Ottinger, E.A.; Shekels, L.L.; Bernlohr, D.A.; Barany, G. Biochem., **1993**, 32, 4354.

10. Compounds **2-5** were characterized by 400 MHz ^{1}H and 100 MHz ^{13}C NMR and mass-spectra. Satisfactory microanalyses for products **4** and **5** were obtained. Peptides were characterized by ^{1}H (COSY), ^{19}F and ^{31}P NMR and FAB mass-spectroscopy.

11. Column: Microsorb 5 mM C18, 4.6 x 250 mm. A: 0.1% TFA in water; B: 0.1% TFA in MeCN. Gradient (B%) 0-60% (amino acids) or 0-30% (peptides) over 30 min; 1.0 ml/min, UV detection at 220 nm.

12. Coupling reagents HBTU/HOBt/DIEA in DMF; cleaved with 90% TFA, 5% EDT and 5% water, r.t., 2 h.

13. Morita, T.; Okamoto, Y.; Sakurai, H. Tetrahedron Lett., **1978**, 28, 2523.

14. Salomon, C.J.; Mata, E.G.; Mascaretti, O.A. Tetrahedron **1993**, 49, 3691.

15. Rabinowitz, R. J. Org. Chem., **1963**, 28, 2975.

16. ^{1}H (400 MHz) NMR data for Ac-Glu-F_2Pmp-Ile-Asn-Gln-NH_2 in D_2O (δ): 7.48 (d, 2 H, J = 8.1 Hz, arom.), 7.28 (d, 2 H, J = 8.1 Hz, arom.), 4.66 (dd, 1 H, J = 7.4 and 6.8 Hz, CH of Asn), 4.62 (dd, 1 H, J = 5.9 and 9.1 Hz, CH of F_2Pmp), 4.23 (m, 1 H, CH of Gln), 4.16 (dd, 1 H, J = 6.6 and 8.1 Hz, CH of Glu), 4.08 (d, 1 H, J = 8.1 Hz, CH of Ile), 3.17 (dd, 1 H, J = 5.9 and 13.9 Hz, CH_AH_B of F_2Pmp), 2.98 (dd, 1 H, J = 9.1 and 13.9 Hz, CH_AH_B of F_2Pmp), 2.82 (dd, 1 H, J = 6.8 and 15.6 Hz, CH_AH_B of Asn), 2.71 (dd, 1 H, J = 7.4 and 15.6 Hz, CH_AH_B of Asn), 2.35-2.05 (m, 5 H, γ-CH_2 of Glu, γ-CH_2 of Gln and β-CH_AH_B of Gln), 2.0 - 1.85 (overlapped with Ac, m, 1 H, β-CH_AH_B of Gln), 1.94 (s, 3 H, Ac), 1.85-1.70 (m, 3 H, β-CH_2 of Glu and β-CH of Ile), 1.37 (m, 1 H, γ-CH_AH_B of Ile); 1.11 (m, 1 H, γ-CH_AH_B of Ile), 0.82-0.77 (m, 6 H, 2 Me of Ile). FABMS $(M+H)^+$ calc. 821.3, found 821.3.

17. Shoelson, S.E.; Chatterjee, S.; Chaudhuri, M.; Burke, T.R. Tetrahedron Lett., **1991**, 32, 6061.

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