Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Stereoselective synthesis of phosphonate pThr mimetic *via* palladium-catalyzed *γ*-C(sp^3)-H activation for peptide preparation

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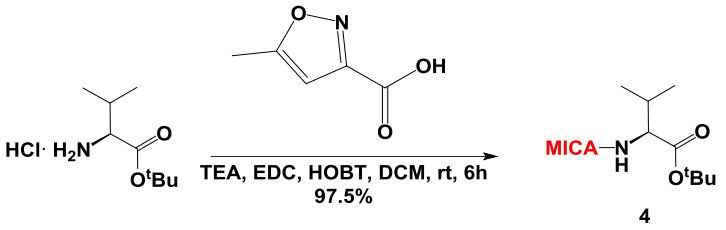
1. General Experimental

Unless otherwise indicated, all solvents, reagents, amino acid derivatives and resins used in reactions were purchased from the trade and used without further purification. All glassware was oven-dried by electric blast drying box after washing with water and acetone. All reactions that require free oxygen and hydrous were carried out under sealed argon protection. Needle syringe and straw were disposable. All the evaporation of solvents was achieved by water-bath rotary evaporation, pump and constant temperature cryocondensation circulator. The separation and purification for products were accomplished by column chromatography with silica gel. The NMR spectra were characterized by a 400 MHz JEOL ECS-400 NMR system at room temperature with deuterochloroform or deuteromethanol. The internal reference of solvent signals are δ 7.26 ppm for ^{1}H NMR spectrum, δ 77.0 ppm for ^{13}C NMR spectrum in deuterochloroform and δ 4.87 ppm for ^{1}H NMR spectrum, δ 49.0 ppm for ^{13}C NMR spectrum in deuteromethanol. All chemical shifts and coupling constants (*J*) were indicated in ppm (δ) and Hertz (Hz) respectively. The multiplicities were showed in abbreviation forms as follows: s (singlet), d (doublet), t (triplet), q (quartet), br.s (broad singlet) and m (multiplet). The ^{31}P NMR spectra were also reported in ppm for chemical shifts with the internal reference of phosphoric acid. All HRMS (High resolution mass spectra) were recorded by using ESI mass spectrometer whose model is LCMS-IT/TOF (Shimadzu, Japan). A Thermo quadrupole mass spectrometer provided Low resolution ESI-MS spectra. Analytical High Performance Liquid Chromatography (HPLC) for products were achieved via a Shimadzu LC-2010A HT HPLC system and a YMC pack C18 column (5 *μ*m, 4.6 x 150 mm). The mixed eluent was 0.6% TFA in water (A) and 80% acetonitrile, 0.6% TFA in water (B). Preparative HPLC was performed on a Shimadzu LC-6AD 230V ASSY HPLC system with an YMC pack C18 column (5 *μ*m, 20 x 250 mm). The eluents of A and B were same as the analytical HPLC. The fluorescence polarization experiment for the peptide and protein was performed by Biotek Synergy 4 Microplate Reader. Optical rotations were obtained by a INESA WZZ-2S Polarimeter instrument and displayed as $[\alpha]_D^T$ (*c* = g/mL, solvent).

2. Synthesis of phosphothreonine (pThr) mimetic

2.1 Tert-butyl (5-methylisoxazole-3-carbonyl)-L-valinate (4)[1]:



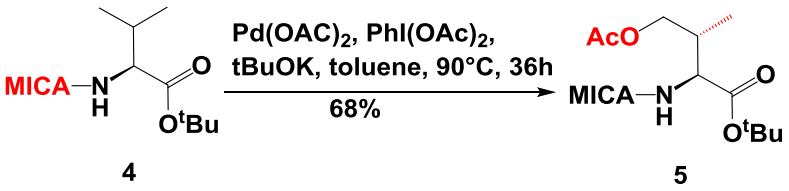
To a stirred mixing of triethylamine (TEA) (4.62 mL, 2.0 eq., 32.2 mmol) in 64 mL (0.25M) of anhydrous dichloromethane (DCM) was added EDC·HCl (3.41 g, 1.1 eq., 17.7 mmol), HOBT (2.17 g, 1.0 eq., 16.1 mmol) and 5-methylisoxazole-3-carboxylic acid (MICA) (2.05 g, 1.0 eq., 16.1 mmol) in the ice bath conditions. Stirred the mixture for 10 min and then added the tert-butyl L-Valine hydrochloride (3.70 g, 1.1 eq., 17.7mmol). After complete dissolution, removed the ice bath and stirred for 6 hours at room temperature. Water was added to stop the reaction and the mixture was washed with water and saturated brine. After dring over anhydrous magnesium and concentrating in vacuo, the residue was purified by silica gel column chromatography (petrol ether/ethyl acetate = 6/1) to afford **4** as yellowish oil in 4.44 g (4.44 g, 15.7 mmol, 97.5% yield). R_f = 0.54 in petrol ether/ethyl acetate = 3/1.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 8.7 Hz, 1H, NH), 6.38 (s, 1H, CH in MICA), 4.55 (dd, J = 9.0, 4.7 Hz, 1H, *α*-CH), 2.44 (s, 3H, CH_3 in MICA), 2.32 – 2.16 (m, 1H, β-CH), 1.45 (s, 9H, tBu), 0.96 (d, J = 6.9 Hz, 3H, γ-CH_3), 0.94 (d, J = 6.9 Hz, 3H, γ-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.07, 170.19, 158.90, 158.40, 101.28, 82.12, 57.38, 31.48, 27.93, 18.89, 17.56, 12.21.

ESI-MS $[M+H]^+$ calcd for $C_{14}H_{23}N_2O_4^+$: 283.2, found 283.1.

2.2 Tert-butyl (2S,3R)-4-acetoxy-3-methyl-2-(5-methylisoxazole-3-carboxamido) butanoate (5):



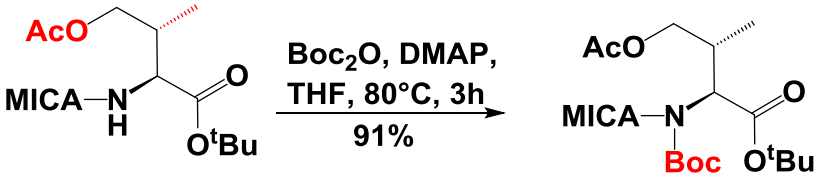
Added $Pd(OAc)_2$ (45 mg, 0.1 eq., 0.2 mmol), $PhI(OAc)_2$ (2.66 g, 4.0 eq., 4 mmol) and potassium tert-butoxide (112mg, 0.5 eq., 1 mmol) into Schlenk flask. The compound **4** (566 mg, 1.0 eq., 2mmol) dissolved by toluene (10 mL, 0.2 M) was added in the sealed flask and then heated the mixture to 90℃ for 36 h. Cooled the reaction mixture to room temperature and concentrated in vacuo. Lastly, the mixture was purified by silica gel column chromatography (petrol ether/ethyl acetate = 10/1) to give the acetoxylated product **5** as brown yellow oil in 0.46 g (1.35 mmol, yield 68%). R_f = 0.28 in petrol ether/ethyl acetate = 3/1.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 8.6 Hz, 1H, NH), 6.37 (s, 1H, CH in MICA), 4.69 (dd, J = 8.7, 4.4 Hz, 1H, *α*-CH), 4.10 (dd, J = 11.4, 6.7 Hz, 1H, one of γ-CH_2), 3.98 (dd, J = 11.4, 5.3 Hz, 1H, one of γ-CH_2), 2.48 – 2.43 (m, 1H, β-CH), 2.42 (s, 3H, CH_3 in MICA), 2.05 (s, 3H, CH_3 in OAc), 1.43 (s, 9H, tBu), 1.00 (d, J = 7.1 Hz, 3H, γ-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.22, 170.80, 169.45, 158.99, 158.31, 101.29, 101.26, 82.70, 65.70, 54.71, 35.36, 27.97, 20.83, 13.70, 12.27.

ESI-MS $[M+H]^+$ calcd for $C_{16}H_{25}N_2O_6^+$: 341.2, found 341.1.

2.3 Tert-butyl (2S,3R)-4-acetoxy-2-(N-(tert-butoxycarbonyl)-5-methylisoxazole-3-carboxamido)-3-methylbutanoate (6):



5 **6**

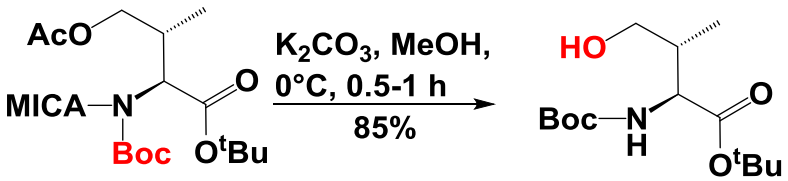
To a stirred solution of compound **5** (1.7 g, 1.0 eq., 5 mmol) in 33 mL of THF (0.15M) was added Boc_2O (2.18 g, 2.0 eq., 10 mmol) and catalytic amount of DMAP, then the mixure was stirred for 3 h at 80℃. The reaction mixture was cooled to room temperature after complete conversion, water and ethyl acetate were added, then the organic layer was washed with water and saturated brine and dried over anhydrous magnesium sulfate and concentrated by rotary evaporator. The residue was then purified by column chromatography (petrol ether/ethyl acetate =8/1) to give desired product **6** (2.0 g, 4.55 mmol, 91 %) as a colorless oil. R_f = 0.48 in petrol ether/ethyl acetate = 3/1.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.19 (s, 1H, CH in MICA), 4.83 (d, J = 9.2 Hz, 1H, *α*-CH), 4.04 (d, J = 4.2 Hz, 2H, γ-CH_2), 2.85 – 2.54 (m, 1H, β-CH), 2.44 (s, 3H, CH_3 in MICA), 1.90 (s, 3H, CH_3 in OAc), 1.43 (s, 9H, Boc or tBu), 1.28 (s, 9H, Boc or tBu), 1.22 (d, J = 6.7 Hz, 3H, γ-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.06, 169.90, 168.07, 163.94, 160.01, 152.25, 101.21, 84.73, 82.24, 66.52, 60.44, 32.96, 27.98, 27.53, 20.86, 16.83, 12.24.

ESI-MS $[M+H]^+$ calcd for $C_{21}H_{33}N_2O_8^+$: 441.2, found 441.2.

2.4 Tert-butyl (2S,3R)-2-((tert-butoxycarbonyl) amino)-4-hydroxy-3-methylbutanoate (7):



6 **7**

Compound **6** (0.81 g, 1.0 eq., 1.84 mmol) was dissolved in MeOH and cooled to 0℃ by ice in a round bottom flask. Then added potassium carbonate (0.25 g, 1.0 eq., 1 .84mmol) into the flask and stirred the mixture for 0.5 ~ 1 h. The excess potassium carbonate was dissolved in water. The mixture was diluted by

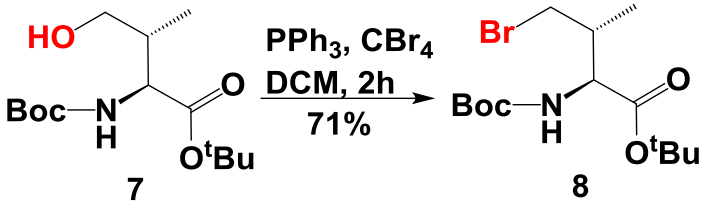
ethyl acetate and washed with water and saturated brine and then concentrated in vacuo, Purified the residue by column chromatography (petrol ether/ethyl acetate = 8/1) to give a colorless-oil product **7** (0.45 g, 1.56 mmol, 85 %). R_f = 0.27 in petrol ether/ethyl acetate = 3/1. $[\alpha]_D^{25}$= 10.5 (*c* = 0.0115, $CDCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 (s, 1H, NH), 4.13 (d, J = 6.9 Hz, 1H, α-CH), 3.67 (dd, J = 11.5, 2.7 Hz, 1H, one of γ-CH_2), 3.50 (d, J = 8.0 Hz, 1H, one of γ-CH_2), 2.19 (br.s, 1H, β-CH), 1.95 (br, 1H, OH), 1.48 (s, 9H, Boc or tBu), 1.44 (s, 9H, Boc or tBu), 1.01 (d, J = 6.8 Hz, 3H, γ-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.55, 156.19, 82.05, 79.85, 64.20, 56.47, 38.83, 28.30, 28.24, 27.99, 13.82.

ESI-MS $[M+H]^+$ calcd for $C_{14}H_{28}NO_5^+$: 290.2, found 290.2.

2.5 Tert-butyl (2S,3R)-4-bromo-2-((tert-butoxycarbonyl)amino)-3-methylbutanoate (8):



To a stirred solution of **7** (0.22 g, 1.0 eq., 0.76 mmol) in 5 mL anhydrous DCM was added carbon tetrabromide (0.28 g, 1.1 eq., 0.84 mmol) at 0℃, and slowly added triphenylphosphine (0.22 g, 1.1 eq., 0.84 mmol) dissolved by DCM. Then the mixture was stirred for 2 h. 10 mL water was added for quenching the reaction. And DCM was added to extracted the mixture. Washed with water and saturated brine and concentrated the mixture to gain the residue. Lastly, it was separated by silica gel column chromatography (petrol ether/ethyl acetate = 12/1) and afforded **8** (0.19 g, 0.54 mmol, 71%) as a colorless oil. R_f = 0.72 (petrol ether/ethyl acetate=4/1).

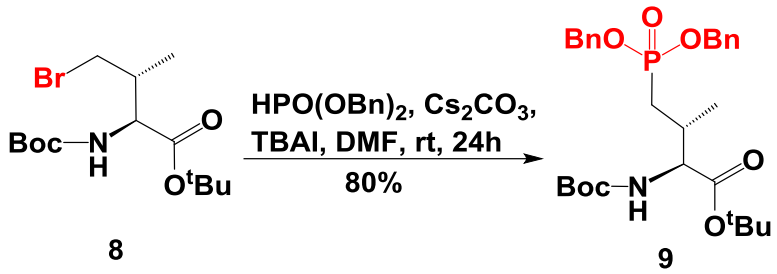
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (s, 1H, NH), 4.29 (s, 1H, α-CH), 3.48 (dd, J = 10.1, 5.5 Hz, 1H, one of γ-CH_2), 3.28 (d, J = 8.5 Hz, 1H, one of γ-CH_2), 2.31 (br.s, 1H, β-CH), 1.48 (s, 9H, Boc or tBu), 1.44 (s, 9H, Boc or tBu), 1.06 (d, J = 6.4 Hz, 3H, γ-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.36, 155.37, 82.80, 80.08, 57.12, 39.61, 35.94, 28.44, 28.18, 15.17.

ESI-MS $[M+H]^+$ calcd for $C_{14}H_{27}BrNO_4^+$: 352.1, found 352.1

HRMS: calculated for $C_{14}H_{26}BrNO_4Na$ $[M+Na]^+$: 374.0937; found: 374.0930:376.0934=1:1 which are the characteristic peaks for monobromide.

2.6 Tert-butyl (2S,3R)-4-(bis(benzyloxy)phosphoryl)-2-((tert-butoxycarbonyl)amino)-3-methylbutanoate (9):



8 **9**

To a stirred suspension of cesium carbonate (1.02 g, 5.0 eq., 3.15 mmol) and tetrabutylammo-nium iodide (1.16 g, 5.0 eq., 3.15 mmol) in 15 mL anhydrous DMF was added dibenzyl phosphite (0.17 g, 1.0 eq., 0.63 mmol). After stirring for 2 h, a solution of **8** (0.22g, 1.0 eq., 0.63 mmol) in DMF (1 mL) was added through a syringe, and the mixture was stirred at room temperature for 24 h. The reaction was quenched by water (20 mL), and then extracted with ethyl acetate (3×20 mL), concentrated and filtered the precipitate tetrabutylammo-nium iodide. Purified the mixture by column chromatography (petrol ether/ethyl acetate = 10/1) to obtain **9** (0.27 g, 0.50 mmol, 80%) as colorless oil. R_f = 0.61 (petrol ether/ethyl acetate = 2/1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 3.4 Hz, 11H, benzene in $(OBn)_2$ and NH), 5.02 (dd, J = 8.9, 3.4 Hz, 2H,CH_2 in $(OBn)_2$), 4.98 – 4.88 (m, 2H,CH_2 in $(OBn)_2$), 4.11 (s, 1H, α-CH), 2.37 (br.s, 1H, β-CH), 1.96 – 1.80 (m, 1H, one of γ-CH_2), 1.75 – 1.54 (m, 1H, one of γ-CH_2), 1.43 (s, 9H,Boc or tBu), 1.40 (s, 9H, Boc or tBu), 1.09 (d, J = 6.7 Hz, 3H, γ-CH_3).

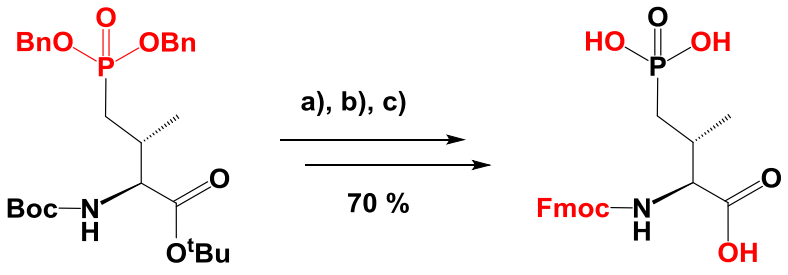
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.76, 155.64, 136.45, 128.71, 128.53, 128.10, 82.47, 79.90, 67.37, 67.30, 67.22, 59.09, 32.17, 28.42, 28.08, 17.36.

^{31}P NMR (243 MHz, $CDCl_3$) δ 32.55(s).

ESI-MS $[M+H]^+$ calcd for $C_{28}H_{41}NO_7P^+$: 534.3, found 534.3.

HRMS: calculated for $C_{28}H_{41}NO_7P$ $[M+H]^+$: 534.2615; found: 534.2614.

2.7 (2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methyl-4-phosphonobutanoic acid (10):



9 **10**

a) H_2, Pd-C, methanol, overnight;
b) TFA, TIS , H_2O, 3h;
c) H2O, $NaHCO_3$, Fmoc-OSu, dioxane, 24h

Compound **9** (0.5 mmol, 1.0 eq., 266 mg) was hydrogenated by hydrogen and palladium on carbon in methanol overnight firstly. Then the palladium carbon was removed by filtration and solvent methanol was removed by rotary evaporation. To a solution of 15 mL of TFA: TIS: H_2O=95:2.5:2.5 was added the residue, stirred for 3 h, and spin-dried with an oil pump. The residue was dissolved in 10 mL of water, and sodium bicarbonate solid was added until the pH reached 8-9, and then Fmoc-Osu (0.5 mmol, 1.0 eq., 168 mg) in dioxane was added. Stirred for 1 d. Dioxane was removed in vacuo, diluted with 10 mL of water, washed

with ethyl acetate (20 mL×2), adjusted the mixture to pH 2 with concentrated hydrochloric acid, extracted with ethyl acetate (20 mL×5), and the organic phases were combined. Washed with water (50 mL×2), sodium chloride solution (50 mL×1), and dried over anhydrous magnesium sulfate. Remove solvent under reduced pressure. The residue was purified by preparative HPLC to afford **10** (145 mg, 0.35 mmol, 70%) as white solid after lyophilization.

^{1}H NMR (400 MHz, METHANOL-D4) δ 7.74 (d, J = 7.5 Hz, 2H, Fluorene 4 and 5 CH in Fmoc), 7.63 (t, J = 6.7 Hz, 2H, Fluorene 1 and 8 CH in Fmoc), 7.34 (t, J = 7.4 Hz, 2H, Fluorene 3 and 6 CH in Fmoc), 7.26 (t, J = 7.4 Hz, 2H, Fluorene 2 and 7 CH in Fmoc), 4.31 (d, J = 7.0 Hz, 2H, Fmoc-CH_2), 4.25 – 4.11 (m, 2H, α-CH and Fmoc-CH), 2.41 (br.s, 1H, β-CH), 1.94 – 1.77 (m, 1H, one of γ-CH_2), 1.64 (m, 1H, one of γ-CH_2), 1.11 (d, J = 6.7 Hz, 3H, γ-CH_3).

^{13}C NMR (100 MHz, METHANOL-D4) δ 174.47, 158.84, 145.33, 142.56, 128.79, 128.19, 126.28, 120.91, 68.11, 61.01, 32.61, 31.69, 30.33, 17.79

^{31}P NMR (243 MHz, METHANOL-D3) δ 28.67 (s).

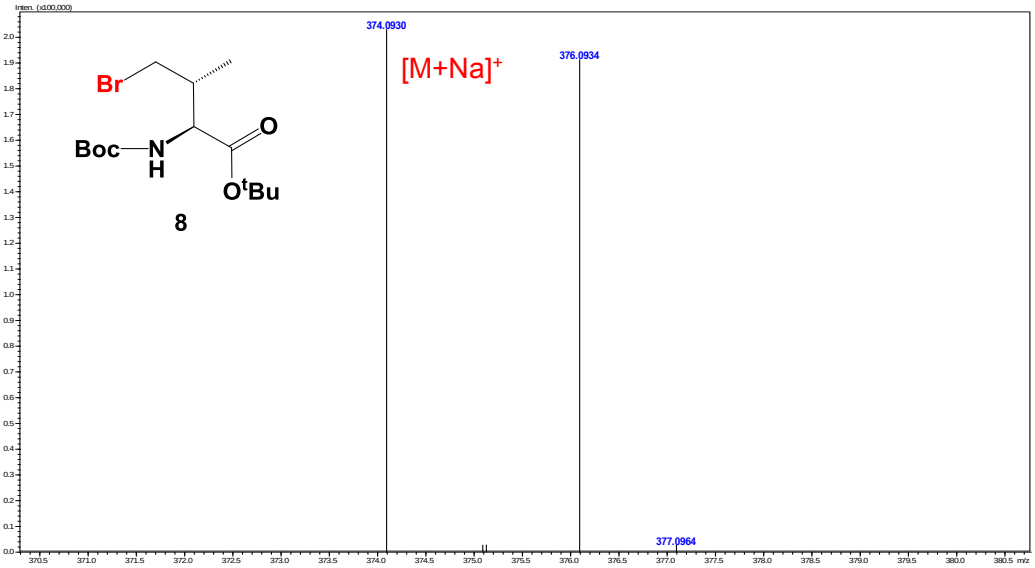
ESI-MS $[M+H]^+$ calcd for $C_{20}H_{23}NO_7P^+$: 420.1, found 420.0.

HRMS: calculated for $C_{20}H_{23}NO_7P$ Na $[M+H]^+$: 420.1207; found: 420.1203.

3. HRMS of compound 8, 9, 10

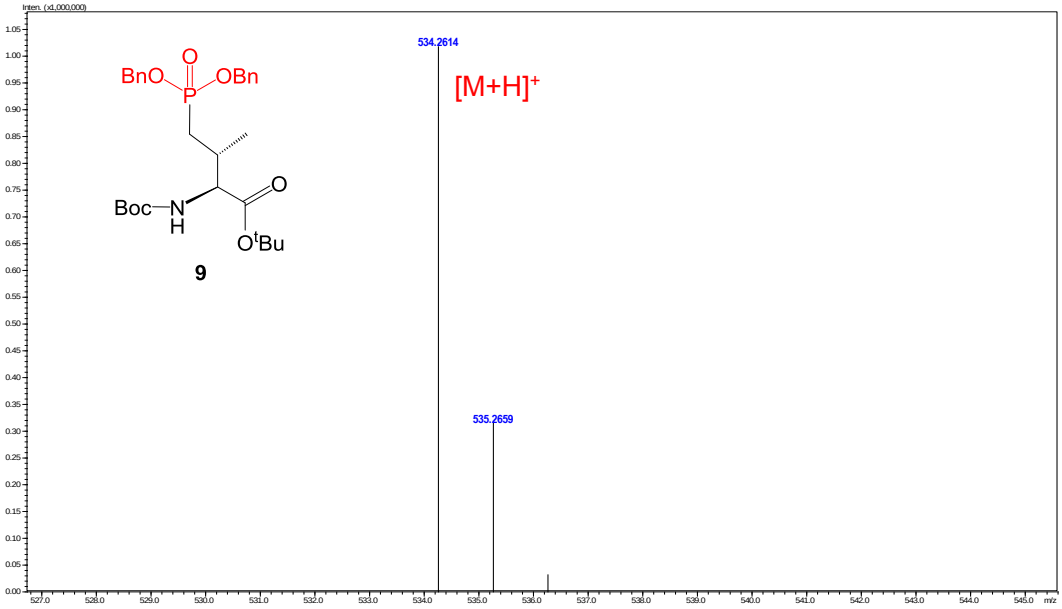
HRMS of compound **8.**

Calculated for $C_{14}H_{26}BrNO_4Na$ $[M+Na]^+$: 374.0937; found: 374.0930:376.0934=1:1 which are the characteristic peaks for monobromide.



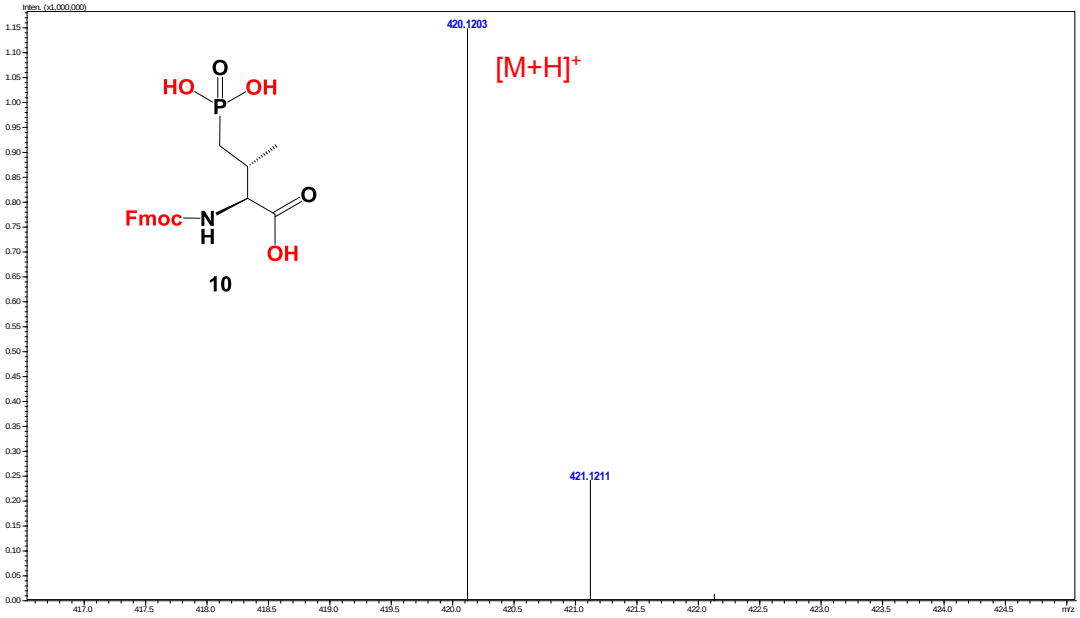
HRMS of compound **9.**

Calculated for $C_{28}H_{41}NO_7P$ $[M+H]^+$: 534.2615; found: 534.2614.



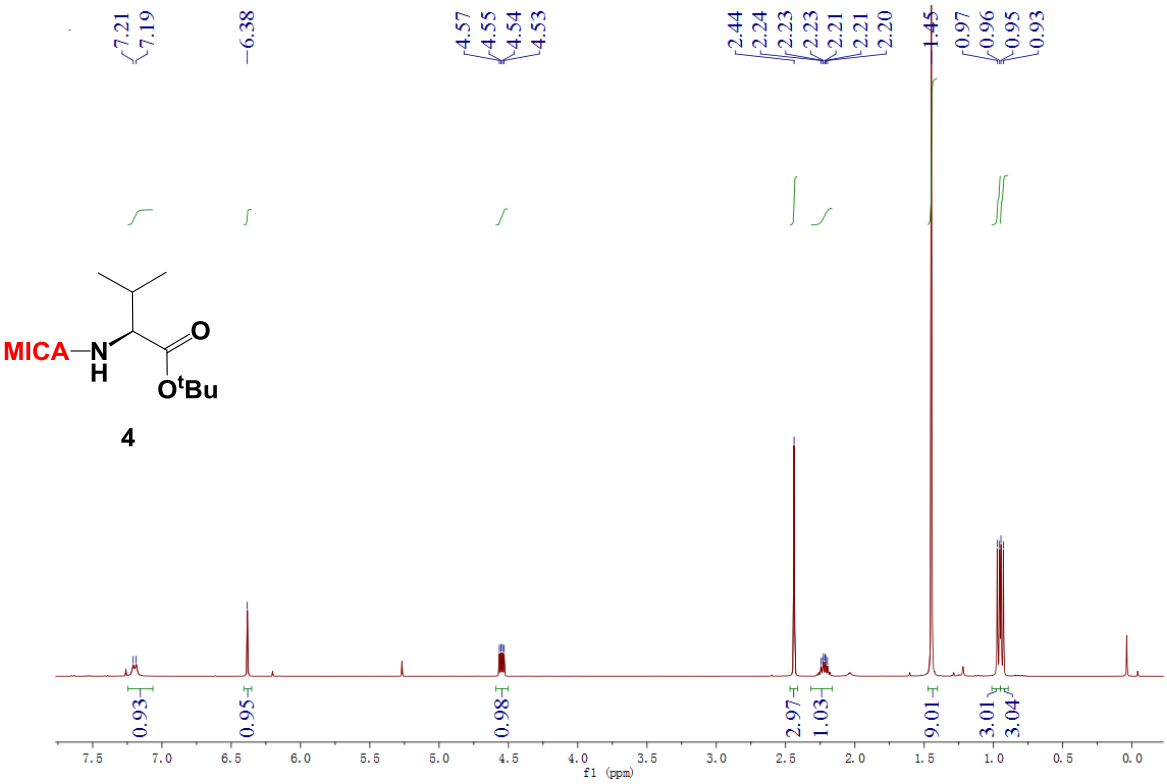
HRMS of compound **10**

Calculated for $C_{20}H_{23}NO_7P$ Na $[M+H]^+$: 420.1207; found: 420.1203.

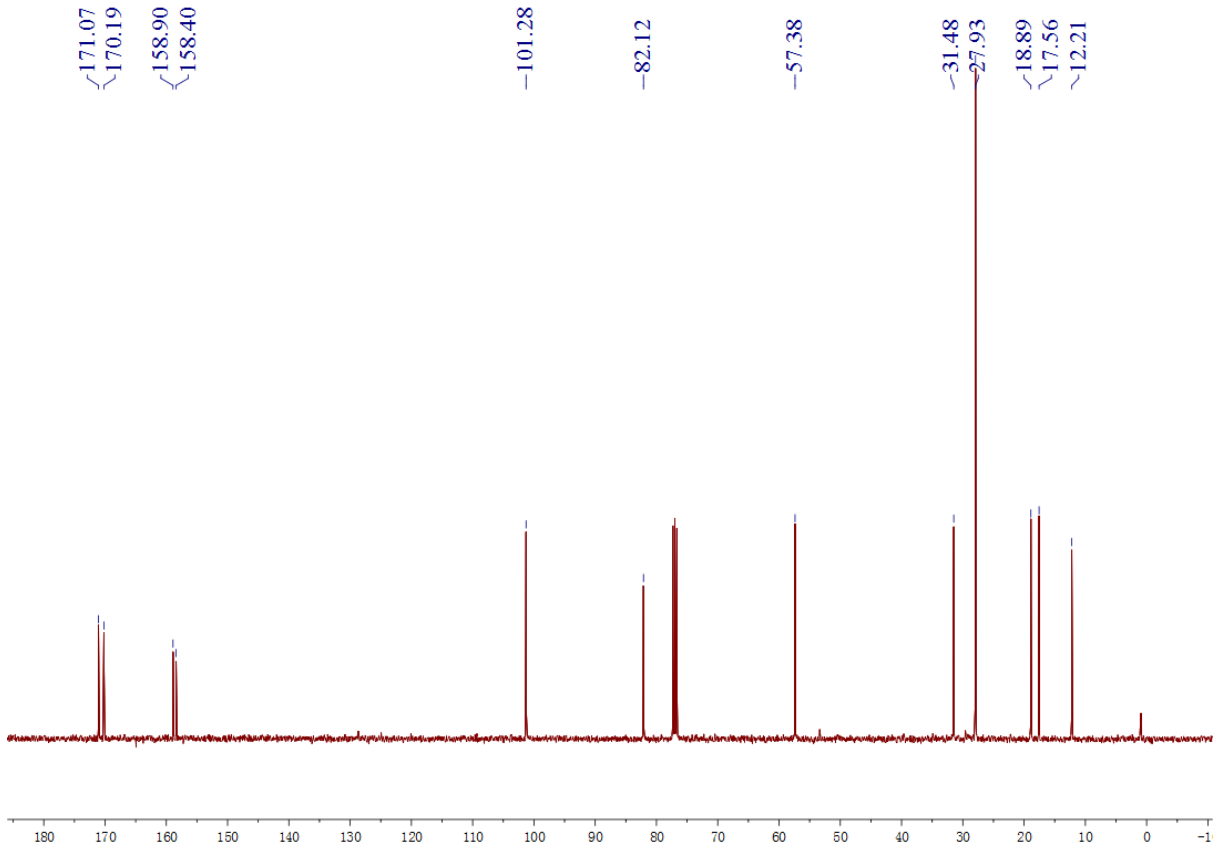

Inten. (x1,000,000)
420.1203
[M+H]+
421.1211
HO
O
OH
P
Fmoc
N
H
O
OH
10
m/z

4. NMR spectra

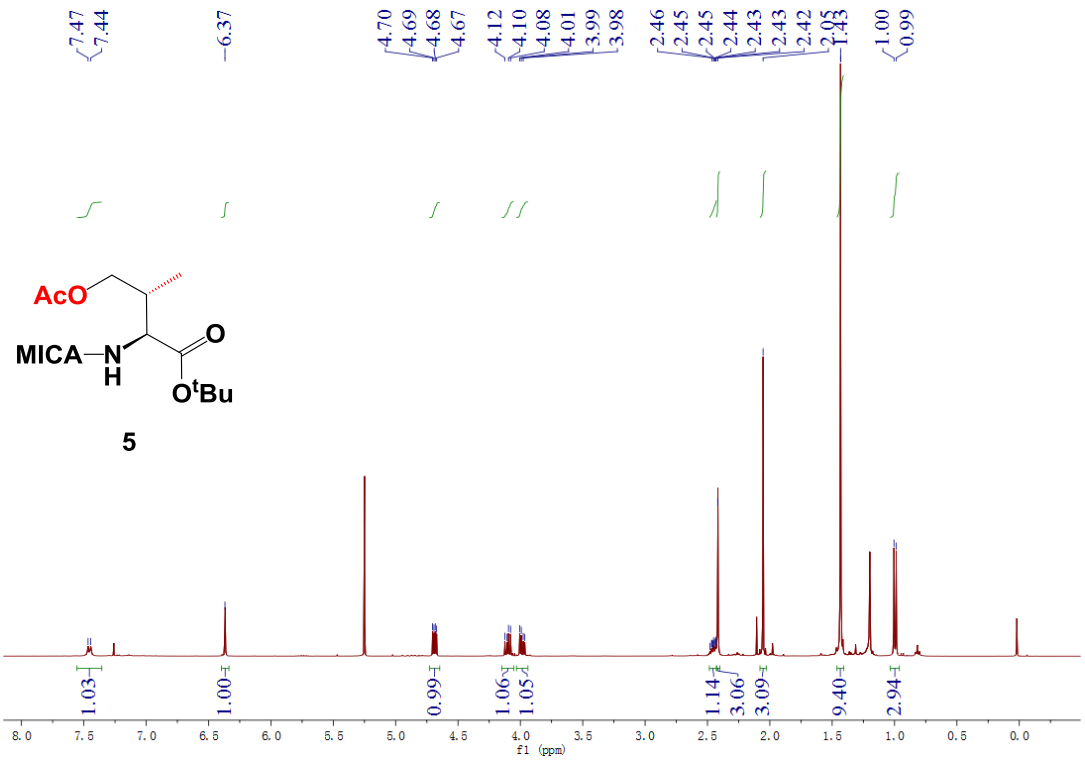
^{1}H NMR of compound **4** (400 MHz, $CDCl_3$)**.**



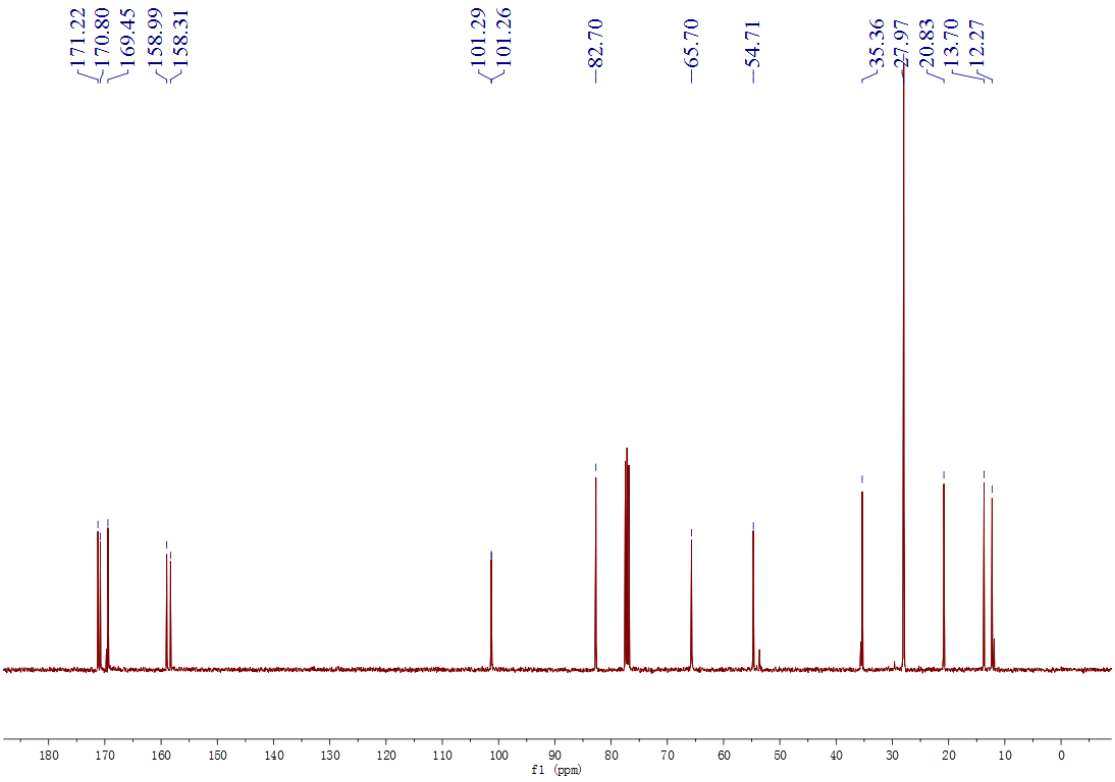
^{13}C NMR of compound **4** (100 MHz, $CDCl_3$)**.**



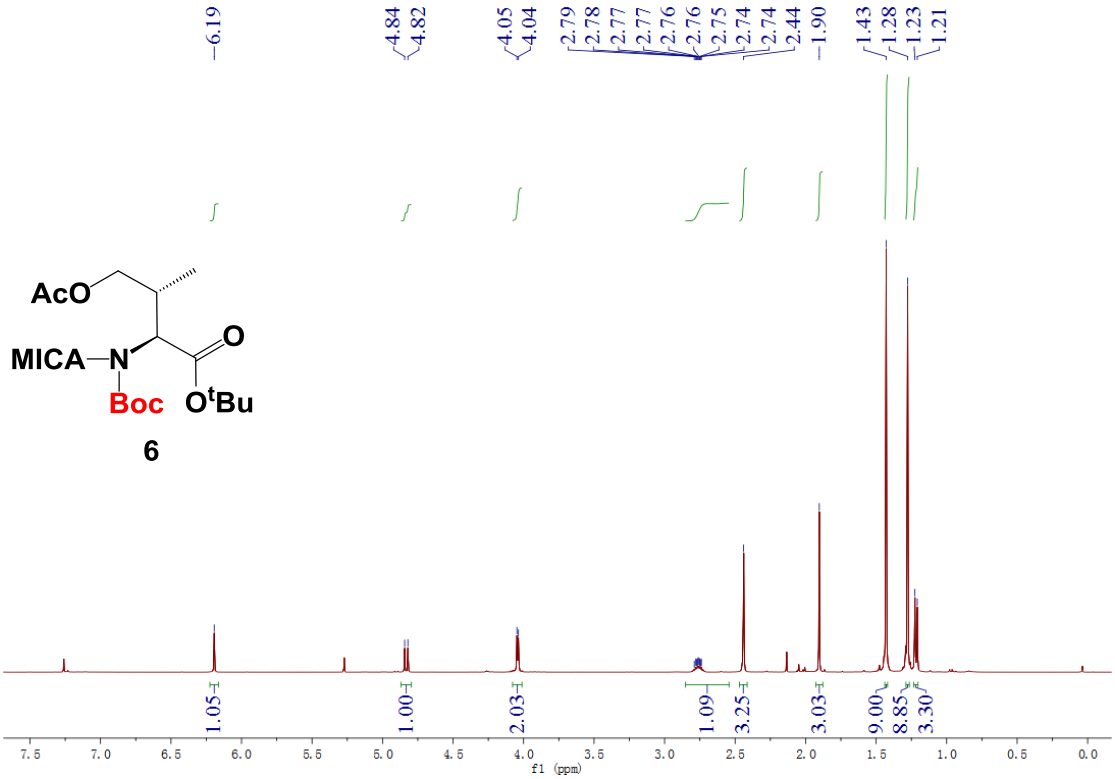
^{1}H NMR of compound **5** (400 MHz, $CDCl_3$)**.**



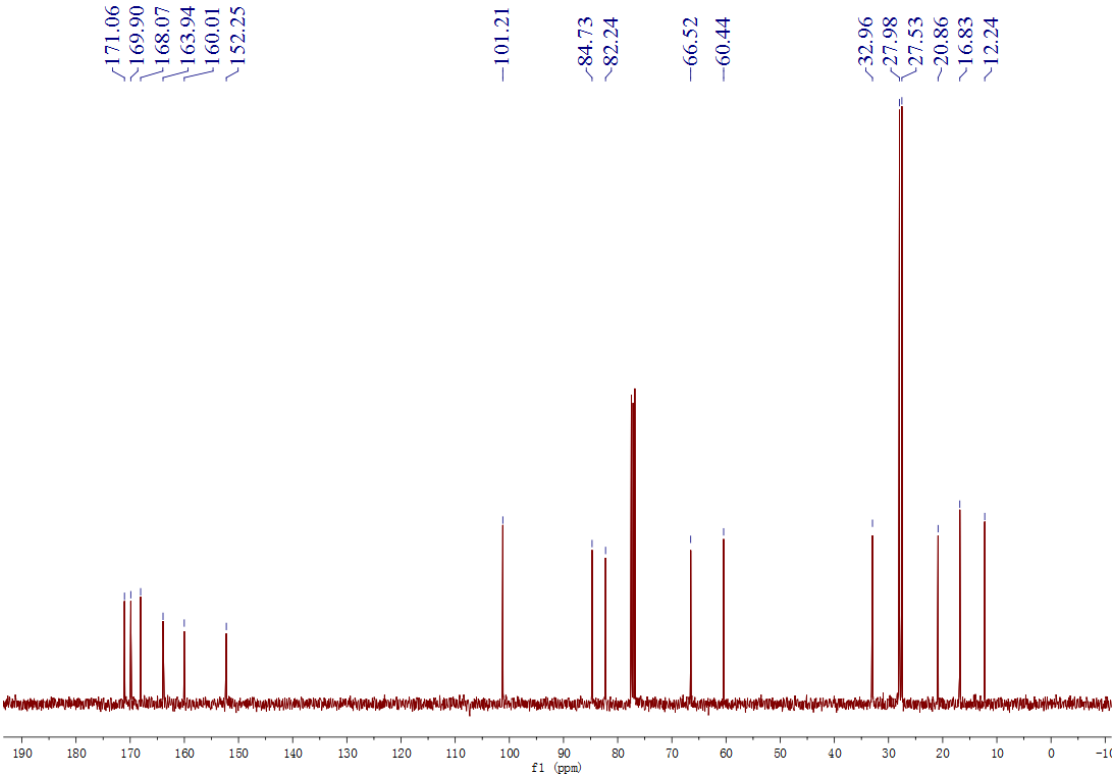
^{13}C NMR of compound **5** (100 MHz, $CDCl_3$)**.**



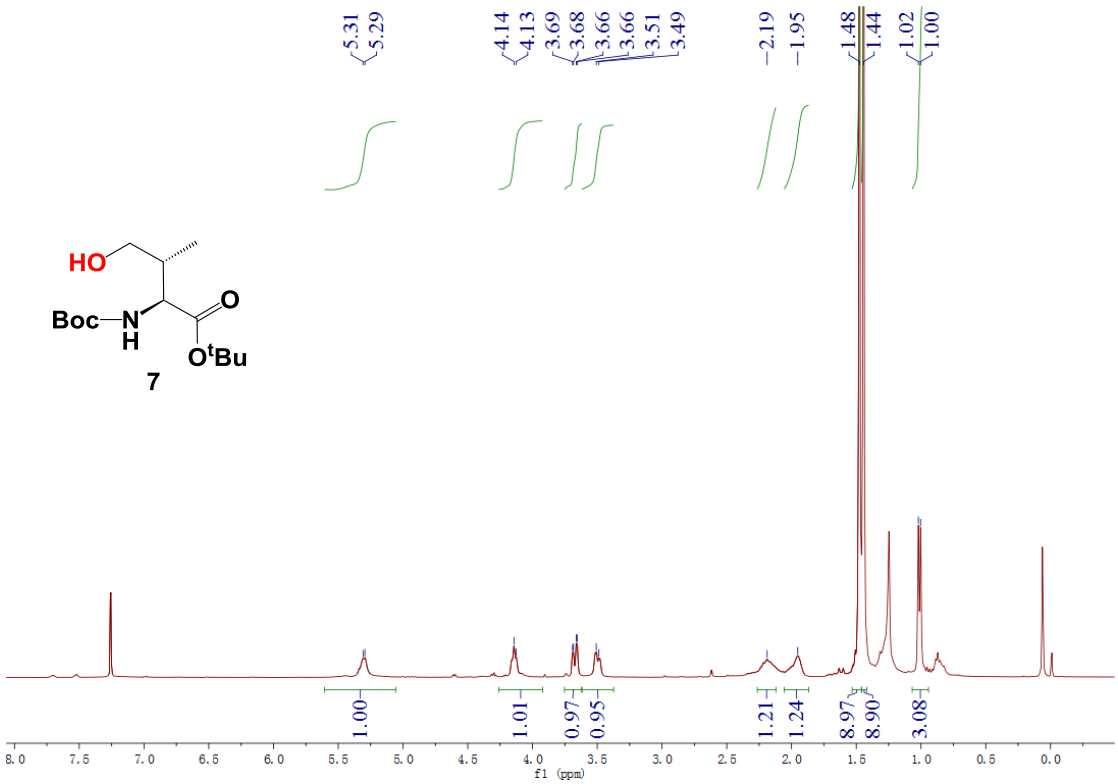
^{1}H NMR of compound **6** (400 MHz, $CDCl_3$)**.**



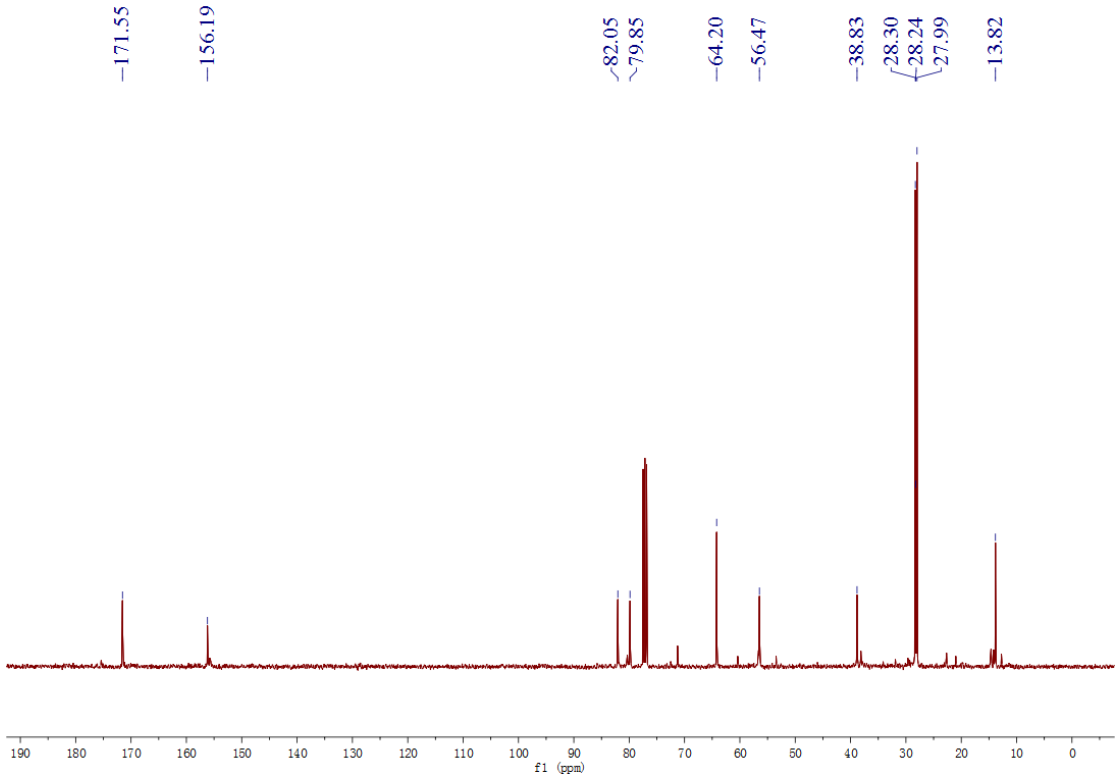
^{13}C NMR of compound **6** (100 MHz, $CDCl_3$)**.**



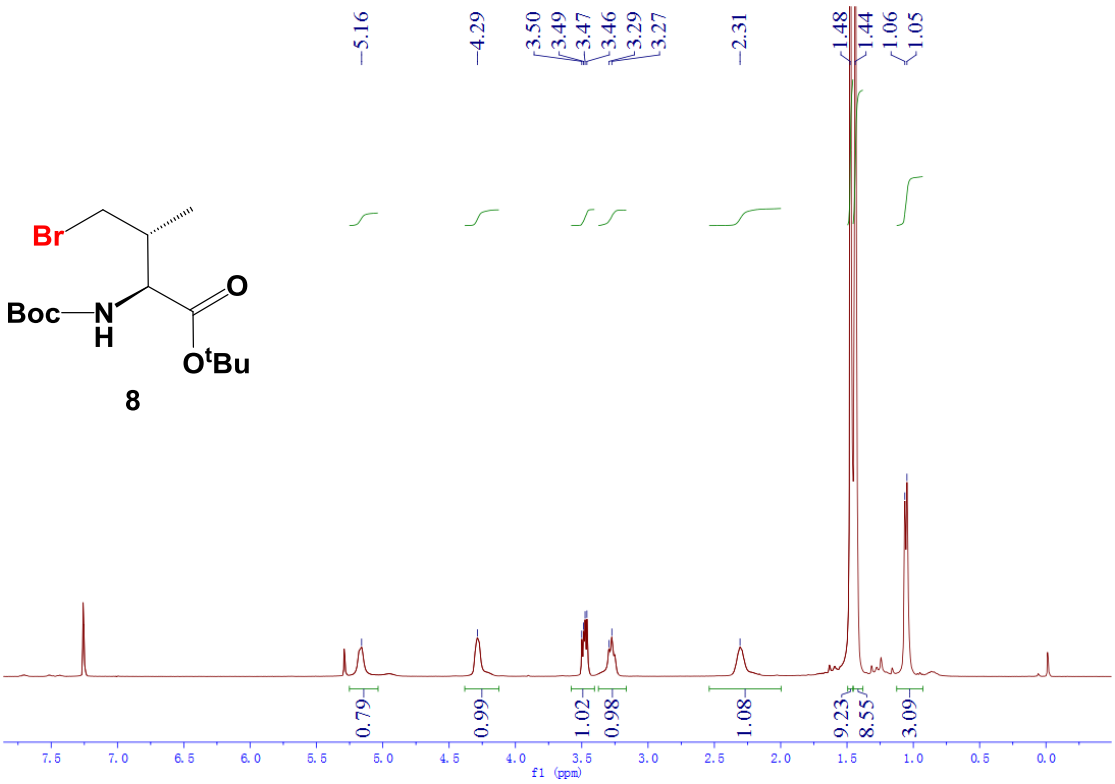
^{1}H NMR of compound **7** (400 MHz, $CDCl_3$)**.**



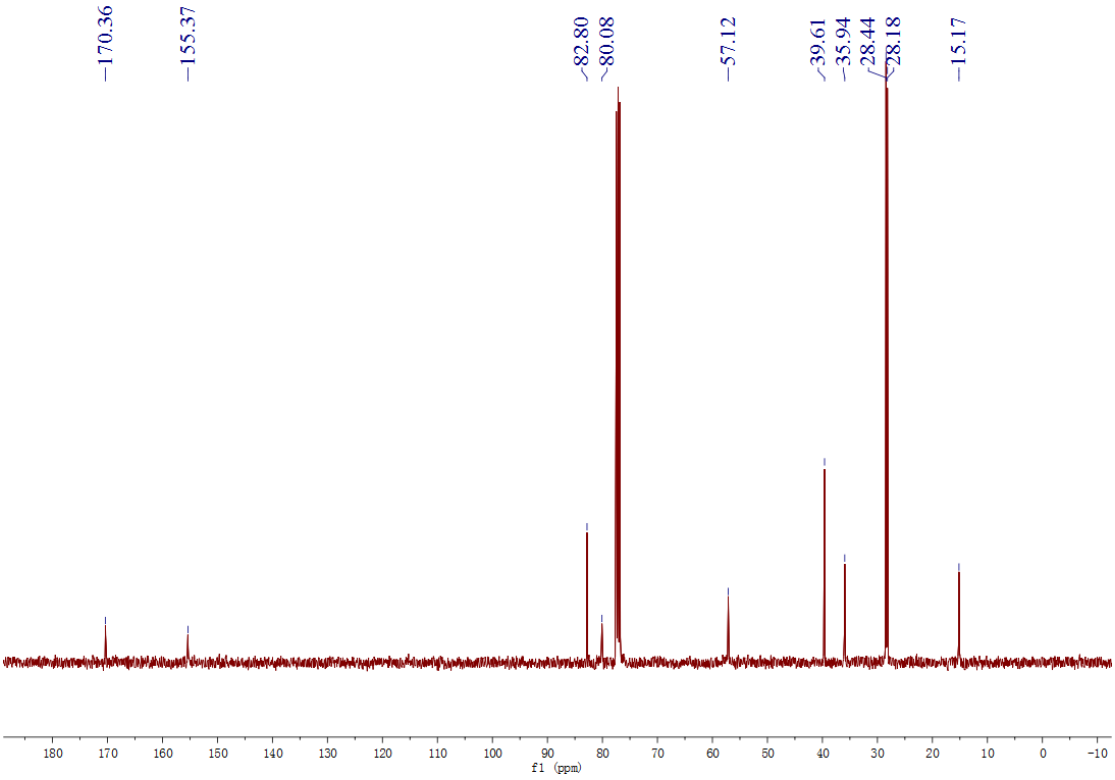
^{13}C NMR of compound **7** (100 MHz, $CDCl_3$)**.**



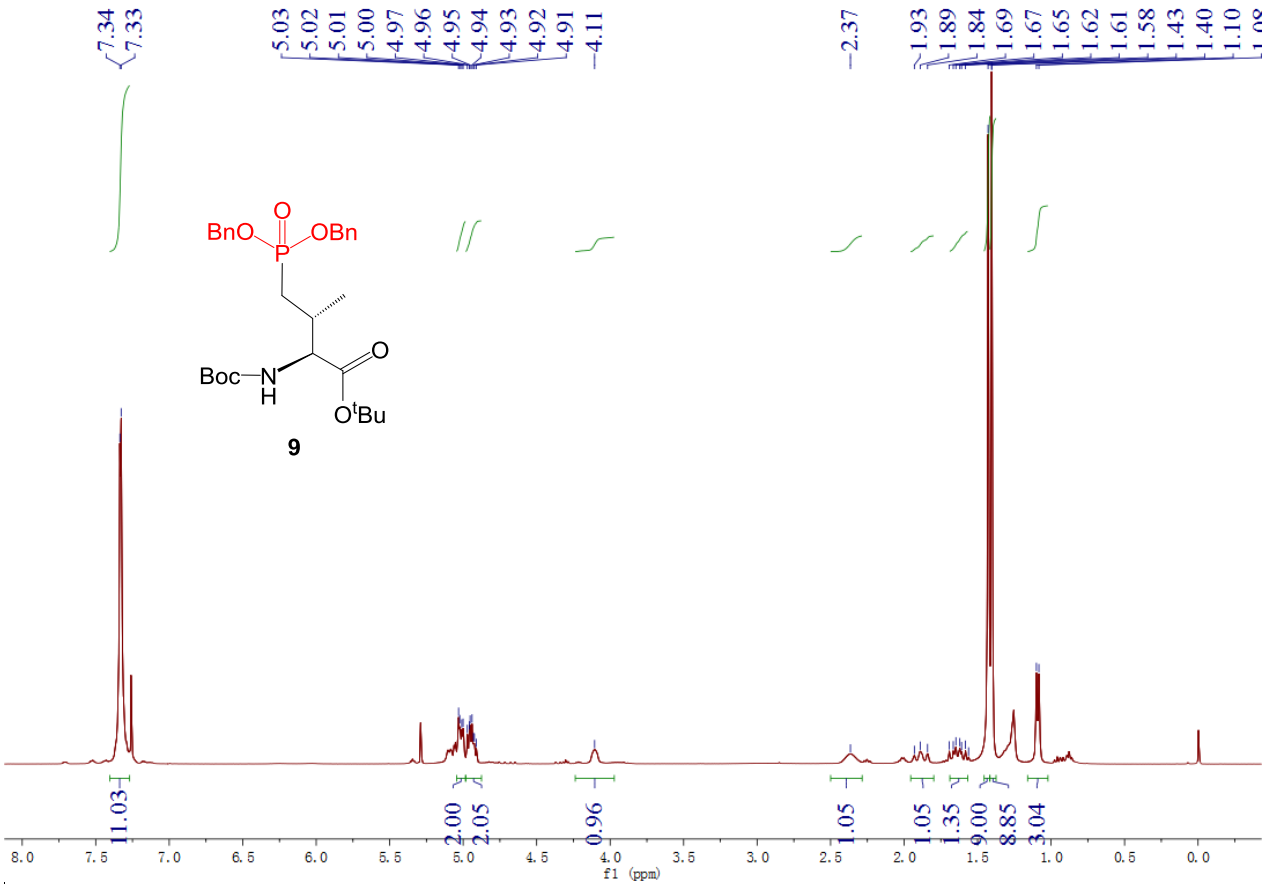
^{1}H NMR of compound **8** (400 MHz, $CDCl_3$)**.**



^{13}C NMR of compound **8** (100 MHz, $CDCl_3$)**.**

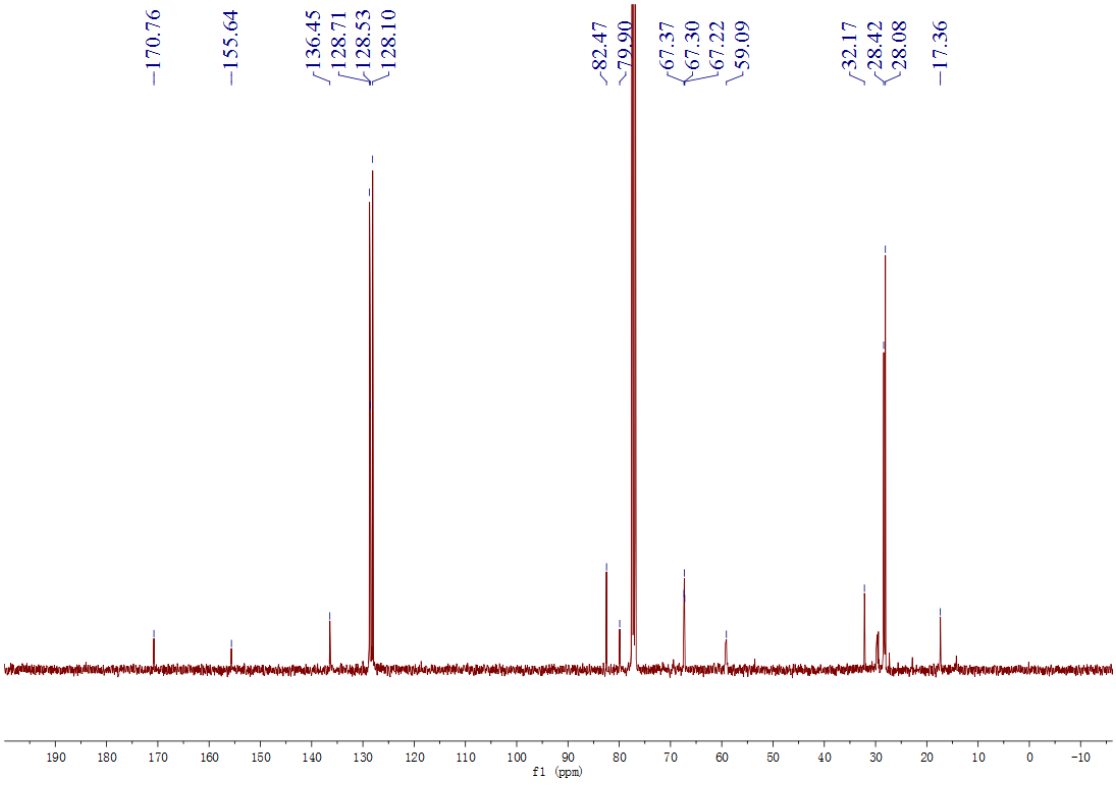


^{1}H NMR of compound **9** (400 MHz, $CDCl_3$).

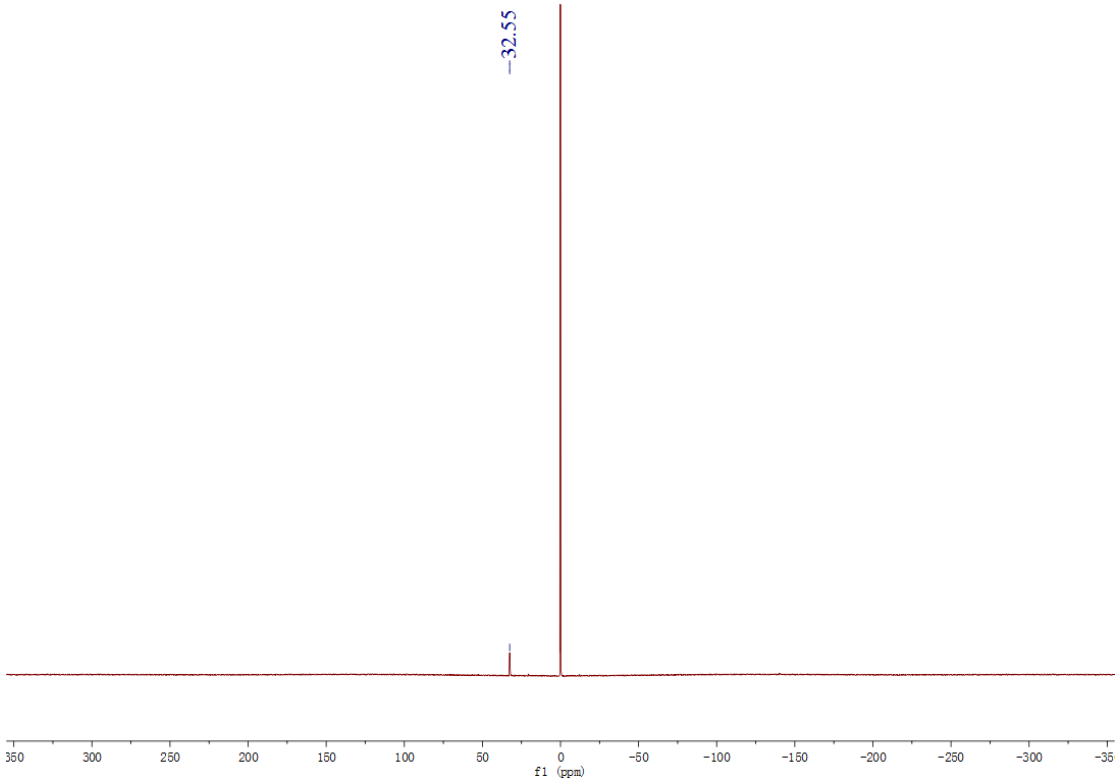


9

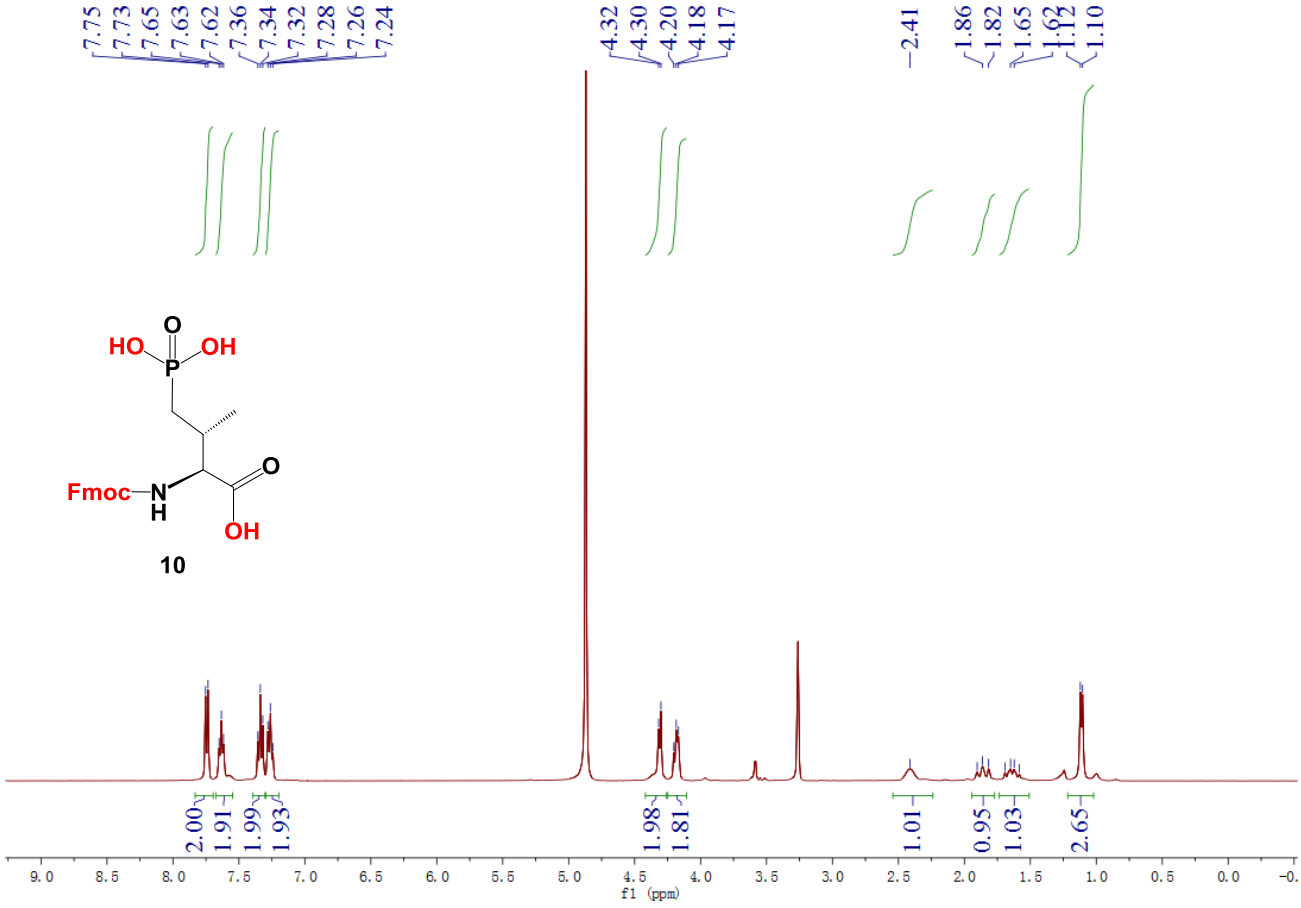
^{13}C NMR of compound **9** (100 MHz, $CDCl_3$).



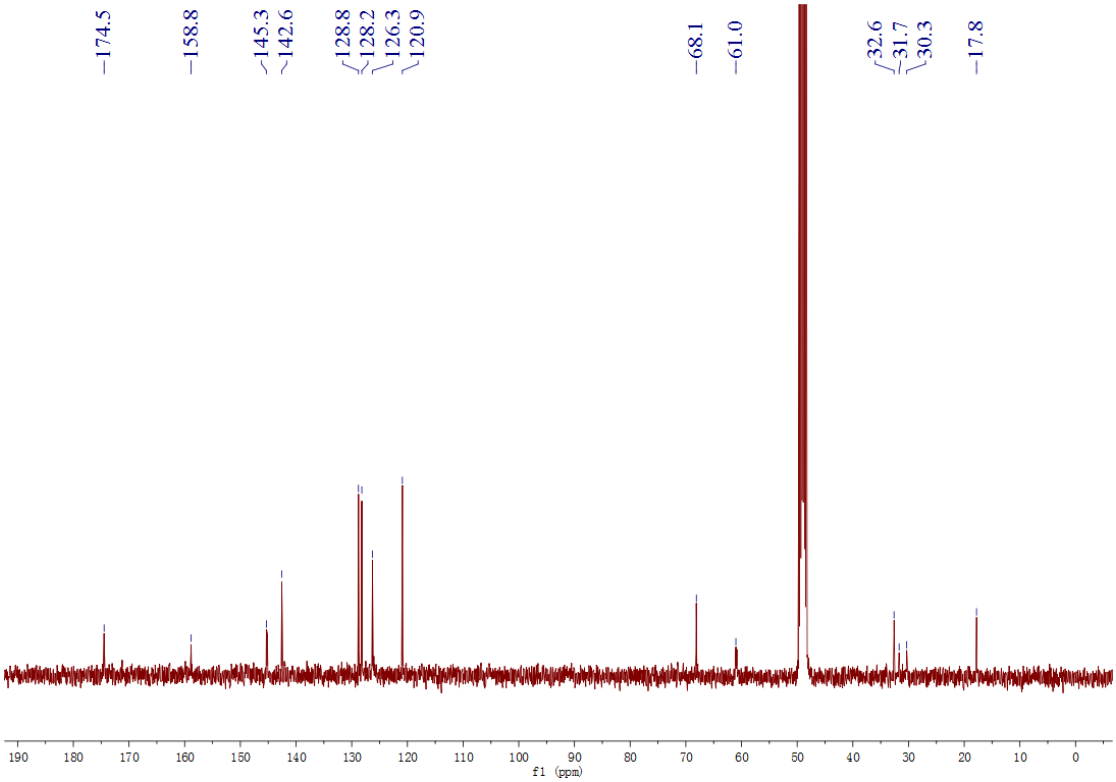
^{31}P NMR of compound **9** (243 MHz, $CDCl_3$)**.**



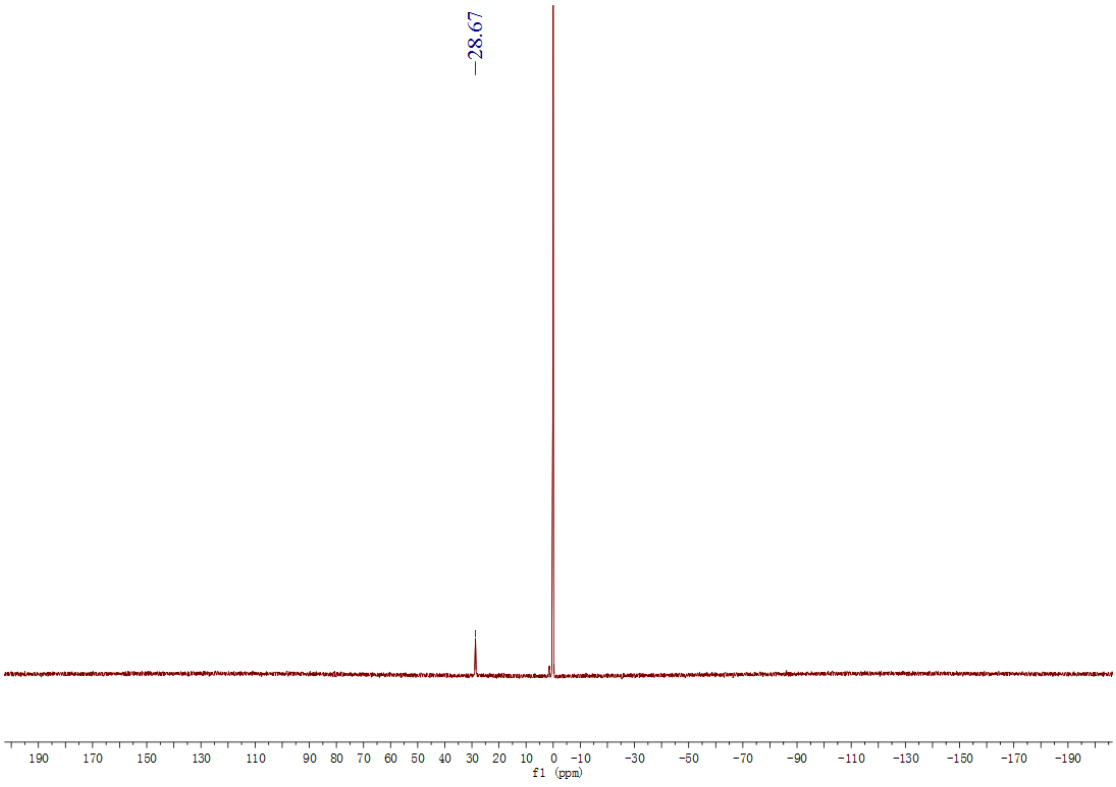
^{1}H NMR of compound **10** (400 MHz, METHANOL-D4)**.**



^{13}C NMR of compound **10** (100 MHz, METHANOL-D4)**.**



^{31}P NMR of compound **10** (243 MHz, METHANOL-D3)**.**

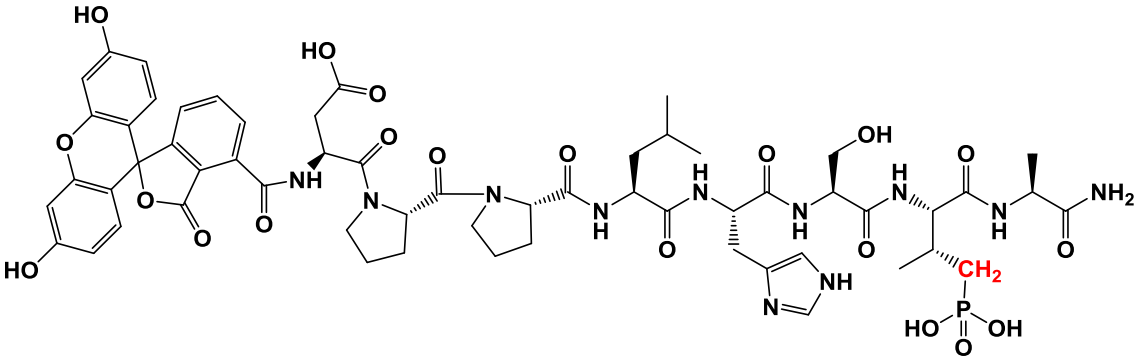


5. Synthesis of peptide inhibitors

The peptide inhibitors were synthesized by use of Fmoc-based solid phase peptide synthesis (SPPS). We used Rink amide MBHA resin for anchoring the peptide as the strategy we reported previously.[2] The swelling of the resin and *N*-Fmoc-protected building blocks condensation were executed in standard SPPS. For peptide inhibitor of 14-3-3 *ζ*, a subsequent linker of PEG and the 5(6)-carboxyfluorescein (5(6)-FAM) were introduced into the N-terminus of the peptide, that is FAM-PEG-RLYH**X**LPA-NH_2 (X = CH_2-pThr, peptide-CH_2-pThr **11**). For peptide inhibitor of Plk1 PBD, its sequence was FAM-DPPLHSpTA labeled with an *N*-terminal 5(6)-FAM. Then, the peptides were cleaved from the resin by mixed reagents of TFA, TIS and water (volume ratio of 95:2.5:2.5) for 3h, as well as all side-chain protecting groups. Finally, we removed the TFA by rotary evaporator and precipitated the residues in ethyl ether. The crude product was purified by preparative HPLC to obtain the pure products. The Peptide-pThr **12** and Peptide-Thr **13** were prepared and characterized by analytical HPLC and ESI-MS in our previous work.[2]

6. Analytical HPLC characterization of Peptide inhibitors.

6.1 Structure and characterization of peptide inhibitor of Plk1 PBD.



Peptide-CH_2-pThr (Plk1 PBD) $C_{58}H_{70}N_{11}O_{20}P$ Exact Mass: 1271.5

Figure S1 The structure of peptide inhibitor of Plk1 PBD.

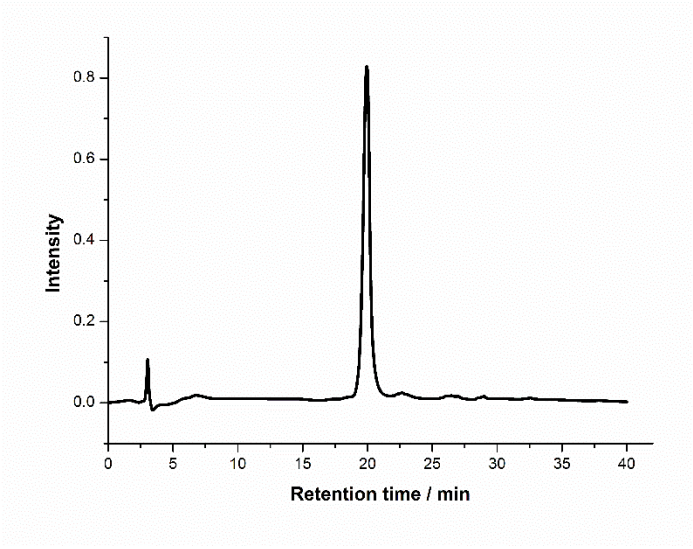


Figure S2 Analytical HPLC chromatogram of peptide inhibitor of Plk1 PBD, monitored at 215 nm. Conditions: C18 reversed-phase column. 5% B to 85% B in 40 min. The eluents of A: 0.06% TFA in water; The eluents of B: 80% acetonitrile and 0.06% TFA in water. Flow rate: 0.8 ml/min.

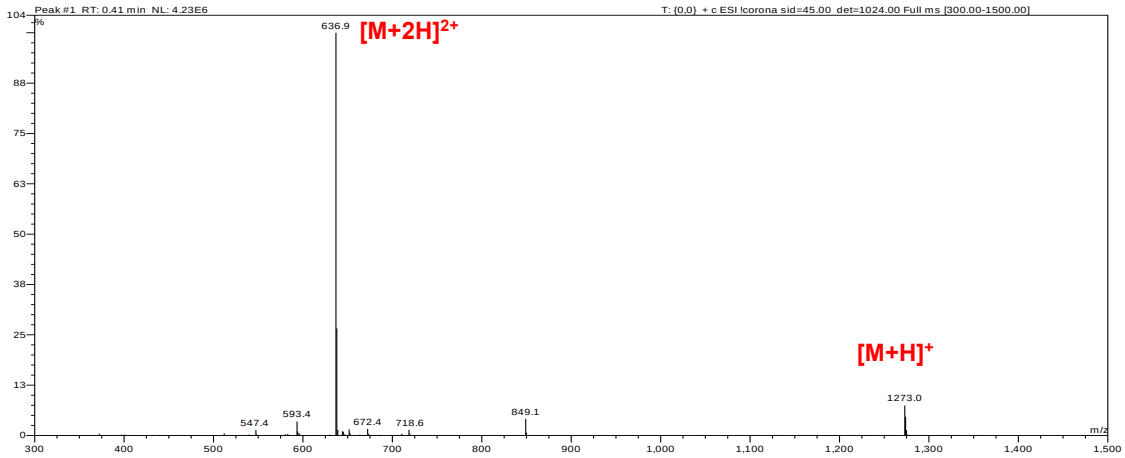


Figure S3 ESI-MS data of peptide inhibitor of Plk1 PBD, calculated for $C_{76}H_{102}N_{15}O_{22}P$: 1271.5, found $[M+H]^+$=1273.0, $[M+2H]^{2+}$=636.9.

6.2 Structure and characterization of Peptide-CH_2-pThr 11.

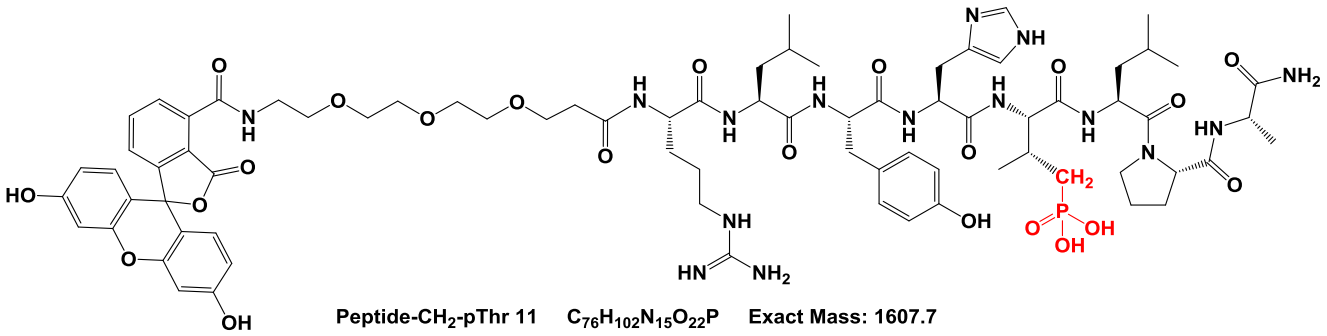


Figure S4 The structure of Peptide-CH_2-pThr **11**.

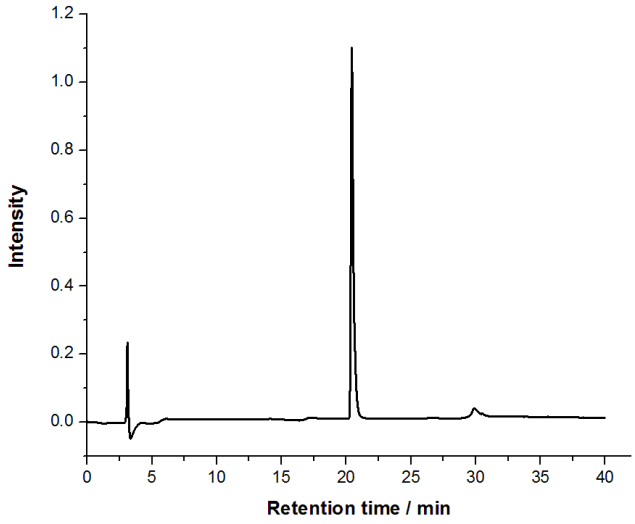


Figure S5 Analytical HPLC chromatogram of Peptide-CH_2-pThr **11**, monitored at 215 nm. The experimental conditions were same as the peptide inhibitor of Plk1 PBD.

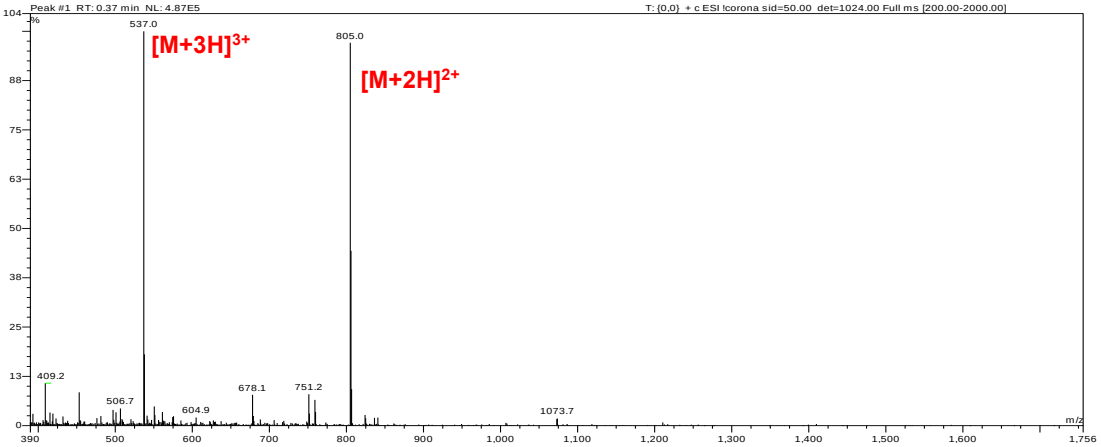


Figure S6 ESI-MS data of Peptide-CH_2-pThr **11**, calculated for $C_{76}H_{102}N_{15}O_{22}P$: 1607.7, found $[M+2H]^{2+}$=805.0, $[M+3H]^{3+}$=537.0.

7. Fluorescence polarization experiment

The fluorescence polarization assay was carried out in the presence of 14-3-3 *ζ* which was obtained by our previously reported procedures.[2-3] Mixed the fluorescent peptide-CH_2-pThr **11** (0.20 *μ*M) in buffer A (50 mM Tris, 100 mM NaCl, pH=7.5) with 14-3-3 *ζ* protein to a total volume of 200 *μ*L, The concentration of 14-3-3 *ζ* was enhanced from 0 to 10 *μ*M gradually. The polarization values was excitated at 494 nm and emission at 520 nm. After full incubation of peptide-CH_2-pThr **11** and 14-3-3 *ζ* at room temperature, the polarization values was measured by enzyme-labeled instrument. The polarization values of the Peptide-CH_2-pThr **11** was fitted to the below **Equation 1**.

$$F = F_{min} + \left(K_d + A_0 + E_0 - \sqrt{(K_d + A_0 + E_0)^2 - 4A_0E_0}\right) * \frac{F_{max} - F_{min}}{2A_0} \quad \textbf{(Equation 1)}$$

In **Equation 1**, the meaning of parameters F, F_{max}, F_{min}, K_d, A_0, and E_0 were stated as our previous work.[2] The K_d value was generated by a Grafit software.

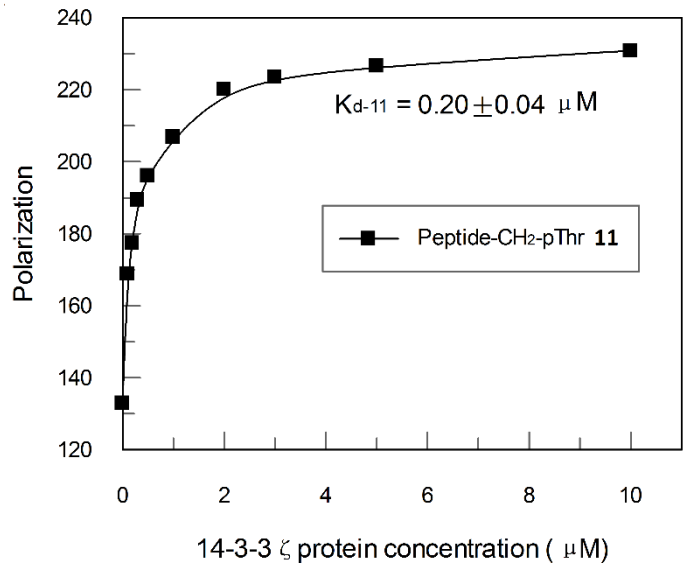


Figure S7 Fluorescence polarization measurement of Peptide-CH_2-pThr **11**. K_d value was generated by fitting to Equation 1.

References:

(1) Pasunooti, K. K.; Yang, R.; Banerjee, B.; Yap, T.; Liu, C. F.; *Org Lett* **2016,** 18, 2696.

(2) Chen, H. X.; Kang, J.; Chang, R.; Zhang, Y. L.; Duan, H. Z.; Li, Y. M.; Chen, Y. X.; *Org Lett* **2018,** 20, 3278.

(3) Kang, J.; Chen, H.-X.; Huang, S.-Q.; Zhang, Y.-L.; Chang, R.; Li, F.-Y.; Li, Y.-M.; Chen, Y.-X.; *Tetrahedron Lett* **2017,** 58, 2551.