Supplementary Material for Chemical Communications

Supplementary data

Stereoselective synthesis of α-monofluorinated phosphonate mimetics of naturally occurring phosphoserine and phosphothreonine, via electrophilic fluorination of lithiated bis-lactim ethers

María Ruiz,* Vicente Ojea,* José M. Quintela and Juan J. Guillín

Departamento de Química Fundamental, Universidade da Coruña, Campus da Zapateira, s/n. 15071 A Coruña, Spain. E-mail: ruizpr@udc.es

General Methods: All moisture-sensitive reactions were performed under an argon atmosphere using oven-dried glassware. Reagents and solvents were purchased and used without further purification unless otherwise stated. THF was distilled from sodium/benzophenone. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and iodine or ninhydrin in a 3% HOAc/*n*-BuOH solution as developing agents. E. Merck silica gel 60 and RP-18 (both 230-400 mesh) were used for liquid chromatography separations. Melting points were determined on a Büchi 510 melting-point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer IR 783 spectrophotometer. Unless otherwise indicated, ^{1}H NMR spectra were recorded at 200 MHz, and ^{31}P, ^{19}F and ^{13}C NMR spectra were recorded at 81, 188 and 50 MHz, respectively, with broad-band ^{1}H decoupling on a Bruker AC 200F spectrometer at 25 °C. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.00) or to HOD in D_2O (δ 4.60) for ^{1}H, relative to internal $CDCl_3$ (δ 77.0) for ^{13}C, relative to 85% phosphoric acid (external) for ^{31}P and relative to $CFCl_3$ (external) for ^{19}F. Recognition of methyl, methylene, methine, and quaternary carbon nuclei in ^{13}C spectra rests on the *J*-modulated spin-echo sequence. ^{1}H or ^{13}C assignments were confirmed with the aid of homonuclear or heteronuclear two-dimensional experiments. MS (FAB) spectra were recorded on a Fisons VG-Quattro mass spectrometer, using thioglycerol as a matrix. Optical rotations were taken at the Na_D-line on a Jasco DIP-1000 automatic digital polarimeter. $[\alpha]_D$ values are given in deg cm^2 g^{-1}, with concentrations in 10^{-2} g cm^{-3}. Elemental analyses were performed at Servicios Xerais de Apoio á Investigación of Universidade da Coruña on a Carlo-Erba EA 1108-CHNS-O analyzer.

(2*S*,5*R*)-3,6-diethoxy-2-[2-(diethoxyphosphoryl)ethyl]-2,5-dihydro-5-isopropyl-pyrazine (3a). A solution of *n*-BuLi (3.77 mL 2.5 M in hexane, 9.42 mmol) was added to a stirred solution of (3*R*)-2,5-diethoxy-3-isopropyl-3,6-dihydropyrazine (2.0 g, 9.42 mmol) in THF (40 mL) at −78 °C and the mixture was stirred for 30 min. Then, a solution of *O*,*O*-diethyl 2-bromoethylphosphonate (2.18 g, 8.90 mmol) and *O*,*O*-diethyl vinylphosphonate (77 mg, 0.47 mmol) in THF (30 mL) was added dropwise. After being stirred at −78 °C for 5 min, the reaction was allowed to reach room temperature, and the solvent was removed in vacuo. The resulting material was diluted with water and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica gel, EtOAc/hexanes 2:1) to yield 3.1 g (85%) of **3a** as a colorless oil. $[\alpha]^{20}_D$ −1.4 (c 0.6, CH_2Cl_2); IR (film) ν 1690 (C=N), 1235 (P=O), 1030 (POC) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.71 (d, *J* = 6.8 Hz, 3 H, $CH(C\underline{H}_3)_2$), 1.02 (d, *J* = 6.8 Hz, 3 H, $CH(C\underline{H}_3)_2$), 1.21-1.38 (m, 12 H, $OCH_2C\underline{H}_3$, $POCH_2C\underline{H}_3$), 1.63-1.81 (m, 2 H, H-2´), 1.83-2.20 (m, 2 H, H-1´), 2.28 (dsp, *J* = 6.8, 3.4 Hz, 1 H, $C\underline{H}(CH_3)_2$), 3.91 (t, *J* = 3.4 Hz, 1 H, H-5), 3.97-4.26 (m, 9 H, $OC\underline{H}_2CH_3$, $POC\underline{H}_2CH_3$, H-2); ^{13}C NMR ($CDCl_3$) δ 14.1 ($OCH_2\underline{C}H_3$), 16.2 (d, *J* = 6.0 Hz, $POCH_2\underline{C}H_3$), 16.5, 18.8 ($CH(\underline{C}H_3)_2$), 20.7 (d, *J* = 142.3 Hz, C-2´), 26.9 (d, *J* = 3.4 Hz, C-1´), 31.8 ($\underline{C}H(CH_3)_2$), 54.6 (d, *J* = 18.9 Hz, C-2), 60.4, 60.5 ($O\underline{C}H_2CH_3$), 60.8 (C-5) 61.2 (d, *J* = 6.4 Hz, $PO\underline{C}H_2CH_3$), 162.1, 163.3 (C=N); ^{31}P NMR ($CDCl_3$) δ 30.9; FABMS (thioglycerol) m/z 377 (MH^+, 100). Anal. Calcd for $C_{17}H_{33}N_2O_5P$: C, 54.24; H, 8.84; N, 7.44. Found: C, 53.95; H, 9.07; N, 7.38.

General procedure for the electrophilic fluorinations of lithiated bis-lactim ethers derived from *cyclo*-[L-AP4-D-Val]. Method I. A solution of **3a,b** (1.92 mmol) in THF (8 mL) was added dropwise to a stirred solution of LDA (4.0 mmol) in THF (20 mL) at −78 °C and the mixture was stirred for 15 min. Then, a solution of the NFSi (0.63 g, 1.92 mmol) in THF (5 mL) was added dropwise, the mixture was stirred for 5 min and the reaction was quenched with AcOH or H_2O (10% in THF). The crude reaction mixture was warmed to room temperature, and the solvent was removed in vacuo. The resulting material was diluted with water and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated, and the residue was purified by gradient flash chromatography to yield substitution products as colorless oils. **Method II.** A solution of LDA (4.0 mmol) in THF (20 mL) was added dropwise to a solution of **3a,b** (1.92 mmol) and NFSi (0.63 g, 1.92 mmol) in THF (15 mL) at −78 °C. After being stirred at −78 °C for 5 min, the reaction was quenched with AcOH or H_2O (10% in THF) and worked up as described in method I.

Electrophilic fluorination of **3a***:* Method I was followed. The crude material was purified by flash chromatography (silica gel, EtOAc/hexanes 1:1) to give 0.55 g (*ca.* 75%) of a mixture of bis-lactims **4a**/**5a**/**6a** in a 8.2:2.7:1.0 ratio. Separation of the components of

this mixture was accomplished by flash chromatography (silica gel, EtOAc/hexanes, from 1:4 to 1:1).

(2*S*,5*R*,2´*S*)-3,6-diethoxy-2-[2-(diethoxyphosphoryl)-2-fluoroethyl]-2,5-dihydro-5-isopropylpyrazine (**4a**): $[\alpha]^{26}_{D}$ –21.6 (c 2.9, CH_2Cl_2); IR (film) ν 1685 (C=N), 1235 (P=O), 1025 (POC) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.72 (d, *J* = 6.9 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.02 (d, *J* = 6.9 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.22-1.39 (m, 12 H, OCH$_2$C<u>H</u>$_3$, POCH$_2$C<u>H</u>$_3$), 1.72 (m, 1 H, H-1´), 2.22 (dsp, *J* = 6.9, 3.4 Hz, 1 H, C<u>H</u>(CH$_3$)$_2$), 2.65 (m, 1 H, H-1´), 3.92 (t, *J* = 3.4 Hz, 1 H, H-5), 4.00-4.30 (m, 9 H, OC<u>H</u>$_2$CH$_3$, POC<u>H</u>$_2$CH$_3$, H-2), 5.34 (ddt, *J* = 47.0, 12.0, 1.5 Hz, 1 H, H-2´); ^{13}C NMR ($CDCl_3$) δ 14.1 (OCH$_2$<u>C</u>H$_3$), 16.3 (d, *J* = 4.5 Hz, POCH$_2$<u>C</u>H$_3$), 16.9 (CH(<u>C</u>H$_3$)$_2$), 18.9 (CH(<u>C</u>H$_3$)$_2$), 32.0 (<u>C</u>H(CH$_3$)$_2$), 35.1 (dd, *J* = 20.0, 2.7 Hz, C-1´), 50.4 (dd, *J* = 14.0, 2.7 Hz, C-2), 60.5 (O<u>C</u>H$_2$CH$_3$), 60.6 (O<u>C</u>H$_2$CH$_3$), 60.7 (C-5), 62.9 (d, *J* = 6.5 Hz, PO<u>C</u>H$_2$CH$_3$), 63.2 (d, *J* = 6.5 Hz, PO<u>C</u>H$_2$CH$_3$), 85.3 (dd, *J* = 179.0, 172.0 Hz, C-2´), 162.5 (C=N), 163.3 (C=N); ^{31}P NMR ($CDCl_3$) δ 19.6 (d, *J* = 74.3 Hz); ^{19}F NMR ($CDCl_3$) δ –213.7 (d, *J* = 74.3 Hz); FABMS (thioglycerol) m/z 396 ($(M+2)^+$, 23), 395 (MH^+, 100). Anal. Calcd for $C_{17}H_{32}FN_2O_5P$: C, 51.77; H, 8.18; N, 7.10. Found: C, 51.85; H, 8.01; N, 7.38.

(2*S*,5*R*,2´*R*)-3,6-diethoxy-2-[2-(diethoxyphosphoryl)-2-fluoroethyl]-2,5-dihydro-5-isopropylpyrazine (**5a**): $[\alpha]^{22}_{D}$ +6.7 (c 0.8, CH_2Cl_2); IR (film) ν 1685 (C=N), 1235 (P=O), 1025 (POC) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.72 (d, *J* = 6.8 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.02 (d, *J* = 6.9 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.24-1.39 (m, 12 H, OCH$_2$C<u>H</u>$_3$, POCH$_2$C<u>H</u>$_3$), 2.13-2.64 (m, 3 H, H-1´, C<u>H</u>(CH$_3$)$_2$), 3.95 (t, *J* = 3.4 Hz, 1 H, H-5), 4.00-4.30 (m, 9 H, OC<u>H</u>$_2$CH$_3$, POC<u>H</u>$_2$CH$_3$, H-2), 5.03 (ddt, *J* = 47.0, 10.0, 3.4 Hz, 1 H, H-2´); ^{13}C NMR ($CDCl_3$) δ 14.1 (OCH$_2$<u>C</u>H$_3$), 14.3 (OCH$_2$<u>C</u>H$_3$), 16.3 (d, *J* = 6.0 Hz, POCH$_2$<u>C</u>H$_3$), 16.6 (CH(<u>C</u>H$_3$)$_2$), 18.9 (CH(<u>C</u>H$_3$)$_2$), 32.0 (<u>C</u>H(CH$_3$)$_2$), 34.4 (dd, *J* = 19.4, 2.7 Hz, C-1´), 55.5 (dd, *J* = 13.0, 3.0 Hz, C-2), 60.6 (O<u>C</u>H$_2$CH$_3$), 60.6 (C-5), 62.8 (d, *J* = 6.5 Hz, PO<u>C</u>H$_2$CH$_3$), 63.1 (d, *J* = 6.5 Hz, PO<u>C</u>H$_2$CH$_3$), 86.0 (dd, *J* = 179.4, 172.5 Hz, C-2´), 162.4 (C=N), 164.3 (C=N); ^{31}P NMR ($CDCl_3$) δ 19.0 (d, *J* = 74.7 Hz); ^{19}F NMR ($CDCl_3$) δ –212.2 (d, *J* = 74.8 Hz); FABMS (thioglycerol) m/z 396 ($(M+2)^+$, 23), 395 (MH^+, 100). Anal. Calcd for $C_{17}H_{32}FN_2O_5P$: C, 51.77; H, 8.18; N, 7.10. Found: C, 51.96; H, 8.31; N, 7.07.

(2*S*,5*R*)-3,6-diethoxy-2-[2-(diethoxyphosphoryl)-2,2-difluoroethyl]-2,5-dihydro-5-isopropylpyrazine (**6a**): $[\alpha]^{26}_{D}$ –7.4 (c 2.4, CH_2Cl_2); IR (film) ν 1685 (C=N), 1225 (P=O), 1015 (POC) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.67 (d, *J* = 6.8 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 0.99 (d, *J* = 6.9 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.18-1.37 (m, 12 H, OCH$_2$C<u>H</u>$_3$, POCH$_2$C<u>H</u>$_3$), 2.12-2.47 (m, 2 H, H-1´, C<u>H</u>(CH$_3$)$_2$), 2.70 (dtt, *J* = 27.3, 14.7, 3.5 Hz, 1 H, H-1´), 3.87 (t, *J* = 3.5 Hz, 1 H, H-5), 4.00-4.38 (m, 9 H, OC<u>H</u>$_2$CH$_3$, POC<u>H</u>$_2$CH$_3$, H-2); ^{13}C NMR ($CDCl_3$) δ 14.2 (OCH$_2$<u>C</u>H$_3$), 16.3 (d, *J* = 5.6 Hz, POCH$_2$<u>C</u>H$_3$), 16.7 (CH(<u>C</u>H$_3$)$_2$), 19.0 (CH(<u>C</u>H$_3$)$_2$), 31.7 (<u>C</u>H(CH$_3$)$_2$),

37.7 (dt, *J* = 19.2, 14.9 Hz, C-1´), 50.4 (t, *J* = 3.9 Hz, C-2), 60.6 (C-5), 60.7 (O<u>C</u>H$_2$CH$_3$), 60.8 (O<u>C</u>H$_2$CH$_3$), 64.4 (d, *J* = 6.4 Hz, PO<u>C</u>H$_2$CH$_3$), 121.0 (dt, *J* = 270.0, 216.0 Hz, C-2´), 161.5 (C=N), 163.1 (C=N); ^{31}P NMR (CDCl$_3$) δ 7.4 (t, *J* = 107.0 Hz); ^{19}F NMR (CDCl$_3$) δ –110.6 (dd, *J* = 298.5, 107.0 Hz), –112.6 (dd, *J* = 298.5, 107.0 Hz); FABMS (thioglycerol) m/z 414 ((M+2)$^+$, 20), 413 (MH$^+$, 100). Anal. Calcd for $C_{17}H_{31}F_2N_2O_5P$: C, 49.51; H, 7.58; N, 6.79. Found: C, 49.35; H, 7.41; N, 6.94.

Electrophilic fluorination of **3b***:* Method I was followed. The crude material was purified by flash chromatography (silica gel, EtOAc/hexanes 1:1) to give 0.58 g (*ca*. 75%) of a mixture of bis-lactims **4b/5b/6b** in a 7:7:1 ratio. Separation of the components of this mixture was accomplished by flash chromatography (silica gel, EtOAc/hexanes, from 1:4 to 1:1).

(2*S*,5*R*,1´R,2´*S*)-3,6-diethoxy-2-[2-(diethoxyphosphoryl)-2-fluoro-1-methylethyl]-2,5-dihydro-5-isopropylpyrazine (**4b**): $[\alpha]^{26}_D$ +32.9 (c 1.6, CH_2Cl_2); IR (film) ν 1700 (C=N), 1245 (P=O), 1040 (POC) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.69 (d, *J* = 6.8 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.03 (d, *J* = 6.8 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.17-1.38 (m, 15 H, CHC<u>H</u>$_3$, OCH$_2$C<u>H</u>$_3$, POCH$_2$C<u>H</u>$_3$), 2.28 (dsp, *J* = 6.8, 3.5 Hz, 1 H, C<u>H</u>(CH$_3$)$_2$), 2.44-2.74 (m, 1 H, H-1´), 3.89 (t, *J* = 3.5 Hz, 1 H, H-5), 4.03-4.28 (m, 9 H, OC<u>H</u>$_2$CH$_3$, POC<u>H</u>$_2$CH$_3$, H-2), 4.90 (ddd, *J* = 47.0, 12.0, 1.5 Hz, 1 H, H-2´); ^{13}C NMR (CDCl$_3$) δ 10.8 (d, *J* = 7.8, 4.3 Hz, CH<u>C</u>H$_3$), 14.3 (OCH$_2$<u>C</u>H$_3$), 16.4 (POCH$_2$<u>C</u>H$_3$), 16.5 (CH(<u>C</u>H$_3$)$_2$), 19.0 (CH(<u>C</u>H$_3$)$_2$), 31.5 (<u>C</u>H(CH$_3$)$_2$), 38.4 (d, *J* = 19.2 Hz, C-1´), 58.4 (dd, *J* = 14.6, 6.0 Hz, C-2), 60.5 (C-5), 60.7 (O<u>C</u>H$_2$CH$_3$), 62.9 (d, *J* = 6.4 Hz, PO<u>C</u>H$_2$CH$_3$), 63.2 (d, *J* = 6.4 Hz, PO<u>C</u>H$_2$CH$_3$), 89.1 (dd, *J* = 183.1, 169.6 Hz, C-2´), 161.8 (C=N), 163.4 (C=N); ^{31}P NMR (CDCl$_3$) δ 18.9 (d, *J* = 69.7 Hz); ^{19}F NMR (CDCl$_3$) δ –218.8 (d, *J* = 76.5 Hz); FABMS (thioglycerol) m/z 410 ((M+2)$^+$, 26), 409 (MH$^+$, 100). Anal. Calcd for $C_{18}H_{34}FN_2O_5P$: C, 52.93; H, 8.39; N, 6.86. Found: C, 53.15; H, 8.01; N, 6.48.

(2*S*,5*R*,1´R,2´*R*)-3,6-diethoxy-2-[2-(diethoxyphosphoryl)-2-fluoro-1-methylethyl]-2,5-dihydro-5-isopropylpyrazine (**5b**): $[\alpha]^{26}_D$ +32.6 (c 0.6, CH_2Cl_2); IR (film) ν 1700 (C=N), 1245 (P=O), 1040 (POC) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.71 (d, *J* = 6.8 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.03 (d, *J* = 6.8 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.09 (d, *J* = 7.3 Hz, 3 H, CH(C<u>H</u>$_3$)$_2$), 1.25-1.40 (m, 12 H, OCH$_2$C<u>H</u>$_3$, POCH$_2$C<u>H</u>$_3$), 2.27 (dsp, *J* = 6.8, 3.4 Hz, 1 H, C<u>H</u>(CH$_3$)$_2$), 2.74-2.92 (m, 1 H, H-1´), 3.92 (t, *J* = 3.4 Hz, 1 H, H-5), 4.05-4.30 (m, 9 H, OC<u>H</u>$_2$CH$_3$, POC<u>H</u>$_2$CH$_3$, H-2), 4.80 (ddd, *J* = 45.4, 11.7, 1.5 Hz, 1 H, H-2´); ^{13}C NMR (CDCl$_3$) δ 12.2 (d, *J* = 8.5 Hz, CH<u>C</u>H$_3$), 14.3 (OCH$_2$<u>C</u>H$_3$), 16.4 (POCH$_2$<u>C</u>H$_3$), 16.7 (CH(<u>C</u>H$_3$)$_2$), 19.0 (CH(<u>C</u>H$_3$)$_2$), 32.0 (<u>C</u>H(CH$_3$)$_2$), 38.2 (d, *J* = 16.3 Hz, C-1´), 56.7 (dd, *J* = 14.6, 6.0 Hz, C-2), 60.6 (O<u>C</u>H$_2$CH$_3$), 60.7 (C-5), 62.8 (d, *J* = 6.0 Hz, PO<u>C</u>H$_2$CH$_3$), 63.2 (d, *J* = 6.0 Hz, PO<u>C</u>H$_2$CH$_3$), 90.4 (dd, *J* = 181.3, 166.4 Hz, C-2´), 161.8 (C=N), 163.4 (C=N); ^{31}P NMR (CDCl$_3$) δ 19.1 (d, *J* = 74.6 Hz); ^{19}F

NMR ($CDCl_3$) δ −205.9 (d, *J* = 74.1 Hz); FABMS (thioglycerol) m/z 410 ($(M+2)^+$, 26), 409 (MH^+, 100). Anal. Calcd for $C_{18}H_{34}FN_2O_5P$: C, 52.93; H, 8.39; N, 6.86. Found: C, 52.85; H, 8.09; N, 7.02.

General procedure for the hydrolysis of the 2´-fluorinated bis-lactim ethers. A solution of **4/5a,b** (1.2 mmol) in THF (12 mL) and 0.25 N HCl (12 mL, 3.0 mmol) was stirred at room temperature for 1.5-10 h. Then, the solution was diluted with water (12 mL) and concentrated to half its initial volume. The aqueous solution was made basic (pH~10) by the addition of $NaHCO_3$ followed by concentrated ammonia. The aqueous layer was extracted with CH_2Cl_2 (7 x 20 mL) and the combined organic layers dried (Na_2SO_4) and evaporated. The crude mixture of ethyl valinate and amino ester was purified by flash chromatography (silica gel, EtOAc to EtOAc/MeOH 6:1) to yield amino esters **7/9a,b** as oils.

Ethyl (2*S*,4*S*)-2-amino-4-diethoxyphosphoryl-4-fluorobutanoate (**7a**): General procedure was followed, stirring the reaction mixture at rt for 1 h. The crude material was purified by flash chromatography (silica gel, EtOAc to EtOAc/MeOH 10:1) to give 0.32 g (93%) of **7a** as a pale yellow oil. $[\alpha]^{26}_D$ −15.0 (c 1.6, CH_2Cl_2); IR (film) ν 3400 (NH), 2980 (CH), 1730 (C=O), 1260 (P=O), 1025 (POC) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, *J* = 7.1 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.28 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.29 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.57 (brs, 2 H, NH_2), 1.64 -2.02 (m, 1 H, H-3), 2.25-2.52 (m, 1 H, H-3), 3.55 (ddd, *J* = 11.0, 3.3, 0.9 Hz, 1 H, H-2), 4.06-4.24 (m, 6 H, $OC\underline{H}_2CH_3$, $POC\underline{H}_2CH_3$), 5.12 (1 H, dddd, *J* = 46.9, 11.6, 2.9, 1.9 Hz, 1 H, H-4); ^{13}C NMR ($CDCl_3$) δ 14.0 ($OCH_2\underline{C}H_3$), 16.3 ($POCH_2\underline{C}H_3$), 16.4 ($POCH_2\underline{C}H_3$), 34.9 (dd, *J* = 20.2, 2.5 Hz, C-3), 50.2 (dd, *J* = 14.2, 2.5 Hz, C-2), 61.2 ($O\underline{C}H_2CH_3$), 62.8 (d, *J* = 6.6 Hz, $PO\underline{C}H_2CH_3$), 63.1 (d, *J* = 6.6 Hz, $PO\underline{C}H_2CH_3$), 85.3 (dd, *J* = 179.9, 172.1 Hz, C-4), 175.4 (C=O); ^{31}P NMR ($CDCl_3$) δ 18.7 (d, *J* = 73.6 Hz); ^{19}F NMR ($CDCl_3$) δ −212.6 (d, *J* = 74.4 Hz); FABMS (thioglycerol) m/z 286 (MH^+, 100). Anal. Calcd for $C_{10}H_{21}FNO_5P$: C, 42.11; H, 7.42; N, 4.91. Found: C, 42.00; H, 7.71; N, 5.20.

Ethyl (2*S*,4*R*)-2-amino-4-diethoxyphosphoryl-4-fluorobutanoate (**9a**): General procedure was followed, stirring the reaction mixture at rt for 1 h. The crude material was purified by flash chromatography (silica gel, EtOAc to EtOAc/MeOH 10:1) to give 0.31 g (89%) of **9a** as a pale yellow oil. $[\alpha]^{26}_D$ +10.0 (c 1.5, CH_2Cl_2); IR (film) ν 3400 (NH), 2980 (CH), 1730 (C=O), 1260 (P=O), 1025 (POC) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (t, *J* = 7.1 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.33 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.34 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.77 (brs, 2 H, NH_2), 2.15-2.40 (m, 2 H, H-3), 3.73 (t, *J* = 6.1 Hz, 1 H, H-2), 4.10-4.27 (m, 6 H, $OC\underline{H}_2CH_3$, $POC\underline{H}_2CH_3$), 4.83-5.21 (m, 1 H, H-4); ^{13}C NMR ($CDCl_3$) δ 14.0 ($OCH_2\underline{C}H_3$), 16.3 ($POCH_2\underline{C}H_3$), 16.4 ($POCH_2\underline{C}H_3$), 35.2 (d, *J* = 19.9 Hz, C-3), 50.9 (d, *J* = 12.8 Hz, C-

2), 61.2 (O<u>C</u>H$_2$CH$_3$), 62.8 (d, *J* = 6.6 Hz, PO<u>C</u>H$_2$CH$_3$), 63.1 (d, *J* = 6.6 Hz, PO<u>C</u>H$_2$CH$_3$), 86.0 (dd, *J* = 179.6, 171.0 Hz, C-4), 174.4 (C=O); ^{31}P NMR (CDCl$_3$) δ 17.9 (d, *J* = 73.6 Hz); ^{19}F NMR (CDCl$_3$) δ –209.4 (d, *J* = 73.4 Hz); FABMS (thioglycerol) m/z 286 (MH$^+$, 100). Anal. Calcd for $C_{10}H_{21}FNO_5P$: C, 42.11; H, 7.42; N, 4.91. Found: C, 42.40; H, 7.51; N, 4.75.

Ethyl (2*S*,3*R*,4*S*)-2-amino-4-diethoxyphosphoryl-4-fluoro-3-methylbutanoate (**7b**): General procedure was followed, stirring the reaction mixture at rt for 10 h. The crude material was purified by flash chromatography (silica gel, EtOAc to EtOAc/MeOH 10:1) to give 0.34 g (95%) of **7b** as a pale yellow oil. [α]$^{26}_D$ –4.6 (c 1.6, CH_2Cl_2); IR (film) ν 3560 (NH), 2990 (CH), 1735 (C=O), 1255 (P=O), 1025 (POC) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.10 (dd, *J* = 7.1, 1.2 Hz, 3 H, CHC<u>H</u>$_3$), 1.23-1.37 (m, 9 H, OCH$_2$C<u>H</u>$_3$), 1.65 (brs, 2 H, NH$_2$), 2.00-2.30 (m, 1 H, H-3), 3.33 (d, *J* = 9.3 Hz, 1 H, H-2), 4.10-4.27 (m, 6 H, OC<u>H</u>$_2$CH$_3$), 5.37 (ddd, *J* = 45.4, 5.9, 2.2 Hz, 1 H, H-4); ^{13}C NMR (CDCl$_3$) δ 10.6 (d, *J* = 5.7 Hz, CH<u>C</u>H$_3$), 14.1 (OCH$_2$<u>C</u>H$_3$), 16.3 (POCH$_2$<u>C</u>H$_3$), 16.4 (POCH$_2$<u>C</u>H$_3$), 38.5 (d, *J* = 20.2 Hz, C-3), 56.8 (d, *J* = 14.2 Hz, C-2), 60.9 (O<u>C</u>H$_2$CH$_3$), 62.8 (d, *J* = 6.7 Hz, PO<u>C</u>H$_2$CH$_3$), 63.0 (d, *J* = 6.7 Hz, PO<u>C</u>H$_2$CH$_3$), 87.8 (dd, *J* = 183.1, 171.0 Hz, C-4), 175.2 (C=O); ^{31}P NMR (CDCl$_3$) δ 18.7 (d, *J* = 77.3 Hz); ^{19}F NMR (CDCl$_3$) δ –224.6 (d, *J* = 77.3 Hz); FABMS (thioglycerol) m/z 300 (MH$^+$, 100). Anal. Calcd for $C_{11}H_{23}FNO_5P$: C, 44.15; H, 7.75; N, 4.68. Found: C, 44.45; H, 7.41; N, 4.83.

Ethyl (2*S*,3*R*,4*R*)-2-amino-4-diethoxyphosphoryl-4-fluoro-3-methylbutanoate (**9b**): General procedure was followed, stirring the reaction mixture at rt for 15 h. The crude material was purified by flash chromatography (silica gel, EtOAc to EtOAc/MeOH 10:1) to give 0.33 g (92%) of **9b** as a pale yellow oil. [α]$^{26}_D$ +14.4 (c 2.9, CH_2Cl_2); IR (film) ν 3560 (NH), 2990 (CH), 1735 (C=O), 1255 (P=O), 1025 (POC) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.12 (d, *J* = 6.8 Hz, 3 H, CHC<u>H</u>$_3$), 1.23-1.38 (m, 9 H, OCH$_2$C<u>H</u>$_3$), 1.68 (brs, 2 H, NH$_2$), 2.63-2.83 (m, 1 H, H-3), 3.58 (d, *J* = 2.9 Hz, 1 H, H-2), 4.10-4.29 (m, 6 H, OC<u>H</u>$_2$CH$_3$), 4.88 (ddd, *J* = 45.4, 10.7, 2.4 Hz, 1 H, H-4); ^{13}C NMR (CDCl$_3$) δ 12.7 (d, *J* = 8.5 Hz, CH<u>C</u>H$_3$), 14.1 (OCH$_2$<u>C</u>H$_3$), 16.1 (POCH$_2$<u>C</u>H$_3$), 16.2 (POCH$_2$<u>C</u>H$_3$), 38.8 (d, *J* = 17.8 Hz, C-3), 55.6 (dd, *J* = 11.1, 5.6 Hz, C-2), 60.9 (O<u>C</u>H$_2$CH$_3$), 62.8 (d, *J* = 6.7 Hz, PO<u>C</u>H$_2$CH$_3$), 63.0 (d, *J* = 6.7 Hz, PO<u>C</u>H$_2$CH$_3$), 89.2 (dd, *J* = 179.6, 167.5 Hz, C-4), 174.1 (C=O); ^{31}P NMR (CDCl$_3$) δ 18.3 (d, *J* = 75.4 Hz); ^{19}F NMR (CDCl$_3$) δ –204.2 (d, *J* = 75.4 Hz); FABMS (thioglycerol) m/z 300 (MH$^+$, 100). Anal. Calcd for $C_{11}H_{23}FNO_5P$: C, 44.15; H, 7.75; N, 4.68. Found: C, 44.01; H, 7.48; N, 4.43.

General procedure for the hydrolysis of the 4-fluorinated triethyl 2-amino-4-phosphonobutanoates. A solution of LiOH (0.75 mmol) in water (3 mL) was added dropwise to a solution of amino ester **7a** or **9a,b** (0.75 mmol) in water (5 mL) and the mixture was stirred at rt for 30 min. The solvent was removed in vacuo and the resulting material was dissolved in H_2O and loaded onto a column (1.5 x 14 cm) of Dowex-50WX8 (H^+ form), the column was washed with H_2O and the product was eluted with 10% aqueous NH_3 solution. Evaporation of the solvent, followed by reversed phase flash chromatography (RP-18, H_2O) afforded the amino acids **8a** or **10a,b**.

(2*S*,4*S*)-2-Amino-4-diethoxyphosphoryl-4-fluorobutanoic acid (**8a**): General procedure was followed, to give 0.18 g (96%) of **8a** as a colorless oil. $[\alpha]^{26}_D$ –5.5 (c 0.7, H_2O); IR (film) ν 3448 (OH), 2990 (CH), 1636 (C=O), 1248 (P=O), 1021 (POC) cm^{-1}; ^1H NMR (D_2O) δ 1.16 (t, *J* = 6.5 Hz, 6 H, $OCH_2C\underline{H}_3$), 2.02-2.55 (m, 2 H, H-3), 3.83 (dd, *J* = 7.6, 4.0 Hz, 1 H, H-2), 4.00-4.17 (m, 4 H, $OC\underline{H}_2CH_3$), 4.96 (dddd, *J* = 46.4, 11.0, 4.9, 2.4 Hz, 1 H, H-4); ^{13}C NMR (D_2O) δ 16.6 ($OCH_2\underline{C}H_3$), 16.7 ($OCH_2\underline{C}H_3$), 31.6 (d, *J* = 18.6 Hz, C-3), 52.7 (d, *J* = 14.8 Hz, C-2), 66.2 (d, *J* = 8.0 Hz, $O\underline{C}H_2CH_3$), 66.4 (d, *J* = 8.0 Hz, $O\underline{C}H_2CH_3$), 86.8 (dd, *J* = 177.5, 173.1 Hz, C-4), 173.7 (C=O); ^{31}P NMR (D_2O) δ 18.4 (d, *J* = 75.3 Hz); ^{19}F NMR (D_2O) δ –210.4 (d, *J* = 75.0 Hz); FABMS (thioglycerol) m/z 258 (MH^+, 100). Anal. Calcd for $C_8H_{17}FNO_5P$: C, 37.36; H, 6.66; N, 5.45. Found: C, 37.05; H, 6.57; N, 5.47.

(2*S*,4*R*)-2-Amino-4-diethoxyphosphoryl-4-fluorobutanoic acid (**10a**): General procedure was followed, to give 0.19 g (98%) of **10a** as a colorless oil. $[\alpha]^{26}_D$ +11.9 (c 1.1, H_2O); IR (film) ν 3445 (OH), 2990 (CH), 1636 (C=O), 1240 (P=O), 1020 (POC) cm^{-1}; ^1H NMR (D_2O) δ 1.13 (t, *J* = 7.3 Hz, 6 H, $OCH_2C\underline{H}_3$), 2.11-2.45 (m, 2 H, H-3), 3.71 (t, *J* = 6.7 Hz, 1 H, H-2), 3.97-4.13 (m, 4 H, $OC\underline{H}_2CH_3$), 5.07 (dddd, *J* = 46.4, 11.0, 4.3, 2.4 Hz, 1 H, H-4); ^{13}C NMR (D_2O) δ 16.6 ($OCH_2\underline{C}H_3$), 16.7 ($OCH_2\underline{C}H_3$), 32.0 (d, *J* = 20.6 Hz, C-3), 52.0 (d, *J* = 15.9 Hz, C-2), 66.2 (d, *J* = 6.6 Hz, $O\underline{C}H_2CH_3$), 66.3 (d, *J* = 6.6 Hz, $O\underline{C}H_2CH_3$), 87.6 (dd, *J* = 177.1, 172.5 Hz, C-4), 174.0 (C=O); ^{31}P NMR (D_2O) δ 18.5 (d, *J* = 75.9 Hz); ^{19}F NMR (D_2O) δ –210.8 (d, *J* = 76.0 Hz); FABMS (thioglycerol) m/z 258 (MH^+, 100). Anal. Calcd for $C_8H_{17}FNO_5P$: C, 37.36; H, 6.66; N, 5.45. Found: C, 37.68; H, 6.37; N, 5.83.

(2*S*,3*R*,4*R*)-2-Amino-4-diethoxyphosphoryl-4-fluoro-3-methylbutanoic acid (**10b**): General procedure was followed, to give 0.19 g (95%) of **10b** as a hygroscopic colorless oil. $[\alpha]^{20}_D$ +22.8 (c 1.9, H_2O); IR (film) ν 3444 (OH), 2965 (CH), 1638 (C=O), 1237 (P=O), 1020 (POC) cm^{-1}; ^1H NMR (D_2O) δ 0.91 (d, *J* = 6.7 Hz, 3 H, $CHC\underline{H}_3$), 1.20 (t, *J* = 7.3 Hz, 6 H, $OCH_2C\underline{H}_3$), 2.37-2.64 (m, 1 H, H-3), 3.83 (d, *J* = 2.4 Hz, 1 H, H-2), 4.12 (qd, *J* = 7.3, 3.1 Hz, 4 H, $OC\underline{H}_2CH_3$), 4.97 (ddd, *J* = 44.6, 11.0, 2.4 Hz, 1 H, H-4); ^{13}C NMR (D_2O) δ 10.9 ($CH\underline{C}H_3$), 16.6 ($OCH_2\underline{C}H_3$), 36.9 (dd, *J* = 17.2, 4.0 Hz, C-3), 55.7 (dd, *J* = 14.6, 6.6

Hz, C-2), 65.9 (d, *J* = 7.4 Hz, O<u>C</u>H$_2$CH$_3$), 66.4 (d, *J* = 7.4 Hz, O<u>C</u>H$_2$CH$_3$), 89.8 (dd, *J* = 179.1, 169.2 Hz, C-4), 173.1 (C=O); ^{31}P NMR (D$_2$O) δ 19.2 (d, *J* = 79.0 Hz); ^{19}F NMR (D$_2$O) δ –203.9 (d, *J* = 79.5 Hz); FABMS (thioglycerol) m/z 272 (MH$^+$, 100). Anal. Calcd for $C_9H_{19}FNO_5P$: C, 39.86; H, 7.06; N, 5.16. Found: C, 40.19; H, 6.85; N, 5.00.

General procedure for the protection of the 4-fluorinated 2-amino-4-diethoxyphosphorylbutanoic acids. A solution of amino acid **8a** or **10a,b** (0.21 mmol), $NaHCO_3$ (20 mg, 0.24 mmol) in acetone (2 mL) and water (2mL) at rt was treated with Fmoc-*O*-*N*-succinimide (0.21 mmol). The mixture was stirred at rt for 14 h, after which it was reduced to half its initial volume and extracted with AcOEt (15 mL). The aqueous layer was made acid (pH = 1) by the addition of 1N HCl and was extracted with AcOEt (2 x 15 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. Flash chromatography (RP-18, MeOH/H$_2$O 1:1) afforded the amino acids **1** or **2a,b** as colorless solids.

(2*S*,4*S*)-4-(Diethoxyphosphoryl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-4-fluoro-butanoic acid (**1a**): General procedure was followed, to give 80 mg (80%) of **1a** as a hygroscopic colorless solid. $[\alpha]^{26}_D$ –2.3 (c 1.0, MeOH); IR (film) ν 3405 (OH), 2983 (CH), 1720 (C=O), 1246 (P=O), 1023 (POC) cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.25 (t, *J* = 7.1 Hz, 6 H, OCH$_2$C<u>H</u>$_3$), 1.87-2.52 (m, 2 H, H-3), 4.03-4.37 (m, 8 H, H-2, C<u>H</u>C<u>H</u>$_2$O, O<u>C</u>H$_2$CH$_3$), 4.65-5.03 (m, 1 H, H-4), 7.13-7.34 (m, 4 H, Ar), 7.48-7.57 (m, 2 H, Ar), 7.62-7.73 (m, 2 H, Ar); ^{13}C NMR (CD$_3$OD) δ 15.2 (OCH$_2$<u>C</u>H$_3$), 15.3 (OCH$_2$<u>C</u>H$_3$), 32.3 (d, *J* = 19.8 Hz, C-3), 46.8 (<u>C</u>HCH$_2$O), 51.6 (C-2), 63.2 (d, *J* = 6.9 Hz, PO<u>C</u>H$_2$CH$_3$), 63.5 (d, *J* = 6.9 Hz, PO<u>C</u>H$_2$CH$_3$), 66.3 (CH<u>C</u>H$_2$O), 85.4 (dd, *J* = 179.3, 172.4 Hz, C-4), 119.4, 124.7, 126.6, 127.2, 141.0, 143.5, 143.8 (Ar), 157.1, 177.5 (C=O); ^{31}P NMR (CD$_3$OD) δ 17.9 (d, *J* = 75.3 Hz); ^{19}F NMR (CD$_3$OD) δ –211.5 (d, *J* = 75.2 Hz); FABMS (thioglycerol) m/z 480 (MH$^+$, 12). Anal. Calcd for $C_{23}H_{27}FNO_7P$: C, 57.62; H, 5.68; N, 2.92. Found: C, 57.98; H, 5.83; N, 2.54.

(2*S*,4*R*)-4-(Diethoxyphosphoryl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-4-fluoro-butanoic acid (**2a**): General procedure was followed, to give 86 mg (86%) of **2a** as a hygroscopic colorless solid. $[\alpha]^{26}_D$ +77.0 (c 1.2, MeOH); IR (film) ν 3400 (NH), 2980 (CH), 1723 (C=O), 1240 (P=O), 1020 (POC) cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.24 (t, *J* = 6.8 Hz, 6 H, OCH$_2$C<u>H</u>$_3$), 2.20-2.60 (m, 2 H, H-3), 4.08-4.34 (m, 8 H, H-2, C<u>H</u>C<u>H</u>$_2$O, O<u>C</u>H$_2$CH$_3$), 4.92-5.29 (m, 1 H, H-4), 7.13-7.34 (m, 4 H, Ar), 7.51-7.60 (m, 2 H, Ar), 7.62-7.73 (m, 2 H, Ar); ^{13}C NMR (CD$_3$OD) δ 15.2 (OCH$_2$<u>C</u>H$_3$), 15.3 (OCH$_2$<u>C</u>H$_3$), 32.8 (d, *J* = 20.6 Hz, C-3), 46.7 (<u>C</u>HCH$_2$O), 52.3 (C-2), 63.1 (d, *J* = 6.9 Hz, PO<u>C</u>H$_2$CH$_3$), 63.4 (d, *J* = 6.9 Hz, PO<u>C</u>H$_2$CH$_3$), 66.4 (CH<u>C</u>H$_2$O), 86.0 (dd, *J* = 178.9, 171.3 Hz, C-4), 119.4, 124.6,

126.6, 127.2, 140.9, 143.6, 143.7 (Ar), 156.4, 176.8 (C=O); ^{31}P NMR (CD_3OD) δ 17.7 (d, *J* = 76.3 Hz); ^{19}F NMR (CD_3OD) δ −211.3 (d, *J* = 76.3 Hz); FABMS (thioglycerol) m/z 480 (MH^+, 20). Anal. Calcd for $C_{23}H_{27}FNO_7P$: C, 57.62; H, 5.68; N, 2.92. Found: C, 57.29; H, 5.79; N, 3.11.

(2*S*,3*R*,4*R*)-4-(Diethoxyphosphoryl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-methyl-4-fluoro-butanoic acid (**2b**): General procedure was followed, to give 88 mg (85%) of **2b** as a hygroscopic colorless solid. $[\alpha]^{28}_D$ +15.6 (c 1.8, MeOH); IR (film) ν 3332 (OH), 2983, 2945 (CH), 1743 (C=O), 1260 (P=O), 1027 (POC) cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.07 (d, *J* = 6.8 Hz, 3 H, CHC$\underline{H}_3$), 1.34 (t, *J* = 6.8 Hz, 6 H, $OCH_2C\underline{H}_3$), 2.64-2.80 (m, 1 H, H-3), 4.15-4.42 (m, 7 H, H-2, $OC\underline{H}_2CH$, $OC\underline{H}_2CH_3$), 4.47-4.53 (m, 1 H, $OCH_2C\underline{H}$), 4.87 (ddd, *J* = 45.0, 10.7, 3.2 Hz, 1 H, H-4), 7.21-7.42 (m, 4 H, Ar), 7.56-7.69 (m, 2 H, Ar); 7.73-7.82 (m, 2 H, Ar); ^{13}C NMR (CD_3OD) δ 12.8 (d, *J* = 8.4 Hz, CH$\underline{C}H_3$), 16.7 (dd, *J* = 5.4, 2.0 Hz, $OCH_2\underline{C}H_3$), 39.0 (d, *J* = 17.5 Hz, C-3), 48.4 ($\underline{C}HCH_2O$), 56.7 (C-2), 64.4 (d, *J* = 6.9 Hz, $PO\underline{C}H_2CH_3$), 65.1 (d, *J* = 6.9 Hz, $PO\underline{C}H_2CH_3$), 68.1 ($CH\underline{C}H_2O$), 90.5 (dd, *J* = 179.3, 168.6 Hz, C-4), 120.9, 126.2, 128.2, 128.8, 142.6, 145.1, 145.3 (Ar), 158.2, 173.9 (C=O); ^{31}P NMR (CD_3OD) δ 18.9 (d, *J* = 88.3 Hz); ^{19}F NMR (CD_3OD) δ −203.8 (d, *J* = 88.0 Hz); FABMS (thioglycerol) m/z 494 (MH^+, 15). Anal. Calcd for $C_{24}H_{29}FNO_7P$: C, 58.42; H, 5.92; N, 2.84. Found: C, 58.80; H, 6.08; N, 2.57.