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Synthesis of 4-Phosphono(difluoromethyl)-D,L-phenylalanine and N-Boc and N-Fmoc Derivatives Suitably Protected for Solid-Phase Synthesis of Nonhydrolyzable Phosphotyrosyl Peptide Analogues

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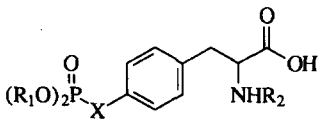
Abstract: Synthesis of 4-phosphono(difluoromethyl)-D,L-phenylalanine as well as its diethyl phosphonate analogues bearing either Boc or Fmoc-amino protection are reported. The latter two derivatives were utilized for the solid-phase synthesis of SH2-related peptides containing nonhydrolyzable phosphotyrosyl mimetics.

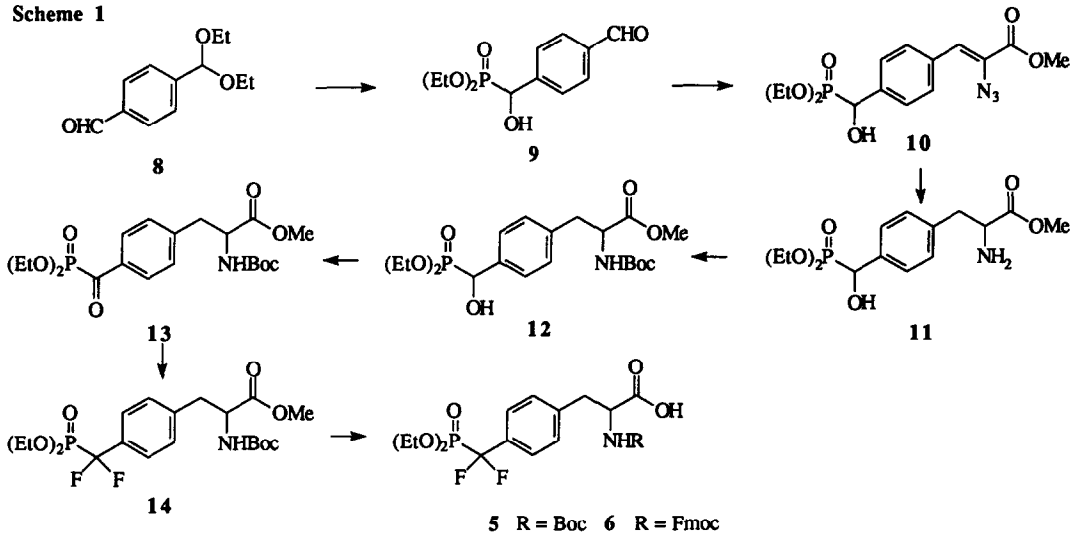
The *O*-Phosphotyrosyl residue is an important structural motif in a variety of cellular signal transduction pathways. We have previously reported the synthesis of N-Fmoc-[(*O*-di-*tert*-butyl)phosphonomethyl]-phenylalanine [N-Fmoc-Pmp(O-*tert*-butyl)$_2$-OH **2**] as an enzymatically and hydrolytically stable analogue of *O*-phosphotyrosine **1** suitable for incorporation into peptides using solid-phase Fmoc techniques.[1,2] However, the second ionization constant of the Pmp phosphonate group (pKa = 7.1) is significantly higher than that of the parent phosphate (pKa = 5.7)[3] and this may be one reason why Pmp-containing peptides are less effective than the corresponding *O*-phosphotyrosyl analogues in antagonizing SH2 phenomena,[3] where the phosphate group is a key component of the interaction. We rationalized that substitution of fluorine at the phosphonate methylene could lower the second pKa and additionally provide hydrogen bonding at the methylene, similar to that displayed by oxygen itself, thereby potentially resulting in Pmp analogues which more closely approximate *O*-phosphotyrosine.



	X	R_1	R_2
1	O	H	H
2	CH_2	*tert*-Bu	Fmoc
3	CHF	*tert*-Bu	Fmoc
4	CF_2	*tert*-Bu	Fmoc
5	CF_2	Et	Boc
6	CF_2	Et	Fmoc
7	CF_2	H	H

We therefore developed methodology for the synthesis of benzylic difluorophosphonates[4] and applied these techniques toward the synthesis of the monofluoro and difluoro N-Fmoc-Pmp(O-*tert*-butyl)$_2$-OH analogues **3** and **4** respectively.[5] While the monofluoro compound **3** proved to be stable and was used to prepare monofluoroPmp-containing peptides, the *tert*-butyl groups of the difluoroPmp **4** proved to be too labile for storage or use in solid-phase synthesis.[5] One reason for this instability was the acid sensitivity of the phosphonate *tert*-butyl protecting groups. We initially believed that *tert*-butyl phosphonate protection was a requisite of our synthesis of difluorophosphonates,[4] but upon further experimentation we have derived reaction conditions compatible with other protecting groups, rendering a synthesis of the more acid stable ethyl-protected difluoroPmp possible. Accordingly, we report herein the synthesis of free difluoroPmp (F_2Pmp-OH) **7** and the respective N-Boc and N-Fmoc diethyl phosphonates (compounds **5** and **6**) as well as their usage in the solid-phase synthesis of difluoroPmp-containing peptides.

Scheme 1



Synthesis of final products **5** - **7** began with commercially available 4-diethoxymethylbenzaldehyde **8**, which upon addition at 0 °C to 1.0 equivalents of sodium diethyl phosphite (generated by the addition of diethyl phosphite to 1.1 equivalents of NaH in THF at 0 °C) provided, after brief acid treatment, the hydroxy phosphonate **9** in 67% yield as a white crystalline solid.[6] Recrystallization from ether removed the slight amount (approximately 6%) of 4-formylbenzaldehyde contaminant. Addition of methanolic sodium methoxide (8 equivalents) to a solution of **9** and ethyl α-azidoacetate (10 equivalents) in methanol at -78 °C, followed by stirring at 0 °C (1 h), provided, after extractive workup (EtOAc/brine), vinyl azide **10** as a light yellow crystalline solid (50% yield).[7] Hydrogenation of **10** (10% Pd•C/MeOH) gave amino ester **11** (95% yield).[8] Compound **11** was therefore obtained using a synthetic approach analogous to our previously reported method[5] for the synthesis of unstable **4**, except that ethyl rather than *tert*-butyl phosphonate protection was utilized.

At this point two fundamental differences were introduced relative to our earlier work, both of which were predicated on the presence of the ethyl phosphonate protection. The first of these differences was in the choice of Boc-amino protection. The original synthesis utilized intermediate CBz-amino protection which was later removed hydrogenolytically. (Boc protection was incompatible with the acid labile *tert*-butyl phosphonate group.) In the present route, the acid stability of the ethyl phosphonate esters allowed the initial use of Boc-amino derivatization, which served both to protect the amino group during subsequent oxidation/fluorination and to directly provide final amino protection for target compound **5**. In this manner, **11** was derivatized with di-*tert*-butyl dicarbonate (1.1 equivalents in THF) to provide **12** (93% yield).[9] The second departure from our original synthesis was in the oxidation of the hydroxyphosphonate to the ketophosphonate. Our previous approach utilized pyridinium dichromate and required *tert*-butyl phosphonate protection.[5] Key to the present synthesis was our finding that Swern oxidation conditions did not require *tert*-butyl phosphonate protection and were compatible with ethyl phosphonate protection. Therefore, standard Swern oxidation (oxalyl chloride, DMSO, CH_2Cl_2, NEt_3) followed by an extractive workup (ice-cold 0.2 N HCl/EtOAc) provided pure ketophosphonate **13** quantitatively.[10] Compound **13** was directly fluorinated by treatment with 5 equivalents of DAST overnight, neat. Extractive workup[5] and silica gel chromatography afforded **14** in 62% yield.[11] Treatment of **14** with LiOH (1.5 equivalents at 0 °C) gave final Boc-protected **5** (96%).[12] Compound **5** was N-deprotected (neat TFA, 1 h) and then reprotected (Fmoc -OSu/ $NaHCO_3$), to provide Fmoc-protected **6** (77%).[13] Finally, refluxing **5** with 3 N HCl (3 h) gave free amino acid **7** (93%).[14]

To examine the utility of these agents in the preparation of difluoroPmp-containing peptides, Boc-protected **5** and Fmoc-protected **6** were utilized to incorporate difluoroPmp into the peptide sequence Gly-(F_2Pmp)-Val-Pro-Met-Leu by Boc-based (Merrifield resin) or Fmoc-based (super acid-labile Rink resin[15]) solid-phase synthesis, respectively.[16] The fully protected peptides were cleaved from the resin and simultaneously deprotected[17] to yield a diastereomeric mixture of L-F_2Pmp- and D-F_2Pmp-containing peptides (**15** and **16** respectively), which were separated and purified by HPLC (Figure 1).[19,20] Configurations were assigned by digestion with amino peptidase M.[5]

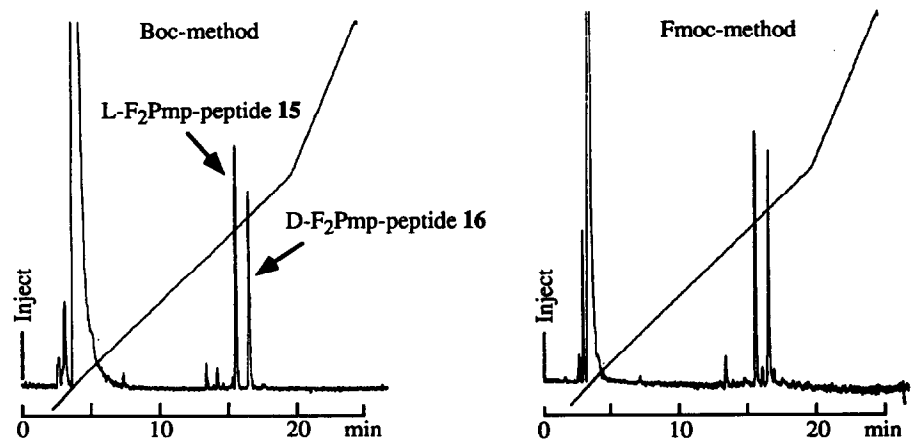


Figure 1. HPLC pattern[19] of crude fully deprotected H-Gly-(D,L-F_2Pmp)-Val-Pro-Met-Leu-OH

References and Notes

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5. Burke, T.R., Jr.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Roller, P.P. *J. Org. Chem.* **1993**, *58*, 1336.
6. Compound **9**: 1H NMR (250 MHz, DMSO-d_6) δ: 10.01 (s, 1H, CHO), 7.90 (d, 2H, J = 8.1 Hz, aromatic), 7.65 (dd, 2H, J = 8.1, 2.0 Hz, aromatic), 6.43 (dd, 1H, J = 14.9, 5.7 Hz, OH), 5.12 (dd, 1H, J = 15.0, 5.6 Hz, P-CH), 3.98 (m, 4H, OCH_2), 1.17 (t, 6H, J = 7.0 Hz, CH_3); mp 78.5-80.5 °C; FABMS *m/z* 271.1 (M-H).
7. Compound **10**: 1H NMR (250 MHz, DMSO-d_6) δ: 7.86 (d, 2H, J = 8.2 Hz, aromatic), 7.48 (dd, 2H, J = 8.2, 1.8 Hz, aromatic), 6.95 (s, 1H, vinylic), 6.30 (dd, 1H, J = 15.3, 5.5 Hz, OH), 5.01 (dd, 1H, J = 14.3, 5.5 Hz, P-CH), 3.98 (m, 4H, OCH_2), 3.87 (s, 3H, CO_2CH_3), 1.18 (t, 3H, J = 7.0 Hz, CH_3), 1.16 (t, 3H, J = 7.0 Hz, CH_3); mp 106-110 °C (dec).
8. Compound **11**: 1H NMR (250 MHz, DMSO-d_6) δ: 7.34 (d, 2H, J = 7.5 Hz, aromatic), 7.15 (d, 2H, J = 7.5 Hz, aromatic), 6.16 (br s, 1H, OH), 4.89 (d, 1H, J = 13.0 Hz, PCH), 3.93 (m, 4H, OCH_2), 3.59 (m, 1H, H_α), 3.57 (s, 3H, CO_2CH_3), 2.87 (dd, 1H, J = 13.4, 6.7 Hz, H_β), 2.76 (dd, 1H, J = 13.4, 7.2 Hz, H_β), 1.17 (t, 3H, J = 7.0 Hz, CH_3), 1.13 (t, 3H, J = 7.0 Hz, CH_3); mp 94-100 °C; FABMS *m/z* 346.1 (M+H)+.
9 Compound **12**: 1H NMR (250 MHz, $CDCl_3$) δ: 7.36 (dd, 2H, J = 8.1, 2.0 Hz, aromatic), 7.07 (d, 2H, J = 8.1 Hz, aromatic), 4.93 (dd, 1H, J = 10.7, 4.8 Hz, P-CH), 4.88 (br s, 1H, NH), 4.51 (m, 1H, H_α), 3.96 (m, 4H, OCH_2), 3.64 (s, 3H, CO_2CH_3), 3.31 (dd, 1H, J = 10.0, 4.8 Hz, OH), 3.02 (m, 2H, H_β), 1.66 (s, 9H, $C(CH_3)_3$), 1.19 (2 t, 6H, J = 7.4, 7.0 Hz, CH_3); FABMS *m/z* 446.1 (M+H)+.
10. Compound **13**: 1H NMR (250 MHz, $CDCl_3$) δ: 8.14 (d, 2H, J = 7.9 Hz, aromatic), 7.21 (d, 2H, J = 7.9 Hz, aromatic), 4.96 (d, 1H, J = 7.7 Hz, NH), 4.56 (m, 1H, H_α), 4.21 (2 q, 4H, J = 7.0 Hz, OCH_2), 3.67 (s, 3H, CO_2CH_3), 3.10 (m, 2H, H_β), 1.35 (s, 9H, $C(CH_3)_3$), 1.32 (t, 6H, J = 7.0 Hz, CH_3); FABMS *m/z* 443.3 (M•)-.
11. Compound **14**: 1H NMR (250 MHz, $CDCl_3$) δ: 7.48 (d, 2H, J = 7.8 Hz, aromatic), 7.16 (d, 2H, J = 7.8 Hz, aromatic), 4.91 (d, 1H, J = 7.7 Hz, NH), 4.53 (m, 1H, H_α), 4.10 (m, 4H, OCH_2), 3.64 (s, 3H, CO_2CH_3), 3.06 (m, 2H, H_β), 1.35 (s, 9H, $C(CH_3)_3$), 1.23 (t, 6H, J = 7.2 Hz, CH_3); FABMS *m/z* 466.1 (M+H)+.
12. Compound **5**: 1H NMR (250 MHz, $CDCl_3$+D_2O) δ: 7.46 (d, 2H, J = 8.1 Hz, aromatic), 7.21 (d, 2H, J = 8.1 Hz, aromatic), 4.57 (m, 1H, H_α), 4.10 (m, 4H, OCH_2), 3.11 (m, 2H, H_β), 1.36 (s, 9H, $C(CH_3)_3$), 1.23 (2 t, 6H, J = 7.1 Hz, CH_3); FABMS *m/z* 452.1 (M+H)+.
13. Compound **6**: 1H NMR (250 MHz, $CDCl_3$) δ: 7.68 (d, 2H, J = 7.3 Hz, fluorenyl $H_{1'}$, $H_{8'}$), 7.49 (d, 2H, J = 7.3 Hz, fluorenyl $H_{4'}$, $H_{5'}$), 7.44 (d, 2H, J = 7.8 Hz, aromatic), 7.33 (t, 2H, J = 7.3 Hz, fluorenyl $H_{3'}$, $H_{6'}$), 7.23 (t, 2H, J = 7.3 Hz, fluorenyl $H_{2'}$, $H_{7'}$), 7.13 (d, 2H, J = 7.8 Hz, aromatic), 5.35 (d, 1H, J = 7.7 Hz, NH), 4.62 (m, 1H, H_α), 4.42 (dd, 1H, J = 10.6, 7.1 Hz, fluorenyl $H_{9'}$), 4.30 (dd, 1H, J = 10.6, 7.1 Hz, NCO_2CH), 4.10 (m, 5H, $POCH_2$ & NCO_2CH), 3.13 (d, 2H, J = 5.5 Hz, H_β), 1.21 (2 t, 6H, J = 7.1 Hz, CH_3); FABMS *m/z* 574.2 (M+H)+.
14. Compound **7**: 1H NMR (250 MHz, DMSO-d_6+D_2O) δ: 8.20 (s, 2H, NH_2), 7.89 (d, 2H, J = 8.0 Hz, aromatic), 7.38 (d, 2H, J = 8.0 Hz, aromatic), 4.16 (t, 1H, J = 6.5 Hz, H_α), 3.15 (d, 2H, J = 6.5 Hz, H_β); mp >300 °C; FABMS *m/z* 296.0 (M+H)+.
15. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
16. Coupling was achieved using diisopropylcarbodiimide/*N*-hydroxybenzotriazole and the N-terminal Gly was as its Boc-derivative.
17. 1 M TMSOTf - 2 M dimethylsulfide/TFA (500 µL to 0.005 mmol resin), ethanedithiol (100 µL), *m*-cresol (25 µL) at 4 °C, 30 min; rt 2 h.[18]
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19. Vydac C_{18} (4.6 x 150 mm) column. A: 0.05% TFA in H_2O; B: 0.05% TFA in 90% aqueous MeCN; gradient (B%): 1-50% over 20 min; 1.0 mL/min, UV detection at 220 nm.
20. Compound **15** FABMS (M-H)- calc. 791.3, found 791.2. Compound **16** FABMS (M-H)- calc. 791.3, found 791.1.

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