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Synthesis of *N*-phosphoryl amino acids using bis(9-fluorenylmethyl)phosphite

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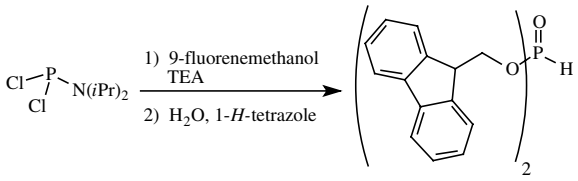
Abstract—Bis(9-fluorenylmethyl)phosphite (BFMP) was found to be an effective reagent for the *N*-phosphorylation of various amino acid methyl esters. BFMP was prepared from *N*,*N*-diisopropyl phosphoramidous dichloride in a one-pot two-step reaction and was obtained as a crystalline solid. *N*-Phosphorylation of the methyl esters of seven representative amino acids with BFMP was high-yielding and generally resulted in crystalline products. Complete deprotection of both the 9-fluorenylmethylphosphophosphate esters and the amino acid methyl esters was accomplished concomitantly with LiOH to give *N*-phosphoryl amino acids.

Phosphoramidates and their derivatives continue to represent an important class of biologically relevant structures. Our group has recently been interested in such compounds as competitive inhibitors of glutamate carboxypeptidases, specifically prostate-specific membrane antigen (PSMA). The most potent inhibitor for this enzyme that we have identified to date is a relatively simple molecule; *N*-phosphoryl glutamic acid which exhibited a K_i value of 70 pM against PSMA.[1] In addition to serving as competitive inhibitors of metallo-peptidases or proteases, *N*-phosphoryl amino acids have also gained a growing interest in the self-assembly of polypeptides.[2] In order to establish a convenient preparation of *N*-phosphoryl glutamic acid as well as other *N*-phosphoryl amino acids, we synthesized bis(9-fluorenylmethyl)phosphite **1** and explored its versatility in the phosphorylation of various amino acid methyl esters.

Our original method for the preparation of *N*-phosphoryl glutamic acids employed 2-cyanoethoxy groups as base-labile protecting ligands on phosphorus that could be concomitantly deprotected under the same condition necessary to hydrolyze the methyl ester of glutamic acid.[3,4] However, this method suffered from chromatographic challenges of the penultimate intermediate.[5] Specifically, the lack of a chromophore or a responsiveness to stains typically used for thin layer chromatography presented a problem in identifying fractions that contained the desired intermediate during purification by flash chromatography. To address these problems, we sought a protecting group for phosphoramidates which could be conveniently removed under mild basic conditions. This constraint was important since the P–N bond is labile under acidic conditions typically used in the deprotection of *tert*-butyl ester protecting groups as well as the neutral conditions of hydrogenolysis in which acidic groups are revealed from benzyl esters. The 9-fluorenylmethyl group was found to be a promising protecting group for phosphate esters[6] and in a previous report, we established that 9-fluorenylmethyl esters of phosphorus could be deptrotected along with the methyl esters of glutamic acid under mild basic conditions.[7] Based upon these results, and the known reaction of H-phosphonate diesters with amines in carbon tetrachloride to form phosphoramidate diesters,[8] we sought to establish a similar methodology for the



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Scheme 1. Synthesis of bis(9-fluorenylmethyl)phosphite **1**.

Keywords: Phosphoryl amino acid; Phosphoramidate; Bis(9-fluorenylmethyl)phosphite.
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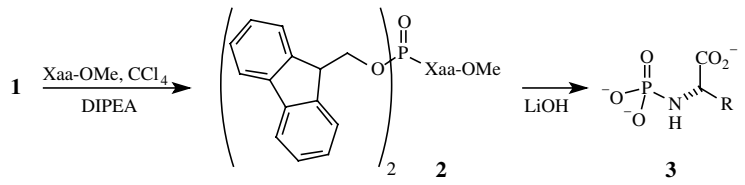
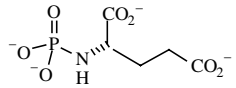
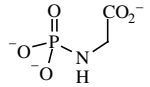
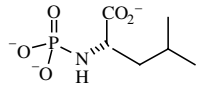
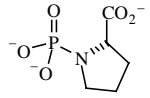
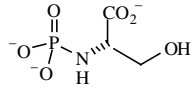
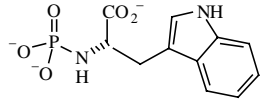
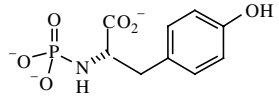
Table 1. Synthesis of *N*-phosphoryl amino acids[10]



Xaa-OMe	Phosphoramidates **2**[11]	Yield (%)	*N*-Phosphoryl amino acids **3**[12]	Yield (%)
H-Glu(OMe)-OMe	**2a**	93	**3a** 	85
H-Gly-OMe	**2b**	98	**3b** 	85
H-Leu-OMe	**2c**	96	**3c** 	89
H-Pro-OMe	**2d**	83	**3d** 	90
H-Ser-OMe	**2e**	80	**3e** 	91
H-Trp-OMe	**2f**	93	**3f** 	78
H-Tyr-OMe	**2g**	86	**3g** 	93

synthesis of *N*-phosphoryl amino acids using bis(9-fluorenylmethyl)phosphite **1**.

From diisopropyl phosphoramidous dichloride, bis(9-fluorenylmethyl)phosphite **1** (BFMP) was prepared as outlined in Scheme 1.[9] From the reaction mixture, BFMP was conveniently purified by crystallization from methylene chloride and hexane. The phosphorylation of various amino acid methyl esters with BFMP to give phosphoramidate precursors **2** (Table 1) occurred in excellent yields. It is noteworthy to mention that the amino acids did not require neutralization prior to use. Phosphoramidates **2** were generally purified by crystallization in high yield. The concomitant deprotection of both the methyl esters and the 9-fluorenylmethyl groups by LiOH proceeded smoothly and in good yield to give the desired *N*-phosphoryl amino acids **3** (Table 1). In summary, bis(9-fluorenylmethyl)phosphite is an excellent reagent for the *N*-phosphorylation of amino acid esters and useful in the preparation of *N*-phosphoryl amino acids.

Acknowledgements

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9. Synthesis of BFMP **1**. To a stirred solution of diisopropyl phosphoramidous dichloride (9 mmol, 1.77 g) in methylene chloride (15 mL) was added a solution of 9-fluorenylmethanol (14.4 mmol, 2.25 g) in methylene chloride (15 mL) via syringe under $Ar_{(g)}$ at 0 °C. The reaction mixture was then stirred for 3 h at room temperature. The solvent was evaporated under reduced pressure to give a viscous residue which was subsequently dissolved in acetonitrile (25 mL) and chilled to 0 °C. A solution of acetonitrile (4 mL), 1-*H*-tetrazole (0.535 g, 7.64 mmol) and distilled water (2 mL) were then added and the reaction mixture stirred for 75 min. The solution was then diluted in ethyl acetate (75 mL) and sequentially washed with 10% HCl (2 × 75 mL), 10% Na_2CO_3 (70 mL), distilled water (75 mL) and brine (75 mL). After drying the organic layer with $MgSO_4$, the solvent was removed in vacuo to give a yellow oil. The crude product was purified by crystallization from methylene chloride (10 mL) and hexane (50 mL) to give white crystals; yield: 64%; mp 99–100 °C. ^{1}H NMR ($CDCl_3$) δ: 4.11 (t, J = 6.4 Hz, 2H), 4.23–4.27 (m, 4H), 6.66 (d, J = 707 Hz, 1H), 7.25–7.53 (m, 12H), 7.68–7.73 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 48.6, 67.7, 120.7, 125.6, 127.8, 128.6, 142.1, 143.6. ^{31}P NMR ($CDCl_3$) δ: 8.42.
10. Anhydrous solvents were used in all reactions. Each amino acid methyl ester was used as supplied in the acid salt form. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer. ^{1}H NMR chemical shifts are relative to TMS (δ = 0.00 ppm), $CDCl_3$ (δ = 7.26 ppm), CD_3OD (δ = 4.87 and 3.31 ppm), or D_2O (δ = 4.87). ^{13}C NMR chemical shifts are relative to CD_3OD (δ = 49.15 ppm) or $CDCl_3$ (δ = 77.23 ppm). ^{31}P NMR chemical shifts in $CDCl_3$ or D_2O were externally referenced to 85% H_3PO_4 (δ = 0.00 ppm) in $CDCl_3$ and D_2O, respectively.
11. General procedure for *N*-bis(9-fluorenylmethyl)phosphoryl amino acids **2**. To a stirred solution of bis(9-fluorenylmethyl)phosphite (0.459 mmol), an amino acid methyl ester (0.505 mmol) in acetonitrile (5 mL) and carbon tetrachloride (5 mL) at 0 °C under $Ar_{(g)}$ was added *N*,*N*-diisopropylethyl amine (1.01 mmol, 180 μL) via syringe. The reaction mixture was stirred for 70 min at room temperature. The reaction mixture was then diluted with ethyl acetate (50 mL) and sequentially washed with 10% HCl (50 mL), 10% $NaHCO_3$ (50 mL), distilled water (50 mL) and brine (50 mL). After drying the organic layer with $MgSO_4$, the solvent was removed in vacuo. Compound **2a**: crystallized from methylene chloride (5 mL) and hexane (35 mL); 93% yield; mp 79–80 °C. ^{1}H NMR ($CDCl_3$) δ: 1.75–1.85 (m, 1H), 1.90–2.01 (m, 1H), 2.25 (t, J = 15 Hz, 2H), 3.24 (t, J = 21 Hz, 1H), 3.60 (s, 3H), 3.62–3.69 (m, 4H), 4.13–4.28 (m, 6H), 7.26–7.54 (m, 14H), 7.70–7.75 (m, 2H). ^{31}P NMR ($CDCl_3$) δ: 7.35. Compound **2b:** 98% yield; mp 58–59 °C. ^{1}H NMR ($CDCl_3$) δ: 3.16–3.24 (m, 1H), 3.44 (dd, J = 6.3, 10.2 Hz, 1H), 3.65 (s, 3H), 3.62–3.69 (t, J = 6.6 Hz, 2H), 4.18–4.34 (m, 4H), 7.22–7.40 (m, 8H), 7.51 (dd, J = 7.5, 12 Hz, 4H), 7.68–7.73 (t, J = 15 Hz, 4H). ^{31}P NMR ($CDCl_3$) δ: 7.89. Compound **2c**: crystallized from methylene chloride (6 mL) and hexane (30 mL); 96% yield; mp 108–109 °C. ^{1}H NMR ($CDCl_3$) δ: 0.80–0.89 (m, 6H), 1.26–1.34 (m, 1H), 1.36–1.51 (m, 1H), 1.56–1.70 (m, 1H), 3.02 (t, J = 21 Hz, 1H), 3.60 (s, 3H), 3.70–3.84 (m, 1H), 4.08–4.31 (m, 6H), 7.22–7.41 (m, 8H), 7.47–7.57 (m, 4H), 7.68–7.74 (m, 4H). ^{31}P NMR ($CDCl_3$) δ: 7.54. Compound **2d**: the product was purified by flash chromatography (hexane–ethyl acetate, 5:2, v/v, R_f = 0.34); 83% yield; mp 50–51 °C. ^{1}H NMR ($CDCl_3$) δ: 1.70–1.85 (m, 2H), 1.85–1.93 (m, 1H), 2.00–2.10 (m, 1H), 3.05–3.09 (m, 2H), 3.62 (s, 3H), 4.09–4.41 (m, 7H), 7.22–7.39 (m, 8H), 7.48–7.52 (m, 2H), 7.57–7.62 (m, 2H), 7.65–7.72 (m, 4H). ^{31}P NMR ($CDCl_3$) δ: 6.52. Compound **2e**: crystallized from methylene chloride (5.5 mL) and hexane (13.5 mL); 80% yield; mp 105–106 °C. ^{1}H NMR ($CDCl_3$) δ: 2.30 (br s, 1H), 3.49–3.67 (m, 6H), 4.06–4.28 (m, 7H), 7.21–7.50 (m, 12H), 7.67–7.69 (m, 4H). ^{31}P NMR ($CDCl_3$) δ: 7.63. Compound **2f**: crystallized from methylene chloride (6.5 mL) and hexane (30 mL); 93% yield; mp 145–146 °C. ^{1}H NMR ($CDCl_3$) δ: 2.95–3.11 (m, 2H), 3.19 (t, J = 20 Hz, 1H), 3.56 (s, 3H), 3.94–4.10 (m, 5H), 4.16–4.26 (m, 2H), 6.74 (d, J = 2.1 Hz, 1H), 7.00–7.13 (m, 2H), 7.21–7.30 (m, 5H), 7.33–7.47 (m, 9H), 7.69–7.76 (m, 4H), 8.05 (s, 1H). ^{31}P NMR ($CDCl_3$) δ: 7.17. Compound **2g**: crystallized from methylene chloride (11 mL) and hexane (33.5 mL); 86% yield; mp 142–143 °C. ^{1}H NMR ($CDCl_3$) δ: 2.70 (d, J = 3.9 Hz, 2H), 3.10 (t, J = 22 Hz, 1H), 3.61 (s, 3H), 3.82–3.88 (m, 1H), 3.91–3.98 (m, 1H), 3.98–4.07 (m, 3H), 4.17–4.27 (m, 2H), 6.61 (d, J = 8.1 Hz, 2H), 6.77 (d, J = 8.1 Hz, 2H), 7.26–7.48 (m, 12H), 7.69–7.73 (m, 4H). ^{31}P NMR ($CDCl_3$) δ: 7.11.
12. General procedure for *N*-phosphoryl amino acids **3**. To a stirred solution of the bis(9-fluorenylmethyl)phosphoryl amino acids **2** (0.106 mmol) in methanol (2 mL) was added an aqueous solution of LiOH (1.2 M, 800 μL for **2b**–**g** or 1.0 mL for **2a**). The reaction mixture was stirred for 15 h at room temperature and then evaporated to dryness under reduced pressure. The residue was dissolved in Milli-Q water, the solids were filtered and the filtrate was again evaporated to dryness under reduced pressure to give the products as white solids in a lithium salt form. Yields were calculated by subtracting the known excess of LiOH in each reaction. Compound **3a**: 85% yield. ^{1}H NMR (D_2O) δ: 1.73–1.88 (m, 2H), 2.02–2.21 (m, 2H), 3.44–3.51 (m, 1H). ^{31}P NMR (D_2O) δ: 8.57. Compound **3b:** 85% yield. ^{1}H NMR (D_2O) δ: 3.08–3.14 (m, 2H). ^{31}P NMR (D_2O) δ: 9.05. Compound **3c**: 89% yield. ^{1}H NMR (D_2O) δ: 0.64–0.67 (m, 6H), 1.15–1.27 (m, 2H), 1.32–1.40 (m, 1H), 3.25 (dd, J = 7.5, 15.9 Hz, 1H). ^{31}P NMR (D_2O) δ: 8.56. Compound **3d**: 90% yield. ^{1}H NMR (D_2O) δ: 1.30–1.51, (m, 3H), 1.69–1.81 (m, 1H), 2.72–2.83 (m, 2H), 3.48–3.56 (m, 1H). ^{31}P NMR (D_2O) δ: 8.15. Compound **3e**: 91% yield. ^{1}H NMR (D_2O) δ: 3.53–3.58 (m, 2H), 3.66–3.73 (m, 1H). ^{31}P NMR (D_2O) δ: 8.59. Compound **3f**: 78% yield. ^{1}H NMR (D_2O) δ: 2.93–3.00 (m, 1H), 2.15–3.19 (m, 1H), 3.75 (br s, 1H), 7.04–7.17 (m, 3H), 7.37 (d, J = 6 Hz, 1H), 7.68 (d, J = 6 Hz, 1H). ^{31}P NMR (D_2O) δ: 8.37. Compound **3g**: 93% yield. ^{1}H NMR (D_2O) δ: 2.61–2.67 (m, 1H), 2.85–2.91 (m, 1H), 3.59–3.66 (m, 1H), 6.49 (d, J = 6 Hz, 2H), 6.93 (d, J = 18 Hz, 2H). ^{31}P NMR (D_2O) δ: 8.27.