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Synthesis of *N*-Fmoc-4-[(diethylphosphono)-2′,2′-difluoro-1′-hydroxyethyl]phenylalanine, a novel phosphotyrosyl mimic for the preparation of signal transduction inhibitory peptides

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Abstract

The synthesis of a novel non-hydrolyzable phosphotyrosyl mimic *N*-Fmoc-4-[(diethylphosphono)-2′,2′-difluoro-1′-hydroxyethyl]-DL-phenylalanine is reported.

Keywords: amino acids and derivatives; phosphorylation; phosphonic acids and derivatives; fluorine and compounds.

The phosphorylation degree of specific tyrosyl residues in a cellular proteins variety serves as key determinant for many biochemical processes both in normal and pathological conditions. The regulation of tyrosine phosphorylation levels appears to be essential for both cellular growth and metabolic control. It is under the influence of protein tyrosine kinases (PTKs), which phosphorylate specific tyrosine residues, and tyrosine phosphatases (PTPs), which catalyze the removal of phosphate from phosphorylated tyrosines. PTPs play essential roles in the regulation of cellular processes including areas of major interest for us, i.e. cell–cell adhesion and cell-matrix contacts.[1,2] Namely they have recently been involved in the regulation of integrin-dependent cell migration.[3]

The search for PTP inhibitors, especially structure-based small molecules, is therefore of great importance. One approach to PTP inhibitors development is based upon the synthesis of non-hydrolyzable analogues of phosphotyrosine and its incorporation into peptides. We have previously described the synthesis of 4-phosphono- and of 4-(phosphonomethyl)-DL-phenylalanine (Pmp).[4] 4-Phosphono (difluoromethyl)-phenylalanine (F_2Pmp) was designed as a Pmp analogue which more closely approximates pTyr. The corresponding *N*-protected diethylphosphonate precursors have been synthesized both in racemic[5,6] and enantiomerically pure L-forms.[7,8] The presence of a fluorine atom at benzylic position increases affinity towards catalytic site and simulates hydrogen bonding interactions similar to the phosphate ester oxygen

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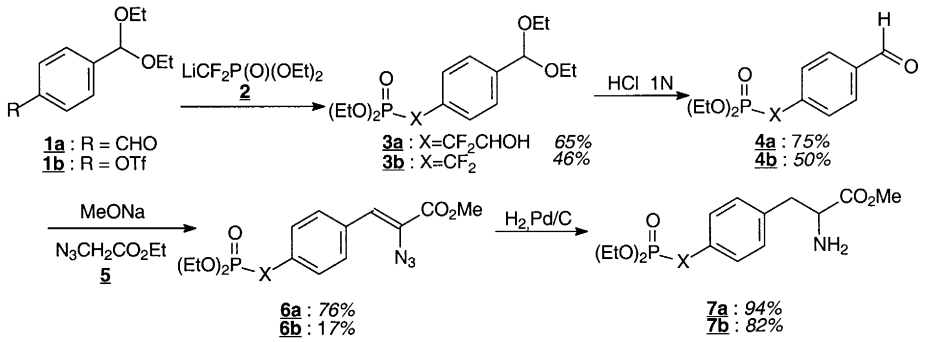
in phosphotyrosine.[9–11] In another approach, the addition of an hydroxyl group was demonstrated to mimic a water molecule found in the reactive catalytic complex, which allows the formation of additional hydrogen bonds, such compounds displayed higher inhibition properties.[12]

We underwent the synthesis of non-hydrolyzable analogues of phosphotyrosine incorporating both of these structural moieties, of which the influence on the binding to the catalytic site was very recently illustrated. We present here our preliminary results dealing with the synthesis of derivatives of 4-[(diethylphosphono)-2′,2′-difluoro-1′-hydroxyethyl]-DL-phenylalanine. Beside the synthesis of this original compound, we illustrated our synthetic scheme with a new route towards F_2Pmp.

The first step of our convergent synthetic approach to (α-difluoroalkyl) phosphonates (Scheme 1) involves the construction of the PCF_2–C bond either via condensation of (diethylphosphinyl)difluoromethyl lithium **2** on aldehydic carbonyl or via triflate displacement.[13,14]

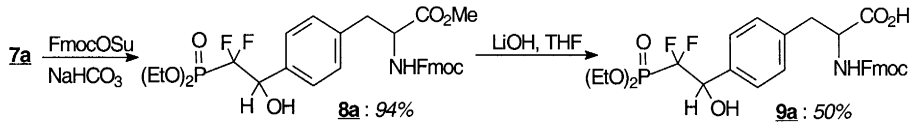


Scheme 1.

Triflate **1b** was first synthesized from 4-hydroxybenzaldehyde and trifluoromethane-sulfonic anhydride (Pyr, CH_2Cl_2, −40°C) and protected as diethyl acetal.[15] Acetal **1b** was obtained in 77% overall yield as a colorless liquid (bp 107°C/1.5 mmHg). Lithium salt **2** was prepared from LDA deprotonation of diethyl difluoromethylphosphonate[17] in THF at −78°C.[14] Addition of commercially available aldehyde **1a** or triflate **1b** to the reagent solution gave **3a**–**b**. Acetals were converted into aldehydes **4a**–**b** by mild acid hydrolysis. Compound **4a** was obtained as a white crystalline solid[18] and **4b** as an oil. Introduction of the amino acid precursor chain was further performed as described by Burke.[5,6,16] First, the Knoevenagel-like condensation of aldehydes **4a**–**b** with a 10-fold excess of ethyl azidoacetate **5**[19] and sodium methylate in methanol at −40°C yielded, after purification, **6a** as a light yellow crystalline solid and **6b** as a light yellow oil. The condensation was in every case quantitatively followed by transesterification towards methyl ester.

Catalytic hydrogenation (3 bars, 10% Pd/C) of **6a**–**b** provided the amino esters **7a**–**b** as colorless solids. It has been found in attempted hydrogenations on other starting materials obtained from similar condensations, that the starting vinyl azide purity is crucial, and that purification of **6a**–**b** by silica gel chromatography and/or recrystallization was necessary. Otherwise impurities may poison the catalyst. Burke claimed[6] that hydrogenation of non-purified **6b** was achieved only once and that it could not be repeated.

Conversion to final orthogonally protected derivative **9a** suitable for utilization in solid phase peptide synthesis was achieved by Fmoc-protection followed by hydrolysis of the methyl ester (Scheme 2). Treatment of **7a** with *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide provided the aminoprotected methyl ester **8a** as a foam. This one was finally saponified with ice-cold diluted lithium hydroxide in THF to yield the target compound **9a**[20] as a white foam.



Scheme 2.

In conclusion, we were able to synthesize 4-[(diethylphosphono)-2′,2′-difluoro-1′-hydroxy-ethyl]phenyl-alanine under a Fmoc protected form convenient for use in solid phase peptide synthesis. Our next goal will be the preparation of stereochemically defined compounds and further incorporation of these molecules into peptides of PTP autophosphorylation sites.

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18. Satisfactory spectroscopic data (^{1}H NMR 200.13 MHz, ^{13}C NMR 50.32 MHz, IR and MS (FAB)) were obtained for all compounds. Compound **4a**, ^{1}H NMR ($CDCl_3$) 1.31 (m, 6H, $^3J_{H-H}$=7.1 Hz, CH_3CH$_2$OP); 4.20 (m, 4H, $^3J_{H-H}$=7.1 Hz, $^3J_{H-P}$=7.3 Hz, $^5J_{H-F}$=2.3 Hz, CH$_3$CH_2OP); 4.41 (d, 1H, $^3J_{H-H}$=4.7 Hz, CHO*H)*; 5.23 (dq, 1H, $^3J_{H-H}$=4.7 Hz, $^3J_{H-F}$=4.6 Hz, $^3J_{H-P}$=20.7 Hz, C*H*OH); 7.65 (d, 2H, $^3J_{H-H}$=8.2 Hz, C$H_{ar3,5}$); 7.88 (d, 2H, $^3J_{H-H}$=8.2 Hz, C$H_{ar2,6}$); 10.0 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$) 16.24 (d, $^3J_{C-P}$=5.2 Hz, *C*H$_3$CH$_2$OP); 65.05, 65.25 (2d, $^2J_{C-P}$=7.2 Hz, CH$_3$*C*H$_2$O); 72.8 (m (ddd), $^2J_{C-P}$=14.5 Hz, J_{C-F}=21.7, 26.2 Hz, *C*HOH); 110.5–125.3 (m (ddd), J_{C-P}=206.3, J_{C-F}=264.7, 273.1 Hz, CF_2); 128.79 (s, $C_{ar3,5}$); 129.3 (s, $C_{ar2,6}$); 136.4 (s, C_{1ar}); 141.5 (d, J_{CP}=6.04 Hz, C_{4ar}); 92.03 (s, *C*HO); IR (cm^{-1}) 3304 (OH); 2994 (ar. C-H); 1695 (C=O); 1608 (ar. C–C); 1247 ((EtO)$_2$P=O); 1208 (C–F); 1062 (P=O); MS (FAB) *m/e* 322.8 [M+1]+, 345.0 [M+23]+, 304.9 [M−18]+, 276.8 [M−45]+, exact: 322.2. Analysis calcd for $C_{13}H_{17}F_20_5P$: C, 48.45; H, 5.32; found: C, 48.41; H, 5.52; mp 93–94°C from Et_2O.
19. Compound **5** was synthesized in 97% yield by halide displacement from the ethyl bromoacetate and sodium azide in water in the presence of tetrabutylammonium bromide as a phase-transfer catalyst.
20. Compound **9a**: ^{1}H NMR (MeOH-D_4) 1.25 (m, 6H, $^3J_{H-H}$=7.2 Hz, CH_3CH$_2$OP); 2.95 (m, 2H, $^3J_{H-H}$=6 Hz, H$_\beta$); 4.10 (m, 4H, $^3J_{H-H}$=7 Hz, $^3J_{H-P}$=7.3 Hz, $^5J_{H-F}$=2.2 Hz, CH$_3$CH_2OP,); 4.22 (t, 1H, $^3J_{H-H}$=7 Hz, fluorenyl $H_{9'}$); 4.35 (dd, 1H, $^3J_{H-H}$=7.10

Hz, NCO_2CH_2); 4.48 (dd, 1H, $^3J_{H-H}$=7.1 Hz, NCO_2CH_2); 4.51 (m, 1H, H_α), 5.03 (m, 1H, CHOH); 7.14 (d, 2H, $^3J_{H-H}$=8 Hz, $CH_{ar2,6}$); 7.30 (dt, 2H, ^{3}J=4.5 Hz, fluorenyl $H_{2',7'}$); 7.34 (d, 2H, $^3J_{H-H}$=7.8 Hz, $CH_{ar3,5}$); 7.39 (t, 2H, ^{3}J=7 Hz, fluorenyl $H_{3',6'}$); 7.59 (dd, 2H, ^{3}J=4.1 Hz, fluorenyl $H_{4',5'}$); 7.76 (d, 2H, ^{3}J=7 Hz, fluorenyl $H_{1',8'}$). ^{13}C NMR (MeOH-D_4) 19.00 (d, $^3J_{H-P}$=5 Hz, CH_3CH_2OP); 40.60 (s, C_β); 51.98 (s, C_α); 55.4 (s, OCH_3); 68.25 (2d, J=7.2 Hz, CH_3CH_2O); 70.15 (s, CH_2O), 76.30 (m, $^2J_{C-P}$=14.5 Hz, $^2J_{CF}$=26.0, $^2J_{CF}$=21.3 Hz, CHOH); 110.6–118.6 (m, $^1J_{CP}$=210.3, $^1J_{CF}$=250.67, 273.11 Hz, CF_2); 123.05–132.50 (4s, fluorenyl CH); 128.53 (s, $CH_{ar2,6}$); 129.06 (s, $CH_{ar3,5}$); 134.03 (s, C_{1ar}); 136.43 (s, C_{4ar}); 141.29–143.78 (4s, fluorenyl C_q); 160.56 (s, NCOO); 174.98 (s, COOH). IR (cm^{-1}) 3411 (NHCOO); 2923 (CH); 2852 (CH_2); 1709 (COOH); 1695(C=O); 1600 (ar. C–C); 1260 ($(EtO)_2P$=O); 1174 (C–F); 1022 (P=O). MS (FAB) *m/e* 626.2 [M+23]+, 604.2 [M+1]+, 382.1 [M−222]+; exact: 603.5.