Electro-induced C-H/S-H Cross-Coupling for the Functionalization/Macrocyclization of Cysteine-Containing Peptides

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Supporting Information

TABLE OF CONTENTS

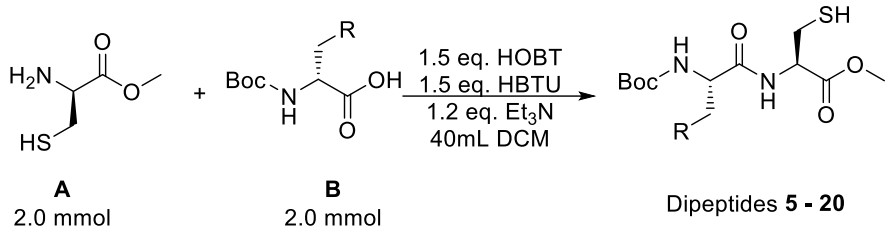
1. General Information

Unless otherwise stated, analytical grade solvents and commercially available reagents were used without further purification. All solvents were analytical reagent or better and were degassed prior to use. The instrument for electrolysis was dual display potentiostat (DJS-292B) (made in China). The anode electrode is platinum plate electrodes (Φ 6mm) and the cathode electrode is platinum plate electrodes (15 mm×15 mm×0.3 mm). Thin layer chromatography (TLC) employed glass 0.25 mm silica gel plates. Flash chromatography columns were packed with 200-300 mesh silica gel in petroleum (boiling point is between 60-90℃). Gradient flash chromatography was conducted eluting with a continuous gradient from petroleum to the indicated solvent, and they are listed as volume/volume ratios. High resolution mass spectra (HRMS) for polypeptides were measured with an Agilent 6224 instrument and accurate masses were reported for the molecular ion + Hydrogen (M+H) or molecular ion + Sodium (M+Na). The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Advance III (400 MHz)

spectrometers with tetramethylsilane as an internal standard. All chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. For ^{1}H NMR, chemical shifts (δ) were given in ppm relatives to internal standard (TMS at 0 ppm, $CDCl_3$ at 7.26 ppm, MeOH-d_4 at 3.31 ppm,DMSO-d6 at 2.50 ppm). For ^{13}C-NMR, chemical shifts (δ) were reported in ppm using solvent as internal standard ($CDCl_3$ at 77.00 ppm, MeOH-d_4 at 49.00 ppm, DMSO-d6 at 39.50 ppm). GC-MS spectra were recorded on a Varian GC-MS 3900-2100T and Shimadzu GCMS-QP2010SE. All the polypeptide precursors used in this article were provided by the PeptiL and Pharmatech CO., LTD.

2. General Experimental Procedures

2.1 Synthesis of starting materials dipeptides[1][2]



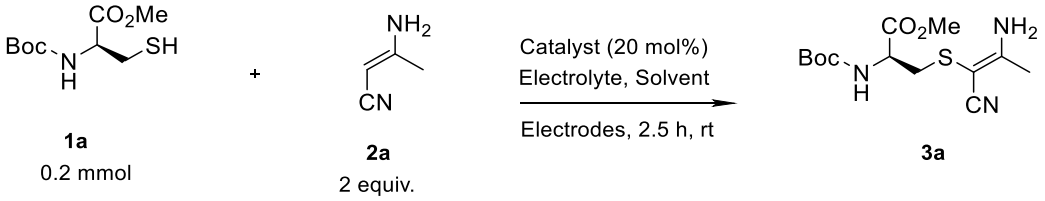
To a solution of L-Cysteine methyl ester hydrochloride **A** (343 mg, 2.0 mmol, 1.0 equiv.) in 40 mL CH_2Cl_2 was added HOBT (1-hydroxybenzotriazole) (3.0 mmol), HBTU (O-benzotriazole-*N, N', N'*-tetramethyluronium-hexafluorophosphate) (3.0 mmol) and triethylamine (2.4 mmol). The mixture was stirred for 30 min at room temperature, and then, peptide **B** (2.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated $NaHCO_3$ solution (40 mL x 3), 2 M hydrochloric acid solution (40 mL x 3) and H_2O (40 mL x 3). The organic layers were combined, dried over Na_2SO_4, and

concentrated. The resulting crude product was purified by flash chromatography (DCM / MeOH) to afford corresponding dipeptides **5-20**.

2.2. Reaction optimization

In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, cysteine residue (0.2 mmol), 3-Aminocrotononitrile(0.4 mmol), $^{n}Bu_4NBr$ (0.2 mmol) and $NiBr_2$•diglyme (0.04 mmol) were combined and added. Then, solvent (7 mL) were injected into the tubes via syringes. The bottle was equipped with platinum plate (15 mm×15 mm×0.3 mm) as the anode and platinum plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current under room temperature. When the reaction was finished, the solvent was removed by reduced pressure and the crude product was purified by flash column chromatography on silica gel (eluent: petroleum ether/ethyl acetate= 1:1). A summary of optimization results is presented in **Table S1** below.

Table S1. Investigation of the reaction conditions

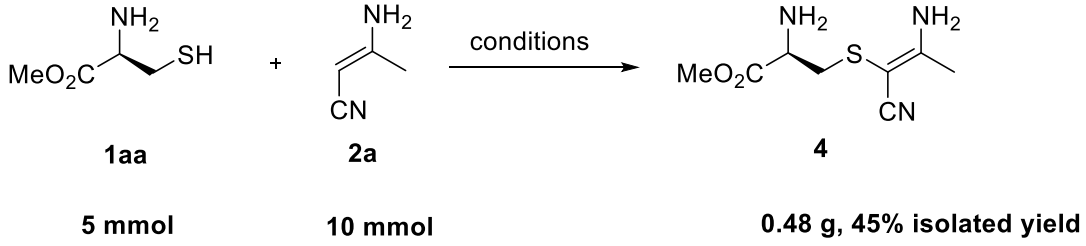


Entry	Electrodes	Electrolyte	Solvent	Catalyst	Halogen source	Current	Yield /%
1	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	CH_3CN	/	/	12 mA	trace
2	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	CH_3CN	/	$MgCl_2$•6 H_2O	12 mA	8
3	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	CH_3CN	/	NaCl	12 mA	14
4	Pt(+)/Pt(-)	$^{n}Bu_4NCl$	CH_3CN	/	$^{n}Bu_4NCl$	12 mA	17
5	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	CH_2Cl_2	/	CH_2Cl_2	12 mA	25
6	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	DCE	/	DCE	12 mA	12

7	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	/	$CHCl_3$	12 mA	30
8	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHBr_3$ = 6/1	/	$CHBr_3$	12 mA	27
9	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$Fe(Cp)_2$	$CHCl_3$	12 mA	38
10	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$Mn(OTf)_2$	$CHCl_3$	12 mA	57
11	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$Ni(acac)_2$	$CHCl_3$	12 mA	42
12	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$Ni(cod)_2$	$CHCl_3$	12 mA	47
13	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	80
14	Pt(+)/Pt(-)	$^{n}Bu_4NI$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	70
15	Pt(+)/Pt(-)	$^{n}Bu_4NBF_4$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	22
16	Pt(+)/Pt(-)	$^{n}Bu_4NPF_6$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	35
17	Pt(+)/Pt(-)	$^{n}Bu_4NCl$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	68
18	C(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	65
19	Pt(+)/Ni(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	42
20	Pt(+)/Pb(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	12 mA	25
21	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	5 mA	36
22	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	8 mA	56
23	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	15 mA	72
24	Pt(+)/Pt(-)	$^{n}Bu_4NBr$	$CH_3CN/CHCl_3$ = 6/1	$NiBr_2$•diglyme	$CHCl_3$	/	n.d.

[a]Reaction conditions: Undivided cell, Pt anode (15 mm×15 mm×0.3 mm), Pt cathode (15 mm×15 mm×0.3 mm), **1a** (0.2 mmol), **2a** (2 equiv.), $^{n}Bu_4NBr$ (1 equiv.), $NiBr_2$•diglyme (0.2 equiv.), 6 mL MeCN, 1 mL $CHCl_3$, air, rt, 12 mA, 2.5 h. Yields of isolated products are shown. N.D. = Not Detected.

2.3 Gram-scale synthesis of 1aa at 5 mmol scale



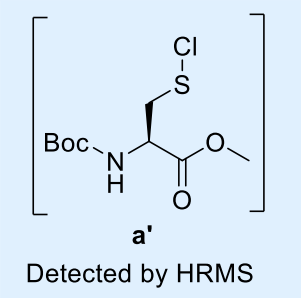
1aa **2a** **4**

5 mmol **10 mmol** **0.48 g, 45% isolated yield**

[b]Reaction conditions: Undivided cell, Pt anode(15 mm×15 mm×0.3 mm), Pt cathode(15 mm×15 mm×0.3 mm), **1aa** (5 mmol), **2a** (10 mmol), $^{n}Bu_4NBr$ (5 mmol), $NiBr_2$•diglyme (1 mmol), K_2CO_3 (5 mmol), 18 mL MeCN, 3 mL $CHCl_3$, air, rt, 12 mA, 12 h. [b]Isolated yields.

3. Mechanistic and control experiments.

3.1 Control experiments



Detected by HRMS of **a′** calcd for $C_9H_{16}ClNO_4S$ $[M+H]^+$: 270.05613; Found: 270.05611

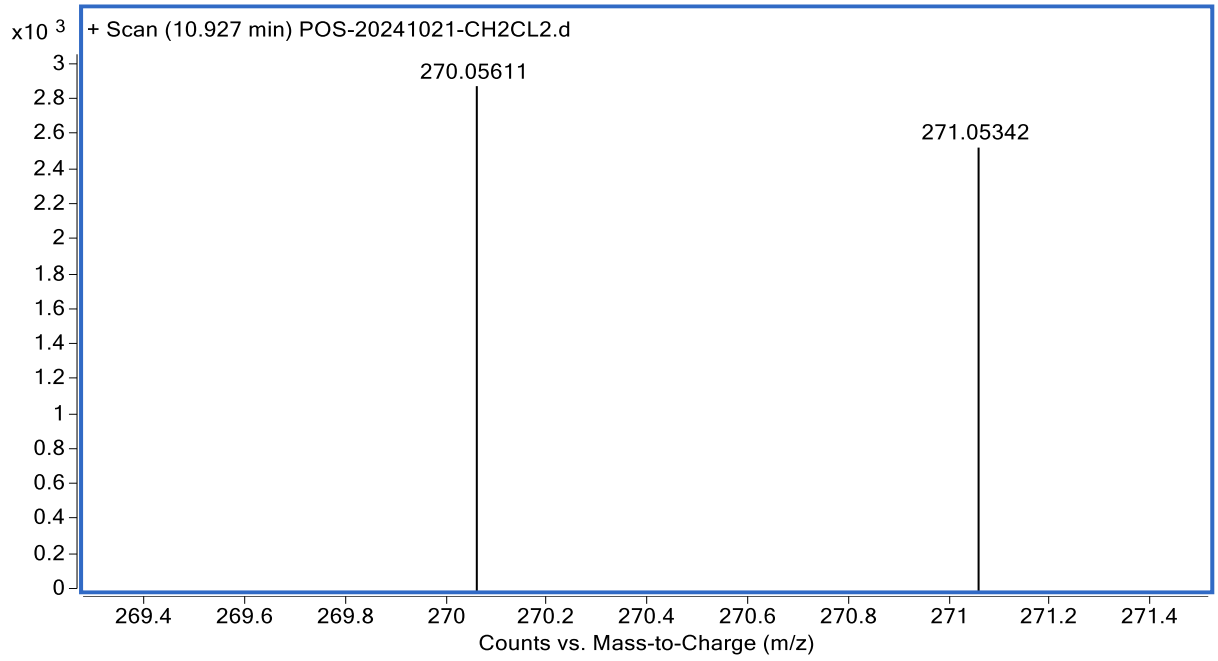
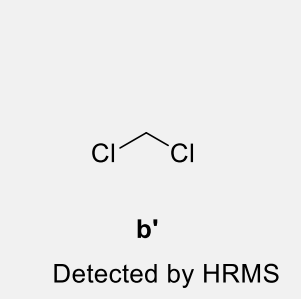


Figure S1. The HRMS spectra of compound **a′**



Detected by HRMS of **b′** calcd for CH_2Cl_2 $[M+H]^+$: 84.96063; Found: 84.96082

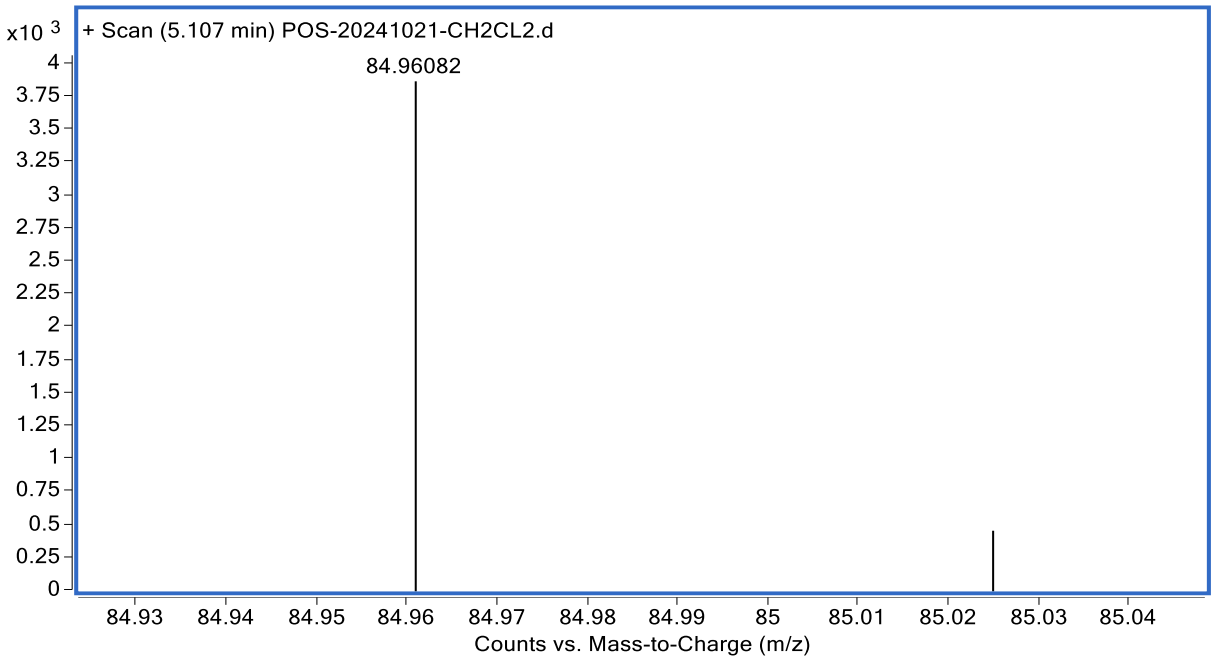
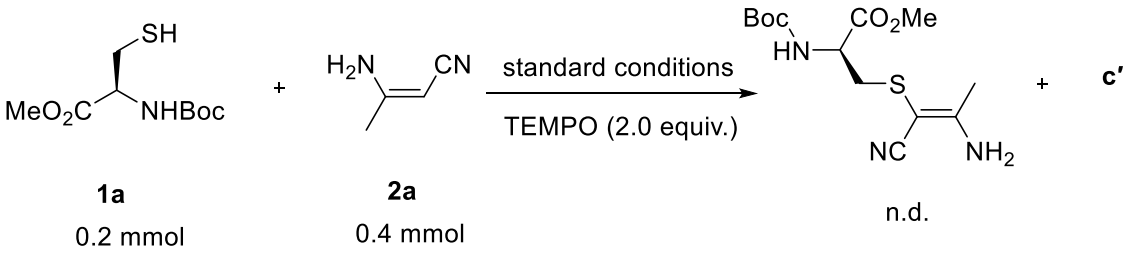
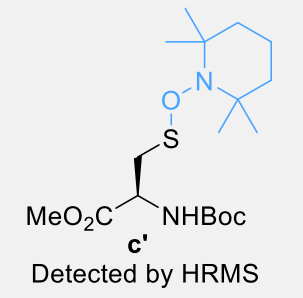


Figure S2. The HRMS spectra of compound **b′**



Reaction conditions: Undivided cell, Pt anode(15 mm×15 mm×0.3 mm), Pt cathode(15 mm×15 mm×0.3 mm), **1a** (0.2 mmol), **2a** (2 equiv.), $^{n}Bu_4NBr$ (1 equiv.), $NiBr_2$•diglyme (0.2 equiv.), TEMPO(2.0 equiv.), 6 mL MeCN, 1 mL $CHCl_3$, air, rt, 12 mA, 2.5 h.



Detected by HRMS of **c′** calcd for $C_{18}H_{34}N_2O_5S$ $[M+H]^+$: 391.22612; Found: 391.22618

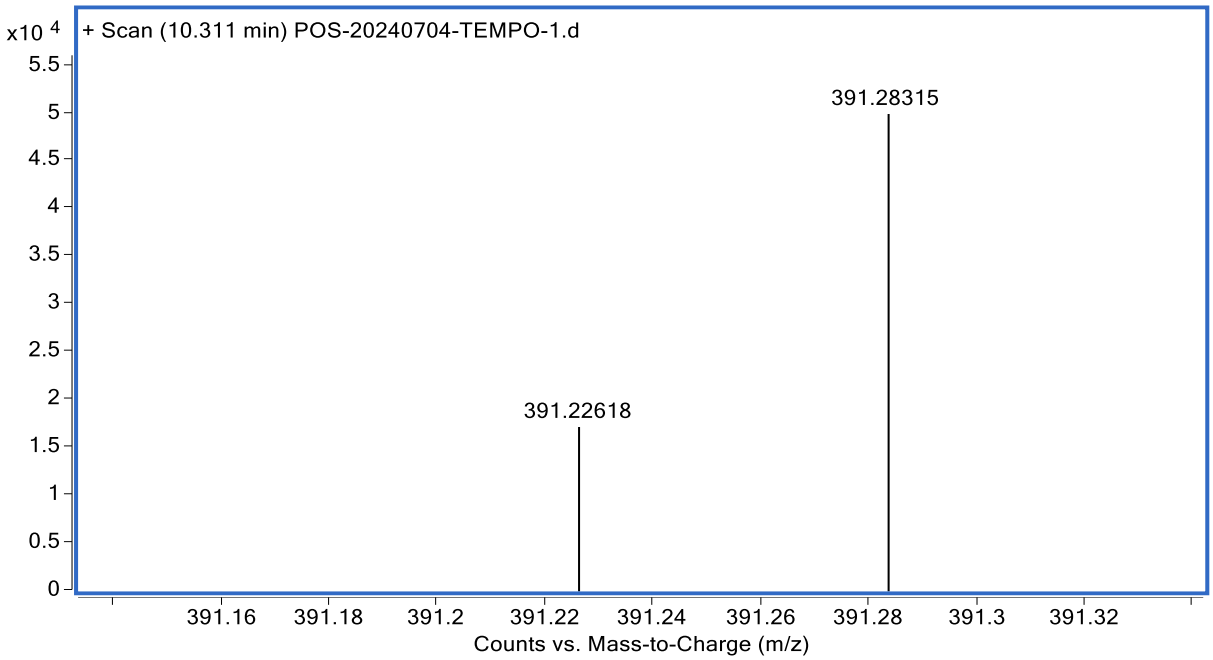


Figure S3. The HRMS spectra of compound **c′**

3.2 General procedure for cyclic voltammetry (CV)

Cyclic voltammetry was performed in a three-electrode cell connected to a schlenk line at room temperature. The working electrode was a steady glassy carbon disk electrode, the counter electrode was a platinum wire. The reference was an Ag/AgCl electrode submerged in saturated aqueous KCl solution and separated from a reaction by a salt bridge.8 mL of CH_3CN containing 0.02 M $^nBu_4NBF_4$ were poured into the electrochemical cell in all experiments. The scan rate is 0.1 V/s. The positive scan range was from -2.0 V to 2.0 V and 0 V to 2.0 V.

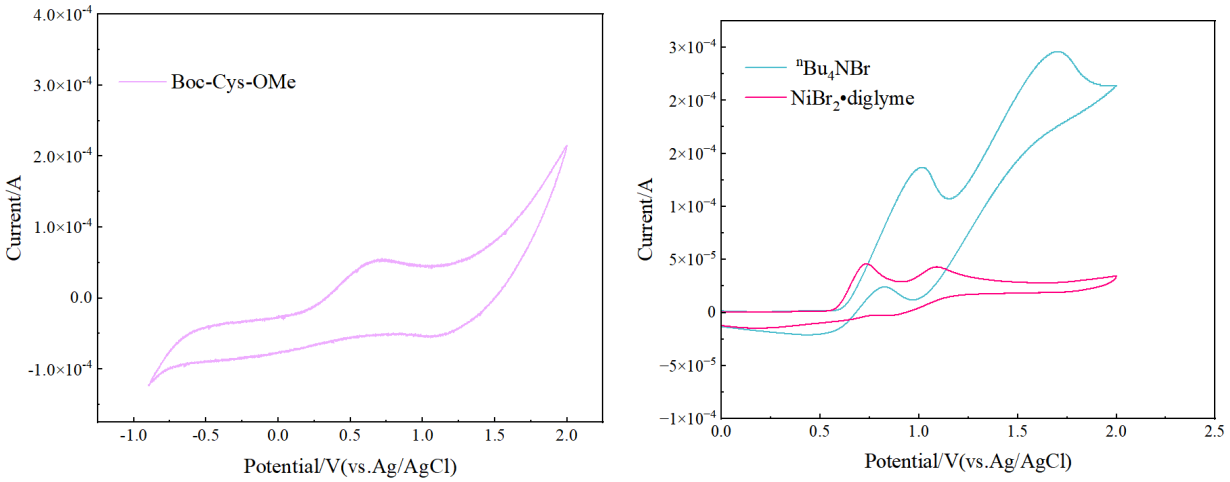
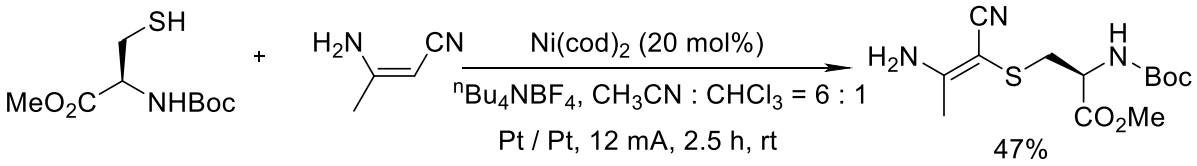


Figure S4. Cyclic voltammograms of substrate **Boc-Cys-OMe, $^{n}Bu_4NBr$, $NiBr_2$•diglyme**



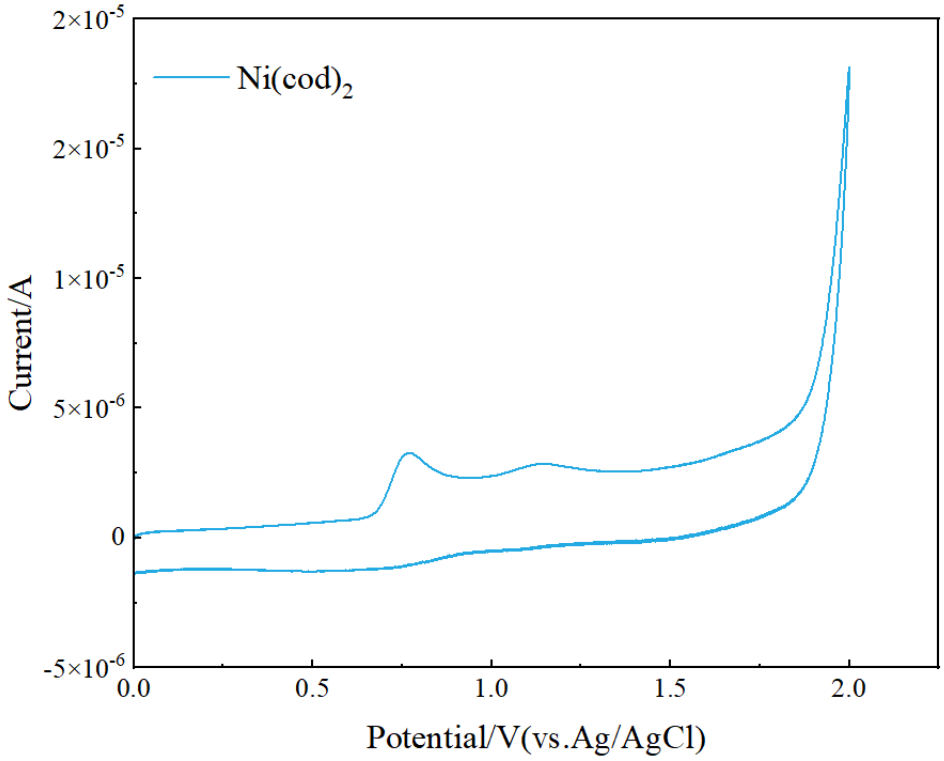
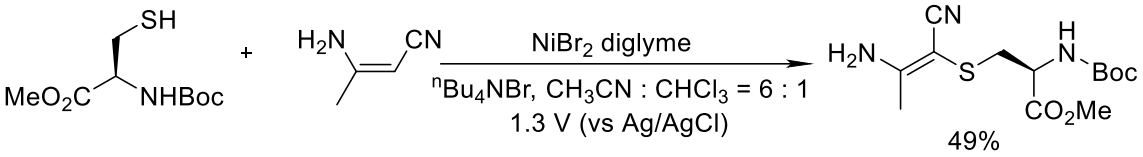


Figure S5. Cyclic voltammograms of substrate **$Ni(cod)_2$**

To avoid the interference of bromide ions in the CV experiment, we choose to use $Ni(cod)_2$

instead of $NiBr_2$•diglyme, and $^nBu_4NBF_4$ instead of $^nBu_4NBr$. The $Ni(cod)_2$ displayed a dual anodic wave with a peak potential of 0.6V and 1.3V, which we attributed to the Ni^0/Ni^I and Ni^I/Ni^{II} redox. Thus, a controlled potential electrolysis was carried out. When the potential of the anode was maintained at 1.3 V, the corresponding product was obtained in 49% yield.

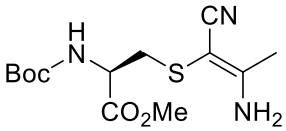


4. Bioactive molecules scope and characterization

4.1 Dipeptides scope and characterization

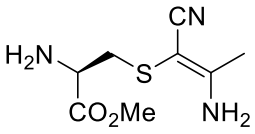
General procedure for bioconjugated product (3): In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, cysteine residue (0.2 mmol), 3-Aminocrotononitrile (0.4 mmol), $^nBu_4NBr$ (0.2 mmol) and $NiBr_2$•diglyme (0.04 mmol) were combined and added. Then, CH_3CN (6 mL) , $CHCl_3$ (1 mL) were injected into the tubes via syringes. The bottle was equipped with platinum plate (15 mm×15 mm×0.3 mm) as the anode and platinum plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at a constant current of 12 mA under room temperature for 2.5 h. After completion of the reaction, as indicated by TLC and LC-MS, the pure product (yield: 80%, 50.4 mg) was obtained by flash column chromatography on silica gel (eluent: petroleum ether/ethyl acetate= 1:1).

Detailed descriptions for products:



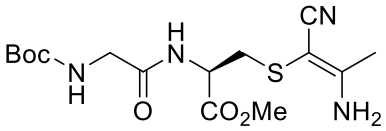
3

Methyl (Z)-S-(2-amino-1-cyanoprop-1-en-1-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (3): yellow solid (Yield: 80%, 50.41 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 5.36 (d, *J* = 8.3 Hz, 1H), 4.46 (d, *J* = 6.4 Hz, 1H), 3.75 (s, 3H), 3.12 – 3.03 (m, 1H), 2.92 (dd, *J* = 14.1, 6.7 Hz, 1H), 2.17 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.27, 164.26, 155.36, 121.23, 80.38, 66.58, 53.66, 52.72, 37.98, 28.28, 20.26. HRMS (ESI) cald. for $(M+Na)^+$ $C_{13}H_{21}N_3O_4S$: 338.1145 found,338.1141.



4

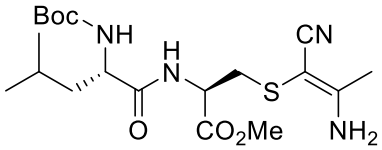
Methyl (Z)-S-(2-amino-1-cyanoprop-1-en-1-yl)-L-cysteinate (4): yellow solid (Yield: 45%, 0.48 g), ^{1}H NMR (400 MHz, Chloroform-d) δ 4.29 (dt, *J* = 7.9, 2.9 Hz, 1H), 3.83 (s, 3H), 3.12 (dd, *J* = 12.7, 3.2 Hz, 1H), 2.81 (dd, *J* = 12.7, 7.9 Hz, 1H), 2.20 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.80, 148.93, 119.00, 65.55, 55.23, 53.31, 25.62, 21.24. HRMS (ESI) cald. for $(M+H)^+$ $C_8H_{13}N_3O_2S$: 216.0801 found,216.0803.



5

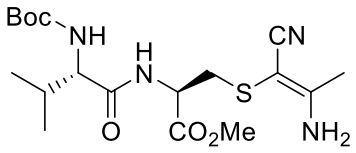
Methyl (Z)-S-(2-amino-1-cyanoprop-1-en-1-yl)-N-((tert-butoxycarbonyl)glycyl)-L-cysteinate (5): brown oil (Yield: 70%, 52.10 mg) , ^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 (d, *J* = 7.8 Hz, 1H), 5.72 (s, 1H), 4.84 – 4.56 (m, 1H), 3.92 – 3.80 (m, 2H), 3.77 (s, 3H), 3.16 – 2.94 (m, 2H), 2.18 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.82, 170.31, 165.02, 156.40,

121.67, 80.49, 65.36, 52.86, 52.02, 44.32, 37.18, 28.33, 20.23. HRMS (ESI) cald. for $(M+Na)^+$ $C_{15}H_{24}N_4O_5S$: 395.1359 found,395.1359.



6

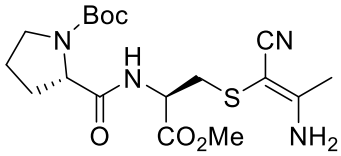
Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((tert-butoxycarbonyl)-L-leucyl)-L-cysteinate (6): yellow solid (Yield: 45%, 38.53 mg) , ^{1}H NMR (400 MHz, Chloroform-d) δ 7.10 (d, *J* = 7.6 Hz, 1H), 5.30 – 5.10 (m, 1H), 4.81 – 4.63 (m, 1H), 4.15 – 4.06 (m, 1H), 3.73 (s, 3H), 3.05 – 2.93 (m, 2H), 2.13 (s, 3H), 1.69 – 1.59 (m, 2H), 1.52 – 1.46 (m, 1H), 1.41 (s, 9H), 0.90 (t, *J* = 6.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.68, 170.60, 164.21, 155.88, 121.14, 80.40, 66.89, 52.77, 52.14, 40.64, 37.23, 28.36, 24.77, 22.93, 21.84, 20.37. HRMS (ESI) cald. for $(M+Na)^+$ $C_{19}H_{32}N_4O_5S$: 451.1985 found,451.1950.



7

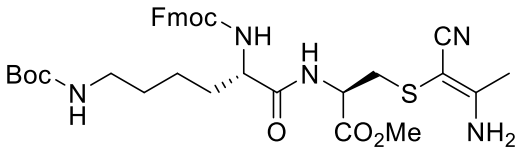
Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((tert-butoxycarbonyl)-L-valyl)-L-cysteinate(7): yellow solid (Yield: 40%, 33.14 mg) , ^{1}H NMR (400 MHz, Chloroform-d) δ 7.04 (d, *J* = 7.6 Hz, 1H), 5.27 (q, *J* = 3.7, 2.9 Hz, 1H), 4.68 (q, *J* = 6.2 Hz, 1H), 3.94 (dd, *J* = 8.8, 6.4 Hz, 1H), 3.74 (d, *J* = 3.5 Hz, 3H), 3.04 (dt, *J* = 18.5, 8.4 Hz, 2H), 2.14 (s, 3H), 1.41 (s, 9H), 1.00 – 0.89 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.15, 170.68, 164.73, 156.12, 121.46, 80.16, 66.02,

60.18, 52.83, 52.17, 37.23, 30.49, 28.36, 20.30, 19.31, 17.91. HRMS (ESI) cald. for $(M+H)^+$ $C_{18}H_{30}N_4O_5S$: 415.2009 found,415.2009.



8

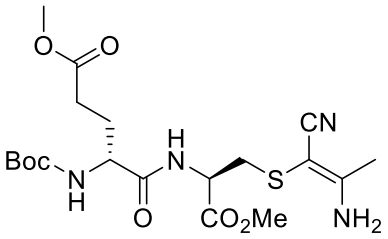
Tert-butyl (S)-2-(((R)-3-(((Z)-2-amino-1-cyanoprop-1-en-1-yl)thio)-1-methoxy-1-oxopropa n-2-y l)carbamoyl)pyrrolidine-1-carboxylate (8): brown oil (Yield: 70%, 57.70 mg) , ^{1}H NMR (400 MHz, Chloroform-d) δ 4.79 – 4.49 (m, 1H), 4.25 (d, *J* = 7.1 Hz, 1H), 3.70 (s, 3H), 3.49 – 3.25 (m, 2H), 2.96 (qd, *J* = 14.1, 11.9, 6.6 Hz, 2H), 2.31 – 2.15 (m, 1H), 2.12 (s, 3H), 2.04 – 1.66 (m, 3H), 1.40 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 172.54, 170.70, 164.93, 155.63, 121.38, 80.56, 59.90, 53.50, 52.66, 51.70, 47.18, 37.17, 28.41, 24.50, 20.32. HRMS (ESI) cald. for $(M+H)^+$ $C_{18}H_{28}N_4O_5S$: 413.1853 found,413.1854.



9

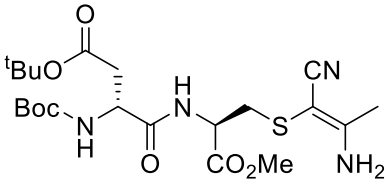
Methyl N-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(tert-butoxycarbonyl)-L-lysyl)-S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-L-cysteinate(9): yellow solid (Yield: 42%, 55.88 mg) ,^{1}H NMR (400 MHz, Chloroform-d) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.41 – 7.29 (m, 2H), 7.22 – 7.04 (m, 1H), 5.81 (d, *J* = 8.0 Hz, 2H), 4.74 (dq, *J* = 12.9, 5.7 Hz, 2H), 4.38 (d, *J* = 7.1 Hz, 2H), 4.21 (t, *J* = 7.0 Hz, 2H), 3.73 (s, 3H), 3.18 – 2.98 (m, 4H), 2.12 (s, 3H), 1.96 – 1.87 (m, 1H), 1.74 – 1.65 (m, 1H), 1.50 (q, *J* = 7.5, 5.9 Hz, 4H), 1.43 (s, 9H). ^{13}C

NMR (101 MHz, $CDCl_3$) δ 172.09, 170.59, 164.57, 156.35, 143.75, 141.29, 127.78, 127.13, 125.12, 121.41, 120.01, 79.32, 67.26, 66.28, 54.88, 52.77, 52.15, 50.64, 47.11, 39.92, 37.05, 31.64, 29.50, 28.45, 22.45, 20.20. HRMS (ESI) cald. for $(M+H)^+$ $C_{34}H_{43}N_5O_7S$: 666.2956 found,666.2956.



10

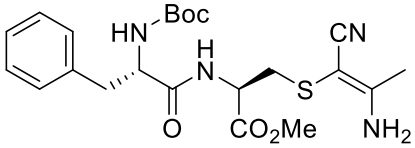
Methyl (R)-5-(((R)-3-(((Z)-2-amino-1-cyanoprop-1-en-1-yl)thio)-1-methoxy-1-oxopropan-2-yl)amino)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoate (10): yellow solid (Yield: 71%, 65.06 mg), ^1H NMR (400 MHz, Chloroform-d) δ 7.45 – 7.00 (m, 1H), 5.63 (dd, *J* = 55.0, 9.0 Hz, 1H), 4.91 – 4.64 (m, 1H), 4.37 – 4.12 (m, 1H), 3.77 (s, 3H), 3.69 (s, 3H), 3.22 – 2.96 (m, 2H), 2.49 (q, *J* = 6.8 Hz, 2H), 2.18 (s, 3H), 1.96 (dt, *J* = 14.5, 7.6 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.98, 171.90, 170.61, 164.62, 155.88, 121.38, 80.39, 66.19, 53.75, 52.87, 52.00, 37.06, 30.19, 28.35, 27.51, 20.35. HRMS (ESI) cald. for $(M+Na)^+$ $C_{19}H_{30}N_4O_7S$: 481.1727 found,481.1724.



11

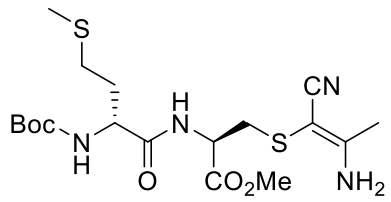
Tert-butyl (R)-4-(((R)-3-(((Z)-2-amino-1-cyanoprop-1-en-1-yl)thio)-1-methoxy-1-oxopropa

n-2-yl)amino)-3-((tert-butoxycarbonyl)amino)-4-oxobutanoate (11): light yellow oil (Yield: 61%, 59.31 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.40 (d, *J* = 7.8 Hz, 1H), 5.92 (d, *J* = 8.5 Hz, 1H), 4.67 (t, *J* = 6.6 Hz, 1H), 4.58 – 4.44 (m, 1H), 3.76 (s, 3H), 3.07 (td, *J* = 12.2, 10.3, 5.5 Hz, 2H), 2.83 – 2.68 (m, 2H), 2.18 (s, 3H), 1.47 (s, 9H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.30, 170.74, 170.46, 164.93, 155.68, 121.46, 81.71, 80.49, 65.33, 53.52, 52.72, 52.09, 51.01, 37.05, 28.30, 27.97, 20.16. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{34}N_4O_7S$: 509.2040,found,509.2015.



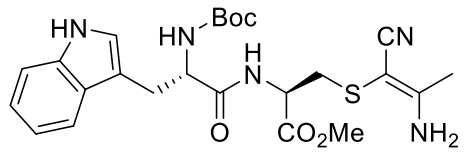
12

Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((tert-butoxycarbonyl)-L-phenylalanyl) -L-cysteinate (12): light yellow solid (Yield: 75%, 69.33 mg),^{1}H NMR (400 MHz, Chloroform-d) δ 7.35 – 7.19 (m, 5H), 6.89 (d, *J* = 7.8 Hz, 1H), 5.14 (d, *J* = 7.5 Hz, 1H), 4.61 (s, 1H), 4.37 (q, *J* = 7.2 Hz, 1H), 3.74 (s, 3H), 3.15 (dd, *J* = 14.2, 6.3 Hz, 1H), 3.04 (p, *J* = 9.8, 7.8 Hz, 2H), 2.93 – 2.72 (m, 1H), 2.16 (s, 3H), 1.40 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 172.03, 170.81, 164.88, 155.74, 136.32, 129.29, 128.82, 127.16, 121.26, 80.77, 65.11, 55.94, 52.83, 51.73, 50.63, 37.78, 36.95, 28.31, 20.40. HRMS (ESI) cald. for $(M+H)^+$ $C_{22}H_{30}N_4O_5S$: 463.2009,found, 463.2007.



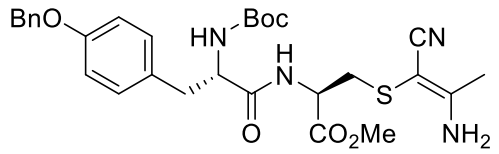
13

Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((tert-butoxycarbonyl)-D-methionyl)-L-cysteinate(13): light yellow solid (Yield: 73%, 65.14 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.13 (d, *J* = 7.7 Hz, 1H), 5.45 (d, *J* = 8.2 Hz, 1H), 4.88 – 4.56 (m, 1H), 4.30 (t, *J* = 7.4 Hz, 1H), 3.77 (d, *J* = 5.7 Hz, 3H), 3.18 – 3.00 (m, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.18 (s, 3H), 2.11 (d, *J* = 3.4 Hz, 4H), 1.96 (dd, *J* = 14.3, 7.2 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.00, 170.61, 164.56, 155.86, 121.36, 80.53, 66.57, 53.70, 52.89, 52.34, 37.27, 31.37, 30.33, 28.45, 20.41, 15.39. HRMS (ESI) cald. for $(M+Na)^+$ $C_{18}H_{30}N_4O_5S_2$: 469.1549,found, 469.1545.



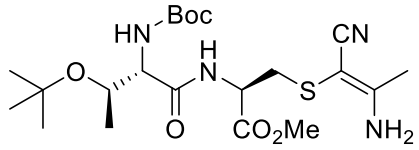
14

Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((tert-butoxycarbonyl)-L-tryptophyl)-L-cysteinate (14): light yellow solid (Yield: 40%, 40.10 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 8.51 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.05 (m, 4H), 6.77 (d, *J* = 7.8 Hz, 1H), 4.74 – 4.64 (m, 1H), 4.46 (q, *J* = 7.0 Hz, 1H), 3.69 (s, 3H), 3.34 – 3.19 (m, 2H), 2.92 (dd, *J* = 13.5, 5.9 Hz, 2H), 2.00 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.06, 170.55, 164.51, 155.73, 136.33, 127.56, 123.69, 122.22, 121.48, 119.72, 118.74, 111.52, 110.17, 80.44, 66.27, 55.43, 52.89, 52.36, 37.46, 28.41, 28.02, 20.20. HRMS (ESI) cald. for $(M+Na)^+$ $C_{24}H_{31}N_5O_5S$: 524.1938,found, 524.1931.



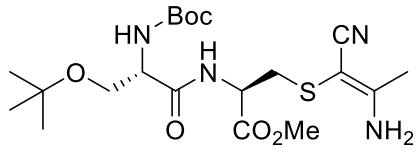
15

Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((S)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)-L-cysteinate (15): light yellow solid (Yield: 47%, 53.41 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.42 – 7.27 (m, 5H), 7.12 (d, *J* = 8.5 Hz, 2H), 7.02 (s, 1H), 6.93 – 6.87 (m, 2H), 5.28 – 5.23 (m, 1H), 5.02 (s, 2H), 4.68 (s, 1H), 4.34 (d, *J* = 7.2 Hz, 1H), 3.73 (s, 3H), 3.13 – 2.91 (m, 4H), 2.13 (s, 3H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.00, 170.51, 164.89, 157.80, 155.74, 136.95, 130.39, 128.76, 128.62, 128.02, 127.51, 121.80, 114.99, 80.41, 69.98, 65.93, 56.01, 52.88, 52.36, 37.63, 36.92, 28.33, 20.31. HRMS (ESI) cald. for $(M+H)^+$ $C_{29}H_{36}N_4O_6S$: 569.2428,found, 569.2428.



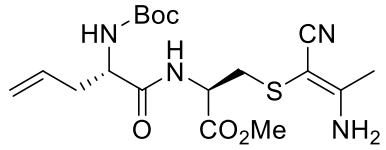
16

Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-(N-(tert-butoxycarbonyl)-O-(tert-butyl)-L-threonyl)-L-cysteinate (16): light yellow solid (Yield: 55%, 51.95 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.97 – 7.77 (m, 1H), 5.60 – 5.52 (m, 1H), 4.71 (d, *J* = 6.5 Hz, 1H), 4.22 – 4.09 (m, 2H), 3.77 (s, 3H), 3.09 (q, *J* = 6.4, 4.8 Hz, 2H), 2.18 (s, 3H), 1.46 (s, 9H), 1.31 (s, 9H), 1.12 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.26, 164.17, 155.52, 120.92, 79.77, 75.65, 67.22, 66.32, 58.32, 52.59, 52.10, 37.19, 28.35, 20.26, 16.97. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{36}N_4O_6S$: 495.2247,found, 495.2200.



17

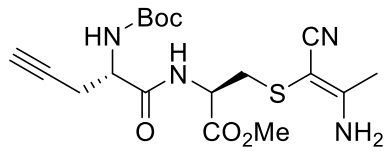
Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-(N-(tert-butoxycarbonyl)-O-(tert-butyl)-L-seryl)-L-cysteinate(17): light yellow solid (Yield: 40%, 36.66 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.45 (d, *J* = 8.3 Hz, 1H), 5.50 – 5.39 (m, 1H), 4.87 – 4.64 (m, 1H), 4.31 – 4.10 (m, 1H), 3.80 (d, *J* = 4.9 Hz, 1H), 3.77 (s, 3H), 3.46 (t, *J* = 7.8 Hz, 1H), 3.05 (ddd, *J* = 52.3, 14.1, 5.6 Hz, 2H), 2.19 (s, 3H), 1.47 (s, 9H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.05, 170.50, 164.41, 155.61, 121.16, 80.32, 74.21, 66.48, 61.62, 54.50, 52.76, 52.36, 37.94, 28.31, 27.38, 20.35. ^{13}C NMR (101 MHz, Chloroform-d) δ 165.89, 165.75, 165.45, 147.78, 147.58, 140.18, 132.22, 130.46, 130.16, 125.80, 123.83, 123.02, 75.96, 68.47, 68.39, 53.49, 53.35, 27.95, 25.70. HRMS (ESI) cald. for $(M+H)^+$ $C_{20}H_{34}N_4O_6S$: 459.2271,found, 459.2277.



18

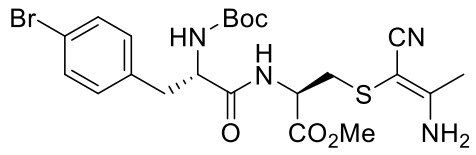
Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((S)-2-((tert-butoxycarbonyl)amino)pent-4-enoyl)-L-cysteinate (18): yellow solid (Yield: 75%, 61.83 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.11 (d, *J* = 7.7 Hz, 1H), 5.84 – 5.73 (m, 1H), 5.26 – 5.11 (m, 3H), 4.83 – 4.64 (m, 1H), 4.20 (q, *J* = 7.1 Hz, 1H), 3.77 (s, 3H), 3.06 (tt, *J* = 14.2, 8.0 Hz, 2H), 2.53 (dp, *J* = 29.1, 7.5 Hz, 2H), 2.18 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.84, 170.52, 164.51,

155.73, 132.92, 121.33, 119.09, 80.44, 66.42, 54.08, 52.75, 52.26, 37.52, 36.26, 28.32, 20.29. HRMS (ESI) cald. for $(M+K)^+$ $C_{18}H_{28}N_4O_5S$: 451.1412,found, 451.1412.



19

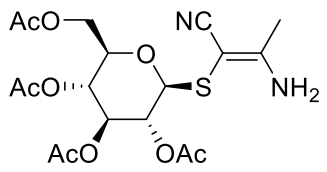
Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((S)-2-((tert-butoxycarbonyl)amino)pent-4-ynoyl)-L-cysteinate (19): light yellow solid (Yield: 53%, 43.48 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.24 (d, *J* = 7.7 Hz, 1H), 5.55 – 5.46 (m, 1H), 4.79 – 4.68 (m, 1H), 4.40 – 4.28 (m, 1H), 3.78 (s, 3H), 3.07 (tt, *J* = 14.2, 8.0 Hz, 2H), 2.73 (qdd, *J* = 16.6, 6.2, 2.5 Hz, 2H), 2.19 (s, 3H), 2.13 (s, 1H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.59, 164.49, 155.58, 121.30, 80.77, 79.26, 71.82, 66.39, 53.43, 52.81, 52.37, 37.44, 28.30, 22.04, 20.28. HRMS (ESI) cald. for $(M+H)^+$ $C_{18}H_{26}N_4O_5S$: 411.1696,found, 411.1696.



20

Methyl S-((Z)-2-amino-1-cyanoprop-1-en-1-yl)-N-((S)-3-(4-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)-L-cysteinate (20): light yellow solid (Yield: 53%, 57.25 mg),^{1}H NMR (400 MHz, Chloroform-d) δ 7.47 – 7.43 (m, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.83 (d, *J* = 7.7 Hz, 1H), 4.95 (d, *J* = 7.3 Hz, 1H), 4.60 (s, 1H), 4.34 (q, *J* = 7.0 Hz, 1H), 3.77 (s, 3H), 3.12 (dd, *J* = 14.0, 6.0 Hz, 2H), 3.02 (dd, *J* = 14.1, 7.3 Hz, 1H), 2.91 – 2.76 (m, 1H), 2.18 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.69, 170.70, 164.66, 155.68, 135.38, 131.80, 131.08,

120.99, 80.89, 65.58, 55.69, 52.87, 51.85, 37.07, 28.29, 20.42. HRMS (ESI) cald. for $(M+H)^+$ $C_{22}H_{29}BrN_4O_5S$: 541.1114,found, 541.1113.



21

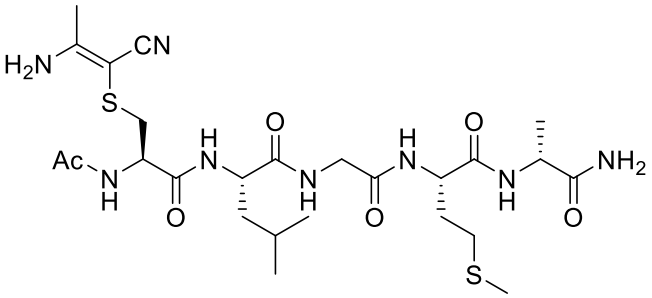
(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(((Z)-2-amino-1-cyanoprop-1-en-1-yl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate(21): yellow solid (Yield: 35%, 31.08 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 5.21 (t, *J* = 9.3 Hz, 1H), 5.07 (t, *J* = 9.7 Hz, 1H), 4.99 – 4.93 (m, 1H), 4.57 (d, *J* = 10.1 Hz, 1H), 4.20 – 4.15 (m, 2H), 3.70 – 3.65 (m, 1H), 2.25 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.66, 170.18, 169.62, 169.43, 166.66, 121.17, 87.23, 76.13, 73.82, 69.66, 68.01, 63.62, 61.79, 20.81, 20.80, 20.65, 20.61, 20.60. HRMS (ESI) cald. for $(M+H)^+$ $C_{18}H_{24}N_2O_9S$: 445.1275,found, 445.1241.

4.2 Polypeptide scope and characterization

The procedure for the bioconjugated product (22): In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar, polypeptides (0.01 mmol), 3-Aminocrotononitrile (2 equiv.), ^{n}Bu_4NBr (2 equiv.), $NiBr_2$•diglyme (20 mol%), MeCN (6 mL), $CHCl_3$ (1 mL) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.3 mm) as the anode and platinum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 4 mA under room temperature for 15 min. After completion of the reaction, the solution was analyzed by TOF-LC/MS spectroscopy. The reaction was analyzed by reversed-phase high-performance liquid chromatography (HPLC) on

an Agilent Zorbax SB-Aq 5 μ m column with a length of 250 mm. A binary high-pressure gradient mode was employed with a total flow rate of 0.5 mL/min, and the concentration of buffer solution B was 100%. The elution time was 20 minutes. HPLC analysis used buffers A (water) and B (acetonitrile + 0.1% TFA). Conversion reported as a % conversion as determined.

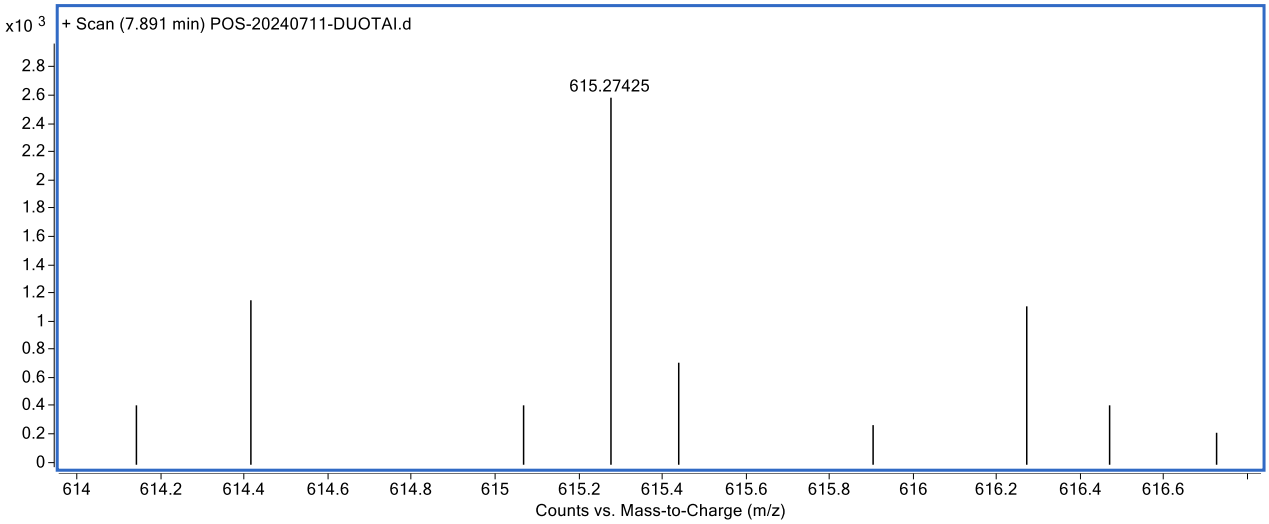
bioconjugated product 22 :

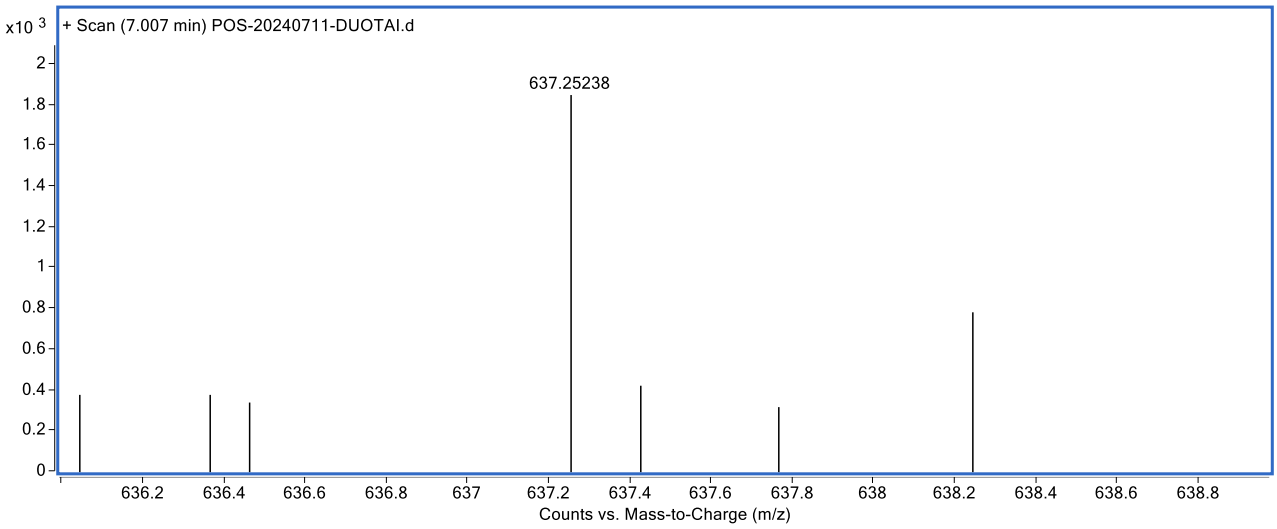


HPLC: >99% conversion.

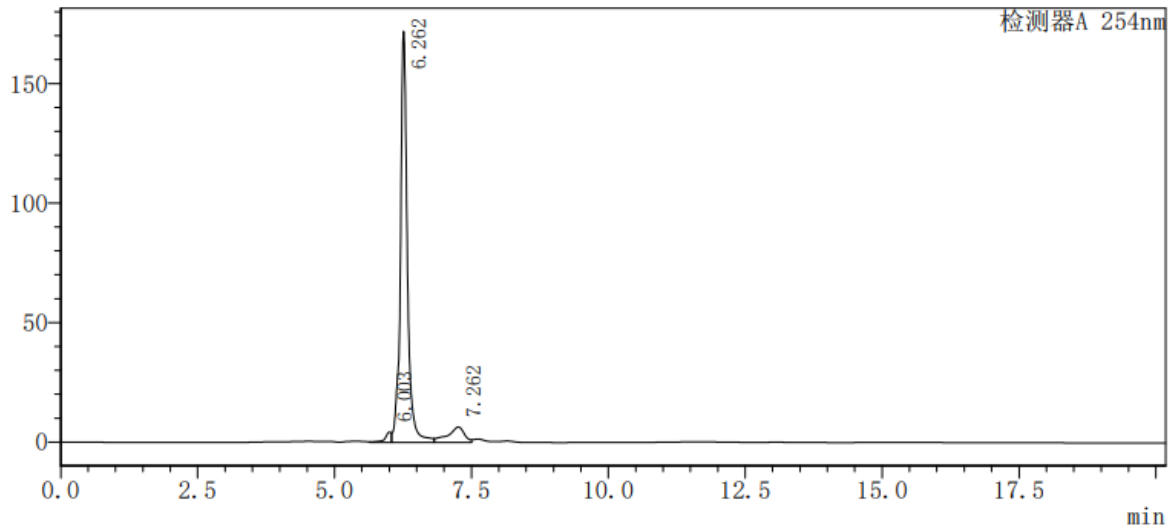
After the reaction finished, there is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 5.189 min. Polypeptide is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 6.262 min.

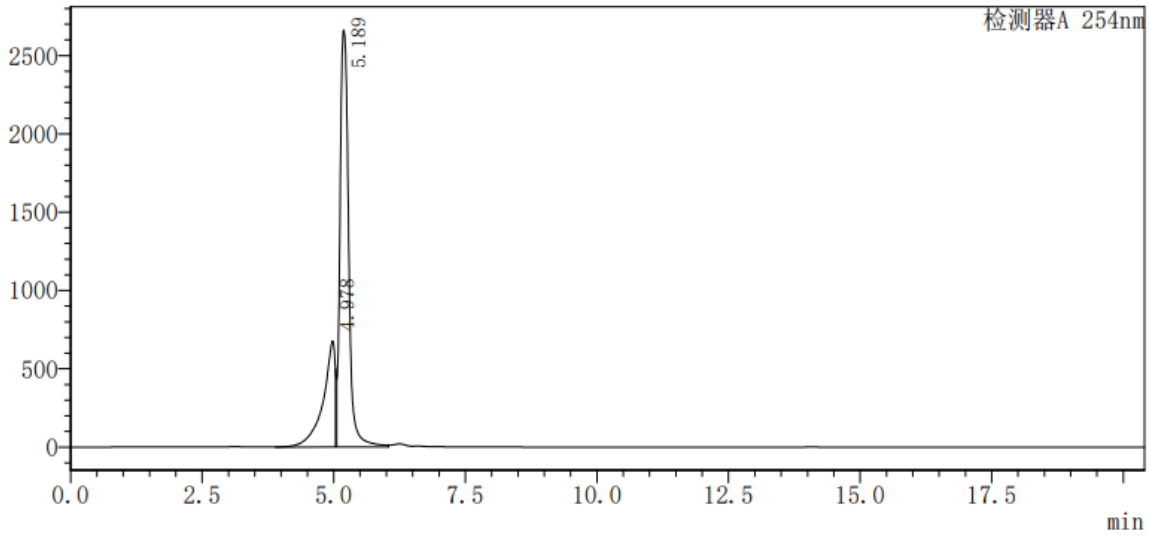
HRMS (ESI-TOF)calcd for $C_{25}H_{42}N_8O_6S_2$, $[M+H]^+$, 615.27415, found 615.27425.calcd for $C_{25}H_{42}N_8O_6S_2$, $[M+Na]^+$, 637.25609, found 637.25238.



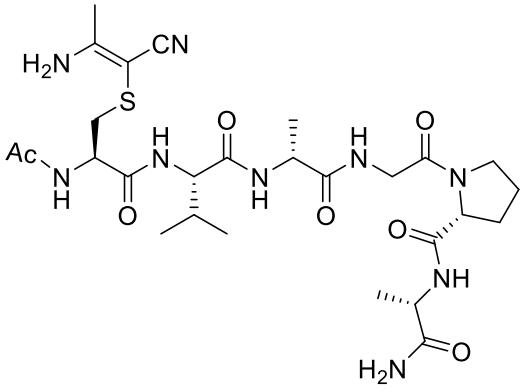


HPLC Spectra:





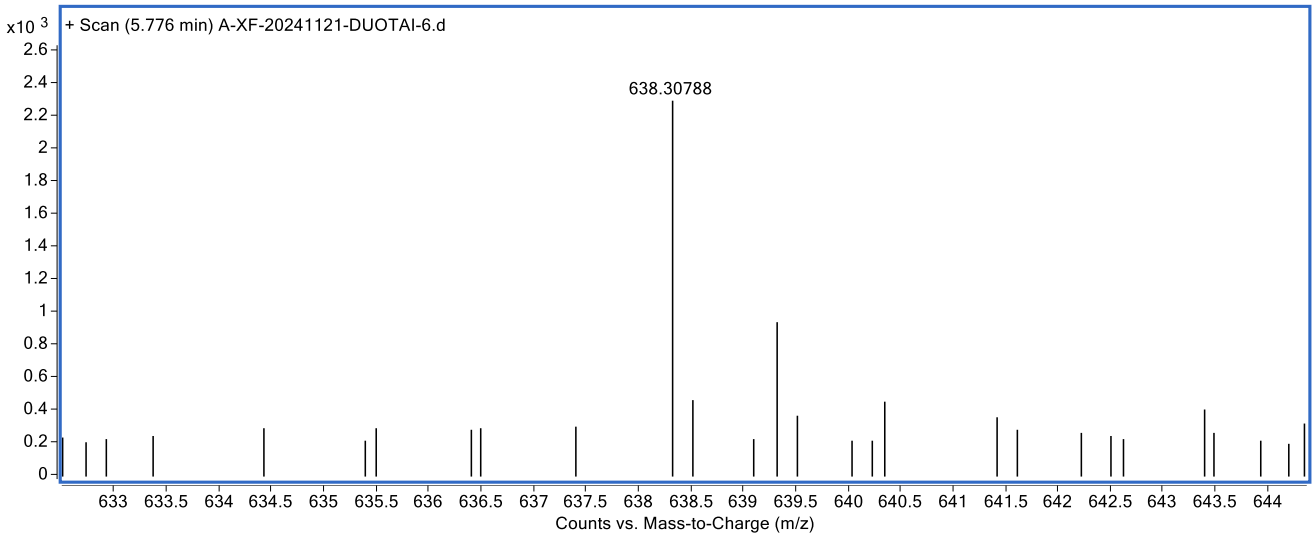
bioconjugated product 23 :

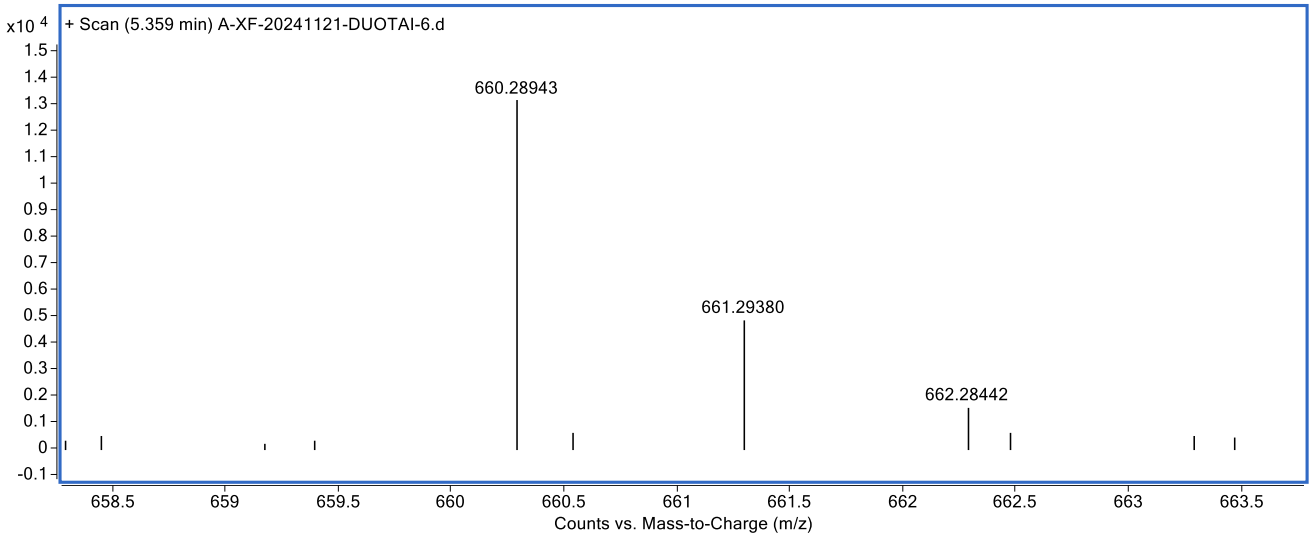


HPLC : 70% conversion.

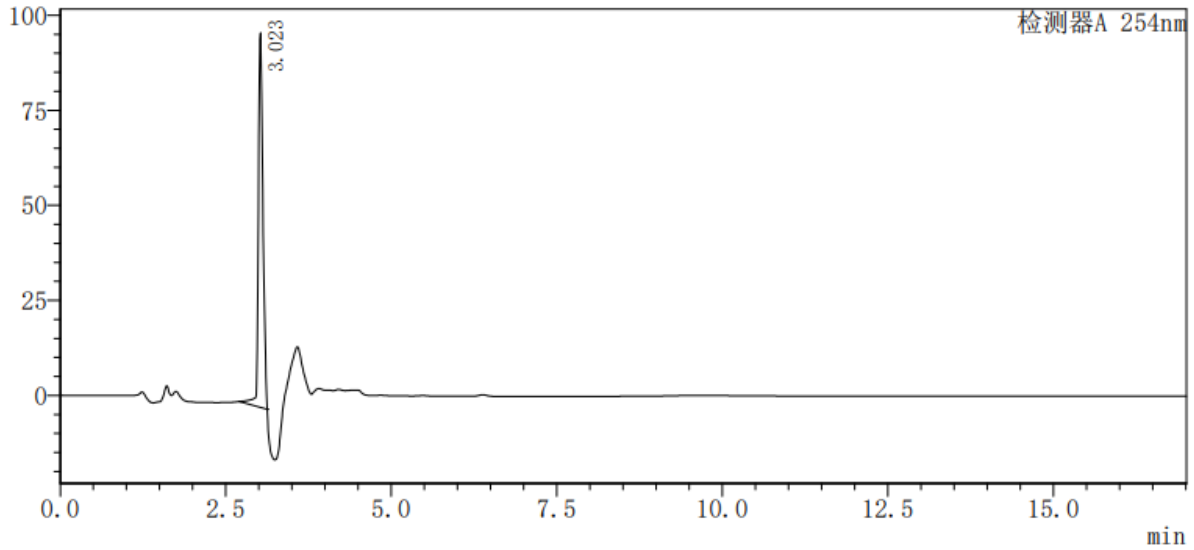
After the reaction finished, there is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.023 min. Polypeptide is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 2.874 min.

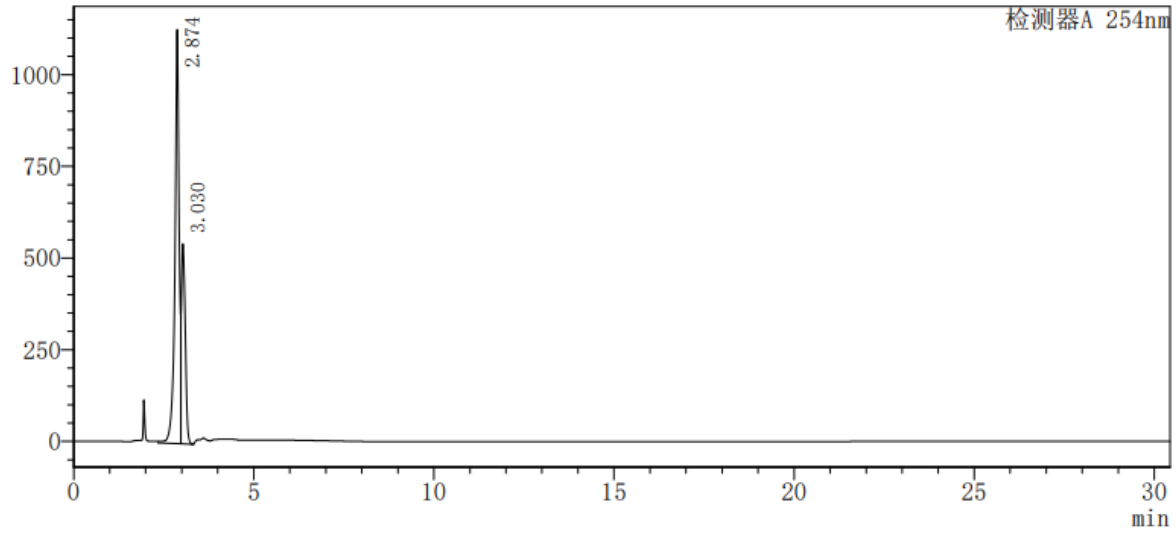
HRMS (ESI-TOF) calcd for $C_{27}H_{43}N_9O_7S$, $[M+H]^+$, 638.30789, found 638.30788.calcd for $C_{27}H_{43}N_9O_7S$, $[M+Na]^+$, 660.28984, found 660.28943.



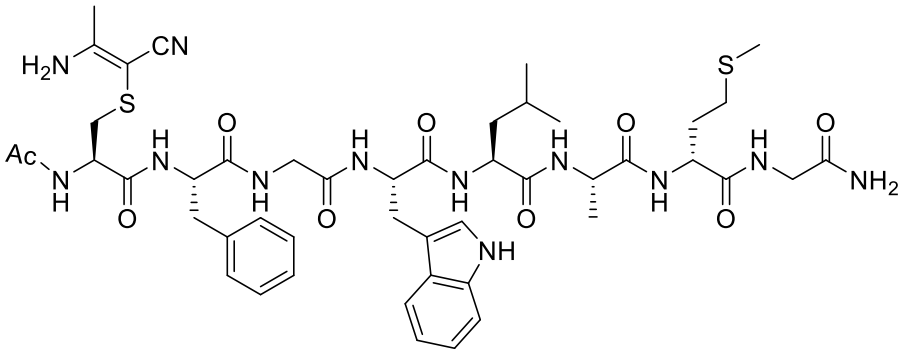


HPLC Spectra:





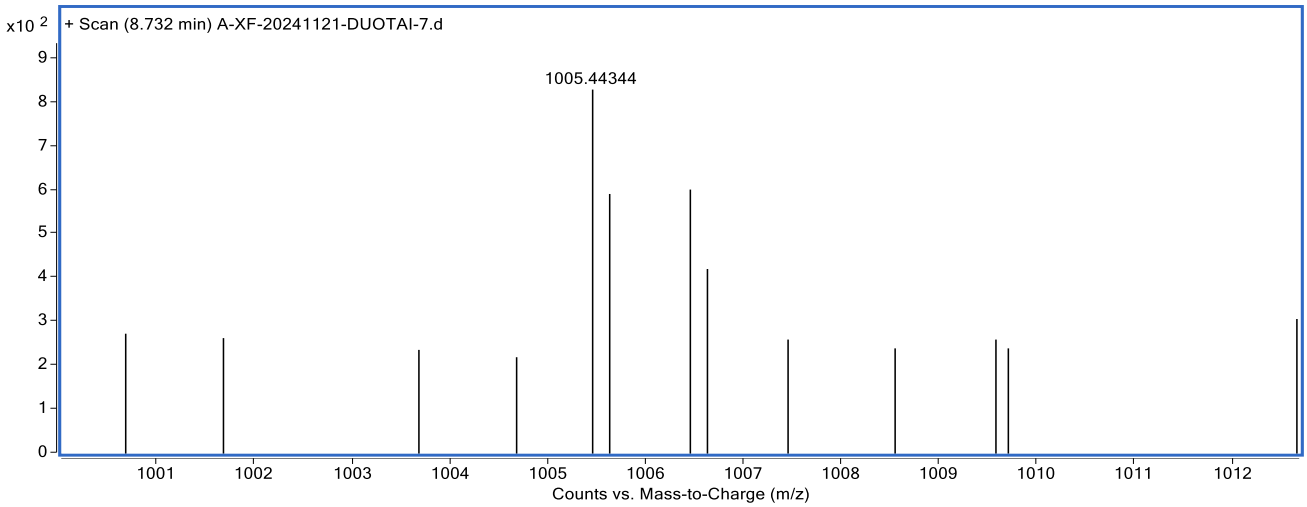
bioconjugated product 24 :

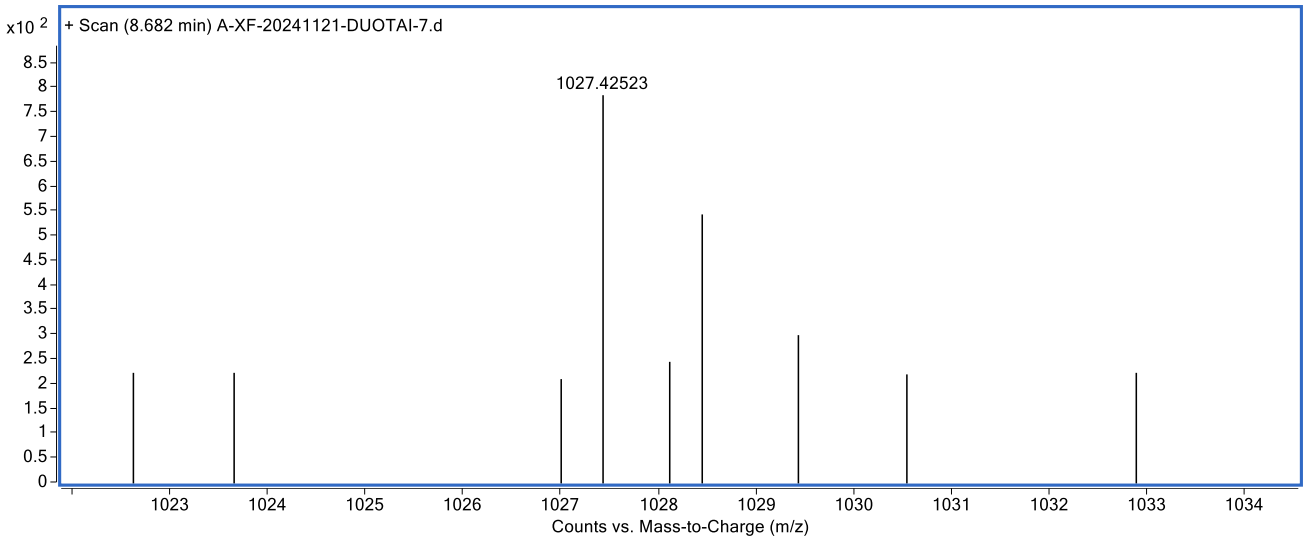


HPLC: 62% conversion.

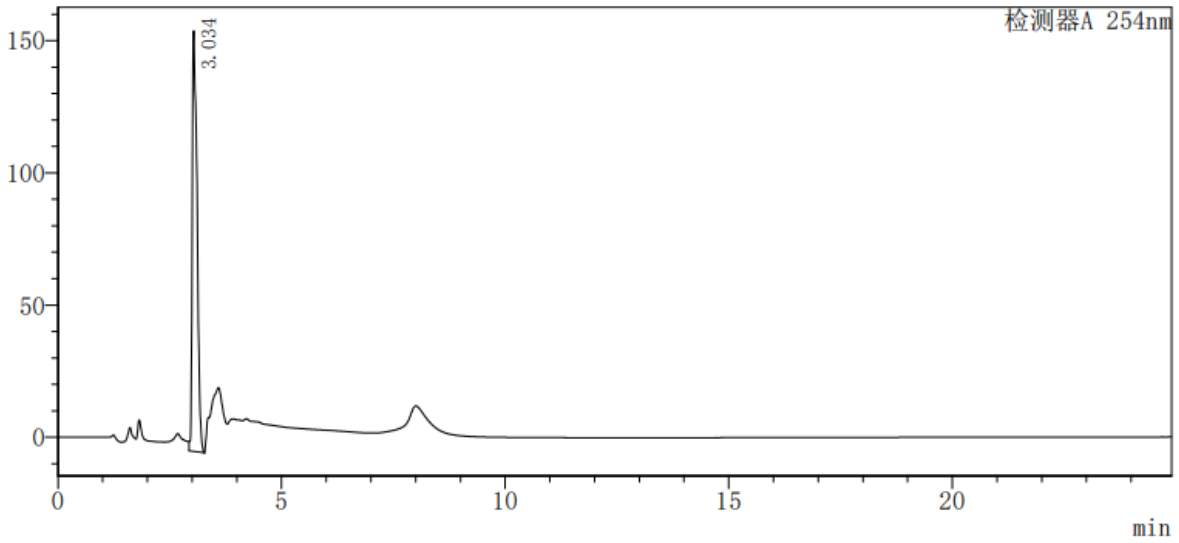
After the reaction finished, there is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.034 min. Polypeptide is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 2.858 min.

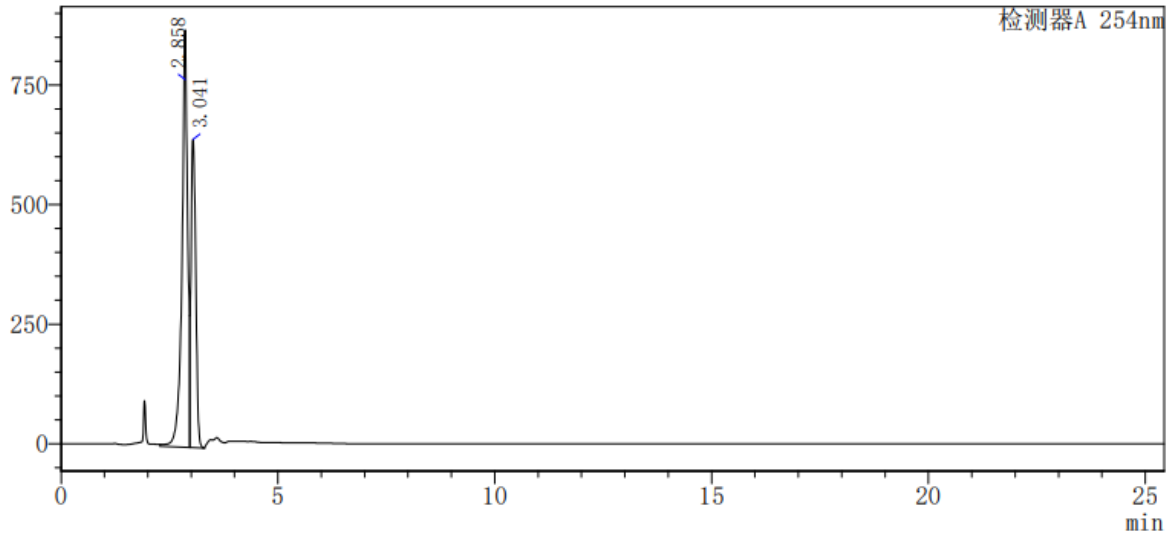
HRMS (ESI-TOF) calcd for $C_{47}H_{64}N_{12}O_9S_2$, $[M+H]^+$, 1005.44334, found 1005.44344. calcd for $C_{47}H_{64}N_{12}O_9S_2$, $[M+Na]^+$, 1027.42528, found 1027.42523.





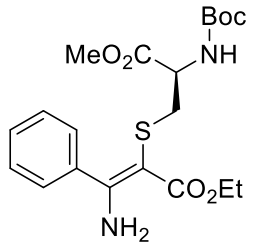
HPLC Spectra:





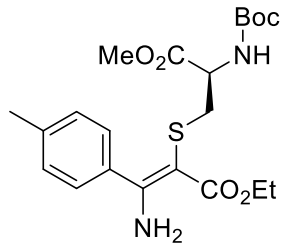
4.3 Enamine and azole scope and characterization

Detailed descriptions for products:



25

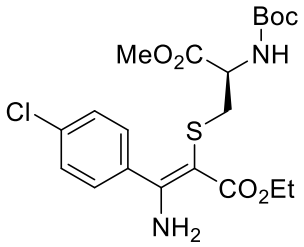
Ethyl (R,E)-3-amino-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-phenylacrylate (25): yellow solid (Yield: 71%, 60.23 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.10 (s, 1H), 7.42 (d, *J* = 2.2 Hz, 5H), 5.31 (d, *J* = 9.2 Hz, 2H), 4.33 – 4.23 (m, 3H), 3.64 (s, 3H), 3.10 (dd, *J* = 13.9, 3.9 Hz, 1H), 2.46 (dd, *J* = 13.9, 5.3 Hz, 1H), 1.43 (d, *J* = 8.3 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.69, 170.33, 168.57, 155.35, 138.09, 129.44, 128.18, 127.93, 84.45, 79.64, 60.48, 53.47, 52.60, 51.84, 37.58, 28.32, 14.41. HRMS (ESI) cald. for $(M+H)^+$ $C_{20}H_{28}N_2O_6S$: 425.1740,found, 425.1732.



26

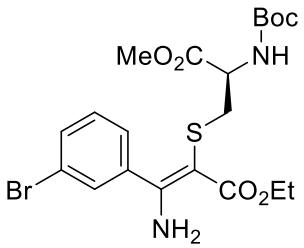
Ethyl (R,E)-3-amino-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(p-tolyl)acrylate (26): yellow oil (Yield: 66%, 57.84 mg),^{1}H NMR (400 MHz, Chloroform-d) δ 9.10 (s, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 5.29 (d, *J* = 8.5 Hz, 2H), 4.36 – 4.22 (m, 3H), 3.65 (s, 3H), 3.14 (dd, *J* = 13.8, 3.7 Hz, 1H), 2.45 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.39 (s, 3H), 1.41 (d, *J* = 3.7 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.73, 170.37, 168.76,

155.40, 139.50, 135.30, 128.86, 127.91, 84.25, 79.60, 60.46, 52.63, 51.79, 37.53, 28.34, 21.43, 14.44. HRMS (ESI) cald. for $(M+H)^+$ $C_{21}H_{30}N_2O_6S$: 439.1897,found, 439.1897.



27

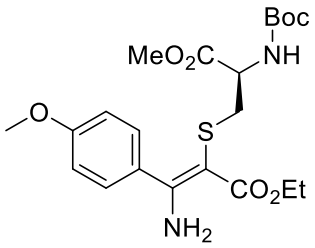
Ethyl (R,E)-3-amino-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(4-chlorophenyl)acrylate (27): light yellow oil (Yield: 61%, 55.89 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.08 (s, 1H), 7.41 (t, *J* = 7.6 Hz, 4H), 5.36 – 5.08 (m, 2H), 4.37 – 4.20 (m, 3H), 3.65 (s, 3H), 3.14 (dd, *J* = 13.9, 3.6 Hz, 1H), 2.48 (dd, *J* = 13.9, 5.3 Hz, 1H), 1.43 (d, *J* = 7.3 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.65, 170.13, 167.17, 155.29, 136.35, 135.48, 129.57, 128.46, 84.73, 79.78, 60.58, 52.66, 51.72, 37.34, 28.33, 14.40. HRMS (ESI) cald. for $(M+H)^+$ $C_{20}H_{27}ClN_2O_6S$: 459.1351,found, 459.1351.



28

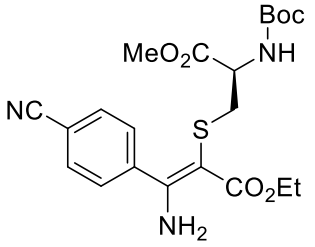
Ethyl (R,E)-3-amino-3-(3-bromophenyl)-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)acrylate (28): light yellow solid (Yield: 68%, 68.27 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.08 (s, 1H), 7.57 (dt, *J* = 5.7, 1.7 Hz, 2H), 7.40 – 7.29 (m, 2H), 5.47 – 5.14 (m, 2H), 4.40 – 4.20 (m, 3H), 3.66 (s, 3H), 3.12 (dd, *J* = 13.9, 3.9 Hz, 1H), 2.50 (dd, *J* = 13.9, 5.3 Hz,

1H), 1.43 (d, *J* = 5.3 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.63, 170.12, 166.60, 155.28, 139.83, 132.48, 130.99, 129.78, 126.70, 122.07, 85.14, 79.78, 60.64, 52.66, 51.86, 37.65, 28.31, 14.39. HRMS (ESI) cald. for $(M+H)^+$ $C_{20}H_{27}BrN_2O_6S$: 503.0846,found, 503.0838.



29

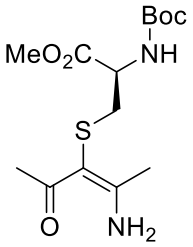
Ethyl (R,E)-3-amino-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(4-methoxyphenyl)acrylate (29): yellow oil (Yield: 84%, 76.30 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.13 (s, 1H), 7.41 – 7.35 (m, 2H), 6.96 – 6.92 (m, 2H), 5.39 – 5.19 (m, 2H), 4.34 – 4.22 (m, 3H), 3.84 (s, 3H), 3.65 (s, 3H), 3.13 (dd, *J* = 13.9, 3.7 Hz, 1H), 2.46 (dd, *J* = 13.9, 5.4 Hz, 1H), 1.43 (d, *J* = 5.5 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.67, 170.44, 168.43, 160.44, 155.35, 130.28, 129.69, 113.40, 84.04, 79.58, 60.40, 55.26, 52.61, 51.75, 37.47, 28.29, 14.43. HRMS (ESI) cald. for $(M+H)^+$ $C_{21}H_{30}N_2O_7S$: 455.1846,found, 455.1885.



30

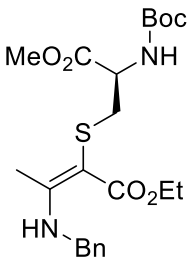
Ethyl (R,E)-3-amino-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(4-cyanophenyl)acrylate (30): yellow solid (Yield: 50%, 44.92 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.08 (s, 1H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 5.42 – 5.02 (m,

2H), 4.39 – 4.24 (m, 3H), 3.66 (s, 3H), 3.11 (dd, *J* = 13.9, 3.8 Hz, 1H), 2.51 (dd, *J* = 13.9, 5.3 Hz, 1H), 1.43 (d, *J* = 7.4 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.61, 169.88, 165.95, 155.19, 142.24, 132.05, 129.01, 118.17, 113.26, 85.46, 79.96, 60.79, 52.72, 51.80, 37.38, 28.35, 14.36. HRMS (ESI) cald. for $(M+H)^+$ $C_{21}H_{27}N_3O_6S$: 450.1693,found, 450.1696.



31

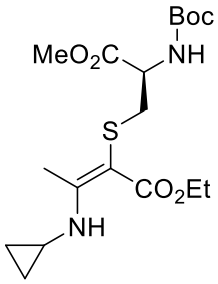
Methyl (E)-S-(2-amino-4-oxopent-2-en-3-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (31): light yellow solid (Yield: 62%, 41.22 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 10.91 – 10.70 (m, 1H), 6.14 (s, 1H), 5.41 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 7.3 Hz, 1H), 3.72 (s, 3H), 2.98 – 2.83 (m, 2H), 2.43 (s, 3H), 2.30 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.14, 171.65, 168.64, 155.22, 127.66, 95.91, 80.18, 52.81, 52.59, 38.99, 28.57, 28.27, 23.32. HRMS (ESI) cald. for $(M+H)^+$ $C_{14}H_{24}N_2O_5S$: 333.1478,found, 333.1473.



32

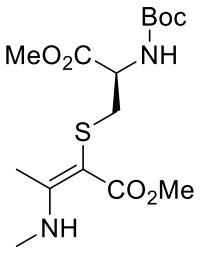
Ethyl (R,E)-3-(benzylamino)-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl) thio)but-2-enoate (32): yellow oil (Yield: 45%, 40.70 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 10.40 (t, *J* = 6.0 Hz, 1H), 7.35 (dd, *J* = 8.0, 6.6 Hz, 2H), 7.30 – 7.25 (m, 3H), 5.80 (d, *J* = 8.5

Hz, 1H), 4.47 (t, *J* = 7.0 Hz, 3H), 4.21 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.63 (s, 3H), 3.16 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.75 (dd, *J* = 13.8, 4.9 Hz, 1H), 2.40 (s, 3H), 1.45 (s, 9H), 1.38 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.96, 170.87, 169.12, 155.47, 137.50, 128.94, 127.68, 126.98, 82.72, 79.84, 60.12, 52.47, 48.22, 38.11, 28.35, 17.55, 14.43. HRMS (ESI) cald. for $(M+H)^+$ $C_{22}H_{32}N_2O_6S$: 453.2053,found, 453.2053.



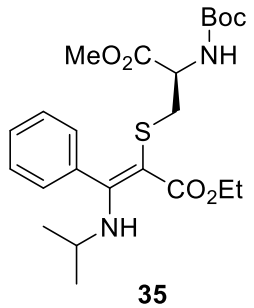
33

Ethyl (R,E)-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(cyclopropylamino)but-2-enoate (33): yellow oil (Yield: 71%, 57.11 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.96 (s, 1H), 5.80 (d, *J* = 8.5 Hz, 1H), 4.44 (dt, *J* = 9.0, 4.8 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.64 (s, 3H), 3.16 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.74 (dd, *J* = 13.8, 4.9 Hz, 1H), 2.64 – 2.59 (m, 1H), 2.51 (s, 3H), 1.46 (s, 9H), 1.37 (t, *J* = 7.1 Hz, 3H), 0.82 (td, *J* = 6.8, 4.9 Hz, 2H), 0.68 – 0.59 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.89, 171.17, 170.65, 155.43, 82.21, 79.79, 60.02, 52.27, 37.79, 28.31, 25.88, 18.31, 14.41, 7.95. HRMS (ESI) cald. for $(M+Na)^+$ $C_{18}H_{30}N_2O_6S$: 425.1716,found, 425.1714.



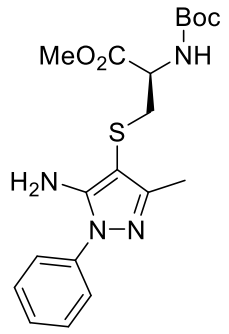
34

Methyl (R,E)-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(methyla mino)but-2-enoate (34): yellow oil (Yield: 65%, 47.08 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 10.07 – 9.91 (m, 1H), 5.82 (d, *J* = 8.4 Hz, 1H), 4.40 (dt, *J* = 9.2, 5.0 Hz, 1H), 3.76 (s, 3H), 3.66 (s, 3H), 3.10 (dd, *J* = 13.8, 5.1 Hz, 1H), 2.98 (d, *J* = 5.1 Hz, 3H), 2.73 (dd, *J* = 13.8, 4.9 Hz, 1H), 2.38 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.97, 171.21, 170.31, 155.53, 81.60, 79.75, 52.41, 51.30, 38.36, 30.91, 28.33, 17.21. HRMS (ESI) cald. for $(M+Na)^+$ $C_{15}H_{26}N_2O_6S$:385.1403,found, 385.1410.



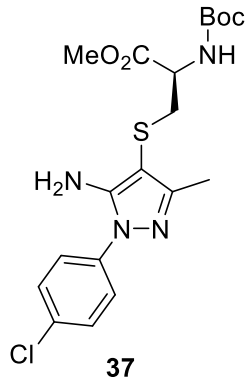
35

Ethyl (R,E)-2-((2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thio)-3-(isopropyla mino)-3-phenylacrylate (35): light yellow solid (Yield: 64%, 59.67 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 9.89 (d, *J* = 9.6 Hz, 1H), 7.50 – 7.43 (m, 3H), 7.31 (d, *J* = 7.2 Hz, 1H), 7.24 (d, *J* = 6.7 Hz, 1H), 5.60 (d, *J* = 8.8 Hz, 1H), 4.37 (dt, *J* = 9.0, 4.6 Hz, 1H), 4.32 – 4.23 (m, 2H), 3.67 (s, 3H), 3.17 (dt, *J* = 9.5, 6.4 Hz, 1H), 3.05 (dd, *J* = 13.8, 3.9 Hz, 1H), 2.38 (dd, *J* = 13.8, 5.2 Hz, 1H), 1.45 (d, *J* = 9.4 Hz, 12H), 1.08 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.89, 171.05, 169.97, 155.50, 135.39, 128.73, 128.43, 128.27, 127.41, 81.76, 79.63, 60.21, 52.57, 51.84, 47.08, 38.15, 28.37, 24.06, 14.46. HRMS (ESI) cald. for (M+H)+ $C_{23}H_{34}N_2O_6S$: 467.2210,found, 467.2207.



36

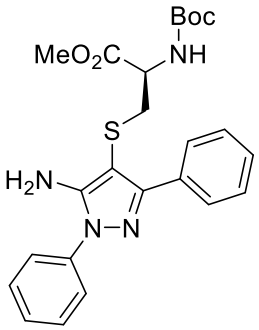
Methyl S-(5-amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (36): yellow oil (Yield: 72%, 58.49 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.54 (d, *J* = 7.4 Hz, 2H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 5.52 (d, *J* = 8.5 Hz, 1H), 4.51 (s, 2H), 3.68 (s, 3H), 3.05 – 2.87 (m, 2H), 2.29 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.50, 155.41, 152.44, 148.47, 138.55, 129.58, 127.43, 123.56, 89.39, 80.28, 53.78, 52.53, 38.80, 28.30, 12.33. HRMS (ESI) cald. for $(M+H)^+$ $C_{19}H_{26}N_4O_4S$: 407.1747,found, 407.1769.



37

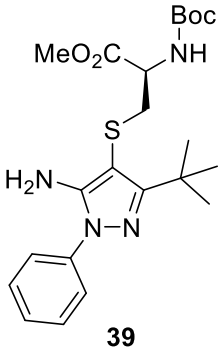
Methyl S-(5-amino-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (37) : yellow oil ((Yield: 62%, 54.58 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.51 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 5.49 (d, *J* = 8.5 Hz, 1H), 4.55 – 4.47 (m, 2H), 3.68 (s, 3H), 2.93 (qd, *J* = 13.9, 5.3 Hz, 2H), 2.27 (s, 3H), 1.44 (d, *J* = 11.3 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.45, 155.40, 152.80, 148.60, 137.13, 132.93, 129.68, 124.62, 89.96,

80.33, 53.79, 52.55, 38.84, 28.29, 12.30. HRMS (ESI) cald. for $(M+H)^+$ $C_{19}H_{25}ClN_4O_4S$: 441.1357,found, 441.1313.



38

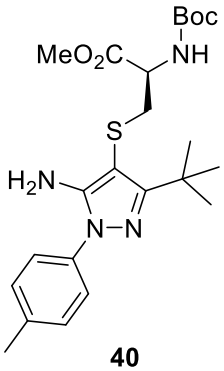
Methyl S-(5-amino-1,3-diphenyl-1H-pyrazol-4-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (38) : yellow oil (Yield: 45%, 42.14 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 8.09 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.51 – 7.32 (m, 6H), 5.50 (d, *J* = 8.4 Hz, 1H), 4.65 – 4.56 (m, 1H), 4.50 – 4.42 (m, 1H), 3.74 (s, 1H), 3.60 (s, 3H), 2.94 (d, *J* = 5.3 Hz, 2H), 1.43 (d, *J* = 17.0 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.35, 155.34, 152.32, 149.48, 138.53, 132.93, 129.63, 128.34, 128.28, 127.73, 127.52, 123.77, 88.31, 80.17, 53.58, 52.48, 38.84, 28.32. HRMS (ESI) cald. for $(M+K)^+$ $C_{24}H_{28}N_4O_4S$: 507.1462,found, 507.1427.



39

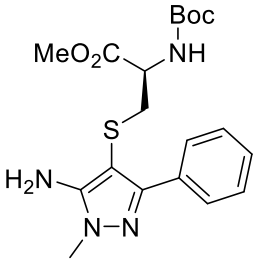
Methyl S-(5-amino-3-(tert-butyl)-1-phenyl-1H-pyrazol-4-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (39) : brown oil (Yield: 40%, 35.86 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 8.09 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.51 – 7.32 (m, 6H), 5.50 (d, *J* = 8.4 Hz, 1H), 4.65 –

4.56 (m, 1H), 4.50 – 4.42 (m, 1H), 3.60 (s, 3H), 2.94 (d, *J* = 5.3 Hz, 2H), 1.43 (d, *J* = 17.0 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.70, 162.05, 155.43, 149.60, 138.75, 129.53, 127.19, 123.52, 87.82, 80.23, 54.26, 52.62, 40.59, 33.71, 29.59, 28.31. HRMS (ESI) cald. for $(M+Na)^+$ $C_{22}H_{32}N_4O_4S$: 471.2036,found, 471.2024.



40

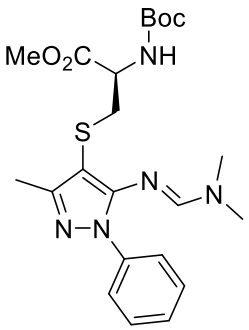
Methyl S-(5-amino-3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-4-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (40) : brown oil (Yield: 53%, 48.99 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.43 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 5.75 (d, *J* = 8.7 Hz, 1H), 4.70 – 4.40 (m, 3H), 3.74 (s, 3H), 3.02 (dd, *J* = 13.6, 4.1 Hz, 1H), 2.93 (dd, *J* = 13.7, 6.4 Hz, 1H), 2.37 (s, 3H), 1.43 (s, 9H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.71, 161.76, 155.42, 149.48, 137.11, 136.27, 130.02, 123.53, 87.44, 80.16, 54.25, 52.57, 40.51, 33.67, 29.62, 28.31, 21.08. HRMS (ESI) cald. for $(M+Na)^+$ $C_{23}H_{34}N_4O_4S$: 485.2193,found, 485.2197.



41

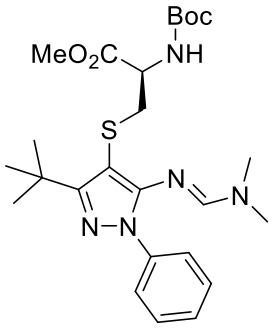
Methyl S-(5-amino-1-methyl-3-phenyl-1H-pyrazol-4-yl)-N-(tert-butoxycarbonyl)-L-cystein

ate (41) : brown oil (Yield: 39%, 31.68 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 8.04 – 7.94 (m, 2H), 7.43 – 7.37 (m, 2H), 7.36 – 7.30 (m, 1H), 5.42 – 5.21 (m, 1H), 4.46 – 4.35 (m, 1H), 4.25 (s, 2H), 3.70 (s, 3H), 3.60 (s, 3H), 2.90 – 2.80 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.42, 155.32, 150.92, 149.54, 133.18, 128.33, 127.97, 127.27, 88.17, 80.18, 53.49, 52.43, 39.18, 35.02, 28.30. HRMS (ESI) cald. for $(M+H)^+$ $C_{19}H_{26}N_4O_4S$: 407.1747,found, 407.1746.



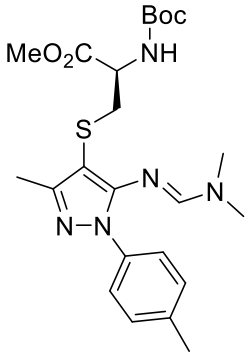
42

Methyl (E)-N-(tert-butoxycarbonyl)-S-(5-((((dimethylamino)methylene)amino)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-L-cysteinate (42) : light yellow oil (Yield: 63%, 58.11 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.81 – 7.60 (m, 3H), 7.28 (t, *J* = 7.7 Hz, 2H), 7.12 (t, *J* = 7.3 Hz, 1H), 5.85 (d, *J* = 8.9 Hz, 1H), 4.47 (dt, *J* = 8.8, 4.3 Hz, 1H), 3.53 (s, 3H), 3.10 (dd, *J* = 14.0, 4.0 Hz, 1H), 3.04 (s, 3H), 2.95 (s, 3H), 2.69 (dd, *J* = 14.0, 4.3 Hz, 1H), 2.25 (s, 3H), 1.32 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.03, 156.35, 155.42, 152.53, 152.13, 139.93, 128.36, 125.78, 123.19, 95.16, 79.66, 53.52, 52.26, 40.29, 38.15, 34.38, 28.32, 12.70. HRMS (ESI) cald. for $(M+K)^+$ $C_{22}H_{31}N_5O_4S$: 500.1728,found, 500.1723.



43

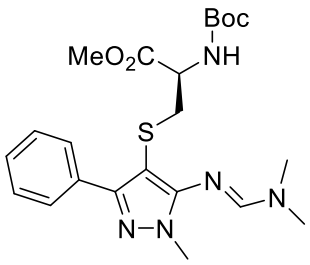
Methyl (E)-N-(tert-butoxycarbonyl)-S-(3-(tert-butyl)-5-(((dimethylamino)methylene)amino)-1-phenyl-1H-pyrazol-4-yl)-L-cysteinate (43) : light yellow solid (Yield: 50%, 50.33 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.84 (d, *J* = 7.9 Hz, 2H), 7.51 (s, 1H), 7.35 (t, *J* = 7.8 Hz, 2H), 7.18 (t, *J* = 7.4 Hz, 1H), 6.69 (d, *J* = 9.5 Hz, 1H), 4.74 – 4.49 (m, 1H), 3.69 (s, 3H), 3.25 (dd, *J* = 14.0, 4.5 Hz, 1H), 3.19 (s, 3H), 3.01 (s, 3H), 2.69 (dd, *J* = 14.0, 3.5 Hz, 1H), 1.47 (s, 9H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.38, 161.56, 157.11, 155.83, 153.91, 140.09, 128.38, 125.60, 123.10, 95.10, 79.43, 53.97, 52.16, 40.77, 40.29, 34.41, 33.98, 29.66, 28.40. HRMS (ESI) cald. for $(M+Na)^+$ $C_{25}H_{37}N_5O_4S$:526.2458,found, 526.2415.



44

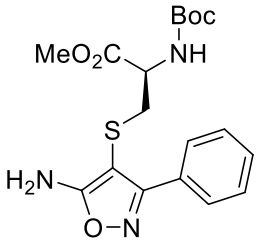
Methyl (E)-N-(tert-butoxycarbonyl)-S-(5-(((dimethylamino)methylene)amino)-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-L-cysteinate (44) : colorless oil (Yield: 40%, 38.02 mg), ^{1}H NMR (400 MHz, DMSO-d6) δ 8.10 (s, 1H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.19 (d, *J* = 6.7 Hz, 3H), 4.03 (d,

J = 9.3 Hz, 1H), 3.58 (s, 3H), 3.04 (s, 3H), 2.94 (s, 3H), 2.77 (s, 2H), 2.31 (s, 3H), 2.18 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 171.95, 156.60, 155.77, 152.79, 151.68, 137.97, 135.08, 129.24, 122.73, 94.30, 78.78, 55.34, 53.84, 52.44, 39.36, 34.24, 28.59, 20.96, 12.94. HRMS (ESI) cald. for $(M+Na)^+$ $C_{23}H_{33}N_5O_4S$: 498.2145,found, 498.2139.



45

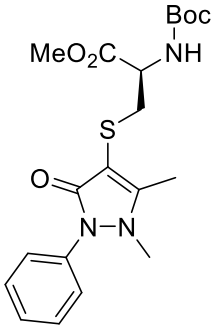
Methyl (E)-N-(tert-butoxycarbonyl)-S-(5-(((dimethylamino)methylene)amino)-1-methyl-3-phenyl-1H-pyrazol-4-yl)-L-cysteinate (45) : yellow oil (Yield: 40%, 36.90 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 8.03 – 7.88 (m, 3H), 7.38 – 7.23 (m, 3H), 5.40 (d, *J* = 8.8 Hz, 1H), 4.30 (d, *J* = 8.2 Hz, 1H), 3.66 (s, 3H), 3.50 (s, 3H), 3.06 (d, *J* = 16.2 Hz, 6H), 2.94 (d, *J* = 13.2 Hz, 1H), 2.63 (d, *J* = 12.6 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.99, 156.35, 155.24, 153.10, 151.19, 133.70, 128.22, 127.69, 127.47, 91.63, 79.63, 53.25, 52.18, 40.39, 38.60, 34.98, 34.19, 28.34. HRMS (ESI) cald. for $(M+H)^+$ $C_{22}H_{31}N_5O_4S$: 462.2169,found, 462.2168.



46

Methyl S-(5-amino-3-phenylisoxazol-4-yl)-N-(tert-butoxycarbonyl)-L-cysteinate (46) : yellow oil (Yield: 55%, 43.25 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.45 (q, *J* = 3.3 Hz,

3H), 7.41 – 7.32 (m, 2H), 7.19 – 6.92 (m, 1H), 6.26 – 5.65 (m, 2H), 4.96 – 4.62 (m, 1H), 3.76 (s, 3H), 3.58 (ddd, *J* = 14.7, 12.7, 3.5 Hz, 1H), 3.11 (td, *J* = 14.1, 7.5 Hz, 1H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.40, 165.47, 159.66, 155.36, 130.13, 128.80, 127.92, 85.57, 80.31, 53.67, 52.69, 40.40, 29.70, 28.36. HRMS (ESI) cald. for $(M+H)^+$ $C_{18}H_{23}N_3O_5S$: 394.1431,found, 394.1430.



47

Methyl N-(tert-butoxycarbonyl)-S-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-L-cysteinate (47) : yellow oil (Yield: 35%, 29.48 mg), ^{1}H NMR (400 MHz, Chloroform-d) δ 7.50 – 7.42 (m, 2H), 7.38 – 7.33 (m, 2H), 7.33 – 7.28 (m, 1H), 6.21 (s, 1H), 4.44 (s, 1H), 3.62 (s, 3H), 3.35 – 3.26 (m, 1H), 3.10 – 2.90 (m, 1H), 2.35 (s, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.59, 165.12, 158.58, 155.55, 134.67, 129.27, 127.09, 124.36, 99.12, 79.67, 53.03, 52.24, 35.79, 28.33, 12.05. HRMS (ESI) cald. for $(M+H)^+$ $C_{20}H_{27}N_3O_5S$: 422.1744,found, 422.1749.

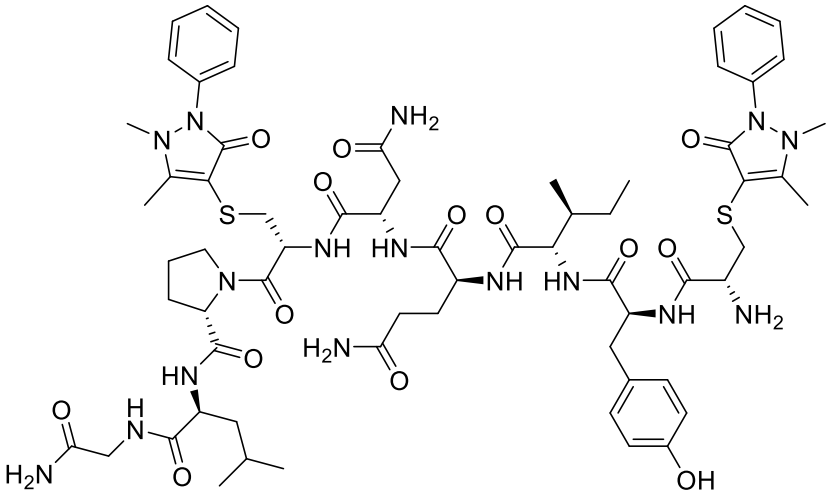
4.4 Electrochemical peptides functionalization[3]

Bioconjugation of Oxytocin Skeleton:

Synthesis of compound (49) : In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar, Oxytocin Skeleton (0.005 mmol), Antipyine (2 equiv.), ^{n}Bu$_4$NBr (2 equiv.),

$NiBr_2$•diglyme (20 mol%), MeCN (6 mL), $CHCl_3$ (1 mL), were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.3 mm) as the anode and platinum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 6 mA under room temperature for 1 h. After completion of the reaction, the solution was analyzed by LC-MS spectroscopy. The reaction was analyzed by reverse phase HPLC using 100% buffer B over 30 minutes on an Agilent Zorbax SB-Aq 5μm column of 250 mm length. HPLC analysis used buffers A (water) and B (acetonitrile + 0.1% TFA). Conversion reported as a % conversion as determined.

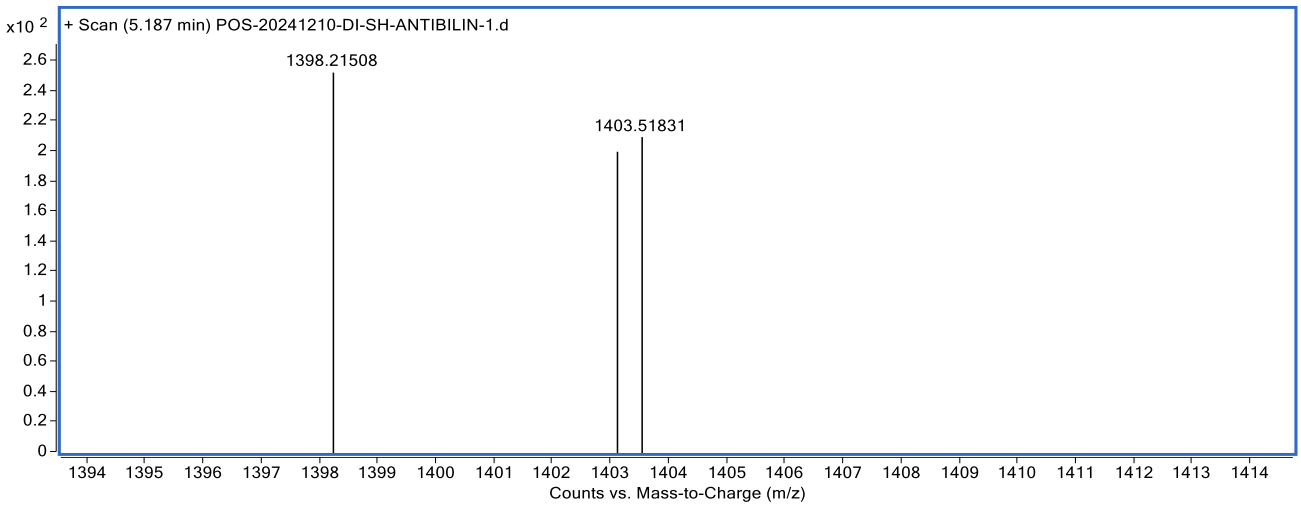
bioconjugated product 49 :



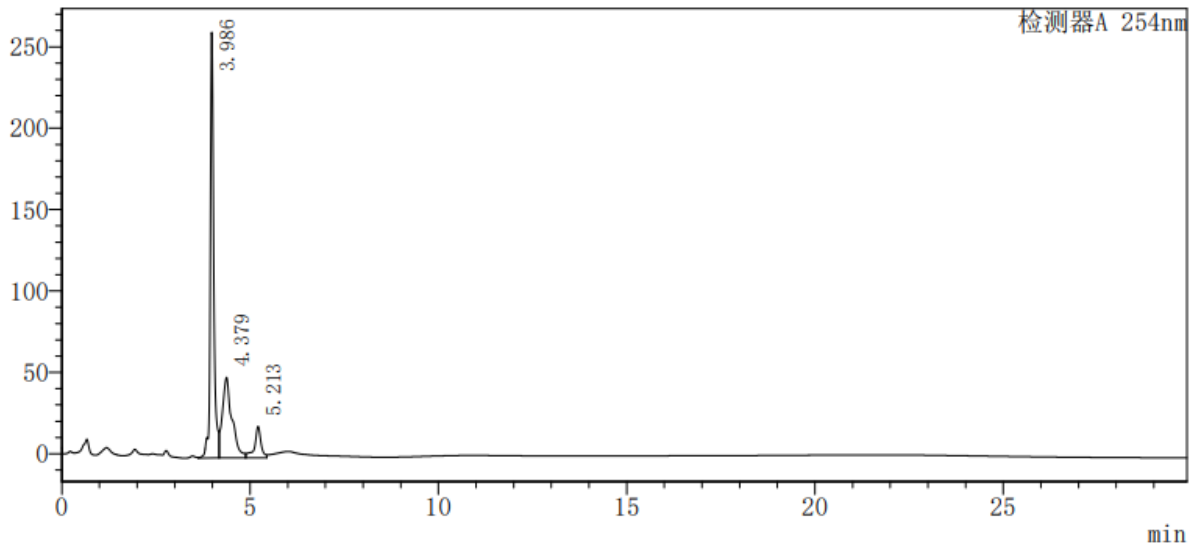
HPLC: 93% conversion.

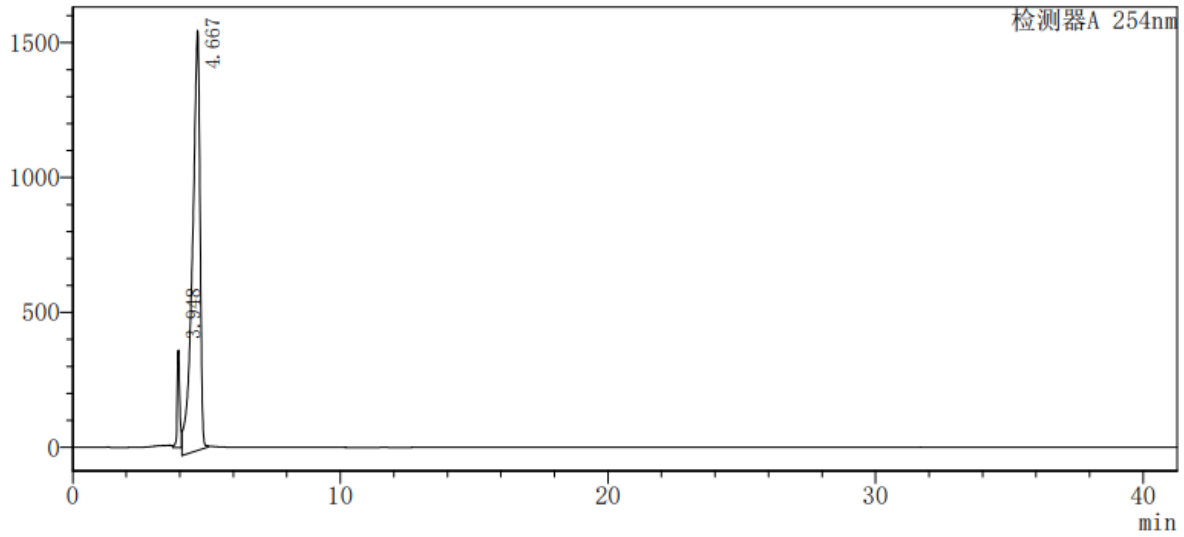
After the reaction finished, there is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 4.667 min. Polypeptide is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.986 min.

HRMS (ESI-TOF) calcd for $C_{65}H_{88}N_{16}O_{14}S_2$, $[M+Na]^+$, 1403.5999, found 1403.51831.



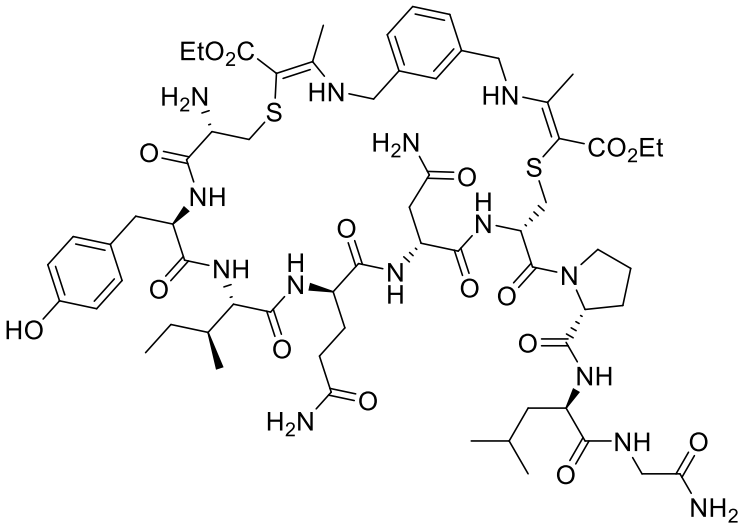
HPLC Spectra:





Synthesis of compound (50) : In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar,Oxytocin Skeleton (0.005 mmol), Bisenamine (4 equiv.), $^{n}Bu_4NBr$ (2 equiv.), $NiBr_2$•diglyme (20 mol%), MeCN (6 mL), $CHCl_3$ (1 mL), were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.3 mm) as the anode and platinum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 6 mA under room temperature for 1.5 h. After completion of the reaction, the solution was analyzed by LC-MS spectroscopy. The reaction was analyzed by reverse phase HPLC using 100% buffer B over 30 minutes on an Agilent Zorbax SB-Aq 5μm column of 250 mm length. HPLC analysis used buffers A (water) and B (acetonitrile + 0.1% TFA). Conversion reported as a % conversion as determined.

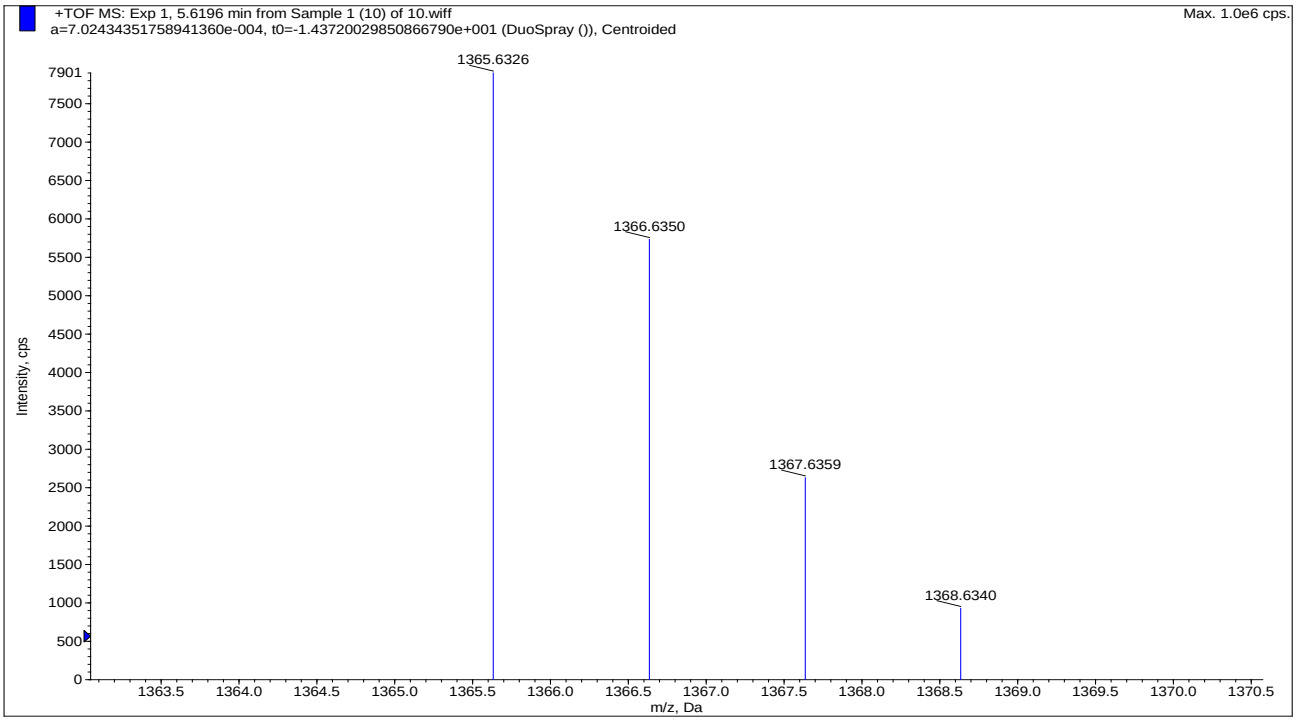
bioconjugated product 50 :



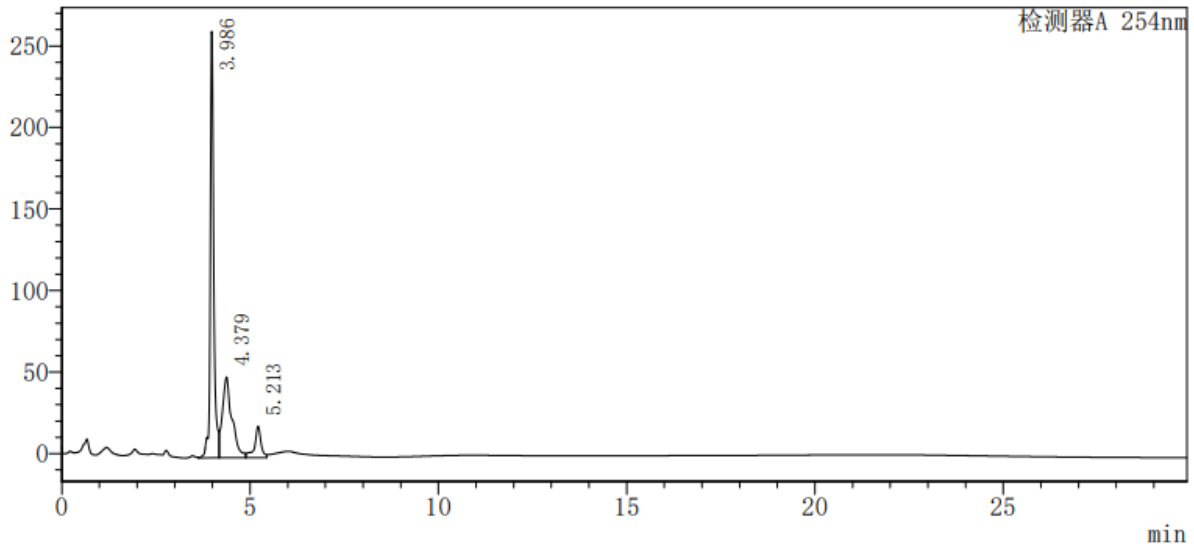
HPLC: 50% conversion.

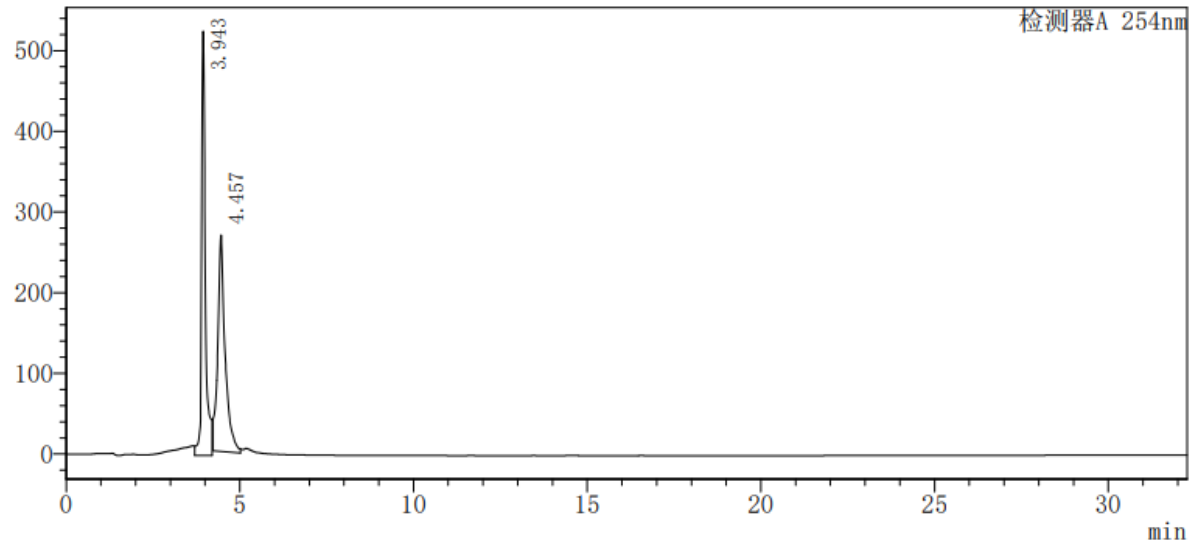
After the reaction finished, there is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 4.457 min. Polypeptide is a peak that elutes at 100% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.986 min.

HRMS (ESI-TOF) calcd for $C_{63}H_{92}N_{14}O_{16}S_2$, $[M+H]^+$, 1365.6330, found 1365.6326.

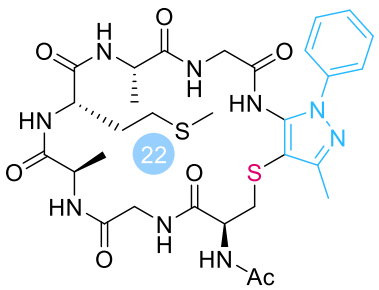


HPLC Spectra:





4.5 Electrochemical peptides cyclization

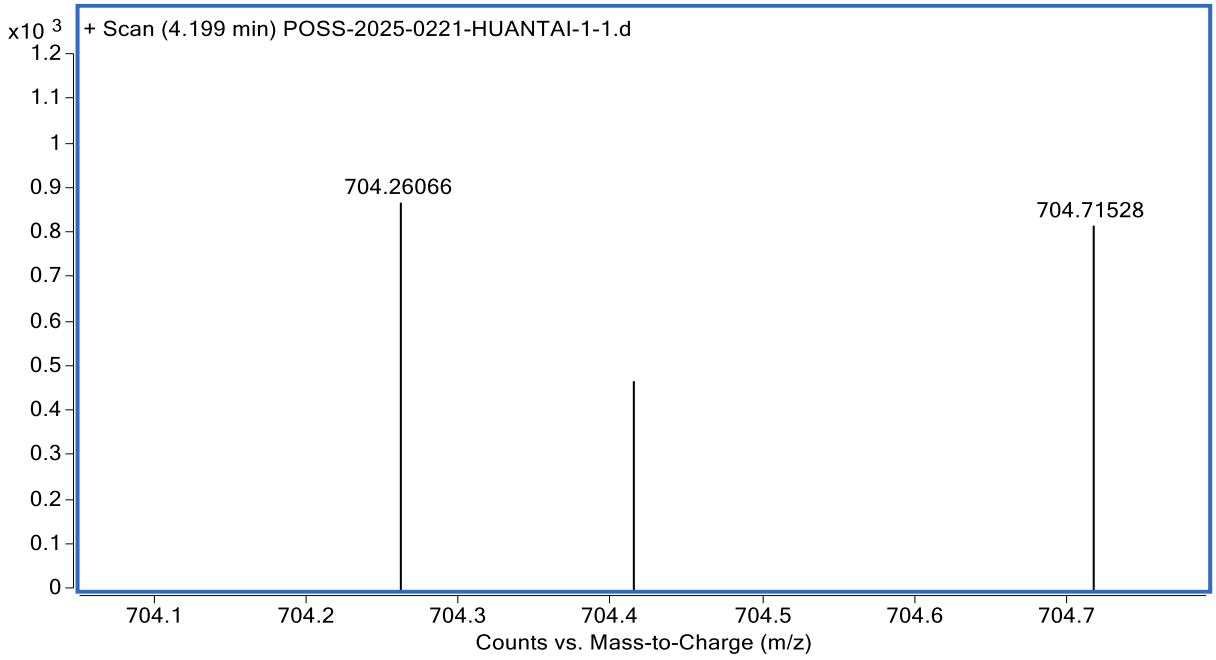


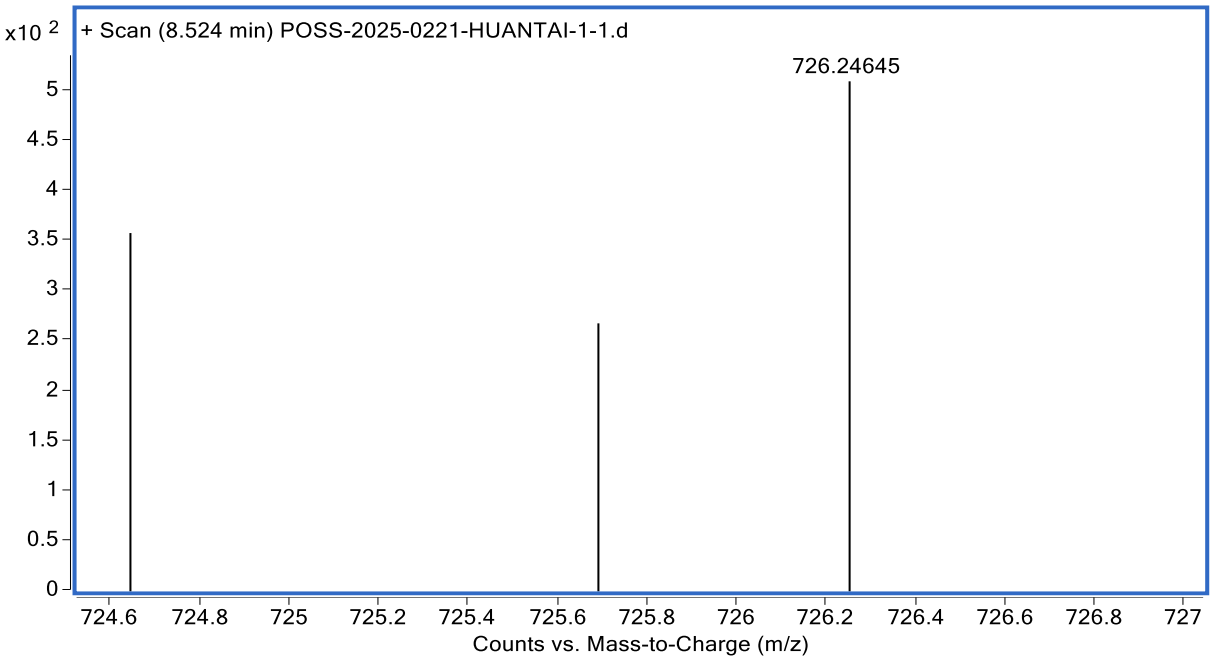
52[e], 85% conv
75% (after HPLC purification)

HPLC: 85% conversion.

After the reaction was completed, a peak was observed with a retention time of 5.88 minutes.The polypeptide corresponds to the peak eluting at a retention time of 5.96 minutes.

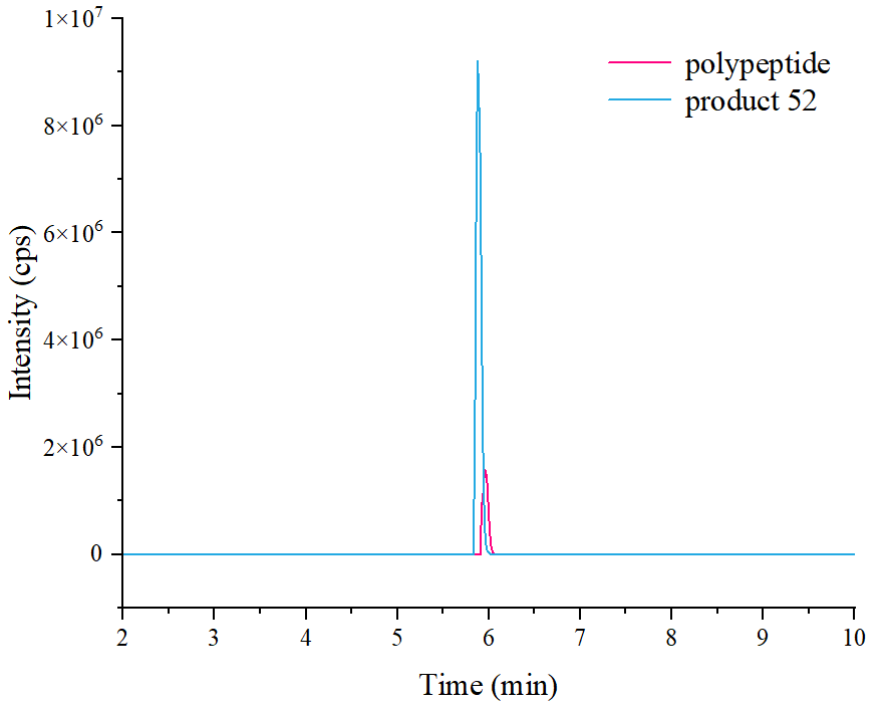
HRMS (ESI-TOF) calcd for $C_{30}H_{41}N_9O_7S_2$, $[M+H]^+$, 704.26431, found 704.26066. calcd for $C_{30}H_{41}N_9O_7S_2$, $[M+Na]^+$, 726.24626, found 726.24645.



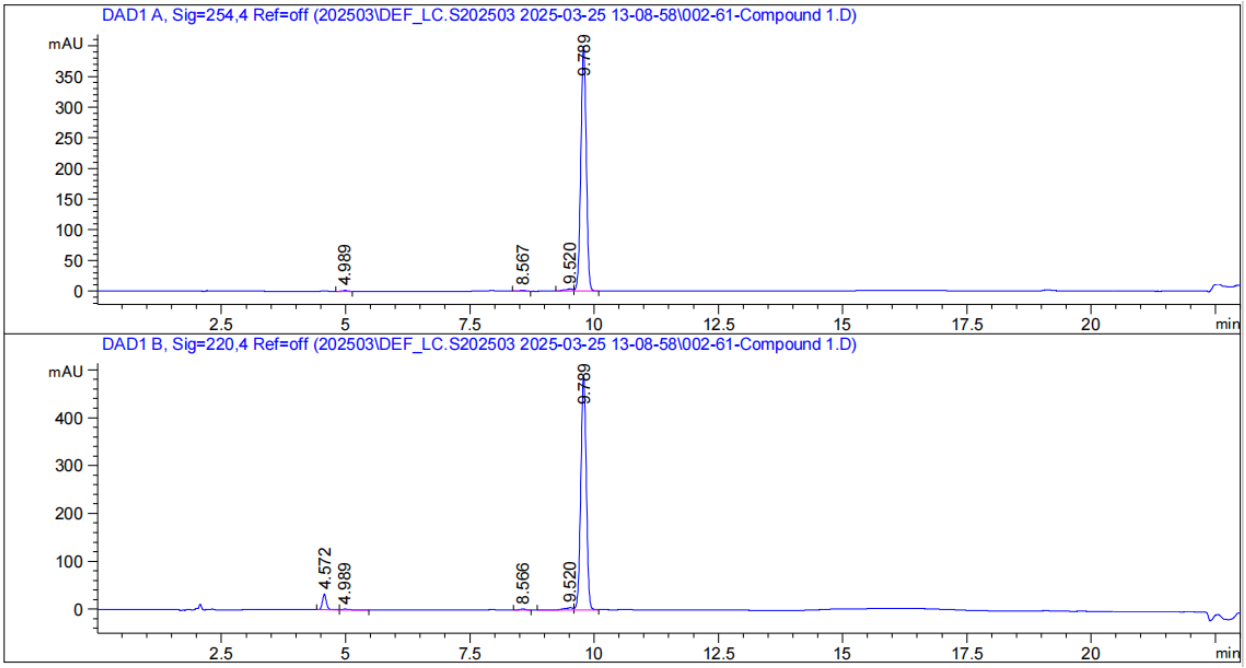


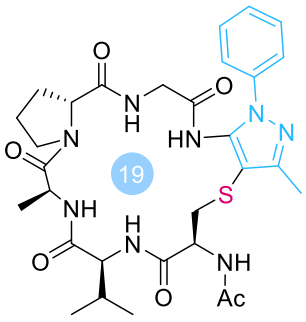
HPLC Spectra:

(1)



(2) After HPLC purification



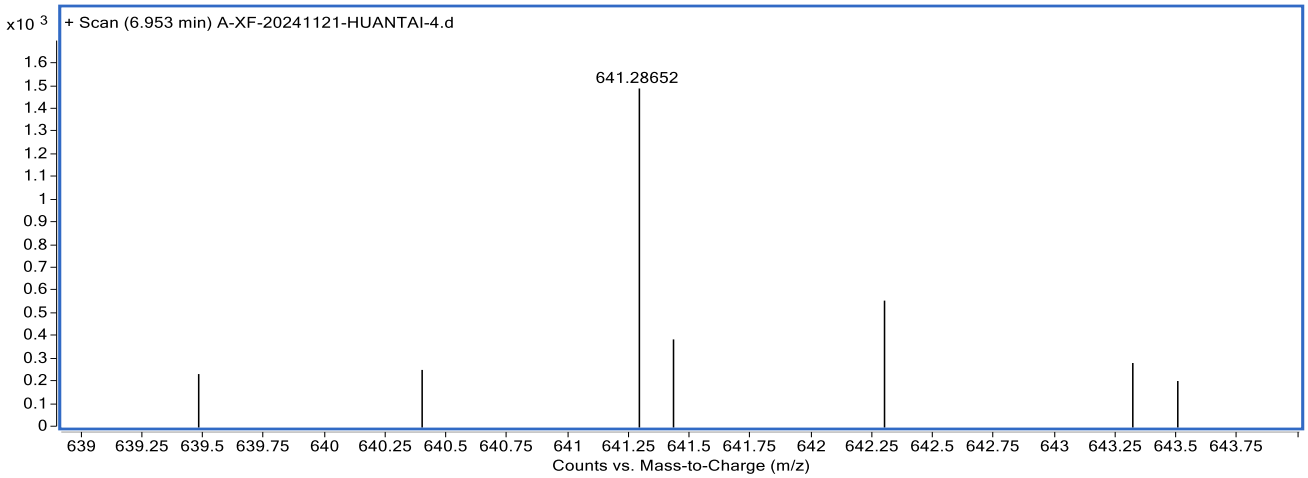


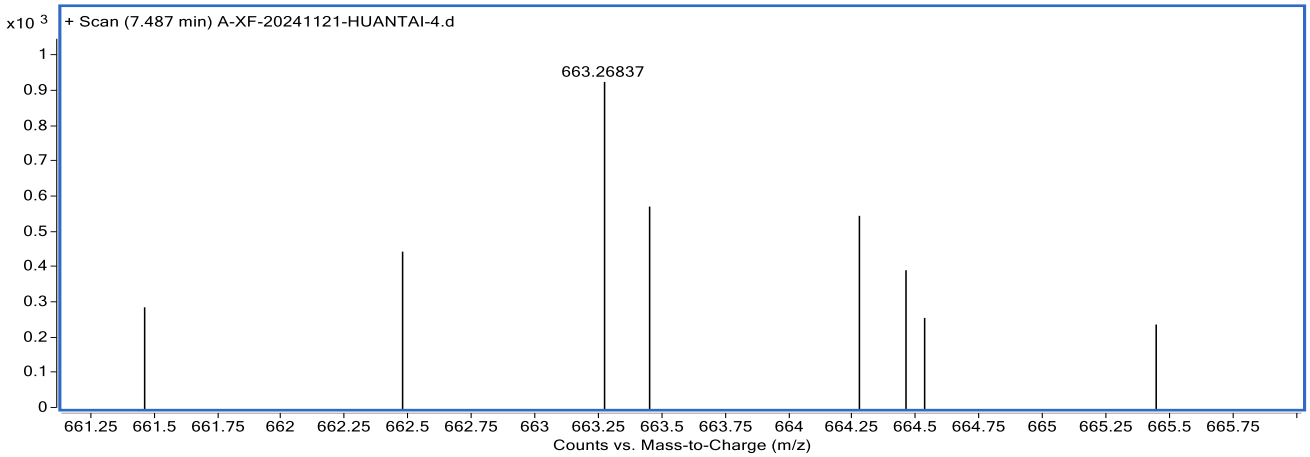
53[e], 32% conv
28% (after HPLC purification)

HPLC: 32% conversion.

After the reaction was completed, a peak was observed with a retention time of 6.04 minutes.The polypeptide corresponds to the peak eluting at a retention time of 6.52 minutes.

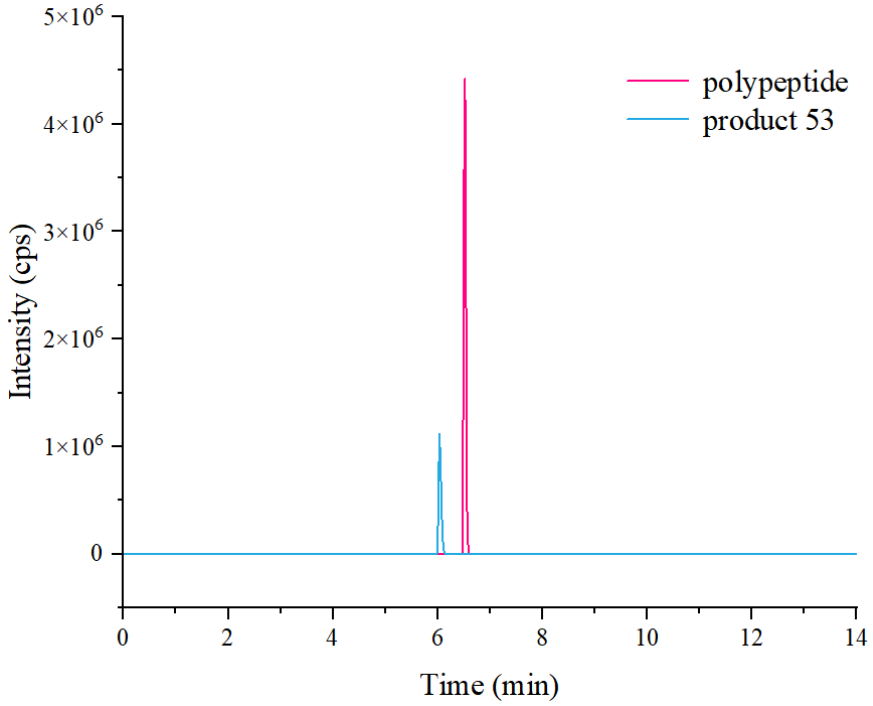
HRMS (ESI-TOF) calcd for $C_{30}H_{40}N_8O_6S$, $[M+H]^+$, 641.28643, found 641.28652.calcd for $C_{30}H_{40}N_8O_6S$, $[M+Na]^+$, 663.26837, found 663.26837.



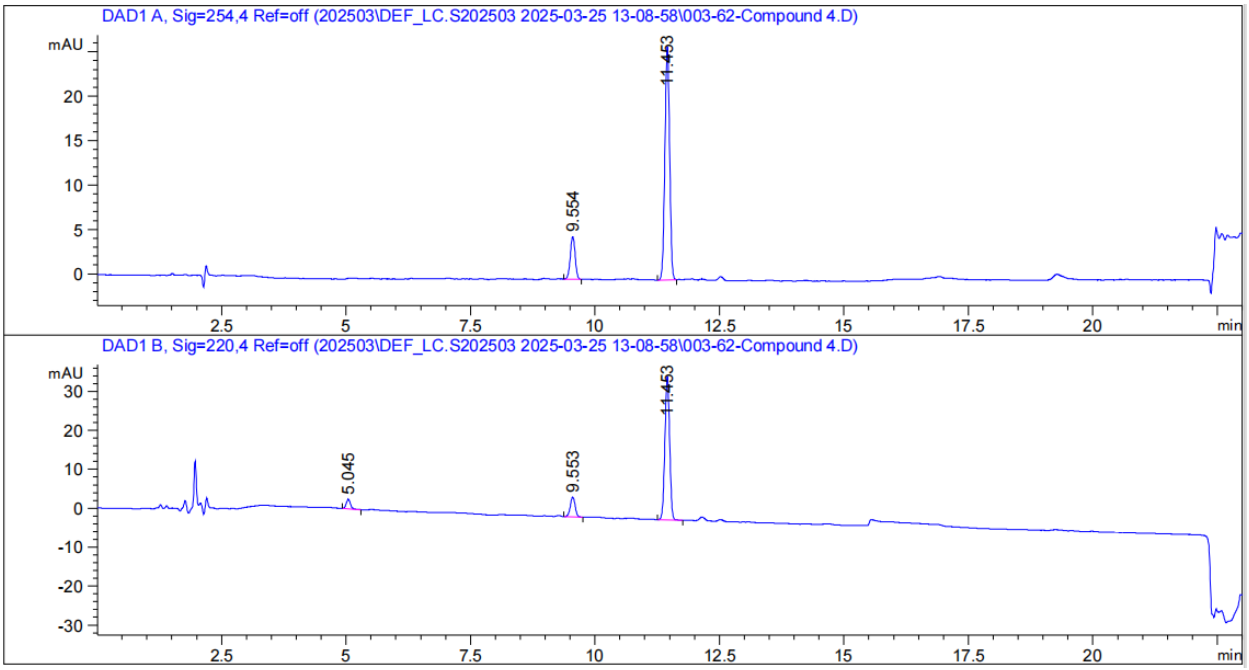


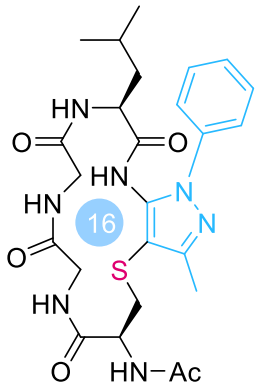
HPLC Spectra:

(1)



(2) After HPLC purification



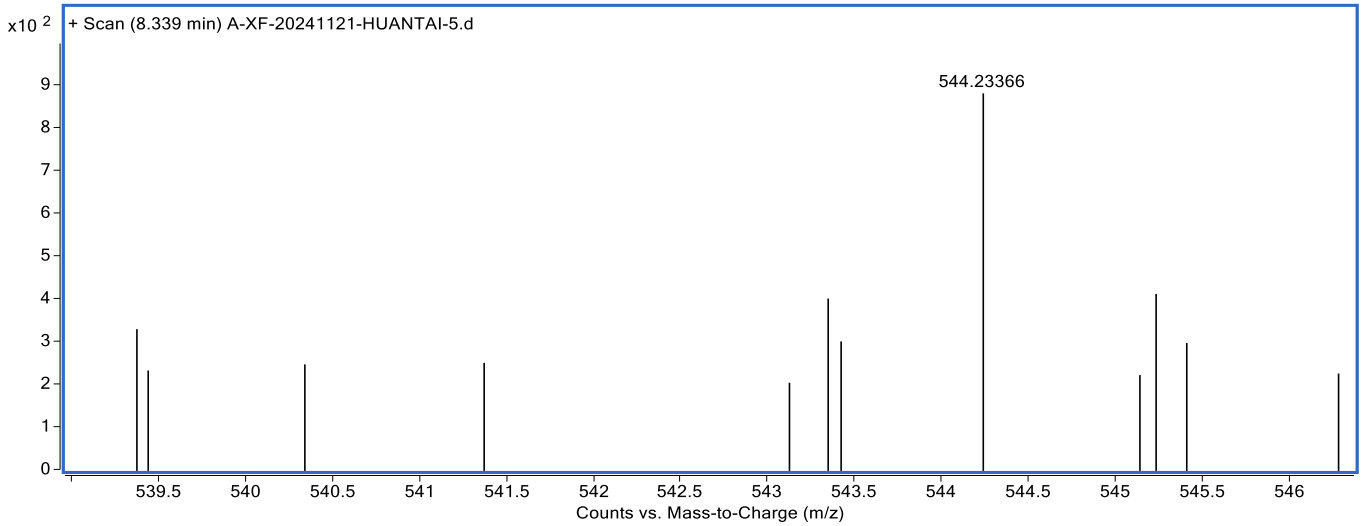


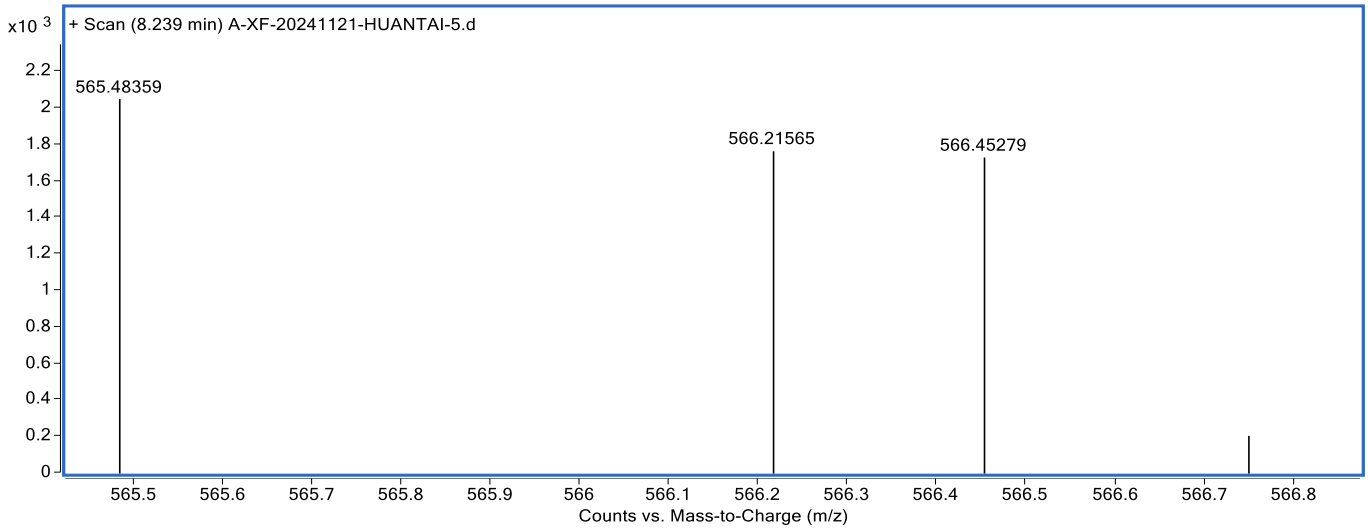
54[e], 98% conv
85% (after HPLC purification)

HPLC: 98% conversion.

After the reaction was completed, a peak was observed with a retention time of 6.26 minutes.The polypeptide corresponds to the peak eluting at a retention time of 6.81 minutes.

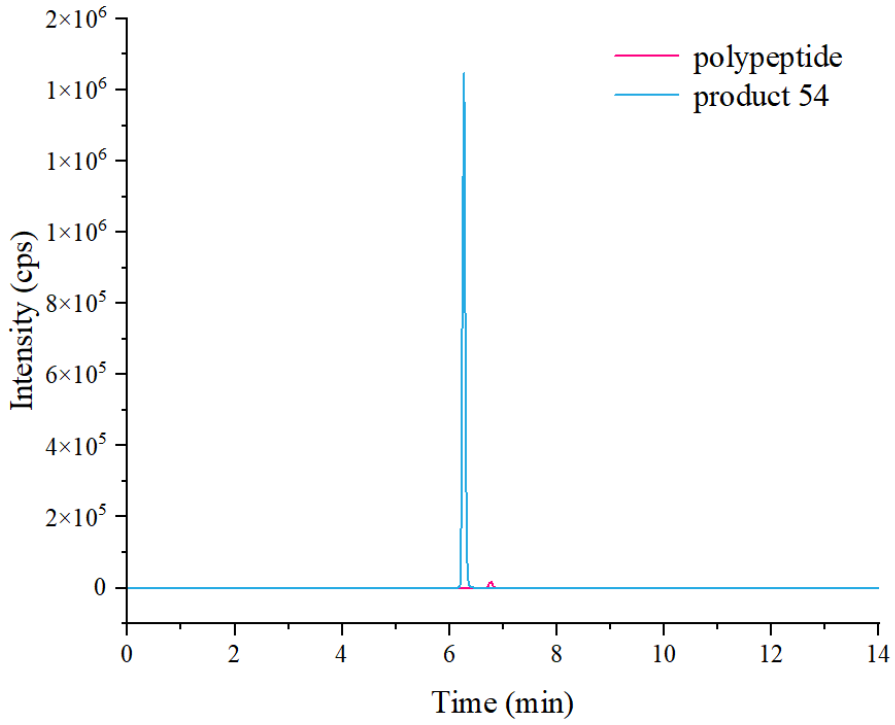
HRMS (ESI-TOF) calcd for $C_{25}H_{33}N_7O_5S$, $[M+H]^+$, 544.23366, found 544.23366.calcd for $C_{25}H_{33}N_7O_5S$, $[M+Na]^+$, 566.21561, found 566.21565



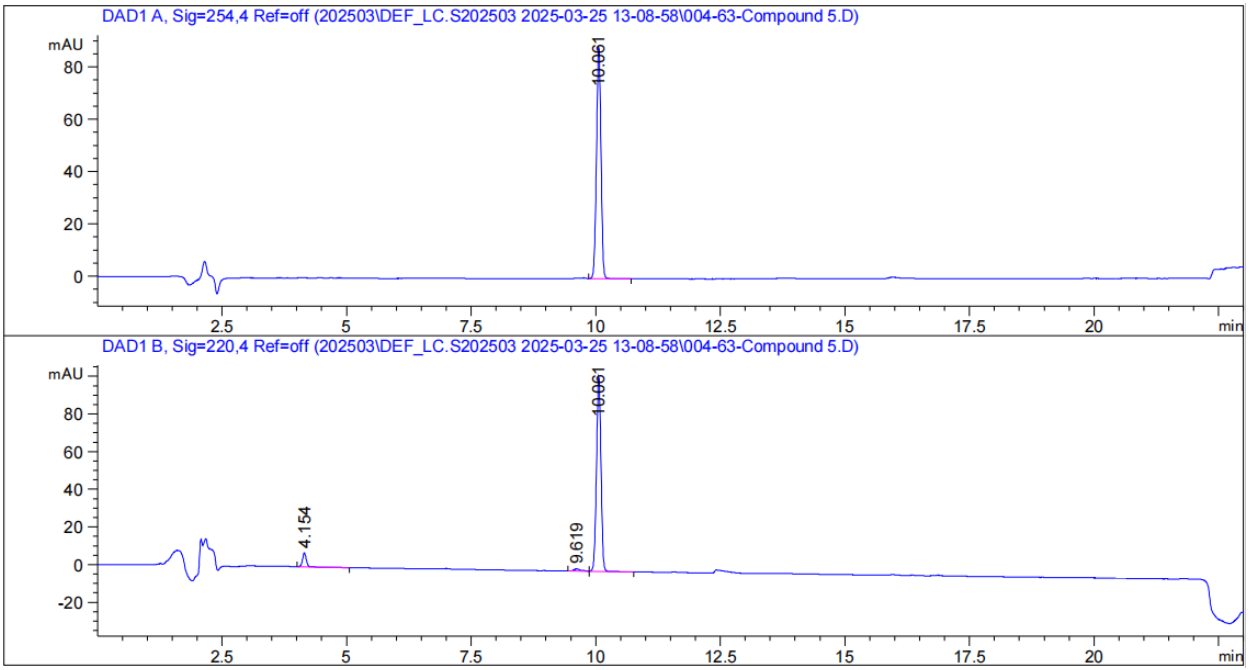


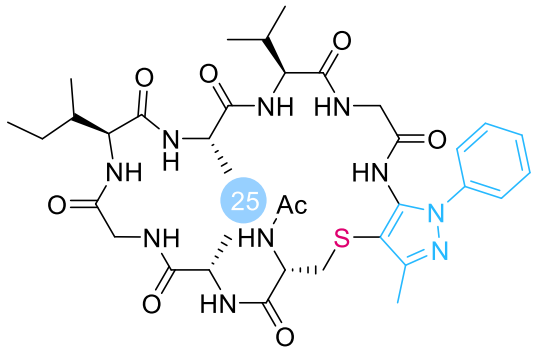
HPLC Spectra:

(1)



(2) After HPLC purification



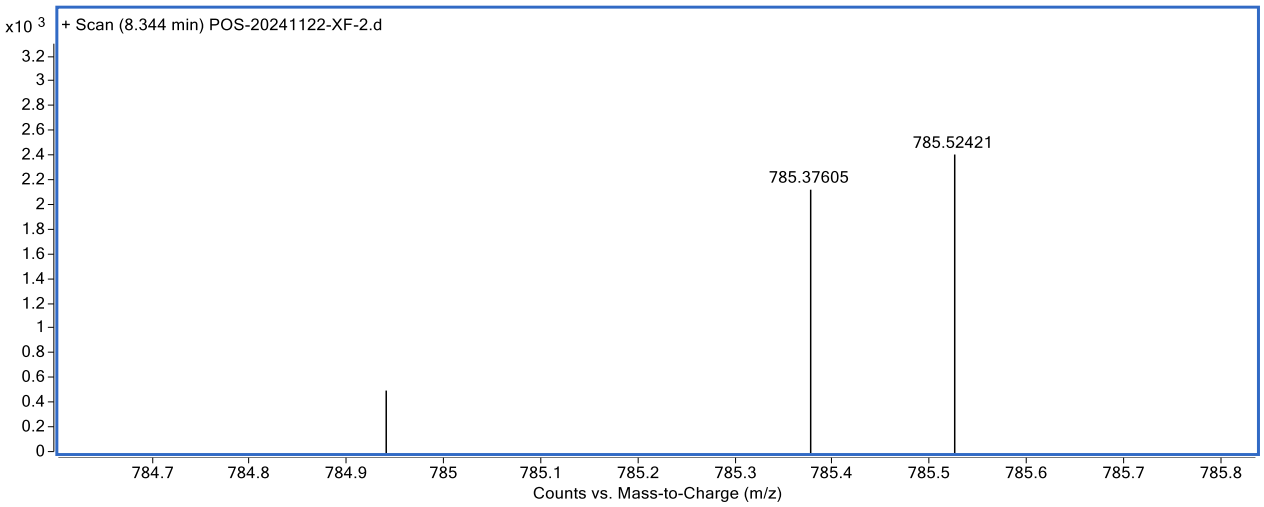


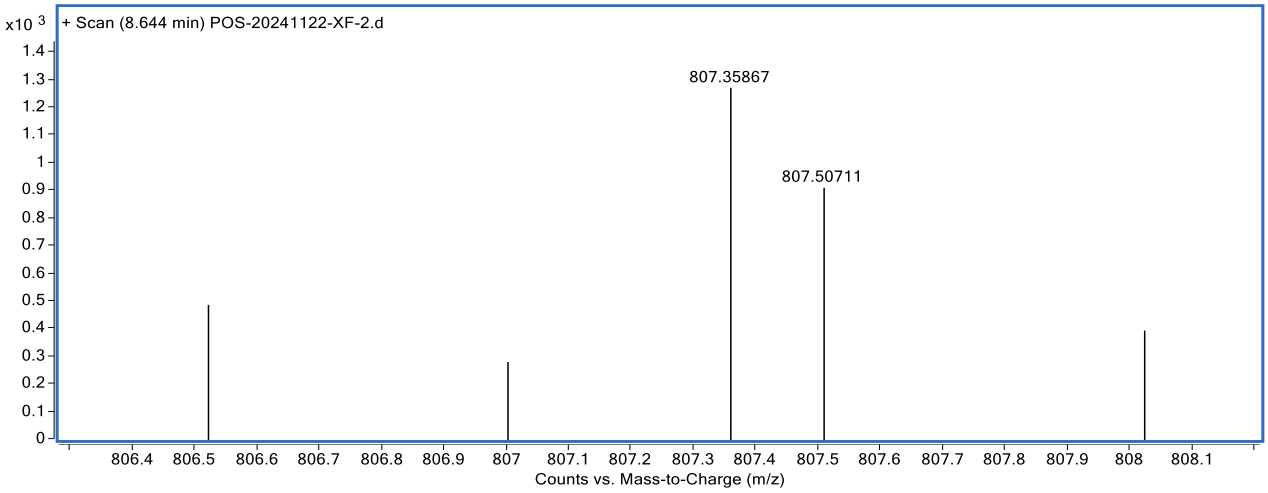
55[f], 91% conv

HPLC: 91% conversion.

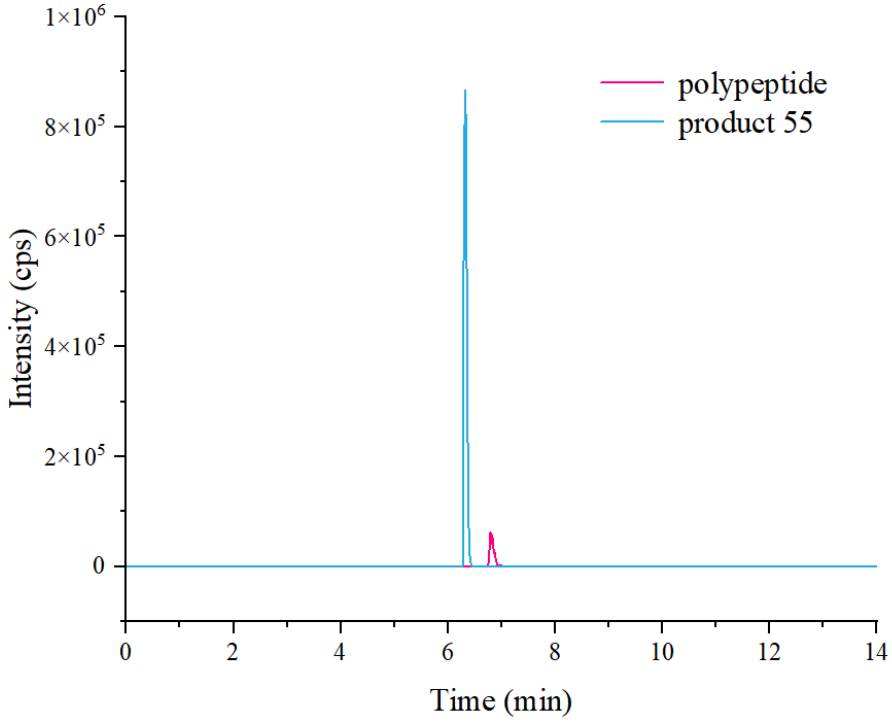
After the reaction was completed, a peak was observed with a retention time of 6.32 minutes.The polypeptide corresponds to the peak eluting at a retention time of 6.79 minutes.

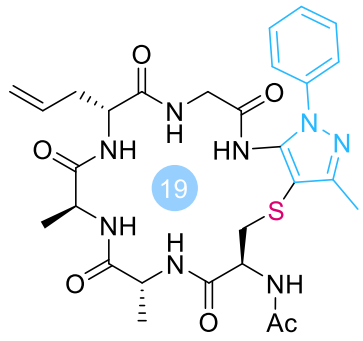
HRMS (ESI-TOF) calcd for $C_{36}H_{52}N_{10}O_8S$, $[M+H]^+$, 785.37631, found 785.37605.calcd for $C_{36}H_{52}N_{10}O_8S$, $[M+Na]^+$, 807.35825, found 807.35867.





HPLC Spectra:



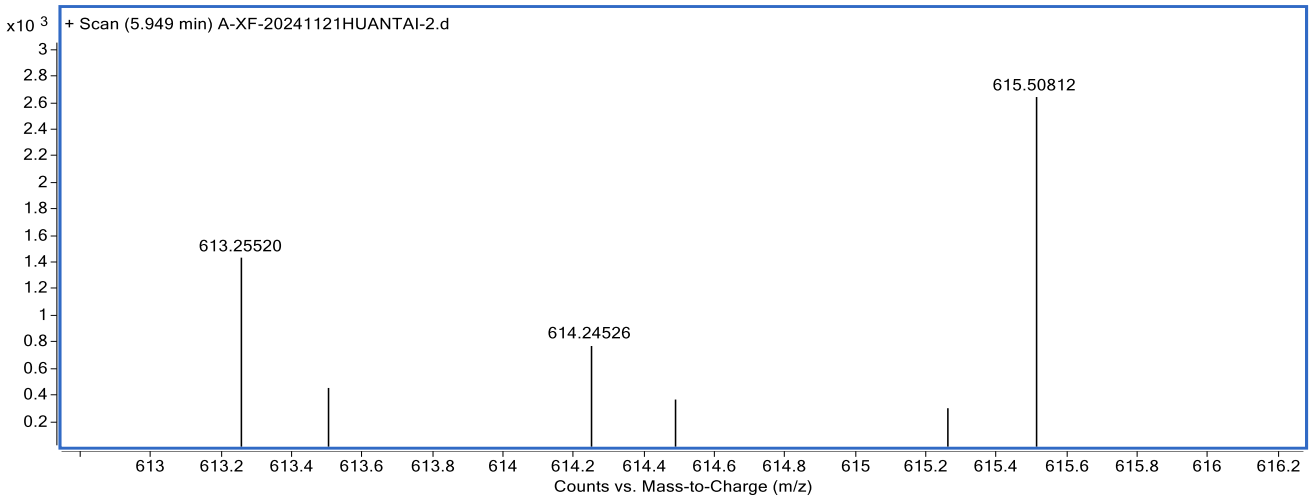


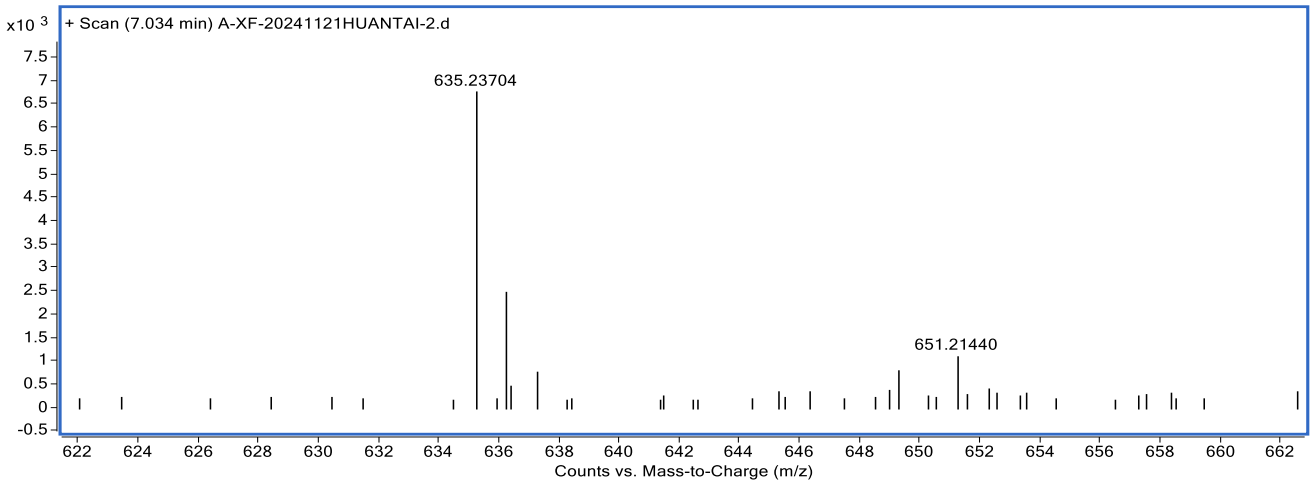
56[f], 60% conv

HPLC: 60% conversion.

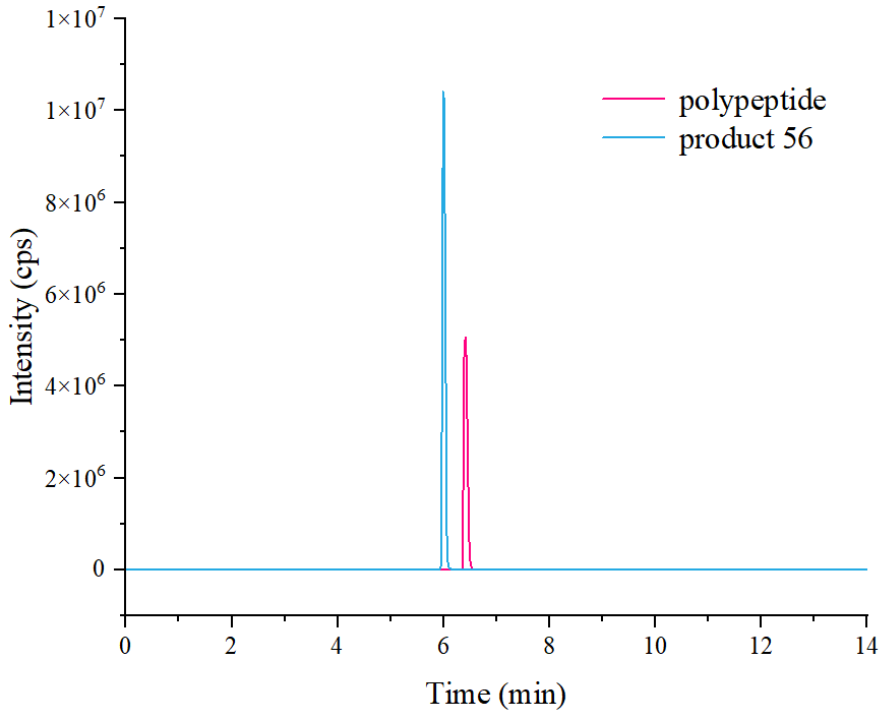
After the reaction was completed, a peak was observed with a retention time of 6.00 minutes.The polypeptide corresponds to the peak eluting at a retention time of 6.41 minutes.

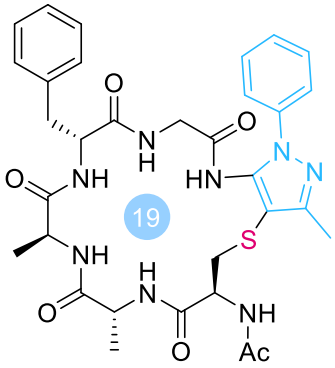
HRMS (ESI-TOF) calcd for $C_{28}H_{36}N_8O_6S$, $[M+H]^+$, 613.25513, found 613.25520.calcd for $C_{28}H_{36}N_8O_6S$, $[M+Na]^+$, 635.23707, found 635.23704.





HPLC Spectra:



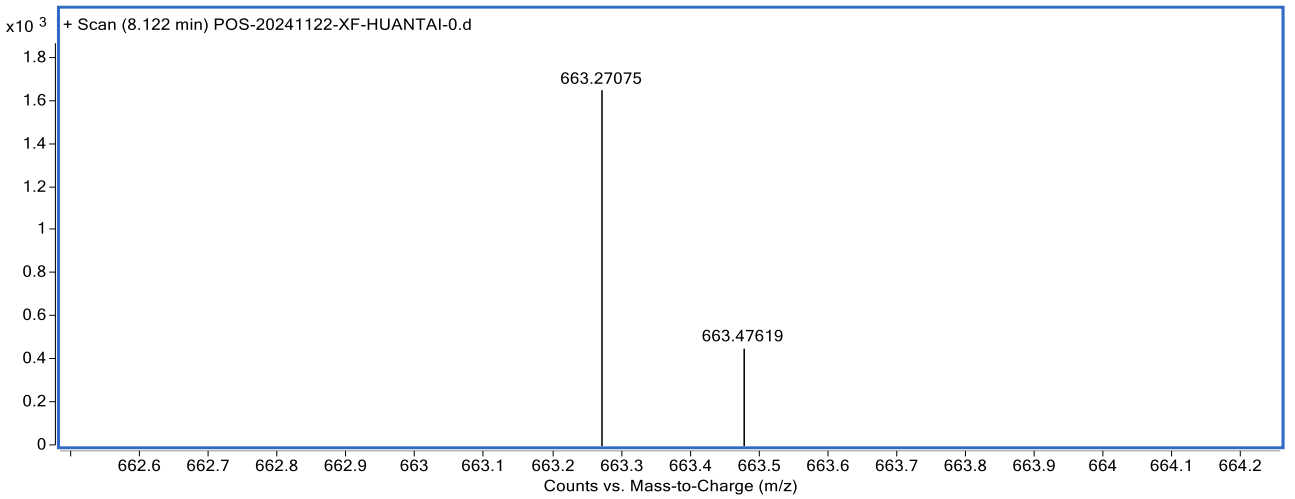


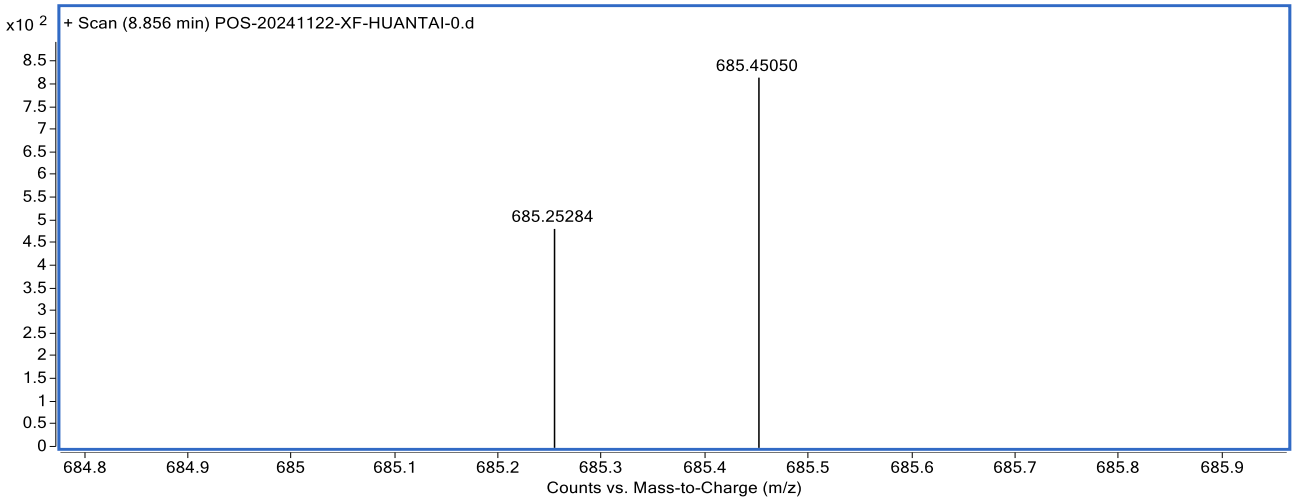
57[f], 50% conv

HPLC: 50% conversion.

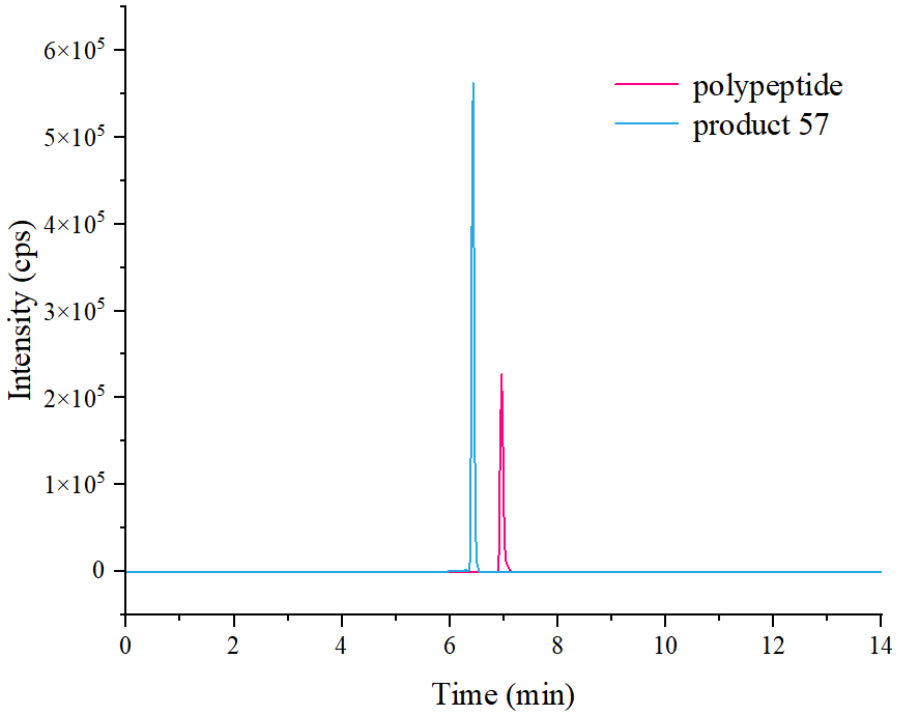
After the reaction was completed, a peak was observed with a retention time of 6.43 minutes.The polypeptide corresponds to the peak eluting at a retention time of 6.96 minutes.

HRMS (ESI-TOF) calcd for $C_{32}H_{38}N_8O_6S$, $[M+H]^+$, 663.27078, found 663.27075.calcd for $C_{32}H_{38}N_8O_6S$, $[M+Na]^+$, 685.25272, found 685.25284.





HPLC Spectra:



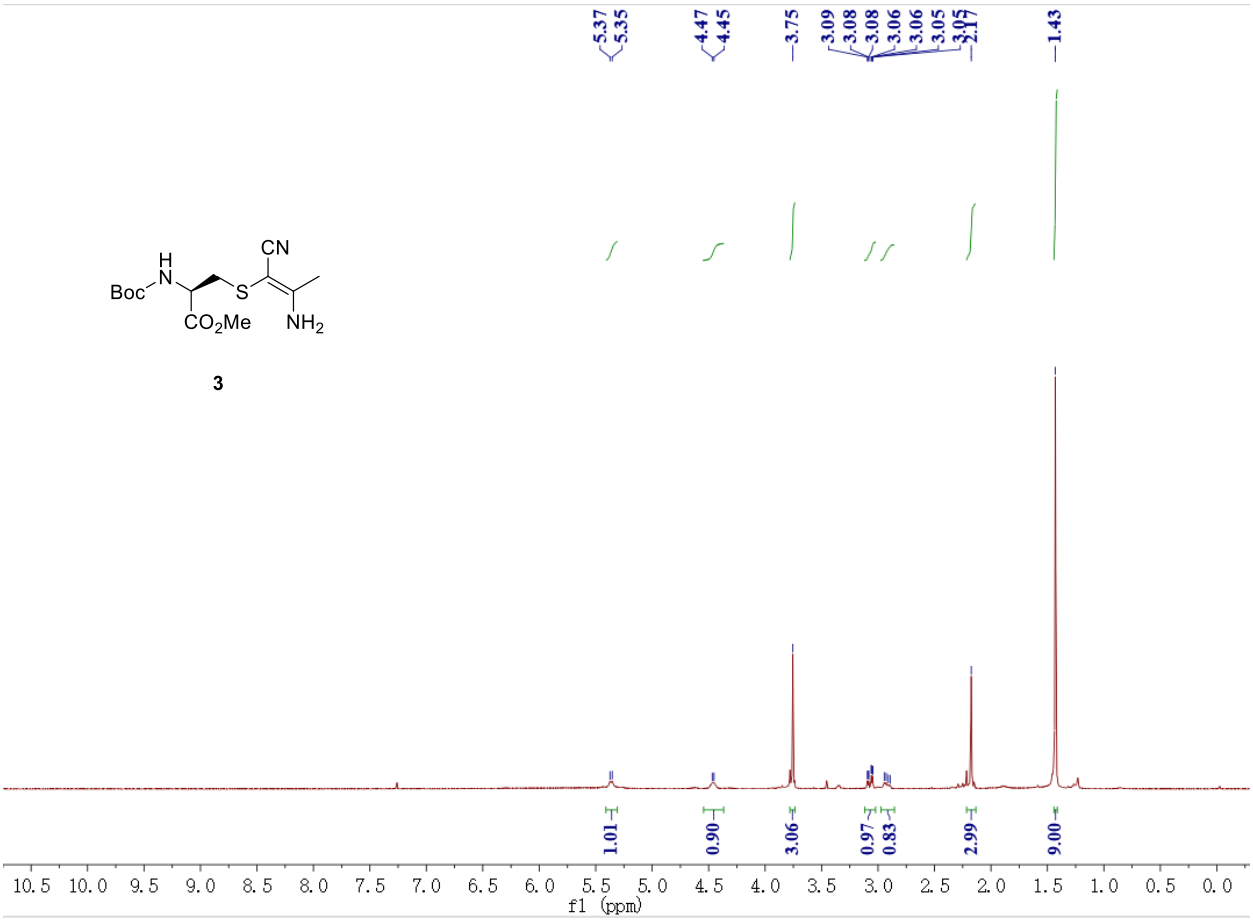
5. References

[1] R. A. Serwa, J.-M. Swiecicki, D. Homann, C. P. R. Hackenberger, Phosphoramidate-peptide synthesis by solution- and solid-phase Staudinger-phosphite reactions. *J. Pept. Sci.* **2010**, *16*, 563–567.

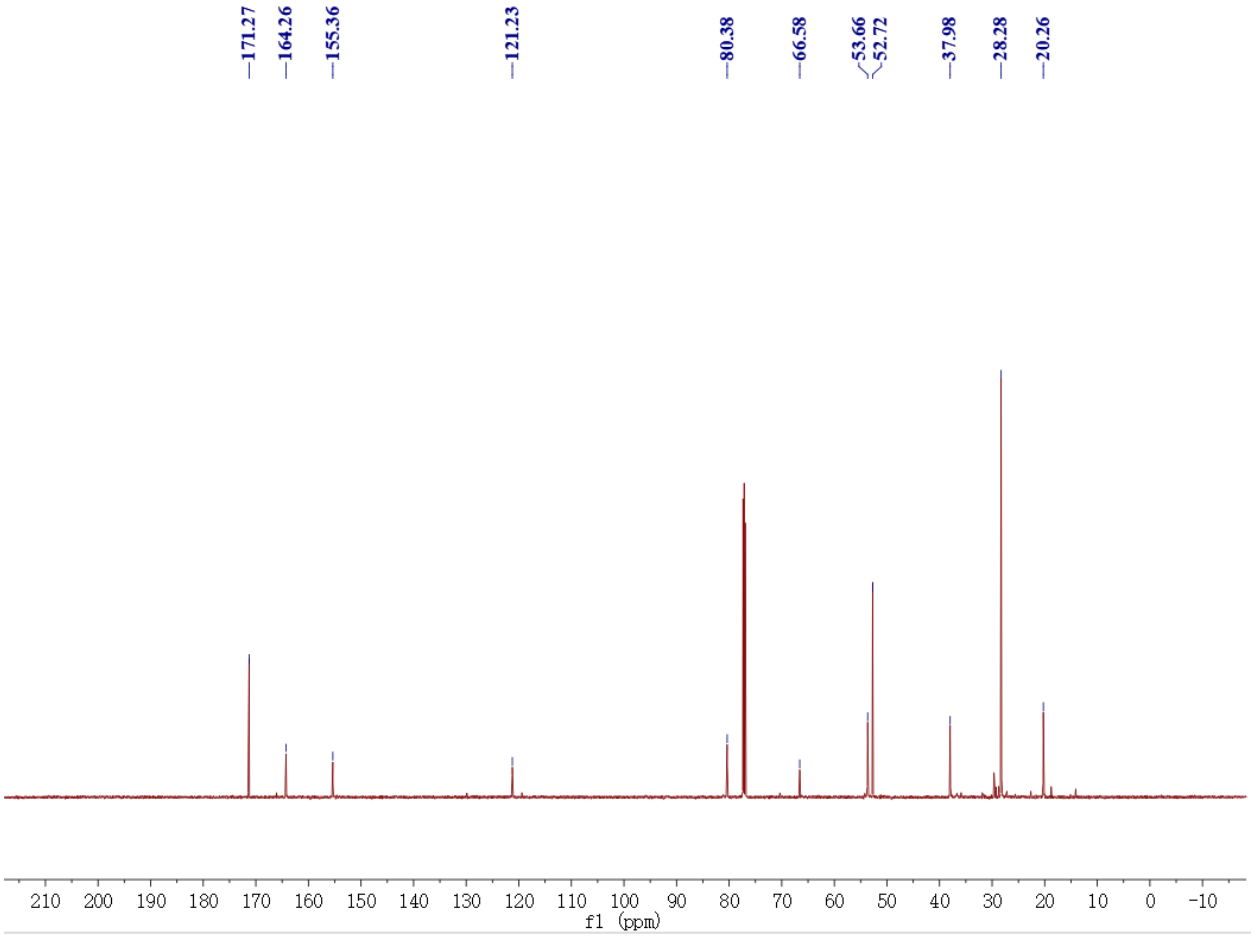
[2] Alam J, Keller T H, Loh T P. Functionalization of peptides and proteins by Mukaiyama aldol reaction. *J. Am. Chem. Soc.* **2010**, *132*, 9546-9548.

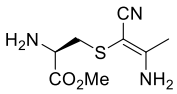
[3] Bottecchia C., Erdmann N., Tijssen P.M.A., et al. Batch and Flow Synthesis of Disulfides by Visible-Light-Induced TiO_2 Photocatalysis [J]. ChemSusChem, **2016**, 9(14): 1781-5.

6. NMR spectra of all products



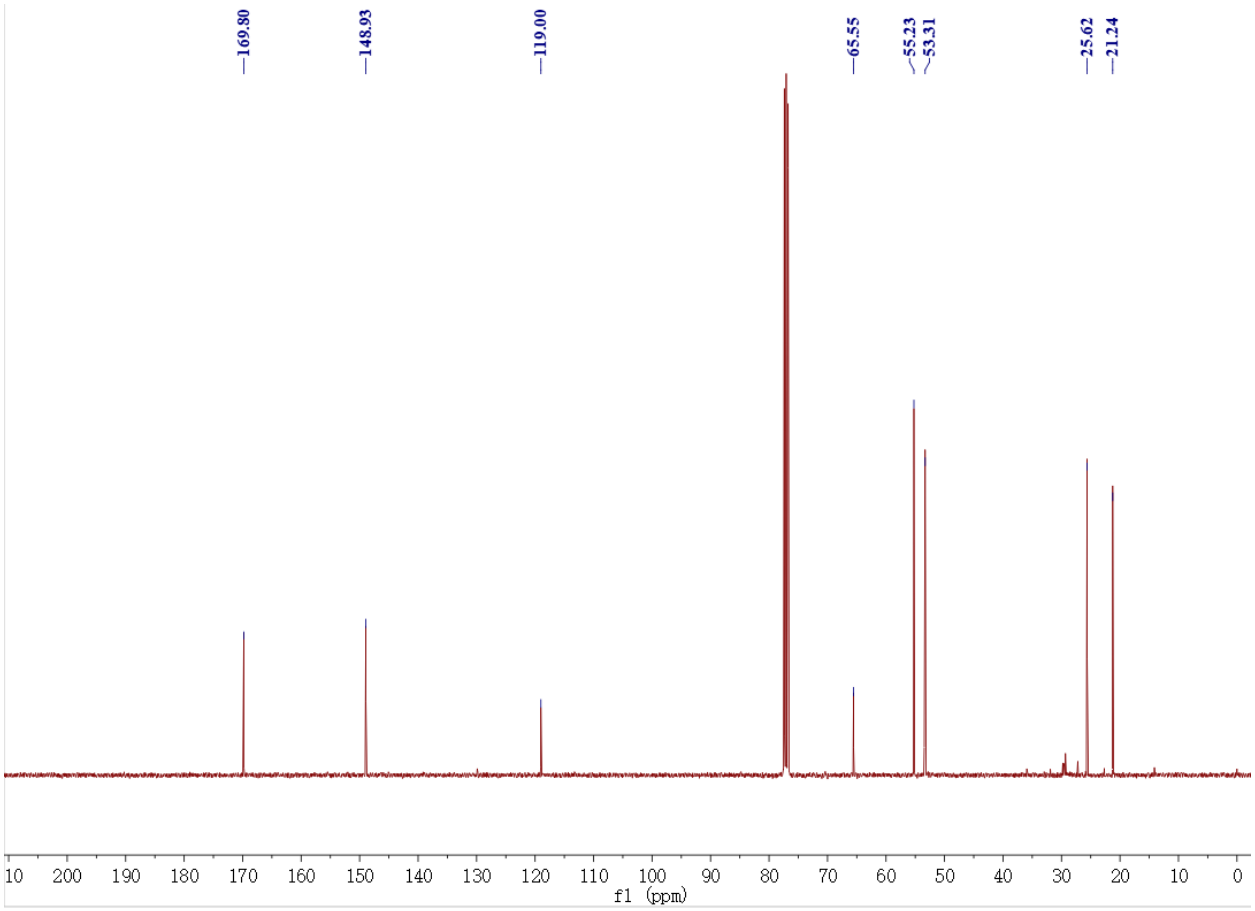
3

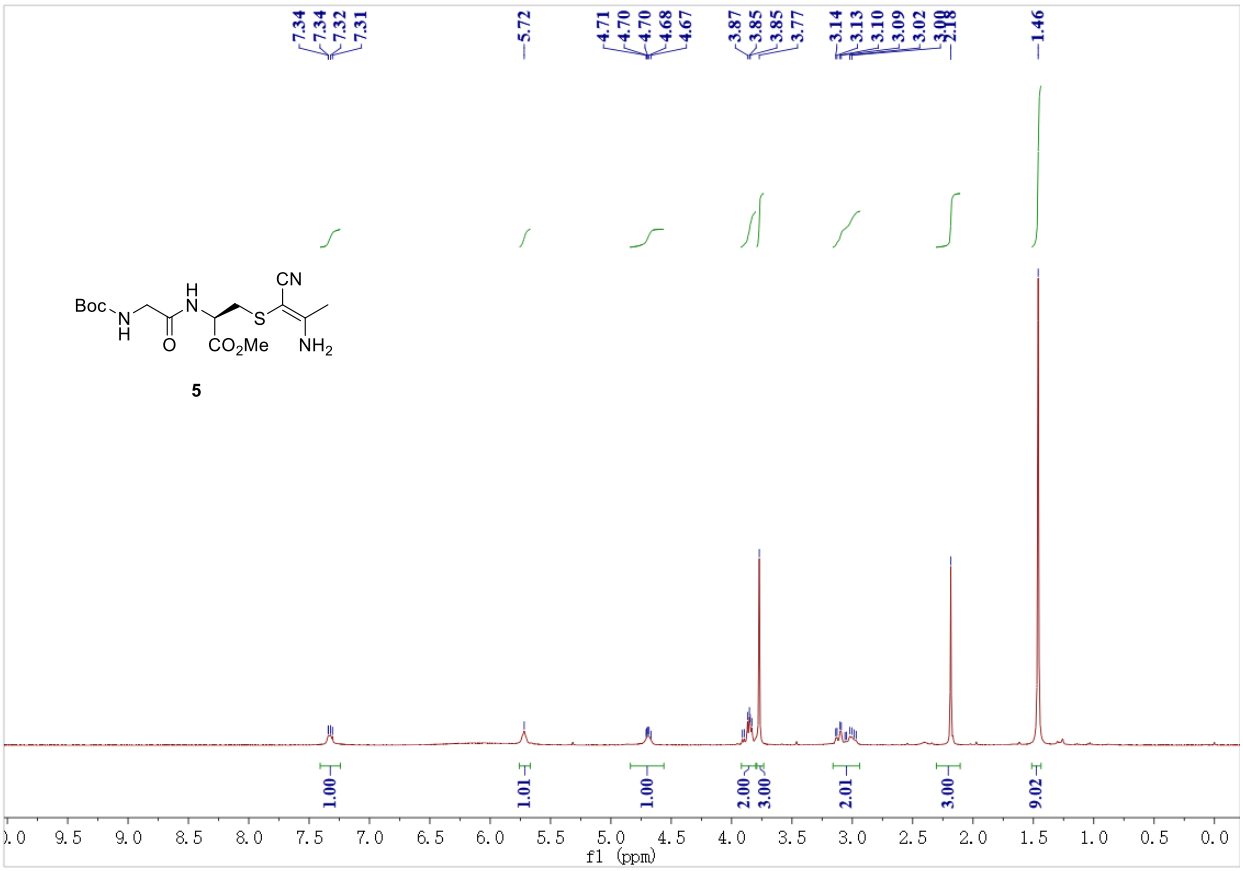

171.27
164.26
155.36
121.23
80.38
66.58
53.66
52.72
37.98
28.28
20.26
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


CN
H2N
S
CO2Me
NH2

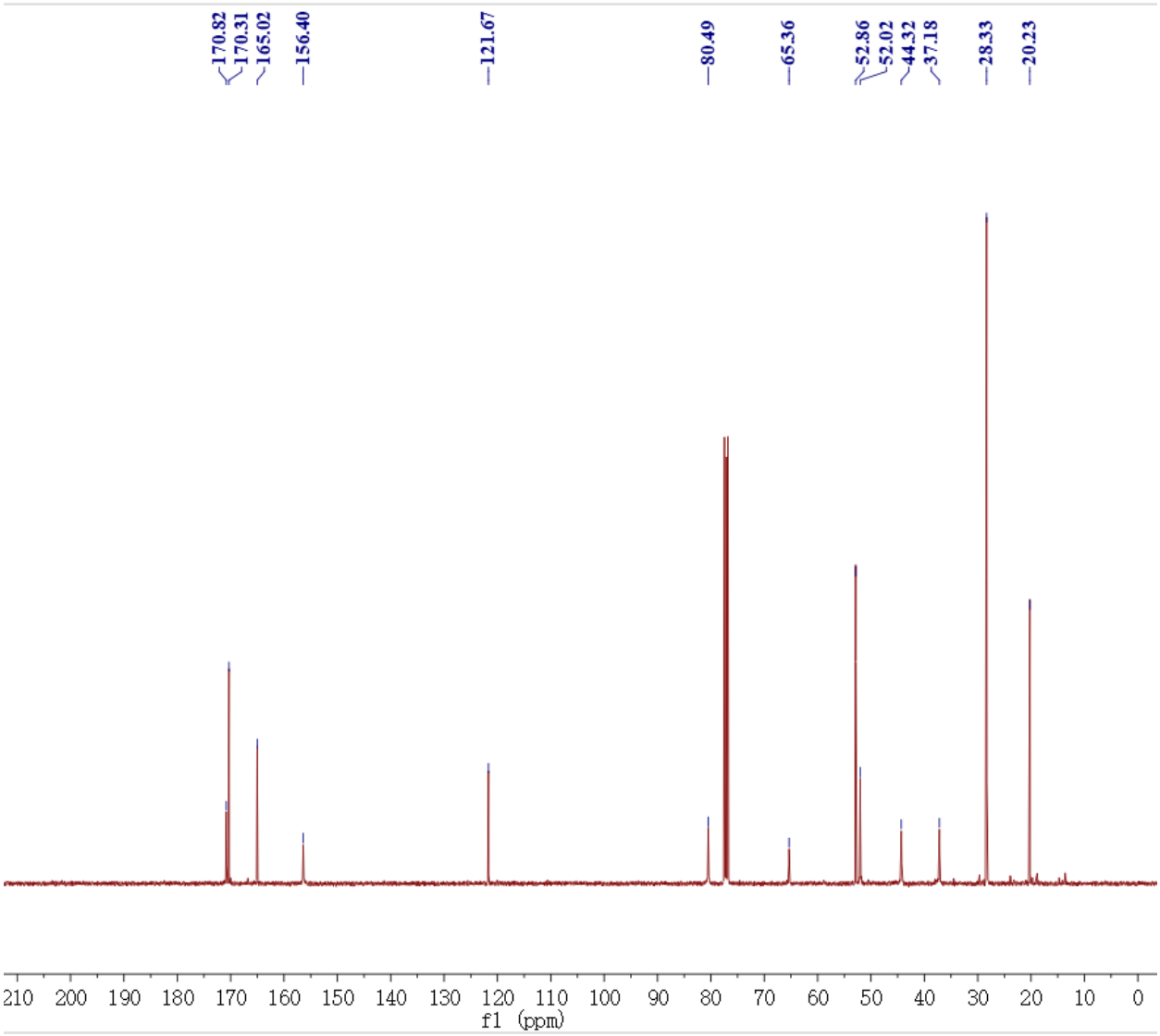
4

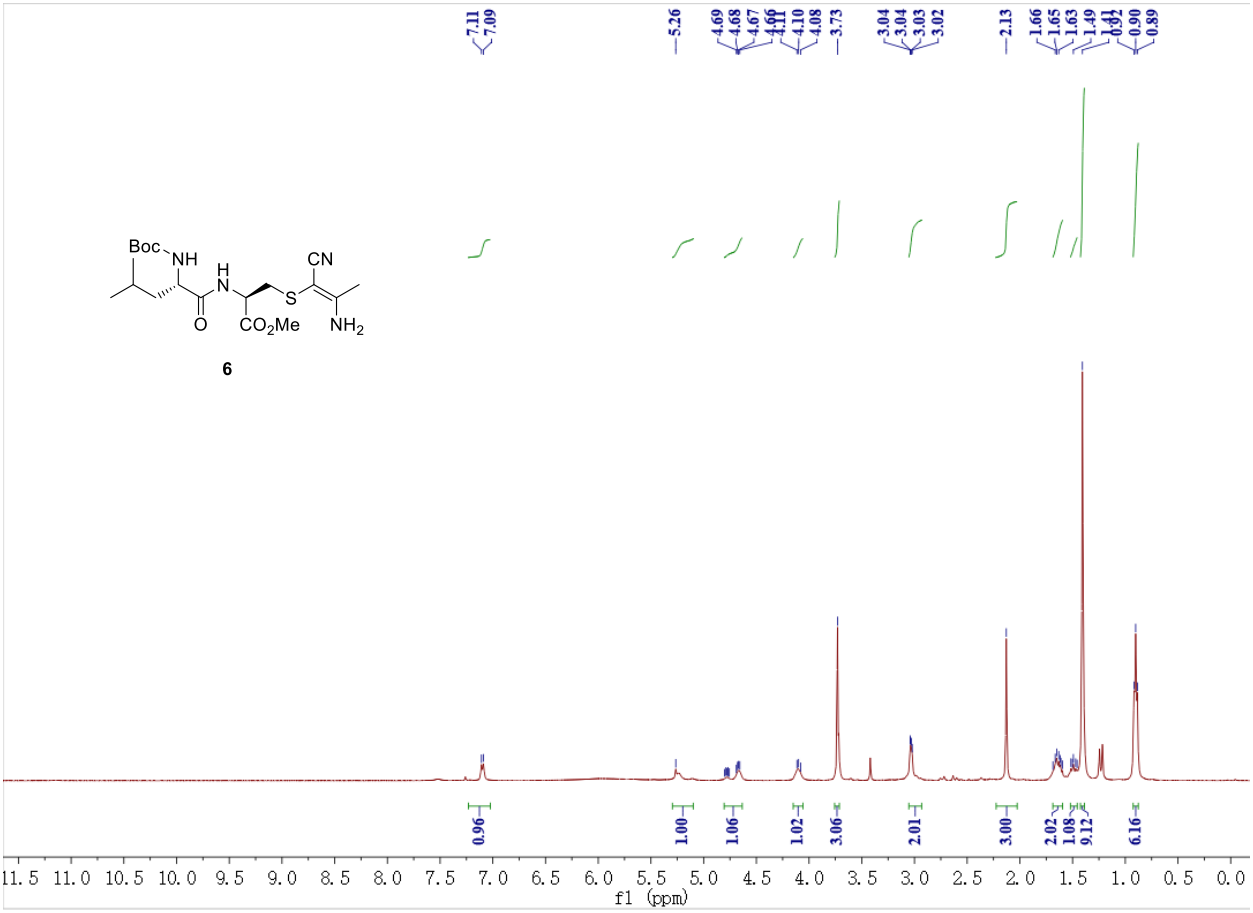
4.31
4.30
4.30
4.29
4.28
4.28
3.83
3.14
3.13
3.11
3.10
2.83
2.81
2.80
2.78
2.20
1.00
3.00
1.00
1.00
3.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

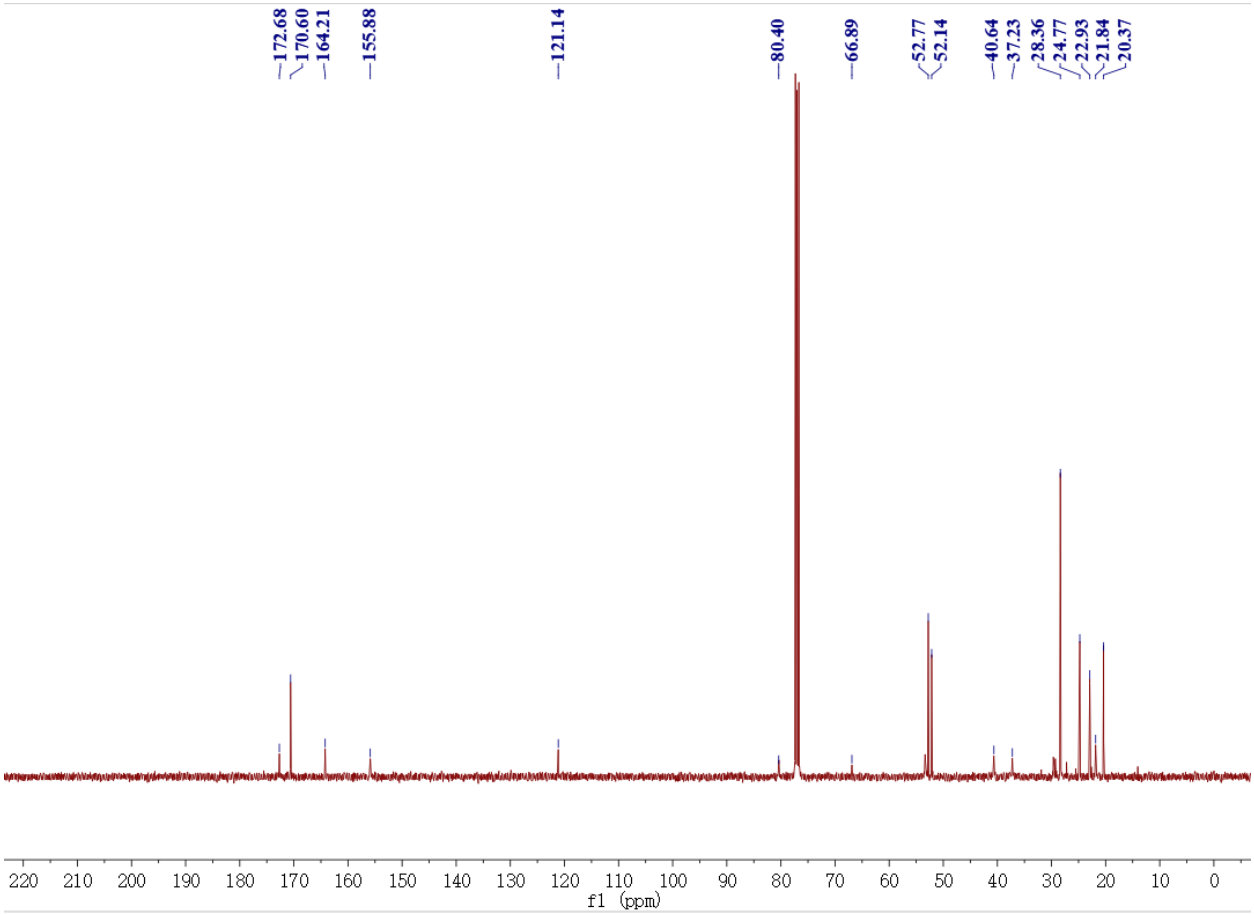

169.80
148.93
119.00
65.55
55.23
53.31
25.62
21.24
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

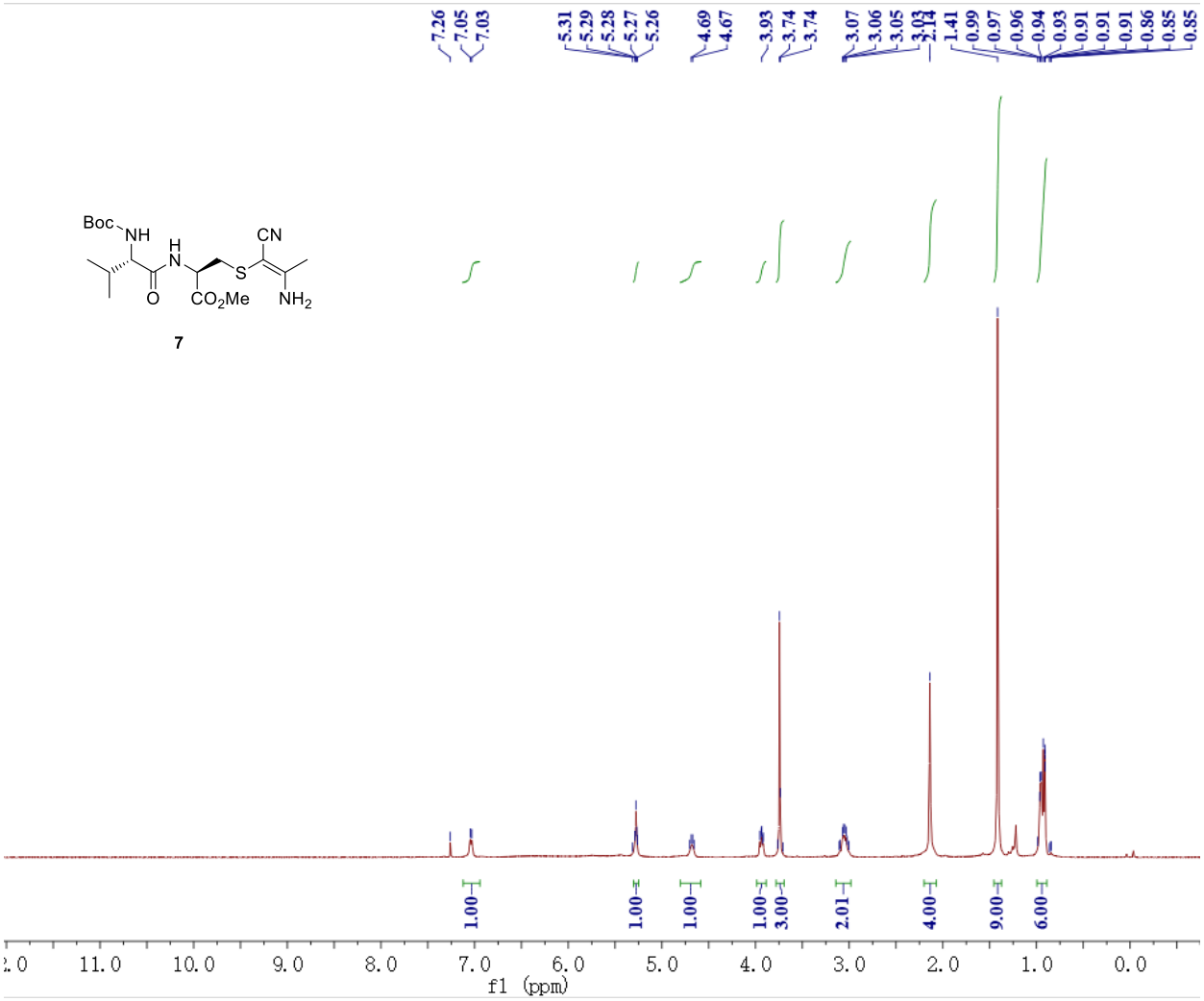

7.34
7.34
7.32
7.31
5.72
4.71
4.70
4.70
4.68
4.67
3.87
3.85
3.85
3.77
3.14
3.13
3.10
3.09
3.02
2.18
1.46
Boc
N
H
O
H
N
S
CN
CO2Me
NH2
1.00
1.01
1.00
2.00
3.00
2.01
3.00
9.02
f1 (ppm)

5

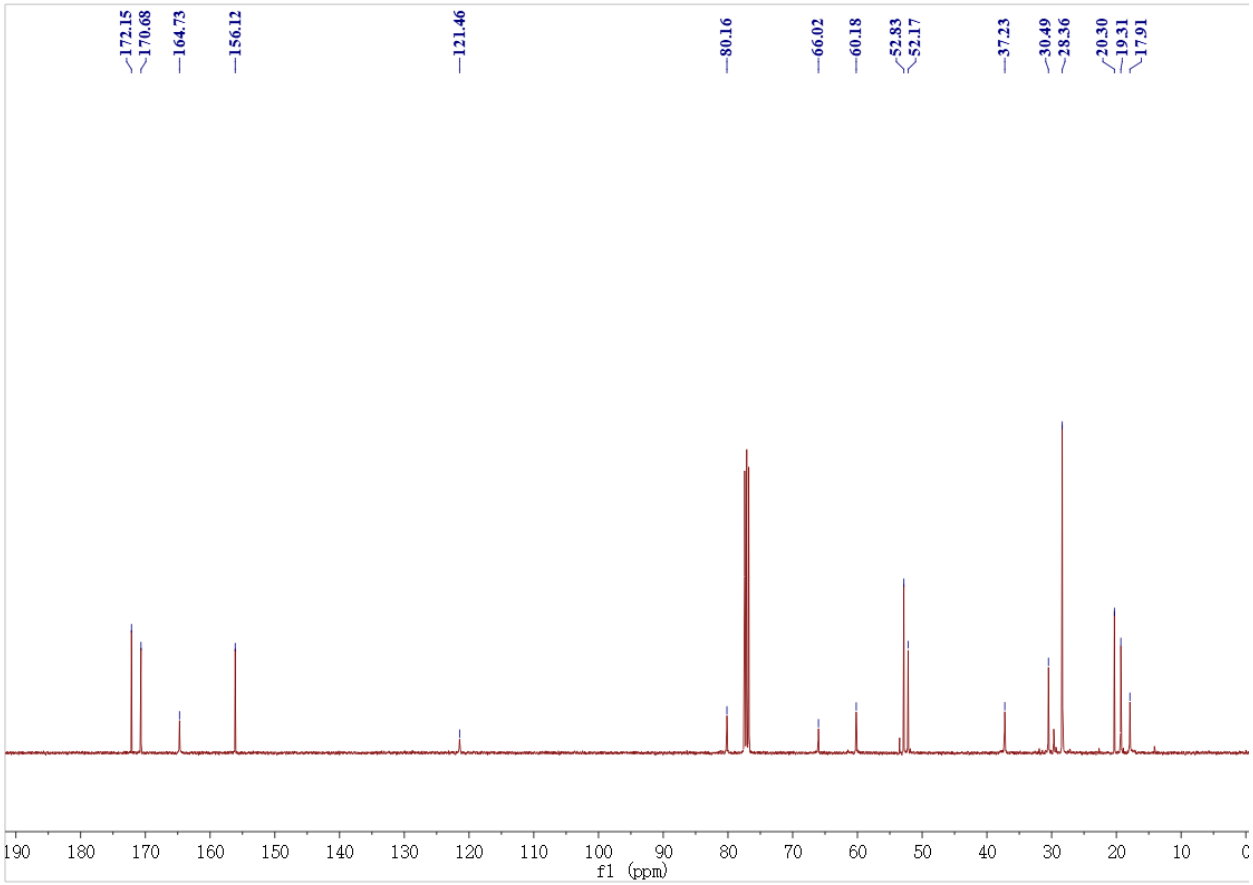

170.82
170.31
165.02
156.40
121.67
80.49
65.36
52.86
52.02
44.32
37.18
28.33
20.23
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

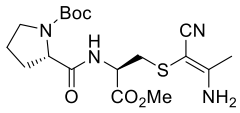

7.11
7.09
5.26
4.69
4.68
4.67
4.66
4.11
4.10
4.08
3.73
3.04
3.04
3.03
3.02
2.13
1.66
1.65
1.63
1.49
1.41
0.92
0.90
0.89
Boc
NH
H
N
O
CN
S
CO2Me
NH2
6
0.96
1.00
1.06
1.02
3.06
2.01
3.00
2.02
1.08
9.12
6.16
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


172.68
170.60
164.21
155.88
121.14
80.40
66.89
52.77
52.14
40.64
37.23
28.36
24.77
22.93
21.84
20.37
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.26
7.05
7.03
5.31
5.29
5.28
5.27
5.26
4.69
4.67
3.93
3.74
3.74
3.07
3.06
3.05
3.03
2.14
1.41
0.99
0.97
0.96
0.94
0.93
0.91
0.91
0.91
0.86
0.85
0.85
Boc
NH
H
N
O
S
CN
CO2Me
NH2
1.00
1.00
1.00
1.00
3.00
2.01
4.00
9.00
6.00
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

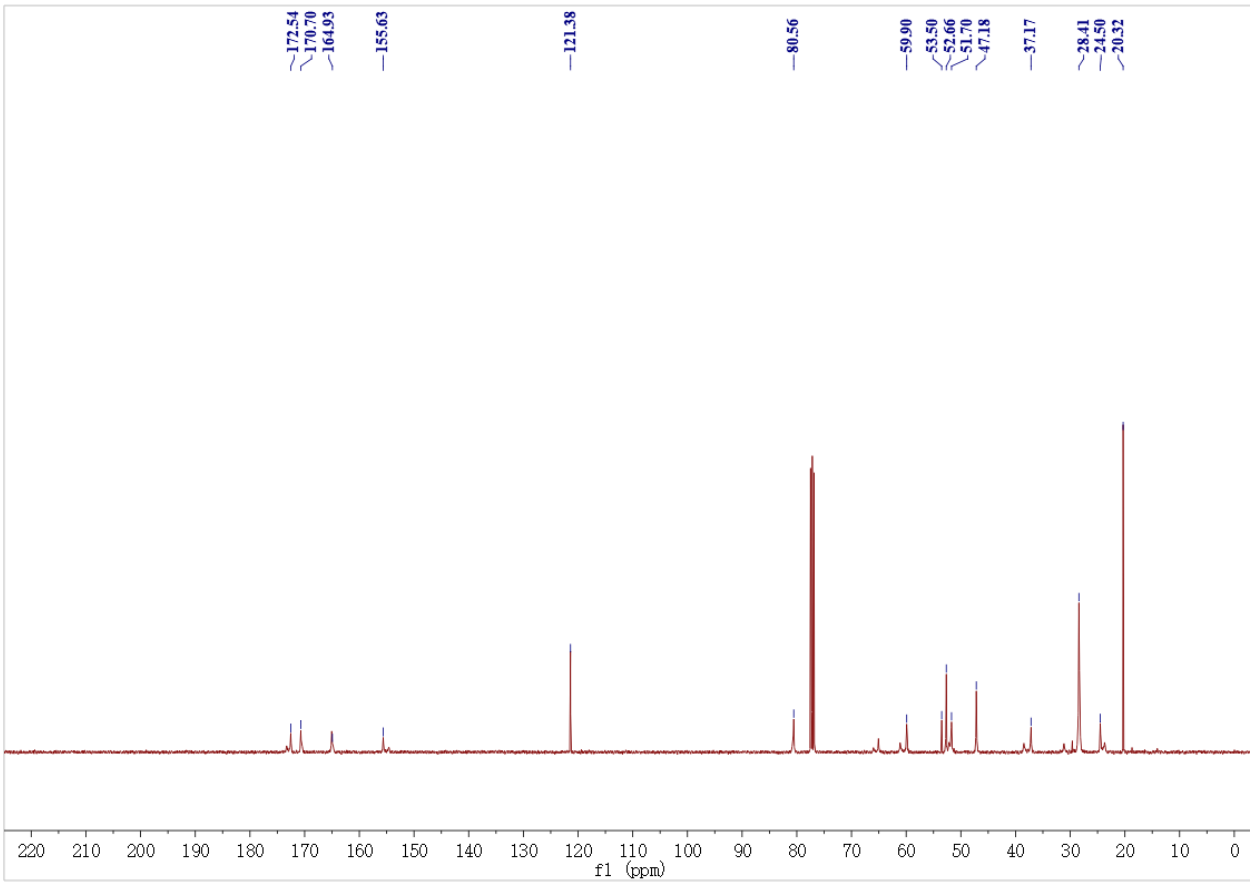
7

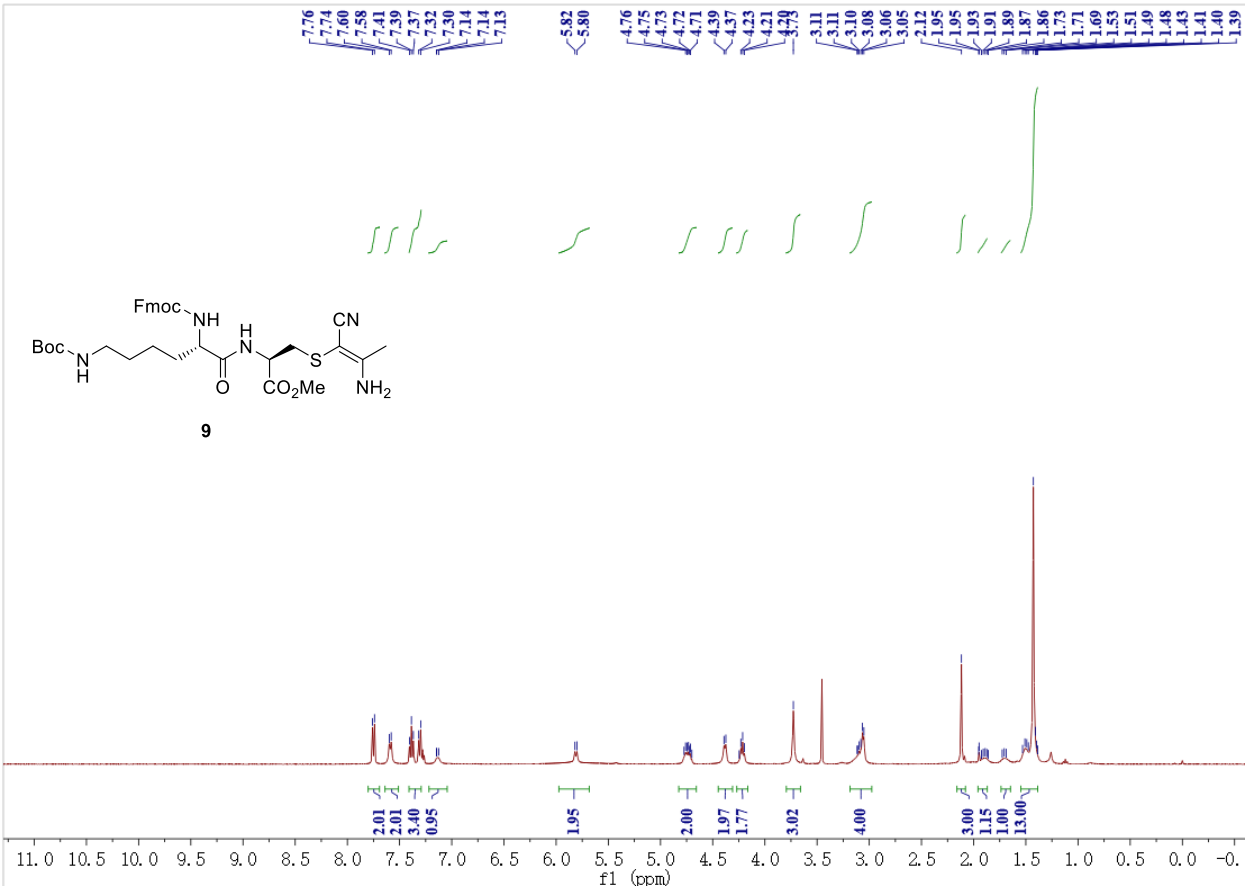

172.15
170.68
164.73
156.12
121.46
80.16
66.02
60.18
52.83
52.17
37.23
30.49
28.36
20.30
19.31
17.91
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

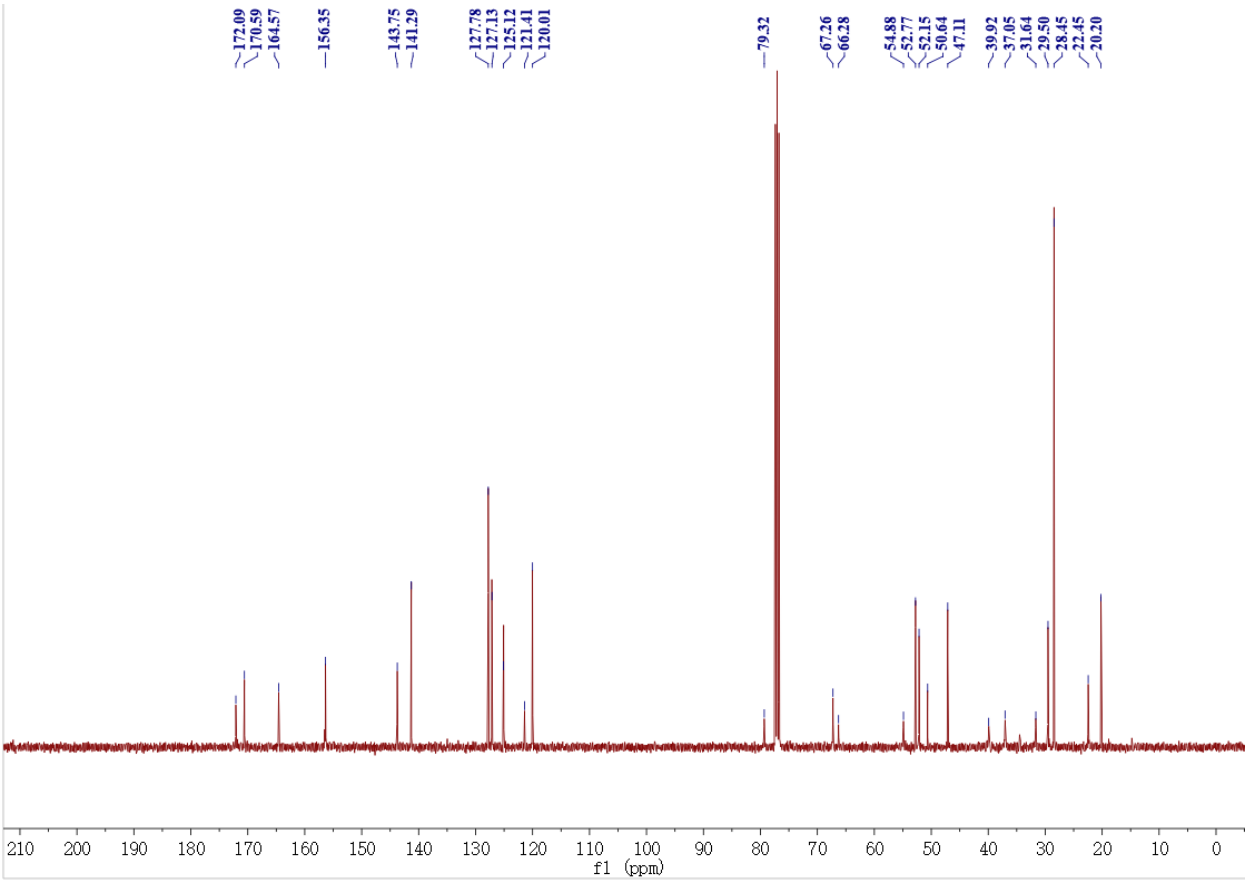

Boc
N
H
N
CN
S
O
CO2Me
NH2

8

4.71
4.61
4.61
4.59
4.26
4.24
3.70
3.43
3.42
2.98
2.97
2.96
2.94
2.12
1.92
1.87
1.85
1.84
1.38
1.30
1.00
1.00
3.00
2.00
2.00
1.03
3.01
2.86
9.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


172.54
170.70
164.93
155.63
121.38
80.56
59.90
53.50
52.66
51.70
47.18
37.17
28.41
24.50
20.32
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.76
7.74
7.60
7.58
7.41
7.39
7.37
7.32
7.30
7.14
7.14
7.13
5.82
5.80
4.76
4.75
4.73
4.72
4.71
4.39
4.37
4.23
4.21
4.20
3.33
3.11
3.11
3.10
3.08
3.06
3.05
2.12
1.95
1.95
1.93
1.91
1.89
1.87
1.86
1.73
1.71
1.69
1.53
1.51
1.49
1.48
1.43
1.41
1.40
1.39
Fmoc
NH
H
N
CN
Boc
N
H
S
O
CO2Me
NH2
9
2.01
2.01
3.40
0.95
1.95
2.00
1.97
1.77
3.02
4.00
3.00
1.15
1.00
13.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.
f1 (ppm)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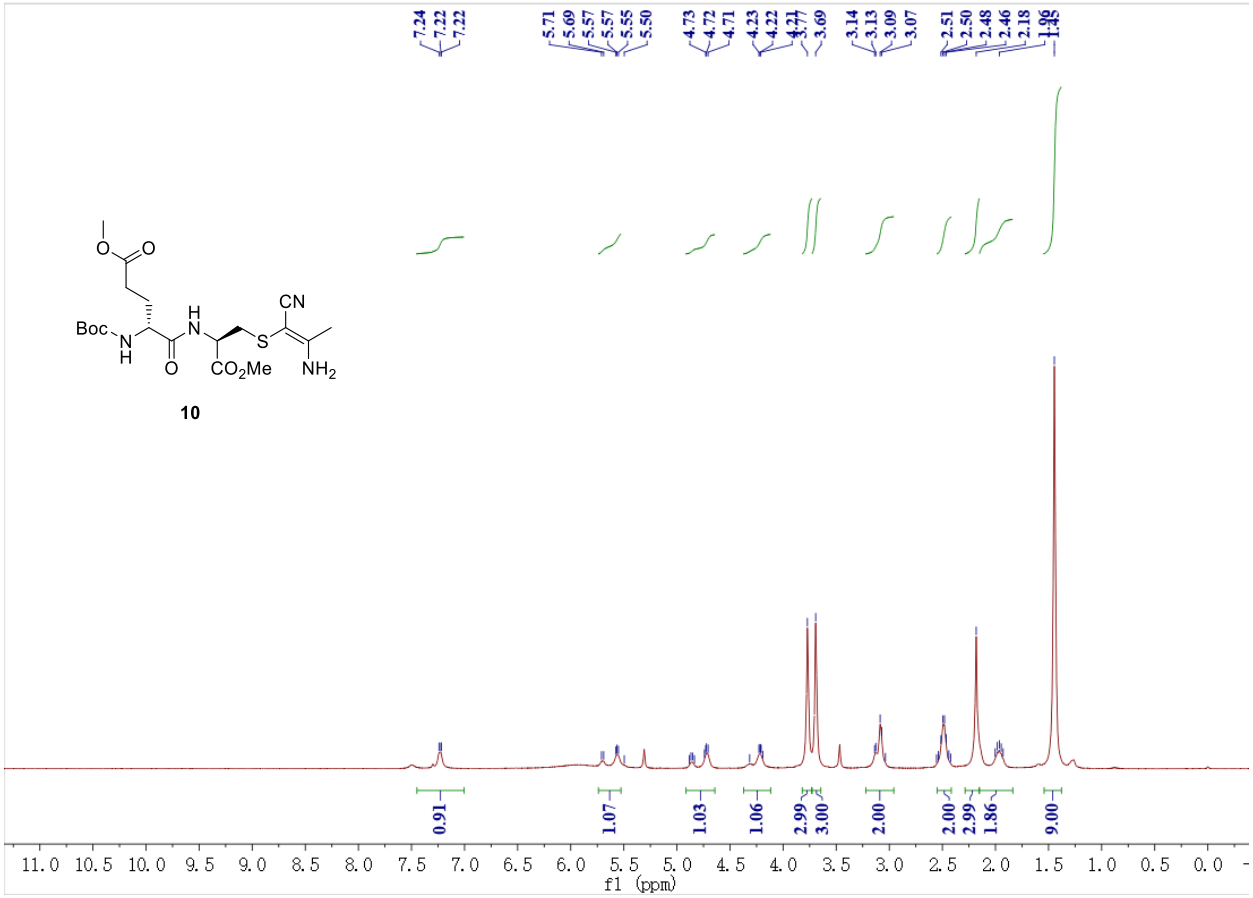
170.59
164.57
156.35
143.75
141.29
127.78
127.13
125.12
121.41
120.01
79.32
67.26
66.28
54.88
52.77
52.15
50.64
47.11
39.92
37.05
31.64
29.50
28.45
22.45
20.20
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.22
7.22
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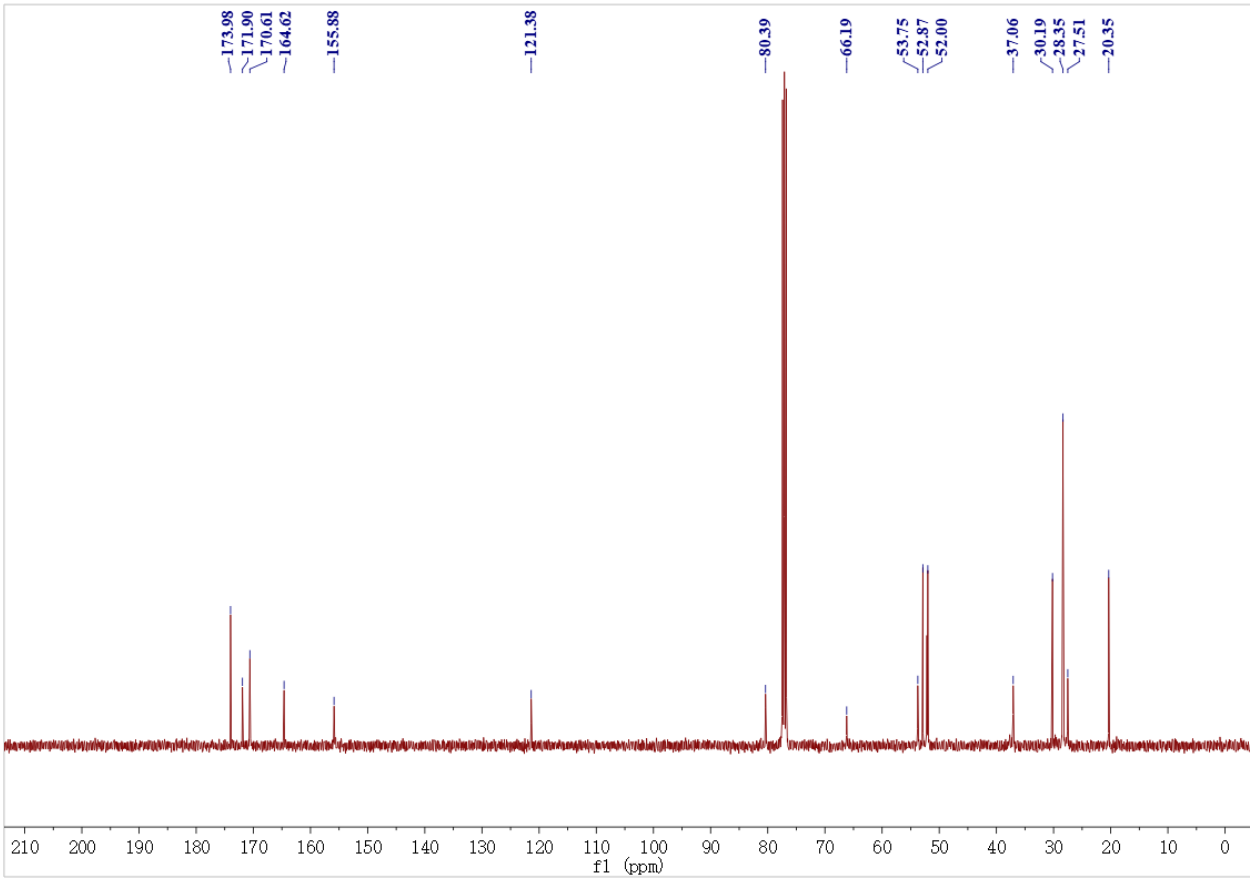
5.57
5.57
5.55
5.50
4.73
4.72
4.71
4.23
4.22
4.21
3.77
3.69
3.14
3.13
3.09
3.07
2.51
2.50
2.48
2.46
2.18
1.96
1.45
0.91
1.07
1.03
1.06
2.99
3.00
2.00
2.00
2.99
1.86
9.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

10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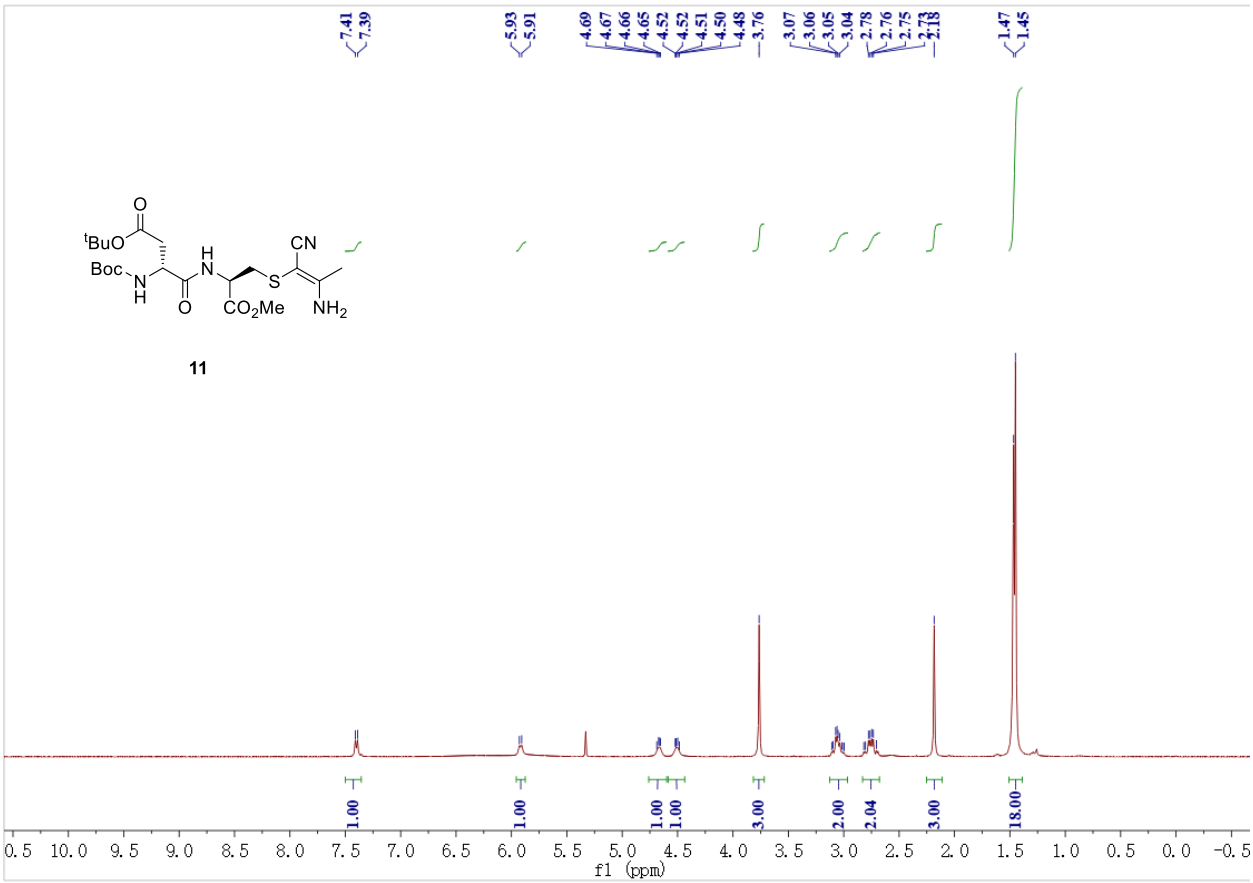
66.19
53.75
52.87
52.00
37.06
30.19
28.35
27.51
20.35
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.39
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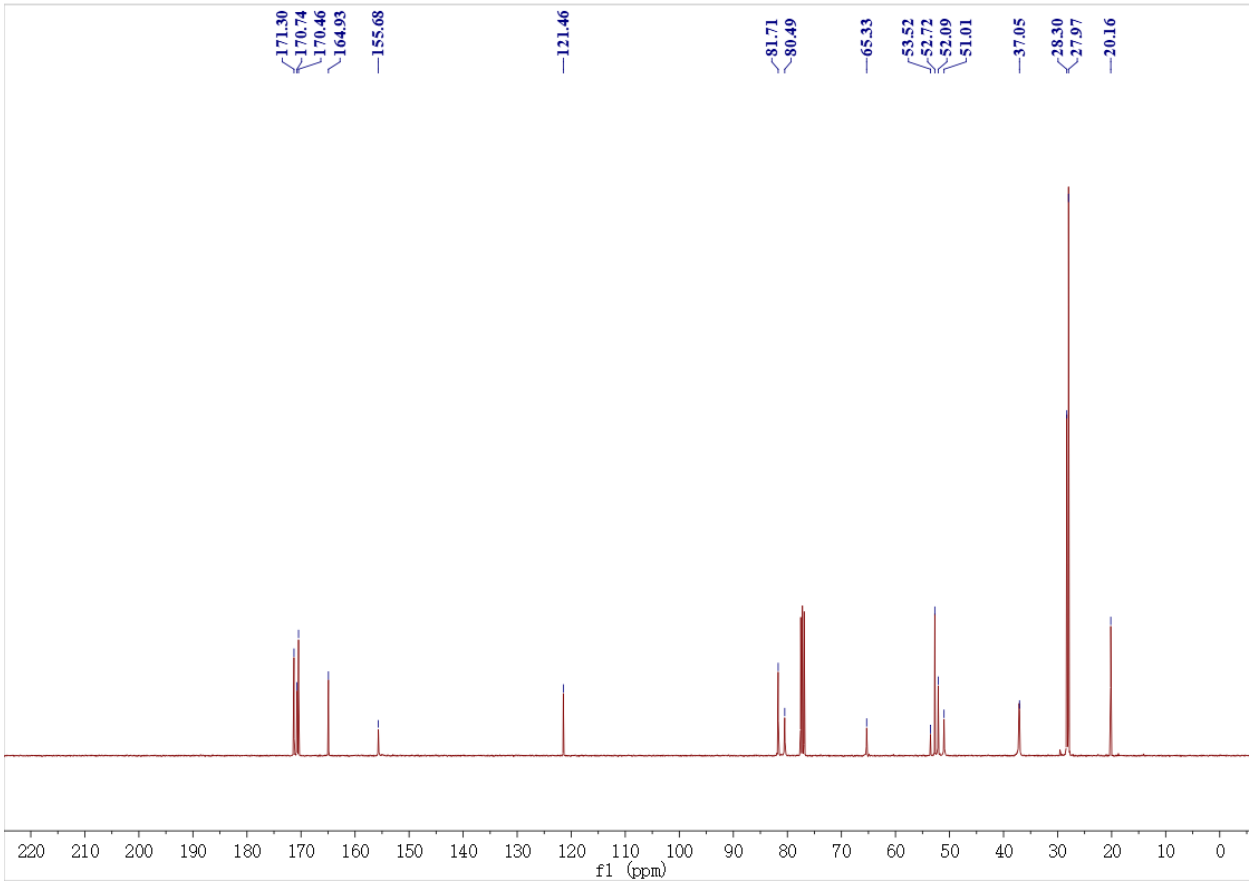
5.91
4.69
4.67
4.66
4.65
4.52
4.52
4.51
4.50
4.48
3.76
3.07
3.06
3.05
3.04
2.78
2.76
2.75
2.73
2.18
1.47
1.45
tBuO
Boc
N
H
H
N
O
O
S
CN
CO2Me
NH2
11
1.00
1.00
1.00
1.00
3.00
2.00
2.04
3.00
18.00
f1 (ppm)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170.74
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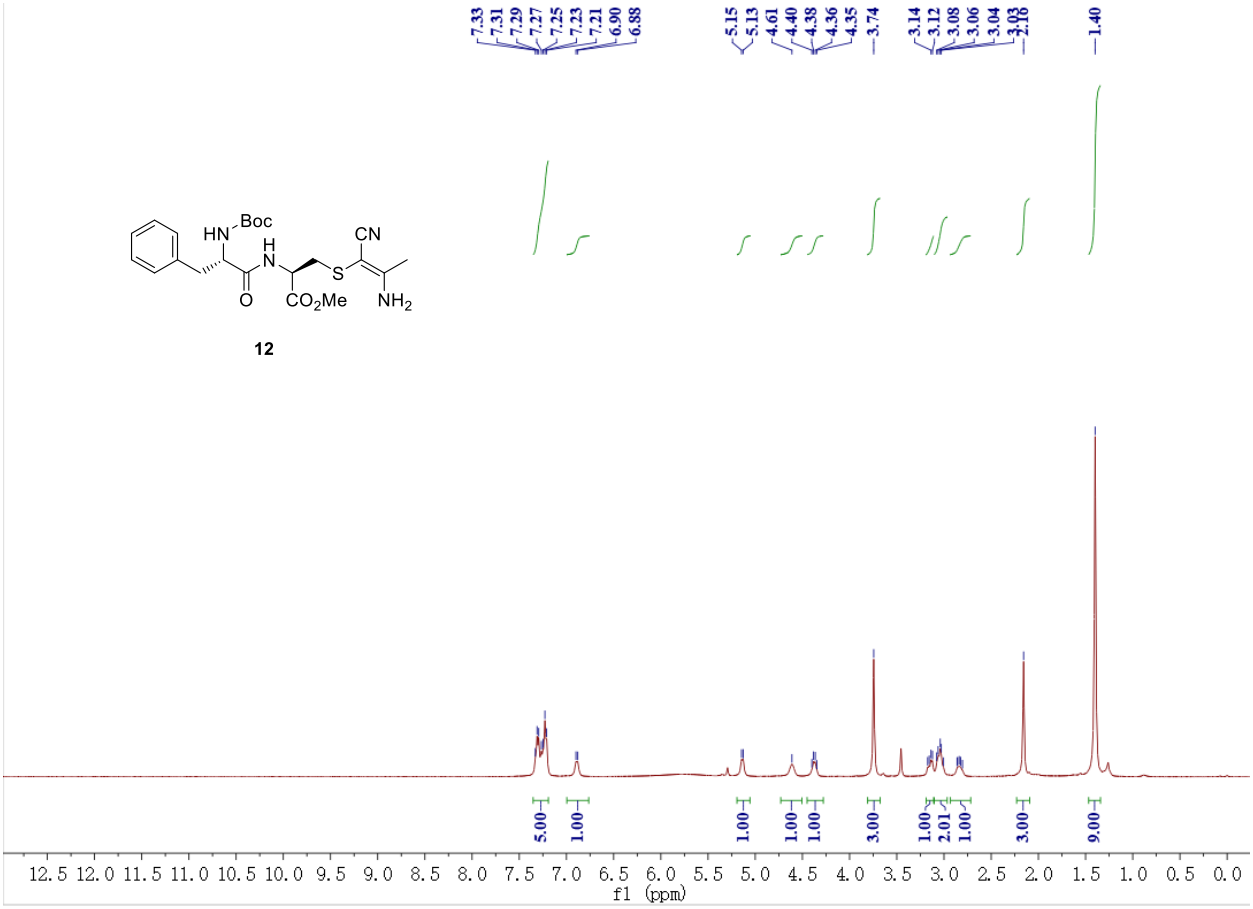
164.93
155.68
121.46
81.71
80.49
65.33
53.52
52.72
52.09
51.01
37.05
28.30
27.97
20.16
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.27
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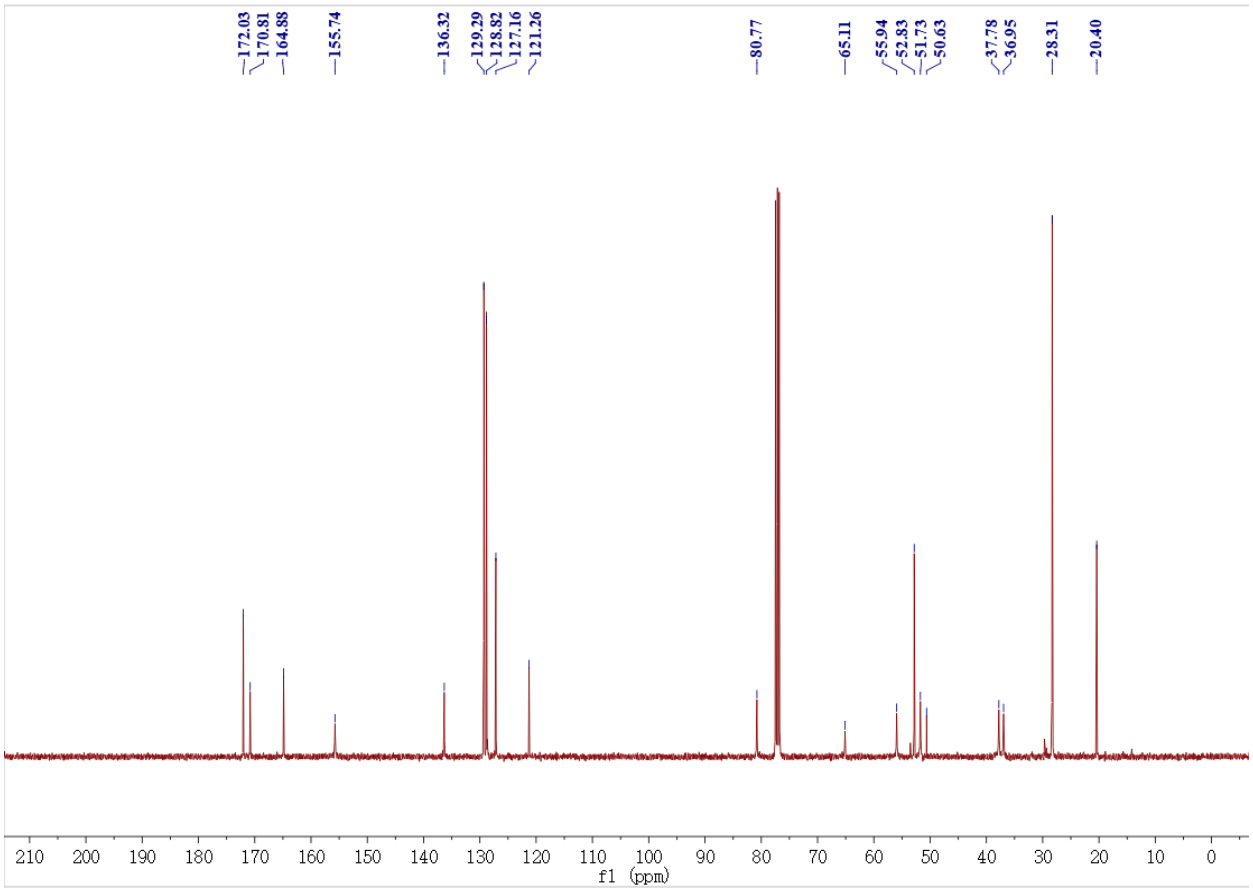
7.23
7.21
6.90
6.88
5.15
5.13
4.61
4.40
4.38
4.36
4.35
3.74
3.14
3.12
3.08
3.06
3.04
3.03
2.10
1.40
HN
Boc
H
N
O
S
CN
NH2
CO2Me
5.00
1.00
1.00
1.00
1.00
3.00
1.00
2.01
1.00
3.00
9.00
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

12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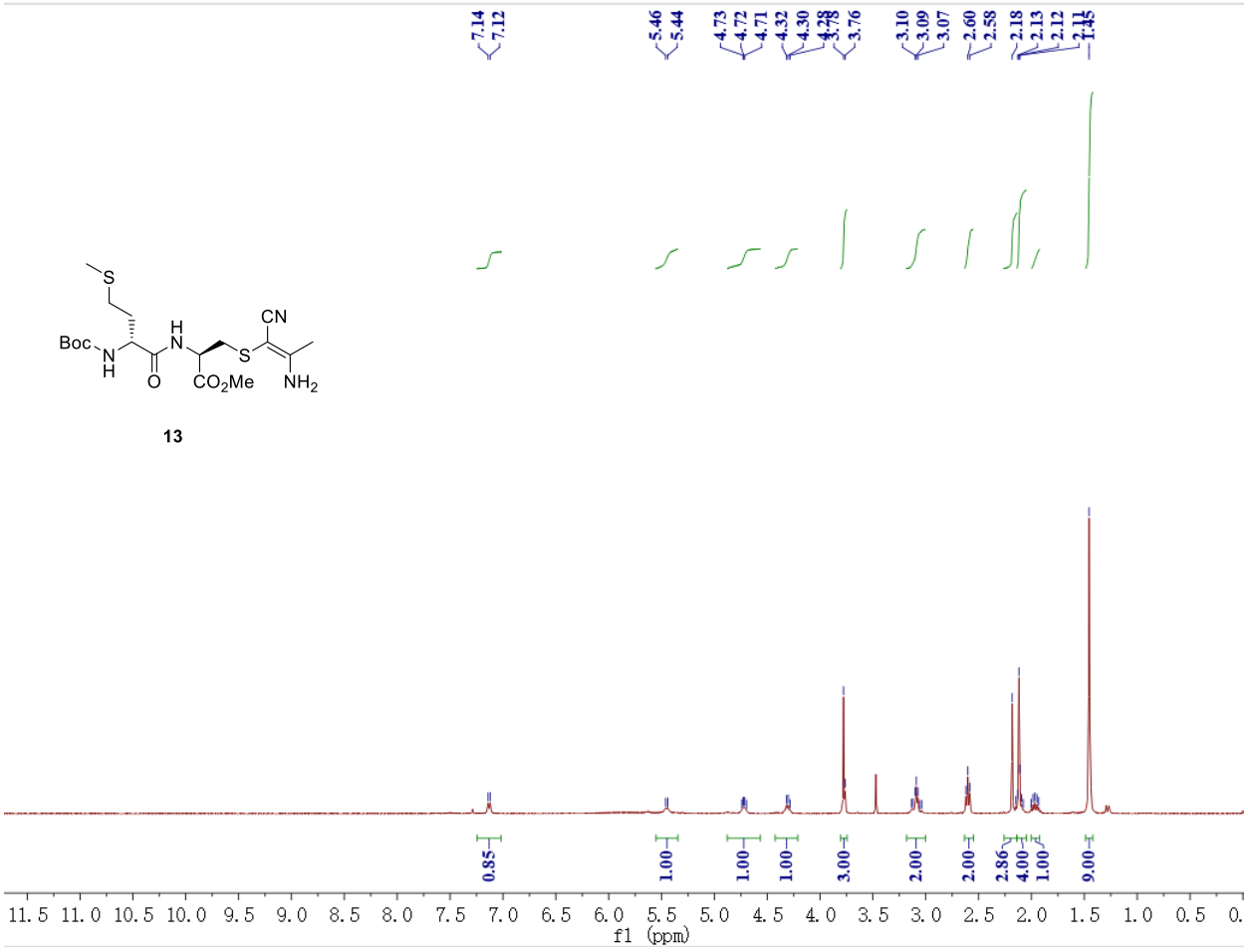
164.88
155.74
136.32
129.29
128.82
127.16
121.26
80.77
65.11
55.94
52.83
51.73
50.63
37.78
36.95
28.31
20.40
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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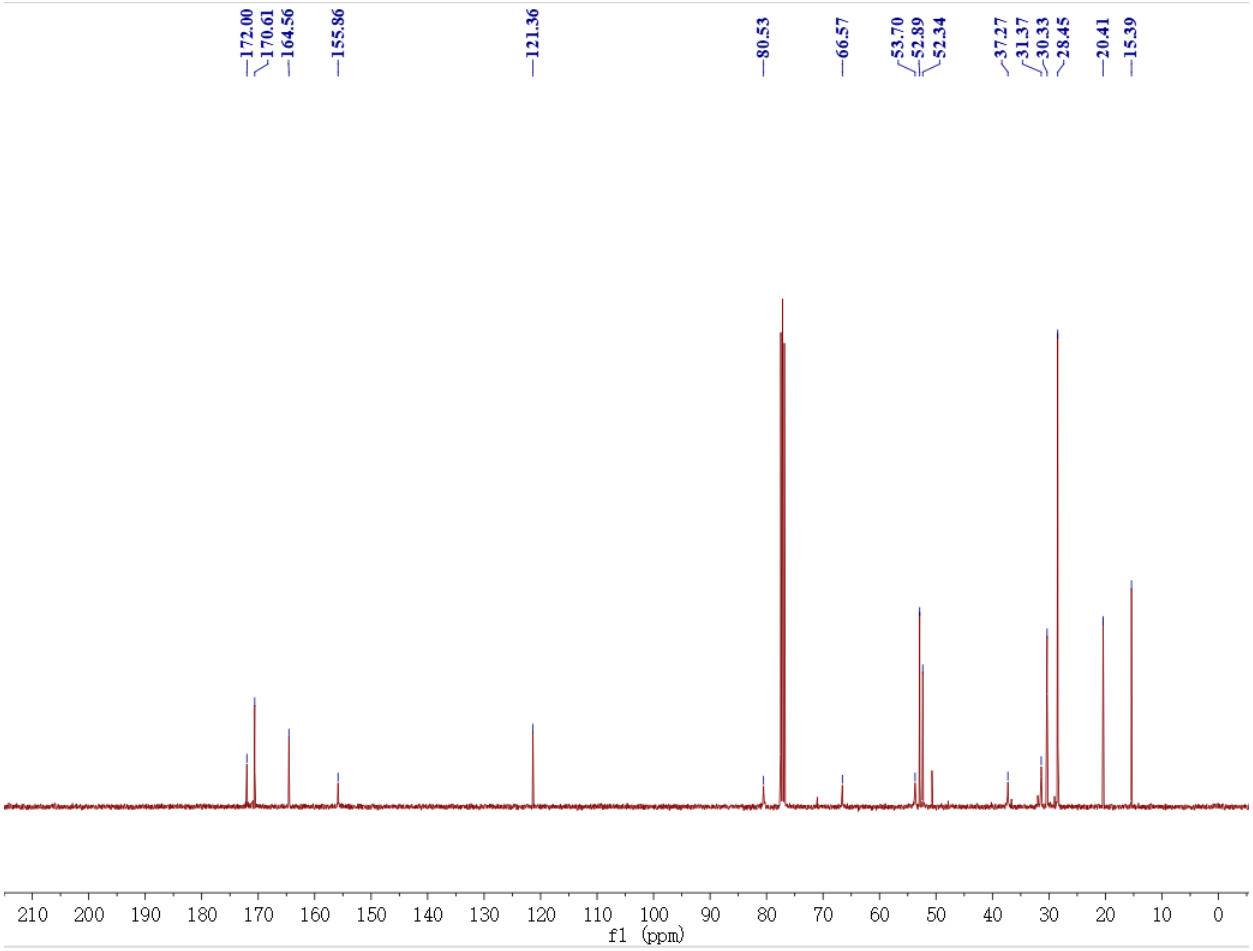
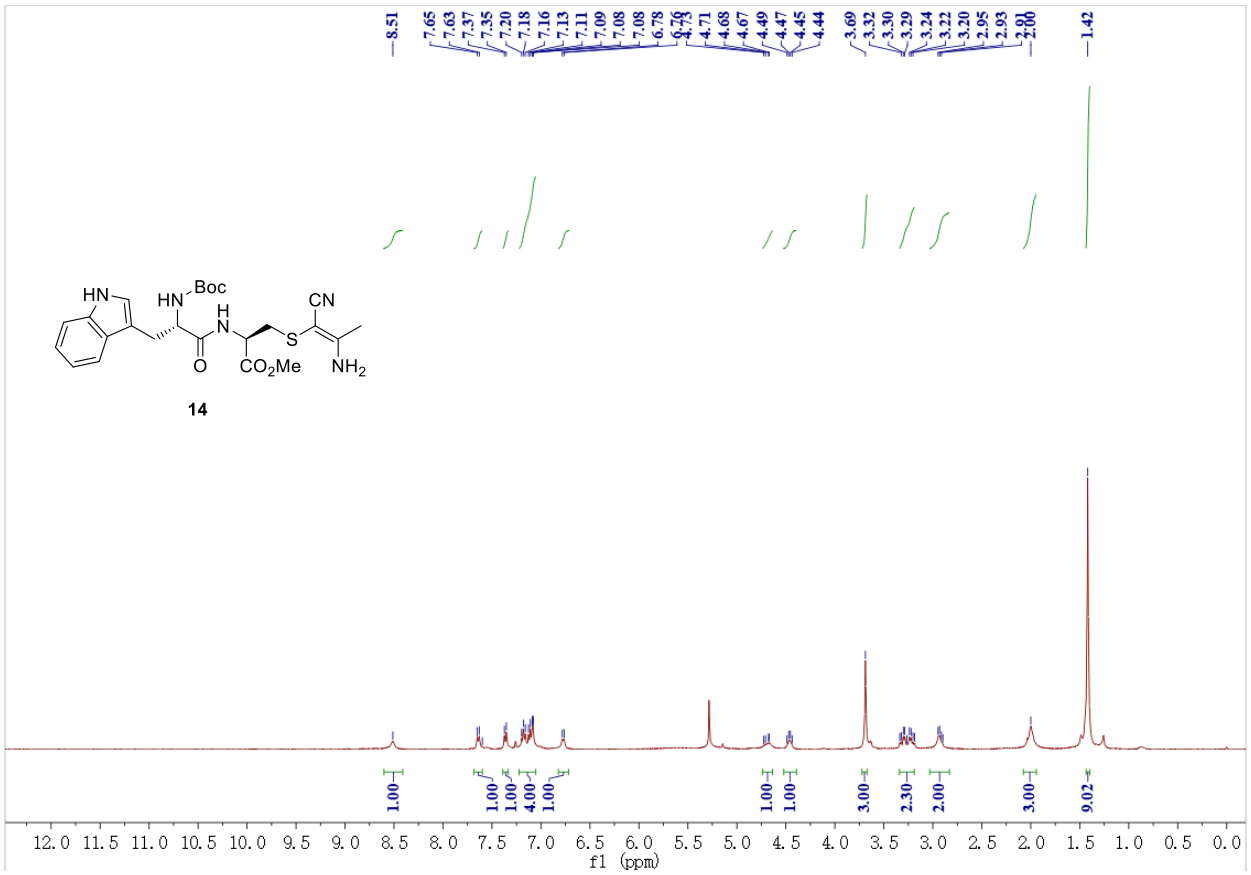
7.12
5.46
5.44
4.73
4.72
4.71
4.32
4.30
4.28
3.78
3.76
3.10
3.09
3.07
2.60
2.58
2.18
2.13
2.12
2.11
1.45
Boc
N
H
S
H
N
O
CO2Me
CN
NH2
0.85
1.00
1.00
1.00
3.00
2.00
2.00
2.86
4.00
1.00
9.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

13


172.00
170.61
164.56
155.86
121.36
80.53
66.57
53.70
52.89
52.34
37.27
31.37
30.33
28.45
20.41
15.39
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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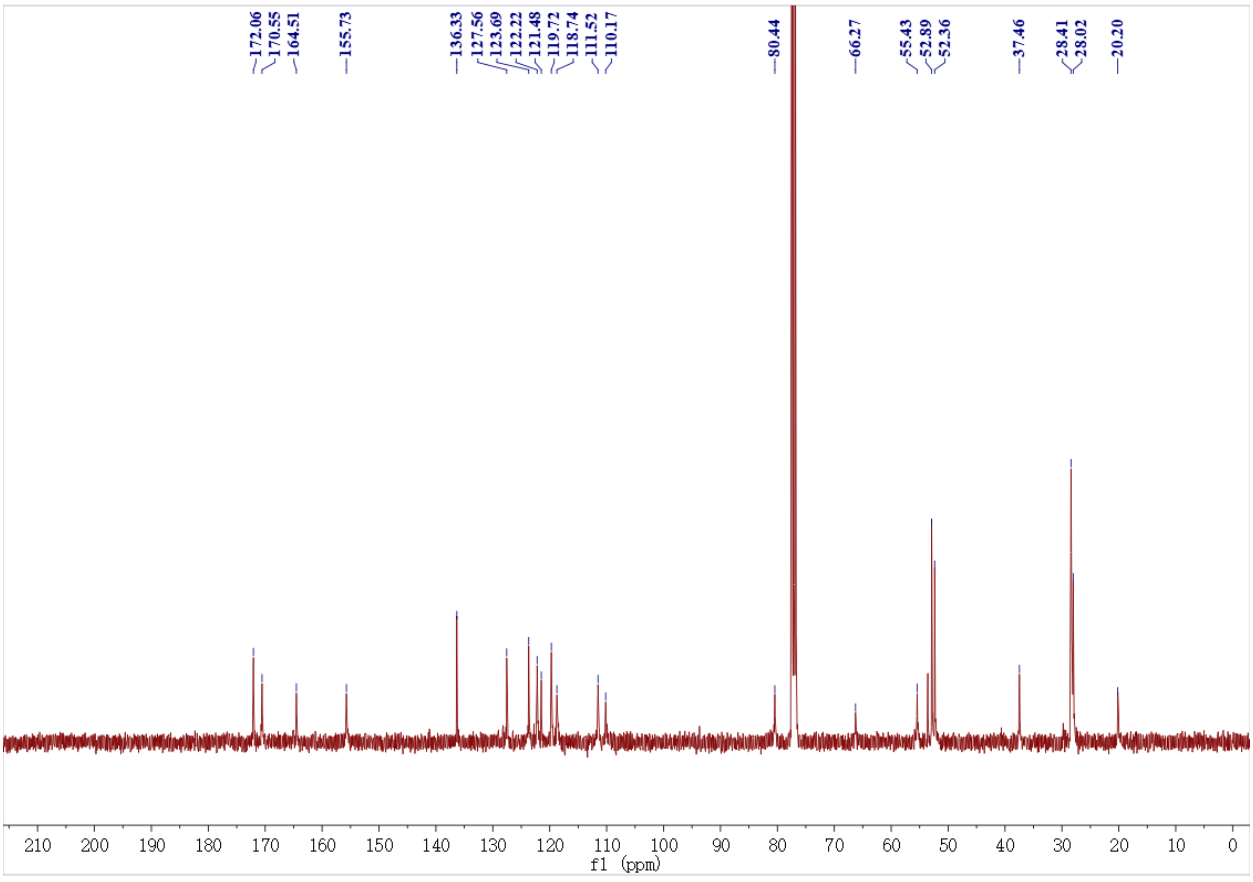
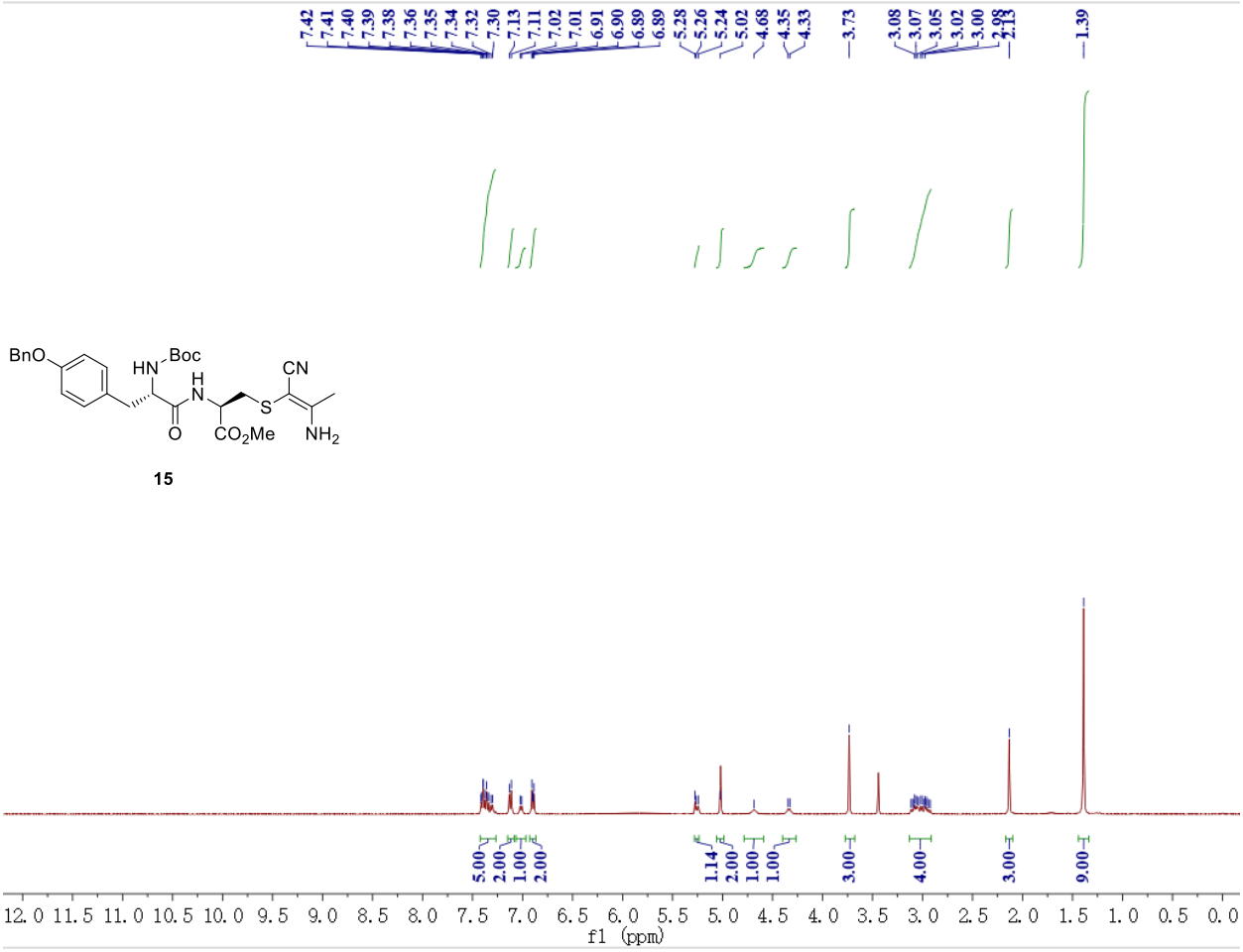
7.63
7.37
7.35
7.20
7.18
7.16
7.13
7.11
7.09
7.08
7.08
6.78
4.71
4.68
4.67
4.49
4.47
4.45
4.44
3.69
3.32
3.30
3.29
3.24
3.22
3.20
2.95
2.93
2.91
2.00
1.42
HN
HN
Boc
H
N
O
CN
S
CO2Me
NH2
1.00
1.00
1.00
4.00
1.00
1.00
3.00
2.30
2.00
3.00
9.02
f1 (ppm)

14


172.06
170.55
164.51
155.73
136.33
127.56
123.69
122.22
121.48
119.72
118.74
111.52
110.17
80.44
66.27
55.43
52.89
52.36
37.46
28.41
28.02
20.20
f1 (ppm)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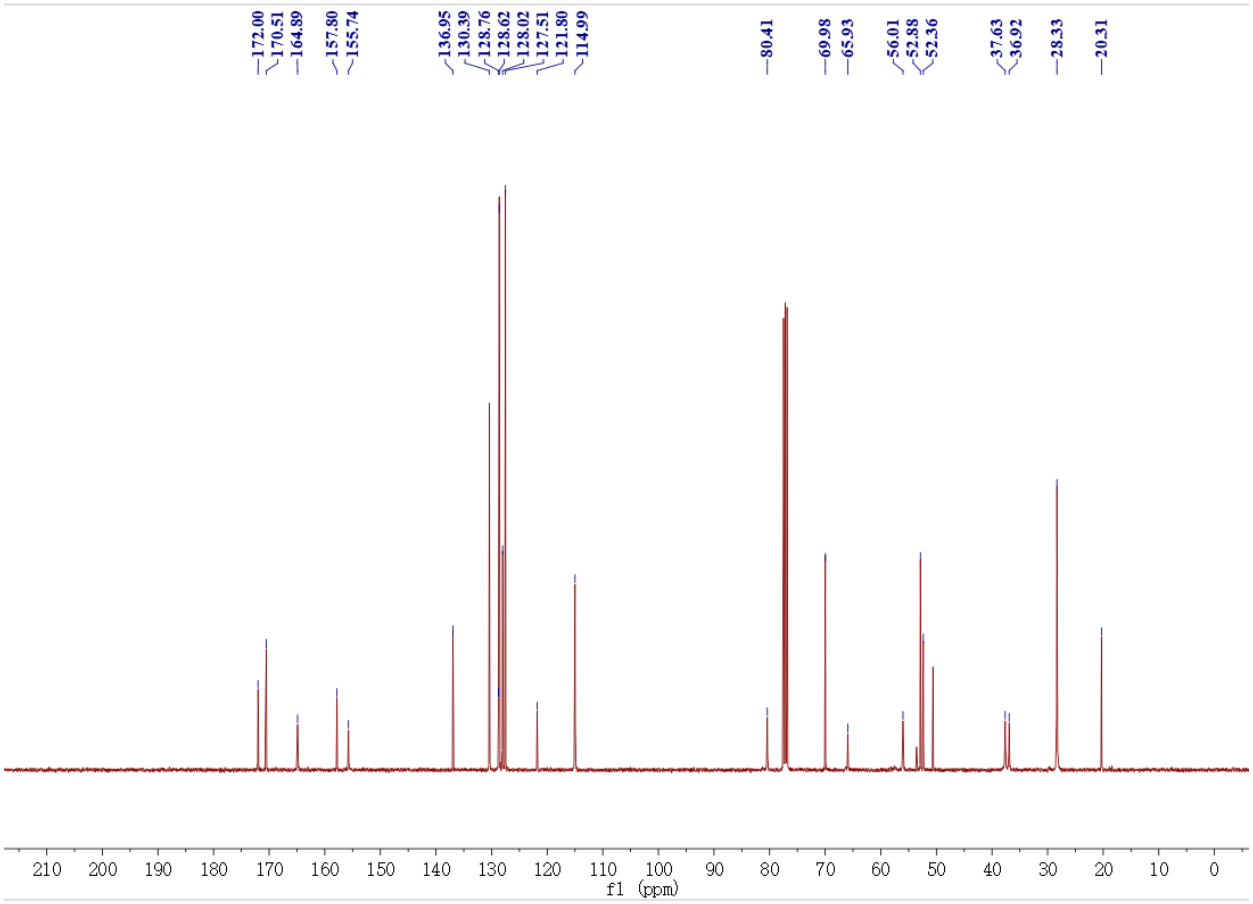
7.41
7.40
7.39
7.38
7.36
7.35
7.34
7.32
7.30
7.13
7.11
7.02
7.01
6.91
6.90
6.89
6.89
5.28
5.26
5.24
5.02
4.68
4.35
4.33
3.73
3.08
3.07
3.05
3.02
3.00
2.98
2.13
1.39
BnO
HN
Boc
H
N
O
CO2Me
S
CN
NH2
15
5.00
2.00
1.00
2.00
1.14
2.00
1.00
1.00
3.00
4.00
3.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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170.51
164.89
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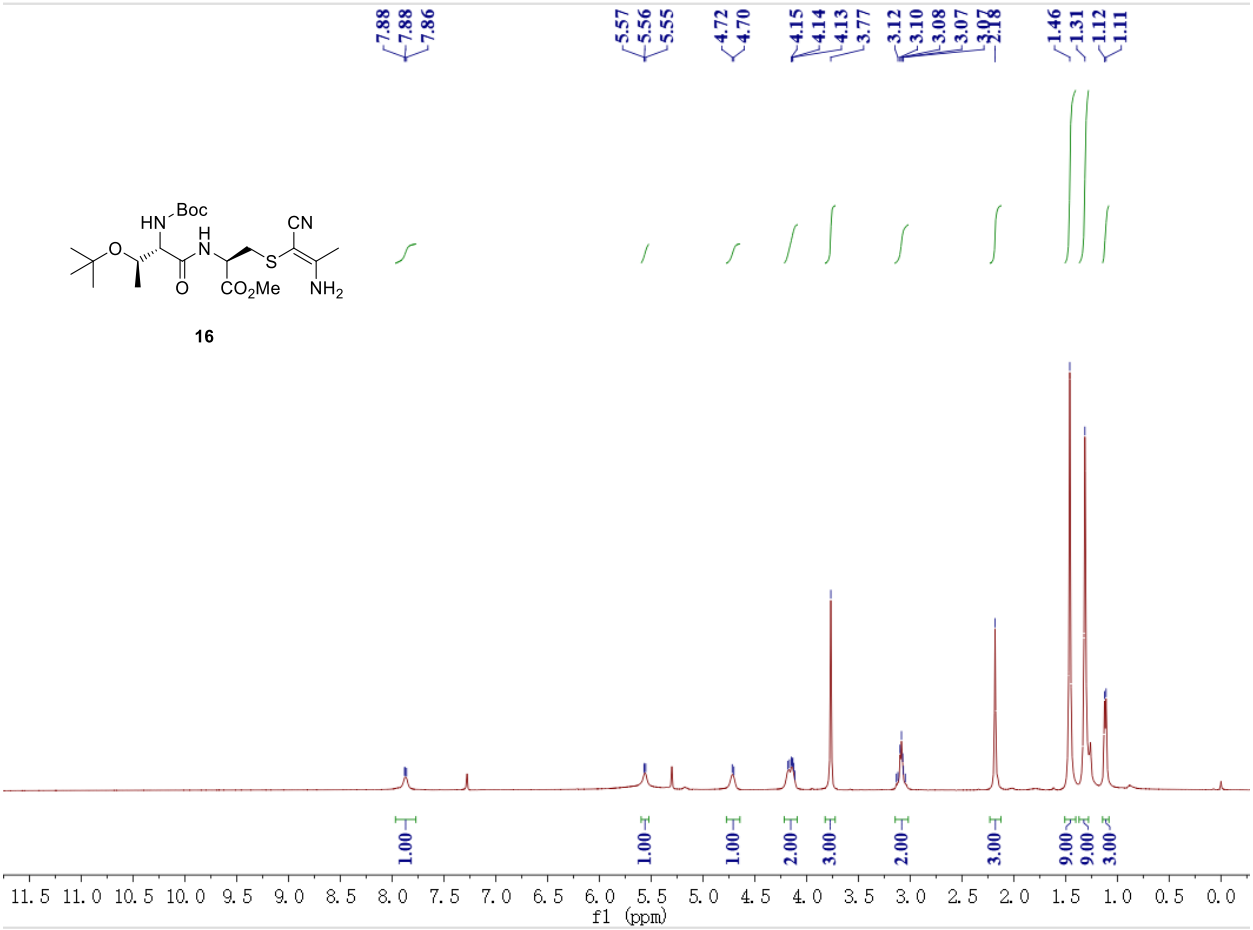
155.74
136.95
130.39
128.76
128.62
128.02
127.51
121.80
114.99
80.41
69.98
65.93
56.01
52.88
52.36
37.63
36.92
28.33
20.31
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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5.56
5.55
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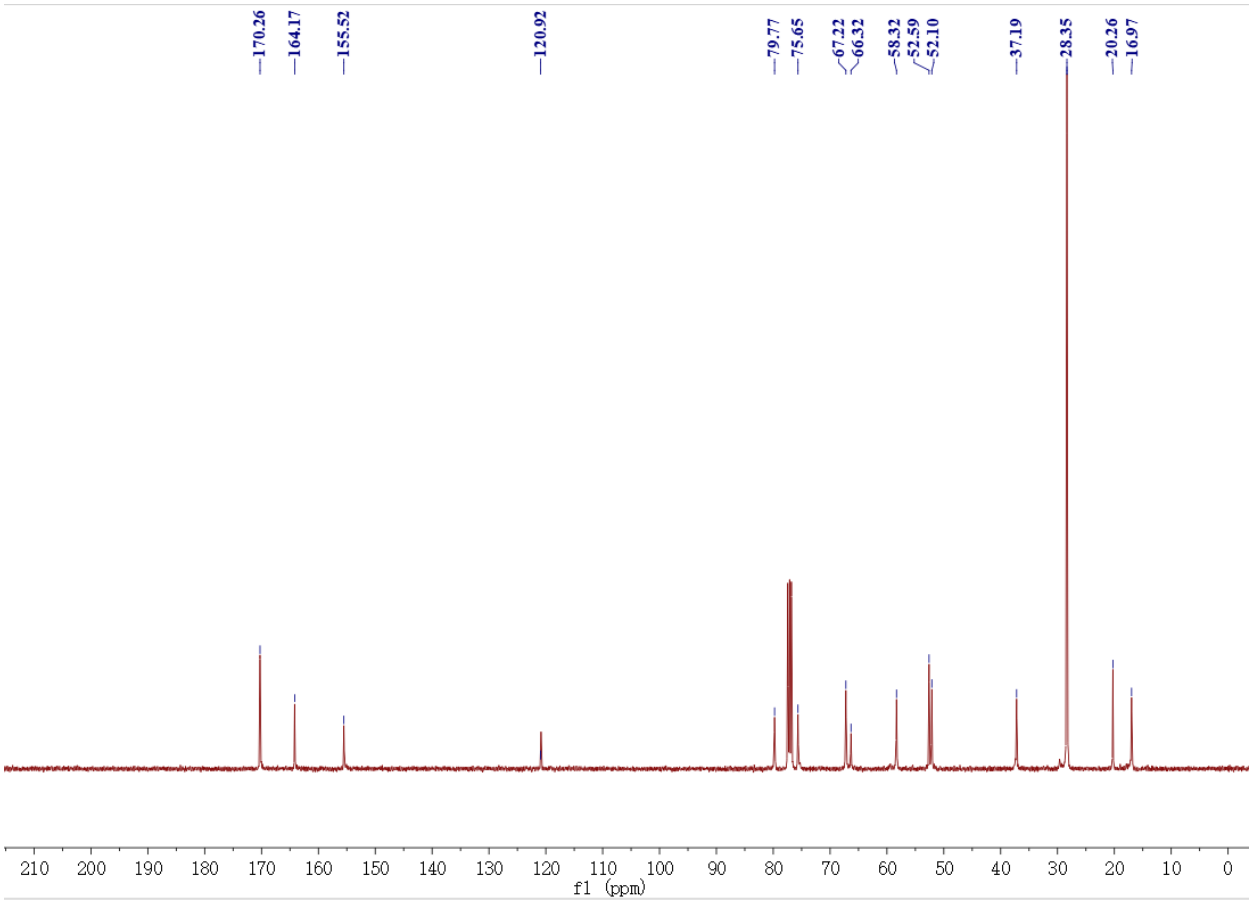
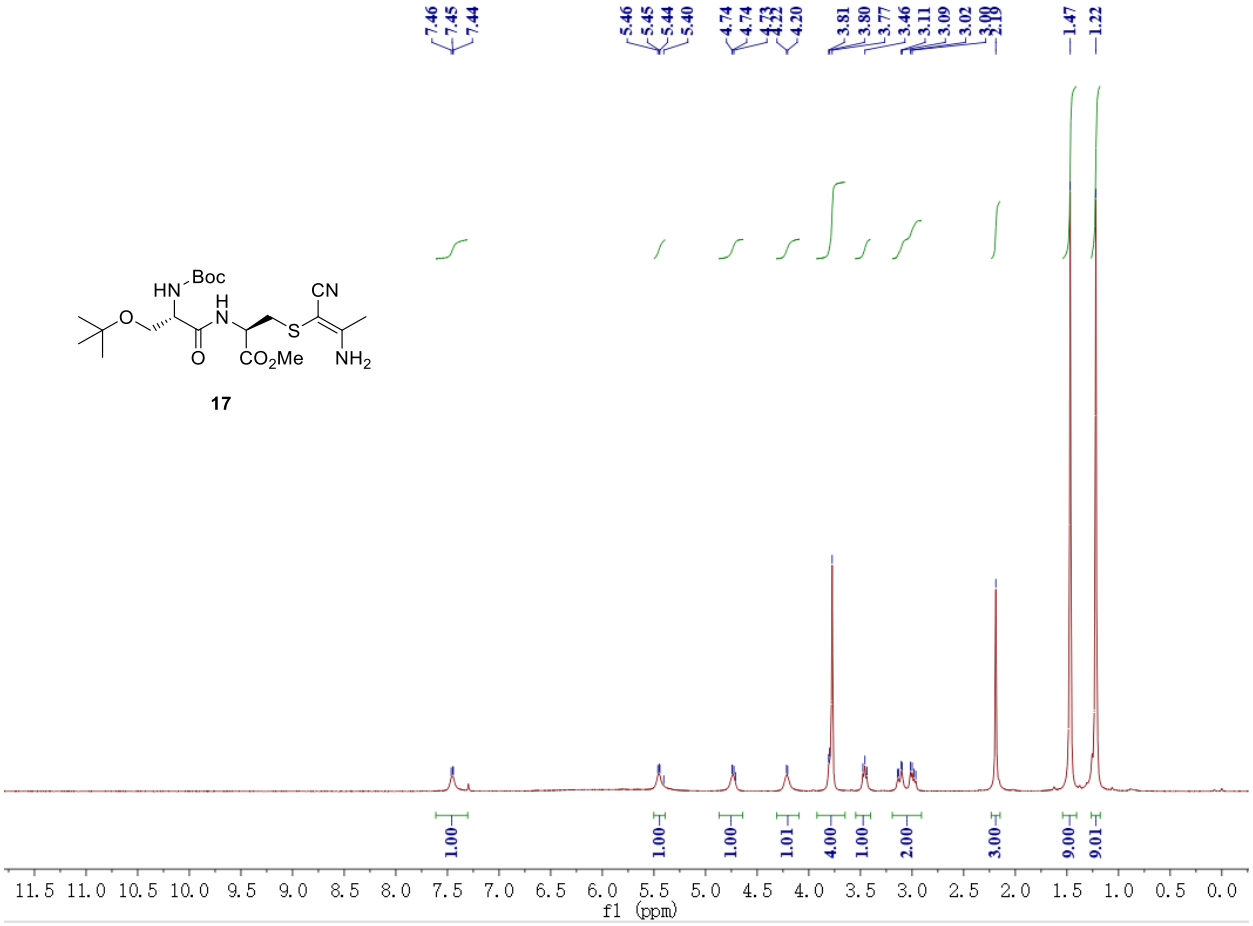
4.70
4.15
4.14
4.13
3.77
3.12
3.10
3.08
3.07
3.07
2.18
1.46
1.31
1.12
1.11
HN
Boc
H
N
O
O
S
CN
NH2
CO2Me
16
1.00
1.00
1.00
2.00
3.00
2.00
3.00
9.00
9.00
3.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


170.26
164.17
155.52
120.92
79.77
75.65
67.22
66.32
58.32
52.59
52.10
37.19
28.35
20.26
16.97
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.45
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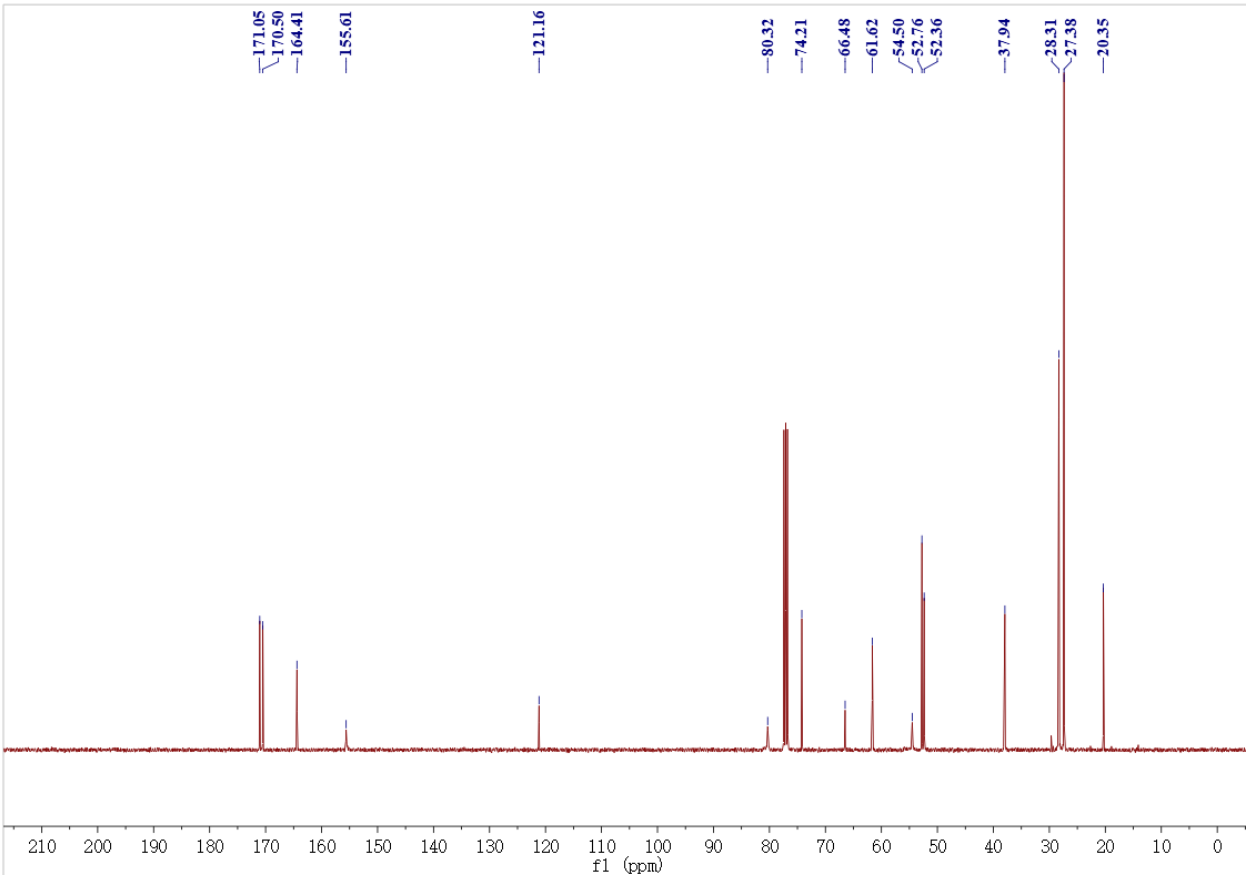
5.45
5.44
5.40
4.74
4.74
4.73
4.22
4.20
3.81
3.80
3.77
3.46
3.11
3.09
3.02
2.19
1.47
1.22
HN
Boc
H
N
O
O
S
CN
CO2Me
NH2
1.00
1.00
1.00
1.01
4.00
1.00
2.00
3.00
9.00
9.01
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

17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170.50
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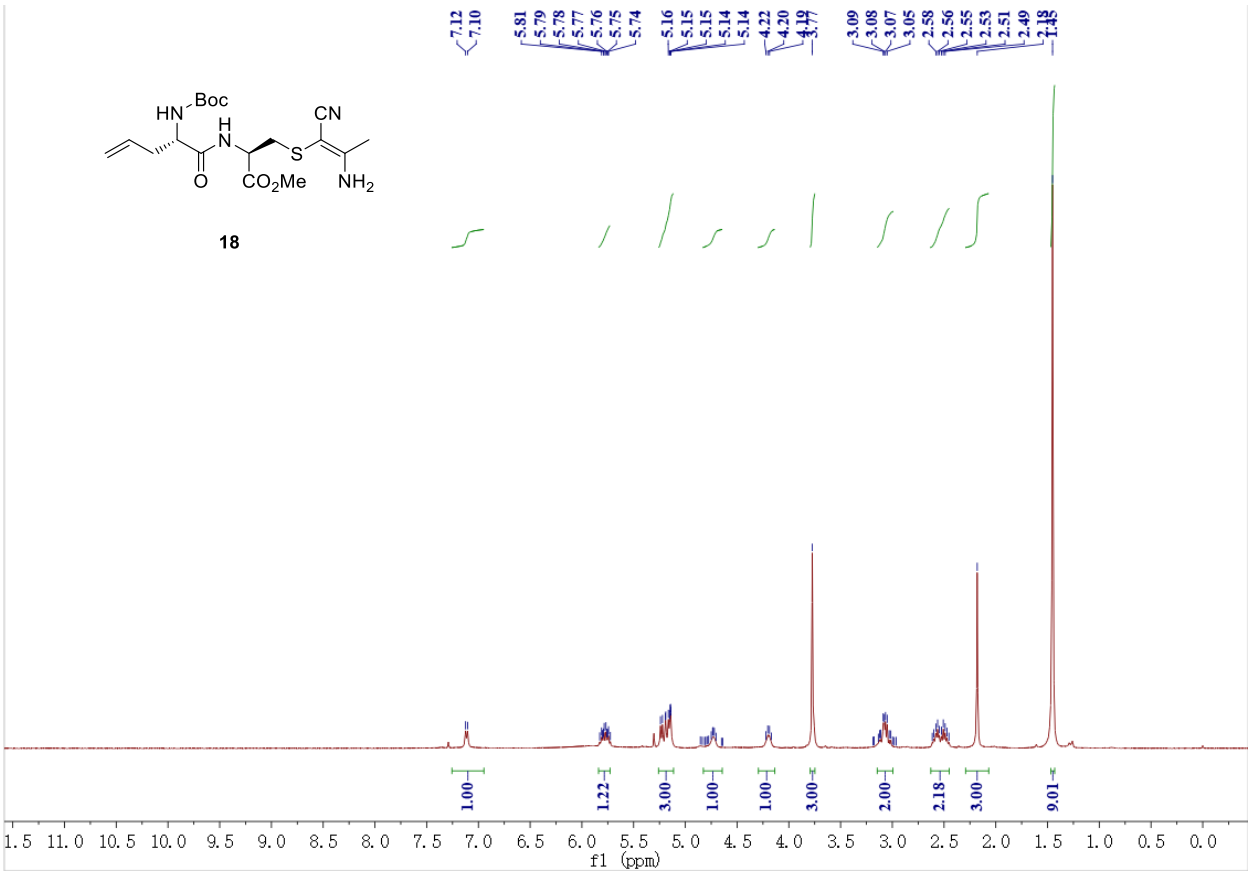
80.32
74.21
66.48
61.62
54.50
52.76
52.36
37.94
28.31
27.38
20.35
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


HN
Boc
H
N
O
CO2Me
S
CN
NH2
18
7.12
7.10
5.81
5.79
5.78
5.77
5.76
5.75
5.74
5.16
5.15
5.15
5.14
5.14
4.22
4.20
4.19
4.17
3.09
3.08
3.07
3.05
2.58
2.56
2.55
2.53
2.51
2.49
2.18
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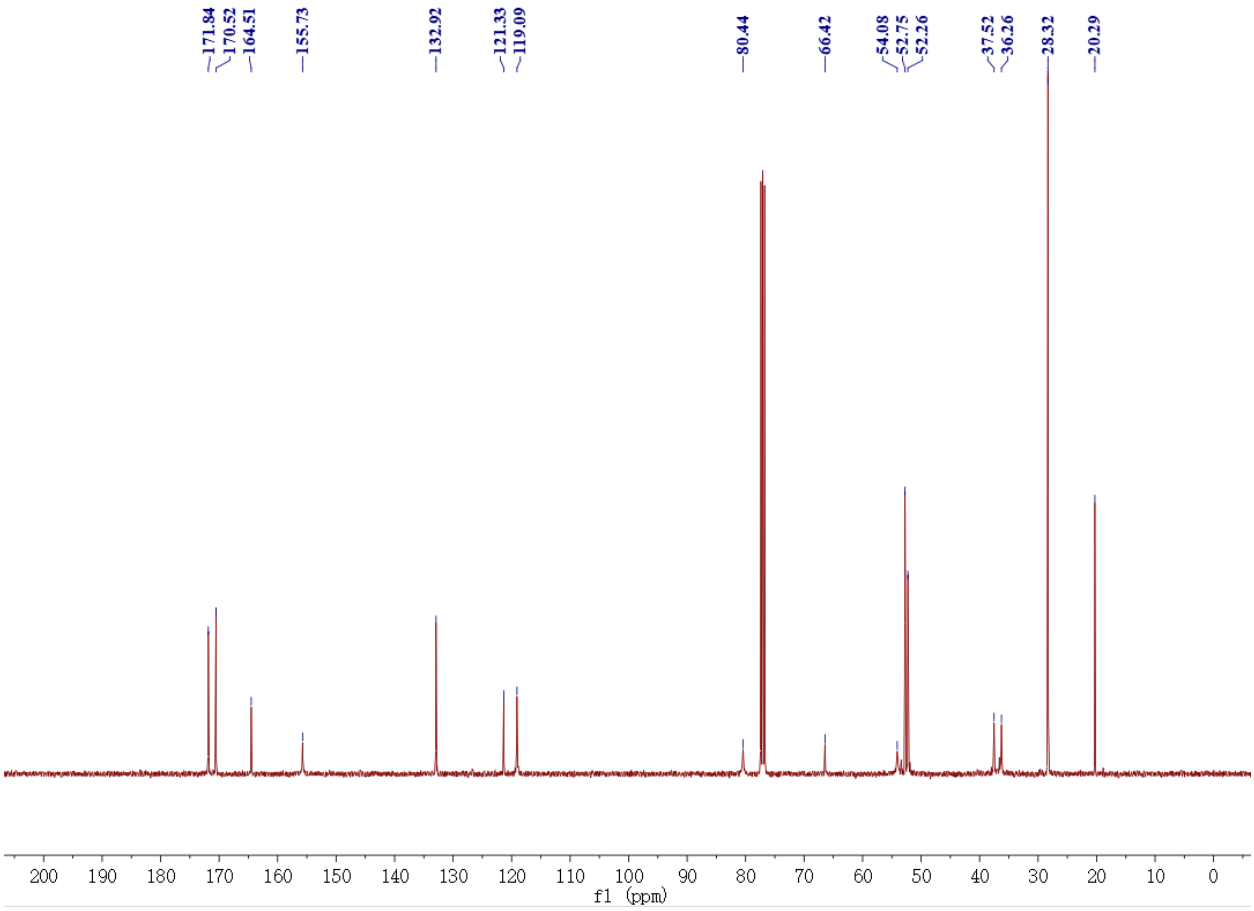
2.00
2.18
3.00
9.01
f1 (ppm)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164.51
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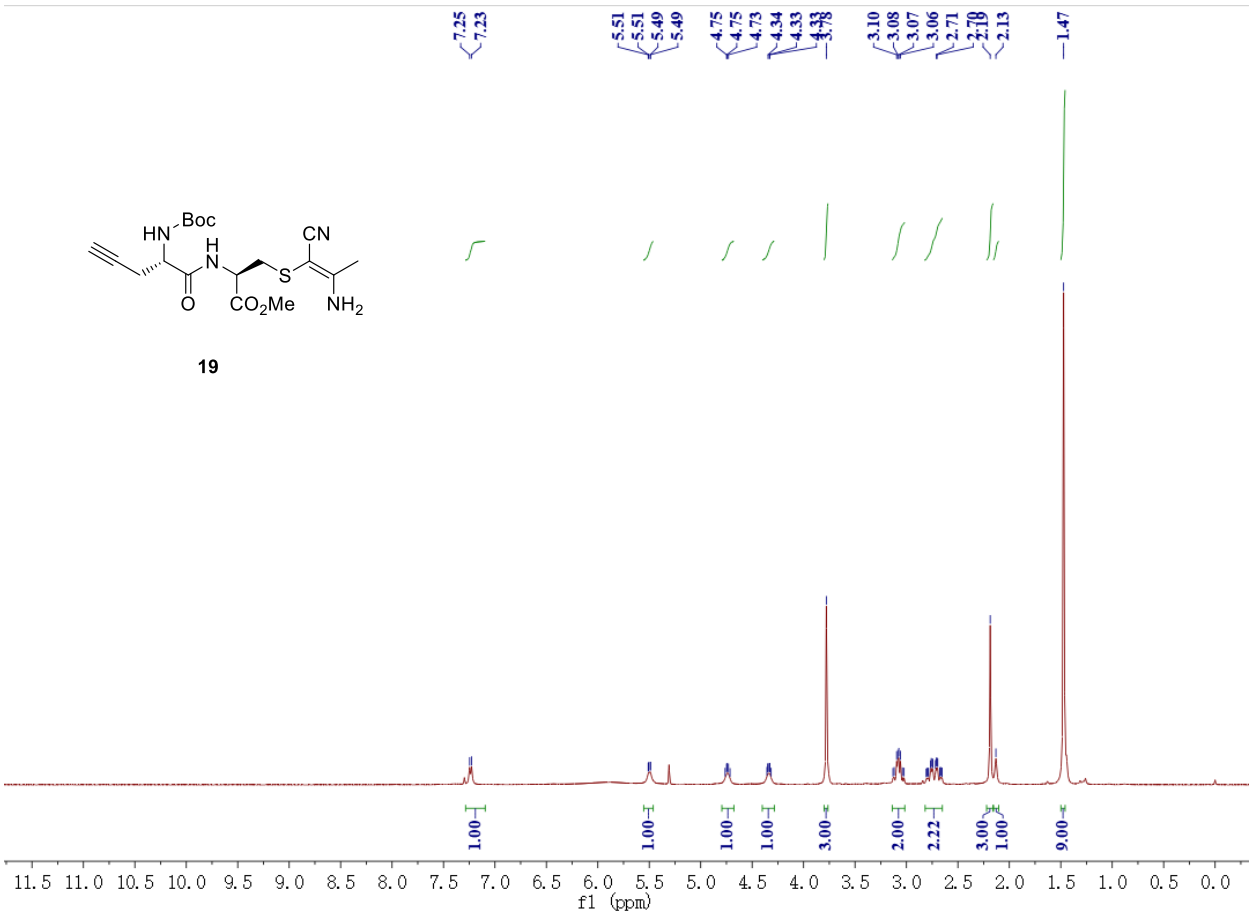
132.92
121.33
119.09
80.44
66.42
54.08
52.75
52.26
37.52
36.26
28.32
20.29
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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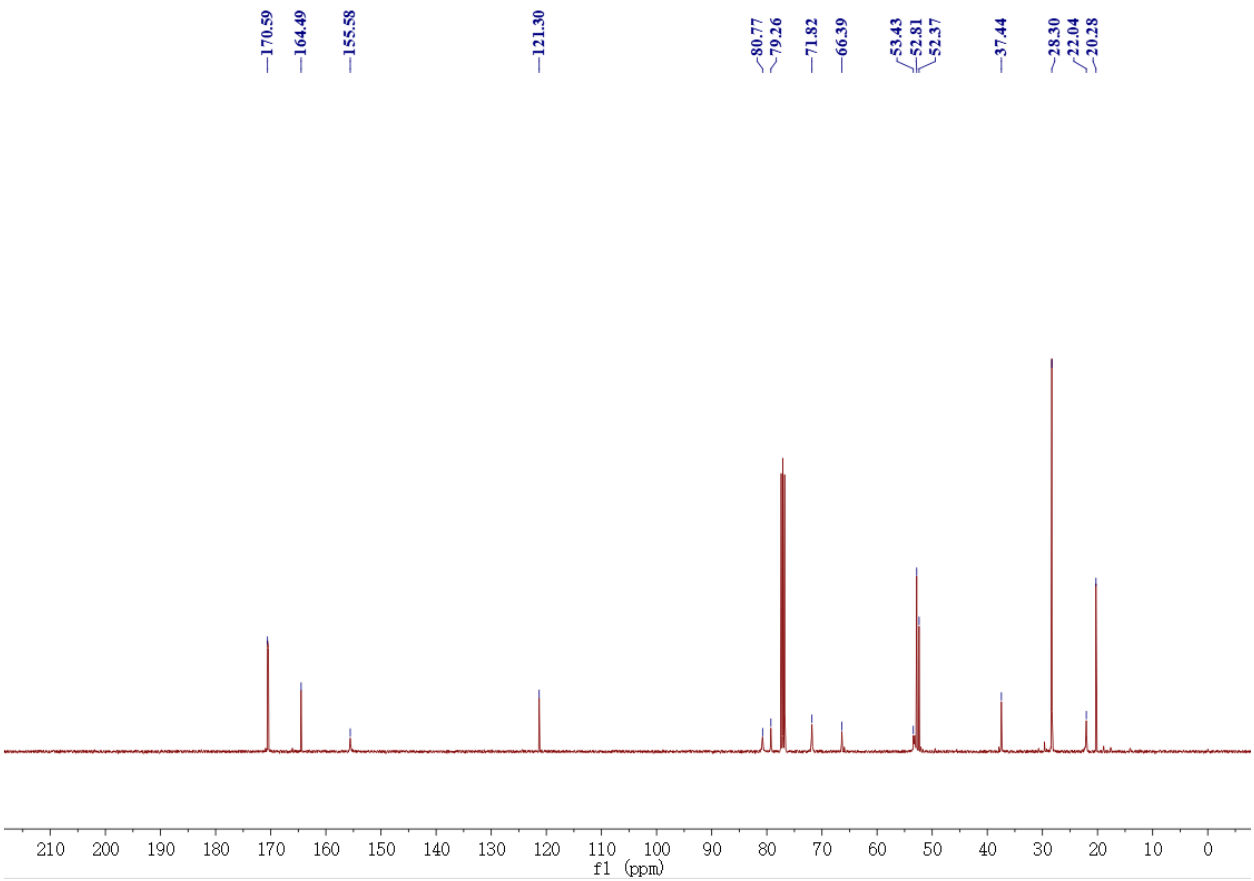
4.75
4.73
4.34
4.33
4.33
3.78
3.10
3.08
3.07
3.06
2.71
2.13
1.47
HN
Boc
H
N
O
S
CN
NH2
CO2Me
1.00
1.00
1.00
1.00
3.00
2.00
2.22
3.00
1.00
9.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

19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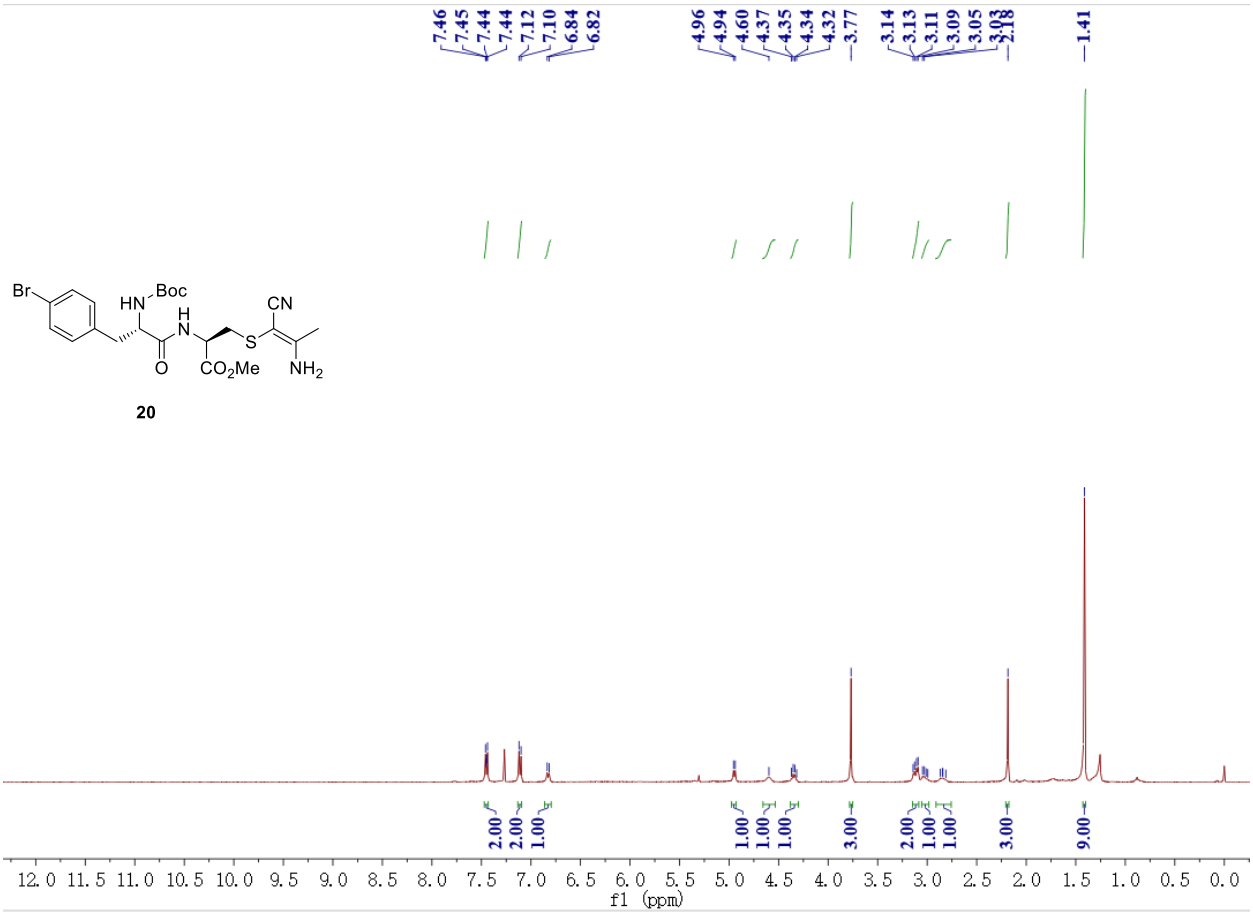
164.49
155.58
121.30
80.77
79.26
71.82
66.39
53.43
52.81
52.37
37.44
28.30
22.04
20.28
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.44
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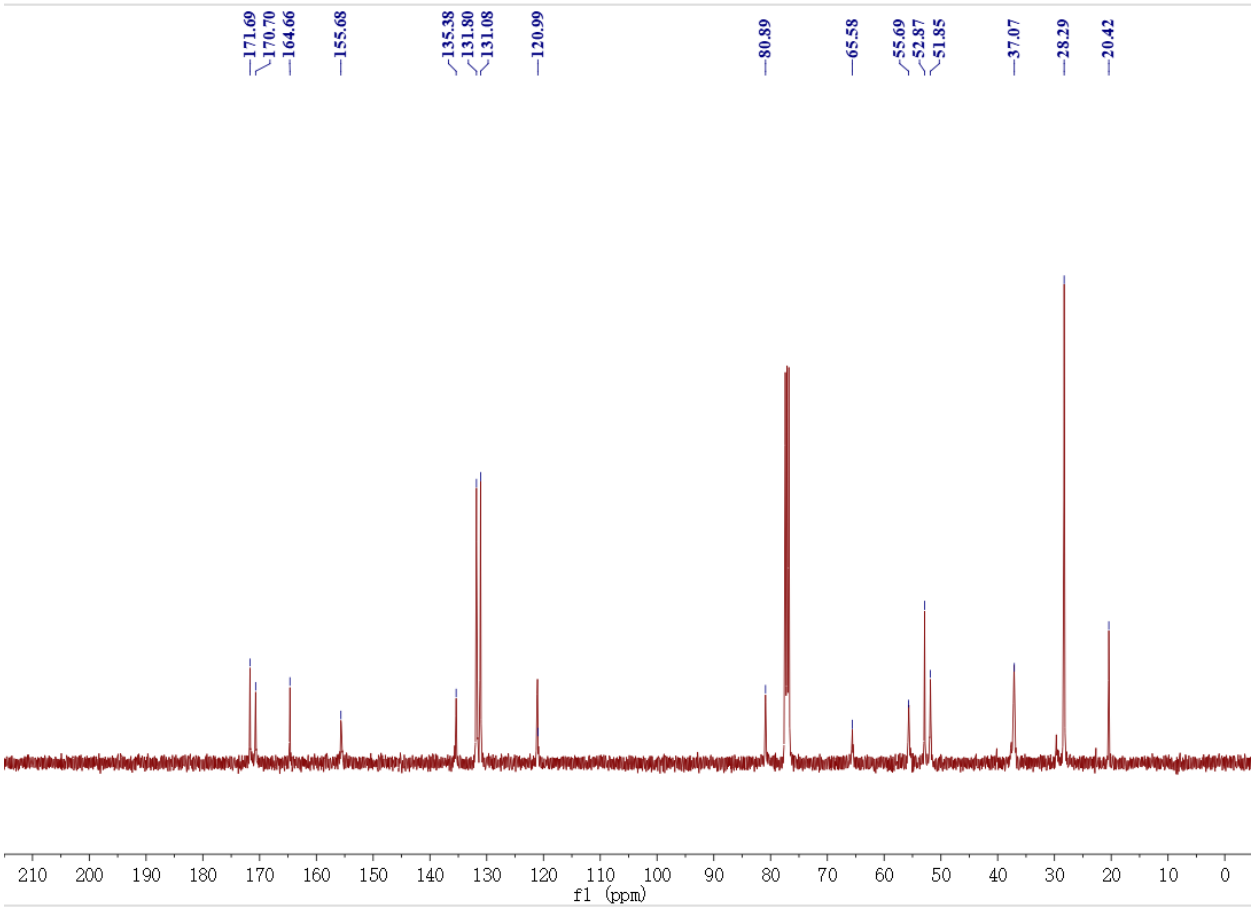
7.10
6.84
6.82
4.96
4.94
4.60
4.37
4.35
4.34
4.32
3.77
3.14
3.13
3.11
3.09
3.05
3.03
2.18
1.41
Br
HN
Boc
H
N
O
CO2Me
S
CN
NH2
2.00
2.00
1.00
1.00
1.00
1.00
3.00
2.00
1.00
1.00
3.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

20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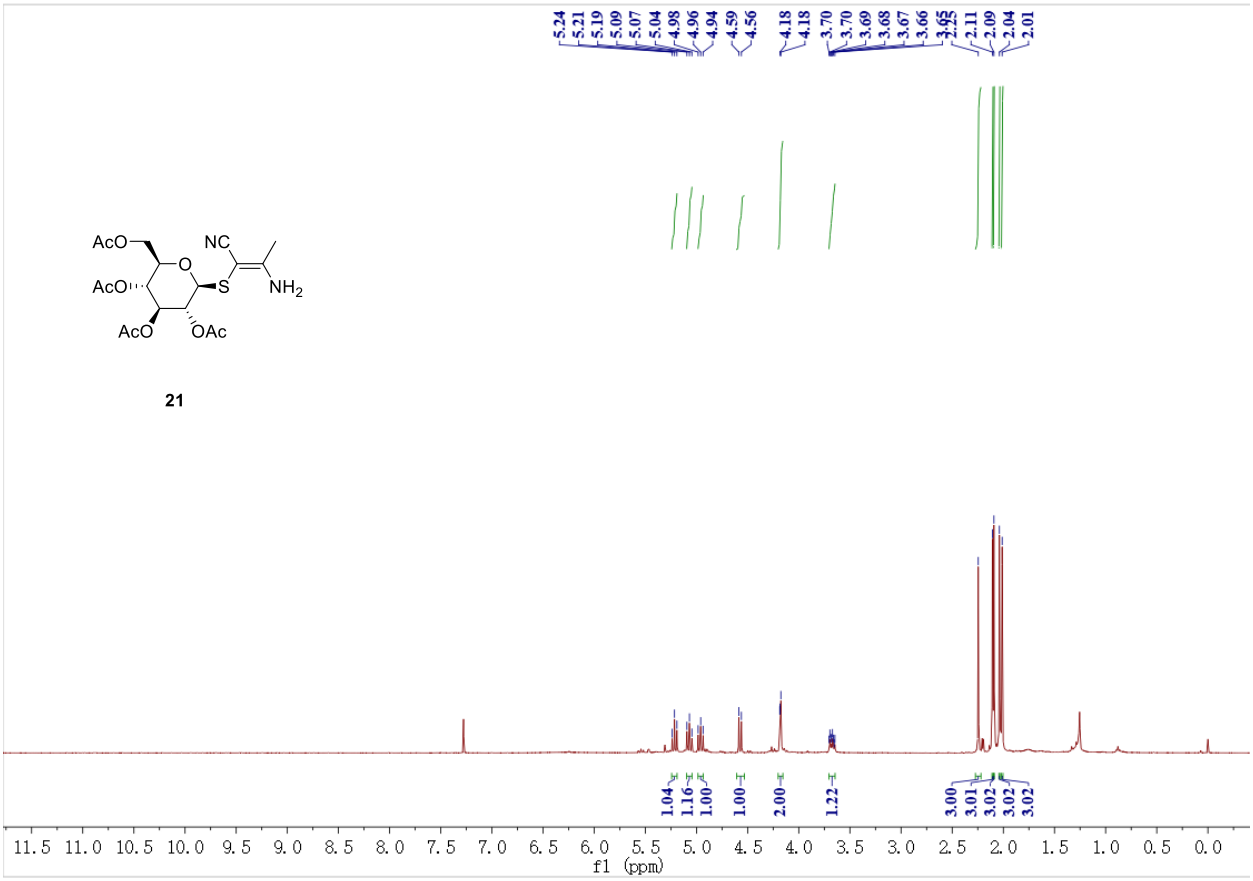
155.68
135.38
131.80
131.08
120.99
80.89
65.58
55.69
52.87
51.85
37.07
28.29
20.42
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


5.24
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5.07
5.04
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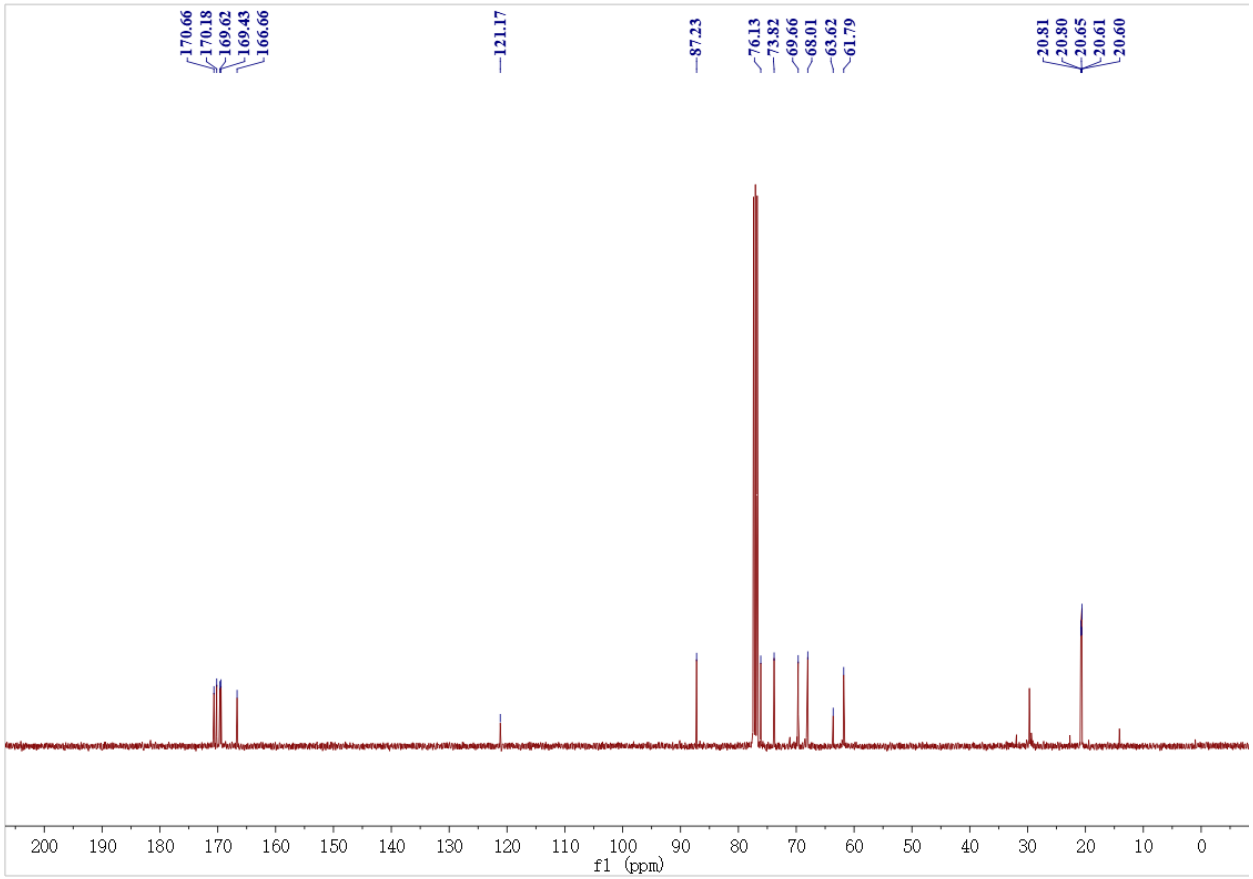
4.96
4.94
4.59
4.56
4.18
4.18
3.70
3.70
3.69
3.68
3.67
3.66
2.11
2.09
2.04
2.01
AcO
AcO
AcO
OAc
NC
O
S
NH2
1.04
1.16
1.00
1.00
2.00
1.22
3.00
3.01
3.02
3.02
3.02
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

21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170.18
169.62
169.43
166.66
121.17
87.23
76.13
73.82
69.66
68.01
63.62
61.79
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20.80
20.65
20.61
20.60
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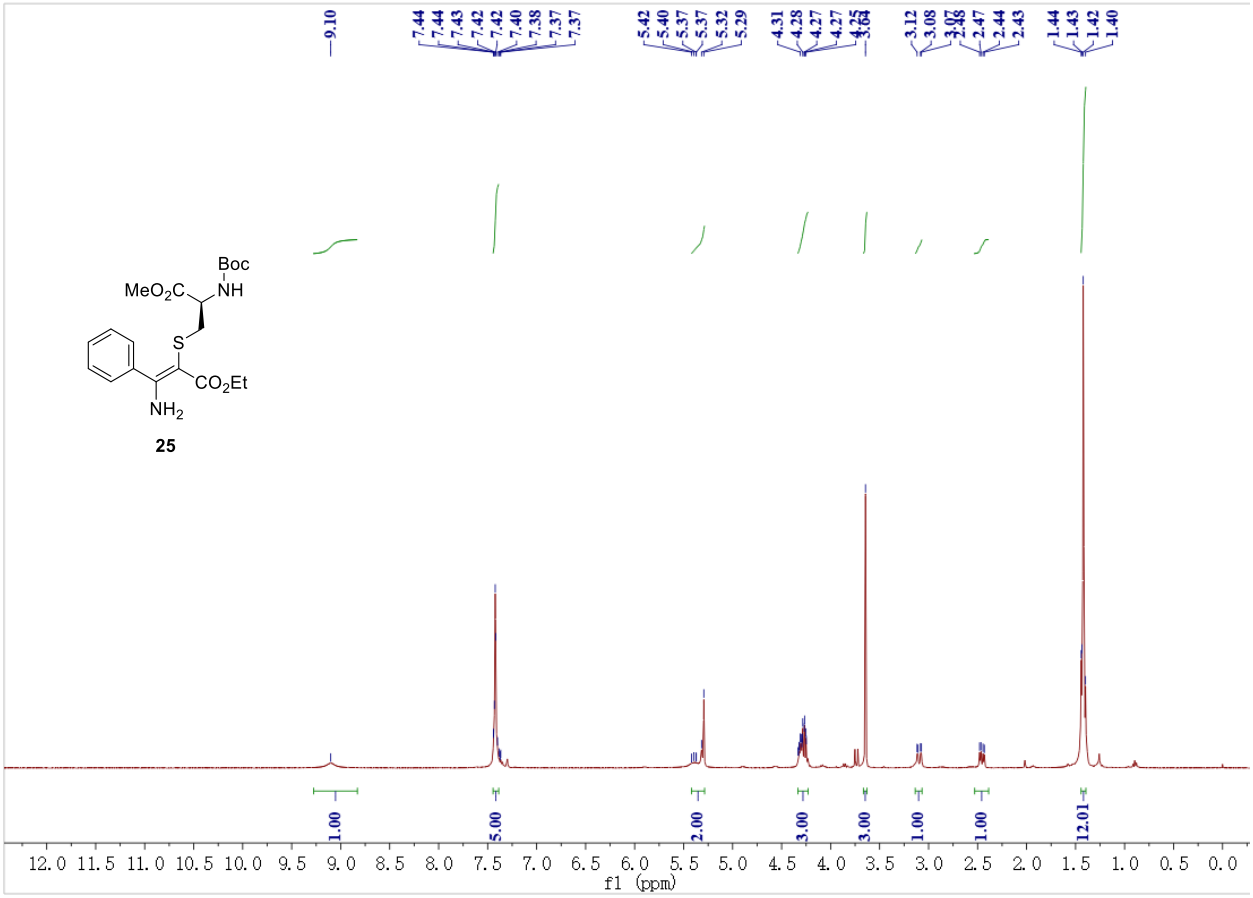
f1 (ppm)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7.44
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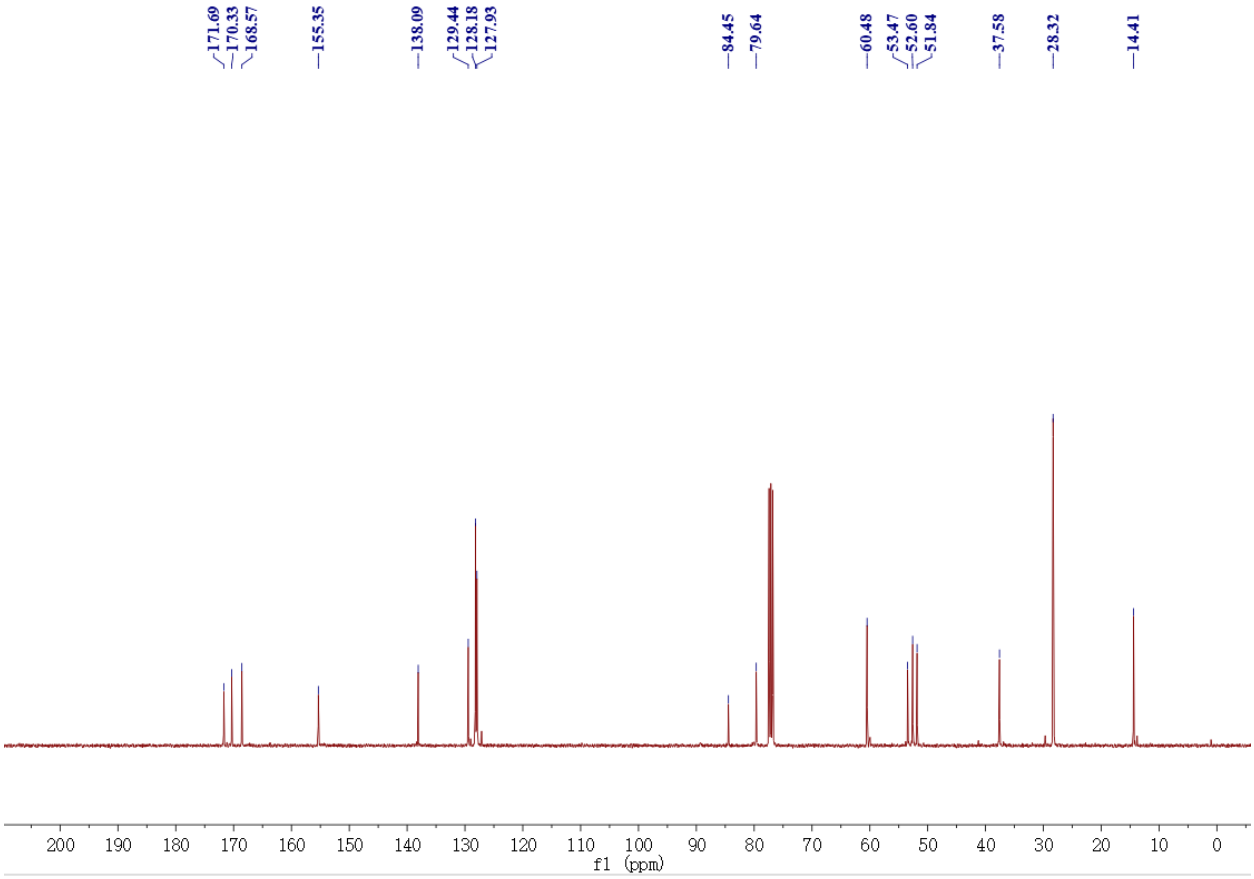
7.38
7.37
7.37
5.42
5.40
5.37
5.37
5.32
5.29
4.31
4.28
4.27
4.27
4.25
3.64
3.12
3.08
3.07
2.48
2.47
2.44
2.43
1.44
1.43
1.42
1.40
Boc
NH
MeO2C
S
CO2Et
NH2
25
1.00
5.00
2.00
3.00
3.00
1.00
1.00
12.01
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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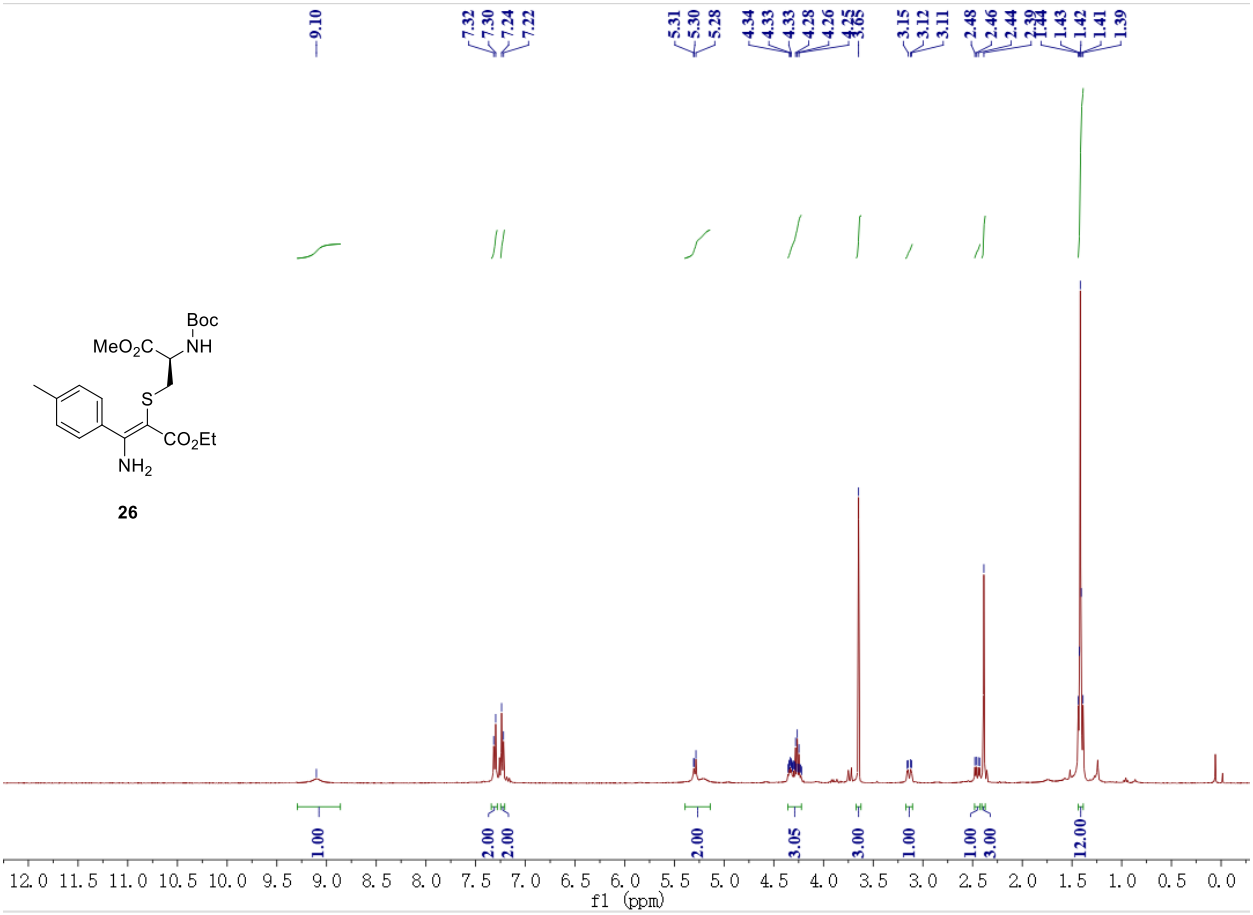
170.33
168.57
155.35
138.09
129.44
128.18
127.93
84.45
79.64
60.48
53.47
52.60
51.84
37.58
28.32
14.41
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.32
7.30
7.24
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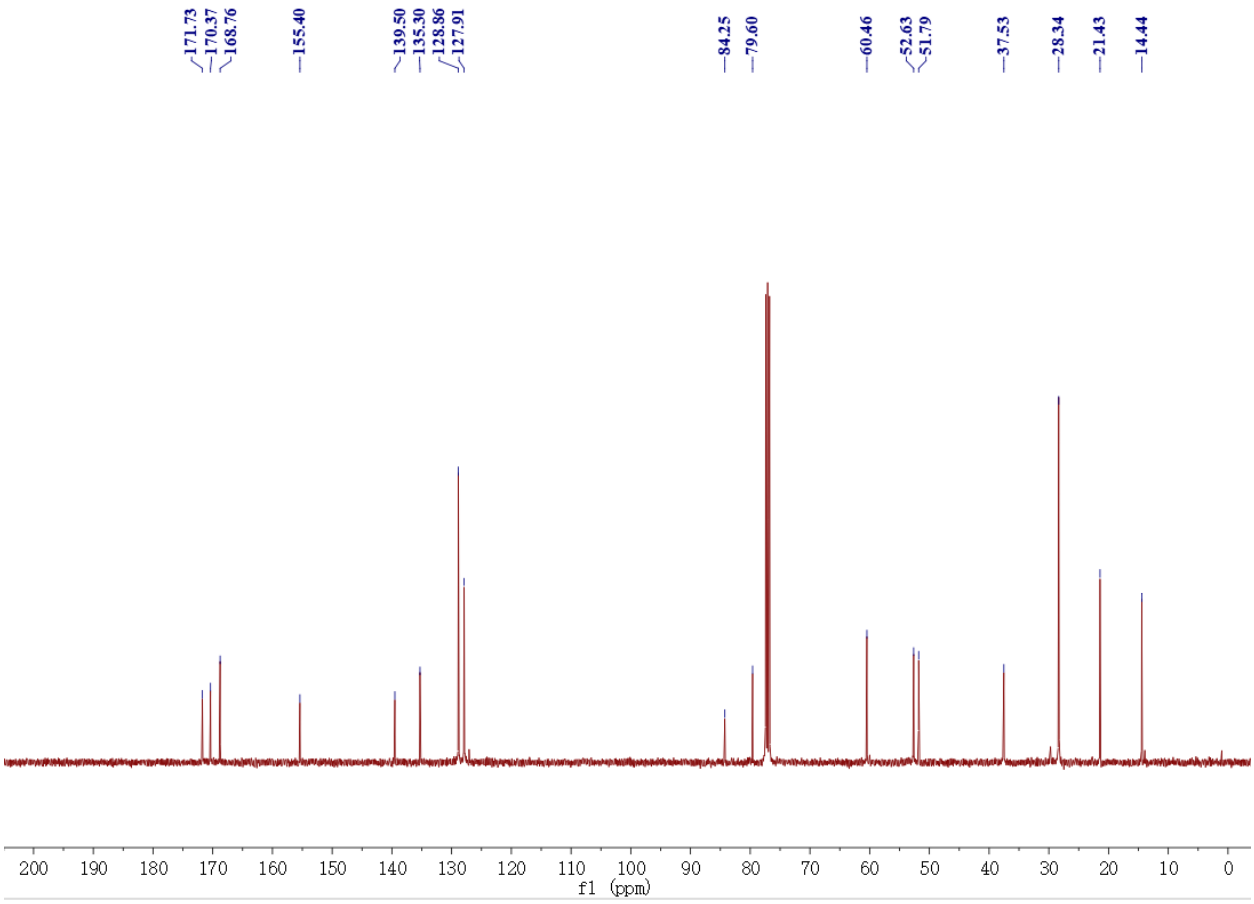
5.31
5.30
5.28
4.34
4.33
4.33
4.28
4.26
3.85
3.15
3.12
3.11
2.48
2.46
2.44
1.43
1.42
1.41
1.39
Boc
MeO2C
NH
S
CO2Et
NH2
1.00
2.00
2.00
2.00
3.05
3.00
1.00
1.00
3.00
12.00
f1 (ppm)

26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127.91
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79.60
60.46
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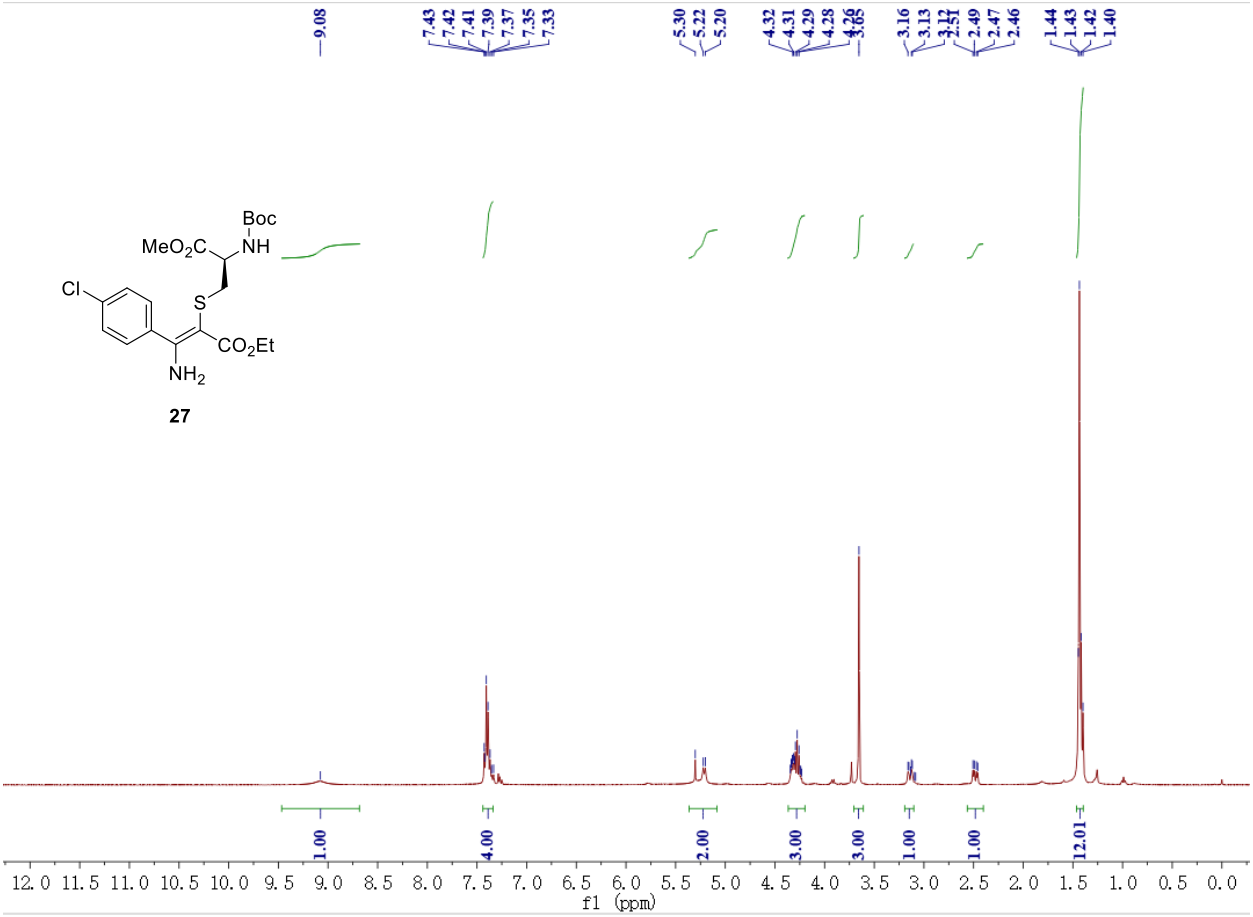
37.53
28.34
21.43
14.44
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.43
7.42
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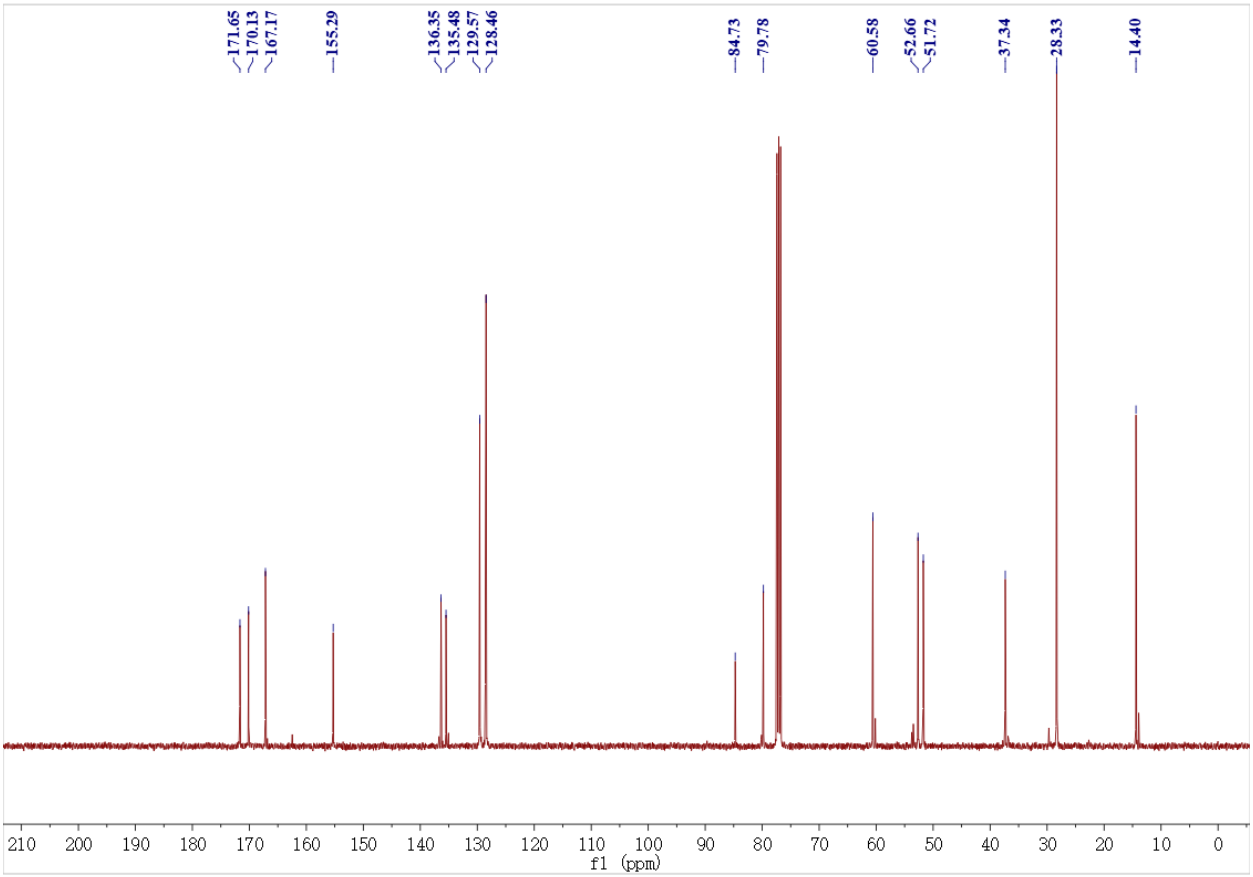
7.37
7.35
7.33
5.30
5.22
5.20
4.32
4.31
4.29
4.28
3.16
3.13
2.49
2.47
2.46
1.44
1.43
1.42
1.40
Boc
NH
MeO2C
Cl
S
CO2Et
NH2
27
1.00
4.00
2.00
3.00
3.00
1.00
1.00
12.01
f1 (ppm)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170.13
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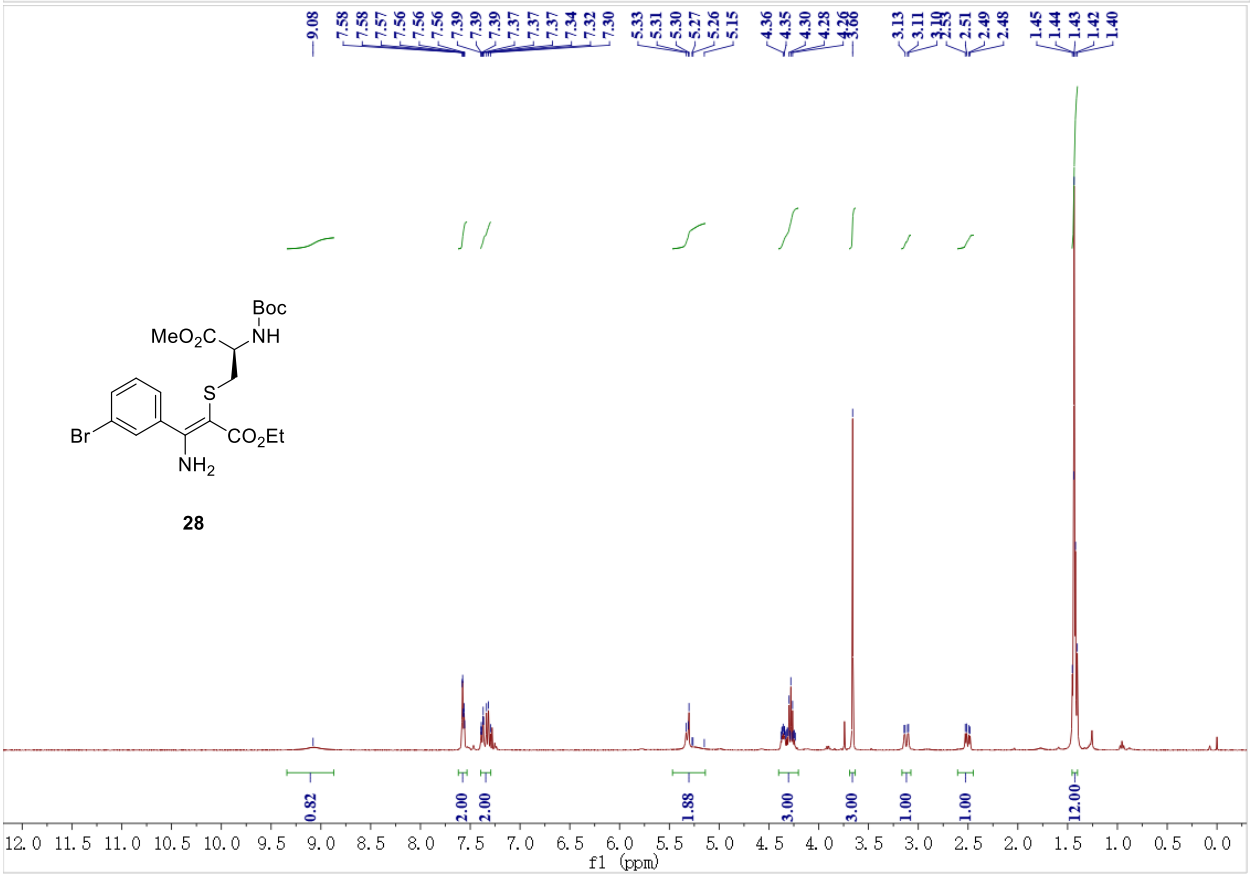
155.29
136.35
135.48
129.57
128.46
84.73
79.78
60.58
52.66
51.72
37.34
28.33
14.40
f1 (ppm)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7.58
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7.56
7.39
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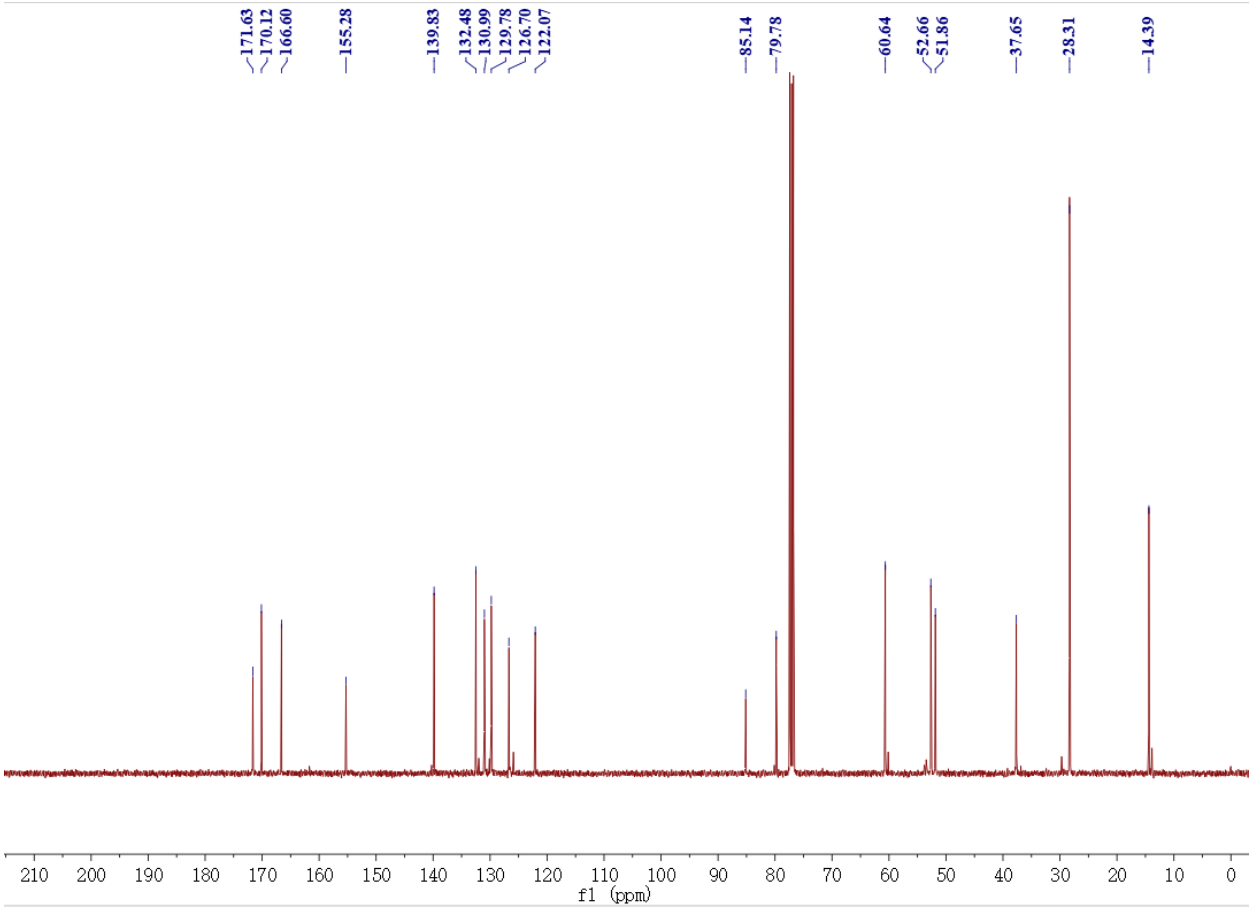
7.39
7.37
7.37
7.37
7.34
7.32
7.30
5.33
5.31
5.30
5.27
5.26
5.15
4.36
4.35
4.30
4.28
3.68
3.13
3.11
2.53
2.51
2.49
2.48
1.45
1.44
1.43
1.42
1.40
Boc
MeO2C
NH
S
Br
CO2Et
NH2
28
0.82
2.00
2.00
1.88
3.00
3.00
1.00
1.00
12.00
f1 (ppm)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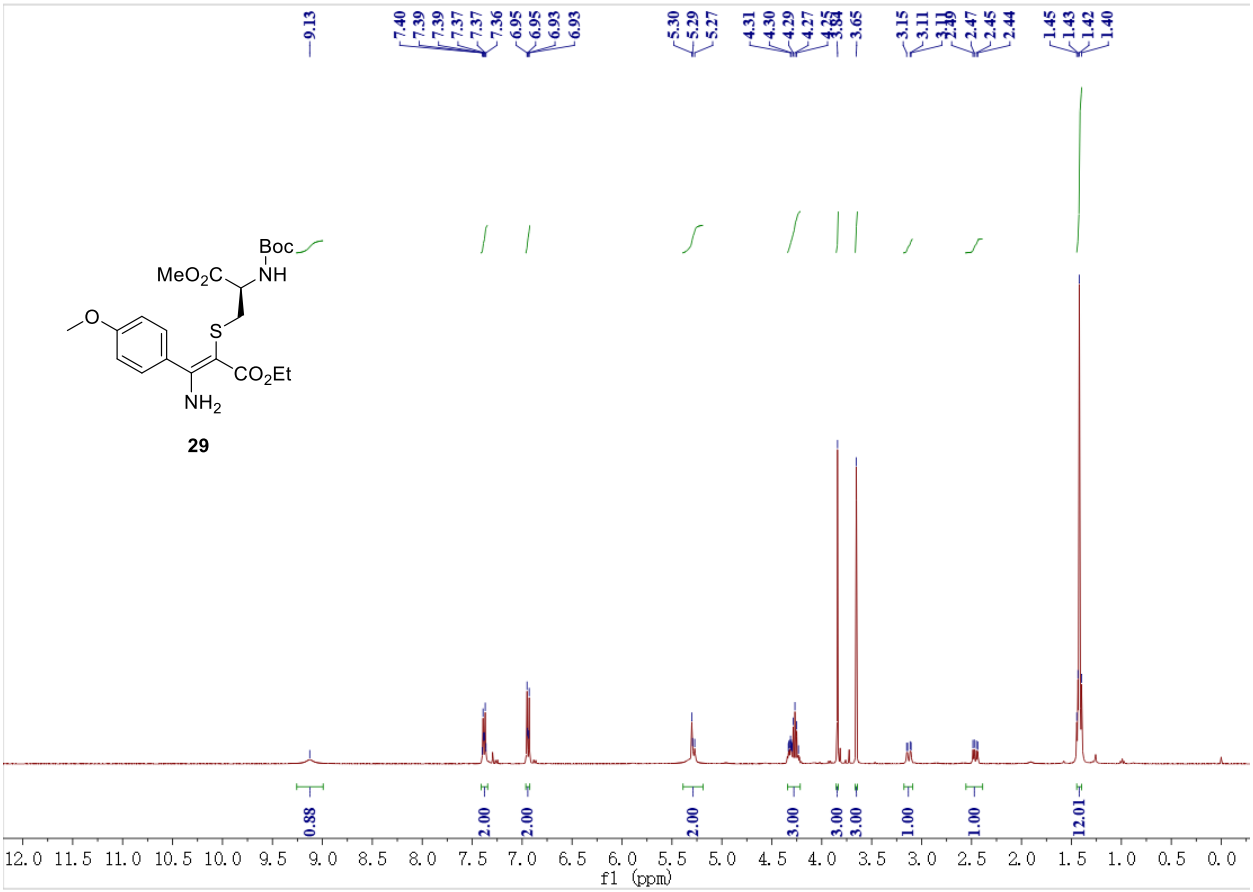
170.12
166.60
155.28
139.83
132.48
130.99
129.78
126.70
122.07
85.14
79.78
60.64
52.66
51.86
37.65
28.31
14.39
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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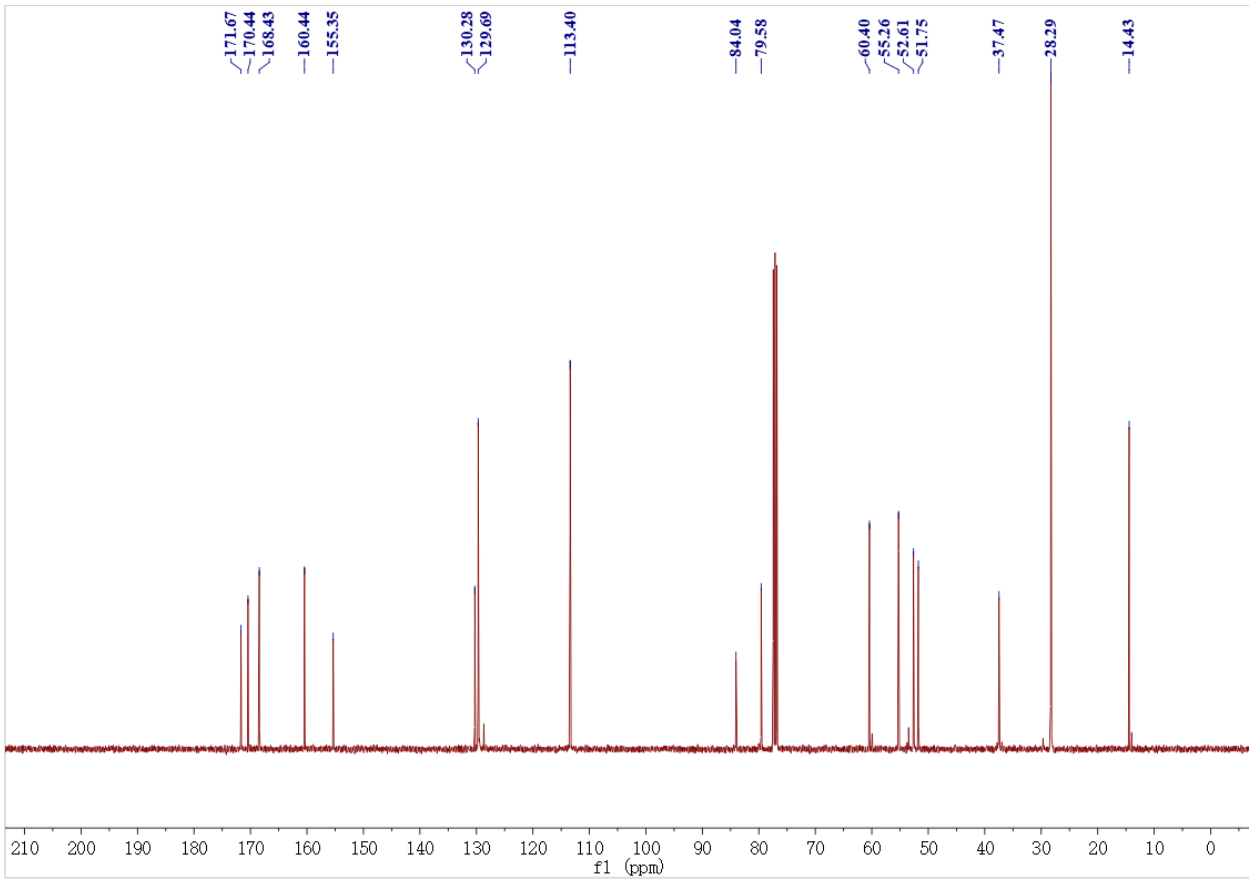
7.40
7.39
7.39
7.37
7.37
7.36
6.95
6.95
6.93
6.93
5.30
5.29
5.27
4.31
4.30
4.29
4.27
3.84
3.65
3.15
3.11
2.47
2.45
2.44
1.45
1.43
1.42
1.40
Boc
MeO2C
NH
S
O
CO2Et
NH2
29
0.88
2.00
2.00
2.00
3.00
3.00
3.00
1.00
1.00
12.01
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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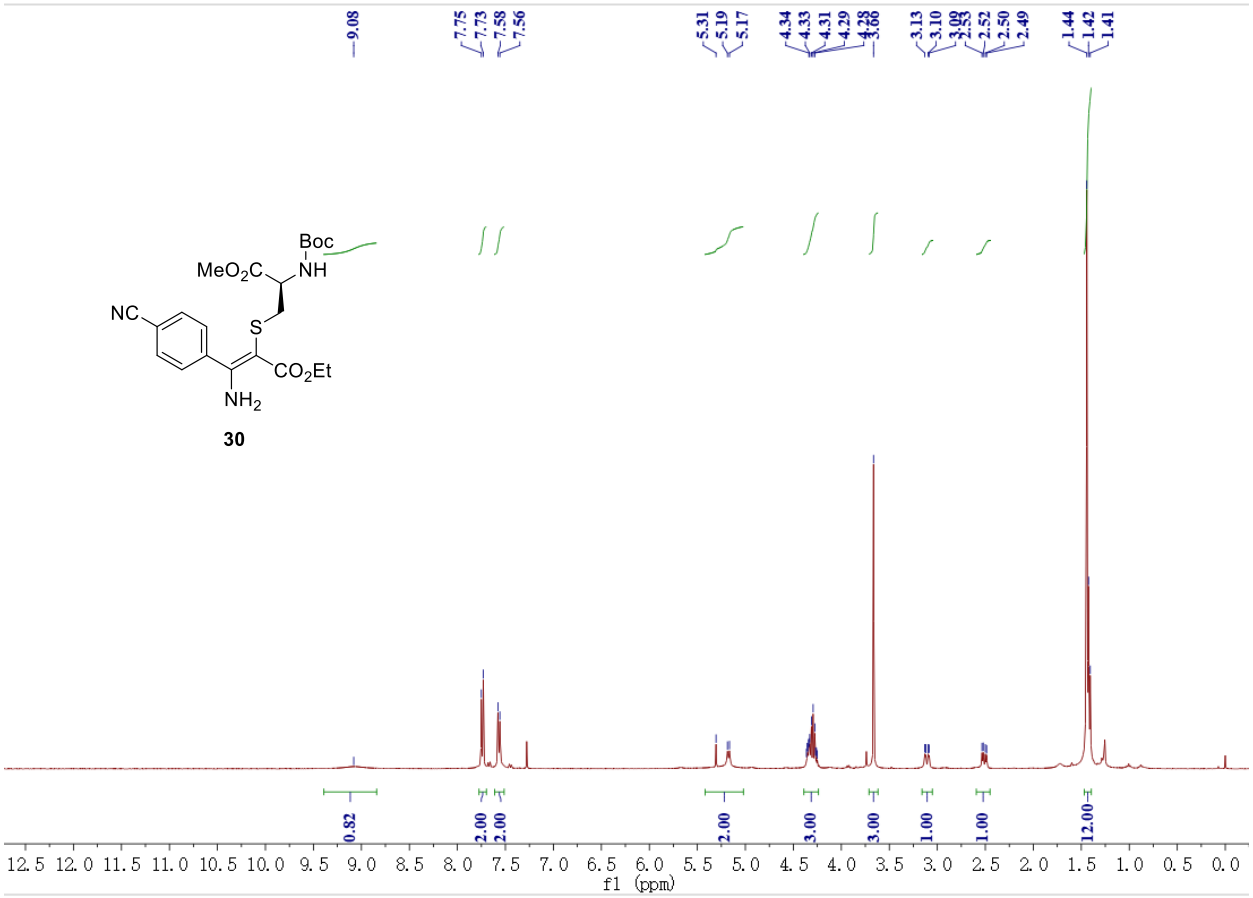
170.44
168.43
160.44
155.35
130.28
129.69
113.40
84.04
79.58
60.40
55.26
52.61
51.75
37.47
28.29
14.43
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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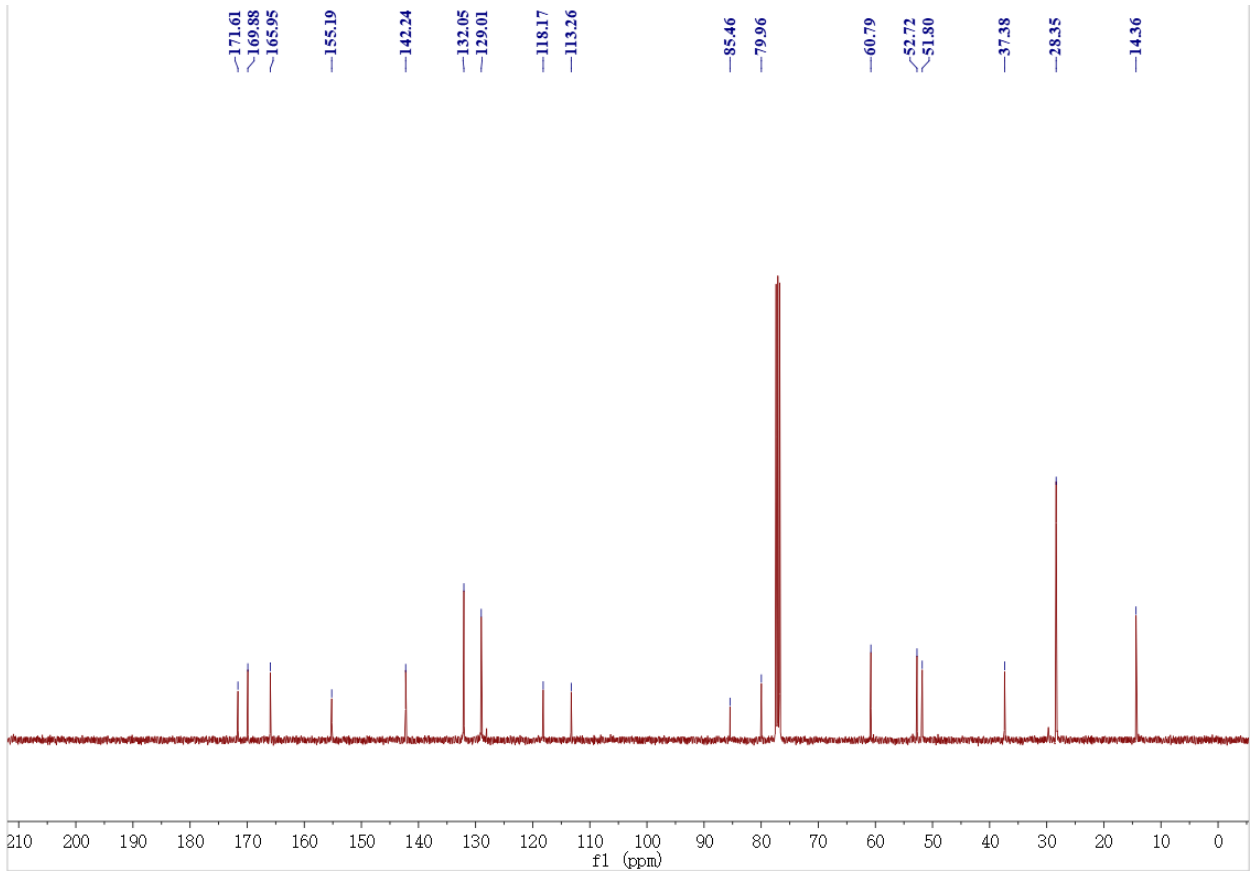
7.75
7.73
7.58
7.56
5.31
5.19
5.17
4.34
4.33
4.31
4.29
4.28
3.68
3.13
3.10
3.09
2.53
2.52
2.50
2.49
1.44
1.42
1.41
Boc
MeO2C
NH
NC
S
CO2Et
NH2
30
0.82
2.00
2.00
2.00
3.00
3.00
1.00
1.00
12.00
f1 (ppm)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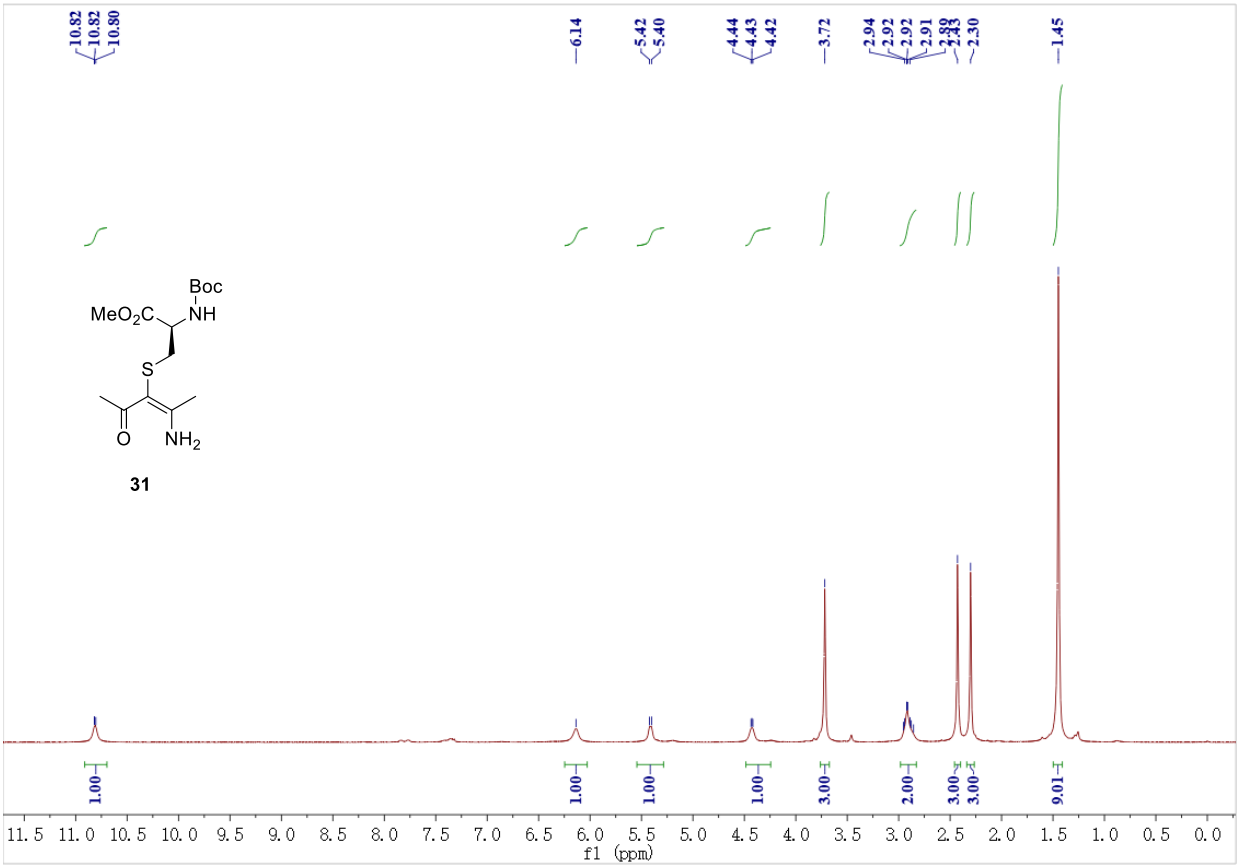
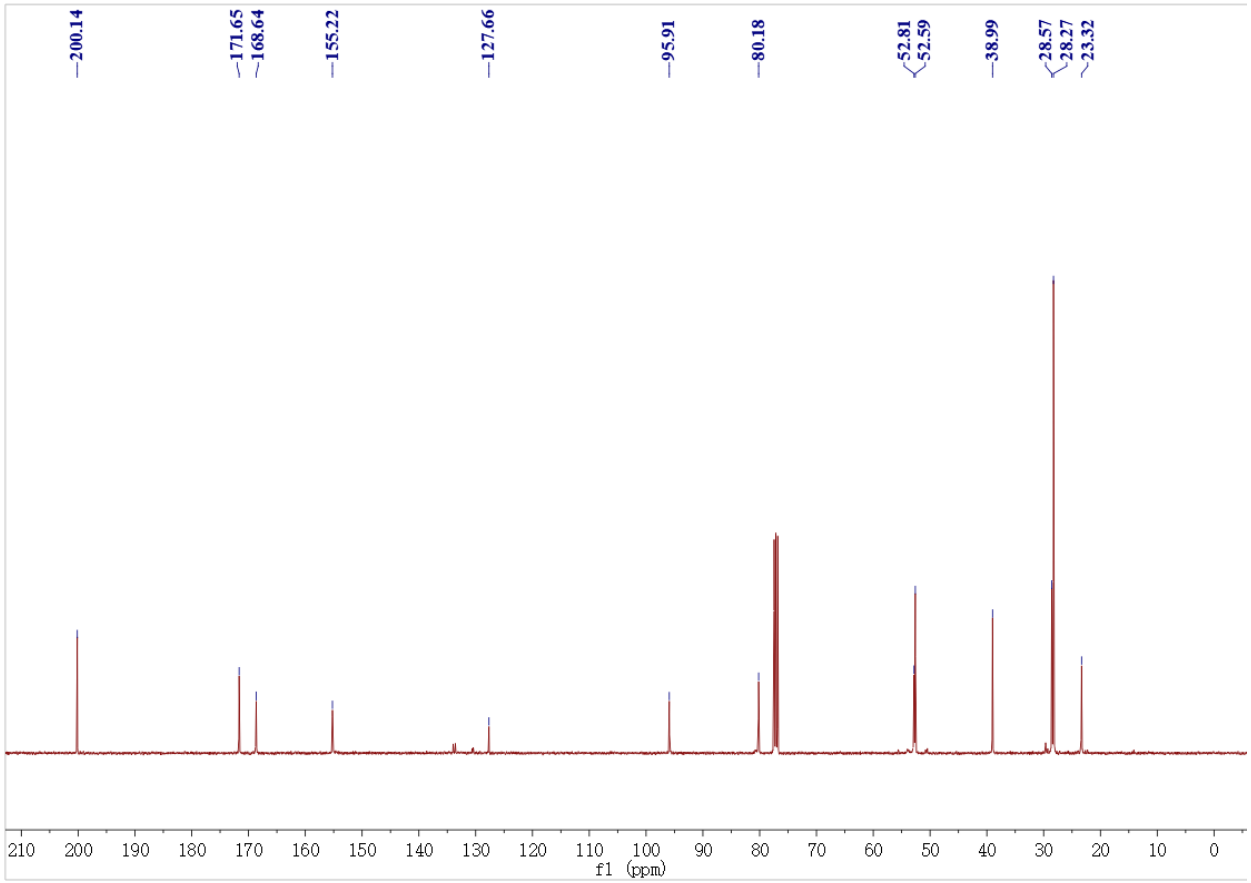
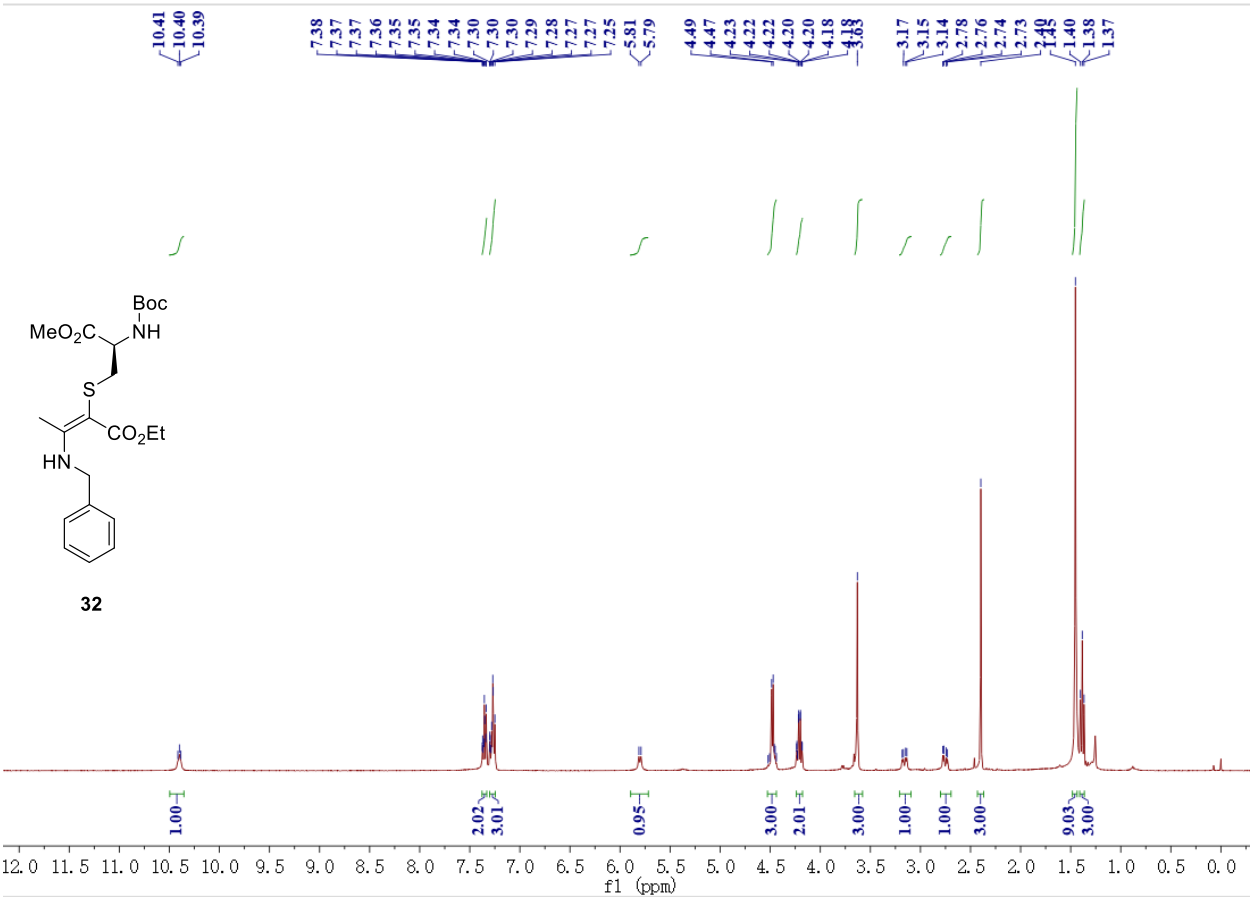
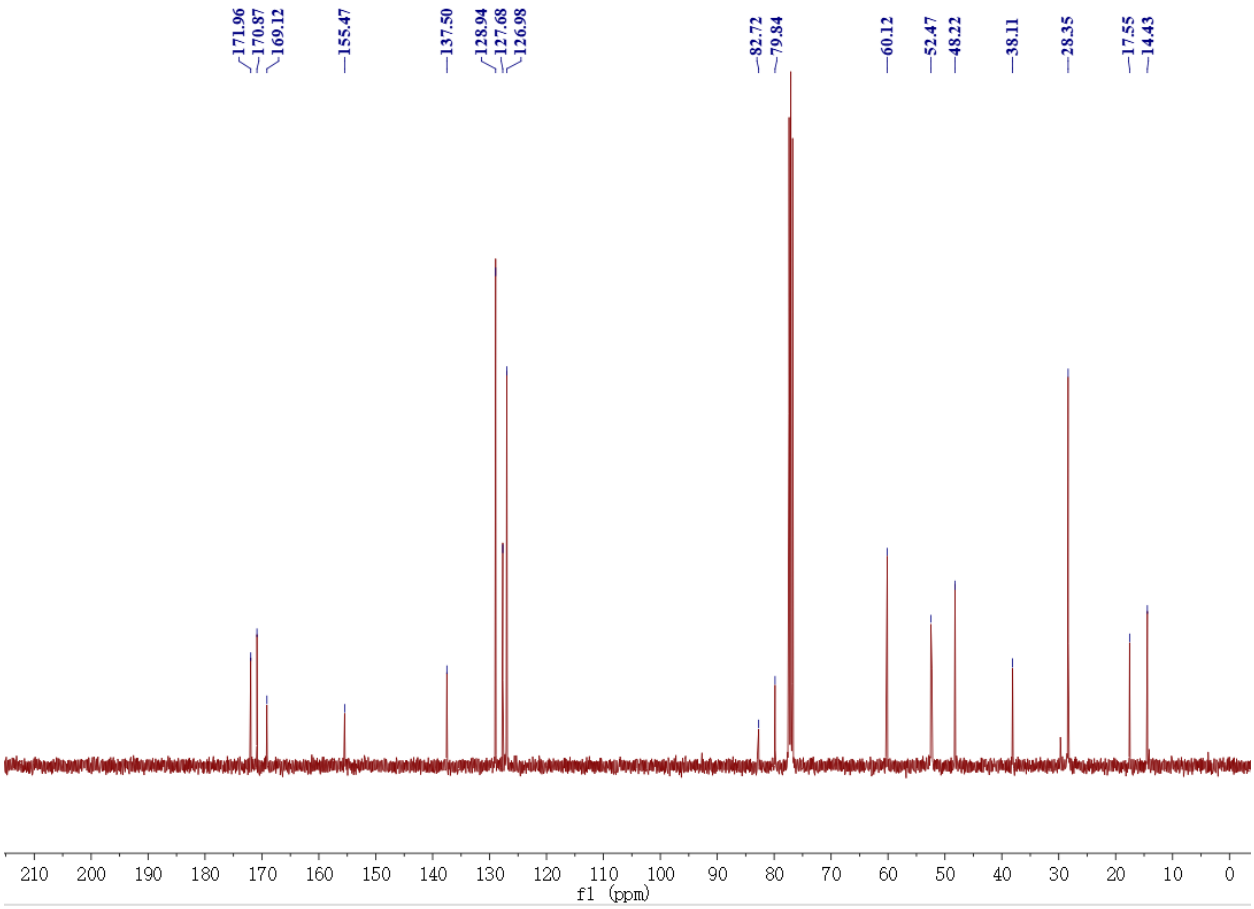
169.88
165.95
155.19
142.24
132.05
129.01
118.17
113.26
85.46
79.96
60.79
52.72
51.80
37.38
28.35
14.36
f1 (ppm)


10.82
10.82
10.80
6.14
5.42
5.40
4.44
4.43
4.42
3.72
2.94
2.92
2.92
2.91
2.30
1.45
Boc
MeO2C
NH
S
O
NH2
31
1.00
1.00
1.00
1.00
3.00
2.00
3.00
3.00
9.01
f1 (ppm)


200.14
171.65
168.64
155.22
127.66
95.91
80.18
52.81
52.59
38.99
28.57
28.27
23.32
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Boc
MeO2C
NH
S
CO2Et
HN
32
10.41
10.40
10.39
7.38
7.37
7.37
7.36
7.35
7.35
7.34
7.34
7.30
7.30
7.30
7.29
7.28
7.27
7.27
7.25
5.81
5.79
4.49
4.47
4.23
4.22
4.22
4.20
4.20
4.18
3.17
3.15
3.14
2.78
2.76
2.74
2.73
1.40
1.38
1.37
1.00
2.02
3.01
0.95
3.00
2.01
3.00
1.00
1.00
3.00
9.03
3.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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169.12
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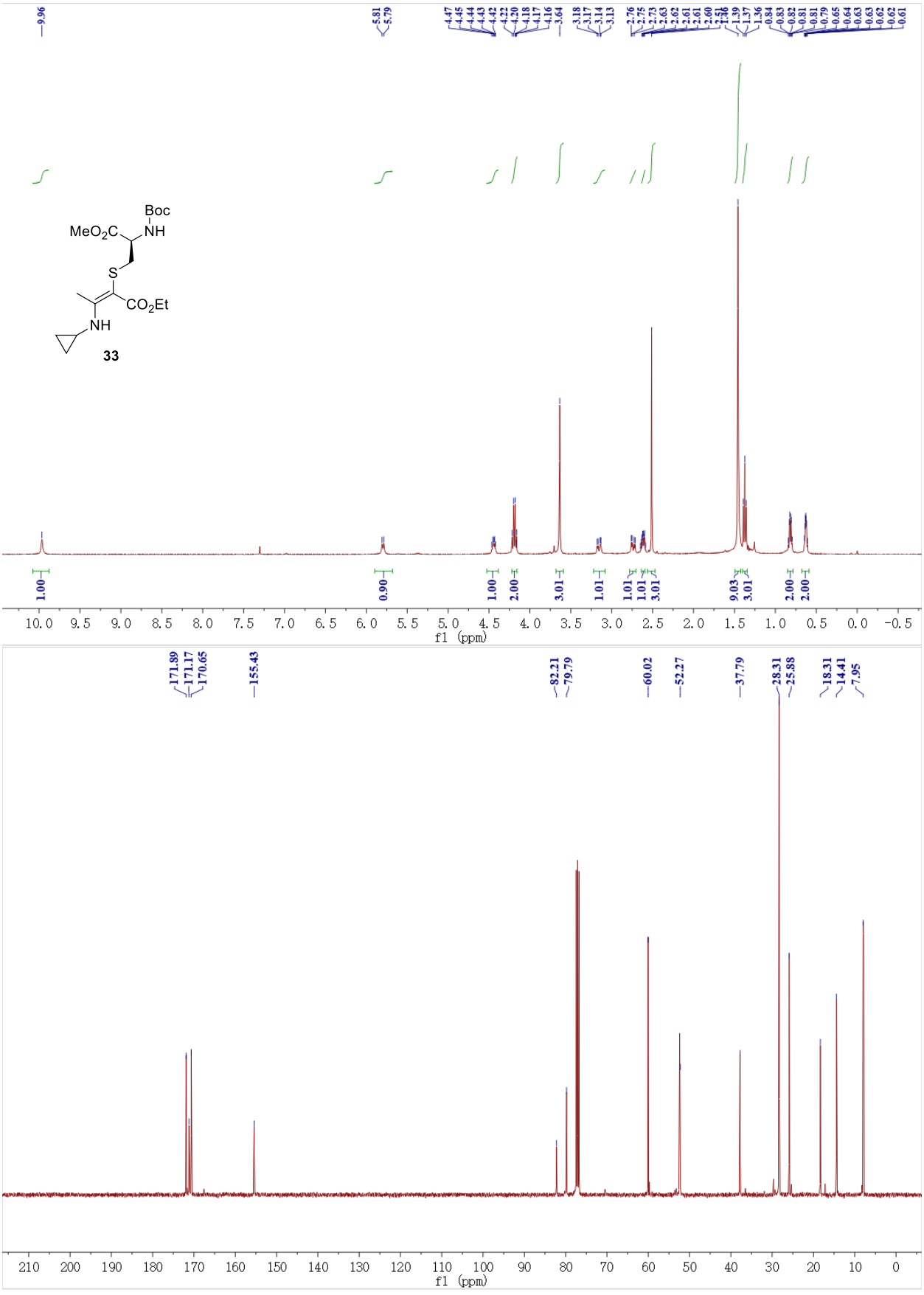
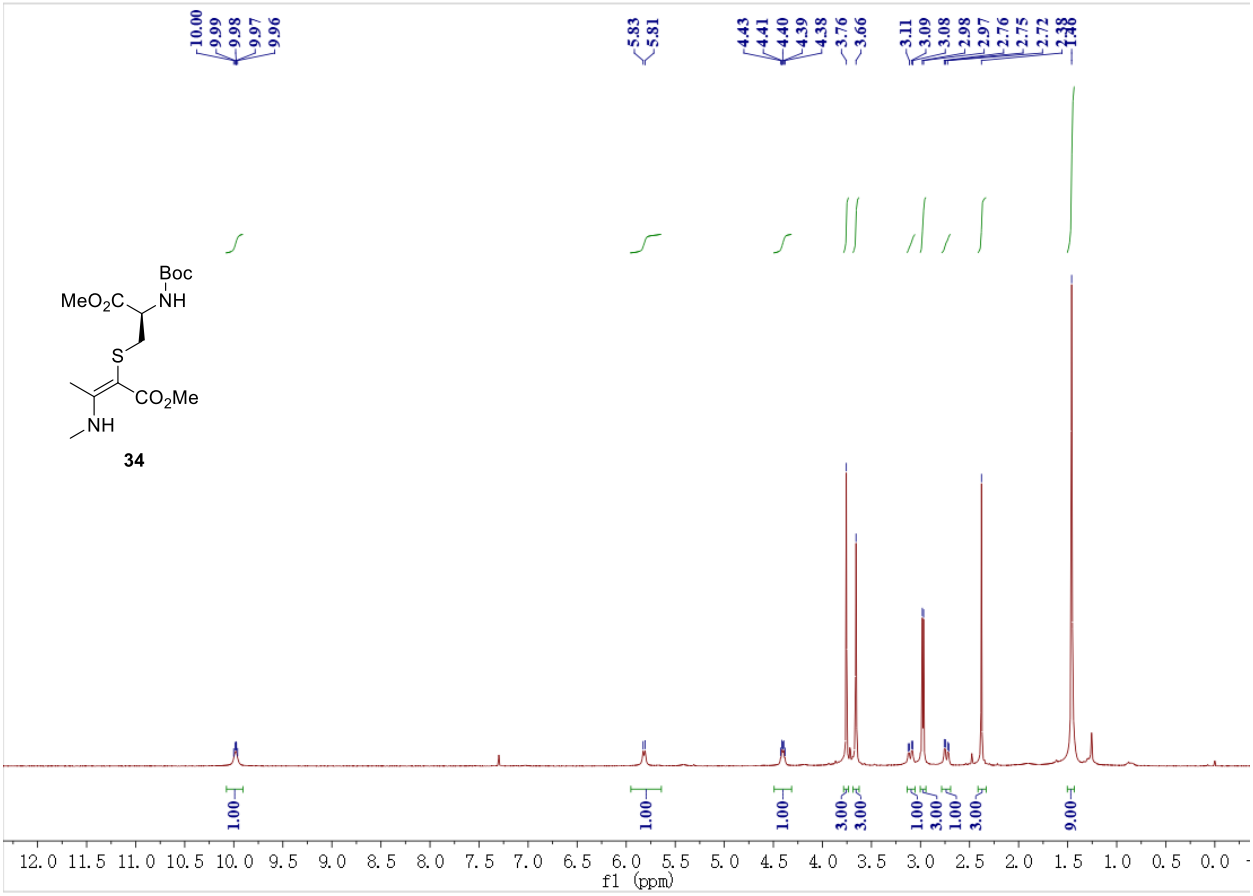
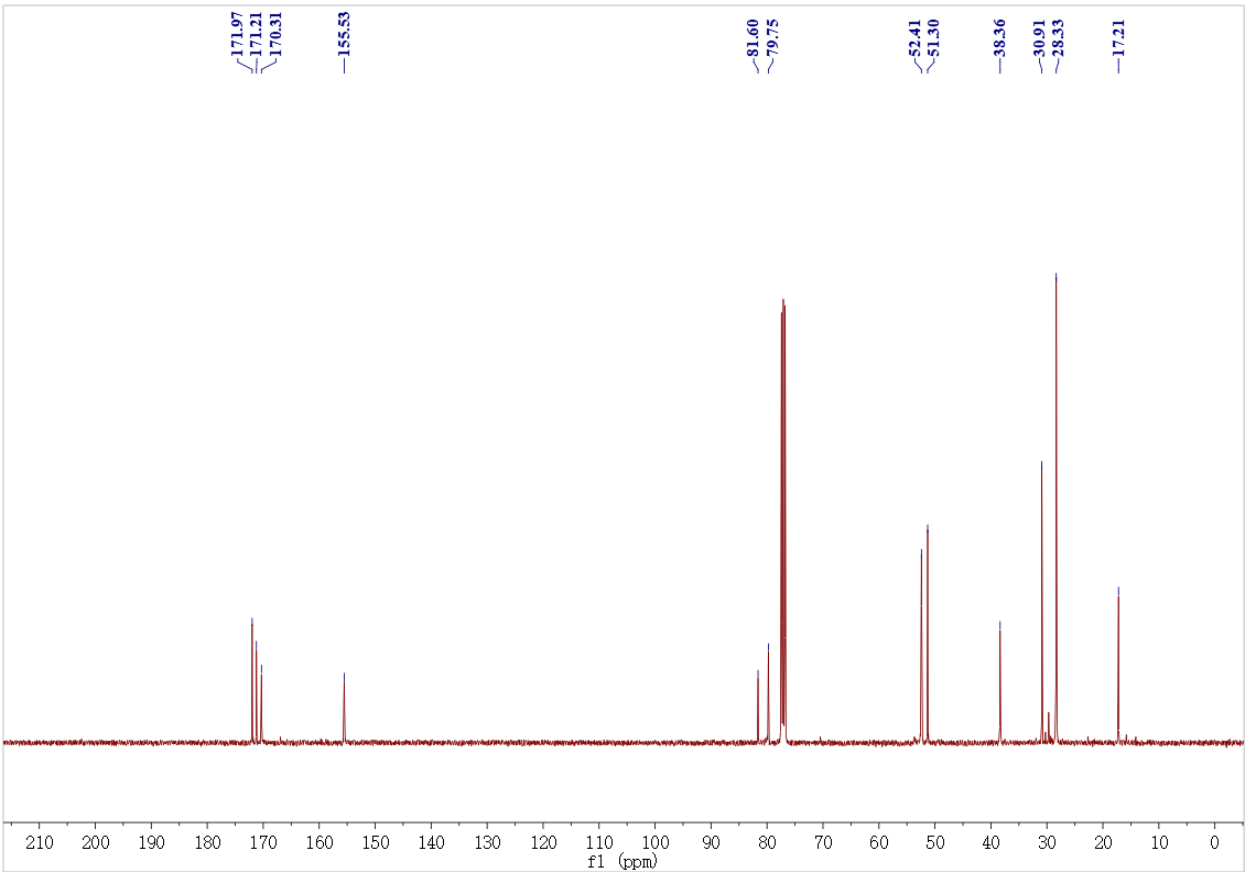
128.94
127.68
126.98
82.72
79.84
60.12
52.47
48.22
38.11
28.35
17.55
14.43
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Boc
MeO2C
NH
S
CO2Et
NH
33
f1 (ppm)
f1 (ppm)


10.00
9.99
9.98
9.97
9.96
5.83
5.81
4.43
4.41
4.40
4.39
4.38
3.76
3.66
3.11
3.09
3.08
2.98
2.97
2.76
2.75
2.72
2.38
1.48
Boc
MeO2C
NH
S
CO2Me
NH
34
1.00
1.00
1.00
3.00
3.00
1.00
3.00
1.00
3.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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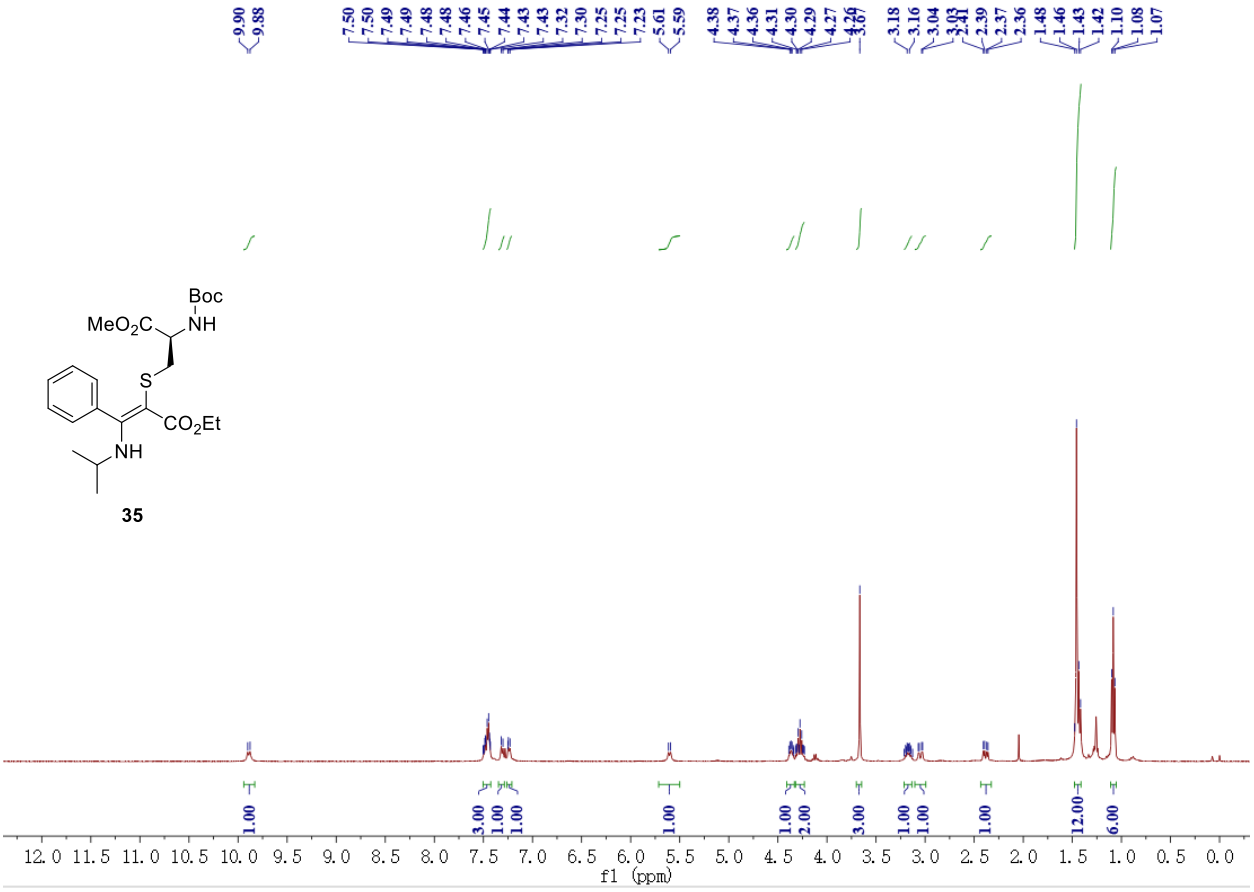
171.21
170.31
155.53
81.60
79.75
52.41
51.30
38.36
30.91
28.33
17.21
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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9.88
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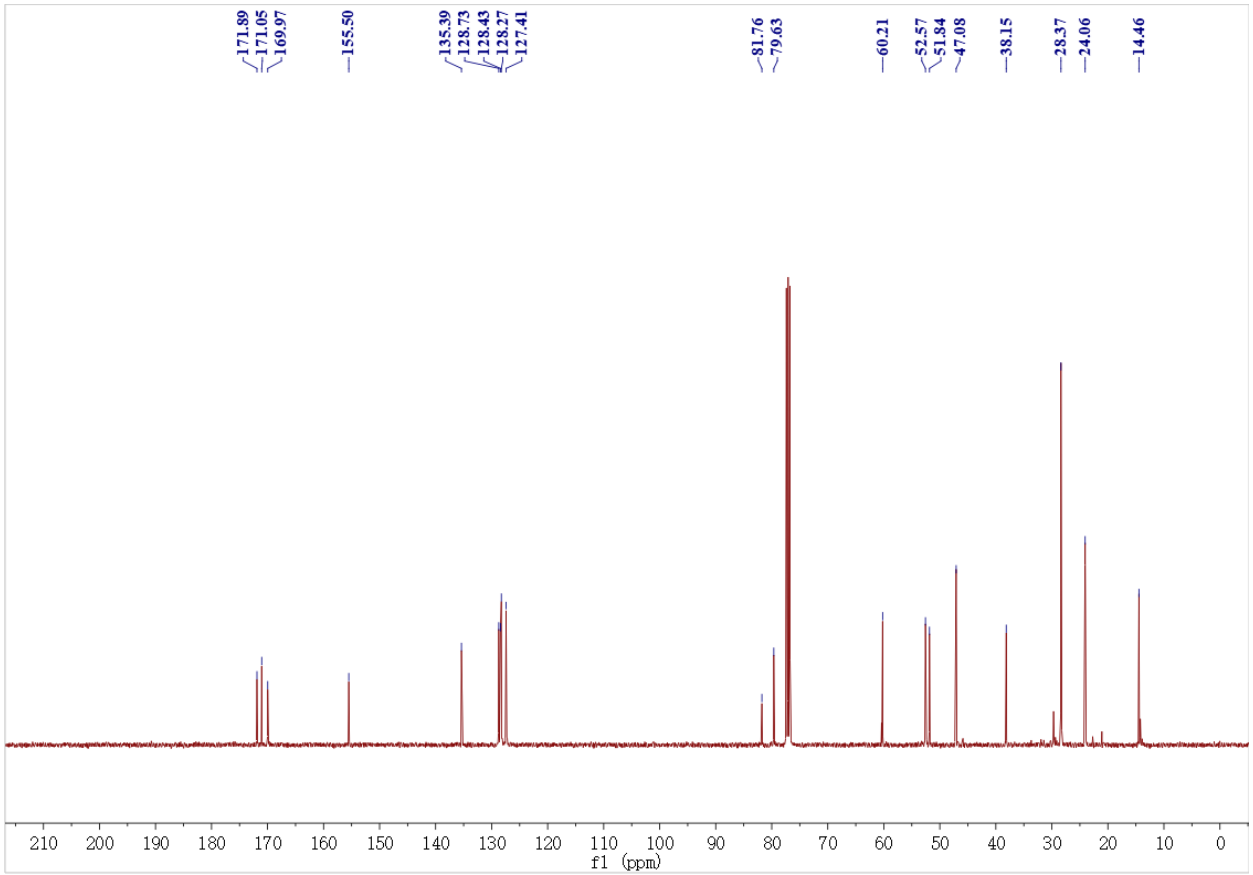
7.48
7.46
7.45
7.44
7.43
7.43
7.32
7.30
7.25
7.25
7.23
5.61
5.59
4.38
4.37
4.36
4.31
4.30
4.29
4.27
4.26
4.07
3.18
3.16
3.04
3.03
2.41
2.39
2.37
2.36
1.48
1.46
1.43
1.42
1.10
1.08
1.07
Boc
MeO2C
NH
S
CO2Et
NH
35
1.00
3.00
1.00
1.00
1.00
1.00
2.00
3.00
1.00
1.00
1.00
12.00
6.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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171.05
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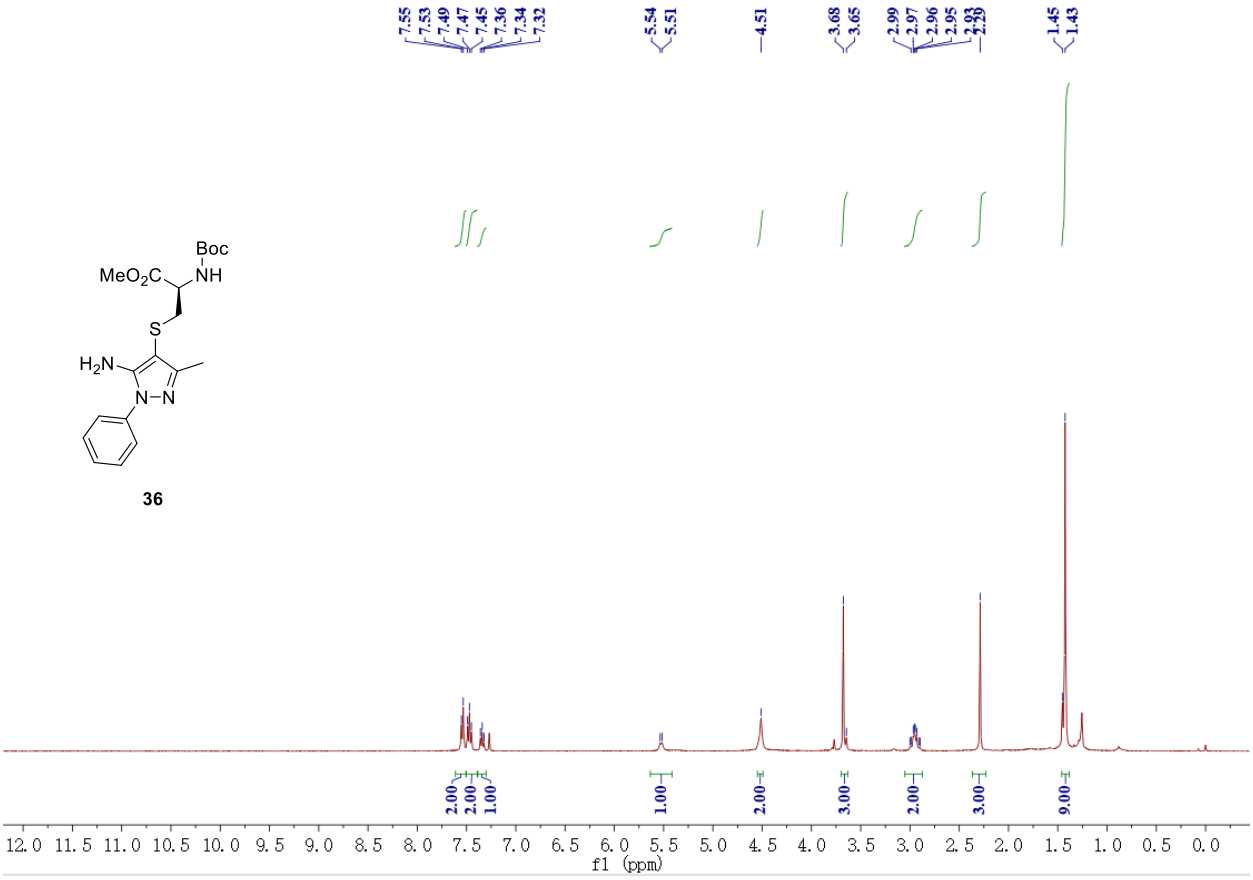
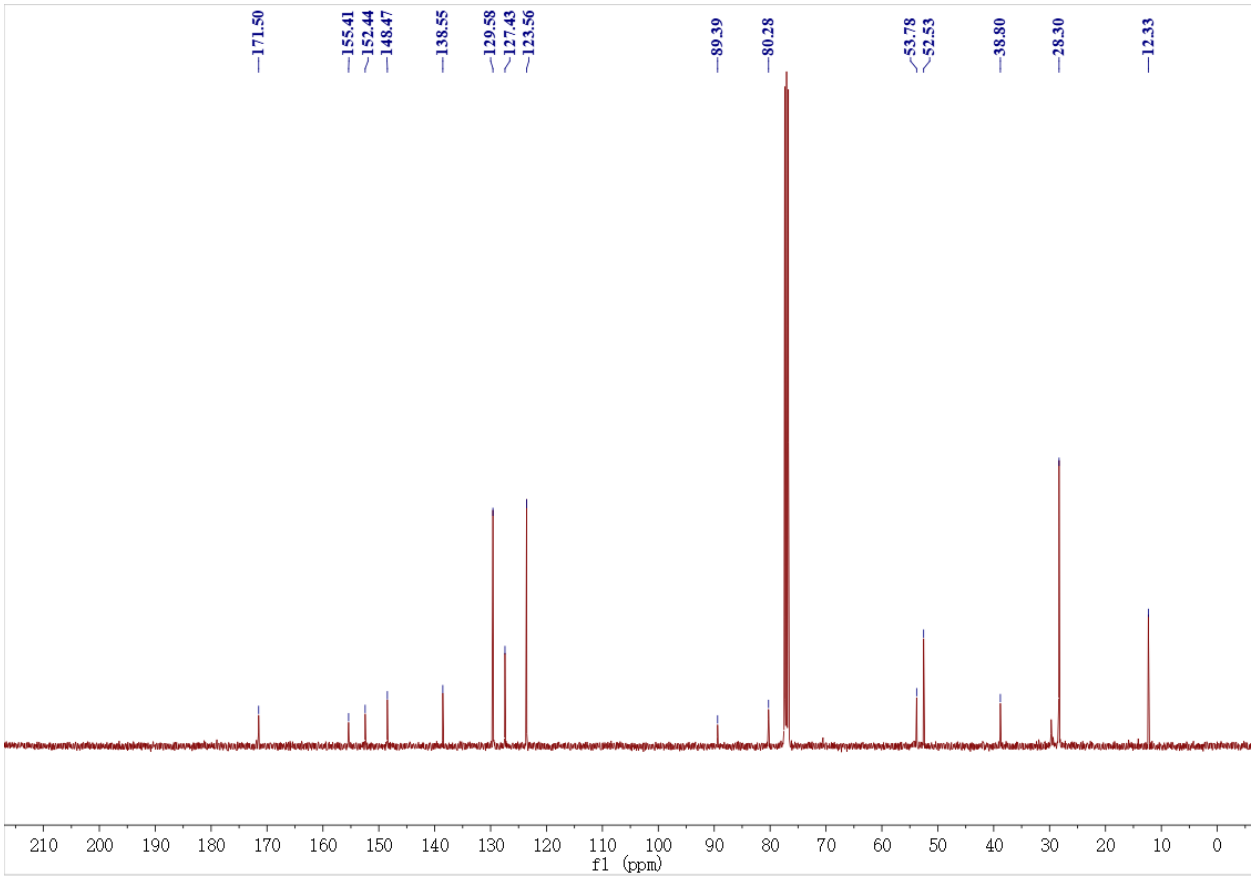
135.39
128.73
128.43
128.27
127.41
81.76
79.63
60.21
52.57
51.84
47.08
38.15
28.37
24.06
14.46
f1 (ppm)


7.55
7.53
7.49
7.47
7.45
7.36
7.34
7.32
5.54
5.51
4.51
3.68
3.65
2.99
2.97
2.96
2.95
1.45
1.43
2.00
2.00
1.00
1.00
2.00
3.00
2.00
3.00
9.00
f1 (ppm)

Boc
MeO2C
NH
S
H2N
N–N

36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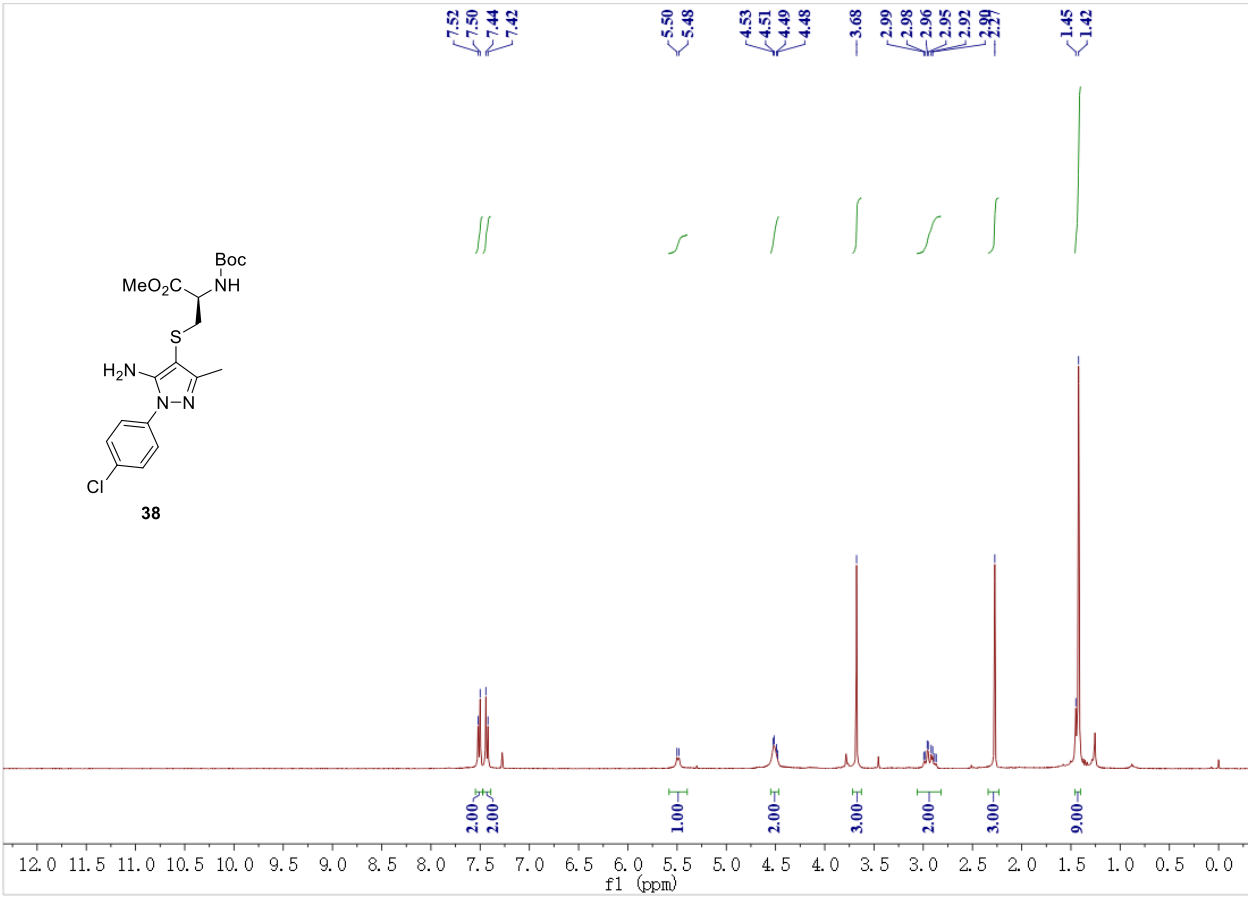
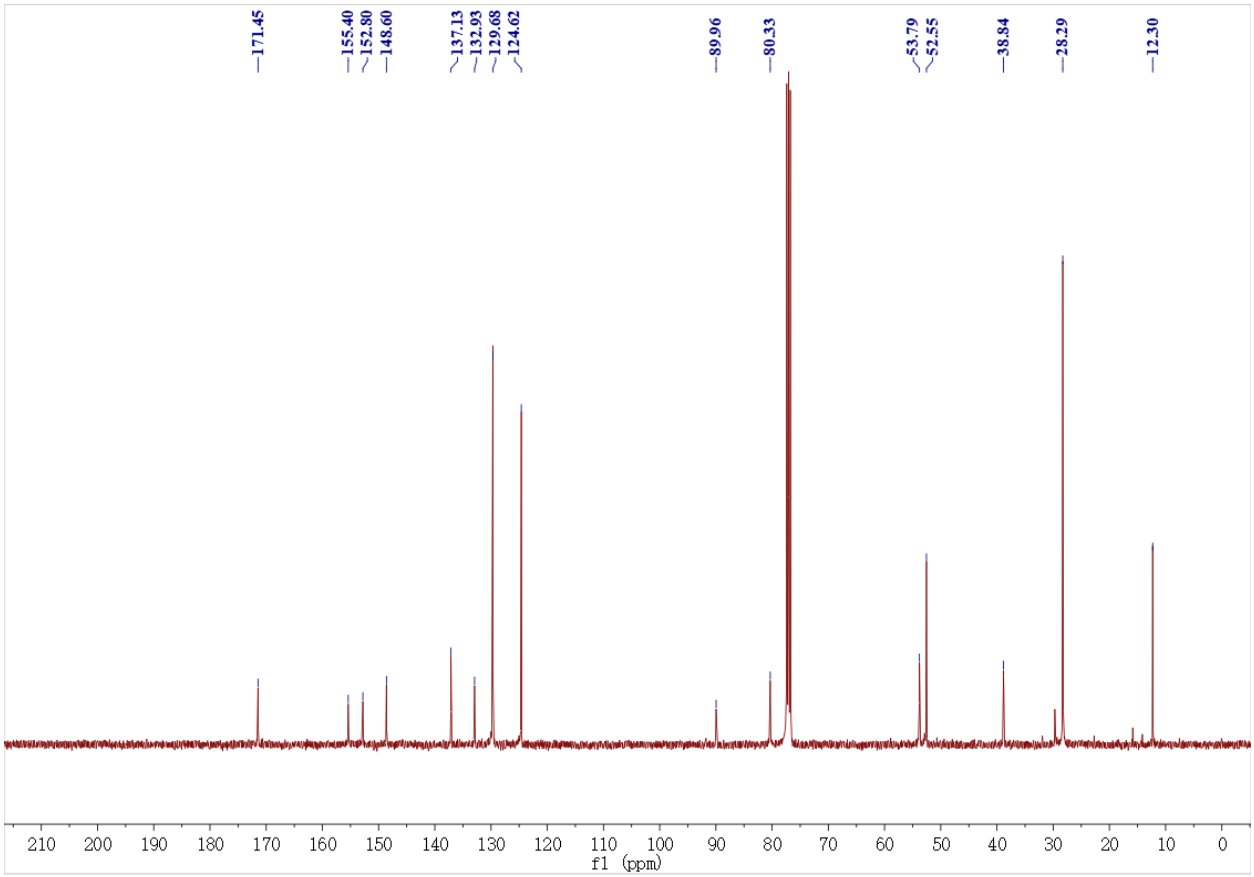
155.41
152.44
148.47
138.55
129.58
127.43
123.56
89.39
80.28
53.78
52.53
38.80
28.30
12.33
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.52
7.50
7.44
7.42
5.50
5.48
4.53
4.51
4.49
4.48
3.68
2.99
2.98
2.96
2.95
2.92
2.90
2.27
1.45
1.42
Boc
MeO2C
NH
S
H2N
N–N
Cl
38
2.00
2.00
1.00
2.00
3.00
2.00
3.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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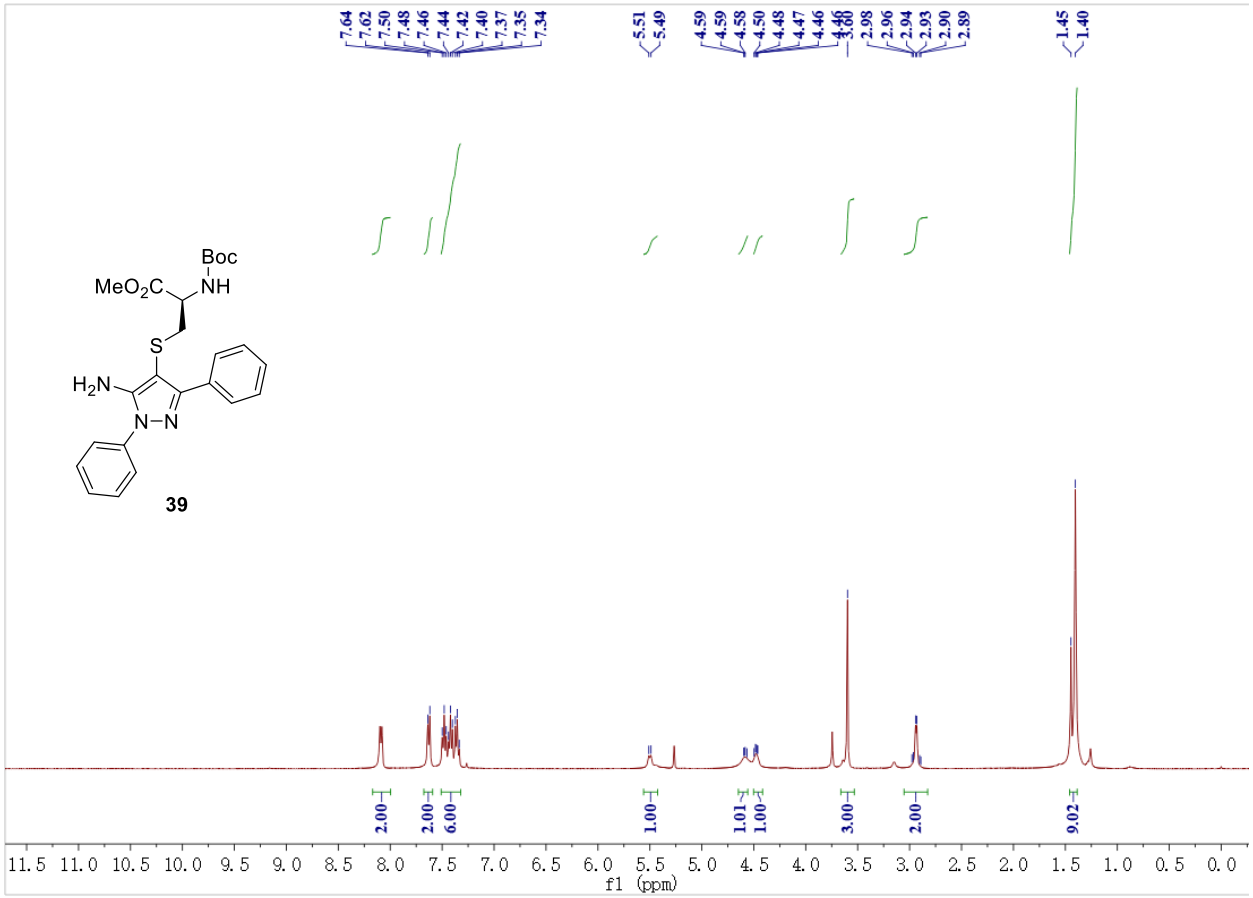
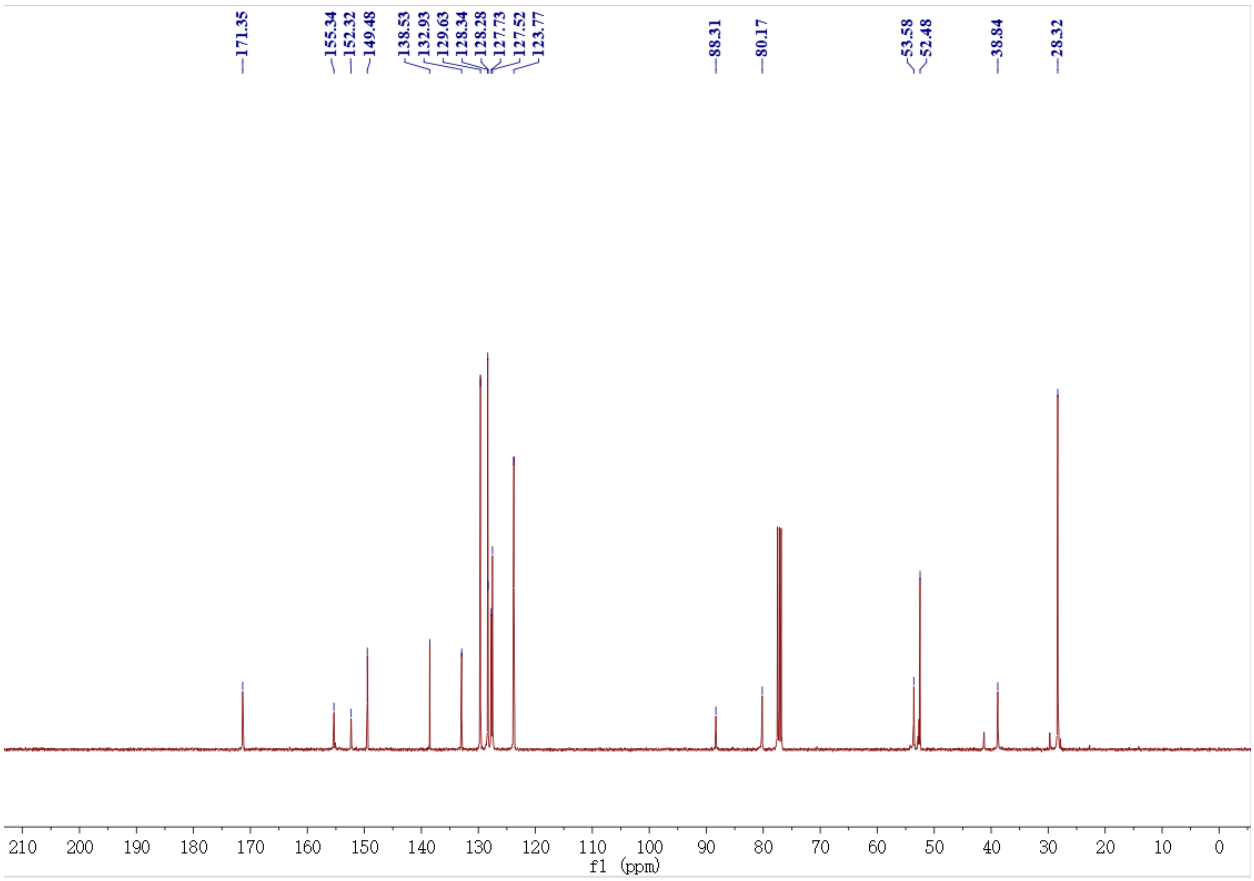
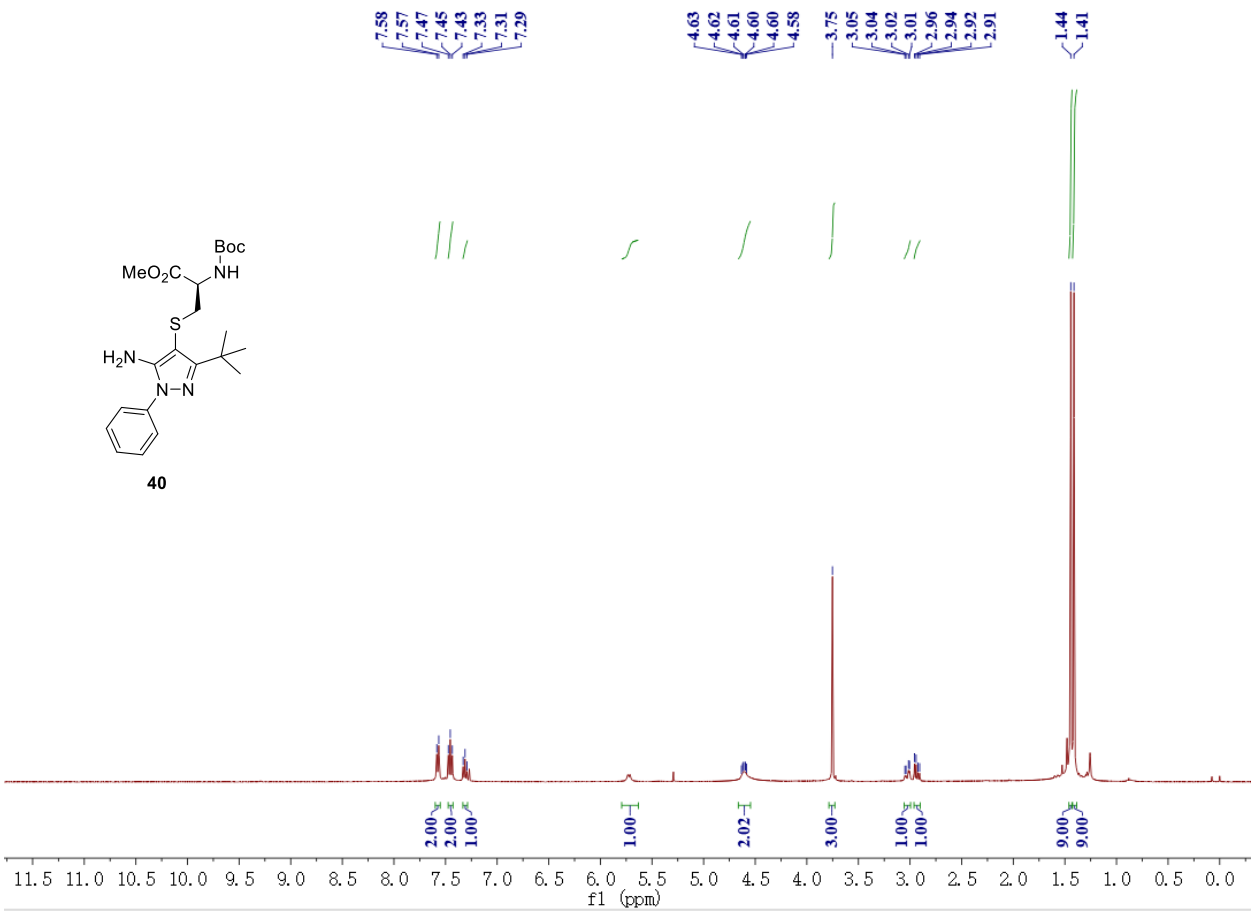
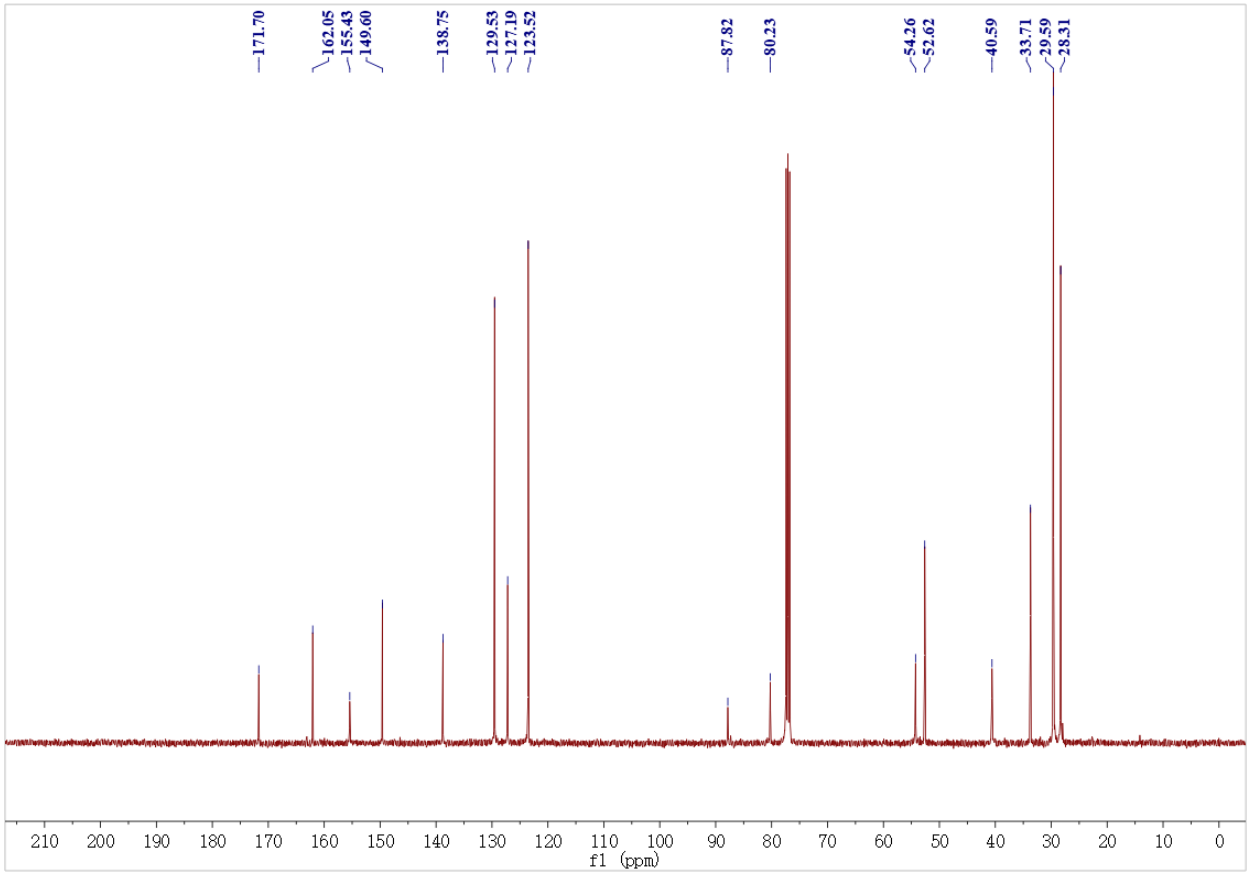
152.80
148.60
137.13
132.93
129.68
124.62
89.96
80.33
53.79
52.55
38.84
28.29
12.30
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

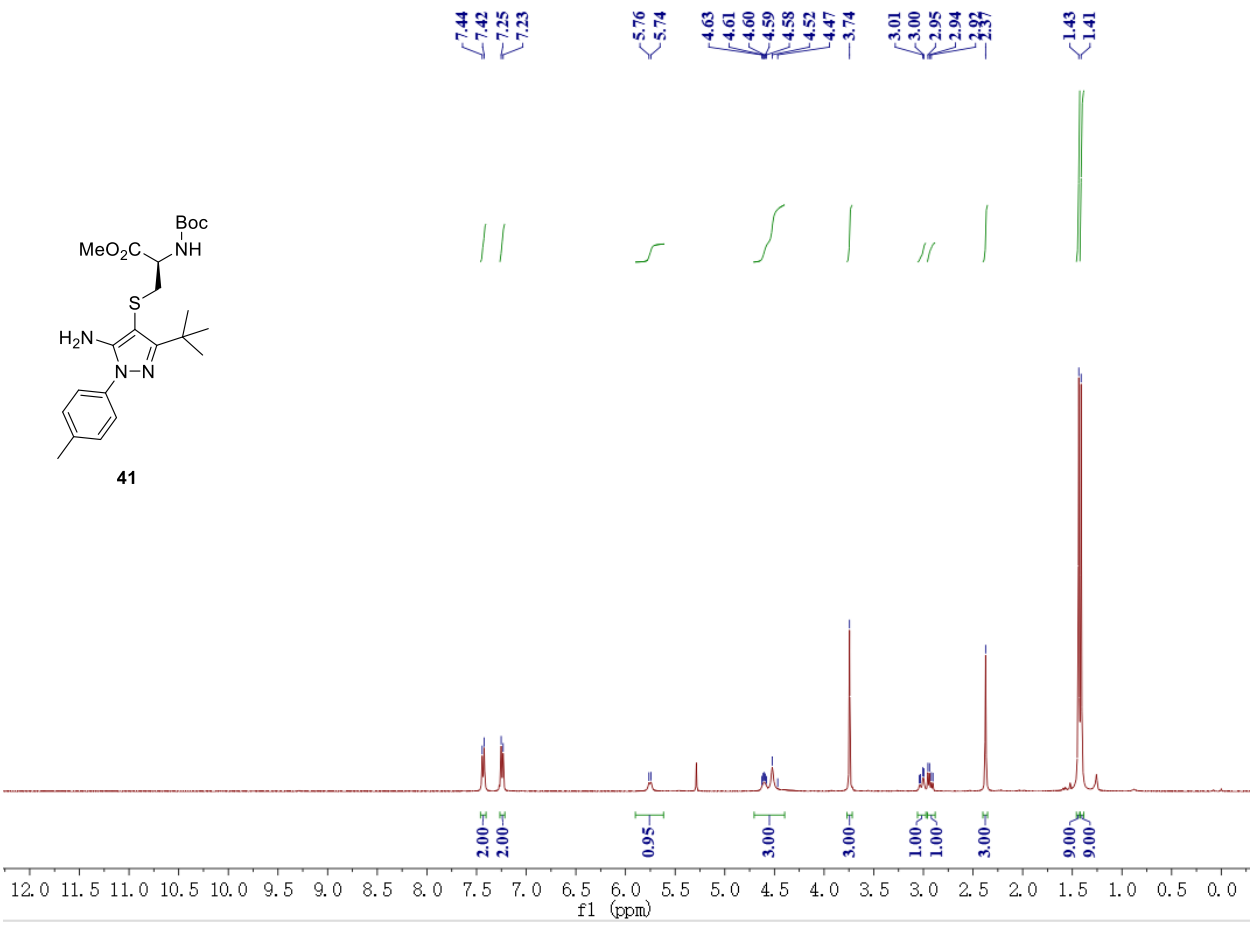

7.64
7.62
7.50
7.48
7.46
7.44
7.42
7.40
7.37
7.35
7.34
5.51
5.49
4.59
4.59
4.58
4.50
4.48
4.47
4.46
3.60
2.98
2.96
2.94
2.93
2.90
2.89
1.45
1.40
Boc
MeO2C
NH
S
H2N
N–N
39
2.00
2.00
6.00
1.00
1.01
1.00
3.00
2.00
9.02
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


171.35
155.34
152.32
149.48
138.53
132.93
129.63
128.34
128.28
127.73
127.52
123.77
88.31
80.17
53.58
52.48
38.84
28.32
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

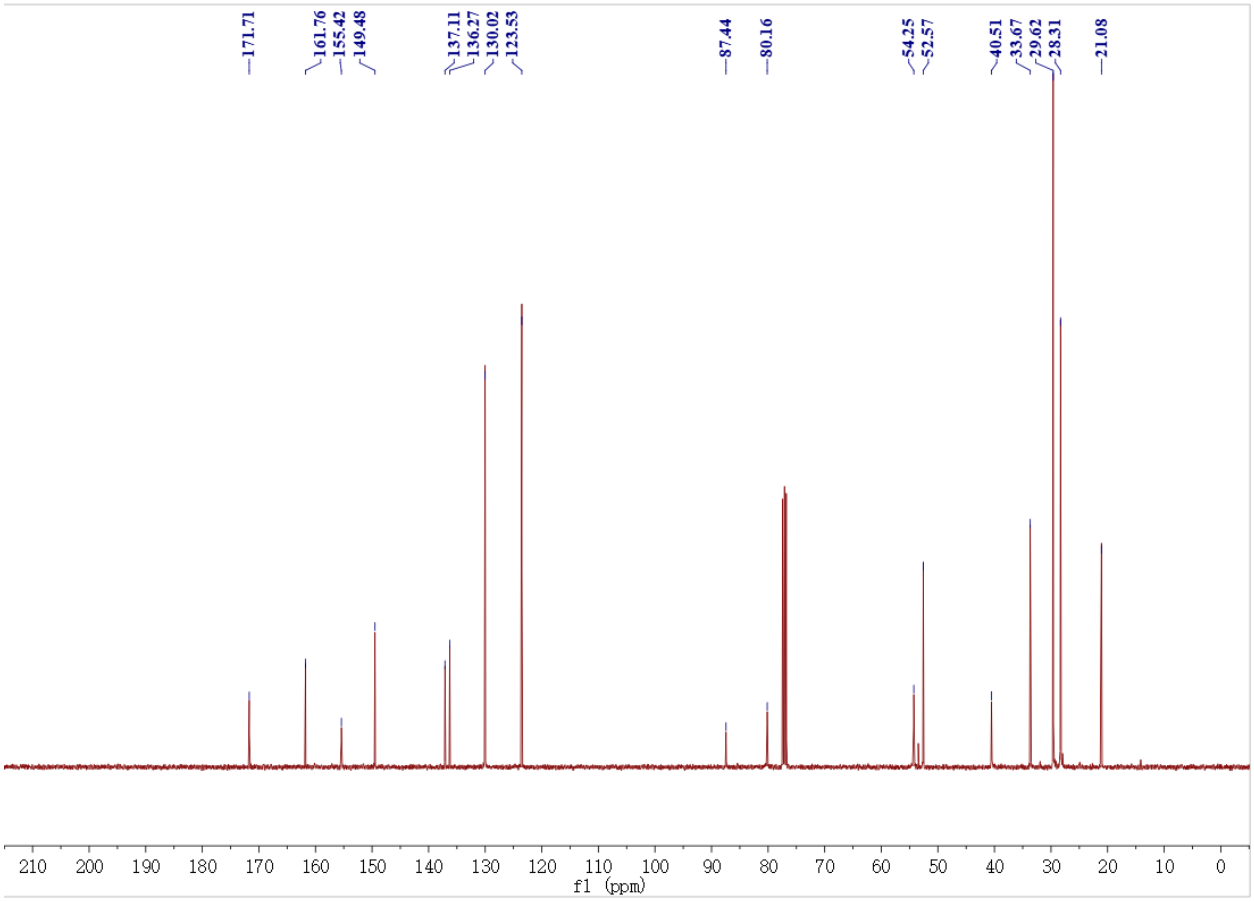

7.58
7.57
7.47
7.45
7.43
7.33
7.31
7.29
4.63
4.62
4.61
4.60
4.60
4.58
3.75
3.05
3.04
3.02
3.01
2.96
2.94
2.92
2.91
1.44
1.41
Boc
MeO2C
NH
S
H2N
N–N
2.00
2.00
1.00
1.00
2.02
3.00
1.00
1.00
9.00
9.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

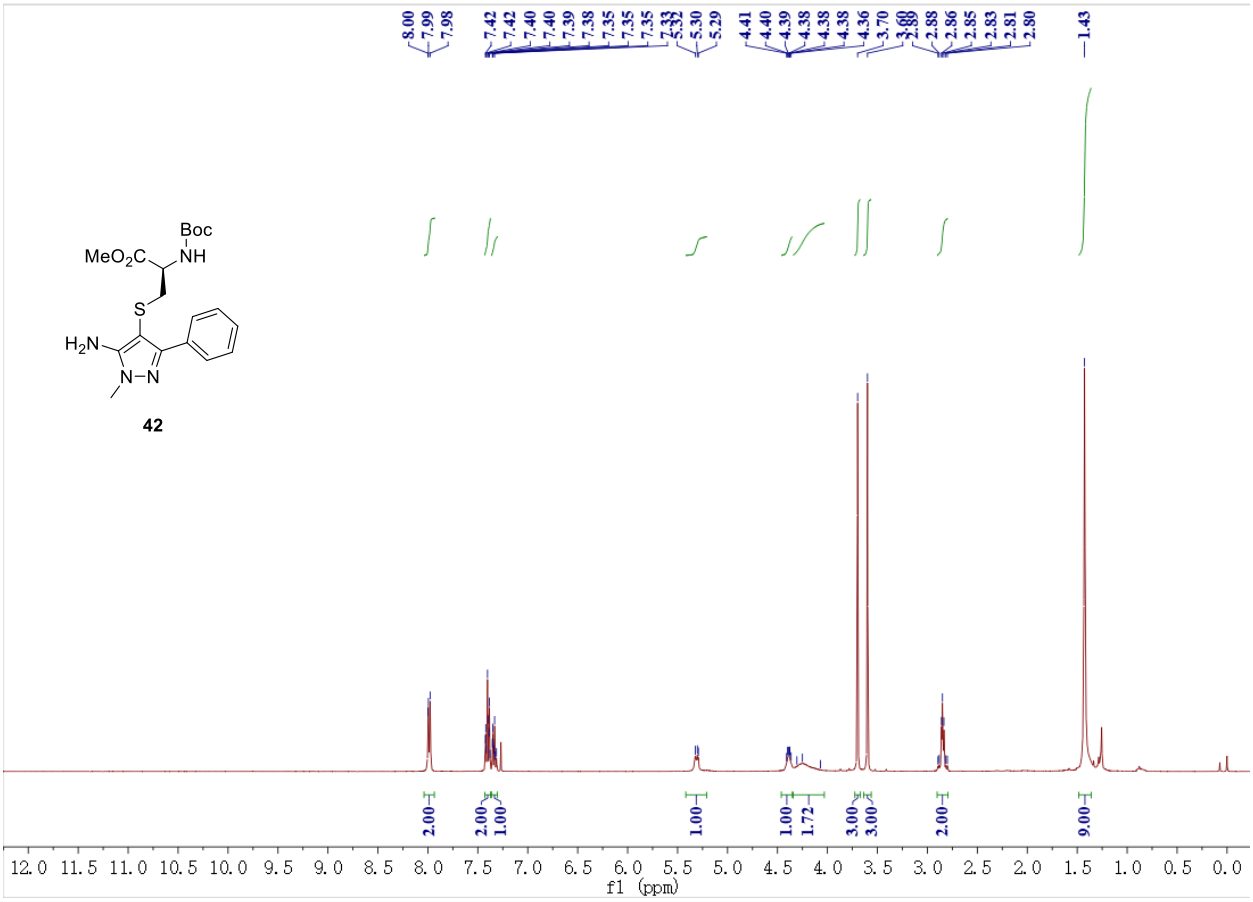
40

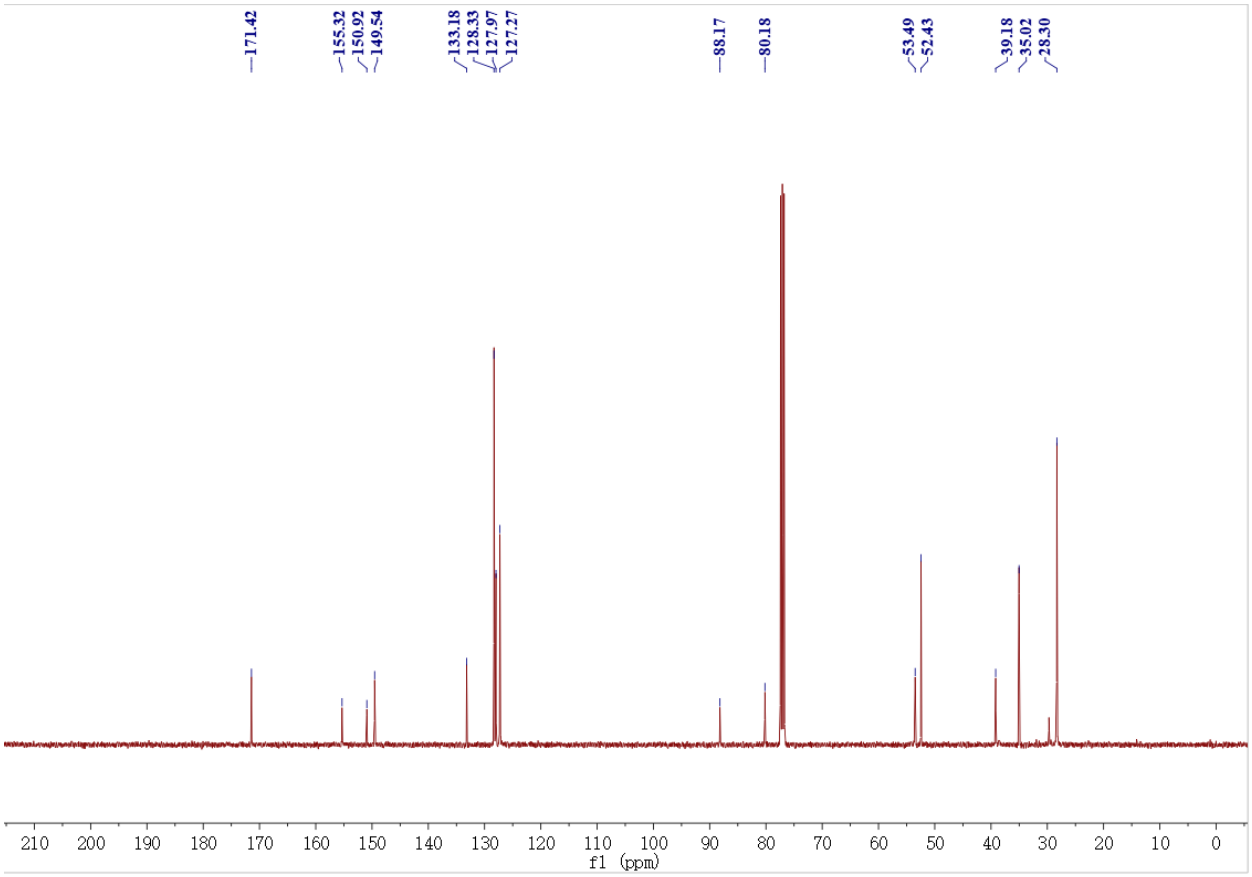

171.70
162.05
155.43
149.60
138.75
129.53
127.19
123.52
87.82
80.23
54.26
52.62
40.59
33.71
29.59
28.31
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

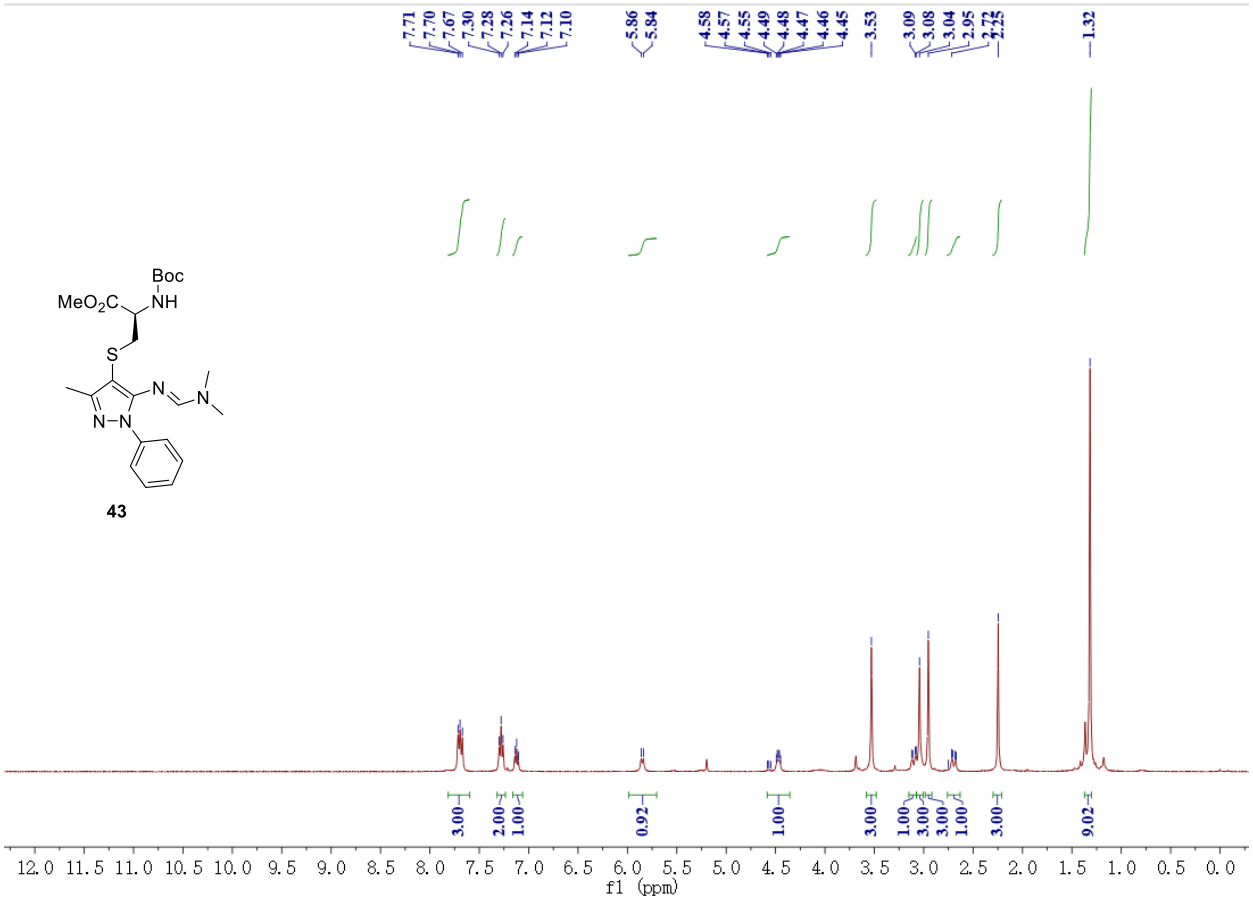

7.44
7.42
7.25
7.23
5.76
5.74
4.63
4.61
4.60
4.59
4.58
4.52
4.47
3.74
3.01
3.00
2.95
2.94
2.92
2.37
1.43
1.41
Boc
NH
MeO2C
S
H2N
N–N
2.00
2.00
0.95
3.00
3.00
1.00
1.00
3.00
9.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

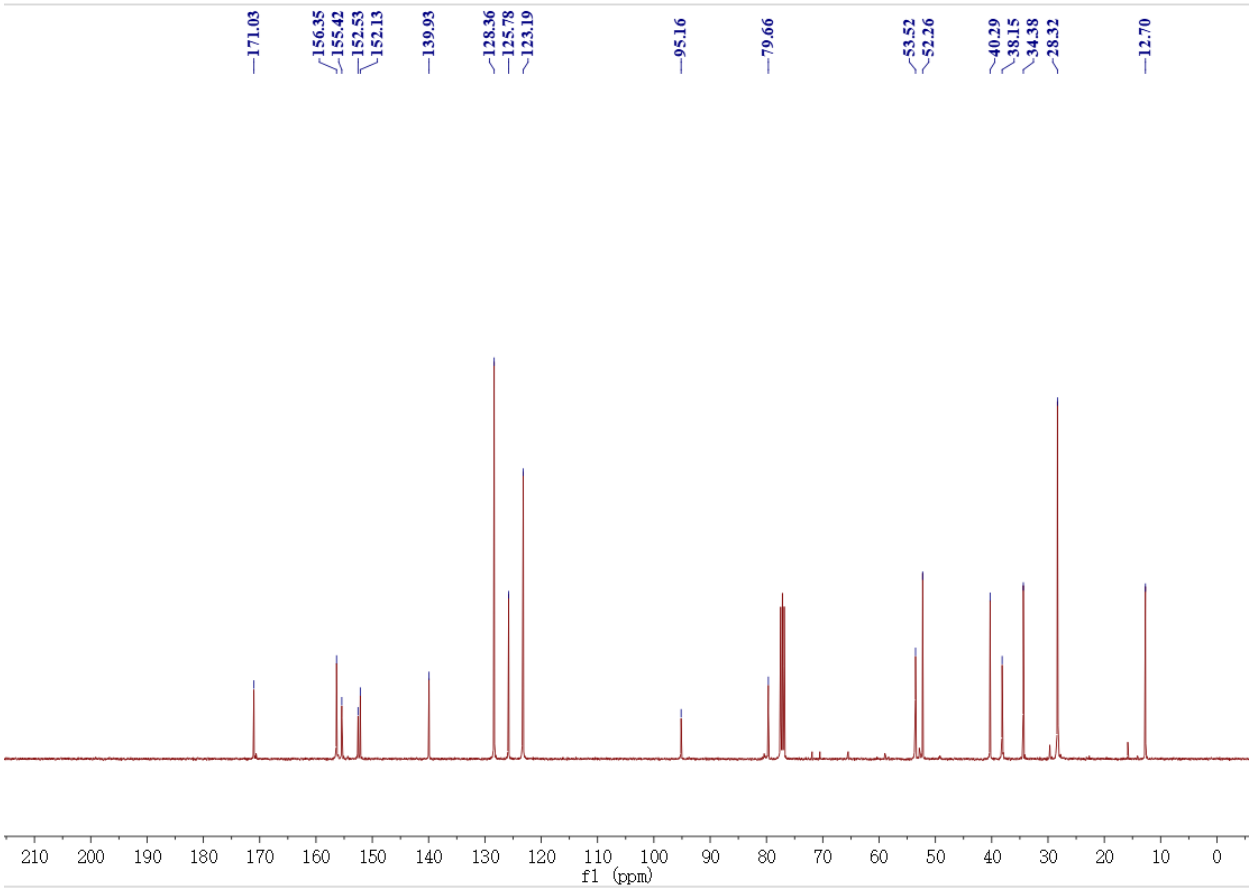
41

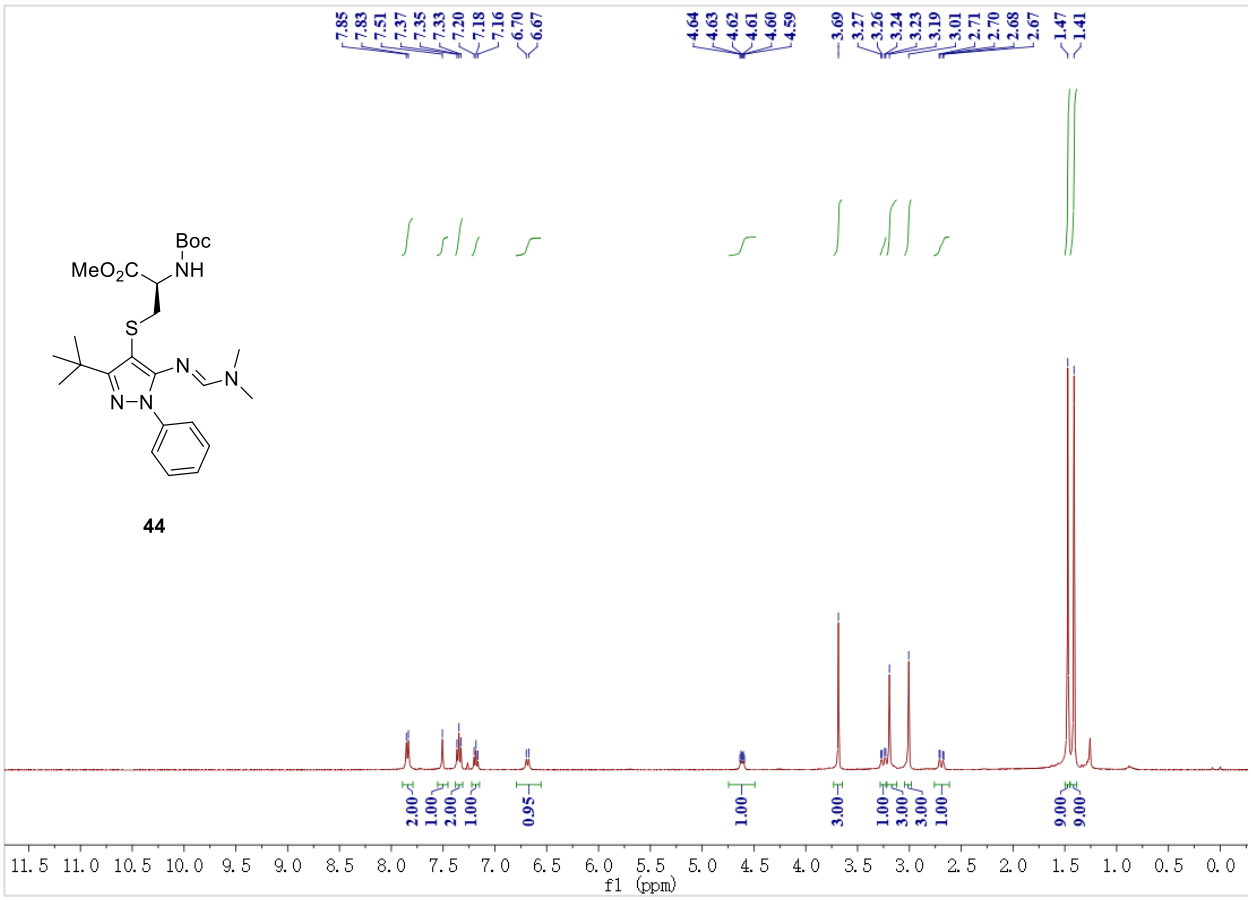

171.71
161.76
155.42
149.48
137.11
136.27
130.02
123.53
87.44
80.16
54.25
52.57
40.51
33.67
29.62
28.31
21.08
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Boc
MeO2C
NH
S
H2N
N–N
42
8.00
7.99
7.98
7.42
7.42
7.40
7.40
7.39
7.38
7.35
7.35
7.35
5.33
5.32
5.30
5.29
4.41
4.40
4.39
4.38
4.38
4.38
4.36
3.70
2.88
2.86
2.85
2.83
2.81
2.80
1.43
2.00
2.00
1.00
1.00
1.00
1.72
3.00
3.00
2.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

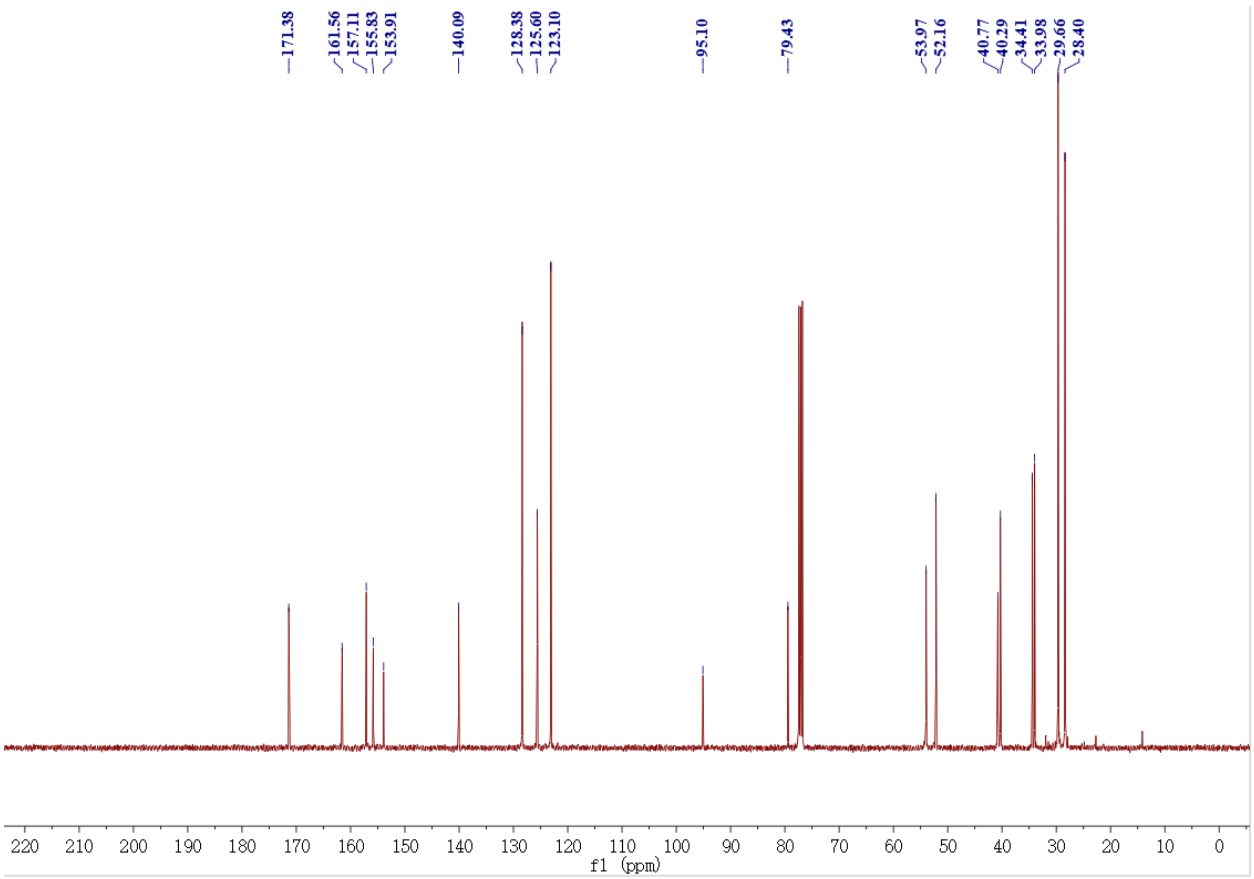

171.42
155.32
150.92
149.54
133.18
128.33
127.97
127.27
88.17
80.18
53.49
52.43
39.18
35.02
28.30
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

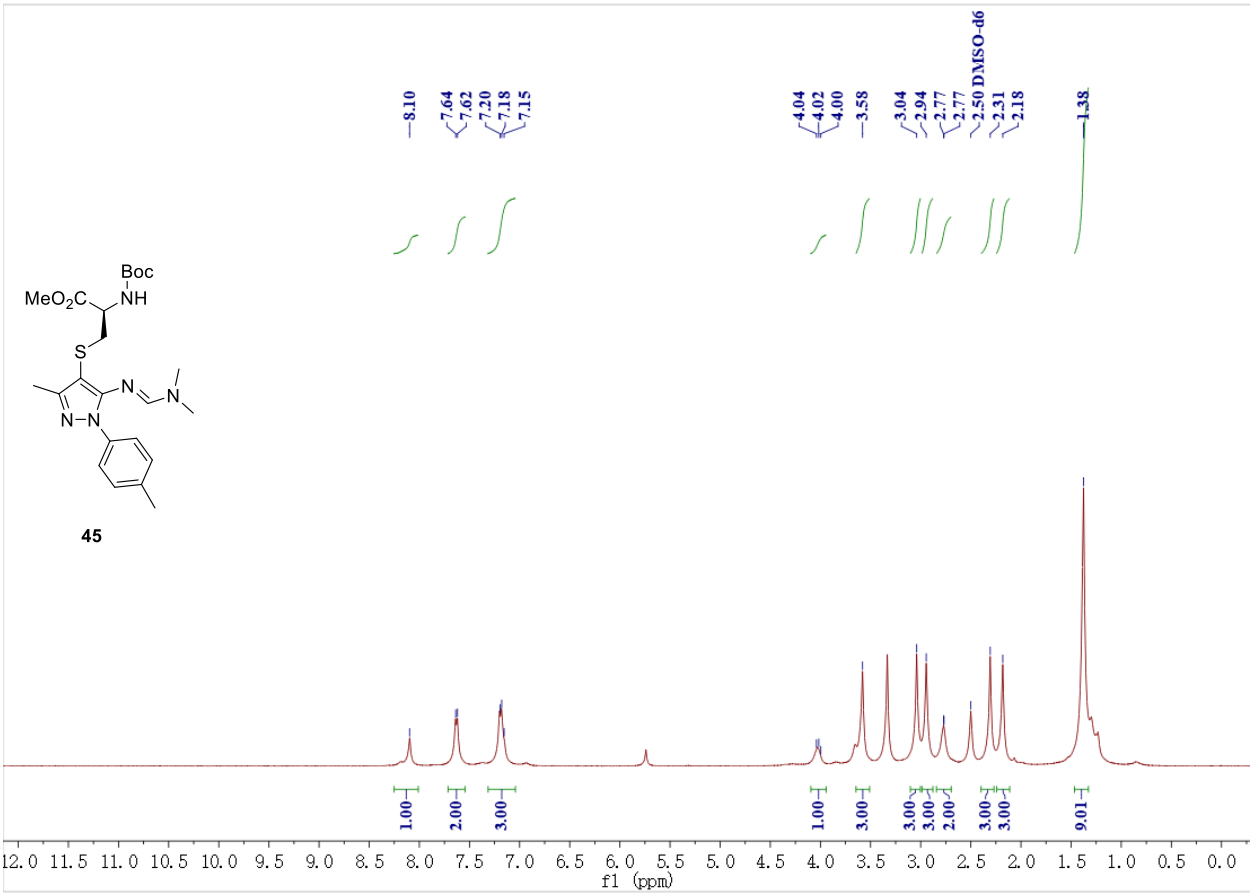

7.71
7.70
7.67
7.30
7.28
7.26
7.14
7.12
7.10
5.86
5.84
4.58
4.57
4.55
4.49
4.48
4.47
4.46
4.45
3.53
3.09
3.08
3.04
2.95
2.72
2.25
1.32
Boc
MeO2C
NH
S
N
N
N
43
3.00
2.00
1.00
0.92
1.00
3.00
1.00
3.00
3.00
1.00
3.00
9.02
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


171.03
156.35
155.42
152.53
152.13
139.93
128.36
125.78
123.19
95.16
79.66
53.52
52.26
40.29
38.15
34.38
28.32
12.70
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

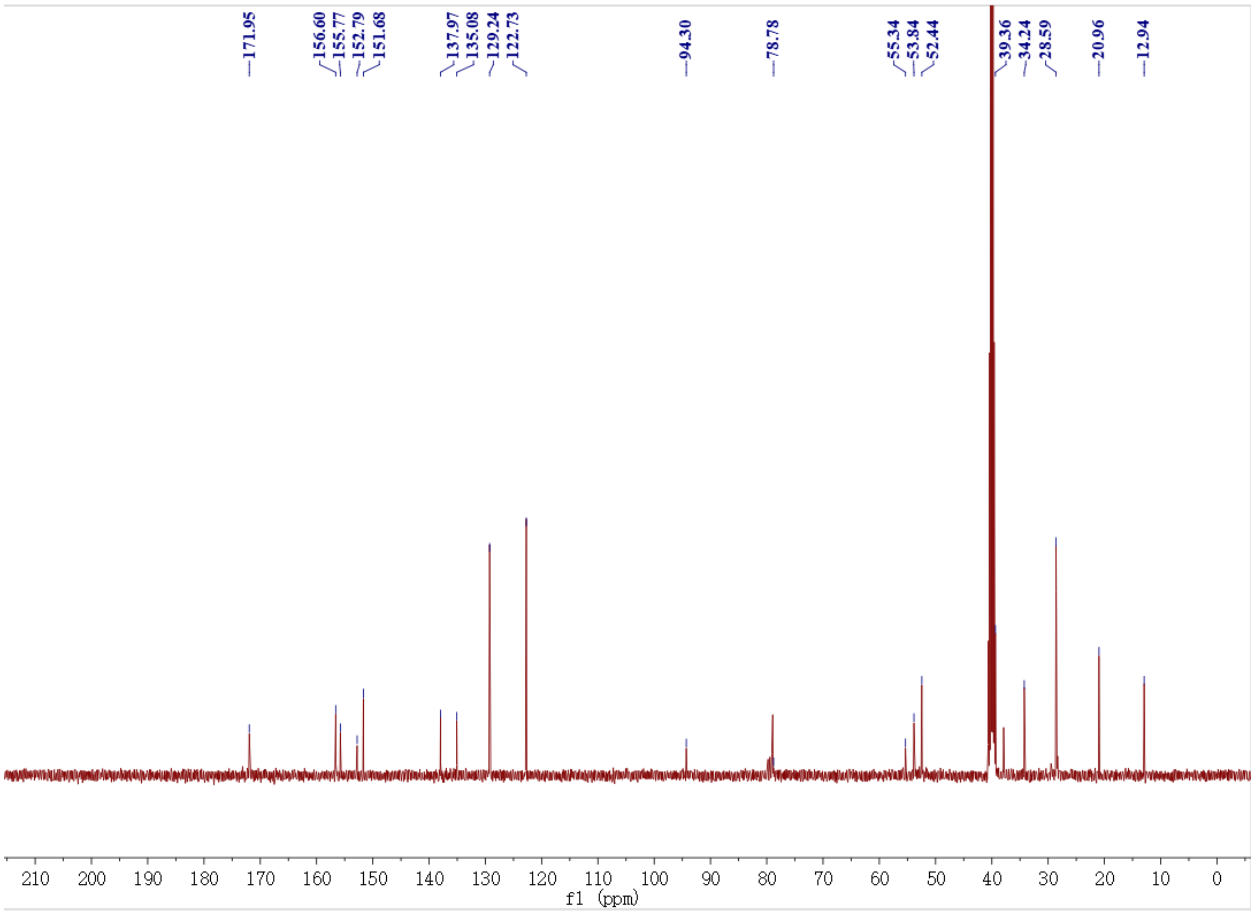

7.85
7.83
7.51
7.37
7.35
7.33
7.20
7.18
7.16
6.70
6.67
4.64
4.63
4.62
4.61
4.60
4.59
3.69
3.27
3.26
3.24
3.23
3.19
3.01
2.71
2.70
2.68
2.67
1.47
1.41
Boc
NH
MeO2C
S
N
N
N
N
2.00
1.00
2.00
1.00
0.95
1.00
3.00
1.00
3.00
3.00
1.00
9.00
9.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

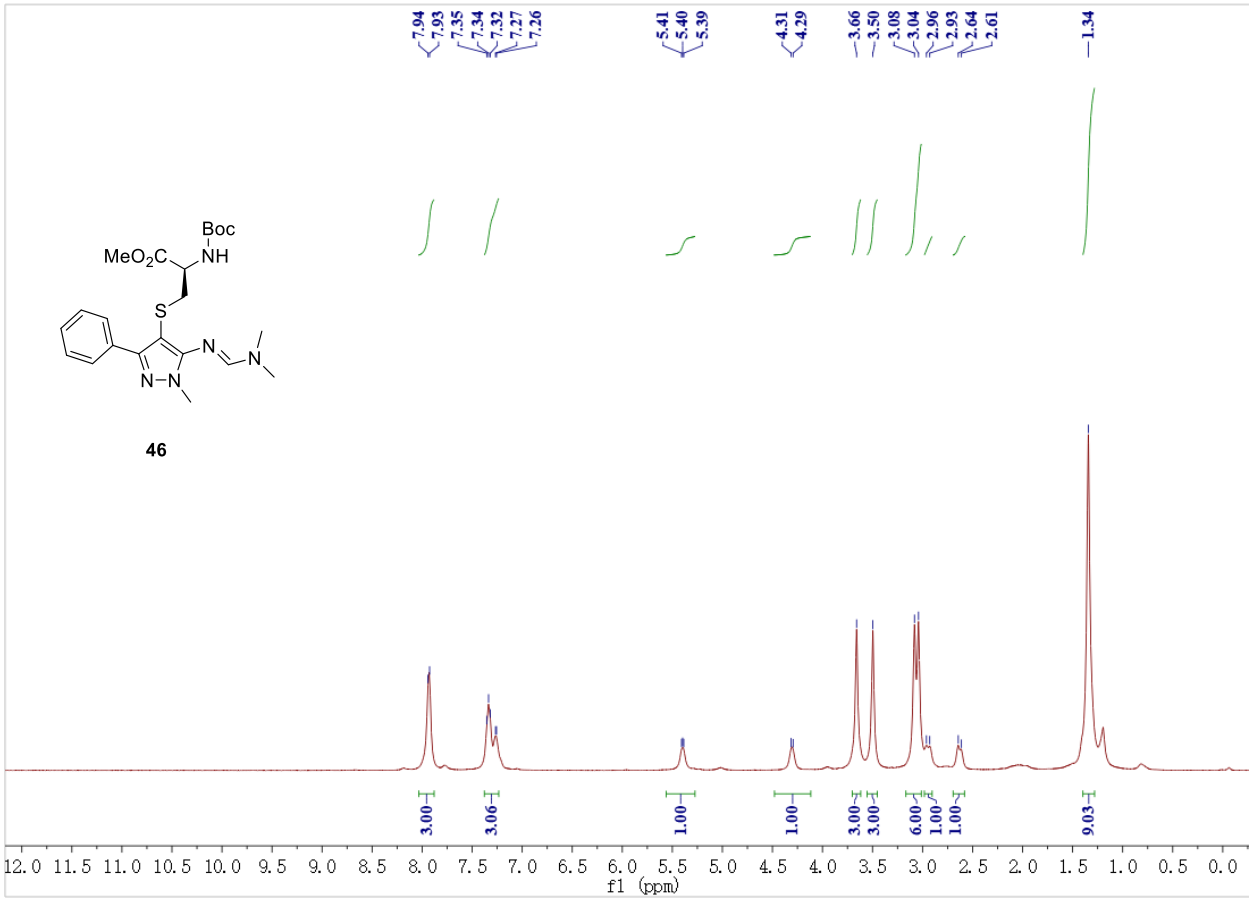
44


171.38
161.56
157.11
155.83
153.91
140.09
128.38
125.60
123.10
95.10
79.43
53.97
52.16
40.77
40.29
34.41
33.98
29.66
28.40
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

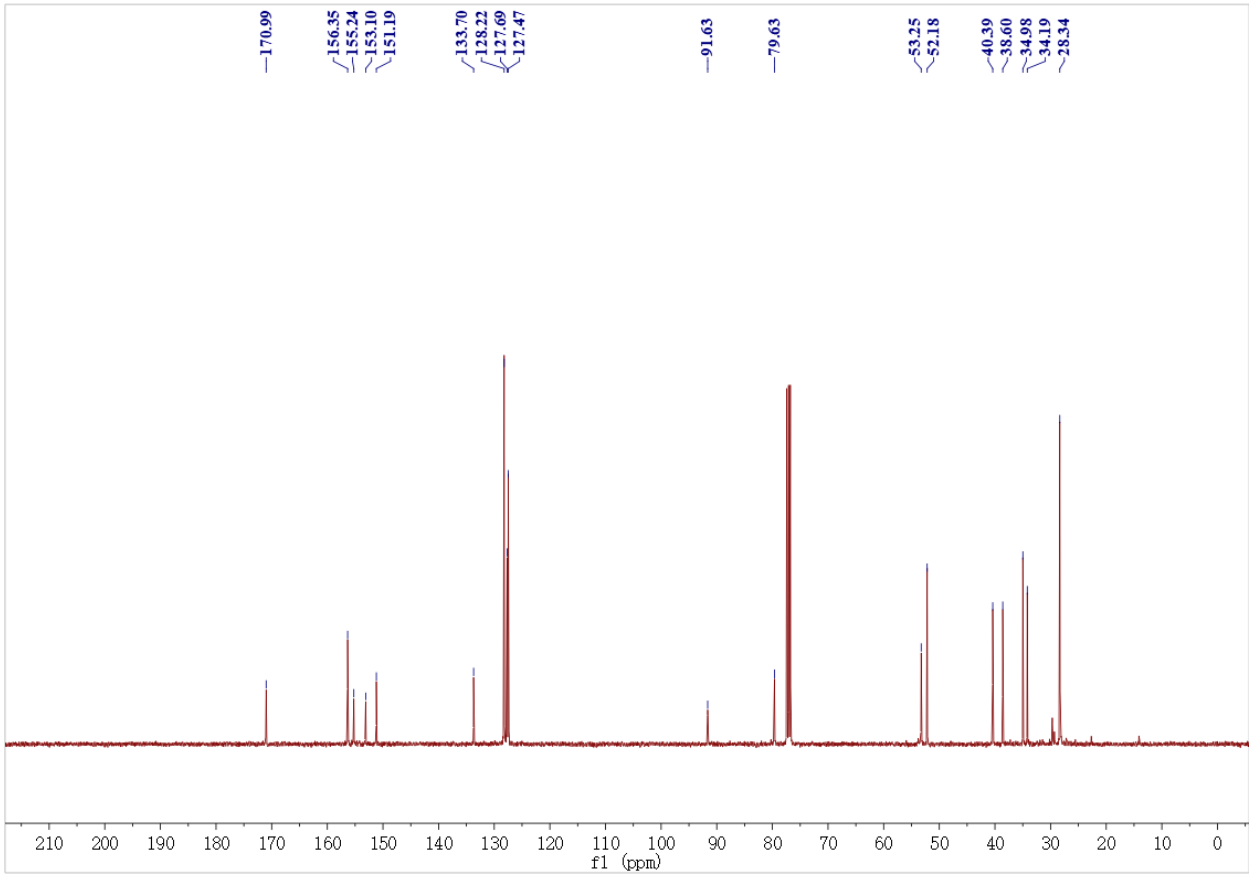

8.10
7.64
7.62
7.20
7.18
7.15
4.04
4.02
4.00
3.58
3.04
2.94
2.77
2.77
2.50 DMSO-d6
2.31
2.18
1.38
Boc
MeO2C
NH
S
N
N
N
1.00
2.00
3.00
1.00
3.00
3.00
3.00
2.00
3.00
3.00
9.01
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

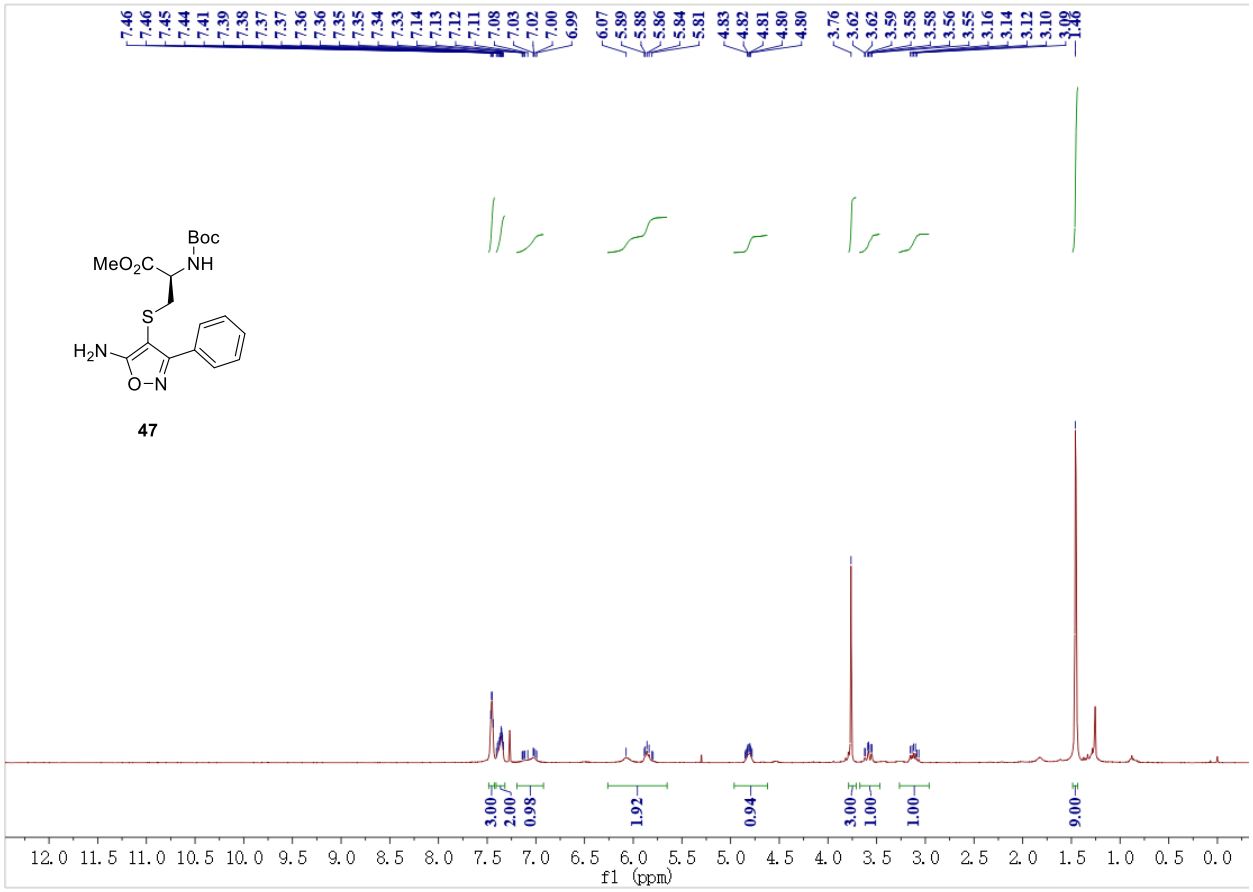
45

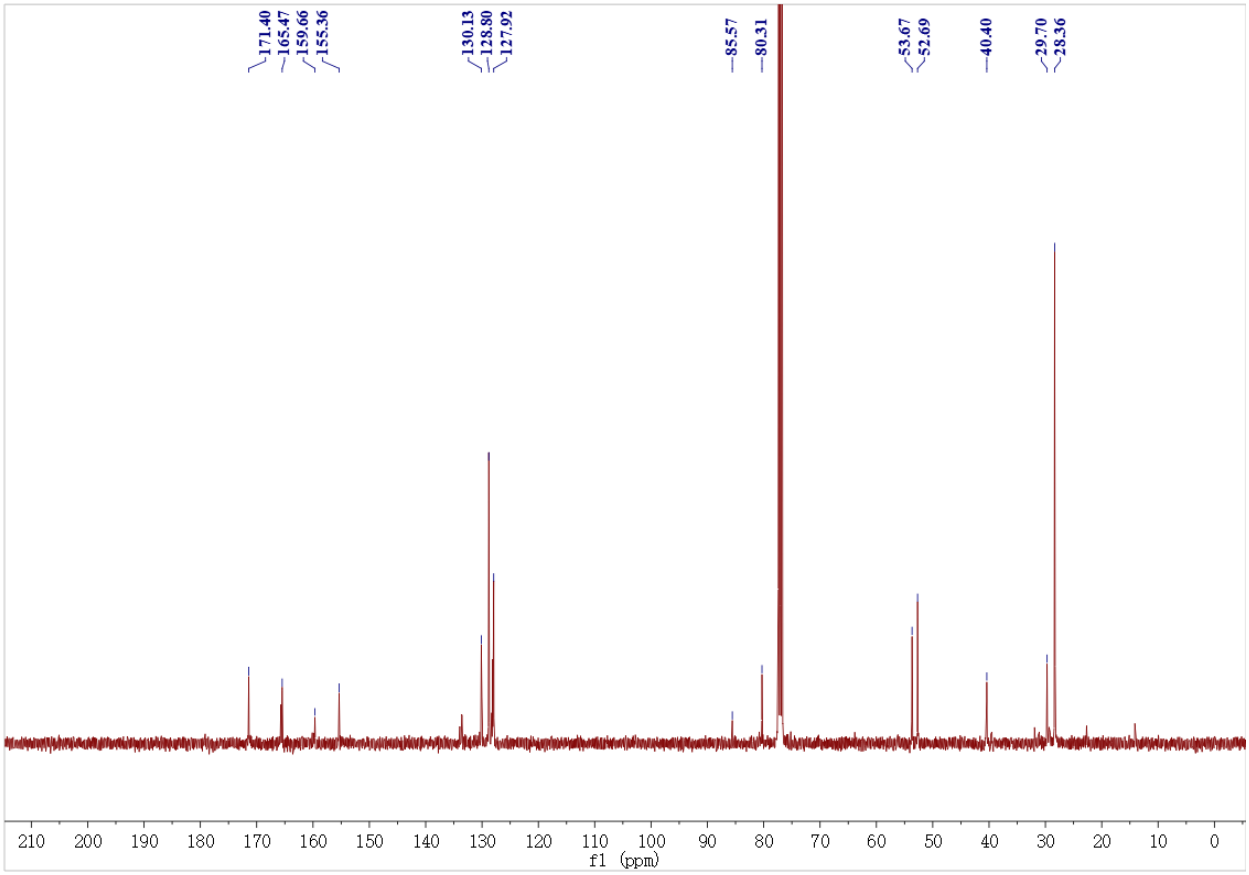

171.95
156.60
155.77
152.79
151.68
137.97
135.08
129.24
122.73
94.30
78.78
55.34
53.84
52.44
39.36
34.24
28.59
20.96
12.94
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

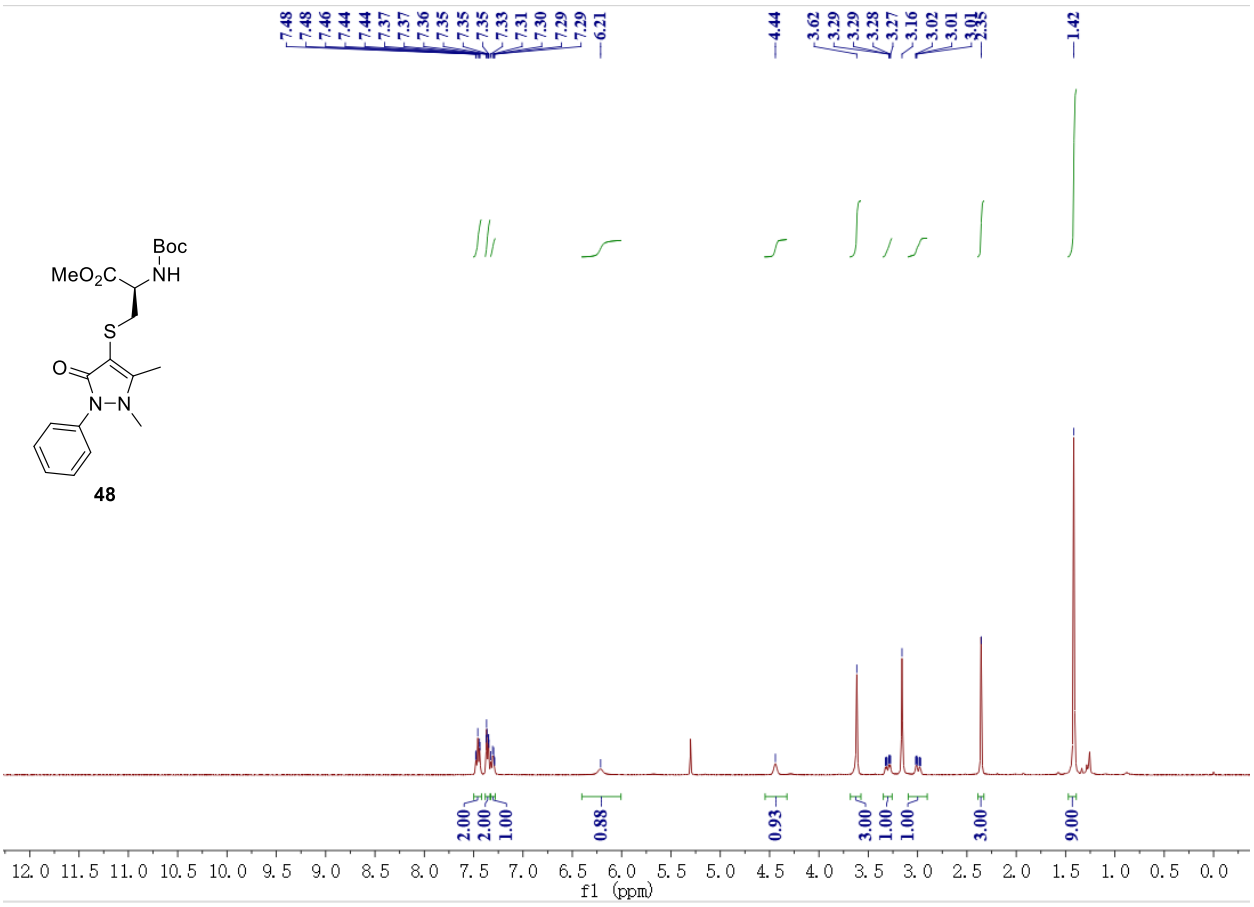

7.94
7.93
7.35
7.34
7.32
7.27
7.26
5.41
5.40
5.39
4.31
4.29
3.66
3.50
3.08
3.04
2.96
2.93
2.64
2.61
1.34
Boc
MeO2C
NH
S
N
N
N
N
3.00
3.06
1.00
1.00
3.00
3.00
6.00
1.00
1.00
9.03
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

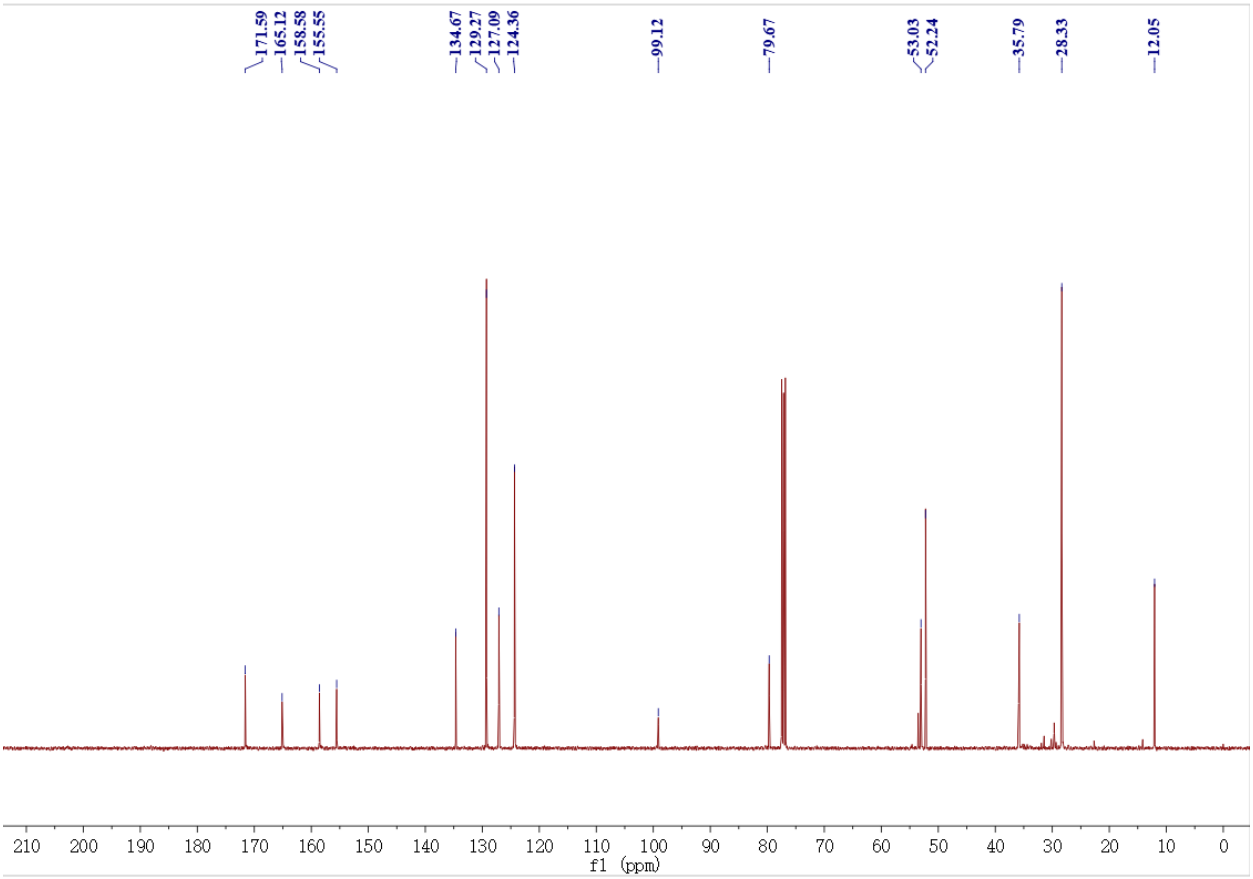
46


170.99
156.35
155.24
153.10
151.19
133.70
128.22
127.69
127.47
91.63
79.63
53.25
52.18
40.39
38.60
34.98
34.19
28.34
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

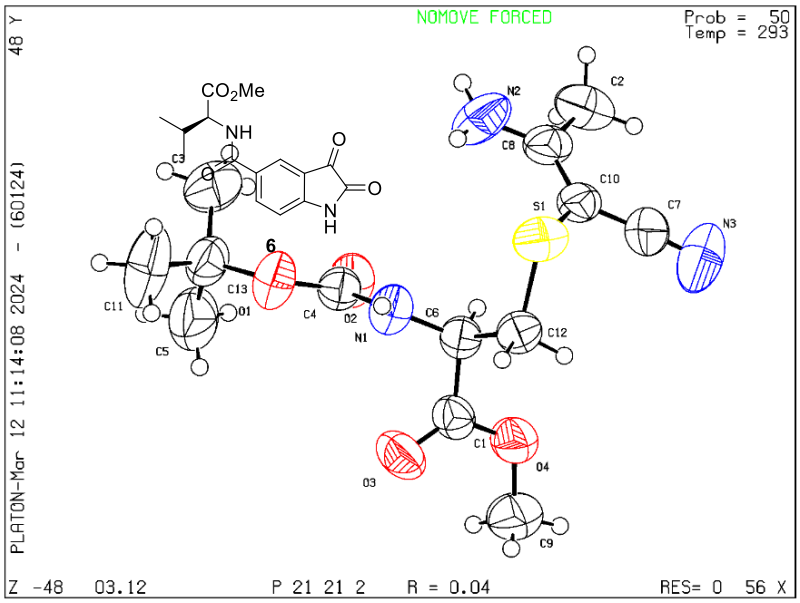

Boc
NH
MeO2C
S
H2N
O–N
47
f1 (ppm)

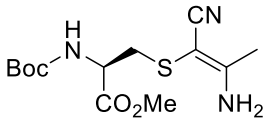

171.40
165.47
159.66
155.36
130.13
128.80
127.92
85.57
80.31
53.67
52.69
40.40
29.70
28.36
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Boc
MeO2C
NH
S
O
N–N
48
7.48
7.48
7.46
7.44
7.44
7.37
7.37
7.36
7.35
7.35
7.35
7.33
7.31
7.30
7.29
7.29
6.21
4.44
3.62
3.29
3.29
3.28
3.27
3.16
3.02
3.01
3.01
2.95
1.42
2.00
2.00
1.00
0.88
0.93
3.00
1.00
1.00
3.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)



7. X-ray single-crystal data





3

Datablock:

Bond precision: C-C = 0.0050 A Wavelength=1.54184

Cell: a=7.2970(1) b=22.7082(4) c=10.4714(2)
alpha=90 beta=90 gamma=90

Temperature: 293 K

	Calculated	Reported
Volume	1735.13(5)	1735.13(5)
Space group	P 21 21 2	P 21 21 2
Hall group	P 2 2ab	P 2 2ab
Moiety formula	C13 H21 N3 O4 S	?
Sum formula	C13 H21 N3 O4 S	C13 H21 N3 O4 S
Mr	315.39	315.39
Dx,g cm-3	1.207	1.207
Z	4	4
Mu (mm-1)	1.818	1.818
F000	672.0	672.0
F000'	675.32	
h,k,lmax	9,28,13	8,28,12
Nref	3519[2043]	3453
Tmin,Tmax	0.790,0.819	
Tmin'	0.790	

Correction method= Not given

Data completeness= 1.69/0.98 Theta(max)= 73.974

R(reflections)= 0.0386(3187) wR2(reflections)= 0.1141(3453)

S = 1.072 Npar= 195

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level A

EXPT005 ALERT 1 A _exptl_crystal_description is missing
Crystal habit description.
The following tests will not be performed.
CRYSR_01

DIFF003 ALERT 1 A _diffrn_measurement_device_type is missing
Diffractometer make and type. Replaces _diffrn_measurement_type.

PLAT183 ALERT 1 A Missing _cell_measurement_reflns_used Value .... Please Do !

PLAT184 ALERT 1 A Missing _cell_measurement_theta_min Value ..... Please Do !

PLAT185 ALERT 1 A Missing _cell_measurement_theta_max Value ..... Please Do !

PLAT699 ALERT 1 A Missing _exptl_crystal_description Value ..... Please Do !

Alert level B

PLAT035 ALERT 1 B _chemical_absolute_configuration Info Not Given Please Do !

Alert level C

PLAT052 ALERT 1 C Info on Absorption Correction Method Not Given Please Do !

PLAT242 ALERT 2 C Low 'MainMol' Ueq as Compared to Neighbors of C7 Check

And 2 other PLAT242 Alerts

More ...

PLAT340 ALERT 3 C Low Bond Precision on C-C Bonds ..... 0.005 Ang.

Alert level G

PLAT007 ALERT 5 G Number of Unrefined Donor-H Atoms ..... 3 Report
H1 H2A H2B

PLAT199 ALERT 1 G Reported _cell_measurement_temperature ..... (K) 293 Check

PLAT200 ALERT 1 G Reported _diffrn_ambient_temperature ..... (K) 293 Check

PLAT883 ALERT 1 G No Info/Value for _atom_sites_solution_primary . Please Do !

PLAT899 ALERT 4 G SHELXL2018 is Deprecated and Succeeded by SHELXL 2019/3 Note

PLAT912 ALERT 4 G Missing # of FCF Reflections Above STh/L= 0.600 12 Note

PLAT969 ALERT 5 G The 'Henn et al.' R-Factor-gap value ..... 3.13 Note
Predicted wR2: Based on SigI**2 3.64 or SHELX Weight 10.96

PLAT978 ALERT 2 G Number C-C Bonds with Positive Residual Density. 0 Info

6 **ALERT level A** = Most likely a serious problem - resolve or explain
1 **ALERT level B** = A potentially serious problem, consider carefully
5 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
8 **ALERT level G** = General information/check it is not something unexpected

11 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
4 ALERT type 2 Indicator that the structure model may be wrong or deficient
1 ALERT type 3 Indicator that the structure quality may be low
2 ALERT type 4 Improvement, methodology, query or suggestion
2 ALERT type 5 Informative message, check