Supporting Information

Visible-light mediated selective phosphonylation modification of tryptophan residues in oligopeptides

Wenfang Xiong,* Junye He, Jinyao Liu, Peiru Chen, Shiqi Xu, Yipeng Liu, Shiting Chen, Yuanyuan You, Zhenyu Chen,* Jinwu Zhao*

School of Pharmacy, Guangdong Medical University, Dongguan, 523808, China.

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A. General methods

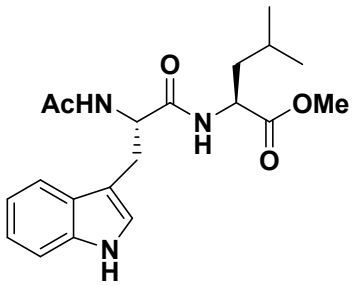
Thin layer chromatography (TLC) employed glass 0.25 mm silica gel plates. Flash chromatography columns were packed with 200-300 mesh silica gel. The separated product is eluted by gradient rapid chromatography using dichloromethane and methanol in corresponding proportions. The known compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR. ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded using a Bruker DRX-400 spectrometer with $CDCl_3$ or CD_3OD or DMSO-d_6 as the solvent and TMS as the internal standard. For ^{1}H-NMR, chemical shifts (δ) were given in ppm relatives to the internal standard (TMS at 0 ppm, CD_3OD at 3.31 ppm, $CDCl_3$ at 7.26 ppm, DMSO-d_6 at 2.50 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, br = broad. Coupling constants are reported as a J value in Hertz (Hz). For ^{13}C-NMR, chemical shifts (δ) were reported in ppm using solvent as internal standard ($CDCl_3$ at 77.16 ppm, CD_3OD at 49.00 ppm, DMSO-d6 at 39.52 ppm). High Resolution Spectra (HRMS) were recorded on an IonSpec FTICR mass spectrometer with Electron Spray Ionization (ESI) resource. LC analyses were performed with a Agilent Technologies 1260 Infinity II using HPLCONE® Packed Column 10C18A (10 μm, 4.6 ID × 250 mm). Linear gradients using A: MeCN (0.1% TFA) and B: H_2O (0.1% TFA) were run over varying periods of time. Semi preparative HPLC was carried out on a Ruihe Tech P2050 using a Ruihe Tech C18 (ID10 × 250 mm) preparative column. Linear gradients using A: MeCN (0.1% TFA) and B: H_2O (0.1% TFA) were run over varying periods of time. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Protected L-tryptophan, Ac-Trp-OMe, Boc-Trp-OEt, Ac-Trp, Fmoc-Trp, Boc-Trp were purchased from Bidepharm. Oligopeptides **S9-S15**, **S17**, **S18** were synthesized according to the literature procedure.[1-2] Modified Endorphin-1(**19)**, Segetalin A (**21)**, Segetalin B (**24)** were purchased from GL Biochem (shanghai) Ltd.

B. General procedures for the synthesis of oligopeptides

In a round-bottomed flask (250 mL), equipped with a stir bar, N-terminal protected amino acid or dipeptide (5.0 mmol), HOBT (1-hydroxybenzotriazole) (7.5 mmol), HBTU (O-benzotriazole-*N*, *N*, *N*', *N*'-tetramethyl-uronium-hexafluorophosphate) (7.5 mmol), dichloromethane (100 mL) and triethylamine (6.0 mmol) were combined and added. The

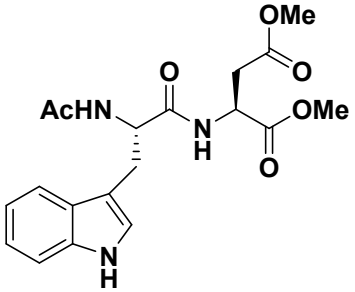
mixture was stirred for 30 min at room temperature, and then, C-terminal protected amino acid hydrochloride (5.0 mmol) and triethylamine (5.0 mmol) were added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated $NaHCO_3$ solution (100 mL × 3), 2 M hydrochloric acid solution (100 mL × 3) and H_2O (100 mL × 3). The organic layers were combined, dried over Na_2SO_4, and concentrated. The resulting crude product was purified by flash column chromatography on silica gel (dichloromethane/ethyl acetate) to afford corresponding dipeptides **S9-S15，S17-S18**.

C. Characterization data for oligopeptides



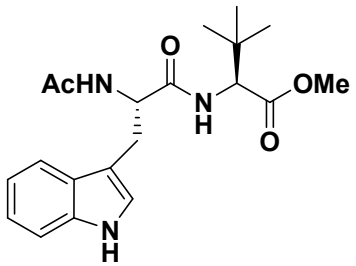
Ac-Trp-Leu-OMe (**S9**)

Compound **S9** (precursor of compound **9**) was prepared as a yellow solid (1.77 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1 H), 8.33 (d, *J* = 7.8 Hz, 1 H), 7.98 (d, *J* = 8.2 Hz, 1 H), 7.62 (d, *J* = 7.9 Hz, 1 H), 7.33 (d, *J* = 8.1 Hz, 1 H), 7.13 (s, 1 H), 7.06 (t, *J* = 6.9 Hz, 1 H), 7.01 – 6.95 (m, 1 H), 4.63 – 4.54 (m, 1 H), 4.38 – 4.27 (m, 1 H), 3.62 (s, 3 H), 3.14 – 3.05 (m, 1 H), 2.93 – 2.85 (m, 1 H), 1.77 (s, 3 H), 1.62 – 1.51 (m, 2 H), 1.18 (t, *J* = 7.1 Hz, 1 H), 0.90 (d, *J* = 6.3 Hz, 3 H), 0.85 (d, *J* = 6.3 Hz, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.3, 172.5, 169.5, 136.5, 127.8, 124.0, 121.3, 118.9, 118.6 111.7, 110.6, 53.5, 52.3, 50.8, 38.7, 28.2, 24.6, 23.2, 23.0.



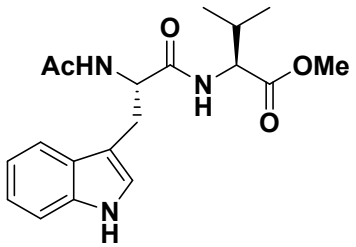
Ac-Trp-L-Asp acid dimethyl (**S10**)

Compound **S10** (precursor of compound **10**) was prepared as a yellow solid (1.63 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1 H), 7.59 (d, *J* = 7.9 Hz, 1 H), 7.30 (d, *J* = 8.1 Hz, 1 H), 7.17 – 7.10 (m, 2 H), 7.09 – 7.00 (m, 2 H), 6.68 (d, *J* = 7.8 Hz, 1 H), 4.80 (q, *J* = 6.8 Hz, 1 H), 4.76 – 4.69 (m, 1 H), 3.63 (s, 3 H), 3.58 (s, 3 H), 3.22 (d, *J* = 6.5 Hz, 2 H), 2.86 (dd, *J* = 17.1, 4.9 Hz, 1 H), 2.75 (dd, *J* = 17.1, 5.0 Hz, 1 H), 1.89 (s, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 171.1, 170.6, 170.4, 136.1, 127.5, 123.4, 121.8, 119.3, 118.4, 111.2, 109.8, 53.7, 52.6, 51.9, 48.6, 35.8, 28.2, 22.9.



Ac-Trp-Tle-OMe (**S11**)

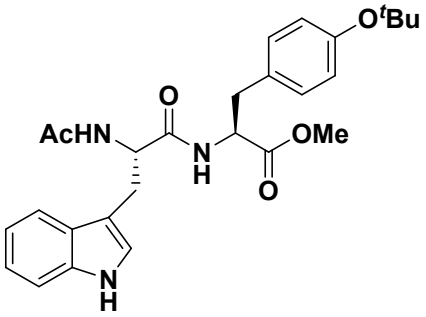
Compound **S11** (precursor of compound **11**) was prepared as a yellow solid (1.68 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1 H), 7.64 (d, *J* = 7.9 Hz, 1 H), 7.31 (d, *J* = 8.1 Hz, 1 H), 7.18 – 7.12 (m, 1 H), 7.09 – 6.99 (m, 2 H), 6.76 – 6.62 (m, 2 H), 4.89 – 4.80 (m, 1 H), 4.29 (d, *J* = 8.9 Hz, 1 H), 3.57 (s, 3 H), 3.26 – 3.19 (m, 1 H), 3.18 – 3.10 (m, 1 H), 1.94 (s, 3 H), 0.84 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.2, 170.4, 136.2, 127.3, 123.4, 122.0, 119.5, 118.6, 111.2, 110.3, 60.4, 54.0, 51.7, 34.5, 28.2, 26.4, 23.0.



Ac-Trp-Val-OMe (**S12**)

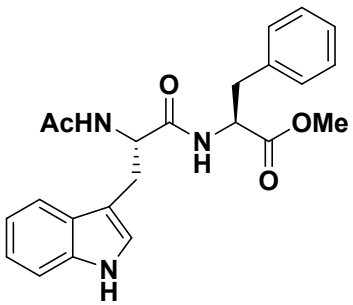
Compound **S12** (precursor of compound **12**) was prepared as a yellow solid (1.65g, 92%). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.60 (s, 1 H), 8.01 (d, *J* = 8.1 Hz, 1 H), 7.83 (d, *J* = 8.2 Hz, 1 H), 7.42 (d, *J* = 7.8 Hz, 1 H), 7.14 (d, *J* = 8.1 Hz, 1 H), 6.95 (s, 1 H), 6.87 (t, *J* = 7.5 Hz, 1 H), 6.82 – 6.75 (m, 1 H), 4.53 – 4.44 (m, 1 H), 4.07 – 3.99 (m, 1 H), 3.43 (s, 3 H), 2.91 (dd, *J* = 14.7, 5.0 Hz, 1 H), 2.73 (dd, *J* = 14.7, 9.0 Hz, 1 H), 1.87 (p, *J* = 6.7 Hz, 1 H), 1.60 (s, 3 H), 0.74

– 0.67 (m, 6 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.5, 170.3, 136.2, 127.4, 123.4, 122.1, 119.7, 118.7, 111.2, 110.4, 57.5, 54.0, 52.0, 31.0, 28.4, 23.1, 18.7, 17.7.



Ac-Trp-Tyr(t-Bu)-OMe (**S13**)

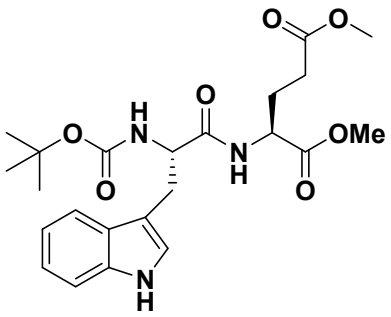
Compound **S13** (precursor of compound **13**) was prepared as a yellow solid (2.23g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1 H), 7.68 (d, *J* = 7.9 Hz, 1 H), 7.34 (d, *J* = 8.0 Hz, 1 H), 7.20 – 7.16 (m, 1 H), 7.13 – 7.09 (m, 1 H), 6.98 (d, *J* = 2.4 Hz, 1 H), 6.80 (d, *J* = 2.5 Hz, 4 H), 6.55 (d, *J* = 7.6 Hz, 1 H), 6.45 (d, *J* = 7.7 Hz, 1 H), 4.80 – 4.74 (m, 1 H), 4.67 (q, *J* = 6.4 Hz, 1 H), 3.60 (s, 3 H), 3.28 (dd, *J* = 14.6, 5.4 Hz, 1 H), 3.13 (dd, *J* = 14.6, 7.8 Hz, 1 H), 2.98 – 2.86 (m, 2 H), 1.95 (s, 3 H), 1.32 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 171.1, 170.2, 154.2, 136.1, 130.4, 129.5, 127.4, 124.1, 123.4, 122.0, 119.5, 118.6, 111.2, 110.2, 78.5, 53.7, 53.5, 52.1, 37.1, 28.7, 28.1, 23.0.



Ac-Trp-Phe-OMe (**S14**)

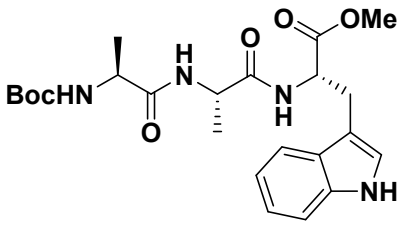
Compound **S14** (precursor of compound **14**) was prepared as a yellow solid (1.95 g, 96%). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.80 (s, 1 H), 8.40 (d, *J* = 7.6 Hz, 1 H), 7.99 (d, *J* = 8.3 Hz, 1 H), 7.61 (d, *J* = 7.9 Hz, 1 H), 7.34 (d, *J* = 8.0 Hz, 1 H), 7.27 (d, *J* = 6.9 Hz, 2 H), 7.22 (d, *J* = 7.5 Hz, 3 H), 7.13 – 7.05 (m, 2 H), 7.01 – 6.97 (m, 1 H), 4.64 – 4.57 (m, 1 H), 4.55 – 4.48 (m, 1 H), 3.59 (s, 3 H), 3.13 – 3.03 (m, 2 H), 2.97 (dd, *J* = 13.8, 8.5 Hz, 1 H), 2.88 (dd, *J* = 14.7, 8.9 Hz, 1 H), 1.77 (s, 3 H). ^{13}C NMR (101 MHz, DMSO-d6) δ 172.3, 172.2, 169.5, 137.6, 136.5,

129.6, 128.7, 127.8, 127.0, 123.9, 121.3, 118.9, 118.6, 111.7, 110.6, 54.1, 53.5, 52.3, 37.1, 28.2, 23.0.



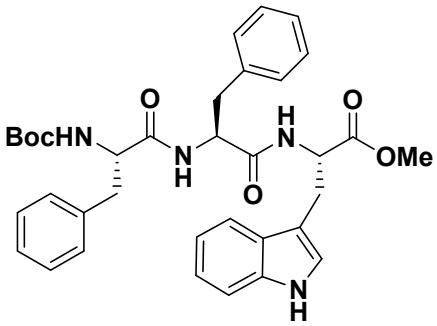
Boc-Trp-L-Glu acid dimethyl ester (**S15**)

Compound **S15** (precursor of compound **15**) was prepared as a yellow solid (2.0 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1 H), 7.64 (d, *J* = 7.8 Hz, 1 H), 7.37 (d, *J* = 8.0 Hz, 1 H), 7.23 – 7.17 (m, 1 H), 7.15 – 7.08 (m, 2 H), 6.60 (s, 1 H), 5.22 (d, *J* = 7.9 Hz, 1 H), 4.56 – 4.42 (m, 2 H), 3.65 (d, *J* = 4.9 Hz, 6 H), 3.39 – 3.30 (m, 1 H), 3.21 (d, *J* = 7.2 Hz, 1 H), 2.27 – 2.06 (m, 3 H), 1.93 – 1.82 (m, 1 H), 1.45 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 171.9, 171.6, 155.5, 136.2, 127.5, 123.4, 122.1, 119.7, 118.6, 111.2, 110.2, 80.3, 55.4, 52.4, 51.8, 51.6, 29.6, 27.2.



Boc-Ala-Ala-Trp-OMe (**S17**)

Compound **S17** (precursor of compound **17**) was prepared as a yellow solid (2.23 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1 H), 7.49 (d, *J* = 7.7 Hz, 1 H), 7.30 (d, *J* = 8.1 Hz, 1 H), 7.21 (d, *J* = 7.7 Hz, 1 H), 7.15 – 7.10 (m, 1 H), 7.07 (t, *J* = 6.9 Hz, 1 H), 7.00 (d, *J* = 16.4 Hz, 1 H), 5.52 (d, *J* = 7.3 Hz, 1 H), 4.92 – 4.80 (m, 1 H), 4.58 – 4.47 (m, 1 H), 4.24 – 4.09 (m, 1 H), 3.69 – 3.55 (m, 3 H), 3.36 – 3.19 (m, 2 H), 1.44 (s, 9 H), 1.30 – 1.25 (m, 3 H), 1.20 (d, *J* = 7.1 Hz, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 172.1, 155.5, 136.1, 136.0, 127.2, 123.5, 121.7, 119.2, 119.1, 118.1, 111.3, 108.9, 80.0, 52.8, 52.3, 50.0, 48.6, 28.2, 27.3, 18.2.



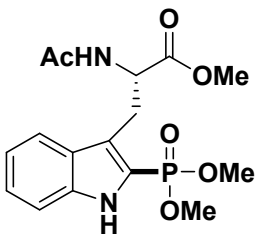
Boc-Phe-Phe-Trp-OMe (**S18**)

Compound **S18** (precursor of compound **18**) was prepared as a yellow solid (2.85 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1 H), 7.44 (d, *J* = 7.9 Hz, 1 H), 7.33 (d, *J* = 8.1 Hz, 1 H), 7.29 – 7.15 (m, 8 H), 7.14 – 7.06 (m, 5 H), 6.88 (d, *J* = 2.4 Hz, 1 H), 6.57 (d, *J* = 8.0 Hz, 2 H), 4.92 – 4.79 (m, 2 H), 4.72 – 4.64 (m, 1 H), 3.65 (s, 3 H), 3.26 – 3.24 (m, 1 H), 2.98 (d, *J* = 6.8 Hz, 2 H), 2.83 (s, 3 H), 1.40 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.1, 170.0, 155.5, 136.3, 136.2, 136.0, 129.3, 129.2, 128.6, 128.5, 127.3, 127.0, 126. 9, 123.3, 122.0, 119.4, 118.3, 111.3, 109.3, 80.5, 55.6, 54.0, 52.8, 52.3, 38.6, 37.8, 28.2, 27.5.

D. General procedures for the synthesis of phosphonylation peptide

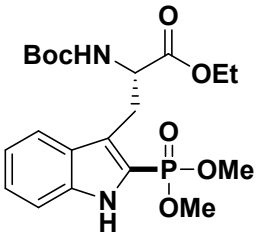
To an oven-dried quarts test-tube (25 mL) equipped with a magnetic stirring bar, the mixture of protected tryptophan or tryptophan oligopeptides (0.2 mmol), trimethyl phosphite or triethyl phosphite (5 eq, 1 mmol), photosensitizer (3 mol%, 0.006 mmol) and 2 mL acetonitrile was added successively. The reaction mixture was stirred at room temperature under the irradiation at 450−460 nm (25 W LED, distance = 8−10 cm, cooling by circulating water) for 18 h. After the reaction was completed, the residual material was quenched with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel to provide the desired products **3-18**.

E. Charaterization data for phosphonylation oligopeptides



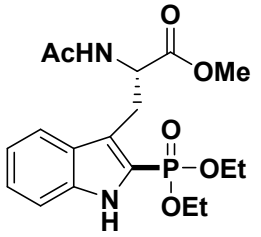
Ac-Trp-OMe-Methyl phosphate (**3**)

The reaction was conducted on a 0.2 mmol scale, affording **3** as a yellow solid (47.8 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1 H), 7.71 (d, *J* = 8.1 Hz, 1 H), 7.64 (d, *J* = 6.6 Hz, 1 H), 7.40 (d, *J* = 8.3 Hz, 1 H), 7.32 (t, *J* = 7.6 Hz, 1 H), 7.18 (t, *J* = 7.5 Hz, 1 H), 4.71 – 4.64 (m, 1 H), 3.86 – 3.77 (m, 6 H), 3.70 (s, 3 H), 3.50 – 3.38 (m, 2 H), 1.92 (s, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.5, 137.6 (d, *J* = 12.1 Hz), 127.2 (d, *J* = 16.2 Hz), 125.6, 122.4 (d, *J* = 19.2 Hz), 120.8, 120.0, 118.6, 112.0 (d, *J* = 2.02 Hz), 53.8, 53.1 (d, *J* = 5.05 Hz), 53.0 (d, *J* = 5.05 Hz), 52.3, 26.7, 22.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.0. HRMS-ESI (*m/z*): calcd for $C_{16}H_{22}N_2O_6P^+$ $[M + H]^+$: 369.1210; found: 369.1204.



Boc-Trp-OEt-Methyl phosphate (**4**)

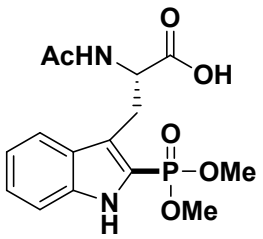
The reaction was conducted on a 0.2 mmol scale, affording **4** as a a yellow solid (55.6mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.73 (dd, *J* = 8.0, 1.0 Hz, 1 H), 7.42 (d, *J* = 8.2 Hz, 1 H), 7.33 (t, *J* = 7.6 Hz, 1 H), 7.19 (t, *J* = 7.6 Hz, 1 H), 5.89 (d, *J* = 7.6 Hz, 1 H), 4.51 – 4.41 (m, 1 H), 4.18 – 4.11 (m, 2 H), 3.83 (d, *J* = 1.9 Hz, 3 H), 3.80 (d, *J* = 1.9 Hz, 3 H), 3.48 – 3.32 (m, 2 H), 1.33 (s, 9 H), 1.21 (d, *J* = 7.2 Hz, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 155.5, 137.5 (d, *J* = 13.1 Hz), 127.5 (d, *J* = 16.2 Hz), 125.3, 122.1 (d, *J* = 19.2 Hz), 120.6, 120.2, 118.7, 111.9, 79.3, 61.2, 54.7, 53.0, 28.2, 27.2, 14.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.4, 14.4, 14.3, 14.2, 14.2. HRMS-ESI (*m/z*): calcd for $C_{20}H_{30}N_2O_7P^+$ $[M + H]^+$: 441.1785; found: 441.1782.



Ac-Trp-OMe-Phosphoryl ethyl ester (**5**)

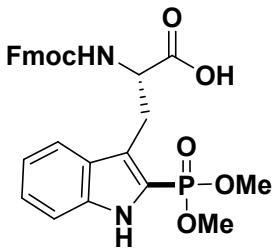
The reaction was conducted on a 0.2 mmol scale, affording **5** as a yellow solid (50 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1 H), 7.86 (d, *J* = 6.3 Hz, 1 H), 7.73 (d, *J* = 8.1 Hz, 1 H),

7.42 (d, *J* = 8.3 Hz, 1 H), 7.33 (t, *J* = 7.6 Hz, 1 H), 7.19 (t, *J* = 7.5 Hz, 1 H), 4.71 – 4.62 (m, 1 H), 4.27 – 4.13 (m, 4 H), 3.72 (s, 3 H), 3.53 – 3.42 (m, 2 H), 1.93 (s, 3 H), 1.42 – 1.33 (m, 6 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.6, 137.5 (d, *J* = 12.1 Hz), 127.2 (d, *J* = 16.2 Hz), 125.4, 121.7 (d, *J* = 19.2 Hz), 120.7, 120.0, 120.0, 112.0, 62.8 (t, *J* = 4.0 Hz), 53.9, 52.3, 26.5, 22.6, 16.2 (q, *J* = 5.1 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.1. HRMS-ESI (*m/z*): calcd for $C_{18}H_{26}N_2O_6P^+$ $[M + H]^+$: 397.1523; found: 397.1521.



Ac-Trp-Methyl phosphate (**6**)

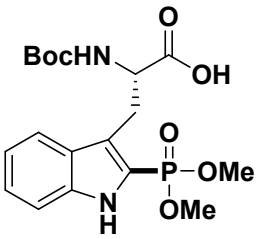
The reaction was conducted on a 0.2 mmol scale, affording **6** as a yellow solid (47.5 mg, 67%). ^{1}H NMR (400 MHz, CD_3OD) δ ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (s, 1 H), 7.76 (d, *J* = 8.2 Hz, 1 H), 7.45 (d, *J* = 8.4 Hz, 1 H), 7.32 – 7.27 (m, 1 H), 7.15 (t, *J* = 7.5 Hz, 1 H), 4.71 – 4.64 (m, 1 H), 3.89 – 3.80 (m, 6 H), 3.65 – 3.60 (m, 1 H), 3.43 – 3.35 (m, 1 H), 1.86 (s, 3 H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.9, 173.1, 139.7 (d, *J* = 13.1 Hz), 128.7 (d, *J* = 16.2 Hz), 126.0, 123.9 (d, *J* = 20.2 Hz), 121.6, 120.9, 119.0, 113.7, 55.4, 53.7 (q, *J* = 4.0 Hz), 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 27.9, 22.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 18.9. HRMS-ESI (*m/z*): calcd for $C_{15}H_{20}N_2O_6P^+$ $[M + H]^+$: 355.1053; found: 355.1050.



Fmoc-Trp-Methyl phosphate (**7**)

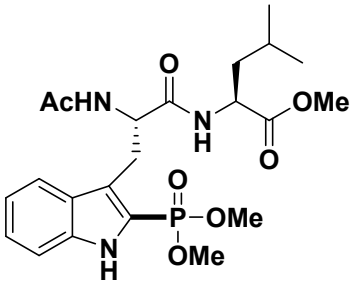
The reaction was conducted on a 0.2 mmol scale, affording **7** as a a yellow solid (69.5 mg, 65%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 – 7.74 (m, 3 H), 7.56 – 7.50 (m, 2 H), 7.46 (d, *J* = 8.3 Hz, 1 H), 7.38 – 7.25 (m, 5 H), 7.23 – 7.18 (m, 1 H), 7.14 (t, *J* = 7.5 Hz, 1 H), 4.53 – 4.46 (m, 1 H), 4.26 – 4.14 (m, 2 H), 4.08 (t, *J* = 7.0 Hz, 1 H), 3.79 (d, *J* = 2.9 Hz, 3 H), 3.76 (d, *J* = 2.8 Hz, 3 H), 3.67 – 3.61 (m, 1 H), 3.46 – 3.39 (m, 1 H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.2,

157.0, 143.8 (d, J = 11.1 Hz), 141.1, 138.4 (d, J = 13.1 Hz), 127.3, 127.3, 127.1, 126.7, 124.8, 124.8, 124.7, 122.9 (d, J = 19.2 Hz), 119.9, 119.6, 119.4, 117.7, 111.9, 66.5, 55.5, 52.3 (d, J = 5.1 Hz), 46. 9, 26.6. ^{31}P NMR (162 MHz, CD_3OD) δ 15.1. HRMS-ESI (*m/z*): calcd for $C_{28}H_{26}N_2O_7P^-$ [M - H]$^-$: 533.1483; found: 533.1490.



Boc-Trp-Methyl phosphate (**8**)

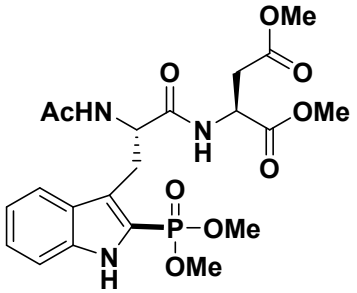
The reaction was conducted on a 0.2 mmol scale, affording **8** as a yellow solid (50.1 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.38 (s, 1 H), 7.79 (d, J = 8.1 Hz, 1 H), 7.45 (d, J = 8.2 Hz, 1 H), 7.34 (t, J = 7.6 Hz, 1 H), 7.19 (t, J = 7.5 Hz, 1 H), 6.08 (d, J = 7.1 Hz, 1 H), 4.57 – 4.50 (m, 1 H), 3.86 – 3.80 (m, 6 H), 3.62 – 3.54 (m, 1 H), 3.44 – 3.36 (m, 1 H), 1.36 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7, 156.2, 137.9 (d, J = 13.2 Hz), 127.5 (d, J = 16.2 Hz), 125.4, 122.5 (d, J = 18.2 Hz), 120.7, 120.2, 118.1, 112.1, 80.0, 54.70, 53.18 (d, J = 5.1 Hz), 28.2, 27.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.5. HRMS-ESI (*m/z*): calcd for $C_{18}H_{24}N_2O_7P^-$ [M - H]$^-$: 411.1327; found: 411.1325.



Ac-Trp-Leu-Methyl phosphate (**9**)

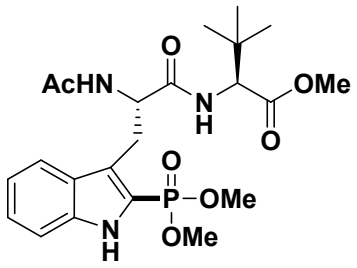
The reaction was conducted on a 0.2 mmol scale, affording **9** as a a yellow solid (54.9 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1 H), 7.88 (d, J = 6.7 Hz, 1 H), 7.73 (d, J = 8.1 Hz, 1 H), 7.40 (d, J = 8.4 Hz, 1 H), 7.33 (t, J = 7.6 Hz, 1 H), 7.29 – 7.24 (m, 1 H), 7.18 (t, J = 7.6 Hz, 1 H), 4.68 (s, 1 H), 4.58 (d, J = 4.7 Hz, 1 H), 3.87 (d, J = 11.0 Hz, 3 H), 3.80 (d, J = 11.7 Hz, 3 H), 3.72 (s, 3 H), 3.60 – 3.52 (m, 1 H), 3.43 – 3.34 (m, 1 H), 1.91 (s, 3 H), 1.66 – 1.54 (m, 3 H), 0.93 (d, J = 5.5 Hz, 6 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 171.4, 171.2, 137.7 (d, J

= 12.1 Hz), 127.3 (d, *J* = 17.2 Hz), 125.5, 123.8 (d, *J* = 19.2 Hz), 120.8, 120.1, 118.3, 111.9, 54.6, 53.2 (d, *J* = 5.1 Hz), 52.8 (d, *J* = 5.1 Hz), 52.1, 50. 9, 41.4, 26.6, 24.8, 22.7, 22.7, 21.9. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.2, 14.1, 14.1, 14.0, 13.9. HRMS-ESI (*m/z*): calcd for $C_{22}H_{33}N_3O_7P^+$ $[M + H]^+$: 482.2051; found: 482.2042.



Ac-Trp-Asp acid dimethyl-Methyl phosphate (**10**)

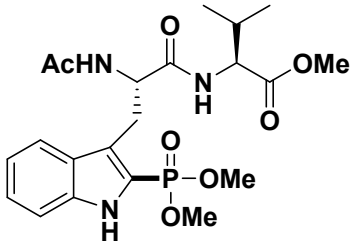
The reaction was conducted on a 0.2 mmol scale, affording **10** as a yellow solid (46.7 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1 H), 7.86 (d, *J* = 6.4 Hz, 1 H), 7.73 (d, *J* = 8.1 Hz, 1 H), 7.43 (d, *J* = 8.0 Hz, 1 H), 7.38 (d, *J* = 8.2 Hz, 1 H), 7.34 – 7.29 (m, 1 H), 7.20 – 7.15 (m, 1 H), 4.82 – 4.75 (m, 1 H), 4.65 – 4.55 (m, 1 H), 3.86 (d, *J* = 11.2 Hz, 3 H), 3.80 (d, *J* = 11.6 Hz, 3 H), 3.72 (s, 3 H), 3.65 (s, 3 H), 3.57 – 3.49 (m, 1 H), 3.42 – 3.34 (m, 1 H), 2.94 – 2.87 (m, 1 H), 2.76 – 2.68 (m, 1 H), 1.91 (s, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 171.1, 171.0, 170.9, 137.6 (d, *J* = 12.1 Hz), 127.3 (d, *J* = 16.2 Hz), 125.7, 123.4 (d, *J* = 19.2 Hz), 120.9, 120.2, 119.4 (d, *J* = 219.2 Hz), 111.8, 54.7, 53.2 (d, *J* = 5.1 Hz), 52.9 (d, *J* = 5.1 Hz), 52.8, 51.9, 48. 7, 36.1, 26.5, 22.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.2, 14.2, 14.1, 14.0, 13.9. HRMS-ESI (*m/z*): calcd for $C_{21}H_{29}N_3O_9P^+$ $[M + H]^+$: 498.1636; found: 498.1629.



Ac-Trp-Tle-OMe-Methyl phosphate (**11**)

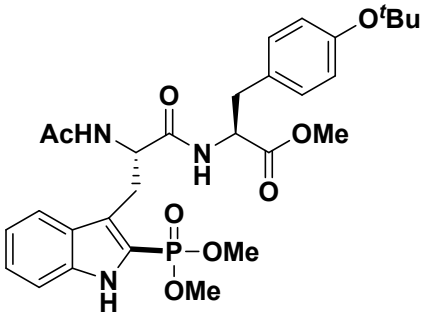
The reaction was conducted on a 0.2 mmol scale, affording **11** as a yellow solid (64.6 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1 H), 7.95 (d, *J* = 6.6 Hz, 1 H), 7.69 (d, *J* = 8.1 Hz, 1 H), 7.44 (d, *J* = 9.0 Hz, 1 H), 7.39 (d, *J* = 8.3 Hz, 1 H), 7.31 (t, *J* = 7.6 Hz, 1 H), 7.16 (t, *J* = 7.5 Hz, 1 H), 4.68 – 4.61 (m, 1 H), 4.36 (d, *J* = 9.0 Hz, 1 H), 3.86 (d, *J* = 11.2 Hz, 3 H), 3.78 (d, *J* =

11.6 Hz, 3 H), 3.69 (s, 3 H), 3.41 – 3.33 (m, 1 H), 3.37 (dd, *J* = 14.7, 11.0 Hz, 1 H), 1.91 (s, 3 H), 0.95 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 171.4, 171.3, 137.6 (d, *J* = 12.1 Hz), 127.2 (d, *J* = 16.2 Hz), 125.6, 123.6 (d, *J* = 19.2 Hz), 120. 8, 120.1, 119.4 (d, *J* = 219.2 Hz), 111.9, 60.5, 54.4, 53.2 (d, *J* = 5.1 Hz), 52.8 (d, *J* = 5.1 Hz), 34.5, 26.5, 26.1, 22.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.3, 14.2, 14.1, 14.1, 14.0. HRMS-ESI (*m/z*): calcd for $C_{22}H_{33}N_3O_7P^+$ [M + H]$^+$: 482.2051; found: 482.2043.



Ac-Trp-Val-OMe-Methyl phosphate (**12**)

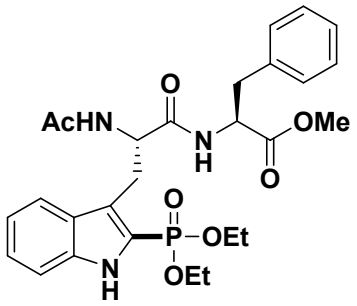
The reaction was conducted on a 0.2 mmol scale, affording **12** as a yellow solid (52.3 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 – 8.64 (m, 1 H), 7.92 (d, *J* = 6.6 Hz, 1 H), 7.70 (d, *J* = 8.1 Hz, 1 H), 7.39 (d, *J* = 8.2 Hz, 1 H), 7.32 (q, *J* = 7.9 Hz, 2 H), 7.16 (t, *J* = 7.5 Hz, 1 H), 4.70 – 4.61 (m, 1 H), 4.50 – 4.44 (m, 1 H), 3.89 – 3.83 (m, 3 H), 3.79 (d, *J* = 11.6 Hz, 3 H), 3.70 (s, 3 H), 3.57 – 3.49 (m, 1 H), 3.42 – 3.33 (m, 1 H), 2.16 – 2.10 (m, 1 H), 1.90 (s, 3 H), 0.91 – 0.86 (m, 6 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 171.7, 171.3, 137.6 (d, *J* = 12.1 Hz), 127.3 (d, *J* = 16.2 Hz), 125.6, 123.7 (d, *J* = 19.2 Hz), 120.8, 120.1, 119.4 (d, *J* = 220.2 Hz), 111.9, 57.4, 54.6, 53.2 (d, *J* = 5.1 Hz), 52.9 (d, *J* = 6.1 Hz), 52.0, 31.1, 26.4, 22.7, 18.9, 17.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.2, 14.1, 14.0, 13.9, 13.9. HRMS-ESI (*m/z*): calcd for $C_{21}H_{31}N_3O_7P^+$ [M + H]$^+$: 468.1894; found: 468.1888.



Ac-Trp-Tyr(tbu)-OMe-Methyl phosphate (**13**)

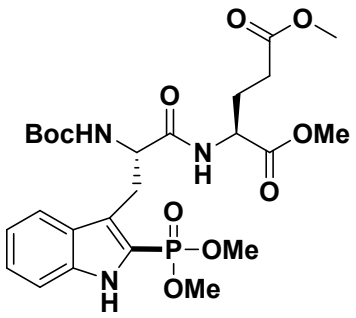
The reaction was conducted on a 0.2 mmol scale, affording **13** as a a yellow solid (63 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1 H), 7.78 – 7.63 (m, 2 H), 7.38 (d, *J* = 8.4 Hz, 1 H),

7.30 (t, *J* = 7.7 Hz, 1 H), 7.19 – 7.11 (m, 2 H), 7.02 – 6.95 (m, 2 H), 6.90 – 6.84 (m, 2 H), 4.76 – 4.70 (m, 1 H), 4.64 – 4.56 (m, 1 H), 3.84 (d, *J* = 11.1 Hz, 3 H), 3.78 (d, *J* = 11.6 Hz, 3 H), 3.63 (s, 3 H), 3.52 – 3.45 (m, 1 H), 3.35 – 3.27 (m, 1 H), 3.01 – 2.94 (m, 2 H), 1.85 (s, 3 H), 1.30 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 171.2, 171.1, 154.3, 137.6 (d, *J* = 12.1 Hz), 130.8, 129.7, 127.3 (d, *J* = 16.2 Hz), 125.6, 124.0, 123.4 (d, *J* = 19.2 Hz), 120.8, 120.2, 119.4 (d, *J* = 220.2 Hz), 111.9, 78.3, 54.6, 53.6, 53.2 (d, *J* = 5.1 Hz), 52.9 (d, *J* = 6.1 Hz), 52.1, 37.4, 28.8, 26.5, 22.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.2, 14.1, 14.1, 14.0, 13.9. HRMS-ESI (*m/z*): calcd for $C_{29}H_{39}N_3O_8P^+$ $[M + H]^+$: 588.2469; found: 588.2468.



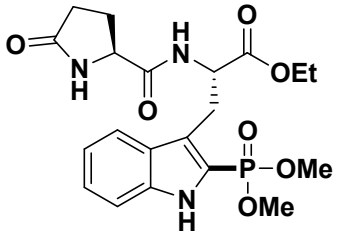
Ac-Trp-Phe-OMe-Ethyl phosphate (**14**)

The reaction was conducted on a 0.2 mmol scale, affording **14** as a yellow solid (60 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1 H), 7.95 (d, *J* = 6.3 Hz, 1 H), 7.71 (d, *J* = 8.1 Hz, 1 H), 7.40 (d, *J* = 8.3 Hz, 1 H), 7.33 (d, *J* = 7.2 Hz, 1 H), 7.28 – 7.15 (m, 4 H), 7.12 (d, *J* = 6.7 Hz, 2 H), 4.82 (q, *J* = 6.8 Hz, 1 H), 4.62 – 4.55 (m, 1 H), 4.28 – 4.09 (m, 4 H), 3.71 (s, 3 H), 3.53 (d, *J* = 14.5 Hz, 1 H), 3.39 – 3.31 (m, 1 H), 3.13 (dd, *J* = 13.9, 5.7 Hz, 1 H), 3.02 (dd, *J* = 13.9, 6.8 Hz, 1 H), 1.85 (s, 3 H), 1.42 (t, *J* = 7.1 Hz, 3 H), 1.34 (t, *J* = 7.1 Hz, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 171.3, 171.3, 137.51 (d, *J* = 12.1 Hz), 136.1, 129.3, 128.4, 127.38 (d, *J* = 17.2 Hz), 126.9, 125.5, 123.01 (d, *J* = 19.2 Hz), 120.8, 120.8 (d, *J* = 218.2 Hz), 120.1, 111.8, 63.0 (d, *J* = 5.1 Hz), 62.8 (d, *J* = 5.1 Hz), 54.5, 53.4, 52.2, 37.9, 26.0, 22.6, 16.4 (d, *J* = 6.1 Hz), 16.2 (d, *J* = 7.1 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.1. HRMS-ESI (*m/z*): calcd for $C_{27}H_{35}N_3O_7P^+$ $[M + H]^+$: 544.2207; found: 544.2198.



Boc-Trp-L-Glu acid dimethyl ester Methyl phosphate (**15**)

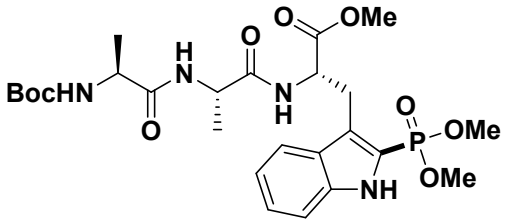
The reaction was conducted on a 0.2 mmol scale, affording **15** as a a yellow solid (49 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1 H), 7.78 (d, *J* = 8.1 Hz, 1 H), 7.43 (d, *J* = 8.3 Hz, 1 H), 7.33 (t, *J* = 7.6 Hz, 1 H), 7.18 (t, *J* = 7.6 Hz, 2 H), 6.39 (d, *J* = 6.7 Hz, 1 H), 4.65 – 4.56 (m, 1 H), 4.43 – 4.34 (m, 1 H), 3.85 (d, *J* = 4.9 Hz, 3 H), 3.82 (d, *J* = 5.0 Hz, 3 H), 3.71 (s, 3 H), 3.67 (s, 3 H), 3.52 (dd, *J* = 15.0, 4.1 Hz, 1 H), 3.35 (dd, *J* = 14.5, 10.1 Hz, 1 H), 2.40 – 2.24 (m, 2 H), 2.22 – 2.09 (m, 1 H), 2.03 – 1.89 (m, 1 H), 1.32 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 172.1, 171.8, 155.8, 137.7 (d, *J* = 12.1 Hz), 127.43 (d, *J* = 16.2 Hz), 125.4 (d, *J* = 19.2 Hz), 120.7, 120.4, 118.1, 111.8, 79.7, 56.0, 53.1, 53.0, 52.4, 51.7, 51.4, 29.7, 28.1, 27.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.3. HRMS-ESI (*m/z*): calcd for $C_{25}H_{37}N_3O_{10}P^+$ $[M + H]^+$: 570.2211; found: 570.2207.



Pyr-Trp-OEt-Methyl phosphate (**16**)

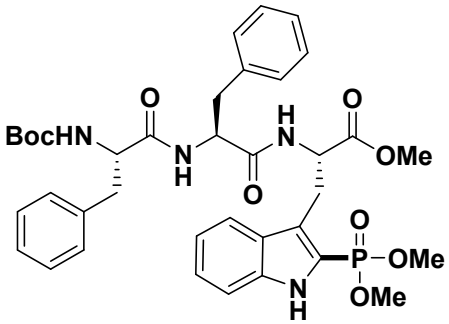
The reaction was conducted on a 0.2 mmol scale, affording **16** as a a yellow solid (55 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1 H), 8.13 (d, *J* = 6.5 Hz, 1 H), 7.69 (d, *J* = 8.1 Hz, 1 H), 7.42 (d, *J* = 8.4 Hz, 1 H), 7.32 (t, *J* = 7.7 Hz, 1 H), 7.18 (t, *J* = 7.6 Hz, 1 H), 6.75 (s, 1 H), 4.67 – 4.61 (m, 1 H), 4.22 – 4.17 (m, 2 H), 4.12 – 4.08 (m, 1 H), 3.92 – 3.86 (m, 3 H), 3.76 – 3.71 (m, 3 H), 3.56 – 3.51 (m, 1 H), 3.43 – 3.36 (m, 1 H), 2.32 – 2.27 (m, 1 H), 2.16 (t, *J* = 8.0 Hz, 2 H), 2.03 (d, *J* = 7.2 Hz, 1 H), 1.28 (t, *J* = 7.1 Hz, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.8, 172.3, 171.5, 137.7 (d, *J* = 13.1 Hz), 127.1 (d, *J* = 16.2 Hz), 125.5, 121.7 (d, *J* = 19.2 Hz), 120.7, 119.7, 120.0 (d, *J* = 220.2 Hz), 112.3, 61.5, 56.6, 53.7, 53.32 (t, *J* = 5.1 Hz), 29.3, 26.5,

24.9, 14.07. ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.8. HRMS-ESI (*m/z*): calcd for $C_{20}H_{27}N_3O_7P^+$ [M + H]$^+$: 452.1581; found: 452.1589.



Boc-Ala-Ala-Trp-OMe-Methyl phosphate (**17**)

The reaction was conducted on a 0.2 mmol scale, affording **17** as a yellow solid (52 mg, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1 H), 8.09 (d, *J* = 6.6 Hz, 1 H), 7.70 (d, *J* = 8.1 Hz, 1 H), 7.40 (d, *J* = 8.3 Hz, 1 H), 7.32 (t, *J* = 7.6 Hz, 1 H), 7.20 – 7.16 (m, 1 H), 6.93 – 6.86 (m, 1 H), 5.08 (d, *J* = 7.6 Hz, 1 H), 4.67 – 4.61 (m, 1 H), 4.48 – 4.42 (m, 1 H), 3.87 (d, *J* = 11.3 Hz, 3 H), 3.77 (d, *J* = 11.6 Hz, 3 H), 3.72 (s, 3 H), 3.53 – 3.42 (m, 2 H), 1.43 (d, *J* = 5.7 Hz, 3 H), 1.37 (d, *J* = 6.5 Hz, 9 H), 1.27 (d, *J* = 3.7 Hz, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.2, 171.6, 155.3, 137.8 (d, *J* = 12.1 Hz), 127.0 (d, *J* = 16.2 Hz), 125.4, 121.9 (d, *J* = 19.2 Hz), 120.6, 119.7, 119.7 (d, *J* = 219.2 Hz), 112.2, 79.8, 53.7, 53.3 (d, *J* = 6.2 Hz), 53.1 (d, *J* = 5.1 Hz), 52.3, 50.4, 48.5, 28.1, 26.6, 19.0, 18.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.1, 14.0, 14.0, 13.9, 13.8. HRMS-ESI (m/z): calcd for $C_{25}H_{36}N_4O_9P^-$ [M - H]$^-$: 567.2225 ; found: 567.2224 .



Boc-Phe-Phe-Trp-OMe-Methyl phosphate (**18**)

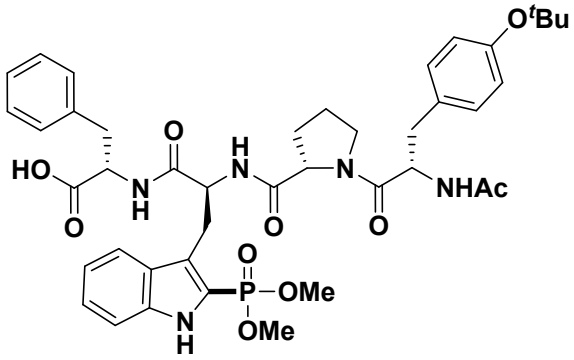
The reaction was conducted on a 0.2 mmol scale, affording **18** as a yellow solid (87 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1 H), 7.88 (d, *J* = 7.1 Hz, 1 H), 7.70 (d, *J* = 8.2 Hz, 1 H), 7.44 (d, *J* = 8.3 Hz, 1 H), 7.36 (t, *J* = 7.7 Hz, 1 H), 7.25 – 7.05 (m, 12 H), 6.75 (d, *J* = 8.2 Hz, 1 H), 4.95 (d, *J* = 8.2 Hz, 1 H), 4.71 – 4.67 (m, 1 H), 4.31 (d, *J* = 13.7 Hz, 1 H), 3.91 – 3.69 (m, 9 H), 3.55 – 3.46 (m, 1 H), 3.45 – 3.30 (m, 1 H), 3.11 – 2.87 (m, 4 H), 1.39 – 1.30 (m, 9 H). ^{13}C

NMR (101 MHz, $CDCl_3$) δ 171.7, 170.7, 170.6, 155.1, 137.6 (d, *J* = 12.1 Hz), 136.6, 136.3, 129.5, 129.2, 128.4, 128.1, 127.20 (d, *J* = 17.2 Hz), 126.7, 126.6, 125.5, 121.7 (d, *J* = 18.2 Hz), 120.8, 119.8, 119.8 (d, *J* = 219.2 Hz), 112.2, 79.9, 55.5, 53.7, 53.6, 53.33 (d, *J* = 6.1 Hz), 53.1 (d, *J* = 5.1 Hz), 52.3, 38.6, 29.6, 28.1, 28.1, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.9. HRMS-ESI (*m/z*): calcd for $C_{37}H_{46}N_4O_9P^+$ $[M + H]^+$: 721.2997; found: 721.2991.

F. General procedures for late-stage functionalization of natural peptides

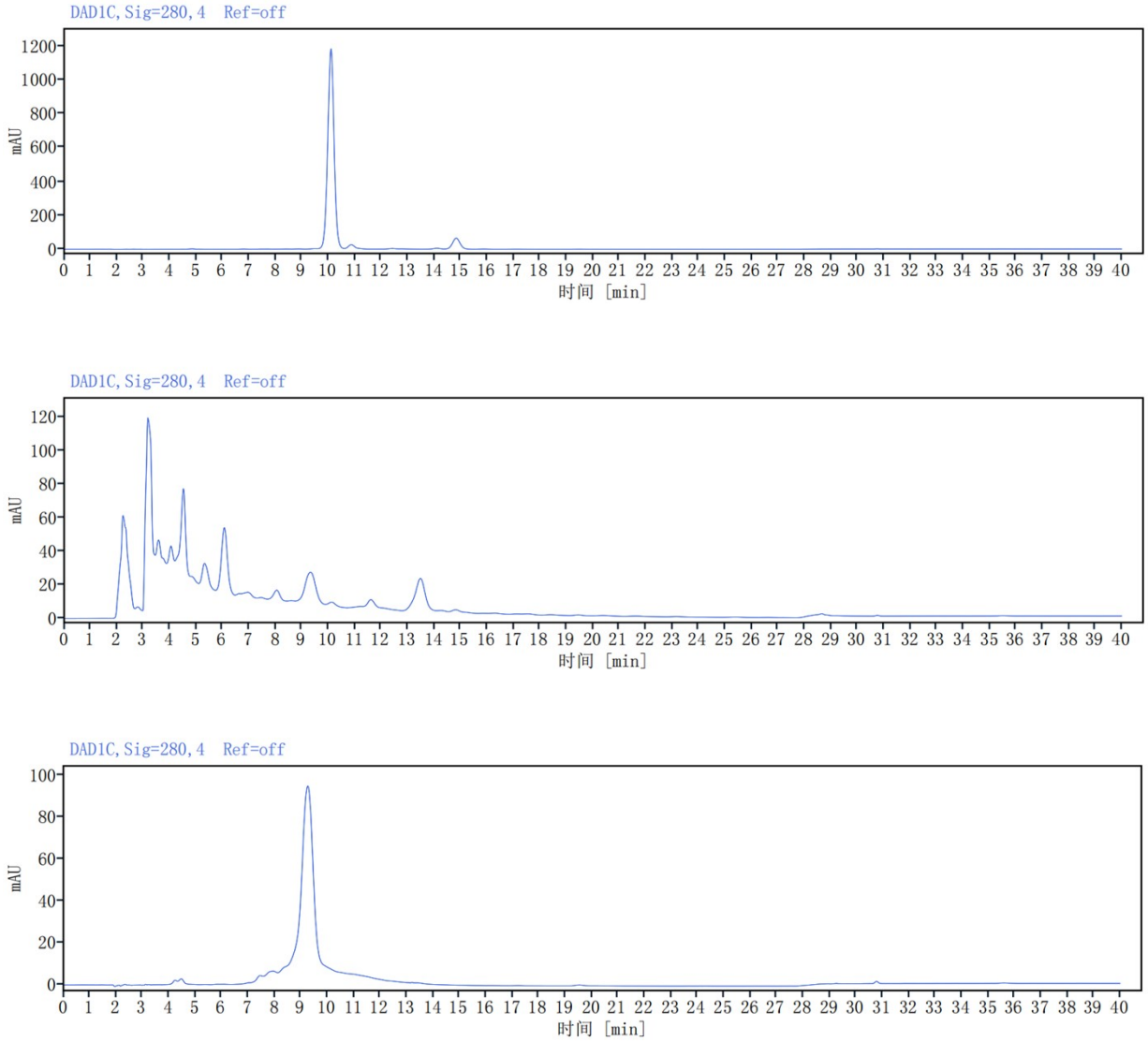
Modified Endorphin-1(**19**), natural peptides Segetalin A(**21)** or Segetalin A(**24)** (0.02 mmol), trimethyl phosphite or triethyl phosphite **2** (5 eq, 0.1 mmol), photosensitizer (3 mol%, 0.0006 mmol) and acetonitrile (1 ml) were was added into a 25 mL quartz tube with a stirring magnet successively. The reaction vessel was then irradiated under blue light for 18 h. Circulating water was used to maintain room temperature. After the reaction, dissolve the mixture in 2 mL of H_2O/CH_3CN (1:1; containing 0.1% TFA) and remove insoluble substances by filtration with a filter membrane. Perform LC analysis on the filtrate and purify it using semi preparative HPLC.

G. Characterization data for phosphonylation natural peptides

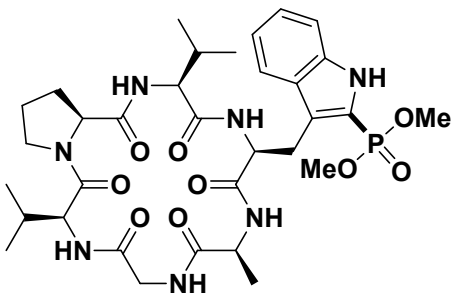


Endomorphin-1-Methyl phosphate (**20**), from Endomorphin-1

Peptide **20** was isolated in 53% yield (8.7 mg) as a yellow flocculent solid. HRMS-ESI (*m/z*): calcd for $C_{42}H_{53}N_5O_{10}P^+$ $[M + H]^+$: 818.3525; found: 818.3521.

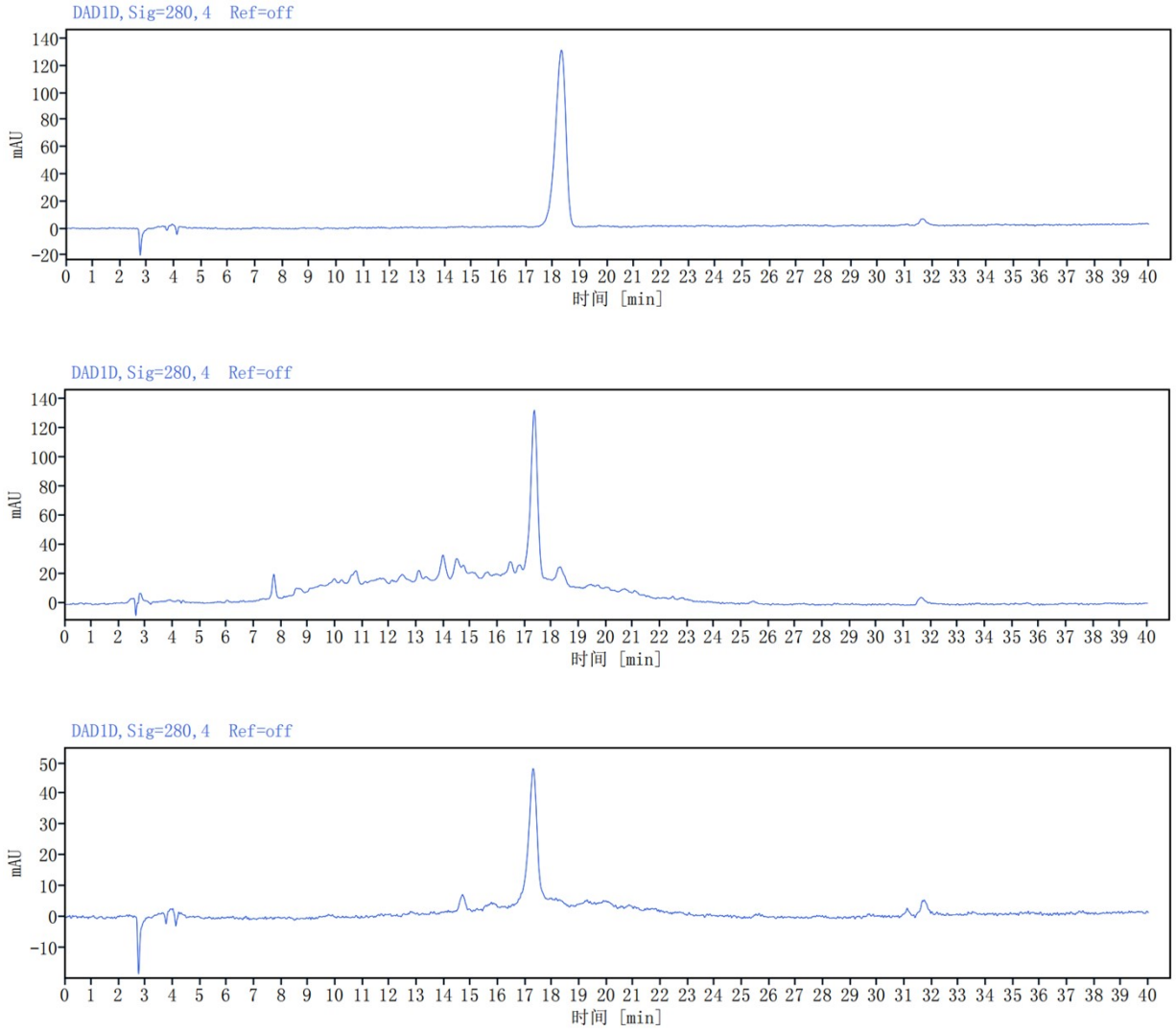


Scheme S1. i) LC trace of compound **19** (10.0 mM). ii) LC trace of reaction mixture of compound **20** (10.0 mM). iii) LC trace of purified compound **20** [1 mL/min, 20% to 100% MeCN for 40 min, λ = 280 nm, HPLCONE® Packed Column 10C18A(10 μm, 4.6 ID × 250 mm) UPLC analytical column].

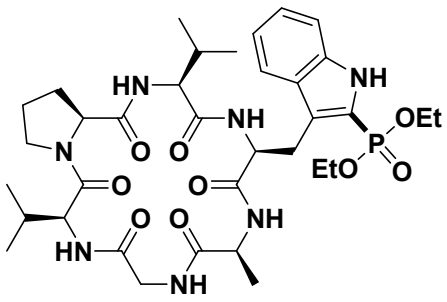


Segetalin A-Methyl phosphate (**22**), from Segetalin A

Peptide **22** was isolated in 50% yield (7.1 mg) as a yellow flocculent solid. HRMS-ESI (*m/z*): calcd for $C_{33}H_{49}N_7O_9P^+$ $[M + H]^+$: 718.3324; found: 718.3332.

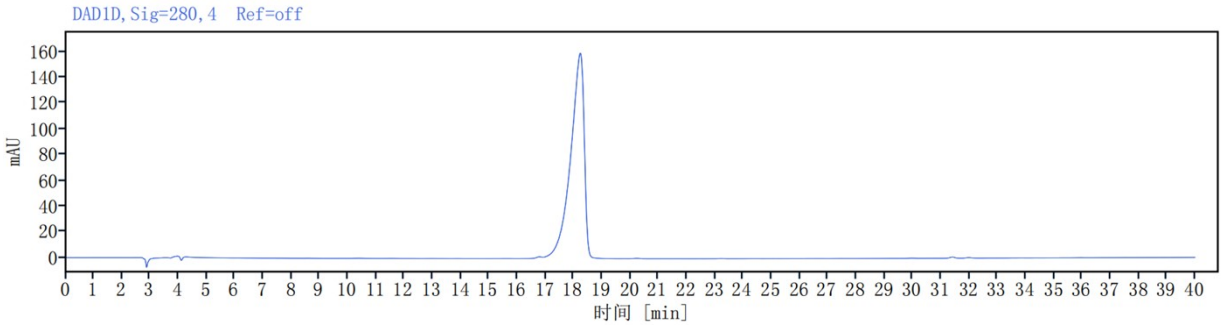


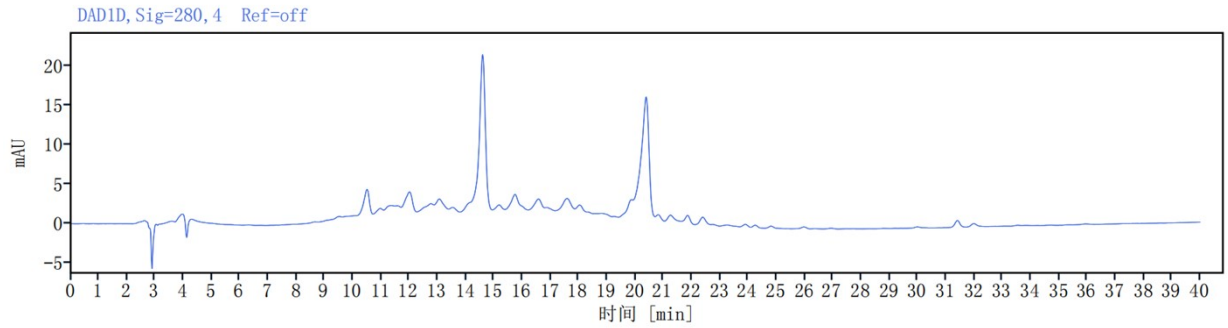
Scheme S2. i) LC trace of compound **21** (10.0 mM). ii) LC trace of reaction mixture of compound **22** (10.0 mM). iii) LC trace of purified compound **22** [1 mL/min, 10% to 100% MeCN for 40 min, λ = 280 nm, HPLCONE® Packed Column 10C18A(10 μm, 4.6 ID × 250 mm) UPLC analytical column].

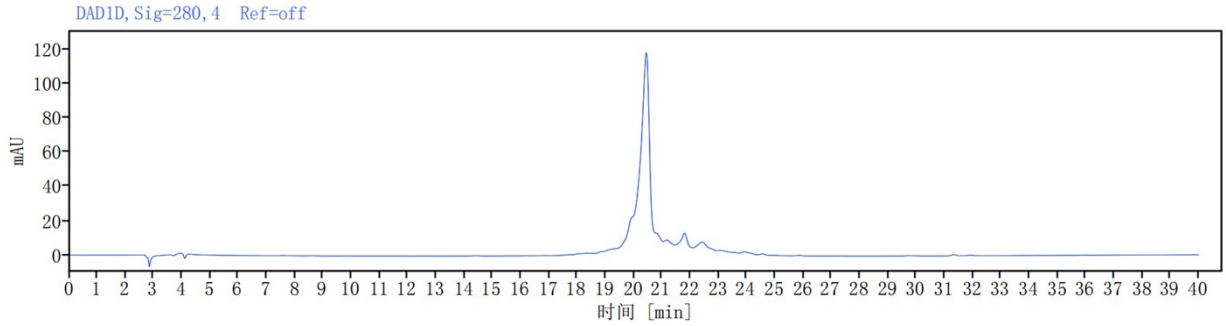


Segetalin A-Phosphoryl ethyl ester (**23**), from Segetalin A

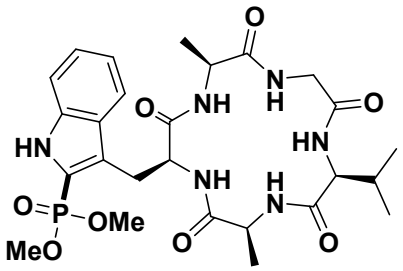
Peptide **23** was isolated in 51% yield (7.6 mg) as a yellow flocculent solid. HRMS-ESI (*m/z*): calcd for $C_{35}H_{53}O_9N_7P^+$ $[M + H]^+$: 746.3637; found: 746.3627.





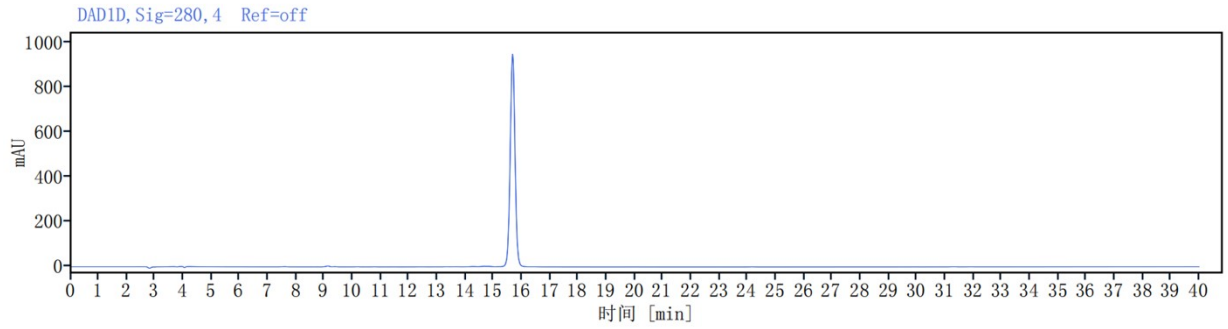


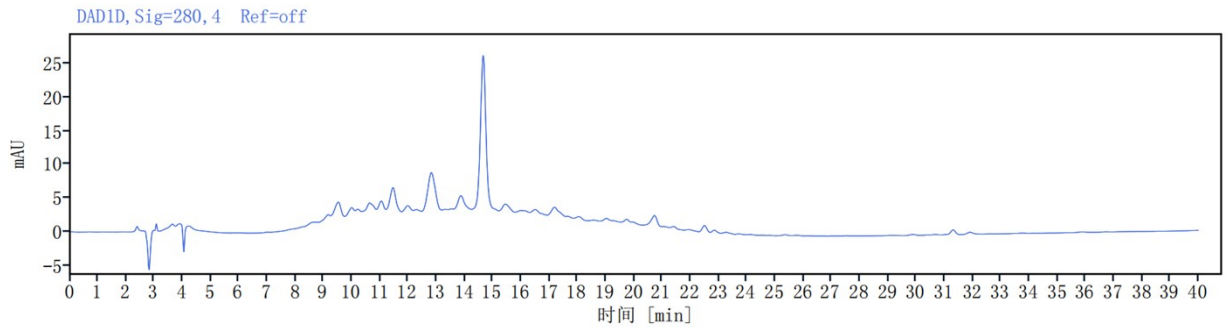
Scheme S3. i) LC trace of compound **21** (10.0 mM). ii) LC trace of reaction mixture of compound **23** (10.0 mM). iii) LC trace of purified compound **23** [1 mL/min, 10% to 100% MeCN for 40 min, λ = 280 nm, HPLCONE® Packed Column 10C18A(10 μm, 4.6 ID × 250 mm) UPLC analytical column].

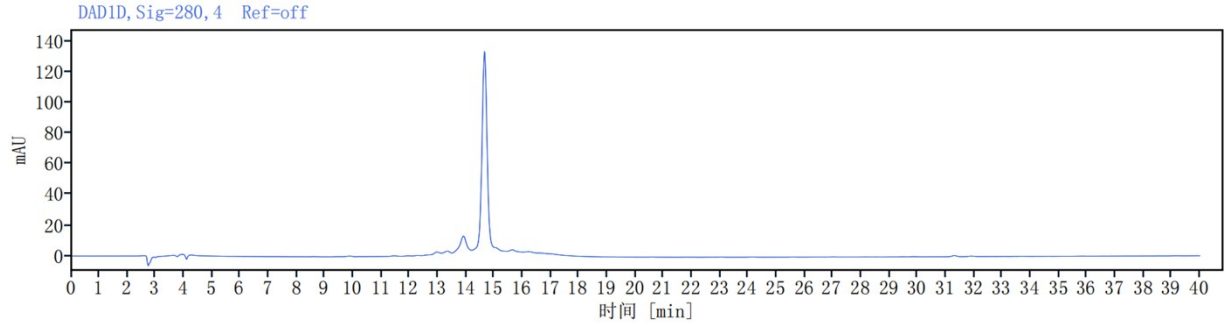


Segetalin B-Methyl phosphate (**25**)

Peptide **25** was isolated in 48% yield (5.7 mg) as a yellow flocculent solid. HRMS-ESI (*m/z*): calcd for $C_{26}H_{38}N_6O_8P^+$ $[M + H]^+$: 593.2483; found: 593.2489.







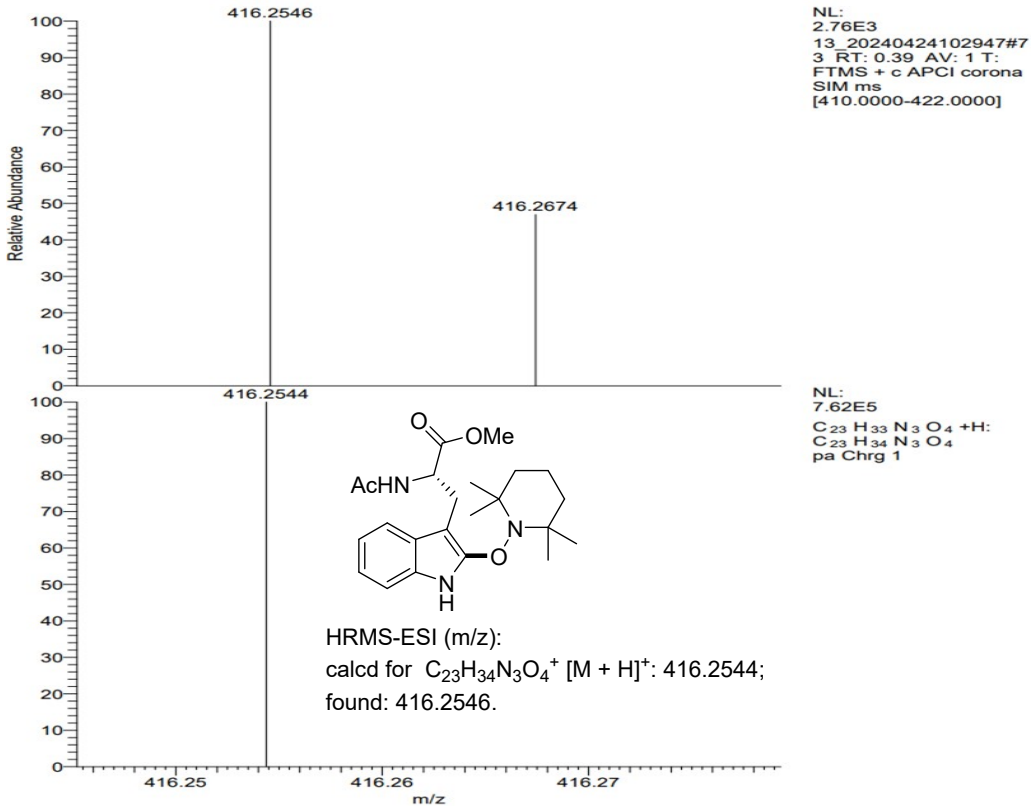
Scheme S4. i) LC trace of compound **24** (10.0 mM). ii) LC trace of reaction mixture of compound **25** (10.0 mM). iii) LC trace of purified compound **25** [1 mL/min, 10% to 100% MeCN for 40 min, λ = 280 nm, HPLCONE® Packed Column 10C18A(10 μm, 4.6 ID × 250 mm) UPLC analytical column].

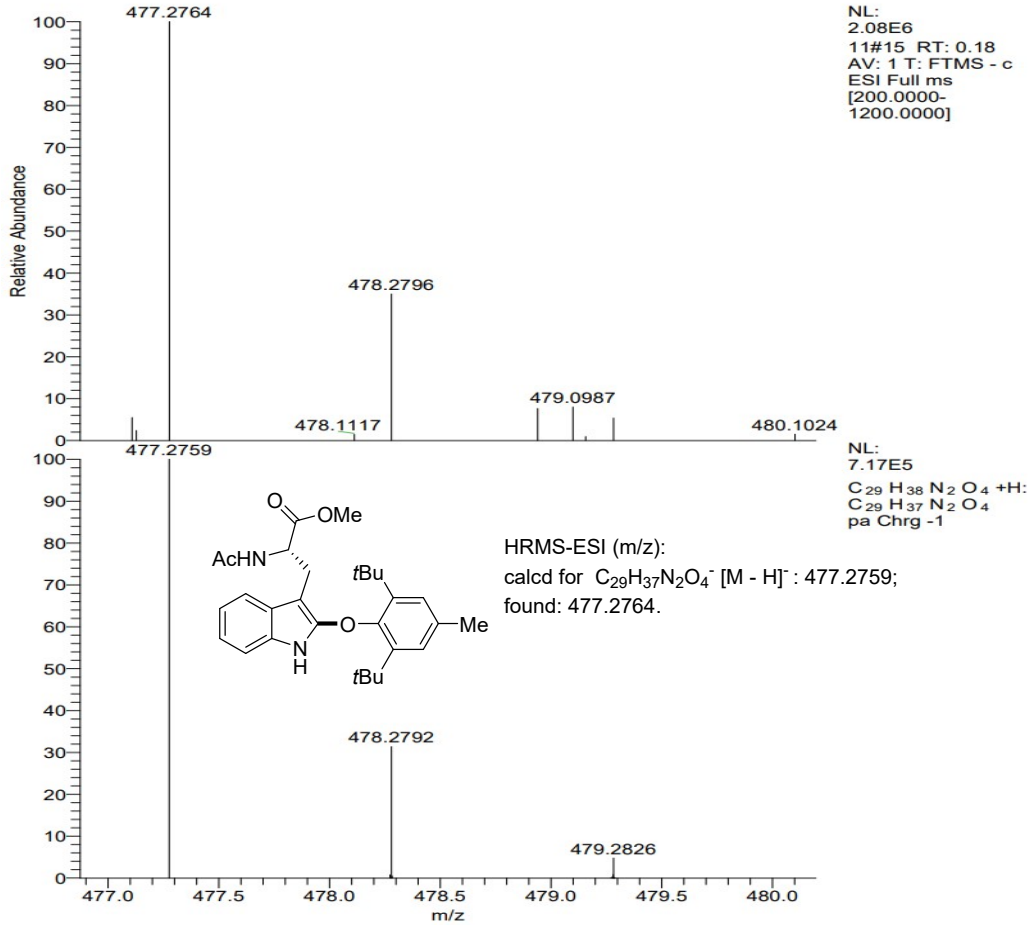
H. General procedures for the scale-up experiments

To an oven-dried quarts test-tube (25 mL) equipped with a magnetic stirring bar, the mixture of methyl acetyl-*L*-tryptophanate (1 mmol), trimethyl phosphite (5 eq, 5 mmol), $[Ir(ppy)_2(dtbbpy)]PF_6$ (3 mol%, 0.03 mmol) and 3 mL acetonitrile was added successively. The reaction mixture was stirred at room temperature under the irradiation at 450−460 nm (25 W LED, distance = 8−10 cm, cooling by circulating water) for 18 h. After the reaction was completed, the residual material was quenched with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel to provide the desired product with 54% isolated yield.

I. General procedures for radical trapping experiments

To an oven-dried quarts test-tube (25 mL) equipped with a magnetic stirring bar, the mixture of methyl acetyl-*L*-tryptophanate (0.2 mmol), trimethyl phosphite (5 eq, 1 mmol), $[Ir(ppy)_2(dtbbpy)]PF_6$ (3 mol%, 0.006 mmol), radical trapping reagent (3 eq, 0.6 mmol) and 2 mL acetonitrile was added successively. The reaction mixture was stirred at room temperature under the irradiation at 450−460 nm (25 W LED, distance = 8−10 cm, cooling by circulating water) for 18 h. After the reaction was completed, the residual material was quenched with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel to provide the desired product. In addition, the trapping products of radical intermediate were detected by HRMS (Scheme S5).





Scheme S5 The HRMS Spectra of the trapping products of radical intermediate.

J. General procedures for $^1O_2$ scavenger experiments

To an oven-dried quarts test-tube (25 mL) equipped with a magnetic stirring bar, the mixture of methyl acetyl-*L*-tryptophanate (0.2 mmol), trimethyl phosphite (5 eq, 1 mmol), $[Ir(ppy)_2(dtbbpy)]PF_6$ (3 mol%, 0.006 mmol), $^1O_2$ scavenger reagent and 2 mL acetonitrile was added successively. The reaction mixture was stirred at room temperature under the irradiation at 450−460 nm (25 W LED, distance = 8−10 cm, cooling by circulating water) for 18 h. After the reaction was completed, the residual material was quenched with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was detected by LCMS.

K. General procedures for $^1O_2$ trapping experiments

To an oven-dried quarts test-tube (25 mL) equipped with a magnetic stirring bar, the mixture of anthracene (0.2 mmol), $[Ir(ppy)_2(dtbbpy)]PF_6$ (3 mol%, 0.006 mmol) and 2 mL acetonitrile was added successively. The reaction mixture was stirred at room temperature under the irradiation at 450−460 nm (25 W LED, distance = 8−10 cm, cooling by circulating water) for 18 h. After the reaction was completed, the residual material was quenched with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was detected by GCMS.

L. General procedures for $^1O_2$ generator experiment

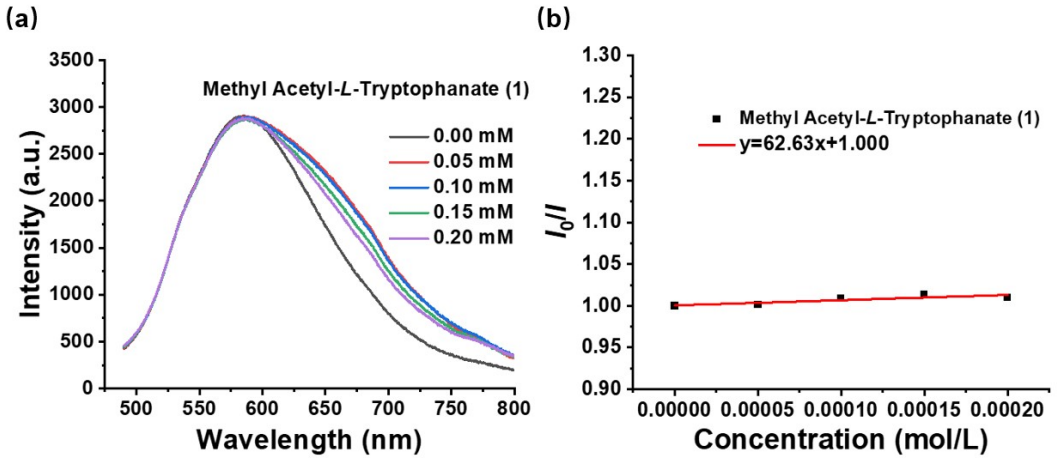
To an oven-dried quarts test-tube (25 mL) equipped with a magnetic stirring bar, the mixture of methyl acetyl-*L*-tryptophanate (0.2 mmol), trimethyl phosphite (5 eq, 1 mmol), TPP (3 mol%, 0.006 mmol) and 2 mL acetonitrile was added successively. The reaction mixture was stirred at room temperature under the irradiation at 450−460 nm (25 W LED, distance = 8−10 cm, cooling by circulating water) for 18 h. After the reaction was completed, the residual material was quenched with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue

was purified by flash chromatography on silica gel to provide the desired product **3** with 53% isolated yield.

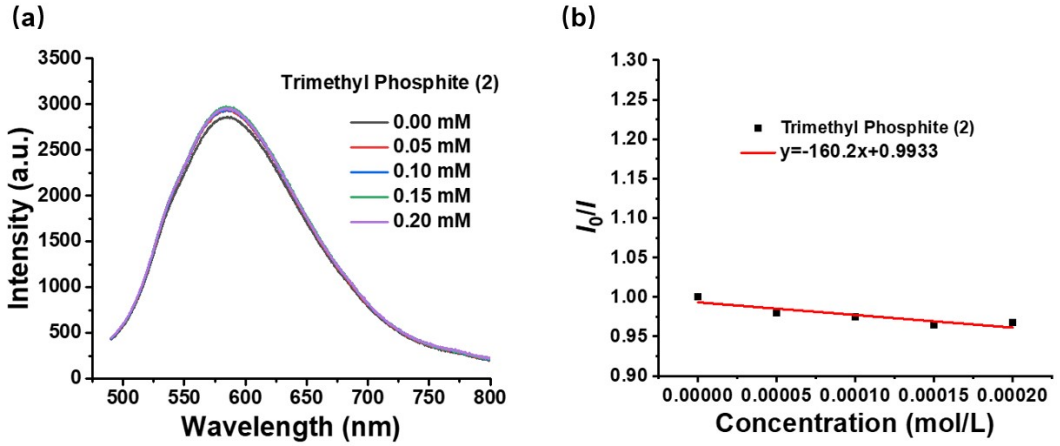
M. Stern-Volmer fluorescence quenching experiments

Formulation solution: Methyl acetyl-*L*-tryptophanate (13.0 mg) was dissolved in MeCN in a 5 mL volumetric flask to set the concentration to be 0.01 M. Trimethyl phosphite (6.2 mg) was dissolved in MeCN in a 5 mL volumetric flask to set the concentration to be 0.01 M. Dissolve the photocatalyst $[Ir(ppy)_2(dtbbpy)]PF_6$ (45.7 mg) in MeCN in a 25 mL volumetric flask, shake well, take out 5 mL of the solution and make up to volume with MeCN in a 25 mL volumetric flask, setting the concentration to 0.4 mM.

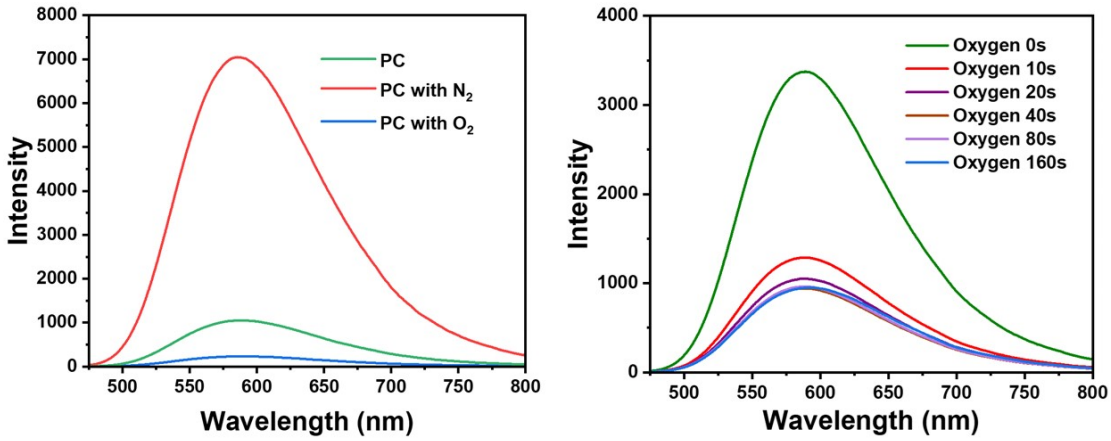
Experimental procedure: The resulting 0.4 mM solution (25 μL) was added to cuvette to obtain different concentrations of catalyst solution. This solution was then diluted to a volume of 2.0 mL by adding MeCN to prepare a 5 μM solution. 0 μL, 10 μL, 20 μL, 30 μL, 40 μL of a quencher solution was successively added and uniformly stirred, and the resulting mixture was irradiated at λ= 460 nm. Follow this method and make changes to the amount to obtain the Stern–Volmer relationship in turn. We performed another Stern–Volmer fluorescence quenching experiment to investigate the influence of oxygen. In a typical experiment, 2 mL of solution of photocatalyst $[Ir(ppy)_2(dtbbpy)]PF_6$ in MeCN was bubbled a stream of oxygen or nitrogen for several seconds. The solution was excited at λ= 460 nm.



Scheme S6. Stern–Volmer quenching experiment of $[Ir(ppy)_2(dtbbpy)]PF_6$ with **1**.
(No measurable luminescence quenching)



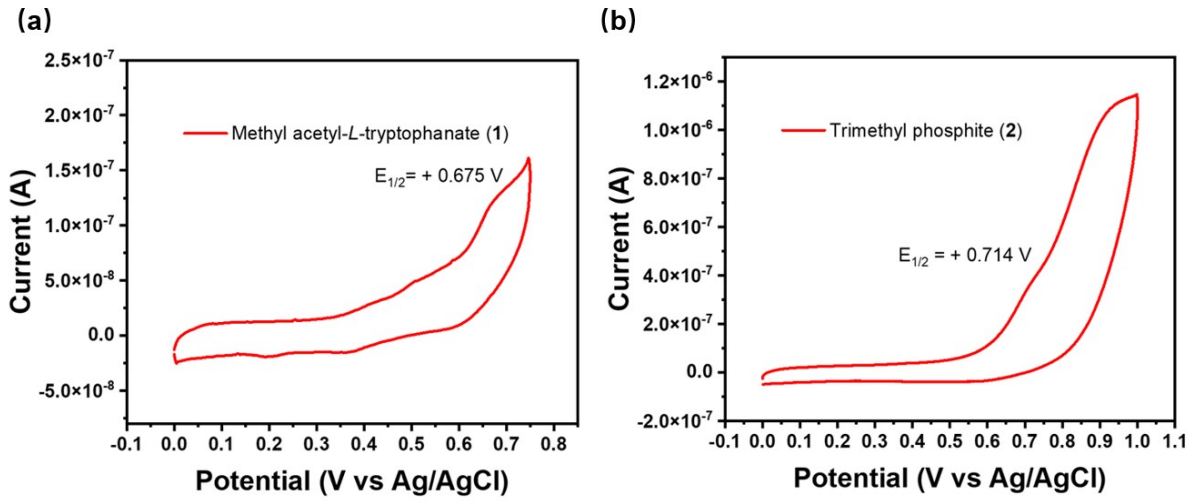
Scheme S7. Stern–Volmer quenching experiment of $[Ir(ppy)_2(dtbbpy)]PF_6$ with **2**.
(No measurable luminescence quenching)



Scheme S8. Stern–Volmer quenching experiment of $[Ir(ppy)_2(dtbbpy)]PF_6$ with O_2.

N. Cyclic Voltammetry experiments

Cyclic voltammetry (CV) experiments were performed using a Bio-Logic VMP-300 multi-channel electrochemical workstation using the three-electrode cell with a rate of 20 mV/s in CH_3CN solution (*N*, *N*, *N*-tributylbutan-1-aminium hexafluorophosphate, 0.1mol/mL) and bubbling with nitrogen for two minutes. In which glassy carbon electrode (GCE) was used as a working electrode, Ag/AgCl and KCl (sat.) worked as the reference electrode, and platinum disk as the counter electrode.

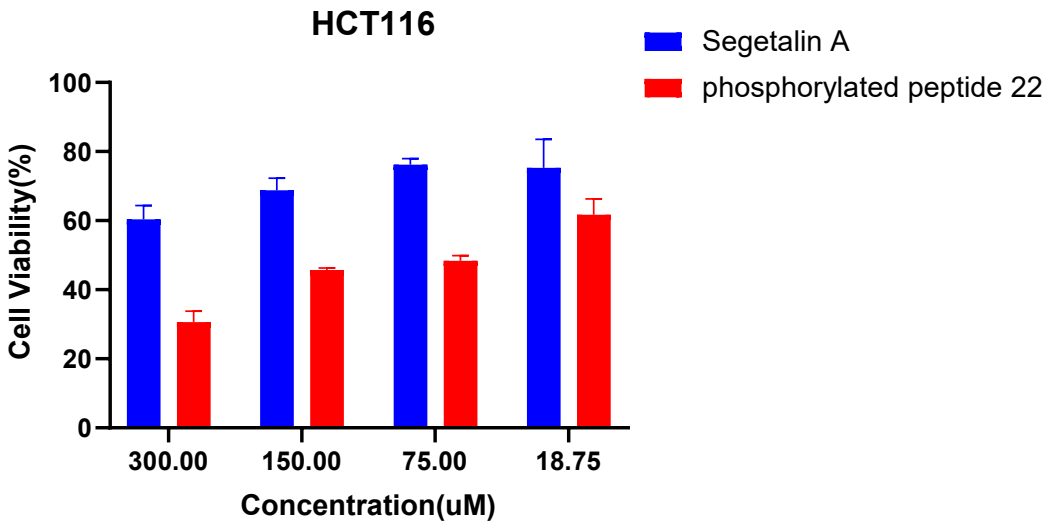


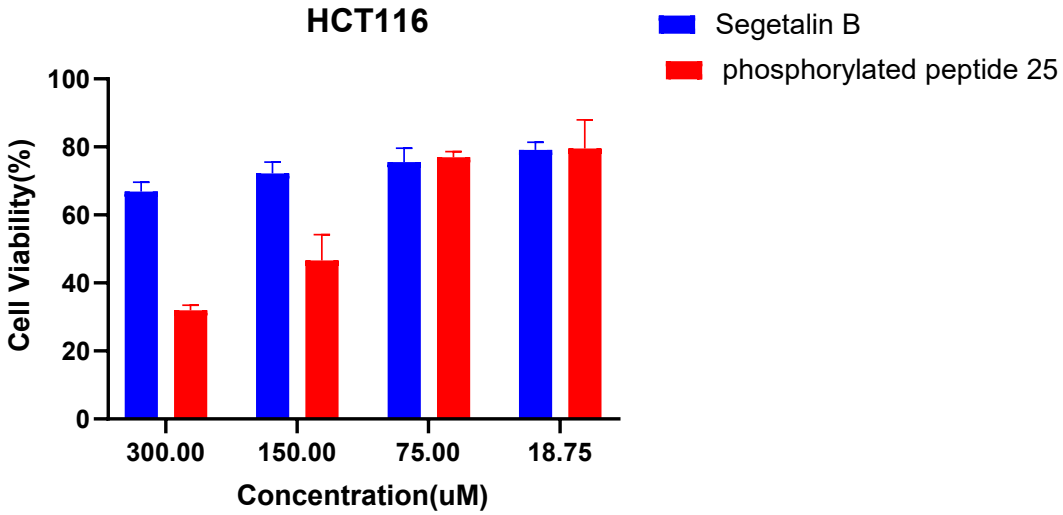
Scheme S9. (a) Cyclic voltammogram of methyl acetyl-*L*-tryptophanate (**1**); (b) Cyclic voltammogram of trimethyl phosphite (**2**).

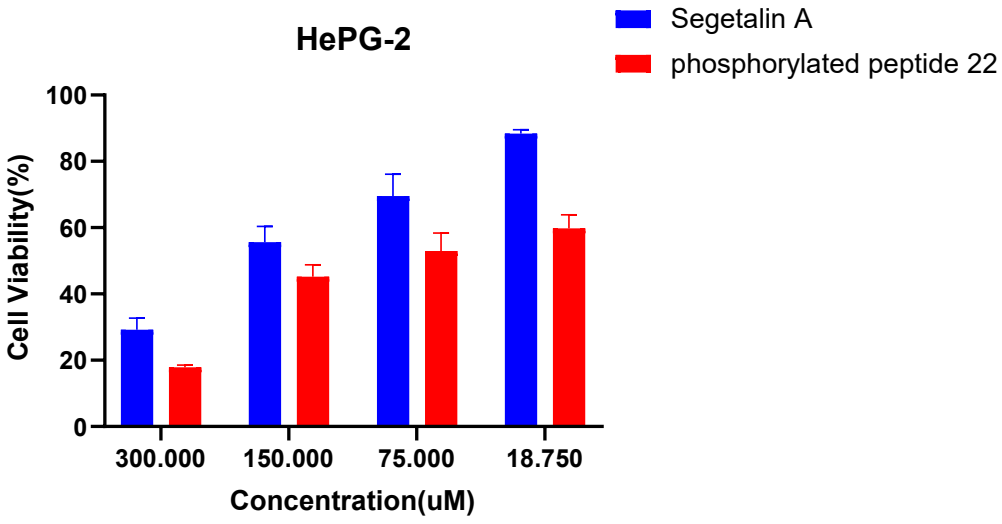
O. General procedures for cell inhibitory assay

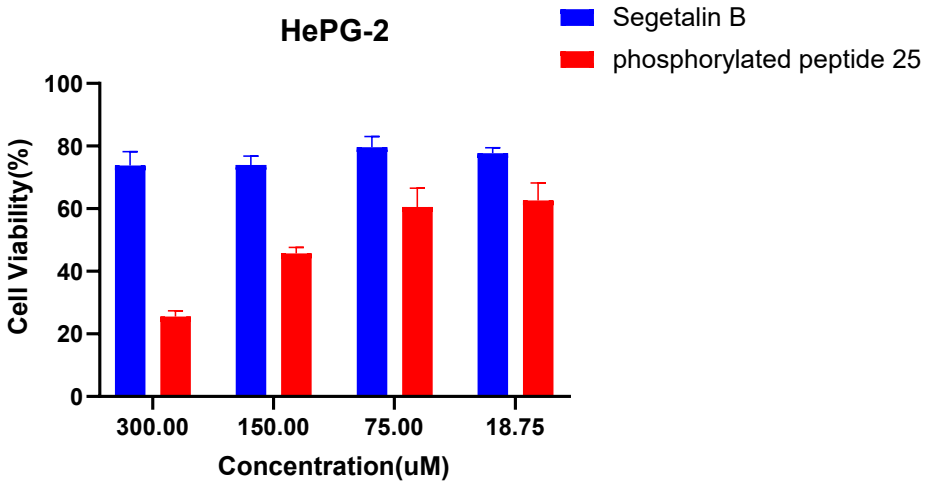
The Cell Counting Kit-8 (CCK-8; Beyotime Biotechnology Inc,, Shanghai, China) was employed to assess the inhibitory effects. HCT 116 and HepG-2 was used to test the cytotoxicity of the phosphonylation natural peptides (**22**, **23** and **25**) respectively. Cells were seeded in 96-well plates at a density of 5000 cells per well and grown for 24 hours at 37 °C in 5% CO_2. Then the cells were incubated with different concentrations (18.75 μM, 75.00 μM, 150.00 μM, 300.00 μM) of the compounds (100 μL) for 24 hours at 37 °C in 5% CO_2. Upon completion, CCK-8 solution was added and the plates were then incubated in a standard

incubator for 2 hours. Subsequently, the absorbance was measured by a microplate reader at 450 nm to evaluate cell viability.









Scheme S10 The Cell Viability of SegetalinA, SegetalinB and the phosphonylation natural peptides (**22** and **25**) towards HCT 116 and HepG-2.

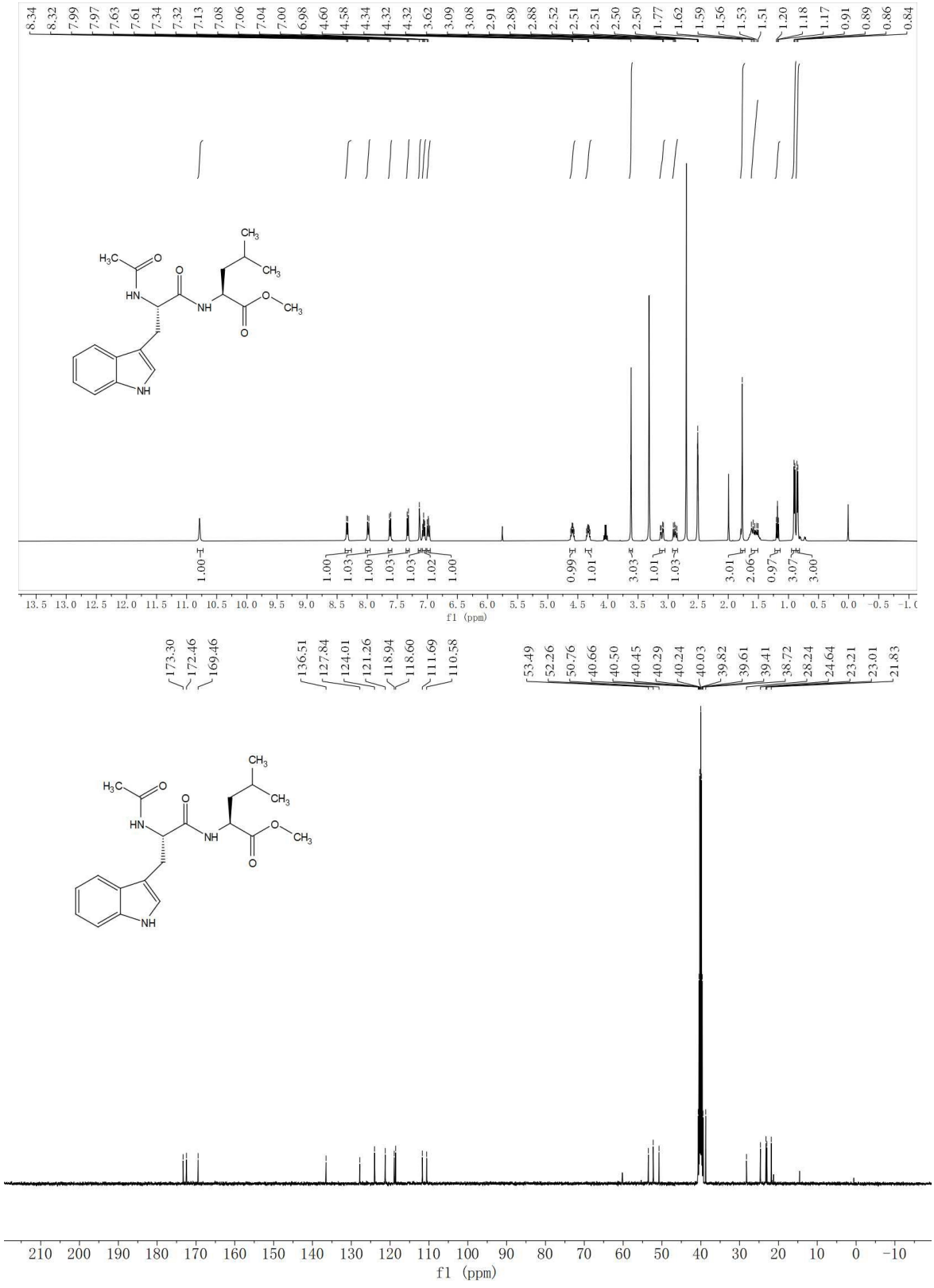
References：

[1] T. J. Williams, A. J. Reay, A. C. Whitwood and I. J. S. Fairlamb, Selective Photoredox Trifluoromethylation of Tryptophan-containing Peptides, *Chem. Commun.*, 2014, **50**, 3052–3054.

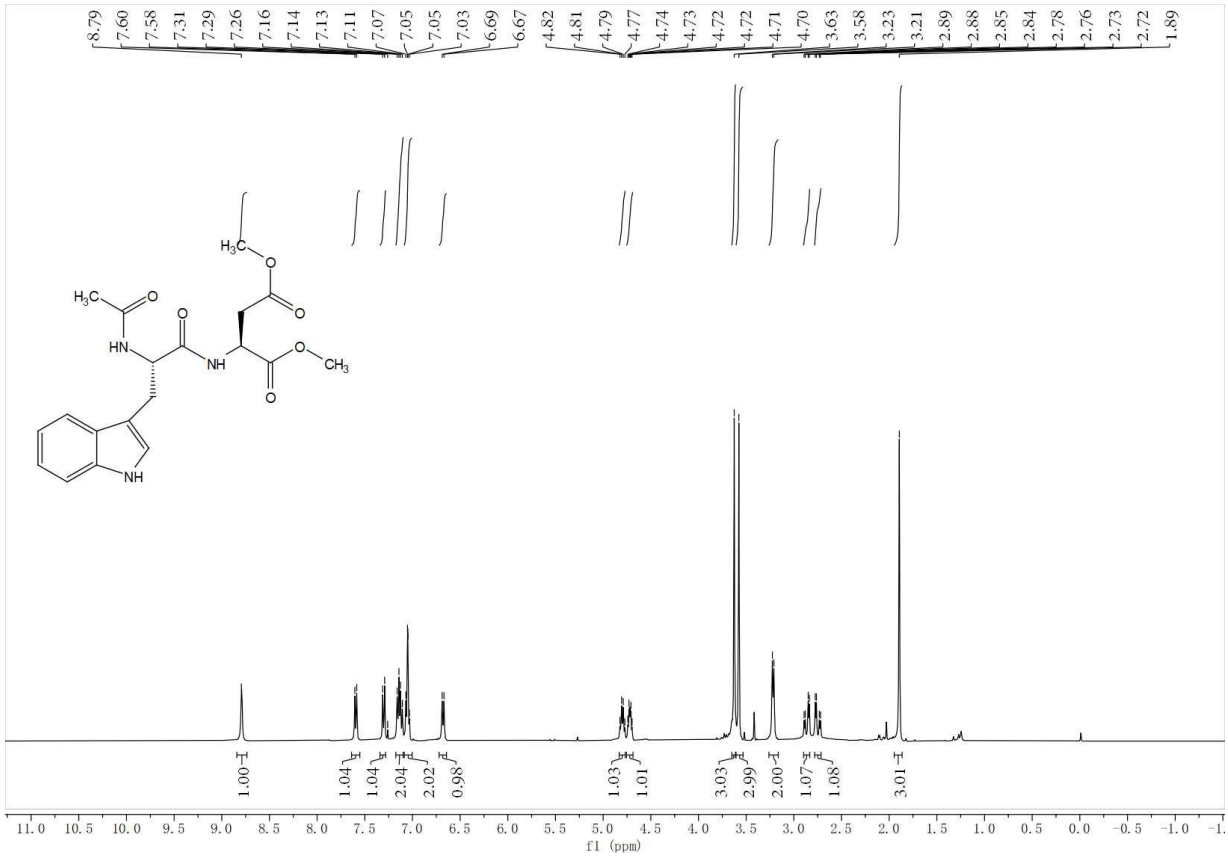
[2] J. Reimler and A. Studer, Visible-Light Mediated Tryptophan Modification in Oligopeptides Employing Acylsilanes, *Chemistry A European J*, 2021, **27**, 15392–15395.

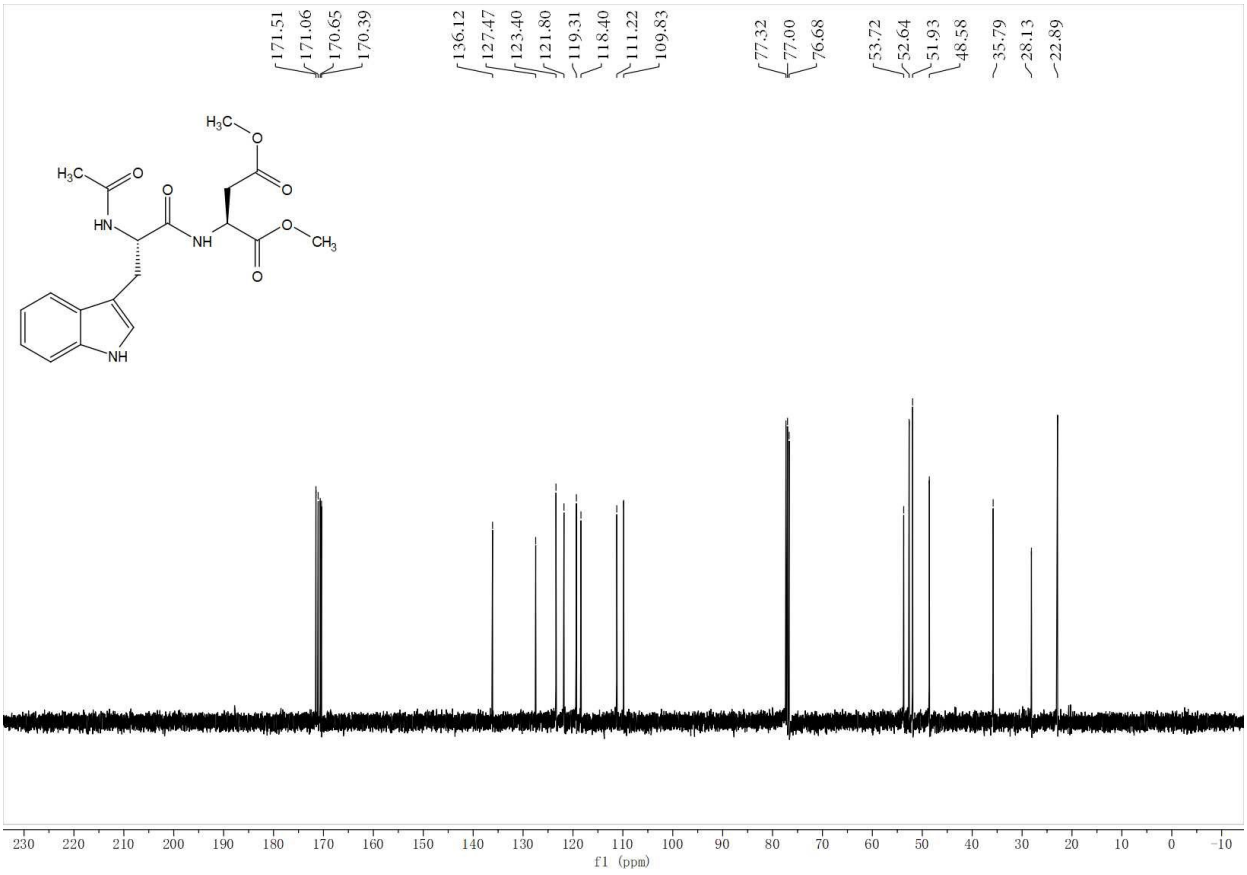
P. NMR Spectra

Ac-Trp-Leu-OMe (**S9**)

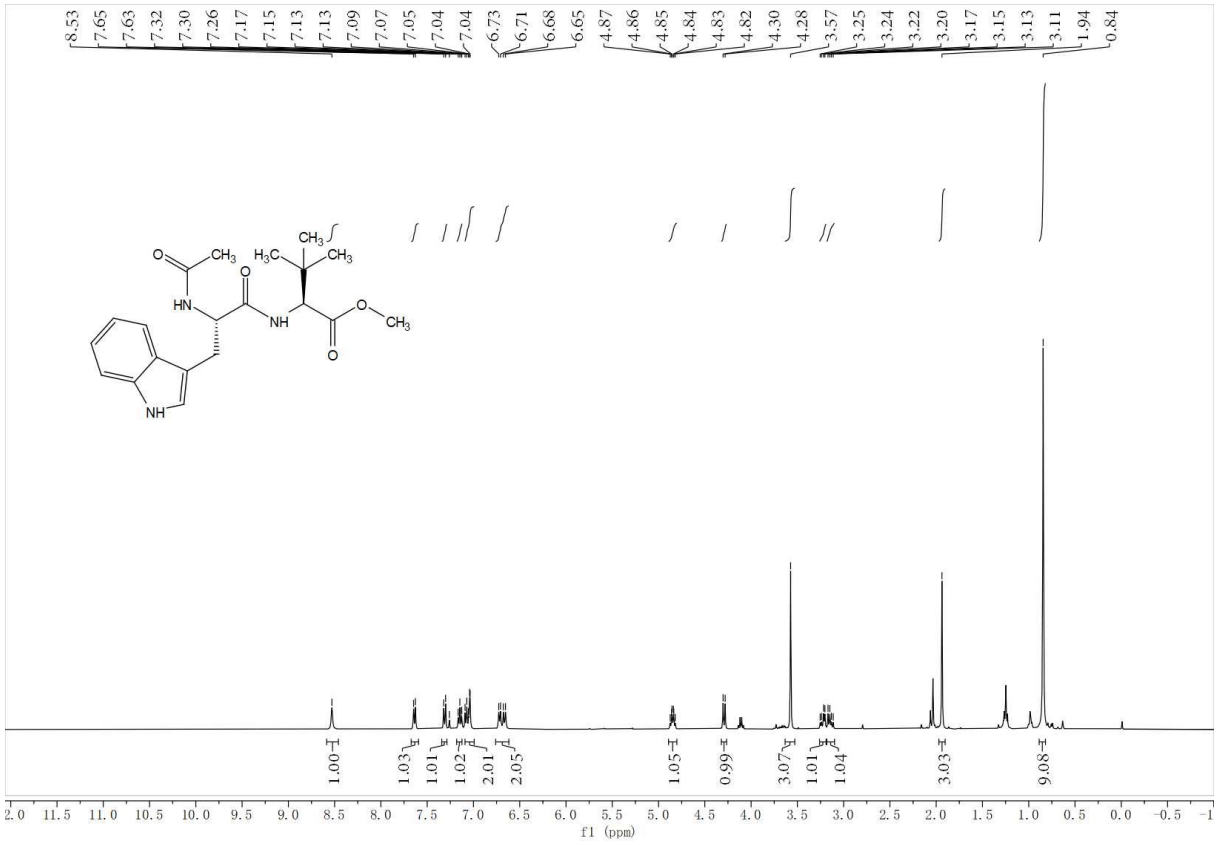


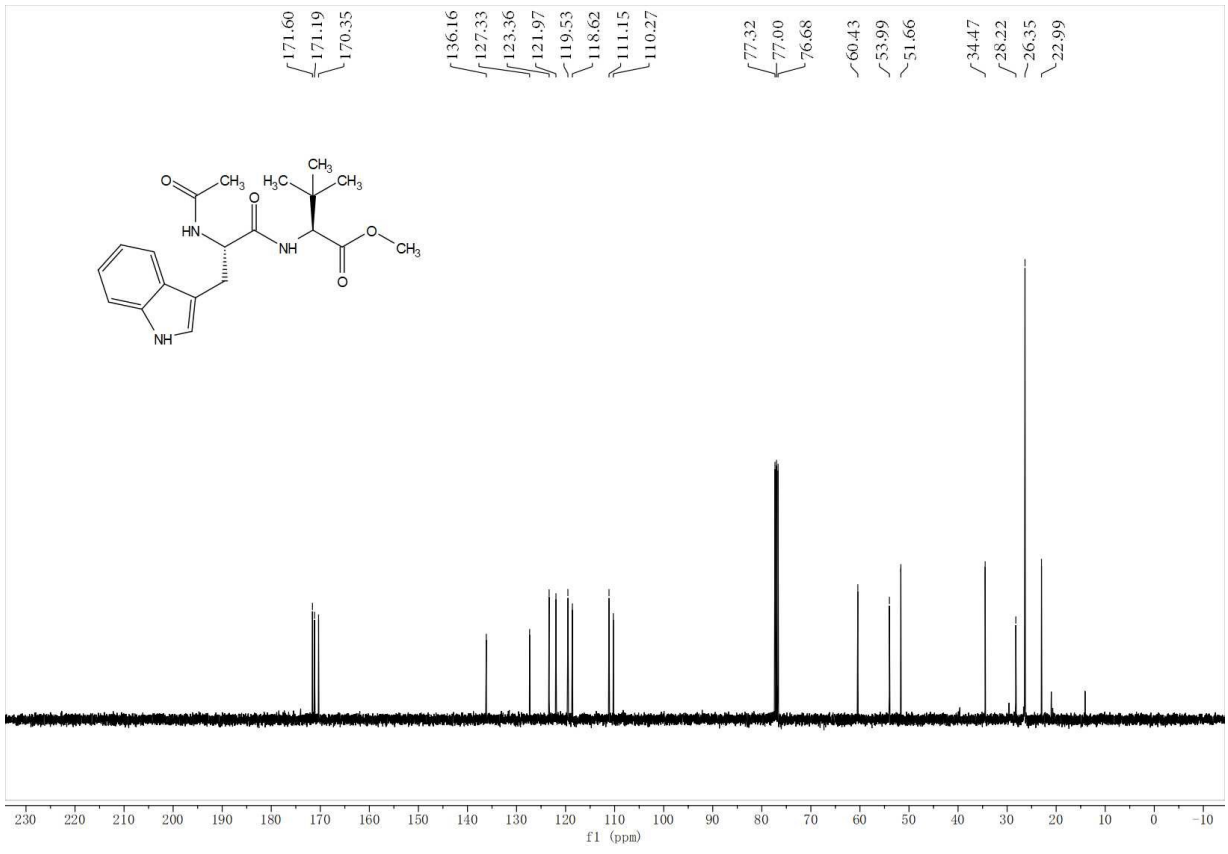
Ac-Trp-L-Asp acid dimethyl (**S10**)



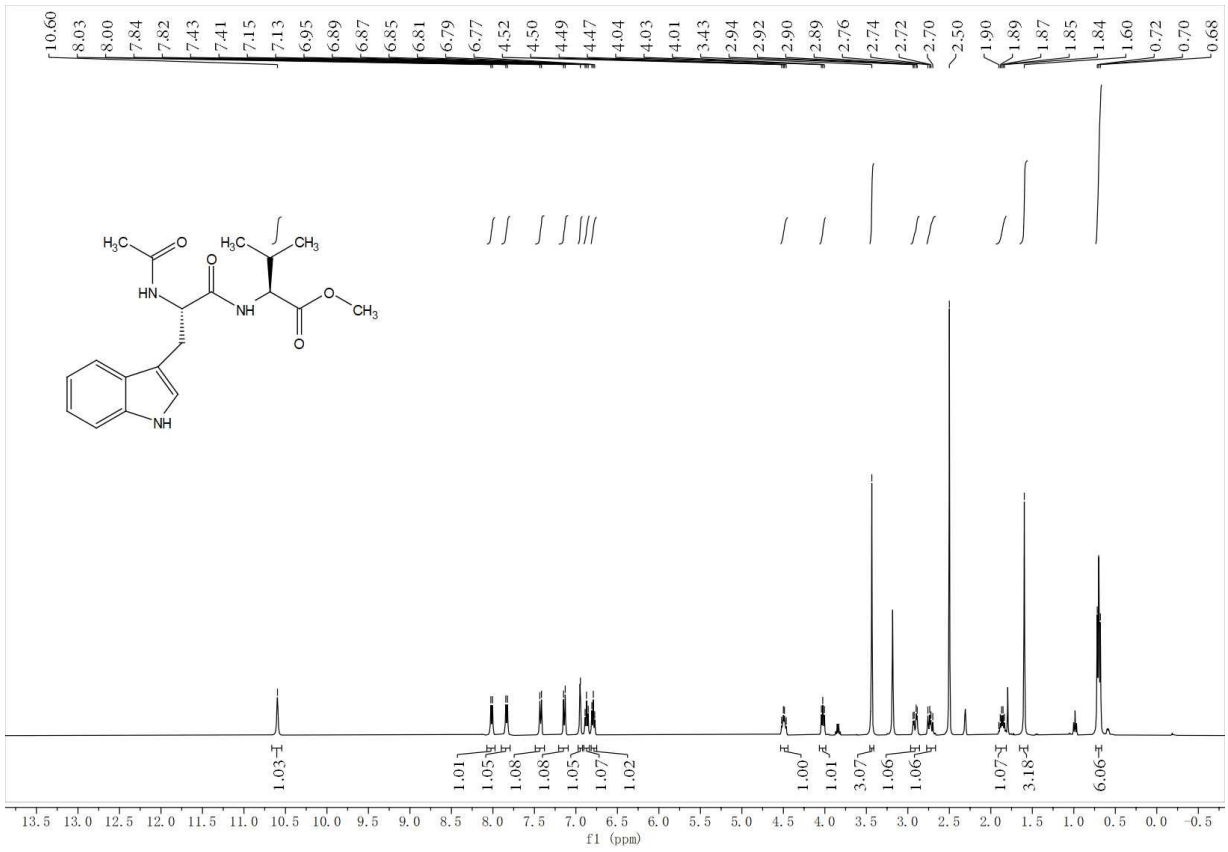


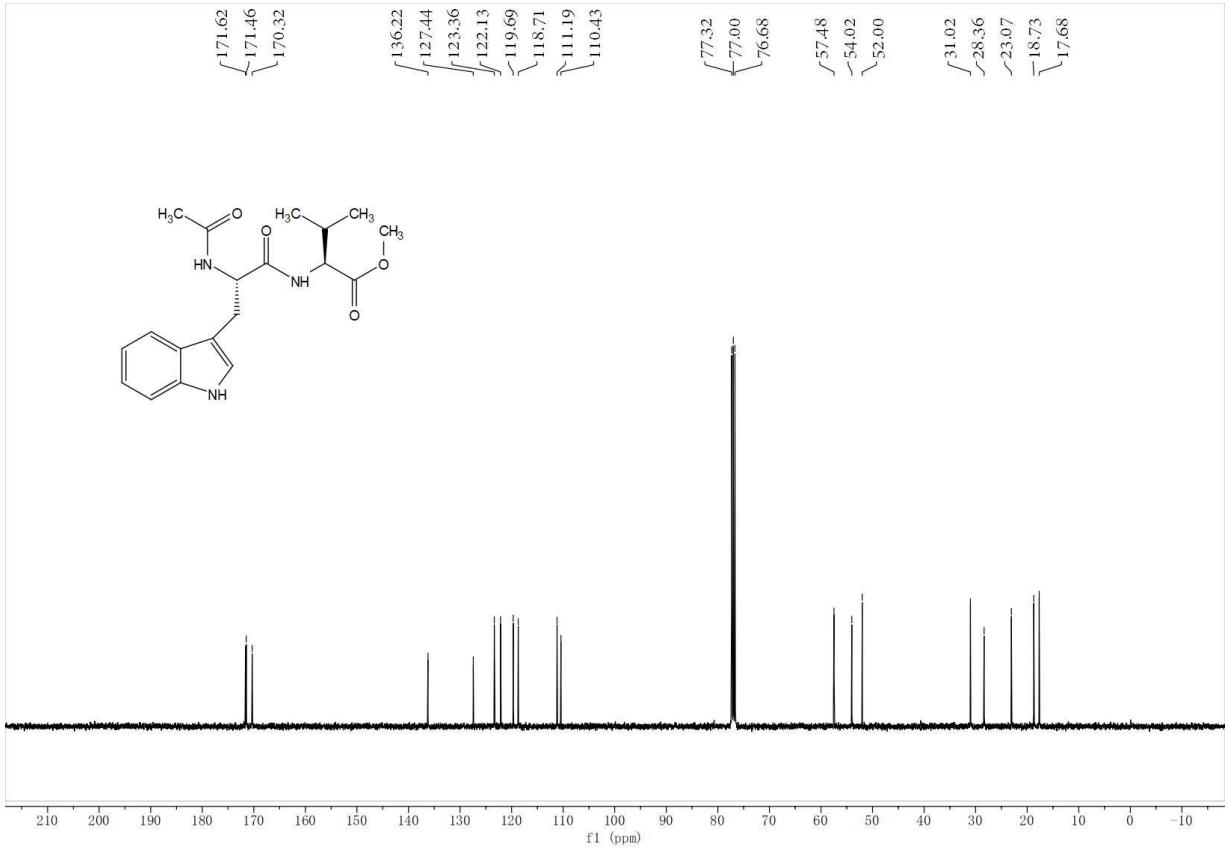
Ac-Trp-Tle-OMe **(S11)**



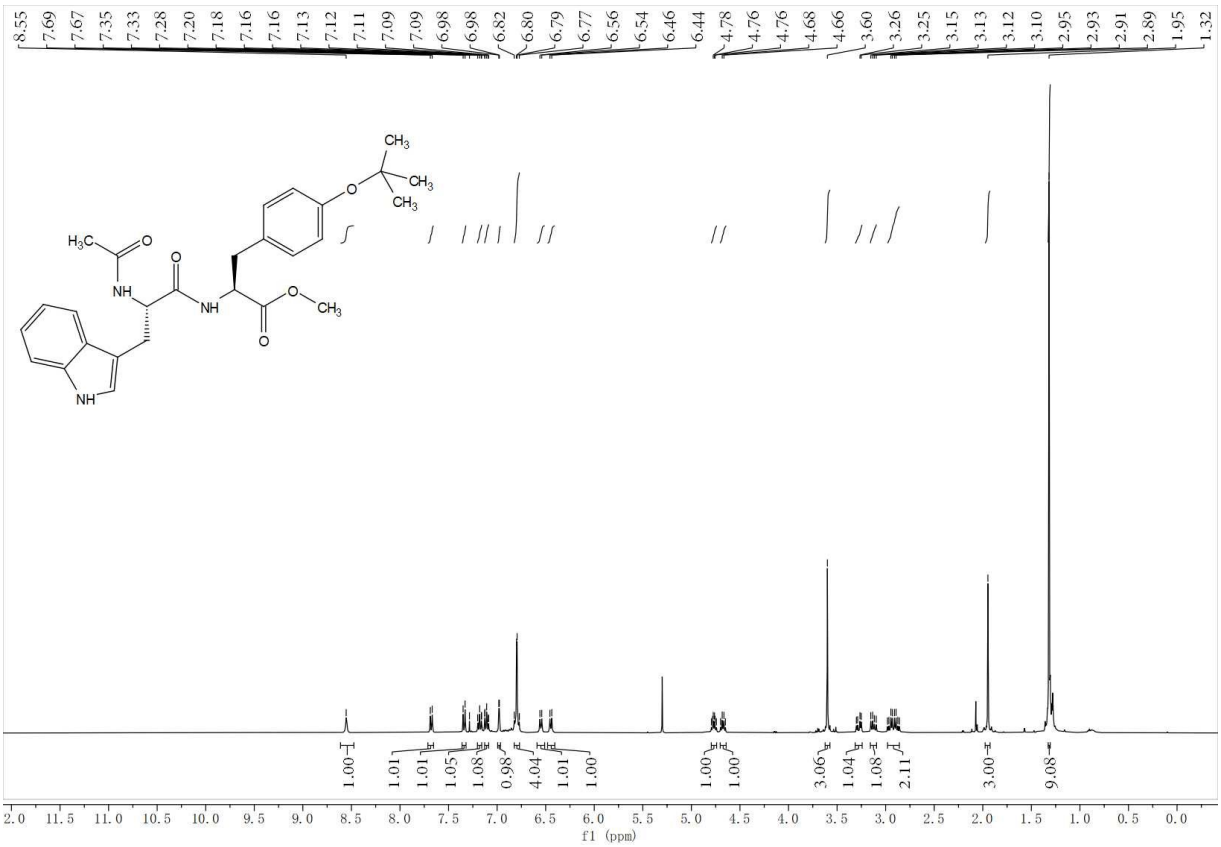


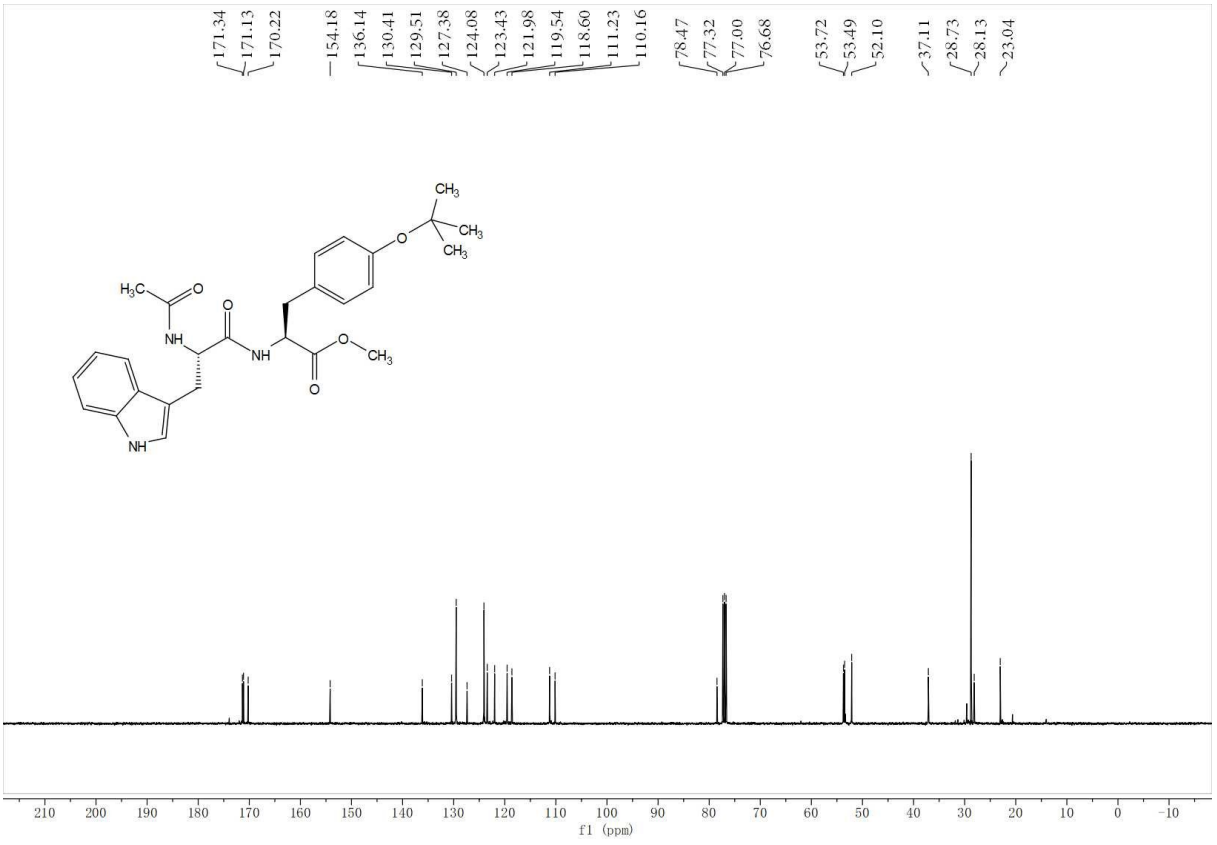
Ac-Trp-Val-OMe **(S12)**



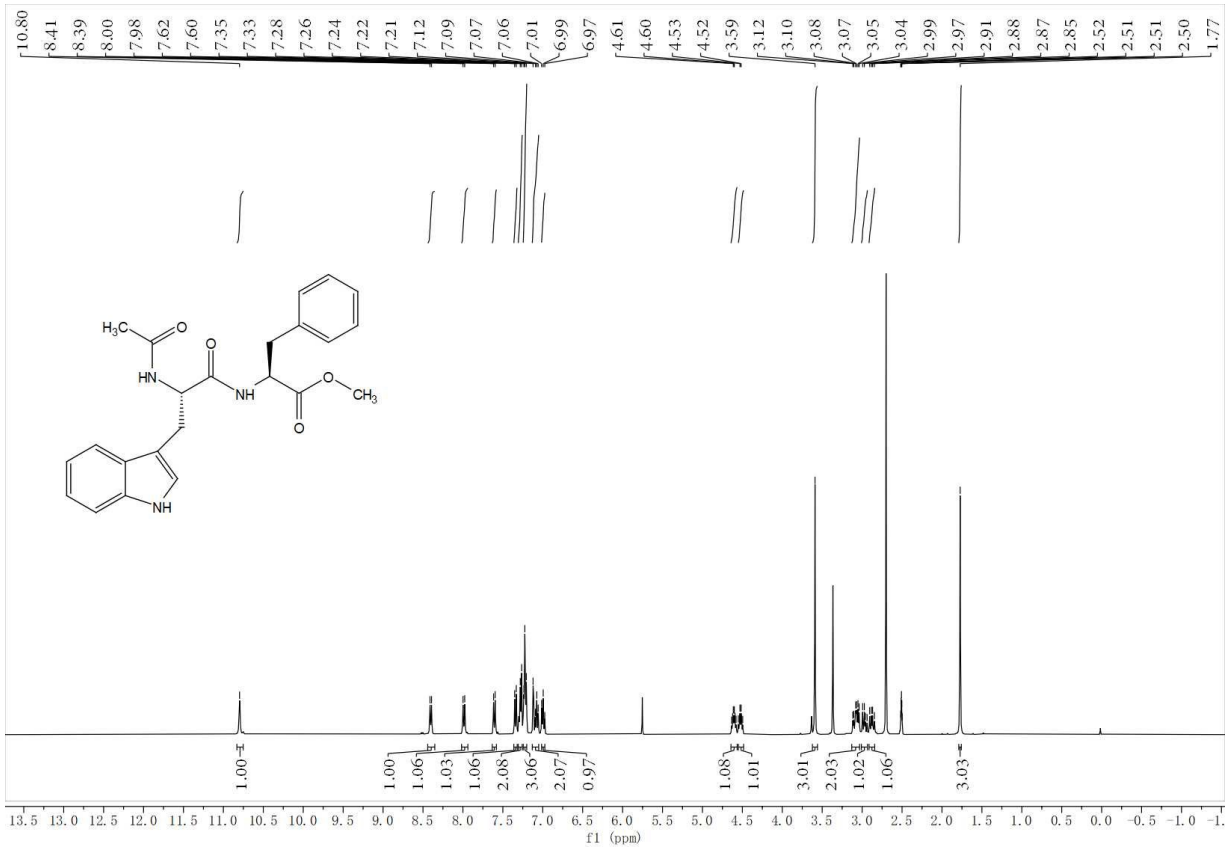


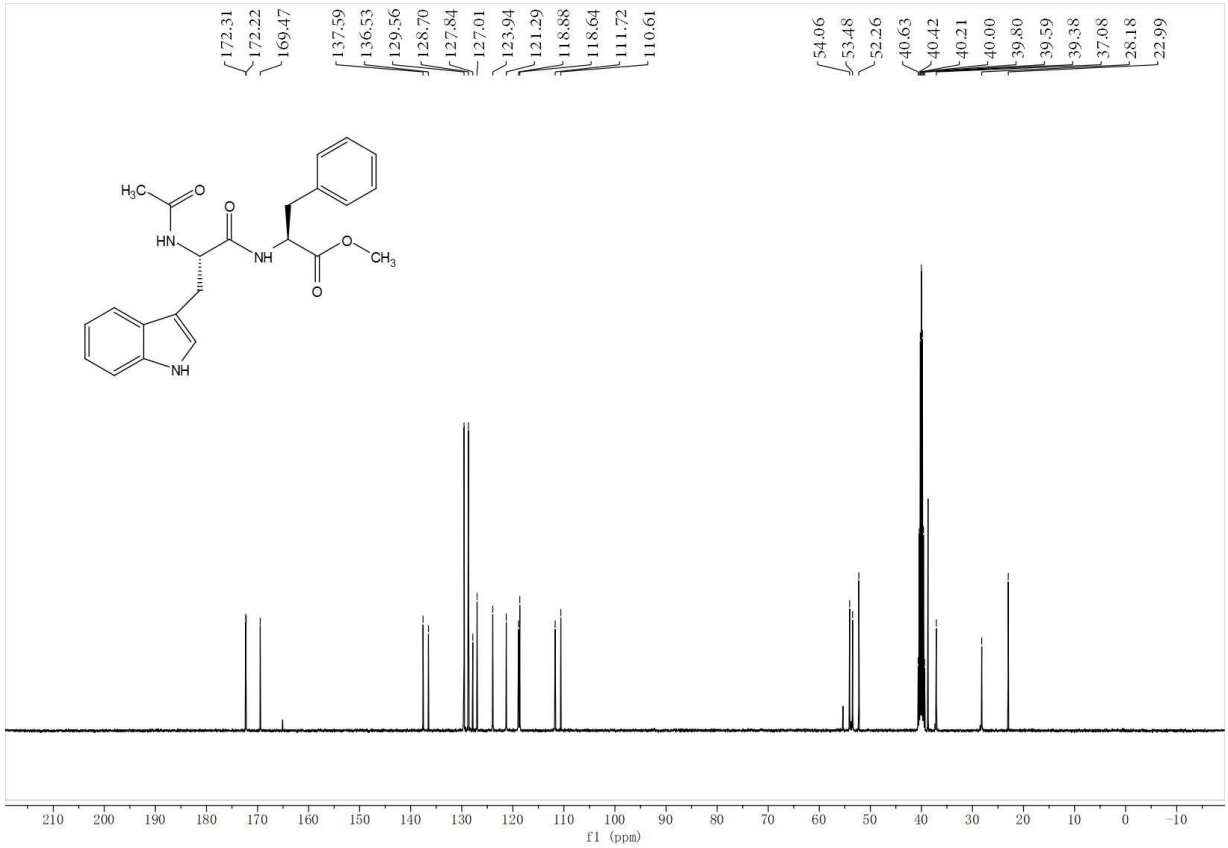
Ac-Trp-Tyr(t-Bu)-OMe (**S13**)



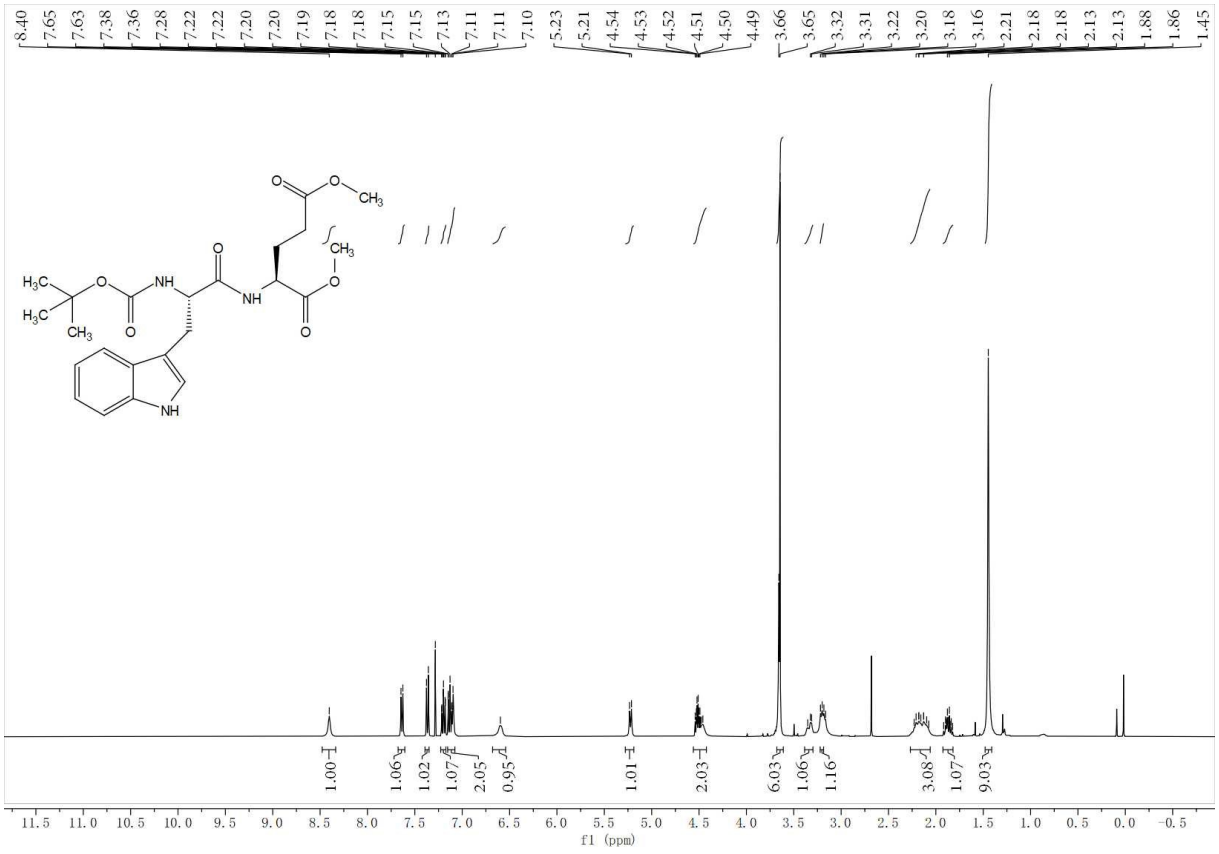


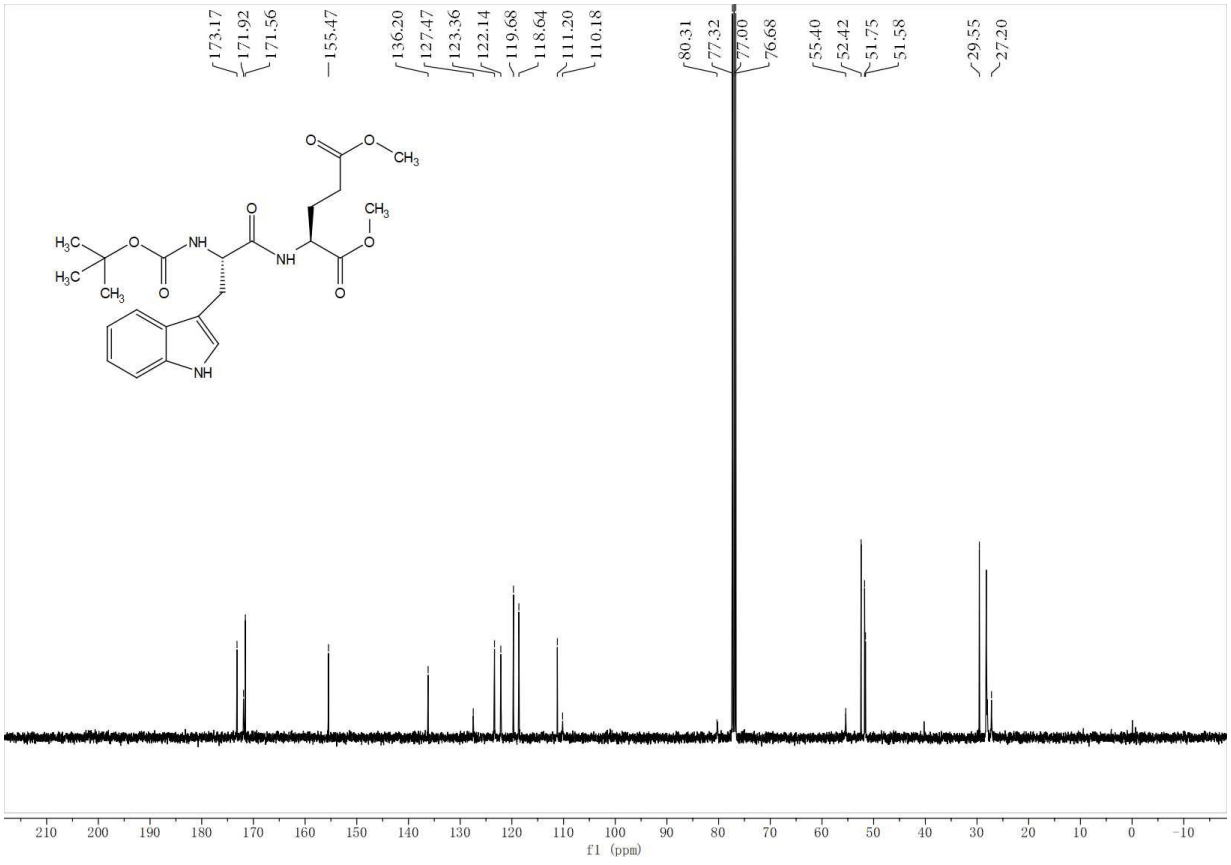
Ac-Trp-Phe-OMe （**S14**）



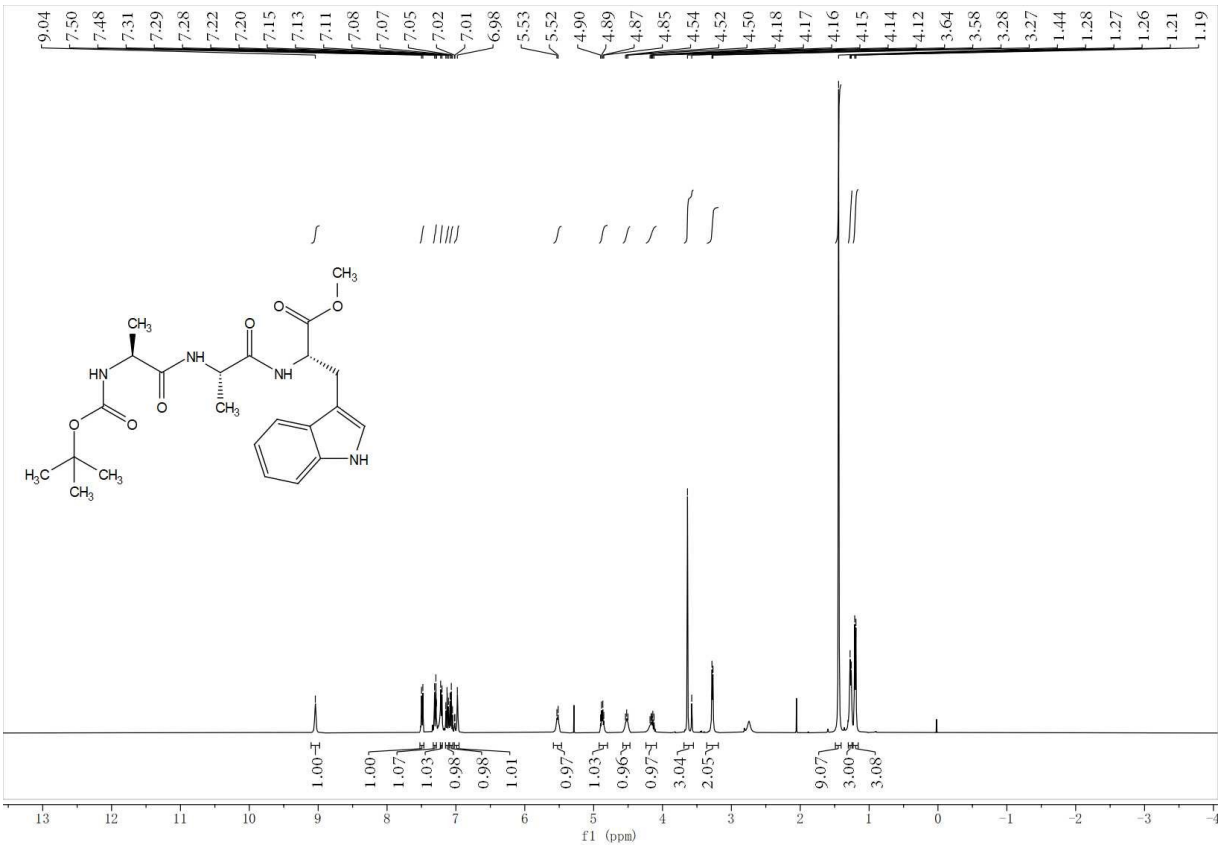


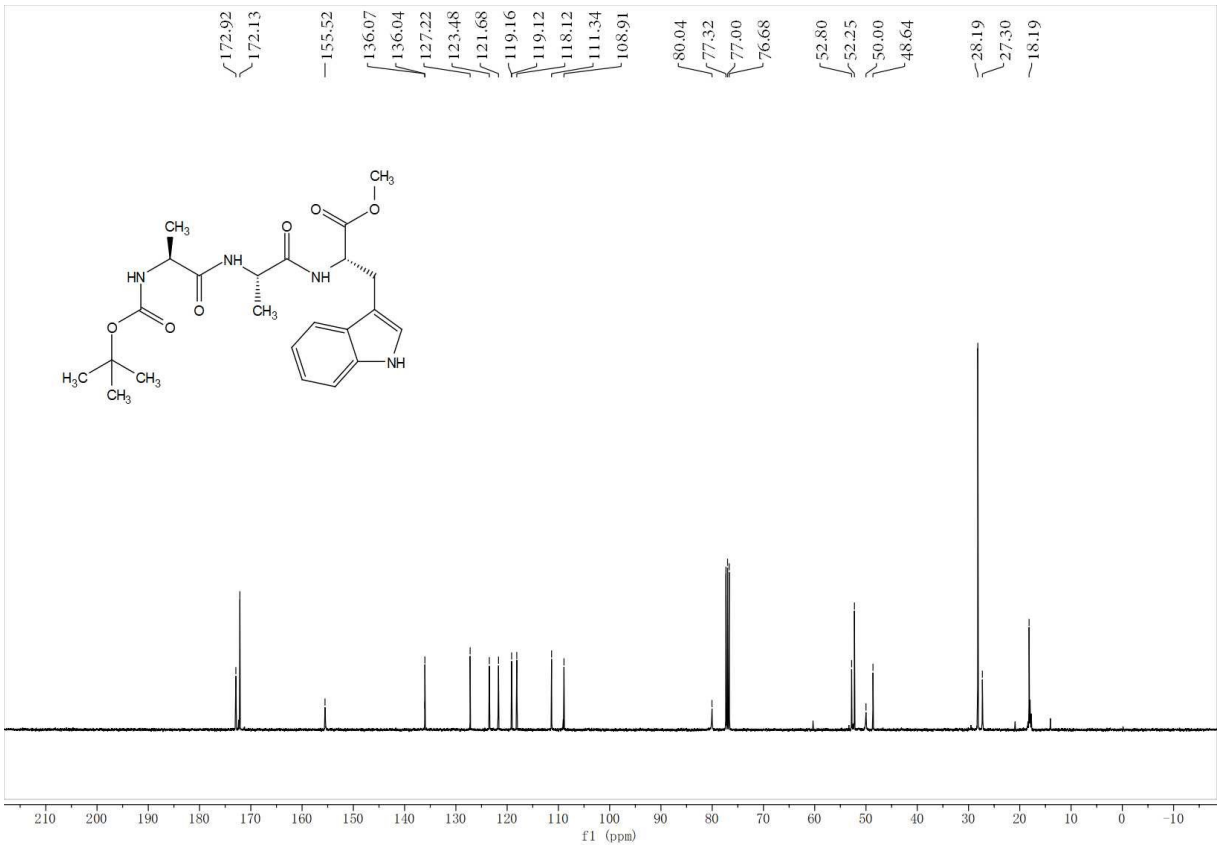
Boc-Trp-L-Glu acid dimethyl ester **(S15)**



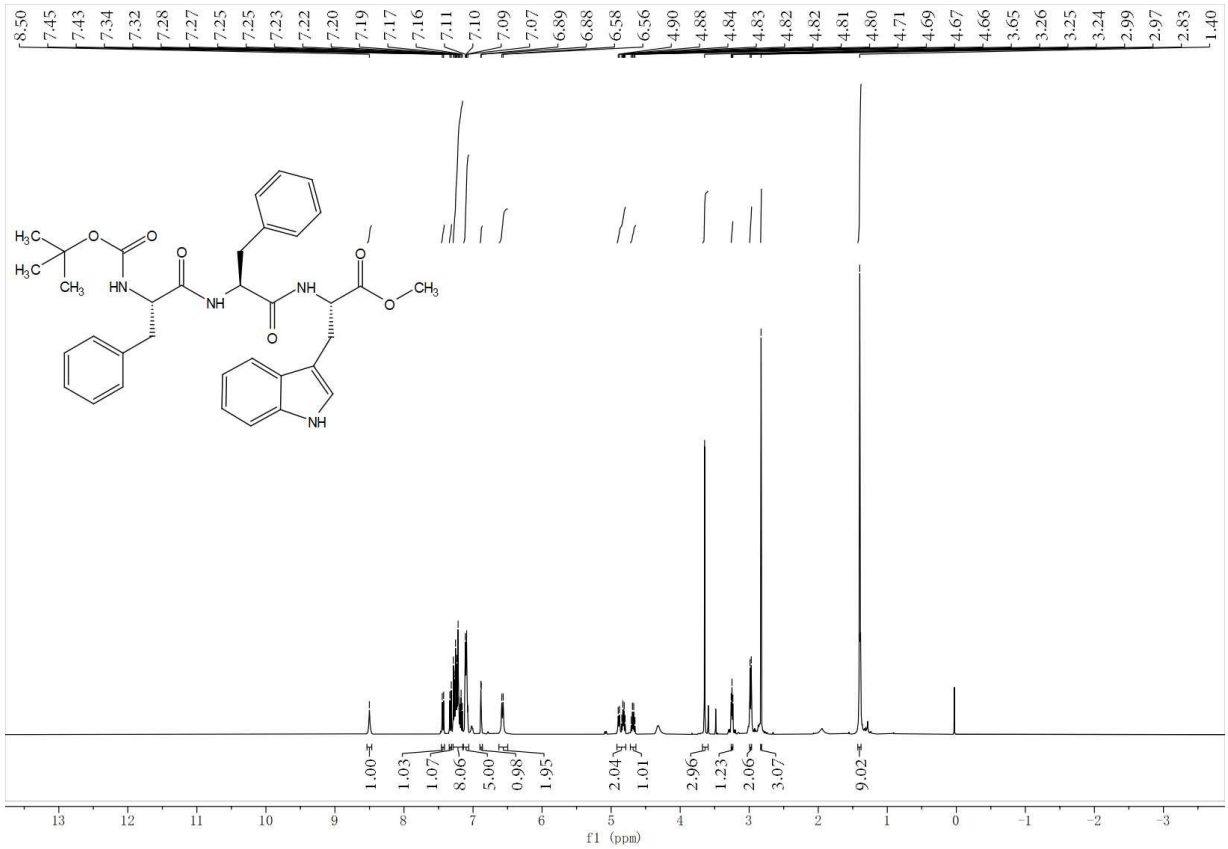


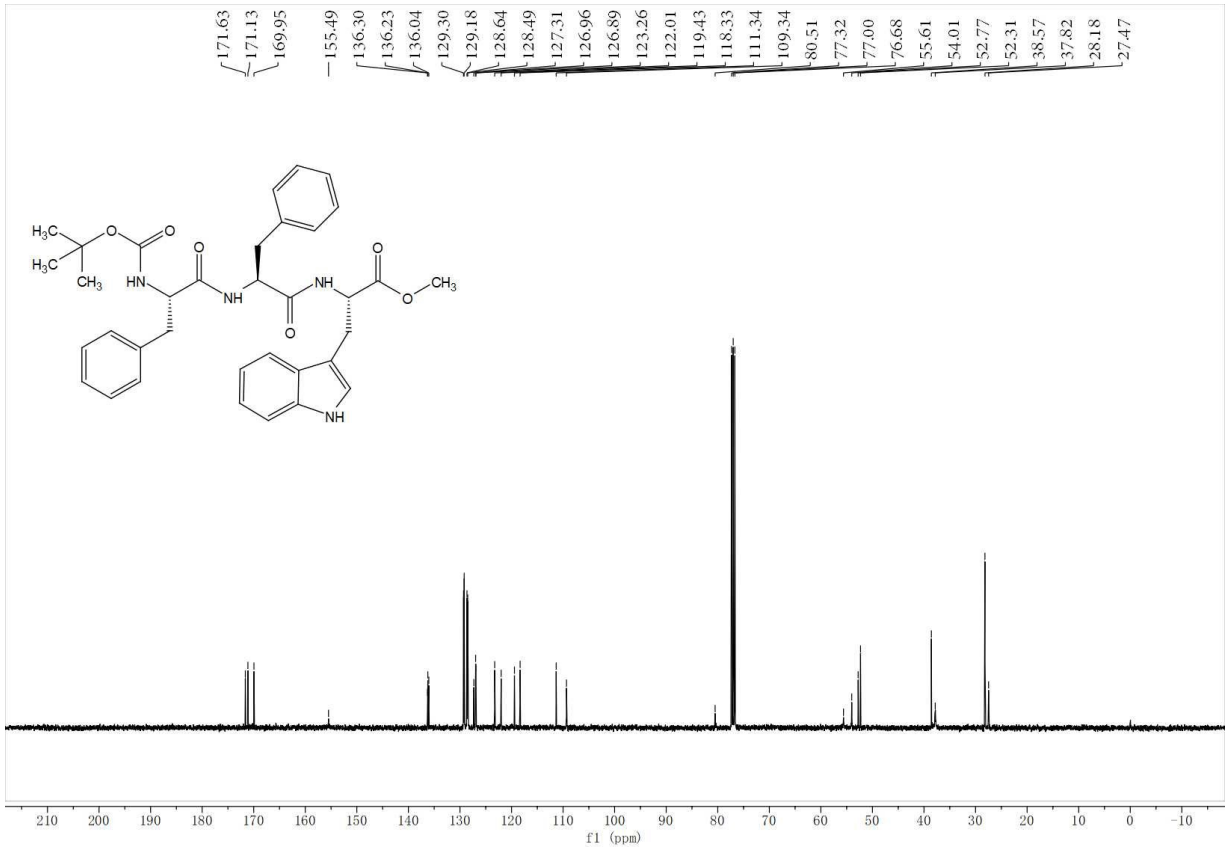
Boc-Ala-Ala-Trp-OMe (**S17**)



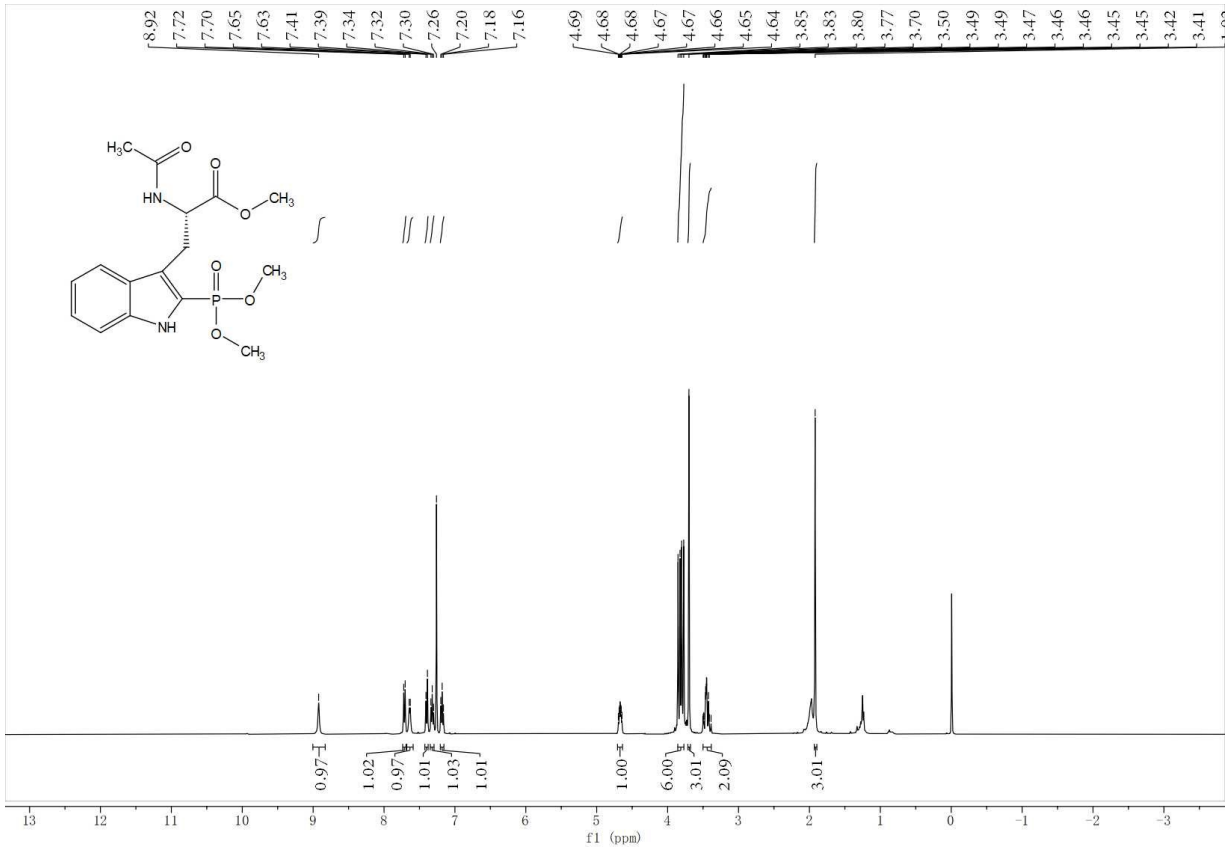


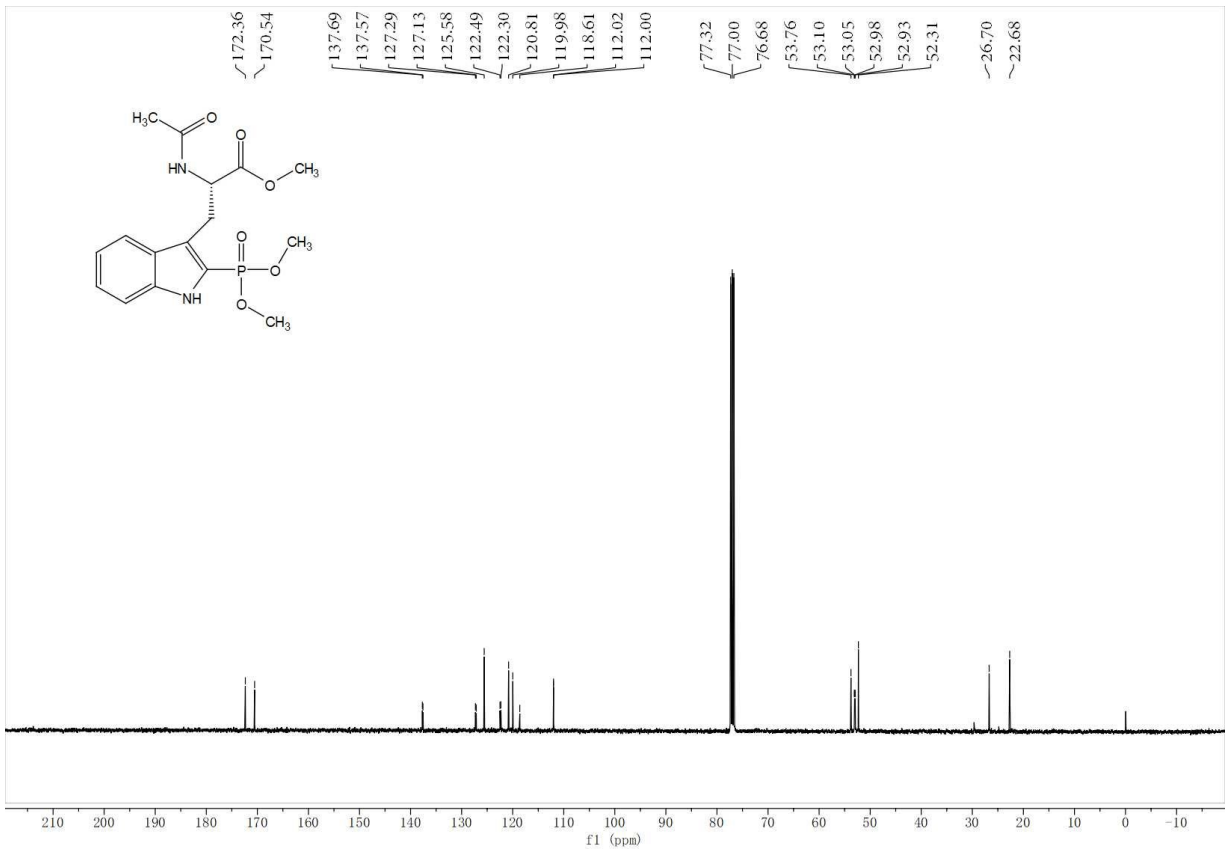
Boc-Phe-Phe-Trp-OMe （**S18**）

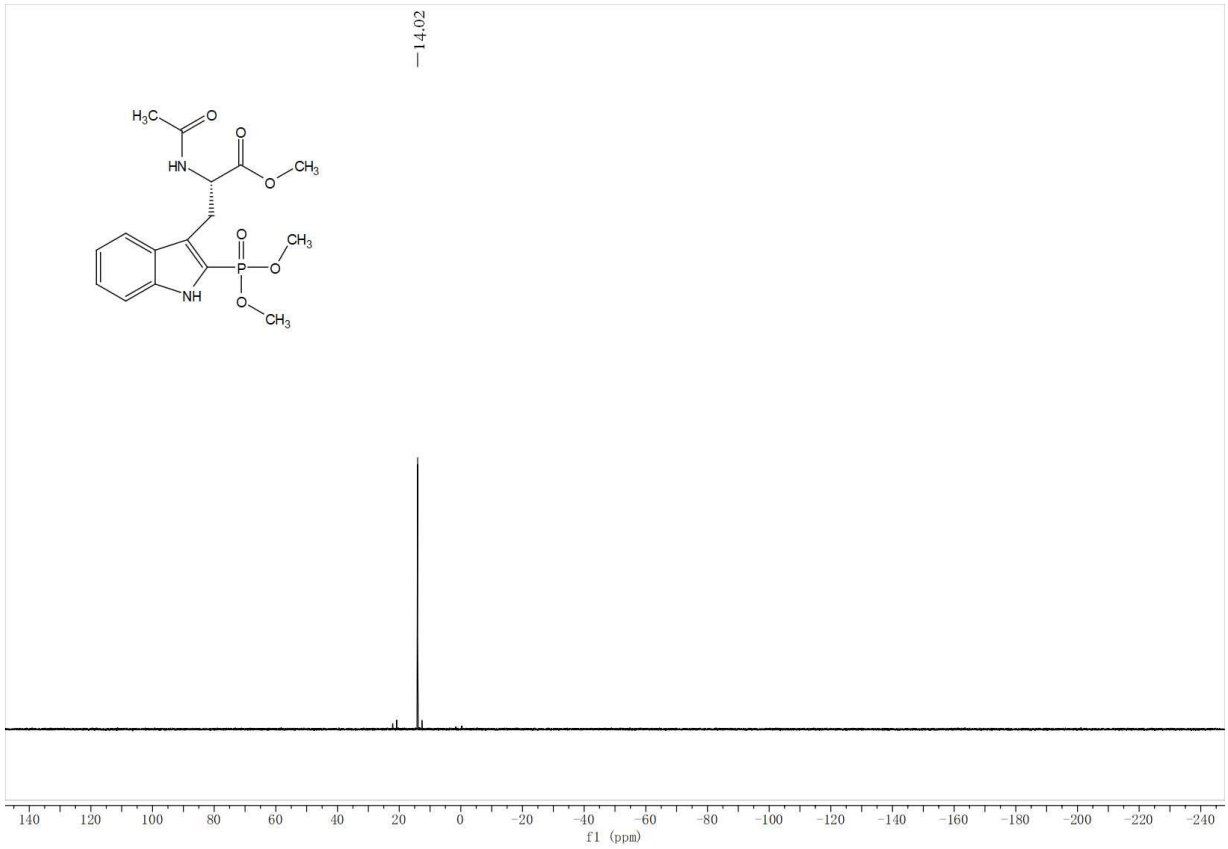




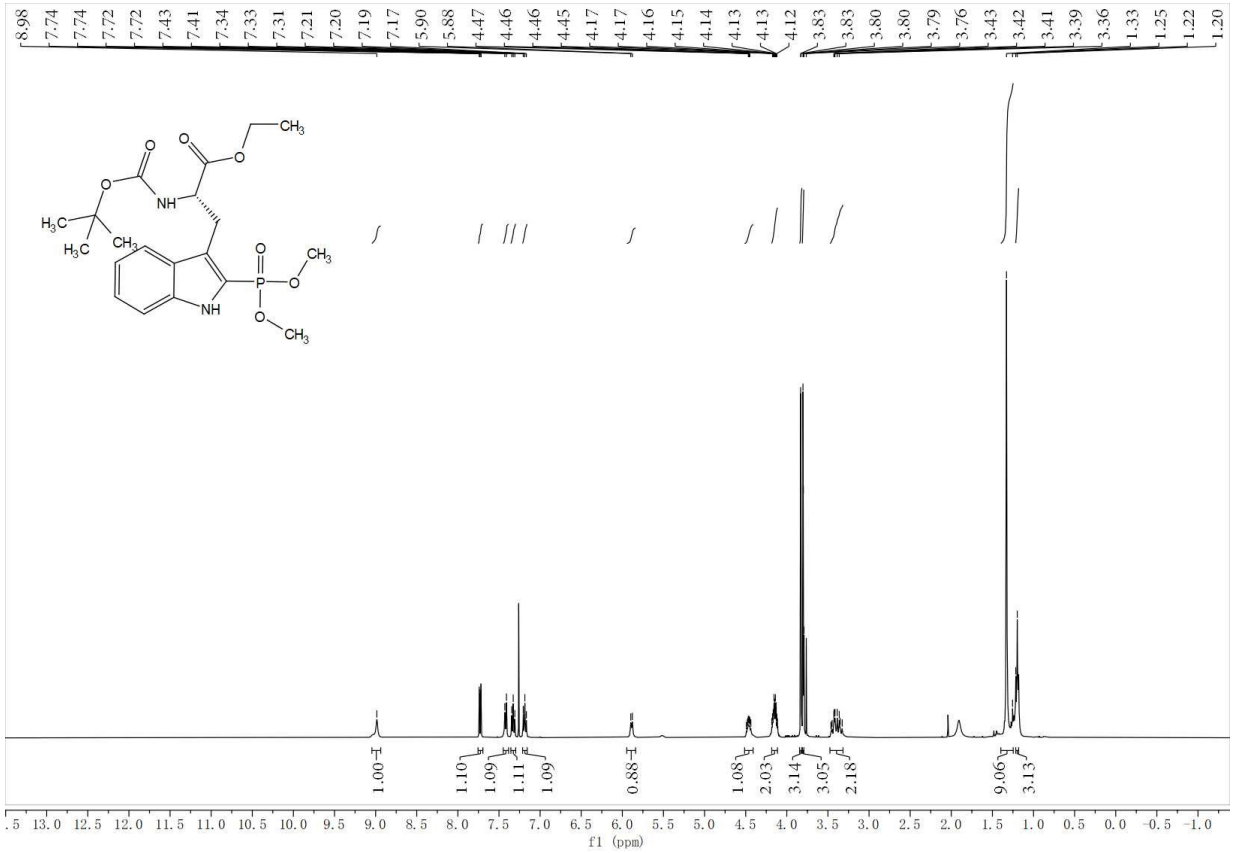
Ac-Trp-OMe-Methyl phosphate (**3**)

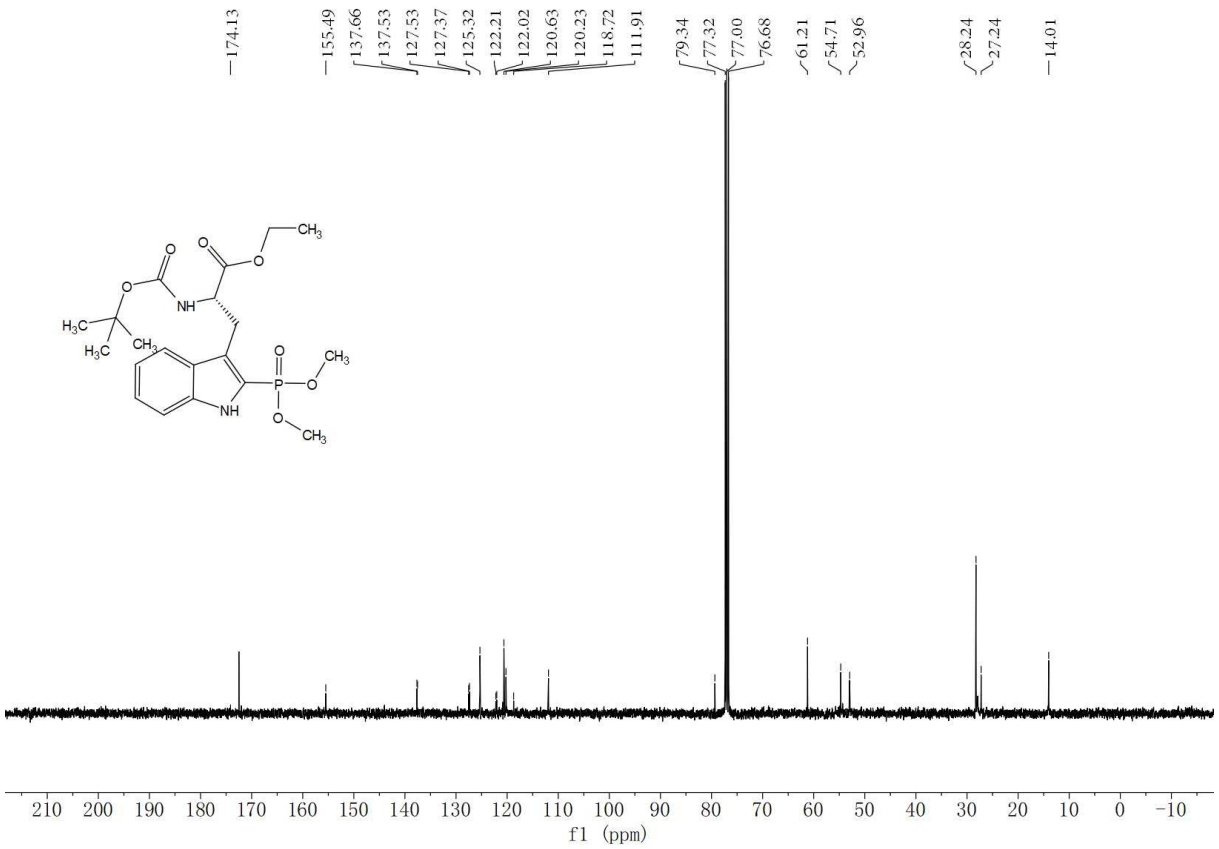


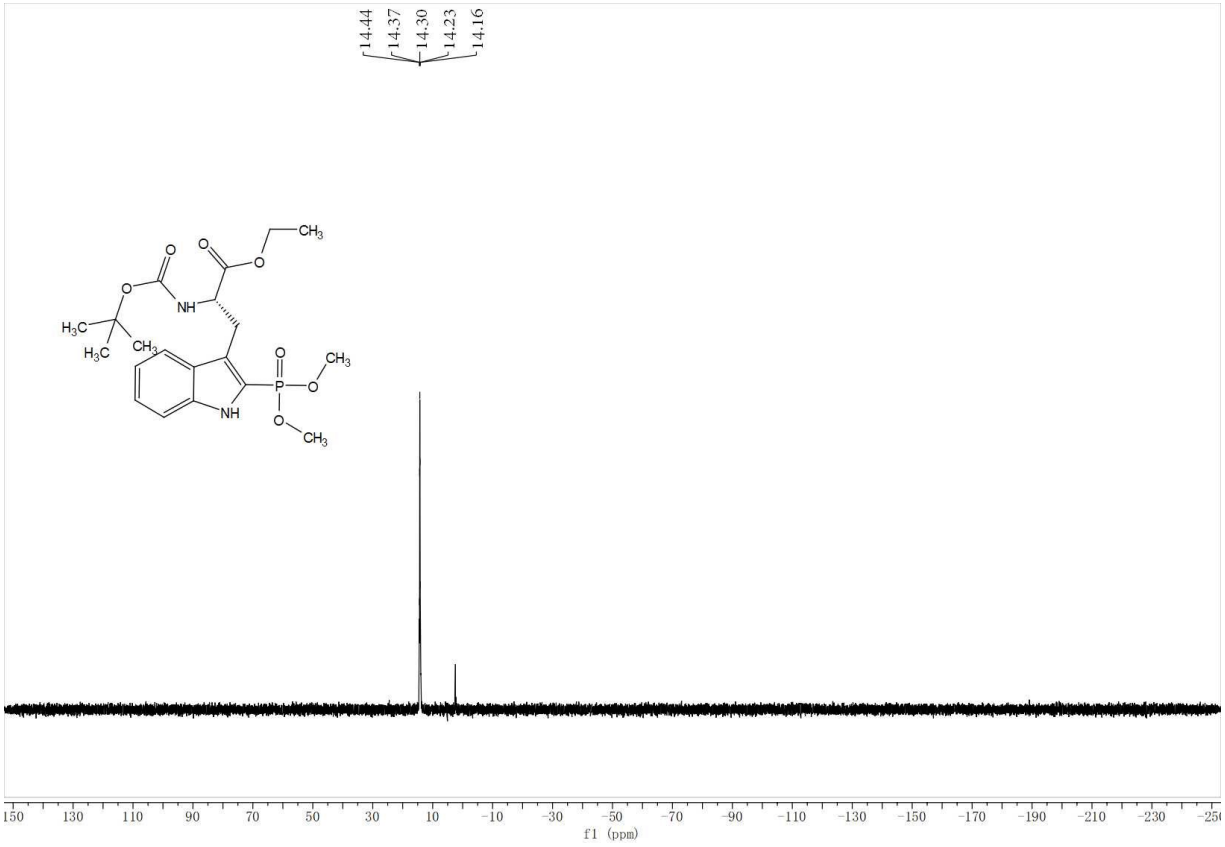




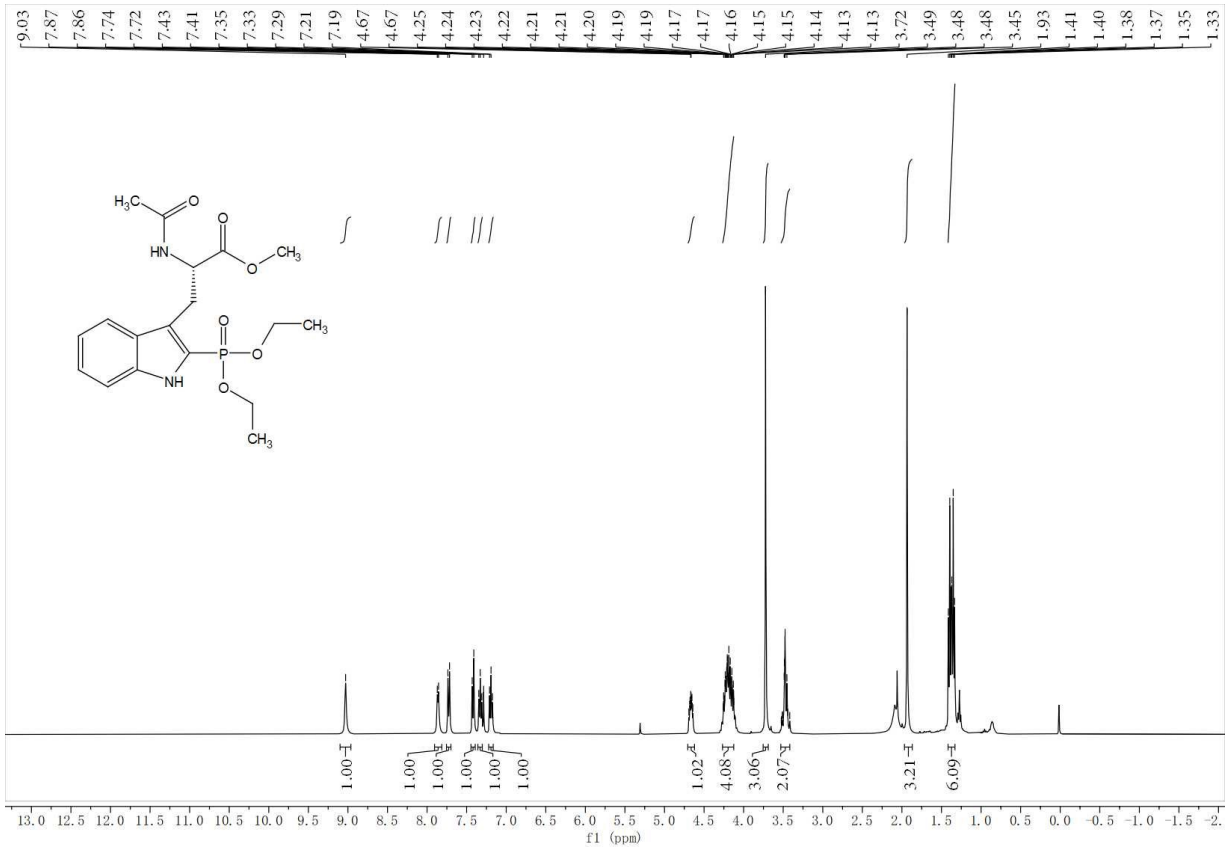
Boc-Trp-OEt-Methyl phosphate (**4**)

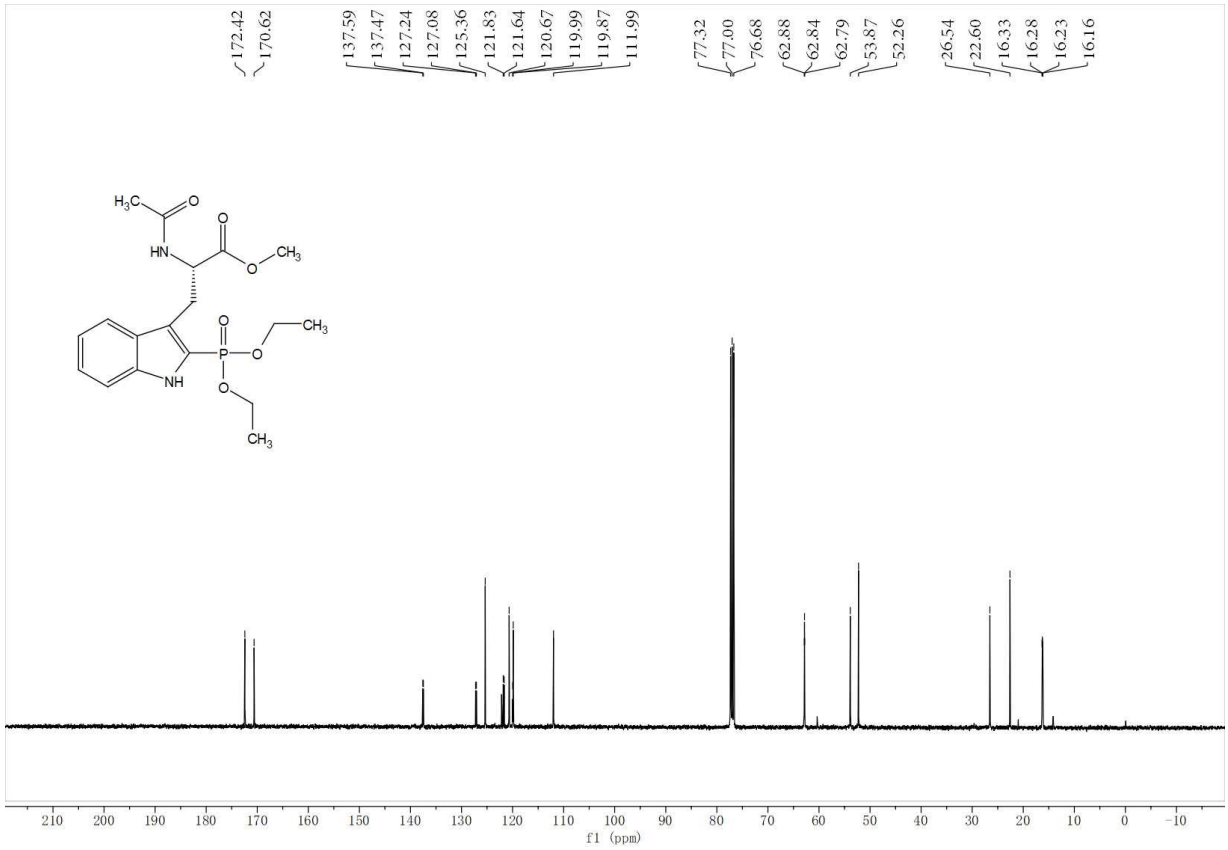


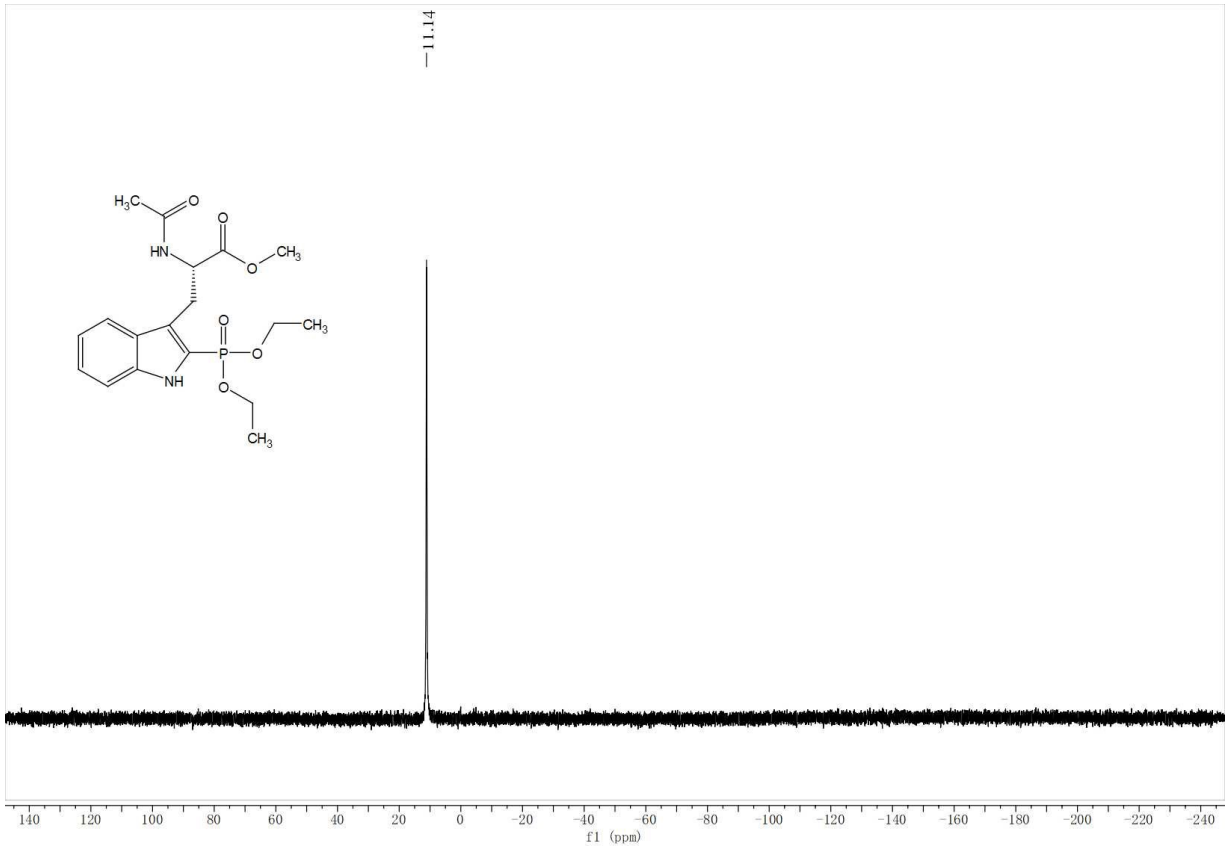

174.13
155.49
137.66
137.53
127.53
127.37
125.32
122.21
122.02
120.63
120.23
118.72
111.91
79.34
77.32
77.00
76.68
61.21
54.71
52.96
28.24
27.24
14.01
NH
NH
f1 (ppm)


14.44
14.37
14.30
14.23
14.16
NH
NH
f1 (ppm)

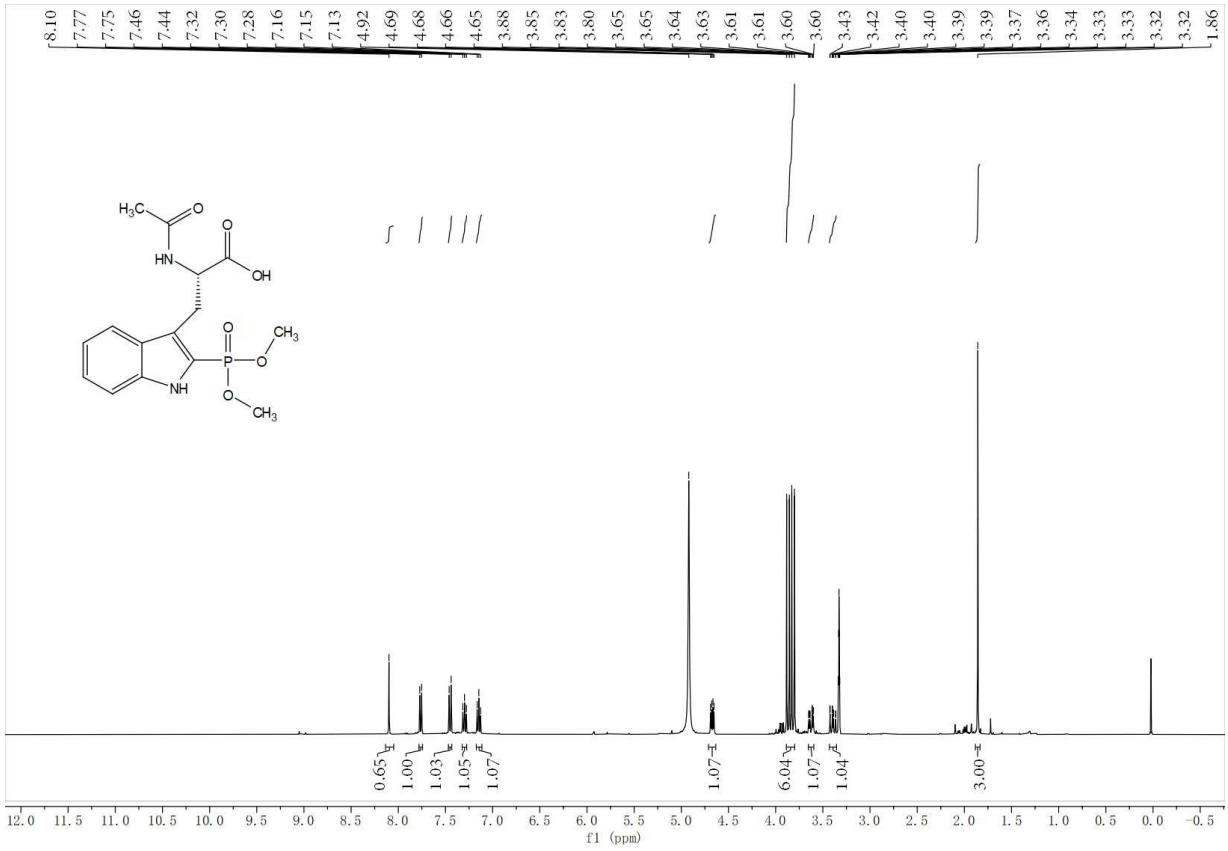
Ac-Trp-OMe-Phosphoryl ethyl ester (**5**)

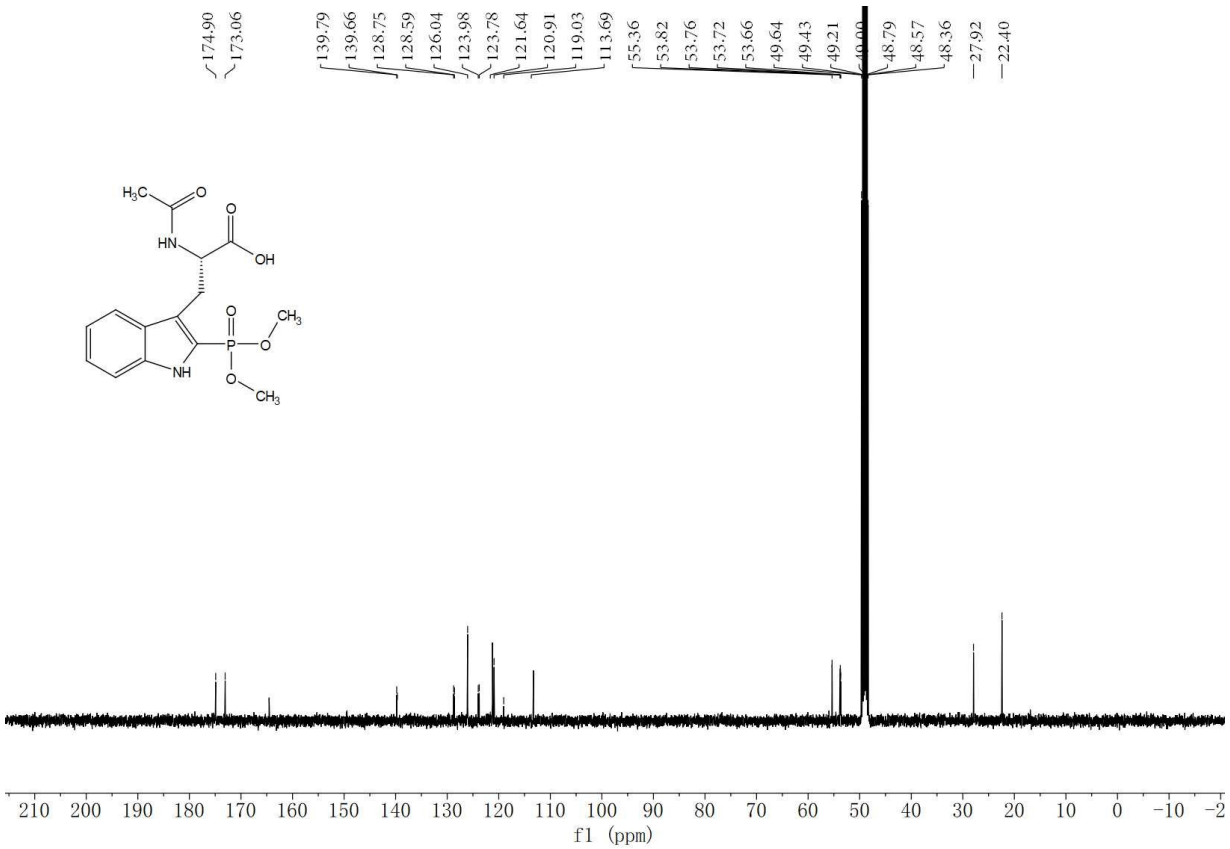


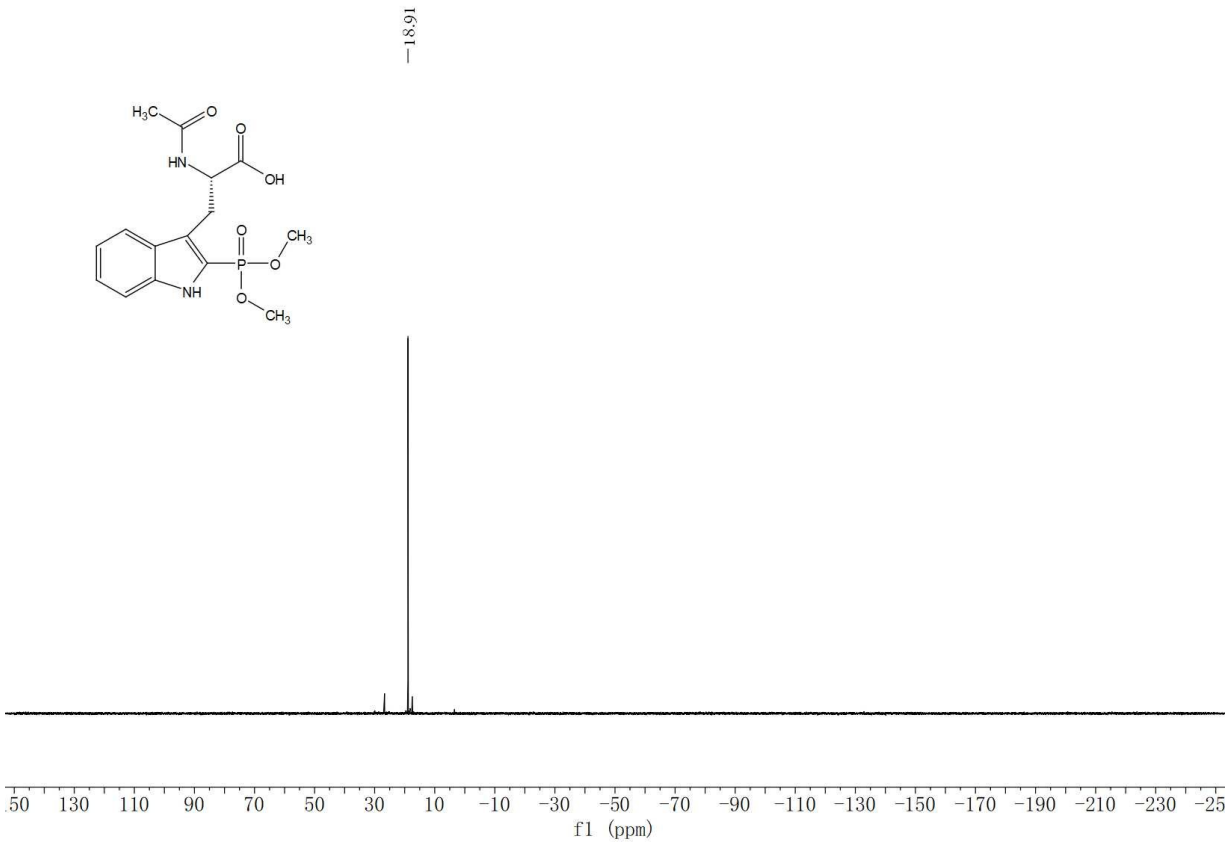




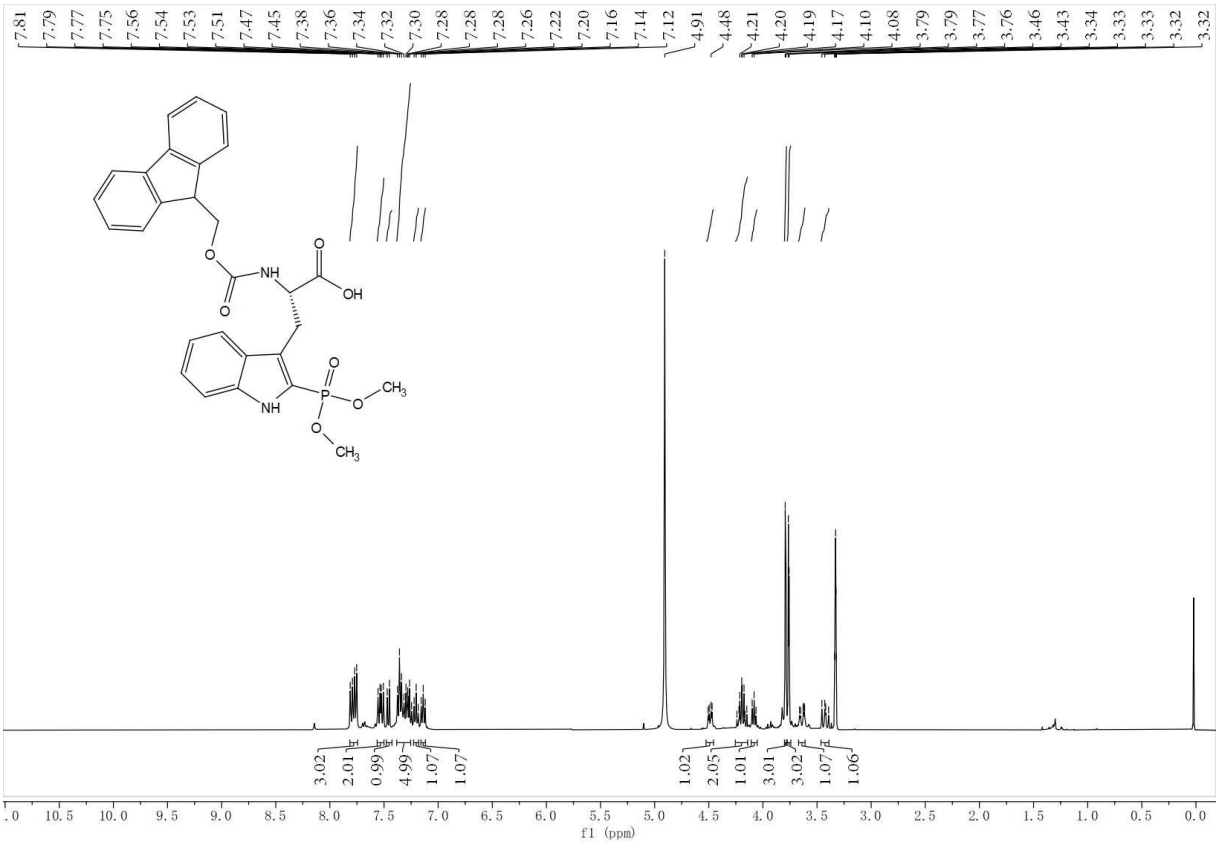
Ac-Trp-Methyl phosphate (**6**)

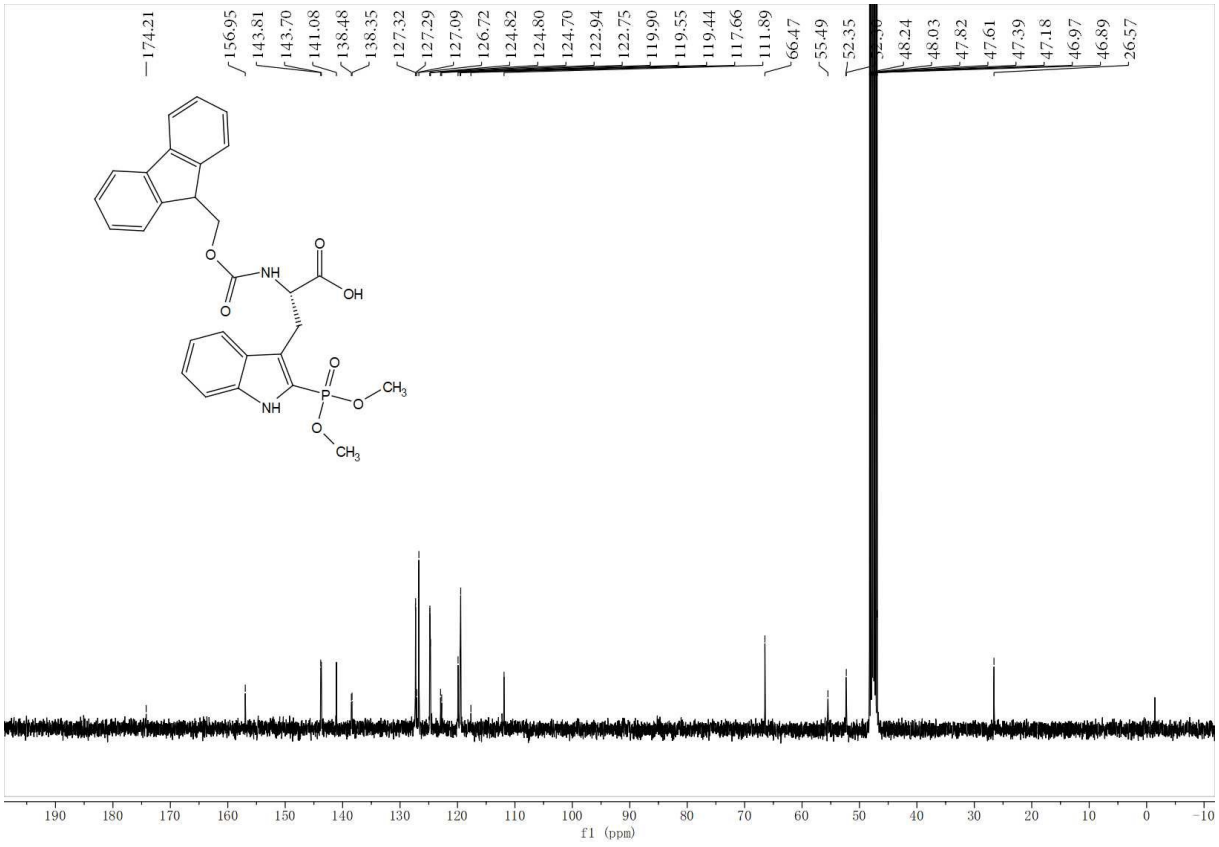


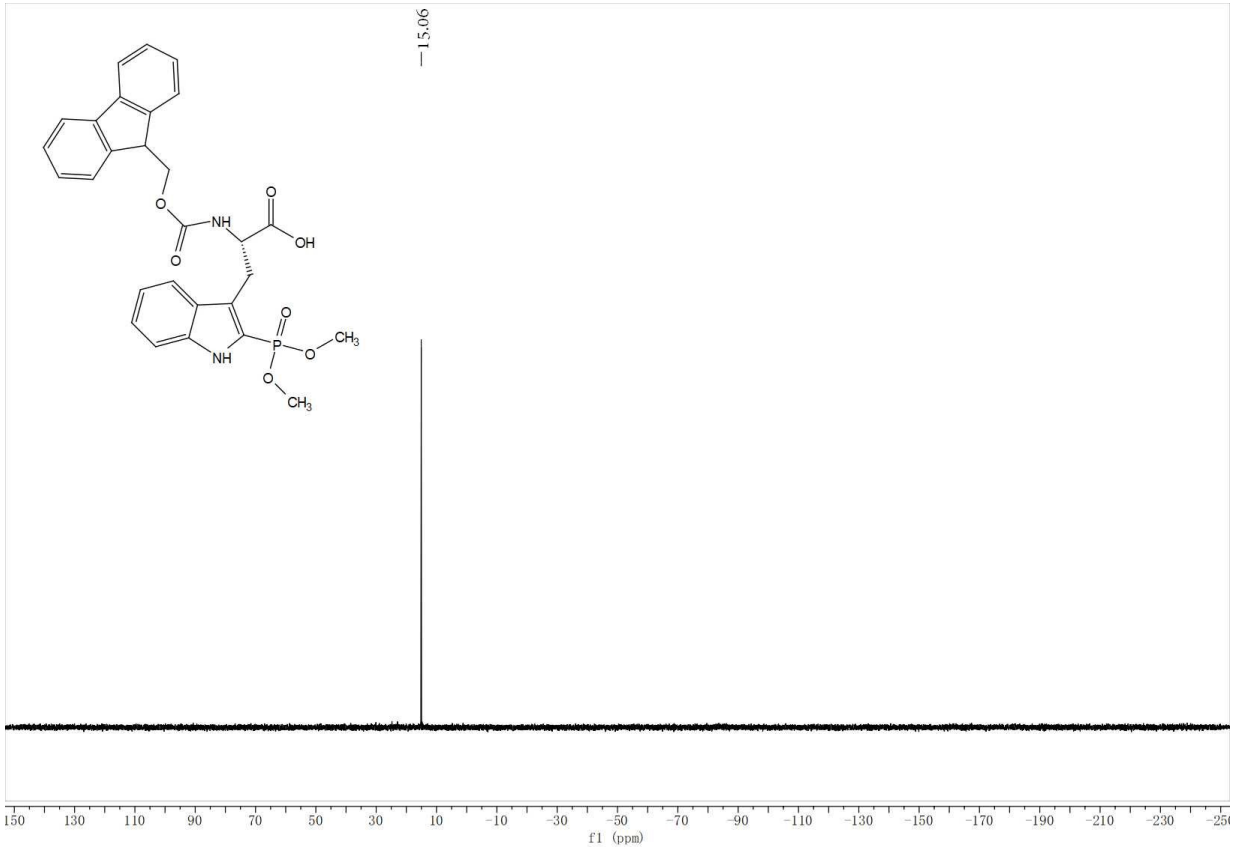

174.90
173.06
139.79
139.66
128.75
128.59
126.04
123.98
123.78
121.64
120.91
119.03
113.69
55.36
53.82
53.76
53.72
53.66
49.64
49.43
49.21
48.79
48.57
48.36
27.92
22.40
H3C
O
HN
OH
CH3
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -2
f1 (ppm)


18.91
H3C
O
HN
OH
CH3
NH
50 130 110 90 70 50 30 10 -10 -30 -50 -70 -90 -110 -130 -150 -170 -190 -210 -230 -25
f1 (ppm)

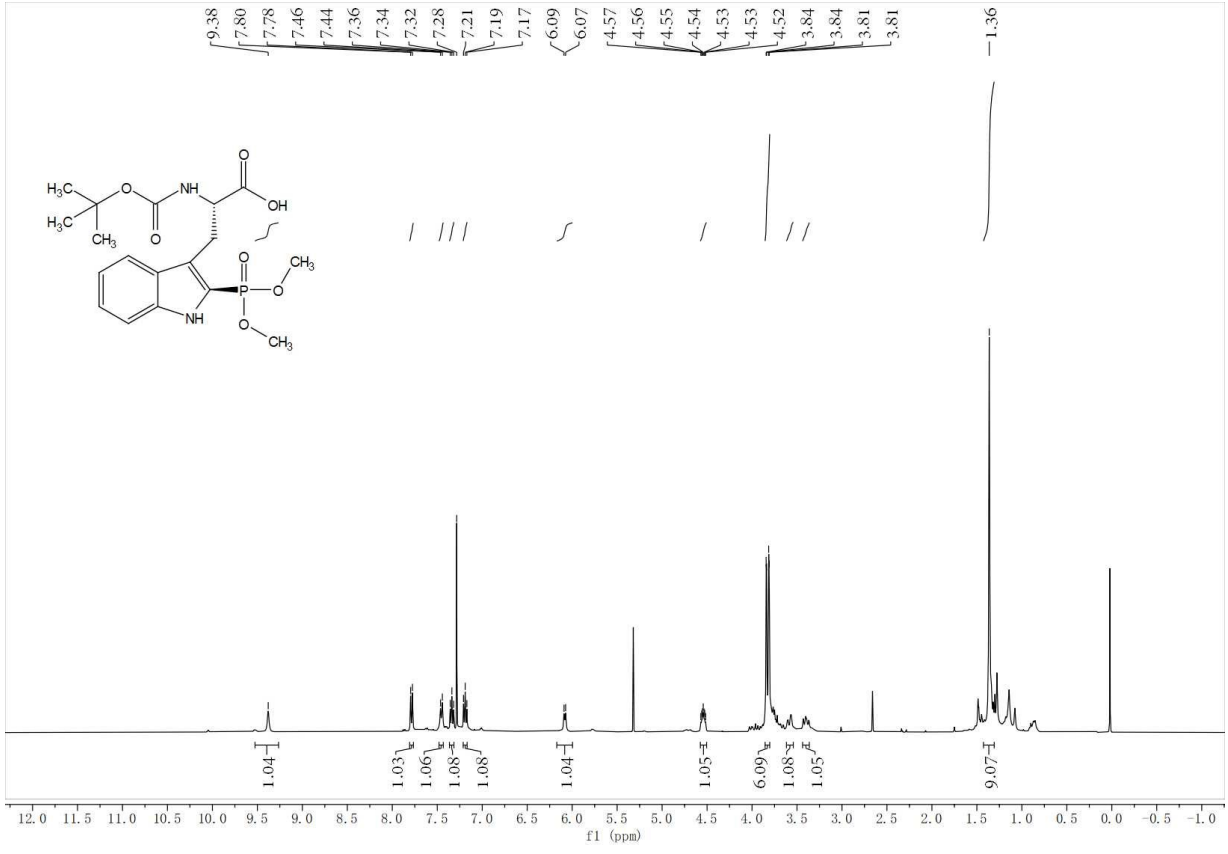
Fmoc-Trp-Methyl phosphate (**7**)

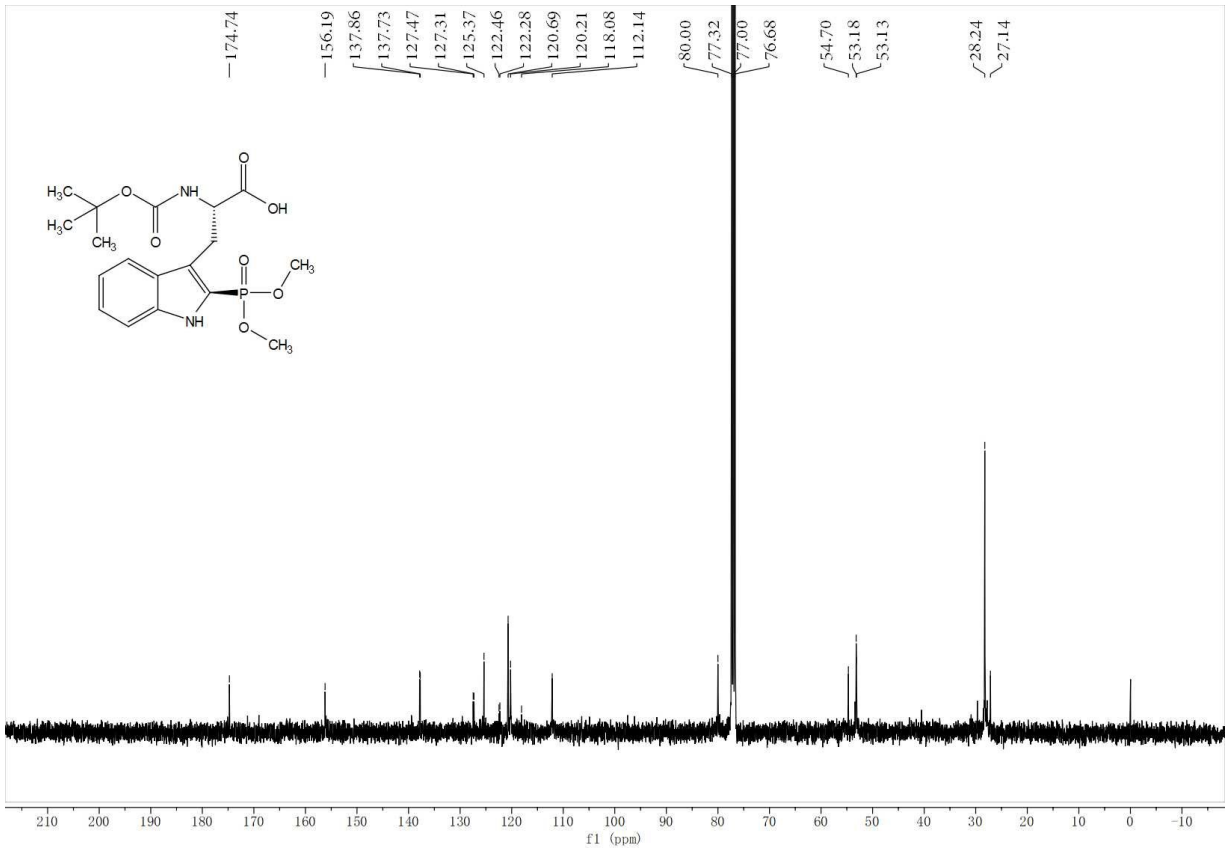


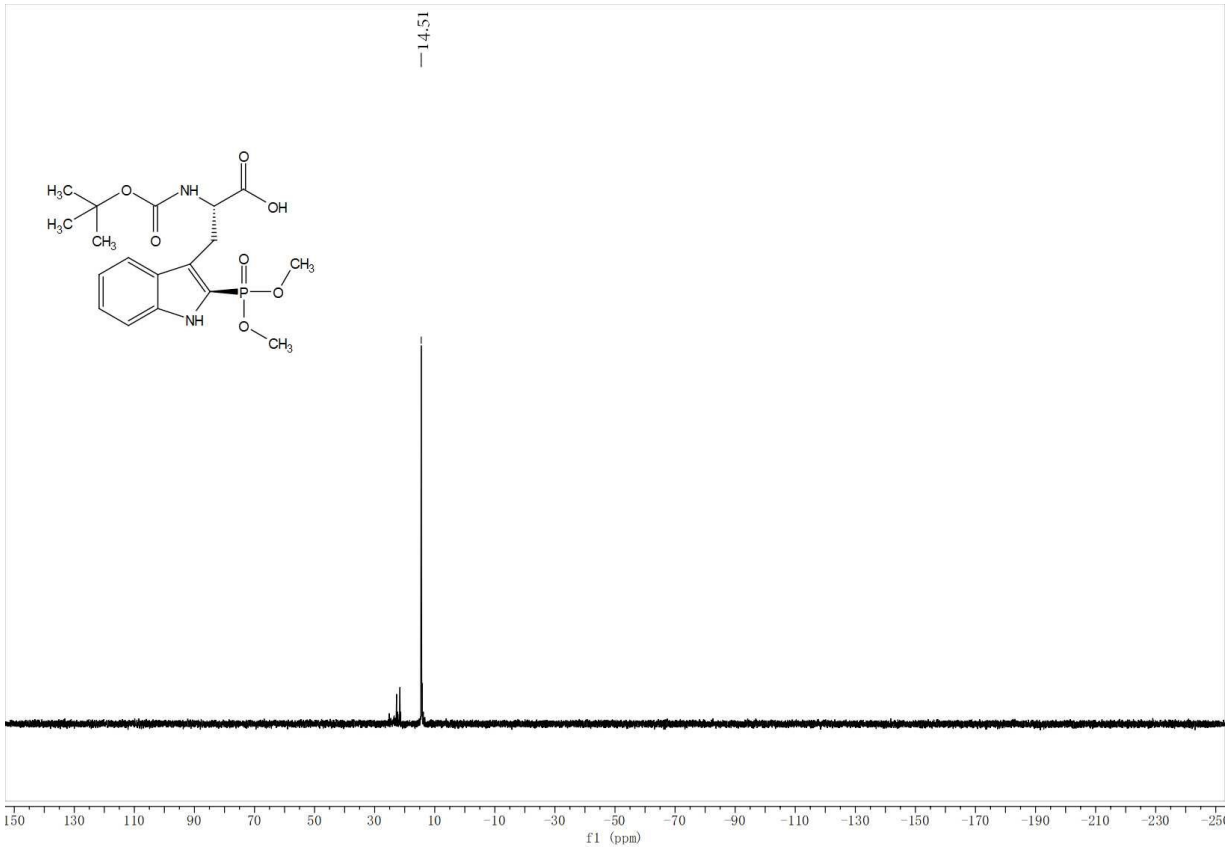




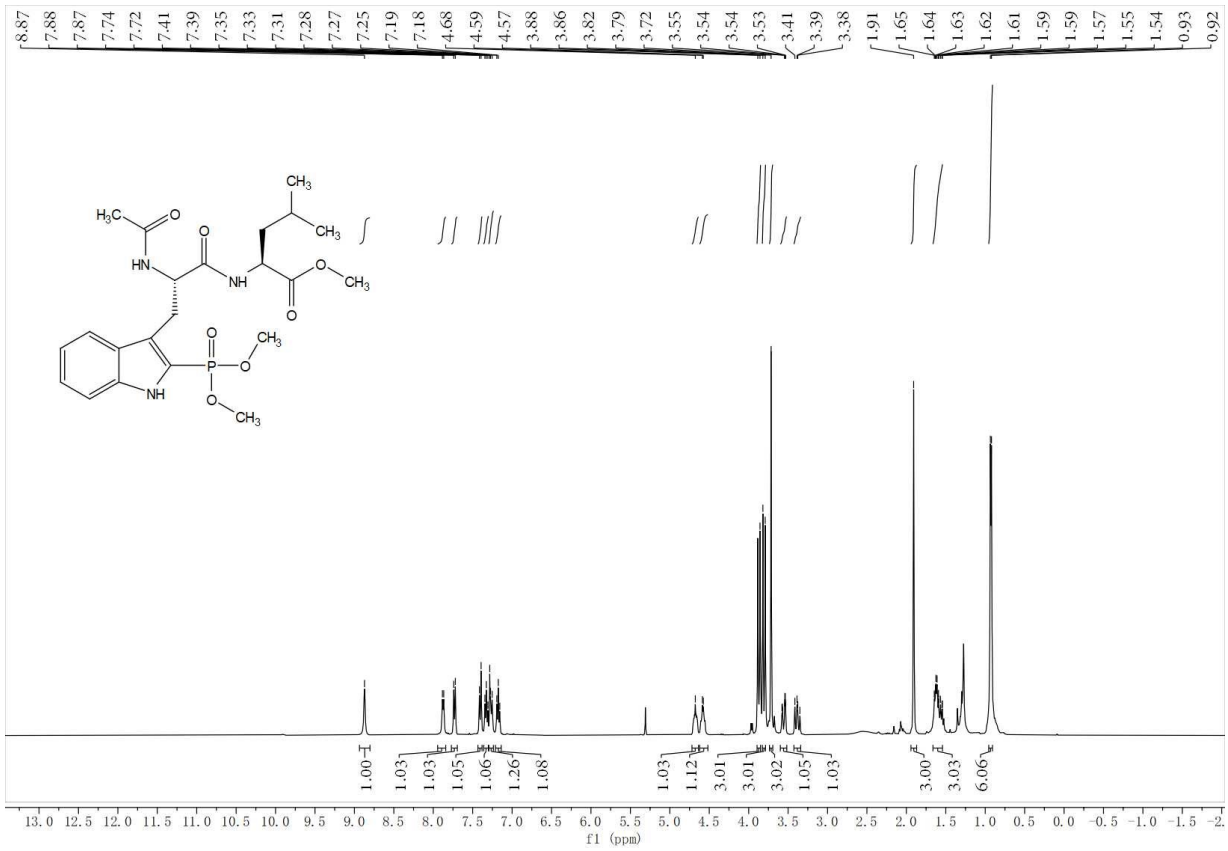
Boc-Trp-Methyl phosphate (**8**)

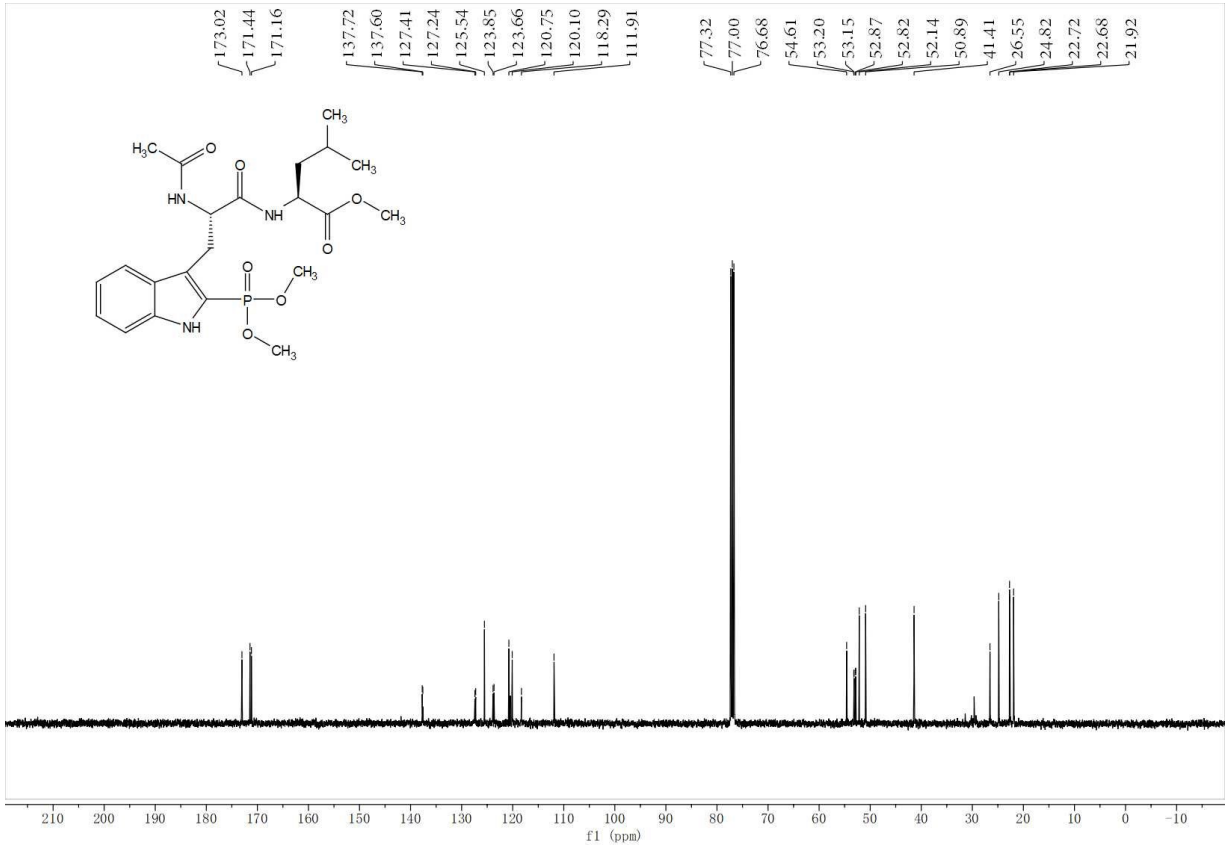


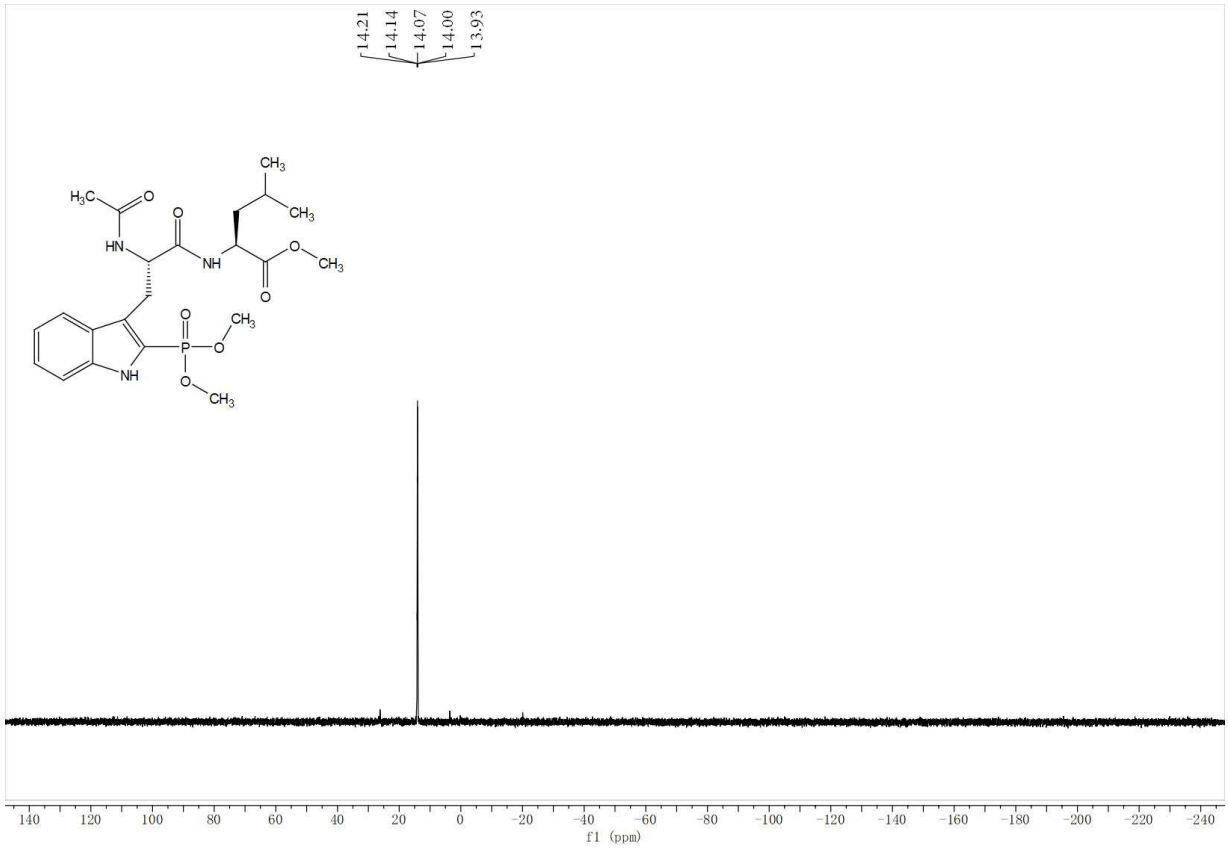

174.74
156.19
137.86
137.73
127.47
127.31
125.37
122.46
122.28
120.69
120.21
118.08
112.14
80.00
77.32
77.00
76.68
54.70
53.18
53.13
28.24
27.14
f1 (ppm)


14.51
f1 (ppm)

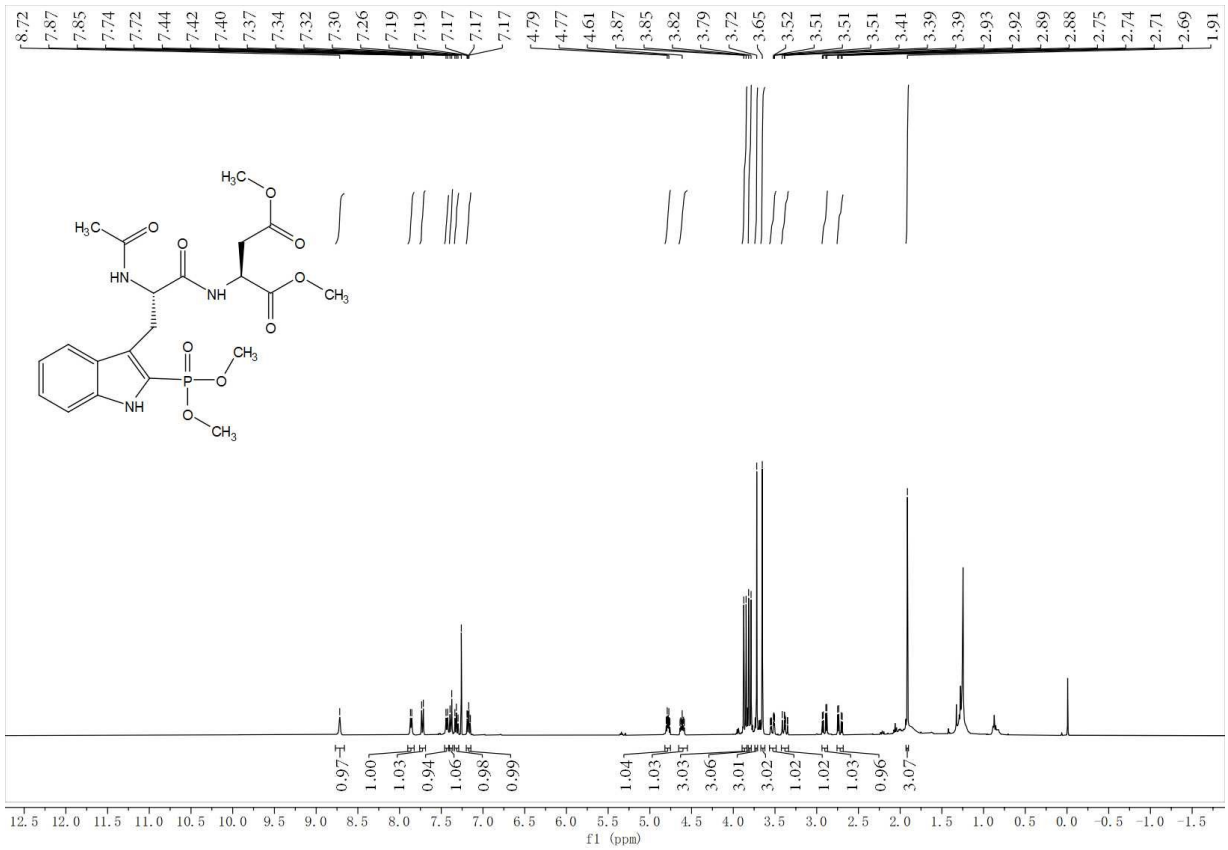
Ac-Trp-Leu-OMe-Methyl phosphate (**9**)

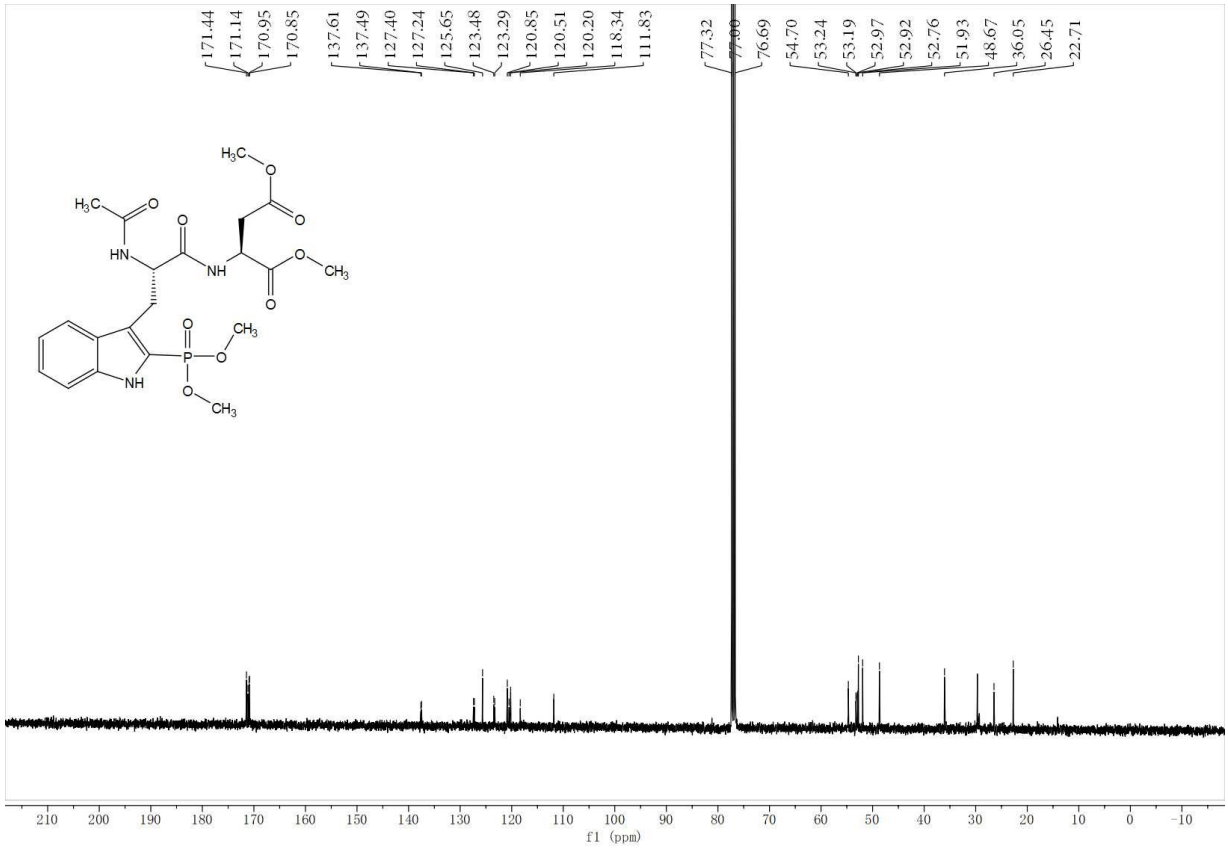


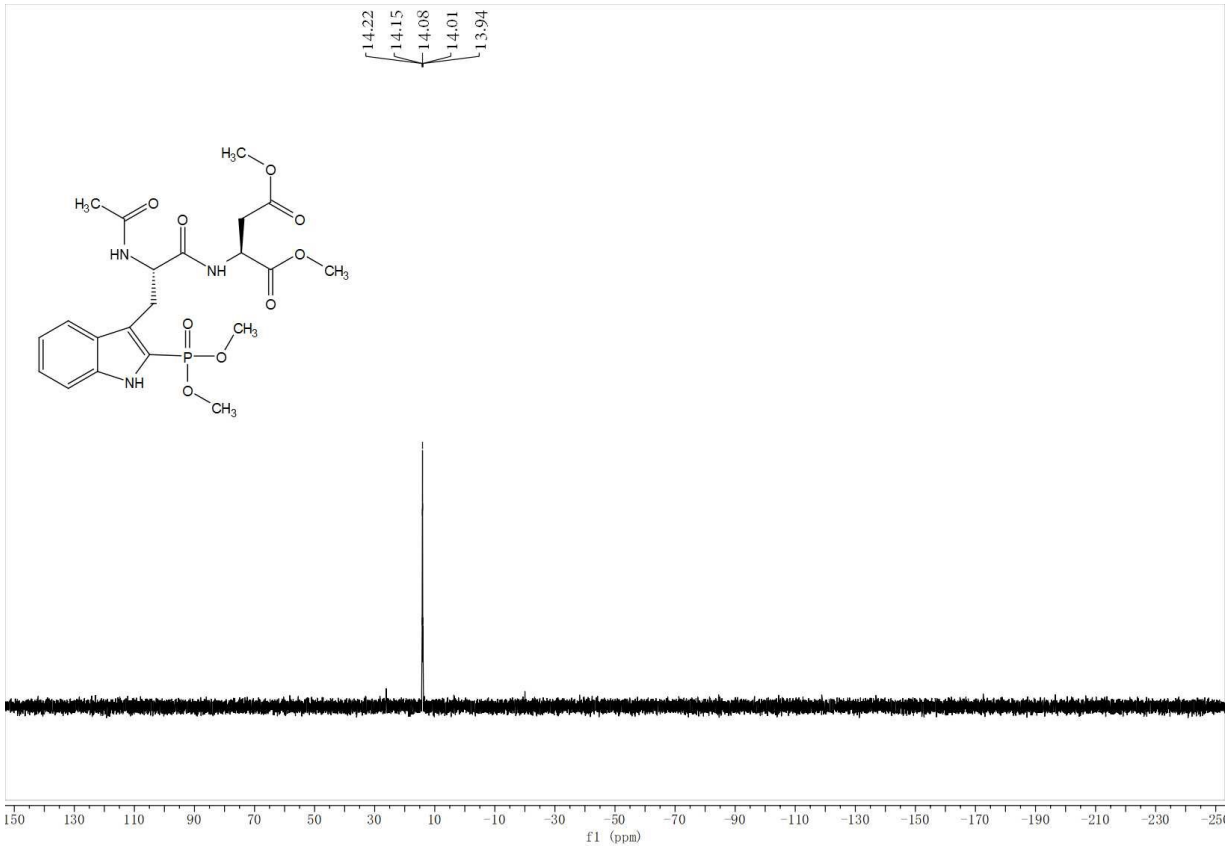




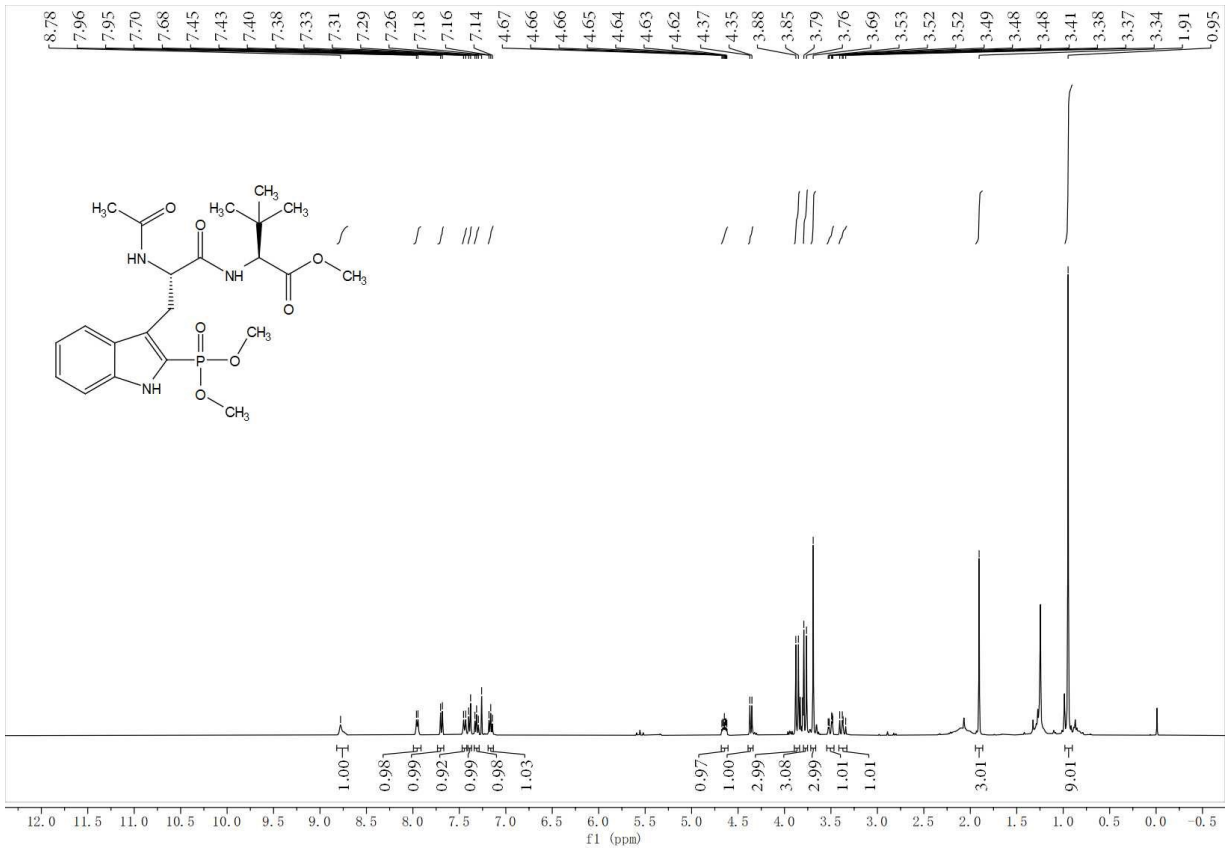
Ac-Trp-L-Asp acid dimethyl-Methyl phosphate (**10**)

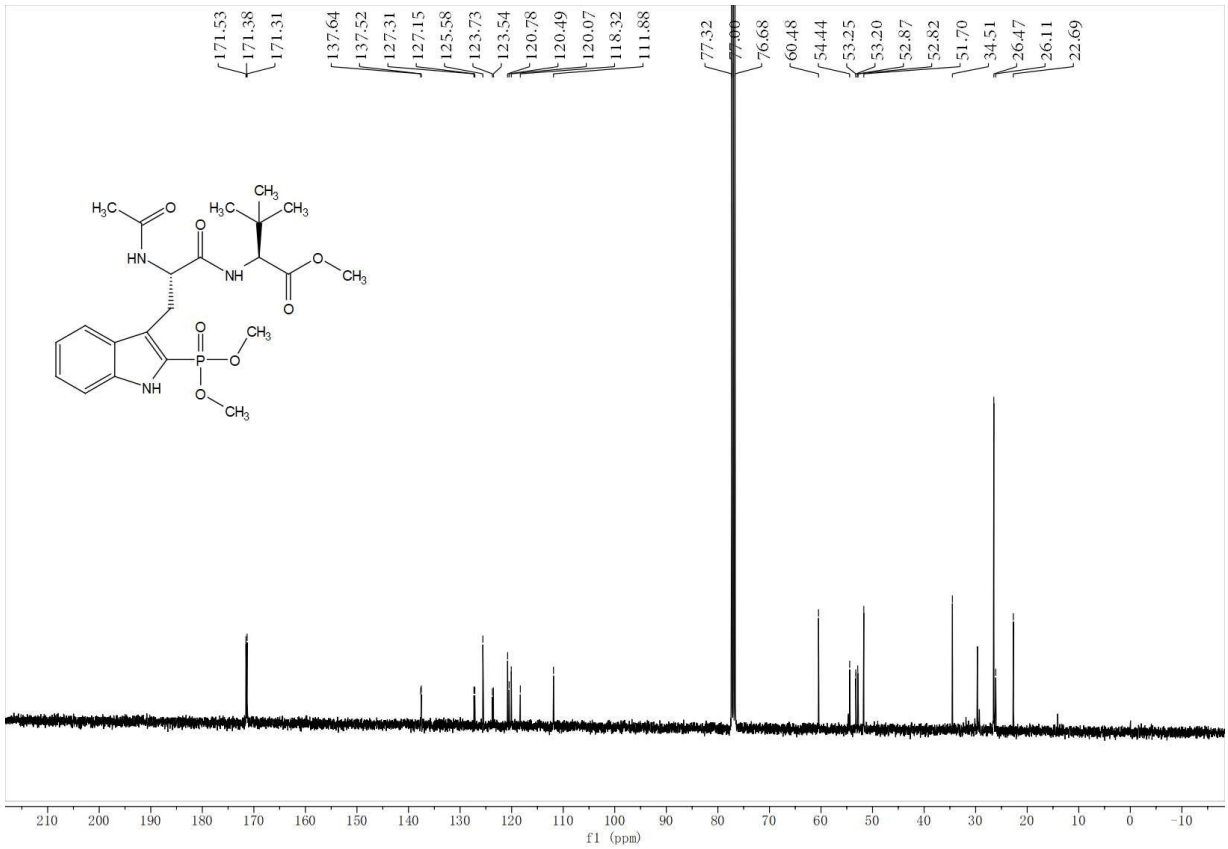


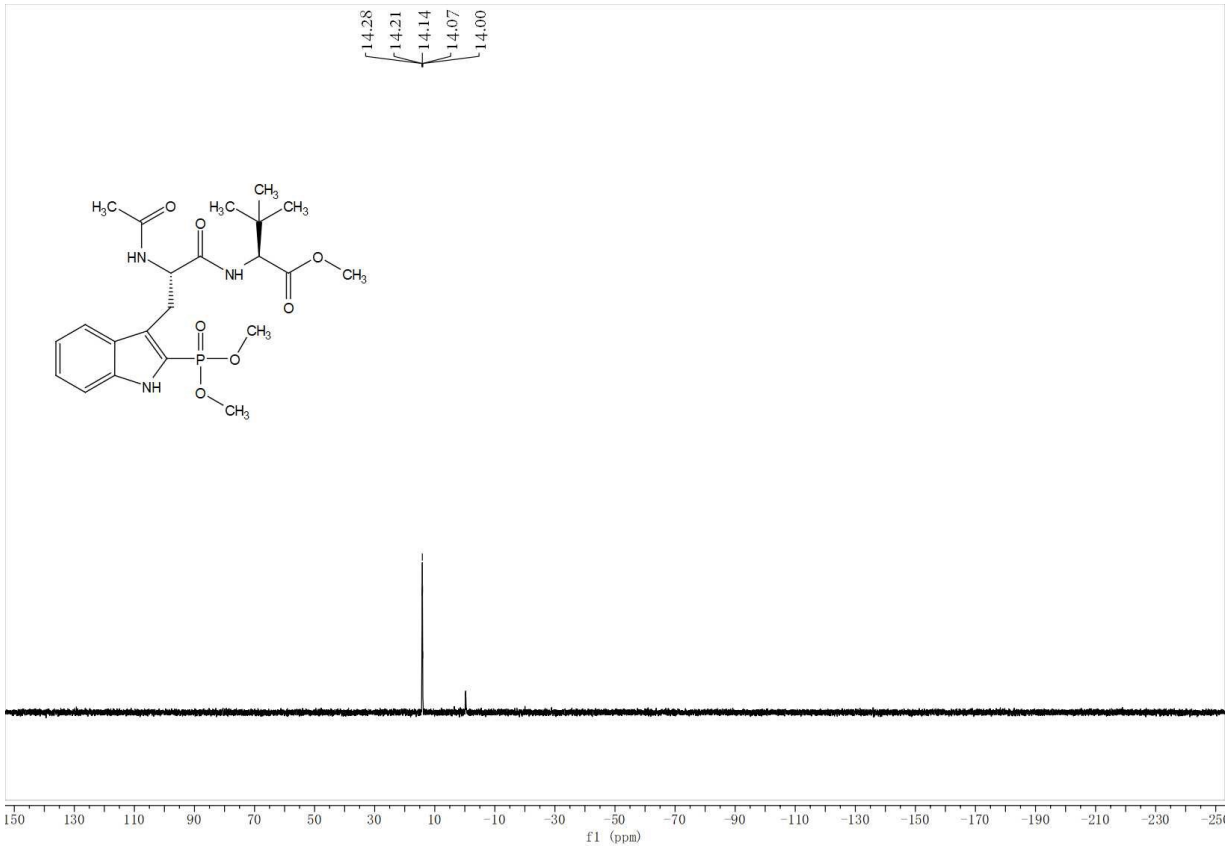

171.44
171.14
170.95
170.85
137.61
137.49
127.40
127.24
125.65
123.48
123.29
120.85
120.51
120.20
118.34
111.83
77.32
77.00
76.69
54.70
53.24
53.19
52.97
52.92
52.76
51.93
48.67
36.05
26.45
22.71
f1 (ppm)


14.22
14.15
14.08
14.01
13.94
f1 (ppm)

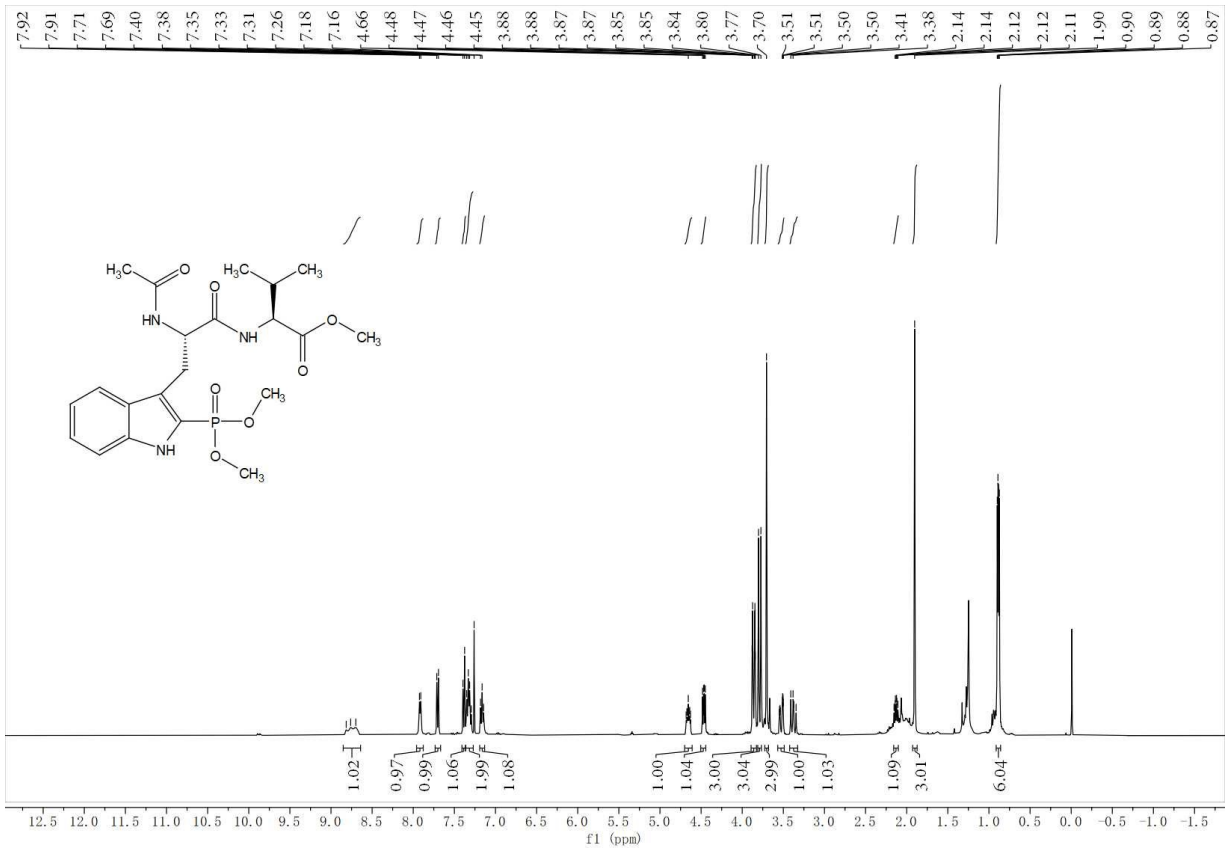
Ac-Trp-Tle-OMe-Methyl phosphate (**11**)

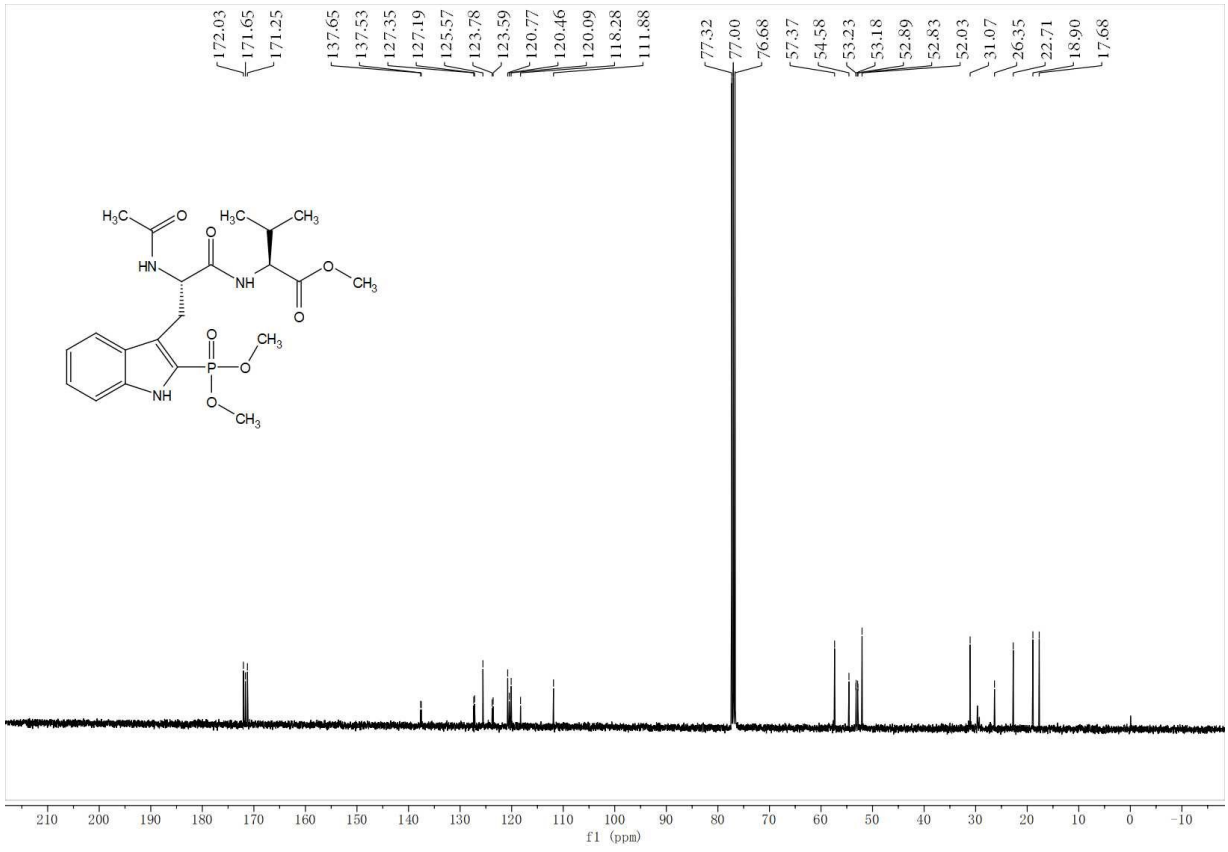


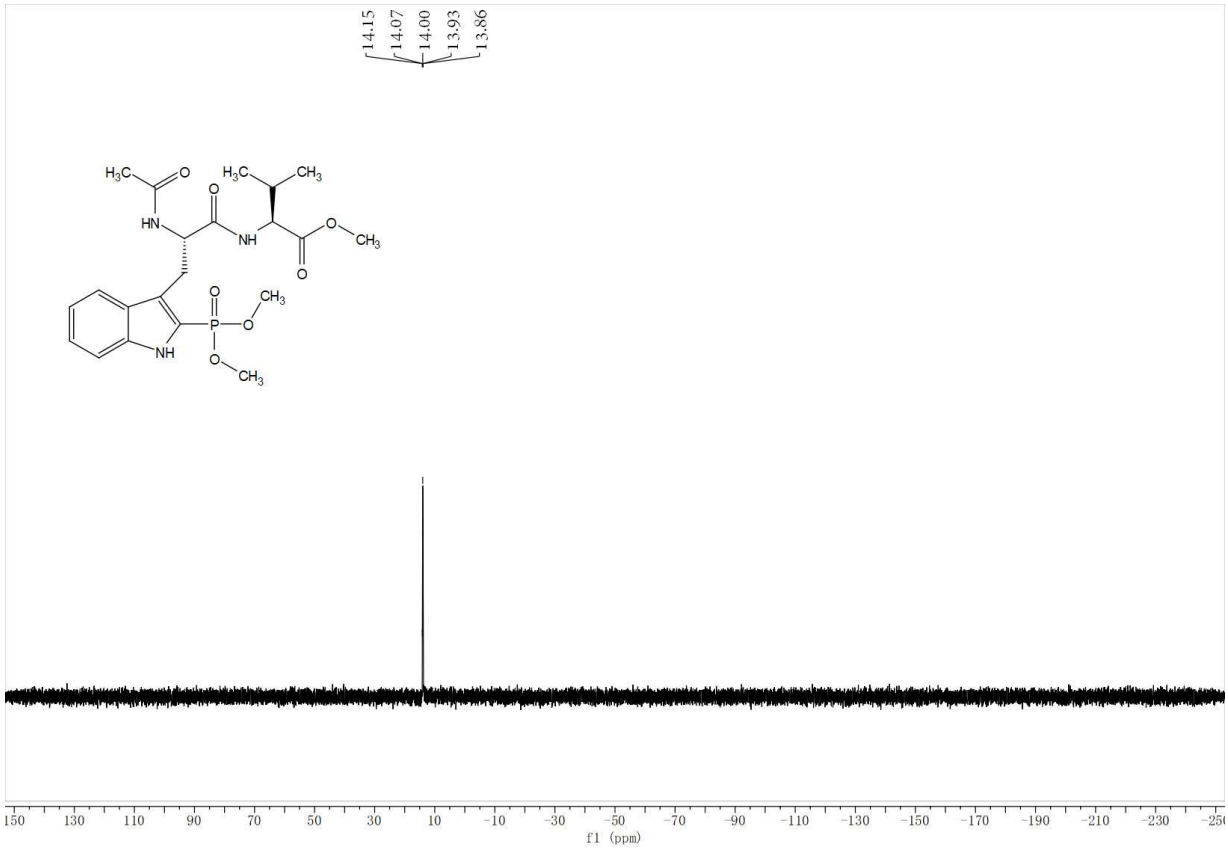




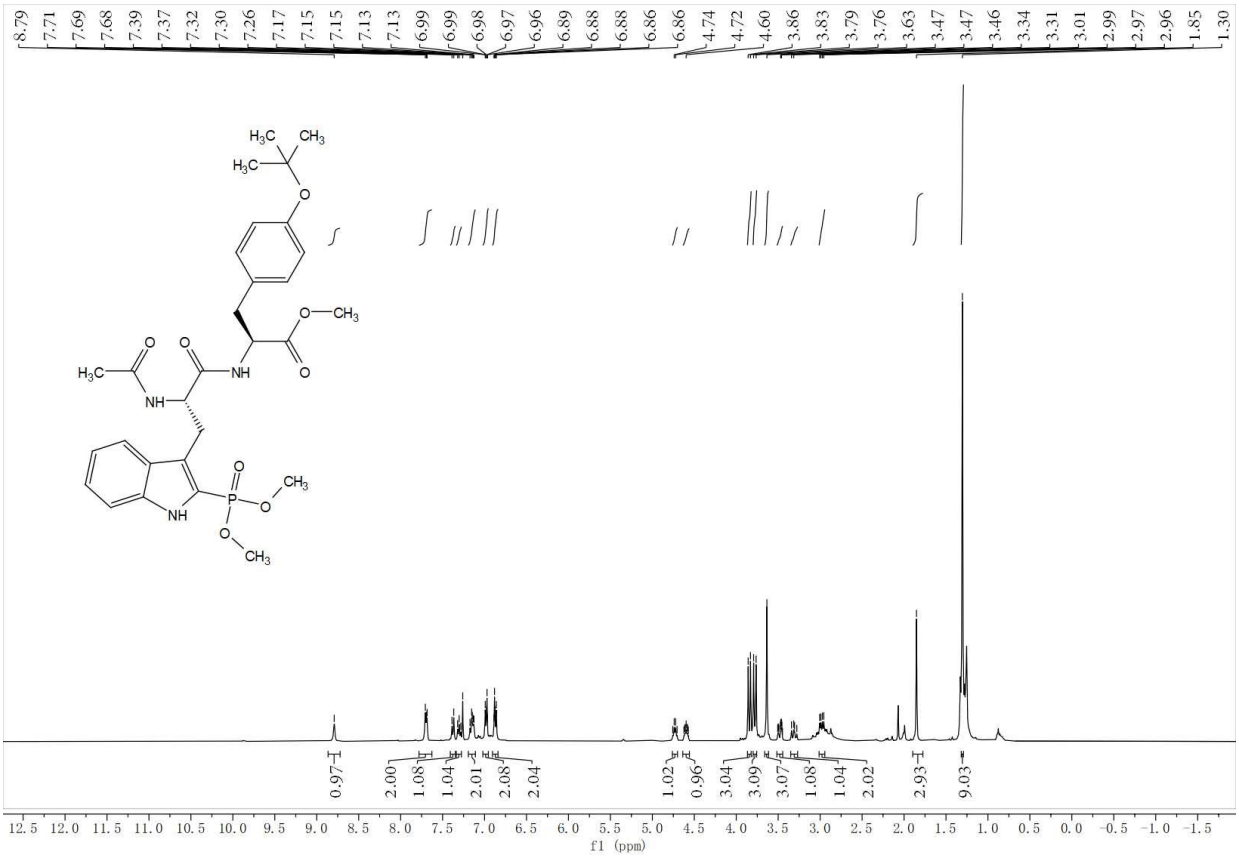
Ac-Trp-Val-OMe-Methyl phosphate (**12**)

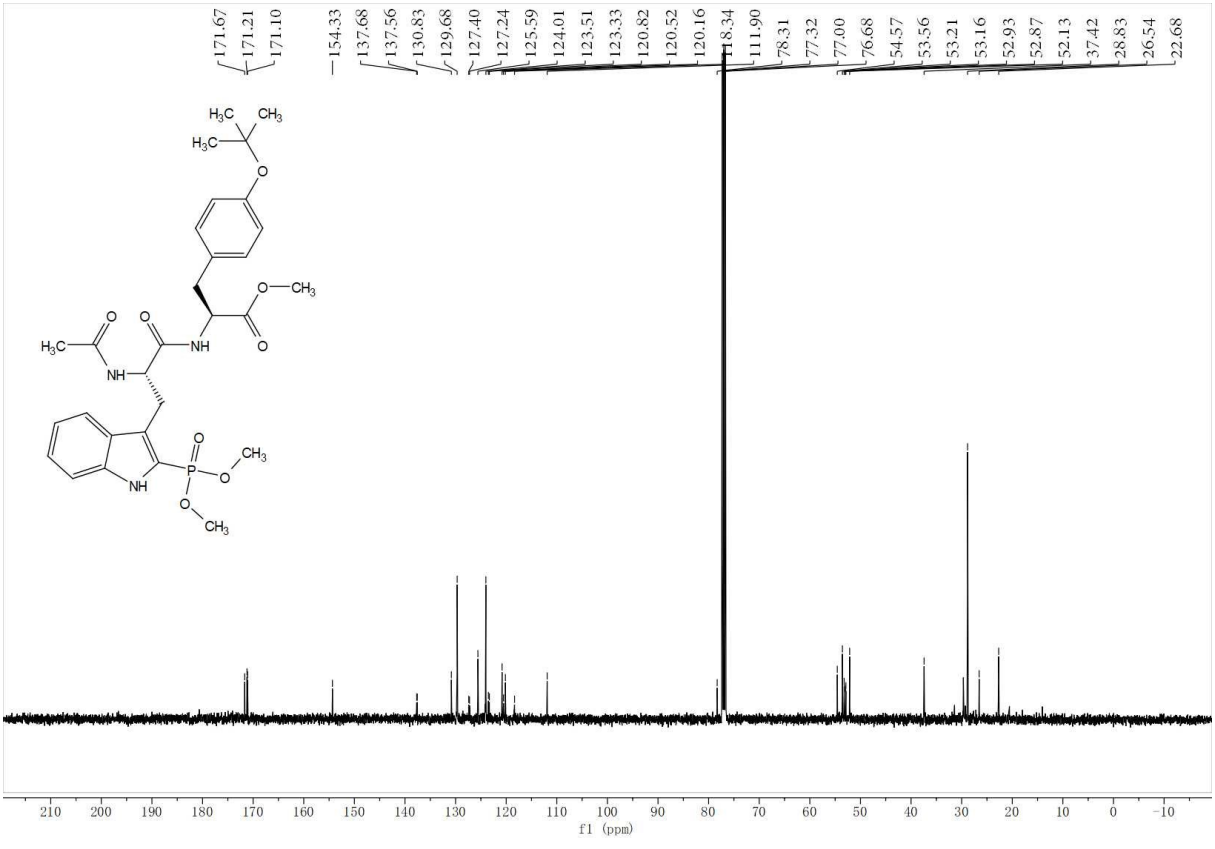


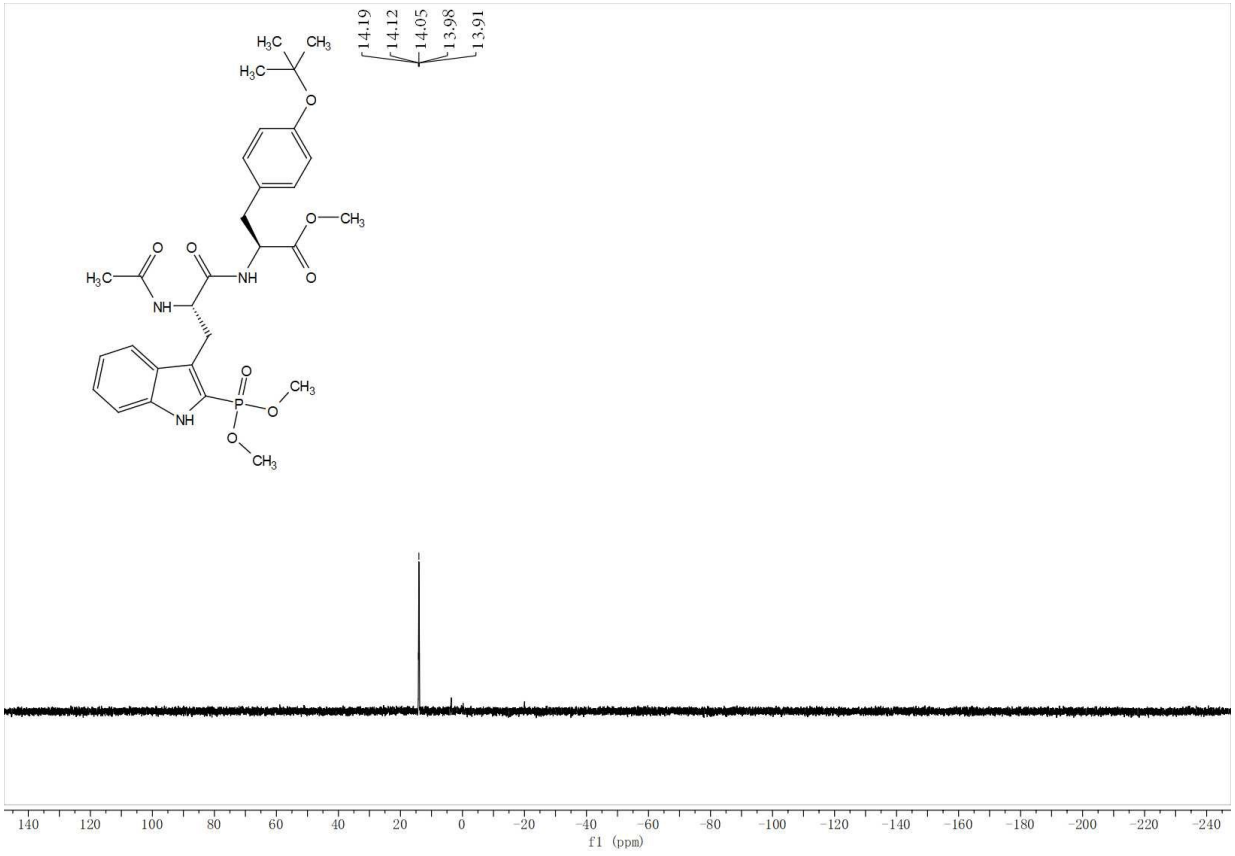

172.03
171.65
171.25
137.65
137.53
127.35
127.19
125.57
123.78
123.59
120.77
120.46
120.09
118.28
111.88
77.32
77.00
76.68
57.37
54.58
53.23
53.18
52.89
52.83
52.03
31.07
26.35
22.71
18.90
17.68
f1 (ppm)


14.15
14.07
14.00
13.93
13.86
f1 (ppm)

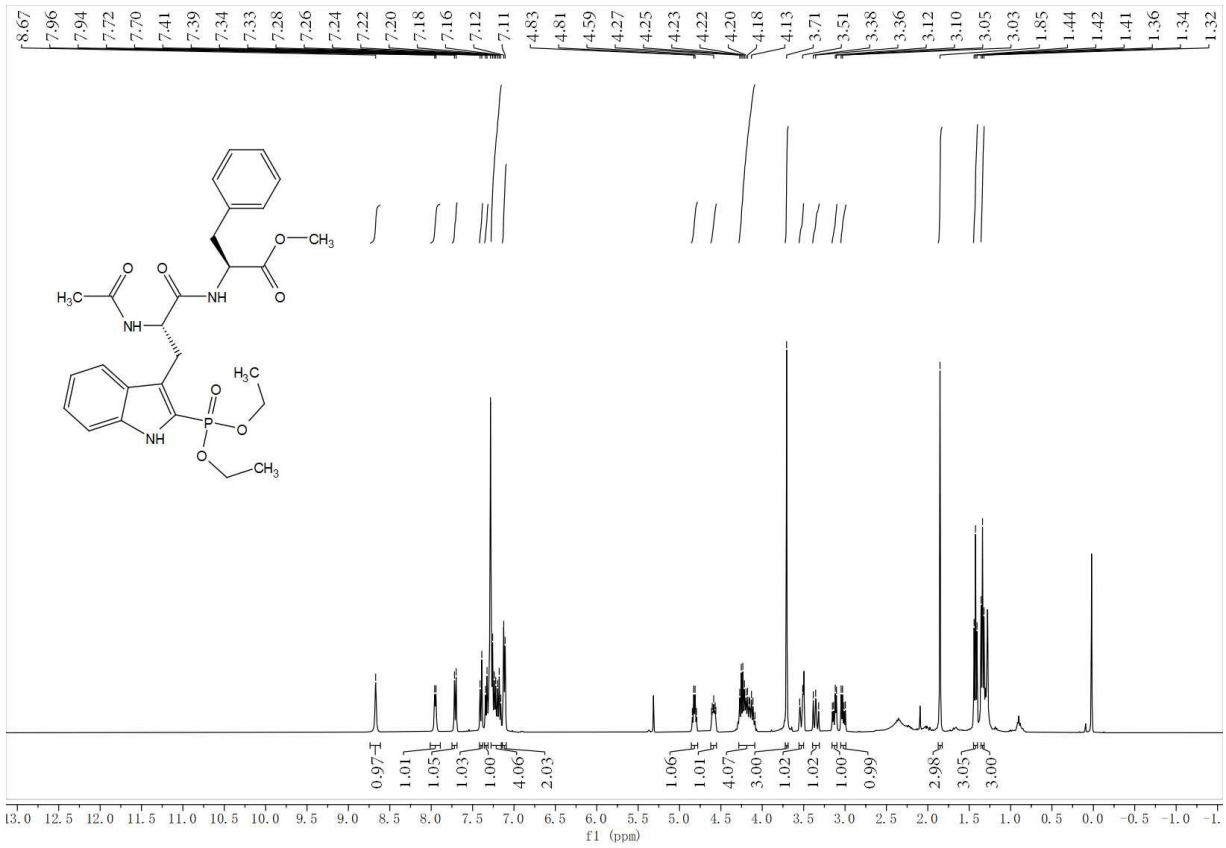
Ac-Trp-Tyr(tbu)-OMe-Methyl phosphate (**13**)

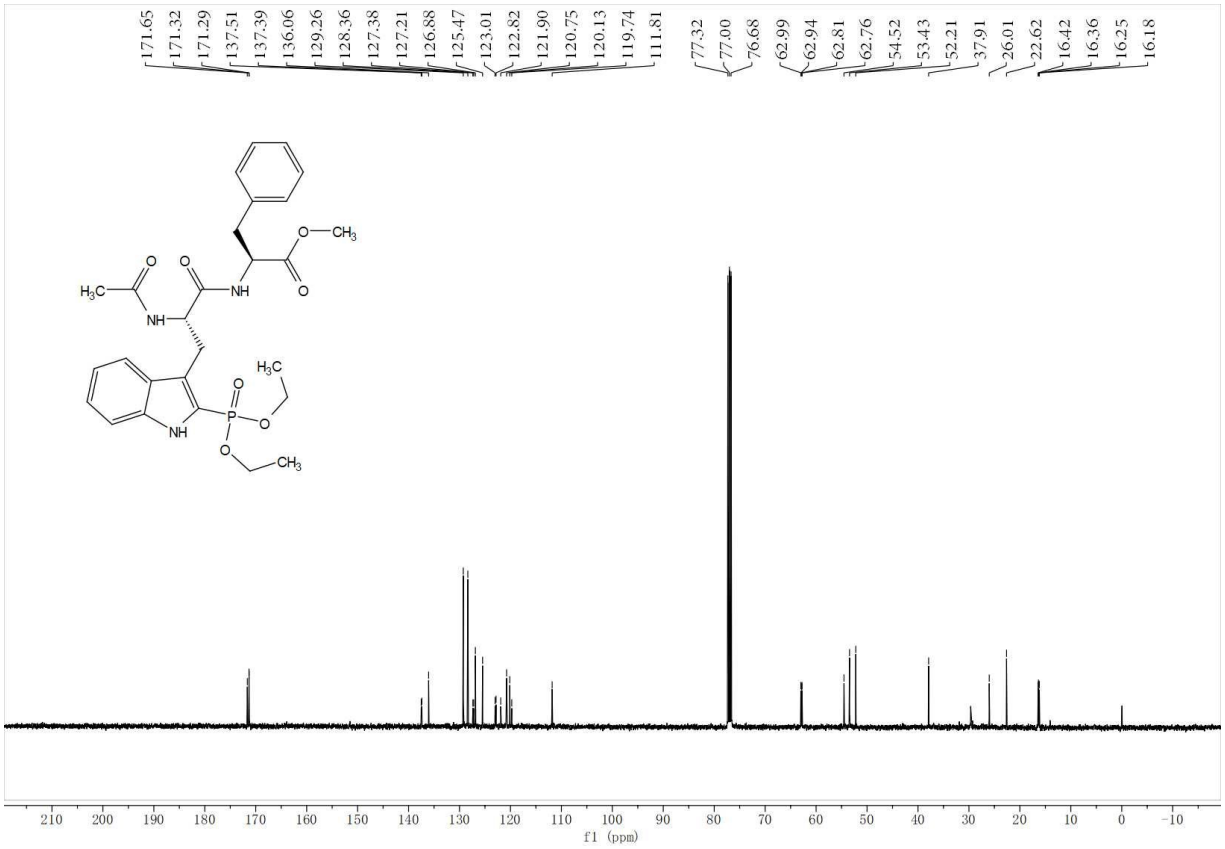


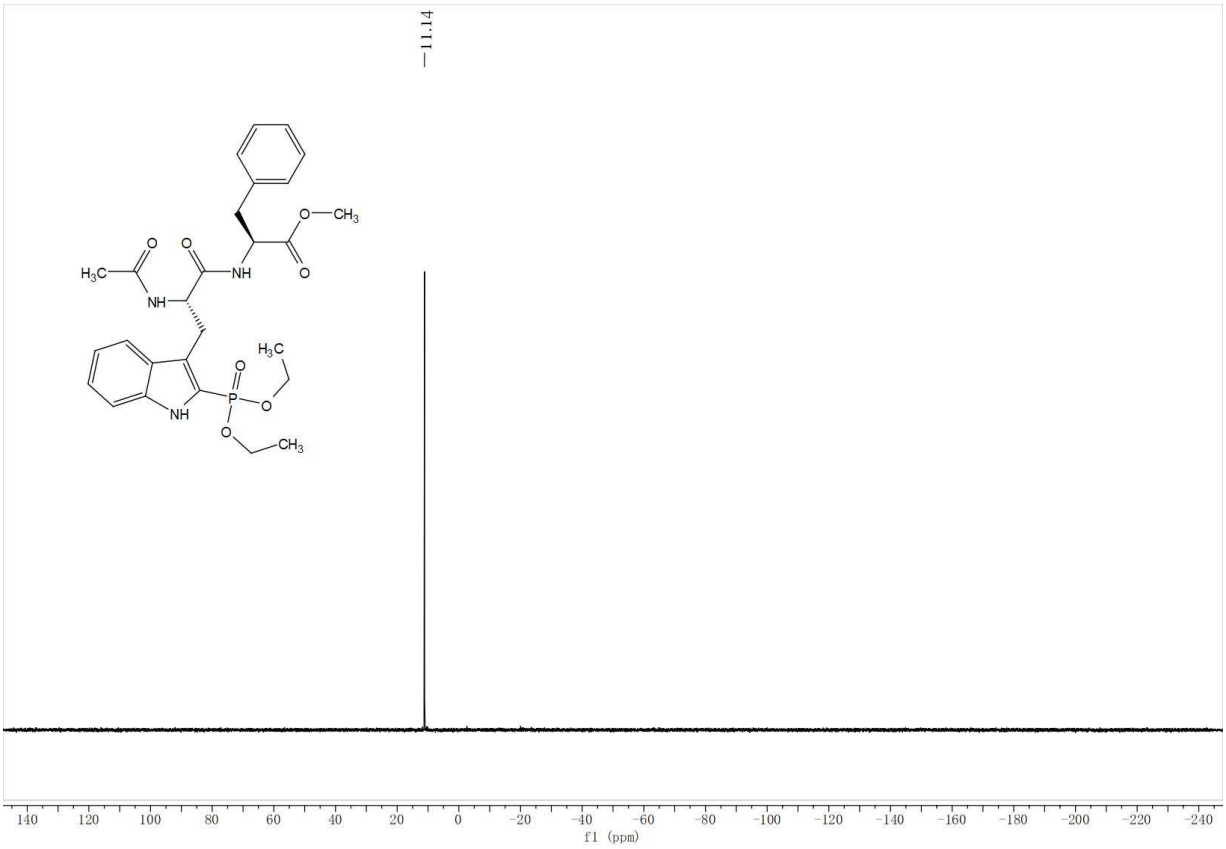




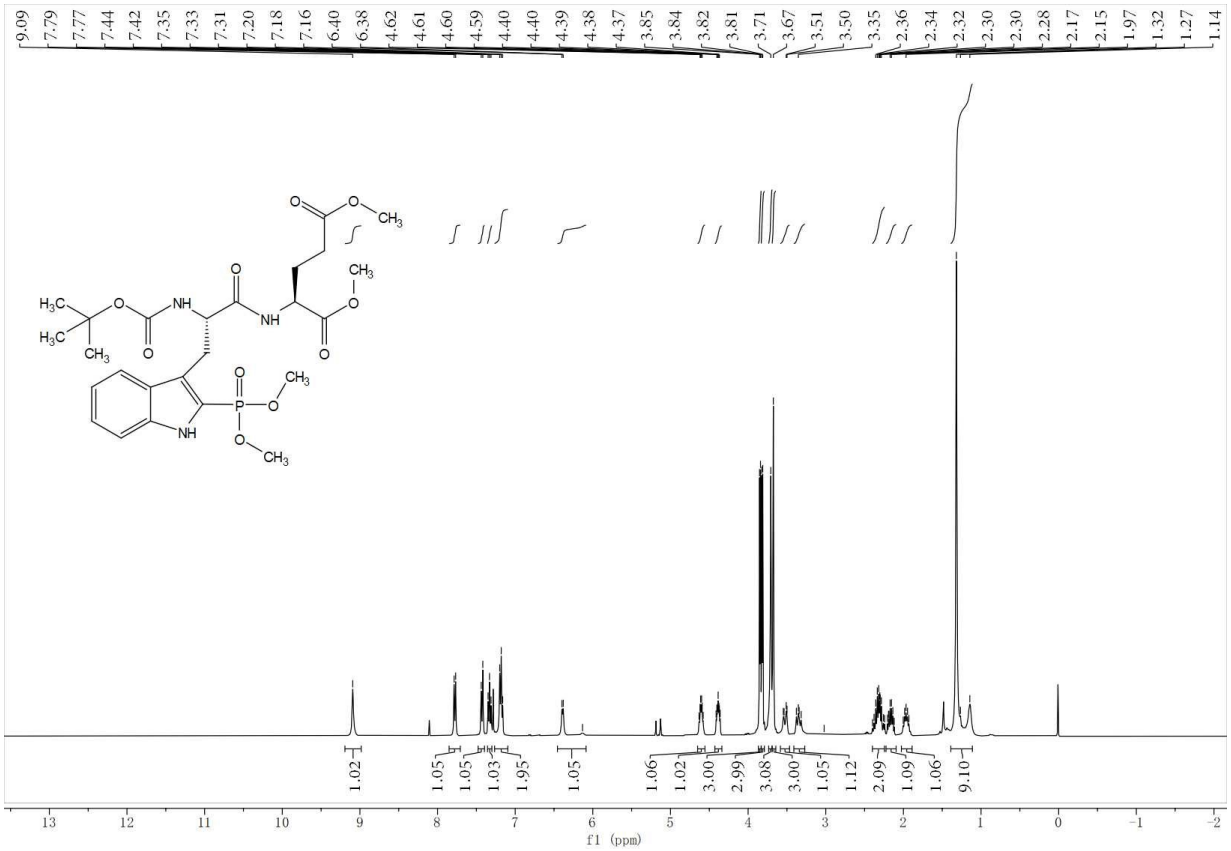
Ac-Trp-Phe-OMe-Ethyl phosphate (**14**)

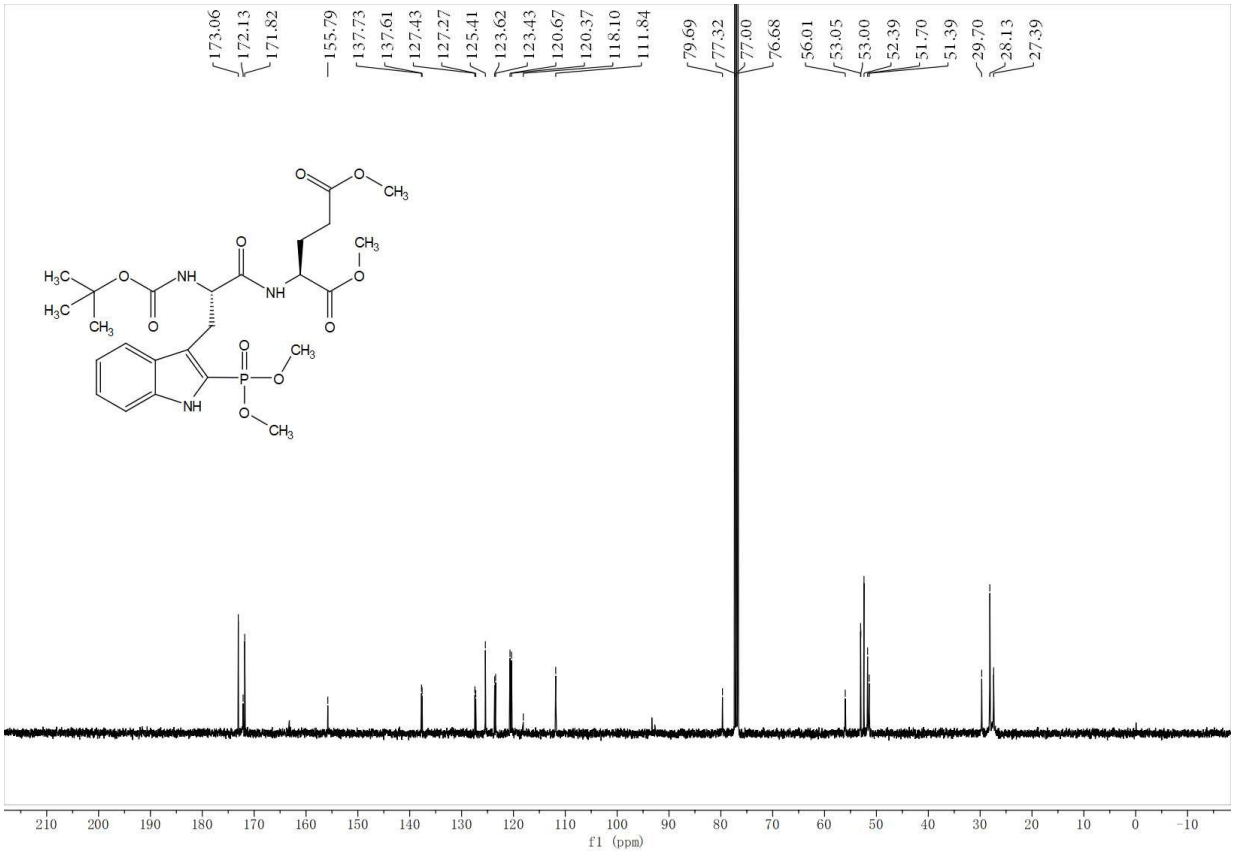


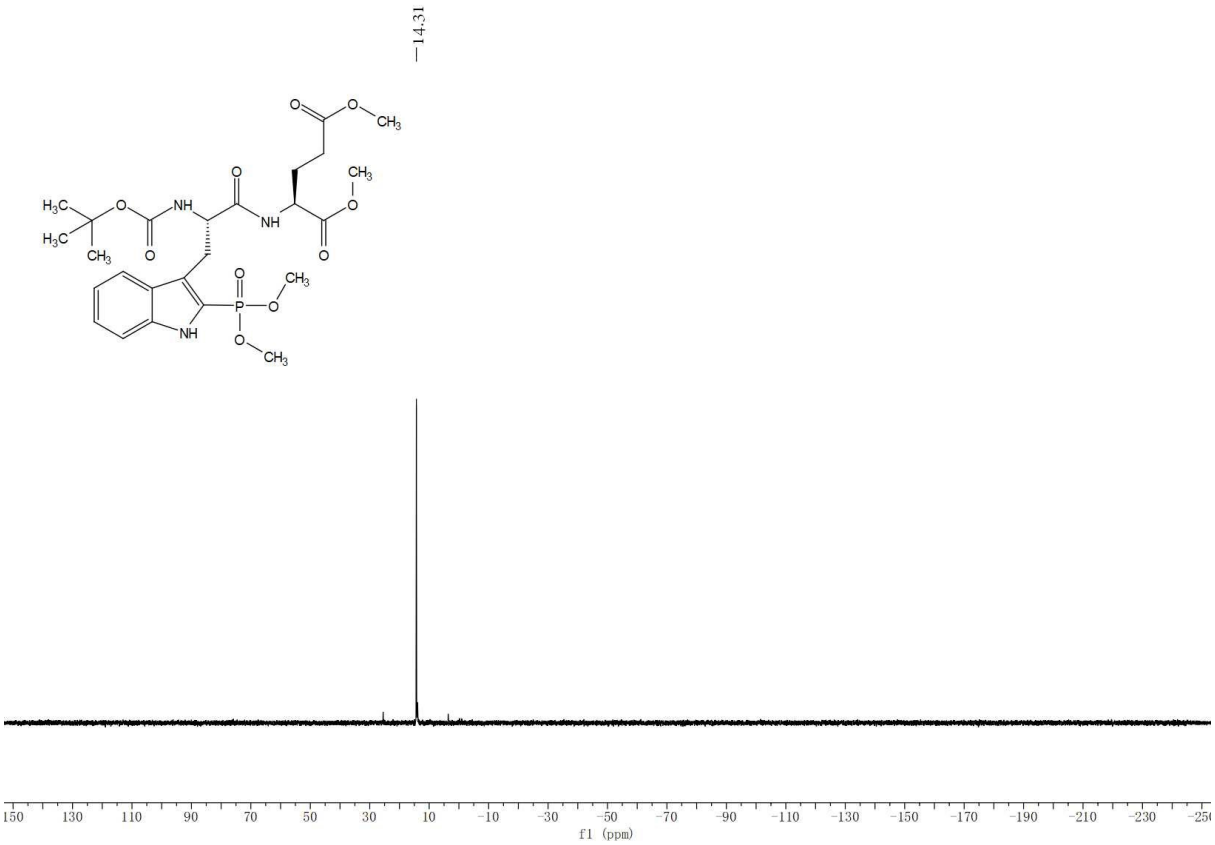

171.65
171.32
171.29
137.51
137.39
136.06
129.26
128.36
127.38
127.21
126.88
125.47
123.01
122.82
121.90
120.75
120.13
119.74
111.81
77.32
77.00
76.68
62.99
62.94
62.81
62.76
54.52
53.43
52.21
37.91
26.01
22.62
16.42
16.36
16.25
16.18
f1 (ppm)


11.14
f1 (ppm)

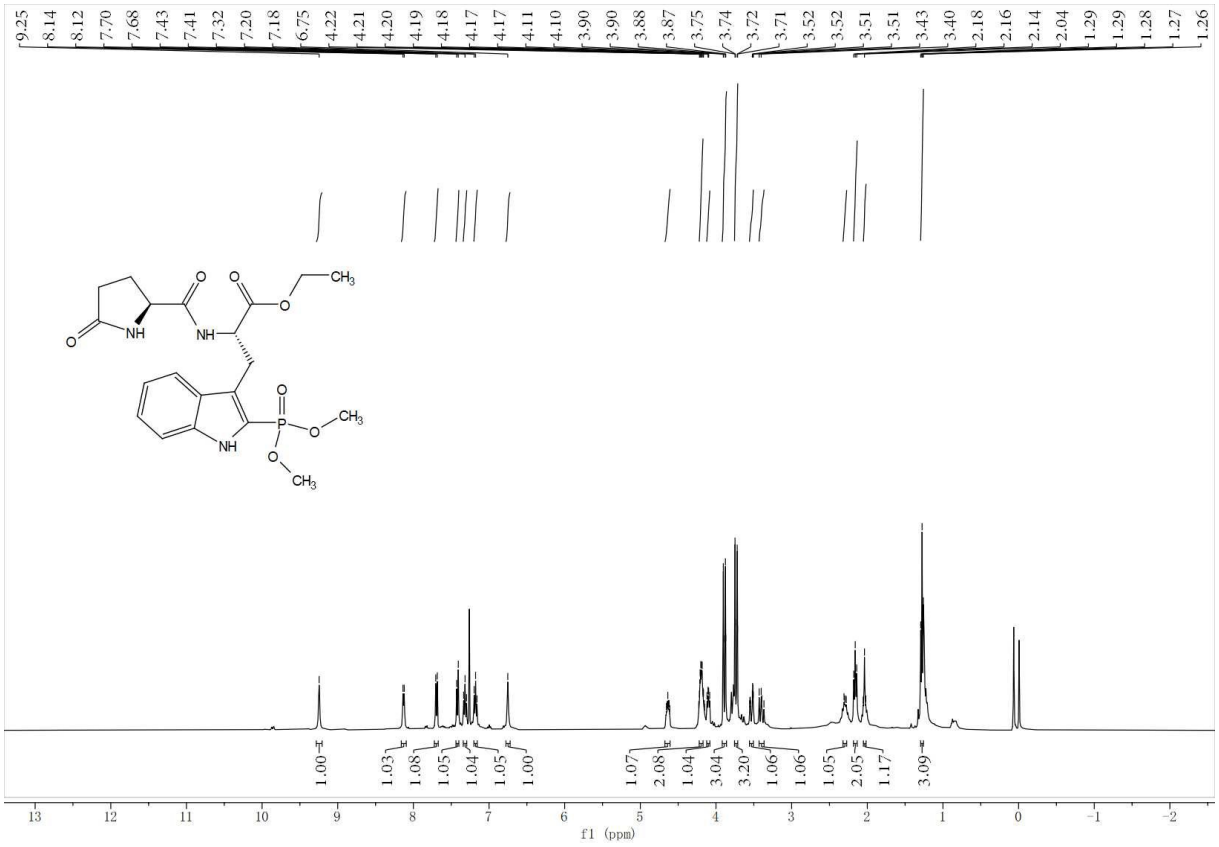
Boc-Trp-L-Glu acid dimethyl ester-Methyl phosphate (**15**)

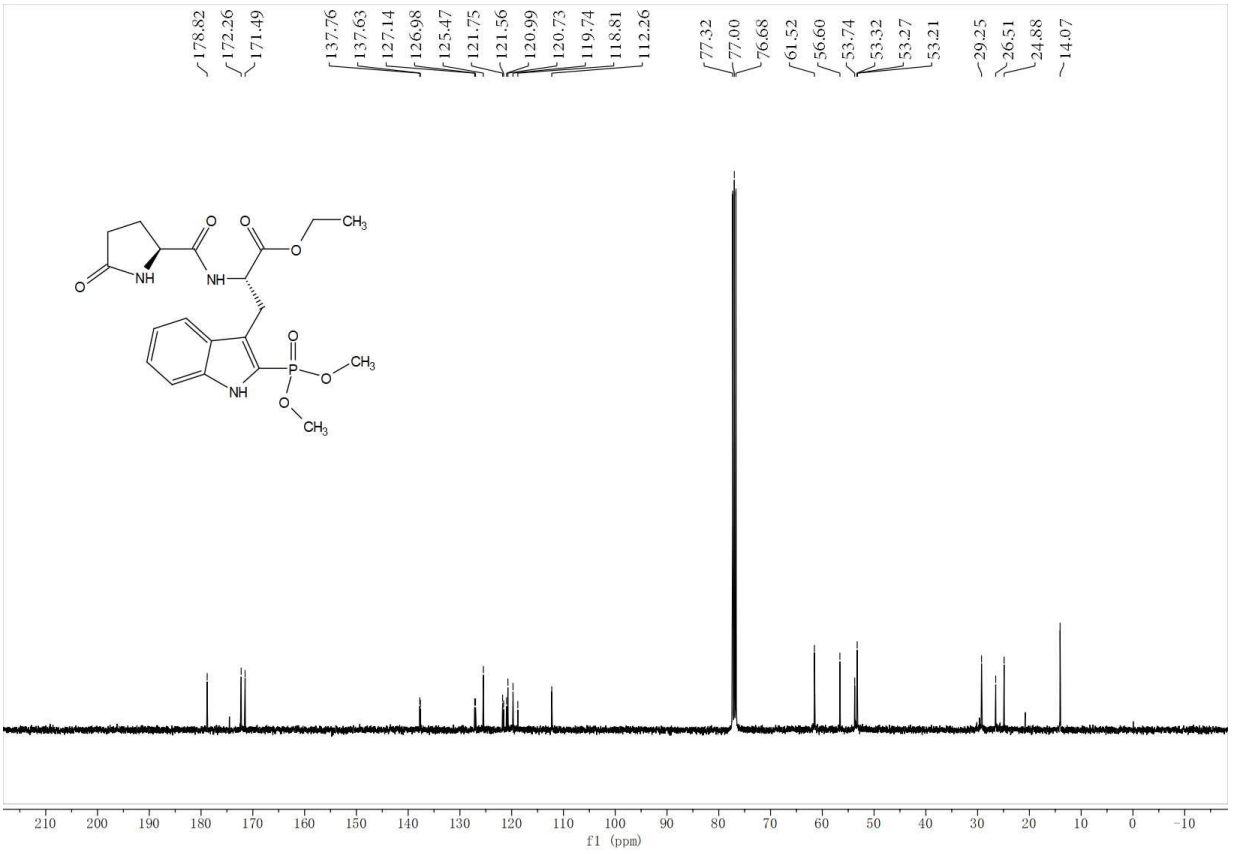


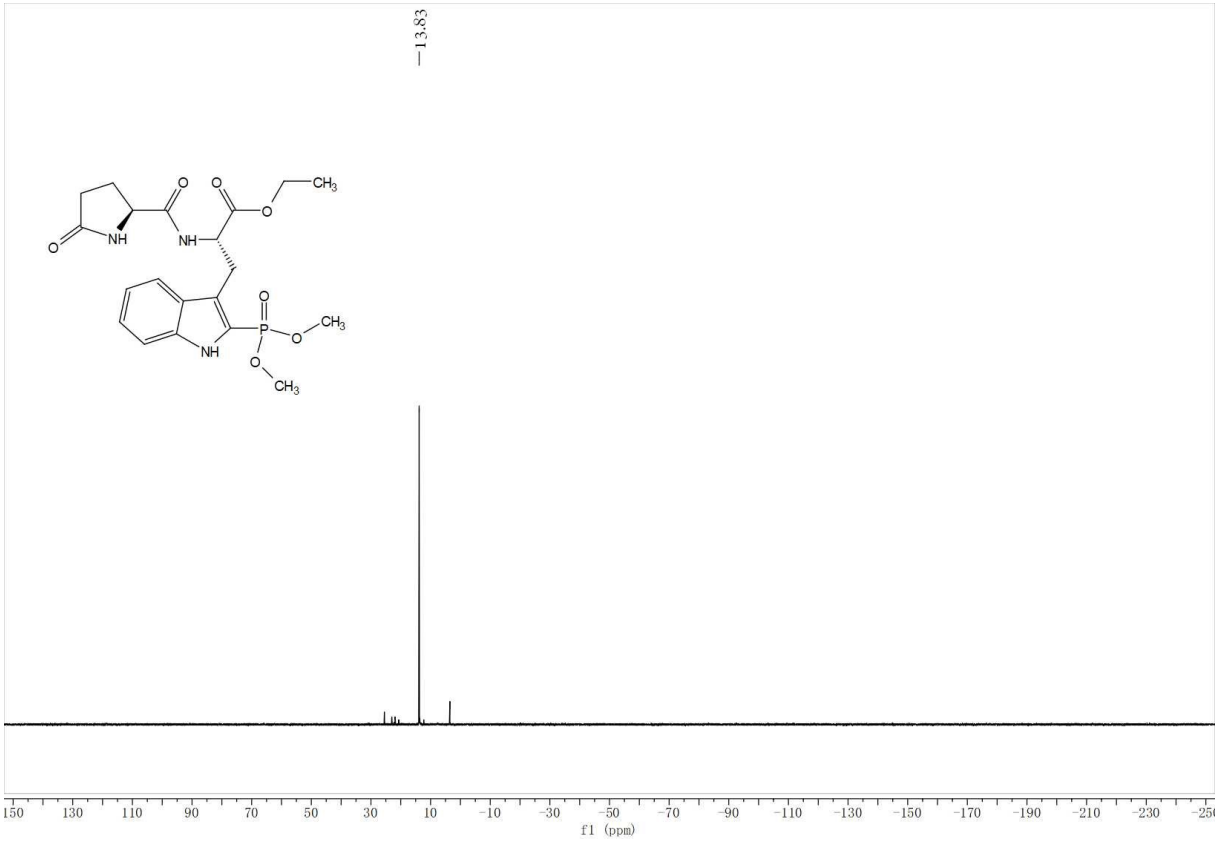




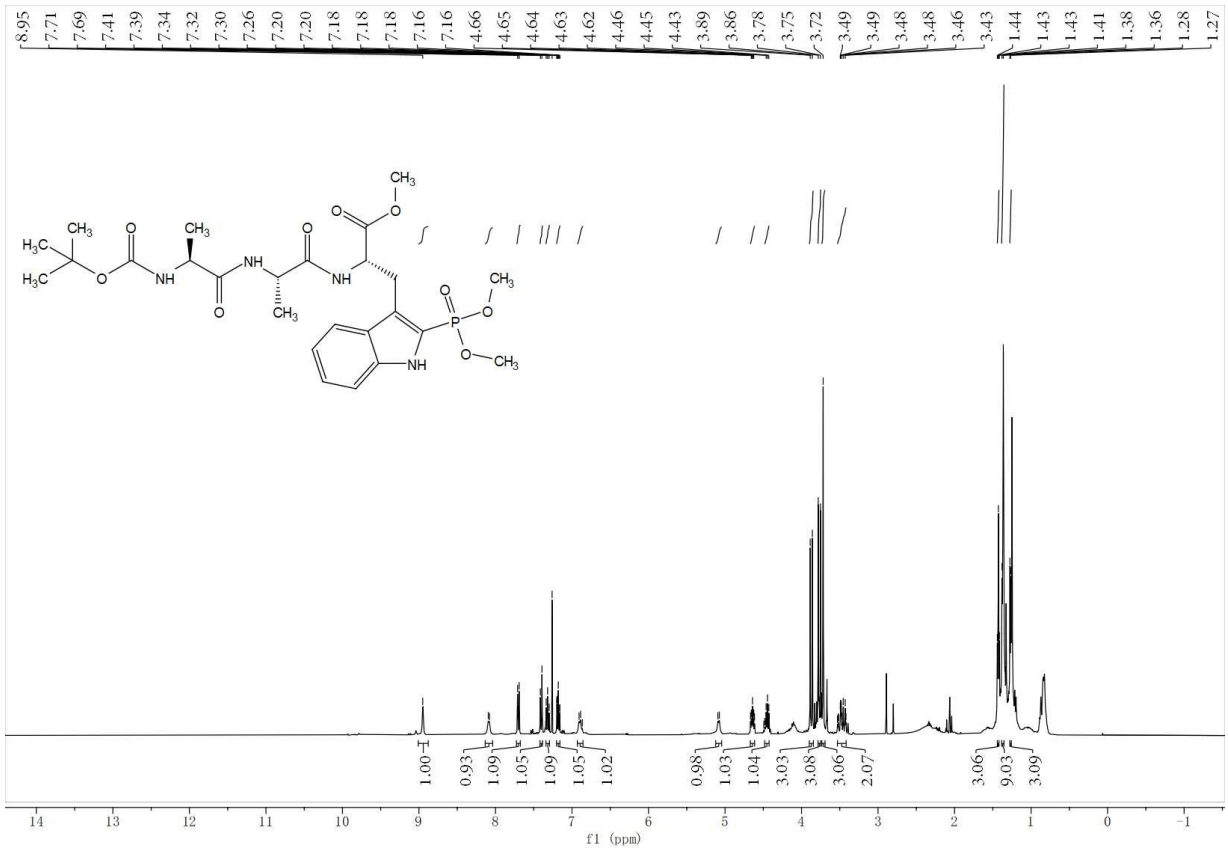
Pyr-Trp-OEt-Methyl phosphate (**16**)

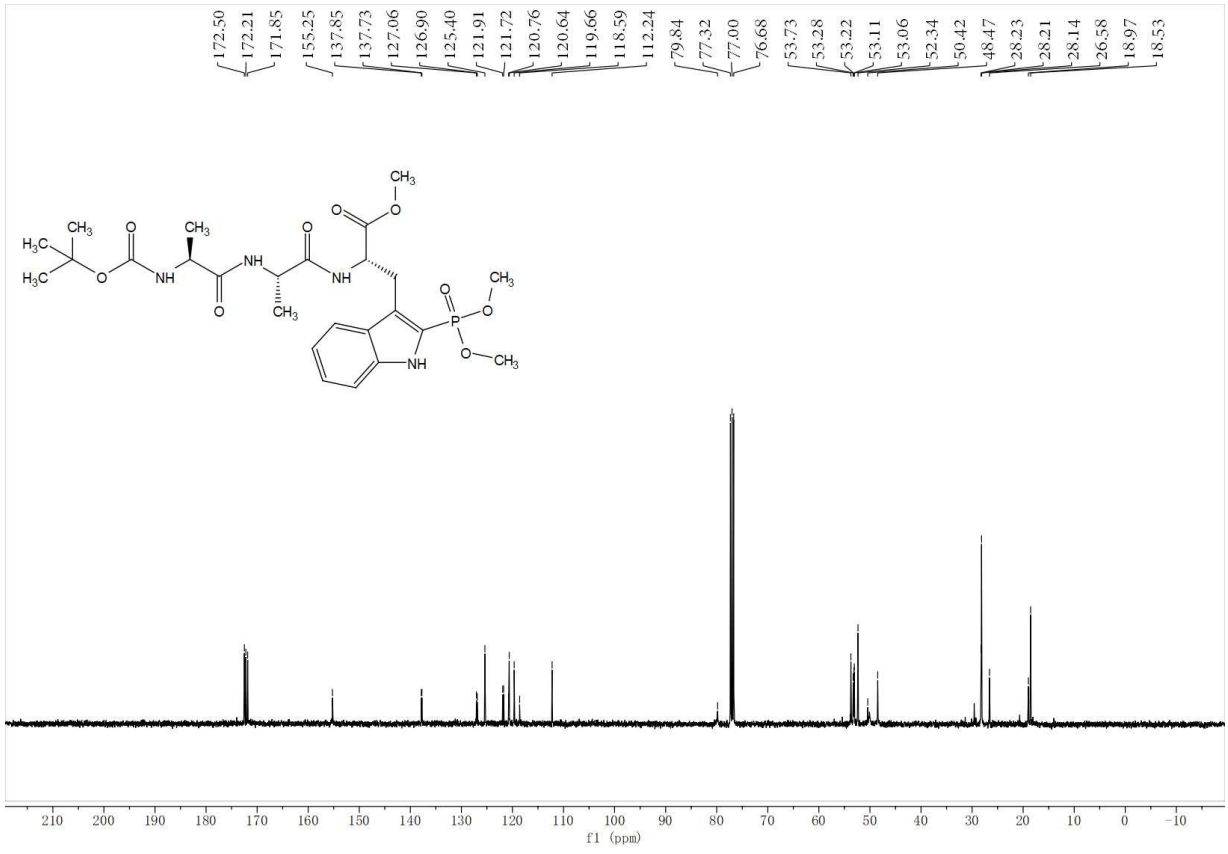


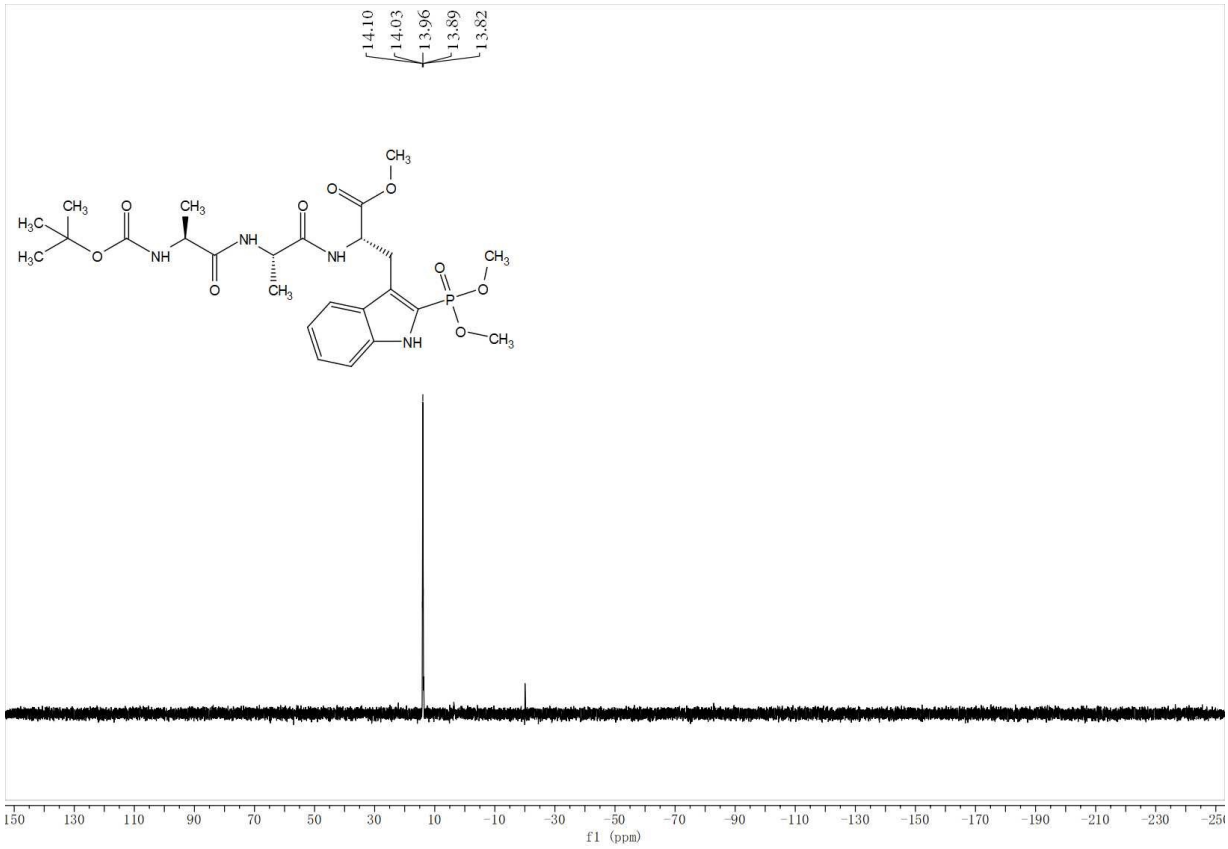

178.82
172.26
171.49
137.76
137.63
127.14
126.98
125.47
121.75
121.56
120.99
120.73
119.74
118.81
112.26
77.32
77.00
76.68
61.52
56.60
53.74
53.32
53.27
53.21
29.25
26.51
24.88
14.07
f1 (ppm)


13.83
f1 (ppm)

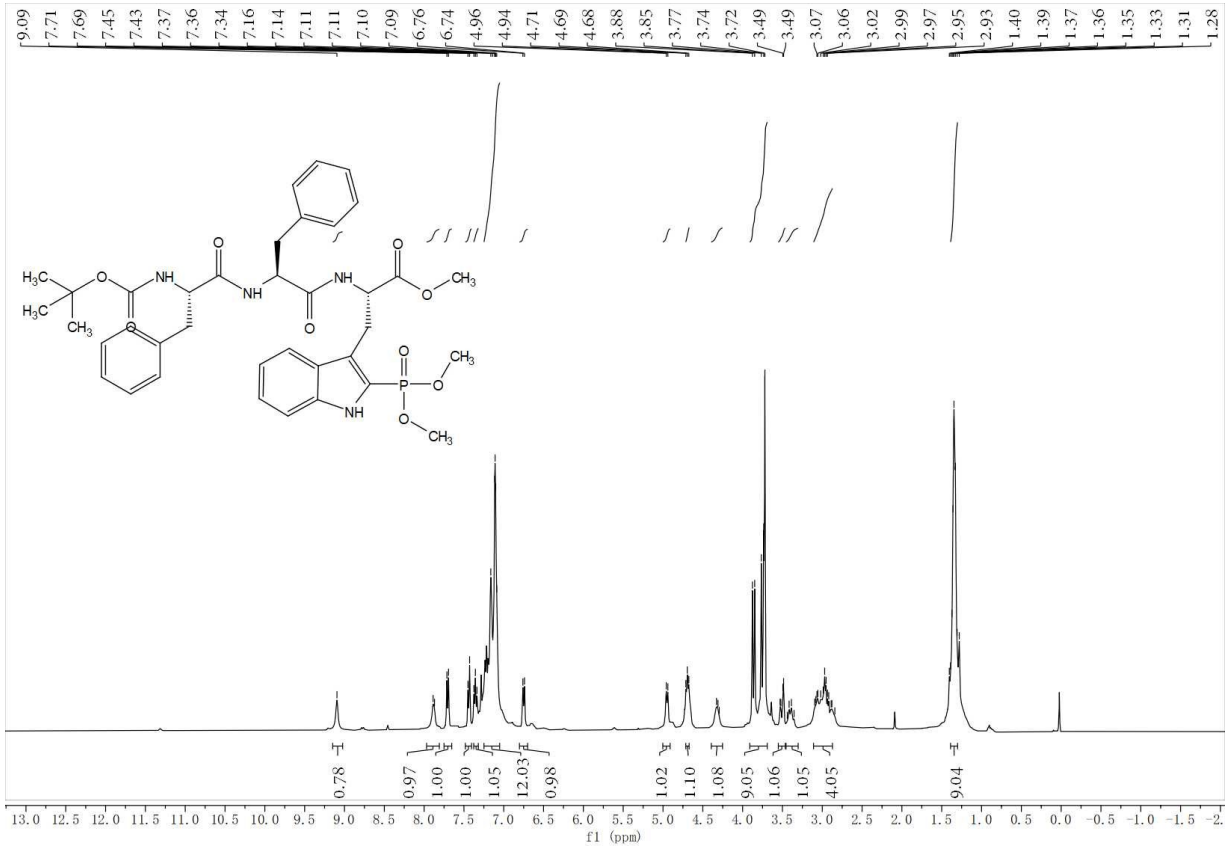
Boc-Ala-Ala-Trp-OMe-Methyl phosphate (**17**)

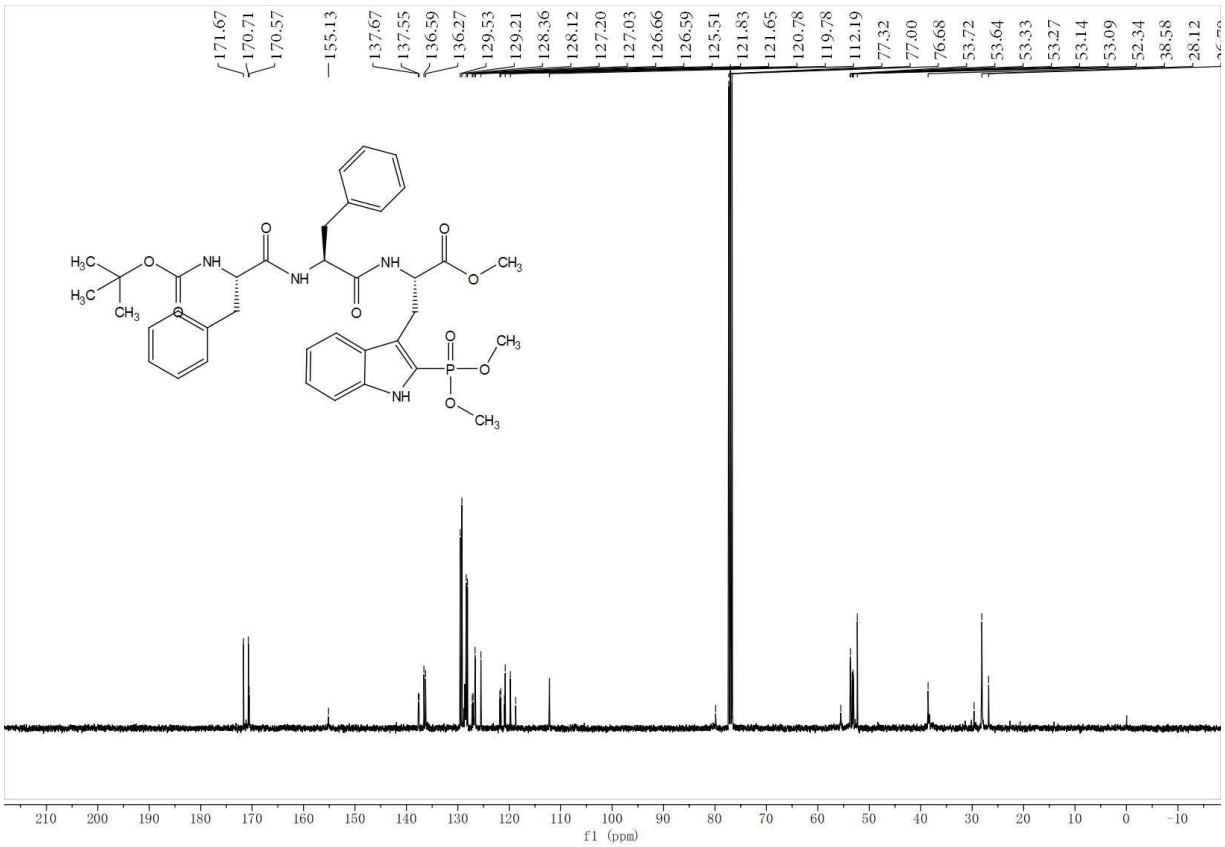


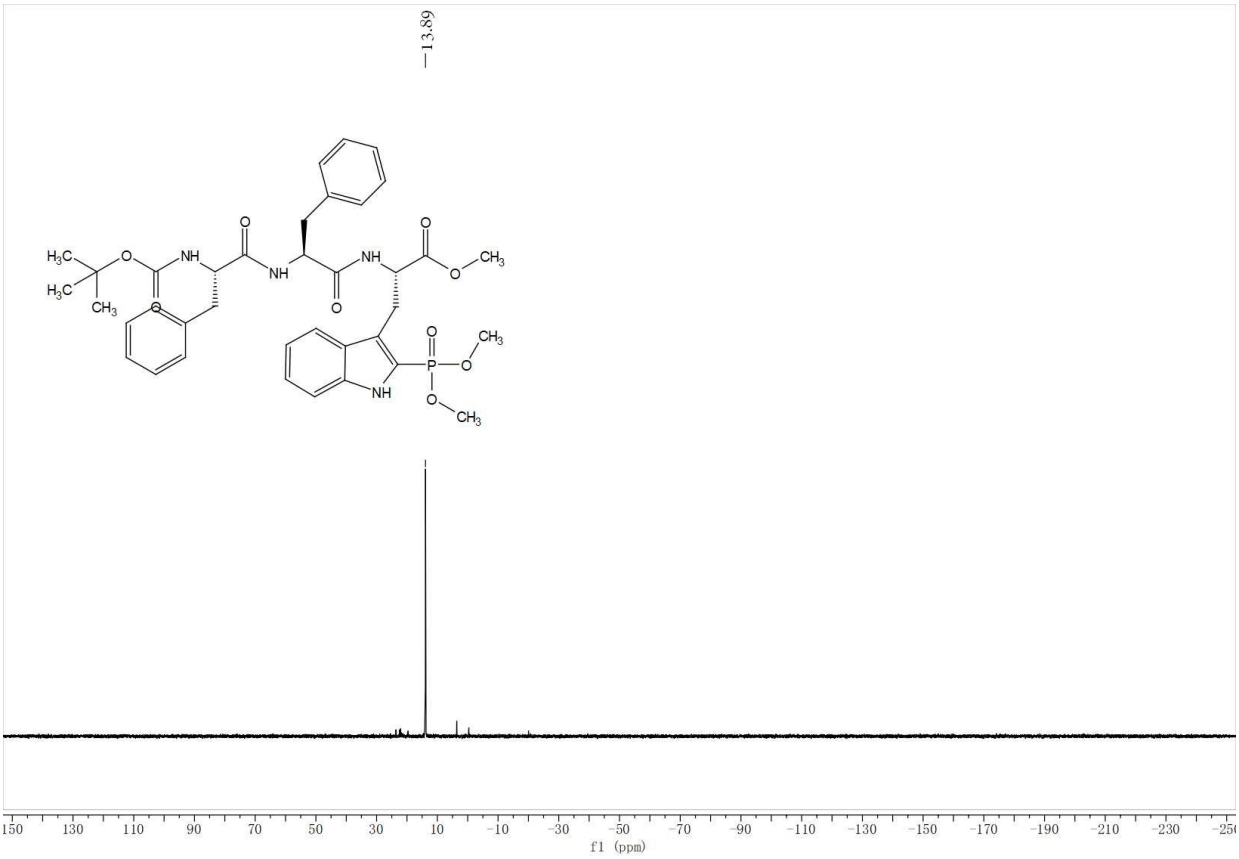




Boc-Phe-Phe-Trp-OMe-Methyl phosphate (**18**)




171.67
170.71
170.57
155.13
137.67
137.55
136.59
136.27
129.53
129.21
128.36
128.12
127.20
127.03
126.66
126.59
125.51
121.83
121.65
120.78
119.78
112.19
77.32
77.00
76.68
53.72
53.64
53.33
53.27
53.14
53.09
52.34
38.58
28.12
f1 (ppm)


13.89
f1 (ppm)