An Efficient Synthesis of *N*-α-Fmoc-4-(Phosphonodifluoromethyl)-L-phenylalanine

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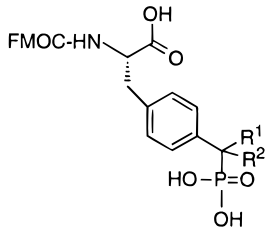
Nonhydrolyzable *O*-phosphotyrosine analogs for use in peptide synthesis have been of great interest over the last several years. This interest stems from thier broad utility in studying various cellular signal transduction pathways. For example, generation of such mimetics and incorporation into suitable peptides would afford systems that could bind to a Src-homology 2 (SH2) domain and potentially prevent interaction with receptor tyrosine kinase molecules. Thus signaling events that can induce changes in cell shape, mobility, or adhesiveness leading to a variety of diseases such as cancer[1] may be modulated.

Much of the interest in nonhydrolyzable phosphotyrosine mimics has centered around phosphonic acids such as (phosphonomethyl)phenylalanine (Pmp) **1a** and its variously substituted analogs such as **1a**−**1c**. The phosphotyrosine isostere **1b** (F_2Pmp), mainly in its phosphonic ester form, has been the focus of much synthetic effort[2−8] because of its lower pK_a value relative to those of **1a** and **1c**. The methods employed to date have resulted in both racemic as well as enantioselective syntheses of F_2Pmp **1b**. Most have involved the stepwise modification of a commercially available starting material. These methods, as mentioned earlier, have resulted in phosphonate ester groups which must be deprotected at a later stage of the peptide synthesis. It has been shown recently[8] that such protection of the phosphonic acid group is unnecessary and the absence of such protection prevents the resulting peptide from being exposed to deprotection conditions which may degrade the final compound. Therefore a convergent enantioselective synthesis, as well as one that would result in a free phosphonic acid group, suitably protected at the nitrogen, would be highly desirable.

One convergent synthesis that has been previously described[7] is one where a modified Fmoc-L-serine ester is used to establish the correct stereochemistry of the final compound. In this case the (phosphonodifluoromethyl)phenyl section is attached via a palladium-catalyzed cross-coupling reaction. This assembles the necessary backbone quickly; however the coupling yield for the desired Fmoc-protected material is not optimal.

Another such synthesis[2] involves the use of the bislactim ether of cyclo(D-Val-Gly) in the asymetric synthesis of Pmp. This methodology requires the chromatographic separation of two amino acids resulting from the hydrolysis of the alkylated lactim to obtain the desired phophonate.



1a: R^1=R^2=H

1b: R^1=R^2=F

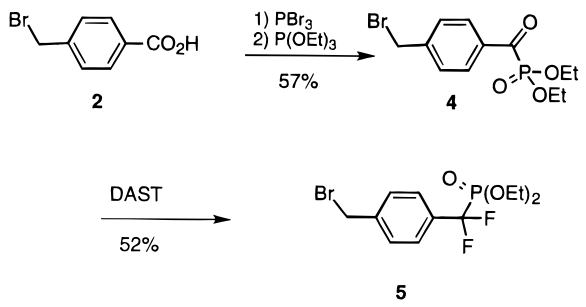
1c: R^1=H, R^2=F

We now report a new asymmetric convergent synthesis of F_2Pmp **1b** using the commercially available imino lactone **3** as the chiral auxiliary.

Results and Discussion

In this synthesis of F_2Pmp **1b** the necessary alkylating reagent **5** (required to react with the imino lactone **3**[9]) was generated from the commercially available α-bromotoluic acid (**2**). Treatment of the acid **2** with phosphorus tribromide afforded the acid bromide. Following low-temperature addition of triethyl phosphite to a toluene solution of the acid bromide, the α-keto phosphonate **4** was obtained in 57% yield. Since the keto phosphonate is rather unstable, rapidly decomposing on silica, it was next treated with a large excess of (diethylamido)sulfur trifluoride (DAST) to yield the desired bromo difluorophosphonate **5** (Scheme 1).

Scheme 1



It should be noted here that the reaction of triethyl phosphite with the acid chloride of **2** was also investigated, but it did not react cleanly, giving instead mainly a polymeric tacky residue. This may be due to a competitve reaction between the aromatic methyl bromide and the acid chloride for the phosphite, even at low temperature, whereas the more reactive acid bromide adds rapidly to the phosphite.

With the desired reagent in hand, the next step involved the alkylation of the imino lactone **3**[9] with bromide **5**, in the presence of HMPA (10%), which afforded a 78% yield of the alkylated material **6**.

Hydrogenation of **6** using H_2/THF:EtOH/$PdCl_2$ gave the amino acid **7** in quantitative yield. In most runs

(1) (a) Brugge, J. S. *Science* **1993,** *260,* 918. (b) Domchek, S. M.; Auger, K. R.; Chatterjee, S.; Burke, T. R.; Shoelson, S. E. *Biochemistry* **1992**, *31*, 9865. (c) Cooper, J. A.; Howell, B. *Cell* **1993**, *73*, 1051.
(2) Cushman, M.; Lee, E.-S. *Tetrahedron Lett.* **1992**, *33*, 1193.
(3) Burke, T. R.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Roller, P. P. *J. Org. Chem.* **1993**, *58*, 1336.
(4) Otaka, A.; Burke, T. R.; Smyth, M. S.; Nomizu, M.; Roller, P. P. *Tetrahedron Lett.* **1993**, *34*, 7039.
(5) Burke, T. R.; Smyth, M. S.; Otaka, A.; Roller, P. P. *Tertrahedron Lett.* **1993**, *35*, 4125.
(6) Wrobel, J.; Dietrich, A. *Tetrahedron Lett.* **1993**, *34*, 3543.
(7) Smyth, M. S.; Burke, T. R. *Tetrahedron Lett.* **1994**, *35*, 551.
(8) Gordeev, M. F.; Patel, D. V.; Barker, P. L.; Gordon, E. M. *Tetrahedron Lett.* **1994**, *35*, 7585.
(9) Williams, R. M.; Im, M.-N. *J. Am. Chem. Soc.* **1991**, *113*, 9276.

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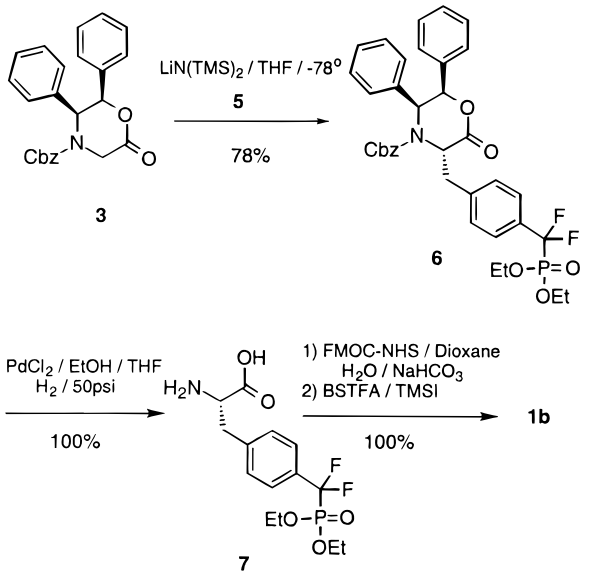
Scheme 2



contamination by palladium salts tended to give slightly higher than quantitative yields, but subsequent reaction steps removed the impurities.

The last operation in the synthesis involved the protection of the amine group using Fmoc-NHS ester followed by deprotection of the phosphonate with a mixture of iodotrimethylsilane (TMSI) and bis(trimethylsilyl)trifluoroacetamide (BSTFA).[8] The overall yield of this two-step reaction was quantitative affording the desired title compound **1b** (Scheme 2).

The stereoselectivity of the akylation was determined by measurement of the optical rotation of the FMOC-protected intermediate of compound **7**. In this synthesis a value of $[\alpha]_D = 44°$ ($c = 1.1$ in chloroform) was obtained which compares well with the literature value[7] of $[\alpha]_D = 42°$ ($c = 1.1$ in chloroform). As well as this information a HPLC of the compound **6** was run and found to be a single peak showing a diastereomeric mix was not present at this stage. Also a HPLC of the final compound **1b** derivatized as an amide with the chiral amine (*R*)-(+)-1-(1-naphthyl)-ethylamine[10] afforded a single peak again demonstrating that a diastereomeric mix was not present.

Conclusion

Utilizing both commercially available α-bromotoluic acid (**2**), as a ready source for the synthesis of the alkylating agent **5**, and the commercially available imino lactone **3**, as the chiral glycine enolate, a new and efficient synthesis of the *N*-α-Fmoc-4-(phosphonodifluoromethyl)-L-phenylalanine (**1b**) has been accomplished. This method allows a quick entry into the preparation of other potential phosphotyrosine isoteres.

Experimental Section

General. ^{1}H and ^{13}C NMR were recorded on a 400 MHz spectrometer in the indicated solvents. Low-resolution mass spectra were obtained via the ESI technique, while high-resolution mass spectra were obtained via the FAB technique. α-Bromotoluic acid and benzyl (2*R*,3*S*)-(−)-6-oxo-2,3-diphenyl-4-morpholinecarboxylate were purchased from Aldrich Chemical Co. and used without further purification. HPLC analysis was performed using a 5 μm 4.6 × 150 mm reverse phase column [eluent A, 0.1% TFA/water and eluent B, 0.1% TFA/CH_3CN; gradient from 30% B to 100% B over 35 min; flow rate 1 mL/min; UV at 260 nm]. Optical purity of **1b** was determined by HPLC of its amide with (*R*)-(+)-1-(1-naphthyl)ethylamine using the known procedure.[10]

Diethyl (4-(α-Bromomethyl)phenyl)oxomethyl)phosphonate (4). A mixture of α-bromotoluic acid (**2**) (2.15 g, 0.01 mol) and PBr_3 (6 mL) was refluxed together for 3 h whereupon a yellow solution with some yellow semisolid was obtained. The solution was decanted from the residue and rotary evaporated at high vacuum to give a yellow semisolid. This residue was taken up into toluene (20 mL), and the solution/suspension was filtered under a blanket of argon. The filtrate was rotary evaporated to dryness at high vacuum affording a white solid. To a solution of the crude acyl bromide (3.1 g) in toluene (50 mL) at 0 °C was added triethyl phosphite (1.9 mL, 0.011 mol) in a dropwise fashion. After addition was completed the mixture was stirred for 40 min at 0 °C. The reaction was then rotary evaporated to dryness at high vacuum, and the gummy residue was dissolved in CH_2Cl_2 (40 mL) and then treated with acid-washed silica (~20 g). After 10 min the mixture was filtered and the filtrate rotary evaporated to a yellow gum which solidified under vacuum to give the keto phosphonate **4** (1.9 g, 57%). ^{1}H NMR ($CDCl_3$): δ 1.4 (t, 6H, $J = 7$ Hz), 4.3 (q, 4H, $J = 4$ Hz), 4.5 (s, 2H), 7.52 (d, $J = 7$ Hz), 8.27 (d, 2H, $J = 7$ Hz). ^{13}C NMR ($CDCl_3$): δ 16.48, 16.54, 45.27, 64.22, 64.29, 128.55, 129.03, 130.39, 131.10, 144.23.

Diethyl ((4-α-Bromomethyl)phenyldifluoromethyl)phosphonate (5). To ice cold keto phosphonate **4** (1.8 g, 0.0054 mol) was added DAST (10 mL, 0.08 mol). After stirring overnight at room temperature the mixture was diluted with CH_2Cl_2 (40 mL) and then added dropwise to an ice cold solution of Na_2CO_3 (100 mL). The CH_2Cl_2 layer was separated and dried (Na_2SO_4), and the solvent was removed via rotary evaporation yielding the desired difluoro phosphonate **5** (1 g, 52%). ^{1}H NMR ($CDCl_3$): δ 1.32 (t, 6H, $J = 7$ Hz), 4.2 (qq, 4H, $J = 7$ Hz), 4.5(s, 2H), 7.46 (d, 2H, $J = 7$ Hz), 7.6 (d, 2H, $J = 7$ Hz). ^{13}C NMR ($CDCl_3$): δ 16.5, 16.6, 32.4, 65.0, 65.1, 119,1, 126.88, 126.9, 129.28, 140.7. ESI MS (M + H) 358.7. Anal. Calcd for $C_{12}H_{16}BrF_2O_3P$: C, 40.36; H, 4.52. Found: C, 40.31; H, 4.66.

Benzyl (2*R*,3*S*)-(−)-6-Oxo-2,3-diphenyl-5-[(4-((diethylphosphono)difluoromethyl)benzyl)]-4-morpholinecarboxylate (6). To a solution of the bromide **5** (1.3 g, 0.0033 mol), the lactone **3** (1.28 g, 0.0036 mol), and HMPA (7 mL) in THF (70 mL) at −78 °C was added in a dropwise fashion 1 M Li-$(TMS)_2$ in THF (3.6 mL, 0.0036 mol). After stirring for 45 min at −78 °C, EtOAc (50 mL) was added and the mixture was washed with water and saturated NaCl and dried (Na_2SO_4) and the solvent removed via rotary evaporation. Column chromatography with 5% EtOAc/CH_2Cl_2 of the crude afforded the desired alkylated lactone **6** (1.7 g, 78%). TLC: $R_f = 0.5$ (10% EtOAc/CH_2Cl_2). $t_R = 28.09$ (100%). ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, $J = 7$ Hz), 1.3 (t, 3H, $J = 7$ Hz), 3.4 (m, 2H), 3.7 (dd, 1H, $J = 4$ Hz), 4.15 (m, 5H), 4.4 (s, 1H), 5.1 (m, 1H), 5.35 (m, 1H), 6.49 (d, 2H, $J = 7$ Hz), 6.6 (m, 3H), 6.8 (d, 2H, $J = 7$ Hz), 7.0−7.65 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 16.38, 16.43, 16.5, 16.55, 38.90, 40.40, 59.21, 60.38, 60.48, 64.92, 64.98, 65.08, 65.14, 68.03, 68.62, 78.85, 79.19, 126.6, 126.69, 127.09, 127.69, 128.03, 128, 13, 128.50, 128.72, 128.96, 129.02, 130.09, 130.28, 133.88, 133.93, 135.16, 135.70, 135.80, 139.36, 154.65, 169.14. ESI MS (M + H): 664.1 Anal. Calcd for $C_{36}H_{36}F_2NO_7P$: C, 65.15; H, 5.47; N, 2.11. Found: C, 65.20; H, 5.65; N, 1.87.

4-[(Diethylphosphono)difluoromethyl]-L-phenylalanine (7). To a solution of $PdCl_2$ (112 mg) in EtOH (8 mL) and THF (4 mL) was added the alkylated lactone **6** (1.4 g, 0.0021 mol) in a small volume of MeOH. After aspiration the mixture was stirred for 20 h under 50 psi of H_2. The reaction was then filtered through Celite and the filtrate rotary evaporated to dryness. The residue was then triturated three times with ether and dried under vacuum affording the desired amino acid **7** (800 mg, quant.). ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 6H, $J = 7$ Hz), 3.3 (d, 2H, $J = 4$ Hz), 4.2 (m, 4H), 4.48 (t, 1H, $J = 6$ Hz), 7.5 (d, 2H, $J = 7$ Hz), 7.61 (d, 2H, $J = 7$ Hz). ^{13}C NMR (CD_3OD): δ 16.63, 16.70, 37.10, 54.84, 66.57, 66.67, 127.86, 127.97, 128.05, 130.84, 139.25, 170.96.

(10) (a) Fujisawa, T.; Sato, T.; Kawara, T.; Ohashi, K. *Tetrahedron Lett.* **1981,** *22,* 4823. (b) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, E. *Tetrahedron Lett.* **1975,** *16* , 1219.

***N*-α-Fmoc-4-(Phosphonodifluoromethyl)-L-phenylalanine (1b).** To a solution of the amino acid **7** (800 mg, 2.1 mmol) in water (5 mL) with $NaHCO_3$ (235 mg, 2.8 mmol) was added dioxane (5 mL). The mixture was then cooled in an ice bath and then treated with FMOC-NHS (944 mg, 2.8 mmol) in a small amount of dioxane. After stirring for 3 h at room temperature, the reaction was diluted with saturated $NaHCO_3$ (30 mL) and then extracted with ether. The aqueous layer was then acidified to pH 2 with 6 N HCl and then extracted with EtOAc. The extracts were then dried (Na_2SO_4), and the solvents were removed yielding the FMOC amino acid (1.29 g) as a white solid. Without further purification the phosphonate in CH_2Cl_2 (20 mL) was then treated with BSTFA (6.4 mL, 0.0242 mol). After stirring for 1 h at room temperature the mix was cooled to −20 °C and TMSI (2.5 mL, 0.0176 mol) was added. After stirring for 1 h at −20 to 0 °C the reaction was stirred for an additional 1 h at room temperature and then rotary evaporated to a viscous brown oil. The oil was treated with CH_3CN (4 mL), TFA (0.5 mL), and water (1 mL) and stirred for 2.5 h at room temperature. The reaction was then rotary evaporated to an oil which was redissolved in EtOAc (50 mL) and washed with 5% $Na_2S_2O_4$ (acidified) followed by a wash with saturated NaCl. The EtOAc layer was then dried (Na_2SO_4) and the solvent removed via rotary evaporation affording the phosphonic acid **1b** (1.19 g, quant. from **6**). $t_R = 11.43$ (100%) as free acid. $t_R = 19.39$ (100% ee) as amide. ^{1}H NMR (DMSO-d_6): δ 2.91 (dd, 1H, $J = 10$ Hz), 3.12 (dd, 1H, $J = 10$ Hz), 4.2 (m, 4H), 7.2−7.9 (m, 12H). ^{13}C NMR (CD_3OD): δ 38.13, 57.63, 67.93, 120.85, 126.85, 126.20, 127.52, 128.15, 128.74, 129.97, 140.61, 142.51, 145.13, 145.19, 158.39, 174.92. ESI MS (M + H): 518. HRMS calcd for $(M + H)^+$: $C_{25}H_{22}F_2NO_7P$ 518.1180, found 518.1168.

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