Electronic Supplementary Information

for

# Phosphinic acid-based inhibitors of tubulin polyglycylation

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## Protein expression and purification

The genes for *Xenopus tropicalis* TTLL10 (Xenbase: 6030649, Amino acids 105-570) and *Homo sapiens* TTLL8 (Swiss-Prot: A6PVC2.4, Amino acids 39-585) were cloned into pCoofy28 (Addgene plasmid #44004) by sequence-specific ligation with RecA[1], and bacmid was produced by transformation of DH10EMBacY (Geneva Biotech). Baculovirus was produced by transfection of and subsequent amplification with ExpiSf9 cells (Thermo Fisher Scientific). Both proteins were expressed in baculovirus-infected Sf9 cells (Thermo Fisher Scientific) with an N-terminal GST tag. Cells were infected at a density of 2.5E6 cells/mL with an MOI of 2 and then grown for 48 hrs before harvest. Cells were resuspended in a buffer containing 300mM NaCl, 50mM Tris pH 8.0, 10mM $MgCl_2$, 2mM TCEP, 1mM ATP, and 1mM PMSF. Cells were lysed using a microfluidizer, and cell lysate was clarified by centrifugation at 450,000 rcf for 60 minutes. The fusion protein was captured using GST affinity chromatography. The TTLL of interest was liberated from its GST tag through incubation with 3C protease at a 1:500 molar ratio and subsequently eluted from the column. TTLL10 was further purified on a heparin column to remove 3C protease and other remaining impurities, followed by size-exclusion chromatography. TTLL10 was flash-frozen in 20mM Tris pH 8.0, 10mM $MgCl_2$, 100mM NaCl, 2mM TCEP, 10% Glycerol. TTLL8 was flash-frozen in 50mM HEPES pH 7.0, 10mM $MgCl_2$, 200mM KCl, 2mM TCEP, 10% Glycerol.

## Microtubule Preparation:

Human tubulin was purified from tsA-201 cells as described previously[2, 3]. Mass spectrometric analysis showed no detectable post-translational modifications. Taxol-stabilized microtubules were assembled using this tubulin as previously described[3], with microtubule stock suspensions being prepared to a concentration of 20uM in BRB80 (80 mM PIPES pH 6.8, 1mM $MgCl_2$, 1mM EGTA) supplemented with 2mM TCEP and 10uM Taxol. These taxol stabilized unmodified human microtubules were used as substrate for TTLL8 inhibition assays.

TTLL8-modified microtubules were used as substrate for TTLL10 inhibition assays. Briefly, unmodified tubulin was thawed on ice and spun at 4°C for 10 minutes at 270,000 rcf to remove aggregated protein. DMSO, GTP, and $MgCl_2$ were added to the supernatant to final concentrations of 10% v/v, 1mM, and 10mm, respectively in 20uM tubulin. After 1hr incubation at 37°C, Taxol

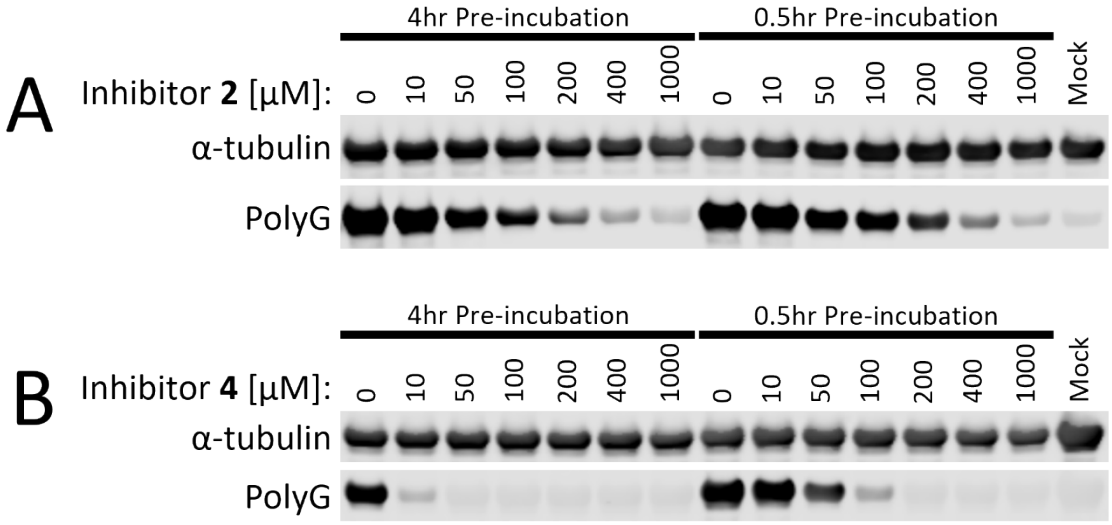
was added to a final concentration of 10uM. After 2hrs additional incubation at 37°C, KCl, TCEP, ATP, Glycine, and TTLL8 are added to final concentrations of 50mM, 2mM, 2mM, 2mM, and 0.1uM, respectively. After, 12hrs glycyl-initiation with 1:200 (molar) TTLL8, the enzyme is removed by a salt wash with 300 mM KCl followed by centrifugation at 37°C for 12 minutes at 110,000 rcf through an equal-volume glycerol cushion consisting of 80mM K-KIPES pH 6.8, 1mM EGTA, 1mM $MgCl_2$, 20uM Taxol, 60% glycerol v/v. The microtubule pellet was washed with warm (37°C) BRB80 supplemented with 2mM TCEP, 10uM Taxol and resuspended in the same buffer to a final tubulin concentration of 20uM.

## Inhibitor assays

Lyophilized inhibitors were reconstituted in water, adjusted to pH 7.0 with HCl, and then diluted to a stock concentration of 50mM. Serial dilutions of inhibitor in enzyme buffer + 1mM ATP were mixed 1:1 (v/v) with enzyme to final inhibitor concentrations of 0.001 – 1mM and then incubated at room temperature or 4°C for 30 minutes – 6hrs. After pre-incubation, the enzyme + inhibitor mix was added to microtubules so that the final assay composition was 4uM tubulin, 0.08uM enzyme (1:50 molar ratio), 1mM ATP, 1mM Glycine, 2mM TCEP, 10mM $MgCl_2$, 10uM Taxol, and 40mM or 20mM KCl for TTLL8 or TTLL10, respectively. Glycylation reactions were performed at 37°C with samples for western blots removed 20 minutes after the addition of enzyme. Reactions were quenched via the addition of denaturing/reducing PAGE buffer. All preincubation reactions and subsequent glycylation reactions were performed in duplicate.

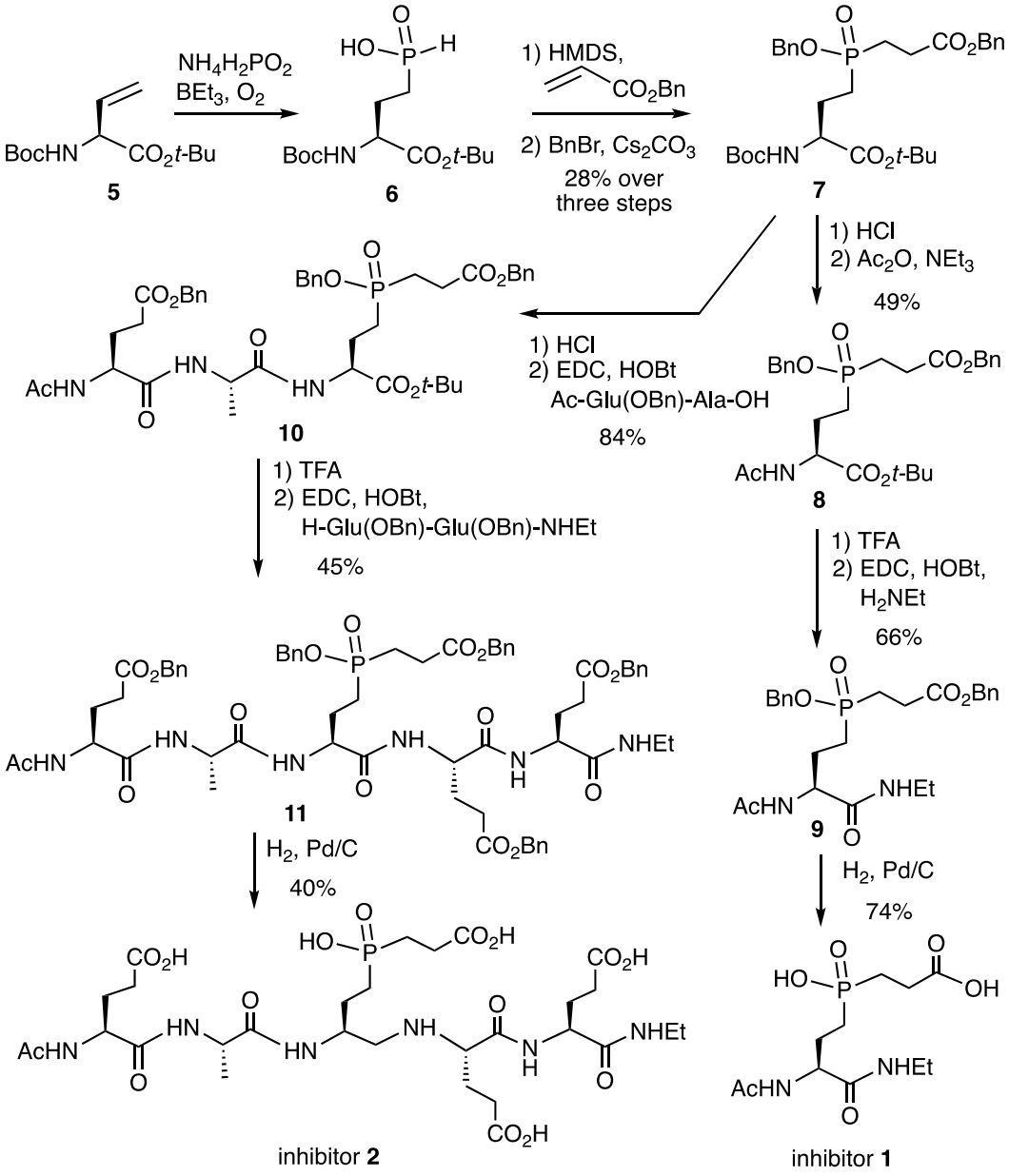
Glycylation was assessed by western blot. 320ng of tubulin from each reaction was run on an SDS-PAGE gel and then transferred to a nitrocellulose membrane which was blocked in PBST + 5% skim milk. Blots were first stained with 1:5,000 mouse anti-α-tubulin (Clone DM1A, Sigma-Aldrich) and 1:10,000 rabbit anti-MonoG (Gly-pep1, Adipogen) or rabbit anti-PolyG (Custom antibody from the research group of Jacek Gaertig, used in [4] and [5]) for TTLL8 or TTLL10 reactions, respectively. Near-IR fluorescent dye-conjugated secondary antibodies (LI-COR) were used in conjunction with a LI-COR Odyssey CLX imaging system, whereupon tubulin and glycylation signals were determined by gel-scanning densitometry using the Image Studio software suite (LI-COR). Normalized glycylation ($G_{N,i}$ for each inhibitor condition, i) was calculated as the glycylation signal divided by the tubulin signal for each condition. Percent

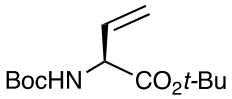
inhibition for each condition ($P_i$) was calculated as $P_i = 100*(1 - (G_{N,i} – G_B)/(G_u – G_B))$, where $G_u$ is the normalized glycylation signal for the uninhibited condition and $G_B$ is the normalized glycylation signal for the mock (No glycine / 100% theoretical inhibition) well.



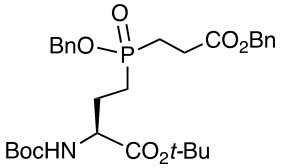

Representative western blots showing (A) inhibition of TTLL8 glycyl-initiation by inhibitor 2 and (B) inhibition of TTLL10 glycyl-elongation by inhibitor 4.

## Synthesis of Inhibitor 1 &2






**Compound 5:** Boc-Glu-OtBu (5 g, 16 mmol) was dissolved in toluene (100 mL), and the solution was treated with copper (II) acetate (0.7 g, 4 mmol). The solution was stirred under Ar for 1 h at rt. Lead (IV) acetate (14.6 g, 33 mmol) was then added and the mixture was stirred for 1 h at rt under Ar. Then the solution was refluxed at 90 °C under Ar for 8 h. The solution was filtered and diluted with EtOAc (150 mL). The filtrate was washed with $H_2O$ (3 x 100 mL). The organic layer was dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (20% EtOAc in petroleum ether) to give compound **5** as a white solid (1.4 g, 35%). The spectra data were identical to that reported in the literature.[6]

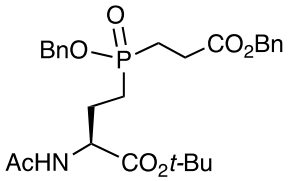



**Compound 7:** Compound **5** (650 mg, 2.53 mmol) was dissolved in methanol (50 mL), and ammonium hypophosphite (630 mg, 7.58 mmol) was added. Triethylborane in THF (1M, 5.05 mmol) was added dropwise and the mixture was stirred open to the air for 2 h at rt. The reaction mixture was concentrated in vacuo, and the residue was dissolved in $KHSO_4$ (1M, 50 mL). The product was extracted with EtOAc (3 x 50 mL), and the combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated in vacuo to give compound **6** as a white solid. This material was used without further purification.[7]

In a double-neck flask, compound **6** (500 mg) and hexamethyldisilazane (1.25 g, 7.75 mmol) were added under Ar. After refluxing at 110 °C for 1h, benzyl acrylate (326 mg, 2.01 mmol) was added dropwise and the mixture was stirred for 3 h at 110 °C. After cooling to 70 °C, ethanol (5 mL) was added to quench the reaction. The reaction mixture was concentrated in vacuo, and partitioned with $NaHCO_3$ (5%, 15 mL) and diethyl ether (15 mL). The aqueous layer was acidified to pH = 1 using 1M HCl, and the product was extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated in vacuo to give the phosphinic acid as a white solid. The phosphinic acid was used without further purification.

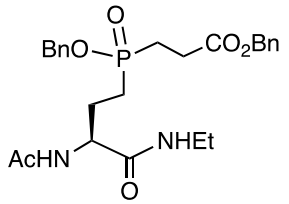
The phosphinic acid (335 mg) was dissolved in ethanol: $H_2O$ (1:1, 10 mL) and cesium carbonate (113 mg, 0.35 mmol) was added. The reaction mixture was stirred for 1 h at rt and concentrated to dryness in vacuo. The residue was dissolved in DMF (10 mL), and benzyl bromide (591 mg, 3.5 mmol) was added. The solution was stirred for 8 h under Ar at rt and then evaporated to dryness in vacuo. The reaction mixture was partitioned with EtOAc (30 mL) and $H_2O$ (30 mL) and the organic layer was dried over $Na_2SO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (80% EtOAc in petroleum ether) to give compound **7** as a white solid (mixture of diastereomers, 203 mg, 28% over three steps).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.42 – 7.31 (m, 10H), 5.12 (s, 2H), [5.04 (d, *J* = 4.6 Hz), 5.02 (d, *J* = 4.6 Hz), 2H], 4.24 – 4.12 (m, 1H), 2.72 – 2.54 (m, 2H), 2.16 – 2.03 (m, 3H), 1.95 – 1.62 (m, 3H), [1,47 (s), 1.46 (s), 9H], 1.45(s, 9H).
**$^{31}$P NMR** (162 MHz, $CDCl_3$) δ 56.56.
**$^{13}$C NMR** (75 MHz, $CDCl_3$) δ [171.99, 171.79], 170.85, 155.41, [136.41, 136.33], 135.54, 128.70, 128.60, 128.53, 128.39, 128.31, 127.97, 82.46, 79.93, 77.45, 77.03, 76.60, 66.85, [66.09, 66.01], 28.30, 27.98, [26.73, 26.69], 25.05, [24.05, 23.84], 22.83.
**HRMS (ESI)**: m/z calcd for $C_{30}H_{42}NO_8P$ $[M+H]^+$ 575.2646, found 575.2648.




**Compound 8:** Compound **7** (200 mg, 0.35 mmol) was dissolved in HCl in dioxane (4 M, 5 mL) and stirred for 30 min at rt. The solution was concentrated in vacuo and redissolved in acetic acid (1%, 5 mL). Acetic anhydride (0.17 mL, 1.74 mmol) was added dropwise while maintaining the pH around 7 using triethylamine. The reaction mixture was stirred for 30 min and concentrated in vacuo. The residue was purified by silica gel chromatography (3% MeOH in DCM) to give compound **8** as a white solid (mixture of diastereomers, 88 mg, 49% over two steps).

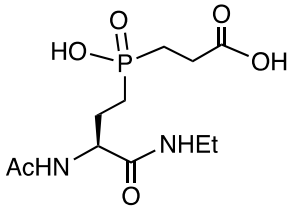
**$^1$H NMR** (400 MHz, MeOD) δ 7.45 – 7.30 (m, 10H), 5.13 (s, 2H), [5.06 (d, *J* = 3.6 Hz), 5.03 (d, *J* = 3.6 Hz), 2H], 4.34 – 4.26 (m, 1H), 2.71 – 2.59 (m, 2H), 2.23 – 2.07 (m, 3H), 1.99 (s, 3H), 1.94 – 1.82 (m, 2H), [1.47 (s), 1.46 (s), 9H].
**$^{31}$P NMR** (162 MHz, MeOD) δ 59.89, 59.87.
**$^{13}$C NMR** (101 MHz, MeOD) δ 172.01, 171.88, 170.55, 136.44, 135.96, 128.36, [128.26, 128.22], 128.16, 127.94, 127.90, [127.79, 127.71], 81.77, 66.40, [66.20, 66.16, 66.14, 66.10], [53.32, 53.21], 26.81, 24.45, 23.54, [22.86, 22.82], [21.95, 21.91], 20.93.
**HRMS (ESI)**: m/z calcd for $C_{27}H_{36}NO_7P$ $[M+H]^+$ 517.2232, found 517.2229.



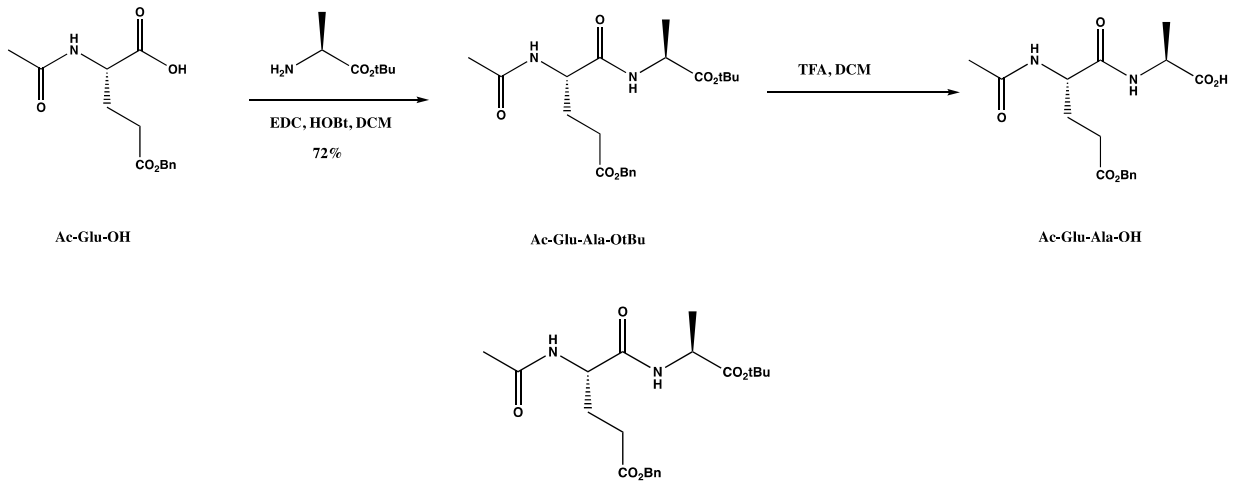

**Compound 9:** Compound **8** (100 mg, 0.19 mmol) was dissolved in DCM (2 mL) and trifluoroacetic acid (2 mL) was added. The reaction mixture was stirred for 3h at rt and then concentrated in vacuo. The residue was dissolved in anhydrous DCM (10 mL) and the solution

was cooled to 0 °C. HOBt (36 mg, 0.23 mmol) and EDC (44 mg, 0.23 mmol) were added and the mixture was stirred under Ar for 20 min at 0 °C. Ethylamine (17 mg, 0.39 mmol) was added dropwise and the mixture was stirred under Ar for 1 h at 0 °C, then 2 h at rt. The reaction mixture was diluted with DCM (10 mL) and then washed with $H_2O$ (3x10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (5% MeOH in DCM) to give compound **9** as a white solid (mixture of diastereomers, 62 mg, 66% yield).

**$^1$H NMR** (400 MHz, MeOD) δ 7.57 – 7.19 (m, 10H), 5.13 (s, 2H), [5.05 (d, *J* = 1.8 Hz), 5.03 (d, *J* = 1.9 Hz), 2H], 4.36 – 4.28 (m, 1H), 3.22 (q, *J* = 7.3 Hz, 2H), 2.69 – 2.56 (m, 2H), 2.23 – 2.12 (m, 2H), 2.06 – 2.03 (m, 1H), 2.00 (s,3H), 1.93 – 1.82 (m, 3H), 1.12 (t, *J* = 7.3 Hz, 3H).
**$^{31}$P NMR** (162 MHz, MeOD) δ 60.05, 60.03.
**$^{13}$C NMR** (101 MHz, MeOD) δ [172.01, 171.88], 171.97, [171.54, 171.52], 136.45, 135.97, 128.34, [128.23, 128.21], 128.16, 127.93, 127.90, [127.76, 127.74], 66.39, [66.18, 66.11], [53.48, 53.35], 33.92, 26.14, [24.39, 23.48], [24.25, 24.22, 24.19, 24.16], [22.84, 22.74, 21.93, 21.82], 21.11, 13.38.
**HRMS (ESI)**: m/z calcd for $C_{25}H_{33}N_2O_6P$ $[M+H]^+$ 488.2080, found 488.2076.

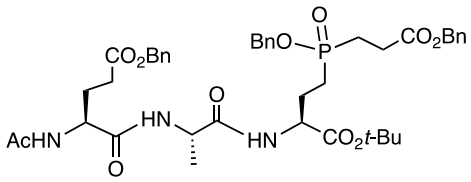



**Inhibitor 1:** To a solution of compound **9** (30 mg, 0.06 mmol) in ethanol (2 mL) was added 10 wt % Pd/C (3 mg), and the mixture was stirred under $H_2$ (1 atm) for 8 h at rt. After filtration, the filtrate was concentrated under vacuum to give a clear oil which was dissolved in $H_2O$ (1 mL). The pH of the solution was adjusted to 10 using NaOH (2 M), and the sample was loaded onto a column of AG 1-X8 resin (formate form, 10 mL, equilibrated with $H_2O$). The column was eluted with with a stepwise gradient of 0.5 M to 4 M formic acid. The fractions containing the desired compound were lyophilized to give inhibitor **1** as a white solid (14 mg, 74%).
**$^1$H NMR** (400 MHz, MeOD) δ 4.33 (dd, *J* = 8.1, 5.5 Hz, 1H), 3.22 (q, *J* = 7.3 Hz, 2H), 2.63 – 2.51 (m, 2H), 2.04 (q, *J* = 5.0 Hz, 2H), 2.01 (s, 3H), 1.98 – 1.82 (m, 2H), 1.82 – 1.70 (m, 2H), 1.13 (t, *J* = 7.2 Hz, 3H).
**$^{31}$P NMR** (162 MHz, MeOD) δ 51.88.
**$^{13}$C NMR** (101 MHz, MeOD) δ 174.41, 172.17, 171.92, 53.74, 34.04, [26.21, 26.18], [25.38, 24.46], [24.60, 24.57], [24.18, 23.25], 21.22, 13.51.
**HRMS (ESI)**: m/z calcd for $C_{11}H_{21}N_2O_6P$ $[M+H]^+$ 308.1140, found 308.1137.




**Ac-Glu(OBn)-Ala-OtBu:** Ac-Glu(OBn)-OH (0.72 g, 2.6 mmol) was dissolved in anhydrous DCM (20 mL) and the solution was cooled to 0 °C. EDC (0.59 g, 3.1 mmol) and HOBt (0.47 g, 3.1 mmol) were added and the mixture was stirred for 20 min under Ar at 0 °C. H-Ala-OtBu (0.37 g, 2.6 mmol) was added and the mixture was stirred for 1 h under Ar at 0 °C and then 2 h at rt. The reaction mixture was diluted with DCM (100 mL) and then washed with $H_2O$ (3x50 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (EtOAc) to give Ac-Glu(OBn)-Ala-OtBu as a white solid (0.75 g, 72% yield).

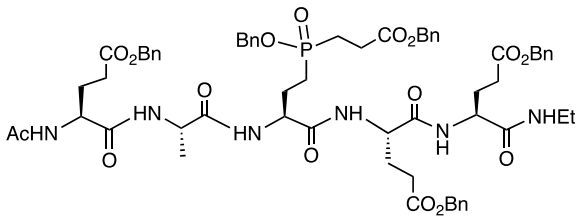
**$^1$H NMR** (300 MHz, $CDCl_3$) δ 7.43 – 7.33 (m, 5H), 6.81 (d, *J* = 7.3 Hz, 1H), 6.46 (d, *J* = 7.7 Hz, 1H), 5.15 (dd, *J*=3.9 Hz, 2H), 4.53 (dt, *J* = 7.9, 5.2 Hz, 1H), 4.41 (dt, *J* = 7.1 Hz, 1H), 2.66 – 2.36 (m, 2H), 2.27 – 2.00 (m, 2H), 1.99 (s, 3H), 1.47 (s, 9H), 1.38 (d, *J* = 7.1 Hz, 3H).
**$^{13}$C NMR** (75 MHz, $CDCl_3$) δ 173.29, 171.60, 170.40, 170.35, 135.68, 128.61, 128.34, 128.29, 82.09, 66.64, 52.41, 48.80, 30.50, 27.95, 27.68, 23.13, 18.33.
**HRMS (ESI)**: m/z calcd for $C_{21}H_{30}N_2O_6$ $[M+H]^+$ 406.2108, found 406.2104.



**Compound 10:** Compound **7** (63 mg, 0.11 mmol) was dissolved in HCl in dioxane (4M, 5mL) and stirred for 30 min at rt. The reaction mixture was concentrated in vacuo to give the ammonium chloride, which was used without further purification.
Ac-Glu(OBn)-Ala-OtBu (0.20 g, 0.48 mmol) was dissolved in DCM (1 mL), and trifluoroacetic acid (1 mL, 13 mmol) was added. The reaction mixture was stirred under rt for 3 h and then concentrated in vacuo to give Ac-Glu(OBn)-Ala-OH, which was used without further purification.

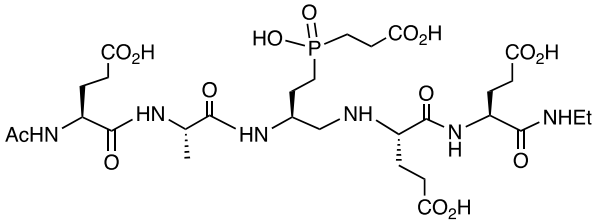
Ac-Glu(OBn)-Ala-OH (39 mg) was dissolved in anhydrous DCM (5 mL) and the solution was cooled to 0 °C. HOBt (20 mg, 0.13 mmol) and EDC (25 mg, 0.13 mmol) were added and the mixture was stirred under Ar for 20 min at 0 °C. The ammonium chloride in anhydrous DCM (2 mL) was added dropwise into the solution and the mixture was stirred under Ar for 1 h at 0 °C, then 2 h at rt. The reaction mixture was diluted with DCM (10 mL) and then washed with $H_2O$ (3x10 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (4% MeOH in DCM) to give compound **10** as a white solid (mixture of diastereomers, 75 mg, 84% yield).

**$^1$H NMR** (400 MHz, MeOD) δ 7.47 – 7.23 (m, 15H), 5.12 – 5.10 (m, 4H), [5.04 (d, *J* = 2.5 Hz), 5.02 (d, *J* = 2.3 Hz), 2H], 4.42 – 4.27 (m, 3H), 2.72 – 2.56 (m, 2H), 2.47 (t, *J* = 7.7 Hz, 2H), 2.22 – 2.04 (m, 5H), 1.96 (s, 3H), 1.94 – 1.84 (m, 3H), 1.46 (s, 9H), 1.38 (d, *J* = 7.1 Hz, 3H).
**$^{31}$P NMR** (162 MHz, MeOD) δ 60.07, 60.02.
**$^{13}$C NMR** (101 MHz, MeOD) δ 173.63, 172.85, 172.14, 171.96, 171.94, 170.23, 136.48, 136.18, 135.97, 128.34, 128.17, 128.16, 127.94, 127.90, 127.85, 127.80, 127.79, 127.70, 81.81, 66.40, [66.12, 66.06], 65.98, [53.13, 52.97], 52.39, 49.11, 29.84, 26.93, 26.82, 26.17, [24.25, 23.34], 23.65, [23.00, 22.09], 21.06, 16.32.
**HRMS (ESI)**: m/z calcd for $C_{42}H_{54}N_3O_{11}P$ $[M+H]^+$ 807.3496, found 807.3496.




**Compound 11:** Compound **10** (70 mg, 0.09 mmol) was dissolved in DCM (3 mL), and trifluoroacetic acid (3 mL, 39 mmol) was added. The reaction mixture was stirred for 3 h at rt and then concentrated in vacuo. After dissolving the residue in anhydrous DCM (5 mL), the solution was cooled to 0 °C. HOBt (16 mg, 0.10 mmol) and EDC (20 mg, 0.10 mmol) were added and the mixture was stirred under Ar for 20 min at 0 °C. N,N-diisopropylethylamine (11 mg, 0.09 mmol) was added. Then the literature known dipeptide H-Glu(OBn)-Glu(OBn)-NHEt[8] (44 mg, 0.09 mmol) in anhydrous DCM (2 mL) was added dropwise and the mixture was stirred under Ar (1atm) for 1 h at 0 °C, then 2 h at rt. The reaction mixture was diluted with DCM (10 mL) and then washed with $H_2O$ (10 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (6% MeOH in DCM) to give compound **11** as a white solid (mixture of diastereomers, 47 mg, 45% yield).

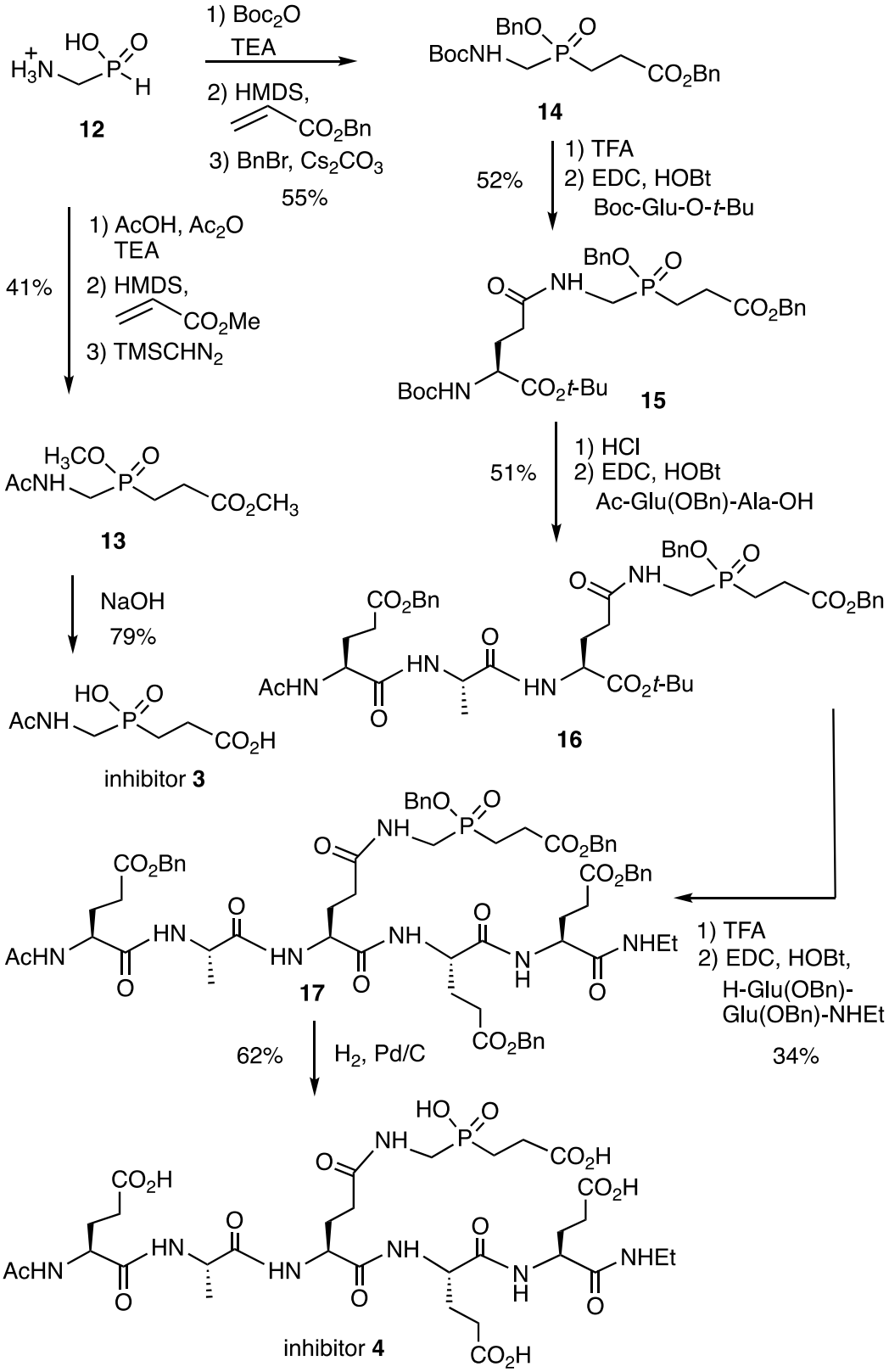
**$^1$H NMR** (400 MHz, MeOD) δ 7.46 – 7.24 (m, 25H), 5.15 – 5.06 (m, 8H), 5.03 (d, *J* = 8.1 Hz, 2H), 4.33 – 4.25 (m, 3H), 4.22 – 4.17 (m, 2H), 3.20 (dq, *J* = 7.3, 1.6 Hz, 2H), 2.69 – 2.55 (m, 2H), 2.53 – 2.35 (m, 7H), 2.23 – 1.96 (m, 11H), 1.95 (s, 3H), 1.33 (d, *J* = 7.2 Hz, 3H), 1.11 (t, *J* = 7.3 Hz, 3H).
**$^{31}$P NMR** (162 MHz, MeOD) δ 60.01.
**$^{13}$C NMR** (101 MHz, MeOD) δ 174.62, 173.21, 172.79, 172.76, 172.68, 172.55, 172.25, 171.69, 136.19, 136.14, 136.11, 135.96, 128.33, 128.17, 127.93, 127.90, 127.85, 127.83, 127.79, 127.74, 66.37, 66.16, 66.10, 66.05, 66.02, 65.95, 56.28, 56.07, 54.25, 53.66, 53.33, 52.85, 50.42, 34.00, 30.13, 30.09, 29.81, 26.70, 26.35, 21.17, 15.72, 13.32.
**HRMS (ESI)**: m/z calcd for $C_{64}H_{77}N_6O_{16}P$ $[M+H]^+$ 1216.5134, found 1216.5314.



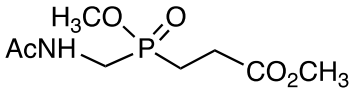

**Inhibitor 2:** To a solution of compound **11** (25 mg, 0.02 mmol) in ethanol (2 mL) was added 10 wt% Pd/C (15 mg), and the mixture was stirred under $H_2$ (1 atm) for 8 h at rt. After filtration, the filtrate was concentrated under vacuum to give a clear oil which was dissolved in $H_2O$ (1 mL). The pH of the solution was adjusted to 10 using NaOH (2 M), and the sample was loaded onto a column of AG 1-X8 resin (formate form, 10 mL, equilibrated with $H_2O$). The column was eluted with a stepwise gradient of 0.5 M to 4 M formic acid. The fractions containing the desired compound were lyophilized to give inhibitor **2** as a white solid (6 mg, 40%).

**$^1$H NMR** (400 MHz, MeOD) δ 4.36 – 4.12 (m, 5H), 3.12 (dq, *J* = 7.1, 2.7 Hz, 2H), 2.58 – 2.46 (m, 2H), 2.45 – 2.32 (m, 6H), 2.11 – 1.96 (m, 4H), 1.95 (s, 3H), 1.93 – 1.80 (m, 6H), 1.72 – 1.54 (m, 2H), 1.31 (d, *J* = 7.2 Hz, 3H), 1.02 (t, *J* = 7.3 Hz, 3H).
**$^{31}$P NMR** (162 MHz, MeOD) δ 48.00.
**$^{13}$C NMR** (101 MHz, $D_2O$) δ 176.96, 176.93, 176.88, 175.16, 174.39, 173.47, 173.28, 173.07, 172.55, 54.47, 54.32, 53.33, 53.07, 52.98, 49.93, 34.53, 29.95, 29.88, 26.95, 26.19, 26.03, 25.90, 25.49, 24.59, 23.83, 23.57, 21.65, 16.28, 13.42.
**HRMS (ESI):** m/z calcd for $C_{29}H_{47}N_6O_{16}P$ $[M+H]^+$ 766.2789, found 766.2789.

**Inhibitor 3 & 4**

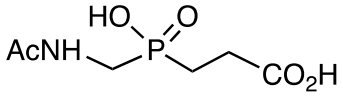



Compound **12** is a literature known compound.[9]



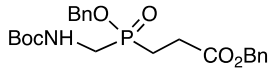
**Compound 13:** Compound **12** (300 mg, 3.16 mmol) was dissolved in acetic acid (0.1 M, 3 mL). Acetic anhydride (1 mL, 17.5 mmol) was added dropwise while maintaining pH around 7 using triethylamine. The reaction mixture was stirred for 30 min and lyophilized to give acetylated compound **12** as a white solid, which was used without further purification.
In a double-neck flask, acetylated compound **12** and hexamethyldisilazane (2.55 g, 15.80 mmol) were added under Ar. After refluxing at 110 °C for 1h, methyl acrylate (353 mg, 4.10 mmol) was added dropwise and the mixture was stirred for 3 h at 110 °C. After cooling to 70 °C, ethanol (5 mL) was added to quench the reaction. The reaction mixture was concentrated in vacuo, and partitioned with $NaHCO_3$ (5%, 15 mL) and diethyl ether (15 mL). The aqueous layer was acidified to pH = 1 using 1M HCl, and the product was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo.
The residue was dissolved in 20% MeOH in toluene (20 mL), and trimethylsilyldiazomethane in hexane (2 M) was added dropwise until yellow color was retained. The reaction mixture was stirred for 1 h under Ar at rt, and then concentrated under vacuum. The residue was purified using silica gel column chromatography (5% MeOH in DCM) to give compound **13** as a white solid (250 mg, 41%).

**$^{1}$H NMR** (300 MHz, MeOD) δ 3.81-3.70 (m, 2H), 3.77 (d, *J* = 10.7 Hz, 3H), 3.71 (s, 3H), 2.76 – 2.52 (m, 2H), 2.19 – 2.06 (m, 2H), 2.01 (s, 3H).
**$^{31}$P NMR** (121 MHz, MeOD) δ 54.87.
**$^{13}$C NMR** (75 MHz, MeOD) δ 172.59, 171.80, 51.10, [51.04, 50.95], [36.33, 34.99], [25.55, 25.51], [21.55, 20.30], 20.84.
**HRMS (ESI)**: m/z calcd for $C_8H_{16}NO_5P$ $[M+H]^+$ 237.0768, found 237.0766.



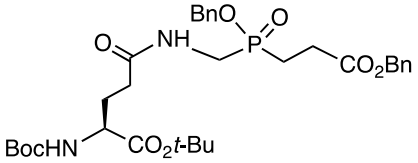
**Inhibitor 3:** Compound **13** (140 mg, 0.59 mmol) was dissolved in NaOH (0.5 M, 5 mL) and the mixture was stirred for 3 h at rt. The solution was acidified to pH 2 by the addition of Amberlite IR-120H ion exchange resin and then the mixture was filtered. The filtrate was concentrated to 1 mL under vacuum and the pH of the solution was readjusted to 10 using 2 M NaOH. The solution was loaded onto a column of AG 1-X8 resin (formate form, 10 mL, equilibrated with $H_2O$) and eluted with a stepwise gradient of 0.5 M to 4 M formic acid. The fractions containing the desired compound were lyophilized to give inhibitor **3** as a white solid (119 mg, 79%).

**$^1$H NMR** (400 MHz, MeOD) δ 3.62 (d, *J* = 9.5 Hz, 2H), 2.67 – 2.58 (m, 2H), 2.09 – 2.02 (m, 2H), 2.01 (s, 3H).
**$^{31}$P NMR** (162 MHz, MeOD) δ 47.79.
**$^{13}$C NMR** (101 MHz, MeOD) δ 174.36, 174.21, [38.18, 37.15], [25.88, 25.85], [22.91, 21.99], 20.89.
**HRMS (ESI)**: m/z calcd for $C_6H_{12}NO_5P$ $[M+H]^+$ 209.0455, found 209.0453.




**Compound 14:** Compound **12** (2.85 g, 30.2 mmol) was dissolved in $H_2O$ (100 mL) and triethylamine (4.60 g, 45.3 mmol) was added. Di-tert-butyl decarbonate (6.60 mg, 30.2 mmol) was added and the mixture was stirred for 8 h under Ar at rt. The solution was diluted with $NaHCO_3$ (5%, 100 mL) and washed with diethyl ether (3 x 100 mL). The aqueous layer was acidified to pH = 1 using 1M HCl and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated in vacuo to give Boc protected compound **12** as a white solid. This material was used without further purification.
In a double-neck flask, Boc protected compound **12** (5.90 g) and hexamethyldisilazane (6.35 g, 39.3 mmol) were added under Ar. After refluxing at 110 °C for 1 h, benzyl acrylate (24.5 g, 151 mmol) was added dropwise into solution and stirred for 3 h at 110 °C. After cooling to 70 °C, ethanol (10 mL) was added to quench the reaction. The reaction mixture was concentrated in vacuo, and partitioned with $NaHCO_3$ (5%, 100 mL) and diethyl ether (100 mL). The aqueous layer was acidified to pH = 1 using 1M HCl, and the product was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated in vacuo to give a phosphinic acid.
The phosphinic acid was dissolved in ethanol: $H_2O$ (1:1, 100 mL) and cesium carbonate (492 mg, 1.51 mmol) was added. The reaction mixture was stirred for 1 h at rt and concentrated to dryness in vacuo. The residue was dissolved in DMF (100 mL), and benzyl bromide (25.8 g, 151 mmol) was added. The solution was stirred for 8 h under Ar at rt and evaporated to dryness in vacuo. The reaction mixture was partitioned with EtOAc (100 mL) and $H_2O$ (100 mL) and the organic layer was dried over $Na_2SO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (80% EtOAc in petroleum ether) to give compound **14** as a yellow oil (7.37 g, 55%).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.44 – 7.32 (m, 10H), 5.14 (s, 2H), 5.09 (d, *J* = 8.3 Hz, 2H), 4.93 (bs, 1H), 3.67 – 3.39 (m, 2H), 2.82 – 2.62 (m, 2H), 2.22 – 2.04 (m, 2H), 1.45 (s, 9H).
**$^{31}$P NMR** (162 MHz, $CDCl_3$) δ 52.15.
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.13, 155.51, [136.17, 136.12], 135.56, 128.77, 128.70, 128.61, 128.39, 128.28, 128.16, 80.42, 66.86, [66.60, 66.54], [38.99, 37.99], 28.27, [26.51, 26.48], [22.39, 21.47].
**HRMS (ESI)**: m/z calcd for $C_{23}H_{30}NO_6P$ $[M+H]^+$ 447.1813, found 447.1811.



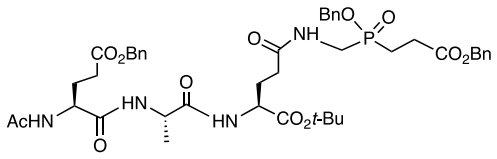

**Compound 15:** Compound **14** (4.95 g, 11.1 mmol) was dissolved in DCM (10 mL), and trifluoroacetic acid (10 mL, 0.13 mol) was added. The mixture was stirred under rt for 3 h at rt and then concentrated under vacuum. After dissolving the residue in anhydrous DCM (50 mL), the solution was cooled to 0 °C. HOBt (3.36 g, 16.6 mmol) and EDC (3.18 g, 16.6 mmol) were added and the mixture was stirred under Ar for 20 min at 0 °C. N,N-diisopropylethylamine (1.43 g, 11.1 mmol) was added. Boc-Glu-OtBu (3.36 g, 11.1 mmol) in anhydrous DCM (10 mL) was added dropwise and the mixture was stirred under Ar for 1 h at 0 °C, then 2 h at rt. The reaction mixture was diluted with DCM (100 mL) and then washed with $H_2O$ (50 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (4% MeOH in DCM) to give compound **15** as a white solid (mixture of diastereomers, 3.62 g, 52% yield).

**$^1$H NMR** (300 MHz, MeOD) δ 7.49 – 7.22 (m, 10H), 5.09 (s, 2H), 5.06 (d, $J$ = 2.2 Hz, 2H), 4.02 – 3.88 (m, 1H), 3.84 – 3.59 (m, 2H), 2.76 – 2.55 (m, 2H), 2.32 (t, $J$ = 7.6 Hz, 2H), 2.25 – 2.04 (m, 3H), 1.95 – 1.74 (m, 1H), 1.45 (s, 9H), 1.43 (s, 9H).

**$^{31}$P NMR** (122 MHz, $CDCl_3$) δ 50.67.

**$^{13}$C NMR** (101 MHz, MeOD) δ 174.81, 173.44, 173.13, 158.10, [137.77, 137.72], 137.39, 129.71, 129.60, 129.55, 129.30, 129.26, 129.17, 82.76, 80.54, [67.89, 67.83], 67.77, 65.23, 55.36, [38.43, 37.43], 32.95, 28.73, 28.25, 27.29, [23.46, 22.53].

**HRMS (ESI)**: m/z calcd for $C_{32}H_{45}N_2O_9P$ $[M+H]^+$ 632.2864, found 632.2863.




**Compound 16:** Compound **15** (1.62 g, 2.56 mmol) was dissolved in HCl in dioxane (4M, 30mL) and stirred for 30 min at rt. The reaction mixture was concentrated in vacuo to give the ammonium chloride which was used without further purification.

Ac-Glu(OBn)-Ala-OtBu (1.07 g, 2.56 mmol) was dissolved in DCM (3 mL), and trifluoroacetic acid (3 mL, 39 mmol) was added. The reaction mixture was stirred under rt for 3 h and then concentrated in vacuo to give Ac-Glu(OBn)-Ala-OH, which was used without further purification.

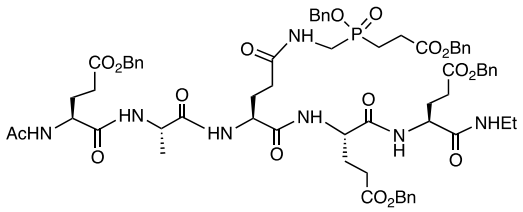
After dissolving Ac-Glu(OBn)-Ala-OH (0.90 g, 2.56 mmol) in anhydrous DCM (50 mL), the solution was cooled to 0 °C. HOBt (0.47 , 3.07 mmol) and EDC (0.59 g, 3.07 mmol) were added and the mixture was stirred under Ar for 20 min at 0 °C. The ammonium chloride in anhydrous DCM (20 mL) was added dropwise and the mixture was stirred under Ar for 1 h at 0 °C, then 2 h at rt. The reaction mixture was diluted with DCM (100 mL) and then washed with $H_2O$ (3x30 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (6% MeOH in DCM) to give compound **16** as a white solid (mixture of diastereomers, 1.13 g, 51% yield).

**$^1$H NMR** (300 MHz, MeOD) δ 7.44 – 7.24 (m, 15H), 5.12 (s, 2H), 5.09 (s, 2H), 5.08 – 5.04 (m, 2H), 4.31 – 4.16 (m, 3H), 3.87 – 3.55 (m, 2H), 2.72 – 2.58 (m, 2H), 2.54 – 2.42 (m, 2H), 2.27 – 2.14 (m, 5H), 2.13 – 1.94 (m, 3H), [1.93 (s), 1.92 (s), 3H], 1.45 (s, 9H), 1.37 (d, $J$ = 6.7 Hz, 3H).

**$^{31}$P NMR** (122 MHz, MeOD) δ 53.13, 53.07.

**$^{13}$C NMR** (151 MHz, MeOD) δ 174.95, [174.73, 174.69], [174.46, 174.43], 174.15, 174.06, 173.75, [172.19, 172.17], 142.69, 137.71, 129.69, [129.58, 129.55], 129.35, [129.17, 129.12], 128.26, 127.99, 83.02, 67.81, 65.22, 54.81, [53.49, 53.47], 51.07, 38.12, 32.74, 30.99, 28.22, 27.89, 27.48, 26.97, 23.14, 22.52, 22.44, 17.60.

**HRMS (ESI)**: m/z calcd for $C_{44}H_{57}N_4O_{12}P$ $[M+H]^+$ 864.3708, found 864.3711.




**Compound 17:** Compound **16** (320 mg, 0.37 mmol) was dissolved in DCM (3 mL), and trifluoroacetic acid (3 mL, 39 mmol) was added. The solution was stirred at rt for 3 h and then concentrated under vacuum. After dissolving the residue in anhydrous DCM (15 mL), the solution was cooled to 0 °C. HOBt (68 mg, 0.44 mmol) and EDC (85 mg, 0.44 mmol) were added and the reaction mixture was stirred under Ar for 20 min at 0 °C. N,N-diisopropylethylamine (48 mg, 0.37 mmol) was added. Then the literature known dipeptide H-Glu(OBn)-Glu(OBn)-NHEt[8] (180 mg, 0.37 mmol) in anhydrous DCM (5 mL) was added dropwise and the mixture stirred under Ar (1atm) for 1 h at 0 °C, then 2 h at rt. The reaction mixture was diluted with DCM (30 mL) and then washed with $H_2O$ (20 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified using silica gel column chromatography (10% MeOH in DCM) to give compound **17** as a white solid (mixture of diastereomers, 160 mg, 34% yield).

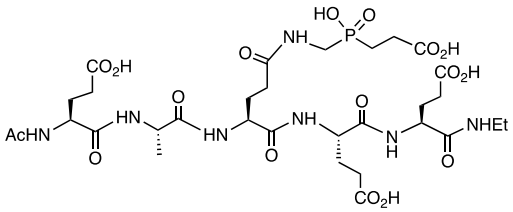
**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.35 – 8.19 (m, 1H), 8.20 – 7.93 (m, 5H), 7.89 – 7.79 (m, 1H), 7.43 – 7.19 (m, 25H), 5.07 (s, 6H), 5.01 – 4.90 (m, 4H), 4.31 – 4.12 (m, 5H), 3.75 – 3.46 (m, 2H),

3.04 (dq, *J* = 13.2, 6.7 Hz, 2H), 2.67 – 2.53 (m, 2H), 2.44 – 2.31 (m, 6H), 2.18 (t, *J* = 7.8 Hz, 2H), 2.12 – 1.99 (m, 2H), 2.01 – 1.86 (m, 5H), 1.83 (s, 3H), 1.82 – 1.72 (m, 3H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.97 (t, *J* = 7.2 Hz, 3H).

**$^{31}$P NMR** (162 MHz, DMSO-$d_6$) δ 51.85, 51.67.

**$^{13}$C NMR** (101 MHz, DMSO) δ 172.72, 172.69, 172.60, 172.23, 172.07, 171.69, 171.60, 171.41, 171.34, 170.80, 170.06, 136.62, 136.45, 128.88, 128.86, 128.72, 128.49, 128.45, 128.39, 128.32, 128.06, 127.09, 126.88, 66.29, 65.97, 65.94, 65.70, 63.35, [52.41, 52.34, 52.27], 48.82, 33.91, 32.08, [30.56, 30.51], 30.38, 28.23, 27.64, 26.58, 22.92, 18.26, 14.99.

**HRMS (ESI)**: m/z calcd for $C_{66}H_{80}N_7O_{17}P$ $[M+H]^+$ 1273.5349, found 1273.5348.




**Inhibitor 4:** To a solution of compound **17** (70 mg, 0.06 mmol) in ethanol (5 mL) was added 10 wt% Pd/C (35 mg), and the solution was stirred under $H_2$ (1 atm) for 8 h at rt. After filtration, the filtrate was concentrated under vacuum to give a clear oil which was dissolved in $H_2O$ (1 mL). The pH of the solution was adjusted to 10 using NaOH (2 M), and the solution was loaded onto a column of AG 1-X8 resin (formate form, 10 mL, equilibrated with $H_2O$). The column was eluted with a stepwise gradient of 0.5 M to 4 M formic acid. The fractions containing the desired compound were lyophilized to give inhibitor **4** as a white solid (28 mg, 62%).

**$^{1}$H NMR** (400 MHz, $D_2O$) δ 4.29 – 4.13 (m, 5H), 3.40 – 3.25 (m, 2H), 3.10 (dq, *J* = 7.2, 2.4 Hz, 2H), 2.48 (q, *J* = 9.3 Hz, 2H), 2.43 – 2.33 (m, 6H), 2.29 (t, *J* = 7.6 Hz, 2H), 2.07 – 1.96 (m, 4H), 1.93 (s, 3H), 1.92 – 1.81 (m, 4H), 1.79 – 1.68 (m, 2H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.00 (t, *J* = 7.3 Hz, 3H).

**$^{31}$P NMR** (162 MHz, $D_2O$) δ 39.04.

**$^{13}$C NMR** (151 MHz, $D_2O$) δ 177.41, 176.98, 176.95, 176.91, 175.11, 174.42, 173.56, 173.37, 173.12, 172.55, 166.09, 53.36, 53.25, 53.10, 53.04, 49.97, 39.19, 38.52, 34.54, 31.72, 29.93, 29.88, 27.12, 26.76, 26.20, 26.10, 25.86, 21.66, 16.22, 13.42.

**HRMS (ESI):** m/z calcd for $C_{31}H_{50}N_7O_{17}P$ $[M+H]^+$ 823.2999, found 823.3001.

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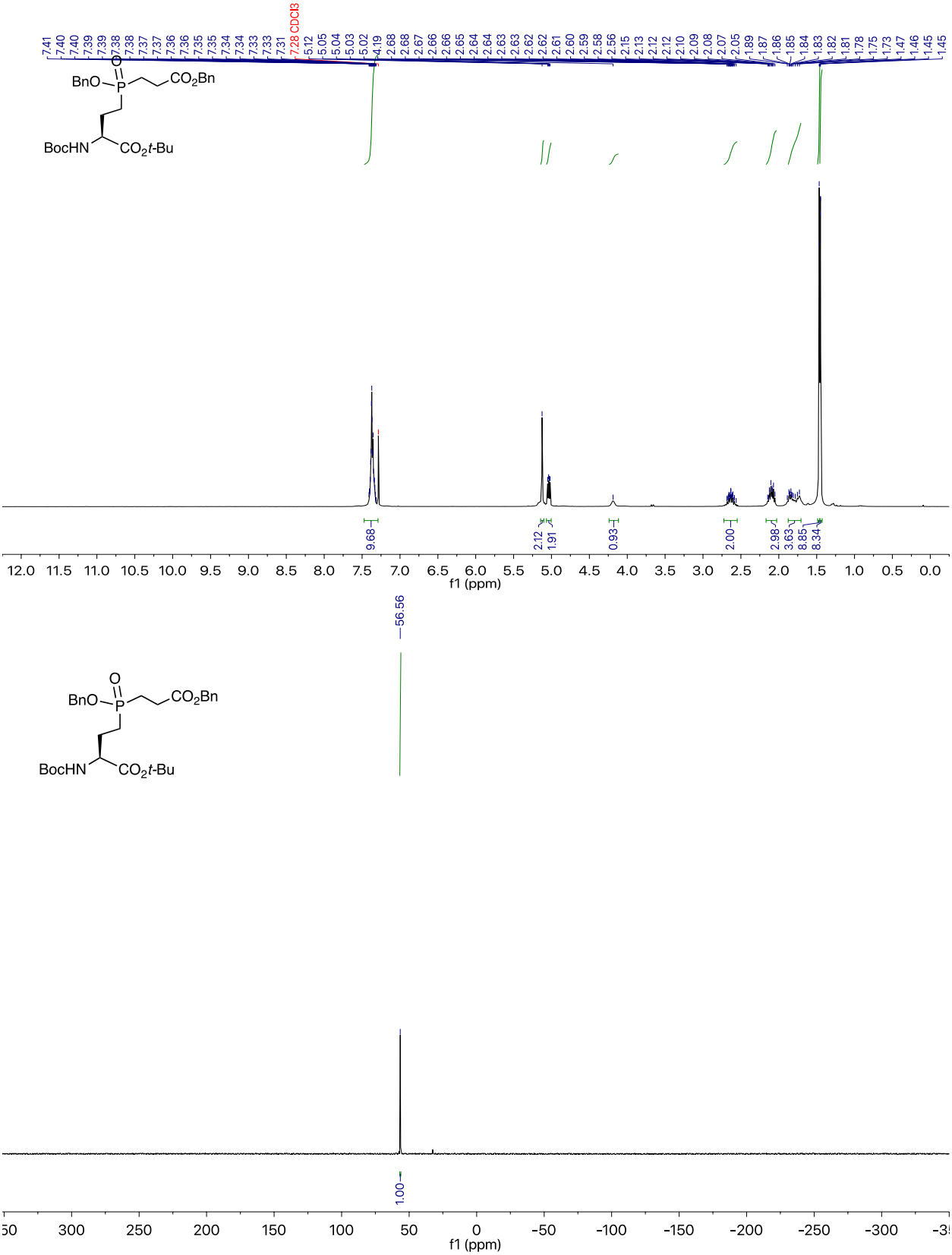
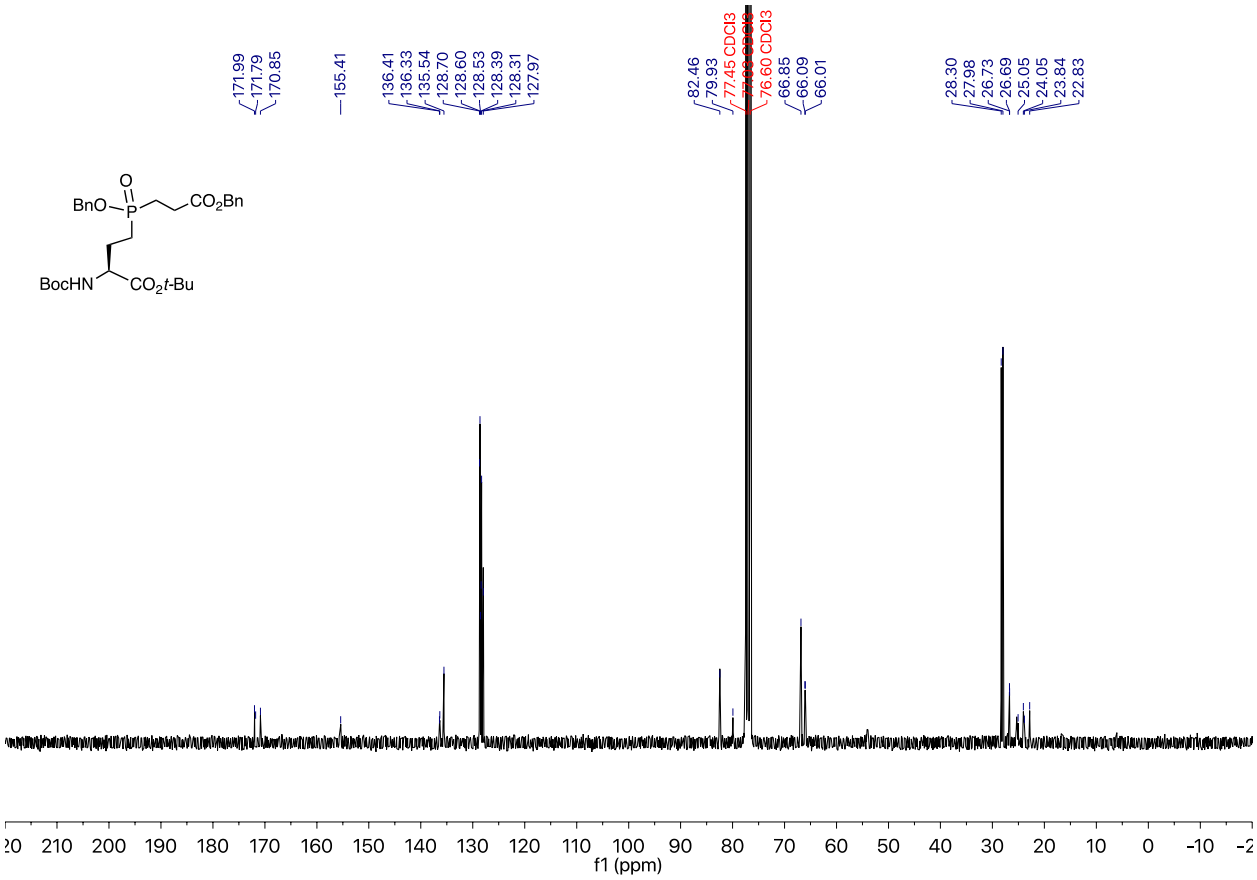
**Figure S1**: $^{1}H$ NMR (400 MHz, $CDCl_3$), $^{31}P$ NMR (162 MHz, $CDCl_3$) and $^{13}C$ NMR (75 MHz, $CDCl_3$) of Compound **7**




171.99
171.79
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155.41
136.41
136.33
135.54
128.70
128.60
128.53
128.39
128.31
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82.46
79.93
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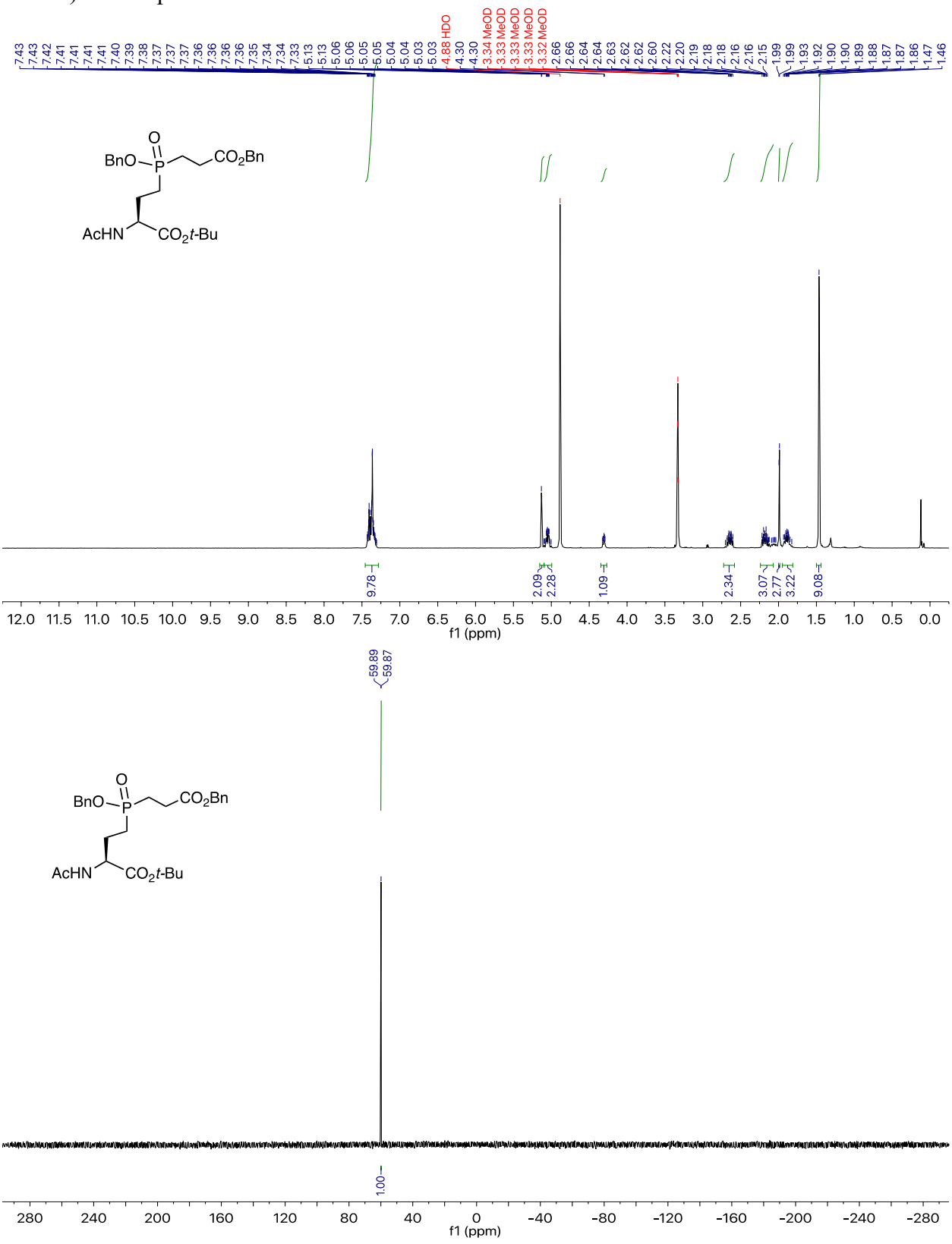
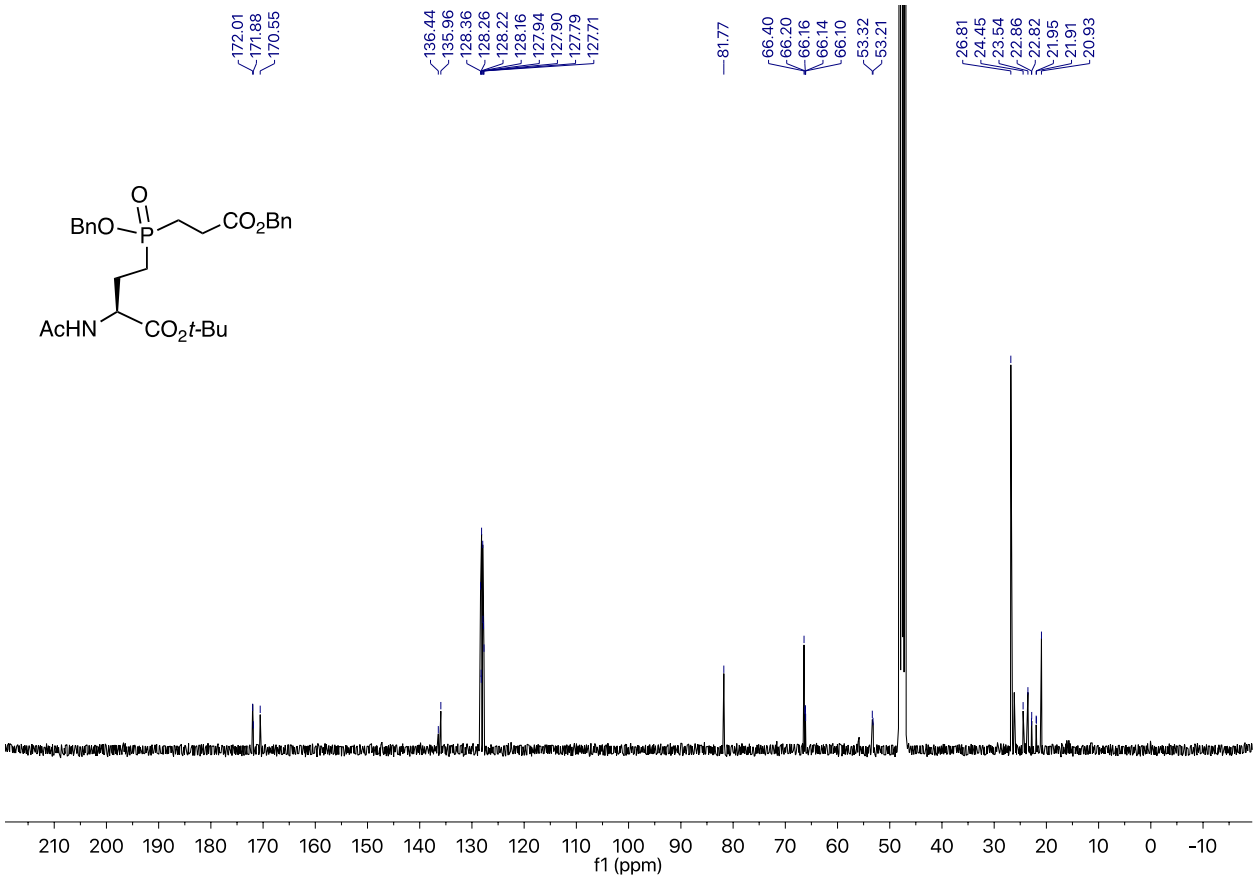
76.60 CDCl3
66.85
66.09
66.01
28.30
27.98
26.73
26.69
25.05
24.05
23.84
22.83
BnO
P
O
CO2Bn
BocHN
CO2t-Bu
f1 (ppm)

**Figure S2**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Compound **8**

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128.22
128.16
127.94
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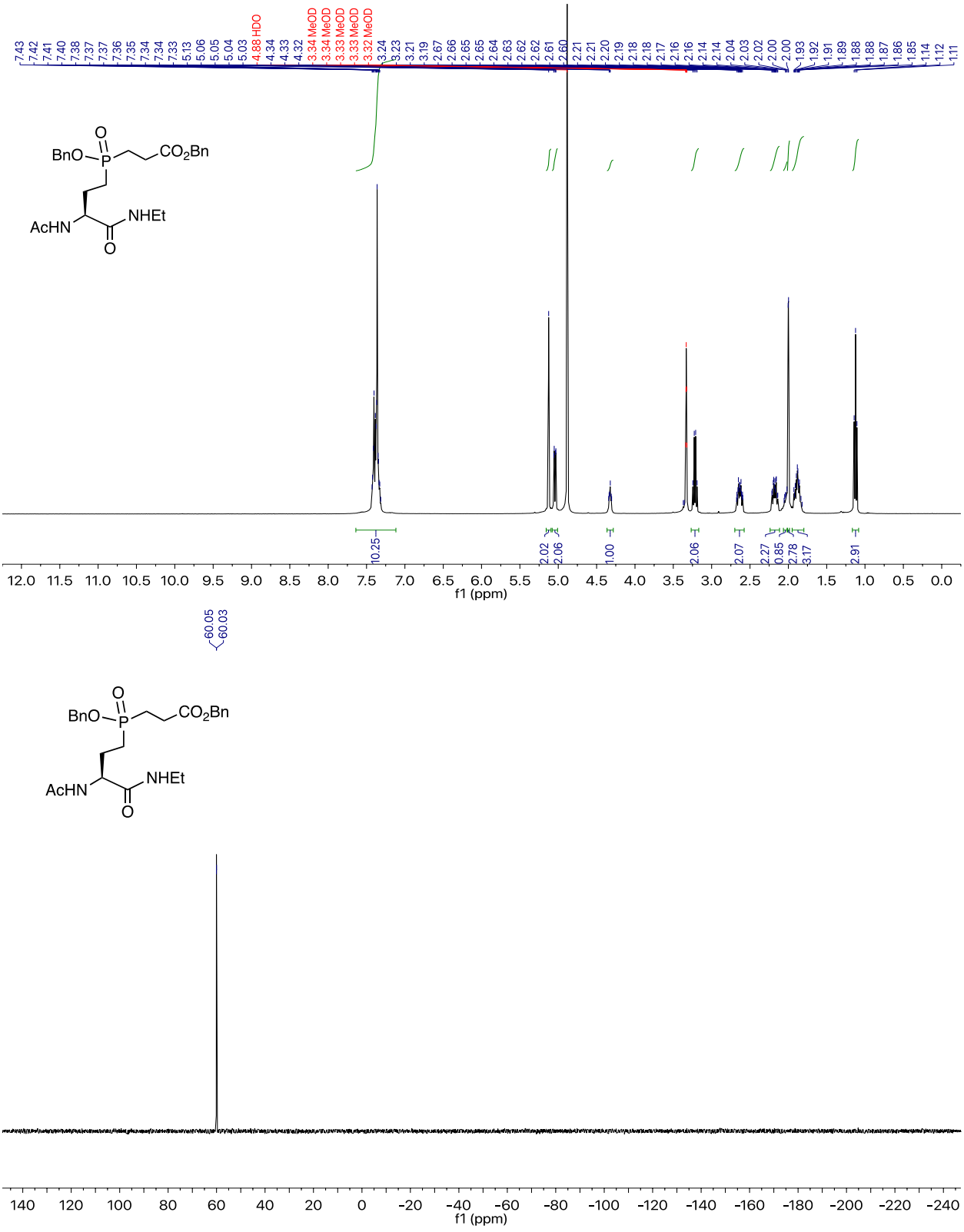
127.79
127.71
81.77
66.40
66.20
66.16
66.14
66.10
53.32
53.21
26.81
24.45
23.54
22.86
22.82
21.95
21.91
20.93
BnO
P
O
CO2Bn
AcHN
CO2t-Bu
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

**Figure S3**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Compound **9**



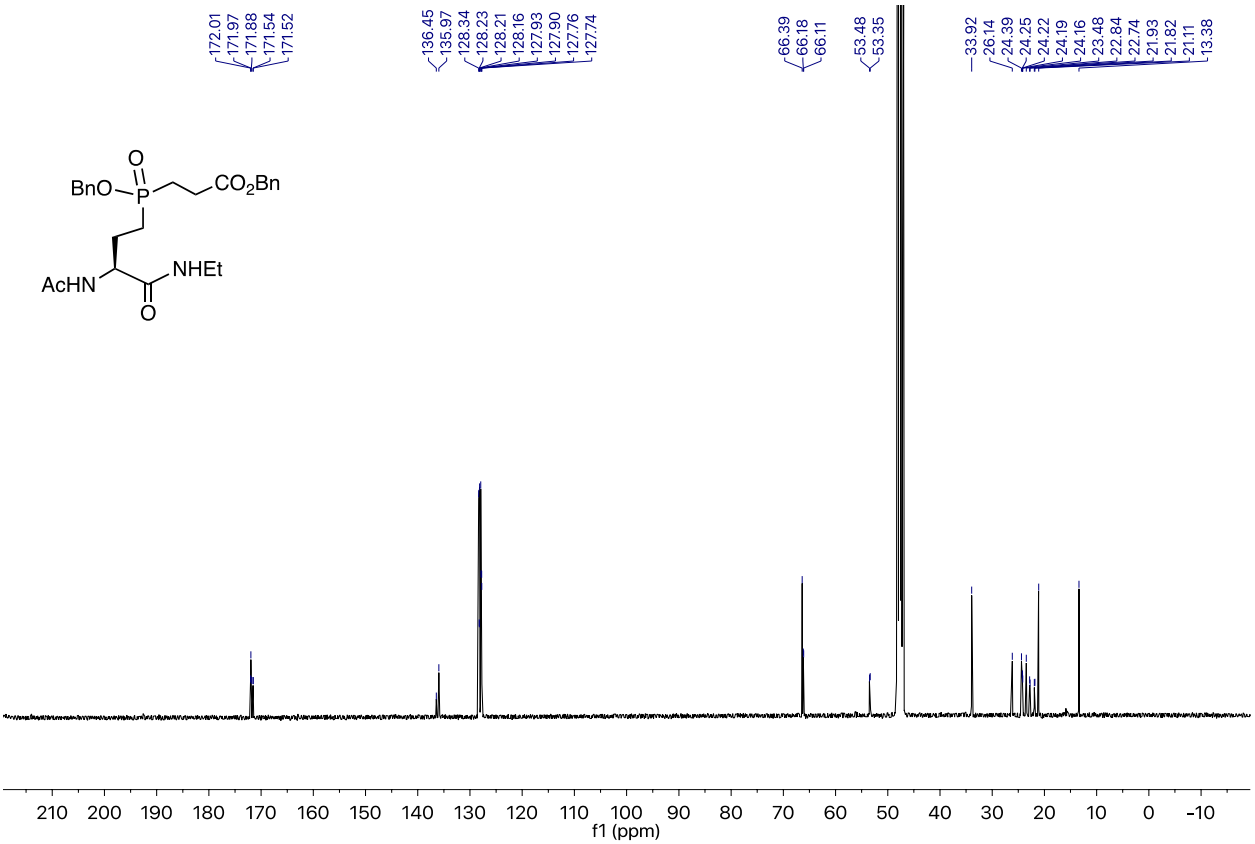
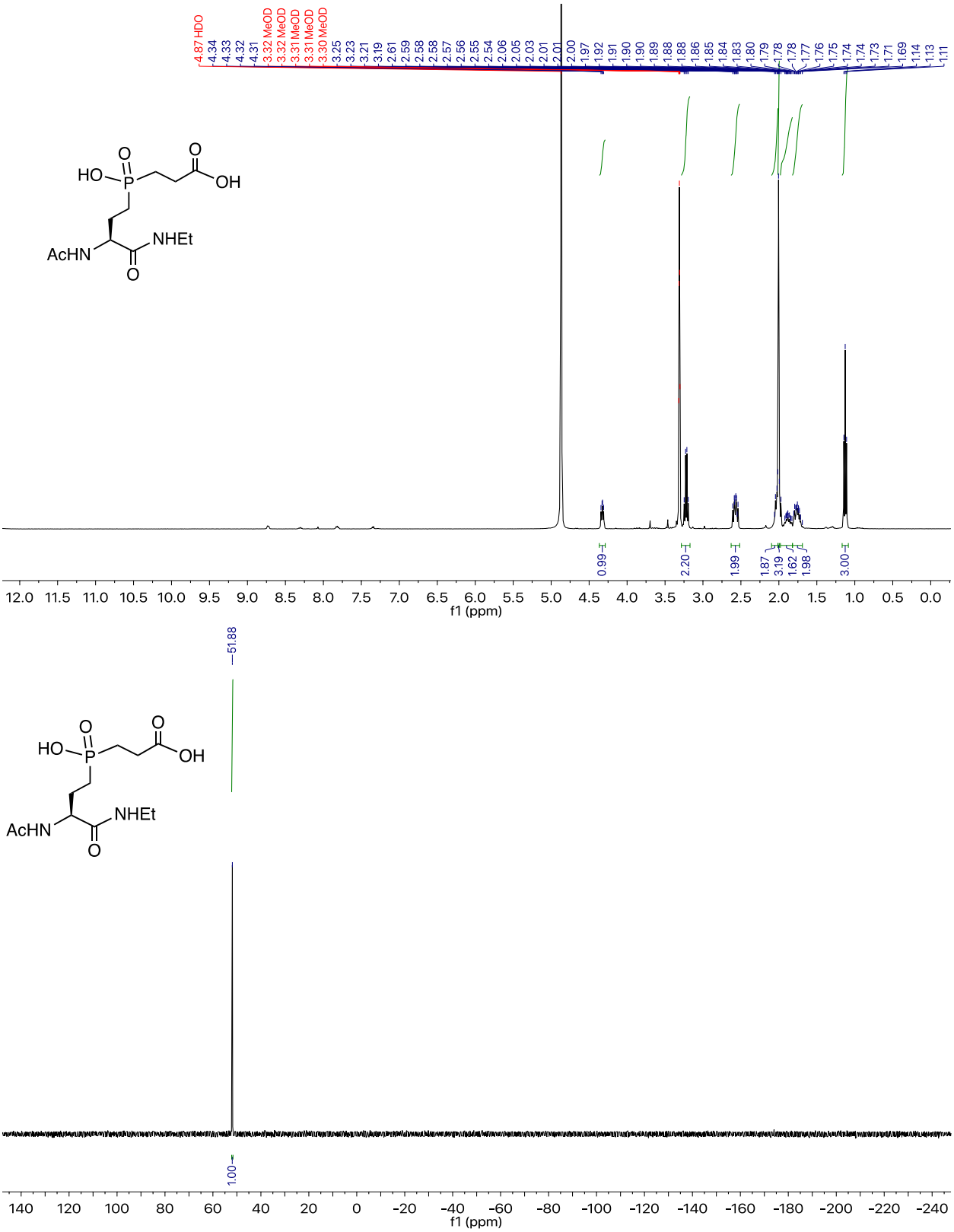

172.01
171.97
171.88
171.54
171.52
136.45
135.97
128.34
128.23
128.21
128.16
127.93
127.90
127.76
127.74
66.39
66.18
66.11
53.48
53.35
33.92
26.14
24.39
24.25
24.22
24.19
24.16
23.48
22.84
22.74
21.93
21.82
21.11
13.38
BnO
P
O
CO2Bn
AcHN
NHEt
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

**Figure S4**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Inhibitor **1**



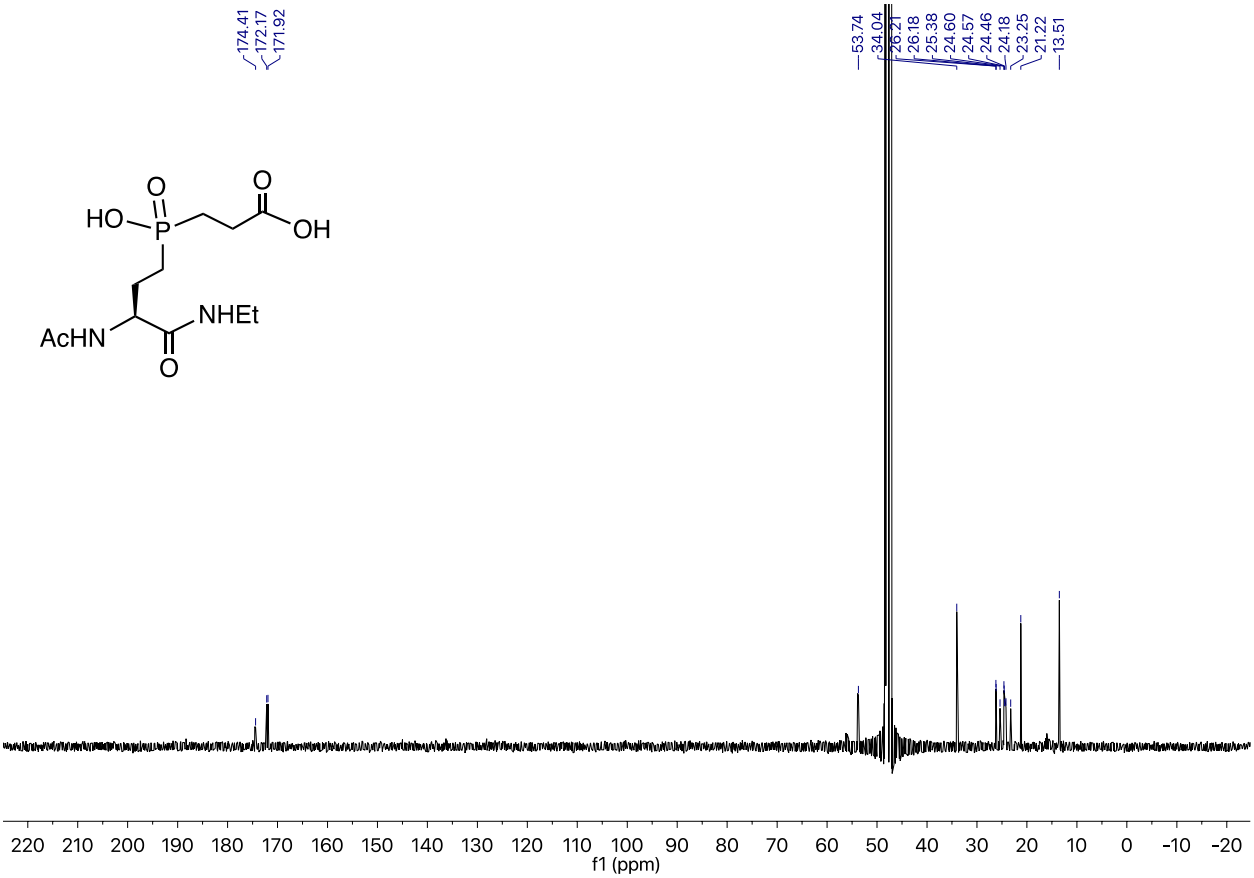
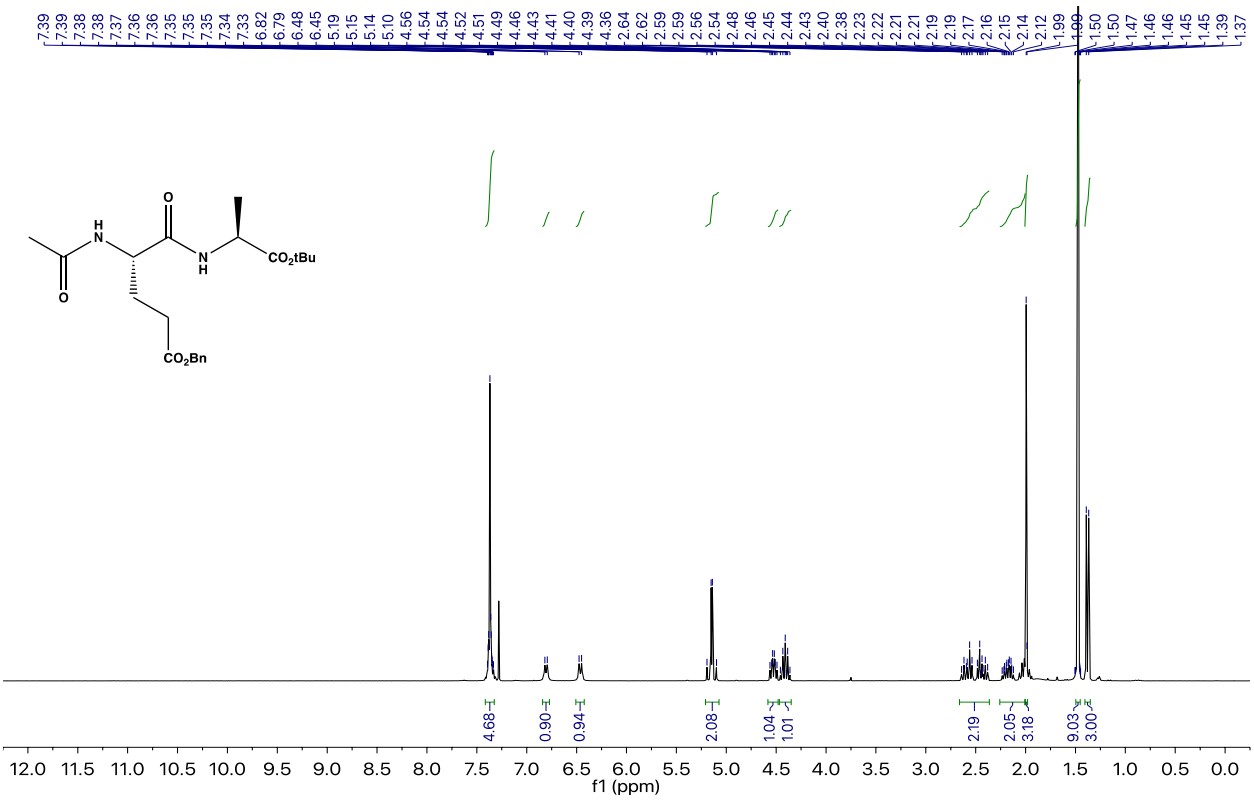

174.41
172.17
171.92
53.74
34.04
26.21
26.18
25.38
24.60
24.57
24.46
24.18
23.25
21.22
13.51
HO
P
O
O
OH
AcHN
NHEt
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

**Figure S5**: $^{1}H$ NMR (300 MHz, $CDCl_3$) and $^{13}C$ NMR (75 MHz, $CDCl_3$) of Ac-Glu(OBn)-Ala-OtBu



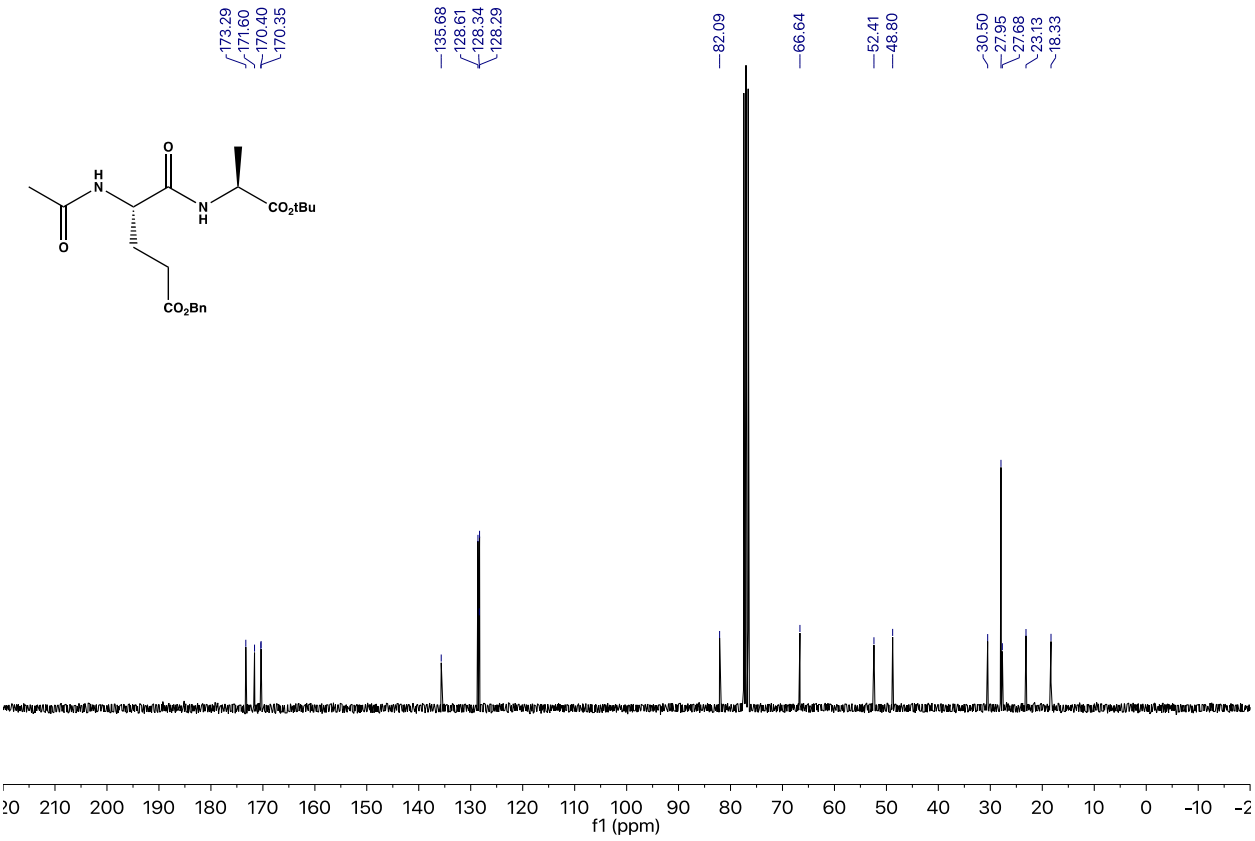
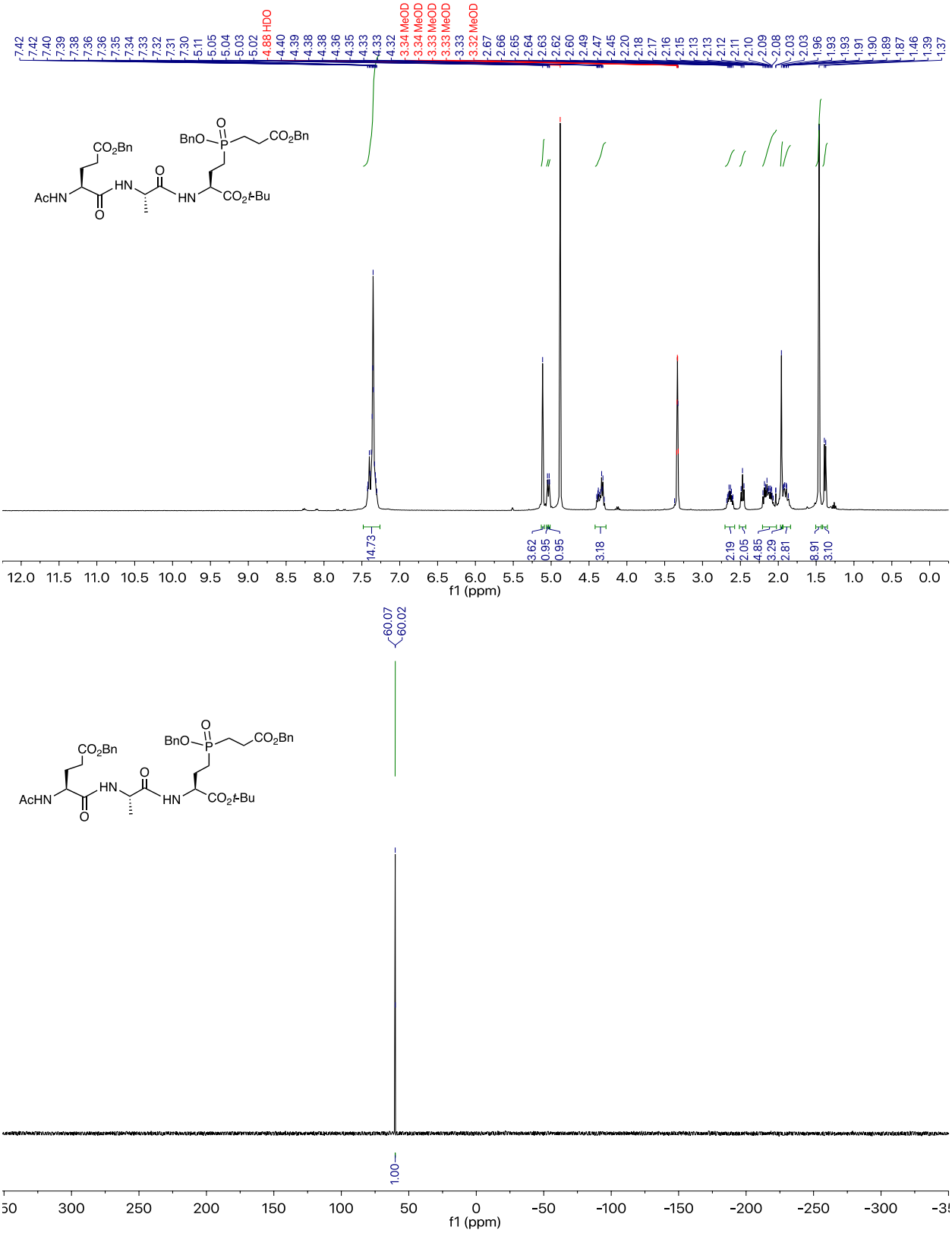

173.29
171.60
170.40
170.35
135.68
128.61
128.34
128.29
82.09
66.64
52.41
48.80
30.50
27.95
27.68
23.13
18.33
H
N
O
O
N
H
O
CO2tBu
CO2Bn
f1 (ppm)

**Figure S6**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Compound **10**



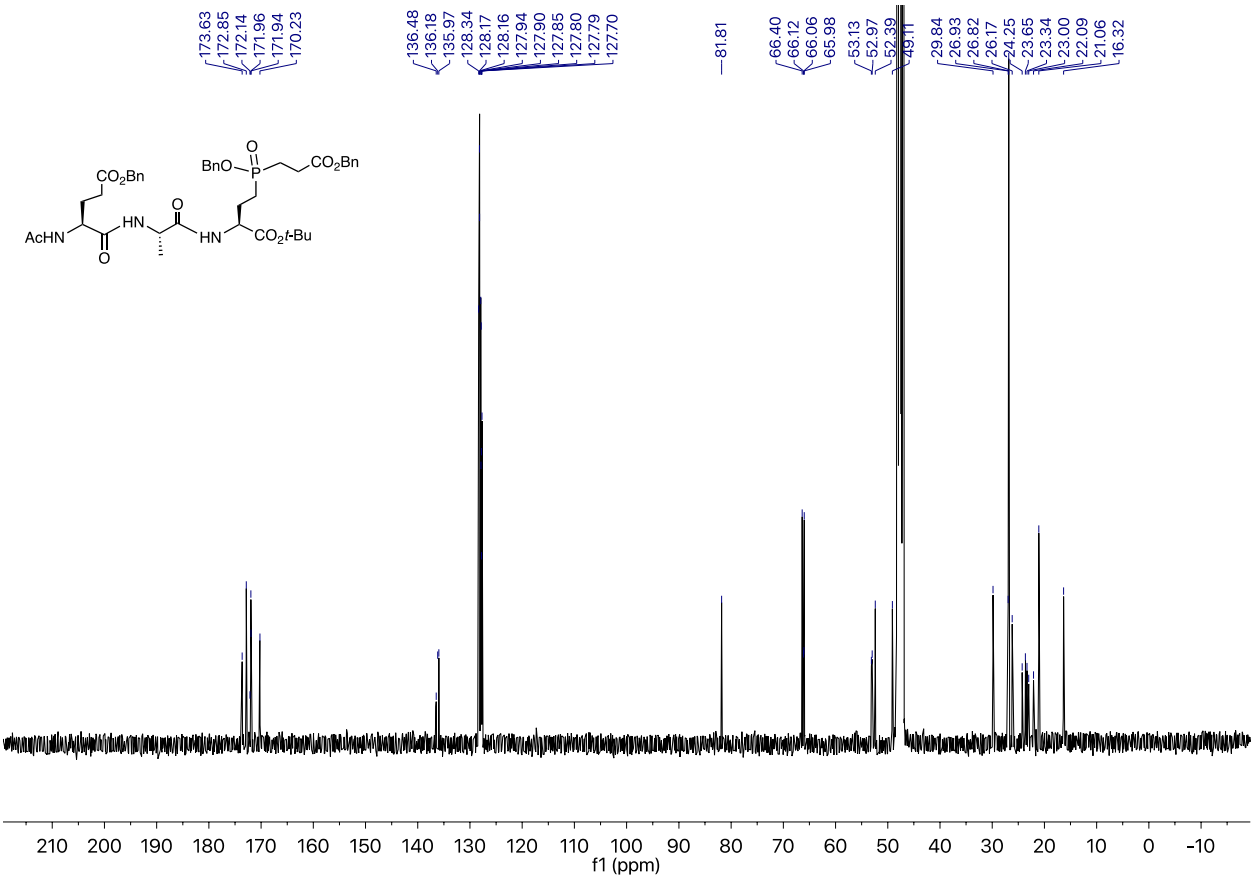
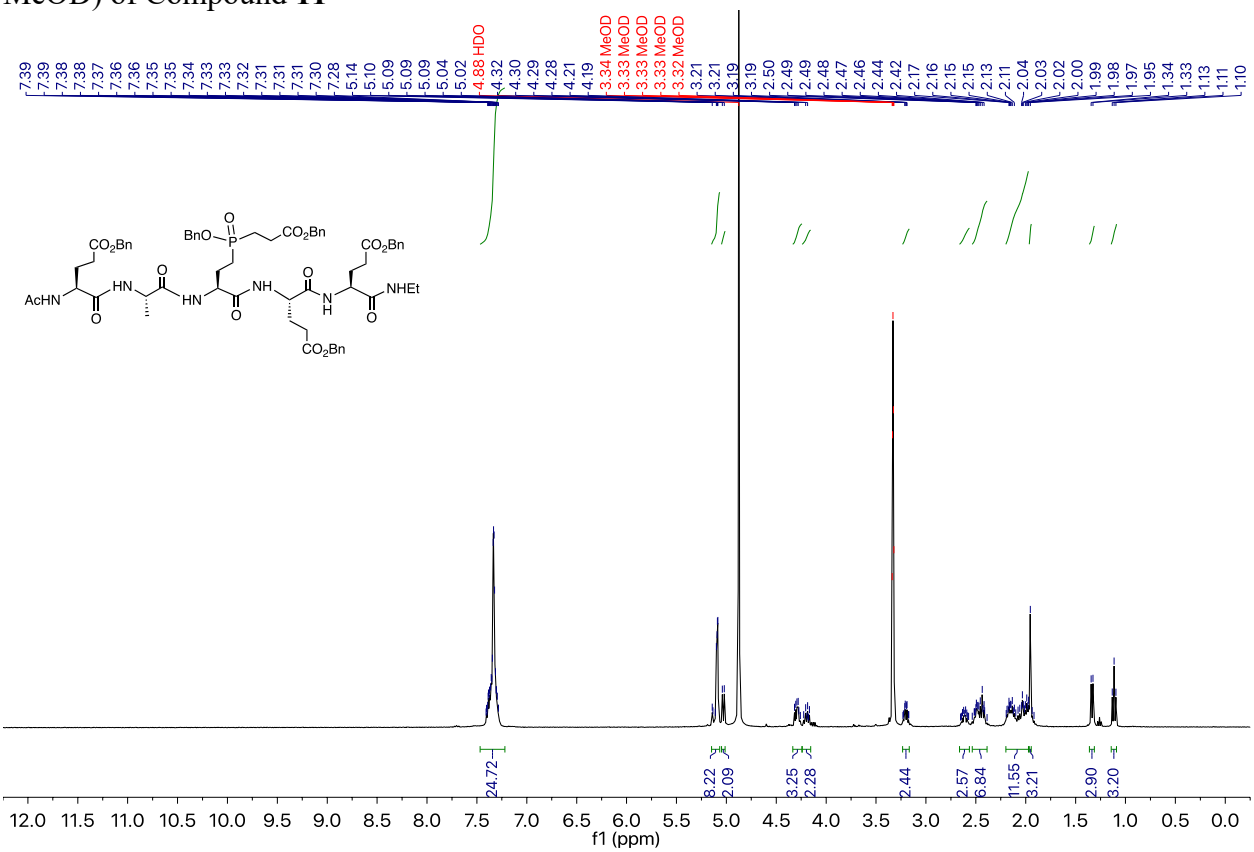
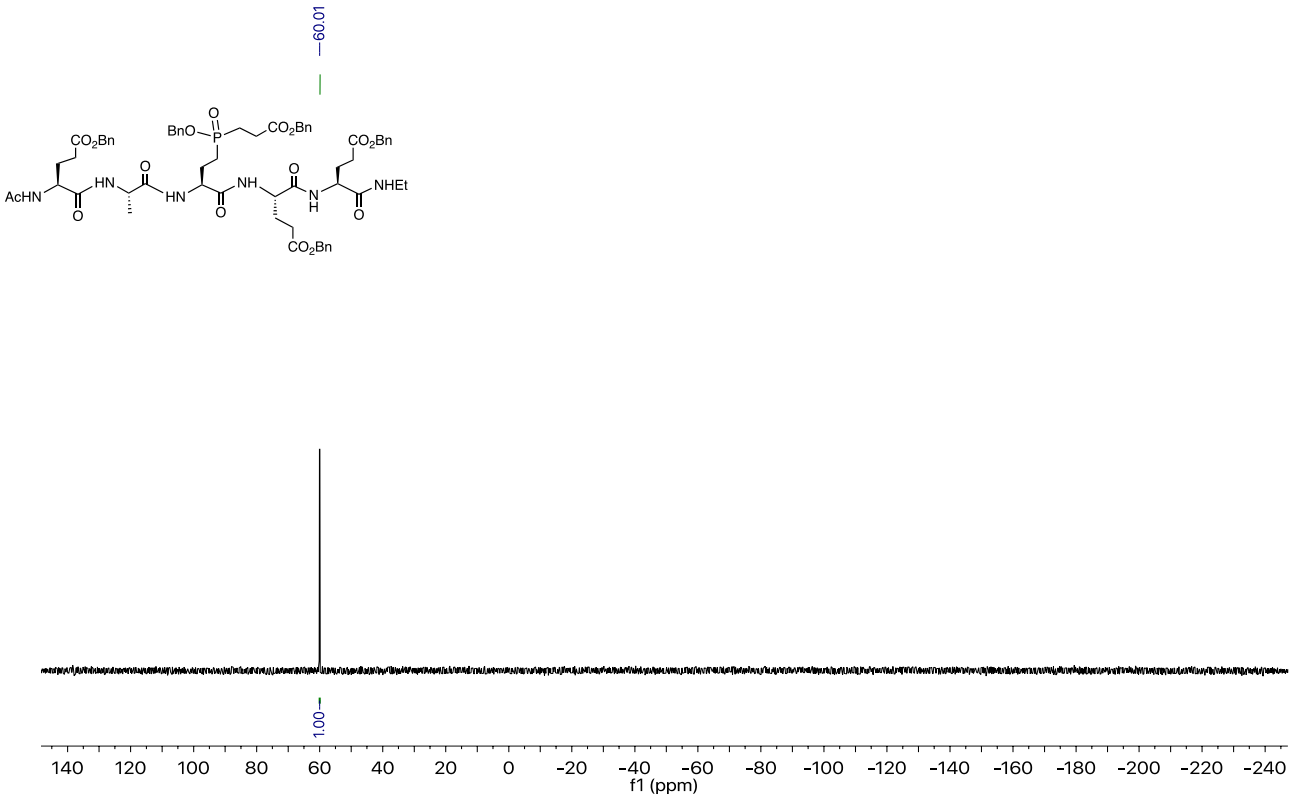

173.63
172.85
172.14
171.96
171.94
170.23
136.48
136.18
135.97
128.34
128.17
128.16
127.94
127.90
127.85
127.80
127.79
127.70
81.81
66.40
66.12
66.06
65.98
53.13
52.97
52.39
49.11
29.84
26.93
26.82
26.17
24.25
23.65
23.34
23.00
22.09
21.06
16.32
CO2Bn
AcHN
HN
O
HN
BnO
P
O
CO2Bn
CO2t-Bu
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

**Figure S7**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Compound **11**



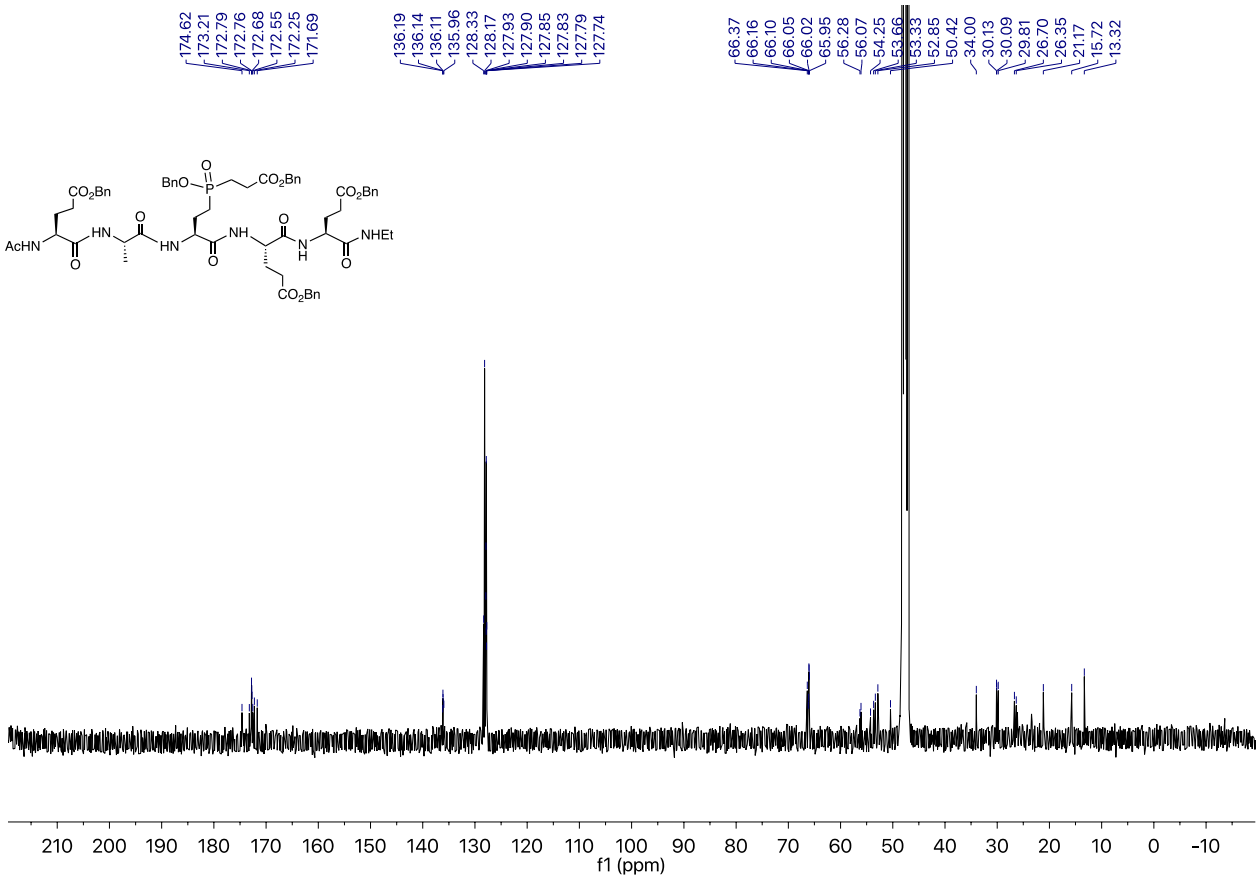
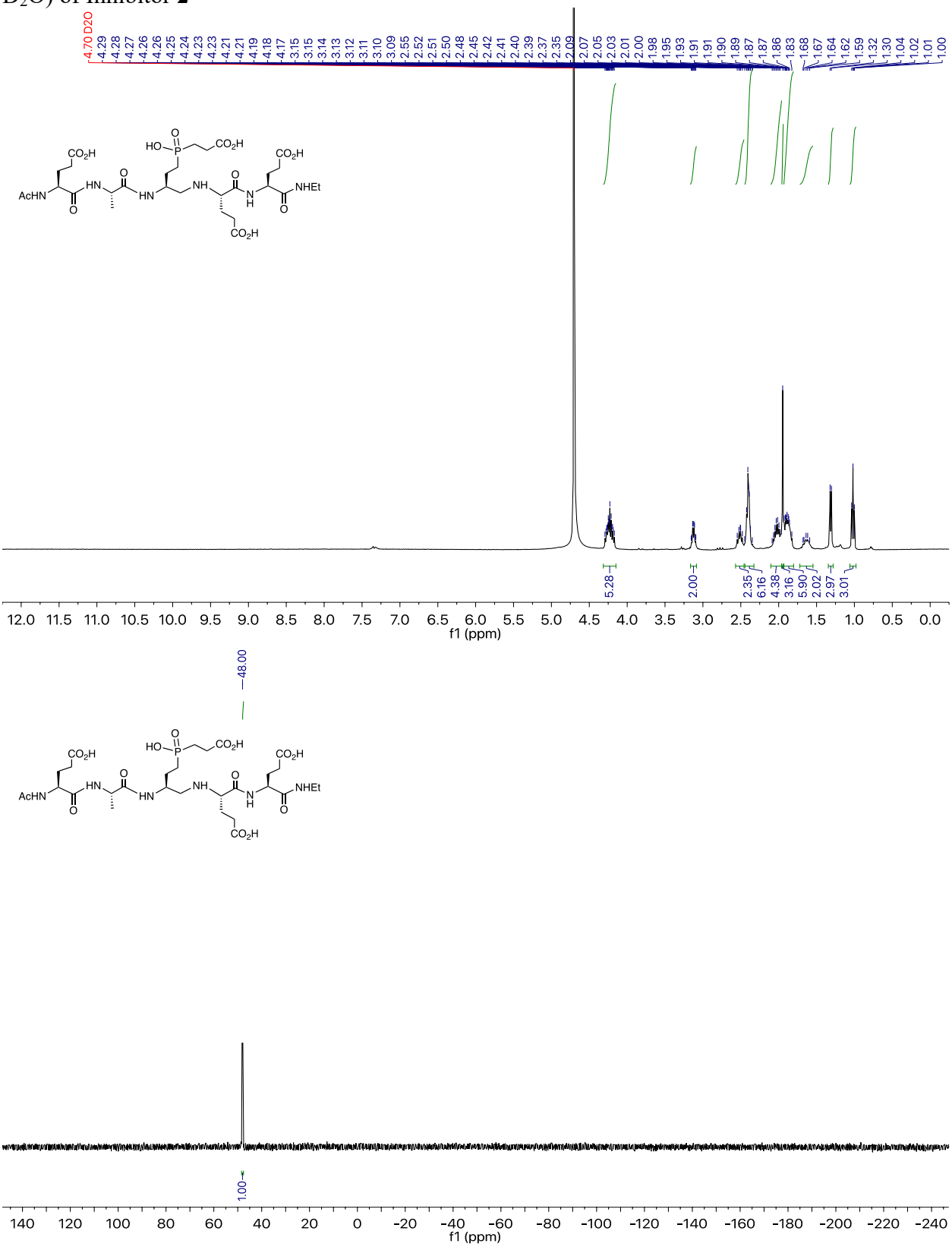

174.62
173.21
172.79
172.76
172.68
172.55
172.25
171.69
136.19
136.14
136.11
135.96
128.33
128.17
127.93
127.90
127.85
127.83
127.79
127.74
66.37
66.16
66.10
66.05
66.02
65.95
56.28
56.07
54.25
53.66
53.33
52.85
50.42
34.00
30.13
30.09
29.81
26.70
26.35
21.17
15.72
13.32
AcHN
HN
HN
NH
N
H
NHEt
BnO
CO2Bn
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)

**Figure S8**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, $D_2O$) of Inhibitor **2**



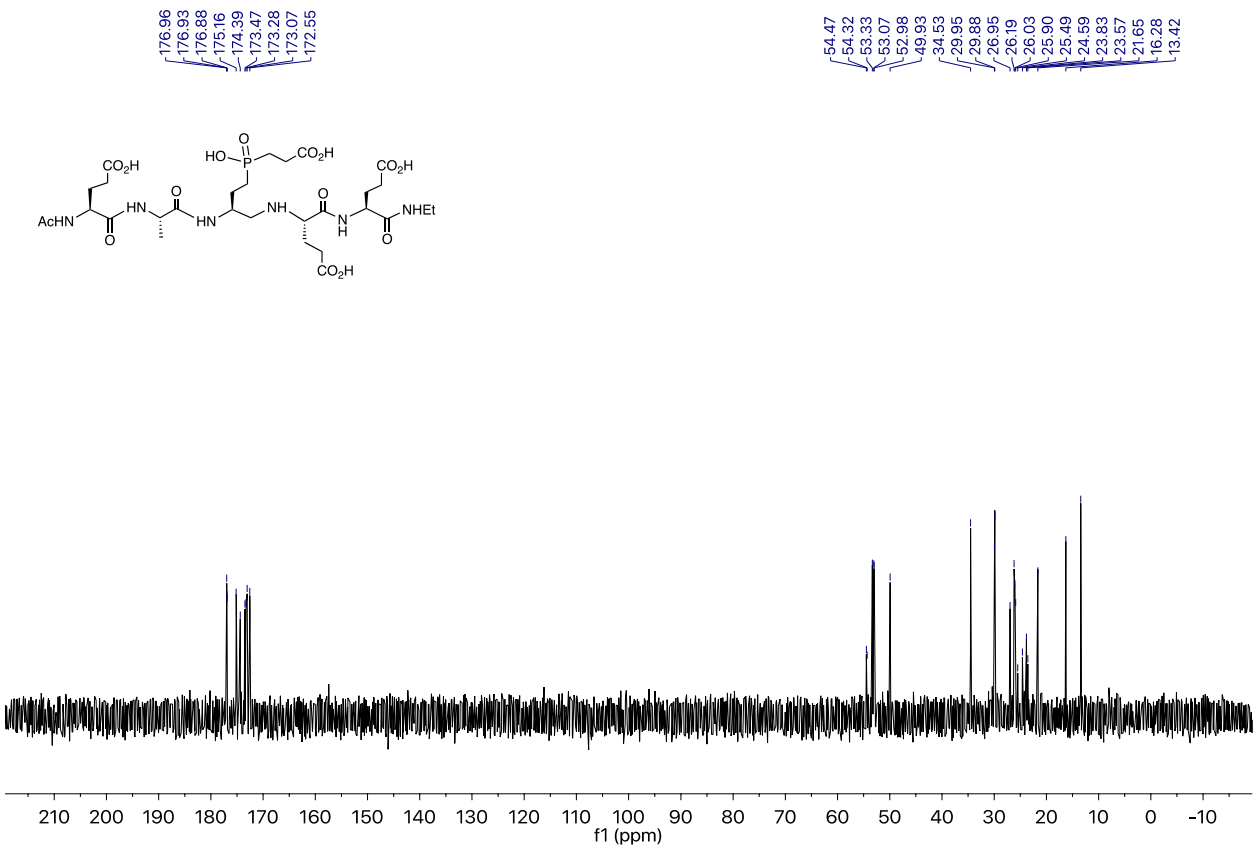
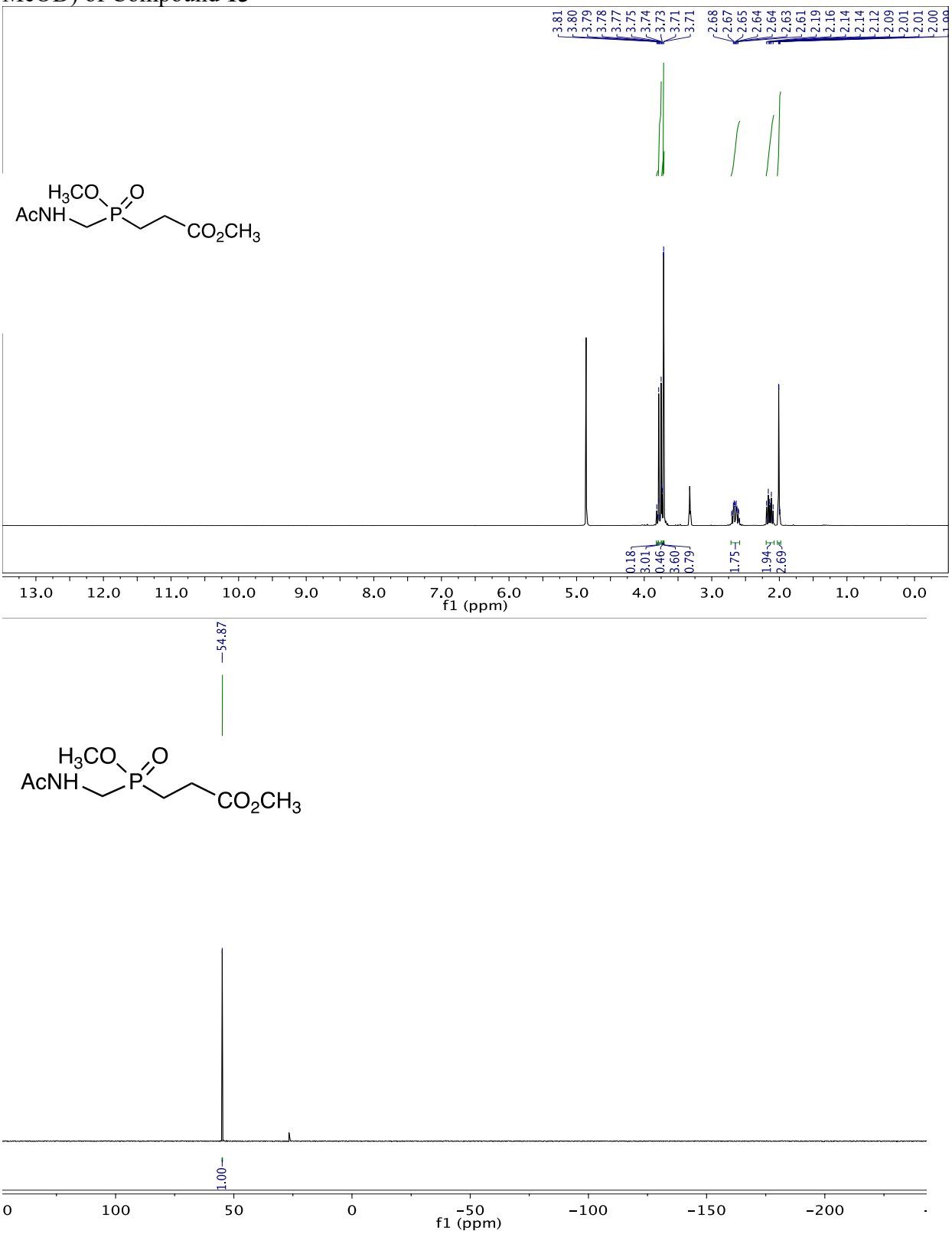

176.96
176.93
176.88
175.16
174.39
173.47
173.28
173.07
172.55
54.47
54.32
53.33
53.07
52.98
49.93
34.53
29.95
29.88
26.95
26.19
26.03
25.90
25.49
24.59
23.83
23.57
21.65
16.28
13.42
AcHN
HN
HN
NH
NHEt
HO
P
CO2H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

**Figure S9**: $^{1}H$ NMR (300 MHz, MeOD), $^{31}P$ NMR (121 MHz, MeOD) and $^{13}C$ NMR (75 MHz, MeOD) of Compound **13**



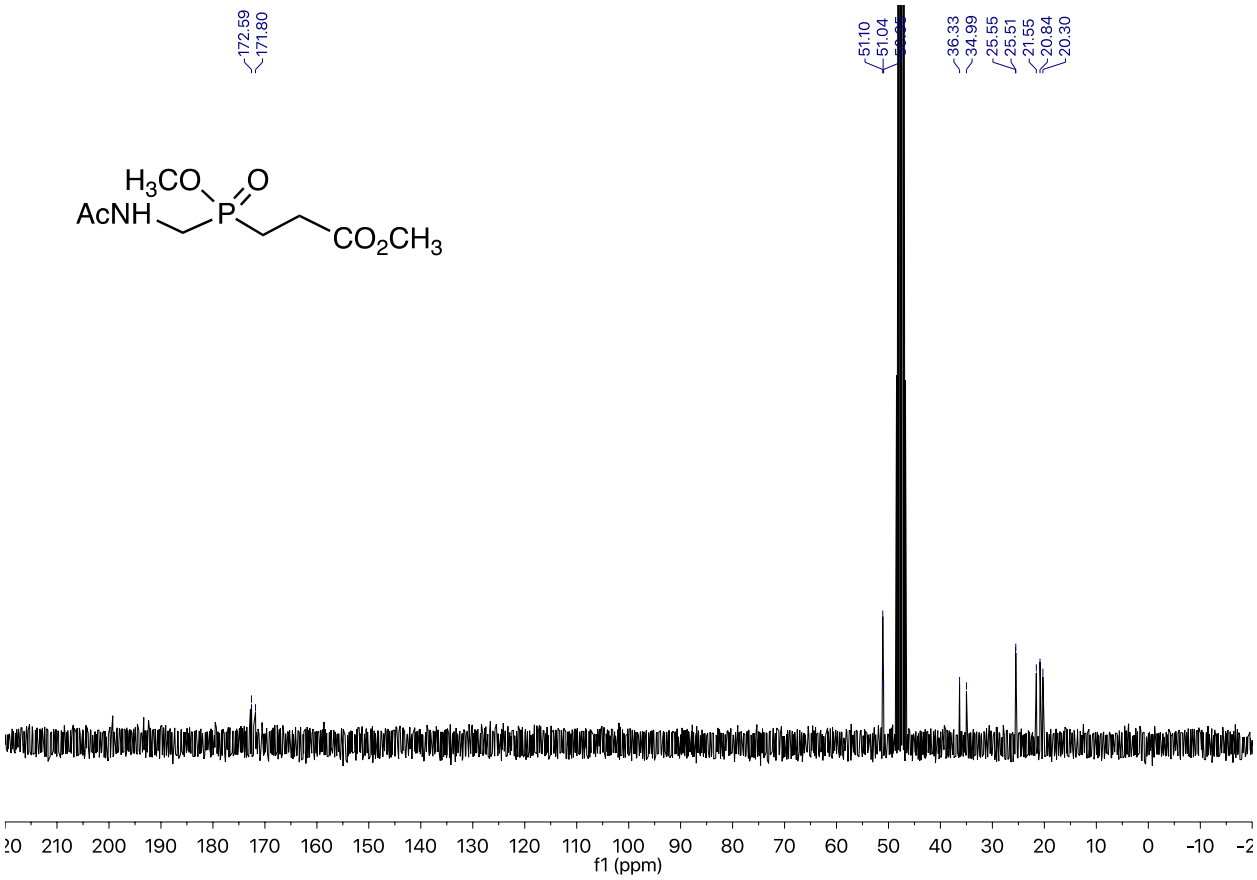
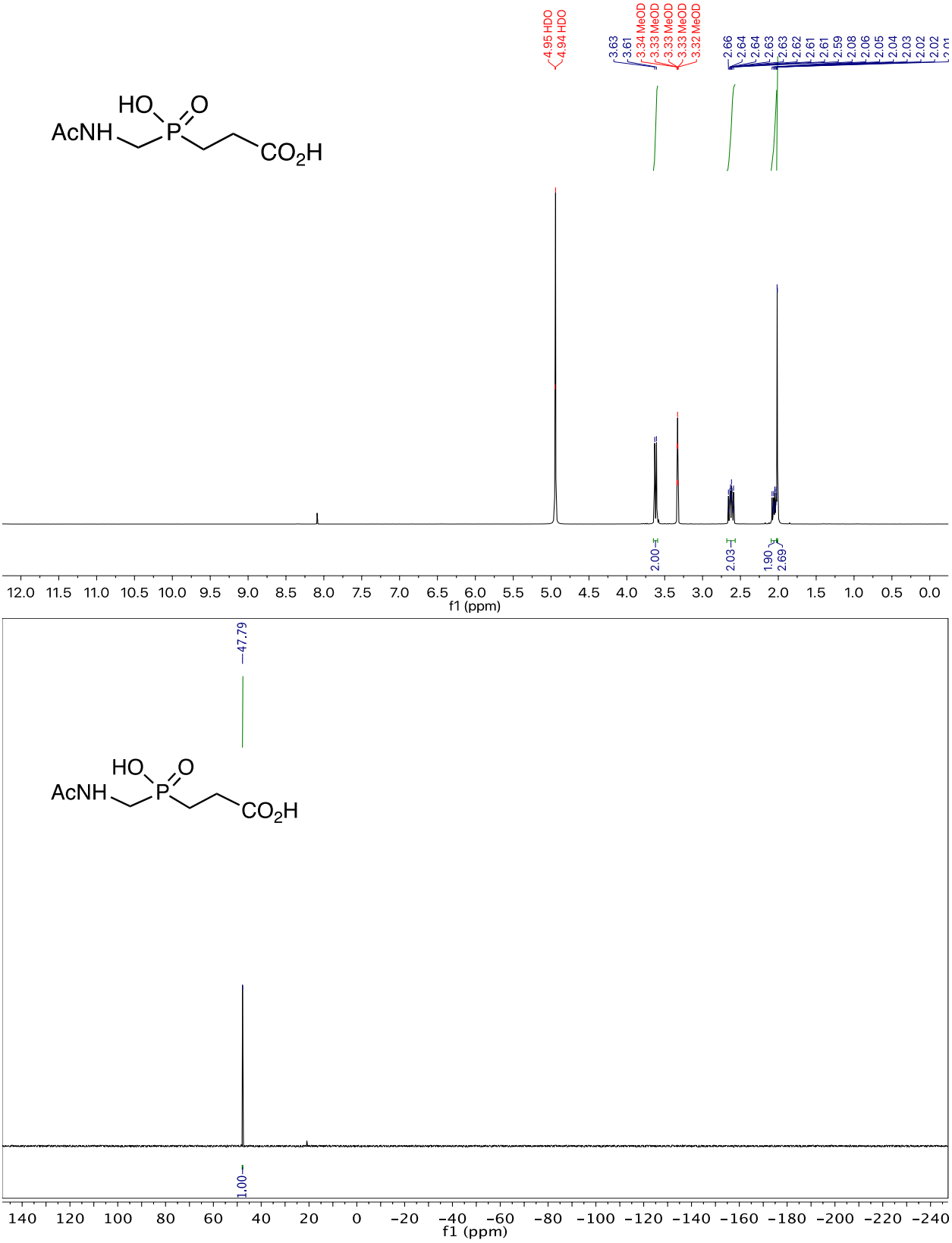

172.59
171.80
51.10
51.04
36.33
34.99
25.55
25.51
21.55
20.84
20.30
H3CO
O
AcNH
P
CO2CH3
f1 (ppm)

**Figure S10**: $^{1}$H NMR (400 MHz, MeOD), $^{31}$P NMR (162 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Inhibitor **3**



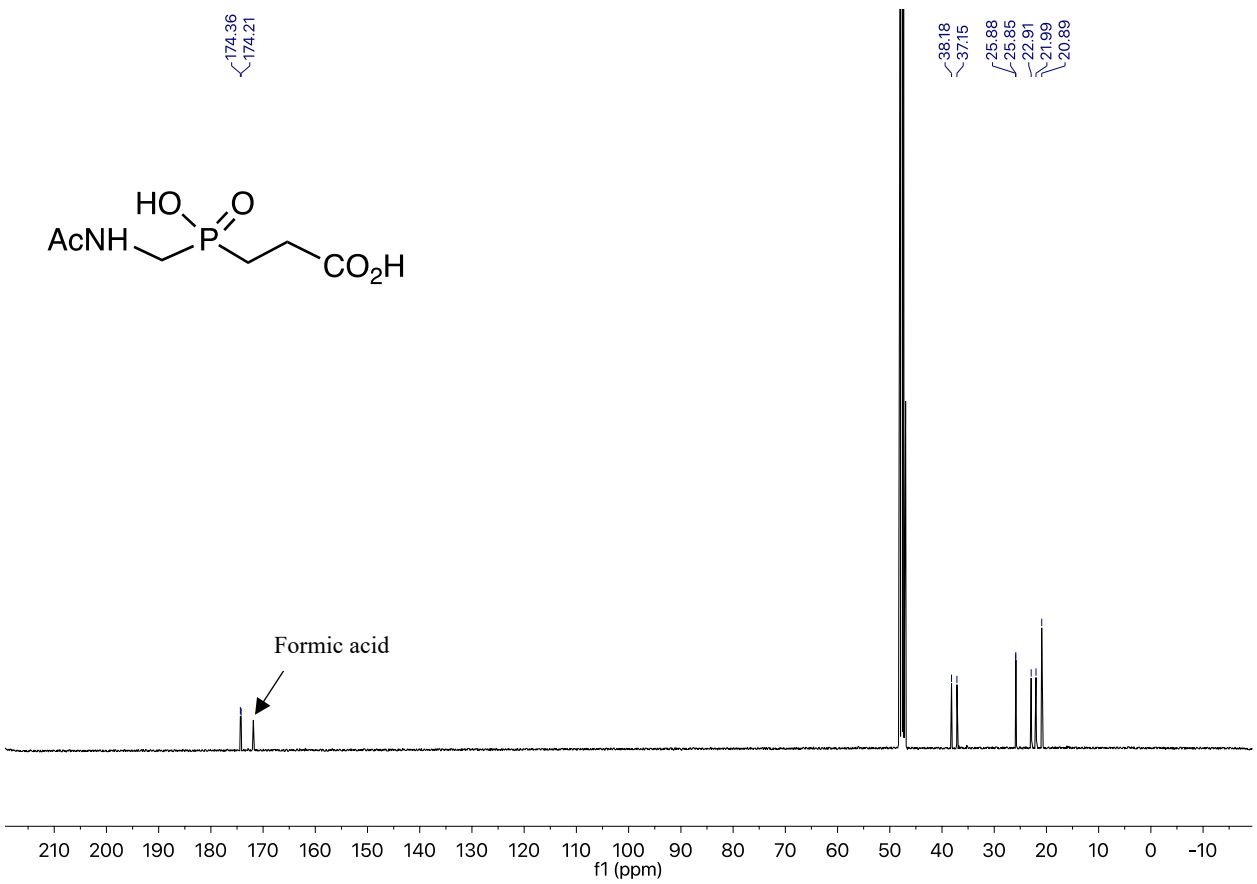
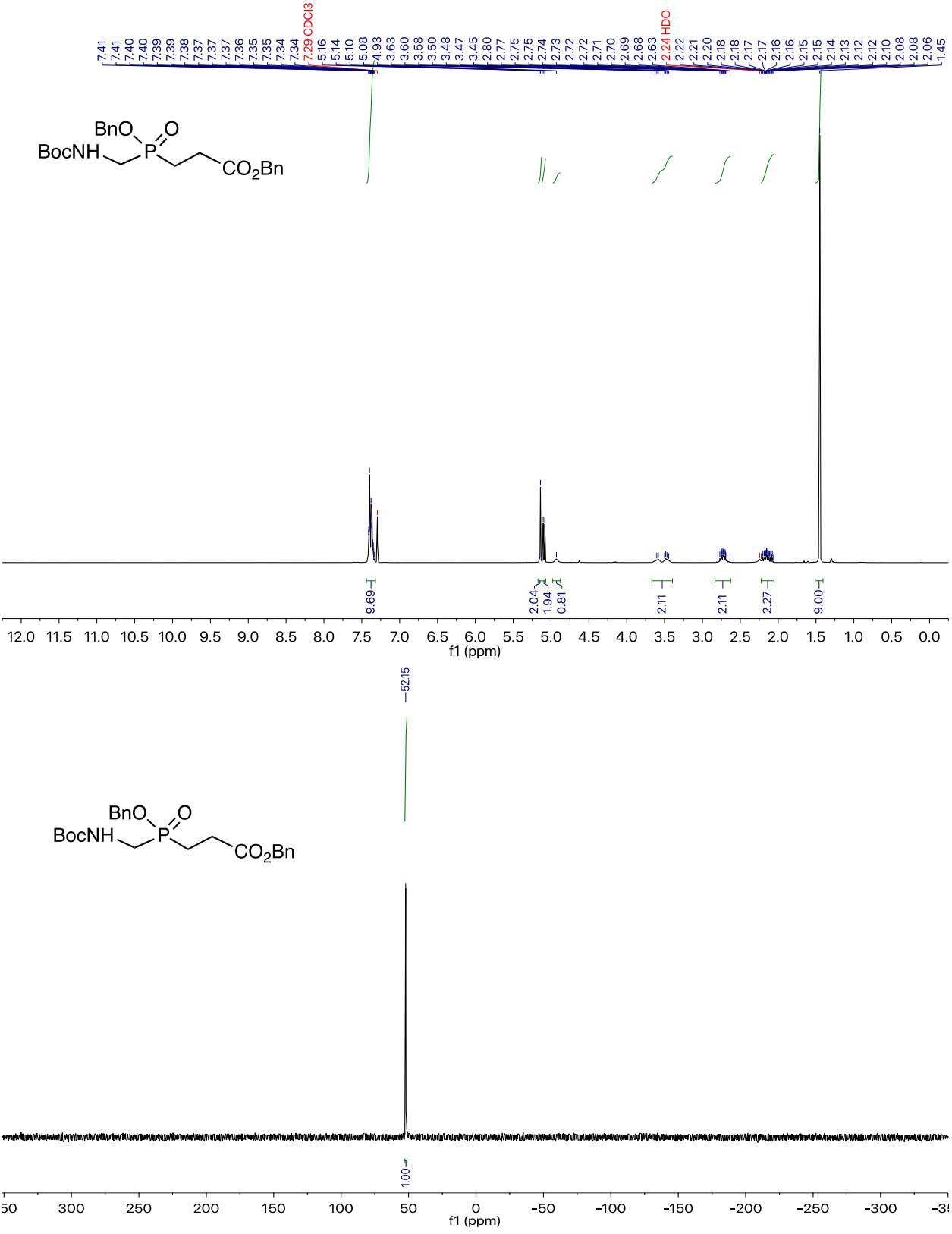

174.36
174.21
38.18
37.15
25.88
25.85
22.91
21.99
20.89
HO
O
AcNH
P
CO2H
Formic acid
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

**Figure S11**: $^{1}H$ NMR (400 MHz, $CDCl_3$), $^{31}P$ NMR (162 MHz, $CDCl_3$) and $^{13}C$ NMR (101 MHz, $CDCl_3$) of Compound **14**



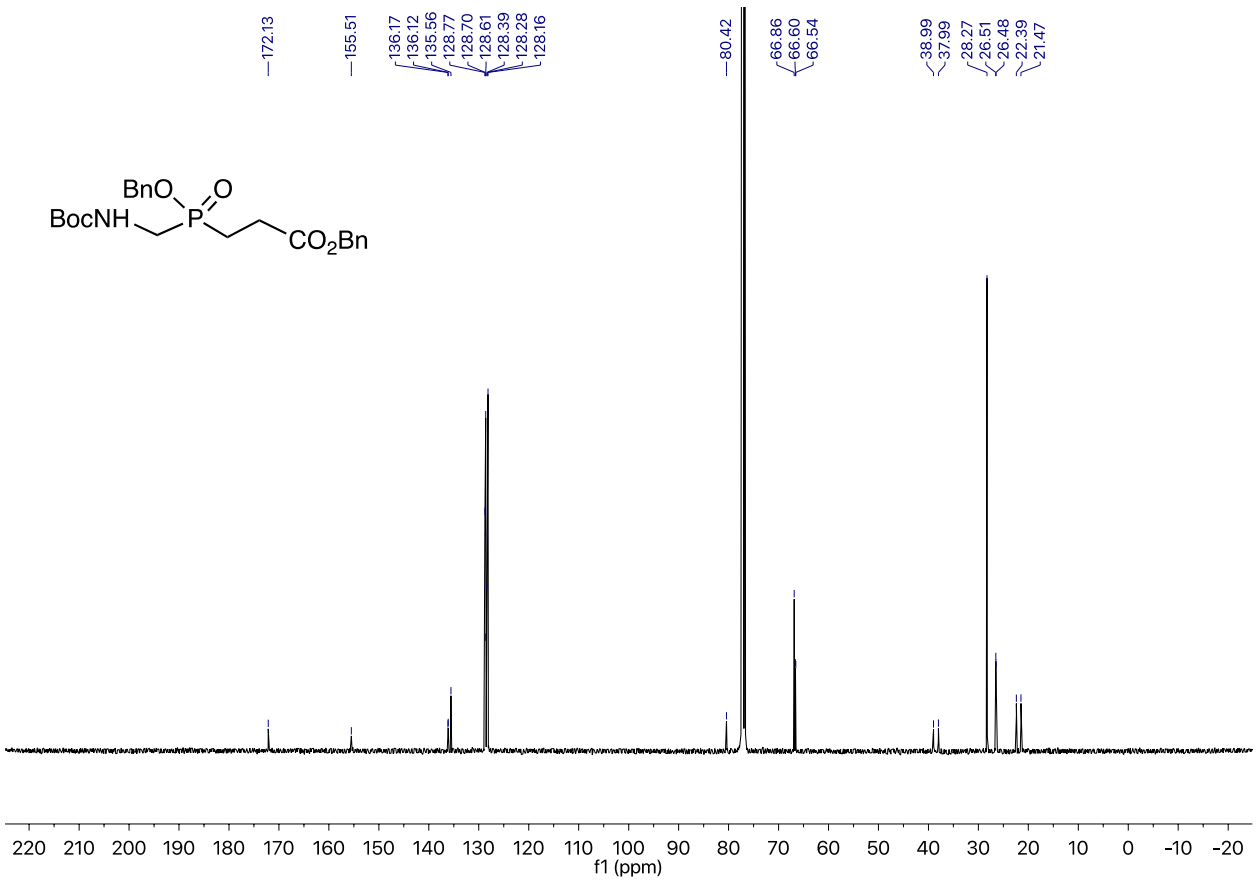
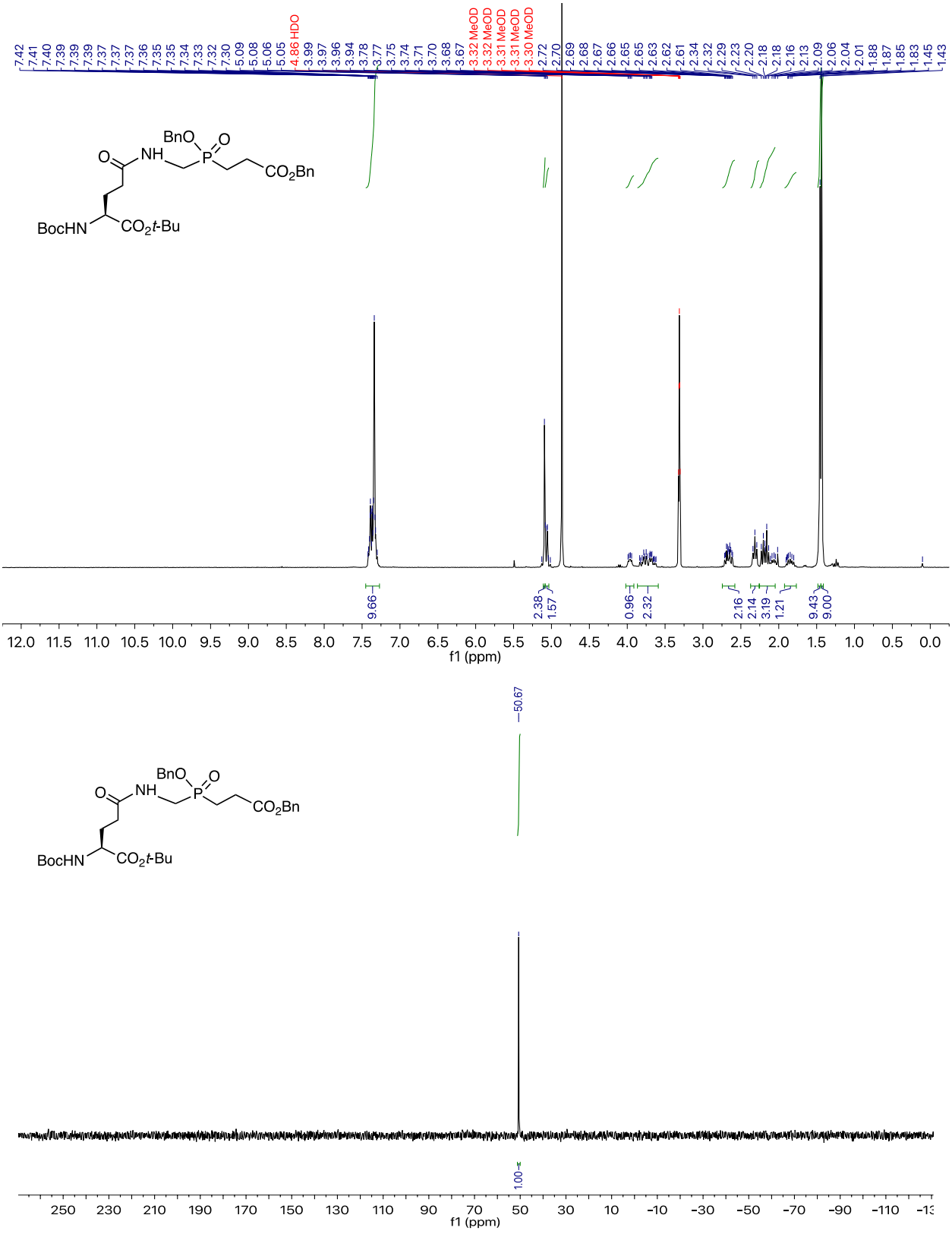

172.13
155.51
136.17
136.12
135.56
128.77
128.70
128.61
128.39
128.28
128.16
80.42
66.86
66.60
66.54
38.99
37.99
28.27
26.51
26.48
22.39
21.47
BnO
O
BocNH
P
CO2Bn
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

**Figure S12**: $^{1}$H NMR (300 MHz, MeOD), $^{31}$P NMR (122 MHz, MeOD) and $^{13}$C NMR (101 MHz, MeOD) of Compound **15**



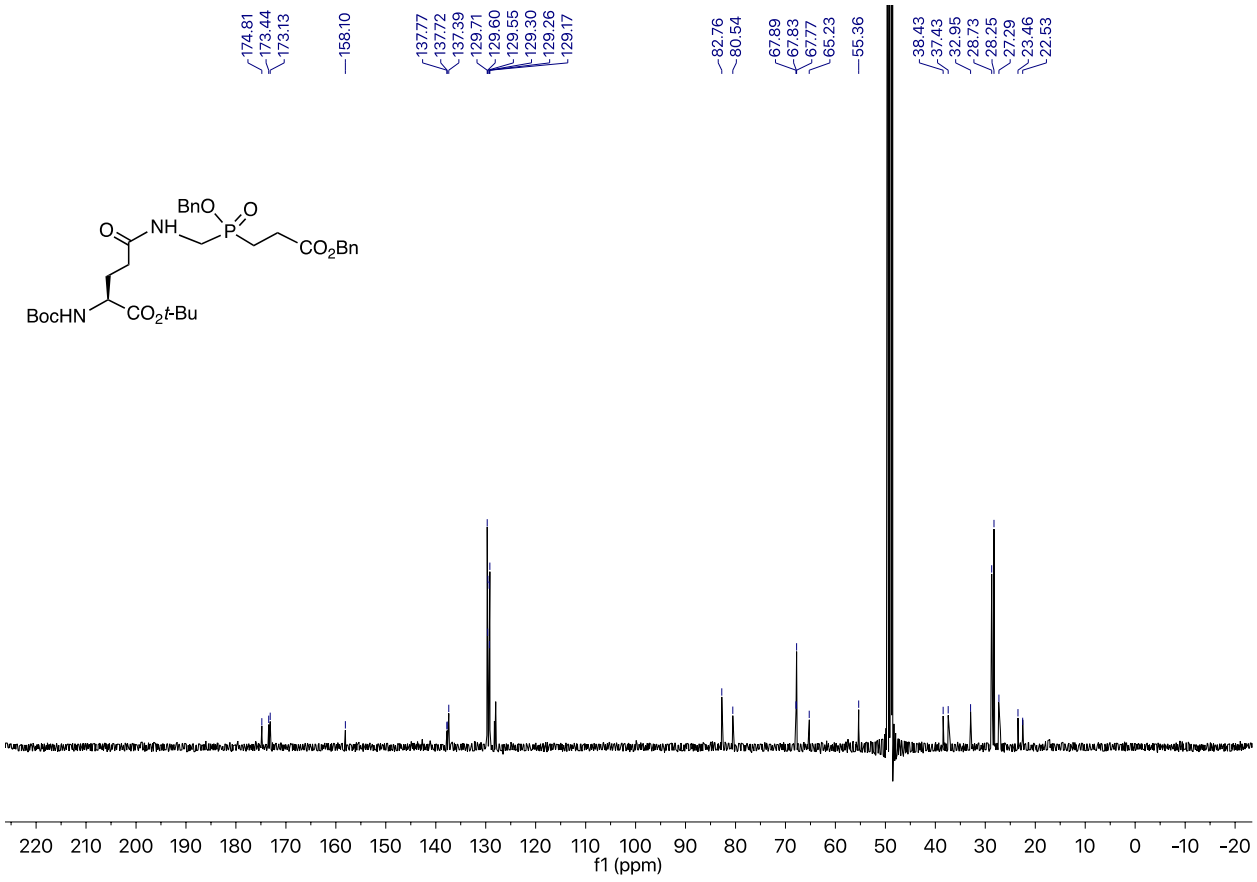
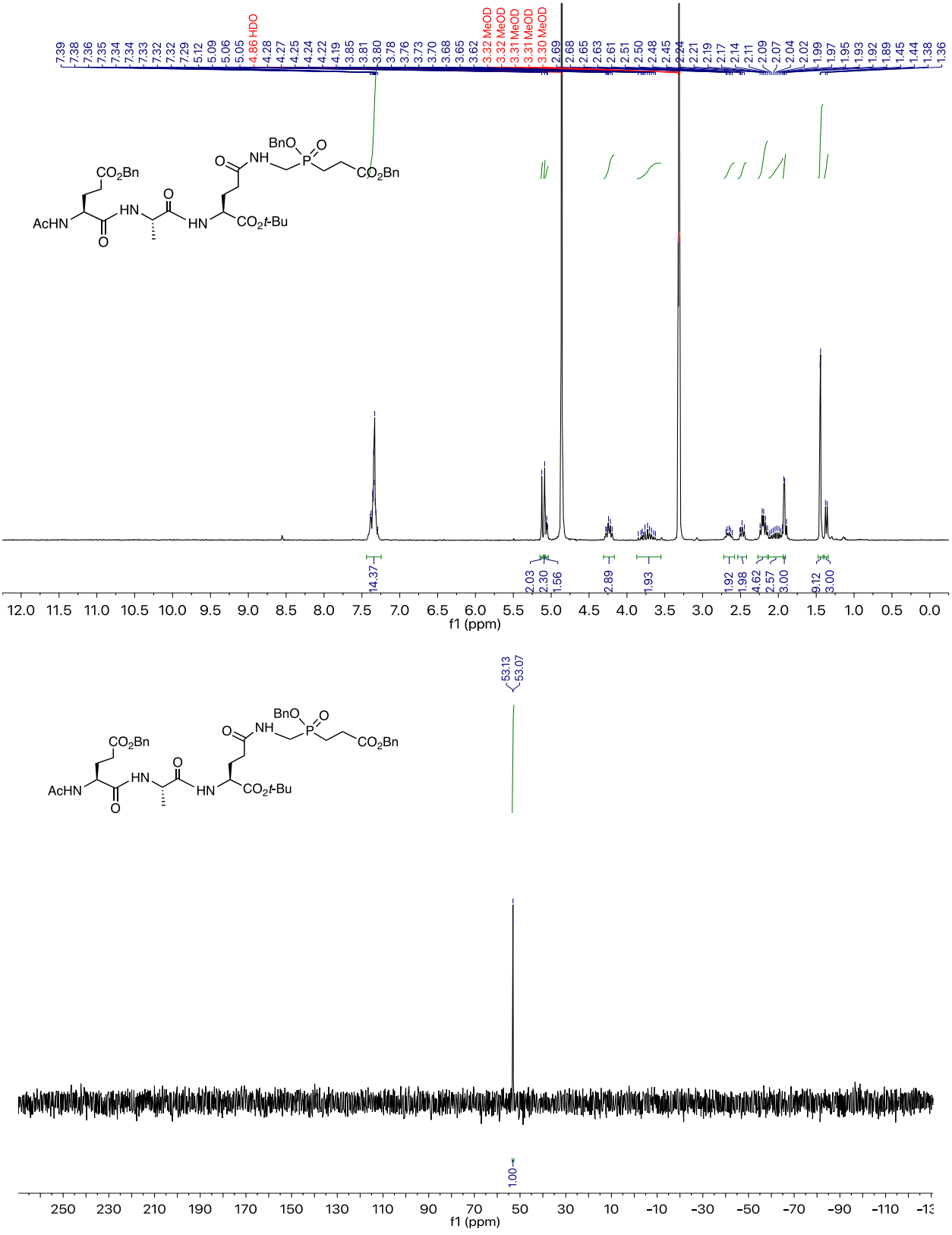

174.81
173.44
173.13
158.10
137.77
137.72
137.39
129.71
129.60
129.55
129.30
129.26
129.17
82.76
80.54
67.89
67.83
67.77
65.23
55.36
38.43
37.43
32.95
28.73
28.25
27.29
23.46
22.53
BnO
O
NH
P
O
CO2Bn
BocHN
CO2t-Bu
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

**Figure S13**: $^{1}$H NMR (300 MHz, MeOD), $^{31}$P NMR (122 MHz, MeOD) and $^{13}$C NMR (151 MHz, MeOD) of Compound **16**



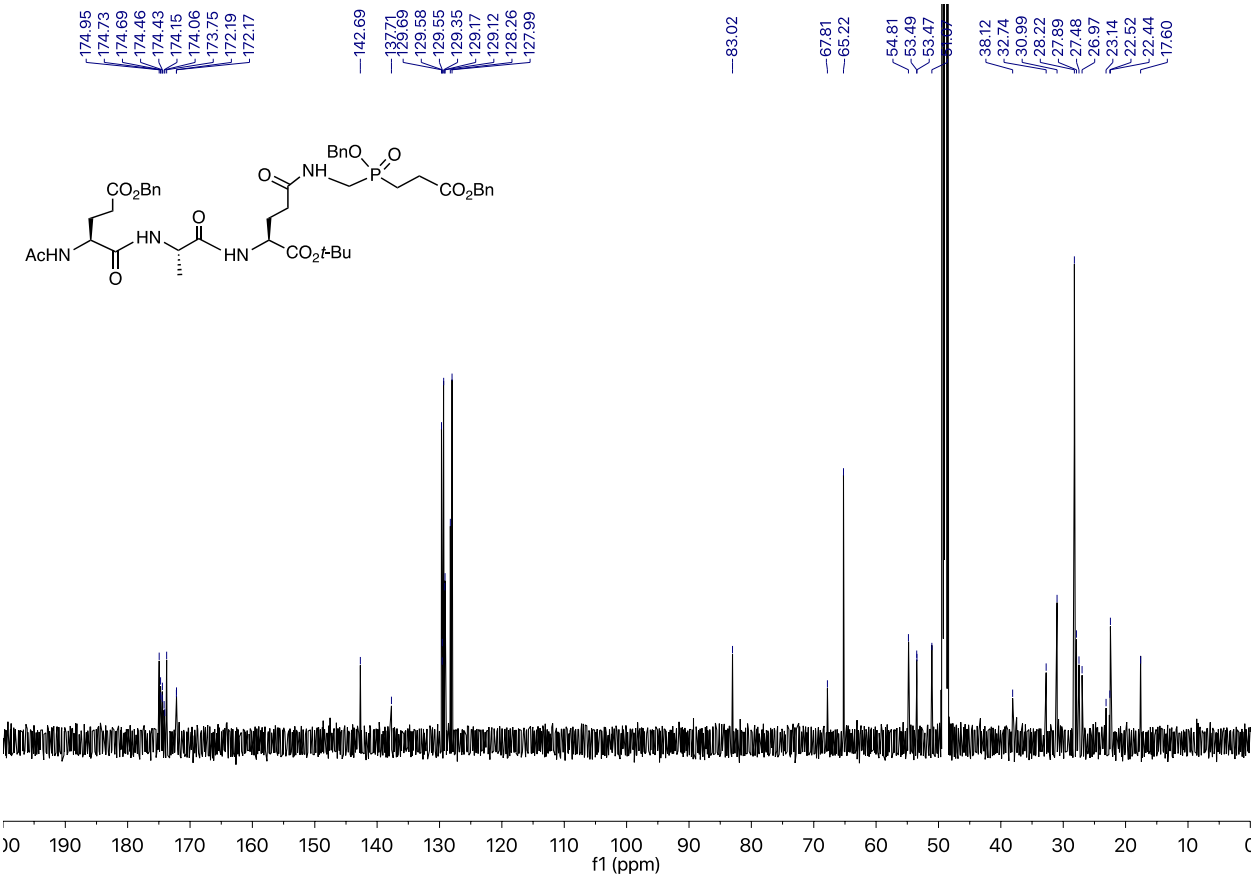
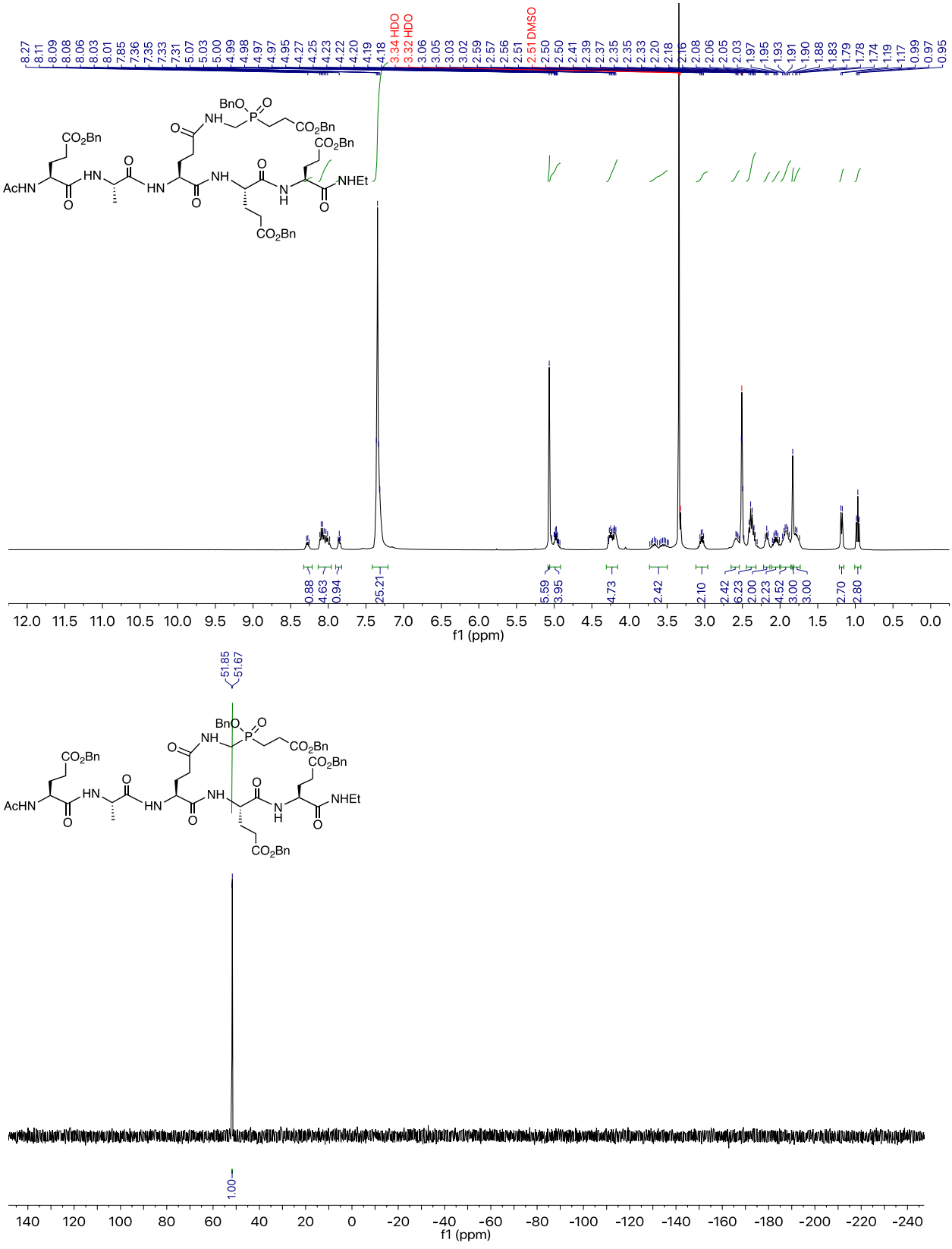

174.95
174.73
174.69
174.46
174.43
174.15
174.06
173.75
172.19
172.17
142.69
137.71
129.69
129.58
129.55
129.35
129.17
129.12
128.26
127.99
83.02
67.81
65.22
54.81
53.49
53.47
38.12
32.74
30.99
28.22
27.89
27.48
26.97
23.14
22.52
22.44
17.60
BnO
O
NH
P
O
CO2Bn
CO2Bn
AcHN
HN
O
HN
CO2t-Bu
O
f1 (ppm)

**Figure S14**: $^{1}$H NMR (400 MHz, DMSO-$d_6$), $^{31}$P NMR (162 MHz, DMSO-$d_6$) and $^{13}$C NMR (101 MHz, DMSO-$d_6$) of Compound **17**



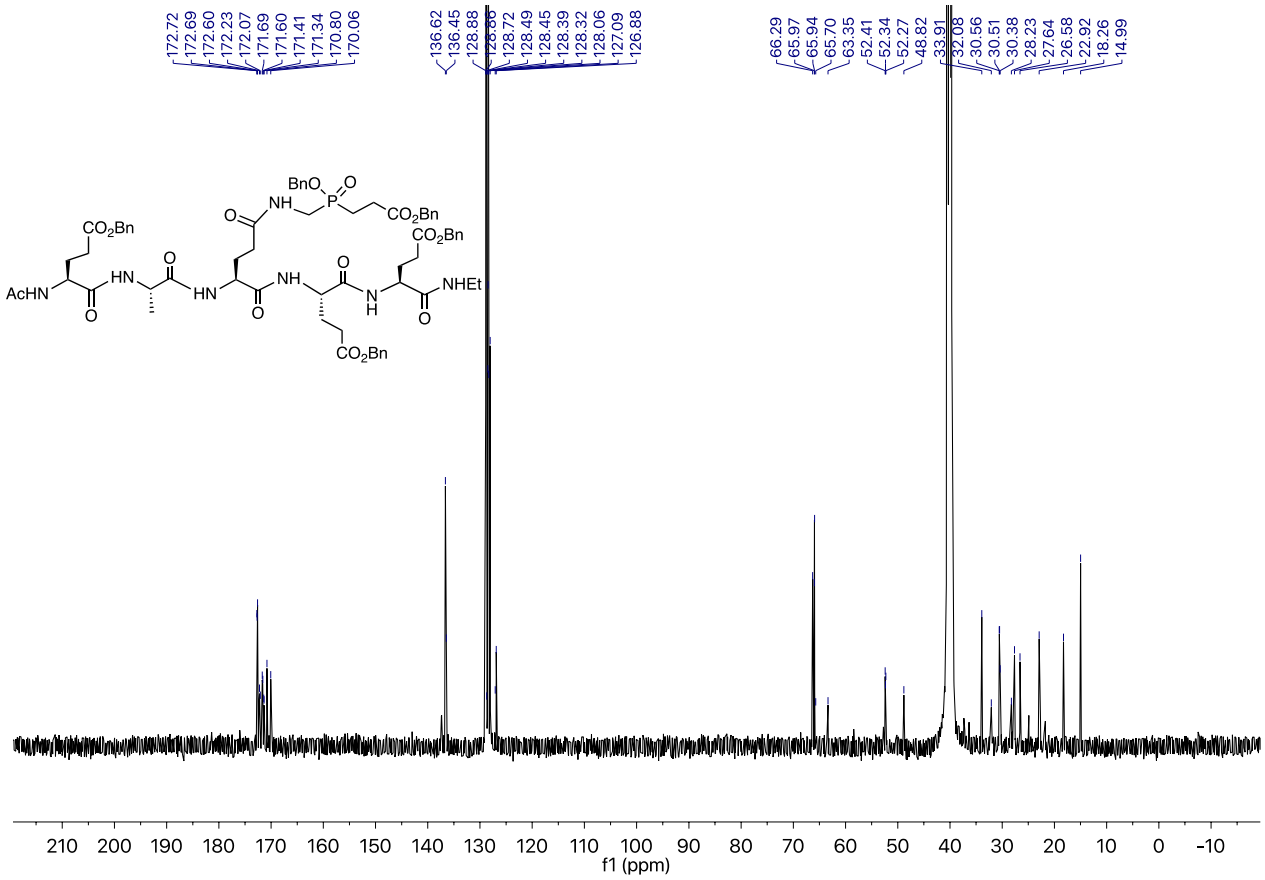
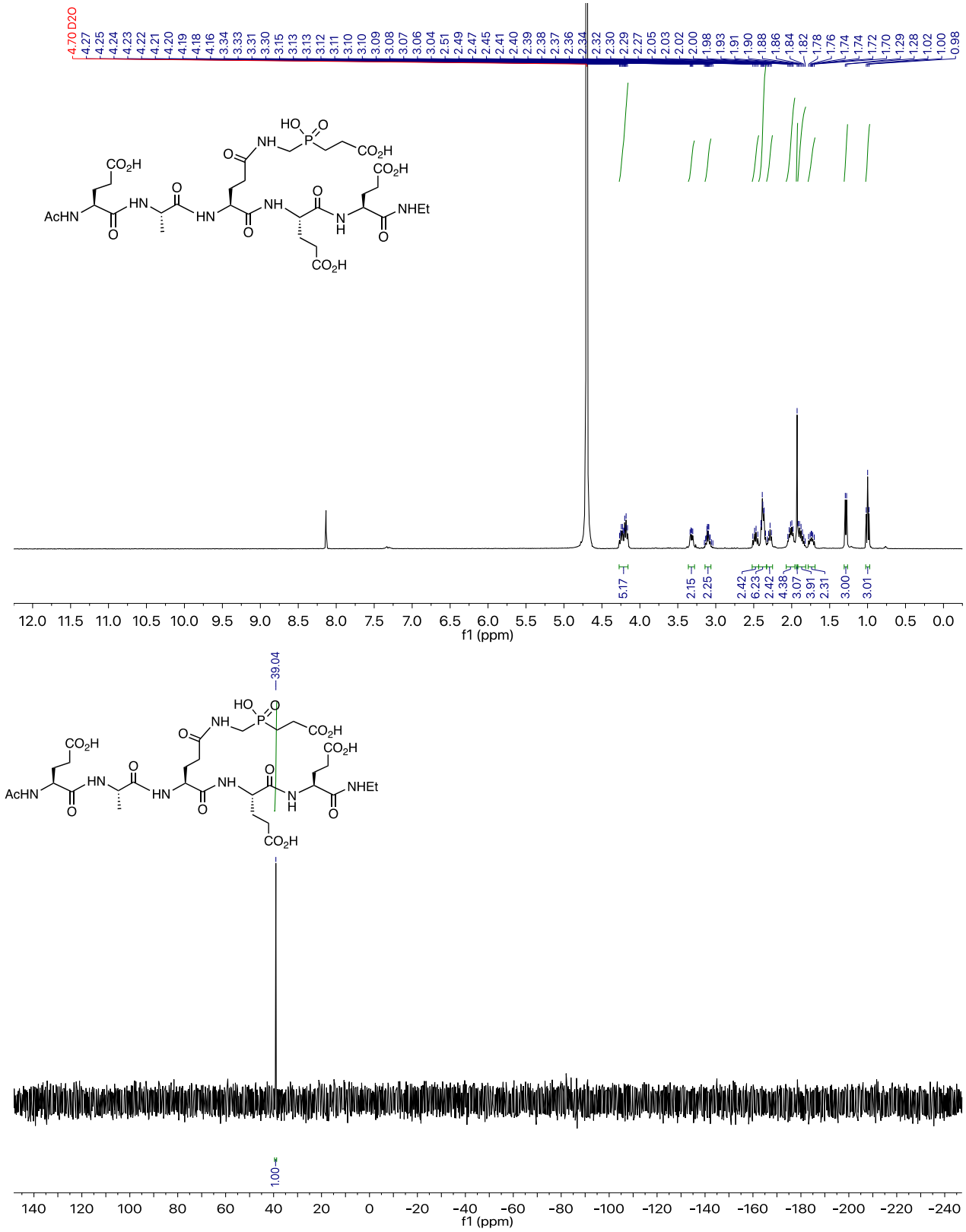
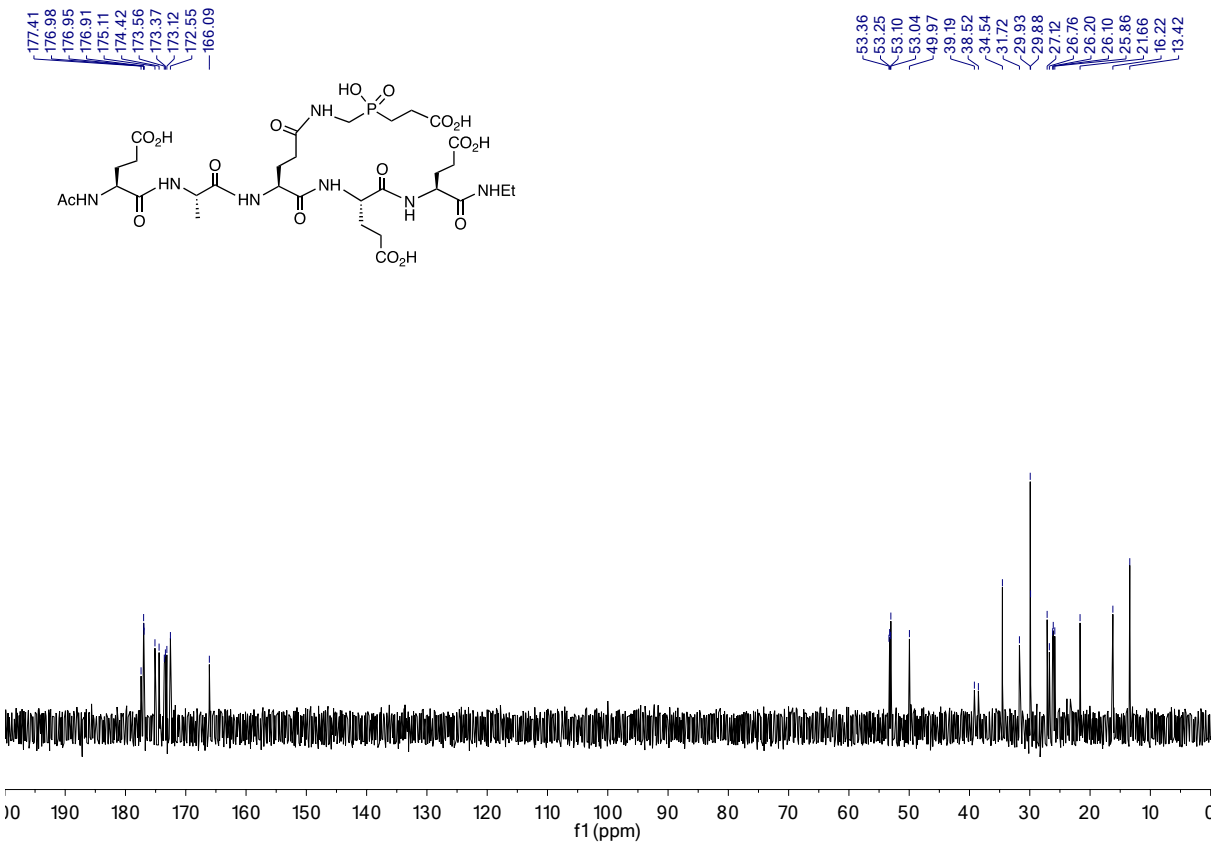

172.72
172.69
172.60
172.23
172.07
171.69
171.60
171.41
171.34
170.80
170.06
136.62
136.45
128.88
128.66
128.72
128.49
128.45
128.39
128.32
128.06
127.09
126.88
66.29
65.97
65.94
65.70
63.35
52.41
52.34
52.27
48.82
33.91
32.08
30.56
30.51
30.38
28.23
27.64
26.58
22.92
18.26
14.99
BnO
P
O
NH
CO2Bn
AcHN
HN
NHEt
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)

**Figure S15**: $^{1}$H NMR (400 MHz, $D_2O$), $^{31}$P NMR (162 MHz, $D_2O$) and $^{13}$C NMR (151 MHz, $D_2O$) of Inhibitor **4**




177.41
176.98
176.95
176.91
175.11
174.42
173.56
173.37
173.12
172.55
166.09
53.36
53.25
53.10
53.04
49.97
39.19
38.52
34.54
31.72
29.93
29.88
27.12
26.76
26.20
26.10
25.86
21.66
16.22
13.42
HO
P
O
NH
CO2H
AcHN
HN
NHEt
f1 (ppm)