Supplementary Information for 'Fmoc -chemistry of a stable phosphohistidine analogue'

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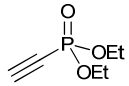
1. Chemical experimental

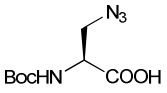
Unless otherwise stated, all reagents were purchased from Sigma Aldrich, Alfa Aesar, Merck, Iris Biochem or Fisher Scientific and were used without further purification. Silica chromatography was perfomed using silica (35-70 mm particles). Thin-layer chromatography was carried out on pre-coated aluminium plates. Mixtures of solvents are *v*/*v*. NMR data were collected using a Bruker Avance 500, Bruker DRX500, or Bruker DPX300 and analysed using MestReNova software. IR spectra were recorded using a PerkinElmer spectrum one FTIR spectrometer. Optical rotations were measured using an AA-5 automatic polarimeter, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. High resolution mass spectrometry (HRMS) was carried out on a Bruker Daltonics micrOTOF by Mrs Tanya Marinko-Covell.

a. Diethyl acetylenylphosphonate *6*[1]



Ethynylmagnesium bromide (0.5M solution in THF; 150 mL, 75 mmol) was added dropwise to a solution of diethyl chlorophosphate (11 mL, 76 mmol) in anhydrous THF (75 mL) at 0 °C. The reaction mixture was warmed to rt and stirred for 3 h, diluted with sat. *aq.* NH_4Cl (50 mL) and extracted with EtOAc (5 × 100 mL). The organic extracts were washed with H_2O (1 × 100 mL), brine (1 × 100 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give a dark brown oil. Column chromatography, eluting with 1:1 EtOAc/Hexane yielded the title compound **6**[1] as a yellow oil (2.975g, 24%); R_f: 0.22 (1:1 EtOAc/Hexane) ν_{max} (film)/cm^{-1} 3172 (CH alkyne), 2988 (CH_3), 2065 (C≡C), 1267 (P=O), 1050 (P-O); δ_H(500 MHz;$CDCl_3$; Me_4Si) 4.22-4.15 (4H, m, 2 × O*C*$H_2$$CH_3$), 3.14 (1H, d, $^3J_{P\text{-}H}$ *J* 13.2, C*CH*), 1.39 (6H, t, $^3J_{H\text{-}H}$ 7.2, 2 × OCH_2CH_3); δ_C(125 MHz; $CDCl_3$; Me_4Si) 88.7 (d, $^2J_{P\text{-}C}$ 50.6, PC*C*H), 74.4 (d, $^1J_{P\text{-}C}$ 288.8, P*C*CH), 63.8 (d, $^2J_{P\text{-}C}$ 5.6, O*C*$H_2$$CH_3$), 16.3 (d, $^3J_{P\text{-}C}$ 7.1, OCH_2CH_3); δ_P(121 MHz, $CDCl_3$) -7.25 (dp, $^3J_{P\text{-}H}$ 13.2, $^3J_{P\text{-}H}$ 8.7); *m/z* (ES) 163.0554 (*M*$^+$-*H*. $C_6H_{12}O_3P$ requires 163.0519), 185.0362 (*MNa*$^+$. $C_6H_{11}NaO_3P$ requires 185.0338).

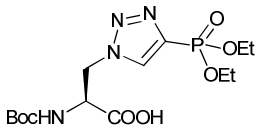
b. (2*S*)-3-azido-2-(tertbutoxylcarbonyl)amino-propionic acid *8*[2]



Freshly distilled triflic anhydride (3 mL, 17.8 mmol) was added dropwise to a vigorously stirred solution of sodium azide (5.76 g, 88.6 mmol) in H_2O (15 mL) and DCM (30 mL) at 0 °C, and stirred for 2 h at room temperature. The organic layer was separated and the aqueous layer extracted with DCM (2 × 15mL). The combined organic extracts were washed with sat *aq.* Na_2CO_3 solution (1 × 25mL), added dropwise to a stirred solution of (2*S*)-3-amino-2-(tertbutoxycarbonyl)amino-propionic acid (1.86 g, 9.11 mmol), K_2CO_3 (1.84 g, 13.3 mmol) and $CuSO_4$ (22 mg, 0.088 mmol) in H_2O (30 mL) and MeOH (45 mL), and stirred at room temperature overnight. The organic solvents were removed *in vacuo* and the remaining aqueous layer acidified to pH 6 with conc. HCl, diluted with potassium phosphate buffer (pH 6.2, 60 mL) and extracted with DCM (4 × 100 mL). The organic layers were combined, washed with brine (1 × 70 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield the title compound **8**[2] as a blue/green oil (1.635 g, 78% yield); $[\alpha]_D^{22}$ +30.5 (*c* 0.79 in MeOH); ν_{max} (film)/cm^{-1} 3328 (OH), 2554 (NH), 2108 (N=N=N), 1693 (C=O acid/carbamate); δ_H(500 MHz;$CDCl_3$; Me_4Si; 298 K) 6.70, 5.42 (1H, s-broad & d, *J* 7.2, N*H*),5.53, 4.35 (1H, 2 × s-broad,

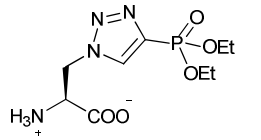
H$_{\alpha}$),3.79, 3. 71 (2H, 2 × s-broad, *H*$_{\beta}$), 1.47 (9H, s, OC(C*H*$_3$)$_3$) rotamer ratio 1:0.4;δ_H(500 MHz;$CDCl_3$; Me_4Si; 268K) **A**: 7.46 (1H, d, $^3J_{H\text{-}H}$ 6.6, N*H*),4.38 (1H, dt, $^3J_{H\text{-}H}$ 6.6, $^3J_{H\text{-}H}$ 4.4, *H*$_{\alpha}$),3.78 (1H, dd, $^2J_{H\text{-}H}$ 12.6, $^3J_{H\text{-}H}$ 4.4, *H*$_{\beta 1}$), 3.68 (1H, dd, $^2J_{H\text{-}H}$ 12.6, $^3J_{H\text{-}H}$ 4.4, *H*$_{\beta 2}$), 1.49 (9H, s, OC(C*H*$_3$)$_3$); **B**: 5.53 (1H, d, $^3J_{H\text{-}H}$ 7.7, N*H*), 4.53 (1H, dt, $^3J_{H\text{-}H}$ 7.7, $^3J_{H\text{-}H}$ 3.6, *H*$_{\alpha}$),3.84 (1H, dd, $^2J_{H\text{-}H}$ *J* 12.7, $^3J_{H\text{-}H}$ 3.6, *H*$_{\beta 1}$), 3.84 (1H, dd, $^2J_{H\text{-}H}$ 12.7, $^3J_{H\text{-}H}$ 3.6, *H*$_{\beta 2}$), 1.46 (9H, s OC(C*H*$_3$)$_3$) rotamer ratio A:B = 1.25:1; δ_C(125 MHz; $CDCl_3$; Me_4Si) 173.8 (*C*OOH), 156.0 (N*C*(O)O), 81.5 (O*C*(CH$_3$)$_3$), 53.8 (*C*$_{\alpha}$), 52.8 (*C*$_{\beta}$),28.6 (OC(*C*H$_3$)$_3$); *m/z* (ES) 253.0919 (*MNa*$^+$. $C_8H_{14}N_4NaO_3$ requires 253.0907).

c. (2*S*)-3-(4-(diethyl phosphoryl)-[1,2,3]triazol-1-yl)-2-(tertbutoxylcarbonyl)amino-propionic acid *4*[3]



(2*S*)-3-azido-2-(tertbutoxycarbonyl)amino-propionic acid **8** (300 mg, 1.30 mmol) and diethyl acetylenylphosphonate **6** (250 mg, 1.56 mmol) were dissolved in a 1:1 mixture of H_2O and ^{t}BuOH (15 mL), generating a pale yellow clear solution. A freshly made solution of $CuSO_4$ (2 mg, 0.013 mmol) and sodium L-ascorbate (15 mg, 0.13 mmol) in H_2O (1 mL) was added in one portion, and the reaction stirred at room temperature overnight. The reaction mixture was titrated to pH 12 with an aqueous solution of Na_2CO_3 (10%), diluted to 50 mL with H_2O and extracted with ether (2 × 20 mL), the aqueous phase was acidified to pH 1 with conc. HCl and extracted with EtOAc (5 × 20 mL). The combined extracts were dried ($MgSO_4$), and concentrated *in vacuo* to yield a yellow oil, Column chromatography (EtOAc) yielded the title compound **4**[3] as a yellow oil (449 mg, 88% yield); R_f : 0.08 (EtOAc); $[\alpha]_D^{22}$ -31.6 (*c* 0.38 in MeOH); ν_{max} (film)/cm^{-1} 3405 (OH), 2579 (NH), 1702 (C=O acid/carbamate), 1236 (P=O), 1010 (P-OEt); δ_H(500 MHz;$CDCl_3$; Me_4Si) 8.29 (1H, s, Trz-*H*$_5$), 5.65 (1H, d, $^3J_{H\text{-}H}$ 6.7, N*H*), 5.01 (1H, dd, $^2J_{H\text{-}H}$ 14.0, $^3J_{H\text{-}H}$ 4.0, C*H*$_{\beta 1}$), 4.95 (1H, dd, $^2J_{H\text{-}H}$ 14.0, $^3J_{H\text{-}H}$ 4.0, C*H*$_{\beta 2}$), 4.81-4.74 (1H, m, C*H*$_{\alpha}$), 4.28-4.15 (4H, m, 2 × OC*H*$_2$CH$_3$), 1.43 (9H, s, OC(C*H*$_3$)$_3$), 1.34 (6H, t, $^3J_{H\text{-}H}$ 7.07, 2 × OCH$_2$C*H*$_3$); δ_C(125 MHz; $CDCl_3$; Me_4Si) 174.3 (s, *C*OOH), 170.7 (NH*C*(O)O)), 136.5 (d, $^1J_{P\text{-}C}$ 241.8, Trz-*C*$_4$),132.8 (d, $^2J_{P\text{-}C}$ 33.8, Trz-C$_5$), 80.7 (s, O*C*(CH$_3$)$_3$), 63.9 (d, $^2J_{P\text{-}C}$ 5.35, O*C*H$_2$CH$_3$), 53.8 (s, *C*$_{\alpha}$), 50.0 (s, *C*$_{\beta}$), 28.4 (s, OC(*C*H$_3$)$_3$), 16.3 (d, $^3J_{P\text{-}C}$ 6.1, OCH$_2$*C*H$_3$); δ_P(121 MHz, $CDCl_3$) 8.24 (p, $^3J_{P\text{-}H}$ 7.9); *m/z* (ES) 393.1536 (*MH*$^+$. $C_{14}H_{26}N_4O_7P$ requires 393.1534), 415.1358 (*MNa*$^+$. $C_{14}H_{25}N_4NaO_7P$ requires 415.1353).

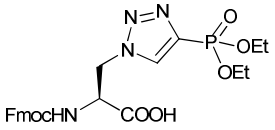
d. (2S)-3-(4-(diethyl phosphoryl)-[1,2,3]triazol-1-yl)-2-amino-propionic acid **9**



*(*2S)-3-[4-(Diethyl-phosphoryl)-[1,2,3]triazol-1-yl]-2-(tertbutoxycarbonyl)amino-propionic acid **4** (380 mg, 0.969 mmol) was dissolved in TFA (15 mL) and stirred at room temperature for 2 hrs. The reaction mixture was poured into H_2O (10 mL), the flask rinsed with H_2O (2 × 10 mL) and the resultant cloudy solution concentrated *in vacuo* to yield a pale brown oil. Excess TFA was removed by dissolution in H_2O (3 × 10 mL), and lyophilisation to yield the *title compound **9*** as a pale brown oil (309 mg, 92% yield); $[\alpha]_D^{22}$ -7.4 (*c* 0.82 in H_2O); δ_H(500 MHz;D_2O) 8.48 (1H, s, Trz-*H*$_5$),5.06 (1H, dd, $^2J_{H\text{-}H}$ 15.7, $^3J_{H\text{-}H}$ 5.2, *H*$_{\beta 1}$)*,5.02 (1H, dd, $^2J_{H\text{-}H}$ 15.7, $^3J_{H\text{-}H}$ 5.2, *H*$_{\beta 2}$)*, 4.60 (1H, t, $^3J_{H\text{-}H}$ 5.2, *H*$_{\alpha}$), 4.06 (4H, p, $^3J_{H\text{-}H,P\text{-}H}$ 7.1, 2 × OC*H*$_2$CH$_3$), 1.17 (6H, t, $^3J_{H\text{-}H}$ 7.1, 2 × OCH$_2$C*H*$_3$) [*H$_{\beta}$ signals overlap];

δ_C125MHz; D_2O) 168.8 (s, *C*OOH), 135.6 (d, $^1J_{P\text{-}C}$ 242.5, Trz-C_4), 133.2 (d, $^2J_{P\text{-}C}$ 33.1, Trz-C_4), 64.7 (d, $^2J_{P\text{-}C}$ 5.6, 2 × O*C*H_2CH$_3$), 52.8 (s, C_α), 49.1 (s, C_β), 15.4 (d, $^3J_{P\text{-}C}$ 6.1, 2 × OCH$_2$*C*H$_3$); δ_P(121Mhz; D_2O) 9.58 (s - broad); *m/z* (ES) 293.1004 (MH^+. $C_9H_{18}N_4O_5P$ requires 293.1009).

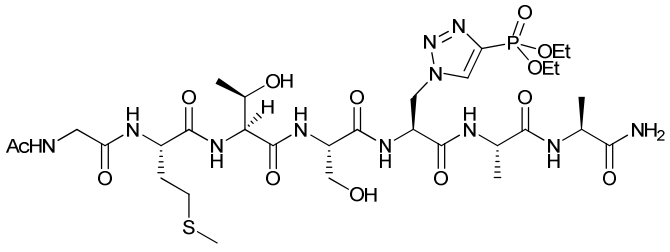
e. (2S)-3-[4-(Diethyl-phosphoryl)-[1,2,3]triazol-1-yl]-2-(9H-fluoren-9-ylmethoxycarbonyl)amino-propionic acid **5**



Diethyl acetylenylphosphonate **6** (111 mg, 0.685 mmol) was dissolved in THF (2 ml) and a solution of $CuSO_4$ (3 mg, 0.02 mmol) and sodium L-ascorbate (30 mg, 0.15 mmol) in H_2O (1 mL) added. (2S)-3-azido-2-(9H-fluoren-9-ylmethoxycarbonyl)amino-propionic acid (200 mg, 0.568 mmol) was added as a solution in THF/H_2O (1:1, 2mL) and the resultant solution stirred at room temperature for 24 h. The reaction mixture was titrated to pH 12 with aqueous Na_2CO_3 (10%), the mixture diluted to 30 mL with H_2O and extracted with ether (3 × 10 mL), the aqueous phase was acidified to pH 1 with conc. HCl and extracted with EtOAc (4 × 20 mL). The combined organic extracts were dried ($MgSO_4$), and concentrated *in vacuo* to yield the *title compound* **5** a yellow foam (283 mg, 96% yield); $[\alpha]_D^{22}$ -38.1 (*c* 0.21 in MeOH); ν_{max} (film)/cm^{-1} 3426 (OH), 2984 (NH), 1717 (C=O acid), 1644 (C=O carbamate), 1266 (P=O), 1052 (P-OEt); δ_H(500MHz; *d*6-DMSO) 8.63 (0.85H, s, Trz-H_5), 7.97 (0.83H, d, $^3J_{H\text{-}H}$ 8.5, N*H*),7.89 (2H, d, $^3J_{H\text{-}H}$ 7.5, 2 × Fmoc-H_5),7.67, 7.65 (2H, 2 × d, $^3J_{H\text{-}H}$ 7.5, 2 × Fmoc-H_2),7.42 (2H, t, $^3J_{H\text{-}H}$ 7.44, 2 × Fmoc-H_4),7.340, 7.336 (2H, 2 × t, $^3J_{H\text{-}H}$ 7.3, 2 × Fmoc-H_3), 4.91 (2H, dd, $^2J_{H\text{-}H}$ 13.8, $^3J_{H\text{-}H}$ 4.6, $H_{\beta1}$), 4.76 (1H, dd, $^2J_{H\text{-}H}$ 13.8, $^3J_{H\text{-}H}$ 4.6, $H_{\beta2}$),4.64 (1H, m, H_α),4.29-4.16 (3H, m, 1 × H_A + 2 × H_B), 4.03 (4H, m, 2 × CH_2CH$_3$), 1.204, 1.200 (6H, 2 × t, $^3J_{H\text{-}H}$ 7.0, 2 × CH$_2$CH_3); δ_C(125MHz; *d*6-DMSO): 171.5 (s, *C*OOH), 156.8 (s, NH*C*(O)O), 144.6 (s, Fmoc-C_1), 141.6 (s, Fmoc-C_6), 137.2 (d, $^1J_{P\text{-}C}$ 237.8, Trz-C$_4$),133.2 (d, $^2J_{P\text{-}C}$ 33.8, Trz-C$_5$),128.7 (s, Fmoc-C_5),128.2 (s, Fmoc-C_2), 126.2 (s, Fmoc-C_3) .,121.1 (s, Fmoc-C_4), 67.0 (s, C_B), 62.2 (d, $^2J_{P\text{-}C}$ 5.5, PO(O*C*H_2CH$_3$)), 55.0 (s, C_A), 50.7 (s, C_β),47.6 (s, C_α), 16.0 (d, $^3J_{P\text{-}C}$ 6.1, PO(OCH$_2$*C*H$_3$)); δ_P(121MHz; *d*6-DMSO): 8.42 (p, $^3J_{P\text{-}H}$ 8.0); *m/z* (ES) 515.1710 (MH^+. $C_{24}H_{28}N_4O_7P$ requires 515.1690.

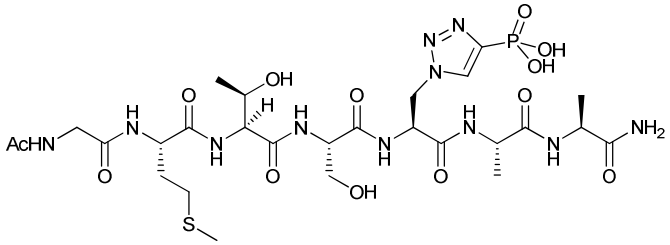
(2S)-3-[4-(Diethyl-phosphoryl)-[1,2,3]triazol-1-yl]-2-amino-propionic acid **4** (as TFA salt, 431 mg, 1.059 mmol) was dissolved in aqueous Na_2CO_3 (10%, 20 mL), dioxane (10 mL) and H_2O (5 mL). This mixture was cooled to 0°C, a solution of Fmoc-Cl (300 mg, 1.16 mmol) in dioxane (5 mL) added dropwise and the mixture stirred at 0°C for 10 min then at room temperature for 1 h. The reaction mixture was extracted with ether (2 × 10 mL), the aqueous phase acidified to pH 1, and extracted with EtOAc (4 × 15 mL). The EtOAc extracts were combined, dried ($MgSO_4$) and concentrated in vacuo to yield **5** as a pale yellow foam (372 mg, 68% yield).

f. Ac-Gly-Met-Thr-Ser-pTz(OEt)$_2$-Ala-Ala-NH$_2$ **11**



Peptides were synthesised according to standard solid phase synthesis protocols using Rink amide Novagel resin (loading: 0.64 mmol/g). Resin (105 mg) was swollen in DMF for 30 min and a solution of Fmoc-Ala-OH (105 mg, 0.34 mmol, 5 eq.), HCTU (139 mg, 0.34 mmol, 5 eq.) and DIPEA (124 µl, 0.68 mmol, 10 eq.) in DMF (2 ml) added to the resin and mixed for 1 h. The resin was washed with DMF (3 × 2 ml, 2 min), 20% piperidine in DMF (5 × 2 ml, 2 min) and DMF (5 × 2 ml, 2 min). Couplings of Fmoc-Ala-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Met-OH and Fmoc-Gly-OH were carried out in the same fashion using 5 eq. amino acid, 5 eq. HCTU and 10 eq. DIPEA in DMF (2 ml) for 1 h. Coupling of the triazole amino acid was carried out using 3 eq. amino acid, 3 eq. HCTU and 6 eq. DIPEA in DMF (1.5 ml) for 1h. The N-terminus was acetylated by addition of a solution of acetic anhydride (32 µl, 0.34 mmol, 5 eq) and DIPEA (62 µl, 0.34 mmol, 5 eq) in DMF (2 ml) for 30 min and the resin washed with DMF (3 × 2 ml, 2 min), DCM (3 × 2 ml, 2 min) and MeOH (3 × 2 ml, 2 min) before drying overnight under a stream of air. The peptide was cleaved from the resin by addition of 2 ml of a cleavage cocktail (TFA (94%), EDT (2.5%), H_2O (2.5%) and TIS (1%)) for 2 h. The solution was added to ice-cold ether (10 ml) and the precipitate collected by centrifugation, and the pellet washed in cold ether (5 × 10 ml). The residual ether was removed under a stream of nitrogen and the resultant gummy white solid dissolved in H_2O/dioxane and lyophilised to give the *title compound* ***11*** as a flocculent colourless solid (24mg, 0.028 mmol, 45%); *m/z* (ES) 852.3455 (MH^+. $C_{31}H_{55}N_{11}O_{13}PS$ requires 852.3438), 868.3387 (MH^+-*Met[O]*. $C_{31}H_{55}N_{11}O_{14}PS$ requires 868.3388), 874.3268 (MNa^+. $C_{31}H_{54}N_{11}NaO_{13}PS$ requires 874.3259), 890.3221 (MNa^+-*Met[O]*. $C_{31}H_{54}N_{11}NaO_{14}PS$ requires 890.3208).

g. Ac-Gly-Met-Thr-Ser-pTz(OH)$_2$-Ala-Ala-NH$_2$ **12**



TMS-Br (120 µl , 0.91 mmol) was added in one portion to a suspension of **11** (15.34 mg, 0.018 mmol) in anhydrous DCM (2.5 ml) and the mixture stirred for 72 h. The solvent was removed *in vacuo* to yield an amorphous colourless solid which was immediately dissolved in MeOH (10 ml) and stirred for 90 min. The solvent was removed *in vacuo* and the resultant colourless oil dissolved in H_2O and lyophilised to yield a brown amorphous solid (14.5 mg, mixture of products); *m/z* (ES) 794.2694 (M^--*acid*. $C_{27}H_{45}N_{11}O_{13}PS$ requires 794.2662), 822.3008 (M^--*monoester*. $C_{29}H_{49}N_{11}O_{13}PS$ requires 822.2975), 850.3306 (M^--*diester*. $C_{31}H_{53}N_{11}O_{13}PS$ requires 850.3288).

2. Modelling electrostatic potential

Calculations were performed in Gaussian_03(e)[4] using the B3LYP functional with 6-311+g(d,p) basis. Full geometry optimizations were performed and converged in all cases. Electrostatic Potential (ESP) maps were plotted using Gaussview 4. Isosurfaces were generated at a total density of 0.0004 e/bohr^3. ESP values range from -0.05 to 0.05 au, i.e. from -31.4 (red) to +31.4 (blue) kcal mol^{-1}.

Output Z-matrices from Gaussian for modelled structures

NATURAL = 3-methylimidazole-1-phosphoramidate

TRIAZOLE = 1-methyl-[1,2,3]-triazole-4-phosphonic acid

NATURAL: C4H7N2O3P1

B3LYP/6-311+g(d,p)

ENERGY = -833.4221803 Hartree

C
C,1,B1
C,1,B2,2,A1
N,1,B3,2,A2,3,D1
H,2,B4,1,A3,4,D2
H,3,B5,1,A4,4,D3
C,1,B6,4,A5,3,D4
H,7,B7,1,A6,4,D5
H,7,B8,1,A7,4,D6
H,7,B9,1,A8,4,D7
P,3,B10,1,A9,4,D8
O,11,B11,3,A10,1,D9
O,11,B12,3,A11,1,D10
H,13,B13,11,A12,3,D11
O,11,B14,3,A13,1,D12
H,15,B15,11,A14,3,D13
N,3,B16,1,A15,4,D14

Variables:
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B3=1.39012312
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B6=1.49314852
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B9=1.09172658
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B11=1.4724792
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D7=179.73857085
D8=-179.4872967
D9=178.22242797
D10=-59.70529758
D11=-167.129144
D12=60.05048621
D13=72.41164846
D14=-179.95249244

TRIAZOLE: C3H6N3O3P1

B3LYP/6-311+g(d,p)

ENERGY = -849.4201555 Hartree

C
N,1,B1
H,1,B2,2,A1
C,2,B3,1,A2,3,D1
H,4,B4,2,A3,1,D2
H,4,B5,2,A4,1,D3
H,4,B6,2,A5,1,D4
P,1,B7,2,A6,4,D5
O,8,B8,1,A7,2,D6
O,8,B9,1,A8,2,D7
H,10,B10,8,A9,1,D8
O,8,B11,1,A10,2,D9
H,12,B12,8,A11,1,D10
C,1,B13,2,A12,4,D11
N,2,B14,1,A13,14,D12
N,1,B15,14,A14,15,D13

Variables:

B1	2.22383
B2	1.07635
B3	2.43557
B4	1.09087
B5	1.08795
B6	1.09107
B7	2.88481
B8	1.47958
B9	1.61749
B10	0.96566
B11	1.61827
B12	0.96555
B13	1.37818
B14	1.29593
B15	1.35101
A1	158.26186
A2	65.80544
A3	122.51435
A4	78.39262
A5	120.72205
A6	97.65807
A7	119.23542
A8	80.46621
A9	112.24874
A10	118.47996
A11	112.35502
A12	69.52957
A13	73.09608
A14	104.2752
D1	0.81947
D2	72.34285
D3	178.33504
D4	-75.62591
D5	-178.64987
D6	86.86402
D7	-158.55934
D8	-150.92664
D9	-59.84733
D10	114.01312
D11	179.66689
D12	-0.06982
D13	0.08534

TRIAZOLE [P=O in plane]: C3H6N3O3P1

B3LYP/6-311+g(d,p)

ENERGY = -849.4161438 Hartree

C
N,1,B1
H,1,B2,2,A1
C,2,B3,1,A2,3,D1
H,4,B4,2,A3,1,D2
H,4,B5,2,A4,1,D3
H,4,B6,2,A5,1,D4
P,1,B7,2,A6,4,D5
O,8,B8,1,A7,2,D6
O,8,B9,1,A8,2,D7
H,10,B10,8,A9,1,D8
O,8,B11,1,A10,2,D9
H,12,B12,8,A11,1,D10
C,1,B13,2,A12,4,D11
N,2,B14,1,A13,14,D12
N,1,B15,14,A14,15,D13

Variables:

B1	2.22383
B2	1.07635
B3	2.43557
B4	1.09087
B5	1.08795
B6	1.09107
B7	2.88481
B8	1.47958
B9	1.61749
B10	0.96566
B11	1.61827
B12	0.96555
B13	1.37818
B14	1.29593
B15	1.35101
A1	158.26186
A2	65.80544
A3	122.51435
A4	78.39262
A5	120.72205
A6	97.65807
A7	138.36526
A8	85.91025
A9	112.24874
A10	93.03903
A11	112.35502
A12	69.52957
A13	73.09608
A14	104.2752
D1	0.81947
D2	72.34285
D3	178.33504
D4	-75.62591
D5	-178.64987
D6	2.00836
D7	127.581
D8	-175.98028
D9	-130.45382
D10	113.18482
D11	179.66689
D12	-0.06982
D13	0.08534

3. References

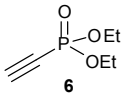
1. B. C. Saunders and P. Simpson, *J. Chem. Soc.* 1963, 3351-3360

2. A. J. Link, M. K. S. Vink and D. A. Tirrell, *J. Amer. Chem. Soc.* 2004, **126**, 10598-10602

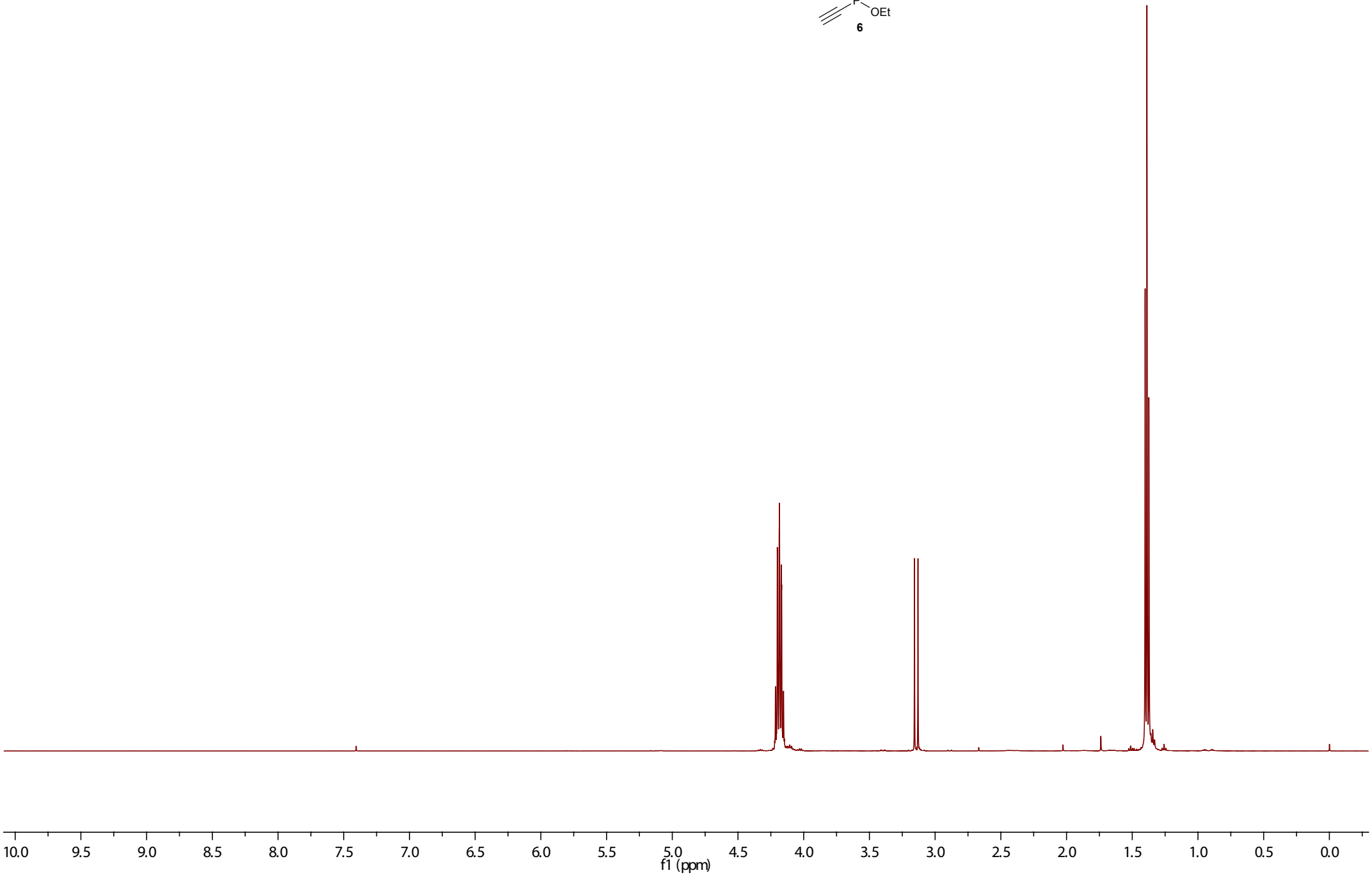
3. J.-M. Kee, B. Villani, L. R. Carpenter and T. W. Muir, *J. Amer. Chem. Soc.*, 2010 DOI:10.1021/ja104393t

4. Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

4. NMR Spectra


O
P
OEt
OEt

6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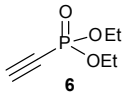
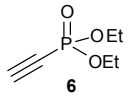
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

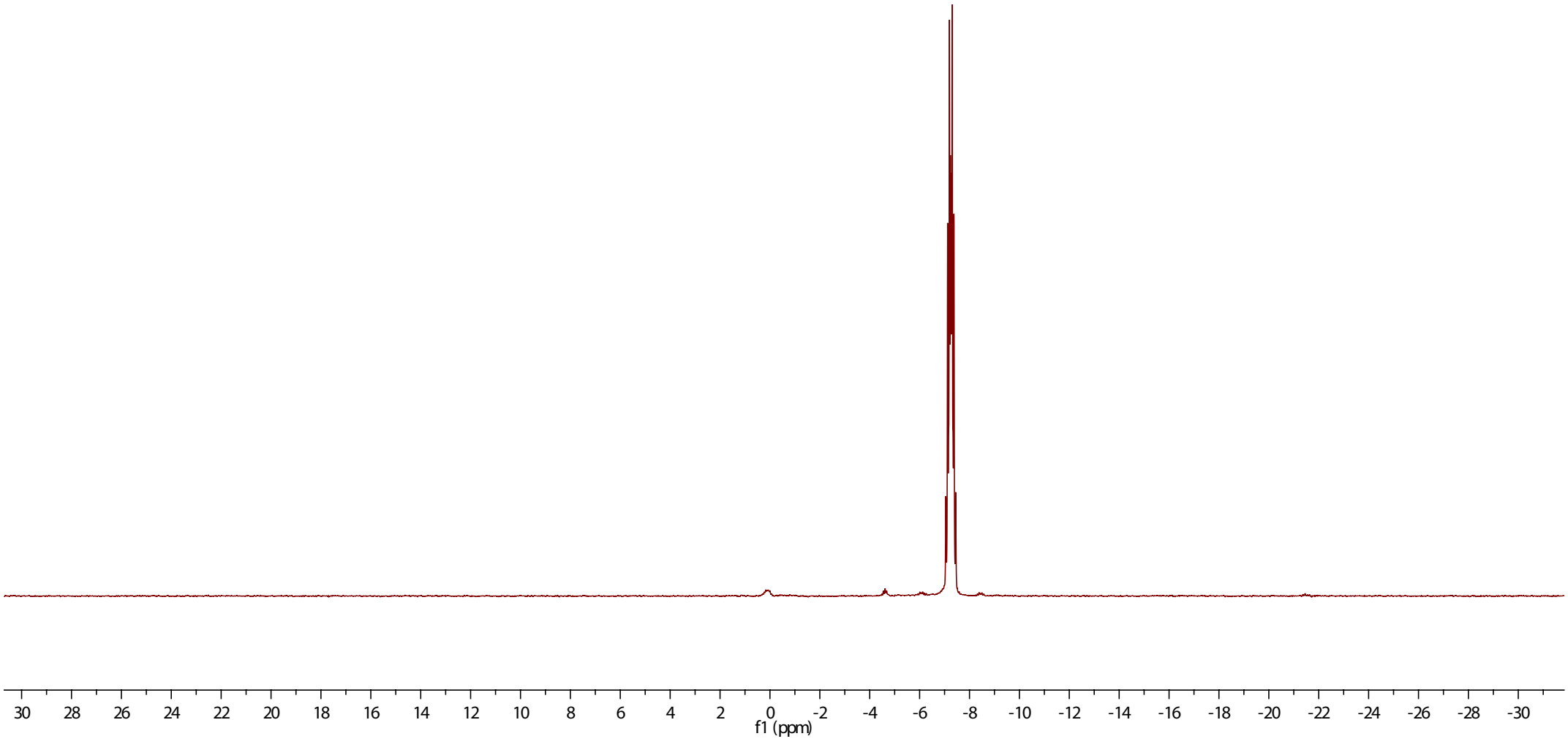

O
P
OEt
OEt

6

110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
f1 (ppm)


O
OEt
P
OEt

6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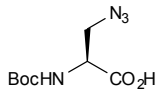
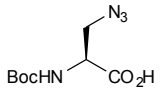
f1 (ppm)

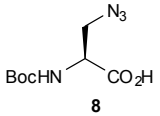


8

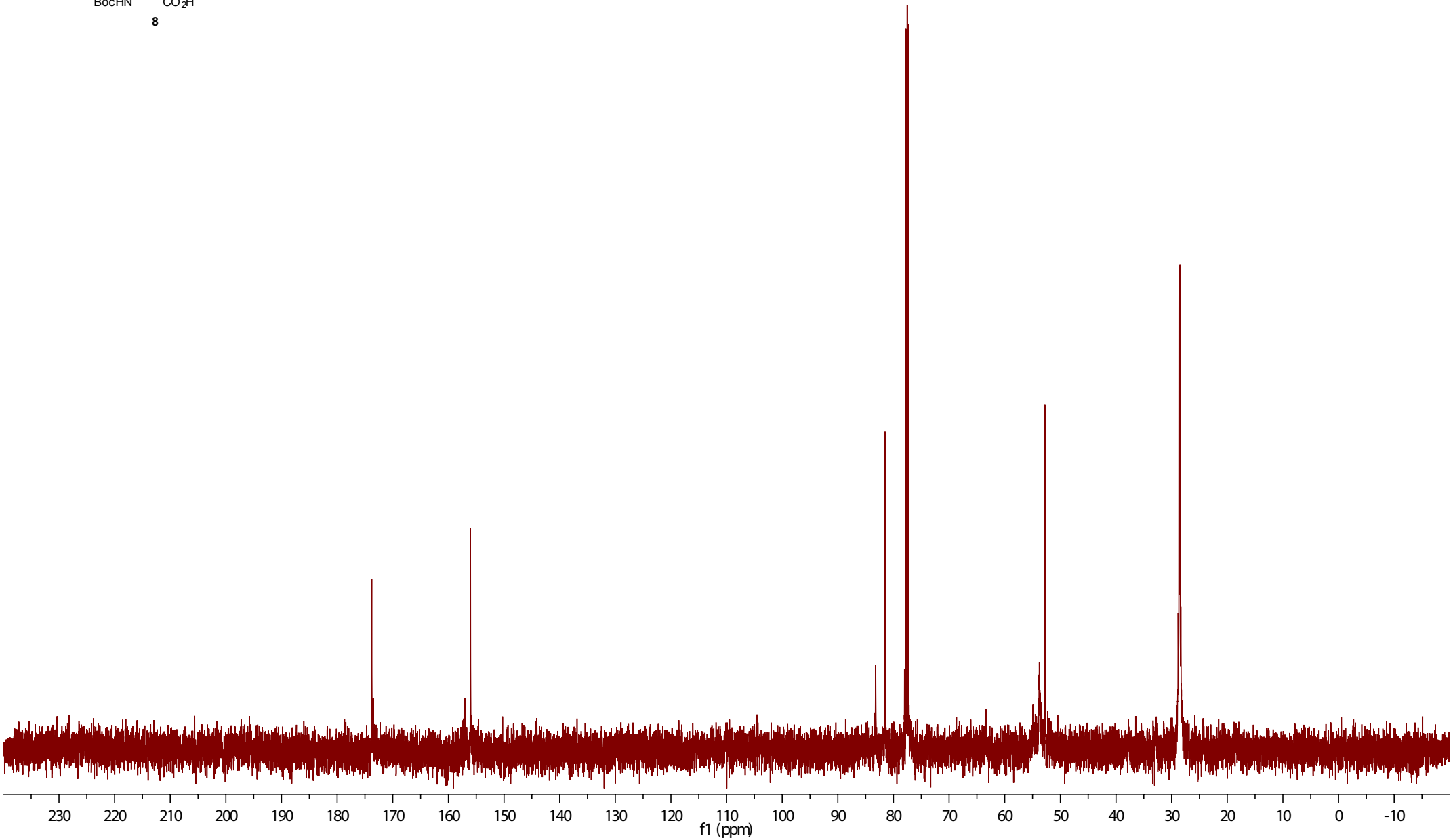

N3
BocHN
CO2H

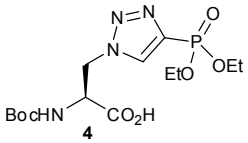
8

9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

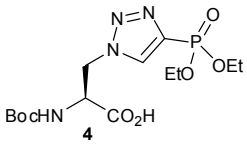

N3
BocHN
CO2H

8


230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

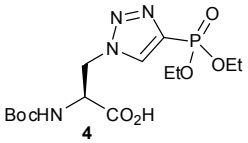


4

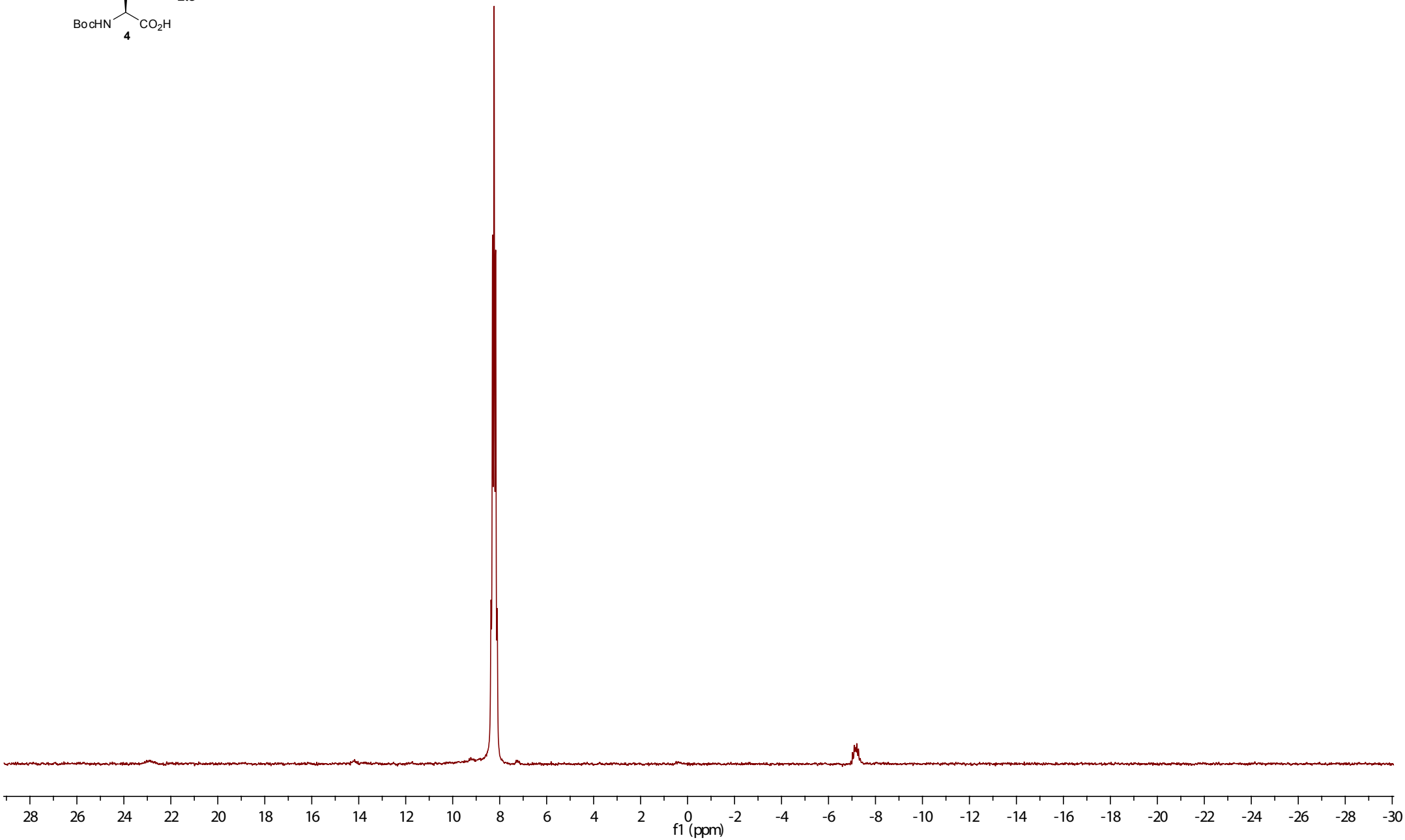

N
N
N
P
O
EtO
OEt
BocHN
CO2H

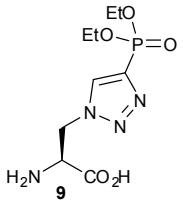
4

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


N=N
N
P
O
EtO
OEt
BocHN
CO2H

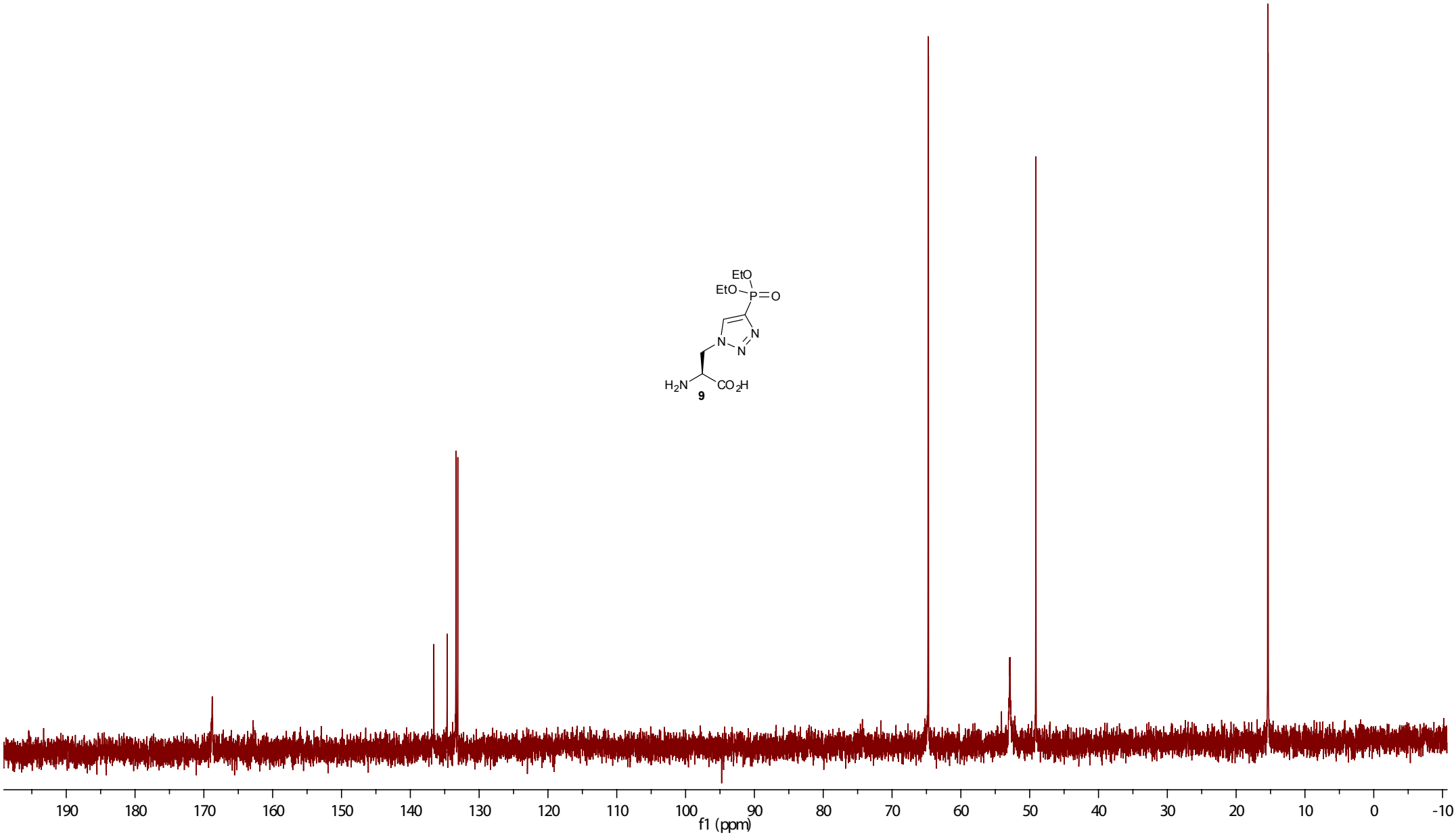
4

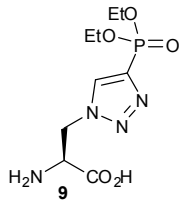

28 26 24 22 20 18 16 14 12 10 8 6 4 2 0 -2 -4 -6 -8 -10 -12 -14 -16 -18 -20 -22 -24 -26 -28 -30
f1 (ppm)


EtO
EtO
P
O
N
N
N
H2N
CO2H

9

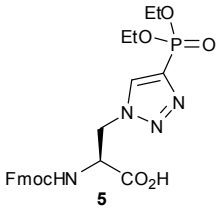
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


EtO
EtO
P=O
N
N
N
H2N
CO2H
9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


EtO
EtO
P=O
N
N
N
H2N
CO2H

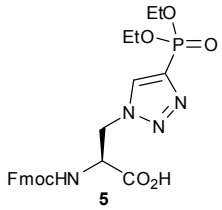
9

100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
f1 (ppm)


EtO
EtO
P=O
N
N
N
FmocHN
CO2H

5

12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
f1 (ppm)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EtO
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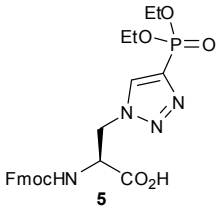
N
N
FmocHN
CO2H

5

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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EtO
P=O
N
N
N
FmocHN
CO2H

5

100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
f1 (ppm)

Analytical HPLC-MS of peptide deprotection reaction

Analytical LC-MS was performed using an Agilent 1200 series LC system comprising a Bruker HCT Ultra ion trap mass spectrometer. Samples were run through a Phenomenex Luna C18 50 × 2 mm 5 µm column using a gradient from 5% to 90% MeCN over 1.8 min. The free phosphonic acid, monoester and diester were separated and detected by negative ion mass spectroscopy as shown below.