Supporting Information

An IEDDA Reaction of Propargyl Sulfonium Salts and Tetrazines: A Versatile [4+2] Modular Assembly

Zheng Wang, [a,b] Qi Zhu,[b] Jiankang Zhang, [a,b] Huajian Zhu, [a,b] Jiaan Shao, *, [a,b] Wenteng Chen*[b]

[a] School of Medicine, Hangzhou City University, Hangzhou, 310015, PR China. E-mail: shaoja@hzcu.edu.cn.

[b] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, PR China. E-mail: wentengchen@zju.edu.cn.

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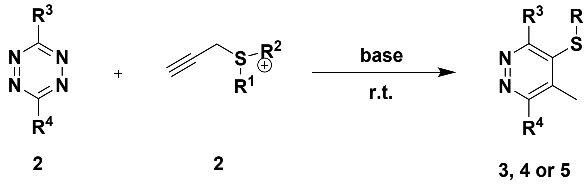
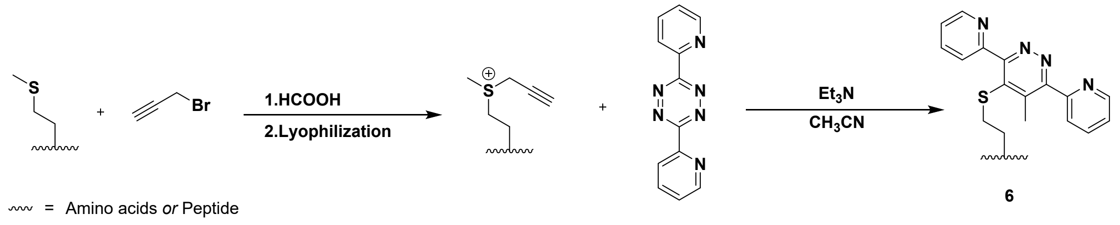
1. General Information

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers and used without further purification. Reaction products were purified by column chromatography on silica gel (200-300 mesh). NMR spectra (^{1}H and ^{13}C) were recorded using either a Bruker AV-500 spectrometer (College of Pharmaceutical Sciences, Zhejiang University) or a Quantum-I Plus 400 MHz NMR spectrometer (School of Medicine, Hangzhou City University). For ^{1}H NMR, tetramethylsilane (TMS, $\delta = 0$) was used as the internal standard, and data are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constants (J) in Hz. For ^{13}C NMR, TMS ($\delta = 0$) or $CDCl_3$ ($\delta = 77.26$) served as the internal standard, and spectra were acquired with full proton decoupling. High-resolution mass spectrometry (HRMS) and HPLC analyses of final compounds were performed on an Agilent 1290 HPLC-6224 Time-of-Flight Mass Spectrometer equipped with a Phenomenex Luna 5 µm C18 column (100 Å, 150 × 4.60 mm). The mobile phase consisted of a linear gradient of solvent B (methanol with 0.1% formic acid) in solvent A (water with 0.1% formic acid) at a flow rate of 0.5 mL/min. The gradient program was as follows: 5% B to 95% B over 7 min, held at 95% B for 2 min, then returned to 5% B over 1 min for column equilibration. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel GF254 plates. The fluorescence emission spectra were acquired on a Shimadzu RF-5301PC spectrofluorometer (excitation at 405 nm, slit width 5 nm), with concurrent UV-vis absorption spectra collected using a Shimadzu UV-2450 spectrophotometer in the 300-800 nm range. The *X*-ray diffraction measurements were carried out on a Rigaku RAXIS-RAPID single-crystal diffractometer. The peptides used in the experiment were obtained by condensation of *N*-BOC-protected amino acids as starting materials under a HATU/DIPEA system, followed by deprotection. The reactions were monitored by thin layer chromatography (TLC) using silica gel GF254.

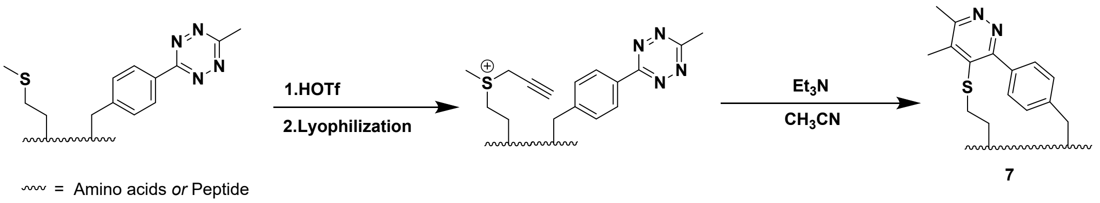
2. General procedure for the synthesis of target compounds 3-7 and tetrazine 2



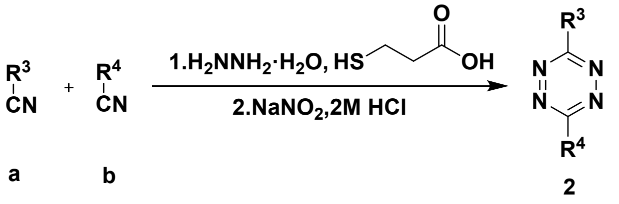
Synthesis of desired products 3, 4 and 5: To a 25 mL reaction tube equipped with a stir bar, tetrazine substrate **1** (1.0 equiv), sulfonium salt substrate **2** (1.5 equiv) and base (K_2CO_3 1.5 equiv) were added in 8 mL CH_3CN. The reaction was stirred at room temperature for 5-30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified on silica column chromatography to afford the desired product.



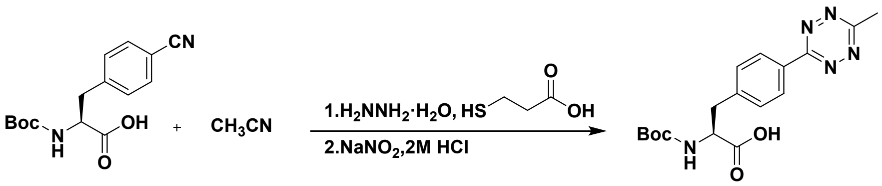
Synthesis of desired products 6: To a 25 mL reaction tube equipped with a stir bar, peptide (1.0 equiv), propargyl bromide (5.0–8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). The reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (1.5 equiv) and Et_3N (1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30-60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified on silica column chromatography to afford the desired product.



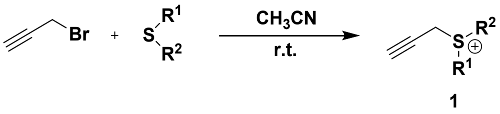
Synthesis of desired products 7: To a 25 mL reaction tube equipped with a stir bar, peptide (1.0 equiv), propargyl bromide (5.0–8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). The reaction was stirred at room temperature for 12-18 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30-60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified on silica column chromatography to afford the desired product.[30]



Synthesis of tetrazine 2: To a 50 mL reaction tube equipped with a stir bar, nitrile substrate **a** (1.0 equiv), nitrile substrate **b** (1.0–8.0 equiv), 3-mercaptopropionic acid (0.2–1.0 equiv), and hydrazine hydrate (16.0 equiv) were added. Under argon, the reaction was stirred at room temperature. Upon completion, the reaction solution was cooled with ice water, and added dichloromethane as cosolvent if necessary. An ice water solution of sodium nitrite (15.0 equiv) was slowly added into the reaction mixture, followed by slow addition of 2 M HCl during which the solution was stirred intensely and turned red, and gas evolved. Addition of 1 M HCl continued until gas evolution ceased and the pH value was 3–4. Then, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified on silica column chromatography to afford the desired product. [31]



Synthesis of tetrazine attached *L*-phenylalanine: To a 50 mL reaction tube equipped with a stir bar, nitrile substrate (*S*)-2-((tert-butoxycarbonyl)amino)-3-(4-cyanophenyl)propanoic acid (1.0 equiv), CH_3CN (8.0 equiv), 3-mercaptopropionic acid (1.0 equiv), and hydrazine hydrate (16.0 equiv) were added. Under argon, the reaction was stirred at room temperature. Upon completion, the reaction solution was cooled with ice water, and added dichloromethane as cosolvent if necessary. An ice water solution of sodium nitrite (15.0 equiv) was slowly added into the reaction mixture, followed by slow addition of 2 M HCl during which the solution was stirred intensely and turned red, and gas evolved. Addition of 1 M HCl continued until gas evolution ceased and the pH value was 3–4. Then, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified on silica column chromatography to afford the desired product.



Synthesis of sulfonium salt: To a 50 mL reaction tube equipped with a stir bar, propargyl bromide (1.0 equiv), thioether (5.0–8.0 equiv) were added in CH_3CN. Under argon, the reaction was stirred at room temperature for 24 h. Upon completion, the reaction solution was filtered to afford the desired solid residue product.

3. Determination of fluorescence intensity of tetrazine probe 3p

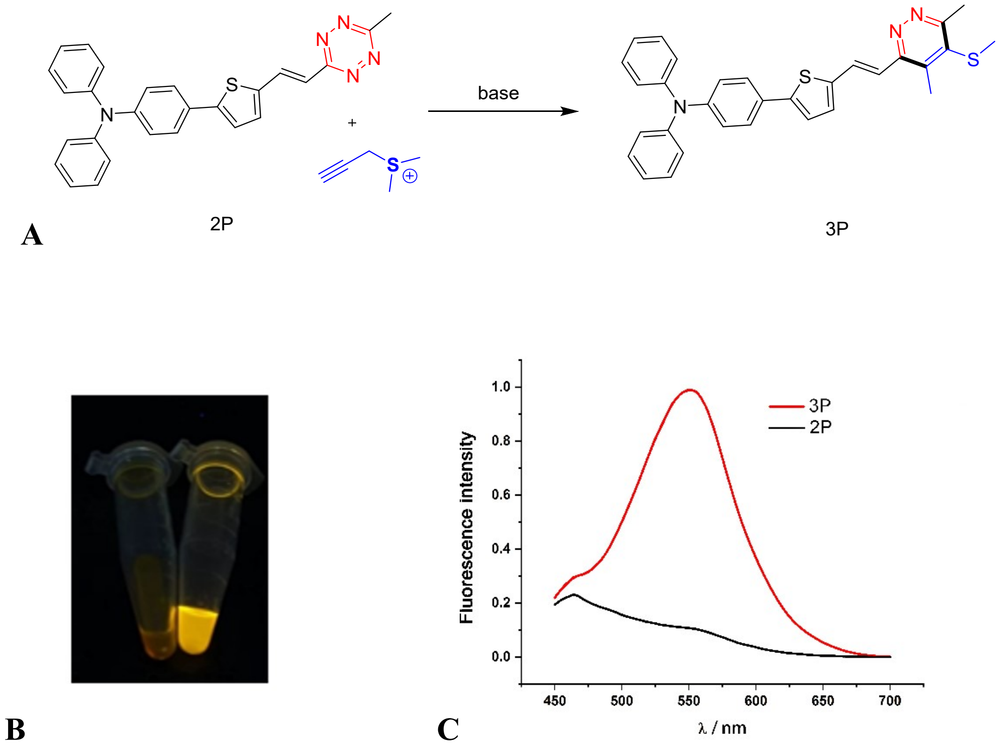


Figure S2. Determination of fluorescence intensity of tetrazine probe **3p**. (a) The Reaction of **2p** with sulfonium salt. (b) Comparison of fluorescence before and after reaction. (c) Comparison of fluorescence intensity after normalization treatment.

The generation of **3p** was obtained with the UV lamp at 365 nm upon adding sulfonium salts **2a** (1.5 mM) into tetrazine **2p** (1.0 mM) in 8 mL CH_3CN at 25 °C. The fluorescence intensity of compound **2p** and **3p** was monitored by a fluorescence spectrometer (λ_{ex} = 405 nm, λ_{em} = 550 nm) under 25 °C. Subsequent spectral analysis revealed a 10-fold enhancement in fluorescence intensity at 550 nm for **3p** compared to the **2p**, as quantified through baseline-corrected intensity normalization.

4. Reaction condition optimization of cyclic peptide synthesis

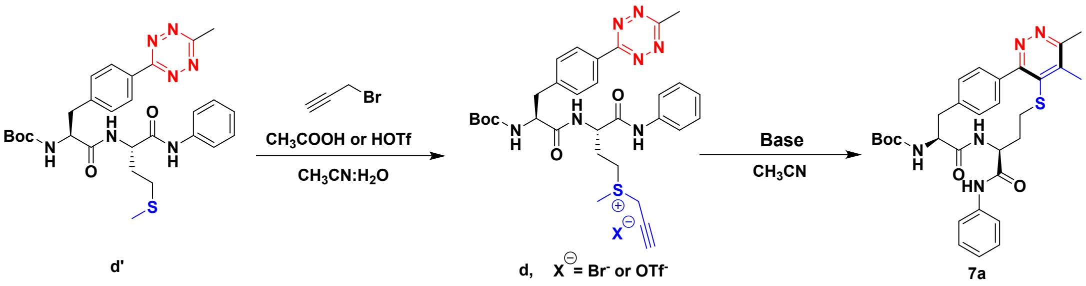


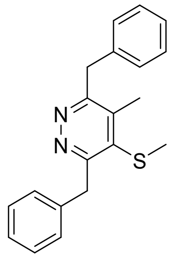
Table S2. Optimization of the reaction conditions

Group	Base (1.1equiv)	Counterion	Yield (%) [a]
1	K_2CO_3	Br^-	21.1%
2	DIPEA	Br^-	8.3%
3	Et_3N	Br^-	27.2%
4	Et_3N	OTf^-	50.0%

[a] Isolated yield based on **d’**.

5. Synthesis and characterization of 3, 4, 5, 6, 7

Synthesis of 3a

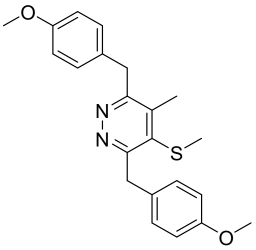


3a

To a 25 mL reaction tube equipped with a stir bar, 3,6-dibenzyl-1,2,4,5-tetrazine (263 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =10: 3) to afford 282 mg of **3a** in 88 % yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J = 7.2 Hz, 2H), 7.28 (dd, J = 9.6, 4.4 Hz, 3H), 7.25 – 7.14 (m, 5H), 4.59 (s, 2H), 4.37 (s, 2H), 2.39 (s, 3H), 1.97 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 163.3, 160.3, 141.7, 138.9, 138.9, 137.7, 129.2, 128.8, 128.6, 128.4, 126.6, 126.4, 41.4, 41.0, 18.1, 16.5. **HRMS** $[M+H]^+$ m/z calcd for $[C_{20}H_{20}N_2S]^+$ 321.1420, found 321.1418.

Synthesis of 3b



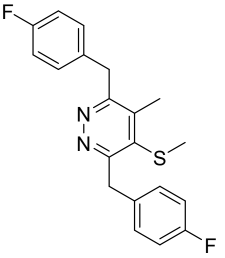
3b

To a 25 mL reaction tube equipped with a stir bar, 3,6-bis(4-methoxybenzyl)-1,2,4,5-tetrazine (322 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =5:1) to afford 305 mg of **3b** in 80 % yield, as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 9.0, 2H), 7.15 (d, J = 9.0 Hz, 2H), 6.84 – 6.78 (m, 4H), 4.54 (s, 2H), 4.32 (s, 2H), 3.76 (d, J = 3.5 Hz, 6H), 2.40 (s, 3H), 2.01 (s, 3H). **^{13}C{^{1}H} NMR** (125

MHz, $CDCl_3$) δ 163.4, 160.4, 158.4, 158.2, 130.6, 130.3, 129.8, 129.3, 114.1, 113.8, 55.3, 55.2, 40.2, 39.8, 18.2, 16.6. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{22}H_{24}N_2O_2S]^+$ 381.1631, found 381.1637.

Synthesis of 3c

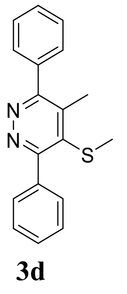


3c

To a 25 mL reaction tube equipped with a stir bar, 3,6-bis(4-fluorobenzyl)-1,2,4,5-tetrazine (298 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =10:3) to afford 271 mg of **3c** in 76 % yield, as a purple oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.23 (dd, *J* = 8.4, 5.2 Hz, 2H), 6.99 (td, *J* = 8.4, 6.4 Hz, 4H), 4.58 (s, 2H), 4.36 (s, 2H), 2.43 (s, 3H), 2.05 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 163.2, 162.9 (162.9, 162.8, d, *J* = 13.2 Hz), 160.5, 160., 160.2, 141.6 , 138.9, 134.5 (134.5, 134.4, d, *J* = 3.0 Hz), 133.3 (133.3, 133.2, d, *J* = 3.0 Hz), 130.7 (130.8, 130.7, d, *J* = 7.8 Hz), 130.2 (130.3, 130.2, d, *J* = 7.9 Hz), 115.6, 115.4 (115.4, 115.3, d, *J* = 11.4 Hz), 115.1, 40.6, 40.1, 18.2, 16.5. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{20}H_{18}F_2N_2S]^+$ 357.1232, found 357.1235.

Synthesis of 3d

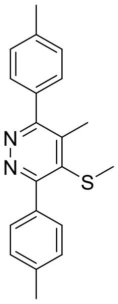


3d

To a 25 mL reaction tube equipped with a stir bar, 3,6-diphenyl-1,2,4,5-tetrazine (234 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 216 mg of **3d** in 80% yield, as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.82 (d, *J* = 1.5 Hz, 2H), 7.61 (d, *J* = 1.5 Hz, 2H), 7.55 – 7.47 (m, 6H), 2.54 (s, 3H), 2.00 (s, 3H). **^{13}C{^{1}H} NMR** (125 MHz, $CDCl_3$) *δ* 160.20, 159.8, 138.6, 137.7, 136.9, 129.5, 129.3, 129.1, 129.1, 128.5, 128.4, 29.7, 18.6, 17.9. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{18}H_{16}N_2S]^+$ 293.1107, found 293.1112.

Synthesis of 3e

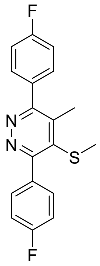


3e

To a 25 mL reaction tube equipped with a stir bar, 3,6-di-p-tolyl-1,2,4,5-tetrazine (262 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 237 mg of **3e** in 74% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.72 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 7.0 Hz, 4H), 2.53 (s, 3H), 2.44 (s, 6H), 2.02 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) *δ* 160.20, 159.8, 138.6, 137.7, 136.9, 129.5, 129.3, 129.1, 129.1, 128.5, 128.4, 29.7, 18.6, 17.9. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{20}H_{20}N_2S]^+$ 321.1420, found 321.1420.

Synthesis of 3f

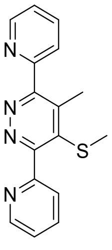


3f

To a 25 mL reaction tube equipped with a stir bar, 3,6-bis(4-fluorophenyl)-1,2,4,5-tetrazine (270 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 263 mg of **3f** in 70% yield, as a purple oil.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.76 (dd, J = 8.5, 5.5 Hz, 2H), 7.52 (dd, J = 8.5, 5.5 Hz, 2H), 7.13 (td, J = 8.5, 2.5 Hz, 4H), 2.46 (s, 3H), 1.95 (s, 3H). **^{13}C{^{1}H} NMR** (125 MHz, CDCl$_3$) δ 164.2 (164.2, 164.2, d, J = 3.7 Hz), 162.2 (162.2, 162.3, d, J = 3.3 Hz), 159.4 (159.5, 159.3, d, J = 15.7 Hz), 139.6, 138.4, 133.9 (133.9, 133.9, d, J = 3.4 Hz), 133.4 (133.3, 133.4, d, J = 3.4 Hz), 131.3 (131.4, 131.4, 131.3, 131.3, dd, J = 18.7, 8.3 Hz), 115.5 (115.6, 115.5, 115.4, 115.4, dd, J = 21.7, 10.4 Hz), 18.6 17.9. **HRMS** [M+H]$^+$ *m/z* calcd for [$C_{18}H_{14}F_2N_2S$]$^+$ 329.0919, found 329.0915.

Synthesis of 3g

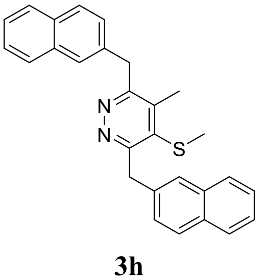


3g

To a 25 mL reaction tube equipped with a stir bar, 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (236 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K$_2$CO$_3$ (206 mg, 1.5 equiv) were added in 8 mL CH$_3$CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 268 mg of **3g** in 84% yield, as a purple oil.

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.77 (dd, J = 13.6, 4.8 Hz, 2H), 8.00 (d, J = 7.6 Hz, 2H), 7.91 (dd, J = 10.8, 4.8 Hz, 2H), 7.42 (dt, J = 6.0, 4.8 Hz, 2H), 2.71 (s, 3H), 2.18 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, CDCl$_3$) δ 159.8, 158.7, 156.3, 148.8, 148.6, 141.5, 134.0, 137.1, 136.8, 125.3, 124.5, 123.7, 18.6. **HRMS** [M+H]$^+$ *m/z* calcd for [$C_{16}H_{14}N_4S$]$^+$ 295.1012, found 295.1011

Synthesis of 3h



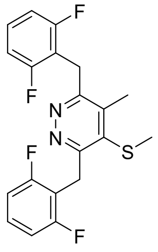
3h

To a 25 mL reaction tube equipped with a stir bar, 3,6-bis(naphthalen-2-ylmethyl)-1,2,4,5-

tetrazine (362 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =5:1) to afford 336 mg of **3h** in 84% yield, as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 8.5 Hz, 1H), 7.79 (t, J = 9.0 Hz, 2H), 7.67 (dd, J = 10.6, 8.4 Hz, 2H), 7.51 – 7.41 (m, 4H), 7.29 – 7.25 (m, 2H), 7.10 (d, J = 6.5 Hz, 1H), 6.95 – 6.91 (m, 1H), 5.00 (s, 2H), 4.80 (s, 2H), 2.32 (s, 3H), 1.94 (s, 3H). **^{13}C{^{1}H} NMR** (125 MHz, $CDCl_3$) δ 162.0, 159.0, 133.7, 132.9, 132.8, 132.2, 131.0, 131.0, 127.8, 127.7, 126.6, 126.3, 125.9, 125.4, 125.2, 125.2, 124.9, 124.7, 124.4, 124.3, 123.0, 122.6, 37.2, 36.7, 17.3, 15.9. **HRMS** $[M+H]^+$ m/z calcd for $[C_{28}H_{24}N_2S]^+$ 421.1733, found 421.1731.

Synthesis of 3i

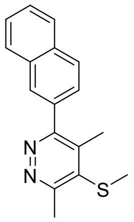


3i

To a 25 mL reaction tube equipped with a stir bar, 3,6-bis(2,6-difluorobenzyl)-1,2,4,5-tetrazine (334 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =5:1) to afford 290 mg of **3i** in 74% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.0 Hz, 2H), 6.85 (dd, J = 14.5, 7.5 Hz, 4H), 4.58 (s, 2H), 4.27 (s, 2H), 2.63 (s, 3H), 2.32 (s, 3H). **^{13}C{1H} NMR** (100 MHz, $CDCl_3$) δ 162.9 (162.9, 162.9, 162.8, 162.8, dd, J = 8.4, 5.4 Hz), 160.74, 160.41 (160.5, 160.4, 160.4, 160.3, dd, J = 8.4, 5.5 Hz), 157.9 , 140.2, 137.7, 128.2 (128.5, 128.4, 128.3, 128.2, 128.1, 128.0, dt, J = 26.9, 10.3 Hz), 114.6 (114.8, 114.6, 114.4, t, J = 19.8 Hz), 113.6 (113.7, 113.6, 113.4, t, J = 19.8 Hz), 111.2 (111.3, 111.2, 111.2, t, J = 5.3 Hz) , 111.0 (111.0, 111.0, 110.9, t, J = 5.3 Hz), 28.2, 27.4, 18.1, 16.4. **HRMS** $[M+H]^+$ m/z calcd for $[C_{20}H_{16}F_4N_2S]^+$ 393.1043, found 393.1042.

Synthesis of 3j

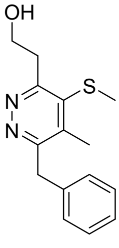


3j

To a 25 mL reaction tube equipped with a stir bar, 3-methyl-6-(naphthalen-2-yl)-1,2,4,5-tetrazine (222 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 15 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 213 mg of **3j** in 66% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.86 (m, 4H), 7.65 (d, J = 8.4 Hz, 1H), 7.57 – 7.51 (m, 2H), 2.95 (s, 3H), 2.55 (s, 3H), 2.42 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 160.8, 160.7, 139.3, 139.2, 135.0, 133.3, 133.2, 129.0, 128.5, 128.1, 127.8, 126.8, 126.5, 22.3, 18.7, 17.7. **HRMS** $[M+H]^+$ m/z calcd for $[C_{17}H_{16}N_2S]^+$ 281.1107, found 281.1104.

Synthesis of 3l^1

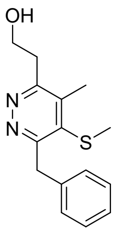


3l^1

To a 25 mL reaction tube equipped with a stir bar, 2-(6-benzyl-4-methyl-5-(methylthio) pyridazin-3-yl) ethan-1-ol (274 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =1:1) to afford 107 mg of **3l1** in 39% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.17 (m, 5H), 4.37 (s, 2H), 4.17 (t, J = 5.2 Hz, 2H), 3.40 (t, J = 5.2 Hz, 2H), 2.44 (s, 3H), 2.26 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 162.7, 160.2, 141.4, 139.4, 137.4, 128.7, 127.6, 126.7, 60.7, 40.9, 36.4, 18.1, 16.8. **HRMS** $[M+H]^+$ m/z calcd for $[C_{15}H_{18}N_2OS]^+$ 275.1213, found 275.1215.

Synthesis of 3l^2

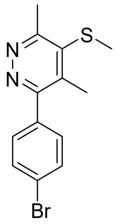


3l2

To a 25 mL reaction tube equipped with a stir bar, 2-(6-benzyl-4-methyl-5-(methylthio) pyridazin-3-yl) ethan-1-ol (274 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =1:1) to afford 94 mg of **3l2** in 34% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J = 6.8 Hz, 2H), 7.29 (t, J = 6.8 Hz, 2H), 7.21 (d, J = 6.8 Hz, 1H), 4.60 (s, 2H), 4.23 (t, 2H), 4.04 (s, 1H), 3.10 (t, 2H), 2.50 (s, 3H), 2.06 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 163.1, 160.0, 142.0, 138.7, 129.2, 128.4, 126.4, 59.9, 41.3, 35.5, 18.2, 16.2. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{15}H_{18}N_2OS]^+$ 275.1213, found 275.1215.

Synthesis of 3m

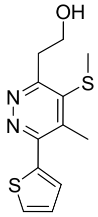


3m

To a 25 mL reaction tube equipped with a stir bar, 3-(4-bromophenyl)-6-methyl-1,2,4,5-tetrazine (250 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 139 mg of **3m** in 54% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 6.0 Hz, 2H), 7.43 (d, J = 6.0 Hz, 2H), 2.91 (s, 3H), 2.49 (s, 3H), 2.40 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 161.0, 159.7, 139.3, 138.9, 136.5, 131.6, 131.0, 123.3, 22.3, 18.5, 17.7. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{13}H_{13}BrN_2S]^+$ 309.0057, found 309.0056.

Synthesis of 3n

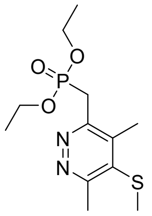


3n

To a 25 mL reaction tube equipped with a stir bar, 2-(5-methyl-4-(methylthio)-6-(thiophen-2-yl) pyridazin-3-yl) ethan-1-ol (266 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 15 min. After the reaction had completed, the residue was purified by silica column (PE: EA =1:1) to afford 136 mg of **3n** in 64% yield, as purple oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.43 (m, 2H), 7.11 (dd, *J* = 5.2, 4.0 Hz, 1H), 5.23 (s, 1H), 4.13 (t, *J* = 5.0 Hz, 2H), 3.39 – 3.34 (m, 2H), 2.72 (s, 3H), 2.31 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 161.7, 154.6, 140.2, 140.1, 138.7, 128.9, 128.8, 127.6, 60.6, 36.6, 19.0, 18.1. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{12}H_{14}N_2OS]^+$ 267.0620, found 267.0623.

Synthesis of 3o



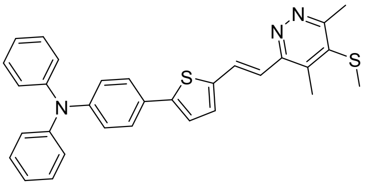
3o

To a 25 mL reaction tube equipped with a stir bar, diethyl ((6-methyl-1,2,4,5-tetrazin-3-yl)methyl)phosphonate (246 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =1:1) to afford 137 mg of **3o** in 47% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 – 4.03 (m, 4H), 3.59 (d, *J* = 22.0 Hz, 2H), 2.82 (d, *J* = 1.6 Hz, 3H), 2.62 (s, 3H), 2.30 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 6H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 161.1 (161.1, 161.1, d, *J* = 3.2 Hz), 153.9 (153.9, 153.8, d, *J* = 9.2 Hz), 141.0 (141.0, 140.9, d, *J* = 4.0 Hz), 138.7 (138.8, 138.7, d, *J* = 2.4 Hz), 62.5 (62.6, 62.5, d, *J* = 6.4 Hz), 32.7(33.4, 32.0, d, *J* = 134.9 Hz), 22.2, 17.8, 17.2,16.4(16.4, 16.3, d, *J* = 6.2 Hz). **HRMS** $[M+H]^+$ *m/z* calcd for

$[C_{12}H_{21}N_2O_3PS]^+$ 305.1083, found 305.1083.

Synthesis of 3p

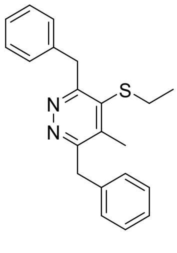


3p

To a 25 mL reaction tube equipped with a stir bar, (*E*)-4-(5-(2-(6-methyl-1,2,4,5-tetrazin-3-yl)vinyl)thiophen-2-yl)-*N,N*-diphenylaniline (447 mg, 1.0 equiv), dimethyl(prop-2-yn-1-yl) sulfonium (270 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =10:3) to afford 272 mg of **3p** in 54% yield, as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 15.2 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 7.6 Hz, 4H), 7.18 – 7.12 (m, 6H), 7.07 (d, J = 8.4 Hz, 4H), 7.00 (s, 1H), 2.87 (s, 3H), 2.63 (s, 3H), 2.33 (s, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 160.1, 155.3, 147.7, 147.4, 144.9, 140.5, 138.1, 132.4, 130.5, 129.4, 128.9, 127.8, 126.6, 124.7, 123.4, 123.3, 122.9, 119.9, 22.4, 18.0, 16.3. **HRMS** $[M+H]^+$ *m/z* calcd for $[C_{31}H_{27}N_3S_2]^+$ 506.1719, found 506.1718.

Synthesis of 4a

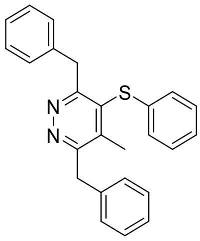


4a

To a 25 mL reaction tube equipped with a stir bar, 3,6-dibenzyl-1,2,4,5-tetrazine (262 mg, 1.0 equiv), diethyl(prop-2-yn-1-yl) sulfonium (310 mg, 1.5 equiv) and K_2CO_3 (206 mg, 1.5 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 15 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 241 mg of **4a** in 72 % yield, as a brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30(d, J = 7.6 Hz, 2H), 7.21 – 7.08 (m, 8H), 4.51 (s, 2H), 4.30 (s, 2H), 2.78 (q, J = 7.6 Hz, 2H), 2.30(s, 3H), 0.93 (t, J = 7.6 Hz, 3H). **^{13}C{^{1}H} NMR** (100 MHz, CDCl$_3$) δ 163.6, 160.1, 142.0, 139.0, 137.7, 137.4, 129.3, 128.7, 128.6, 128.3, 126.6, 126.3, 41.4, 41.0, 29.7, 16.8, 14.5. **HRMS** [M+H]$^+$ *m/z* calcd for [C$_{21}$H$_{22}$N$_2$S]$^+$ 335.1576, found 335.1578.

Synthesis of 4b

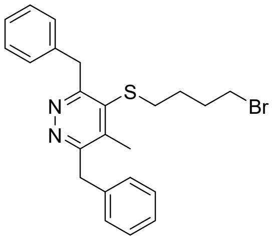


4b

To a 10 mL reaction tube equipped with a stir bar, 3,6-dibenzyl-1,2,4,5-tetrazine (262 mg, 1.0 equiv), methyl(phenyl)(prop-2-yn-1-yl) sulfonium (363 mg, 1.5 equiv) and K$_2$CO$_3$ (206 mg, 1.5 equiv) were added in 8 mL CH$_3$CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 308 mg of **4d** in 81 % yield, as a white oil.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.25 (d, J = 7.0 Hz, 2H), 7.21 – 7.01 (m, 11H), 6.75 (dd, J = 7.5, 1.5 Hz, 2H), 4.45 (s, 2H), 4.31 (s, 2H), 2.05 (s, 3H). **^{13}C{1H} NMR** (125 MHz, CDCl$_3$) δ 163.3, 160.8, 142.2, 138.4, 137.6, 135.8, 134.6, 129.4, 129.3, 128.7, 128.4, 127.7, 126.6, 126.5, 126.4, 41.2, 40.9, 17.0. **HRMS** [M+H]$^+$ *m/z* calcd for [C$_{25}$H$_{22}$N$_2$S]$^+$ 381.1631, found 381.1637.

Synthesis of 4d

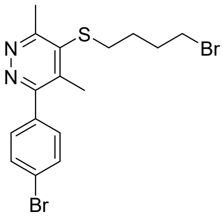


4d

To a 25 mL reaction tube equipped with a stir bar, 3,6-dibenzyl-1,2,4,5-tetrazine (262 mg, 1.0 equiv), 1-(prop-2-yn-1-yl) tetrahydro-1*H*-thiophen-1-ium (309 mg, 1.5 equiv) and K$_2$CO$_3$ (206 mg, 1.5 equiv) were added in 8 mL CH$_3$CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 357 mg of **4d** in 81 % yield, as a brown oil.

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38 (d, J = 7.2 Hz, 2H), 7.34 – 7.19 (m, 8H), 4.62 (s, 2H), 4.41 (s, 2H), 3.26 (t, *J* = 6.4 Hz, 2H), 2.47 – 2.39 (m, 5H), 1.81 – 1.73 (m, 2H), 1.57 – 1.40 (m, 2H). **^{13}C{^{1}H} NMR** (100 MHz, CDCl$_3$) *δ* 163.4, 160.2, 141.9, 138.9, 137.6, 137.5, 129.2, 128.8, 128.7, 128.4, 126.7, 126.4, 41.5, 41.0, 34.6, 32.7, 31.4, 28.0, 16.9. **HRMS** [M+H]$^+$ *m/z* calcd for [C$_{23}$H$_{25}$BrN$_2$S]$^+$441.0995, found 441.0992.

a) Synthesis of 4e

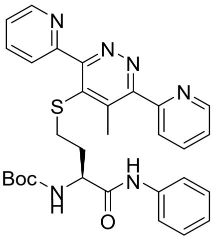


4e

To a 25 mL reaction tube equipped with a stir bar, 3-(4-bromophenyl)-6-methyl-1,2,4,5-tetrazine (250 mg, 1.0 equiv), 1-(prop-2-yn-1-yl) tetrahydro-1H-thiophen-1-ium (309 mg, 1.5 equiv) and K$_2$CO$_3$ (206 mg, 1.5 equiv) were added in 8 mL CH$_3$CN. The reaction mixture was stirred at ambient temperature for 5 min. After the reaction had completed, the residue was purified by silica column (PE: EA =3:1) to afford 300 mg of **4e** in 70 % yield, as a purple oil.

^{1}H NMR (400 MHz, CDCl$_3$) *δ* 7.89 (t, *J* = 10.4 Hz, 2H), 7.68 (t, *J* = 10.4 Hz, 2H), 3.66 (dt, J = 8.4, 5.6 Hz, 2H), 3.23 – 3.16 (m, 3H), 3.13 (dd, *J* = 16.4, 9.2 Hz, 2H), 2.84 – 2.72 (m, 3H), 2.25 (dd, *J* = 14.0, 6.8 Hz, 2H), 2.02 (dd, *J* = 14.0, 6.8 Hz, 2H). **^{13}C{^{1}H} NMR** (100 MHz, CDCl$_3$) *δ* 160.9, 131.8, 131.1, 124.2, 34.2, 32.7, 31.4, 28.5, 19.5. **HRMS** [M+H]$^+$ m/z calcd for [C$_{16}$H$_{18}$Br$_2$N$_2$S]$^+$ 430.9630, found 430.9633.

b) Synthesis of 5a



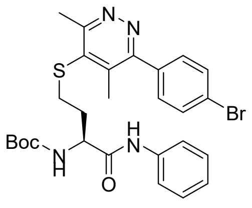
5a

To a 25 mL reaction tube equipped with a stir bar, 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (236 mg, 1.0 equiv), ((S)-3-((tert-butoxycarbonyl)amino)-4-oxo-4-(henyla n,j mino)butyl)(methyl) (prop-2-yn-1-yl) sulfonium (544 mg, 1.5 equiv) and Et$_3$N (111 mg, 1.1 equiv) were added in 8 mL

CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =1:1) to afford 372 mg of **5a** in 71% yield, as a brown solid.

^{1}H NMR (400 MHz, DMSO-*$_{d6}$)* δ 9.99 (s, 1H), 8.77 (s, 1H), 8.65 (s, 1H), 7.92 (t, *J* = 7.2 Hz, 1H), 7.86 (d, *J* = 9.6 Hz, 2H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.43 (s, 1H), 7.38 (d, *J* = 7.6 Hz, 3H), 7.14 (t, *J* = 7.6 Hz, 2H), 6.94 (t, *J* = 6.8 Hz, 1H), 5.26 (s, 1H), 4.51 (s, 1H), 2.88 (s, 1H), 2.80 (s, 1H), 2.64 (s, 3H), 1.84 (s, 1H), 1.76 (s, 1H), 1.35 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 170.2, 160.9, 159.5, 155.8, 156.2, 155.9, 155.8, 148.8, 148.7, 141.7, 138.1, 137.6, 137.1, 128.9, 125.3, 125.2,124.2, 124.1, 123.8, 119.6, 80.0, 52.9, 33.6, 31.5, 28.3, 18.7. **HRMS** $[M+H]^+$ m/z calcd for $[C_{30}H_{32}O_6N_3S]^+$557.2329, found 557.2326.

c) Synthesis of 5b

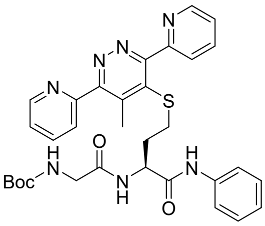


5b

To a 25 mL reaction tube equipped with a stir bar, 3-(4-bromophenyl)-6-methyl-1,2,4,5-tetrazine (250 mg, 1.0 equiv), ((S)-3-((tert-butoxycarbonyl) amino)-4-oxo-4-(henylamino)butyl) (methyl)(prop-2-yn-1-yl) sulfonium (544 mg, 1.5 equiv) and Et_3N (111 mg, 1.1 equiv) were added in 8 mL CH_3CN. The reaction mixture was stirred at ambient temperature for 30 min. After the reaction had completed, the residue was purified by silica column (PE: EA =1:1) to afford 194 mg of **5c** in 32% yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47-8.45 (m, *2*H), 7.76 (d, *J* = 7.9 Hz, *2*H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.30 (t, *J* = 7.7 Hz, 2H), 7.11 (d, *J* = 7.4 Hz, 1H), 5.40 (s, 1H), 4.6-4.35(m, 1H), 3.09 (s, 3H), 2.71 – 2.47 (m, 2H), 2.19 (dd, *J* = 13.9, 6.9 Hz, 1H), 2.13 (s, *3*H), 2.09 – 1.92 (m, 1H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ *1*69.9, 167.5, 163.6, 156, 137.6, 132.6, 131.8, 131.6, 130.7, 129.4, 129.1, 127.8, 124.6, 119.9, 80.8, 54.2, 31.2, 30.4, 28.3, 21.2, 15.4. **HRMS** $[M+H]^+$ m/z calcd for $[C_{27}H_{31}BrO_4N_3S]^+$: 570.1348, found: 570.1352.

d) Synthesis of 6a

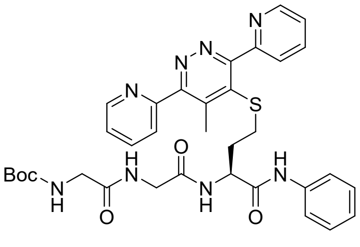


6a

To a 25 mL reaction tube equipped with a stir bar, tert-butyl (S)-(2-((4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)carbamate (38.1 mg,1.0 equiv), propargyl bromide (59.5 mg, 5.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified on silica column (PE: EA =2:1) to afford 41 mg of **6a** in 67 % yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (s, 1H), 8.87 (d, J = 4.4 Hz, 1H), 8.76 (d, J = 4.4 Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.97 (ddd, J = 13.6, 7.2, 4.4 Hz, 2H), 7.79 (d, J = 7.6 Hz, 1H), 7.57 – 7.43 (m, 5H), 7.25 (t, J = 7.6 Hz, 2H), 7.06 (t, J = 7.2 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 5.65 (s, 1H), 4.91 (d, J = 4.8 Hz, 1H), 3.81 (t, J = 4.8 Hz, 2H), 2.90 (dd, J = 13.2, 7.2 Hz, 2H), 2.72 (s, 3H), 1.93 (dt, J = 14.8, 7.2 Hz, 2H), 1.48 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 169.9, 169.4, 160.9, 159.4, 156.0, 155.8, 148.6, 148.6, 141.6, 138.4, 138.0, 137.7, 137.3, 128.9, 125.4, 124.3, 123.9, 119.8, 80.4, 51.5, 44.4, 33.1, 31.5, 28.3, 18.8. **HRMS** $[M+H]^+$ m/z calcd for $[C_{32}H_{35}N_7O_4S]^+$: 614.2544, found: 614.2552.

e) Synthesis of 6b

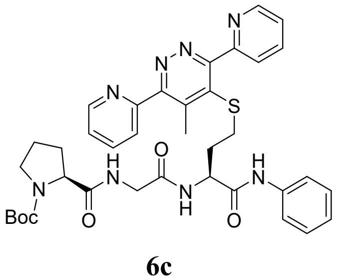


6b

To a 25 mL reaction tube equipped with a stir bar, tert-butyl (S)-(2-((2-((4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamate (43.8 mg,1.0 equiv), propargyl bromide (59.5 mg, 5.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt water. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 42.3 mg of **6b** in 63% yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.88 (s, 1H), 8.81 (s, 2H), 8.19-8.12 (m, 1H), 8. 1-7.95 (m, 5H), 7.70-7.65 (m, 1H), 7.60-7.50 (m, 4H), 7.30 – 7.21 (m, 2H), 7.06 (t, *J* = 7.4 Hz, 1H), 5.54 (s, 1H), 4.56 (s, 1H), 4.1-3.99 (m, 1H), 3.83-3.70 (m, 3H), 2.76 (d, *J* = 6.3 Hz, 2H), 2.68 (s, 3H), 2.20-2.0(m, 1H),1.32 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 171.6, 169.8, 169.3, 158.7, 156.6, 154.3, 153.2, 147.8, 146.7, 142.5, 140.3, 138.9, 138.8, 137.8, 128.84, 128.79, 126.5, 126.0, 125.4, 124.8, 124.4, 120.1, 80.7, 52.4, 44.7, 43.2, 32.2, 31.7, 28.3, 18.6. **HRMS** $[M+H]^+$ m/z calcd for $[C_{34}H_{38}N_8O_5S]^+$: 671.2759, found: 671.2658.

f) Synthesis of 6c

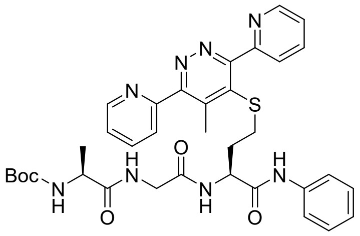


6c

To a 25 mL reaction tube equipped with a stir bar, tert-butyl (S)-2-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate(47.8 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt water. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =2:1) to afford 46.2 mg of **6c** in 65 % yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12-8.95 (m 2H), 8.94-8.88 (m, 1H), 8.73 (s, 1H), 8.35-8.15 (m, 4H), 7.92 – 7.69 (m, 5H), 7.55-7.40 (m, 3H), 7.28 (d, *J* = 6.6 Hz, 1H), 4.71 (t, *J* = 10.4 Hz, 1H), 4.60-4.41 (m, *1*H), 4.40 (s, 1H), 3.75-3.61 (m, 3H), 3.0-2.8 (m, *5*H), 2.70-2.55(m, 1H), 2.54-2.40 (m, 1H), 2.37-2.24 (m, 2H), 2.21-2.05 (m, 2H), 1.38 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 174.9, 169.9, 168.9, 159.3, 158.8, 155.3, 154.9, 154.3, 148., 147.7, 141.9, 140.1, 138.9, 138.2, 138.0, 128.6, 125.95,125.90, 124.9, 124.4, 124.0, 119.9, 80.4, 60.4, 52.3, 47.5, 43.5, 32.4, 31.5, 30.2, 28.2 24.8, 18.7. **HRMS** $[M+H]^+$ m/z calcd for $[C_{37}H_{42}N_8O_5S]^+$: 711.2999, found: 711.2998.

g) Synthesis of 6d

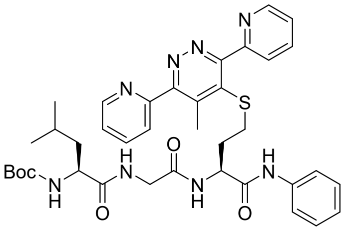


6d

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-1-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate (45.2 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt water. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 42.5 mg of **6d** in 62 % yield, as a brown solid.

^{1}H NMR ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.82 – 8.72 (m, 2H), 8.70 (d, *J* = 4.4 Hz, 1H), 7.99 – 7.87 (m, 4H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.45 (dd, *J* = 10.8, 6.2 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 2H), 7.00 (t, *J* = 7.2 Hz, 1H), 5.07 (d, *J* = 3.2 Hz, 1H), 4.47 (d, *J* = 7.6 Hz, 1H), 4.07 (d, *J* = 5.2 Hz, 1H), 3.63 (dd, *J* = 17.2, 4.4 Hz, 1H), 3.47 (q, *J* = 7.2 Hz, 1H), 2.66 (d, *J* = 7.2 Hz, 2H), 2.63 (s, 3H), 2.27 (s, 2H), 1.36 (d, *J* = 6.8 Hz, 3H), 1.15 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, CDCl$_3$) *δ* 174.8, 169.8, 169.0, 160.7, 159.1, 155.8, 149.0, 148.4, 141.3, 139.2, 138.0, 137.5, 137.3, 128.7, 125.2, 124.1, 119.8, 80.5, 52.3, 51.0, 43.3, 31.8, 31.8, 28.1, 18.8, 17.6. **HRMS** [M+H]$^+$ m/z calcd for [$C_{35}H_{40}N_8O_5S$]$^+$: 685.2815, found: 685.2820.

h) Synthesis of 6e



6e

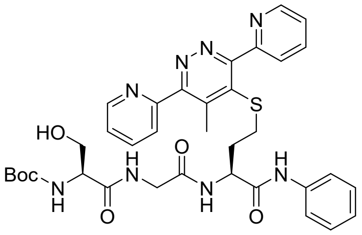
To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-4-methyl-1-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-1-oxopentan-2-yl)carbamate (49.4 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH$_3$CN: H$_2$O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et$_3$N (11.1 mg, 1.1 equiv) in 8 mL CH$_3$CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =2:1) to afford 30.5 mg of **6e** in 42 % yield, as a brown solid.

^{1}H NMR (400 MHz, CDCl$_3$) *δ* 8.78 (s, 1H), 8.71 (d, *J* = 4.8 Hz, 1H), 8.67 (s, 1H), 8.59 (s, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.96 – 7.85 (m, 4H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.44 (dd, *J* = 11.6, 6.8 Hz, 3H), 7.37 – 7.31 (m, 1H), 7.22 (d, *J* = 7.2 Hz, 2H), 7.00 (t, *J* = 7.2 Hz, 1H), 4.99 (d, *J* = 4.0 Hz, 1H), 4.47

(d, *J* = 8.0 Hz, 1H), 4.10 – 4.00 (m, 2H), 3.68 (dd, *J* = 16.4, 4.0 Hz, 1H), 2.64 (s, 2H), 2.04 (s, 3H), 1.78 (d, *J* = 4.0 Hz, 1H), 1.68 (dt, *J* = 19.6, 6.4 Hz, 1H), 1.37 (dd, *J* = 17.2, 10.0 Hz, 1H), 1.27 – 1.24 (m, 2H), 1.10 (s, 9H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.7, 169.8, 168.8, 160.6, 159.0, 156.1, 149.1, 148.5, 138.0, 137.4, 137.1, 128.7, 125.7, 125.2, 124.2, 123.9, 119.8, 80.5, 53.9, 52.4, 43.4, 40.6, 31.8, 31.8, 28.1, 23.0, 21.9, 18.8.

HRMS $[M+H]^+$ m/z calcd for $[C_{38}H_{46}N_8O_5S]^+$: 727.3312, found: 727.3306.

i) Synthesis of 6f



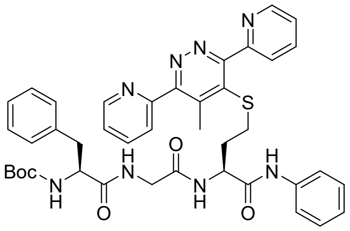
6f

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-3-hydroxy-1-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate (46.8 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:2) to afford 26.6 mg of **6f** in 38 % yield, as a brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J = 6.8 Hz, 2H), 7.29 (t, J = 6.8 Hz, 2H), 7.21 (d, J = 6.8 Hz, 1H), 4.60 (s, 2H), 4.23 (t, 2H), 4.04 (s, 1H), 3.10 (t, 2H), 2.50 (s, 3H), 2.06 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.2, 170.4, 169.6, 160.8, 160.4, 157.8, 157.1, 155.9, 152.4, 150.9, 146.9, 145.0, 143.3, 142.7, 140.7, 137.4, 137.2, 130.2, 128.9, 127.7, 126.7, 126.4, 125.6, 124.8, 122.1, 120.4, 80.6, 63.1, 56.2, 52.6, 43.4, 32.3, 31.5, 28.2, 18.5. **HRMS** $[M+H]^+$ m/z calcd for $[C_{35}H_{40}N_8O_6S]^+$:

701.2864, found: 701.2865.

j) Synthesis of 6g

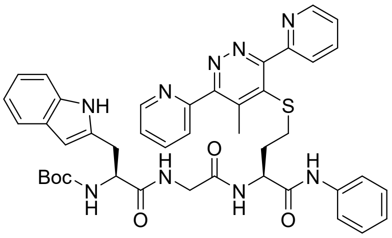


6g

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-1-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (52.8 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 45.6 mg of **6g** in 60 % yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 – 8.60 (m, 2H), 8.54 (s, 1H), 8.46 (s, 1H), 7.97 – 7.73 (m, 5H), 7.49 (d, J = 7.6 Hz, 2H), 7.44 – 7.30 (m, 4H), 7.20 (dd, J = 16.4, 8.8 Hz, 4H), 6.99 (t, J = 7.2 Hz, 1H), 4.96 (s, 1H), 4.43 (d, J = 8.4 Hz, 1H), 4.25 (s, 2H), 3.57 (d, J = 13.2 Hz, 1H), 3.45 (q, J = 6.8 Hz, 2H), 3.12 (dd, J = 13.6, 6.4 Hz, 1H), 2.88 (dd, J = 13.2, 8.8 Hz, 1H), 2.59 (s, 3H), 2.04 (s, 2H), 1.04 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 173.6, 169.8, 168.7, 160.7, 159.0, 156.1, 155.8, 149.1, 148.5, 141.5, 138.9, 137.9, 137.3, 135.9, 129.3, 128.8, 127.2, 125.6, 125.1, 123.9, 119.9, 80.6, 65.9, 56.4, 52.5, 43.4, 37.6, 31.9, 28.1, 18.8, 15.3. **HRMS** $[M+H]^+$ m/z calcd for $[C_{41}H_{44}N_8O_5S]^+$: 761.3155, found: 761.3150.

k) Synthesis of 6h

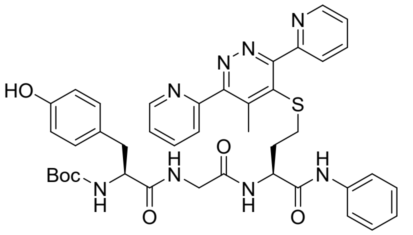


6h

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-3-(1H-indol-2-yl)-1-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate (56.7 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 52 mg of **6h** in 60 % yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 – 8.71 (m, 3H), 8.67 (d, J = 4.4 Hz, 1H), 8.34 (s, 2H), 7.88 – 7.77 (m, 5H), 7.65 – 7.50 (m, 4H), 7.40 (dd, J = 8.8, 4.2 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 7.04 (dt, J = 20.8, 7.6 Hz, 3H), 5.13 (s, 1H), 4.46 (s, 1H), 4.31 (d, J = 6.4 Hz, 1H), 4.11 (dd, J = 16.8, 6.4 Hz, 1H), 3.48 (t, J = 7.2 Hz, 2H), 3.19 (d, J = 6.8 Hz, 1H), 3.15 – 3.02 (m, 2H), 2.64 (s, 2H), 2.58 (s, 3H), 2.24 (s, 2H), 1.12 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 172.8, 169.7, 162.7, 162.3, 161.1, 159.5, 155.7, 155.6, 150.0, 148.9, 148.8, 141.8, 138.0, 137.5, 137.3, 128.8, 125.2, 125.1, 124.5, 124.3, 124.2, 124.1, 120.0, 118.2, 115.3, 80.2, 63.2, 52.6, 43.4, 31.9, 31.6, 29.7, 28.3, 18.6. **HRMS** $[M+H]^+$ m/z calcd for $[C_{43}H_{45}N_9O_5S]^+$: 800.3264, found: 800.3272.

l) Synthesis of 6i



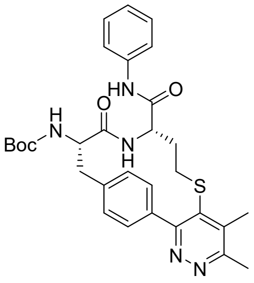
6i

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-3-(4-hydroxyphenyl)-1-((2-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate (54.4 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HCOOH (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. The above obtained propargyl sulfonium-peptide was then mixed with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 2 (63 mg, 1.5 equiv) and Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 24.8 mg of **6i** in 32 % yield, as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.75 (t, J = 6.2 Hz, 1H), 8.68 (d, J = 4.4 Hz, 2H), 8.52 (d, J = 3.6 Hz, 1H), 8.31 (s, 1H), 7.86 (t, J = 7.2 Hz, 5H), 7.53 (d, J = 8.0 Hz, 2H), 7.43 – 7.34 (m, 4H), 7.21 (d, J = 7.8 Hz, 2H), 7.02 (d, J = 7.2 Hz, 1H), 6.90 (d, J = 8.0 Hz, 2H), 6.62 (d, J = 7.6 Hz, 2H), 5.15 (s, 1H), 4.48 (s, 1H), 4.13– 4.02 (m, 2H), 3.66 – 3.53 (m, 1H), 3.52 – 3.43 (m, 1H), 3.09 (q, J = 7.2 Hz, 1H), 2.92 (dd, J = 13.6, 7.2 Hz, 1H), 2.79 (dd, J = 13.6, 7.2 Hz, 1H), 2.60 (s, 3H), 2.26 (s, 1H), 1.75 (s, 1H), 1.11 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.8, 169.8, 169.1, 160.8, 159.0, 155.9, 149.7, 149.1, 148.5, 141.7, 137.9, 137.6, 130.2, 128.8, 126.7, 125.6, 125.1, 124.2, 121.9, 119.9, 115.8, 80.6, 65.9, 56.8, 52.5, 43.3, 31.8, 29.7, 28.1, 18.8.

HRMS $[M+H]^+$ m/z calcd for $[C_{41}H_{44}N_8O_6S]^+$: 777.3105, found: 777.3107.

m) Synthesis of 7a

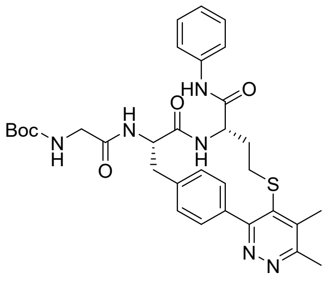


7a

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)carbamate (56.5 mg, 1.0 equiv), propargyl bromide (59.5 mg, 5.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt water. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =2:1) to afford 30.9 mg of **7a** in 50 % yield, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.55 (t, *J* = 7.6 Hz, 3H), 7.41 (d, *J* = 7.2 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 3H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.21 (d, *J* = 7.6 Hz, 1H), 5.39 (d, *J* = 6.0 Hz, 1H), 4.30 (s, 1H), 4.21 – 4.11 (m, 1H), 3.49 (s, 1H), 3.32 (dd, *J* = 12.0, 5.6 Hz, 1H), 2.97 – 2.82 (m, 2H), 2.70 (s, 3H), 2.46 (d, *J* = 7.6 Hz, 3H), 2.09 (d, *J* = 11.2 Hz, 1H), 1.58 (d, *J* = 9.2 Hz, 1H), 1.39 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 171.8, 168.1, 159.6, 158.3, 155.6, 139.0, 137.6, 136.9, 136.5, 130.2, 129.9, 128.7, 128.5, 124.5, 120.4, 80.7, 58.2, 52.5, 50.9, 38.5, 29.9, 29.7, 28.2, 28.1, 21.2, 16.9. **HRMS** $[M+H]^+$ m/z calcd for $[C_{30}H_{35}N_5O_4S]^+$: 562.2283, found: 562.2291.

n) Synthesis of 7b

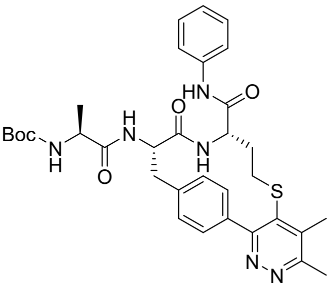


7b

To a 25 mL reaction tube equipped with a stir bar, tert-butyl (2-(((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)carbamate (62.2 mg, 1.0 equiv), propargyl bromide (59.5 mg, 5.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt water. The extract was combined, dried over sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =2:1) to afford 31.5 mg of **7b** in 51 % yield, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.03 (s, 1H), 8.05 (s, 1H), 7.79 (d, *J* = 7.2 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.59 – 7.51 (m, 3H), 7.26 (t, *J* = 8.0 Hz, 3H), 7.07 (t, *J* = 7.2 Hz, 1H), 6.75 (s, 1H), 5.68 (s, 1H), 4.69 (s, 1H), 4.54 (s, 1H), 4.07 (d, *J* = 13.6 Hz, 1H), 3.95 (d, *J* = 15.6 Hz, 1H), 3.47 (s, 1H), 2.96 (dd, *J* = 21.2, 9.2 Hz, 1H), 2.73 (s, 3H), 2.48 (s, 3H), 2.07 (d, *J* = 12.8 Hz, 2H), 1.76 (s, 2H), 1.47 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* 170.7, 170.3, 169.2, 168.0, 159.6, 156.3, 147.5, 139.3, 137.6, 136.6, 130.2, 130.0, 129.2, 128.9, 124.6, 120.3, 80.4, 52.5, 44.1, 44.0, 39.6, 30.8, 28.4, 27.4, 21.3, 17.0. **HRMS** $[M+H]^+$ m/z calcd for $[C_{32}H_{38}N_6O_5S]^+$: 619.2697, found: 619.2694.

o) Synthesis of 7c

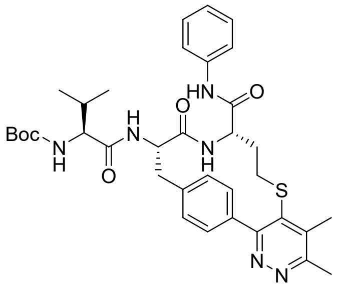


7c

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-1-(((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (63.6 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 12 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 30 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 26.0 mg of **7c** in 40 % yield, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.45 (d, J = 7.2 Hz, 2H), 7.73 (s, 1H), 7.59 (d, J = 7.6 Hz, 3H), 7.40 (d, J = 7.2 Hz, 2H), 7.30 (t, J = 6.8 Hz, 3H), 7.10 (t, J = 7.2 Hz, 1H), 5.48 (s, 1H), 4.87 (s, 2H), 4.34 (d, J = 6.8 Hz, 1H), 3.22 (d, J = 6.0 Hz, 2H), 3.09 (s, 3H), 2.84 (s, 3H), 2.44 (s, 2H), 2.15 (s, 1H), 2.06 (s, 3H), 1.99 (d, J = 7.8 Hz, 1H), 1.40 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 172.8, 171.1, 168.4, 159.7, 158.1, 155.6, 139.1, 138.0, 136.8, 136.4, 130.3, 129.9, 129.7, 128.7, 128.6, 124.3, 120.2, 80.7, 59.6, 57.6, 52.7, 47.2, 37.8, 29.8, 29.6, 29.3, 28.4, 24.6, 22.7, 21.1, 16.8. **HRMS** $[M+H]^+$ m/z calcd for $[C_{33}H_{40}N_6O_5S]^+$: 633.2854, found: 633.2845.

p) Synthesis of 7d

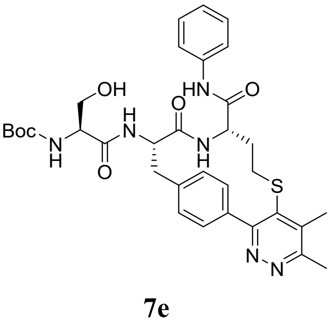


7d

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-3-methyl-1-(((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxobutan-2-yl)carbamate (66.4 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 15 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 17.8 mg of **7d** in 27 % yield, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.41 (d, J = 7.6 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 7.6 Hz, 2H), 7.34 (s, 1H), 7.28 (s, 1H), 7.07 (t, J = 7.2 Hz, 1H), 5.34 (s, 1H), 4.90 (s, 2H), 4.12 (s, 1H), 3.20 (d, J = 5.6 Hz, 2H), 3.06 (s, 3H), 2.41 (s, 2H), 2.12 (dd, J = 13.6, 6.4 Hz, 1H), 2.02 (s, 3H), 1.99 – 1.91 (m, 2H), 1.45 (s, 3H), 1.38 (s, 9H), 0.80 (t, J = 7.2 Hz, 6H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 171.5, 171.1, 169.0, 167.2, 163.7, 156.5, 141.2, 137.8, 130.8, 130.2, 128.9, 128.2, 124.4, 120.0, 80.6, 60.2, 54.3, 53.0, 38.6, 31.2, 30.1, 28.4, 21.2, 19.2, 17.9 15.4. **HRMS** $[M+H]^+$ m/z calcd for $[C_{35}H_{44}N_6O_5S]^+$: 661.3163, found: 661.3153.

q) Synthesis of 7e

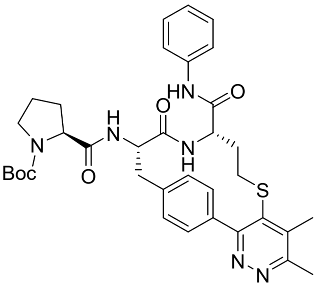


7e

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-3-hydroxy-1-(((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (65.2 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 18 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 18.1 mg of **7e** in 28 % yield, as a white solid.

^{1}H NMR (400 MHz, DMSO) δ 9.87 (s, 1H), 8.45 (d, *J* = 7.6 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 2H), 8.25 (d, *J* = 7.2 Hz, 1H), 7.94 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.69 (d, *J* = 7.6 Hz, 1H), 4.63 – 4.54 (m, 1H), 4.41 (dd, *J* = 12.4, 9.2 Hz, 1H), 4.03 (d, *J* = 6.8 Hz, 1H), 3.49 (d, *J* = 5.6 Hz, 2H), 3.14 – 3.02 (m, 2H), 2.88 (s, 3H), 2.72 (s, 3H), 2.21 (t, *J* = 7.2 Hz, 2H), 1.93 (s, 4H), 1.85 – 1.70 (m, 1H), 1.35 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, DMSO-*d_6*) *δ* 176.0, 175.9, 175.1, 172.2, 168.4, 167.5, 160.4, 156.4, 147.5, 143.9, 135.6, 135.2, 133.9, 132.4, 128.7, 126.0, 124.6, 83.5, 67.3, 62.0, 59.5, 57.8, 41.0, 36.0, 34.7, 33.3, 26.0, 19.7. **HRMS** [M+H]$^+$ m/z calcd for [$C_{33}H_{40}N_6O_6S$]$^+$: 649.2803, found: 649.2794.

r) Synthesis of 7f



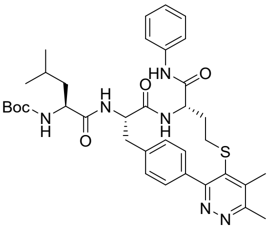
7f

To a 25 mL reaction tube equipped with a stir bar, tert-butyl (S)-2-(((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (66.2 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 18 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 27 mg of **7f** in 41 %

yield, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.61 (dd, J = 22.4, 12.0 Hz, 4H), 7.41 (s, 1H), 7.07 (t, J = 7.2 Hz, 1H), 6.42 (s, 1H), 4.36 (s, 2H), 4.25 (d, J = 7.2 Hz, 1H), 3.44 (d, J = 33.2 Hz, 2H), 3.30 (dd, J = 11.8, 5.2 Hz, 1H), 2.94 (d, J = 11.2 Hz, 1H), 2.73 (s, 3H), 2.47 (s, 3H), 2.16 (s, 5H), 1.86 (dd, J = 17.6, 8.4 Hz, 4H), 1.51 (s, 9H), 1.29 (s, 2H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 172.8, 171.1, 168.4, 159.7, 158.1, 139.1, 138.0, 136.8, 136.4, 130.3, 129.9, 129.7, 128.7, 128.6, 124.3, 120.2, 80.7, 59.6, 57.6, 52.7, 47.2, 37.8, 29.8, 29.3, 28.4, 24.6, 22.7, 21.1, 16.8. **HRMS** $[M+H]^+$ m/z calcd for $[C_{35}H_{42}N_6O_5S]^+$: 659.3010, found: 659.3007.

s) Synthesis of 7g

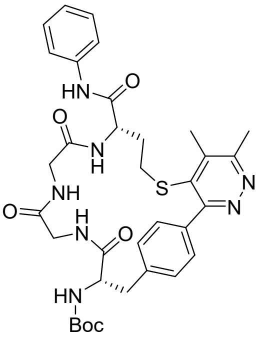


7g

To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((S)-4-methyl-1-(((S)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1-(((S)-4-(methylthio)-1-oxo-1-(phenylamino)butan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopentan-2-yl)carbamate (67.8 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 18 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:1) to afford 37.7 mg of **7g** in 55 % yield, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.12 (s, 1H), 7.88 – 7.49 (m, 6H), 7.28 (d, J = 7.6 Hz, 2H), 7.18 – 7.05 (m, 1H), 6.63 (s, 1H), 5.60 (s, 1H), 4.82 (d, J = 22.8 Hz, 1H), 4.48 (s, 1H), 4.36 (t, J = 6.8 Hz, 1H), 3.54 (s, 1H), 3.08 (dd, J = 25.6, 14.0 Hz, 1H), 2.77 (s, 3H), 2.50 (s, 3H), 2.18 (s, 1H), 2.00 (d, J = 12.0 Hz, 1H), 1.49 (s, 9H), 1.03 (q, J = 7.6 Hz, 2H), 0.95 – 0.91 (m, 2H), 0.82 (d, J = 6.0 Hz, 6H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 172.5, 170.5, 168.1, 167.8, 159.3, 158.0, 156.0, 139.1, 137.7, 137.1, 136.5, 132.3, 131.0, 130.4, 130.0, 129.1, 128.9, 124.5, 120.0, 80.1, 65.6, 56.1, 52.9, 52.0, 43.3, 40.2, 32.0, 31.4, 30.6, 29.8, 29.4, 28.6, 27.4, 24.6, 22.74 22.2, 21.0, 19.2, 17.0, 14.2, 13.8. **HRMS** $[M+H]^+$ m/z calcd for $[C_{36}H_{46}N_6O_5S]^+$: 675.3323, found: 675.3327.

t) Synthesis of 7i

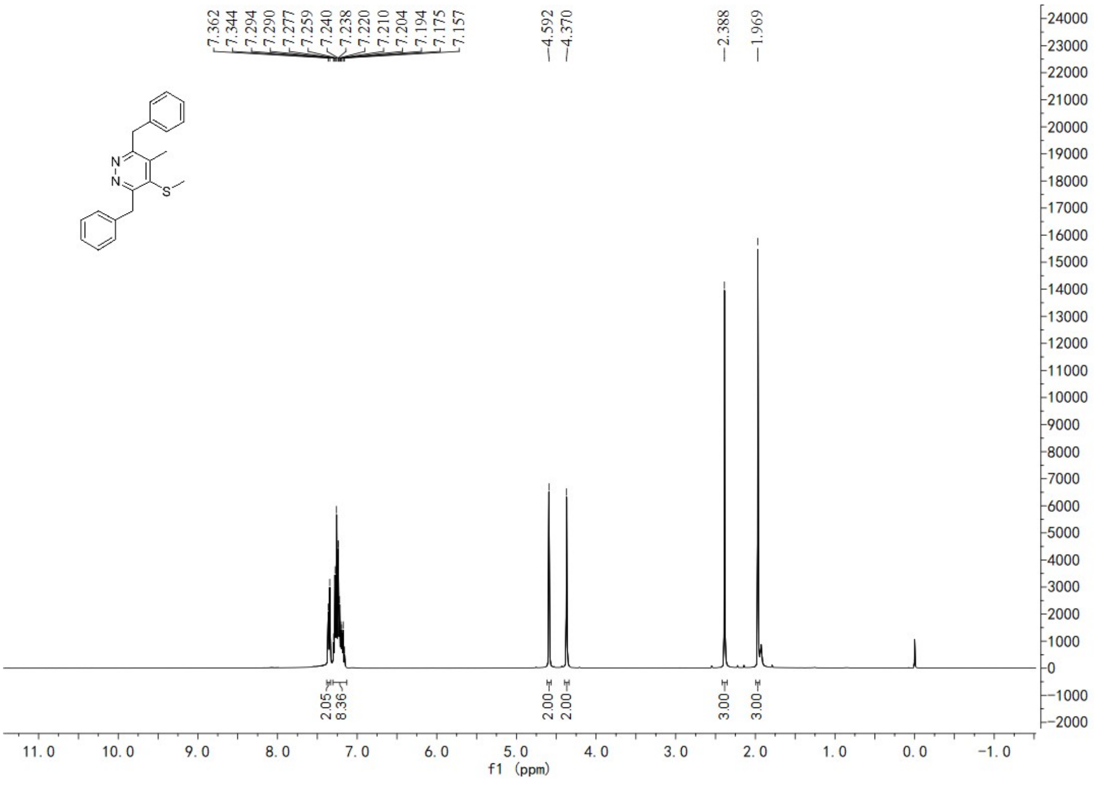


7i

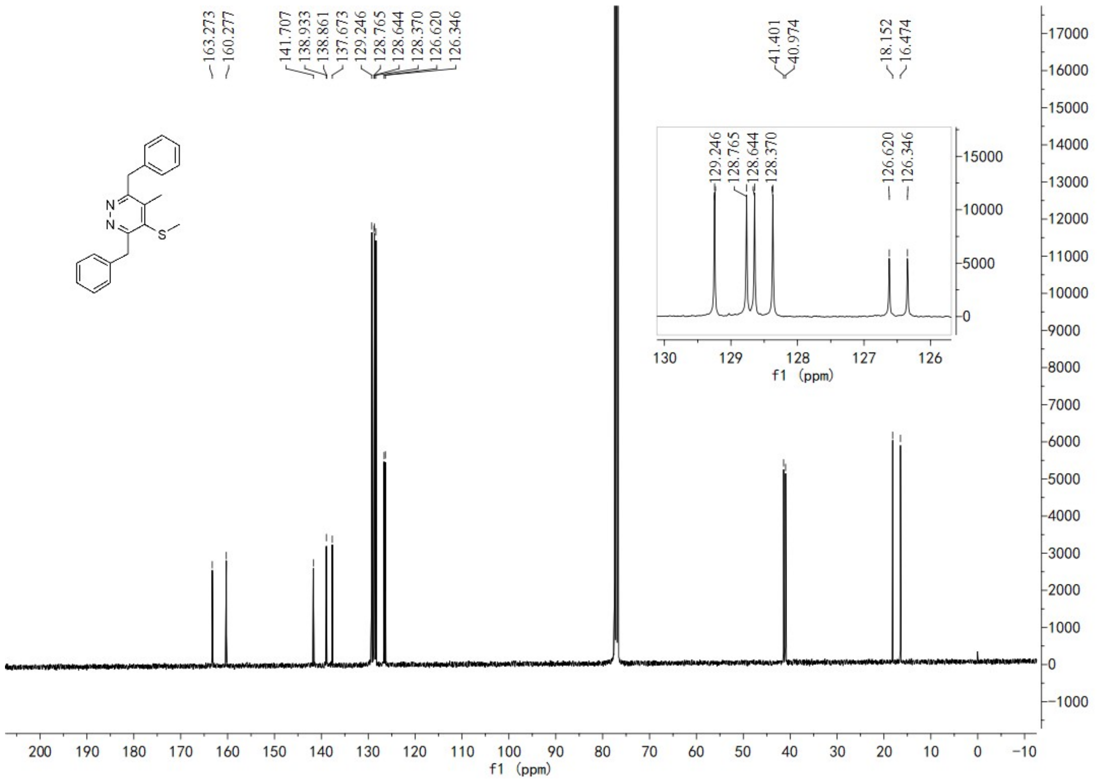
To a 25 mL reaction tube equipped with a stir bar, tert-butyl ((5S,14S)-15-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-7,10,13-trioxo-5-(phenylcarbamoyl)-2-thia-6,9,12-triazapentadecan-14-yl)carbamate (67.9 mg, 1.0 equiv), propargyl bromide (95.2 mg, 8.0 equiv), HOTf (1%) were added in 8 mL CH_3CN: H_2O (1:1). Under argon, the reaction was stirred at room temperature for 18 h. Upon completion, lyophilized to dryness to give the propargyl sulfonium-peptide as a white or brown solid. To a 25 mL reaction tube equipped with a stir bar, the above obtained propargyl sulfonium-peptide was then mixed with Et_3N (11.1 mg, 1.1 equiv) in 8 mL CH_3CN. The reaction was stirred at room temperature for 60 min. Upon completion, the reaction mixture was extracted with ethyl acetate before washing with saturated salt solution. The extract was combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude reaction mixture was purified by silica column (PE: EA =1:2) to afford 10.1 mg of **7i** in 15 % yield, as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.45 (d, J = 7.6 Hz, 3H), 8.25 (d, J = 7.4 Hz, 2H), 7.73 (t, J = 10.8 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.39 (t, J = 7.6 Hz, 2H), 7.15 (dd, J = 13.2, 6.8 Hz, 2H), 4.61 (dd, J = 13.2, 8.4 Hz, 1H), 4.41 (d, J = 12.4 Hz, 1H), 3.89 (s, 4H), 3.26 (d, J = 5.2 Hz, 1H), 3.08 (s, 3H), 2.64 (t, J = 6.4 Hz, 1H), 2.60 (s, 2H), 2.54 (dd, J = 13.2, 7.2 Hz, 1H), 2.15 (s, 3H), 1.37 (s, 9H). **^{13}C{^{1}H} NMR** (100 MHz, DMSO-d_6) δ 172.5, 170.8, 170.5, 169.9, 169.4, 167.5, 163.8, 155.8, 143.7, 139.2, 132.0, 130.8, 130.4, 129.2, 127.6, 124.0, 119.9, 78.7, 65.5, 60.2, 55.9, 53.2, 49.1, 42.8, 42.6, 37.8, 32.1, 30.5, 30.1, 28.6, 21.3, 21.2, 19.1, 15.1, 14.6, 14.0. **HRMS** $[M+H]^+$ m/z calcd for $[C_{34}H_{41}N_7O_6S]^+$: 676.2912, found: 676.2911.

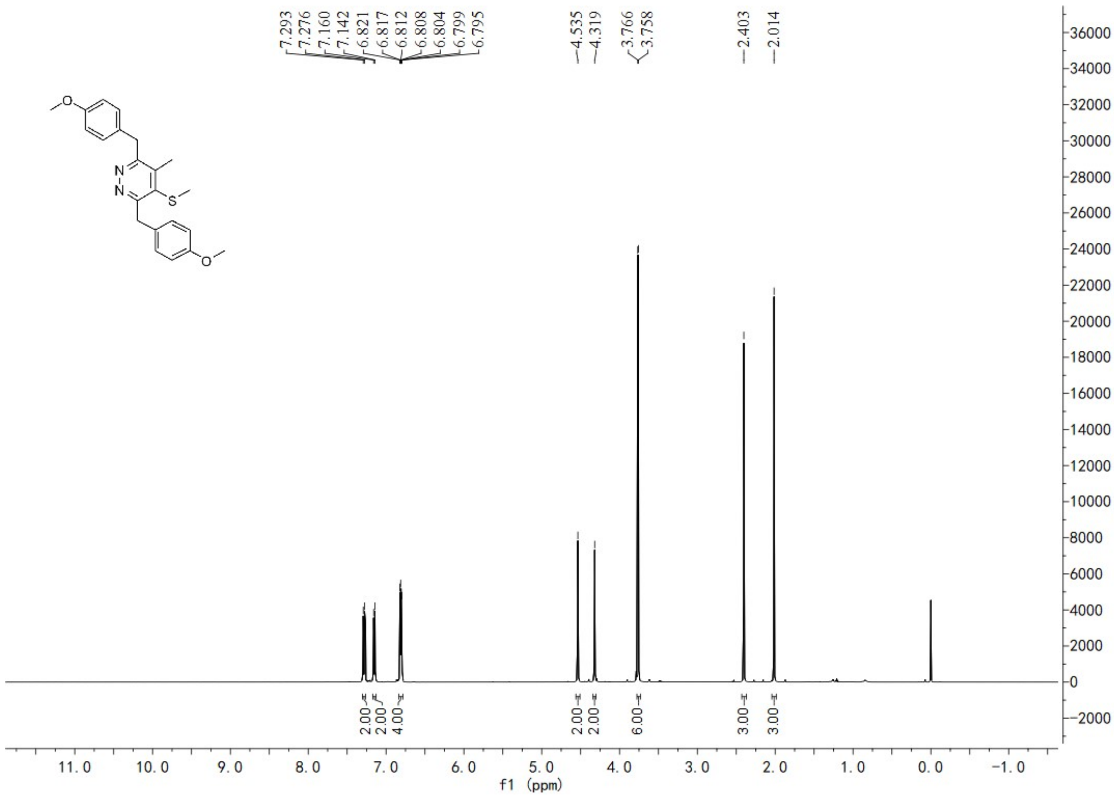
6. ^1H and $^{13}C\{^1H\}$ NMR spectra of compounds **3**, **4**, **5**, **6**, **7**



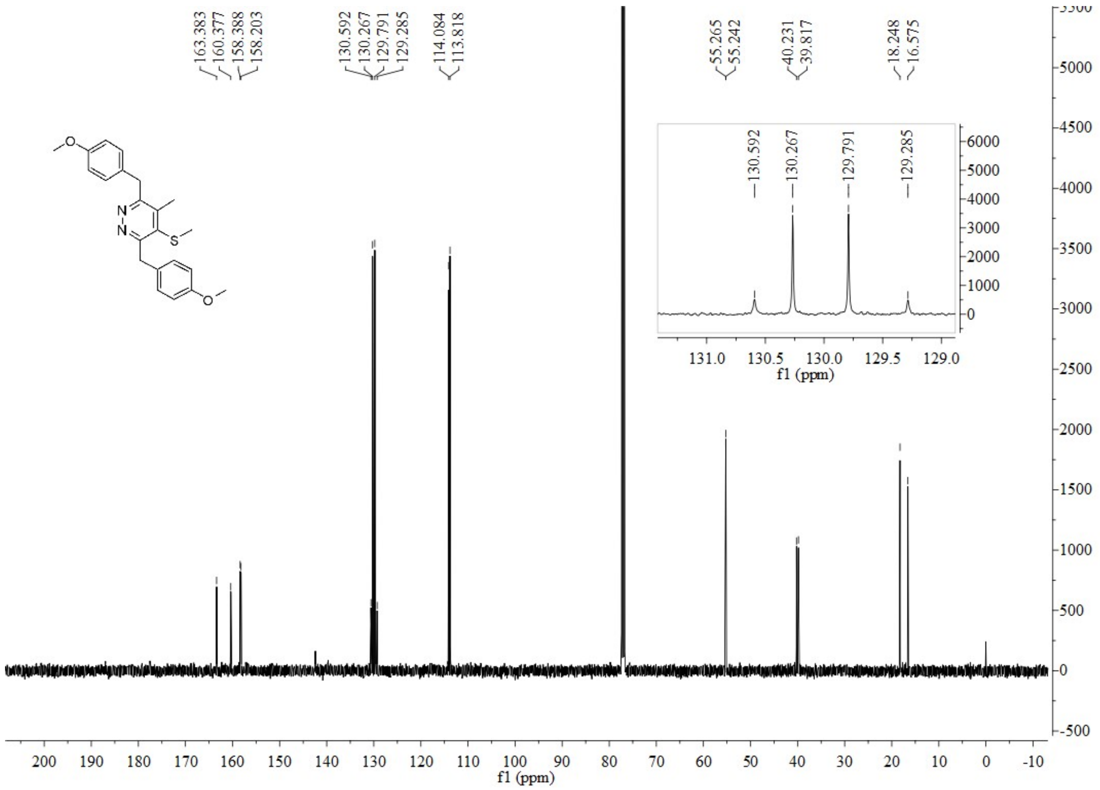
^1H NMR (400 MHz, $CDCl_3$) spectrum of compound **3a**



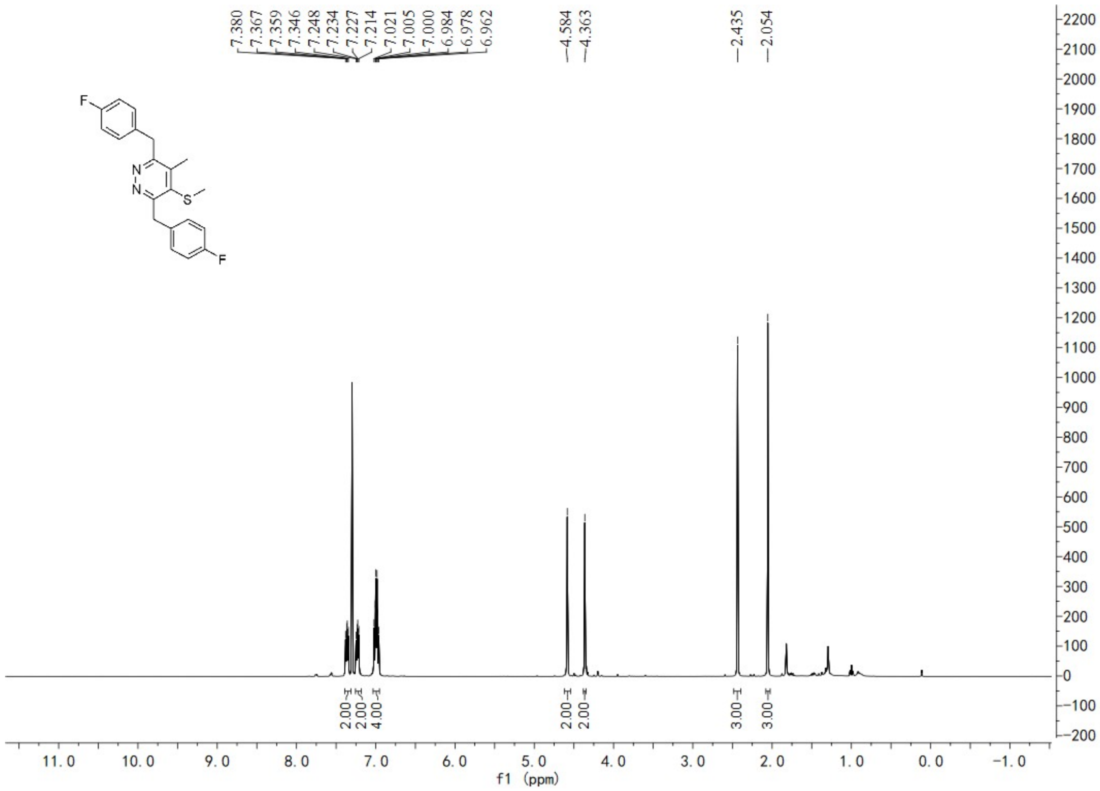
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3a**



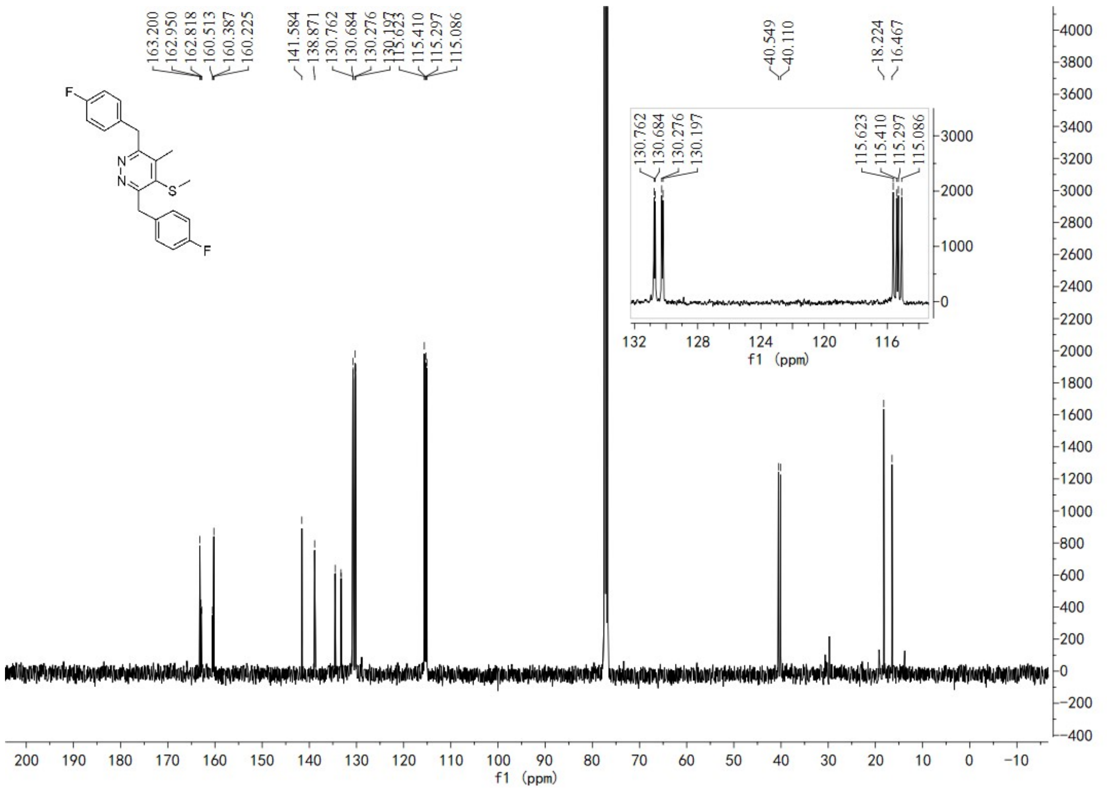
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3b**



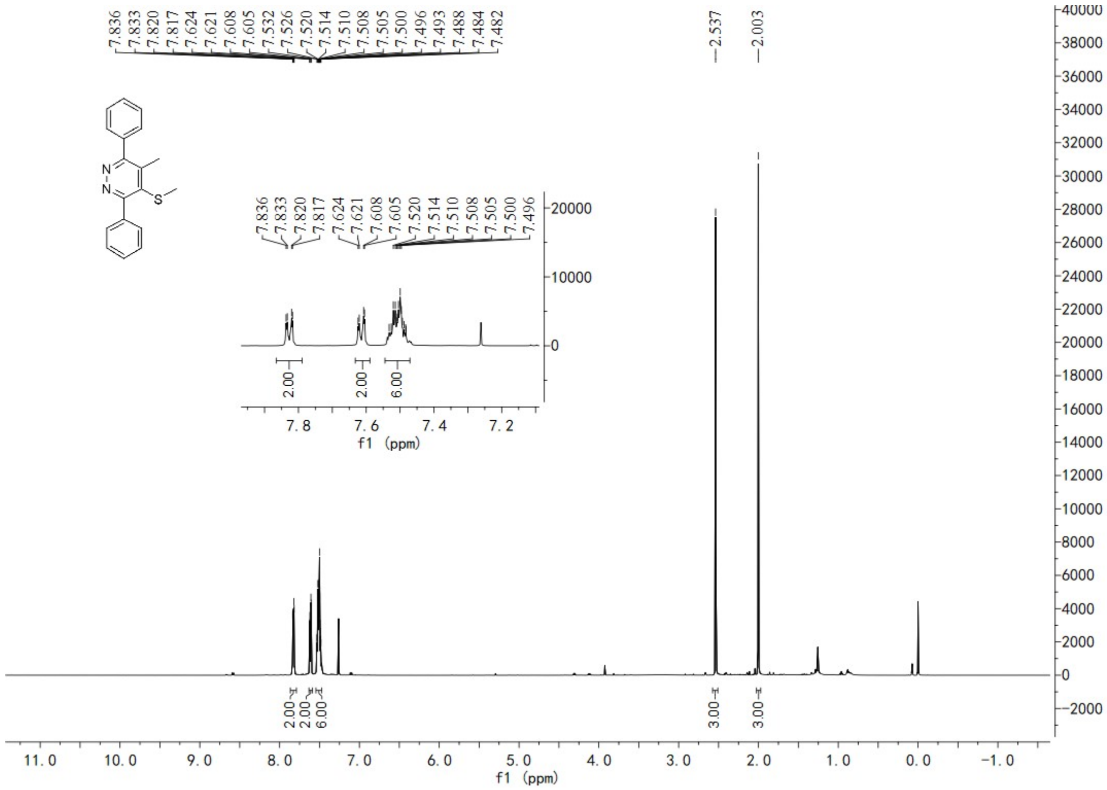
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3b**



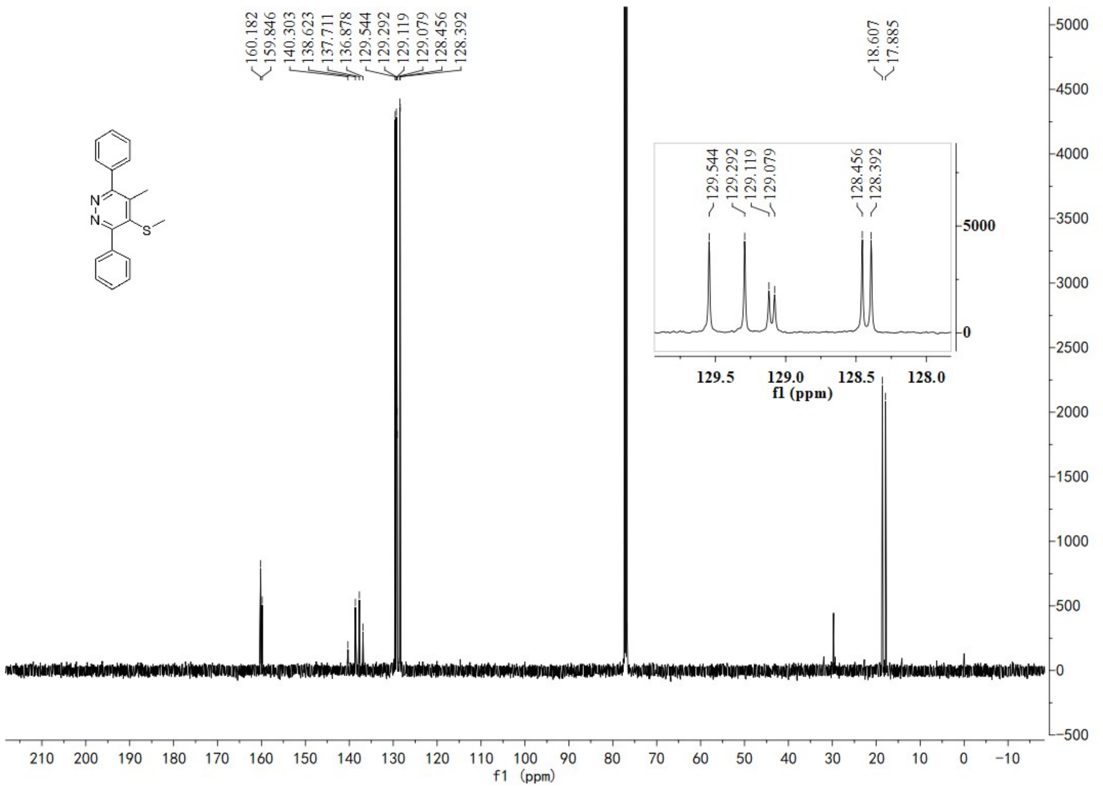
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3c**



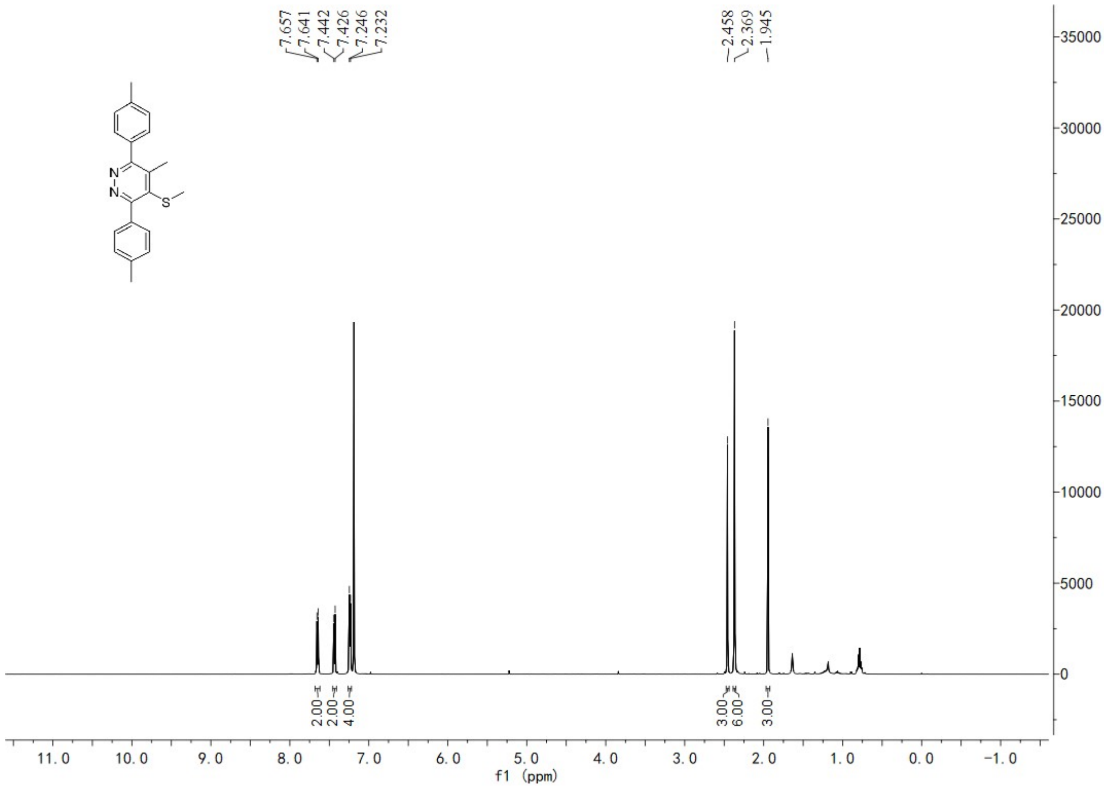
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3c**



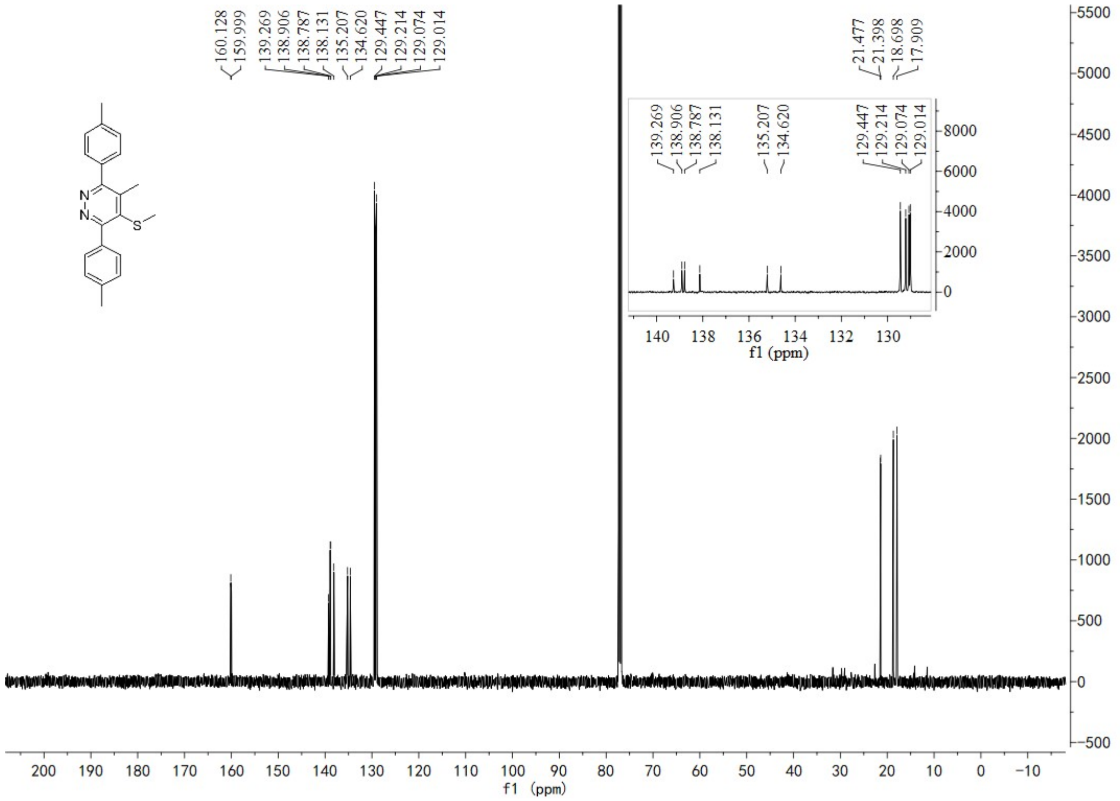
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3d**



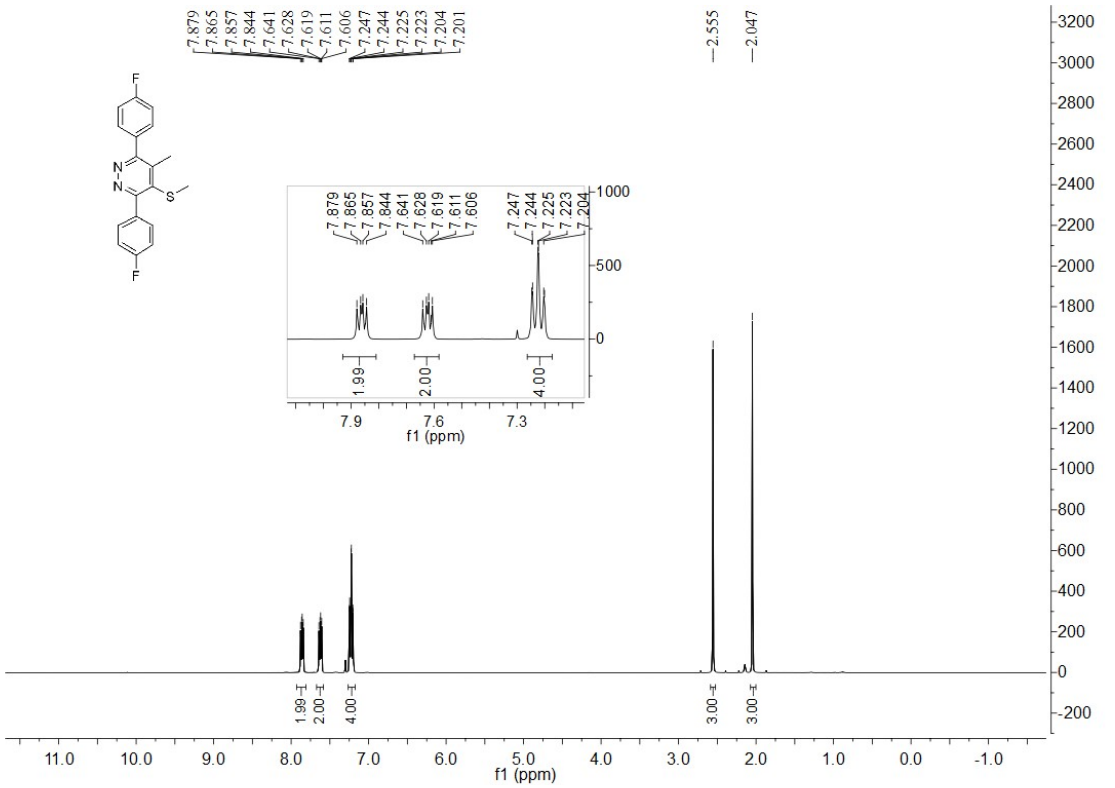
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3d**



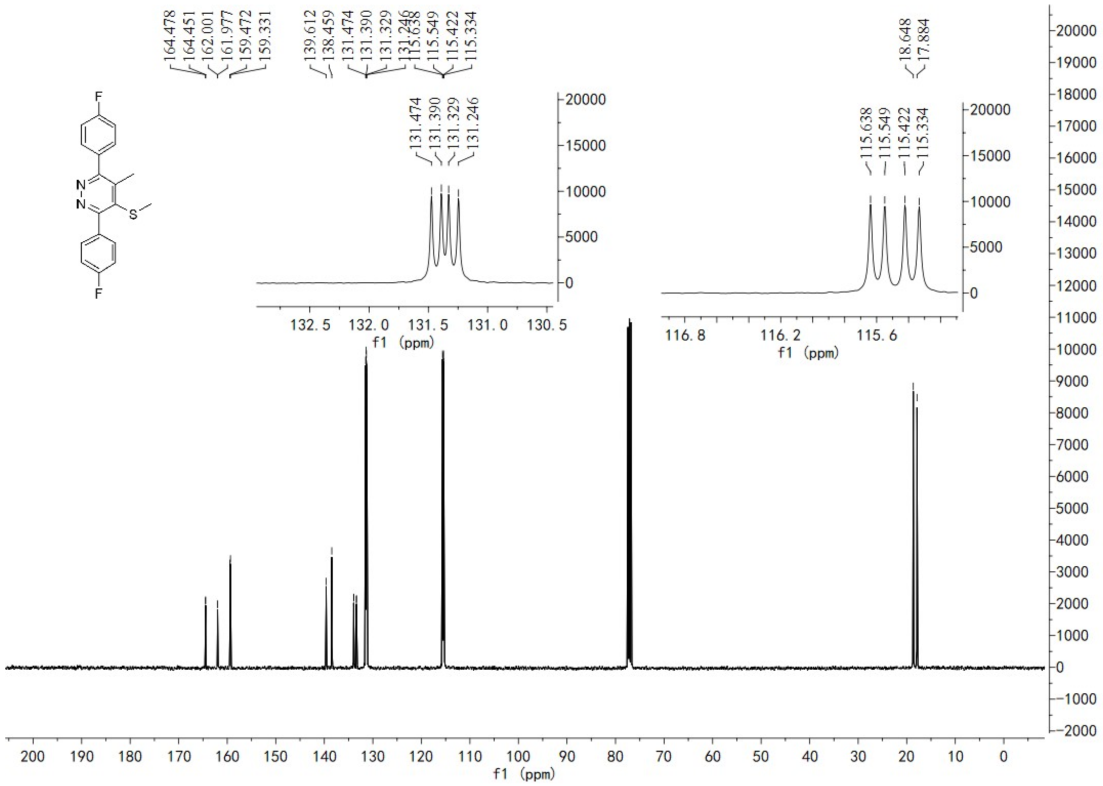
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3e**



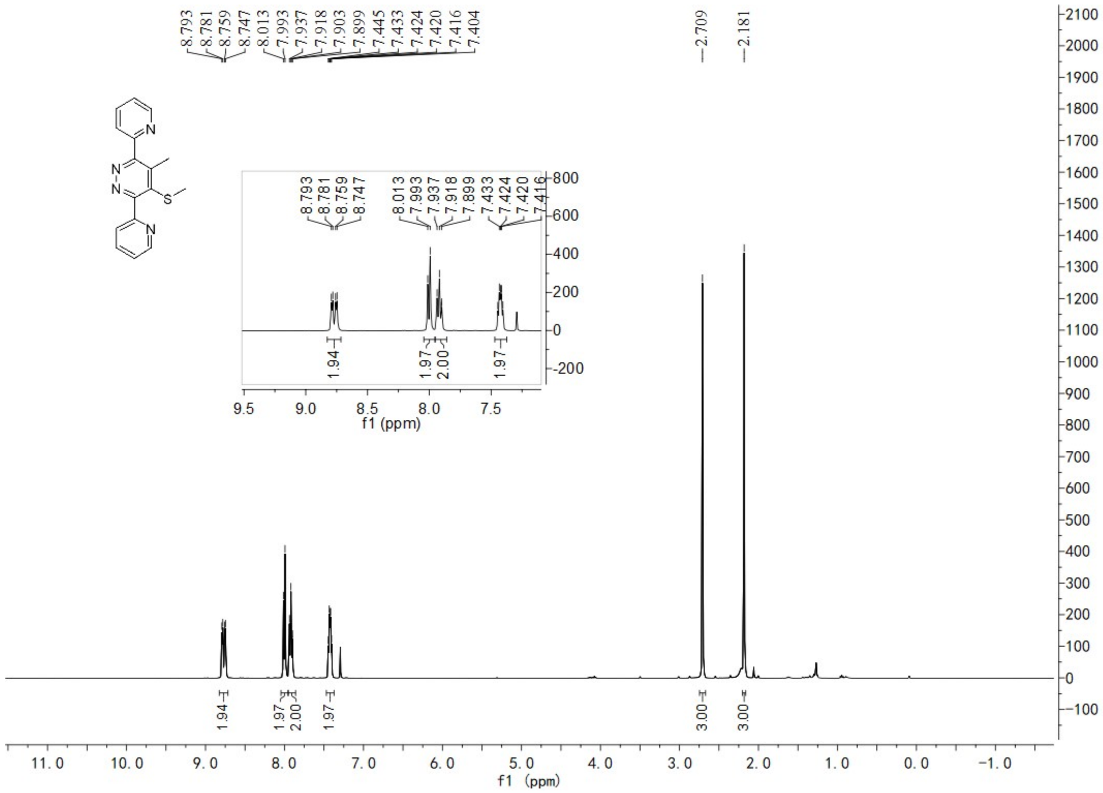
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3e**



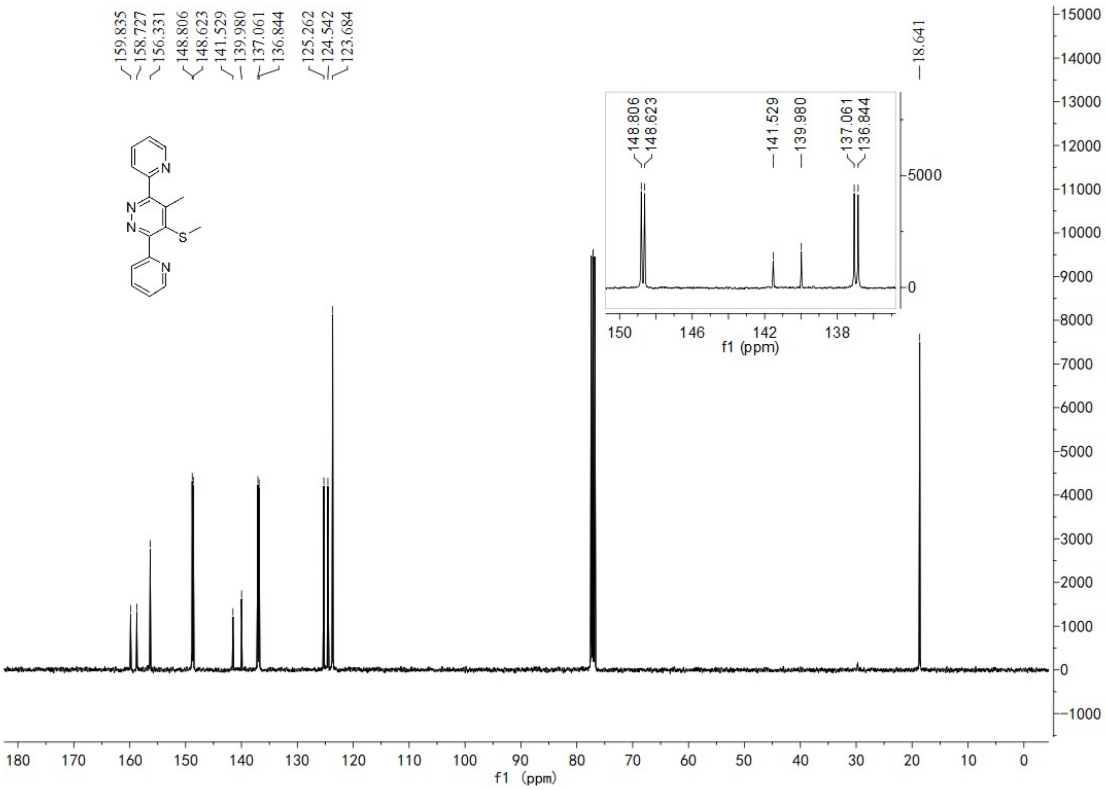
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3f**



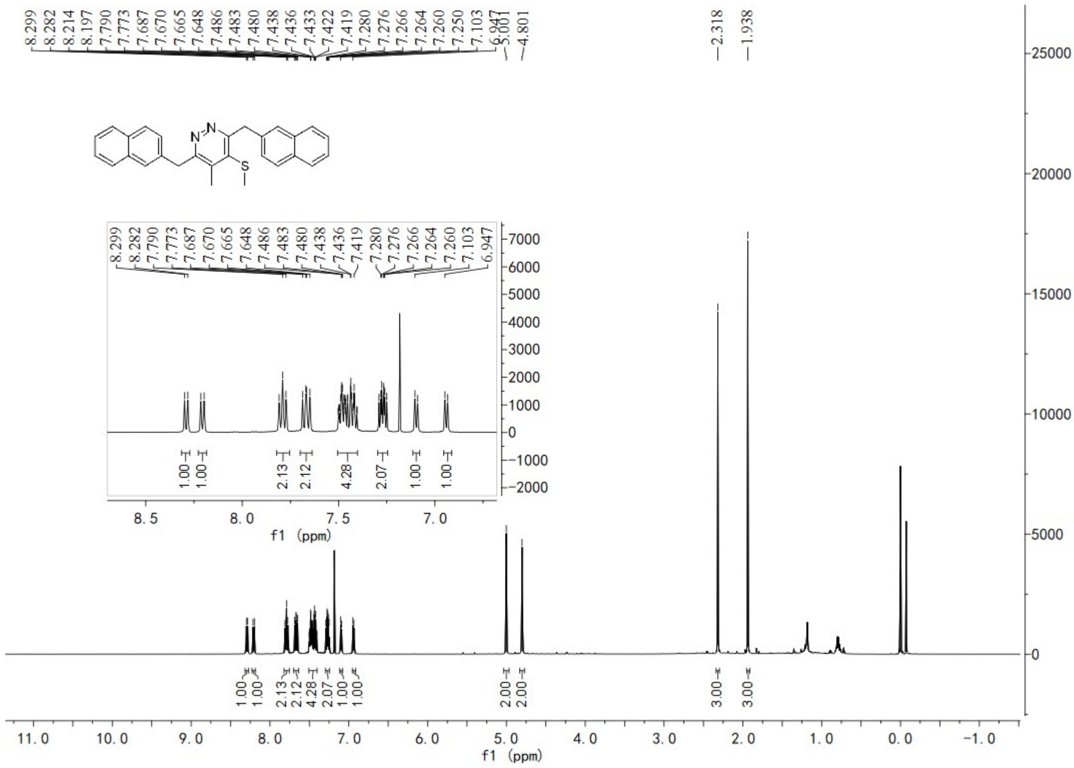
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3f**



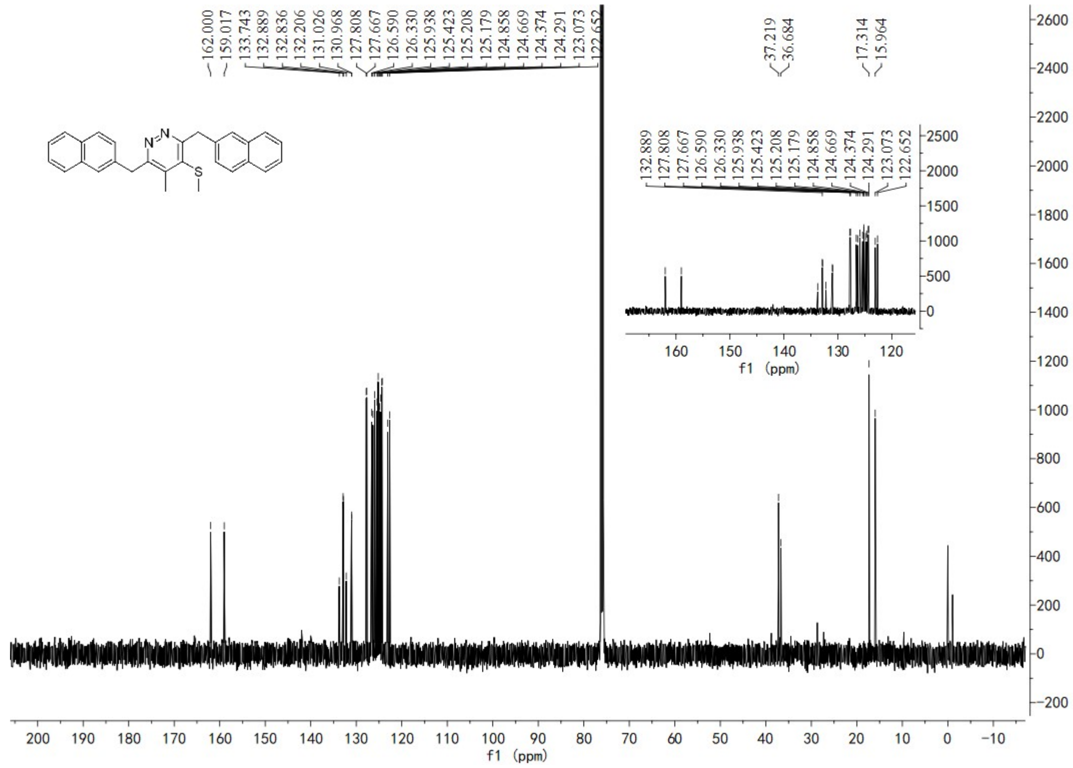
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3g**



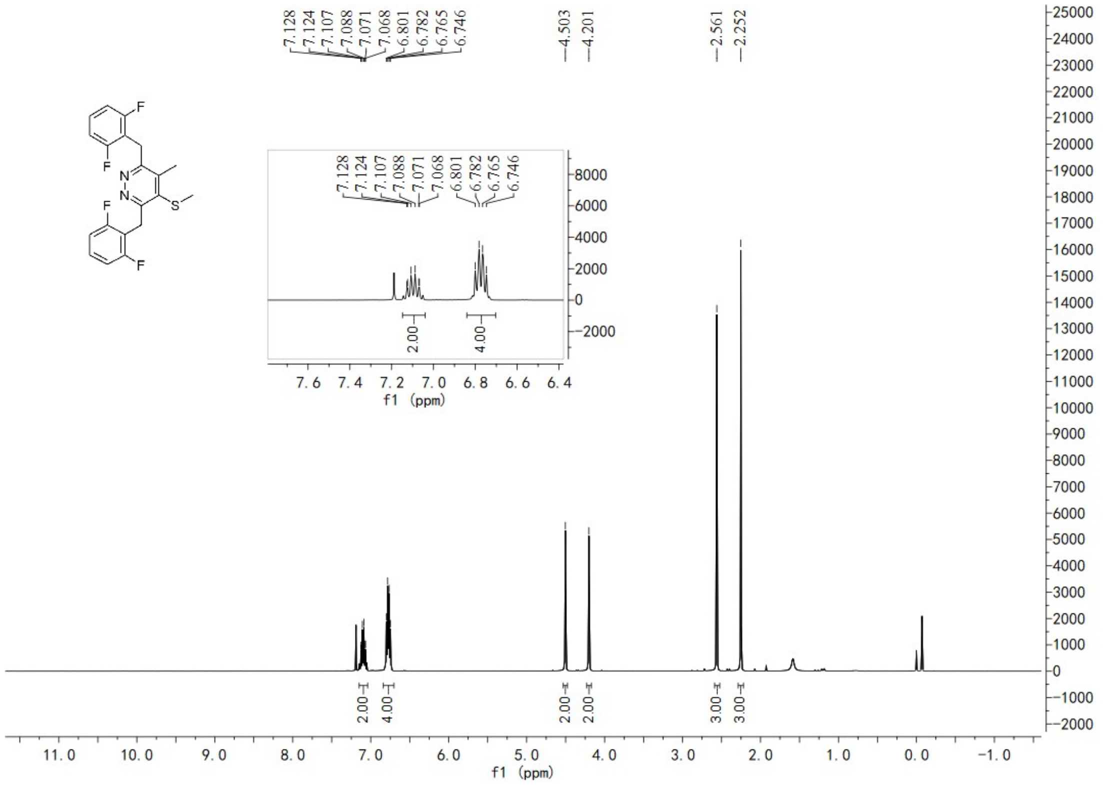
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3g**



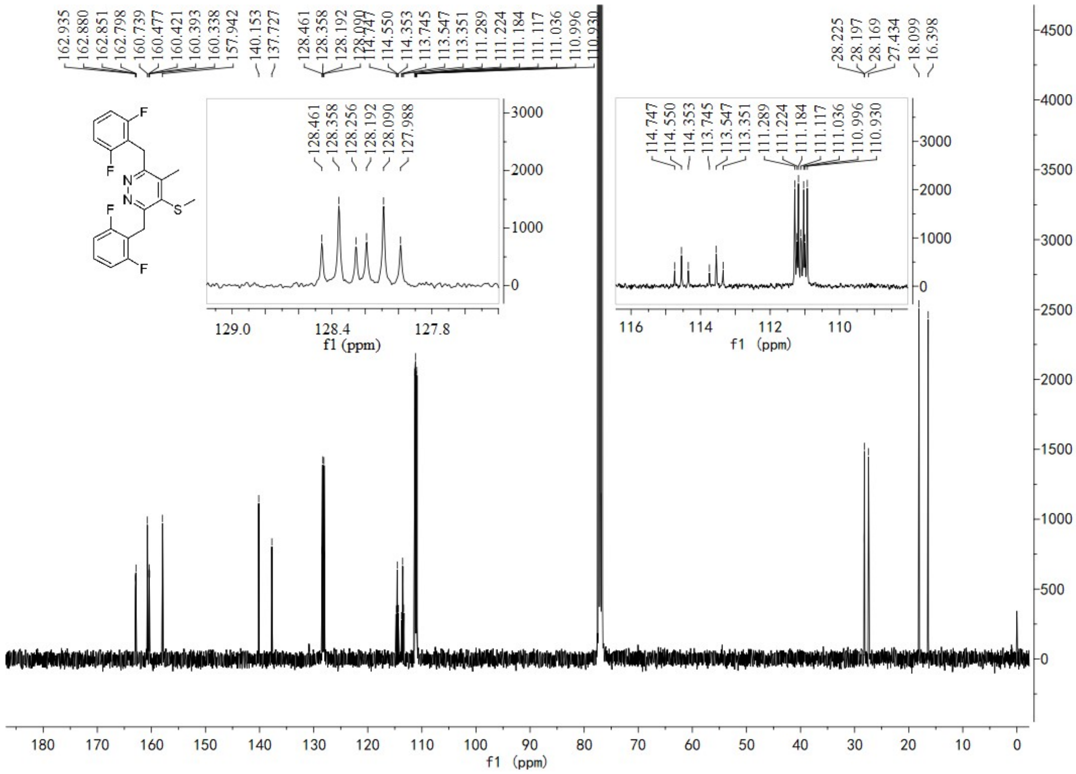
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3h**



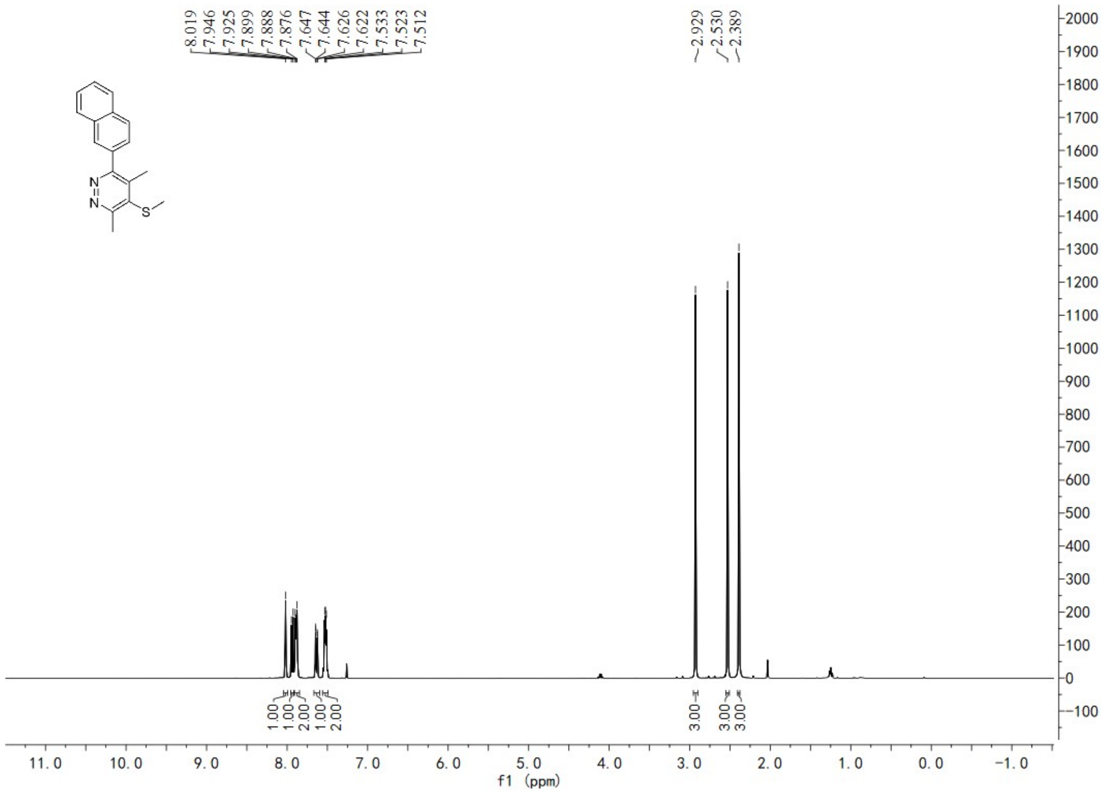
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3h**



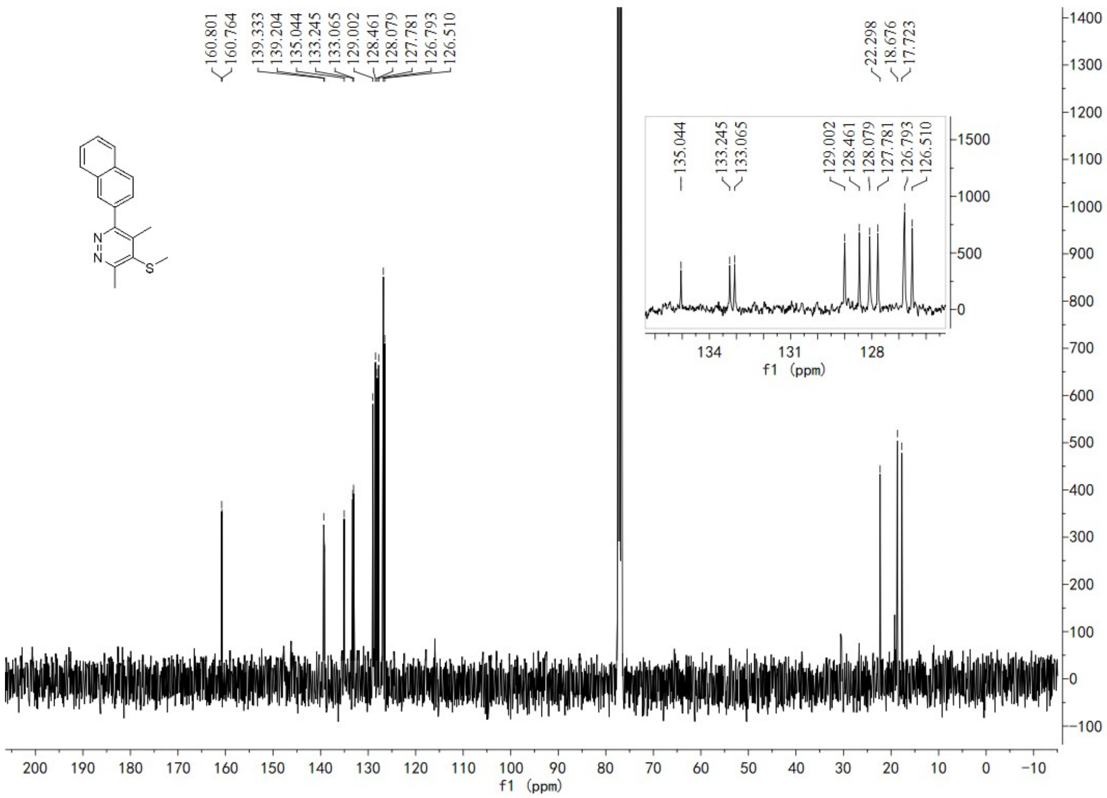
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3i**



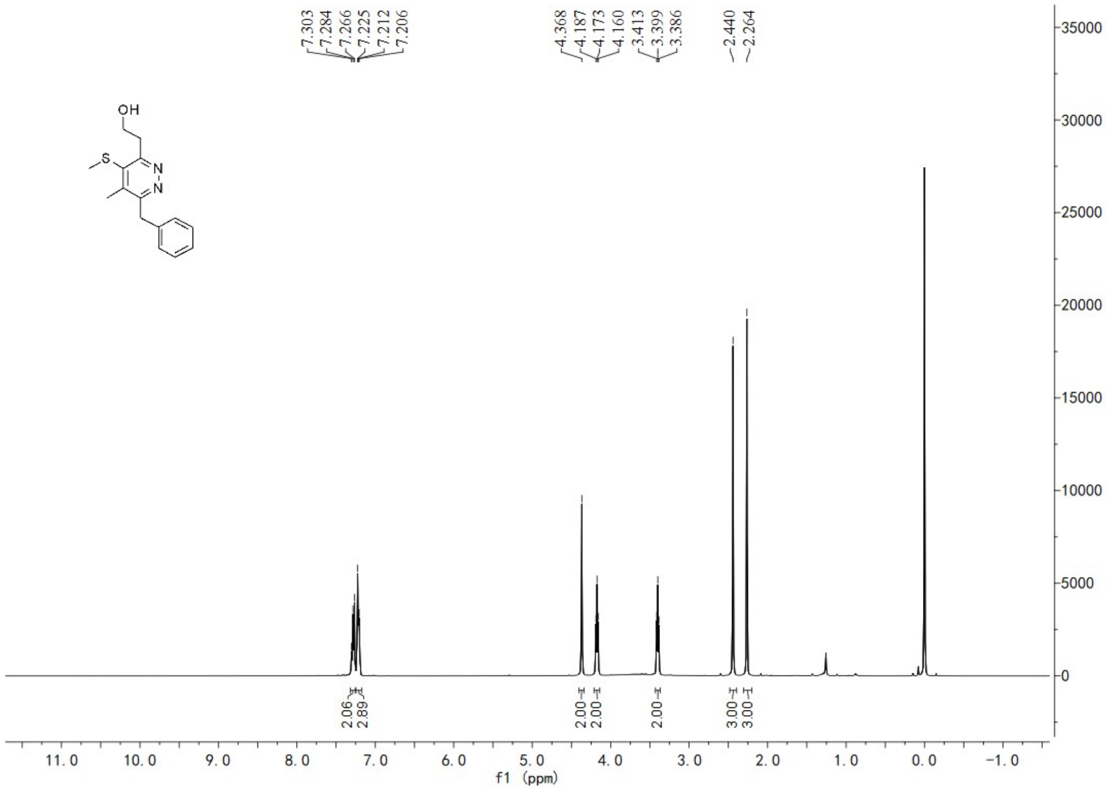
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3i**



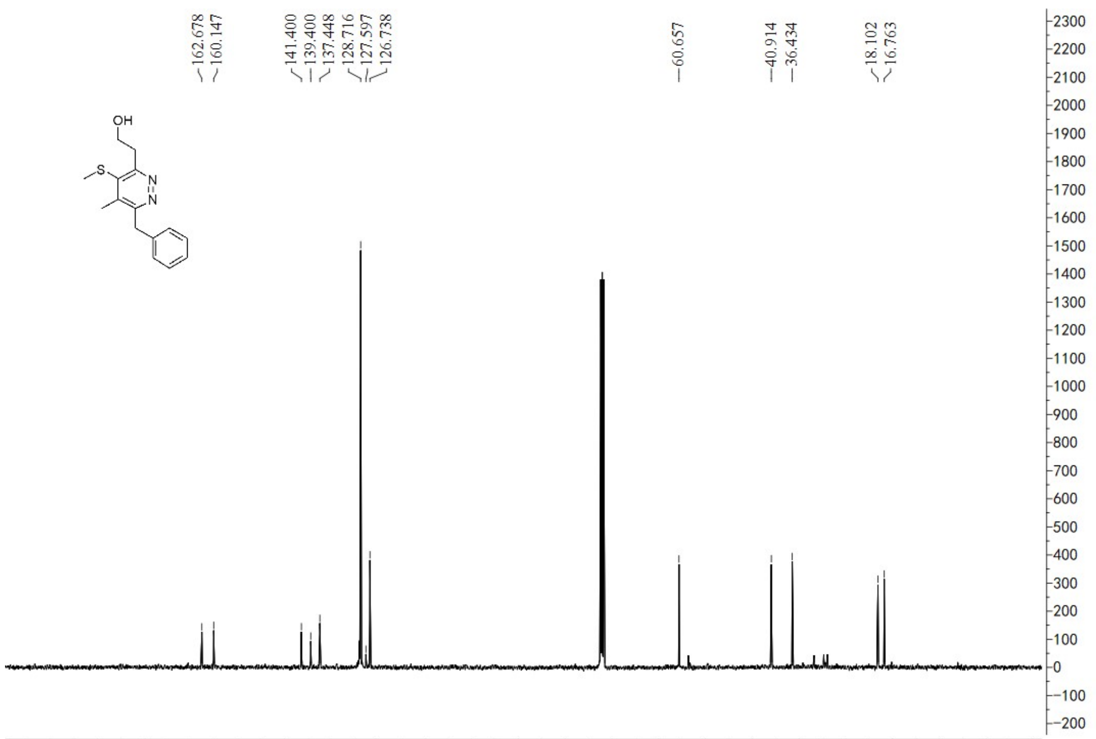
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3j**



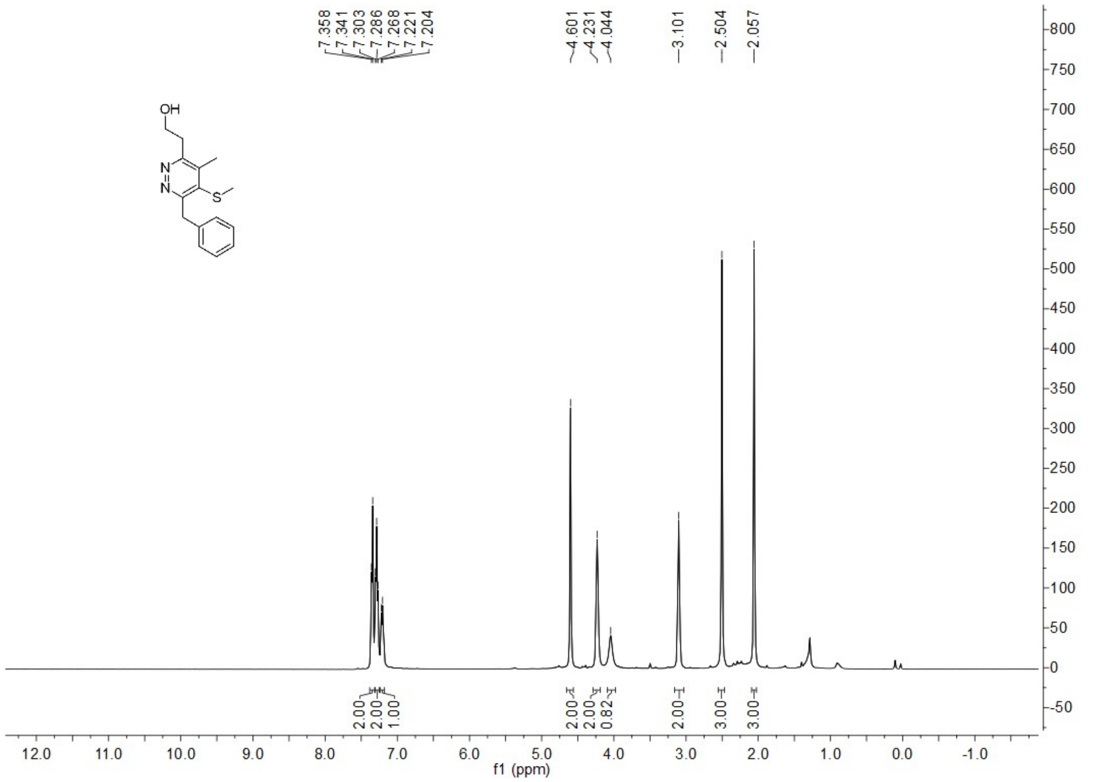
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3j**



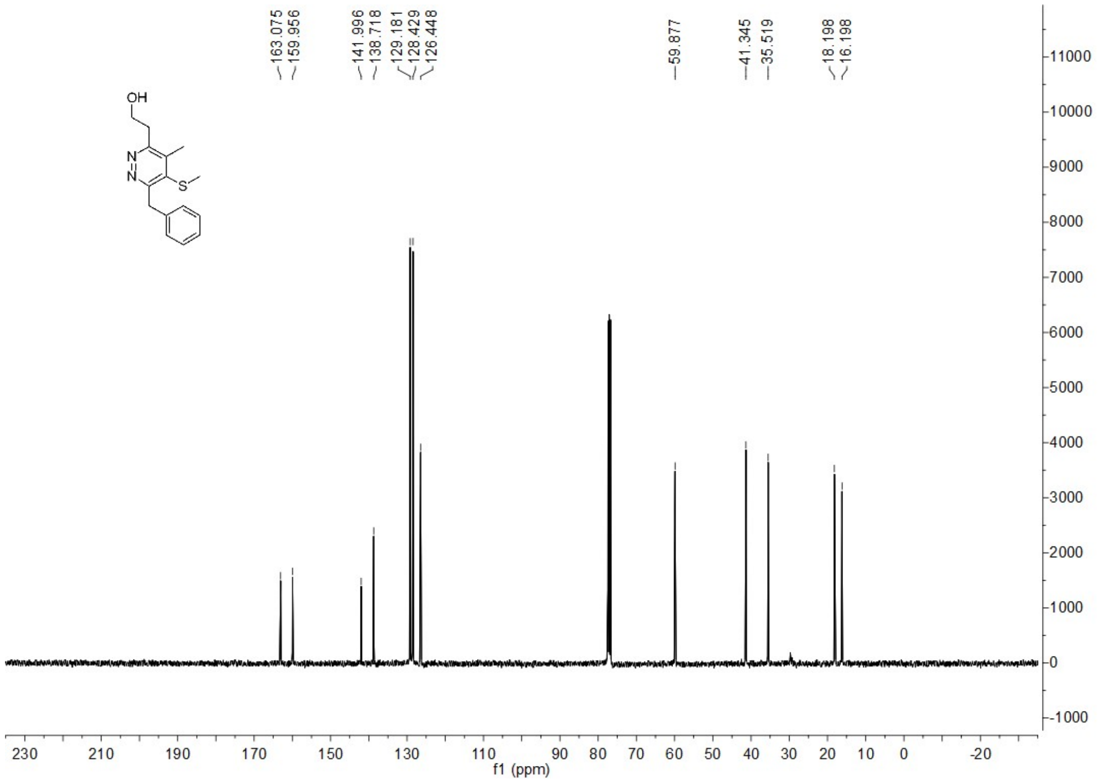
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3l1**



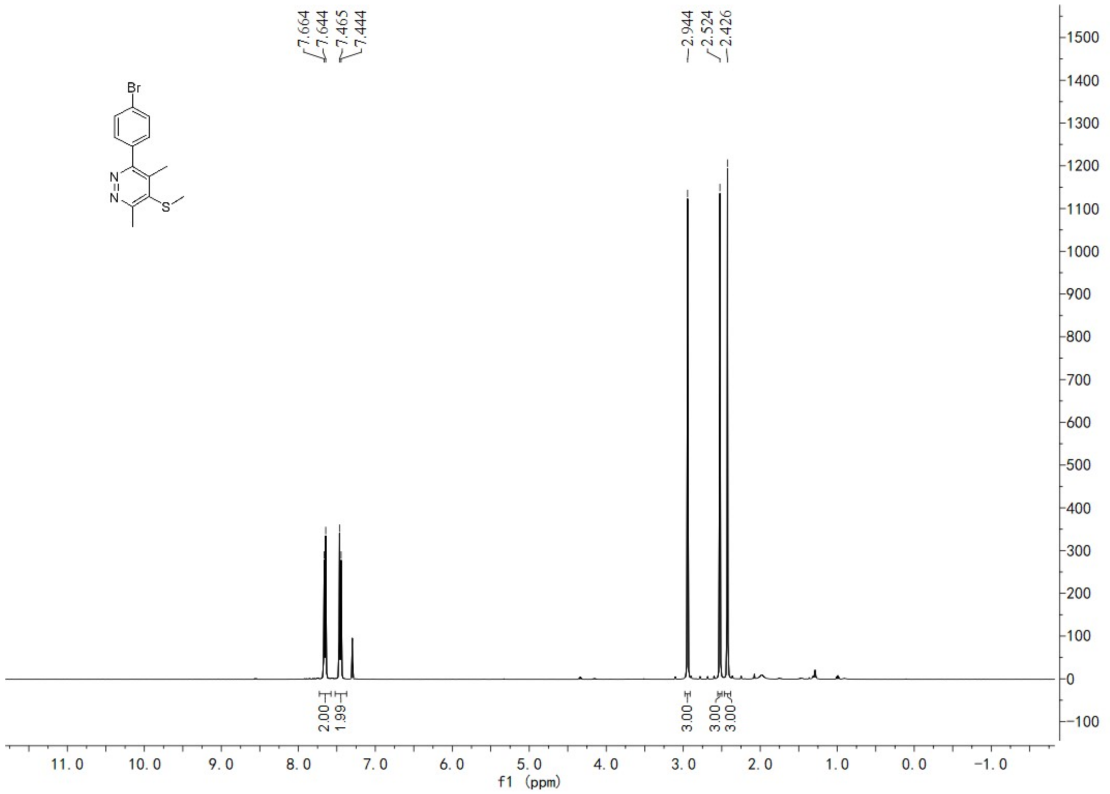
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3l1**



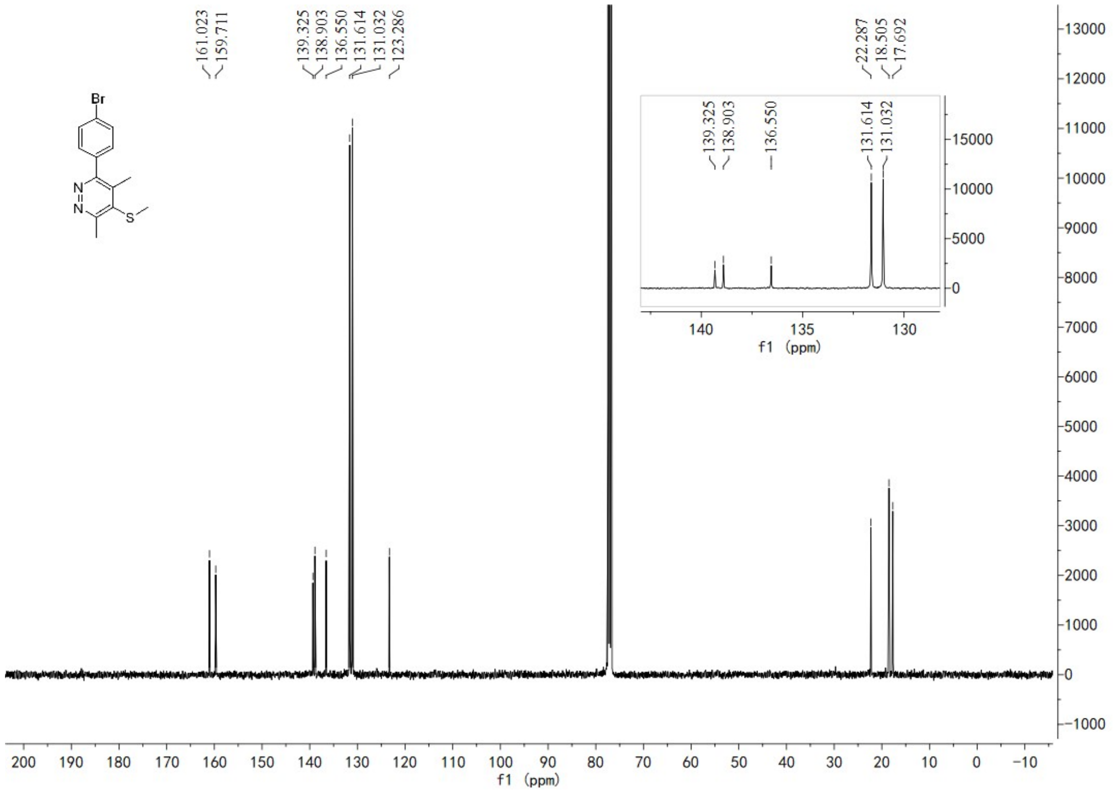
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3l2**



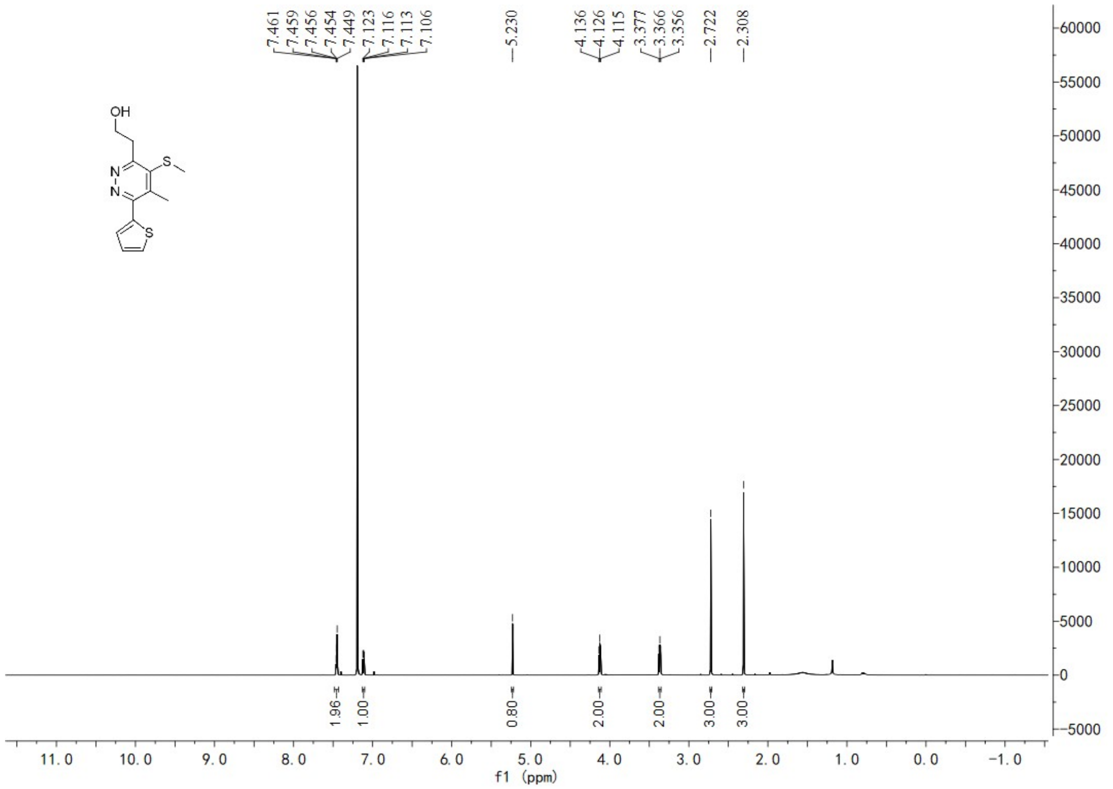
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3l2**



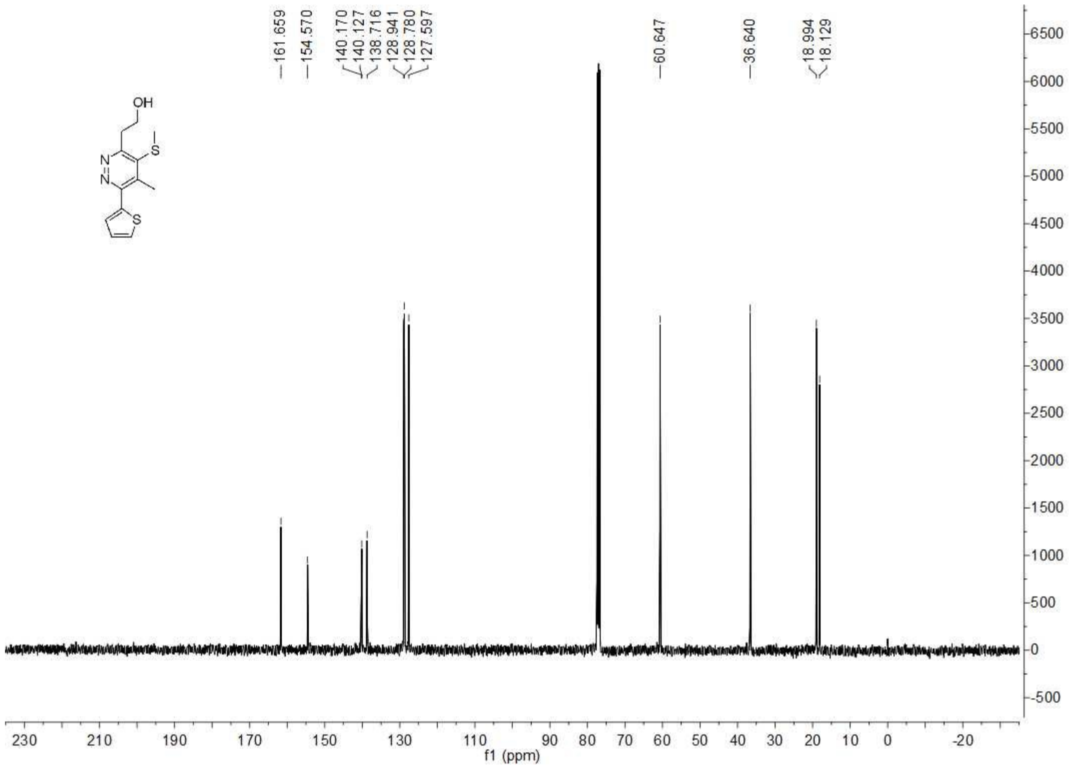
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3m**



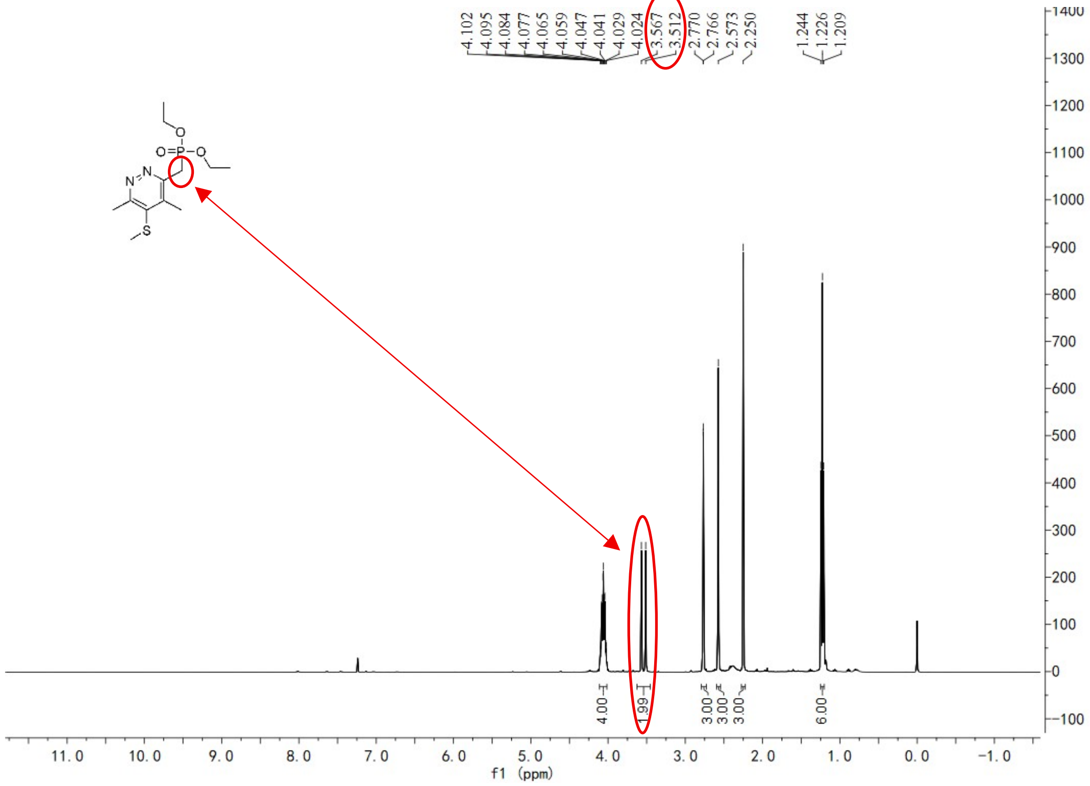
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3m**



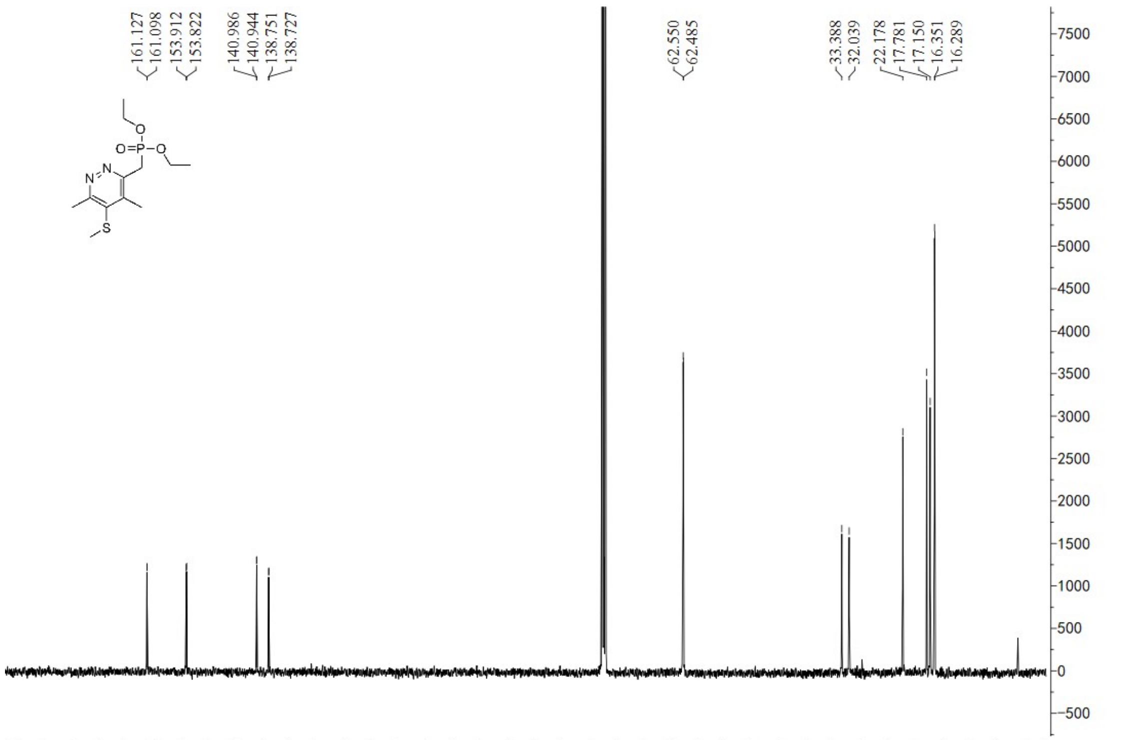
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3n**



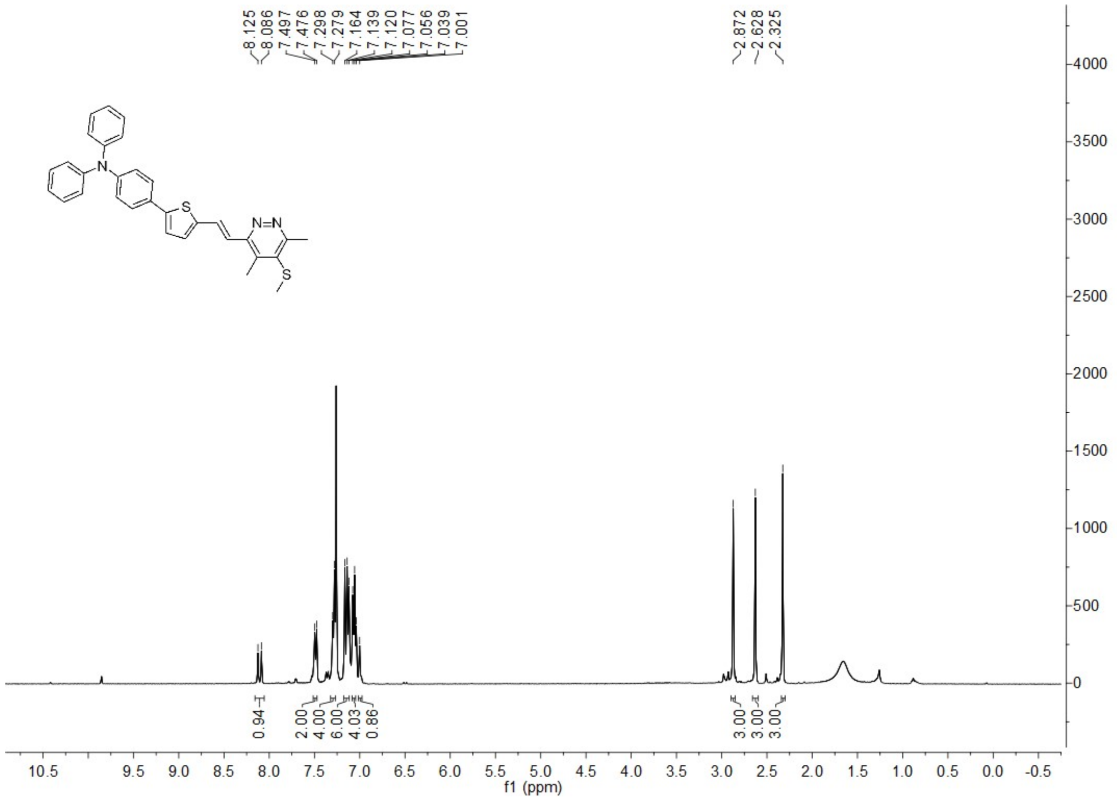
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3n**



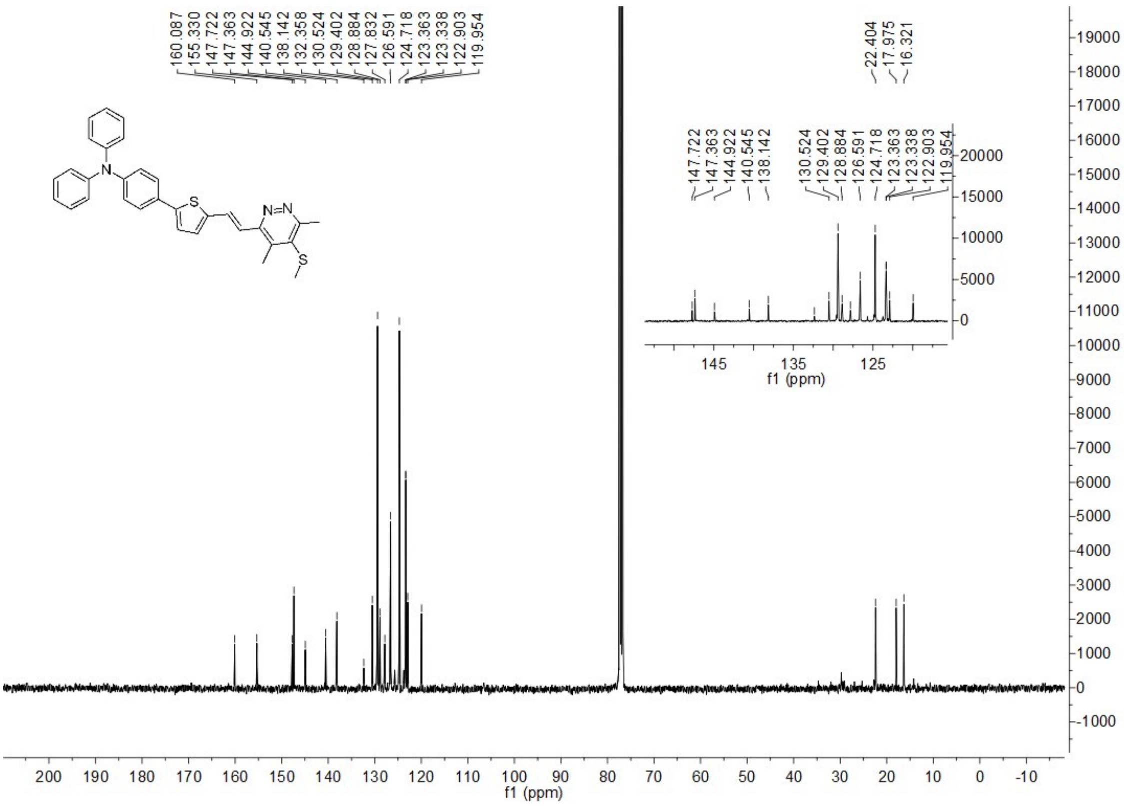
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3o**



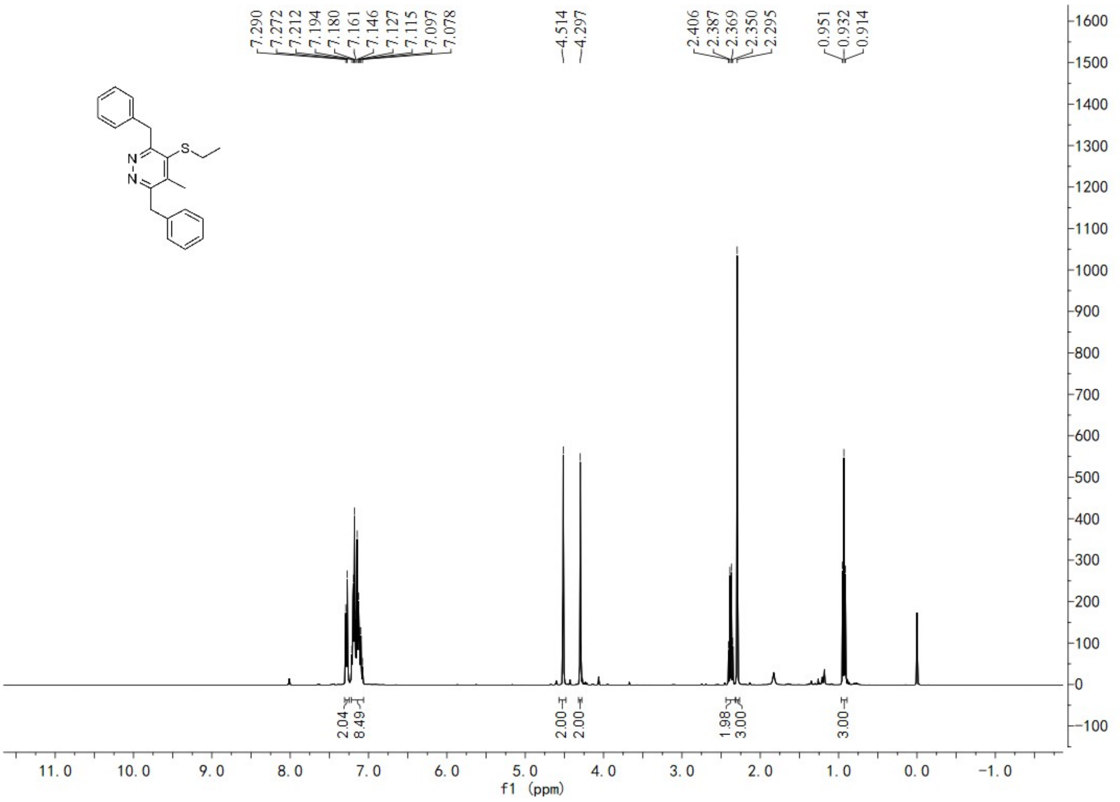
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3o**



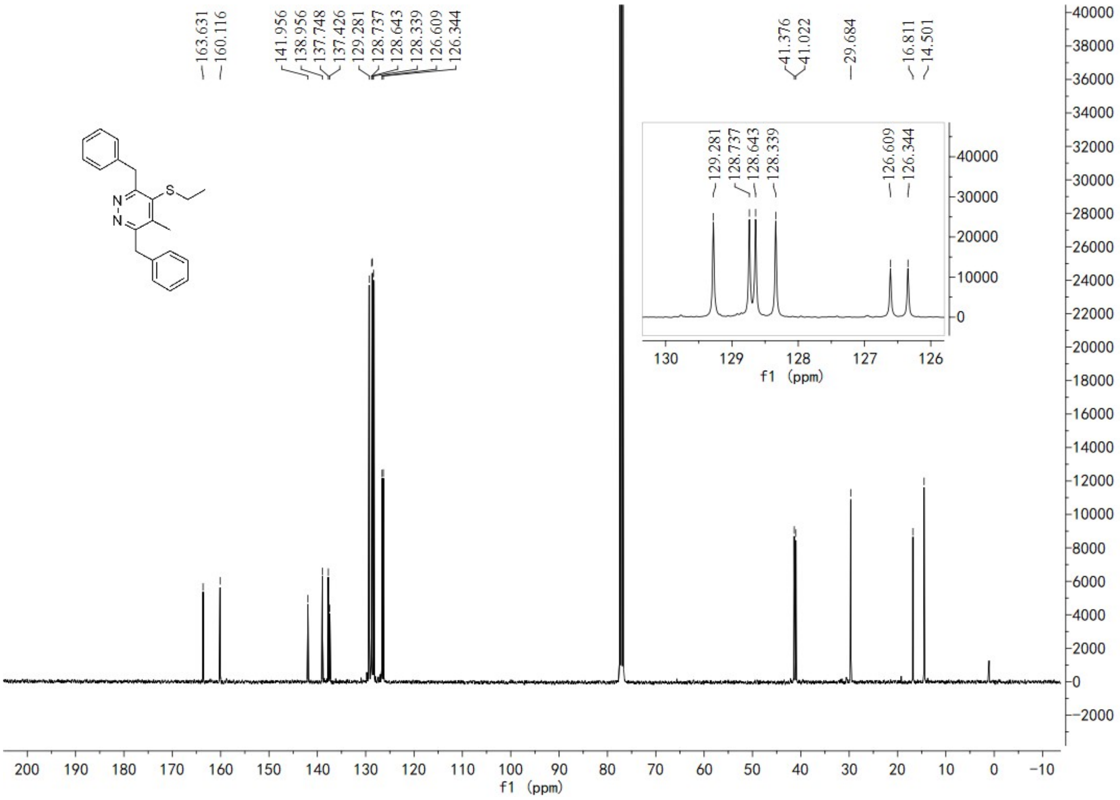
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **3p**



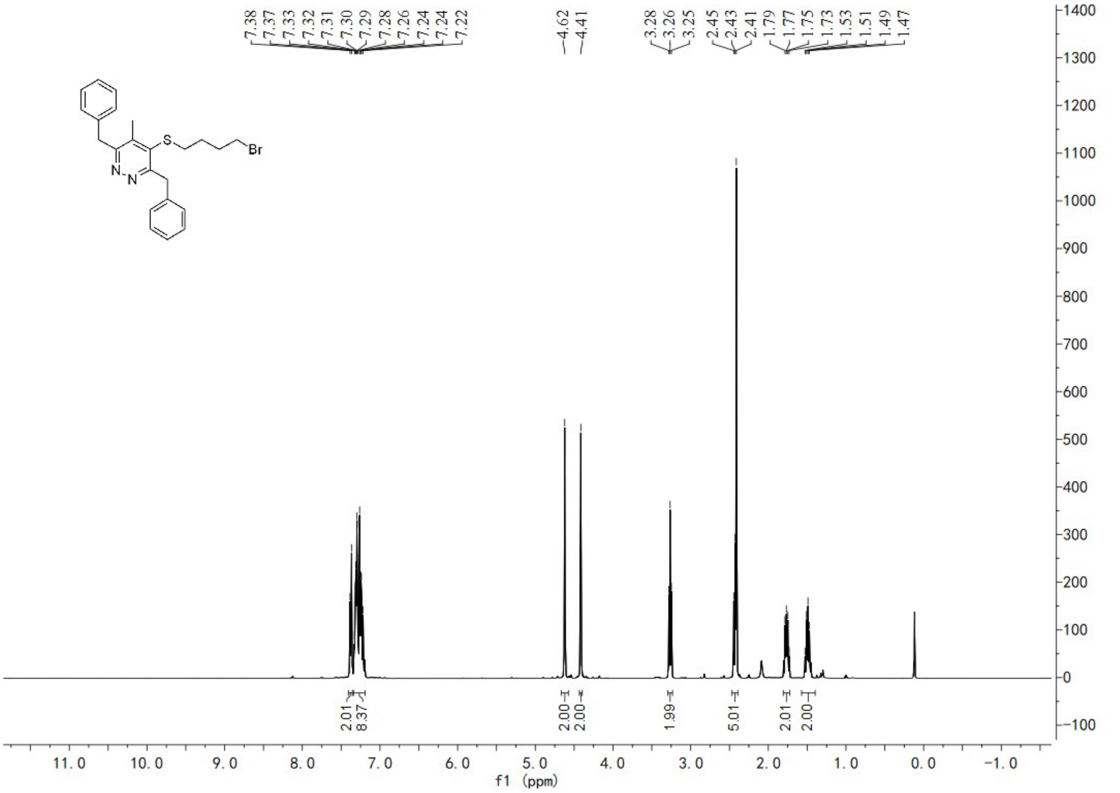
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **3p**



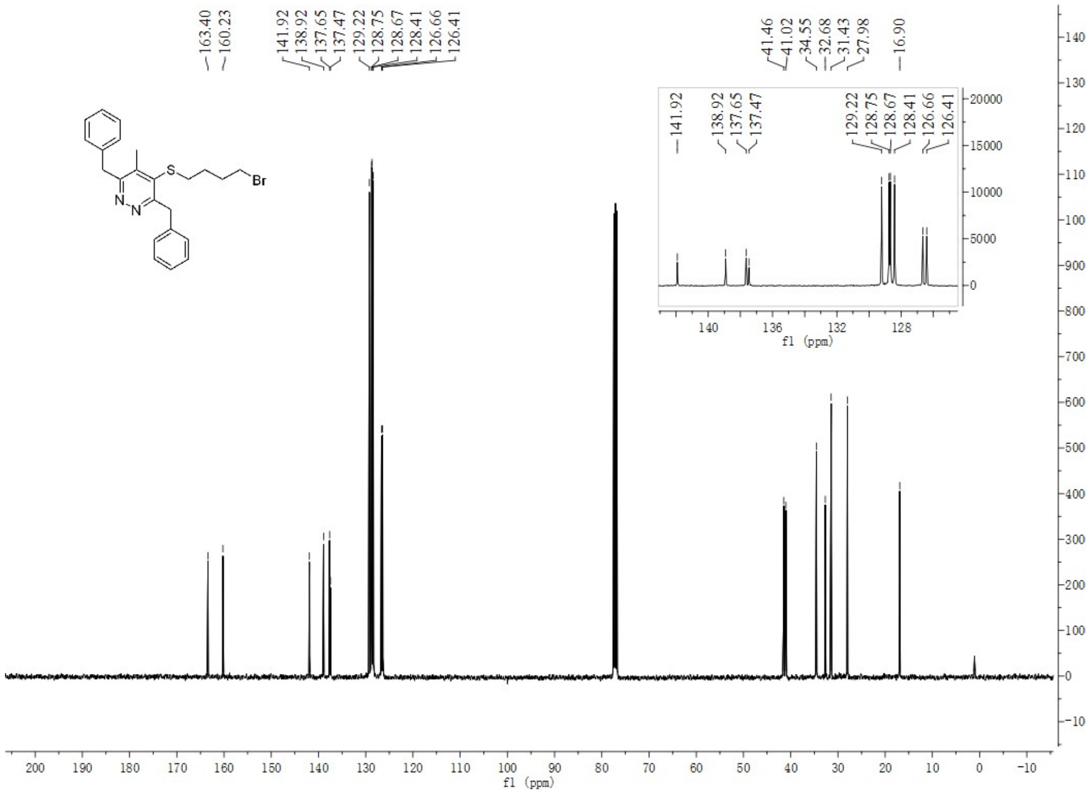
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **4a**



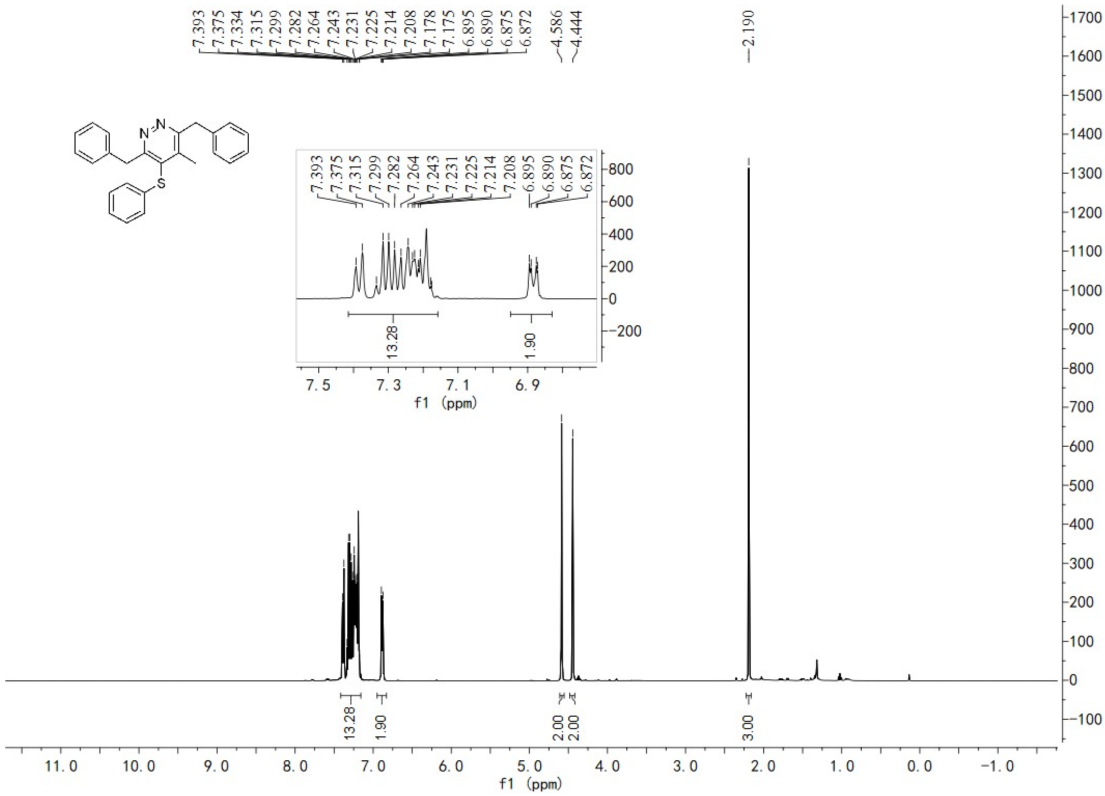
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **4a**



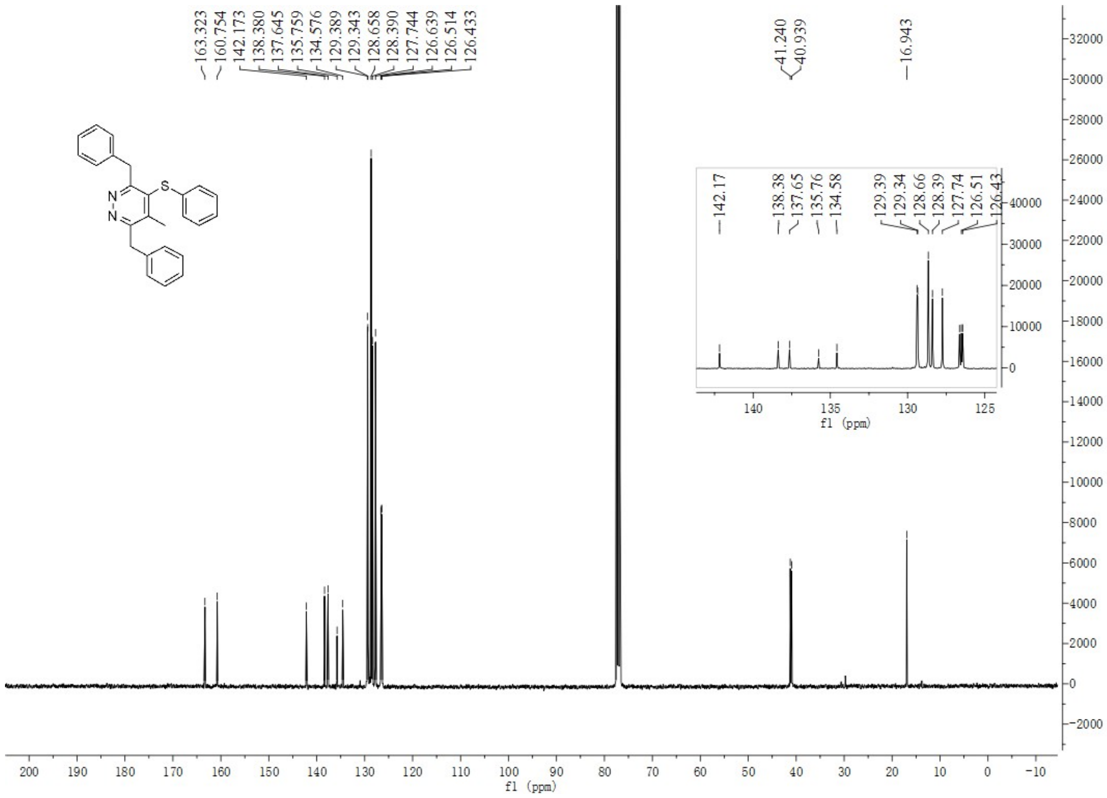
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **4b**



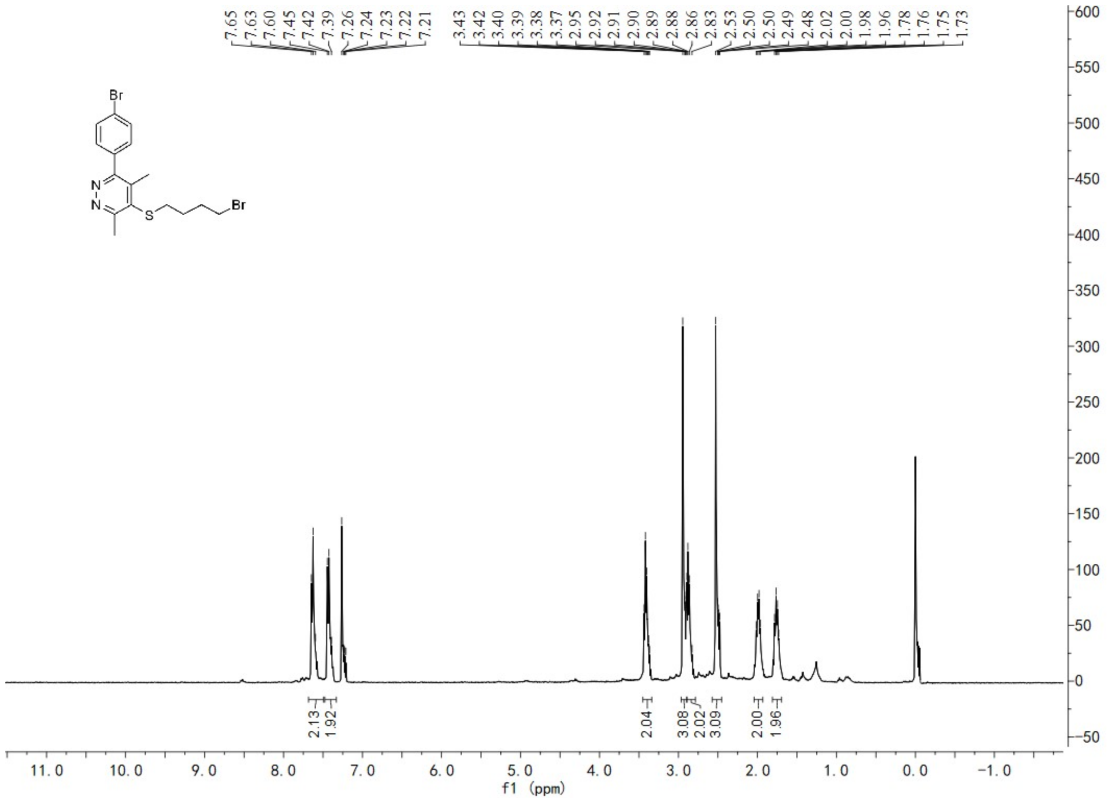
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **4b**



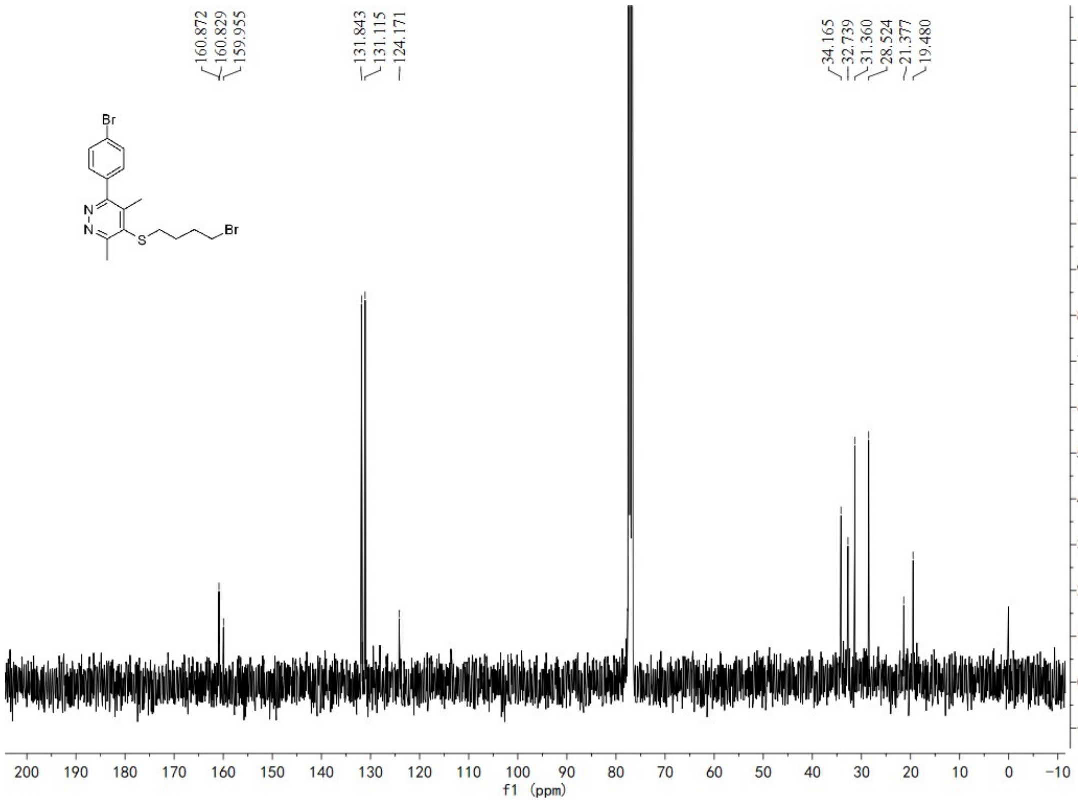
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **4d**



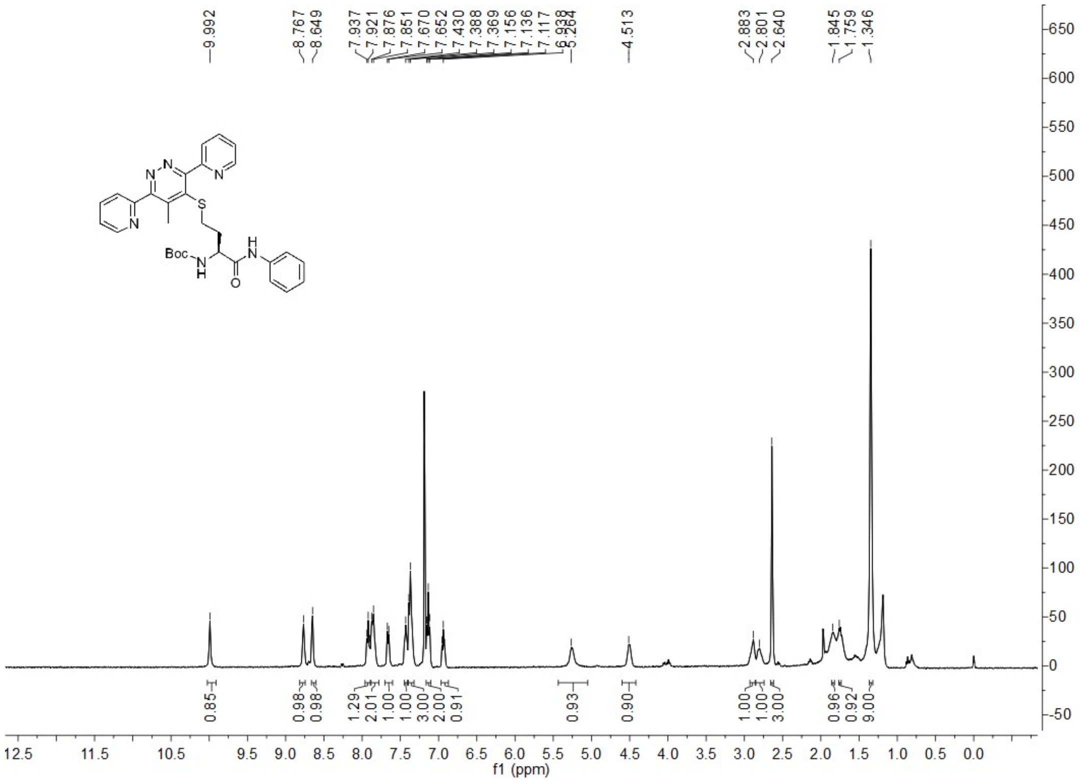
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **4d**



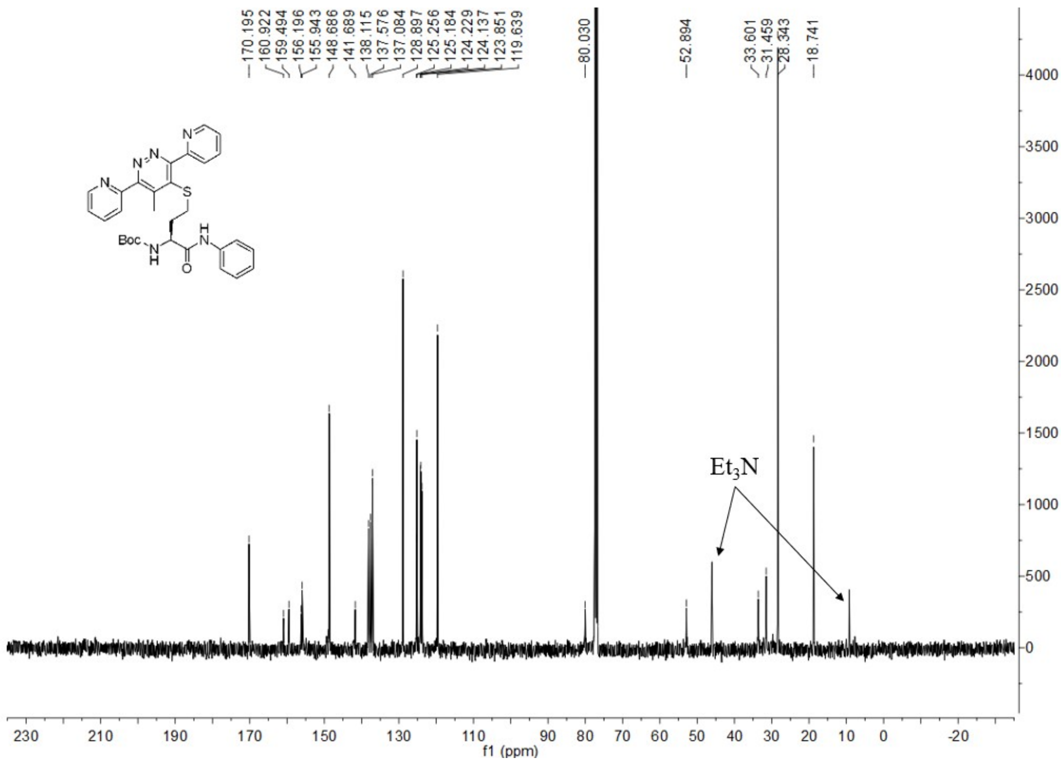
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **4e**



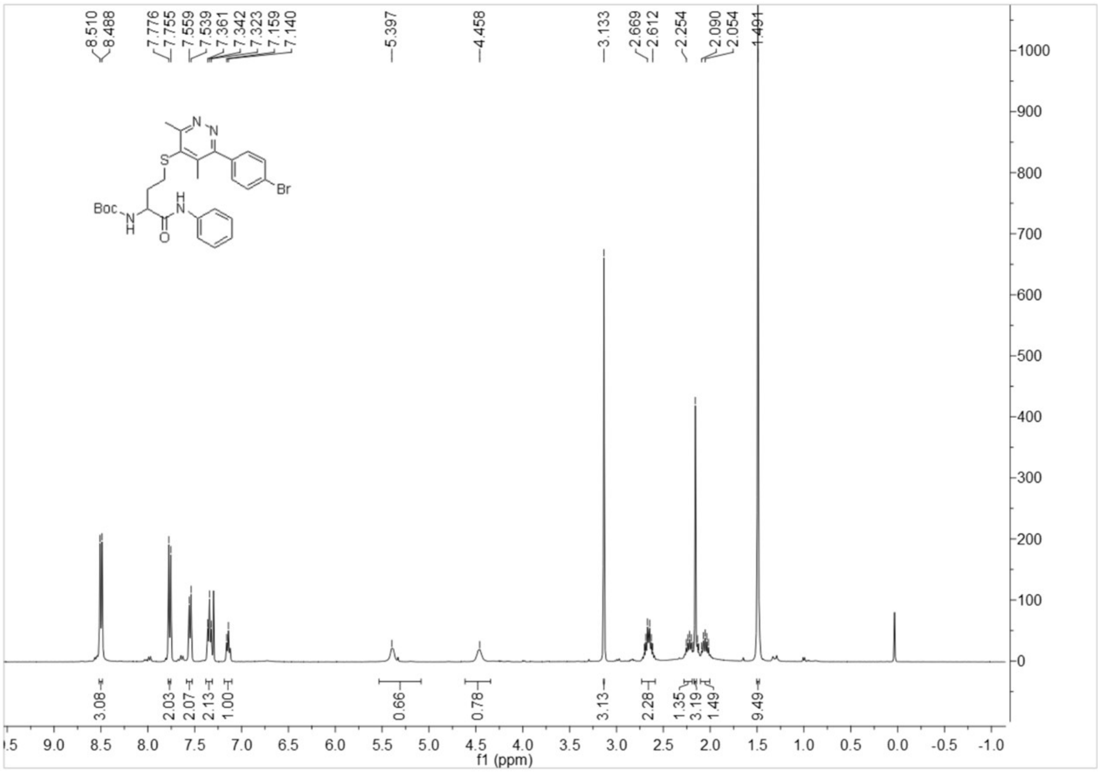
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **4e**



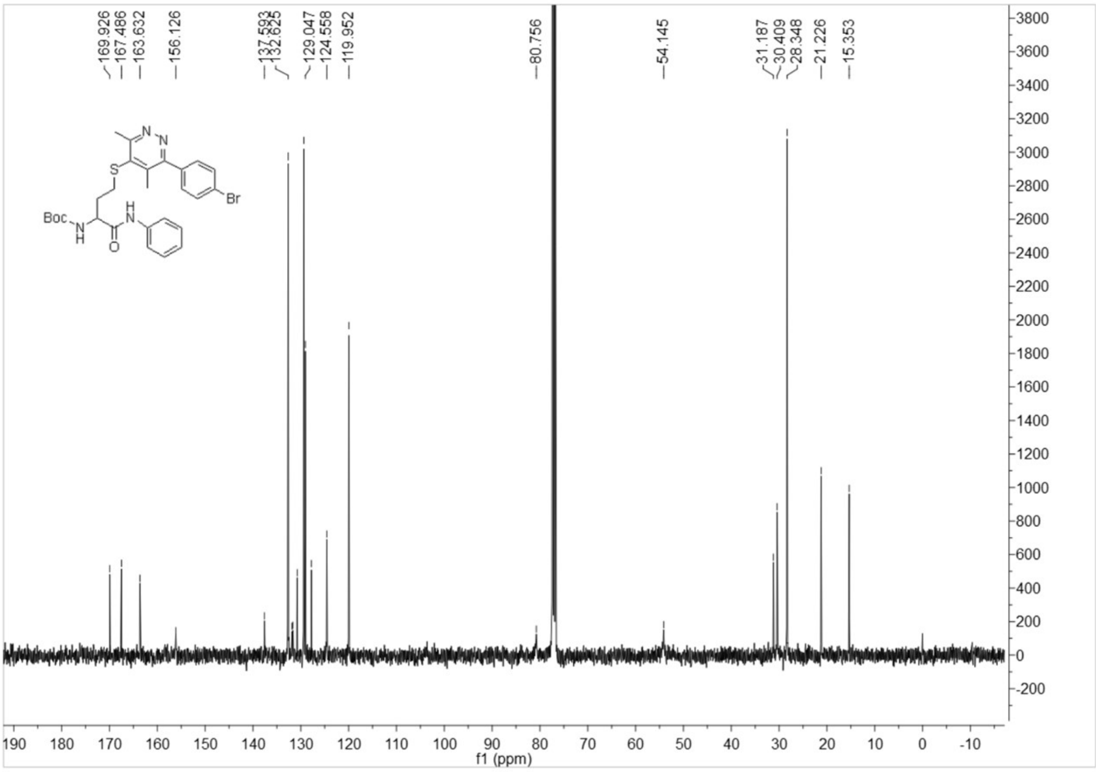
^{1}H NMR (400 MHz, DMSO-$_{d6}$) spectrum of compound **5a**



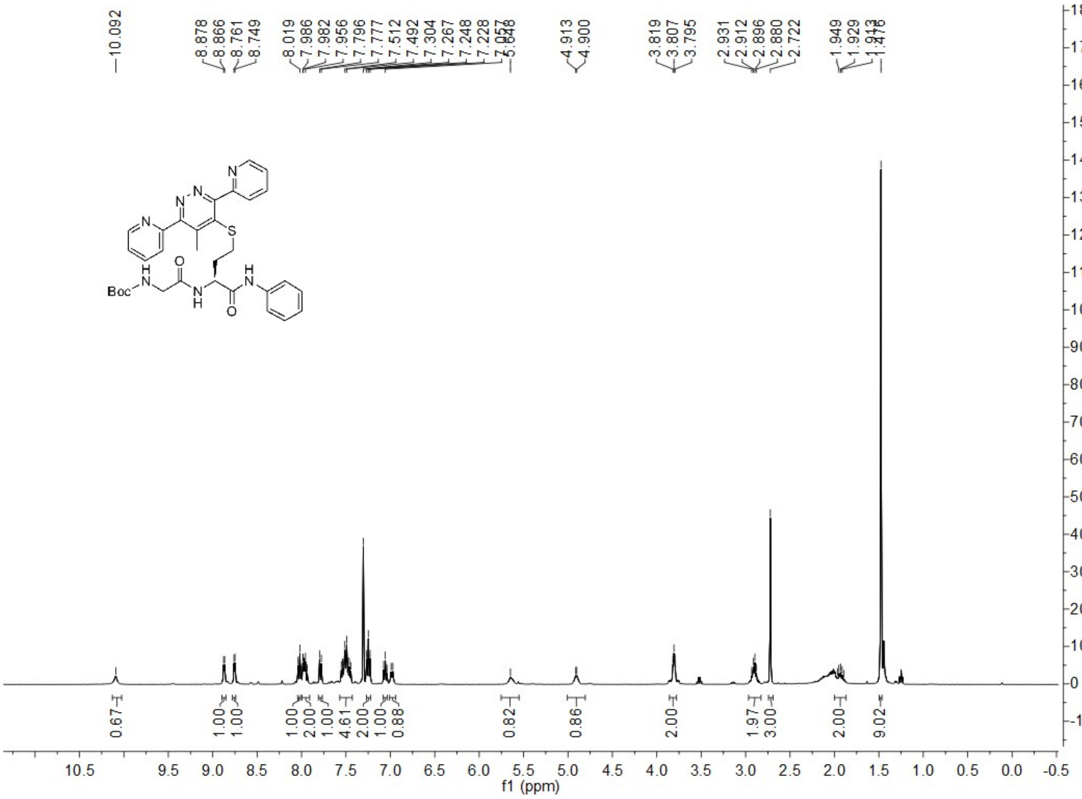
^{13}C NMR (100 MHz, DMSO-$_{d6}$) spectrum of compound **5a**



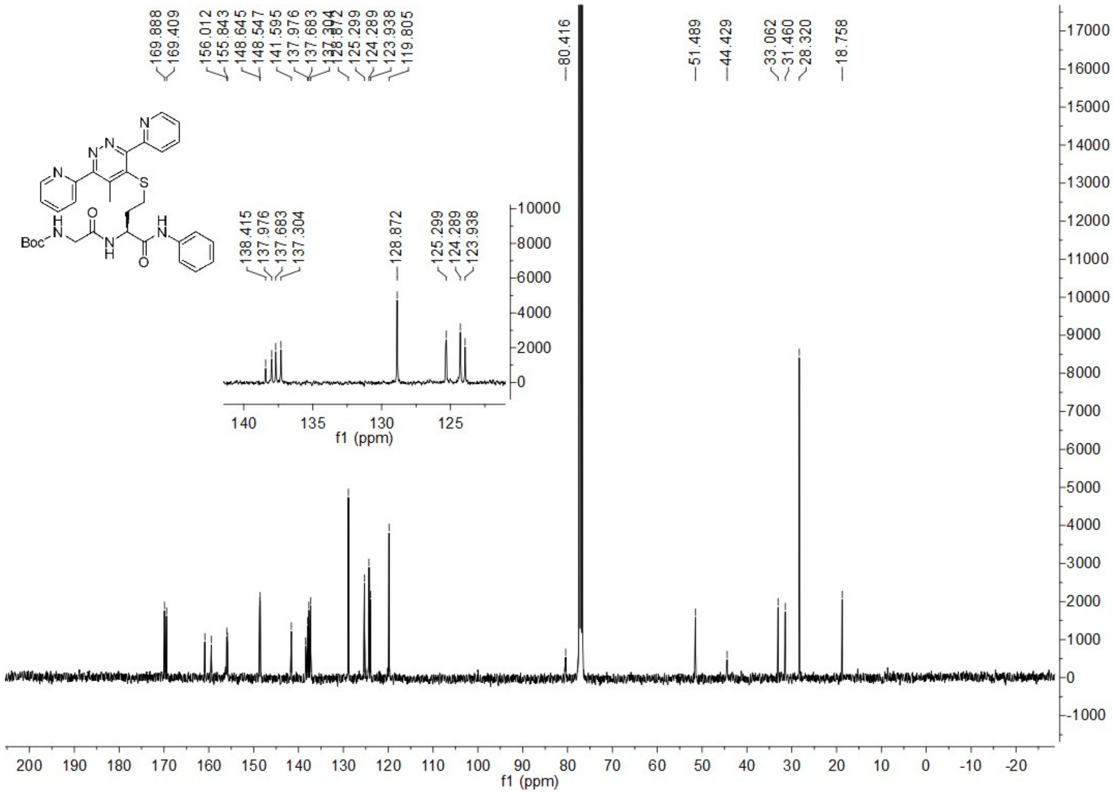
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **5b**



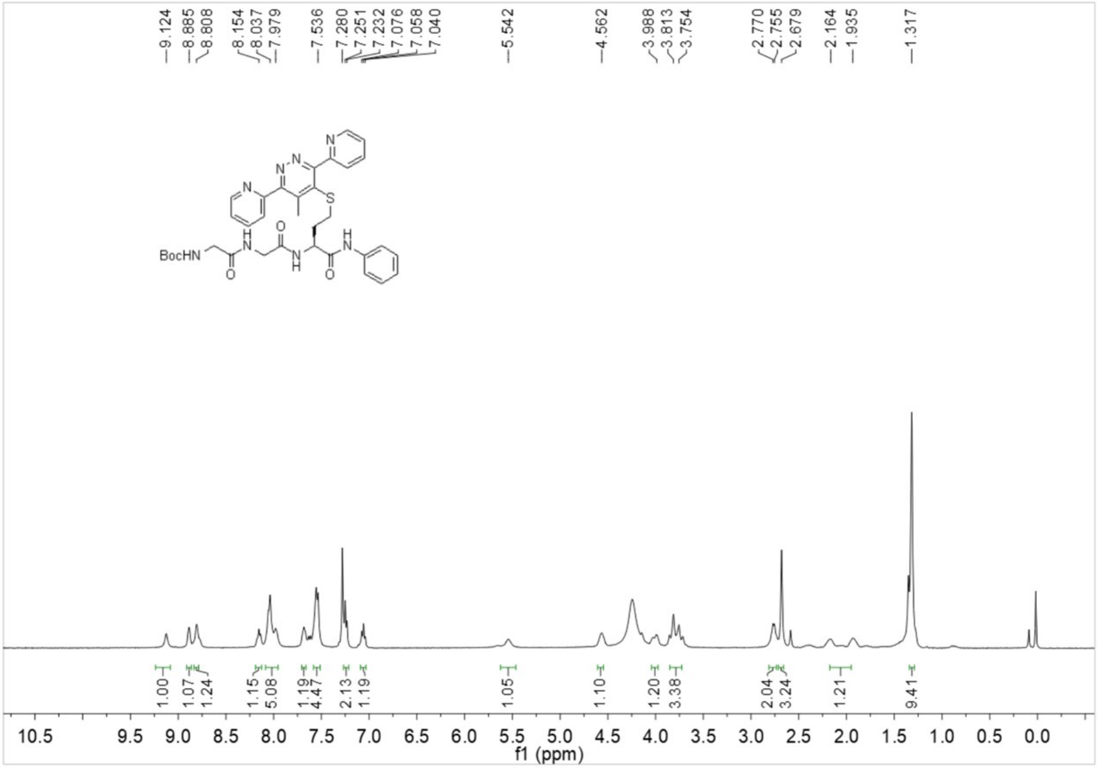
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **5b**



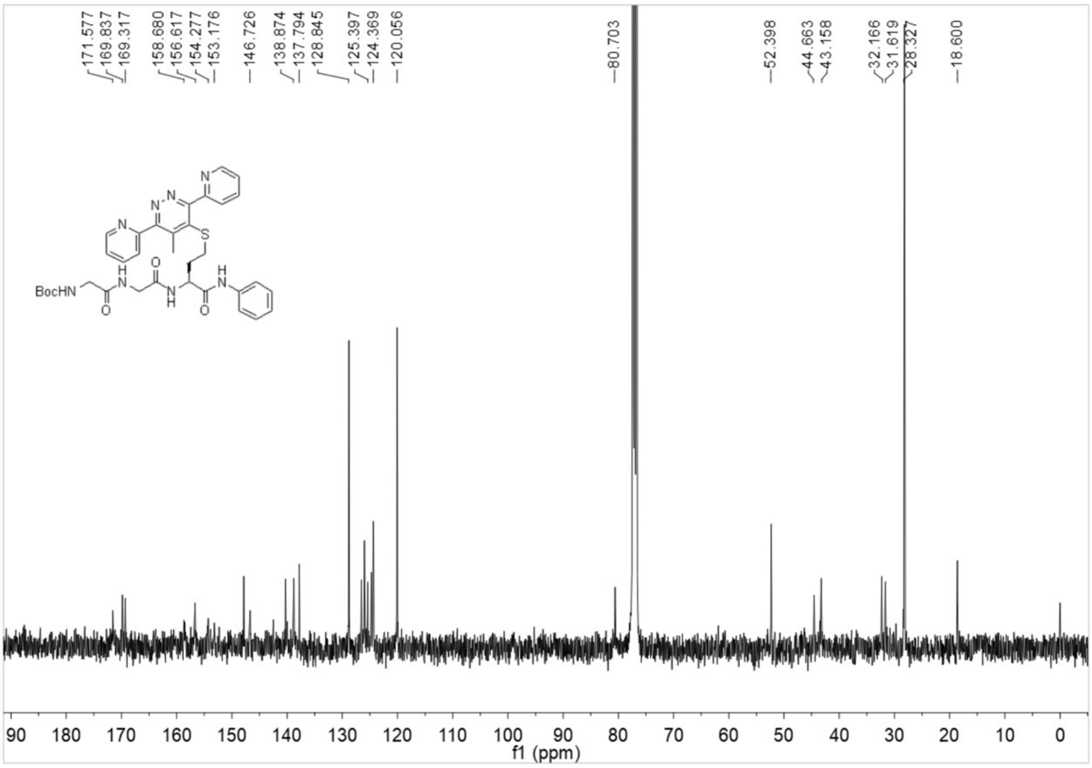
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **6a**



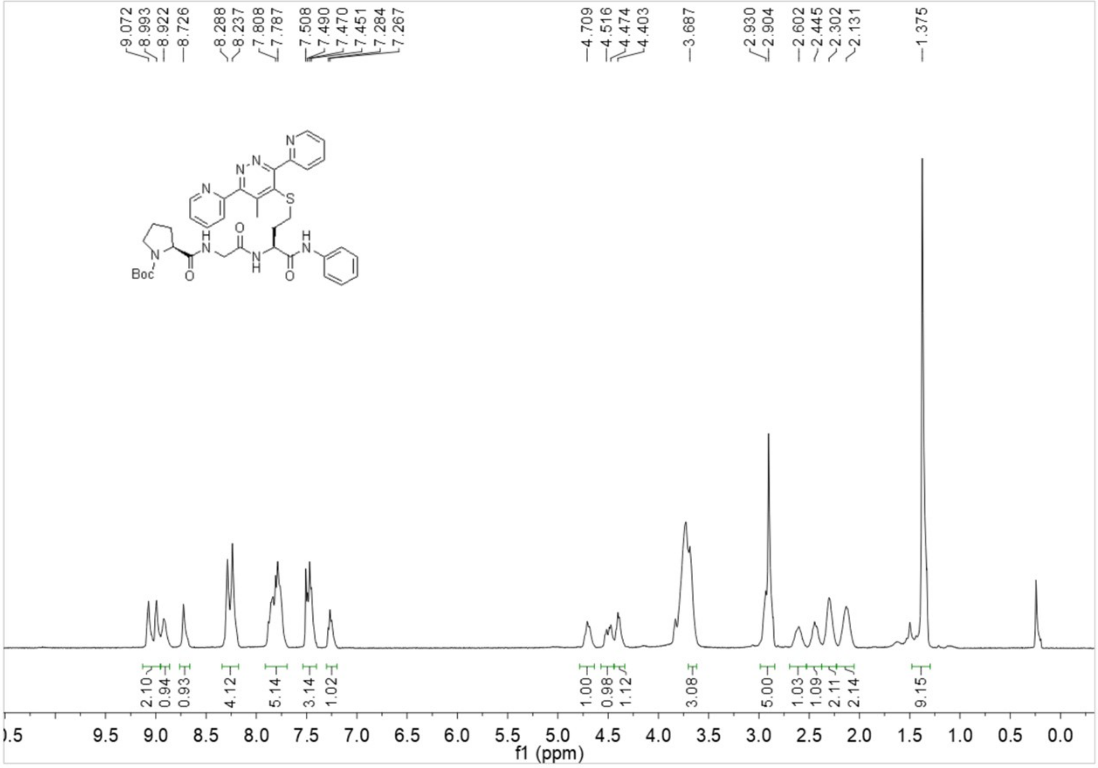
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **6a**



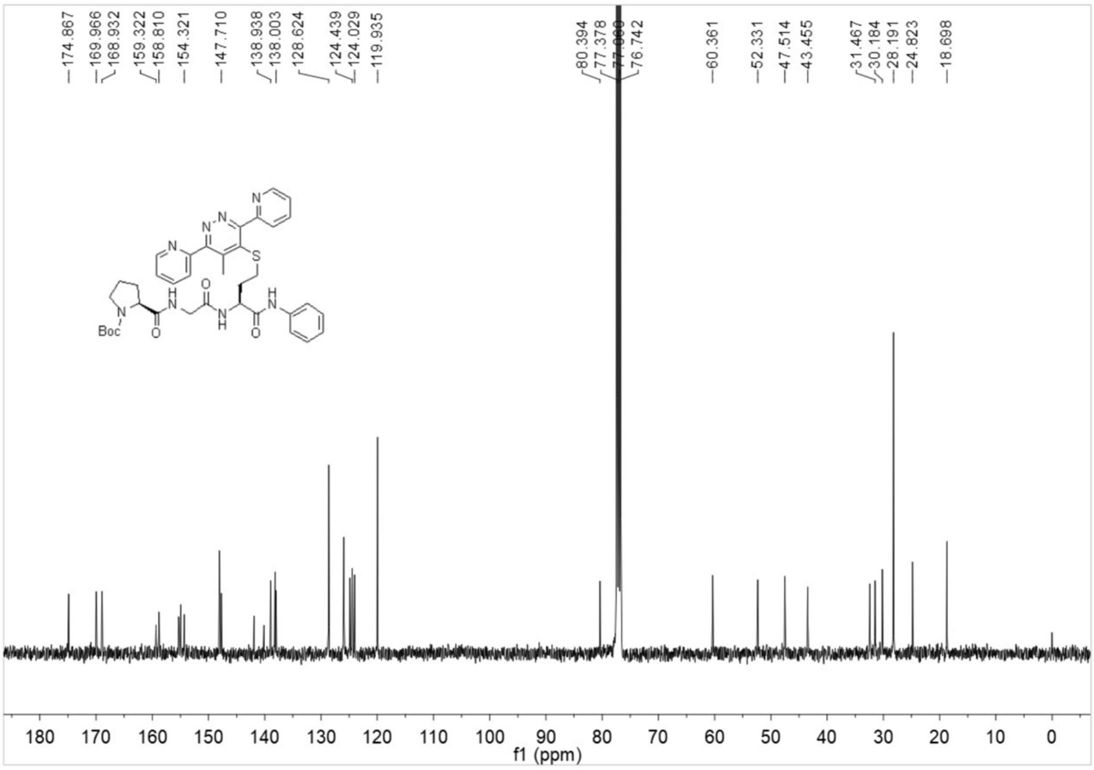
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **6b**



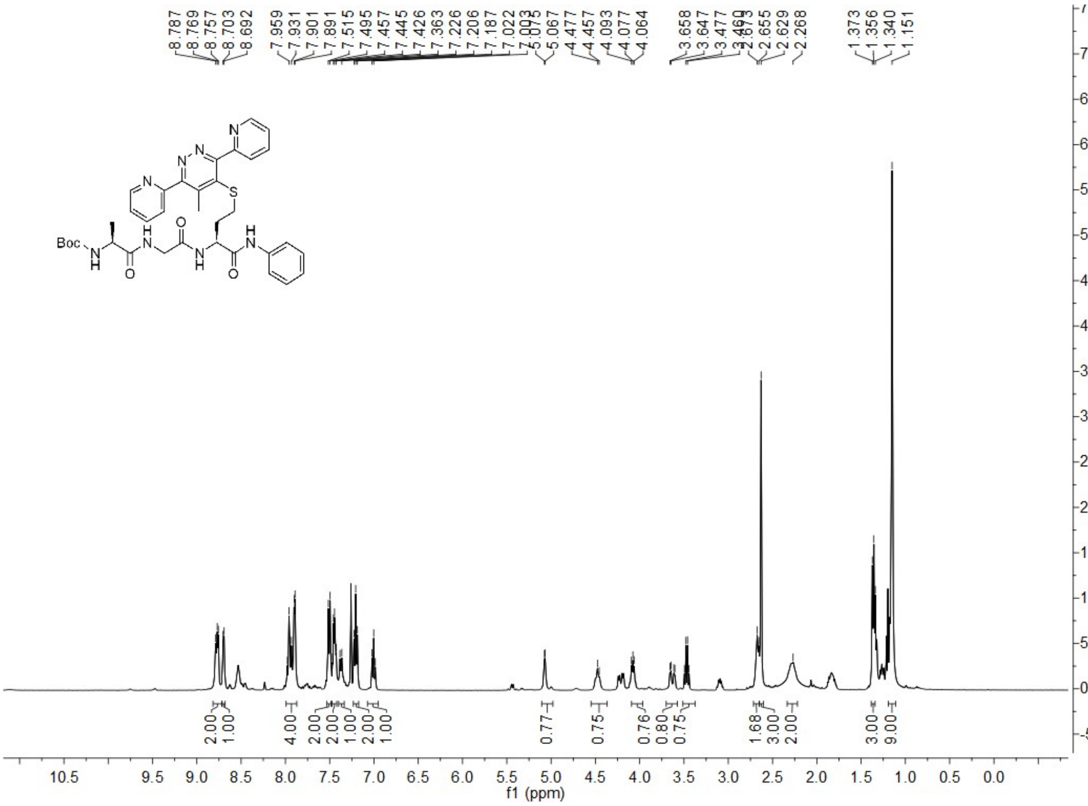
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **6b**



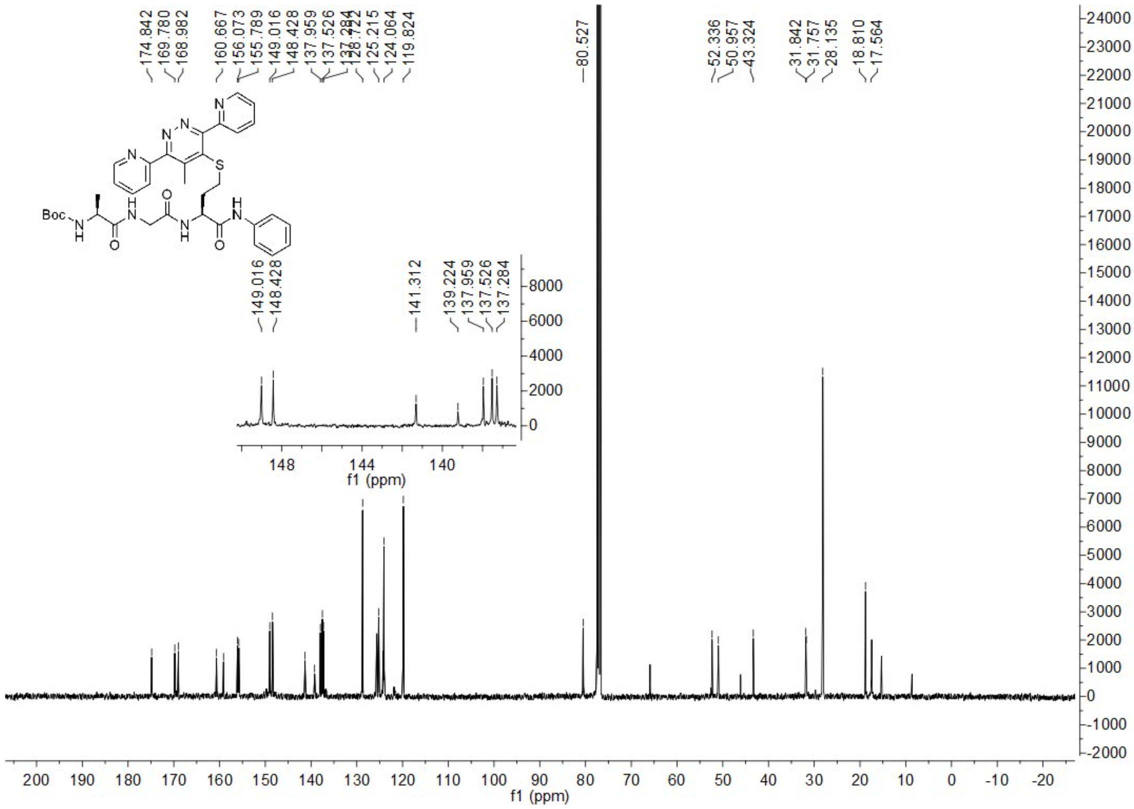
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **6c**



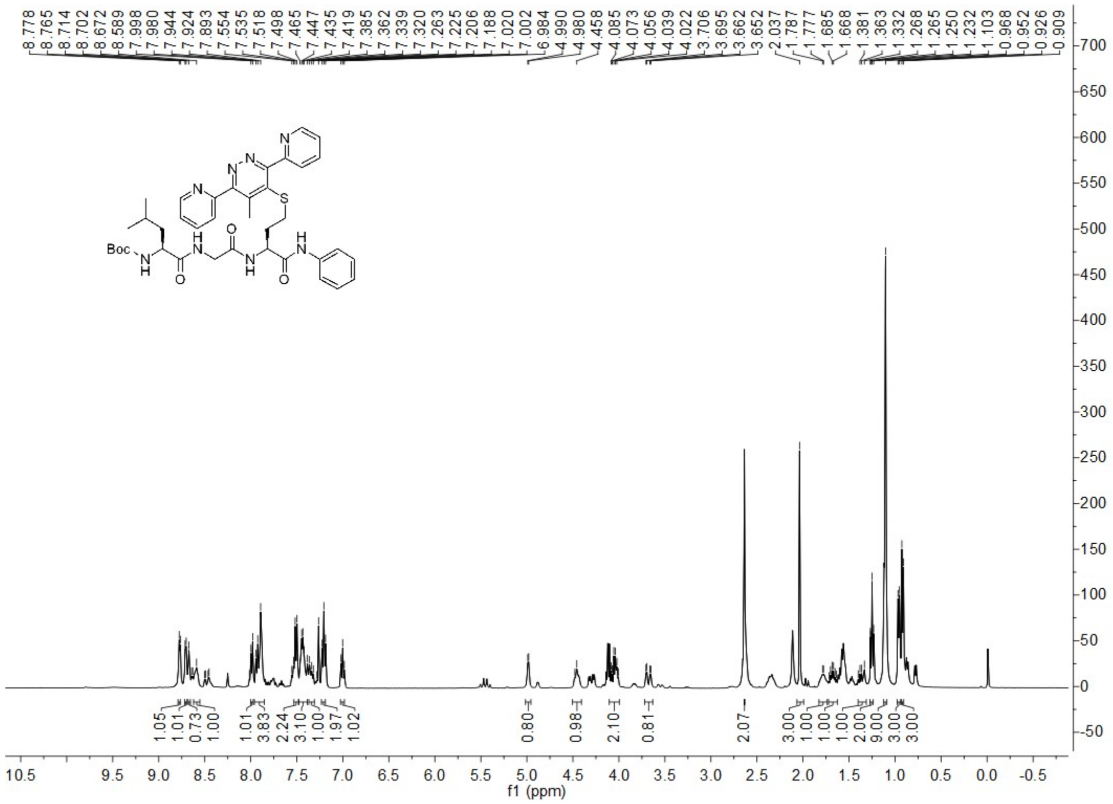
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **6c**



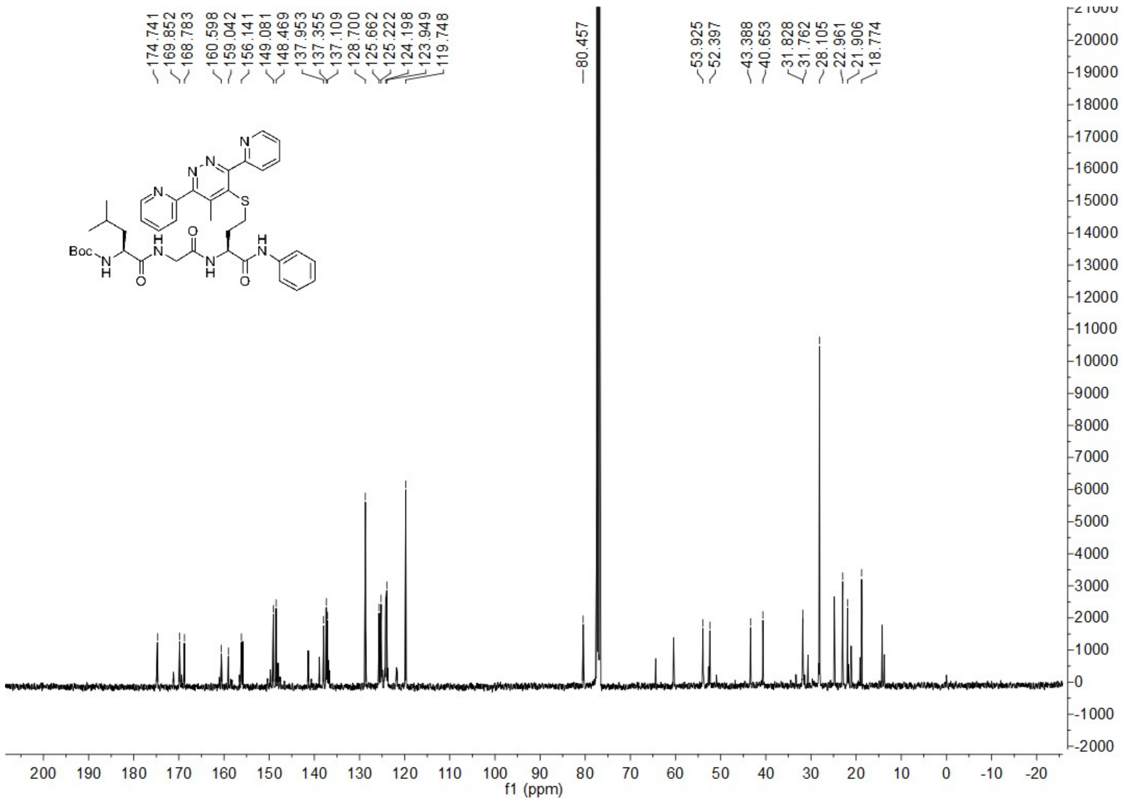
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **6d**



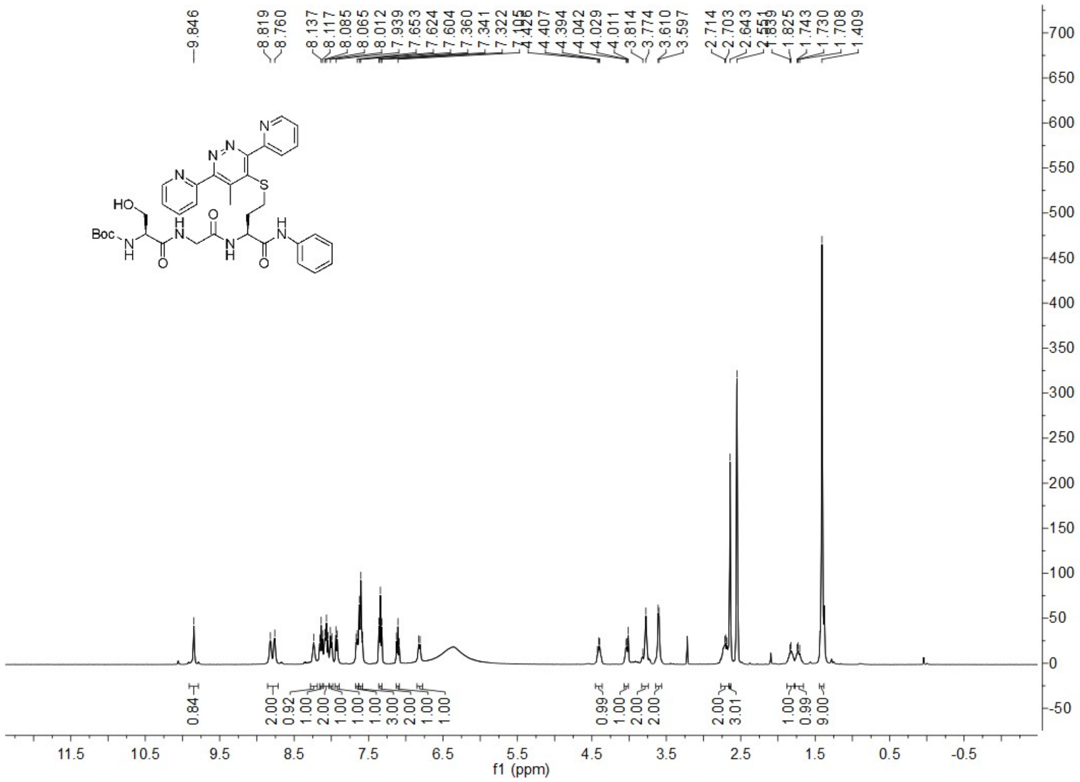
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **6d**



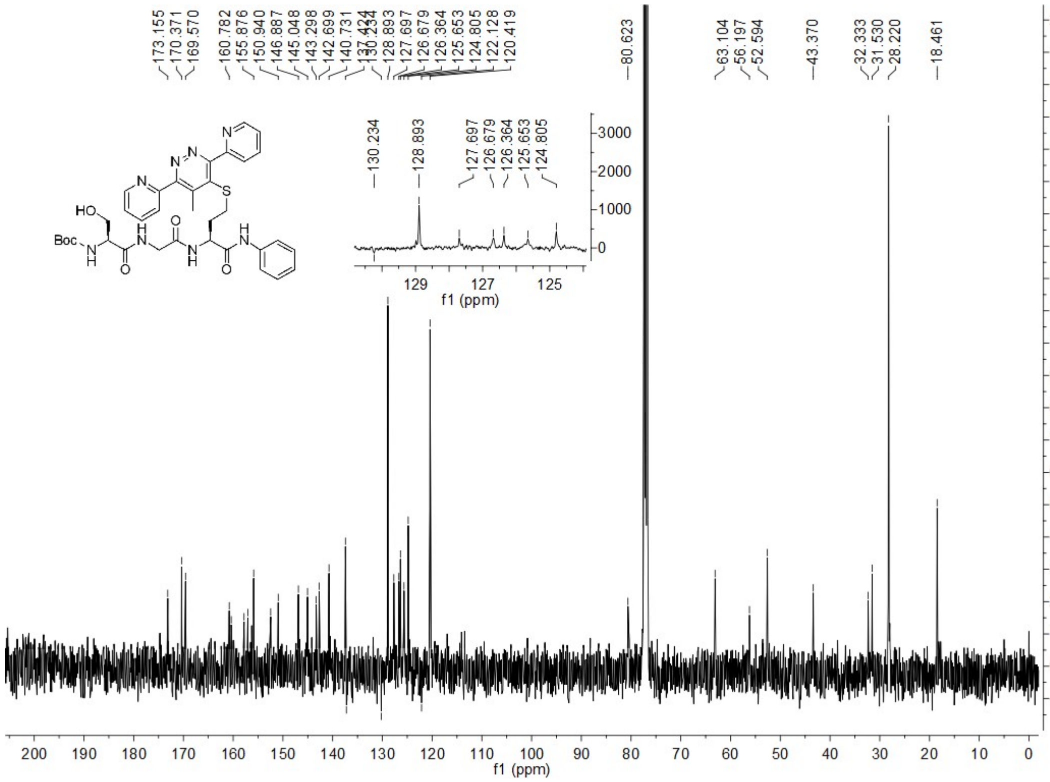
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **6e**



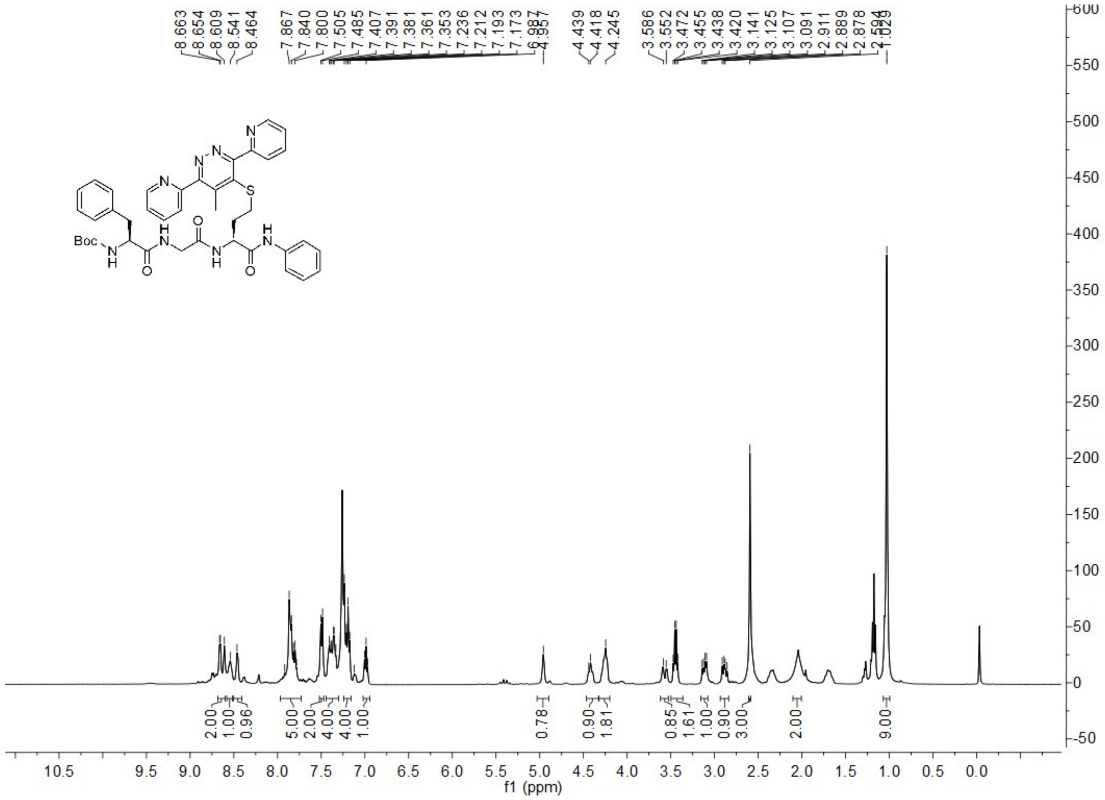
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **6e**



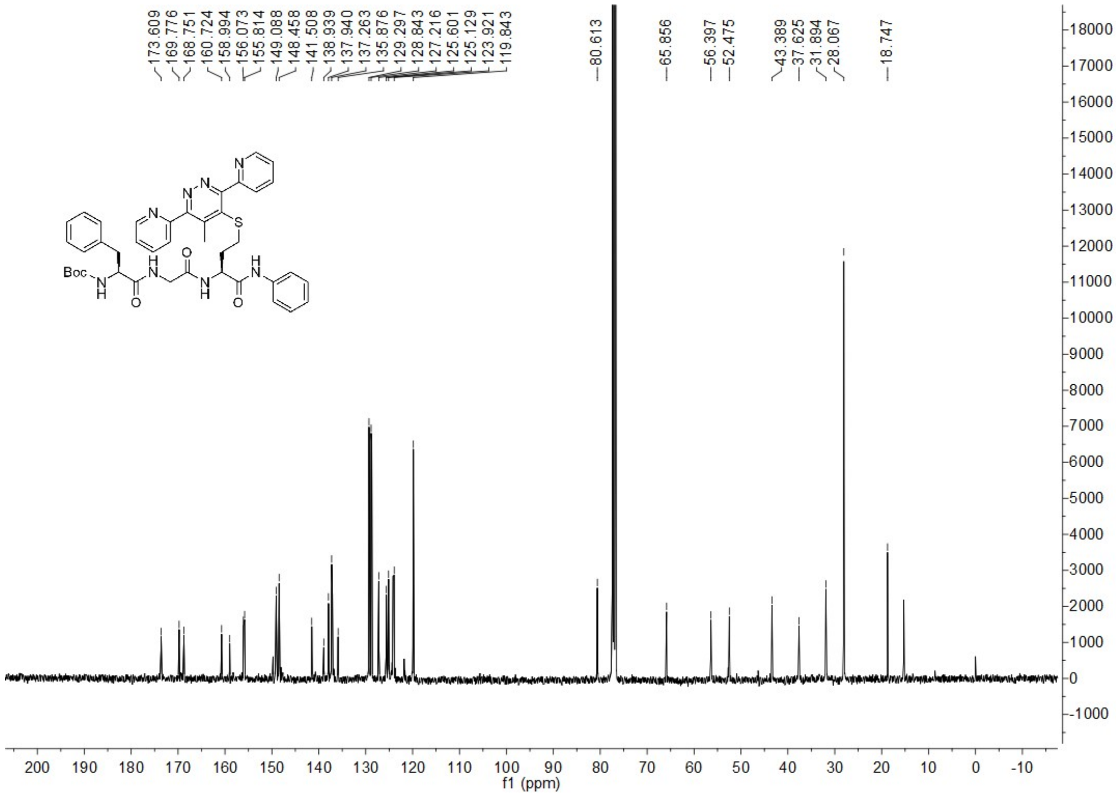
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **6f**



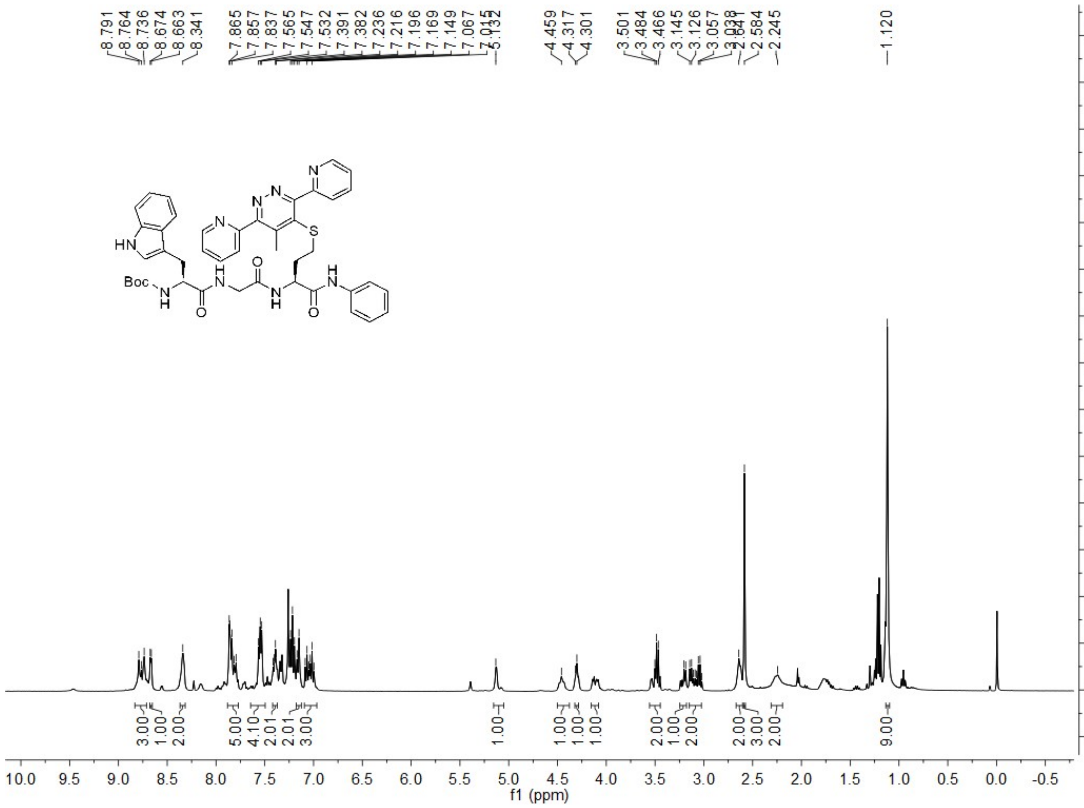
¹³C NMR (100 MHz, CDCl$_3$) spectrum of compound **6f**



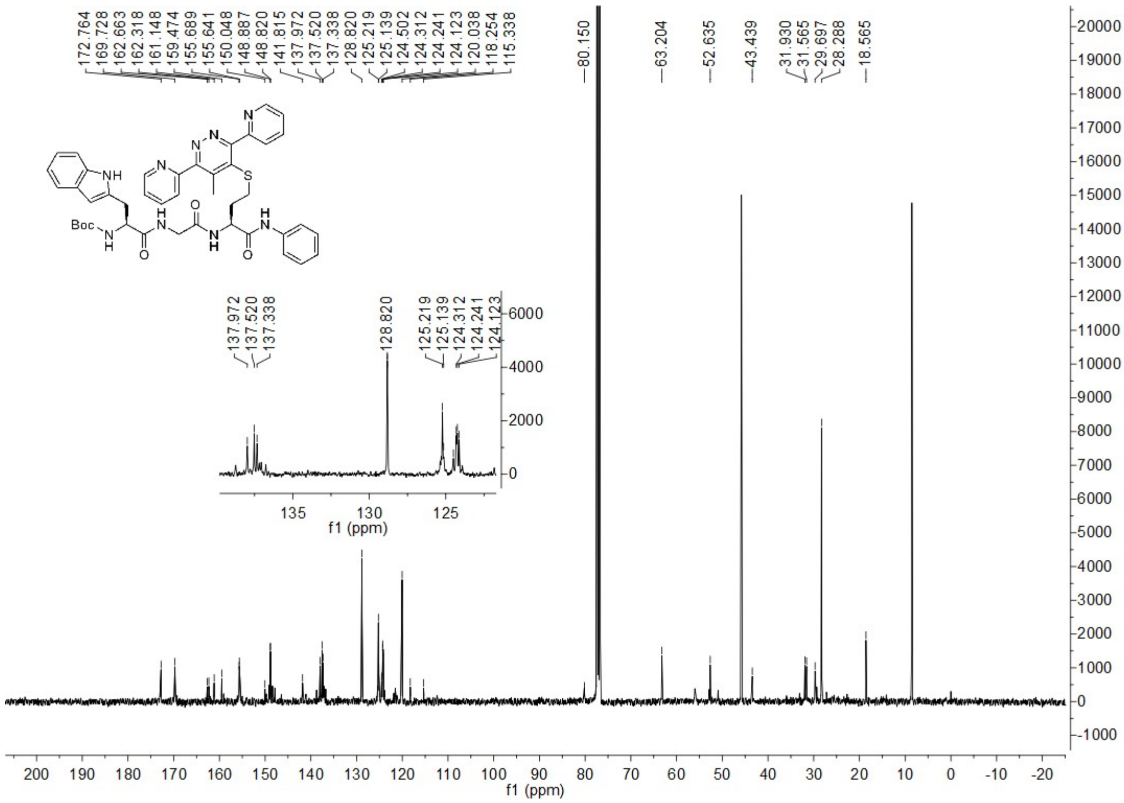
¹H NMR (400 MHz, CDCl$_3$) spectrum of compound **6g**



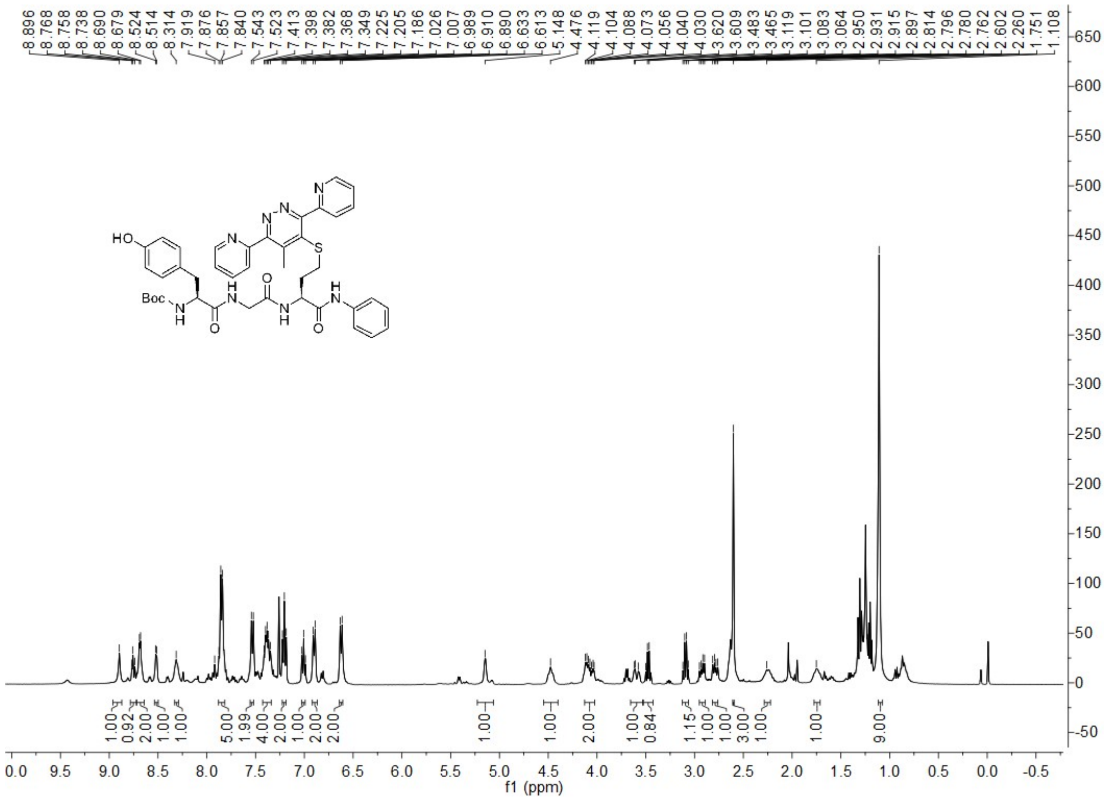
¹³C NMR (100 MHz, CDCl$_3$) spectrum of compound **6g**



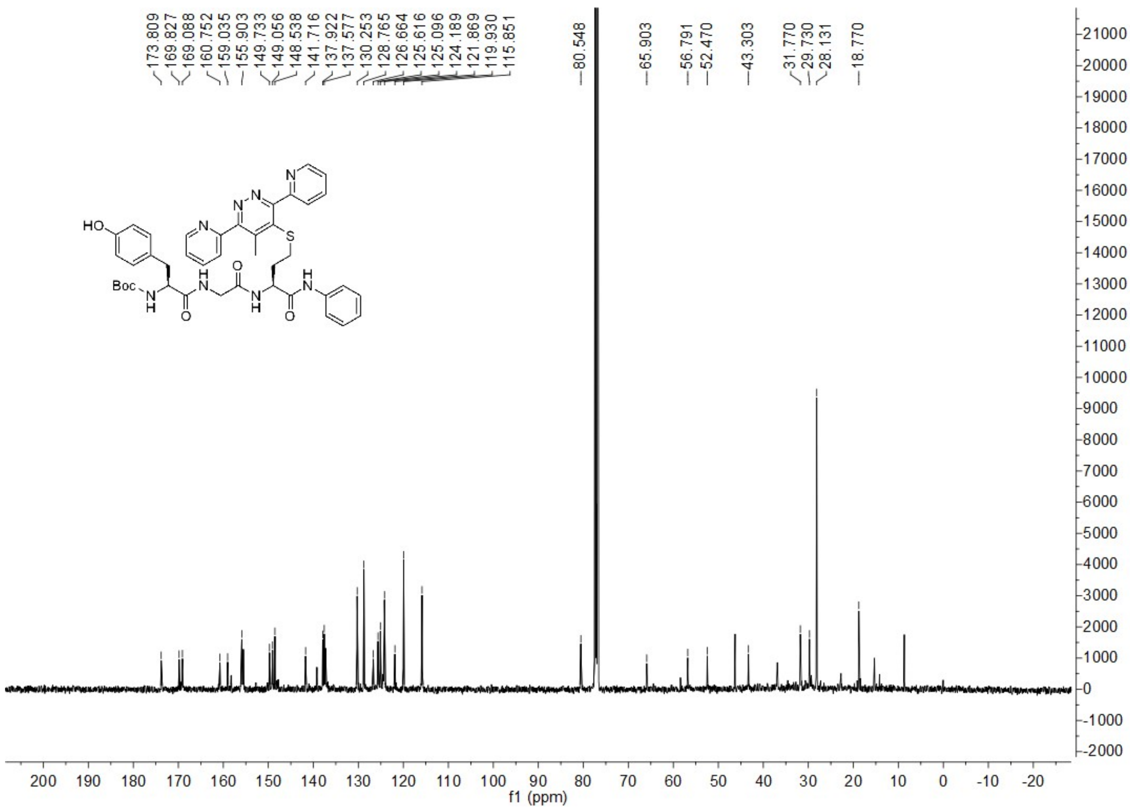
¹H NMR (400 MHz, CDCl$_3$) spectrum of compound **6h**



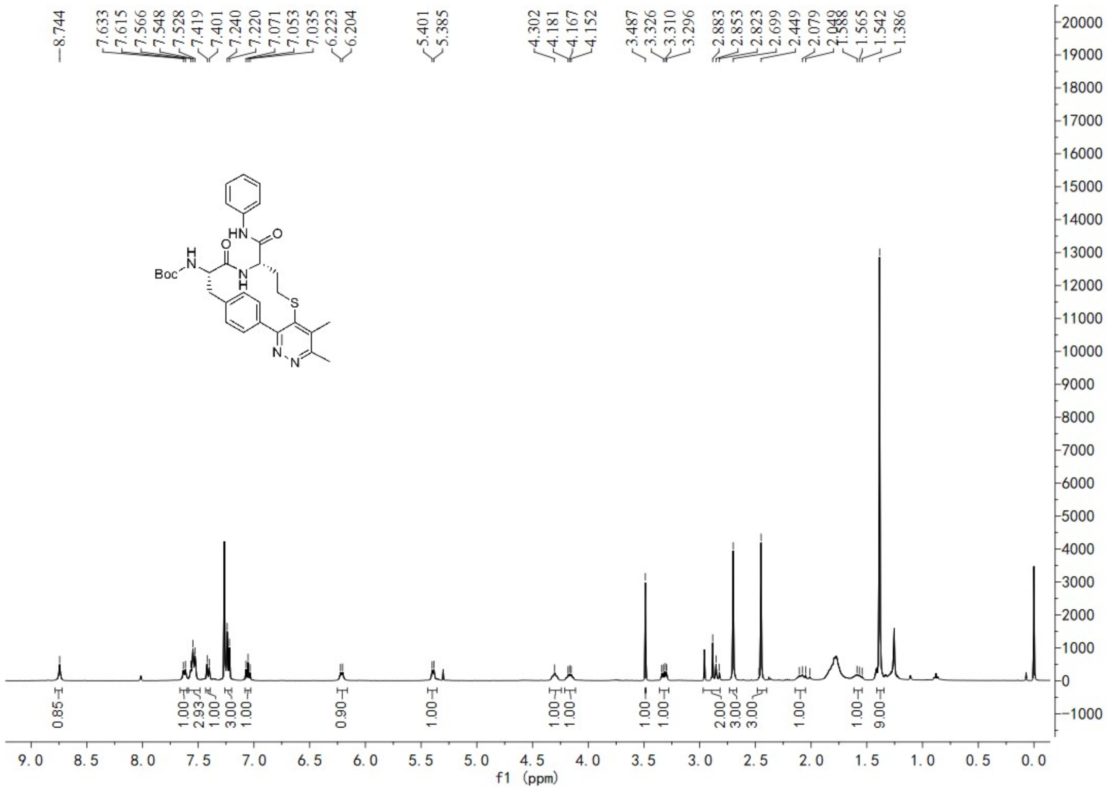
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **6h**



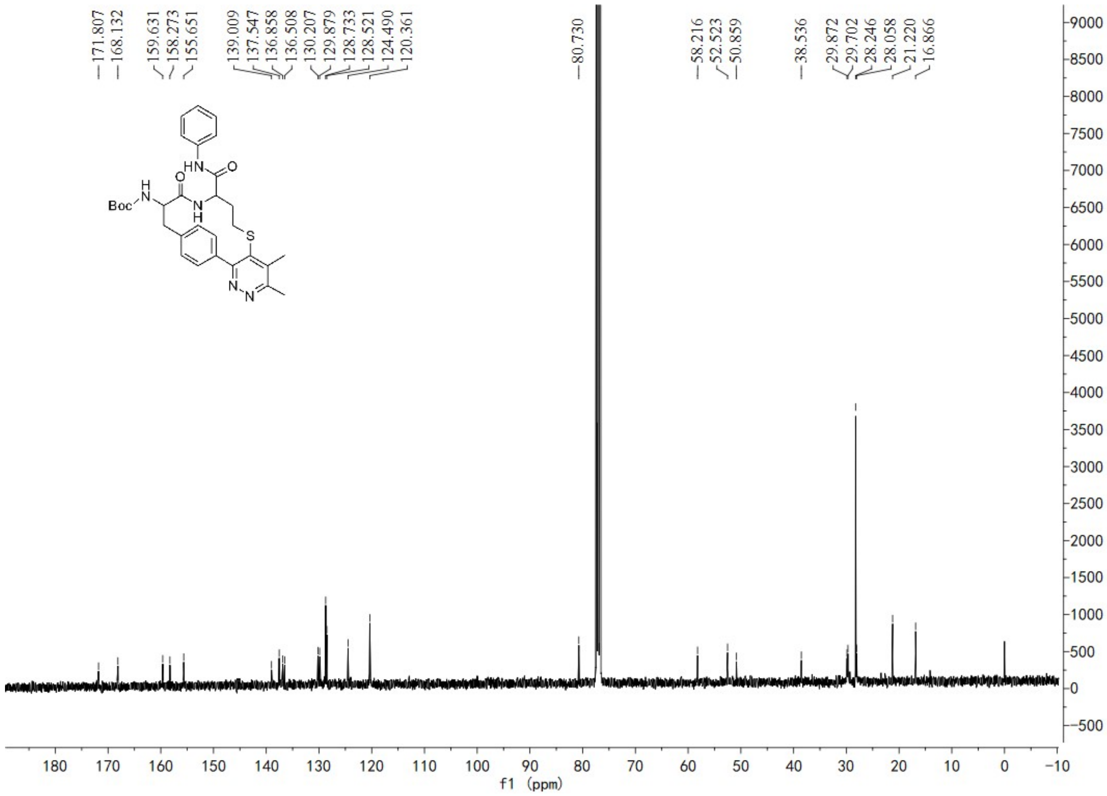
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **6i**



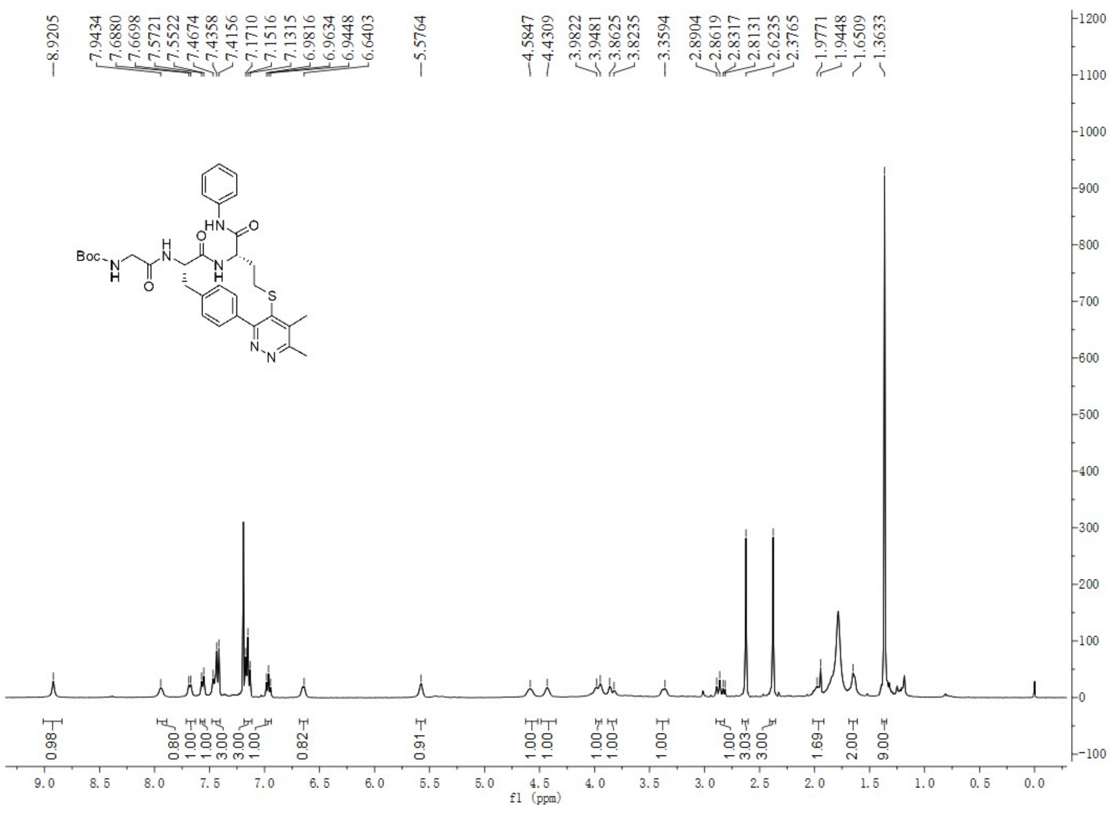
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **6i**



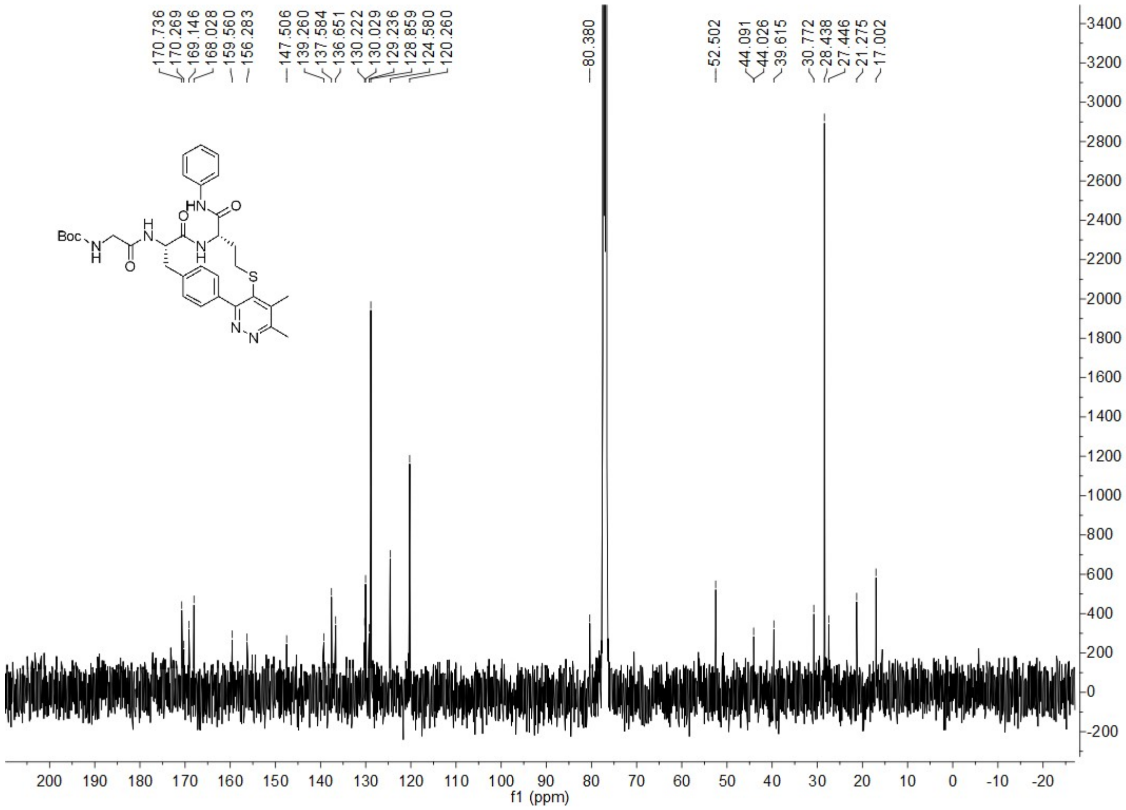
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7a**



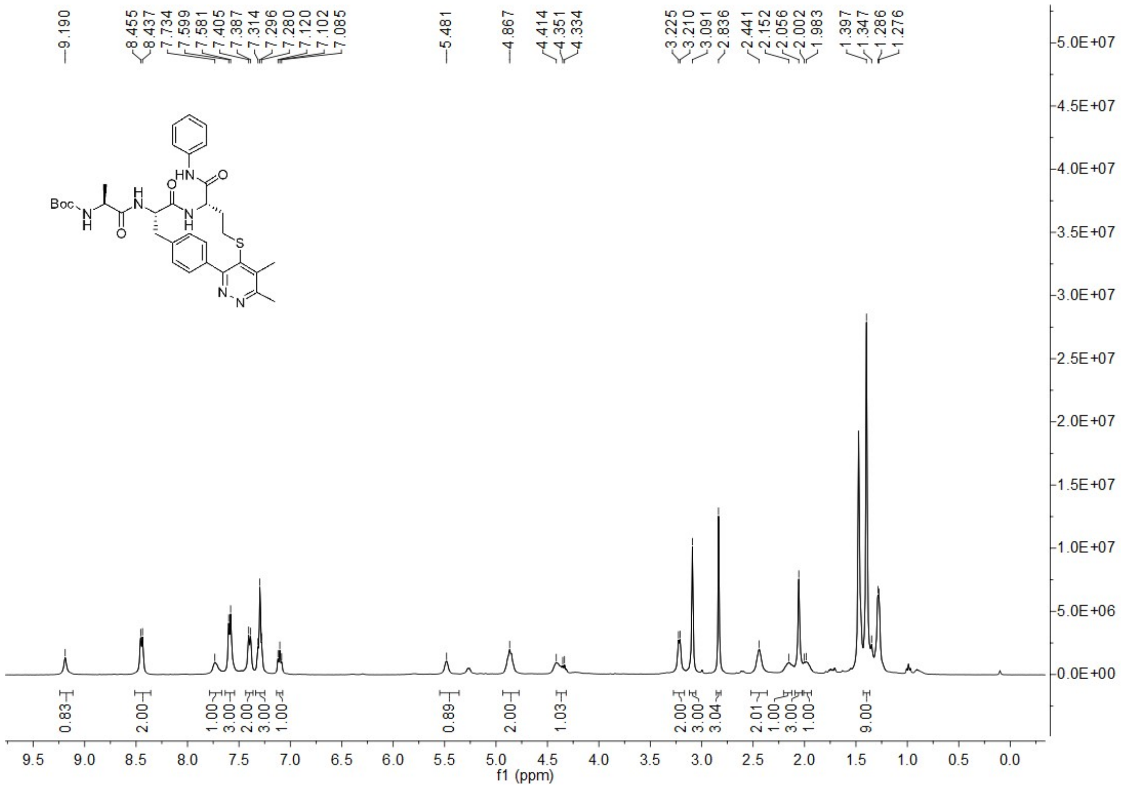
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7a**



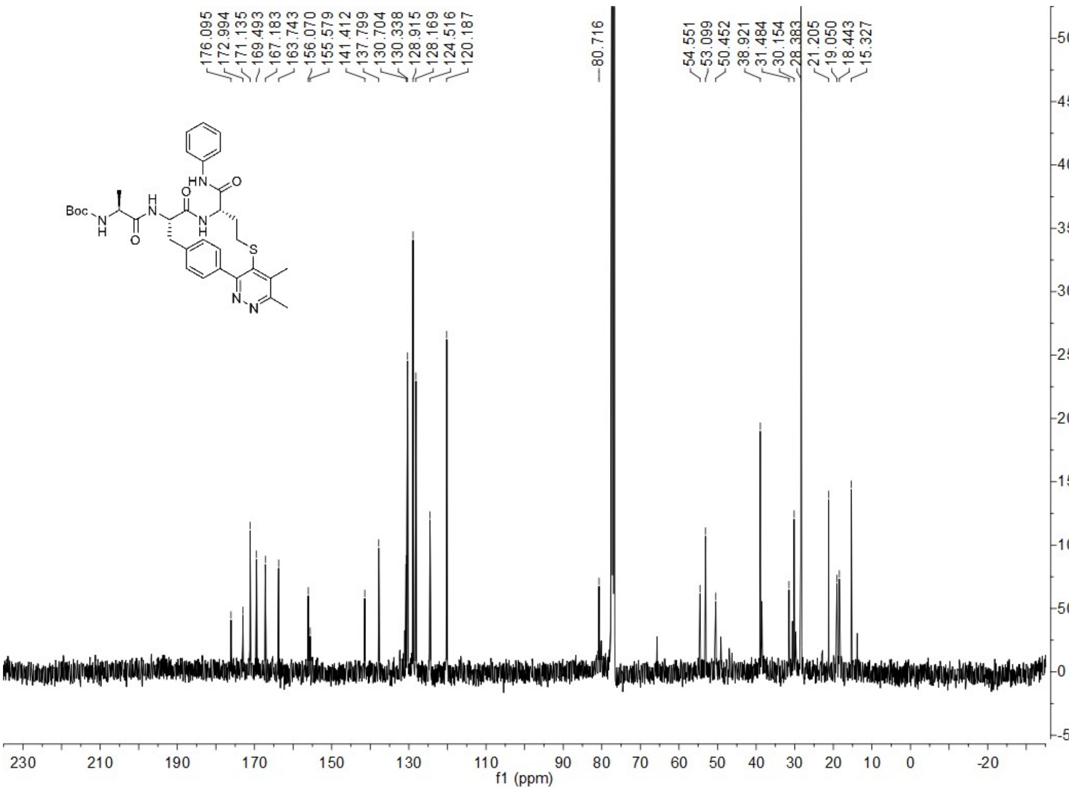
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7b**



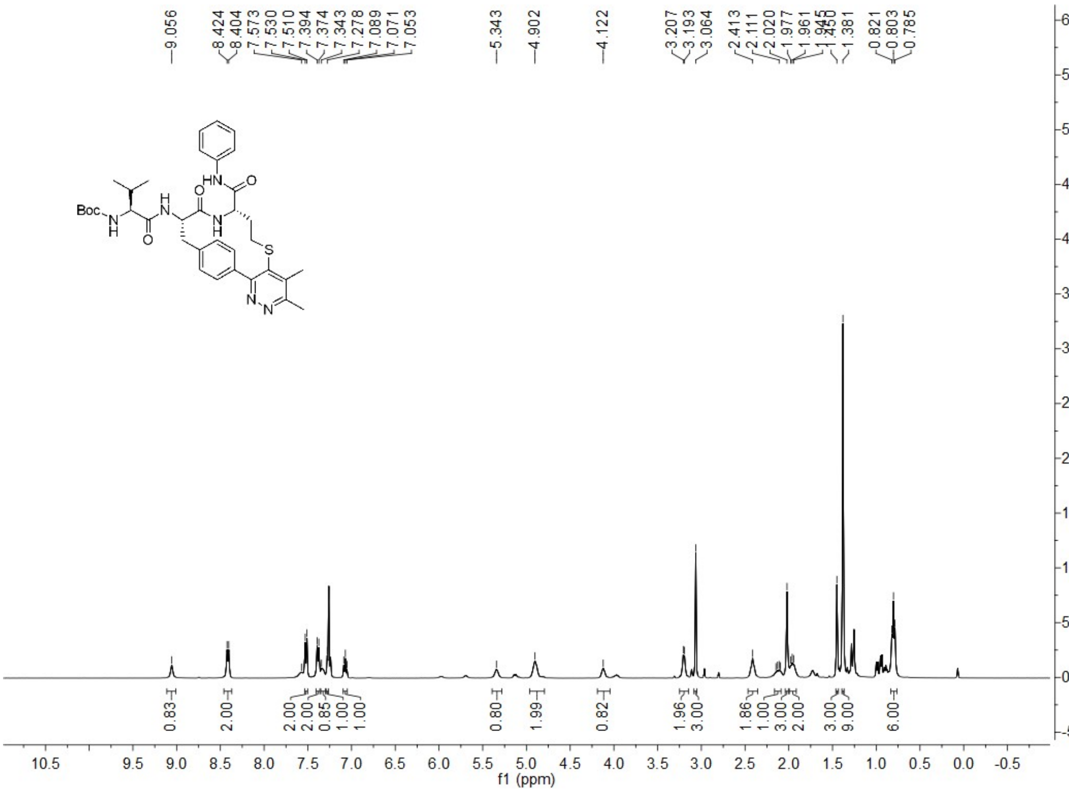
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7b**



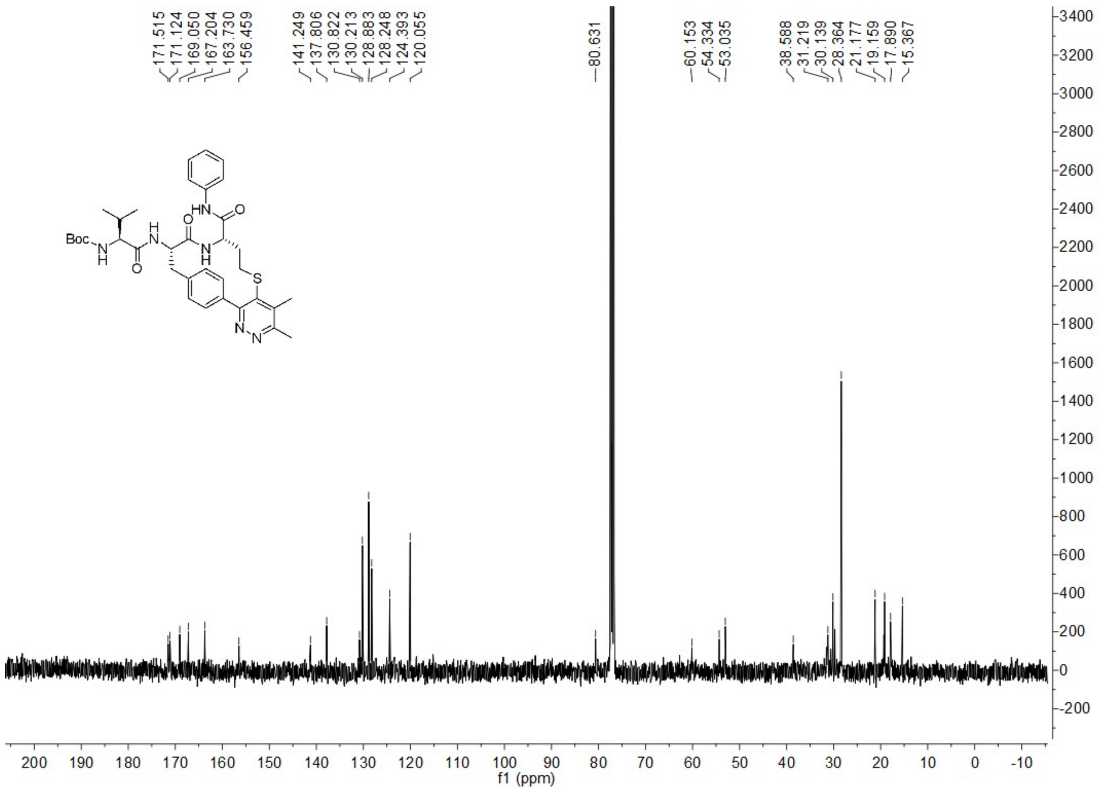
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7c**



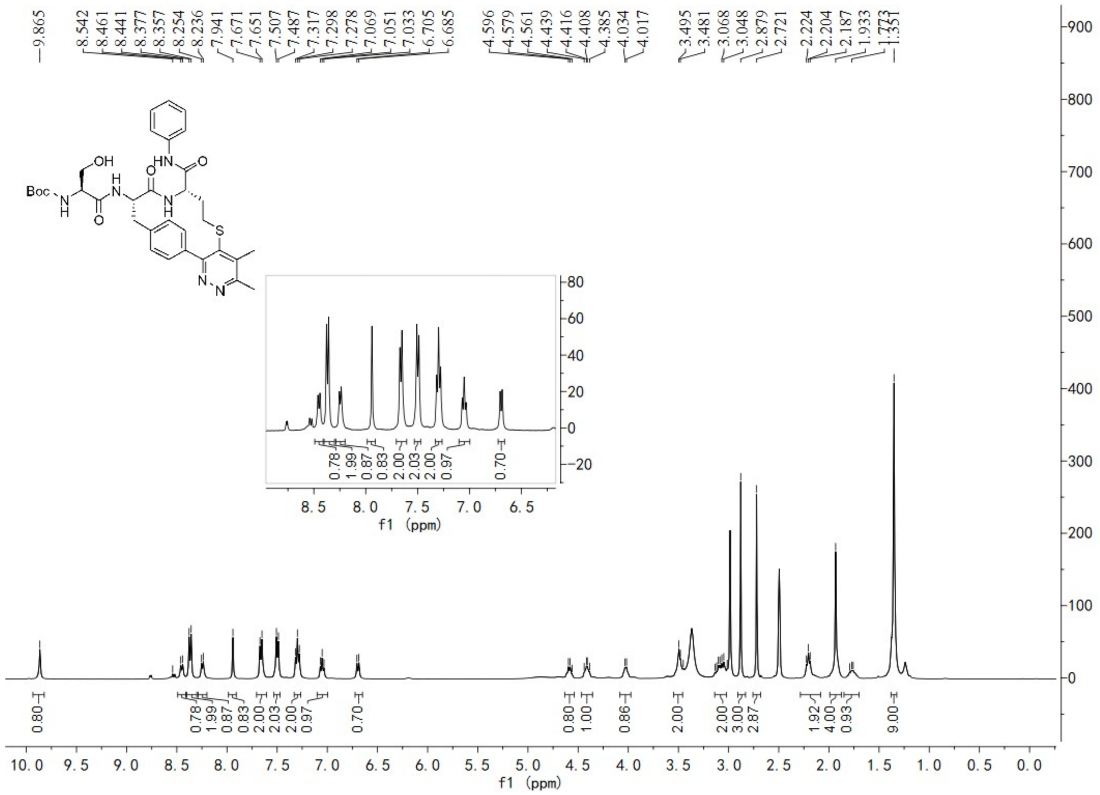
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7c**



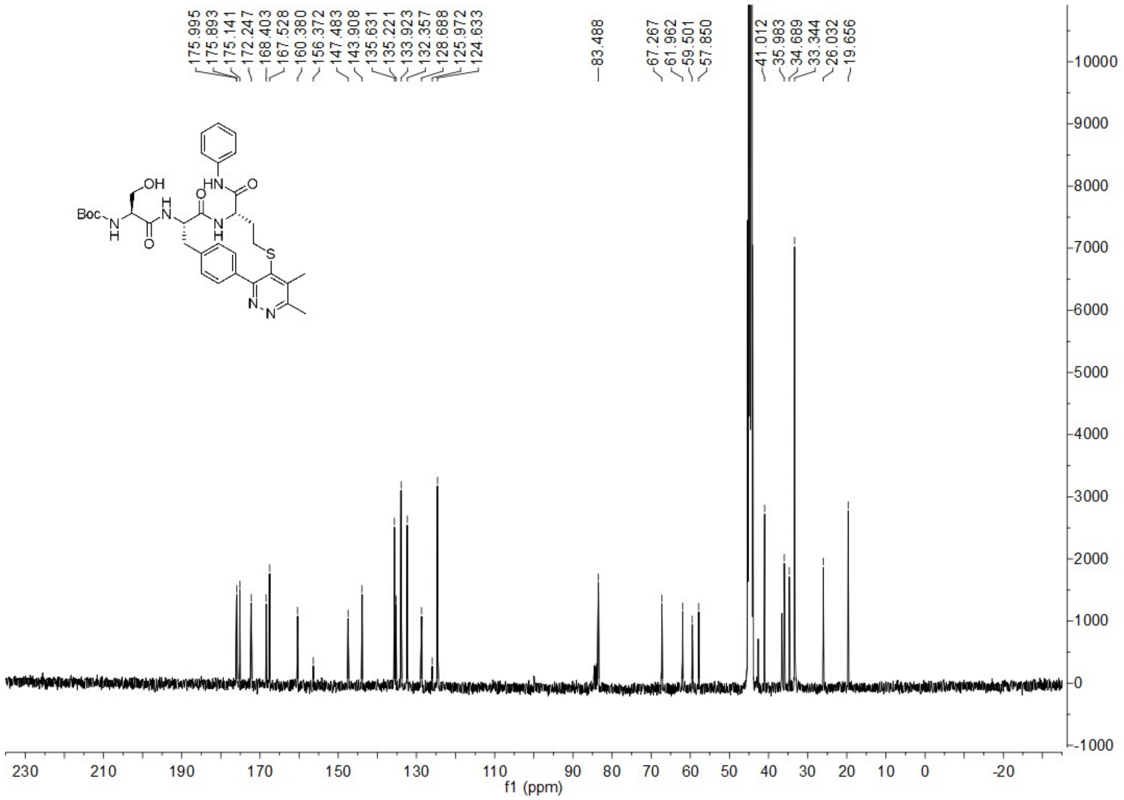
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7d**



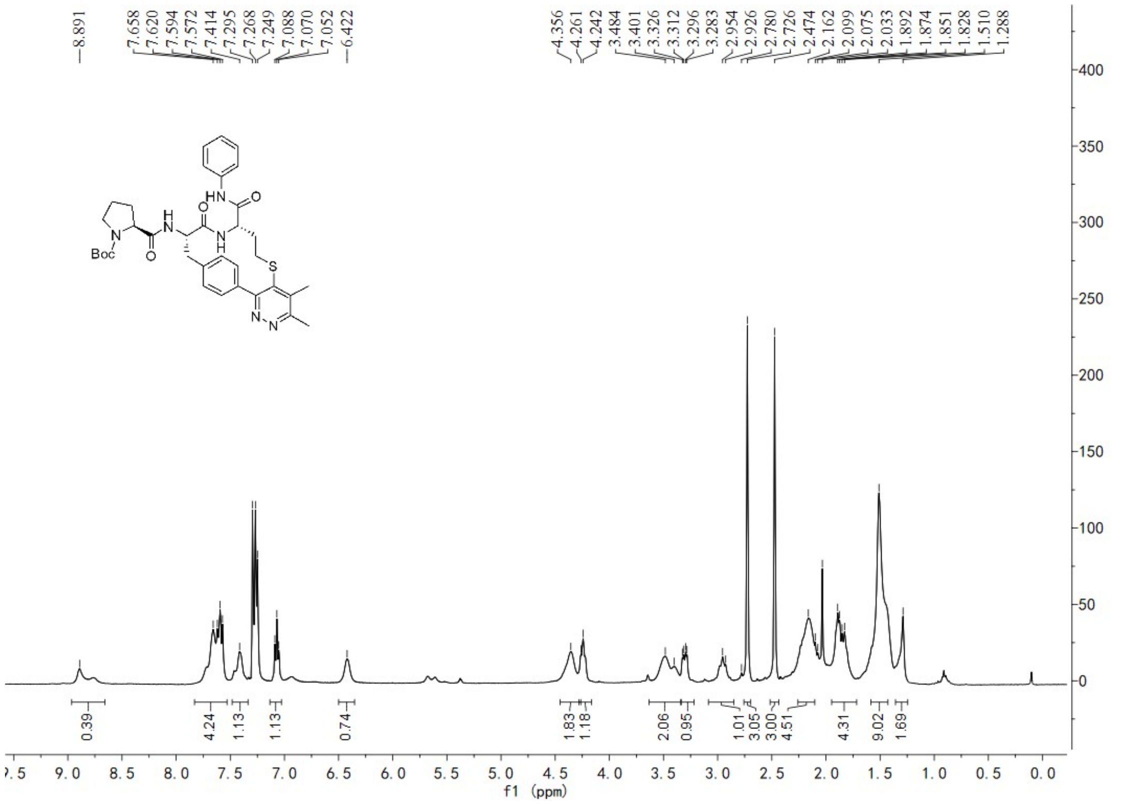
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7d**



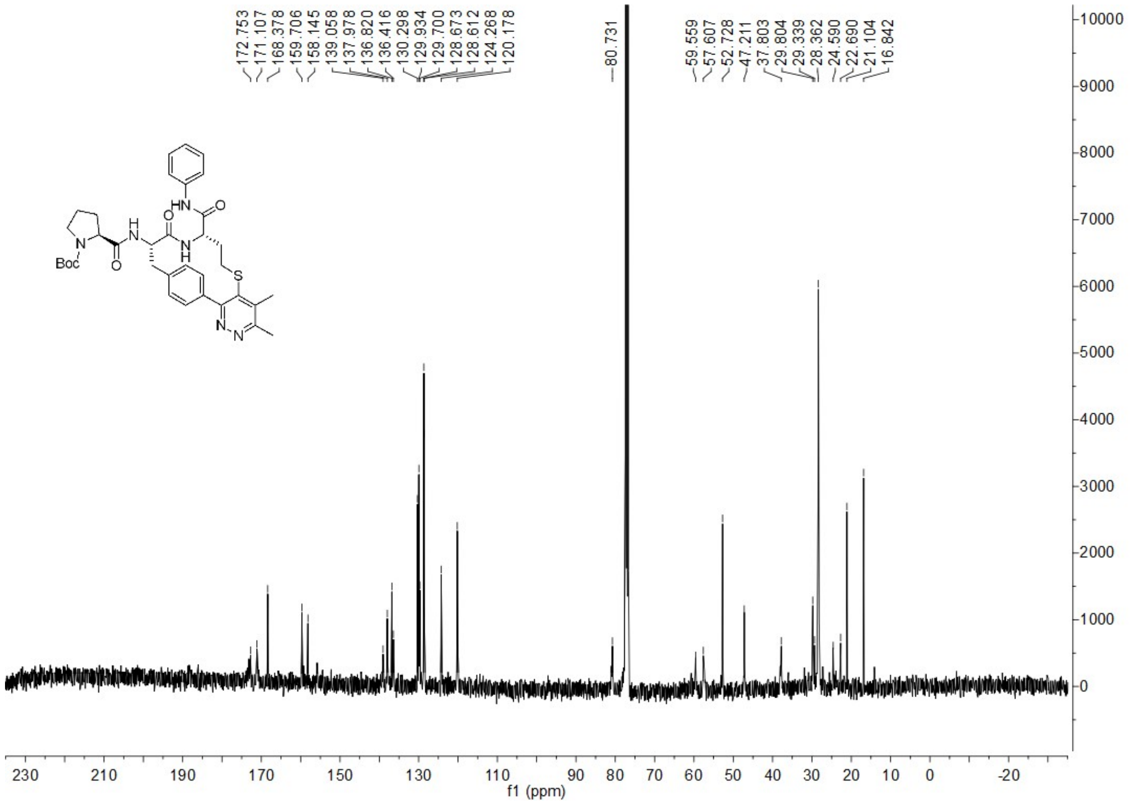
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7e**



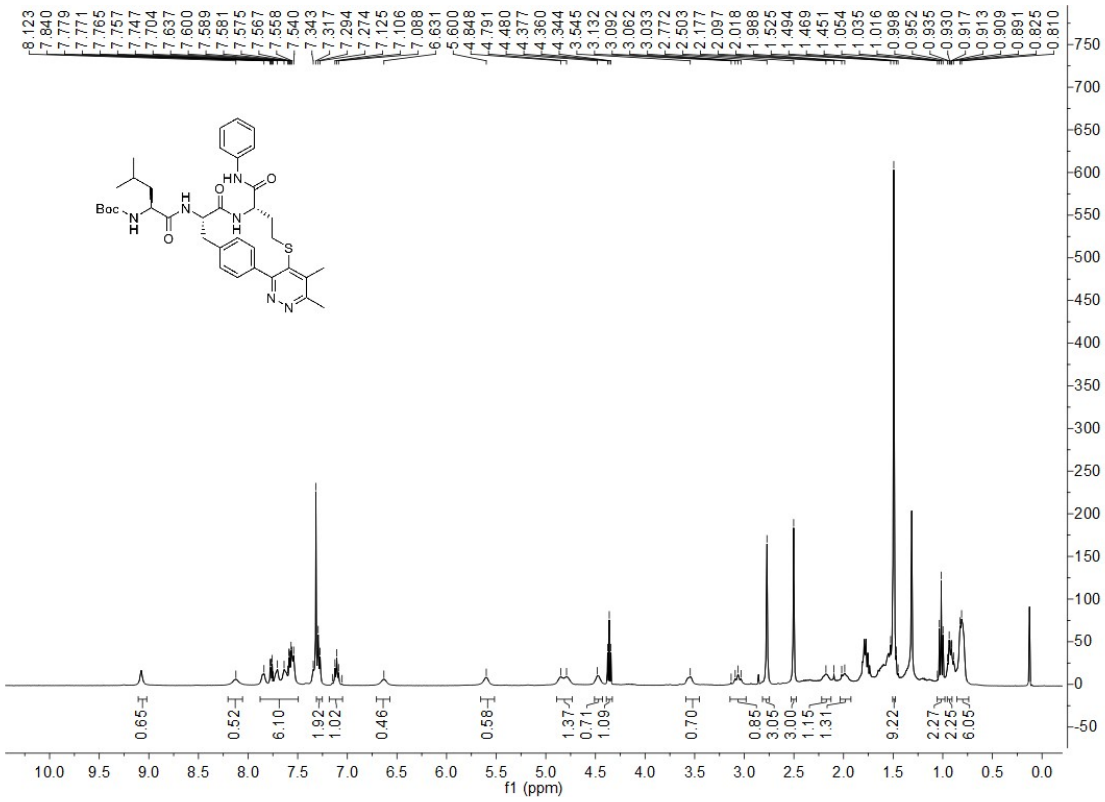
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7e**



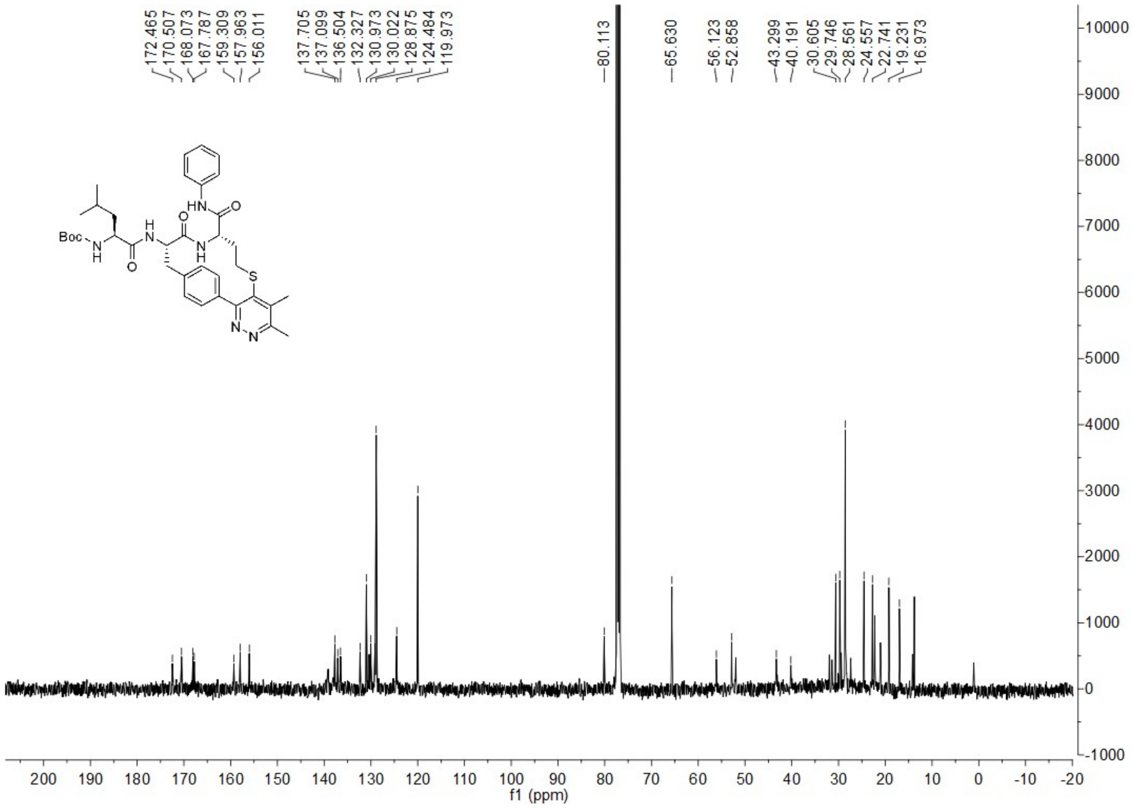
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7f**



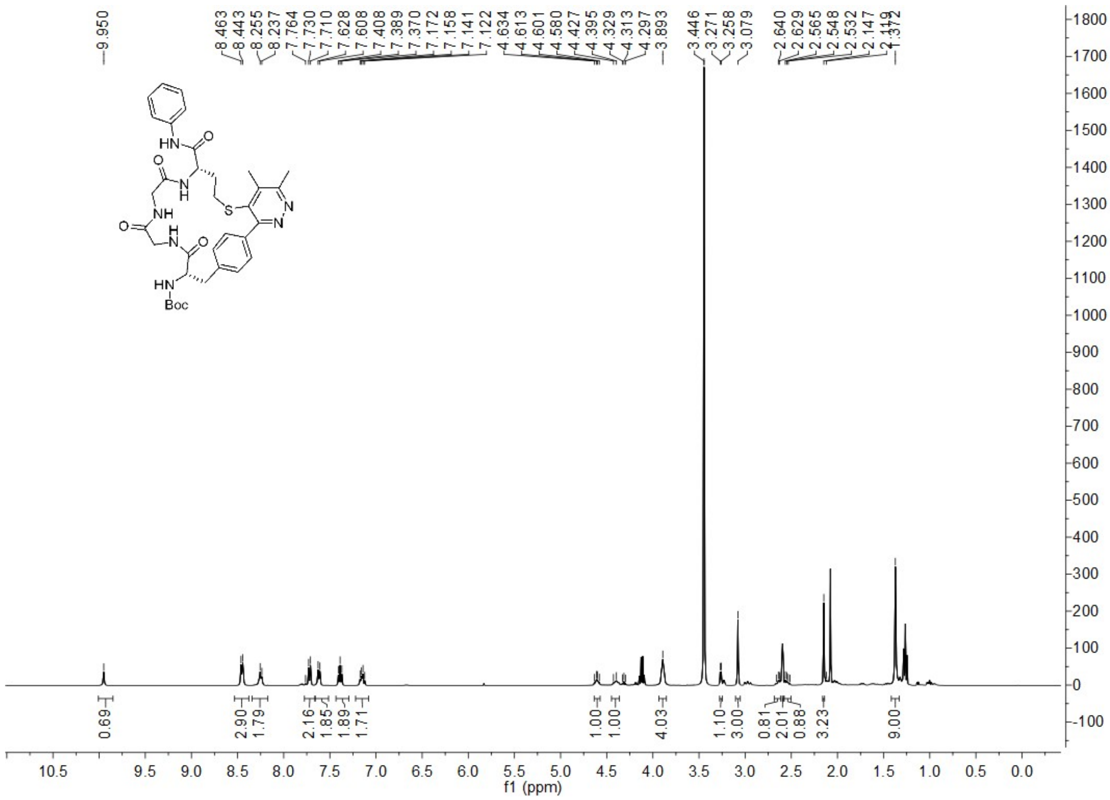
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7f**



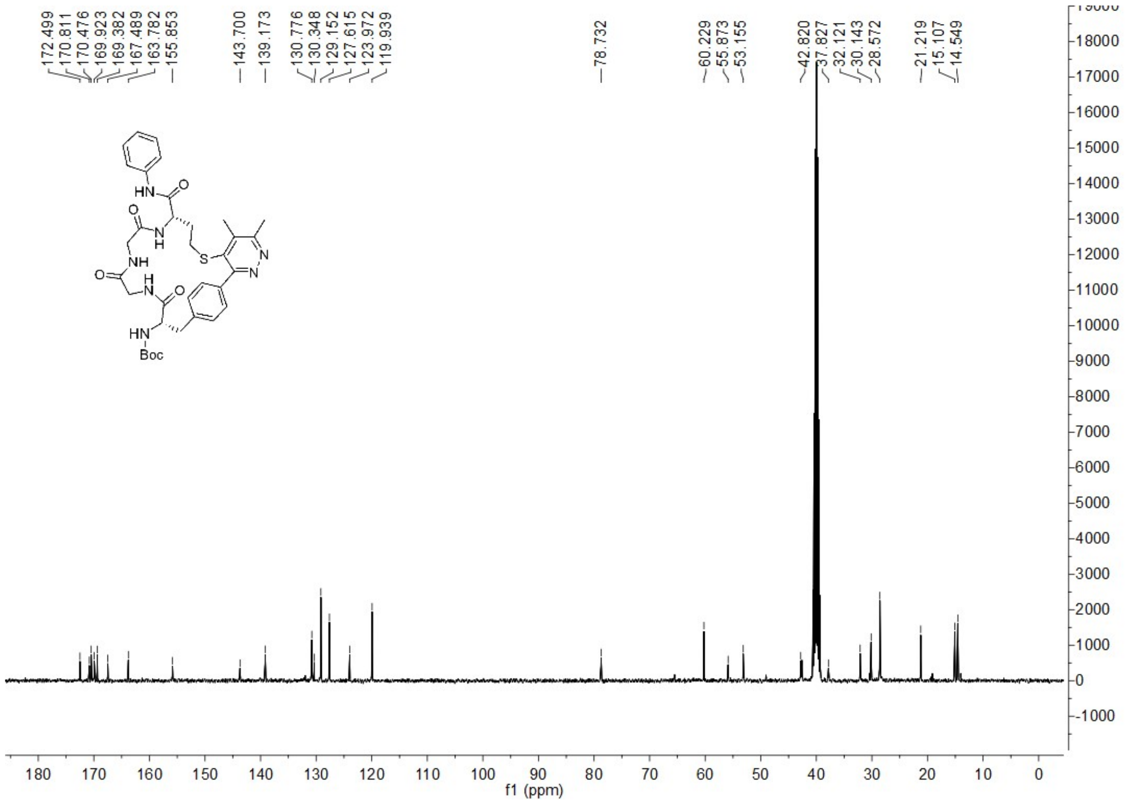
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7g**



^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7g**



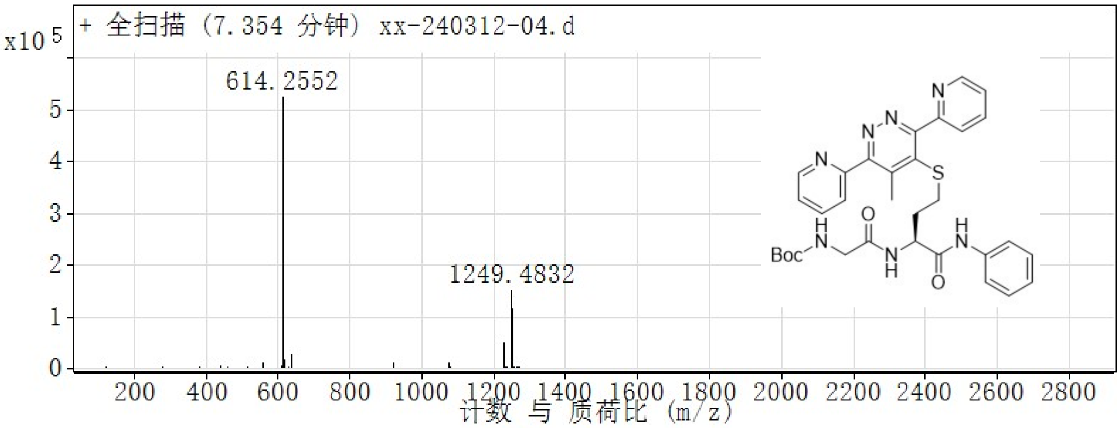
^{1}H NMR (400 MHz, CDCl$_3$) spectrum of compound **7i**



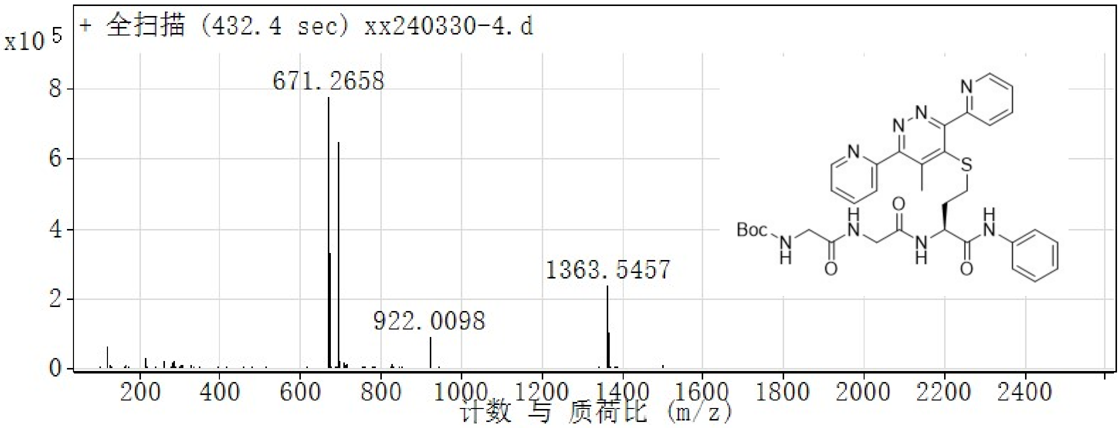
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound **7i**

7. The HRMS spectrum for confirmation of compounds 6a-7g

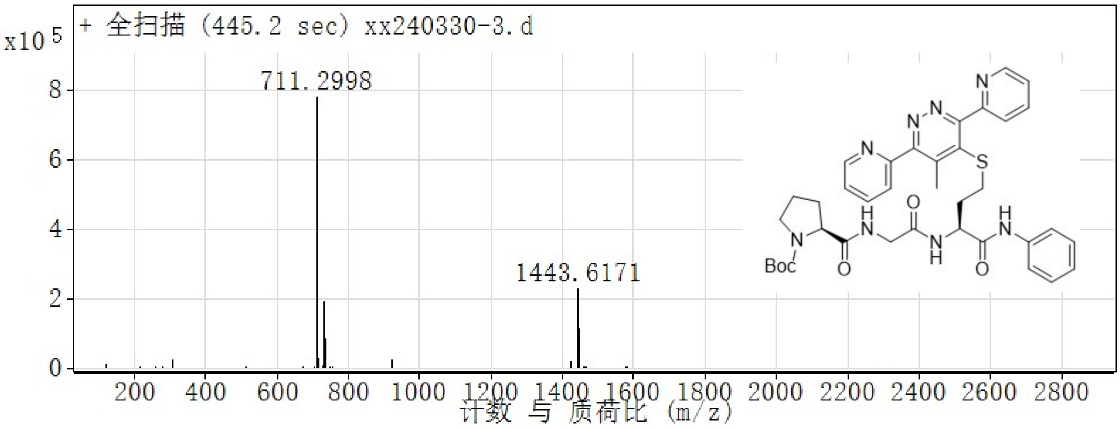
6a (HRMS)



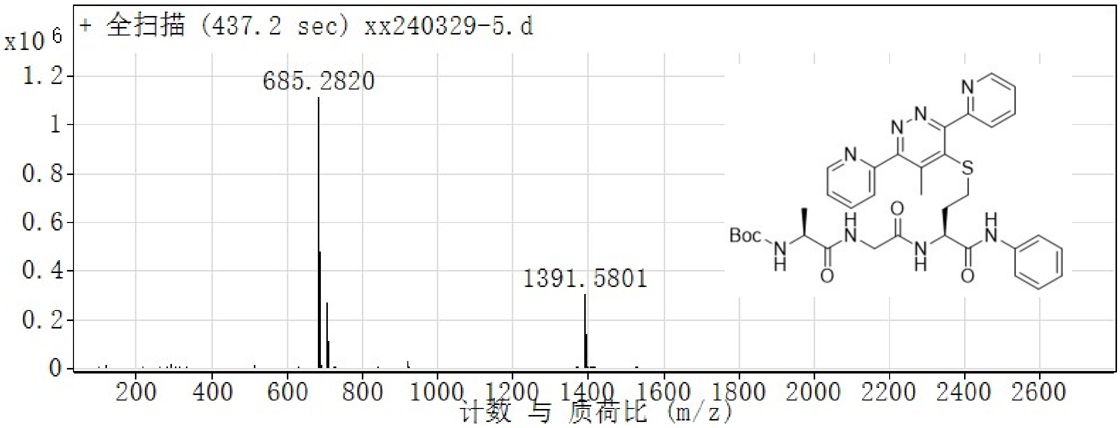
6b (HRMS)



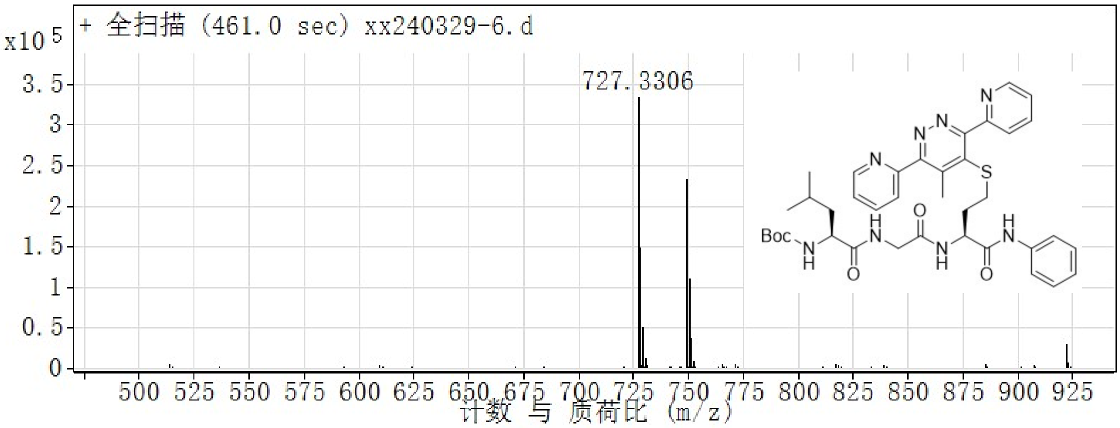
6c (HRMS)



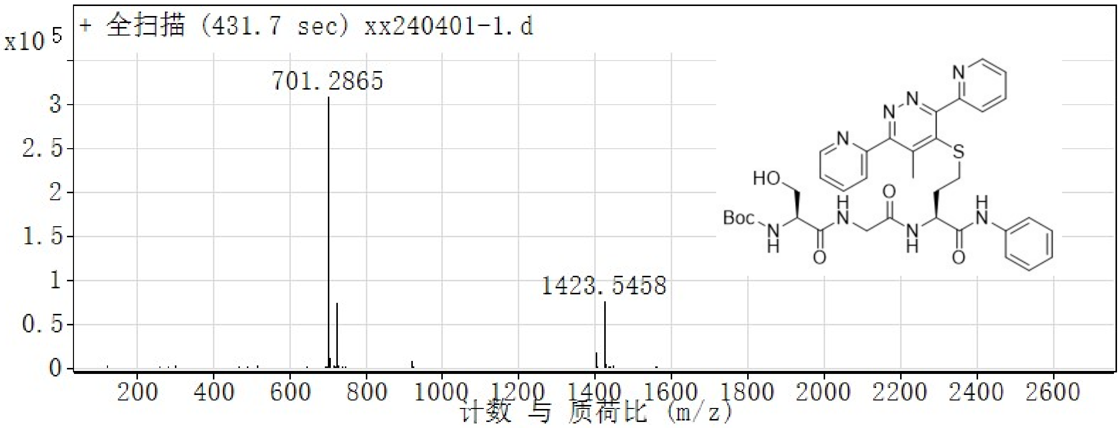
6d (HRMS)



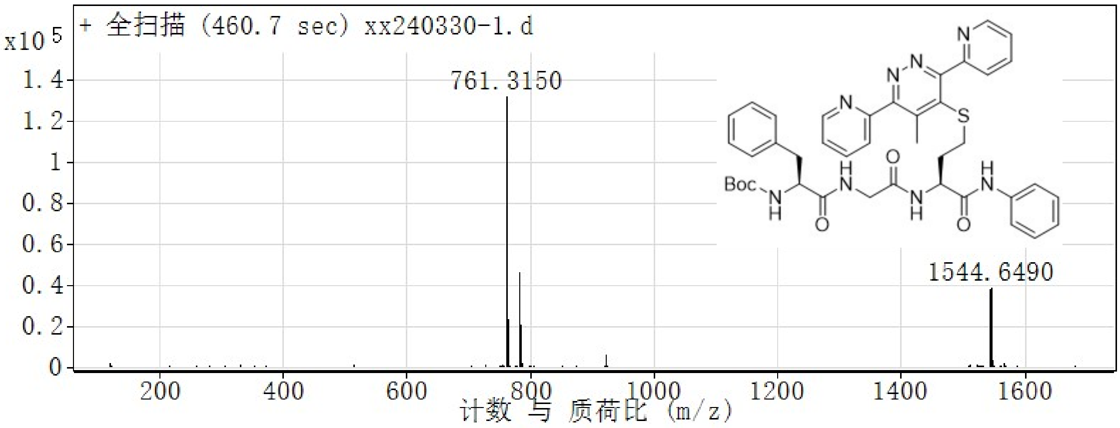
6e (HRMS)



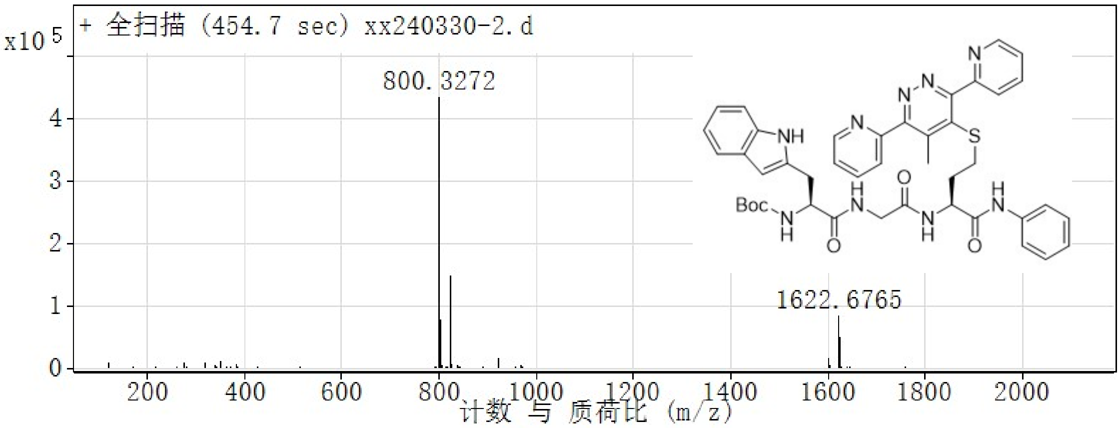
6f (HRMS)



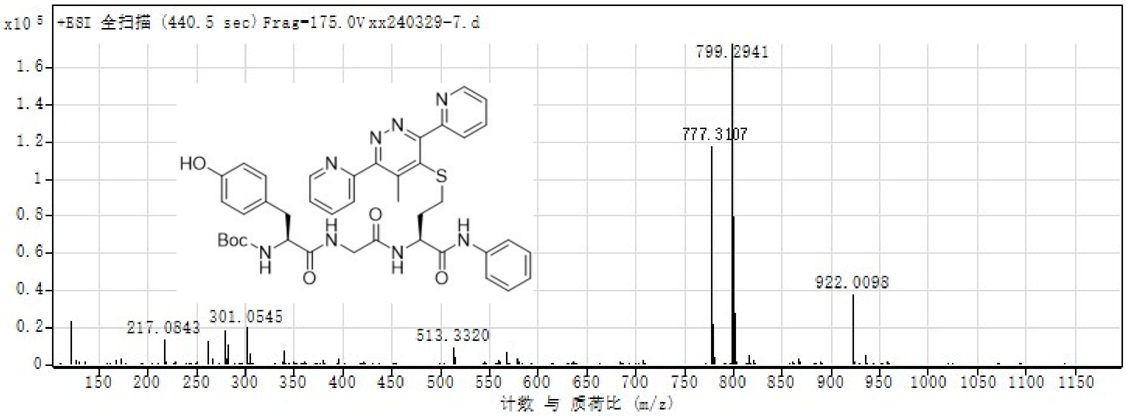
6g (HRMS)



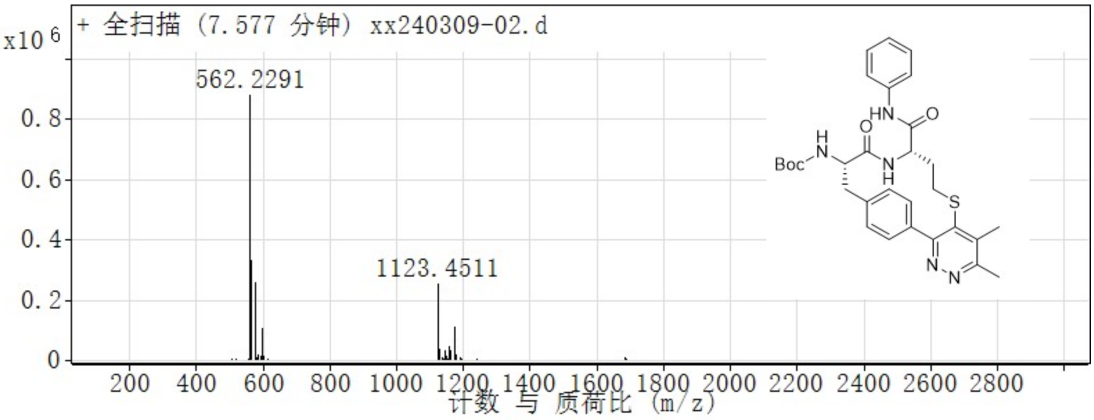
6h (HRMS)



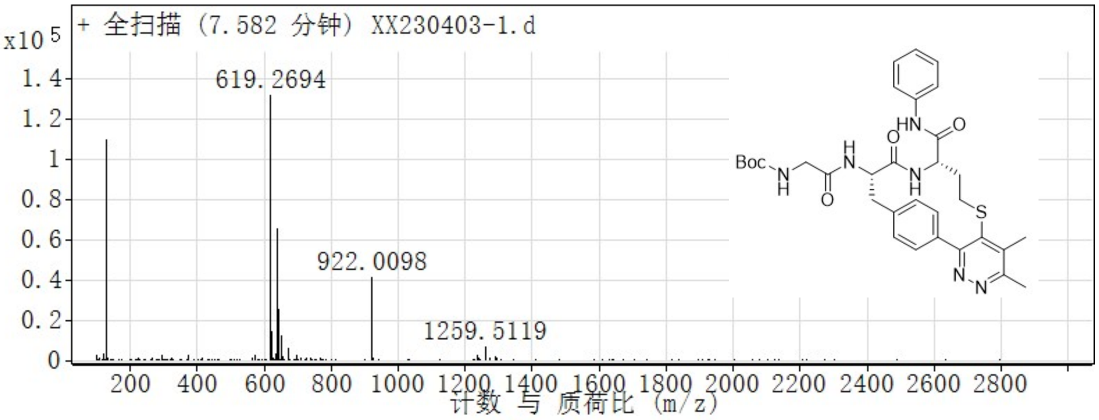
6i (HRMS)



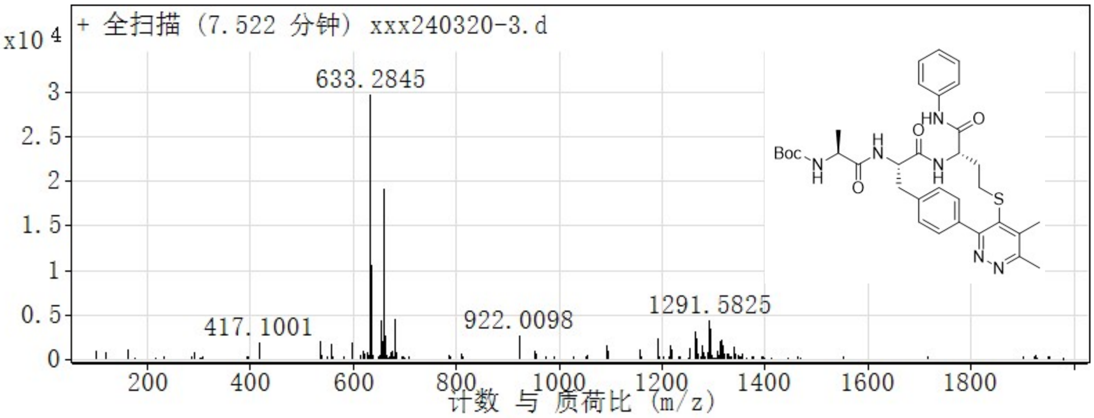
7a (HRMS)



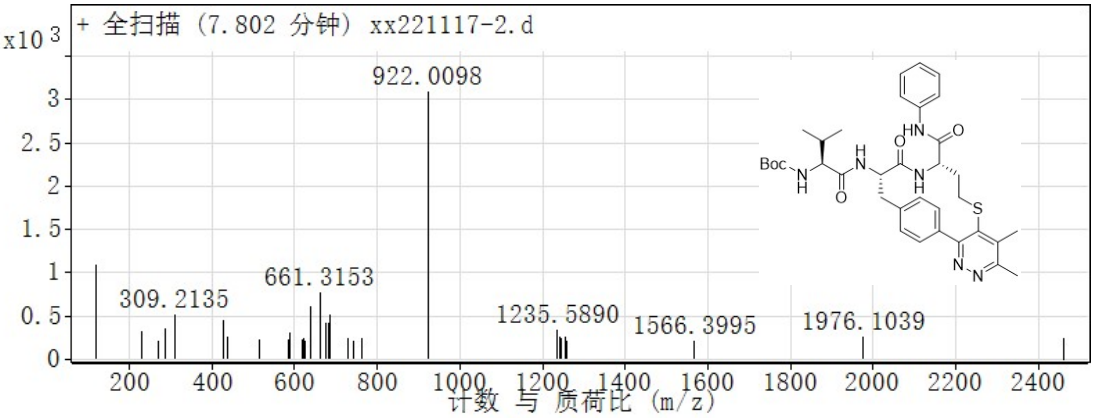
7b (HRMS)



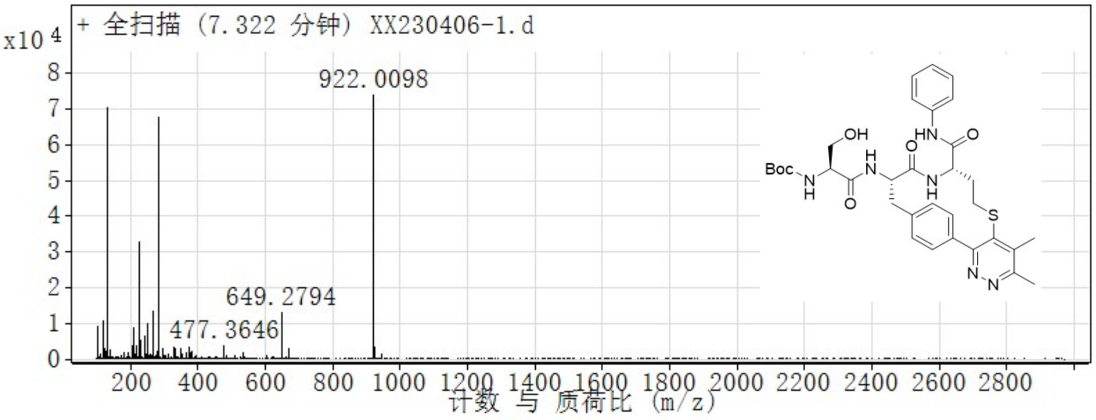
7c (HRMS)



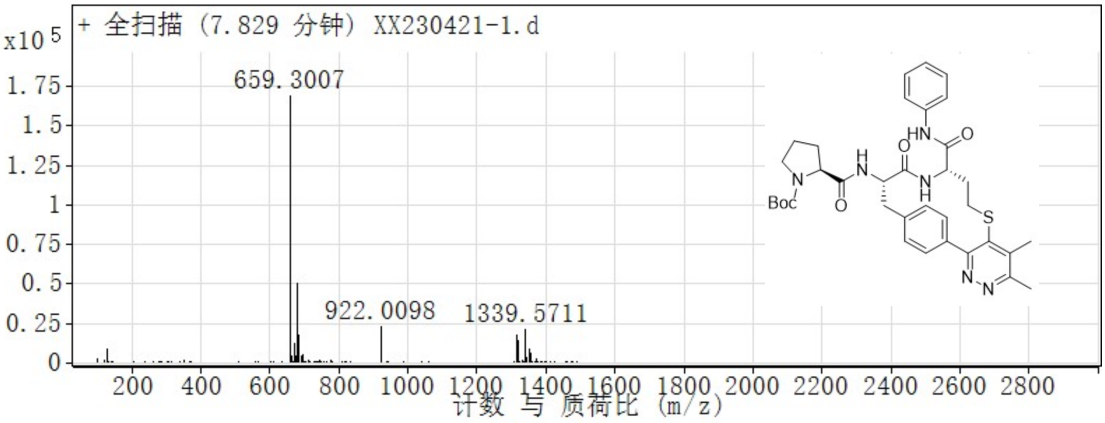
7d (HRMS)



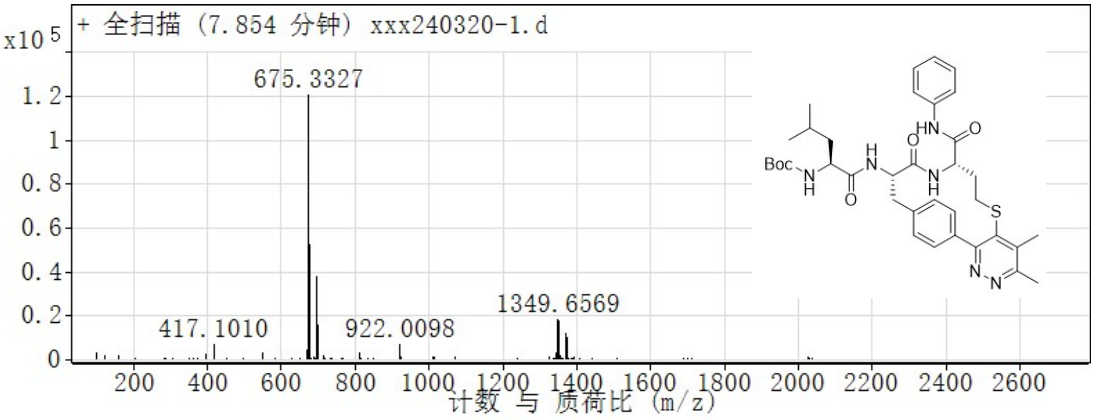
7e (HRMS)



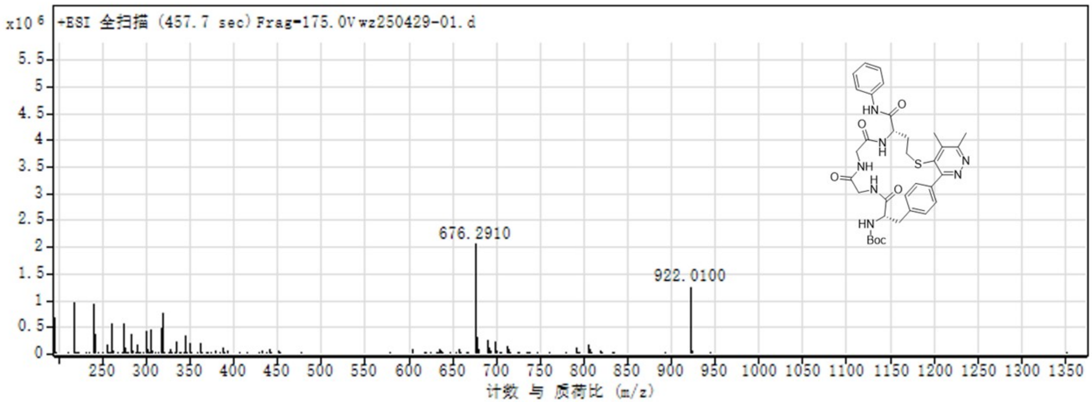
7f (HRMS)



7g (HRMS)

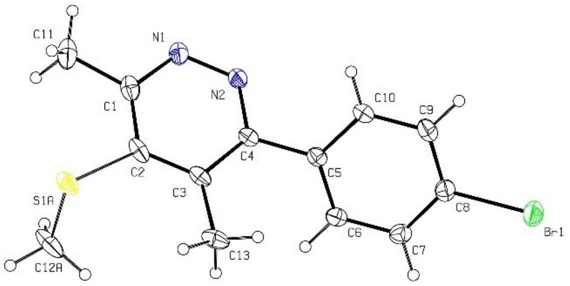


7i (HRMS)



8. X-ray crystallography Data of 3m

Single crystals of compound **3m** was measured on a Rigaku PAXIS–PAPID single–crystal diffractometer. The recrystallization solvent of **3m** was methanol.



X–ray crystallography of **3m**

Figure S3 X–ray crystallography of **3m** (the ellipsoid contour probability level is 50%)

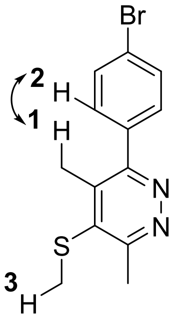
Table S3 X–ray crystallography data of **3m**

Formula moiety	$C_{13}H_{13}BrN_2S$
Formula sum	$C_{13}H_{13}BrN_2S$
Formula weight	309.22
Temperature	170K
Crystal system	monoclinic
Space group	$P2_{1/c}$
Unit cell dimensions	a=12.2575(10)
	b=7.9358(6)
	c=13.1764(10)
	alpha=90
	beta=95.726(3)
	gamma=90
Volume	1275.31(17)
Z	4
Calculated density	1.611 g/cm^3
Absorption coefficient	3.365

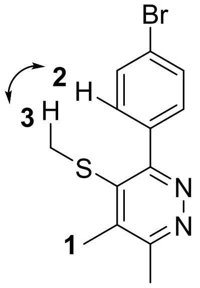
F(000)	624.0
Crystal size	0.32×0.23×0.05 mm
Theta range for data collection	6 to 55.002 deg.
Reflections collected	18037
Independent reflections	2913 [R_{int} = 0.0554, R_{sigma} = 0.0380]
Data / restraints / parameters	2913/51/177
Goodness–of–fit on F^2	1.056
Final R indexes [I>=2σ (I)]	R_1 = 0.0296, wR_2 = 0.0666
Final R indexes [all data]	R_1 = 0.0403, wR_2 = 0.0721
Largest diff. peak/hole / e Å^{-3}	0.44/-0.44

9. The ^{1}H-^{1}H NOESY spectrum for confirmation of structure of 3m and 3n

According to the chemical shifts and the coupling constants in ^1H NMR spectra, the signals of the key H-1, H-2, can be identified. As shown in NOESY spectra of 3m, correlations between H-1 and H-2 were observed, instead of H-2 with H-3. Which indicated that the structure can't be 3m'. Therefore, the structure of representative product should be 3m. The single crystal results of 3m are consistent with the inference.



3m



3m'

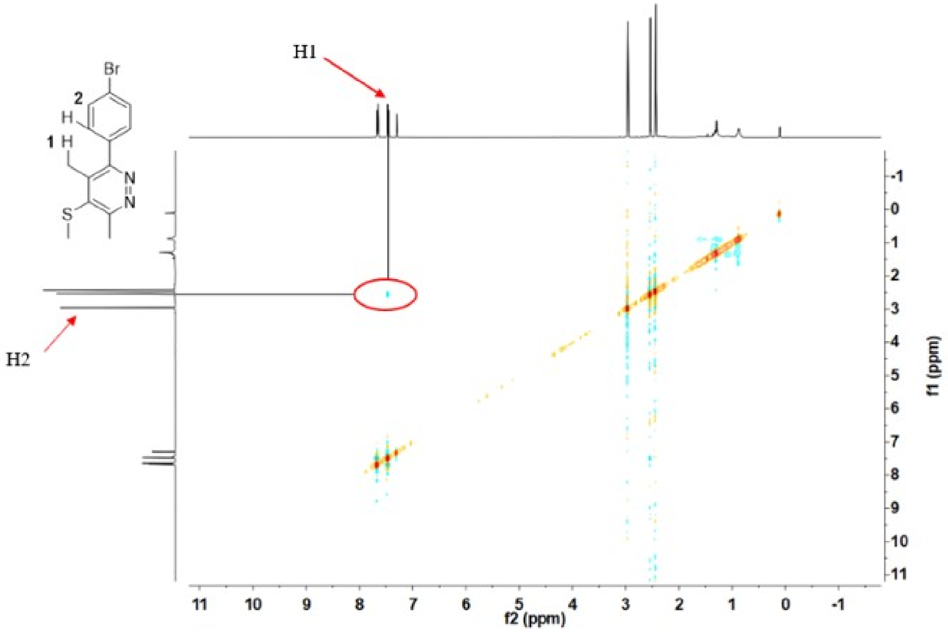


Figure S4 ^1H-^1H-NOESY spectrum of **3m**

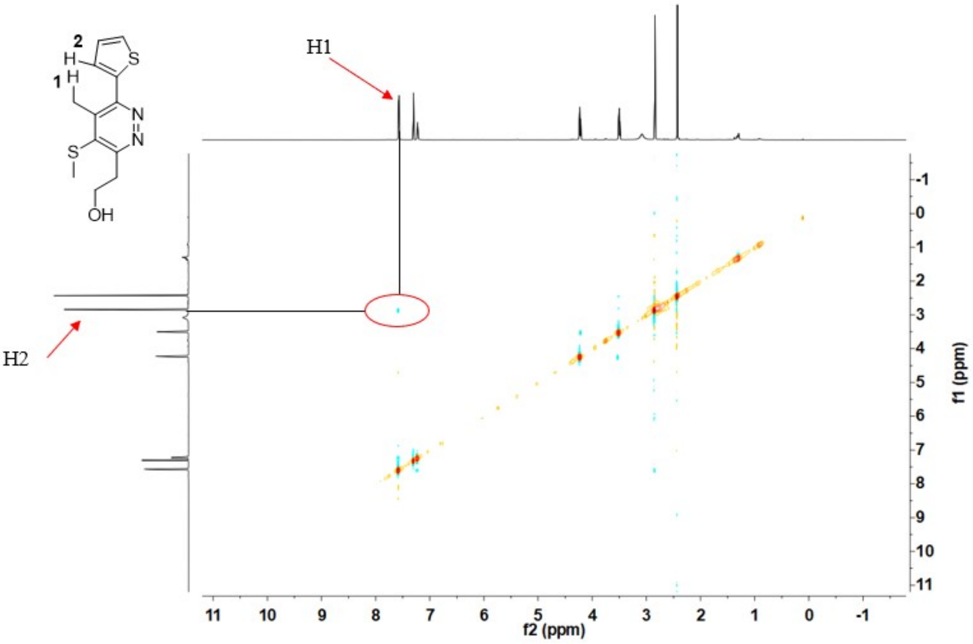


Figure S5 ^{1}H-^{1}H-NOESY spectrum of **3n**