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Supporting Information

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for

Design, Synthesis and Analysis of Inhibitors of Bacterial Aspartate Semialdehyde Dehydrogenase

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Experimental Section

Nuclear Magnetic Resonance (NMR) spectra were recorded on Jeol *d*270, *d*300, *L*300, or *d*400 spectrophotometers at the indicated frequency. Coupling constants (*J*) are quoted in Hz and *d* values in ppm. NMR samples were dissolved in $CDCl_3$ using tetramethylsilane as the internal standard or in D_2O using sodium 3-(trimethylsilyl)propionate as the internal standard. ^{19}F and ^{31}P spectra are referenced to CF_3CO_2H and phosphoric acid, respectively. ^{13}C and ^{31}P spectra were obtained under broad-band proton decoupled conditions {^1H} unless otherwise stated.

Electron ionisation (EI) at a potential of 70 eV and chemical ionisation (CI) mass spectra were obtained on a VG analytical Autospec mass spectrometer. Electrospray (ESI) mass spectra were obtained on a Waters/Micromass system comprising of a Waters 600 LC system, equipped with both Waters 996 photodiode array and platform MS detectors running in ES^+ mode. Chromatographic separations were achieved using Phenomenex C_8 reverse phase column (4.6×250 mm) run at 1 mL/min. Solvent A, 0.1 % TFA in water; Solvent B, 0.05 % TFA in acetonitrile. Samples (20 µL) of ca. 1 mg/mL

were injected. The gradient was as follows: 0 min, 5 % B; 13 min, 99 % B; 17 min, 99 % B; 18 min, 5 % B; 20 min, 0 % B. The void volume of the system was 540 µL. Data analysis was performed with MassLynx v.3.3 software.

Elemental analyses were carried out in the microanalytal laboratories of the University of Bristol. IR spectra were obtained with a Perkin Elmer FT-IR Paragon 1000 machine, with oil or solid samples mounted directly over the diamond cell. Signal intensities are quoted as, either weak (w), medium (m) or strong (s) signals.

Melting points were obtained on an Electrothermal Melting Point Apparatus and they uncorrected. Flash chromatography was performed using silica gel 60 (230-400 mesh, Merck). TLC was carried out on glass plates coated with 0.2 mm silica gel 60 purchased from Merck, and were visualized under ultraviolet light (254 nm) or sprayed with potassium permanganate and developed by heating with a hot air gun. Commercially available reagents were used as received without further purification unless otherwise stated.

(*S*)-2-Amino-5-fluoro-4-oxo-5-phosphono-pentanoic acid 7: (*S*)-2-[*bis*(*tert*-butoxycarbonyl)amino]-5-(dimethoxy-phosphoryl)-5-fluoro-4-oxo-pentanoic acid methyl ester **17** (450 mg, 0.95 mmol) was dissolved in dry acetoniltrile (5 cm^3). Chlorotrimethylsilane (4.7 mmol, 0.61 cm^3) and NaI (4.7 mmol, 716 mg) were added. The resulting mixture was stirred at RT for 5 h. The reaction mixture was filtered and the filtrate concentrated *in vacuo*. The resulting residue was dissolved in water (10 cm^3) and extracted with ethyl acetate (4 × 10 cm^3). KOH (2.0 mmol, 112 mg) was added to the aqueous layer, which was stirred at RT overnight. Lyophilisation afforded the potassium salt of *(S)-2-Amino-5-fluoro-4-oxo-5-phosphono-pentanoic acid* **7** as a hygroscopic solid (202 mg, 70 %) and a 1:1 mixture of diastereoisomers (**A**+**B**); n_{max} (solid)/cm^{-1} 1136 (P=O, m), 1734 (C=O, s), 2980 (CH, w), 3132 (NH, COOH, broad m); d_H(400 MHz, D_2O) 3.37-3.55 (2 H, m, *b*CH_2, **A**+**B**), 4.81 (1 H, m, *a*CH, **A**+**B**), 5.49 (1 H, dd, $^2J_{HF}$ 70, $^2J_{HP}$ 20, **A**+**B**); d_P(121.5 MHz, D_2O {^1H}) 5.71 (d, $^2J_{FP}$ 56, **A**), 5.73 (d, $^2J_{FP}$ 60, **B**); d_F(282.6 MHz, D_2O) -207.1 (1 F, dd, $^2J_{HF}$ 72, $^2J_{FP}$ 56, **A**), -206.9 (1 F, dd, $^2J_{HF}$ 70, $^2J_{FP}$ 60, **B**); d_C(75.5 MHz, D_2O) 41.8/42.5 (*b*CH_2, **A**+**B**), 54.4 (*a*CH, **A**+**B**), 93.3 (dd, $^1J_{CF}$ 196, $^1J_{CP}$ 152, CHF, **A**+**B**), 170.9/171.1 (*a*C=O **A**+**B**), 200.9 (dd, $^2J_{CF}$ 169.8, $^2J_{CP}$ 22.2, *d*C=O, **A**+**B**); *m/z* (ESI) 230 (100 %, $[M+H]^+$).

(*E*)-(*S*)-2-Amino-5-phosphono-pent-4-enoic acid 8.: (*E*)-(*S*)-2-[*bis*(*tert*-butoxycarbonyl)amino]-5-(diethoxyphosphoryl)pent-4-enoic acid methyl ester **25** (100 mg, 0.22 mmol) was refluxed in aqueous HCl (5 M, 4 cm^3) overnight. The reaction mixture was concentrated in vacuo, the residue taken up in water (~3 cm^3) and the resulting mixture extracted with ethyl acetate (3×5 cm^3). Lyophilisation of the aqueous layer gave (*E*)-(*S*)-2-Amino-5-phosphono-pent-4-enoic acid **8** (39 mg, 0.20 mmol, 99 %) as a solid; m.p 80-84 °C; R_f(*i*PrOH:NH_3 1:1) 0.13; $[a]_D^{19}$ +0.72 (c 6.10 in D_2O); n_{max} (solid)/cm^{-1} 2849 (OH, NH_2, CH, broad, s), 1727 (C=O, s), 1500 (m), 1127 (s); d_H(400 MHz, D_2O) 2.52-2.71 (2 H, m, *b*CH_2), 4.00 (2 H, t, $^3J_{HH}$ 6.2, *a*CH), 5.82 (1 H, dd, $^2J_{HP}$ 17, $^3J_{HH}$ 17, C=C<u>H</u>P), 6.25 (1 H, ddt, $^3J_{HH}$ 21.6, $^3J_{HP}$ 17, $^3J_{HH}$ 17, $^3J_{HH}$ 7.3, <u>H</u>C=CP); d_C(100.5 MHz, D_2O) 34.3 (d, $^3J_{CP}$ 23.6, *b*CH_2), 52.3 (*a*CH), 126.5 (d, $^1J_{CP}$ 179 6, C=<u>C</u>P), 142.7 (d, $^2J_{CP}$ 4.9, <u>C</u>=CP), 183.7 (C=O); d_P(121 MHz, D_2O, {^{1}H}) 16.1; *m/z* (CI) 71 (100 %), 196 (44, $[M+H]^+$); HRMS (CI, $[M+H]^+$) Found: 196.0450 Calc. for $C_5H_{11}O_5N_1P_1$ 196.0453.

(*Z*)-(*S*)-2-Amino-5-phosphono-pent-4-enoic acid 9: (*Z*)-(*S*)-5-(Dimethoxy-phosphoryl)-2-(3,3-dimethyl-1-methylene-butylamino)-pent-4-enoic acid methyl ester **22** (220 mg, 0.65 mmol) was dissolved in HCl (aq. 5 M, 10 cm^3) and heated at reflux overnight. The reaction mixture was concentrated *in vacuo*. The resulting oil was taken up in water (3 cm^3) and extracted with EtOAc (10 cm^3 × 5). Lyophillisation of the aqueous layer gave *(Z)-(S)-2-Amino-5-phosphono-pent-4-enoic acid* **9** (150 mg, quant.); $[a]_D^{20}$ -1.3 (c 2.3 in H_2O); n_{max} (solid)/cm^{-1} 2849 (NH, OH, CH, broad, s), 2224 (CH, m), 1732 (C=O, s), 1627 (C=C, m), 1073 (O-C, s); (300 MHz, $CDCl_3$) δ_H=2.80-3.19 (2 H, m, *b*CH_2), 4.20 (1 H, dd, $^3J_{HH}$ 6.9, $^3J_{HH}$ 5.8, *a*CH), 5.99 (1 H, dd, $^3J_{HP}$ 18.2, $^3J_{HH}$ 13.1, =C<u>H</u>P), 6.32 (1 H, dddd, $^3J_{HP}$ 48.1, $^3J_{HH}$ 13.1, $^3J_{HH}$ 8.8, $^3J_{HH}$ 7.7, C<u>H</u>=CHP); (100.5 MHz, $CDCl_3$) d_C=30.6 (d, $^3J_{CP}$ 9.2, *b*CH_2), 51.9 (d, $^4J_{CP}$ 2.3, *a*CH), 125.5 (d, $^1J_{CP}$ 174.5, =<u>C</u>HP), 140.9 (d, $^2J_{CP}$ 1.5, <u>C</u>H=CHP), 171.1 (C=O); (121 MHz, $CDCl_3$) d_P=13.2; *m/z* (ESI) 196 (100 %, $[M+H]^+$); HRMS (ESI, $[M+H]^+$) Found: 196.0367 Calc. for $C_5H_{11}O_5N_1P_1$ 196.0369.

(*S*)-2-Amino-5-phosphono-pent-4-ynoic acid 10: (*S*)-2-[*bis*(*tert*-butoxycarbonyl)amino]-5-(dimethoxy-phosphoryl)-pent-4-ynoic acid methyl ester **20** (370 mg, 0.85 mmol) was dissolved in HCl (aq. 5 M, 8 cm^3) and heated at reflux overnight. The reaction mixture was concentrated *in vacuo* and the resulting residue dissolved in H_2O (4 cm^3) and washed with EtOAc (10 cm^3 × 5). Lyophilisation of the aqueous layer gave (*S*)-2-Amino-

5-phosphono-pent-4-ynoic acid **10** (0.85 mmol, 100 %) as a hygroscopic solid; $[a]_D^{20}$ -36.2 (c 2.2 in H_2O); n_{max} (solid)/cm^{-1} 2836 (CH, OH, NH, s), 2217 (C=C, s), 1731 (C=O, s), 1591 (s), 1501 (s); d_H(300 MHz, D_2O) 2.90 (2 H, m, *b*CH_2), 4.17 (1 H, dt, $^3J_{HH}$ 5.5, $^5J_{HP}$ 1.1, *a*CH); d_P(121 MHz, D_2O, {^{1}H}) -10.5; d_C(100.5 MHz, D_2O) 20.6 (d, $^3J_{CP}$ 3.8, *b*CH_2), 51.2 (d, $^4J_{CP}$ 2.3, *a*CH), 79.5 (d, $^1J_{CP}$ 269, =<u>C</u>P), 90.3 (d, $^2J_{CP}$ 48.4, <u>C</u>=CP), 170.9 (C=O); *m/z* (ESI) 387 (45 %, $[M_2]H^+$), 194 (100, $[M+H]^+$).

(*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-5-(dimethoxy-phosphoryl)-4-oxo-pentanoic acid methyl ester 15: Dimethyl methylphosphonate (2.10 cm^3, 19.4 mmol) was dissolved in dry THF (45 cm^3) and cooled to -78 °C. *n*-Butyllithium (2.5 M in hexanes, 8.53 cm^3, 21.3 mmol) was added dropwise *via* syringe. After 1 h the reaction mixture was added to a solution of (*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-succinic acid dimethyl ester **14**[1] (3.50 g, 9.7 mmol) in dry THF (20 cm^3) at -78 °C. The reaction was slowly allowed to reach room temperature over 2.5 h the reaction, then quenched with glacial acetic acid (3.0 cm^3) and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (100 $cm^3 \times 3$). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography eluting with 70:30 EtOAc/hexane, to give the phosphonate **15** (1.30 g, 2.86 mmol, 29 %) as an oil; R_f(60:40, EtOAc/hexane) 0.13; d_H(400 MHz, $CDCl_3$) 1.51 (18 H, s, CCH_3), 2.93 (1 H, dd, $^2J_{HH}$ 17.8, $^3J_{HH}$ 5.1, *b*CH), 3.18 (1 H, dd, $^2J_{HP}$ 45, $^2J_{HH}$ 22.6, *d*CH), 3.26 (1 H, dd, $^2J_{HP}$ 45, $^2J_{HH}$ 22.6, *d*CH), 3.60 (1 H, dd, $^2J_{HH}$ 17.8, $^3J_{HH}$ 7.6, *b*CH), 3.71 (3 H, s, *a*OCH_3), 3.78 (3 H, d, $^3J_{HP}$ 11.3, $POCH_3$), 3.80 (3 H, d, $^3J_{HP}$ 11.4, $POCH_3$), 5.51 (1 H, dd, $^3J_{HH}$ 7.6, $^3J_{HH}$ 5.1, *a*CH); d_P(61 MHz, $CDCl_3$, {^{1}H}) 22.8 (s); d_C(75.5 MHz, $CDCl_3$) 27.9 ($C(\underline{C}H_3)_3$), 41.8 (d, $^2J_{CP}$ 128.6, *d*CH_2), 44.9 (*b*CH_2), 52.5 (OCH_3), 52.9 (d, $^2J_{CP}$ 6.5, $POCH_3$), 54.1 (*a*CH), 53.1 (d, $^2J_{CP}$ 6.5, $POCH_3$), 83.6 ($\underline{C}(CH_3)_3$), 151.6 (NCO), 170.5 (*a*C=O), 198.3 (d, $^2J_{CP}$ 6, *d*C=O); *m/z* (ESI) 454 (56 %, $[M+H]^+$), 398 (100, $[M\text{-}C(CH_3)_3+H]H^+$), 354 (32, [M-Boc+H]$H^+$), 298 (22, [M-Boc-$C(CH_3)_3$+2H]$H^+$), 254 (41, [M-2Boc+2H]$H^+$).

(*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-5-(dimethoxy-phosphoryl)-5,5-difluoro-4-oxo-pentanoic acid methyl ester 16 and (*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-5-(dimethoxy-phosphoryl)-5-fluoro-4-oxo-pentanoic acid methyl ester 17: A solution

of (*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-5-(dimethoxy-phosphoryl)-4-oxo-pentanoic acid methyl ester **15** (3.20 g, 7.1 mmol) in dry THF (20 cm^3) was added to a suspension of NaH (282 mg, 7.1 mmol) in dry THF (10 cm^3) at 0 °C. After 20 minutes the reaction mixture was added to a solution of Selectfluor (5.02 g, 14.2 mmol, in CH_3CN (20 cm^3) at -10 °C. The reaction mixture was allowed to reach RT overnight. The reaction mixture was then partitioned between EtOAc and water and the aqueous layer extracted with ethyl acetate (150 $cm^3 \times 3$). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was purified by flash chromatography, eluting with 60:40 EtOAc:hexane, to afford (*S*)-2-[bis(*tert*-butoxy-carbonyl)amino]-5-(dimethoxy-phosphoryl)-5-fluoro-4-oxo-pentanoic acid methyl ester **17** as a 1:1 mixture of diastereoisomers (1.60 g, 3.5 mmol, 50 %) as an oil and (*S*)-2-[bis(*tert*-butoxycarbonyl)amino]-5-(dimethoxyphosphoryl)-5,5-difluoro-4-oxopentanoic acid methyl ester **16** (520 mg, 1.1 mmol, 16 %) as an oil.

(*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-5-(dimethoxy-phosphoryl)-5,5-difluoro-4-oxo-pentanoic acid methyl ester 16: (Found C 44.50; H 6.46; N 2.46; $C_{18}H_{30}F_2NO_{10}P$ requires C 44.17; H 6.18; N 2.86); R_f(60:40, EtOAc:hexane) 0.74; $[\alpha]_D^{19}$ –0.27 (c 6.15 in EtOAc); n_{max} (oil)/cm^{-1} 2980 (CH, m), 1744 (C=O, s), 1699 (NC=O, s), 1368 ($C(CH_3)_3$, s), 1244 (CF, s), 1141 (P=O, s); δ_H(399.7 MHz, $CDCl_3$) 1.51 (18 H, s, $C(CH_3)_3$), 3.12 (1 H, dd, $^2J_{HH}$ 19, $^3J_{HH}$ 5.5, *b*CH), 3.73 (3 H, s, OCH_3), 3.84 (1 H, dd, $^2J_{HH}$ 19, $^3J_{HH}$ 7.2, *b*H), 3.94 (3 H, d, $^3J_{HP}$ 9.2, $POCH_3$), 3.95 (3 H, d, $^3J_{HP}$ 9.2, $POCH_3$), 5.55 (1 H, dd, $^3J_{HH}$ 7.2, $^3J_{HH}$ 5.5, *a*CH); δ_P (121.5 MHz, $CDCl_3$, $\{^1H\}$) 5.91 (t, $^2J_{PF}$ 96); δ_F(283 MHz, $CDCl_3$) -116.82 (dd, $^2J_{FF}$ 319.8, $^2J_{FP}$ 96), -118.17 (dd, $^2J_{FF}$ 319.8, $^2J_{FP}$ 96); δ_C(100.5 MHz, $CDCl_3$) 27.9 ($C(\underline{C}H_3)_3$), 39.2 (*b*CH_2), 52.7 (*a*OCH_3), 53.3 (*a*CH), 55.4 (d, $^2J_{CP}$ 7.6, $POCH_3$), 83.8 ($\underline{C}(CH_3)_3$), 110.1-117.0 (m, CF_2), 151.5 (NCO), 169.8 (OC=O), 195.5 (m, *g*C=O); *m/z* (CI) 490 (88 %, $[M+H]^+$), 434 (100, $[M-C(CH_3)_3+H]H^+$), 390 (22, $[M-Boc+H]H^+$), 334 (30, $[M-Boc-C(CH_3)_3+2H]H^+$), 290 (86, $[M-2Boc+2H]H^+$).

(*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-5-(dimethoxy-phosphoryl)-5-fluoro-4-oxo-pentanoic acid methyl ester 17: R_f(60:40, EtOAc:hexane) 0.45; (Found C 45.93; H 6.72; N 2.87; $C_{18}H_{31}FNO_{10}P$ requires C 44.86; H 6.63; N 2.97); n_{max} (oil)/cm^{-1} 2980 (CH, m), 1734 (C=O, s), 1368 ($C(CH_3)_3$, s), 1239 (CF, s), 1136 (P=O, m), 1012 (P-OR, m); d_H(400 MHz, $CDCl_3$) 1.51 (36 H, s, CCH_3, **A**+**B**), 2.86-2.98 (1 H, ddd, $^2J_{HH}$ 18.1, $^3J_{HH}$ 5.4,

$^4J_{HF}$ 2.0, *b*CH, **A**), 2.98-3.0 (1 H, ddd, $^2J_{HH}$ 17.6, $^3J_{HH}$ 5.4, $^4J_{HF}$ 2.0, *b*CH, **B**), 3.65 (1 H, dd, $^2J_{HH}$ 17.6, $^3J_{HH}$ 7.8, *b*CH, **B**), 3.72 (6 H, s, *a*OCH_3, **A**+**B**), 3.72-3.86 (1 H, ddd, $^2J_{HH}$ 18.1, $^3J_{HH}$ 7.8, $^4J_{HF}$ 4.0, *b*CH, **A**), 3.85 (3 H, d, $^3J_{HP}$ 11.2, $POCH_3$, **A**), 3.87 (3 H, d, $^3J_{HP}$ 10.6, $POCH_3$, **B**), 3.89 (3 H, d, $^3J_{HP}$ 10.5, $POCH_3$, **A**), 3.91 (3 H, d, $^3J_{HP}$ 10.5, $POCH_3$, **B**), 5.26 (1 H, dd, $^2J_{HF}$ 47.5, $^2J_{HP}$ 14.2, *d*CHF, **A**), 5.44 (1 H, dd, $^2J_{HF}$ 47, $^2J_{HP}$ 14.3, *d*CHF, **B**), 5.56 (2 H, m, *a*CH, **A**+**B**); d_P(121.4 MHz, $CDCl_3$, {^{1}H}) 12.6 (d, $^2J_{PF}$ 71, **A**), 13.2 (d, $^2J_{PF}$ 69, **B**), 7.2 (d, $^2J_{PF}$ 96, enol **174**); d_F(282.6 MHz, $CDCl_3$) -209.7 (ddt, $^2J_{FP}$ 69, $^2J_{FH}$ 47, $^4J_{HF}$ 2.0, **B**), -210.1 (dddd, $^2J_{FP}$ 71, $^2J_{FH}$ 47.5, $^4J_{HF}$ 4, $^4J_{HF}$ 2, **A**), -117 (d, $^2J_{FP}$ 96, enol **174**); d_C(100.5 MHz, $CDCl_3$) 28.0 (CCH_3, **A**+**B**), 40.0 (*b*CH_2, **A**), 40.5 (*b*CH_2, **B**), 52.5 (*a*OCH_3, **A**+**B**), 53.5 (d, $^4J_{CF}$ 1.0, *a*CH, **A**), 54.1 (d, $^4J_{CF}$ 1.5, *a*CH, **B**), 54.4 (d, $^2J_{CP}$ 7, $POCH_3$, **A**+**B**), 54.5 (d, $^2J_{CP}$ 7.7, $POCH_3$, **A**+**B**), 83.6 ($\underline{C}CH_3$, **A**+**B**), 89.5-93.0 (m, CHF, **A**+**B**), 151.6 (NCO, **A**+**B**), 170.2/170.3 (*a*C=O, **A**+**B**), 198.2-200.1 (dd, $^2J_{CF}$ 172.2, $^2J_{CP}$ 19.2, *d*C=O, **A**+**B**); *m/z* (ESI) 494 (15 %, $[M]Na^+$), 472 (28, $[M+H]^+$), 416 (55, $[M-C(CH_3)_3+H]H^+$), 372 (18, $[M-Boc+H]H^+$), 316 (22, $[M-Boc-C(CH_3)_3+2H]H^+$), 272 (100, $[M-2Boc+2H]H^+$), 254 (10, $[M-2Boc-F+3H]H^+$).

(*S*)-2-[*bis*(*tert*-butoxycarbonyl)-amino]-pent-4-ynoic acid methyl ester 19: K_2CO_3 (923 mg, 6.7 mmol) was stirred in dry methanol (70 cm^3) for 1 h then cooled to 0 °C. Diazophoshonate[2] (773 mg, 4.0 mmol) was added and the reaction stirred at 0 °C for 30 min. The aldehyde **18**[1] (1.11 g, 3.4 mmol) was then added and the reaction mixture slowly allowed to reach RT. Stirring was continued overnight, then the reaction mixture was partitioned between EtOAc and brine. The aqueous layer was extracted with EtOAc (50 $cm^3 \times 3$). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 80:20 hexane/EtOAc, to yield (*S*)-2-[bis(*tert*-butoxycarbonyl)-amino]-pent-4-ynoic acid methyl ester **19** (150 mg, 0.4 mmol, 12 %) as a solid, m.p. 70-71 °C (Found: C 58.8; H 7.9; N 4.5; $C_{16}H_{25}NO_6$ requires C 58.7; H 7.7; N 4.3 %); R_f(Hexane: EtOAc, 80:20) 0.41; $[a]_D^{23}$ 1.28 (c 1.56 in EtOAc); n_{max} (solid)/cm^{-1} 3258 (=CH, m), 2985 (CH, m), 2940 (CH, w), 1743 (C=O, s), 1712 (NC=O, s), 1393 ($C(CH_3)_3$, w), 1368 ($C(CH_3)_3$, s), 1270 (s), 1257 (s), 1145 (C-O, s), 1111 (C-O, s), 1014 (s); d_H(400 MHz, $CDCl_3$) 1.51 (18 H, s, $C(CH_3)_3$), 1.97 (1 H, t, $^4J_{HH}$ 2.6, =CH), 2.94-2.96 (2 H, m, *b*CH_2), 3.71 (3 H, s OCH_3), 5.18 (1 H, dd, $^3J_{HH}$ 8.8, $^3J_{HH}$ 6.8, *a*CH); d_H(100.5 MHz, $CDCl_3$) 20.5 (*b*CH_2), 28.1 ($C(\underline{C}H_3)_3$), 52.4

(OCH_3), 56.6 (αCH), 70.4 (=CH), 79.9 (-C=CH), 83.3 ($C(CH_3)_3$), 151.8 (NCO), 169.9 (C(O)O); *m/z* (CI) 272 (10 %, $[M\text{-}C(CH_3)_3+H]H^+$), 172 (20, $[M\text{-Boc-}C(CH_3)_3+2H]H^+$), 128 (65, $[M\text{-}2Boc+2H]H^+$), 57 (100, $[C(CH_3)_3]^+$).

(*S*)-2-[*bis*(*tert*-butoxycarbonyl)amino]-5-(dimethoxy-phosphoryl)-pent-4-ynoic acid methyl ester 20: Phosphonate **15** (2.0 g, 4.4 mmol) was dissolved in dry CH_2Cl_2 (15 cm^3) and cooled to 0 °C. Diisopropylethyl amine (3.1 cm^3, 17.7 mmol) was added followed by triflic anhydride (0.96 cm^3, 5.7 mmol). The reaction mixture was stirred at 0 °C for 3 h, then allowed to reach RT. Stirring was continued overnight then diluted with Et_2O and washed with brine (50 cm^3 × 2). The organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was purified by flash chromatography eluting with EtOAc/hexane (1:1) to yield (*S*)-2-[bis(*tert*-butoxycarbonyl)amino]-5-(dimethoxy-phosphoryl)pent-4-ynoic acid methyl ester **20** (1.12 g, 2.5 mmol, 57 %) as an oil; R_f(EtOAc/hexane, 1:1) 0.21; $[\alpha]_D^{20}$ -3.4 (c 5.5 in CH_2Cl_2); (Found C 49.71; H 7.03; N 3.05; $C_{18}H_{30}NO_9P$ requires C 49.65; H 6.94; N 3.22); ν_{max} (oil)/cm^{-1} 2981 (CH, w), 2955 (CH, w), 2211 (C=C, m), 1737 (C=O, s), 1696 (NC=O, s), 1367 (*t*Bu, s), 1268 (P=O, s), 140 (C-O, s), 1020 (C-O, s); δ_H(400 MHz, $CDCl_3$) 1.51 (9 H, s, $C(CH_3)_3$), 3.13-3.16 (2 H, m, βCH_2), 3.74 (3 H, s, OCH_3), 3.77 (3 H, d, $^3J_{HP}$ 12.2, $POCH_3$), 3.78 (3 H, d, $^3J_{HP}$ 12.8, $POCH_3$), 5.22 (1 H, dd, $^2J_{HH}$ 8.6, $^2J_{HH}$ 6.3, αCH); δ_C(100.6 MHz, $CDCl_3$) 21.3 (d, $^3J_{CP}$ 4.62, βCH_2), 27.9 ($C(\underline{C}H_3)_3$), 52.7 (OCH_3), 53.2 (d, $^2J_{CP}$ 5.6, $POCH_3$), 53.3 (d, $^2J_{CP}$ 5.6, $POCH_3$), 55.6 (d, $^4J_{CP}$ 2.4, αCH), 71.5 (d, $^1J_{CP}$ 301.5, =C-P), 83.9 ($\underline{C}(CH_3)_3$), 98.9 (d, $^2J_{CP}$ 53.0, C=C-P), 151.7 (NC=O), 169.3 (C=O); δ_P(121.4 MHz, $CDCl_3$, $\{^1H\}$) -3.18; *m/z* (CI) 671 (70 %, $[M_2\text{-}2Boc+2H]H^+$), 336 (35, $[M\text{-Boc}+H]H^+$), 280 (100, $[M\text{-Boc-}C(CH_3)_3+2H]H^+$), 236 (83, $[M\text{-}2Boc+2H]H^+$), 57 (66, $[C(CH_3)_3]^+$).

(*S*)-2-*tert*-butoxycarbonylamino-5-(dimethoxy-phosphoryl)pent-4-ynoic acid methyl ester 21: (*S*)-2-[*bis*(*tert*-butoxycarbonyl)amino]-5-(dimethoxy-phosphoryl)-pent-4-ynoic acid methyl ester **20** (550 mg, 1.21 mmol) was dissolved in dry CH_2CL_2 (15 cm^3) and CF_3CO_2H (140 µL, 1.5 eq.) was added. The reaction mixture was stirred at room temperature overnight, then concentrated *in vacuo*. The resulting residue was purified by flash chromatography eluting with EtOAc/hexane (60:40) to give (*S*)-2-*tert*-butoxycarbonylamino-5-(dimethoxyphosphoryl)pent-4-ynoic acid methyl ester **21** (290 mg, 0.82

mmol, 68 %) as an oil; R_f(EtOAc/hexane; 1:1) 0.21; n_{max} (oil)/cm^{-1} 3297 (NH, w), 2977 (CH, w), 2211 (C=C, m), 1710 (C=O, s), 1519 (m), 1252 (P=O, s) 1160 (s), 1025 (C-OR, s); d_H(400 MHz, $CDCl_3$)1.46 (9 H, s, $C(CH_3)_3$), 2.92 (2 H, m, *b*CH_2), 3.76 (3 H, d, $^3J_{HP}$ 12, $POCH_3$), 3.78 (3 H, d, $^3J_{HP}$ 12, $POCH_3$), 3.08 (3 H, s, OCH_3), 4.54 (1 H, m, *a*CH), 5.36 (1 H, d, $^3J_{HH}$ 7.8, NH); d_C(75 MHz, $CDCl_3$) 23.8 (d, $^3J_{CP}$ 5, *b*CH_2), 28.3 ($C(\underline{C}H_3)_3$), 51.5 (*a*CH), 52.9 (OCH_3), 53.4 (d, $^2J_{CP}$ 6, $POCH_3$), 73.5 (d, $^1J_{CP}$ 299, =$\underline{C}$-P), 80.6 ($\underline{C}(CH_3)_3$), 97.5 (d, $^2J_{CP}$ 53, $\underline{C}$=C-P), 154.9 (NC=O), 170.4 (OC=O); d_P(121 MHz, $CDCl_3$) -3.5; *m/z* (CI) 671 (22 %, $[M_2]H^+$), 571 (100, $[M_2\text{-Boc+H}]H^+$), 336 (55, $[M+H]^+$), 280 (60, $[M\text{-}C(CH_3)_3\text{+H}]H^+$), 236 (22, $[M\text{-Boc+H}]H^+$); HRMS (CI, $[M+H]^+$) Found 336.1212 Calc. for $C_{13}H_{23}O_7N_1P$ 336.1209.

(*Z*)-(*S*)-5-(Dimethoxy-phosphoryl)-2-(3,3-dimethyl-1-methylene-butylamino)-pent-4-enoic acid methyl ester 22 and (*R*)-2-*tert*-butoxycarbonylamino-5-(dimethoxy-phosphoryl)-pentanoic acid methyl ester 23: (*S*)-2-*tert*-butoxycarbonylamino-5-(dimethoxyphosphoryl)pent-4-ynoic acid methyl ester **21** (530 mg, 1.6 mmol) was dissolved in methanol (5 cm^3). Quinoline (14 µL) was added followed by Pd-$BaSO_4$ (15 mg). The reaction mixture was placed under an atmosphere of hydrogen and stirred at RT for 45 minutes. The resulting mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The resulting residue was purified by flash chromatography eluting with EtOAc/hexane (3:2) to yield (*Z*)-(*S*)-5-(dimethoxyphosphoryl)-2-(3,3-dimethyl-1-methylenebutylamino)-pent-4-enoic acid methyl ester **22** (404 mg, 1.2 mmol, 75 %) as an oil and (*R*)-2-*tert*-butoxycarbonylamino-5-(dimethoxyphosphoryl)pentanoic acid methyl ester **23** (113 mg, 0.34 mmol, 21 %) as an oil which solidified upon standing.

(*Z*)-(*S*)-5-(Dimethoxy-phosphoryl)-2-(3,3-dimethyl-1-methylene-butylamino)-pent-4-enoic acid methyl ester 22; R_f(EtOAc/hexane; 1:1) 0.47; $[a]_D^{20}$ +3.4 (c 2 in EtOAc); n_{max} (oil)/cm^{-1} 3294 (NH, m), 2956 (CH, m), 1708 (C=O, s), 1627 (C=C, m), 1238 (P=O, s) 1020 (O-R, s); d_H(300 MHz, $CDCl_3$) 1.42 (9 H, s, $C(CH_3)_3$), 2.92-3.12 (2 H, m, *b*CH_2), 3.72 (3 H, d, $^3J_{HP}$ 11.5, $POCH_3$), 3.74 (3 H, d, $^3J_{HP}$ 11.2, $POCH_3$), 3.75 (3 H, s, $C(O)OCH_3$), 4.37 (1 H, m, *a*CH), 5.67 (1 H, d, $^3J_{HH}$ 7.8, NH), 5.74 (1 H, dd, $^2J_{HP}$ 18.3, $^3J_{HH}$ 13, CH=C$\underline{H}$(P)), 5.44 (1 H, dddd, $^3J_{HP}$ 52.4, $^3J_{HH}$ 13, $^3J_{HH}$ 7.1, $^3J_{HH}$ 8.7, C$\underline{H}$=CH(P)); d_C(67 MHz, $CDCl_3$) 28.3 ($C(\underline{C}H_3)_3$), 33.1 (d, $^3J_{CP}$ 8.5, *b*CH_2), 52.0 (d, $^2J_{CP}$ 4, $POCH_3$), 52.1 (d, $^2J_{CP}$ 6.2, $POCH_3$), 52.3 ($C(O)O\underline{C}H_3$), 52.7 (*a*CH), 79.8 ($\underline{C}(CH_3)_3$), 118.5 (d, $^1J_{CP}$

183.7, =CP), 150.2 (d, $^2J_{CP}$ 2.3, C=CP), 172.1 (C=O); d_P(121 MHz, $CDCl_3$) 19.8; *m/z* (CI) 338 (42 %, $[M+H]^+$), 238, (100, $[M\text{-Boc}+H]H^+$), 178 (30, $[M\text{-Boc-}OCH_3\text{-}OCH_3+3H]H^+$); HRMS (CI, $[M+H]^+$) Found 338.1370 Calc. for $C_{13}H_{24}O_7N_1P$ 338.1368.

(*R*)-2-*tert*-butoxycarbonylamino-5-(dimethoxyphosphoryl)pentanoic acid methyl ester 23; m.p. 60-61 °C; R_f(EtOAc/hexane 1:1) 0.29; $[\alpha]_D^{22}$ -9.6 (c 5.5 in EtOAc); ν_{max} (solid)/cm^{-1} 3279 (NH, m), 2956 (CH, m), 1739 (C=O, s), 1705 (NC=O, s), 1277 (P=O, m), 1218 (m), 1161 (P-OR, s), 1023 (C-OR, s); d_H(270 MHz, $CDCl_3$)1.45 (9 H, s, $C(CH_3)_3$), 1.61-1.72 (6 H, m, 3 CH_2), 3.74 (6 H, d, $^3J_{HP}$ 10.8, 2 × $POCH_3$), 3.75 (3 H, s, αOCH_3), 4.32 (1 H, m, αCH), 5.12 (1 H, d, $^3J_{HH}$ 7.7, NH); d_C(67 MHz, $CDCl_3$) 18.5 (d, J_{CP} 4.8, CH_2), 24.1 (d, $^1J_{CP}$ 141.2, CH_2), 28.3 (s, $C(\underline{C}H_3)_3$), 33.3 (d, J_{CP} 16.2, CH_2), 52.4 (m, 3 × OCH_3), 52.9m (s, αCH), 80.1 ($\underline{C}(CH_3)_3$), 154.2 (NC=O), 172.9 (αC=O); d_P(121 MHz; $CDCl_3$) 35.1; *m/z* (CI) 340 (55 %, $[M+H]^+$), 240 (100, $[M\text{-Boc}+H]H^+$); HRMS (CI, $[M+H]^+$) Found 340.1527 Calc. for $C_{13}H_{26}O_7N_1P$ 340.1525.

L-2-Amino-5-phosphonopentanoic acid 24.[3]: (*R*)-2-*tert*-butoxycarbonylamino-5-(dimethoxy-phosphoryl)-pentanoic acid methyl ester **23** (88 mg, 0.24 mmol) was dissolved in HCl (aq. 5 M) and heated at reflux overnight. The resulting mixture was concentrated in vacuo and partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (10 cm^3 × 5). Lypophilisation of the aqueous layer gave the amino acid **24** (57 mg, 0.24 mmol , 100 %) as a solid; d_H(400 MHz, D_2O) 1.65-1.90 (4 H, m, CH_2CH_2), 1.95-2.01 (2 H, m, CH_2), 3.95 (1 H, t, $^3J_{HH}$ 6.3, αCH); d_C(100.5 MHz, D_2O) 18.6 (d, $^3J_{CP}$ 3, CH_2), 25.9 (d, $^1J_{CP}$ 136.1, CH_2) 30.8 (d, $^2J_{CP}$ 16.9, CH_2), 53.0 (αCH), 172.1 (αC=O); d_P(121 MHz, D_2O) 30.7; *m/z* (ESI) 198 (100 %, $[M+H]^+$).

(*E*)-(*S*)-2-[bis(*tert*-butoxycarbonyl)-amino]-5-(diethoxy-phosphoryl)pent-4-enoic acid methyl ester 28: Tetraethylmethylene diphosphonate **31** (818 mg, 2.80 mmol) in dry THF (5 cm^3) was added to a suspension of sodium hydride (170 mg, 4.2 mmol) in dry THF (5 cm^3) at 0 °C. After 30 minutes a solution of aldehyde **18**[1] (940 mg, 2.80 mmol) was added and the reaction mixture stirred at 0 °C for 40 minutes. The reaction was quenched with saturated ammonium chloride (~20 cm^3). Then partitioned between EtOAc and water, the aqueous layer was extracted with ethyl acetate (3×30 cm^3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and con-

centrated in vacuo. The resulting oil was purified by flash chromatography eluting with EtOAc/hexane (3:1), to give (*E*)-(*S*)-2-[bis(*tert*-butoxycarbonyl)-amino]-5-(diethoxyphosphoryl)pent-4-enoic acid methyl ester **28** as an oil, which crystallized upon standing (830 mg, 1.8 mmol, 65 %); (Found: C 51.89; H 7.97; N 2.66 $C_{20}H_{36}NO_9P$ requires C 51.61; H 7.80; N 3.01); m.p. 56-60 °C; R_f(EtOAc/hexane, 1:1) 0.29; $[a]_D^{25}$ -3.22 (c 1.06 in CH_2Cl_2); n_{max}(film)/cm^{-1} 2980 (C-H, m), 1746 (C=O, s), 1699 (NC=O, s), 1636 (C=C, w), 1367 (C(CH_3)$_3$, s), 1247 (P=O, s), 1139 (C-O, s); d_H(400 MHz, $CDCl_3$) 1.31 (6 H, t, $^2J_{HH}$ 7.1, POCH$_2$C<u>H</u>$_3$), 1.50 (18 H, s, C(C<u>H</u>$_3$)$_3$), 2.87 (1 H, dddt, $^2J_{HH}$ 16.6, $^3J_{HH}$ 9.8, $^3J_{HH}$ 8.3, $^4J_{HH}$ 1.5, *b*CH), 3.03 (1 H, m, *b*CH), 3.73 (3 H, s, OCH_3), 4.03 (4 H, m, POCH$_2$), 5.05 (1 H, dd, $^3J_{HH}$ 9.8, $^3J_{HH}$ 5.4, *a*CH), 5.74 (1 H, ddt, $^2J_{HP}$ 20.0, $^3J_{HH}$ 17.1, $^4J_{HH}$ 1.5, HC=C<u>H</u>P), 6.70 (1 H, dddd, $^3J_{HP}$ 21.5, $^3J_{HH}$ 17.1, $^3J_{HH}$ 8.3, $^3J_{HH}$ 5.9, <u>H</u>C=CHP); d_C(100.5 MHz, $CDCl_3$) 16.3 (d, $^3J_{CP}$ 6.2, POCH$_2$<u>C</u>H$_3$), 23.0 (C(<u>C</u>H$_3$)$_3$), 34.8 (d, $^2J_{CP}$ 23.1, POCH$_2$), 52.4 (OCH_3), 56.8 (*a*CH), 61.8 (d, $^3J_{CP}$ 5.4, *b*CH$_2$), 83.5 (<u>C</u>(CH$_3$)$_3$), 120.5 (d, $^1J_{CP}$ 186, HC=<u>C</u>HP), 148.2 (m, H<u>C</u>=CHP), 151.8 (NCO), 170.3 (OC=O); d_P(121.5 MHz, $CDCl_3$, {^{1}H}) 17.93; *m/z* (CI) 494 (11 %, [M]$C_2H_5^+$), 394 (24, [M-Boc+H]$C_2H_5^+$), 366 (15, [MH-Boc]H$^+$), 338 (12, [M-Boc-C(CH_3)$_3$+2H]$C_2H_5^+$), 310 (89, [M-Boc-C(CH_3)$_3$+2H]H$^+$), 266 (38, [M-2Boc+2H]H$^+$), 57 (100, [C(CH_3)$_3$]$^+$).

(*E*)-(*S*)-2-[bis(*tert*-butoxycarbonyl)-amino]-5-(diethoxyphosphoryl) -5-fluoro-pent-4-enoic acid methyl ester 29: Tetraethyl fluoromethylene diphosphonate **32** (500 mg, 7.7 mmol) in dry THF (20 cm^3) was added to a suspension of sodium hydride (60 % *w/w*, 99 mg in THF 5 cm^3) at 0 °C. After 30 min aldehyde **18**[1] (548 mg, 7.7 mmol in THF 15 cm^3) was added. The reaction mixture was stirred at 0 °C for 3 h then at RT for 72 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride then partitioned between EtOAc and water. The aqueous layer was extracted with ethyl acetate (3 × 50 cm^3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The resulting residue was purified by flash chromatography, eluting with EtOAc/hexane (3:2) to give (*E*)-(*S*)-2-[bis(*tert*-butoxycarbonyl)-amino]-5-(diethoxyphosphoryl)-5-fluoro-pent-4-enoic acid methyl ester **29** as an oil (286 mg, 0.6 mmol, 8 %); R_f(EtOAc/hexane, 3:2) 0.5; $[a]_D^{25}$ -3.72 (c 4.3 in EtOAc); n_{max}(film)/cm^{-1} 2891 (CH, m), 1747 (C=O, s), 1700 (NC=O, s), 1367 (C(CH_3)$_3$, s) 1254 (CF, s), 1123 (P=O, m); d_H(400 MHz, $CDCl_3$) 1.34 (3 H, t, $^3J_{HH}$ 6.9, POCH$_2$C<u>H</u>$_3$) 1.35 (3

H, t, $^3J_{HH}$ 6.9, $POCH_2C\underline{H}_3$), 1.50 (18 H, s, $C(CH_3)_3$), 2.88-3.06 (2 H, m, *b* CH_2), 3.74 (3 H, s, *a* OCH_3), 4.09-4.20 (4 H, m, $POCH_2$), 5.01 (1 H, dd, $^3J_{HH}$ 9.7, $^3J_{HH}$ 5.7, *a* CH), 5.96 (1 H, ddt, $^3J_{FH}$ 38.4, $^3J_{HH}$ 16.1, $^3J_{HP}$ 6.9, HC=CFP); d_P(121 MHz, $CDCl_3$; {^{1}H}) 5.05 (d, $^2J_{FP}$ 101); d_F(282 MHz, $CDCl_3$) -128.5 (dd, $^2J_{FP}$ 101, $^3J_{FH}$ 38.4); d_C(75.5 MHz, $CDCl_3$) 17.0 (d, $^3J_{CP}$ 6.1, $CH_2\underline{C}H_3$), 25.7 (dd, $^3J_{CF}$ 10.8, $^3J_{CP}$ 6.2, *b* CH_2), 28.7 (s, $C(\underline{C}H_3)_3$), 53.2 (s, OCH_3), 57.5 (s, *a* CH), 84.4 (s, $\underline{C}(CH_3)_3$), 122.2 (dd, $^2J_{CF}$ 29.2, $^2J_{CP}$ 15.4, $\underline{C}$=CFP), 152.4 (s, NCO), 153.0 (dd, $^1J_{CF}$ 277.5, $^1J_{CP}$ 233.7, C=$\underline{C}$FP), 171.0 (s, *a* C=O); *m/z* (CI) 484 (3 %, $[M+H]^+$), 412 (24, $[M\text{-}OC(CH_3)_3+H]H^+$), 384 (28, $[M\text{-Boc}+H]H^+$), 356 (34, $[M\text{-Boc-}CH_2CH_3+2H]H^+$), 328 (60, $[M\text{-Boc-}2(CH_2CH_3)+2H]H^+$), 310 (14, $[M\text{-Boc-}2(CH_2CH_3)\text{-F}+4H]H^+$), 284 (100, $[M\text{-}2Boc+2H]H^+$), 57 (36, $[C(CH_3)_3]^+$); HRMS (CI, $[M+H]^+$), found 484.2122 Calc. for $C_{20}H_{35}O_9N_1P_1F_1$ 484.2112.

(*E*)-(*S*)-2-Amino-5-fluoro-5-phosphono-pent-4-enoic acid 30: (*E*)-(*S*)-2-[bis(*tert*-butoxycarbonyl)amino]-5-(diethoxyphosphoryl)-5-fluoro-pent-4-enoic acid methyl ester **29** (220 mg, 0.45 mmol) was dissolved in HCl (aq. 5 M, 8 cm^3) and heated at reflux overnight. The reaction mixture was concentrated *in vacuo* and the residue dissolved in water (5 cm^3). The aqueous layer was extracted with EtOAc (10 cm^3 × 5). Lyophillisation of the aqueous layer gave (*E*)-(*S*)-2-Amino-5-fluoro-5-phosphonopent-4-enoic acid **30** (96 mg, 0.45 mmol, quant.) as a hygroscopic solid; $[a]_D^{23}$ +0.52 (c 6.15 in D_2O); n_{max} (solid)/cm^{-1} 2903 (COOH, NH_2, CH, broad, s), 1725 (C=O, s), 1501 (m), 1509 (m), 1001 (O-C, s); d_H(300 MHz, D_2O) 2.57 (2 H, m, *b* CH_2), 3.95 (1 H, t, $^3J_{HH}$ 6.3, *a* CH), 5.40 (1 H, dq, $^3J_{HF}$ 39, $^3J_{HH}$ 7, $^3J_{HP}$ 7, $\underline{H}$C=CPF); d_C(75.5 MHz, D_2O) 24.9 (d, $^3J_{CF}$ 9.8, *b* CH_2), 52.4 (*a* CH), 112.5 (dd, $^2J_{CP}$ 11.8, $^2J_{CF}$ 27, $\underline{C}$=C(F)P), 159.8 (dd, $^1J_{CF}$ 276, $^1J_{CP}$ 221, =$\underline{C}$(F)P), 171.6 (C=O); d_P(121 MHz, D_2O) 0.5 (d, $^2J_{FP}$ 104); d_F(282 MHz, D_2O) -123.3 (dd, $^2J_{FP}$ 104, $^3J_{FH}$ 39).

Tetraethyl fluoromethylene diphosphonate 32:[4] Tetraethylmethylene diphosphonate **31** (2.5 g, 8.7 mmol) was dissolved in dry THF (10 cm^3) and added to a suspension of NaH (382 mg, 9.5 mmol) in dry THF (5 cm^3) at 0 °C. After 30 minutes the resulting mixture was added to a solution of Selectfluor® (3.1 g, 8.7 mmol) *via* cannula at 0 °C. The reaction mixture was stirred for a further 1 h, then quenched with cold NH_4Cl (aq.) and partitioned between EtOAc and brine. The aqueous layer was extracted with EtOAc (70 cm^3 × 3). The combined organic extracts were dried over anhydrous sodium sulfate,

filtered and concentrated *in vacuo*. The resulting residue was purified by flash chromatography eluting with 100 % EtOAc to yield the fluorophosphonate **32** (1.4 g, 4.6 mmol, 53 %) as an oil; R_f(100 % EtOAc) 0.22; d_H(300 MHz, $CDCl_3$) 1.36 (12 H, t, $^3J_{HH}$ 3.6, $POCH_2C\underline{H}_3$), 4.31 (8 H, m, $POCH_2$), 5.00 (1 H, dt, $^2J_{HF}$ 45, $^2J_{HP}$ 13.6, CHFPP); d_P(121 MHz, $CDCl_3$, $\{^1H\}$) 11.75 (d, $^2J_{FP}$ 63); d_F(282 MHz, $CDCl_3$) 23.6 (dt, $^2J_{FP}$ 63, $^2J_{HF}$ 45); *m/z* (CI) 307 (100 %, $[M+H]^+$), 279 (42, $[M-CH_2CH_3+H]H^+$), 261 (18, $[M-CH_2CH_3-F+2H]H^+$), 251 (19, $[M-2(CH_2CH_3)+2H]H^+$).

Enzyme assays: Stock solutions were made with Milli–Q water and ACS grade reagents. ASA–DH was expressed and purified from recombinant *E. coli*. The decrease or increase in b–NADPH concentration was observed at 340 nm over 600 seconds in a Pharmacia–LKB Ultrospec III spectrophotometer equipped with a water-heated (37°C) cuvette holder. The buffer solutions were prewarmed to 37°C before use by immersion in a water bath. The other stock solutions (vide infra) were stored on ice.

The assay was performed in the following way: Buffer solution (910 µL, 0.2 M Tris, 1.0 mM EDTA, pH 8.6), phosphate solution (10 µL, 1.5 M) ASA-DH solution (20 µL, 14 µg/mL) and ASA **3**[5] (30 µL, 11.5 mM) were introduced, in that order, into a 1000 µL quartz cuvette which was placed in the spectrophotometer. Then, $NADP^+$ (30 µL, 5 mM) was added, and the subsequent reaction was monitored at 340nm over 600 s. Data points were collected every 2 s and the plots were analysed using Microsoft Excel.

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