A Convenient Synthetic Approach to New α-(9-Fluorenylmethoxycarbonylamino)alkylphosphonic Acid Derivatives

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Benzyl hydrogen α-(9-fluorenylmethoxycarbonylamino)alkylphosphonates **4**, obtained by the sodium metaperiodate oxidation of the corresponding phosphinates **3**, were converted to the chloridates **5** and coupled with the N^{ε}-protected lysine benzyl ester to afford peptides **6** containing a phosphonamide bond.

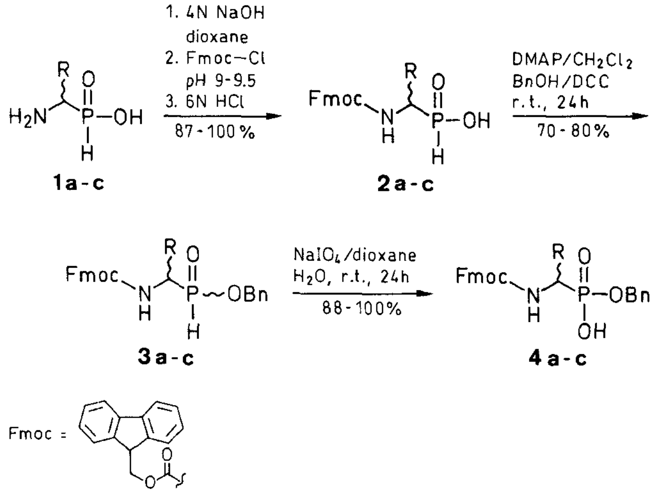
The monoesters of *N*-protected α-aminophosphonic acids are crucial intermediates in the synthesis of phosphonamide substituted peptides. Synthetic approaches have been reported using α-aminophosphonic acids or their diesters, as precursors of *N*-protected (benzyloxycarbonyl) derivatives.[1] However, besides the difficulties encountered during partial hydrolysis of such diesters[2] to yield the monoester derivatives or conversely during monoesterification of the diacidic phosphonic acid function using dicyclohexylcarbodiimide (DCC)[3] or trichloroacetonitrile,[4] it needs to be considered that the choice of the protecting groups, as required for such trifunctional compounds, can be significantly restricted under the reaction conditions used.

We propose here a more general synthetic strategy using α-aminophosphinic acid derivatives as precursors of suitably protected α-aminophosphonic acids (Scheme 1), and we describe the synthesis of a series of novel 9-fluorenylmethoxycarbonyl (Fmoc)[5] substituted α-aminophosphonic acid benzyl monoesters, as well as their use in the synthesis of phosphonamide substituted dipeptides.

The α-aminophosphinic acids **1** are prepared according to a procedure adapted from Baylis et al.[6] The corresponding *N*-Fmoc derivatives **2** are obtained in quantitative yields by addition of 9-fluorenylmethyl chloroformate (Fmoc-Cl) to a solution of the α-aminophosphinic acids **1** in water/dioxane mixture at controlled pH (9–9.5 using 4 M sodium hydroxide).

Esterification of **2** with benzyl alcohol in the presence of DCC (1.1 equiv) and 4-(dimethylamino)pyridine (DMAP) (0.1–0.5 equiv) in dichloromethane (instead of tetrahydrofuran as initially proposed Karanewski and Badia[7] owing to the poor solubility of compounds **2**) leads to **3**, as a mixture of two diastereoisomers in nearly equal quantities, with yields ranging between 70% and 80%.

Finally, the oxidation of the monoesters **3** using sodium metaperiodate, in water/dioxane mixture, affords the expected α-aminophosphonic monoesters **4** in quantitative yields. The structures of compounds **2**, **3**, and **4** were confirmed by ^{1}H and ^{31}P NMR spectra (Table 1). Compounds **2** and **3** display ^{31}P chemical shifts in the 27–35 ppm range, and, as expected large $^1J_{\mathrm{P,H}}$ coupling constants (530–560 Hz).

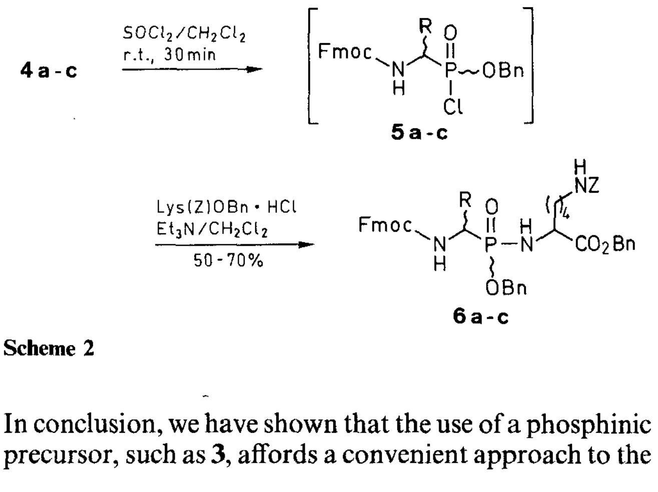


1–4	a	b	c
R	Me	CH_2Ph	*i*-Pr

Scheme 1

The occurence of two diastereoisomers for compounds **3** is inferred from the observation of two distinct ^{31}P resonances with chemical shifts, however, not differing by more than 2 ppm (Table 1).

A first example of the usefulness of compounds **4** for synthesizing peptide structures including a phosphonamide bond is illustrated in the case of a series of dipeptide derivatives **6**, prepared according to the reaction pathway in Scheme 2, with a central phosphonamide bond and both *N*- and *C*-terminii selectively blocked by a Fmoc group and an ester group, respectively. As shown in Table 2, all compounds **6a–c** synthesized were satisfactorily characterized by ^{1}H, ^{31}P NMR and FAB mass spectrometry.



Scheme 2

In conclusion, we have shown that the use of a phosphinic precursor, such as **3**, affords a convenient approach to the

Table 1. Compounds **2**–**4** Prepared

Product	Yield (%)	mp (°C) (Et_2O)	Molecular Formula[a]	^1H NMR (solvent/TMS)[b] δ, J (Hz)	^{31}P NMR δ, $^1J_{P,H}$ (Hz) (solvent)
2a	92	181	$C_{17}H_{18}NO_4P$ (331.3)	DMSO-d_6: 1.2 (dd, 3 H, $J = 7.3$, 16.2, CH_3), 3.6 (m, 1 H, $C\underline{H}CH_3$), 4.3 (m, 3 H, $CH_{fluorenyl} + CH_2$), 6.8 (d, 1 H, $J = 533$, PH), 7.3 (t, 2 $H_{fluorenyl}$, $J = 7.4$), 7.4 (t, 2 $H_{fluorenyl}$, $J = 7.4$), 7.6 (d, 1 H, $J = 8.4$, NH), 7.7 (d, 2 $H_{fluorenyl}$, $J = 7.4$), 7.9 (d, 2 $H_{fluorenyl}$, $J = 7.5$)	28.6, $J = 533$ (DMSO-d_6)
2b	87	184–185	$C_{23}H_{22}NO_4P$ (407.4)	DMSO-d_6: 2.8, 3.0 (2 m, 1 H each, $C\underline{H}_2Ph$), 3.7 (m, 1 H, $C\underline{H}CH_2Ph$), 4.1 (m, 3 H, $CH_{fluorenyl} + CH_2$), 6.9 (d, 1 H, $J = 532$, PH), 7.3 (m, 9 H, C_6H_5 + 4 $H_{fluorenyl}$), 7.6 (t, 2 $H_{fluorenyl}$, $J = 7.4$), 7.8 (d, 1 H, $J = 9.9$, NH), 7.9 (d, 2 $H_{fluorenyl}$, $J = 7.4$)	27.2, $J = 532$ (DMSO-d_6)
2c	~100	166–168	$C_{19}H_{22}NO_4P$ (359.4)	DMSO-d_6: 0.9, 1.0 [2 d, 3 H each, $J = 6.7$, $CH(C\underline{H}_3)_2$], 2.0 [m, 1 H, $C\underline{H}(CH_3)_2$], 3.4 (m, 1 H, $C\underline{H}CH(CH_3)_2$], 4.2 (3 H, $CH_{fluorenyl} + CH_2$), 6.8 (d, 1 H, $J = 554$, PH), 7.3 (t, 2 $H_{fluorenyl}$, $J = 7.4$), 7.4 (t, 2 $H_{fluorenyl}$, $J = 7.4$), 7.6 (d, 1 H, $J = 9.6$, NH), 7.7 (d, 2 $H_{fluorenyl}$, $J = 7.6$), 7.9 (d, 2 $H_{fluorenyl}$, $J = 7.4$)	31.8, $J = 554$ (MeOH)
3a	79	116–127	$C_{24}H_{24}NO_4P$ (421.4)	$CDCl_3$: 1.3 (dd, 3 H, $J = 6.2$, 16.2, CH_3), 4.1 (m, 1 H, $C\underline{H}CH_3$), 4.3 (m, 3 H, $CH_{fluorenyl} + CO_2CH_2$), 5.0 (br s, 1 H, NH), 5.1 (m, 2 H, $C\underline{H}_2Ph$), 7.0 (br d, 1 H, $J = 555$, PH), 7.2 (t, 2 $H_{fluorenyl}$, $J = 7.4$), 7.3 (s, 5 H, C_6H_5), 7.4 (t, 2 $H_{fluorenyl}$, $J = 7.6$), 7.6 (d, 2 $H_{fluorenyl}$, $J = 7.5$), 7.7 (d, 2 $H_{fluorenyl}$, $J = 7.5$)	33.3, 35.3, $J = 555$, 556 ($CDCl_3$)
3b	75	–[c]	$C_{30}H_{28}NO_4P$ (497.5)	$CDCl_3$: 2.9, 3.2 (2 m, 1 H each, $C\underline{H}_2Ph$), 4.1 (m, 1 H, $CH_{fluorenyl}$), 4.2 (m, 1 H, $C\underline{H}CH_2Ph$), 4.3 (m, 2 H, CO_2CH_2), 4.9 (m, 1 H, NH), 5.0 (m, 2 H, $OC\underline{H}_2Ph$), 7.1 (br d, 1 H, $J = 562$, PH), 7.3 (m, 16 H, C_6H_5 + 11 $H_{fluorenyl}$), 7.7 (d, 2 $H_{fluorenyl}$, $J = 7.6$)	33.0, 33.7, $J = 560$, 562 ($CDCl_3$)
3c	80	–[c]	$C_{26}H_{28}NO_4P$ (449.5)	DMSO-d_6: 0.9, 1.0 [2 d, 3 H each, $CH(C\underline{H}_3)_2$], 2.1 [m, 1 H, $C\underline{H}(CH_3)_2$], 3.6 [m, 1 H, $J = 17.7$, 9.3, $C\underline{H}CH(CH_3)_2$], 4.2 (m, 3 H, $CH_{fluorenyl} + CO_2CH_2$), 5.1 (m, 2 H, $C\underline{H}_2Ph$), 7.0 (br d, 1 H, $J = 549$, PH), 7.3 (d, 2 $H_{fluorenyl}$, $J = 7.3$), 7.4 (m, 7 H, C_6H_5 + 2 $H_{fluorenyl}$), 7.7 (d, 2 $H_{fluorenyl}$, $J = 7.3$), 7.8 (d, 1 H, $J = 9.3$, NH), 7.9 (d, 2 $H_{fluorenyl}$, $J = 7.4$)	34.7, 35.1, $J = 549$ ($CDCl_3$)
4a	96	135	$C_{24}H_{24}NO_5P$ (437.4)	$CDCl_3$: 1.4 (dd, 3 H, $J = 7$, 16, CH_3), 4.1 (m, 1 H, $C\underline{H}CH_3$), 4.3 (m, 3 H, $CH_{fluorenyl} + CO_2CH_2$), 5.0 (m, 2 H, $C\underline{H}_2Ph$), 7.1 (br s, 1 H, NH), 7.3 (m, 7 H, C_6H_5 + 2 $H_{fluorenyl}$), 7.4 (t, 2 $H_{fluorenyl}$, $J = 6.7$), 7.6 (d, 2 $H_{fluorenyl}$, $J = 6.8$), 7.7 (d, 2 $H_{fluorenyl}$, $J = 6.7$), 10.5 (br s, 1 H, OH)	25.9 ($CDCl_3$)
4b	88	154–156	$C_{30}H_{28}NO_5P$ (513.5)	DMSO-d_6: 2.9, 3.1 (2 m, 1 H each $C\underline{H}_2Ph$), 4.1 (m, 4 H, $CH_{fluorenyl}$ + $C\underline{H}CH_2Ph + CO_2CH_2$), 5.0 (m, 2 H, OCH_2Ph), 7.3 (m, 14 H, C_6H_5 + 9 $H_{fluorenyl}$), 7.6 (t, 2 $H_{fluorenyl}$, $J = 7.8$), 7.8 (d, 1 H, $J = 9.7$, NH), 7.9 (d, 2 $H_{fluorenyl}$, $J = 7.8$)	26.4 (MeOH)
4c	98	172	$C_{26}H_{28}NO_5P$ (465.5)	DMSO-d_6: 0.9, 1.0 [2 d, 3 H each, $J = 6.9$, 7.0, $CH(C\underline{H}_3)_2$], 2.1 [m, 1 H, $C\underline{H}(CH_3)_2$], 3.8 [ddd, 1 H, $J = 10.3$, 6, 17.7, $C\underline{H}CH(CH_3)_2$], 4.2 (m, 3 H, $CH_{fluorenyl} + CO_2CH_2$), 5.1 (m, 2 H, $OC\underline{H}_2Ph$), 7.3 (m, 8 H, C_6H_5 + OH + 2 $H_{fluorenyl}$), 7.4 (t, 2 $H_{fluorenyl}$, $J = 7.3$), 7.6 (d, 1 H, $J = 10.3$, NH), 7.7 (d, 2 $H_{fluorenyl}$, $J = 7.3$), 7.9 (d, 2 $H_{fluorenyl}$, $J = 7.4$)	22.9 (DMSO-d_6)

[a] Satisfactory microanalyses obtained (for **4a**–**c**): C ±0.1, H ±0.26, N ±0.13.

[b] A signal for acidic P(O)OH proton was not observed except for compounds **4a** and **4c**.

[c] Amorphous powder.

synthesis of suitably derivatized α-aminophosphonic acids **4**, which can be subsequently reacted for generating a phosphonamide bond in peptide structures. Work is now in progress to demonstrate the usefulness of such an approach in the synthesis of pseudopeptides differing by the number of their residues, as well as by the position of the phosphonamide bond in the peptide chain, with the aim of introducing a phosphonamide moiety as an analog of the transition state associated with carboxamide hydrolysis in peptides.[1,8]

Materials were obtained from commercial suppliers and used without further purification. CH_2Cl_2 was distilled from CaH_2. All melting points are uncorrected. NMR spectra were recorded on high field instruments (> 200 MHz). ^1H chemical shifts are reported in ppm on δ scale relative to TMS. ^1H NMR data are presented as follows: chemical shift (multiplicity, number of protons, coupling in Hz). ^{31}P chemical shift in ppm are reported downfield to external 80% H_3PO_4.

α-(9-Fluorenylmethoxycarbonylamino)alkylphosphinic Acids 2a–c; General Procedure:

Compound **1** (50 mmol) was dissolved in water (50 mL), the pH of the solution adjusted to 9.5 with 4 N NaOH and the mixture cooled to 4 °C. Fmoc-Cl (14.3 g, 55 mmol) dissolved in dioxane (50 mL) was added over 10 min and the mixture was stirred for further 6 h while the pH was maintained at 9–9.5 by periodic addition of 4 N NaOH. The mixture was then allowed to warm to r. t. and washed with Et_2O. The aqueous solution was slowly added to a solution of 6 N HCl (200 mL) at 0 °C. The solid product was collected by filtration and dried under vacuum. The purity of compound **2** thus prepared was checked by ^1H, ^{31}P NMR and TLC (silica gel; MeOH/CH_2Cl_2, 1:1) (Table 1).

Benzyl α-(9-Fluorenylmethoxycarbonylamino)alkylphosphinates 3a–c; General Procedure:

A solution of DCC (8.9 g, 43 mmol) and benzyl alcohol (4.2 mL, 41 mmol) in anhydrous CH_2Cl_2 (20 mL) was added over 1 h to a solution of compound **2** (39 mmol) and DMAP (2.6 g, 22 mmol) in anhydrous CH_2Cl_2 (70 mL) at r. t. After stirring at r. t. for 24 h, the mixture was filtered, diluted with EtOAc (100 mL) and washed

Table 2. Compounds **6a–c** Prepared

Product	Yield[a] (%)	Molecular Formula[b]	^1H NMR (DMSO-d_6/TMS) δ, J (Hz)	^{31}P NMR (DMSO-d_6) δ	MS (FAB) m/z
6a	58	$C_{45}H_{48}N_3O_8P$ (789.9)	1.2 (m, 3H, CH_3), 1.3 (m, 4H, CH_2), 1.6 (m, 2H, CH_2), 2.9 (m, 2H, CH_2), 4.0 (m, 2H, $C\underline{H}CH_3$ + $C\underline{H}CO_2CH_2Ph$), 4.2 (m, 3H, $CH_{fluorenyl}$ + Fluorenyl-OCH_2), 5.0 [m, 7H, P(O)NH + 3 × $C\underline{H}_2Ph$], 7.2 (m, 1H, $N\underline{H}CO_2Ph$), 7.3 (m, 19H, C_6H_5, $4H_{fluorenyl}$), 7.5 (d, 1H, $J = 7.2$, NH), 7.7 (m, $2H_{fluorenyl}$), 7.8 (d, $2H_{fluorenyl}$, $J = 7.6$)	28.8, 29.7, 30.6, 30.7	790 ($M^+ + H$), 656 ($M^+ - Z - CO_2 + 2$)
6b	68	$C_{51}H_{52}N_3O_8P$ (865.9)	1.3 (m, 4H, CH_2), 1.6 (m, 2H, CH_2), 2.8, 3.0 (2m, 1H each, $CHC\underline{H}_2Ph$), 2.9 (m, 2H, CH_2), 4.1 (m, 5H, $C\underline{H}CH_2Ph$ + $C\underline{H}CO_2CH_2Ph$ + $CH_{fluorenyl}$ + Fluorenyl OCH_2), 5.1 [m, 7H, P(O)NH + 3 × $OC\underline{H}_2Ph$], 7.1 (m, 1H, $N\underline{H}CO_2Ph$), 7.3 (m, 24H, 4 × C_6H_5 + $4H_{fluorenyl}$), 7.5 (m, $2H_{fluorenyl}$), 7.6 (m, 1H, NH), 7.7 (m, $2H_{fluorenyl}$), 7.8 (d, $2H_{fluorenyl}$, $J = 7.5$)	27.4, 28.2, 29.1, 29.3	866 ($M^+ + H$), 732 ($M^+ - Z - CO_2 + 2$), 644 (M^+ − Fmoc − $CO_2 + 2$)
6c	51	$C_{47}H_{52}N_3O_8P$ (817.9)	0.9 [m, 6H, $CH(C\underline{H}_3)_2$], 1.3 (m, 4H, CH_2), 1.6 (m, 2H, CH_2), 2.2 [m, 1H, $C\underline{H}(CH_3)_2$], 2.9 (m, 2H, CH_2), 3.9 [m, 2H, $C\underline{H}CH(CH_3)_2$ + $C\underline{H}CO_2CH_2Ph$), 4.1 (m, 3H, $CH_{fluorenyl}$ + Fluorenyl-$OC\underline{H}_2$), 4.4 [m, 1H, P(O)NH], 5.0 (m, 6H, 3 × $OC\underline{H}_2Ph$), 6.4 (m, 1H, NH), 7.2 (m, 17H, 3 × C_6H_5 + $2H_{fluorenyl}$), 7.3 (t, $2H_{fluorenyl}$, $J = 7.5$), 7.5 (m, $2H_{fluorenyl}$), 7.7 (d, $2H_{fluorenyl}$, $J = 7.6$)	27.5, 28.9, 29.0, 29.4	818 ($M^+ + H$), 684 ($M^+ - Z - CO_2 + 2$), 594 (M^+ − Fmoc − $CO_2 + 2$)

[a] Yield of pure chromatographed product.

[b] Satisfactory microanalyses obtained: C ±0.1, H ±0.24, N ±0.26.

successively with 2% $KHSO_4$, sat. K_2CO_3 and brine, dried (Na_2SO_4) and evaporated to dryness. The purity of compound **3** as a diastereoisomeric mixture thus prepared was checked by ^1H, ^{31}P NMR and TLC (silica gel; EtOAc/CH_2Cl_2, 1:1) (Table 1).

Benzyl Hydrogen α-(9-Fluorenylmethoxycarbonylamino)alkylphosphonates 4a–c; General Procedure:

Compound **3** (24 mmol) was dissolved in dioxane (200 mL) and reacted with a solution of $NaIO_4$ (5.8 g, 26 mmol) in water (30 mL). After stirring for 10 h, the colored mixture was diluted with EtOAc (100 mL)/2% $KHSO_4$ (100 mL) and filtered. The organic phase was washed with 2% $NaHSO_3$ (2 × 50 mL) and brine, dried (Na_2SO_4) and evaporated to dryness under vacuum. The residue was precipitated with Et_2O then filtered to give a white powder. The purity of compounds **4** was checked by ^1H, ^{31}P NMR; MS (FAB) and TLC (silica gel; MeOH/CH_2Cl_2/AcOH, 20:80:3) (Table 1).

Benzyl α-(9-Fluorenylmethoxycarbonylamino)alkylphosphonochloridates 5a–c; General Procedure:

To a stirred suspension of **4** (1 mmol) in anhydrous CH_2Cl_2 (5 mL) was added $SOCl_2$ (100 μL, 1.4 mmol). After 30 min the solvent was removed under vacuum to afford the oily phosphonochloridates **5**. Compound **5** thus prepared was checked by ^{31}P NMR.

Benzyl α-(9-Fluorenylmethoxycarbonylamino)alkylphosphonopeptides 6a–c; General Procedure:

To a suspension of Lys(Z)OBn · HCl (366 mg, 0.9 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C was added Et_3N (300 μL, 2.2 mmol) under N_2. The appropriate crude phosphonochloridate **5**, dissolved in anhydrous CH_2Cl_2, was then added via a cannula. The flask containing **5** was rinsed with additional CH_2Cl_2 (5 mL), which was added to the reaction mixture. After stirring for 1 h the solvent was removed under vacuum to provide a light yellow powder which was purified directly by chromatography on a column of silica gel (15 g of silica gel gradient of EtOAc in CH_2Cl_2). The fractions were pooled to give a white solid which contained the desired mixture of the expected diastereoisomers **6**. The homogeneity of compounds **6** thus prepared was checked by ^1H, ^{31}P NMR, TLC (silica gel; EtOAc/CH_2Cl_2, 1:1).

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