Supporting Information

Synthesis and characterization of L-3-(pentafluorophosphato-difluoromethyl)-alanine, a structural and functional mimetic of phosphoserine

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General methods

All chemicals used for synthesis were purchased from Sigma (Merck), ABCR, and Roth and were used without further purification. Solvents were obtained from VWR chemicals and Fisher scientific chemicals. Dry solvents were obtained from a column-based solvent purification system (MBraun, MB-SPS-800).

Moisture sensitive reactions were carried out using Schlenk technology in glassware that was heat-dried in vacuum and flushed with nitrogen.

Volatile solvents were removed by rotary evaporators from the brand Heidolph using a water bath of 40°C to 53°C. High vacuum of up to 10^{-3} mbar was generated by an oil pump by Vacuubrand. For lyophilization of aqueous samples frozen in liquid nitrogen Christ Alpha 2-4 LD plus was used.

For MPLC purification the Biotage Isolera One flash chromatography system was used with Biotage Sfär Silica HC D and Biotage Sfär C18 D cartridges. For normal phase chromatography, hexane/ethyl acetate was used as mobile phase, for reversed phase 10 mM aqueous NH_4HCO_3 and acetonitrile was used.

For HPLC-MS measurements the Agilent UPLC-System 1290 Infinity II was used. Samples were run on a Zorbax C18 RRHT (2.1 x 50 mm, 1.8 µm, 80 Å) column, coupled with an ESI single quadrupole mass spectrometer LC/MSD (Model# G6125C, Serial# SG2236N102) from Agilent. The mobile phase, consisting of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), was run as a gradient starting from 5% B to 95% B.

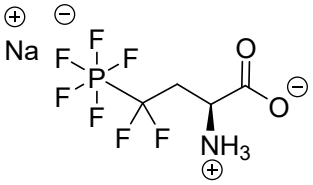
High resolution mass spectra were measured with an analytical HPLC system (Agilent Technologies, Infinity II 1290), Zorbax Eclipse plus C18 RRHD (2.1 x 50 mm, 1.8 µM, 95 Å) column, coupled with an ESI-Q-TOF iFunnel mass spectrometer (Agilent Technologies, 6550).

^{1}H-, ^{13}C- and ^{19}F-NMR and ^{31}P-spectra of synthesized molecules were measured on JEOL ECZLS400 and JEOL ECZ600 spectrometers. Chemical shifts (δ) were referenced to the solvent peaks and are given in ppm, coupling constants (*J*) in Hz.

Biological activity data analysis was performed in GraphPad Prism (Version 5.0). IC_{50} values were calculated with the log(inhibitor) vs. normalized response – variable slope function.

Chemical synthesis

Sodium 3-(pentafluorophosphato-difluoromethyl)-L-alanine 3



Compound **11** (100 mg, 0.2 mmol, 1 eq.) was dissolved in MeCN (1.8 mL), and 200 µL piperidine were added. After 1 h, HPLC-MS showed complete deprotection of the amino group. Volatiles were evaporated and the crude product was purified via MPLC (RP-C18, 10 mM NH_4HCO_3/acetonitrile). The obtained residue was redissolved in H_2O and ion exchange was performed using the Amberlite™ IRC-120 resin sodium form (CAS: 68441-33-8) as described previously.[7] After lyophilization, compound **3** was obtained as off-white solid (47 mg, 86% yield).

^{1}H NMR (600 MHz, D_2O) δ 3.83 (dd, *J* = 10.0, 2.8 Hz, 1H), 2.59 – 2.43 (m, 1H), 2.33 – 2.17 (m, 1H).

^{13}C NMR (151 MHz, D_2O) δ 174.00 (C=O carboxylic acid), 50.03 (C_α), 36.91 ($C_\beta H_2$).

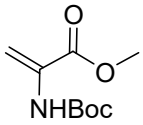
^{19}F NMR (376 MHz, D_2O) δ -69.24 (dp, *J* = 698.6, 43.4 Hz), -74.34 (ddt, *J* = 854.0, 43.2, 10.7 Hz), -99.83 – -103.89 (m).

^{31}P NMR (162 MHz, D_2O) δ -125.33 – -165.83 (m).

HRMS (ESI/Q-TOF**)**: $[M]^-$ Calcd for $C_4H_6F_7NO_2P^-$ 264.0024 Da; Found 264.0034 m/z.

$[\alpha]^{20}_D$ = - 6.6 ° (c = 1.4, H_2O)

N-(*tert*-Butoxycarbonyl)-dehydroalanine methyl ester 5



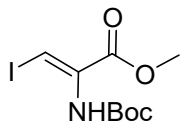
To a stirred solution of *N*-(tert-butoxycarbonyl)-L-serine methyl ester (3.0 g, 13.7 mmol, 1 eq.) in dry DCM (30 mL), triethylamine (2.5 mL, 17.8 mmol, 1.3 eq.) was added. The resulting solution was cooled to 0 °C and methanesulfonyl chloride (1.4 mL, 17.8 mmol, 1.3 eq.) was added dropwise. The mixture was allowed to reach room temperature and stirred overnight. The reaction mixture was diluted with DCM and washed with water (2x) and with saturated $NaHCO_3$ (2x). The organic phase was dried over Na_2SO_4 and concentrated in vacuo to approximately 30 mL. DBU (2.5 mL, 16.4 mmol, 1.2 eq.) was added and the reaction mixture was stirred for 2 h at room temperature. The DCM phase was washed with 1M $KHSO_4$ (1x) and brine (1x) and dried over Na_2SO_4. After filtration, DCM was removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexane/ethyl acetate) yielding product **5** as colorless liquid (2.4 g, 86%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.01 (s, 1H, NHBoc), 6.15 (s, 1H, $CH_{2\alpha}$), 5.72 (s, 1H, $CH_{2\beta}$), 3.82 (s, 3H, CH_3 methyl ester), 1.48 (s, 9H, $(CH_3)_3$ Boc).

^{13}C NMR (151 MHz, $CDCl_3$) δ 164.55 (C=O methyl ester), 152.65 (C=O Boc), 131.37 ($C_α$), 105.26 ($C_α$=C), 80.78 (C(CH_3) Boc), 52.96 (CH_3 methyl ester), 28.32 (*C*(CH3) Boc).

HRMS (ESI/Q-TOF**)**: [M – Boc + H]$^+$ Calcd for $C_4H_8NO_2$ 102.0555 Da; Found 102.0554 m/z.

***N*-(*tert*-Butoxycarbonyl)-3-iodo-deyhdroalanine methyl ester 6**



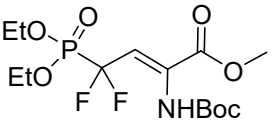
To a stirred suspension of *N*-iodosuccinimide (2.5 g, 10.9 mmol, 1.1 eq.) in DCM (30 mL), compound **5** (2.0 g, 9.9 mmol, 1 eq.) was added in DCM (5 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight and monitored with TLC. After consumption of **5**, triethylamine (2.8 mL, 19.9 mmol, 2 eq.) was added and the reaction was stirred for another 15 min. Following, the reaction was diluted with DCM and washed with water (1x) and brine (1x). The organic phase was dried over Na_2SO_4, filtrated, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexane/ethyl acetate) yielding product **6** as yellow oil (2.11 g, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (s, 1H, NHBoc), 6.10 (s, 1H, C=CH), 3.82 (s, 3H, CH_3 methyl ester), 1.48 (s, 9H, $(CH_3)_3$ Boc).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.17 (C=O methyl ester), 151.99 (C=O Boc), 137.60 ($C_α$), 83.40 ($C_α$=C), 81.94 (C*(CH$_3$)* Boc), 52.98 (CH_3 methyl ester), 28.25 (*C*(CH_3) Boc).

HRMS (ESI/Q-TOF**)**: [M – Boc + H]$^+$ Calcd for $C_4H_7INO_2$ 227.9522 Da; Found 227.9522 m/z.

***N*-(*tert*-Butoxycarbonyl)-4-(diethoxyphosphoryl)-dehydroalanine methyl ester 7**



Previously activated cadmium powder[7] (3.6 g, 32.0 mmol, 6 eq.) was added to a heat- and vacuum-dried Schlenk flask and stirred with dry DMF (3 mL) under protective gas environment at rt. Upon dropwise addition of diethyl bromo-difluoromethyl phosphonate (3.1 mL, 17.6 mmol, 3.3 eq.), the reaction mixture grew warm and was stirred for 3 h. Compound **6** (1.74 g, 5.3 mmol, 1 eq.) was dried for several hours under high vacuum in a separate flask. Previously dried CuBr (2.2 g, 16.0 mmol, 3 eq.) was added to compound **6** together with 2 mL of DMF. Following, the formed organocadmium reagent was transferred to the second Schlenk flask containing compound **6** and CuBr with a syringe and slowly added under protective gas atmosphere. The resulting reaction mixture was stirred overnight and monitored via TLC. After 18 h, ethyl acetate was added, which led to the formation of a white precipitate, which was filtered off through a bed of Celite. The filtrate was washed with saturatedNH_4Cl solution (3x) and brine (1x). The organic phase was dried over Na_2SO_4, filtrated, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexane/ethyl acetate) yielding product **7** as colorless liquid (1.38 g, 67%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (s, 1H, NH), 5.63 (td, *J* = 13.8, 4.9 Hz, 1H, C=C-H), 4.36 – 4.21 (m, 4H, CH_2 ethyl ester), 3.83 (s, 3H, CH_3 methyl ester), 1.45 (s, 9H, CH_3 Boc), 1.38 (t, *J* = 7.1 Hz, 6H, CH_3 ethyl ester).

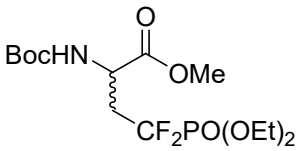
^{13}C NMR (151 MHz, $CDCl_3$) δ 164.73 (C=O methyl ester), 152.50 (C=O Boc), 137.23 (C_α), 119.36 – 116.19 (m, CF_2), 111.63 (C_α=C), 82.08 (C*(CH$_3$)* Boc), 65.83 (CH_2 phosphonate ester), 53.23 (CH_3 methyl ester), 28.38 (*C*(CH_3) Boc), 16.72 (CH_3 phosphonate ester).

^{19}F NMR (565 MHz, $CDCl_3$) δ -107.03 (d, *J* = 108.1 Hz, CF_2).

^{31}P NMR (243 MHz, $CDCl_3$) δ 7.72 (t, *J* = 111.5 Hz, *Z*-isomer), 5.53 (t, *J* = 91.1 Hz, *E*-isomer).

HRMS (ESI/Q-TOF**)**: $[M + H]^+$ Calcd for $C_{14}H_{25}F_2NO_7P^+$ 388.1337 Da; Found 388.1337 m/z.

***N*-(*tert*-butoxycarbonyl)-4-(diethoxyphosphoryl)-alanine methyl ester 8-rac**



49 mg of **7** (0.13 mmol, 1 eq.) were dissolved in 5 mL MeOH and 10 wt% of Pd/C catalyst (5 mg) was added to the solution. H_2 was filled into a balloon and bubbled through the reaction mixture with a needle and the flask was set under H_2 atmosphere with a second balloon. After 2 h LC-MS showed full conversion to the product. The reaction mixture was filtered through a 0.2 µM regenerated cellulose filter to remove the catalyst. The volatiles were evaporated and the product was obtained as light-yellow oil (42.9 mg, 87% yield).

^{1}H NMR (600 MHz, MeOH-*D*$_4$) δ 4.51 (dd, *J* = 8.9, 3.9 Hz, 1H, $C_\alpha H$), 4.34 – 4.25 (m, 4H, CH_2 phosphonate ester), 3.74 (s, 3H, CH_3 methyl ester), 2.74 – 2.61 (m, 1H, $C_\beta H_{2\alpha}$), 2.58 – 2.44 (m, 1H, $C_\beta H_{2\beta}$), 1.44 (s, 9H, s, 9H, $(CH_3)_3$ Boc), 1.39 (t, *J* = 7.1 Hz, 6H, $CH_3)_2$ phosphonate ester).

^{13}C NMR (151 MHz, MeOH-*D*$_4$) δ 171.78 (C=O methyl ester), 156.21 (C=O Boc), 119.58 (d, *J* = 218.9 Hz, CF_2), 79.47 (C*(CH)$_3$* Boc), 65.13 (CH_2 phosphonate ester), 51.77 (CH_3 methyl ester), 48.23 (C_α), 34.80 ($C_\beta H_2$), 27.33 (*C*(CH_3) Boc), 15.38 (CH_3 phosphonate ester).

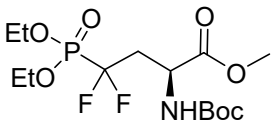
^{19}F NMR (565 MHz, MeOH-*D*$_4$) δ -103.76 – -120.13 (m, CF_2).

^{31}P NMR (243 MHz, MeOH-*D*$_4$) δ 6.71 (t, *J* = 107.3 Hz).

HRMS (ESI/Q-TOF**)**: $[M + H]^+$ Calcd for $C_{14}H_{27}F_2NO_7P^+$ 390.1493 Da; Found 390.1491 m/z.

$[\alpha]^{20}_D$ = - 0.3 ° (c = 1.7, $CHCl_3$)

***N*-(*tert*-Butoxycarbonyl)-3-(diethoxyphosphoryl-difluoromethyl)-L-alanine methyl ester 8-(+)**



A solution of **7** (1.66 g, 4.28 mmol) in dry MeOH (8 mL) and a solution of (*S*,*S*)-Et-DUPHOS-Rh (CAS: 213343-64-7) (42.40 mg, 64.21 µmol, 1.5 mol%) in dry MeOH (8 mL) were combined in a stainless-steel high-pressure reaction vessel (130 mL) under Argon atmosphere. The reaction mixture was stirred at 22 °C under 20 bar of hydrogen gas for 3 d. After reaction control using LC-MS, the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (SiO_2, hexane/ethyl acetate) yielding product **8-(+)** as a colorless liquid (1.45 g, 87% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.34 (d, *J* = 8.9 Hz, 1H, NH Boc), 4.64 – 4.56 (m, 1H, $C_\alpha H$), 4.33 – 4.22 (m, 4H, CH_2 phosphonate ester), 3.76 (s, 3H, CH_3 methyl ester), 2.78 – 2.49 (m, 2H), 1.44 (s, 9H, $(CH_3)_3$ Boc), 1.38 (td, *J* = 6.8, 1.2 Hz, 6H, $(CH_3)_2$ phosphonate ester).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.61 (C=O methyl ester), 155.13 (C=O Boc), 122.86 – 118.80 (m, CF_2), 80.36 (C*(CH)$_3$* Boc), 64.99 (CH_2 phosphonate ester), 52.84 (CH_3 methyl ester), 48.49 (C_α), 36.10 – 35.24 (m, $C_\beta H_2$), 28.40 (*C*(CH_3) Boc), 16.48 (CH_3 phosphonate ester).

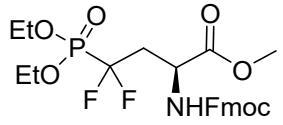
^{19}F NMR (565 MHz, $CDCl_3$) δ -110.30 – -112.01 (m, CF_2).

^{31}P NMR (243 MHz, $CDCl_3$) δ 6.47 (t, *J* = 104.7 Hz).

HRMS (ESI/Q-TOF**)**: $[M + H]^+$ Calcd for $C_{14}H_{27}F_2NO_7P^+$ 390.1493 Da; Found 390.1491 m/z.

$[\alpha]^{20}_D$ = + 7.6 ° (c = 2.1, $CHCl_3$)

N-(Fluorenyl-9H-methoxy-carbonyl)-3-(diethoxyphosphoryl-difluoromethyl)-L-alanine methyl ester 9



500 mg of **8** were dissolved in 5 mL of DCM and 5 mL of TFA and 100 µL of triethylsilane were added. The reaction was stirred for 3 h after which time LC-MS showed full conversion to the deprotected amino acid. The solvents were evaporated and TFA was co-evaporated three times with 50 mL of toluene. 10 mL of water were added to the brown oil, and the pH was tuned to 8-9 using $NaHCO_3$. Following, Fmoc-OSu (500 mg, 1.48 mmol, 1.2 eq) was added in 10 mL dioxane and the reaction was stirred at rt. After 20 h LC-MS showed full conversion to product **9**. Dioxane was evaporated and 20 mL of water were added. The solution was acidified to pH = 1 using conc. HCl. The aqueous phase was extracted with EtOAc 3 times and the combined organic phases were washed with brine. The organic phase was dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by MPLC (SiO_2, hexane/EtOAc). Compound **9** was obtained as light-yellow oil (489 mg, 75%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.61 (t, *J* = 7.1 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.4, 1.3 Hz, 2H) (8x Fmoc Ar-H), 5.74 (d, *J* = 8.3 Hz, 1H, NH Fmoc), 4.74 – 4.66 (m, 1H, $C_\alpha H$), 4.38 (d, *J* = 7.1 Hz, 2H, CH_2 Fmoc), 4.34 – 4.21 (m, 5H, CH Fmoc, CH_2 phosphonate ester), 3.79 (s, 3H, CH_3 methyl ester), 2.84 – 2.57 (m, 2H, $C_\beta H_2$), 1.38 (td, *J* = 7.1, 2.5 Hz, 6H, CH_3 phosphonate ester).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.23 (C=O methyl ester), 155.74 (C=O Fmoc), 143.95, 143.88, 141.42, 127.86, 127.23, 125.32 (d, *J* = 6.5 Hz, CF_2), 120.12 (12x Ar-C), 67.49 (CH_2 Fmoc), 65.27 – 64.93 (m, CH_2 phosphonate ester), 53.04 (CH_3 methyl ester), 48.93 (C_α), 47.20 (CH Fmoc), 35.64 (q, *J* = 19.5 Hz, $C_\beta H_2$), 16.54 (CH_3 phosphonate ester).

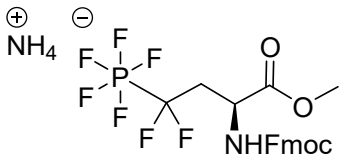
^{19}F NMR (565 MHz, $CDCl_3$) δ -109.71 – -111.70 (m, CF_2).

^{31}P NMR (243 MHz, $CDCl_3$) δ 6.39 (t, *J* = 105.6 Hz).

HRMS (ESI/Q-TOF): $[M + H]^+$ Calcd for $C_{24}H_{29}F_2NO_7P^+$ 512.1650 Da; Found 512.1653 m/z.

$[\alpha]^{20}_D$ = + 3.3 ° (c = 1.1, $CHCl_3$)

Ammonium *N*-(fluorenyl-9H-methoxy-carbonyl)-3-(pentafluorophosphato-difluoromethyl)-L-alanine methyl ester 10



Method A

Compound **9** (478 mg, 0.93 mmol, 1 eq.) was placed in a heat- and vacuum-dried Schlenk flask under inert gas atmosphere and 6 mL of dry MeCN were added. TMSBr (0.62 mL, 4.7 mmol, 5 eq.) was added dropwise and the reaction was heated to 60 °C for 1.5 h. The reaction was cooled to rt and then oxalyl chloride (0.8 mL, 9.3 mmol, 10 eq.) and DMF (0.4 mL, 4.7 mmol, 5 mL) were added. The reaction was heated to 40 °C and stirred for 1 h. Following, the reaction mixture was cooled with an ice-bath and TMAF (983 mg, 9.3 mmol, 10 eq.) was added under inert gas atmosphere and the reaction was allowed to reach rt. After 1.5 h the reaction was quenched by adding 40 mL of sat. $NaHCO_3$ solution and the solvents were evaporated. The crude product was purified by MPLC (RP C18, 10 mM NH_4HCO_3/MeCN). The product fractions were evaporated and dried by lyophilization. Compound **10** was obtained as white solid (346 mg, 74%).

Method B

Compound **9** (1065 mg, 2.08 mmol, 1 eq.) was placed in a PFA (perfluoralkoxy polymer) round bottom flask equipped with a PTFE (polytetrafluorethylene polymer) plug. Olah's reagent (70:30 HF:pyridine) (3 mL, 104 mmol, 50 eq.) was added slowly. The yellow reaction mixture was stirred with a magnetic stir bar at room temperature. Samples of the reaction mixture (1 µL) were diluted in acetonitrile-water (1:1, 499 µL) and analyzed by HPLC-MS. Full conversion was observed after 4 h. The reaction mixture was cooled with an ice bath and quenched by adding dropwise TMSOMe until a sample of the reaction mixture on wet pH paper indicated a pH of 7-8. All volatiles were evaporated and the crude yellow oil purified by MPLC (RP-C18, 10 mM NH_4HCO_3/acetonitrile). Compound **10** was obtained as white solid (876 mg, 81% yield).

^{1}H NMR (600 MHz, DMSO-D6) δ 7.89 (d, *J* = 7.6 Hz, 2H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.42 (td, *J* = 7.5, 1.3 Hz, 2H), 7.33 (td, *J* = 7.4, 1.2 Hz, 2H) (8x Ar-H Fmoc), 4.34 (td, *J* = 7.8, 4.7 Hz, 1H, $C_\alpha H$), 4.28 – 4.19 (m, 3H, CH Fmoc, CH_2 Fmoc), 3.61 (s, 3H, CH_3 methyl ester), 2.39 – 2.17 (m, 2H, $C_\beta H_2$).

^{13}C NMR (151 MHz, DMSO-D_6) δ 173.43, 156.18, 144.36 (d, *J* = 9.4 Hz), 141.24, 128.18, 127.62, 125.87 (d, *J* = 4.3 Hz, CF_2), 120.63 (12x Ar-C), 66.24 (CH_2 Fmoc), 52.52 (C_α), 47.13 (CH Fmoc), 38.15 (d, *J* = 19.5 Hz, $C_\beta H_2$).

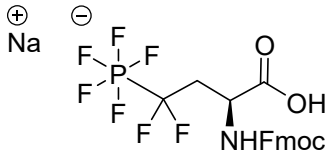
^{19}F NMR (565 MHz, DMSO-D_6) δ -67.90 (dp, *J* = 704.1, 44.5 Hz, 1F, F_{ax} PF_5), -72.72 (ddt, *J* = 853.5, 44.5, 11.1 Hz, 4F, F_{eq} PF_5), -102.19 – -104.05 (m, 2F, CF_2).

^{31}P NMR (243 MHz, DMSO-D6) δ -143.44 (pdt, *J* = 853.2, 701.6, 109.0 Hz).

HRMS (ESI/Q-TOF**)**: $[M]^-$ Calcd for $C_{20}H_{18}F_7NO_4P^-$ 500.0862 Da; Found 500.0866 m/z.

$[\alpha]^{20}_D$ = - 11.4 ° (c = 1.4, CH_3CN)

Sodium *N*-(fluorenyl-9H-methoxy-carbonyl)-3-(pentafluorophosphato-difluoromethyl)-L-alanine 11



100 mg of **10** were dissolved in 20 mL aqueous 10 mM NH_4HO_3 buffer. A spatula tip of *B. licheniformis* protease (CAS: 9014-01-1) was added, and the reaction mixture was stirred for 16 h after which LC-MS showed full conversion. The water was evaporated, and the crude product was purified by MPLC (RP-C18, 10 mM NH_4HCO_3/acetonitrile). The obtained residue was redissolved in 50:50 H_2O:MeCN and ion exchange was performed using Amberlite™ IRC-120 resin in its sodium form (CAS: 68441-33-8) as described previously.[7] After lyophilization, compound **11** was obtained as off-white solid (86.1 mg, 89% yield).

^{1}H NMR (600 MHz, D_2O) δ 7.92 (d, *J* = 8.0 Hz, 2H), 7.75 – 7.68 (m, 2H), 7.50 (t, *J* = 7.5 Hz, 2H), 7.47 – 7.40 (m, 2H) (8x Fmoc Ar-H), 4.57 (dd, *J* = 10.8, 6.4 Hz, 1H, $C_\alpha H$), 4.47 – 4.31 (m, 2H, CH_2 Fmoc), 4.24 (dd, *J* = 9.4, 3.1 Hz, 1H, CH Fmoc), 2.53 – 2.40 (m, 1H, $C_\beta H_{2\alpha}$), 2.33 – 2.19 (m, 1H, $C_\beta H_{2\beta}$).

^{13}C NMR (151 MHz, D_2O) δ 179.12 (C=O carboxylic acid), 157.49 (C=O Fmoc), 144.06, 143.82, 140.92, 128.09, 127.58 (d, *J* = 6.2 Hz), 125.21 (d, *J* = 5.9 Hz, CF_2), 120.20 (8x Ar-C), 66.37 (CH_2 Fmoc), 51.60 – 51.34 (m, C_α), 47.01 (CH Fmoc), 38.74 – 36.71 (m, C_β).

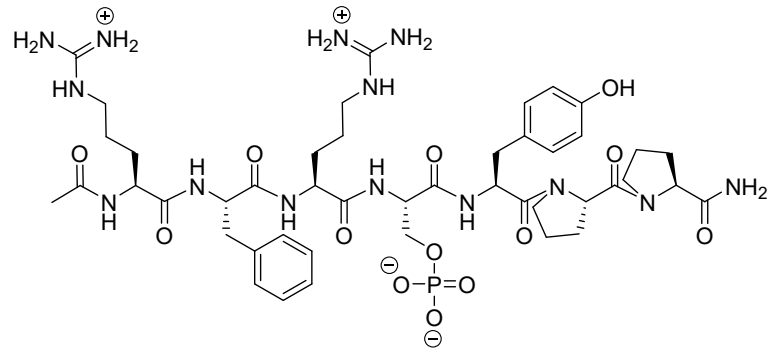
^{19}F NMR (565 MHz, D_2O) δ -68.18 (dp, *J* = 691.3, 42.7 Hz, $1F_{ax}PF_5$), -74.49 (ddt, *J* = 862.5, 42.4, 10.9 Hz, 4F, F_{eq}, PF_5), -101.63 – -103.38 (m, 2F, CF_2).

^{31}P NMR (243 MHz, D_2O) δ -142.59 (pdt, *J* = 862.3, 691.4, 114.7 Hz).

HRMS (ESI/Q-TOF**)**: $[M]^-$ Calcd for $C_{19}H_{16}F_7NO_4P^-$ 486.0705 Da; Found 486.0712 m/z.

$[\alpha]^{20}_D$ = + 9.7 ° (c = 1.4, H_2O)

Ac-RFRpSYPP-NH$_2$ 15



Phosphoserine peptide amide **15** was synthesized by standard solid phase peptide synthesis in a 10 mL syringe. 200 mg of Rink amide resin (low loading, 0.058 mmol) were weighed into

the syringe and swollen for 1 h in DMF. Cleavage of N-terminal protecting groups was conducted by adding piperidine:DMF (20:80) (2 x 5 min) and coupling steps were done by premixing amino acid with coupling reagent and base for 5 min in 3 mL DMF and then adding the mixture to the resin.

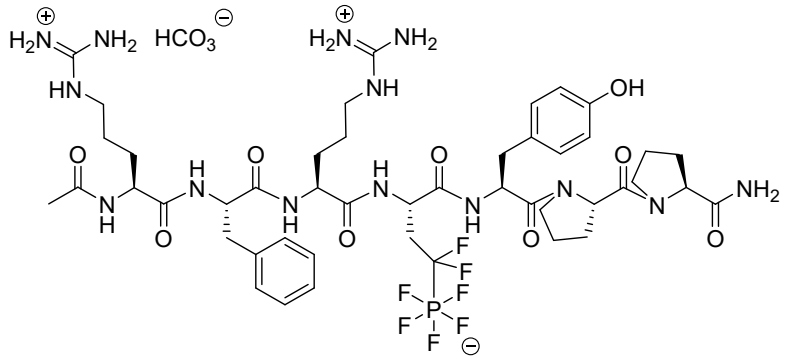
Table S1. Reagents used for the individual coupling steps in the order of addition.

Fmoc-L-Pro-OH (5 eq.)	HATU (4.9 eq.)	Trimethylpyridine (10 eq.)
Fmoc-L-Pro-OH (5 eq.)	HATU (4.9 eq.)	Trimethylpyridine (10 eq.)
Fmoc-L-Tyr(tBu)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Ser(PO(OBzl)OH)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Arg(Pbf)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Phe-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Arg(Pbf)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)

Couplings were reacted for 2 h or overnight. After the reaction, the success of coupling was determined via the Kaiser test.[22] In the last step, the peptide was acetylated by adding an acetic anhydride:pyridine (50:50) mixture (1 mL in 2 mL DMF) for 1 hour. The resin was washed with DMF, THF and DCM and dried under high vacuum. The peptide was cleaved from the resin by addition of 2 mL 95:5 TFA:H_2O for 90 min. The solution was added dropwise to 40 mL of ice-cold diethyl ether to precipitate the peptide. The formed precipitate was washed 3 times with ice-cold diethyl ether and purified by HPLC (C18 column, 10 mM NH_4HCO_3/MeCN). Product fractions were evaporated and dried by lyophilization, yielding a white powder (34.6 mg, 57% yield).

HRMS (ESI/Q-TOF): $[M + H]^+$ Calcd for $C_{45}H_{68}N_{14}O_{13}P^+$ 1043.4828 Da; Found 1043.4849 m/z.

Ac-RFR(PF_5CF_2Ala)YPP-NH_2 16



PF_5-serine peptide amide **16** was synthesized under the same conditions as compound **15**. 200 mg of Rink amide resin (low loading, 0.058 mmol) were used. Amino acids were coupled as described above.

Table S2. Reagents used for the individual coupling steps in the order of addition.

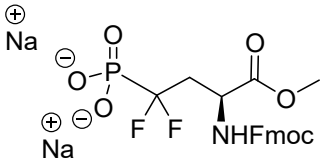
Fmoc-L-Pro-OH (5 eq.)	HATU (4.9 eq.)	Trimethylpyridine (10 eq.)
Fmoc-L-Pro-OH (5 eq.)	HATU (4.9 eq.)	Trimethylpyridine (10 eq.)
Fmoc-L-Tyr(tBu)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-PF_5CF_2Ala-OH (Na^+) (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Arg(Pbf)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Phe-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)
Fmoc-L-Arg(Pbf)-OH (3 eq.)	TBTU (2.9 eq.)	DIPEA (3 eq.)

The cleavage was done by addition of Olah's reagent (1 mL) with 10% anisole for 1 h and the peptide was added dropwise to an ice-cold saturated sodium hydrogen carbonate solution and evaporated. Purification was done by HPLC (C18 column, 10 mM NH_4HCO_3/MeCN). After the first column a large amount of ammonium fluoride was observed in the ^{19}F NMR, and the purification was repeated. The product fractions were lyophilized and the clean product was obtained as a white powder (8.5 mg, 13% yield).

^{19}F NMR (565 MHz, DMSO-*D*6) δ -69.44 (dp, *J* = 698.7, 44.2 Hz), -75.34 (ddt, *J* = 858.3, 42.9, 10.3 Hz), -103.00 – -106.11 (m).

HRMS (ESI/Q-TOF): $[M + 2H]^+$ Calcd for $C_{46}H_{67}F_7N_{14}O_9P^+$ 1123.4841 Da; Found 1123.4872 m/z.

Disodium N-(fluorenyl-9H-methoxy-carbonyl)-4-(phosphato-difluoromethyl)-L-alanine methyl ester 17



43 mg (0.08 mmol, 1 eq.) of compound **10** were stirred in 10% TFA in MeCN/H_2O (50/50, total 1 mL) for 20 h, after which LC-MS showed full conversion to structure **17**. Volatiles were evaporated and the product was purified via MPLC (RP C18, 10 mM NH_4HCO_3/MeCN). After evaporation of product fractions, the residue was re-dissolved in 50:50 H_2O:MeCN and ion exchange was performed using the Amberlite™ IRC-120 resin sodium form (CAS: 68441-33-8) as described previously.[7] The lyophilized product was obtained as light yellow solid (28 mg, 67% yield).

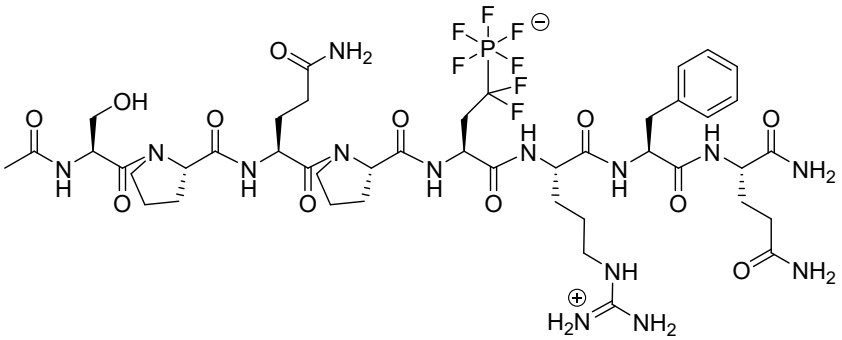
^1H NMR (600 MHz, DMSO-D_6) δ 7.89 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.72 (dd, *J* = 7.6, 5.3 Hz, 2H, Ar-H), 7.41 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.33 (t, *J* = 7.7 Hz, 2H, Ar-H), 4.38 – 4.34 (m, 1H, $C_\alpha H$), 4.26 – 4.17 (m, 3H, CH Fmoc, CH_2 Fmoc), 3.62 (s, 3H, methyl ester), 2.48 – 2.36 (m, 2H, $C_\beta H_2$).

^{19}F NMR (376 MHz, DMSO-D_6) δ -108.27 – -111.53 (m).

^{31}P NMR (162 MHz, DMSO-D_6) δ 1.12 (t, *J* = 81.7 Hz).

HRMS (ESI/Q-TOF**)**: $[M + H]^+$ Calcd for $C_{20}H_{21}F_2NO_7P^+$ 456.1023 Da; Found 456.1021 m/z.

Ac-SPQP(PF_5CF_2Ala)RFQ-NH_2 18



Compound **18** was synthesized using the microwave peptide synthesizer Liberty Blue 2.0 (serial number: BA001137). 300 mg of Rink amide resin (low loading, 0.087 mmol) were weighed into the reaction tube and swollen for 1 h in DMF. Cleavage of N-terminal protecting groups was conducted by adding piperidine:DMF (20:80) for 1 minute at 90°C. Coupling steps for canonical amino acids were performed at 90 °C for 2 minutes, Fmoc-PF_5CF_2Ala-OH was coupled at 75 °C for 20 minutes. All incoming amino acids after proline were coupled twice. In the last step, the peptide was acetylated by adding an acetic anhydride:pyridine (50:50) mixture (1 mL in 2 mL DMF) for 1 hour.

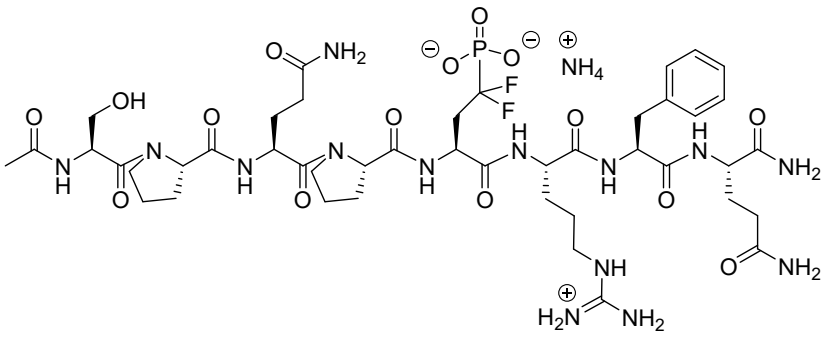
Table S3. Reagents used for the individual coupling steps in the order of addition.

Fmoc-L-Gln-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Phe-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Arg(Pbf)-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-PF_5CF_2Ala-OH (Na^+) (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Pro-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Gln-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Pro-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Ser-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)

The cleavage was done by addition of Olah's reagent (2 mL) with 10% anisole for 1 h and the peptide was added dropwise to an ice-cold saturated sodium hydrogen carbonate solution and evaporated. Purification was done by HPLC (C18 column, 10 mM NH_4HCO_3/MeCN). The product fractions were lyophilized and the clean product was obtained as a white powder (37 mg, 38% yield).

HRMS (ESI/Q-TOF**)**: $[M]^-$ Calcd for $C_{44}H_{65}F_7N_{14}O_{12}P$ 1145.4532 Da; Found 1145.4534 m/z.

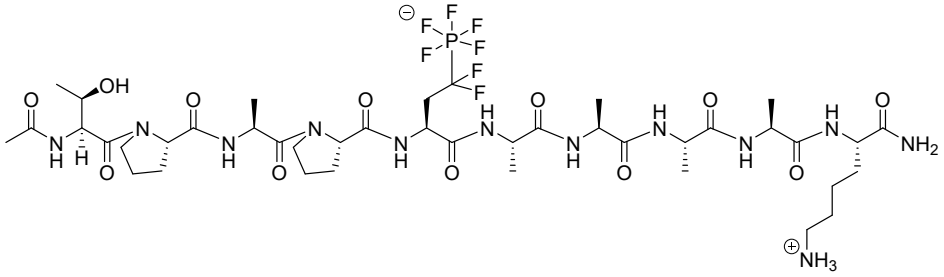
Ac-SPQP(PO_3CF_2Ala)RFQ-NH_2 19



10 mg of compound **18** were treated with TFA:H_2O:MeCN (1:1:1, 1.5 mL) for 20 hours. All volatiles were evaporated. The crude product was purified by MPLC (C18 column, 10 mM NH_4HCO_3/MeCN). The product fractions were lyophilized and the clean product was obtained as a white powder (7.3 mg, 75% yield).

HRMS (ESI/Q-TOF**)**: $[M-H]^-$ Calcd for $C_{44}H_{66}F_2N_{14}O_{15}P$ 1099.4538 Da; Found 1099.4540 m/z.

Ac-TPAP(PF_5CF_2Ala)AAAAK-NH_2 20



Compound **20** was synthesized using the microwave peptide synthesizer Liberty Blue 2.0 (serial number: BA001137). 300 mg of Rink amide resin (low loading, 0.087 mmol) were weighed into the reaction tube and swollen for 1 h in DMF. Cleavage of N-terminal protecting groups was conducted by adding piperidine:DMF (20:80) for 1 minute at 90°C. Coupling steps for canonical amino acids were performed at 90 °C for 2 minutes, Fmoc-PF_5CF_2Ala-OH was coupled at 75 °C for 20 minutes. All incoming amino acids after proline were coupled twice. In the last step, the peptide was acetylated by adding an acetic anhydride:pyridine (50:50) mixture (1 mL in 2 mL DMF) for 1 hour.

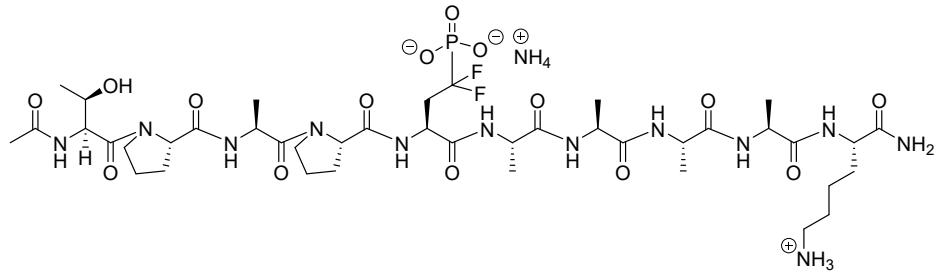
Table S4. Reagents used for the individual coupling steps in the order of addition.

Fmoc-L-Lys(Boc)-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Ala-OH (5 eq.) (4x)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-PF_5CF_2Ala-OH (Na^+) (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Pro-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Ala-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Pro-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)
Fmoc-L-Thr(tBu)-OH (5 eq.)	DIC (5 eq.)	Oxyma (5 eq.)	DIPEA (0.5 eq.)

The cleavage was done by addition of Olah's reagent (2 mL) with 10% anisole for 1 h and the peptide was added dropwise to an ice-cold saturated sodium hydrogen carbonate solution and evaporated. Purification was done by HPLC (C18 column, 10 mM NH_4HCO_3/MeCN). The product fractions were lyophilized and the clean product was obtained as a white powder (34 mg, 36% yield).

HRMS (ESI/Q-TOF**)**: $[M]^-$ Calcd for $C_{41}H_{67}F_7N_{12}O_{12}P$ 1083.4627 Da; Found 1083.4627 m/z.

Ac-TPAP(PO_3CF_2Ala)AAAAK-NH_2 21



10 mg of compound **18** were treated with TFA:H_2O:MeCN (1:1:1, 1.5 mL) for 20 hours. All volatiles were evaporated. The crude product was purified by MPLC (C18 column, 10 mM NH_4HCO_3/MeCN). The product fractions were lyophilized and the clean product was obtained as a white powder (7.6 mg, 79% yield).

HRMS (ESI/Q-TOF**)**: $[M-H]^-$ Calcd for $C_{41}H_{69}F_2N_{12}O_{15}P$ 1038.4711 Da; Found 1038.4467 m/z.

Chiral SFC-MS

Compounds **8-rac** and **8-(+)** were analyzed on a chiral column (Agilent Poroshell Chiral-T, 4.6x150 mm, 2.7 µm particle size, part number: AG683975-603), under supercritical fluid chromatography (SFC, G4301A) conditions and subsequently identified via single quadrupole mass spectrometry (Agilent 6130A).

Settings: Isocratic pump: flow 0.3 mL/min, solvent: methanol + 3.5% aq. 10 mM NH_4Ac; pump valve cluster: flow: 1.2 mL/min, solvent A) CO_2 (pre-compressed), B) MeOH; timetable: 9 min 75% A, 25% B, 10 min 65% A 35% B, 11 min 65% A, 35% B, 12 min 90% A, 10% B; SFC BPR pressure: 150 bar, BPR temperature: 60 °C; MS ionization mode: API-ES, polarity positive, gas temperature 350 °C, drying gas: 12.0 L/min, nebulizer pressure: 35 psig, quadrupole temperature: 100 °C, VCap: 3000 V.

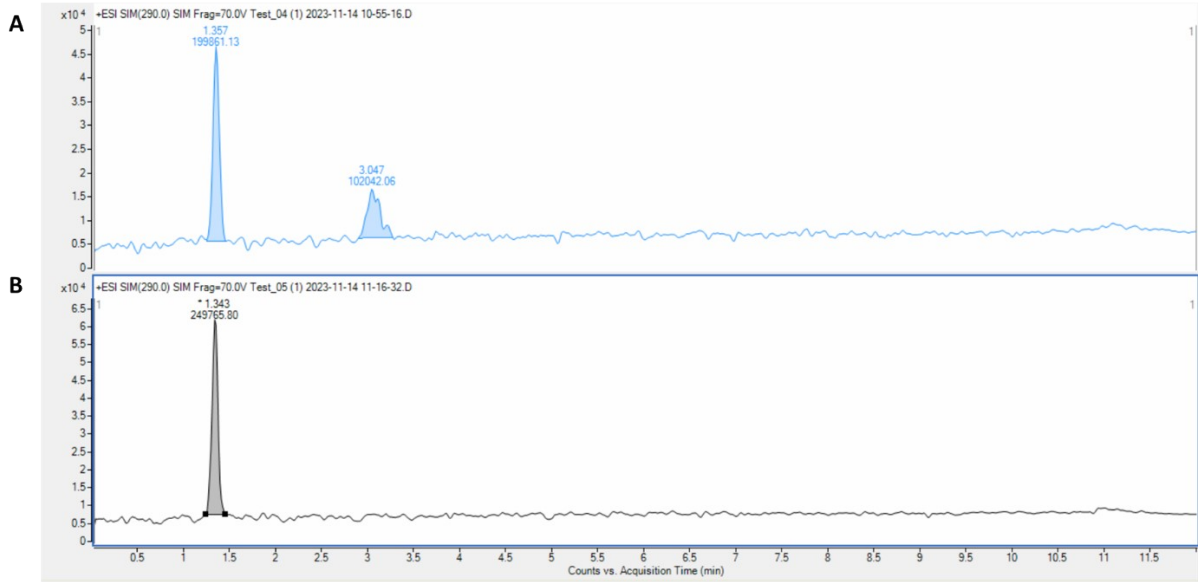


Figure S1. A) Extracted ion chromatogram (EIC) of Pd/C hydrogenated compound **8-rac** after separation on a chiral column, showing two distinct peaks with retention times of 1.357 min and peak integral of 199861.13 and 3.047 min and peak integral of 102042.06, respectively. **B)** EIC of asymmetrically hydrogenated compound **8-(+)**, showing only one enantiomer at a retention time of 1.343 min and peak integral of 249765.8.

Stability testing

Compound **11** was dissolved in DMSO at a concentration of 40 mM and the compound was diluted 1:40 (1 mM final concentration) with the respective test solvents. After the set time points, the sample was diluted 1:50 in LCMS buffer (50:50 H_2O:MeCN) and measured. Decomposition products were identified via LC-MS by their mass and quantified by the area under the curve (AUC) of the extracted ion chromatograms of individual products relative to the total AUC of all measured products.

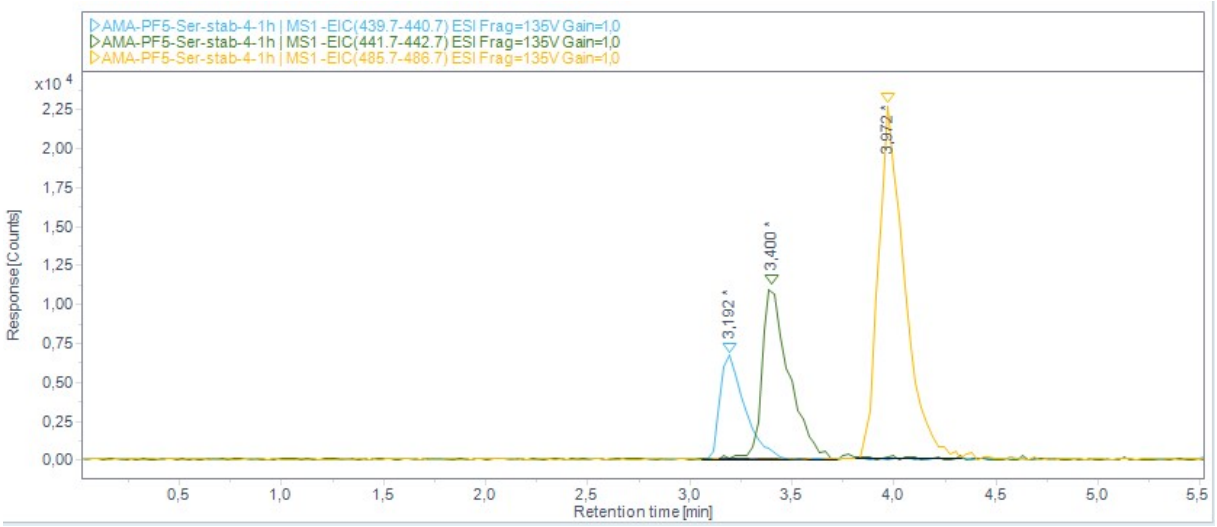


Figure S2. Overlaid extracted ion chromatograms of structures **11** (retention time: 3.972 min, m/z = 486.0), **12** (retention time: 3.400 min, m/z = 442.0) and **13** (retention time: 3.192 min, m/z = 440.0) after 1 h in TFA:H_2O (50:50).

Table S4. Stability of **11** under different conditions. No decomposition was observed in a pH range of 3-12 for 48 h or by treating **11** with hexamethyldisilazane (HMDS). Strong Brønsted and Lewis acids like TFA and TMSBr effectively cleaved the PF_5-group resulting in monofluorophosphonate, that slowly hydrolyzed to the free phosphonate.

	pH 1	pH 3	pH 7	pH 12	TFA:H_2O (50:50)	TFA:MeCN (50:50)	TMSBr in MeCN (50 eq.)	HMDS in MeCN (50 eq.)
1 h	stable	stable	stable	stable	44% decomp.	100% decomp.	1% decomp.	stable
2 h	stable	stable	stable	stable	72% decomp.	-	2% decomp.	stable
3 h	stable	stable	stable	stable	91% decomp.	-	3% decomp.	stable
24 h	5% decomp.	stable	stable	stable	100% decomp.	-	13% decomp.	stable
48 h	8% decomp.	stable	stable	stable	-	-	25% decomp.	stable

Biological activity

Fluorescence polarization assay against 14-3-3 σ

The 14-3-3 σ protein was expressed as GST-fusion protein in-house. The expression vector was purchased from MRC PPU Reagents and Services (item number DU8298) and the expression was carried out following the protocol by Li et al. in *E coli* BL21(DE3) cells.[23] After purification using glutathione agarose, 2.8 mg of purified protein were obtained from 200 mL *E. coli* suspension culture in 100 mM HEPES-NaOH pH 8.0 / 0.5 mM DTT buffer. Aliquots were stored at -80 °C.

The fluorescent peptide 5-TMR-GG-RLSHpSLPG-NH_2, with a 5-carboxytetramethylrhodamin (5-TMR) group at the N-terminus, was purchased from Biosyntan GmbH, Berlin, at 95% purity and was used without further purification.

Assay buffer: 10 mM HEPES, 150 mM NaCl, Tween-20 (0.05%), pH = 7.4; DTT (0.5 mM, added freshly prior to each measurement)

The assay was carried out in a Corning 4514 384 well plate with a total assay volume of 20 µL. Protein, tracer and inhibitors were diluted in the assay buffer.

The K_D value of the tracer was determined by incubating a dilution series of the protein with a fixed concentration of fluorescent peptide (10 nM final concentration).

Inhibitor assays were carried out by incubating the protein (final assay concentration 3 µM) with dilution series of the inhibitors (dissolved in DMSO, 5% final assay concentration) for 30 min at room temperature. After that, the tracer was added and incubated for 10 min at room temperature. Following, the plate was centrifuged and measured.

Fluorescence polarization measurements were taken on a Tecan Safire 2 microplate reader (serial number: 712001018) with the following settings: measurement mode: fluorescence polarization; excitation wavelength: 530 nm; emission wavelength: 578 nm; emission bandwidth: 20 nm; gain (manual): 57 nm; number of reads: 10; Z-position: 13200 µm; time between move and flash: 100 ms; G-factor: 1.1097.

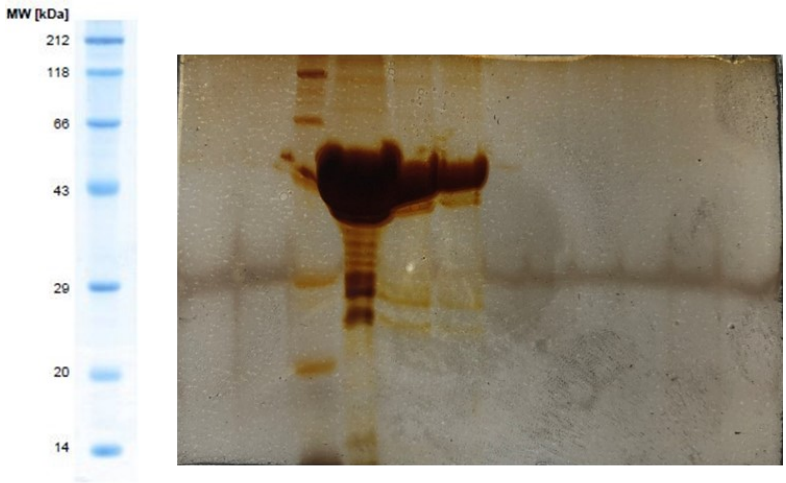


Figure S3. SDS-Page of purified fractions of GST-fused 14-3-3 σ protein (MW = 54.6 kDa).

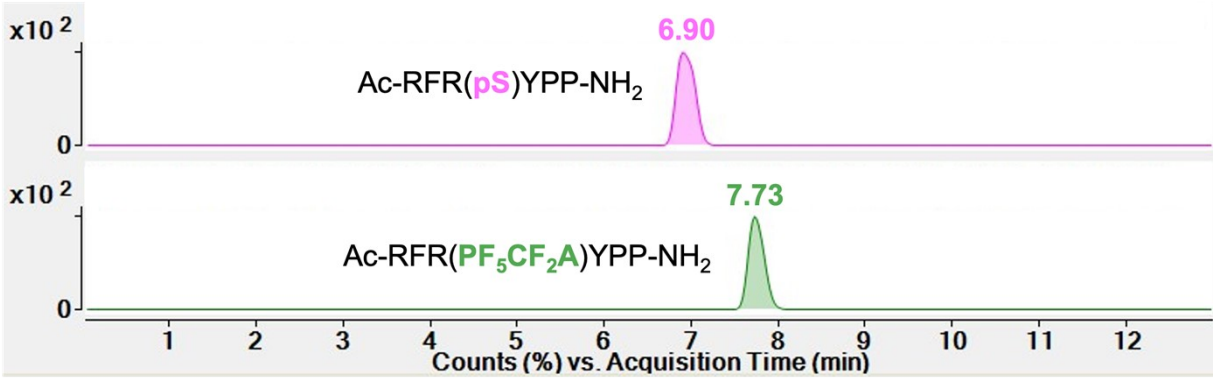


Figure S4. Extracted ion chromatograms of pS- and PF_5CF_2-Ala-peptides (**15** and **16**, respectively) in an LC-MS run with a retention time shift of Δt = 0.83 min on a C18 column, suggesting stronger hydrophobicity of the PF_5-peptide.

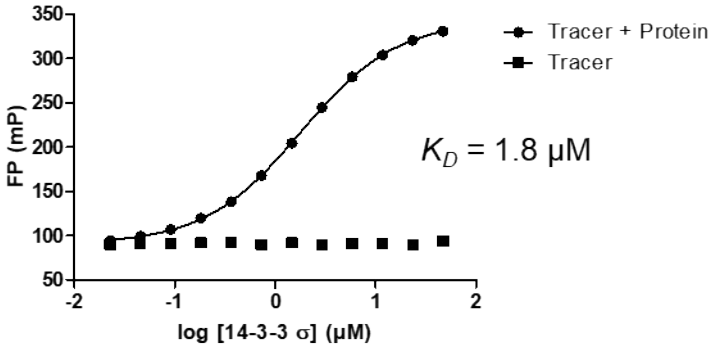


Figure S5. Determination of K_D of 5-TMR-GGRLSH-pS-LPG-NH_2 (commercial) binding to 14-3-3 σ protein.

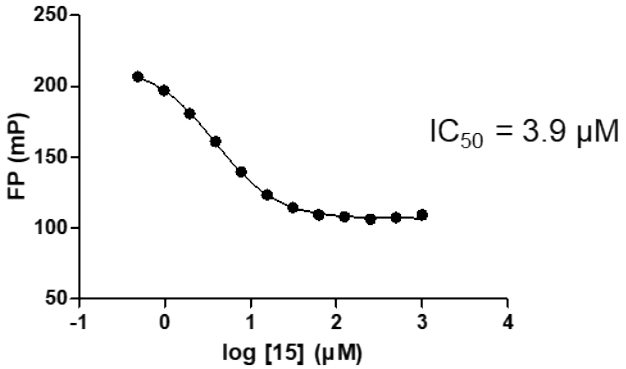


Figure S6. IC_{50} determination of pSer peptide **15** against 14-3-3 σ. A final concentration of 5% DMSO was used to avoid precipitation of the peptide. The measured IC_{50} was 3.9 µM.

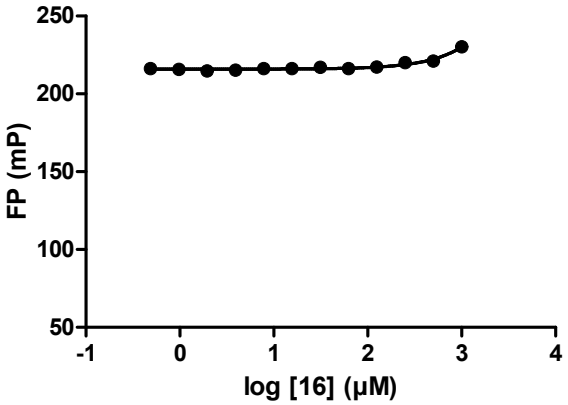


Figure S7. IC_{50} determination of PF_5CF_2Ala peptide **16** against 14-3-3 σ. A final concentration of 5% DMSO was used to avoid precipitation of the peptide. Peptide **16** did not show any binding to 14-3-3 σ in the measured concentration range.

Phosphatase assay against PPP2CA using DiFMUP as substrate

Activity testing against the catalytic domain of PPP2A was done following the protocol of Wegner et al.[24] The protein phosphatase 2A C subunit (L309 deletion mutant; human, recombinant) was purchased from Cayman Chemical and used without further purification (item number 10011237, batch number 0689188-1). Aliquots were stored at -80 °C.

Assay buffer: 50 mM Tris/HCl buffer + 0.1 mM $CaCl_2$

Enzyme solution: Enzyme in buffer (540 µL), 40 mM $NiCl_2$ in water (50 µL), 5 mg/mL bovine serine albumin in buffer (50 µL); 38 nM final enzyme assay concentration; freshly prepared prior to each experiment.

DiFMUP: 20 µL of 20 mM DMSO stock diluted with buffer to a final assay concentration of 100 µM; freshly prepared prior to each experiment.

Inhibitor: 40 mM stock solution in DMSO diluted 1:1 with buffer and serially diluted with 1:1 buffer:DMSO

The assay was carried out in a Corning 3766 plate with a total volume of 20 µL, containing 16 µL enzyme solution, 2 µL inhibitor or buffer/DMSO and 2 µL DiFMUP. The enzyme solution was preincubated with inhibitor or buffer/DMSO as control for 15 min at room temperature. For peptides **18** and **19** the incubation was conducted at 37 °C for 30 min to ensure sufficient solubility and reaction time. Following, the substrate DiFMUP was added and incubated for 30 seconds. After centrifugation the measurement was conducted at 37 °C for 10 min on an infinite M1000 pro plate reader (serial number: 1404007767) with the following settings: kinetic cycle, kinetic interval: 00:01:00, excitation wavelength: 360 nm, excitation bandwith: 20 nm, emission wavelength: 460 nm, emission bandwidth: 20 nm, lag time: 0 µs, integration time: 40 µs, gain: manual, gain value: 60, Z-position: manual, Z-position height: 24000 µm. Each data point was measured in triplicate.

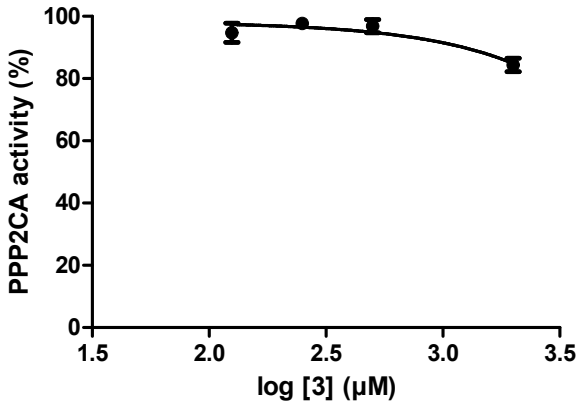


Figure S8. Incubation of amino acid PF_5CF_2Ala **3** (Na^+) with PPP2CA did not lead to a significant inhibition of the phosphatase up to 2 mM.

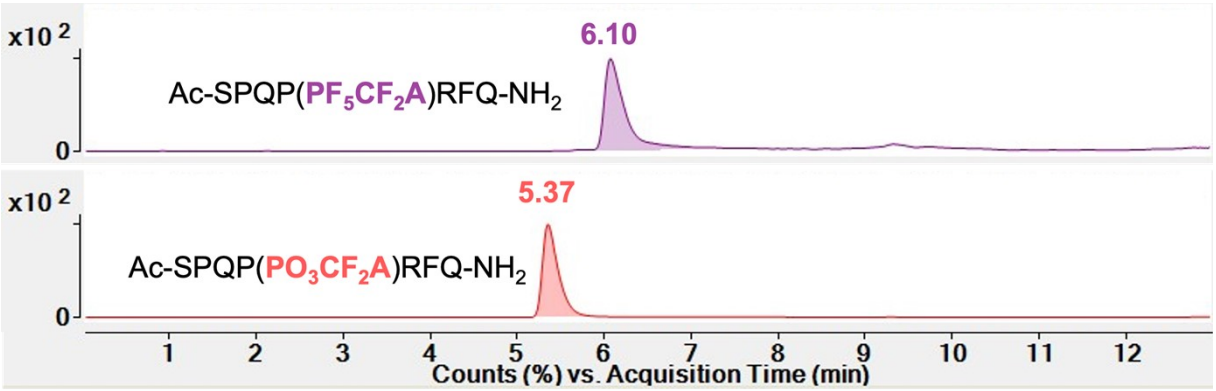


Figure S9. Extracted ion chromatograms of PO_3CF_2- and PF_5CF_2-Ala-peptides (**18** and **19**, respectively) in an LC-MS run with a retention time shift of $\Delta t = 0.73$ min on a C18 column, suggesting stronger hydrophobicity of the PF_5-peptide.

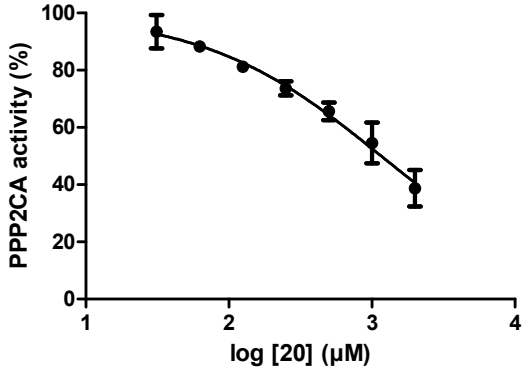


Figure S10. Incubation of peptide Ac-TPAP(PF_5CF_2Ala)AAAAK-NH_2 (**20**) with PPP2CA led to a concentration-dependent inhibition of the phosphatase with an approximated IC_{50} of 1000 µM.

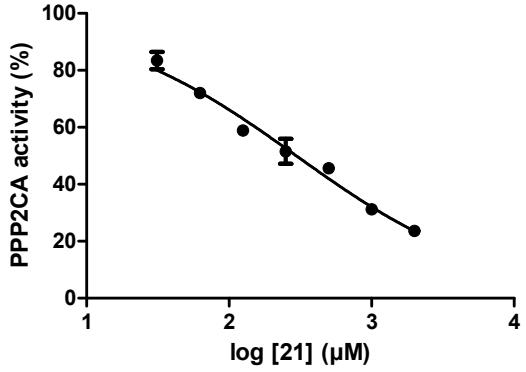


Figure S11. Incubation of peptide Ac-TPAP(PO_3CF_2Ala)AAAAK-NH_2 (**21**) with PPP2CA led to a concentration-dependent inhibition of the phosphatase with an approximated IC_{50} of 300 µM.

Molecular docking experiments

The protein structure with PDB entry 2IE4 was obtained from the Protein Data Bank (https://www.rcsb.org/). The scaffolding subunit was removed, as it binds to the opposite side of the active site relative to the catalytic subunit used for docking studies. Steric clashes in the catalytic subunit were resolved by energy minimization of the affected residues using the AmberEHT force field. Structural warnings and issues were addressed using the Structure Preparation tool, and water molecules were removed. All preparation steps were performed in MOE (v2024.0601, Molecular Operating Environment, Chemical Computing Group). The catalytic subunit was subsequently protonated at pH 7.4 using the Protonate 3D algorithm in MOE.[25]

All ligands were constructed in MOE using SMILES notation as input in the molecule builder. Appropriate formal charges were assigned to the PF_5 and PO_3 moieties using the Atom Manager, and the structures were energy-minimized using the Amber10 force field.

Molecular docking was performed using GOLD (v2020, Genetic Optimization for Ligand Docking, Cambridge Crystallographic Data Center, UK).[26] The active site was defined as a 10 Å sphere centered on the binding site of okadaic acid. For each ligand, 20 poses using diverse solutions were generated. A distance constraint to Arg214 was applied during docking of the PF_5-containing ligand. The resulting poses were visually inspected in LigandScout (v4.4.3, Inte:Ligand GmbH, Vienna, Austria)[27,28]), and 3D pharmacophore models were generated to analyze potential binding modes.

NMR spectra

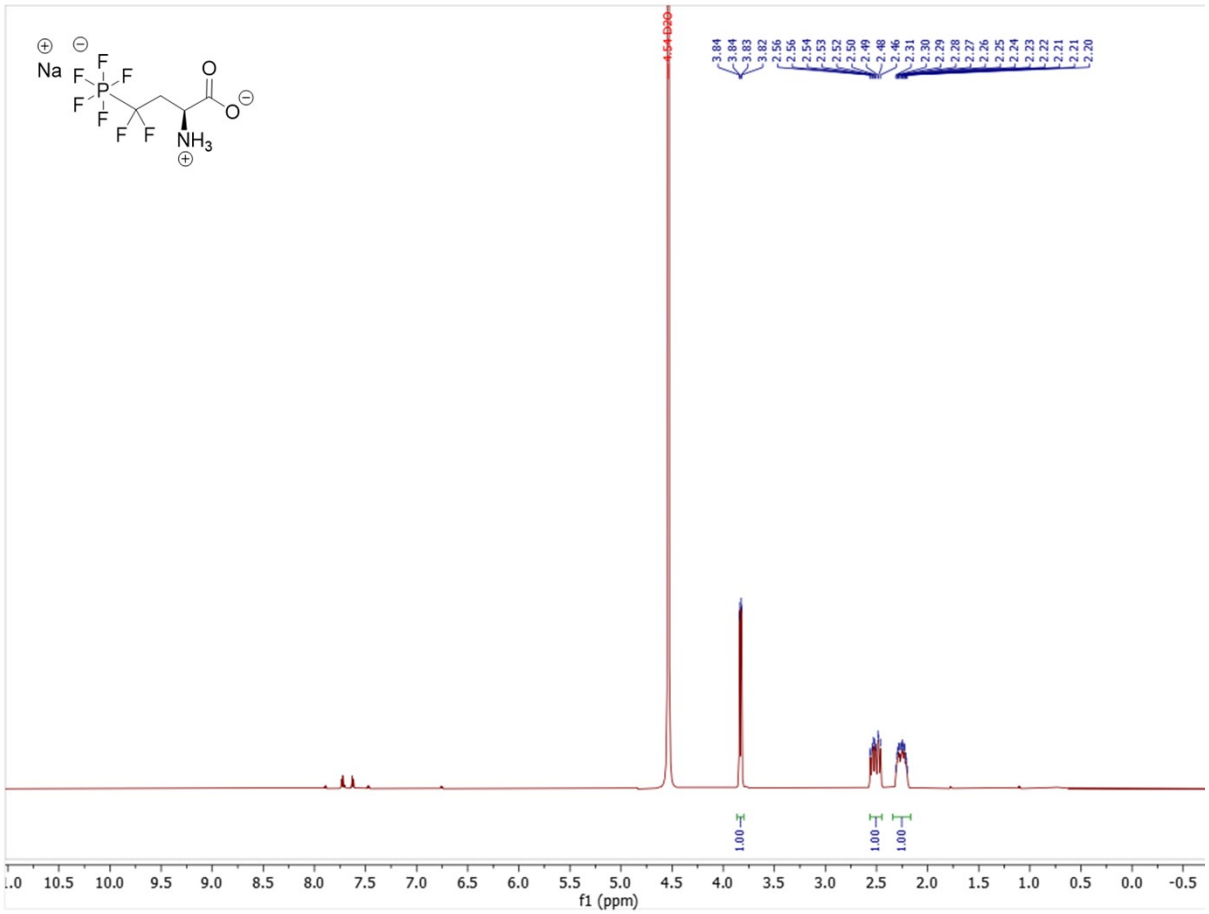


Figure S12. ^{1}H NMR (600 MHz, D_2O) of **3**

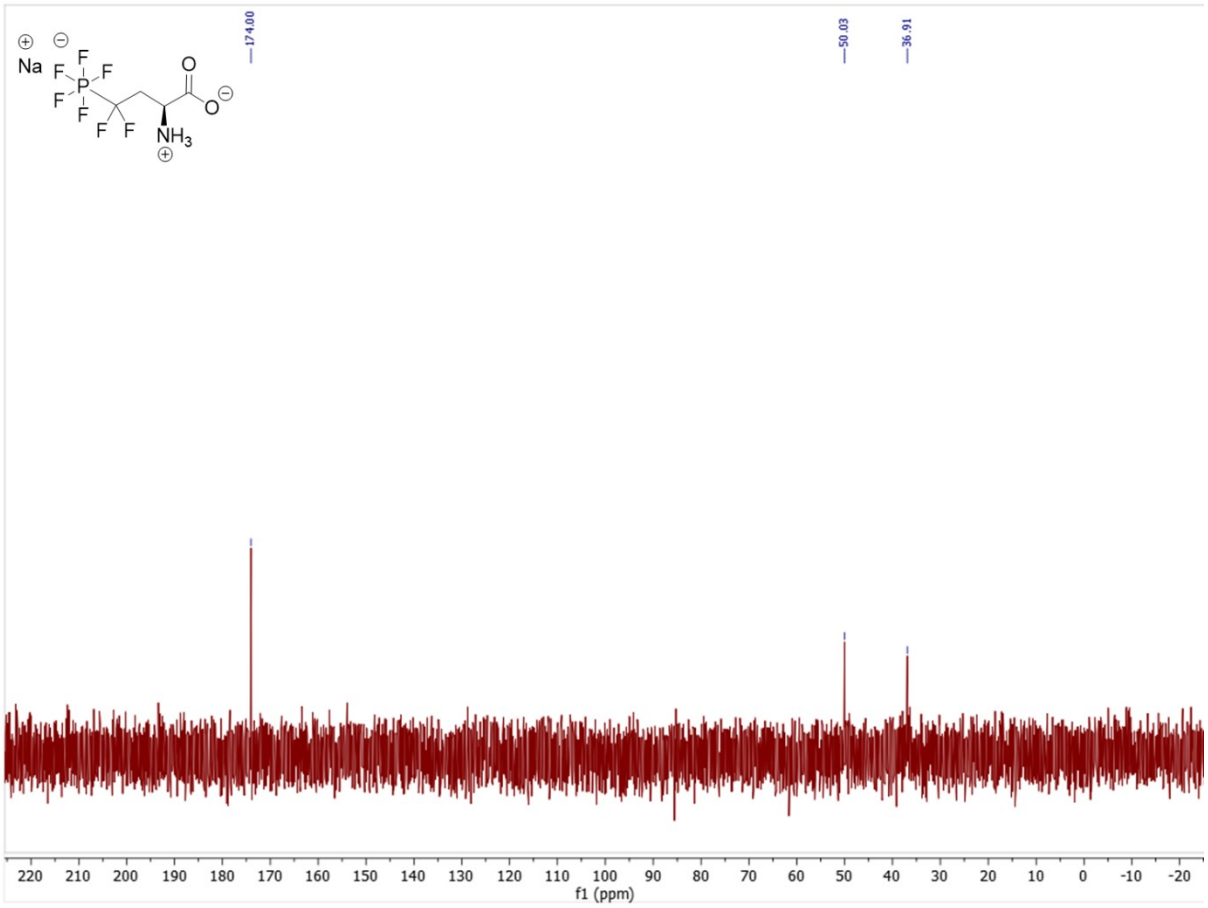


Figure S13. ^{13}C NMR (151 MHz, D_2O) of **3**

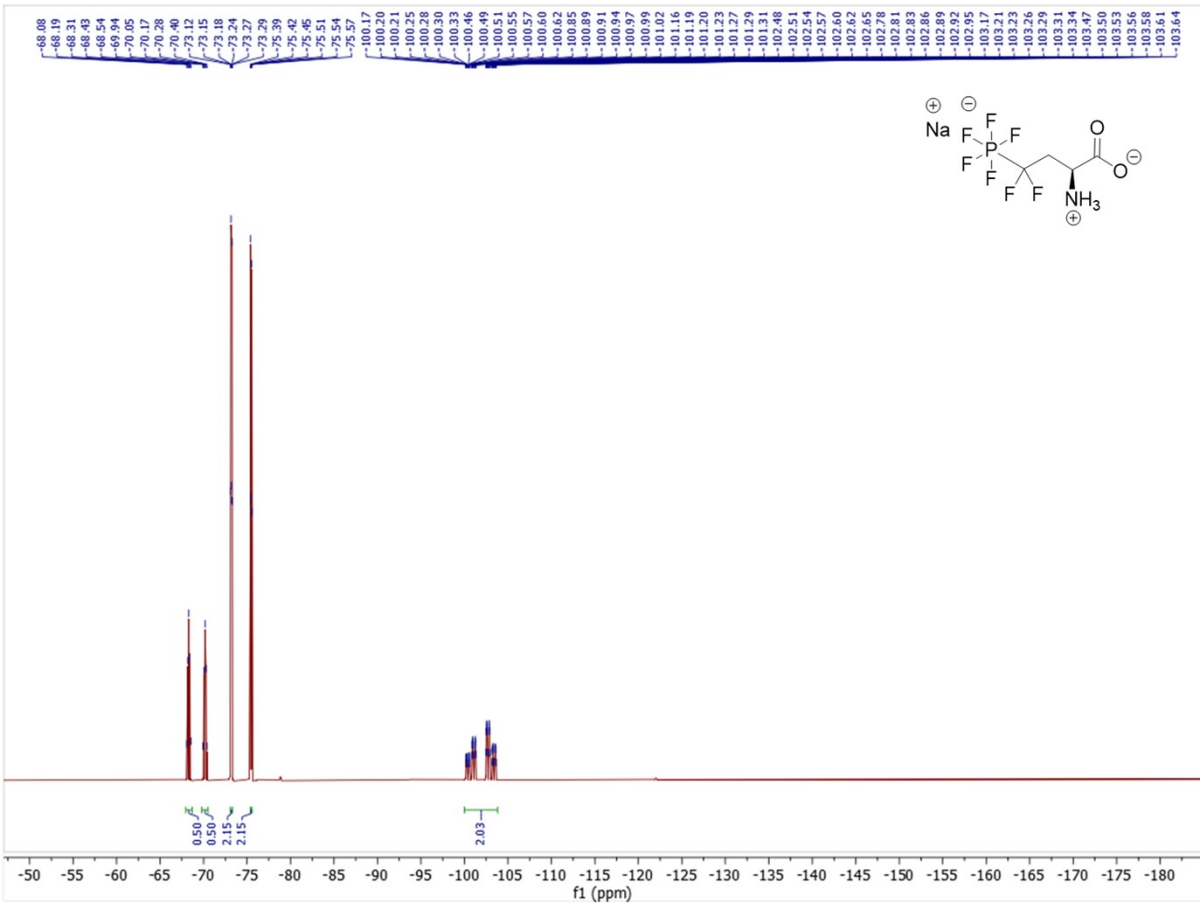


Figure S14. ^{19}F NMR (376 MHz, D_2O) of **3**

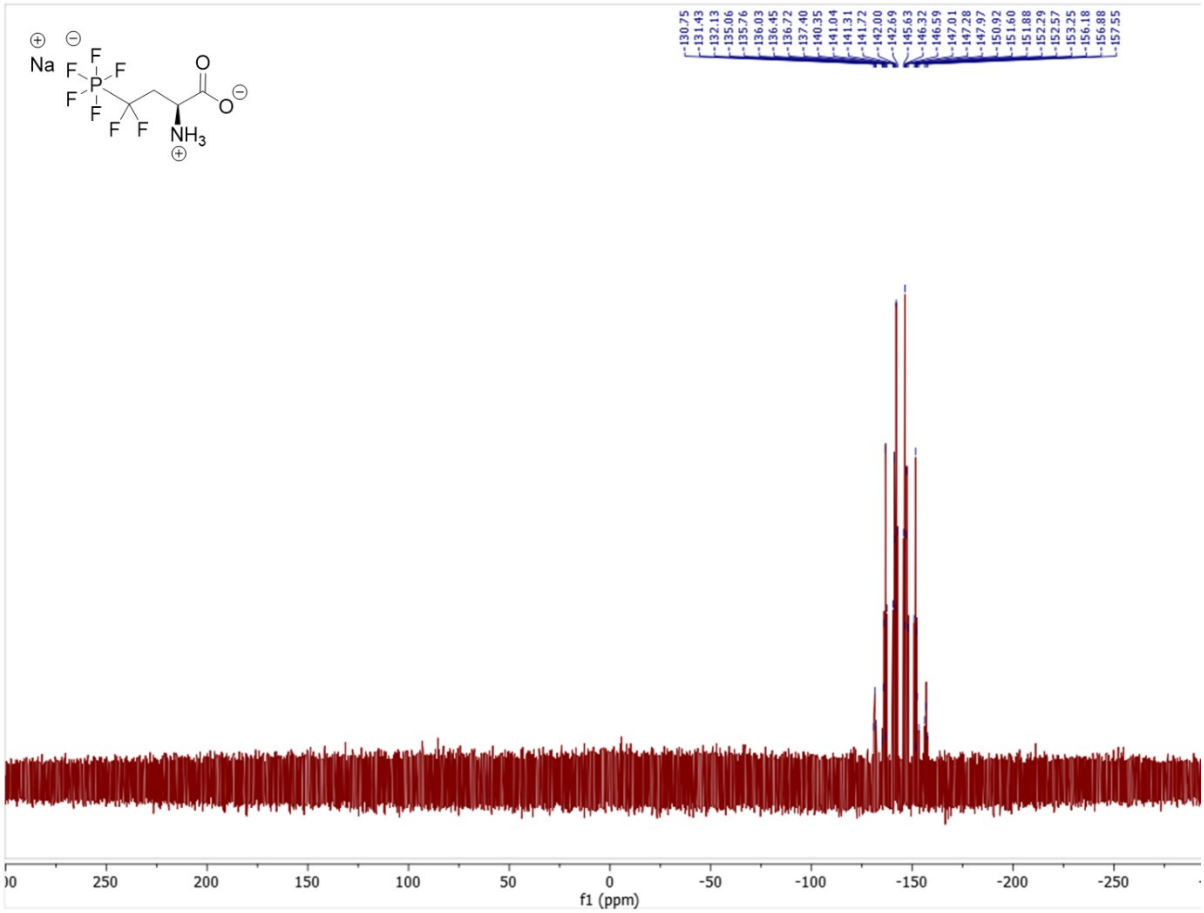


Figure S15. ^{31}P NMR (376 MHz, D_2O) of **3**

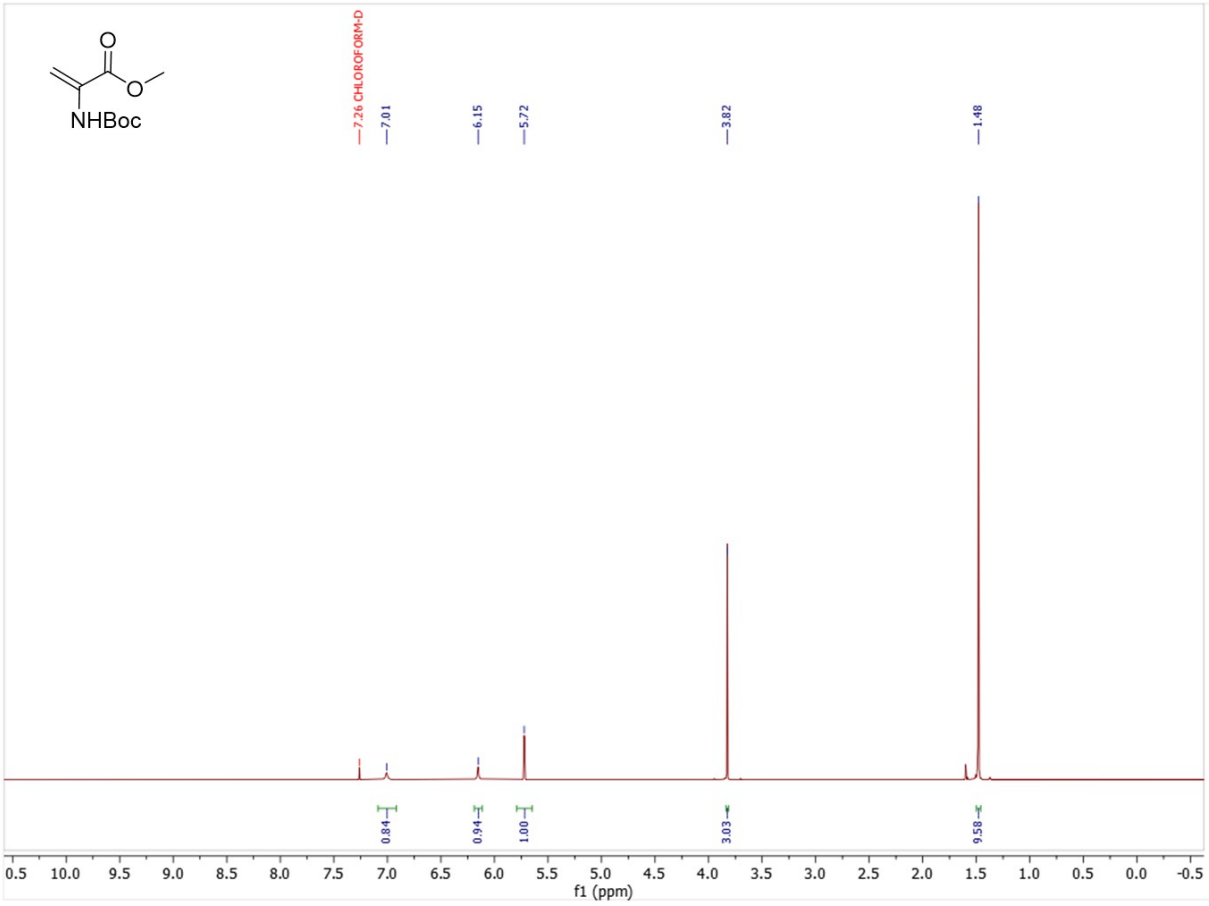


Figure S16. ^{1}H NMR (600 MHz, $CDCl_3$) of **5**

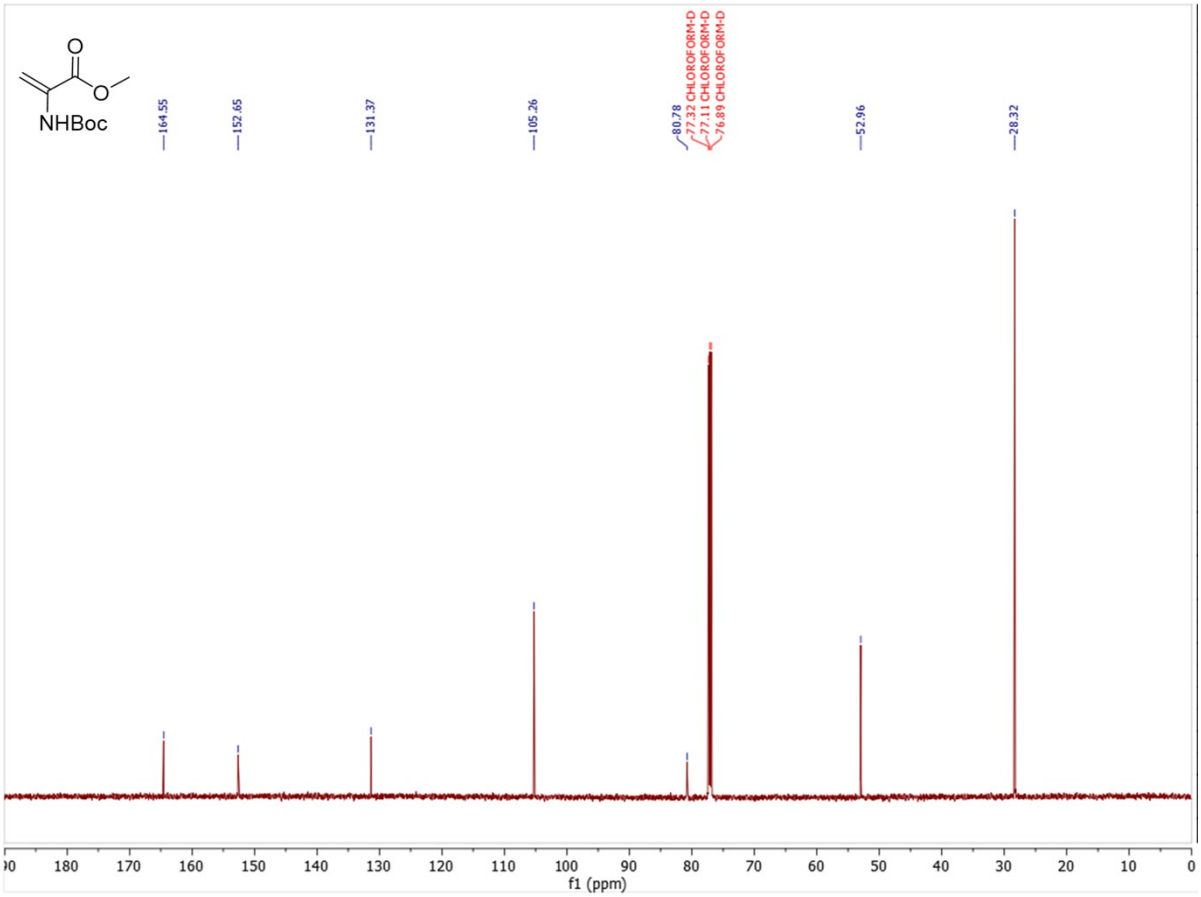


Figure S17. ^{13}C NMR (151 MHz, $CDCl_3$) of **5**

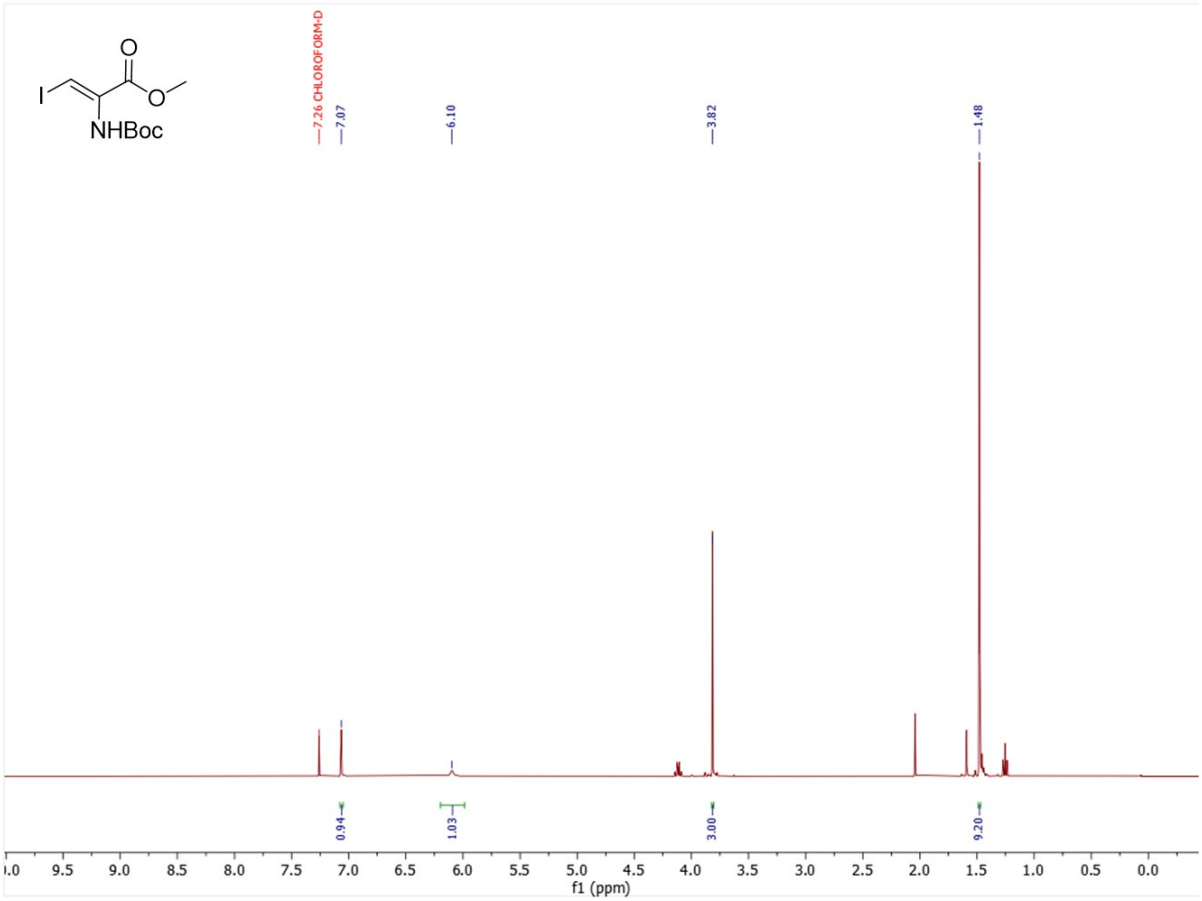


Figure S18. ^{1}H NMR (600 MHz, $CDCl_3$) of **6**

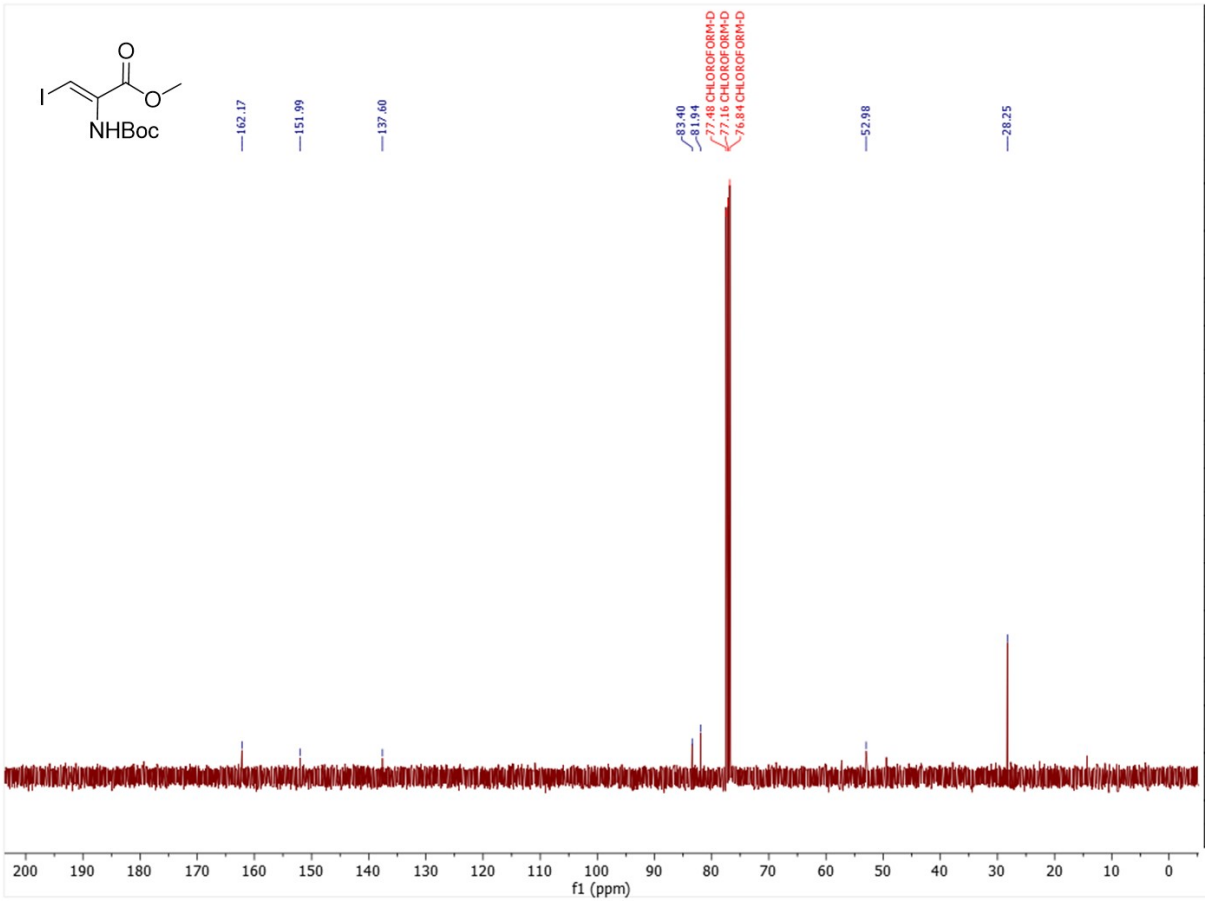


Figure S19. ^{13}C NMR (151 MHz, $CDCl_3$) of **6**

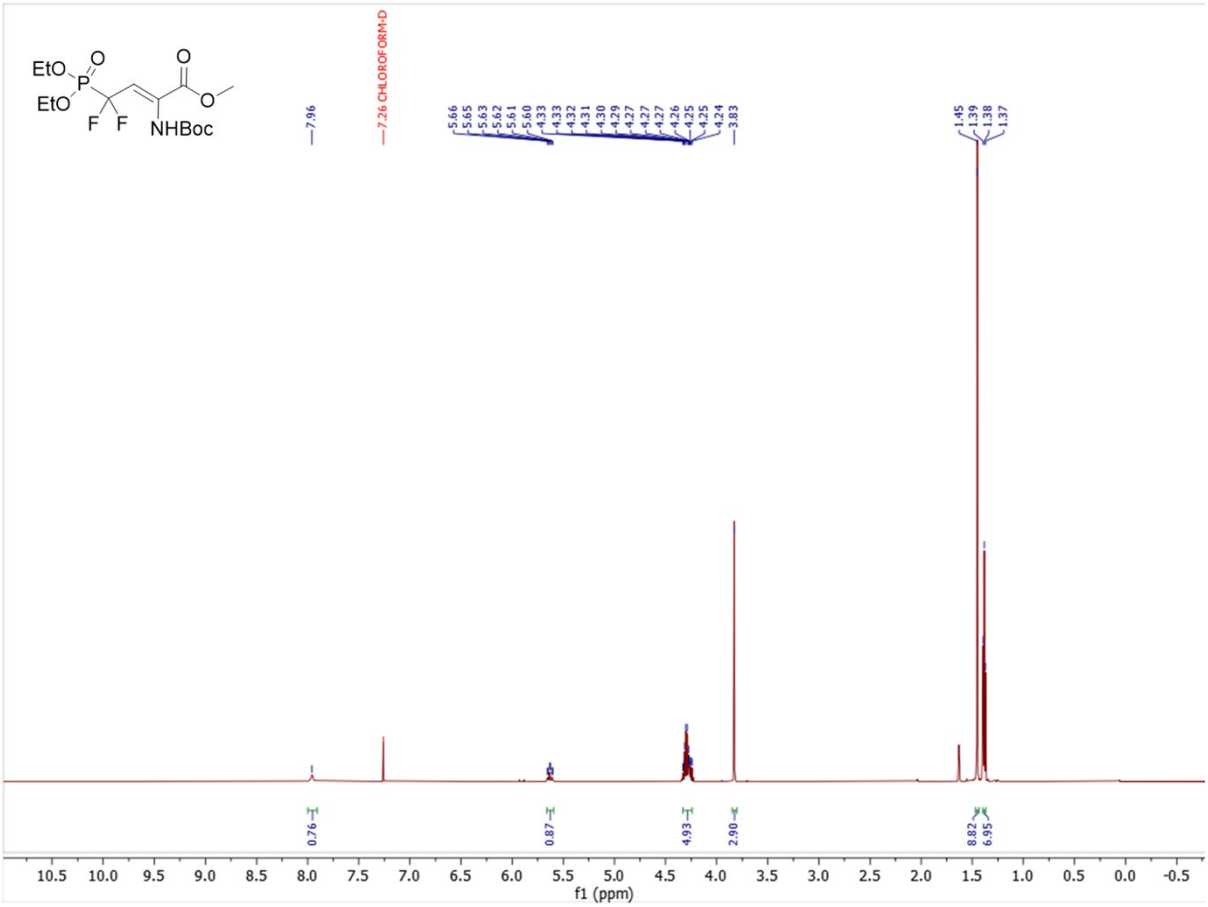


Figure S20. ^{1}H NMR (600 MHz, $CDCl_3$) of **7**

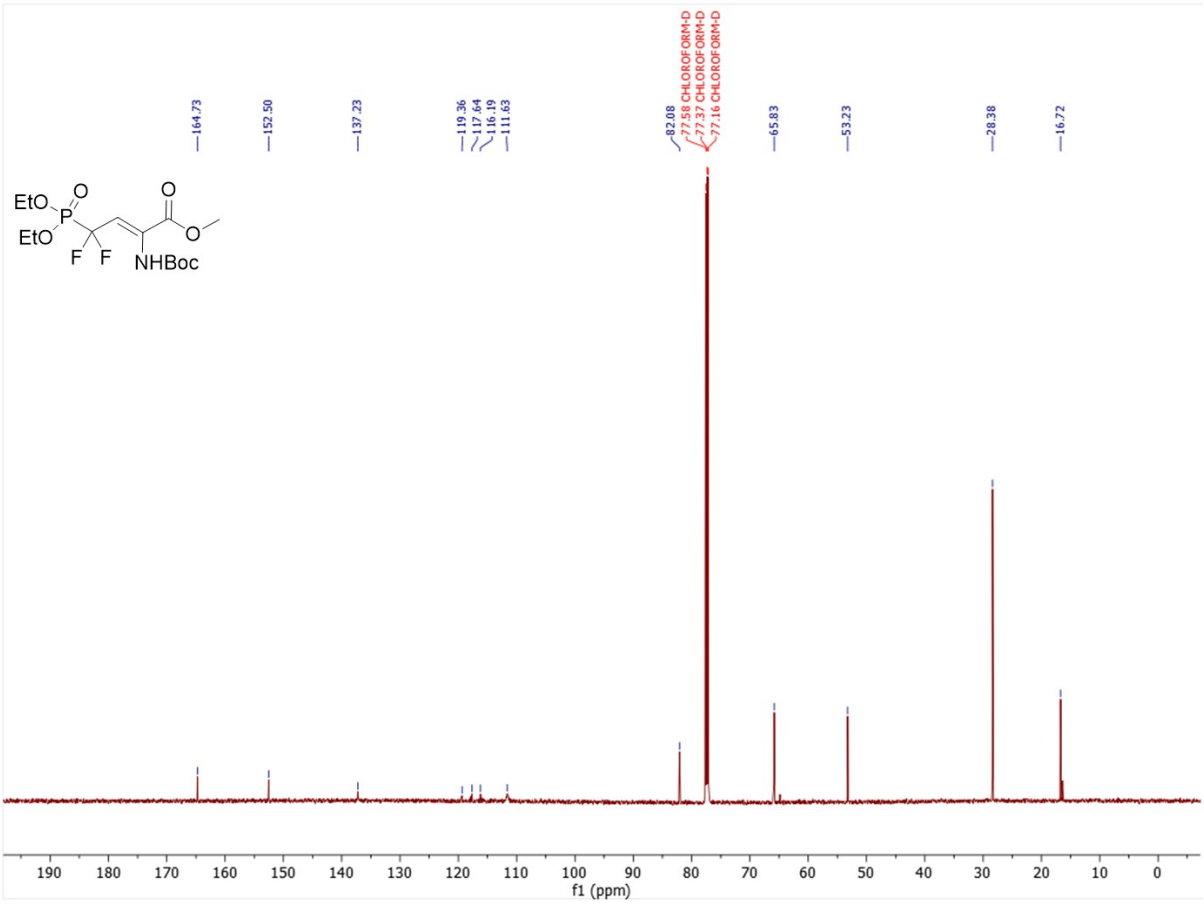


Figure S21. ^{13}C NMR (151 MHz, $CDCl_3$) of **7**

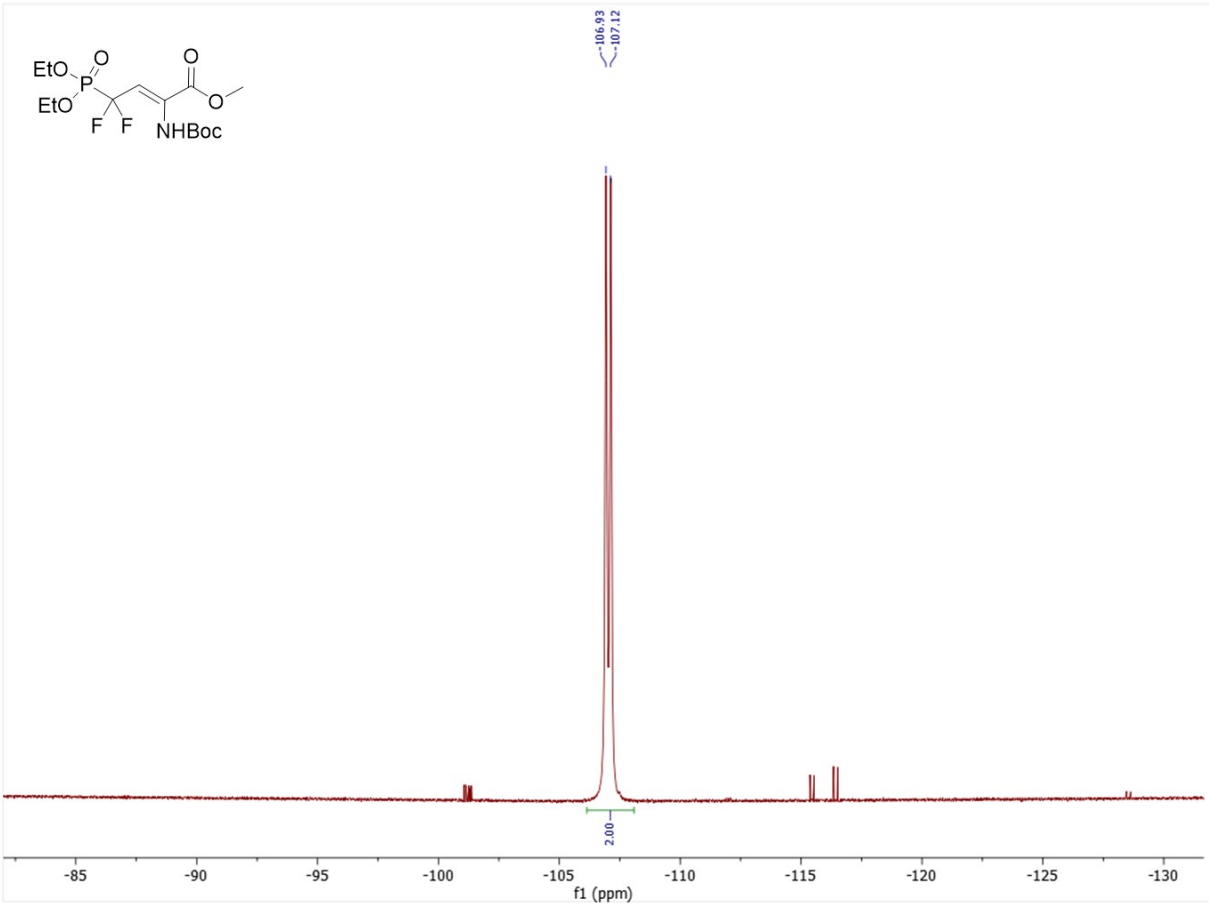


Figure S22. ^{19}F NMR (565 MHz, $CDCl_3$) of **7**

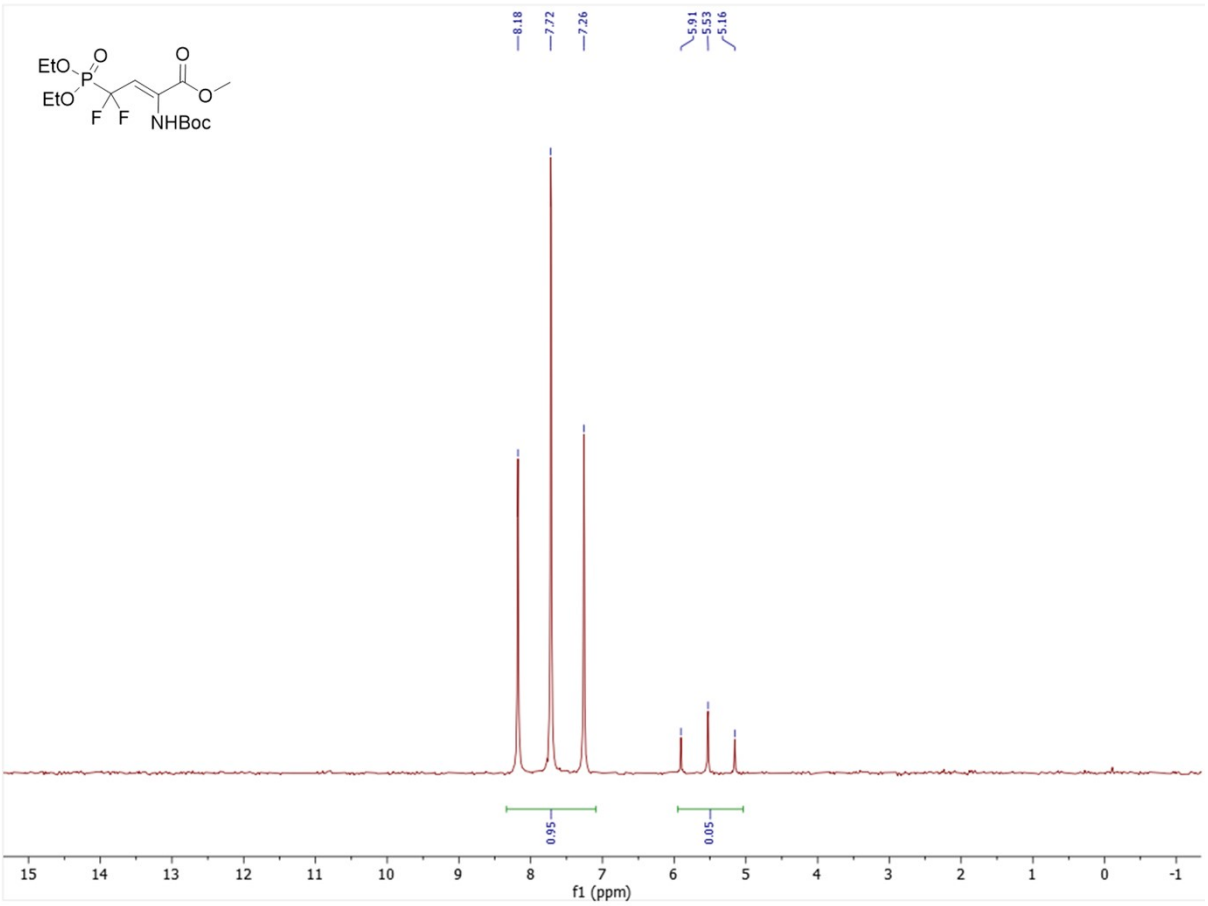


Figure S23. ^{31}P NMR (243 MHz, $CDCl_3$) of **7**

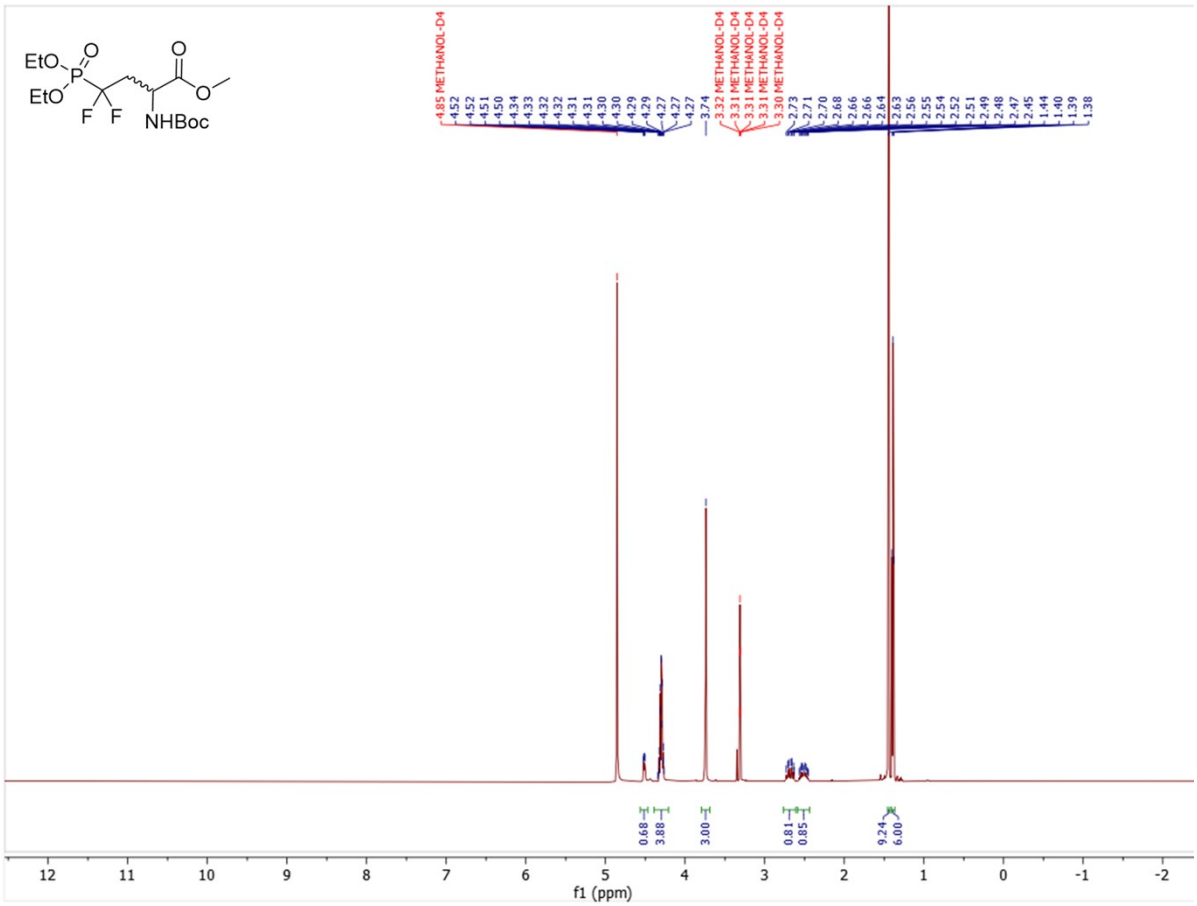


Figure S24. ^{1}H NMR (600 MHz, MeOD-D_4) of **8-rac**

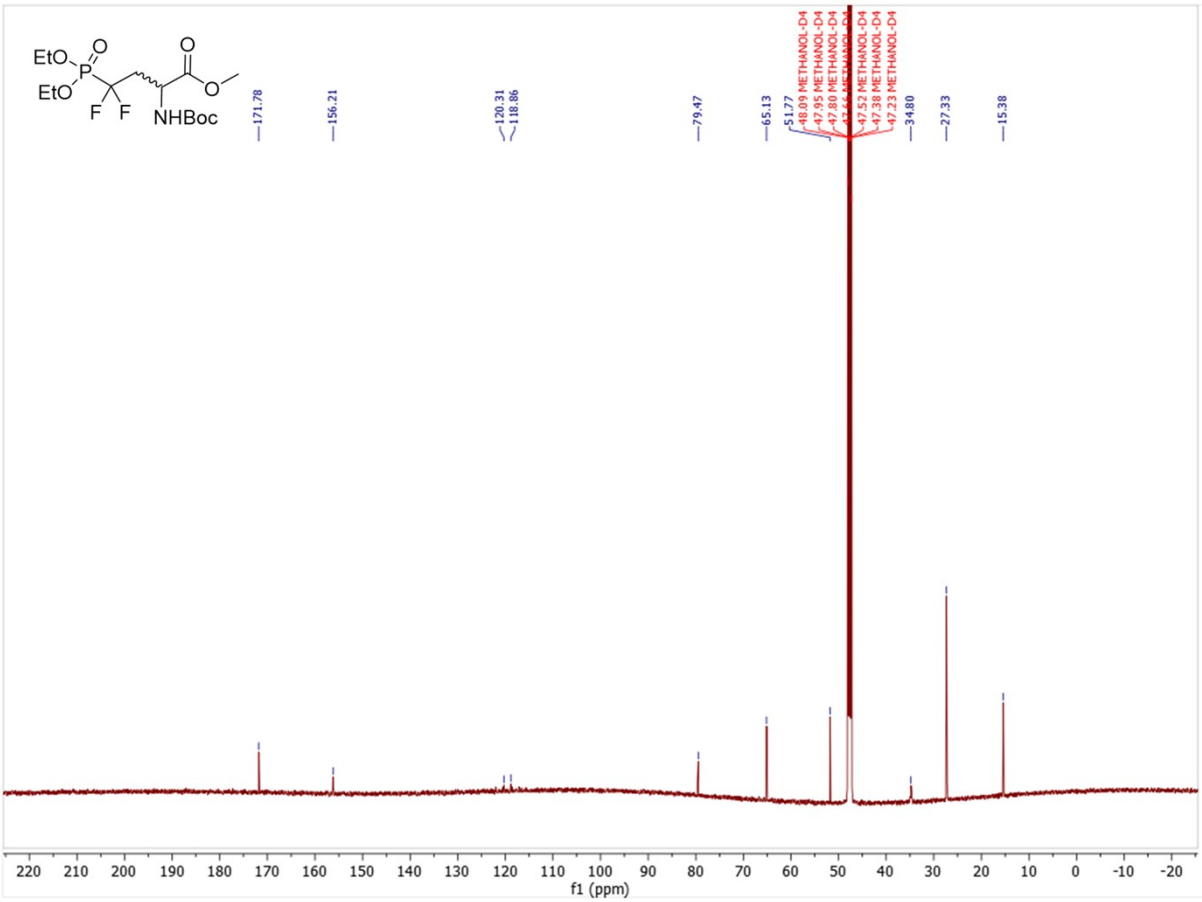


Figure S25. ^{13}C NMR (151 MHz, MeOD-D_4) of **8-rac**

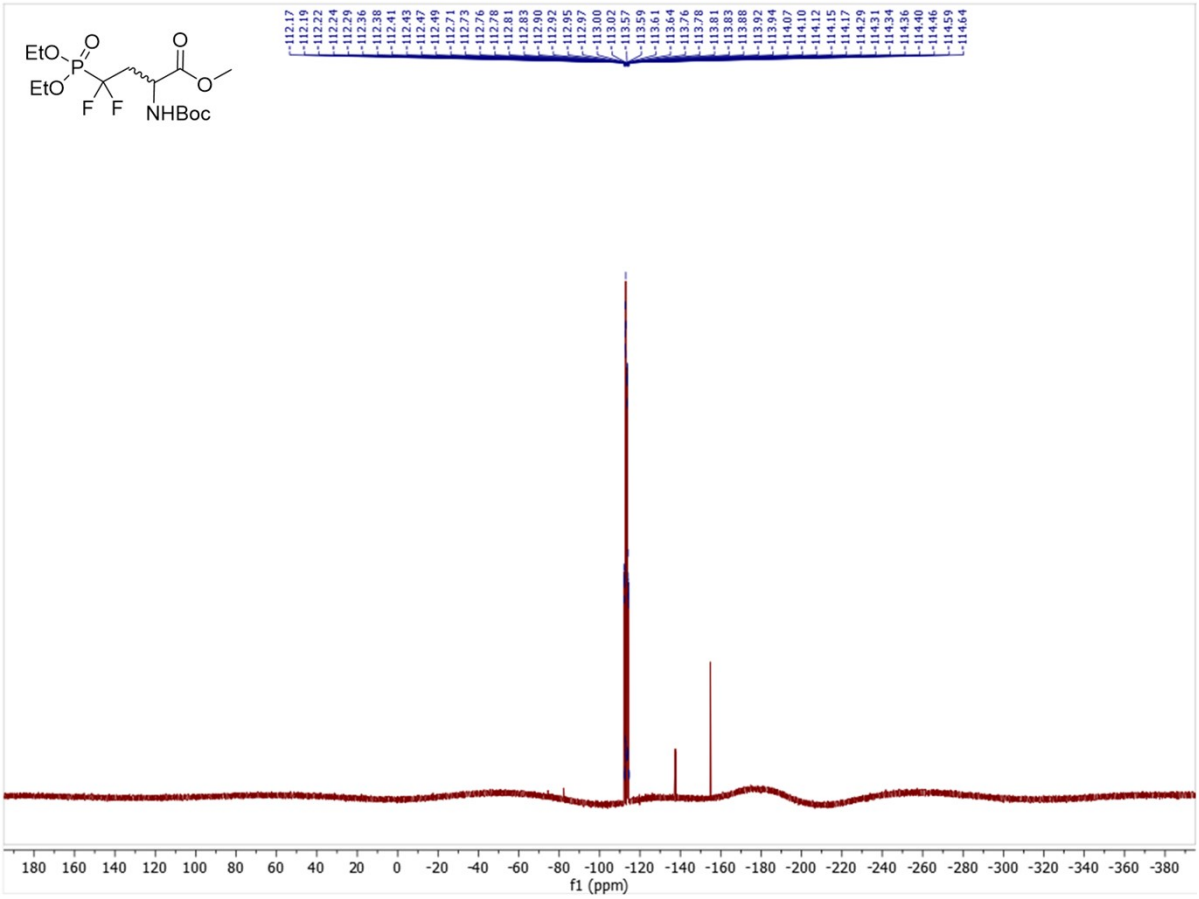


Figure S26. ^{19}F NMR (565 MHz, MeOD-D_4) of **8-rac**

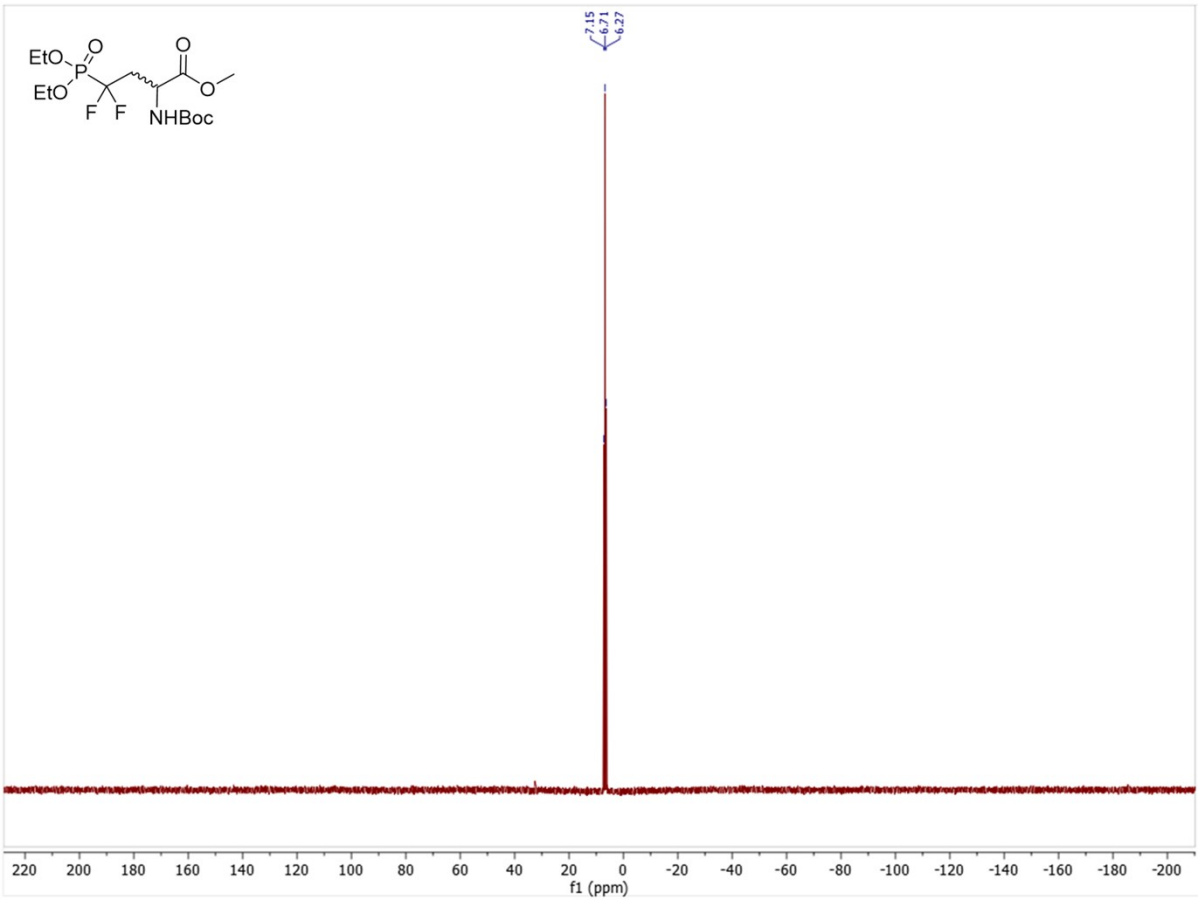


Figure S27. ^{31}P NMR (243 MHz, MeOD-D_4) of **8-rac**

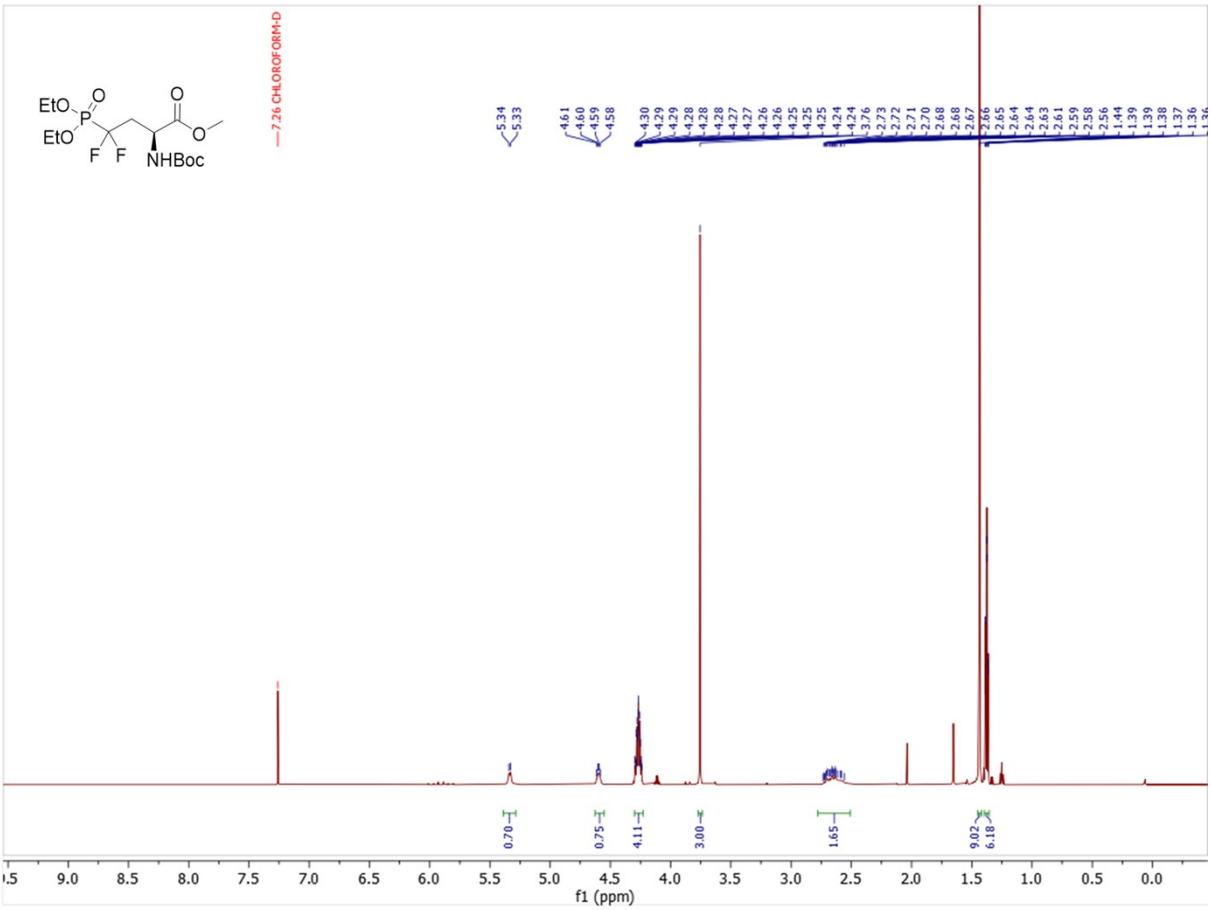


Figure S28. ^{1}H NMR (600 MHz, $CDCl_3$) of **8-(+)**

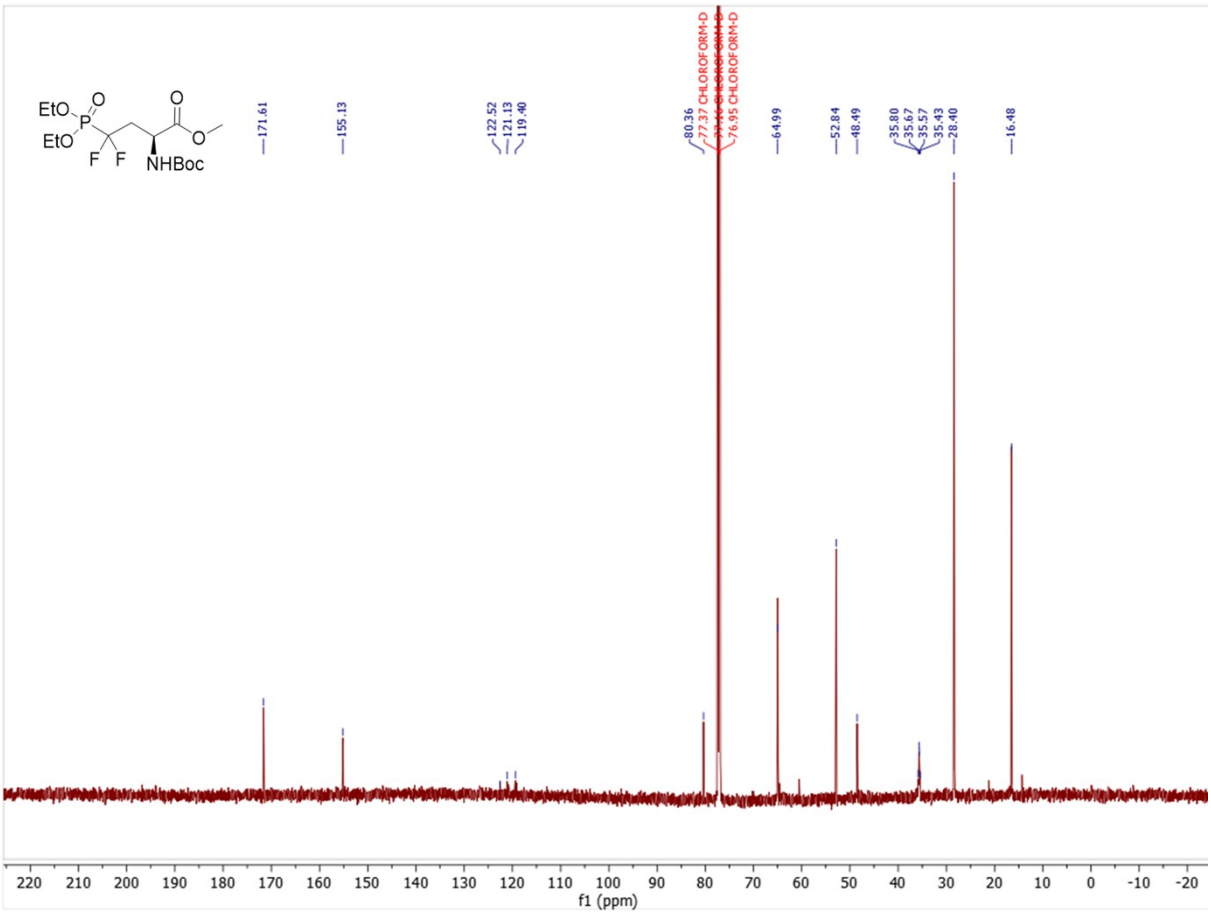


Figure S29. ^{13}C NMR (151 MHz, $CDCl_3$) of **8-(+)**

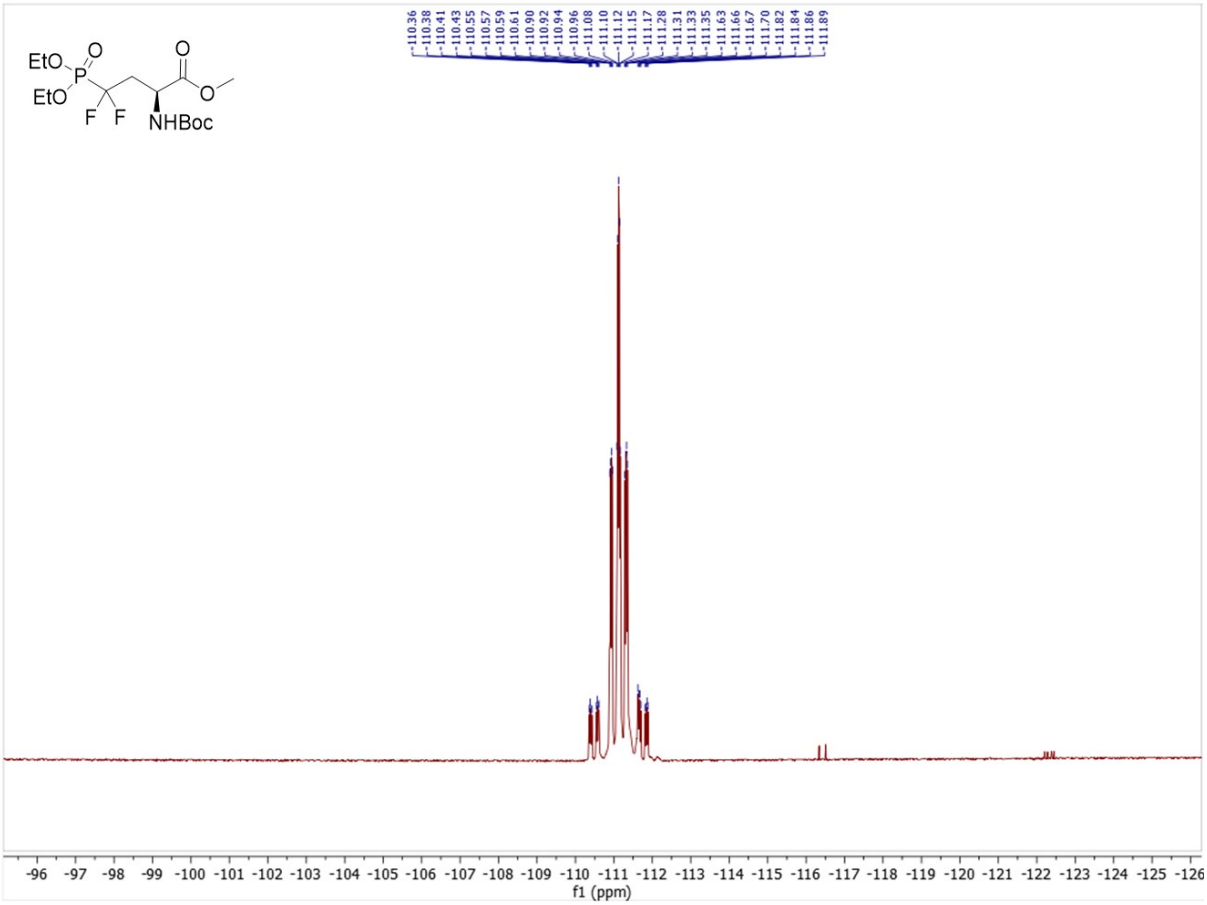


Figure S30. ^{19}F NMR (565 MHz, $CDCl_3$) of **8-(+)**

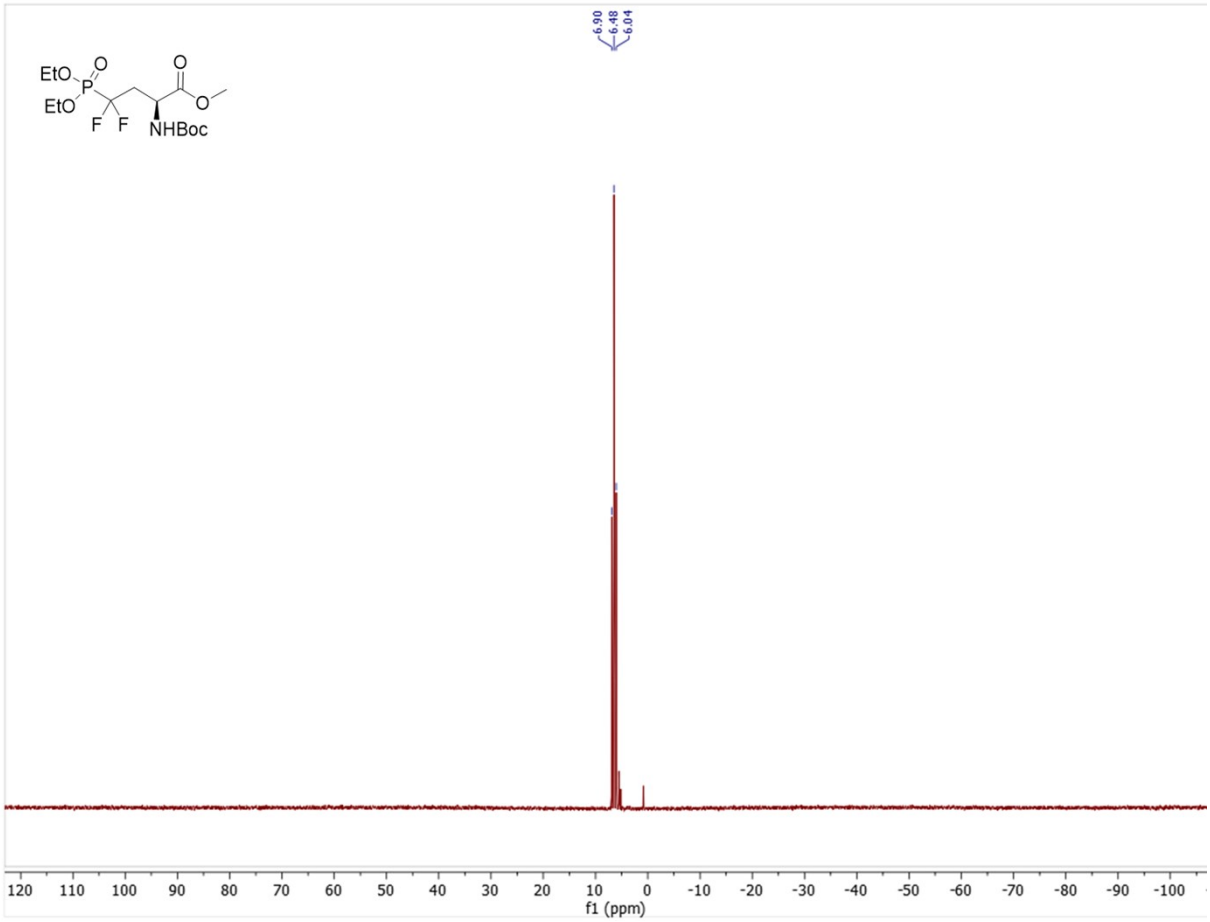


Figure S31. ^{31}P NMR (243 MHz, $CDCl_3$) of **8-(+)**

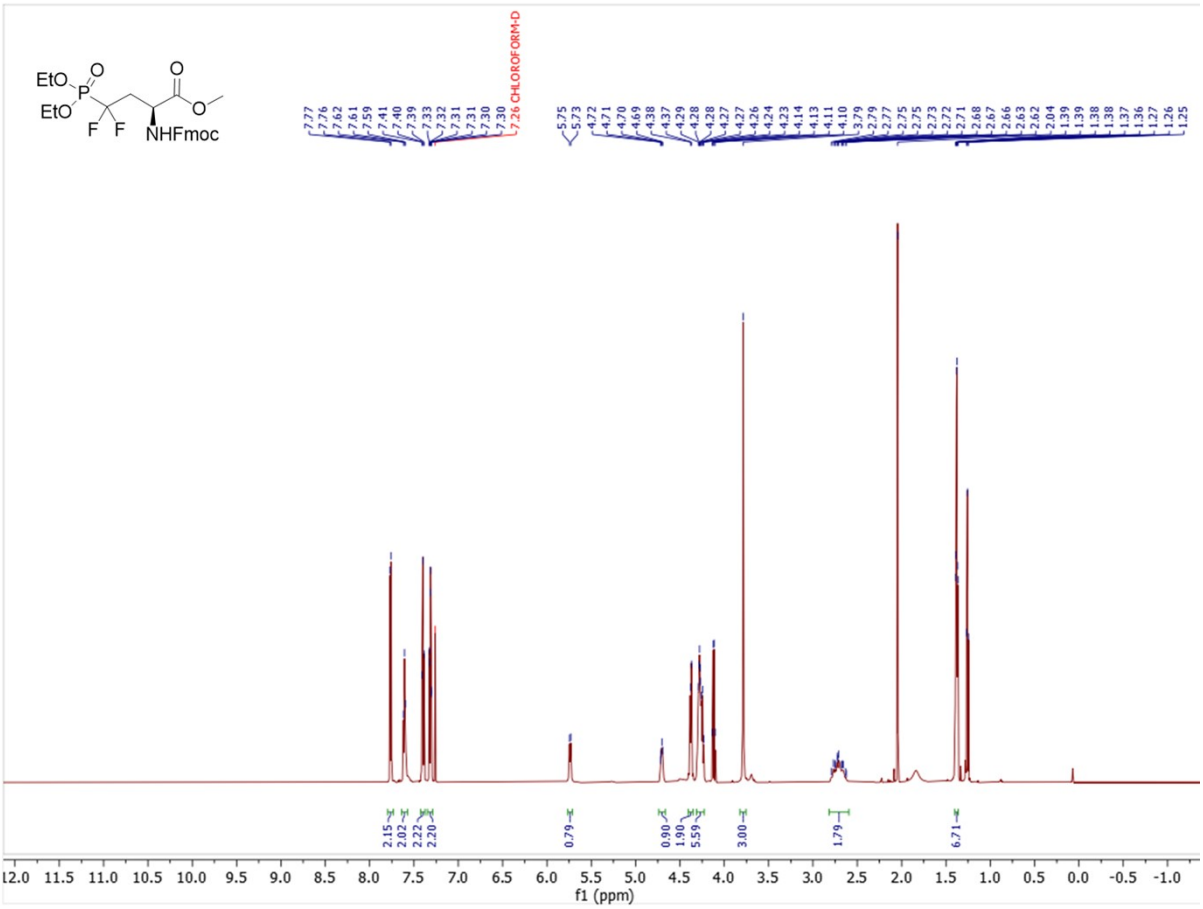


Figure S32. ^{1}H NMR (600 MHz, $CDCl_3$) of **9**

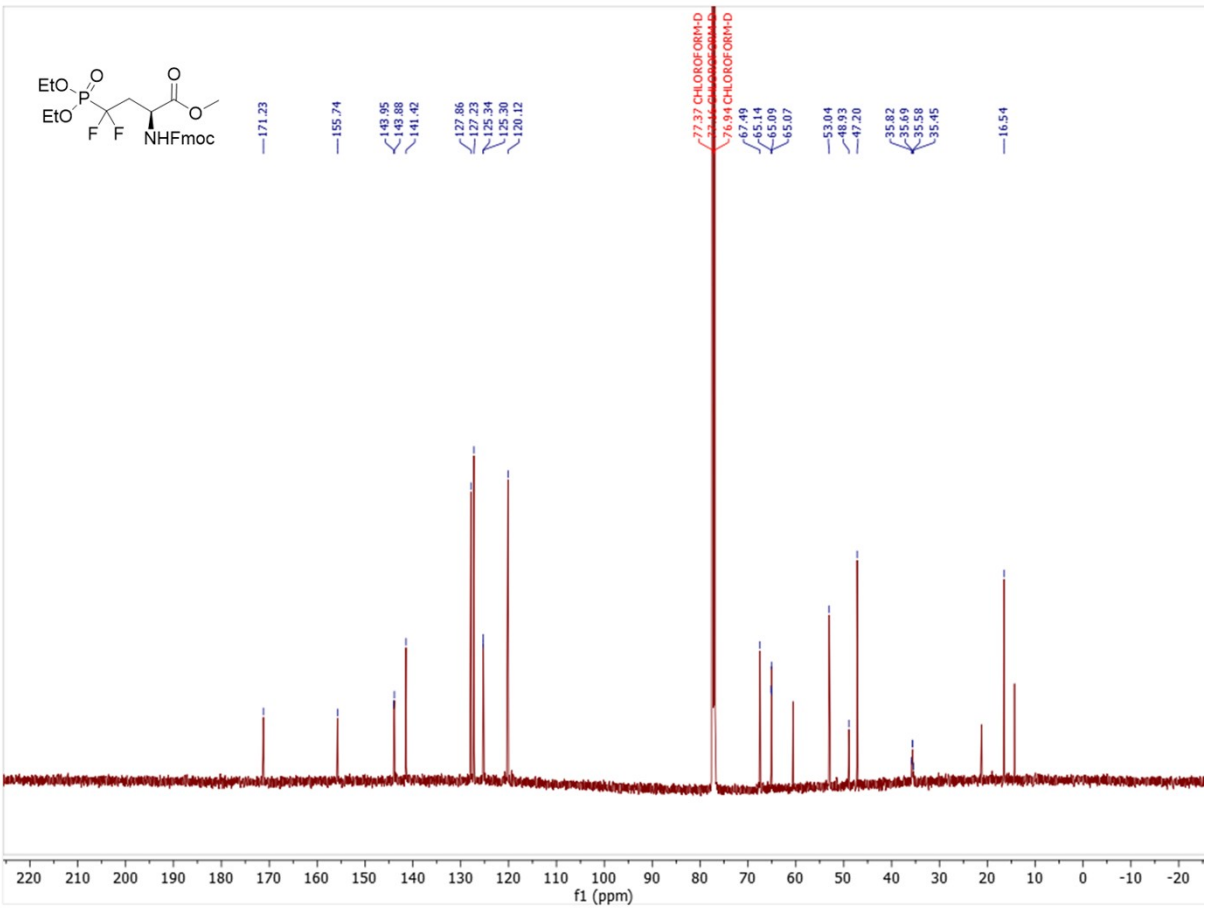


Figure S33. ^{13}C NMR (151 MHz, $CDCl_3$) of **9**

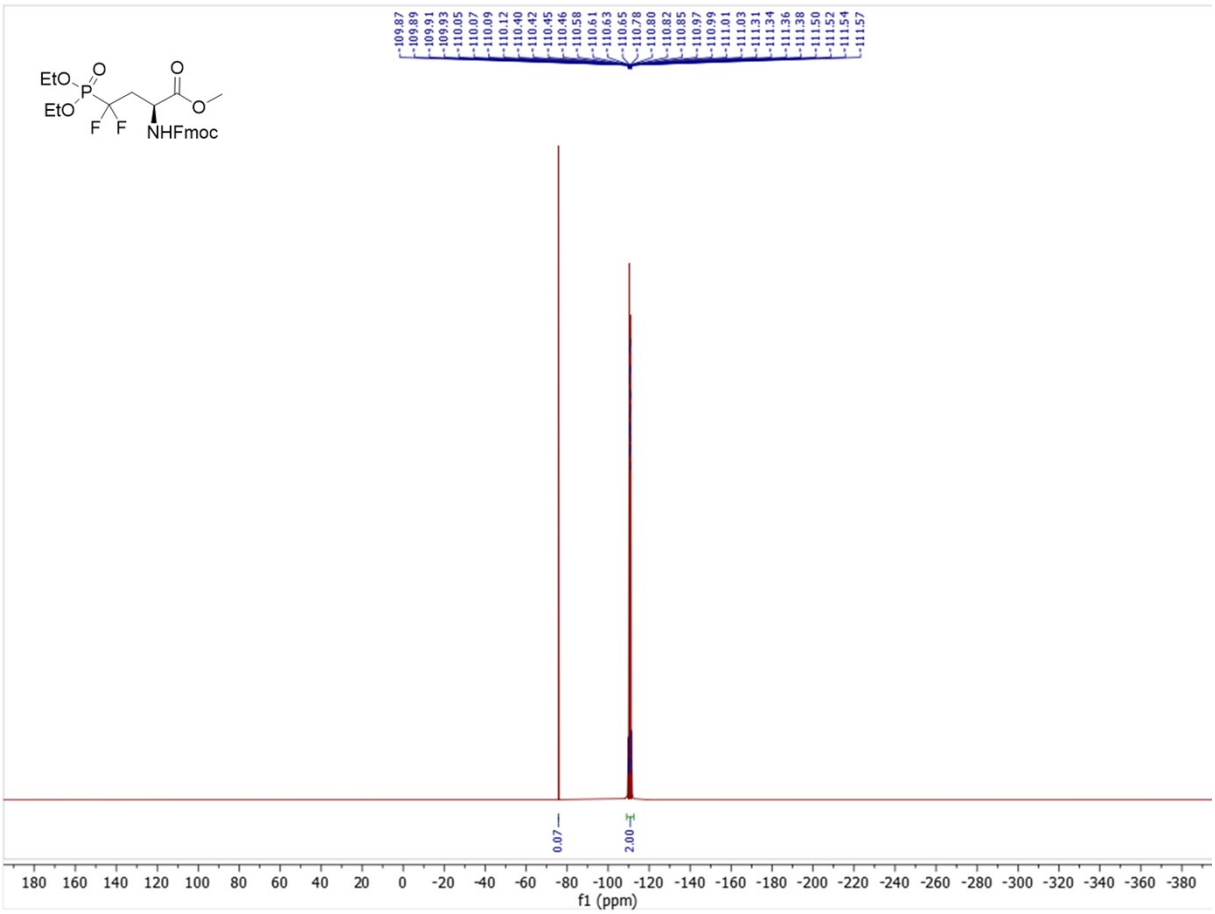


Figure S34. ^{19}F NMR (565 MHz, $CDCl_3$) of **9**

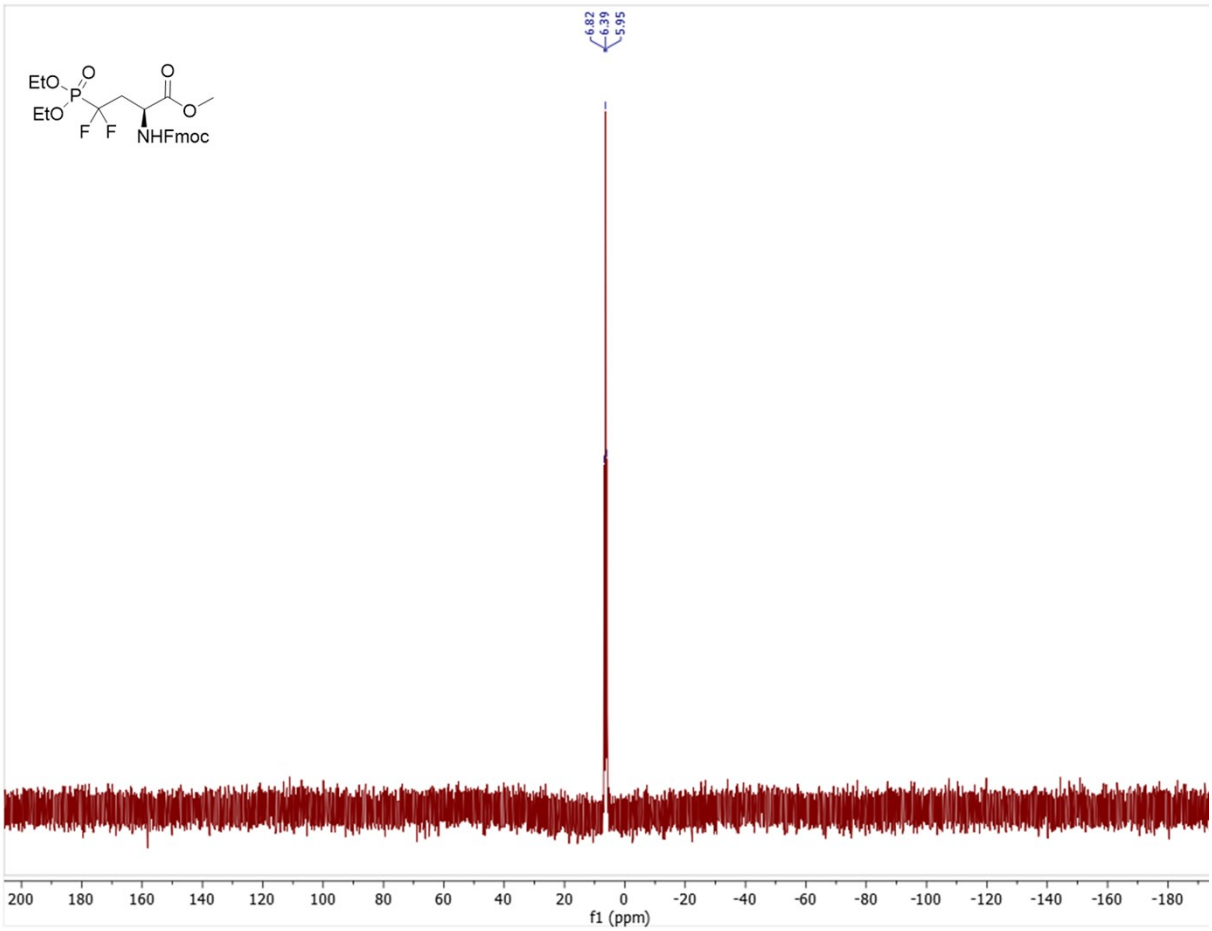


Figure S35. ^{31}P NMR (243 MHz, $CDCl_3$) of **9**

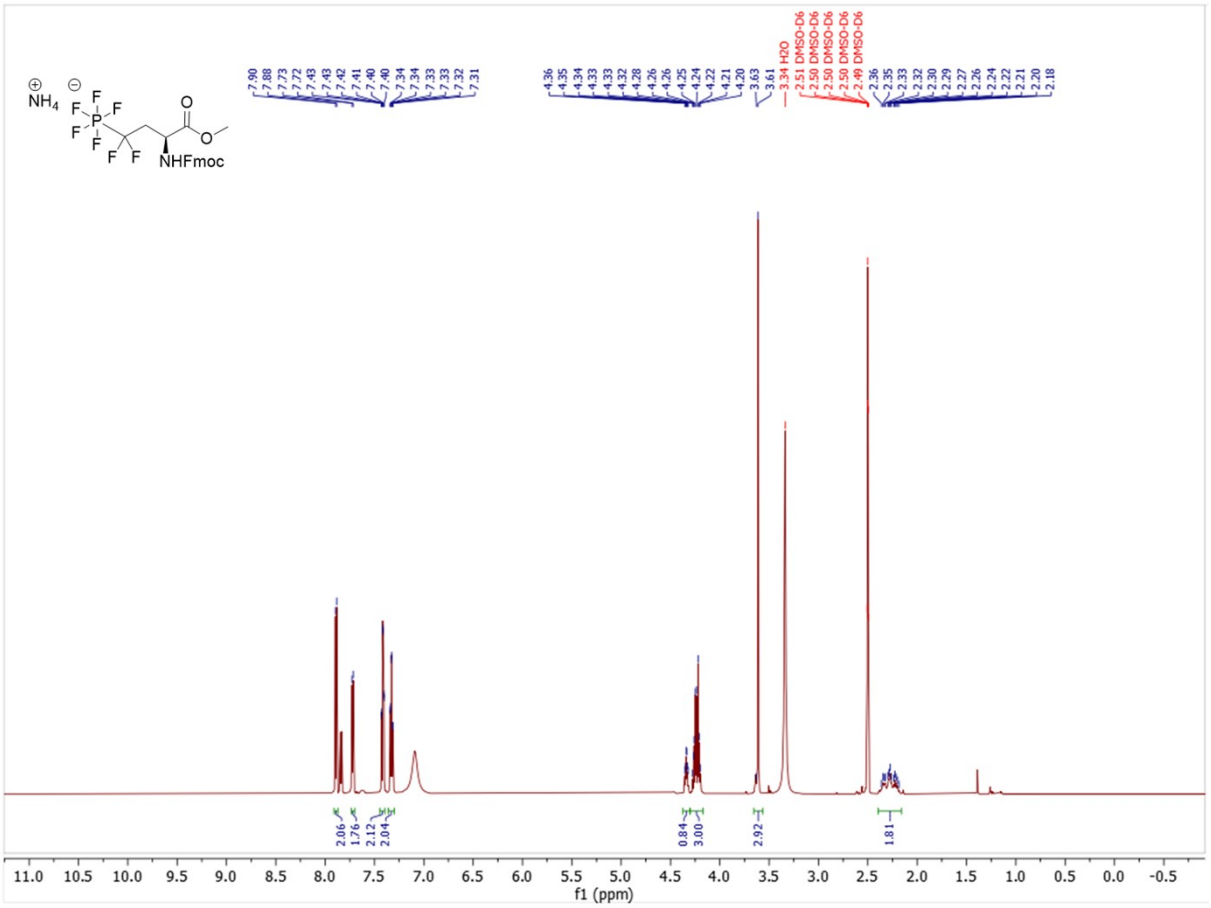


Figure S36. ^{1}H NMR (600 MHz, DMSO-D_6) of **10**

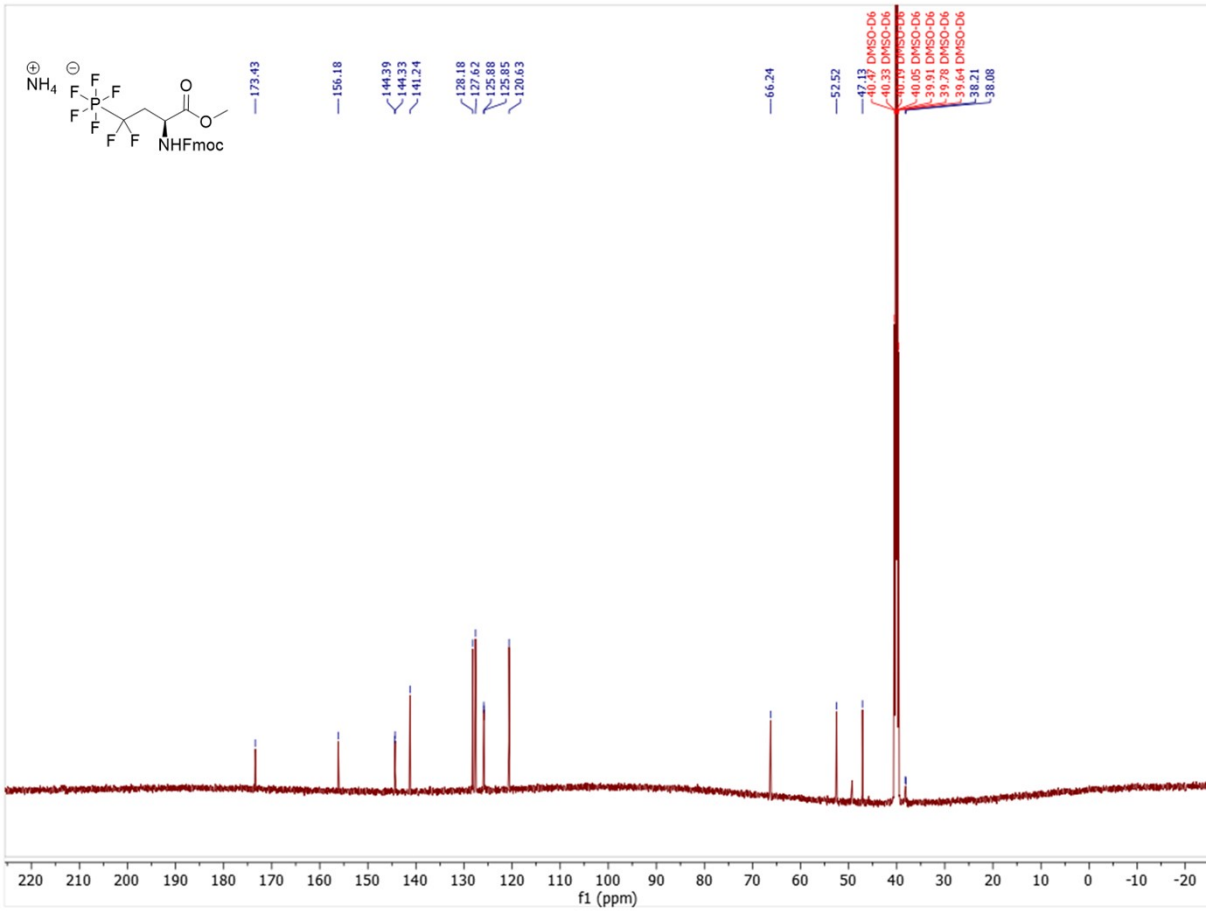


Figure S37. ^{13}C NMR (151 MHz, DMSO-D_6) of **10**

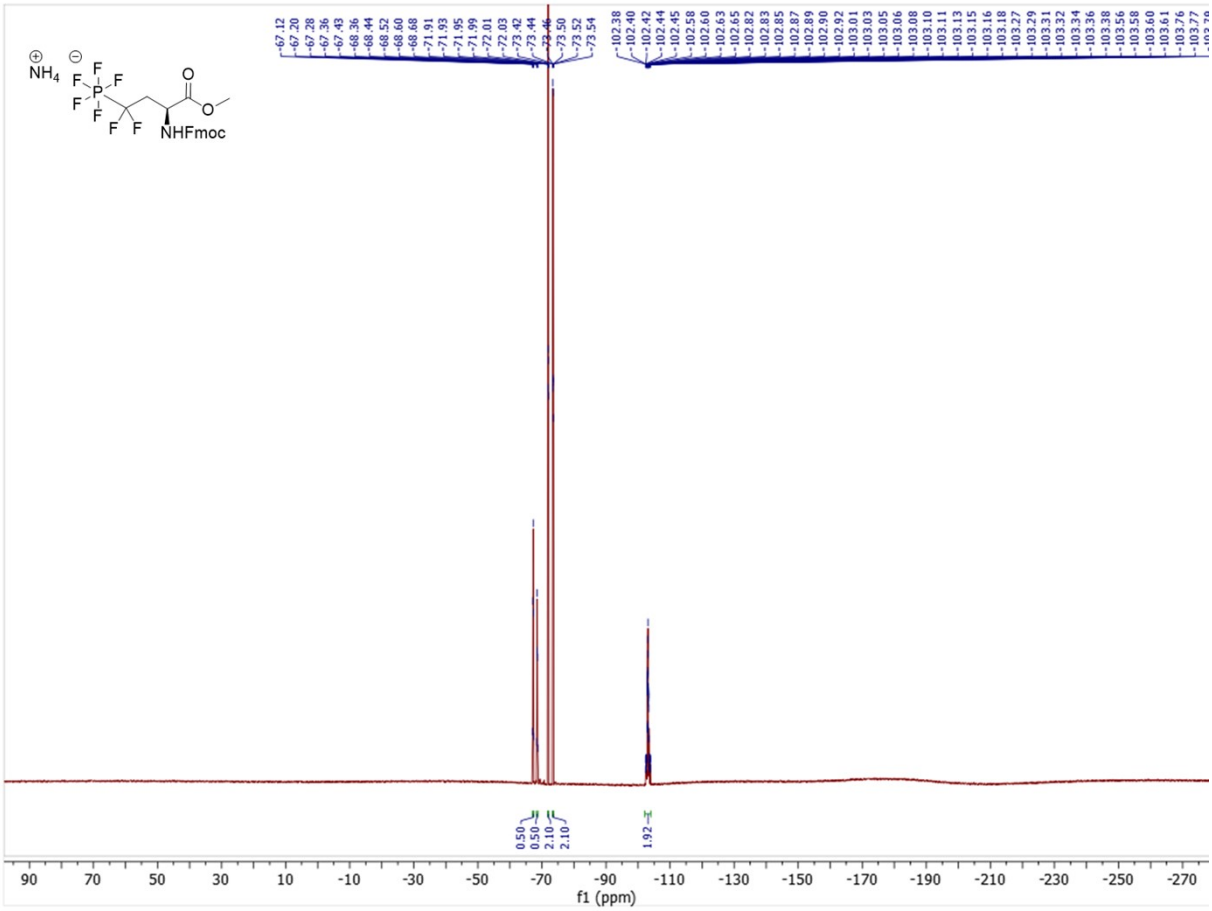


Figure S38. ^{19}F NMR (565 MHz, DMSO-D_6) of **10**

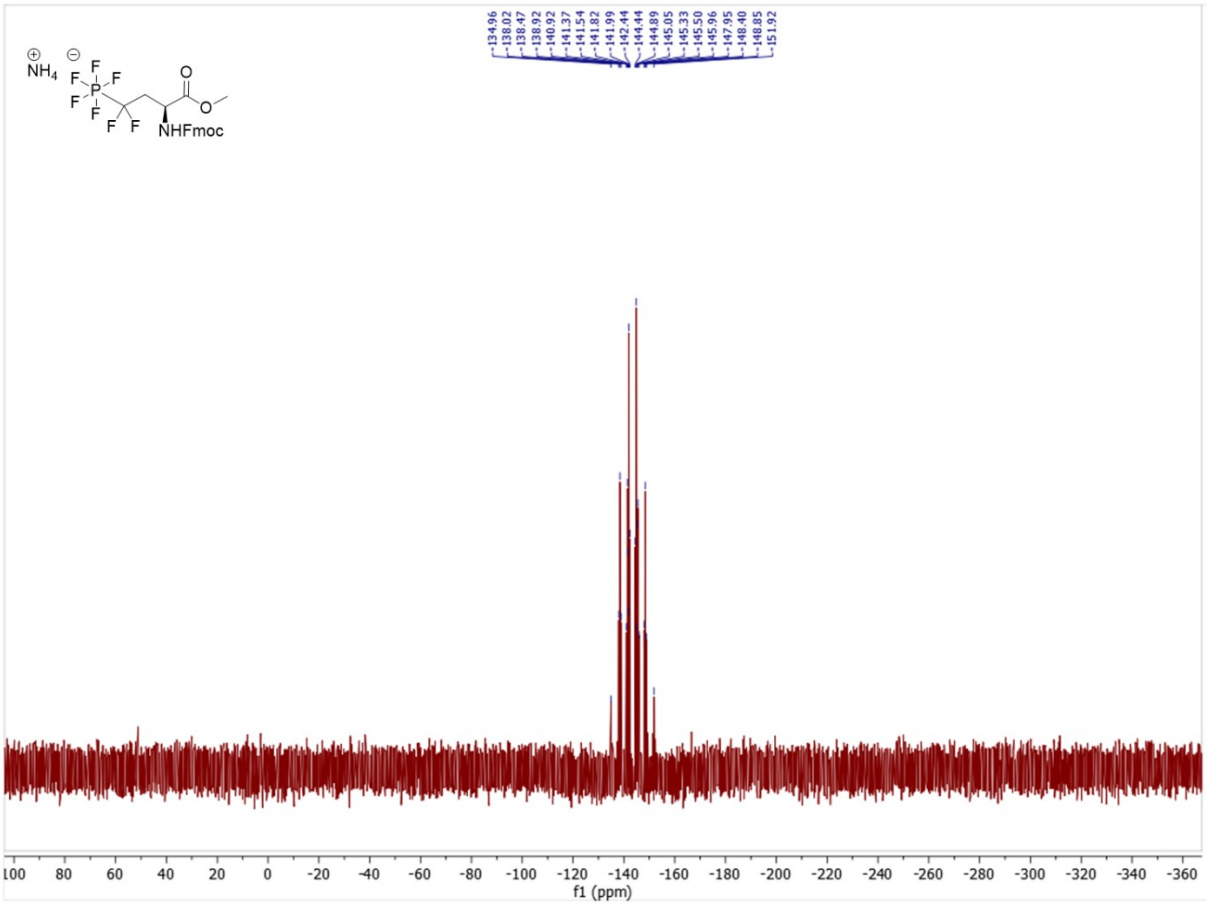


Figure S39. ^{31}P NMR (243 MHz, DMSO-D_6) of **10**

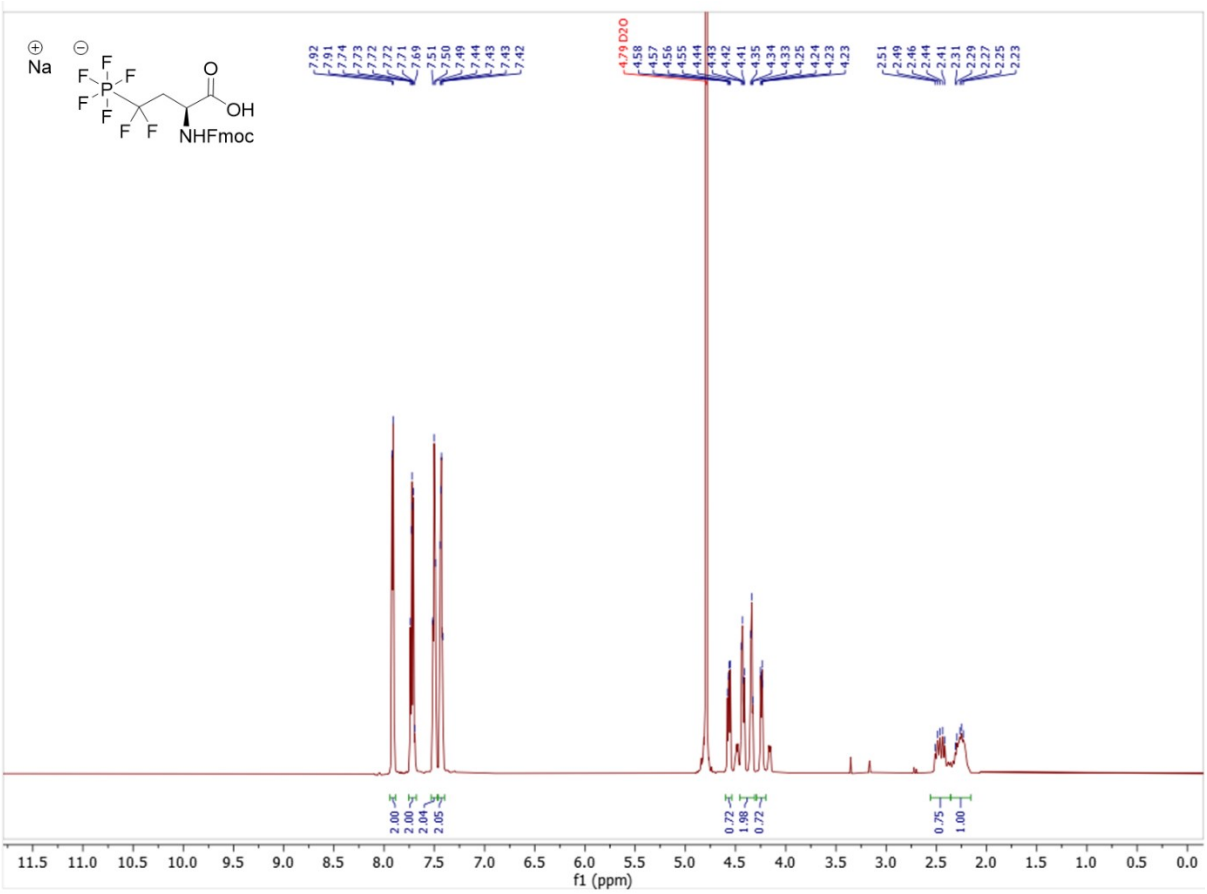


Figure S40. ^{1}H NMR (600 MHz, D_2O) of **11**

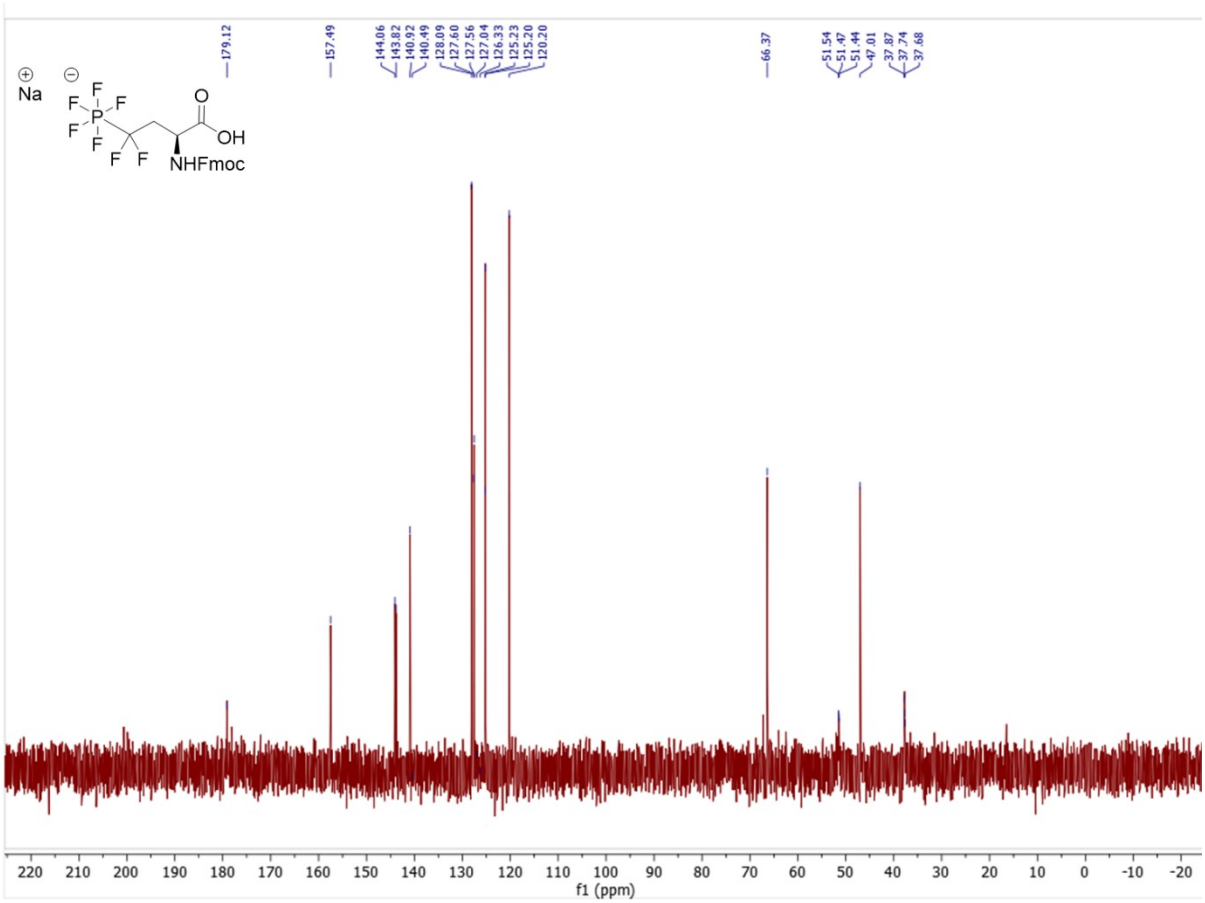


Figure S41. ^{13}C NMR (151 MHz, D_2O) of **11**

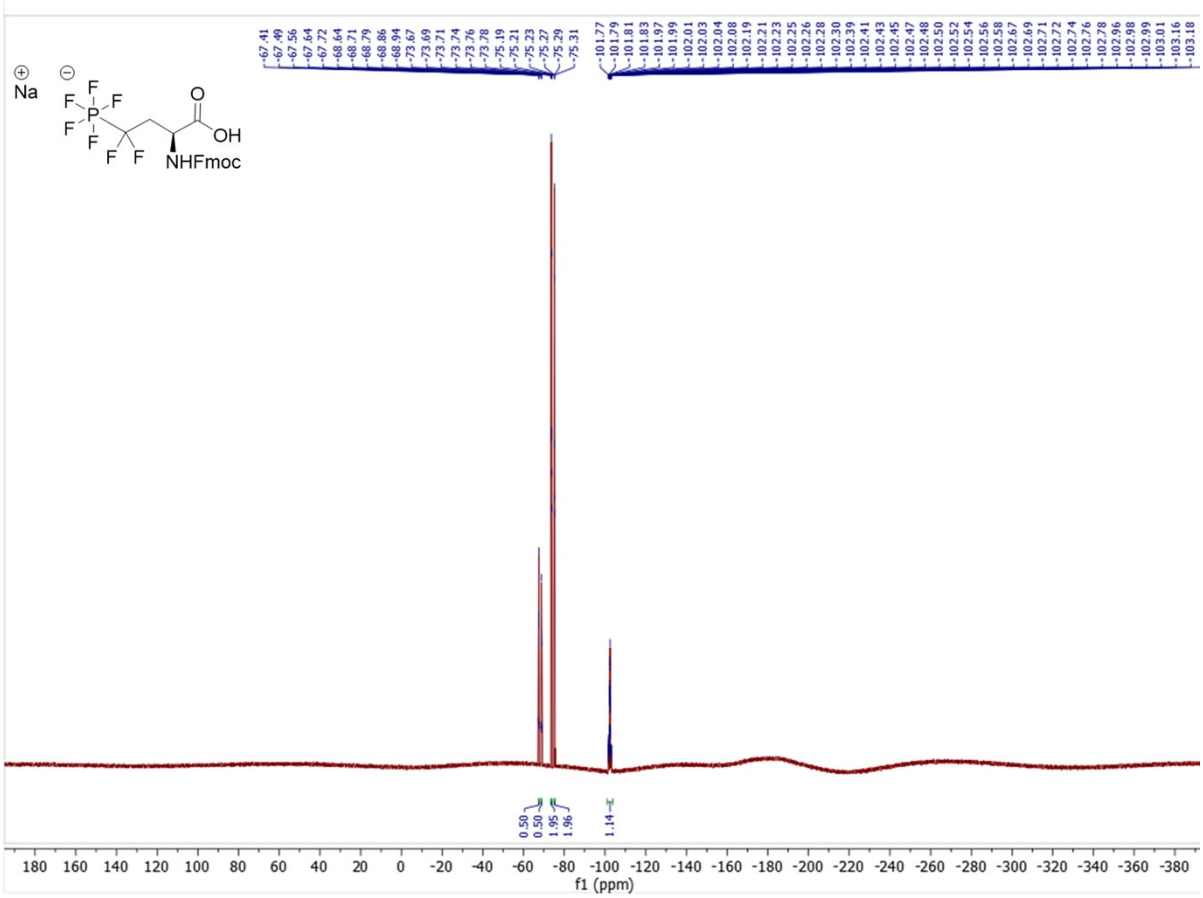


Figure S42. ^{19}F NMR (565 MHz, D_2O) of **11**

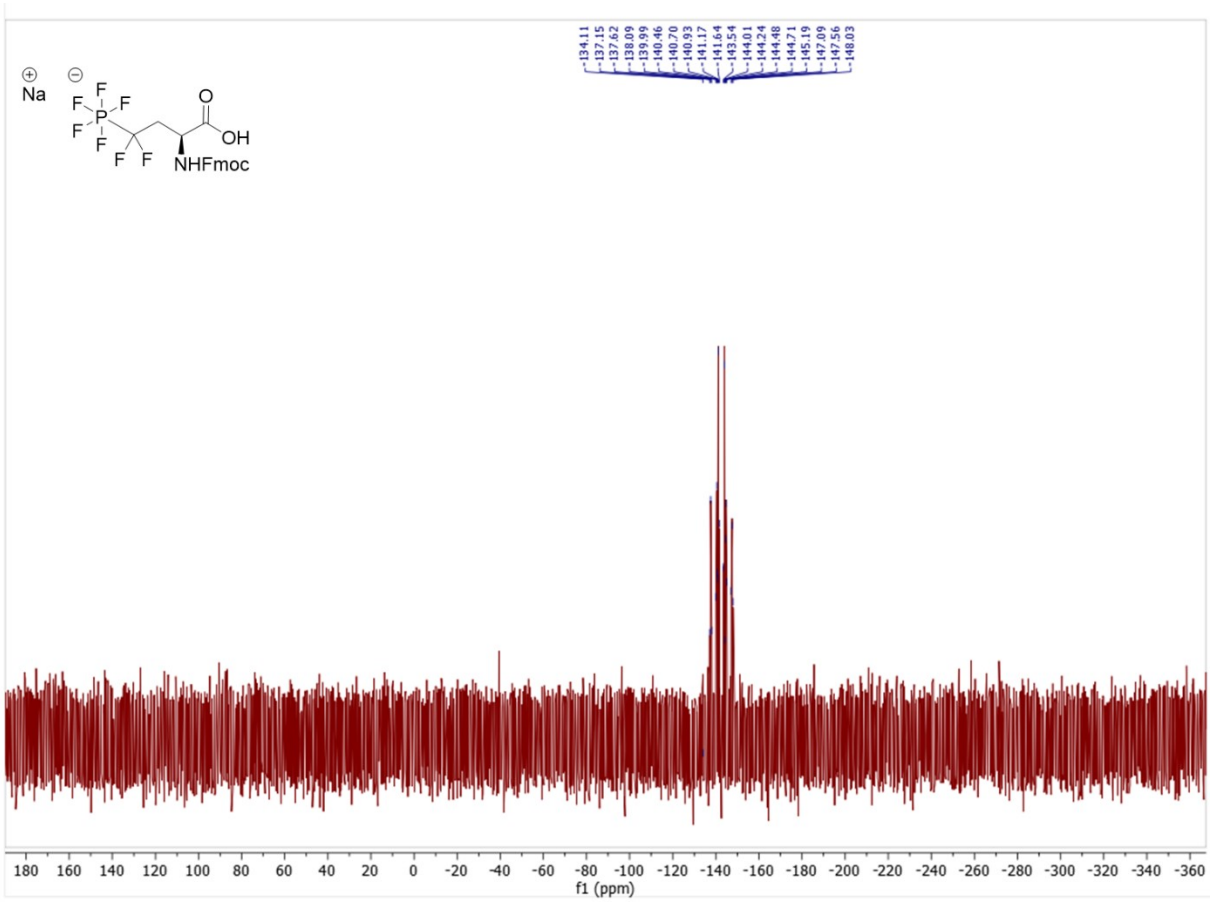


Figure S43. ^{31}P NMR (243 MHz, D_2O) of **11**

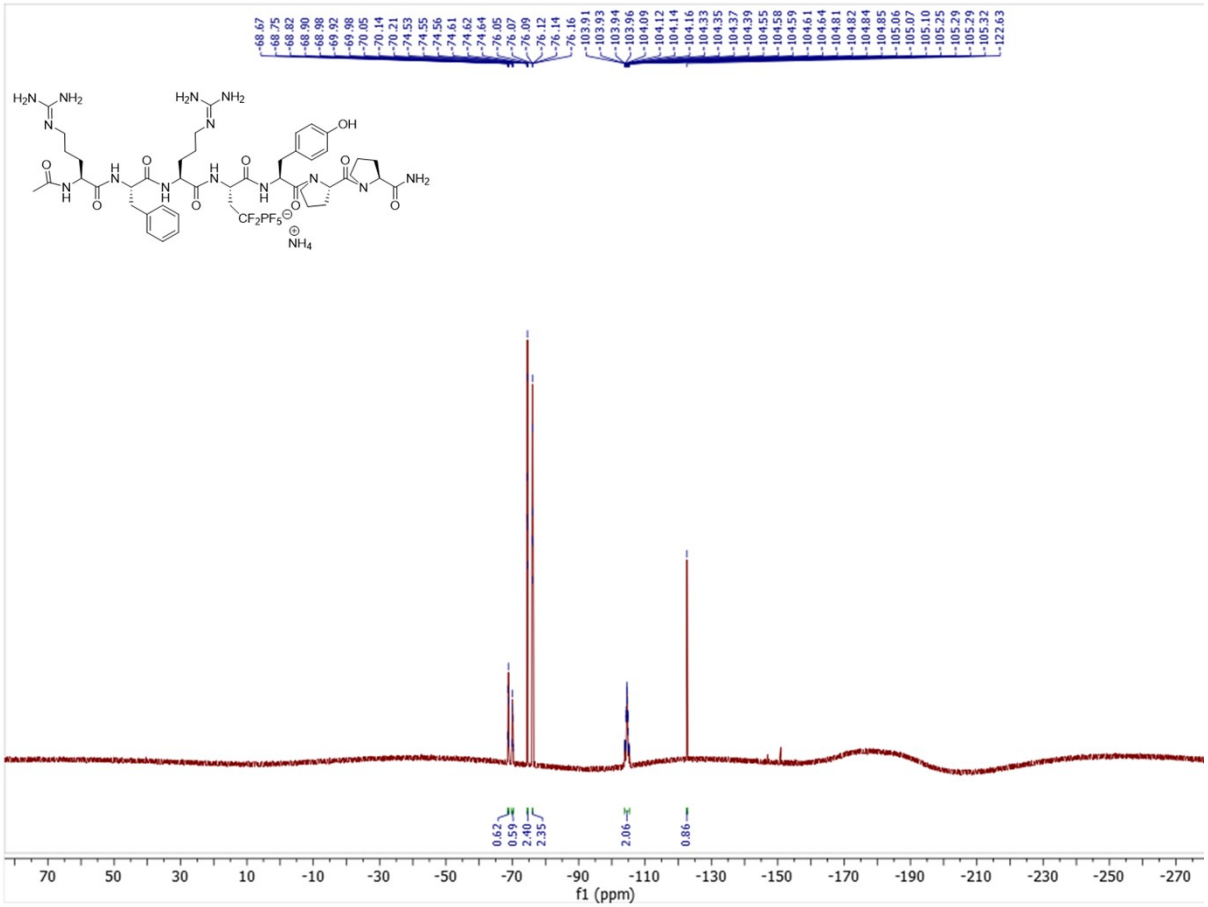


Figure S44. ^{19}F NMR (565 MHz, DMSO-D_6) of **16**

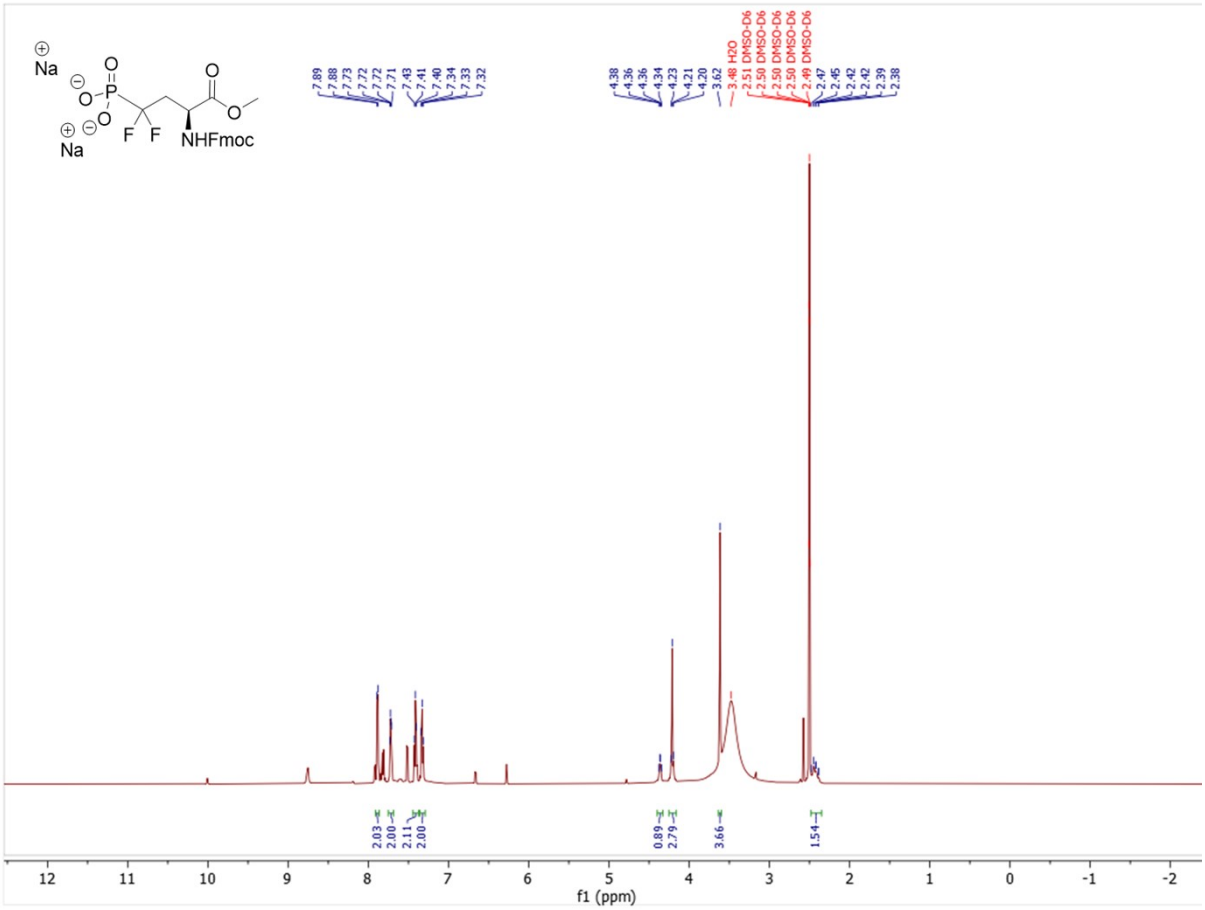


Figure S45. ^{1}H NMR (600 MHz, DMSO-D_6) of **17**

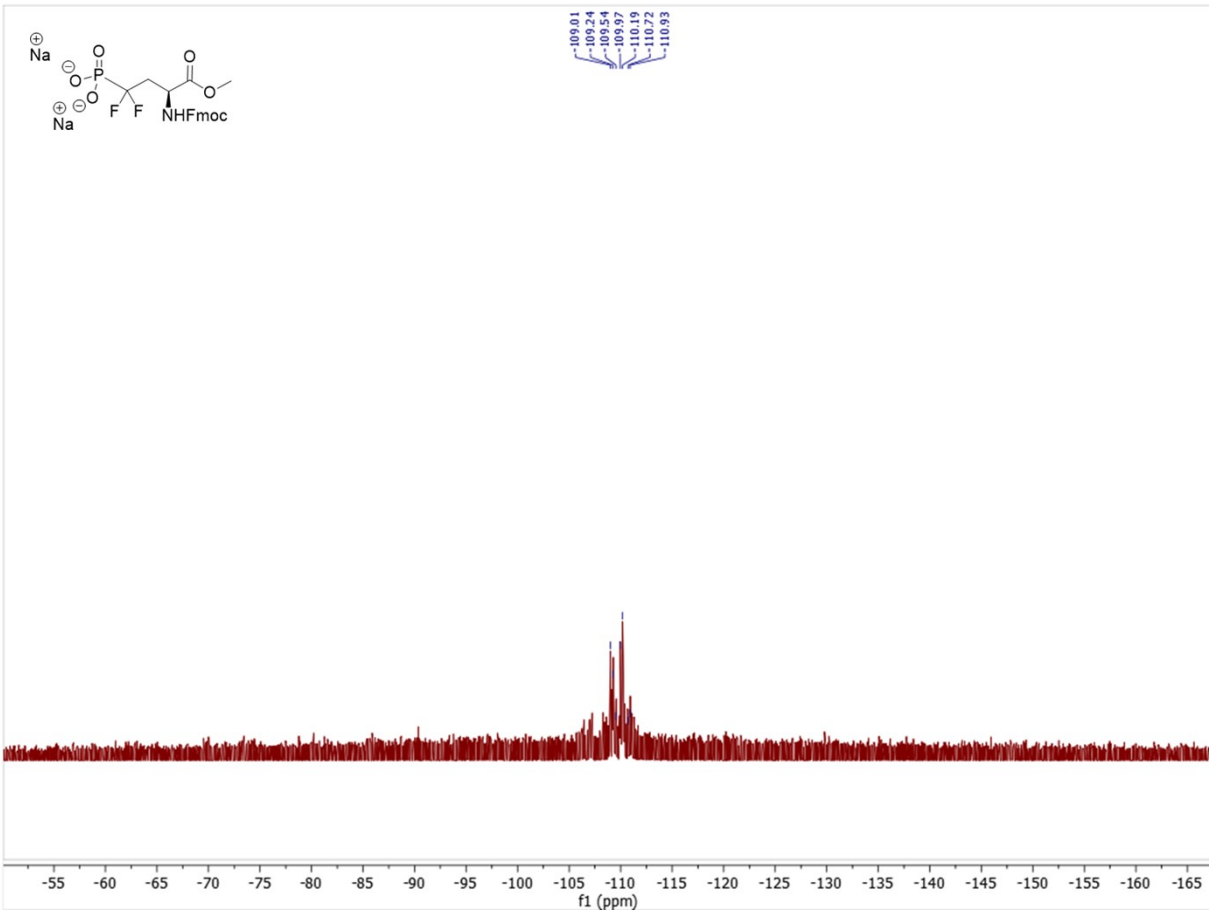


Figure S46. ^{19}F NMR (376 MHz, DMSO-D_6) of **17**

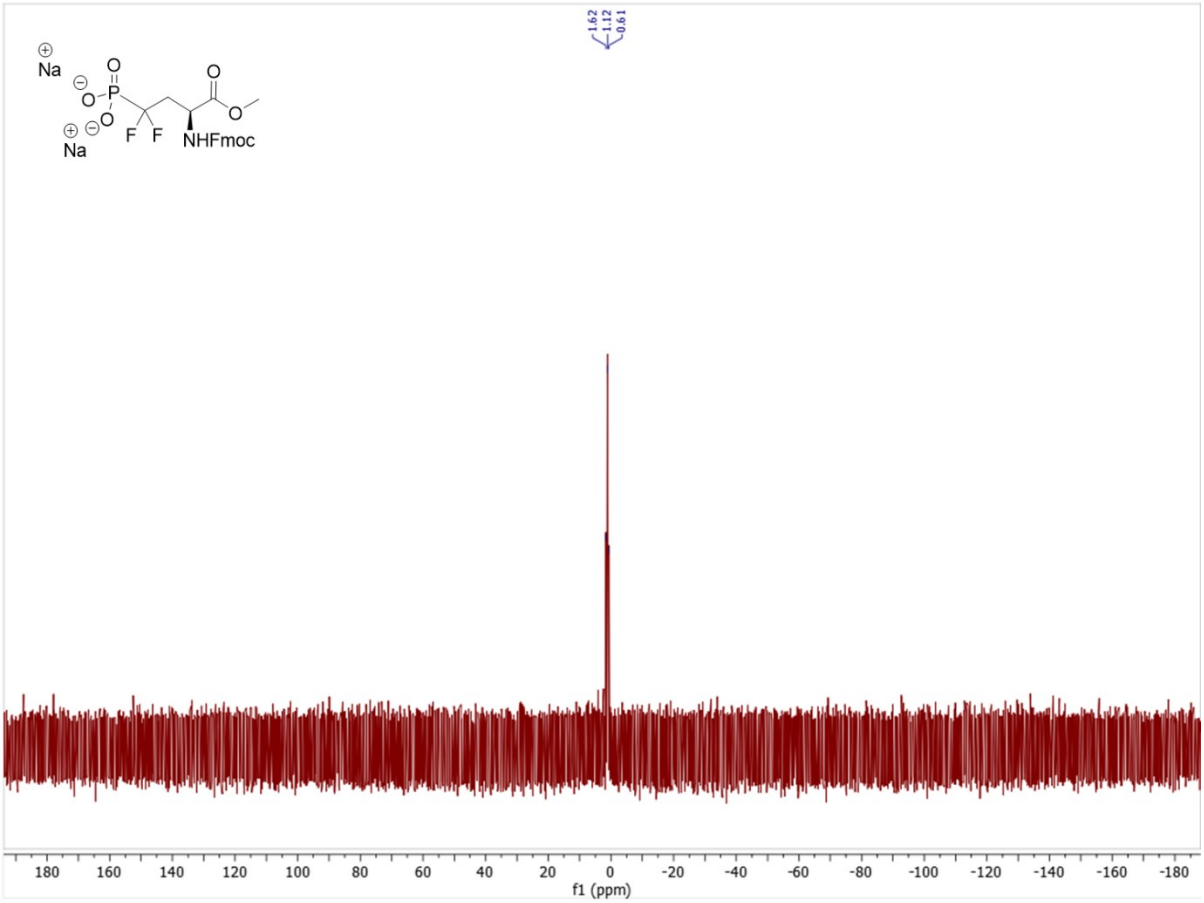


Figure S47. ^{31}P NMR (162 MHz, DMSO-D_6) of **17**

References

22 E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595

23 Y. Li, X. Lin, R. T. Kilani, J. C. R. Jones and A. Ghahary, *J. Cell. Physiol.*, 2007, **212**, 675-681.

24 A. M. Wegner, J. L. McConnell, R. D. Blakely and B. E. Wadzinski, *Methods Mol. Biol.,* 2007, **365**, 61-69.

25 P. Labute, *Proteins Struct. Funct. Bioinf.*, 2009, **75**, 187-205.

26 G. Jones, P. Willett, R. C. Glen, A. R. Leach and R. Taylor, *J. Mol. Biol.,* 1997, **267**, 727-748.

27 G. Wolber and T. Langer, *J. Chem. Inf. Model.*, 2005, **45**, 160-169.

28 G. Wolber, A. A. Dornhofer and T. Langer, *J. Comput. Aided. Mol. Des.,* 2006, **20**, 773-788.