Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Mechanism-Based Inhibitors of the Human Sirtuin 5 Deacylase: Structure–Activity Relationship, Biostructural, and Kinetic Insight

*Nima Rajabi, Marina Auth, Kathrin R. Troelsen, Martin Pannek, Dhaval P. Bhatt, Martin Fontenas, Matthew D. Hirschey, Clemens Steegborn, Andreas S. Madsen, and Christian A. Olsen**

anie_201709050_sm_miscellaneous_information.pdf

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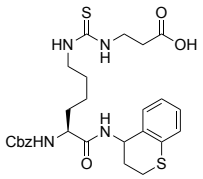
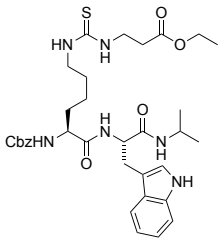
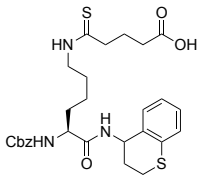


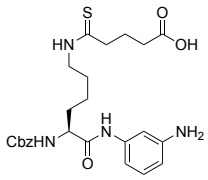
V, 8 ± 1 μM
Rate inhib. assay:
K_i = 13 ± 1 μM



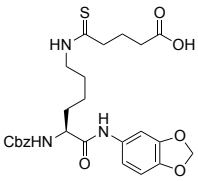
Et-29, 54% [100 μM]



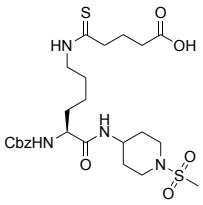
S1, 87% [100 μM]



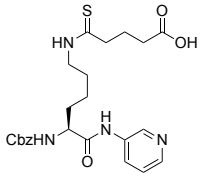
S2, 82% [100 μM]



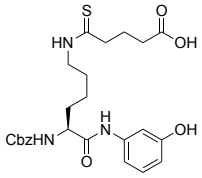
S3, 80% [100 μM]



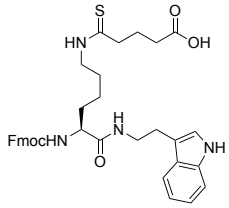
S4, 72% [100 μM]



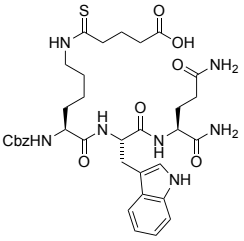
S5, 63% [100 μM]



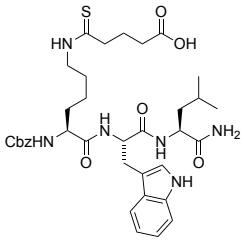
S6, 71% [100 μM]



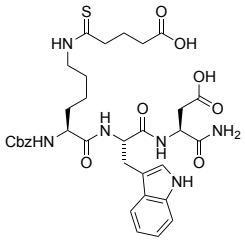
S7, 71% [100 μM]



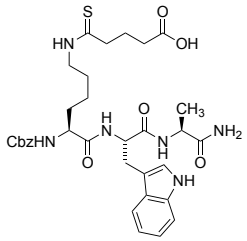
S8, 0.98 ± 0.02 μM



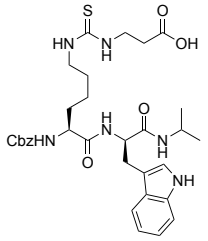
S9, 0.45 ± 0.03 μM



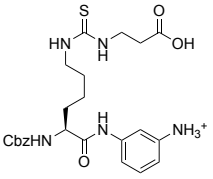
S10, 76% [10 μM]



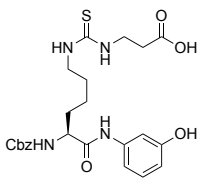
S11, 86% [10 μM]



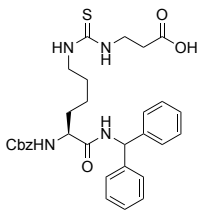
S12, 92% [100 μM]



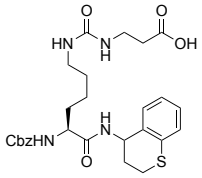
S13, 78% [100 μM]



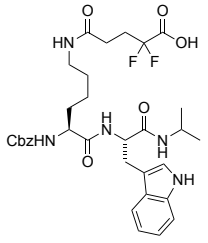
S14, 52% [100 μM]



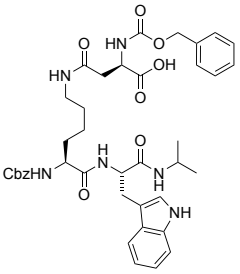
S15, 76% [100 μM]



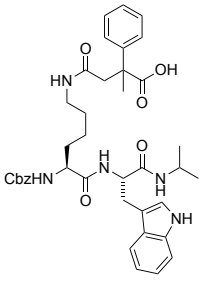
S16, 53% [100 μM]



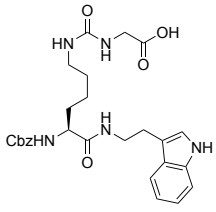
S17, 84% [100 μM]



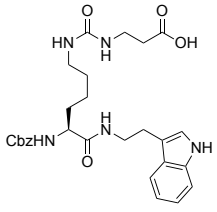
S18, 24% [100 μM]



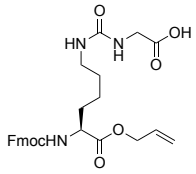
S19, 240 ± 10 μM



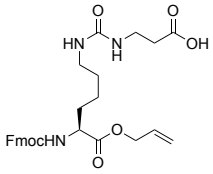
S20, 14% [100 μM]



S21, 63% [100 μM]

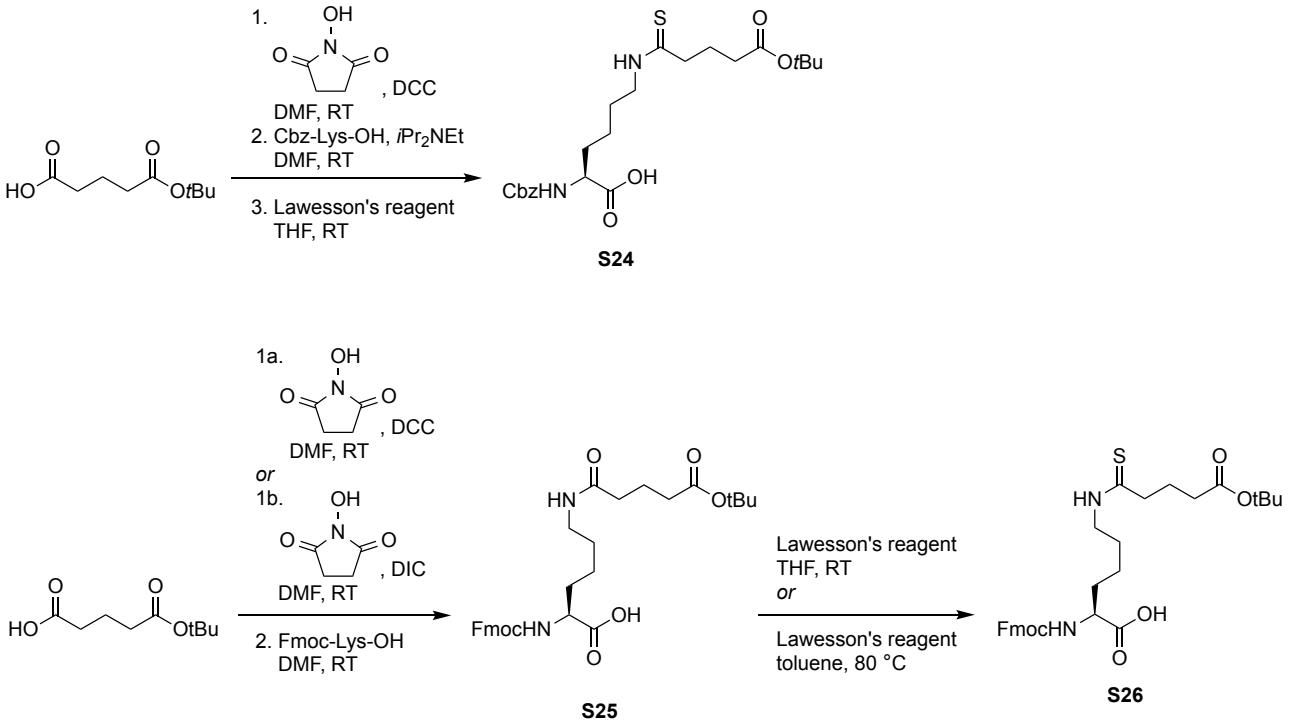


S22, 18% [100 μM]

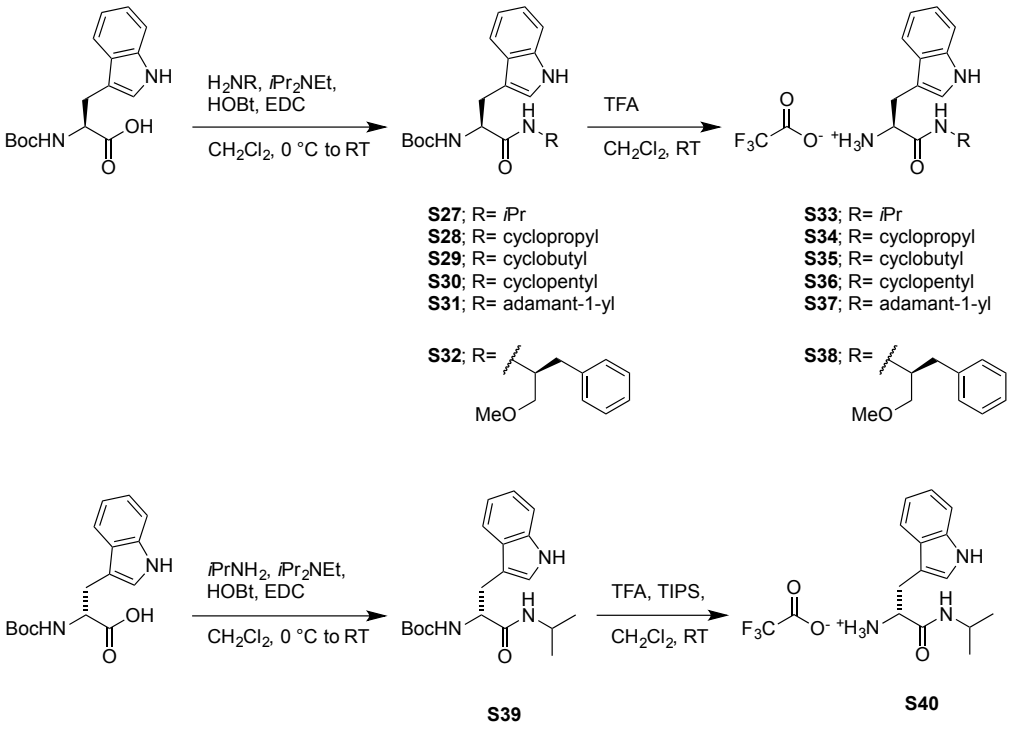


S23, 32% [100 μM]

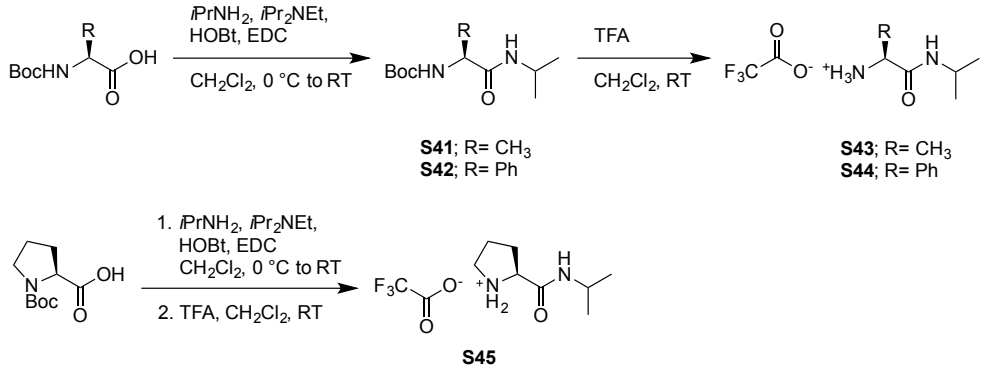
Scheme S1. Additional inhibitors for the structure–activity relationship study. Potencies are given as IC_{50} values or inhibition (%) at the highest tested concentration (given in brackets)



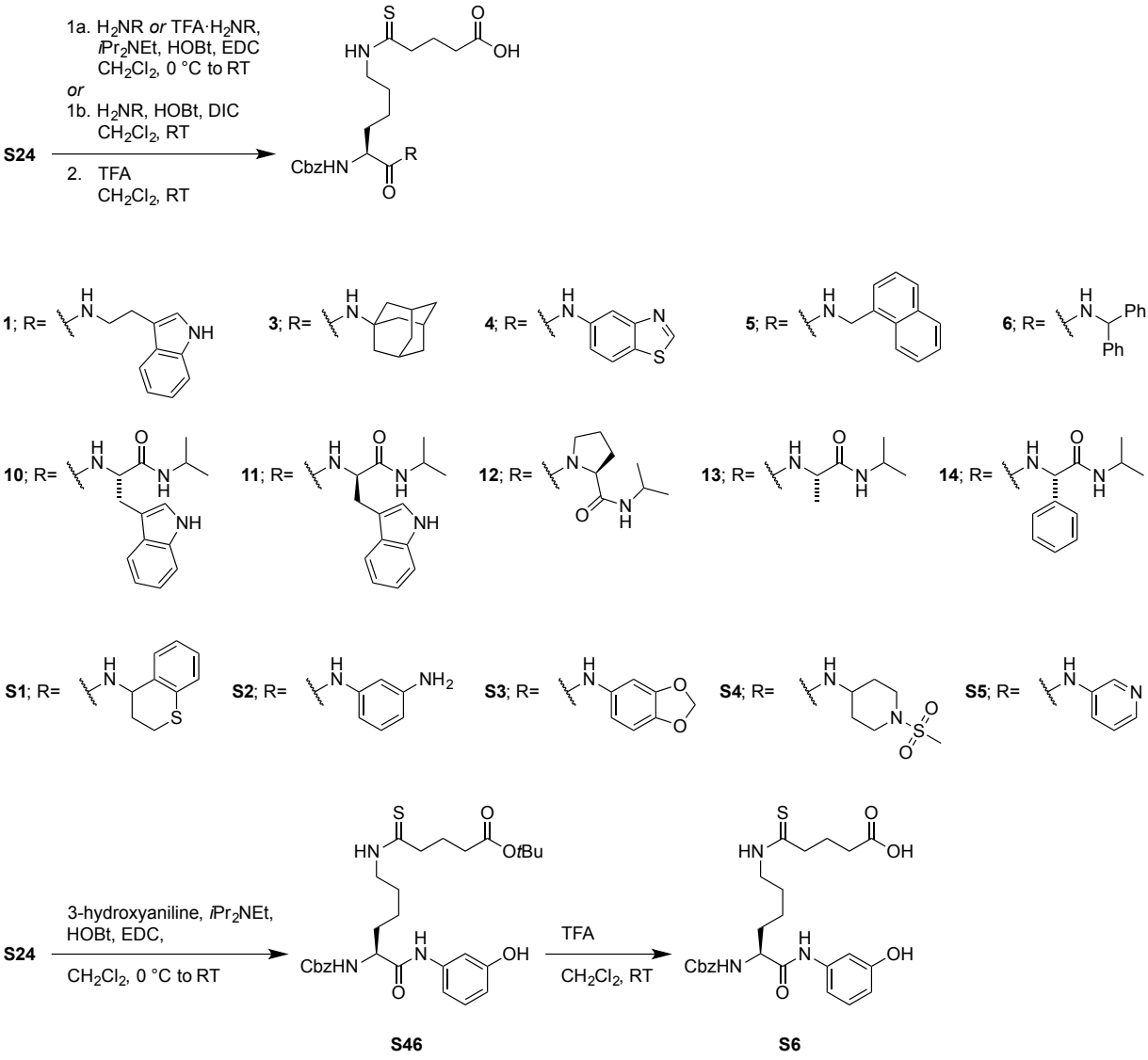
Scheme S2. Synthesis of thioamide building blocks



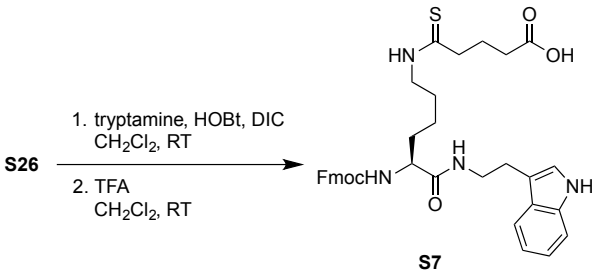
Scheme S3. Synthesis of tryptophan building blocks



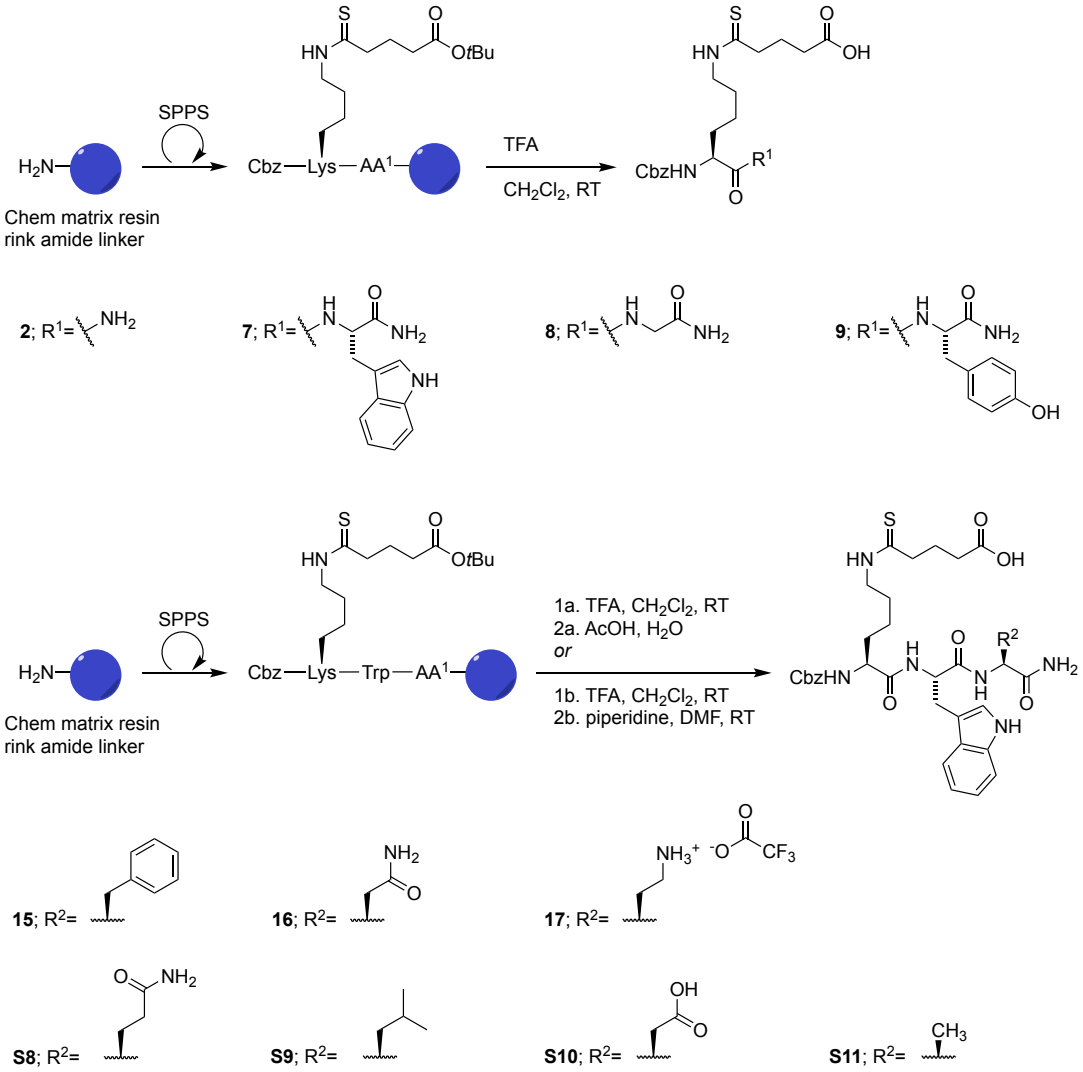
Scheme S4. Synthesis of other amino acid building blocks



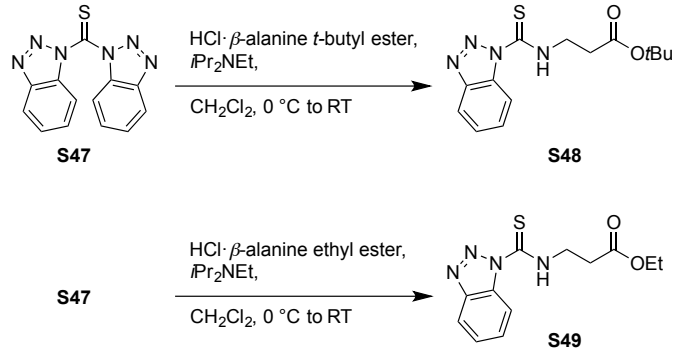
Scheme S5. Synthesis of thioamide inhibitors, part I



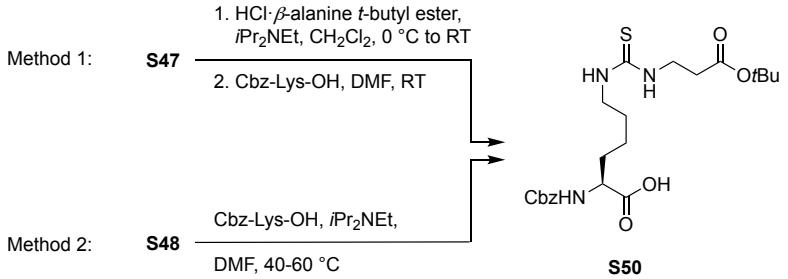
Scheme S6. Synthesis of thioamide inhibitor, part II



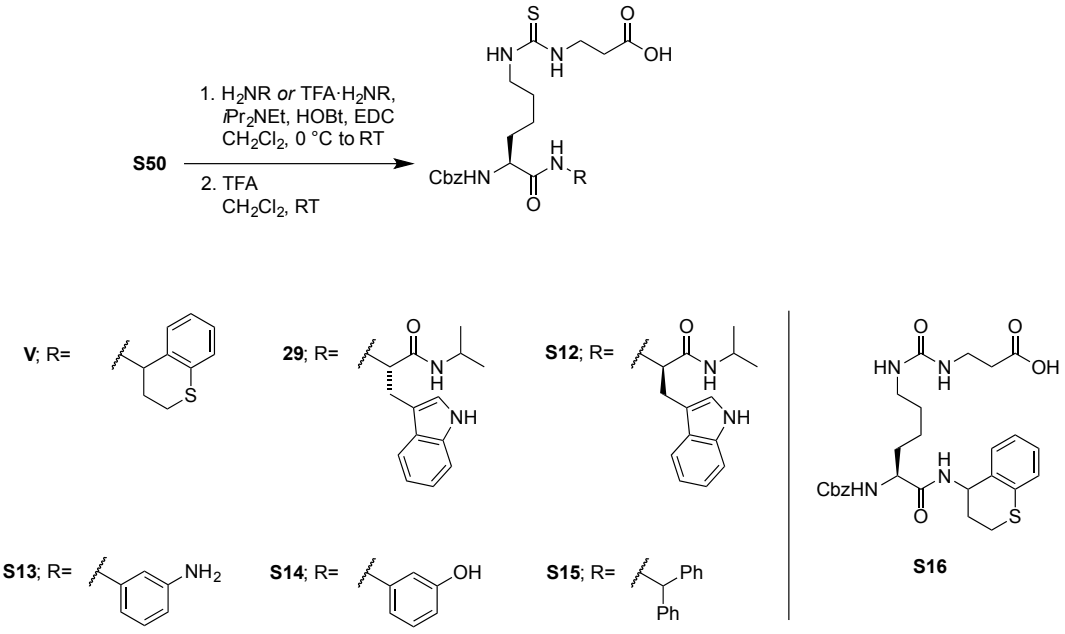
Scheme S7. Solid phase synthesis of tripeptide thioamide inhibitors



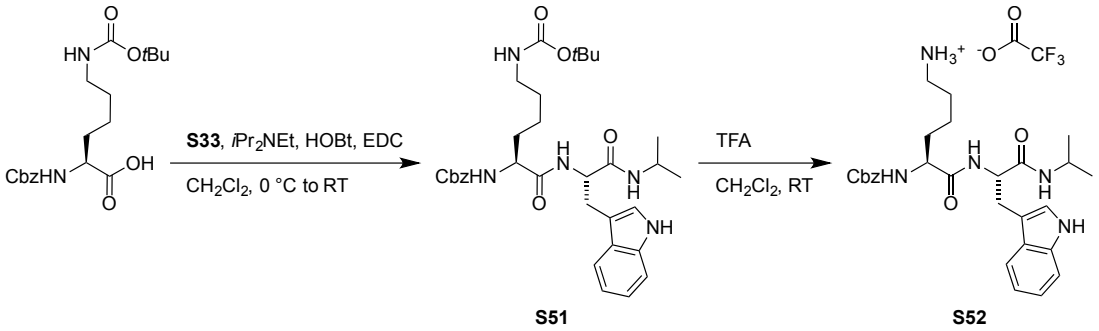
Scheme S8. Synthesis of benotriazole carbothioamido reagents



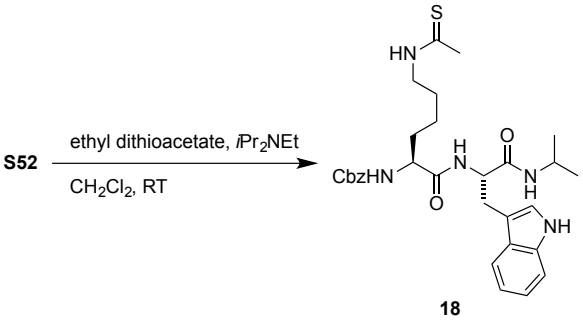
Scheme S9. Synthesis of thiourea building block **S50**



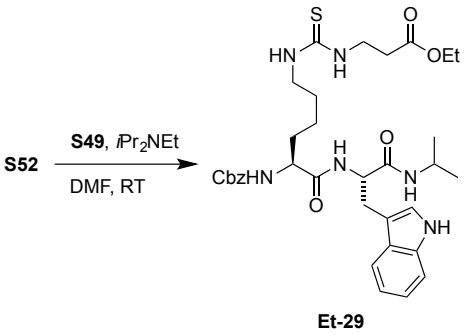
Scheme S10. Synthesis of thiourea inhibitors, part I



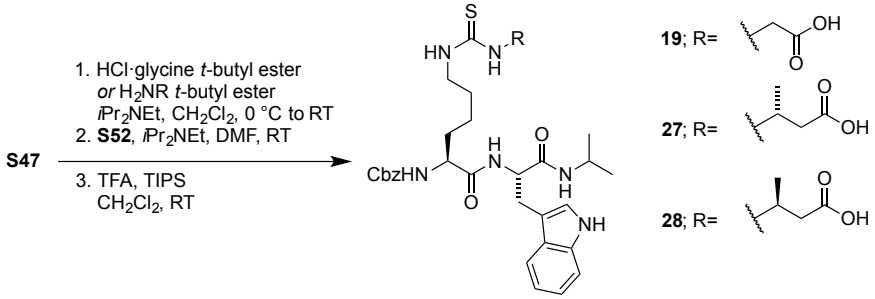
Scheme S11. Synthesis of common intermediate **S52**



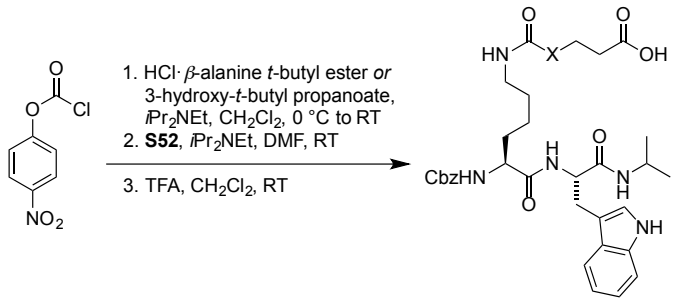
Scheme S12. Synthesis of thioacetamide **18**



Scheme S13. Synthesis of compound **Et-29**



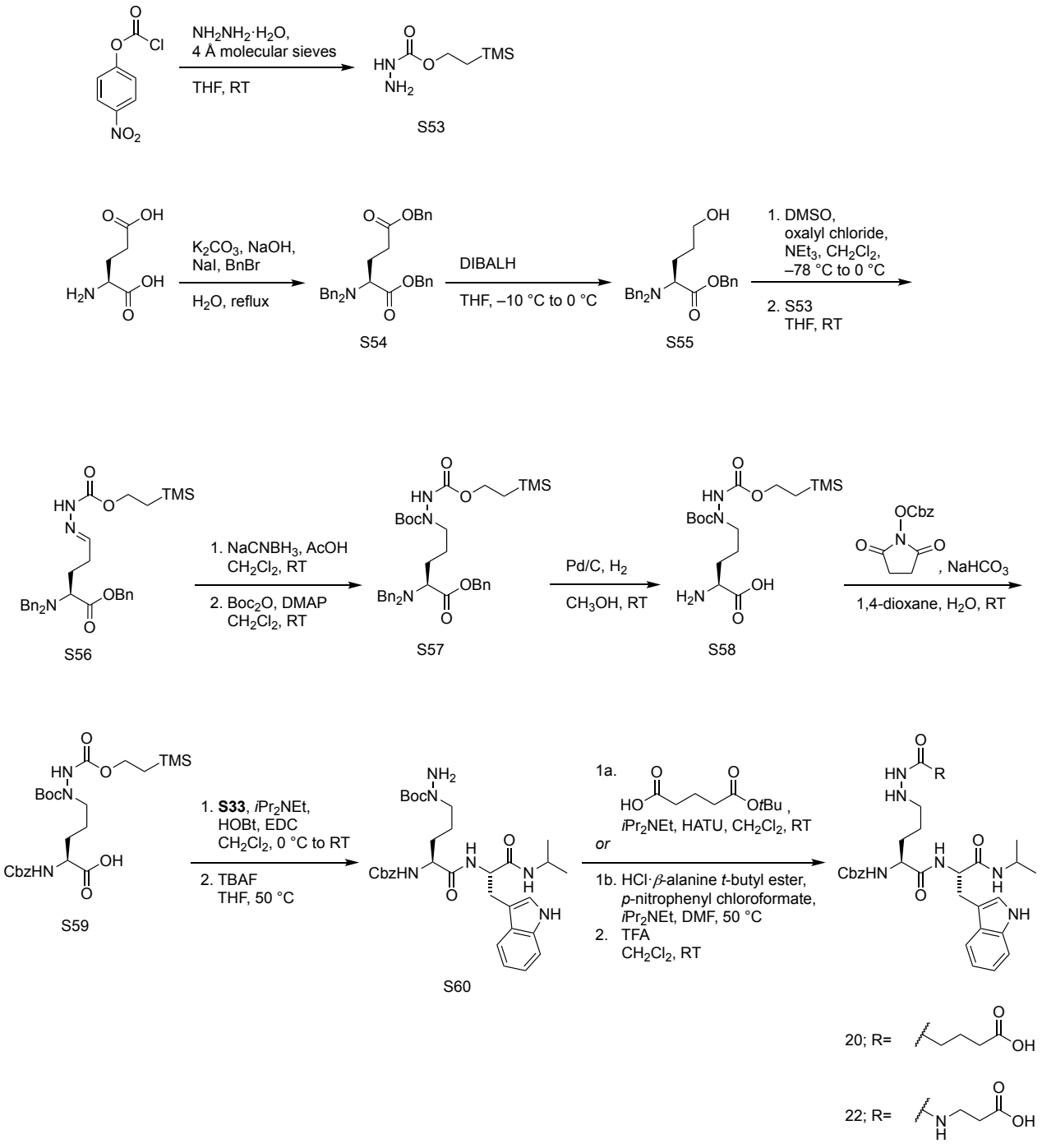
Scheme S14. Synthesis of thiourea inhibitors, part II



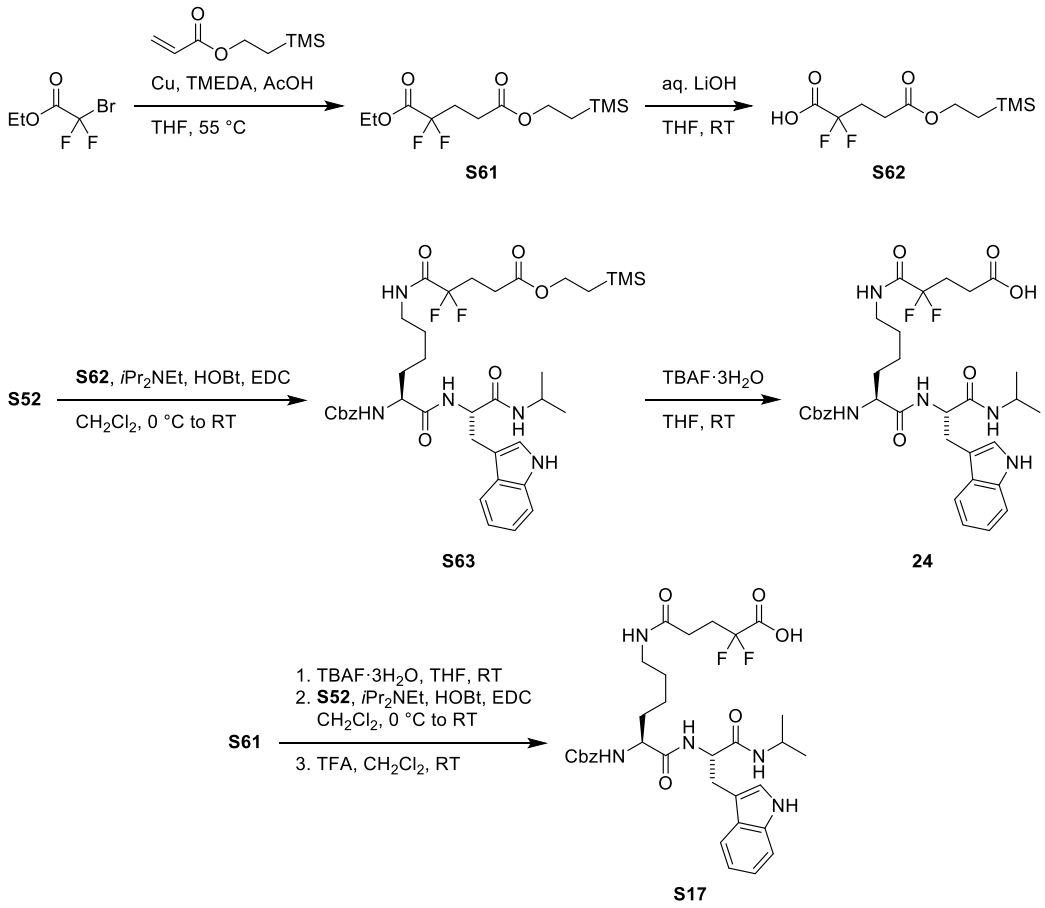
21; X= NH

23; X= O

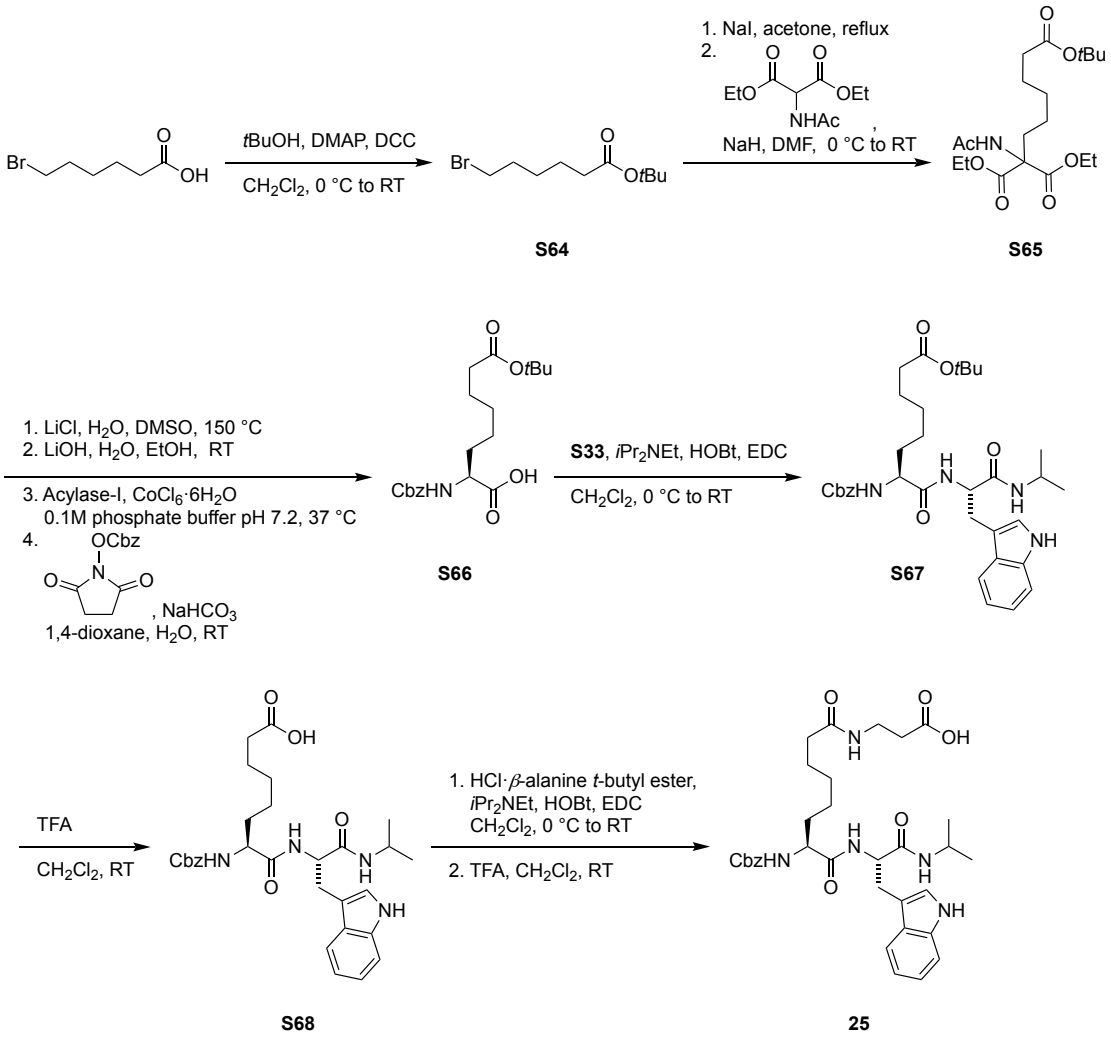
Scheme S15. Synthesis of inhibitors **21** and **23**



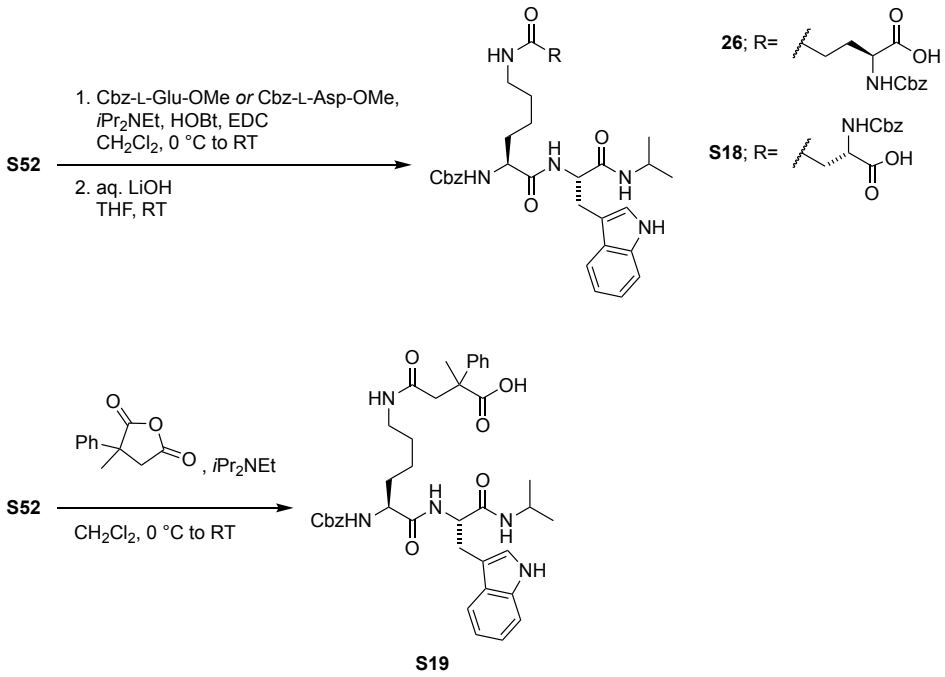
Scheme S16. Synthesis of hydrazide and semicarbazide inhibitors



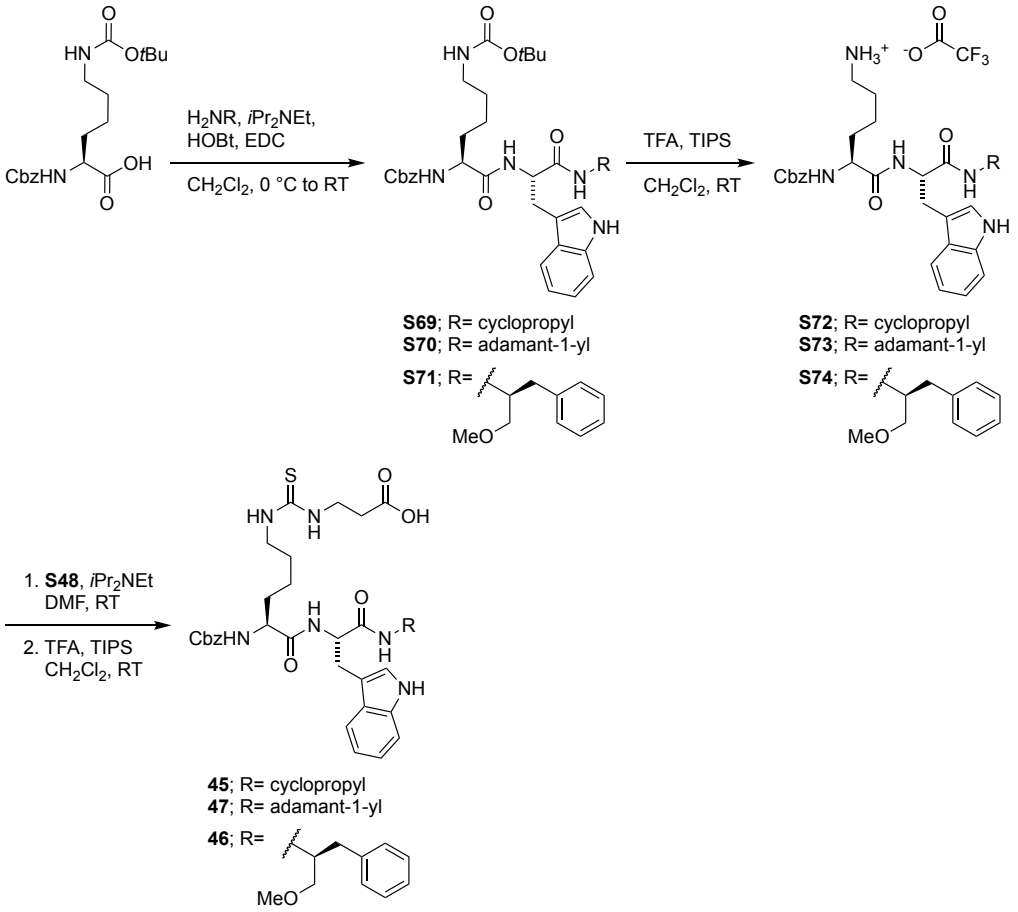
Scheme S17. Synthesis of fluorinated inhibitors **24** and **S17**



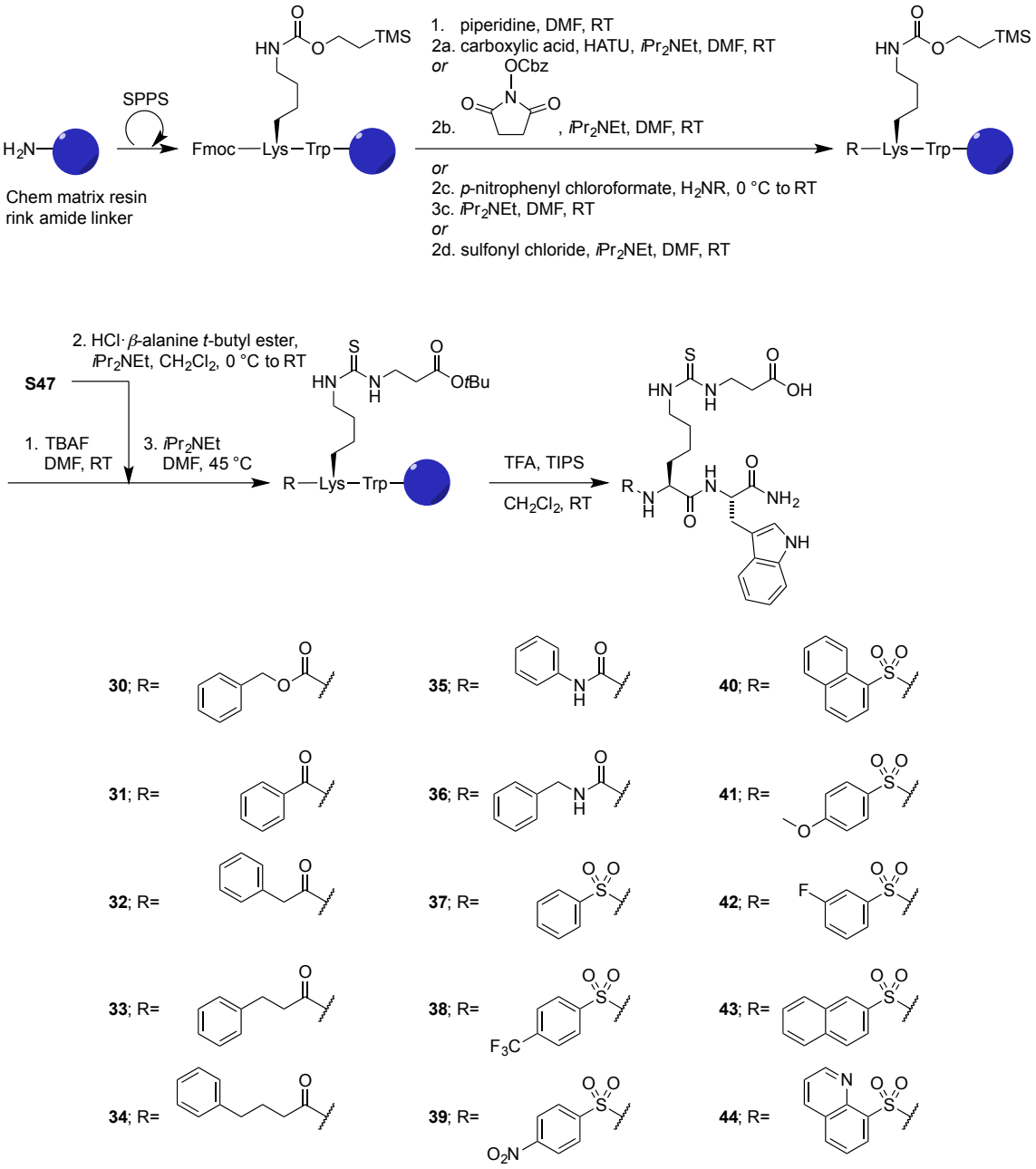
Scheme S18. Synthesis of inverted amide inhibitor **25**



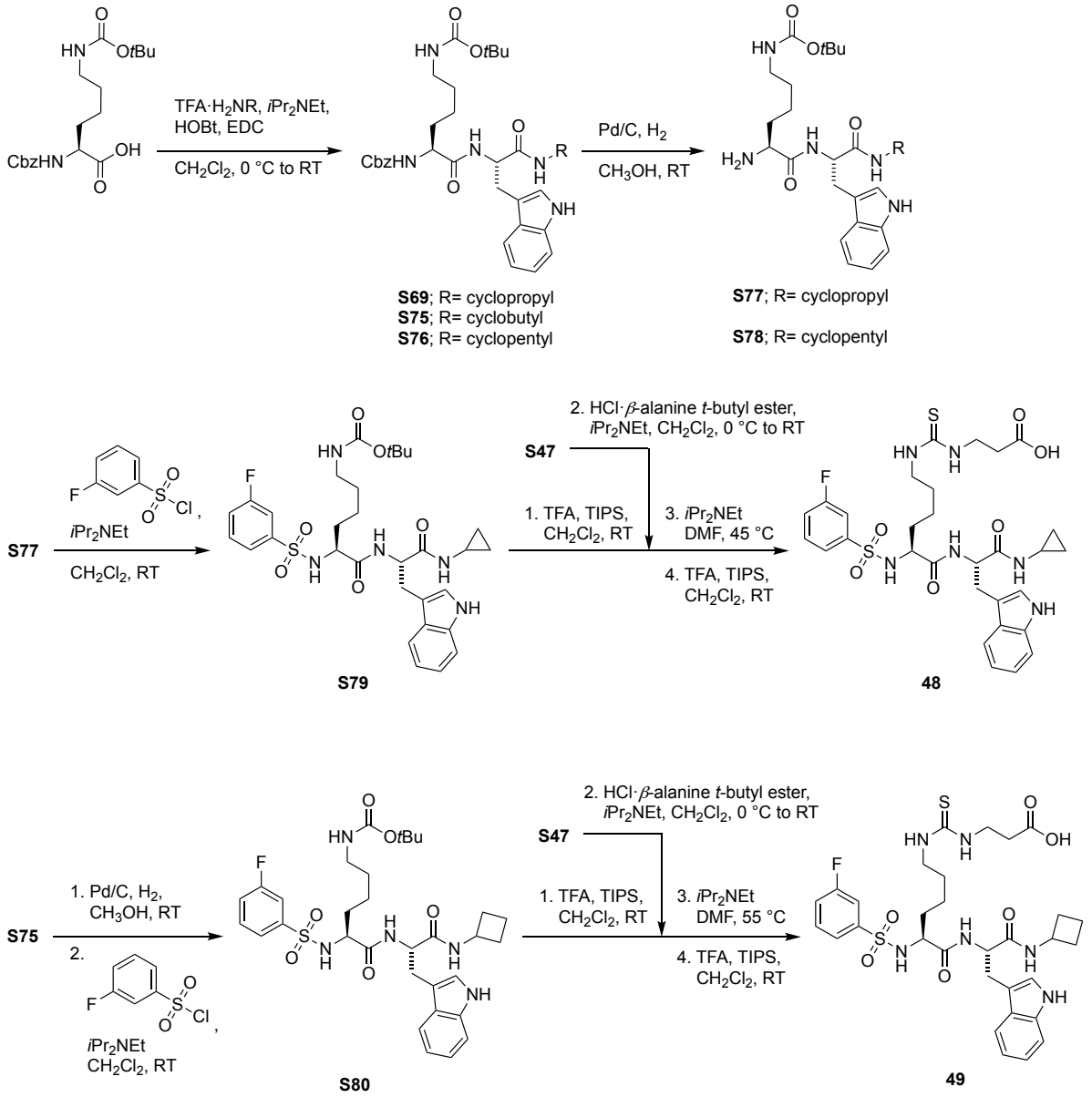
Scheme S19. Synthesis of amide inhibitors



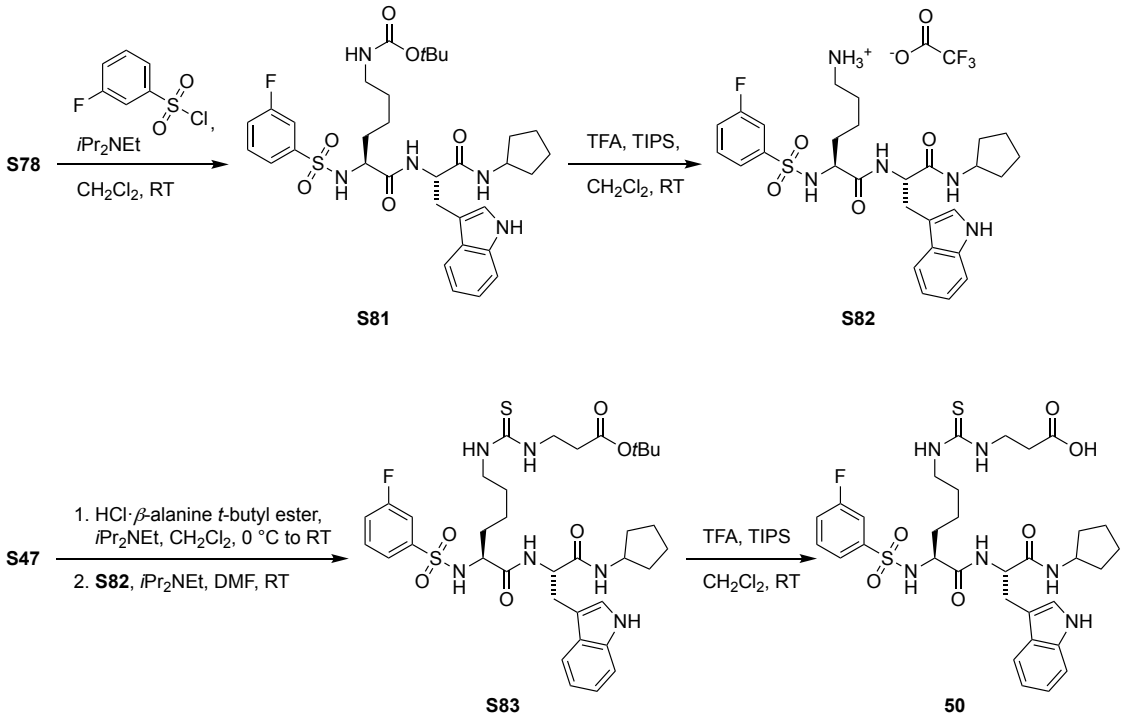
Scheme S20. Synthesis of thiourea inhibitors, part III



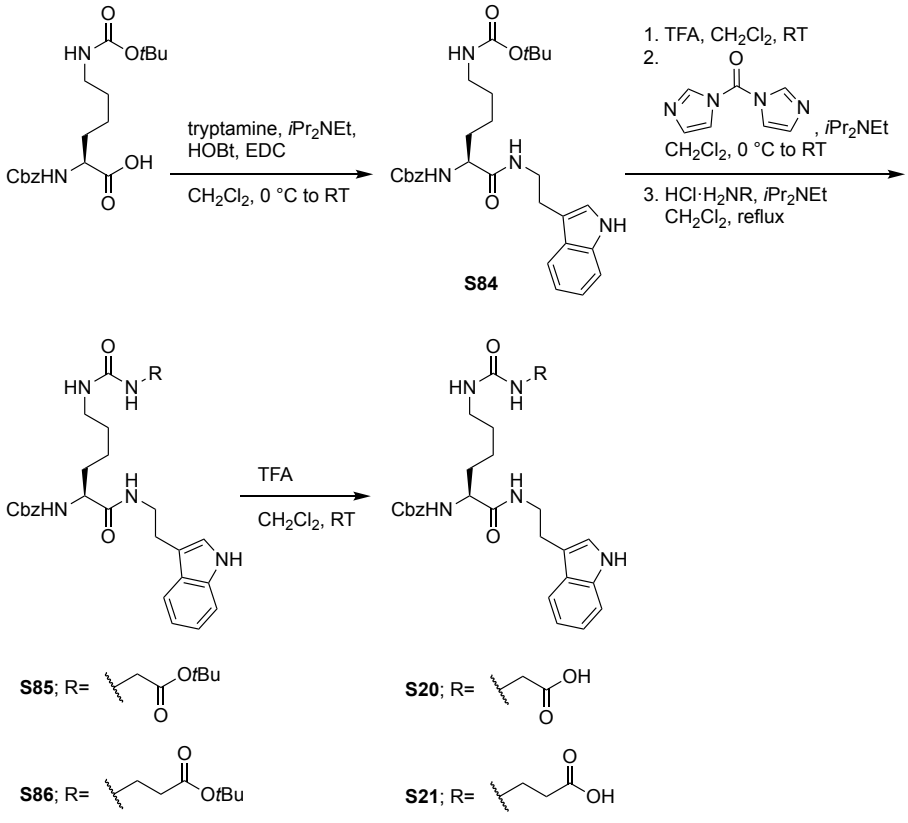
Scheme S21. Solid-phase synthesis of N-terminal modified thiourea inhibitors



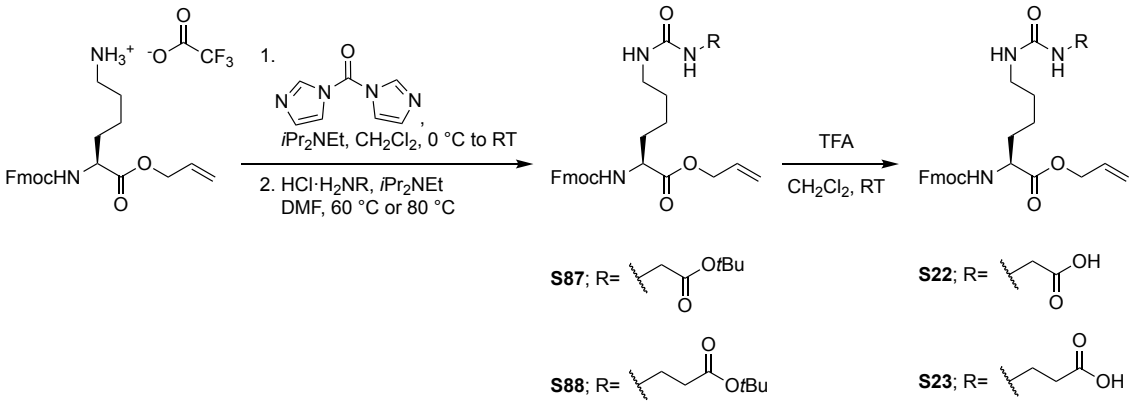
Scheme S22. Synthesis of thiourea inhibitors **48** and **49**



Scheme S23. Synthesis of thiourea inhibitor **50**



Scheme S24. Synthesis of urea inhibitors, part I



Scheme S25. Synthesis of urea inhibitors, part II

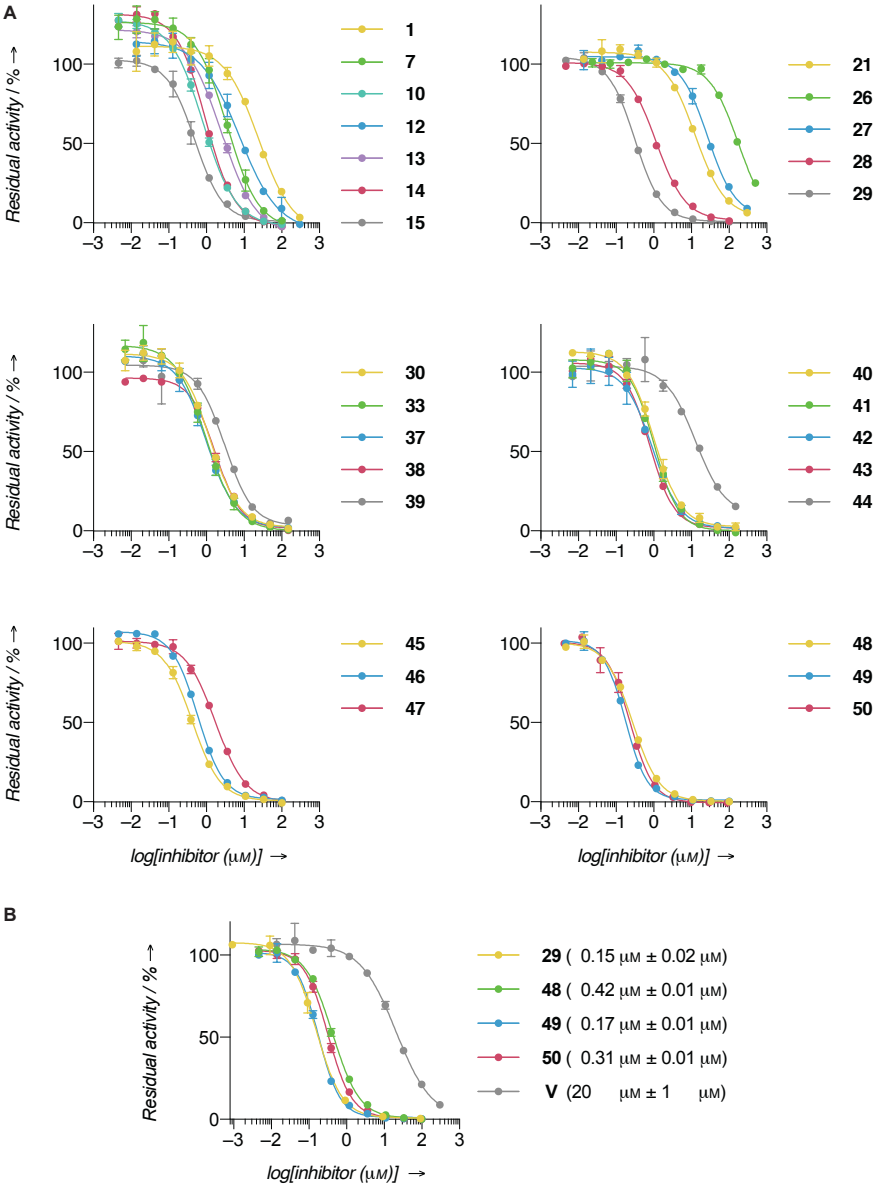


Figure S1. Concentration–response curves for SIRT5 inhibition of representative compounds using (A) Ac-LGKglu-AMC or (B) Ac-LGKsuc-AMC as substrate, respectively.

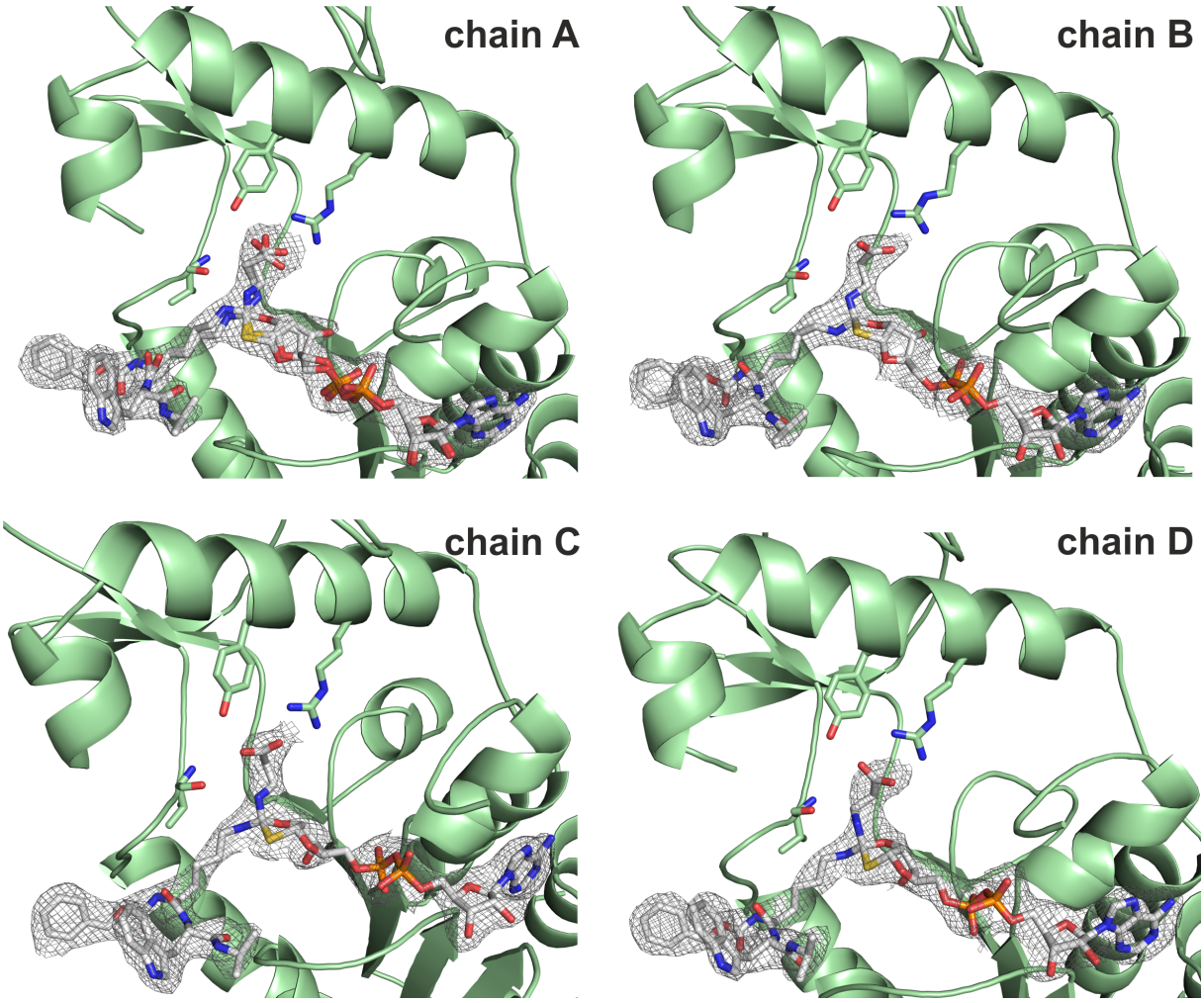


Figure S2. Co-crystal structures of **29**:zSIRT5.The complex crystal structure of **29**:zSIRT5 is composed of four protein chains (chain A to D) per asymmetric unit, one of which comprised an indistinguishable mixture of peptidyl-thioimidate and bicyclic intermediate. $2F_o$-F_c electron density (σ = 1.0) is shown for the intermediate ligand of each protein chain. SIRT5 is represented as green cartoon with amino acids of interest in stick style, while the intermediates are shown in white cpk colored stick representation.

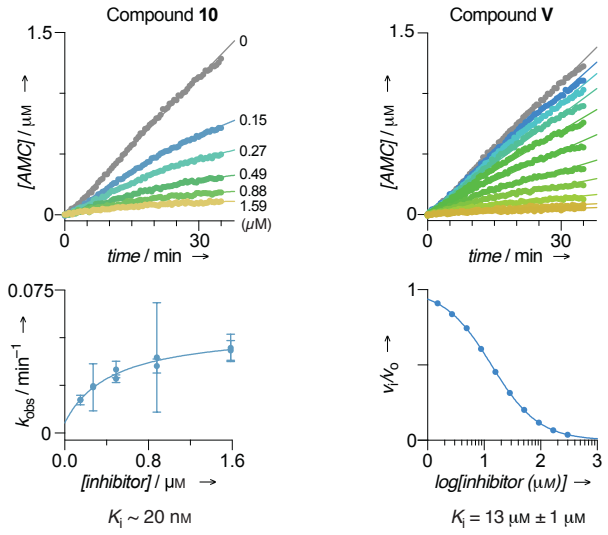


Figure S3. Progression curves and data fitting for SIRT5 inhibition by compound **10** and **V**.

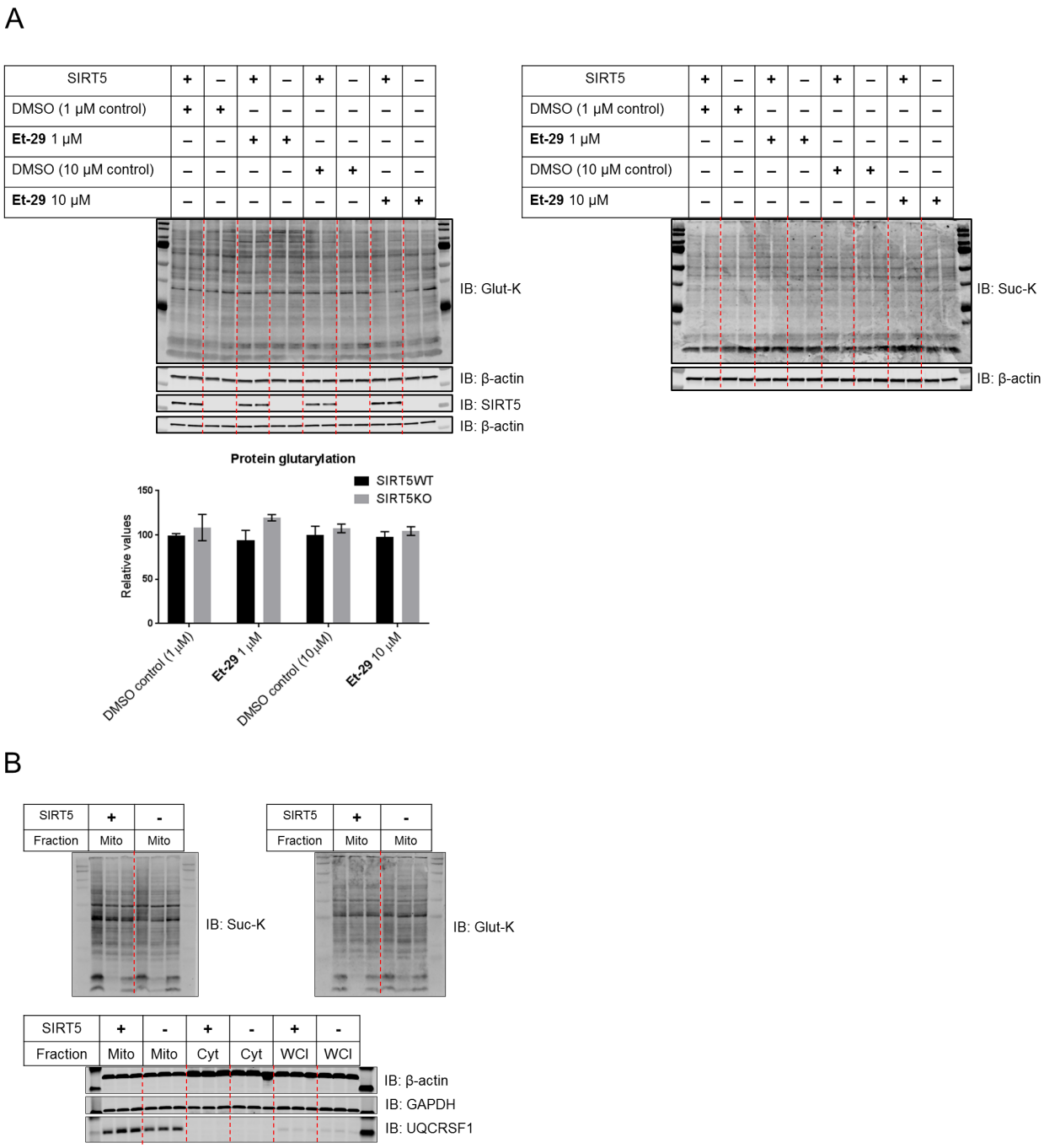


Figure S4. Western blot analysis of whole cell lysates after **Et-29** treatment or mitochondrial enrichment. (A) Effects of **Et-29** on lysine glutarylation and malonylation in HEK293T WT and crSIRT5KO cells (whole cell lysates). (B) Analysis of lysine succinylation and glutarylation in mitochondria enriched fractions of HEK293T WT and crSIRT5KO cells (Mito=mitochondrial fraction, Cyt=cytosolic fraction, WCl=Whole cell lysate). Anti-UQCRSF1 was used to analyze mitochondrial enrichment.

To assess the effect of **Et-29** in cells, we treated HEK293T WT and CRISPR/Cas9 SIRT5 knock out (crSIRT5KO) whole cell lysates, analyzing for changes in lysine glutarylation and succinylation levels. However, we observed no significant changes in the levels of either glutarylation or succinylation (Fig. S4A). Interestingly, no significant difference could be observed between untreated WT and crSIRT5KO cells either. Since succinyl and glutaryl modification of lysines are found on many mitochondrial proteins, we hypothesized that a larger difference in acylation levels between WT and crSIRT5KO cells could be detectable when analyzing this fraction—potentially making this a more suitable assay for evaluation of **Et-29**. However, mitochondrial enrichment of WT and crSIRT5KO cells did not show any significant changes in acylation levels between the cell lines either (Fig S4B).

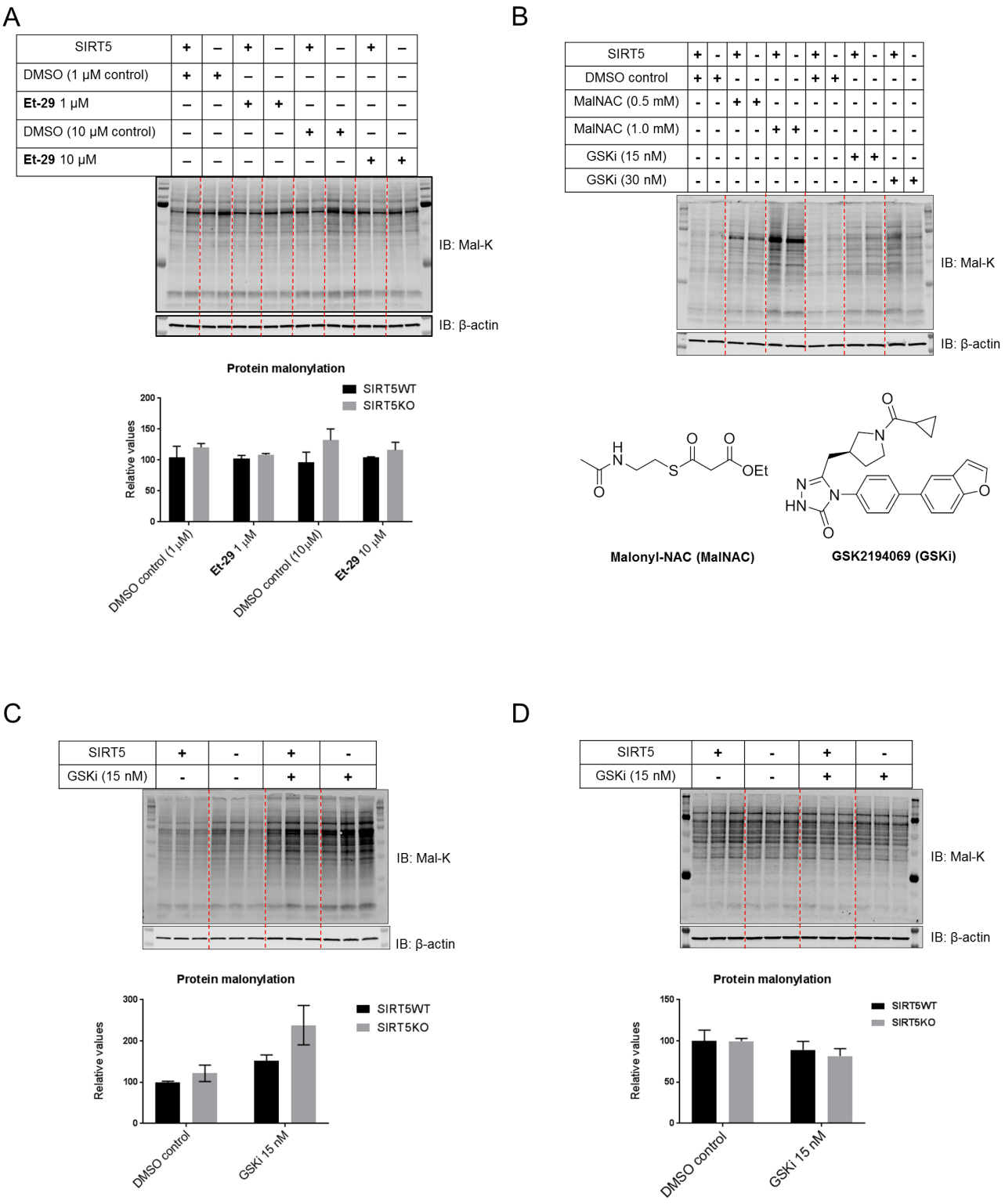


Figure S5. Western blot analysis of whole cell lysates after **Et-29**, MalNAC or GSKi treatments. (A) Effects of **Et-29** on lysine malonylation in HEK293T WT and crSIRT5KO cells. (B) Effects of MalNAC and GSKi on lysine malonylation in HEK293T WT and crSIRT5KO cells (initial experiment, one sample of the triplicate loaded). (C) Effects of GSKi on lysine malonylation in HEK293T WT and crSIRT5KO cells (initial experiment, all samples of the triplicate loaded). (D) Effects of GSKi on lysine malonylation in HEK293T WT and crSIRT5KO cells (second experiment, all samples of the triplicate loaded).

Since our initial efforts were unable to detect any significant differences in glutarylation or succinylation between WT and crSIRT5KO cells, we turned our attention to lysine malonylation, which is also regulated by SIRT5. We treated cells with **Et-29** and analyzed whole cell lysates for changes in malonyl-lysine levels. The results showed no significant changes in malonylation, nor were there any differences between the two cell lines (Figure S5A). A recent report by Kulkarni et al. have demonstrated that treating cells with an acylating reagent, Malonyl-NAC (MalNAC, Figure S5B), could considerably increase lysine malonylation in cells.[1] Additionally, they showed a similar effect when treating cells with a fatty acid synthase inhibitor (orlistat), which leads to increased availability of malonyl-CoA, the acyl donor in lysine malonylation. Inspired by this study, we hypothesized that increasing the on-rate of acylation by using MalNAC (obtained from Jordan L. Meier's lab) or the fatty acid synthase inhibitor GSK2194059 (GSKi, Figure S5B, Sigma-Aldrich, #SML1259-5MG) would increase our chances of observing changes in acylation levels between WT and crSIRT5KO cells. In our initial assay, both treatment with MalNAC and GSKi increased malonylation significantly in both cell lines (Figure S5B). However, no significant difference in malonylation was observed between the two cell lines, except when treated with a low concentration of GSKi (Figure S5C). Although these results were promising, the levels of malonylation upon GSKi treatment turned out to be considerably variable. In subsequent assays only minor or no apparent increase in malonylation could be observed (one example, Figure S5D, compare with Figure S5C).

The variability in cell culture acylation response and lack of observable changes between WT and crSIRT5KO cells prevented accurate evaluation of the effect of **Et-29** on SIRT5 regulated acylation in cells. Furthermore, western blot quantification of complete lanes is not an accurate representation of acylation. Consequently, probing specific SIRT5 targets or utilizing quantitative proteomics to measure changes in acylation may be more suitable assays to evaluate the effect of SIRT5 inhibitors, including prodrugs like **Et-29**.

Table S1. Kinetic parameters and dissociation constants for slow-binding inhibitors **29** and **49** derived from rate inhibition assays.

		10	**29**	**49**
k_{-2}	(min^{-1})	0.053 ± 0.0050	0.0080 ± 0.0098	0.0042 ± 0.0051
k_2	(min^{-1})	0.050 ± 0.003	0.32 ± 0.08	0.41 ± 0.08
$K_{i,1}$	(μM)	0.23 ± 0.10	1.5 ± 0.8	0.62 ± 0.19
K_i	(μM)	~0.022	~0.037	~0.006

Table S2. Data collection and refinement statistics

	zSirt5/ 10-ADPr-1′-thioimidate	**zSirt5/ 29-ADPr-1′-thioimidate**	**hSirt5/ 29-ADPr-1′-thioimidate**
Space group	C2	$P2_1$	P1
Unit cell constants (Å; °)	116.5 / 38.3 / 75.6; 90.0 / 122.5 / 90.0	64.9 / 113.6 / 72.3; 90.0 / 103.2 / 90.0	40.3 / 56.0 / 123.0; 97.4 / 99.3 / 90.5
Resolution[a] (Å)	50.00–1.95 (2.00–1.95)	50.00–2.40 (2.50–2.40)	43.77–1.32 (1.40–1.32)
Unique reflections	20704 (1518)	38453 (4467)	227669 (35569)
Multiplicity	4.1 (4.1)	3.8 (3.8)	2.0 (1.9)
Completeness (%)	99.1 (99.2)	96.2 (97.3)	91.5 (88.1)
R_{meas} (%)	21.3 (156.5)	25.2 (170.2)	5.9 (95.9)
CC1/2 (%)	98.9 (46.3)	98.9 (51.7)	99.9 (72.3)
$I/\sigma I$	6.8 (1.1)	6.1 (0.9)	6.8 (1.1)
Protein atoms	2088	8579	8501
Ligand atoms	80	400	640
Solvent atoms	177	193	1244
Resolution (Å)	49.11–1.95 (2.00–1.95)	48.41–2.40 (2.46–2.40)	43.77–1.32 (1.35–1.32)
R_{cryst}/R_{free}[b,c] (%)	19.5 / 24.2	25.3 / 30.5	16.0 / 20.9
Average B-factors ($Å^2$)			
protein	26.8	53.5	20.5
ligands	20.4	49.3	17.4
solvent	34.4	44.2	40.3
RMSD bond lengths	0.016	0.013	0.031
RMSD bond angles	2.0	2.0	2.9

[a] Values in parentheses refer to outermost shell.

[b] $R_{cryst} = \frac{\sum||F_{obs}|-k|F_{calc}||}{\sum|F_{obs}|}$. $|F_{obs}|$ is the observed and $|F_{calc}|$ the calculated structure factor amplitude.

[c] R_{free} was calculated from 5% (zSirt5 complexes) or 1.1% (hSirt5 complex) of reflections omitted from refinement.

General experimentals

All reagents and solvents were of analytical grade and used without further purification as obtained from commercial suppliers. Anhydrous solvents were obtained from a PureSolv-system. Reactions were conducted under an atmosphere of argon or nitrogen whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates (analytical SiO_2-60, F-254). TLC plates were visualized under UV light and by dipping in either (a) a solution of potassium permanganate (10 g/L), potassium carbonate (67 g/L) and sodium hydroxide (0.83 g/L) in water, (b) a solution of ninhydrin (3 g/L) in 3% acetic acid in water (v/v), or (c) a solution of molybdato-phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% conc. sulfuric acid in water (v/v) followed by heating with a heat gun. Vacuum liquid chromatography (VLC) was performed with silica gel 60 (particle size 15–40 µm). After column chromatography, appropriate fractions were pooled and dried at high vacuum (<2 mbar) for at least 12 hours to give obtained products in high purity (>95%) unless otherwise stated. Evaporation of solvents was carried out under reduced pressure at a temperature below 40 °C. UPLC−MS analyses were performed on a Phenomenex Kinetex column (1.7 µm, 50×2.10 mm) using a Waters Acquity ultra high–performance liquid chromatography (UPLC) system. Gradient A with eluent I (0.1% HCOOH in H_2O) and eluent II (0.1% HCOOH in MeCN) rising linearly from 0% to 95% of II during t = 0.00–5.20 min was applied at a flow rate of 0.6 mL/min. Preparative reversed-phase HPLC purification was performed on a C18 Phenomenex Luna column (5 µm, 100 Å, 250×20 mm) using an Agilent 1260 LC system equipped with a diode array UV detector and an evaporative light scattering detector (ELSD). Gradient B with eluent III (H_2O/MeCN/TFA, 95:5:0.1) and eluent IV (0.1% TFA in MeCN) rising linearly from 0% to 95% of IV during t = 5−45 min, then isocratically at 95% during t = 45–50 min was applied at a flow rate of 20 mL/min; *or* 0% to 95% of IV during t = 5−65 min, then isocratically at 95% during t = 65–70 min was applied at a flow rate of 20 mL/min. Analytical HPLC was performed on a C18 phenomenex Luna column (3 µm, 100 Å, 150×4.60 mm) using an Agilent 1100 series system equipped with a diode array UV detector. Gradient C using eluent III and eluent IV, rising linearly from 0% to 95% of IV during t = 5−35 min was applied at a flow rate of 1 mL/min. High-resolution mass spectrometry (HRMS) measurements were recorded either on a maXis G3 quadrupole time-of-flight (TOF) mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray (ESI) source or on an Agilent 1290 UHPLC equipped with a diode array detector and coupled to Agilent 6550 QTOF mass spectrometer operated in positive electrospray or on a Bruker Solarix WR by either matrix assisted laser desorption/ionization, or electrospray ionization (ESI). Nuclear magnetic resonance (NMR) spectra were recorded either on a Bruker Avance III HD equipped with a cryogenically cooled probe (^{1}H NMR and ^{13}C NMR recorded at 600 and 151 MHz, respectively), a Bruker Avance III (^{1}H NMR, ^{13}C NMR and ^{19}F NMR recorded at 400, 101, and 377 MHz, respectively), or a Bruker Ascend 400 MHz (^{1}H NMR and ^{13}C NMR recorded at 400 and 101 MHz, respectively). All spectra were recorded at 298 K unless otherwise stated. Chemical shifts are reported in ppm relative to deuterated solvent as internal standard (δ_H DMSO-d_6 2.50 ppm; δ_C DMSO-d_6 39.52 ppm; δ_H $CDCl_3$ 7.26 ppm; δ_C $CDCl_3$ 77.16 ppm; δ_H Methanol-d_4 3.31 ppm; δ_C Methanol-d_4 49.0 ppm). Assignments of NMR spectra are based on 2D correlation spectroscopy (COSY, HSQC, and HMBC spectra). Melting point measurements were performed on a Stanford Research Systems DigiMelt MPA161 apparatus.

Synthesis of compounds 1-50, V, Et-29, and S1-S88

General solid phase peptide synthesis procedure (SPPS)

The peptides were synthesized on a ChemMatrix resin using a rink amide linker by standard solid-phase peptide synthesis. The resin loading was determined spectrophotometrically, quantifying the amount of Fmoc released upon cleavage of a small sample.[2] Each elongation step was performed by applying the relevant amino acid (3 equiv.), HATU (2.9 equiv.) and *i*Pr_2NEt (6 equiv.). Fmoc-deprotection was performed by treatment with DMF/piperidine (4:1 (v/v), 4.0 mL; 2 min then 15 min), followed by washing with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL). The reaction progress of each elongeation was evaluated by Kaiser's tests.

General procedure for global deprotection and cleavage from resin

Peptides were cleaved from the resin by CH_2Cl_2/TFA/TIPS (1:1:0.05 (v/v/v), 2.0 mL; 2×1 hour), the resulting crude was purified by preparative reversed-phase HPLC, and yields were determined based on resin loading.

General procedure for on-resin acylation

The relevant acid (3 equiv.) and HATU (2.9 equiv.) *or* Cbz-OSuc (3 equiv.) were dissolved in DMF (2.0 mL/0.1 mmol resin) and *i*Pr_2NEt (6 equiv.) was added. The solution was added to the fritted syringe containing the resin bound dipeptide and the reaction mixture was agitated for 2 hours. After washing with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL) the reaction progress was evaluated with a Kaiser's test. If the Kaiser's test indicated presence of unreacted amine, the acylation procedure was repeated.

General procedure for on-resin sulfonamide formation

The relevant sulfonyl chloride (3 equiv.) and *i*Pr_2NEt (6 equiv.) were dissolved in DMF (2.0 mL/0.1 mmol resin) and added to the fritted syringe containing the resin-bound dipeptide and the reaction mixture was agitated overnight. After washing with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL) the reaction progress was evaluated with a Kaiser's test. If the Kaiser's test indicated presence of unreacted amine, the sulfonamide formation procedure was repeated.

General procedure for on-resin urea formation

The relevant amine (1.5 equiv.) and *i*Pr_2NEt (1.5 equiv.) were added dropwise to a solution of p-nitrophenylchloroformate in anhydrous CH_2Cl_2 (15 mL) at 0°C. The reaction mixture was stirred for 30 minutes at ambient temperature and was then concentrated under reduced pressure. The resulting crude residue and *i*Pr_2NEt (3 equiv.) were dissolved in DMF (2 mL/0.1 mmol resin), added to the fritted syringe containing the resin bound dipeptide, and the reaction mixture was agitated overnight. After washing with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL) the reaction progress was evaluated with a Kaiser's test. If the Kaiser's test indicated presence of unreacted amine, the urea formation procedure was repeated.

General procedure for on-resin Teoc deprotection

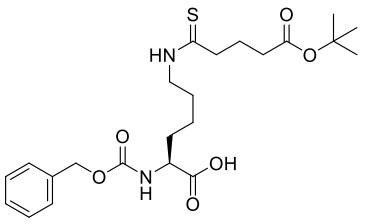
A solution of TBAF trihydrate (4 equiv.) in DMF (2.0 mL/0.1 mmol resin) was added to the fritted syringe containing the resin bound dipeptide and the reaction mixture was agitated for 2 hours. The procedure was repeated and the resin was then washed with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL).

General on-resin thiouea formation procedure

A solution of HCl·β-alanine *t*-butyl ester (2 equiv.) and *i*Pr_2NEt (3 equiv.) in CH_2Cl_2 (3.0 mL) was added dropwise over 5 minutes to a solution of compound **S47** (2 equiv.) in CH_2Cl_2 (1.5 mL) at 0°C. The reaction mixture was concentrated under reduced pressure and the resulting crude residue and *i*Pr_2NEt (2 equiv.)

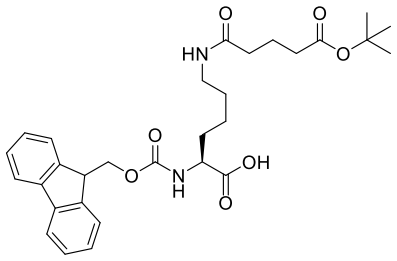
were dissolved in DMF (2.0 mL/0.1 mmol resin), added to the fritted syringe containing the resin bound dipeptide and the reaction mixture was agitated overnight. After washing with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL) the reaction progress was evaluated with a Kaiser's test. If the Kaiser's test indicated presence of unreacted amine, the thiourea formation procedure was repeated.

N^2-((Benzyloxy)carbonyl)-N^6-(5-(tert-butoxy)-5-oxopentanethioyl)-L-lysine (S24).



A solution of DCC (3.91 g, 18.9 mmol) in anhydrous DMF (12.0 mL) was added to a solution of N-hydroxysuccinimide (2.18 g, 18.9 mmol) and mono *t*-butyl glutarate (3.9 g, 21 mmol) in anhydrous DMF (12.0 mL). The solution was stirred at ambient temperature for 3.5 hours and solids were filtered off. The filtrate was added to a solution of Cbz-Lys-OH (4.47 g, 16.0 mmol) and *i*Pr_2NEt (8.0 mL, 45.9 mmol) and the mixture was stirred at ambient temperature overnight. The reaction was acidified with aq. HCl (1 M, 50 mL) and extracted with EtOAc (4×75 mL). The combined organic phases were washed with brine (3×75 mL) and dried over Na_2SO_4 and was then concentrated under reduced pressure. Column chromatography (**1**. 0→1.5% CH_3OH and 0.25% AcOH in CH_2Cl_2/MeCN (1:1), **2**. 0→5% CH_3OH and 0.25% AcOH in CH_2Cl_2/MeCN (1:1), **3**. 0→7% CH_3OH and 0.25% AcOH in CH_2Cl_2/MeCN (1:1)) of the crude residue afforded a clear oil, tentatively assigned as Cbz-Lys(5-*O*-*t*-butyl glutaryl)-OH (3.82 g, UPLC-MS $[M+H]^+$, *m*/*z* 451.1; $[M+H]^+$, $C_{23}H_{35}N_2O_7^+$ Calcd 451.2). The clear oil (3.82 g) and Lawesson's reagent (3.42 g, 8.46 mmol) were dissolved in anhydrous THF (22 mL) and the reaction mixture was stirred under N_2 atmosphere at ambient temperature. After 2 hours the solvent was removedand the crude residue was purified by column chromatography (**1**. 0→2% CH_3OH and 0.25% AcOH in CH_2Cl_2, **2**. 0→2% CH_3OH and 0.25% AcOH in CH_2Cl_2, **3**. 50→75% EtOAc and 0.25% AcOH in heptane, **4**. 50→75% EtOAc and 0.25% AcOH in heptane) affording the desired thioamide **S24** (2.37 g, 32% from Cbz-Lys-OH), as a dark green oil. TLC (4% CH_3OH, 0.25% AcOH in CH_2Cl_2): R_f = 0.29. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.55 (s, 1H, CO_2H), 9.90 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.53 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Lys}$), 7.42–7.26 (m, 5H, $H_{Ar,Cbz}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 3.92 (ddd, *J* = 9.6, 7.9, 4.6 Hz, 1H, $H_{\alpha,Lys}$), 3.51–3.39 (m, 2H, $H_{\varepsilon,Lys}$), 2.55–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2tBu$, overlap with solvent peak), 2.18 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2tBu$), 1.86 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2tBu$), 1.76–1.65 (m, 1H, $H_{\beta,Lys,A}$), 1.64–1.46 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.39 (s, 11H, $C(CH_3)_3$, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 173.9 (CO_{Lys}), 171.8 (CO_2tBu), 156.1 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 79.5 ($\underline{C}(CH_3)_3$), 65.4 ($CH_{2,Cbz}$), 53.7 ($C_{\alpha,Lys}$), 44.9 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2tBu$), 33.76 ($\underline{C}H_2CO_2tBu$), 30.4 ($C_{\beta,Lys}$), 27.8 ($C(\underline{C}H_3)_3$), 26.7 ($C_{\delta,Lys}$), 24.3 ($\underline{C}H_2CH_2CO_2tBu$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.09 min, *m*/*z* 467.2 ($[M+H]^+$, $C_{23}H_{35}N_2O_6S^+$ Calcd 467.2); HRMS *m*/*z* 467.2216 ($[M+H]^+$,$C_{23}H_{35}N_2O_6S^+$ Calcd 467.2210).

N^6-(5-(*tert*-Butoxy)-5-oxopentanoyl)-N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-lysine (S25).

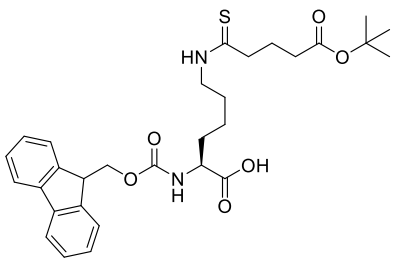


Method 1—DCC (126 mg, 0.61 mmol) was added to a solution of *N*-hydroxysuccinimide (70 mg, 0.61 mmol) and mono *t*-butyl glutarate in anhydrous DMF (3.0 mL). The solution was stirred at ambient temperature for 2 hours and solids were filtered off and the filtrate (6.0 mL) was used without further purification. The filtrate (3.0 mL) was added to a solution of Fmoc-Lys-OH (155 mg, 0.42 mmol) and *i*Pr_2NEt (0.22 mL, 1.26 mmol) and the mixture was stirred at ambient temperature overnight. The reaction was acidified with aq. HCl (1 M, 5 mL) and H_2O (15 mL) and extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine (3×75 mL) and dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→1% MeOH and 0.25% AcOH in CH_2Cl_2) affording the desired amide **S25** (104 mg, 46%) as a slightly yellow oil.

Method 2—*N*-Hydroxysuccinimide (968 mg, 8.41 mmol) and DIC (1.31 mL, 8.40 mmol) was added to a solution of mono *t*-butyl glutarate (1.70 g, 9.01 mmol) in anhydrous DMF (15 mL) and stirred at ambient temperature. After stirring for 3 hours, the mixture was filtered and the filtrate added to a suspension of N^{α}-Fmoc-lysine (2.25 g, 6.10 mmol) in anhydrous DMF (20 mL) and *i*Pr_2NEt (3.0 mL, 17.2 mmol) at ambient

temperature. After stirring for 2.5 hours, 10% brine (20 mL) was added to the resulting yellow solution, and the reaction mixture was stirred for 15 min. aq. HCl (1 M, 15 mL) was added and the solution extracted with EtOAc (4×100 mL). The combined organic phases were washed with 10% brine (2×100 mL), dried over $MgSO_4$, the precipitate filtered off and the filtrate was concentrated under reduced pressure. The residue was suspended in EtOAc–heptane (25 mL, 60:40), resulting in formation of a white precipitate. The suspension was filtered and the filtrate was concentrated under reduced pressure. The crude residue was was purified by column chromatography (50% MeCN in CH_2Cl_2, then 0→10% MeOH and 0.25% AcOH in MeCN/CH_2Cl_2 (50:50)). The appropriate fractions were pooled, and excess AcOH was removed by coevaporations: toluene/MeCN (1:1, 3×10 mL) and MeCN (3×10 mL), affording the desired amide **S25** (2.23 g, 68%) as a white solid. TLC (5% MeOH and 0.25% AcOH in CH_2Cl_2): R_f = 0.2. ^{1}H NMR (DMSO-d_6) δ 12.56 (br s, 1H, CO_2H), 7.89 (d, J = 7.5, 2H, $H4_{Fmoc}$, $H5_{Fmoc}$), 7.77 (t, J = 5.6, 1H, $NH_{\varepsilon,Lys}$), 7.73 (d, J = 7.5, 2H, $H1_{Fmoc}$, $H8_{Fmoc}$), 7.59 (d, J = 8.0, 1H, $NH_{\alpha,Lys}$), 7.42 (t, J = 7.4, 2H, $H3_{Fmoc}$, $H6_{Fmoc}$), 7.37–7.28 (m, 2H, $H2_{Fmoc}$, $H7_{Fmoc}$), 4.31–4.25 (m, 2H, $CH_{2,Fmoc,A}$, $H9_{Fmoc}$), 4.25–4.18 (m, 1H, $CH_{2,Fmoc,B}$), 3.90 (ddd, J = 9.6, 7.9, 4.6, 1H, $H_{\alpha,Lys}$), 3.02 (q, J = 6.4, 2H, $H_{\varepsilon,Lys}$), 2.16 (t, J = 7.4, 2H, $C\underline{H}_2CO_2tBu$), 2.06 (t, J = 7.4, 2H, $C\underline{H}_2(CH_2)_2CO_2tBu$), 1.76–1.53 (m, 4H, $H_{\beta,Lys}$, $C\underline{H}_2CH_2CO_2tBu$), 1.45–1.24 (m, 13H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (DMSO-d_6) δ 174.0 (CO_2H), 172.0 (CO_2tBu), 171.3 ($NH_\varepsilon CO$), 156.1 (CO_{Fmoc}), 143.9 ($C8a_{Fmoc}$/$C9a_{Fmoc}$), 143.8 ($C8a_{Fmoc}$/$C9a_{Fmoc}$), 140.73 ($C4a_{Fmoc}$,$C4b_{Fmoc}$), 140.71 ($C4a_{Fmoc}$,$C4b_{Fmoc}$), 127.6 ($C3_{Fmoc}$,$C6_{Fmoc}$), 127.1 ($C2_{Fmoc}$,$C7_{Fmoc}$), 125.30 ($C1_{Fmoc}$/$C8_{Fmoc}$), 125.28 ($C1_{Fmoc}$/$C8_{Fmoc}$), 120.12 ($C4_{Fmoc}$/$C5_{Fmoc}$), 120.10 ($C4_{Fmoc}$/$C5_{Fmoc}$), 79.5 ($\underline{C}(CH_3)_3$), 65.6 ($CH_{2,Fmoc}$), 53.8 ($C_{\alpha,Lys}$), 46.7 ($C9_{Fmoc}$), 38.2 ($C_{\varepsilon,Lys}$), 34.3 ($\underline{C}H_2(CH_2)_2CO_2tBu$), 34.2 ($\underline{C}H_2CO_2tBu$), 30.5 ($C_{\beta,Lys}$), 28.7 ($C_{\delta,Lys}$), 27.8 ($C(\underline{C}H_3)_3$), 23.1 ($C_{\gamma,Lys}$), 20.8 ($\underline{C}H_2CH_2CO_2tBu$). Trace amounts (<3%) of diisopropylurea could be detected. UPLC-MS t_R 2.20 min, m/z 539.3 ([M+H]$^+$, $C_{30}H_{39}N_2O_7^+$ Calcd 539.3); HRMS m/z 539.2759 ([M+H]$^+$, $C_{30}H_{39}N_2O_7^+$ Calcd 539.2752).

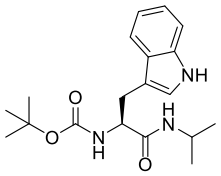
N^6-(5-(*tert*-Butoxy)-5-oxopentanethioyl)-N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-lysine (S26).



Method 1—Amide **S25** (41.5 mg, 0.08 mmol) and Lawesson's reagent (28.4 mg, 0.07 mmol) was dissolved in anhydrous THF (2.5 mL) and stirred at ambient temperature. The reaction was followed by TLC, and after 2 hours the solvent was removed. The crude residue was purified by column chromatography (0.5% MeOH, 0.25% AcOH in CH_2Cl_2) affording the desired thioamide **S26** (18.5 mg, 43%) as a dark oil.

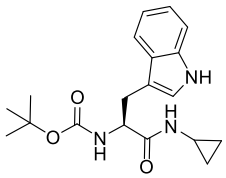
Method 2—Amide **S25** (1.33 g, 2.48 mmol) was dissolved in anhydrous toluene (50 mL) and heated to 80 °C. Lawesson's reagent (128 mg, 127 mg, 142 mg and 111 mg, 1.25 mmol total) was added after 0, 45, 75 and 105 min, respectively. After stirring for a total of 2 hours, the reaction mixture was allowed to reach ambient temperature and was concentrated under reduced pressure. The crude residue was purified by column chromatography (0→3% MeOH and 0.25% AcOH in CH_2Cl_2), to afford desired thioamide **S26** (171 mg, 12%) and a byproduct tentatively assigned as the corresponding thioamide thiocarboxylic acid (66 mg, 5%, UPLC-MS m/z 571.2 ([M+H]$^+$, $C_{30}H_{39}N_2O_5S_2^+$ Calcd 571.2)). TLC (5% MeOH and 0.25% AcOH in CH_2Cl_2): R_f = 0.25. ^{1}H NMR ($CDCl_3$) δ 7.89–7.80 (m, 1H, $NH_{\varepsilon,Lys}$), 7.76 (d, J = 7.3, 2H, $H4_{Fmoc}$, $H5_{Fmoc}$), 7.64–7.53 (m, 2H, $H1_{Fmoc}$, $H8_{Fmoc}$), 7.39 (t, J = 7.3, 2H, $H3_{Fmoc}$, $H6_{Fmoc}$), 7.30 (td, J = 7.4, 1.2, 2H, $H2_{Fmoc}$, $H7_{Fmoc}$), 5.54 (d, J = 8.0, 1H, $NH_{\alpha,Lys}$), 4.57–4.34 (m, 3H, $H_{\alpha,Lys}$, $CH_{2,Fmoc}$), 4.21 (t, J = 6.9, 1H, $H9_{Fmoc}$), 3.73–3.56 (m, 2H, $H_{\varepsilon,Lys}$), 2.66 (t, J = 7.3, 2H, $C\underline{H}_2(CH_2)_2CO_2tBu$), 2.26 (t, J = 7.1, 2H, $C\underline{H}_2CO_2tBu$), 2.09–1.97 (m, 2H, $C\underline{H}_2CH_2CO_2tBu$), 1.97–1.85 (m, 1H, $H_{\beta,Lys,A}$), 1.85–1.56 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.56–1.34 (m, 11H, $H_{\gamma,Lys}$, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$) δ 204.5 (NHCS), 175.2 (CO_2H), 173.9 (CO_2tBu), 156.3 (CO_{Fmoc}), 143.9 ($C8a_{Fmoc}$/$C9a_{Fmoc}$), 143.7 ($C8a_{Fmoc}$/$C9a_{Fmoc}$), 141.4 ($C4a_{Fmoc}$, $C4b_{Fmoc}$), 127.9 ($C3_{Fmoc}$, $C6_{Fmoc}$), 127.2 ($C2_{Fmoc}$, $C7_{Fmoc}$), 125.2 ($C1_{Fmoc}$,$C8_{Fmoc}$), 120.2 ($C4_{Fmoc}$,$C5_{Fmoc}$), 81.5 ($\underline{C}(CH_3)_3$), 67.3 ($CH_{2,Fmoc}$), 53.5 ($C_{\alpha,Lys}$), 47.2 ($C9_{Fmoc}$), 45.8 ($\underline{C}H_2(CH_2)_2CO_2tBu$), 45.6 ($C_{\varepsilon,Lys}$), 34.3 ($\underline{C}H_2CO_2tBu$), 31.9 ($C_{\beta,Lys}$), 28.2 ($C(\underline{C}H_3)_3$), 27.1 ($C_{\delta,Lys}$), 24.9 ($\underline{C}H_2CH_2CO_2tBu$), 22.4 ($C_{\gamma,Lys}$). Trace amounts (<5%) of diisopropylurea could be detected. UPLC-MS t_R 2.44 min, m/z 555.3 ([M+H]$^+$, $C_{30}H_{39}N_2O_6S^+$ Calcd 555.3); HRMS m/z 555.2534 ([M+H]$^+$, $C_{30}H_{39}N_2O_6S^+$ Calcd 555.2524).

***tert*-Butyl (*S*)-(3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)carbamate (S27).**



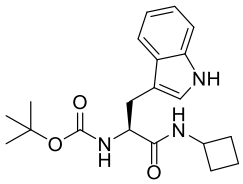
Boc-Trp-OH (1.09 g, 3.58 mmol), HOBt (729 mg, 5.40 mmol), *i*PrNH$_2$ (0.61 mL, 7.10 mmol) and *i*Pr$_2$NEt (0.61 mL, 3.50 mmol) were dissolved in anhydrous CH_2Cl_2 (12 mL) and cooled to 0 °C. EDC (1.03 g, 5.37 mmol) was added and the reaction mixture was stirred at 0°C for 5 minutes and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was dissolved in EtOAc (150 mL) and washed with aq. $KHSO_4$ (5%, 3×100 mL), saturated aq. $NaHCO_3$ (3×100 mL), and brine (2×100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, affording the desired amide **S27** as a colorless solid (1.25 g, >99%), which was used without further purification.[1]H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH_{Indole}), 7.63 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.31 (d, J = 8.0 Hz, 1H, $H7_{Indole}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.0, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.61 (d, J = 8.4 Hz, 1H, $NH_{α,Trp}$), 4.12 (td, J = 8.6, 5.4 Hz, 1H, $H_{α,Trp}$), 3.86–3.76 (m, 1H, $CH_{i Pr}$), 3.00 (m_{ABX}, J = 14.5, 5.4 Hz, 1H, $H_{β,Trp,A}$), 2.88 (m_{ABX}, J = 14.5, 8.7 Hz, 1H, $H_{β,Trp,B}$), 1.31 (s, 9H, $C(CH_3)_3$), 1.03 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,A}$), 0.96 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.2 (CO_{Trp}), 155.5 (CO_{Boc}), 136.5 ($C7a_{Indole}$), 127.9 ($C3a_{Indole}$), 124.0 ($C2_{Indole}$), 121.2 ($C6_{Indole}$), 119.0 ($C4_{Indole}$), 118.5 ($C5_{Indole}$), 111.7 ($C7_{Indole}$), 110.6 ($C3_{Indole}$), 78.4 ($\underline{C}(CH_3)_3$), 55.6 ($C_{α,Trp}$), 40.8 ($CH_{i Pr}$), 28.6 ($C(\underline{C}H_3)_3$), 28.5 ($C_{β,Trp}$), 22.7 ($(CH_3)_{2,iPr}$). UPLC-MS t_R 2.00 min, m/z 346.2 ($[M+H]^+$, $C_{19}H_{28}N_3O_3^+$ Calcd 346.2).

***tert*-Butyl (*S*)-(1-(cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (S28).**



By the method described for compound **S27**, the title compound was synthesized using Boc-Trp-OH (308 mg, 1.01 mmol), HOBt (210 mg, 1.55 mmol), cyclopropylamine (0.11 mL, 1.59 mmol), *i*Pr$_2$NEt (0.35 mL, 2.01 mmol), anhydrous CH_2Cl_2 (7.0 mL), and EDC (292 mg, 1.52 mmol) affording the desired amide **S28** (277 mg, 80%) as a colorless solid, which was used without further purification. TLC (3% CH_3OH in CH_2Cl_2): R_f = 0.4. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH_{Indole}), 7.92 (d, J = 4.2 Hz, 1H, $CO_{Trp}NH$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.31 (d, J = 8.0 Hz, 1H, $H7_{Indole}$), 7.09 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.05 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.63 (d, J = 8.2 Hz, 1H, $NH_{α,Trp}$), 4.10 (td, J = 8.5, 5.4 Hz, 1H, $H_{α,Trp}$), 2.99 (m_{ABX}, J = 14.5, 5.4 Hz, 1H, $H_{β,Trp,A}$), 2.87 (m_{ABX}, J = 14.5, 8.7 Hz, 1H, $H_{β,Trp,B}$), 2.64–2.56 (m, 1H, $H1_{Cyclopropyl}$), 1.31 (s, 9H, $C(CH_3)_3$), 0.65–0.48 (m, 2H, $H2_{Cyclopropyl}$), 0.43–0.25 (m, 2H, $H3_{Cyclopropyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.0 (CO_{Trp}), 155.0 (CO_{Boc}), 136.0 ($C7a_{Indole}$), 127.4 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.1 ($C3_{Indole}$), 77.9 ($\underline{C}(CH_3)_3$), 54.9 ($C_{α,Trp}$), 28.2 ($C(\underline{C}H_3)_3$), 27.9 ($C_{β,Trp}$), 22.3 ($C1_{Cyclopropyl}$), 5.7 ($C2_{Cyclopropyl}$), 5.5 ($C3_{Cyclopropyl}$). UPLC-MS t_R 1.79 min, m/z 344.1 ($[M+H]^+$, $C_{19}H_{26}N_3O_3^+$ Calcd 344.2); HRMS m/z 366.1784 ($[M+Na]^+$,$C_{19}H_{x25}N_3O_3Na^+$ Calcd 366.1788). In accordance with previously reported data.[3]

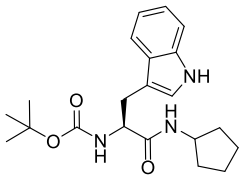
***tert*-Butyl (*S*)-(1-(cyclobutylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (S29).**



By the method described for compound **S27**, the title compound was synthesized using Boc-Trp-OH (998 mg, 3.28 mmol), HOBt (665 mg, 4.92 mmol), cyclobutylamine (0.42 mL, 4.92 mmol), *i*Pr$_2$NEt (1.14 mL, 6.56 mmol), anhydrous CH_2Cl_2 (24 mL), and EDC (942.9 mg, 4.92 mmol) affording the desired amide **S29** (1.16 g, >99%), which was used without further purification. TLC (3% CH_3OH in CH_2Cl_2): R_f = 0.35. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.8 (s, 1H, NH_{Indole}), 8.06 (d, J = 7.8 Hz, 1H, $CO_{Trp}NH$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.31 (d, J = 8.0 Hz, 1H, $H7_{Indole}$), 7.09 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.08–7.02 (m, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.62 (d, J = 8.4 Hz, 1H, $NH_{α,Trp}$), 4.24–4.08 (m, 2H, $H_{α,Trp}$, $CH_{Cyclobutyl}$), 3.01 (m_{ABX}, J = 14.5, 5.3 Hz, 1H, $H_{β,Trp,A}$), 2.88 (m_{ABX}, J = 14.5, 8.7 Hz, 1H, $H_{β,Trp,B}$), 2.16–2.03 (m, 2H, $H2_{Cyclobutyl}$), 1.88 (p, J = 9.7 Hz, 1H, $H4_{Cyclobutyl,A}$), 1.79 (p, J = 9.8 Hz, 1H, $H4_{Cyclobutyl,B}$), 1.65–1.52 (m, 2H, $H3_{Cyclobutyl}$), 1.31 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.1 (CO_{Trp}), 155.5 (CO_{Boc}), 136.5 ($C7a_{Indole}$), 127.9 ($C3a_{Indole}$), 124.0 ($C2_{Indole}$), 121.2 ($C6_{Indole}$), 119.0 ($C4_{Indole}$), 118.6 ($C5_{Indole}$), 111.7 ($C7_{Indole}$), 110.6 ($C3_{Indole}$), 78.4 ($\underline{C}(CH_3)_3$), 55.4 ($C_{α,Trp}$), 44.4 ($C1_{Cyclobutyl}$), 30.7

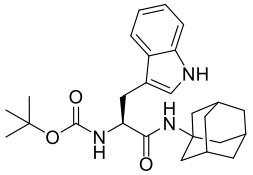
($C2_{Cyclobutyl}$), 30.5 ($C4_{Cyclobutyl}$), 28.6 ($C(\underline{C}H_3)_3$), 28.5 ($C_{\beta,Trp}$), 15.1 ($C3_{Cyclobutyl}$). UPLC-MS t_R 1.99 min, *m*/*z* 358.2 ($[M+H]^+$, $C_{20}H_{28}N_3O_3^+$ Calcd 358.2); HRMS *m*/*z* 380.1941 ($[M+Na]^+$,$C_{20}H_{27}N_3O_3Na^+$ Calcd 380.1945).

tert-Butyl (*S*)-(1-(cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (S30).[3]



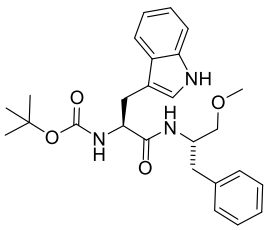
By the method described for compound **S27**, the title compound was synthesized using Boc-Trp-OH (952 mg, 3.13 mmol), HOBt (634 mg, 4.69 mmol), cyclopentylamine (0.46 mL, 4.69 mmol), *i*Pr_2NEt (1.09 mL, 6.26 mmol), anhydrous CH_2Cl_2 (24 mL), EDC (899.5 mg, 4.69 mmol). The crude product was purified by column chromatography (0→3% CH_3OH in CH_2Cl_2), affording the desired amide **S30** (1.15 g, 99%) as a colorless solid.TLC (3% CH_3OH in CH_2Cl_2): R_f = 0.35. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.84–10.75 (m, 1H, NH_{Indole}), 7.71 (d, *J* = 7.3 Hz, 1H, $CO_{Trp}NH$), 7.57 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.31 (d, *J* = 8.0 Hz, 1H, $H7_{Indole}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.08–7.02 (m, 1H, $H6_{Indole}$), 6.97 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 6.61 (d, *J* = 8.4 Hz, 1H, $NH_{\alpha,Trp}$), 4.15 (td, *J* = 8.5, 5.4 Hz, 1H, $H_{\alpha,Trp}$), 3.97 (p, *J* = 6.8 Hz, 1H, $CH_{Cyclopentyl}$), 3.00 (m_{ABX}, *J* = 14.5, 5.3 Hz, 1H, $H_{\beta,Trp,A}$), 2.89 (m_{ABX}, *J* = 14.5, 8.6 Hz, 1H, $H_{\beta,Trp,B}$), 1.82–1.66 (m, 2H, $H2_{Cyclopentyl}$), 1.66–1.52 (m, 2H, $H3_{Cyclopentyl}$), 1.53–1.22 (m, 13H, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl}$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO) δ 171.2 (CO_{Trp}), 155.0 (CO_{Boc}), 136.0 ($C7a_{Indole}$), 127.4 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.1 ($C3_{Indole}$), 77.9 ($\underline{C}(CH_3)_3$), 55.0 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 32.2 ($C2_{Cyclopentyl}$), 32.0 ($C5_{Cyclopentyl}$), 28.1 ($C(\underline{C}H_3)_3$), 28.0 ($C_{\beta,Trp}$), 23.5 ($C3_{Cyclopentyl}$), 23.4 ($C4_{Cyclopentyl}$). UPLC-MS t_R 1.97 min, *m*/*z* 372.2 ($[M+H]^+$, $C_{21}H_{30}N_3O_3^+$ Calcd 372.2).

((*S*)-1-((Adamantan-1-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-_tert_-butyl-carbamate (S31).



By the method described for compound **S27**, the title compound was synthesized using Boc-Trp-OH (203 mg, 0.67 mmol), HOBt (137 mg, 1.01 mmol), adamantylamine (154 mg, 1.02 mmol), *i*Pr_2NEt (0.34 mL, 1.95 mmol), anhydrous CH_2Cl_2 (7 mL), and EDC (192 mg, 1.00 mmol) affording the desired amide **S31** (286 mg, 69%) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (s, 1H, NH_{Indole}), 7.57 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.31 (d, *J* = 8.0 Hz, 1H, $H7_{Indole}$), 7.15 (s, 1H, $CO_{Trp}NH$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.05 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 6.60–6.51 (m, 1H, $NH_{\alpha,Trp}$), 4.13 (td, *J* = 8.5, 5.2 Hz, 1H, $H_{\alpha,Trp}$), 3.01 (m_{ABX}, *J* = 14.7, 5.4 Hz, 1H, $H_{\beta,Trp,A}$), 2.87 (m_{ABX}, *J* = 14.6, 8.6 Hz, 1H, $H_{\beta,Trp,B}$), 2.03–1.97 (m, 3H, $H3_{Ada}$, $H5_{Ada}$, $H7_{Ada}$), 1.93–1.86 (m, 6H, $H2_{Ada}$, $H8_{Ada}$, $H9_{Ada}$), 1.65–1.57 (m, 6H, $H4_{Ada}$, $H6_{Ada}$, $H10_{Ada}$), 1.32 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.9 (CO_{Trp}), 155.0 (CO_{Boc}), 136.0 ($C7a_{Indole}$), 127.5 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.7 ($C6_{Indole}$), 118.6 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.2 ($C3_{Indole}$), 78.0 ($\underline{C}(CH_3)_3$), 55.4 ($C_{\alpha,Trp}$), 50.7 ($C1_{Ada}$), 40.9 ($C3_{Ada}$, $C5_{Ada}$, $C7_{Ada}$), 36.0 ($C2_{Ada}$, $C8_{Ada}$, $C9_{Ada}$), 28.8 ($C4_{Ada}$, $C6_{Ada}$, $C10_{Ada}$), 28.1 ($C(\underline{C}H_3)_3$), 28.0 ($C_{\beta,Trp}$). UPLC-MS t_R 2.43 min, *m*/*z* 438.2 ($[M+H]^+$, $C_{26}H_{36}N_3O_3^+$ Calcd 438.3); HRMS *m*/*z* 438.2745 ($[M+H]^+$,$C_{26}H_{36}N_3O_3^+$ Calcd 438.2751). Ada=adamant-1-yl.

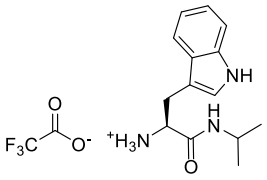
tert-Butyl ((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (S32).



By the method described for compound **S27**, the title compound was synthesized using Boc-Trp-OH (219 mg, 0.72 mmol), HOBt (148 mg, 1.09 mmol), (*S*)-(+)-1-Methoxy-3-phenyl-2-propylamine hydrochloride (224 mg, 1.11 mmol), *i*Pr_2NEt (0.25 mL, 1.43 mmol), anhydrous CH_2Cl_2 (5 mL), and EDC (208 mg, 1.09 mmol) affording the desired amide **S32** (310 mg, 69%) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (s, 1H, NH_{Indole}), 7.74 (d, *J* = 8.5 Hz, 1H, $CO_{Trp}NH$), 7.54 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.32 (d, *J* = 8.0 Hz, 1H, $H7_{Indole}$), 7.29–7.15 (m, 5H, H_{Ph}), 7.10–7.01 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 7.01–6.94 (m, 1H, $H5_{Indole}$), 6.65 (d, *J* = 8.4 Hz, 1H, $NH_{\alpha,Trp}$), 4.15 (td, *J* = 8.6, 5.2 Hz, 1H, $H_{\alpha,Trp}$), 4.10–4.00 (m, 1H, $CO_{Trp}NHC\underline{H}$), 3.26–3.13 (m, 5H, CH_2OCH_3), 2.95 (m_{ABX}, *J* = 14.6, 5.3 Hz, 1H, $H_{\beta,Trp,A}$),

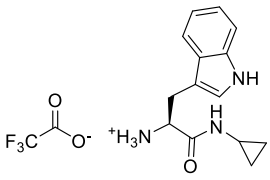
2.84 (m_{ABX}, J = 14.7, 8.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.78 (m_{ABX}, J = 13.7, 6.0 Hz, 1H, $CHCH_{2,A}Ph$), 2.69 (m_{ABX}, J = 13.7, 8.0 Hz, 1H, $CHCH_{2,B}Ph$), 1.32 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (CO_{Trp}), 155.0 (CO_{Cbz}), 138.6 ($C1_{Ph}$), 136.0 ($C7a_{Indole}$), 129.2 ($C2_{Ph}$, $C6_{Ph}$), 128.1 ($C3_{Ph}$, $C5_{Ph}$), 127.4 ($C3a_{Indole}$), 126.0 ($C4_{Ph}$), 123.5 ($C2_{Indole}$), 120.8 ($C4_{Indole}$), 118.4 ($C6_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.1 ($C3_{Indole}$), 78.0 ($\underline{C}(CH_3)_3$), 73.1 ($\underline{C}H_2OCH_3$), 58.2 ($CH_2O\underline{C}H_3$), 55.4 ($C_{\alpha,Trp}$), 49.8 ($CO_{Trp}NH\underline{C}H$), 36.7 (CH_2Ph), 28.1 ($C(\underline{C}H_3)_3$), 27.9 ($C_{\beta,Trp}$). UPLC-MS t_R 2.19 min, m/z 452.2 ($[M+H]^+$, $C_{26}H_{34}N_3O_4^+$ Calcd 452.3); HRMS m/z 474.2356 ($[M+Na]^+$,$C_{26}H_{33}N_3O_4Na^+$ Calcd 474.2363).

(*S*)-2-Amino-3-(1*H*-indol-3-yl)-*N*-isopropylpropanamide·TFA salt (S33).



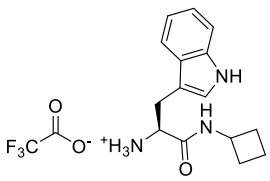
TFA (5.0 mL, 65.29 mmol) was added to a solution of compound **S27** (905 mg, 2.03 mmol) in CH_2Cl_2 (8 mL). The reaction was stirred at ambient temperature for 1 hour and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 2×100 mL), CH_2Cl_2/heptane/CH_3OH (1:1:0.06, 2×100 mL) and CH_2Cl_2/CH_3OH (1:0.06, 100 mL) affording the desired TFA salt **S33** (723 mg, 99%) as a colorless solid, which was used without further purification. ^1H NMR (400 MHz, Methanol-d_4) δ 7.62 (dt, J = 7.9, 1.0 Hz, 1H, $H4_{Indole}$), 7.37 (dt, J = 8.2, 1.0 Hz, 1H, $H7_{Indole}$), 7.18 (s, 1H, $H2_{Indole}$), 7.13 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H, $H6_{Indole}$), 7.05 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 3.97 (t, J = 7.4 Hz, 1H, $H_{\alpha,Trp}$), 3.86 (p, J = 6.6 Hz, 1H, $CH_{i\mathrm{Pr}}$), 3.36–3.28 (m, 2H, $H_{\beta,Trp,A}$, overlap with solvent peak), 3.22 (m_{ABX}, J = 14.4, 7.2 Hz, 1H, $H_{\beta,Trp,B}$),1.09 (d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.85 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (101 MHz, Methanol-d_4) δ 169.1 (CO_{Trp}), 163.1 (q, J = 34.3 Hz, CO_{TFA}), 138.2 ($C7a_{Indole}$), 128.4 ($C3a_{Indole}$), 125.4 ($C2_{Indole}$), 122.8 ($C6_{Indole}$), 120.2 ($C5_{Indole}$), 119.1 ($C4_{Indole}$), 118.3 (q, J = 293.6 Hz, CF_3), 112.5 ($C7_{Indole}$), 108.3 ($C3_{Indole}$), 55.4 ($C_{\alpha,Trp}$), 42.9 ($CH_{i\mathrm{Pr}}$), 28.9 ($C_{\beta,Trp}$), 22.4 ($CH_{3,i\mathrm{Pr},A}$), 22.0 ($CH_{3,i\mathrm{Pr},B}$). UPLC-MS t_R 1.22 min, m/z 246.1 ($[M+H]^+$, $C_{14}H_{20}N_3O^+$ Calcd 246.1); HRMS m/z 246.1599 ($[M+H]^+$,$C_{14}H_{20}N_3O^+$ Calcd 246.1601).

(*S*)-2-Amino-*N*-cyclopropyl-3-(1*H*-indol-3-yl)propanamide·TFA salt (S34).



By the method described for compound **S33**, the title compound was synthesized using TFA (2.6 mL, 33.95 mmol), **S28** (243 mg, 0.70 mmol), and CH_2Cl_2 (3.6 mL) affording the desired TFA salt **S34** (250 mg, 99%) as a colorless solid, which was used without further purification. ^1H NMR (600 MHz, DMSO-d_6) δ 11.04 (d, J = 2.5 Hz, 1H, NH_{Indole}), 8.52 (d, J = 4.1 Hz, 1H, $CO_{Trp}NH$), 8.15 (s, 3H, NH_3), 7.61 (dd, J = 7.9, 1.0 Hz, 1H, $H4_{Indole}$), 7.36 (dt, J = 8.2, 0.9 Hz, 1H, $H7_{Indole}$), 7.17 (d, J = 2.4 Hz, 1H, $H2_{Indole}$), 7.09 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.00 (ddd, J = 7.9, 6.9, 1.0 Hz, 1H, $H5_{Indole}$), 3.88–3.79 (m, 1H, $H_{\alpha,Trp}$), 3.17 (m_{ABX}, J = 14.7, 6.5 Hz, 1H, $H_{\beta,Trp,A}$), 3.09 (m_{ABX}, J = 14.7, 7.4 Hz, 1H, $H_{\beta,Trp,B}$), 2.61 (tq, J = 7.7, 3.9 Hz, 1H, $H1_{Cyclopropyl}$), 0.67–0.55 (m, 2H, $H2_{Cyclopropyl}$), 0.41–0.23 (m, 2H, $H3_{Cyclopropyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4 (CO_{Trp}), 158.2 (q, J = 32.2 Hz, CO_{TFA}), 136.2 ($C7a_{Indole}$), 127.0 ($C3a_{Indole}$), 124.7 ($C2_{Indole}$), 121.1 ($C6_{Indole}$), 118.4 ($C4_{Indole}$, $C5_{Indole}$), 116.9 (q, J = 298.0 Hz, CF_3), 111.5 ($C7_{Indole}$), 106.9 ($C3_{Indole}$), 52.8 ($C_{\alpha,Trp}$), 27.2 ($C_{\beta,Trp}$), 22.3 ($C1_{Cyclopropyl}$), 5.4 ($C2_{Cyclopropyl}$, $C3_{Cyclopropyl}$). UPLC-MS t_R 0.85 min, m/z 244.1 ($[M+H]^+$, $C_{14}H_{18}N_3O^+$ Calcd 244.1).

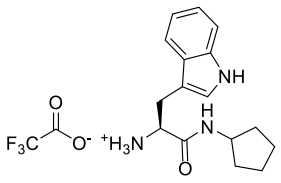
(*S*)-2-Amino-*N*-cyclobutyl-3-(1*H*-indol-3-yl)propanamide·TFA salt (S35).



By the method described for compound **S33**, the title compound was synthesized using TFA (5.8 mL, 75.7 mmol), **S29** (1.16 g, 3.25 mmol), and anhydrous CH_2Cl_2 (20 mL) affording the desired TFA salt **S35** (1.16 mg, 97%) as an off-white solid, which was used without further purification. TLC (4% CH_3OH in CH_2Cl_2): R_f = 0.45. ^1H NMR (600 MHz, DMSO-d_6) δ 11.05 (d, J = 2.5 Hz, 1H, NH_{Indole}), 8.68 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 8.13 (s, 3H, NH_3), 7.60 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.36 (d, J = 8.1 Hz, 1H, $H7_{Indole}$), 7.17 (d, J = 2.4 Hz, 1H, $H2_{Indole}$), 7.08 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.00 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 4.17 (h, J = 8.1 Hz, 1H, $H1_{Cyclobutyl}$), 3.85 (t, J = 6.9 Hz, 1H, $H_{\alpha,Trp}$), 3.17 (m_{ABX}, J = 14.6, 6.5 Hz, 1H, $H_{\beta,Trp,A}$), 3.11 (m_{ABX}, J = 14.7, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.19–2.10 (m, 1H, $H2_{Cyclobutyl,A}$), 2.10–2.00 (m, 1H, $H2_{Cyclobutyl,B}$), 1.93–1.83 (m, 1H, $H4_{Cyclobutyl,A}$), 1.77–1.67 (m, 1H,

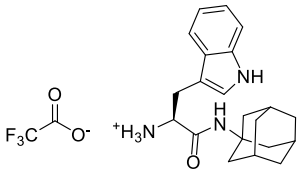
$H4_{Cyclobutyl,B}$), 1.64–1.55 (m, 2H, $H3_{Cyclobutyl}$). ^{13}C NMR (151 MHz, DMSO) δ 167.2 (CO_{Trp}), 158.1 (q, *J* = 31.0 Hz, CO_{TFA}), 136.2 ($C7a_{Indole}$), 127.1 ($C3a_{Indole}$), 124.8 ($C2_{Indole}$), 121.1 ($C6_{Indole}$), 118.41 ($C4_{Indole}$), 118.39 ($C5_{Indole}$), 117.3 (q, *J* = 300.2 Hz, CF_3), 111.4 ($C7_{Indole}$), 106.9 ($C3_{Indole}$), 52.9 ($C_{α,Trp}$), 44.0 ($C1_{Cyclobutyl}$), 29.90 ($C2_{Cyclobutyl}$), 29.85 ($C4_{Cyclobutyl}$), 27.3 ($C_{β,Trp}$), 14.7 ($C3_{Cyclobutyl}$). UPLC-MS t_R 1.09 min, *m*/*z* 258.1 ($[M+H]^+$, $C_{15}H_{21}N_3O^+$ Calcd 258.2); HRMS *m*/*z* 258.1599 ($[M+H]^+$,$C_{15}H_{20}N_3O^+$ Calcd 258.1601).

(*S*)-2-Amino-*N*-cyclopentyl-3-(1*H*-indol-3-yl)propanamide·TFA salt (S36).



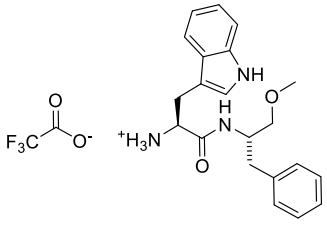
By the method described for compound **S33**, the title compound was synthesized using TFA (5.5 mL, 71.8 mmol), **S30** (ALA27, 1.07 g, 2.89 mmol), and CH_2Cl_2 (10 mL) affording the desired TFA salt **S36** (1.08 g, 97%) as an off-white solid, which was used without further purification. ^1H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H, NH_{Indole}), 8.31 (d, *J* = 7.2 Hz, 1H, $CO_{Trp}NH$), 8.14 (s, 3H, NH_3), 7.61 (dd, *J* = 7.7, 1.1 Hz, 1H, $H4_{Indole}$), 7.36 (dt, *J* = 8.2, 0.9 Hz, 1H, $H7_{Indole}$), 7.18 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.09 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 7.00 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 4.01–3.93 (m, 1H, $H1_{Cyclopentyl}$), 3.88 (t, *J* = 7.1 Hz, 1H, $H_{α,Trp}$), 3.16 (m_{ABX}, *J* = 14.6, 6.9 Hz, 1H, $H_{β,Trp,A}$), 3.10 (m_{ABX}, *J* = 14.6, 7.3 Hz, 1H, $H_{β,Trp,B}$), 1.82–1.74 (m, 1H, $H2_{Cyclopentyl,A}$), 1.68–1.34 (m, 6H, $H2_{Cyclopentyl,B}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl,A}$), 1.20–1.11 (m, 1H, $H5_{Cyclopentyl,B}$). ^{13}C NMR (151 MHz, DMSO) δ 167.6 (CO_{Trp}), 158.0 (q, *J* = 30.8 Hz, CO_{TFA}), 136.2 ($C7a_{Indole}$), 127.1 ($C3a_{Indole}$), 124.7 ($C2_{Indole}$), 121.1 ($C6_{Indole}$), 118.41 ($C4_{Indole}$), 118.36 ($C5_{Indole}$), 117.3 (q, *J* = 300.7 Hz, CF_3), 111.4 ($C7_{Indole}$), 107.0 ($C3_{Indole}$), 52.9 ($C_{α,Trp}$), 50.5 ($C1_{Cyclopentyl}$), 31.97 ($C2_{Cyclopentyl}$), 31.94 ($C5_{Cyclopentyl}$), 27.3 ($C_{β,Trp}$), 23.4 ($C3_{Cyclopentyl}$), 23.3 ($C4_{Cyclopentyl}$). UPLC-MS t_R 1.17 min, *m*/*z* 272.2 ($[M+H]^+$, $C_{16}H_{22}N_3O^+$ Calcd 272.2).

(*S*)-*N*-(Adamantan-1-yl)-2-amino-3-(1*H*-indol-3-yl)propanamide·TFA salt (S37).



By the method described for compound **S33**, the title compound was synthesized using TFA (2.6 mL, 33.95 mmol), **S31** (248 mg, 0.57 mmol), and CH_2Cl_2 (3.6 mL) affording the desired TFA salt **S37** (253 mg, 99%) as a colorless solid, which was used without further purification. ^1H NMR (600 MHz, DMSO-d_6) δ 11.03 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 8.04 (s, 3H, NH_3), 7.81 (s, 1H, $CO_{Trp}NH$), 7.67 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.37 (d, *J* = 8.1 Hz, 1H, $H7_{Indole}$), 7.19 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.12–7.05 (m, 1H, $H6_{Indole}$), 7.01 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 3.97–3.86 (m, 1H, $H_{α,Trp}$), 3.16 (m_{ABX}, *J* = 14.6, 6.4 Hz, 1H, $H_{β,Trp,A}$), 3.07 (m_{ABX}, *J* = 14.6, 7.7 Hz, 1H, $H_{β,Trp,B}$), 2.04–1.95 (m, 3H, $H3_{Adamantyl}$, $H5_{Adamantyl}$, $H7_{Adamantyl}$), 1.86 (br s, 6H, $H2_{Adamantyl}$, $H8_{Adamantyl}$, $H9_{Adamantyl}$), 1.64–1.55 (m, 6H, $H4_{Adamantyl}$, $H6_{Adamantyl}$, $H10_{Adamantyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.3 (CO_{Trp}), 158.0 (q, *J* = 31.1 Hz, CO_{TFA}),136.2 ($C7a_{Indole}$), 127.1 ($C3a_{Indole}$), 124.8 ($C2_{Indole}$), 121.1 ($C6_{Indole}$), 118.6 ($C4_{Indole}$), 118.3 ($C5_{Indole}$), 117.2 (q, *J* = 300.3 Hz, CF_3), 111.4 ($C7_{Indole}$), 107.1 ($C3_{Indole}$), 53.0 ($C_{α,Trp}$), 51.3 ($C1_{Adamantyl}$), 40.7 ($C3_{Adamantyl}$, $C5_{Adamantyl}$, $C7_{Adamantyl}$), 35.9 ($C2_{Adamantyl}$, $C8_{Adamantyl}$, $C9_{Adamantyl}$), 28.7 ($C4_{Adamantyl}$, $C6_{Adamantyl}$, $C10_{Adamantyl}$), 27.4 ($C_{β,Trp}$). UPLC-MS t_R 1.55 min, *m*/*z* 338.2 ($[M+H]^+$, $C_{21}H_{28}N_3O^+$ Calcd 338.2); HRMS *m*/*z* 338.2223 ($[M+H]^+$,$C_{21}H_{28}N_3O^+$ Calcd 338.2227).

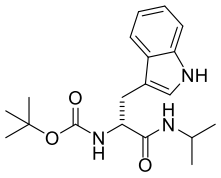
(*S*)-2-Amino-3-(1*H*-indol-3-yl)-*N*-((*S*)-1-methoxy-3-phenylpropan-2-yl)propanamide·TFA salt (S38).



By the method described for compound **S33**, the title compound was synthesized using TFA (2.6 mL, 33.95 mmol), **S32** (276 mg, 0.61 mmol), and CH_2Cl_2 (3.6 mL) affording the desired TFA salt **S38** (295 mg, >99%) as a colorless solid, which was used without further purification. ^1H NMR (600 MHz, DMSO-d_6) δ 11.05 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.50 (d, *J* = 8.2 Hz, 1H, $CO_{Trp}NH$), 8.10 (s, 3H, NH_3), 7.67 (dd, *J* = 7.9, 1.1 Hz, 1H, $H4_{Indole}$), 7.37 (dt, *J* = 8.1, 0.9 Hz, 1H, $H7_{Indole}$), 7.32–7.26 (m, 2H, $H2_{Ar,Ph}$, $H6_{Ar,Ph}$), 7.25–7.17 (m, 4H, $H3_{Ar,Ph}$, $H4_{Ar,Ph}$, $H5_{Ar,Ph}$, $H2_{Indole}$), 7.09 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.01 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 4.09–4.00 (m, 1H, $CO_{Trp}NHC\underline{H}$), 3.99–3.92 (m, 1H, $H_{α,Trp}$), 3.23–3.05 (m, 7H, CH_2OCH_3, $H_{β,Trp}$), 2.80 (m_{ABX}, *J* = 13.7, 6.7 Hz, 1H, $PhCH_{2,A}$), 2.73 (m_{ABX}, *J* = 13.7, 7.3 Hz, 1H, $PhCH_{2,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.3 (CO_{Trp}), 157.7 (q, *J* = 30.4 Hz, CO_{TFA}), 138.3

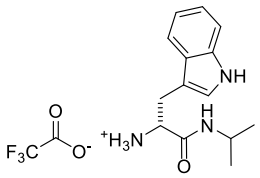
($C1_{Ph}$), 136.3 ($C7a_{Indole}$), 129.1 ($C2_{Ph}$, $C6_{Ph}$), 128.3 ($C3_{Ph}$, $C5_{Ph}$), 127.1 ($C3a_{Indole}$), 126.2 ($C4_{Ph}$), 124.9 ($C2_{Indole}$), 121.1 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.4 ($C5_{Indole}$), 117.4 (q, J = 301.3 Hz, CF_3),111.5 ($C7_{Indole}$), 106.9 ($C3_{Indole}$), 72.4 ($\underline{C}H_2OCH_3$), 58.2 ($CH_2O\underline{C}H_3$), 52.7 ($C_{\alpha,Trp}$), 50.5 ($CO_{Trp}NH\underline{C}H$), 36.5 (CH_2Ph), 27.6 ($C_{\beta,Trp}$). UPLC-MS t_R 1.38 min, m/z 352.1 ($[M+H]^+$, $C_{21}H_{26}N_3O_2^+$ Calcd 352.2); HRMS m/z 352.2016 ($[M+H]^+$,$C_{21}H_{26}N_3O_2^+$ Calcd 352.2020).

***tert*-Butyl (*R*)-(3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)carbamate (S39).**



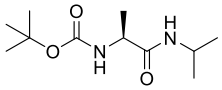
By the method described for compound **S27**, the title compound was synthesized using Boc-D-Trp-OH (202 mg, 0.66 mmol), HOBt (141 mg, 1.04 mmol), iPrNH$_2$ (0.11 mL, 1.28 mmol), iPr$_2$NEt (0.17 mL, 0.98 mmol), CH_2Cl_2 (4.0 mL), and EDC (193 mg, 1.0 mmol) affording the desired amide **S39** (197 mg, 86%) as a colorless solid, which was used without further purification. TLC (50% EtOAc in heptane): R_f = 0.4. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH_{Indole}), 7.64 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.31 (d, J = 8.1 Hz, 1H, $H7_{Indole}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.61 (d, J = 8.4 Hz, 1H, $NH_{\alpha,Trp}$), 4.13 (td, J = 8.5, 5.3 Hz, 1H, $H_{\alpha,Trp}$), 3.90–3.74 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.01 (m_{ABX}, J = 14.5, 5.4 Hz, 1H, $H_{\beta,Trp,A}$), 2.89 (m_{ABX}, J = 14.5, 8.7 Hz, 1H, $H_{\beta,Trp,B}$), 1.32 (s, 9H, $C(CH_3)_3$), 1.03 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.97 (d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.7 (CO_{Trp}), 155.0 (CO_{Boc}), 136.0 ($C7a_{Indole}$), 127.4 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.1 ($C3_{Indole}$), 77.9 ($\underline{C}(CH_3)_3$), 55.1 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 28.1 ($C(\underline{C}H_3)_3$), 28.0 ($C_{\beta,Trp}$), 22.27 ($CH_{3,i\mathrm{Pr,A}}$), 22.25 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 2.00 min, m/z 346.3 ($[M+H]^+$, $C_{19}H_{28}N_3O_3^+$ Calcd 364.2); HRMS m/z 346.2130 ($[M+H]^+$,$C_{19}H_{28}N_3O_3^+$ Calcd 346.2125).

(*R*)-2-Amino-3-(1*H*-indol-3-yl)-*N*-isopropylpropanamide·TFA salt (S40).



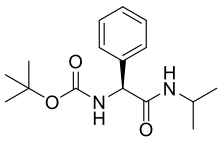
TFA (4.0 mL, 52.1 mmol) and TIPS (250 µL, 1.20 mmol) were added to a solution of **S39** (82 mg, 0.24 mmol) in CH_2Cl_2 (6.0 mL). The reaction mixture was stirred at ambient temperature for 2 hours and was then concentrated under reduced pressure. The excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 3×30 mL), CH_2Cl_2/heptane (1:1, 2×30 mL), and CH_2Cl_2 (30 mL), affording the desired TFA salt **S40** (120 mg, >99%) as an off-white solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.05 (s, 1H, NH_{Indole}), 8.27 (d, J = 7.2 Hz, 1H, $CO_{Trp}NH$), 8.24–8.05 (br s, 3H, NH_3), 7.63 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.36 (dd, J = 8.0, 1.0 Hz, 1H, $H7_{Indole}$), 7.19 (d, J = 2.4 Hz, 1H, $H2_{Indole}$), 7.08 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 7.00 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 3.86 (t, J = 7.1 Hz, 1H, $NH_{\alpha,Trp}$), 3.85–3.76 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.17 (m_{ABX}, J = 14.5, 6.7 Hz, 1H, $H_{\beta,Trp,A}$), 3.09 (m_{ABX}, J = 14.5, 7.5 Hz, 1H, $H_{\beta,Trp,B}$), 1.06 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.89 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO) δ 167.30 (CO_{Trp}), 158.1 (q, J = 30.0 Hz, CO_{TFA}), 136.2 ($C7a_{Indole}$), 127.1 ($C3a_{Indole}$), 124.8 ($C2_{Indole}$), 121.1 ($C6_{Indole}$), 118.42 ($C4_{Indole}$), 118.36 ($C5_{Indole}$), 117.3 (q, J = 300.5 Hz, CF_3), 111.4 ($C7_{Indole}$), 107.1 ($C3_{Indole}$), 52.9 ($C_{\alpha,Trp}$), 40.9 ($CH_{i\mathrm{Pr}}$), 27.4 ($C_{\beta,Trp}$), 22.1 ($CH_{3,i\mathrm{Pr,A}}$), 21.9 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 1.24 min, m/z 246.3 ($[M+H]^+$, $C_{14}H_{20}N_3O^+$ Calcd 246.2); HRMS m/z 246.1600 ($[M+H]^+$,$C_{14}H_{20}N_3O^+$ Calcd 246.1601).

***tert*-Butyl (*S*)-(1-(isopropylamino)-1-oxopropan-2-yl)carbamate (S41).[4]**



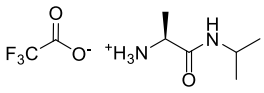
By the method described for compound **S27**, the title compound was synthesized using Boc-Ala-OH (201 mg, 1.06 mmol), HOBt (289 mg, 2.14 mmol), iPrNH$_2$ (0.20 mL, 2.33 mmol), iPr$_2$NEt (0.40 mL, 2.30 mmol), CH_2Cl_2 (6.0 mL), and EDC (408 mg, 2.13 mmol) affording the desired amide **S41** (122 mg, 50%) as a colorless solid, which was used without further purification. TLC (50% EtOAc in heptane): R_f = 0.3. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.87 (s, 1H, $NH_{\alpha,Ala}$), 4.95 (s, 1H, $CO_{Ala}NH$), 4.18–3.96 (m, 2H, $H_{\alpha,Ala}$, $CH_{i\mathrm{Pr}}$), 1.45 (s, 9H, $C(CH_3)_3$), 1.33 (d, J = 7.1 Hz, 3H, $H_{\beta,Ala}$), 1.15 (d, J = 4.3 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 1.14 (d, J = 4.4 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.7 (CO_{Ala}), 155.7 (CO_{Boc}), 80.3 ($\underline{C}(CH_3)_3$), 50.3 ($C_{\alpha,Ala}$), 41.6 ($CH_{i\mathrm{Pr}}$), 28.5 ($C(\underline{C}H_3)_3$), 22.8 ($CH_{3,i\mathrm{Pr}}$), 18.3 ($C_{\beta,Ala}$). UPLC-MS t_R 1.69 min, m/z 231.2 ($[M+H]^+$, $C_{11}H_{23}N_2O_3^+$ Calcd 231.2).

***tert*-Butyl (*S*)-(2-(isopropylamino)-2-oxo-1-phenylethyl)carbamate (S42).**



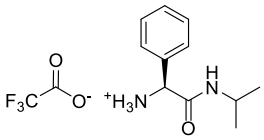
By the method described for compound **S27**, the title compound was synthesized using N^{α}-Boc-phenylglycine (203 mg, 0.81 mmol), HOBt (218 mg, 1.61 mmol), *i*PrNH$_2$ (0.15 mL, 1.75 mmol), *i*Pr$_2$NEt (0.20 mL, 1.15 mmol), CH_2Cl_2 (4.0 mL), EDC (312 mg, 1.62 mmol) affording the desired amide **S42** (189 mg, 80%) as a colorless solid, which was used without further purification. TLC (50% EtOAc in heptane): R_f = 0.6. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38–7.29 (m, 5H, $H_{Ar,Phg}$), 5.78 (s, 1H, $NH_{\alpha,Phg}$), 5.37 (s, 1H, $CO_{Phg}NH$), 5.04 (s, 1H, $H_{\alpha,Phg}$), 4.12–4.00 (m, 1H, $CH_{i Pr}$), 1.41 (s, 9H, $C(CH_3)_3$), 1.15 (d, J = 6.5 Hz, 3H, $CH_{3,iPr,A}$), 1.03 (d, J = 6.5 Hz, 3H $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.2 (CO_{Phg}), 155.3 (CO_{Boc}), 138.8 ($C1_{Ar,Phg}$), 129.2 ($C2_{Ph}$, $C6_{Ph}$), 128.4 ($C4_{Ph}$), 127.4 ($C3_{Ph}$, $C5_{Ph}$), 80.1 ($\underline{C}(CH_3)_3$), 58.8 ($C_{\alpha,Phg}$), 42.1 ($CH_{i Pr}$), 28.5 ($C(\underline{C}H_3)_3$), 22.8 ($CH_{3,iPr,A}$), 22.6 ($CH_{3,iPr,B}$).

(*S*)-2-Amino-*N*-isopropylpropanamide·TFA salt (S43).



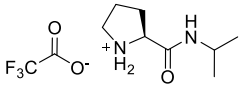
By the method described for compound **S40**, the title compound was synthesized using TFA (4.0 mL, 52.2 mmol), **S41** (113 mg, 0.49 mmol), TIPS (250 µL, 1.20 mmol), and CH_2Cl_2 (3.0 mL) affording the desired TFA salt **S43** (134 mg, >99%) as an off-white solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23 (d, J = 7.5 Hz, 1H, $CO_{Ala}NH$), 8.06 (s, 3H, NH_3), 3.92–3.80 (m, 1H, $CH_{i Pr}$), 3.76–3.69 (m, 1H, $H_{\alpha,Ala}$), 1.31 (d, J = 7.0 Hz, 3H, $H_{\beta,Ala}$), 1.09 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,A}$), 1.07 (d, J = 6.5 Hz, 3H, $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, DMSO) δ 168.3 (CO_{Ala}), 157.9 (q, J = 30.2 Hz, CO_{TFA}), 117.3 (q, J = 300.6 Hz, $CF_{3,TFA}$), 48.2 ($C_{\alpha,Ala}$), 40.8 ($CH_{i Pr}$), 22.14 ($CH_{3,iPr,A}$), 22.12 ($CH_{3,iPr,B}$), 17.26 ($C_{\beta,Ala}$). Triisopropylsilane (6%) could be detected as an impurity. HRMS *m*/*z* 131.1179 ($[M+H]^+$,$C_6H_{15}N_2O^+$ Calcd 131.1179).

(*S*)-2-Amino-*N*-isopropyl-2-phenylacetamide·TFA salt (S44).



By the method described for compound **S33**, the title compound was synthesized using TFA (2.0 mL, 26.1 mmol), **S42** (189 mg, 0.65 mmol), and CH_2Cl_2 (3.0 mL) affording the desired TFA salt **S44** (206 mg, >99%) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (s, 3H, NH_3), 8.45 (d, J = 7.6 Hz, 1H, $CO_{Phg}NH$), 7.53–7.48 (m, 2H, $H2_{Ph}$, $H6_{Ph}$), 7.47–7.37 (m, 3H, $H3_{Ph}$, $H4_{Ph}$, $H5_{Ph}$), 4.85 (s, 1H, $H_{\alpha,Phg}$), 3.89–3.79 (m, 1H, $CH_{i Pr}$), 1.10 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,A}$), 0.94 (d, J = 6.5 Hz, 3H, $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.1 (CO_{Phg}), 158.1 (q, J = 30.9 Hz, CO_{TFA}), 134.3 ($C1_{Ph}$), 129.1 ($C4_{Ph}$), 128.8 ($C2_{Ph}$, $C6_{Ph}$), 127.6 ($C3_{Ph}$, $C5_{Ph}$), 117.3 (q, J = 300.2 Hz, $CF_{3,TFA}$), 55.5 ($C_{\alpha,Phg}$), 41.1 ($CH_{i Pr}$), 22.0 ($CH_{3,iPr,A}$), 21.9 ($CH_{3,iPr,B}$). UPLC-MS t_R 1.43 min, *m*/*z* 193.1 ($[M+H]^+$, $C_{11}H_{17}N_2O^+$ Calcd 193.1); HRMS *m*/*z* 193.1334 ($[M+H]^+$,$C_{11}H_{17}N_2O^+$ Calcd 193.1335).

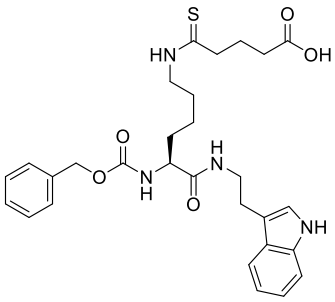
(*S*)-*N*-Isopropylpyrrolidine-2-carboxamide·TFA salt (S45).



Boc-Pro-OH (201 mg, 0.93 mmol), HOBt (191 mg, 1.41 mmol), *i*PrNH$_2$ (0.16 mL, 1.86 mmol) and *i*Pr$_2$NEt (0.24 mL, 1.38 mmol) were dissolved in anhydrous CH_2Cl_2 (4.0 mL) and cooled to 0 °C. EDC (270 mg, 1.41 mmol) was added and the reaction mixture was stirred at 0°C for 5 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (50 mL) and washed with aq. $KHSO_4$ (5%, 3×50 mL), saturated aq. $NaHCO_3$ (3×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure, affording a colorless solid (140 mg). TFA (2.0 mL, 26.1 mmol) was added to a solution of the colorless solid (140 mg) in CH_2Cl_2 (3.0 mL). The reaction mixture was stirred at ambient temperature for 35 minutes and was then concentrated under reduced pressure. The excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 2×40 mL), CH_2Cl_2/MeCN (1:1, 40 mL), CH_2Cl_2/heptane (1:1, 40 mL) and CH_2Cl_2 (40 mL) affording the desired TFA salt **S45** (157 mg, >99%) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.95 (s, 1H, $NH_{\alpha,Pro,A}$), 7.88–7.60 (m, 2H, $NH_{\alpha,Pro,B}$, $CO_{Pro}NH$), 4.63–4.50 (m, 1H, $H_{\alpha,Pro}$), 4.05–3.92 (m, 1H, $CH_{i Pr}$), 3.51–3.33 (m, 2H, $H_{\delta,Pro}$), 2.51–2.38 (m, 1H, $H_{\beta,Pro,A}$), 2.14–1.93 (m, 3H, $H_{\beta,Pro,B}$, $H_{\gamma,Pro}$), 1.15

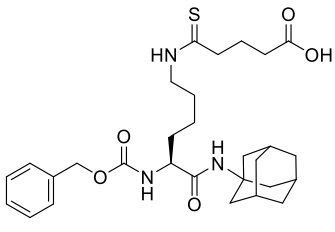
(d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 1.14 (d, J = 6.7 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.6 (CO_{Pro}), 161.9 (q, J = 36.9 Hz, CO_{TFA}), 116.3 (q, J = 291.1 Hz, CF_3), 59.7 ($C_{\alpha,\mathrm{Pro}}$), 46.7 ($C_{\delta,\mathrm{Pro}}$), 42.7 ($CH_{i\mathrm{Pr}}$), 30.5 ($C_{\beta,\mathrm{Pro}}$), 24.7 ($C_{\gamma,\mathrm{Pro}}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.0 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 0.45 min, m/z 157.1 ($[M+H]^+$, $C_8H_{17}N_2O^+$ Calcd 157.1); HRMS m/z 157.1335 ($[M+H]^+$,$C_8H_{17}N_2O^+$ Calcd 157.1335).

(*S*)-5-((6-((2-(1*H*-Indol-3-yl)ethyl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (1).



DIC (11.5 µL, 0.07 mmol) was added to a solution of the carboxylic acid **S24** (31 mg, 0.07 mmol) and HOBt (13 mg, 0.08 mmol) in anhydrous CH_2Cl_2 (5.0 mL). The reaction mixture was stirred at ambient temperature for 15 minutes after which tryptamine (13 mg, 0.08 mmol) was added. After one hour the reaction mixture was diluted with EtOAc (50 mL) and washed with aq. HCl (2 M, 2×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography (0→1.25% CH_3OH in CH_2Cl_2) of the crude residue afforded a clear oil (30 mg), tentatively assigned as *tert*-butyl-(*S*)-5-((6-((2-(1*H*-indol-3-yl)ethyl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoate (UPLC-MS t_R 2.41 min, m/z 609.3; $[M+H]^+$, $C_{33}H_{45}N_4O_5S^+$ Calcd 609.3), which was used without further purification. TFA (2.0 mL, 26.1 mmol) was added to a solution of the clear oil (30 mg) in anhydrous CH_2Cl_2 (3.0 mL). The mixture was stirred for 1.5 hours and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **1** (14 mg, 37% from **S24**), as a colorless fluffy material after liophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, J = 2.4 Hz, 1H, NH_{Indole}), 9.91 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,\mathrm{Lys}}$), 7.97 (t, J = 5.7 Hz, 1H, $CO_{\mathrm{Lys}}NH$), 7.54 (d, J = 7.9 Hz, 1H, $H4_{\mathrm{Indole}}$), 7.48–7.19 (m, 7H, $H_{\mathrm{Ar,Cbz}}$, $NH_{\alpha,\mathrm{Lys}}$, $H7_{\mathrm{Indole}}$), 7.13 (d, J = 2.3 Hz, 1H, $H2_{\mathrm{Indole}}$), 7.08–7.03 (m, 1H, $H6_{\mathrm{Indole}}$), 7.00–6.95 (m, 1H, $H5_{\mathrm{Indole}}$), 5.08–4.97 (m, 2H, $CH_{2,\mathrm{Cbz}}$), 3.99–3.90 (m, 1H, $H_{\alpha,\mathrm{Lys}}$, overlap with residual water), 3.50–3.40 (m, 1H, $H_{\varepsilon,\mathrm{Lys}}$), 3.40–3.26 (m, 2H, $CO_{\mathrm{Lys}}NHC\underline{H}_2$), 2.81 (t, J = 7.5 Hz, 2H, $CO_{\mathrm{Lys}}NHCH_2C\underline{H}_2$), 2.53 (t, J = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.66–1.44 (m, 4H, $H_{\beta,\mathrm{Lys}}$, $H_{\delta,\mathrm{Lys}}$), 1.36–1.21 (m, 2H, $H_{\gamma,\mathrm{Lys}}$). ^{13}C NMR (151 MHz, DMSO-d6) δ 202.8 (C=S), 174.1 (CO_2H), 171.7 (CO_{Lys}), 156.0 (CO_{Cbz}), 137.1 ($C1_{\mathrm{Ar,Cbz}}$), 136.3 ($C7a_{\mathrm{Indole}}$), 128.4 ($C3_{\mathrm{Ar,Cbz}}$, $C5_{\mathrm{Ar,Cbz}}$), 127.8 ($C4_{\mathrm{Ar,Cbz}}$), 127.7 ($C2_{\mathrm{Ar,Cbz}}$, $C6_{\mathrm{Ar,Cbz}}$), 127.2 ($C3a_{\mathrm{Indole}}$), 122.7 ($C2_{\mathrm{Indole}}$), 120.9 ($C6_{\mathrm{Indole}}$), 118.3 ($C4_{\mathrm{Indole}}$), 118.2 ($C5_{\mathrm{Indole}}$), 111.7 ($C3_{\mathrm{Indole}}$), 111.4 ($C7_{\mathrm{Indole}}$), 65.4 ($CH_{2,\mathrm{Cbz}}$), 54.7 ($C_{\alpha,\mathrm{Lys}}$), 45.1 ($C_{\varepsilon,\mathrm{Lys}}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 39.4 ($CO_{\mathrm{Lys}}NH\underline{C}H_2$ overlap with solvent peak), 32.7 ($\underline{C}H_2CO_2H$), 31.7 ($C_{\beta,\mathrm{Lys}}$), 26.9 ($C_{\delta,\mathrm{Lys}}$), 25.2 ($CO_{\mathrm{Lys}}NHCH_2\underline{C}H_2$), 24.3 ($\underline{C}H_2CH_2CO_2H$), 23.1 ($C_{\gamma,\mathrm{Lys}}$). UPLC-MS t_R 2.25 min, m/z 553.4 ($[M+H]^+$, $C_{29}H_{37}N_4O_5S^+$ Calcd 553.2); HRMS m/z 553.2481 ($[M+H]^+$, $C_{29}H_{37}N_4O_5S^+$ Calcd 553.2479).

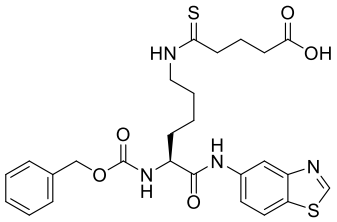
5-(((*S*)-6-((Adamantan-1-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (3).



The carboxylic acid **S24** (79 mg, 0.17 mmol), HOBt (36 mg, 0.26 mmol), 1-adamantylamine (52 mg, 0.34 mmol) and iPr$_2$NEt (0.10 mL, 0.57 mmol) were dissolved in anhydrous CH_2Cl_2 (4.0 mL) and cooled to 0°C. EDC (41 mg, 0.21 mmol) was added and the reaction mixture was stirred at 0°C for 10 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (40 mL) and washed with aq. $KHSO_4$ (5%, 2×40 mL), saturated aq. $NaHCO_3$ (4x40 mL), and brine (2×40 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The residue was dissolved in in anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) was added. The mixture was stirred at ambient temperature for 40 minutes and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **3** (26 mg, 29% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 9.90 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,\mathrm{Lys}}$), 7.41–7.27 (m, 5H, $H_{\mathrm{Ar,Cbz}}$), 7.22 (s, 1H, $NH_{\mathrm{Adamantyl}}$), 7.16 (d, J = 8.3 Hz, 1H, $NH_{\alpha,\mathrm{Lys}}$), 5.02 (s, 2H, $CH_{2,\mathrm{Cbz}}$), 3.96–3.83 (m, 1H, $H_{\alpha,\mathrm{Lys}}$), 3.50–

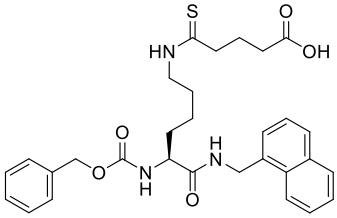
3.40 (m, J = 6.5 Hz, 2H, $H_{\varepsilon,Lys}$), 2.55–2.50 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.99 (s, 3H, $H3_{Adamantyl}$, $H5_{Adamantyl}$, $H7_{Adamantyl}$), 1.94–1.80 (m, 8H, $H2_{Adamantyl}$, $H8_{Adamantyl}$, $H9_{Adamantyl}$, $C\underline{H}_2CH_2CO_2H$), 1.66–1.43 (m, 10H, $H4_{Adamantyl}$, $H6_{Adamantyl}$, $H10_{Adamantyl}$, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.36–1.20 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 170.9 (CO_{Lys}), 155.7 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 65.3 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 50.7 ($C1_{Adamantyl}$), 45.0 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 40.9 ($C3_{Adamantyl}$, $C5_{Adamantyl}$, $C7_{Adamantyl}$), 36.0 ($C2_{Adamantyl}$, $C8_{Adamantyl}$, $C9_{Adamantyl}$), 32.7 ($\underline{C}H_2CO_2H$), 32.1 ($C_{\beta,Lys}$), 28.8 ($C4_{Adamantyl}$, $C6_{Adamantyl}$, $C10_{Adamantyl}$), 26.9 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 22.9 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.22 min, m/z 544.2 ($[M+H]^+$, $C_{29}H_{42}N_3O_5S^+$ Calcd 544.3); HRMS m/z 544.2846 ($[M+H]^+$, $C_{29}H_{42}N_3O_5S^+$ Calcd 544.2840).

(*S*)-5-((6-(Benzo[d]thiazol-5-ylamino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (4).



By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (27 mg, 0.06 mmol), HOBt (17 mg, 0.12 mmol), 1,3-benzothiazol-5-amine (18 mg, 0.12 mmol), iPr$_2$NEt (0.10 mL, 0.57 mmol), CH_2Cl_2 (4.0 mL), and EDC (23 mg, 0.12 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **4** (4 mg, 12% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 10.27 (s, 1H, $CO_{Lys}NH$), 9.92 (s, 1H, $NH_{\varepsilon,Lys}$), 9.37 (s, 1H, $H2_{Bth}$), 8.48 (d, J = 2.0 Hz, 1H, $H4_{Bth}$), 8.07 (d, J = 8.7 Hz, 1H, $H7_{Bth}$), 7.66–7.57 (m, 2H, $H6_{Bth}$, $NH_{\alpha,Lys}$), 7.43–7.12 (m, 5H, $H_{Ar,Cbz}$), 5.04 (s, 2H, $CH_{2,Cbz}$), 4.23–4.12 (m, 1H, $H_{\alpha,Lys}$), 3.53–3.44 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 2.56–2.52 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.19 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.86 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.78–1.50 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.50–1.31 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.1 (CO_2H), 171.4 (CO_{Lys}), 157.0 (CO_{Cbz}), 156.1 ($C2_{Bth}$), 153.6 ($C3a_{Bth}$), 137.5 ($C5_{Bth}$), 137.0 ($C1_{Ar,Cbz}$), 128.4, ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.0 ($C7a_{Bth}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 122.8 ($C7_{Bth}$), 118.6 ($C6_{Bth}$), 113.3 ($C4_{Bth}$), 65.9 ($CH_{2,Cbz}$), 55.9 ($C_{\alpha,Lys}$), 45.4 ($C_{\varepsilon,Lys}$), 44.3 ($\underline{C}H_2(CH_2)_2CO_2H$), 33.1 ($\underline{C}H_2CO_2H$), 32.0 ($C_{\beta,Lys}$), 27.3 ($C_{\delta,Lys}$), 24.7 ($\underline{C}H_2CH_2CO_2H$), 23.7 ($C_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO) δ 202.8, 174.1, 171.4, 157.0, 156.1, 153.6, 137.5, 137.0, 128.4, 128.0, 127.8, 127.7, 122.4, 118.1, 112.9, 65.5, 55.5, 45.0, 43.9, 40.4, 32.7, 31.5, 26.9, 24.3, 23.2. UPLC-MS t_R 1.78 min, m/z 541.1 ($[M-H]^-$, $C_{26}H_{29}N_4O_5S_2^-$ Calcd 541.2); HRMS m/z 543.1737 ($[M+H]^+$, $C_{26}H_{31}N_4O_5S_2^+$Calcd 543.1730). Bth=1,3-benzothiazol-5-yl

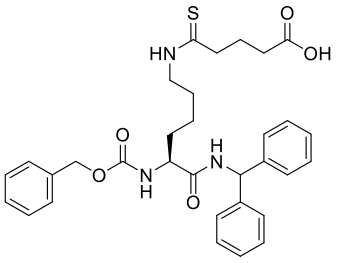
(*S*)-5-((5-(((Benzyloxy)carbonyl)amino)-6-((naphthalen-1-ylmethyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (5).



By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (52 mg, 0.11 mmol), HOBt (17 mg, 0.13 mmol), naphthalen-1-yl-methaneamine (20 µL, 0.14 mmol), iPr$_2$NEt (40 µL, 0.23 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (33 mg, 0.17 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **5** (15 mg, 24% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 9.90 (t, J = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.44 (t, J = 5.7 Hz, 1H, $CO_{Lys}NH$), 8.06–7.99 (m, 1H, $H8_{Naph}$), 7.98–7.92 (m, 1H, $H5_{Naph}$, 7.85 (dd, J = 6.9, 2.5 Hz, 1H, $H4_{Naph}$), 7.59–7.25 (m, 10H, $H2_{Naph}$, $H3_{Naph}$, $H6_{Naph}$, $H7_{Naph}$, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.81–4.66 (m, 2H, CH_2Naph), 4.04 (td, J = 8.6, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.50–3.35 (m, 2H, $H_{\varepsilon,Lys}$), 2.55–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.71–1.43 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.39–1.22 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 171.9 (CO_{Lys}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 134.4 ($C1_{Naph}$), 133.2 ($C4a_{Naph}$), 130.8 ($C8a_{Naph}$), 128.4 ($C5_{Naph}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.73 ($C4_{Ar,Cbz}$), 127.65 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C4_{Naph}$), 126.1 ($C6_{Naph}$), 125.8 ($C7_{Naph}$), 125.4 ($C3_{Naph}$), 125.2 ($C2_{Naph}$), 123.4 ($C8_{Naph}$), 65.4 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.7 ($\underline{C}H_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS

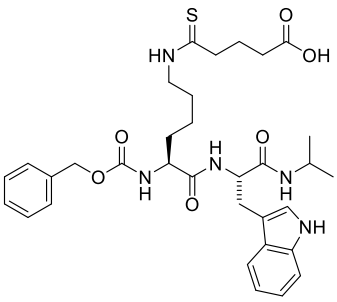
t_R 2.03 min, *m*/*z* 550.2 ([M+H]$^+$, $C_{30}H_{36}N_3O_5S^+$ Calcd 550.2); HRMS *m*/*z* 550.2376 ([M+H]$^+$, $C_{30}H_{36}N_3O_5S^+$ Calcd 550.2370).

(*S*)-5-((6-(Benzhydrylamino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (6).



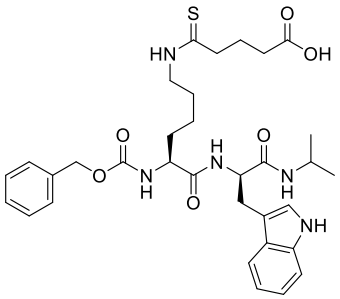
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (45 mg, 0.10 mmol), HOBt (20 mg, 0.15 mmol), benzhydrylamine (33 µL, 0.19 mmol), *i*Pr$_2$NEt (0.10 mL, 0.57 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (28 mg, 0.15 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **6** (17 mg, 30% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (br s, 1H, CO_2H), 9.90 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.78 (d, *J* = 8.5 Hz, 1H, $CO_{Lys}NH$), 7.46–7.14 (m, 16H, $H_{Ar,Cbz}$, $H_{Ar,Bh,}$ $NH_{\alpha,Lys}$), 6.09 (d, *J* = 8.4 Hz, 1H, $CH(Ph)_2$), 5.02 (s, 2H, $CH_{2,Cbz}$), 4.22–4.07 (m, 1H, $H_{\alpha,Lys}$), 3.52–3.38 (m, 2H, $H_{\varepsilon,Lys}$), 2.56–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.70–1.44 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.39–1.19 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) *δ* 202.7 (C=S), 174.0 (CO_2H), 171.3 (CO_{Lys}), 155.9 (CO_{Cbz}), 142.4 ($C1_{Ar,Bh,A}$), 142.2 ($C1_{Ar,Bh,B}$), 137.0 ($C1_{Ar,Cbz}$), 128.4 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 128.3 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.8 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.7 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.4 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.1 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.0 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 126.9 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 65.4 ($CH_{2,Cbz}$), 55.9 ($CH(Ph)_2$), 54.5 ($C_{\alpha,Lys}$), 45.0 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.7 ($\underline{C}H_2CO_2H$), 31.7 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.14 min, *m*/*z* 576.2 ([M+H]$^+$, $C_{32}H_{38}N_3O_5S^+$ Calcd 576.3); HRMS *m*/*z* 576.2533 ([M+H]$^+$, $C_{32}H_{38}N_3O_5S^+$ Calcd 576.2527). Bh=Benzhydryl

5-(((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (10).



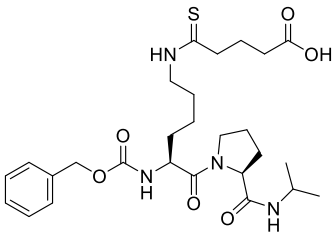
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (56 mg, 0.12 mmol), HOBt (24 mg, 0.18 mmol), the TFA salt **S33** (78 mg, 0.21 mmol), *i*Pr$_2$NEt (46 µL, 0.26 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (35 mg, 0.18 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affordeding the desired carboxylic acid **10** (27 mg, 35% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 10.78 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 9.88 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.85 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.65 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.42 (d, *J* = 7.9 Hz, 1H, $NH_{\alpha,Lys}$), 7.39–7.21 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.2 Hz, 1H, $H2_{Indole}$), 7.04 (t, *J* = 7.5 Hz, 1H, $H6_{Indole}$), 6.95 (t, *J* = 7.3 Hz, 1H, $H5_{Indole}$), 5.08–4.97 (m, 2H, $CH_{2,Cbz}$), 4.50–4.41 (m, 1H, $H_{\alpha,Trp}$), 3.95 (td, *J* = 8.3, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.82–3.71 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.43–3.38 (m, $H_{\varepsilon,Lys}$, overlap with residual water), 3.06 (m_{ABX}, *J* = 14.6, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, *J* = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.52 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.60–1.43 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.24 (m, 2H, $H_{\gamma,Lys}$), 0.99 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 171.4 (CO_{Lys}), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 40.4 ($CH_{i\mathrm{Pr}}$), 32.6 ($\underline{C}H_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 27.9 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 1.94 min, *m*/*z* 638.4 ([M+H]$^+$, $C_{33}H_{44}N_5O_6S^+$ Calcd 638.3); HRMS *m*/*z* 638.3013 ([M+H]$^+$,$C_{33}H_{44}N_5O_6S^+$ Calcd 638.3007).

5-(((*S*)-6-(((*R*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (11).



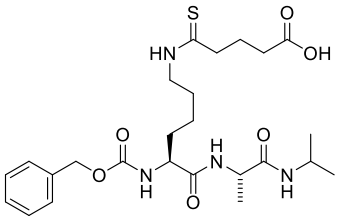
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (66 mg, 0.14 mmol), HOBt (32 mg, 0.24 mmol), TFA salt **S40** (140 mg, 0.35 mmol), *i*Pr_2NEt (75 µL, 0.43 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (35 mg, 0.18 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **11** (36 mg, 40% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (br s, 1H, CO_2H), 10.74 (d, J = 2.4 Hz, 1H, NH_{Indole}), 9.85 (t, J = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.13 (d, J = 8.3 Hz, 1H, $NH_{\alpha,Trp}$), 7.65–7.53 (m, 2H, $CO_{Trp}NH$, $H4_{Indole}$), 7.42 (d, J = 7.3 Hz, 1H, $NH_{\alpha,Lys}$), 7.38–7.21 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.08 (d, J = 2.2 Hz, 1H, $H2_{Indole}$), 7.06–7.00 (m, 1H, $H6_{Indole}$), 6.99–6.93 (m, 1H $H5_{Indole}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 4.42 (td, J = 8.7, 4.9 Hz, 1H, $H_{\alpha,Trp}$), 3.97–3.89 (m, $H_{\alpha,Lys}$), 3.88–3.76 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.58–3.28 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.15 (m_{ABX}, J = 14.6, 4.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.89 (m_{ABX}, J = 14.6, 9.2 Hz, 1H, $H_{\beta,Trp,B}$), 2.57–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.21 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.50–1.29 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.16–0.91 (m, 8H, $H_{\gamma,Lys}$, $(CH_3)_{2,i\mathrm{Pr}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 171.7 (CO_{Lys}), 170.0 (CO_{Trp}), 156.1 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.2 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.2 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Trp}$), 44.9 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 40.4 ($CH_{i\mathrm{Pr}}$), 32.6 ($\underline{C}H_2CO_2H$), 31.0 ($C_{\beta,Lys}$), 27.7 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 22.7 ($C_{\gamma,Lys}$), 22.19 ($CH_{3,i\mathrm{Pr,A}}$), 22.17 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 1.93 min, m/z 638.4 ($[M+H]^+$, $C_{33}H_{44}N_5O_6S^+$ Calcd 638.3); HRMS m/z 638.3013 ($[M+H]^+$, $C_{33}H_{44}N_5O_6S^+$ Calcd 638.3007).

5-(((*S*)-5-(((Benzyloxy)carbonyl)amino)-6-((*S*)-2-(isopropylcarbamoyl)pyrrolidin-1-yl)-6-oxohexyl)amino)-5-thioxopentanoic acid (12).



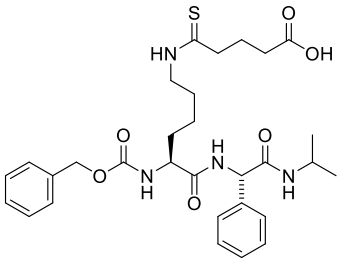
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (48 mg, 0.10 mmol), HOBt (24 mg, 0.19 mmol), TFA salt **S45** (78 mg, 0.22 mmol), *i*Pr_2NEt (54 µL, 0.31 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (35 mg, 0.18 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **12** (36 mg, 63% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.56 (d, J = 7.8 Hz, 1H, $CO_{Pro}NH$), 7.44 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Lys}$), 7.40–7.23 (m, 5H, $H_{Ar,Cbz}$), 5.04–4.97 (m, 2H, $CH_{2,Cbz}$), 4.26–4.18 (m, 2H, $H_{\alpha,Lys}$, $H_{\alpha,Pro}$, overlap with residual water), 3.82–3.74 (m, 2H, $CH_{i\mathrm{Pr}}$, overlap with residual water), 3.68–3.51 (m, 2H, $CH_{2,\delta,Pro}$ overlap with residual water), 3.50–3.39 (m, 2H, $H_{\varepsilon,Lys}$), 2.54 (t, J = 7.4 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 2.06–1.45 (m, 10H, $H_{\beta,Lys}$, $C\underline{H}_2CH_2CO_2H$, $H_{\gamma,Pro}$, $H_{\beta,Pro}$, $H_{\delta,Lys}$), 1.44–1.31 (m, 2H, $H_{\gamma,Lys}$), 1.03 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 1.01 (d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.0 (CO_2H), 170.44 (CO_{Lys}), 170.35 (CO_{Pro}), 156.0 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 65.4 ($CH_{2,Cbz}$), 59.5 ($C_{\alpha,Pro}$), 52.4 ($C_{\alpha,Lys}$), 46.8 ($C_{\delta,Pro}$), 45.0 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 40.3 ($CH_{i\mathrm{Pr}}$), 32.6 ($\underline{C}H_2CO_2H$), 30.5 ($C_{\beta,Pro}$), 29.1 ($C_{\beta,Lys}$), 26.9 ($C_{\delta,Lys}$), 24.5 ($C_{\gamma,Pro}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 22.7 ($C_{\gamma,Lys}$), 22.30 ($CH_{3,i\mathrm{Pr,A}}$), 22.28 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 1.77 min, m/z 549.4 ($[M+H]^+$, $C_{27}H_{41}N_4O_6S^+$ Calcd 549.3); HRMS m/z 571.2567 ($[M+Na]^+$,$C_{27}H_{40}N_4O_6SNa^+$ Calcd 571.2561).

5-(((*S*)-5-(((Benzyloxy)carbonyl)amino)-6-(((*S*)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (13).



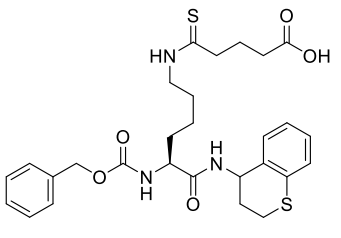
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (54 mg, 0.12 mmol), HOBt (27 mg, 0.20 mmol), TFA salt **S43** (45 mg, 0.18 mmol), *i*Pr$_2$NEt (60 µL, 0.34 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (57 mg, 0.30 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **13** (31 mg, 51% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.02 (br s, 1H, CO_2H), 9.90 (t, J = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.88 (d, J = 7.6 Hz, 1H, $NH_{\alpha,Ala}$), 7.63 (d, J = 7.7 Hz, 1H, $CO_{Ala}NH$), 7.42 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Lys}$), 7.40–7.24 (m, 5H, $H_{Ar,Cbz}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.19 (p, J = 7.1 Hz, 1H, $H_{\alpha,Ala}$), 3.96 (td, J = 8.4, 4.9 Hz, 1H, $H_{\alpha,Lys}$), 3.80 (h, J = 6.7 Hz, 1H, $CH_{i\mathrm{Pr}}$), 3.49–3.35 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 2.53 (t, J = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, J = 7.6 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.68–1.46 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.39–1.24 (m, 2H, $H_{\gamma,Lys}$), 1.17 (d, J = 7.0 Hz, 3H, $CH_{3,\ Ala}$), 1.06–0.99 (m, 6H, $(CH_3)_{2,i\mathrm{Pr}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 171.4 (CO_{Lys}), 171.0 (CO_{Ala}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 65.4 ($CH_{2,Cbz}$), 54.6 ($C_{\alpha,Lys}$), 48.0 ($C_{\alpha,Ala}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($C\underline{H}_2(CH_2)_2CO_2H$), 40.6 ($CH_{i\mathrm{Pr}}$), 32.6 ($C\underline{H}_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($C\underline{H}_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$), 22.3 ($CH_{3,i\mathrm{Pr,A}}$), 22.2 ($CH_{3,i\mathrm{Pr,B}}$), 18.4 ($CH_{3,Ala}$). UPLC-MS t_R 1.87 min, *m*/*z* 523.4 ([M+H]$^+$, $C_{25}H_{39}N_4O_6S^+$ Calcd 523.3); HRMS *m*/*z* 523.2590 ([M+H]$^+$,$C_{25}H_{39}N_4O_6S^+$ Calcd 523.2585).

5-(((*S*)-5-(((Benzyloxy)carbonyl)amino)-6-(((*S*)-2-(isopropylamino)-2-oxo-1-phenylethyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (14).



By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (49 mg, 0.11 mmol), HOBt (26 mg, 0.19 mmol), TFA salt **S44** (67 mg, 0.22 mmol), *i*Pr$_2$NEt (55 µL, 0.32 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (57 mg, 0.30 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **14** (29 mg, 43% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 9.90 (t, J = 5.4 Hz, 1H $NH_{\varepsilon,Lys}$), 8.30 (d, J = 8.0 Hz, 1H, $NH_{\alpha,Phg}$), 8.16 (d, J = 7.6 Hz, 1H, $CO_{Phg}NH$), 7.53 (d, J = 8.3 Hz, 1H, $NH_{\alpha,Lys}$), 7.44–7.21 (m, 10H, $CH_{Ar,Phg}$, $H_{Ar,Cbz}$), 5.39 (d, J = 8.1 Hz, 1H, $H_{\alpha,Phg}$), 5.09–4.91 (m, 2H, $CH_{2,Cbz}$), 4.10 (td, J = 8.8, 4.9 Hz, 1H, $H_{\alpha,Lys}$), 3.87–3.74 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.52–3.37 (m, 2H, $H_{\varepsilon,Lys}$), 2.53 (t, J = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.71–1.45 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.39–1.22 (m, 2H, $H_{\gamma,Lys}$), 1.08 (d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.96 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 171.4 (CO_{Lys}), 168.5 (CO_{Phg}), 156.0 (CO_{Cbz}), 138.9 ($C1_{Ar,Phg}$), 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C_{Ar,Phg}$, $C_{Ar,Cbz}$), 128.2 ($C_{Ar,Phg}$, $C_{Ar,Cbz}$), 127.7 ($C_{Ar,Phg}$, $C_{Ar,Cbz}$), 127.6 ($C_{Ar,Phg}$, $C_{Ar,Cbz}$), 127.3 ($C_{Ar,Phg}$, $C_{Ar,Cbz}$), 126.7 ($C_{Ar,Phg}$, $C_{Ar,Cbz}$), 65.4 ($CH_{2,Cbz}$), 55.9 ($C_{\alpha,Phg}$), 54.5 ($C_{\alpha,Lys}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($C\underline{H}_2(CH_2)_2CO_2H$), 40.6 ($CH_{i\mathrm{Pr}}$), 32.7 ($C\underline{H}_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($C\underline{H}_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 1.91 min, *m*/*z* 585.4 ([M+H]$^+$, $C_{30}H_{41}N_4O_6S^+$ Calcd 585.3); HRMS *m*/*z* 585.2747 ([M+H]$^+$,$C_{30}H_{41}N_4O_6S^+$ Calcd 585.2741).

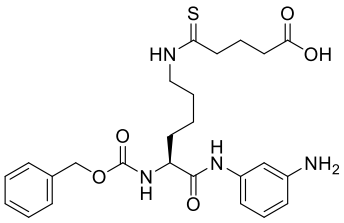
5-(((5*S*)-5-(((Benzyloxy)carbonyl)amino)-6-oxo-6-(thiochroman-4-ylamino)hexyl)amino)-5-thioxopentanoic acid (S1).



By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (50 mg, 0.11 mmol), HOBt (29 mg, 0.22 mmol), thiochroman-4-amine[5] (35 mg, 0.21 mmol), *i*Pr$_2$NEt (0.10 mL, 0.57 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (58 mg, 0.30 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording a diasteroisomeric mixture (ratio ≈1:1 based on NMR and analytical HPLC, termed I and II when distinguishable in NMR assignment) of the

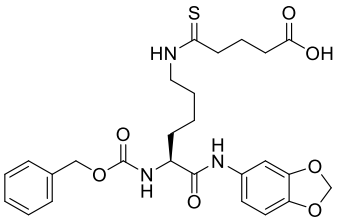
desired carboxylic acid **S1** (23 mg, 39% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) 12.05 (br s, 1H, CO_2H), 9.92 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.44 (d, J = 8.3 Hz, 1H_I, $CO_{Lys}NH_I$), 8.34 (d, J = 8.2 Hz, 1H_{II}, $CO_{Lys}NH_{II}$), 7.42–7.25 (m, 6H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$), 7.22–6.96 (m, 4H, $H_{Ar,Tc}$), 5.09–4.92 (m, 3H, $CH_{2,Cbz}$, $H4_{Tc}$), 4.05–3.94 (m, 1H, $H_{\alpha,Lys}$), 3.56–3.42 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 3.15–2.90 (m, 2H, $H2_{Tc}$), 2.57–2.51 (m, 2H, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 2.21 (t, J = 7.4 Hz, 2H, C$\underline{H}_2$CO$_2$H), 2.15–1.95 (m, 2H, $H3_{Tc}$), 1.88 (p, J = 7.5 Hz, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 1.68–1.47 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.41–1.21 (m, 2H $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.1 (CO_2H), 171.22 ($CO_{Lys,I}$), 171.18 ($CO_{Lys,II}$), 155.95 ($CO_{Cbz,I}$), 155.91 ($CO_{Cbz,II}$), 137.1 ($C1_{Ar,Cbz,I}$), 137.0 ($C1_{Ar,Cbz,II}$), 133.74 ($C4a_{Tc,I}$), 133.68 ($C4a_{Tc,II}$), 133.13 ($C8a_{Tc,I}$), 133.11 ($C8a_{Tc,II}$), 130.0 ($C5_{Tc,I}$), 129.8 ($C5_{Tc,II}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.80 ($C4_{Ar,Cbz,I}$), 127.78 ($C4_{Ar,Cbz,II}$), 127.72 ($C2_{Ar,Cbz,I}$, $C6_{Ar,Cbz,I}$), 127.67 ($C2_{Ar,Cbz,II}$, $C6_{Ar,Cbz,II}$), 127.6 ($C8_{Tc}$), 126.3 ($C7_{Tc,I}$), 126.2 ($C7_{Tc,II}$), 124.21 ($C6_{Tc,I}$), 124.15 ($C6_{Tc,II}$), 65.41 ($CH_{2,Cbz,I}$), 65.36 ($CH_{2,Cbz,II}$), 54.7 ($C_{\alpha,Lys,I}$), 54.5 ($C_{\alpha,Lys,II}$), 45.6 ($C4_{Tc,I}$), 45.5 ($C4_{Tc,II}$), 45.1 ($C_{\varepsilon,Lys}$), 43.9 (C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 32.7 (C$\underline{H}_2$CO$_2$H), 31.8 ($C_{\beta,Lys,I}$), 31.7 ($C_{\beta,Lys,II}$), 28.7 ($C3_{Tc}$), 26.9 ($C_{\delta,Lys}$), 24.3 (C$\underline{H}_2$CH$_2$CO$_2$H), 23.1 ($C_{\gamma,Lys}$), 22.70 ($C2_{Tc,I}$), 22.65 ($C2_{Tc,II}$). UPLC-MS t_R 2.05 min, m/z 558.2 ([M+H]$^+$, $C_{28}H_{36}N_3O_5S_2^+$ Calcd 558.2); HRMS m/z 558.2096 ([M+H]$^+$, $C_{28}H_{36}N_3O_5S_2^+$ Calcd 558.2091). Tc=thiochroman-4-yl.

(*S*)-5-((6-((3-Aminophenyl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S2).



By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (50 mg, 0.11 mmol), HOBt (22 mg, 0.17 mmol), *tert*-butyl-(3-aminophenyl)carbamate[6] (45 mg, 0.21 mmol), *i*Pr$_2$NEt (0.10 mL, 0.57 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (32 mg, 0.17 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S2** (15 mg, 23% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 9.85 (s, 1H, $CO_{Lys}NH$), 7.51 (d, J = 7.7 Hz, 1H, $NH_{\alpha,Lys}$), 7.43–6.83 (m, 8H, $H_{Ar,Cbz}$, $H6_{APh}$, $H5_{APh}$, $H4_{APh}$), 6.49 (s, 1H, $H2_{APh}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.19–4.03 (m, 1H, $H_{\alpha,Lys}$), 3.53–3.37 (m, 2H, $H_{\varepsilon,Lys}$), 2.55–2.50 (m, 2H, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, C$\underline{H}_2$CO$_2$H), 1.87 (p, J = 7.5 Hz, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 1.73–1.48 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.49–1.27 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 170.9 (CO_{Lys}), 156.0 (CO_{Cbz}), 139.7 ($C1_{APh}$/$C3_{APh}$), 137.0 ($C1_{Ar,Cbz}$), 129.2 ($C5_{APh}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C2_{Ar,Cbz}$, $C4_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 126.9 ($C1_{APh}$/$C3_{APh}$), 111.8 ($C6_{APh}$), 110.4 ($C4_{APh}$), 107.6 ($C2_{APh}$), 65.4 ($CH_{2,Cbz}$), 55.3 ($C_{\alpha,Lys}$), 44.9 ($C_{\varepsilon,Lys}$), 43.8 (C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 32.6 (C$\underline{H}_2$CO$_2$H), 31.5 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 (C$\underline{H}_2$CH$_2$CO$_2$H), 23.1 ($C_{\gamma,Lys}$). Peaks for $C2_{APh}$, $C4_{APh}$, and $C6_{APh}$ were broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.43 min, m/z 501.2 ([M+H]$^+$, $C_{25}H_{33}N_4O_5S^+$ Calcd 501.2); HRMS m/z 501.2172 ([M+H]$^+$, $C_{25}H_{33}N_4O_5S^+$ Calcd 501.2166). APh=3-aminophenyl.

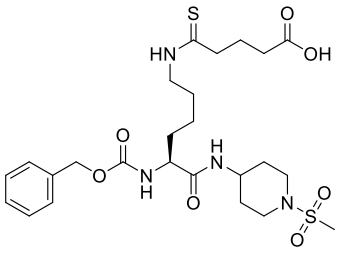
(*S*)-5-((6-(Benzo[d][1,3]dioxol-5-ylamino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S3).



By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (40 mg, 0.09 mmol), HOBt (24 mg, 0.18 mmol), 3,4-(methylenedioxy)aniline (25 mg, 0.18 mmol), *i*Pr$_2$NEt (0.10 mL, 0.57 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (34 mg, 0.18 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S3** (11 mg, 24% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 9.99–9.85 (m, 2H, $NH_{\varepsilon,Lys}$, $CO_{Lys}NH$), 7.52 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Lys}$), 7.43–7.15 (m, 6H, $H_{Ar,Cbz}$, $H4_{Ar,Bd}$), 6.97 (dd, J = 8.4, 2.1 Hz, 1H, $H6_{Ar,Bd}$), 6.84 (d, J = 8.4 Hz, 1H, $H7_{Ar,Bd}$), 5.97 (d, J = 2.6 Hz, 2H, $H2_{Bd}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.11–4.02 (m, 1H, $H_{\alpha,Lys}$), 3.51–3.41 (m, 2H, $H_{\varepsilon,Lys}$), 2.54–2.51 (m, 2H, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, C$\underline{H}_2$CO$_2$H), 1.87 (p, J = 7.5 Hz, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 1.71–1.49 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.44–1.26 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.0 (CO_2H), 170.6 (CO_{Lys}), 156.0 (CO_{Cbz}), 147.0 ($C3a_{Bd}$/$C7a_{Bd}$), 142.9 ($C3a_{Bd}$/$C7a_{Bd}$), 137.0

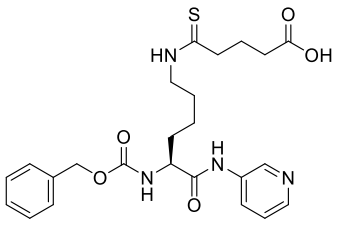
($C1_{Ar,Cbz}$), 133.3 ($C5_{Bd}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 112.0 ($C6_{Bd}$), 108.0 ($C7_{Bd}$), 101.4 ($C4_{Bd}$), 100.9, ($C2_{Bd}$), 65.4 ($CH_{2,Cbz}$), 55.3 ($C_{\alpha,Lys}$), 44.9 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.6 ($\underline{C}H_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.84 min, *m*/*z* 530.2 ($[M+H]^+$, $C_{26}H_{32}N_3O_7S^+$ Calcd 530.2); HRMS *m*/*z* 530.1961 ($[M+H]^+$, $C_{26}H_{32}N_3O_7S^+$ Calcd 530.1955); HRMS *m*/*z* 552.1781 ($[M+Na]^+$, $C_{26}H_{31}N_3NaO_7S^+$ Calcd 552.1775). Bd= benzo[d][1,3]dioxol-5-yl.

(*S*)-5-((5-(((Benzyloxy)carbonyl)amino)-6-((1-(methylsulfonyl)piperidin-4-yl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S4).



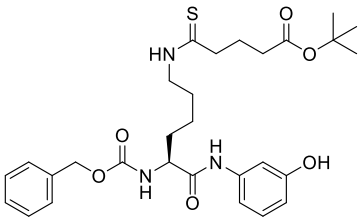
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (42 mg, 0.09 mmol), HOBt (25 mg, 0.19 mmol), 1-(methylsulfonyl)piperidin-4-amine (33 mg, 0.19 mmol), *i*Pr_2NEt (0.10 mL, 0.57 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (36 mg, 0.19 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S4** (21 mg, 40% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (br s, 1H, CO_2H), 9.90 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.91 (d, *J* = 7.7 Hz, 1H, $CO_{Lys}NH$), 7.43–7.25 (m, 6H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$), 5.02 (s, 2H, $CH_{2,Cbz}$), 3.95–3.90 (m, 1H, $H_{\alpha,Lys}$), 3.72–3.62 (m, 1H, $H4_{Msp}$), 3.54–3.40 (m, 4H, $H_{\varepsilon,Lys}$, $H2_{Msp}$/$H6_{Msp}$), 2.89–2.77 (m, 5H, SO_2CH_3, $H2_{Msp}$/$H6_{Msp}$), 2.55–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.83–1.75 (m, 2H, $H3_{Msp}$/$H5_{Msp}$), 1.63–1.21 (m, 8H, $H_{\beta,Lys}$, $H_{\delta,Lys}$, $H_{\gamma,Lys}$, $H3_{Msp}$/$H5_{Msp}$). ^{13}C NMR (151 MHz, DMSO-d_6) *δ* 202.7 (C=S), 174.0 (CO_2H), 171.2 (CO_{Lys}), 155.9 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 65.31 ($CH_{2,Cbz}$), 54.5 ($C_{\alpha,Lys}$), 45.01 ($C2_{Msp}$/$C4_{Msp}$/$C6_{Msp}$), 44.97 ($C2_{Msp}$/$C4_{Msp}$/$C6_{Msp}$), 44.4 ($C_{\varepsilon,Lys}$/$C2_{Msp}$/$C6_{Msp}$), 44.3 ($C_{\varepsilon,Lys}$/$C2_{Msp}$/$C6_{Msp}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 34.3 ($SO_2CH_3$), 32.6 ($\underline{C}H_2CO_2H$), 31.8 ($C_{\beta,Lys}$), 30.8 ($C3_{Msp}$/$C5_{Msp}$), 30.7 ($C3_{Msp}$/$C5_{Msp}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.61 min, *m*/*z* 571.2 ($[M+H]^+$, $C_{25}H_{39}N_4O_7S_2^+$ Calcd 571.2); HRMS *m*/*z* 571.2261 ($[M+H]^+$, $C_{25}H_{39}N_4O_7S_2^+$ Calcd 571.2255). Msp=1-(methylsulfonyl)piperidin-4-yl.

(*S*)-5-((5-(((Benzyloxy)carbonyl)amino)-6-oxo-6-(pyridin-3-ylamino)hexyl)amino)-5-thioxopentanoic acid (S5).



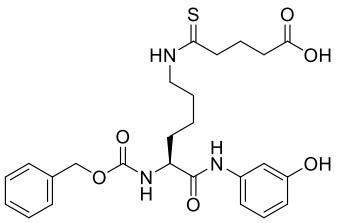
By the method described for compound **3**, the title compound was synthesized using carboxylic acid **S24** (66 mg, 0.14 mmol), HOBt (22 mg, 0.16 mmol), 3-aminopyridine (17 mg, 0.18 mmol), *i*Pr_2NEt (40 µL, 0.23 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (41 mg, 0.21 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S5** (13 mg, 19% from **S24**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.05 (br s, 1H, CO_2H), 10.48 (s, 1H, $CO_{Lys}NH$), 9.92 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.90 (s, 1H, $H2_{pyridinyl}$), 8.38 (dd, *J* = 4.9, 1.4 Hz, 1H, $H6_{pyridinyl}$), 8.18 (d, *J* = 8.4 Hz, 1H, $H4_{pyridinyl}$), 7.66 (d, *J* = 7.6 Hz, 1H, $NH_{\alpha,Lys}$), 7.55 (dd, *J* = 8.5, 5.0 Hz, 1H, $H5_{pyridinyl}$), 7.44–7.13 (m, 5H, $H_{Ar,Cbz}$), 5.07–5.00 (m, 2H, $CH_{2,Cbz}$), 4.20–4.08 (m, 1H, $H_{\alpha,Lys}$), 3.47 (m, 2H, $H_{\varepsilon,Lys}$), 2.54–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.19 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.86 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.77–1.29 (m, 6H, $H_{\beta,Lys}$, $H_{\delta,Lys}$, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.0 (CO_2H), 171.9 (CO_{Lys}), 156.1 (CO_{Cbz}), 142.0 ($C6_{pyridinyl}$), 138.2 ($C2_{pyridinyl}$), 136.9 ($C1_{Ar,Cbz}$), 136.3 ($C3_{pyridinyl}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$, $C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 124.8 ($C5_{pyridinyl}$), 65.5 ($CH_{2,Cbz}$), 55.4 ($C_{\alpha,Lys}$), 44.9 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.6 ($\underline{C}H_2CO_2H$), 31.2 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.46 min, *m*/*z* 487.2 ($[M+H]^+$, $C_{24}H_{31}N_4O_5S^+$ Calcd 487.2); HRMS *m*/*z* 487.2015 ($[M+H]^+$, $C_{24}H_{31}N_4O_5S^+$ Calcd 487.2010).

***tert*-Butyl (*S*)-5-((5-(((benzyloxy)carbonyl)amino)-6-((3-hydroxyphenyl)amino)-6-oxohexyl)amino)-5-thioxopentanoate (S46).**



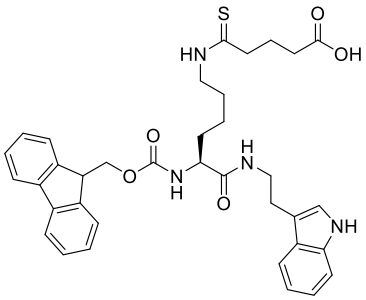
Carboxylic acid **S24** (64 mg, 0.14 mmol), HOBt (30 mg, 0.22 mmol), 3-aminophenol (30 mg, 0.27 mmol) and *i*Pr_2NEt (0.05 mL, 0.29 mmol) were dissolved in anhydrous CH_2Cl_2 (4.0 mL) and cooled to 0°C. EDC (40 mg, 0.21 mmol) was added and the reaction mixture was stirred at 0°C for 10 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (40 mL) and washed with aq. $KHSO_4$ (5%, 3×40 mL), saturated aq. $NaHCO_3$ (3×40 mL), and brine (2×40 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (heptane/EtOAc, 1:1), affording the desired amide **S46** (34 mg, 44%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H, $NH_{\varepsilon,Lys}$), 7.41–7.28 (m, 5H, $H_{Ar,Cbz}$), 7.14 (t, *J* = 7.9 Hz, 1H, $H5_{HPh}$), 6.64–6.55 (m, 1H, $H6_{HPh}$), 6.49 (d, *J* = 8.6 Hz, 2H, $H4_{HPh}$, $H2_{HPh}$), 5.52 (d, *J* = 8.2 Hz, 1H, $NH_{\alpha,Lys}$), 5.13 (s, 2H, $CH_{2,Cbz}$), 4.63–4.50 (m, 1H, $H_{\alpha,Lys}$), 3.73–3.55 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 2.68 (t, *J* = 7.3 Hz, 2H, C$\underline{H}_2(CH_2)_2CO_2t$Bu), 2.25 (t, *J* = 7.0 Hz, 2H, C$\underline{H}_2CO_2$H), 2.09–1.96 (m, *J* = 7.1 Hz, 3H, C$\underline{H}_2CH_2CO_2t$Bu, $H_{\beta,Lys,A}$), 1.92–1.80 (m, 1H, $H_{\beta,Lys,B}$), 1.79–1.63 (m, 2H, $H_{\gamma,Lys}$), 1.61–1.47 (m, 2H, $H_{\delta,Lys}$), 1.43 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 204.6 (C=S), 173.3 (CO_2tBu), 171.1 (CO_{Lys}), 156.3 (CO_{Cbz}), 151.4 ($C1_{HPh}$), 146.6 ($C3_{HPh}$), 136.2 ($C1_{Ar,Cbz}$), 130.3 ($C5_{HPh}$), 128.7 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.4 ($C4_{Ar,Cbz}$), 128.2 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 113.8 ($C6_{HPh}$), 111.9 ($C4_{HPh}$), 108.7 ($C2_{HPh}$), 80.9 ($\underline{C}(CH_3)_3$), 67.3 ($CH_{2,Cbz}$), 53.8 ($C_{\alpha,Lys}$), 45.8 ($C_{\varepsilon,Lys}$), 45.7 ($\underline{C}H_2(CH_2)_2CO_2t$Bu), 34.2 ($\underline{C}H_2CO_2t$Bu), 32.5 ($C_{\beta,Lys}$), 28.2 ($C(\underline{C}H_3)_3$), 27.4 ($C_{\gamma,Lys}$), 24.9 ($\underline{C}H_2CH_2CO_2t$Bu), 22.8 ($C_{\delta,Lys}$). UPLC-MS t_R 2.29 min, *m*/*z* 558.2 ($[M+H]^+$, $C_{29}H_{40}N_3O_6S^+$ Calcd 558.3). HPh=hydroxyphen-3-yl.

(*S*)-5-((5-(((Benzyloxy)carbonyl)amino)-6-((3-hydroxyphenyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S6).



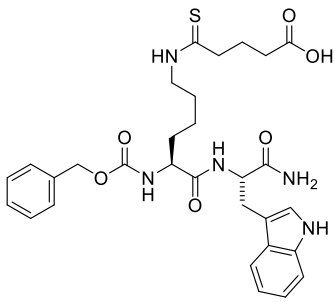
t-Butyl ester **S46** (33.8, 0.06 mmol) was dissolved in in anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) was added. The mixture was stirred at ambient temperature for 1 hour and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 30 mL), CH_2Cl_2/heptane (1:1, 30 mL) and CH_2Cl_2/MeCN (1:1, 12 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **S6** (8 mg, 27%), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.94 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.90 (d, *J* = 7.3 Hz, 1H, $NH_{\alpha,Lys}$), 7.40–7.27 (m, 5H, $H_{Ar,Cbz}$), 7.07 (t, *J* = 8.0 Hz, 1H, $H5_{HPh}$), 6.52 (d, *J* = 8.2 Hz, 1H, $H4_{HPh}$/6_{HPh}), 6.36 (s, 1H, $H2_{HPh}$), 6.27 (d, *J* = 7.8 Hz, 1H, $H4_{HPh}$/6_{HPh}), 5.07 (s, 2H, $CH_{2,Cbz}$), 4.24–4.16 (m, 1H, $H_{\alpha,Lys}$), 3.60–3.38 (m, 2H, $H_{\varepsilon,Lys}$), 2.54 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2(CH_2)_2CO_2$H), 2.21 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2CO_2$H), 1.92–1.69 (m, 4H, C$\underline{H}_2CH_2CO_2$H, $H_{\beta,Lys}$), 1.67–1.54 (m, 2H, $H_{\delta,Lys}$), 1.50–1.38 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) *δ* 202.8 (C=S), 174.0 (CO_2H), 171.2 (CO_{Lys}), 156.2 (CO_{Cbz}), 151.2 ($C1_{HPh}$), 148.1 ($C3_{HPh}$), 136.9 ($C1_{Ar,Cbz}$), 129.7 ($C5_{HPh}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.9 ($C4_{Ar,Cbz}$), 127.8 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 112.5 ($C6_{HPh}$), 109.6 ($C4_{HPh}$), 107.7 ($C2_{HPh}$), 65.6 ($CH_{2,Cbz}$), 54.1 ($C_{\alpha,Lys}$), 44.8 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2$H), 32.6 ($\underline{C}H_2CO_2$H), 30.2 ($C_{\beta,Lys}$), 26.7 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2$H), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.75 min, *m*/*z* 502.2 ($[M+H]^+$, $C_{25}H_{32}N_3O_6S^+$ Calcd 502.2); HRMS *m*/*z* 502.2012 ($[M+H]^+$, $C_{25}H_{32}N_3O_6S^+$ Calcd 502.2006). HPh=hydroxyphen-3-yl.

(*S*)-5-((6-((2-(1*H*-Indol-3-yl)ethyl)amino)-5-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S7).



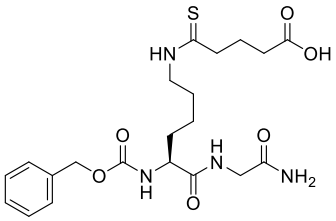
The carboxylic acid **S26** (19 mg, 0.03 mmol) and HOBt (60 mg, 0.44 mmol) was dissolved in CH_2Cl_2 (4.0 mL). DIC (5.7 µL, 0.04 mmol) was added and the solution was stirred for 10 minutes before tryptamine (7 mg, 0.05 mmol) was added. The reaction mixture was stirred at ambient temperature for 70 minutes and was then diluted with EtOAc (50 mL) and washed with 2 M HCl (2×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (0→2% CH_3OH in CH_2Cl_2) of the crude residue afforded a clear oil (13 mg), tentatively assigned as the *t*-butyl ester of **S7** (UPLC-MS t_R 2.68 min, *m*/*z* 697.2; $[M+H]^+$, $C_{40}H_{48}N_4O_5S^+$ Calcd 697.3). The clear oil (11 mg) was dissolved in anhydrous CH_2Cl_2 (2.0 mL) and TFA (1.3 mL, 17.0 mmol) was added. The mixture was stirred at ambient temperature for 1h and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the carboxylic acid **S7** (3 mg, 16% from **S26**), as a colorless fluffy material after liophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 10.78 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 9.92 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.97 (t, *J* = 5.7 Hz, 1H, $CO_{Lys}NH$), 7.89 (d, *J* = 7.6 Hz, 2H, $H4_{Fmoc}$, $H5_{Fmoc}$), 7.74 (dd, *J* = 7.6, 4.4 Hz, 2H, $H1_{Fmoc}$, $H8_{Fmoc}$), 7.54 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.47–7.39 (m, 3H, $H3_{Fmoc}$, $H6_{Fmoc}$, $NH_{\alpha,Lys}$), 7.37–7.27 (m, 3H, $H2_{Fmoc}$, $H7_{Fmoc}$, $H7_{Indole}$), 7.13 (d, *J* = 2.2 Hz, 1H, $H2_{Indole}$), 7.05 (t, *J* = 7.5 Hz, 1H, $H6_{Indole}$), 6.97 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 4.35–4.18 (m, 3H, $CH_{2,Fmoc}$, $H9_{Fmoc}$), 3.93 (td, *J* = 8.6, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.51–3.24 (m, 4H, $H_{\varepsilon,Lys}$, $CO_{Lys}NHC\underline{H}_2$, overlap with residual water), 2.81 (t, *J* = 7.5 Hz, 2H, $CO_{Lys}NHCH_2C\underline{H}_2$), 2.56–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$), 2.20 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.95–1.81 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 1.66–1.44 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.39–1.16 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 171.7 (CO_{Lys}), 155.9 (CO_{Fmoc}), 143.9 ($C8a_{Fmoc}$/$C9a_{Fmoc}$), 143.8 ($C8a_{Fmoc}$/$C9a_{Fmoc}$), 140.7 ($C4a_{Fmoc}$, $C4b_{Fmoc}$), 136.2 ($C3a_{Indole}$), 127.6 ($C3_{,Fmoc}$, $C6_{Fmoc}$), 127.2 ($C7a_{Indole}$), 127.0 ($C2_{Fmoc}$, $C7_{Fmoc}$), 125.3 ($C8_{Fmoc}$, $C1_{Fmoc}$), 122.6 ($C2_{Indole}$), 120.9 ($C6_{Indole}$), 120.1 ($C4_{Fmoc}$, $C5_{Fmoc}$), 118.2 ($C5_{Indole}$, $C4_{Indole}$), 111.7 ($C2_{Indole}$), 111.3 ($C7_{Indole}$), 65.6 ($CH_{2,Fmoc}$), 54.6 ($C_{\alpha,Lys}$), 46.7 ($C9_{Fmoc}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 39.5 ($CO_{Lys}NH\underline{C}H_2$ overlap with solvent peak), 32.6 ($\underline{C}H_2CO_2H$), 31.7 ($C_{\beta,Lys}$), 26.9 ($C_{\delta,Lys}$), 25.1 ($CO_{Lys}NHCH_2\underline{C}H_2$), 24.3 ($\underline{C}H_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.23 min, *m*/*z* 641.2 ($[M+H]^+$, $C_{36}H_{41}N_4O_5S^+$ Calcd 641.3); HRMS *m*/*z* 641.2792 ($[M+H]^+$, $C_{36}H_{41}N_4O_5S^+$ Calcd 641.2792).

5-(((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (7).



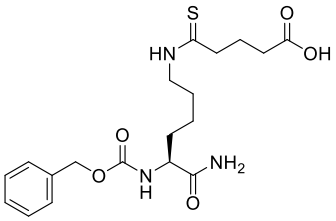
The title compound was synthesized from Fmoc-Trp-OH and **S24** by the general procedures for SPSS and global deprotection and cleavage from resin, affording the desired carboxylic acid **7** (5 mg, 11% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 10.78 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 9.89 (t, *J* = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.84 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.58 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.48–7.19 (m, 8H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$, $CONH_{2,A}$), 7.12 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 2H, $H6_{Indole}$, $CONH_{2,B}$), 6.98–6.92 (m, 1H, $H5_{Indole}$), 5.08–4.95 (m, 2H, $CH_{2,Cbz}$), 4.48 (td, *J* = 7.9, 5.5 Hz, 1H, $H_{\alpha,Trp}$), 3.95 (td, *J* = 8.8, 5.0 Hz, 1H, $H_{\alpha,Lys}$), 3.60–3.33 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.11 (m_{ABX}, *J* = 14.7, 5.4 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, *J* = 14.7, 7.9 Hz, 1H, $H_{\beta,Trp,B}$), 2.56–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.67–1.37 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.33–1.11 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 173.2 (CO_{Trp}), 171.5 (CO_{Lys}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.74 ($C4_{Ar,Cbz}$), 127.65 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.0 ($C_{\alpha,Trp}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.6 ($\underline{C}H_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 27.7 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.76 min, *m*/*z* 596.4 ($[M+H]^+$, $C_{30}H_{38}N_5O_6S^+$ Calcd 596.3); HRMS *m*/*z* 596.2543 ($[M+H]^+$,$C_{30}H_{38}N_5O_6S^+$ Calcd 596.2537).

(S)-5-((6-((2-Amino-2-oxoethyl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (8).



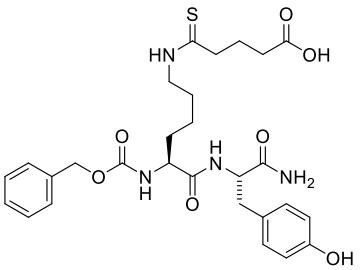
The title compound was synthesized from Fmoc-Gly-OH and **S24** by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **8** (1 mg, 10% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (s, 1H, CO_2H), 9.90 (t, J = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.09 (t, J = 5.7 Hz, 1H, $NH_{\alpha,Gly}$), 7.48 (d, J = 7.6 Hz, 1H, $NH_{\alpha,Lys}$), 7.42–7.26 (m, 5H, $H_{Ar,Cbz}$), 7.18 (s, 1H, $CONH_{2,A}$), 7.06 (s, 1H, $CONH_{2,B}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 3.96 (td, J = 8.4, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.70–3.57 (m, 2H, $H_{\alpha,Gly}$), 3.49–3.39 (m, J = 6.8 Hz, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 2.52 (t, J = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.72–1.61 (m, 1H, $H_{\beta,Lys,A}$), 1.60–1.45 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.42–1.20 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.1 (CO_2H), 172.1 (CO_{Lys}), 170.8 (CO_{Gly}), 156.2 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 45.0 ($C_{\varepsilon,Lys}$), 43.85 ($\underline{C}H_2(CH_2)_2CO_2H$), 41.9 ($C_{\alpha,Gly}$), 32.7 ($\underline{C}H_2CO_2H$), 31.3 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($\underline{C}H_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.64 min, m/z 467.1 ($[M+H]^+$, $C_{21}H_{31}N_4O_6S^+$ Calcd 467.2); HRMS m/z 467.1968 ($[M+H]^+$,$C_{21}H_{31}N_4O_6S^+$ Calcd 467.1959).

(*S*)-5-((6-Amino-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (2).



The title compound was isolated as a by-product in the synthesis of compound **8,** by the general procedures for SPPS and global deprotection and cleavage from resin, affording carboxylic acid **2** (1 mg, 10% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.02 (br s, 1H, CO_2H), 9.92 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.40–7.21 (m, 7H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$, $CONH_{2,A}$), 7.00–6.93 (s, 1H, $CONH_{2,B}$), 5.02 (s, 2H, $CH_{2,Cbz}$), 3.90 (td, J = 8.8, 4.9 Hz, 1H, $H_{\alpha,Lys}$), 3.50–3.38 (m, 2H, $H_{\varepsilon,Lys}$), 2.52 (t, J = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.68–1.45 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.40–1.19 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.8 (C=S), 174.1 (CO_2H), 173.9 (CO_{Lys}), 155.9 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.74 ($C4_{Ar,Cbz}$), 127.65 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 65.3 ($CH_{2,Cbz}$), 54.4 ($C_{\alpha,Lys}$), 45.0 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.7 ($\underline{C}H_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($\underline{C}H_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.62 min, m/z 410.2 ($[M+H]^+$, $C_{19}H_{28}N_3O_5S^+$ Calcd 410.2); HRMS m/z 410.1750 ($[M+H]^+$,$C_{19}H_{28}N_3O_5S^+$ Calcd 410.1744).

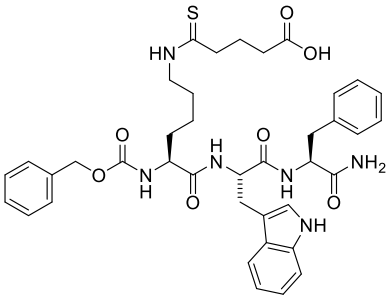
5-(((*S*)-6-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (9).



The title compound was synthesized from Fmoc-Tyr(*t*Bu)-OH and **S24** by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **9** (3 mg, 27% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.02 (s, 1H, CO_2H), 9.89 (t, J = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 9.12 (s, 1H, OH_{Tyr}), 7.75 (d, J = 8.2 Hz, 1H, $NH_{\alpha,Tyr}$), 7.46–7.22 (m, 7H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$, $CONH_{2,A}$), 7.08–6.92 (m, 3H, $CONH_{2,B}$, $H2_{Ar,Tyr}$, $H6_{Ar,Tyr}$), 6.68–6.55 (m, 2H, $H3_{Ar,Tyr}$, $H5_{Ar,Tyr}$), 5.09–4.96 (m, 2H, $CH_{2,Cbz}$), 4.35 (td, J = 8.3, 5.2 Hz, 1H, $H_{\alpha,Tyr}$), 3.96–3.87 (m, 1H, $H_{\alpha,Lys}$, overlap with residual water), 3.48–3.36 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 2.86 (m_{ABX}, J = 13.9, 5.2 Hz, 1H, $H_{\beta,Tyr,A}$), 2.71 (m_{ABX}, J = 13.9, 8.4 Hz, 1H, $H_{\beta,Tyr,B}$), 2.55–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.62–1.41 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.35–1.15 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 172.8 (CO_{Tyr}), 171.4 (CO_{Lys}), 155.9 (CO_{Cbz}), 155.7 ($C4_{Ar,Tyr}$), 136.9 ($C1_{Ar,Cbz}$), 130.1 ($C2_{Ar,Tyr}$, $C6_{Ar,Tyr}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.67 ($C1_{Ar,Tyr}$), 127.65 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 114.8 ($C3_{Ar,Tyr}$, $C5_{Ar,Tyr}$), 65.5 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.8 ($C_{\alpha,Tyr}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 36.8 ($C_{\beta,Tyr}$), 32.7 ($\underline{C}H_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($\underline{C}H_2CH_2CO_2H$), 23.0

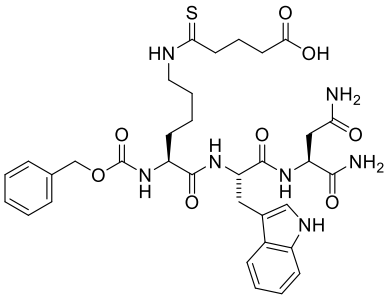
($C_{\gamma,Lys}$). UPLC-MS t_R 1.74 min, *m/z* 573.2 ($[M+H]^+$, $C_{28}H_{37}N_4O_7^+$ Calcd 573.2); HRMS *m/z* 573.2387 ($[M+H]^+$,$C_{28}H_{37}N_4O_7S^+$ Calcd 573.2377).

5-(((*S*)-6-(((*S*)-1-(((*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (15).



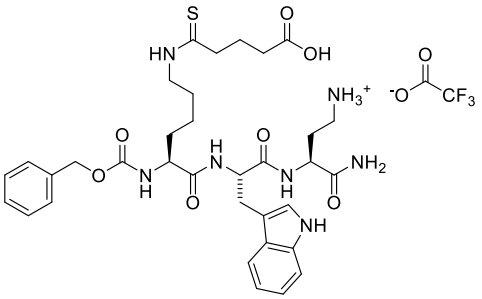
The title compound was synthesized from Fmoc-Phe-OH, Fmoc-Trp-OH, and **S24** by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **15** (4 mg, 10% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (br s, 1H, CO_2H), 10.77 (s, 1H, NH_{Indole}), 9.89 (t, *J* = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.97–7.88 (m, 2H, $NH_{\alpha,Trp}$, NH_{Phe}), 7.53 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.38–7.13 (m, 13H, $NH_{\alpha,Lys}$, $CONH_2$, $H_{Ar,Cbz}$, $CH_{Ar,Phe}$, $H7_{Indole}$), 7.12–7.01 (m, 3H, $CONH_2$, $H2_{Indole}$, $H6_{Indole}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 5.06–4.95 (m, 2H, $CH_{2,Cbz}$), 4.53–4.46 (m, 1H, $H_{\alpha,Trp}$), 4.43 (td, *J* = 8.1, 5.3 Hz, 1H, $H_{\alpha,Phe}$), 3.94 (td, *J* = 8.8, 4.9 Hz, 1H, $H_{\alpha,Lys}$), 3.36 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.06 (m_{ABX}, *J* = 14.9, 5.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, *J* = 13.9, 5.3 Hz, 1H, $H_{\beta,Phe,A}$), 2.92 (m_{ABX}, *J* = 14.9, 8.5 Hz, 1H, $H_{\beta,Trp,B}$), 2.82 (m_{ABX}, *J* = 13.9, 8.4 Hz, 1H, $H_{\beta,Phe,B}$), 2.52 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.55–1.35 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.33–1.11 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.1 (CO_2H), 172.6 ($CONH_2$), 171.9 (CO_{Lys}), 171.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.7 ($C56_{Ar,Phe}$), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 129.2 ($C58/62_{Ar,Phe}$), 128.3 ($C59/61_{Ar,Phe}$), 128.0 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 126.2 ($C60_{Ar,Phe}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.3 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.7 ($C_{\alpha,Phe}$), 53.4 ($C_{\alpha,Trp}$), 45.1 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 37.5 ($C_{\beta,Phe}$), 32.7 ($C\underline{H}_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 27.5 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($C\underline{H}_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.12 min, *m/z* 743.1 ($[M+H]^+$, $C_{39}H_{47}N_6O_7S^+$ Calcd 743.3); HRMS *m/z* 765.3044 ($[M+Na]^+$, $C_{39}H_{46}N_6O_7SNa^+$ Calcd 765.3041).

5-(((*S*)-5-(((Benzyloxy)carbonyl)amino)-6-(((*S*)-1-(((*S*)-1,4-diamino-1,4-dioxobutan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (16).



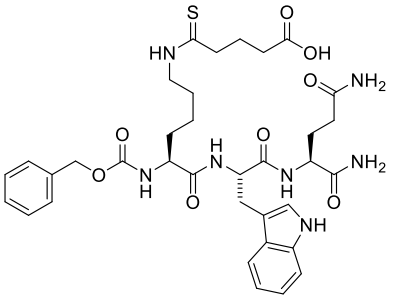
The title compound was synthesized from Fmoc-Asn(Trt)-OH, Fmoc-Trp-OH, and **S24**by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **16** (11 mg, 31% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (br s, 1H, CO_2H), 10.78 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 9.89 (t, *J* = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.09 (d, *J* = 7.9 Hz, 1H, $NH_{\alpha,Asn}$), 8.03 (d, *J* = 7.4 Hz, 1H, $NH_{\alpha,Trp}$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.43–7.21 (m, 8H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$, $CONH_{2,\gamma,Asn,A}$), 7.16 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.08–7.00 (m, 2H, $H6_{Indole}$, $CONH_{2,\alpha,Asn,A}$), 6.99–6.89 (m, 2H, $CONH_{2,\alpha,Asn,B}$, $H5_{Indole}$), 6.84 (s, 1H, $CONH_{2,\gamma,Asn,B}$), 5.08–4.96 (m, 2H, $CH_{2,Cbz}$), 4.54–4.41 (m, 2H, $H_{\alpha,Trp}$, $H_{\alpha,Asn}$), 3.95 (td, *J* = 8.6, 4.7 Hz, 1H, $H_{\alpha,Lys}$), 3.60–3.29 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.15 (m_{ABX}, *J* = 14.8, 4.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.97 (m_{ABX}, *J* = 14.9, 8.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.56–2.41 (m, 4H, $C\underline{H}_2(CH_2)_2CO_2H$, $H_{\beta,Asn}$ overlap with solvent peak), 2.21 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.62–1.41 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.32–1.14 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.1 (CO_2H), 172.7 ($CONH_{2,\alpha,Asn}$), 172.2 (CO_{Lys}), 171.7 ($CONH_{2,\gamma,Asn}$), 171.1 (CO_{Trp}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.3 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.7 ($C_{\alpha,Trp}$), 49.6 ($C_{\alpha,Asn}$), 45.1 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 36.8 ($C_{\beta,Asn}$), 32.7 ($C\underline{H}_2CO_2H$), 31.4 ($C_{\beta,Lys}$), 27.3 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($C\underline{H}_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.82 min, *m/z* 710.0 ($[M+H]^+$, $C_{34}H_{44}N_7O_8S^+$ Calcd : 710.3); HRMS *m/z* 732.2786 ($[M+Na]^+$, $C_{34}H_{43}N_7O_8SNa^+$ Calcd 732.2786).

5-(((*S*)-5-(((Benzyloxy)carbonyl)amino)-6-(((*S*)-1-(((*S*)-1,4-diamino-1-oxobutan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid·TFA salt (17).



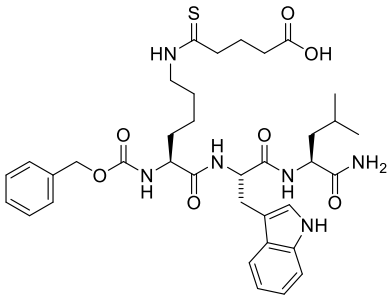
The title compound was synthesized from N^{α}-Fmoc,N^{γ}-Boc-2,4-diaminobutanoic acid, Fmoc-Trp-OH, and **S24** by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **17** (4 mg, 10% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.07 (br s, 1H, CO_2H), 10.82 (s, 1H, NH_{Indole}), 9.98–9.88 (m, 1H, $NH_{\varepsilon,Lys}$), 8.19 (d, J = 8.1 Hz, 1H, NH_{Dab}), 7.96 (d, J = 7.4 Hz, 1H, $NH_{\alpha,Trp}$), 7.80–7.66 (m, 3H, $NH_{3,\delta,Dab}$), 7.56 (d, J = 8.0 Hz, 1H, $H4_{Indole}$), 7.45–7.17 (m, 9H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$, $CONH_2$), 7.14 (s, 1H, $H2_{Indole}$), 7.05 (t, J = 7.8 Hz, 1H, $H6_{Indole}$), 6.96 (t, J = 7.7 Hz, 1H, $H5_{Indole}$), 5.12–4.91 (m, 2H, $CH_{2,Cbz}$), 4.61–4.46 (m, 1H, $H_{\alpha,Trp}$), 4.34–4.23 (m, 1H, $H_{\alpha,Dab}$), 4.00–3.88 (m, 1H, $H_{\alpha,Lys}$), 3.70–3.22 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 3.15 (m_{ABX}, J = 15.0, 4.8 Hz, 1H, $H_{\beta,Trp,A}$), 3.00 (m_{ABX}, J = 15.0, 8.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.85–2.70 (m, 2H, $H_{\gamma,Dab}$), 2.58–2.47 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, J = 7.6 Hz, 2H, $C\underline{H}_2CO_2H$), 2.04–1.73 (m, 4H, $H_{\beta,Dab}$, $C\underline{H}_2CH_2CO_2H$), 1.62–1.35 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.35–1.12 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.1 (CO_2H), 172.3 ($CONH_2$), 172.0 (CO_{Lys}), 171.6 (CO_{Trp}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.8 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.9 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.3 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Trp}$), 50.1 ($C_{\alpha,Dab}$), 45.1 ($C_{\varepsilon,Lys}$), 43.9 ($C\underline{H}_2(CH_2)_2CO_2H$), 36.2 ($C_{\gamma,Dab}$), 32.7 ($C\underline{H}_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 30.0 ($C_{\beta,Dab}$), 27.4 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($C\underline{H}_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.66 min, m/z 696.1 ($[M+H]^+$, $C_{34}H_{46}N_7O_7S^+$ Calcd 696.3); HRMS m/z 696.3178 ($[M+H]^+$, $C_{34}H_{46}N_7O_7S^+$ Calcd 696.3174).

5-(((*S*)-5-(((Benzyloxy)carbonyl)amino)-6-(((*S*)-1-(((*S*)-1,5-diamino-1,5-dioxopentan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S8).



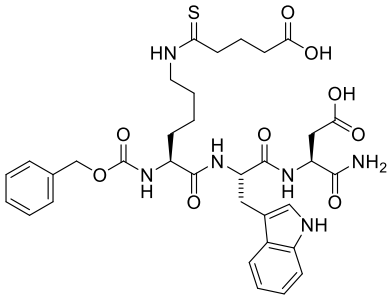
The title compound was synthesized from Fmoc-Gln(Trt)-OH, Fmoc-Trp-OH, and **S24** by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **S8** (6 mg, 17% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (br s, 1H, CO_2H), 10.79 (d, J = 2.4 Hz, 1H, NH_{Indole}), 9.89 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.02–7.91 (m, 2H, $NH_{\alpha,Trp}$, $NH_{\alpha,Gln}$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.45–7.17 (m, 9H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$, $CONH_{2,\alpha,Gln,A}$, $CONH_{2,\delta,Gln,A}$), 7.14 (d, J = 2.4 Hz, 1H, $H2_{Indole}$), 7.08–7.00 (m, 2H, $H6_{Indole}$, $CONH_{2,\alpha,Gln,B}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.74 (s, 1H, $CONH_{2,\delta,Gln,B}$), 5.07–4.95 (m, 2H, $CH_{2,Cbz}$), 4.54 (td, J = 8.1, 4.9 Hz, 1H, $H_{\alpha,Trp}$), 4.17 (td, J = 8.1, 5.3 Hz, 1H, $H_{\alpha,Gln}$), 3.97–3.89 (m, 1H, $H_{\alpha,Lys}$), 3.44–3.37 (m, 2H, $H_{\varepsilon,Lys}$), 3.17 (m_{ABX}, J = 14.8, 4.8 Hz, 1H, $H_{\beta,Trp,A}$), 2.99 (m_{ABX}, J = 14.9, 8.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.53 (t, J = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.21 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 2.07 (t, J = 8.2 Hz, 2H, $H_{\gamma,Gln}$), 1.88 (p, J = 7.4 Hz, 3H, $C\underline{H}_2CH_2CO_2H$, $H_{\beta,Gln,A}$), 1.79–1.70 (m, 1H, $H_{\beta,Gln,B}$), 1.62–1.40 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.31–1.14 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.1 (CO_2H), 173.7 ($CONH_{2,\delta,Gln}$), 173.1 (CO_{Gln}), 172.0 (CO_{Lys}), 171.1 (CO_{Trp}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C5_{Indole}$), 118.2 ($C4_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Trp}$), 52.2 ($C_{\alpha,Gln}$), 45.0 ($C_{\varepsilon,Lys}$), 43.9 ($C\underline{H}_2(CH_2)_2CO_2H$), 32.7 ($C\underline{H}_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 31.4 ($C_{\gamma,Gln}$), 28.0 ($C_{\beta,Gln}$), 27.4 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($C\underline{H}_2CH_2CO_2H$), 23.1 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.83 min, m/z 724.1 ($[M+H]^+$, $C_{35}H_{46}N_7O_8S^+$ Calcd 724.3); HRMS m/z 746.2944 ($[M+Na]^+$, $C_{35}H_{45}N_7O_8SNa^+$ Calcd 746.2943).

5-(((*S*)-6-(((*S*)-1-(((*S*)-1-Amino-4-methyl-1-oxopentan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S9).



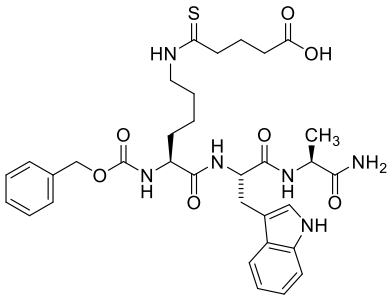
The title compound was synthesized from Fmoc-Ile-OH, Fmoc-Trp-OH, and **S24**by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **S9** (7 mg, 21% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 10.79 (d, J = 2.5 Hz, 1H, NH_{Indole}), 9.88 (t, J = 5.3 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.00 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Trp}$), 7.84 (d, J = 8.3 Hz, 1H, $NH_{\alpha,Leu}$), 7.56 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.44–7.19 (m, 7H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.16–7.07 (m, 2H, $CONH_2$, $H2_{Indole}$), 7.07–7.00 (m, 1H, $H6_{Indole}$), 7.00–6.87 (m, 2H, $H5_{Indole}$, $CONH_2$), 5.11–4.92 (m, 2H, $CH_{2,Cbz}$), 4.55 (td, J = 8.1, 5.3 Hz, 1H, $H_{\alpha,Trp}$), 4.27–4.17 (m, 1H, $H_{\alpha,Leu}$), 3.95 (td, J = 8.6, 5.0 Hz, 1H, $H_{\alpha,Lys}$), 3.44–3.37 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 3.14 (m_{ABX}, J = 14.8, 5.3 Hz, 1H, $H_{\beta,Trp,A}$), 2.97 (m_{ABX}, J = 14.8, 8.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.58–2.46 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$ overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.87 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.61–1.39 (m, 7H, $H_{\beta,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Leu}$, $H_{\gamma,Leu}$), 1.32–1.14 (m, 2H, $H_{\gamma,Lys}$), 0.85 (d, J = 6.6 Hz, 3H, $CH_{3,\delta,Leu,A}$), 0.81 (d, J = 6.5 Hz, 3H, $CH_{3,\delta,Leu,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.0 (CO_2H), 173.9 ($CONH_2$), 171.9 (CO_{Lys}), 171.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 50.9 ($C_{\alpha,Leu}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 41.0 ($C_{\gamma,Leu}$), 32.6 ($C\underline{H}_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 27.2 ($C_{\beta,Trp}$), 26.9 ($C_{\delta,Lys}$), 24.2 ($C\underline{H}_2CH_2CO_2H$), 24.1 ($C_{\beta,Leu}$), 23.0 ($CH_{3,\delta,Leu,A}$), 23.0 ($C_{\gamma,Lys}$), 21.6 ($CH_{3,\delta,Leu,B}$). UPLC-MS t_R 1.81 min, m/z 709.1 ($[M+H]^+$, $C_{36}H_{49}N_6O_7S^+$ Calcd 709.3); HRMS m/z 709.3389 ($[M+H]^+$, $C_{36}H_{49}N_6O_7S^+$ Calcd 709.3378).

(5*S*,8*S*,11*S*)-8-((1*H*-Indol-3-yl)methyl)-11-carbamoyl-5-(4-(4-carboxybutanethioamido)butyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazatridecan-13-oic acid (S10).



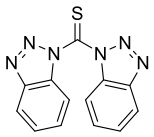
The title compound was synthesized from Fmoc-Asp(tBu)-OH, Fmoc-Trp-OH, and **S24** by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **S10** (8 mg, 24% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.16 (br s, 1H, CO_2H), 10.79 (d, J = 2.4 Hz, 1H, NH_{Indole}), 9.88 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 8.10 (d, J = 8.0 Hz, 1H, NH_{Asp}), 8.00 (d, J = 7.5 Hz, 1H, $NH_{\alpha,Trp}$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.48–7.21 (m, 7H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.18–6.90 (m, 5H, $CONH_2$, $H2_{Indole}$, $H6_{Indole}$, $H5_{Indole}$), 5.08–4.95 (m, 2H, $CH_{2,Cbz}$), 4.56–4.41 (m, 2H, $H_{\alpha,Trp}$, $H_{\alpha,Asp}$), 3.97–3.90 (m, 1H, $H_{\alpha,Lys}$), 3.46–3.37 (m, 2H, $H_{\varepsilon,Lys}$), 3.15 (m_{ABX}, J = 14.8, 5.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, J = 14.8, 8.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.66–2.43 (m, 4H, $C\underline{H}_2(CH_2)_2CO_2H$, $H_{\beta,Asp}$, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $CH_2C\underline{H}_2CO_2H$), 1.88 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.59–1.41 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.30–1.13 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.1 ($CH_2CH_2\underline{C}O_2H$), 172.3 ($CONH_2$), 172.1 ($CO_{Lys}$), 171.9 ($CO_2H_{Asp}$), 171.3 ($CO_{Trp}$), 156.1 ($CO_{Cbz}$), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.80 ($C4_{Ar,Cbz}$), 127.75 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.9 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.3 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.6 ($C_{\alpha,Trp}$), 49.4 ($C_{\alpha,Asp}$), 45.0 ($C_{\varepsilon,Lys}$), 43.9 ($\underline{C}H_2(CH_2)_2CO_2H$), 36.0 ($C_{\beta,Asp}$), 32.7 ($C\underline{H}_2CO_2H_{Thioglutaryl}$), 31.4 ($C_{\beta,Lys}$), 27.3 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.3 ($C\underline{H}_2CH_2CO_2H$), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.89 min, m/z 711.1 ($[M+H]^+$, $C_{34}H_{43}N_6O_9S^+$ Calcd 711.3); HRMS m/z 733.2620 ($[M+Na]^+$, $C_{34}H_{42}N_6O_9SNa^+$ Calcd 733.2626).

5-(((*S*)-6-(((*S*)-1-(((*S*)-1-Amino-1-oxopropan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-5-thioxopentanoic acid (S11).



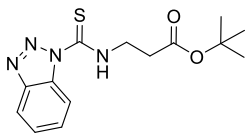
The title compound was synthesized from Fmoc-Ala-OH, Fmoc-Trp-OH, and **S24**by the general procedures for SPPS and global deprotection and cleavage from resin, affording the desired carboxylic acid **S11** (2 mg, 6% base on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br s, 1H, CO_2H), 10.80 (d, J = 2.5 Hz, 1H, NH_{Indole}), 9.89 (t, J = 5.4 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.95 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Trp}$), 7.89 (d, J = 7.5 Hz, 1H, NH_{Ala}), 7.56 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.43–7.25 (m, 7H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.20–7.12 (m, 2H, $H2_{Indole}$, $CONH_2$), 7.04 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.99 (s, 1H, $CONH_2$), 6.98–6.93 (m, 1H, $H5_{Indole}$), 5.09–4.94 (m, 2H, $CH_{2,Cbz}$), 4.53 (td, J = 8.1, 5.0 Hz, 1H, $H_{\alpha,Trp}$), 4.19 (p, J = 7.1 Hz, 1H, $H_{\alpha,Ala}$), 3.94 (td, J = 8.7, 5.0 Hz, 1H, $H_{\alpha,Lys}$), 3.47–3.36 (m, 2H, $H_{\varepsilon,Lys}$), 3.15 (m_{ABX}, J = 14.8, 5.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, J = 14.8, 8.4 Hz, 1H, $H_{\beta,Trp,B}$), 2.55–2.51 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$, overlap with solvent peak), 2.20 (t, J = 7.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.88 (p, J = 7.5 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.61–1.40 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.31–1.15 (m, 5H, $H_{\gamma,Lys}$, $H_{\beta,Ala}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 202.7 (C=S), 174.04 (CO_2H), 173.96 ($CONH_2$), 171.9 (CO_{Lys}), 170.8 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.6 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 48.0 ($C_{\alpha,Ala}$), 45.0 ($C_{\varepsilon,Lys}$), 43.8 ($\underline{C}H_2(CH_2)_2CO_2H$), 32.7 ($C\underline{H}_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 27.3 ($C_{\beta,Trp}$), 26.8 ($C_{\delta,Lys}$), 24.2 ($CH_2\underline{C}H_2CO_2H$), 23.0 ($C_{\gamma,Lys}$), 18.3 ($C_{\beta,Ala}$). UPLC-MS t_R 1.80 min, m/z 667.1 ($[M+H]^+$, $C_{33}H_{43}N_6O_7S^+$ Calcd 667.3); HRMS m/z 667.2906 ($[M+H]^+$, $C_{33}H_{43}N_6O_7S^+$ Calcd 667.2908).

Bis(benzotriazol-1-yl)methanethione (S47).[7]



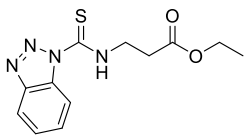
Benzotriazole (3.16 g, 26.7 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C. Thiophosgene (0.50 mL, 6.52 mmol) was added dropwise over 4 minutes and the reaction mixture was stirred at 0 °C for 2 hours. The precipitate was filtered off and the filtrate was washed with saturated aq. $NaHCO_3$ (3×100 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude solid was recrystallized from CH_2Cl_2, affording the desired compound **S47** (1.62 g, 88%) as yellow micro-crystals. TLC (50% EtOAc in heptane): R_f = 0.7. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (dt, J = 8.4, 1.0 Hz, 2H, $H7_{Bt}$), 8.22 (dt, J = 8.3, 1.0 Hz, 2H, $H4_{Bt}$), 7.73 (ddd, J = 8.3, 7.1, 1.0 Hz, 2H, $H6_{Bt}$), 7.60 (ddd, J = 8.2, 7.1, 1.0 Hz, 2H, $H5_{Bt}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.9 (C=S), 147.0 ($C3a_{Bt}$), 133.3 ($C7a_{Bt}$), 130.7 ($C6_{Bt}$), 127.1 ($C5_{Bt}$), 121.2 ($C4_{Bt}$), 114.1 ($C7_{Bt}$). mp 169.5 °C (lit.[8] mp 170−172 °C). Bt=1-Benzotriazol

***tert*-Butyl 3-(1*H*-benzo[d][1,2,3]triazole-1-carbothioamido)propanoate (S48).**



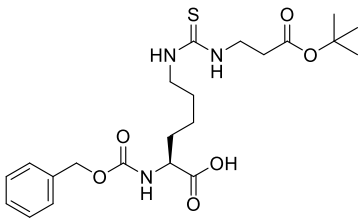
HCl·β-alanine *t*-butyl ester (389 mg, 2.14 mmol) and **S47** (600 mg, 2.13 mmol) were dissolved in anhydrous CH_2Cl_2 (20 mL). iPr$_2$NEt (0.40 mL, 2.30 mmol) was added and the bright yellow solution was stirred in ambient temperature overnight after which the solution turned clear. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography (0→10% EtOAc in heptane) affording the desired compound **S48** (365 mg, 56 %) as an off-white solid. TLC (25% EtOAc in heptane): R_f = 0.4. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.62 (s, 1H, NH), 8.90 (d, J = 8.5 Hz, 1H, $H7_{Bt}$), 8.09 (d, J = 8.3 Hz, 1H, $H4_{Bt}$), 7.63 (t, J = 7.8 Hz, 1H, $H6_{Bt}$), 7.47 (t, J = 7.7 Hz, 1H, $H5_{Bt}$), 4.12 (q, J = 6.0 Hz, 2H, $C\underline{H}_2CH_2CO_2t$Bu), 2.74 (t, J = 6.0 Hz, 2H, $C\underline{H}_2CO_2t$Bu), 1.48 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7 (C=S), 171.2 (CO_2tBu), 147.2 ($C3a_{Bt}$), 132.6 ($C7a_{Bt}$), 130.4 ($C6_{Bt}$), 125.8 ($C5_{Bt}$), 120.4 ($C4_{Bt}$), 116.2 ($C7_{Bt}$), 82.0 ($\underline{C}(CH_3)_3$), 40.6 ($\underline{C}H_2CH_2CO_2t$Bu), 33.9 ($\underline{C}H_2CO_2t$Bu), 28.3 ($C(\underline{C}H_3)_3$). UPLC-MS t_R 2.69 min, m/z 329.0 ($[M+Na]^+$, $C_{14}H_{18}N_4O_2SNa^+$ Calcd 329.1); HRMS m/z 329.1044 ($[M+Na]^+$, $C_{14}H_{18}N_4O_2SNa^+$ Calcd 329.1043). Bt=1-Benzotriazol

Ethyl 3 (1H-benzo[d][1,2,3]triazole-1-carbothioamido)propanoate (S49).



A solution of HCl·β-alanine ethyl ester (275 mg, 1.79 mmol) and *i*Pr_2NEt (0.30 mL, 1.78 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise to a solution of compound **S47** (500 mg, 1.78 mmol) in anhydrous CH_2Cl_2 (15 mL). The reaction mixture was stirred at ambient temperature overnight and then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→10% EtOAc in heptane), affording the desired compound **S49** (300 mg, 60%) as a colorless solid. TLC (25% EtOAc in heptane): R_f = 0.3. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.60 (s, 1H, NH), 8.89 (dt, *J* = 8.5, 0.8 Hz, 1H, $H7_{Bt}$), 8.09 (dt, *J* = 8.3, 0.9 Hz, 1H, $H4_{Bt}$), 7.63 (ddd, *J* = 8.3, 7.0, 1.0 Hz, 1H, $H6_{Bt}$), 7.47 (ddd, *J* = 8.1, 7.0, 0.9 Hz, 1H, $H5_{Bt}$), 4.24–4.14 (m, 4H, $C\underline{H}_2CH_2CO_2Et$, $C\underline{H}_2CH_3$), 2.84 (t, *J* = 6.1 Hz, 2H, $C\underline{H}_2CO_2Et$), 1.28 (t, *J* = 7.2 Hz, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7 (C=S), 172.0 (CO_2Et), 147.2 ($C3a_{Bt}$), 132.6 ($C7a_{Bt}$), 130.4 ($C6_{Bt}$), 125.8 ($C5_{Bt}$), 120.4 ($C4_{Bt}$), 116.1 ($C7_{Bt}$), 61.3 ($\underline{C}H_2CH_3$), 40.4 ($\underline{C}H_2CH_2CO_2Et$), 32.7 ($\underline{C}H_2CO_2Et$), 14.3 ($CH_3$). UPLC-MS t_R 2.37 min, *m*/*z* 301.0 ([M+Na]$^+$, $C_{12}H_{14}N_4O_2Na^+$ Calcd 301.1); HRMS *m*/*z* 279.0911 ([M+H]$^+$,$C_{12}H_{15}N_4O_2S^+$ Calcd 279.0910). Bt=1-Benzotriazol

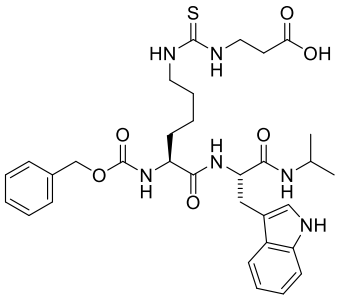
N^2-((Benzyloxy)carbonyl)-N^6-((3-(*tert*-butoxy)-3-oxopropyl)carbamothioyl)-L-lysine (S50).



Method 1—A solution of HCl·β-alanine *t*-butyl ester (66 mg, 0.36 mmol) and *i*Pr_2NEt (95 µL, 0.54 mmol) in anhydrous CH_2Cl_2 (8.0 mL) was added dropwise over 5 minutes to a solution of compund **S47** (98 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (4.0 mL) at 0 °C. The reaction mixture was stirred for 15 minutes at at 0 °C and was then concentrated under reduced pressure. The crude residue and Cbz-Lys-OH (58 mg, 0.21 mmol) were dissolved in anhydrous DMF (2.0 mL). *i*Pr_2NEt (70 µL, 0.40 mmol) was added and the mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure and the crude residue was redissolved in EtOAc (25 mL) and washed with aq. HCl (1 M, 2×25 mL) and brine (2×25 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxlic acid **S50** (33 mg, 34% from Cbz-Lys-OH), as a colorless fluffy material after lyophilization.

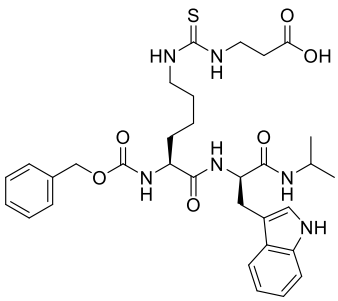
Method 2—Cbz-Lys-OH (546 mg, 1.95 mmol) and compound **S48** (298 mg, 0.97 mmol) were suspended in anhydrous DMF (30 mL). *i*Pr_2NEt (0.40 mL, 2.30 mmol) and the reaction mixture was stirred overnight at 40 °C after which the temperature of the reaction was increased to 60 °C and was stirred overnight. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL) and washed with aq. HCl (1 M, 2×100 mL) and brine (2×100 mL), dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→75% EtOAc in heptane) affording the desired carboxylic acid **S50** (251 mg, 55%) as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.61–7.46 (m, 2H, $NH_{\alpha,Lys}$, $NH_{\varepsilon,Lys}$), 7.40–7.27 (m, 6H, $H_{Ar,Cbz}$, $N\underline{H}(CH_2)_2CO_2tBu$), 5.03 (s, 2H, $CH_{2,Cbz}$), 3.96–3.88 (m, 1H, $H_{\alpha,Lys}$), 3.55 (br s, 2H, $C\underline{H}_2CH_2CO_2tBu$), 3.32 (br s, 2H, $H_{\varepsilon,Lys}$), 2.45 (t, *J* = 6.7 Hz, 2H, CH_2CO_2tBu), 1.73–1.63 (m, 1H, $H_{\beta,Lys,A}$), 1.63–1.53 (m, 1H, $H_{\beta,Lys,B}$), 1.40 (s, 13H, $C(CH_3)_3$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.9 (CO_2H), 170.9 (CO_2tBu), 156.2 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 80.0 ($\underline{C}(CH_3)_3$), 65.4 ($CH_{2,Cbz}$), 53.8 ($C_{\alpha,Lys}$), 43.4 ($C_{\varepsilon,Lys}$), 39.2 ($\underline{C}H_2CH_2CO_2tBu$, overlap with solvent peak), 34.8 ($\underline{C}H_2CO_2tBu$), 30.5 ($C_{\beta,Lys}$), 28.3 ($C_{\delta,Lys}$), 27.8 ($C(\underline{C}H_3)_3$), 23.1 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.26 min, *m*/*z* 468.3 ([M+H]$^+$, $C_{22}H_{34}N_3O_6S^+$ Calcd 468.2); HRMS *m*/*z* 468.2158 ([M+H]$^+$,$C_{22}H_{34}N_3O_6S^+$ Calcd 468.2163).

(5*S*,8*S*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2-methyl-4,7-dioxo-14-thioxo-3,6,13,15-tetraazaoctadecan-18-oic acid (29).



Carboxylic acid **S50** (29 mg, 0.06 mmol), HOBt (14 mg, 0.10 mmol), TFA salt **S33** (44 mg, 0.12 mmol) and *i*Pr_2NEt (48 µL, 0.28 mmol) were dissolved in anhydrous CH_2Cl_2 (4.0 mL) and cooled to 0°C. EDC (19 mg, 0.10 mmol) was added and the reaction mixture was stirred at 0°C for 20 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (60 mL) and washed with aq. $KHSO_4$ (5%, 3×40 mL), saturated aq. $NaHCO_3$ (3×40 mL), and brine (2×40 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The residue was dissolved in in anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) was added. The mixture was stirred at ambient temperature for 50 minutes and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **29** (16 mg, 41% from **S50**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (d, J = 2.5 Hz, 1H, NH_{Indole}), 7.85 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.65 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.51–7.21 (m, 8H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\alpha,Lys}$, $NH_{\varepsilon,Lys}$), 7.10 (s, 1H, $H2_{Indole}$), 7.04 (t, J = 7.4 Hz, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 5.07–4.97 (m, 2H, $CH_{2,Cbz}$), 4.52–4.39 (m, 1H, $H_{\alpha,Trp}$), 3.94 (td, J = 8.4, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.82–3.72 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$), 3.28 (br s, 2H, $H_{\varepsilon,Lys}$), 3.06 (m_{ABX}, J = 14.6, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, J = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, J = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$), 1.60–1.32 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.31–1.13 (m, 2H, $H_{\gamma,Lys}$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.91 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.6 (C=S), 173.1 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C4_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 43.4 ($C_{\varepsilon,Lys}$), 40.38 ($CH_{i\mathrm{Pr}}$), 39.0 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 22.9 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr},A}$), 22.1 ($CH_{3,i\mathrm{Pr},B}$). The peak for C=S was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.91 min, m/z 639.3 ($[M+H]^+$, $C_{32}H_{43}N_6O_6S^+$ Calcd 639.3); HRMS m/z 639.2965 ($[M+H]^+$, $C_{32}H_{43}N_6O_6S^+$ Calcd 639.2959).

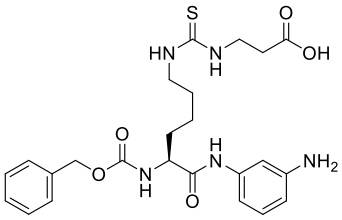
(5*R*,8*S*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2-methyl-4,7-dioxo-14-thioxo-3,6,13,15-tetraazaoctadecan-18-oic acid (S12).



By the method described for compound **29**, the title compound was synthesized using carboxylic acid **S50** (24 mg, 0.05 mmol), HOBt (11 mg, 0.08 mmol), TFA salt **S40** (35 mg, 0.1 mmol), *i*Pr_2NEt (26 µL, 0.15 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (18 mg, 0.09 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S12** (13 mg, 41% from **S50**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (br s, 1H, CO_2H), 10.75 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.13 (d, J = 8.4 Hz, 1H, $NH_{\alpha,Trp}$), 7.62–7.54 (m, 2H $CO_{Trp}NH$, $H4_{Indole}$), 7.42 (d, J = 7.3 Hz, 2H, $NH_{\alpha,Lys}$), 7.39–7.24 (m, 7H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\varepsilon,Lys}$), 7.08 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 1H, $H6_{Indole}$), 6.99–6.93 (m, 1H, $H5_{Indole}$), 5.12–4.92 (m, 2H, $CH_{2,Cbz}$), 4.41 (td, J = 8.8, 4.9 Hz, 1H, $H_{\alpha,Trp}$), 3.98–3.88 (m, 1H, $H_{\alpha,Lys}$), 3.88–3.78 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.66–3.50 (m, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 3.35–3.07 (m, 4H, $H_{\varepsilon,Lys}$, $H_{\beta,Trp,A}$ overlap with residual water), 2.88 (m_{ABX}, J = 14.6, 9.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.48 (t, J = 6.8 Hz, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 1.40–1.24 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.14–0.86 (m, 8H, $H_{\gamma,Lys}$, $(CH_3)_{2,i\mathrm{Pr}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.2 (C=S), 173.1 (CO_2H), 171.7 (CO_{Lys}), 170.0 (CO_{Trp}), 156.1 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.2 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 110.15 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Trp}$), 43.4 ($C_{\varepsilon,Lys}$), 40.5 ($CH_{i\mathrm{Pr}}$), 39.1 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 31.1 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.7 ($C_{\beta,Trp}$), 22.7 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr}}$). The peak for C=S was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei.

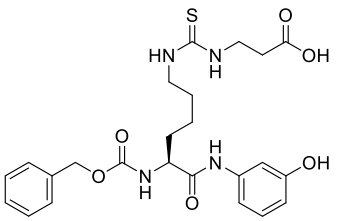
UPLC-MS t_R 1.90 min, *m*/*z* 639.4 ([M+H]$^+$, $C_{32}H_{43}N_6O_6S^+$ Calcd 639.3); HRMS *m*/*z* 639.2966 ([M+H]$^+$,$C_{32}H_{43}N_6O_6S^+$ Calcd 639.2959).

(*S*)-5-((3-Aminophenyl)carbamoyl)-3-oxo-1-phenyl-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (S13).



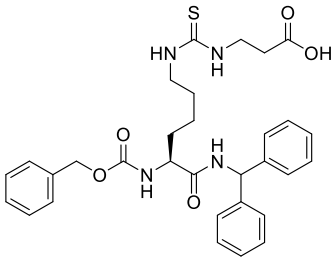
By the method described for compound **29**, the title compound was synthesized using carboxylic acid **S50** (42 mg, 0.09 mmol), HOBt (18 mg, 0.13 mmol), *tert*-butyl (3-aminophenyl)carbamate[6] (37 mg, 0.18 mmol), *i*Pr$_2$NEt (31 µL, 0.18 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (28 mg, 0.15 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S13** (22 mg, 41% from **S50**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.07 (s, 1H, $CO_{Lys}NH$), 7.64–7.03 (m, 11H, $H_{Ar,Cbz}$, $H6_{APh}$, $H5_{APh}$, $H2_{APh}$, $NH_{\alpha,Lys}$, $NH_{\varepsilon,Lys}$, N<u>H</u>$(CH_2)_2CO_2H$), 6.74 (d, *J* = 7.8 Hz, 1H, $H4_{APh}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.15–4.06 (m, 1H, $H_{\alpha,Lys}$), 3.55 (s, 2H, C<u>H</u>$_2$CH_2CO_2H), 3.33 (s, 2H, $H_{\varepsilon,Lys}$), 2.47 (t, *J* = 6.7 Hz, 2H, C<u>H</u>$_2CO_2H$ overlap with solvent peak), 1.72–1.55 (m, 2H, $H_{\beta,Lys}$), 1.53–1.23 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.2 (C=S), 173.1 (CO_2H), 171.3 (CO_{Lys}), 156.1 (CO_{Cbz}), 139.9 ($C1_{APh}$), 138.5 ($C3_{APh}$), 137.0 ($C1_{Ar,Cbz}$), 129.7 ($C5_{APh}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 114.5 ($C4_{APh}$/6_{APh}), 114.2 ($C4_{APh}$/6_{APh}), 110.3 ($C2_{APh}$), 65.4 ($CH_{2,Cbz}$), 55.5 ($C_{\alpha,Lys}$), 43.4 ($C_{\varepsilon,Lys}$), 39.5 (C<u>H</u>$_2$CH_2CO_2H overlap with solvent peak), 33.6 (<u>C</u>H_2CO_2H), 31.5 ($C_{\beta,Lys}$), 28.5 ($C_{\delta,Lys}$), 23.1 ($C_{\gamma,Lys}$). The peak for C=S was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.33 min, *m*/*z* 502.3 ([M+H]$^+$, $C_{24}H_{32}N_5O_5S^+$ Calcd 502.2); HRMS *m*/*z* 502.2124 ([M+H]$^+$, $C_{24}H_{32}N_5O_5S^+$ Calcd 502.2119). APh=3-aminophenyl

(*S*)-5-((3-Hydroxyphenyl)carbamoyl)-3-oxo-1-phenyl-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (S14).



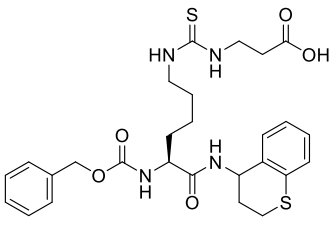
By the method described for compound **29**, the title compound was synthesized using carboxylic acid **S50** (46 mg, 0.10 mmol), HOBt (21 mg, 0.15 mmol), 3-aminophenol (22 mg, 0.20 mmol), *i*Pr$_2$NEt (34 µL, 0.20 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (29 mg, 0.15 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S14** (15 mg, 30% from **S50**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (d, *J* = 7.2 Hz, 1H, $NH_{\alpha,Lys}$), 7.52 (s, 1H, $NH_{\varepsilon,Lys}$), 7.40–7.29 (m, 6H, $H_{Ar,Cbz}$, N<u>H</u>$(CH_2)_2CO_2H$), 7.13 (t, *J* = 8.0 Hz, 1H, $H5_{HPh}$), 6.62 (d, *J* = 8.1 Hz, 1H, $H4_{HPh}$), 6.45 (s, 1H, $H2_{HPh}$), 6.38 (d, *J* = 8.0 Hz, 1H, $H6_{HPh}$), 5.07 (s, 2H, $CH_{2,Cbz}$), 4.25–4.14 (m, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, C<u>H</u>$_2$CH_2CO_2H), 3.36 (br s, 2H, $H_{\varepsilon,Lys}$), 2.47 (t, *J* = 6.7 Hz, 2H, C<u>H</u>$_2CO_2H$ overlap with solvent peak), 1.89–1.69 (m, 2H, $H_{\beta,Lys}$), 1.57–1.30 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.5 (C=S), 173.1 (CO_2H), 171.2 (CO_{Lys}), 156.2 (CO_{Cbz}), 151.2 ($C1_{HPh}$), 146.3 ($C3_{HPh}$), 136.8 ($C1_{Ar,Cbz}$), 129.8 ($C5_{HPh}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C4_{Ar,Cbz}$), 113.5 ($C4_{HPh}$), 111.0 ($C6_{HPh}$), 108.8 ($C2_{HPh}$), 65.6 ($CH_{2,Cbz}$), 54.1 ($C_{\alpha,Lys}$), 43.3 ($C_{\varepsilon,Lys}$), 39.5 (C<u>H</u>$_2$CH_2CO_2H overlap with solvent peak), 33.6 (<u>C</u>H_2CO_2H), 30.3 ($C_{\beta,Lys}$), 28.3 ($C_{\delta,Lys}$), 22.9 ($C_{\gamma,Lys}$). The peak for C=S was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.74 min, *m*/*z* 503.4 ([M+H]$^+$, $C_{24}H_{31}N_4O_6S^+$ Calcd 503.2); HRMS *m*/*z* 503.1964 ([M+H]$^+$, $C_{24}H_{31}N_4O_6S^+$ Calcd 503.1959). HPh=hydroxyphen-3-yl

(*S*)-5-(Benzhydrylcarbamoyl)-3-oxo-1-phenyl-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (S15).



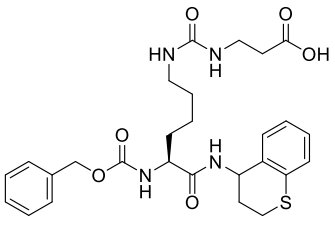
By the method described for compound **29**, the title compound was synthesized using carboxylic acid **S50** (40 mg, 0.09 mmol), HOBt (18 mg, 0.13 mmol), benzhydrylamine (30 µL, 0.17 mmol), *i*Pr_2NEt (30 µL, 0.17 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (26 mg, 0.13 mmol) followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording the desired carboxylic acid **S15** (25 mg, 49% from **S50**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.78 (d, J = 8.4 Hz, 1H, $CO_{Lys}NH$), 7.69–6.86 (m, 17H, $H_{Ar,Cbz}$, $H_{Ar,Bh}$, $NH_{\varepsilon,Lys}$, $N\underline{H}(CH_2)_2CO_2H$), 6.09 (d, J = 8.4 Hz, 1H, $CH(Ph)_2$), 5.02 (s, 2H, $CH_{2,Cbz}$), 4.20–4.05 (m, 1H, $NH_{\alpha,Lys}$), 3.55 (s, 2H, $H_{\varepsilon,Lys}$), 3.32 (br s, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 2.47 (t, J = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$), 1.70–1.50 (m, 2H, $H_{\beta,Lys}$), 1.50–1.37 (m, 2H, $H_{\delta,Lys}$), 1.37–1.16 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.3 (C=S), 173.1 (CO_2H), 171.3 (CO_{Lys}), 155.9 (CO_{Cbz}), 142.4 ($C1_{Ar,Bh,A}$), 142.2 ($C1_{Ar,Bh,B}$), 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.7 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.4 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 127.1 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 126.9 ($C_{Ar,Cbz}$/$C_{Ar,Bh}$), 65.3 ($CH_{2,Cbz}$), 55.9 ($CH(Ph)_2$), 54.5 ($C_{\alpha,Lys}$), 43.4 ($C_{\varepsilon,Lys}$), 39.2 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 31.8 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 23.0 ($C_{\gamma,Lys}$). The peak for C=S was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.05 min, *m*/*z* 577.4 ($[M+H]^+$, $C_{31}H_{37}N_4O_5S^+$ Calcd 577.2); HRMS *m*/*z* 577.2485 ($[M+H]^+$,$C_{32}H_{37}N_4O_5S^+$ Calcd 577.2479). Bh=Benzhydryl

(5*S*)-3-Oxo-1-phenyl-5-(thiochroman-4-ylcarbamoyl)-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (V).



By the method described for compound **29**, the title compound was synthesized using carboxylic acid **S50** (59 mg, 0.13 mmol), HOBt (28 mg, 0.21 mmol), thiochroman-4-amine (39 mg, 0.24 mmol), *i*Pr_2NEt (44 µL, 0.25 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (36 mg, 0.19 mmol), followed by anhydrous CH_2Cl_2 (2.4 mL) and TFA (1.6 mL, 20.9 mmol) affording a diastereomeric mixture (ratio ≈3:2 based on NMR and analytical HPLC, termed I and II when distinguishable in NMR assignment) of the desired carboxylic acid **V** (40 mg, 49% from **S50**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.43 (d, J = 8.3 Hz, $1H_I$, $CO_{Lys}NH_I$), 8.33 (d, J = 8.3 Hz, $1H_{II}$, $CO_{Lys}NH_{II}$), 7.56–7.41 (m, 1H, $NH_{\varepsilon,Lys}$), 7.42–7.27 (m, 7H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$, $N\underline{H}(CH_2)_2CO_2H$), 7.23–7.00 (m, 4H, $H_{Ar,Tc}$), 5.12–4.88 (m, 3H, $CH_{2,Cbz}$, $H4_{Tc}$), 4.05–3.93 (m, 1H, $H_{\alpha,Lys}$, overlap with residual water), 3.55 (br s, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.31 (br s, 2H, $H_{\varepsilon,Lys}$), 3.12–2.93 (m, 2H, $H2_{Tc}$), 2.47 (t, J = 6.8 Hz, 2H, $C\underline{H}_2CO_2H$), 2.16–1.92 (m, 2H, $H3_{Tc}$), 1.66–1.51 (m, 2H, $H_{\beta,Lys}$), 1.50–1.39 (m, 2H, $H_{\delta,Lys}$), 1.38–1.20 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.4 (C=S), 173.1 (CO_2H), 171.2 (CO_{Lys}), 155.9 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz,II}$), 137.0 ($C1_{Ar,Cbz,II}$), 133.7 ($C4a_{Tc}$), 133.1 ($C8a_{Tc}$), 130.0 ($C5_{Tc,I}$), 129.7 ($C5_{Tc,II}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.74 ($C4_{Ar,Cbz}$), 127.65 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C8_{Tc,I}$), 127.1 ($C8_{Tc,I}$), 126.2 ($C7_{Tc,II}$), 126.1 ($C7_{Tc,\ II}$), 124.2 ($C6_{Tc,I}$), 124.1 ($C6_{Tc,II}$), 65.3 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 54.6 ($C_{\alpha,Lys}$), 45.5 ($C4_{Tc,I}$), 45.4 ($C4_{Tc\ II}$), 43.5 ($C_{\varepsilon,Lys}$), 39.0 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 31.8 ($C_{\beta,Lys}$), 28.7 ($C3_{Tc}$), 28.4 ($C_{\delta,Lys}$), 23.0 ($C_{\gamma,Lys}$), 22.6 ($C2_{Tc}$). The peak for C=S was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.96 min, *m*/*z* 559.3 ($[M+H]^+$, $C_{27}H_{35}N_4O_5S_2^+$ Calcd 559.2); HRMS *m*/*z* 559.2049 ($[M+H]^+$,$C_{27}H_{35}N_4O_5S_2^+$ Calcd 559.2043). Tc=thiochroman-4-yl

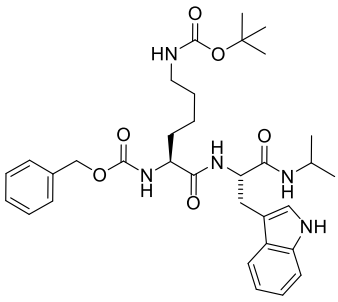
(5*S*)-3,11-Dioxo-1-phenyl-5-(thiochroman-4-ylcarbamoyl)-2-oxa-4,10,12-triazapentadecan-15-oic acid (S16).



The title compound was isolated as a by-product in the synthesis of compound **V,** affording a diastereomeric mixture (ratio ≈3:2 based on NMR and analytical HPLC, termed I and II when distinguishable in NMR assignment) of carboxylic acid **S16** (7 mg, 10% from **S50**) as colorless fluffy materials after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.15 (br s, 1H, CO_2H), 8.42 (d, J = 8.3 Hz,

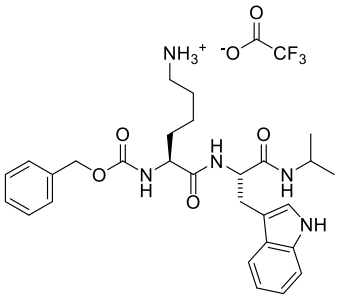
$1H_I$, $CO_{Lys}NH_I$), 8.32 (d, *J* = 8.3 Hz, $1H_{II}$, $CO_{Lys}NH_{II}$), 7.44–7.27 (m, 6H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$), 7.24–6.94 (m, 4H, $CH_{Ar,Tc}$), 6.00–5.69 (m, 2H, N<u>H</u>$(CH_2)_2CO_2H$, $NH_{\varepsilon,Lys}$), 5.24–4.73 (m, 3H, $CH_{2,Cbz}$, H_{Tc}), 4.04–3.90 (m, 1H, $H_{\alpha,Lys}$), 3.22–3.12 (m, 2H, C<u>H</u>$_2CH_2CO_2H$), 3.11–2.96 (m, 2H, $H2_{Tc}$), 2.96–2.86 (m, 2H, $H_{\varepsilon,Lys}$), 2.31 (t, *J* = 6.5 Hz, 2H, C<u>H</u>$_2CO_2H$), 2.16–1.92 (m, 2H, $H3_{Tc}$), 1.64–1.49 (m, 2H, $H_{\beta,Lys}$), 1.39–1.18 (m, 4H, $H_{\delta,Lys}$, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4 (CO_2H), 171.2 (CO_{Lys}), 157.9 (NHCONH), 155.9 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 133.7 ($C4a_{Tc}$), 133.1 ($C8a_{Tc}$), 130.0 ($C5_{Tc,I}$), 129.7 ($C5_{Tc,II}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C4_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C8_{Tc,I}$), 127.1 ($C8_{Tc,II}$), 126.1 ($C7_{Tc}$), 124.1 ($C6_{Tc}$), 65.3 ($CH_{2,Cbz}$), 54.6 ($C_{\alpha,Lys}$), 45.4 ($C4_{Tc}$), 39.0 ($C_{\varepsilon,Lys}$ overlap with solvent peak), 35.3 (C<u>H</u>$_2CH_2CO_2H$), 35.0 (<u>C</u>H_2CO_2H), 31.7 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 28.7 ($C3_{Tc}$), 22.9 ($C_{\gamma,Lys}$), 22.6 ($C2_{Tc}$). UPLC-MS t_R 1.88 min, *m*/*z* 543.4 ($[M+H]^+$, $C_{27}H_{35}N_4O_6S^+$ Calcd 543.2); HRMS *m*/*z* 543.2278 ($[M+H]^+$,$C_{27}H_{35}N_4O_6S^+$ Calcd 543.2272). Tc=thiochroman-4-yl

Benzyl *tert*-butyl ((*S*)-6-(((*S*)-3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S51).



Cbz-Lys(Boc)-OH (922 mg, 2.42 mmol), HOBt (497 mg, 3.68 mmol), TFA salt **S33** (1.3 g, 3.63 mmol) and *i*Pr_2NEt (0.90 mL, 5.17 mmol) were dissolved in anhydrous CH_2Cl_2 (14 mL) and cooled to 0 °C. EDC (703 mg, 3.67 mmol) was added and the reaction mixture was stirred at 0°C for 5 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (100 mL) and washed with aq. $KHSO_4$ (5%, 3×100 mL), saturated aq. $NaHCO_3$ (3×100 mL), and brine (2×100 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→5% CH_3OH in CH_2Cl_2), affording the desired amide **S51** (1.18 g, 80%) as a colorless solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.4. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.58 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.38–7.25 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.11–7.06 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 7.01 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 5.12–4.95 (m, 2H, $CH_{2,Cbz}$), 4.57 (t, *J* = 7.0 Hz, 1H, $H_{\alpha,Trp}$), 4.01–3.91 (m, 1H, $H_{\alpha,Lys}$), 3.92–3.82 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.25 (m_{ABX}, *J* = 14.5, 6.7 Hz, 1H, $H_{\beta,Trp,A}$), 3.16 (m_{ABX}, *J* = 14.5, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 3.00–2.91 (m, 2H, $H_{\varepsilon,Lys}$), 1.67–1.49 (m, 2H, $H_{\beta,Lys}$), 1.42 (s, 11H, $C(CH_3)_3$, $H_{\delta,Lys}$), 1.29–1.13 (m, 2H, $H_{\gamma,Lys}$), 1.01 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.92 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.6 (CO_{Lys}), 172.5 (CO_{Trp}), 158.8 (CO_{Cbz}), 158.6 (CO_{Boc}), 138.0 ($C1_{Ar,Cbz}$, $C7a_{Indole}$), 129.5 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 129.1 ($C4_{Ar,Cbz}$), 128.9 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 128.8 ($C3a_{Indole}$), 124.6 ($C2_{Indole}$), 122.5 ($C6_{Indole}$), 119.9 ($C5_{Indole}$), 119.5 ($C4_{Indole}$), 112.3 ($C7_{Indole}$), 110.8 ($C3_{Indole}$), 79.9 (<u>C</u>$(CH_3)_3$), 67.8 ($CH_{2,Cbz}$), 57.1 ($C_{\alpha,Lys}$), 55.6 ($C_{\alpha,Trp}$), 42.6 ($CH_{i\mathrm{Pr}}$), 40.9 ($C_{\varepsilon,Lys}$), 32.4 ($C_{\beta,Lys}$), 30.6 ($C_{\delta,Lys}$), 28.8 (C(<u>C</u>$H_3)_3$), 28.7 ($C_{\beta,Trp}$), 23.9 ($C_{\gamma,Lys}$), 22.5 ($CH_{3,i\mathrm{Pr,A}}$), 22.3 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 2.22 min, *m*/*z* 608.5 ($[M+H]^+$, $C_{33}H_{46}N_5O_6^+$ Calcd 608.3); HRMS *m*/*z* 608.3441 ($[M+H]^+$,$C_{33}H_{46}N_5O_6^+$ Calcd 608.3443).

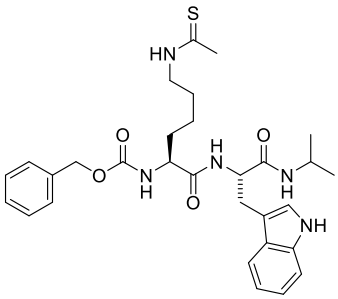
Benzyl ((*S*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-amino-1-oxohexan-2-yl)carbamate·TFA salt (S52).



TFA (4.0 mL, 52.2 mmol) was added to a solution of carbamate **S51** (910 mg, 1.50 mmol) in CH_2Cl_2 (6 mL). The mixture was stirred at ambient temperature for 45 minutes. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 2×100 mL), CH_2Cl_2/heptane (1:1, 3×100 mL), CH_2Cl_2 (100 mL) and toluene (100 mL). The crude residue was dissolved in CH_3OH (1 mL) and triturated with ice-cold diethyl ether (50 mL), affording the desired TFA salt **S52** (849 mg, 91%), as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 7.86 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.76–7.59 (m, 4H, $CO_{Trp}NH$, NH_3), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.44 (d, *J* = 7.9 Hz, 1H, $NH_{\alpha,Lys}$), 7.38–7.24 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.11 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H, $H6_{Indole}$), 6.99–6.92 (m, 1H, $H5_{Indole}$), 5.06–4.97 (m, 2H, $CH_{2,Cbz}$), 4.46 (td, *J* = 7.8, 6.0 Hz, 1H, $H_{\alpha,Trp}$), 3.95 (td, *J* = 8.5, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.82–3.72 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.06 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.97 (m_{ABX}, *J* = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.70 (t, *J* = 7.6 Hz, 2H, $H_{\varepsilon,Lys}$), 1.59–1.40 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.30–1.17 (m, 2H, $H_{\gamma,Lys}$), 1.00 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz,

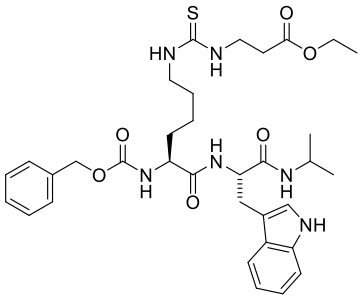
DMSO-d_6) δ 171.4 (CO_{Lys}), 170.0 (CO_{Trp}), 157.9 (q, J = 30.5 Hz, CO_{TFA}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 120.3 ($C5_{Indole}$), 118.5 ($C4_{Indole}$), 117.3 (q, J = 300.7 Hz, $CF_{3,TFA}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{α,Lys}$), 53.3 ($C_{α,Trp}$), 40.3 ($CH_{i}Pr$), 38.6 ($C_{ε,Lys}$), 31.2 ($C_{β,Lys}$), 27.9 ($C_{δ,Lys}$), 26.6 ($C_{β,Trp}$), 22.2 ($C_{γ,Lys}$, $CH_{3,iPr,A}$), 22.1 ($CH_{3,iPr,B}$). UPLC-MS t_R 1.77 min, m/z 508.2 ($[M+H]^+$, $C_{28}H_{38}N_5O_4^+$ Calcd 508.3); HRMS m/z 508.2913 ($[M+H]^+$,$C_{28}H_{38}N_5O_4^+$ Calcd 508.2918).

Benzyl ((*S*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-ethanethioamido-1-oxohexan-2-yl)carbamate (18).



Ethyl dithioacetate (7.5 µL, 0.07 mmol) was added to a solution of TFA salt **S52** (35 mg, 0.06 mmol) and iPr$_2$NEt (15 µL, 0.09 mmol) in anhydrous CH_2Cl_2 (2 mL). The reaction mixture was for 4 hours at ambient temperature and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired thioamide **18** (11.5 mg, 36%), as a colorless fluffy material after lyophilization. H NMR (600 MHz, DMSO-d_6) δ10.79 (d, J = 2.4 Hz, 1H, NH_{Indole}), 9.91 (t, J = 5.3 Hz, 1H, $NH_{ε,Lys}$), 7.86 (d, J = 8.1 Hz, 1H, $NH_{α,Trp}$), 7.65 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.56 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.45–7.22 (m, 7H, $NH_{α,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 6.96 (td, J = 7.4, 6.9, 1.0 Hz, 1H, $H5_{Indole}$), 5.08–4.98 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, J = 7.8, 6.1 Hz, 1H, $H_{α,Trp}$), 3.95 (td, J = 8.5, 5.1 Hz, 1H, $H_{α,Lys}$), 3.82–3.72 (m, 1H, $CH_{i}Pr$), 3.40 (td, J = 7.2, 5.2 Hz, 2H, $H_{ε,Lys}$, overlap with residual water), 3.06 (m_{ABX}, J = 14.5, 6.1 Hz, 1H, $H_{β,Trp,A}$), 2.96 (m_{ABX}, J = 14.6, 7.6 Hz, 1H, $H_{β,Trp,B}$), 2.37 (s, 3H, $CSCH_3$), 1.61–1.41 (m, 4H, $H_{β,Lys}$, $H_{δ,Lys}$), 1.36–1.16 (m, 2H, $H_{γ,Lys}$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,A}$), 0.91 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ198.7 (C=S), 171.4 (CO_{Lys}), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.8 ($C_{α,Lys}$), 53.3 ($C_{α,Trp}$), 45.3 ($C_{ε,Lys}$), 40.4 ($CH_{i}Pr$), 32.8 ($CS\underline{C}H_3$), 31.6 ($C_{β,Lys}$), 27.9 ($C_{β,Trp}$), 26.9 ($C_{δ,Lys}$), 23.0 ($C_{γ,Lys}$), 22.2 ($CH_{3,iPr,A}$), 22.1 ($CH_{3,iPr,B}$). UPLC-MS t_R 1.90 min, m/z 566.1 ($[M+H]^+$, $C_{30}H_{40}N_5O_4S^+$ Calcd 566.3); HRMS m/z 566.2794 ($[M+H]^+$, $C_{41}H_{51}N_6O_9^+$ Calcd 566.2796).

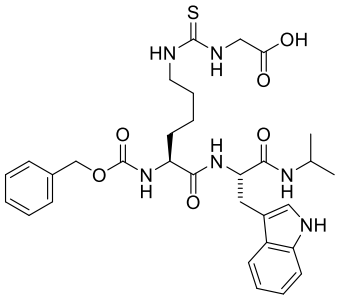
Ethyl-(5*S*,8*S*)-5-((1*H*-indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2-methyl-4,7-dioxo-14-thioxo-3,6,13,15-tetraazaoctadecan-18-oate (Et-29).



TFA salt **S52** (49 mg, 0.08 mmol), **S49** (25 mg, 0.09 mmol), and iPr$_2$NEt (20 µL, 0.11 mmol) were dissolved in anhydrous DMF (4.0 mL) and the mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure. Preparative reversed-phase HPLC purification of crude residue afforded the desired ethyl ester **Et-29** (30 mg, 58% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH_{Indole}), 7.86 (d, J = 8.1 Hz, 1H, $H4_{Indole}$), 7.66 (d, J = 7.7 Hz, 1H, $NH_{α,Trp}$), 7.60–7.22 (m, 10H, $NH_{ε,Lys}$, $CO_{Trp}NH$, $N\underline{H}(CH_2)_2CO_2H$, $NH_{α,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.11 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 7.00–6.93 (m, 1H, $H5_{Indole}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, J = 7.8, 6.1 Hz, 1H, $H_{α,Trp}$), 4.06 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 3.95 (td, J = 8.6, 5.2 Hz, 1H, $H_{α,Lys}$), 3.83–3.73 (m, 1H, $CH_{i}Pr$), 3.59 (br s, 2H, $C\underline{H}_2CH_2CO_2Et$, overlap with residual water), 3.28 (br s, 2H, $H_{ε,Lys}$), 3.06 (m_{ABX}, J = 14.6, 6.1 Hz, 1H, $H_{β,Trp,A}$), 2.97 (m_{ABX}, J = 14.6, 7.7 Hz, 1H, $H_{β,Trp,B}$), 2.55 (t, J = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$), 1.61–1.33 (m, 4H, $H_{β,Lys}$, $H_{δ,Lys}$), 1.18 (t, J = 7.1 Hz, 5H, $H_{γ,Lys}$, $CH_2C\underline{H}_3$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,iPr,A}$), 0.91 (d, J = 6.5 Hz, 3H, $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (CO_2Et), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 59.9 ($\underline{C}H_2CH_3$), 54.9 ($C_{α,Lys}$), 53.3 ($C_{α,Trp}$), 43.5 ($C_{ε,Lys}$), 40.4 ($CH_{i}Pr$), 33.6 ($\underline{C}H_2CO_2H$), 31.6 ($C_{β,Lys}$), 28.4 ($C_{δ,Lys}$), 27.9 ($C_{β,Trp}$), 22.9 ($C_{γ,Lys}$), 22.2 ($CH_{3,iPr,A}$), 22.1 ($CH_{3,iPr,B}$), 14.1 ($CH_2\underline{C}H_3$). The peak for C=S was not visible in ^{13}C NMR,

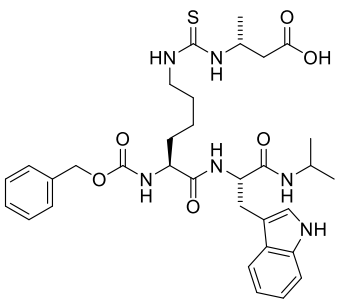
probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.01 min, *m*/*z* 667.2 ([M+H]$^+$, $C_{34}H_{47}N_6O_6S^+$ Calcd 667.3); HRMS *m*/*z* 689.3100 ([M+Na]$^+$,$C_{34}H_{46}N_6O_6SNa^+$ Calcd 689.3092).

(((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)carbamothioyl)glycine (19).



A solution of HCl·glycine *t*-butyl ester (27 mg, 0.16 mmol) and *i*Pr$_2$NEt (43 µL, 0.24 mmol) in anhydrous CH_2Cl_2 (4.0 mL) was added dropwise over 6 minutes to a solution of compound **S47** (46 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (2.0 mL) at 0 °C. The reaction mixture was stirred for 35 minutes at ambient temperature and then additional HCl·glycine *t*-butyl ester (5 mg, 0.03 mmol) was added. After stirring for an additional 5 minutes the reaction mixture was concentrated under reduced pressure. The crude residue and TFA salt **S52** (51 mg, 0.08 mmol) was dissolved in anhydrous DMF (2.0 mL). *i*Pr$_2$NEt (29 µL, 0.17 mmol) was added and the mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure and the crude residue was redissolved in EtOAc (50 mL) and washed with H_2O (2×50 mL), saturated aq. $NaHCO_3$ (2×50 mL), aq. HCl (1 M, 2×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in anhydrous CH_2Cl_2 (3.0 mL) and TIPS (0.20 mL, 0.98 mmol) and TFA (1.0 mL, 13.0 mmol) were added. The mixture was stirred at ambient temperature for 2 hours and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 3×10 mL), CH_2Cl_2/heptane (1:1, 2×10 mL) and CH_2Cl_2 (10 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **19** (18 mg, 34% from TFA salt **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.54 (s, 1H, CO_2H), 10.79 (s, 1H, NH_{Indole}), 7.86 (d, *J* = 8.2 Hz, 1H, $NH_{\alpha,Trp}$), 7.78–7.62 (m, 2H, $NH_{\varepsilon,Lys}$, $CO_{Trp}NH$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.52–7.18 (m, 8H, N<u>H</u>CH_2CO_2H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 7.01–6.91 (m, 1H, $H5_{Indole}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, *J* = 7.8, 6.1 Hz, 1H, $H_{\alpha,Trp}$), 4.14 (s, 2H, CH_2CO_2H), 3.94 (td, *J* = 8.5, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.83–3.72 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.32 (br s, 2H, $H_{\varepsilon,Lys}$), 3.06 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, *J* = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 1.61–1.35 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.34–1.13 (m, 2H, $H_{\gamma,Lys}$), 1.00 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, *J* = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (CO_{Lys}), 171.4 (CO_2H), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 45.4 ($C_{\alpha,Gly}$), 43.7 ($C_{\varepsilon,Lys}$), 40.4 ($CH_{i\mathrm{Pr}}$), 31.6 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 22.9 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.94 min, *m*/*z* 625.3 ([M+H]$^+$, $C_{31}H_{41}N_6O_6S^+$ Calcd 625.3); HRMS *m*/*z* 625.2810 ([M+H]$^+$, $C_{31}H_{41}N_6O_6S^+$ Calcd 625.2803).

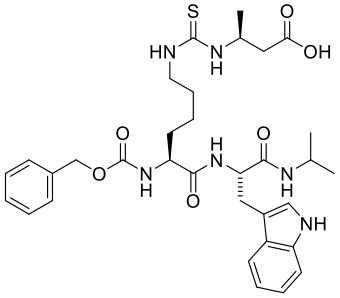
(5*S*,8*S*,16*R*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2,16-dimethyl-4,7-dioxo-14-thioxo-3,6,13,15-tetraazaoctadecan-18-oic acid (27).



By the method described for compound **19**, the title compound was synthesized using *tert*-butyl-(R)-3-aminobutanoate (26 mg, 0.19 mmol), *i*Pr$_2$NEt (14 µL, 0.08 mmol), anhydrous CH_2Cl_2 (4.0 mL), and compound **S47** (46 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (2.0 mL), then TFA salt **S52** (52 mg, 0.08 mmol), anhydrous DMF (2.0 mL), and *i*Pr$_2$NEt (29 µL, 0.17 mmol), then anhydrous CH_2Cl_2 (3.0 mL), TIPS (0.20 mL, 0.98 mmol) and TFA (1.0 mL, 13.0 mmol) affording the desired carboxylic acid **27** (20 mg, 37% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.18 (br s, 1H, CO_2H), 10.79 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 7.85 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.65 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.46–7.15 (m, 9H, $NH_{\varepsilon,Lys}$, C(=S)N<u>H</u>CH, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 6.99–6.93 (m, 1H, $H5_{Indole}$), 5.07–4.98 (m,

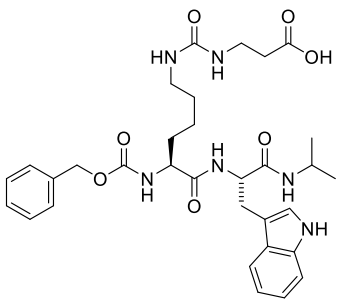
2H, $CH_{2,Cbz}$), 4.60–4.38 (m, 2H, $H_{\alpha,Trp}$, C<u>H</u>CH_2CO_2H), 4.15–3.46 (m, 2H, $H_{\alpha,Lys}$, $CH_{i\mathrm{Pr}}$, overlap with residual water), 3.27 (br s, 2H, $H_{\varepsilon,Lys}$), 3.06 (m_{ABX}, J = 14.6, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, J = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.55 (m_{ABX}, J = 15.6, 5.3 Hz, 1H, C<u>H</u>$_{2,A}CO_2H$), 2.34 (m_{ABX}, J = 15.6, 7.5 Hz, 1H, C<u>H</u>$_{2,B}CO_2H$), 1.59–1.33 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.31–1.08 (m, 5H, $H_{\gamma,Lys}$, C<u>H</u>$_3CHCH_2$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.6 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 46.1 (<u>C</u>HCH_2CO_2H), 43.4 ($C_{\varepsilon,Lys}$), 40.4 ($CH_{i\mathrm{Pr}}$), 40.2 (<u>C</u>H_2CO_2H), 31.6 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 22.9 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$), 19.8 (<u>C</u>H_3CHCH_2). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.97 min, m/z 653.3 ($[M+H]^+$, $C_{33}H_{45}N_6O_6S^+$ Calcd 653.3); HRMS m/z 653.3125 ($[M+H]^+$, $C_{33}H_{45}N_6O_6S^+$ Calcd 653.3116).

(5*S*,8*S*,16*S*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2,16-dimethyl-4,7-dioxo-14-thioxo-3,6,13,15-tetraazaoctadecan-18-oic acid (28).



By the method described for compound **19**, the title compound was synthesized using *tert*-butyl-(*S*)-3-aminobutanoate (26 mg, 0.19 mmol) and $i\mathrm{Pr_2NEt}$ (14 µL, 0.08 mmol), anhydrous CH_2Cl_2 (4.0 mL), compound **S47** (45 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (2.0 mL), then TFA salt **S52** (50 mg, 0.08 mmol), anhydrous DMF (2.0 mL), and $i\mathrm{Pr_2NEt}$ (28 µL, 0.16 mmol), then anhydrous CH_2Cl_2 (3.0 mL), TIPS (0.20 mL, 0.98 mmol), and TFA (1.0 mL, 13.0 mmol) affording the desired carboxylic acid **28** (20 mg, 38% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.17 (s, 1H, CO_2H), 10.79 (d, J = 2.5 Hz, 1H, NH_{Indole}), 7.86 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.65 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.47–7.14 (m, 9H, $NH_{\varepsilon,Lys}$, C(=S)N<u>H</u>CH, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 6.95 (t, J = 7.6 Hz, 1H, $H5_{Indole}$), 5.08–4.97 (m, 2H, $CH_{2,Cbz}$), 4.60–4.40 (m, 2H, $H_{\alpha,Trp}$, C<u>H</u>CH_2CO_2H, overlap with residual water), 3.98–3.91 (m, 1H, $H_{\alpha,Lys}$, overlap with residual water), 3.83–3.72 (m, 1H, $CH_{i\mathrm{Pr}}$, overlap with residual water), 3.27 (br s, 2H, $H_{\varepsilon,Lys}$), 3.06 (m_{ABX}, J = 14.6, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, J = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.55 (m_{ABX}, J = 15.6, 5.4 Hz, 1H, C<u>H</u>$_{2,A}CO_2H$), 2.34 (m_{ABX}, J = 15.6, 7.5 Hz, 1H, C<u>H</u>$_{2,B}CO_2H$), 1.61–1.33 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.31–1.10 (m, 5H, $H_{\gamma,Lys}$, C<u>H</u>$_3CHCH_2$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, J = 6.5 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.6 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 46.1 (<u>C</u>HCH_2CO_2H), 43.4 ($C_{\varepsilon,Lys}$), 40.4 ($CH_{i\mathrm{Pr}}$), 40.2 (<u>C</u>H_2CO_2H), 31.6 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 22.9 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$), 19.8 (<u>C</u>H_3CHCH_2). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.02 min, m/z 653.3 ($[M+H]^+$, $C_{33}H_{45}N_6O_6S^+$ Calcd 653.3); HRMS m/z 653.3127 ($[M+H]^+$, $C_{33}H_{45}N_6O_6S^+$ Calcd 653.3116).

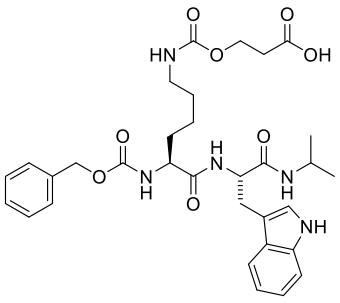
(5*S*,8*S*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2-methyl-4,7,14-trioxo-3,6,13,15-tetraazaoctadecan-18-oic acid (21).



A solution of HCl·β-alanine *t*-butyl ester (96 mg, 0.53 mmol) and $i\mathrm{Pr_2NEt}$ (136 µL, 0.78 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise over 15 minutes to a solution of p-nitrophenyl chloroformate (260 mg, 1.29 mmol) in anhydrous CH_2Cl_2 (4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 minutes and was then stirred at ambient temperature. After 1 hour and 20 minutes the reaction mixture was concentrated under reduced pressure. Column chromatography (0→20% EtOAc in heptane) of the crude residue, afforded the a off-white solid (148 mg), tentatively assigned as *tert*-butyl 3-(((4-nitrophenoxy)carbonyl)amino)propanoate (UPLC-MS t_R 2.12 min, m/z

255.1; [M+H-*t*Bu]$^+$, $C_{10}H_{11}N_2O_6^+$ Calcd 255.1), which was used without further purification. The off-white solid (58 mg) was dissolved in DMF (2.0 mL) and was added to a mixture of TFA salt **S52** (51 mg, 0.08 mmol) and *i*Pr$_2$NEt (30 µL, 0.17 mmol) in DMF (2.0 mL). The reaction mixture was stirred at ambient temperature for 1.5 hours and was then concentrated under reduced pressure. The residue was redissolved in EtOAc (50 mL) and washed with aq. NaOH (1 M, 5×50 mL) and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (3.6 mL) and TFA (2.4 mL, 31.3 mmol) was added. The reaction mixture was stirred at ambient temperature for 50 minutes and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 50 mL), CH_2Cl_2/heptane (1:1, 50 mL) and CH_2Cl_2/MeCN (1:1, 10 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **21** (21 mg, 40% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.16 (br s, 1H, CO_2H), 10.81 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 7.84 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.65 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.47–7.18 (m, 7H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 6.99–6.92 (m, 1H, $H5_{Indole}$), 5.92 (t, *J* = 5.5 Hz, 1H, $NH_{\varepsilon,Lys}$), 5.84 (t, *J* = 6.0 Hz, 1H, N<u>H</u>$(CH_2)_2CO_2H$), 5.10–4.97 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, *J* = 8.0, 6.0 Hz, 1H, $H_{\alpha,Trp}$), 3.91 (td, *J* = 8.4, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.83–3.72 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.22–3.14 (m, 2H, C<u>H</u>$_2CH_2CO_2H$), 3.07 (m_{ABX}, *J* = 14.6, 5.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, *J* = 14.6, 7.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.93–2.84 (m, 2H, $H_{\varepsilon,Lys}$), 2.32 (t, *J* = 6.6 Hz, 2H, C<u>H</u>$_2CO_2H$), 1.56–1.39 (m, 2H, $H_{\beta,Lys}$), 1.32–1.09 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 1.00 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.92 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 157.9 (NHCONH), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 55.0 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.9 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 35.4 (<u>C</u>$H_2CH_2CO_2H$), 35.0 (<u>C</u>H_2CO_2H), 31.5 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 2.05 min, *m*/*z* 623.1 ([M+H]$^+$, $C_{32}H_{43}N_6O_7^+$ Calcd 623.3); HRMS *m*/*z* 645.3006 ([M+Na]$^+$, $C_{32}H_{42}N_6O_7Na^+$ Calcd 645.3007).

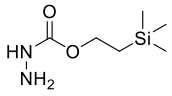
(5*S*,8*S*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2-methyl-4,7,14-trioxo-15-oxa-3,6,13-triazaoctadecan-18-oic acid (23).



A solution of *tert*-butyl-3-hydroxypropanoate (78 µL, 0.53 mmol) and *N*-methylmorpholine (175 µL, 1.59 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise over 15 minutes to a solution of p-nitrophenyl chloroformate (267 mg, 1.33 mmol) in anhydrous CH_2Cl_2 (4.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 minutes and was then stirred at ambient temperature. After 1 hour, H_2O (9 mL) was added to the reaction mixture and the aqueous phase was extracted with CH_2Cl_2 (4×10 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography (0→10% EtOAc in heptane) of the crude residue, afforded a clear oil (185 mg), tentatively assigned as *tert*-butyl 3-(((4-nitrophenoxy)carbonyl)oxy)propanoate (UPLC-MS t_R 2.33 min, *m*/*z* 256.2; [M+H-*t*Bu]$^+$, $C_{10}H_{10}NO_7^+$ Calcd 256.0), which was used without further purification. The clear oil (68 mg) was dissolved in DMF (2.0 mL) and was added to a mixture of TFA salt **S52** (50 mg, 0.08 mmol) and *i*Pr$_2$NEt (30 µL, 0.17 mmol) in DMF (2.0 mL). The reaction mixture was stirred at ambient temperature for 1.5 hours and was then concentrated under reduced pressure. The residue was redissolved in EtOAc (50 mL) and washed with aq. NaOH (1 M, 5×50 mL) and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (3.6 mL) and TFA (2.4 mL, 31.3 mmol) was added. The reaction mixture was stirred at ambient temperature for 45 minutes and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **23** (21 mg, 43% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (br s, 1H, CO_2H), 10.78 (s, 1H, NH_{Indole}), 7.84 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.64 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.46–7.19 (m, 7H, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.12–7.07 (m, 2H, $H2_{Indole}$, $NH_{\varepsilon,Lys}$), 7.04 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.99–6.93 (m, 1H, $H5_{Indole}$), 5.09–4.97 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, *J* = 7.9, 6.0 Hz, 1H, $H_{\alpha,Trp}$), 4.10 (t, *J* = 6.2 Hz, 2H, C<u>H</u>$_2CH_2CO_2H$), 3.92 (td, *J* = 8.4, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.84–3.71 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.06 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,B}$), 2.96 (m_{ABX}, *J* =

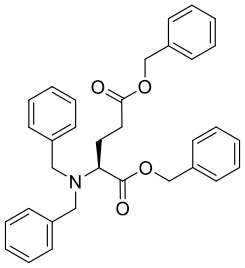
14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.93–2.81 (m, 2H, $H_{\varepsilon,Lys}$), 2.53–2.47 (m, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 1.56–1.38 (m, 2H, $H_{\beta,Lys}$), 1.38–1.10 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.91 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.2 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 155.99 (CO_{Cbz}), 155.96 ($\underline{C}O_2(CH_2)_2CO_2H$), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 59.8 ($\underline{C}H_2CH_2CO_2H$), 55.0 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 39.8 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 34.0 ($\underline{C}H_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 29.1 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$), 22.7 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr},A}$), 22.1 ($CH_{3,i\mathrm{Pr},B}$). UPLC-MS t_R 2.06 min, m/z 624.1 ([M+H]$^+$, $C_{32}H_{42}N_5O_8^+$ Calcd 624.3); HRMS m/z 624.3023 ([M+H]$^+$, $C_{32}H_{42}N_5O_8^+$ Calcd 624.3028).

2-(trimethylsilyl)ethyl hydrazinecarboxylate (S53).



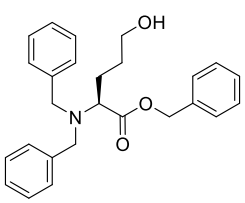
A solution of 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate (10.0 g, 35.4 mmol) in anhydrous THF was added dropwise to a solution of hydrazine hydrate (10 mL, 174.0 mmol) in anhydrous THF with 4 Å molecular sieves over 15 minutes. The reaction mixture was stirred at ambient temperature for 2 hours and was the concentrated under reduced pressure. The crude residue was redissolved in EtOAc (200 mL) and washed with aq. NaOH (2 M, 9×200 mL) and brine (2×100 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure, affording the desired hydrazide **S53**[9] (5.3 g, 85%) as a clear oil which was used without further purification. TLC (50% EtOAc in heptane): R_f = 0.2. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 (s, 1H, $NH_2N\underline{H}$), 4.09–4.01 (m, 2H, OCH_2), 3.97 (s, 2H, NH_2), 0.91 (t, J = 8.4 Hz, 2H, $OCH_2C\underline{H}_2$), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.4 (C=O), 61.7 (OCH_2), 17.5 ($OCH_2\underline{C}H_2$), −1.5 ($Si(CH_3)_3$).

Dibenzyl-*N*,*N*-dibenzyl-L-glutamate (S54).[10]



L-Glutamic acid (10.05 g, 68.3 mmol), K_2CO_3 (47.04 g, 340 mmol), NaOH (5.48 g, 137 mmol), and NaI (10.7 g, 71.4 mmol) were dissolved in H_2O (120 mL). The mixture was heated to reflux and BnBr (40 mL, 340 mmol) was added dropwise over 40 minutes. After 1 hour, the mixture was allowed to reach ambient temperature and the phases were separated. The aqueous phase was extracted with Et_2O (2×100 mL) and the combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (0→88% EtOAc in heptane) affording the desired ester **S54** (20.4 g, 59%), as a clear oil. TLC (20% EtOAc in heptane): R_f = 0.4. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60–7.23 (m, 20H, $H_{Ar,Bn}$), 5.37–5.32 (m_{ABX}, J = 12.2 Hz, 1H, $CH_{2,O\text{-}Bn,\alpha,Glu,A}$), 5.27–5.19 ($m_{ABX}$, J = 12.2 Hz, 1H, $CH_{2,O\text{-}Bn,\alpha,Glu,B}$), 5.11–5.01 (m, 2H, $CH_{2,O\text{-}Bn,\delta,Glu}$), 3.97 ($m_{ABX}$, J = 13.7 Hz, 2H, $CH_{2,N\text{-}Bn,A}$), 3.59 (m_{ABX}, J = 13.8 Hz, 2H, $CH_{2,N\text{-}Bn,B}$), 3.50 (t, J = 7.8 Hz, 1H, $H_{\alpha,Glu}$), 2.64–2.54 (m, 1H, $H_{\gamma,Glu,A}$), 2.49–2.39 (m, 1H, $H_{\gamma,Glu,B}$), 2.25–2.08 (m, 2H, $H_{\beta,Glu}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.0 ($CO_{\delta,Glu}$), 172.3 ($CO_{\alpha,Glu}$), 139.2 ($C1_{Ar,N\text{-}Bn,A}$, $C1_{Ar,N\text{-}Bn,B}$), 136.0 ($C1_{Ar,O\text{-}Bn,A}$), 135.9 ($C1_{Ar,O\text{-}Bn,B}$), 129.0 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.7 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.7 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.6 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.5 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.5 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.4 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.3 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 127.2 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 66.4 ($CH_{2,O\text{-}Bn,\delta,Glu}$), 66.3 ($CH_{2,O\text{-}Bn,\alpha,Glu}$), 59.6 ($C_{\alpha,Glu}$), 54.4 ($CH_{2,N\text{-}Bn,A}$, $CH_{2,N\text{-}Bn,B}$), 30.7 ($C_{\gamma,Glu}$), 24.2 ($C_{\beta,Glu}$). UPLC-MS t_R 2.83 min, m/z 508.3 ([M+H]$^+$, $C_{33}H_{33}NO_4^+$ Calcd 508.2).

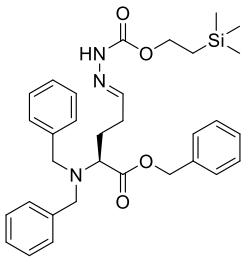
Benzyl-(*S*)-2-(dibenzylamino)-5-hydroxypentanoate (S55).[10]



Benzyl ester **S54** (4.6 g, 8.1 mmol) was dissolved in THF (40 mL) and cooled to −10 °C. Then DIBAl-H (1 M in heptane, 24.3 mmol) was added dropwise over 30 min. H_2O (2.9 mL) was added and the reaction mixture was stirred at 0 °C for 30 min. Na_2SO_4 (2.4 g) was added, the solids were filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (0→40% EtOAc in heptane), affording the desired alcohol **S55** (3.11 g, 95%), as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08–7.31 (m, 15H, $H_{Ar,N\text{-}Bn}$, $H_{Ar,O\text{-}Bn}$), 5.14 (m_{ABX}, J = 12.2 Hz, 1H, $CH_{2,Ar,O\text{-}Bn,A}$), 5.03 (m_{ABX}, J = 12.2 Hz, 1H, $CH_{2,Ar,O\text{-}Bn,B}$), 3.80 (m_{ABX}, J = 13.8 Hz, 2H, $CH_{2,Ar,N\text{-}Bn,A}$), 3.41 (m_{ABX},

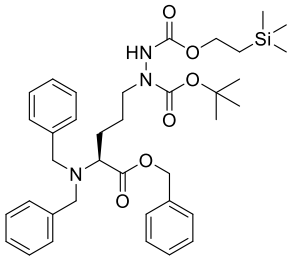
J = 13.8 Hz, 2H, $CH_{2,Ar,N\text{-}Bn,B}$), 3.32 (t, J = 6.4 Hz, 2H, C$\underline{H}_2$OH), 3.27 (t, J = 7.5 Hz, 1H, C$\underline{H}$CO$_2$Bn), 1.75–1.64 (m, 2H, C$\underline{H}_2$CH$_2$OH), 1.63–1.50 (m, 1H, C$\underline{H}_{2,A}$(CH$_2$)$_2$OH), 1.41–1.27 (m, 1H, C$\underline{H}_{2,B}$(CH$_2$)$_2$OH). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.9 (CO$_2$Bn), 139.5 ($C1_{Ar,N\text{-}Bn,A}$, $C1_{Ar,N\text{-}Bn,B}$), 136.1 ($C1_{Ar,O\text{-}Bn,A}$), 129.0 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.7 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.60 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.55 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.4 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.3 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 127.1 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 127.0 ($C_{Ar,N\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 66.1 ($CH_{2,Ar,O\text{-}Bn}$), 62.3 (C$\underline{H}$CO$_2$Bn), 60.7 (C$\underline{H}_2$OH), 54.6 ($CH_{2,Ar,N\text{-}Bn,A}$, $CH_{2,Ar,N\text{-}Bn,B}$), 29.4 (C$\underline{H}_2$(CH$_2$)$_2$OH), 25.8 (C$\underline{H}_2$CH$_2$OH). UPLC-MS t_R 2.54 min, m/z 404.3 ([M+H]$^+$, $C_{26}H_{30}NO_3^+$ Calcd 404.5).

2-(Trimethylsilyl)ethyl (*S*)-2-(5-(benzyloxy)-4-(dibenzylamino)-5-oxopentylidene)hydrazine-1-carboxylate (S56).



A solution of anhydrous DMSO (3.3 mL, 46.2 mmol) in anhydrous CH_2Cl_2 (22 mL) was slowly added to a solution of oxalyl-chloride (2.0 mL, 24.1 mmol) in anhydrous CH_2Cl_2 (45 mL) at −78 °C. The mixture was stirred for 15 min, and then a solution of alcohol **S55** (7.8 g, 19.3 mmol) in anhydrous CH_2Cl_2 (12 mL) was slowly added. After stirring for 15 min, anhydrous NEt_3 (6.5 mL, 46.6 mmol) was added and the mixture was stirred at −78 °C for 15 min. Additional NEt_3 (6.5 mL, 46.6 mmol) was added, and the reaction was stirred at 0 °C for 15 min. H_2O (60 mL) was added, and the reaction mixture was allowed to reach ambient temperature. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3×75 mL). The combined organic phases were washed with aq. HCl (1 M, 2×250 mL), saturated aq. $NaHCO_3$ (250 mL), and brine (250 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure affording a yellow oil (7.5 g), tentatively assigned as the corresponding aldehyde of **S55** (benzyl-(*S*)-2-(dibenzylamino)-5-oxopentanoate)[10] (UPLC-MS t_R 2.69 min, m/z 402.4; [M+H]$^+$, $C_{26}H_{28}NO_3^+$ Calcd 402.5). The yellow oil (5.0 g) and hydrazide **S53** (4.4 g, 24.8 mmol) was dissolved in anhydrous THF (25 mL). The reaction mixture was stirred at ambient temperature overnight and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→40% EtOAc in heptane), affording the desired hydrazone **S56** (5.5 g, 78% from **S55**), as a yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.20–7.39 (m, 15H, $H_{Ar,O\text{-}Bn}$, $H_{Ar,N\text{-}Bn}$), 6.75 (br s, 1H, C$\underline{H}$=NNH), 5.30–5.20 (m, 1H, $CH_{2,Ar,O\text{-}Bn,A}$), 5.21–5.09 (m, 1H, $CH_{2,Ar,O\text{-}Bn,A}$), 4.34–4.18 (m, 2H, C$\underline{H}_2$CH$_2$Si(CH$_3$)$_3$), 3.84 (m, 2H, $CH_{2,Ar,N\text{-}Bn,A}$), 3.50 (m, 2H, $CH_{2,Ar,N\text{-}Bn,B}$), 3.34–3.28 (m, 1H, C$\underline{H}$CO$_2$Bn), 2.49–2.34 (m, 1H, C$\underline{H}_{2,A}$CH=NNH), 2.28–2.14 (m, 1H, C$\underline{H}_{2,B}$CH=NNH), 1.91 (m, 2H, C$\underline{H}_2$CHCO$_2$Bn), 1.22–0.95 (m, 2H, C$\underline{H}_2$Si(CH$_3$)$_3$), 0.02 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.3/171.2 (CO$_2$Bn)*, 153.9 (CO_{Teoc}), 146.9 (C$\underline{H}$=NNH), 139.3/139.0 ($C1_{Ar,N\text{-}Bn}$/$C1_{Ar,N\text{-}Bn}$)*, 136.0/135.8 ($C1_{Ar,O\text{-}Bn}$), 129.1 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.9 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.8 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.72 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.71 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.63 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 127.61 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.51 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.48 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.4 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 127.4/127.2 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$)*, 66.6/66.3 ($CH_{2,Ar,O\text{-}Bn}$)*, 64.2 (C$\underline{H}_2$CH$_2$Si(CH$_3$)$_3$)*, 60.2/59.9 (C$\underline{H}$CO$_2$Bn)*, 54.8/54.5 ($CH_{2,Ar,N\text{-}Bn,A}$, $CH_{2,Ar,N\text{-}Bn,B}$)*, 28.7 (C$\underline{H}_2$CH=NNH), 26.5 (C$\underline{H}_2$CHCO$_2$Bn), 17.9/17.8 (C$\underline{H}_2$Si(CH$_3$)$_3$)*, −1.4 (Si(CH$_3$)$_3$). EtOAc (3%) could be detected as an impurity. Peaks for CO_{Teoc} and C$\underline{H}$=NNH were broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. *Rotamers gave rise to splitting of signals and line broadening of (C$\underline{H}_2$CH$_2$Si(CH$_3$)$_3$) in ^{13}C NMR. UPLC-MS t_R 2.99 min, m/z 560.2 ([M+H]$^+$, $C_{32}H_{42}N_3O_4Si^+$ Calcd 560.8).

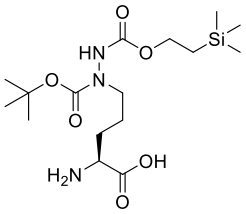
1-(*tert*-butyl)-2-(2-(trimethylsilyl)ethyl)-(*S*)-1-(5-(benzyloxy)-4-(dibenzylamino)-5-oxopentyl)hydrazine-1,2-dicarboxylate (S57).



Hydrazone **S56** (5.5 g, 9.8 mmol) was dissolved in CH_3OH (100 mL) and acidified by AcOH (10.8 mL, 176.4 mmol), then $NaCNBH_3$ (2.5 g, 39.2 mmol) was added. After stirring for 2h at ambient temperature the reaction was poured into saturated aq. $NaHCO_3$ (150 mL) and extracted with CH_2Cl_2 (3×100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, affording a residue, tentatively assigned as 2-(trimethylsilyl)ethyl (*S*)-2-(5-(benzyloxy)-4-(dibenzylamino)-5-oxopentyl)hydrazine-1-carboxylate (UPLC-MS t_R 3.00 min, m/z 562.2; [M+H]$^+$, $C_{32}H_{44}N_3O_4Si^+$ Calcd 562.8), which was used

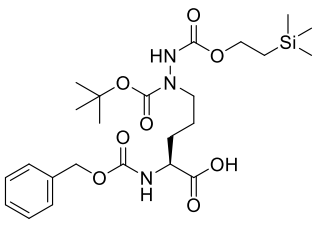
without further purification. The crude residue, Boc_2O (4.3 g, 19.6 mmol) and DMAP (cat.) were dissolved in CH_2Cl_2 (100 mL). The reaction mixture was stirred at ambient temperature overnight and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→60% EtOAc in heptane), affording the desired carbamate **S57** (5.5 g, 85% from **S56**), as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.10 (m, 15H, $H_{Ar,N\text{-}Bn}$, $H_{Ar,O\text{-}Bn}$), 5.22 (m_{ABX}, J = 12.2 Hz, 1H, $CH_{2,O\text{-}Bn,A}$), 5.11 (m_{ABX}, J = 12.2 Hz, 1H, $CH_{2,O\text{-}Bn,B}$), 4.19–4.11 (m, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 3.86 ($m_{ABX}$, J = 13.9 Hz, 2H, $CH_{2,N\text{-}Bn,A}$), 3.48 (m_{ABX}, J = 13.9 Hz, 2H, $CH_{2,N\text{-}Bn,B}$), 3.39–3.23 (m, 3H, $H_{\alpha,Azalys}$, $H_{\delta,Azalys}$), 1.78–1.62 (m, 3H, $H_{\beta,Azalys,A}$, $H_{\gamma,Azalys}$), 1.37 (s, 10H, $H_{\beta,Azalys,B}$, $C(CH_3)_3$), 1.01–0.92 (m, 2H, $C\underline{H}_2Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7 ($CO_{\alpha,Azalys}$), 156.5 (CO_{Boc}), 155.3 (CO_{Teoc}), 139.6 ($C1_{Ar,N\text{-}Bn}$), 136.1 ($C1_{Ar,O\text{-}Bn}$), 128.9 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.7 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.6 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.44 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 128.35 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 127.10 ($C_{Ar,O\text{-}Bn}$/$C_{Ar,N\text{-}Bn}$), 81.4 ($\underline{C}(CH_3)_3$), 66.1 ($CH_{2,O\text{-}Bn}$), 64.2 ($\underline{C}H_2CH_2Si(CH_3)_3$), 60.7 ($C_{\alpha,Azalys}$), 54.6 ($CH_{2,N\text{-}Bn}$), 49.8 ($C_{\delta,Azalys}$), 28.3 ($C(\underline{C}H_3)_3$), 26.6 ($C_{\beta,Azalys}$), 24.3 ($C_{\gamma,Azalys}$), 17.8 ($\underline{C}H_2Si(CH_3)_3$), −1.4 ($Si(CH_3)_3$). Peaks for CO_{Boc}, CO_{Teoc}, $\underline{C}(CH_3)_3$, $\underline{C}H_2CH_2Si(CH_3)_3$, $C_{\alpha,Azalys}$, and $C_{\delta,Azalys}$ were broad and of low intensity *Rotamers gave rise to splitting of signals and line broadening of ($\underline{C}H_2CH_2Si(CH_3)_3$) in ^{13}C NMR. in ^{13}C NMR, probably due to rotamers and fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 3.23 min, m/z 662.1 ($[M+H]^+$, $C_{37}H_{52}N_3O_6Si^+$ Calcd 662.9).

(*S*)-2-Amino-5-(2,2,11,11-tetramethyl-6,9-dioxo-5,10-dioxa-7,8-diaza-2-siladodecan-8-yl)pentanoic acid (S58).



Palladium on carbon (300 mg) was added to a solution of benzyl ester **S57** in CH_3OH (80 mL) and the reaction was stirred under H_2 at ambient temperature overnight. The reaction mixture was filtered through celite, and the filtrate was concentrated under reduced pressure, affording the desired amino acid **S58** (1.25 g, >99%), as a colorless solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.28–4.15 (m, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 3.58 (t, J = 6.0 Hz, 1H, $H_{\alpha,Azalys}$), 3.47 (br s, 2H, $H_{\delta,Azalys}$), 2.01–1.76 (m, 2H, $H_{\beta,Azalys}$), 1.76–1.57 (m, 2H, $H_{\gamma,Azalys}$), 1.46 (s, 9H, $C(CH_3)_3$), 1.07–0.97 (m, 2H, $SiCH_2$), 0.06 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.2 ($CO_{\alpha,Azalys}$), 158.8 (CO_{Boc}), 157.1 (CO_{Teoc}), 82.3 ($\underline{C}(CH_3)_3$), 65.2/64.9 ($\underline{C}H_2CH_2Si(CH_3)_3$)*, 55.9 ($C_{\alpha,Azalys}$), 51.1 ($C_{\delta,Azalys}$), 30.2/29.4 ($C_{\beta,Azalys}$)*, 28.7/28.5 ($C(\underline{C}H_3)_3$)*, 25.0/24.5 ($C_{\gamma,Azalys}$)*, 18.7 ($\underline{C}H_2Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). Peaks for CO_{Boc}, CO_{Teoc}, $\underline{C}(CH_3)_3$, $\underline{C}H_2CH_2Si(CH_3)_3$, and $C_{\delta,Azalys}$ were broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. *Rotamers gave rise to splitting of signals in ^{13}C NMR UPLC-MS t_R 1.57 min, m/z 392.2 ($[M+H]^+$, $C_{16}H_{33}N_3O_6Si^+$ Calcd 392.6); HRMS m/z 392.2206 ($[M+H]^+$,$C_{16}H_{34}N_3O_6Si^+$ Calcd 392.2211).

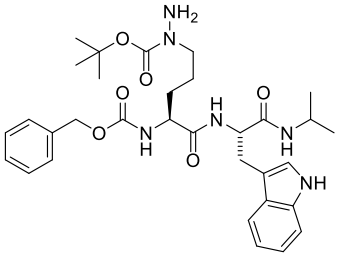
(*S*)-2-(((Benzyloxy)carbonyl)amino)-5-(2,2,11,11-tetramethyl-6,9-dioxo-5,10-dioxa-7,8-diaza-2-siladodecan-8-yl)pentanoic acid (S59).



$NaHCO_3$ (1.1 g, 12.9 mmol) was added to a solution of amino acid **S58** (758 mg, 1.94 mmol) in H_2O/Dioxane (1:1, 20 mL). The pH of the mixture was adjusted with aq. NaOH to pH 9 and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure and the resulting suspension was acidified to pH 2 with aq. HCl and extracted with EtOAc (4×75 mL). The combined organic phases were washed with brine (2×50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (0→ 3.5% CH_3OH and 0.25% AcOH in CH_2Cl_2) affording the desired carboxylic acid **S59** (1.06 g, >99%), as a clear oil. TLC (5% CH_3OH and 0.25% AcOH in CH_2Cl_2): R_f = 0.4. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.58 (s, 1H, CO_2H), 9.19 (d, J = 9.3 Hz, 1H, $N(CO_2tBu)N\underline{H}$), 7.47 (d, J = 8.0 Hz, 1H, $NH_{\alpha,Azalys}$), 7.41–7.24 (m, 5H, $H_{Ar,Cbz}$), 5.06–4.98 (m, 2H, $CH_{2,Cbz}$), 4.09 (br s, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 3.92 (br s, 1H, $H_{\alpha,Azalys}$), 3.32 (br s, 2H, $H_{\delta,Azalys}$), 1.71 (br s, 1H, $H_{\beta,Azalys,A}$), 1.61–1.29 (m, 12H, $H_{\beta,Azalys,B}$, $H_{\gamma,Azalys}$, $C(CH_3)_3$), 0.97–0.88 (m, 2H, $C\underline{H}_2Si(CH_3)_3$), 0.02 (d, J = 8.1 Hz, 9H, $Si(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.9 (CO_2H), 156.1 (CO_{Cbz}), 155.9/155.8 (CO_{Boc})*, 155.0/154.5 (CO_{Teoc})*, 137.0 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 80.1/79.6 ($\underline{C}(CH_3)_3$)*, 65.4 ($CH_{2,Cbz}$), 62.9/62.6 ($\underline{C}H_2CH_2Si(CH_3)_3$)*,

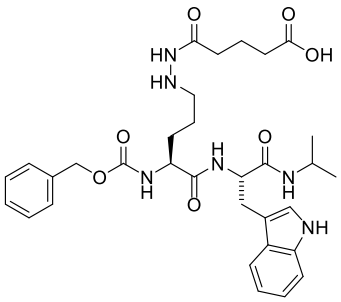
53.7/53.4 ($C_{\alpha,Azalys}$)*, 49.0/47.8 ($C_{\delta,Azalys}$)*, 27.8 ($C_{\beta,Azalys}$, $C(\underline{C}H_3)_3$), 23.7 ($C_{\gamma,Azalys}$), 17.3 ($\underline{C}H_2Si(CH_3)_3$), −1.45 ($Si(CH_3)_3$). ^{1}H NMR (400 MHz, 340 K, DMSO-d_6) δ 8.96 (s, 1H, $N(CO_2tBu)N\underline{H}$), 7.40–7.27 (m, 5H, $H_{Ar,Cbz}$), 7.20 (br s, 1H, $NH_{\alpha,Azalys}$), 5.05 (s, 2H, $CH_{2,Cbz}$), 4.16–4.07 (m, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 3.96 (td, J = 8.5, 4.6 Hz, 1H, $H_{\alpha,Azalys}$), 3.42–3.23 (m, 2H, $H_{\delta,Azalys}$), 1.82–1.66 (m, 1H, $H_{\beta,Azalys,A}$), 1.66–1.46 (m, 3H, $H_{\beta,Azalys,B}$, $H_{\gamma,Azalys}$), 1.39 (s, 9H, $C(CH_3)_3$), 1.00–0.90 (m, 2H, $C\underline{H}_2Si(CH_3)_3$), 0.03 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (101 MHz, 340 K, DMSO-d_6) δ 174.6 (CO_2H), 156.9 (CO_{Cbz}), 138.1 ($C1_{Ar,Cbz}$), 129.2 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.6 ($C4_{Ar,Cbz}$), 128.5 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 80.8 ($\underline{C}(CH_3)_3$), 66.4 ($CH_{2,Cbz}$), 63.6 ($\underline{C}H_2CH_2Si(CH_3)_3$), 54.8 ($C_{\alpha,Azalys}$), 29.1 ($C_{\beta,Azalys}$), 28.9 ($C(\underline{C}H_3)_3$), 24.7 ($C_{\gamma,Azalys}$), 18.4 ($\underline{C}H_2Si(CH_3)_3$), −0.5 ($Si(CH_3)_3$). *Rotamers gave rise to splitting of signals. Peaks for CO_{Boc}, CO_{Teoc}, and $C_{\delta,Azalys}$ were broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.32 min, m/z 524.5 ($[M-H]^-$, $C_{24}H_{38}N_3O_8Si^-$ Calcd 524.2); HRMS m/z 548.2396 ($[M+Na]^+$,$C_{24}H_{39}N_3O_8SiNa^+$ Calcd 548.2399).

tert-Butyl 1-((*S*)-5-(((*S*)-3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-4-(((benzyloxy)carbonyl)amino)-5-oxopentyl)hydrazine-1-carboxylate (S60).



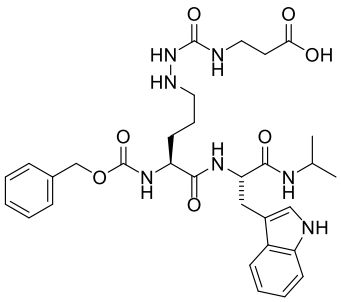
Carboxylic acid **S59** (503 mg, 0.96 mmol), HOBt (259 mg, 1.90 mmol), TFA salt **S33** (691 mg, 1.90 mmol) and iPr$_2$NEt (0.50 mL, 2.87 mmol) were dissolved in anhydrous CH_2Cl_2 (8.0 mL) and cooled to 0 °C. EDC (374 mg, 1.95 mmol) was added and the reaction mixture was stirred at 0°C for 5 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (100 mL) and washed with aq. $KHSO_4$ (5%, 3×75 mL), saturated aq. $NaHCO_3$ (3×75 mL), and brine (2×75 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. Column chromatography (0→2% CH_3OH in CH_2Cl_2) of the crude residue afforded a colorless solid (618 mg), tentatively assigned as the Teoc-protected derivative of compound **S60** (UPLC-MS t_R 2.62 min, m/z 753.2; $[M+H]^+$, $C_{38}H_{57}N_6O_8Si^+$ Calcd 753.4), which was used without further purification. The colorless solid (618 mg) and TBAF (1 M in THF, 2.5 mL, 2.50 mmol) were dissolved in THF and stirred at 50 °C for 1 hour and the reaction mixture was then allowed to reach ambient temperature. Excess TBAF was removed by the addition of $CaCO_3$ (959 mg, 9.59 mmol), DOWEX Monosphere 650C resin (2.83 g) and CH_3OH (6 mL) and the mixture was stirred overnight at ambient temperature. Additional $CaCO_3$ (2.06 g, 20.60 mmol), DOWEX Monosphere 650C resin (3.49 g) and CH_3OH (8 mL) was added.[11] After stirring at ambient temperature for an additional 4 hours the reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (0→5% CH_3OH in CH_2Cl_2), affording the desired hydrazine **S60** (309 mg, 62%), as a colorless solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.4. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (s, 1H, NH_{Indole}), 7.63 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 741–7.27 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.19–7.13 (m, 1H, $H6_{Indole}$), 7.13–7.07 (m, 1H, $H5_{Indole}$), 7.01 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 6.93 (br s, 1H, $NH_{\alpha,Trp}$), 5.90 (br s, 1H, $CO_{Trp}NH$), 5.76 (d, J = 6.4 Hz, 1H, $NH_{\alpha,Azalys}$), 5.10–4.93 (m, 2H, $CH_{2,Cbz}$), 4.68–4.57 (m, 1H, $H_{\alpha,Trp}$), 4.22–4.12 (m, 1H, $H_{\alpha,Azalys}$), 3.99–3.86 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.34 (t, J = 5.7 Hz, 2H, $H_{\delta,Azalys}$), 3.28 (m_{ABX}, J = 14.9, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 3.16 (m_{ABX}, J = 14.8, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 1.77–1.31 (m, 14H, $H_{\beta,Azalys}$, $H_{\gamma,Azalys}$, $C(CH_3)_3$), 0.96 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.92 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.6 (CO_{Azalys}), 170.1 (CO_{Trp}), 156.7 (CO_{Boc}), 156.6 (CO_{Cbz}), 136.34 ($C1_{Ar,Cbz}$), 136.28 ($C7a_{Indole}$), 128.7 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.4 ($C4_{Ar,Cbz}$), 128.2 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.4 ($C6_{Indole}$), 119.8 ($C5_{Indole}$), 118.9 ($C4_{Indole}$), 111.4 ($C7_{Indole}$), 110.7 ($C3_{Indole}$), 81.3 ($\underline{C}(CH_3)_3$), 67.2 ($CH_{2,Cbz}$), 55.3 ($C_{\alpha,Azalys}$), 54.0 ($C_{\alpha,Trp}$), 49.2 ($C_{\delta,Azalys}$), 41.6 ($CH_{i\mathrm{Pr}}$), 29.4 ($C_{\beta,Azalys}$), 28.5 ($C(\underline{C}H_3)_3$), 28.0 ($C_{\beta,Trp}$), 23.9 ($C_{\gamma,Azalys}$), 22.5 ($CH_{3,i\mathrm{Pr,A}}$), 22.3 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 2.08 min, m/z 609.2 ($[M+H]^+$, $C_{32}H_{45}N_6O_6^+$ Calcd 609.3); HRMS m/z 609.3394 ($[M+H]^+$, $C_{32}H_{45}N_6O_6^+$ Calcd 609.3395).

5-(2-((*S*)-5-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-4-(((benzyloxy)carbonyl)amino)-5-oxopentyl)hydrazinyl)-5-oxopentanoic acid (20).



HATU (97 mg, 0.25 mmol), mono *t*-butyl glutarate (38 mg, 0.20 mmol), and *i*Pr_2NEt (0.10 mL, 0.57 mmol) were dissolved in anhydrous CH_2Cl_2 (1.5 mL). After stirring for 15 miuntes at ambient temperature, a solution of hydrazine **S60** (62 mg, 0.10 mmol) in CH_2Cl_2 (3.0 mL) was added. The reaction mixture was stirred at ambient temperature overnight and was then concentrated under reduced pressure. The residue was redissolved in EtOAc (60 mL) and washed with HCl (0.1 M, 2×40 mL), saturated aq. $NaHCO_3$ (2×40 mL), and brine (2×40 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (3.6 mL) and TFA (2.4 mL, 31.3 mmol) was added. The reaction mixture was stirred at ambient temperature for 45 minutes and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **20** (12 mg, 11% from **S60**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.07 (br s, 1H, CO_2H), 10.80 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 10.11 (br s, 1H, $CH_2N\underline{H}NH$ *or* $CH_2NHN\underline{H}$), 7.87 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.68 (d, *J* = 7.6 Hz, 1H, $CO_{Trp}NH$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.44 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Azalys}$), 7.40–7.21 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 1H, $H6_{Indole}$), 6.95 (t, *J* = 7.3 Hz, 1H, $H5_{Indole}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 4.52–4.40 (m, 1H, $H_{\alpha,Trp}$), 3.97 (td, *J* = 8.5, 4.8 Hz, 1H, $H_{\alpha,Azalys}$), 3.83–3.70 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.05 (m_{ABX}, *J* = 14.5, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.97 (m_{ABX}, *J* = 14.6, 7.5 Hz, 1H, $H_{\beta,Trp,B}$), 2.86–2.70 (m, 2H, $H_{\delta,Azalys}$), 2.22 (t, *J* = 7.4 Hz, 2H, $C\underline{H}_2CO_2H$), 2.14 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_2CO_2H$), 1.73 (p, *J* = 7.4 Hz, 2H, $C\underline{H}_2CH_2CO_2H$), 1.65–1.38 (m, 4H, $H_{\beta,Azays}$, $H_{\gamma,Azalys}$), 0.99 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.90 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.0 (CO_2H), 171.3 (CO_{Azalys}), 170.7 ($NHNHCO_{Azalys}$), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.5 ($C_{\alpha,Azalys}$), 53.4 ($C_{\alpha,Trp}$), 50.0 ($C_{\delta,Azalys}$), 40.4 ($CH_{i\mathrm{Pr}}$), 32.8 ($\underline{C}H_2CO_2H$), 32.3 ($\underline{C}H_2(CH_2)_2CO_2H$), 29.0 ($C_{\beta,Azalys}$), 28.0 ($C_{\beta,Trp}$), 22.3 ($C_{\gamma,Azalys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$), 20.3 ($\underline{C}H_2CH_2CO_2H$). UPLC-MS t_R 1.68 min, *m*/*z* 623.1 ($[M+H]^+$, $C_{32}H_{43}N_6O_7^+$ Calcd 623.3); HRMS *m*/*z* 623.3186 ($[M+H]^+$, $C_{32}H_{43}N_6O_7^+$ Calcd 623.3188).

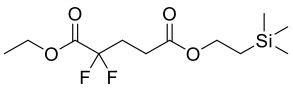
(5*S*,8*S*)-5-((1*H*-Indol-3-yl)methyl)-8-(((benzyloxy)carbonyl)amino)-2-methyl-4,7,14-trioxo-3,6,12,13,15-pentaazaoctadecan-18-oic acid (22).



The off-white solid tentatively assigned as *tert*-butyl 3-(((4-nitrophenoxy)carbonyl)amino)propanoate (prepared as described in the synthesis of compound **21**, 65 mg), hydrazine **S60** (50 mg, 0.08 mmol) and *i*Pr_2NEt (43 µL, 0.25 mmol) were dissolved in DMF (4.0 mL). The reaction mixture was stirred at 50 °C overnight and additional *tert*-butyl 3-(((4-nitrophenoxy)carbonyl)amino)propanoate (31 mg) was added. After stirring at 50 °C overnight the reaction mixture was concentrated under reduced pressure. The residue was redissolved in EtOAc and washed with aq. NaOH (1 M, 4×50 mL) and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (6 mL) and TFA (4.0 mL, 52.2 mmol) was added. The reaction mixture was stirred at ambient temperature for 1 hour and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/heptane (1:1, 100 mL) and CH_2Cl_2/MeCN (1:1, 12 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **22** (17 mg, 33% from **S60**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 7.89 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.69 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.47 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Azalys}$), 7.42–7.21 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.99–6.92 (m, 1H, $H5_{Indole}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.51–4.40 (m, 1H, $H_{\alpha,Trp}$), 3.99 (td, *J* = 8.3, 4.4 Hz, 1H, $H_{\alpha,Azalys}$), 3.83–3.70 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.32–3.27 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 3.14–2.74 (m, 4H, $H_{\beta,Trp,}$ $H_{\delta,Azalys}$), 2.40 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$), 1.69–1.43 (m, 4H, $H_{\beta,Azalys}$, $H_{\gamma,Azalys}$), 0.99 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.89 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz,

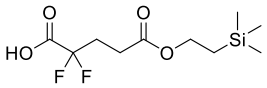
DMSO-d_6) δ 172.9 (CO_2H), 171.2 (CO_{Azalys}), 169.9 (CO_{Trp}), 156.6 (NHNHCONH), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.3 ($C_{\alpha,Azalys}$), 53.4 ($C_{\alpha,Trp}$), 49.8 ($C_{\delta,Azalys}$), 40.4 ($CH_{i\mathrm{Pr}}$), 35.5 ($\underline{C}H_2CH_2CO_2H$), 34.3 ($\underline{C}H_2CO_2H$), 28.8 ($C_{\beta,Azalys}$), 28.0 ($C_{\beta,Trp}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$), 21.2 ($C_{\gamma,Azalys}$ overlap with solvent peak). The peak for NHNHCONH was broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.68 min, *m*/*z* 624.1 ($[M+H]^+$, $C_{31}H_{42}N_7O_7^+$ Calcd 624.3); HRMS *m*/*z* 624.3137 ($[M+H]^+$, $C_{31}H_{42}N_7O_7^+$ Calcd 624.3140).

1-Ethyl-5-(2-(trimethylsilyl)ethyl) 2,2-difluoropentanedioate (S61).



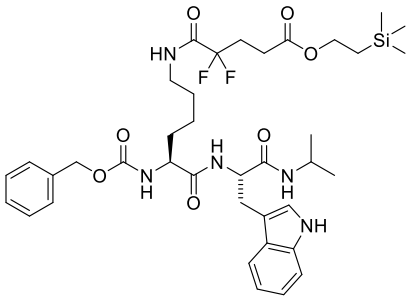
Ethyl bromodifluoroacetate (338 µL, 2.64 mmol), 2-(trimethylsilyl)ethyl acrylate[12] (253 mg, 1.47 mmol) and copper powder (236 mg, 3.71 mmol) were heated at 55 °C in THF (4.0 mL) under vigorous stirring. TMEDA (110 µL, 0.73 mmol) was added to the reaction mixture, followed by addition of AcOH (75 µL, 1.31 mmol). The reaction mixture was stirred vigorously at 55 °C for 1.5 hours and was then allowed to reach ambient temperature. Aq. ammonium chloride (10%, 4.0 mL) and diethylether (3.0 mL) was added and the mixture was stirred at ambient temperature for 30 minutes. The reaction mixture was filtered through celite and the aqueous phase was extracted with diethylether (4×20 mL). The organic phase was washed with ammonium chloride (10%, 2×50 mL) and brine (50 mL) and was then dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (0→10% EtOAc in heptane), affording the desired ester **S61** (306 mg, 70%) as a clear oil. TLC (25% EtOAc in heptane): R_f = 0.6. ^1H NMR (400 MHz, $CDCl_3$) δ 4.31 (q, *J* = 7.1 Hz, 2H, $CH_3C\underline{H}_2$), 4.25–4.13 (m, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 2.60–2.32 (m, 4H, $CF_2C\underline{H}_2C\underline{H}_2$), 1.34 (t, *J* = 7.2 Hz, 3H, $C\underline{H}_3CH_2$), 1.04–0.92 (m, 2H, $C\underline{H}_2Si(CH_3)_3$), 0.03 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8 ($\underline{C}O_2(CH_2)_2Si(CH_3)_3$), 163.9 (t, *J* = 32.6 Hz, CO_2Et), 115.5 (t, *J* = 251 Hz, CF_2), 63.4 ($\underline{C}H_2CH_2Si(CH_3)_3$), 63.1 ($CH_3\underline{C}H_2$), 30.0 (t, *J* = 24.0 Hz, $CF_2\underline{C}H_2$), 26.9 (t, *J* = 4.7 Hz, $CF_2CH_2\underline{C}H_2$), 17.4 ($\underline{C}H_2Si(CH_3)_3$), 14.0 ($CH_3$), −1.40 $Si(CH_3)_3$. ^{19}F NMR (376 MHz, $CDCl_3$) δ −106.86 (t, *J* = 16.4 Hz, CF_2). UPLC-MS t_R 1.84 min, *m*/*z* 269.2 ($[M+H-Et]^+$, $C_{10}H_{19}F_2O_4Si^+$ Calcd 269.1); HRMS *m*/*z* 319.1149 ($[M+H]^+$,$C_{12}H_{22}F_2O_4NaSi^+$ Calcd 319.1148).

2,2-Difluoro-5-oxo-5-(2-(trimethylsilyl)ethoxy)pentanoic acid (S62).



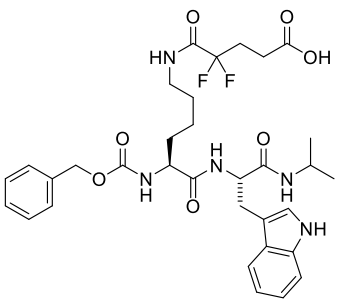
Aq. LiOH (1 M, 0.54 mL, 0.54 mmol) was added to a solution of ester **S61** (160 mg, 0.54 mmol) in THF (2.0 mL) and the mixture was stirred at ambient temperature for 2 hours. The reaction mixture was acidified to pH 2 with aq. HCl and diluted with H_2O to a final volume of 10 mL. The aqueous phase was extracted with CH_2Cl_2 (4×10 mL) and the organic phase was washed with brine (2×40 mL) and was then dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (0→8% CH_3OH, 0.25% AcOH in CH_2Cl_2), affording the desired carboxylic acid **S62** (122 mg, 84 %) as a clear oil. TLC (5% CH_3OH, 0.25% AcOH in CH_2Cl_2): R_f = 0.2. ^1H NMR (600 MHz, $CDCl_3$) δ 4.25–4.14 (m, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 2.60-2.48 (m, 2H, $CF_2CH_2C\underline{H}_2$), 2.48-2.34 (m, 2H, CF_2CH_2), 1.04–0.96 (m, 2H, $C\underline{H}_2Si(CH_3)_3$), 0.04 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.3 ($\underline{C}O_2(CH_2)_2Si(CH_3)_3$), 169.4 (t, *J* = 32.4 Hz, CO_2H), 117.22 (t, *J* = 250.6 Hz, CF_2), 65.3 ($\underline{C}H_2CH_2Si(CH_3)_3$), 31.3 (t, *J* = 24.2 Hz, $CF_2\underline{C}H_2$), 28.56 (t, *J* = 4.6 Hz, $CF_2CH_2\underline{C}H_2$), 18.8 ($\underline{C}H_2Si(CH_3)_3$), 0.00 ($Si(CH_3)_3$). ^{19}F NMR (376 MHz, $CDCl_3$) δ −106.80 (s, CF_2). UPLC-MS t_R 1.76 min, *m*/*z* 267.2 ($[M-H]^-$, $C_{10}H_{17}F_2O_4Si^-$ Calcd 267.1); HRMS *m*/*z* 291.0842 ($[M+H]^+$, $C_{10}H_{18}F_2O_4SiNa^+$ Calcd 291.0835).

2-(Trimethylsilyl)ethyl 5-(((*S*)-6-(((*S*)-3-(1*H*-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-4,4-difluoro-5-oxopentanoate (S63).



TFA salt **S52** (52 mg, 0.08 mmol), HOBt (17 mg, 0.13 mmol), carboxylic acid **S62** (34 mg, 0.12 mmol) and *i*Pr_2NEt (29 µL, 0.17 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to 0 °C. EDC (25 mg, 0.13 mmol) was added and the reaction mixture was stirred at 0°C for 10 minutes and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (60 mL) and washed with aq. $KHSO_4$ (5%, 3×40 mL), saturated aq. $NaHCO_3$ (3×40 mL), and brine (2×40 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The residue was purified by column chromatography (0→2% CH_3OH in CH_2Cl_2), affording the desired fluorinated amide **S63** (39 mg, 62%) as a colorless solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.45. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.71 (t, J = 5.8 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.83 (d, J = 8.2 Hz, 1H, $NH_{\alpha,Trp}$), 7.64 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.45–7.21 (m, 7H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H, $H6_{Indole}$), 7.00–6.91 (m, 1H, $H5_{Indole}$), 5.07–4.97 (m, 2H, $CH_{2,Cbz}$), 4.53–4.40 (m, 1H, $H_{\alpha,Trp}$), 4.14–4.08 (m, 2H, $C\underline{H}_2CH_2Si(CH_3)_3$), 3.93 (td, J = 8.4, 5.0 Hz, 1H, $H_{\alpha,Lys}$), 3.83–3.71 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.09–3.01 (m, 3H, $H_{\beta,Trp,A}$, $H_{\varepsilon,Lys}$), 2.96 (m_{ABX}, J = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,A}$), 2.41 (t, J = 7.7 Hz, 2H, $CF_2CH_2C\underline{H}_2$), 2.38–2.25 (m, 2H, $CF_2C\underline{H}_2$), 1.59–1.10 (m, 6H, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 1.02–0.88 (m, 8H, $(CH_3)_{2,i\mathrm{Pr}}$, $C\underline{H}_2Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (CO_{Lys}), 171.2 ($\underline{C}O_2(CH_2)_2Si(CH_3)_3$), 170.0 ($CO_{Trp}$), 162.9 (t, J = 28.4 Hz, $NH_{\varepsilon,Lys}CO$), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.7 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 117.4 (t, J = 251.3 Hz, CF_2), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 62.3 ($\underline{C}H_2CH_2Si(CH_3)_3$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.6 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 29.2 (t, J = 24.2 Hz, $CF_2\underline{C}H_2$), 28.3 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 26.5 (t, J = 4.5 Hz, $CF_2CH_2\underline{C}H_2$), 22.7 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$), 16.7 ($\underline{C}H_2Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.62 (t, J = 17.0 Hz, CF_2). UPLC-MS t_R 2.61 min, m/z 780.2 ($[M+Na]^+$, $C_{38}H_{53}N_5F_2O_7SiNa^+$ Calcd 780.3); HRMS m/z 780.3580 ($[M+Na]^+$, $C_{38}H_{53}N_5F_2O_7SiNa^+$ Calcd 780.3575).

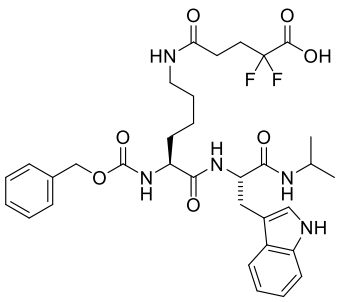
5-(((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-4,4-difluoro-5-oxopentanoic acid (24).



TBAF trihydrate (39 mg, 0.12 mmol) was added to a solution of trimethylsilyl ethyl ester **S63** in THF (2.0 mL) and the mixture was stirred at ambient temperature for 8 hours. Excess TBAF was removed by the addition of $CaCO_3$ (53 mg, 0.53 mmol), DOWEX Monosphere resin (124 mg) and CH_3OH (0.50 mL) mixture was stirred at ambient temperature overnight.[11] The suspension was filtered through celite and the filtrate was concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **24** (16 mg, 60%), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.35 (br s, 1H, CO_2H), 10.78 (d, J = 2.5 Hz, 1H, NH_{Indole}), 8.70 (t, J = 5.8 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.84 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.64 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.46–7.21 (m, 7H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 7.00–6.91 (m, 1H, $H5_{Indole}$), 5.09–4.96 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, J = 7.8, 6.1 Hz, 1H, $H_{\alpha,Trp}$), 3.93 (td, J = 8.5, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.84–3.71 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.10–3.01 (m, 3H, $H_{\beta,Trp,A}$, $H_{\varepsilon,Lys}$), 2.96 (m_{ABX}, J = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.41–2.22 (m, 4H, $CF_2C\underline{H}_2C\underline{H}_2$), 1.60–1.30 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.30–1.12 (m, 2H, $H_{\gamma,Lys}$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.6 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 163.0 (t, J = 28.7 Hz, $NH_{\varepsilon,Lys}CO$), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 117.5 (t, J = 251.1 Hz, CF_2), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.6 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 29.3 (t, J = 24.1 Hz, $CF_2\underline{C}H_2$), 28.3 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 26.4 (t, J = 4.5 Hz, $CF_2CH_2\underline{C}H_2$), 22.7 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). ^{19}F NMR (376

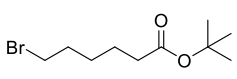
MHz, DMSO-d_6) δ −74.74 (s, $CF_{3,TFA}$), −106.38 (t, J = 16.7 Hz, CF_2). UPLC-MS t_R 2.08 min, m/z 658.2 ($[M+H]^+$, $C_{33}H_{42}N_5F_2O_7^+$ Calcd 658.3); HRMS m/z 658.3056 ($[M+H]^+$, $C_{33}H_{42}N_5F_2O_7^+$ Calcd 658.3047).

5-(((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-2,2-difluoro-5-oxopentanoic acid (S17).



TBAF trihydrate (601 mg, 1.90 mmol) was added to a solution of trimethylsilyl ethyl ester **S61** (200 mg, 0.67 mmol) in THF (4.0 mL) and the mixture was stirred at ambient temperature for 1.5 hours. Excess TBAF was removed by the addition of $CaCO_3$ (412 mg, 4.11 mmol), DOWEX Monosphere resin (1.33 g) and CH_3OH (4.0 mL) mixture was stirred at ambient temperature overnight. The suspension was filtered through celite and the filtrate was concentrated under reduced pressure.[11] Column chromatography (0→2% CH_3OH, 0.25% AcOH in CH_2Cl_2) of the crude residue afforded a clear oil (35 mg), tentatively assigned as 5-ethoxy-4,4-difluoro-5-oxopentanoic acid (UPLC-MS t_R 1.71 min, m/z 195.0; $[M-H]^-$, $C_7H_9F_2O_4^-$ Calcd 195.0), which was used without further purification. The crude oil (35 mg), TFA salt **S52** (74 mg, 0.12 mmol), HOBt (24 mg, 0.18 mmol) and iPr$_2$NEt (42 µL, 0.24 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to 0 °C. EDC (36 mg, 0.19 mmol) was added and the reaction mixture was stirred at 0°C for 15 minutes and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (60 mL) and washed with aq. $KHSO_4$ (5%, 3×40 mL), saturated aq. $NaHCO_3$ (3×40 mL), and brine (2×40 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. Column chromatography (0→3% CH_3OH in CH_2Cl_2) of the crude residue afforded a white solid (74 mg), tentatively assigned as the ethyl ester of **S17**, which was used without further purification. The white solid (40 mg) was dissolved in THF (1.3 mL) and aq. LiOH (1 M, 0.30 mL, 0.30 mmol) was added. The reaction mixture was stirred at ambient temperature for 1 hour and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **S17** (17 mg, 40% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, J = 2.4 Hz, 1H, NH_{Indole}), 7.90 (t, J = 5.6 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.84 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.65 (d, J = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.56 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.45–7.21 (m, 7H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 7.00–6.93 (m, 1H, $H5_{Indole}$), 5.08–4.97 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, J = 7.9, 6.0 Hz, 1H, $H_{\alpha,Trp}$), 3.93 (td, J = 8.4, 5.2 Hz, 1H, $H_{\alpha,Lys}$, overlap with residual water), 3.82–3.73 (m, 1H, $CH_{i\mathrm{Pr}}$, overlap with residual water), 3.06 (m_{ABX}, J = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 3.01–2.92 (m, 3H, $H_{\beta,Trp,B}$, $H_{\varepsilon,Lys}$), 2.35–2.21 (m, 4H, $CF_2C\underline{H}_2C\underline{H}_2$), 1.57–1.40 (m, 2H, $H_{\beta,Lys}$), 1.37–1.27 (m, 2H, $H_{\delta,Lys}$), 1.26–1.11 (m, 2H, $H_{\gamma,Lys}$), 1.00 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,A}}$), 0.91 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 169.6 ($NH_{\varepsilon,Lys}CO$), 164.90 (t, J = 31.7 Hz, CO_2H), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 116.3 (t, J = 248.2 Hz, CF_2), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.5 ($C_{\varepsilon,Lys}$), 31.5 ($C_{\beta,Lys}$), 29.7 (t, J = 23.7 Hz, $CF_2\underline{C}H_2$), 28.7 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 27.37 (t, J = 4.0 Hz, $CF_2CH_2\underline{C}H_2$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −75.30 ($CF_{3,TFA}$), −106.45 (t, J = 16.7 Hz, CF_2). UPLC-MS t_R 1.91 min, m/z 658.2 ($[M+H]^+$, $C_{33}H_{42}F_2N_5O_7^+$ Calcd 658.3); HRMS m/z 658.3055 ($[M+H]^+$, $C_{33}H_{42}F_2N_5O_7^+$ Calcd 658.3047).

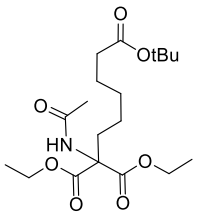
***tert*-Butyl 6-bromohexanoate (S64).**



DMAP (1.25 g, 0.01 mol) and *t*BuOH (48 mL, 0.51 mol) were added to a solution of 6-bromohexanoic acid (20.05 g, 0.1 mol) in anhydrous CH_2Cl_2 (350 mL) and the solution was cooled to 0 °C. DCC (23.38 g, 0.11 mol) was added and the solution was stirred at 0 °C for 15 minutes and was then stirred at ambient temperature overnight. The reaction mixture was filtered and the filtrate was partitioned with H_2O. The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→10% EtOAc in heptane), affording desired ester **S64** as a clear oil (12.25 g, 47%). TLC (10% EtOAc in heptane):

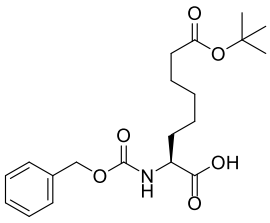
R_f = 0.3. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.41 (t, J = 6.7 Hz, 2H, $BrCH_2$), 2.23 (t, J = 7.4 Hz, 2H, CH_2CO_2tBu), 1.87 (p, J = 6.9 Hz, 2H, $BrCH_2CH_2$), 1.61 (p, J = 7.5 Hz, 2H, $CH_2CH_2CO_2tBu$), 1.52–1.39 (m, 11H, $BrCH_2CH_2CH_2$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.0 (CO_2tBu), 80.3 ($C(CH_3)_3$), 35.5 (CH_2CO_2tBu), 33.7 ($BrCH_2$), 32.6 $BrCH_2CH_2$), 28.3 ($C(CH_3)_3$), 27.8 ($BrCH_2CH_2CH_2$), 24.4 ($CH_2CH_2CO_2tBu$). In accordance with previously reported data.[13]

6-(*tert*-Butyl) 1,1-diethyl 1-acetamidohexane-1,1,6-tricarboxylate (S65).



tert-Butyl 6-hexanoate (12.25g, 48.8 mmol) and NaI (14.72 g, 98.2 mmol) were suspended in acetone (200 mL) and refluxed overnight. The reaction mixture was allowed to reach ambient temperature and was then concentrated under reduced pressure. The residue was dissolved in diethyl ether (200 mL) and washed with H_2O (2×50 mL), sat. $Na_2S_2O_3$ (50 mL), and brine (50 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure affording the a clear oil (11.8 g) tentively assigned as *tert*-butyl 6-iodohexanoate (UPLC-MS t_R 2.40 min, m/z 243.0; $[M+H-tBu]^+$, $C_6H_{12}IO_2^+$ Calcd 242.9), which was used without further purification. NaH (1.45 g, 60% dispersion in mineral oil, 36.2 mmol) was added to a solution of diethyl acetamidomalonate (7.87 g, 36.2 mmol) in anhydrous DMF (20 mL) at 0 °C. A solution of *tert*-butyl 6-iodohexanoate (11.84 g, 39.7 mmol) in anhydrous DMF (50 mL) was added dropwise to the reaction mixture over and the mixture was stirred at 0 °C for 10 minutes and was then stirred at ambient temperature overnight. The reaction mixture was diluted with diethyl ether (1.2 L) and was washed with H_2O (3×200 mL), aq. HCl (0.1 M, 200 mL), saturated aq. $NaHCO_3$ (200 mL), and brine (200 mL). The organic phase was dried over $NaSO_4$ and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→30% EtOAc in heptane) affording the desired ester **S65** (11.13 g, 79 % from diethyl acetamidomalonate) as a clear oil. TLC (50% EtOAc in heptane): R_f = 0.4. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 (s, 1H, NH), 4.23 (q, J = 7.1 Hz, 4H, CH_2CH_3), 2.34–2.27 (m, 2H, H_β), 2.17 (t, J = 7.5 Hz, 2H, H_ζ), 2.02 (s, 3H, $CH_{3,acetyl}$), 1.59–1.50 (m, 2H, H_ε), 1.43 (s, 9H, $C(CH_3)_3$), 1.35–1.27 (m, 2H, H_δ), 1.25 (t, J = 7.1 Hz, 6H, CH_2CH_3), 1.15–1.06 (m, 2H, H_γ).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.1 (CO_2tBu), 169.0 (CO_{Acetyl}), 168.3 (CO_2Et), 80.2 ($C(CH_3)_3$), 66.7 (C_α), 62.6 (CH_2CH_3), 35.5 (C_β), 32.1 (C_ζ), 28.9 (C_δ), 28.3 ($C(CH_3)_3$), 25.0 (C_ε), 23.6 (C_γ), 23.2 ($COCH_3$), 14.1 (CH_2CH_3). UPLC-MS t_R 2.23 min, m/z 410.2 ($[M+Na]^+$, $C_{19}H_{33}NO_7Na^+$ Calcd 410.2). In accordance with previously reported data.[14]

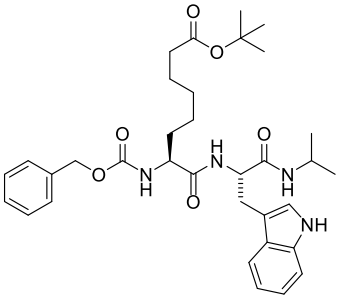
(*S*)-2-(((Benzyloxy)carbonyl)amino)-8-(*tert*-butoxy)-8-oxooctanoic acid (S66).



LiCl (1.83 g, 43.3 mmol) and H_2O (1.0 mL, 55.6 mmol) were added to a solution of the ester **S65** (11.13 g, 28.7 mmol) in DMSO (80 mL) and the mixture was stirred at 150 °C overnight. The reaction mixture was allowed to reach ambient temperature and was extracted with diethyl ether (3×300 mL) and the organic phase was washed with H_2O (2×200 mL) and brine (2×200 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure, affording an orange oil (8.4 g). Aq. LiOH (1 M, 27 mL, 27.0 mmol) and H_2O (38 mL, 2.11 mol) were added to a solution of the orange oil (8.4 g) in EtOH (65 mL). After stirring in ambient temperature for 2 hours the mixture was concentrated under reduced pressure and the remaining aqueous phase was acidified to pH 3 and extracted with EtOAc (3×100 mL). The organic phase was washed with brine (150 mL), dried over Na_2SO_4 and was then concentrated under reduced pressure, affording an off-white solid (7.28 g). *Aspergillus* amino acylase-I (685 mg) and $CoCl_6$•$6H_2O$ (136 mg, 0.57 mmol) were added to a solution of the off-white solid (7.28 g) in aq. phosphate buffer (0.1 M, pH 7.2, 600 mL). The mixture was stirred at 37 °C overnight and followed by ^{1}H NMR until a 1:1 mixture of the acetamide (starting material, **S65**) and the amine (product) was detected. The reaction mixture was concentrated under reduced pressure, to a final volume of 310 mL. A solution of Cbz-OSuc (3.29 g, 13.2 mmol) in 1,4-dioxane (155 mL) and $NaHCO_3$ (5.75g, 68.5 mmol) were added to 155 mL of the concentrated mixture and was stirred overnight under ambient temperature. The pH of the mixture was adjusted from pH 8 to pH 9 with aq. NaOH and after stirring at ambient temperature for 2 hours the reaction was acidified to pH 2 with aq. HCl and was then concentrated under reduced pressure. The aqueous phase was extracted with EtOAc (3×400 mL) and the organic phase

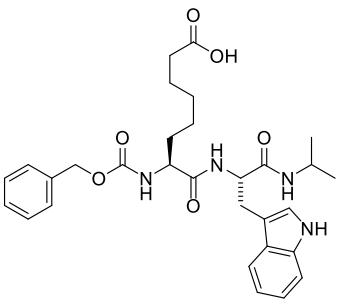
was dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→5% CH_3OH in CH_2Cl_2, 0.25% AcOH), affording the desired carboxylic acid **S66** (2.19 g, 40% from **S65**), as a clear oil. TLC (5% CH_3OH, 0.25% AcOH in CH_2Cl_2): R_f = 0.5. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.55 (d, J = 8.0 Hz, 1H, $NH_{\alpha,Asu}$), 7.40–7.26 (m, 5H, $H_{Ar,Cbz}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 3.97–3.89 (m, 1H, $H_{\alpha,Asu}$), 2.21–2.10 (m, 2H, $H_{\zeta,Asu}$), 1.71–1.62 (m, 1H, $H_{\beta,Asu,A}$), 1.61–1.53 (m, 1H, $H_{\beta,Asu,B}$), 1.52–1.42 (m, 2H, $H_{\varepsilon,Asu}$), 1.39 (s, 9H, $C(CH_3)_3$), 1.35–1.20 (m, 4H, $H_{\gamma,Asu}$, $H_{\delta,Asu}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.0 (CO_{Asu}), 172.2 (CO_2*t*Bu), 156.2 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 79.4 ($\underline{C}(CH_3)_3$), 65.4 ($CH_{2,Cbz}$), 53.8 ($C_{\alpha,Asu}$), 34.7 ($C_{\zeta,Asu}$), 30.6 ($C_{\beta,Asu}$), 27.9 ($C_{\delta,Asu}$), 27.8 ($C(\underline{C}H_3)_3$), 25.2 ($C_{\gamma,Asu}$), 24.4 ($C_{\varepsilon,Asu}$). UPLC-MS t_R x.xx min, m/z 378.3 ($[M-H]^-$, $C_{20}H_{28}NO_6^-$ Calcd 378.2); HRMS m/z 402.1887 ($[M+Na]^+$, $C_{20}H_{29}NO_6Na^+$ Calcd 402.1887).

tert-Butyl (_S_)-8-(((_S_)-3-(1_H_-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-7-(((benzyloxy)carbonyl)amino)-8-oxooctanoate (S67).



Carboxylic acid **S66** (158 mg, 0.42 mmol), HOBt (115 mg, 0.85 mmol), TFA salt **S33** (233 mg, 0.65 mmol) and iPr$_2$NEt (0.20 mL, 1.15 mmol) were dissolved in anhydrous CH_2Cl_2 and cooled to 0 °C. EDC (120 mg, 0.63 mmol) was added and the reaction mixture was stirred at 0°C for 5 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (100 mL) and washed with aq. $KHSO_4$ (5%, 3×75 mL), saturated aq. $NaHCO_3$ (3×75 mL), and brine (2×75 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→2.5% CH_3OH in CH_2Cl_2), affording the desired amide **S67** (223 mg, 88%) as a colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.24 (s, 1H, NH_{Indole}), 7.65 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.42–7.29 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.19 (t, J = 7.3 Hz, 1H, $H6_{Indole}$), 7.12 (t, J = 7.5 Hz, 1H, $H5_{Indole}$), 7.02 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 6.58 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Trp}$), 5.74 (d, J = 7.8 Hz, 1H, $CO_{Trp}NH$), 5.08–4.97 (m, 3H, $CH_{2,Cbz}$, $NH_{\alpha,Asu}$), 4.68–4.53 (m, 1H, $H_{\alpha,Trp}$), 4.05 (td, J = 7.2, 5.4 Hz, 1H, $H_{\alpha,Asu}$), 4.00–3.91 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.30 (m_{ABX}, J = 14.6, 6.3 Hz, 1H, $H_{\beta,Trp,A}$), 3.18 (m_{ABX}, J = 14.5, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.16 (t, J = 7.4 Hz, 2H, $H_{\zeta,Asu}$), 1.73–1.40 (m, 14H, $H_{\beta,Asu}$, $H_{\varepsilon,Asu}$, $C(CH_3)_3$), 1.32–1.08 (m, 4H, $H_{\gamma,Asu}$, $H_{\delta,Asu}$), 1.00 (d, 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.95 (d, 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.4 (CO_2*t*Bu), 171.4 (CO_{Asu}), 169.9 (CO_{Trp}), 156.3 (CO_{Cbz}), 136.4 ($C7a_{Indole}$), 136.2 ($C1_{Ar,Cbz}$), 128.8 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.5 ($C4_{Ar,Cbz}$), 128.3 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.6 ($C3a_{Indole}$), 123.1 ($C2_{Indole}$), 122.5 ($C6_{Indole}$), 120.0 ($C5_{Indole}$), 118.9 ($C4_{Indole}$), 111.5 ($C7_{Indole}$), 111.0 ($C3_{Indole}$), 80.4 ($\underline{C}(CH_3)_3$), 67.3 ($CH_{2,Cbz}$), 55.7 ($C_{\alpha,Asu}$), 54.1 ($C_{\alpha,Trp}$), 41.7 ($CH_{i\mathrm{Pr}}$), 35.5 ($C_{\zeta,Asu}$), 32.2 ($C_{\beta,Asu}$), 28.7 ($C_{\delta,Asu}$), 28.3 ($C(\underline{C}H_3)_3$), 27.9 ($C_{\beta,Trp}$), 25.0 ($C_{\gamma,Asu}$), 24.8 ($C_{\varepsilon,Asu}$), 22.6 ($CH_{3,i\mathrm{Pr},A}$), 22.4 ($CH_{3,i\mathrm{Pr},B}$). UPLC-MS t_R 2.87 min, m/z 607.2 ($[M+H]^+$, $C_{34}H_{47}N_4O_6^+$ Calcd 607.3).

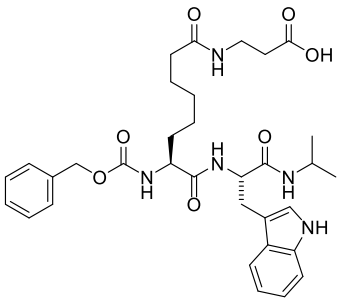
(_S_)-8-(((_S_)-3-(1_H_-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-7-(((benzyloxy)carbonyl)amino)-8-oxooctanoic acid (S68).



TFA (4.0 mL, 52.2 mmol) was added to a solution of *t*-butyl ester **S67** (219 mg, 0.36 mmol) in CH_2Cl_2 (6 mL). The mixture was stirred at ambient temperature for 3 hours, then TFA (1.0 mL, 13.1 mmol) was added. After stirring for an additional 45 minutes the reaction mixture was concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 3×100 mL), CH_2Cl_2/heptane (1:1, 2×100 mL) and CH_2Cl_2 (2×100 mL), affording the desired carboxylic acid **S68** (196 mg, 99%) as an off-white solid. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.58 (d, J = 8.0 Hz, 1H, $H4_{Indole}$), 7.39–7.24 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.11–7.05 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 7.01 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 5.11–4.96 (m, 2H, $CH_{2,Cbz}$), 4.57 (t, J = 7.0 Hz, 1H, $H_{\alpha,Trp}$), 3.99–3.92 (m, 1H, $H_{\alpha,Asu}$), 3.91–3.81 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.25 (m_{ABX}, J = 14.6, 7.0 Hz, 1H, $H_{\beta,Trp,A}$), 3.16 (m_{ABX}, J = 14.5, 7.0 Hz, 1H, $H_{\beta,Trp,B}$), 2.23 (t, J = 7.5 Hz, 2H, $H_{\zeta,Asu}$), 1.67–1.46 (m, 4H, $H_{\beta,Asu}$, $H_{\varepsilon,Asu}$), 1.36–1.14 (m, 4H, $H_{\gamma,Asu}$, $H_{\delta,Asu}$), 1.02 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.93 (d, J = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (151 MHz, Methanol-d_4) δ 177.9 (CO_2H), 174.6 (CO_{Asu}), 172.6 (CO_{Trp}), 158.8 (CO_{Cbz}), 138.1 ($C1_{Ar,Cbz}$), 138.0

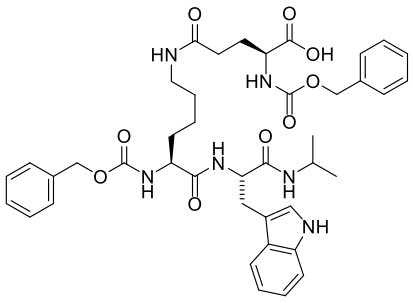
($C7a_{Indole}$), 129.5 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 129.1 ($C4_{Ar,Cbz}$), 128.8 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$, $C3a_{Indole}$), 124.6 ($C2_{Indole}$), 122.5 ($C6_{Indole}$), 119.9 ($C5_{Indole}$), 119.4 ($C4_{Indole}$), 112.3 ($C7_{Indole}$), 110.9 ($C3_{Indole}$), 67.8 ($CH_{2,Cbz}$), 57.1 ($C_{\alpha,Asu}$), 55.6 ($C_{\alpha,Trp}$), 42.7 ($CH_{i Pr}$), 35.1 ($C_{\zeta,Asu}$), 32.6 ($C_{\beta,Asu}$), 29.8 ($C_{\delta,Asu}$), 28.7 ($C_{\beta,Trp}$), 26.3 ($C_{\gamma,Asu}$), 25.9 ($C_{\varepsilon,Asu}$), 22.4 ($CH_{3,iPr,A}$), 22.3 ($CH_{3,iPr,B}$). UPLC-MS t_R 1.91 min, *m*/*z* 551.1 ($[M+H]^+$, $C_{30}H_{39}N_4O_6^+$ Calcd 551.3); HRMS *m*/*z* 551.2861 ($[M+H]^+$,$C_{30}H_{39}N_4O_6^+$ Calcd 551.2864).

3-((*S*)-8-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-7-(((benzyloxy)carbonyl)amino)-8-oxooctanamido)propanoic acid (25).



Carboxylic acid **S68** (52 mg, 0.09 mmol), HOBt (20 mg, 0.14 mmol), HCl·β-alanine *t*-butyl ester (34 mg, 0.19 mmol) and iPr_2NEt (50 µL, 0.29 mmol) were dissolved in anhydrous CH_2Cl_2 (4.0 mL) and cooled to 0°C. EDC (28 mg, 0.14 mmol) was added and the reaction mixture was stirred at 0°C for 10 minutes and was then stirred overnight at ambient temperature. The reaction mixture was diluted with EtOAc (60 mL) and washed with aq. $KHSO_4$ (5%, 3×50 mL), saturated aq. $NaHCO_3$ (3×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure. The residue was dissolved in in anhydrous CH_2Cl_2 (3.0 mL) and TFA (2.0 mL, 26.1 mmol) was added. The mixture was stirred at ambient temperature for 35 minutes and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **25** (15 mg, 30% from **S68**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.17 (br s, 1H, CO_2H), 10.79 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.92–7.77 (m, 2H, $NH_{\alpha,Trp}$, N<u>H</u>$(CH_2)_2CO_2H$), 7.64 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.41 (d, *J* = 7.8 Hz, 1H, $NH_{\alpha,Asu}$), 7.39–7.22 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 1H, $H6_{Indole}$), 6.95 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 5.08–4.97 (m, 2H, $CH_{2,Cbz}$), 4.45 (td, *J* = 7.9, 6.1 Hz, 1H, $H_{\alpha,Trp}$), 3.92 (td, *J* = 8.4, 5.3 Hz, 1H, $H_{\alpha,Asu}$), 3.83–3.70 (m, 1H, $CH_{i Pr}$), 3.22 (td, *J* = 6.9, 5.4 Hz, 2H, C<u>H</u>$_2CH_2CO_2H$), 3.06 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, *J* = 14.6, 7.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.36 (t, *J* = 7.0 Hz, 2H, C<u>H</u>$_2CO_2H$), 2.01 (t, *J* = 7.5 Hz, 2H, $H_{\zeta,Asu}$), 1.57–1.33 (m, 4H, $H_{\beta,Asu}$, $H_{\varepsilon,Asu}$), 1.28–1.09 (m, 4H, $H_{\gamma,Asu}$, $H_{\delta,Asu}$), 1.00 (d, *J* = 6.6 Hz, 3H, $CH_{3,iPr,A}$), 0.91 (d, *J* = 6.6 Hz, 3H, $CH_{3,iPr,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.9 (CO_2H), 172.2 ($CO_{\zeta,Asu}$), 171.5 ($CO_{\alpha,Asu}$), 170.0 (CO_{Trp}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 55.0 ($C_{\alpha,Asu}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i Pr}$), 35.2 ($C_{\zeta,Asu}$), 34.7 (C<u>H</u>$_2CH_2CO_2H$), 34.0 (<u>C</u>H$_2CO_2H$), 31.8 ($C_{\beta,Asu}$), 28.3 ($C_{\delta,Asu}$), 27.9 ($C_{\beta,Trp}$), 25.1 ($C_{\gamma,Asu}$, $C_{\varepsilon,Asu}$), 22.2 ($CH_{3,iPr,A}$), 22.1 ($CH_{3,iPr,B}$). UPLC-MS t_R 1.81 min, *m*/*z* 622.2 ($[M+H]^+$, $C_{33}H_{44}N_5O_7^+$ Calcd 622.3); HRMS *m*/*z* 622.3232 ($[M+H]^+$, $C_{33}H_{44}N_5O_7^+$ Calcd 622.3235).

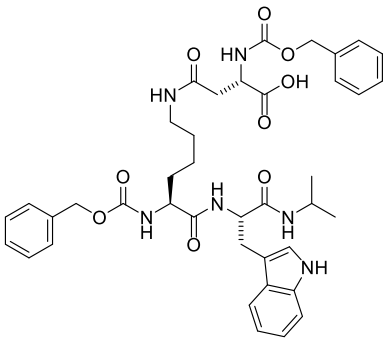
N^5-((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)-N^2-((benzyloxy)carbonyl)-L-glutamine (26).



TFA salt **S52** (48 mg, 0.08 mmol), HOBt (16 mg, 0.12 mmol), Cbz-L-Glu-OMe (35 mg, 0.12 mmol) and iPr_2NEt (20 µL, 0.11 mmol) were dissolved in anhydrous CH_2Cl_2 (4.0 mL) and cooled to 0 °C. EDC (22 mg, 0.12 mmol) was added and the reaction mixture was stirred at 0°C for 15 minutes and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (50 mL) and washed with aq. $KHSO_4$ (5%, 3×50 mL), saturated aq. $NaHCO_3$ (3×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and was then concentrated under reduced pressure, affording a colorless solid (57 mg). The colorless solid (33 mg) was dissolved in THF (1.3 mL) and aq. LiOH (1 M, 0.20 mL, 0.20 mmol) was added. The reaction mixture was stirred at ambient temperature for 75 minutes and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **26** (3 mg, 9% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6)

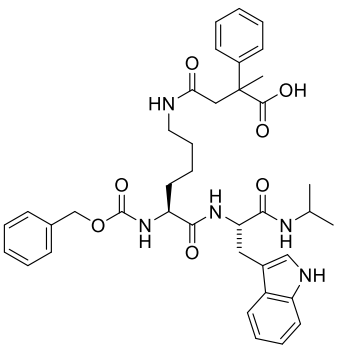
δ 12.58 (br s, 1H, CO_2H), 10.79 (s, 1H, NH_{Indole}), 7.83 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.77 (t, *J* = 5.6 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.64 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.60–7.52 (m, 2H, $H4_{Indole}$, $NH_{\alpha,Glu}$), 7.47–7.18 (m, 12H, $H_{Ar,Cbz,Glu}$, $H_{Ar,Cbz,Lys}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.00 (m, 1H, $H6_{Indole}$), 6.99–6.92 (m, 1H, $H5_{Indole}$), 5.10–4.96 (m, 4H, $CH_{2,Cbz,Glu}$, $CH_{2,Cbz,Lys}$), 4.45 (td, *J* = 7.9, 6.1 Hz, 1H, $H_{\alpha,Trp}$), 3.99–3.88 (m, 2H, $H_{\alpha,Lys}$, $H_{\alpha,Glu}$), 3.81–3.73 (m, 1H, $CH_{i\mathrm{Pr}}$, overlap with residual water), 3.06 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 3.01–2.90 (m, 3H, $H_{\beta,Trp,B}$, $H_{\varepsilon,Lys}$), 2.15 (t, *J* = 7.7 Hz, 2H, $H_{\gamma,Glu}$), 2.03–2.91 (m, 1H, $H_{\beta,Glu,A}$), 1.81–1.69 (m, 1H, $H_{\beta,Glu,B}$), 1.56–1.39 (m, 2H, $H_{\beta,Lys}$), 1.36–1.09 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 0.99 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.91 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.6 (CO_2H), 171.5 (CO_{Lys}), 171.0 ($CO_{\delta,Glu}$), 170.0 (CO_{Trp}), 156.1 ($CO_{Cbz,Lys}$), 156.0 ($CO_{Cbz,Glu}$), 136.96 ($C1_{Ar,Cbz,Lys}$), 136.95 ($C1_{Ar,Cbz,Glu}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz,Lys}$, $C3_{Ar,Cbz,Glu}$, $C5_{Ar,Cbz,Glu}$), 127.78 ($C4_{Ar,Cbz,Lys}$), 127.75 ($C4_{Ar,Cbz,Glu}$), 127.69 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz,Lys}$), 127.65 ($C2_{Ar,Cbz,Glu}$, $C6_{Ar,Cbz,Glu}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.42 ($CH_{2,Cbz,Lys}$), 65.38 ($CH_{2,Cbz,Glu}$), 55.0 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Glu}$), 53.3 ($C_{\alpha,Trp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.4 ($C_{\varepsilon,Lys}$), 31.7 ($C_{\gamma,Glu}$), 31.5 ($C_{\beta,Lys}$), 28.8 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$), 26.8 ($C_{\beta,Glu}$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr},A}$), 22.1 ($CH_{3,i\mathrm{Pr},B}$). UPLC-MS t_R 1.96 min, *m*/*z* 771.2 ($[M+H]^+$, $C_{41}H_{51}N_6O_9^+$ Calcd 771.3); HRMS *m*/*z* 771.3706 ($[M+H]^+$, $C_{41}H_{51}N_6O_9^+$ Calcd 771.3712).

N^4-((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)-N^2-((benzyloxy)carbonyl)-L-asparagine (S18).



By the method described for compound **26**, the title compound was synthesized using TFA salt **S52** (50 mg, 0.08 mmol), HOBt (17 mg, 0.13 mmol), Cbz-L-Asp-OMe (36 mg, 0.13 mmol), $i\mathrm{Pr}_2$NEt (20 µL, 0.11 mmol), anhydrous CH_2Cl_2 (4.0 mL), and EDC (23 mg, 0.12 mmol), affording a colorless solid (47 mg). Then the colorless solid (29 mg), THF (1.3 mL), and aq. LiOH (1 M, 0.20 mL, 0.20 mmol) affording the desired carboxylic acid **S18** (6 mg, 9% from **S52**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.64 (br s, 1H, CO_2H), 10.79 (s, 1H, NH_{Indole}), 7.88–7.79 (m, 2H, $NH_{\alpha,Trp}$, $NH_{\varepsilon,Lys}$), 7.65 (d, *J* = 7.7 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.48–7.17 (m, 13H, $NH_{\alpha,Asp}$, $NH_{\alpha,Lys}$, $H_{Ar,Cbz,Lys}$, $H_{Ar,Cbz,Asp}$, $H7_{Indole}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.00 (m, 1H, $H6_{Indole}$), 6.95 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 5.08–4.97 (m, 4H, $CH_{2,Cbz,Lys}$, $CH_{2,Cbz,Asp}$), 4.50–4.41 (m, 1H, $H_{\alpha,Trp}$), 4.40–4.30 (m, 1H, $H_{\alpha,Asp}$), 3.92 (td, *J* = 8.4, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.84–3.70 (m, 1H, $CH_{i\mathrm{Pr}}$), 3.06 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 3.01–2.87 (m, 3H, $H_{\beta,Trp,B}$, $H_{\varepsilon,Lys}$), 2.59–2.53 (m, 1H, $H_{\beta,Asp,A}$), 2.47–2.40 (m, 1H, $H_{\beta,Asp,B}$), 1.58–1.39 (m, 2H, $H_{\beta,Lys}$), 1.36–1.10 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 0.99 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},A}$), 0.91 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr},B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.1 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 168.8 ($CO_{\gamma,Asp}$), 156.0 ($CO_{Cbz,Lys}$), 155.8 ($CO_{Cbz,Asp}$), 136.9 ($C1_{Ar,Cbz,Lys}$, $C1_{Ar,Cbz,Asp}$), 136.0 ($C7a_{Indole}$), 128.33 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz,Lys}$), 128.31 ($C3_{Ar,Cbz,Asp}$, $C5_{Ar,Cbz,Asp}$), 127.8 ($C4_{Ar,Cbz,Asp}$, $C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz,Asp}$, $C6_{Ar,Cbz,Asp}$, $C2_{Ar,Cbz}$, $C6_{Ar,Cbz,Lys}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.44 ($CH_{2,Cbz,Lys}$), 65.39 ($CH_{2,Cbz,Asp}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 50.7 ($C_{\alpha,Asp}$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.5 ($C_{\varepsilon,Lys}$), 37.0 ($C_{\beta,Asp}$), 31.5 ($C_{\beta,Lys}$), 28.7 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr},A}$), 22.1 ($CH_{3,i\mathrm{Pr},B}$). UPLC-MS t_R 1.96 min, *m*/*z* 757.1 ($[M+H]^+$, $C_{40}H_{49}N_6O_9^+$ Calcd 757.3); HRMS *m*/*z* 757.3550 ($[M+H]^+$, $C_{40}H_{49}N_6O_9^+$ Calcd 757.3556).

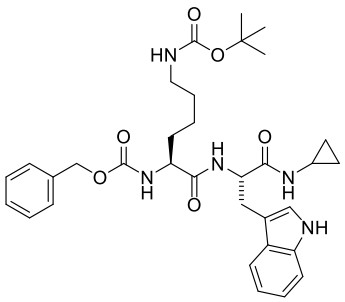
4-(((*S*)-6-(((*S*)-3-(1*H*-Indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)amino)-2-methyl-4-oxo-2-phenylbutanoic acid (S19).



TFA salt **S52** (27 mg, 0.04 mmol) and 3-methyl-3-phenyldihydrofuran-2,5-dione (5 mg, 0.03 mmol) were dissolved in anhydrous DMF (2.0 mL) and cooled to 0 °C. $i\mathrm{Pr}_2$NEt (9.5 µL, 0.05 mmol) was added and the reaction mixture was stirred at 0 °C for 2 hours and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **S19** (3 mg, 14% from 3-methyl-3-phenyldihydrofuran-2,5-dione), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.44 (br s, 1H, CO_2H), 10.79

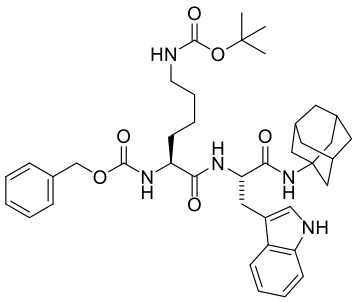
(s, 1H, NH_{Indole}), 7.89–7.75 (m, 2H, $NH_{\alpha,Trp}$, $NH_{\varepsilon,Lys}$), 7.66 (d, *J* = 7.8 Hz, 1H, $CO_{Trp}NH$), 7.55 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.46–7.15 (m, 13H, $NH_{\alpha,Lys}$, $H_{Ar,Ph}$, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (t, *J* = 7.6 Hz, 1H, $H6_{Indole}$), 6.95 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 5.10–4.97 (m, 2H, $CH_{2,Cbz}$), 4.51–4.40 (m, 1H, $H_{\alpha,Trp}$), 3.97–3.85 (m, 1H, $H_{\alpha,Lys}$, overlap with residual water), 3.83–3.71 (m, 1H, $CH_{i\mathrm{Pr}}$, overlap with residual water), 3.12–2.85 (m, 5H, $H_{\beta,Trp,}$ $H_{\varepsilon,Lys}$, $NH_{\varepsilon,Lys}COCH_{2,A}$), 2.70–2.63 (m, 1H, $NH_{\varepsilon,Lys}COCH_{2,B}$), 1.56–1.38 (m, 5H, CH_3CCO_2H, $H_{\beta,Lys}$), 1.34–1.06 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 0.99 (dd, *J* = 6.7, 2.1 Hz, 3H $CH_{3,i\mathrm{Pr,A}}$), 0.90 (d, *J* = 6.6 Hz, 3H, $CH_{3,i\mathrm{Pr}}$ $CH_{3,i\mathrm{Pr,B}}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.0 (CO_2H), 171.5 (CO_{Lys}), 170.0 (CO_{Trp}), 169.8 ($NH_{\varepsilon,Lys}CO$), 156.0 ($CO_{Cbz,Lys}$), 143.9 ($C1_{Ph}$), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.2 ($C2_{Ph}$, $C6_{Ph}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 126.5 ($C4_{Ar,Ph}$), 125.7 ($C3_{Ph}$, $C5_{Ph}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.3 ($C_{\alpha,Trp}$), 47.9 ($PhCCO_2H$), 43.8 ($NH_{\varepsilon,Lys}COCH_2$), 40.4 ($CH_{i\mathrm{Pr}}$), 38.2 ($C_{\varepsilon,Lys}$), 31.5 ($C_{\beta,Lys}$), 28.7 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 23.7 ($CH_3CCO_2H$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CH_{3,i\mathrm{Pr,A}}$), 22.1 ($CH_{3,i\mathrm{Pr,B}}$). UPLC-MS t_R 1.97 min, *m*/*z* 698.2 ($[M+H]^+$, $C_{39}H_{48}N_5O_7^+$ Calcd 698.3); HRMS *m*/*z* 698.3545 ($[M+H]^+$, $C_{39}H_{48}N_5O_7^+$ Calcd 698.3548).

Benzyl *tert*-butyl ((*S*)-6-(((*S*)-1-(cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S69).



Cbz-Lys(Boc)-OH (100 mg, 0.26 mmol), HOBt (54 mg, 0.40 mmol), TFA salt of **S34** (141 mg, 0.39 mmol), and *i*Pr_2NEt (90 µL, 0.52 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to 0 °C. EDC (77 mg, 0.40 mmol) was added and the reaction mixture was stirred at 0°C for 5 minutes and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (50 mL) and washed with aq. $KHSO_4$ (5%, 3×50 mL), saturated aq. $NaHCO_3$ (3×50 mL), and brine (2×50 mL). The crude residue was purified by column chromatography (0→4% CH_3OH in CH_2Cl_2) affording the desired amide **S69** (145 mg, 90%) as a colorless solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.4. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.90 (d, *J* = 4.3 Hz, 1H, $CO_{Trp}NH$), 7.85 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.54 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.43–7.22 (m, 7H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.08 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 1H, $H6_{Indole}$), 7.00–6.92 (m, 1H, $H5_{Indole}$), 6.73 (t, *J* = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 5.09–4.89 (m, 2H, $CH_{2,Cbz}$), 4.49–4.37 (m, 1H, $H_{\alpha,Trp}$), 3.92 (td, *J* = 8.4, 4.9 Hz, 1H, $H_{\alpha,Lys}$), 3.05 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.95 (m_{ABX}, *J* = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.91–2.79 (m, 2H, $H_{\varepsilon,Lys}$), 2.60–2.53 (m, 1H, $H1_{Cyclopropyl}$), 1.58–1.08 (m, 16H, $H_{\beta,Lys}$, $C(CH_3)_3$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 0.62–0.48 (m, 2H, $H2_{Cyclopropyl}$), 0.36–0.21 (m, 2H, $H2_{Cyclopropyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.2 (CO_{Trp}), 171.5 (CO_{Lys}), 156.0 (CO_{Cbz}), 155.5 (CO_{Boc}), 136.9 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 77.3 ($C(CH_3)_3$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.1 ($C_{\alpha,Trp}$), 39.3 ($C_{\varepsilon,Lys}$ overlap with solvent peak), 31.5 ($C_{\beta,Lys}$), 29.2 ($C_{\delta,Lys}$), 28.3 ($C(CH_3)_3$), 27.8 ($C_{\beta,Trp}$), 22.7 ($C_{\gamma,Lys}$), 22.2 ($C1_{Cyclopropyl}$), 5.52 ($C2_{Cyclopropyl}$), 5.46 ($C3_{Cyclopropyl}$). UPLC-MS t_R 2.18 min, *m*/*z* 606.2 ($[M+H]^+$, $C_{33}H_{44}N_5O_6^+$ Calcd 606.3); HRMS *m*/*z* 628.3098 ($[M+Na]^+$,$C_{33}H_{43}N_5O_6Na^+$ Calcd 628.3106).

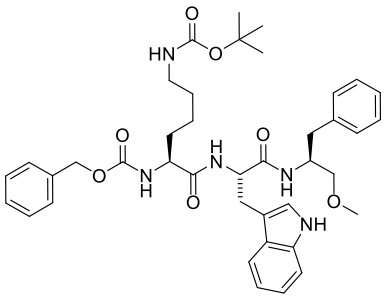
Benzyl *tert*-butyl ((*S*)-6-(((*S*)-1-((adamantan-1-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S70).



By the method described for compound **S69**, the title compound was synthesized using Cbz-Lys(Boc)-OH (100 mg, 0.26 mmol), HOBt (56 mg, 0.42 mmol), TFA salt **S37** (178 mg, 0.39 mmol), *i*Pr_2NEt (90 µL, 0.52 mmol), anhydrous CH_2Cl_2 (5 mL), and EDC (77 mg, 0.40 mmol). The crude residue was purified by column chromatography (0→3% CH_3OH in CH_2Cl_2) affording the desired amide **S70** (166 mg, 90%) as a colorless solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.5. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47 (s, 1H, NH_{Indole}), 7.66 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.39–7.27 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.17 (t, *J* = 7.5 Hz, 1H, $H6_{Indole}$), 7.11 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 7.03 (s, 1H, $H2_{Indole}$),

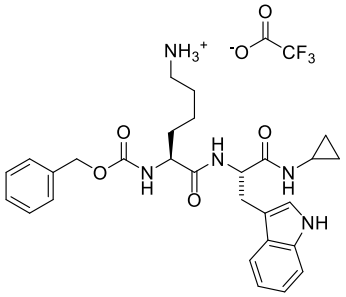
6.91 (br s, 1H, $NH_{\alpha,Trp}$), 5.43 (br s, 1H, $NH_{\alpha,Lys}$), 5.05 (s, 2H, $CH_{2,Cbz}$), 4.68–4.57 (m, 1H, $H_{\alpha,Trp}$), 4.15–4.01 (m, 1H, $H_{\alpha,Lys}$), 3.27 (m_{ABX}, J = 14.8, 6.6 Hz, 1H, $H_{\beta,Trp,A}$), 3.17 (m_{ABX}, J = 14.7, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 3.01 (t, J = 6.9 Hz, 2H, $H_{\varepsilon,Lys}$), 2.07–1.93 (m, 3H, $H3_{Ada}$, $H5_{Ada}$, $H7_{Ada}$), 1.81 (br s, 6H, $H2_{Ada}$, $H8_{Ada}$, $H9_{Ada}$), 1.70–1.50 (m, 8H, $H4_{Ada}$, $H6_{Ada}$, $H10_{Ada}$, $H_{\beta,Lys}$), 1.43 (s, 11H, $C(CH_3)_3$, $H_{\delta,Lys}$), 1.20–1.08 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.0 (CO_{Lys}), 170.4 (CO_{Trp}), 156.6 (CO_{Cbz}), 156.4 (CO_{Boc}), 136.4 ($C1_{Ar,Cbz}$, $C7a_{Indole}$), 128.7 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.4 ($C4_{Ar,Cbz}$), 128.3 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.4 ($C6_{Indole}$), 119.9 ($C5_{Indole}$), 119.0 ($C4_{Indole}$), 111.5 ($C7_{Indole}$), 110.8 ($C3_{Indole}$), 79.7 ($\underline{C}(CH_3)_3$), 67.2 ($CH_{2,Cbz}$), 55.4 ($C_{\alpha,Lys}$), 54.5 ($C_{\alpha,Trp}$), 52.7 ($C1_{Ada}$), 41.2 ($C3_{Ada}$, $C5_{Ada}$, $C7_{Ada}$), 40.1 ($C_{\varepsilon,Lys}$), 36.3 ($C2_{Ada}$, $C8_{Ada}$, $C9_{Ada}$), 31.8 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 29.5 ($C4_{Ada}$, $C6_{Ada}$, $C10_{Ada}$), 28.6 ($C(\underline{C}H_3)_3$), 28.1 ($C_{\beta,Trp}$), 22.2 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.50 min, m/z 700.3 ($[M+H]^+$, $C_{40}H_{54}N_5O_6^+$ Calcd 700.4); HRMS m/z 722.3872 ($[M+Na]^+$,$C_{40}H_{53}N_5O_6Na^+$ Calcd 722.3888). Ada=adamant-1-yl

Benzyl *tert*-butyl ((*S*)-6-(((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S71).



By the method described for compound **S69**, the title compound was synthesized using Cbz-Lys(Boc)-OH (101 mg, 0.26 mmol), HOBt (58 mg, 0.40 mmol), TFA salt **S38** (185 mg, 0.39 mmol), iPr$_2$NEt (90 µL, 0.52 mmol), anhydrous CH_2Cl_2 (5.0 mL), and EDC (77 mg, 0.40 mmol). The crude residue was purified by column chromatography (0→2.5% CH_3OH in CH_2Cl_2) affording the desired amide **S71** (96 mg, 51%) as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, J = 2.5 Hz, 1H, NH_{Indole}), 7.87 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.81 (d, J = 8.3 Hz, 1H, $CO_{Trp}NH$), 7.54 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.40–7.12 (m, 11H, $H_{Ar,Cbz}$, H_{Ph}, $H7_{Indole}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.98–6.93 (m, 1H, $H5_{Indole}$), 6.74 (t, J = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 5.07–4.96 (m, 2H, $CH_{2,Cbz}$), 4.57–4.44 (m, 1H, $H_{\alpha,Trp}$), 4.05–3.88 (m, 2H, $H_{\alpha,Lys}$, $CO_{Trp}NHC\underline{H}$), 3.17 (s, 3H, $CH_2OC\underline{H}_3$), 3.11–3.05 (m, 2H, $C\underline{H}_2OCH_3$), 3.03 (m_{ABX}, J = 14.6, 6.3 Hz, 1H, $H_{\beta,Trp,A}$), 2.93 (m_{ABX}, J = 14.7, 7.5 Hz, 1H, $H_{\beta,Trp,B}$), 2.90–2.79 (m, J = 6.5 Hz, 2H, $H_{\varepsilon,Lys}$), 2.74 (m_{ABX}, J = 13.9, 6.1 Hz, 1H, $CHC\underline{H}_{2,A}Ph$), 2.63 (m_{ABX}, J = 13.9, 8.0 Hz, 1H, $CHC\underline{H}_{2,B}Ph$), 1.36 (s, 15H, $C(CH_3)_3$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (CO_{Lys}), 170.7 (CO_{Trp}), 155.9 (CO_{Cbz}), 155.5 (CO_{Boc}), 138.5 ($C1_{Ph}$), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 129.0 ($C2_{Ph}$, $C6_{Ph}$), 128.3 ($C3_{Ph}$, $C5_{Ph}$), 128.1 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.73 ($C4_{Ar,Cbz}$), 127.67 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 126.0 ($C4_{Ph}$), 123.5 ($C2_{Indole}$), 120.7 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_3$), 72.8 ($\underline{C}H_2OCH_3$), 65.4 ($CH_{2,Cbz}$), 58.1 ($CH_2O\underline{C}H_3$), 54.8 ($C_{\alpha,Lys}$), 53.4 ($C_{\alpha,Trp}$), 49.8 ($CO_{Trp}NH\underline{C}H$), 39.4 ($C_{\varepsilon,Lys}$ overlap with solvent peak), 36.6 ($CH\underline{C}H_2Ph$), 31.6 ($C_{\beta,Lys}$), 29.2 ($C_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_3$), 27.9 ($C_{\beta,Trp}$), 22.8 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.49 min, m/z 714.3 ($[M+H]^+$, $C_{40}H_{52}N_5O_7^+$ Calcd 714.4); HRMS m/z 736.3663 ($[M+Na]^+$,$C_{40}H_{51}N_5O_7Na^+$ Calcd 736.3681).

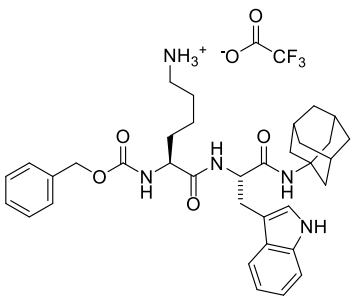
Benzyl ((*S*)-6-amino-1-(((*S*)-1-(cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)carbamate·TFA salt (S72).



TFA (1.25 mL, 16.3 mmol) was added to a solution of carbamate **S69** (115 mg, 0.19 mmol) and TIPS (0.25 mL, 1.22 mmol) in anhydrous CH_2Cl_2 (3.5 mL). The reaction mixture was stirred at ambient temperature for 45 minutes and was then concentrated under reduced pressure. Excess TFA was removed with by coevaporations: CH_2Cl_2/tolune (1:1, 2×25 mL), CH_2Cl_2/heptane (1:1, 2×25 mL) and CH_3OH /CH_2Cl_2 (10 mL) affording the crude TFA salt **S72** (97 mg, 83%) as an off-white solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.83 (d, J = 2.4 Hz, 1H, NH_{Indole}), 7.95 (d, J = 4.3 Hz, 1H, $CO_{Trp}NH$), 7.89 (d, J = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.74 (br s, 3H, NH_3), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.50–7.18 (m, 7H, $H_{Ar,Cbz}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.10 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.05 (t, J = 7.4 Hz, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 5.08–4.95 (m, 2H, $CH_{2,Cbz}$), 4.49–4.38 (m, 1H, $H_{\alpha,Trp}$), 3.95 (td, J = 8.5, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 3.06 (m_{ABX}, J = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, J = 14.6, 7.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.77–2.66 (m, 2H, $H_{\varepsilon,Lys}$), 2.57 (td, J = 7.3, 3.7 Hz, 1H,

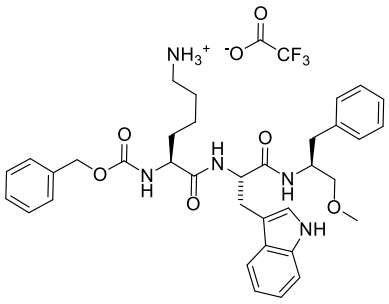
$H1_{Cyclopropyl}$), 1.59–1.41 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.31–1.15 (m, 2H, $H_{\gamma,Lys}$), 0.62–0.48 (m, 2H, $H2_{Cyclopropyl}$), 0.36–0.22 (m, 2H, $H3_{Cyclopropyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.2 (CO_{Trp}), 171.4 (CO_{Lys}), 158.2 (q, J = 31.9 Hz, CO_{TFA}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 117.9 (q, J = 297.8 Hz, $CF_{3,TFA}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 38.6 ($C_{\varepsilon,Lys}$), 31.2 ($C_{\beta,Lys}$), 27.8 ($C_{\beta,Trp}$), 26.6 ($C_{\delta,Lys}$), 22.3 ($C1_{Cyclopropyl}$), 5.53 ($C2_{Cyclopropyl}$), 5.47 ($C2_{Cyclopropyl}$). UPLC-MS t_R 1.75 min, m/z 506.2 ($[M+H]^+$, $C_{28}H_{36}N_5O_4^+$ Calcd 506.3); HRMS m/z 506.2754 ($[M+H]^+$,$C_{28}H_{36}N_5O_4^+$ Calcd 506.2762).

Benzyl ((*S*)-1-(((*S*)-1-((adamantan-1-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-amino-1-oxohexan-2-yl)carbamate·TFA salt (S73).



By the method described for compound **S72**, the title compound was synthesized using TFA (1.0 mL, 13.1 mmol), carbamate **S70** (129 mg, 0.19 mmol), TIPS (0.15 mL, 0.73 mmol), and anhydrous CH_2Cl_2 (2.1 mL) affording the desired TFA salt **S73** (141 mg, >99%) as an off-white solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (d, J = 2.4 Hz, 1H, NH_{Indole}), 7.81 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.69 (s, 3H, NH_3), 7.57 (d, J = 8.0 Hz, 1H, $H4_{Indole}$), 7.44 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Lys}$), 7.39–7.28 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.23 (s, 1H, $CO_{Trp}NH$), 7.11 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.05 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.98–6.92 (m, 1H, $H5_{Indole}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 4.48 (td, J = 7.9, 5.9 Hz, 1H, $H_{\alpha,Trp}$), 3.97–3.91 (m, 1H, $H_{\alpha,Lys}$), 3.06 (m_{ABX}, J = 14.7, 5.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.95 (m_{ABX}, J = 14.6, 7.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.70 (t, J = 7.4 Hz, 2H, $H_{\varepsilon,Lys}$), 2.02–1.92 (m, 3H, $H3_{Ada}$, $H5_{Ada}$, $H7_{Ada}$), 1.91–1.80 (m, 6H, $H2_{Ada}$, $H8_{Ada}$, $H9_{Ada}$), 1.65–1.39 (m, 12H, $H4_{Ada}$, $H6_{Ada}$, $H10_{Ada}$, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.30– 1.16 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.3 (CO_{Lys}), 170.2 (CO_{Trp}), 157.9 (q, J =30.5 Hz, CO_{TFA}), 156.0 (CO_{Cbz}), 136.9 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.4 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.6 ($C5_{Indole}$), 118.1 ($C4_{Indole}$), 117.3 (q, J = 300.0 Hz, $CF_{3,TFA}$), 111.1 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.5 ($CH_{2,Cbz}$), 54.7 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Trp}$), 50.8 ($C1_{Ada}$), 40.8 ($C3_{Ada}$, $C5_{Ada}$, $C7_{Ada}$), 38.6 ($C_{\varepsilon,Lys}$), 36.0 ($C2_{Ada}$, $C8_{Ada}$, $C9_{Ada}$), 31.2 ($C_{\beta,Lys}$), 28.8 ($C4_{Ada}$, $C6_{Ada}$, $C10_{Ada}$), 27.9 ($C_{\beta,Trp}$), 26.6 ($C_{\delta,Lys}$), 22.3 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.06 min, m/z 600.2 ($[M+H]^+$, $C_{35}H_{46}N_5O_4^+$ Calcd 600.4); HRMS m/z 600.3537 ($[M+H]^+$,$C_{35}H_{46}N_5O_4^+$ Calcd 600.3544). Ada=adamant-1-yl

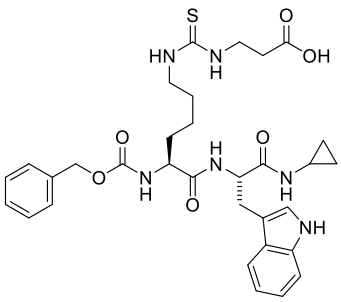
Benzyl ((*S*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)amino)-6-amino-1-oxohexan-2-yl)carbamate·TFA salt (S74).



By the method described for compound **S72**, the title compound was synthesized using TFA (0.75 mL, 9.79 mmol), carbamate **S71** (67 mg, 0.09 mmol), TIPS (0.15 mL, 0.73 mmol), and anhydrous CH_2Cl_2 (2.1 mL) affording the desired TFA salt **S74** (60 mg, 89%) as an off-white solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.82 (d, J = 2.6 Hz, 1H, NH_{Indole}), 7.94–7.84 (m, 2H, $NH_{\alpha,Trp}$, $CO_{Trp}NH$), 7.69 (s, 3H, NH_3), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.44–7.13 (m, 12H, $H_{Ar,Cbz}$, $H_{Ar,Mpp}$, $H7_{Indole}$, $NH_{\alpha,Lys}$), 7.11 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.08–7.02 (m, 1H, $H6_{Indole}$), 6.96 (t, J = 7.5 Hz, 1H, $H5_{Indole}$), 5.07–4.98 (m, 2H, $CH_{2,Cbz}$), 4.57–4.46 (m, 1H, $H_{\alpha,Trp}$), 4.05–3.92 (m, 2H, $H_{\alpha,Lys}$, $CO_{Trp}NHC\underline{H}$), 3.17 (s, 3H, OCH_3), 3.10–2.91 (m, 4H, $C\underline{H}_2OCH_3$, $H_{\beta,Trp}$), 2.79–2.59 (m, 4H, $H_{\varepsilon,Lys}$, $CHC\underline{H}_2Ph$), 1.56–1.38 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.29–1.15 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.4 (CO_{Lys}), 170.8 (CO_{Trp}), 157.9 (q, J = 31.2 Hz, CO_{TFA}), 156.0 (CO_{Cbz}), 138.5 ($C1_{Ar,MPP}$), 136.9 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 129.1 ($C2_{Ar,Mpp}$, $C6_{Ar,Mpp}$), 128.3 ($C3_{Ar,Mpp}$, $C5_{Ar,Mpp}$), 128.1 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 126.0 ($C4_{Ar,Mpp}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 117.3 (q, J = 301.8 Hz, $CF_{3,TFA}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 72.8 ($\underline{C}H_2OCH_3$), 65.5 ($CH_{2,Cbz}$), 58.2 (OCH_3), 54.6 ($C_{\alpha,Lys}$), 53.4 ($C_{\alpha,Trp}$), 49.8 ($CO_{Trp}NH\underline{C}H$), 38.7 ($C_{\varepsilon,Lys}$), 36.6 ($CH\underline{C}H_2Ph$), 31.3 ($C_{\beta,Lys}$), 28.0 ($C_{\beta,Trp}$), 26.6 ($C_{\delta,Lys}$), 22.3 ($C_{\gamma,Lys}$). UPLC-MS

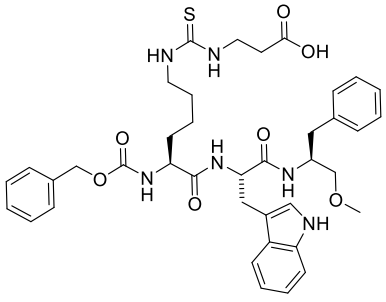
t_R 1.98 min, *m*/*z* 614.2 ([M+H]$^+$, $C_{35}H_{44}N_5O_5^+$ Calcd 614.3); HRMS *m*/*z* 614.3325 ([M+H]$^+$,$C_{35}H_{44}N_5O_5^+$ Calcd 614.3337). Mpp=(*S*)-(1-methoxy-3-phenylpropan-2-yl)

(*S*)-5-(((*S*)-1-(Cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3-oxo-1-phenyl-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (45).



TFA salt **S72** (50 mg, 0.08 mmol), compound **S48** (30 mg, 0.10 mmol), and *i*Pr$_2$NEt (28 µL, 0.16 mmol) were dissolved in anhydrous DMF (2.0 mL) and the mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (50 mL) and washed with H_2O (2×50 mL), saturated aq. $NaHCO_3$ (2×50 mL), aq. HCl (1 M, 2×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (3.0 mL) and TIPS (0.20 mL, 0.98 mmol) and TFA (1.0 mL, 13.0 mmol) were added. The mixture was stirred at ambient temperature for 2 hours and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **45** (32 mg, 62% from **S72**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.23 (br s, 1H, CO_2H), 10.79 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.02–7.81 (m, 2H, $CO_{Trp}NH$, $NH_{\alpha,Trp}$), 7.62–7.19 (m, 10H, $NH_{\varepsilon,Lys}$, $N\underline{H}(CH_2)_2CO_2H$, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$, $H4_{Indole}$), 7.09 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 1H, $H6_{Indole}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 5.08–4.97 (m, 2H, $CH_{2,Cbz}$), 4.48–4.38 (m, 2H, $H_{\alpha,Trp}$, overlap with residual water), 4.00–3.90 (m, 2H, $H_{\alpha,Lys}$, overlap with residual water), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$), 3.29 (br s, 2H, $H_{\varepsilon,Lys}$), 3.05 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.95 (m_{ABX}, *J* = 14.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.60–2.52 (m, 1H, $H1_{Cyclopropyl}$), 2.47 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$, overlap with solvent peak), 1.62–1.32 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.32–1.10 (m, 2H, $H_{\gamma,Lys}$), 0.61–0.48 (m, 2H, $H2_{Cyclopropyl}$), 0.35–0.17 (m, 2H, $H3_{Cyclopropyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.1 (CO_2H), 172.2 (CO_{Trp}), 171.5 (CO_{Lys}), 156.0 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 43.5 ($C_{\varepsilon,Lys}$), 39.2 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 33.7 (CH_2CO_2H), 31.6 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$), 22.9 ($C_{\gamma,Lys}$), 22.3 ($C1_{Cyclopropyl}$), 5.53 ($C2_{Cyclopropyl}$), 5.48 ($C3_{Cyclopropyl}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.98 min, *m*/*z* 637.2 ([M+H]$^+$, $C_{32}H_{41}N_6O_6S^+$ Calcd 637.3); HRMS *m*/*z* 637.2810 ([M+H]$^+$, $C_{32}H_{41}N_6O_6S^+$ Calcd 637.2803).

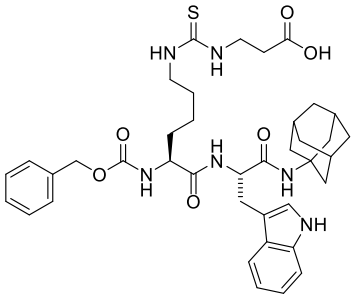
(4*S*,7*S*,10*S*)-7-((1*H*-Indol-3-yl)methyl)-4-benzyl-10-(((benzyloxy)carbonyl)amino)-6,9-dioxo-16-thioxo-2-oxa-5,8,15,17-tetraazaicosan-20-oic acid (46).



By the method described for compound **45**, the title compound was synthesized using TFA salt **S74** (45 mg, 0.06 mmol), compound **S48** (23 mg, 0.07 mmol), *i*Pr$_2$NEt (22 µL, 0.13 mmol), and anhydrous DMF (2.0 mL), followed by anhydrous CH_2Cl_2 (3.0 mL), TIPS (0.20 mL, 0.98 mmol), and TFA (1.0 mL, 13.0 mmol) affording the desired carboxylic acid **46** (6 mg, 13% from **S74**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.23 (br s, 1H, CO_2H), 10.79 (d, *J* = 2.5 Hz, 1H, NH_{Indole}), 7.89 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.82 (d, *J* = 8.3 Hz, 1H, $CO_{Trp}NH$), 7.54 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.48 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.42–7.12 (m, 13H, $N\underline{H}(CH_2)_2CO_2H$, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H_{Ar,Mpp}$, $H7_{Indole}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.99–6.92 (m, 1H, $H5_{Indole}$), 5.07–4.97 (m, 2H, $CH_{2,Cbz}$), 4.57–4.43 (m, 1H, $H_{\alpha,Trp}$), 4.03–3.92 (m, 2H, $H_{\alpha,Lys}$, $CO_{Trp}NHC\underline{H}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 3.29 (br s, 2H, $H_{\varepsilon,Lys}$), 3.17 (s, 3H, $CH_2OC\underline{H}_3$), 3.10–3.05 (m, 2H, $C\underline{H}_2OCH_3$), 3.03 (m_{ABX}, *J* = 14.6, 6.2 Hz, 1H, $H_{\beta,Trp,A}$), 2.93 (m_{ABX}, *J* = 14.6, 7.5 Hz, 1H, $H_{\beta,Trp,B}$), 2.74 (m_{ABX}, *J* = 13.7, 6.1 Hz, 1H, $CHC\underline{H}_{2,A}Ph$), 2.63 (m_{ABX}, *J* = 13.7, 7.7 Hz, 1H, $CHC\underline{H}_{2,B}Ph$), 2.47 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$), 1.58–1.33 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.32–1.11 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H), 171.5 (CO_{Lys}), 170.7 (CO_{Trp}), 156.0 (CO_{Cbz}), 138.5

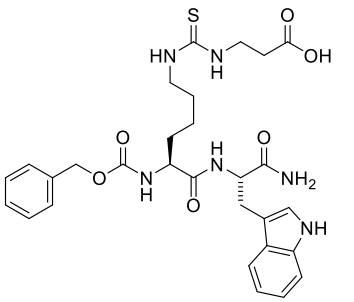
($C1_{Ar,Mpp}$), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 129.0 ($C2_{Ar,Mpp}$, $C6_{Ar,Mpp}$), 128.3 ($C3_{Ar,Mpp}$, $C5_{Ar,Mpp}$), 128.1 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.8 ($C4_{Ar,Cbz}$), 127.7 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 126.0 ($C4_{Ar,Mpp}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 72.8 ($\underline{C}H_2OCH_3$), 65.4 ($CH_{2,Cbz}$), 58.2 ($CH_2O\underline{C}H_3$), 54.8 ($C_{\alpha,Lys}$), 53.4 ($C_{\alpha,Trp}$), 49.8 ($CO_{Trp}NH\underline{C}H$), 43.5 ($C_{\varepsilon,Lys}$), 39.0 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 36.6 ($\underline{C}H_2Ph_{Mpp}$), 33.7 (CH_2CO_2H), 31.7 ($C_{\beta,Lys}$), 28.5 ($C_{\delta,Lys}$), 27.9 ($C_{\beta,Trp}$), 23.0 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.21 min, *m*/*z* 745.2 ($[M+H]^+$, $C_{39}H_{49}N_6O_7S^+$ Calcd 745.3); HRMS *m*/*z* 767.3210 ($[M+Na]^+$, $C_{39}H_{48}N_6O_7SNa^+$ Calcd 767.3197). Mpp=(*S*)-(1-methoxy-3-phenylpropan-2-yl)

(*S*)-5-(((*S*)-1-((Adamantan-1-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3-oxo-1-phenyl-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (47).



By the method described for compound **45**, the title compound was synthesized using TFA salt **S73** (52 mg, 0.07 mmol), compound **S48** (27 mg, 0.09 mmol), *i*Pr_2NEt (25 µL, 0.14 mmol), and anhydrous DMF (2.0 mL), followed CH_2Cl_2 (3.0 mL), TIPS (0.20 mL, 0.98 mmol), and TFA (1.0 mL, 13.0 mmol) affording the desired carboxylic acid **47** (18 mg, 34% from **S73**), as a colorless fluffy material after lyophilization. ^1H NMR (600 MHz, DMSO-d_6) δ 12.23 (br s, 1H, CO_2H), 10.78 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.83 (d, *J* = 8.2 Hz, 1H, $NH_{\alpha,Trp}$), 7.60–7.20 (m, 10H, $NH_{\varepsilon,Lys}$, $N\underline{H}(CH_2)_2CO_2H$, $NH_{\alpha,Lys}$, $H_{Ar,Cbz}$, $H7_{Indole}$, $H4_{Indole}$), 7.16 (s, 1H, $NH_{Adamantyl}$), 7.11 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, *J* = 8.1, 6.8, 1.1 Hz, 1H, $H6_{Indole}$), 6.96 (t, *J* = 7.2 Hz, 1H, $H5_{Indole}$), 5.03 (s, 2H, $CH_{2,Cbz}$), 4.47 (td, *J* = 7.8, 5.9 Hz, 1H, $H_{\alpha,Trp}$, overlap with residual water), 3.94 (td, *J* = 8.5, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$), 3.28 (br s, 2H, $H_{\varepsilon,Lys}$), 3.05 (m_{ABX}, *J* = 14.7, 5.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.94 (m_{ABX}, *J* = 14.7, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7 Hz, 2H, CH_2CO_2H), 2.01–1.92 (m, 3H, $H3_{Adamantyl}$, $H5_{Adamantyl}$, $H7_{Adamantyl}$), 1.89–1.77 (m, 6H, $H2_{Ada}$, $H8_{Ada}$, $H9_{Ada}$), 1.64–1.50 (m, 7H, $H4_{Ada}$, $H6_{Ada}$, $H10_{Ada}$, $H_{\beta,Lys,A}$), 1.50–1.33 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.32–1.12 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.1 (CO_2H), 171.5 (CO_{Lys}), 170.2 (CO_{Trp}), 155.9 (CO_{Cbz}), 137.0 ($C1_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.5 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 65.4 ($CH_{2,Cbz}$), 54.9 ($C_{\alpha,Lys}$), 53.5 ($C_{\alpha,Trp}$), 50.8 ($C1_{Ada}$), 43.5 ($C_{\varepsilon,Lys}$), 40.8 ($C3_{Ada}$, $C5_{Ada}$, $C7_{Ada}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 36.0 ($C2_{Ada}$, $C8_{Ada}$, $C9_{Ada}$), 33.7 ($C\underline{H}_2CO_2H$), 31.7 ($C_{\beta,Lys}$), 28.8 ($C4_{Ada}$, $C6_{Ada}$, $C10_{Ada}$), 28.5 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$), 22.9 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.36 min, *m*/*z* 731.2 ($[M+H]^+$, $C_{39}H_{51}N_6O_6S^+$ Calcd 731.3); HRMS *m*/*z* 753.3416 ($[M+Na]^+$, $C_{39}H_{50}N_6O_6SNa^+$ Calcd 753.3405). Ada=adamant-1-yl

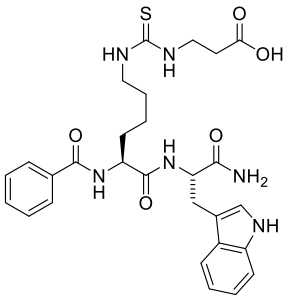
(*S*)-5-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3-oxo-1-phenyl-11-thioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (30).



Starting from Fmoc-Lys(Teoc)-Trp-resin (199 mg, estimated loading: 0.29 mmol/g) synthesized by SPPS, the title compound was synthesized using the general acylation procedure with Cbz-OSuc, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **30** (1 mg, 3% based on resin loading), as a colorless fluffy material after lyophilization. ^1H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.84 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.58 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.47 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.42–7.22 (m, 9H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$, $H7_{Indole}$), 7.12 (d, *J* = 2.3, 1H, $H2_{Indole}$), 7.06–7.02 (m, 2H, $H6_{Indole}$, $CONH_{2,B}$), 6.99–6.93 (m, 1H, $H5_{Indole}$), 5.07–4.97 (m, 2H, $CH_{2,Cbz}$), 4.48 (td, *J* = 8.0, 5.4 Hz, 1H, $H_{\alpha,Trp}$), 3.94 (td, *J* = 8.8, 5.0 Hz, 1H, $H_{\alpha,Lys}$), 3.85–3.39 (m, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.28 (br s, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 3.11 (m_{ABX}, *J* = 14.7, 5.4 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, *J* = 14.7, 8.0 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7

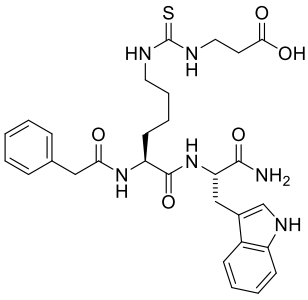
Hz, 2H, C$\underline{H}_2$CO$_2$H), 1.61–1.32 (m, 4H, H$_{\beta,Lys}$, H$_{\delta,Lys}$), 1.32–1.10 (m, 2H, H$_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO$_2$H), 173.2 (CO$_{\alpha,Trp}$), 171.6 (CO$_{\alpha,Lys}$), 156.0 (CO$_{Cbz}$), 137.0 (C1$_{Ar,Cbz}$), 136.0 (C7a$_{Indole}$), 128.3 (C3$_{Ar,Cbz}$, C5$_{Ar,Cbz}$), 127.8 (C4$_{Ar,Cbz}$), 127.7 (C2$_{Ar,Cbz}$, C6$_{Ar,Cbz}$), 127.4 (C3a$_{Indole}$), 123.5 (C2$_{Indole}$), 120.8 (C6$_{Indole}$), 118.5 (C4$_{Indole}$), 118.2 (C5$_{Indole}$), 111.2 (C7$_{Indole}$), 110.0 (C3$_{Indole}$), 65.5 (CH$_{2,Cbz}$), 54.9 (C$_{\alpha,Lys}$), 53.0 (C$_{\alpha,Trp}$), 39.1 (C$\underline{H}_2$CH$_2$CO$_2$H overlap with solvent peak), 33.7 (C$\underline{H}_2$CO$_2$H), 31.6 (C$_{\beta,Lys}$), 28.4 (C$_{\delta,Lys}$), 27.7 (C$_{\beta,Trp}$), 23.0 (C$_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.81 min, *m*/*z* 597.3 ([M+H]$^+$, $C_{29}H_{37}N_6O_6S^+$ Calcd 597.2); HRMS *m*/*z* 597.2499 ([M+H]$^+$, $C_{29}H_{37}N_6O_6S^+$ Calcd 597.2490).

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-benzamido-6-oxohexyl)thioureido)propanoic acid (31).



Starting from Fmoc-Lys(Teoc)-Trp-resin (147 mg, estimated loading: 0.40 mmol/g) synthesized by SPPS, the title compound was synthesized using the general acylation procedure with benzoic acid, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **31** (3 mg, 9% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, *J* = 2.4 Hz, 1H, NH$_{Indole}$), 8.41 (d, *J* = 7.7 Hz, 1H, NH$_{\alpha,Lys}$), 7.90 (d, *J* = 8.0 Hz, 1H, NH$_{\alpha,Trp}$), 7.88–7.82 (m, 2H, H2$_{Bz}$, H6$_{Bz}$), 7.57 (d, *J* = 7.9 Hz, 1H, H4$_{Indole}$), 7.56–7.52 (m, 1H, H4$_{Bz}$), 7.50–7.44 (m, 3H, H3$_{Bz}$, H5$_{Bz,}$ NH$_{\varepsilon\ Lys}$), 7.37–7.32 (m, 2H, N$\underline{H}$(CH$_2$)$_2$, CONH$_{2,A}$), 7.30 (d, *J* = 8.1 Hz, 1H, H7$_{Indole}$), 7.13 (d, *J* = 2.3, 1H, H2$_{Indole}$), 7.04 (ddd, *J* = 8.0, 5.4, 1.2 Hz, 2H, H6$_{Indole}$, CONH$_{2,B}$), 6.97–6.91 (m, 1H, H5$_{Indole}$), 4.48 (td, *J* = 8.0, 5.3 Hz, 1H, H$_{\alpha,Trp}$), 4.41–4.31 (m, 1H, H$_{\alpha,Lys}$), 3.55 (br s, 2H, C$\underline{H}_2$CH$_2$CO$_2$H overlap with residual water), 3.31 (br s, 2H, H$_{\varepsilon,Lys}$), 3.13 (m$_{ABX}$, *J* = 14.7, 5.3 Hz, 1H, H$_{\beta,Trp,A}$), 3.00 (m$_{ABX}$, *J* = 14.7, 8.1 Hz, 1H, H$_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7 Hz, 2H, C$\underline{H}_2$CO$_2$H), 1.77–1.60 (m, 2H, H$_{\beta,Lys}$), 1.48–1.38 (m, 2H, H$_{\delta,Lys}$), 1.37–1.27 (m, 2H, H$_{\gamma,Lys,A}$), 1.27–1.18 (m, 2H, H$_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO$_2$H), 173.2 (CO$_{\alpha,Trp}$), 171.6 (CO$_{\alpha,Lys}$), 166.7 (CO$_{Bz}$), 136.0 (C7a$_{Indole}$), 134.2 (C1$_{Ar,Bz}$), 131.3 (C4$_{Bz}$), 128.2 (C3$_{Bz}$, C5$_{Bz}$), 127.6 (C2$_{Bz}$, C6$_{Bz}$), 127.4 (C3a$_{Indole}$), 123.4 (C2$_{Indole}$), 120.8 (C6$_{Indole}$), 118.4 (C4$_{Indole}$), 118.2 (C5$_{Indole}$), 111.2 (C7$_{Indole}$), 110.0 (C3$_{Indole}$), 53.9 (C$_{\alpha,Lys}$), 53.1 (C$_{\alpha,Trp}$), 43.2 (C$_{\varepsilon,Lys}$), 39.1 (C$\underline{H}_2$CH$_2$CO$_2$H overlap with solvent peak), 33.7 (C$\underline{H}_2$CO$_2$H), 31.2 (C$_{\beta,Lys}$), 28.5 (C$_{\delta,Lys}$), 27.6 (C$_{\beta,Trp}$), 23.2 (C$_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.64 min, *m*/*z* 567.2 ([M+H]$^+$, $C_{28}H_{35}N_6O_5S^+$ Calcd 567.2); HRMS *m*/*z* 567.2395 ([M+H]$^+$, $C_{28}H_{35}N_6O_5S^+$ Calcd 567.2384). Bz=benzoyl

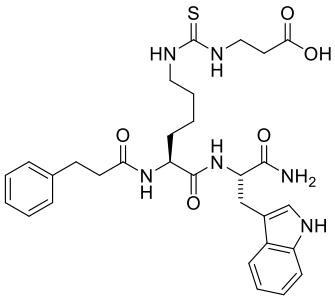
3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-(2-phenylacetamido)hexyl)thioureido)propanoic acid (32).



Starting from Fmoc-Lys(Teoc)-Trp-resin (158 mg, estimated loading: 0.40 mmol/g) synthesized by SPPS, the title compound was synthesized using the general acylation procedure with 2-phenylacetic acid, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **32** (3 mg, 9% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, *J* = 2.4 Hz, 1H, NH$_{Indole}$), 8.17 (d, *J* = 7.8 Hz, 1H, NH$_{\alpha,Lys}$), 7.86 (d, *J* = 8.0 Hz, 1H, NH$_{\alpha,Trp}$), 7.58 (d, *J* = 7.9 Hz, 1H, H4$_{Indole}$), 7.46 (br s, 1H, NH$_{\varepsilon,Lys}$), 7.38–7.17 (m, 8H, N$\underline{H}$(CH$_2$)$_2$CO$_2$H, CONH$_{2,A}$, H7$_{Indole}$, H$_{Ar,Ph}$), 7.10 (d, *J* = 2.3, 1H, H2$_{Indole}$), 7.07–7.02 (m, 2H, H6$_{Indole}$, CONH$_{2,B}$), 6.98–6.94 (m, 1H, H5$_{Indole}$), 4.45 (td, *J* = 8.0, 5.4 Hz, 1H, H$_{\alpha,Trp}$), 4.19 (td, *J* = 8.3, 5.2 Hz, 1H, H$_{\alpha,Lys}$), 3.56 (br s, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 3.52–3.38 (m, 2H, PhCH$_2$), 3.31 (br s, 2H, H$_{\varepsilon,Lys}$), 3.11 (m$_{ABX}$, *J* = 14.7, 5.4 Hz, 1H, H$_{\beta,Trp,A}$), 2.97 (m$_{ABX}$, *J* = 14.7, 8.0 Hz, 1H, H$_{\beta,Trp,B}$), 2.48 (t, *J* = 6.7 Hz, 2H, C$\underline{H}_2$CO$_2$H overlap with solvent peak), 1.64–1.54 (m, 1H, H$_{\beta,Lys,A}$), 1.52–1.33 (m, 3H, H$_{\beta,Lys,B}$, H$_{\delta,Lys}$),1.27–1.12 (m, 2H, H$_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO$_2$H), 173.2 (CO$_{\alpha,Trp}$), 171.4 (CO$_{\alpha,Lys}$), 170.3 (C$\underline{O}$CH$_2$Ph), 136.4 (C1$_{Ph}$), 136.0 (C7a$_{Indole}$), 129.0

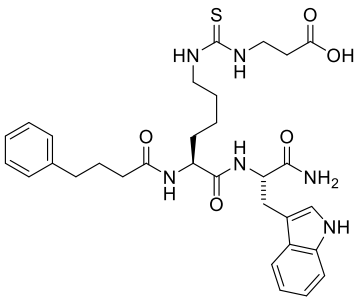
($C2_{Ph}$, $C6_{Ph}$), 128.2 ($C3_{Ph}$, $C5_{Ph}$), 127.4 ($C3a_{Indole}$), 126.3 ($C4_{Ph}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.0 ($C3_{Indole}$), 53.1 ($C_{\alpha,Trp}$), 52.8 ($C_{\alpha,Lys}$), 43.4 ($C_{\varepsilon,Lys}$), 42.05 ($PhCH_2$), 39.1 ($\underline{C}H_2CH_2CO_2H$), 33.7 ($\underline{C}H_2CO_2H$), 31.6 ($C_{\beta,Lys}$), 28.4 ($C_{\delta,Lys}$), 27.6 ($C_{\beta,Trp}$), 22.7 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.62 min, *m*/*z* 581.3 ($[M+H]^+$, $C_{29}H_{37}N_6O_5S^+$ Calcd 581.3); HRMS *m*/*z* 581.2550 $[M+H]^+$, $C_{29}H_{37}N_6O_5S^+$ Calcd 581.2541).

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-(3-phenylpropanamido)hexyl)thioureido)propanoic acid (33).



Starting from Fmoc-Lys(Teoc)-Trp-resin (153 mg, estimated loading: 0.40 mmol/g) synthesized by SPPS, the title compound was synthesized using the general acylation procedure with 2-phenylacetic acid, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **33** (3 mg, 8% based on resin loading), as a colorless fluffy material after lyophilization. ^1H NMR (600 MHz, DMSO-d_6) δ 10.81 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.96 (d, *J* = 7.7 Hz, $NH_{\alpha,Lys}$), 7.82 (d, *J* = 8.0 Hz, $NH_{\alpha,Trp}$), 7.57 (d, *J* = 7.8 Hz, 1H, $H4_{Indole}$), 7.49 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.42–7.33 (m, 2H, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H7_{Indole}$), 7.28–7.22 (m, 2H, $H3_{Ph}$, $H5_{Ph}$), 7.21–7.13 (m, 3H, $H2_{Ph}$, $H4_{Ph}$, $H6_{Ph}$), 7.11 (d, *J* = 2.3, 1H, $H2_{Indole}$), 7.08–7.02 (m, 2H, $H6_{Indole}$, $CONH_{2,B}$), 6.96 (ddd, *J* = 7.9, 7.0, 0.8 Hz, 1H, $H5_{Indole}$), 4.44 (td, *J* = 8.1, 5.2 Hz, 1H, $H_{\alpha,Trp}$), 4.17 (td, *J* = 8.3, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 3.31–3.17 (m, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.12 (m_{ABX}, *J* = 14.7, 5.2 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, *J* = 14.7, 8.2 Hz, 1H, $H_{\beta,Trp,B}$), 2.83–2.72 (m, 2H, $PhCH_2$), 2.49–2.33 (m, 4H, $C\underline{H}_2CO_2H$, $PhCH_2C\underline{H}_2$), 1.60–1.51 (m, 1H, $H_{\beta,Lys,A}$), 1.47–1.33 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.20–1.07 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4 (CO_2H), 173.3 ($CO_{\alpha,Trp}$), 171.7 ($CO_{\alpha,Lys}$), 171.5 ($\underline{C}O(CH_2)_2Ph$), 141.3 ($C1_{Ph}$), 136.0 ($C7a_{Indole}$), 128.24 ($C3_{Ph}$, $C5_{Ph}$), 128.18 ($C2_{Ph}$, $C6_{Ph}$), 127.4 ($C3a_{Indole}$), 125.8 ($C4_{Ph}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.0 ($C3_{Indole}$), 53.1 ($C_{\alpha,Trp}$), 52.8 ($C_{\alpha,Lys}$), 43.5 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 36.7 ($PhCH_2\underline{C}H_2$), 33.9 ($\underline{C}H_2CO_2H$), 31.5 ($C_{\beta,Lys}$), 31.1 ($Ph\underline{C}H_2$), 28.4 ($C_{\delta,Lys}$), 27.5 ($C_{\beta,Trp}$), 22.7 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.66 min, *m*/*z* 595.4 ($[M+H]^+$, $C_{30}H_{39}N_6O_5S^+$ Calcd 595.3); HRMS *m*/*z* 595.2707 ($[M+H]^+$, $C_{30}H_{39}N_6O_5S^+$ Calcd 595.2697).

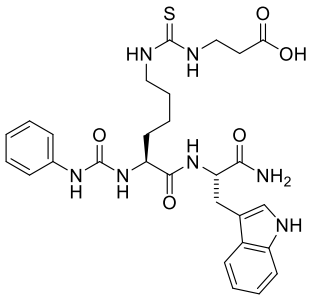
3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-(4-phenylbutanamido)hexyl)thioureido)propanoic acid (34).



Starting from Fmoc-Lys(Teoc)-Trp-resin (148 mg, estimated loading: 0.40 mmol/g) synthesized by SPPS, the title compound was synthesized using the general acylation procedure with 4-phenylbutanoic acid, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **34** (3 mg, 8% based on resin loading), as a colorless fluffy material after lyophilization. ^1H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.93 (d, *J* = 7.6 Hz, 1H, $NH_{\alpha,Lys}$), 7.75 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.56 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.47 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.38–7.23 (m, 5H, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$, $H7_{Indole}$, $H3_{Ph}$, $H5_{Ph}$), 7.19–7.14 (m, 3H, $H2_{Ph}$, $H4_{Ph}$, $H6_{Ph}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 7.07–7.01 (m, 2H, $H6_{Indole}$, $CONH_{2,B}$), 6.95 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 4.44 (td, *J* = 8.0, 5.4 Hz, 1H, $H_{\alpha,Trp}$), 4.15 (td, *J* = 8.3, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.55 (br s, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.30 (br s, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.11 (m_{ABX}, *J* = 14.7, 5.3 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, *J* = 14.7, 8.0 Hz, 1H, $H_{\beta,Trp,B}$), 2.53 (t, *J* = 7.7 Hz, 2H, $Ph(CH_2)_2C\underline{H}_2$), 2.47 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$), 2.18–2.07 (m, 2H, $PhC\underline{H}_2$), 1.82–1.70 (m, 2H, $PhCH_2C\underline{H}_2$), 1.62–1.53 (m, 1H, $H_{\beta,Lys,A}$), 1.51–1.36

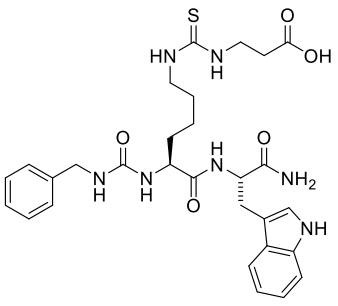
(m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.30–1.15 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO_2H), 173.2 ($CO_{\alpha,Trp}$), 172.3 ($\underline{C}O(CH_2)_3Ph$), 171.6 ($CO_{\alpha,Lys}$), 141.8 ($C1_{Ph}$), 136.0 ($C7a_{Indole}$), 128.4 ($C3_{Ph}$, $C5_{Ph}$), 128.3 ($C2_{Ph}$, $C6_{Ph}$), 127.4 ($C3a_{Indole}$), 125.7 ($C4_{Ph}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.0 ($C3_{Indole}$), 53.1 ($C_{\alpha,Trp}$), 52.9 ($C_{\alpha,Lys}$), 43.4 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 34.68 ($Ph(CH_2)_2\underline{C}H_2$), 34.65 ($Ph\underline{C}H_2$), 33.7 ($\underline{C}H_2CO_2H$), 31.4 ($C_{\beta,Lys}$), 28.5 ($C_{\delta,Lys}$), 27.5 ($C_{\beta,Trp}$), 27.0 ($PhCH_2\underline{C}H_2$), 22.9 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.75 min, *m*/*z* 609.5 ($[M+H]^+$, $C_{31}H_{41}N_6O_5S^+$ Calcd 609.3); HRMS *m*/*z* 609.2862 ($[M+H]^+$,$C_{31}H_{41}N_6O_5S^+$ Calcd 609.2854).

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-(3-phenylureido)hexyl)thioureido)propanoic acid (35).



Starting from Fmoc-Lys(Teoc)-Trp-resin (201 mg, estimated loading: 0.29 mmol/g) synthesized by SPPS, the title compound was synthesized using the general urea formation procedure with aniline, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **35** (2 mg, 6% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ12.23 (br s, 1H, CO_2H), 10.77 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.11 (d, *J* = 8.1 Hz, 1H, NHPh), 8.11 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.60 (d, *J* = 7.7 Hz, 1H, $H4_{Indole}$), 7.48 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.40 (s, 1H, $CONH_{2,A}$), 7.37–7.32 (m, 3H, $H2_{Ph}$, $H6_{Ph}$, $N\underline{H}(CH_2)_2CO_2H$), 7.32–7.28 (m, 1H, $H7_{Indole}$), 7.25–7.18 (m, 2H, $H3_{Ph}$, $H5_{Ph}$,), 7.12 (d, *J* = 2.3, 1H, $H2_{Indole}$), 7.07–7.00 (m, 2H, $H6_{Indole}$, $CONH_{2,B}$), 6.96 (ddd, *J* = 7.9, 6.9, 1.1 Hz, 1H, $H5_{Indole}$), 6.89 (tt, *J* = 7.3, 1.2 Hz, 1H, $H4_{Ph}$), 6.33 (d, *J* = 7.7 Hz, 1H, $NH_{\alpha,Lys}$), 4.50 (td, *J* = 8.4, 5.3 Hz, 1H, $H_{\alpha,Trp}$), 4.17 (td, *J* = 7.6, 5.2 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.30 (br s, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 3.14 (m_{ABX}, *J* = 14.7, 5.2 Hz, 1H, $H_{\beta,Trp,A}$), 2.98 (m_{ABX}, *J* = 14.7, 8.6 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$, overlap with solvent peak), 1.66–1.52 (m, 1H, $H_{\beta,Lys,A}$), 1.51–1.38 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.28–1.20 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d6) δ 173.4 (CO_2H), 173.2 ($CO_{\alpha,Trp}$), 171.9 ($CO_{\alpha,Lys}$), 157.7 (NHCONH), 140.3 ($C1_{Phenyl}$), 136.0 ($C7a_{Indole}$), 128.7 ($C3_{Ph}$, $C5_{Ph}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 121.1 ($C4_{Ar,Ph}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 117.5 ($C2_{Ph}$, $C6_{Ph}$), 111.2 ($C7_{Indole}$), 110.2 ($C3_{Indole}$), 53.2 ($C_{\alpha,Lys}$), 52.8 ($C_{\alpha,Trp}$), 43.4 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.9 ($C_{\beta,Lys}$), 28.6 ($C_{\delta,Lys}$), 27.6 ($C_{\beta,Trp}$), 22.5 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.76 min, *m*/*z* 582.3 ($[M+H]^+$, $C_{28}H_{36}N_7O_5S^+$ Calcd 582.2); HRMS *m*/*z* 582.2502 ($[M+H]^+$, $C_{28}H_{36}N_7O_5S^+$ Calcd 582.2493).

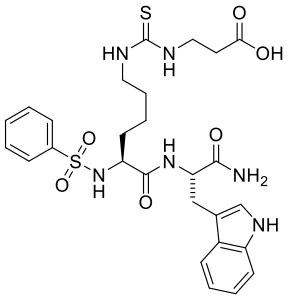
(*S*)-5-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3-oxo-1-phenyl-11-thioxo-2,4,10,12-tetraazapentadecan-15-oic acid (36).



Starting from Fmoc-Lys(Teoc)-Trp-resin (155 mg, estimated loading: 0.40 mmol/g) synthesized by SPPS, the title compound was synthesized using the general urea formation procedure with benzylamine, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **36** (3 mg, 8% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 7.90 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.59 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.47 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.42–7.15 (m, 8H, $CONH_{2,A}$, $N\underline{H}(CH_2)_2CO_2H$, H_{Ph}, $H7_{Indole}$), 7.11 (d, *J* = 2.3, 1H, $H2_{Indole}$), 7.06–7.01 (m, 2H, $H6_{Indole}$, $CONH_{2,B}$), 6.96 (td, *J* = 7.3, 1.0, 1H, $H5_{Indole}$), 6.49 (t, *J* = 5.9 Hz, 1H, $N\underline{H}CH_2Ph$), 6.15 (d, *J* = 7.6 Hz, 1H, $NH_{\alpha,Lys}$) 4.47 (td, *J* = 8.1, 5.4 Hz, 1H, $H_{\alpha,Trp}$), 4.25–4.13 (m, 2H, CH_2Ph, overlap with residual water), 4.07 (td, *J* = 7.6, 5.1 Hz, 1H, $H_{\alpha,Lys}$, overlap with residual water), 3.51 (br s, 2H, $C\underline{H}_2CH_2CO_2H$), 3.28 (br s, 2H, $H_{\varepsilon,Lys}$), 3.13 (m_{ABX}, *J* = 14.6,

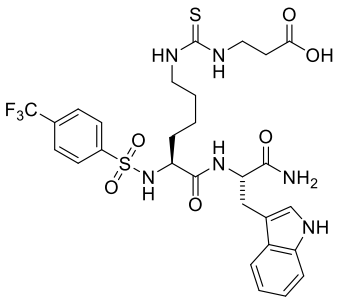
5.3 Hz, 1H, $H_{\beta,Trp,A}$), 2.99 (m_{ABX}, J = 14.7, 8.1 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, J = 6.6 Hz, 2H, $C\underline{H}_2CO_2H$), 1.61–1.50 (m, 1H, $H_{\beta,Lys,A}$), 1.48–1.35 (m, 3H, $H_{\delta,Lys}$, $H_{\beta,Lys,B}$), 1.28–1.13 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO_2H), 173.2 ($CO_{\alpha,Trp}$), 172.3 ($CO_{\alpha,Lys}$), 157.7 (NHCONH), 140.6 ($C1_{Ph}$), 136.0 ($C7a_{Indole}$), 128.2 ($C3_{Ph}$, $C5_{Ph}$), 127.4 ($C3a_{Indole}$), 126.9 ($C2_{Ph}$, $C6_{Ph}$), 126.5 ($C4_{Ph}$), 123.4 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 110.1 ($C3_{Indole}$), 53.3 ($C_{\alpha,Lys}$), 53.0 ($C_{\alpha,Trp}$), 43.4 ($C_{\varepsilon,Lys}$), 42.8 ($\underline{C}H_2Ph$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.7 ($C_{\beta,Lys}$), 28.5 ($C_{\delta,Lys}$), 27.7 ($C_{\beta,Trp}$), 22.6 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.59 min, m/z 596.4 ($[M+H]^+$, $C_{29}H_{38}N_7O_5S^+$ Calcd 596.3); HRMS m/z 596.2659 ($[M+H]^+$, $C_{29}H_{38}N_7O_5S^+$ Calcd 596.2650).

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-(phenylsulfonamido)hexyl)thioureido)propanoic acid (37).



Starting from Fmoc-Lys(Teoc)-Trp-resin (201 mg, estimated loading: 0.27 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure with benzenesulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **37** (4 mg, 12% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.23 (br s, 1H, CO_2H), 10.81 (d, J = 2.3 Hz, 1H, NH_{Indole}), 7.98 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Trp}$), 7.90 (d, J = 8.2 Hz, 1H, $NH_{\alpha,Lys}$), 7.66 (dd, J = 7.4, 1.0 Hz, 2H, $H2_{Ph}$, $H6_{Ph}$), 7.56 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.47 (t, J = 7.4 Hz, 1H, $H4_{Ph}$), 7.40 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.37–7.29 (m, 5H, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$, $H7_{Indole}$, $H3_{Ph}$, $H5_{Ph}$), 7.09–7.04 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 7.02–6.95 (m, 2H, $H5_{Indole}$, $CONH_{2,B}$), 4.27 (td, J = 7.7, 5.7 Hz, 1H, $H_{\alpha,Trp}$), 3.66 (td, J = 8.4, 5.3 Hz, 2H, $H_{\alpha,Lys}$, overlap with residual water), 3.60–3.37 (m, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 3.18 (br s, 2H, $H_{\varepsilon,Lys}$), 2.99 (m_{ABX}, J = 14.6, 5.8 Hz, 1H, $H_{\beta,Trp,A}$), 2.83 (m_{ABX}, J = 14.6, 7.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, J = 6.6 Hz, 2H, $C\underline{H}_2CO_2H$, overlap with solvent peak), 1.48–1.39 (m, 1H $H_{\beta,Lys,A}$), 1.38–1.20 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.18–1.07 (m, 1H, $H_{\gamma,Lys,A}$), 1.06–0.94 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d6) δ 173.2 (CO_2H), 173.1 ($CO_{\alpha,Trp}$), 170.4 ($CO_{\alpha,Lys}$), 140.7 ($C1_{Ph}$), 136.0 ($C7a_{Indole}$), 132.2 ($C4_{Ph}$), 128.8 ($C3_{Ph}$, $C5_{Ph}$), 127.4 ($C3a_{Indole}$), 126.4 ($C2_{Ph}$, $C6_{Ph}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 56.3 ($C_{\alpha,Lys}$), 53.0 ($C_{\alpha,Trp}$), 43.4 ($H_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.4 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 27.6 ($C_{\beta,Trp}$), 22.4 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.57 min, m/z 603.2 ($[M+H]^+$, $C_{xx}H_{xx}N_xO_x^+$ Calcd 603.2); HRMS m/z 603.2063 ($[M+H]^+$, $C_{27}H_{35}N_6O_6S_2^+$ Calcd 603.2054).

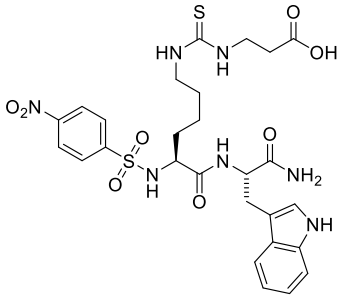
3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-((4-(trifluoromethyl)phenyl)sulfonamido)hexyl)thioureido)propanoic acid (38).



Starting from Fmoc-Lys(Teoc)-Trp-resin (201 mg, estimated loading: 0.48 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure with 4-(trifluoromethyl)benzenesulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **38** (1 mg, 2% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.24 (br s, 1H, CO_2H), 10.87 (s, 1H, NH_{Indole}), 8.25–8.04 (m, 2H, $NH_{\alpha,Trp}$, $NH_{\alpha,Trp}$), 7.70–7.58 (m, 3H, $H2_{Tfm}$, $H6_{Tfm}$, $H4_{Indole}$), 7.52–7.29 (m, 6H, $H3_{Tfm}$, $H5_{Tfm}$, $NH_{\varepsilon,Lys}$, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$, $H7_{Indole}$), 7.15 (s, 1H, $H2_{Indole}$), 7.07 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H, $H6_{Indole}$), 7.02–6.94 (m, 2H, $H5_{Indole}$, $CONH_{2,B}$), 4.25–4.12 (m, 1H, $H_{\alpha,Trp}$), 3.85–3.80 (m, 1H, $H_{\alpha,Lys}$), 3.53 (br s, 2H, $C\underline{H}_2CH_2CO_2H$), 3.40–3.12 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 2.98 (m_{ABX}, J = 14.5, 5.4 Hz, 1H, $H_{\beta,Trp}$), 2.80 (m_{ABX}, J = 14.5, 8.5 Hz, 1H, $H_{\beta,Trp}$), 2.57–2.41 (m, 2H, $C\underline{H}_2CO_2H$

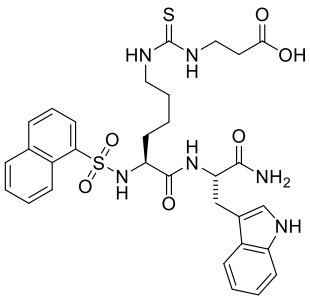
overlap with solvent peak), 1.56–1.27 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.26–1.21 (m, 1H, $H_{\gamma,Lys,A}$), 1.18–1.07 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO_2H), 173.2 ($CO_{\alpha,Trp}$), 170.1 ($CO_{\alpha,Lys}$), 144.9 ($C1_{Tfm}$), 136.0 ($C7a_{Indole}$), 131.8 (d, J = 30.8 Hz, $C4_{Tfm}$), 127.2 ($C3a_{Indole}$, $C2_{Tfm}$, $C6_{Tfm}$), 125.7 ($C3_{Tfm}$, $C5_{Tfm}$), 123.8 ($C2_{Indole}$), 123.4 (q, J = 274 Hz, CF_3), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 56.4 ($C_{\alpha,Lys}$), 53.1 ($C_{\alpha,Trp}$), 43.2 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.5 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 27.6 ($C_{\beta,Trp}$), 22.6 ($C_{\gamma,Lys}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −61.4 (s, $CF_{3,Tfm}$), −73.4 (s, $CF_{3,TFA}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.94 min, m/z 671.3 ($[M+H]^+$, $C_{28}H_{34}N_6O_6S_2F_3^+$ Calcd 671.2); HRMS m/z 671.1935 ($[M+H]^+$,$C_{28}H_{34}N_6O_6S_2F_3^+$ Calcd 671.1928). Tfm= 4-(trifluoromethyl)phen-1-yl

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((4-nitrophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoic acid (39).



Starting from Fmoc-Lys(Teoc)-Trp-resin (206 mg, estimated loading: 0.48 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure 4-nitrobenzenesulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **39** (2 mg, 3% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.24 (br s, 1H, CO_2H), 10.86 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.32 (d, J = 8.8 Hz, 1H, $NH_{\alpha,Lys}$), 8.11 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Trp}$), 7.98–7.92 (m, 2H, $H3_{Np}$, $H5_{Np}$), 7.75–7.69 (m, 2H, $H2_{Np}$, $H6_{Np}$), 7.57 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.44 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.39–7.31 (m, 3H, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$, $H7_{Indole}$), 7.12 (d, J = 2.4 Hz, 1H, $H2_{Indole}$), 7.08 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H6_{Indole}$), 6.99 (t, J = 14.9 Hz, 1H, $H5_{Indole}$), 6.95 (s, 1H, $CONH_{2,B}$), 4.15 (td, J = 8.2, 5.7 Hz, 1H, $H_{\alpha,Trp}$), 3.83 (td, J = 8.8, 5.3 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$), 3.25 (br s, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 2.97 (m_{ABX}, J = 14.5, 5.6 Hz, 1H, $H_{\beta,Trp,A}$), 2.76 (m_{ABX}, J = 14.5, 8.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.49–2.44 (m, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 1.57–1.27 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.26–1.19 (m, 1H, $H_{\gamma,Lys,A}$), 1.19–1.08 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H), 173.1 ($CO_{\alpha,Trp}$), 169.9 ($CO_{\alpha,Lys}$), 149.1 ($C4_{Np}$), 146.6 ($C1_{Np}$), 136.0 ($C7a_{Indole}$), 127.9 ($C2_{Np}$, $C6_{Np}$), 127.2 ($C3a_{Indole}$), 123.9 ($C3_{Np}$, $C5_{Np}$), 123.7 ($C2_{Indole}$), 120.9 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 56.4 ($C_{\alpha,Lys}$), 53.1 ($C_{\alpha,Trp}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.4 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 27.6 ($C_{\beta,Trp}$), 22.4 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.77 min, m/z 648.3 ($[M+H]^+$, $C_{27}H_{34}N_7O_8S_2^+$ Calcd 648.2); HRMS m/z 648.1914 ($[M+H]^+$,$C_{27}H_{34}N_7O_8S_2^+$ Calcd 648.1905). Np=4-nitrophen-1-yl

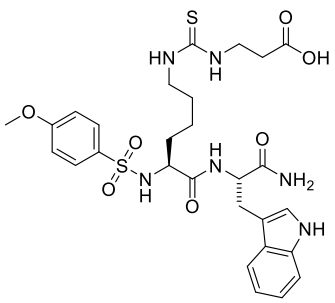
3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-(naphthalene-1-sulfonamido)-6-oxohexyl)thioureido)propanoic acid (40).



Starting from Fmoc-Lys(Teoc)-Trp-resin (204 mg, estimated loading: 0.48 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure naphthalene-1-sulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **40** (1 mg, 2% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.24 (br s, 1H, CO_2H), 10.80 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.67 (dd, J = 8.5, 1.1 Hz, 1H, $H8_{Naph}$), 8.26 (d, J = 8.5 Hz, 1H, $NH_{\alpha,Lys}$), 8.15–8.08 (m, 1H, $H4_{Naph}$), 8.05 (dd, J = 7.3, 1.2 Hz, 1H, $H2_{Naph}$), 8.03–7.98 (m, 1H, $H5_{Naph}$), 7.95 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Trp}$), 7.66 (ddd, J = 8.5, 6.8, 1.4 Hz, 1H, $H7_{Naph}$), 7.61 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Naph}$), 7.51–7.43 (m, 2H, $H4_{Indole}$, $H3_{Naph}$), 7.34–7.31 (m, 1H, $H7_{Indole}$) 7.30–7.23 (m, 3H, $N\underline{H}(CH_2)_2CO_2H$, $CONH_{2,A}$, $NH_{\varepsilon,Lys}$), 7.09–7.03 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 6.99–6.94 (m, 2H, $H5_{Indole}$, $CONH_{2,B}$), 4.26 (td, J = 7.5, 6.3 Hz, 1H, $H_{\alpha,Trp}$), 3.65 (td, J = 8.7, 5.3 Hz, 2H, $H_{\alpha,Lys}$, overlap

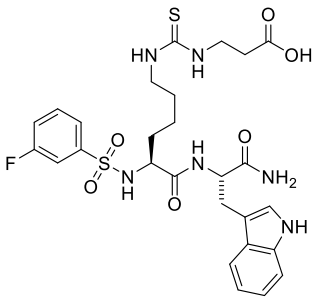
with residual water), 3.61–3.31 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H, overlap with residual water), 3.00–2.83 (m, 3H, H$_{\beta,Trp,A}$, H$_{\varepsilon,Lys}$), 2.73 (m$_{ABX}$, *J* = 14.6, 7.3 Hz, 1H, H$_{\beta,Trp,B}$), 2.46 (t, *J* = 6.7 Hz, 2H, C$\underline{H}_2$CO$_2$H), 1.41–1.27 (m, 2H, H$_{\beta,Lys}$), 1.14–1.06 (m, 1H, H$_{\delta,Lys,A}$), 1.03–0.91 (m, 2H, H$_{\gamma,Lys,A}$, H$_{\delta,Lys,B}$), 0.82–0.73 (m, 1H, H$_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-*d*$_6$) δ 173.2 (CO$_2$H), 173.0 (CO$_{\alpha,Trp}$), 170.6 (CO$_{\alpha,Lys}$), 136.0 (C7a$_{Indole}$), 135.6 (C1$_{Naph}$), 133.7 (C4a$_{Naph}$), 133.7 (C4$_{Naph}$), 128.7 (C5$_{Naph}$), 128.5 (C2$_{Naph}$), 127.61 (C8a$_{Naph}$), 127.56 (C7$_{Naph}$), 127.4 (C3a$_{Indole}$), 126.7 (C6$_{Naph}$), 125.0 (C8$_{Naph}$), 124.2 (C3$_{Naph}$), 123.4 (C2$_{Indole}$), 120.8 (C6$_{Indole}$), 118.5 (C4$_{Indole}$), 118.1 (C5$_{Indole}$), 111.1 (C7$_{Indole}$), 109.8 (C3$_{Indole}$), 56.1 (C$_{\alpha,Lys}$), 52.9 (C$_{\alpha,Trp}$), 43.1 (C$_{\varepsilon,Lys}$), 39.1 (C$\underline{H}_2$CH$_2$CO$_2$H overlap with solvent peak), 33.6 (C$\underline{H}_2$CO$_2$H), 32.2 (C$_{\beta,Lys}$), 28.7 (C$_{\delta,Lys}$), 27.5 (C$_{\beta,Trp}$), 22.2 (C$_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.82 min, *m*/*z* 653.3 ([M+H]$^+$, C$_{31}$H$_{37}$N$_6$O$_6$S$^+$ Calcd 653.2); HRMS *m*/*z* 653.2221 ([M+H]$^+$,C$_{31}$H$_{37}$N$_6$O$_6$S$^+$ Calcd 653.2211).

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((4-methoxyphenyl)sulfonamido)-6-oxohexyl)thioureido)propanoic acid (41).



Starting from Fmoc-Lys(Teoc)-Trp-resin (192 mg, estimated loading: 0.43 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure 4-methoxybenzenesulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **41** (3 mg, 6% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 12.25 (br s, 1H, CO$_2$H), 10.83 (d, *J* = 2.4 Hz, 1H, NH$_{Indole}$), 7.98 (d, *J* = 7.9 Hz, 1H, NH$_{\alpha,Trp}$), 7.72 (d, *J* = 8.2 Hz, 1H, NH$_{\alpha,Lys}$), 7.59–7.53 (m, 3H, H4$_{Indole}$, H2$_{MPh,}$ H6$_{MPh}$), 7.41 (br s, 1H, NH$_{\varepsilon,Lys}$), 7.37–7.28 (m, 3H, N$\underline{H}$(CH$_2$)$_2$CO$_2$H, CONH$_{2,A}$, H7$_{Indole}$), 7.12 (d, *J* = 2.4 Hz, 1H, H2$_{Indole}$), 7.06 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 2H, H6$_{Indole}$), 7.0 (s, 1H, CONH$_{2,B}$), 6.98 (t, *J* = 7.5 Hz, 1H, H5$_{Indole}$), 6.84–6.77 (m, 2H, H3$_{MPh,}$ H5$_{MPh}$), 4.26 (td, *J* = 7.8, 5.8 Hz, 1H, H$_{\alpha,Trp}$), 3.69 (s, 3H, OCH$_3$), 3.61 (td, *J* = 8.4, 5.4 Hz, 2H, H$_{\alpha,Lys}$), 3.59–3.50 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 3.18 (br s, 2H, H$_{\varepsilon,Lys}$), 3.01 (m$_{ABX}$, *J* = 14.6, 5.7 Hz, 1H, H$_{\beta,Trp,A}$), 2.84 (m$_{ABX}$, *J* = 14.6, 7.8 Hz, 1H, H$_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7 Hz, 2H, C$\underline{H}_2$CO$_2$H overlap with solvent peak), 1.49–1.37 (m, 1H, H$_{\beta,Lys,A}$), 1.36–1.19 (m, 3H, H$_{\beta,Lys,B}$, H$_{\delta,Lys}$), 1.18–1.08 (m, 1H, H$_{\gamma,Lys,A}$), 1.06–0.95 (m, 1H, H$_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-*d*$_6$) δ 173.2 (CO$_2$H), 173.1 (CO$_{\alpha,Trp}$), 170.5 (CO$_{\alpha,Lys}$), 161.9 (C4$_{MPh}$), 136.0 (C7a$_{Indole}$), 132.4 (C1$_{MPh}$), 128.7 (C2$_{MPh,}$ C6$_{MPh}$), 127.4 (C3a$_{Indole}$), 123.6 (C2$_{Indole}$), 120.8 (C6$_{Indole}$), 118.5 (C4$_{Indole}$), 118.2 (C5$_{Indole}$), 113.6 (C3$_{MPh,}$ C5$_{MPh}$), 111.2 (C7$_{Indole}$), 109.9 (C3$_{Indole}$), 56.4 (C$_{\alpha,Lys}$), 55.5 (OCH$_3$), 53.1 (C$_{\alpha,Trp}$), 43.4 (C$_{\varepsilon,Lys}$), 39.1 (C$\underline{H}_2$CH$_2$CO$_2$H overlap with solvent peak), 33.7 (C$\underline{H}_2$CO$_2$H), 32.4 (C$_{\beta,Lys}$), 28.2 (C$_{\delta,Lys}$), 27.6 (C$_{\beta,Trp}$), 22.4 (C$_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.67 min, *m*/*z* 633.3 ([M+H]$^+$, C$_{28}$H$_{37}$N$_6$O$_7$S$_2^+$ Calcd 633.2); HRMS *m*/*z* 633.2168 ([M+H]$^+$,C$_{28}$H$_{37}$N$_6$O$_7$S$_2^+$ Calcd 633.2160). MPh=4-methoxyphen-1-yl

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoic acid (42).

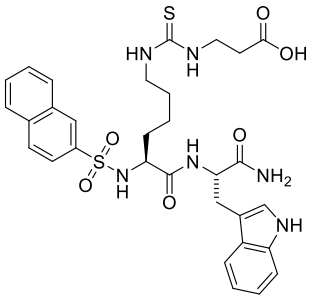


Starting from Fmoc-Lys(Teoc)-Trp-resin (201 mg, estimated loading: 0.48 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure 3-fluorobenzenesulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **42** (1 mg, 2% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 12.24 (s, 1H, CO$_2$H), 10.81 (d, *J* = 2.4 Hz, 1H, NH$_{Indole}$), 8.09 (d, *J* = 8.3 Hz, 1H, NH$_{\alpha,Lys}$), 8.04 (d, *J* = 8.2 Hz, 1H, NH$_{\alpha,Trp}$), 7.57–7.49 (m, 2H, H4$_{Indole}$, H2$_{FPh}$), 7.48–7.38 (m, 2H, H6$_{FPh}$, NH$_{\varepsilon,Lys}$), 7.37–7.28 (m, 5H, N$\underline{H}$(CH$_2$)$_2$CO$_2$H, CONH$_2$, H7$_{Indole}$, H4$_{FPh,}$ H5$_{FPh}$), 7.10–7.04 (m, 2H, H2$_{Indole}$,

$H6_{Indole}$), 7.02–6.95 (m, 2H, $H5_{Indole}$, $CONH_2$), 4.30–4.23 (m, 1H, $H_{\alpha,Trp}$), 3.76 (td, *J* = 8.6, 5.4 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.21 (br s, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 2.96 (m_{ABX}, *J* = 14.6, 6.3 Hz, 1H, $H_{\beta,Trp,A}$), 2.77 (m_{ABX}, *J* = 14.6, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.6 Hz, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 1.53–1.40 (m, 1H, $H_{\beta,Lys,A}$), 1.40–1.29 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.22–1.13 (m, 1H, $H_{\gamma,Lys,A}$), 1.12–1.00 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H), 172.9 ($CO_{\alpha,Trp}$), 170.5 ($CO_{\alpha,Lys}$), 162.28 (d, *J* = 244.3 Hz, $C3_{FPh}$), 143.02 (d, *J* = 6.3 Hz, $C1_{FPh}$), 136.0 ($C7a_{Indole}$), 131.1 (d, *J* = 8.1 Hz, $C5_{FPh}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.6 (d, *J* = 3.3 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.2 (d, *J* = 21.1 Hz, $C4_{FPh}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 113.7 (d, *J* = 24.2 Hz, $C2_{FPh}$), 111.2 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 56.2 ($C_{\alpha,Lys}$), 53.0 ($C_{\alpha,Trp}$), 43.2 ($C_{\varepsilon,Lys}$), 39.1 ($C\underline{H}_2CH_2CO_2H$), 33.7 ($C\underline{H}_2CO_2H$), 32.5 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 27.6($C_{\beta,Trp}$), 22.4 ($C_{\gamma,Lys}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −74.5 (s, $CF_{3,TFA}$), −111.0 (s, 3_{FPh}-F). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei.

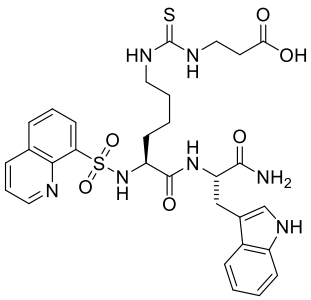
UPLC-MS t_R 1.70 min, *m*/*z* 621.2 ($[M+H]^+$, $C_{27}H_{34}N_6O_6S_2F^+$ Calcd 621.2); HRMS *m*/*z* 621.1968 ($[M+H]^+$,$C_{27}H_{34}N_6O_6S_2F^+$ Calcd 621.1960). FPh=3-fluorophen-1-yl

3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-(naphthalene-2-sulfonamido)-6-oxohexyl)thioureido)propanoic acid (43).



Starting from Fmoc-Lys(Teoc)-Trp-resin (214 mg, estimated loading: 0.29 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure naphthalene-2-sulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **43** (2 mg, 5% based on resin loading), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (br s, 1H, CO_2H), 10.81 (d, *J* = 2.3 Hz, 1H, NH_{Indole}), 8.34 (d, *J* = 1.8 Hz, 1H, $H1_{Naph}$), 8.08–7.97 (m, 3H, $NH_{\alpha,Trp}$, $H4_{Naph}$, $NH_{\alpha,\ Lys}$), 7.86 (d, *J* = 8.1 Hz, 1H, $H5_{Naph}$), 7.67 (d, J = 8.7 Hz, 1H, $H8_{Naph}$), 7.65–7.55 (m, 3H, $H3_{Naph}$, $H7_{Naph}$, $H6_{Naph}$), 7.47 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.43–7.24 (m, 4H, $N\underline{H}(CH_2)_2CO_2H$, $NH_{\varepsilon,Lys}$, $CONH_{2,A}$, $H7_{Indole}$), 7.12–7.04 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 7.01–6.95 (m, 2H, $H5_{Indole}$, $CONH_{2,B}$), 4.21 (td, *J* = 7.8, 6.1 Hz, 1H, $H_{\alpha,Trp}$ overlap with residual water), 3.75 (td, *J* = 8.5, 5.3 Hz, 2H, $H_{\alpha,Lys}$ overlap with residual water), 3.55 (br s, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 3.14 (br s, 2H, $H_{\varepsilon,Lys}$), 2.90 (m_{ABX}, *J* = 14.6, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.72 (m_{ABX}, *J* = 13.6, 7.7 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$, overlap with solvent peak), 1.49–1.40 (m, 1H, $H_{\beta,Lys,A}$), 1.41–1.32 (m, 1H, $H_{\beta,Lys,B}$), 1.30–1.23 (m, 2H, $H_{\delta,Lys}$), 1.21–1.10 (m, 1H, $H_{\gamma,Lys,A}$), 1.07–0.96 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H), 173.1 ($CO_{\alpha,Trp}$), 170.5 ($CO_{\alpha,Lys}$), 137.9 ($C4a_{Naph}$), 136.0 ($C7a_{Indole}$), 134.0 ($C2_{Naph}$), 131.5 ($C8a_{Naph}$), 129.1 ($C4_{Naph}$), 128.9 ($C8_{Naph}$), 128.5 ($C5_{Naph}$), 127.7 ($C6_{Naph}$), 127.4 ($C7_{Naph}$), 127.3 ($C3a_{Indole}$), 127.1 ($C1_{Naph}$), 123.5 ($C2_{Indole}$), 122.3 ($C3_{Naph}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.2 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.9 ($C3_{Indole}$), 56.4 ($C_{\alpha,Lys}$), 53.0 ($C_{\alpha,Trp}$), 43.3 ($C_{\varepsilon,Lys}$ from HSQC), 39.1 ($C\underline{H}_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($C\underline{H}_2CO_2H$), 32.6 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 27.6($C_{\beta,Trp}$), 22.6 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.85 min, *m*/*z* 653.3 ($[M+H]^+$, $C_{31}H_{37}N_6O_6S_2^+$ Calcd 653.2); HRMS *m*/*z* 653.2220 ($[M+H]^+$,$C_{31}H_{37}N_6O_6S_2^+$ Calcd 653.2211).

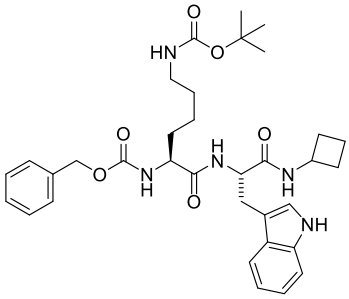
3-(3-((*S*)-6-(((*S*)-1-Amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxo-5-(quinoline-8-sulfonamido)hexyl)thioureido)propanoic acid (44).



Starting from Fmoc-Lys(Teoc)-Trp-resin (207 mg, estimated loading: 0.43 mmol/g) synthesized by SPPS, the title compound was synthesized using the general sulfonamide formation procedure quinoline-8-sulfonyl chloride, followed by Teoc deprotection, and thiourea formation as described in the general procedures. Global deprotection and cleavage from resin, followed by preparative reversed-phase HPLC purification afforded the desired carboxylic acid **44** (1 mg, 2% based on resin loading), as a colorless fluffy material after

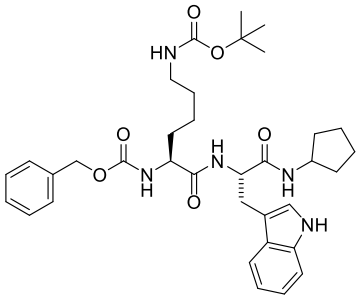
lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.76 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.93 (dd, J = 4.2, 1.7 Hz, 1H, $H2_{Qui}$), 8.42 (dd, J = 8.4, 1.7 Hz, 1H, $H4_{Qui}$), 8.20–8.15 (m, 2H, $H7_{Qui}$, $H5_{Qui}$), 7.95 (d, J = 7.9 Hz, 1H, $NH_{\alpha,Trp}$), 7.58 (t, J = 7.7 Hz, 1H, $H6_{Qui}$), 7.54 (dd, J = 8.3, 4.2 Hz, 1H, $H3_{Qui}$), 7.46–7.28 (m, 5H, $NH_{\alpha,Lys}$, $NH_{\varepsilon,Lys}$, $N\underline{H}(CH_2)_2CO_2H$, $H4_{Indole}$ $H7_{Indole}$), 7.23 (s, 1H, $CONH_{2,A}$), 7.07 (t, J = 7.5 Hz, 1H, $H6_{Indole}$), 6.96 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.93 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 6.90 (s, 1H, $CONH_{2,B}$), 4.16–4.10 (m, 1H, $H_{\alpha,Trp}$), 3.99–3.87 (m, 1H, $H_{\alpha,Lys}$ overlap with residual water), 3.90–3.30 (m, 2H, $C\underline{H}_2CH_2CO_2H$ overlap with residual water), 3.12 (br s, 2H, $H_{\varepsilon,Lys}$), 2.79 (m_{ABX}, J = 14.6, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.55 (m_{ABX}, J = 14.6, 7.6 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, J = 6.6 Hz, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 1.48–1.32 (m, 2H, $H_{\beta,Lys}$), 1.32–1.14 (m, 2H, $H_{\delta,Lys}$), 1.14–0.91 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H), 172.8 ($CO_{\alpha,Trp}$), 170.2 ($CO_{\alpha,Lys}$), 151.0 ($C2_{Qui}$), 142.6 ($C8a_{Qui}$), 136.8 ($C4_{Qui}$), 136.7 ($C8_{Qui}$), 136.0 ($C7a_{Indole}$), 133.5 ($C5_{Qui}$), 129.9 ($C7_{Qui}$), 128.3 ($C4a_{Qui}$), 127.3 ($C3a_{Indole}$), 125.4 ($C6_{Qui}$), 123.4 ($C2_{Indole}$), 122.3 ($C3_{Qui}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 56.7 ($C_{\alpha,Lys}$), 52.9 ($C_{\alpha,Trp}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.9 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 27.5 ($C_{\beta,Trp}$), 22.1 ($C_{\gamma,Lys}$). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.69 min, m/z 654.3 ($[M+H]^+$, $C_{30}H_{36}N_7O_6S_2^+$ Calcd 654.2); HRMS m/z 654.2173 ($[M+H]^+$,$C_{30}H_{36}N_7O_6S_2^+$ Calcd 654.2163). Qui=quinolin-8-yl

Benzyl *tert*-butyl ((*S*)-6-(((*S*)-1-(cyclobutylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S75).



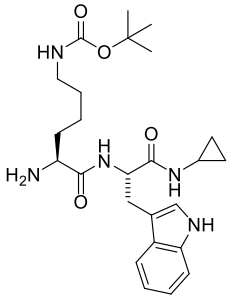
Cbz-Lys(Boc)-OH (306 mg, 0.82 mmol), TFA salt **S35** (447 mg, 1.20 mmol), HOBt (161 mg, 1.19 mmol), and *i*Pr_2NEt (280 µL, 1.62 mmol) were dissolved in anhydrous CH_2Cl_2 (6.0 mL) and cooled to 0°C. EDC (256 mg, 1.33 mmol) was added and the reaction mixture was stirred for 15 minutes at 0°C. The reaction mixture was stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (100 mL) and washed with aq. $KHSO_4$ (5%, 3×80 mL), saturated aq. $NaHCO_3$ (3×80 mL), and brine (2×80 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, affording the desired amide **S75** (652 mg, >99%) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.84 (d, J = 2.6 Hz, 1H, NH_{Indole}), 8.09 (d, J = 4.3 Hz, 1H, $CO_{Trp}NH$), 7.89 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.53 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.42 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Lys}$), 7.39–7.27 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.08 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.03 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.98–6.90 (m, 1H, $H5_{Indole}$), 6.74 (t, J = 5.8 Hz, 1H, $NH_{\varepsilon,Lys}$), 5.09–4.97 (m, 2H, $CH_{2,Cbz}$), 4.42 (td, J = 7.9, 5.9 Hz, 1H, $H_{\alpha,Trp}$), 4.19–4.06 (m, 1H, $H1_{Cyclobutyl}$), 3.91 (td, J = 8.3, 4.9 Hz, 1H, $H_{\alpha,Lys}$), 3.06 (m_{ABX}, J = 14.6, 5.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.97 (m_{ABX}, J = 14.6, 7.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.90–2.76 (m, 2H, $H_{\varepsilon,Lys}$), 2.14–1.97 (m, 2H, $H2_{Cyclobutyl}$), 1.89–1.70 (m, 2H, $H4_{Cyclobutyl}$), 1.61–1.08 (m, 17H, $H_{\beta,Lys}$, $H_{\delta,Lys}$, $H_{\gamma,Lys}$, $H3_{Cyclobutyl}$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 ($CO_{\alpha,Lys}$), 169.8 ($CO_{\alpha,Trp}$), 156.0 (CO_{Cbz}), 155.5 (CO_{Boc}), 137.0 ($C1_{Ar,Cbz}$), 136.0 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.7 ($C4_{Ar,Cbz}$), 127.6 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.7 ($C6_{Indole}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_3$), 65.4 ($CH_{2,Cbz}$), 55.0 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 43.8 ($C1_{Cyclobutyl}$), 39.4 ($C_{\varepsilon,Lys}$ overlap with solvent peak), 31.5 ($C_{\beta,Lys}$), 30.0 ($C2_{Cyclobutyl}$, $C4_{Cyclobutyl}$), 29.2 ($H_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_3$), 27.8 ($C_{\beta,Trp}$), 22.7 ($C_{\gamma,Lys}$), 14.6 ($C3_{Cyclobutyl}$). UPLC-MS t_R 2.18 min, m/z 620.3 ($[M+H]^+$, $C_{34}H_{46}N_5O_6^+$ Calcd 620.3); HRMS m/z 642.3253 ($[M+Na]^+$,$C_{34}H_{45}N_5O_6Na^+$ Calcd 642.3262).

Benzyl *tert*-butyl ((*S*)-6-(((*S*)-1-(cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S76).



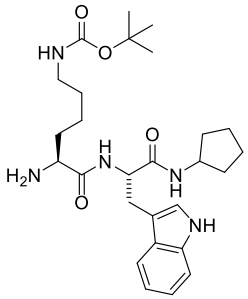
By the method described for compound **S69**, the title compound was synthesized using Cbz-Lys(Boc)-OH (444 mg, 1.17 mmol), HOBt (210 mg, 1.56 mmol), TFA salt **S36** (300 mg, 0.78 mmol), *i*Pr_2NEt (271 µL, 1.56 mmol), anhydrous CH_2Cl_2 (6.0 mL), and EDC (298 mg, 1.56 mmol) The crude residue was purified by column chromatography (0→3.0% CH_3OH in CH_2Cl_2), affording the desired amide **S76** (309 mg, 63%), as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, J = 2.4 Hz, 1H, NH_{Indole}), 7.83 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Trp}$), 7.71 (d, J = 7.3 Hz, 1H, $CO_{\alpha,Trp}NH$), 7.55 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.40 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Lys}$), 7.38–7.19 (m, 6H, $H7_{Indole}$, $H_{Ar,Cbz}$), 7.09 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.99–6.93 (m, $H5_{Indole}$), 6.73 (t, J = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 5.08–4.97 (m, 2H, $CH_{2,Cbz}$), 4.47 (q, J = 7.7, 1H, $H_{\alpha,Trp}$), 3.89–3.98 (m, 2H, $H1_{Cyclopentyl}$, $H_{\alpha,Lys}$), 3.06 (m_{ABX}, J = 14.6, 6.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.96 (m_{ABX}, J = 14.6, 7.6 Hz, 1H, $H_{\beta,Trp,B}$), 2.90–2.78 (m, 2H $H_{\varepsilon,Lys}$), 1.77–1.10 (m, 23H, $H2_{Cyclopentyl}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 ($CO_{\alpha,Trp}$), 170.4 ($CO_{\alpha,Lys}$), 156.0 (CO_{Cbz}), 155.5 (CO_2*t*Bu), 136.9 ($C_{Ar,Cbz}$), 135.9 ($C7a_{Indole}$), 128.3 ($C_{Ar,Cbz}$), 127.8 ($C_{Ar,Cbz}$), 127.6 ($C_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 120.7 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_3$), 65.4 ($CH_{2,Cbz}$), 55.0 (DCM), 54.9 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 39.4 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 32.1 ($C2_{Cyclopentyl}$), 32.0 ($C5_{Cyclopentyl}$), 31.5 ($C_{\beta,Lys}$), 29.2 ($C_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_3$), 27.9 ($C_{\beta,Trp}$), 23.4 ($C4_{Cyclopentyl}$), 23.4 ($C3_{Cyclopentyl}$), 22.7 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.47 min, m/z 634.4 ($[M+H]^+$, $C_{35}H_{48}N_5O_6^+$ Calcd 634.4); HRMS m/z 656.3420 ($[M+Na]^+$,$C_{35}H_{47}N_5O_6Na^+$ Calcd 656.3419).

***tert*-Butyl ((*S*)-5-amino-6-(((*S*)-1-(cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (S77).**



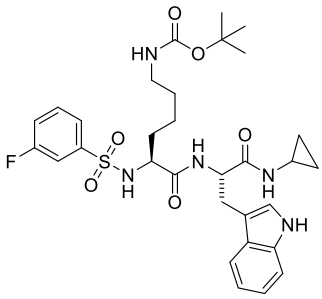
Pd/C (57 mg, 10% wt) was added to a solution of carbamate **S69** (489 mg, 0.81 mmol) in anhydrous CH_3OH (30 mL). The reaction mixture was stirred overnight under H_2 atmosphere and was then filtered through celite. The filtrate was concentrated under reduced pressure, affording the desired H-Lys(Nε-Boc)-Trp-NH-cyclopropyl (391 mg, >99%) as a colorless oil, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.05–7.93 (m, 2H, $NH_{\alpha,Trp}$, $CO_{Trp}NH$), 7.54 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.30 (d, J = 8.0 Hz, 1H, $H7_{Indole}$), 7.10–7.01 (m, 2H, $H2_{Indole}$, $H6_{Indole}$), 6.96 (t, J = 7.7 Hz, 1H, $H5_{Indole}$), 6.72 (t, J = 5.6 Hz, 1H, $NH_{\varepsilon,Lys}$), 4.50–4.38 (m, 1H, $H_{\alpha,Trp}$), 3.15–3.06 (m, 1H, $H_{\alpha,Lys}$), 3.03 (m_{ABX}, J = 14.5, 6.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.95 (m_{ABX}, J = 14.5, 7.4 Hz, 1H, $H_{\beta,Trp,B}$), 2.90–2.78 (m, 2H, $H_{\varepsilon,Lys}$), 2.60–2.53 (m, 1H, $H1_{Cyclopropyl}$), 1.57–1.07 (m, 15H, $H_{\beta,Lys}$, $C(CH_3)_3$, $H_{\delta,Lys}$, $H_{\gamma,Lys}$), 0.64–0.51 (m, 2H, $H2_{Cyclopropyl}$), 0.36–0.20 (m, 2H, $H3_{Cyclopropyl}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.3 ($CO_{\alpha,Lys}$), 172.3 ($CO_{\alpha,Trp}$), 155.5 (CO_{Boc}), 136.0 ($C7a_{Indole}$), 127.4 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.8 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole,Trp}$), 77.3 ($\underline{C}(CH_3)_3$), 54.4 ($C_{\alpha,Lys}$), 52.7 ($C_{\alpha,Trp}$), 39.4 ($C_{\varepsilon,Lys}$ overlap with solvent peak), 34.3 ($C_{\beta,Lys}$), 29.4 ($C_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_3$, $C_{\beta,Trp}$), 22.4 ($C_{\gamma,Lys}$), 22.2 ($C1_{Cyclopropyl}$), 5.52 ($C2_{Cyclopropyl}$), 5.45 ($C3_{Cyclopropyl}$). UPLC-MS t_R 1.48 min, m/z 472.3 ($[M+H]^+$, $C_{25}H_{38}N_5O_4^+$ Calcd 472.3); HRMS m/z 472.2911 ($[M+H]^+$,$C_{25}H_{38}N_5O_4^+$ Calcd 472.2918).

***tert*-Butyl ((*S*)-5-amino-6-(((*S*)-1-(cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (S78).**



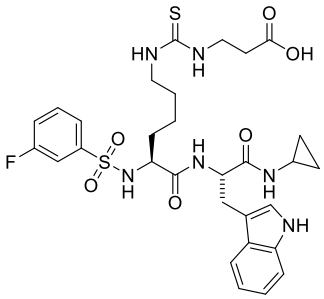
By the method described for compound **S77**, the title compound was synthesized using Pd/C (28 mg, 10% wt), carbamate **S76** (277 mg, 0.44 mmol), and anhydrous CH_3OH (20 mL) affording the desired amine **S78** (208 mg, 95%) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (d, J = 2.4 Hz, 1H, NH_{Indole}), 7.99 (d, J = 8.5 Hz, 1H, $NH_{\alpha,Trp}$), 7.82 (d, J = 7.1 Hz, 1H, $CO_{\alpha,Trp}NH$), 7.55 (d, J = 7.8 Hz, 1H, $H4_{Indole}$), 7.33–7.26 (m, 1H, $H7_{Indole}$), 7.07 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 7.04 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 6.95 (ddd, J = 7.9, 6.9, 1.0 Hz, 1H, $H5_{Indole}$), 6.72 (t, J = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 4.55–4.45 (m, 1H, $H_{\alpha,Trp}$), 3.93 (h, J = 7.1 Hz, 1H, $H1_{Cyclopentyl}$), 3.10–3.05 (m, 1H, $H_{\alpha,Lys}$), 3.02 (m_{ABX}, J = 14.5, 6.2 Hz, 1H, $H_{\beta,Trp,A}$), 2.95 (m_{ABX}, J = 14.5, 7.3 Hz, 1H, $H_{\beta,Trp,B}$), 2.90–2.78 (m, 2H, $H_{\varepsilon,Lys}$), 1.77–1.10 (m, 23H, $H2_{Cyclopentyl}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.5 ($CO_{\alpha,Lys}$), 170.6 ($CO_{\alpha,Trp}$), 155.5 (CO_2tBu), 136.0 ($C7a_{Indole}$), 127.5 ($C3a_{Indole}$), 123.5 ($C2_{Indole}$), 120.7 ($C6_{Indole}$), 118.5 ($C4_{Indole}$), 118.0 ($C5_{Indole}$), 111.1 ($C7_{Indole}$), 109.8 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_3$), 54.5 ($C_{\alpha,Lys}$), 52.7 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 39.5 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 34.5 ($C_{\beta,Lys}$), 32.1 ($C2_{Cyclopentyl}$), 32.0 ($C5_{Cyclopentyl}$), 29.4 ($C_{\delta,Lys}$), 28.5 ($C_{\beta,Trp}$), 28.3 ($C(\underline{C}H_3)_3$), 23.42 ($C4_{Cyclopentyl}$), 23.35 ($C3_{Cyclopentyl}$), 22.5 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.65 min, m/z 500.4 ($[M+H]^+$, $C_{27}H_{42}N_5O_4^+$ Calcd 500.3); HRMS m/z 500.3230 ($[M+H]^+$,$C_{27}H_{42}N_5O_4^+$ Calcd 500.3231).

***tert*-Butyl ((*S*)-6-(((*S*)-1-(cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)carbamate (S79).**



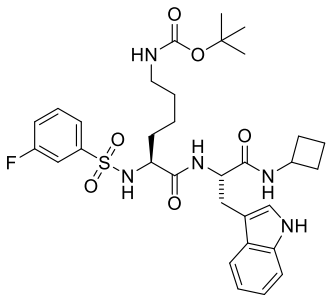
Amine **S77** (392 mg, 0.83 mmol), m-fluorophenylsulfonyl chloride (223 µL, 1.66 mmol), and iPr$_2$NEt (578 µL, 3.32 mmol) were dissolved in anhydrous CH_2Cl_2 (15 mL) and stirred for 1 hour at ambient temperature. Additional anhydrous CH_2Cl_2 (10 mL) was added and reaction mixture was stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was purified with chromatography column (0→3.5% CH_3OH in CH_2Cl_2), affording the desired sulfonamide **S79** (246 mg, 47%) as a colorless solid. TLC (5 % CH_3OH in CH_2Cl_2): R_f = 0.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.80 (d, J = 2.4 Hz, 1H, NH_{Indole}), 8.09–8.03 (m, 2H, $NH_{\alpha,Lys}$, $NH_{\alpha,Trp}$), 7.84 (d, J = 4.2 Hz, 1H, $CO_{Trp}NH$), 7.53 (dt, J = 8.4, 2.1 Hz, 1H, $H2_{FPh}$), 7.51–7.46 (m, 2H, $H6_{FPh}$, $H4_{Indole}$), 7.39–7.33 (m, 2H, $H4_{FPh,}$ $H5_{FPh}$),7.31 (dt, J = 8.1, 0.9 Hz, 1H, $H7_{Indole}$), 7.05 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.01 (d, J = 2.3 Hz, 1H, $H2_{Indole}$), 6.97 (ddd, J = 7.9, 6.9, 1.0 Hz, 1H, $H5_{Indole}$), 6.69 (t, J = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 4.22–4.16 (m, 1H, $H_{\alpha,Trp}$), 3.78–3.64 (m, 1H, $H_{\alpha,Lys}$), 2.90 (m_{ABX}, J = 14.4, 7.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.84–2.75 (m, 2H, $H_{\varepsilon,Lys}$), 2.71 (m_{ABX}, J = 14.5, 6.9 Hz, 1H, $H_{\beta,Trp,B}$), 2.55–2.50 (m, 1H, $H1_{Cyclopropyl}$, overlap with solvent peak), 1.46–1.31 (m, 11H, $H_{\beta,Lys}$, $C(C\underline{H}_3)_3$), 1.27–1.18 (m, 2H, $H_{\delta,Lys}$), 1.19–1.09 (m, 1H, $H_{\gamma,Lys,A}$), 1.06–0.95 (m, 1H, $H_{\gamma,Lys,B}$), 0.59–0.43 (m, 2H, $H2_{Cyclopropyl}$), 0.33–0.22 (m, 1H, $H3_{Cyclopropyl,A}$), 0.21–0.13 (m, 1H, $H3_{Cyclopropyl,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.8 ($CO_{\alpha,Trp}$), 170.2 ($CO_{\alpha,Lys}$), 161.5 (d, J = 248.5 Hz, $C3_{FPh}$), 155.5 (CO_{Boc}), 143.0 (d, J = 6.3 Hz, $C1_{FPh}$), 135.9 ($C7a_{Indole}$), 131.1 (d, J = 7.4 Hz, $C5_{FPh}$), 127.3 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.3 (d, J = 2.6 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.2 (d, J = 21.2 Hz, $C4_{FPh}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 113.6 (d, J = 21.8 Hz, $C2_{FPh}$), 111.2 ($C7_{Indole}$), 109.6 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_{Boc}$), 56.2 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 39.5 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 32.5 ($C_{\beta,Lys}$), 28.2 ($C_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_{Boc}$), 27.6 ($C_{\beta,Trp}$), 22.3 ($C_{\gamma,Lys}$), 22.2 ($C1_{Cyclopropyl}$), 5.5 ($C2_{Cyclopropyl}$), 5.4 ($C3_{Cyclopropyl}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −111.0 (s, 3_{FPh}-F). UPLC-MS t_R 1.93 min, m/z 630.3($[M+H]^+$, $C_{31}H_{40}FN_5O_6S^+$ Calcd 630.3); HRMS m/z 652.2563 ($[M+Na]^+$,$C_{31}H_{40}N_5O_6SFNa^+$ Calcd 652.2576). FPh=3-fluorophen-1-yl

3-(3-((*S*)-6-(((*S*)-1-(Cyclopropylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoic acid (48).



TFA (2.0 mL, 26.1 mmol) was added to a solution of carbamate **S79** (105 mg, 0.17 mmol) and TIPS (125 µL, 0.61 mmol) in anhydrous CH_2Cl_2 (5.0 mL). The reaction mixture was stirred for 1 hour at ambient temperatureand was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toulene (1:1, 3x20 mL), affording an off-white solid, tentatively assigned as the TFA salt of the Boc-deprotected derivative of compound **S79** (UPLC-MS t_R 1.22 min, *m*/*z* 530.2; $[M+H]^+$, $C_{26}H_{33}FN_5O_4S^+$ Calcd 530.2), which was used without further purification. A solution of HCl·β-alanine *t*-butyl ester (63 mg, 0.35 mmol) and *i*Pr_2NEt (182 µL, 1.04 mmol) in anhydrous CH_2Cl_2 (6.0 mL) was added dropwise to a solution of **S47** (98 mg, 0.35 mol) in anhydrous CH_2Cl_2 (3.0 mL) at 0°C over 5 minutes. The reaction mixture was stirred at 0°C for 10 minutes and was then concentrated under reduced pressure. The crude residue and the crude TFA salt of the Boc-deprotected derivative of compound **S79** (105 mg) were dissolved in anhydrous DMF (20.0 mL). *i*Pr_2NEt was added (129 µL, 0.56 mmol) and the reaction mixture was stirred overnight at 45°C. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (30 mL) and washed with aq. HCl (1 M, 2x30 mL), and brine (2x30 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure affording an orange oil, tentatively assigned as the *tert*-butyl ester of compound **48** (UPLC-MS t_R 1.94 min, *m*/*z* 717.2; $[M+H]^+$, $C_{34}H_{46}FN_6O_6S_2^+$ Calcd 717.3), which was used without further purification. TFA (3.0 mL, 39.2 mmol) and TIPS (300 µL, 1.46 mmol) were added to a solution of the orange oil in CH_2Cl_2 (4.0 mL). The reaction mixture was stirred for 2 hours and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH_2Cl_2/toluene (1:1, 3x10 mL) and heptane (10 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **48** (6 mg, 5% from **S79**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.80 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.12–8.04 (m, 2H, $NH_{\alpha,Lys}$, $NH_{\alpha,Trp}$), 7.86 (d, *J* = 4.2 Hz, 1H, $CO_{Trp}NH$), 7.58–7.52 (m, 1H, $H2_{FPh}$), 7.51–7.46 (m, 2H, $H4_{Indole}$, $H6_{FPh}$), 7.43 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.35 (ddt, *J* = 7.7, 4.4, 2.0 Hz, 3H, $H4_{FPh,}$ $H5_{FPh}$, N<u>H</u>$(CH_2)_2CO_2H$), 7.32 (dt, *J* = 8.0, 1.0 Hz, 1H, $H7_{Indole}$), 7.06 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.01 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 6.97 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H, $H5_{Indole}$), 4.24–4.14 (m, 1H, $H_{\alpha,Trp}$), 3.77 (td, *J* = 8.6, 5.5 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, C<u>H</u>$_2$$CH_2CO_2H$), 3.12 (br s, 2H, $H_{\varepsilon,Lys}$), 2.90 (m_{ABX}, *J* = 14.4, 7.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.70 (m_{ABX}, *J* = 14.5, 6.9 Hz, 1H, $H_{\beta,Trp,B}$), 2.54–2.51 (m, 1H, $H1_{Cyclopropyl}$, overlap with solvent peak), 2.47 (t, *J* = 6.6 Hz, 2H, C<u>H</u>$_2$$CO_2H$ overlap with solvent peak), 1.50–1.41 (m, 1H, $H_{\beta,Lys,A}$), 1.40–1.28 (m, 3H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$), 1.26–1.12 (m, 1H, $H_{\gamma,Lys,A}$), 1.12–1.00 (m, 1H, $H_{\gamma,Lys,B}$), 0.57–0.43 (m, 2H, $H2_{Cyclopropyl}$), 0.30–0.21 (m, 1H, $H3_{Cyclopropyl,A}$), 0.20–0.12 (m, 1H, $H3_{Cyclopropyl,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H), 171.8 ($CO_{\alpha,Trp}$), 170.0 ($CO_{\alpha,Lys}$), 161.5 (d, *J* = 247.9 Hz, $C3_{FPh}$), 143.0 (d, *J* = 7.1 Hz, $C1_{FPh}$), 135.9 ($C7a_{Indole}$), 131.1 (d, *J* = 7.1 Hz, $C5_{FPh}$), 127.3 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.6 (d, *J* = 3.5 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.2 (d, *J* = 21.3 Hz, $C2_{FPh}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 113.6 (d, *J* = 24.1 Hz, $C2_{FPh}$), 111.2 ($C7_{Indole}$), 109.6 ($C3_{Indole}$), 56.1 ($C_{\alpha,Lys}$), 53.1 ($C_{\alpha,Trp}$), 43.4 ($C_{\varepsilon,Lys}$), 39.1 (<u>C</u>$H_2CH_2CO_2H$, overlap with solvent peak), 33.7 (<u>C</u>H_2CO_2H), 32.6 ($C_{\beta,Lys}$), 28.1 ($C_{\delta,Lys}$), 27.7 ($C_{\beta,Trp}$), 22.5 ($C_{\gamma,Lys}$), 22.2 ($C1_{Cyclopropyl}$), 5.5 ($C2_{Cyclopropyl}$), 5.4 ($C3_{Cyclopropyl}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −74.3 (s, $CF_{3,TFA}$), −111.0 (s, 3_{FPh}-F). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.59 min, *m*/*z* 661.2 ($[M+H]^+$, $C_{30}H_{37}FN_5O_6S_2^+$ Calcd 661.2); HRMS *m*/*z* 661.2281 ($[M+H]^+$,$C_{30}H_{38}N_6O_6S_2F^+$ Calcd 661.2273). FPh=3-fluorophen-1-yl

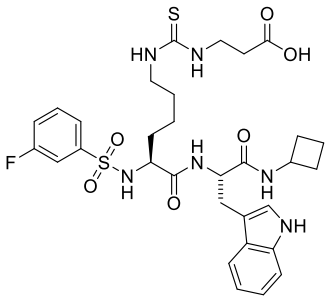
***tert*-Butyl ((*S*)-6-(((*S*)-1-(cyclobutylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)carbamate (S80).**



Pd/C (61 mg, 10% wt) was added to a solution of carbamate **S75** (652.3 mg, 1.07 mmol) in anhydrous CH_3OH (40 mL). The reaction mixture was stirred overnight under H_2 atmosphere and was then filtered through celite. The filtrate was concentrated under reduced pressure affording a colorless solid (576 mg) tentatively assigned as H-Lys(Boc)-Trp-NHCH$(CH_2)_3$ (UPLC-MS t_R 1.66 min, *m*/*z* 486.3; $[M+H]^+$, $C_{26}H_{40}FN_5O_4^+$ Calcd 486.3), was used without further purification. The colorless solid (576 mg), m-fluorophenylsulfonyl

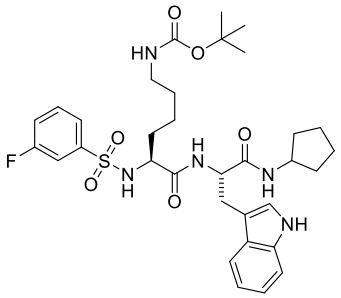
chloride (223 µL, 1.66 mmol), and *i*Pr_2NEt (578 µL, 3.32 mmol) were dissolved in anhydrous CH_2Cl_2 (15 mL) and stirred for 2 hours at ambient temperature. Additional anhydrous CH_2Cl_2 (6 mL) was added and the mixture was stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by chromatography column (0→4% CH_3OH in CH_2Cl_2), affording the sulfonamide **S80** (73 mg, 11% from **S75**), as a colorless solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.80 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.07 (d, *J* = 8.5 Hz, 1H, $NH_{\alpha,Lys}$), 8.05–7.95 (m, 2H, $NH_{\alpha,Trp}$, $CO_{Trp}NH$), 7.53 (dt, *J* = 8.4, 2.1 Hz, 1H, $H2_{FPh}$), 7.51–7.46 (m, 2H, $H6_{FPh}$, $H4_{Indole}$), 7.43–7.33 (m, 2H, $H4_{FPh,}$ $H5_{FPh}$), 7.32–7.29 (m, 1H, $H7_{Indole}$), 7.05 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.01 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 6.96 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 6.68 (t, *J* = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 4.21 (td, *J* = 8.1, 7.0 Hz, 1H, $H_{\alpha,Trp}$), 4.09 (h, *J* = 8.2 Hz, 1H, $H1_{Cyclobutyl}$), 3.73 (td, *J* = 8.5, 5.4 Hz, 1H, $H_{\alpha,Lys}$), 2.90 (m_{ABX}, *J* = 14.6, 6.8 Hz, 1H, $H_{\beta,Trp,A}$), 2.79–2.70 (m, 3H, $H_{\beta,Trp,B}$, $H_{\varepsilon,Lys}$), 2.17–2.04 (m, 1H, $H2_{Cyclobutyl,A}$), 2.04–1.91 (m, 1H, $H2_{Cyclobutyl,B}$), 1.85–1.73 (m, 1H, $H4_{Cyclobutyl,A}$), 1.71–1.60 (m, 1H, $H4_{Cyclobutyl,B}$), 1.59–1.48 (m, 2H, $H3_{Cyclobutyl}$), 1.45–1.30 (m, 11H, $H_{\beta,Lys}$, $C(C\underline{H}_3)_3$), 1.26–1.19 (m, 2H, $H_{\delta,Lys}$), 1.16–1.06 (m 1H, $H_{\gamma,Lys,A}$), 1.06–0.94 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.2 ($CO_{\alpha,Lys}$), 169.8 ($CO_{\alpha,Trp}$), 161.5 (d, *J* = 246.6 Hz, $C3_{FPh}$), 155.5 (CO_{Boc}), 143.0 (d, *J* = 6.5 Hz, $C1_{FPh}$), 135.9 ($C7a_{Indole}$), 131.1 (d, *J* = 8.0 Hz, $C5_{FPh}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.6 (d, *J* = 2.5 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.2 (d, *J* = 22.6 Hz, $C4_{FPh}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 113.6 (d, *J* = 24.5 Hz, $C2_{FPh}$), 111.2 ($C7_{Indole}$), 109.6 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_{Boc}$), 56.2 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 43.8 ($C1_{Cyclobutyl}$), 39.4 ($C_{\varepsilon,Lys}$), 32.5 ($C_{\beta,Lys}$), 29.99 ($C2_{Cyclobutyl}$), 29.92 ($C4_{Cyclobutyl}$), 28.9 ($C_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_3$), 27.7 ($C_{\beta,Trp}$), 22.3 ($C_{\gamma,Lys}$), 14.6 ($C3_{Cyclobutyl}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −111.0 (s, 3_{FPh}-F). UPLC-MS t_R 2.01 min, *m*/*z* 644.3 ($[M+H]^+$, $C_{32}H_{42}FN_5O_6S^+$ Calcd 644.3); HRMS *m*/*z* 666.2720 ($[M+Na]^+$,$C_{32}H_{42}N_5O_6SFNa^+$ Calcd 666.2738). FPh=3-fluorophen-1-yl

3-(3-((*S*)-6-(((*S*)-1-(Cyclobutylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoic acid (49).



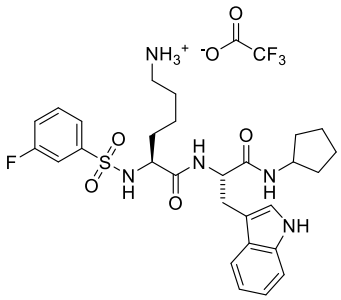
By the method described for compound **48**, the title compound was synthesized using TFA (2.0 mL, 26.1 mmol), carbamate **S80** (73 mg, 0.11 mmol), TIPS (125 µL, 0.61 mmol), and anhydrous CH_2Cl_2 (5.0 mL), then HCl·β-alanine *t*-butyl ester (63 mg, 0.35 mmol) and *i*Pr_2NEt (182 µL, 1.04 mmol) in anhydrous CH_2Cl_2 (6.0 mL), **S47** (98 mg, 0.35 mol) in anhydrous CH_2Cl_2 (2.0 mL), followed by anhydrous DMF (20 mL), and *i*Pr_2NEt (129 µL, 0.56 mmol). Then TFA (2.0 mL, 26.1 mmol), TIPS (150 µL, 0.73 mmol), and CH_2Cl_2 (3.0 mL), affording the desired carboxylic acid **49** (3 mg, 4% from **S80**), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.24 (s, 1H, CO_2H), 10.80 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.10 (d, *J* = 8.6 Hz, 1H, $NH_{\alpha,Lys}$), 8.05 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 8.00 (d, *J* = 8.0 Hz, 1H, $CO_{Trp}NH$), 7.56–7.51 (m, 1H, $H2_{FPh}$), 7.51–7.46 (m, 2H, $H4_{Indole}$, $H6_{FPh}$), 7.42 (br s, 1H, $NH_{\varepsilon,Lys}$), 7.39–7.32 (m, 3H, $H4_{FPh,}$ $H5_{FPh}$, $N\underline{H}(CH_2)_2CO_2H$), 7.32–7.30 (m, 1H, $H7_{Indole}$), 7.05 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.01 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 6.97 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 4.24–4.17 (m, 1H, $H_{\alpha,Trp}$), 4.08 (h, *J* = 8.0 Hz, 1H, $H1_{Cyclobutyl}$), 3.76 (td, *J* = 8.6, 5.4 Hz, 1H, $H_{\alpha,Lys}$), 3.56 (br s, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.20 (br s, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 2.90 (m_{ABX}, *J* = 14.4, 6.9 Hz, 1H, $H_{\beta,Trp,A}$), 2.72 (m_{ABX}, *J* = 14.4, 6.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.6 Hz, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 2.11–2.04 (m, 1H, $H2_{Cyclobutyl,A}$), 2.03–1.93 (m, 1H, $H2_{Cyclobutyl,B}$), 1.84–1.74 (m, 1H, $H4_{Cyclobutyl,A}$), 1.69–1.60 (m, 1H, $H4_{Cyclobutyl,B}$), 1.58–1.49 (m, 2H, $H3_{Cyclobutyl}$), 1.49–1.27 (m, 4H, $H_{\beta,Lys}$, $H_{\delta,Lys}$), 1.22–1.12 (m, 1H, $H_{\gamma,Lys,A}$), 1.09–1.00 (m, 1H, $H_{\gamma,Lys,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.1 (CO_2H), 170.1 ($CO_{\alpha,Lys}$), 169.3 ($CO_{\alpha,Trp}$), 161.4 (d, *J* = 249.0 Hz, $C3_{FPh}$), 142.9 (d, *J* = 6.6 Hz, $C1_{FPh}$), 135.9 ($C7a_{Indole}$), 131.1 (d, *J* = 8.5 Hz, $H5_{FPh}$), 127.4 ($C3a_{ndole}$), 123.4 ($C2_{Indole}$), 122.6 (d, *J* = 2.8 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.2 (d, *J* = 20.6 Hz, $C4_{FPh}$), 118.4 ($C4_{Indole}$), 118.1 ($C5_{Indole}$), 113.6 (d, *J* = 24.4 Hz, $C2_{FPh}$), 111.1 ($C7_{Indole}$), 109.6 ($C3_{Indole}$), 56.1 ($C_{\alpha,Lys}$), 53.1 ($C_{\alpha,Trp}$), 43.8 ($CH_{Cyclobutyl}$), 43.4 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$ overlap with solvent peak), 33.7 ($\underline{C}H_2CO_2H$), 32.6 ($C_{\beta,Lys}$), 29.98 ($C2_{Cyclobutyl}$), 43.92 ($C4_{Cyclobutyl}$), 28.2 ($C_{\delta,Lys}$), 27.7 ($C_{\beta,Trp}$), 22.5 ($C_{\gamma,Lys}$), 14.6 ($C3_{Cyclobutyl}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −73.5 (s, $CF_{3,TFA}$), −111.0 (s, 3_{FPh}-F). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 1.95 min, *m*/*z* 675.4 ($[M+H]^+$,$C_{31}H_{40}N_6O_6S_2F^+$ Calcd 675.2); HRMS *m*/*z* 675.2440 ($[M+H]^+$,$C_{31}H_{40}N_6O_6S_2F^+$ Calcd 675.2429). FPh=3-fluorophen-1-yl

***tert*-Butyl ((*S*)-6-(((*S*)-1-(cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)carbamate (S81).**



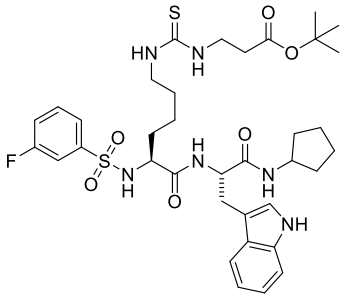
3-Fluorobenzenesulfonyl chloride (104 µL, 0.77 mmol) and *i*Pr_2NEt (269 µL, 1.46 mmol) were added to a solution of amine **S78** (193 mg, 0.39 mmol) in anhydrous CH_2Cl_2 (15 mL). The mixture was stirred at ambient temperature overnight and was then concentrated under reduced pressure. The crude residue was purified by column chromatography (0→2.5% CH_3OH in CH_2Cl_2) affording the desired sulfonamide **S81** (207 mg, 81%) as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.07 (s, 1H, $NH_{\alpha,Lys}$), 8.03 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.67 (d, *J* = 7.3 Hz, 1H, $CO_{\alpha,Trp}NH$), 7.54 (dt, *J* = 8.6, 2.1 Hz, 1H, $H2_{FPh}$), 7.52–7.46 (m, 2H, $H4_{Indole}$, $H6_{FPh}$), 7.41–7.33 (m, 2H, $H4_{FPh}$, $H5_{FPh}$), 7.33–7.29 (m, 1H, $H7_{Indole}$), 7.05 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.02 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 6.97 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H, $H5_{Indole}$), 6.69 (t, *J* = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 4.30–4.23 (m, 1H, $H_{\alpha,Trp}$), 3.89 (h, *J* = 6.9 Hz, 1H, $H1_{Cyclopentyl}$), 3.79–3.71 (m, 1H, $H_{\alpha,Lys}$), 2.90 (m_{ABX}, *J* = 14.5, 7.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.82–2.68 (m, 3H, $H_{\beta,Trp,B}$, $H_{\varepsilon,Lys}$), 1.75–1.65 (m, 1H, $H2_{Cyclopentyl,A}$), 1.65–0.95 (m, 22H, $H2_{Cyclopentyl,B}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.2 ($CO_{\alpha,Lys}$), 170.0 ($CO_{\alpha,Trp}$), 161.5 (d, *J* = 247.7 Hz, $C3_{FPh}$), 155.5 ($\underline{C}O_2tBu$), 143.0 ($C1_{FPh}$), 135.9 ($C7a_{Indole}$), 131.1 (d, *J* = 7.7 Hz, $C5_{FPh}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.6 (d, *J* = 2.8 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.3 (d, *J* = 21.2 Hz, $C4_{FPh}$), 118.4 ($C4_{Indole}$), 118.1($C5_{Indole}$), 113.6 (d, *J* = 24.2 Hz, $C2_{FPh}$), 111.1 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 77.3 ($\underline{C}(CH_3)_3$), 56.2 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 39.4 ($C_{\varepsilon,Lys}$), 32.5 ($C_{\beta,Lys}$), 32.0 ($C2_{Cyclopentyl}$), 31.9 ($C5_{Cyclopentyl}$), 28.9 ($C_{\delta,Lys}$), 28.2 ($C(\underline{C}H_3)_3$), 27.7 ($C_{\beta,Trp}$), 23.4 ($C4_{Cyclopentyl}$), 23.3 ($C3_{Cyclopentyl}$), 22.3 ($C_{\gamma,Lys}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −111.0 (s, 3_{FPh}-F). UPLC-MS t_R 2.36 min, *m*/*z* 658.3 ($[M+H]^+$,$C_{33}H_{45}N_5O_6SF^+$ Calcd 658.3); HRMS *m*/*z* 680.2877 ($[M+Na]^+$, $C_{33}H_{44}N_5O_6SFNa^+$ Calcd 680.2889). FPh=3-fluorophen-1-yl

(*S*)-6-Amino-*N*-((*S*)-1-(cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-2-((3-fluorophenyl)sulfonamido)hexanamide·TFA salt (S82).



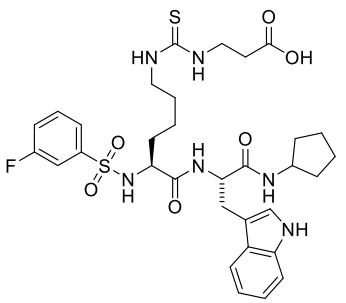
Carbamate **S81** (189 mg, 0.29 mmol) was suspended in anhydrous CH_2Cl_2 (7 mL) and TFA (4.5 mL, 58.77 mmol) and TIPS (300 µL, 1.46 mmol) were added. The mixture was stirred at ambient temperature for 1 hour and was then concentrated under reduced pressure. Excess TFA was removed through co-evaporations: CH_2Cl_2/toluene (1:1, 5×20 mL), the pink solid was dissolved in CH_3OH (1 mL) and co-evaporated with CH_2Cl_2/heptane (1:1, 4×20 mL) and CH_2Cl_2 (20 mL) affording the desired TFA salt **S82** (206 mg, >99%) as an off-white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.82 (d, *J* = 2.4 Hz, 1H, NH_{Indole}), 8.11 (d, *J* = 8.6 Hz, 1H, $NH_{\alpha,Lys}$), 8.07 (d, *J* = 7.9 Hz, 1H, $NH_{\alpha,Trp}$), 7.75–7.64 (m, 4H, $CO_{\alpha,Trp}NH$, NH_3), 7.55–7.51 (m, 1H, $H2_{FPh}$), 7.51–7.46 (m, 2H, $H4_{Indole}$, $H6_{FPh}$), 7.40–7.34 (m, 2H, $H5_{FPh}$, $H7_{Indole}$), 7.32 (dd, *J* = 8.1, 0.9 Hz, 1H, $H4_{FPh}$), 7.06 (td, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H6_{Indole}$), 7.01 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 6.97 (td, *J* = 7.9, 6.9, 1.0 Hz, 1H, $H5_{Indole}$), 4.28–4.20 (m, 1H, $H_{\alpha,Trp}$), 3.93–3.83 (m, 1H, $H1_{Cyclopentyl}$), 3.79 (td, *J* = 8.4, 5.4 Hz, 1H, $H_{\alpha,Lys}$), 2.89 (m_{ABX}, *J* = 14.4, 7.0 Hz, 1H, $H_{\beta,Trp,A}$), 2.75–2.63 (m, 3H, $H_{\beta,Trp,B}$, $H_{\varepsilon,Lys}$), 1.76–1.65 (m, 1H, $H2_{Cyclopentyl,A}$), 1.63–1.17 (m, 12H, $H2_{Cyclopentyl,B}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl,A}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$), 1.16–1.08 (m, 1H, $H5_{Cyclopentyl,B}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.1 ($CO_{\alpha,\ Lys}$), 170.0 ($CO_{\alpha,\ Trp}$), 161.5 (d, *J* = 247.7 Hz, $C3_{FPh}$), 143.0 (d, *J* = 6.6 Hz, $C1_{FPh}$), 136.0 ($C7a_{Indole}$), 131.1 (d, *J* = 8.0 Hz, $C5_{FPh}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.6 (d, *J* = 3.2 Hz, $C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.3 (d, *J* = 21.1 Hz, $C4_{FPh}$), 118.4 ($C4_{Indole}$), 118.1($C5_{Indole}$), 113.6 (d, *J* = 24.2 Hz, $C2_{FPh}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 56.0 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 38.6 ($C_{\varepsilon,Lys}$), 32.3 ($C_{\beta,Lys}$), 32.0 ($C2_{Cyclopentyl}$), 31.9 ($C5_{Cyclopentyl}$), 27.8 ($C_{\beta,Trp}$), 26.4 ($C_{\delta,Lys}$), 23.4 ($C4_{Cyclopentyl}$), 23.3 ($C3_{Cyclopentyl}$), 21.9 ($C_{\gamma,Lys}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −111.0 (s, 3_{FPh}-F). UPLC-MS t_R 1.89 min, *m*/*z* 558.3 ($[M+H]^+$,$C_{28}H_{37}N_5O_4SF^+$ Calcd 558.3); HRMS *m*/*z* 558.2548 ($[M+H]^+$, $C_{28}H_{37}N_5O_4SF^+$ Calcd 558.2545). FPh=3-fluorophen-1-yl

***tert*-Butyl 3-(3-((*S*)-6-(((*S*)-1-(cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoate (S83).**



A solution of HCl·β-alanine *t*-butyl ester (41 mg, 0.23) and *i*Pr_2NEt (59 µL, 0.34 mmol) in anhydrous CH_2Cl_2 (6 mL) was added dropwise to a solution **S47** (64 mg, 0.23 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C. The solution was stirred for 35 min then additional HCl·β-alanine *t*-butyl ester (4 mg, 0.02) was added. The reaction mixture was stirred for 15 min and was then concentrated under reduced pressure affording a yellow oil. The yellow oil and TFA salt **S82** (75 mg, 0.11 mmol) were dissolved in anhydrous DMF (3 mL). *i*Pr_2NEt (39 µL, 0.22 mmol) was added and the mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in EtOAc (75 mL) and washed with H_2O (2×75 mL), saturated aq. $NaHCO_3$ (2×75 mL), aq. HCl (1 M, 2×75 mL), and brine (2×75 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (0→2.75% CH_3OH in CH_2Cl_2) affording the desired thiourea **S83** (46 mg, 53%) as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH_{Indole}), 8.09 (d, *J* = 8.6 Hz, 1H, $NH_{\alpha,Lys}$), 8.06 (d, *J* = 7.9 Hz, 1H, $NH_{\alpha,Trp}$), 7.68 (d, *J* = 7.3 Hz, 1H, $CO_{\alpha,Trp}NH$), 7.57–7.26 (m, 8H, $H2_{FPh}$, $H4_{Indole}$, $H6_{FPh}$, $NH_{\varepsilon,Lys}$, $N\underline{H}(CH_2)_2CO_2tBu$, $H4_{FPh}$, $H5_{FPh}$, $H7_{Indole}$), 7.05 (td, *J* = 8.1, 7.0, 1.1 Hz, 1H, $H6_{Indole}$), 7.02 (d, *J* = 2.3 Hz, 1H, $H2_{Indole}$), 6.99–6.94 (m, 1H, $H5_{Indole}$), 4.30–4.22 (m, 1H, $H_{\alpha,Trp}$), 3.88 (h, *J* = 6.8 Hz, 1H, $H1_{Cyclopentyl}$), 3.81–3.74 (m, 1H, $H_{\alpha,Lys}$), 3.55 (s, 2H, $C\underline{H}_2CH_2CO_2tBu$), 3.27–3.14 (m, 2H, $H_{\varepsilon,Lys}$ overlap with residual water), 2.90 (m_{ABX}, *J* = 14.5, 7.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.71 (m_{ABX}, *J* = 14.4, 6.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.45 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2tBu$), 1.77–0.99 (m, 23H, $H2_{Cyclopentyl}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.9 (CO_2tBu), 170.1 ($CO_{\alpha,Trp}$), 170.1 ($CO_{\alpha,Lys}$), 161.5 (d, *J* = 247.8 Hz, $C3_{FPh}$), 157.9 (q, *J* = 30.8 Hz, CO_{TFA}), 143.0 (d, *J* = 6.6 Hz $C1_{FPh}$), 135.9 ($C7a_{Indole}$), 131.1 (d, *J* = 8.1 Hz, $C5_{FPh}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.6 ($C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.3 (d, *J* = 20.9 Hz, $C4_{FPh}$), 118.4 ($C4_{Indole}$), 118.1($C5_{Indole}$), 117.3 (q, *J* = 300.4 Hz, $CF_{3,TFA}$), 113.6 (d, *J* = 24.3 Hz, $C2_{FPh}$), 111.1 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 79.9 ($C(C\underline{H}_3)_3$), 56.2 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 43.3 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2tBu$), 34.8 ($\underline{C}H_2CO_2tBu$), 32.6 ($C_{\beta,Lys}$), 32.0 ($C2_{Cyclopentyl}$), 31.9 ($C5_{Cyclopentyl}$), 28.1 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$, $C(\underline{C}H_3)_3$), 23.4 ($C4_{Cyclopentyl}$), 23.3 ($C3_{Cyclopentyl}$), 22.4 ($C_{\gamma,Lys}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −111.0 (s, 3_{FPh}-F). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.30 min, *m*/*z* 745.4 ($[M+H]^+$,$C_{36}H_{50}N_6O_6S_2F^+$ Calcd 745.3); HRMS *m*/*z* 767.3038 ($[M+Na]^+$, $C_{36}H_{49}N_6O_6S_2FNa^+$ Calcd 767.3031). FPh=3-fluorophen-1-yl

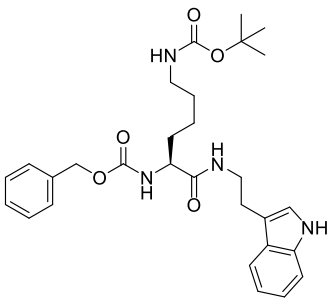
3-(3-((*S*)-6-(((*S*)-1-(Cyclopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoic acid (50).



TIPS (125 µL, 0.61 mmol) and TFA (2.0 mL, 26.1 mmol) were added to a suspension of ester **S83** (40 mg, 0.05 mmol) in CH_2Cl_2 (3.0 mL). The mixture was stirred at ambient temperature for 1.5 hours and was then concentrated under reduced pressure. Excess TFA was removed by co-evaporations: CH_2Cl_2/toluene (1:1, 2×20 mL), CH_3OH/CH_2Cl_2/CH_3CN (0.05:1:1, 20 mL), and CH_3OH/CH_3CN (0.05:1, 20 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **50** (9 mg, 24%) as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.79 (s, 1H, NH_{Indole}), 8.09 (d, *J* = 8.6 Hz, 1H, $NH_{\alpha,Lys}$), 8.06 (d, *J* = 8.0 Hz, 1H, $NH_{\alpha,Trp}$), 7.67 (d, *J* = 7.0 Hz, 1H, $CO_{\alpha,Trp}NH$), 7.54 (d, *J* = 8.6 Hz, 1H, $H2_{FPh}$), 7.51–7.47 (m, 2H, $H4_{Indole}$, $H6_{FPh}$), 7.43 (s, 1H $NH_{\varepsilon,Lys}$), 7.40–7.33 (m, 3H, $N\underline{H}(CH_2)_2CO_2H$, $H4_{FPh}$, $H5_{FPh}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H7_{Indole}$), 7.05 (t, *J* = 7.5 Hz, 1H, $H6_{Indole}$), 7.02 (s, 1H, $H2_{Indole}$), 6.97 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 4.30–4.21 (m, 1H, $H_{\alpha,Trp}$), 3.88 (h, *J* = 7.0 Hz, 1H, $H1_{Cyclopentyl}$), 3.80–3.73 (m, 1H, $H_{\alpha,Lys}$), 3.55 (br s, 2H, $C\underline{H}_2CH_2CO_2H$, overlap with residual water), 3.21 (br s, 2H, $H_{\varepsilon,Lys}$, overlap with residual water), 2.90 (m_{ABX}, *J* = 14.4, 7.1 Hz, 1H, $H_{\beta,Trp,A}$), 2.71 (m_{ABX}, *J* = 14.4, 6.8 Hz, 1H, $H_{\beta,Trp,B}$), 2.47 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2CO_2H$ overlap with solvent peak), 1.77–0.98 (m, 14H, $H2_{Cyclopentyl}$, $H3_{Cyclopentyl}$, $H4_{Cyclopentyl}$, $H5_{Cyclopentyl}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2 (CO_2H),

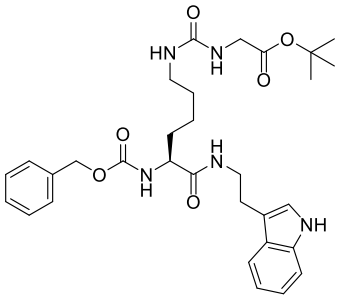
170.1 ($CO_{\alpha,Lys}$), 170.1 ($CO_{\alpha,Trp}$), 161.5 (d, J = 247.7 Hz, $C3_{FPh}$), 143.0 ($C1_{FPh}$), 136.0 ($C7a_{Indole}$), 131.1 (d, J = 8.2 Hz, $C5_{FPh}$), 127.4 ($C3a_{Indole}$), 123.4 ($C2_{Indole}$), 122.7 ($C6_{FPh}$), 120.8 ($C6_{Indole}$), 119.3 (d, J = 20.4 Hz, $C4_{FPh}$), 118.5 ($C4_{Indole}$), 118.1($C5_{Indole}$), 113.6 (d, J = 24.2 Hz, $C2_{FPh}$), 111.2 ($C7_{Indole}$), 109.7 ($C3_{Indole}$), 56.2 ($C_{\alpha,Lys}$), 53.2 ($C_{\alpha,Trp}$), 50.2 ($C1_{Cyclopentyl}$), 43.4 ($C_{\varepsilon,Lys}$), 39.1 ($\underline{C}H_2CH_2CO_2H$), 33.7 ($\underline{C}H_2CO_2H$), 32.6 ($C_{\beta,Lys}$), 32.03 ($C2_{Cyclopentyl}$), 31.96 ($C5_{Cyclopentyl}$), 28.2 ($C_{\delta,Lys}$), 27.8 ($C_{\beta,Trp}$), 23.4 ($C4_{Cyclopentyl}$), 23.3 ($C3_{Cyclopentyl}$), 22.5 ($C_{\gamma,Lys}$). ^{19}F NMR (376 MHz, DMSO-d_6) δ −111.0 (s, 3_{FPh}-F). The peak for C=S was not visible in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei. UPLC-MS t_R 2.00 min, m/z 689.4 ($[M+H]^+$,$C_{32}H_{42}N_6O_6S_2F^+$ Calcd 689.3); HRMS m/z 689.2595 ($[M+H]^+$, $C_{32}H_{42}N_6O_6SF^+$ Calcd 689.2586). FPh=3-fluorophen-1-yl

Benzyl *tert*-butyl-(6-((2-(1*H*-indol-3-yl)ethyl)amino)-6-oxohexane-1,5-diyl)(*S*)-dicarbamate (S84).



By the method described for compound **S75**, the title compound was synthesized using Cbz-Lys(Boc)-OH (1.0 g, 2.66 mmol), HOBt (397 mg, 2.94 mmol), tryptamine (472 mg, 2.94 mmol), *i*Pr_2NEt (0.90 mL, 5.17 mmol), anhydrous CH_2Cl_2 (9 mL), and EDC (769 mg, 4.01 mmol) affording the desired amide **S84** (1.3 g, 93% from Cbz-Lys(Boc)-OH) as a colorless solid, which was used without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.49 (s, 1H, NH_{Indole}), 7.58 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.39–7.28 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.19 (t, J = 7.5 Hz, 1H, $H6_{Indole}$), 7.11 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.98 (s, 1H, $H2_{Indole}$), 5.90 (s, 1H, $CO_{Lys}NH$), 5.40 (s, 1H, $NH_{\alpha,Lys}$), 5.14–5.00 (m, 2H, $CH_{2,Cbz}$), 4.57 (s, 1H, $NH_{\varepsilon,Lys}$), 4.06–3.96 (m, 1H, $H_{\alpha,Lys}$), 3.76–3.45 (m, 2H, $CO_{Lys}NHC\underline{H}_2$), 3.12–2.89 (m, 4H, $H_{\varepsilon,Lys}$, $CO_{Lys}NHCH_2C\underline{H}_2$), 1.79–1.68 (m, 1H, $H_{\beta,Lys,A}$), 1.54–1.12 (m, 14H, $H_{\beta,Lys,B}$, $C(CH_3)_3$, $H_{\delta,Lys}$, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.4 (CO_{Lys}), 156.5 (CO_2tBu), 156.2 (CO_{Cbz}), 136.6 ($C7a_{Indole}$), 136.4 ($C1_{Ar,Cbz}$), 128.7 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.4 ($C4_{Ar,Cbz}$), 128.3 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.3 ($C3a_{Indole}$), 122.6 ($C2_{Indole}$), 122.3 ($C6_{Indole}$), 119.5 ($C5_{Indole}$), 118.7 ($C4_{Indole}$), 112.4 ($C3_{Indole}$), 111.5 ($C7_{Indole}$), 79.6 ($\underline{C}(CH_3)_3$), 67.1 ($CH_{2,Cbz}$), 55.0 ($C_{\alpha,Lys}$), 40.2 ($C_{\varepsilon,Lys}$), 39.6 ($CO_{Lys}NH\underline{C}H_2$), 32.5 ($C_{\beta,Lys}$), 29.9 ($C_{\delta,Lys}$), 28.6 ($C(\underline{C}H_3)_3$), 25.1 ($CO_{Lys}NHCH_2\underline{C}H_2$), 22.4 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.31 min, m/z 523.3 ($[M+H]^+$, $C_{29}H_{39}N_4O_5^+$ Calcd 523.3).

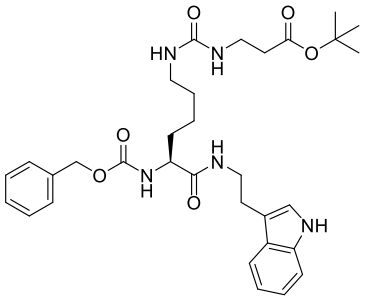
***tert*-Butyl (*S*)-((6-((2-(1*H*-indol-3-yl)ethyl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)carbamoyl)glycinate (S85).**



TFA (3.0 mL, 39.2 mmol) was added to a solution of carbamate **S84** (193 mg, 0.37 mmol) in CH_2Cl_2 (4.5 mL). The reaction mixture was stirred at ambient temperature for 1 hour and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations with CH_2Cl_2/acetone (1:1, 2×50 mL), CH_2Cl_2/heptane (1:1, 2×50 mL) and CH_2Cl_2 (50 mL). The crude residue was dissolved in anhydrous CH_2Cl_2 (10 mL) and *i*Pr_2NEt (0.10 mL, 0.57 mmol) was added. The mixture was added dropwise to a solution of carbonyldiimidazole (54 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (5.0 mL) at 0°C, over 20 minutes. After the addition was complete, the reaction mixture was allowed to reach ambient temperature and stirred for 2 hours. HCl·glycine *t*-butyl ester (63 mg, 0.37 mmol) was added and the reaction mixture was heated to reflux. After one hour an additional HCl·glycine *t*-butyl ester (63 mg, 0.37 mmol) was added and the solution was stirred at reflux overnight. The reaction mixture was allowed to reach ambient temperature and was diluted with EtOAc (50 mL) and washed with aq. HCL (0.1 M, 2×50 mL), saturated aq. $NaHCO_3$ (2×50 mL), and brine (2×50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (0→3% CH_3OH in CH_2Cl_2) affording the desired urea **S85** (59 mg, 27% from **S84**) as a white solid. TLC (3% CH_3OH in CH_2Cl_2): R_f = 0.2. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.75 (s, 1H, NH_{Indole}), 7.56 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.38–7.28 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.17 (t, J = 7.6 Hz, 1H, $H6_{Indole}$), 7.09 (t, J = 7.4 Hz, 1H, $H5_{Indole}$), 6.98 (s, 1H, $H2_{Indole}$), 6.15 (br s, 1H, $CO_{Lys}NH$), 5.66 (d, J = 7.7 Hz, 1H, $NH_{\alpha,Lys}$), 5.11–5.01 (m, 2H, $CH_{2,Cbz}$), 4.96 (t, J = 5.4 Hz, 1H, $N\underline{H}CH_2CO_2t$Bu), 4.69 (br s, 1H, $NH_{\varepsilon,Lys}$), 4.07–3.97 (m, 1H, $H_{\alpha,Lys}$), 3.95–3.80 (m, 2H, CH_2CO_2tBu), 3.74–3.63 (m, 1H, $CO_{Lys}NHC\underline{H}_{2,A}$), 3.54–3.44 (m,

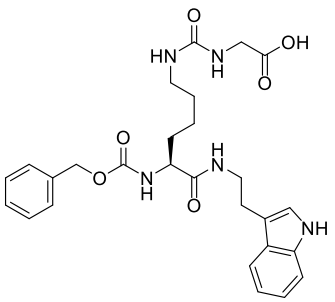
1H, $CO_{Lys}NHC\underline{H}_{2,B}$), 3.15–3.03 (m, 2H, $H_{\varepsilon,Lys}$), 3.02–2.88 (m, 2H, $CO_{Lys}NHCH_2C\underline{H}_2$), 1.72–1.61 (m, 1H, $H_{\beta,Lys,A}$), 1.55–1.23 (m, 12H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$, $C(CH_3)_3$), 1.19–1.09 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.6 (CO_{Lys}), 170.9 (CO_2tBu), 158.3 (NHCONH), 156.4 (CO_{Cbz}), 136.7 ($C7a_{Indole}$), 136.5 ($C1_{Ar,Cbz}$), 128.7 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.3 ($C4_{Ar,Cbz}$), 128.2 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 122.8 ($C2_{Indole}$), 122.1 ($C6_{Indole}$), 119.4 ($C5_{Indole}$), 118.6 ($C4_{Indole}$), 112.3 ($C3_{Indole}$), 111.6 ($C7_{Indole}$), 82.3 ($\underline{C}(CH_3)_3$), 67.1 ($CH_{2,Cbz}$), 55.0 ($C_{\alpha,Lys}$), 43.0 ($C_{\alpha,Gly}$), 40.0 ($C_{\varepsilon,Lys}$), 39.6 ($CO_{Lys}NH\underline{C}H_2$), 32.4 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 28.2 ($C(\underline{C}H_3)_3$), 25.1 ($CO_{Lys}NHCH_2\underline{C}H_2$), 22.3 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.16 min, *m*/*z* 580.3 ($[M+H]^+$, $C_{31}H_{42}N_5O_6^+$ Calcd 580.3).

tert-Butyl (*S*)-5-((2-(1*H*-indol-3-yl)ethyl)carbamoyl)-3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecan-15-oate (S86).



By the method described for compound **S85**, the title compound was synthesized using TFA (3.0 mL, 39.2 mmol), carbamate **S84** (212 mg, 0.41 mmol) in CH_2Cl_2 (4.5 mL), then anhydrous CH_2Cl_2 (10 mL), *i*Pr_2NEt (0.10 mL, 0.57 mmol), and carbonyldiimidazole (58 mg, 0.36 mmol) in anhydrous CH_2Cl_2 (5 mL), followed by HCl·β-alanine *t*-butyl ester (2×73 mg, 0.80 mmol). The crude residue was purified by column chromatography (0→3% CH_3OH in CH_2Cl_2) affording the desired urea **S86** (68 mg, 28% from **S84**) of as a white solid. TLC (5% CH_3OH in CH_2Cl_2): R_f = 0.3. ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 8.75 (s, 1H, NH_{Indole}), 7.58 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.40–7.29 (m, 6H, $H_{Ar,Cbz}$, $H7_{Indole}$), 7.19 (t, *J* = 7.6 Hz, 1H, $H6_{Indole}$), 7.11 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 6.99 (s, 1H, $H2_{Indole}$), 5.98 (br s, 1H, $CO_{Lys}NH$), 5.58 (d, *J* = 7.6 Hz, 1H, $NH_{\alpha,Lys}$), 5.11–5.01 (m, 2H, $CH_{2,Cbz}$), 4.86 (t, *J* = 6.1 Hz, 1H, $N\underline{H}(CH_2)_2CO_2t$Bu), 4.41 (br s, 1H, $NH_{\varepsilon,Lys}$), 4.03–3.92 (m, 1H, $H_{\alpha,Lys}$), 3.76–3.62 (m, 1H, $CO_{Lys}NHC\underline{H}_{2,A}$), 3.55–3.47 (m, 1H, $CO_{Lys}NHC\underline{H}_{2,B}$), 3.45–3.33 (m, 2H, $C\underline{H}_2CH_2CO_2t$Bu), 3.13–3.03 (m, 2H, $H_{\varepsilon,Lys}$), 3.03–2.92 (m, 2H, $CO_{Lys}NHCH_2C\underline{H}_2$), 2.40 (t, *J* = 5.9 Hz, 2H, $C\underline{H}_2CO_2t$Bu), 1.77–1.65 (m, 1H, $H_{\beta,Lys,A}$), 1.55–1.28 (m, 12H, $H_{\beta,Lys,B}$, $H_{\delta,Lys}$, $C(CH_3)_3$), 1.27–1.11 (m, 2H, $H_{\gamma,Lys}$). ^{13}C NMR (151 MHz, $CDCl_3$) *δ* 172.7 (CO_2tBu), 171.5 (CO_{Lys}), 158.4 (NHCONH), 156.3 (CO_{Cbz}), 136.7 ($C7a_{Indole}$), 136.5 ($C1_{Ar,Cbz}$), 128.7 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 128.3 ($C4_{Ar,Cbz}$), 128.3 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.4 ($C3a_{Indole}$), 122.7 ($C2_{Indole}$), 122.2 ($C6_{Indole}$), 119.4 ($C5_{Indole}$), 118.7 ($C4_{Indole}$), 112.3 ($C3_{Indole}$), 111.6 ($C7_{Indole}$), 81.3 ($\underline{C}(CH_3)_3$), 67.1 ($CH_{2,Cbz}$), 55.0 ($C_{\alpha,Lys}$), 39.8 ($C_{\varepsilon,Lys}$), 39.6 ($CO_{Lys}NH\underline{C}H_2$), 36.3 ($\underline{C}H_2CH_2CO_2t$Bu), 36.0 ($\underline{C}H_2CO_2t$Bu), 32.3 ($C_{\beta,Lys}$), 29.9 ($C_{\delta,Lys}$), 28.3 ($C(\underline{C}H_3)_3$), 25.1 ($CO_{Lys}NHCH_2\underline{C}H_2$), 22.3 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.22 min, *m*/*z* 594.2 ($[M+H]^+$, $C_{32}H_{44}N_5O_6^+$ Calcd 594.3).

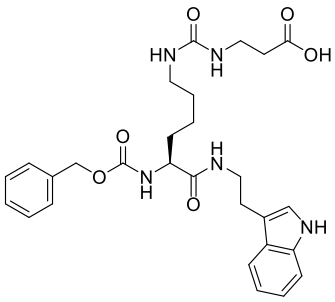
(*S*)-((6-((2-(1*H*-Indol-3-yl)ethyl)amino)-5-(((benzyloxy)carbonyl)amino)-6-oxohexyl)carbamoyl)glycine (S20).



TFA (2.4 mL, 31.3 mmol) was added to a solution of *t*-butyl ester **S85** (55 mg, 0.09 mmol) in CH_2Cl_2 (3.6 mL). The reaction mixture was stirred at ambient temperature for 2 hours and the reaction mixture was concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired carboxylic acid **S20** (28 mg, 55%) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.36 (br s, 1H, CO_2H), 10.80 (s, 1H, NH_{Indole}), 7.95 (t, *J* = 5.7 Hz, 1H, $CO_{Lys}NH$), 7.54 (d, *J* = 7.9 Hz, 1H, $H4_{Indole}$), 7.45–7.24 (m, 7H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$, $H7_{Indole}$), 7.13 (s, 1H, $H2_{Indole}$), 7.06 (t, *J* = 7.5 Hz, 1H, $H6_{Indole}$), 6.97 (t, *J* = 7.4 Hz, 1H, $H5_{Indole}$), 6.11 (br s, 1H $NH_{\varepsilon,Lys}$), 6.02 (br s, 1H, $N\underline{H}CH_2CO_2H$), 5.09–4.98 (m, 2H, $CH_{2,Cbz}$), 3.96–3.85 (m, 1H, $H_{\alpha,Lys}$), 3.68 (s, 2H, $C\underline{H}_2CO_2H$), 3.41–3.25 (m, 2H, $CO_{Lys}NHC\underline{H}_2$), 3.00–2.88 (m, 2H, $H_{\varepsilon,Lys}$), 2.81 (t, *J* = 7.4 Hz, 2H, $CO_{Lys}NHCH_2C\underline{H}_2$), 1.62–1.42 (m, 2H, $H_{\beta,Lys}$), 1.39–1.14 (m, 4H, $H_{\gamma,Lys,}$ $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) *δ* 172.6 (CO_2H), 171.7 (CO_{Lys}), 157.9 (NHCONH), 155.9 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 136.2 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.73 ($C4_{Ar,Cbz}$), 127.68 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.15 ($C3a_{Indole}$), 122.7 ($C2_{Indole}$), 120.85 ($C6_{Indole}$), 118.22 ($C4_{Indole}$), 118.16 ($C5_{Indole}$), 111.7 ($C3_{Indole}$), 111.3 ($C7_{Indole}$), 65.3 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 41.4 ($C_{\alpha,Gly}$), 39.1 ($CO_{Lys}NH\underline{C}H_2$, overlap with solvent peak), 39.0 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 31.8 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 25.1 ($CO_{Lys}NHCH_2\underline{C}H_2$), 22.9 ($C_{\gamma,Lys}$). UPLC-MS

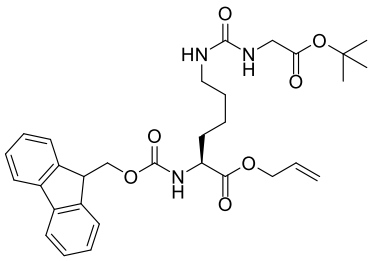
t_R 1.77 min, *m*/*z* 524.2 ([M+H]$^+$, $C_{27}H_{34}N_5O_6^+$ Calcd 524.3); HRMS *m*/*z* 546.2343 ([M+H]$^+$, $C_{27}H_{33}N_5O_6Na^+$ Calcd 546.2323).

(*S*)-5-((2-(1*H*-Indol-3-yl)ethyl)carbamoyl)-3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecan-15-oic acid (S21).



By the method described for compound **S20**, the title compound was synthesized using TFA (4.0 mL, 52.2 mmol), ester **S86** (65 mg, 0.11 mmol), and CH_2Cl_2 (6.0 mL) affording the desired carboxylic acid **S21** (32 mg, 54%) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.16 (br s, 1H, CO_2H), 10.8 (s, 1H, NH_{Indole}), 7.95 (t, J = 5.7 Hz, 1H, $CO_{Lys}NH$), 7.54 (d, J = 7.9 Hz, 1H, $H4_{Indole}$), 7.43–7.24 (m, 7H, $H_{Ar,Cbz}$, $NH_{\alpha,Lys}$, $H7_{Indole}$), 7.13 (s, 1H, $H2_{Indole}$), 7.09–7.03 (m, 1H, $H6_{Indole}$), 7.00–6.94 (m, 1H, $H5_{Indole}$), 5.92 (t, J = 5.7 Hz, 1H, $NH_{\varepsilon,Lys}$), 5.83 (t, J = 5.9 Hz, 1H, $N\underline{H}(CH_2)_2CO_2H$), 5.09–4.96 (m, 2H, $CH_{2,Cbz}$), 3.96–3.85 (m, 1H, $H_{\alpha,Lys}$), 3.42–3.25 (m, 2H, $CO_{Lys}NHC\underline{H}_2$, overlap with residual water), 3.22–3.13 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.97–2.87 (m, 2H, $H_{\varepsilon,Lys}$), 2.81 (t, J = 7.4 Hz, 2H, $CO_{Lys}NHCH_2C\underline{H}_2$), 2.32 (t, J = 6.5 Hz, 2H, $C\underline{H}_2CO_2H$), 1.64–1.43 (m, 2H, $H_{\beta,Lys}$), 1.38–1.11 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3 (CO_2H), 171.7 (CO_{Lys}), 157.9 (NHCONH), 155.9 (CO_{Cbz}), 137.1 ($C1_{Ar,Cbz}$), 136.2 ($C7a_{Indole}$), 128.3 ($C3_{Ar,Cbz}$, $C5_{Ar,Cbz}$), 127.73 ($C4_{Ar,Cbz}$), 127.68 ($C2_{Ar,Cbz}$, $C6_{Ar,Cbz}$), 127.2 ($C3a_{Indole}$), 122.7 ($C2_{Indole}$), 120.9 ($C6_{Indole}$), 118.22 ($C4_{Indole}$), 118.16 ($C5_{Indole}$), 111.7 ($C3_{Indole}$), 111.3 ($C7_{Indole}$), 65.3 ($CH_{2,Cbz}$), 54.8 ($C_{\alpha,Lys}$), 39.3 ($CO_{Lys}NH\underline{C}H_2$, overlap with solvent peak), 39.0 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 35.3 ($\underline{C}H_2CH_2CO_2H$), 35.0 ($\underline{C}H_2CO_2H$), 31.8 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 25.1 ($CO_{Lys}NHCH_2\underline{C}H_2$), 22.9 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.78 min, *m*/*z* 538.3 ([M+H]$^+$, $C_{28}H_{36}N_5O_6^+$ Calcd 538.3); HRMS *m*/*z* 560.2495 ([M+H]$^+$, $C_{28}H_{35}N_5O_6Na^+$ Calcd 560.2480).

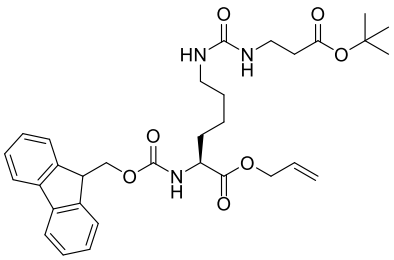
Allyl N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^6-((2-(*tert*-butoxy)-2-oxoethyl)carbamoyl)-L-lysinate (S87).



Carbonyldiimidazole (90 mg, 0.55 mmol) was added dropwise over 10 minutes to a suspension of TFA·Fmoc-Lys-OAll[15] (138 mg, 0.26 mmol) and *i*Pr_2NEt (140 µL, 0.80 mmol) in anhydrous CH_2Cl_2 (4 mL) 0 °C. The reaction mixture was stirred at 0 °C for 5 minutes and was then stirred at ambient temperature for 3 hours. The reaction mixture was washed with aq. HCl (0.1 M, 2×10 mL) and the aqueous phase was extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure affording a clear oil (130 mg), tentatively assigned as Fmoc-Lys(1H-imidazole-1-carbonyl)-OAll (UPLC-MS t_R 1.95 min, *m*/*z* 503.3; [M+H]$^+$ $C_{28}H_{31}N_4O_5^+$ Calcd 503.2). The clear oil (61 mg) and the HCl salt of tert-butyl glycine (24 mg, 0.13 mmol) were suspended in anhydrous DMF (5.0 mL) and *i*Pr_2NEt (5 µL, 0.03 mmol) was added. The reaction mixture was stirred at ambient temperature overnight and was then stirred at 80 °C for 1 hour. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in CH_2Cl_2 (25 mL). The organic phase was washed with aq. HCl (1 M, 25 mL), saturated aq. $NaHCO_3$ (25 mL), and brine (25 mL) and was then dried over over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (0→1.5% CH_3OH in CH_2Cl_2), affording the desired urea **S87** (28 mg, 40% from TFA·Fmoc-Lys-OAll), as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.5 Hz, 2H, $H1_{Ar,Fmoc}$, $H8_{Ar,Fmoc}$), 7.61 (d, J = 7.5 Hz, 2H, $H4_{Ar,Fmoc}$, $H5_{Ar,Fmoc}$), 7.39 (t, J = 7.4 Hz, 2H, $H3_{Ar,Fmoc}$, $H6_{Ar,Fmoc}$), 7.34–7.27 (m, 2H, $H2_{Ar,Fmoc}$, $H7_{Ar,Fmoc}$), 5.96–5.82 (m, 1H, $C\underline{H}=CH_2$), 5.75 (d, J = 8.1 Hz, 1H, $NH_{\alpha,Lys}$), 5.37–5.18 (m, 2H, $CH=C\underline{H}_2$), 4.64 (d, J = 5.8 Hz, 2H, $C\underline{H}_2CH=CH_2$), 4.47–4.29 (m, 3H, $CH_{2,Fmoc}$, $H_{\alpha,Lys}$), 4.23 (t, J = 7.1 Hz, 1H, $H9_{Fmoc}$), 3.95–3.79 (m, 2H, CH_2CO_2tBu), 3.17 (t, J = 6.7 Hz, 2H, $H_{\varepsilon,Lys}$), 1.92–1.63 (m, 2H, $H_{\beta,Lys}$), 1.42 (s, 13H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3 (CO_{Lys}), 170.8 (CO_2tBu), 158.3 (NHCONH), 156.4 (CO_{Fmoc}), 144.0 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 143.9 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 141.4 ($C4a_{Ar,Fmoc}$, $C4b_{Ar,Fmoc}$), 131.7 ($\underline{C}H=CH_2$), 127.8 ($C3_{Ar,Fmoc}$, $C6_{Ar,Fmoc}$), 127.23 ($C2_{Ar,Fmoc}$/$C7_{Ar,Fmoc}$), 127.21 ($C2_{Ar,Fmoc}$/$C7_{Ar,Fmoc}$), 125.3 ($C4_{Ar,Fmoc}$, $C5_{Ar,Fmoc}$), 120.10 ($C1_{Ar,Fmoc}$/$C8_{Ar,Fmoc}$), 120.08 ($C1_{Ar,Fmoc}$/$C8_{Ar,Fmoc}$), 119.1 ($CH=\underline{C}H_2$), 82.1 ($\underline{C}(CH_3)_3$), 67.1 ($CH_{2,Fmoc}$),

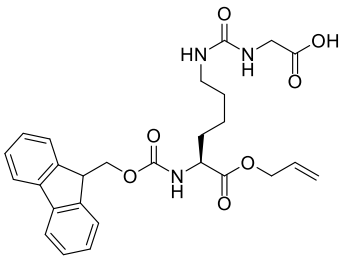
66.1 ($\underline{C}H_2CH=CH_2$), 53.9 ($C_{\alpha,Lys}$), 47.3 ($C9_{Fmoc}$), 43.0 ($C_{\alpha,Gly}$), 40.0 ($C_{\varepsilon,Lys}$), 32.1 ($C_{\beta,Lys}$), 29.6 ($C_{\delta,Lys}$), 28.1 ($C(\underline{C}H_3)_3$), 22.5 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.39 min, *m*/*z* 566.3 ($[M+H]^+$, $C_{31}H_{40}N_3O_7^+$ Calcd 566.3).

tert-Butyl (*S*)-5-((allyloxy)carbonyl)-1-(9*H*-fluoren-9-yl)-3,11-dioxo-2-oxa-4,10,12-triazapentadecan-15-oate (S88).



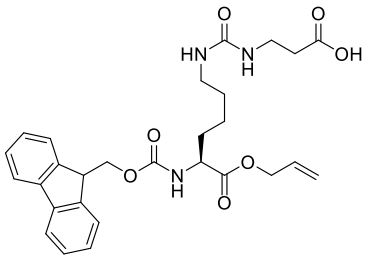
By the method described for compound **S87**, the title compound was synthesized using the remaining clear oil (69 mg) tentatively assigned as as Fmoc-Lys(1H-imidazole-1-carbonyl)-OAll, HCl·β-alanine *t*-butyl ester (72 mg, 0.39 mmol), and anhydrous DMF (4 mL). The crude residue was purified by column chromatography (2% CH_3OH in CH_2Cl_2), affording the desired urea **S88** (47 mg, 58% from TFA·Fmoc-Lys-OAll), as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H, $H1_{Ar,Fmoc}$, $H8_{Ar,Fmoc}$), 7.60 (dd, *J* = 7.0, 3.8 Hz, 2H, $H4_{Ar,Fmoc}$, $H5_{Ar,Fmoc}$), 7.39 (t, *J* = 7.4 Hz, 2H, $H3_{Ar,Fmoc}$, $H6_{Ar,Fmoc}$), 7.30 (t, *J* = 7.4 Hz, 2H, $H2_{Ar,Fmoc}$, $H7_{Ar,Fmoc}$), 5.89 (ddt, *J* = 16.4, 10.9, 5.8 Hz, 1H, $C\underline{H}=CH_2$), 5.66 (d, *J* = 8.1 Hz, 1H, $NH_{\alpha,Lys}$), 5.35–5.20 (m, 2H, $CH=C\underline{H}_2$), 4.63 (d, *J* = 5.6 Hz, 2H, $C\underline{H}_2CH=CH_2$), 4.49–4.28 (m, 3H, $CH_{2,Fmoc}$, $H_{\alpha,Lys}$), 4.22 (t, *J* = 6.9 Hz, 1H, $H9_{Fmoc}$), 3.39 (t, *J* = 4.9 Hz, 2H, $C\underline{H}_2CH_2CO_2tBu$), 3.14 (t, *J* = 6.6 Hz, 2H, $H_{\varepsilon,Lys}$), 2.40 (t, *J* = 5.9 Hz, 2H, $C\underline{H}_2CO_2tBu$), 1.94–1.63 (m, 2H, $H_{\beta,Lys}$), 1.55–1.24 (m, 13H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6 (CO_{Lys}), 172.3 (CO_2tBu), 158.4 (NHCONH), 156.3 (CO_{Fmoc}), 144.0 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 143.9 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 141.4 ($C4a_{Ar,Fmoc}$, $C4b_{Ar,Fmoc}$), 131.6 ($C\underline{H}=CH_2$), 127.8 ($C3_{Ar,Fmoc}$, $C6_{Ar,Fmoc}$), 127.2 ($C2_{Ar,Fmoc}$, $C7_{Ar,Fmoc}$), 125.24 ($C4_{Ar,Fmoc}$/$C5_{Ar,Fmoc}$), 125.22 ($C4_{Ar,Fmoc}$/$C5_{Ar,Fmoc}$), 120.1 ($C1_{Ar,Fmoc}$, $C8_{Ar,Fmoc}$), 119.1 ($CH=C\underline{H}_2$), 81.0 ($\underline{C}(CH_3)_3$), 67.1 ($CH_{2,Fmoc}$), 66.1 ($\underline{C}H_2CH=CH_2$), 53.9 ($C_{\alpha,Lys}$), 47.3 ($C9_{Fmoc}$), 39.9 ($C_{\varepsilon,Lys}$), 36.1 ($\underline{C}H_2CO_2tBu$), 36.0 ($\underline{C}H_2CH_2CO_2tBu$), 32.1 ($C_{\beta,Lys}$), 29.7 ($C_{\delta,Lys}$), 28.2 ($C(\underline{C}H_3)_3$), 22.5 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.43 min, *m*/*z* 580.4 ($[M+H]^+$, $C_{32}H_{42}N_3O_7^+$ Calcd 580.3).

(*S*)-((5-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-6-(allyloxy)-6-oxohexyl)carbamoyl)glycine (S22).



By the method described for compound **S20**, the title compound was synthesized using TFA (0.8 mL, 10.5 mmol), ester **S87** (28 mg, 0.05 mmol), and CH_2Cl_2 (1.2 mL) affording the desired carboxylic acid **S22** (7 mg, 28%), as a colorless fluffy material after lyophilization. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, *J* = 7.5 Hz, 2H, $H1_{Ar,Fmoc}$, $H8_{Ar,Fmoc}$), 7.79 (d, *J* = 7.7 Hz, 1H, $NH_{\alpha,Lys}$), 7.72 (d, *J* = 7.4 Hz, 2H, $H4_{Ar,Fmoc}$, $H5_{Ar,Fmoc}$), 7.42 (t, *J* = 7.4 Hz, 2H, $H3_{Ar,Fmoc}$, $H6_{Ar,Fmoc}$), 7.33 (t, *J* = 7.4 Hz, 2H, $H2_{Ar,Fmoc}$, $H7_{Ar,Fmoc}$), 6.14 (s, 1H, $NH_{\varepsilon,Lys}$), 6.03 (s, 1H, $N\underline{H}CH_2CO_2H$), 5.89 (ddt, *J* = 17.0, 10.5, 5.3 Hz, 1H, $C\underline{H}=CH_2$), 5.30 (dd, *J* = 17.2, 1.5 Hz, 1H, $CH=C\underline{H}_{2,trans}$), 5.19 (dd, *J* = 10.5, 1.4 Hz, 1H, $CH=C\underline{H}_{2,cis}$), 4.57 (d, *J* = 5.1 Hz, 2H, $C\underline{H}_2CH=CH_2$), 4.42–4.16 (m, 3H, $H9_{Fmoc}$, $CH_{2,Fmoc}$), 4.09–3.95 (m, 1H, $H_{\alpha,Lys}$), 3.68 (s, 2H, $C\underline{H}_2CO_2H$, overlap with residual water), 2.97 (br s, 2H, $H_{\varepsilon,Lys}$), 1.81–1.55 (m, 2H, $H_{\beta,Lys}$), 1.46–1.18 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.6 (CO_2H), 172.2 (CO_{Lys}), 158.0 (NHCONH), 156.2 (CO_{Fmoc}), 143.82 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 143.79 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 140.8 ($C4a_{Ar,Fmoc}$, $C4b_{Ar,Fmoc}$), 132.4 ($C\underline{H}=CH_2$), 127.7 ($C3_{Ar,Fmoc}$, $C6_{Ar,Fmoc}$), 127.1 ($C2_{Ar,Fmoc}$, $C7_{Ar,Fmoc}$), 125.3 ($C4_{Ar,Fmoc}$, $C5_{Ar,Fmoc}$), 120.2 ($C1_{Ar,Fmoc}$, $C8_{Ar,Fmoc}$), 117.7 ($CH=C\underline{H}_2$), 65.7 ($CH_{2,Fmoc}$), 64.8 ($\underline{C}H_2CH=CH_2$), 54.0 ($C_{\alpha,Lys}$), 46.6 ($C9_{Fmoc}$), 41.5 ($C_{\alpha,Gly}$), 39.0 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 30.4 ($C_{\beta,Lys}$), 29.5 ($C_{\delta,Lys}$), 23.0 ($C_{\gamma,Lys}$). UPLC-MS t_R 1.95 min, *m*/*z* 510.2 ($[M+H]^+$, $C_{27}H_{32}N_3O_7^+$ Calcd 510.2); HRMS *m*/*z* 532.2063 ($[M+H]^+$, $C_{27}H_{31}N_3O_7Na^+$ Calcd 532.2054).

(*S*)-5-((Allyloxy)carbonyl)-1-(9*H*-fluoren-9-yl)-3,11-dioxo-2-oxa-4,10,12-triazapentadecan-15-oic acid (S23).



By the method described for compound **S20**, the title compound was synthesized using TFA (2 mL, 26.1 mmol), ester **S88** (47 mg, 0.08 mmol), and CH_2Cl_2 (3 mL) affording the desired carboxylic acid **S23** (13 mg, 30%), as a colorless fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.16 (br s, 1H, CO_2H), 7.89 (d, J = 7.6 Hz, 2H, $H1_{Ar,Fmoc}$, $H8_{Ar,Fmoc}$), 7.78 (d, J = 7.8 Hz, 1H, $NH_{\alpha,Lys}$), 7.71 (ddd, J = 7.5, 2.4, 1.1 Hz, 2H, $H4_{Ar,Fmoc}$, $H5_{Ar,Fmoc}$), 7.42 (t, J = 7.4 Hz, 2H, $H3_{Ar,Fmoc}$, $H6_{Ar,Fmoc}$), 7.33 (td, J = 7.5, 1.1 Hz, 2H, $H2_{Ar,Fmoc}$, $H7_{Ar,Fmoc}$), 5.93 (br s, 1H, $NH_{\varepsilon,Lys}$), 5.92–5.85 (m, 1H, C<u>H</u>=CH_2), 5.83 (br s, 1H, N<u>H</u>$(CH_2)_2CO_2H$), 5.30 (ddd, J = 17.2, 3.2, 1.6 Hz, 1H, CH=C<u>H</u>$_{2,trans}$), 5.19 (ddd, J = 10.5, 3.0, 1.5 Hz, 1H, CH=C<u>H</u>$_{2,cis}$), 4.57 (ddd, J = 5.3, 3.0, 1.5 Hz, 2H, C<u>H</u>$_2$CH=CH_2), 4.35–4.20 (m, 3H, $H9_{Fmoc}$, $CH_{2,Fmoc}$), 4.04–3.98 (m, 1H, $H_{\alpha,Lys}$), 3.21–3.14 (m, 2H, C<u>H</u>$_2CH_2CO_2H$), 2.99–2.91 (m, 2H, $H_{\varepsilon,Lys}$), 2.32 (t, J = 6.6 Hz, 2H, C<u>H</u>$_2CO_2H$), 1.74–1.58 (m, 2H, $H_{\beta,Lys}$), 1.38–1.24 (m, 4H, $H_{\gamma,Lys}$, $H_{\delta,Lys}$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4 (CO_2H), 172.1 (CO_{Lys}), 157.9 (NHCONH), 156.1 (CO_{Fmoc}), 143.8 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 143.7 ($C8a_{Ar,Fmoc}$/$C9a_{Ar,Fmoc}$), 140.7 ($C4a_{Ar,Fmoc}$, $C4b_{Ar,Fmoc}$), 132.4 (<u>C</u>H=CH_2), 127.6 ($C3_{Ar,Fmoc}$, $C6_{Ar,Fmoc}$), 127.0 ($C2_{Ar,Fmoc}$, $C7_{Ar,Fmoc}$), 125.2 ($C4_{Ar,Fmoc}$, $C5_{Ar,Fmoc}$), 120.1 ($C1_{Ar,Fmoc}$, $C8_{Ar,Fmoc}$), 117.7 (CH=<u>C</u>H_2), 65.6 ($CH_{2,Fmoc}$), 64.7 (<u>C</u>H_2CH=CH_2), 53.9 ($C_{\alpha,Lys}$), 46.6 ($C9_{Fmoc}$), 38.9 ($C_{\varepsilon,Lys}$, overlap with solvent peak), 35.3 (<u>C</u>$H_2CH_2CO_2H$), 35.0 (<u>C</u>H_2CO_2H), 30.3 ($C_{\beta,Lys}$), 29.6 ($C_{\delta,Lys}$), 22.9 ($C_{\gamma,Lys}$). UPLC-MS t_R 2.20 min, m/z 524.2 ($[M+H]^+$, $C_{28}H_{34}N_3O_7^+$ Calcd 524.2); HRMS m/z 546.2231 ($[M+H]^+$, $C_{28}H_{33}N_3O_7Na^+$ Calcd 546.2211).

Fluorescence-based *in vitro* sirtuin deacylase assays

Materials—SIRT1 (aa 193–741 with N-terminal GST-tag, >60% purity), SIRT2 (aa 50–356 with C-terminal His-tag, >90% purity), and SIRT6 (full length with N-terminal GST-tag, >75% purity) were purchased from BPS Biosciences (San Diego, CA); SIRT3 (aa 102–399 with N-terminal His-tag; ~75% purity) and SIRT5 (aa 37–310 with N-terminal His-tag, >90% purity) were purchased from Enzo Life Sciences (Farmingdale, NY). Purities were based on SDS-PAGE and Coomassie blue stain according to the supplier, and all enzyme concentrations given are based on stock concentrations according to the supplier. Assay buffer was prepared as described in Biomol International product sheets BML-KI-143 [http://www.enzolifesciences.com/BML-AK500/fluor-de-lys-hdac-fluorometric-activity-assay-kit/] [TRIS HCl (50 mM), NaCl (137 mM), KCl (2.7 mM), $MgCl_2$ (1 mM), pH 8.0] with addition of BSA (1.0 mg/mL). Trypsin (10,000 units/mg, TPCK treated from bovine pancreas, T1426) was purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals and solvents were of analytical grade were and used without further purification as obtained from commercial suppliers.

Fluorescence-based sirtuin deacylase assays—All reactions were performed in black low binding 96-well microtiter plates (Corning half area wells), with duplicate series in each assay and each assay performed at least twice. Control wells without enzyme were included in each plate. All reactions were performed in assay buffer, with appropriate concentrations of substrates and inhibitors obtained by dilution from 3.8–50 mM stock solutions in either water or DMSO, and appropriate concentration of enzyme obtained by dilution of the stock provided by the supplier. DMSO concentration in the final assay solution did not exceed 2% (v/v) and control wells without either enzyme (negative control) or inhibitor (positive control) were included in each plate. All plates were analyzed using a Perkin Elmer Enspire plate reader with excitation at 360 nm and detecting emission at 460 nm. Fluorescence measurements (RFU) were converted to [AMC] concentrations based on a [AMC]–fluorescence standard curve, and all data analysis was performed using GraphPad Prism.

End-point sirtuin 5 inhibition assays, initial screening—Relevant substrate and inhibitor was added to each well, and the experiment initiated by addition of a freshly prepared solution of sirtuin 5, for a final volume of 25 µL per well. The following final concentrations were used: SIRT5 (172 nM), Ac-LGKglu-AMC (50 µM), NAD^+ (500 µM), and inhibitor (100 µM or 10 µM). The plate was incubated at 37 °C for 30 min, then a solution of trypsin and nicotinamide (25 µL, 0.4 mg/mL and 8 mM, respectively; final concentration 0.2 mg/mL and 4 mM, respectively) was added and the assay development was allowed to proceed for 15 min at room temperature, before fluorescence measurement and calculation of residual activity.

End-point sirtuin 5 inhibition assays, concentration–response—Relevant substrate and inhibitor was added to each well, and the experiment initiated by addition of a freshly prepared solution of sirtuin 5, for a final volume of 25 µL per well. The following final concentrations were used: SIRT5 (172 nM), Ac-LGKglu-AMC (50 µM) or Ac-LGKsuc-AMC (50 µM), NAD^+ (500 µM), and inhibitor (3-fold dilution series). The plate was incubated at 37 °C for 30 min, then a solution of trypsin and nicotinamide (25 µL, 0.4 mg/mL and 4 mM, respectively; final concentration 0.2 mg/mL and 2 mM, respectively) was added and the assay development was allowed to proceed for 15 min at room temperature, before fluorescence measurement. Residual activity was calculated, and assuming a standard fast-on/fast-off mechanism, IC_{50} values were obtained by fitting the resulting data to the concentration–response equation (**Eq. 1**).

$$\textbf{Eq. 1} \qquad v_i = v_{bottom} + \frac{v_{top} - v_{bottom}}{1 + 10^{(\log IC_{50} - \log[I])h}}$$

End-point sirtuin inhibition assays, sirtuin selectivity—Using the protocol described above, SIRT1–3 (SIRT1: 250 nM; SIRT2 250 µM; SIRT3: 500 nM) and Ac-QPKKac-AMC (50 µM), SIRT5 (100 nM) and Ac-QPKKsuc-AMC (50 µM) or Ac-QPKKglu-AMC (50 µM), or SIRT6 (500 nM) and Ac-QPKKdec-AMC (50 µM) were incubated with NAD^+ (500 µM) and inhibitor (100 µM or 10 µM).[16] The plate was incubated at 37 °C for 60 min, then a solution of trypsin and nicotinamide (25 µL, 5 mg/mL and 4 mM, respectively; final concentration 2.5 mg/mL and 2 mM, respectively) was added and the assay development was allowed to proceed for 90 min at room temperature, before fluorescence measurement and calculation of residual activity.

End-point sirtuin 5 pre-incubation assays—Sirtuin 5 and inhibitor was pre-incubated with or without NAD^+ for 5 min at rt or 15 or 30 min at 37 °C in a total volume of 40 µL, prior to addition of substrate (and NAD^+ if excluded in pre-incubation), for a final volume of 45 µL. For pre-incubation excluding NAD^+, the following concentrations were used: SIRT5 (113 nM during pre-incubation, giving 100 nM after substrate and NAD^+ addition), inhibitor (113/100 µM, 11.3/10 µM, 1.13/1.0 µM, or 0.11/0.10 µM), substrate (0/50 µM), NAD^+ (0/500 µM); For pre-incubation including NAD^+, the following concentrations were used: SIRT5 (113 nM during pre-incubation, giving 100 nM after substrate addition), inhibitor (113/100 µM, 11.3/10 µM, 1.13/1.0 µM, or 0.11/0.10 µM), NAD^+ (563/500 µM), and substrate (0/50 µM). The plate was incubated at 37 °C for 30 min, then a solution of trypsin and nicotinamide (5 µL, 2.0 mg/mL and 40 mM, respectively; final concentration 0.2 mg/mL and 4 mM, respectively) was added and the assay development was allowed to proceed for 15 min at room temperature, before fluorescence measurement and calculation of residual activity.

Rate inhibition assays, concentration–response—Rate experiments for determination of kinetic parameters were evaluated under varying inhibitor concentrations. Sirtuin 5 was incubated with the relevant substrate, inhibitor and trypsin in assay buffer in a total volume of 50 µL per well using the following final concentrations: SIRT5 (100 nM); Ac-LGKglu-AMC (23 µM), NAD^+ (500 µM) and trypsin (0.125 ng/µL). *In situ* fluorophore release was monitored immediately by fluorescence readings recorded every 30 seconds for 35 min at 25 °C. The data were fitted to the relevant equations (**Eq. 2** or **3**) to obtain either initial linear rates (v) or apparent first-order rate constant (k_{obs}) for each inhibitor concentration. Secondary plots were then fitted to the relevant equations (**Eq. 1**, **4**, or **5**) to obtain the desired dissociation constants (K_i and $K_{i,1}$) and/or kinetic parameters (k_1, k_{-1}, k_2 and k_{-2}).

Eq. 2 $[P] = vt$

Eq. 3 $$[P] = v_{ss}t + \frac{v_{in} - v_{ss}}{k_{obs}}\left(1 - e^{-k_{obs}t}\right)$$

Eq. 4 $$k_{obs} = k_1\left(1 + \frac{[S]}{K_M}\right)[I] + k_{-1}$$

Eq. 5 $$k_{obs} = \frac{k_2}{[I] + K_{i,1}\left(1 + \frac{[S]}{K_M}\right)}[I] + k_{-2}$$

Expression and purification of hSirt5 and zSirt5

hSirt5(34-302) and zSirt5(30-298) proteins were expressed in *E. coli* Rosetta2 (DE3) cells and *E. coli* BL21-CodonPlus™ (DE3) cells, respectively, and purified through Co-Talon affinity chromatography, TEV-proteolysis, reverse affinity chromatography and gel filtration as previously reported[17], except that zSirt5 gel filtration buffer was 20 mM TRIS HCl, pH 8.5, 150 mM NaCl, 0.5 mM TCEP.

Crystallization and structure solution

10 mg/mL zSirt5 or hSirt5 protein, 1 mM compound **10** or **29** and 5 mM NAD^+ were mixed and pre-incubated for 30 min on ice (DMSO concentration 10% during incubation; compound stocks 20 mM in DMSO, NAD^+ stock 50 mM in sodium phosphate buffer pH 8.0) before setting up sitting-drop vapor-diffusion crystallization experiments at 20 °C. Co-crystallization was achieved by using 20% PEG8000, 0.2 M $MgCl_2$, 0.1 M TRIS HCl, pH 8.5, 0.1 M glycine for zSirt5 complexes and 30% PEG3350, 0.2 M NaCl, 0.1 M BIS-TRIS, pH 5.5, 5% 1,3-butandiol for the hSirt5 complex as reservoir solution. 2 µL crystallization drops were prepared with a 1:1 ratio of protein/ligand and reservoir solution, which were equilibrated against 200 µL reservoir solution in MRC Maxi 48-well crystallization plates (Hampton Research, Aliso Viejo, USA, # HR3-179). After 2–4 days, tiny crystal cubes (zSirt5) or large cubes, rods and bundles of rods (hSirt5) appeared. The crystals were transferred to a cryo-solution drop composed of reservoir and ligands supplemented with 25% glycerol prior to shock-freezing in liquid nitrogen and data collection. hSirt5 diffraction was mostly anisotropic, but one of the rods showed clearer diffraction images and was used for structure solution. Diffraction data collection was performed at 100 K at BESSY II beamline MX14.1[18] (operated by the Helmholtz-Zentrum Berlin, Germany) with a Pilatus 6M detector (Dectris, Baden, Switzerland) or at Swiss Light Source beamline

X06DA - PXIII (Paul Scherrer Institut, Villigen, Switzerland) with a Pilatus 2M-F detector (Dectris, Baden, Switzerland). Indexing, scaling and merging of diffraction data were done using XDS.[19] Structures were solved by Phaser[20] molecular replacement with search models PDB 3RIY (hSirt5/succinyl-H3K9/NAD^+ complex) for the hSirt5 complex and PDB 4UTV (zSirt5/3-phenyl-succinyl-CPS1) for the zSirt5 complexes. Refinement was performed using Refmac5[21] and manual modeling as well as structure validation were done with Coot.[22] Geometry parameters for compounds and intermediates were generated using PRODRG[23] and structure figures created with PyMOL Molecular Graphics System (Version 1.7 Schrödinger, LLC).

Cell culture and generation of crSIRT5KO cells

HEK293T were obtained from Dr. Eric Verdin's lab. The HEK293T crSIRT5KO cell line was generated with Dharmacon's Edit-R gene engineering system that uses plasmid-driven Cas9 nuclease expression, synthetic tracrRNA and crRNA for gene of interest as per manufacturer's protocol. Briefly, an Edit-R hCMV-Puro-Cas9 (#U-005100-120) was co-transfected with Edit-R tracrRNA (#U-002000-120) and Edit-R crRNA's for SIRT5 into HEK293T cells using the DharmaFECT Duo (#T-2010-03) transfection reagent. After 48 hours of transfection, positively transfected cells were selected using 2 µg/mL puromycin for 3 days and mono-clonal populations were selected using serial dilution technique. The guide sequence targeting the sense strand at sequence 5'-GAT GAG CTG CAC CGC AAG GC-3' in Exon 5 of human SIRT5 was selected from Dharmacon's online CRISPR RNA configurator tool (http://dharmacon.gelifesciences.com/gene-editing/crispr-rna-configurator/). Control cells underwent the same procedure as the crSIRT5KO cells, except they did not receive the gene-specific crRNA. Each clonal population was cultured and analyzed for loss of SIRT5 protein by Western blotting. Both cell lines were maintained at 37 °C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific #11965118) supplemented with 10% (v/v) fetal bovine serum (FBS, Thermo Scientific #26140079).

Western blotting and Antibodies

Equal amounts of denatured protein samples (30–60 µg) were uniformly loaded and run on BioRad AnykD Criterion TGX Precast Midi Protein Gels, 14 well (#5671123), 18 well (#5671124), or 26 well (#5671125), 15–40 uL at 150–180 V for 45–60 minutes. The proteins were wet-transferred to a 0.45 µm nitrocellulose membrane in a BioRad Criterion™ Blotter at 100 V for 90 minutes at 4 °C. The membranes were blocked for 1 hour in LI-COR blocking buffer (20 mM TRIS base, 150 mM NaCl, 0.45% fish gelatin, 0.1% casein and 0.02% azide, pH 7.5). Primary antibodies (1:1000–1:2000) were diluted in LI-COR blocking buffer (containing 0.1–0.2% Tween-20) overnight at 4 °C. Membranes were washed with TRIS-buffered saline (TBS, 20 mM TRIS base, 150 mM NaCl, containing 0.1% Tween-20), 4–6 times for 5–10 minutes each. Infra-red dye-conjugated secondary antibodies were diluted 1:10,000 in LI-COR blocking buffer containing 0.1–0.2% Tween-20 and incubated for 1 hour at room temperature. Western blots were visualized on a LI-COR Odyssey CLx imager. Un-stripped blots were re-probed for loading control and image analysis to quantify band-intensity was performed using the LI-COR Image Studio software (version 3.0.12). Antibodies: anti-SIRT5 (Sigma-Aldrich #HPA022002), anti-β-actin (Cell Signaling Technologies, #3700), anti-GAPDH (Santa Cruz Biotechnology, #sc-32233), anti-UQCRFS1 (Abcam, #ab14746), anti-glutaryl-lysine (Cell Signaling Technologies, non-commercial and PTM-Biolabs #1151), anti-succinyl-lysine (Cell Signaling Technologies, non-commercial and PTM-Biolabs #401), anti-malonyl-lysine (Cell Signaling Technologies, #14942), IRDye 680RD Donkey anti-mouse IgG (LI-COR, #926-68072), and IRDye 800CW Donkey anti-rabbit IgG (LI-COR, #926-32213).

Drug treatment of HEK293T WT and crSIRT5KO cells

Et-29 *treatment*—The crSIRT5KO and WT cells at passage 11 were treated with 1 or 10 µM of **Et-29** or respective volume of DMSO as control, in duplicate. Cells were plated at a density of 120,000 cell per well in 6-well plates and the SIRT5 inhibitor/DMSO were included in the plating media. After incubation for 48 hours, cells were washed in phosphate-buffered saline (PBS, pH 7.4, Thermo Scientific #10010023) and collected in ice-cold radio immunoprecipitation assay buffer (RIPA buffer, 20 mM TRIS base, 150 mM NaCl, 0.5%

sodium deoxycholate, 0.1% sodium dodecyl sulphate, 0.1% triton-X-100, 1 mM EDTA, 1 mM EGTA, pH 7.4) buffer containing protease- (Sigma-Aldrich, #P8340) and/or phosphatase (Sigma-Aldrich, #P0044 and P5726) inhibitors, 10 μM Trichostatin A (Selleckchem, #S1045), 10 mM nicotinamide (Sigma-Aldrich, #N5535) and 10 mM sodium butyrate (Sigma-Aldrich, #303410). Cells were lysed by sonication (10 intermittent pulses with a probe sonicator) after which samples were vortexed and centrifuged at 14,000×g for 1 hour. Supernatants were collected and protein concentration was determined using a Bicinchoninic Acid Protein Assay Kit (BCA assay, Sigma-Aldrich # BCA1). Western blot analysis of lysine glutarylation was performed as described above.

Malonyl-NAC[1] *and GSK2194069 treatement*—Malonyl-NAC (MalNAC) was obtained from Jordan L. Meier's lab. Fatty acid synthase inhibitor GSK2194069 (GSKi) was purchase from Sigma-Aldrich (#SML1259-5MG). The crSIRT5KO and WT cells at passage 11 were plated at a density of 200,000 cell per well in 6-well plates, in triplicate. After 48 hours cells were treated with MalNAC (0.5 or 1.0 mM) or GSKi (30 or 15 nM) or respective volume of DMSO as control. After incubation for 24 hours, cells were collected in RIPA buffer and western blot analysis of lysine malonylation was performed, as described above.

Mitochondrial enrichment

crSIRT5KO and WT cells at passage 11 were plated at a density of 2,200,000 cells in tissue-culture dishes (100×20 mm), in triplicate. After 48 hours in culture, media was aspirated and cells were collected in ice-cold PBS (12 mL). The cell suspension was centrifuged at 1000×g for 5 minutes at 4 °C and the resulting supernatant was aspirated. The pellet was resuspended in MHS buffer (220 mM mannitol, 70 mM sucrose, 5 mM potassium-HEPES, 10 mM nicotinamide, pH 7.5, 750 μL). An aliquot (50 μL) was mixed with aq. triton-X-100 (10%, containing Halt protease inhibitors 10× (Halt, Thermo Scientific, #78441), 5 μL), representing the whole cell lysate. The remaining cell lysate was transferred to a dounce homogenizer, and stroked 50 times. The dounced suspension was pelleted at 750×g for 5 minutes at 4 °C and the supernatant was collected. The pellet was resuspended in MHS buffer (300 μL) and transferred to a dounce homogenizer and stroked 50 times. The homogenate was centrifuged at 750×g for 5 minutes at 4 °C and the resulting supernatant was pooled with the previously collected supernatant. The pooled supernatants were pelleted at 14000×g for 10 minutes at 4 °C and an aliquot (100 μL) of the supernatant was mixed with aq. Triton-X-100 (10%, containing Halt (10×), 10 μL), representing the cytosolic fraction. The remaining supernatant was aspirated and the pellet was washed with MHS buffer (200 μL) and pelleted at 14000×g for 10 minutes at 4 °C and the supernatant was aspirated. This washing step was repeated 3 times, and after the last wash the pellet was resuspended in isolation buffer (30 μL) and mixed with aq. Triton-X-100 (10%, containing Halt (10×), 3 μL), representing the mitochondrial fraction. The three fractions (whole cell lysate, cytosolic and mitochondrial) were vortexed and protein concentration was determined using a BCA assay. Western blot analysis of lysine succinylation and lysine glutarylation was performed, as described above.

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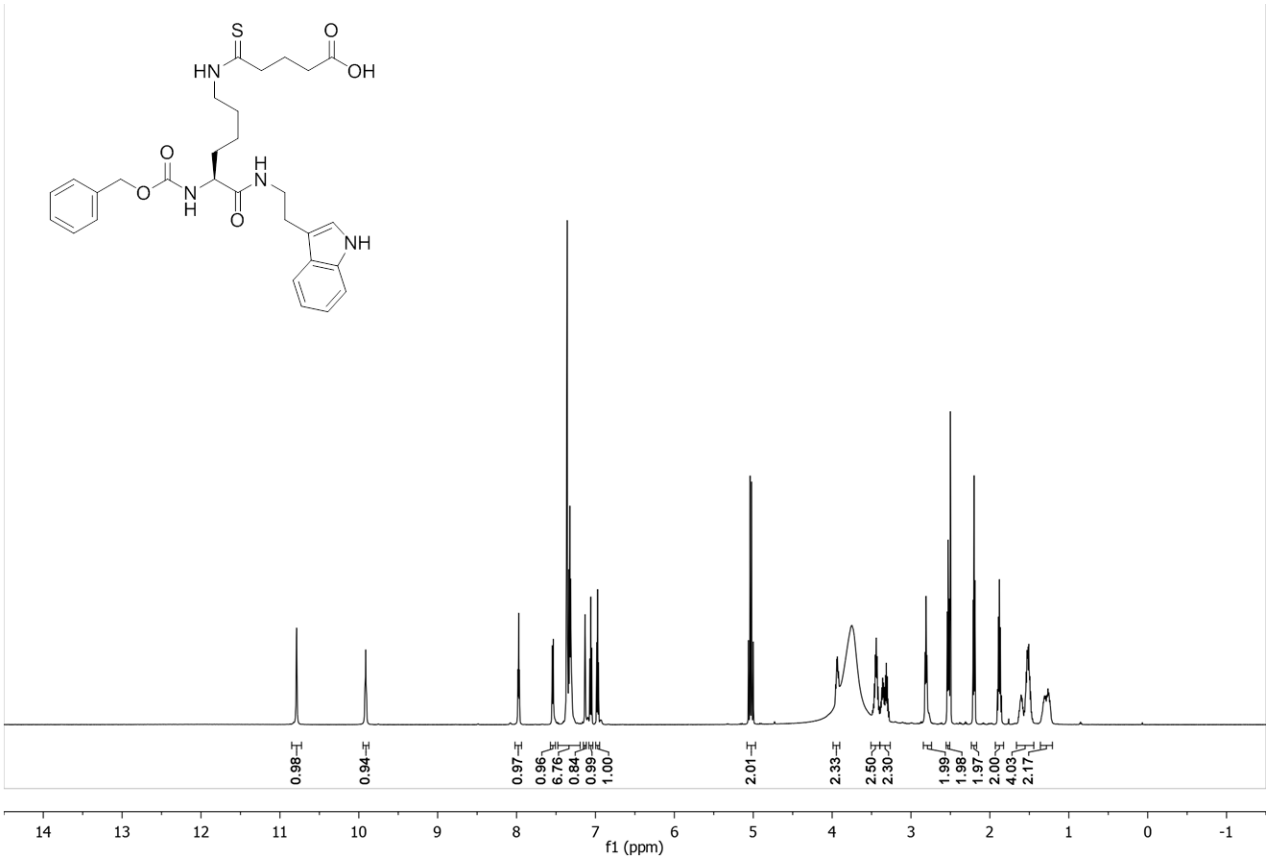
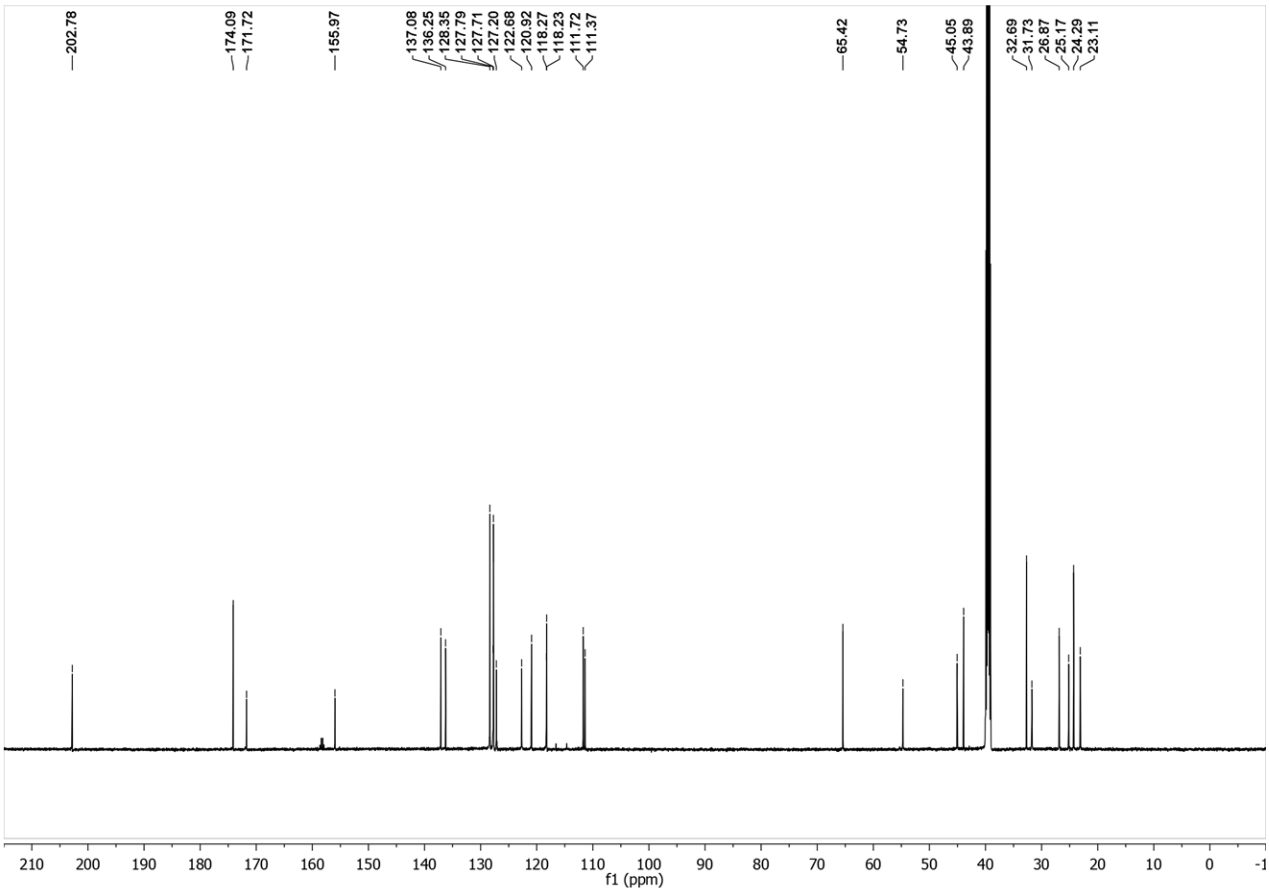
NMR spectra of compounds 1-50, V, Et-29, and S1-S88

^{1}H and ^{13}C spectra of compound 1.





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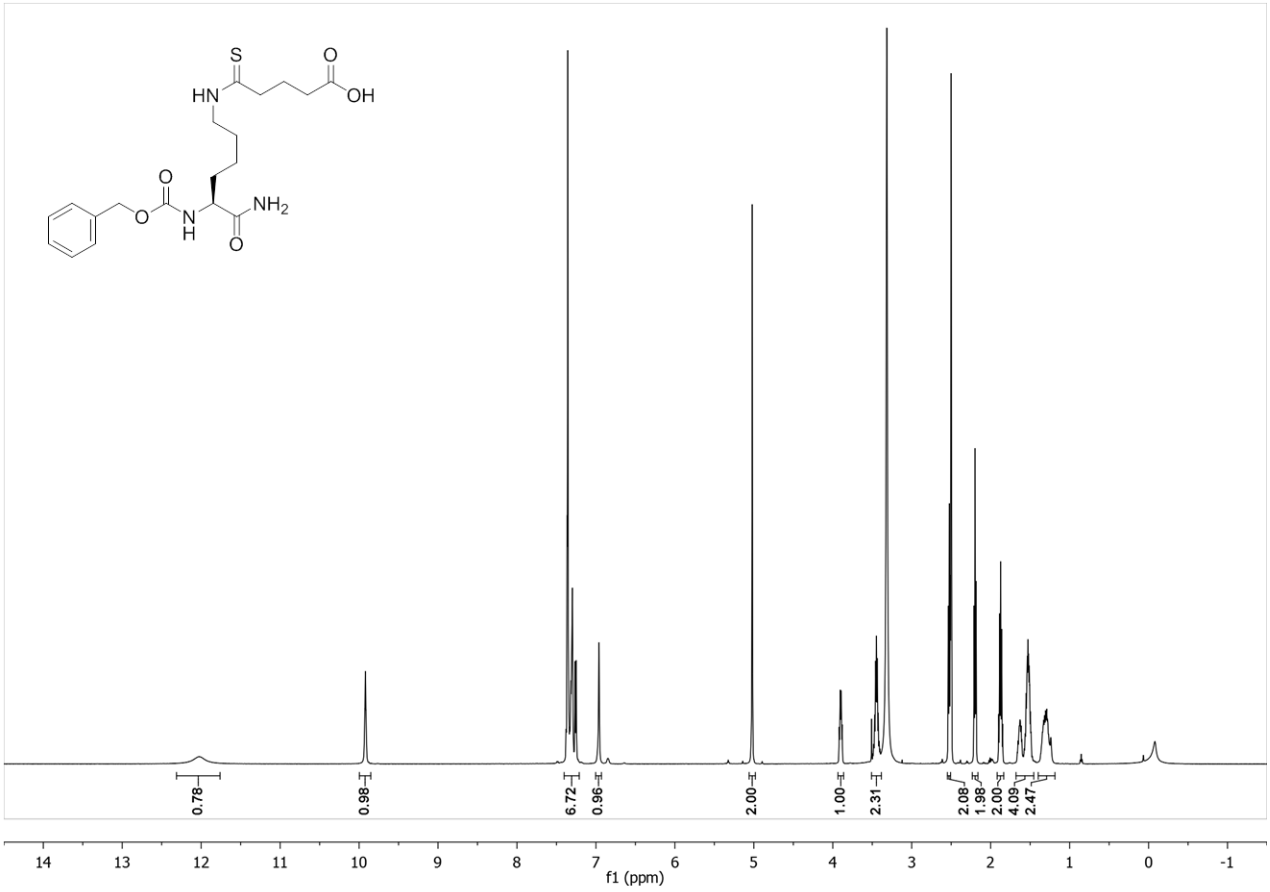

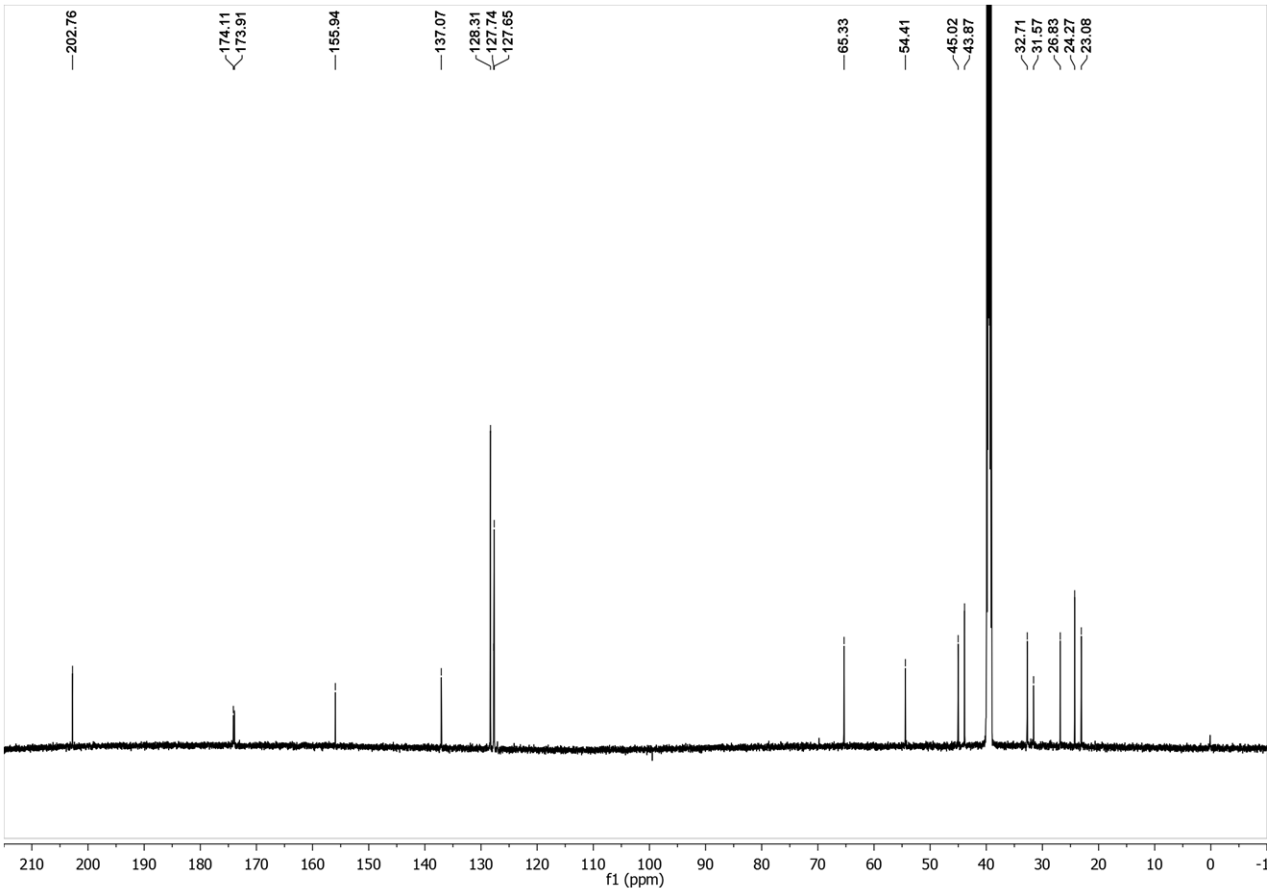

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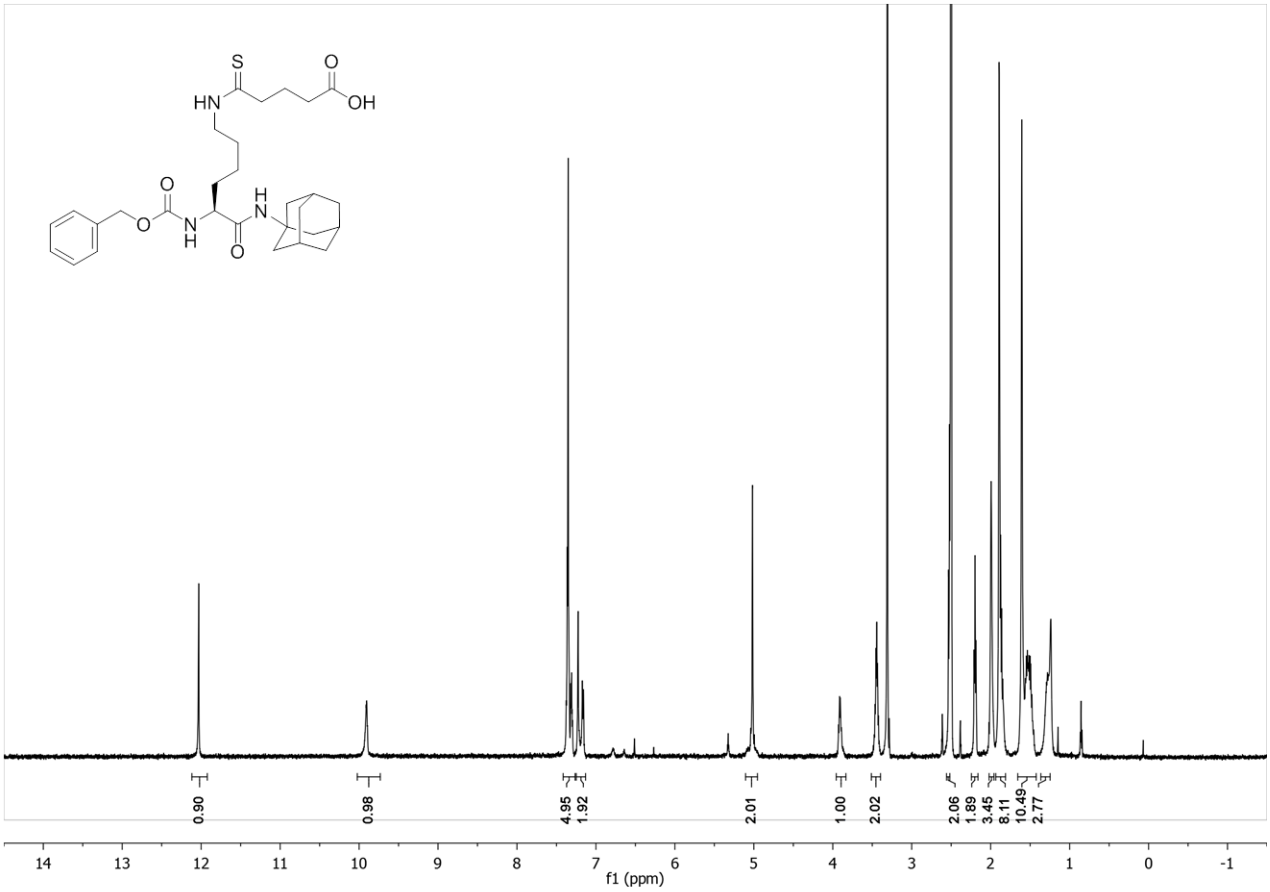

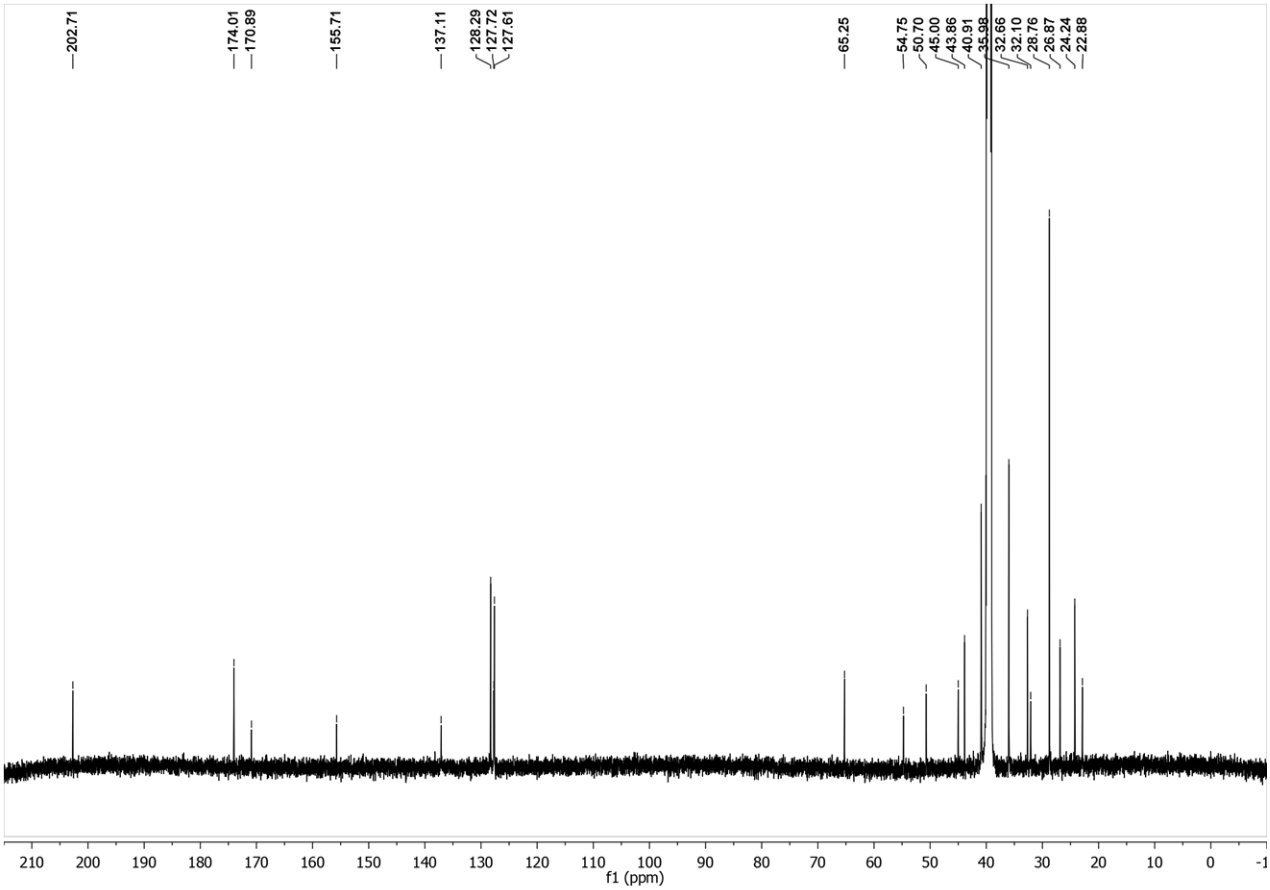

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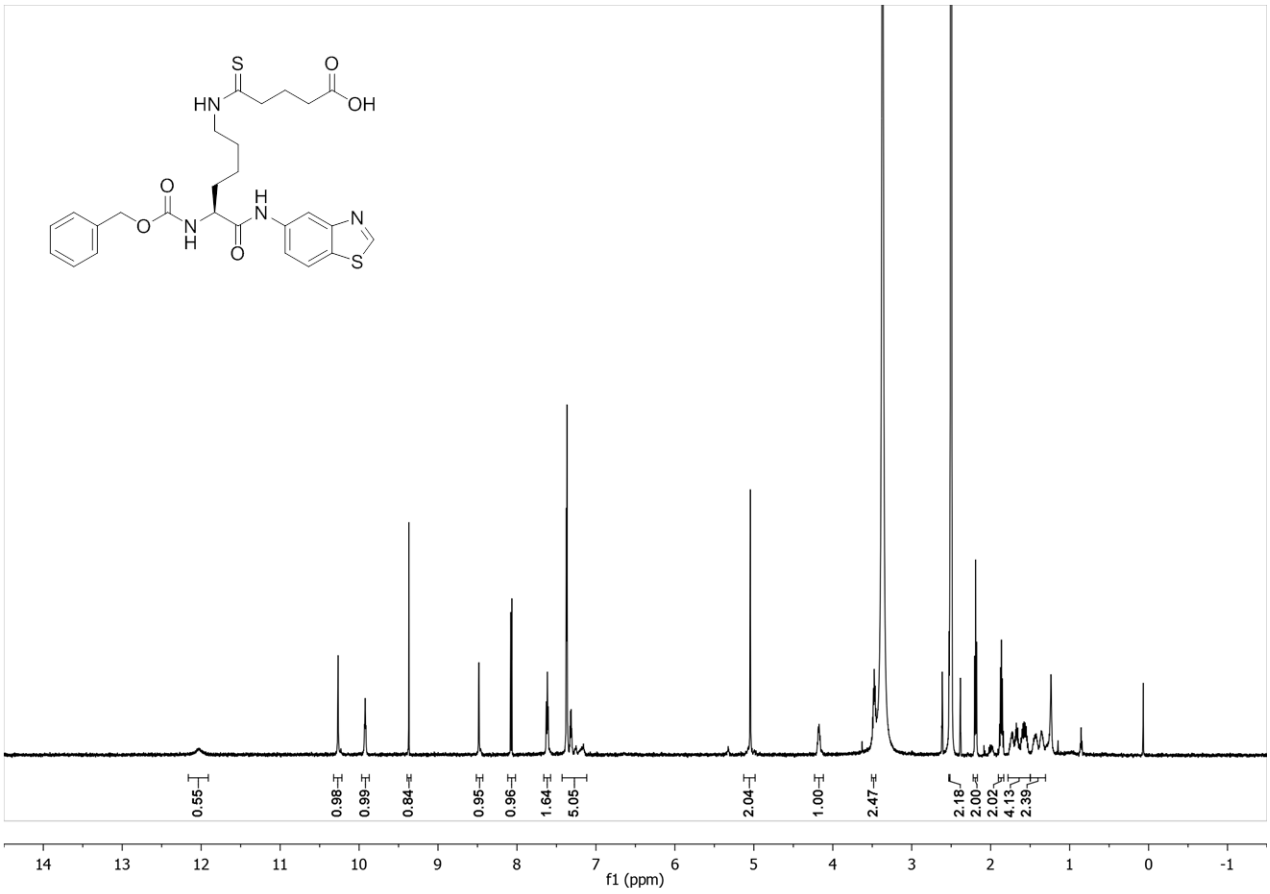

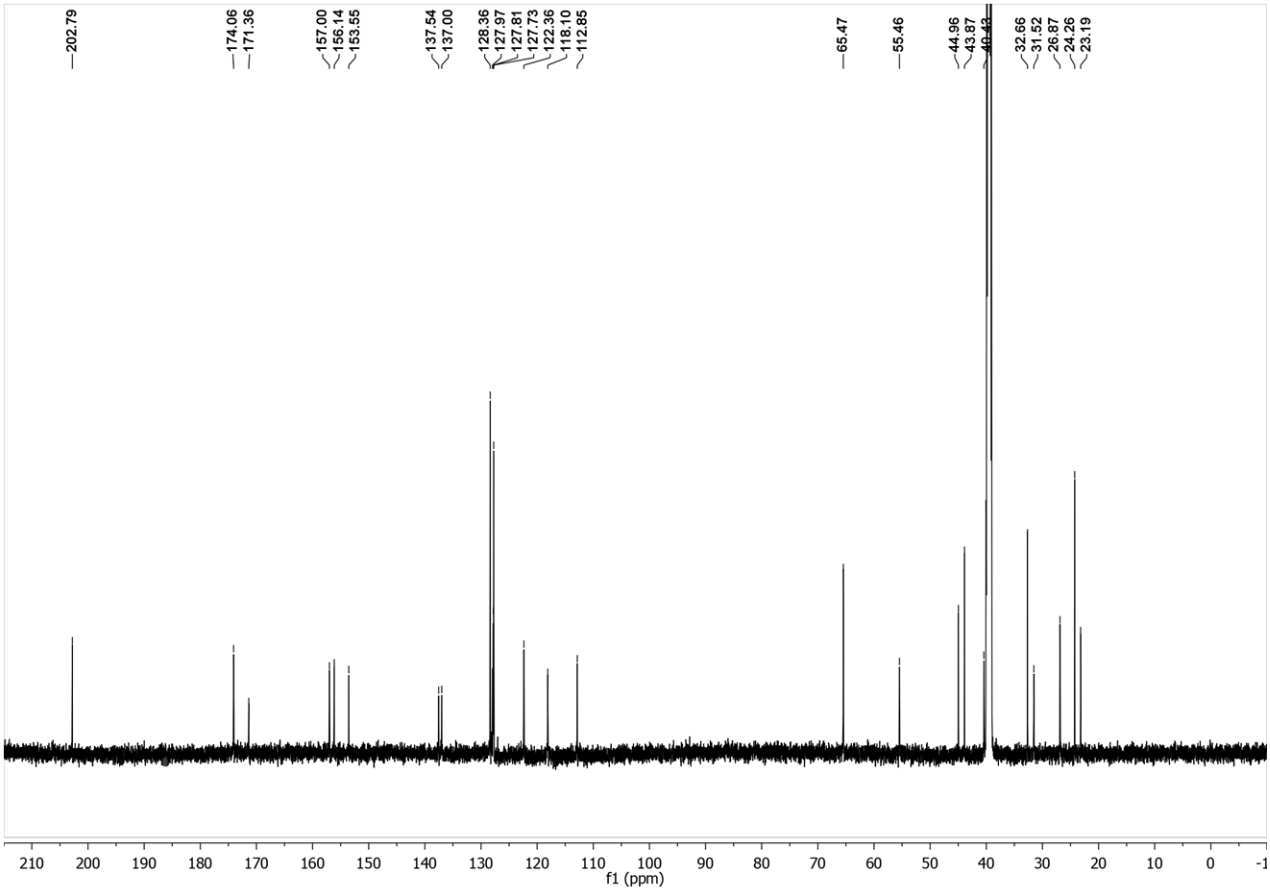

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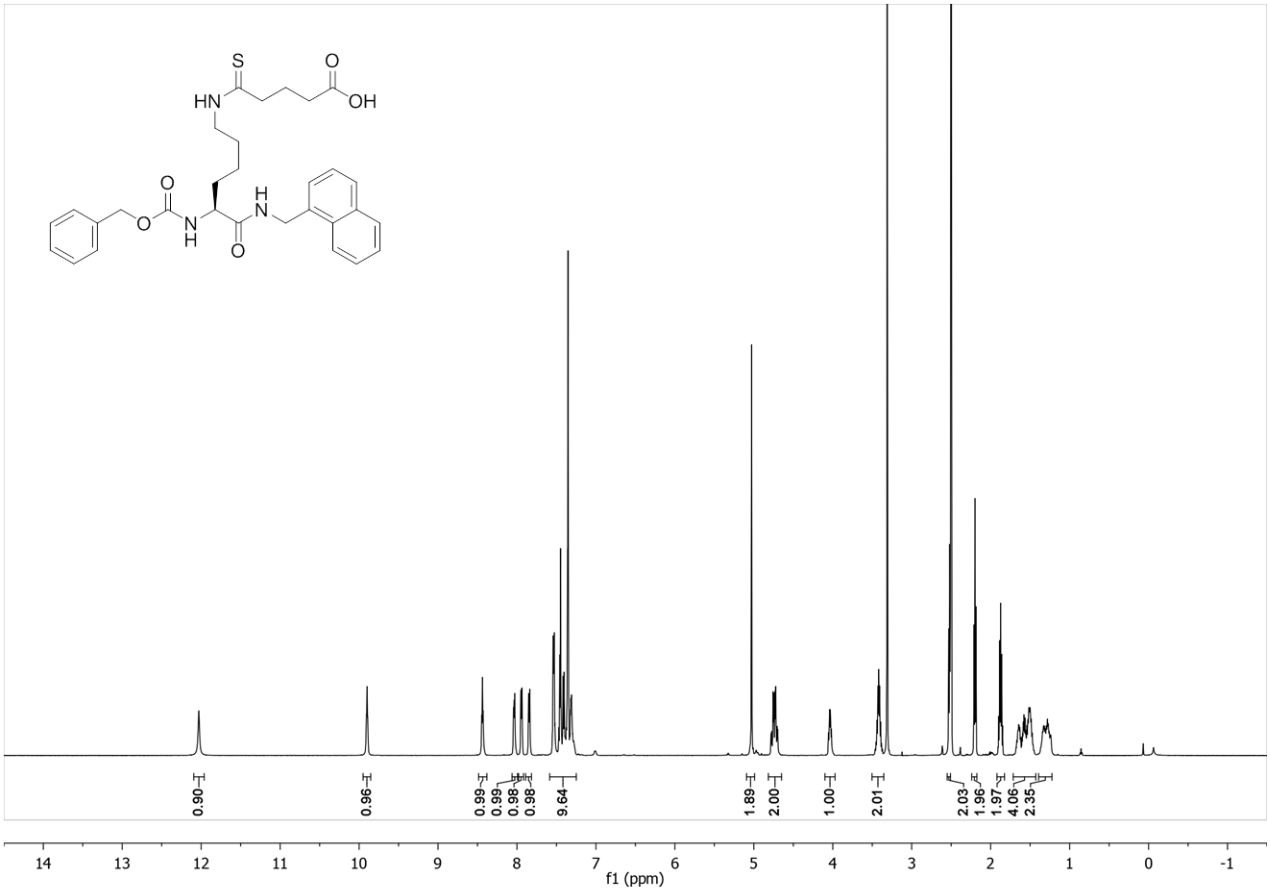

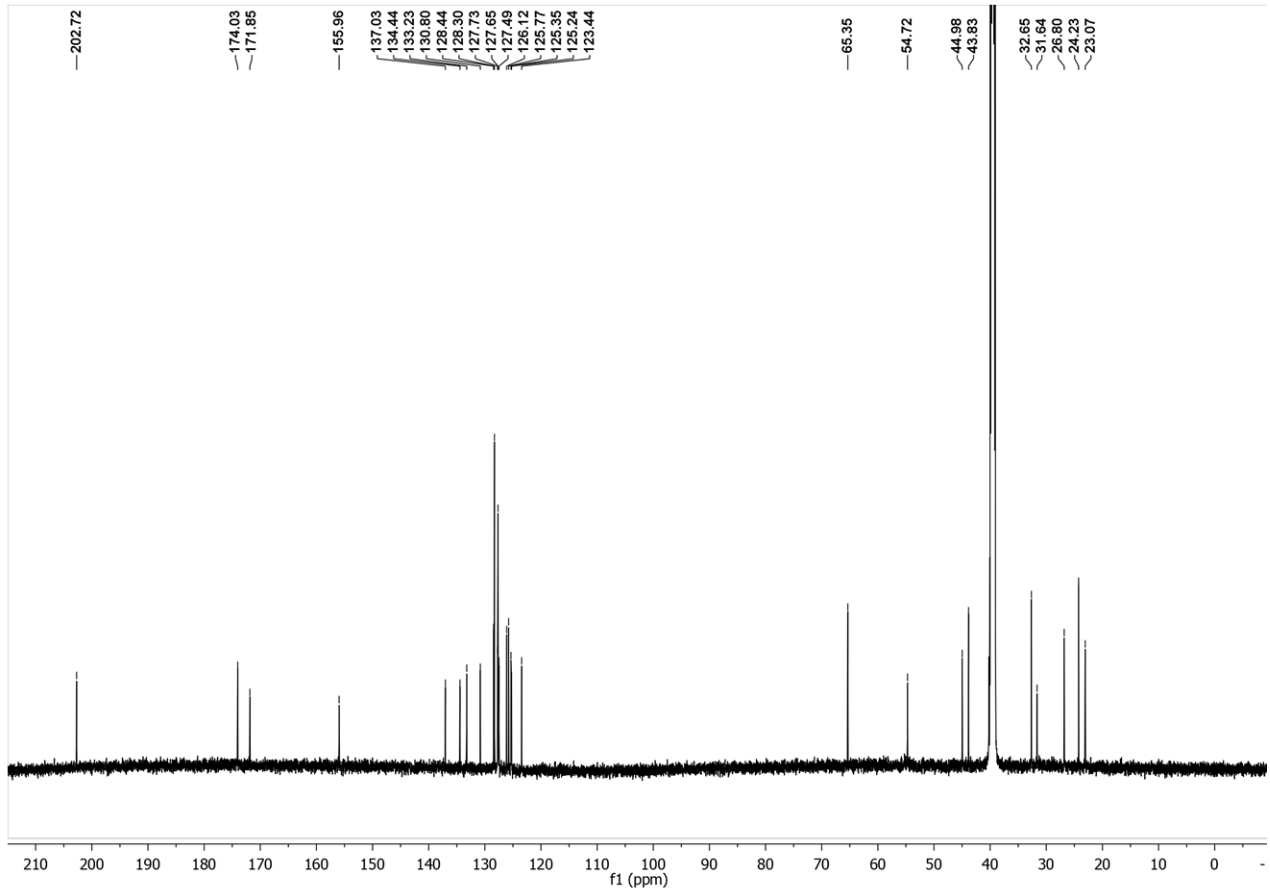

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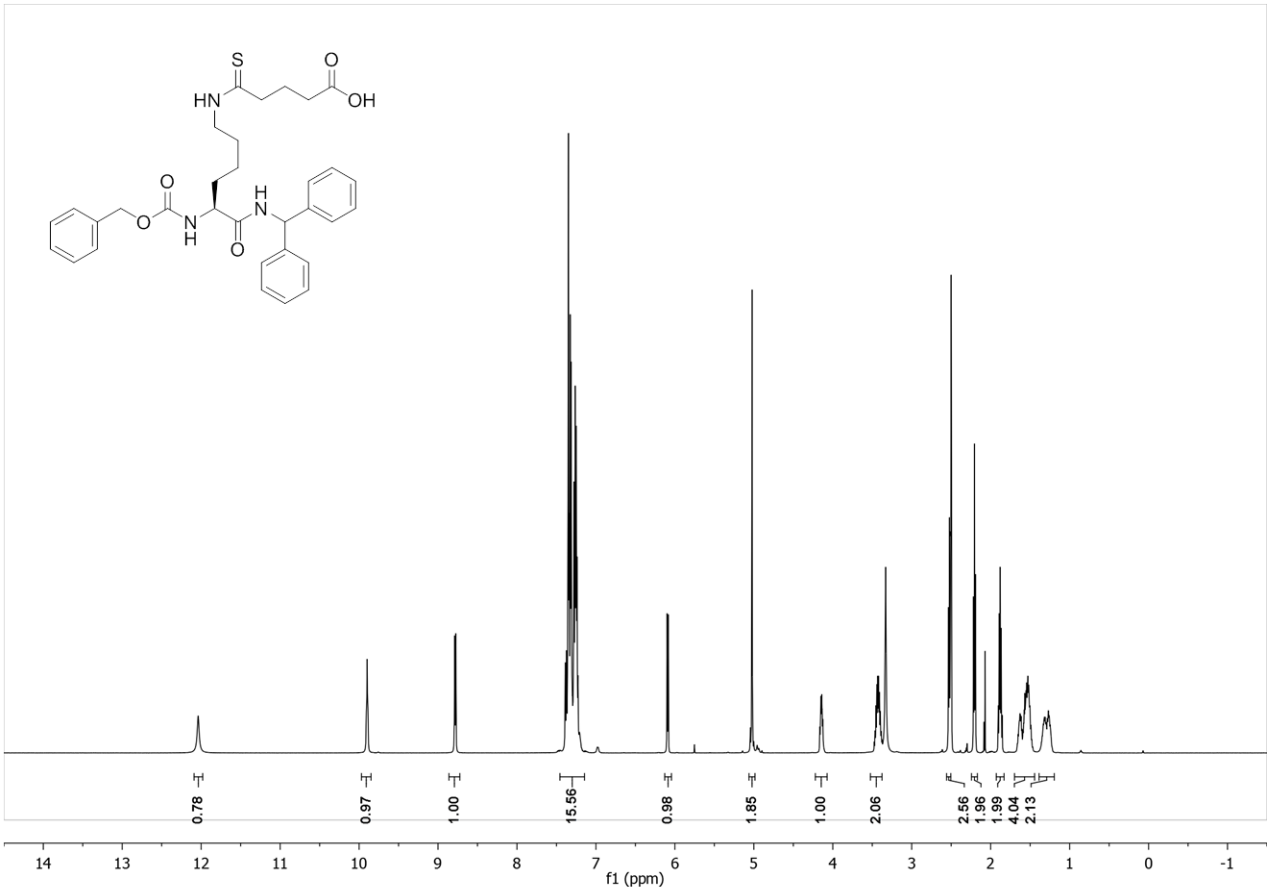

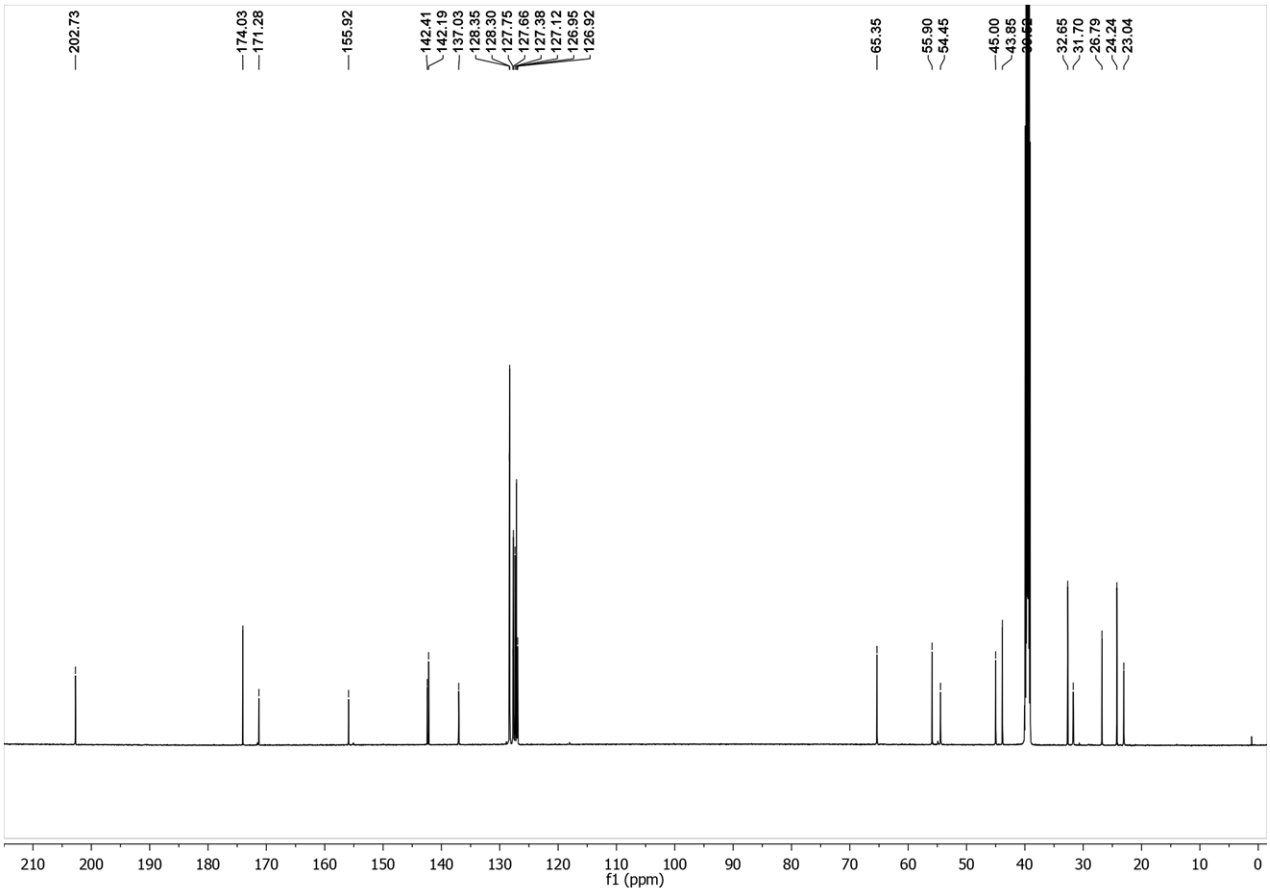

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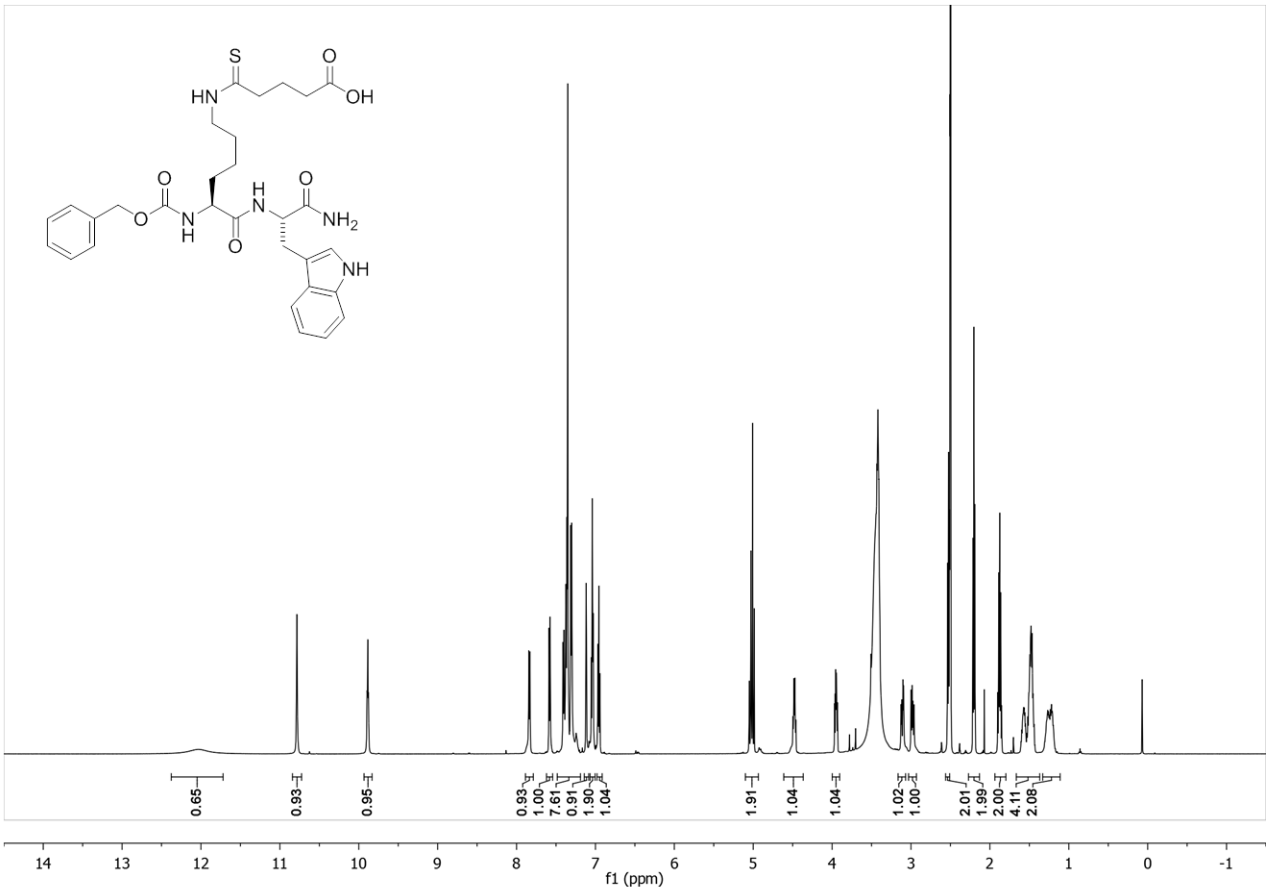

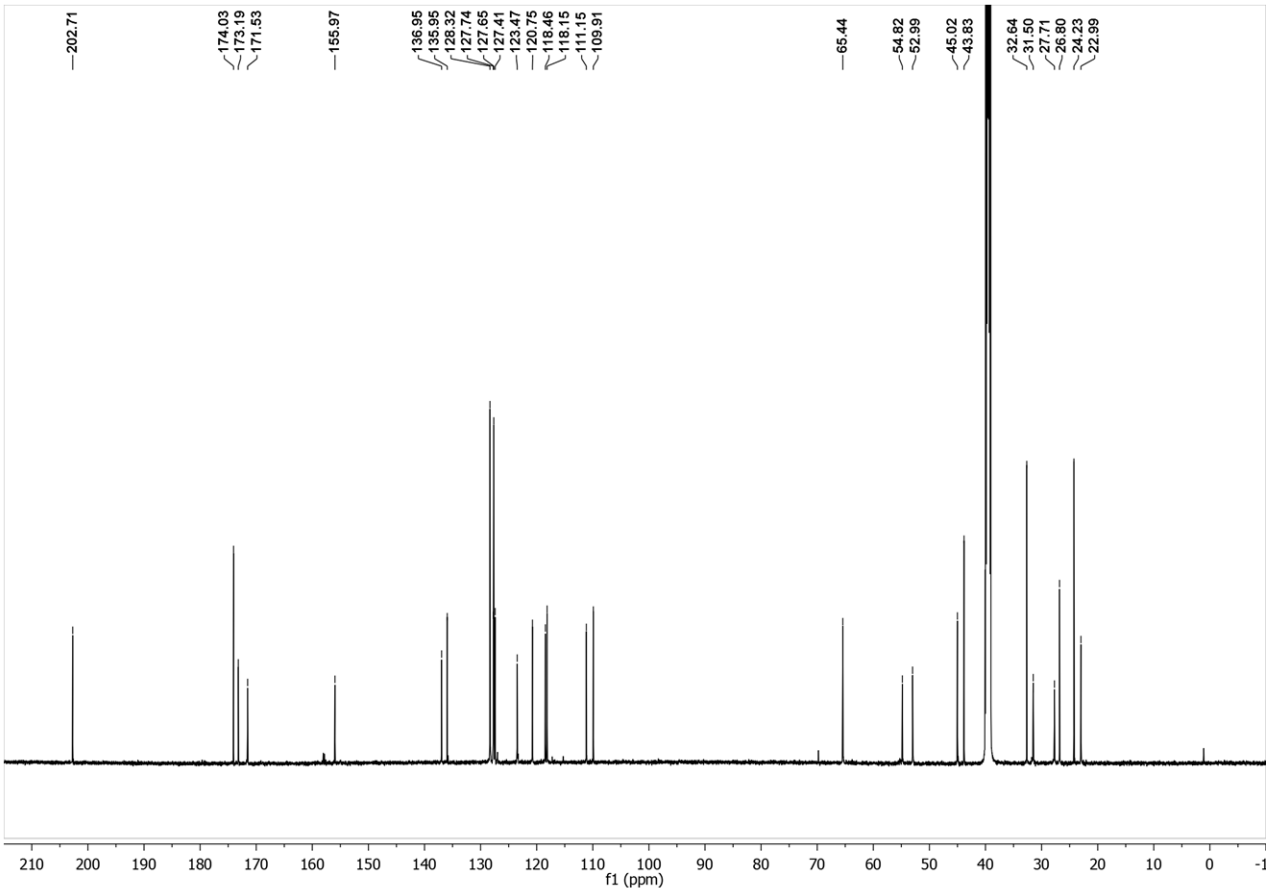

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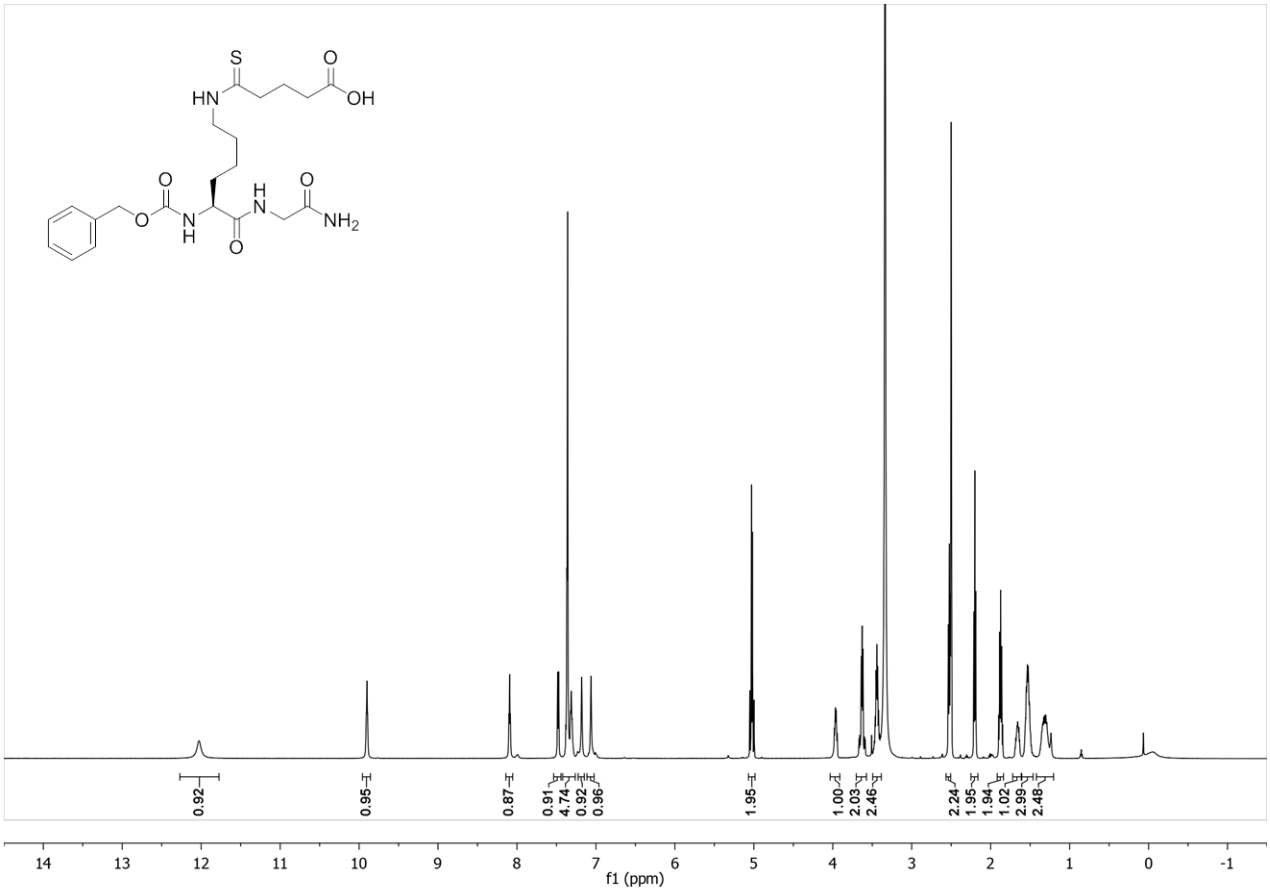

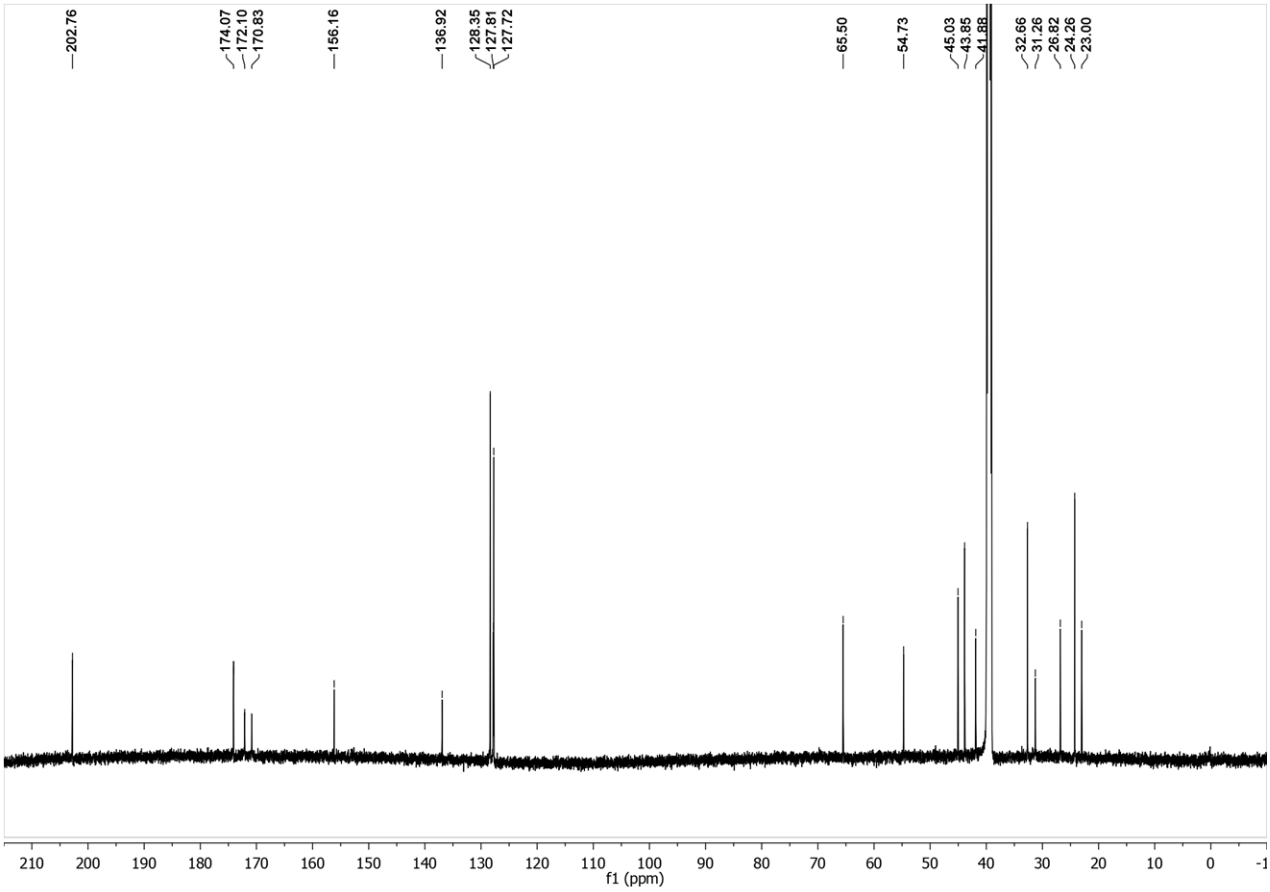

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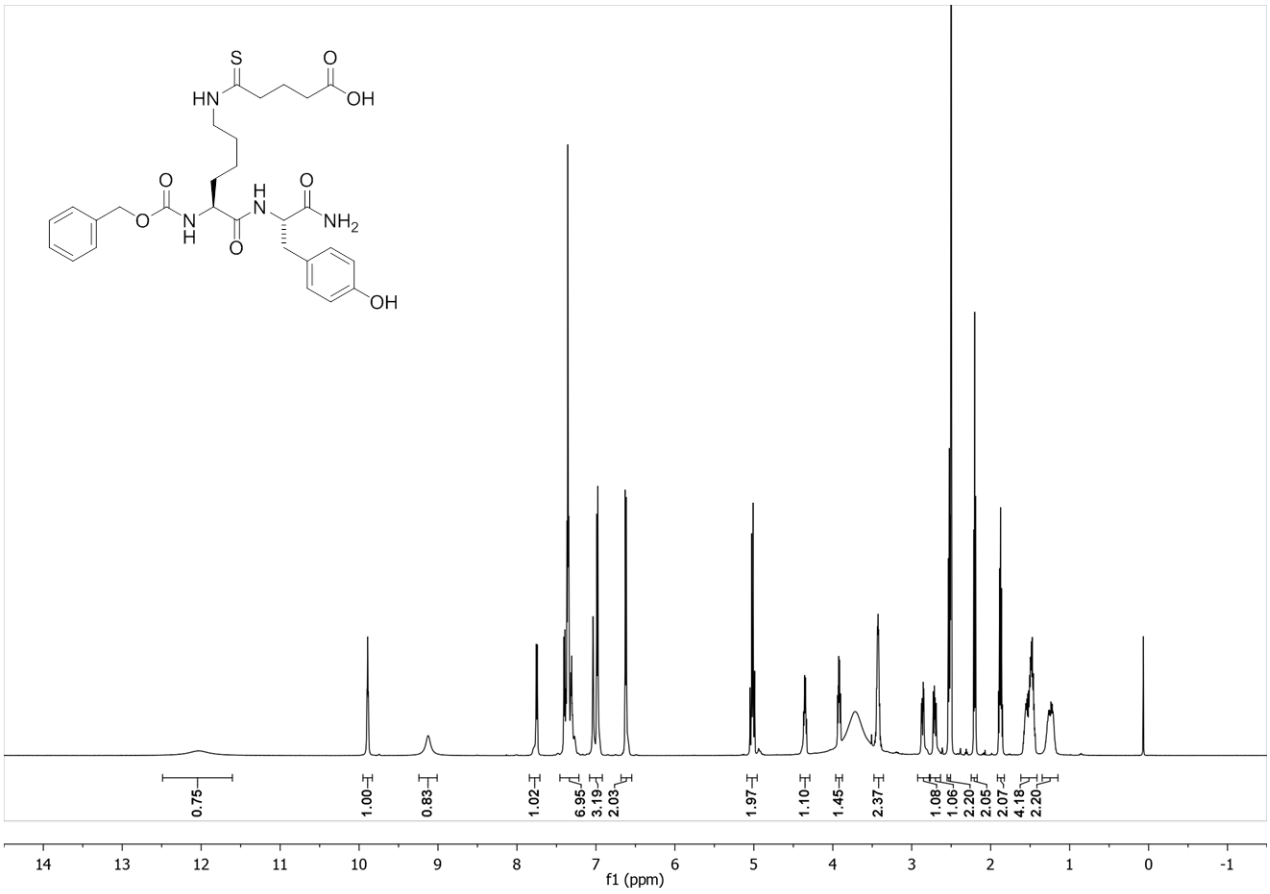

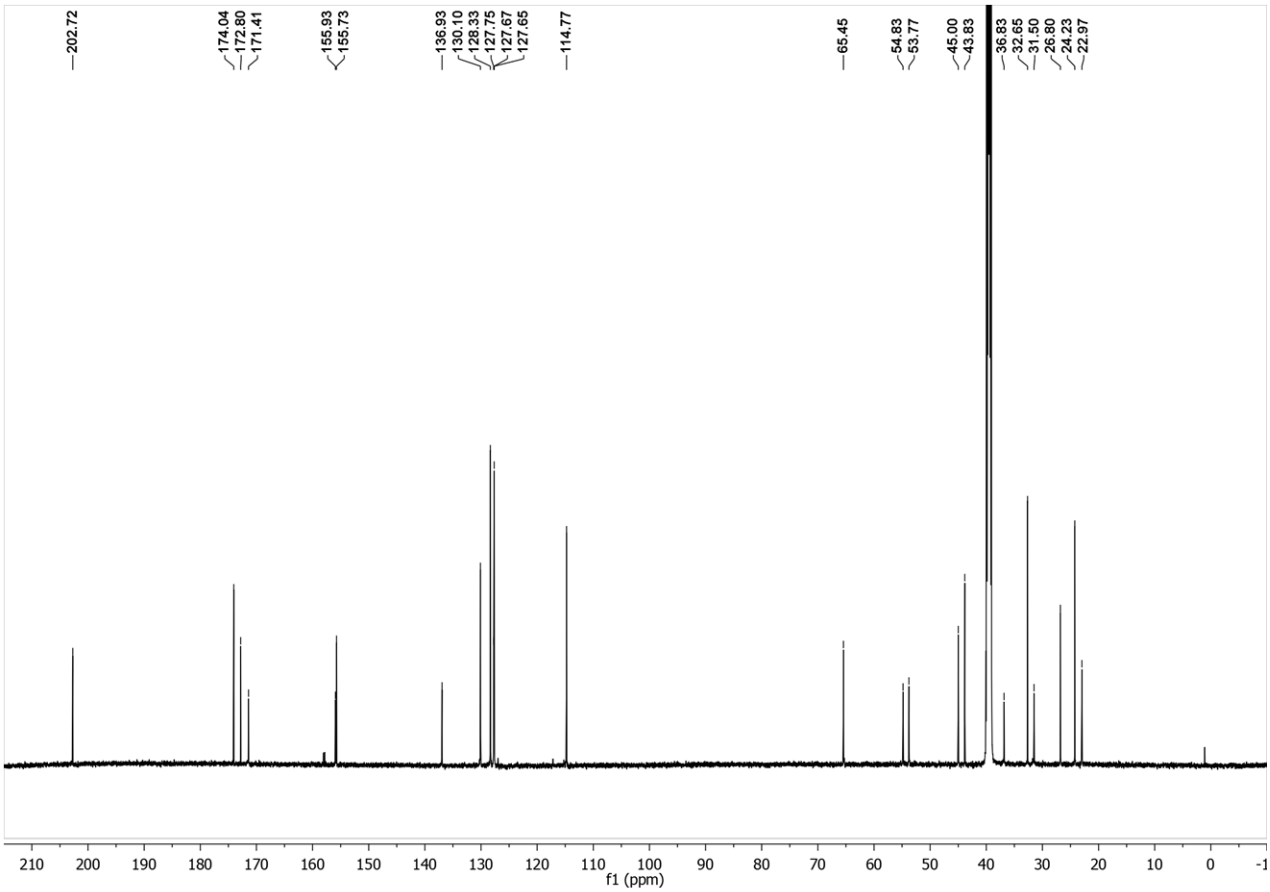

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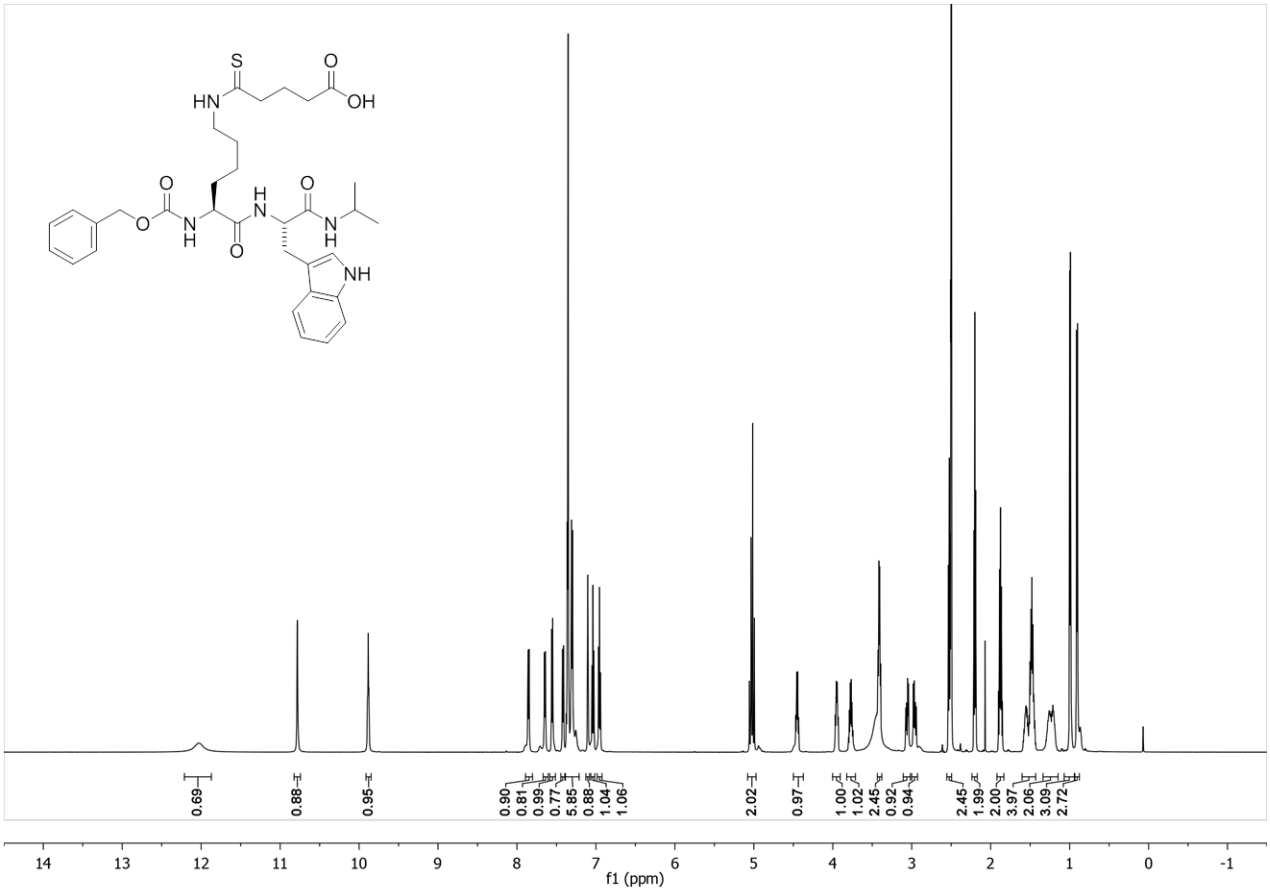

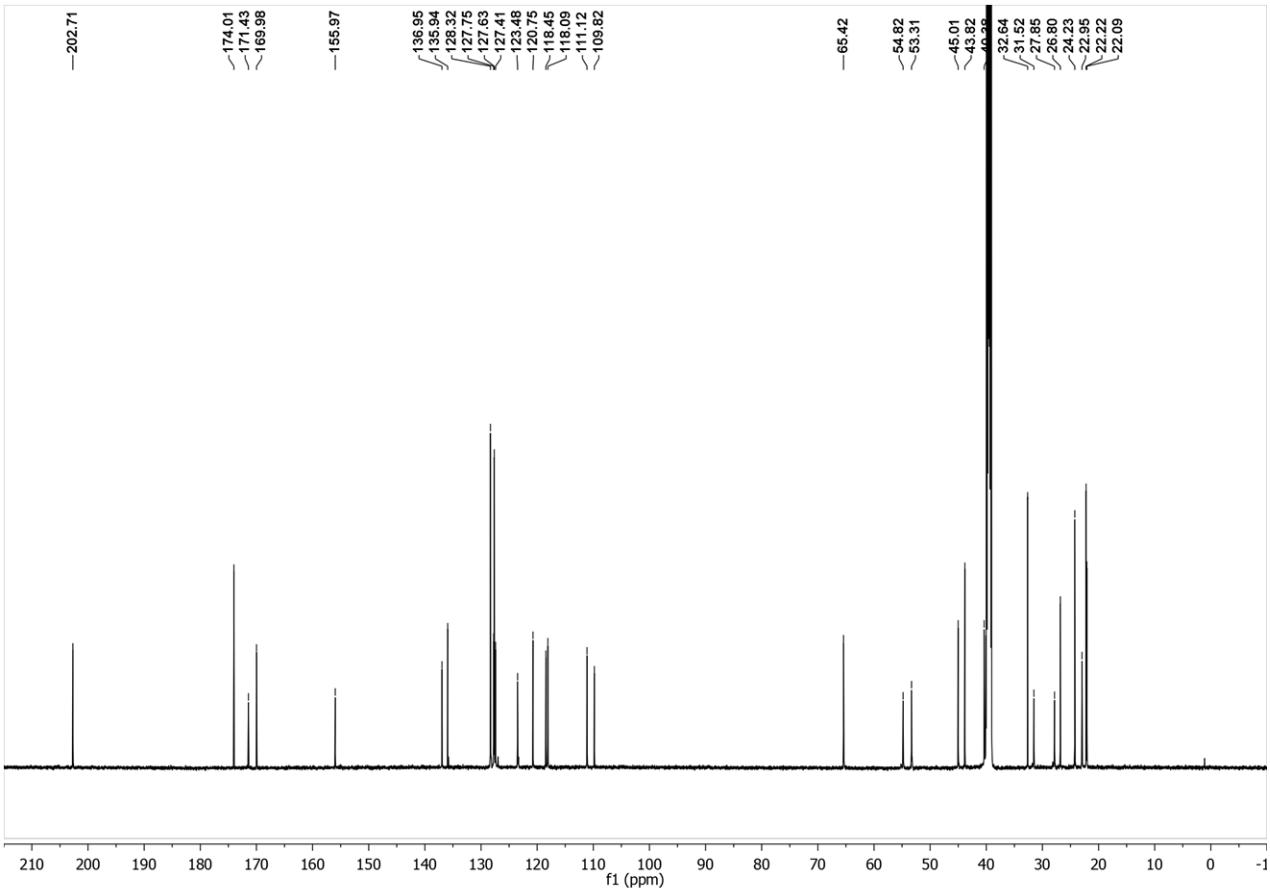

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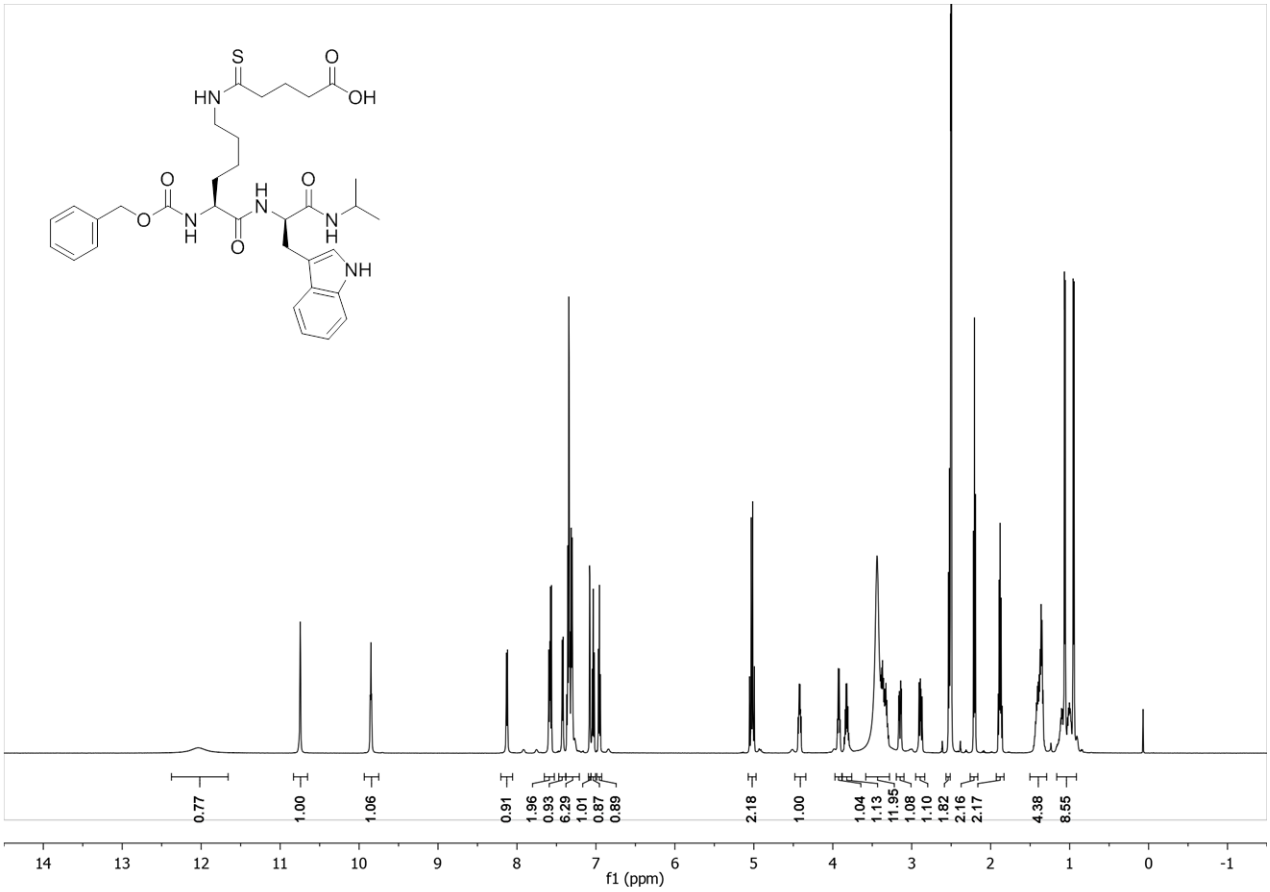
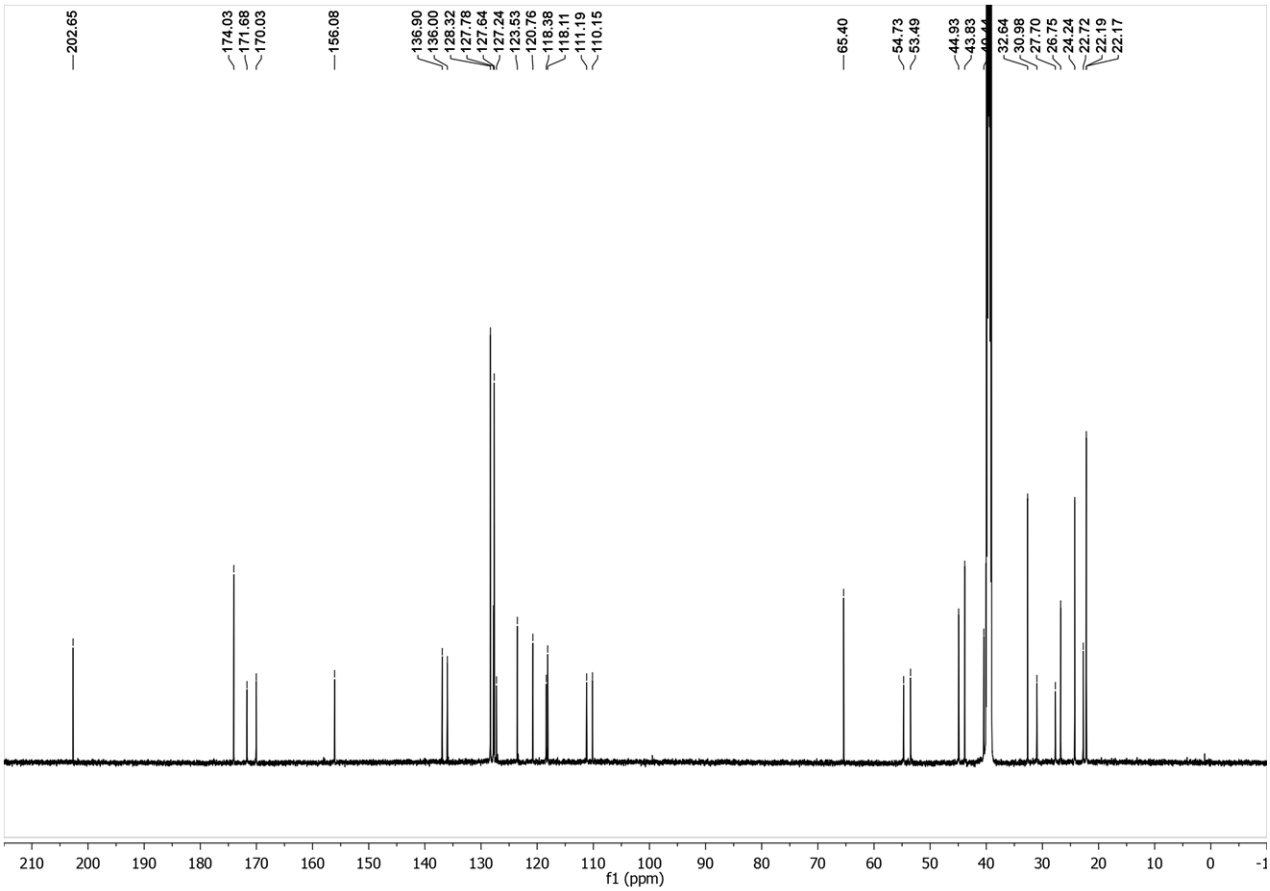
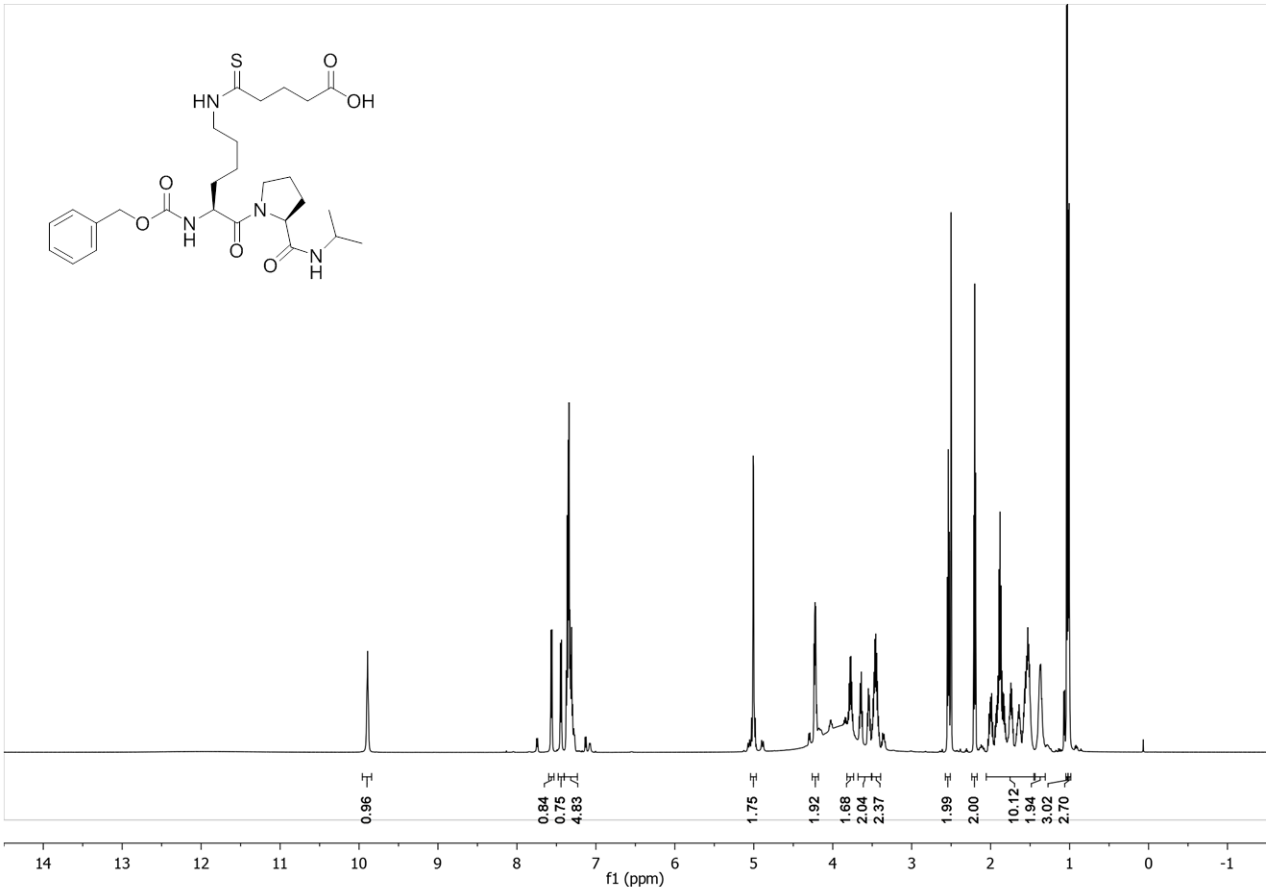
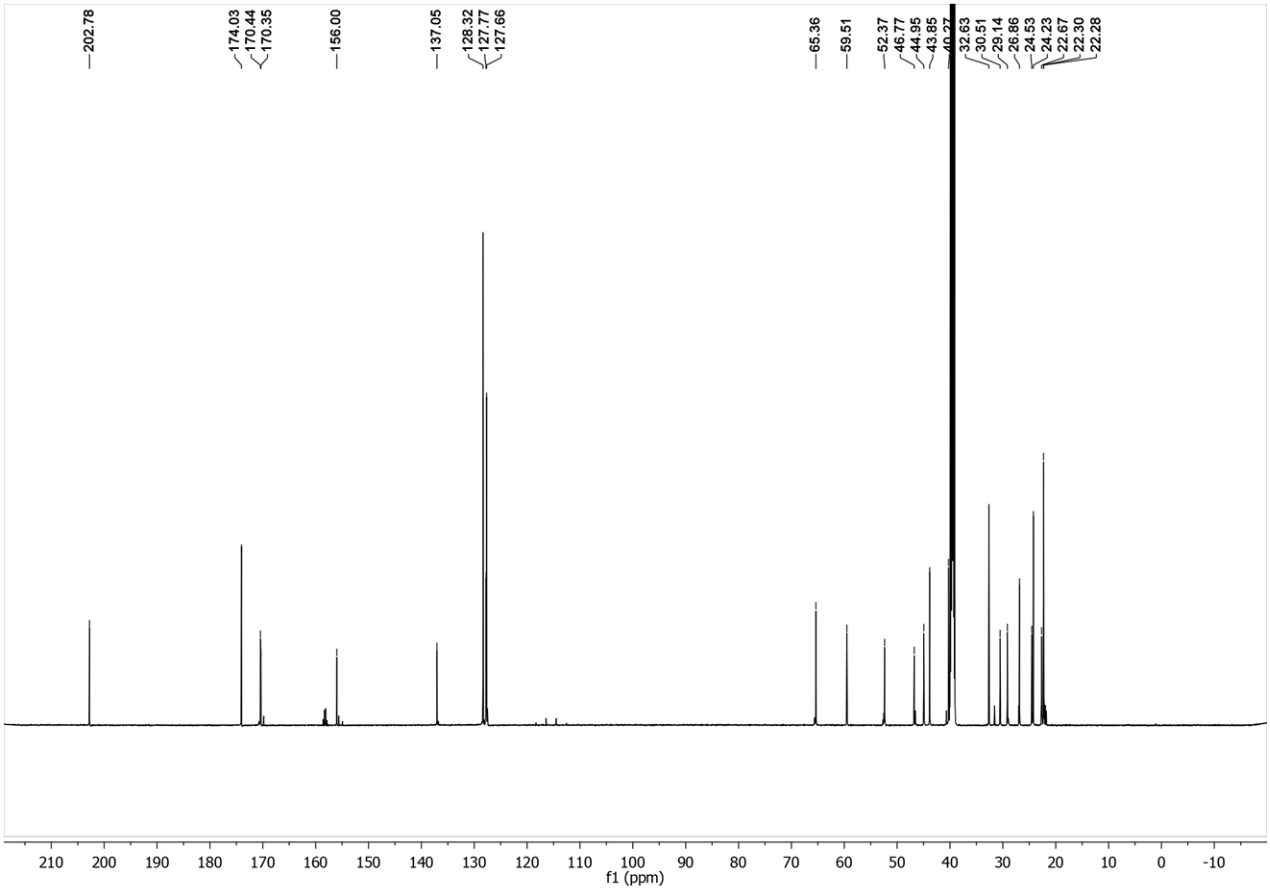


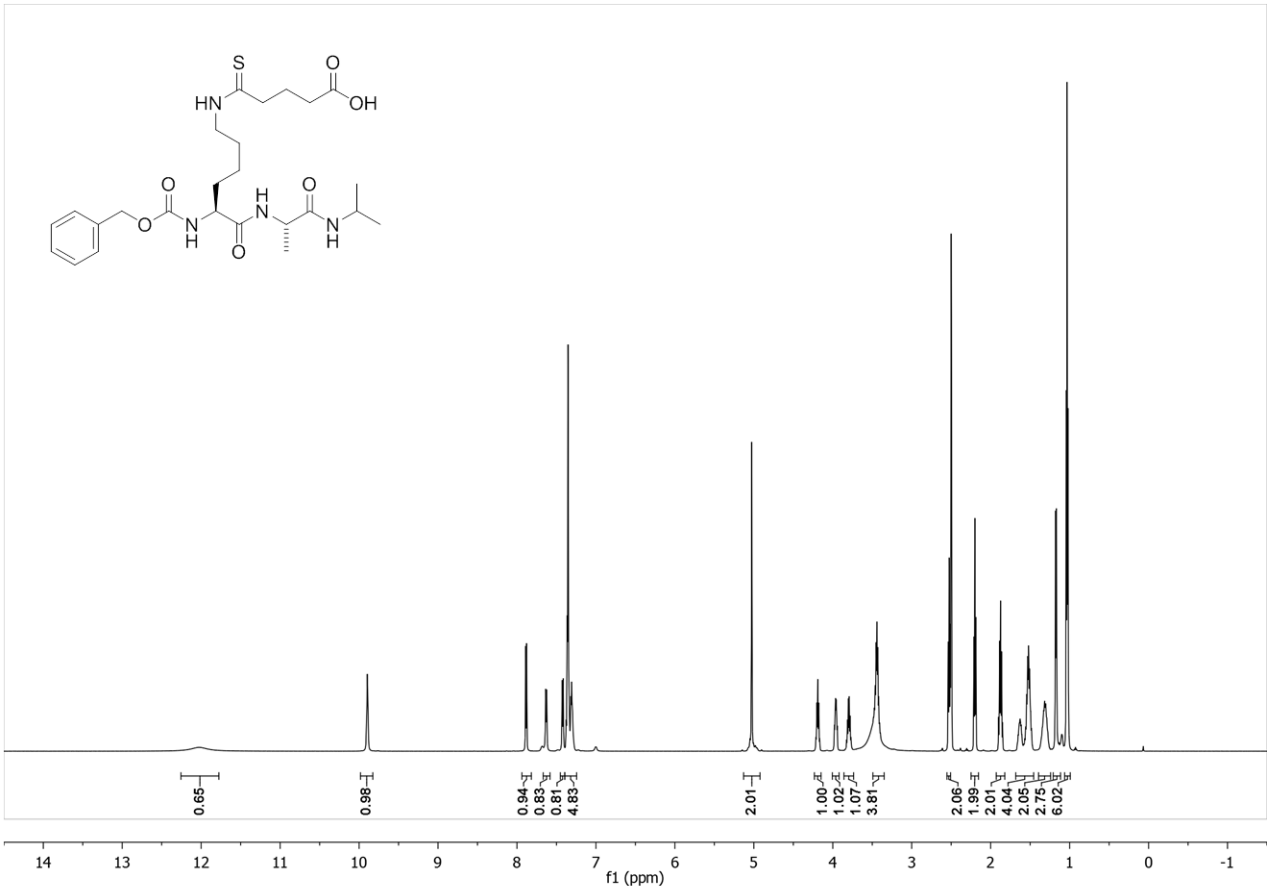


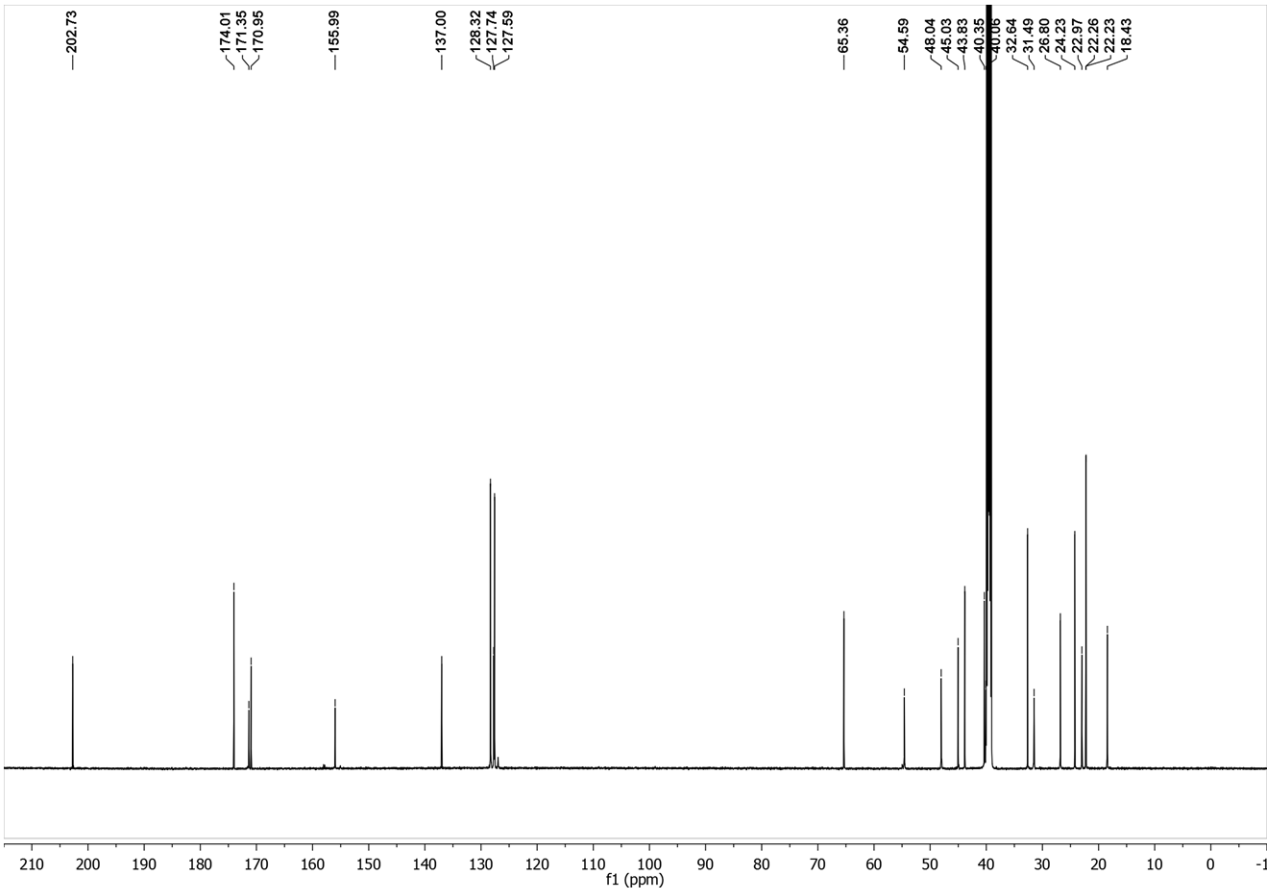
^{1}H and ^{13}C spectra of compound 12.



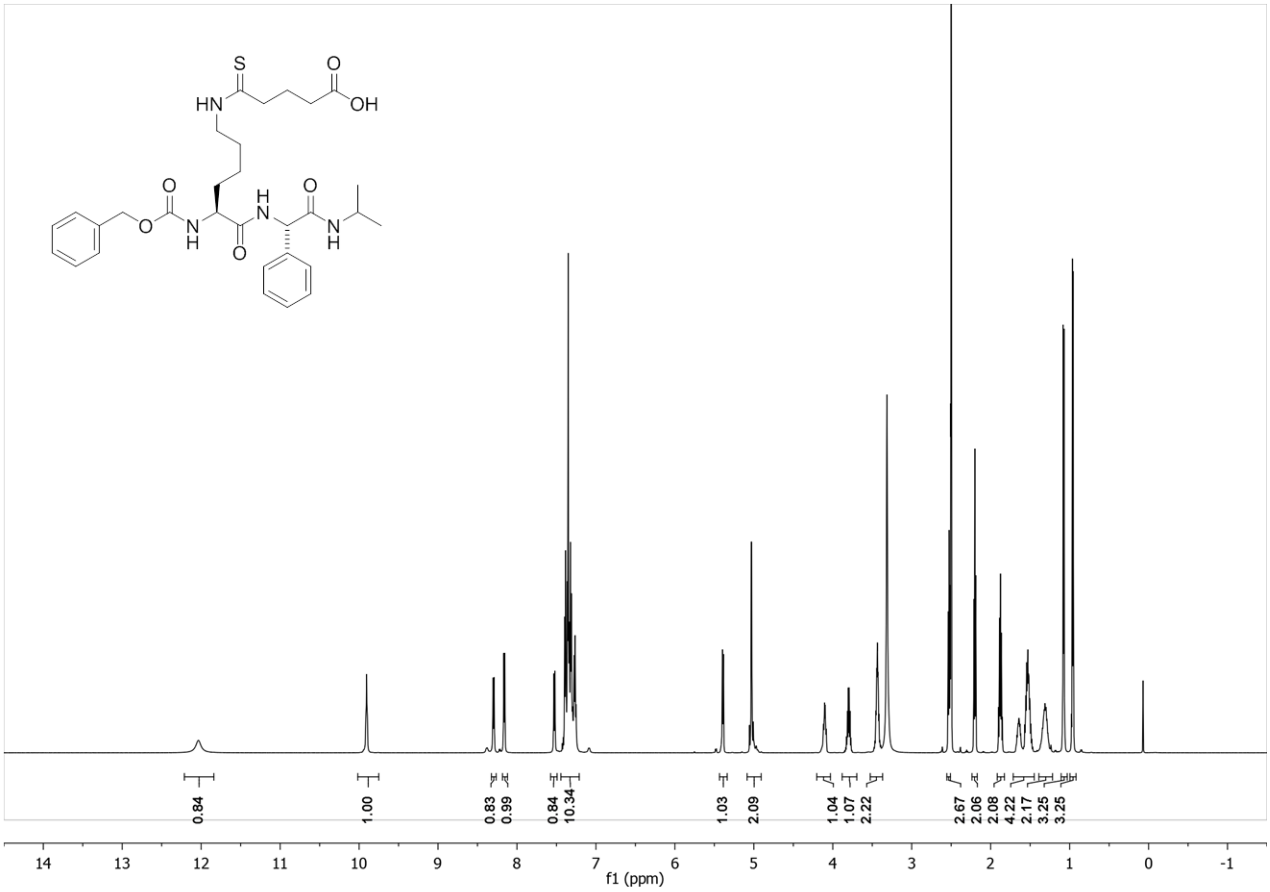


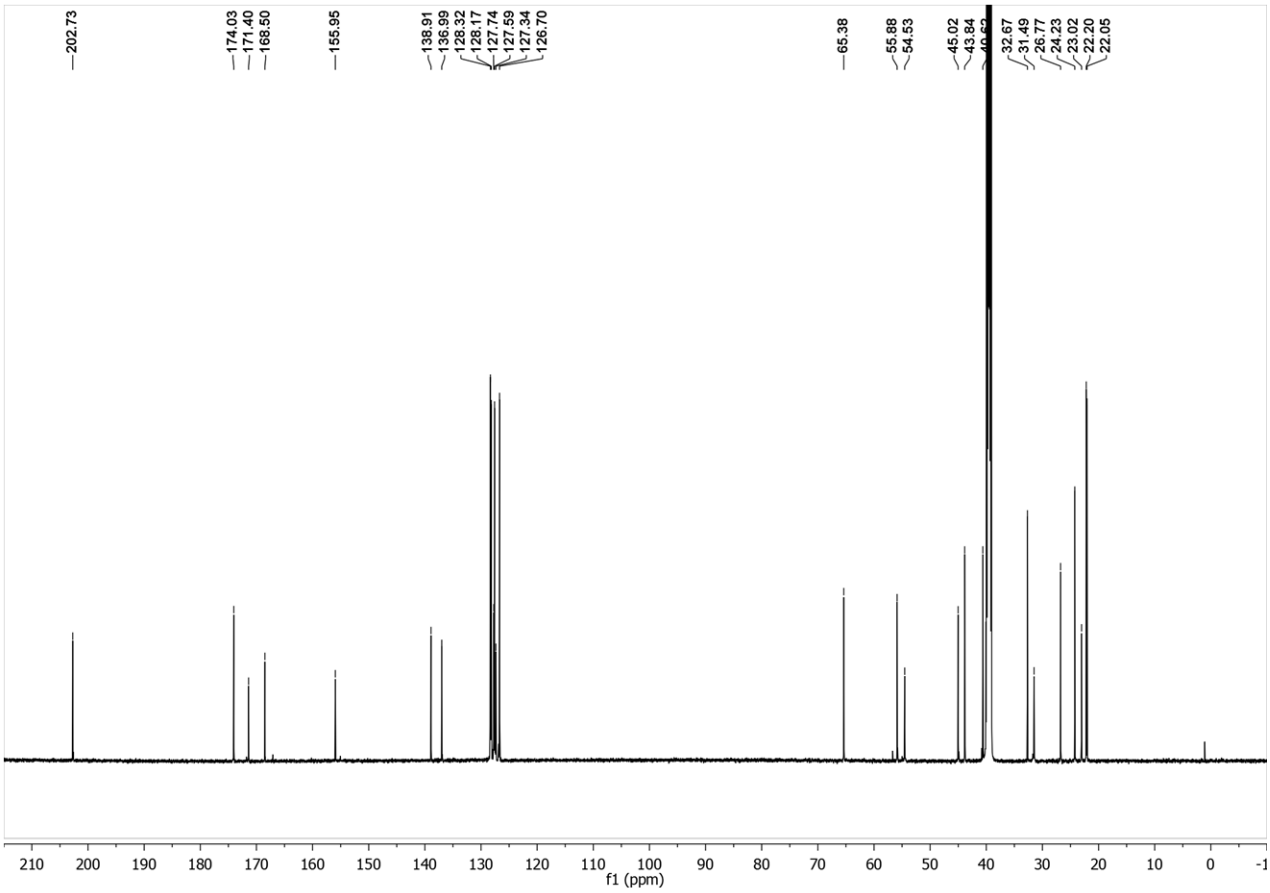
^{1}H and ^{13}C spectra of compound 13.



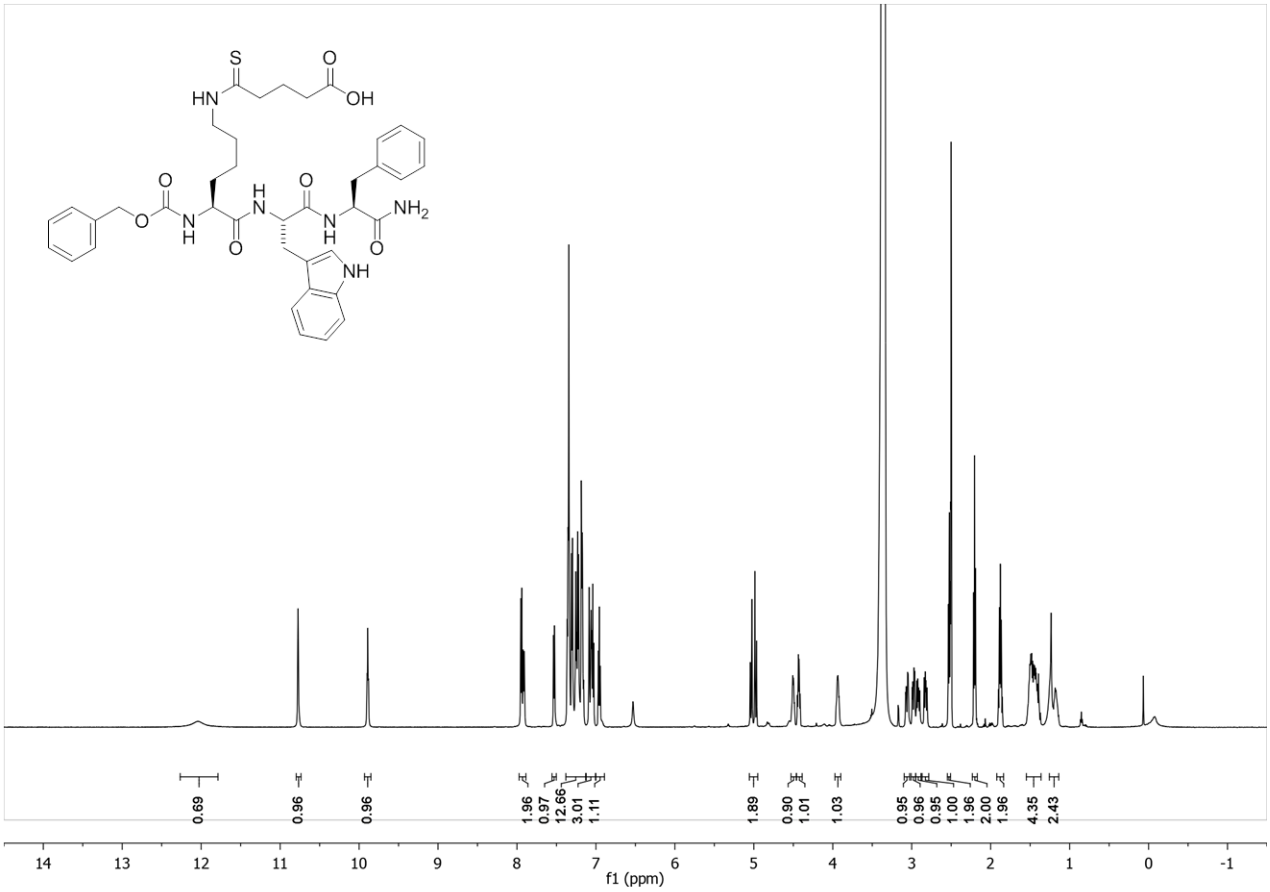


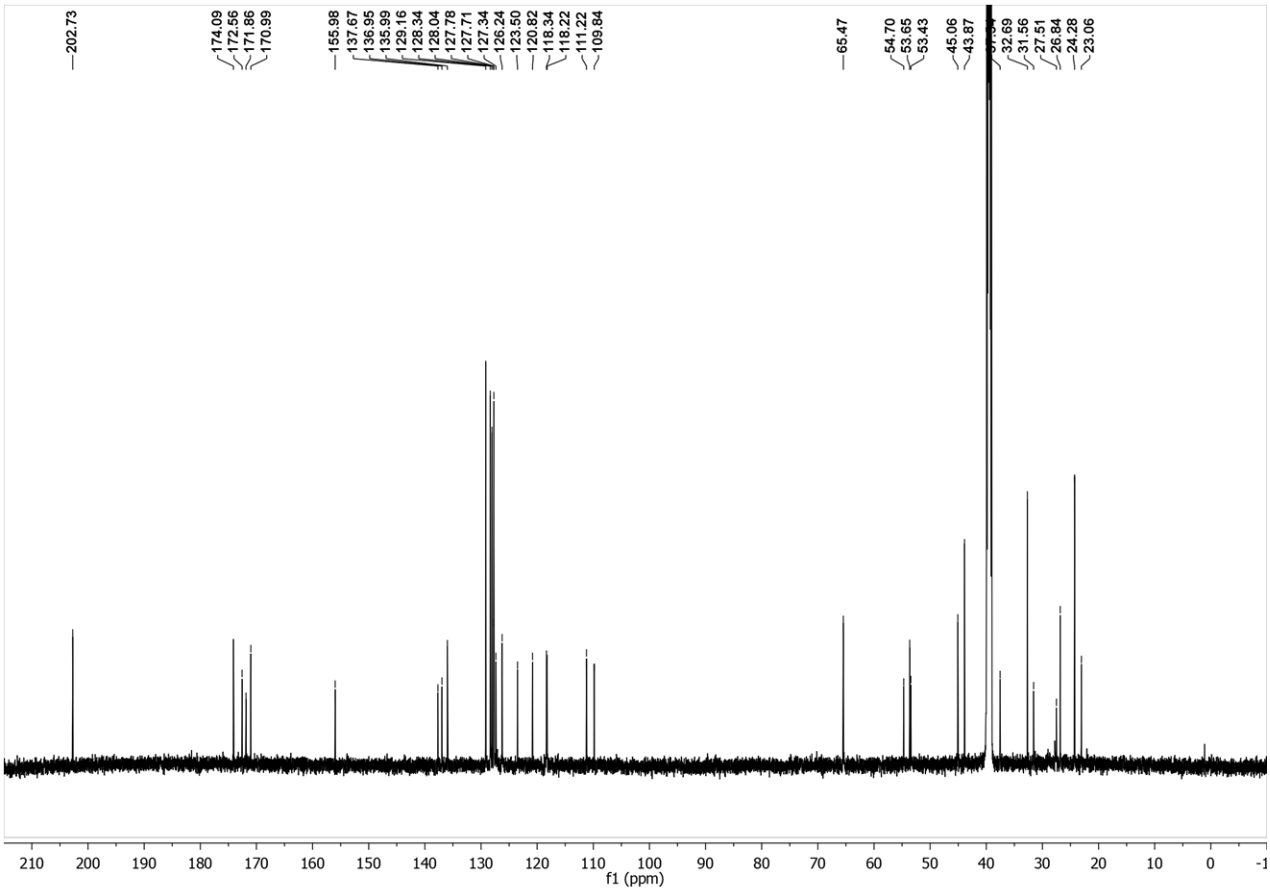
^{1}H and ^{13}C spectra of compound 14.



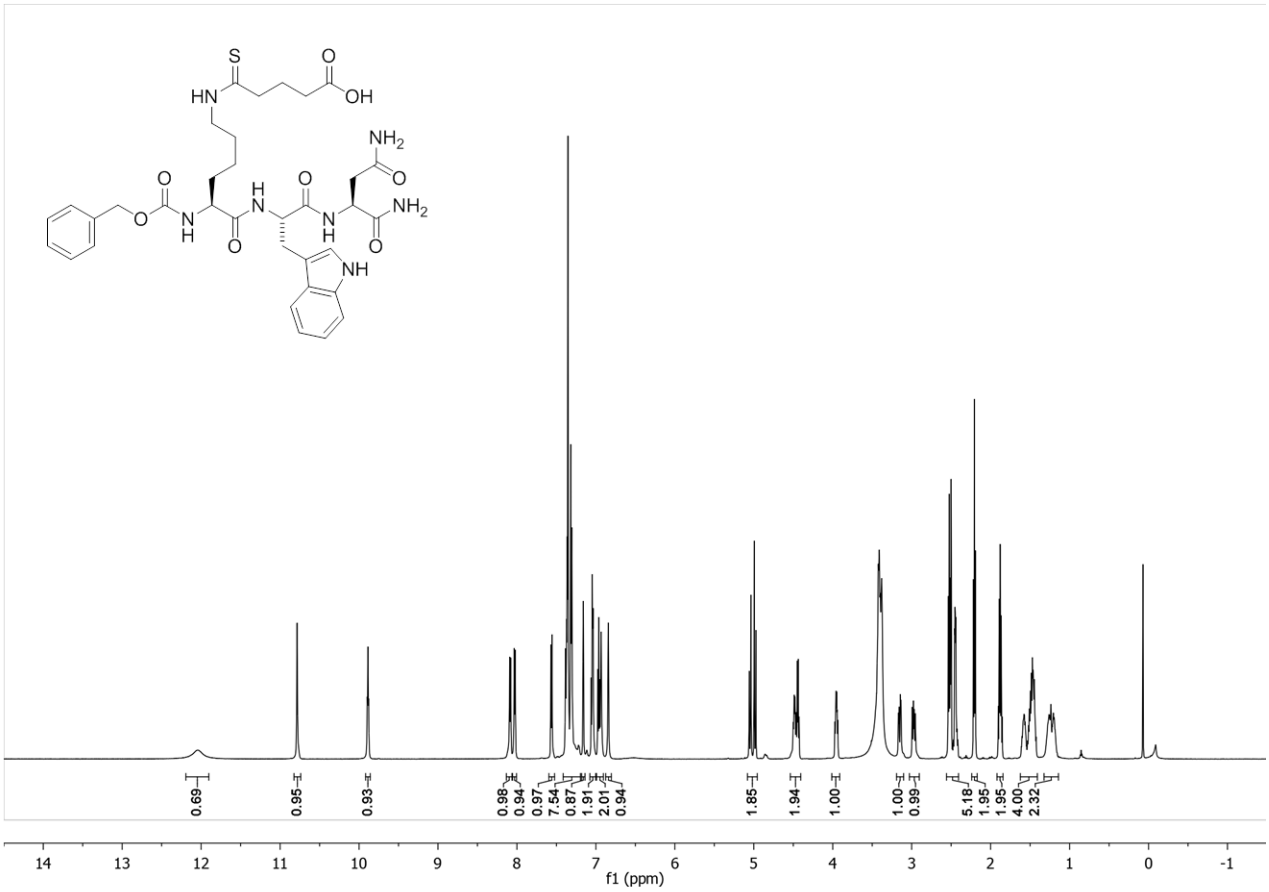


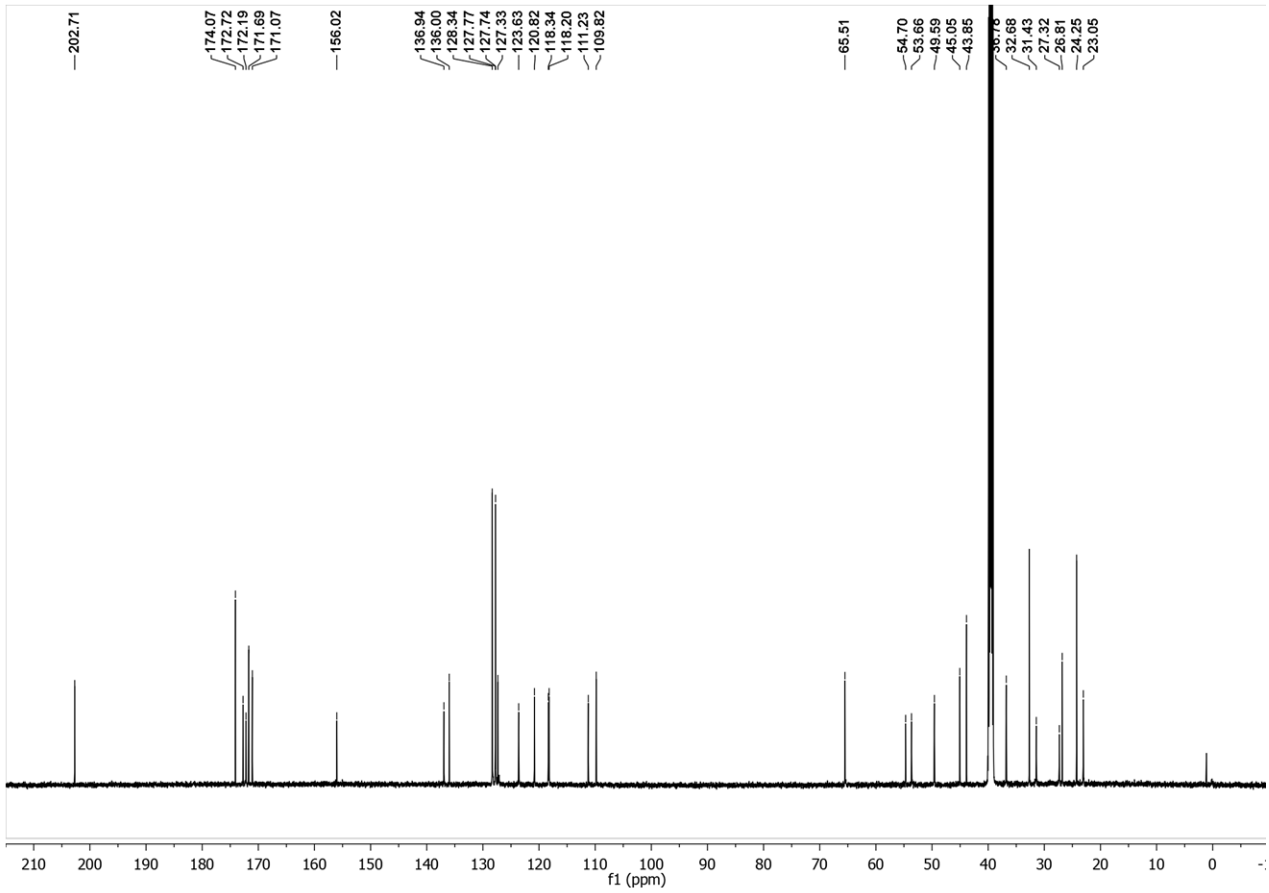
^{1}H and ^{13}C spectra of compound 15.



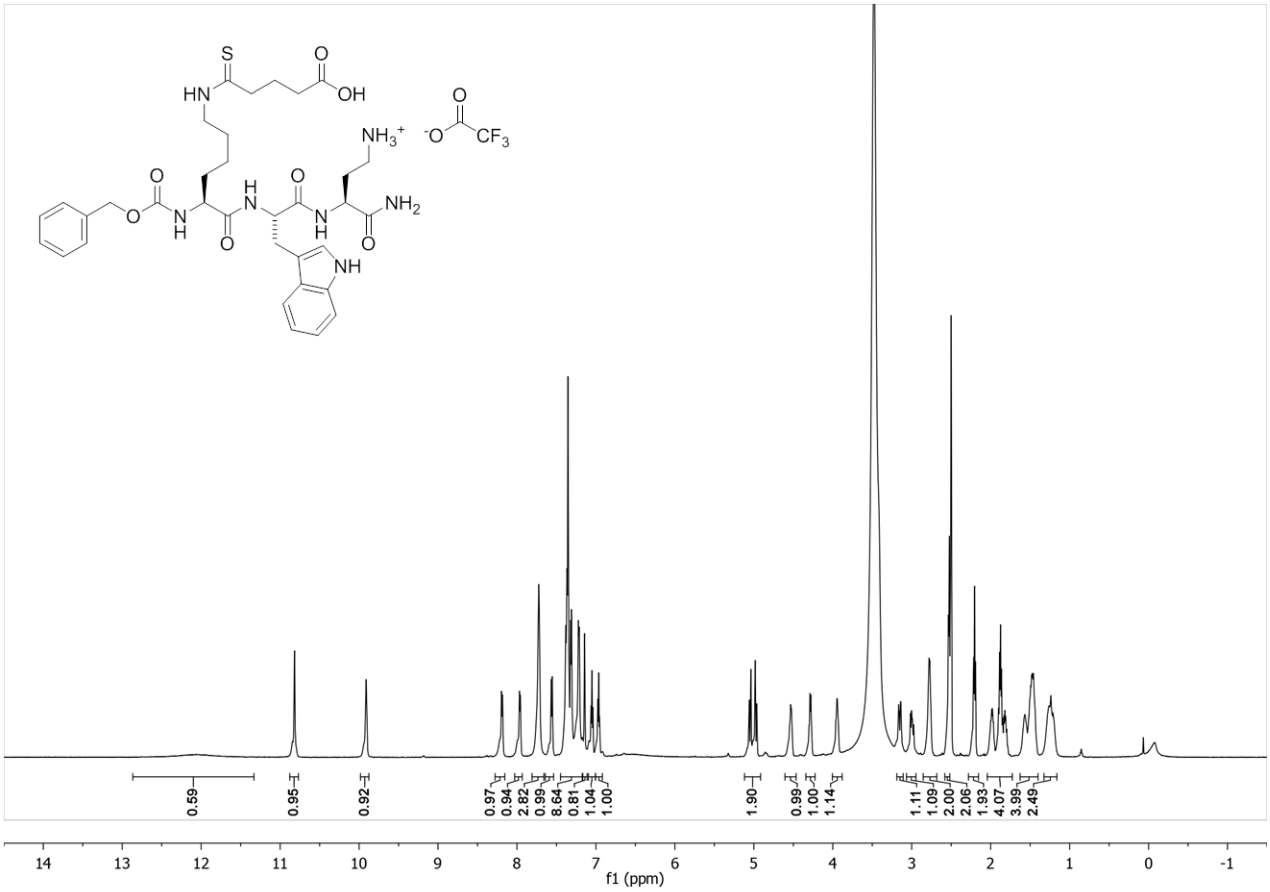


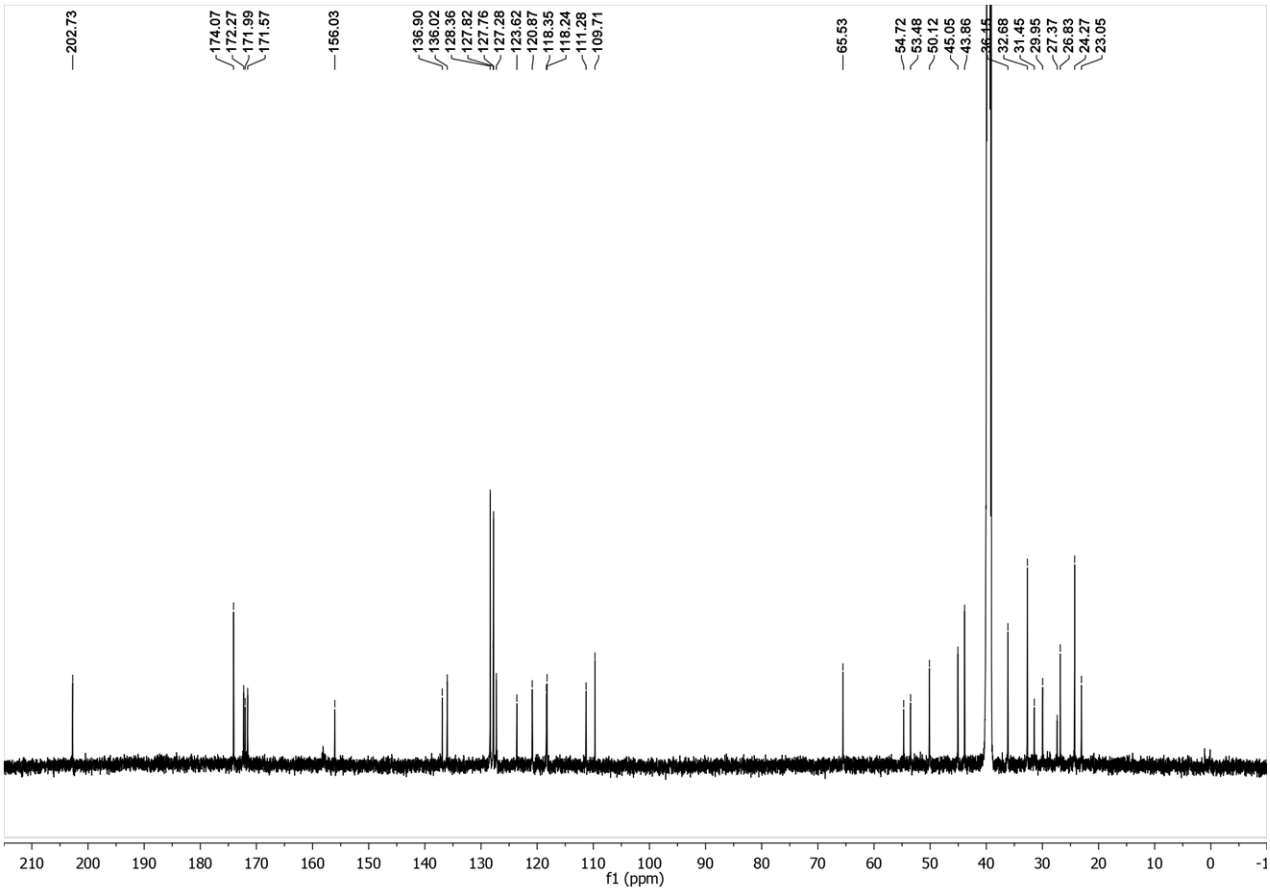
^{1}H and ^{13}C spectra of compound 16.



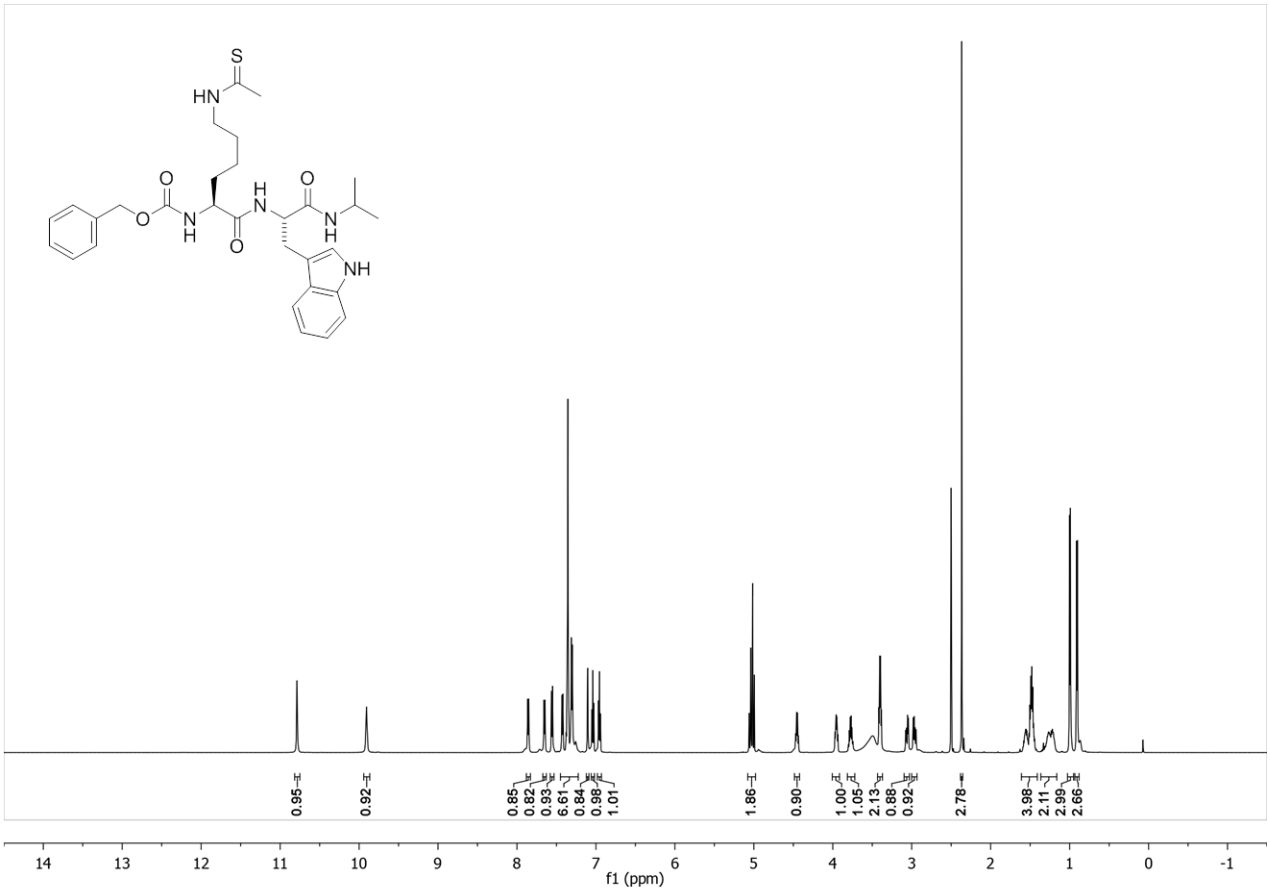


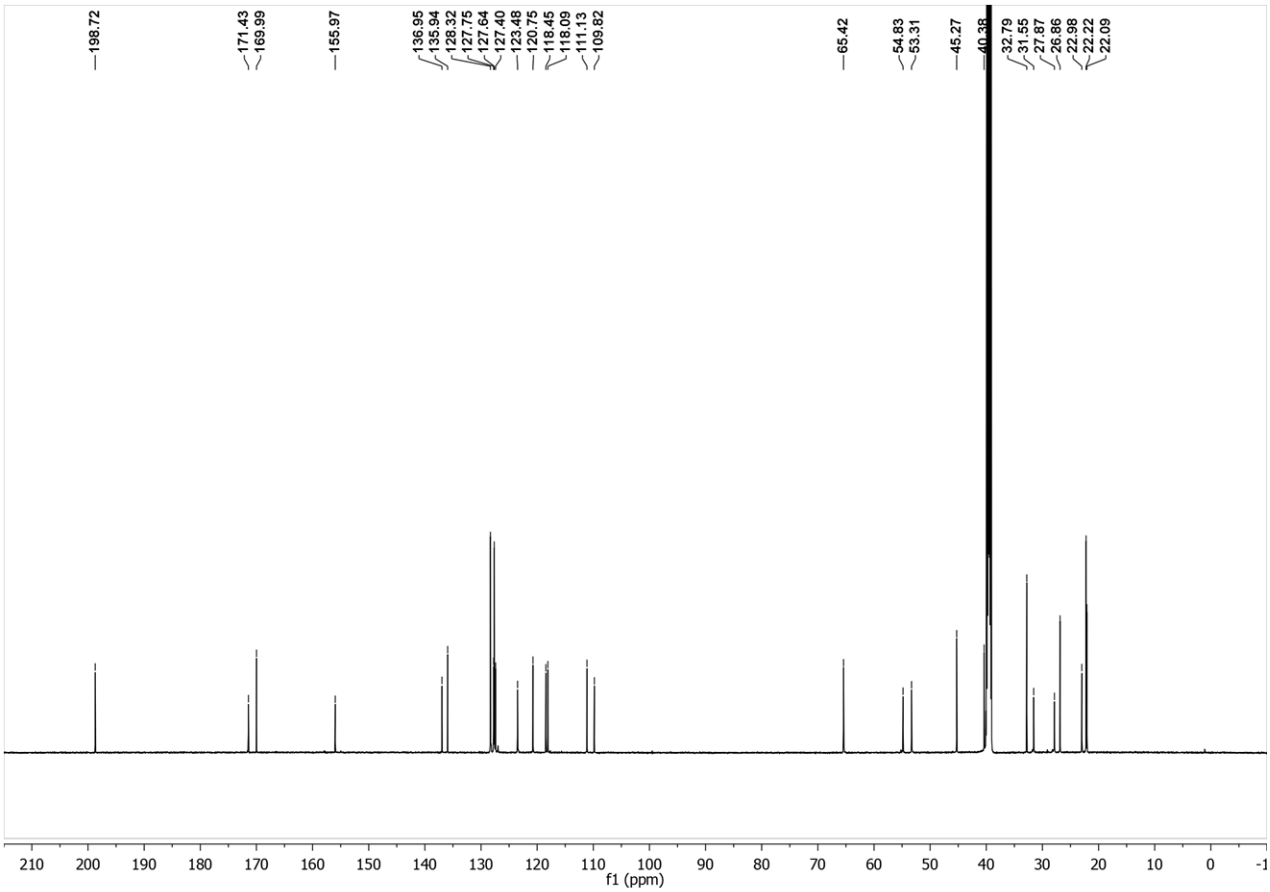
^{1}H and ^{13}C spectra of compound 17.



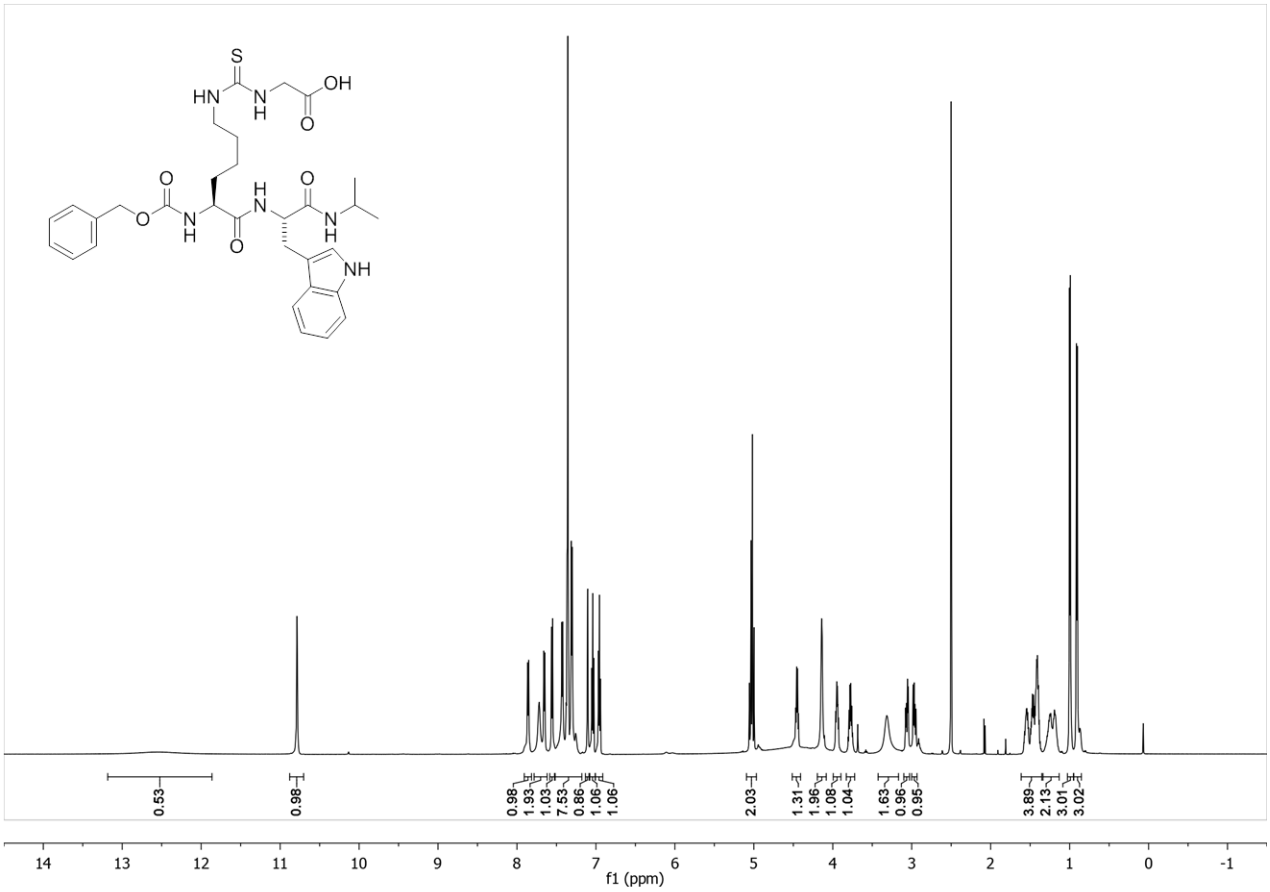


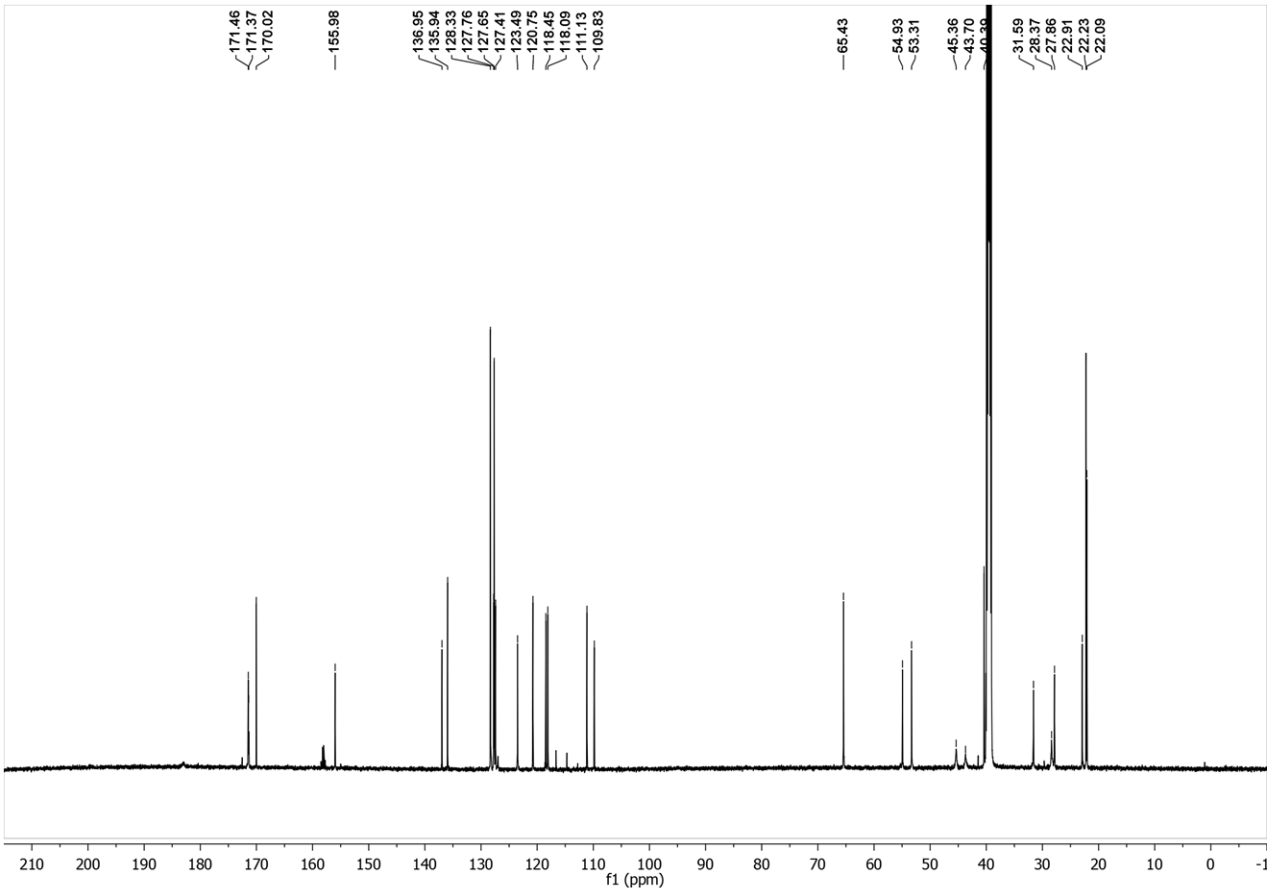
^{1}H and ^{13}C spectra of compound 18.



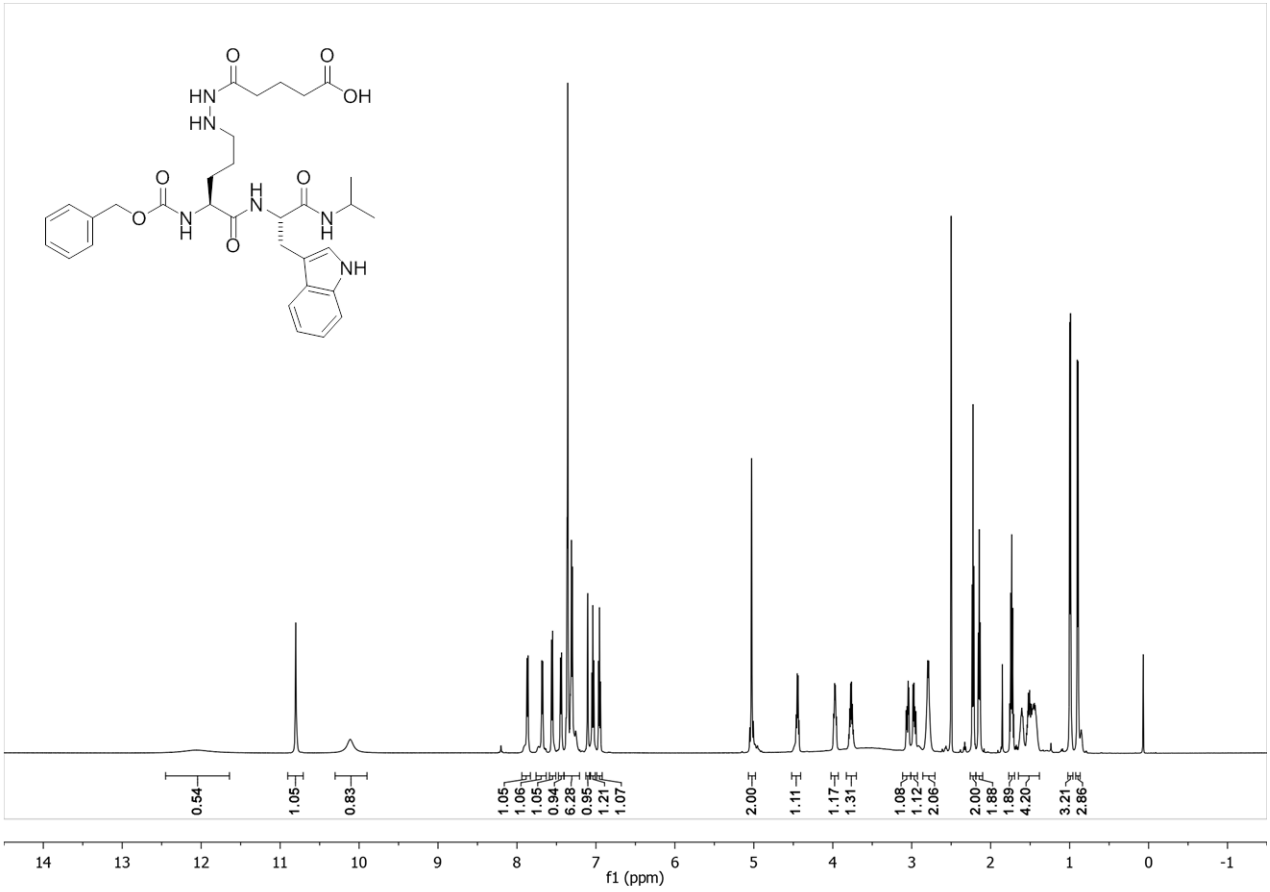


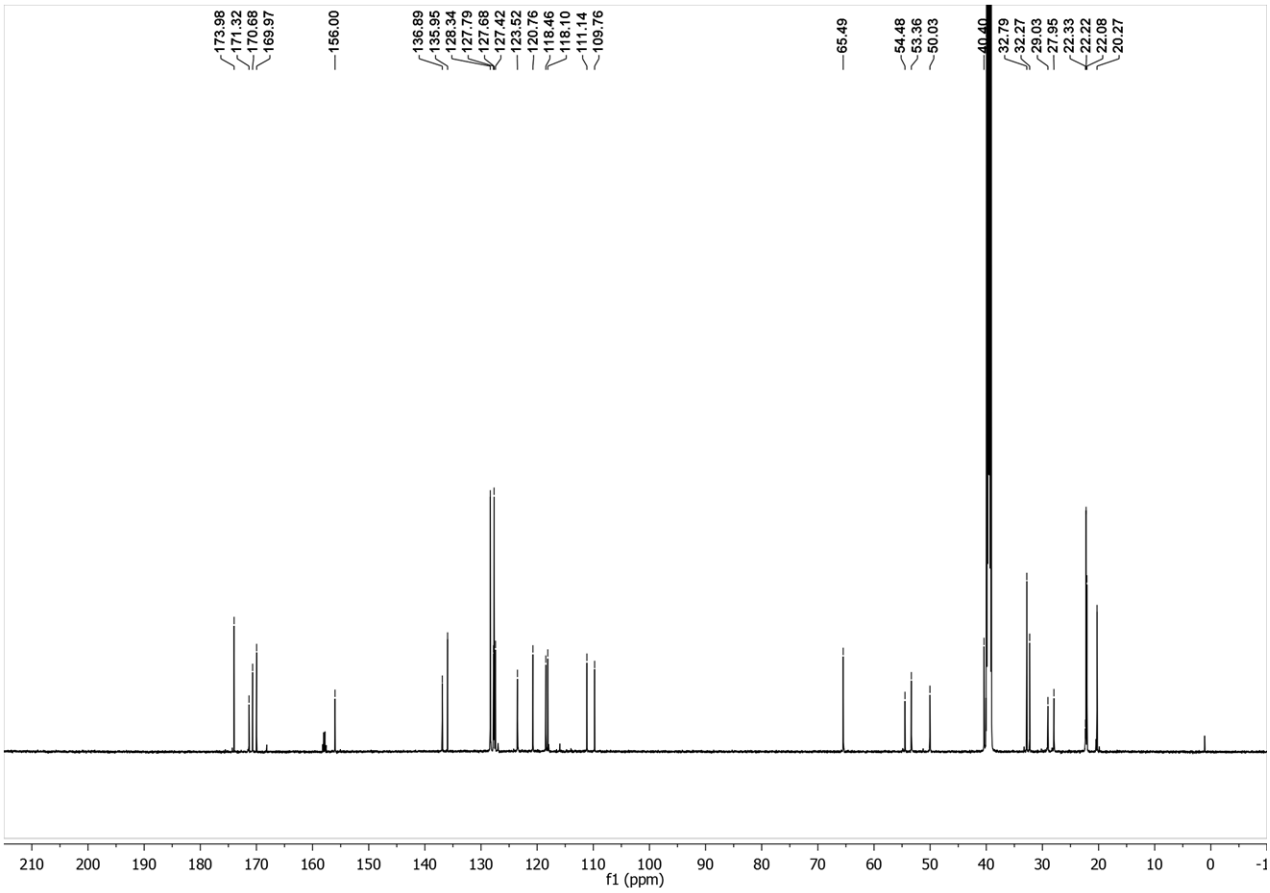
^{1}H and ^{13}C spectra of compound 19.



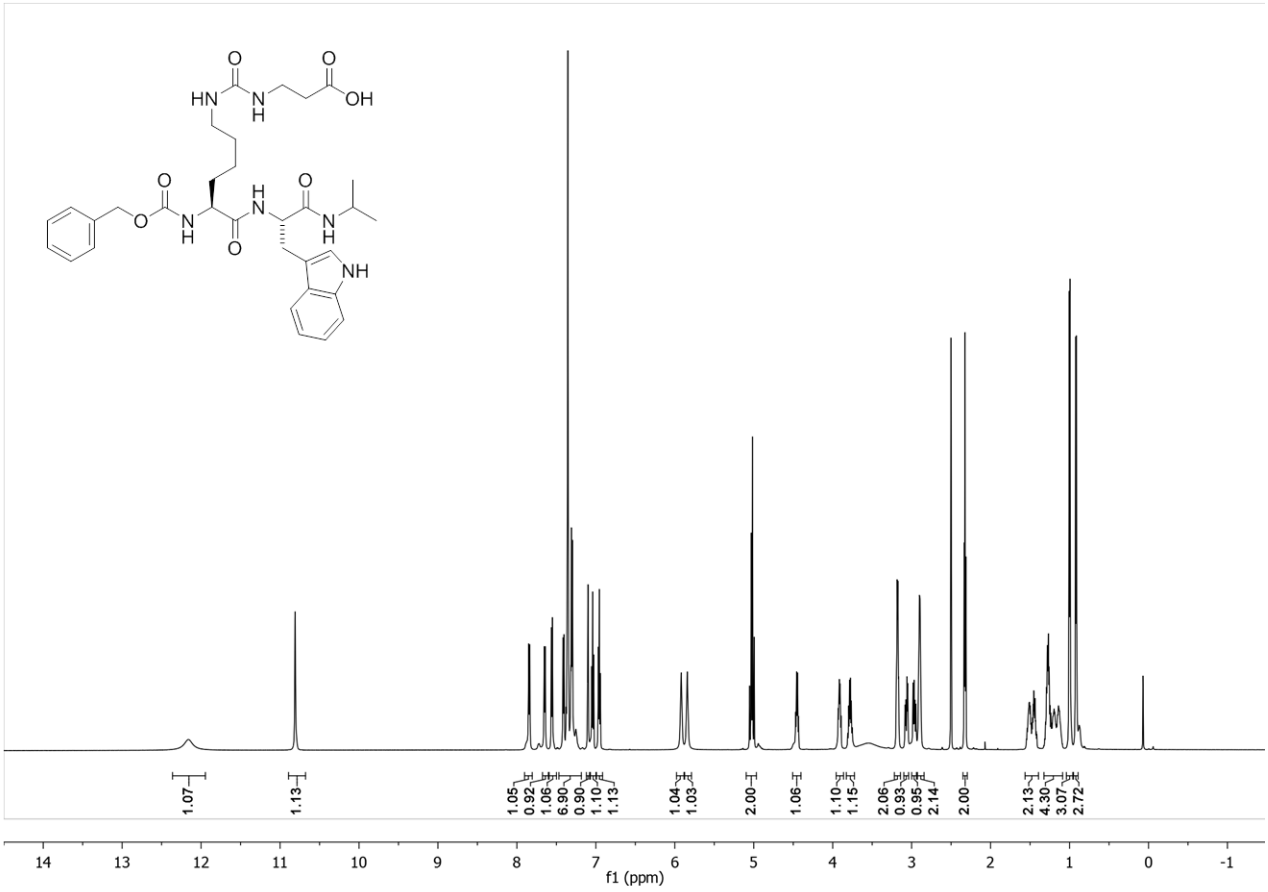


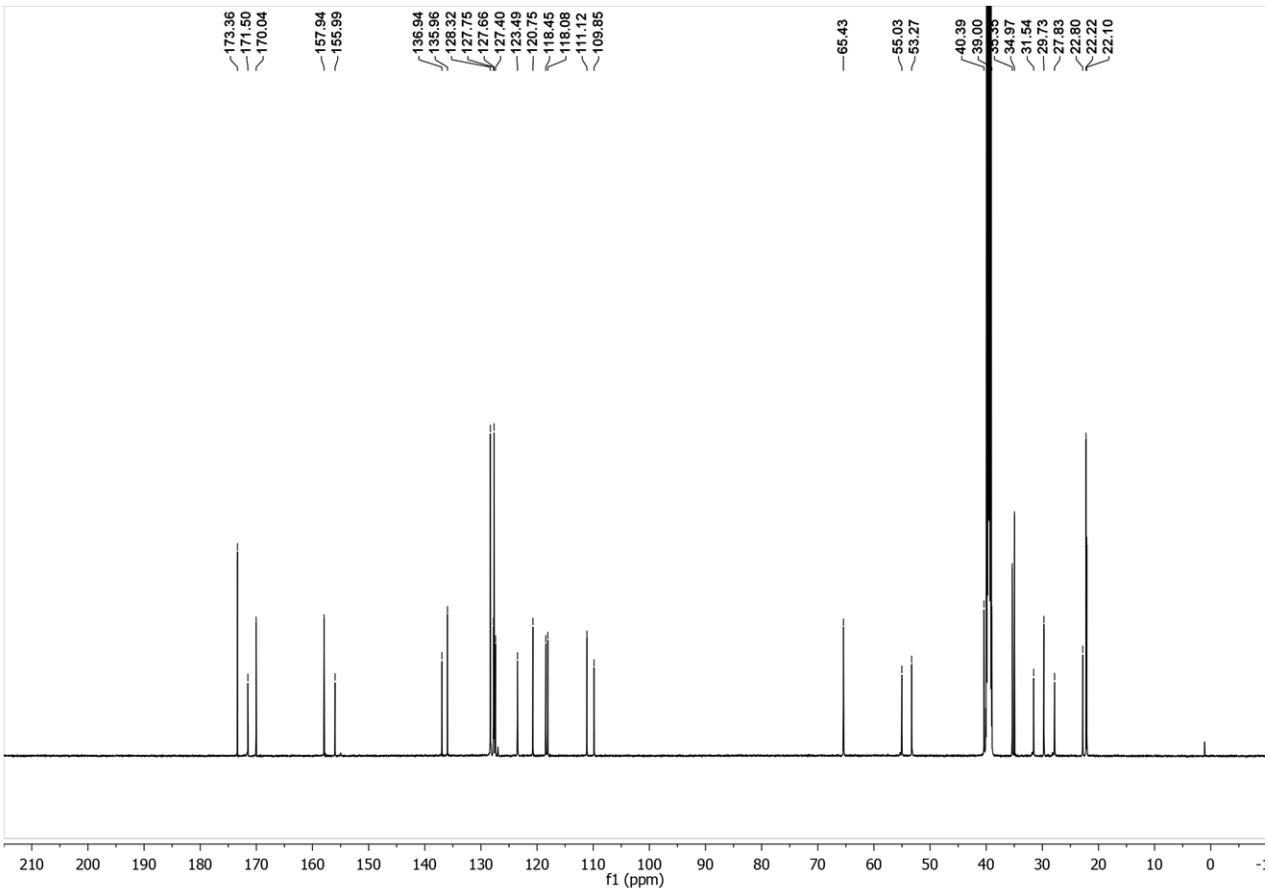
^{1}H and ^{13}C spectra of compound 20.



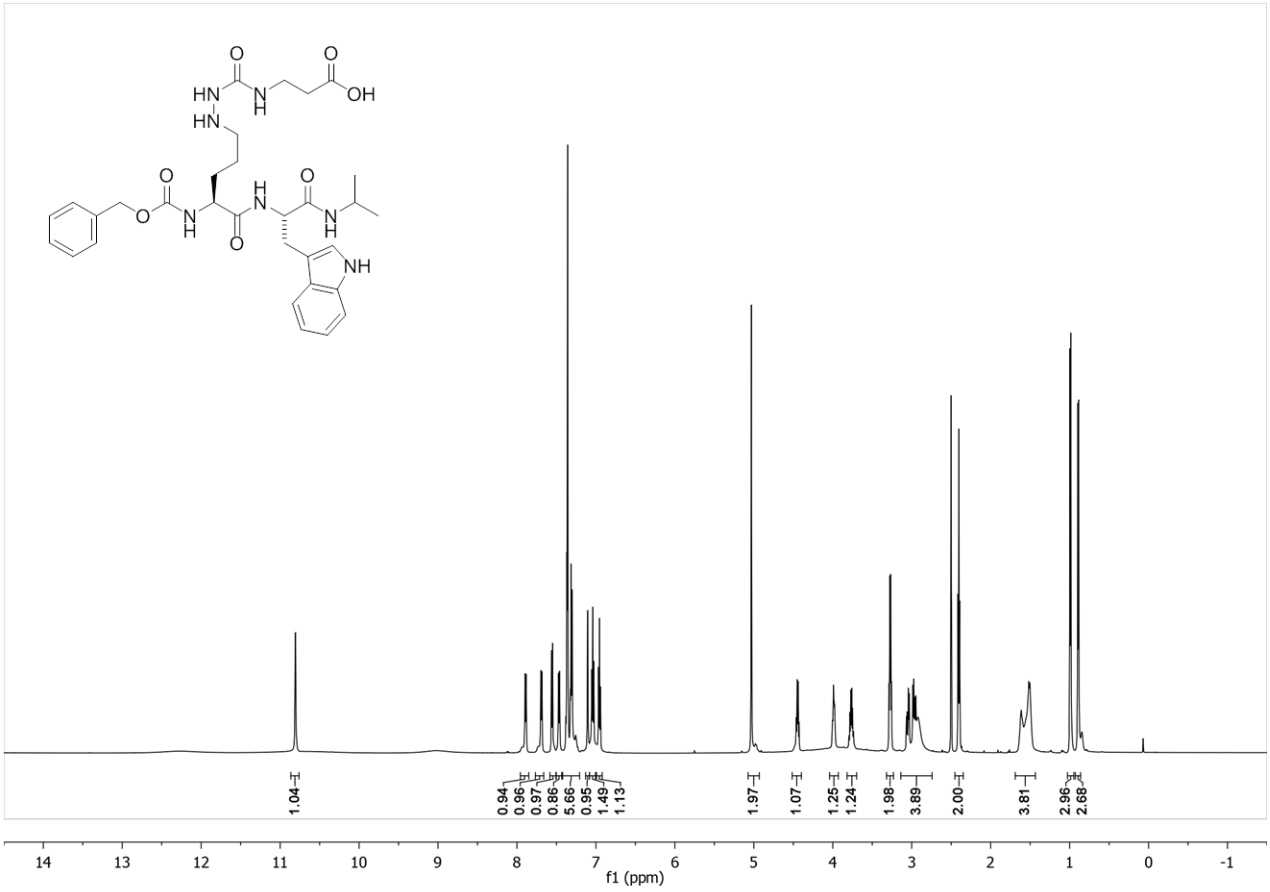


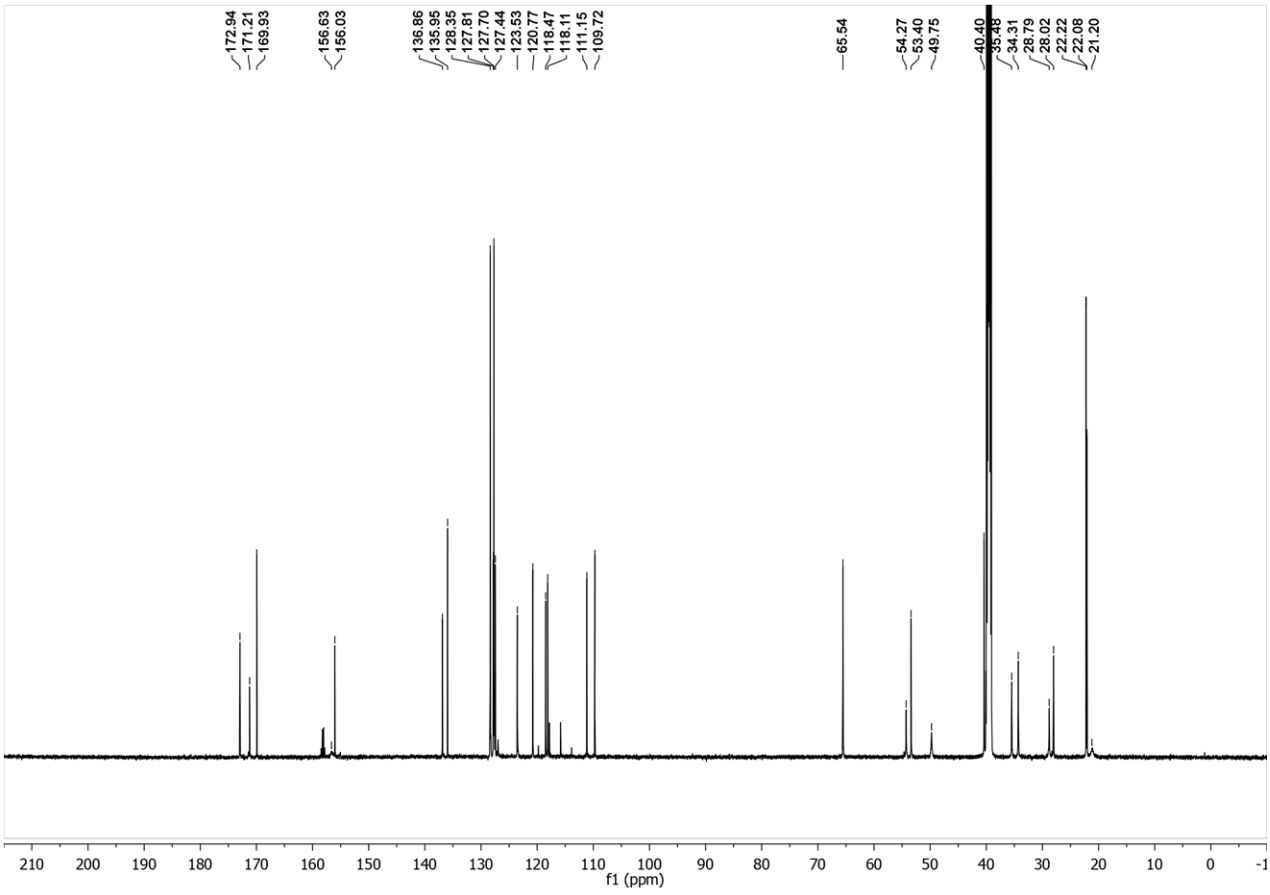
^{1}H and ^{13}C spectra of compound 21.



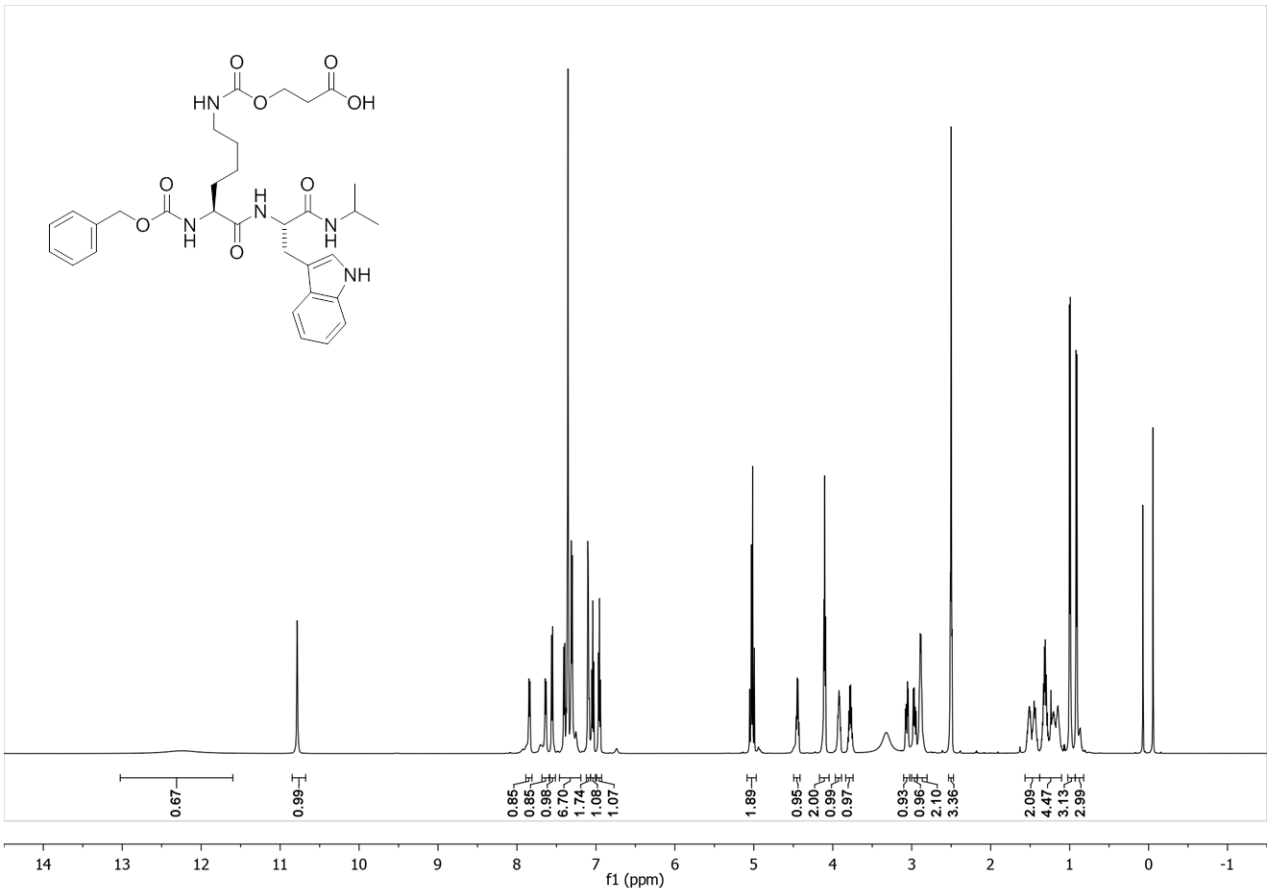


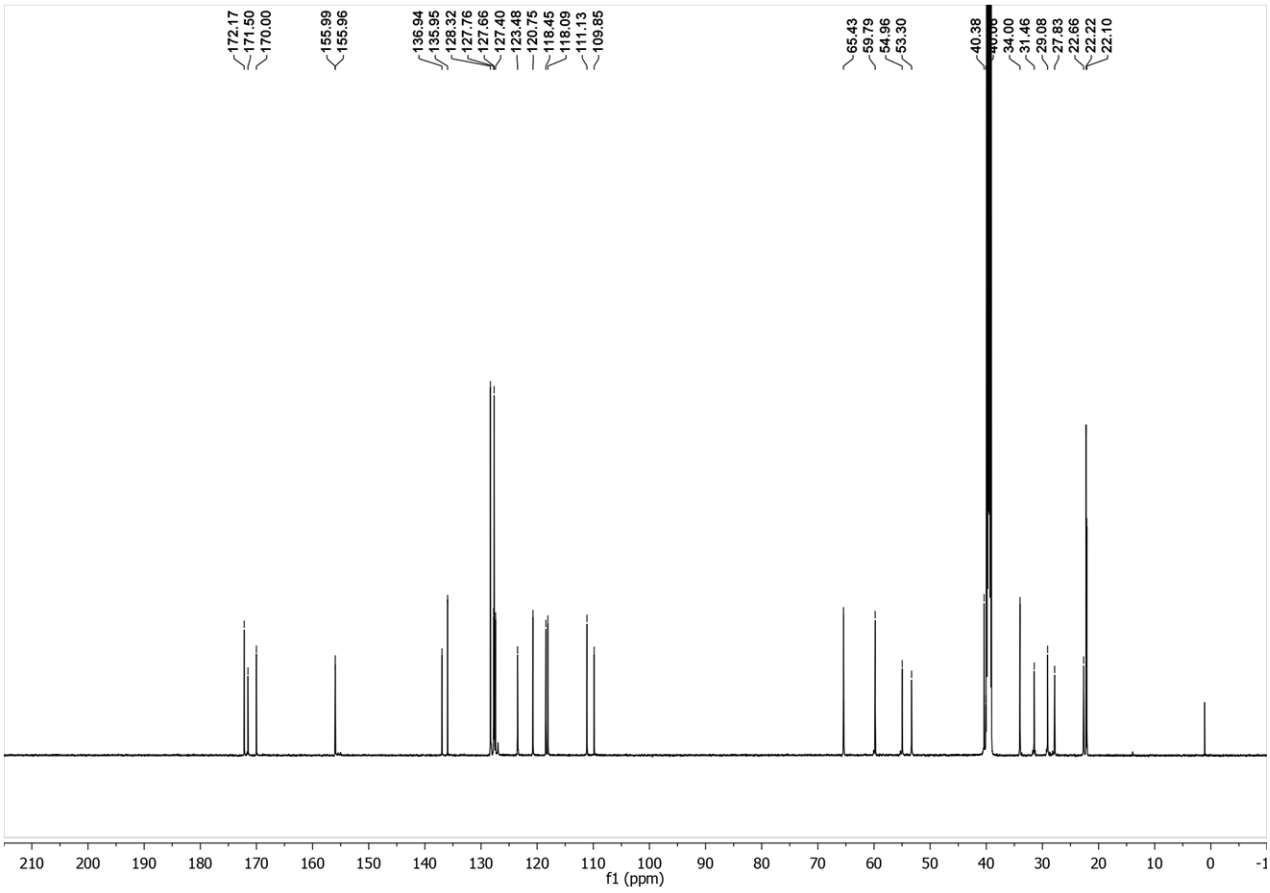
^{1}H and ^{13}C spectra of compound 22.



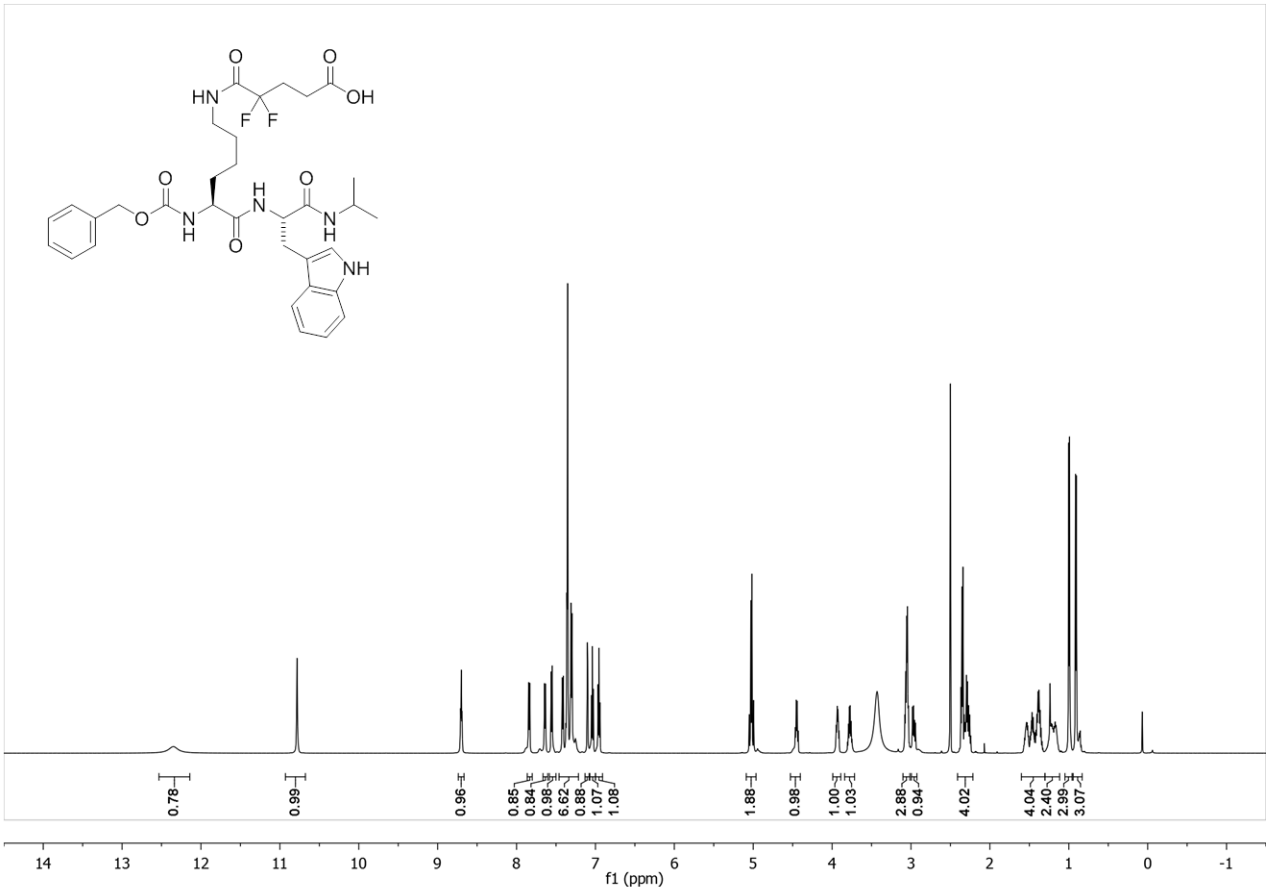


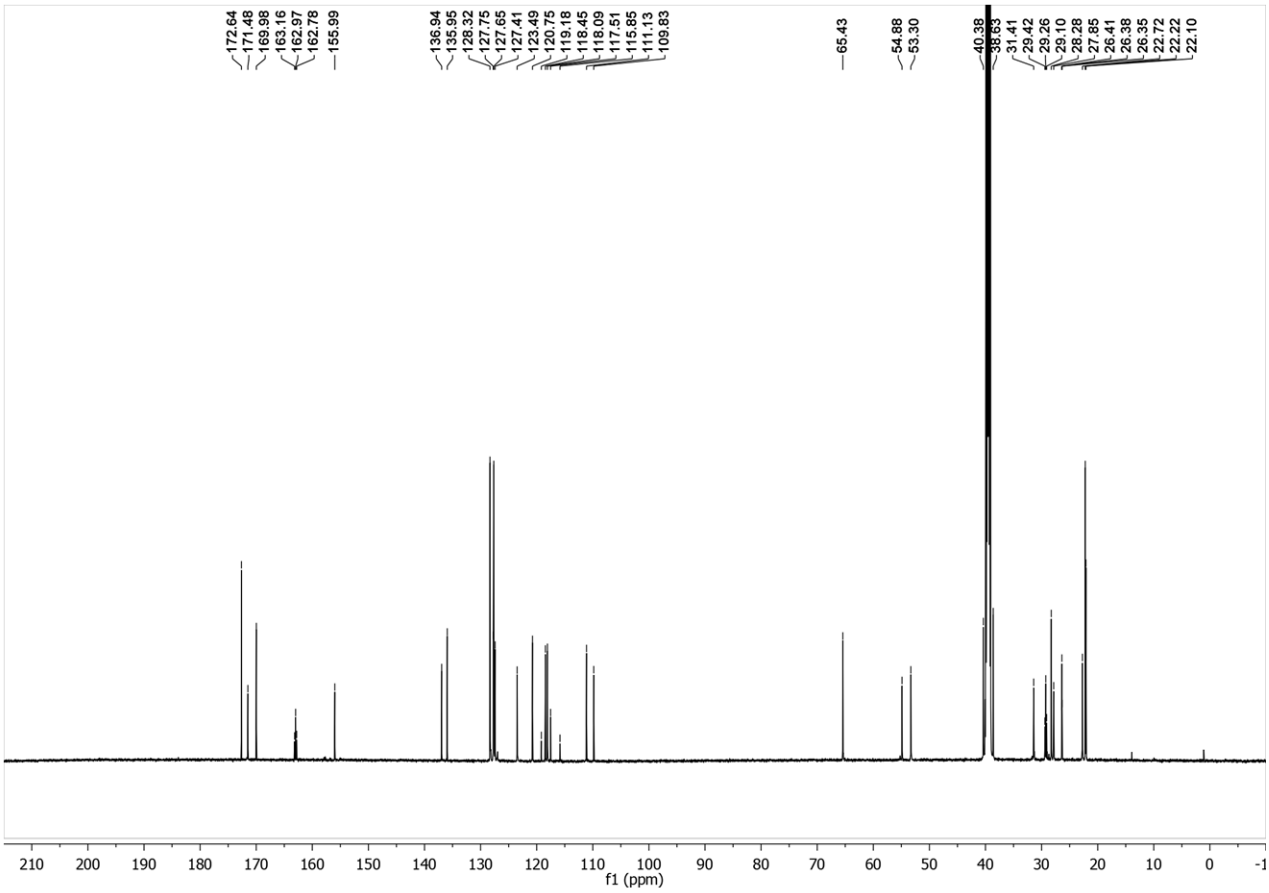
^{1}H and ^{13}C spectra of compound 23.

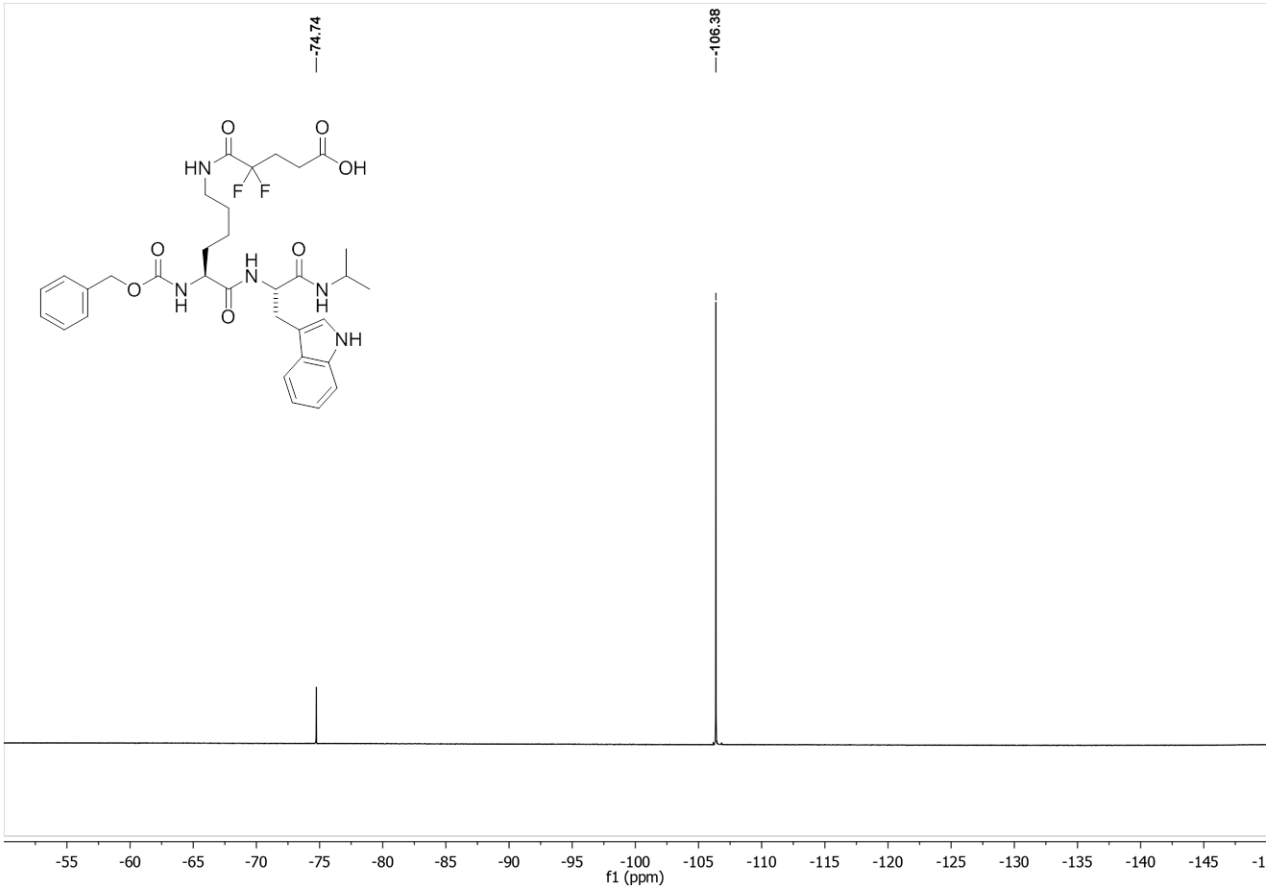




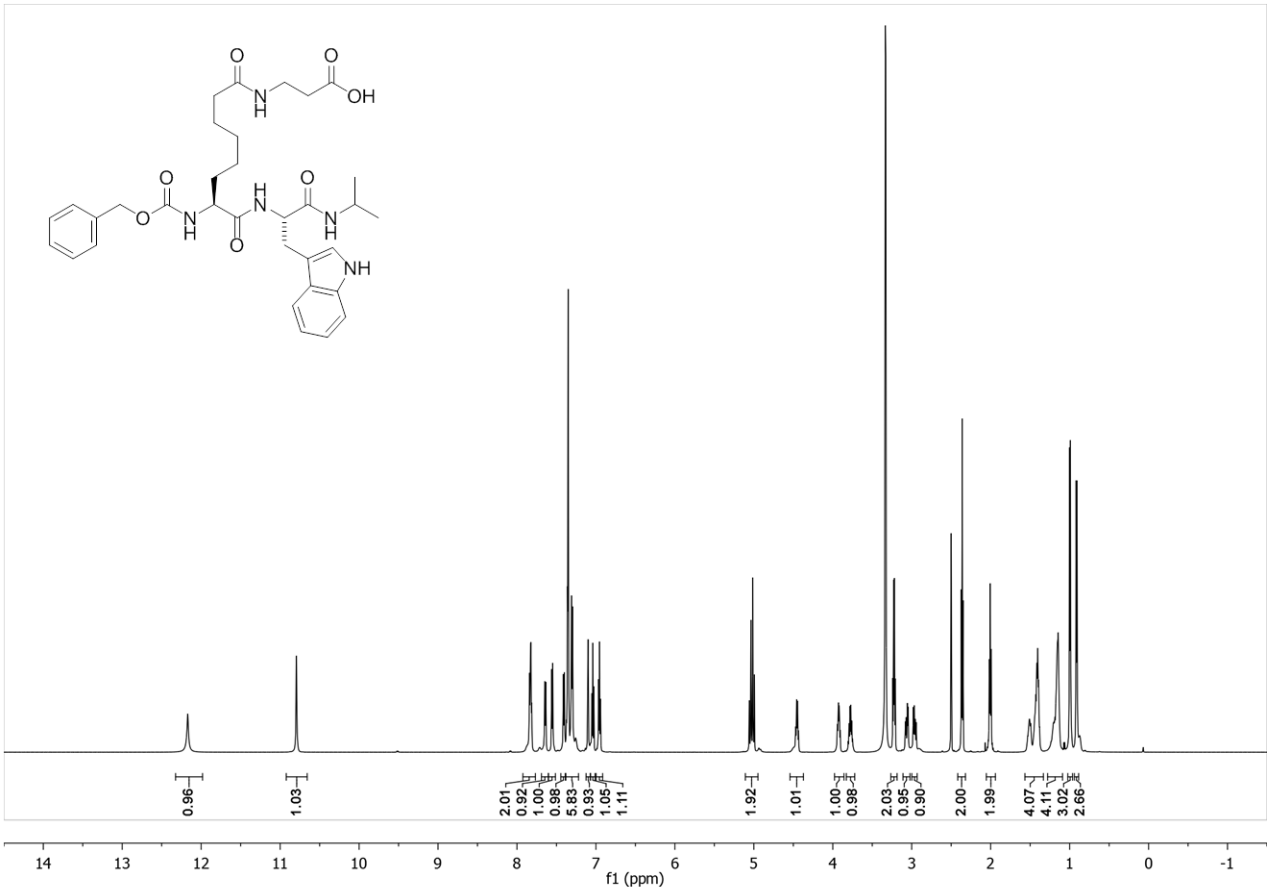
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound 24.

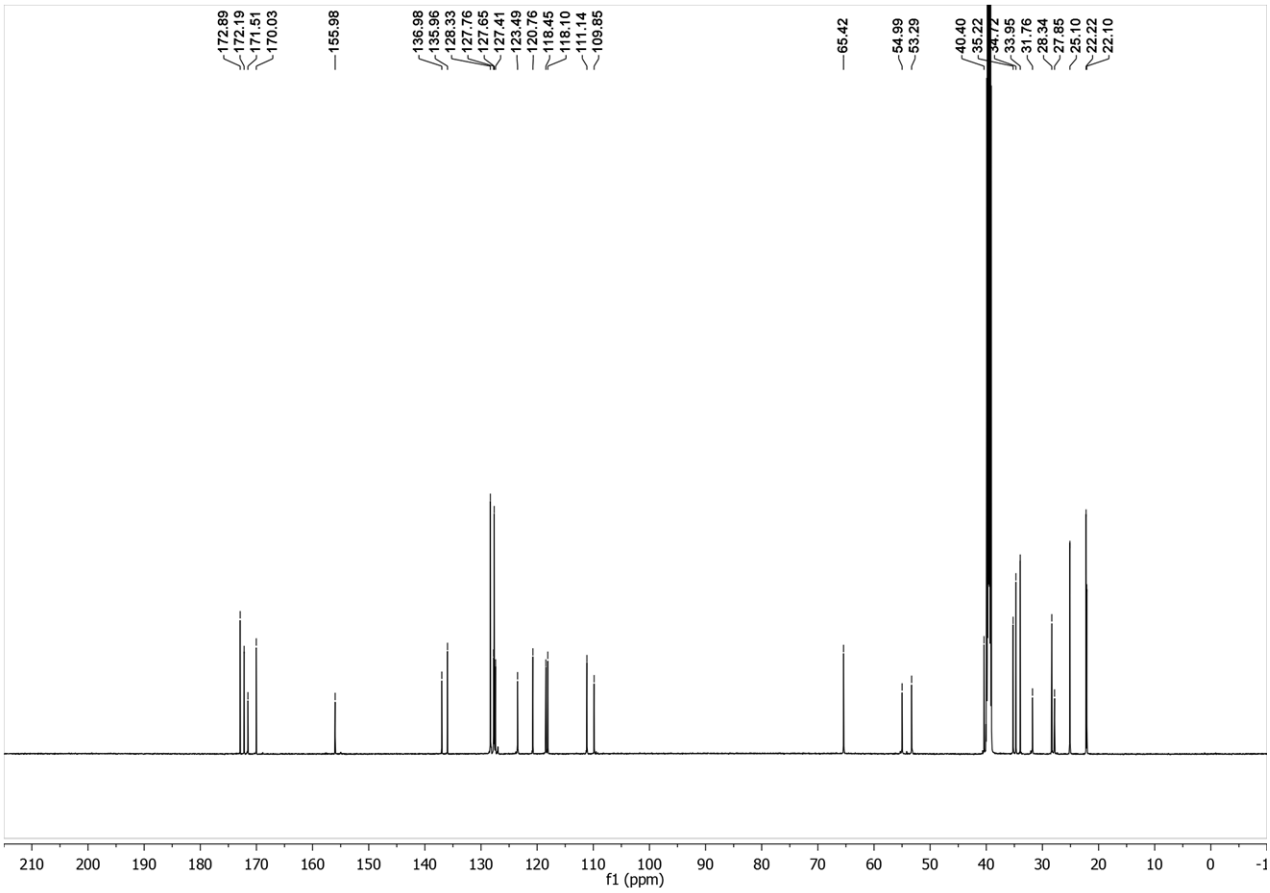




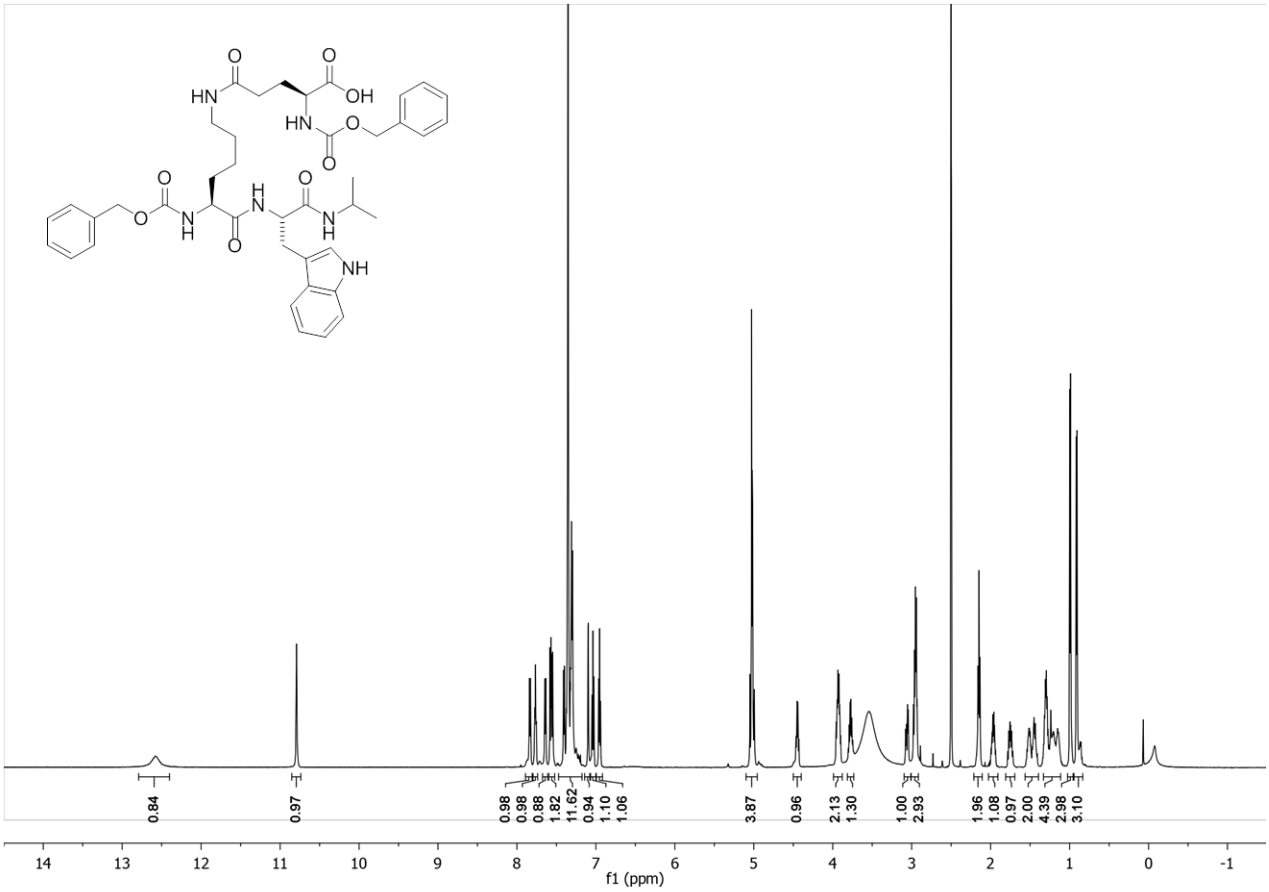

-74.74
-106.38
O
O
HN
OH
F
F
O
O
H
N
O
N
H
N
H
O
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

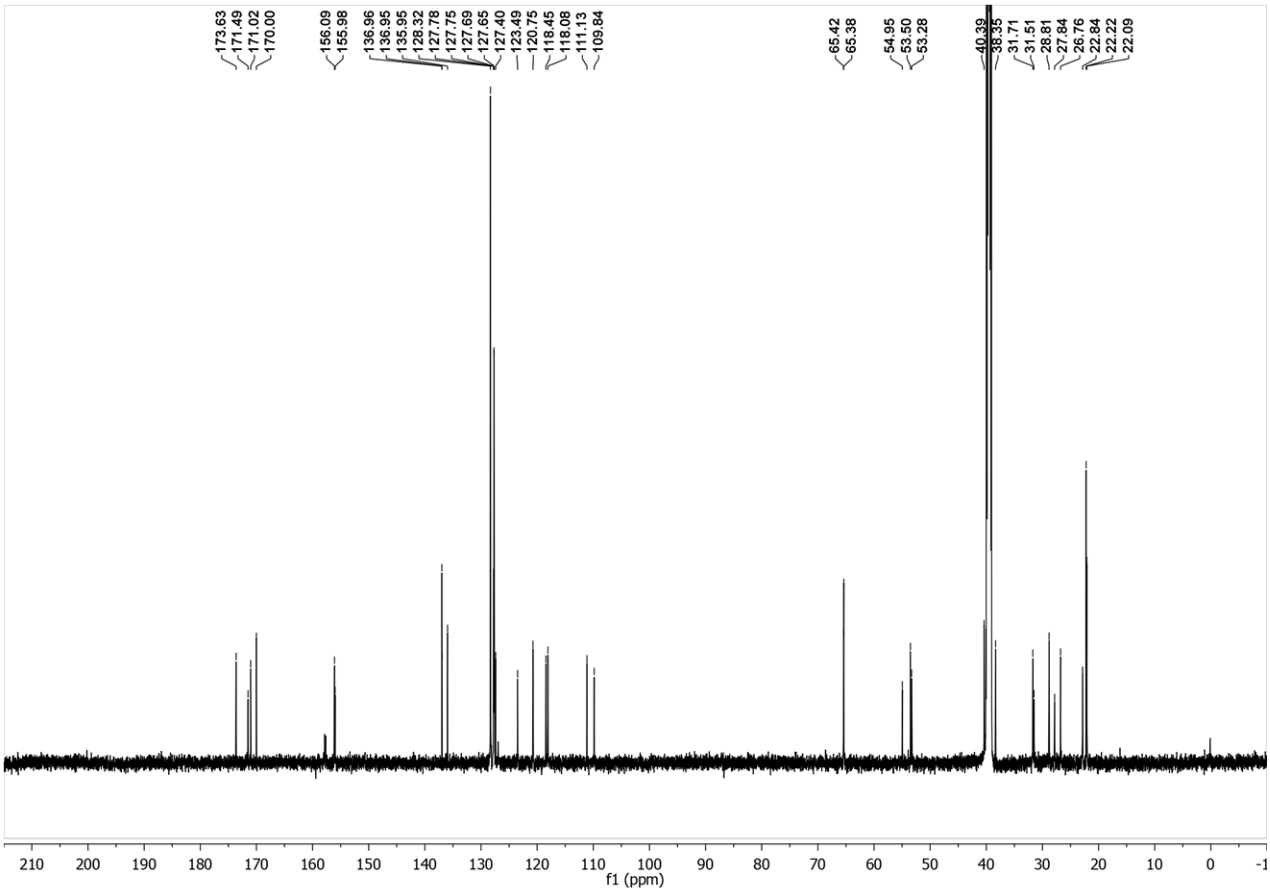
^{1}H and ^{13}C spectra of compound 25.



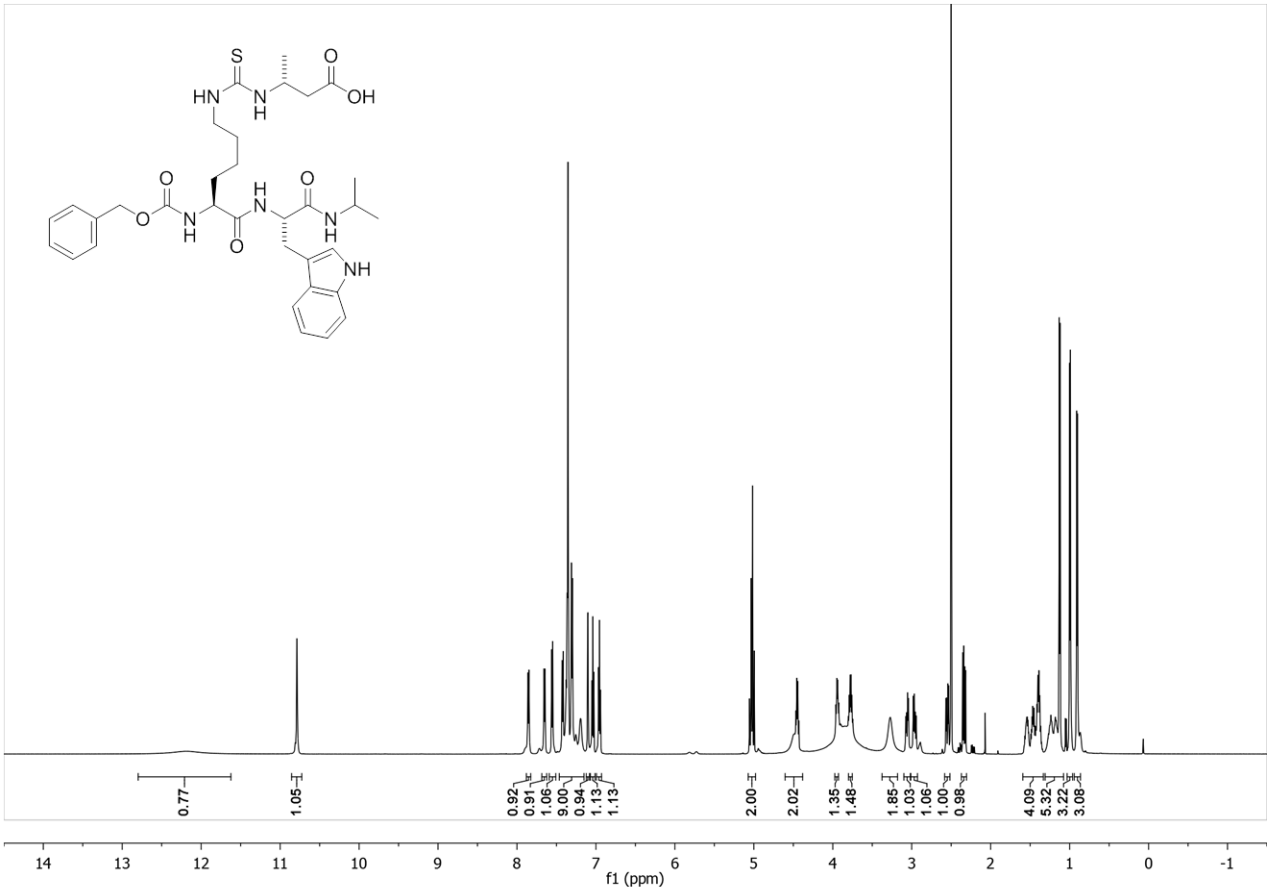


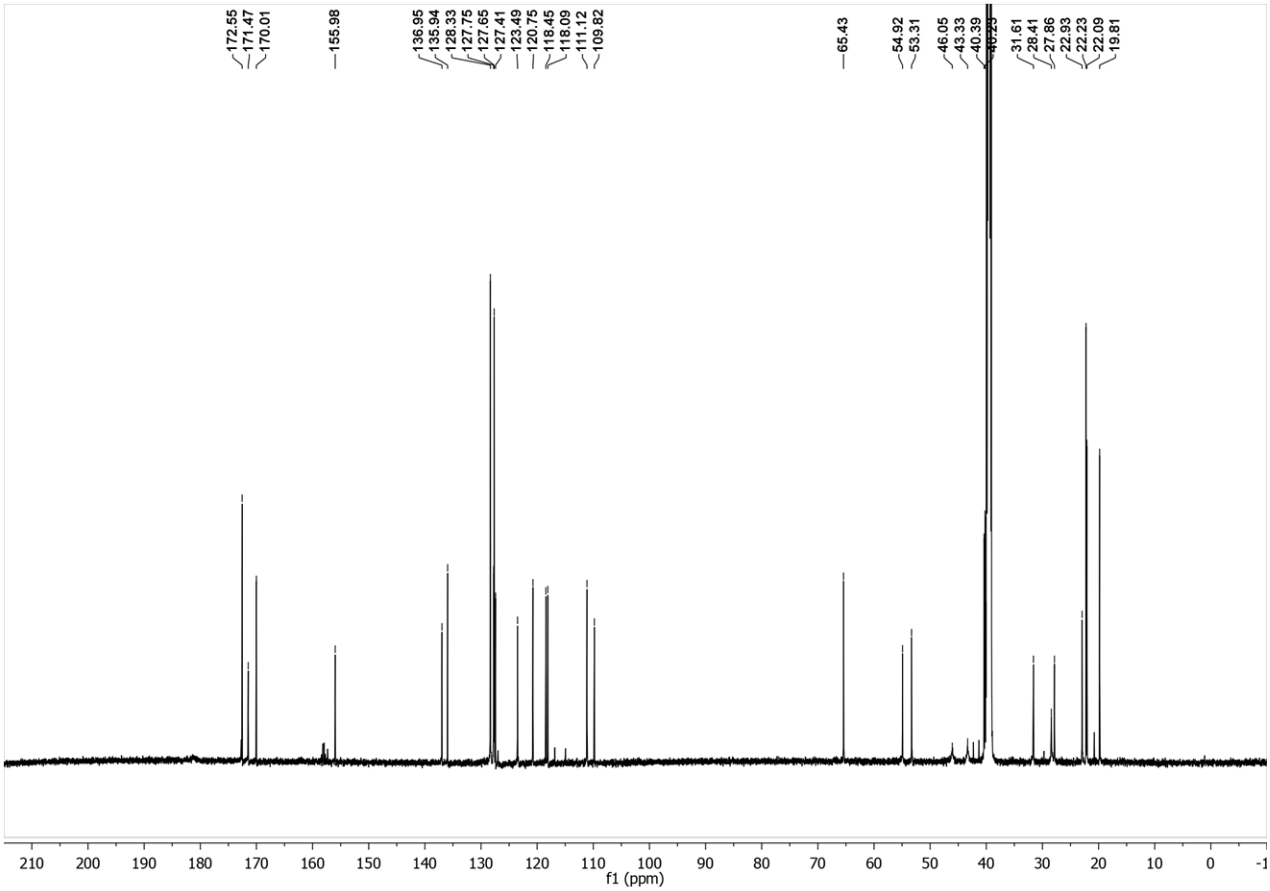
^{1}H and ^{13}C spectra of compound 26.



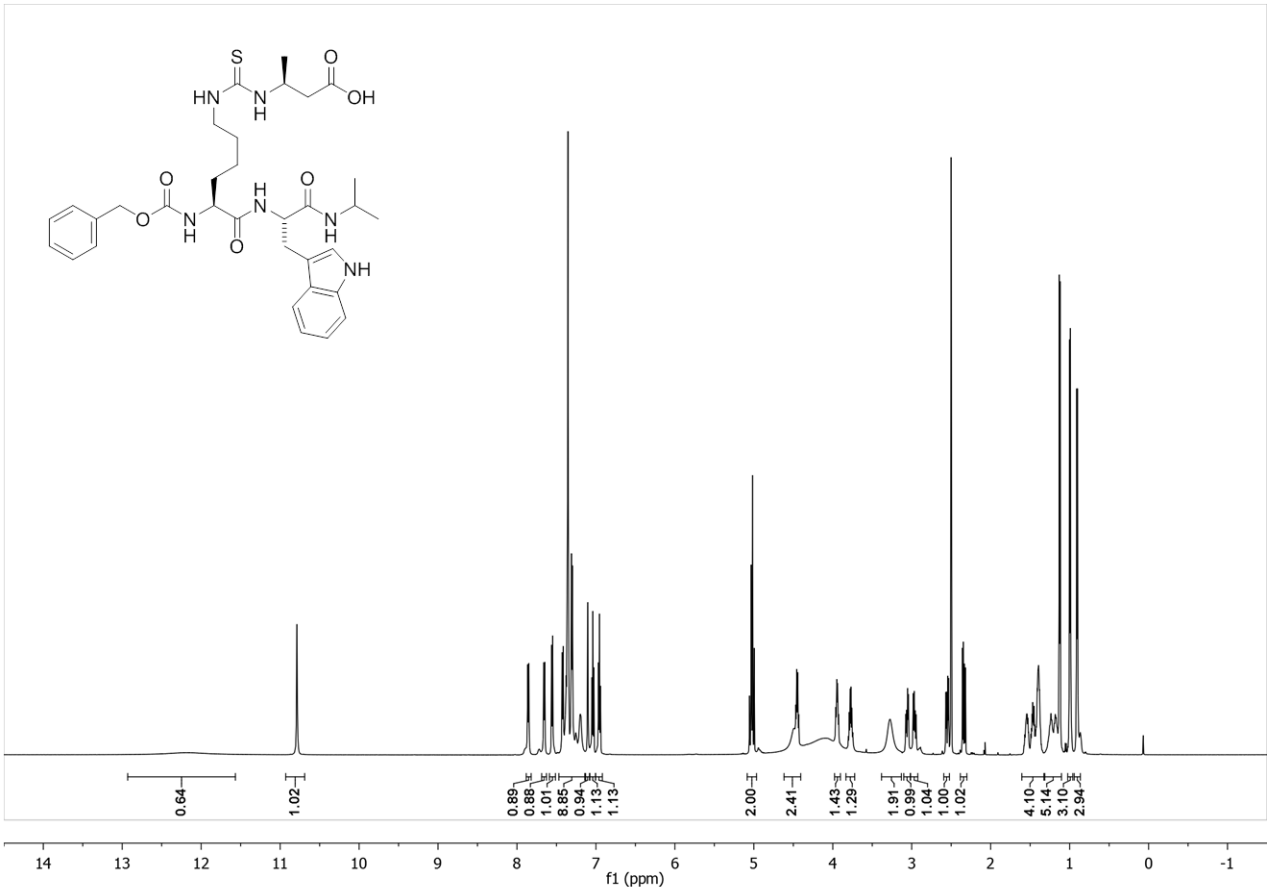


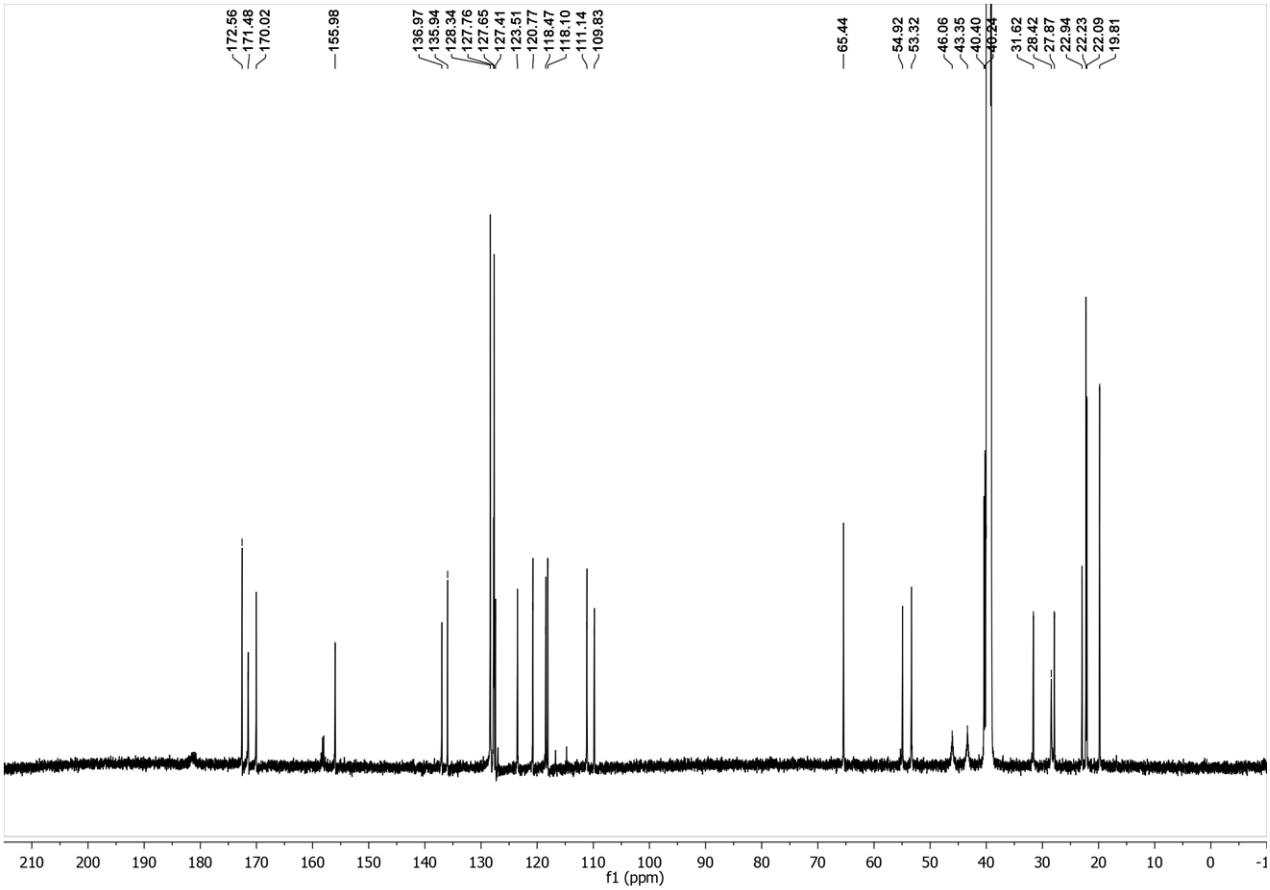
^{1}H and ^{13}C spectra of compound 27.



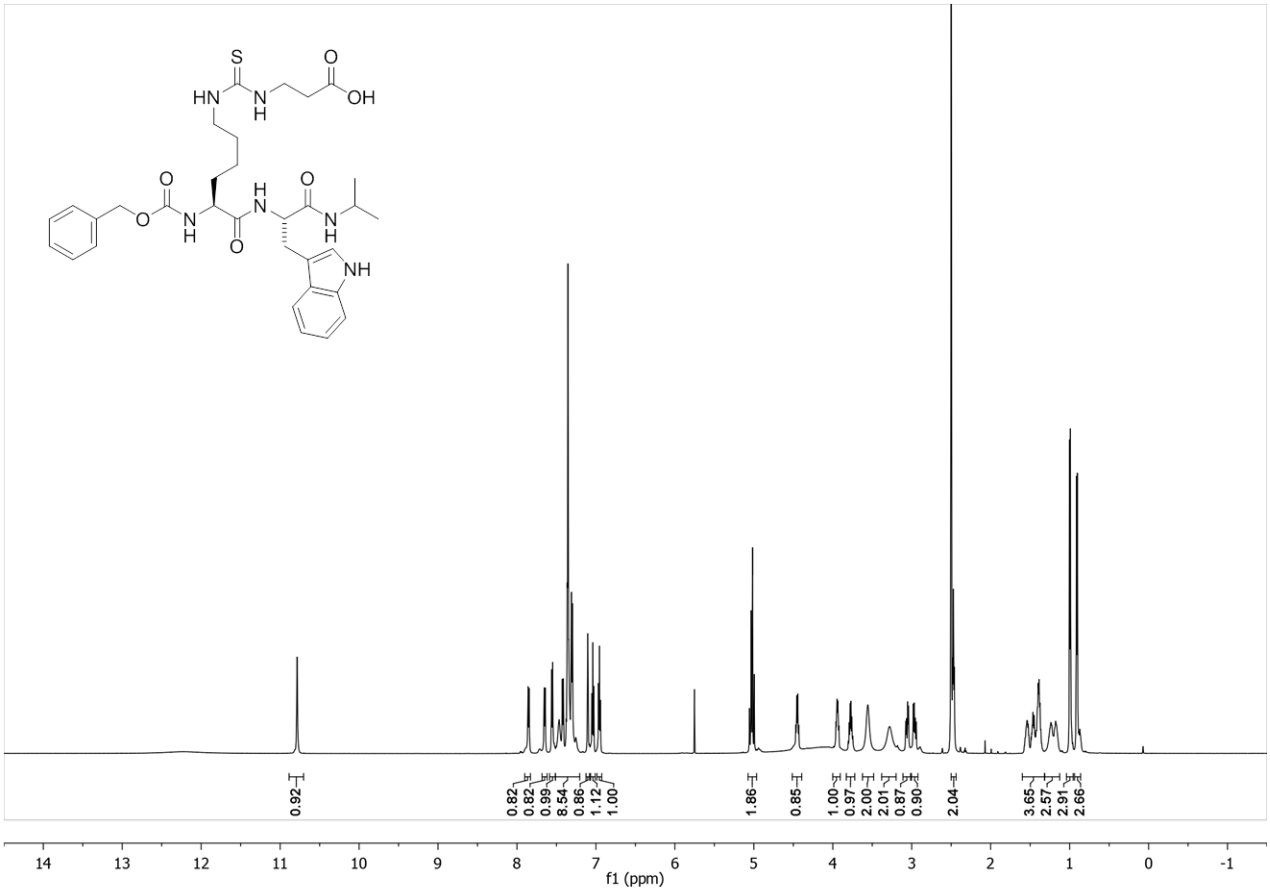


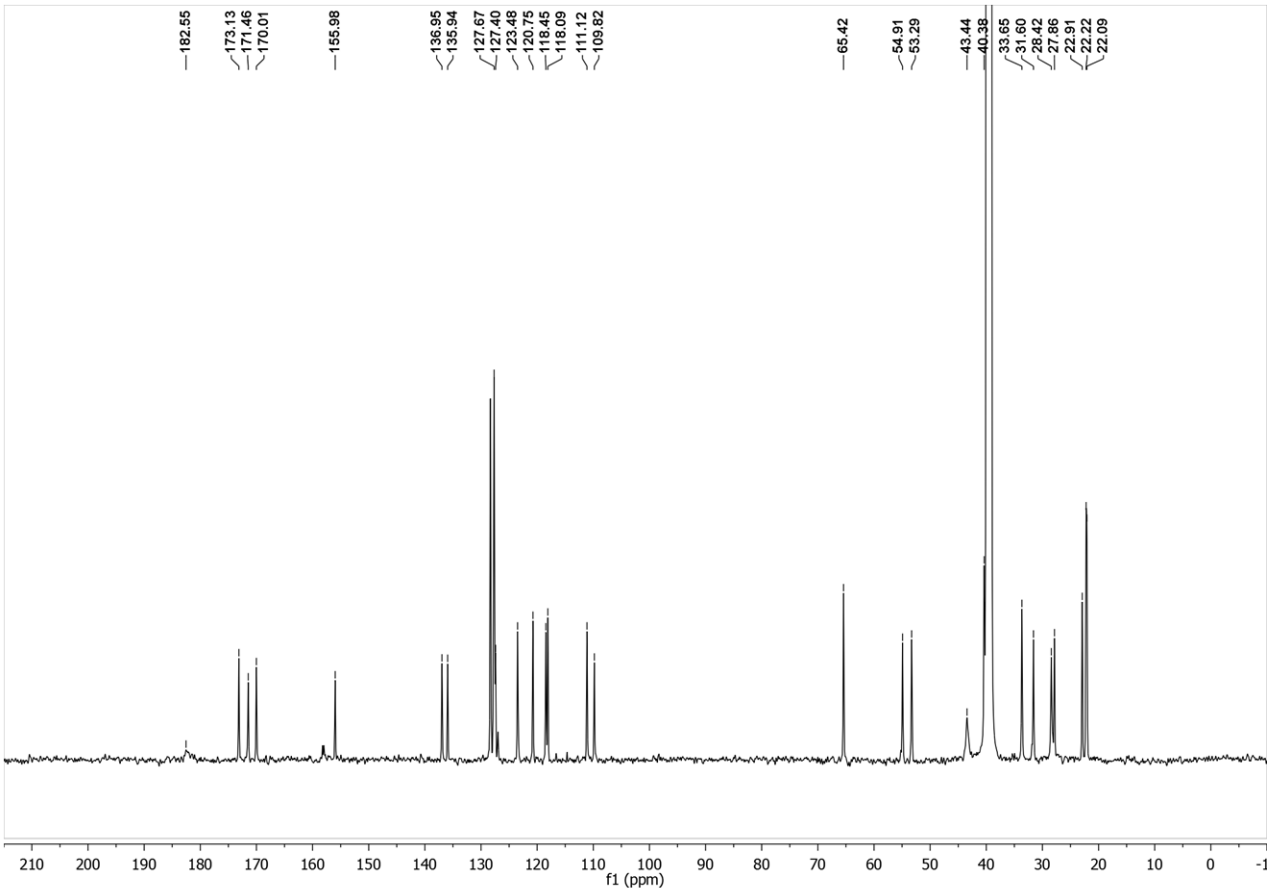
^{1}H and ^{13}C spectra of compound 28.



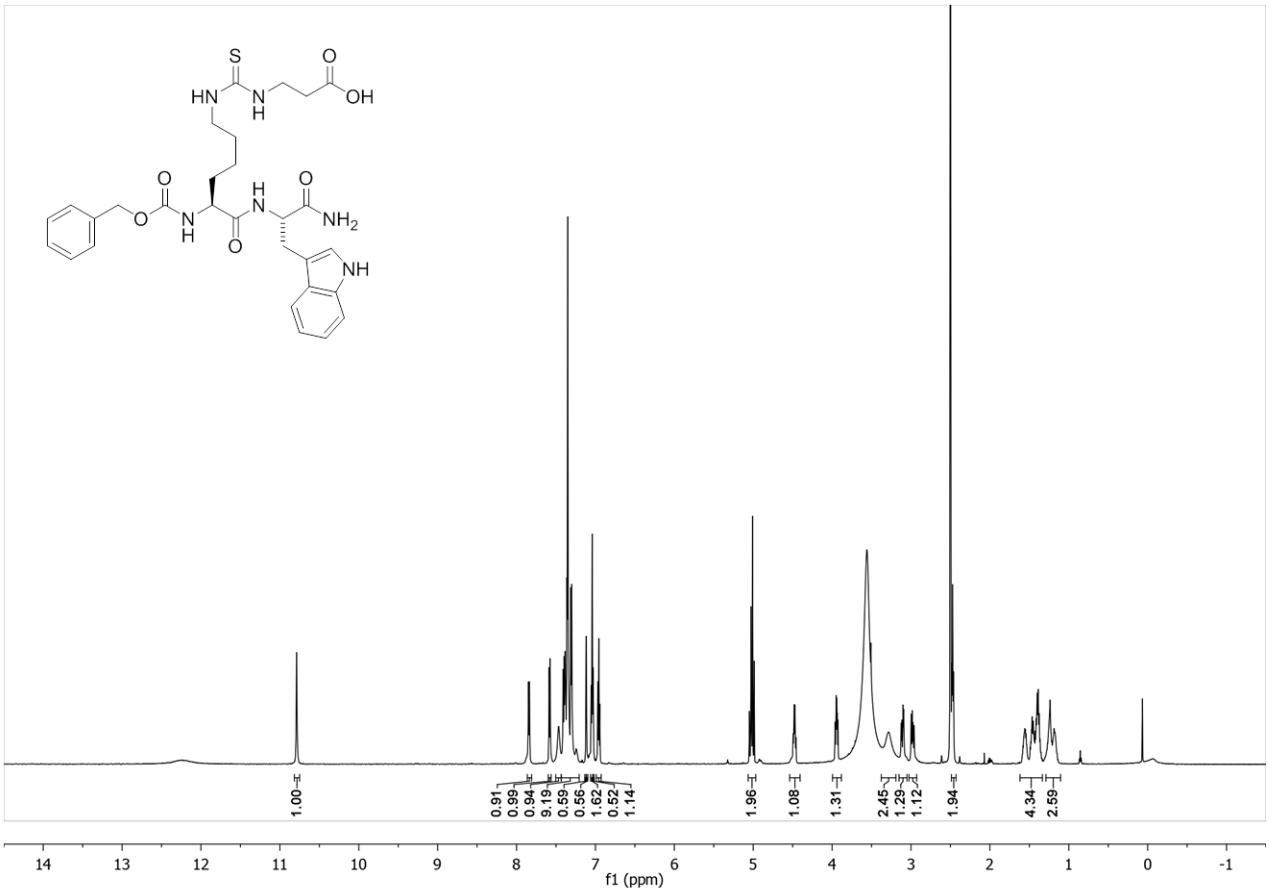


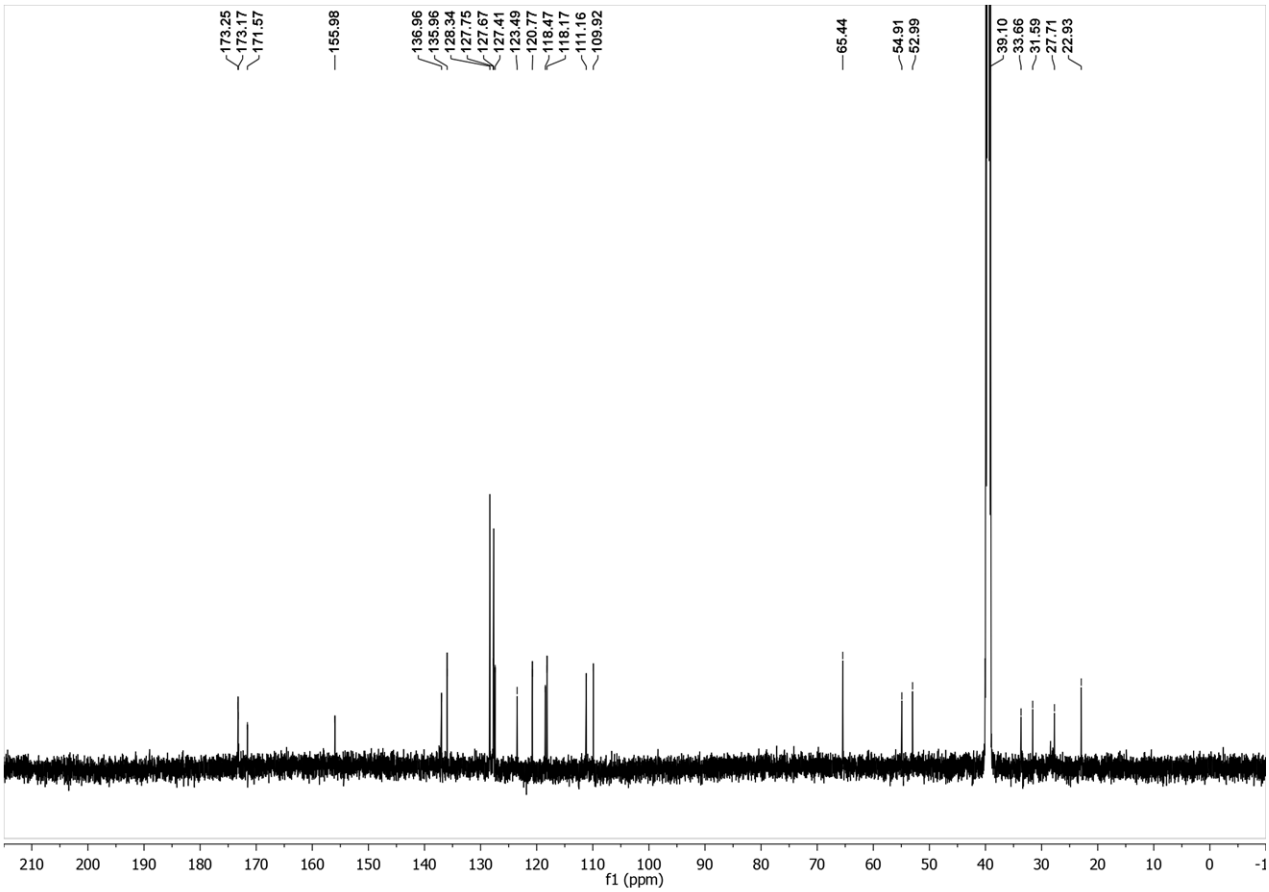
^{1}H and ^{13}C spectra of compound 29.



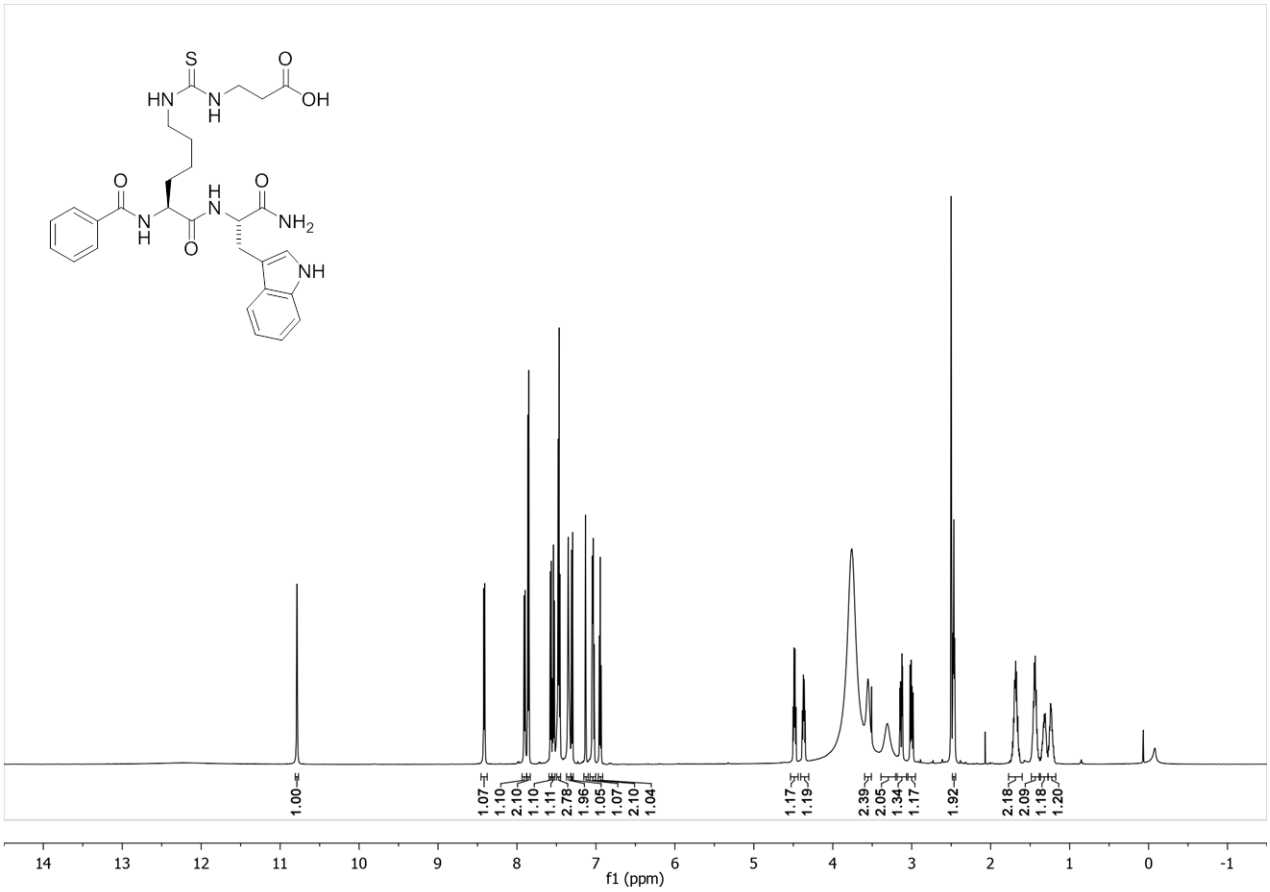


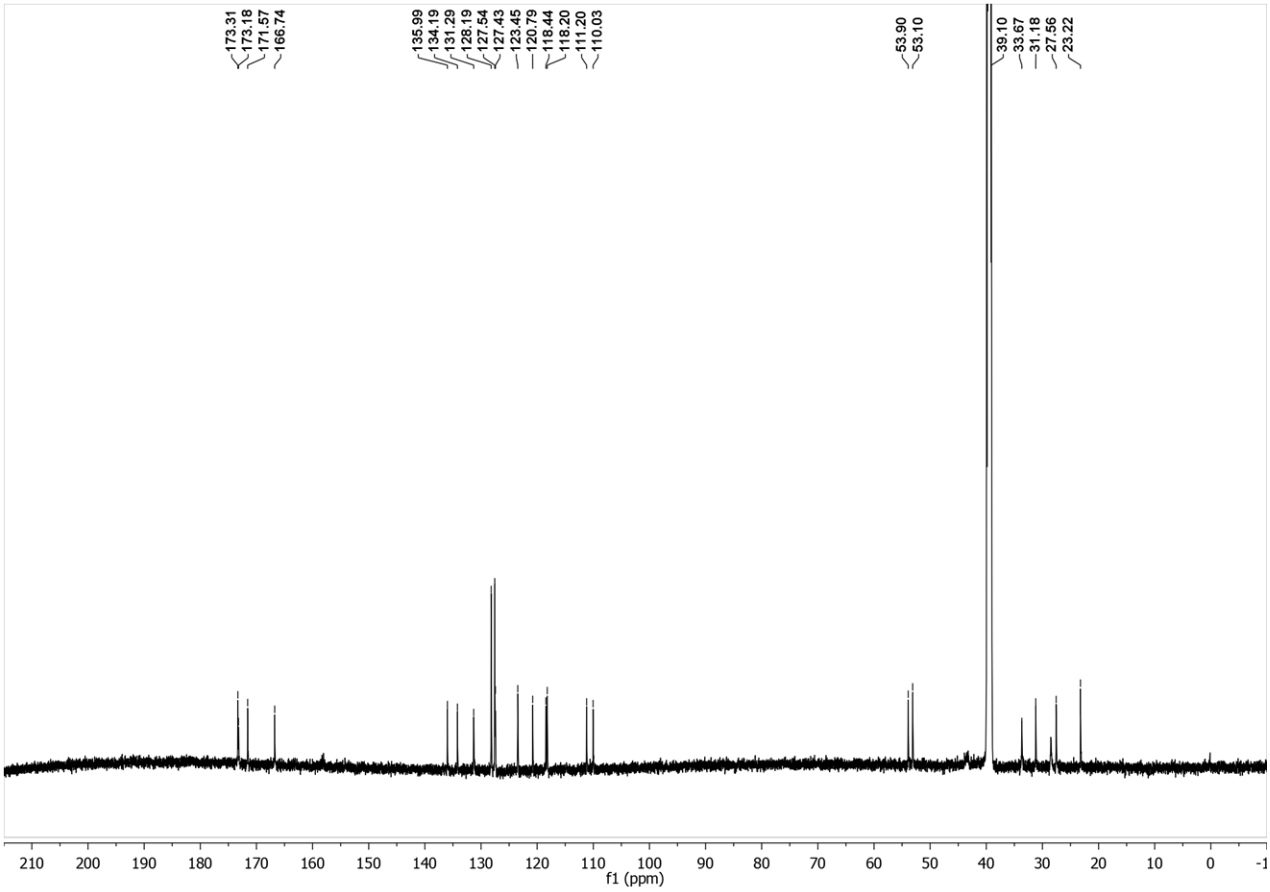
^{1}H and ^{13}C spectra of compound 30.



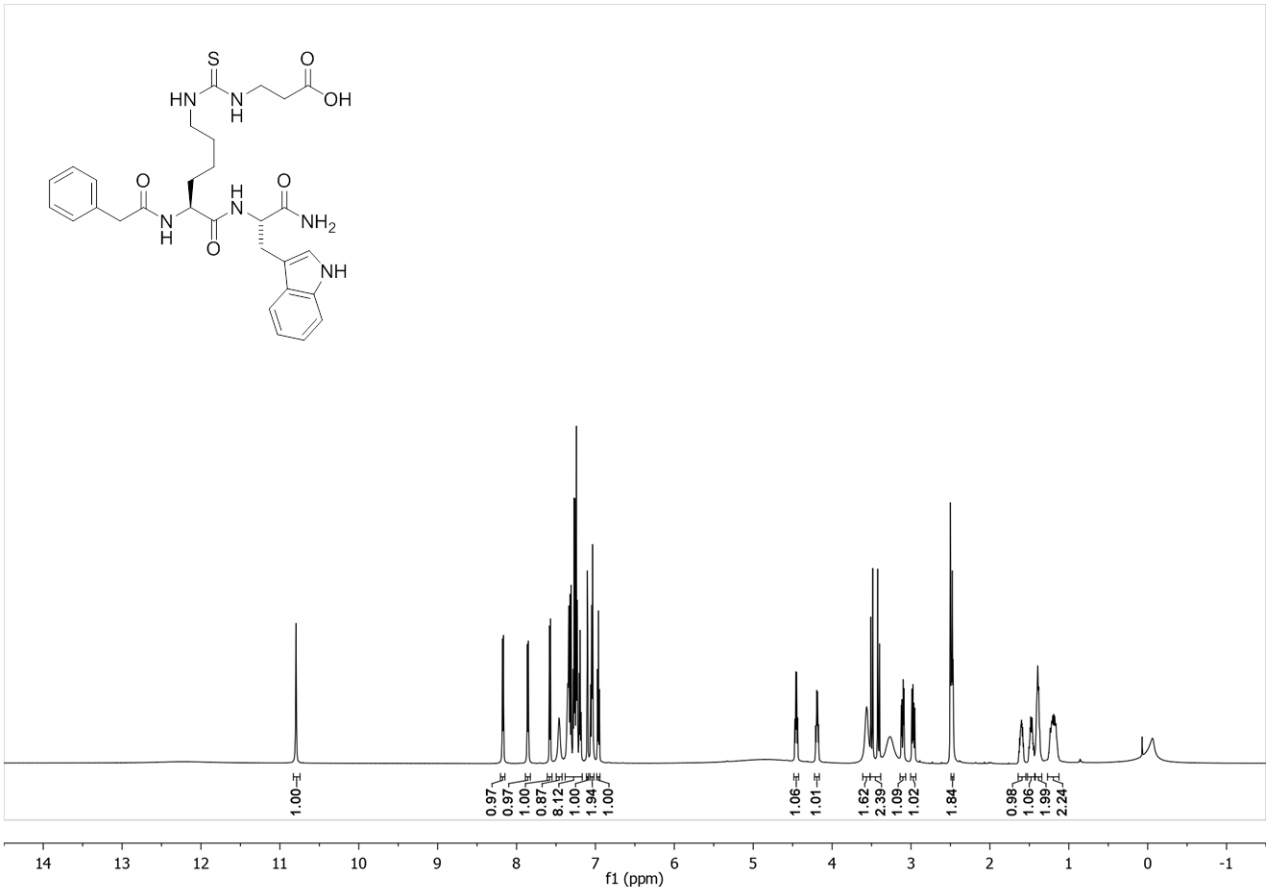


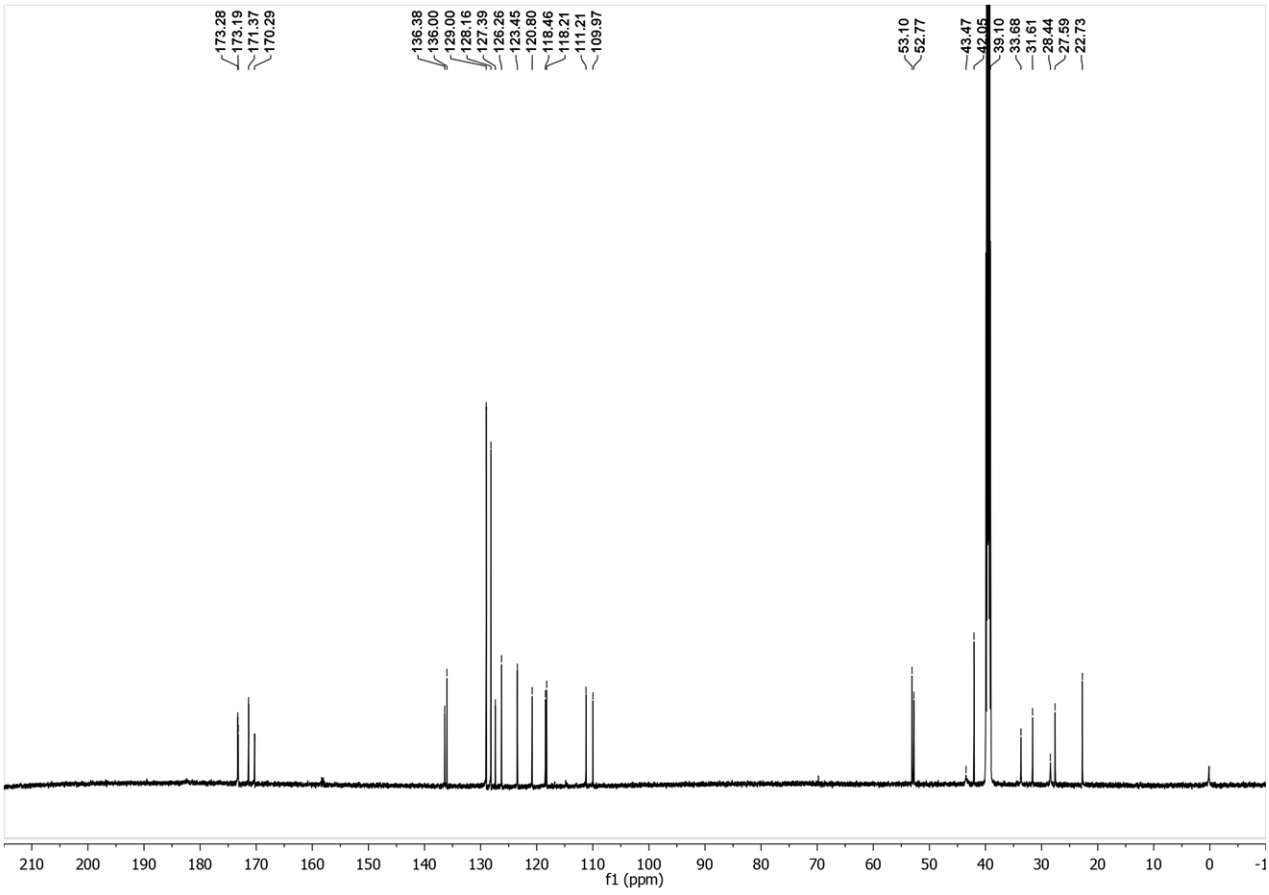
^{1}H and ^{13}C spectra of compound 31.



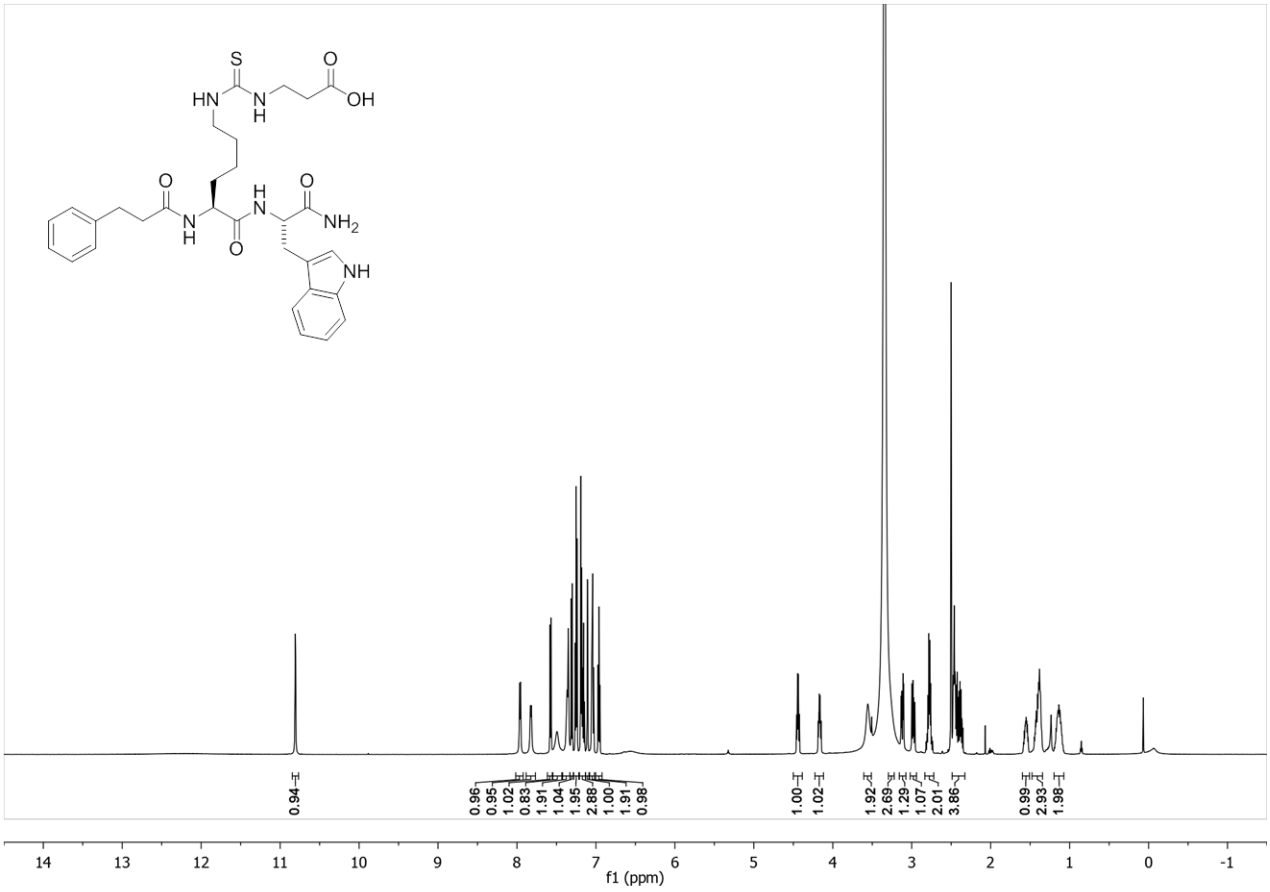


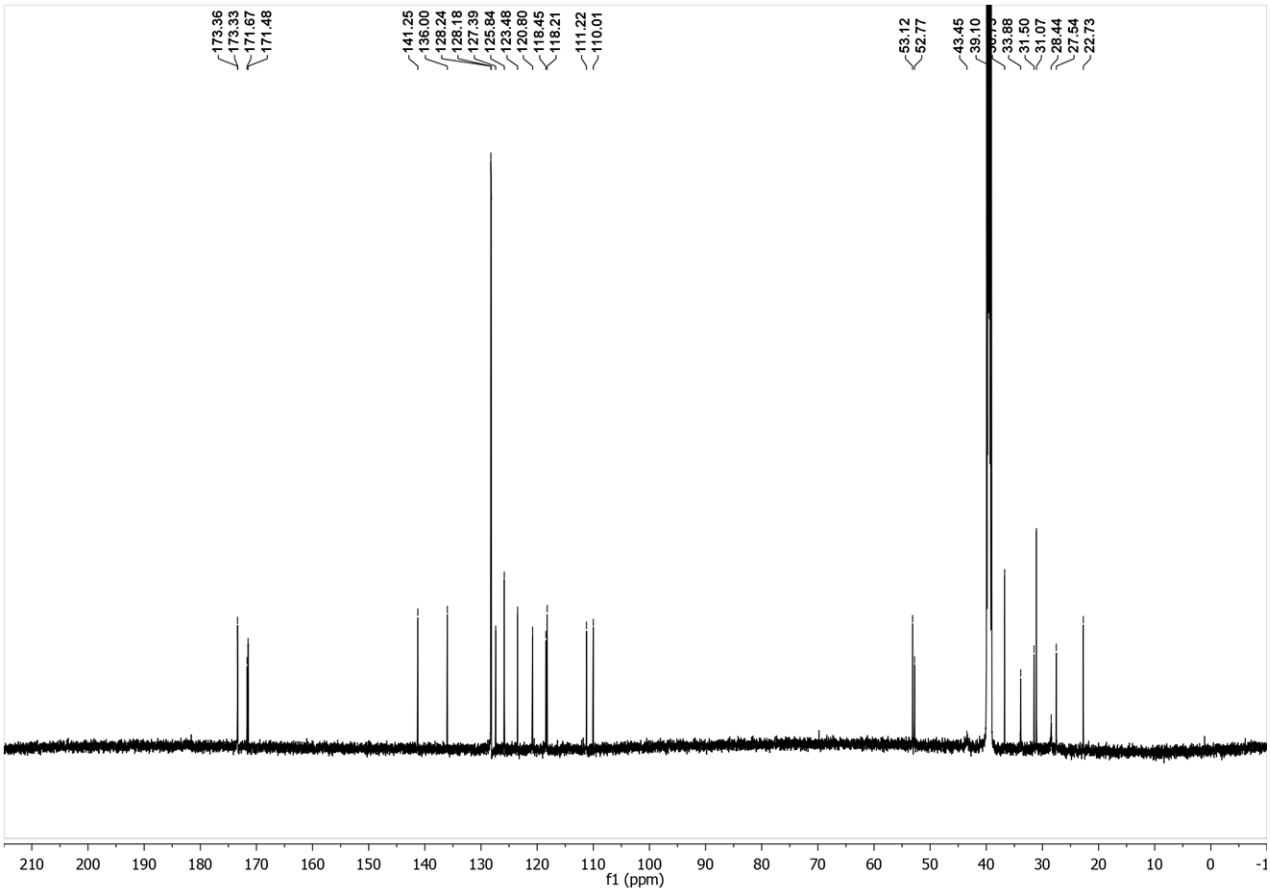
^{1}H and ^{13}C spectra of compound 32.



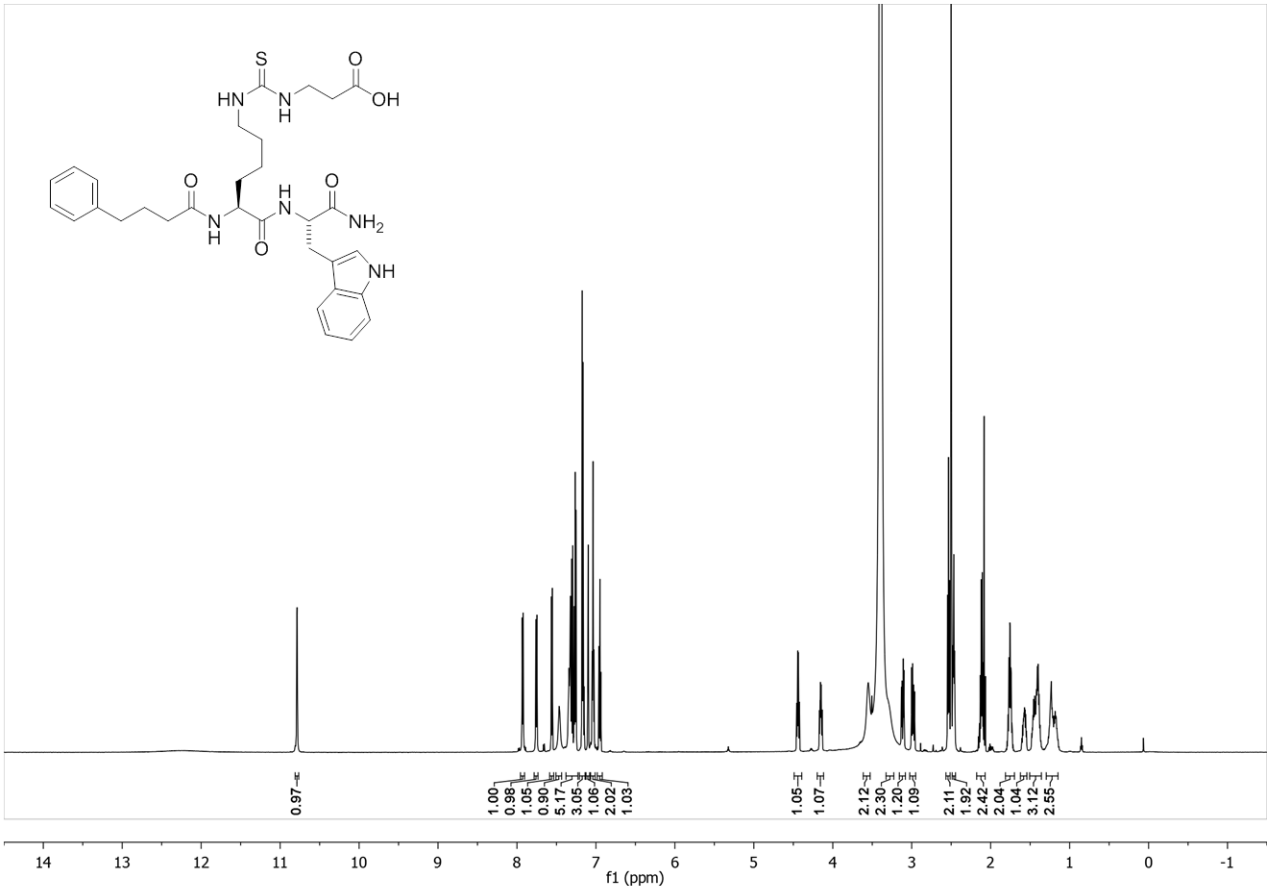


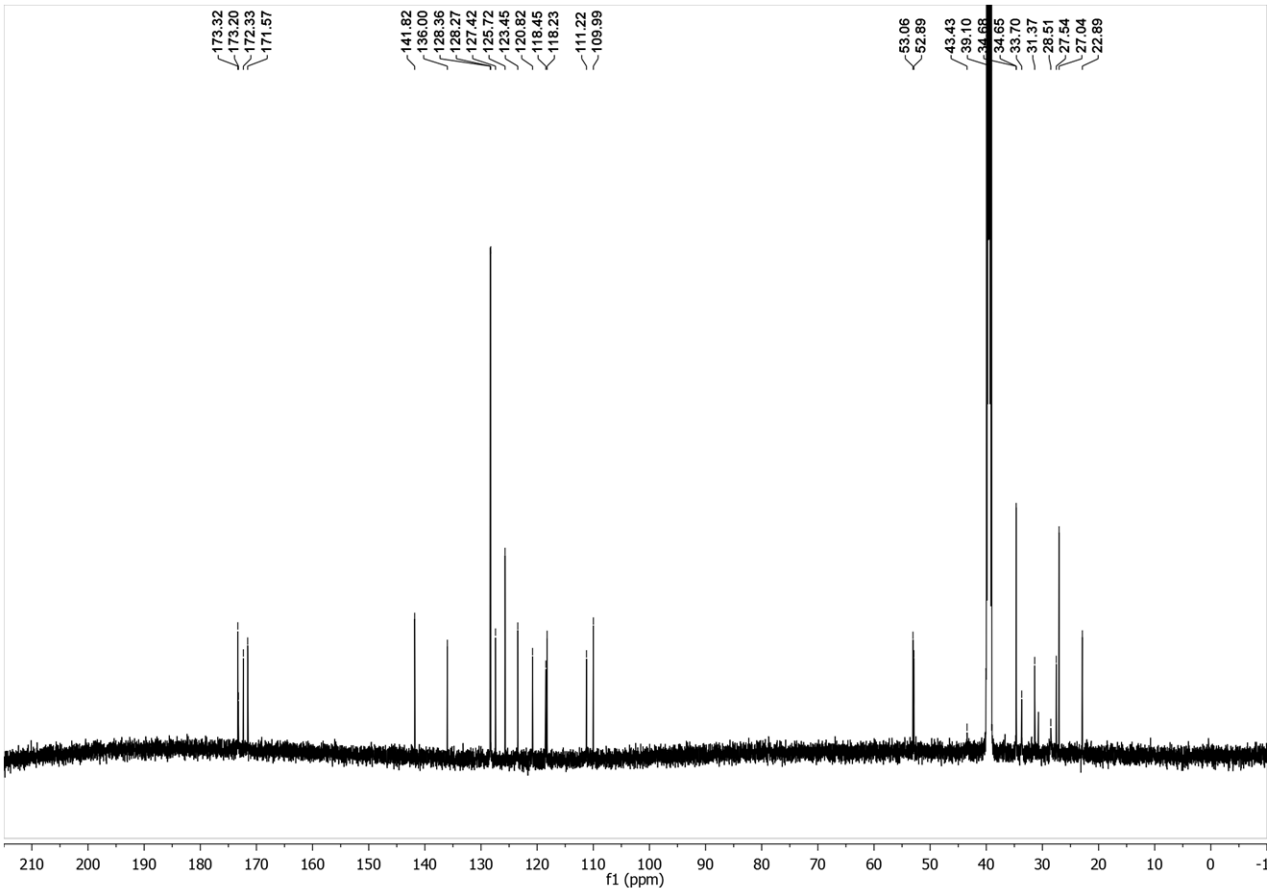
^{1}H and ^{13}C spectra of compound 33.



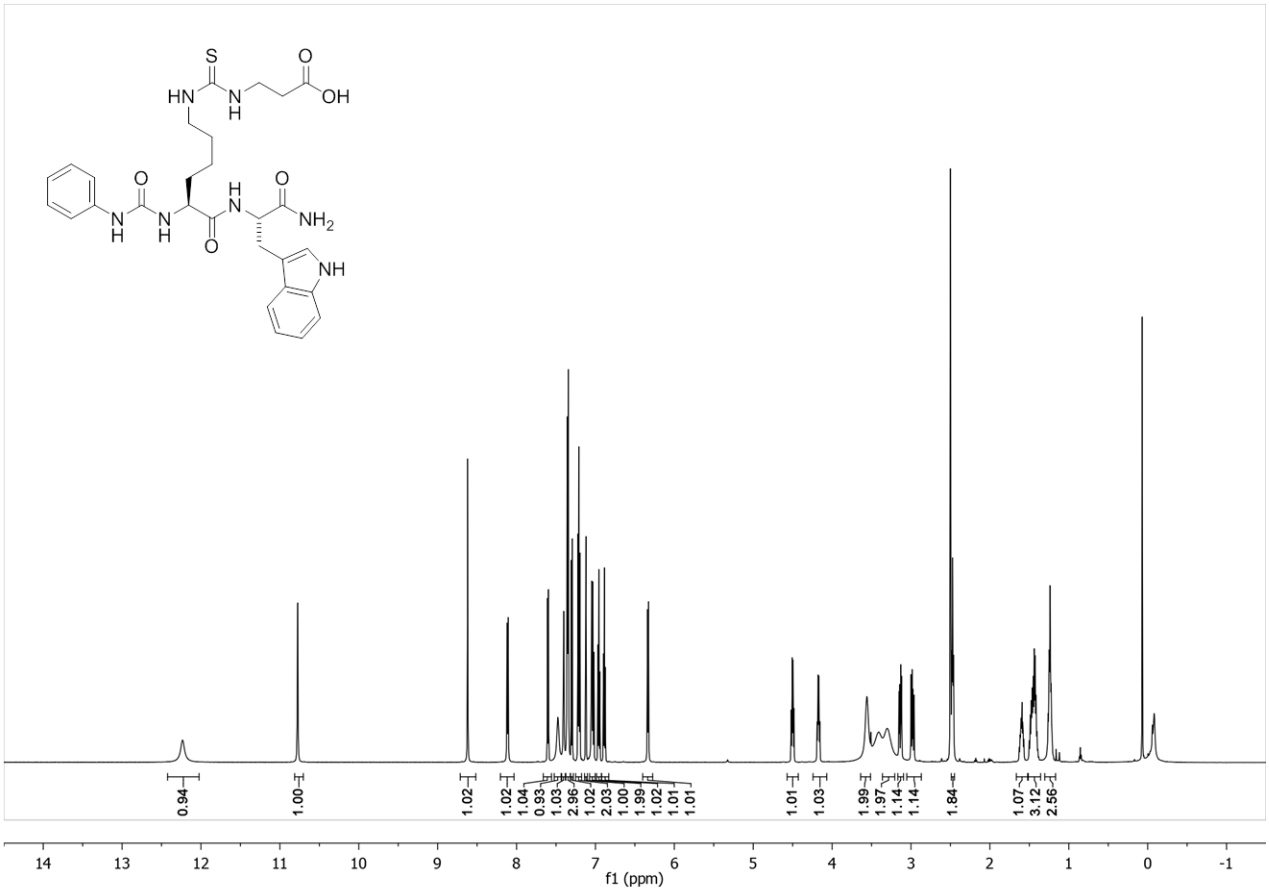


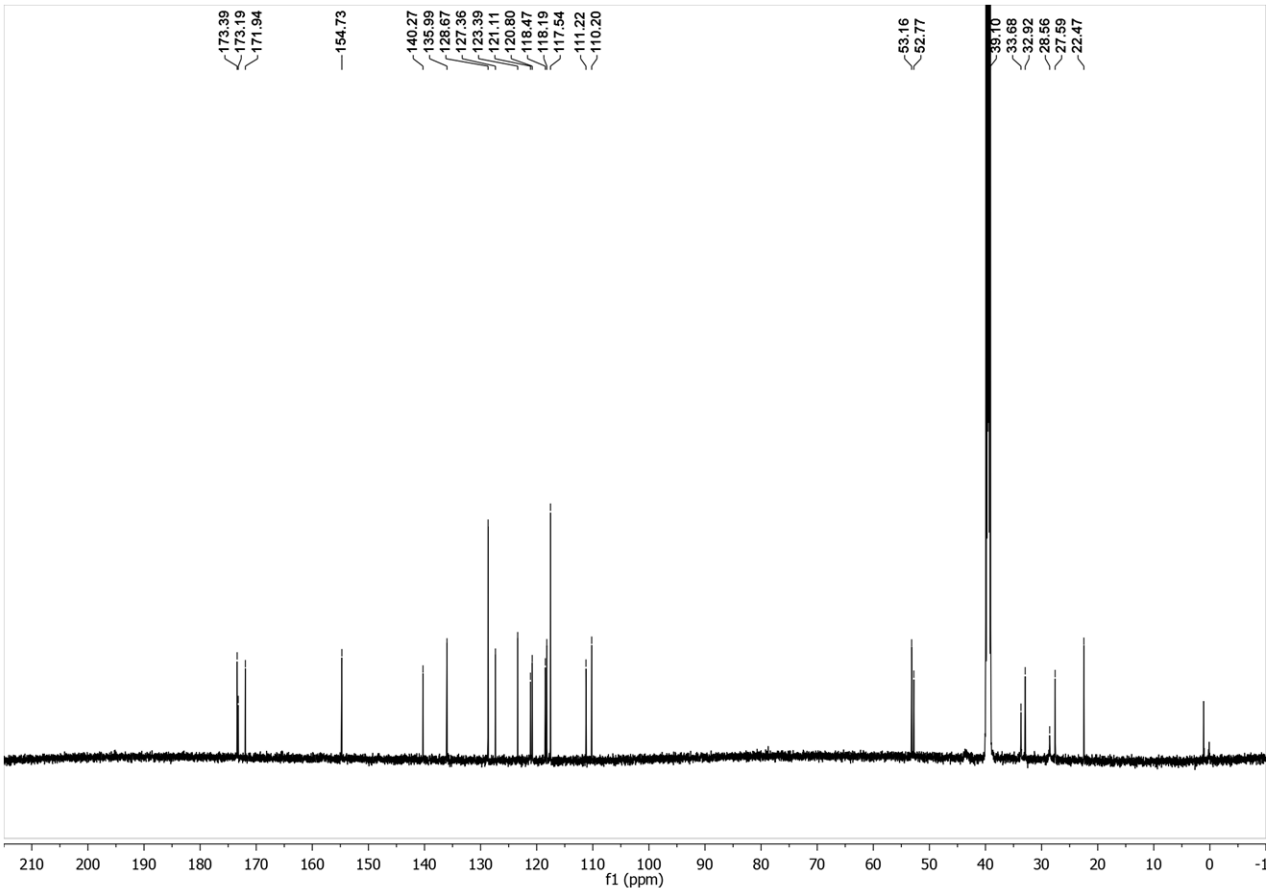
^{1}H and ^{13}C spectra of compound 34.



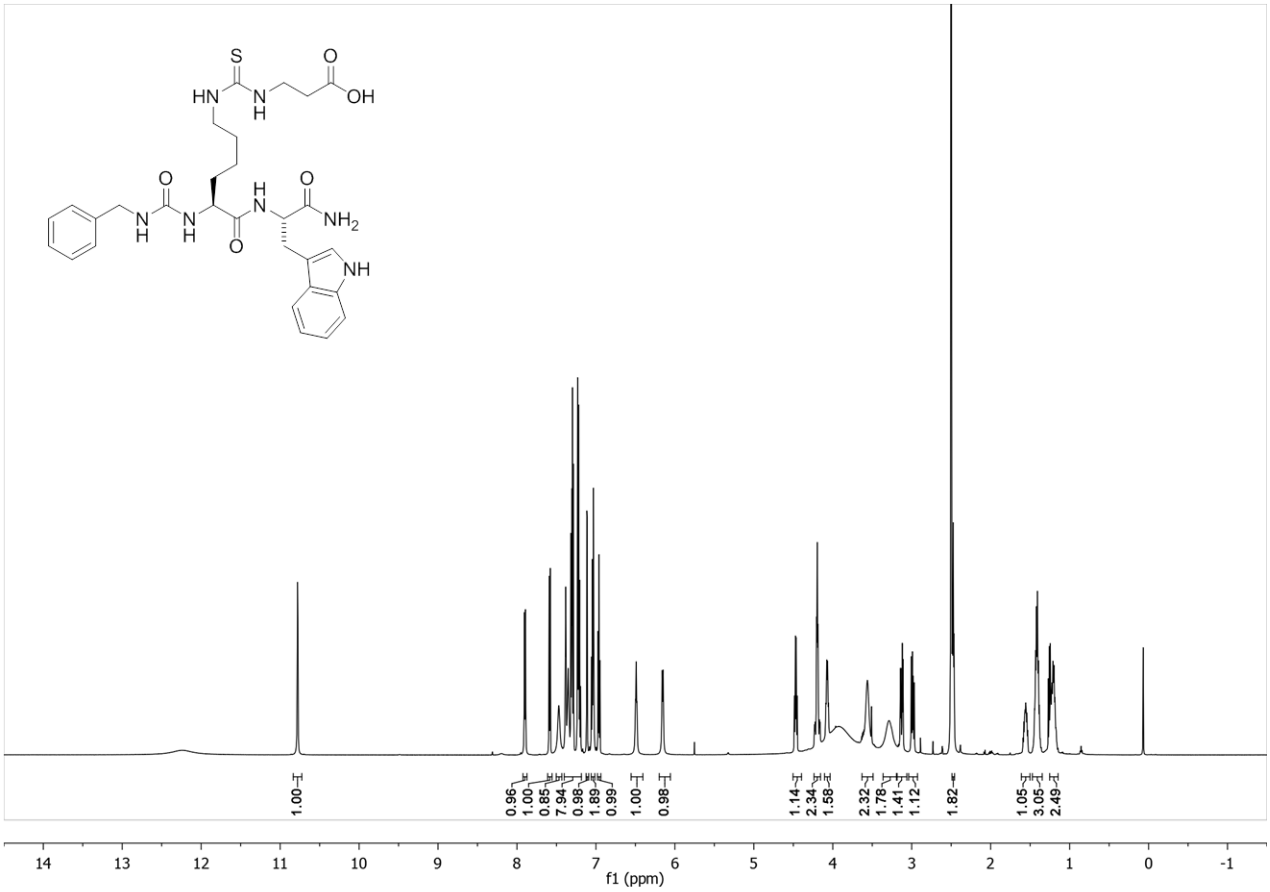


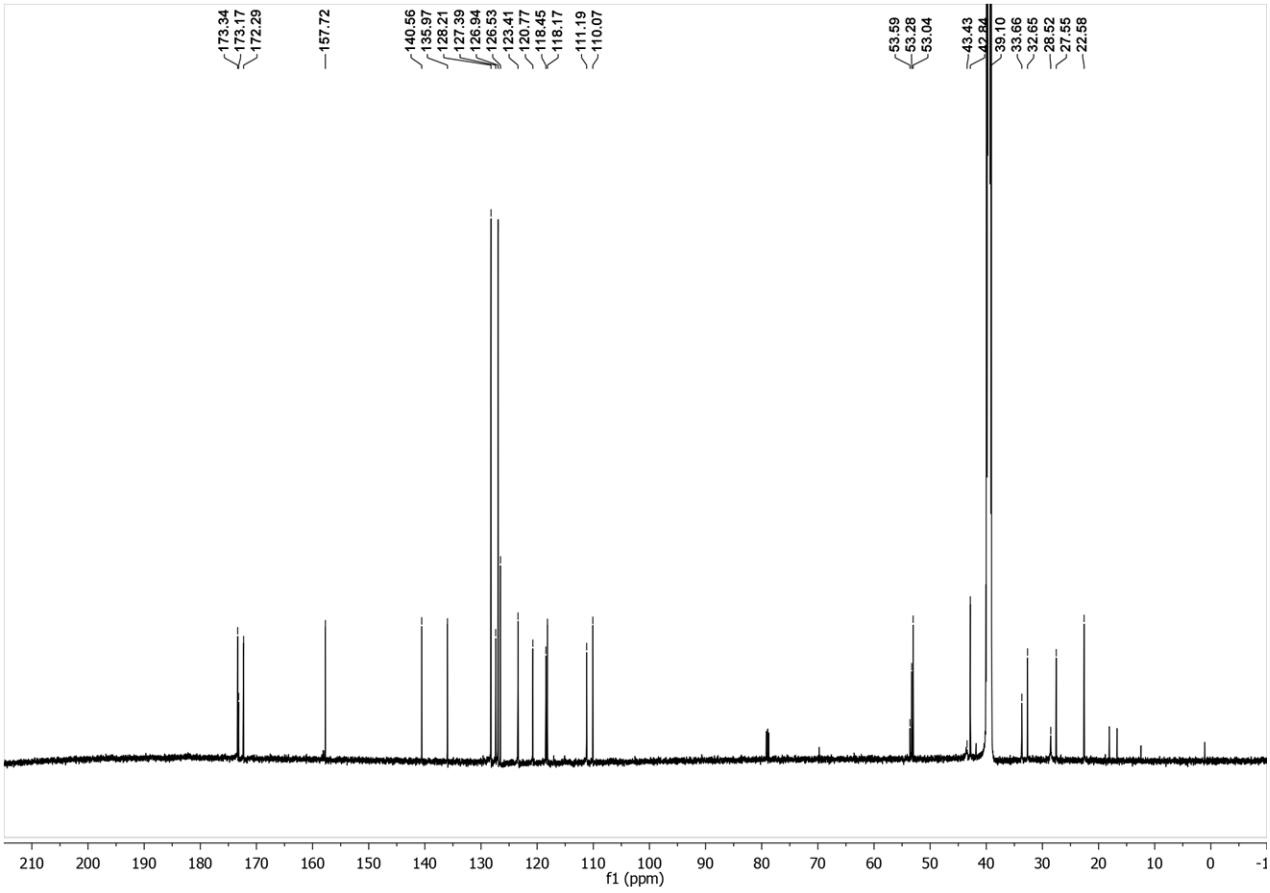
^{1}H and ^{13}C spectra of compound 35.



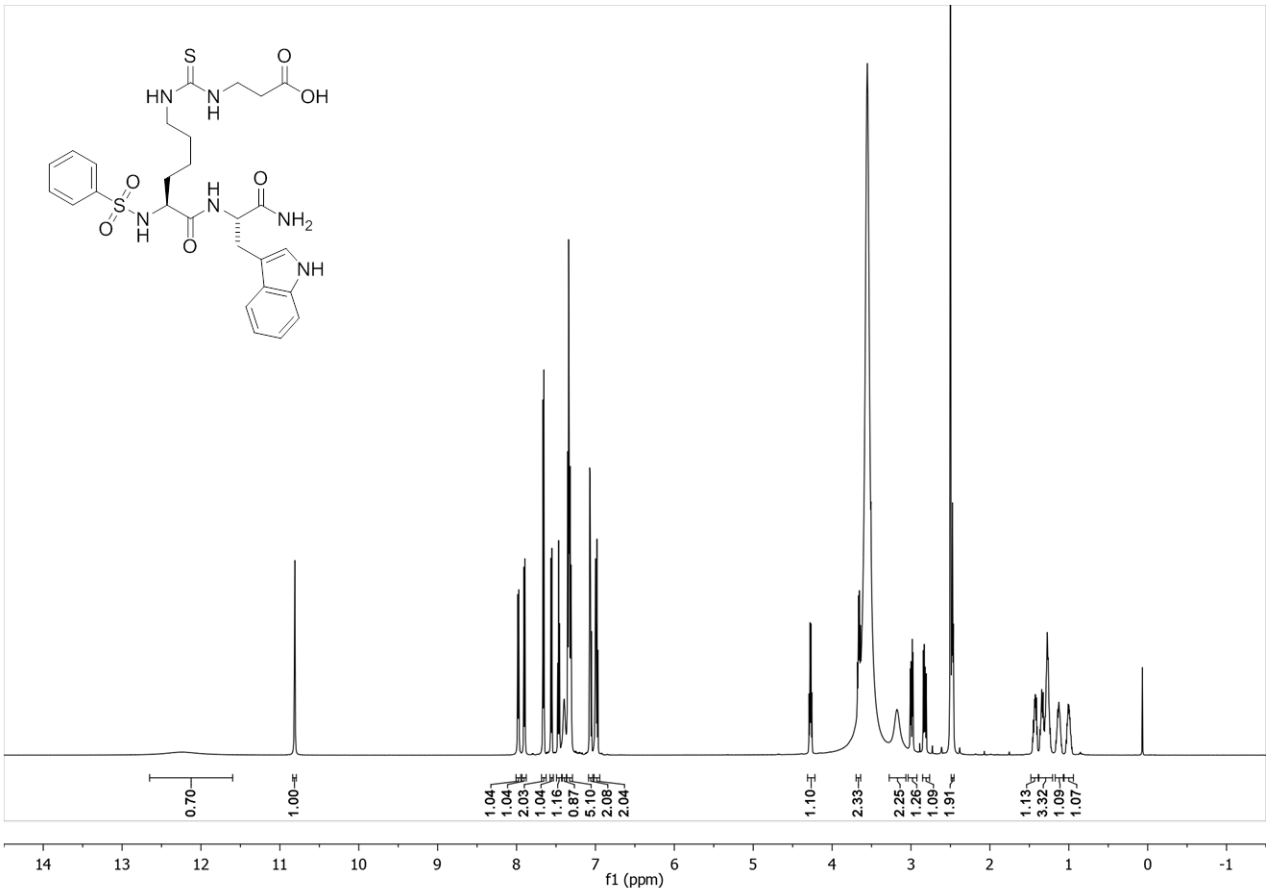


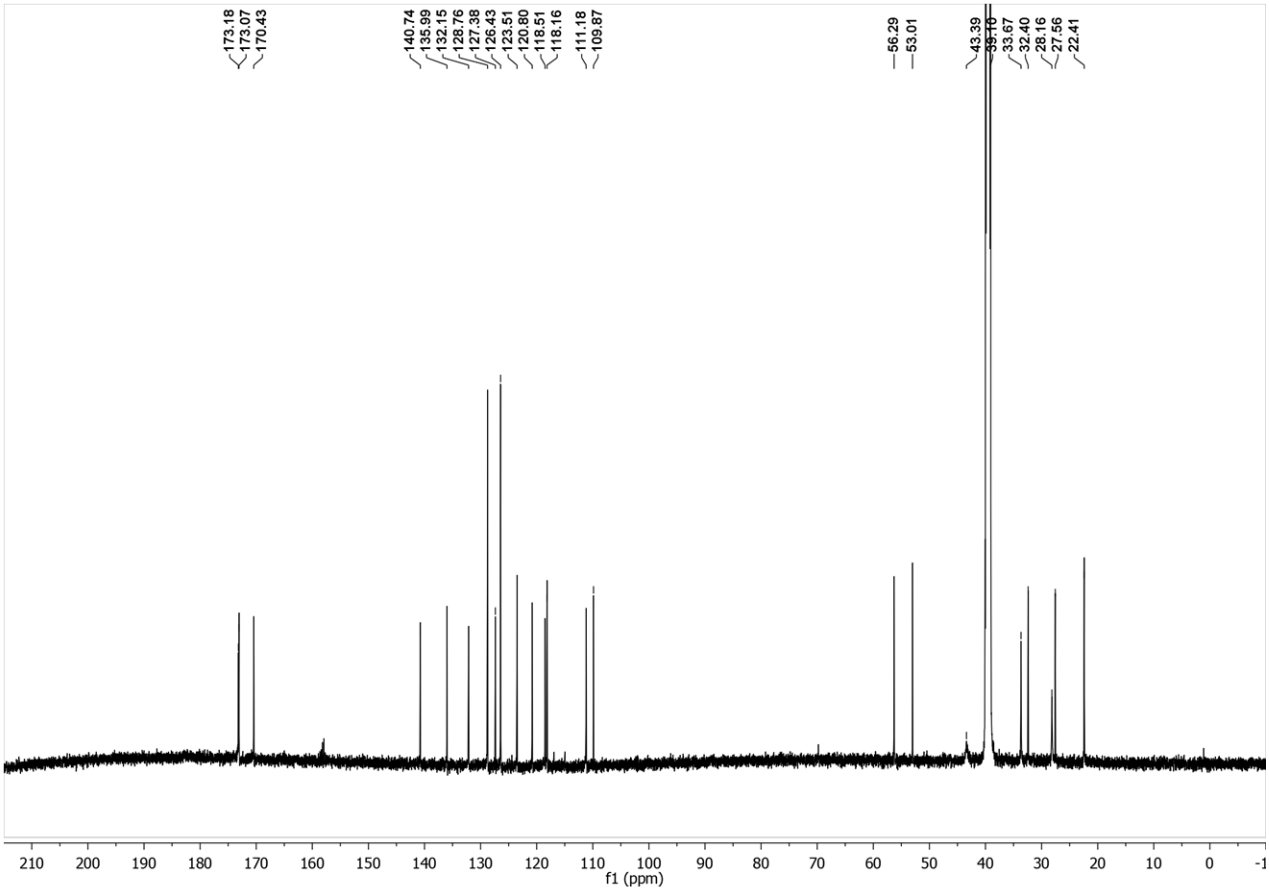
^{1}H and ^{13}C spectra of compound 36.



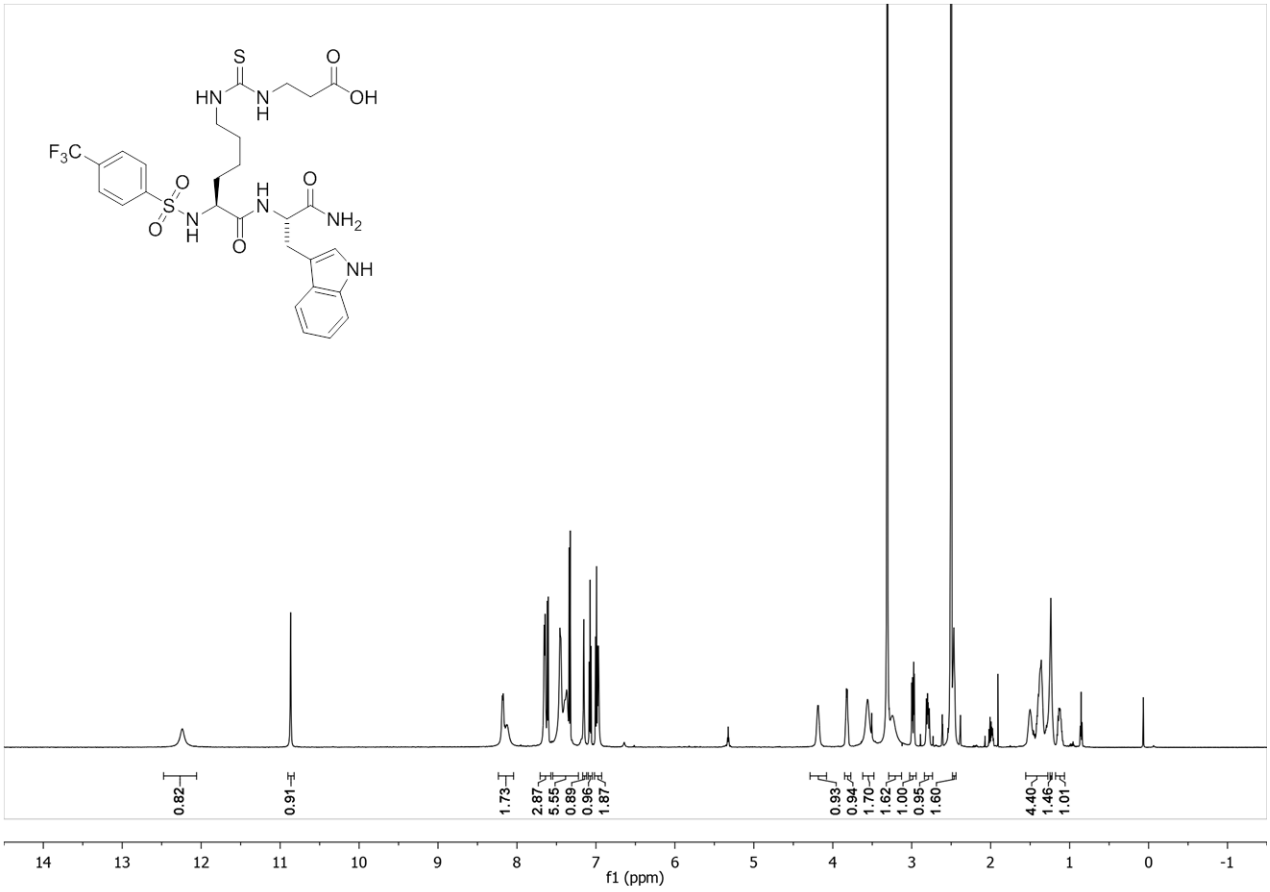


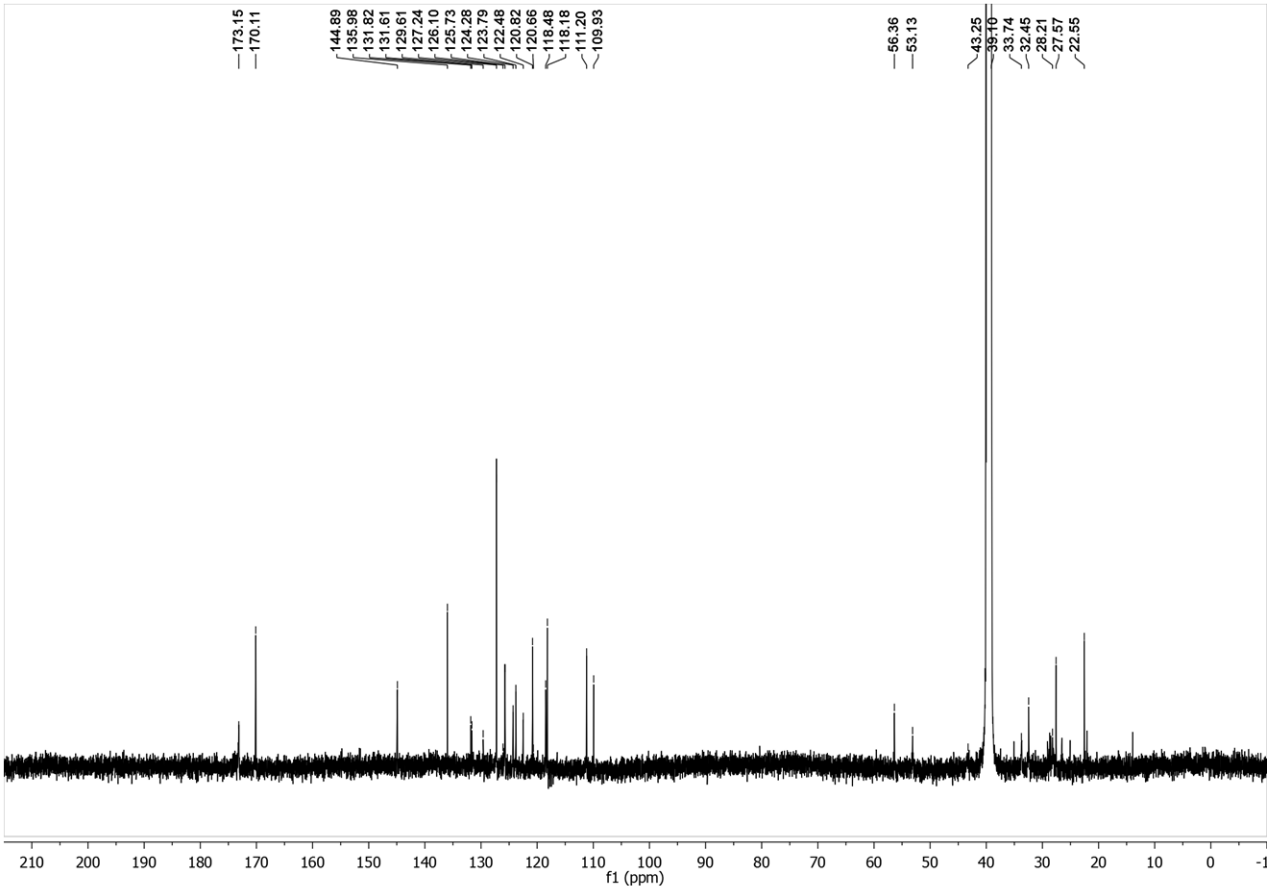
^{1}H and ^{13}C spectra of compound 37.

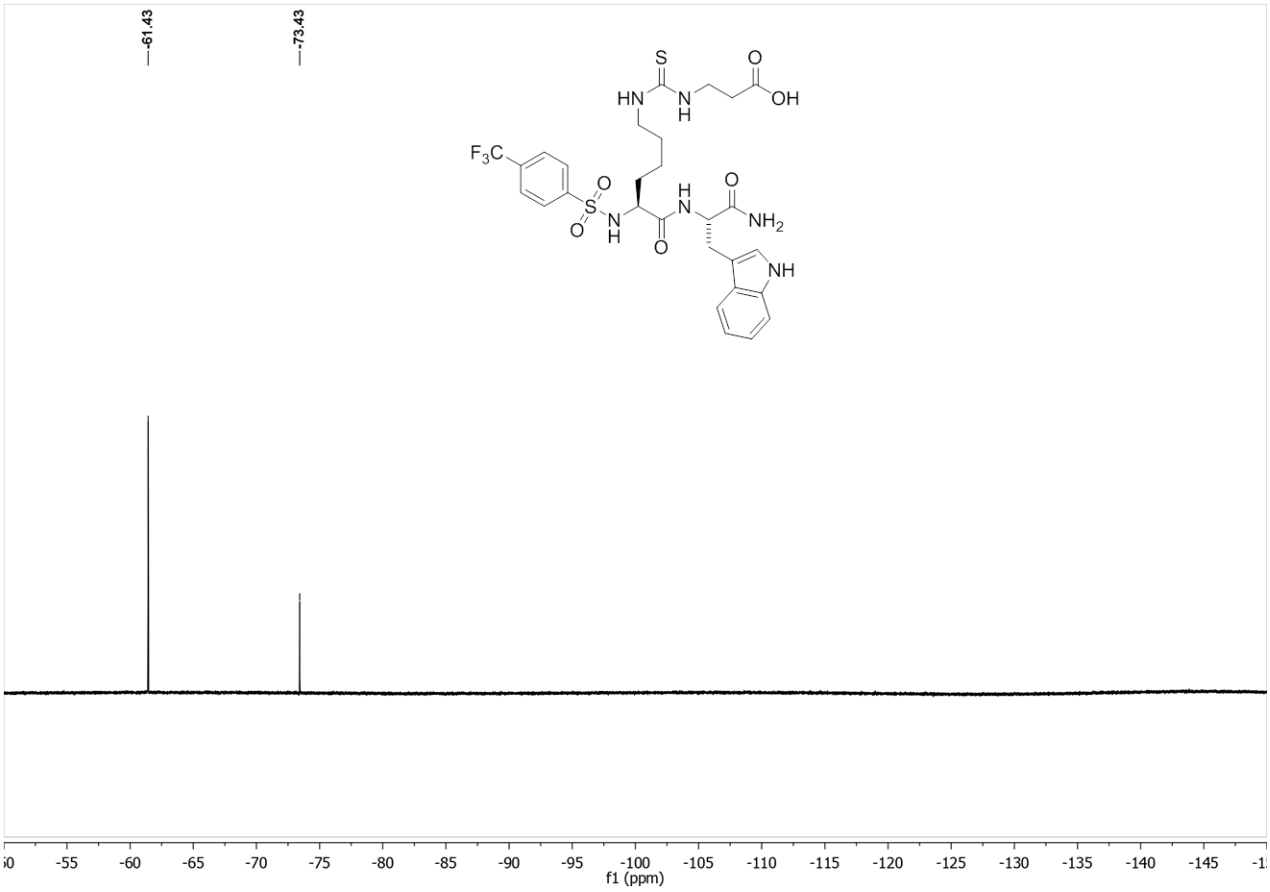




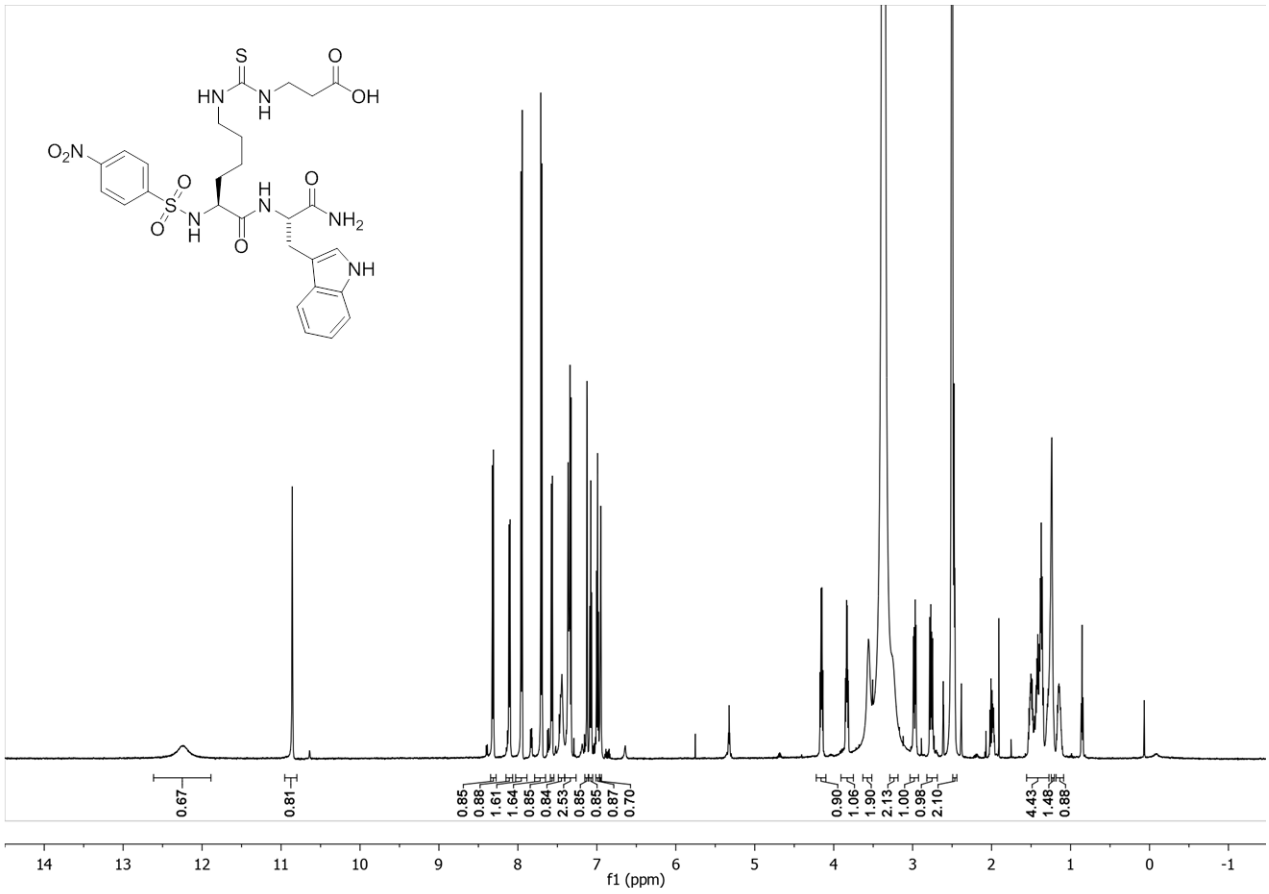
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound 38.

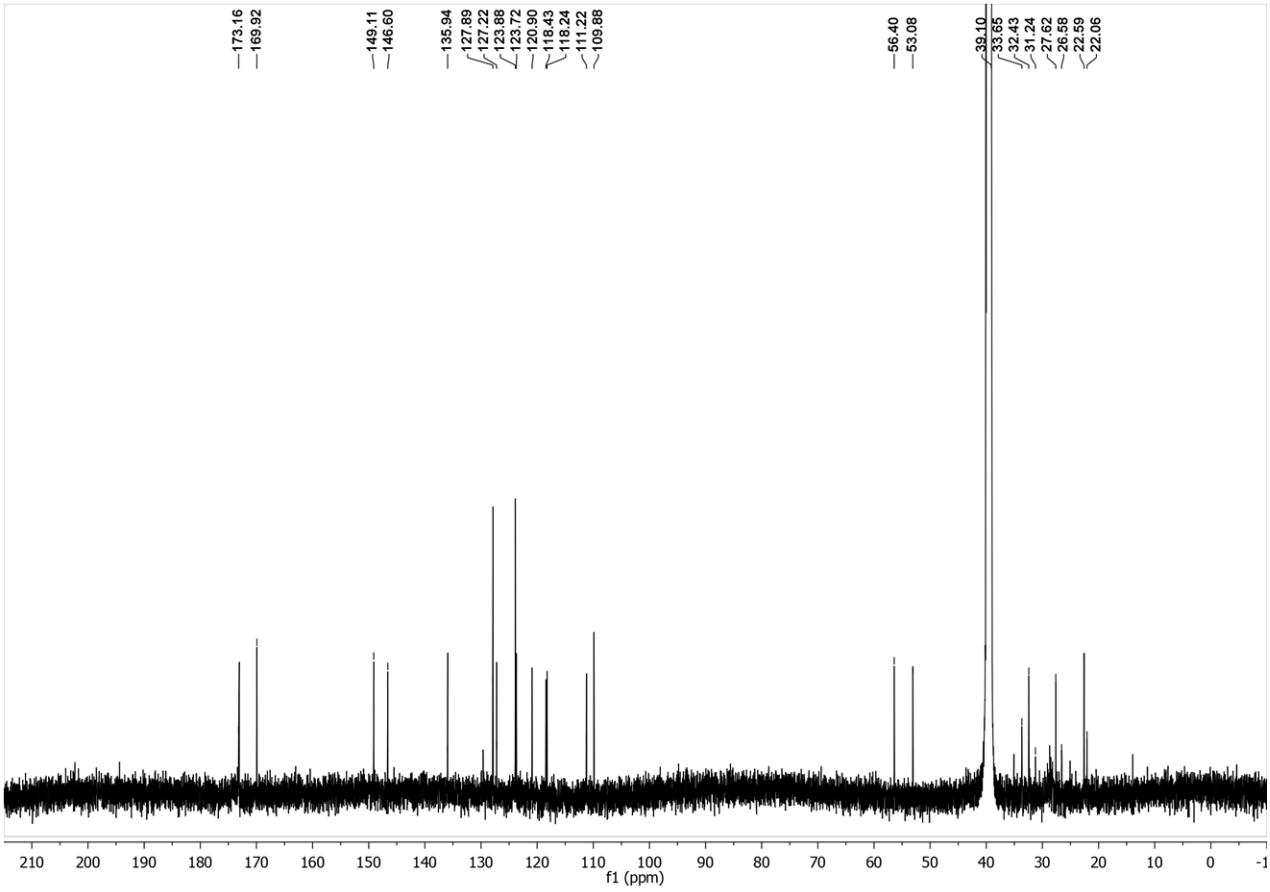




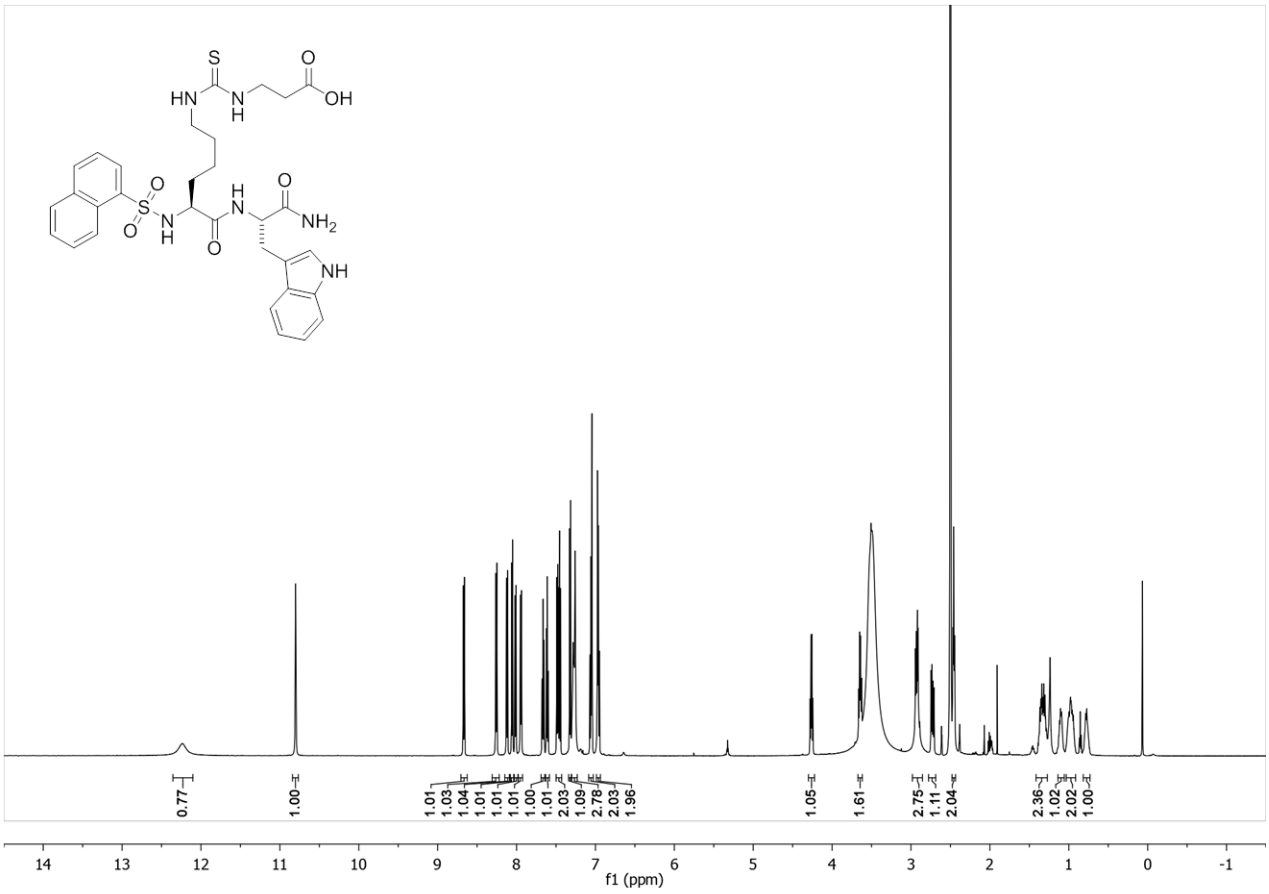

-61.43
-73.43
S
O
HN
N
H
OH
F3C
O
S
O
N
H
H
N
O
O
NH2
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

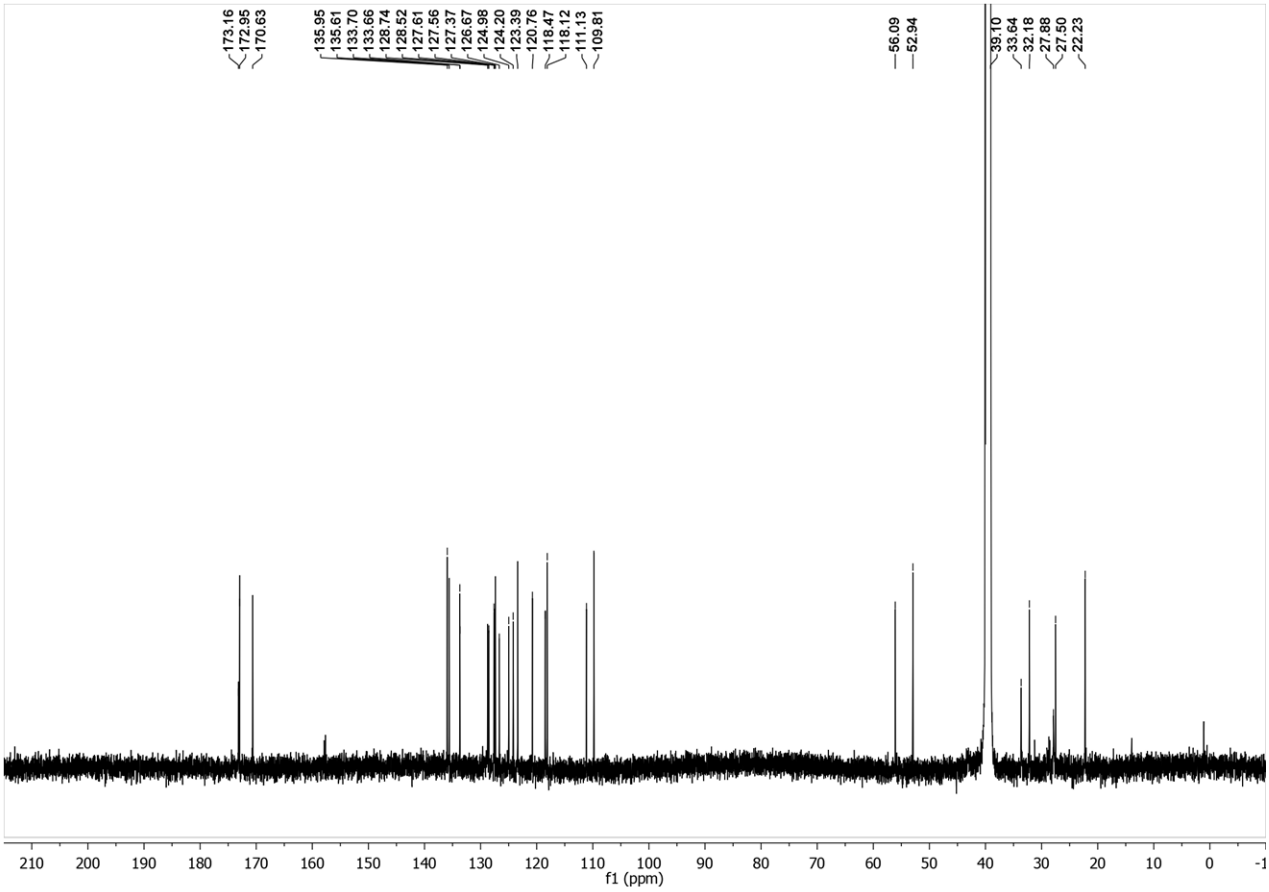
^{1}H and ^{13}C spectra of compound 39.



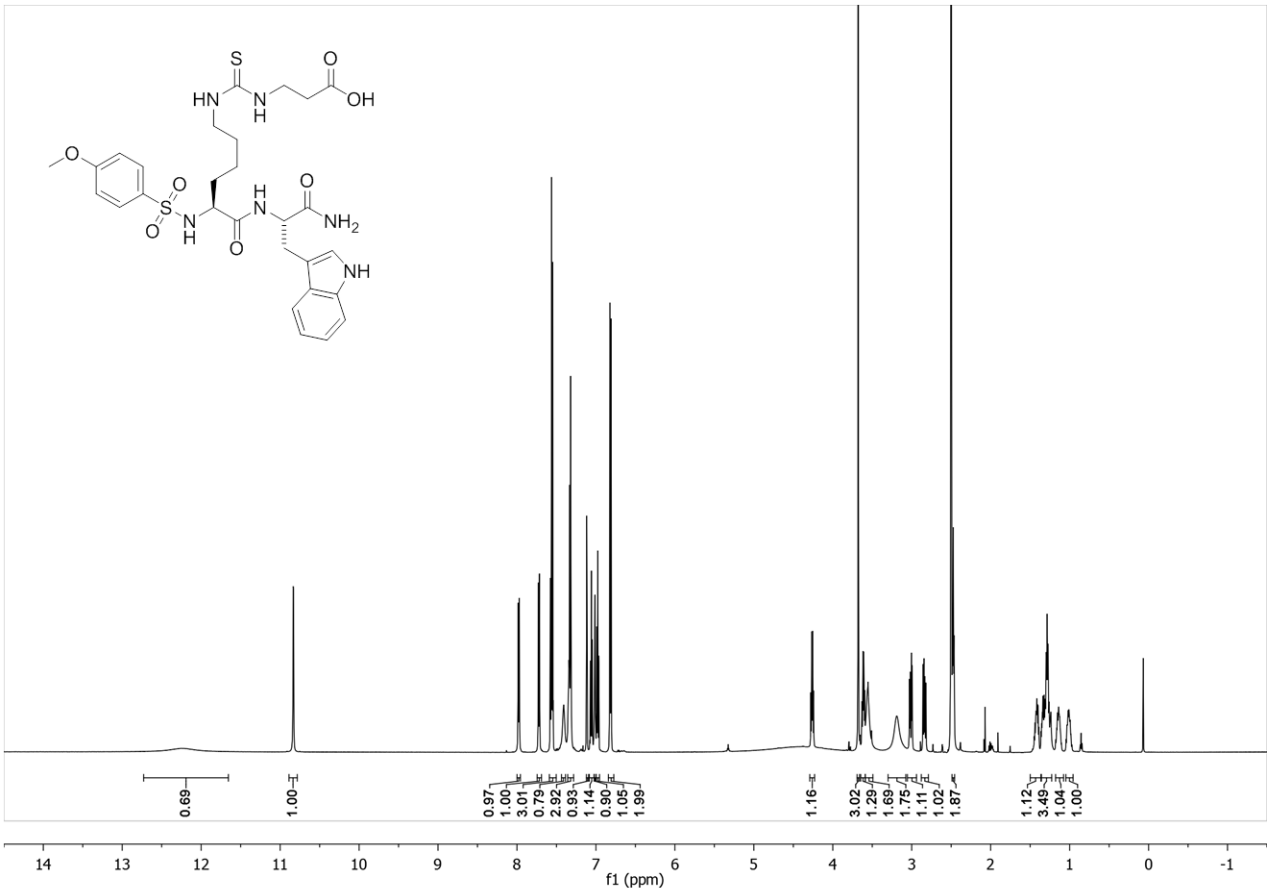


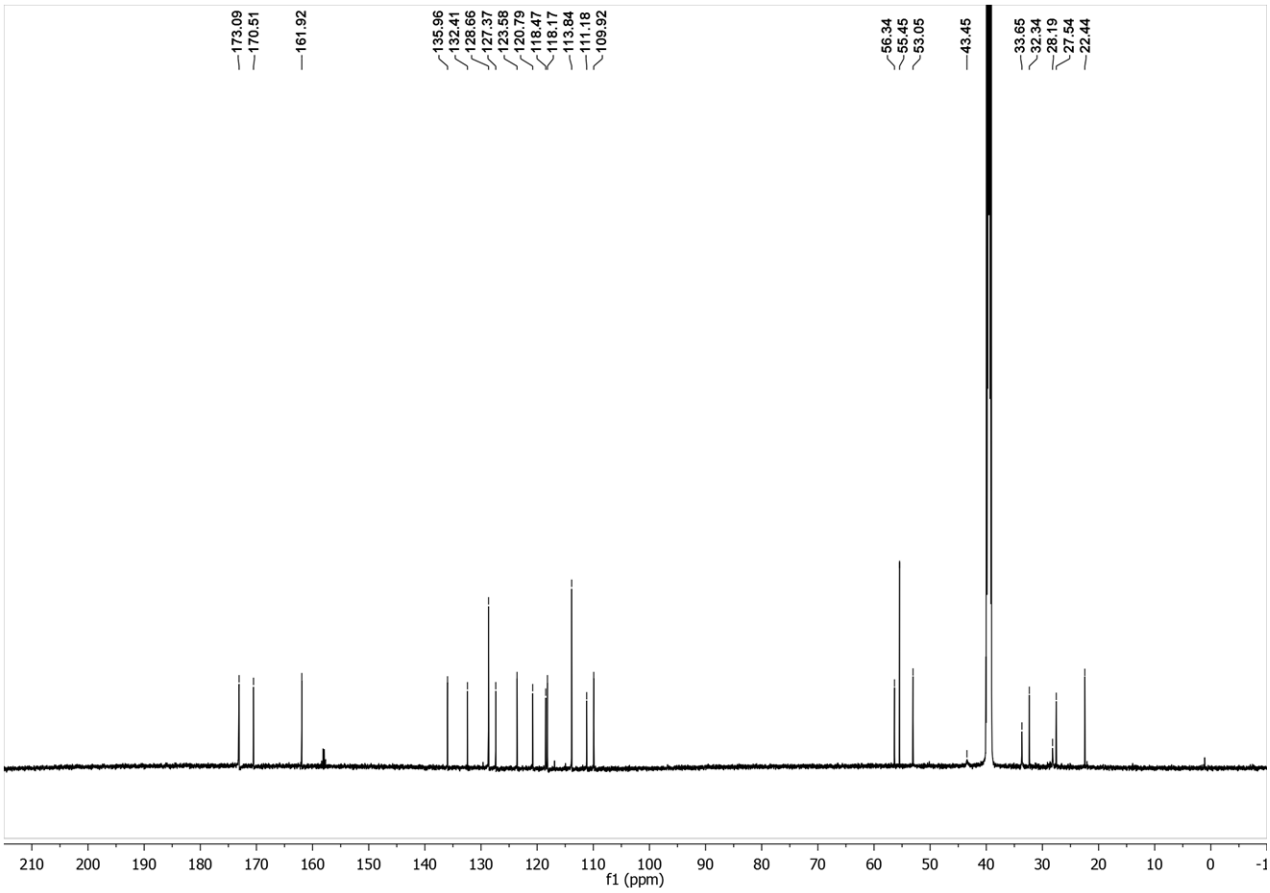
^{1}H and ^{13}C spectra of compound 40.



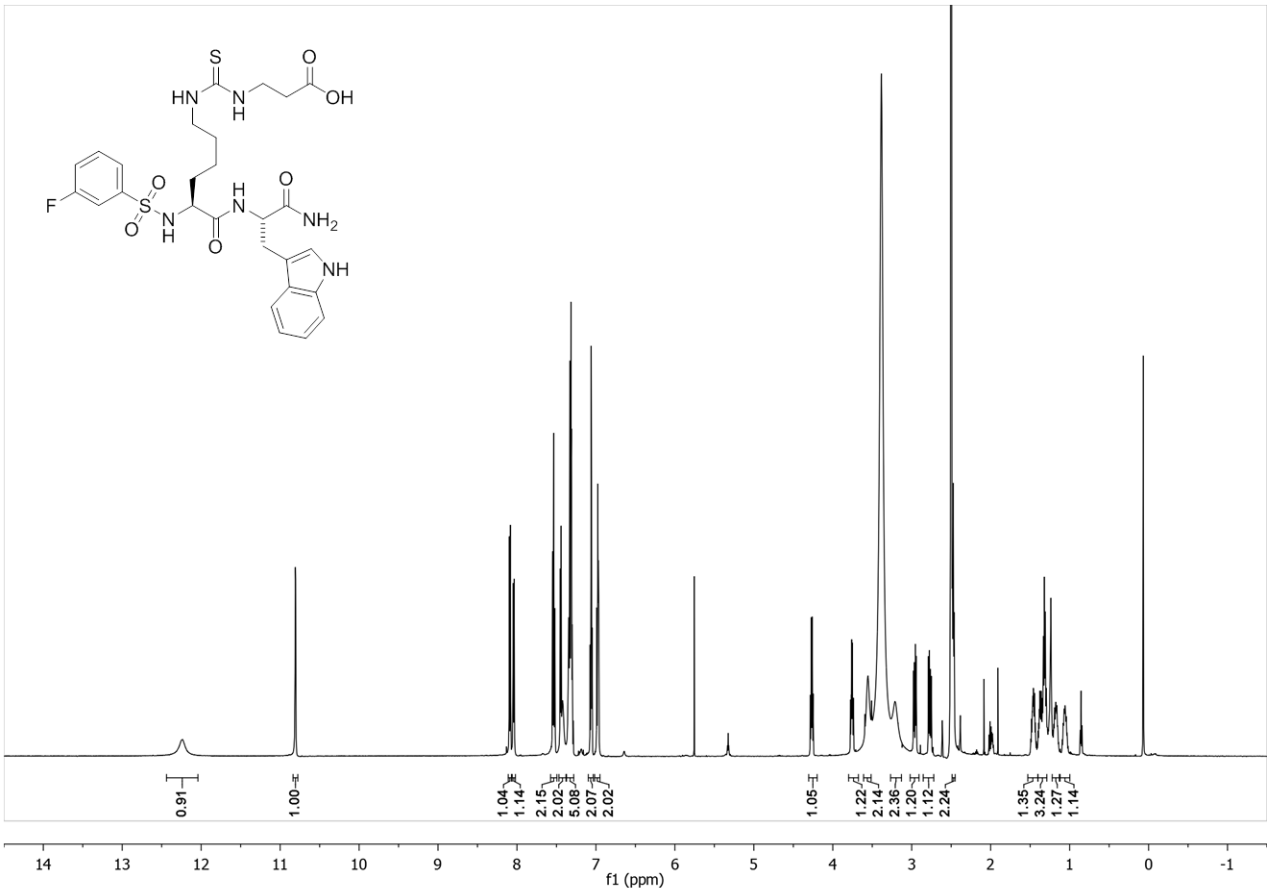


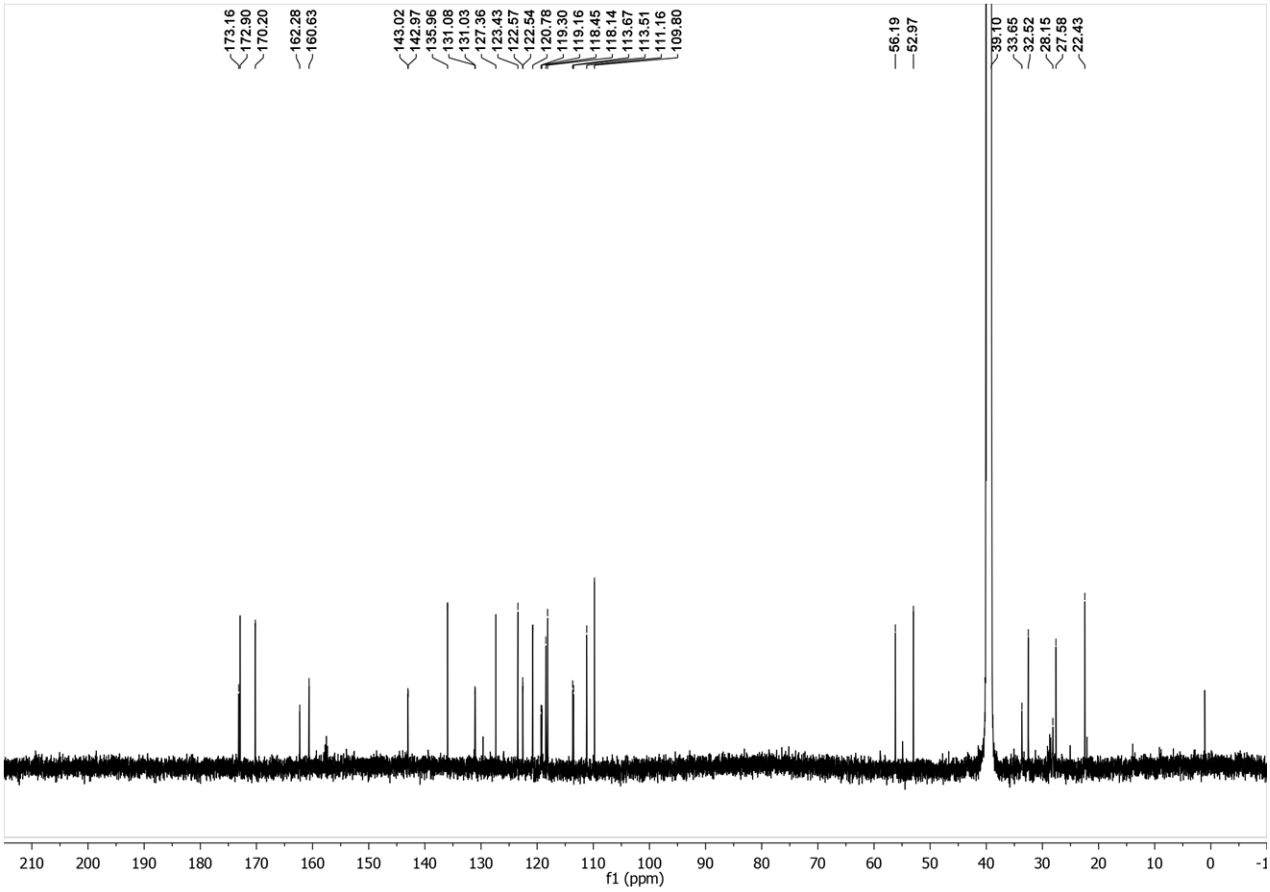
^{1}H and ^{13}C spectra of compound 41.

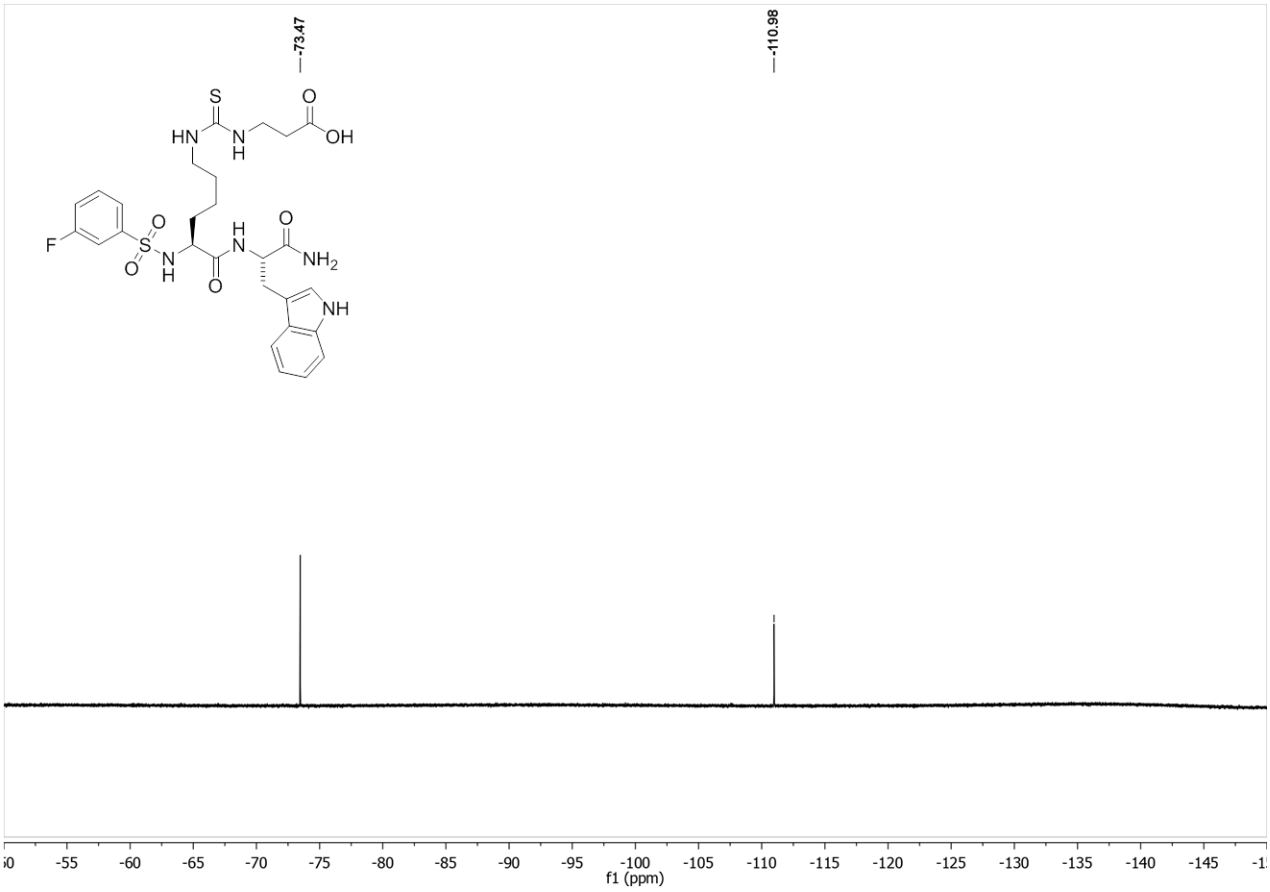




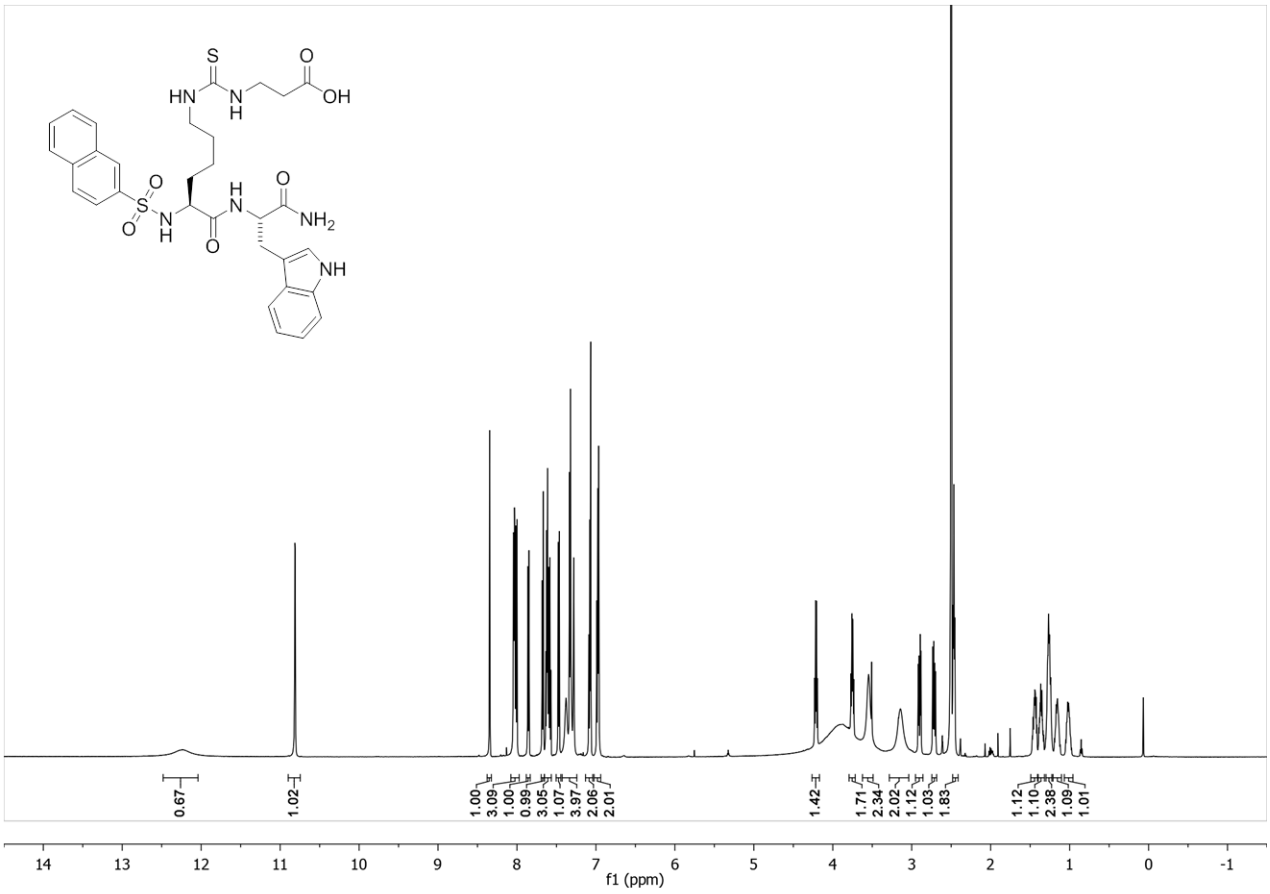
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound 42.

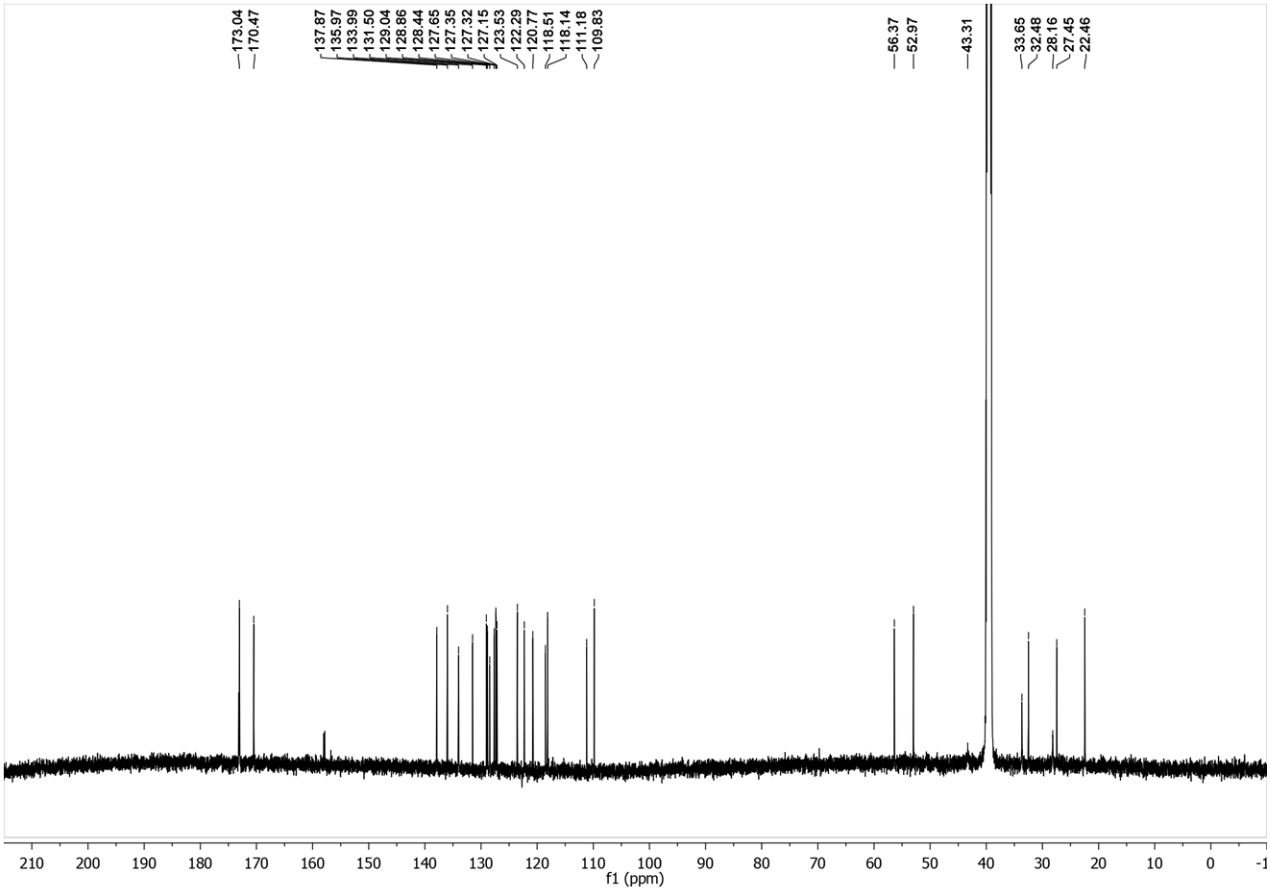




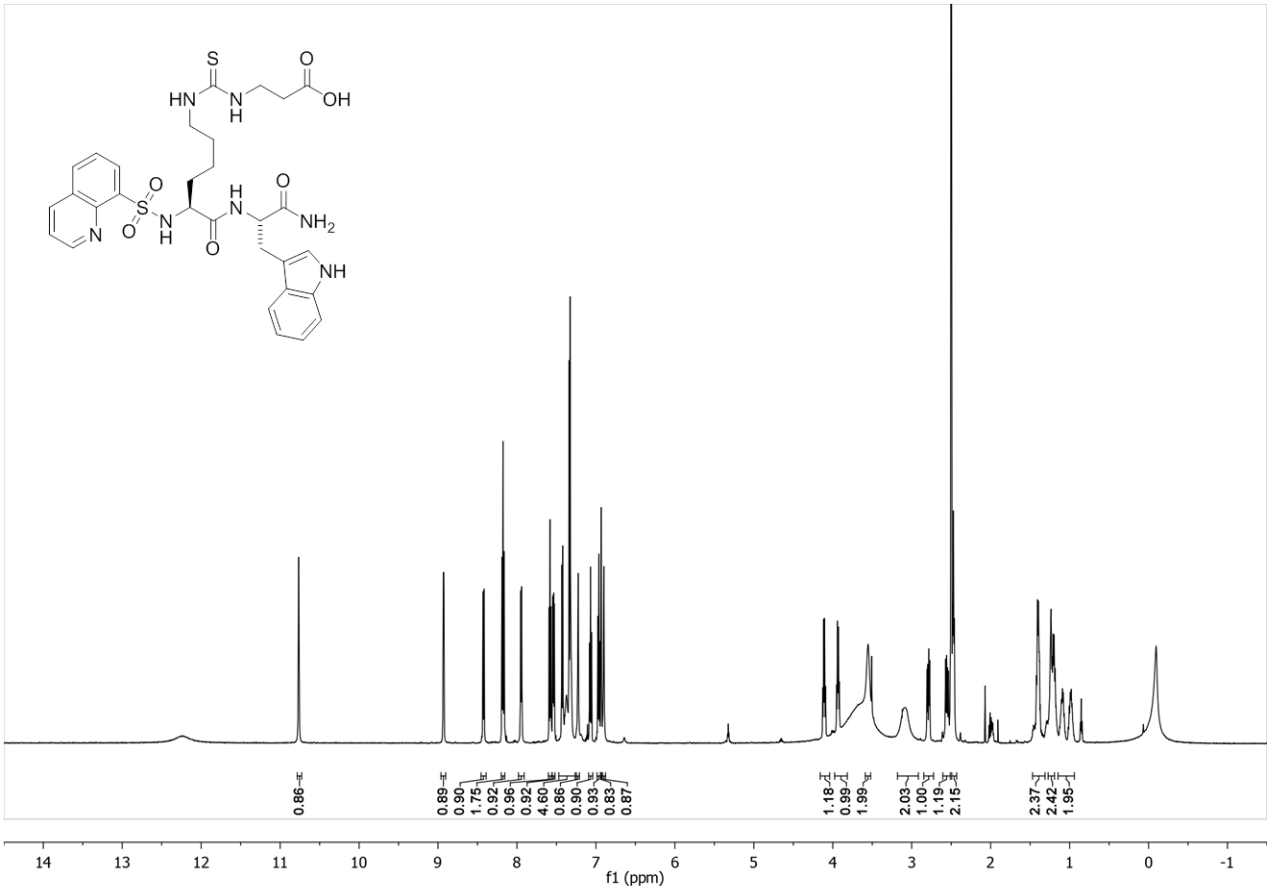

-73.47
-110.98
S
O
HN
N
H
OH
F
O
S
O
N
H
H
N
O
O
NH2
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

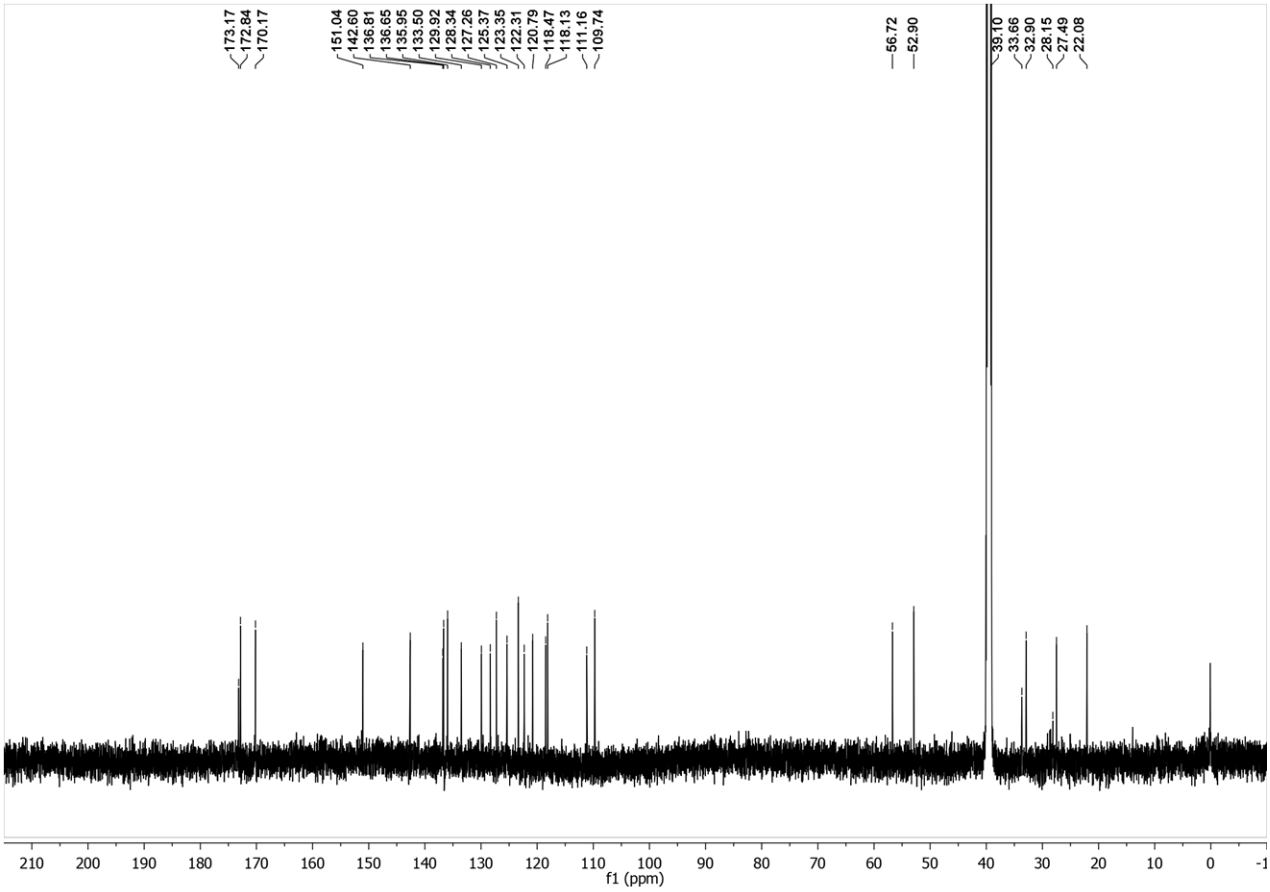
^{1}H and ^{13}C spectra of compound 43.



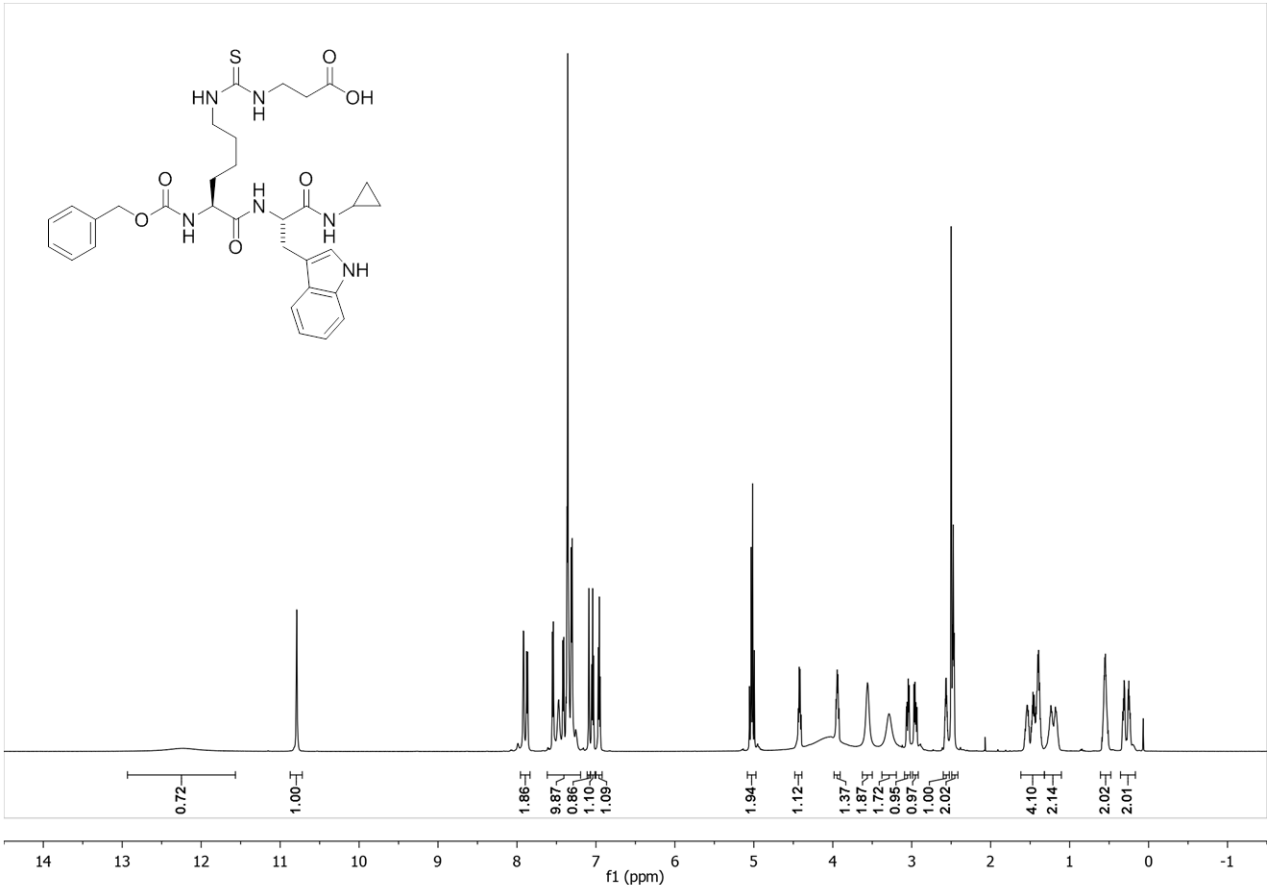


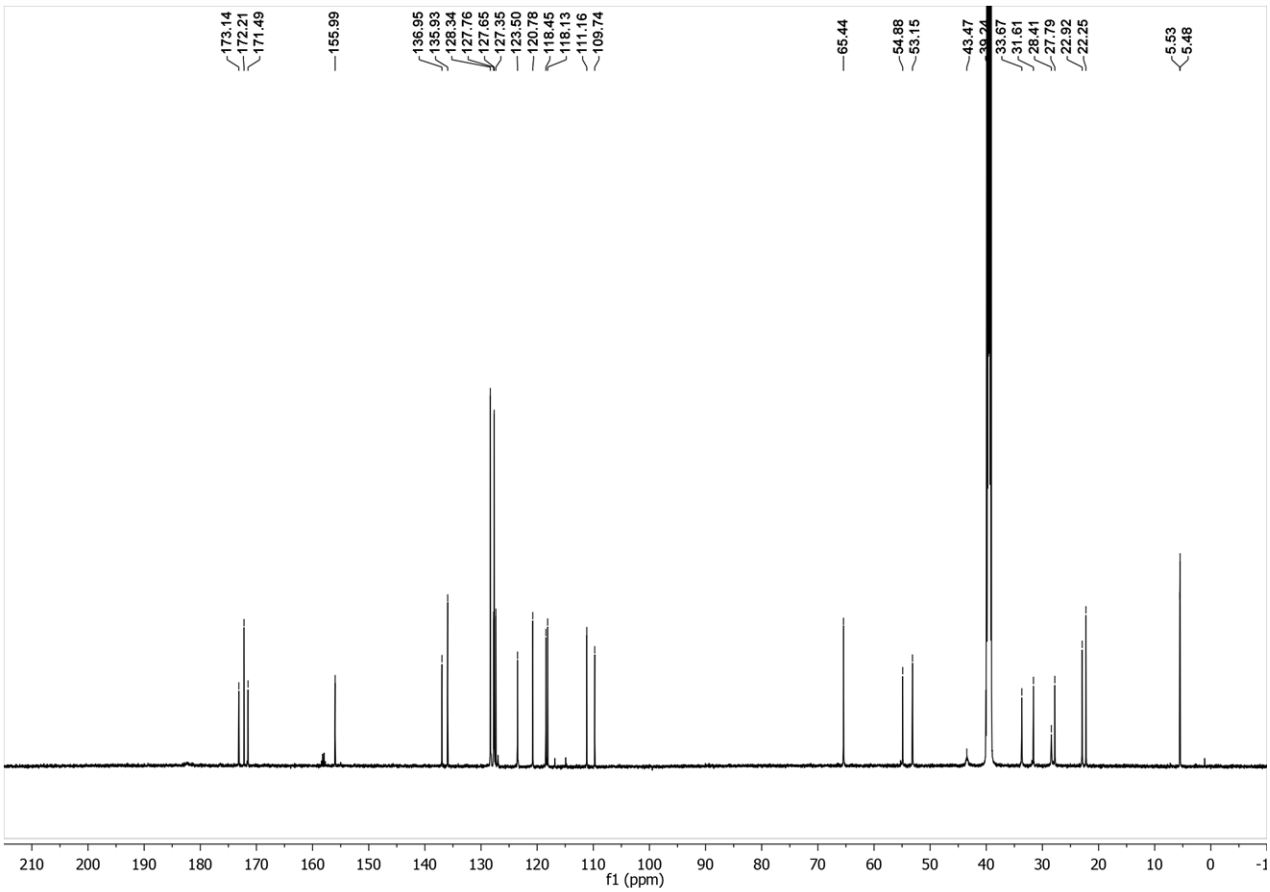
^{1}H and ^{13}C spectra of compound 44.



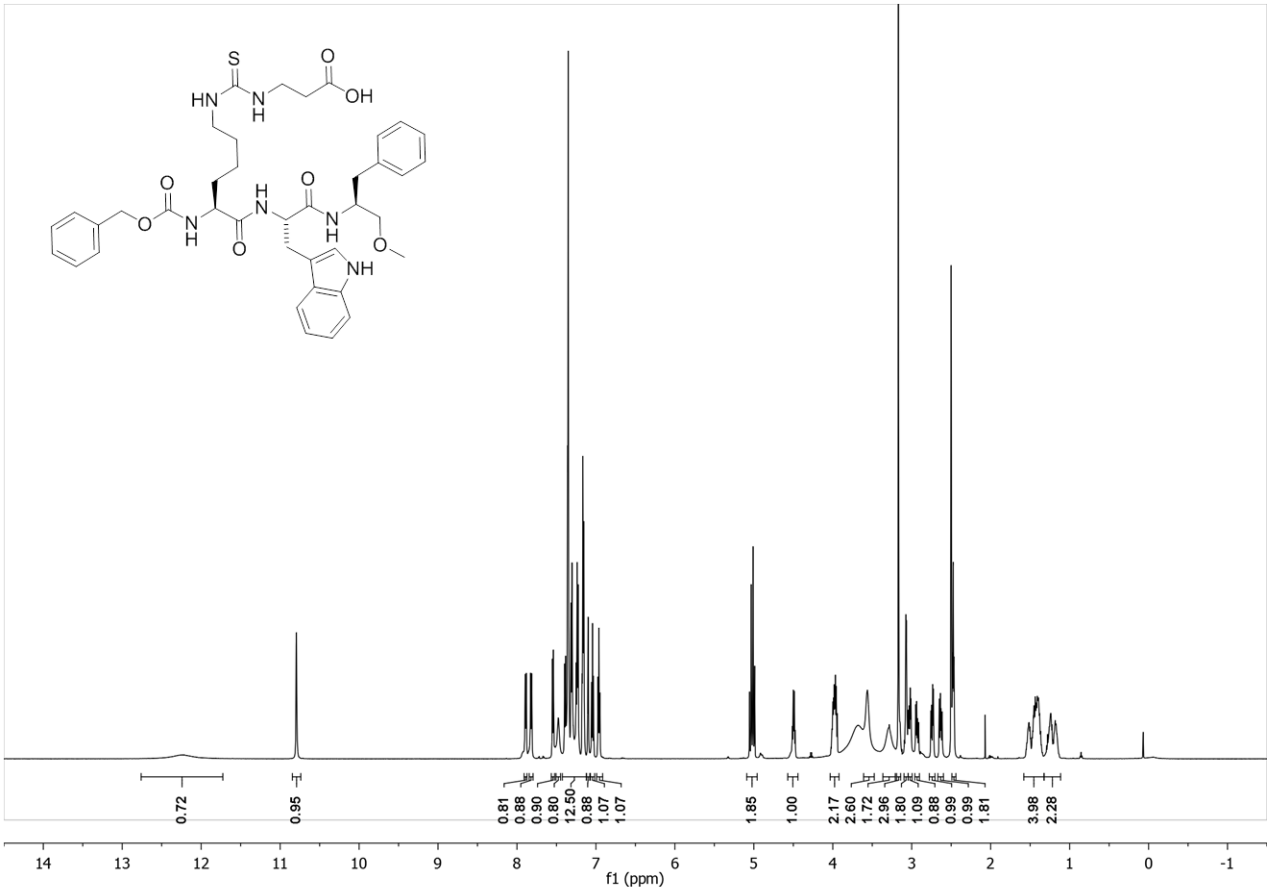


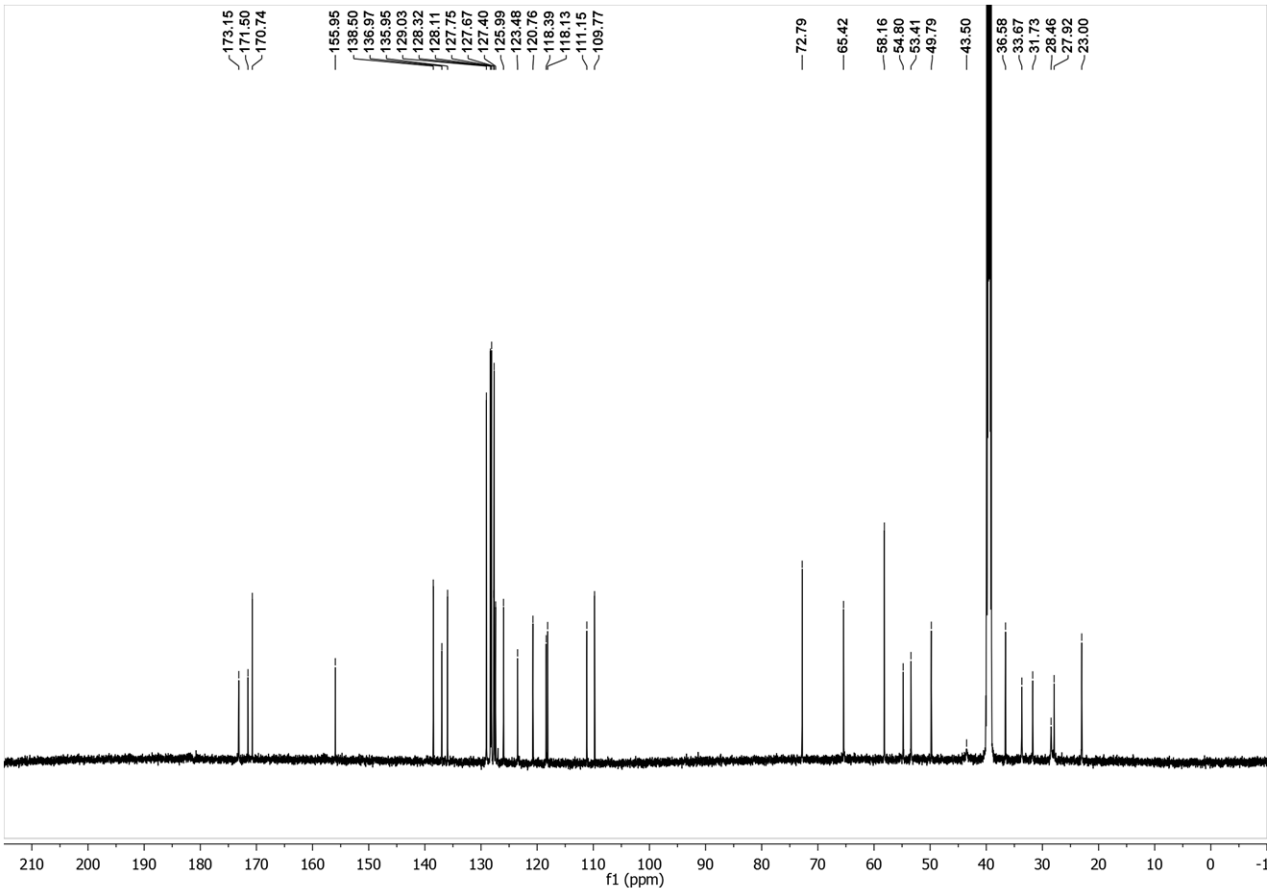
^{1}H and ^{13}C spectra of compound 45.



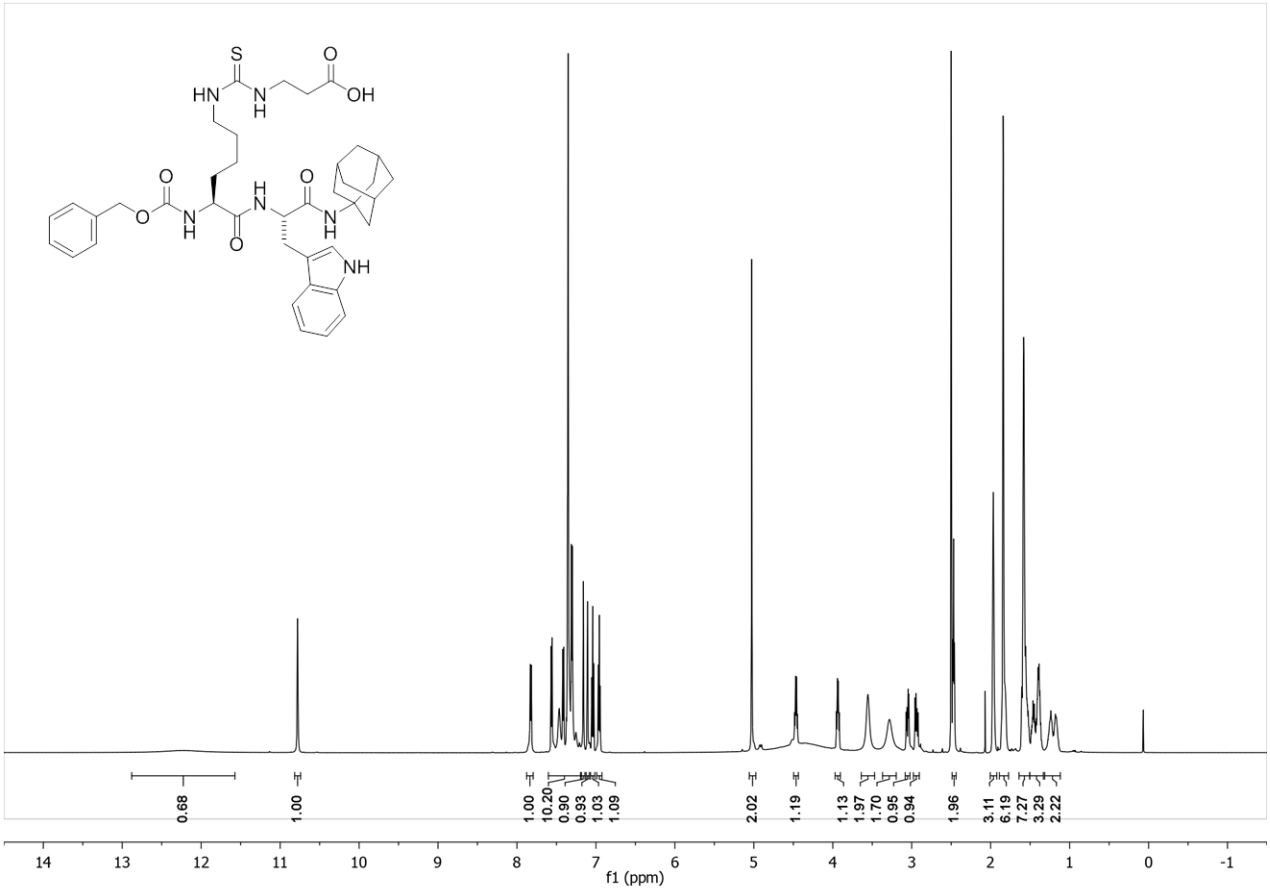


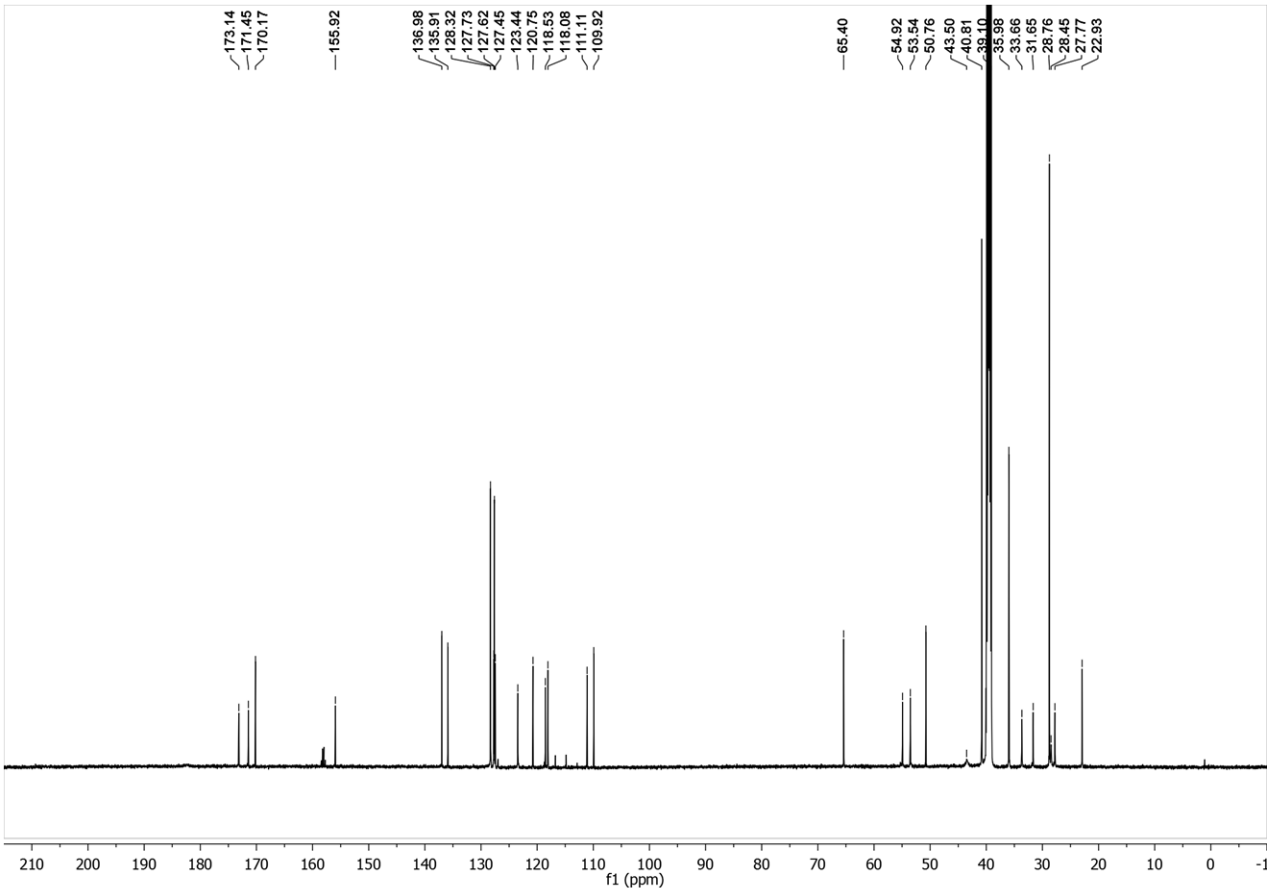
^{1}H and ^{13}C spectra of compound 46.



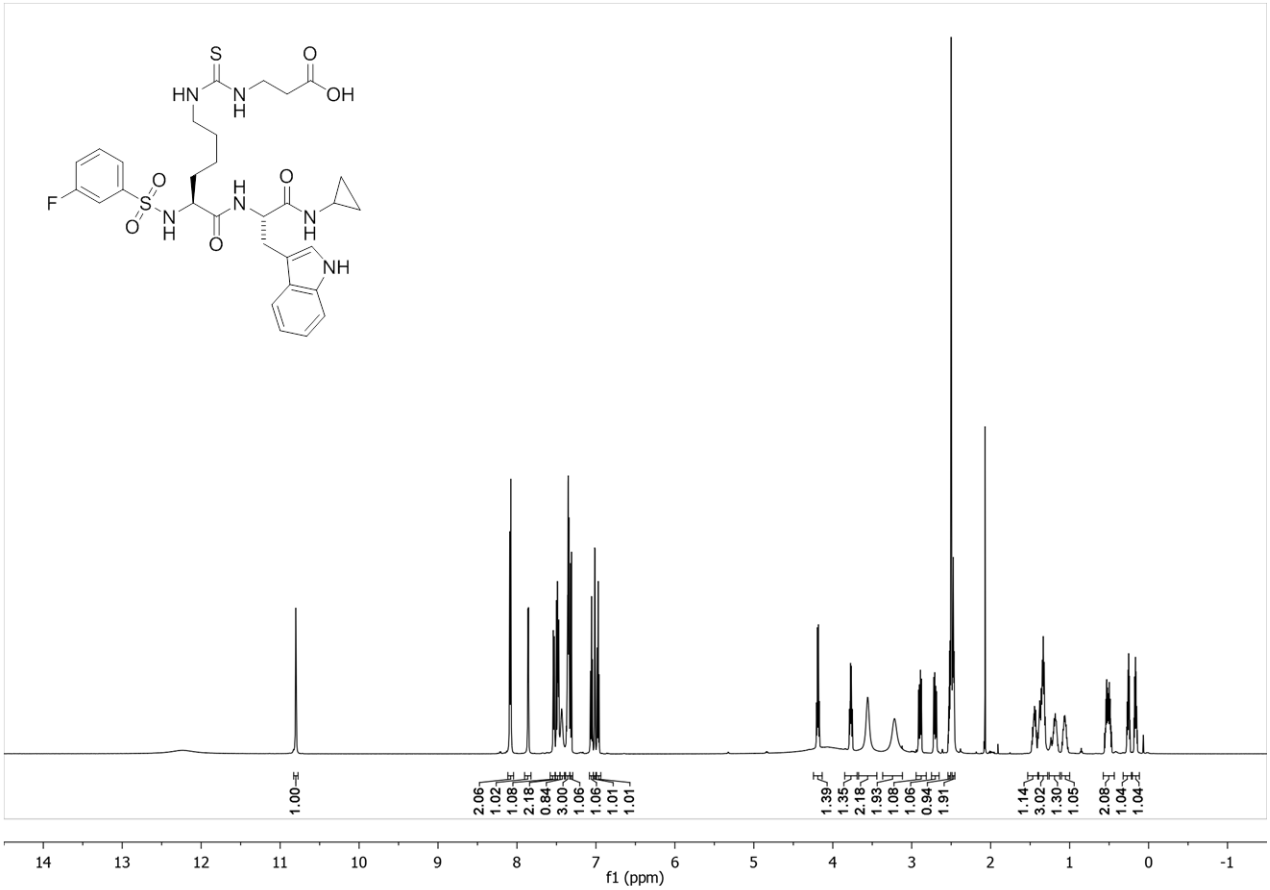


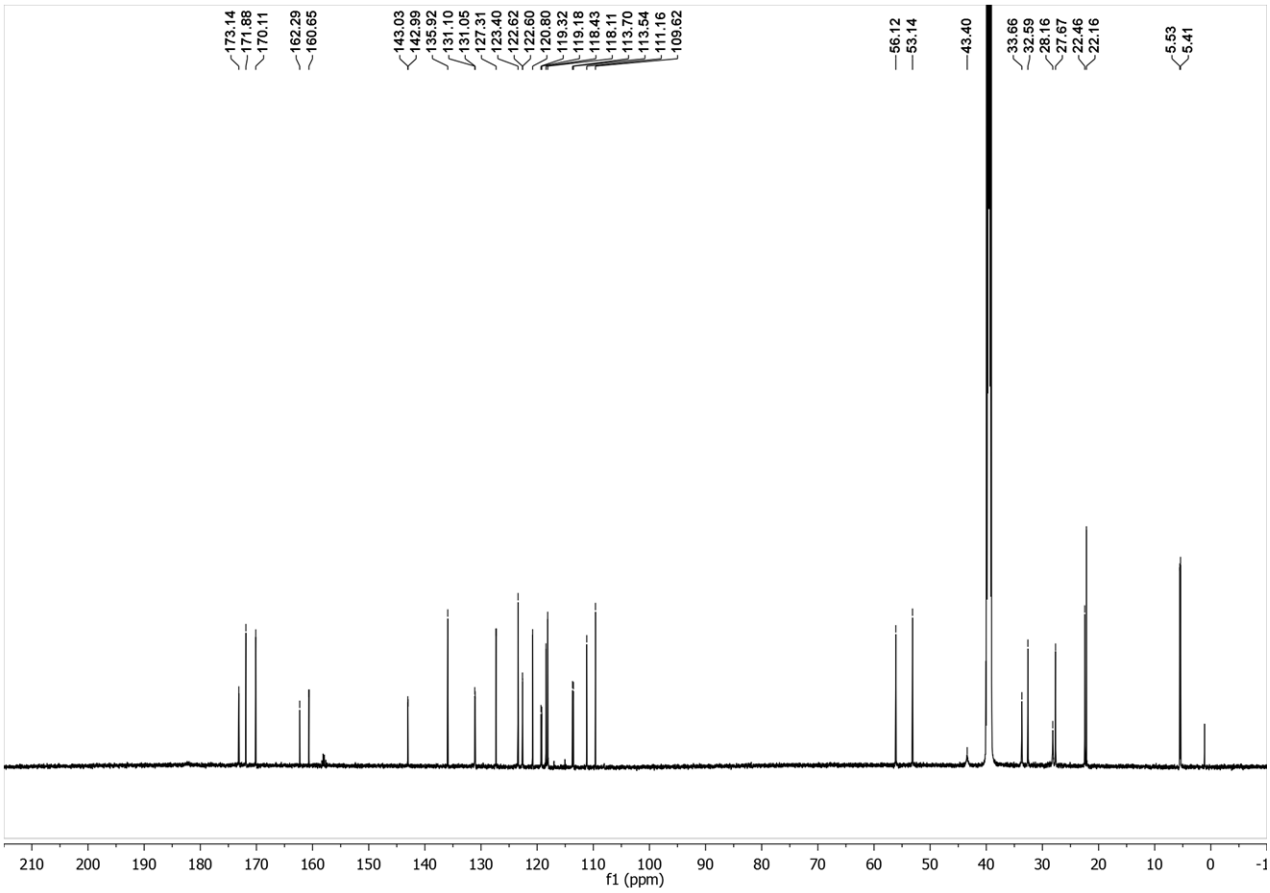
^{1}H and ^{13}C spectra of compound 47.

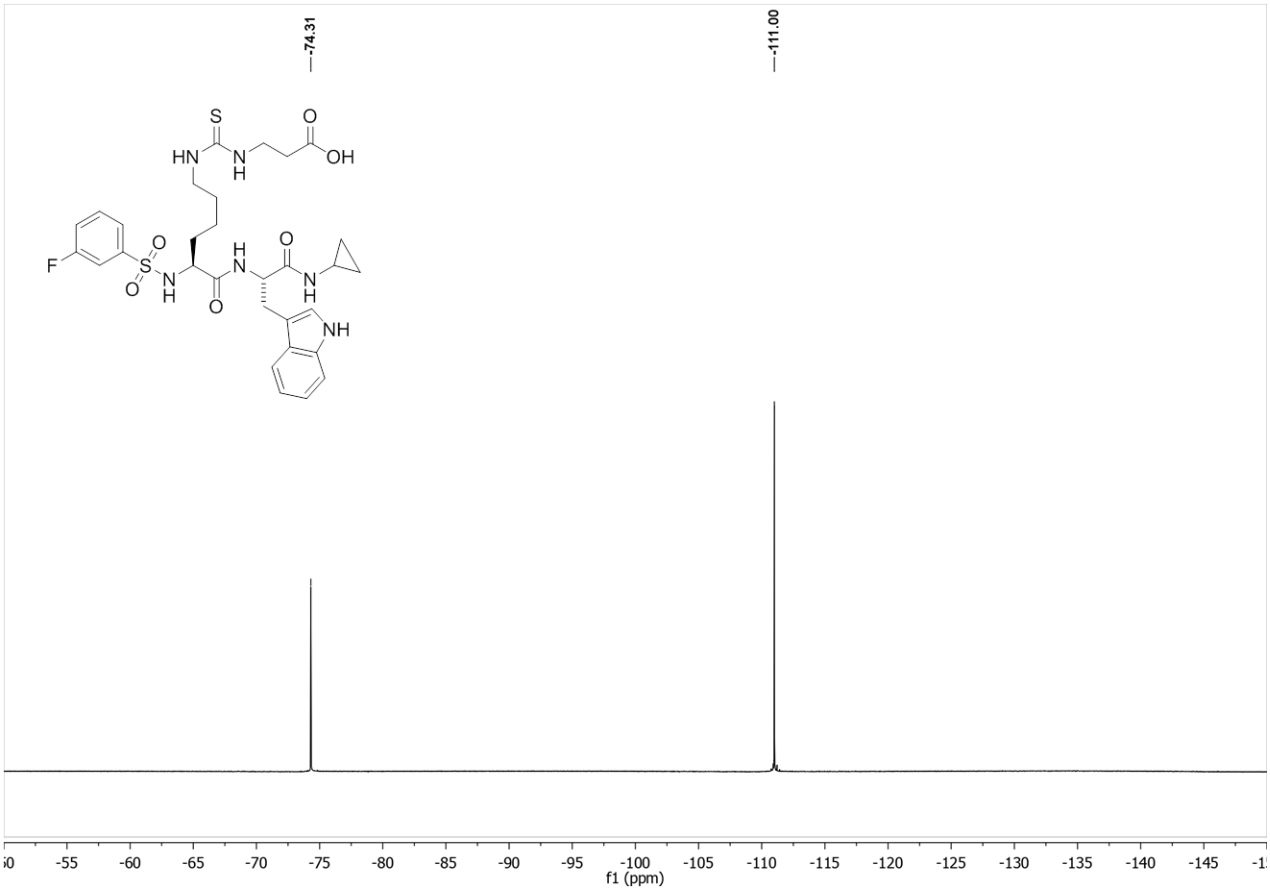




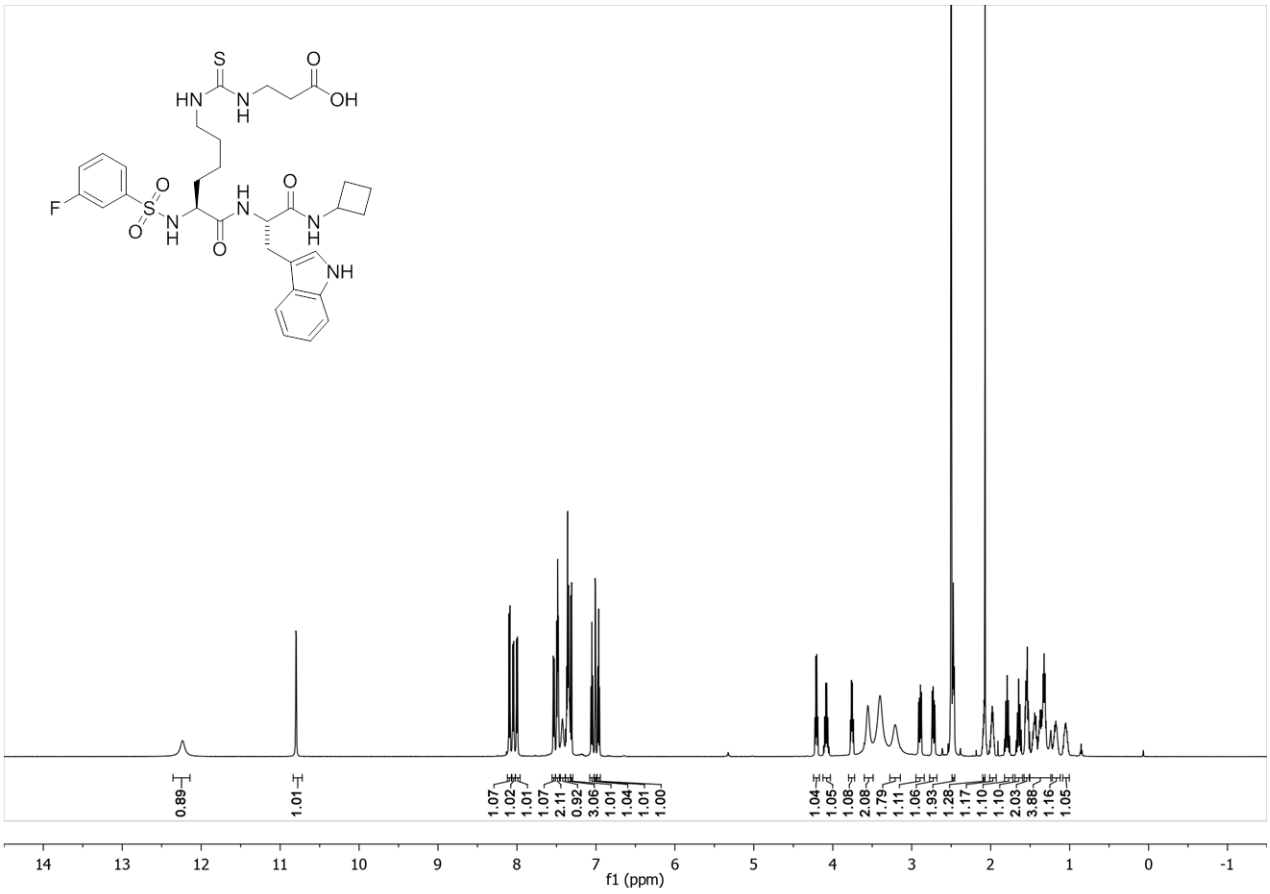
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound 48.

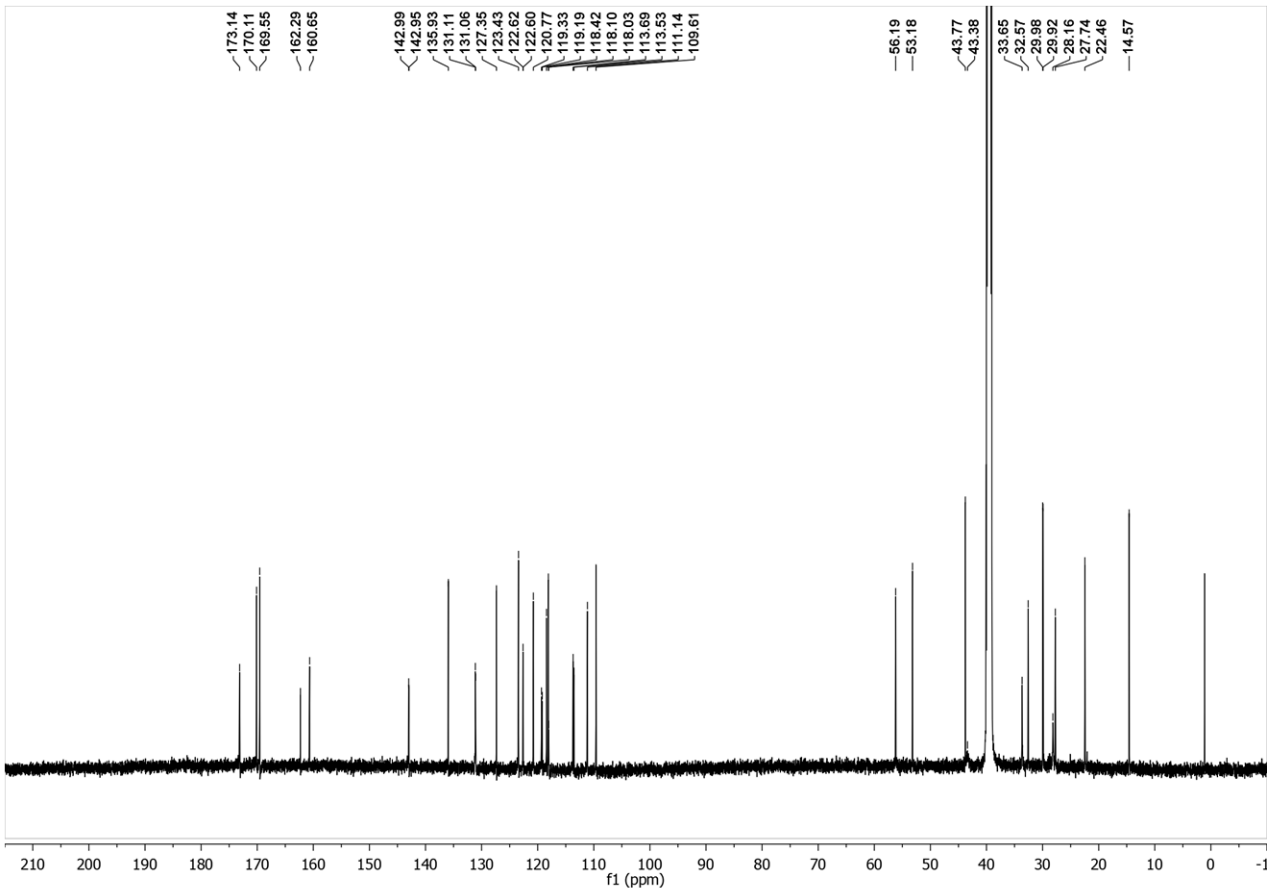


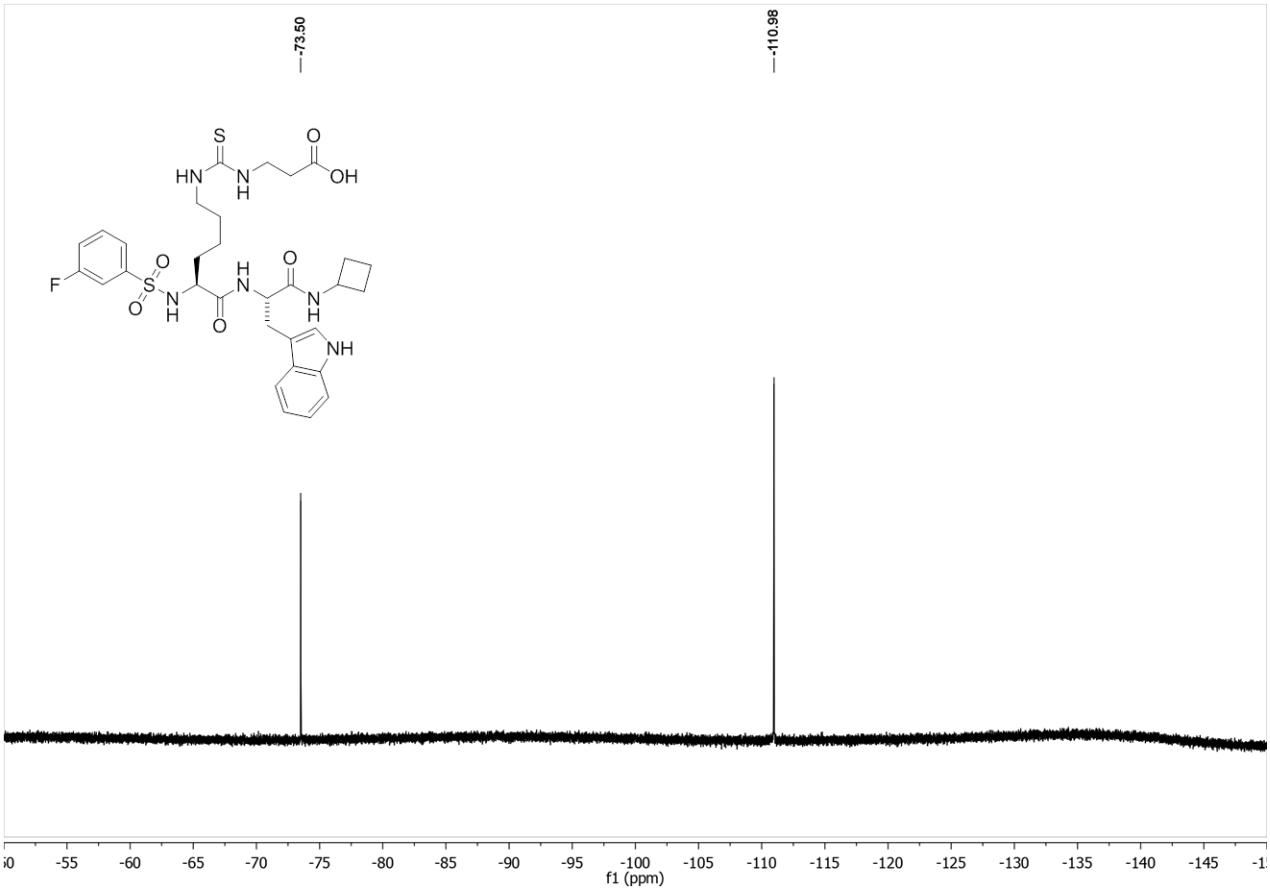



-74.31
-111.00
S
O
HN
N
H
OH
F
O
S
O
N
H
H
N
O
O
N
H
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

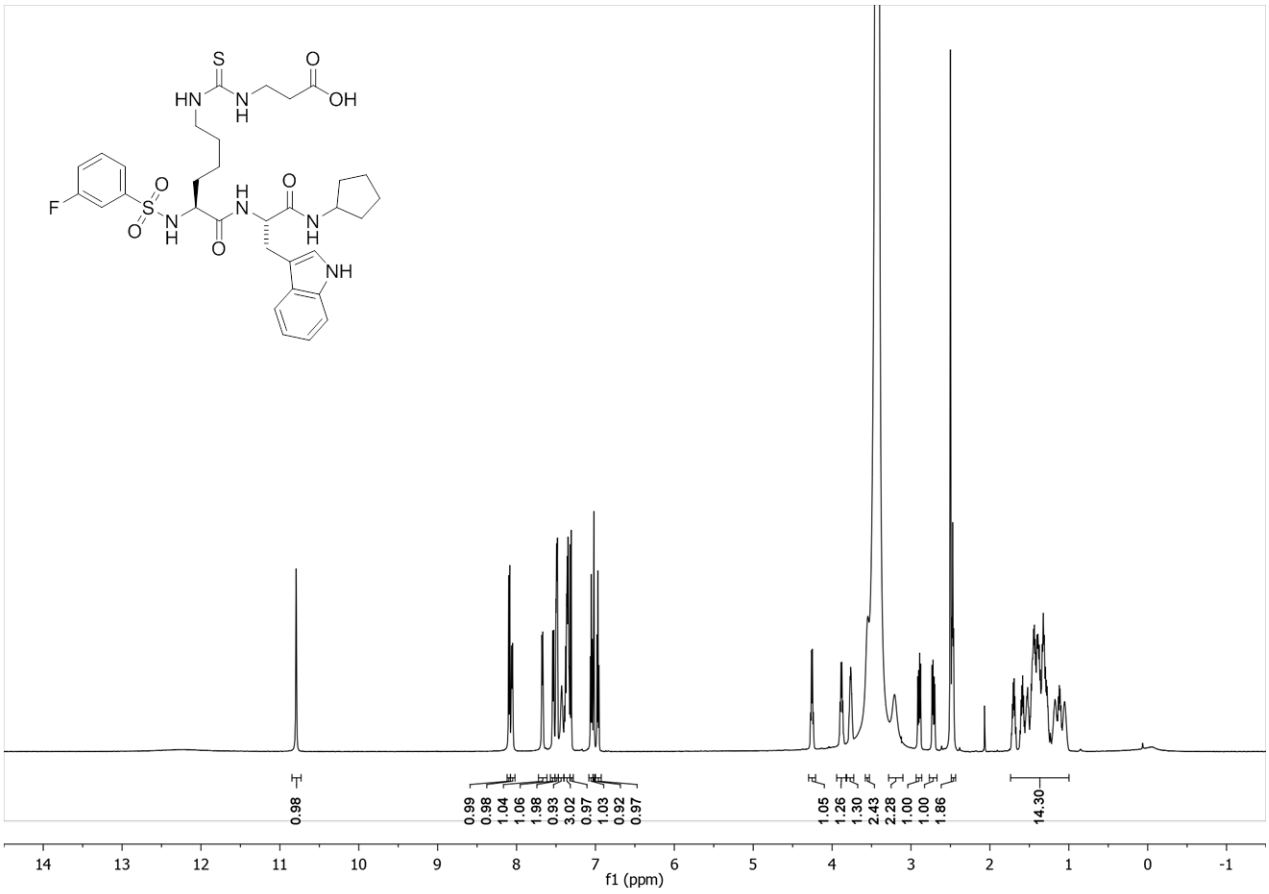
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound 49.

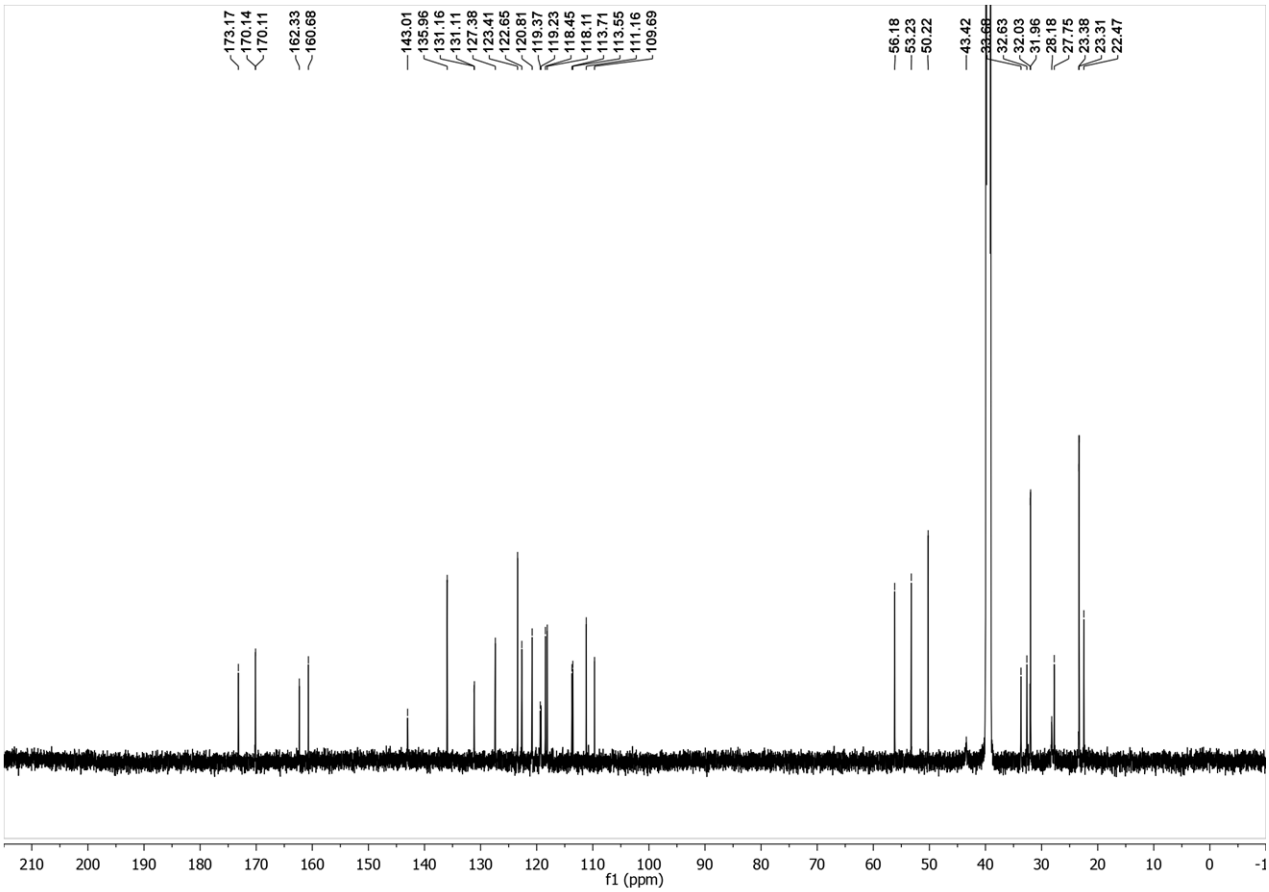


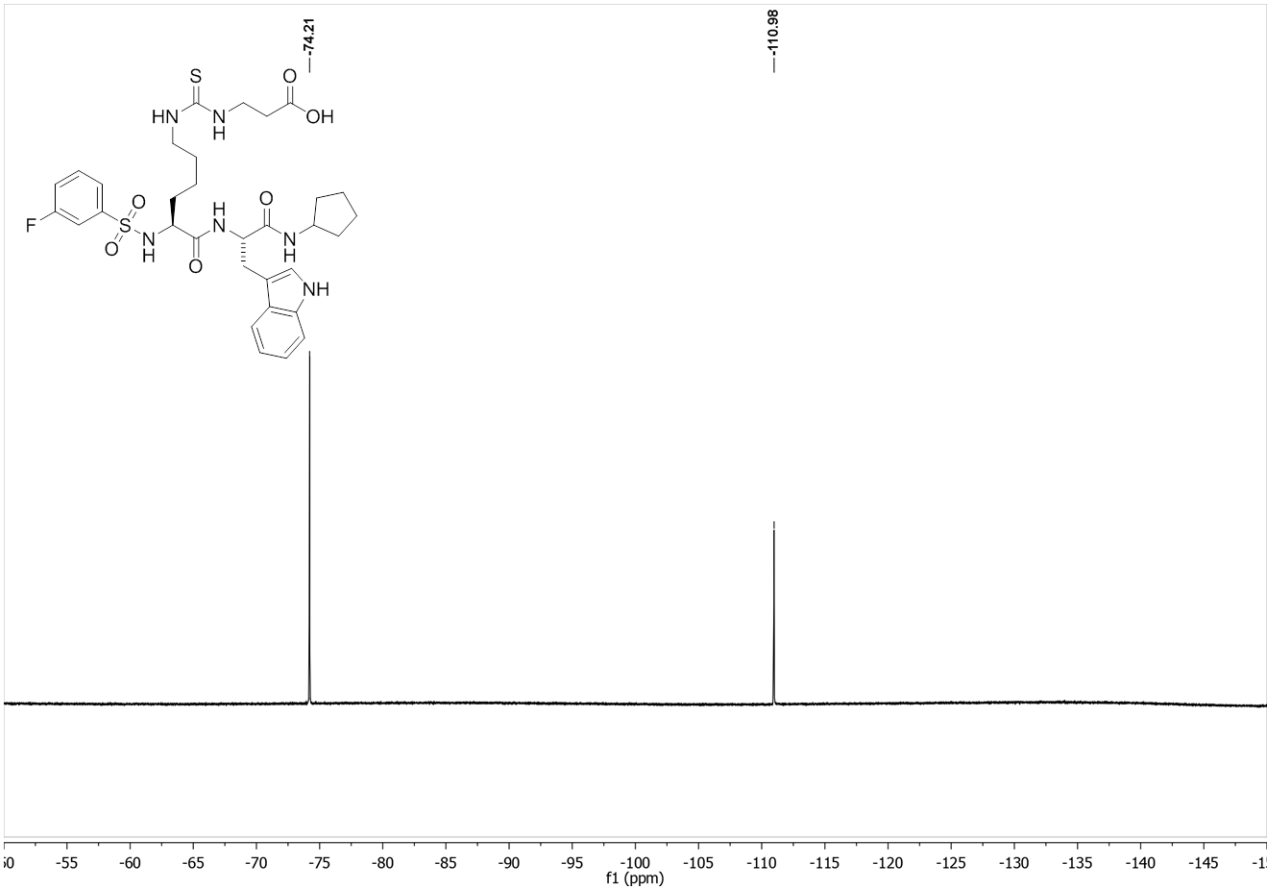



-73.50
-110.98
S
O
HN
N
H
OH
F
O
S
O
N
H
H
N
O
O
N
H
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

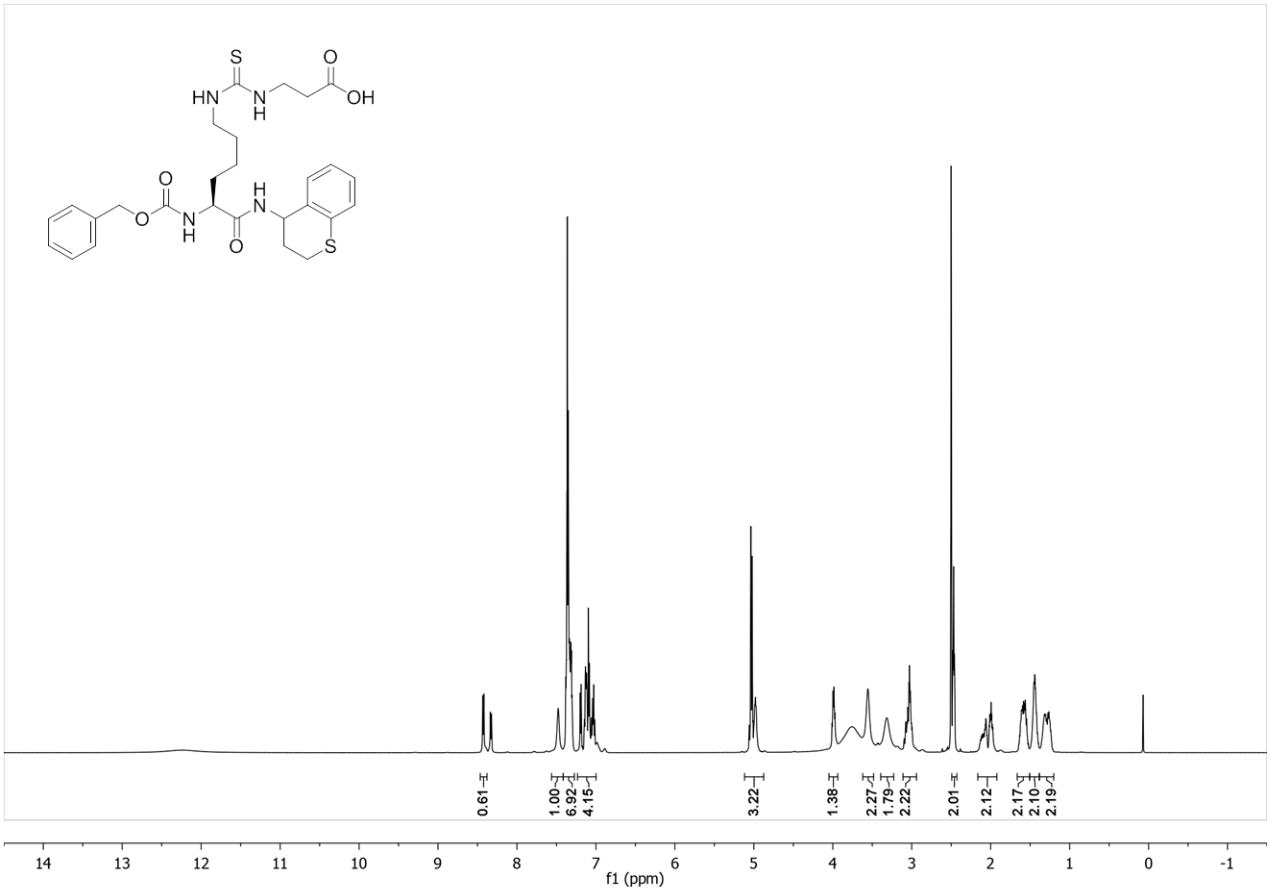
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound 50.

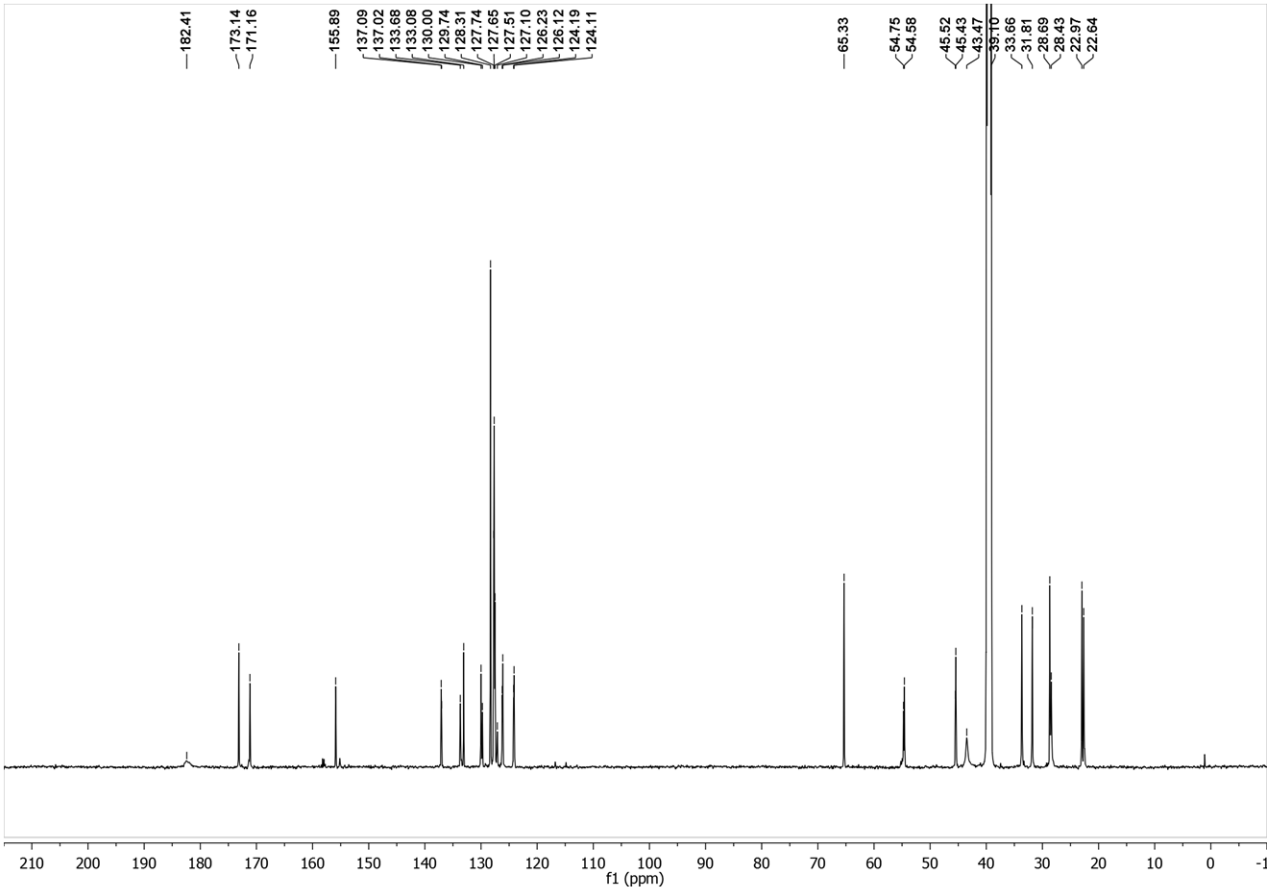




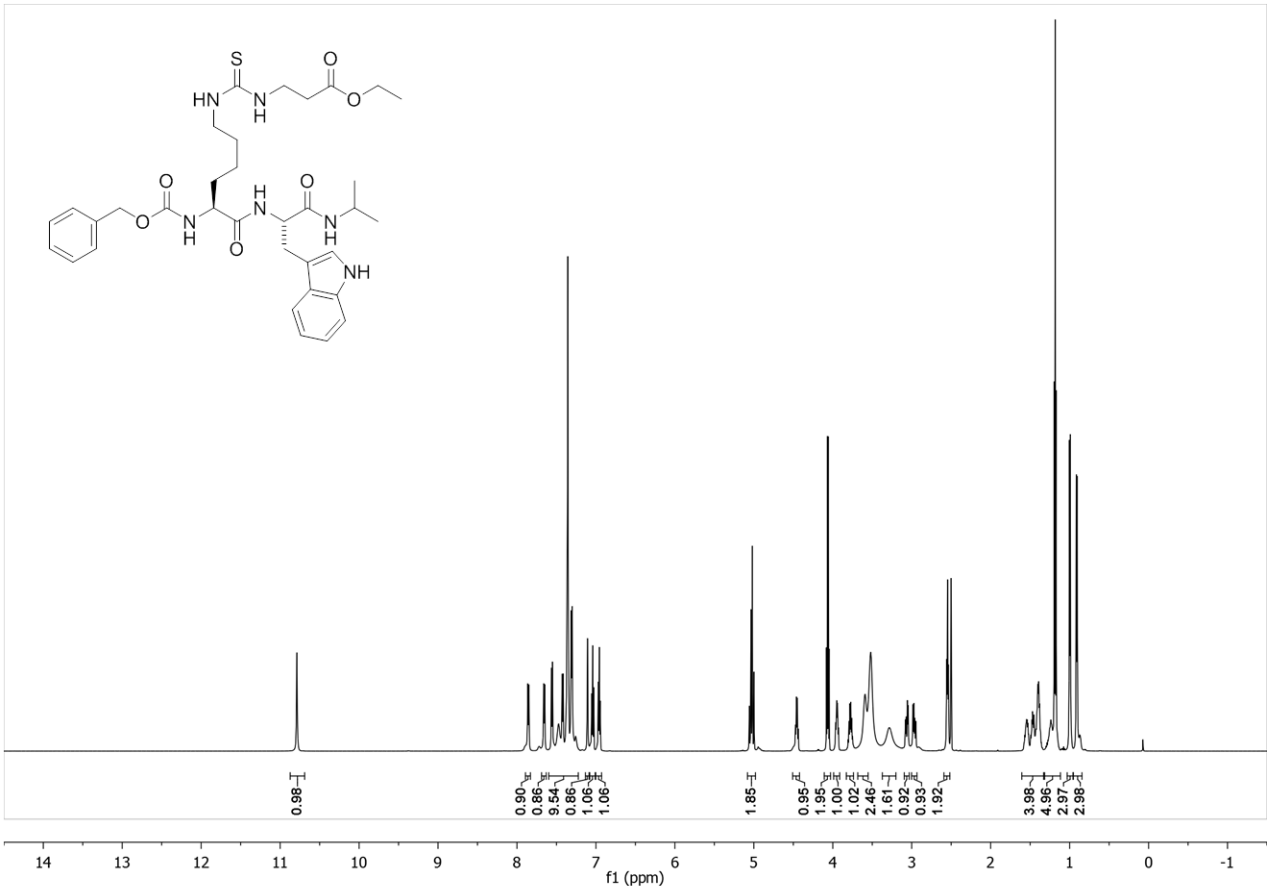

-74.21
-110.98
S
O
HN
N
H
OH
F
O
S
O
N
H
H
N
O
O
N
H
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

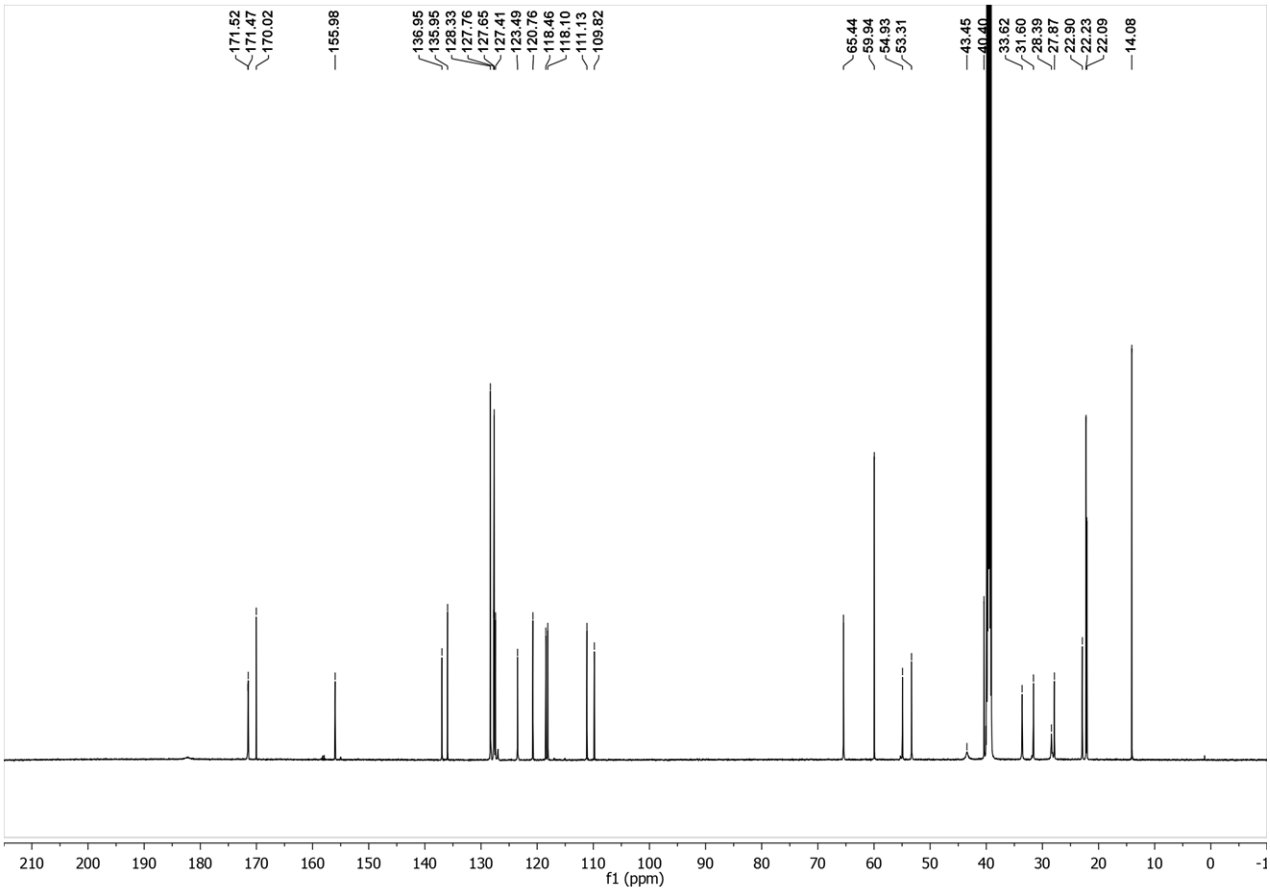
^{1}H and ^{13}C spectra of compound V.



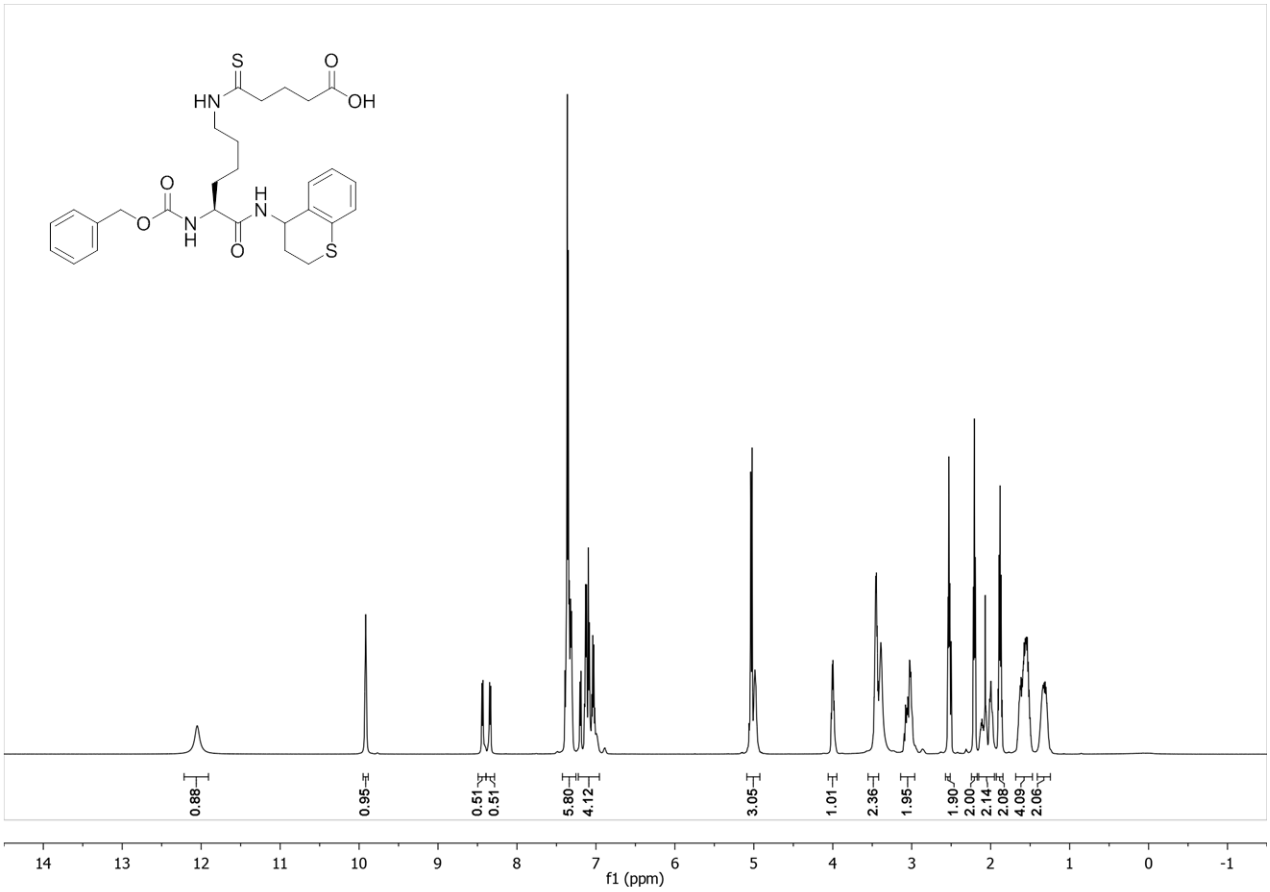


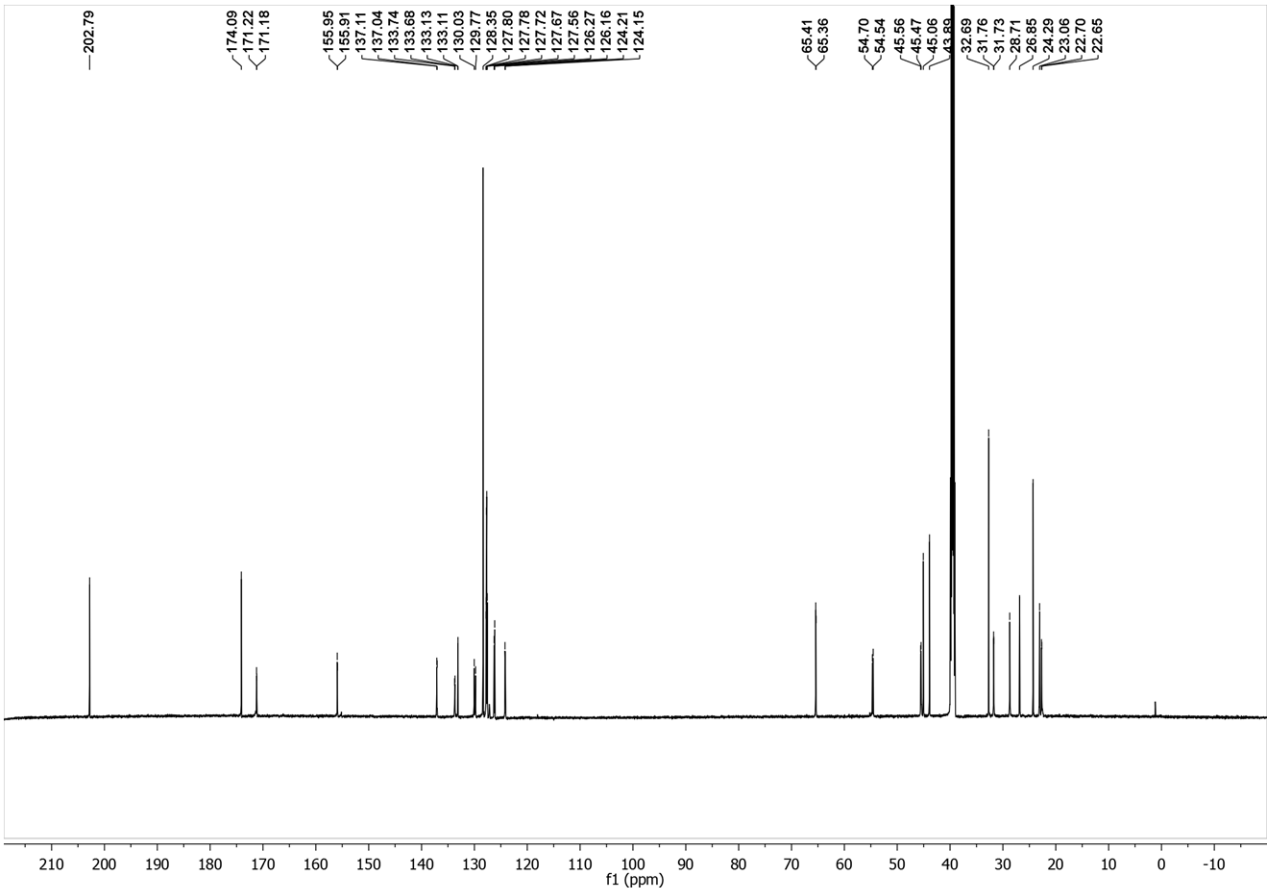
^{1}H and ^{13}C spectra of compound Et-29.



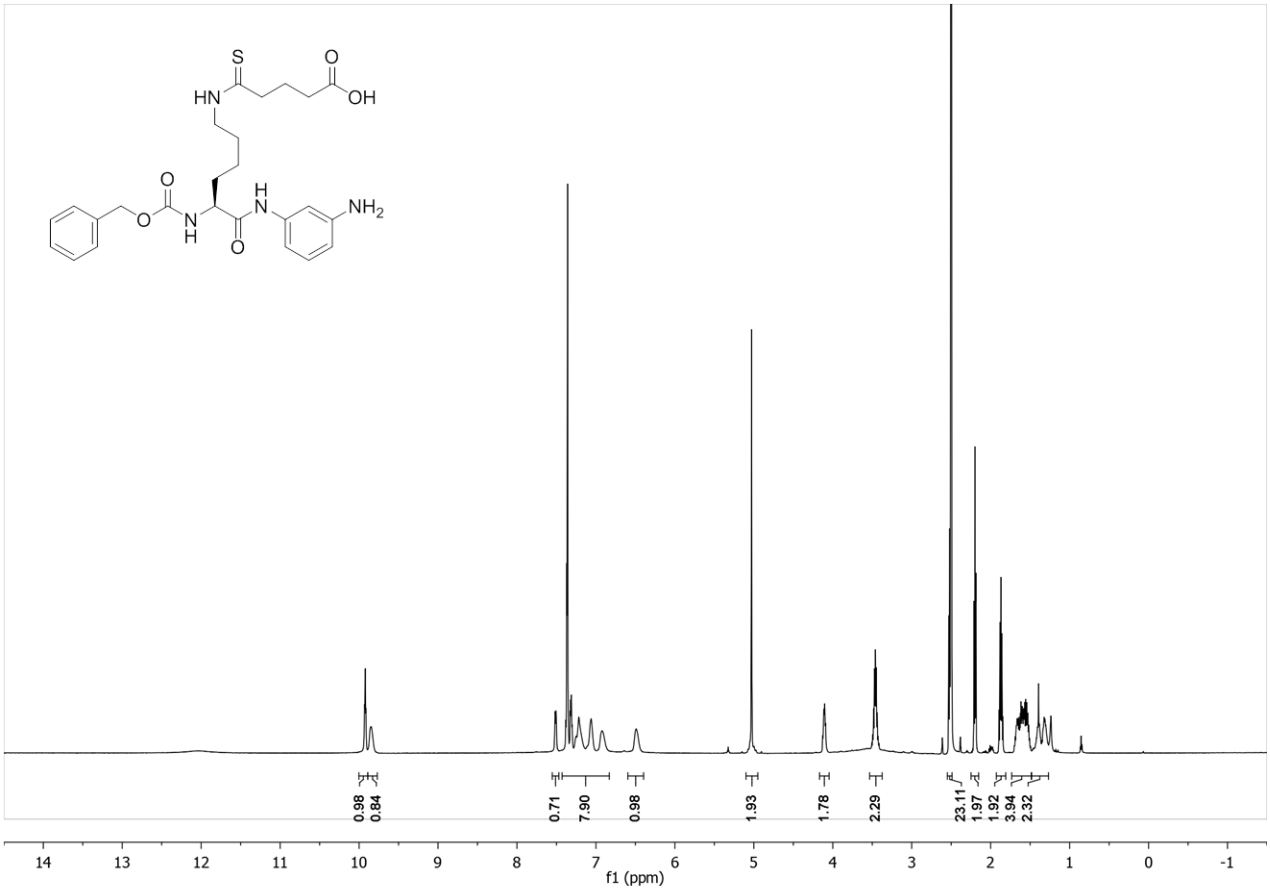


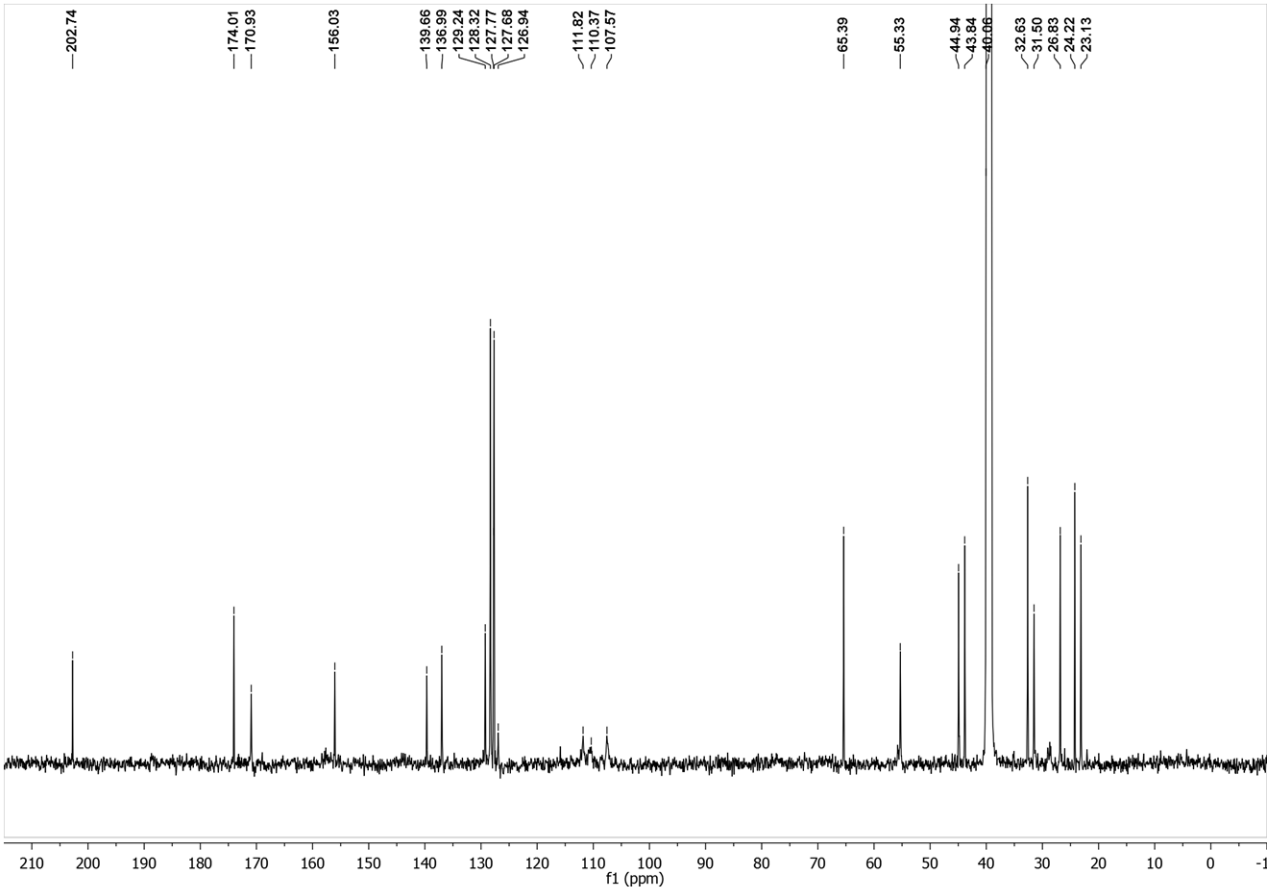
^{1}H and ^{13}C spectra of compound S1.



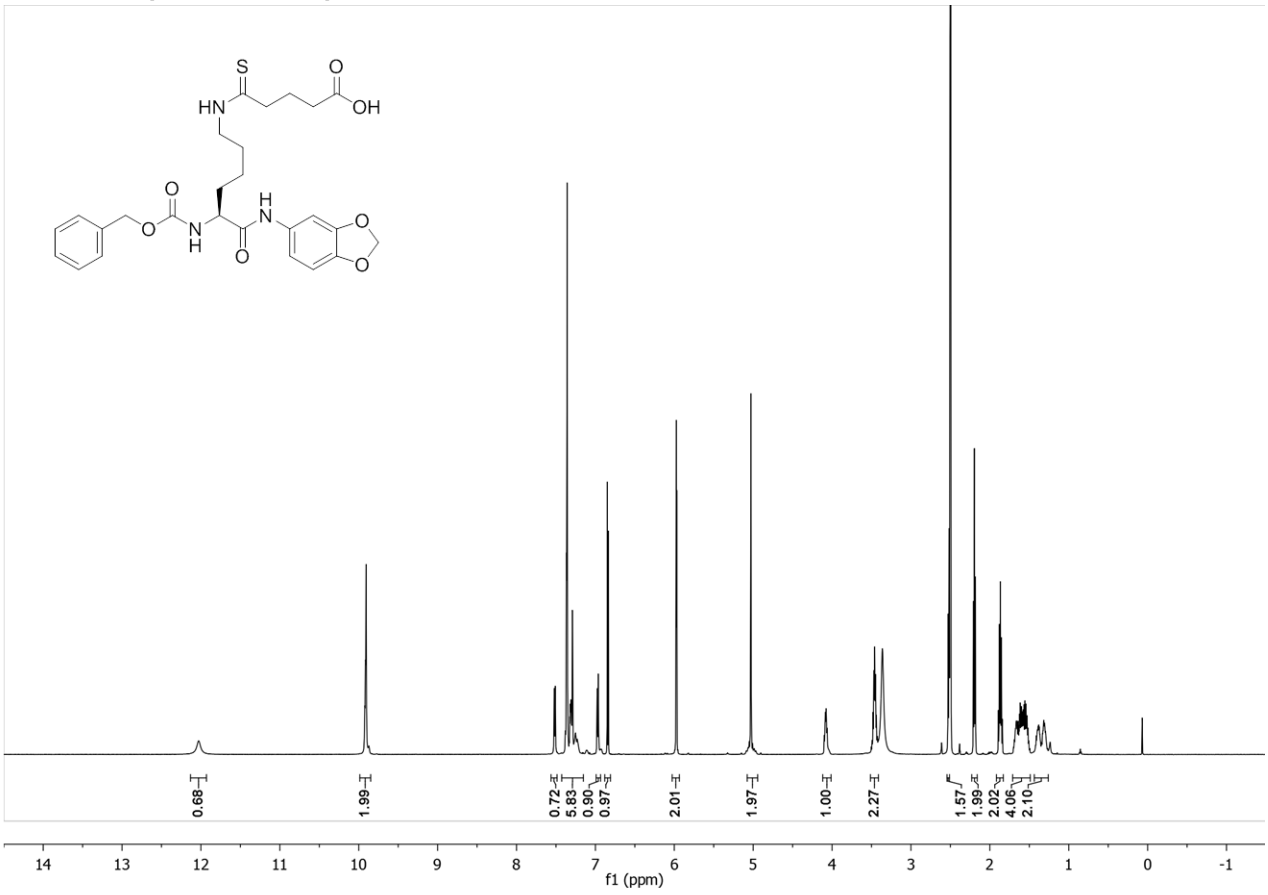


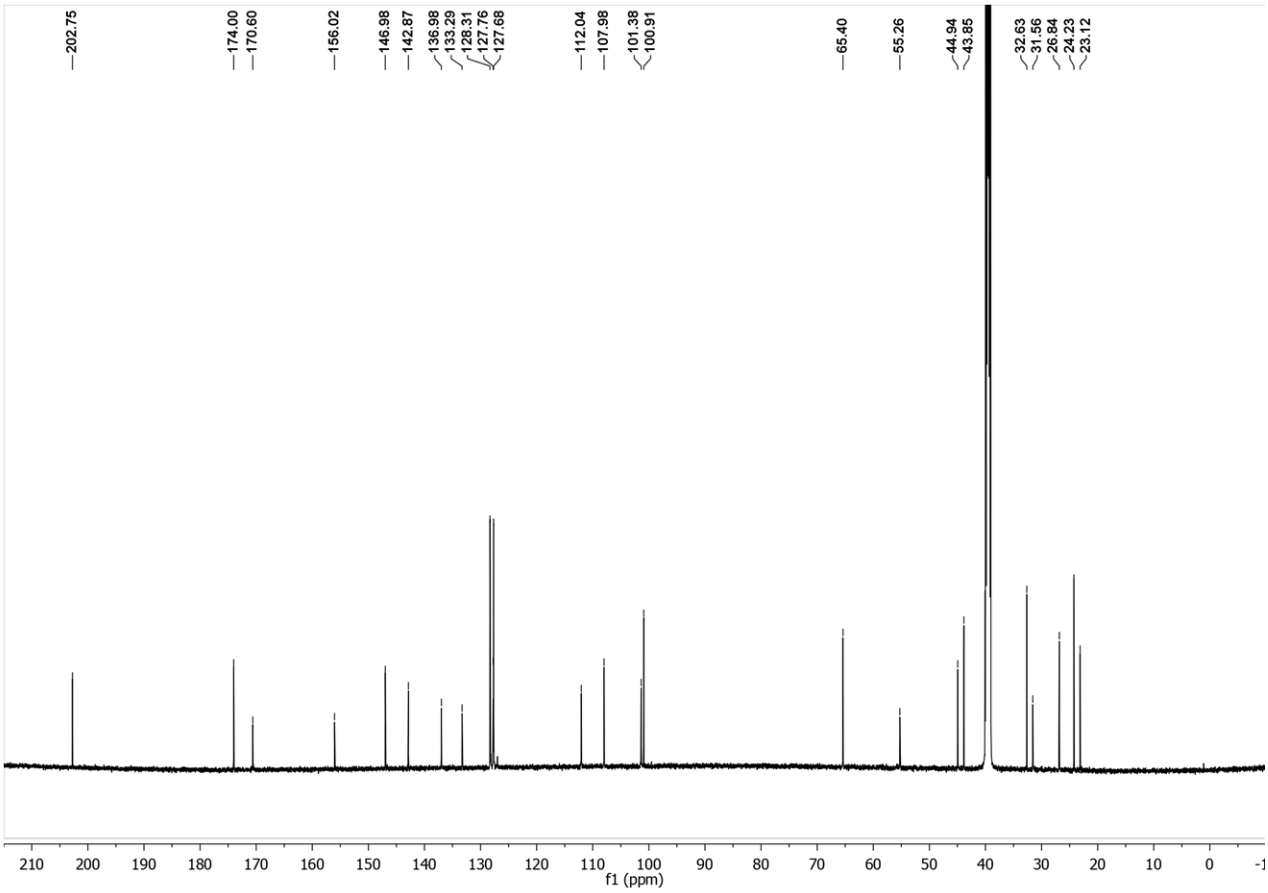
^{1}H and ^{13}C spectra of compound S2.



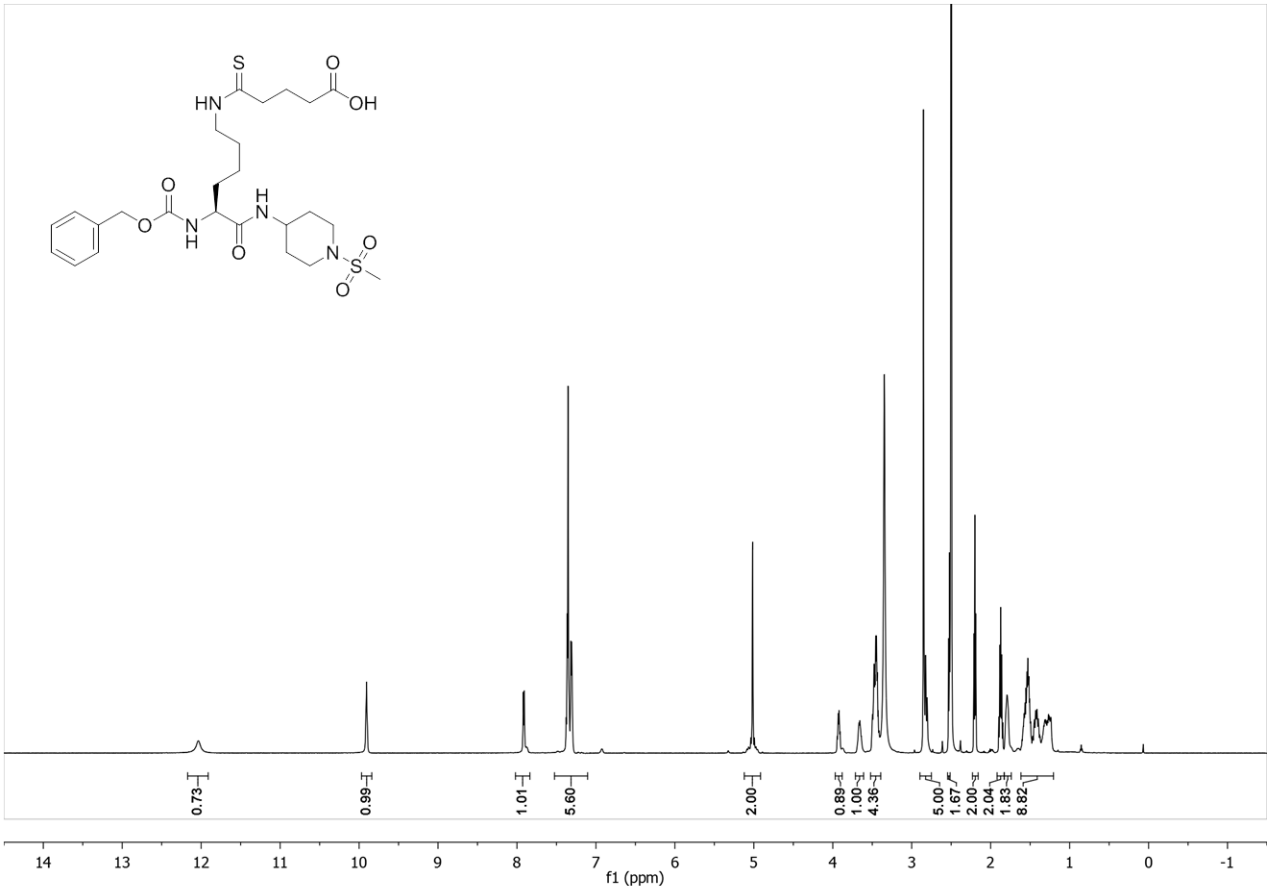


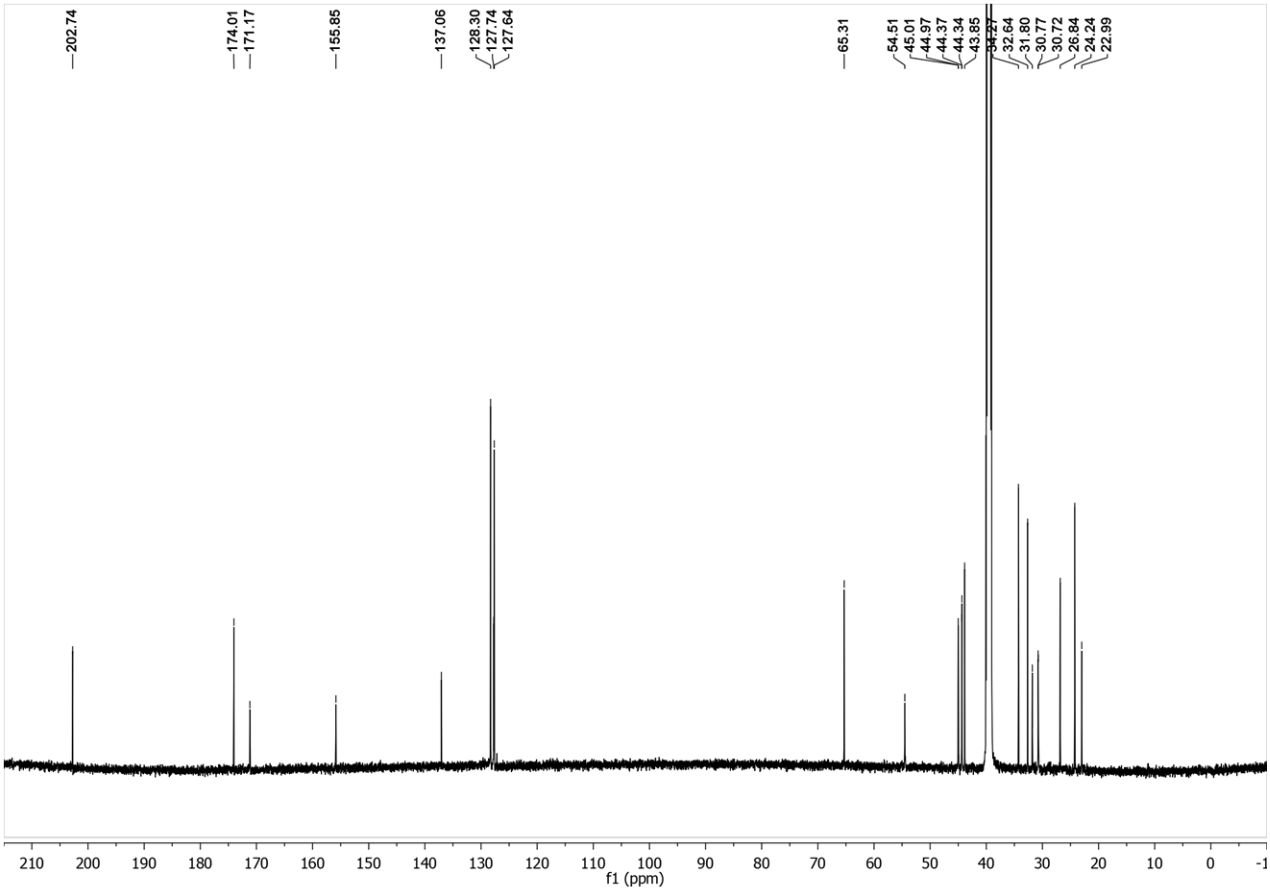
^{1}H and ^{13}C spectra of compound S3.



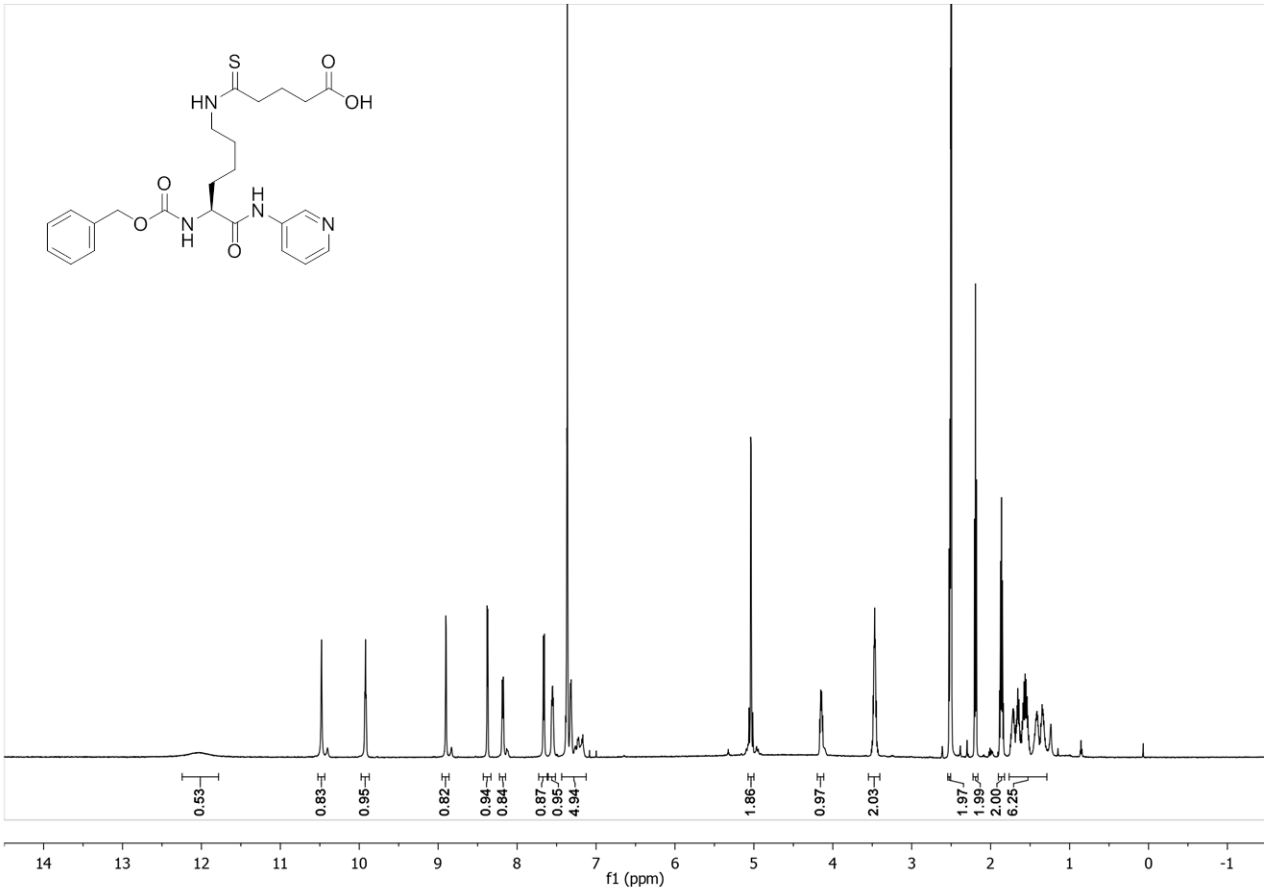


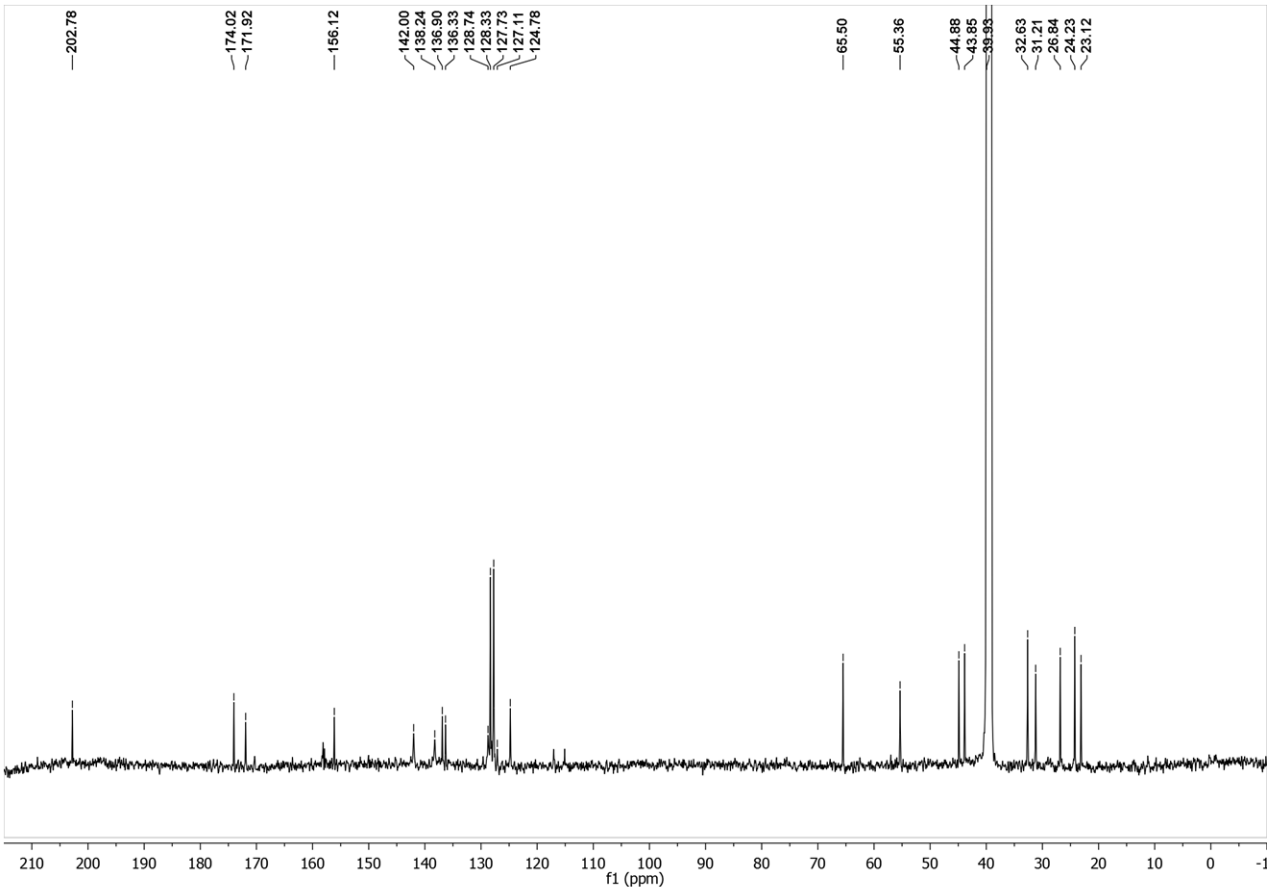
^{1}H and ^{13}C spectra of compound S4.



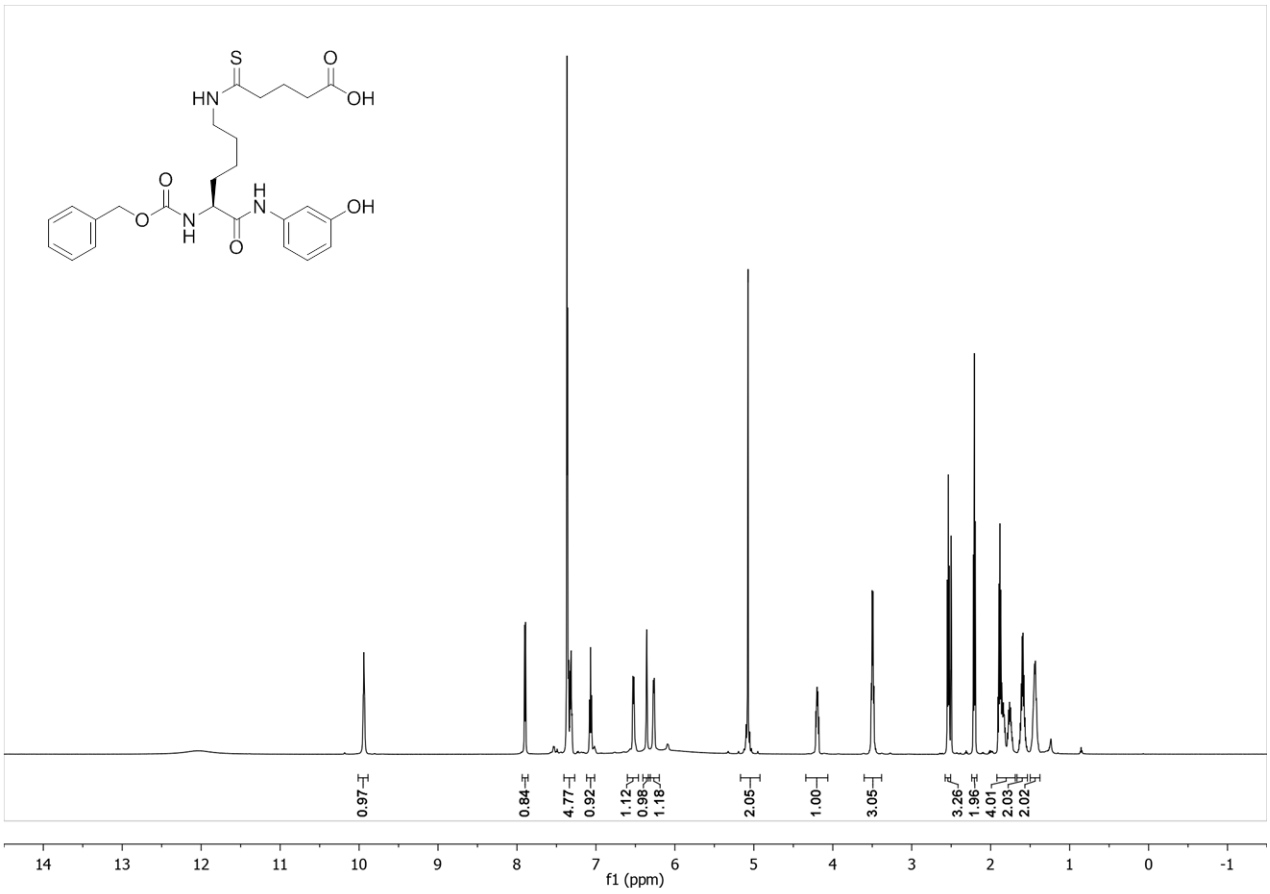


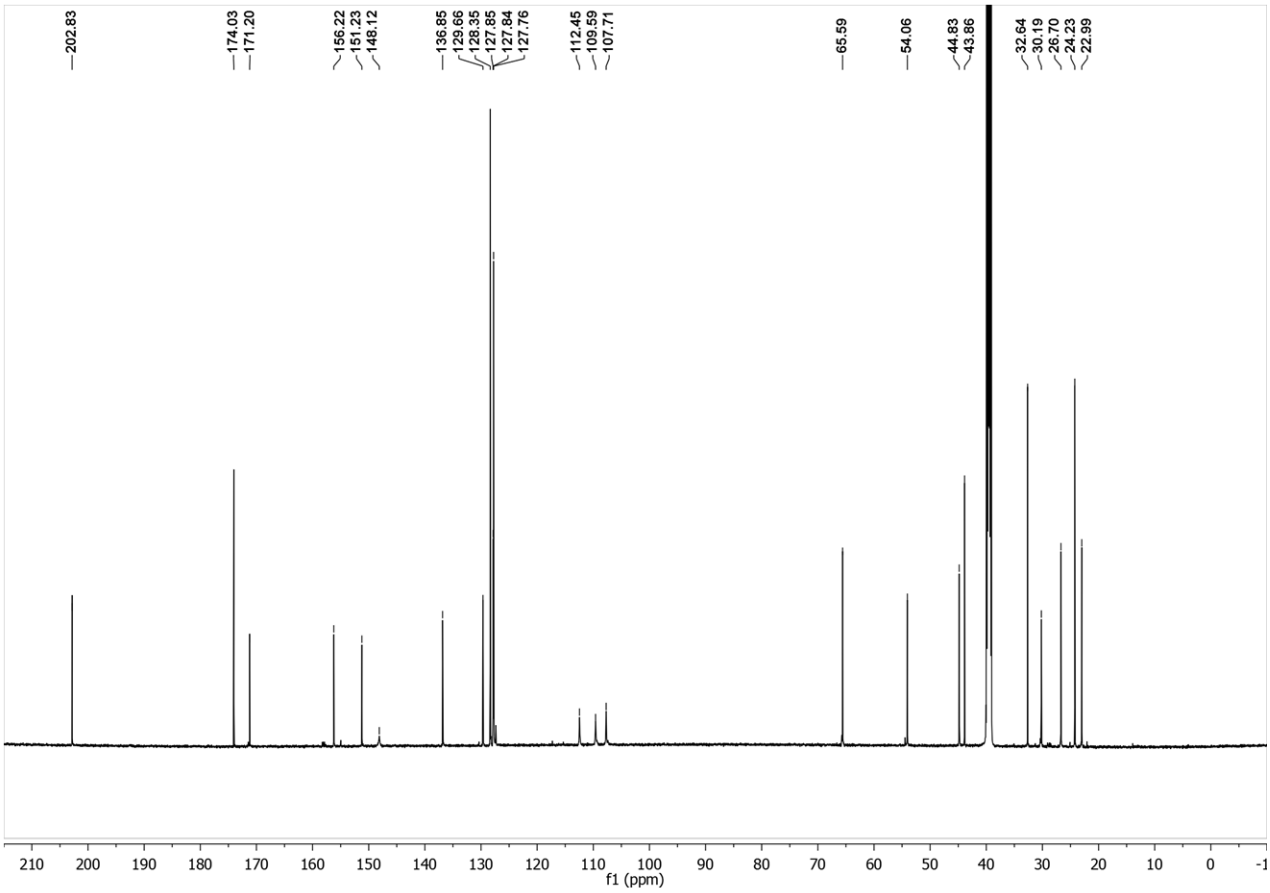
^{1}H and ^{13}C spectra of compound S5.



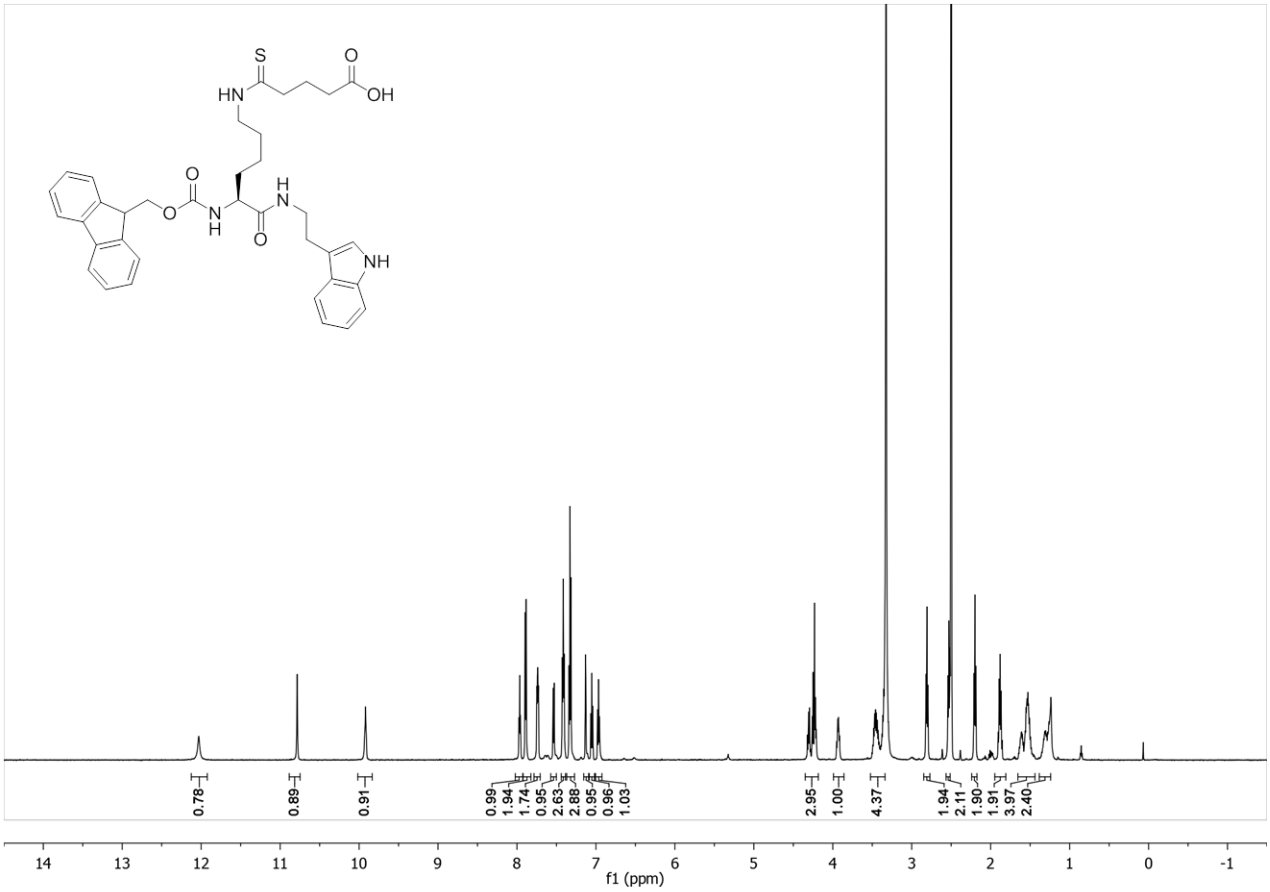


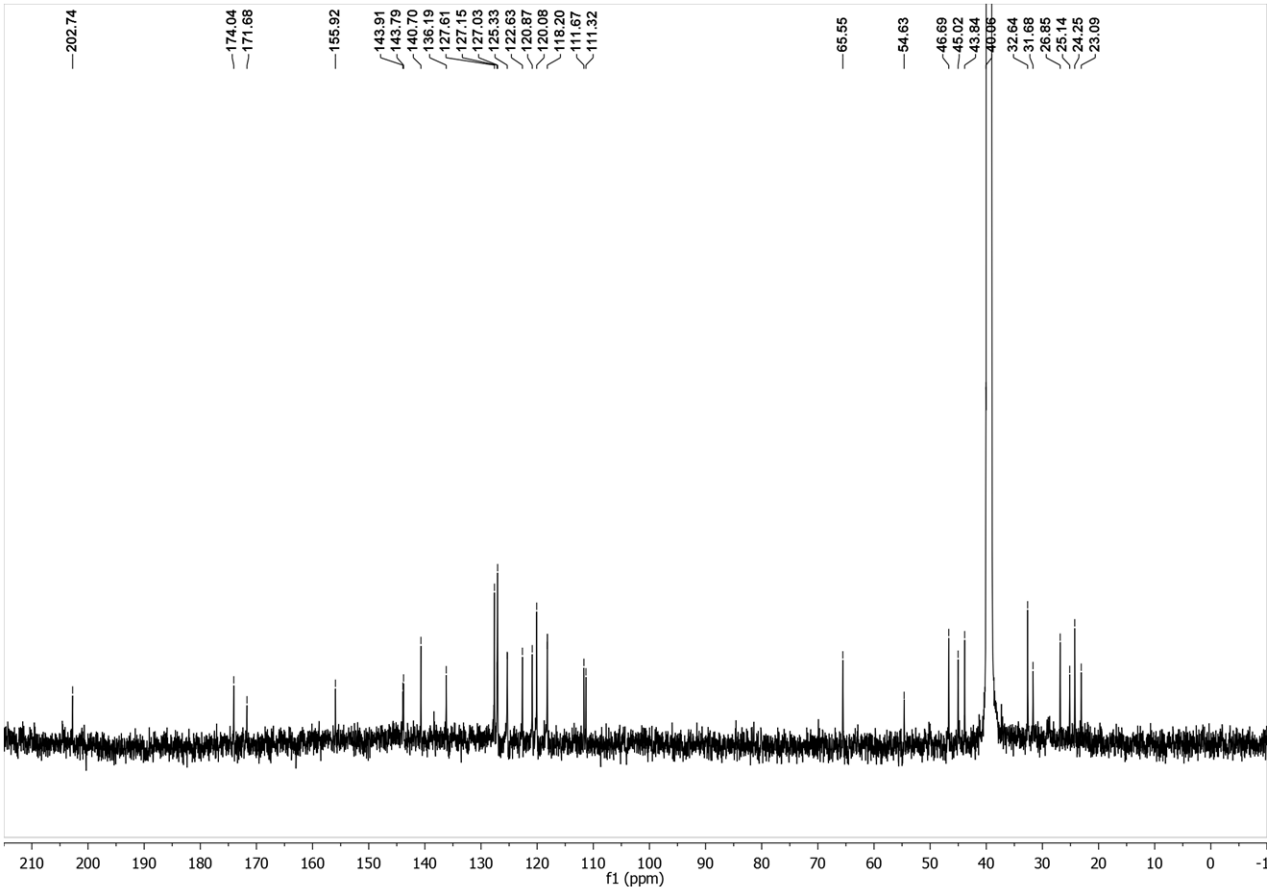
^{1}H and ^{13}C spectra of compound S6.



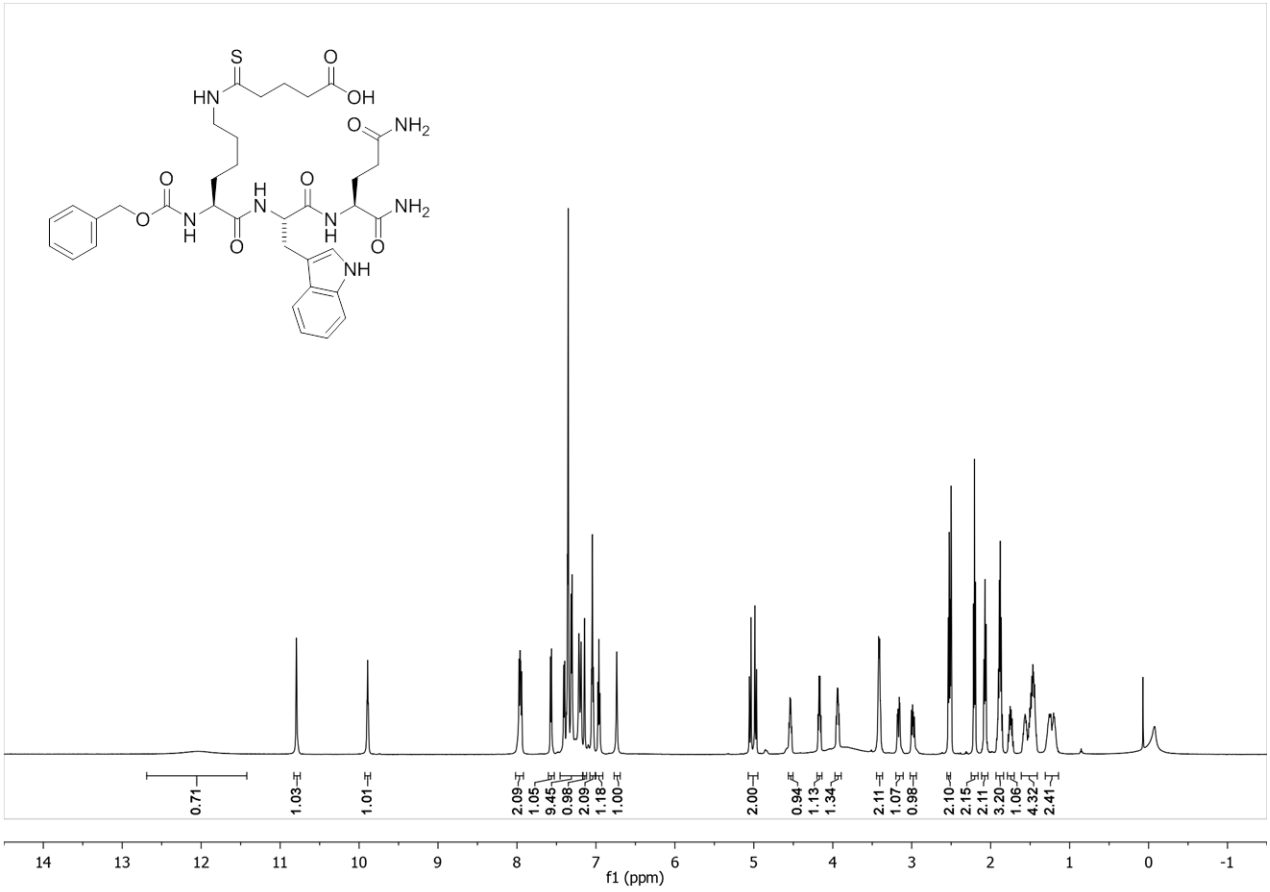


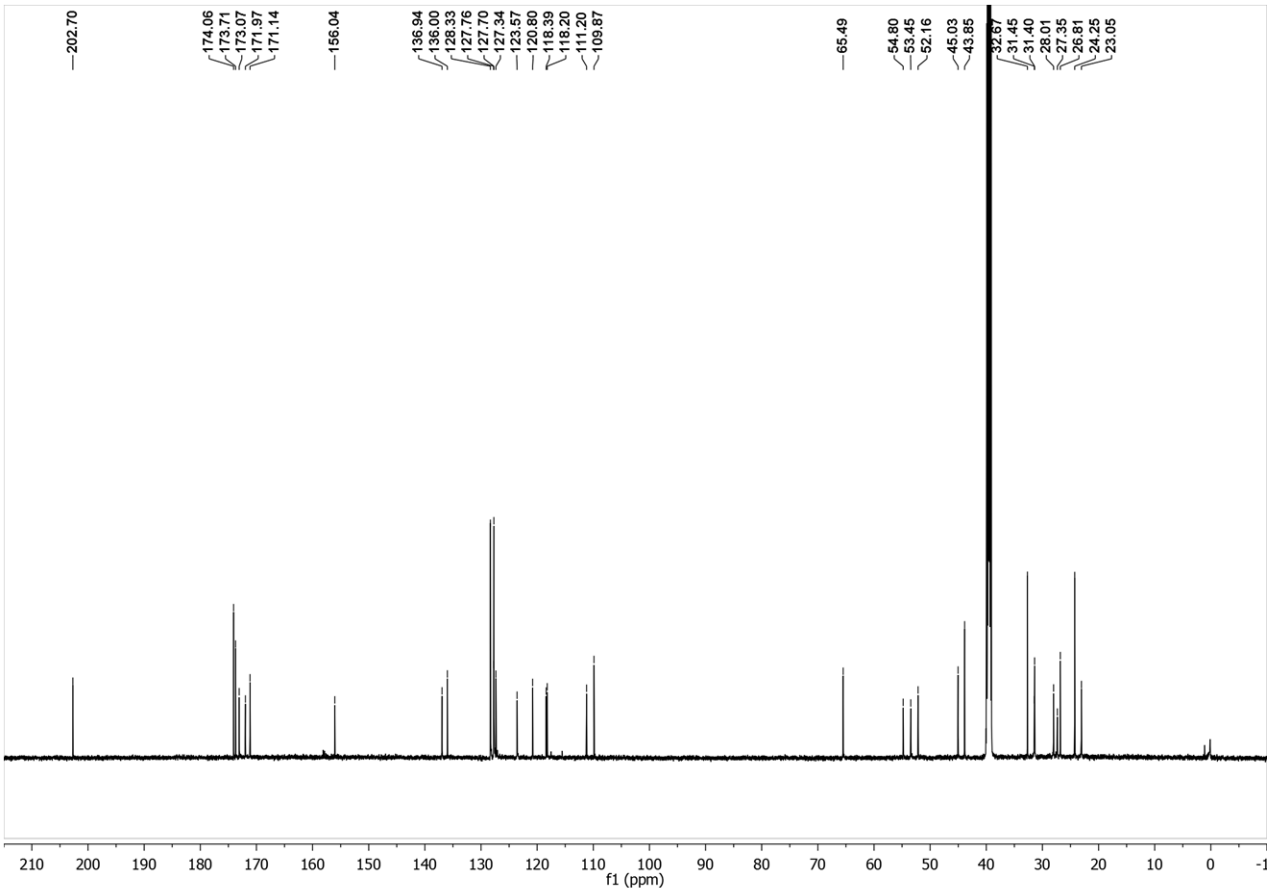
^{1}H and ^{13}C spectra of compound S7.



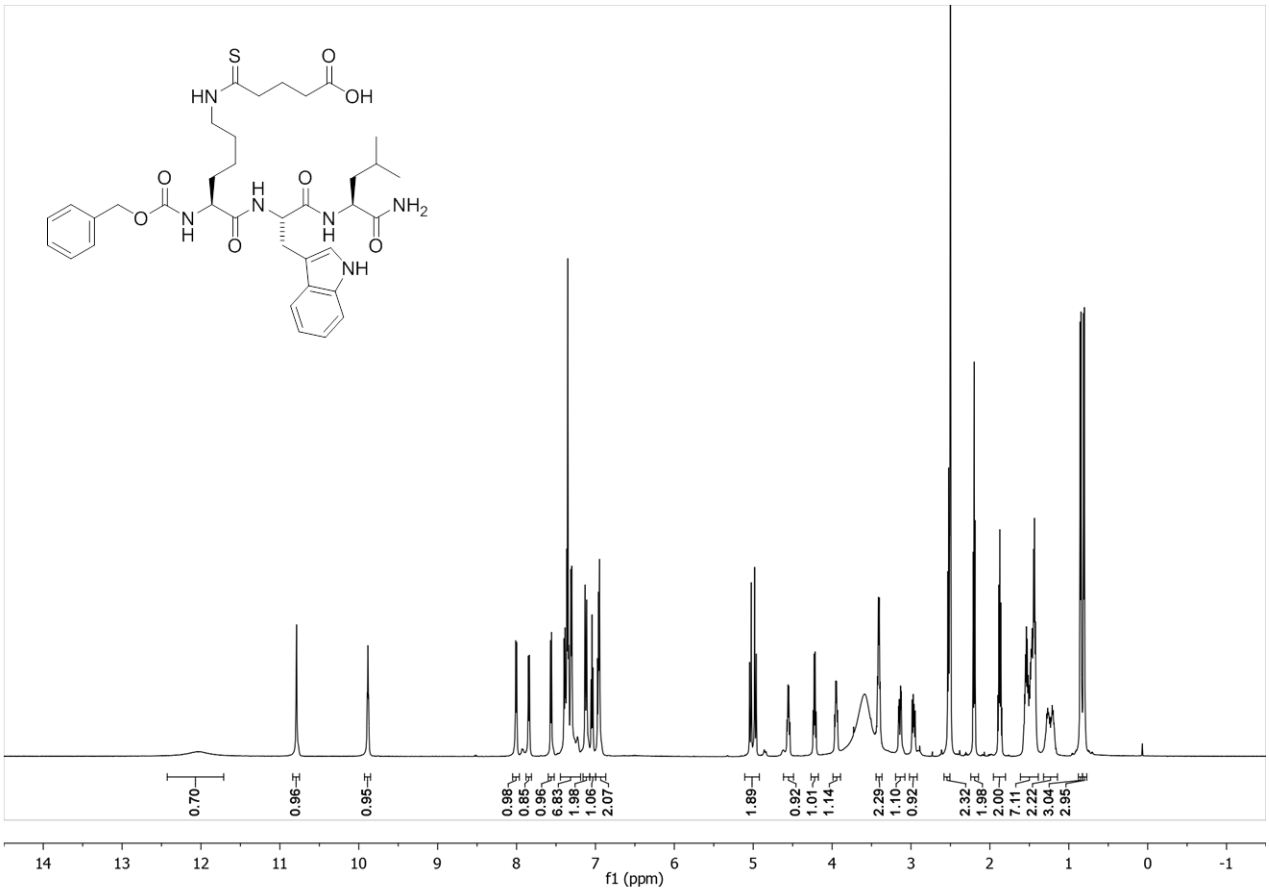


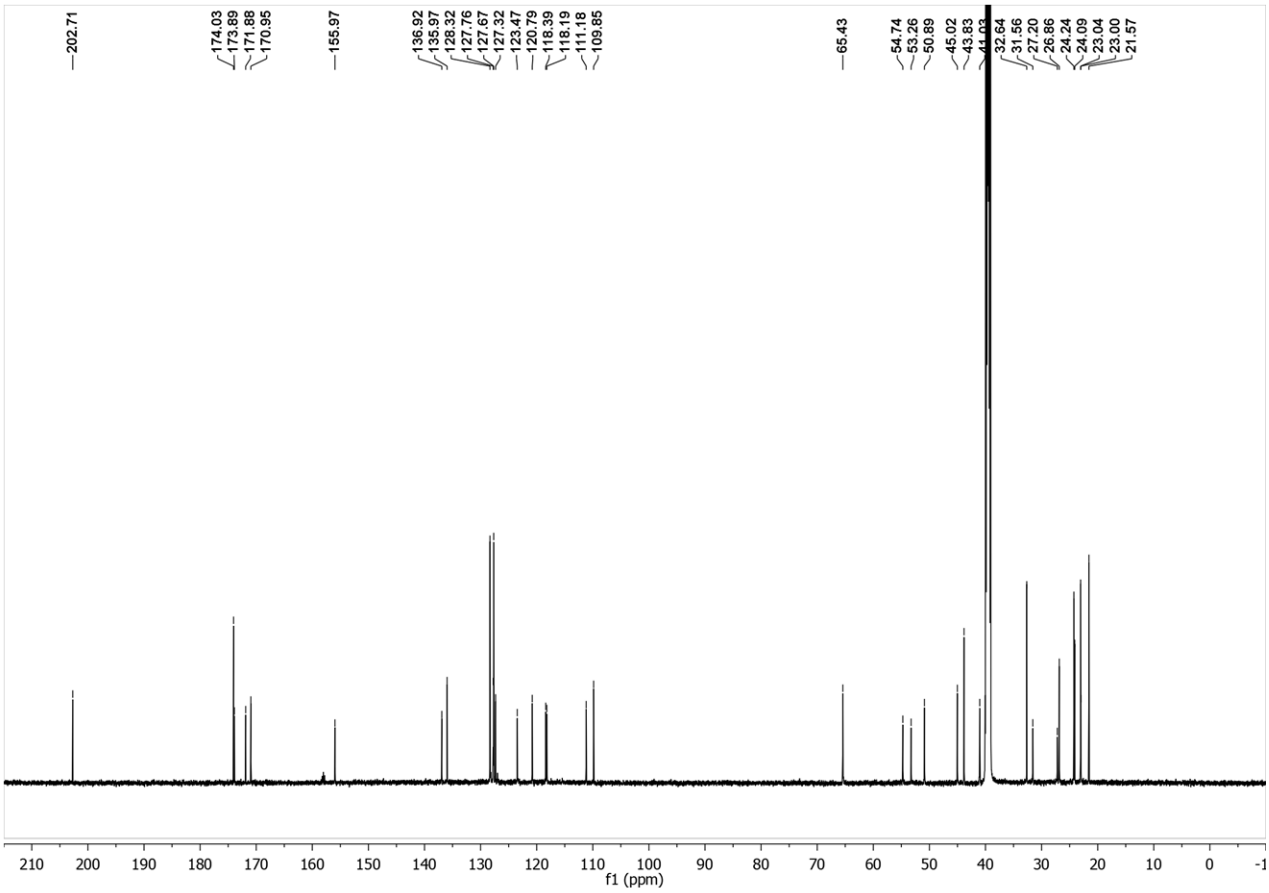
^{1}H and ^{13}C spectra of compound S8.



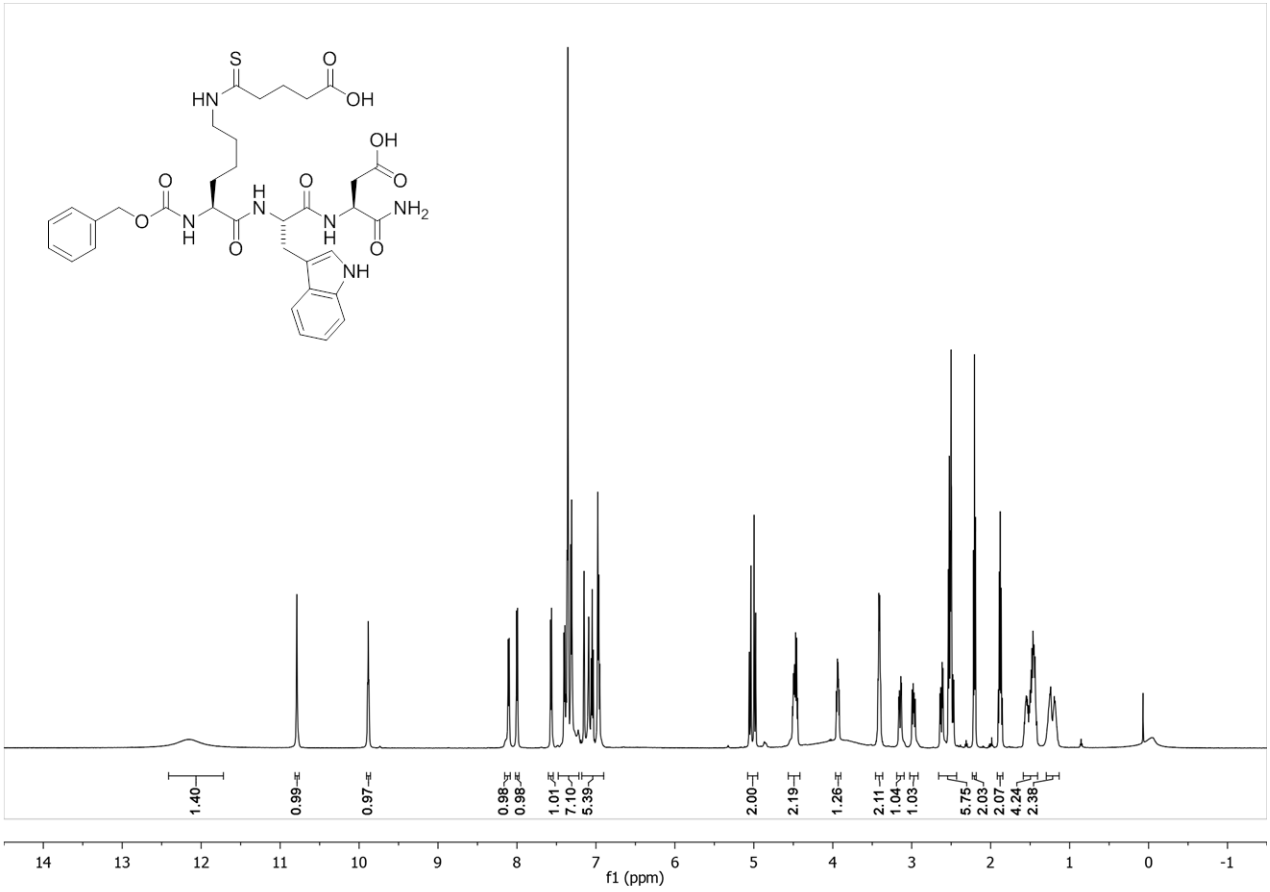


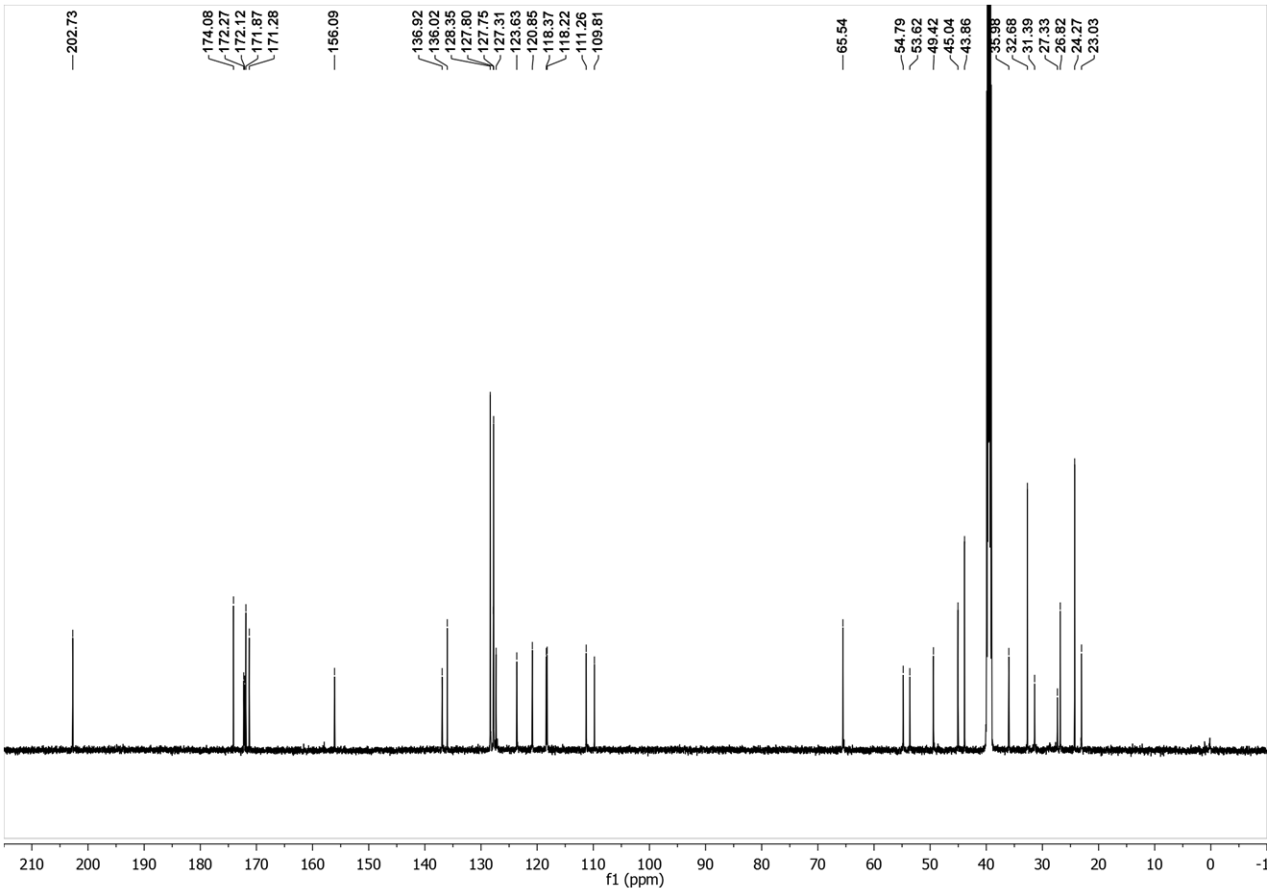
^{1}H and ^{13}C spectra of compound S9.



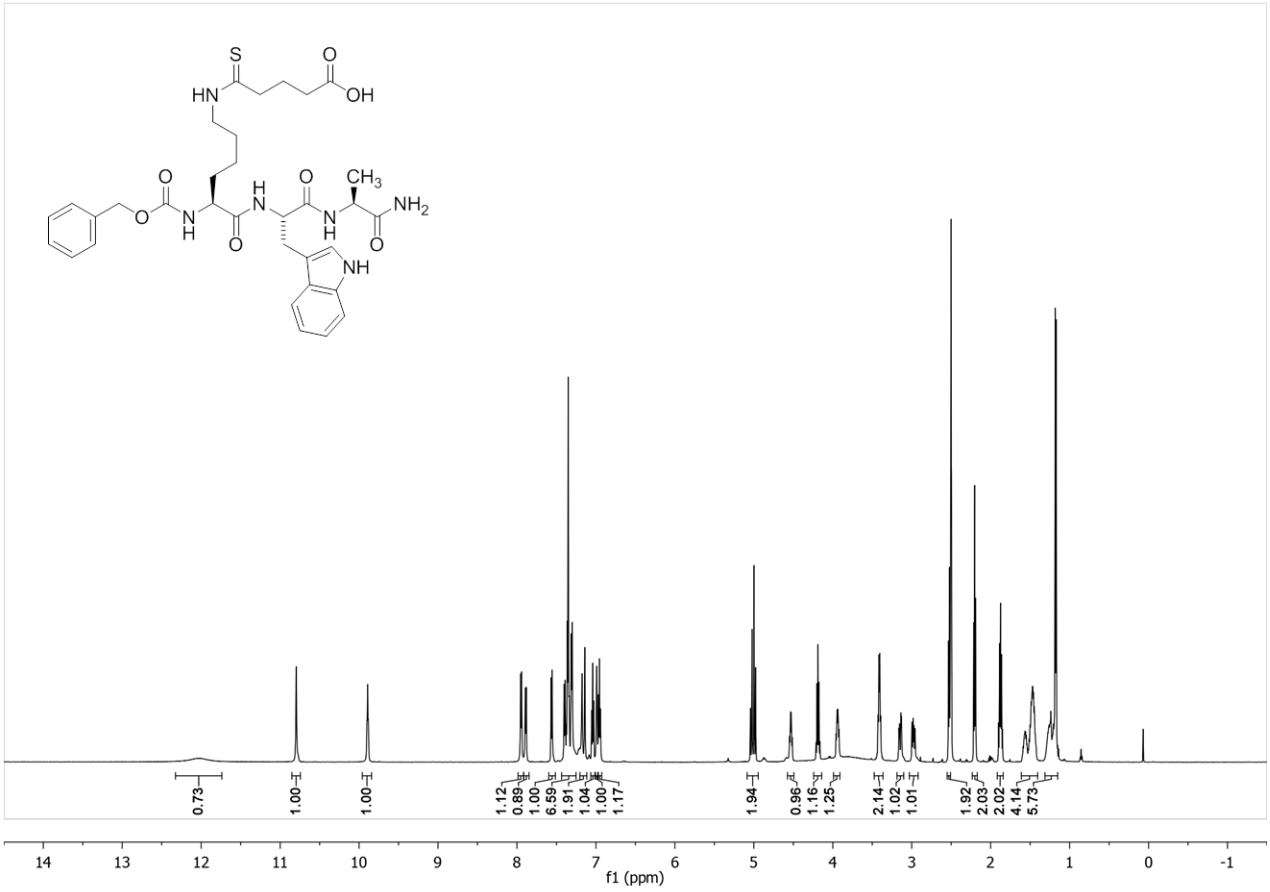


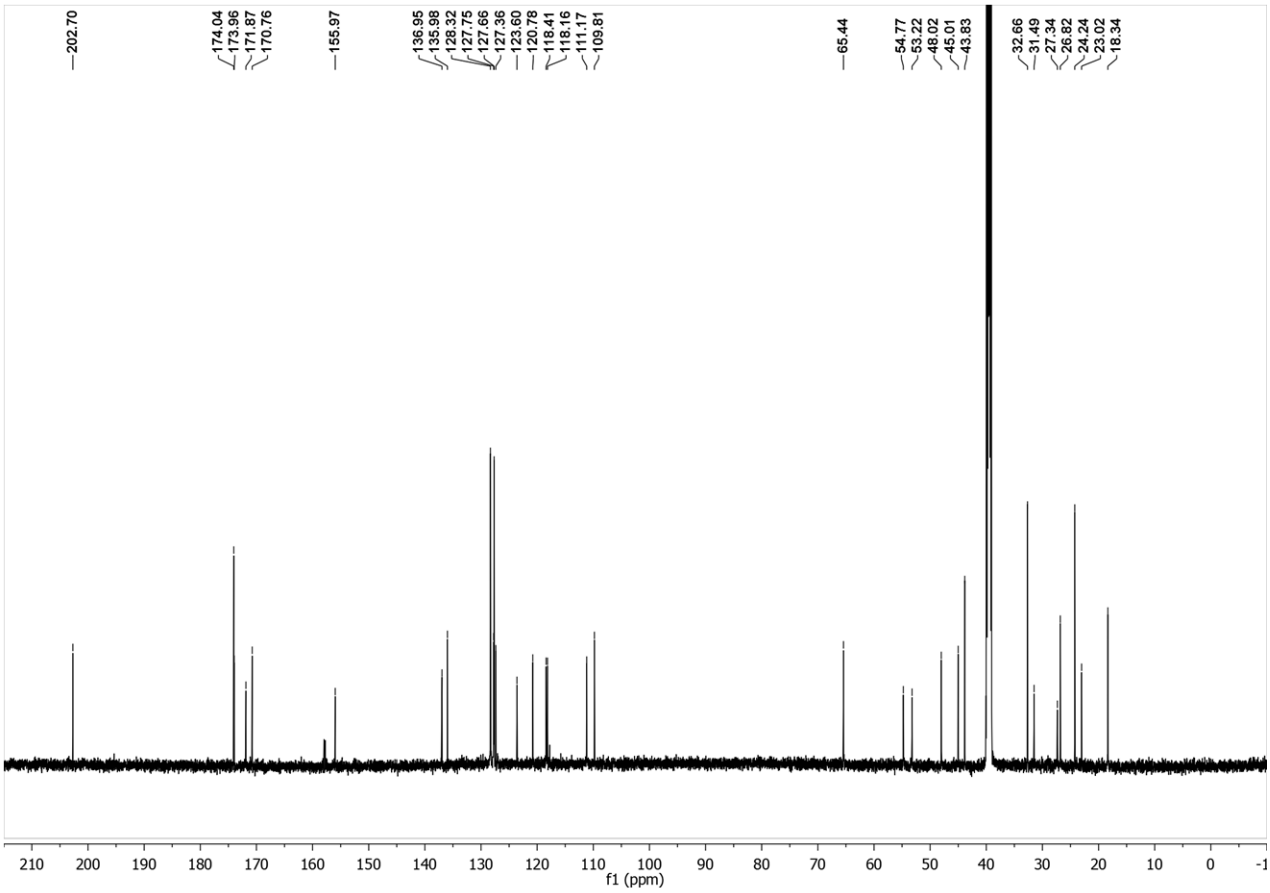
^{1}H and ^{13}C spectra of compound S10.



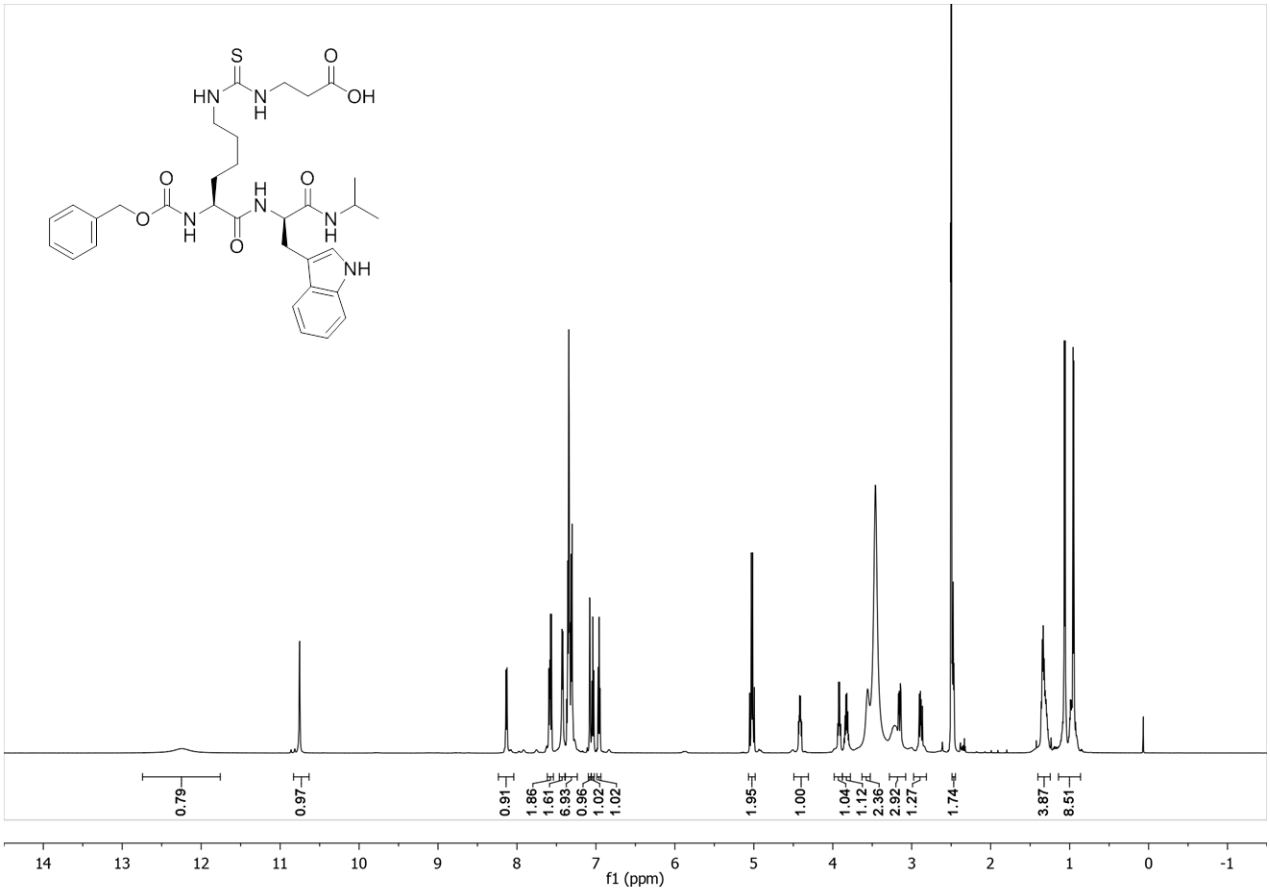


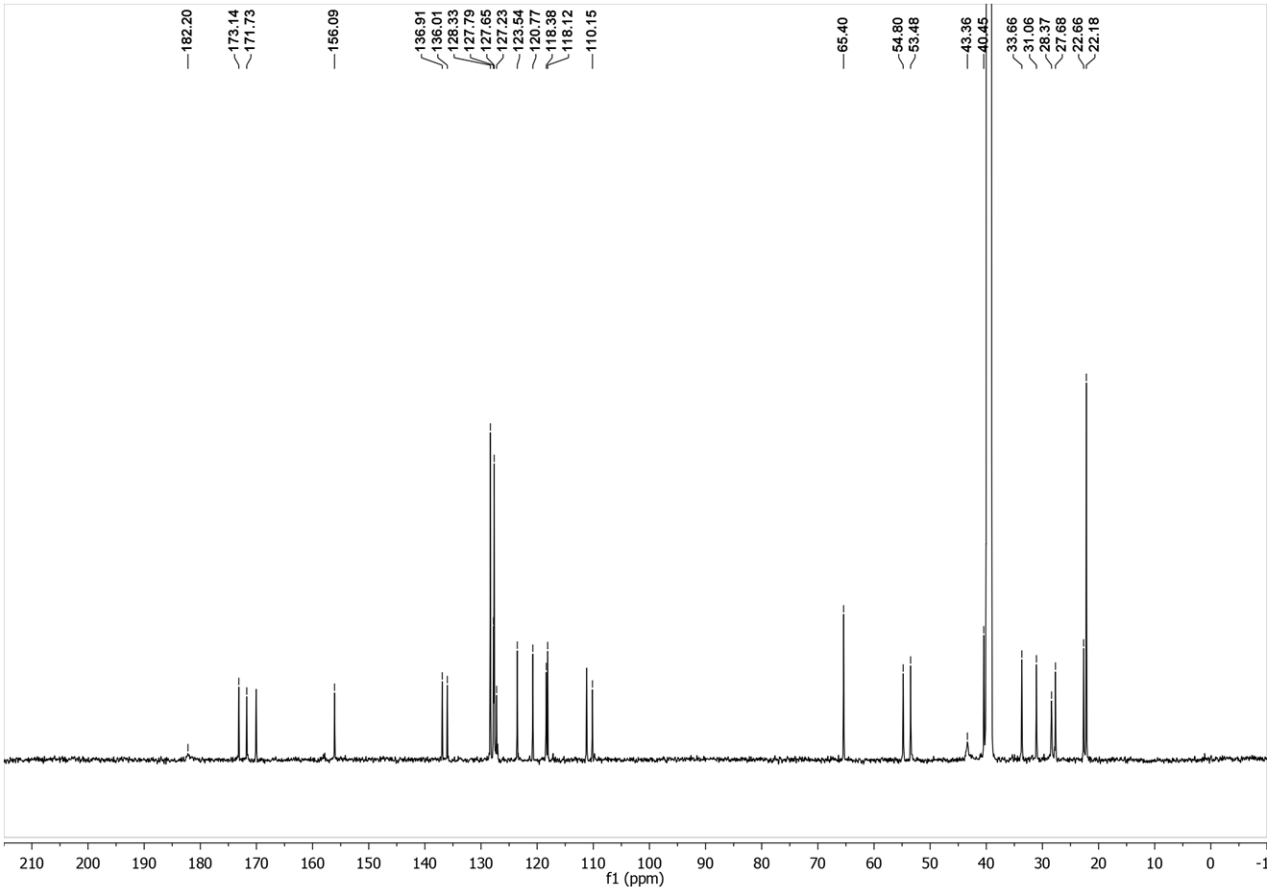
^{1}H and ^{13}C spectra of compound S11.



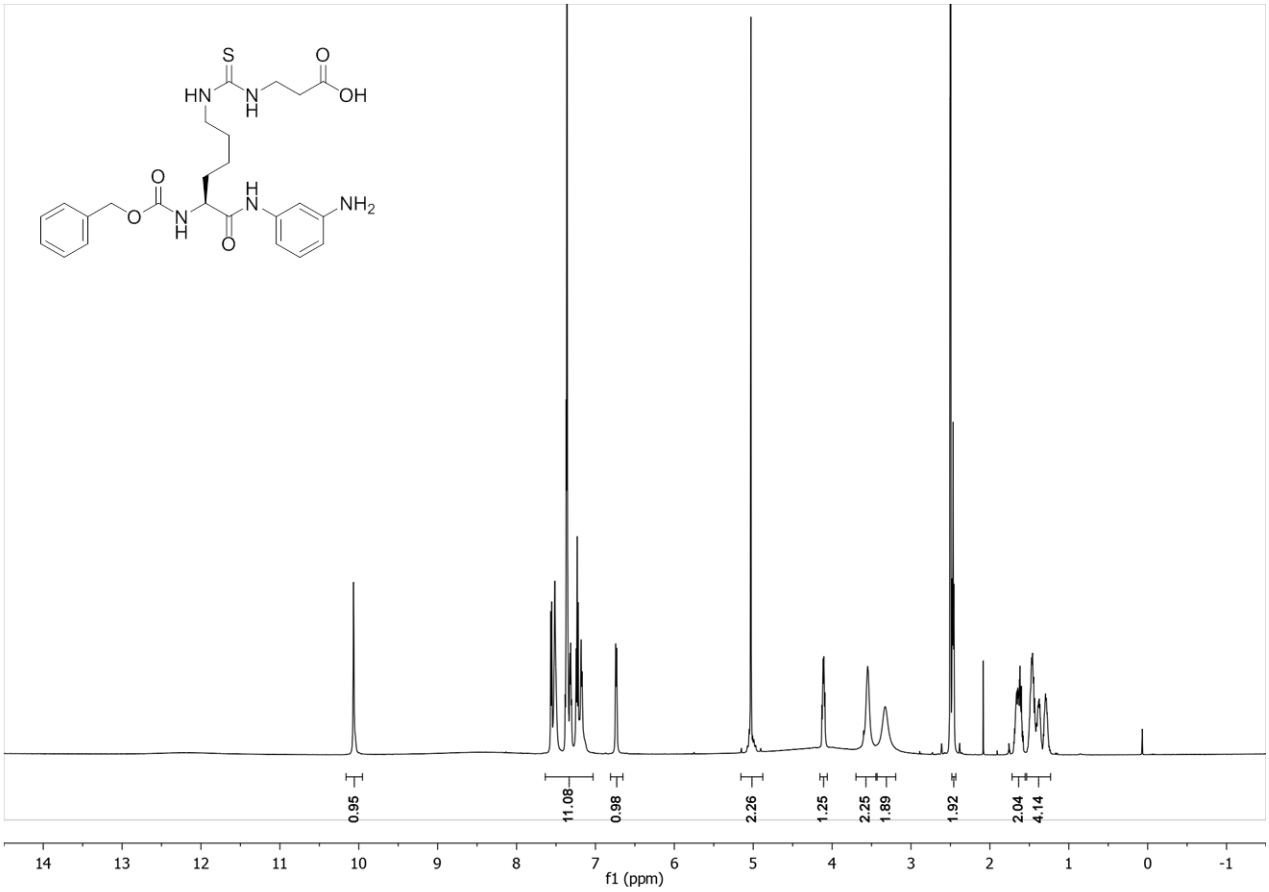


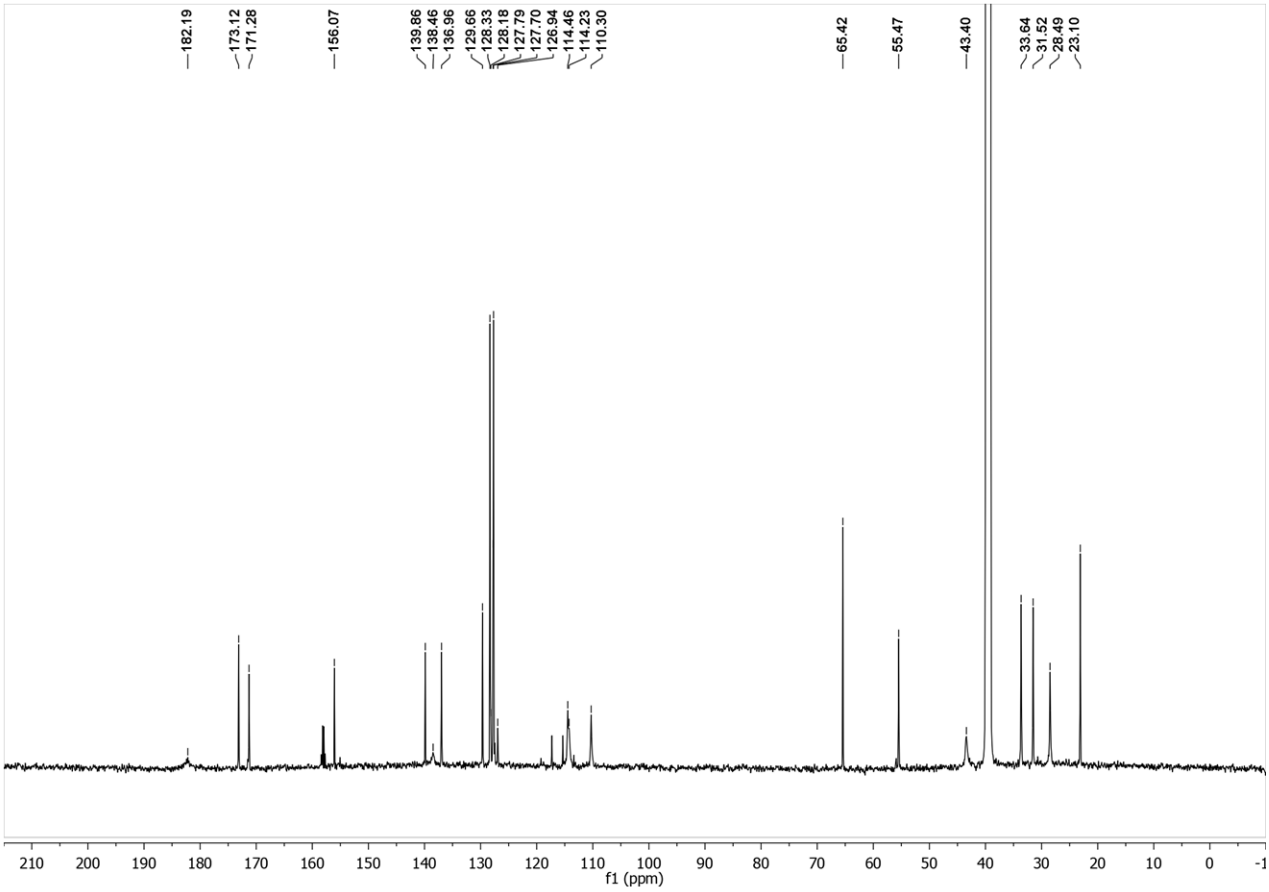
^{1}H and ^{13}C spectra of compound S12.



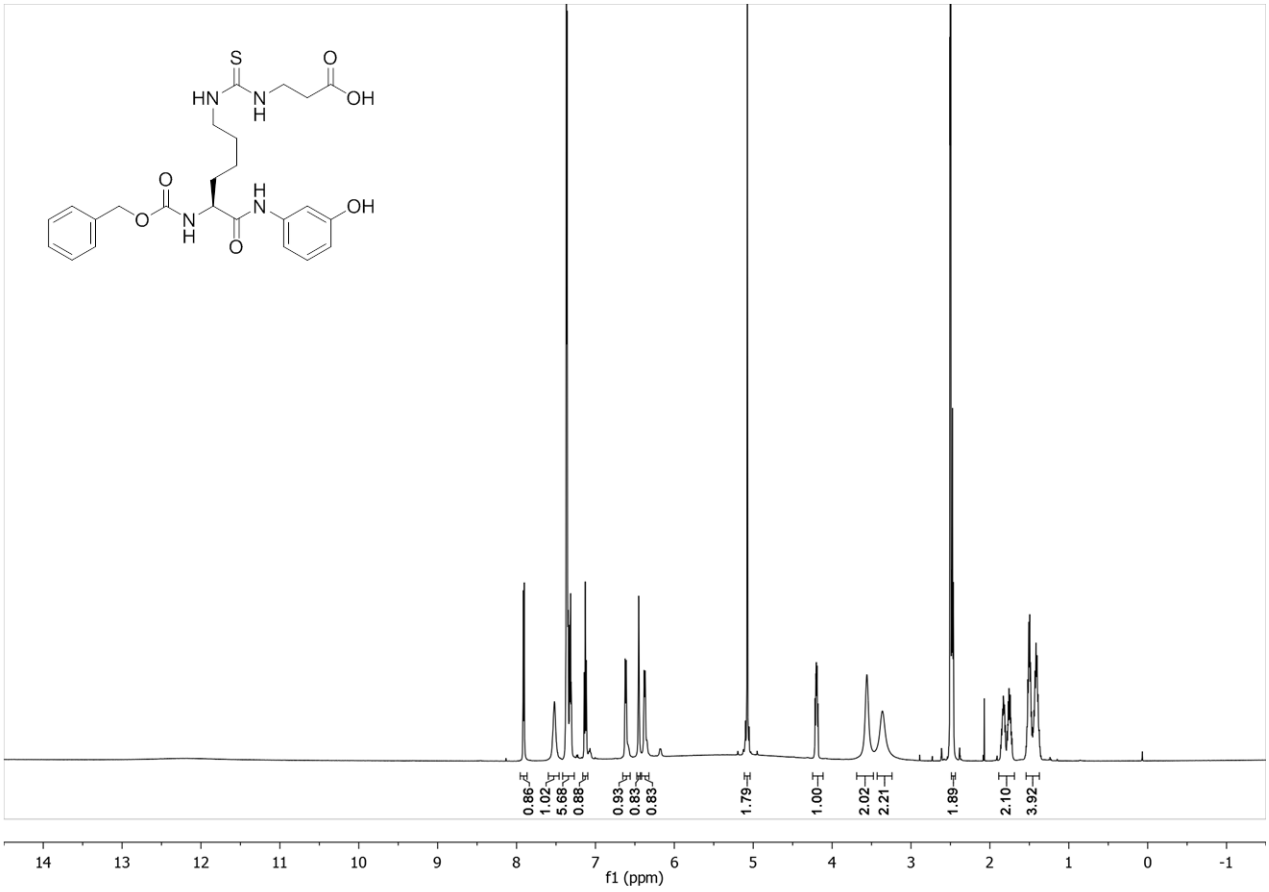


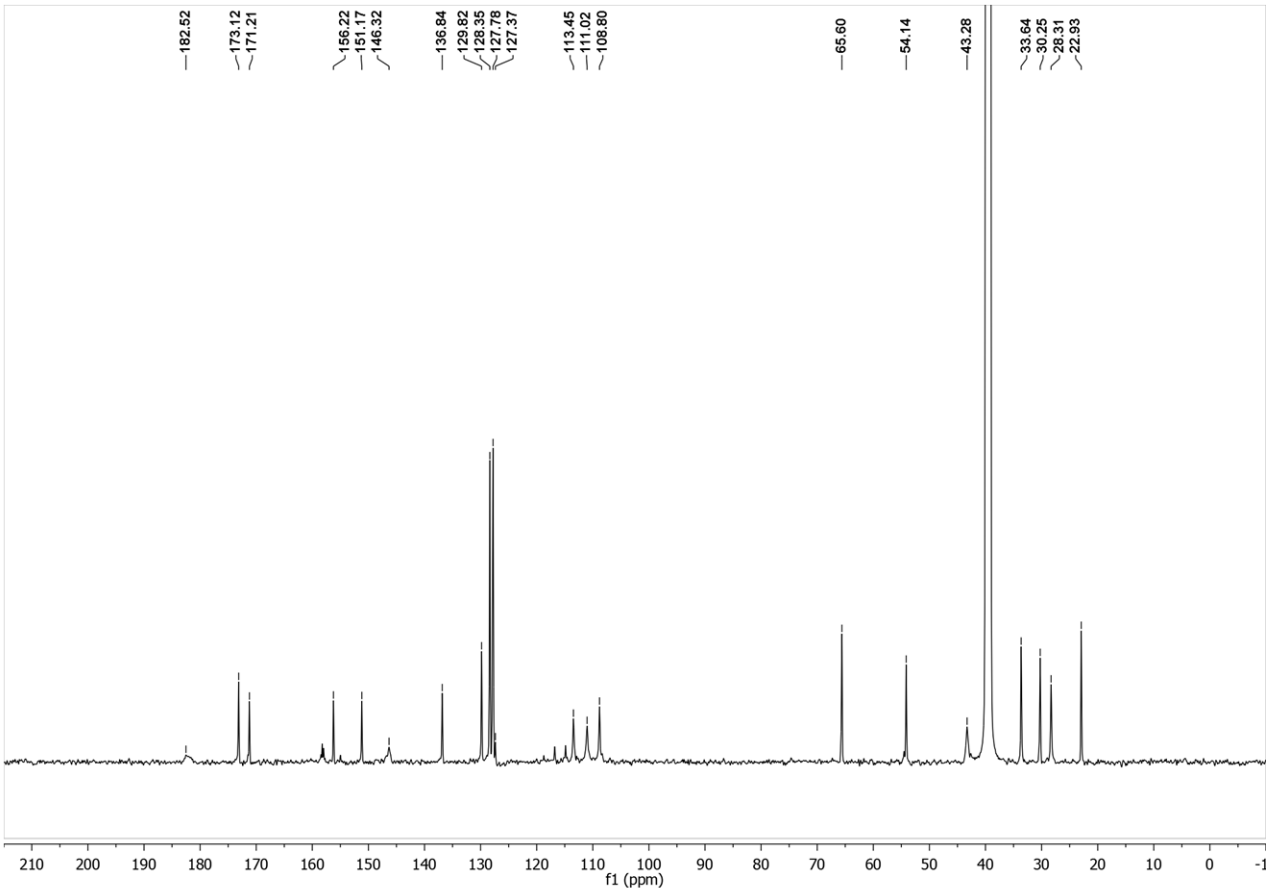
^{1}H and ^{13}C spectra of compound S13.



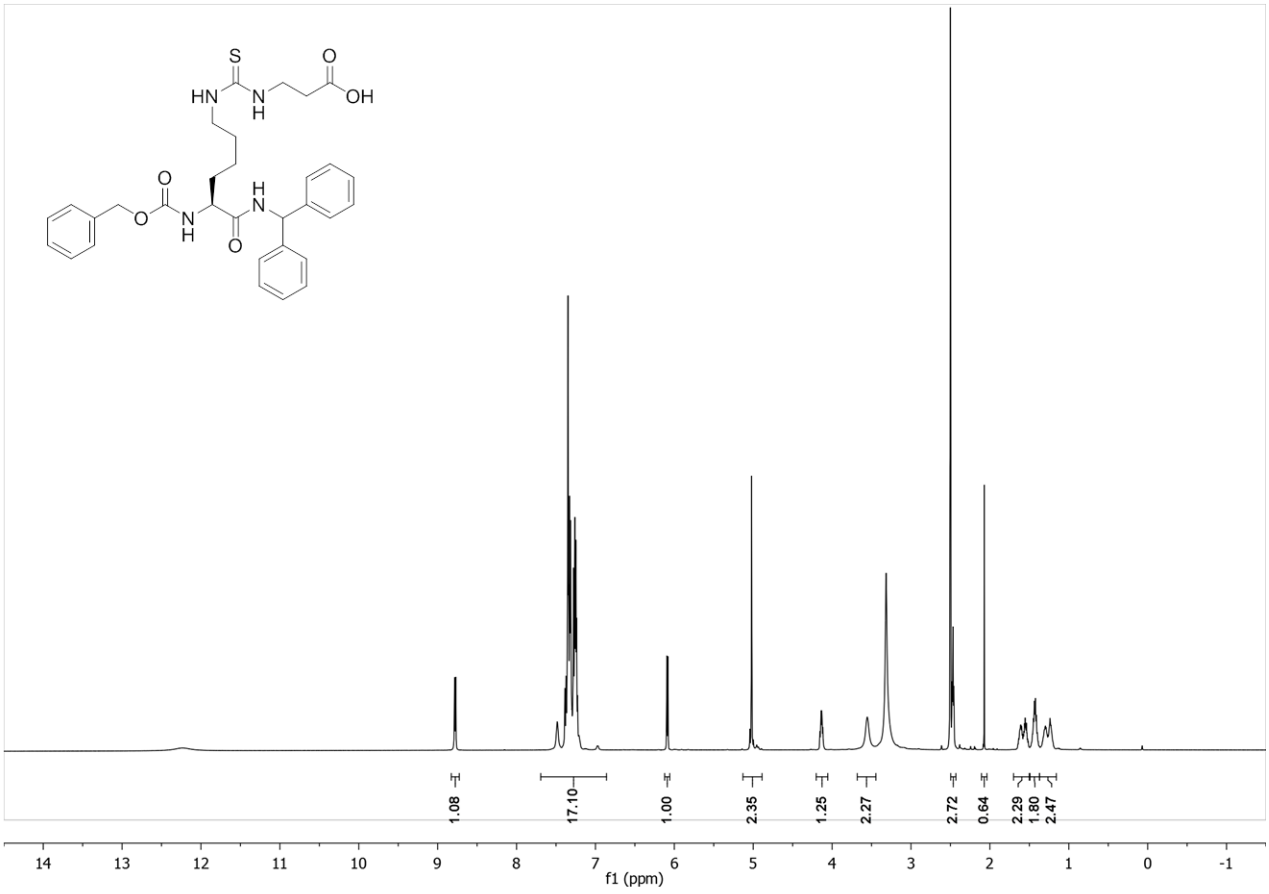


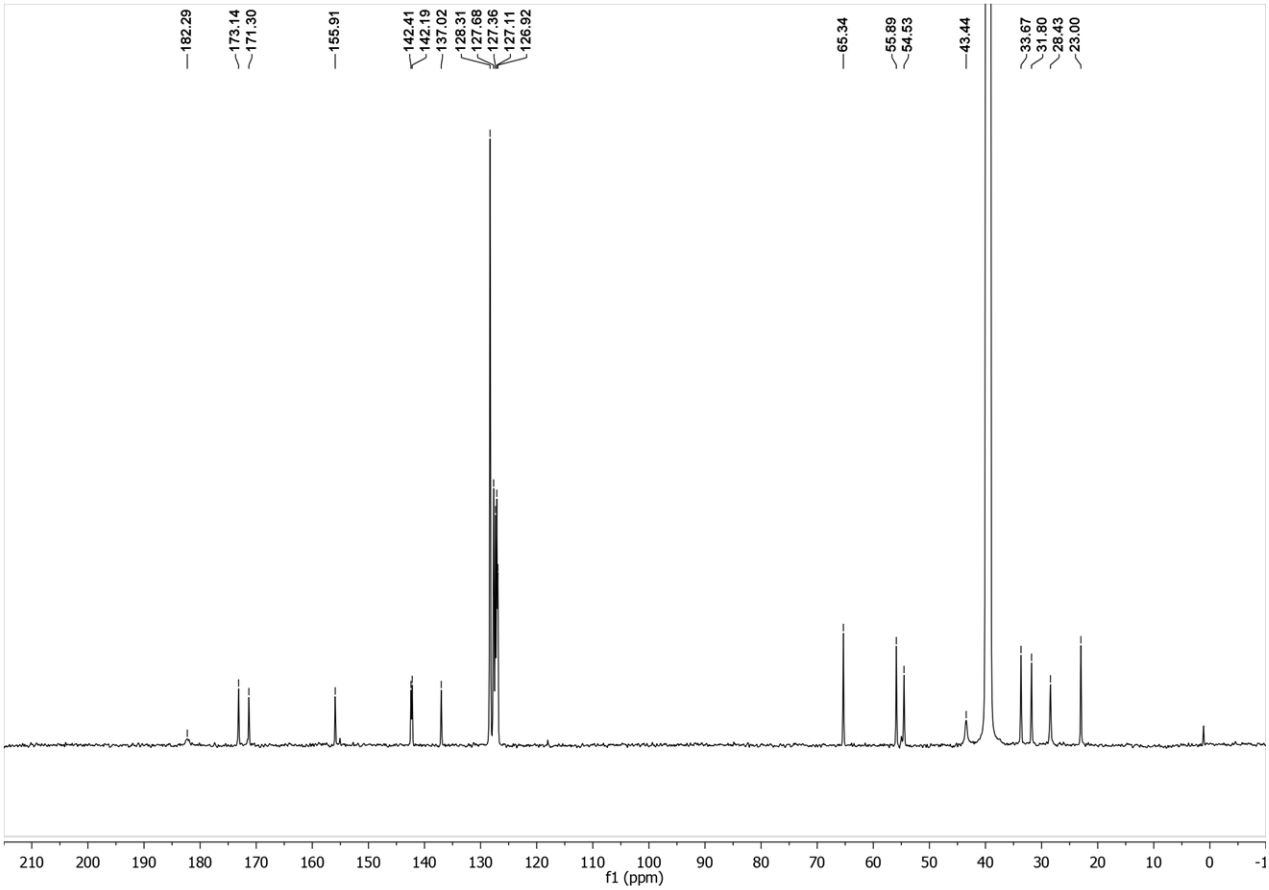
^{1}H and ^{13}C spectra of compound S14.



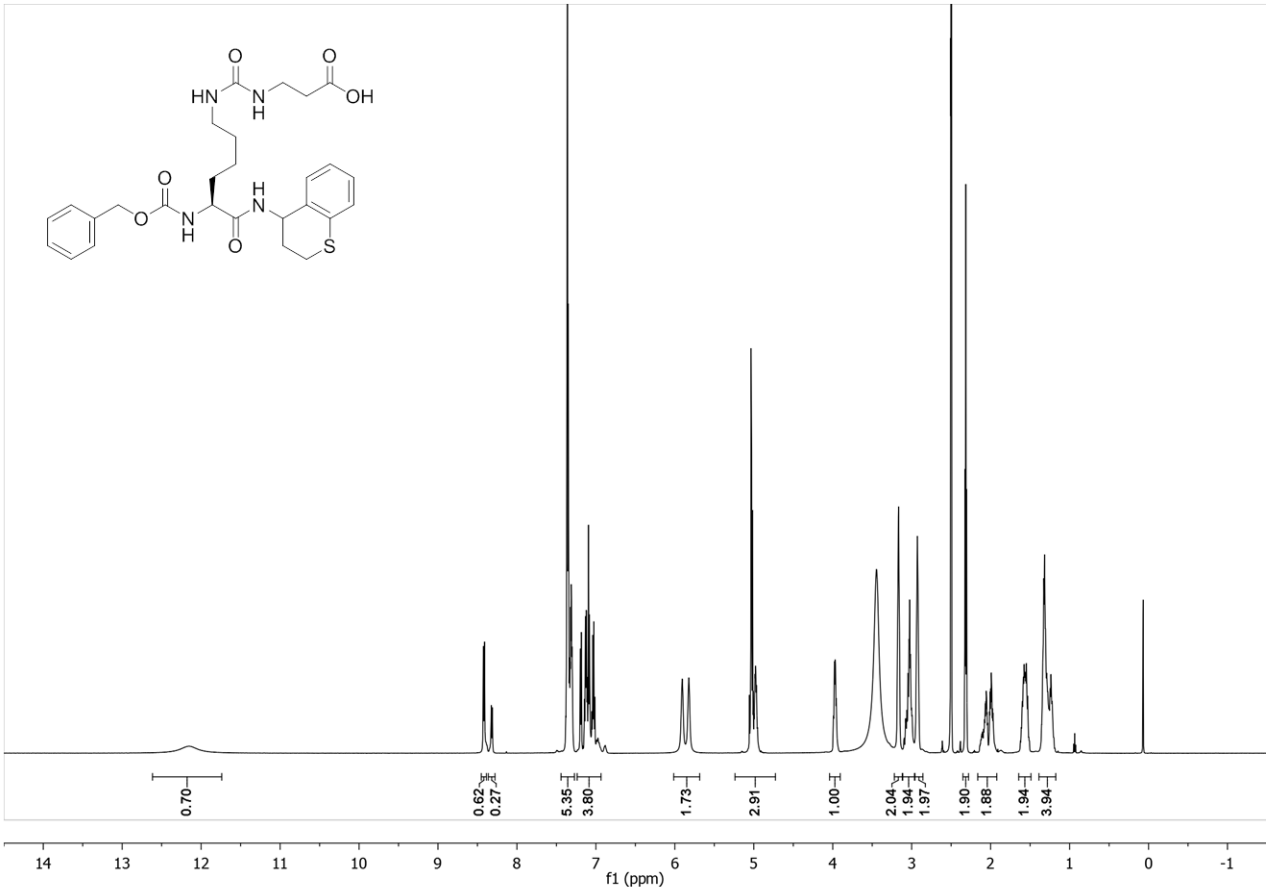


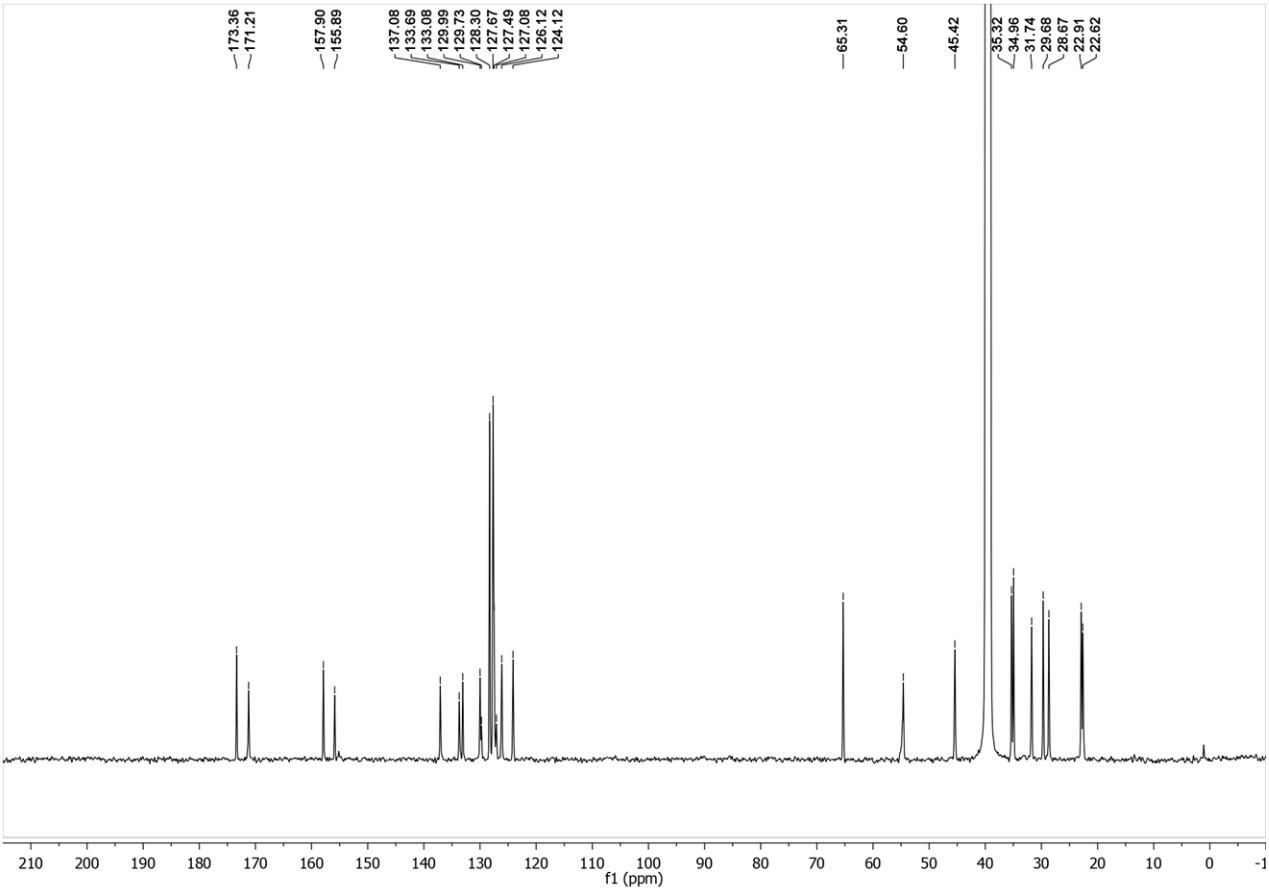
^{1}H and ^{13}C spectra of compound S15.



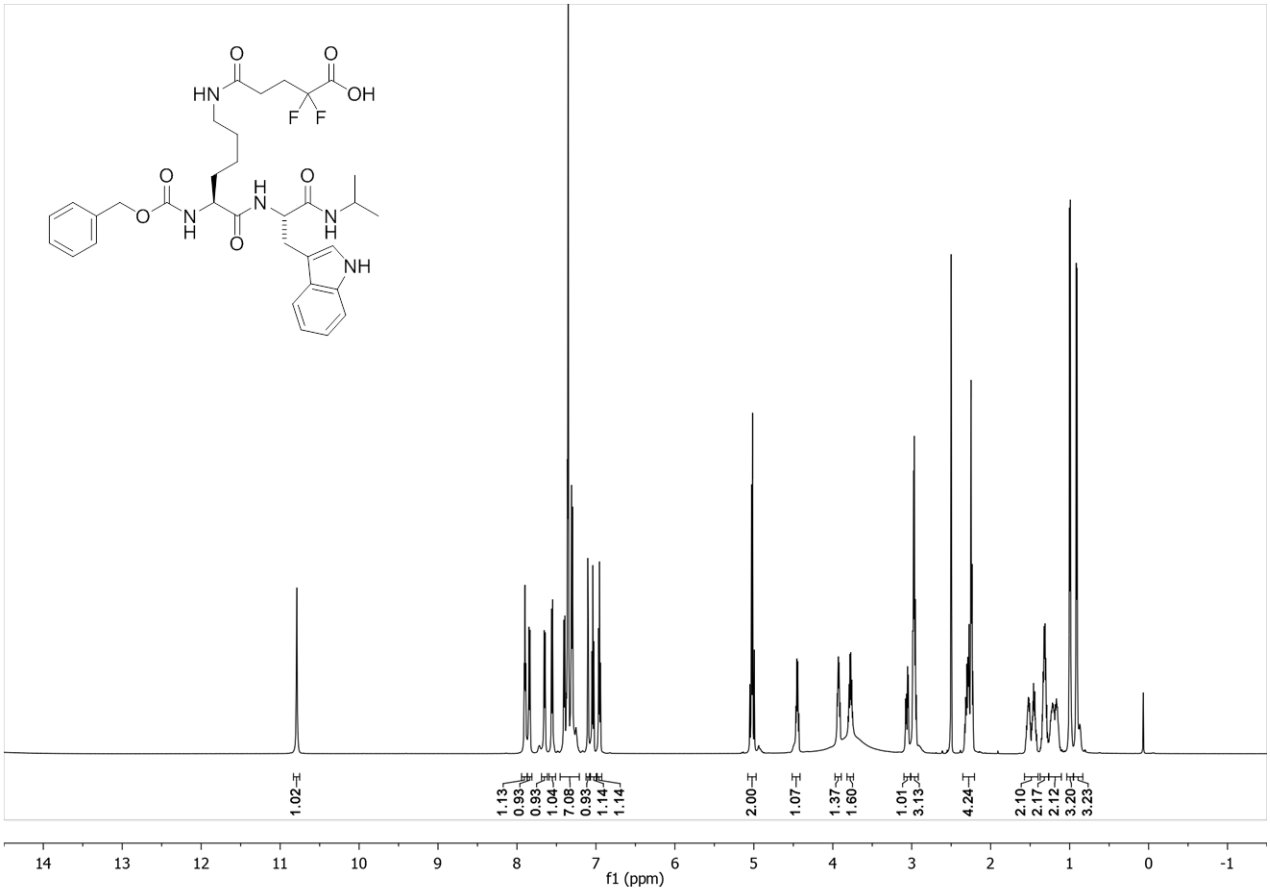


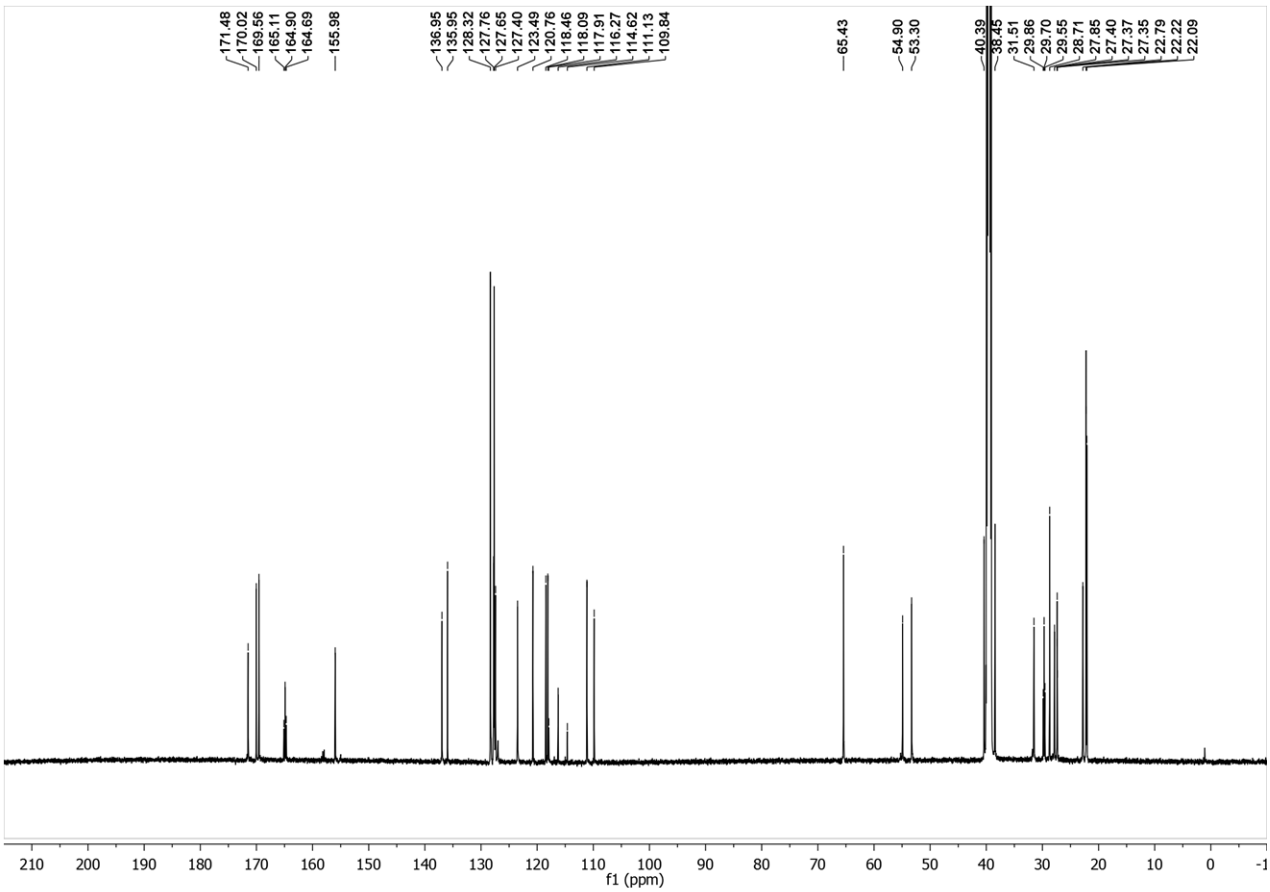
^{1}H and ^{13}C spectra of compound S16.

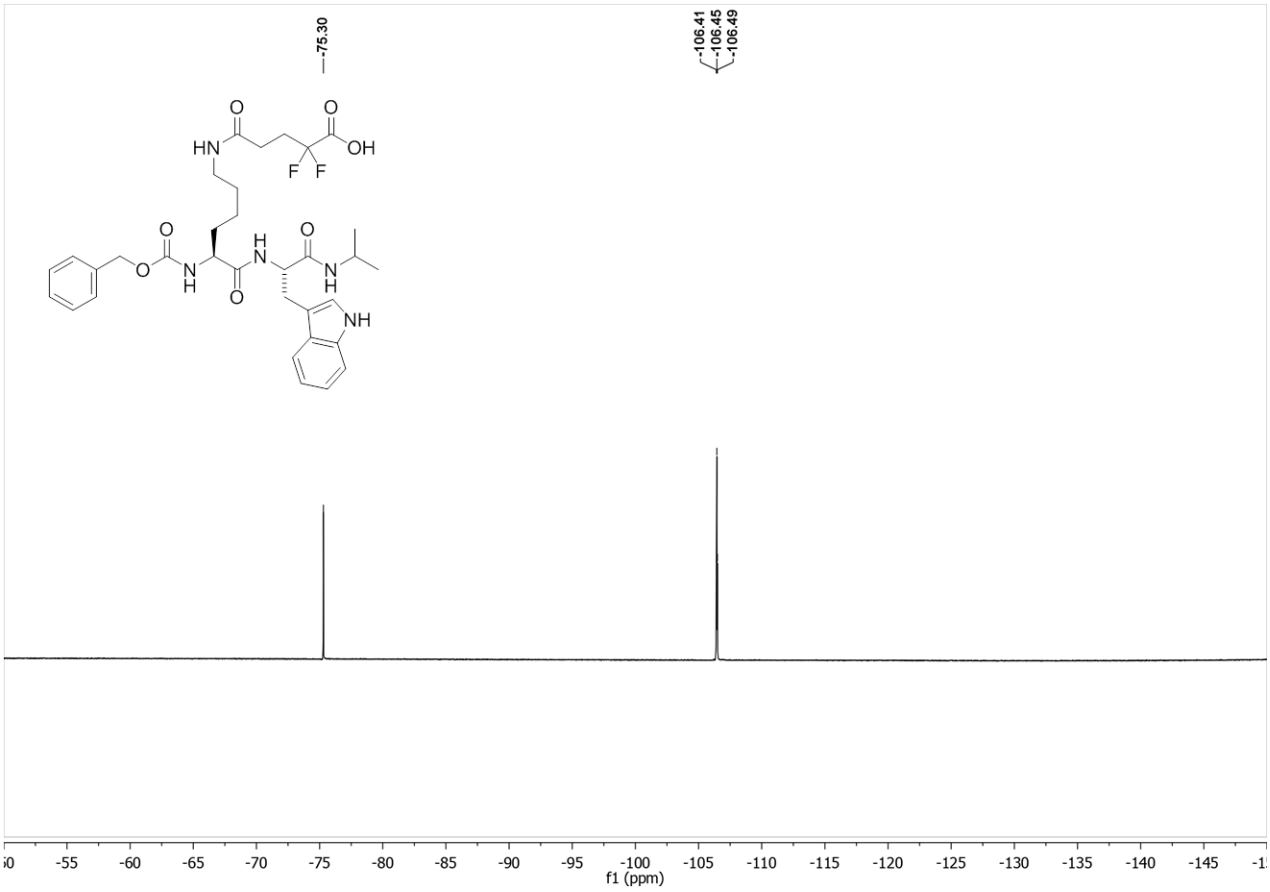




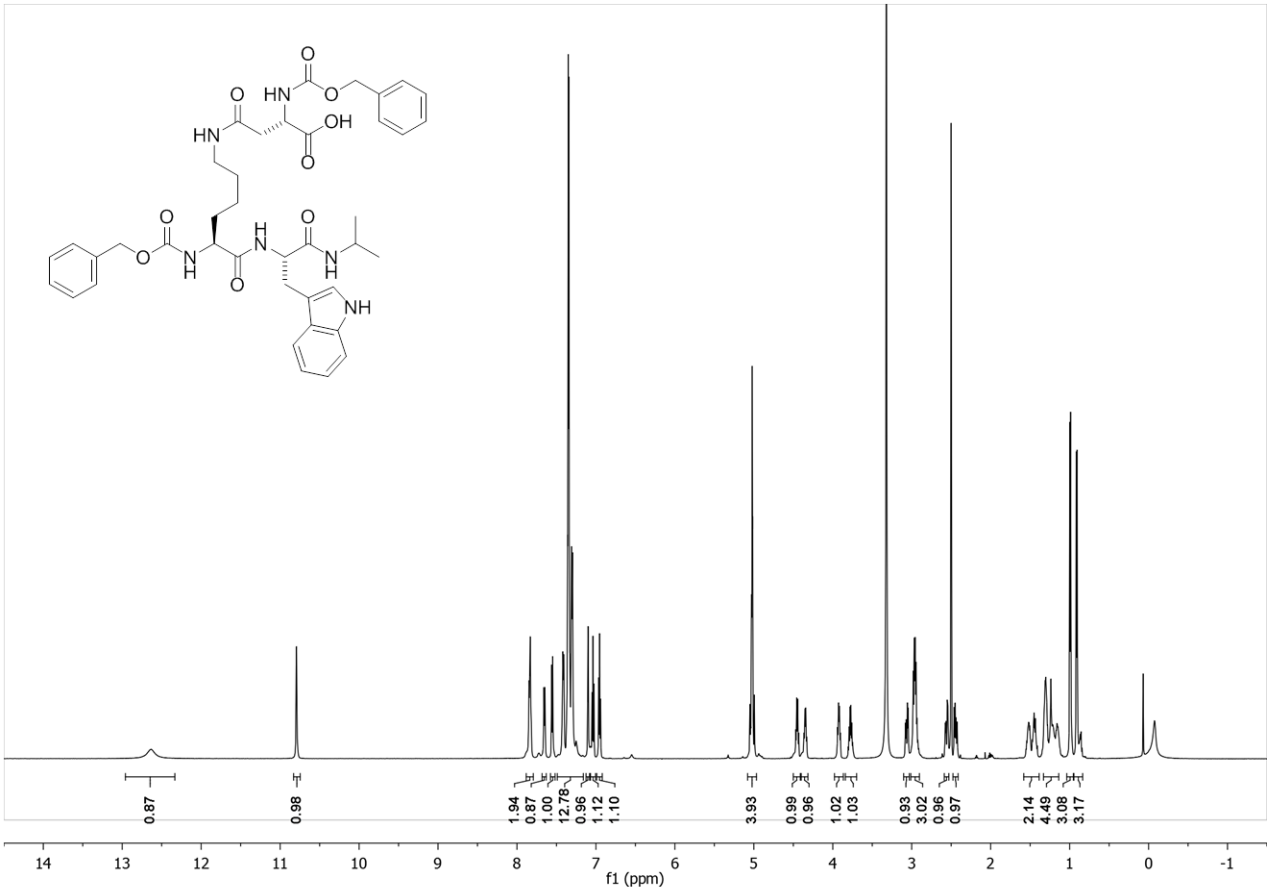
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S17.

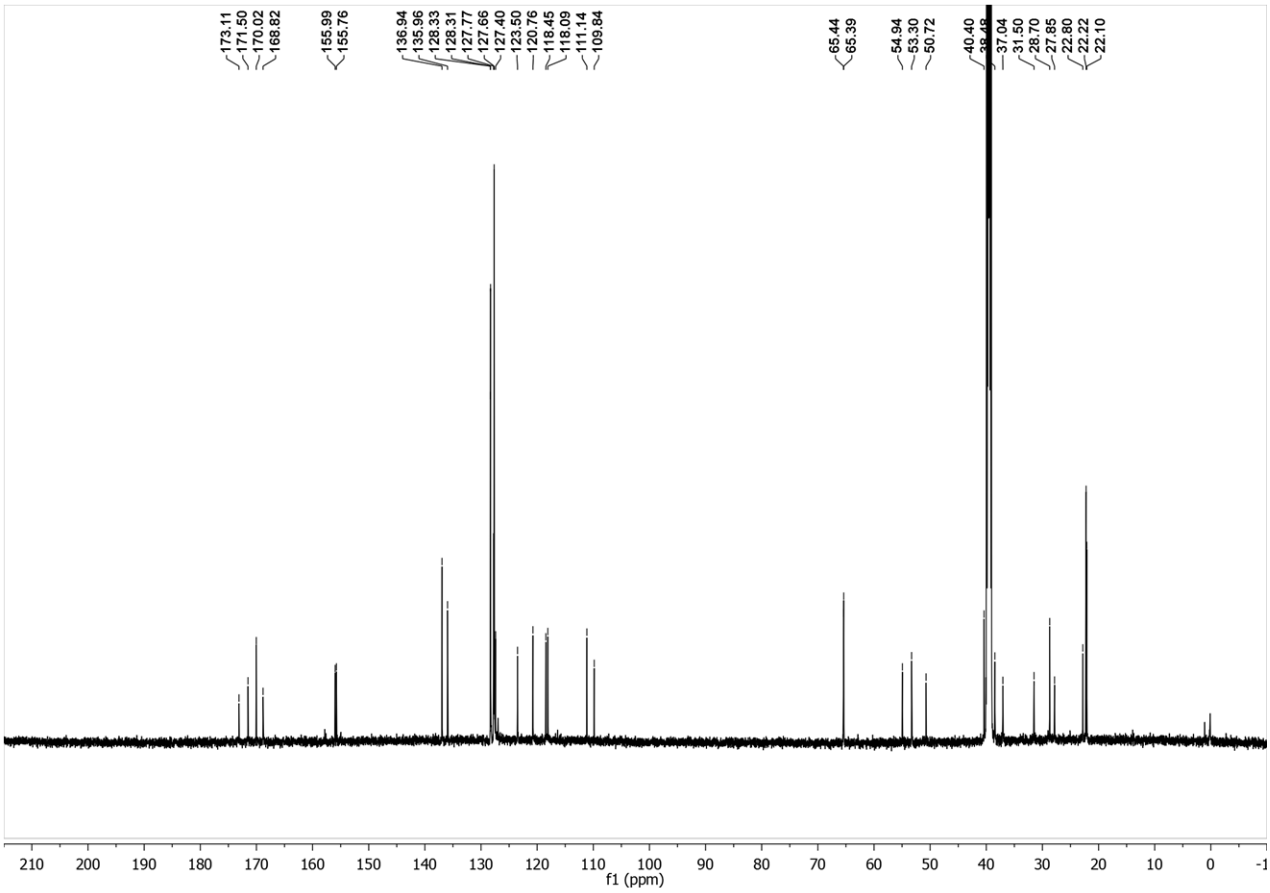




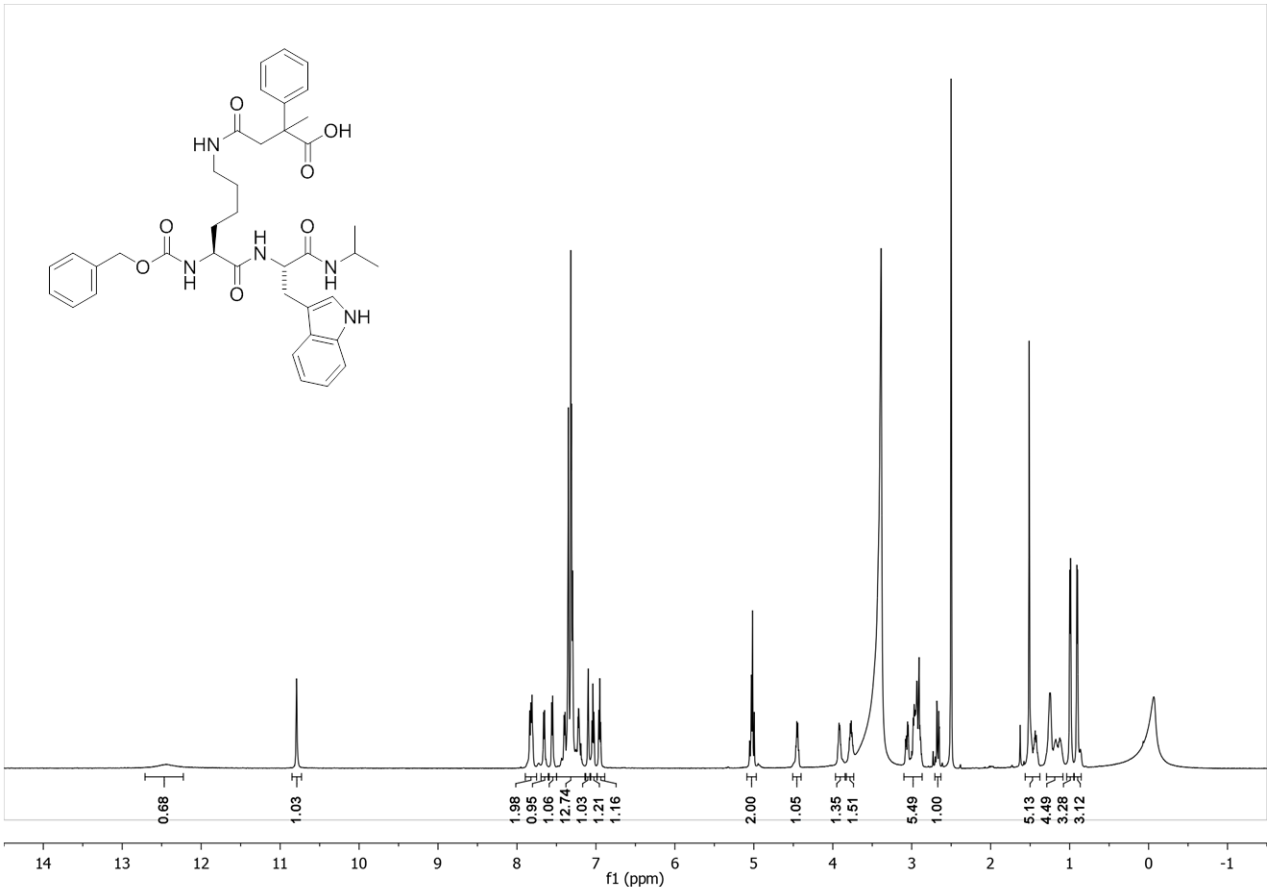

-75.30
-106.41
-106.45
-106.49
HN
O
O
OH
F F
O
O
N
H
H
N
O
O
N
H
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

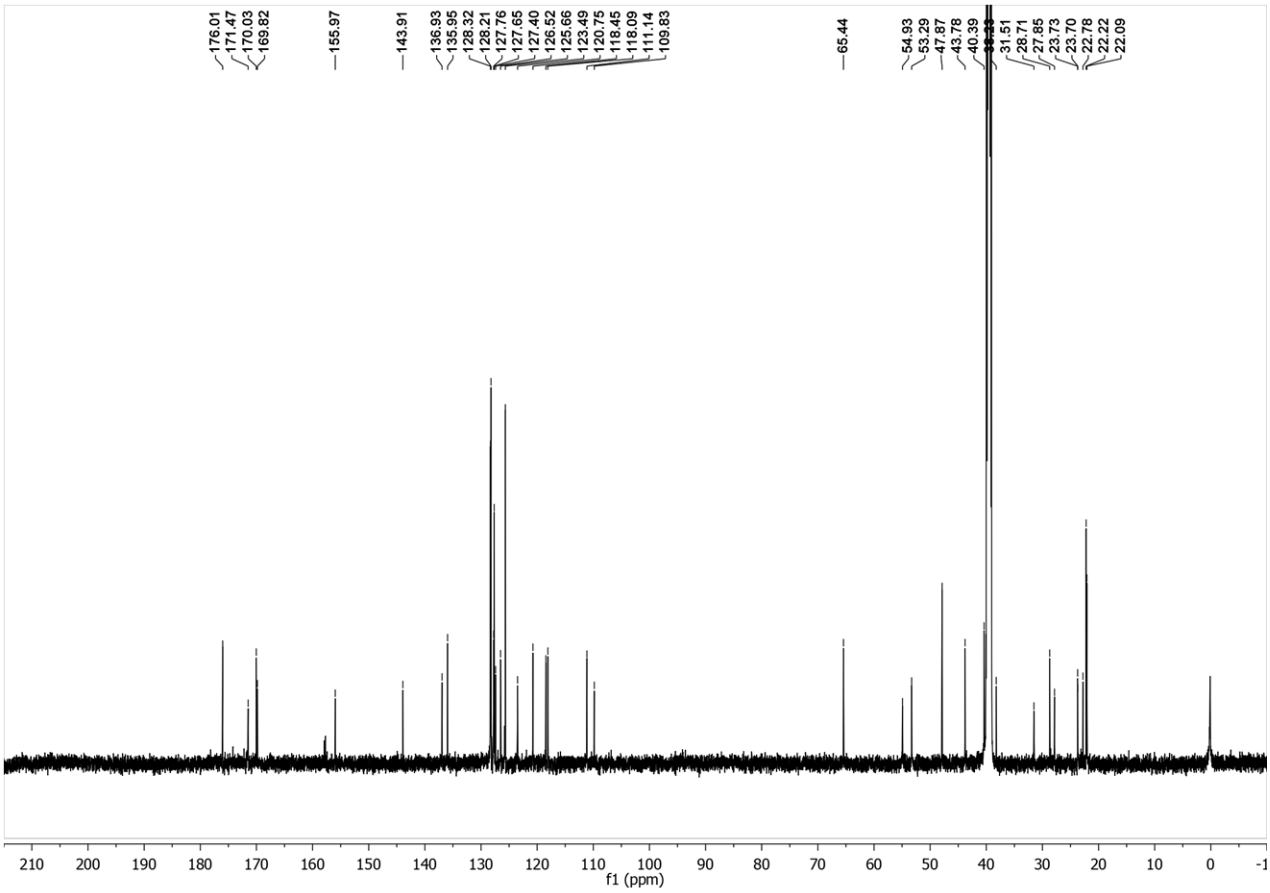
^{1}H and ^{13}C spectra of compound S18.



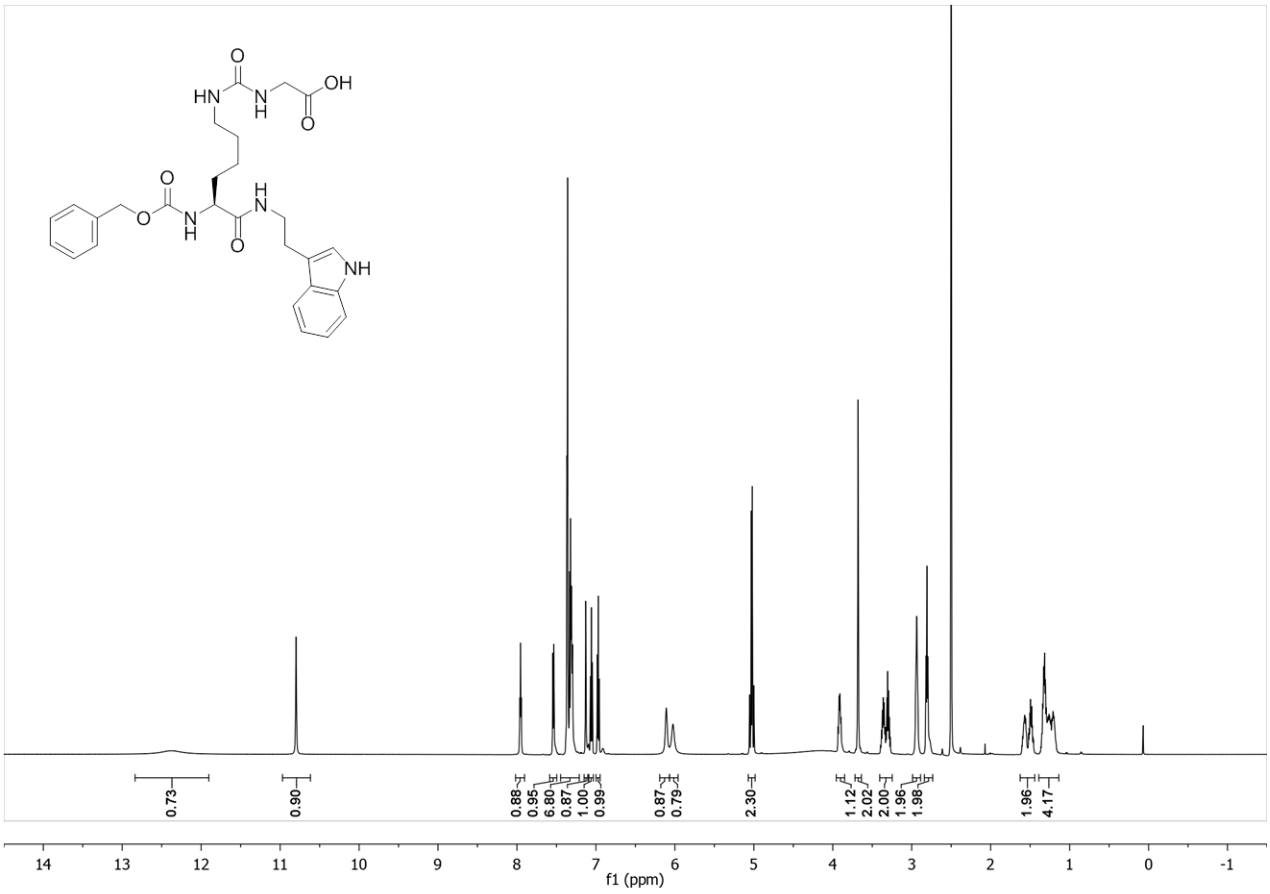


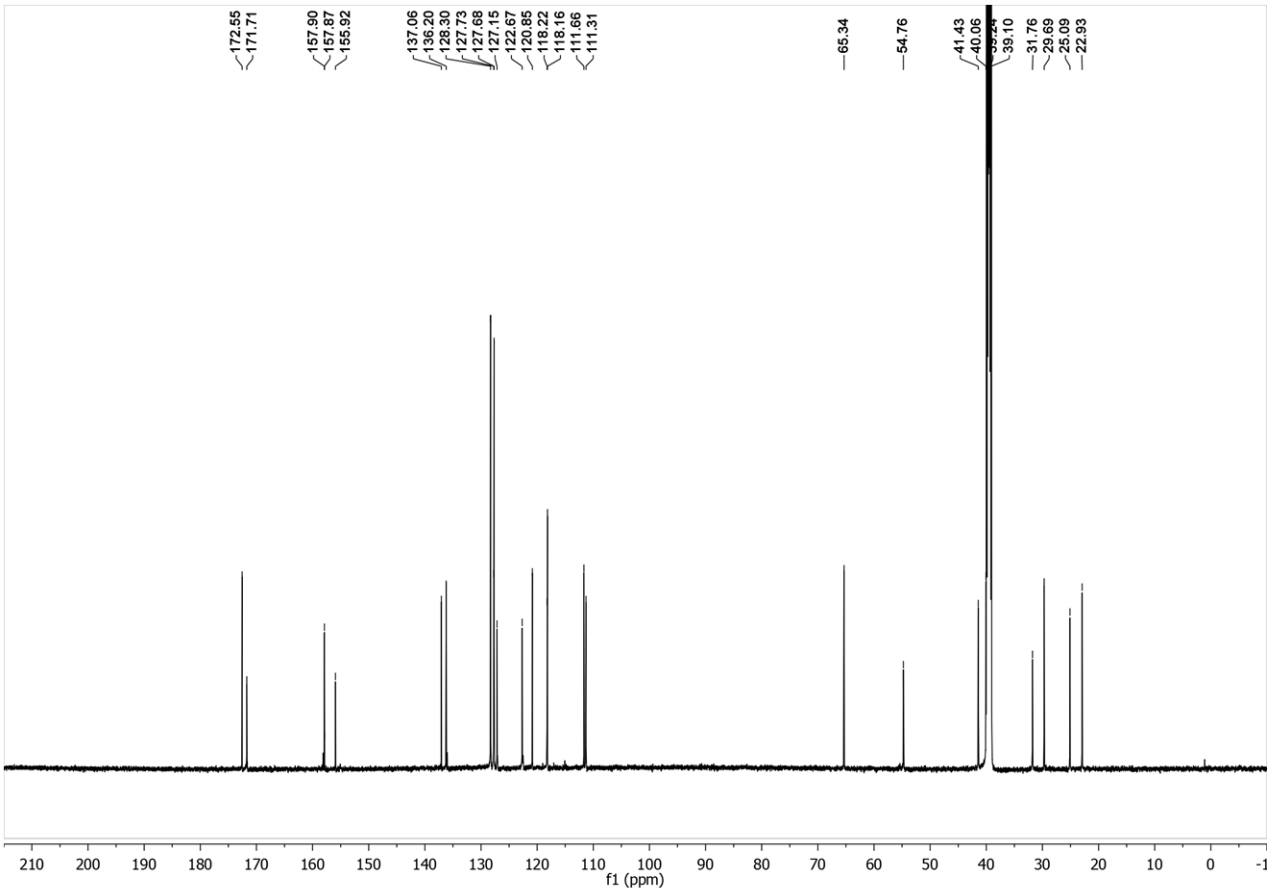
^{1}H and ^{13}C spectra of compound S19.



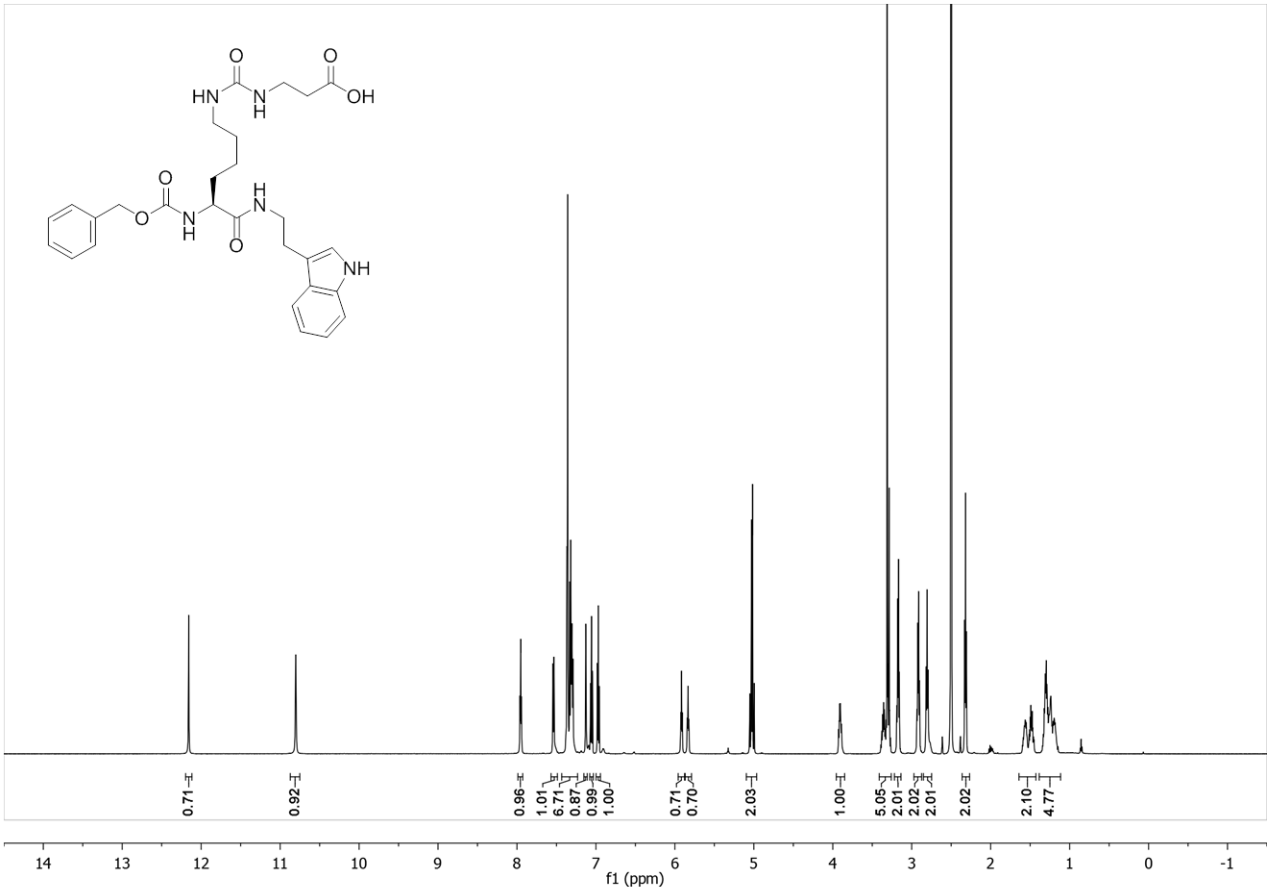


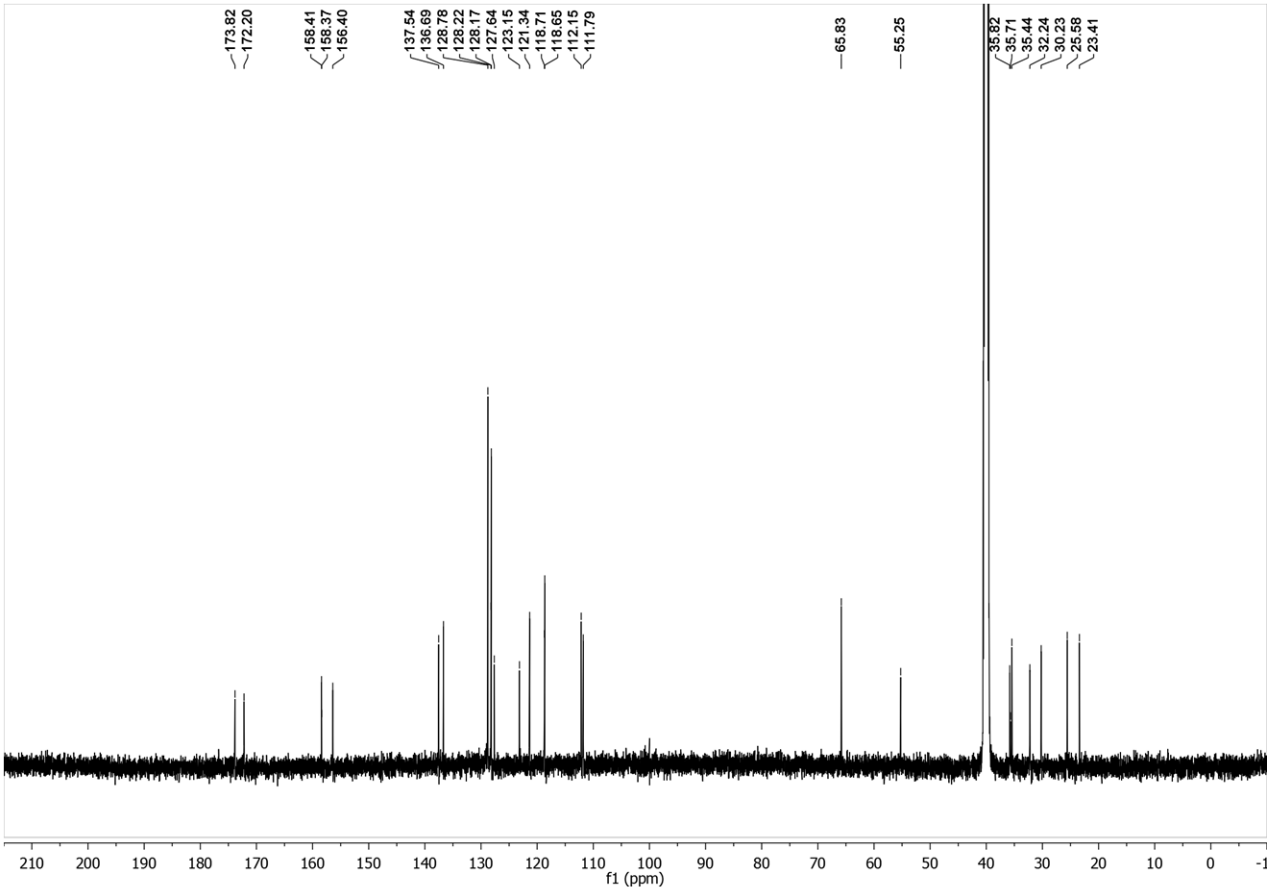
^{1}H and ^{13}C spectra of compound S20.



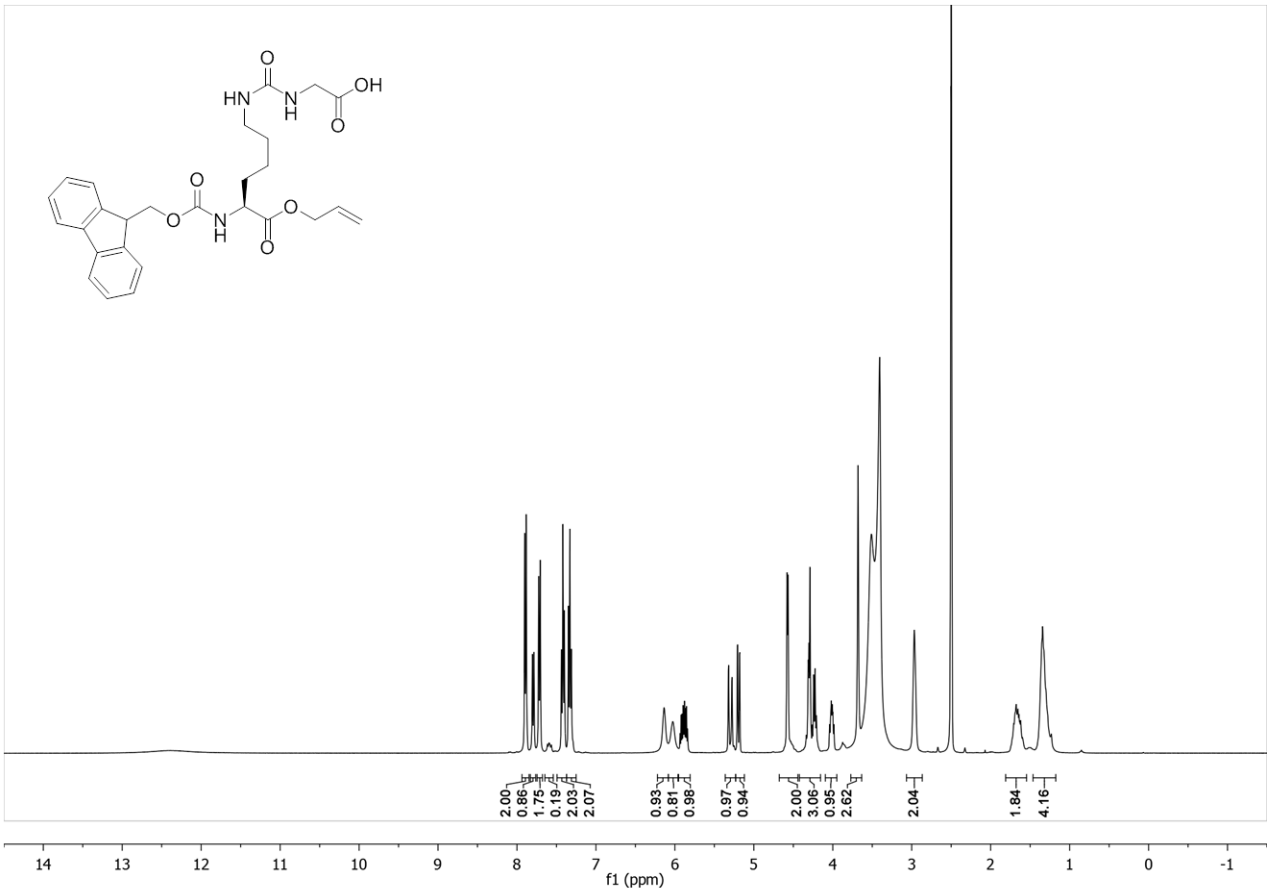


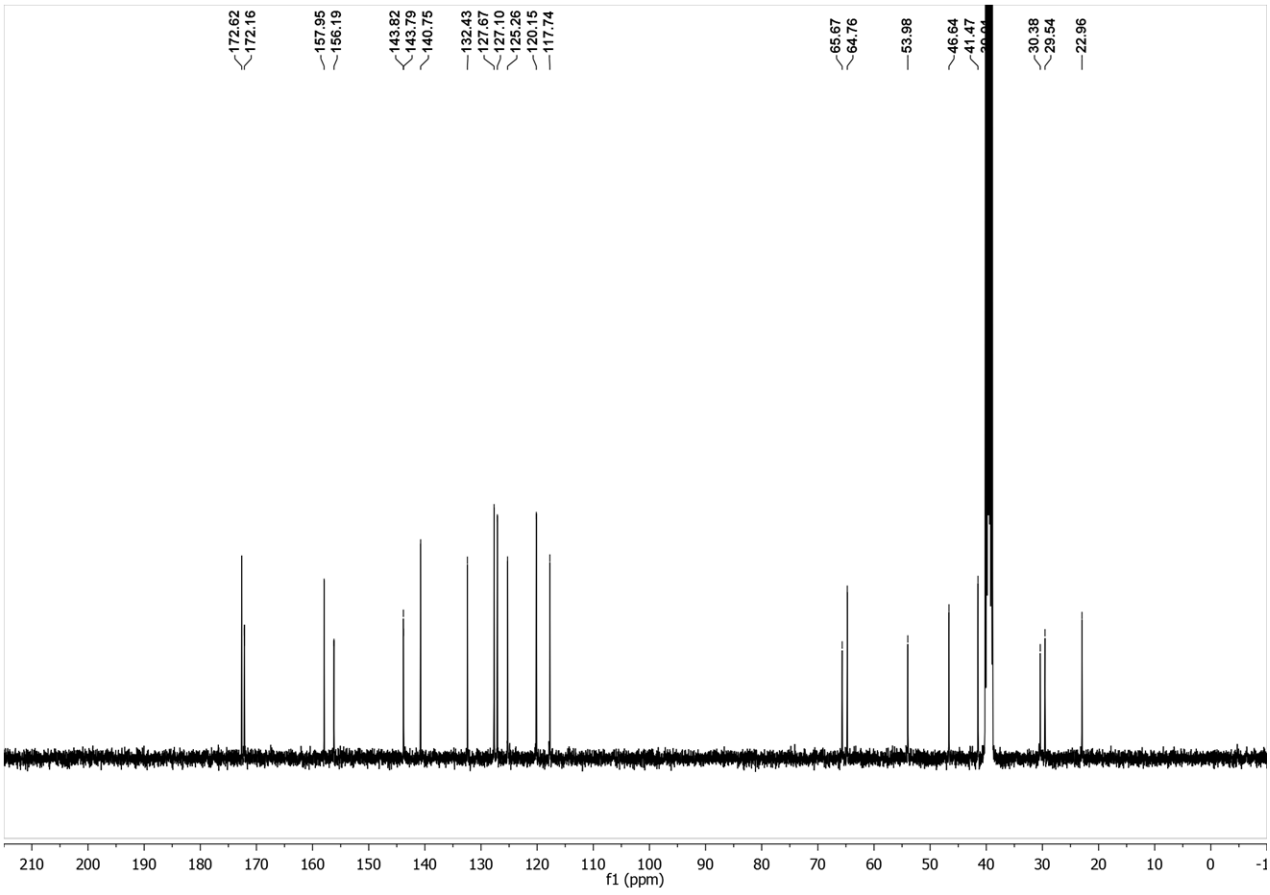
^{1}H and ^{13}C spectra of compound S21.



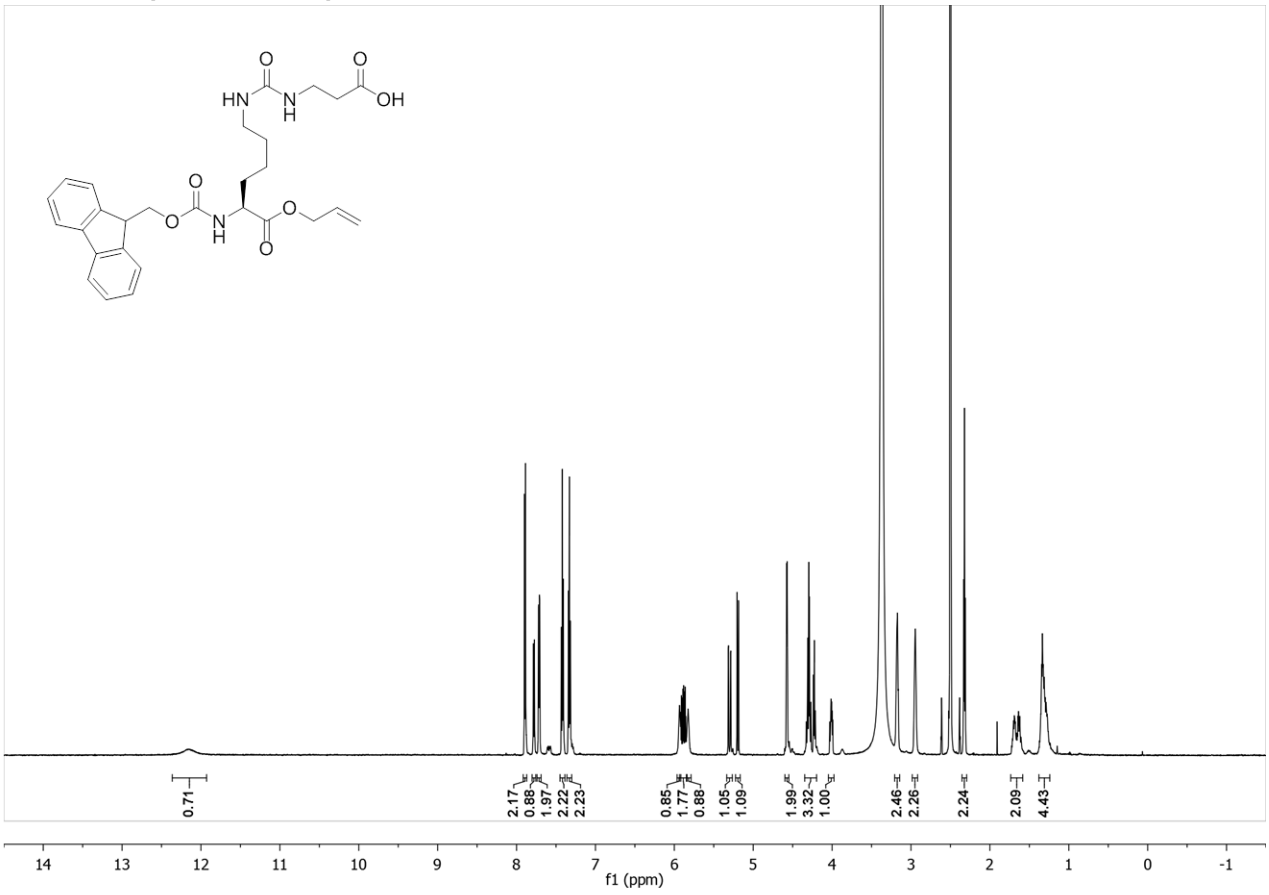


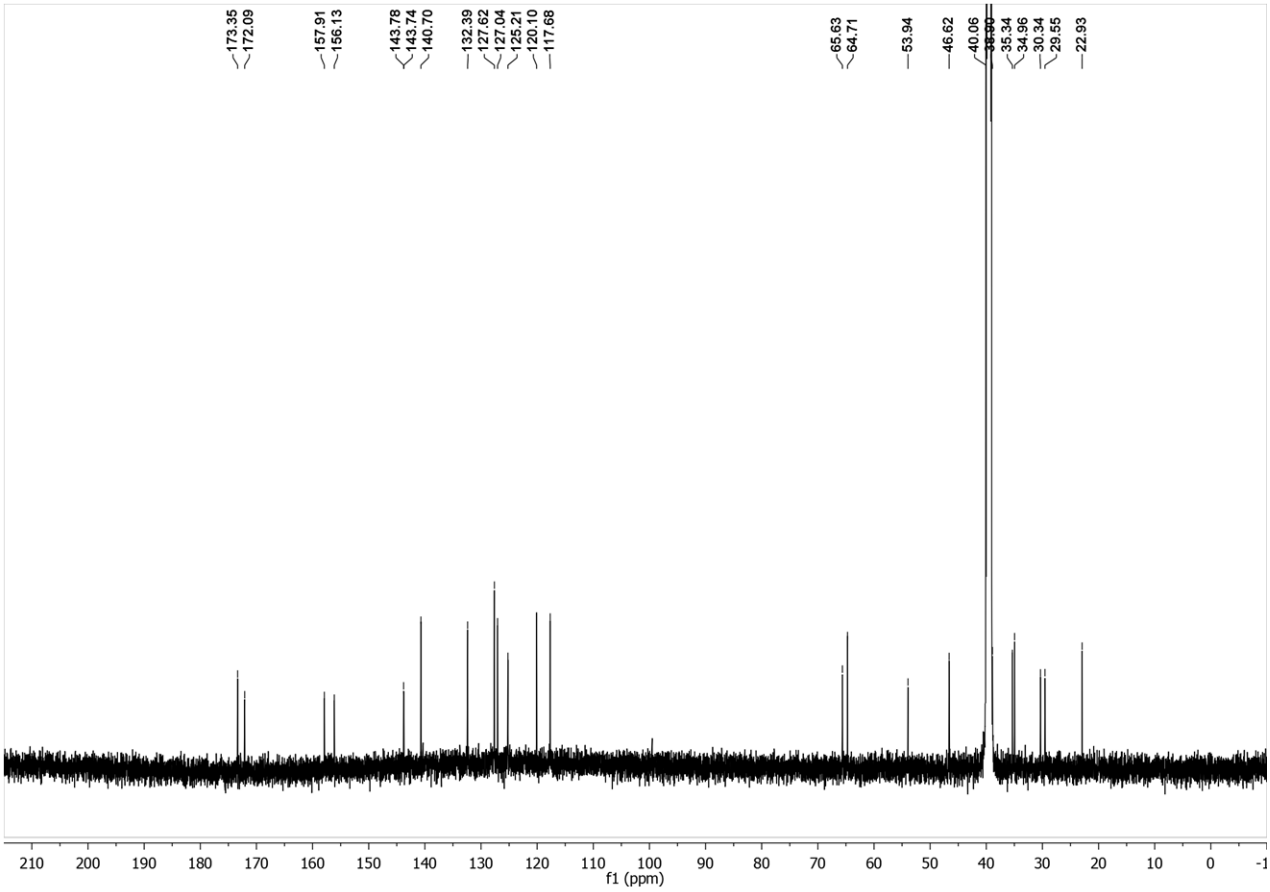
^{1}H and ^{13}C spectra of compound S22.



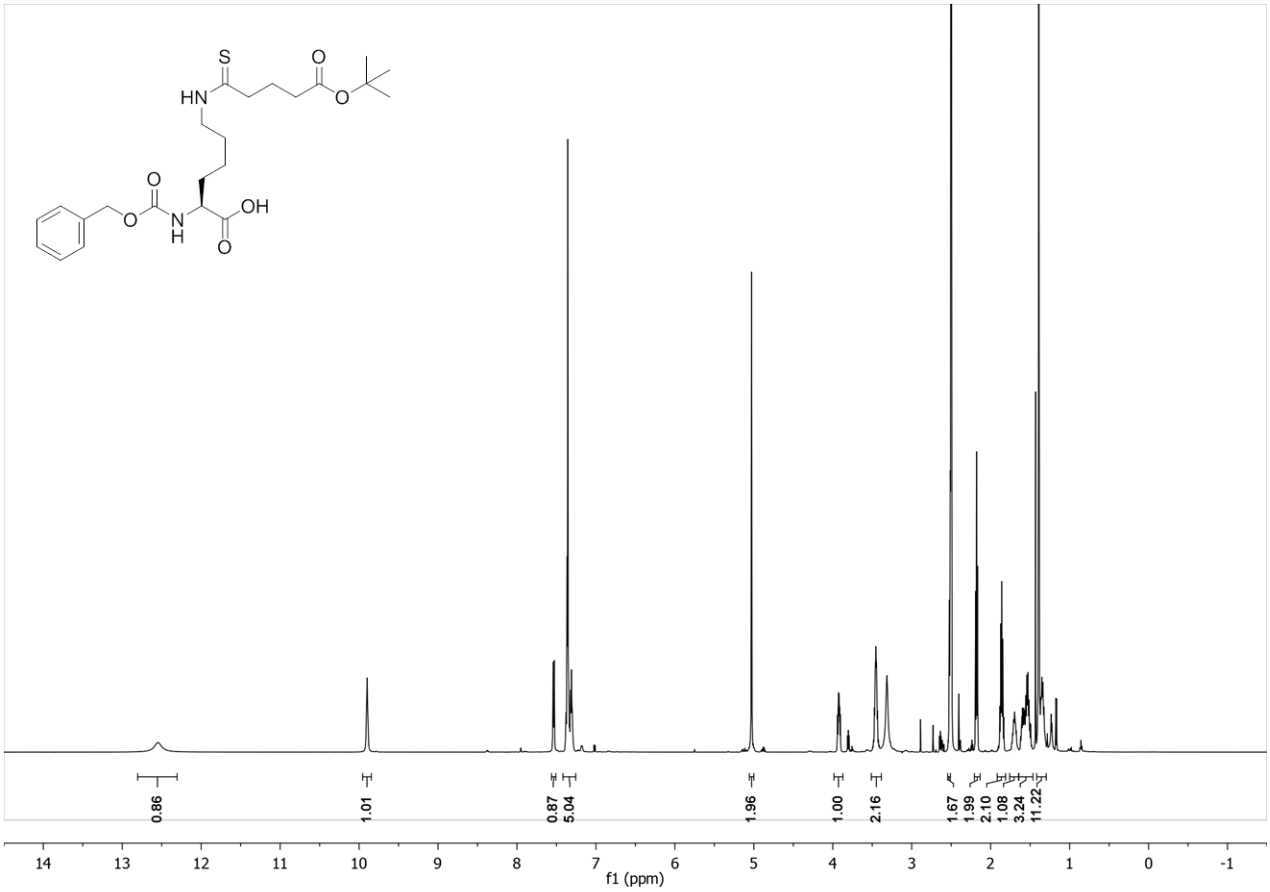


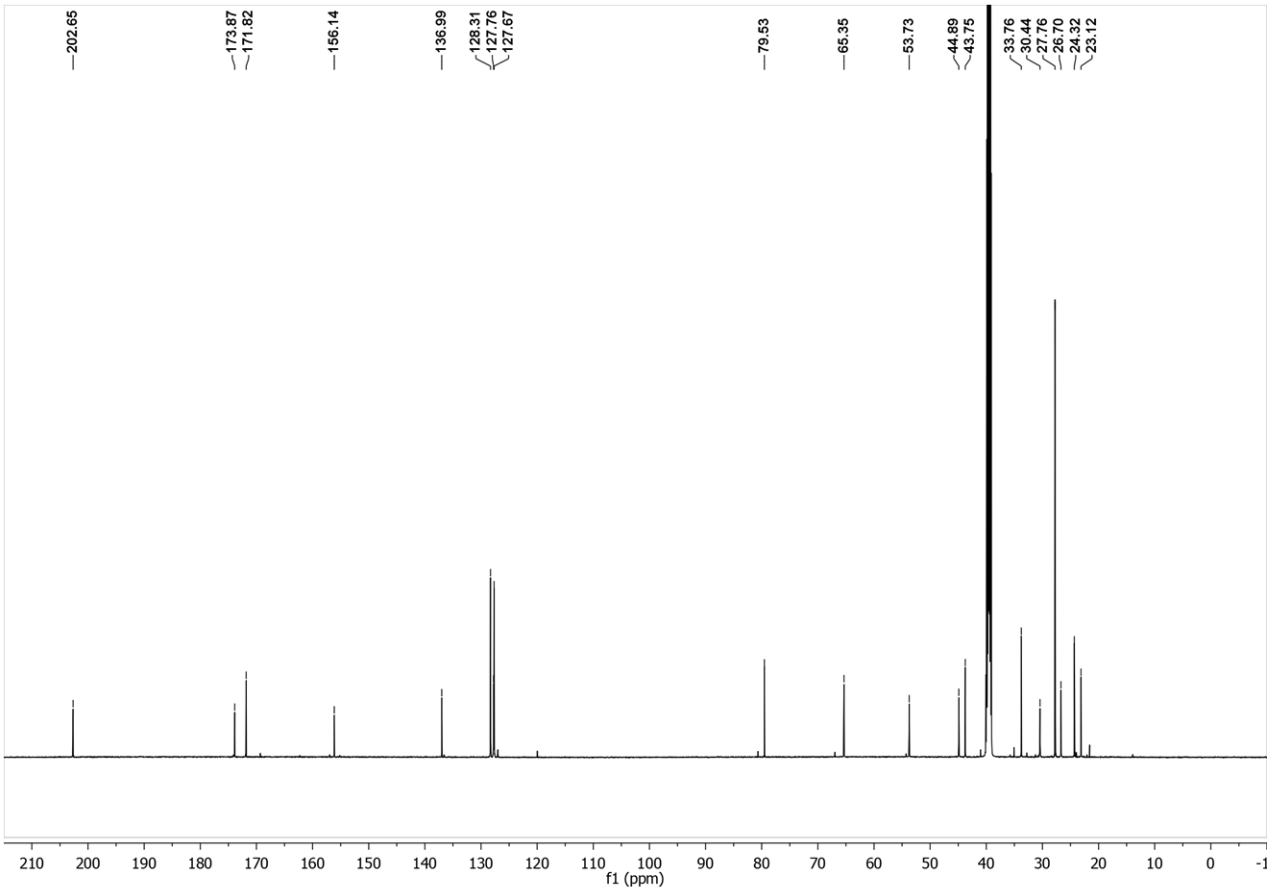
^{1}H and ^{13}C spectra of compound S23.



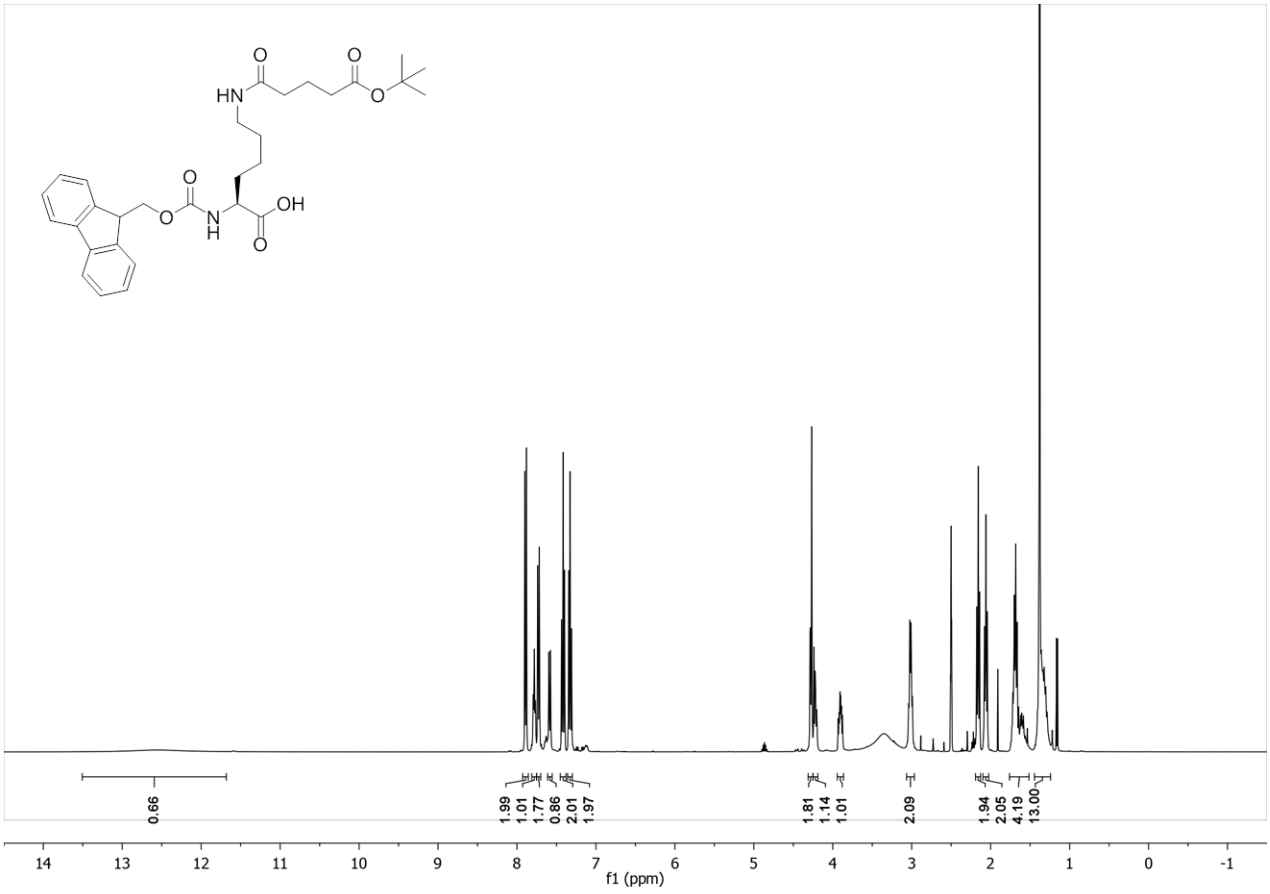


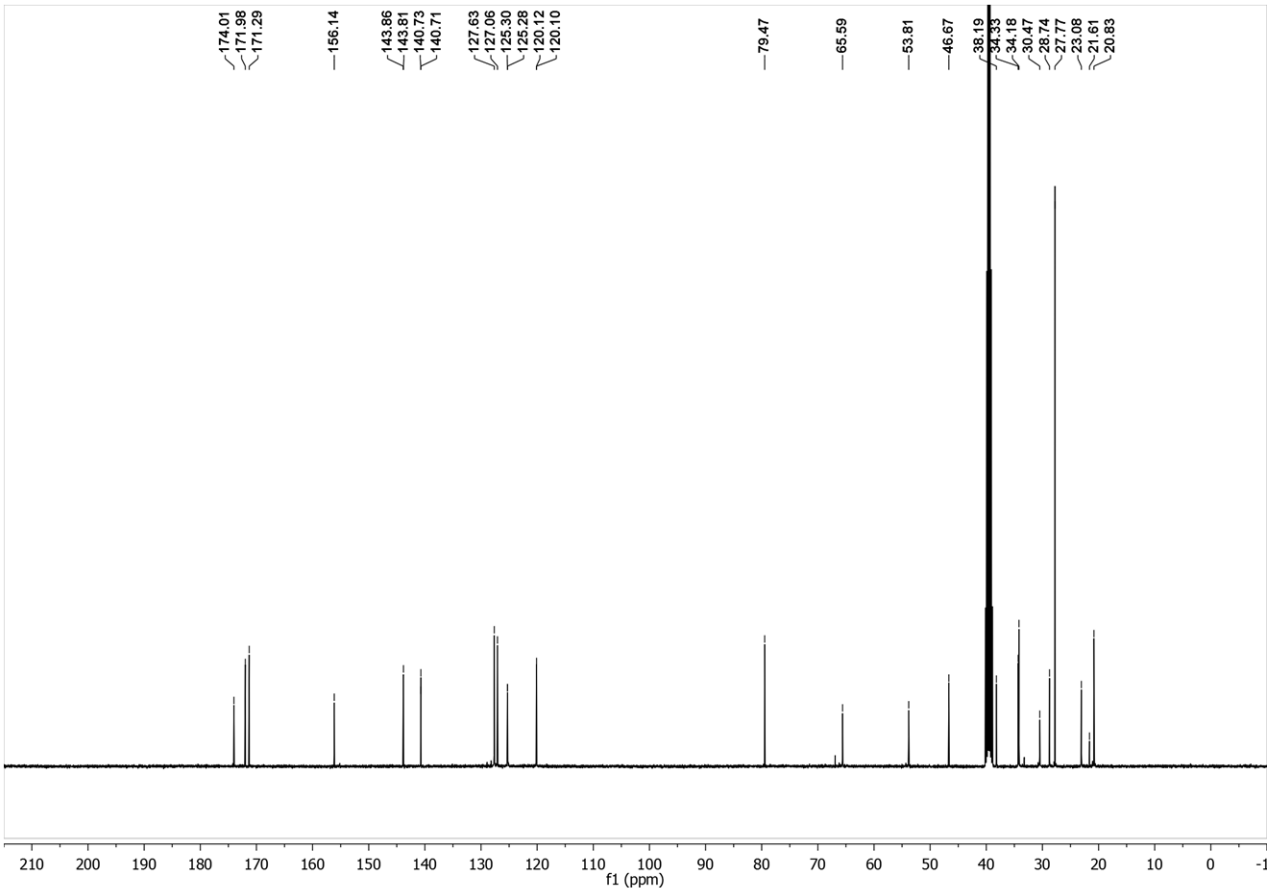
^{1}H and ^{13}C spectra of compound S24.



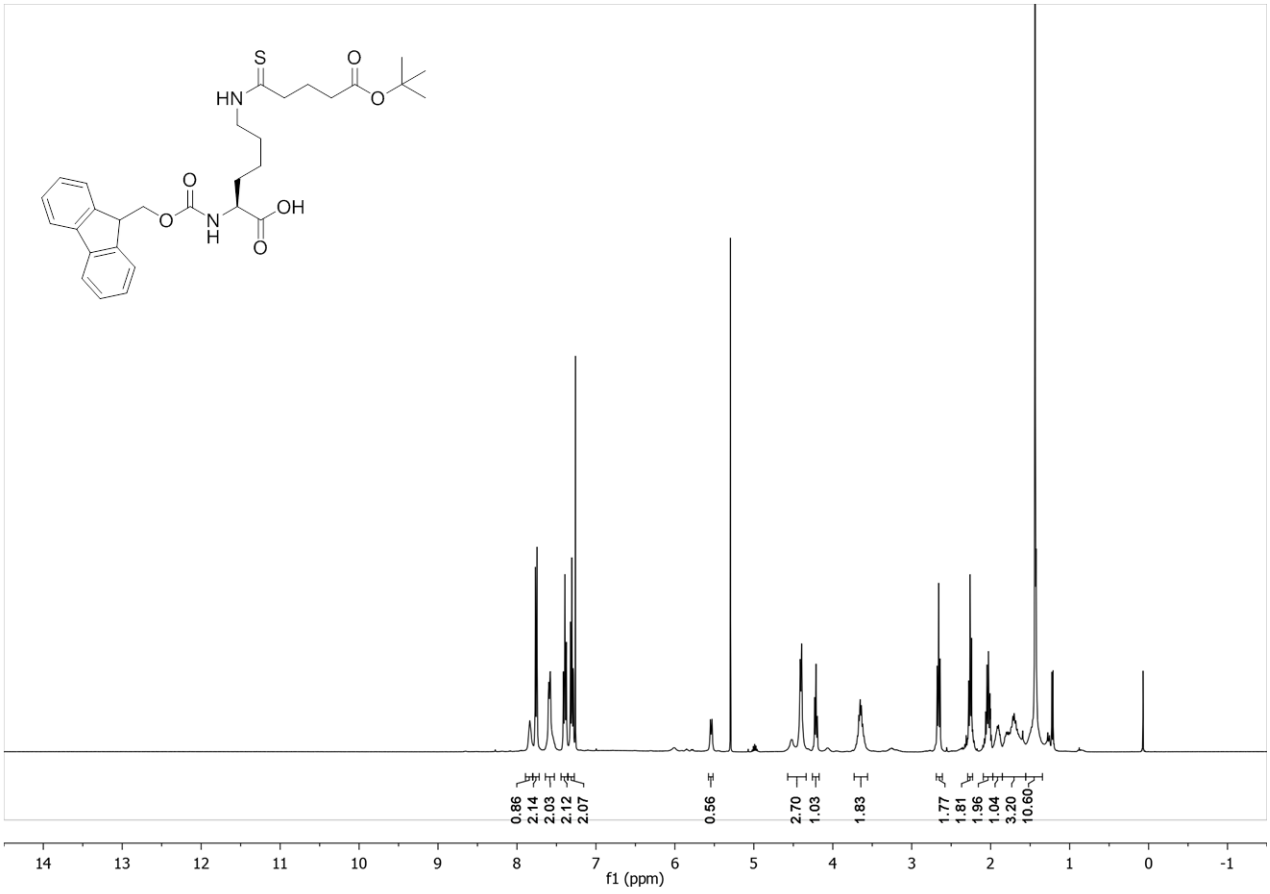


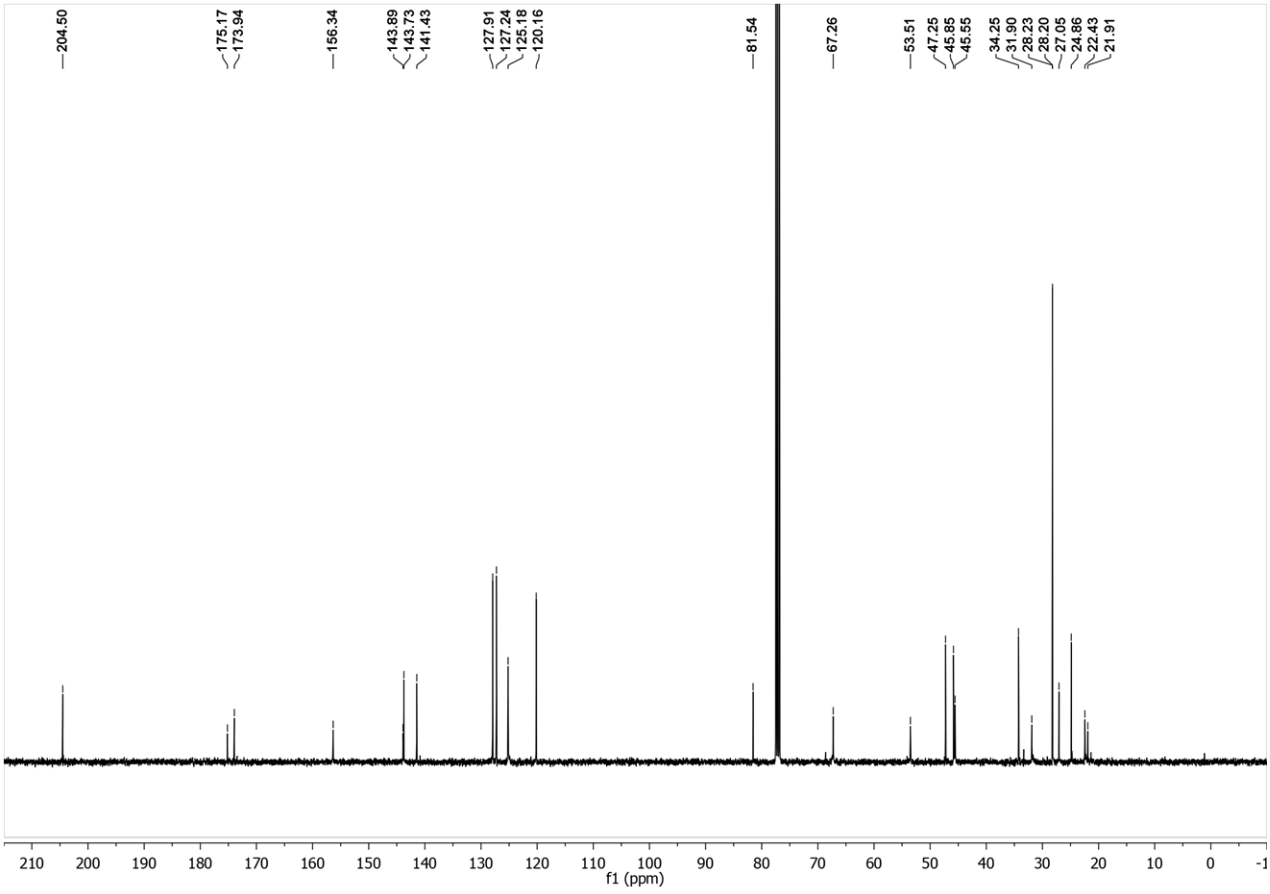
^{1}H and ^{13}C spectra of compound S25.



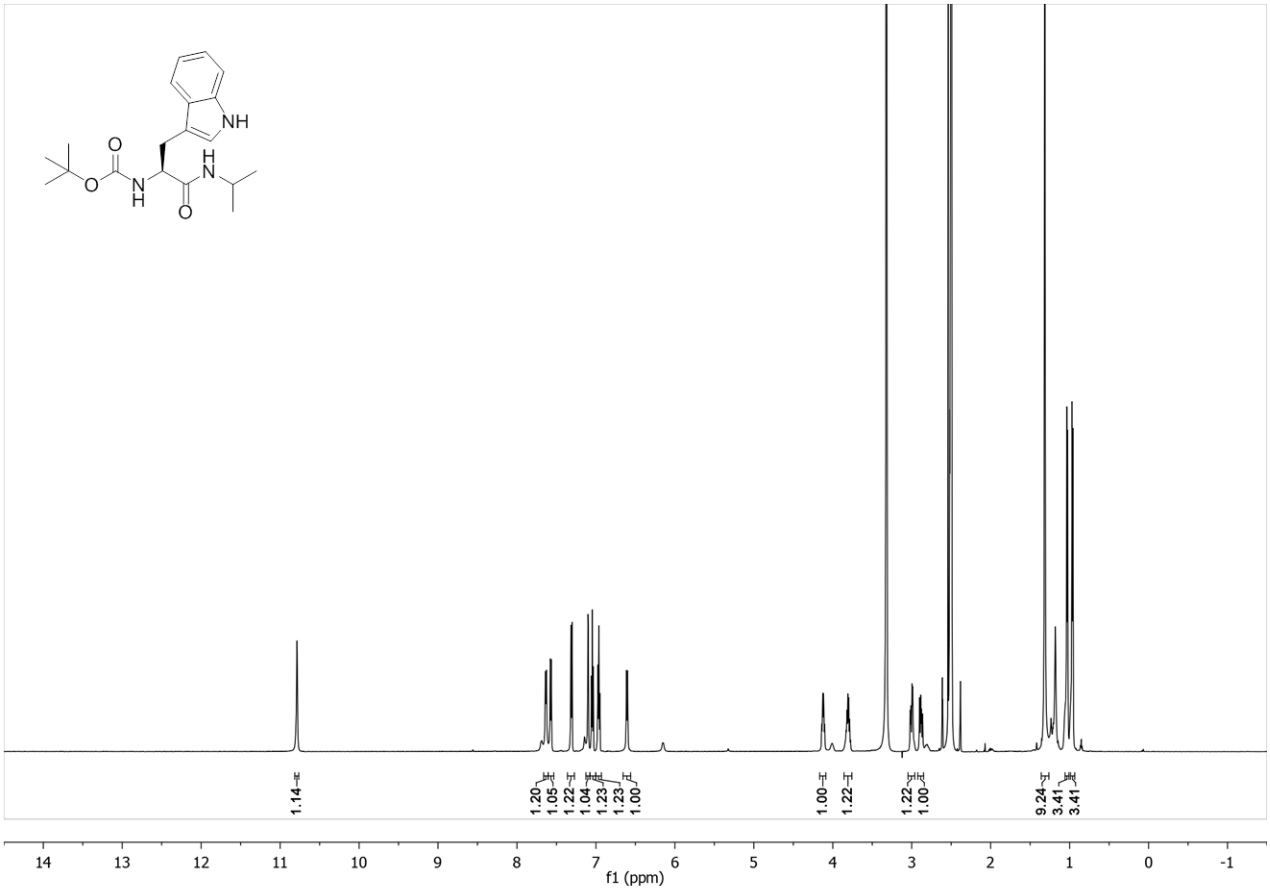


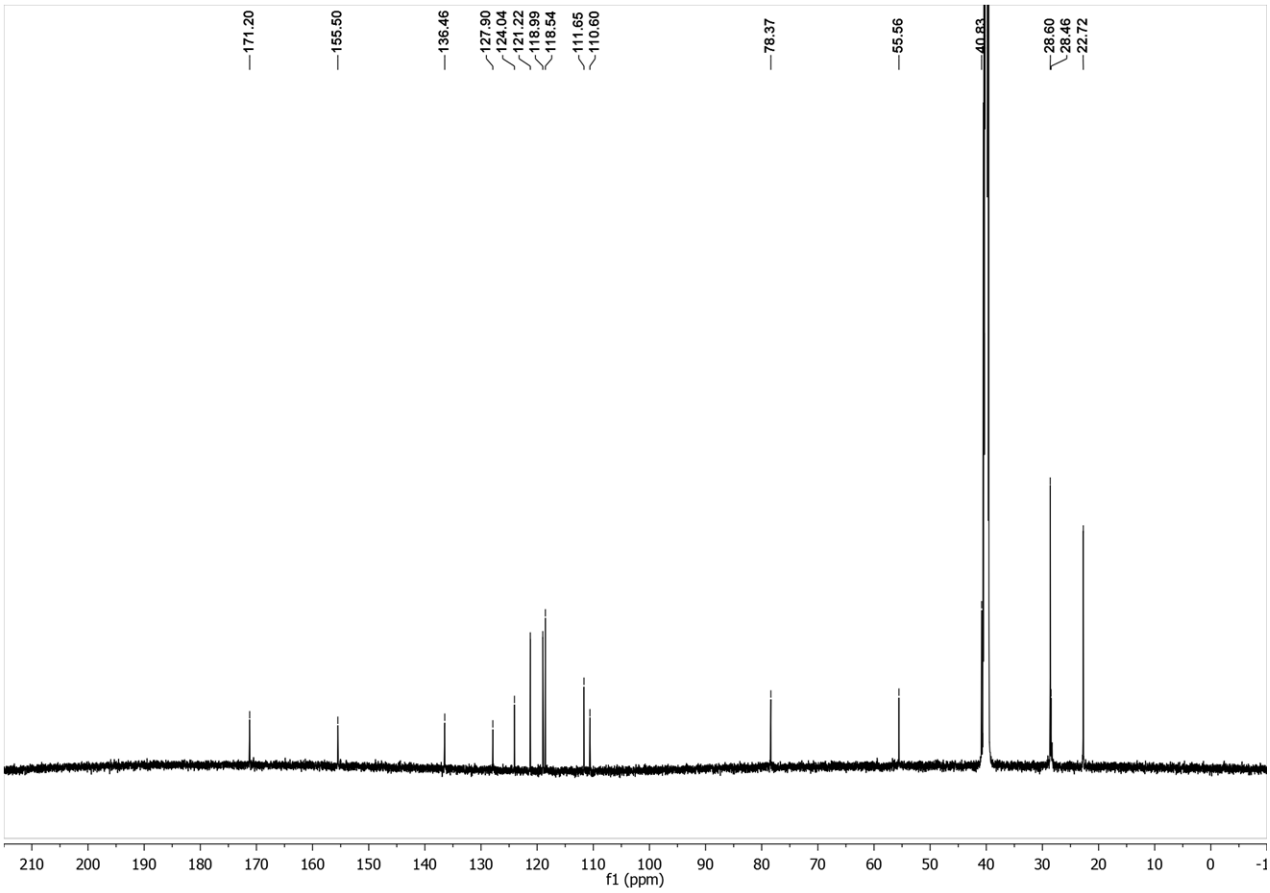
^{1}H and ^{13}C spectra of compound S26.



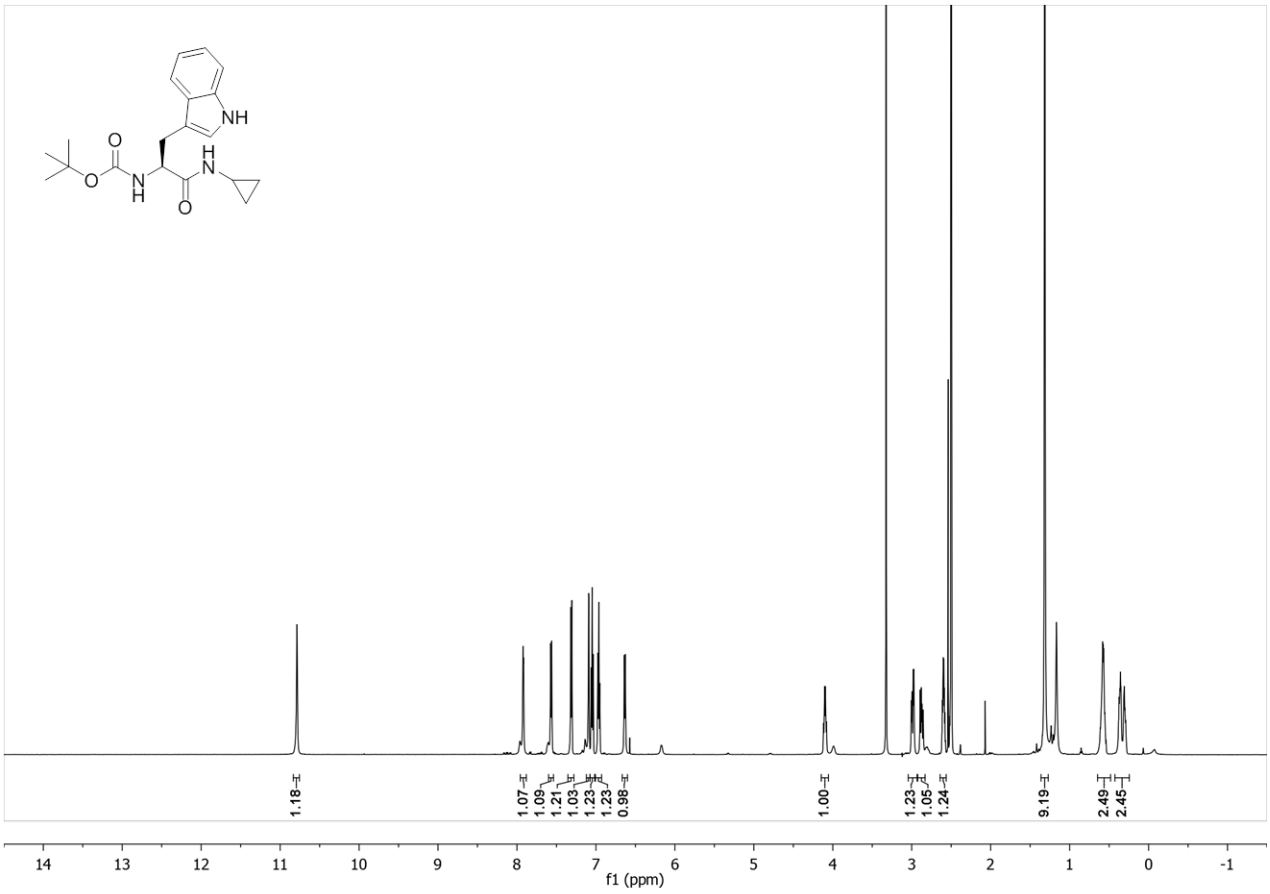


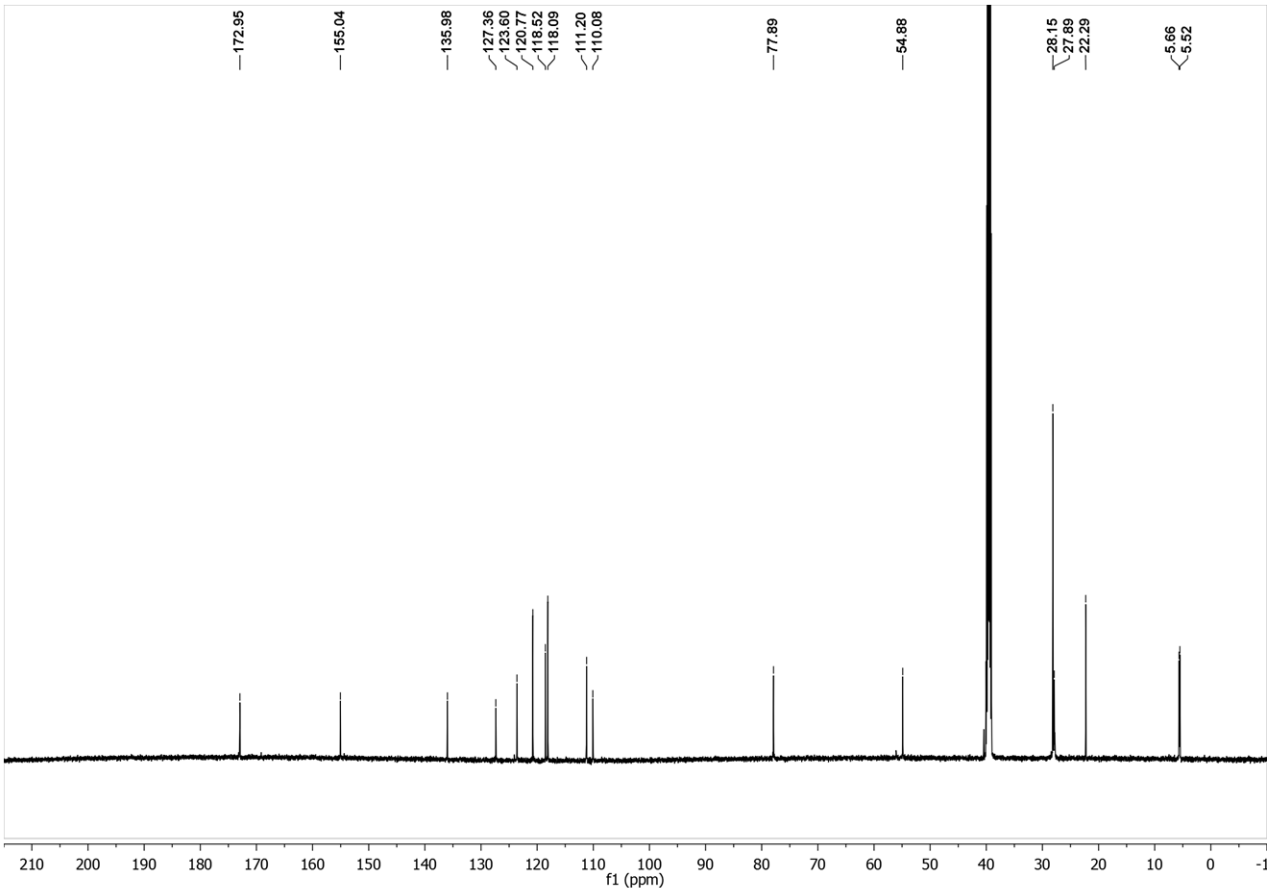
^{1}H and ^{13}C spectra of compound S27.



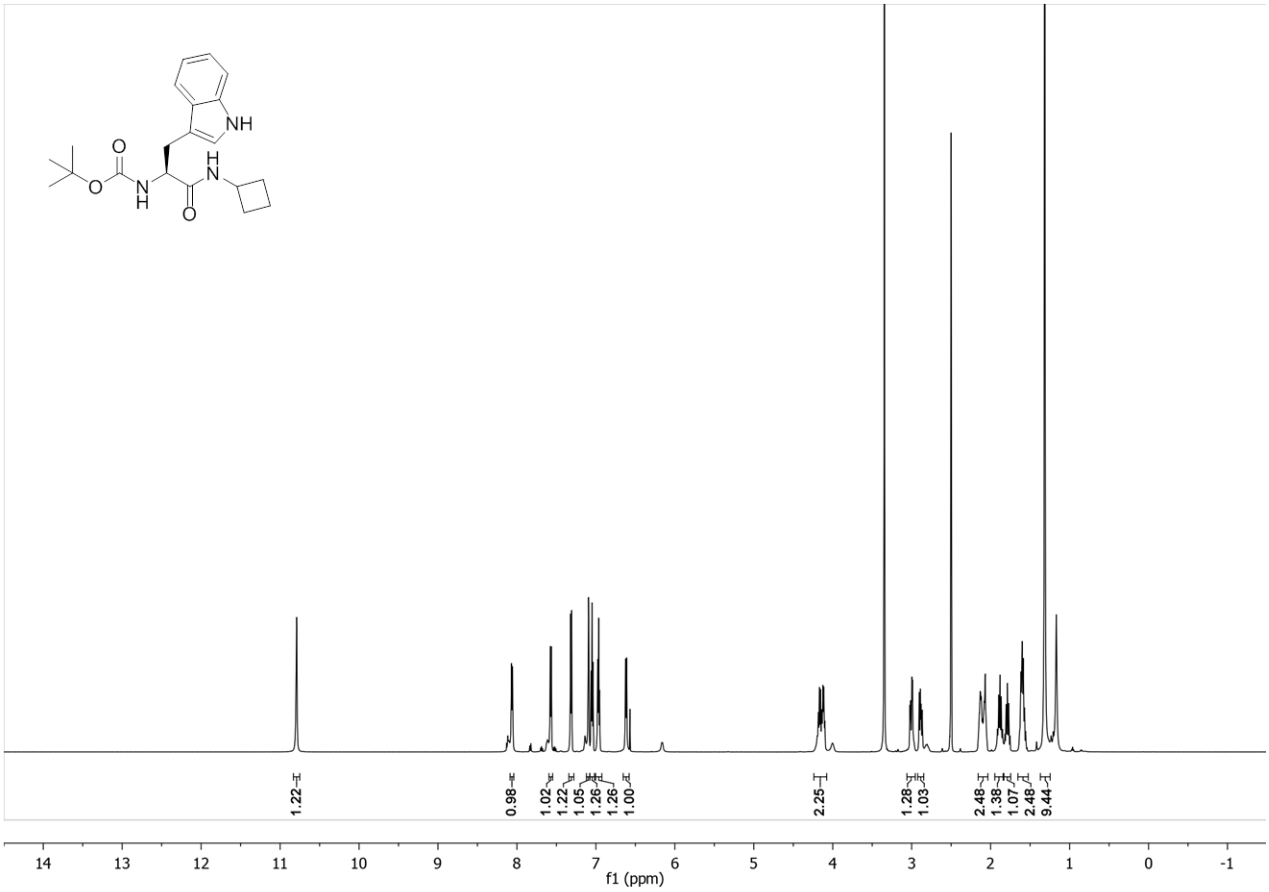


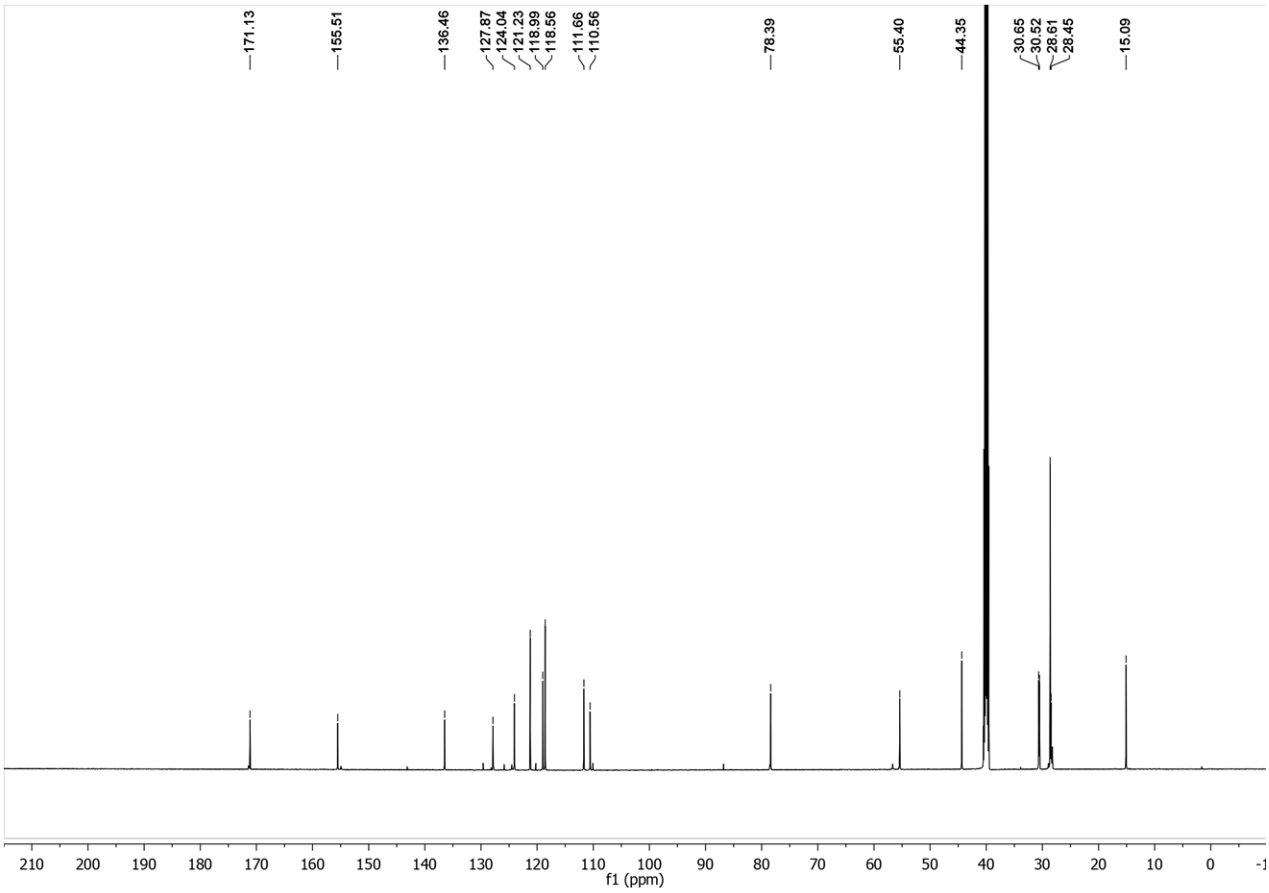
^{1}H and ^{13}C spectra of compound S28.



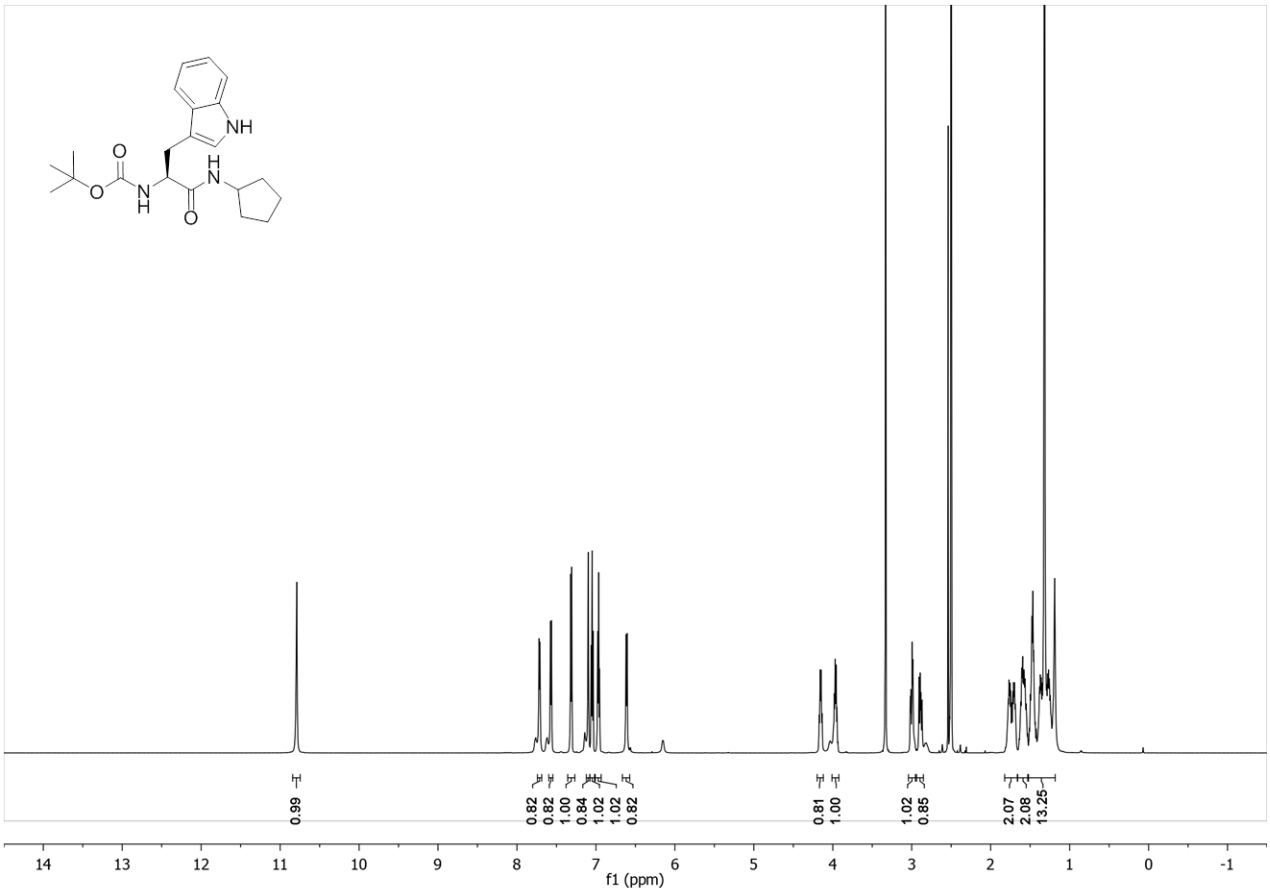


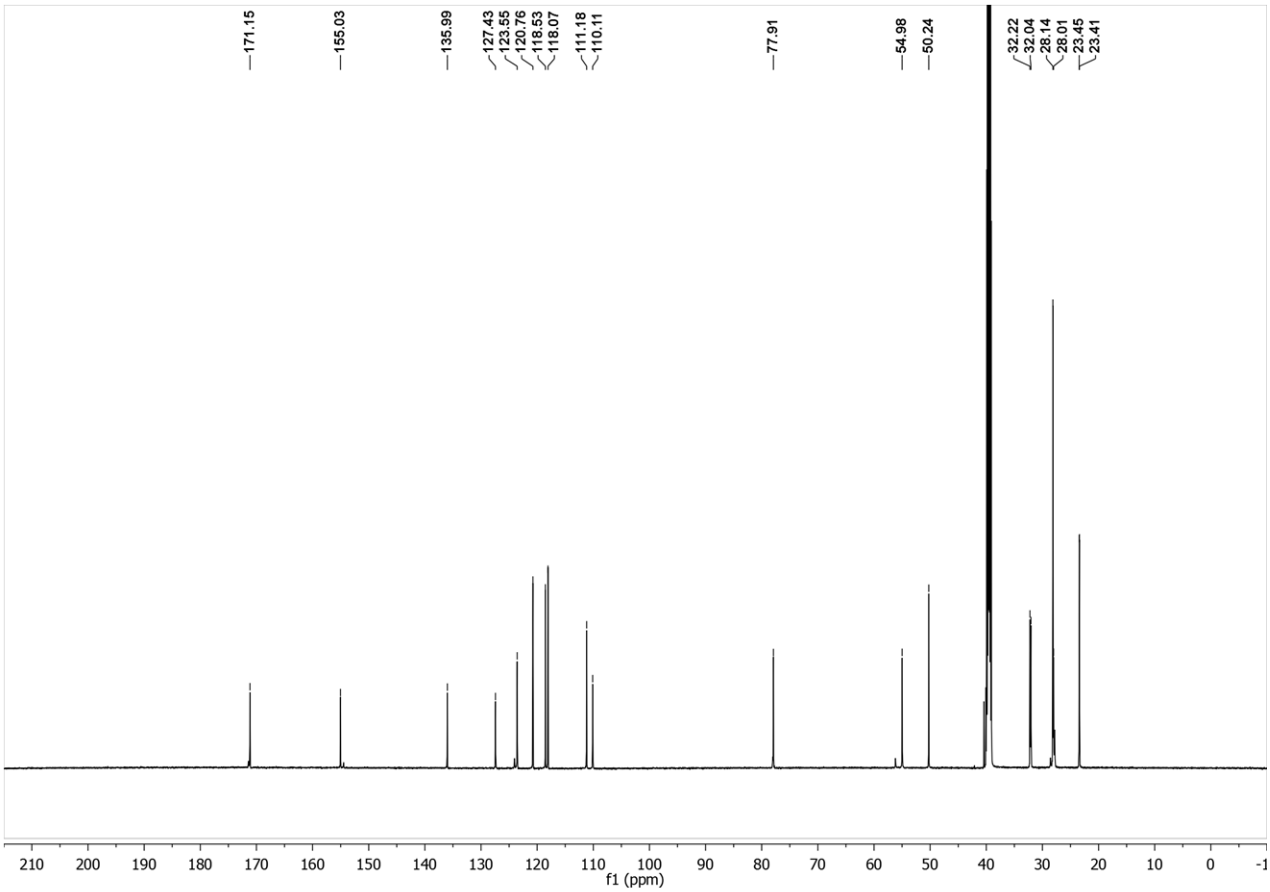
^{1}H and ^{13}C spectra of compound S29.



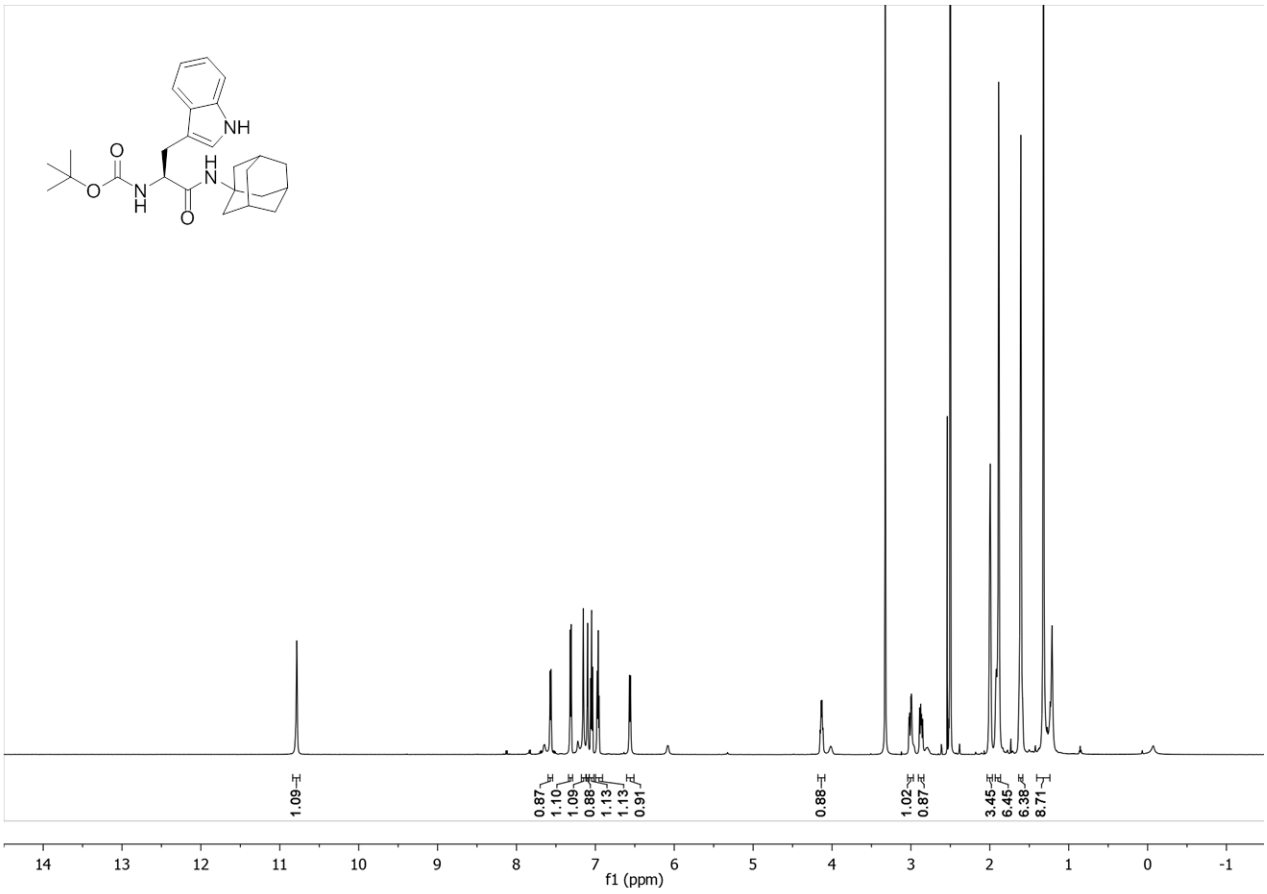


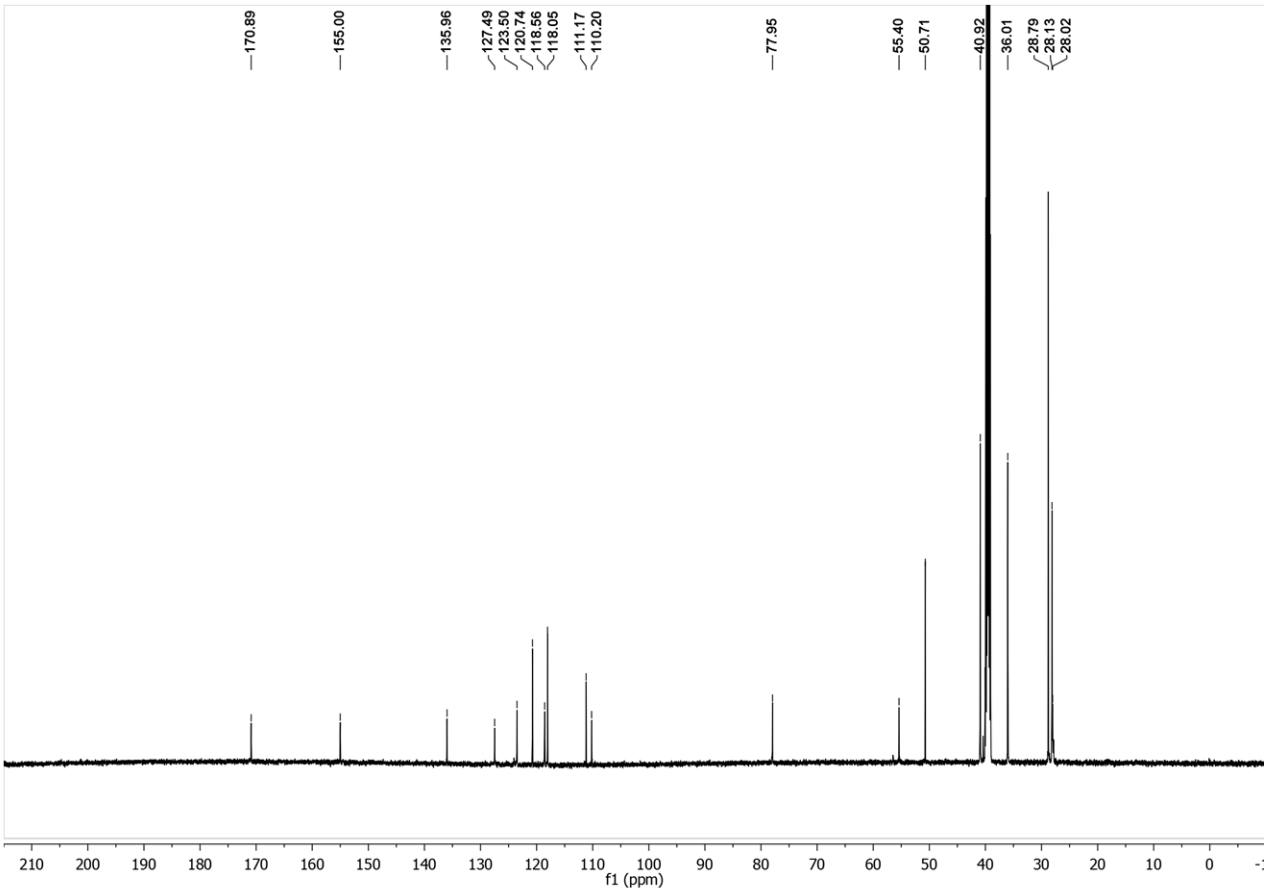
^{1}H and ^{13}C spectra of compound S30.



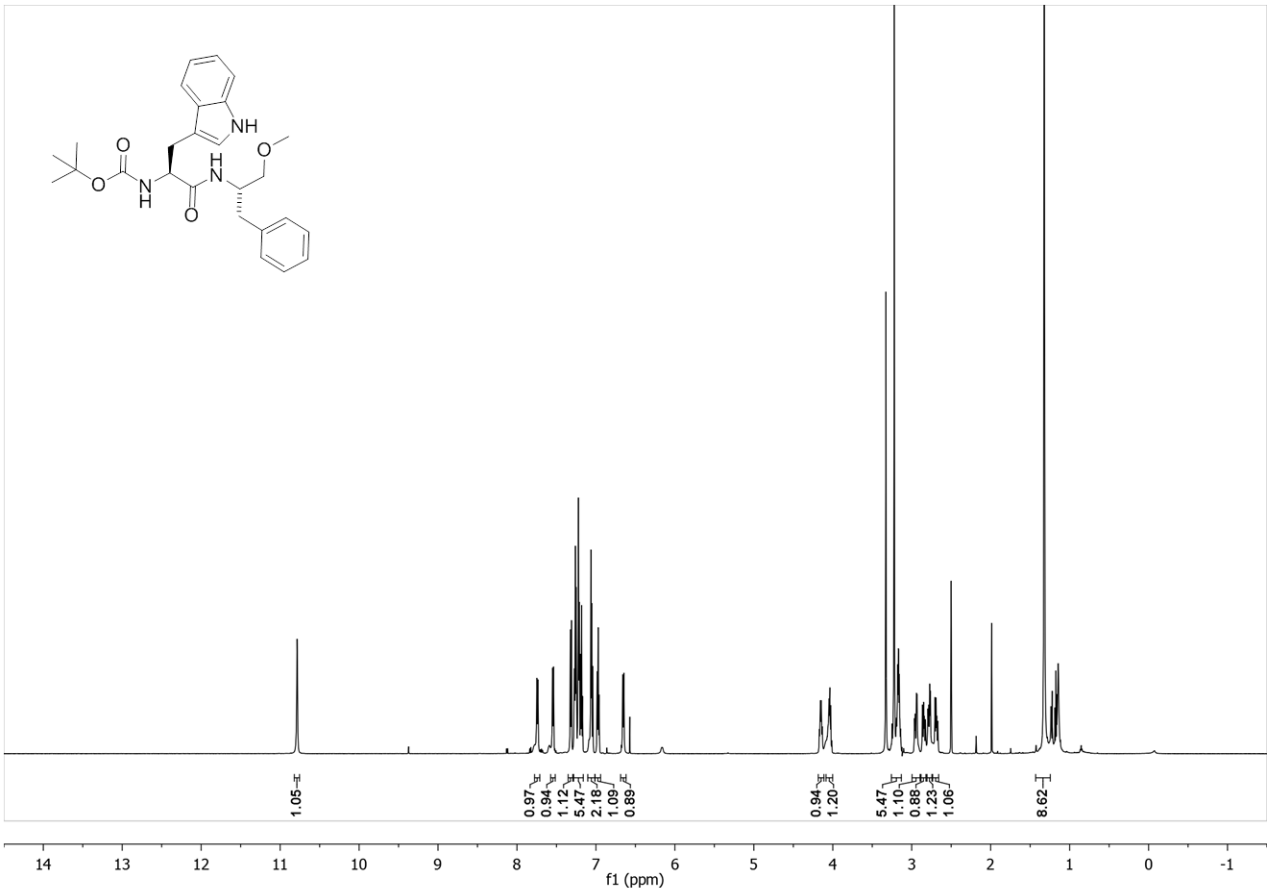


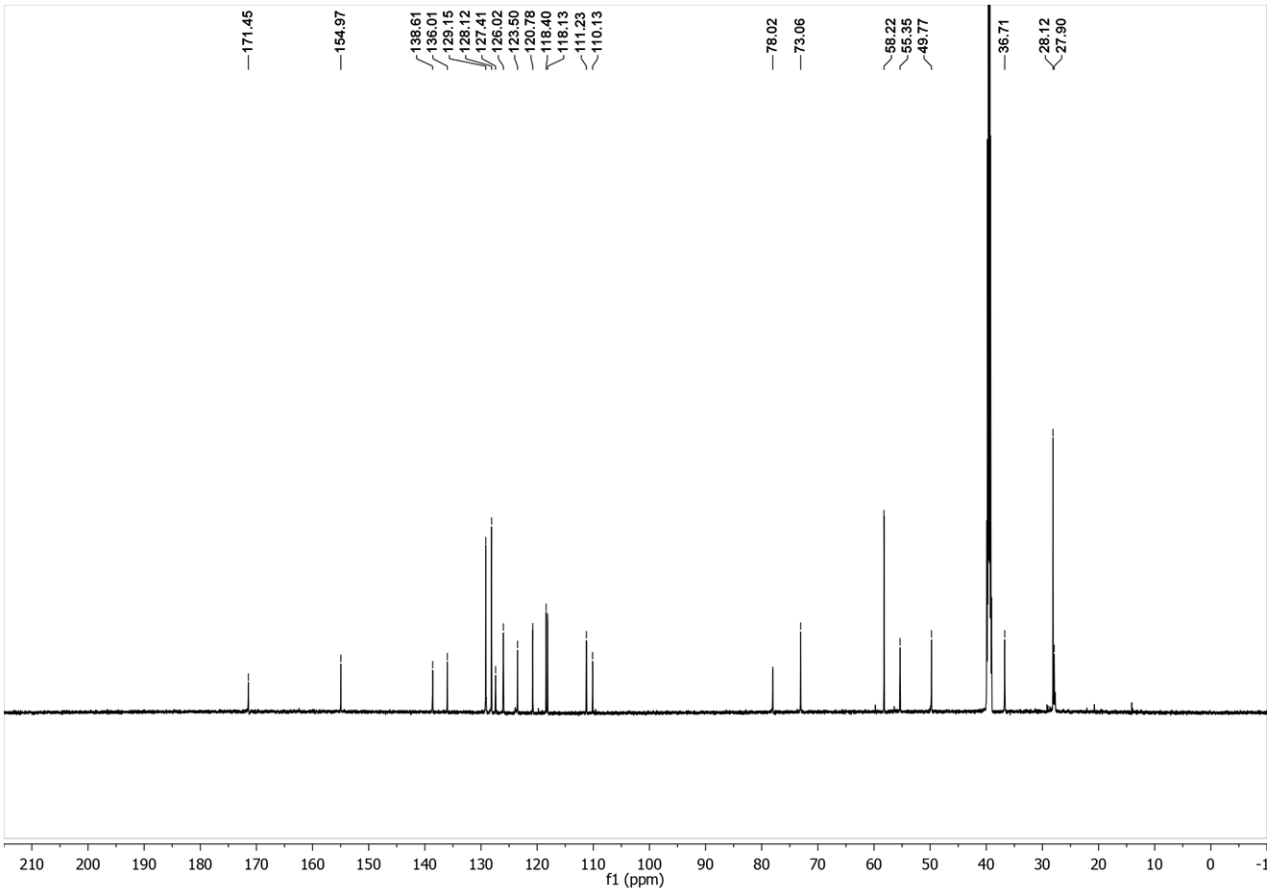
^{1}H and ^{13}C spectra of compound S31.



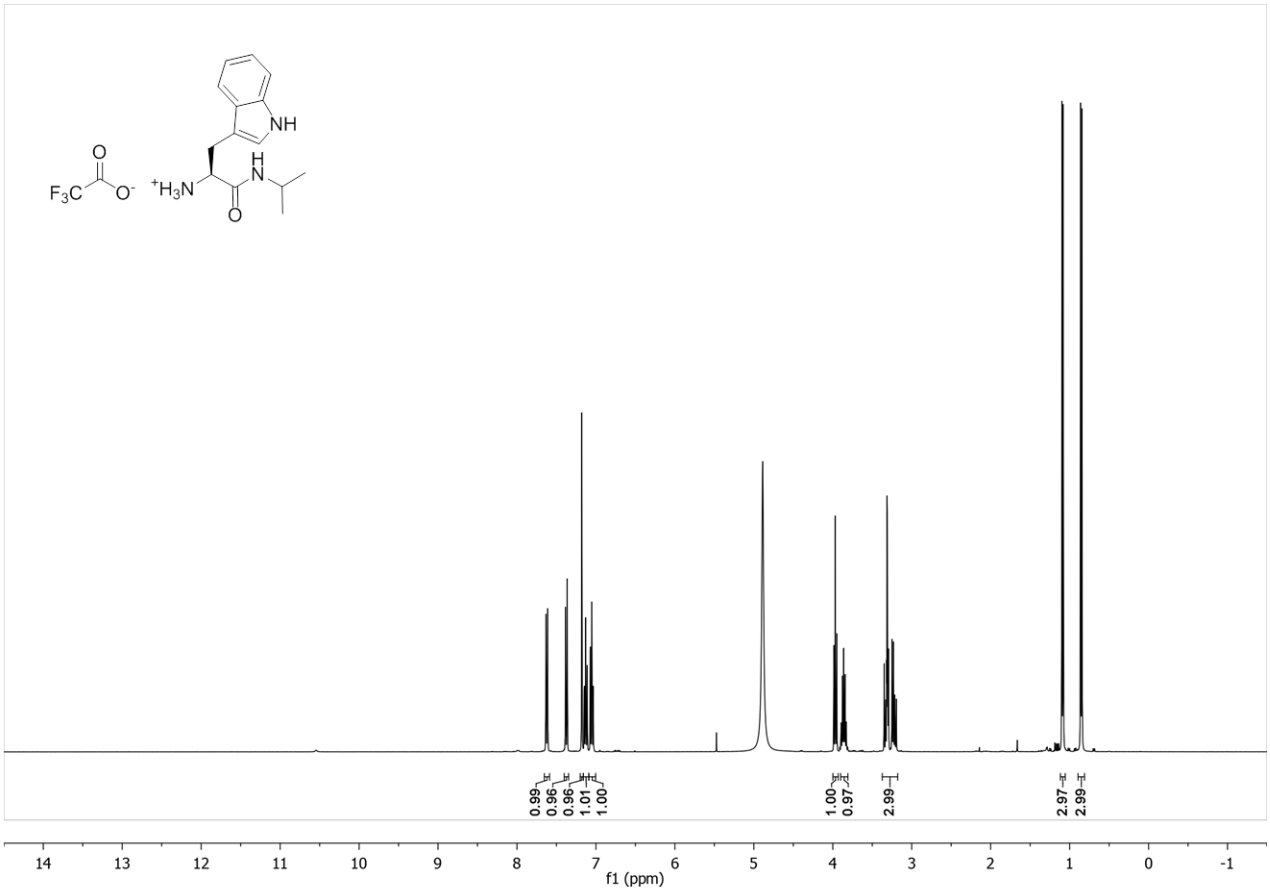


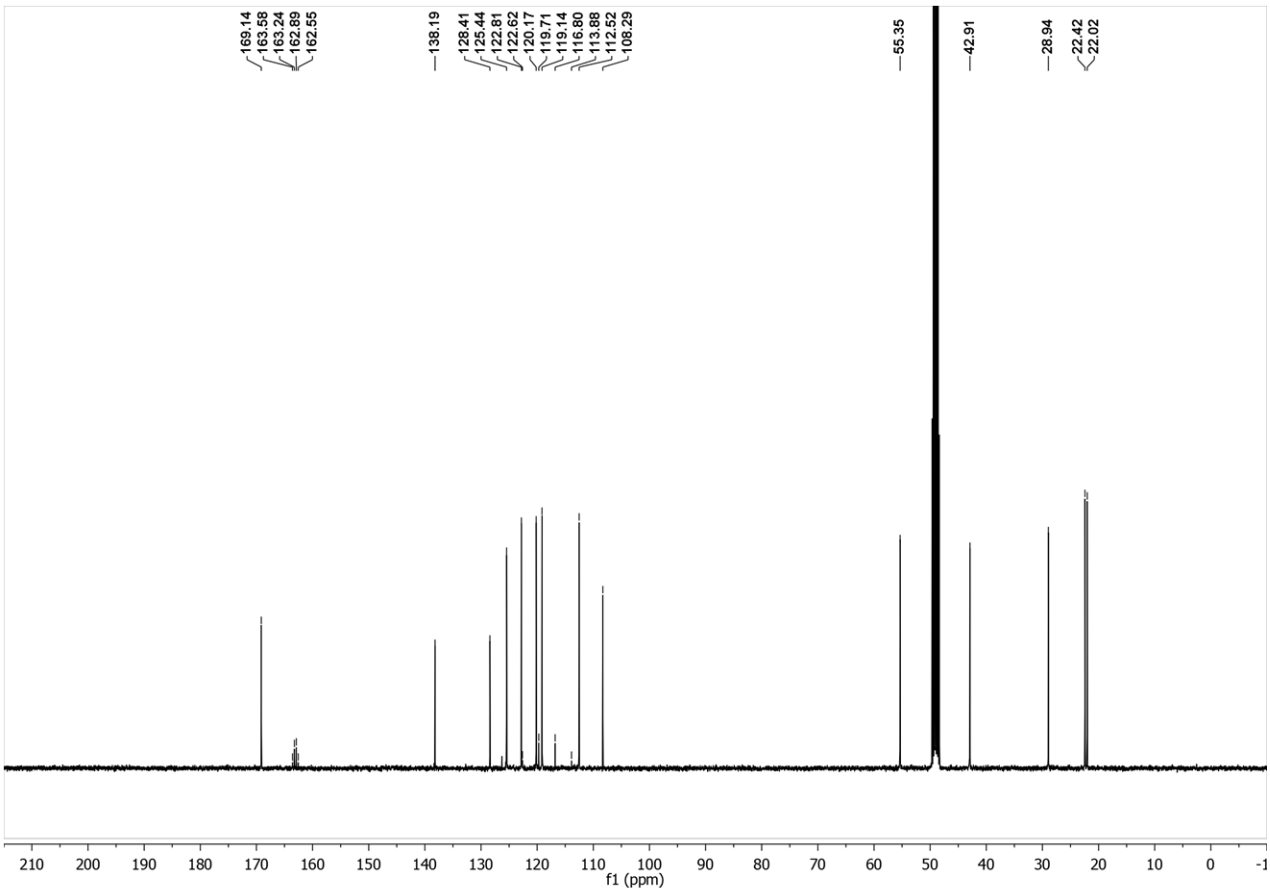
^{1}H and ^{13}C spectra of compound S32.



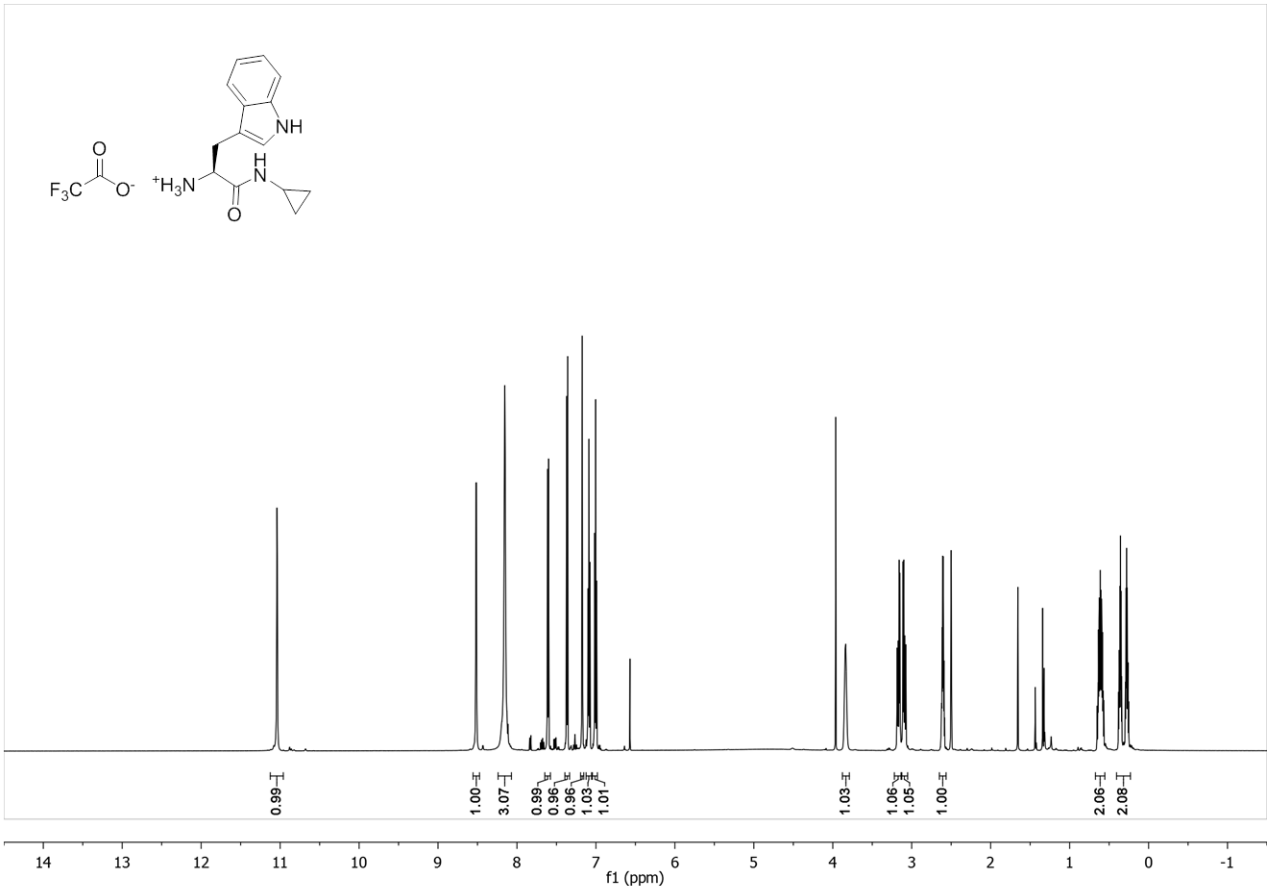


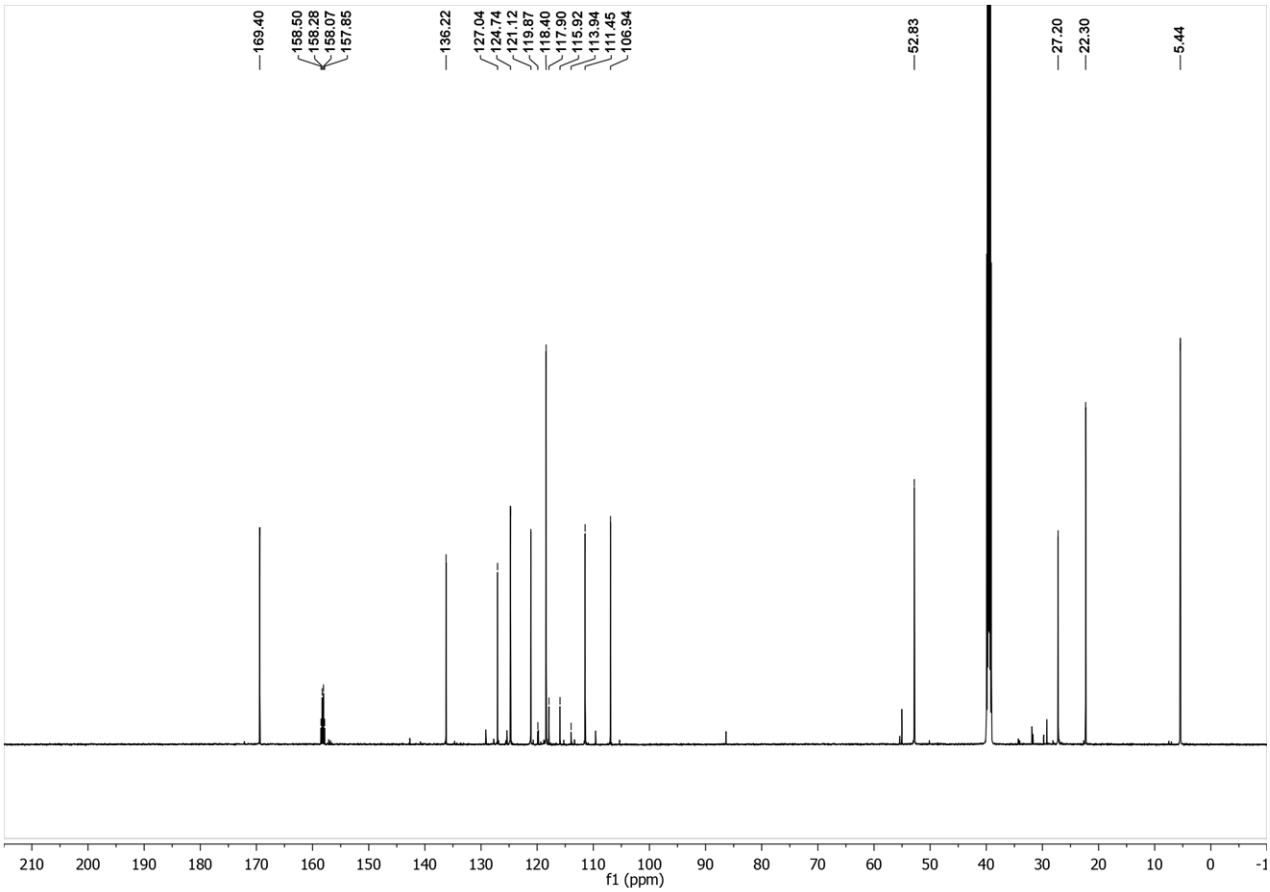
^{1}H and ^{13}C spectra of compound S33.



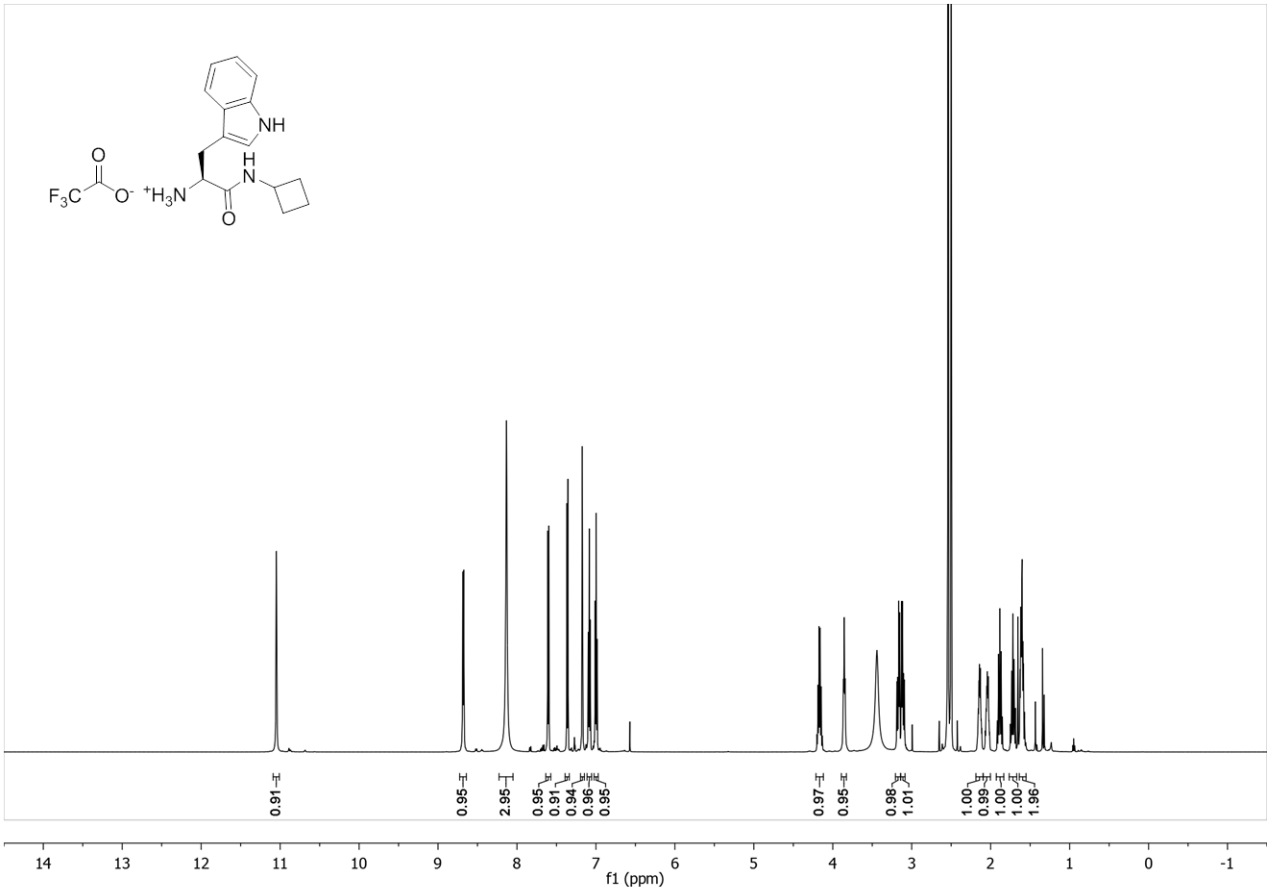


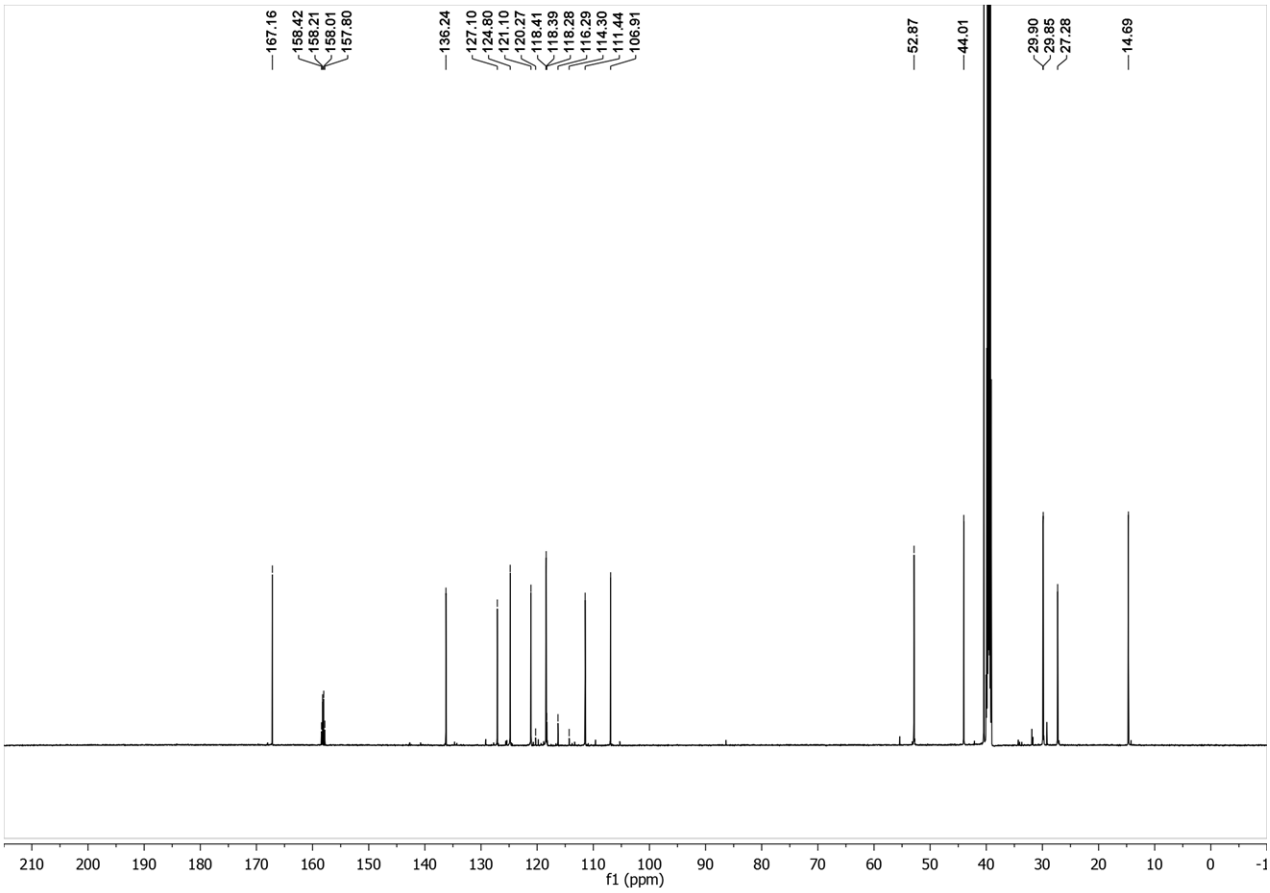
^{1}H and ^{13}C spectra of compound S34.



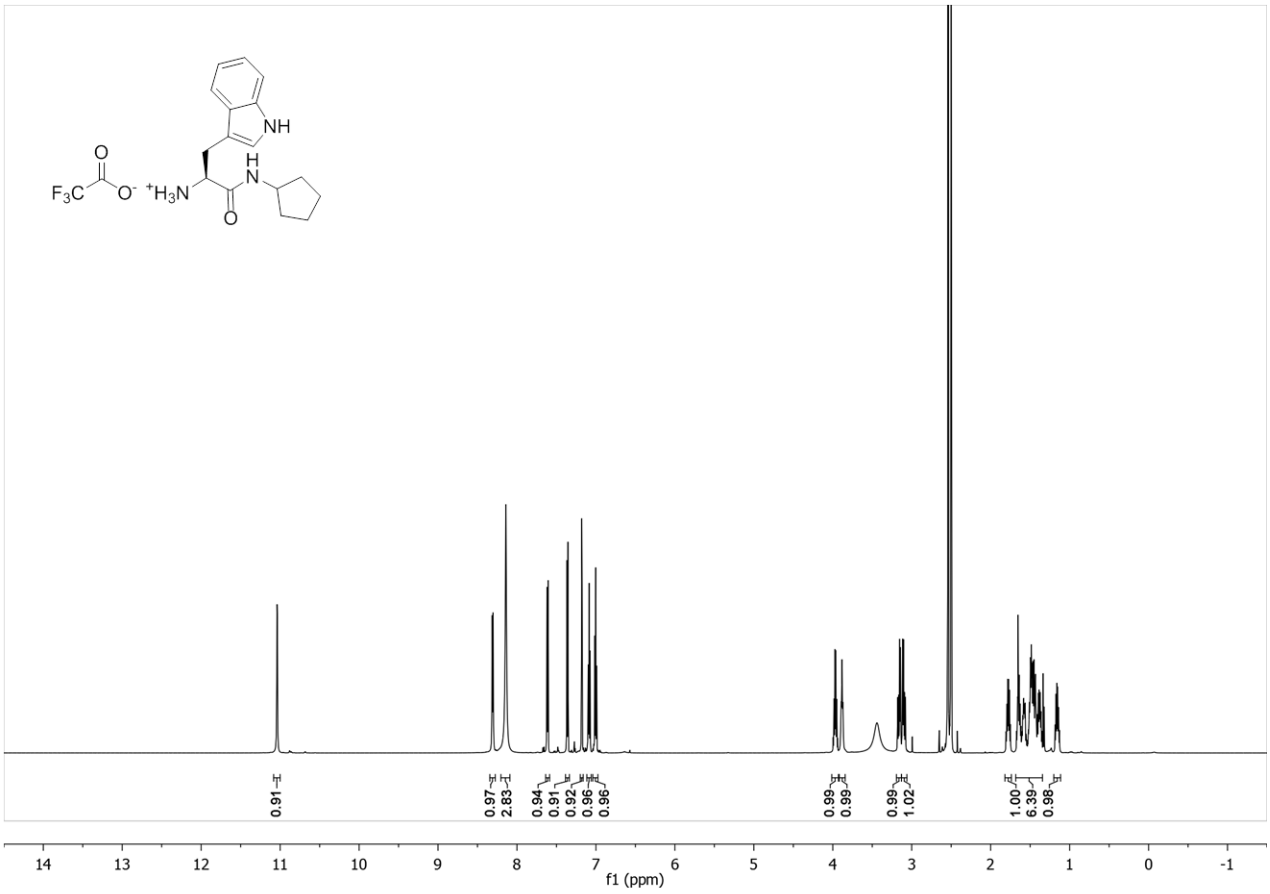


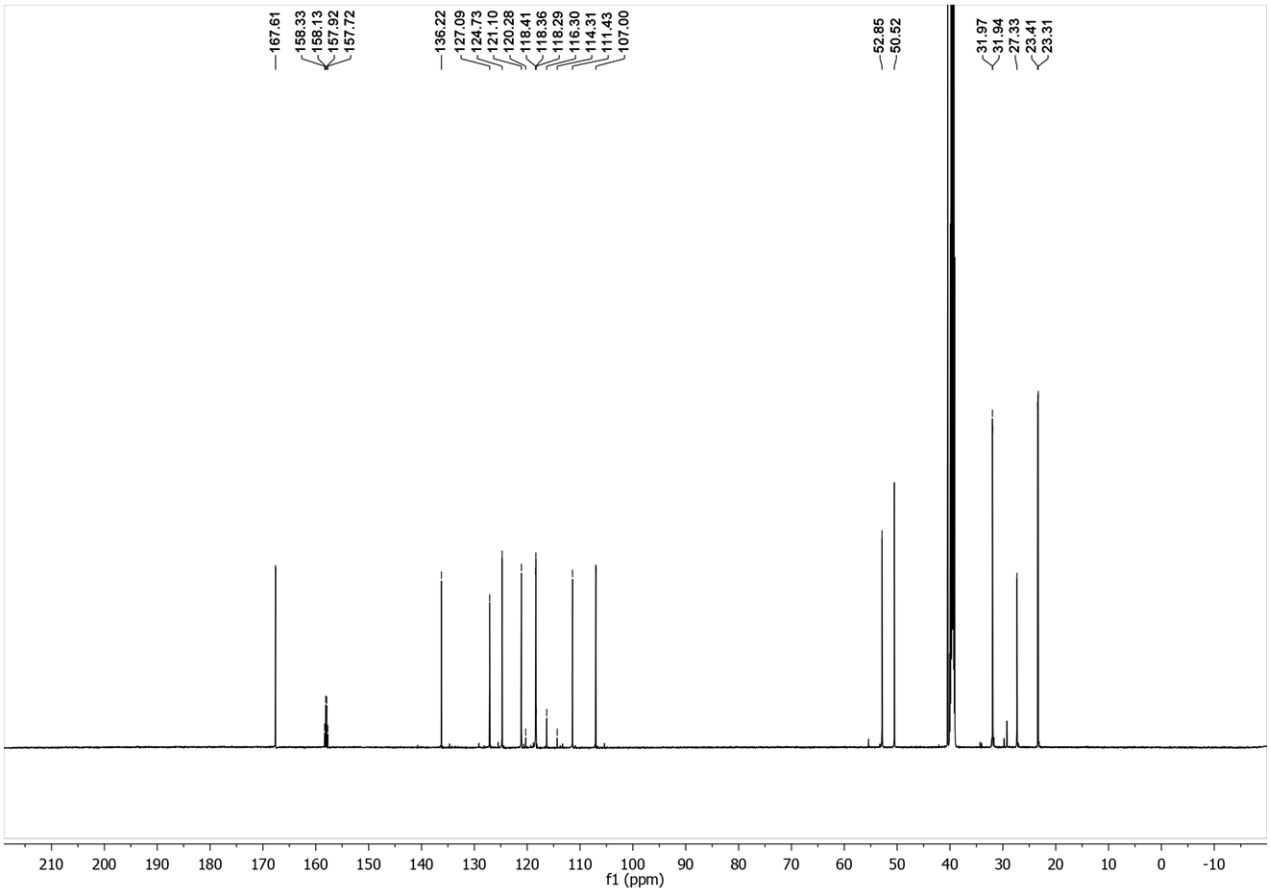
^{1}H and ^{13}C spectra of compound S35.



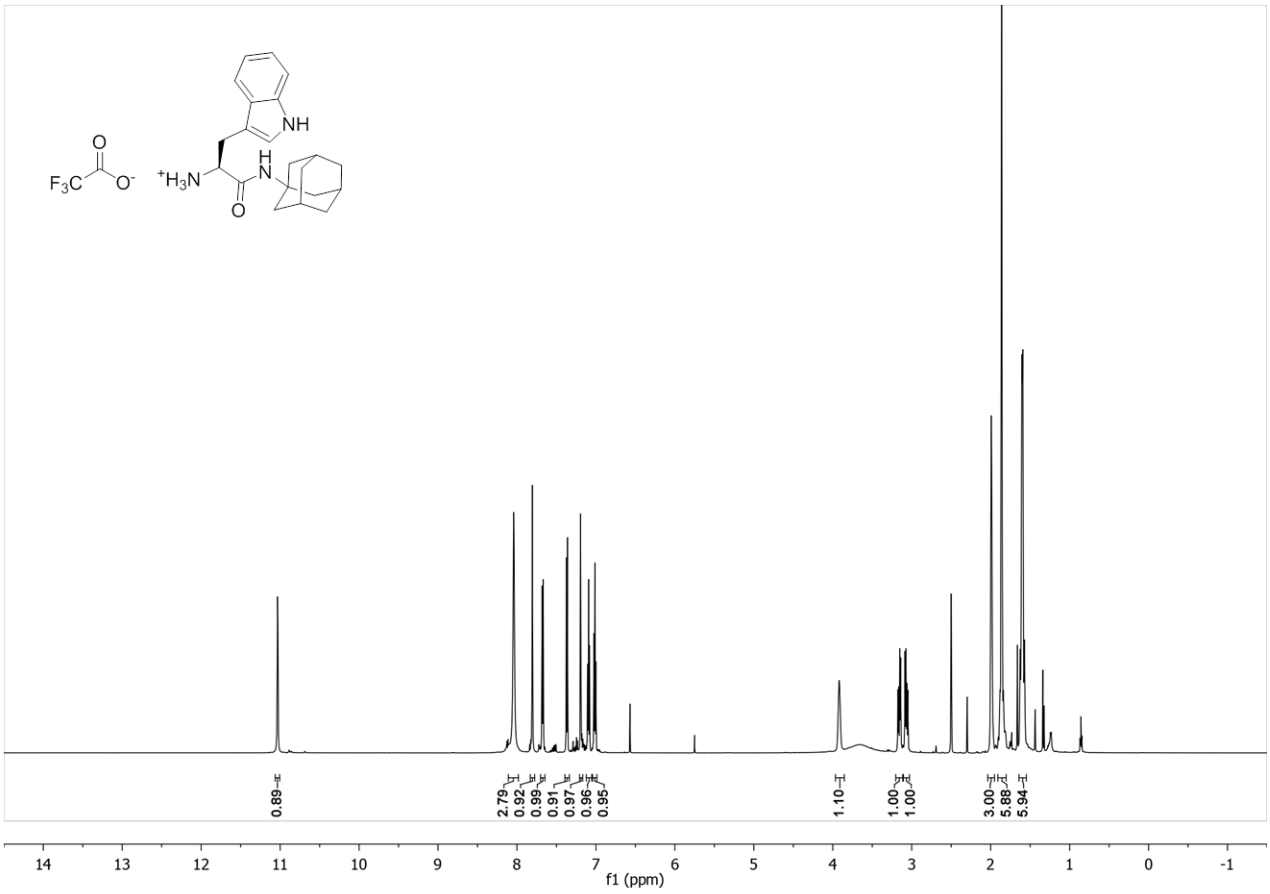


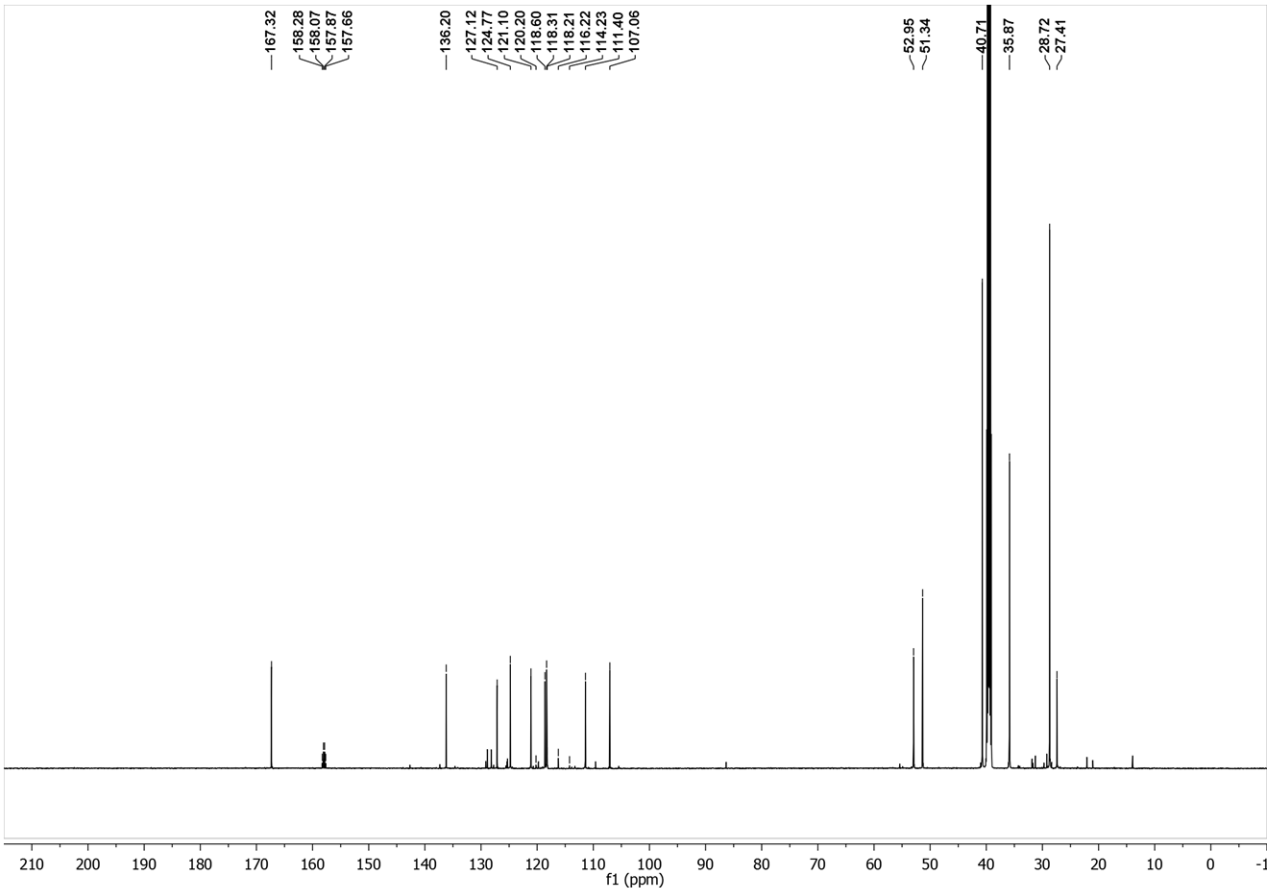
^{1}H and ^{13}C spectra of compound S36.



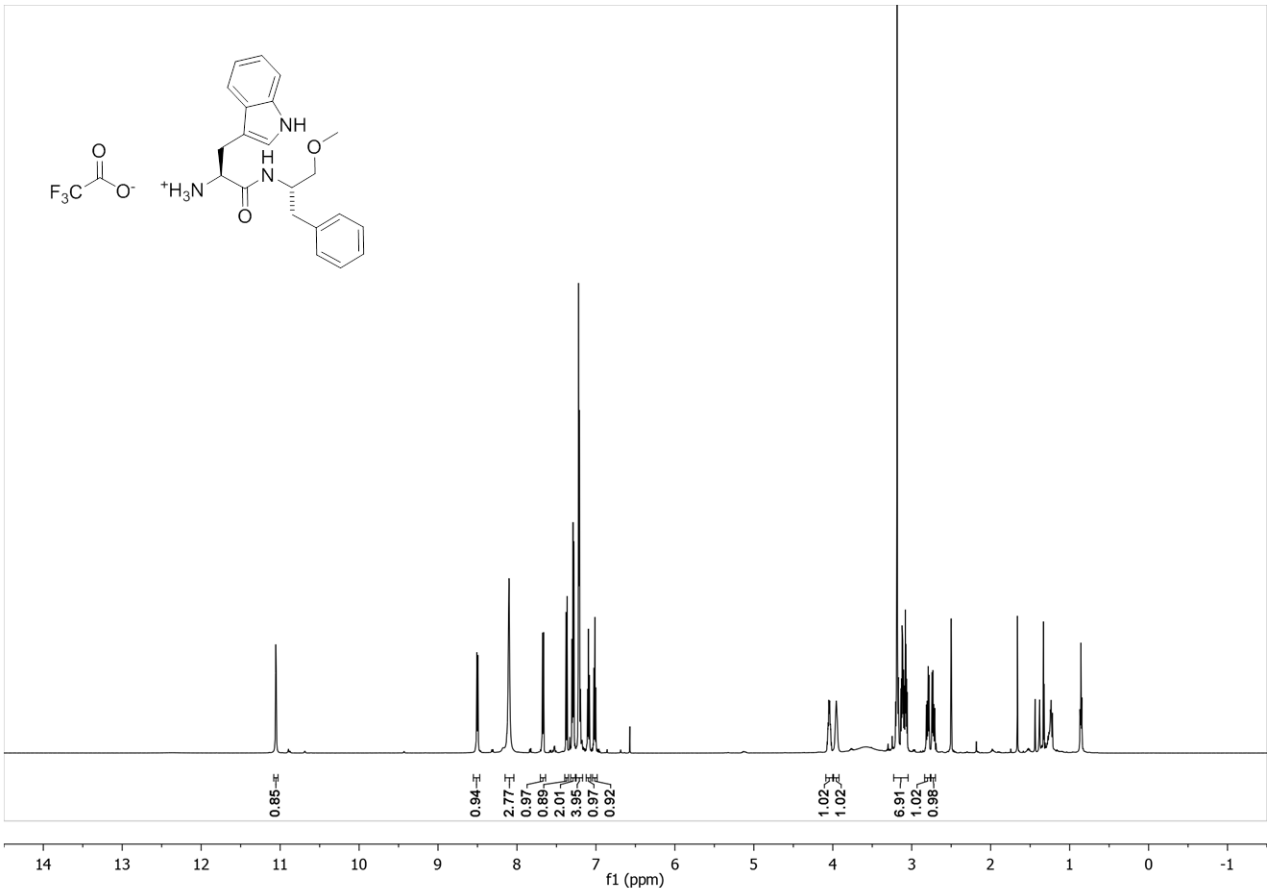


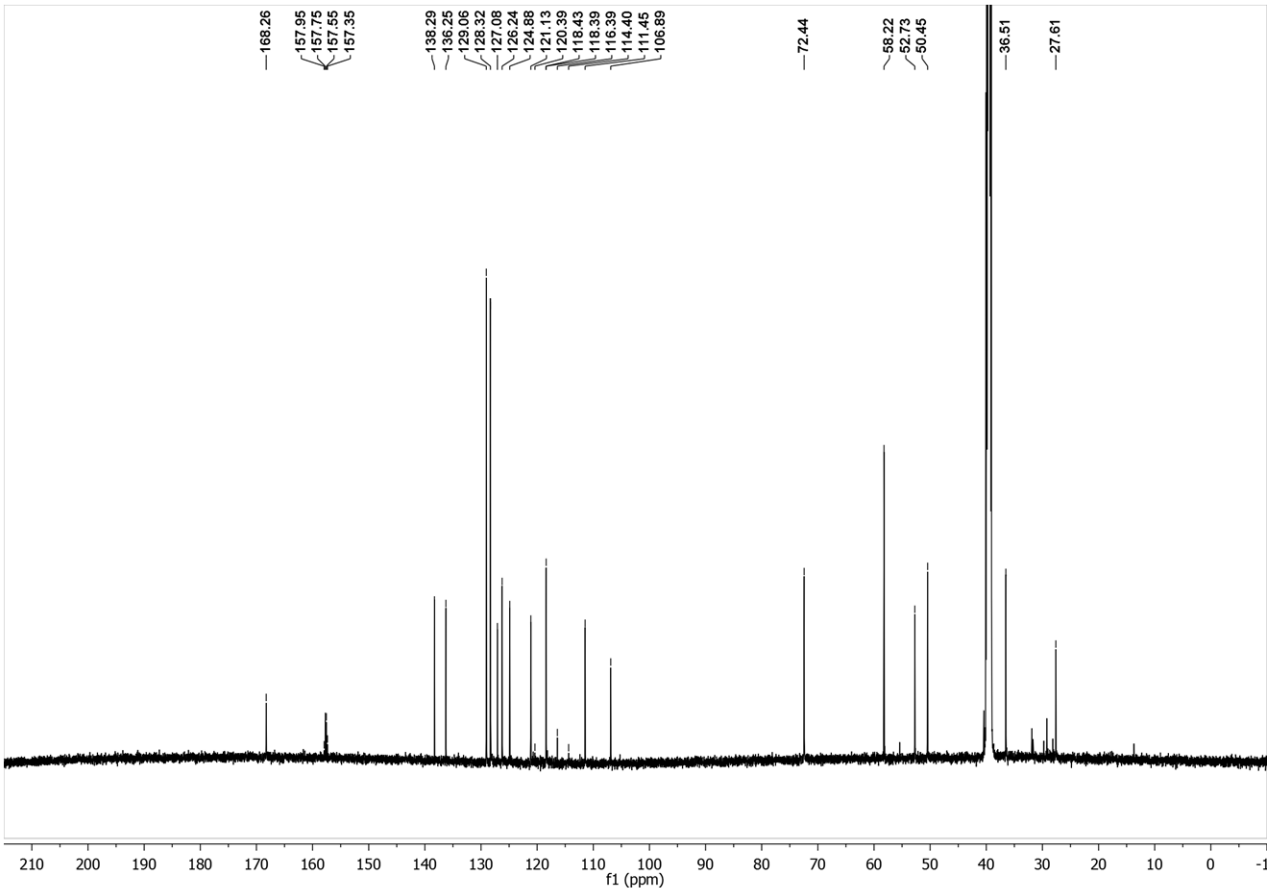
^{1}H and ^{13}C spectra of compound S37.



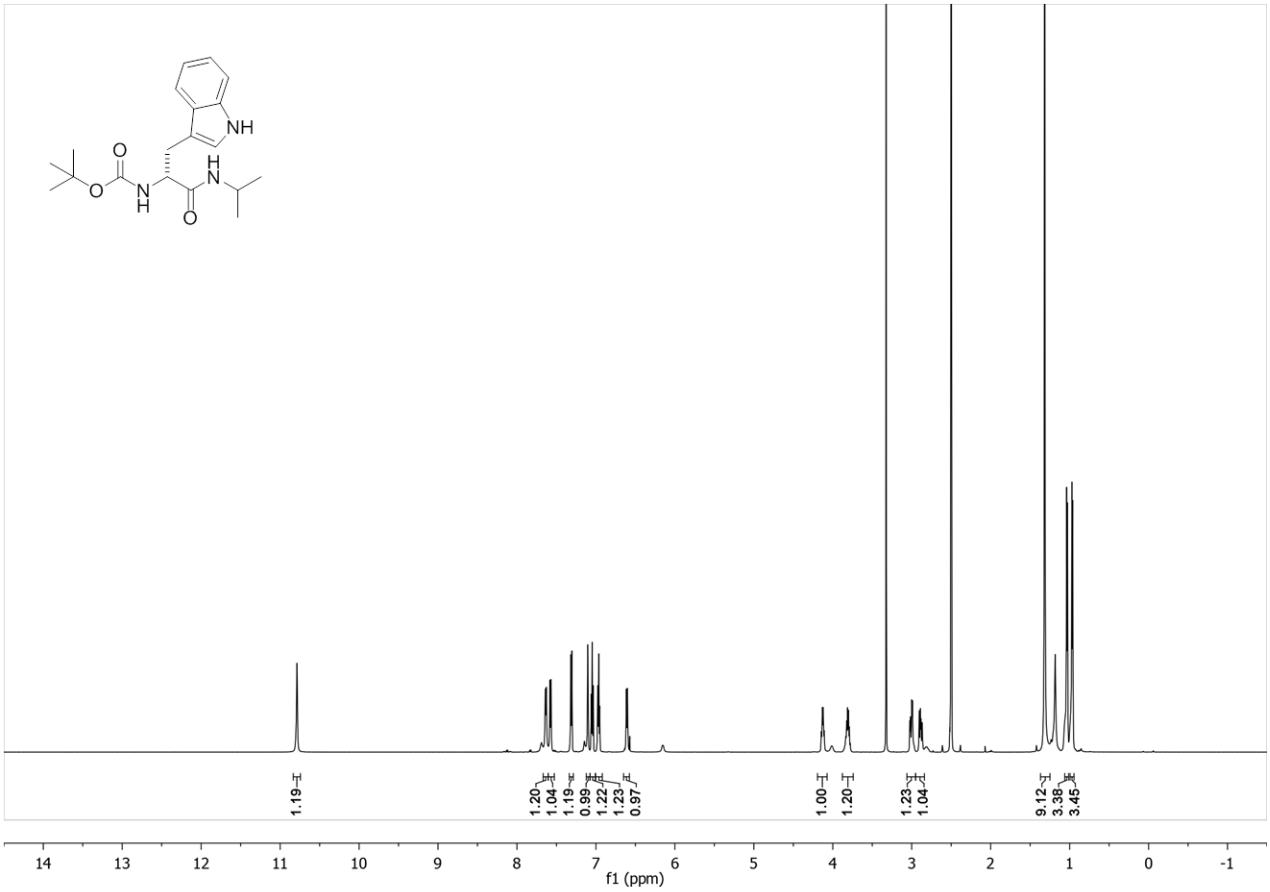


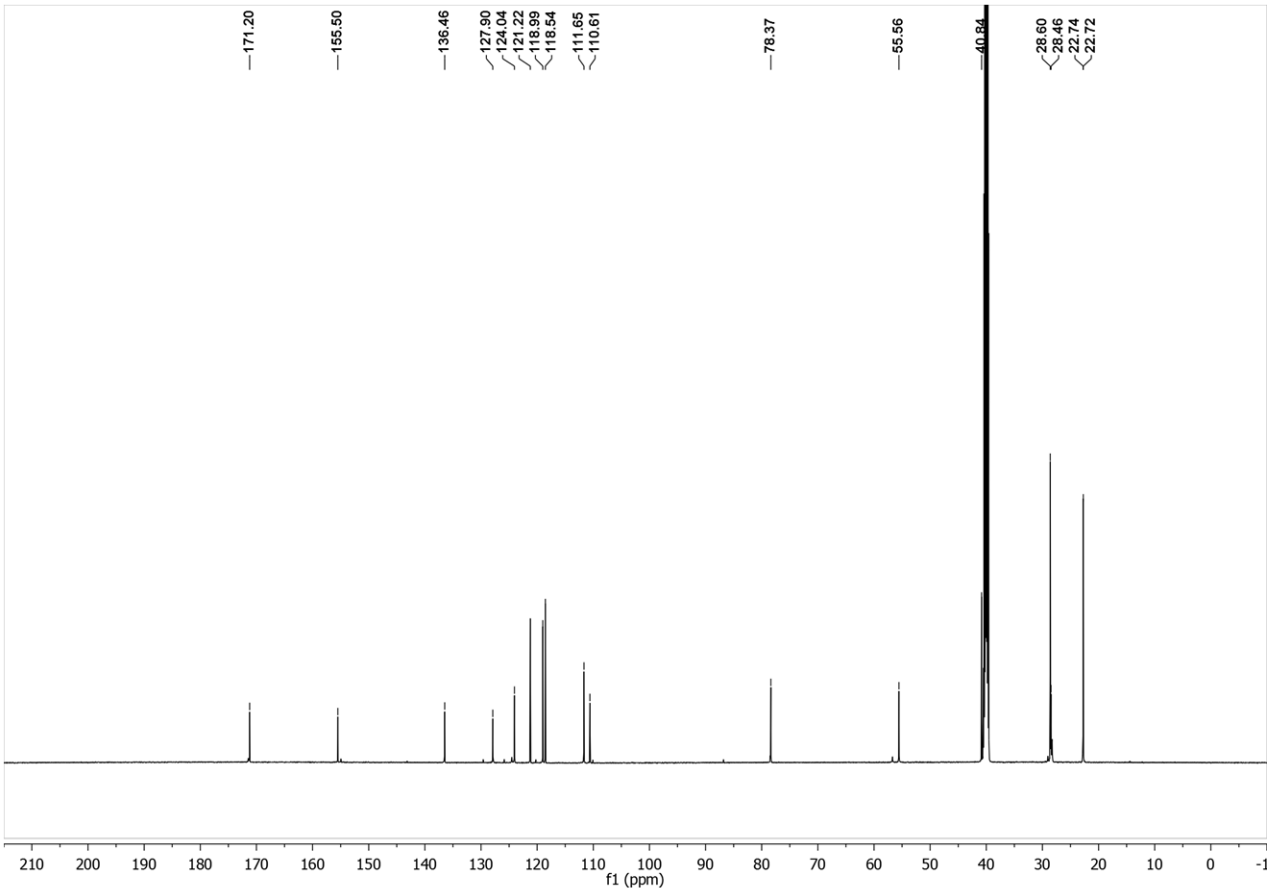
^{1}H and ^{13}C spectra of compound S38.



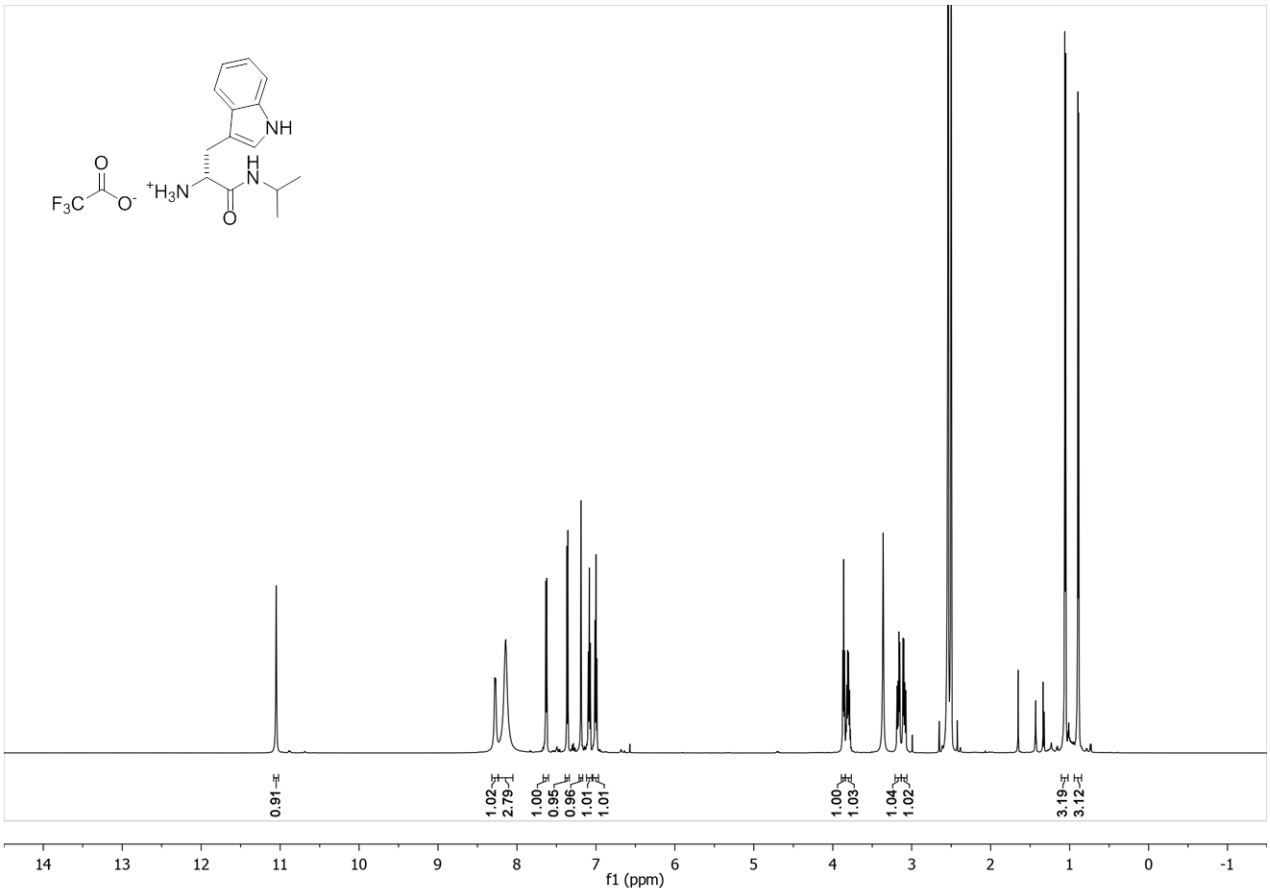


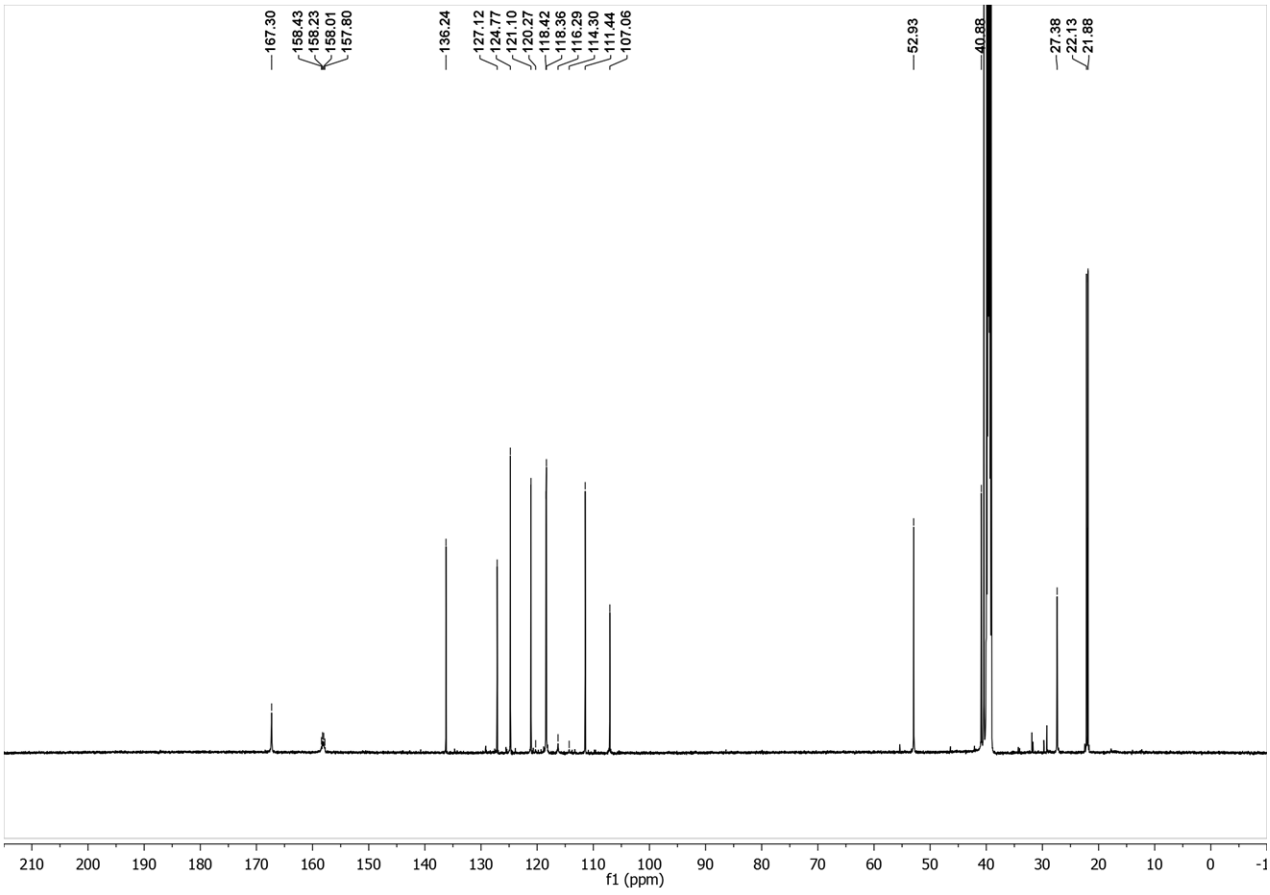
^{1}H and ^{13}C spectra of compound S39.



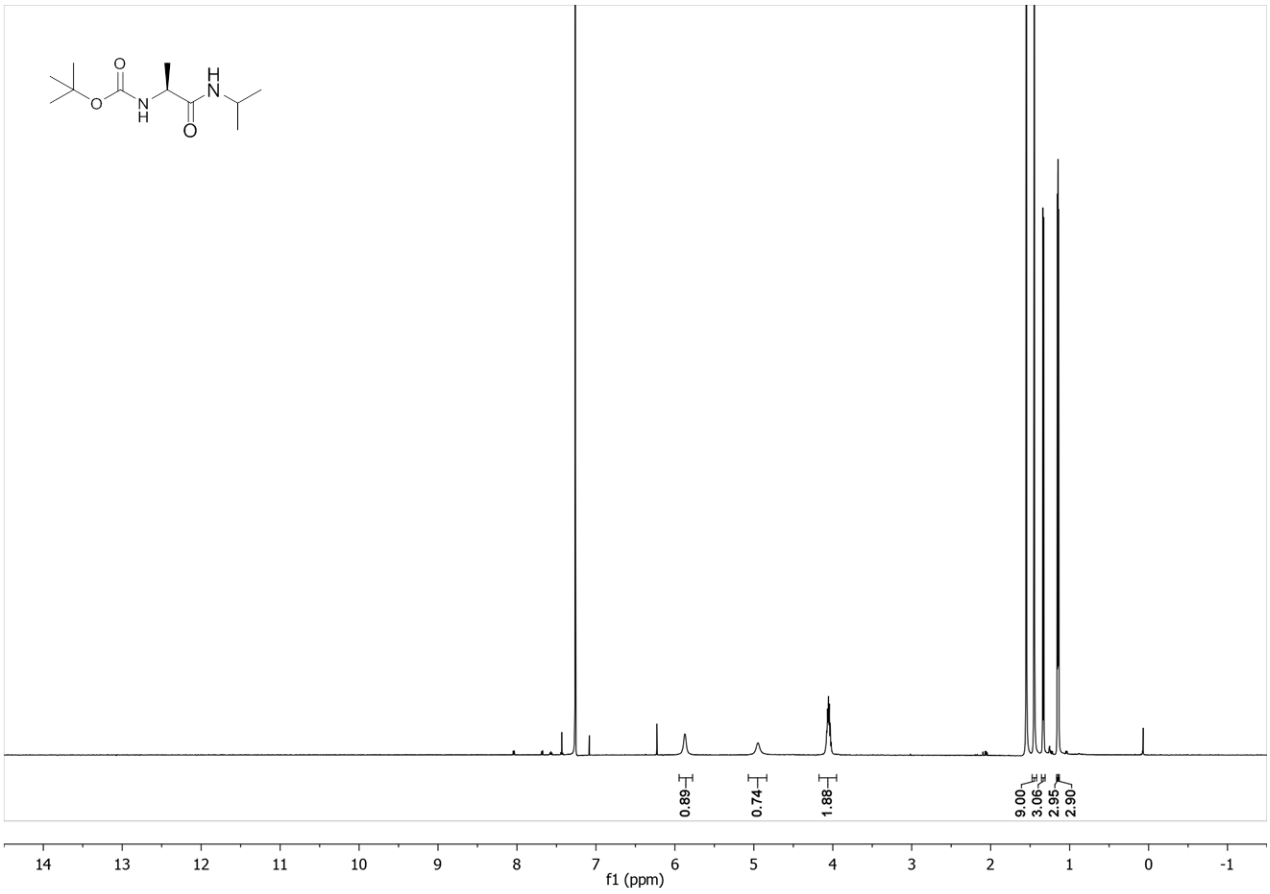


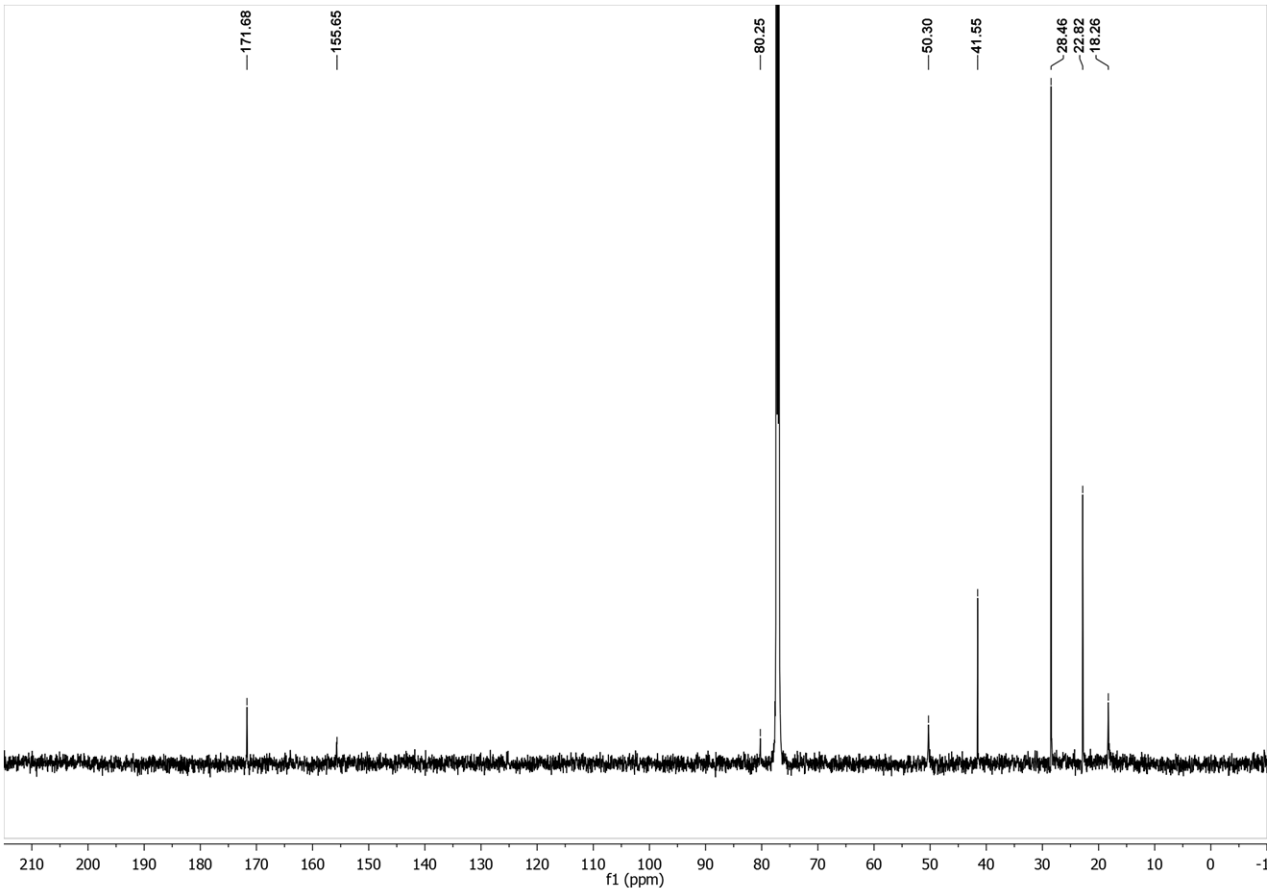
^{1}H and ^{13}C spectra of compound S40.



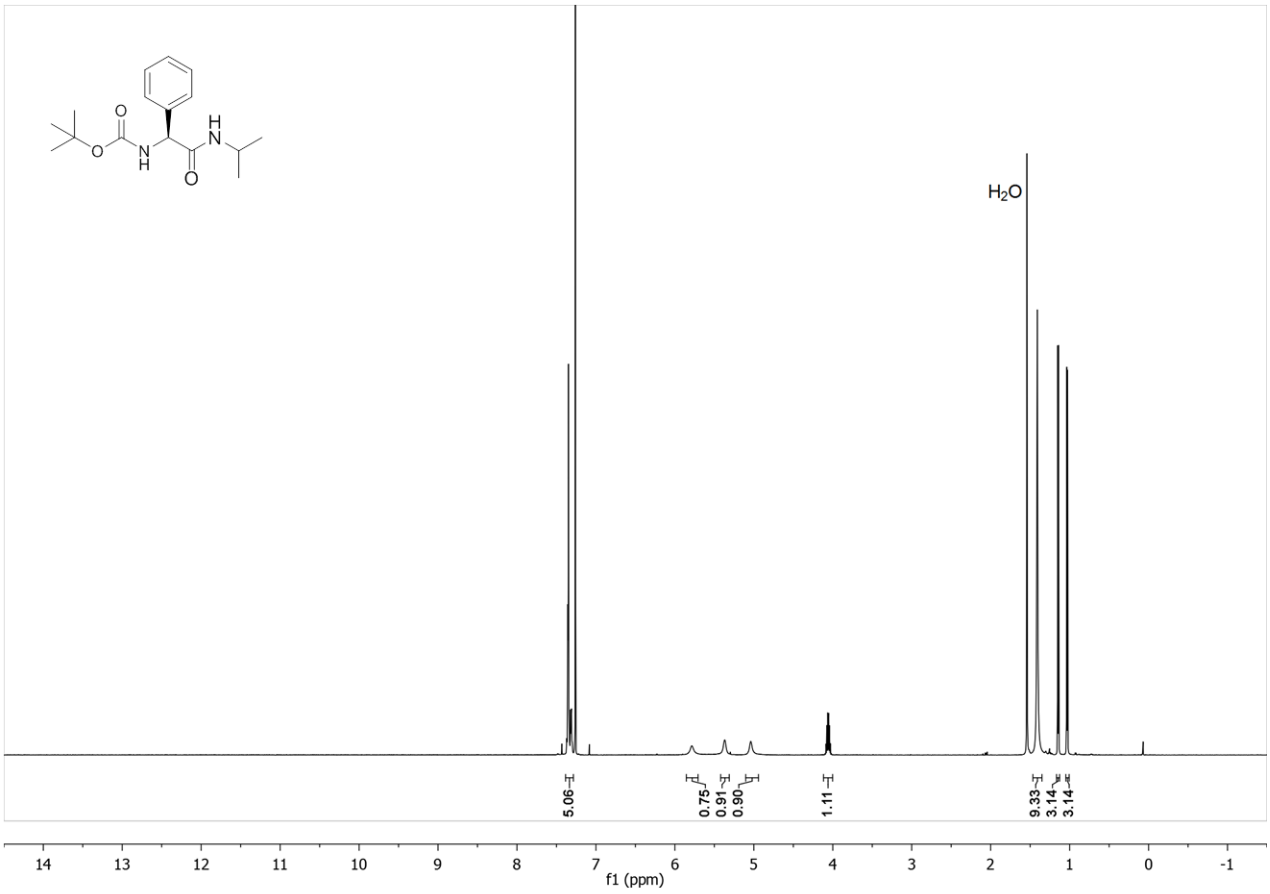


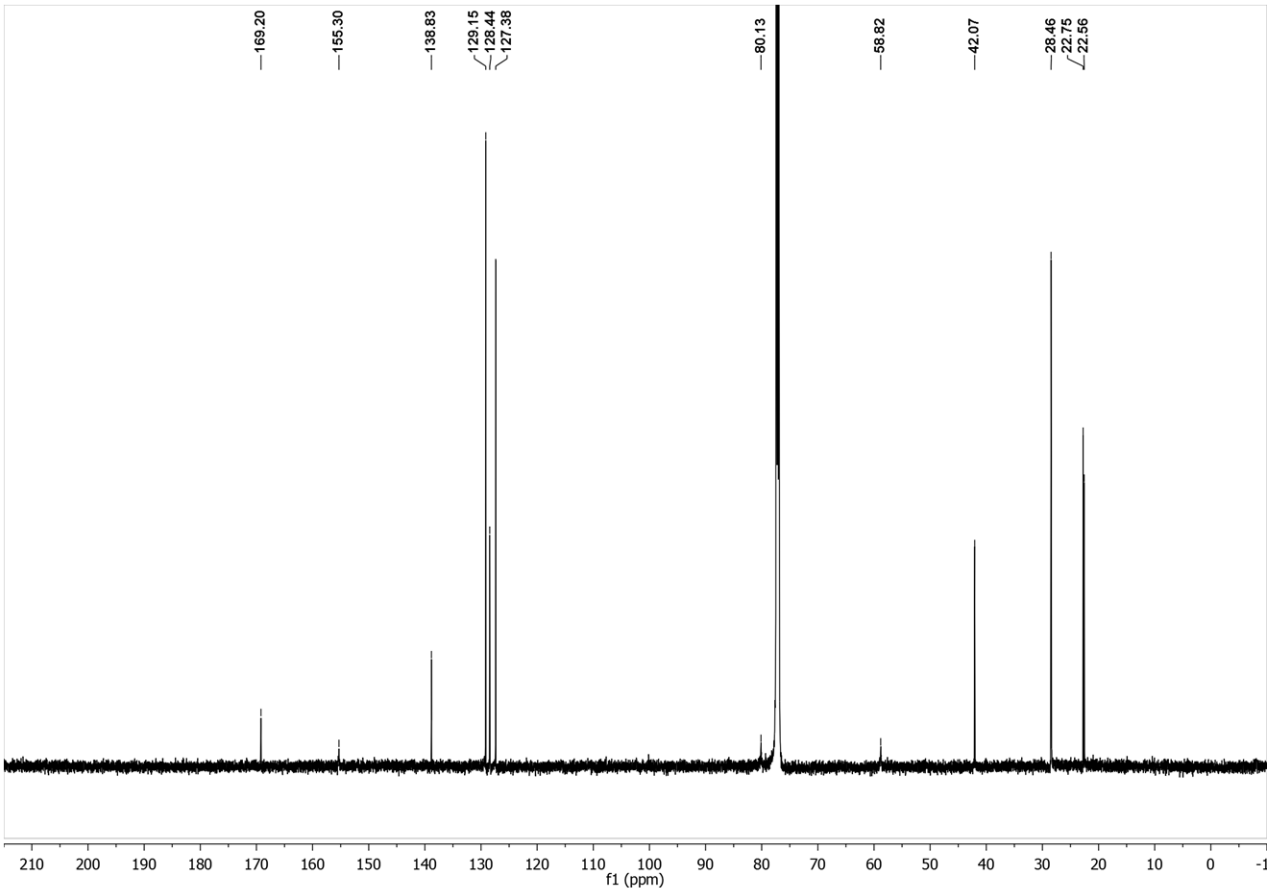
^{1}H and ^{13}C spectra of compound S41.



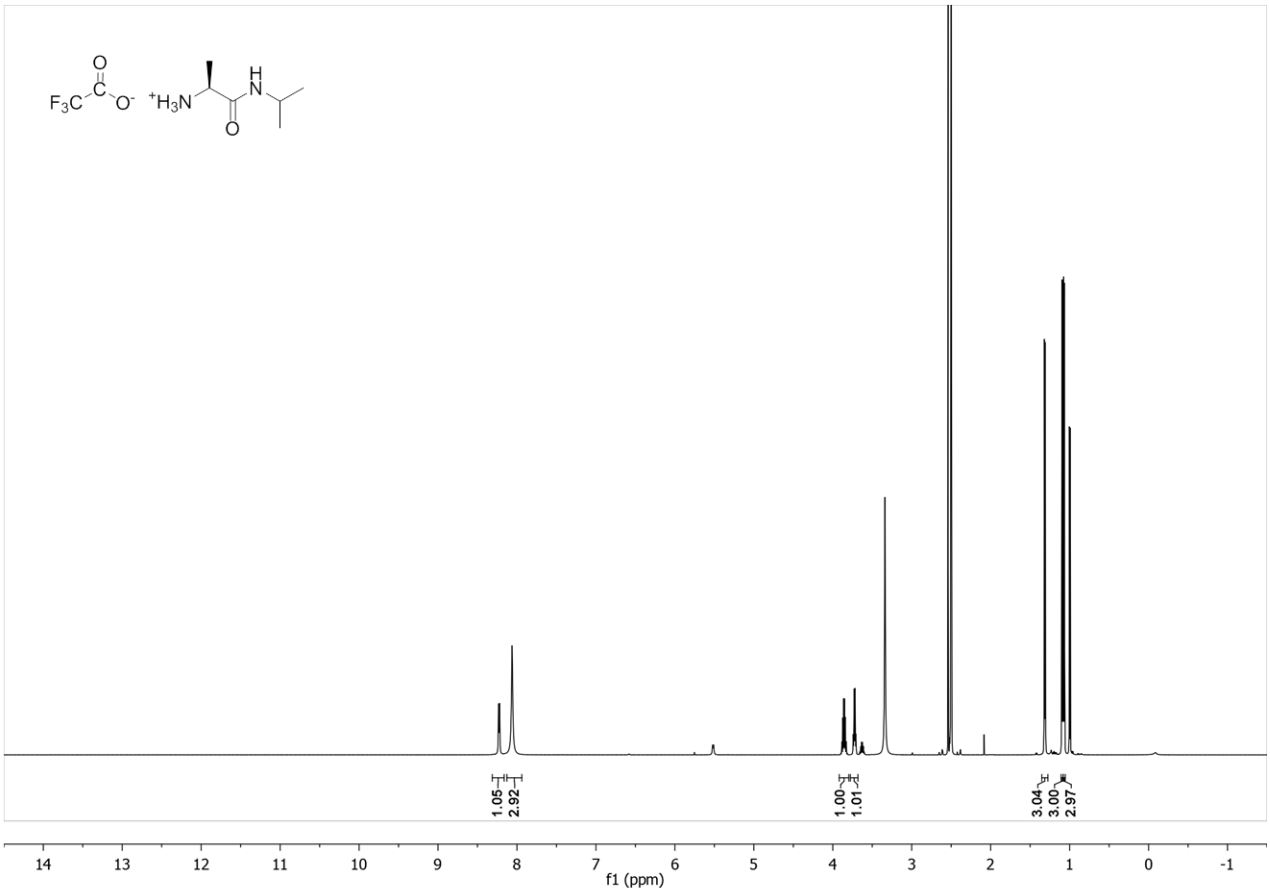


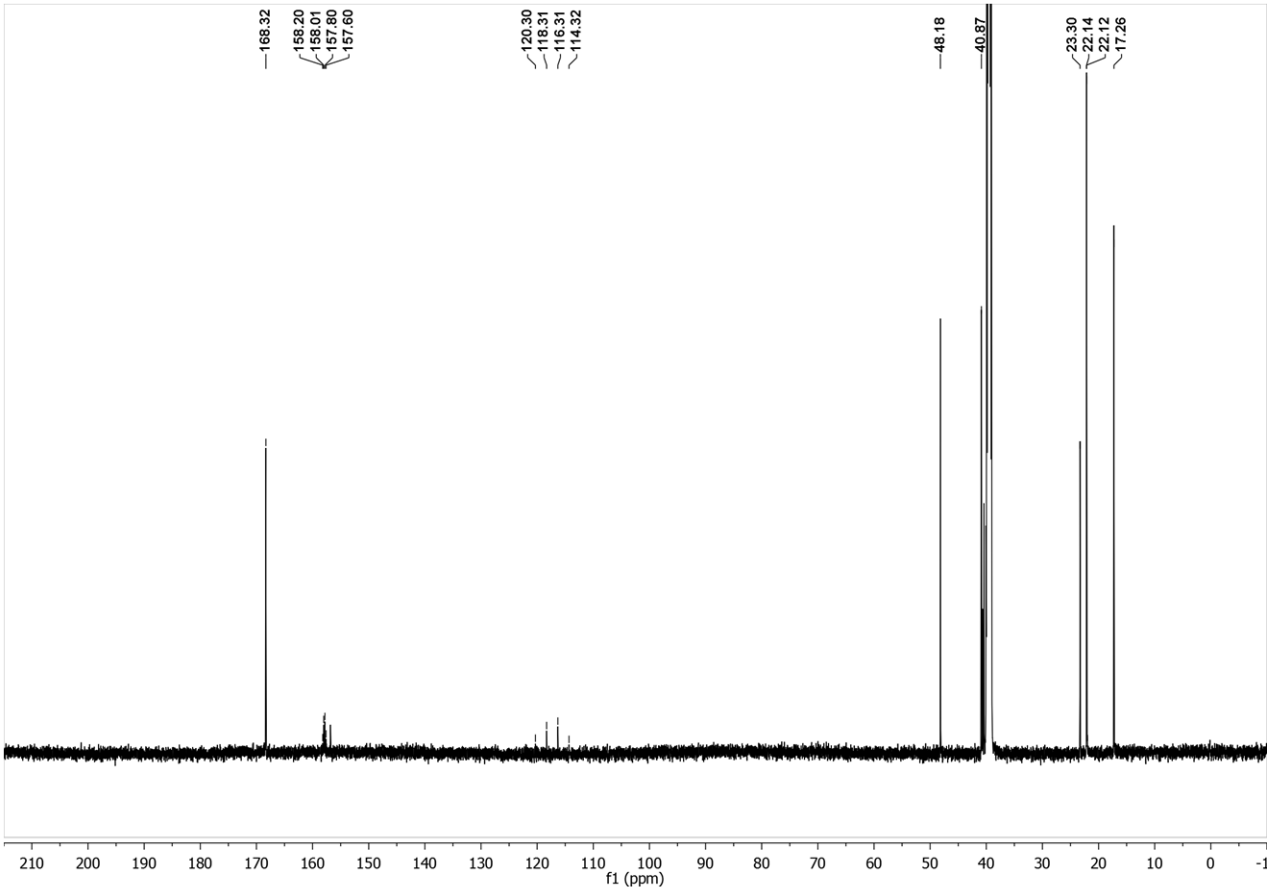
^{1}H and ^{13}C spectra of compound S42.



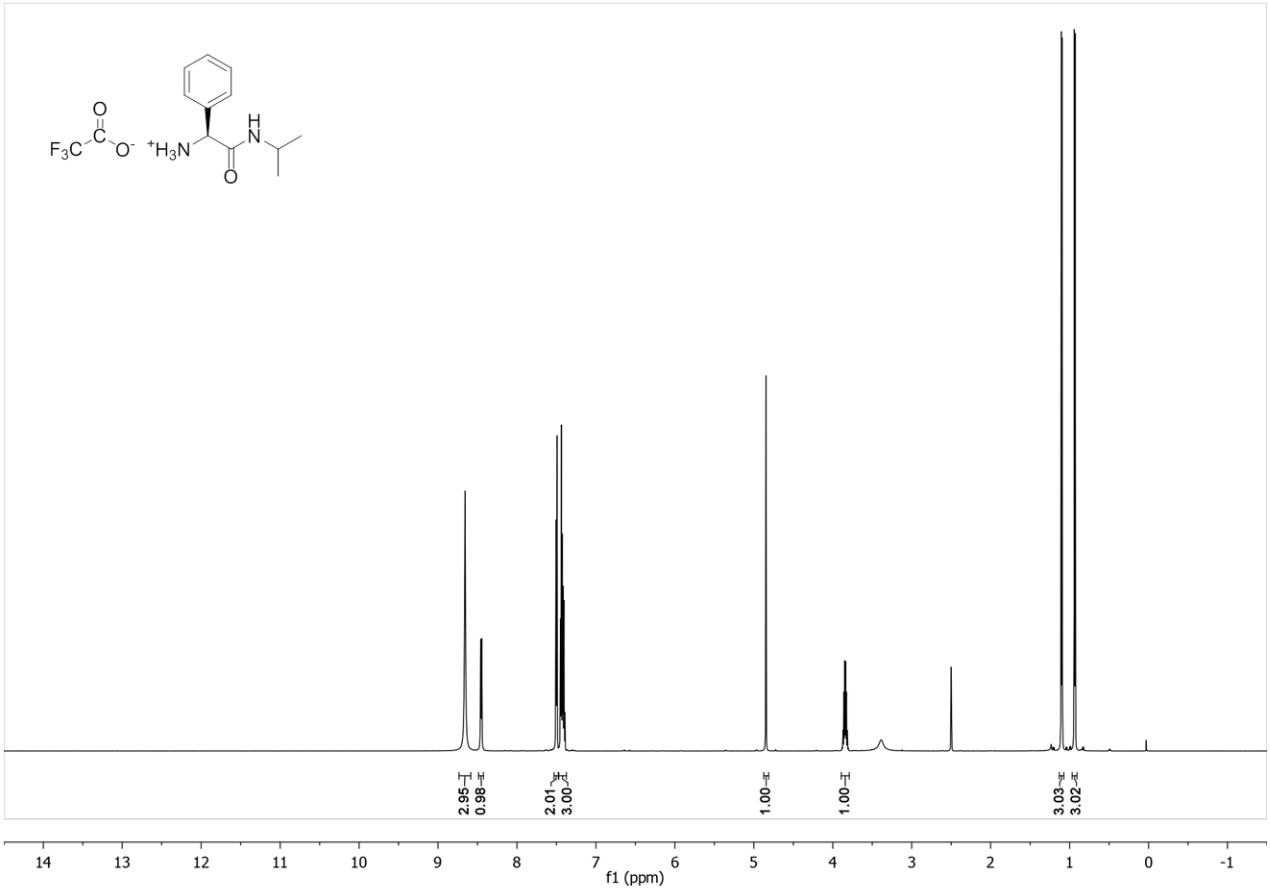


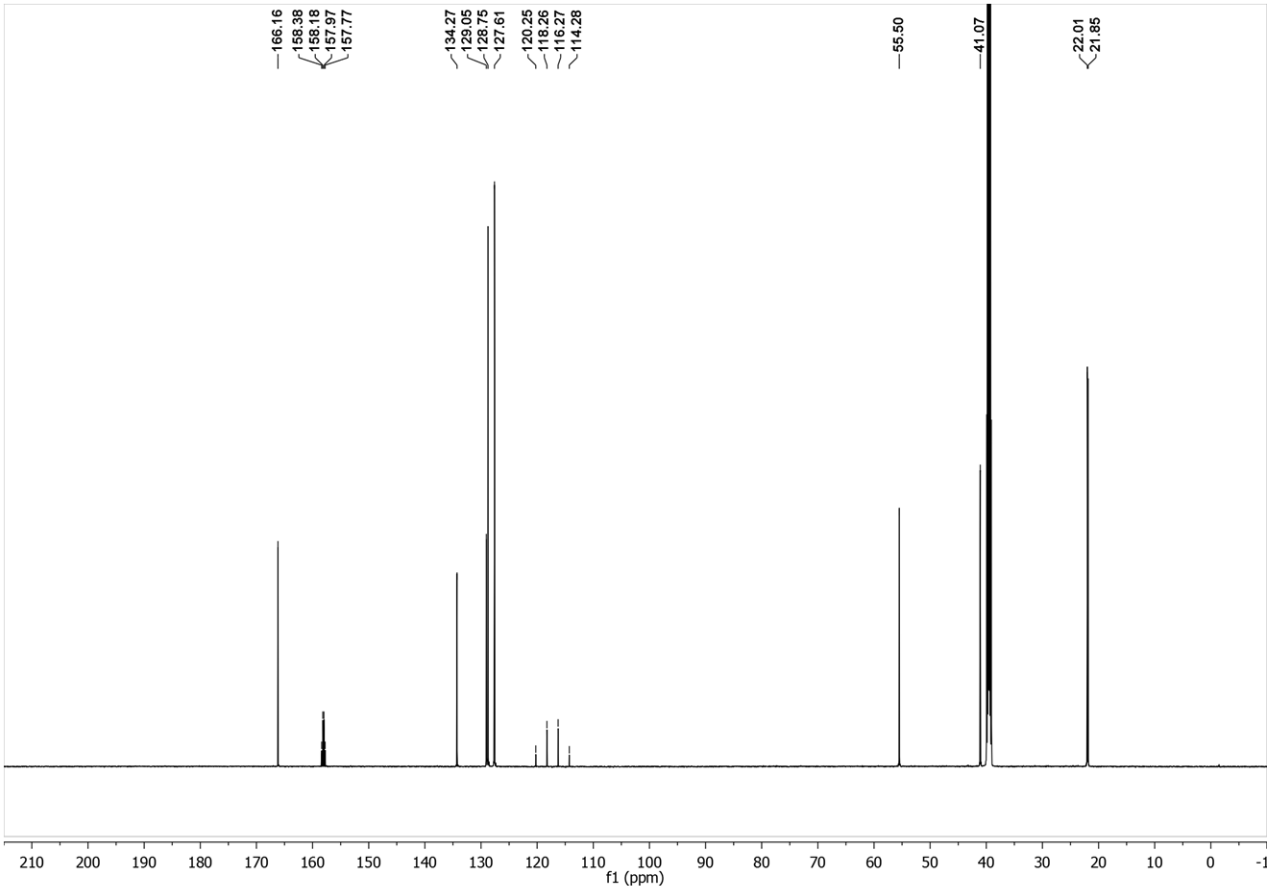
^{1}H and ^{13}C spectra of compound S43.



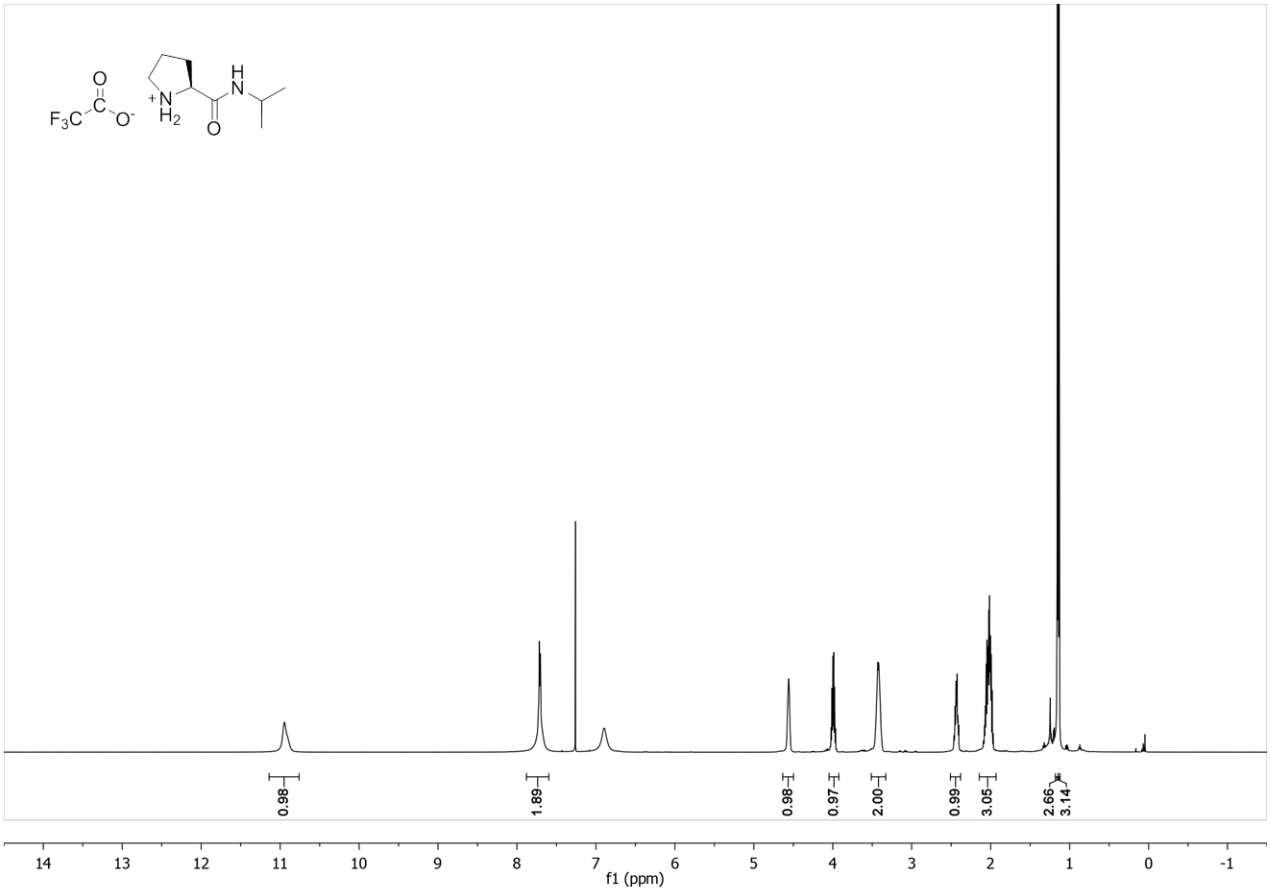


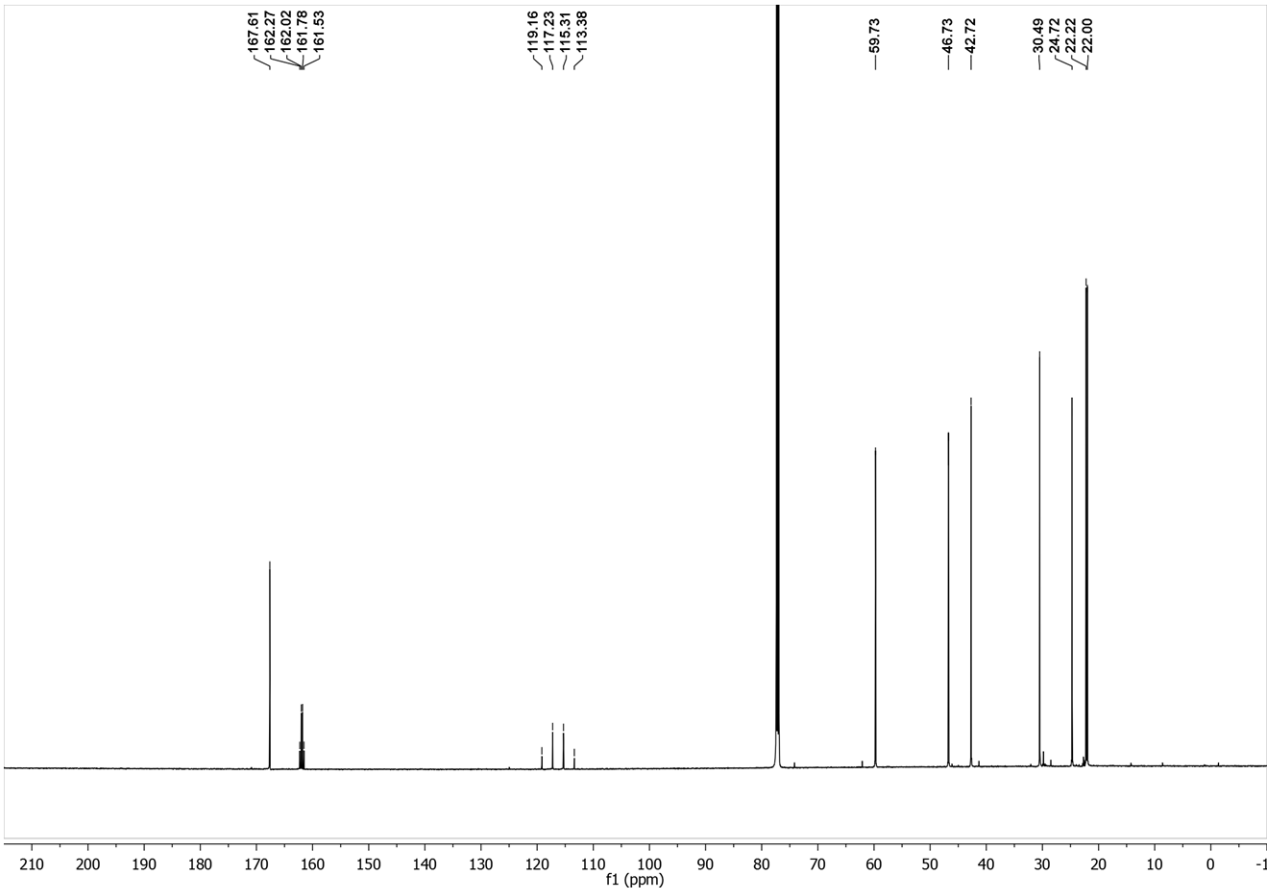
^{1}H and ^{13}C spectra of compound S44.



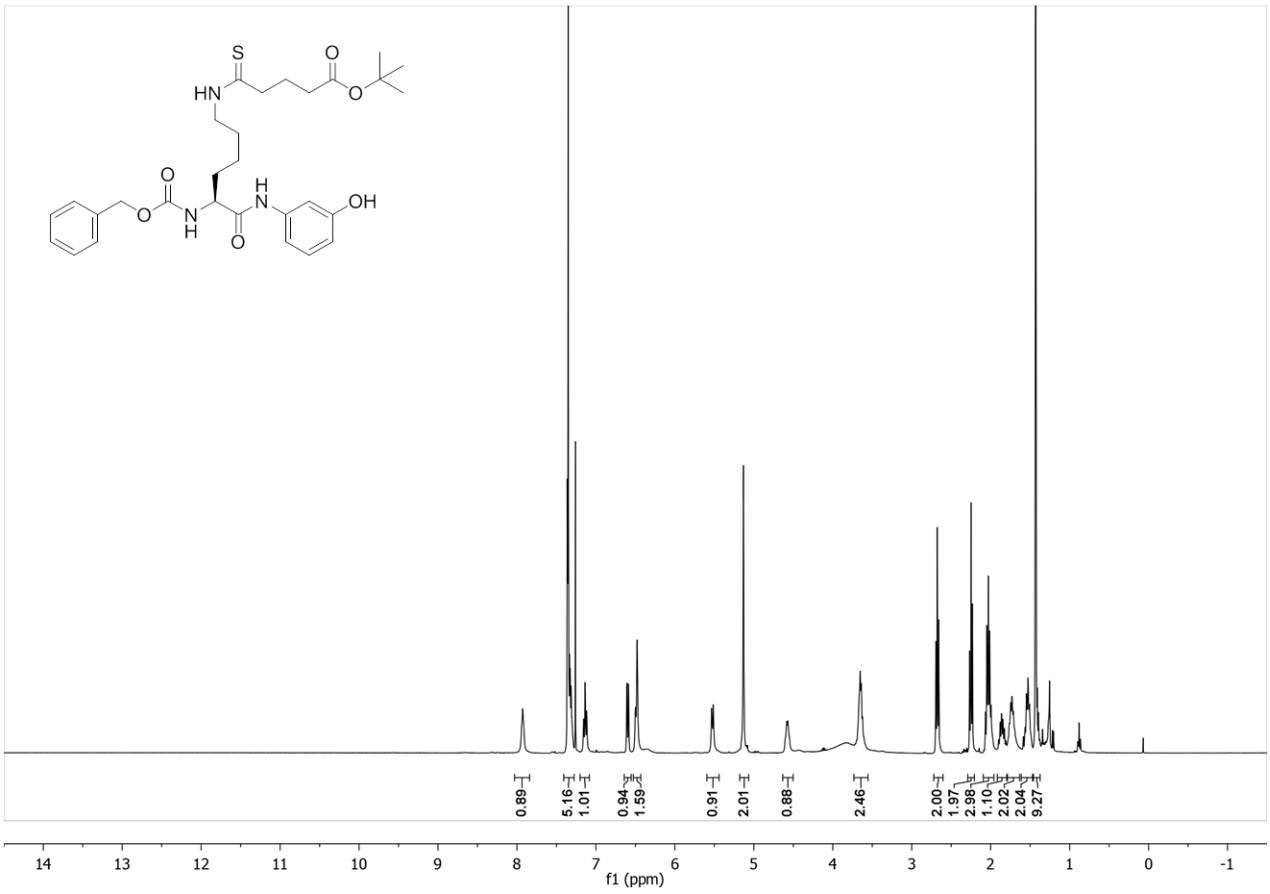


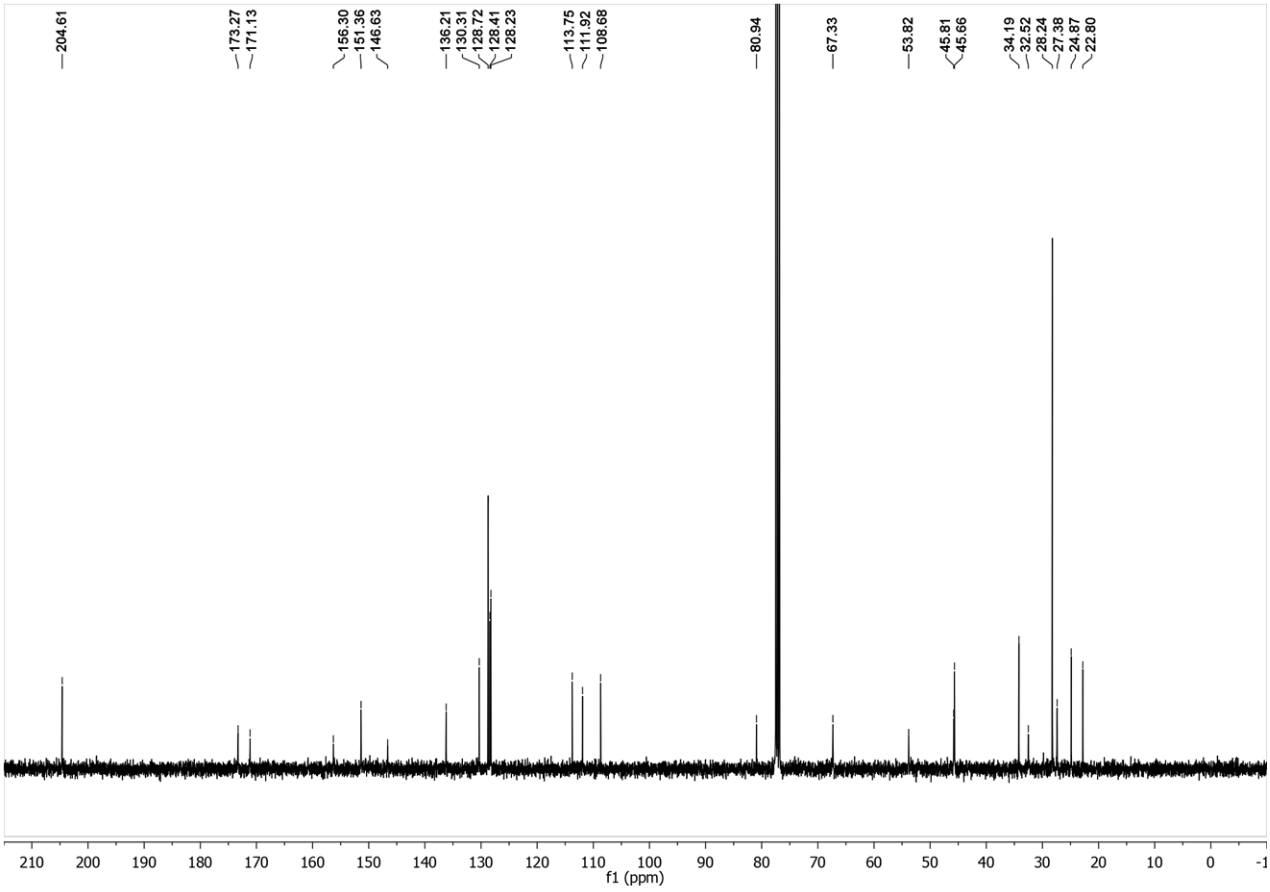
^{1}H and ^{13}C spectra of compound S45.



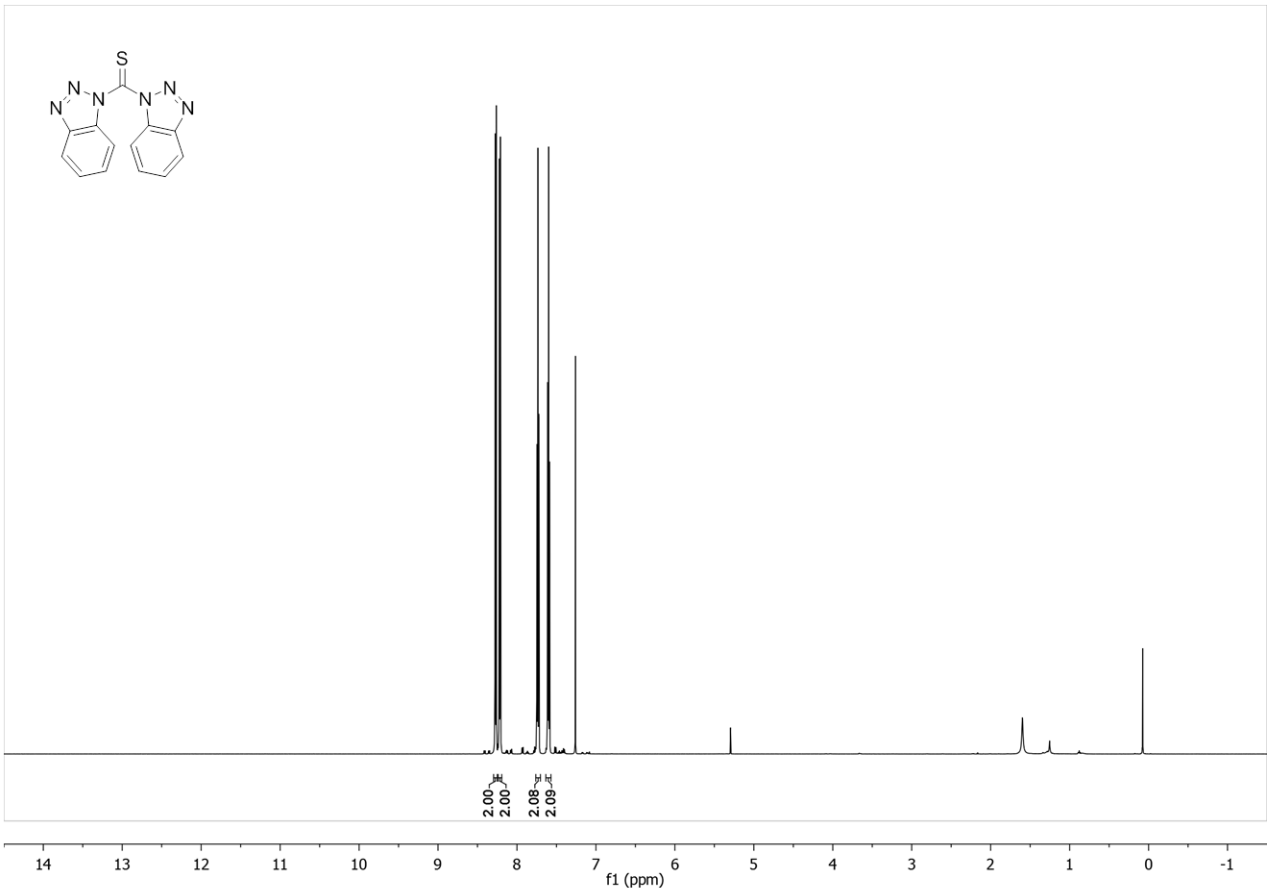


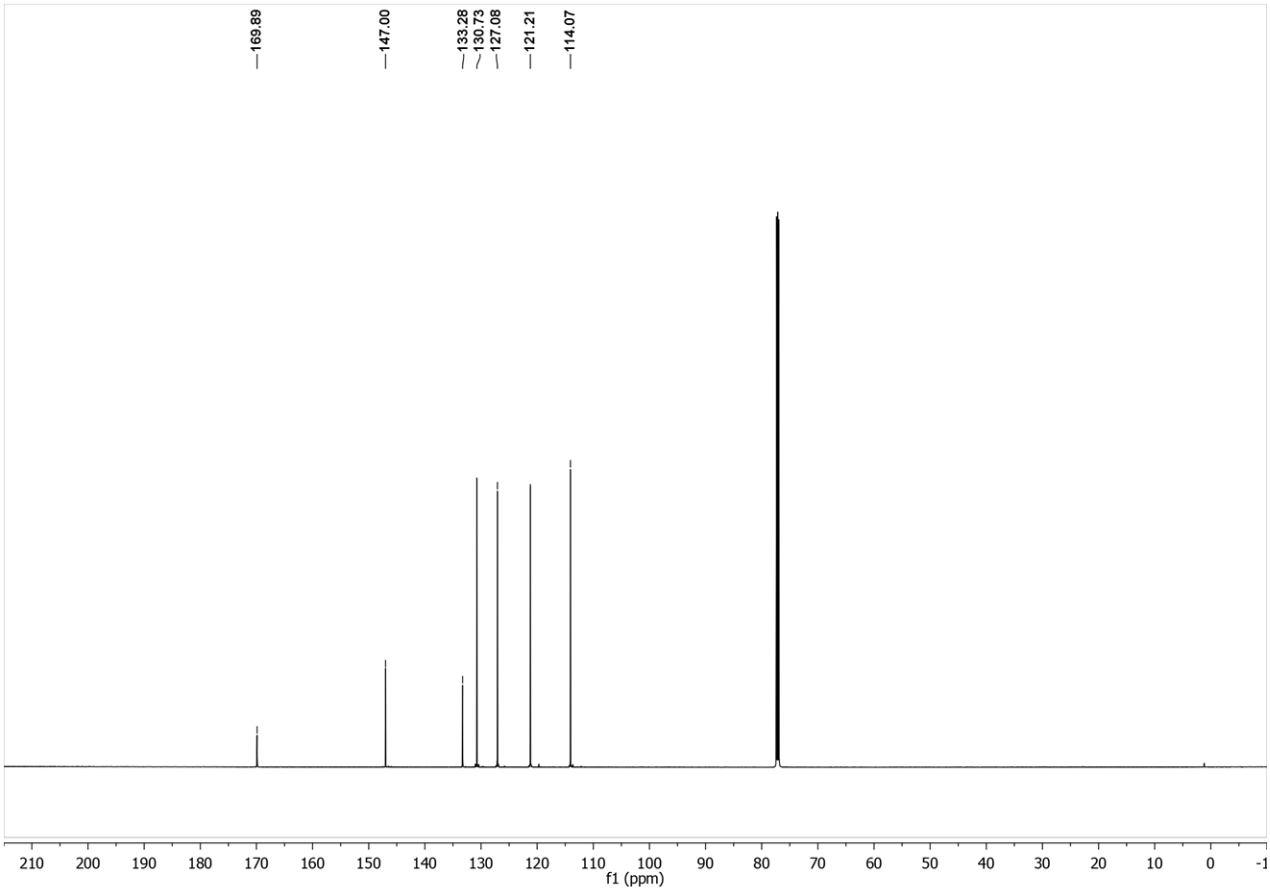
^{1}H and ^{13}C spectra of compound S46.



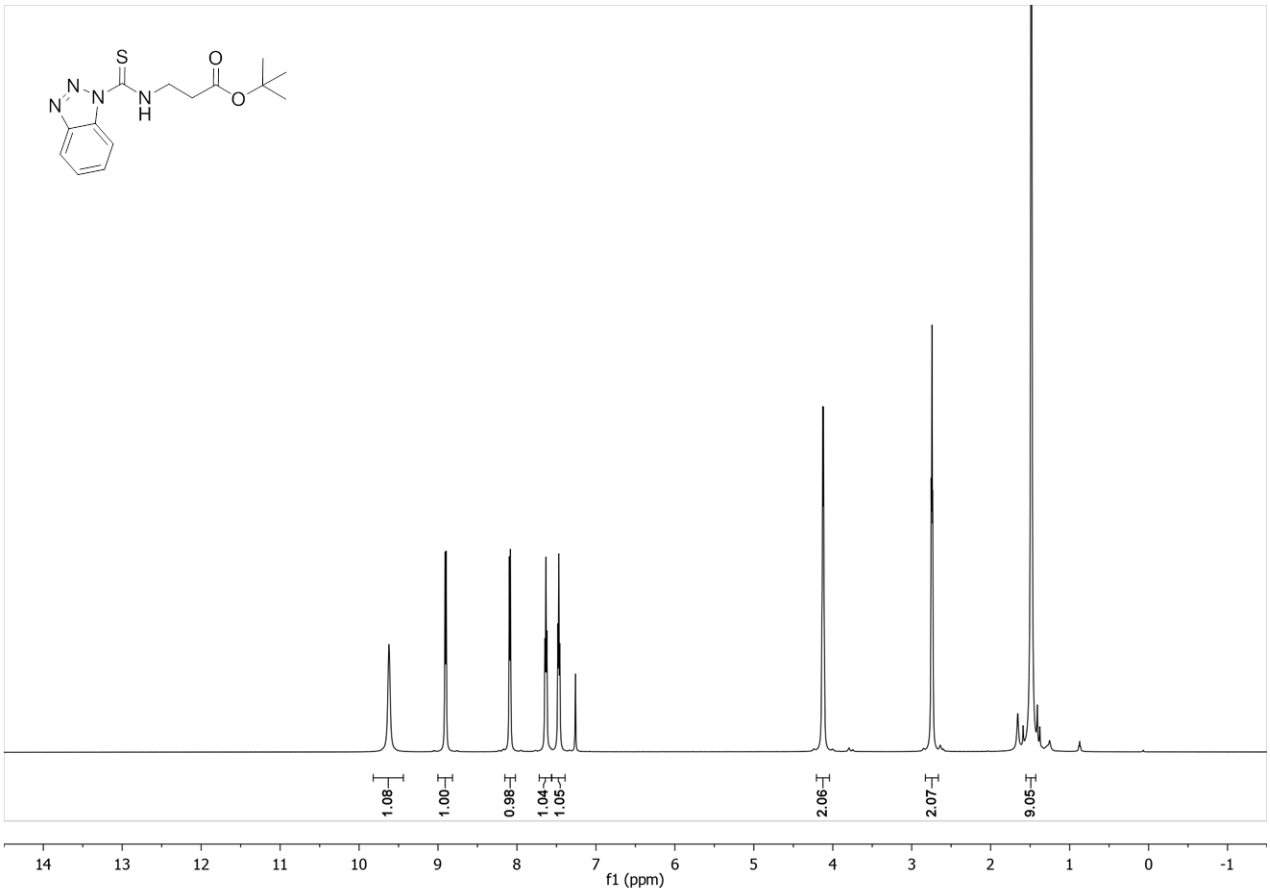


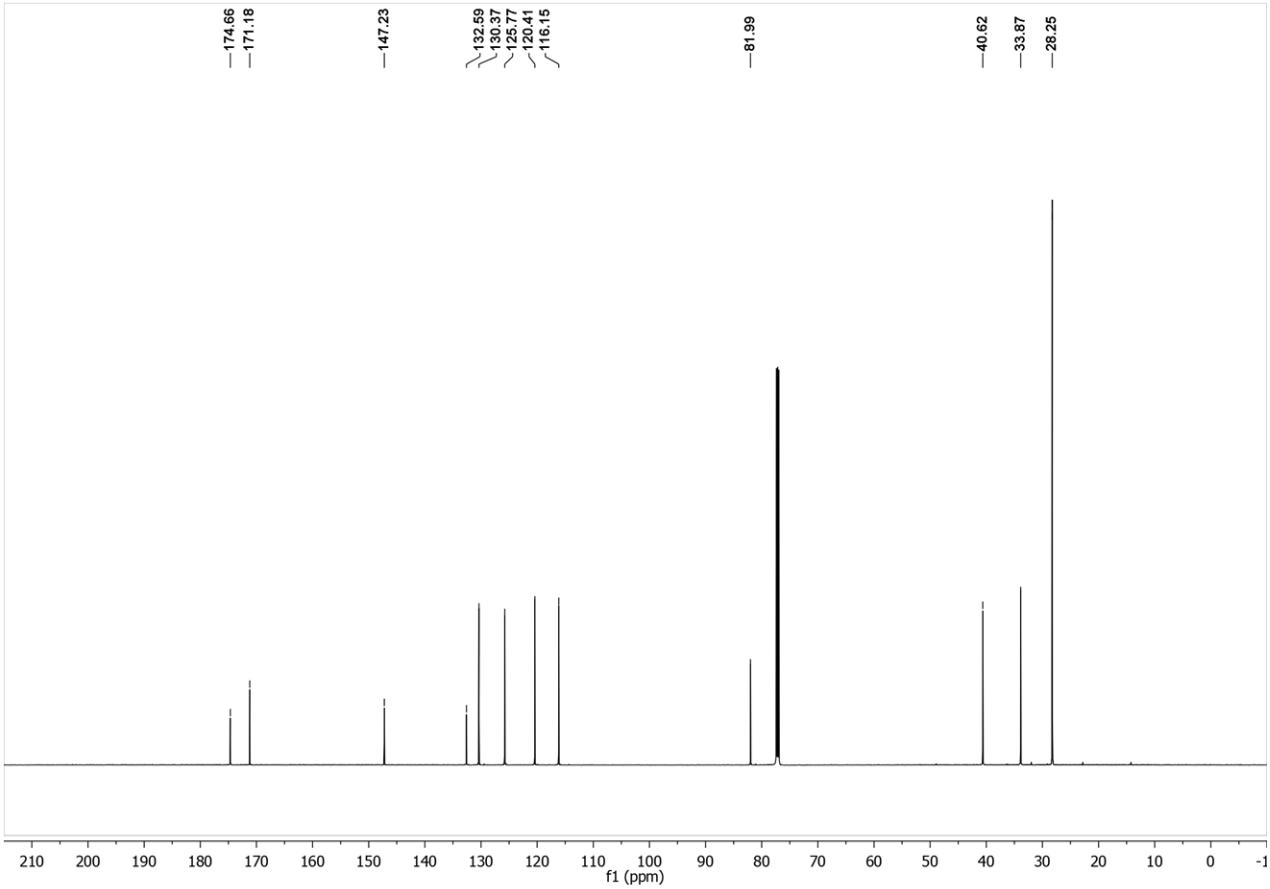
^{1}H and ^{13}C spectra of compound S47.



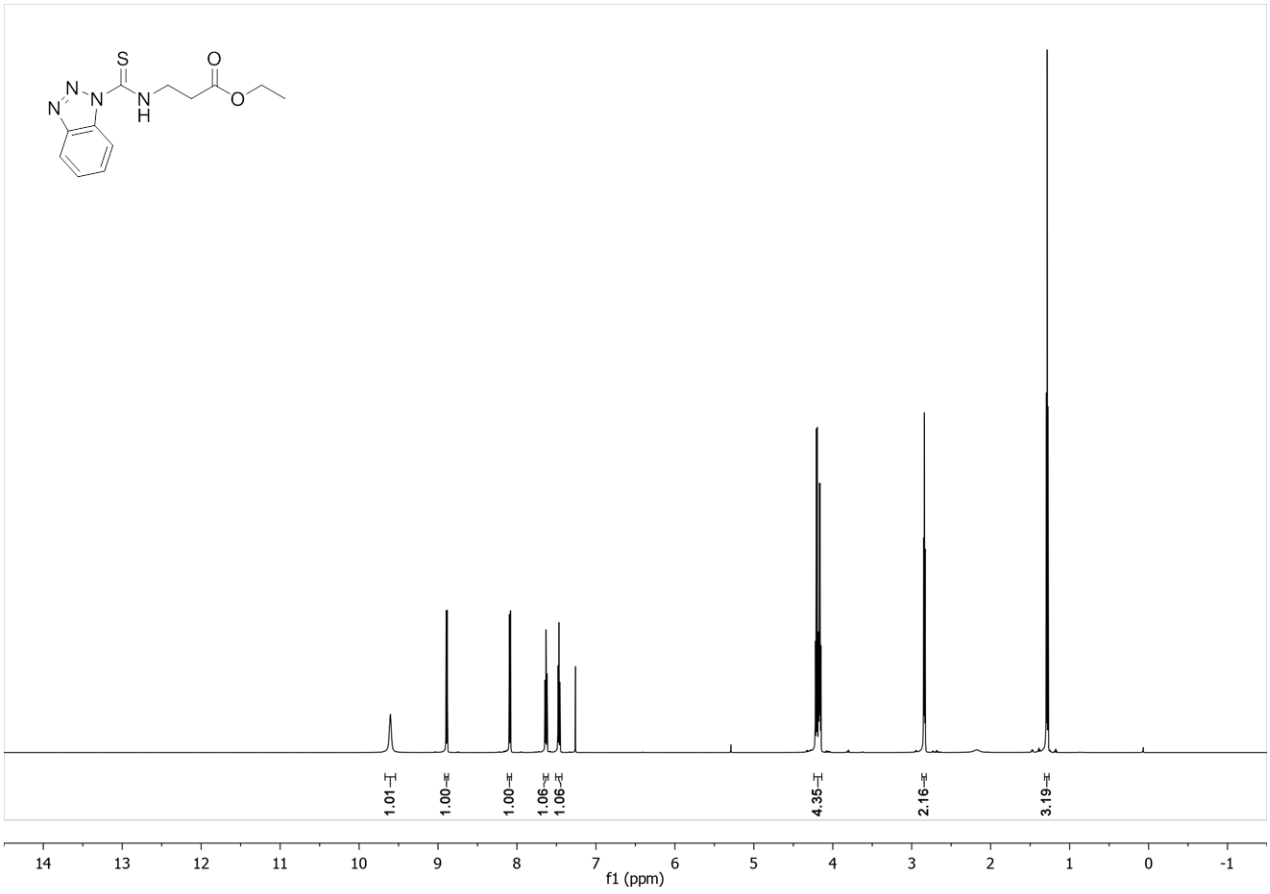


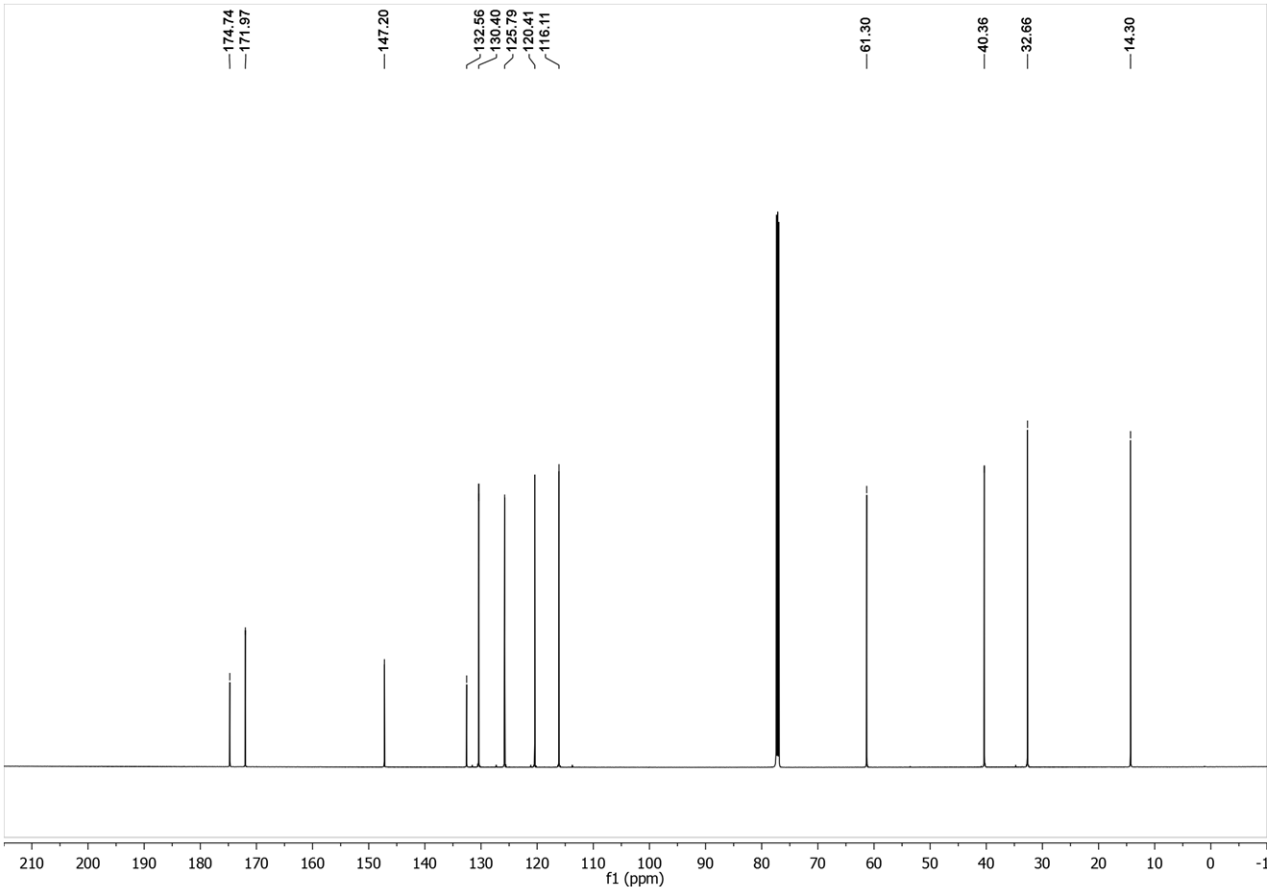
^{1}H and ^{13}C spectra of compound S48.



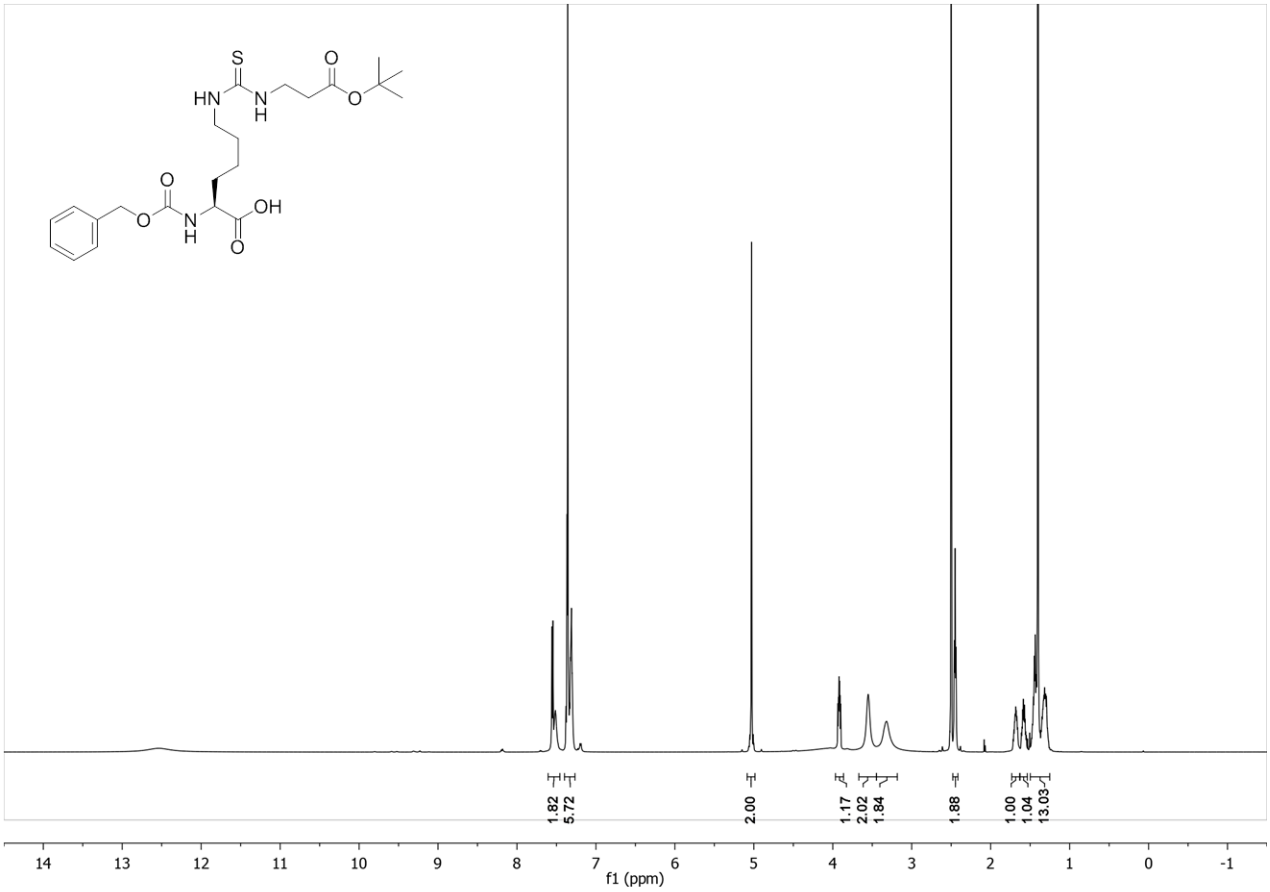


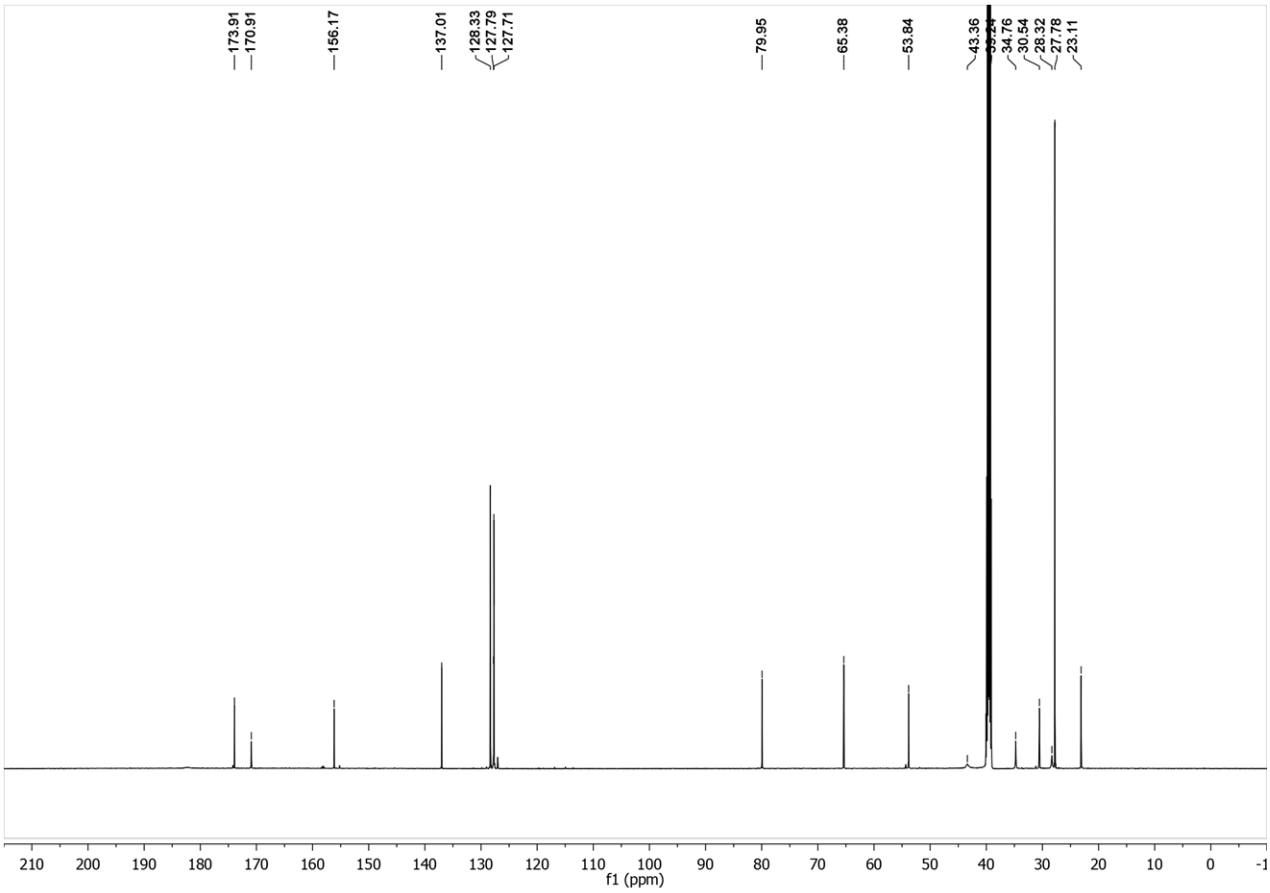
^{1}H and ^{13}C spectra of compound S49.



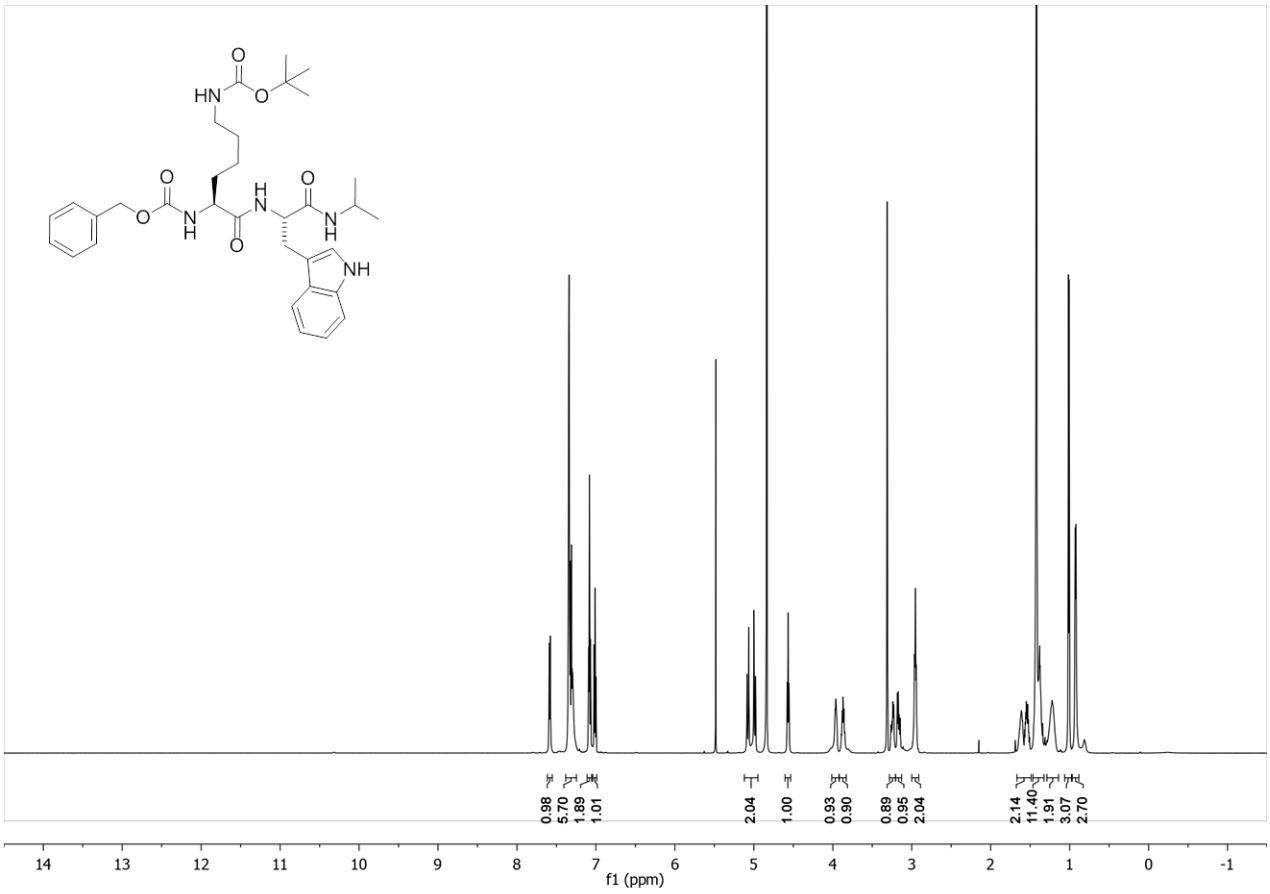


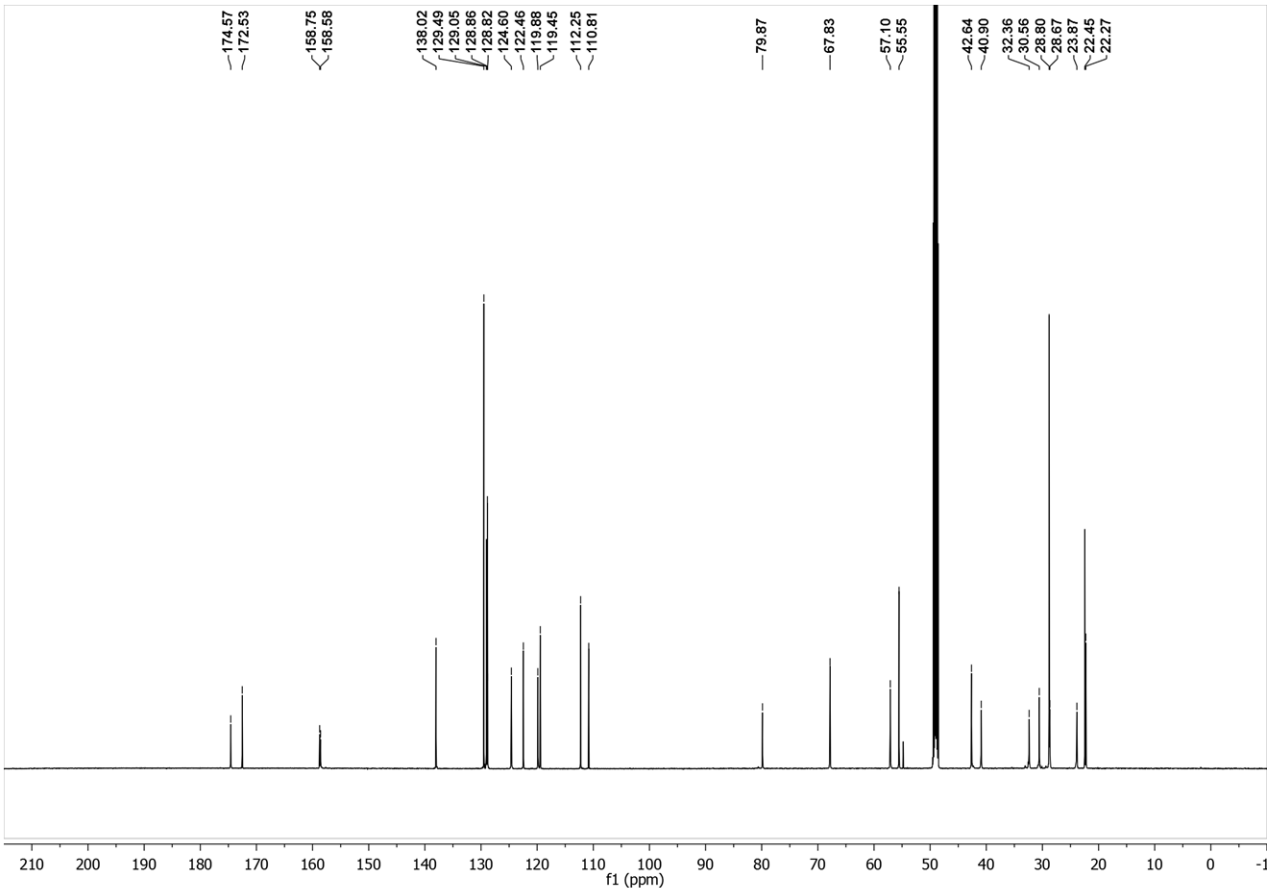
^{1}H and ^{13}C spectra of compound S50.



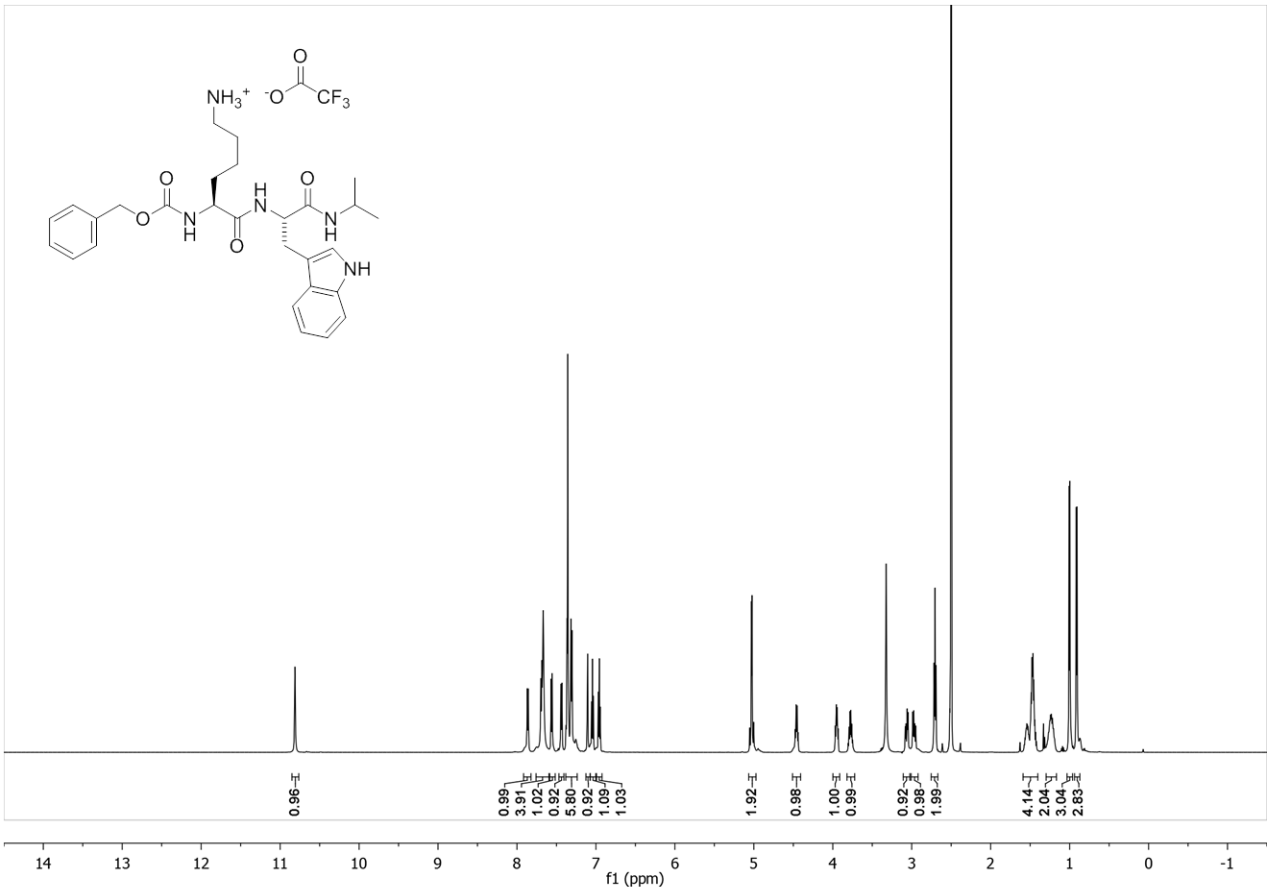


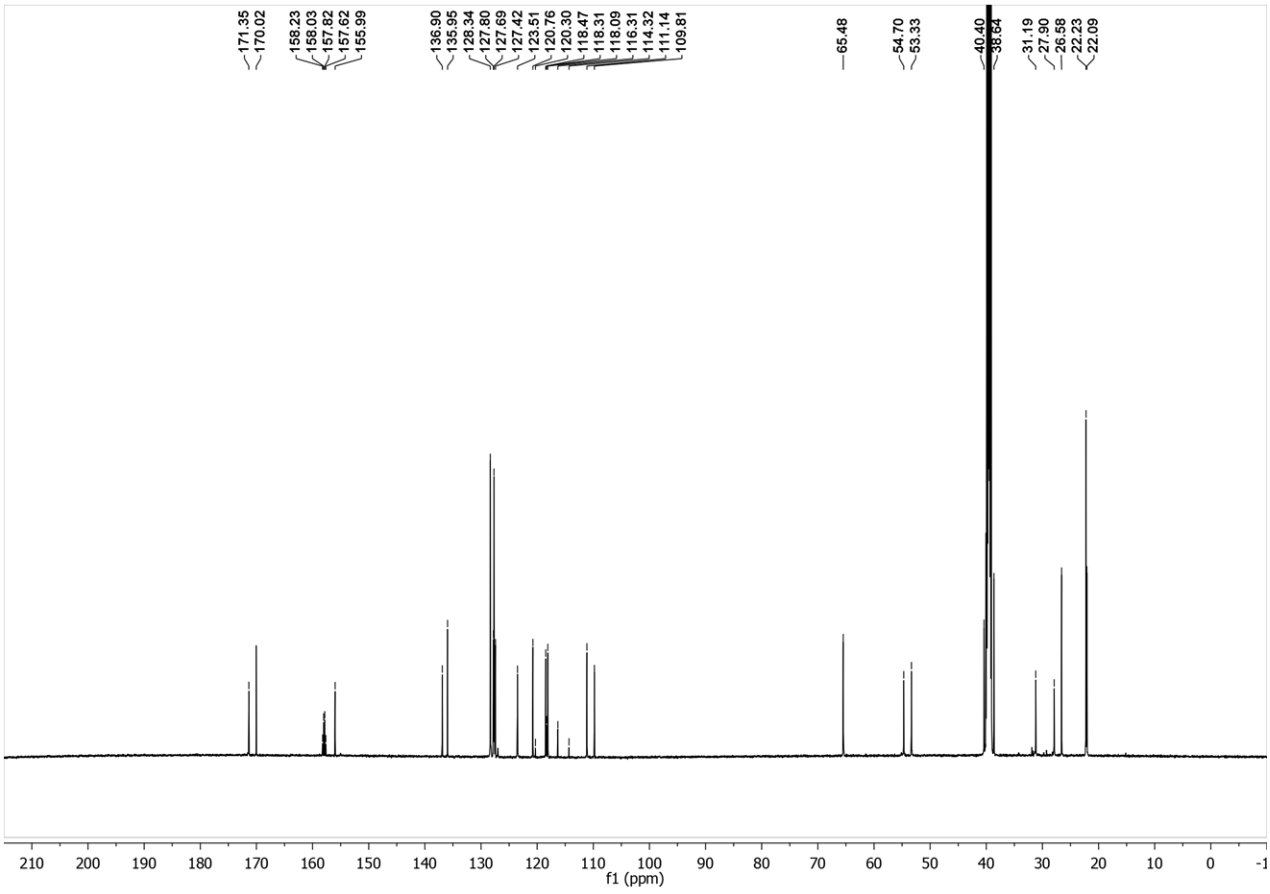
^{1}H and ^{13}C spectra of compound S51.



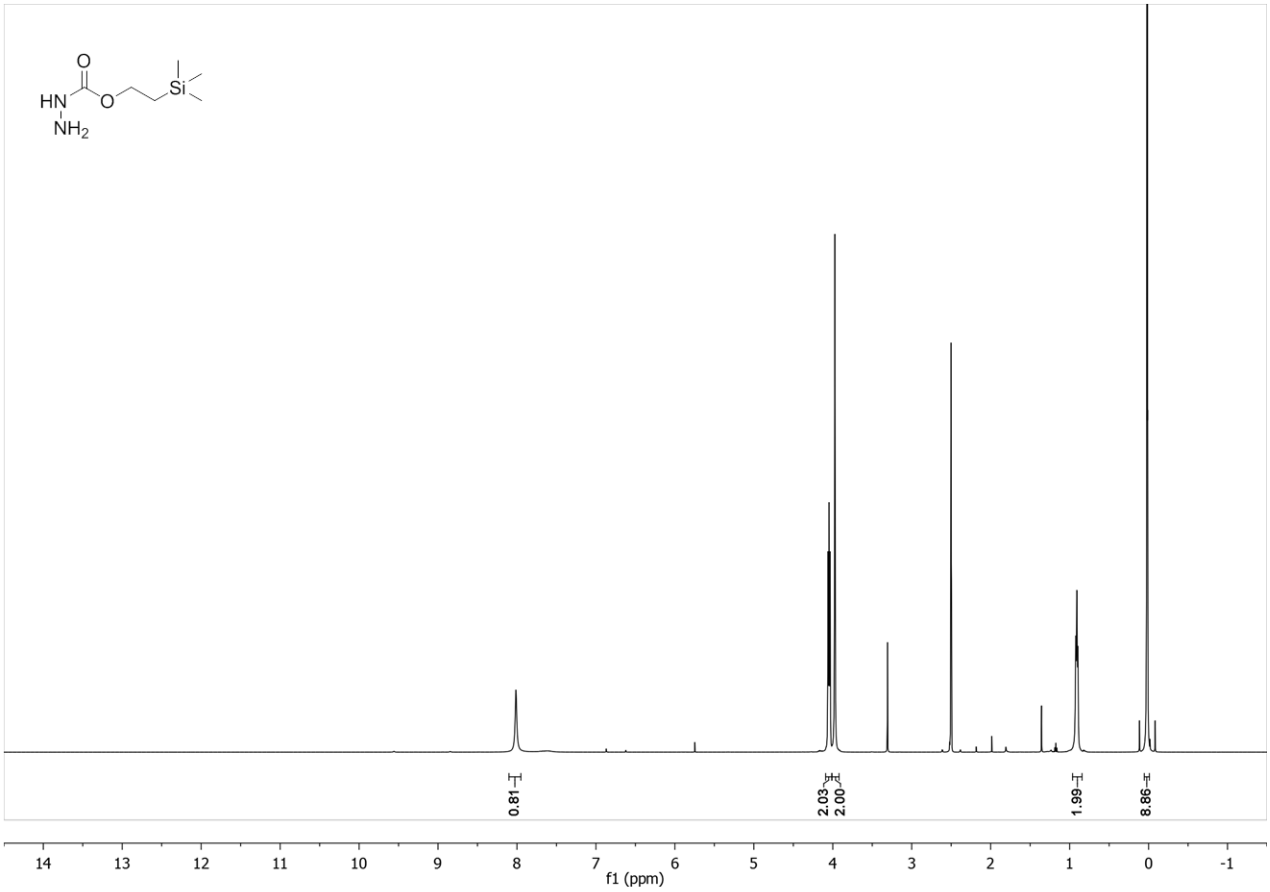


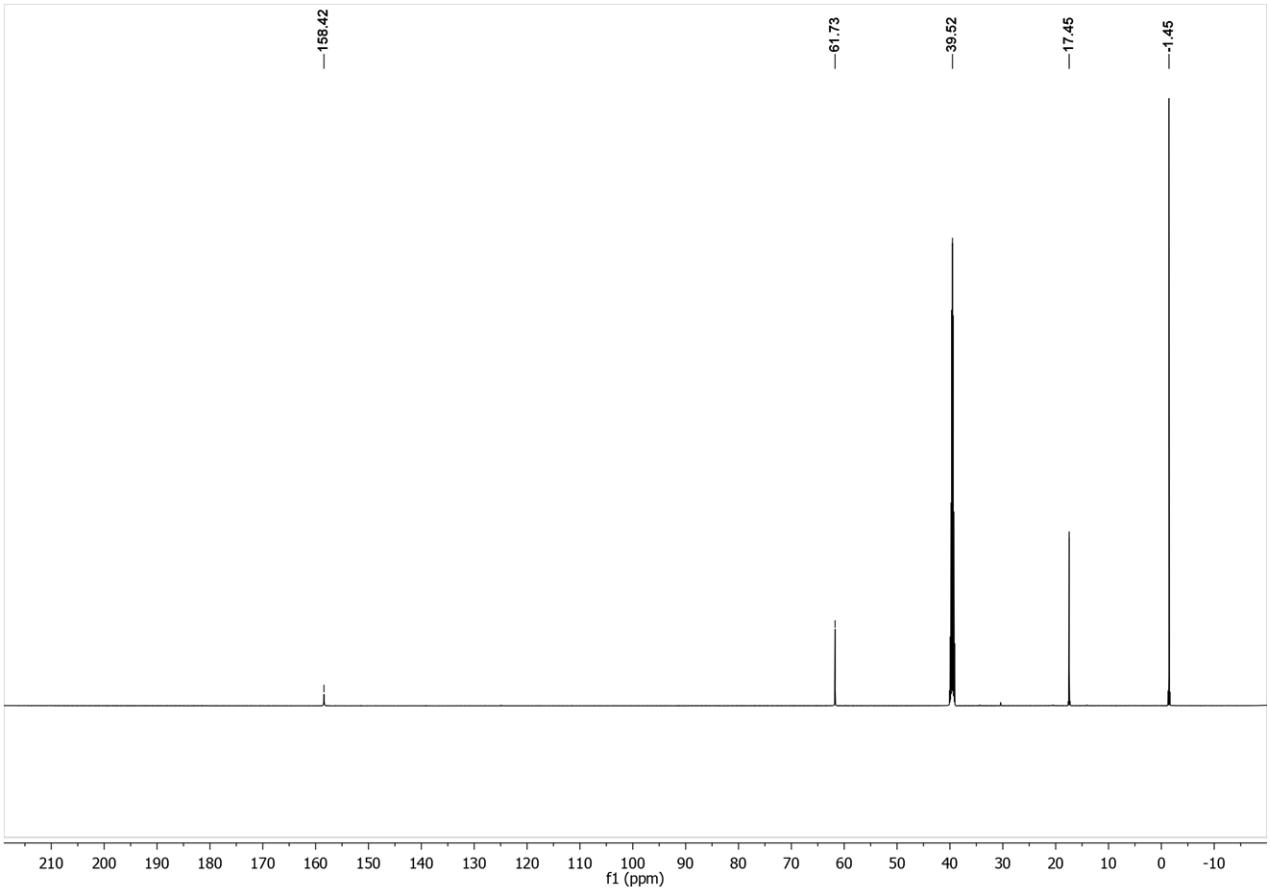
^{1}H and ^{13}C spectra of compound S52.



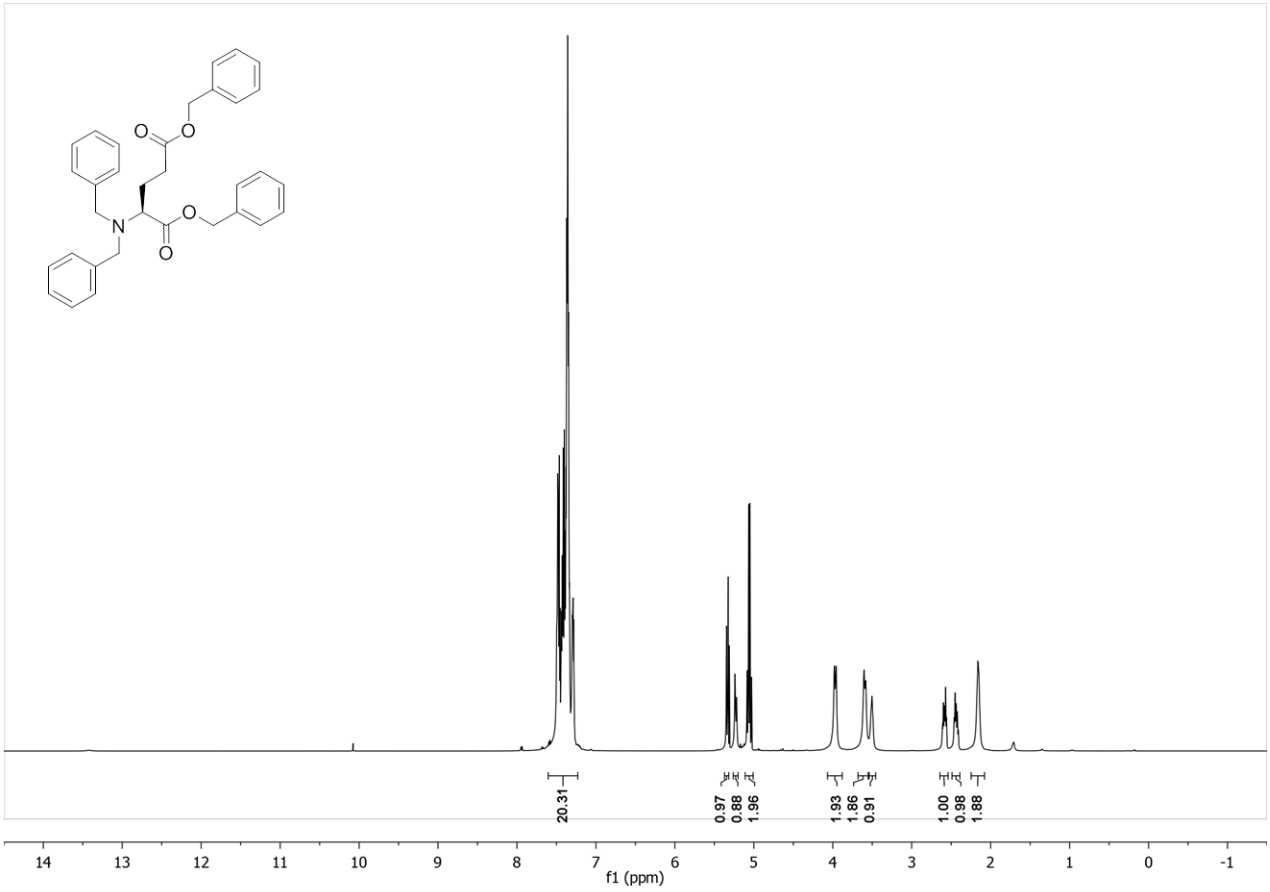


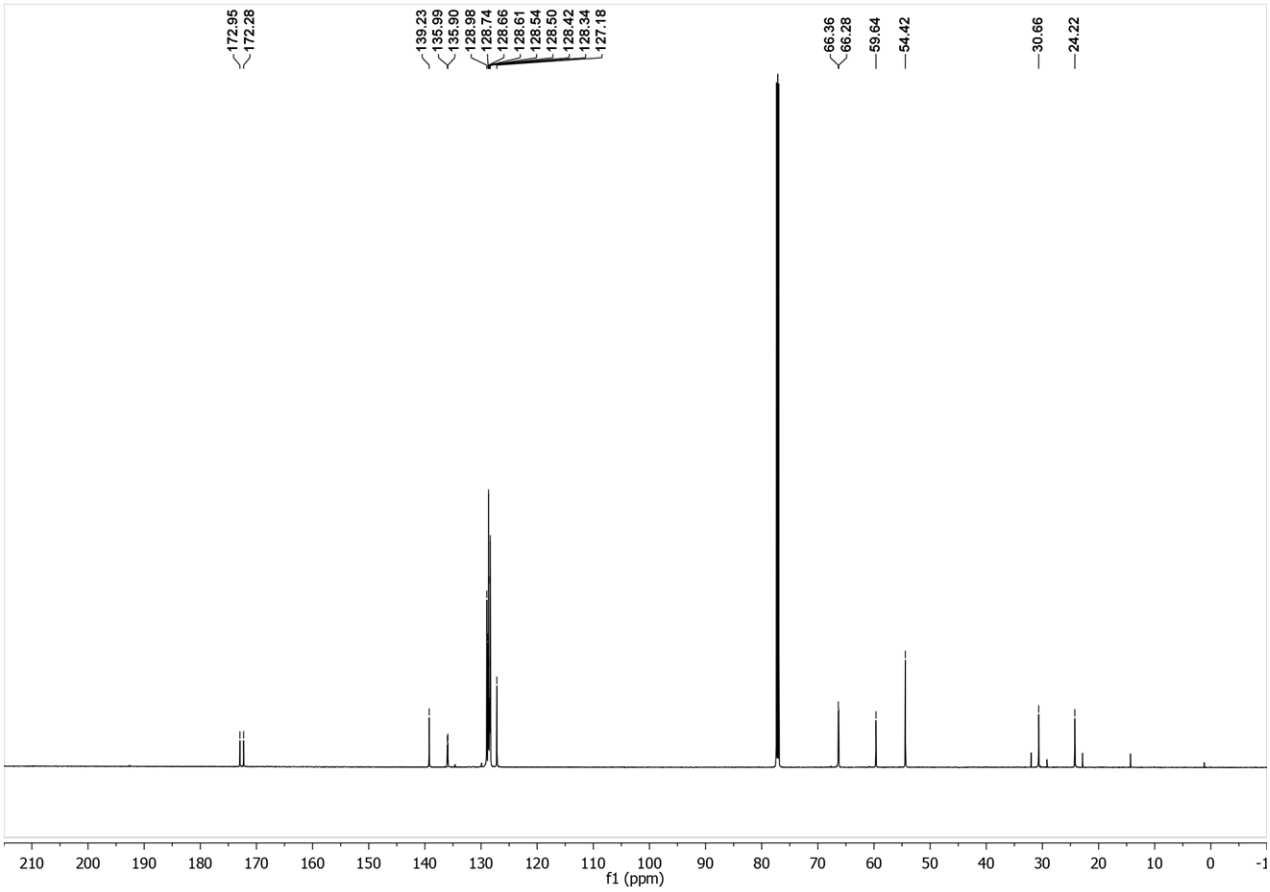
^{1}H and ^{13}C spectra of compound S53.



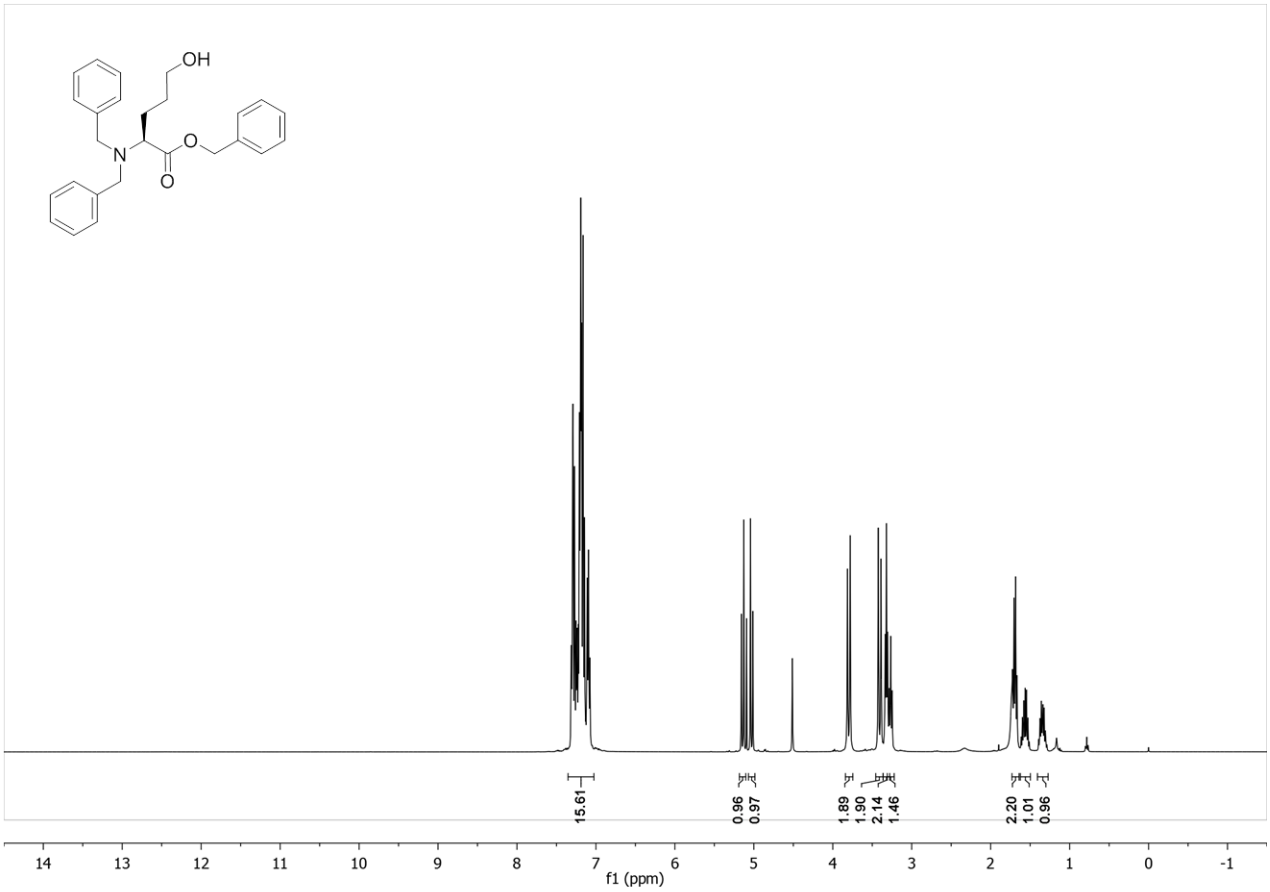


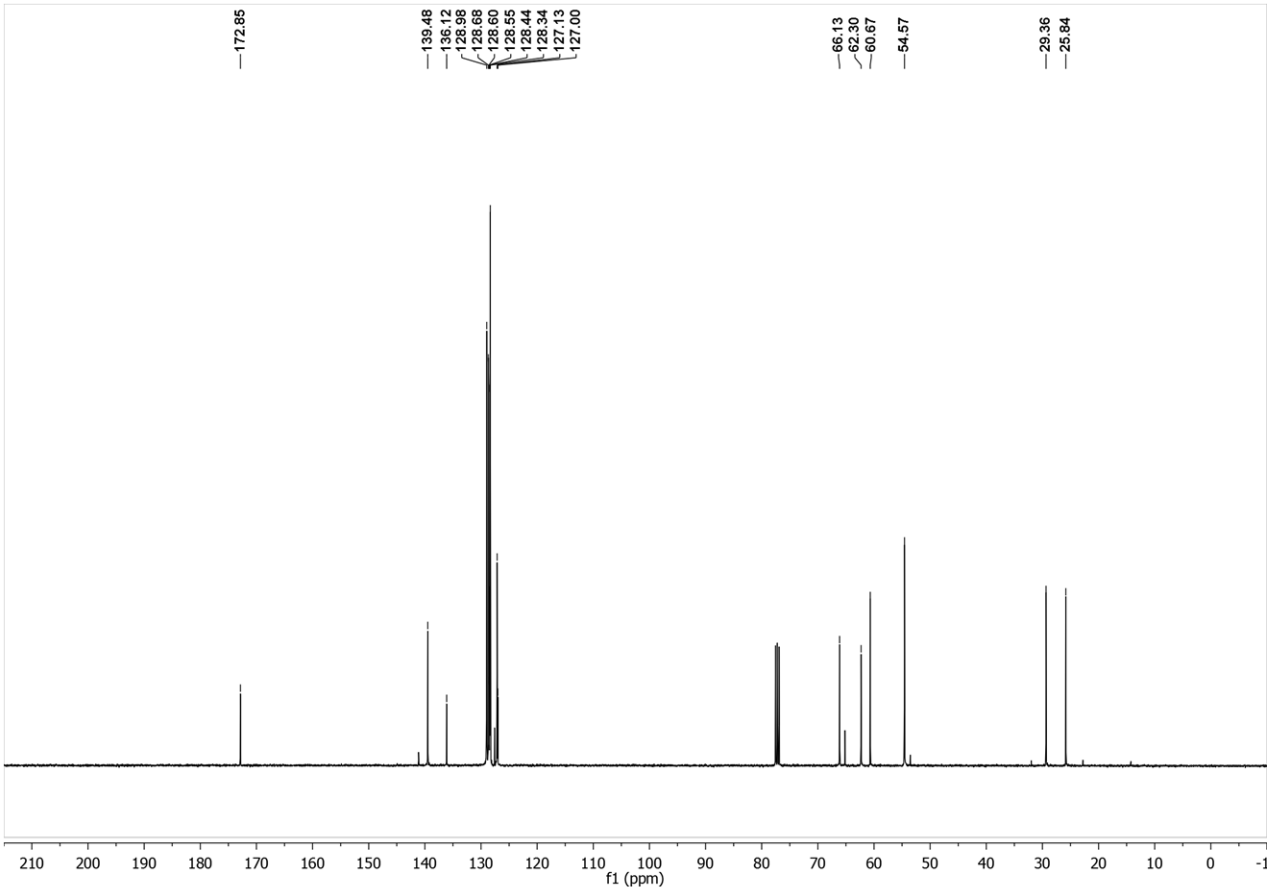
^{1}H and ^{13}C spectra of compound S54.



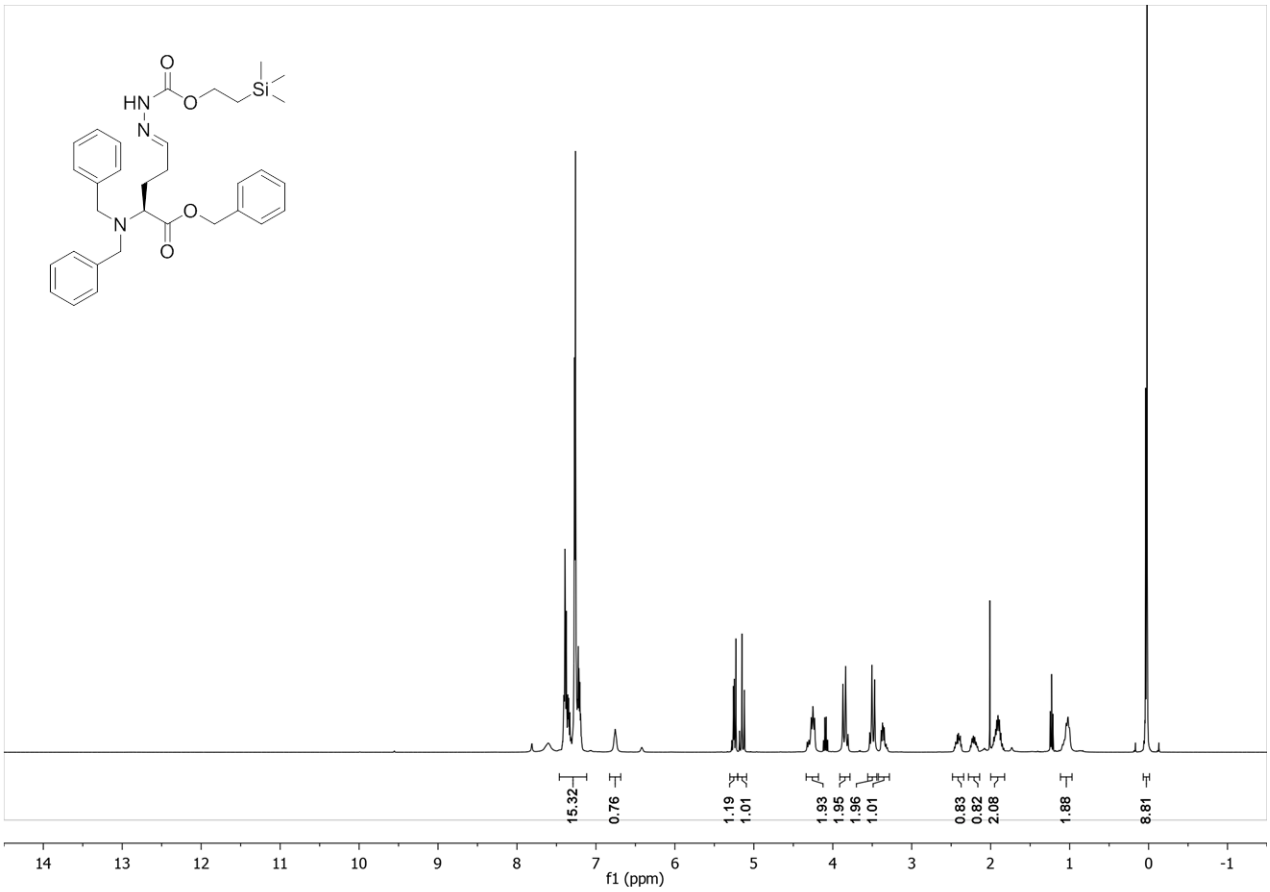


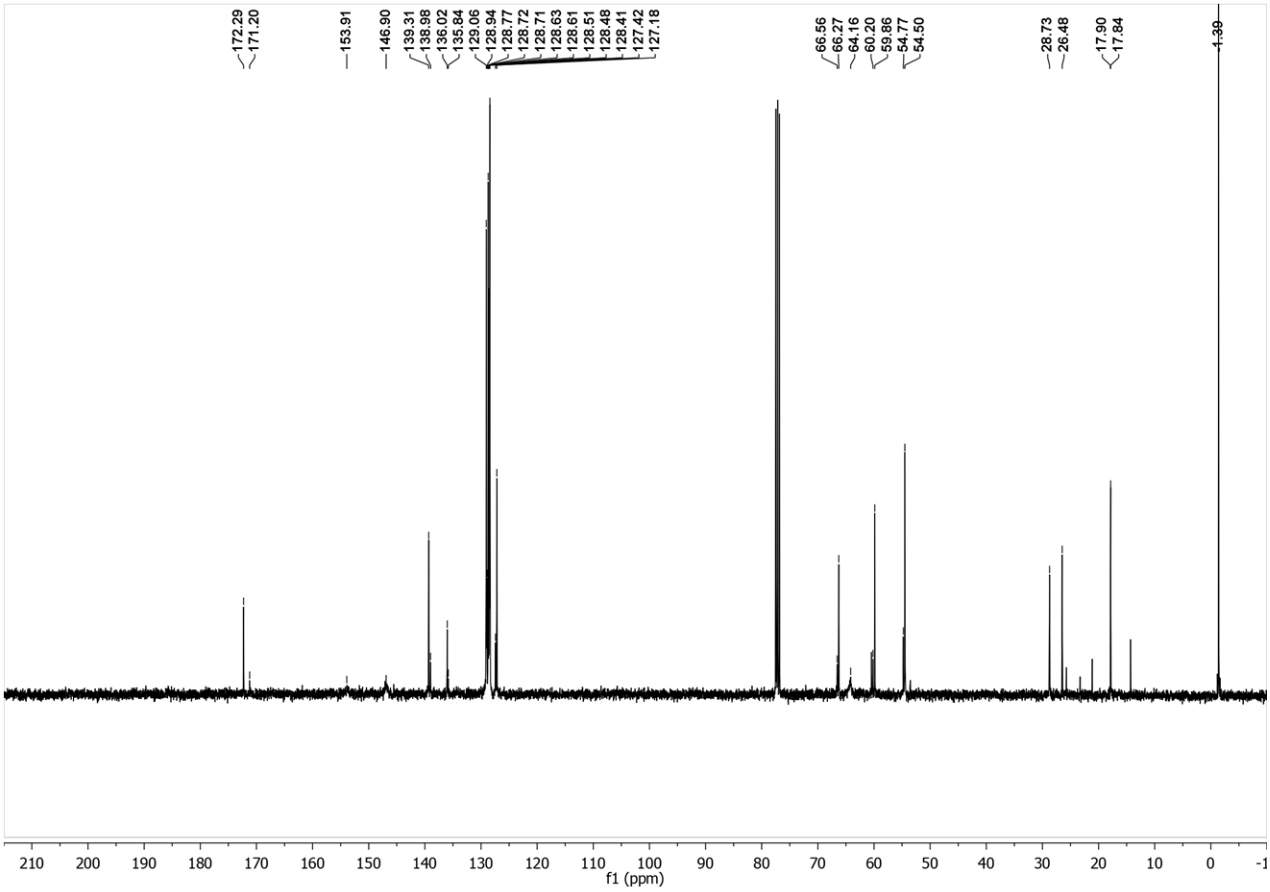
^{1}H and ^{13}C spectra of compound S55.



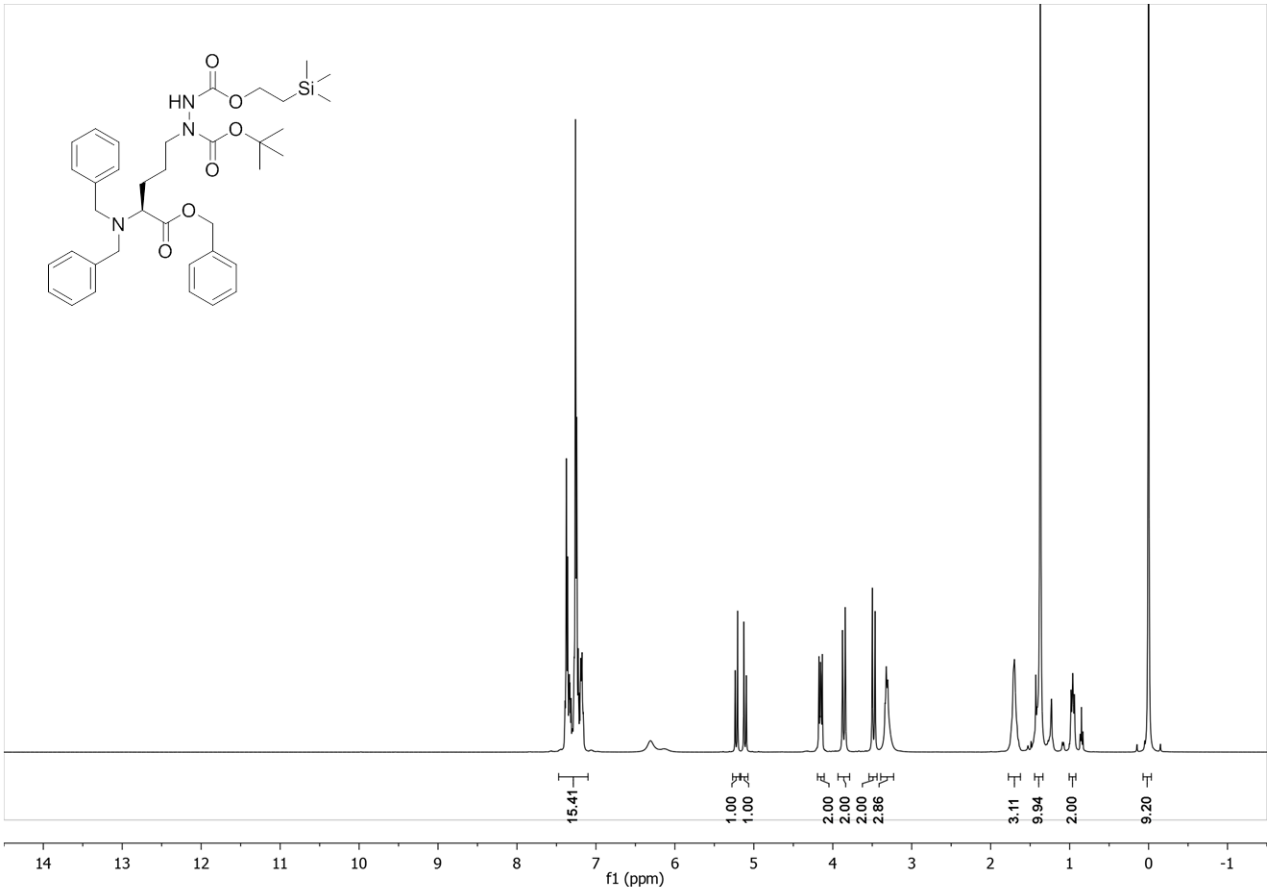


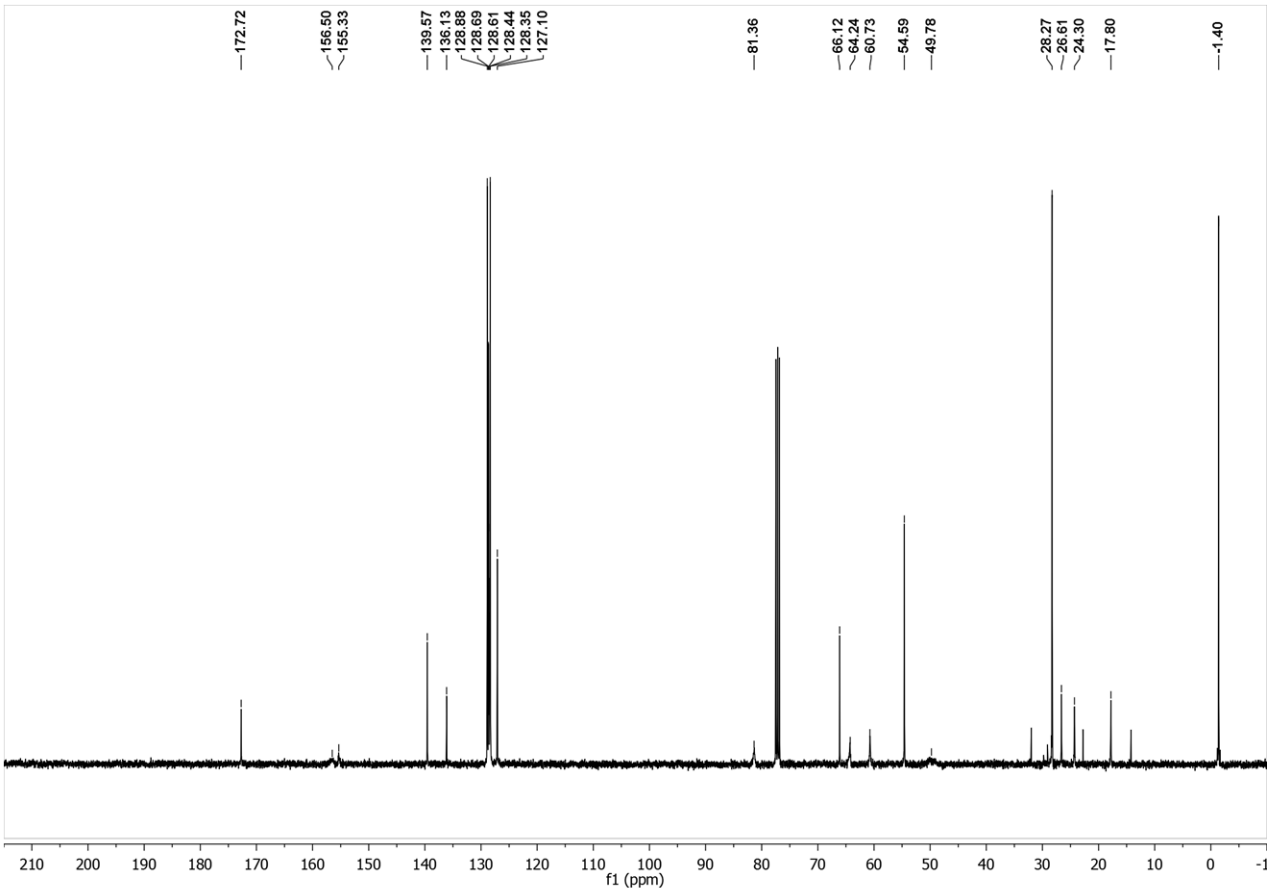
^{1}H and ^{13}C spectra of compound S56.



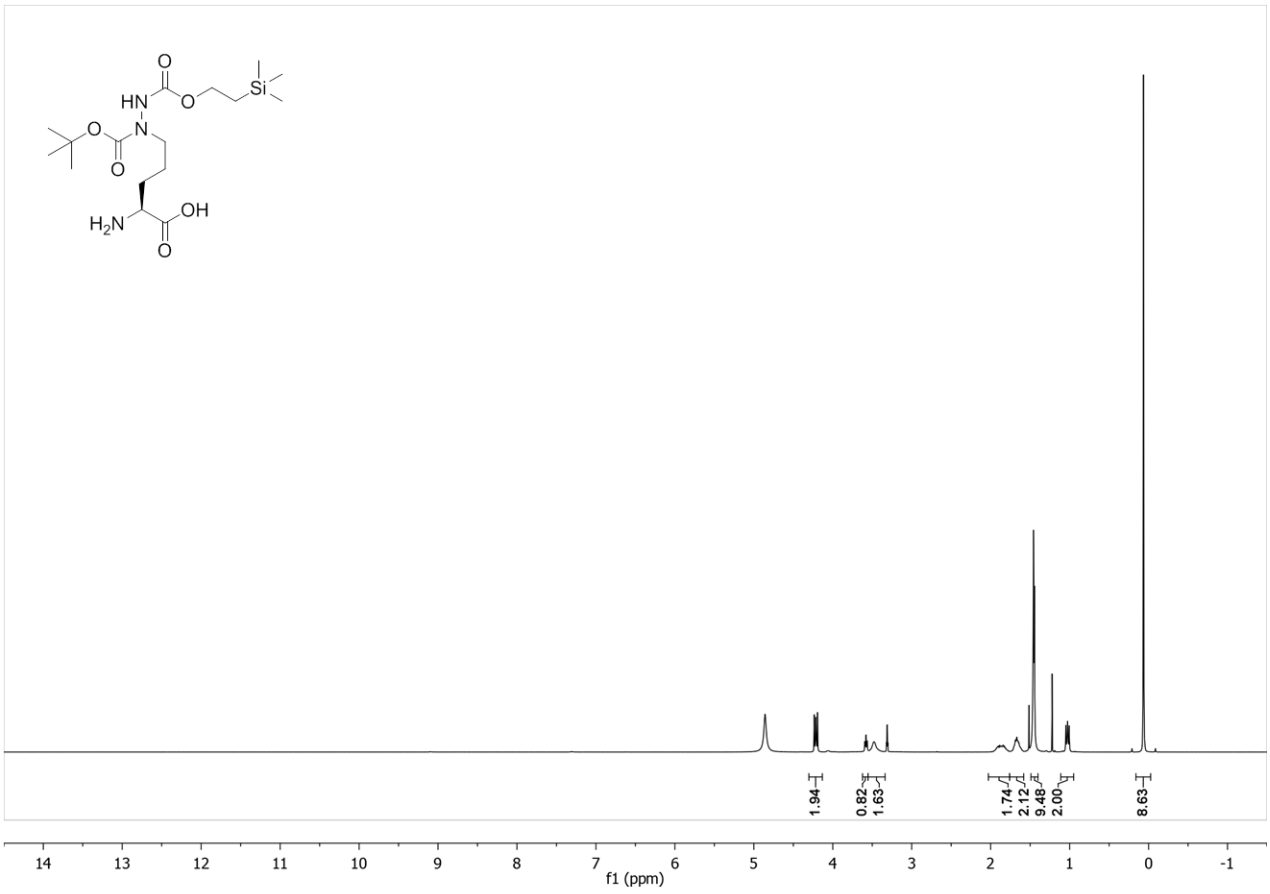


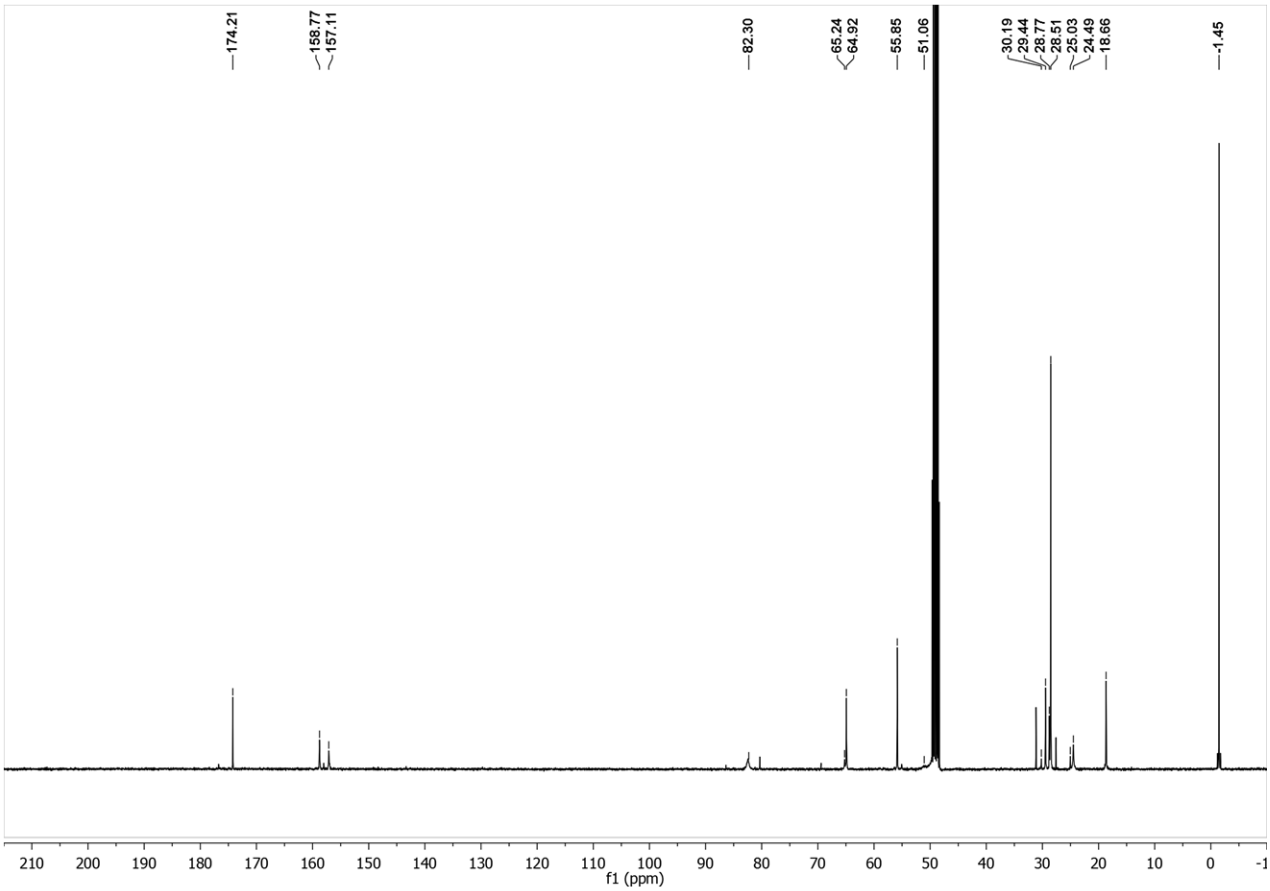
^{1}H and ^{13}C spectra of compound S57.



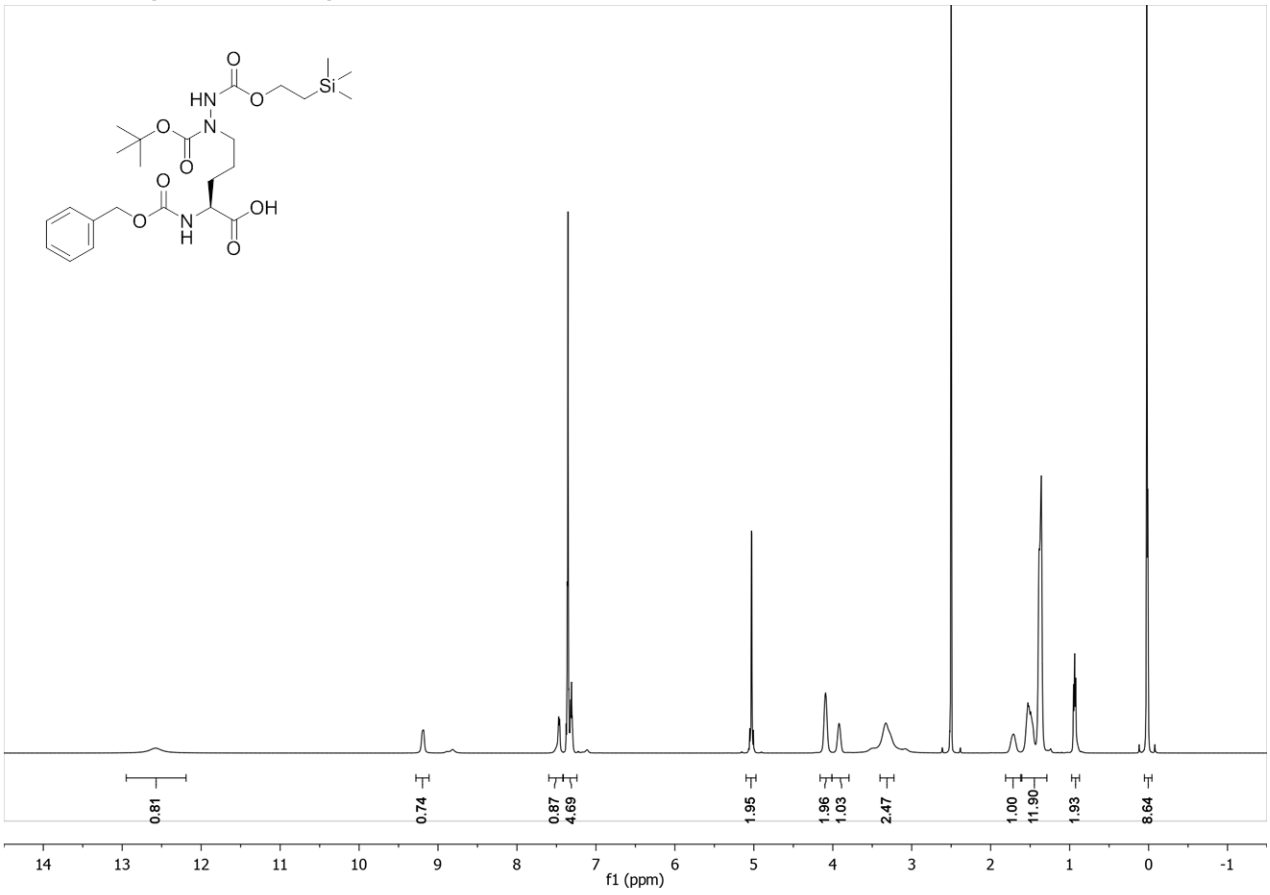


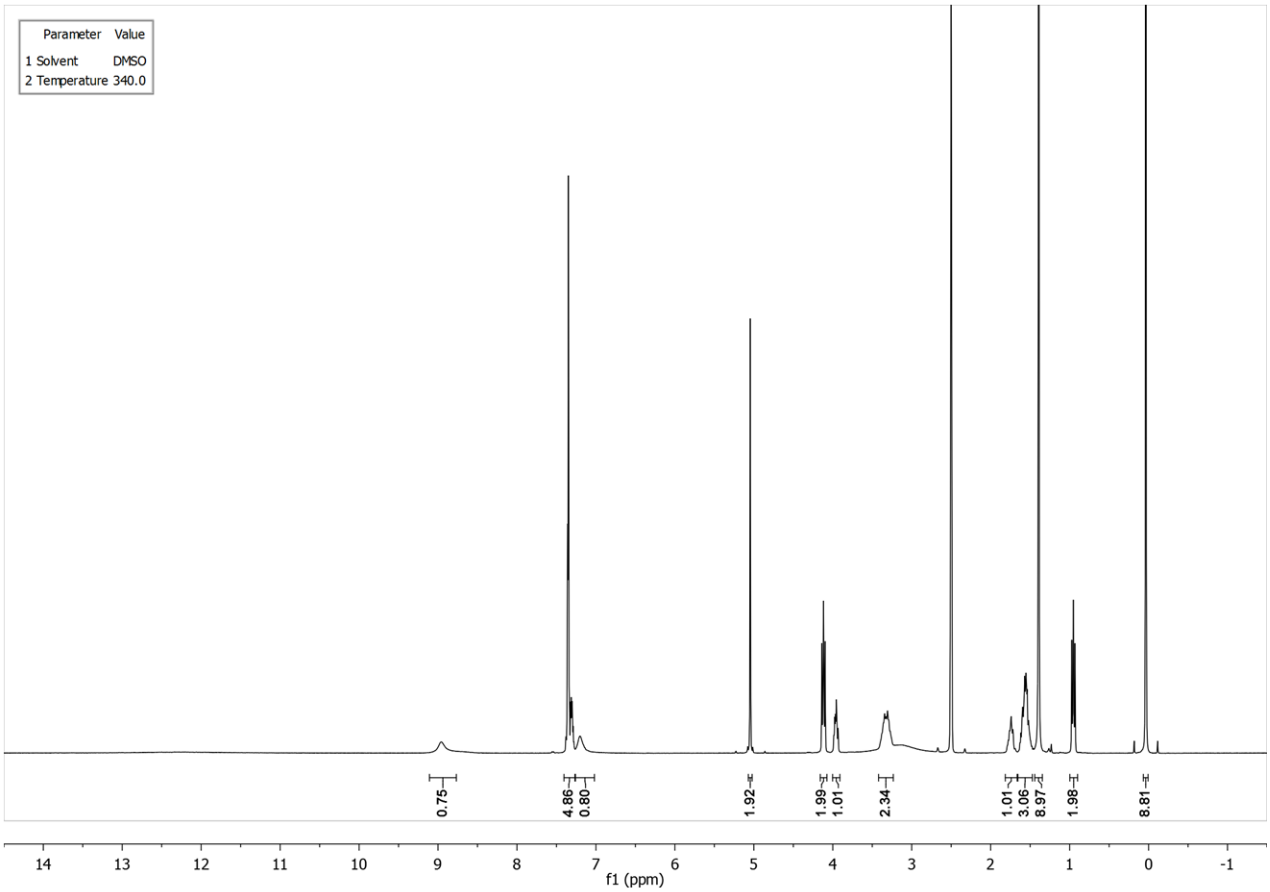
^{1}H and ^{13}C spectra of compound S58.

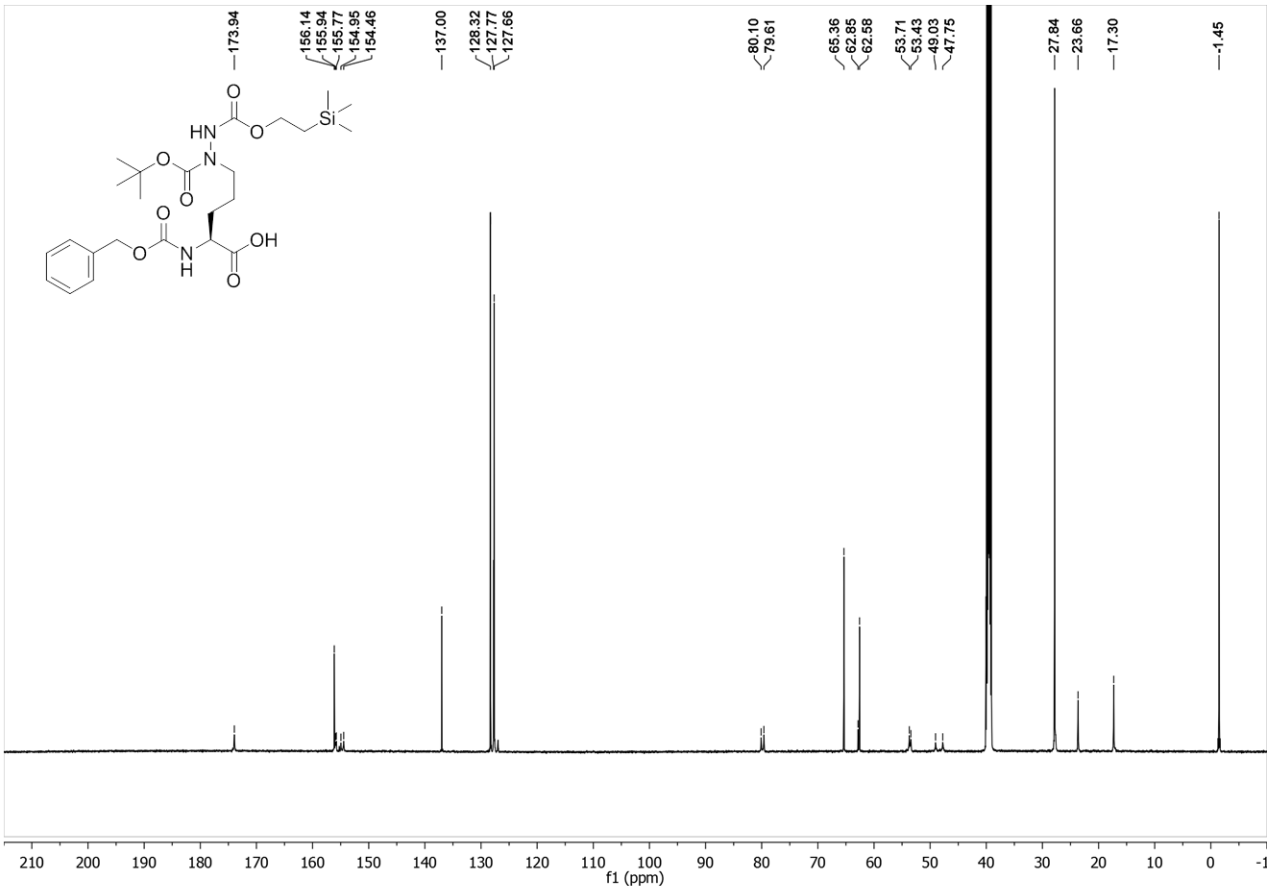


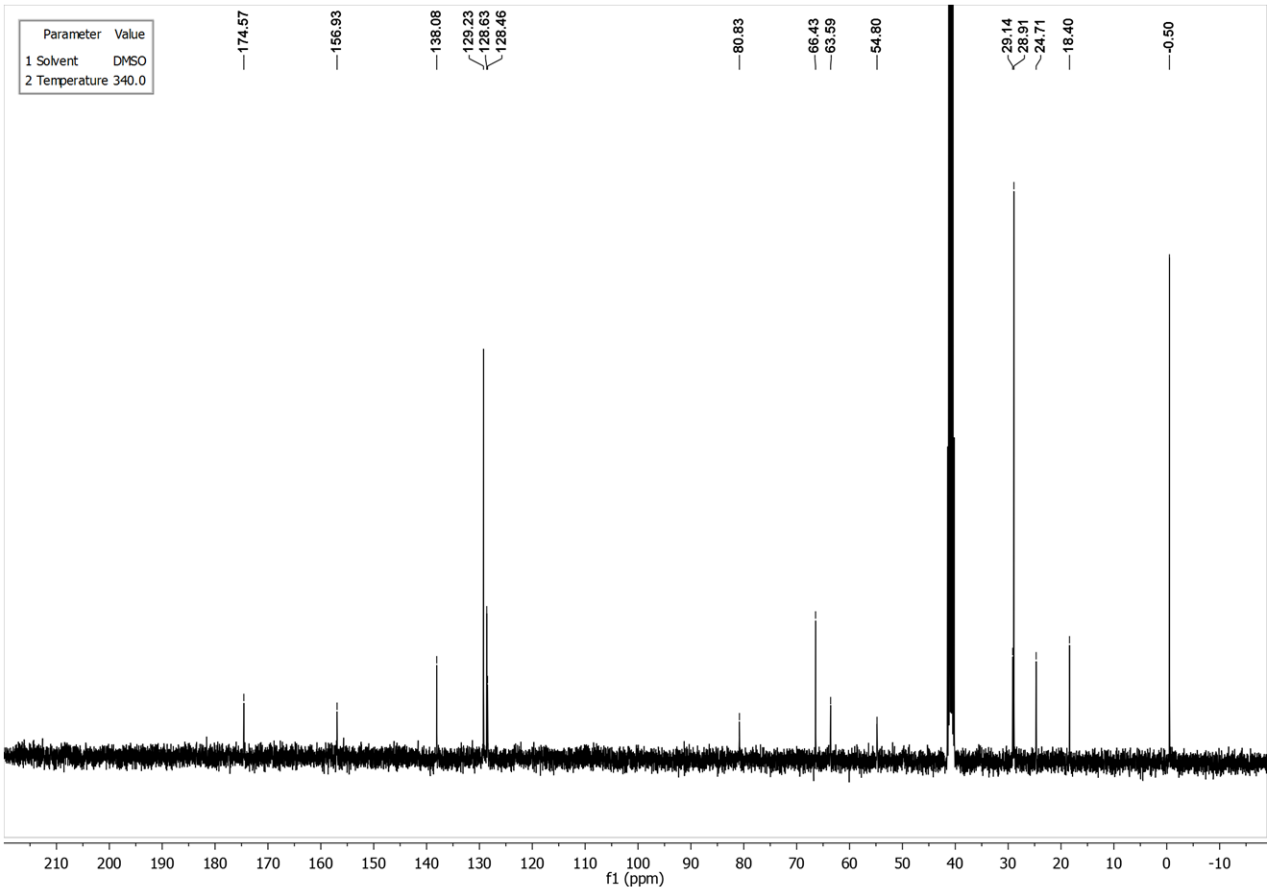


^{1}H and ^{13}C spectra of compound S59.

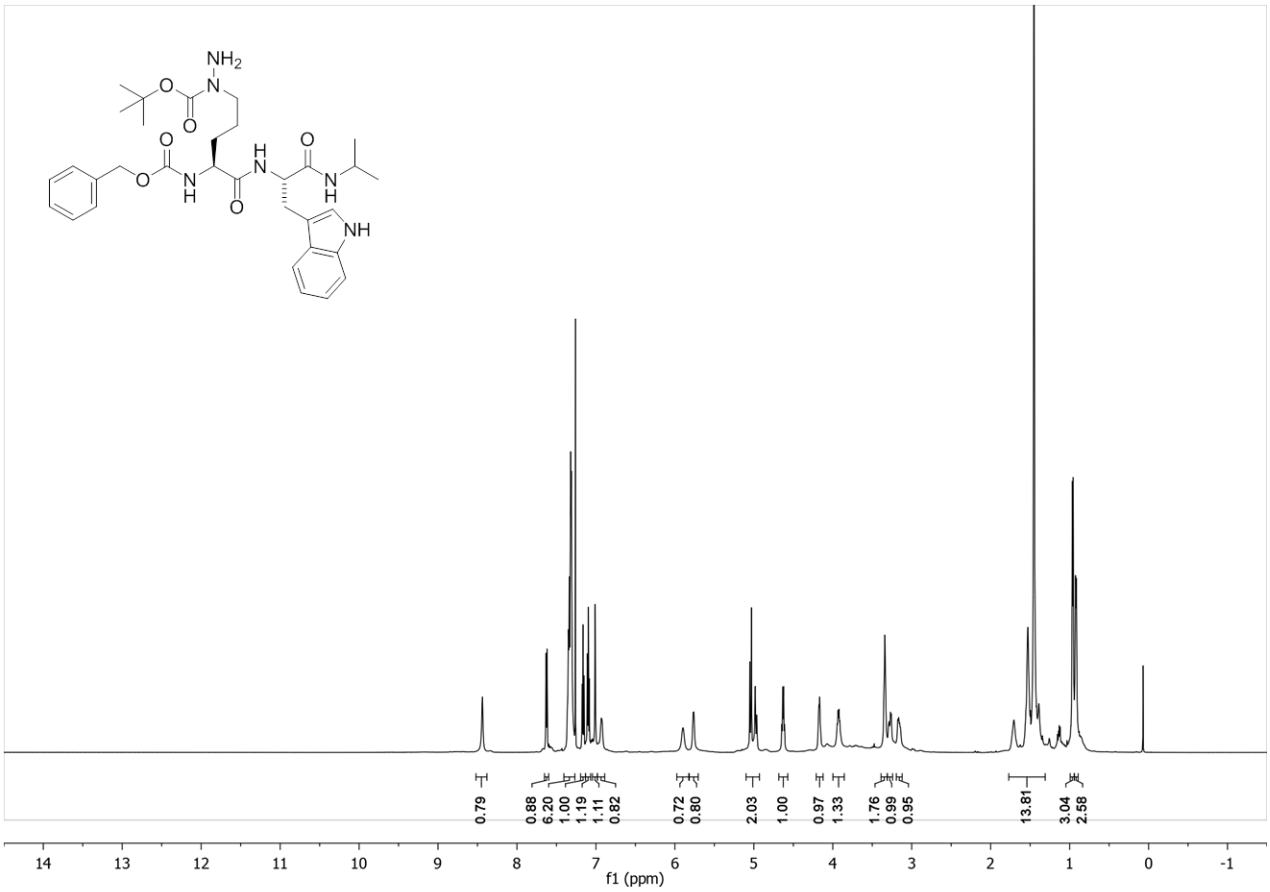


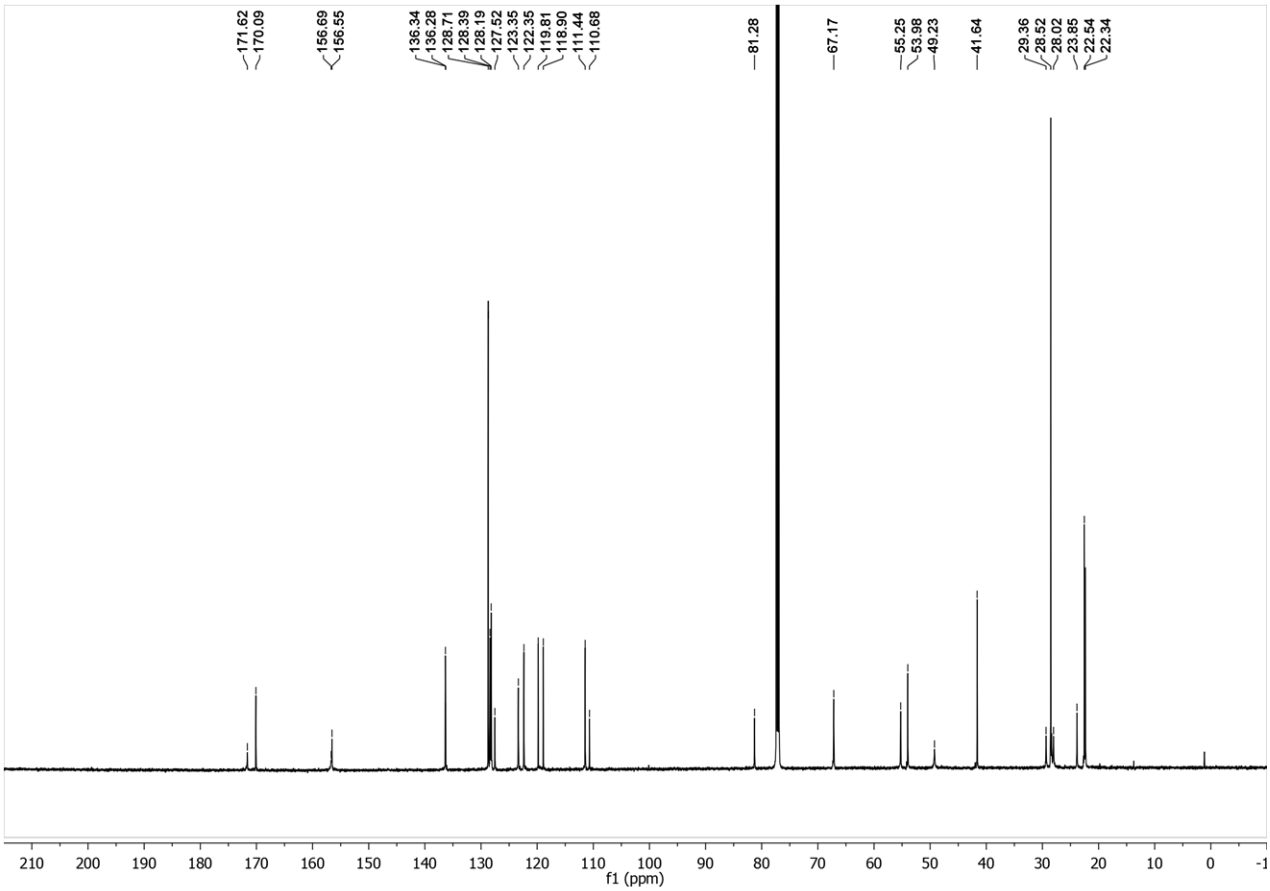



173.94
156.14
155.94
155.77
154.95
154.46
137.00
128.32
127.77
127.66
80.10
79.61
65.36
62.85
62.58
53.71
53.43
49.03
47.75
27.84
23.66
17.30
1.45
HN
Si
O
N
OH
H
f1 (ppm)

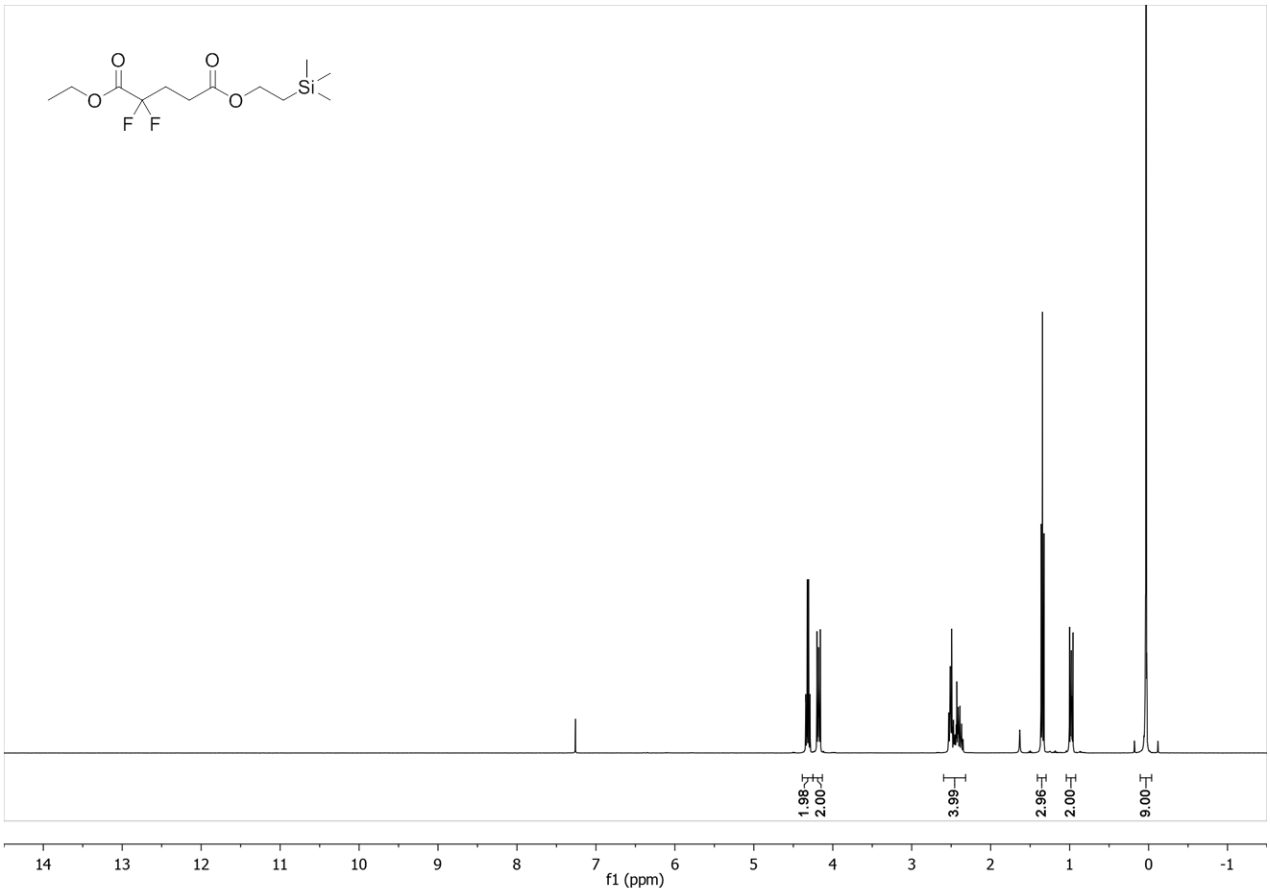

Parameter Value
1 Solvent DMSO
2 Temperature 340.0
174.57
156.93
138.08
129.23
128.63
128.46
80.83
66.43
63.59
54.80
29.14
28.91
24.71
18.40
0.50
f1 (ppm)

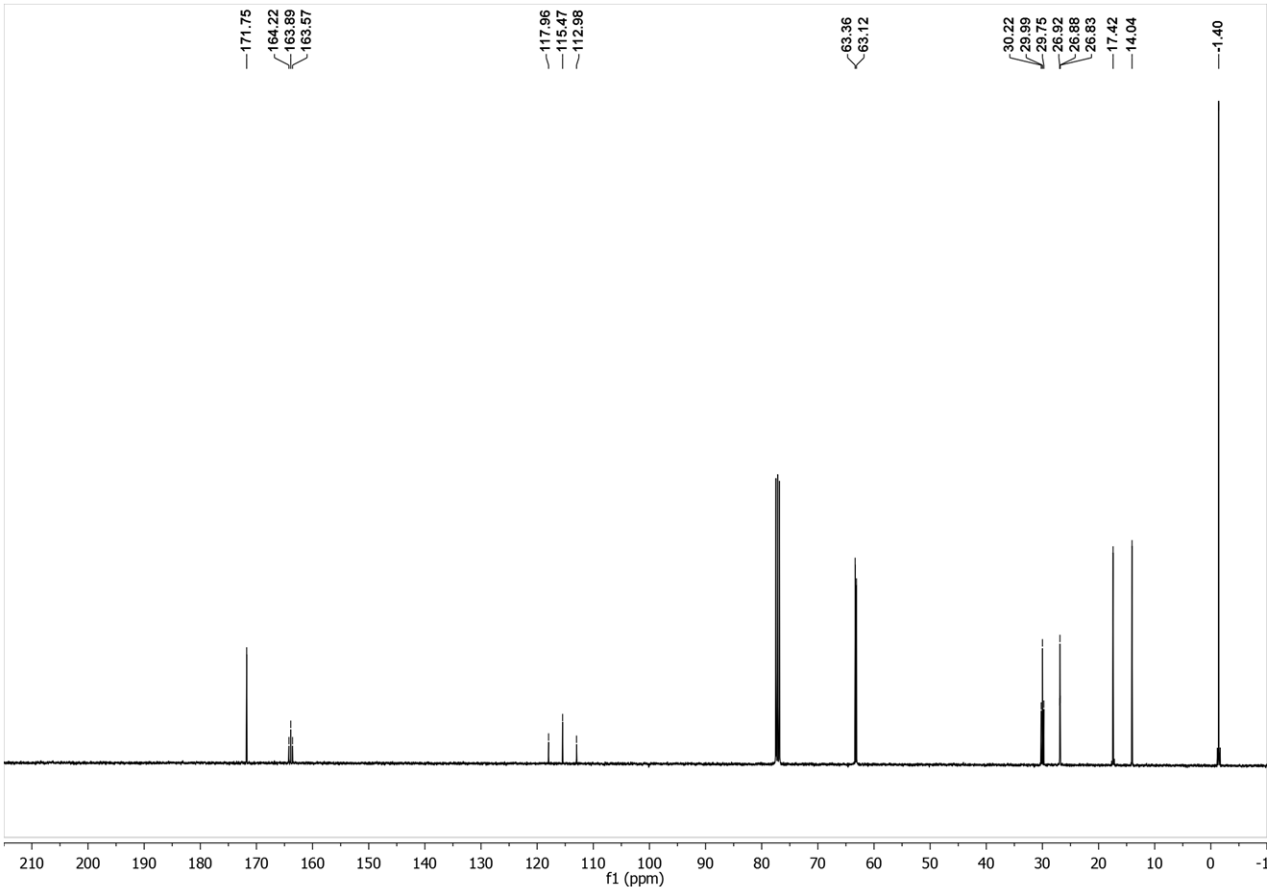
^{1}H and ^{13}C spectra of compound S60.

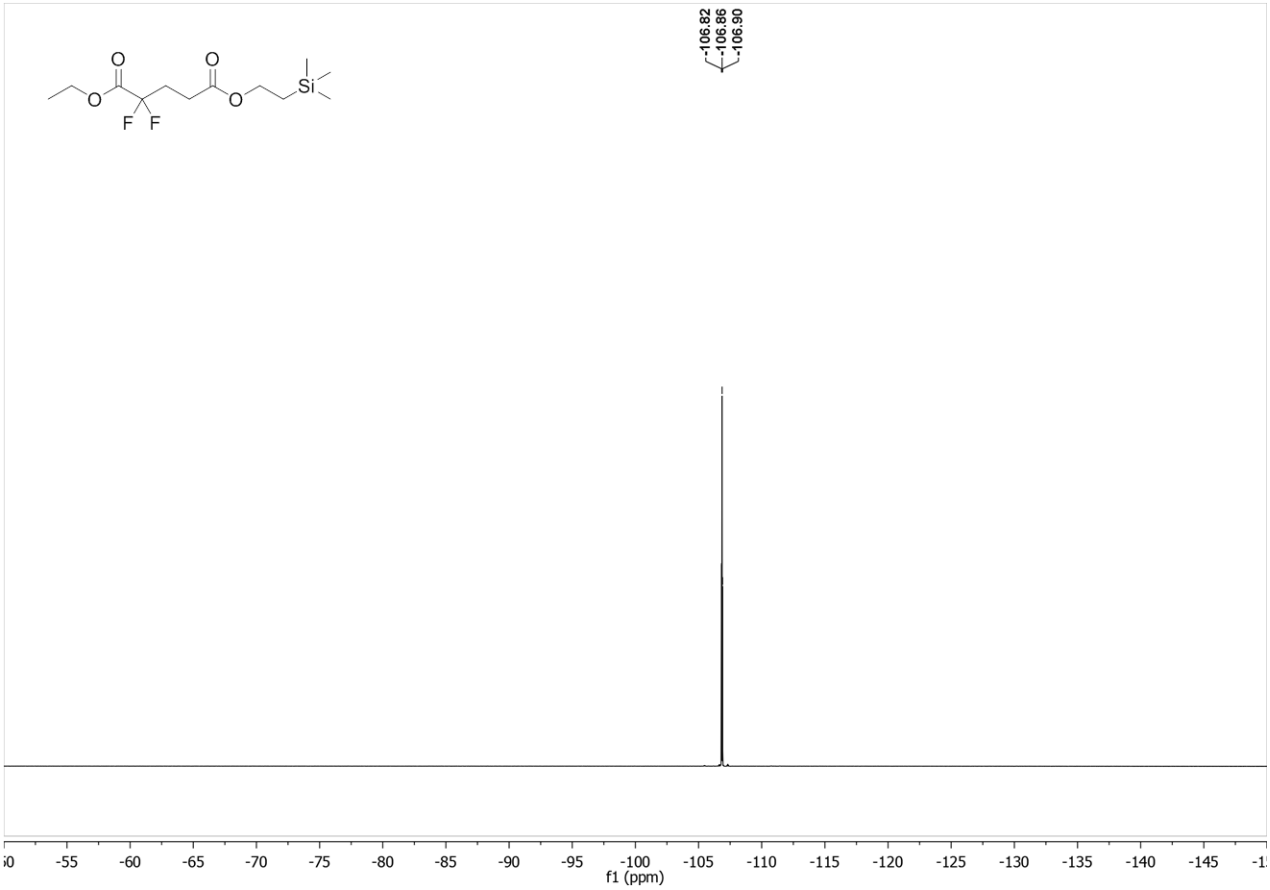




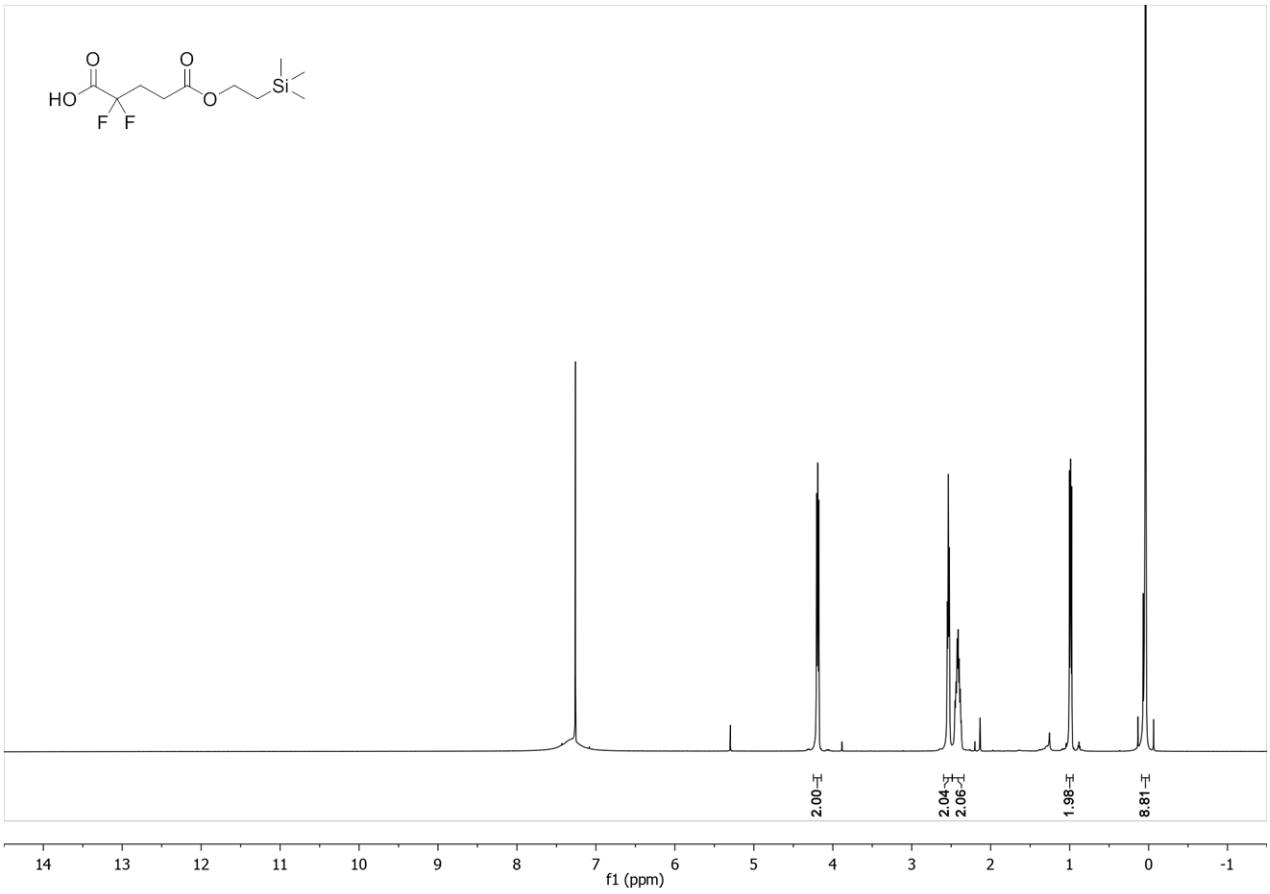
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S61.

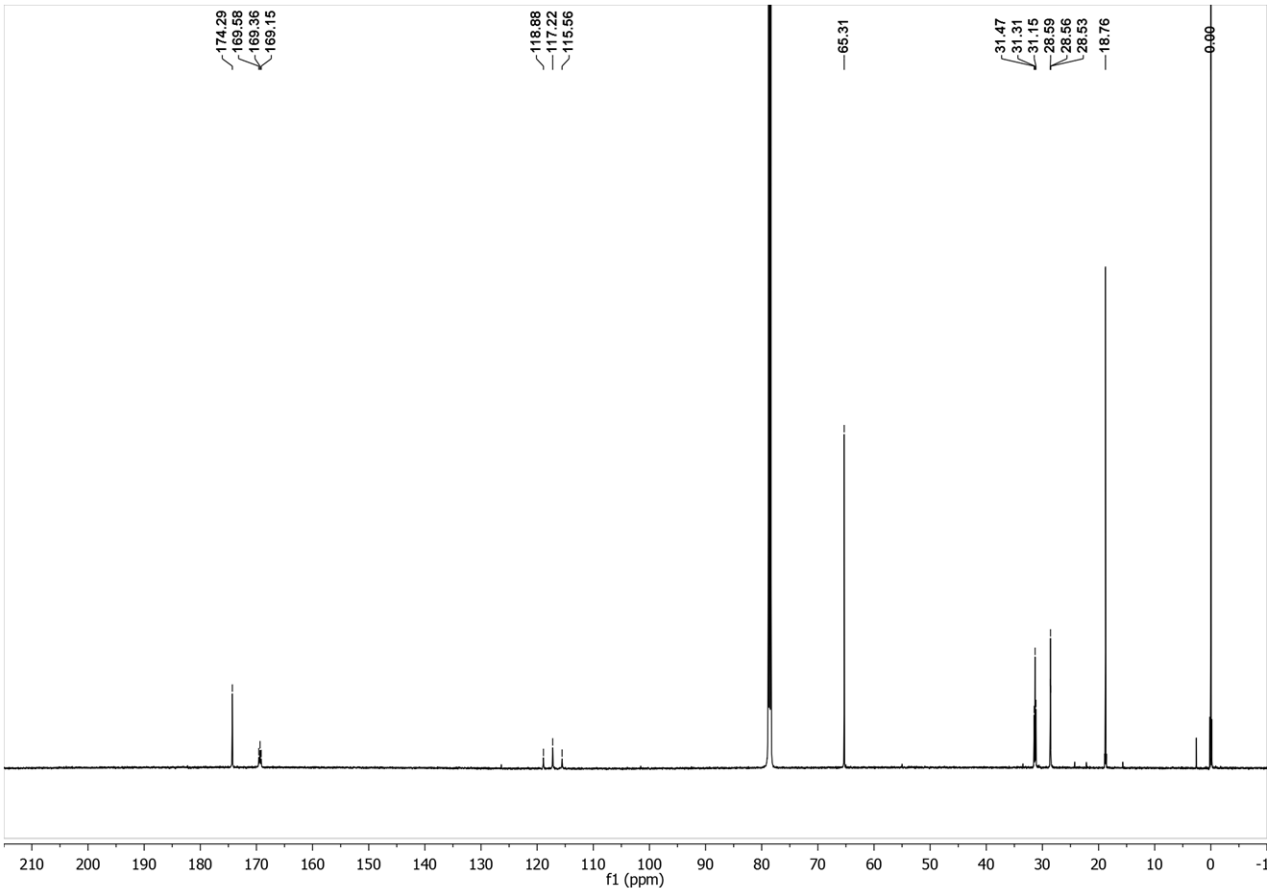


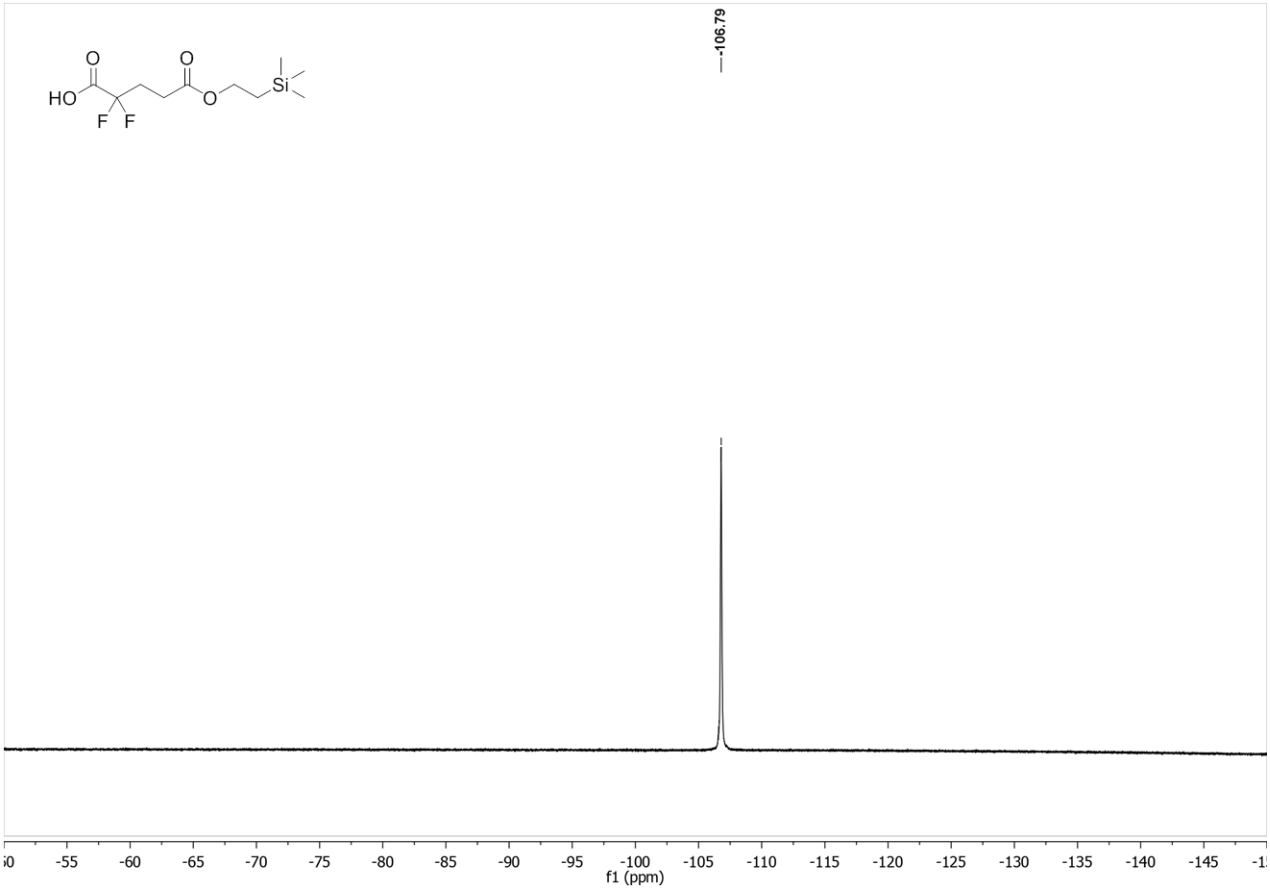



-106.82
-106.86
-106.90
O
O
O
Si
F F
-55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145
f1 (ppm)

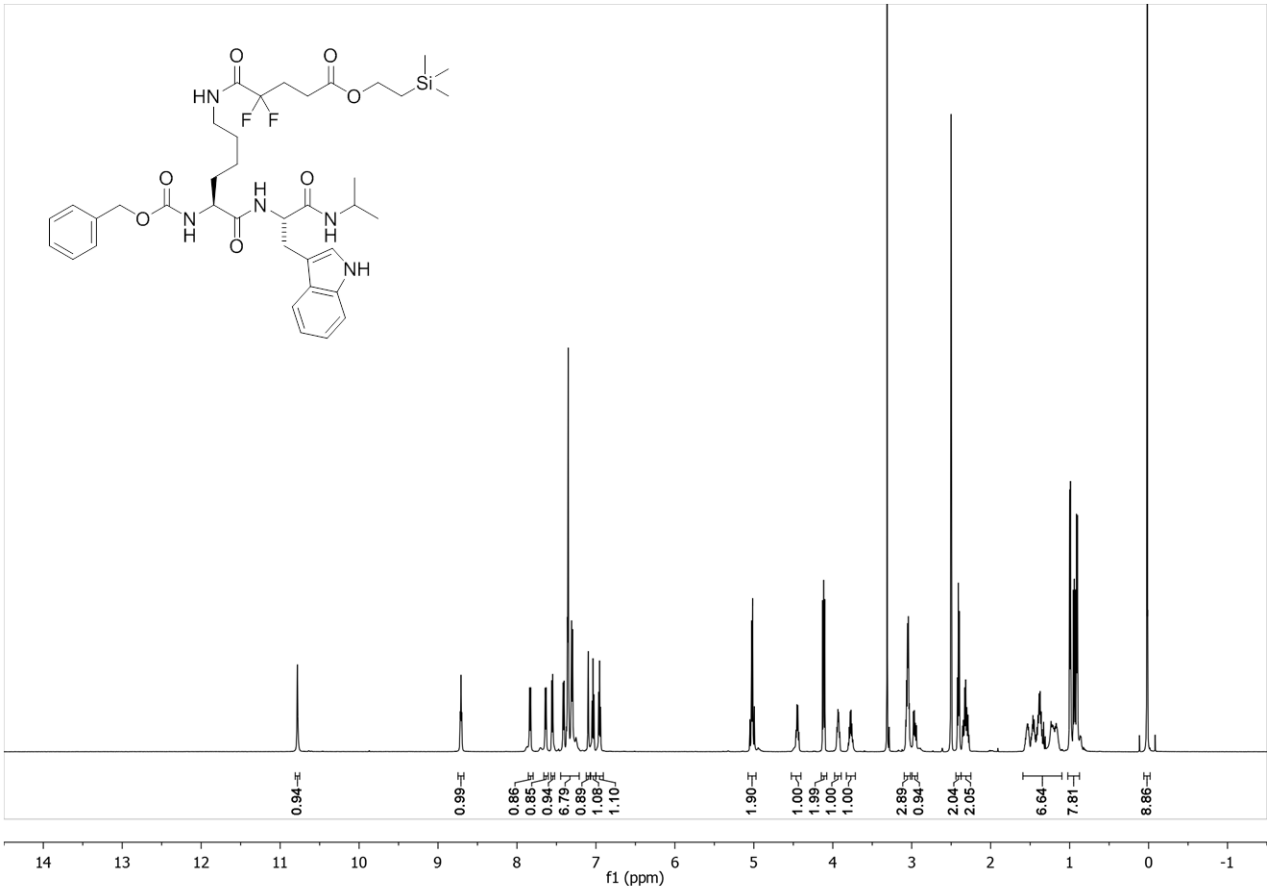
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S61.

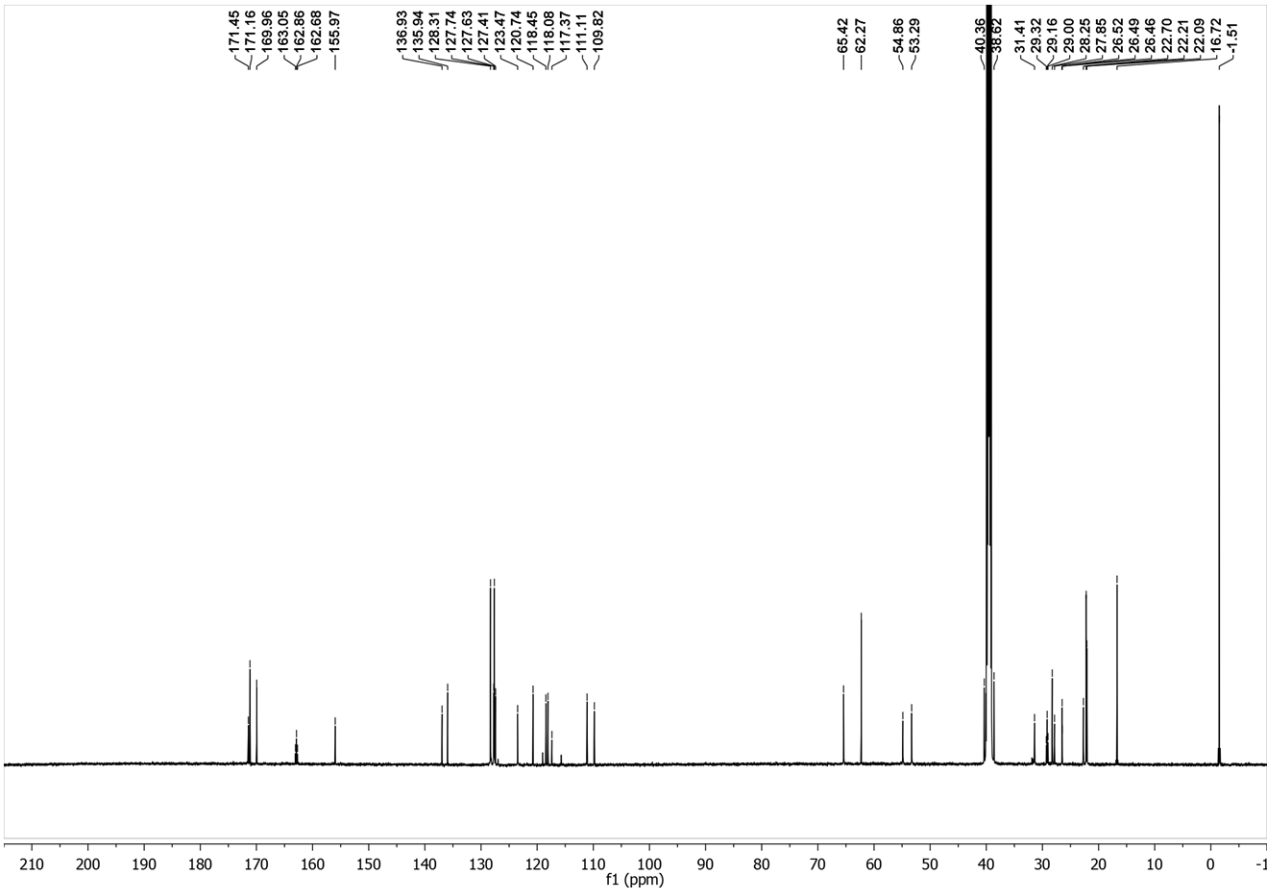


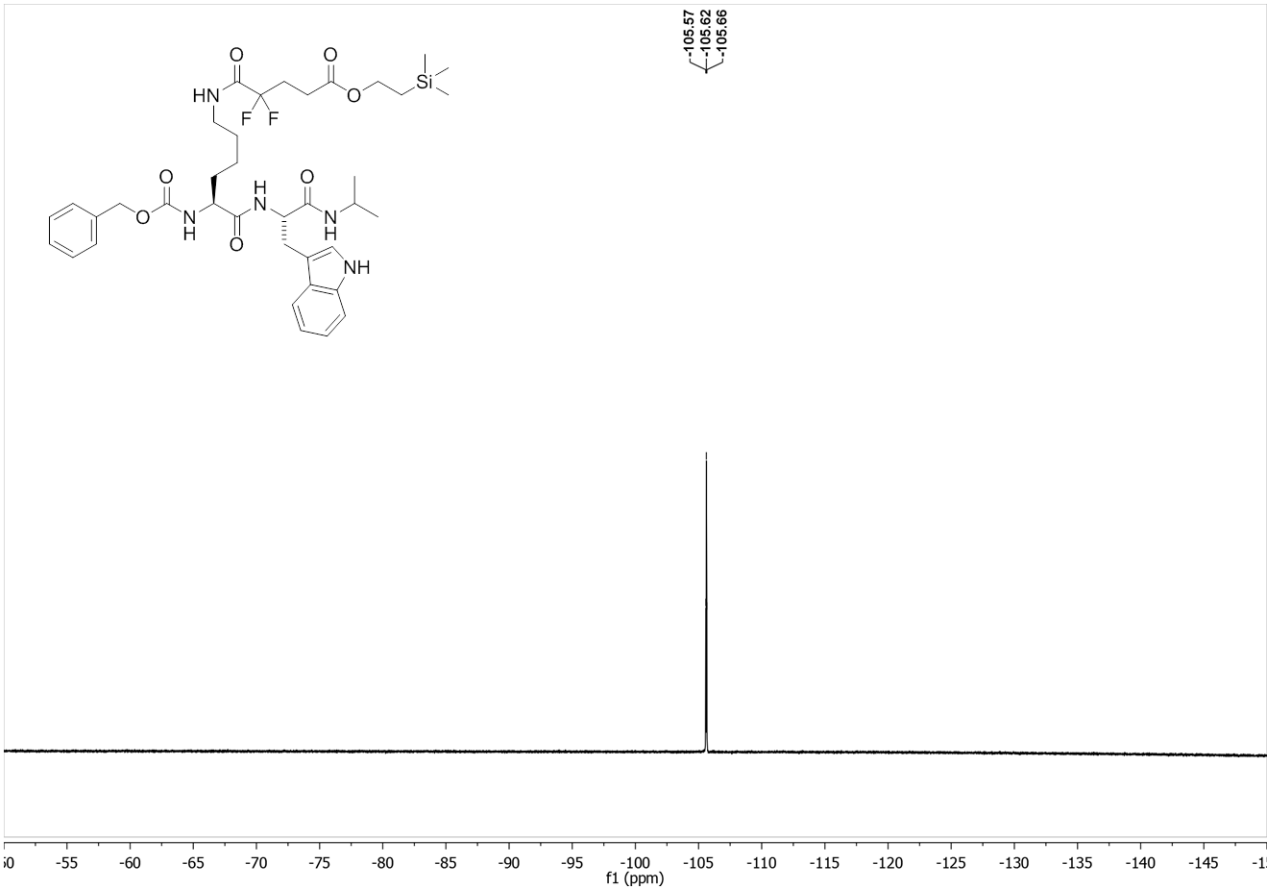



-106.79
HO
O
O
F F
O
Si
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

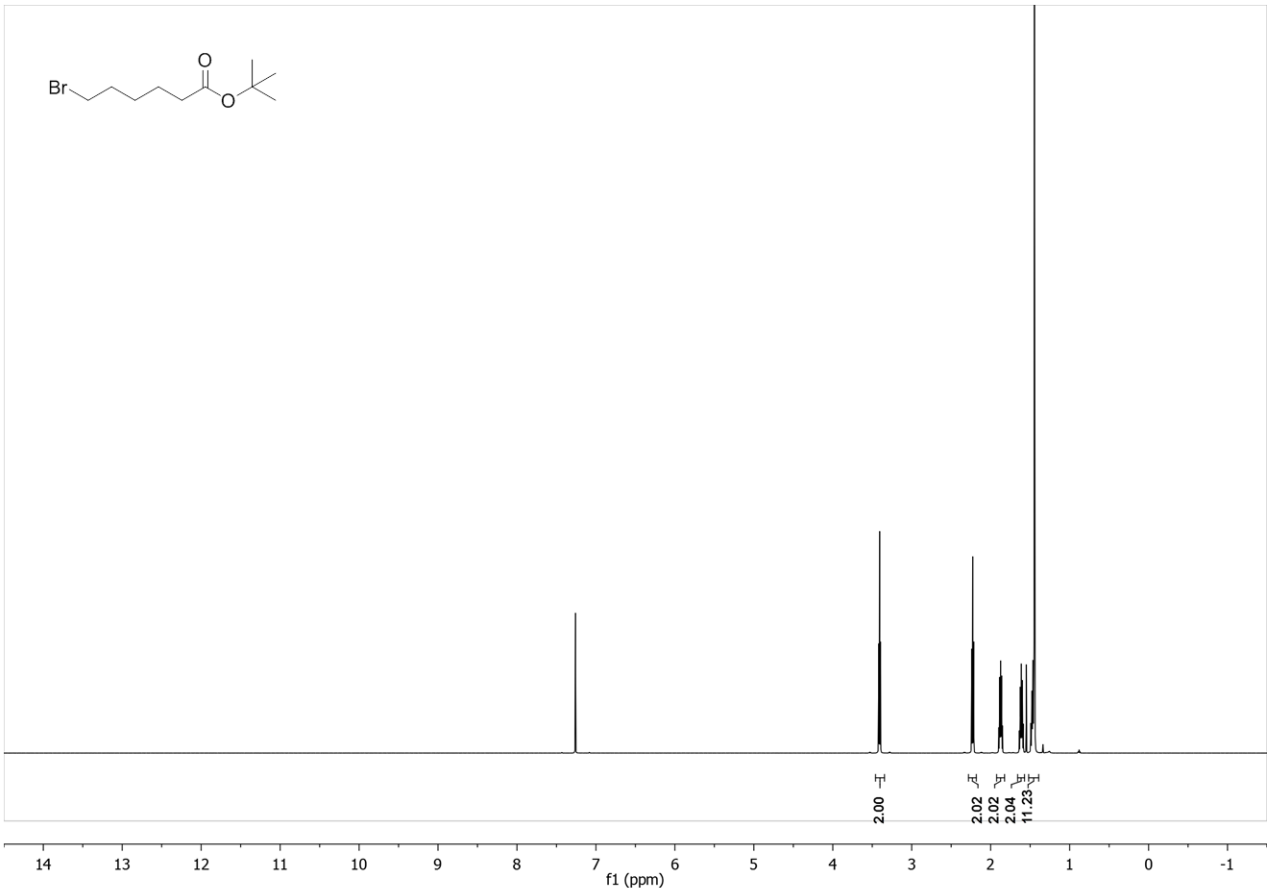
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S63.

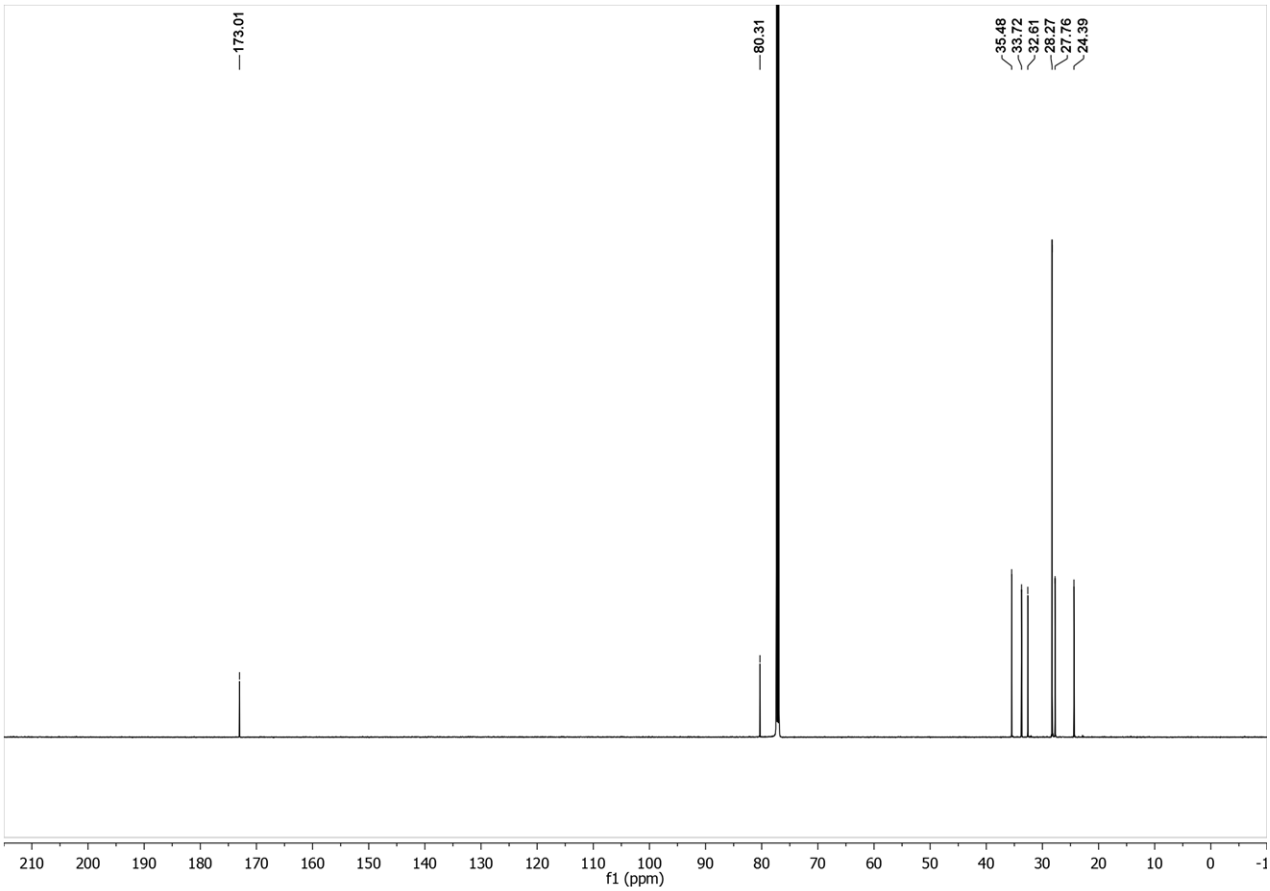




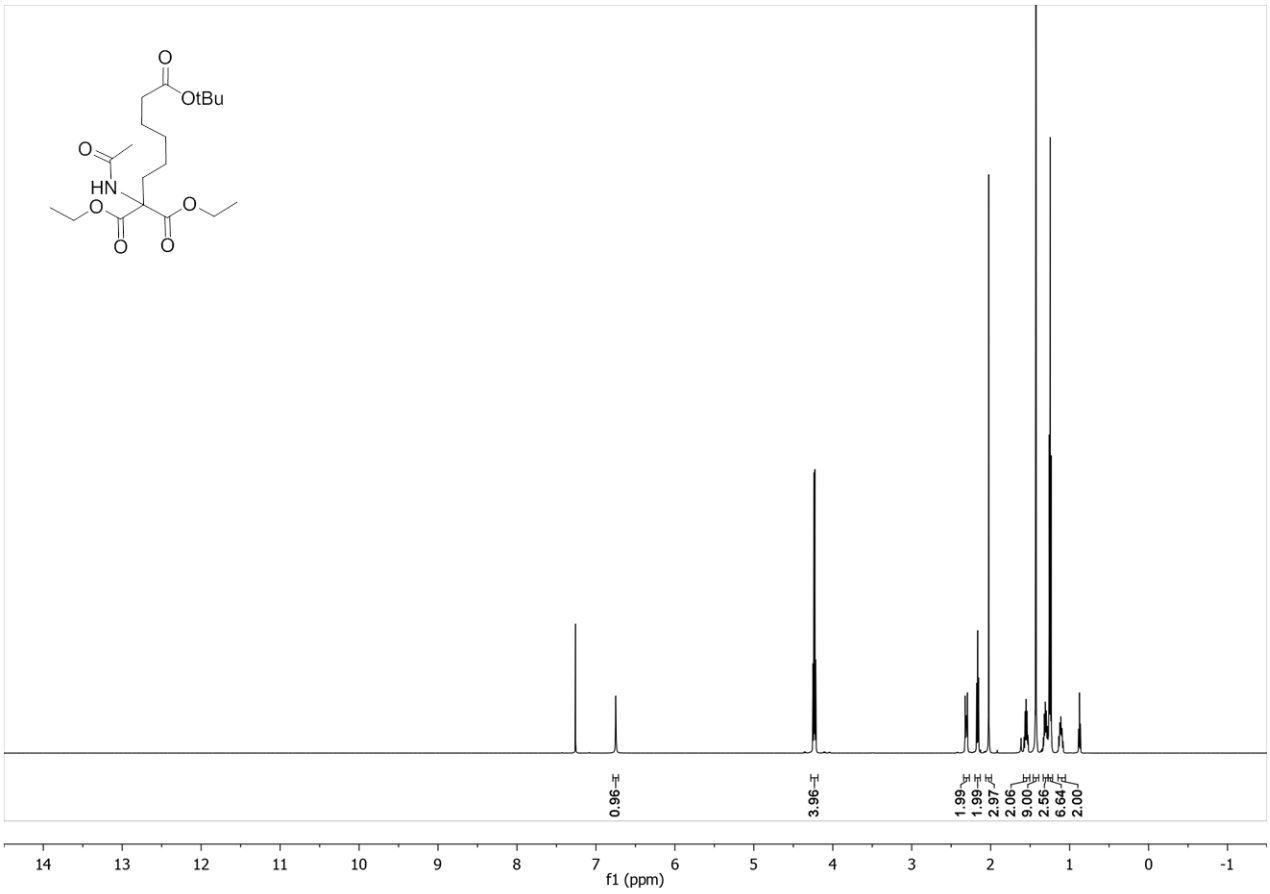

-105.57
-105.62
-105.66
HN
F F
O
O
Si
O
O
N
H
H
N
O
O
N
H
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

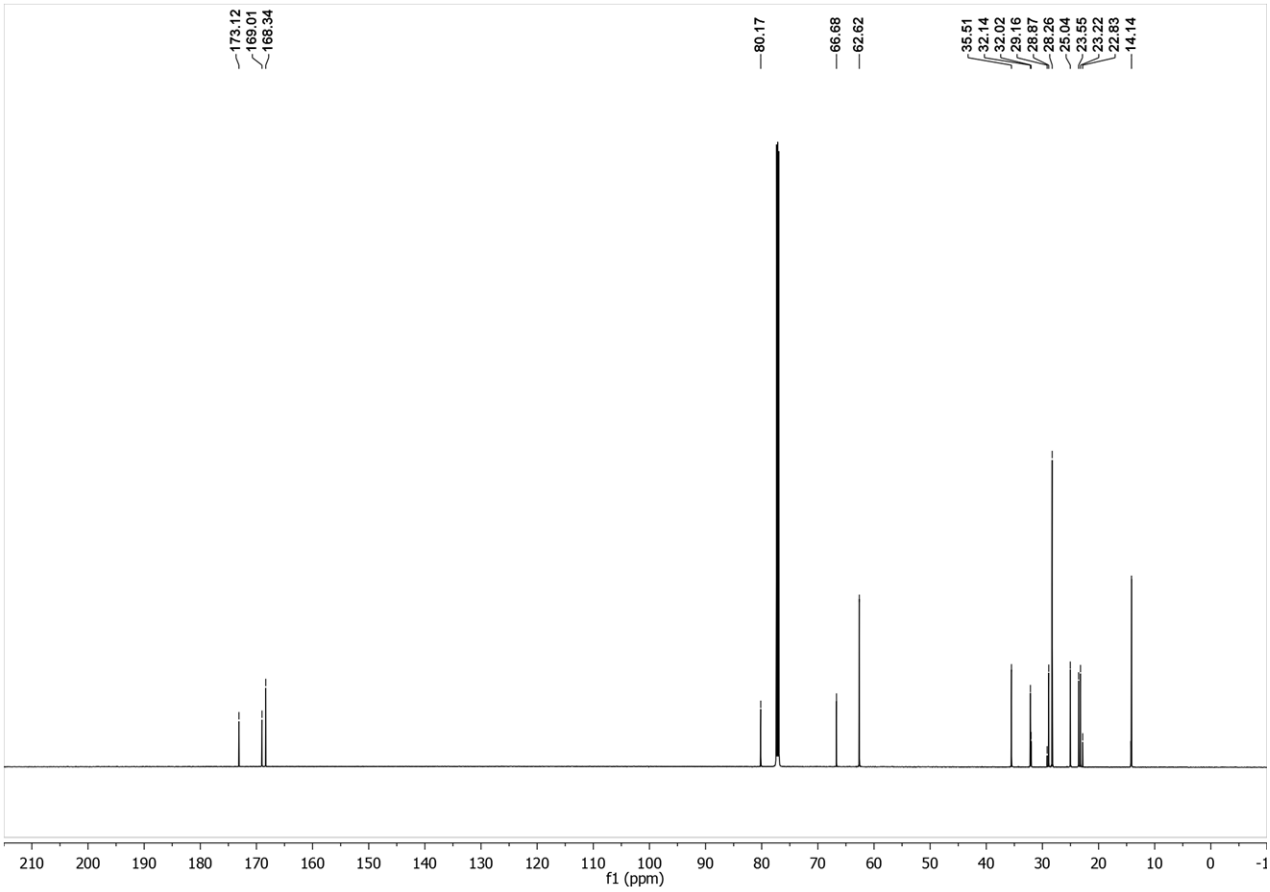
^{1}H and ^{13}C spectra of compound S64.



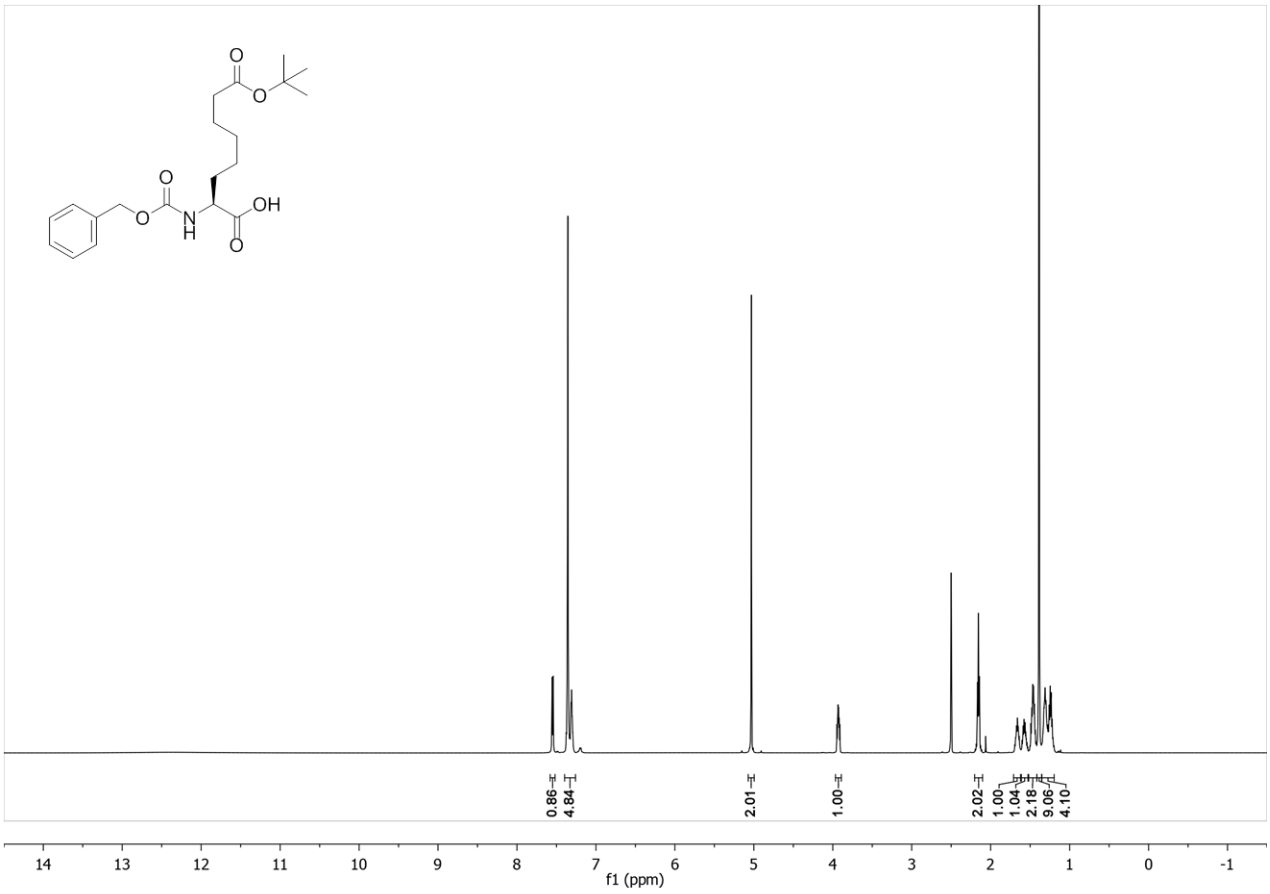


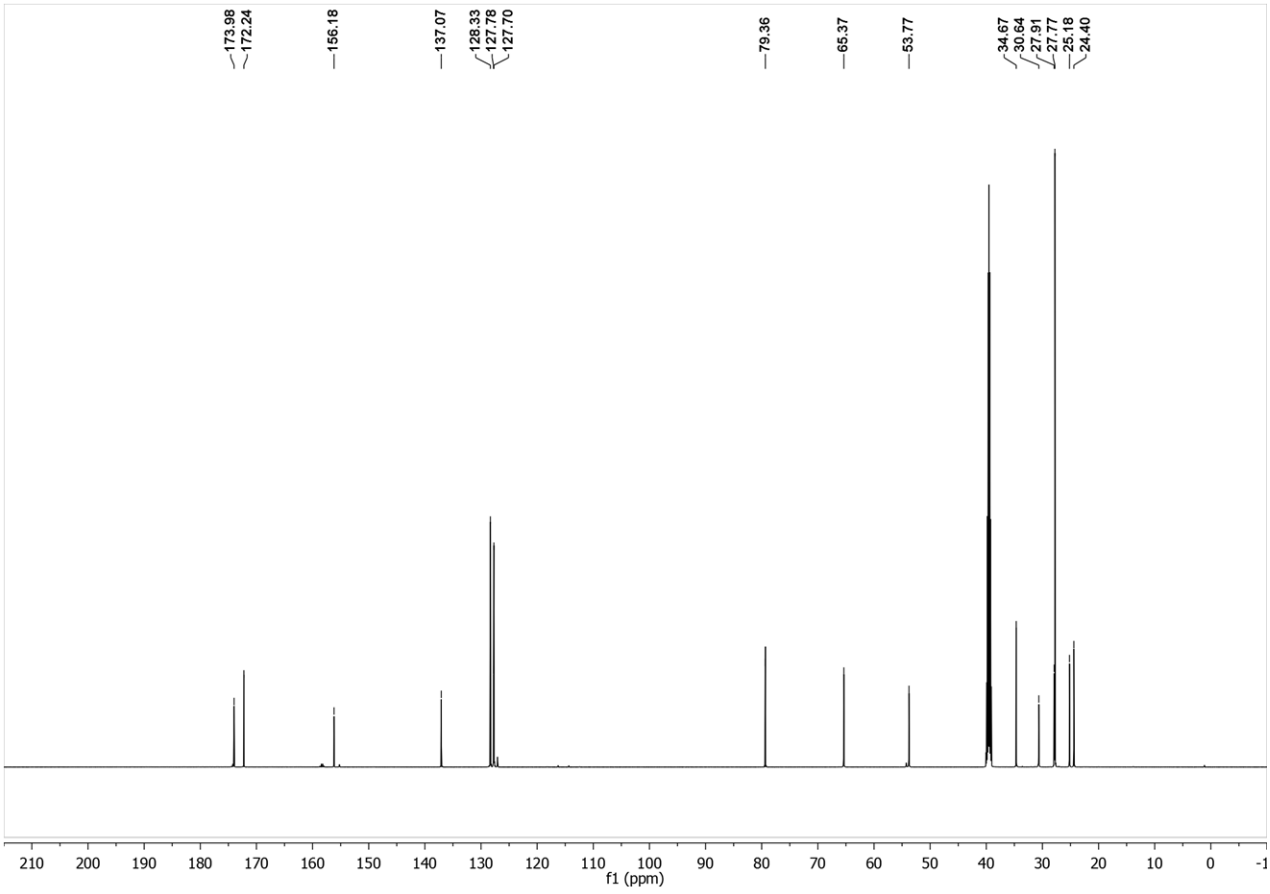
^{1}H and ^{13}C spectra of compound S65.



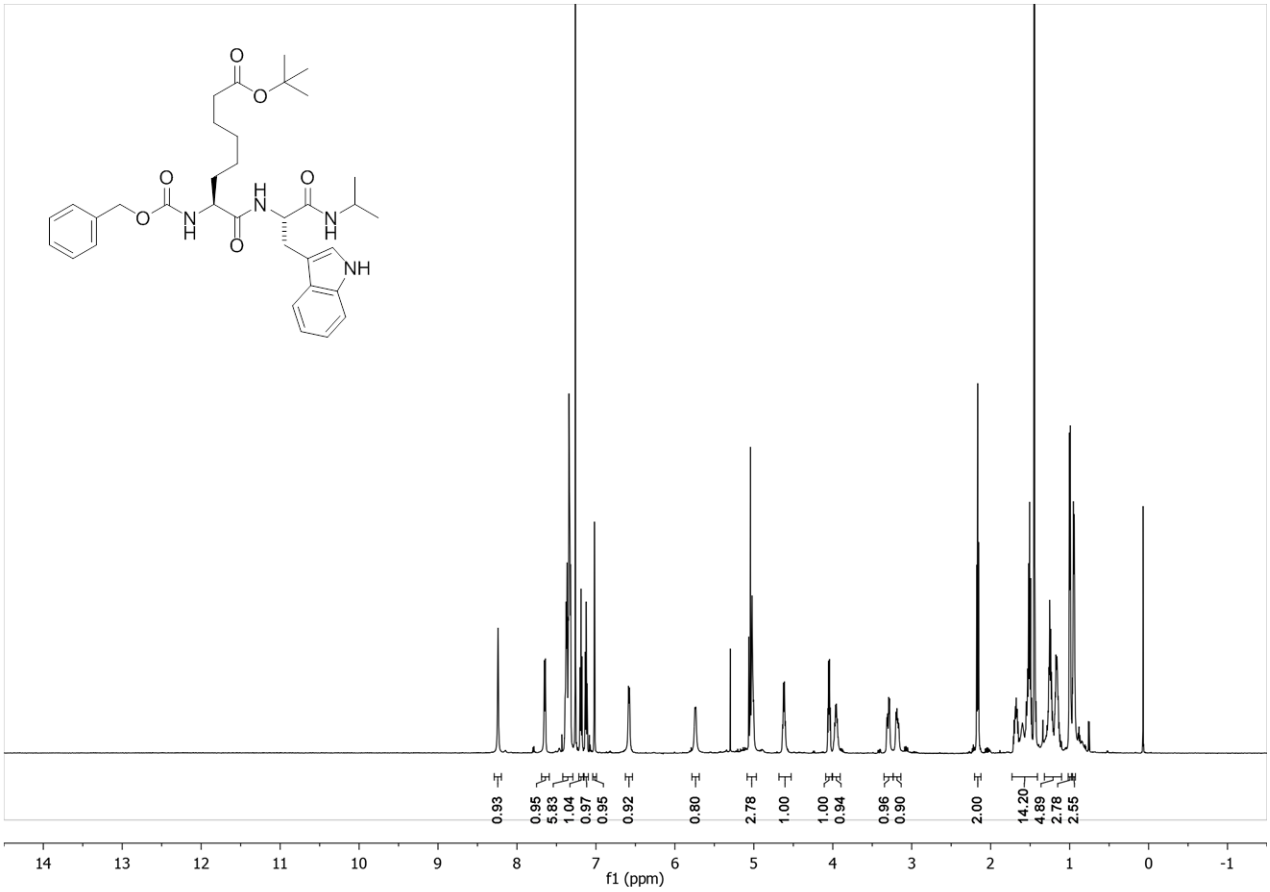


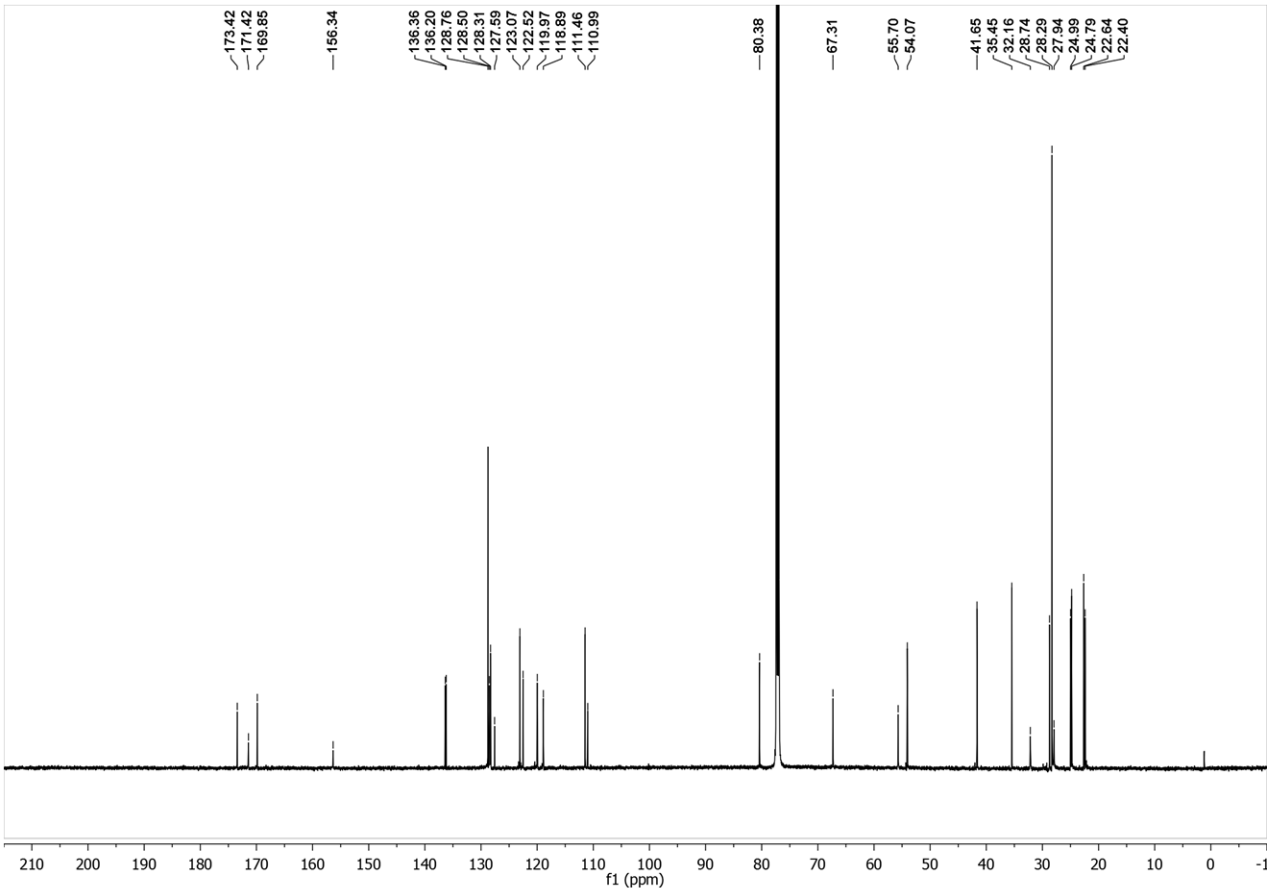
^{1}H and ^{13}C spectra of compound S66.



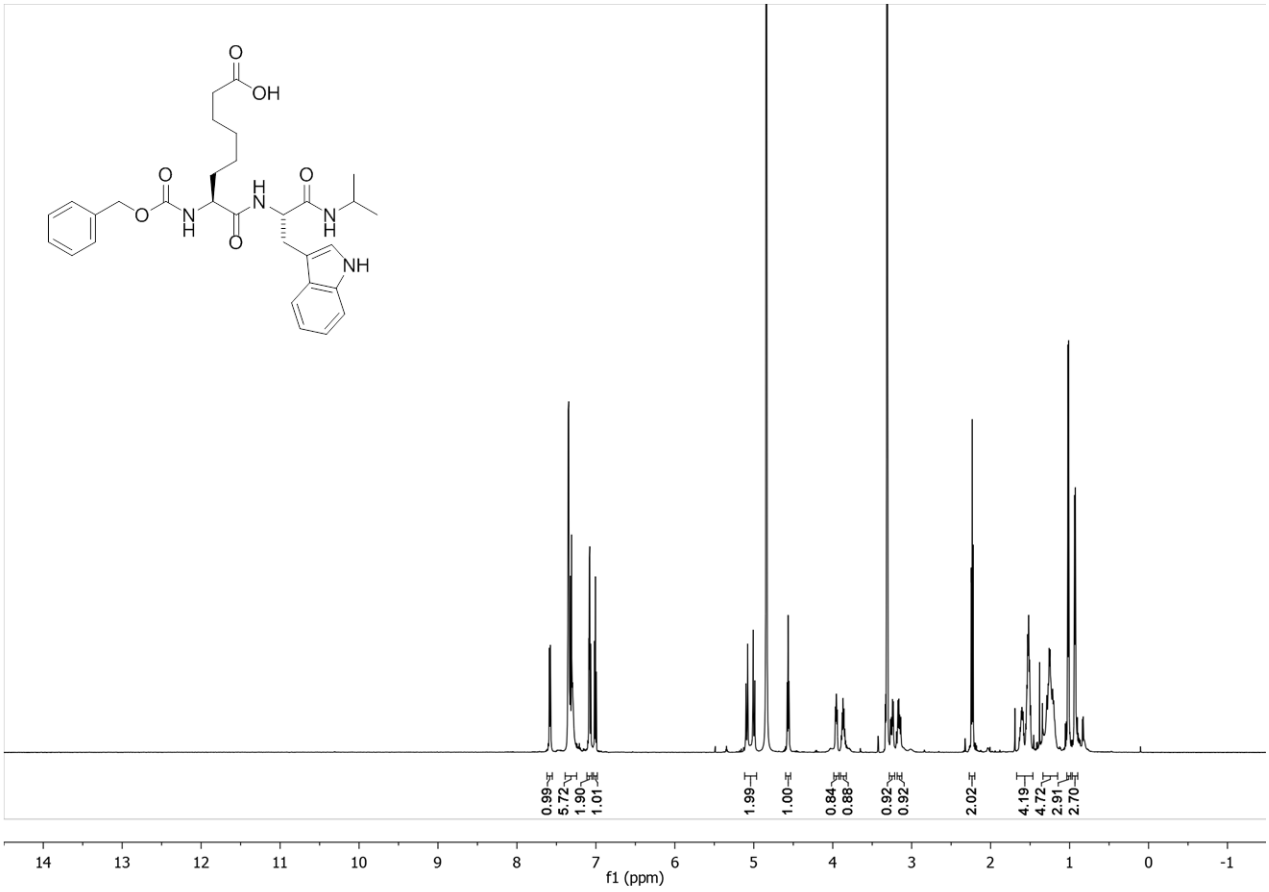


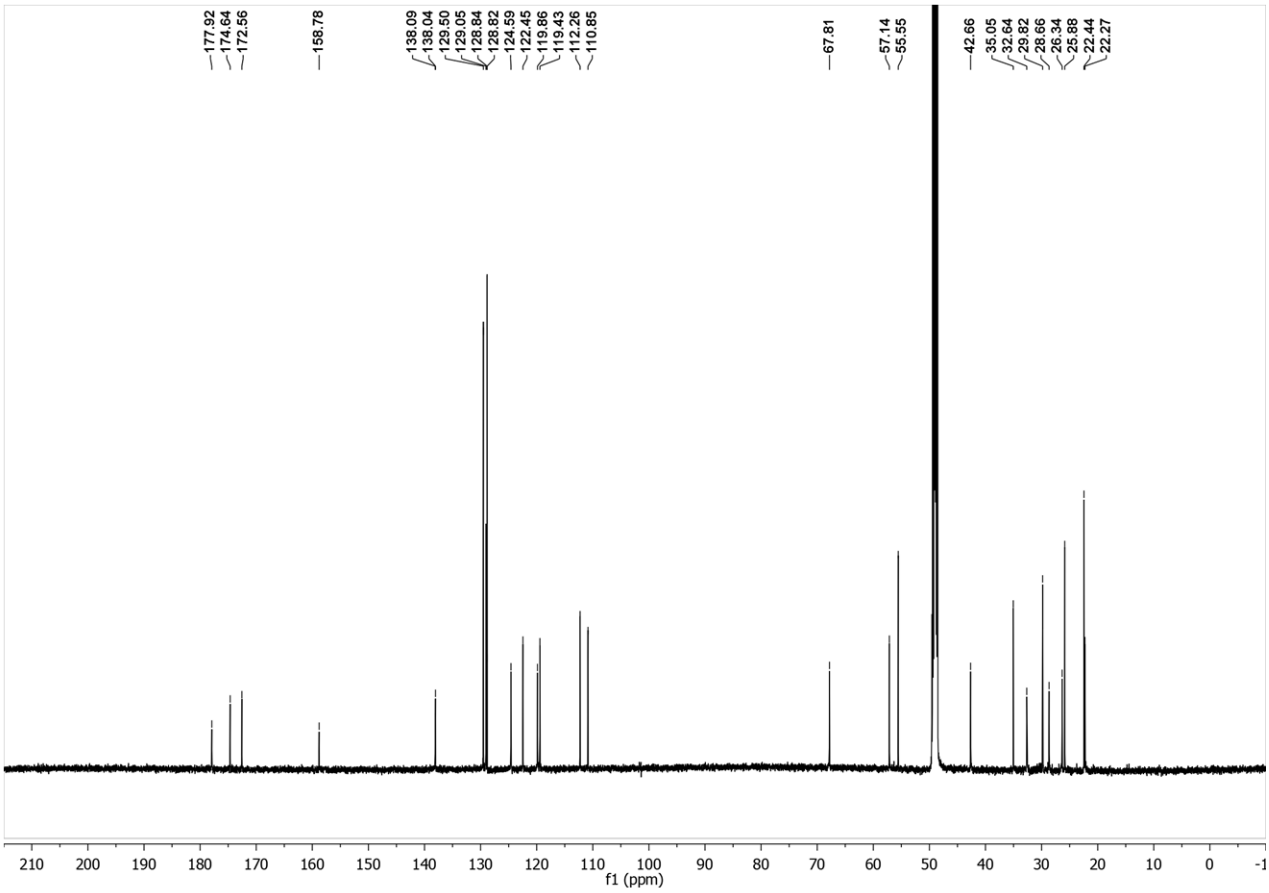
^{1}H and ^{13}C spectra of compound S67.



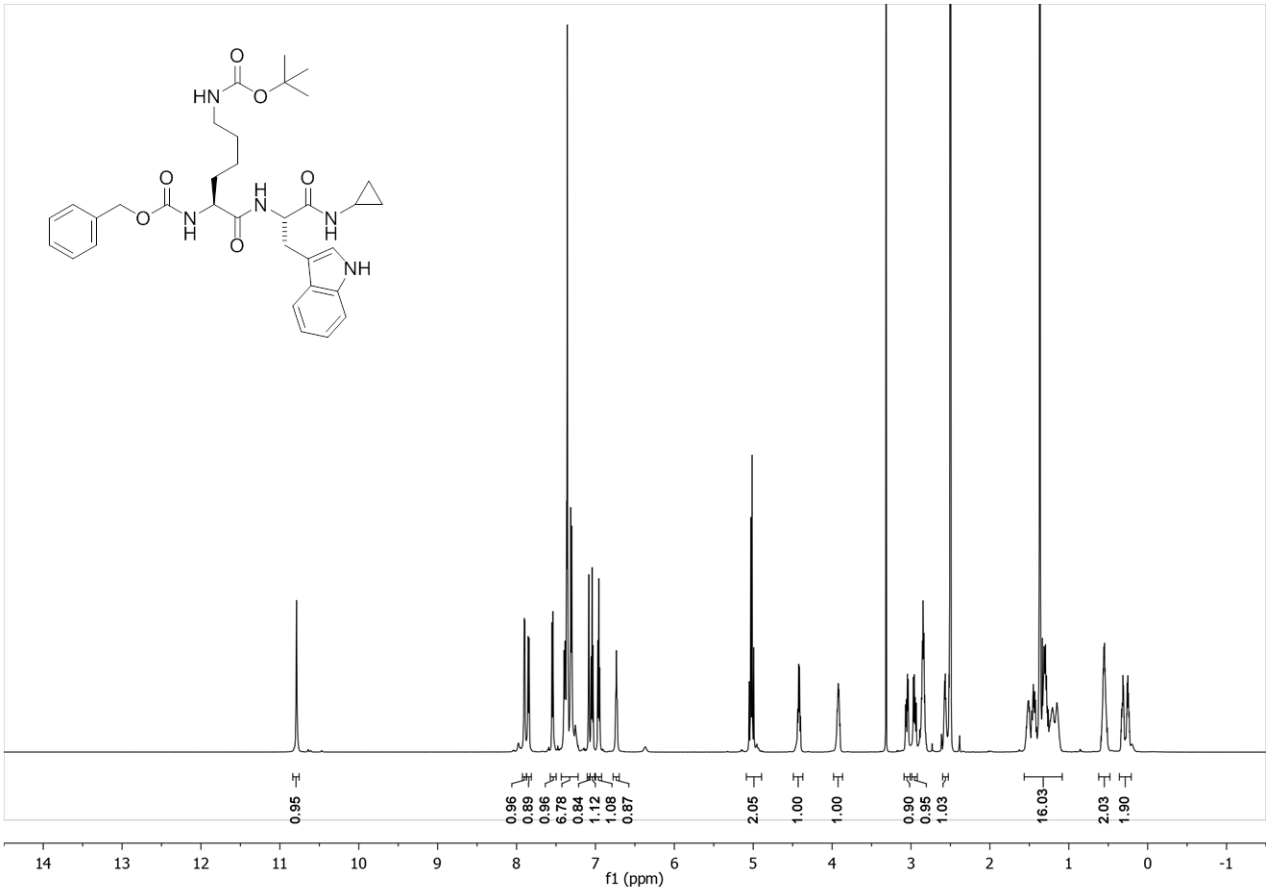


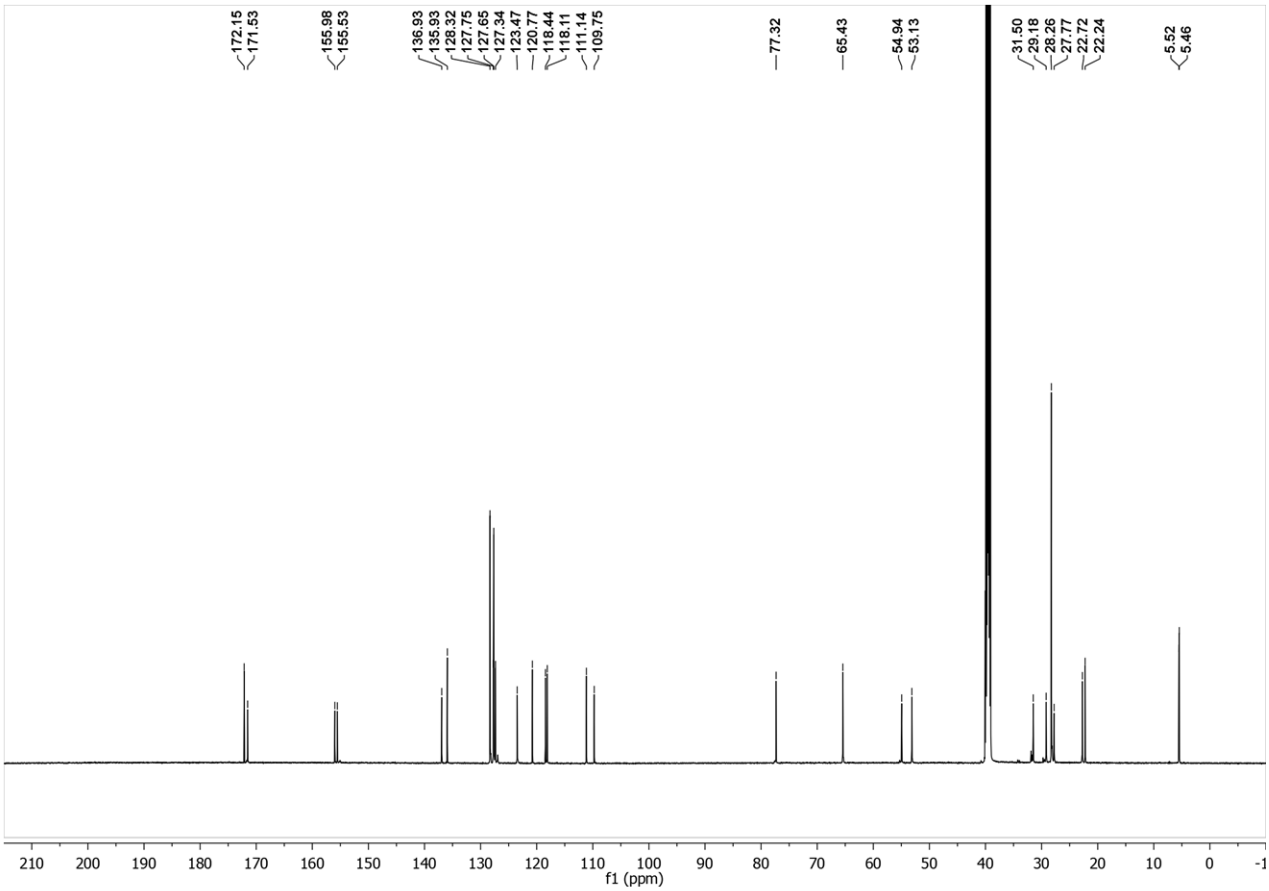
^{1}H and ^{13}C spectra of compound S68.



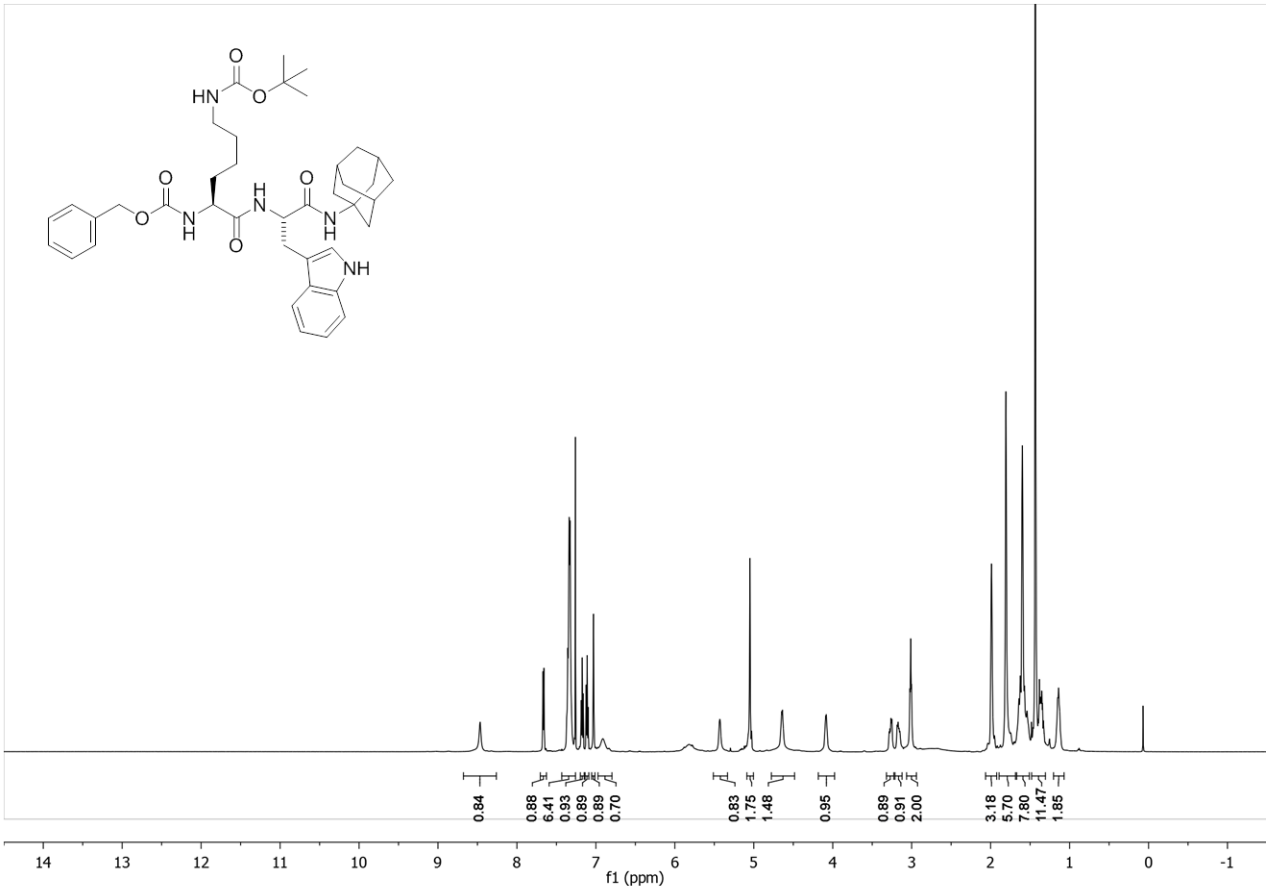


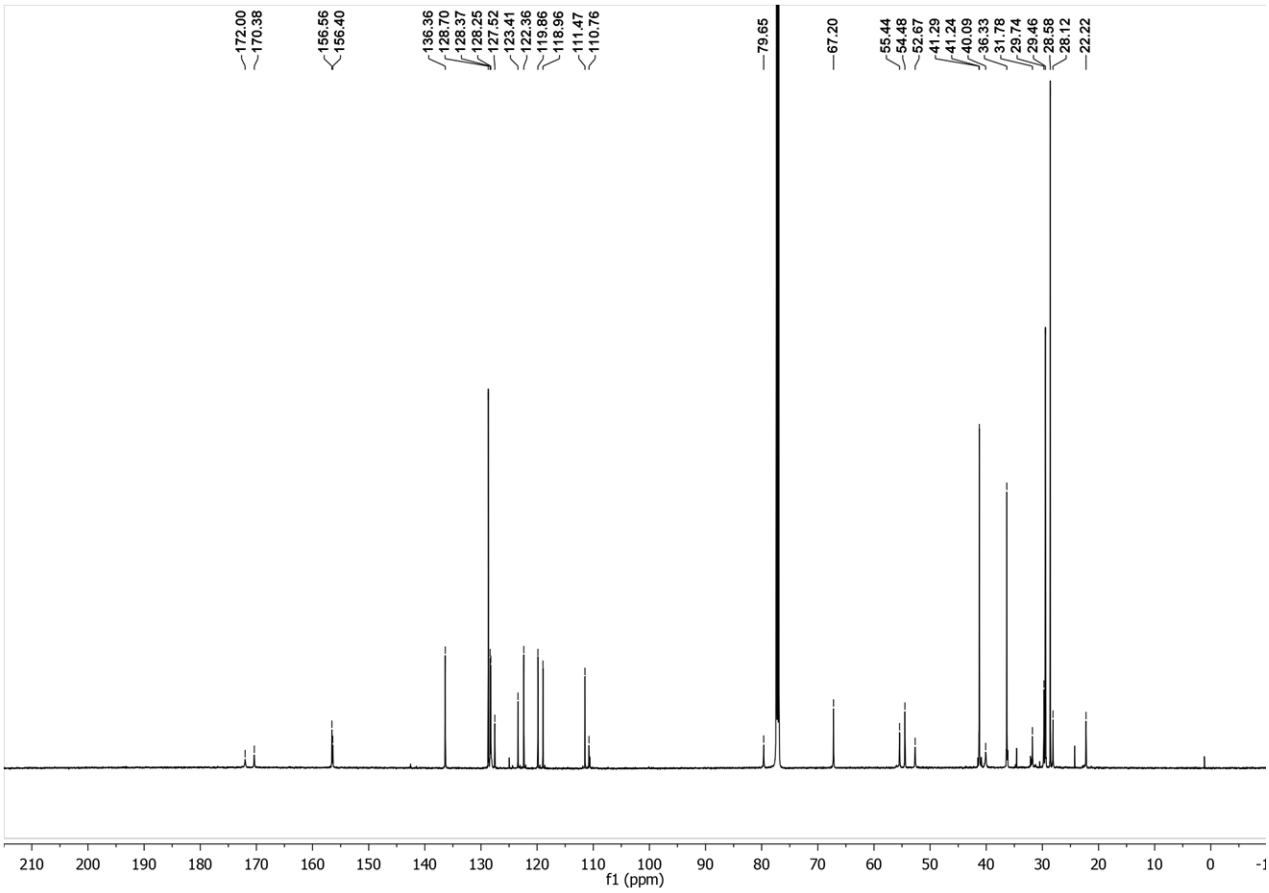
^{1}H and ^{13}C spectra of compound S69.



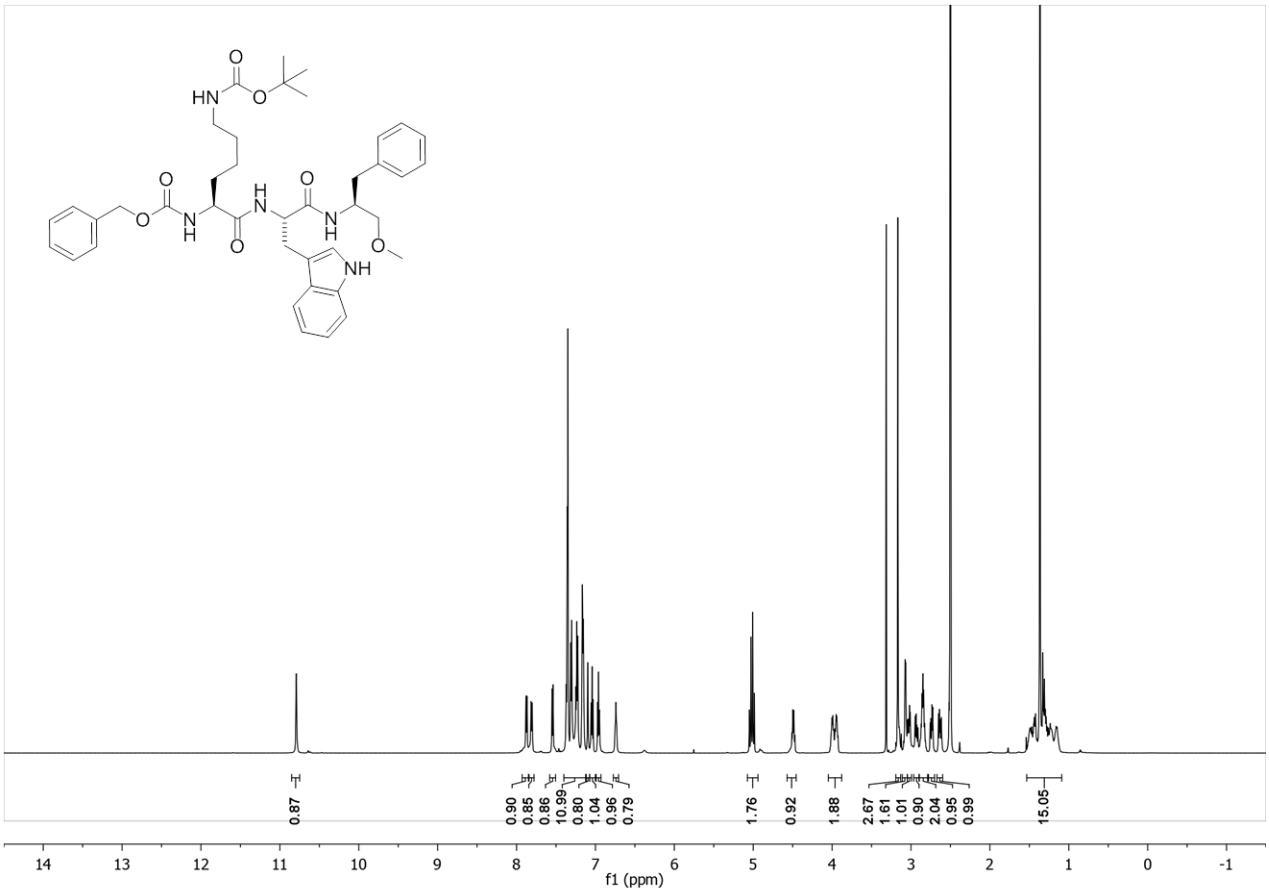


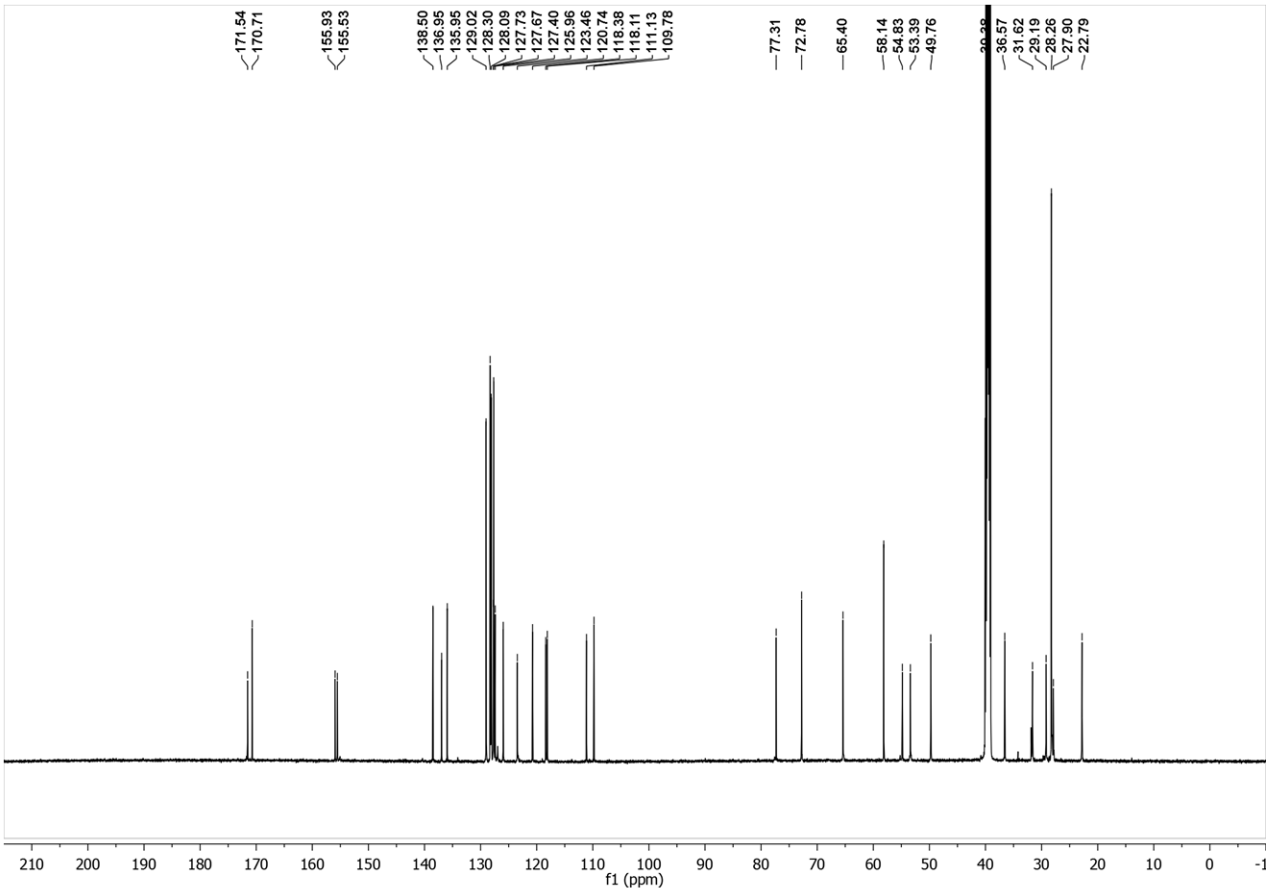
^{1}H and ^{13}C spectra of compound S70.



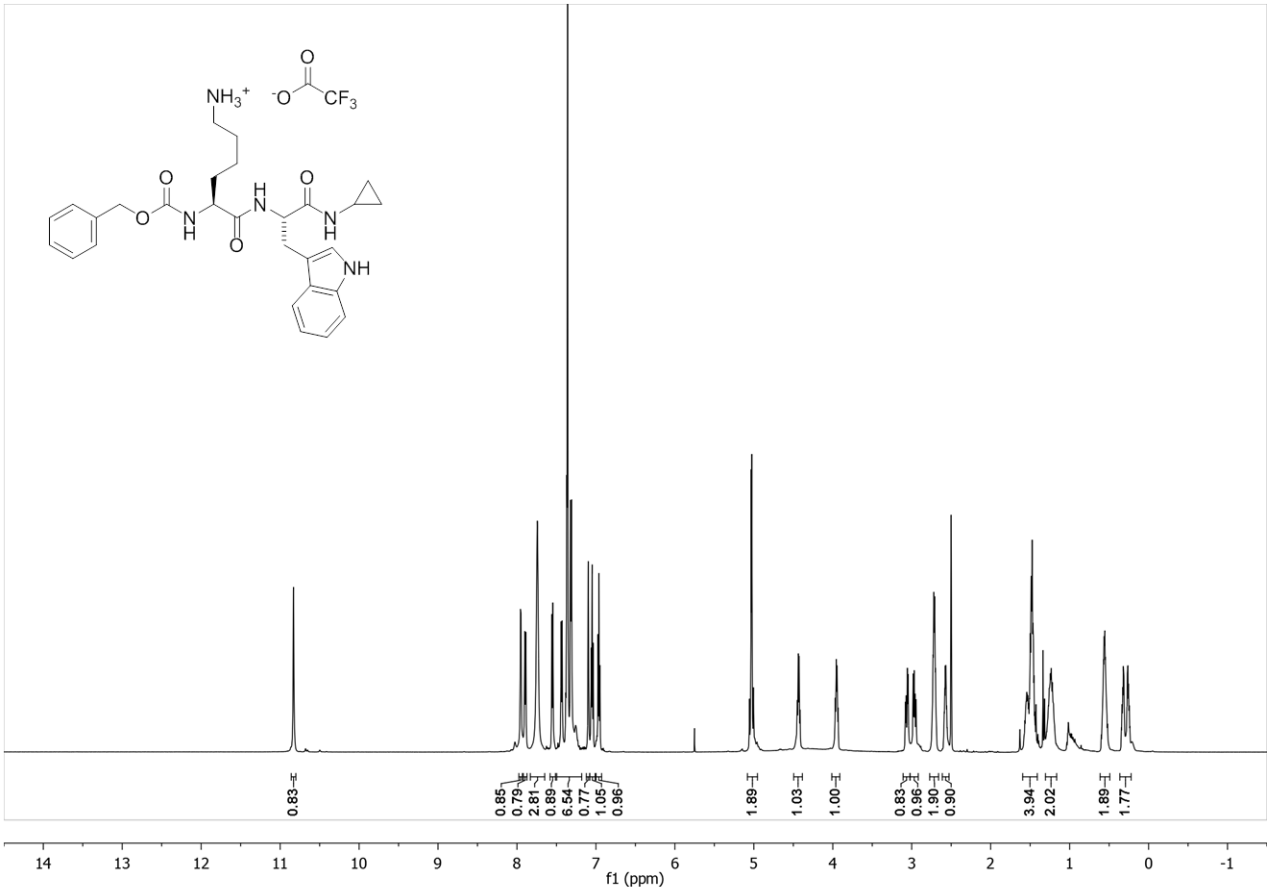


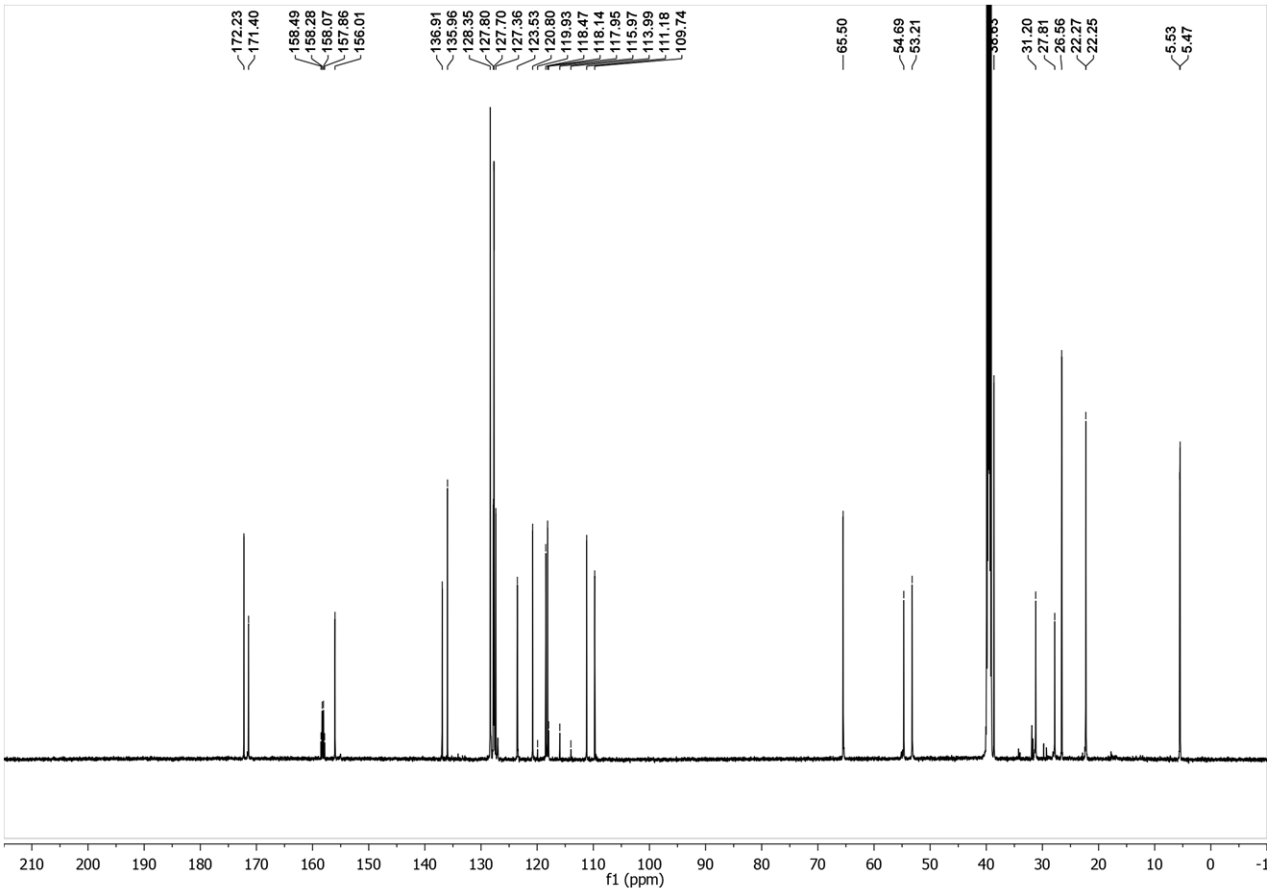
^{1}H and ^{13}C spectra of compound S71.



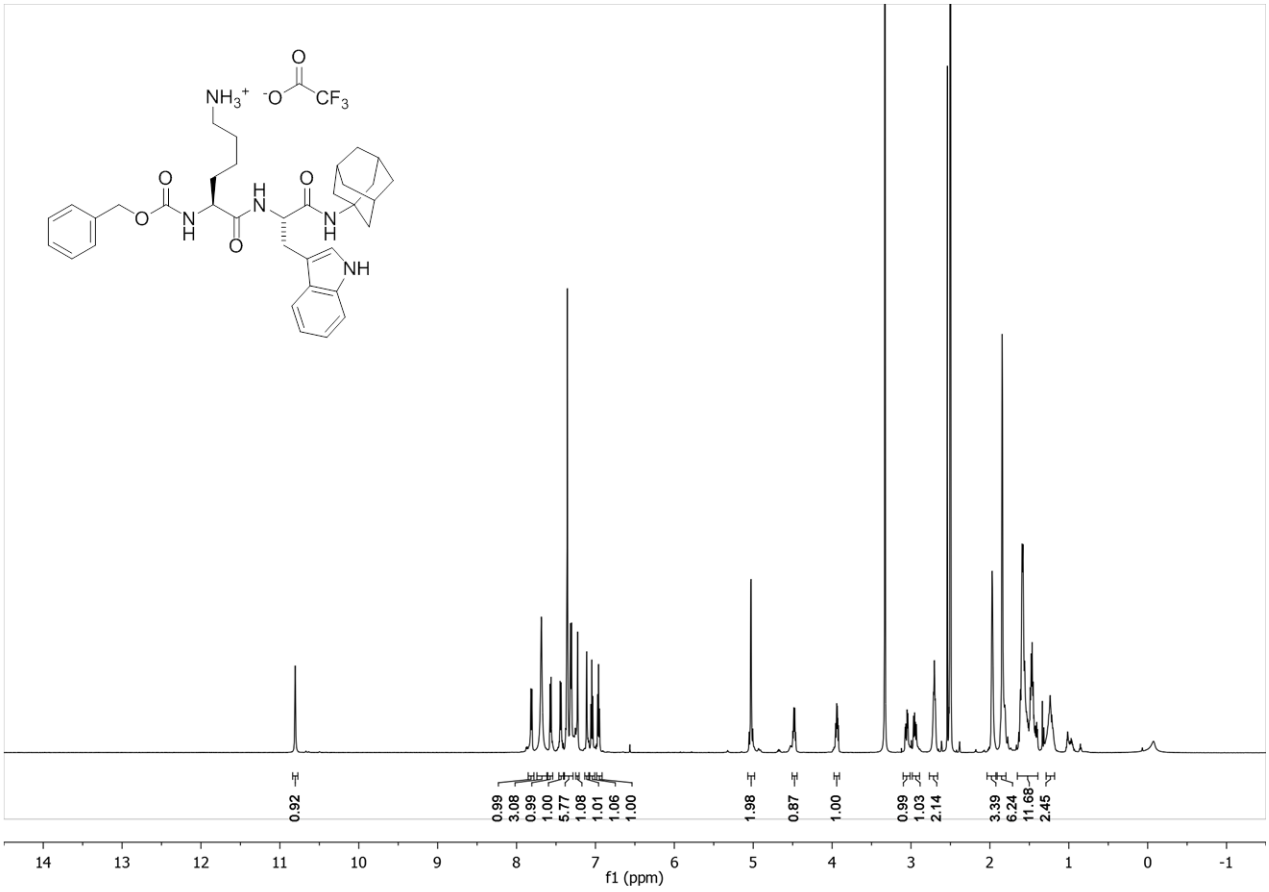


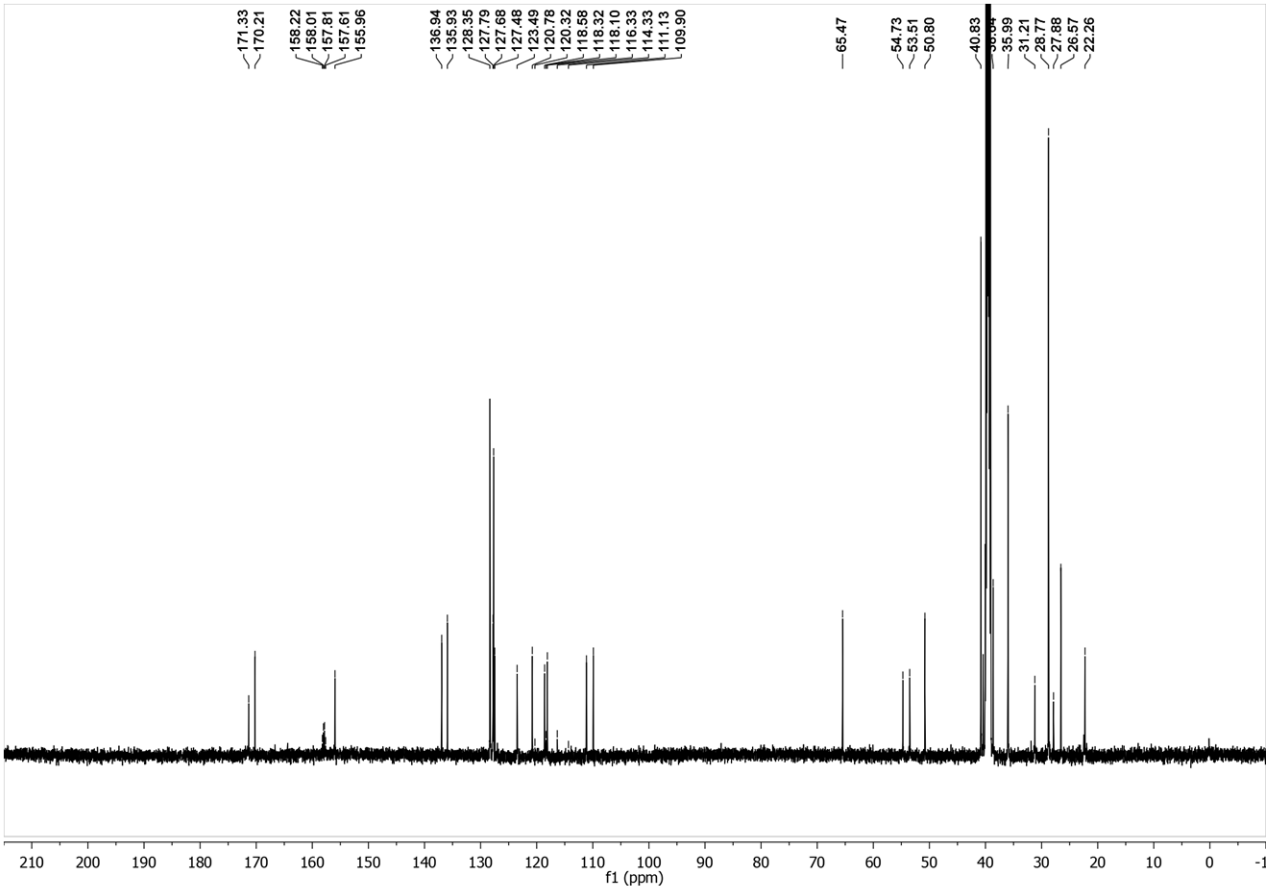
^{1}H and ^{13}C spectra of compound S72.



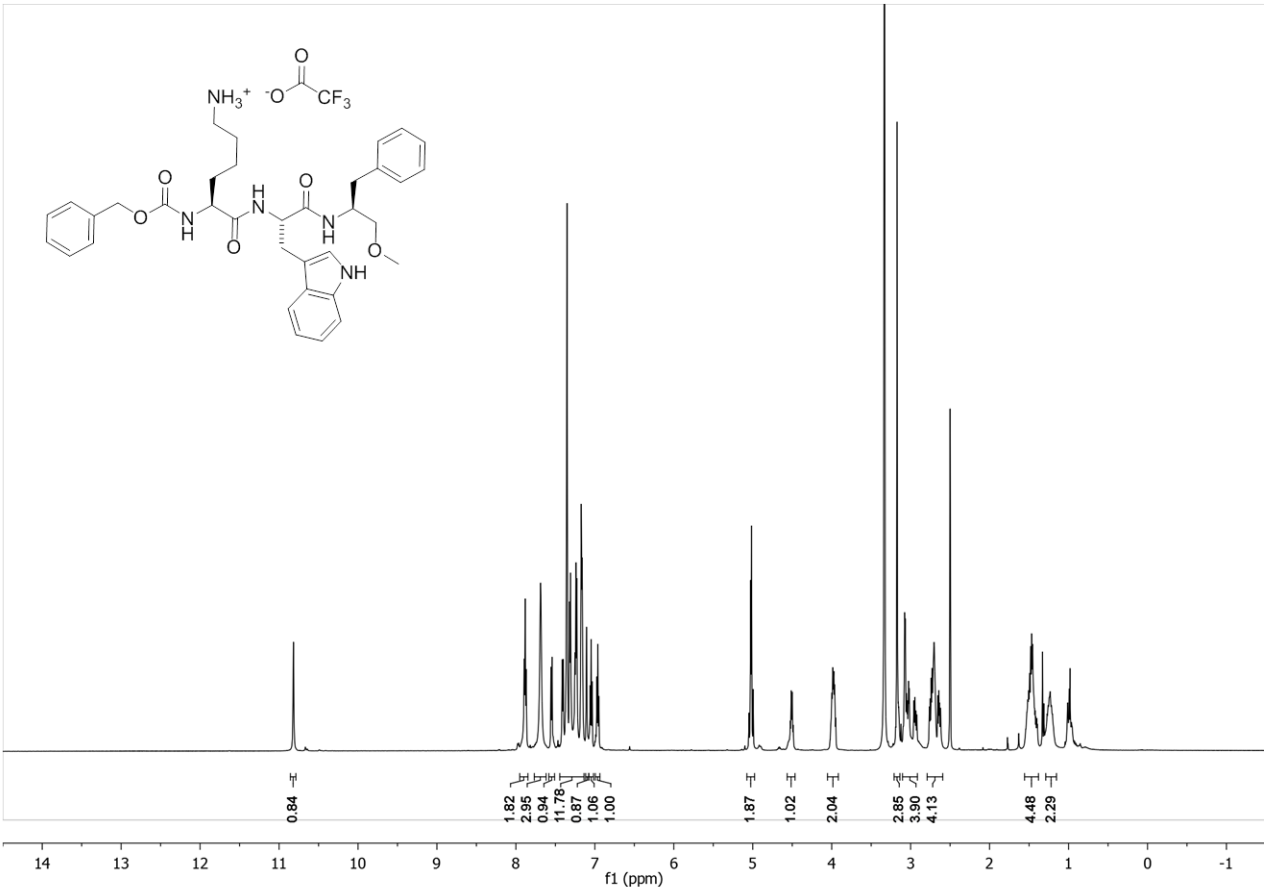


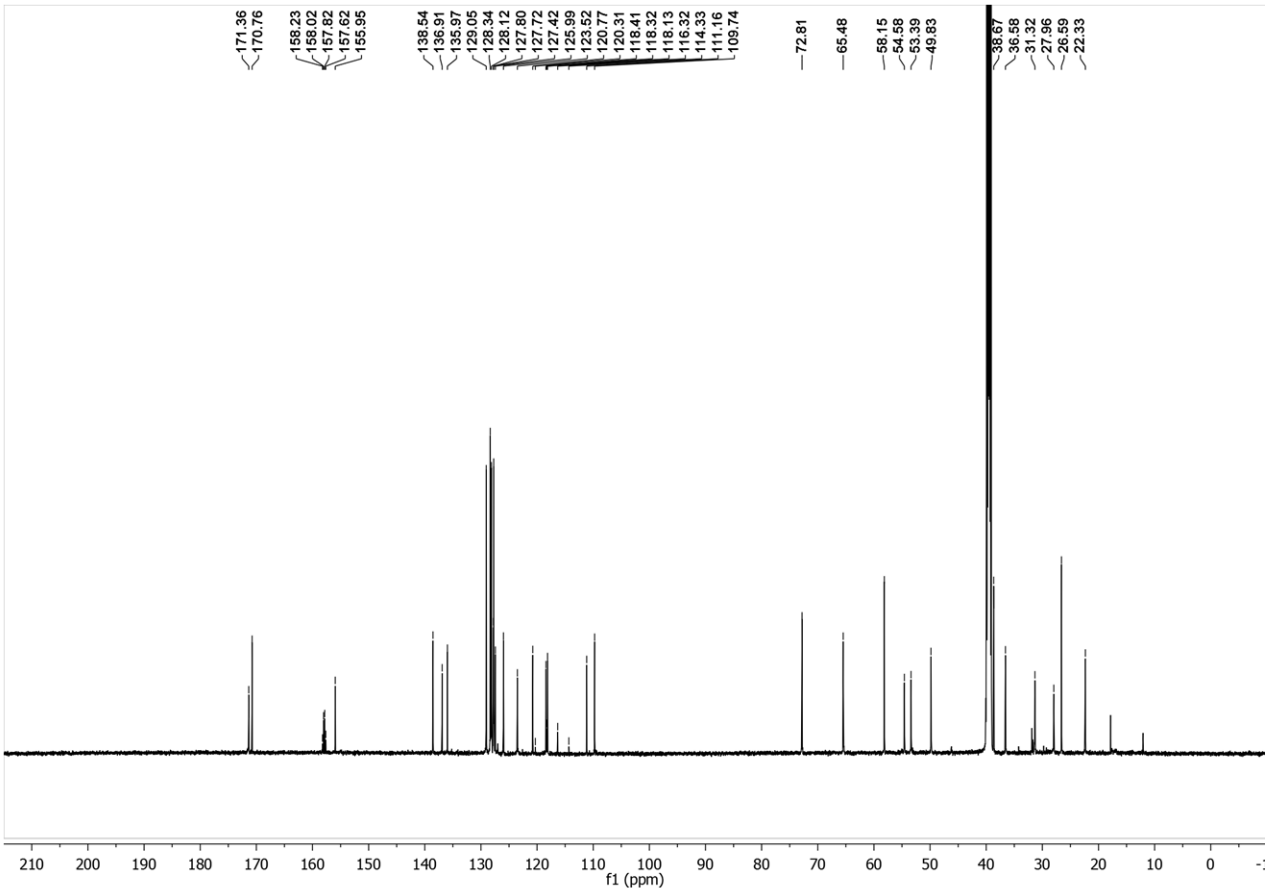
^{1}H and ^{13}C spectra of compound S73.



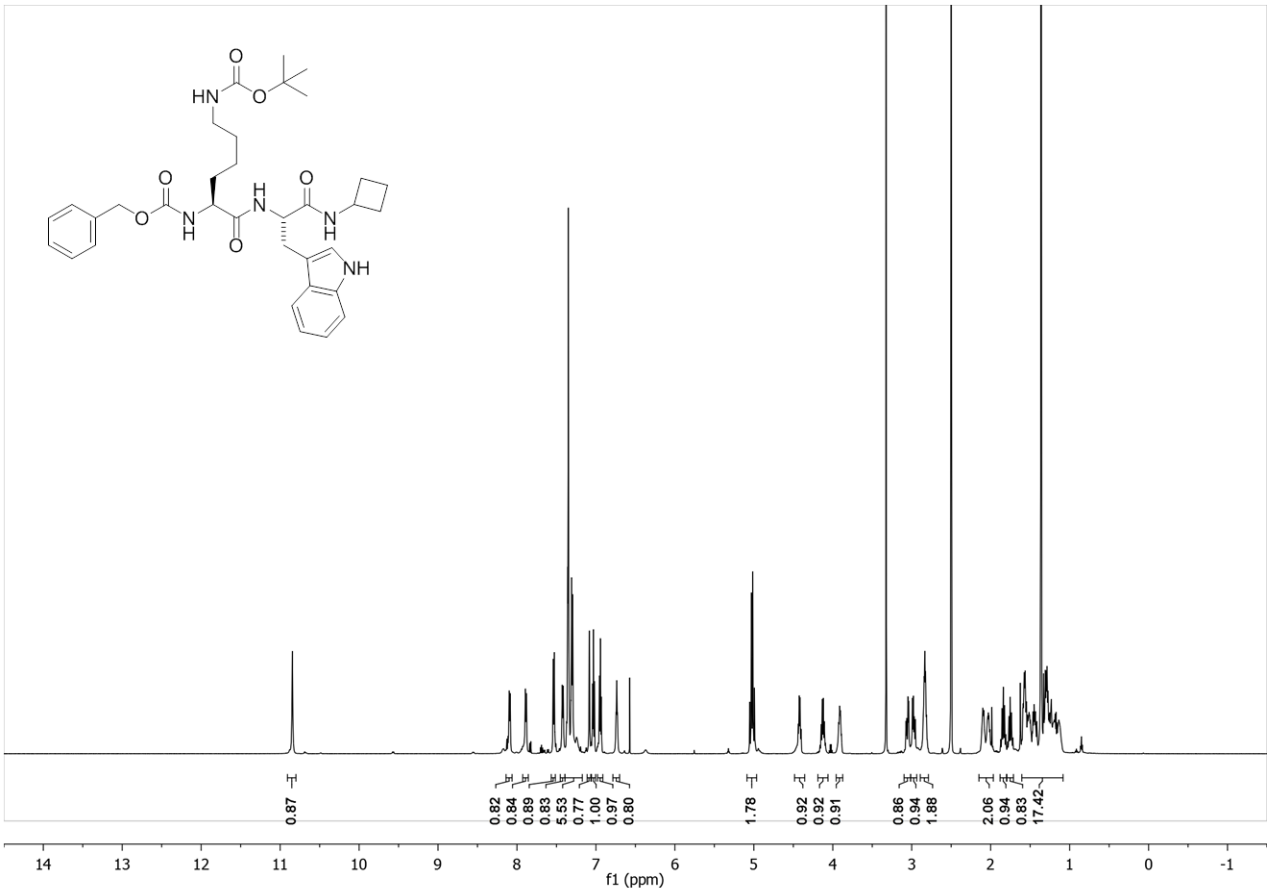


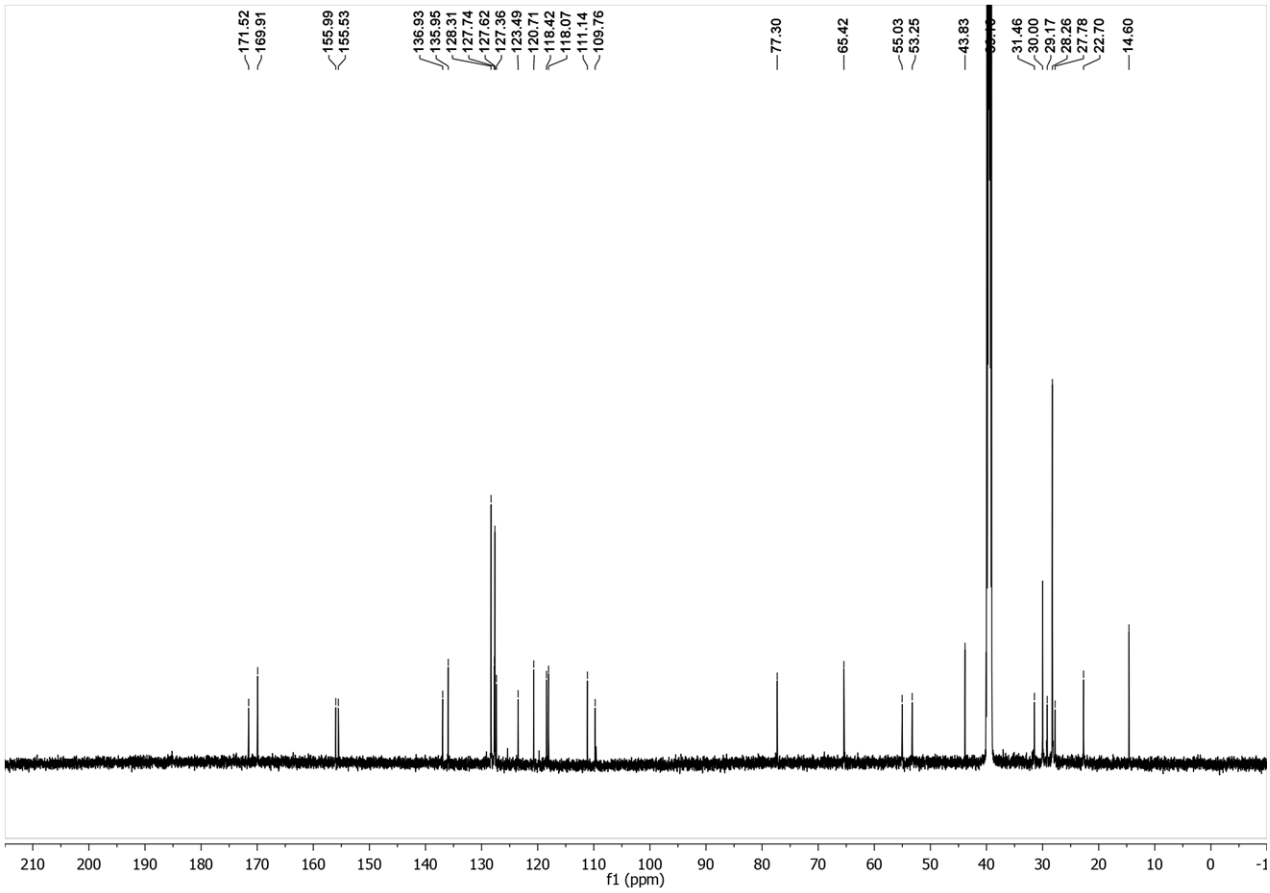
^{1}H and ^{13}C spectra of compound S74.



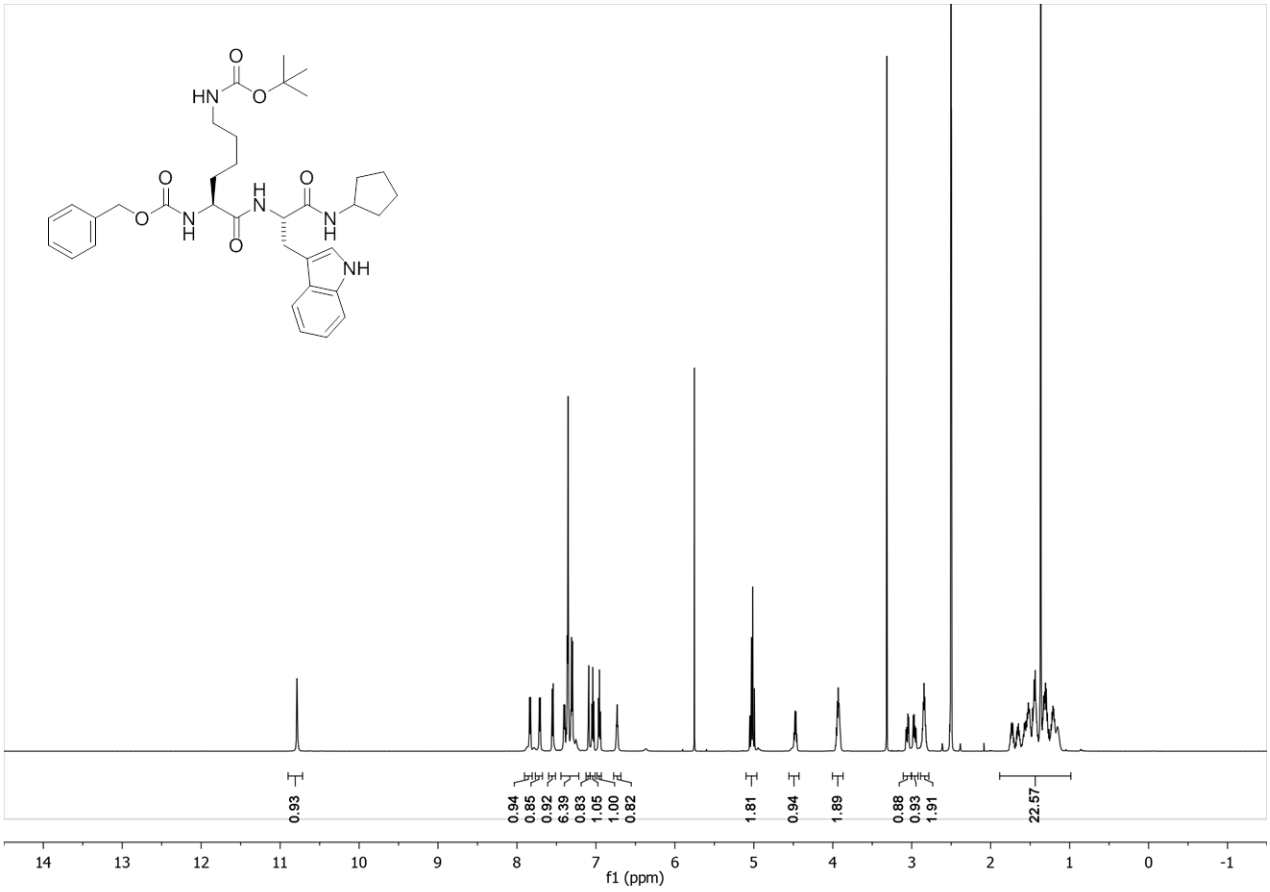


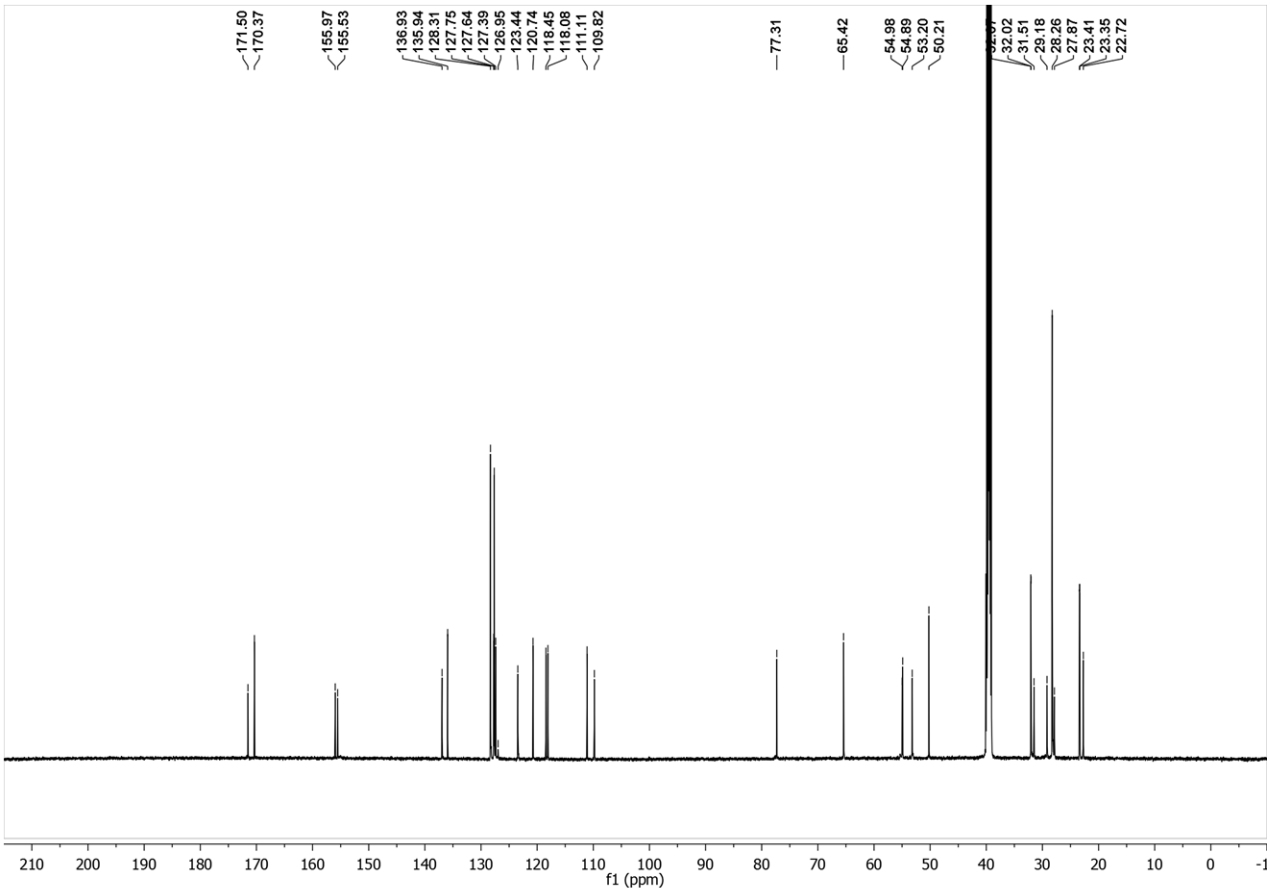
^{1}H and ^{13}C spectra of compound S75.



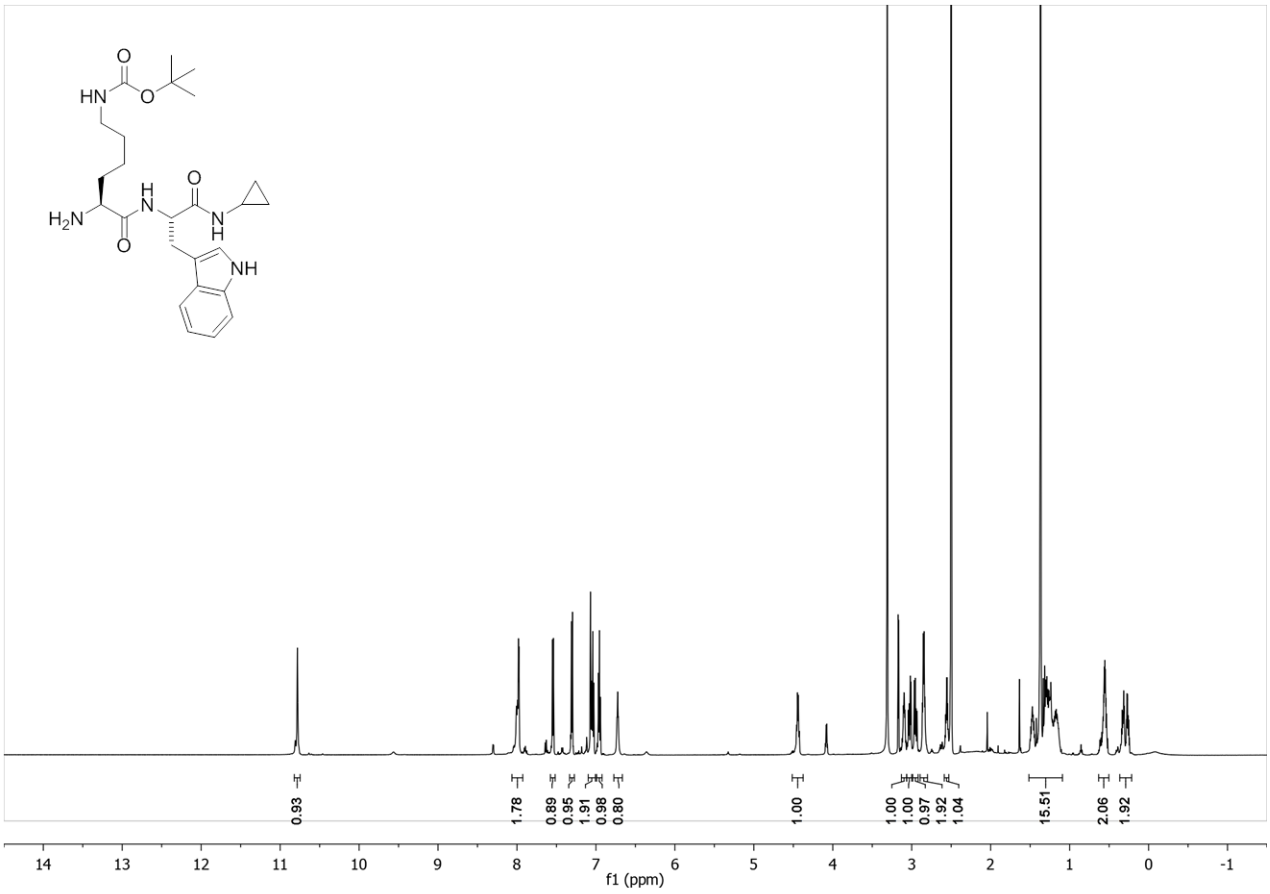


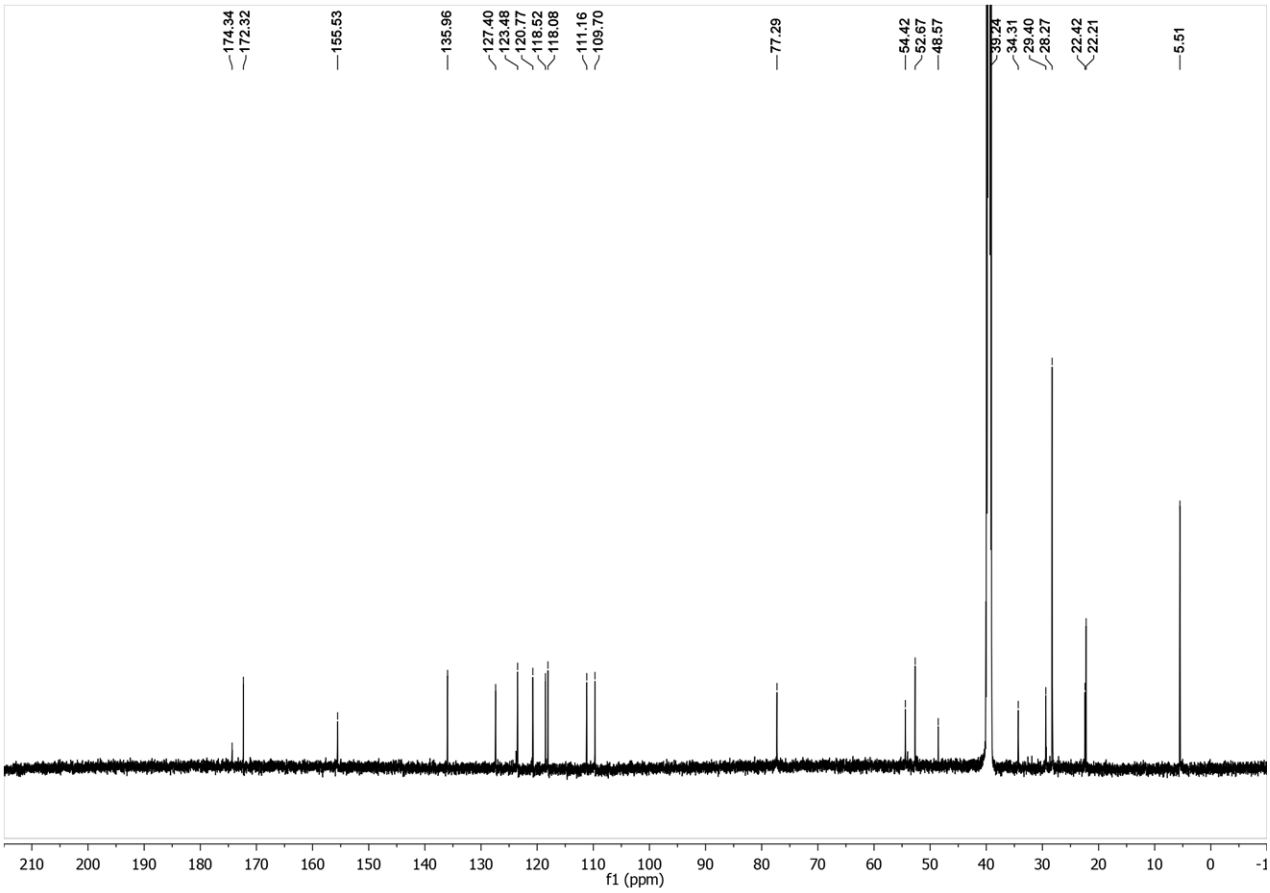
^{1}H and ^{13}C spectra of compound S76.



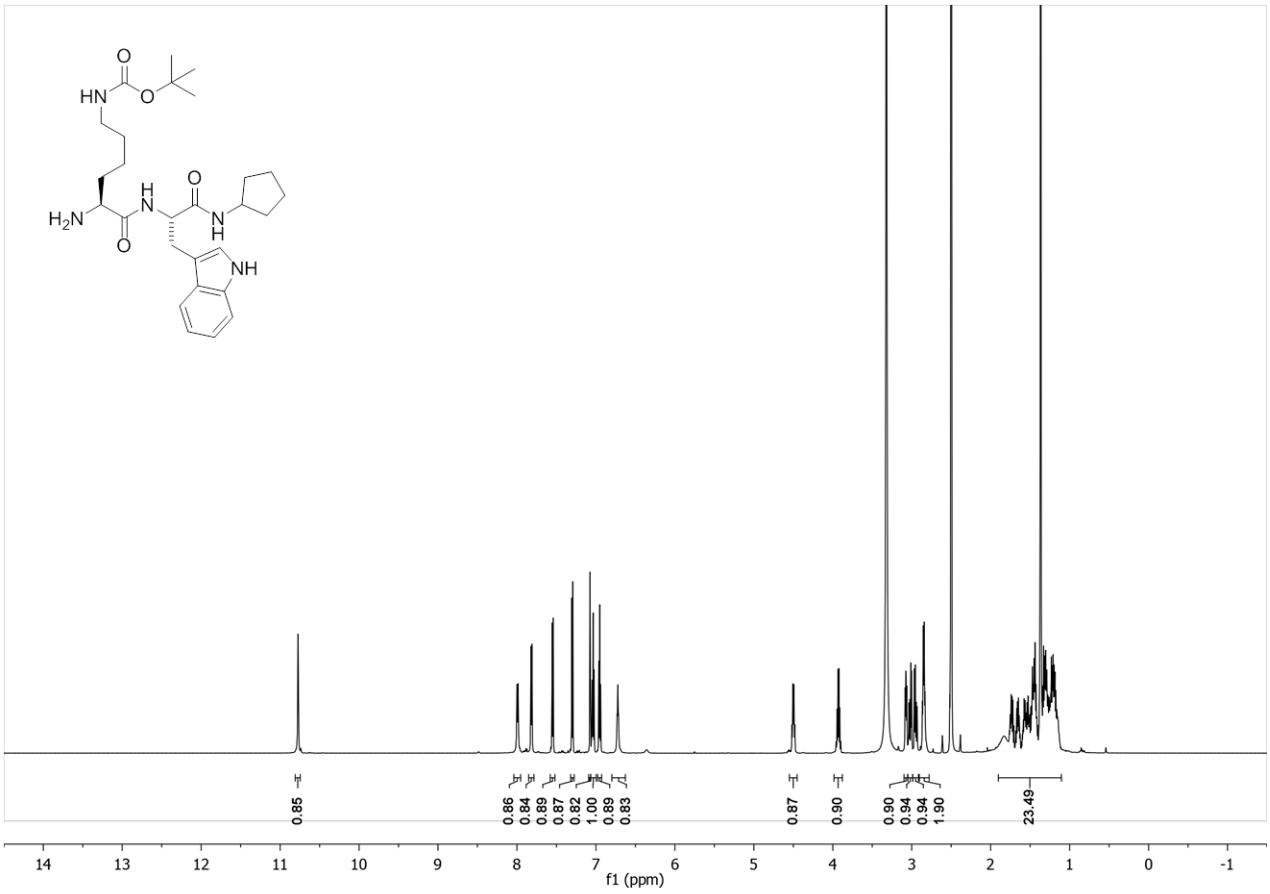


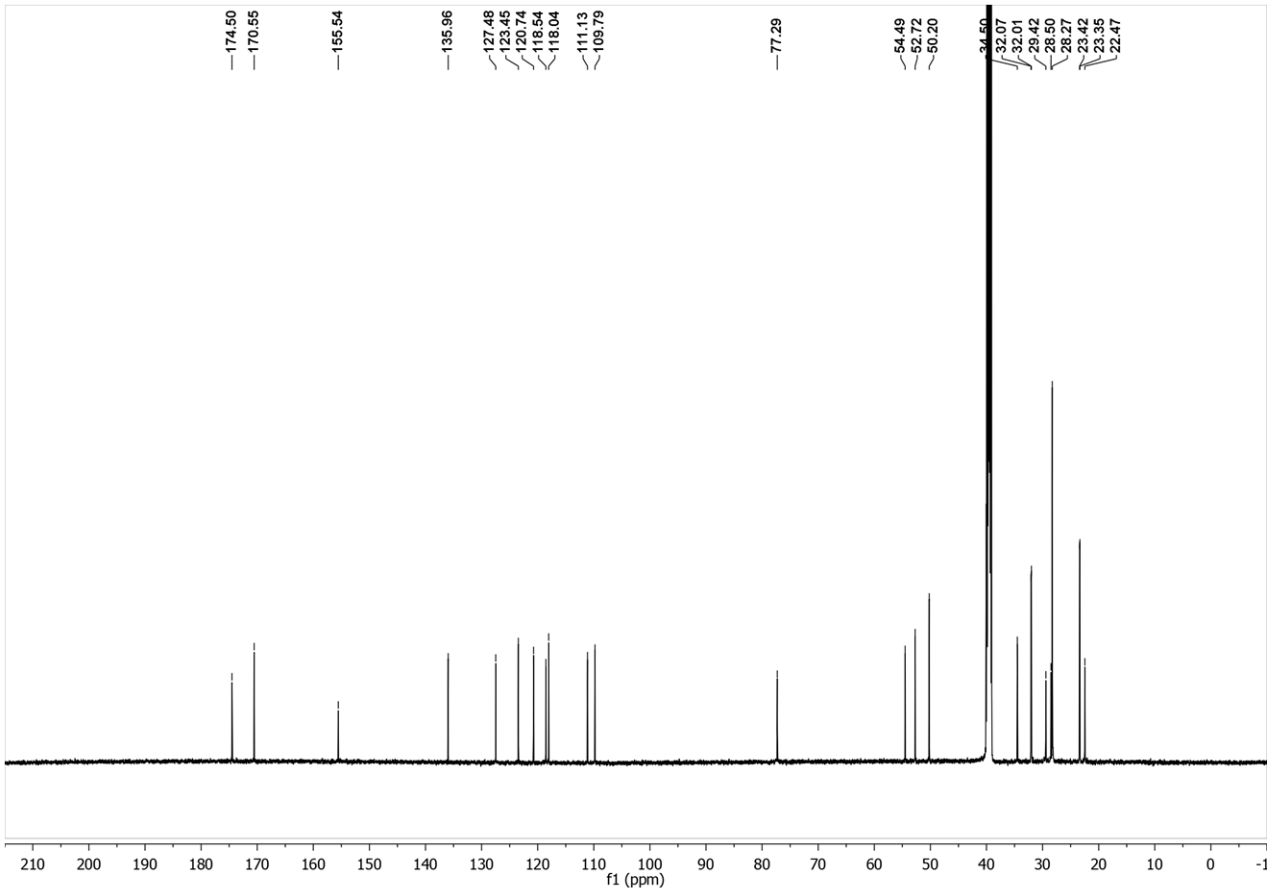
^{1}H and ^{13}C spectra of compound S77.



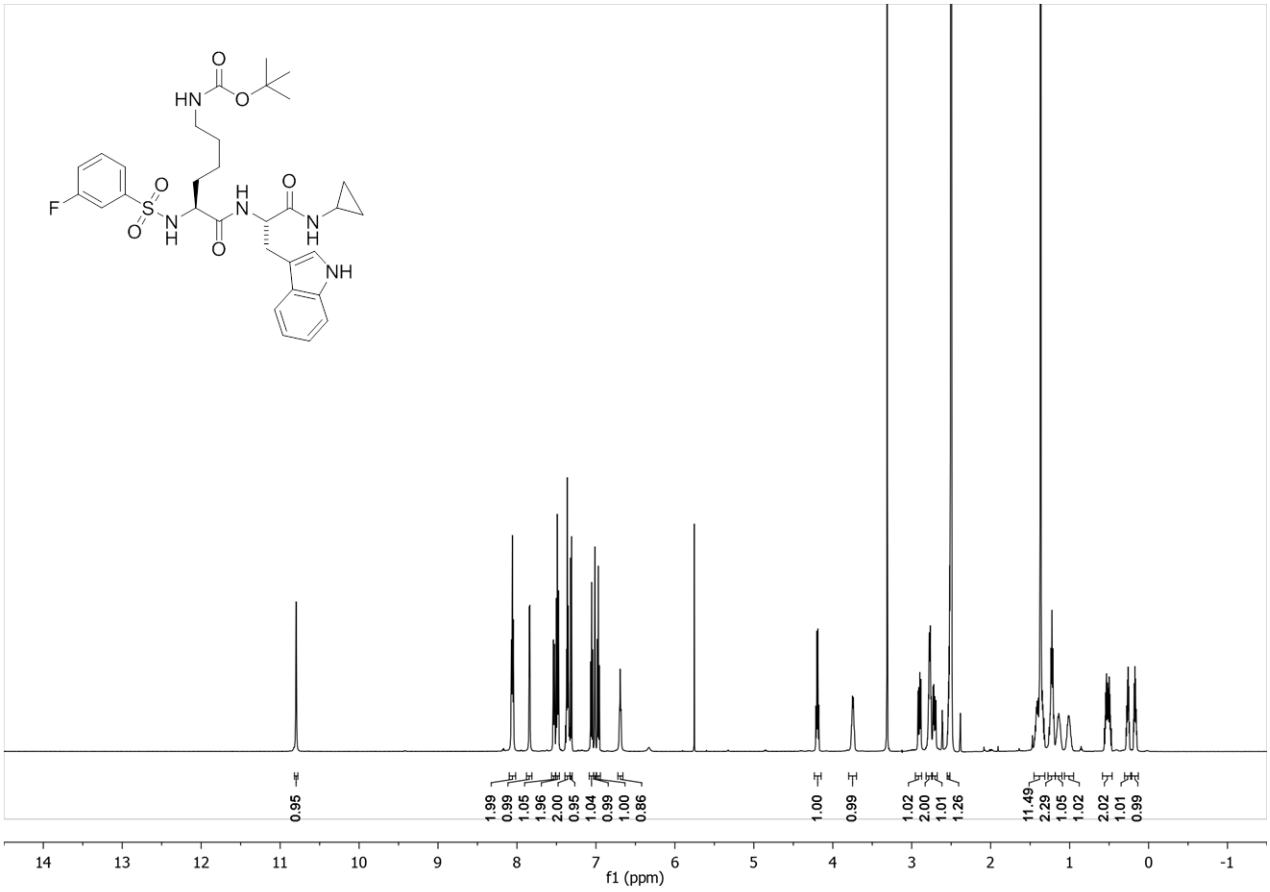


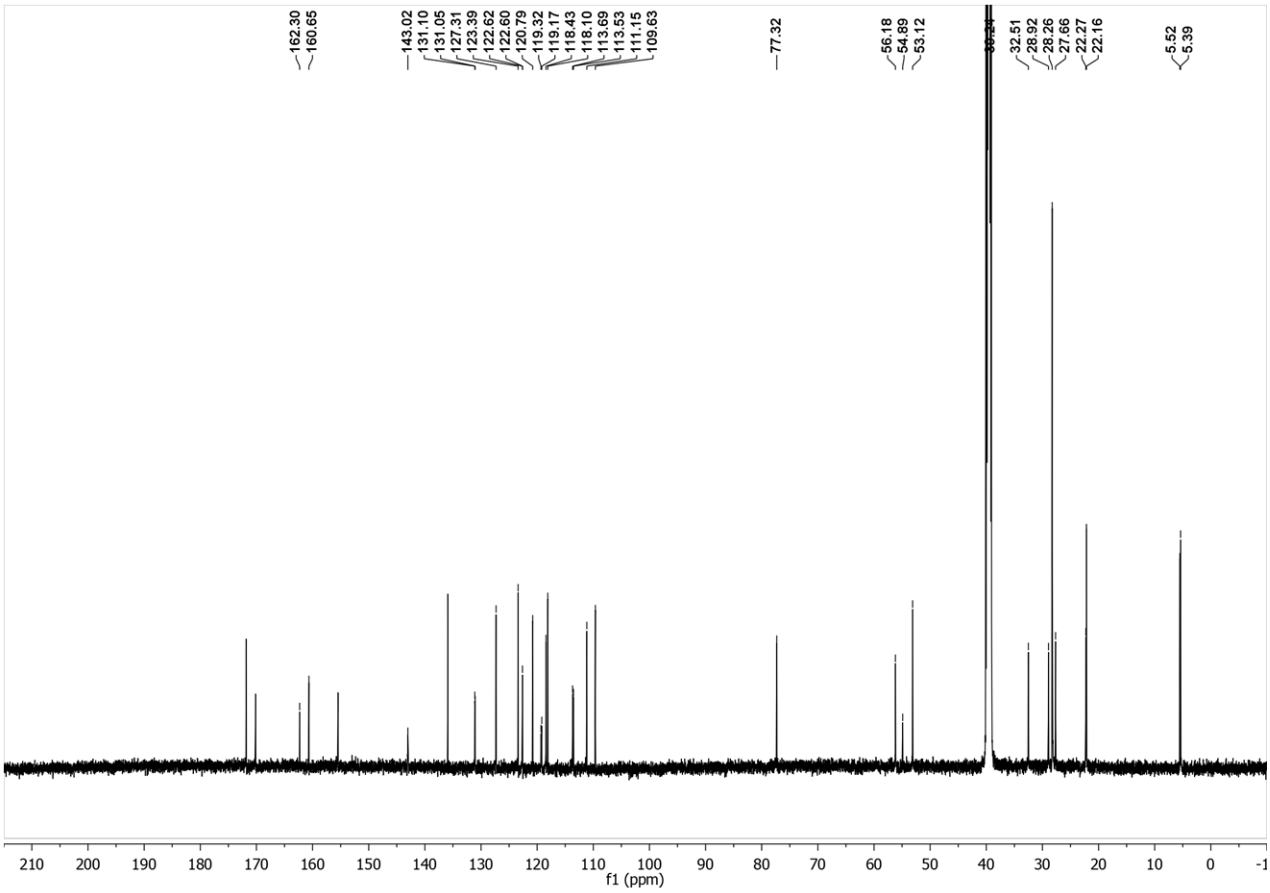
^{1}H and ^{13}C spectra of compound S78.

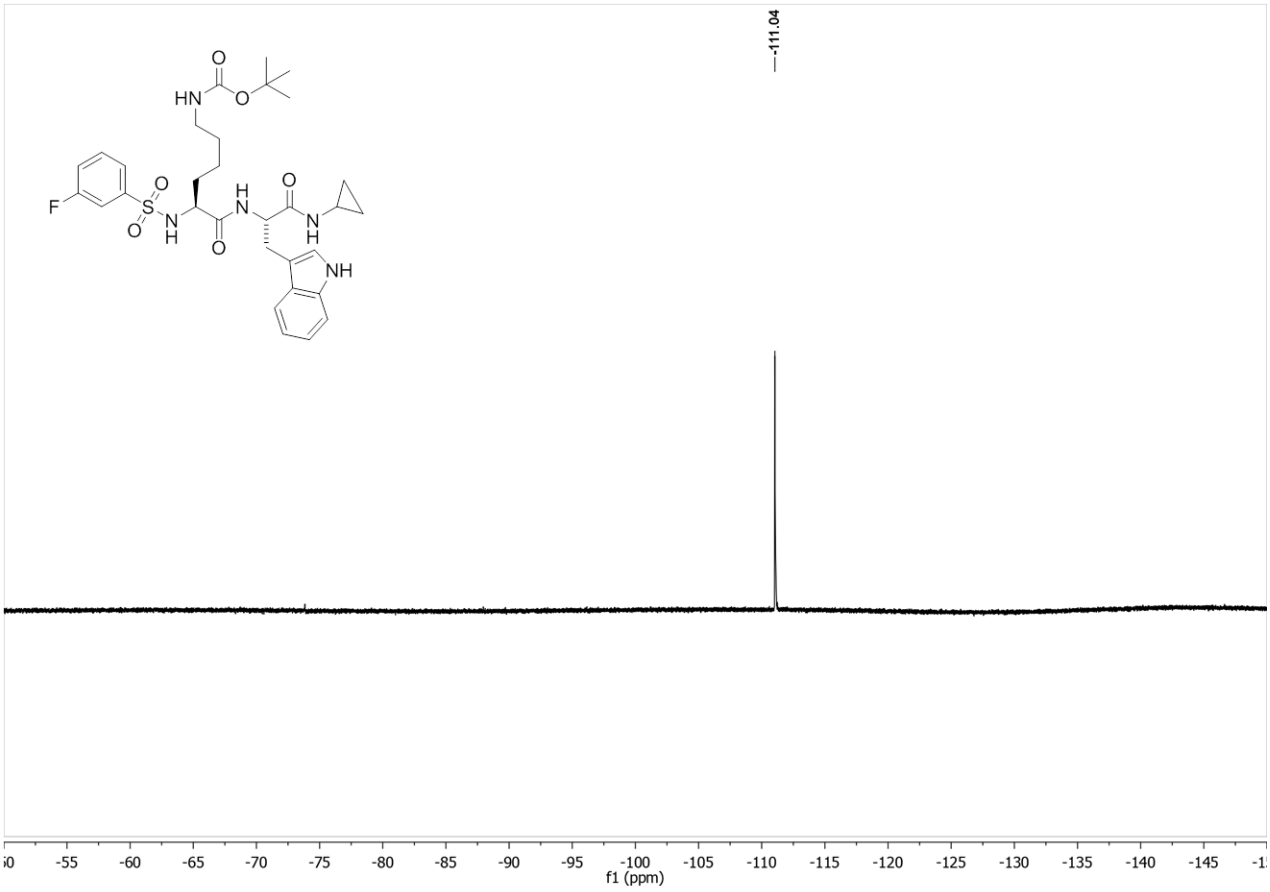




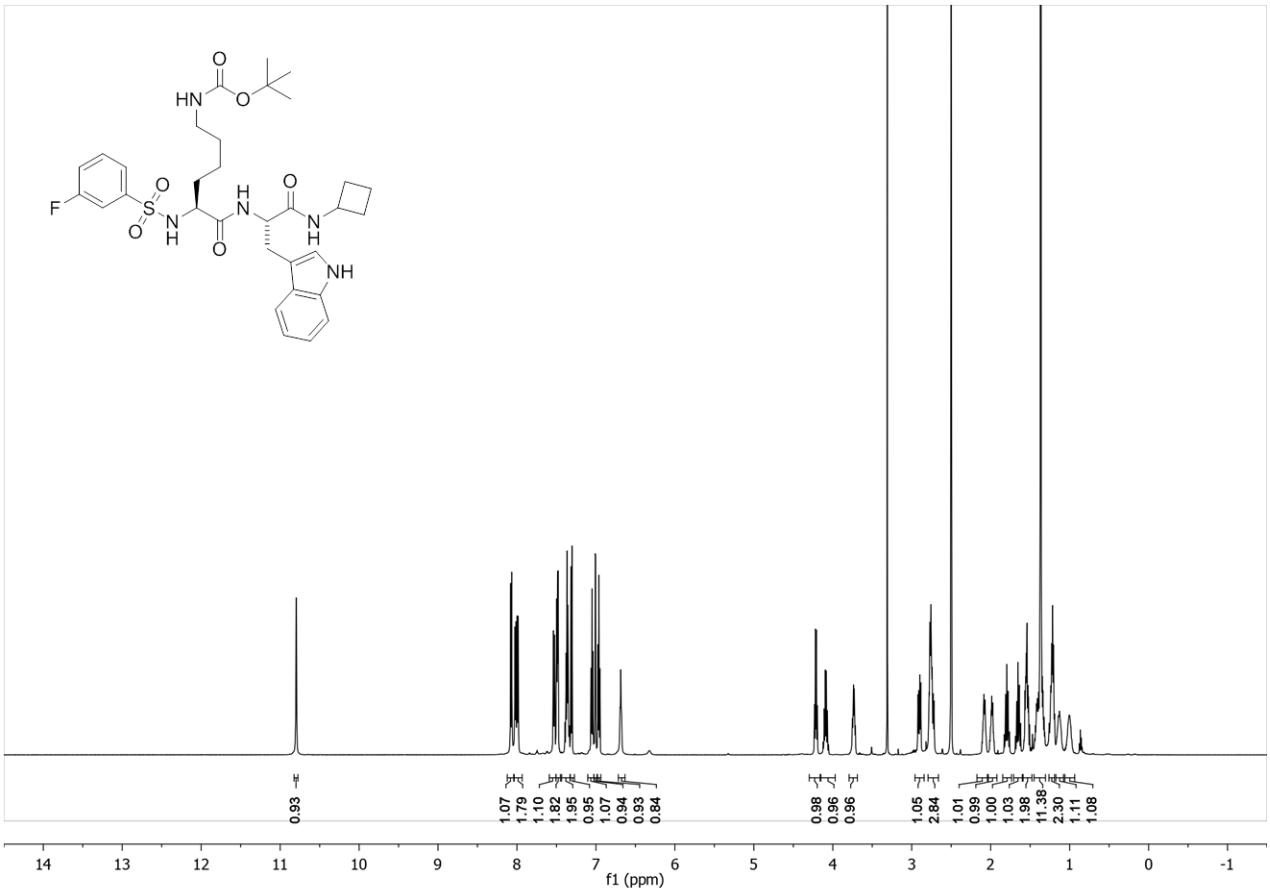
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S79.

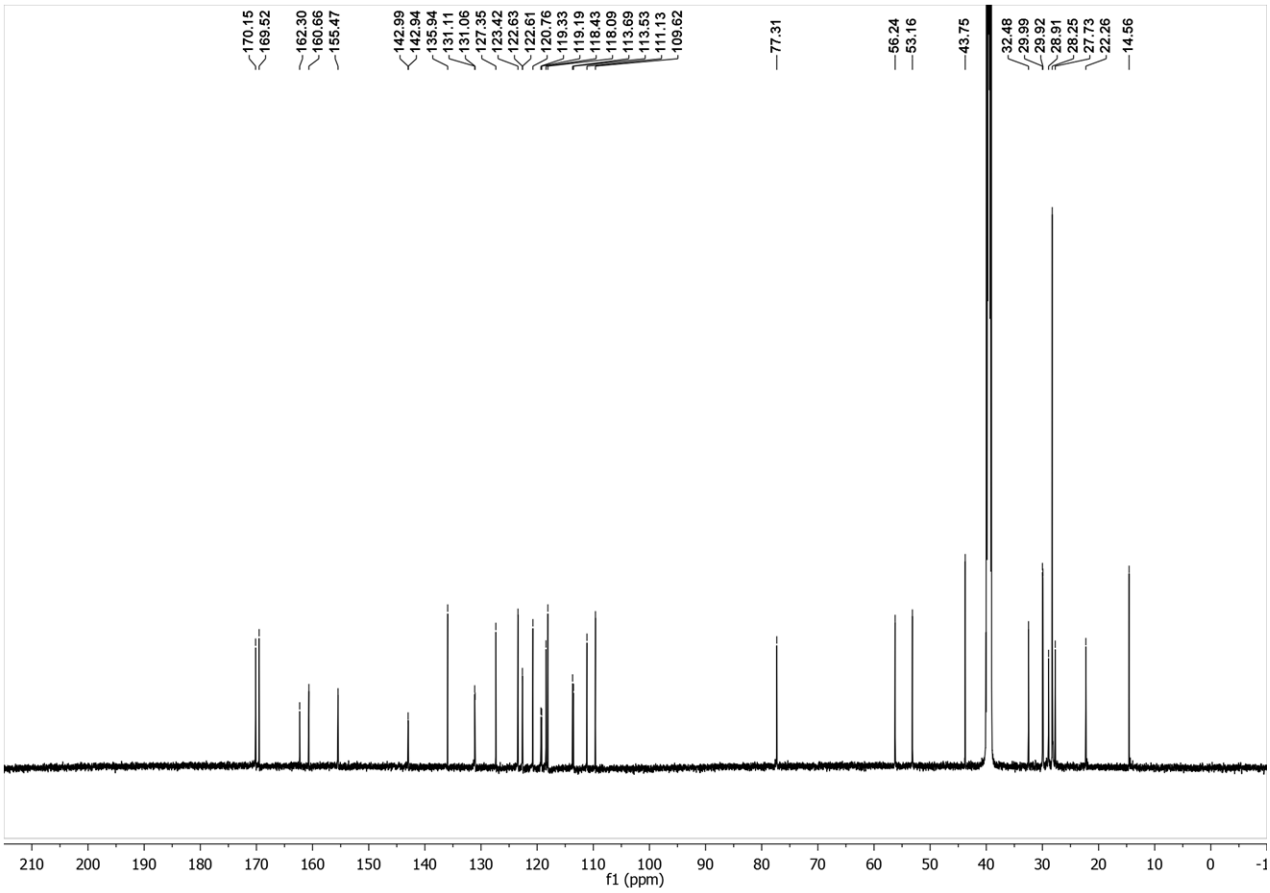


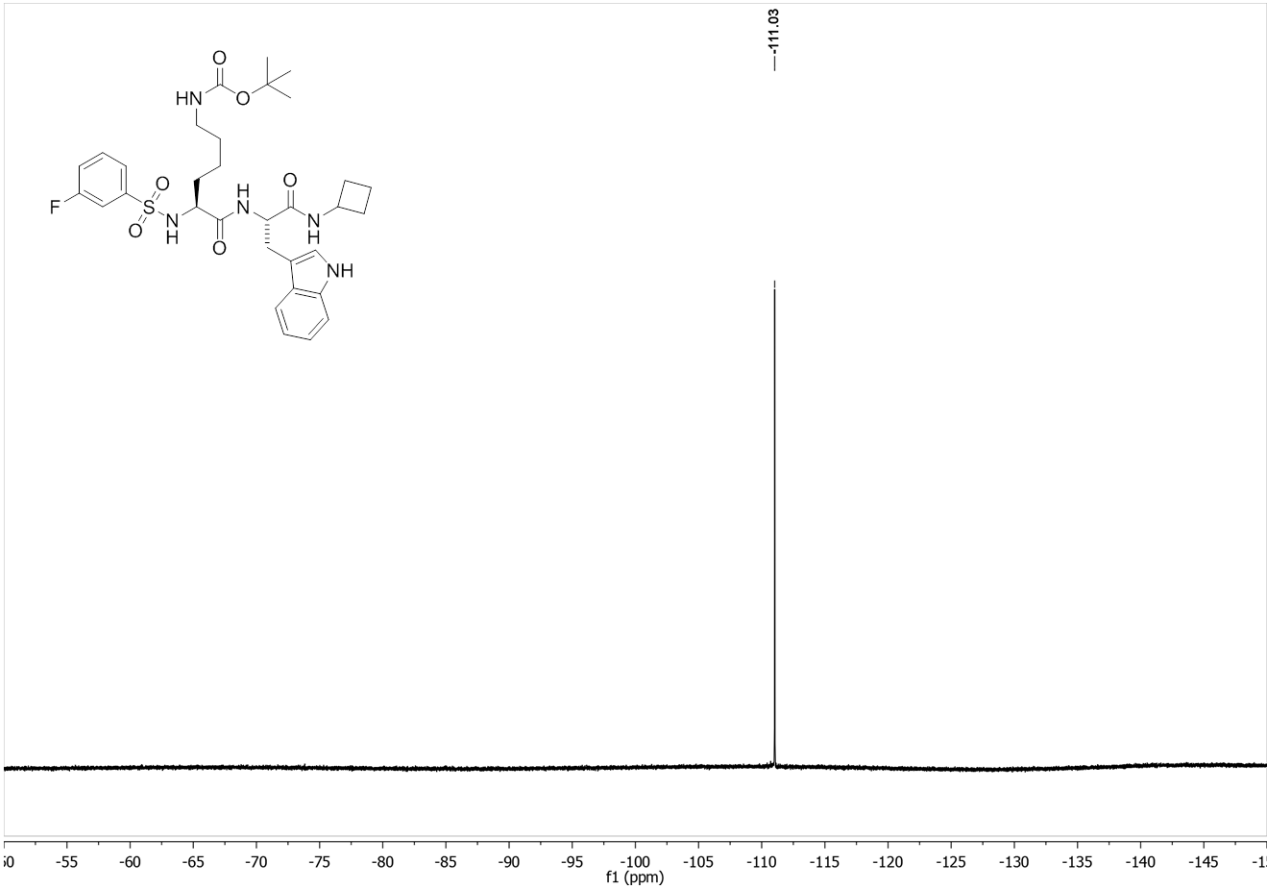



-111.04
F
S
O
HN
NH
NH
f1 (ppm)

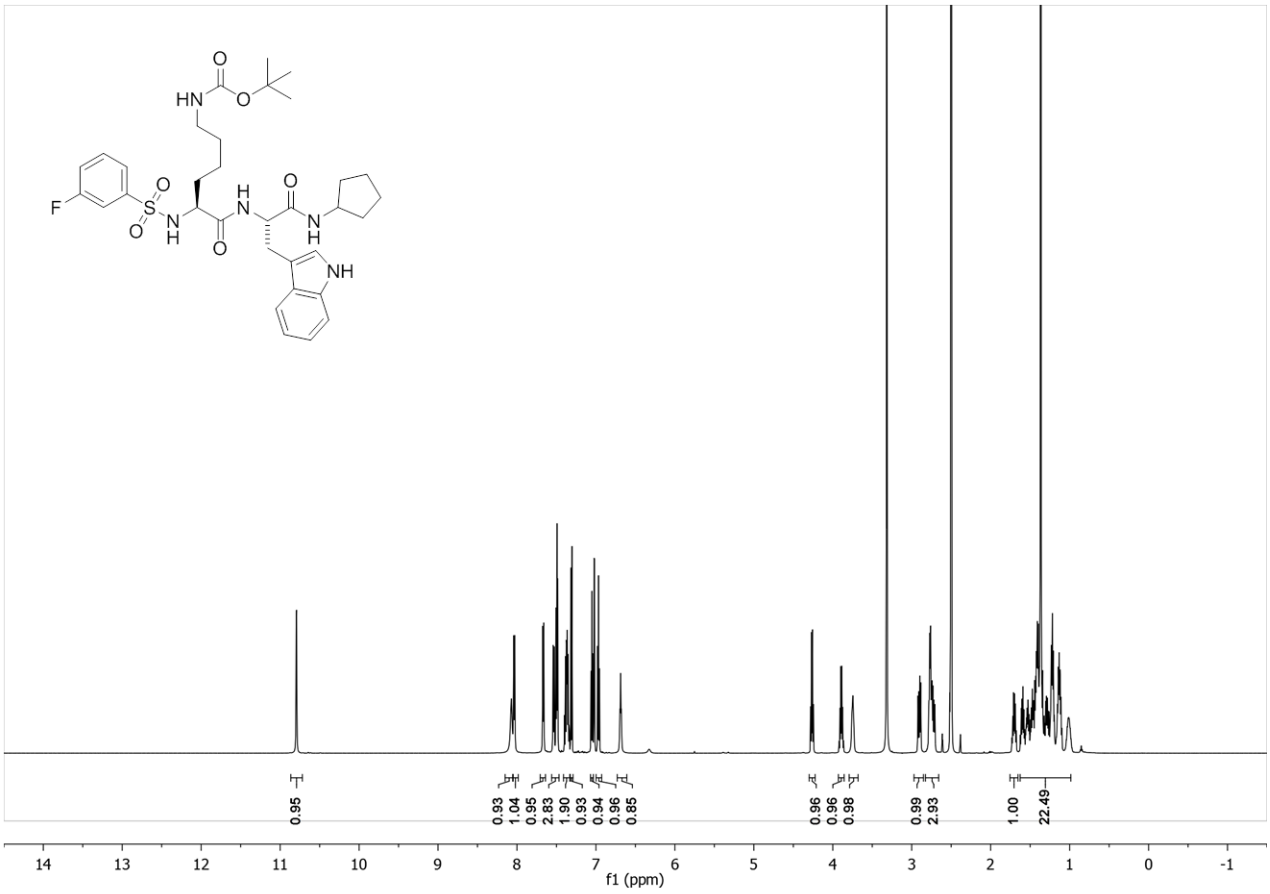
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S80.

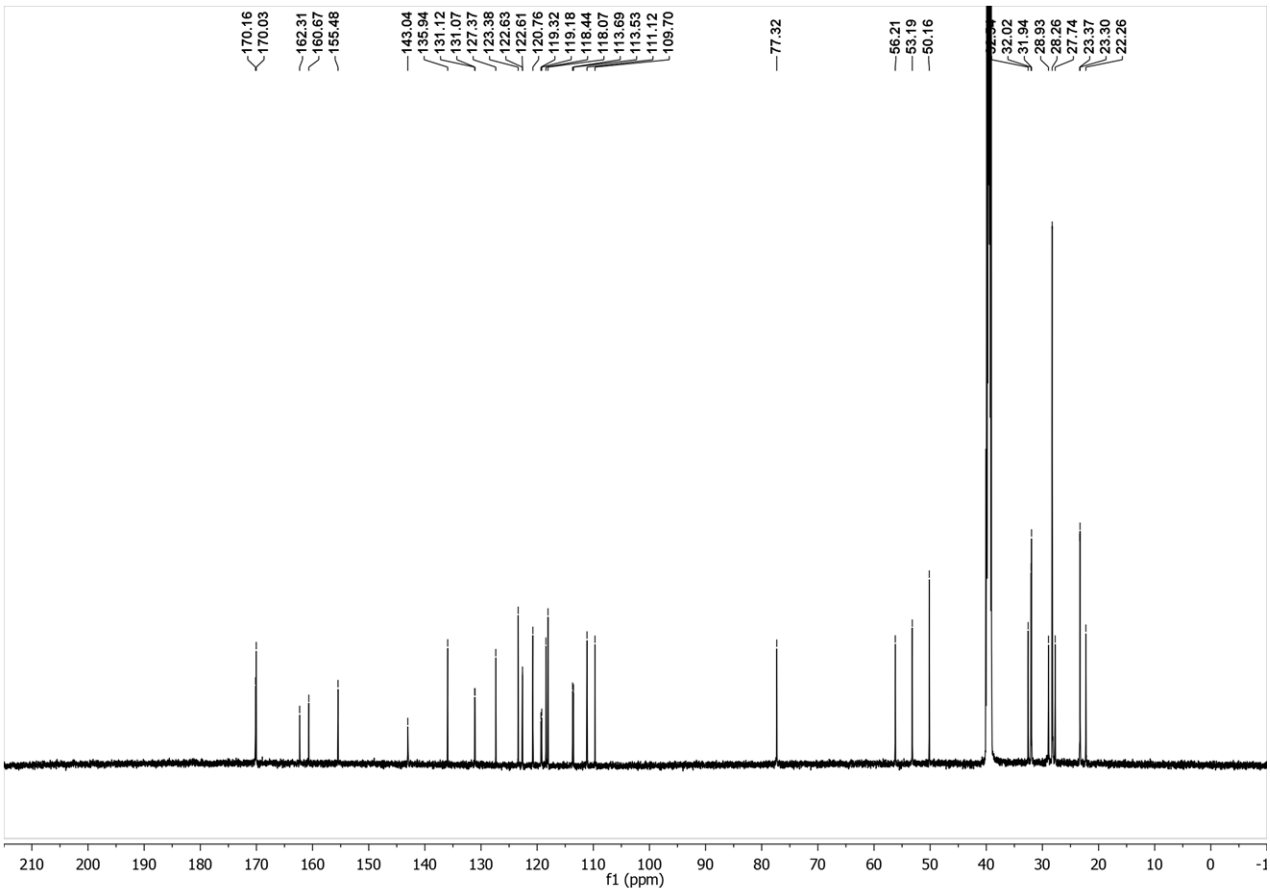


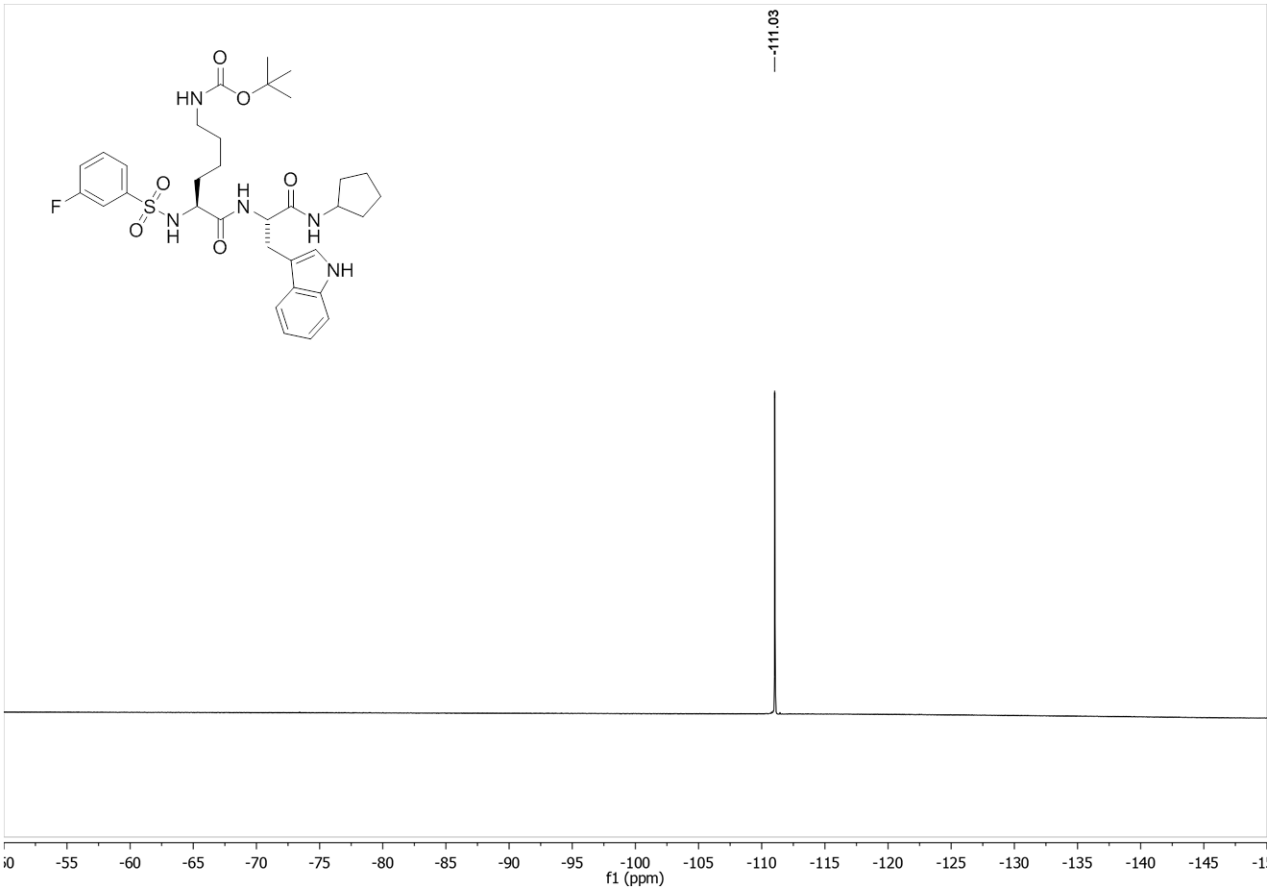



-111.03
F
O
HN
O
O
S
O
N
H
O
H
N
O
N
H
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

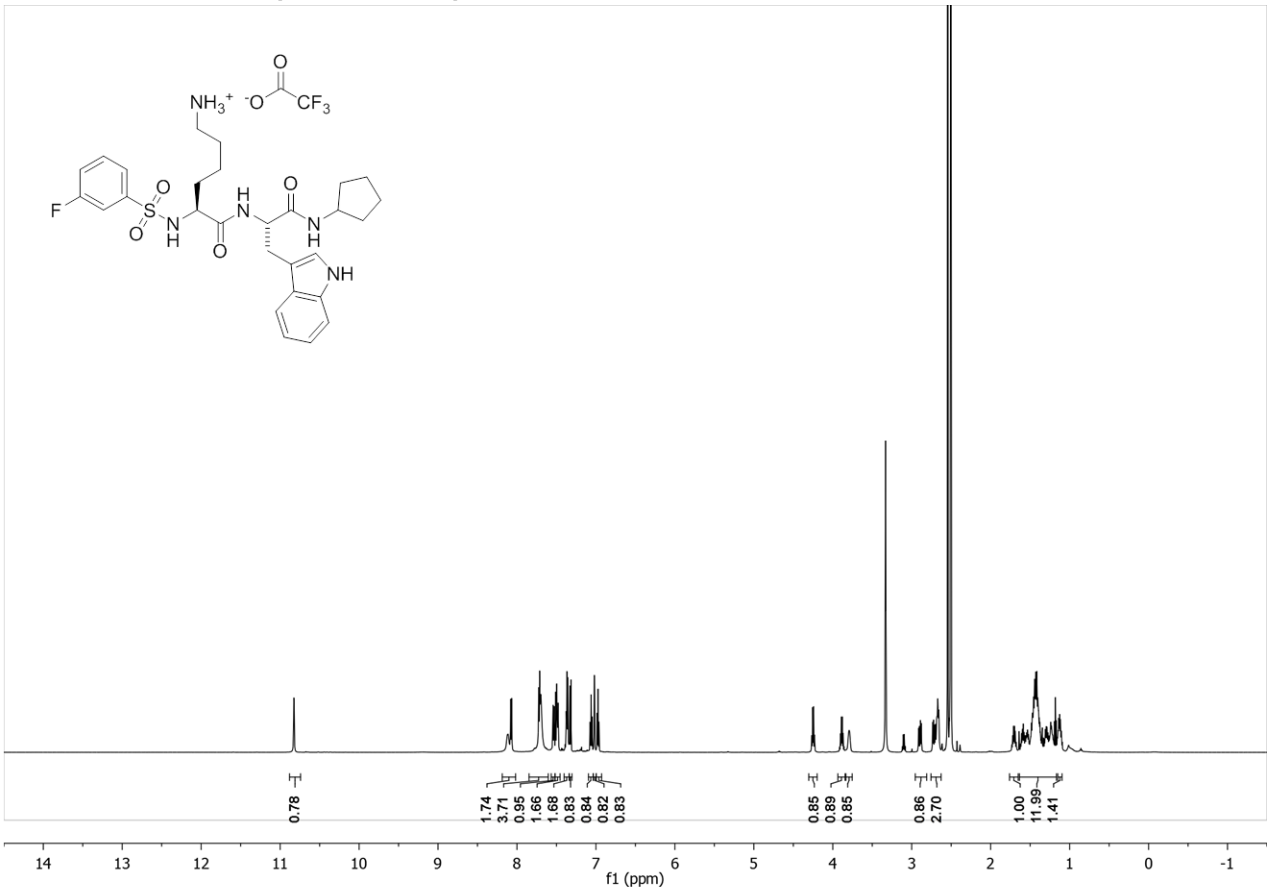
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S81.

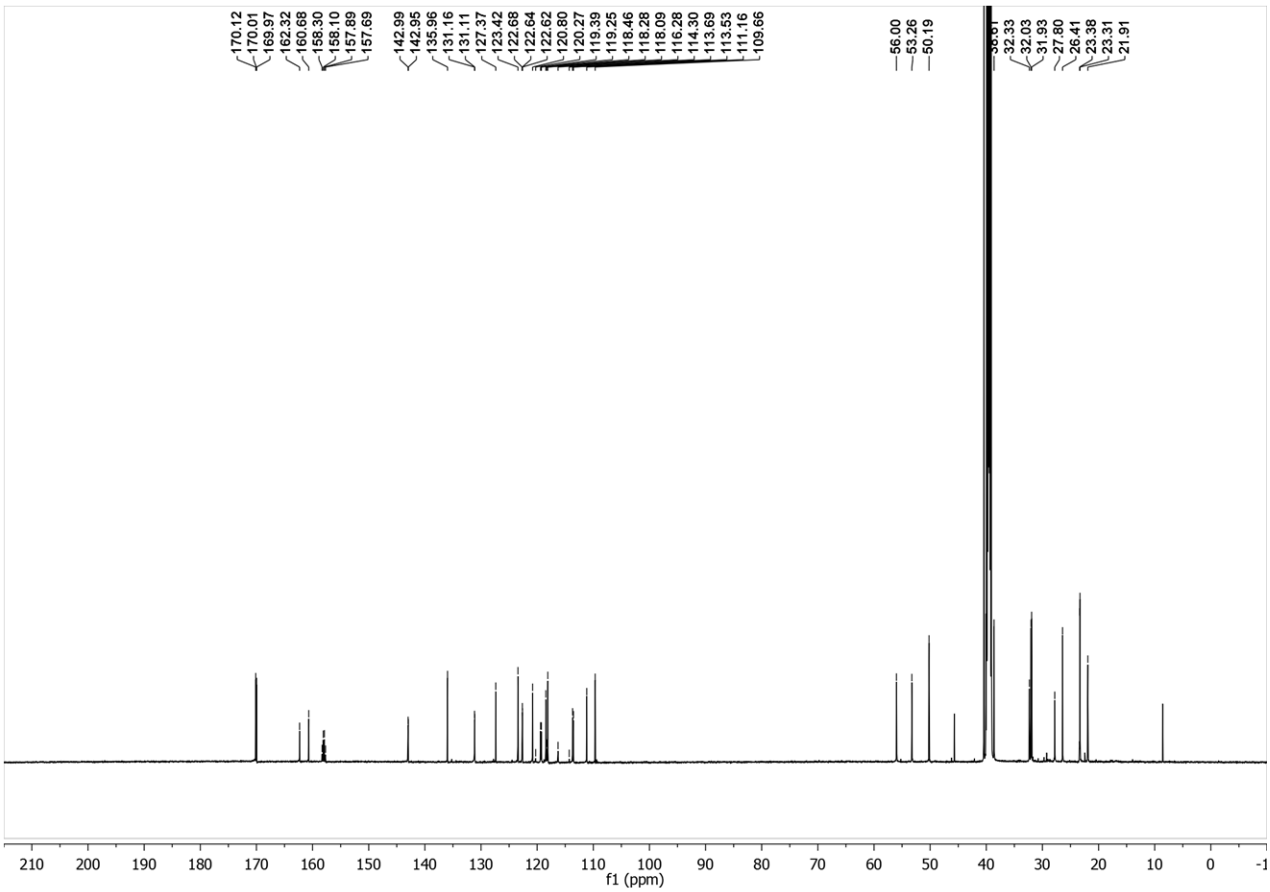


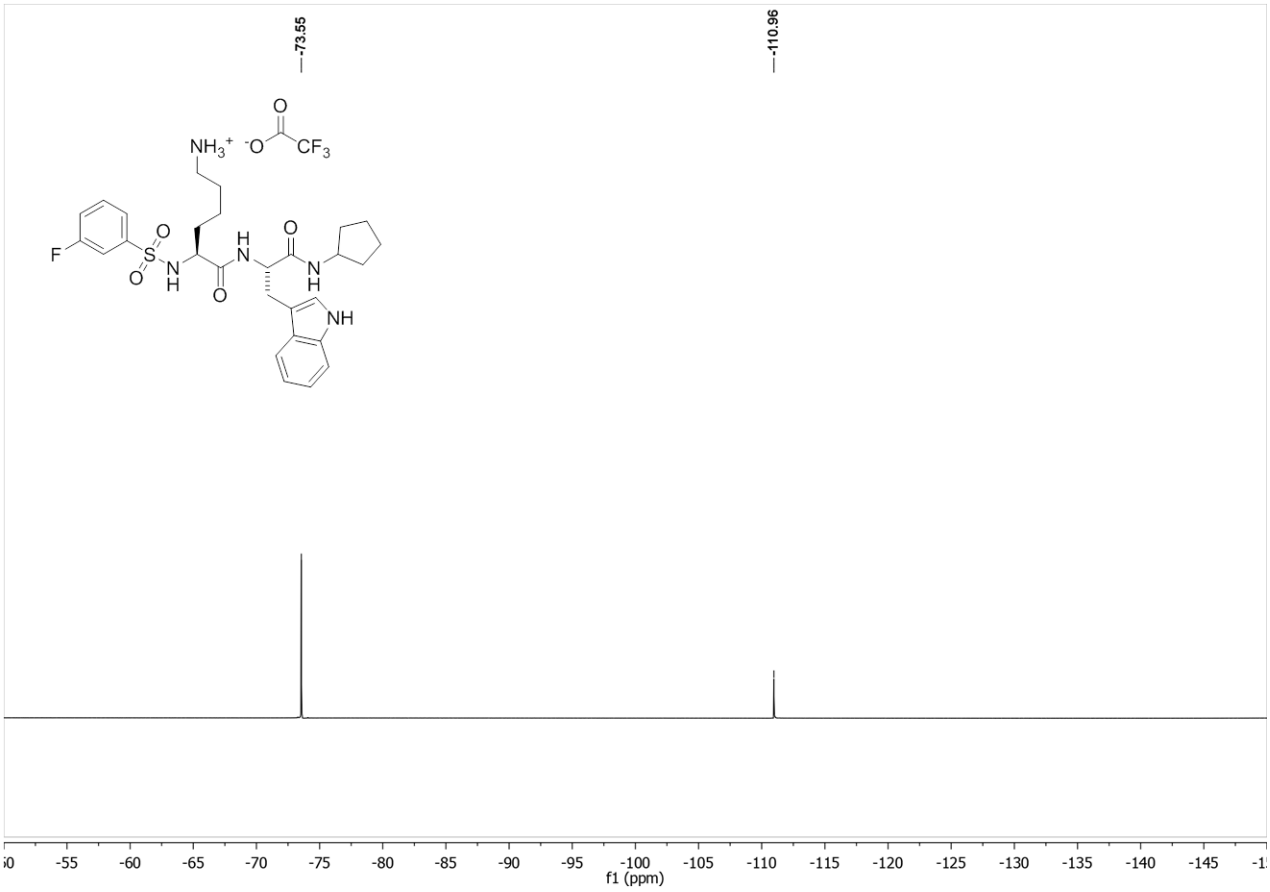



-111.03
HN
O
O
F
S
O
O
N
H
H
N
O
N
H
O
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

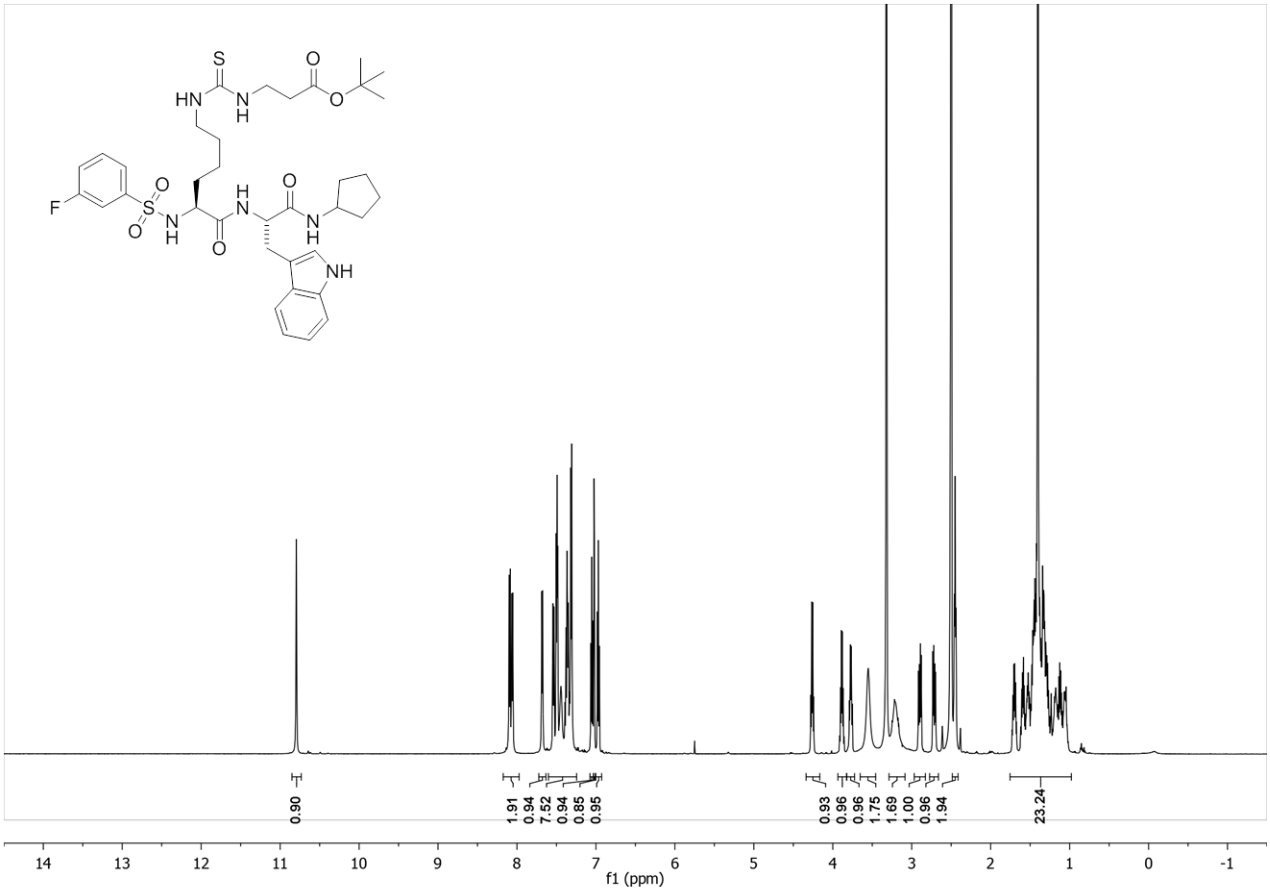
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S82.

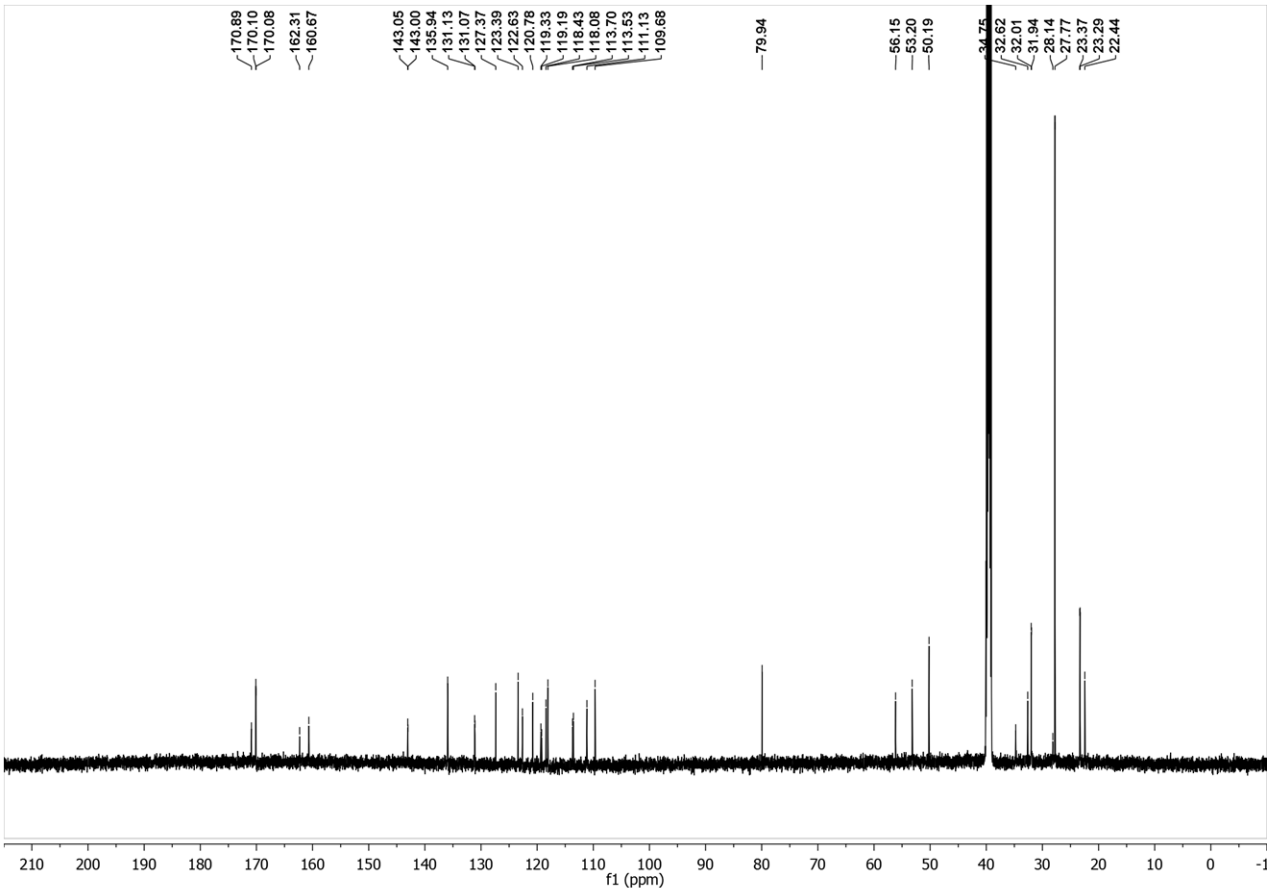


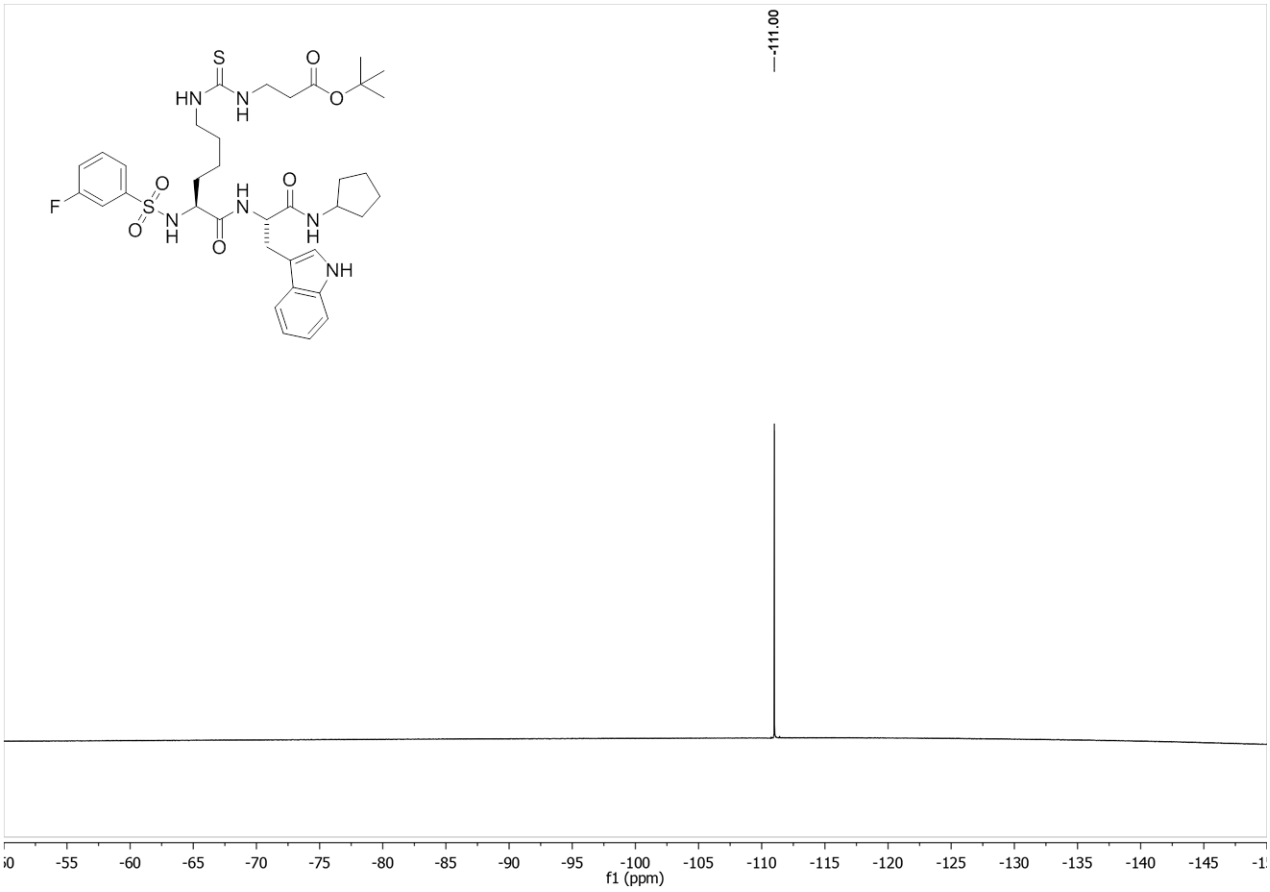



-73.55
-110.96
NH3+
-O
CF3
F
S
N
H
NH
f1 (ppm)

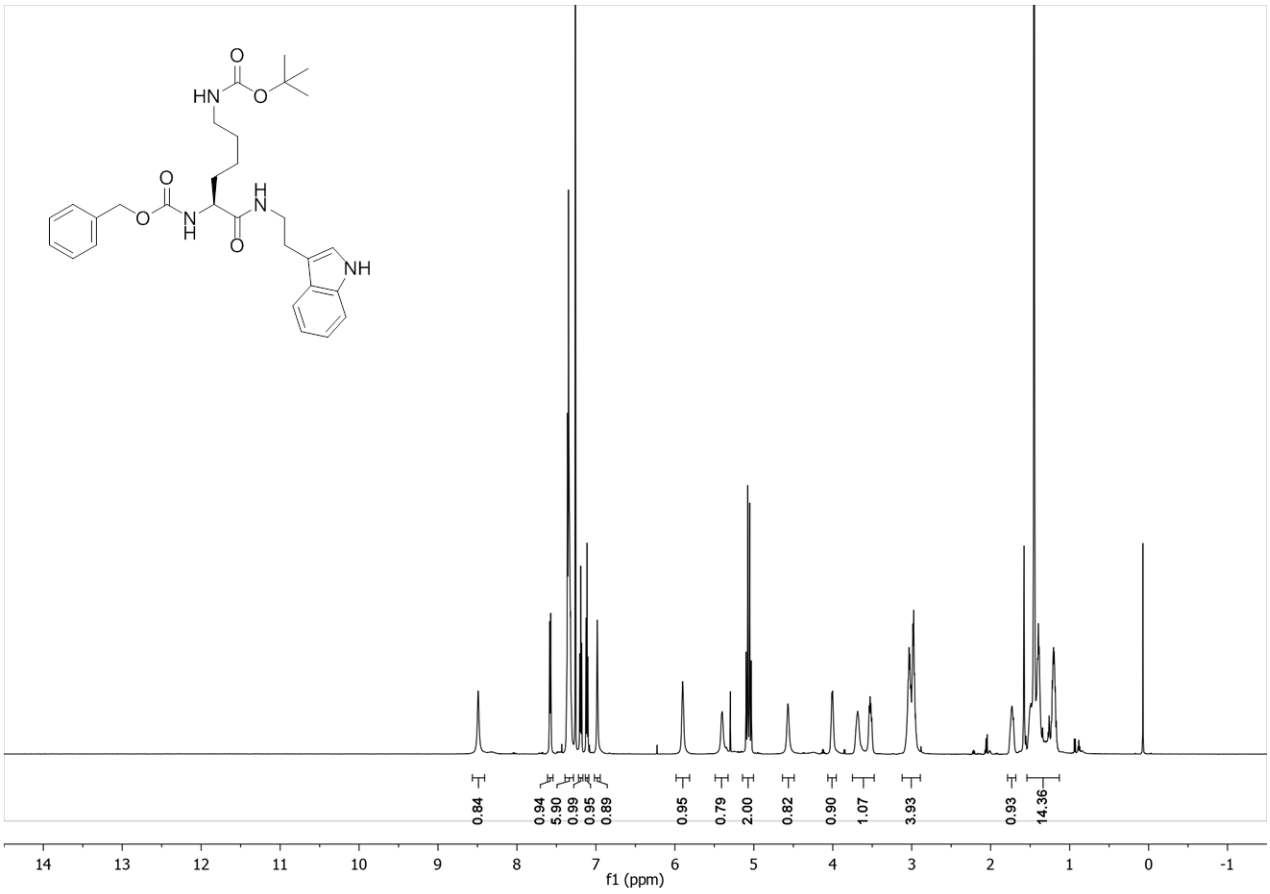
^{1}H, ^{13}C, and ^{19}F NMR spectra of compound S83.

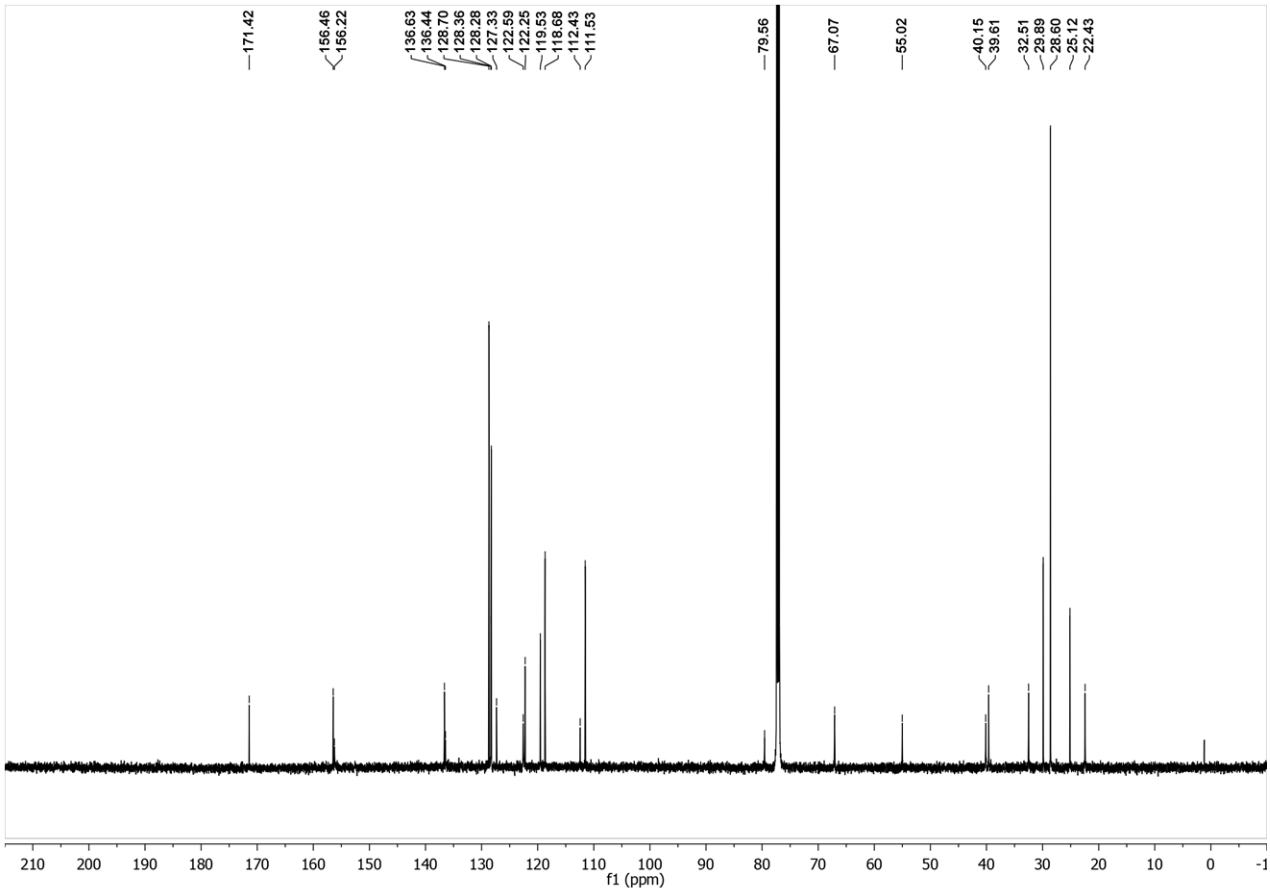




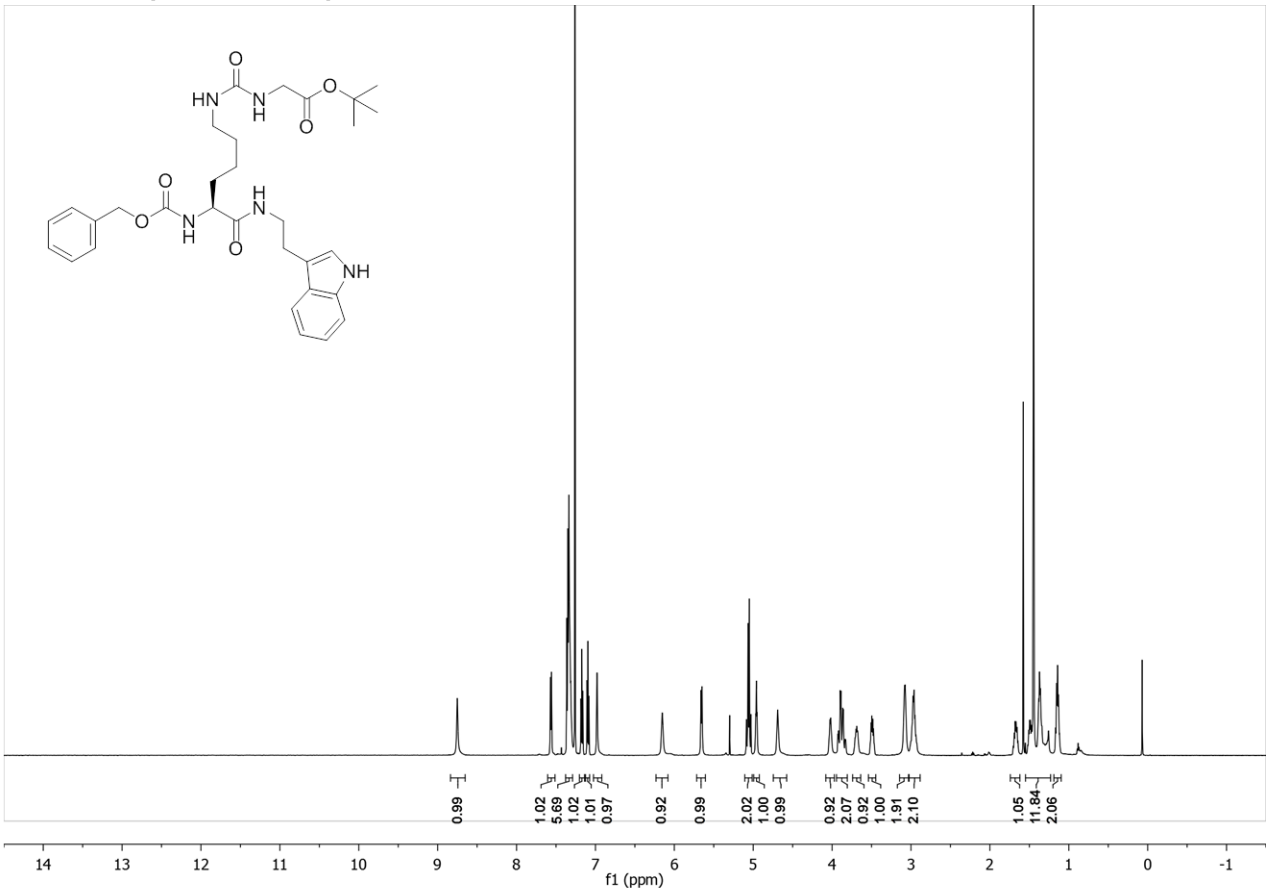

-111.00
F
S
O
O
HN
N
H
O
O
H
N
O
N
H
O
NH
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
f1 (ppm)

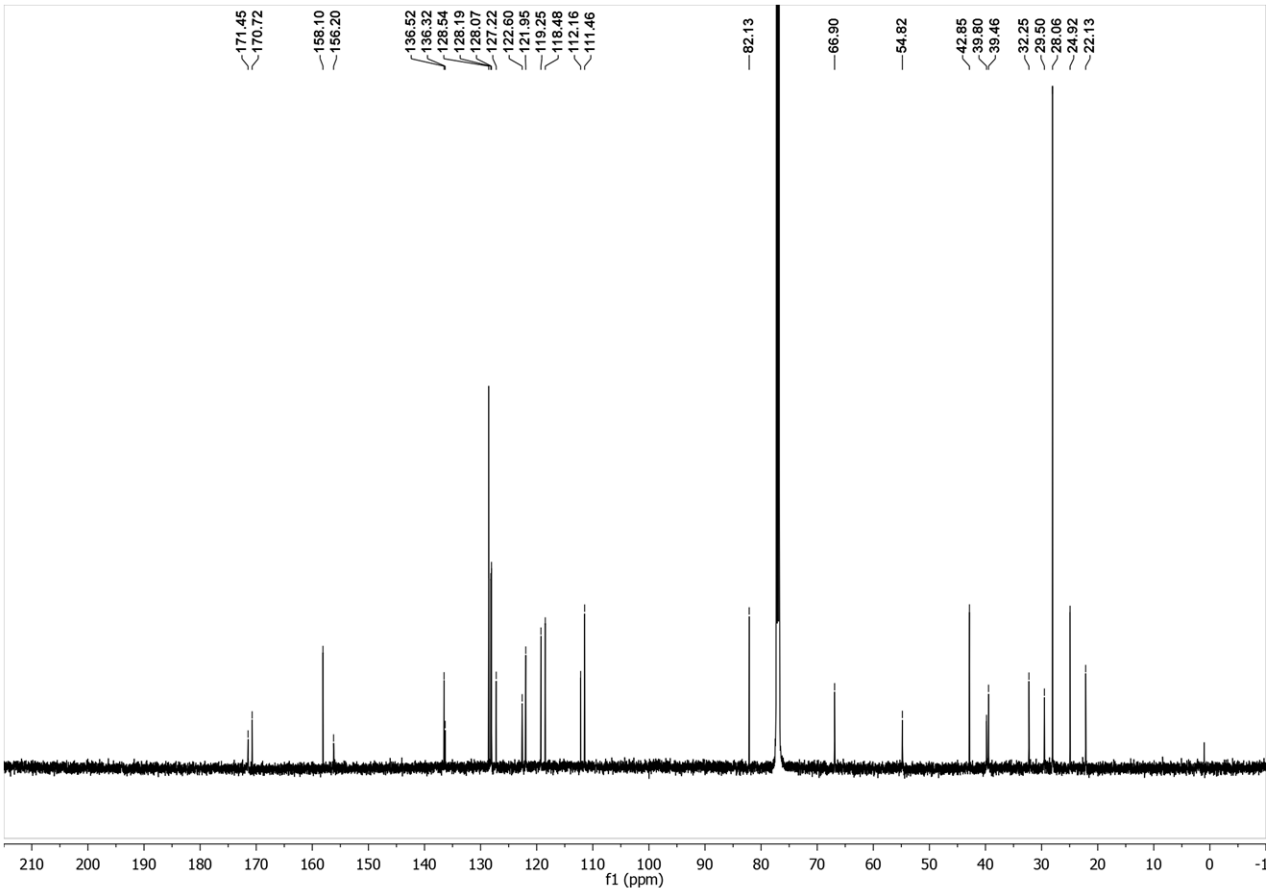
^{1}H and ^{13}C spectra of compound S84.



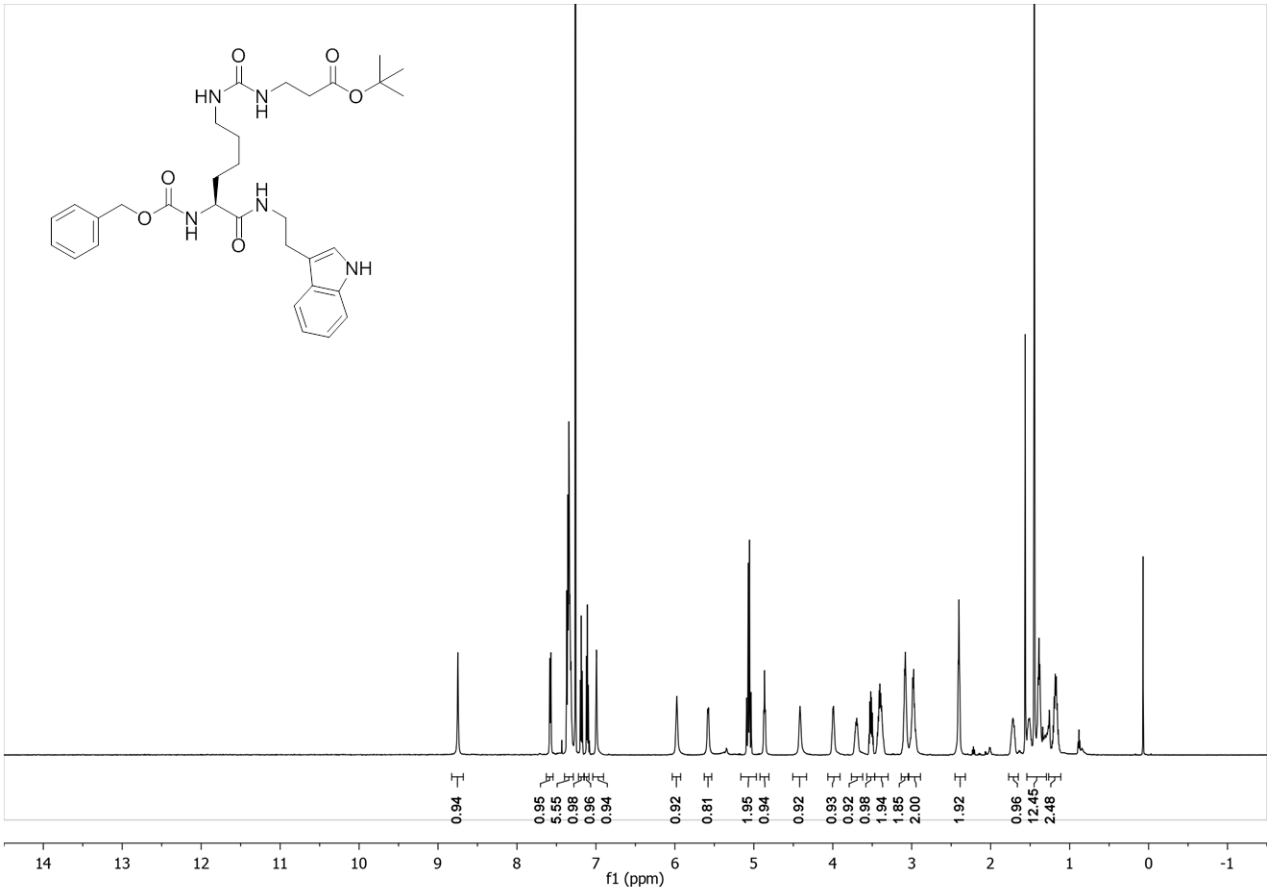


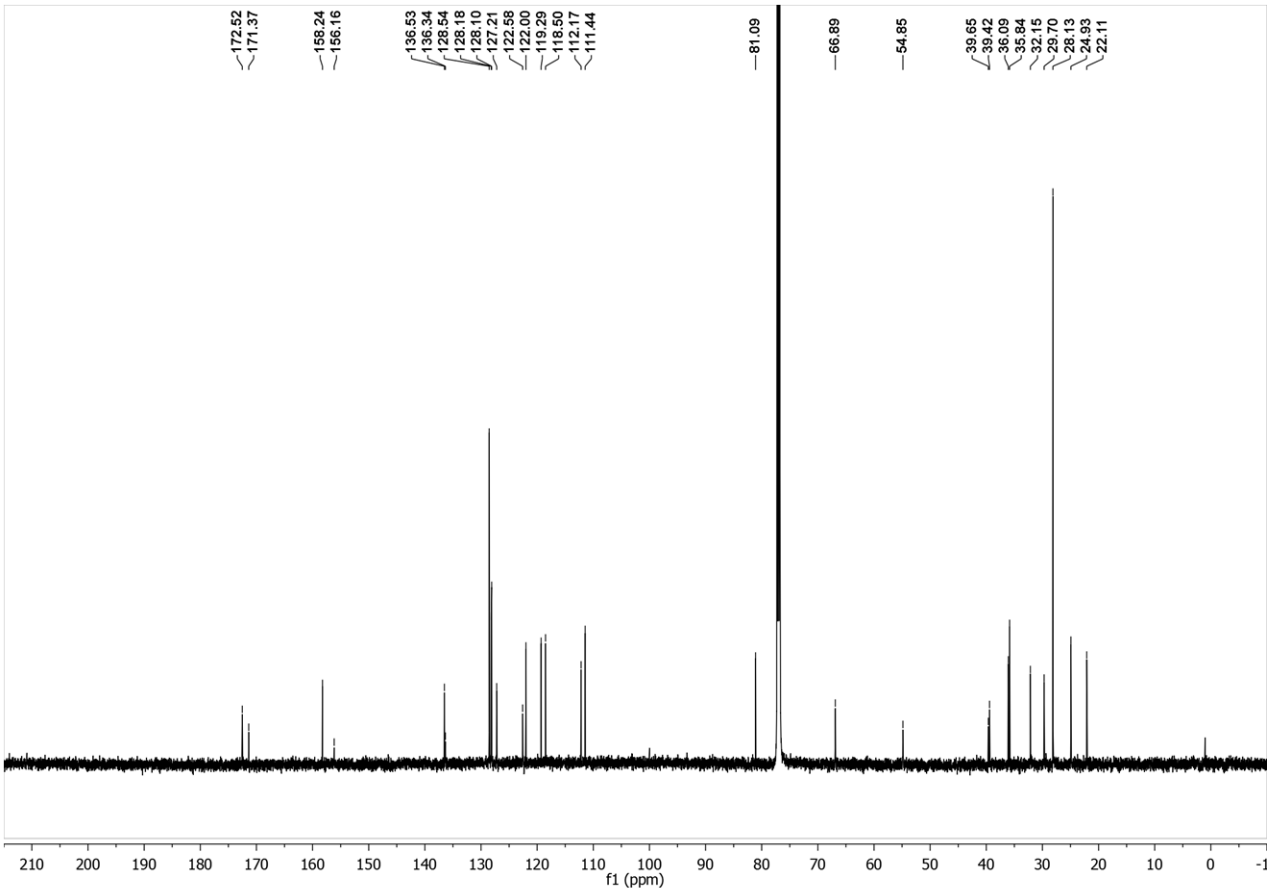
^{1}H and ^{13}C spectra of compound S85.



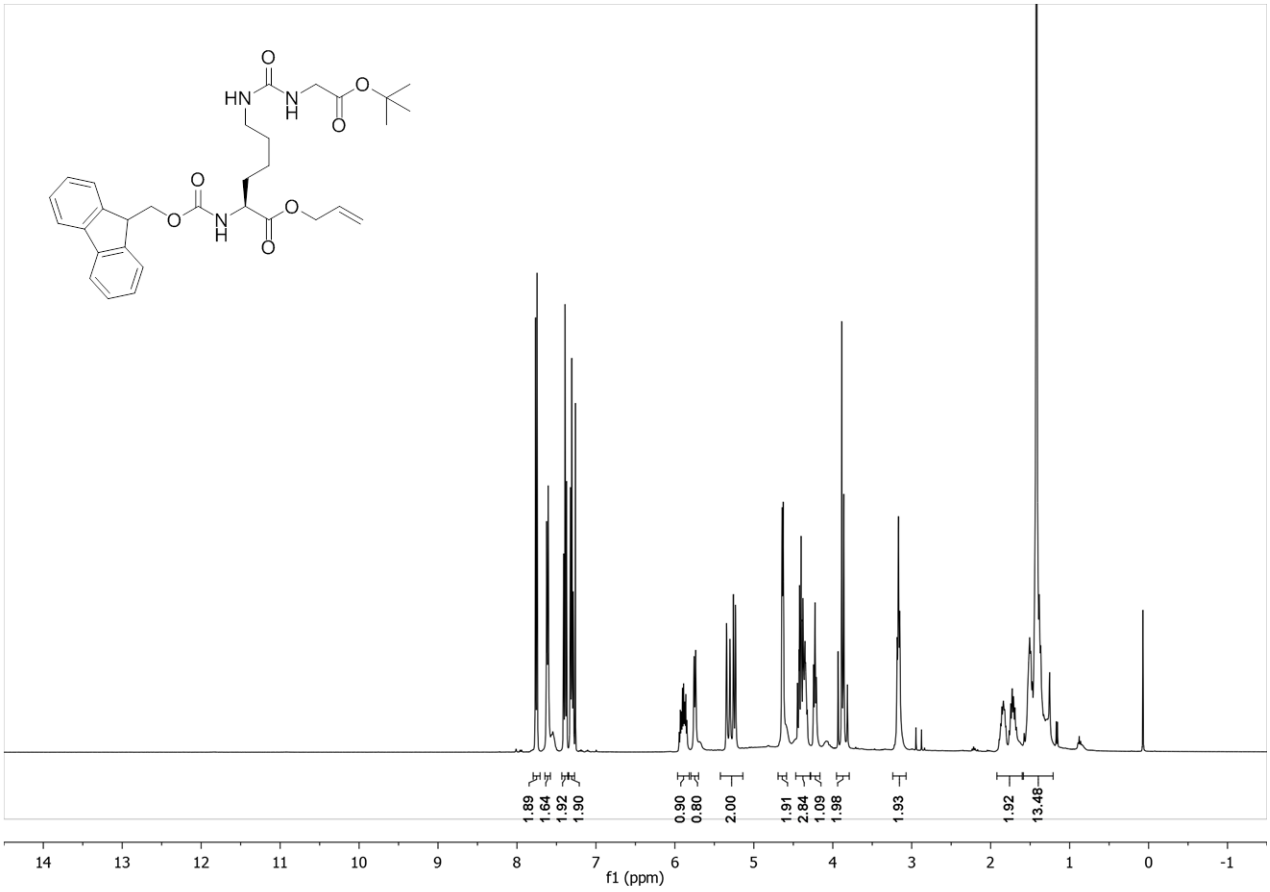


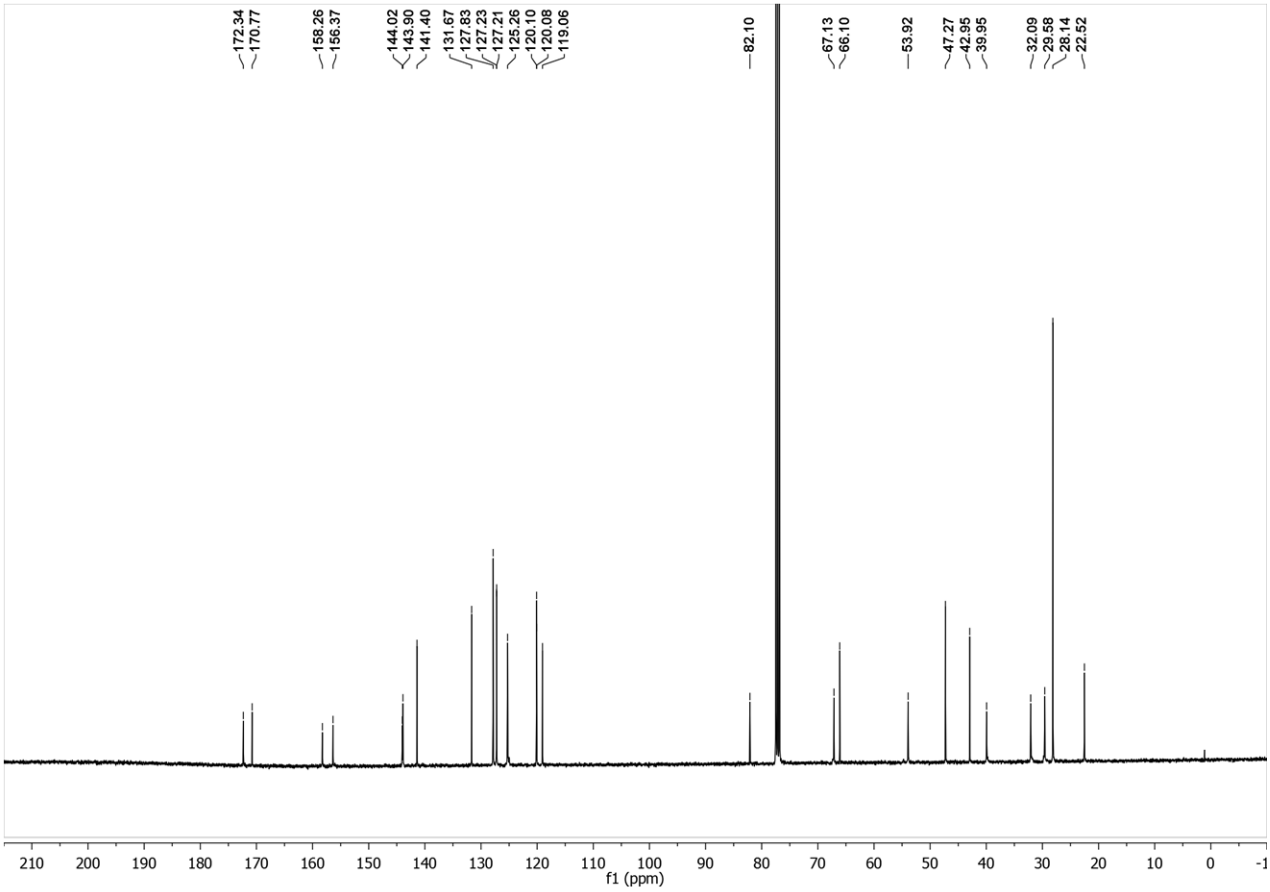
^{1}H and ^{13}C spectra of compound S86.





^{1}H and ^{13}C spectra of compound S87.





^{1}H and ^{13}C spectra of compound S88.