Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Synthesis of Macrocyclic Thiolactone Peptides via Intramolecular Radical Acyl Thiol-Ene

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1. General Experimental Information

All commercially available chemicals were purchased from Merck KGaA, Fluorochem Ltd or Tokyo Chemical Industry (TCI) and used without further purification unless otherwise stated. Solvents for synthesis and purification were supplied by Sigma-Aldrich at HPLC grade. Dry solvents were obtained from a PureSolv MD-4EN solvent purification system. Room-temperature (rt) is classified in this work as 18 – 21 °C. Thin Layer Chromatography (TLC) was performed using Merck 60 F254 silica gel plates (pre-coated, 0.2 mm thick) and visualised by UV illumination (254 nm), ninhydrin staining (1.5 g ninhydrin, 3 mL acetic acid in 100 mL *n*-butanol), permanganate staining (1.6 g potassium permanganate, 10 g potassium carbonate and 1.25 mL 10% NaOH in 200 mL H_2O) or molybdenum staining (5.0 g ammonium molybdate, 5.3 mL concentrated H_2SO_4 in 100 mL H_2O). Melting points are uncorrected and were measured with a Stuart SP-10 melting point apparatus. All UV reactions were carried out in a Luzchem photoreactor, LZC-EDU (110 V/60 Hz), containing 14 UVA lamps centred at 354 nm.

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra were recorded using Bruker DPX 400 (400.13 MHz for ^{1}H NMR and 100.61 MHz for ^{13}C NMR), Bruker AV 400 (400.13 MHz for ^{1}H NMR and 100.61 MHz for ^{13}C NMR), Agilent MR400 (400.13 MHz for ^{1}H NMR and 100.61 MHz for ^{13}C NMR) or Bruker AV 600 (600.13 MHz for ^{1}H NMR and 150.90 MHz for ^{13}C NMR) instruments. Deuterated solvents for NMR were purchased from Sigma Aldrich (Merck). Chemical shifts, δ, are in ppm and referenced to the internal solvent signals for chloroform (δ_H 7.26 and δ_C 77.0) or DMSO (δ_H 7.26 and δ_C 39.7). NMR descriptions: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Coupling constants, *J*, are reported in Hertz (Hz). NMR data was processed using MestReNova software (version 14.1.1-24571) by MestReNova Lab Research S. L.

High Resolution Mass Spectrometry (HRMS)

ESI mass spectra were acquired using a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC in positive and negative modes as required. Agilent ESI-L Low Concentration Tuning Mix was used to calibrate the system, and this was also used as an internal lock mass. Masses were recorded over the range 100 – 2000 m/z. Operating conditions were as follows: end-plate offset 500 V, capillary 4500 V, nebulizer 2.0 bar, dry gas 8.0 L min^{-1}, and dry temperature 180 °C.

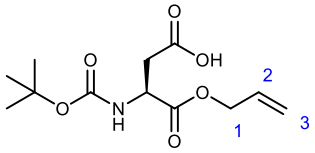
APCI experiments were carried out on a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC or direct insertion probe in positive or negative mode as required. Agilent APCI-TOF Tuning Mix was used to calibrate the system. Masses were recorded over a range of 100 – 2000 m/z. Operating conditions were as follows: capillary voltage 4000 V, corona 4000 nA, nebulizer gas 2.0 bar, dry gas 3.0 L min^{-1}, dry gas temperature 100 – 200 °C, vap. temperature 100 – 400 °C. Compass HyStar version 3.2 and micrOTOF Control version 3.2 software were used to analyse the data.

High-performance Liquid Chromatography (HPLC)

HPLC was carried out using a Shimadzu Nexera lite Low Pressure Gradient system. For analytical HPLC, an Ascentis® Express 90 Å C18 100 mm x 4.6 mm, 5 µm column with a flow rate of 1.0 mL min^{-1} was used. For semi-preparative HPLC purification, an Ascentis® C18 250 mm x 10 mm, 5 µm column with a flow rate of 5 mL min^{-1} was used. Gradient elution with Solvent A (0.1% TFA in H_2O) and Solvent B (0.1% TFA in MeCN) was used for both analytical and semi-preparative HPLC. Eluting compounds were detected with an SPD-M40 PDA detector at 220 nm.

2. Synthesis of Boc-Asp(STrt)-OTMS (1)

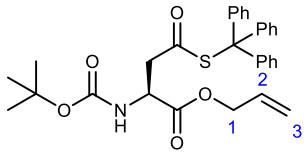
1-allyl 4-(tert-butyl) (tert-butoxycarbonyl)-L-aspartate (3)



Boc-Asp-OAll (**3**) was prepared from Boc-Asp(O^{t}Bu)-OH following the procedure published by Kapernitcou and Taylor using MeCN instead of DMF.[1] The product was isolated as a pale yellow oil (XX g) in an overall yield of 90% over 3 steps from Boc-Asp(O^{t}Bu)-OH. The isolated product was in good agreement with the literature.[2]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.14 (brs, 1H, COOH), 5.91 (ddt, *J* = 16.4, 10.9, 5.7 Hz, 1H, H2), 5.54 (d, *J* = 8.7 Hz, 1H, NH), 5.34 (dd, *J* = 17.2, 1.1 Hz, 1H, H3), 5.25 (dd, *J* = 10.5, 1.1 Hz, 1H, H3), 4.69 – 4.56 (m, 3H, Asp αCH, H1), 3.09 (dd, *J* = 17.4, 4.6 Hz, 1H, Asp βCH_2), 2.90 (dd, *J* = 17.4, 4.6 Hz, 1H, Asp βCH_2), 1.47 (s, 9H, ^{t}Bu).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 176.1 (C=O), 170.6 (C=O), 155.5 (Boc C=O), 131.4 (C2), 118.8 (C3), 80.4 (tBu qC), 66.4 (C1), 49.8 (Asp αC), 36.5 (Asp βC), 28.2 (tBu CH_3).
HRMS APCI-MS m/z: $[M-H]^-$ calculated for $C_{12}H_{18}NO_6$ 272.1140; found 272.1139.

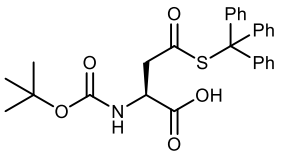
allyl (S)-2-((tert-butoxycarbonyl)amino)-4-oxo-4-(tritylthio)butanoate (4)



To a stirring solution of **3** (2.00 g, 7.32 mmol) in DCM (60 mL) at 0 °C was added EDC.HCl (2.10 g, 10.98 mmol) and stirred for 5 min. Then DMAP (0.134 g, 1.10 mmol) and triphenylmethanethiol (2.02 g, 7.32 mmol) were added and stirred at rt under nitrogen for 3 h. The reaction mixture was diluted with DCM (100 mL) and water (100 mL) and the organic layer was extracted, washed with brine (100 mL) and dried over anhydrous magnesium sulfate. The solvent was then removed in vacuo. The residue was dry-loaded on Celite and purified by silica gel flash chromatography (0 to 20% (v/v) EtOAc/hexane). Fractions containing pure product were combined and the solvent was evaporated in vacuo to give the product as a white foam (2.45 g, 63%).

R_f (30:70 EtOAc:Hex): 0.63.
m.p. 92 – 94 °C
^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.34 – 7.25 (m, 10 H, Ph, NH), 7.15 – 7.10 (m, 6 H, Ph), 5.86 (ddt, *J* = 17.2, 10.3, 5.2 Hz, 1H, H2), 5.30 (dd, *J* = 17.2, 1.9 Hz, 1H, H3), 5.19 (dd, *J* = 10.4, 1.9 Hz, 1H, H3), 4.55 (d, *J* = 5.2 Hz, 2H, H1), 4.33 (m, 1 H, Asp αCH), 3.01 (dd, *J* = 15.8, 5.7 Hz, 1H, Asp βCH_2), 2.94 (dd, *J* = 15.8, 8.2 Hz, 1H, Asp βCH_2), 1.38 (s, 9H, ^{t}Bu).
^{13}C-NMR (101 MHz, DMSO-d_6): δ 193.4 (SC=O), 170.7 (C=O), 155.3 (Boc C=O), 143.4 (Ar qC), 132.4 (C2), 129.5 (Ar C), 128.0 (Ar C), 127.4 (Ar C), 117.8 (C3), 78.7 (^{t}Bu qC), 70.1 (Trt qC), 65.3 (C1), 50.3 (Asp αC), 44.3 (Asp βC), 28.3 (^{t}Bu CH_3).
HRMS ESI m/z: $[M+Na]^+$ calculated for $C_{31}H_{33}NO_5SNa$ 554.1972; found 554.1972

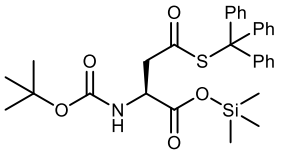
(S)-2-((tert-butoxycarbonyl)amino)-4-oxo-4-(tritylthio)butanoic acid (5)



To a stirring solution of Boc-Asp(STrt)-OAll **4** (2.0 g, 3.76 mmol) in DCM (60 mL) under nitrogen was added tetrakis(triphenylphosphine)palladium(0) (0.020 g, 17.3 µmol) and phenylsilane (2.2 mL, 17.8 mmol) and stirred at rt for 24 h. The reaction mixture was concentrated in vacuo. The residue was dry-loaded on Celite and purified by silica gel flash chromatography (0 to 7% (v/v) DCM/MeOH). Fractions containing pure product were combined and the solvent was evaporated in vacuo to give the product as a pale-yellow foam (1.66 g, 90%).

R_f (80:20 EtOAc:Hex): 0.40.
m.p. 172 – 174 °C
^{1}H-NMR (400 MHz, $CDCl_3$:CD_3OD 98:2 v/v): δ 7.23 – 7.09 (m, 15 H, Ph), 5.62 (d, *J* = 6.9 Hz, 1H, NH), 4.16 – 4.07 (m, 1H, Asp αCH), 3.14 (dd, *J* = 15.5, 5.2 Hz, 1H, Asp βCH_2), 2.98 (dd, *J* = 15.5, 5.2 Hz, 1H, Asp βCH_2), 1.37 (s, 9H, ^{t}Bu).[3]
^{13}C-NMR (101 MHz, $CDCl_3$:CD_3OD 98:2 v/v): δ 196.5 (SC=O), 176.0 (COOH), 155.6 (Boc C=O), 143.4 (Ar qC), 129.6 (Ar C), 127.6 (Ar C), 127.0 (Ar C), 79.6 (^{t}Bu qC), 70.7 (Trt qC), 51.7 (Asp αC), 44.8 (Asp βC), 28.1 (^{t}Bu CH_3).
HRMS ESI-MS m/z: $[M+Na]^+$ calculated for $C_{28}H_{29}NO_5SNa$ 514.1659; found 514.1655.

trimethylsilyl (S)-2-((tert-butoxycarbonyl)amino)-4-oxo-4-(tritylthio)butanoate (1)



To a stirring solution of Boc-Asp(STrt)-OH **5** (1.5 g, 3.05 mmol) in dry DCM (10 mL) with 4 Å molecular sieves under nitrogen was added trimethylsilyl chloride (4 mL) slowly. The mixture was stirred at rt for 30 min. The solvent was removed in vacuo and the residue was dried under high vacuum until a constant weigh was achieved. The product was isolated as a tan-coloured foam (1.72 g, quant %). Used without further purification.

m.p. 155 – 157 °C
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30 – 7.17 (m, 15H, Ph), 5.29 (d, *J* = 8.0 Hz, 1H, NH), 4.52 – 4.32 (m, 1H, Asp αCH), 3.20 (dd, *J* = 17.2, 5.5 Hz, 1H, Asp βCH_2), 3.05 (dd, *J* = 16.3, 5.1 Hz, 1H, Asp βCH_2), 1.44 (s, 9H, ^{t}Bu), 0.07 (s, 9H, $Si(CH_3)_3$).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 195.3 (SC=O), 174.3 (C=O), 155.6 (Boc C=O), 143.3 (Ar qC), 129.7 (Ar C), 127.9 (Ar C), 127.3 (Ar C), 80.6 (^{t}Bu qC), 71.1 (Trt qC), 50.5 (Asp αC), 44.2 (Asp βC), 28.3 (^{t}Bu CH_3), 1.9 ($Si(CH_3)_3$).

3. Fmoc Solid Phase Peptide Synthesis (SPPS) General Procedure

SPPS was performed manually in polypropylene syringe reaction vessels (10 mL; Torviq, MI, USA) fitted with a polystyrene frit. All reactions were performed at room temperature under continuous agitation on an LBX Orb-B2 shaker set to 180 rpm.

Coupling of First Amino Acid: Fmoc-allylglycine-OH (Fmoc-Agl-OH)

Novabiochem® Rink amide aminomethyl resin 100-200 mesh (0.13 g, 0.1 mmol) was swollen in DMF (5 mL) for 20 min and then drained. The resin bound Fmoc group was removed using 20% (v/v) piperidine in DMF (2 x 10 min; 5 mL) and the resin was then washed with DMF (3 x 5 mL), DCM (3 x 5mL) and DMF (3 x 5 mL). Fmoc-Agl-OH (4 equiv.), HATU (3.9 equiv.) and DIPEA (8 equiv.) were dissolved in DMF (0.2 M) and transferred immediately to the syringe containing the resin and agitated for 45 min. Excess reagents were drained and the resin was washed with DMF (3 x 5 mL), DCM (3 x 5 mL) and DMF (3 x 5 mL). Then, the synthesis was continued via the following protocols, unless otherwise stated.

Coupling up to Penultimate Amino Acid

Subsequent amino acid coupling cycles consisted of: **(i)** Fmoc deprotection with 20% (v/v) piperidine in DMF (2 x 10 min; 5 mL). **(ii)** Resin washes with DMF (3 x 5 mL), DCM (3 x 5 mL) and DMF (3 x 5 mL). **(iii)** Coupling with a solution of Fmoc-AA-OH (4 equiv.), HATU (3.9 equiv.) and DIPEA (8 equiv.) in DMF (0.2 M) for 45 min. The coupling was repeated for all subsequent amino acids in the sequence after the incorporation of Leu or Ile. The resin was washed with DMF (3 x 5 mL) between the repeat coupling steps to remove excess reagents. **(iv)** Resin washes with DMF (3 x 5 mL), DCM (3 x 5 mL) and DMF (3 x 5 mL).

Coupling of Final Amino Acid: Boc-Asp(STrt)-OTMS 1

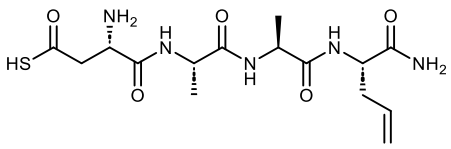
The Fmoc group was removed using 20% (v/v) piperidine in DMF (2 x 10 min; 5 mL) and the resin was then washed with DMF (3 x 5 mL), DCM (3 x 5mL) and DMF (3 x 5 mL). The resin was subsequently washed with dry DCM (3 x 5 mL). Boc-Asp(STrt)-OTMS **1** (3 equiv.) was added to a solution containing DIC (3 equiv.), Oxyma Pure (3 equiv.) and DIPEA (3 equiv.) in dry DCM (0.2 M), and the resulting mixture was immediately added to the resin and agitated for 45 min. The coupling procedure was then repeated after washing the resin with dry DCM (3 x 5 mL) to remove any excess reagent. The resin was washed with DCM (3 x 5 mL), DMF (3 x 5 mL) and DCM (3 x 5 mL). The resin was then dried under reduced pressure.

Global Deprotection and Resin Cleavage

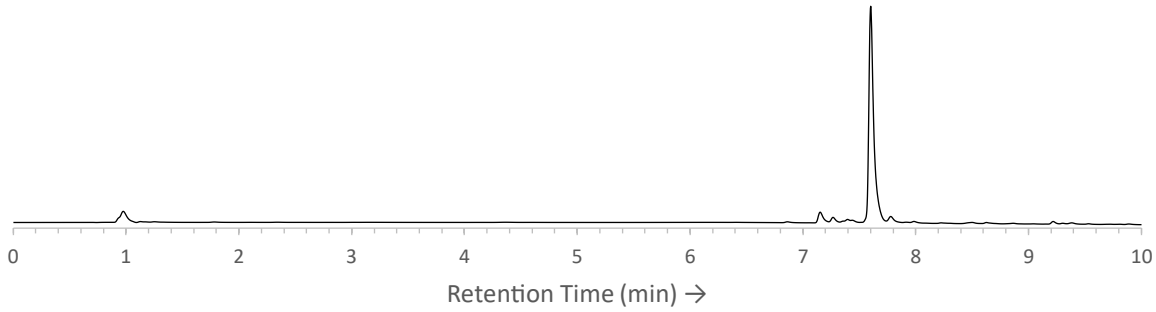
The dried resin was immersed in the cleavage cocktail (TFA:DCM:H_2O:TES; 90:5:2.5:2.5 v/v, 3 mL) unless otherwise stated, capped tightly and agitated for 2 h. The syringe was then drained, and the filtrate was collected. The resin was then washed with another portion of the cleavage cocktail (1 mL) and the washings were combined with the initial filtrate. The combined filtrate was concentrated under a stream of N_2 to form an oily residue which was precipitated with ice-cold diethyl ether (10 mL). The suspension was allowed to rest at 0 °C for 10 min, centrifuged and the supernatant was decanted. The pellet was resuspended in ice-cold diethyl ether (10 mL), centrifuged and the supernatant was decanted twice. After the final wash, the pellet was dried under a stream of N_2 and lyophilised to furnish the peptide.

4. Synthesis of Linear Peptides

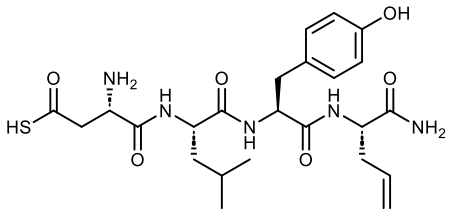
H-Asp(SH)-Ala-Ala-Agl-NH_2 (6)



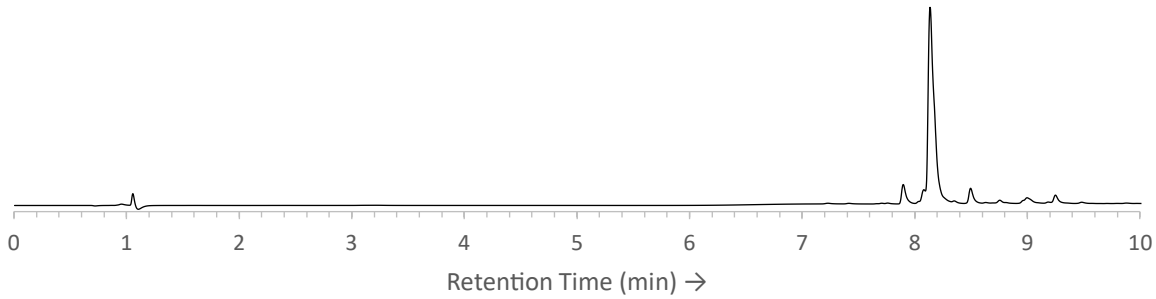
Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Ala-OH and Boc-Asp(STrt)-OTMS **1**. Isolated yield: 32 mg (83%).
HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 7.60 min
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{15}H_{26}N_5O_5S$ 388.1649, found 388.1650.



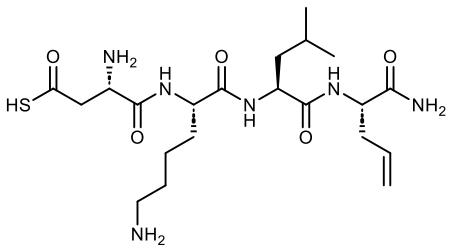
H-Asp(SH)-Leu-Tyr-Agl-NH_2 (S1)



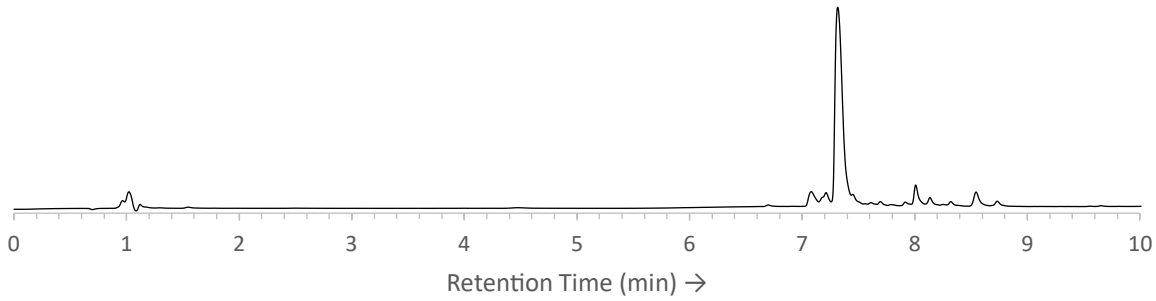
Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Tyr(O^tBu)-OH, Fmoc-Leu-OH and Boc-Asp(STrt)-OTMS **1**. Isolated yield: 34 mg (65%).
HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 8.13 min.
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{24}H_{36}N_5O_6S$ 522.2381, found 522.2387.



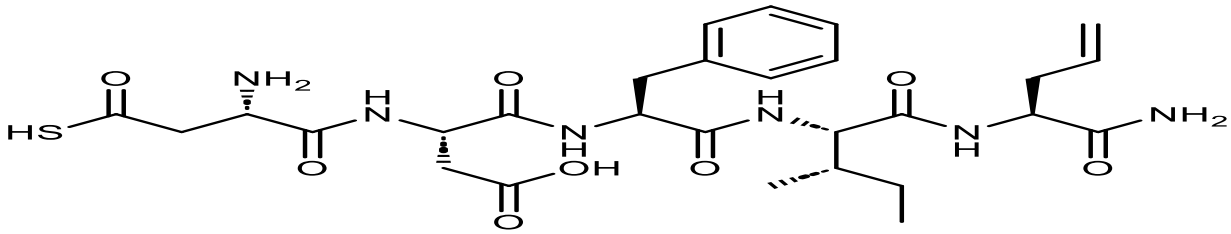
H-Asp(SH)-Lys-Leu-Agl-NH_2 (S2)



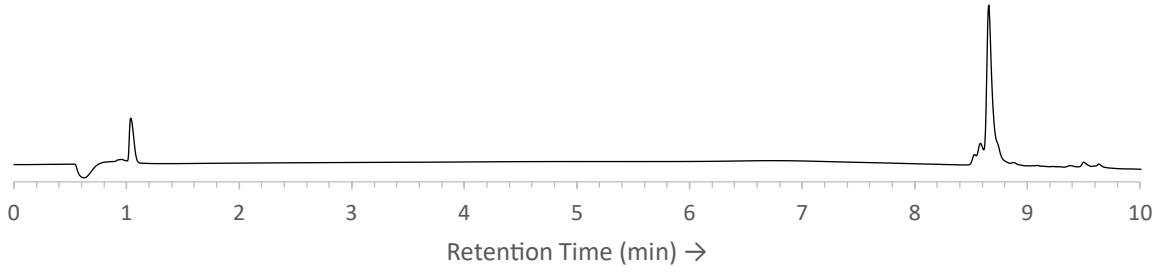
Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Leu- OH, Fmoc-Lys(Boc)-OH and Boc-Asp(STrt)-OTMS **1**. Isolated yield: 45 mg (93%).
HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 7.32 min.
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{21}H_{39}N_6O_5S$ 487.2697, found 487.2694.



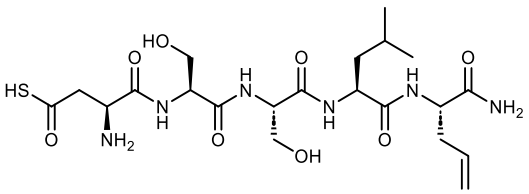
H-Asp(SH)-Asp-Phe-Ile-Agl-NH_2 (S3)



Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Ile-OH, Fmoc-Phe-OH, Fmoc-Asp(O^{t}Bu)-OH and Boc-Asp(STrt)-OTMS **1**. Isolated yield: 26 mg (42%).
HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 8.66 min.
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{28}H_{41}N_6O_8S$ 621.2701, found 621.2698.



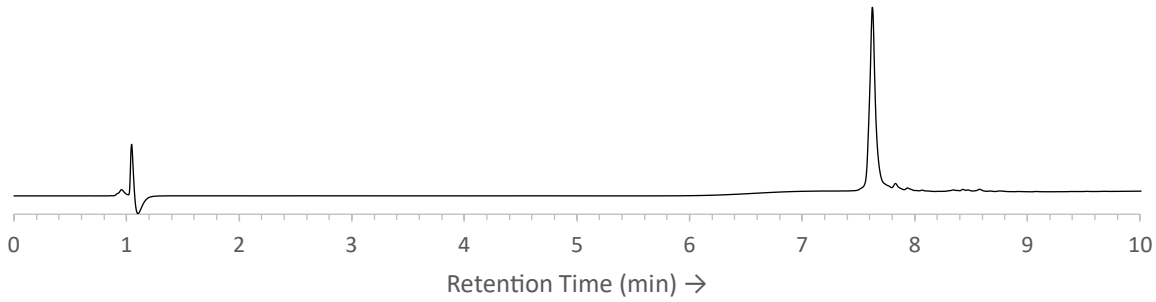
H-Asp(SH)-Ser-Ser-Leu-Agl-NH_2 (S4)



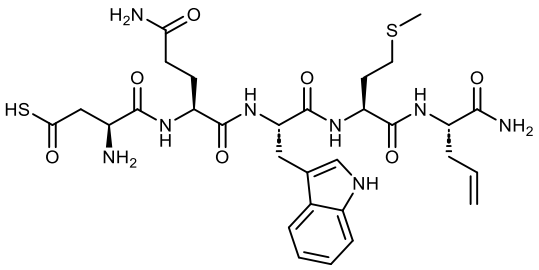
Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Leu-OH, Fmoc-Ser(O^{t}Bu)-OH and Boc-Asp(STrt)-OTMS **1**. Isolated yield: 35 mg (66%).

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 7.62 min.

HRMS ESI m/z: $[M+H]^+$ calculated for $C_{21}H_{37}N_6O_8S$ 533.2388, found 533.2388.



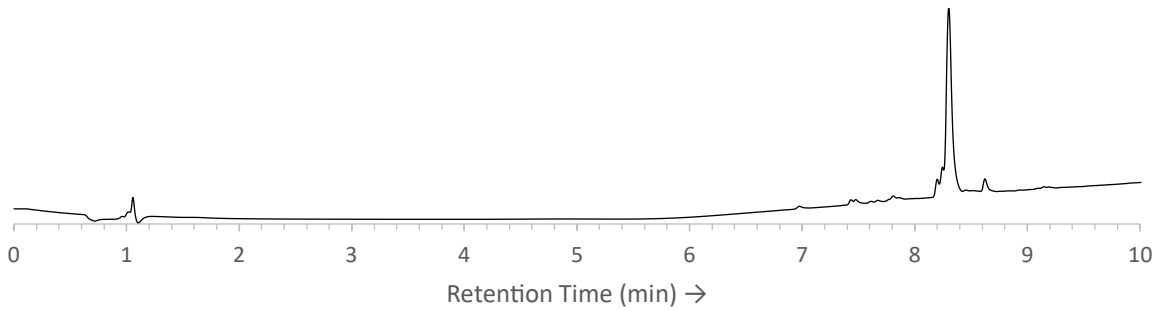
H-Asp(SH)-Gln-Trp-Met-Agl-NH_2 (S5)



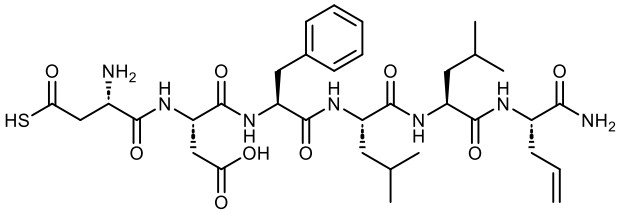
Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Met-OH, Fmoc-Trp(Boc)-OH, Fmoc-Gln(Trt)-OH and Boc-Asp(STrt)-OTMS **1**. The cleavage cocktail consisted of TFA:EDT:TES:H_2O 94:2.5:2.5:1 v/v. Isolated yield: 32 mg (46%).

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 8.30 min.

HRMS ESI m/z: $[M+H]^+$ calculated for $C_{30}H_{43}N_8O_7S_2$ 691.2691, found 691.2691.

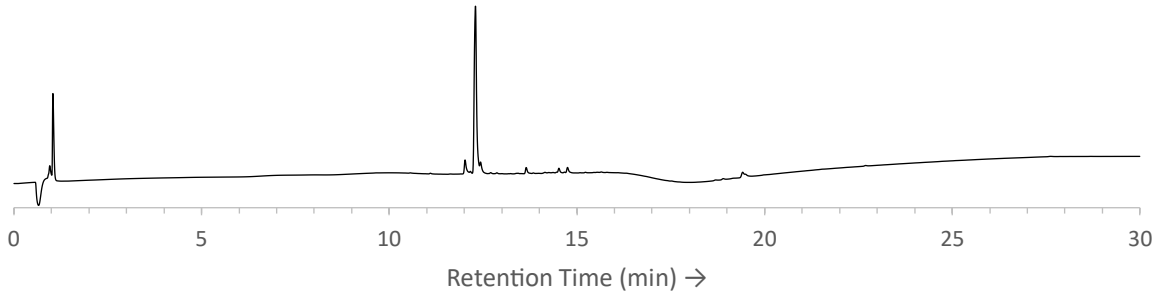


H-Asp(SH)-Asp-Phe-Leu-Leu-Agl-NH_2 (S6)



Prepared using the SPPS general procedures using Fmoc-Agl-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(O^{t}Bu)-OH and Boc-Asp(STrt)-OTMS **1**. Isolated yield: 69 mg (95%).

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 30 min, 220 nm): 12.30 min.
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{34}H_{53}N_7O_9S$ 734.3541, found 734.3546.

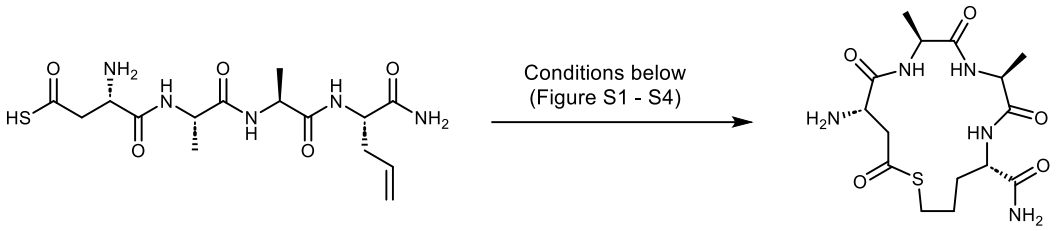


5. Peptide Cyclisation & Reaction Condition Screening

General Cyclisation Procedure

Linear peptide (10 mg) and 2,2-dimethoxy-2-phenylacetophenone (DPAP, 1 equiv.) was dissolved in 1:1 H_2O:MeCN containing 0.1% TFA at 5 mM concentration in a vial and the resulting solution was stirred at rt under UV light illumination for 15 min. 200 µL of the reaction mixture was taken and analysed by analytical HPLC to confirm complete consumption of the linear peptide. The reaction mixture was then purified by semi-preparative HPLC as described followed by lyophilisation to yield the desired cyclic peptide.

Reaction Conditions Screening



6 **7**

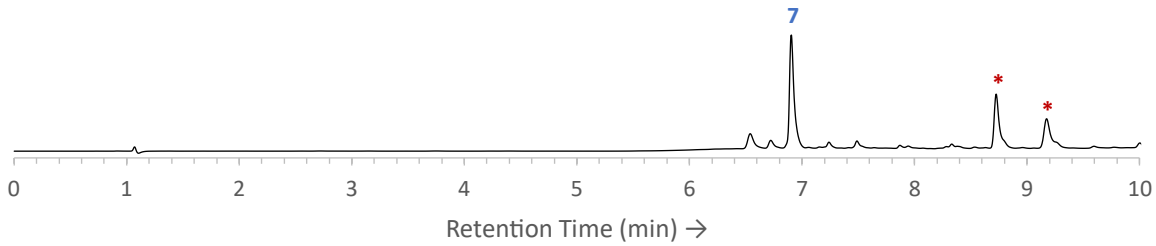


Figure S1. HPLC trace (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm) of the cyclisation of linear peptide **6** (5 mM) to form thiolactone **7** with DPAP (1 equiv.) in 1:1 H_2O:MeCN containing 0.1% TFA under UV light (354 nm) for **15 min**. *DPAP byproducts.

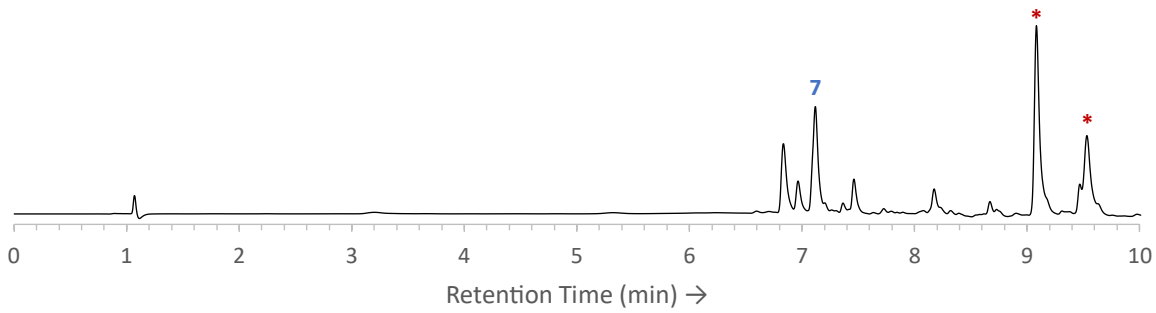


Figure S2. HPLC trace (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm) of the cyclisation of linear peptide **6** (5 mM) to form thiolactone **7** with DPAP (1 equiv.) in 1:1 H_2O:MeCN **without 0.1% TFA** under UV light (354 nm) for 15 min. *DPAP byproducts.

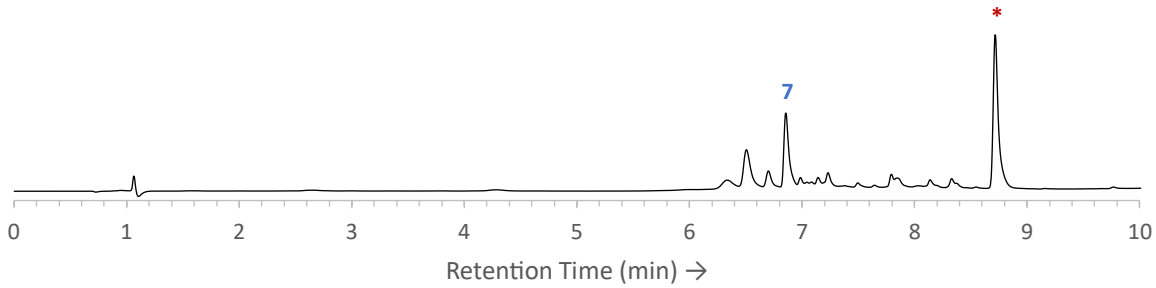


Figure S3. HPLC trace (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm) of the cyclisation of linear peptide **6** (5 mM) to form thiolactone **7** with DPAP (1 equiv.) in 1:1 H_2O:MeCN containing 0.1% TFA under UV light (354 nm) for **1 min**. *DPAP byproduct.

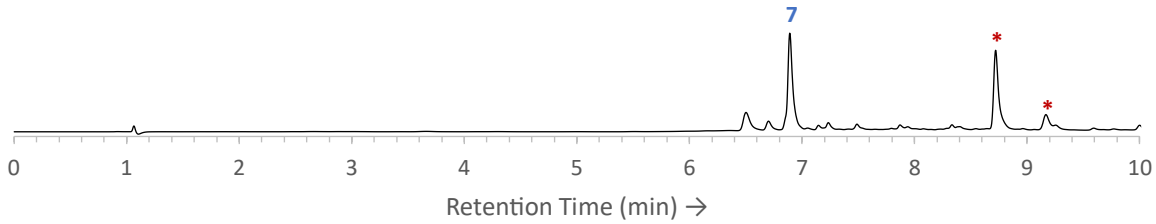


Figure S4. HPLC trace (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm) of the cyclisation of linear peptide **6** (5 mM) to form thiolactone **7** with DPAP (1 equiv.) in 1:1 H_2O:MeCN containing 0.1% TFA under UV light (354 nm) for **5 min**. *DPAP byproducts.

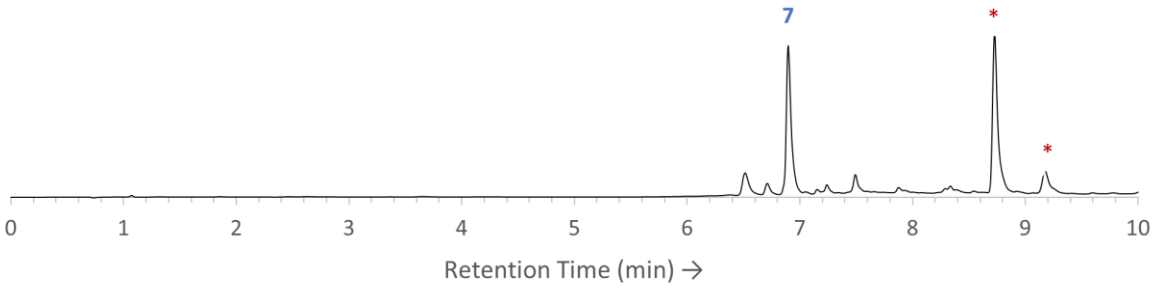
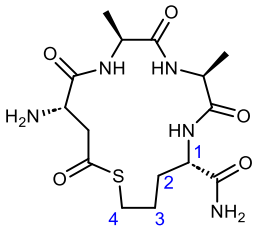


Figure S5. HPLC trace (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm) of the cyclisation of linear peptide **6** (**25 mM**) to form thiolactone **7** with DPAP (1 equiv.) in 1:1 H_2O:MeCN containing 0.1% TFA under UV light (354 nm) for 15 min. *DPAP byproducts.

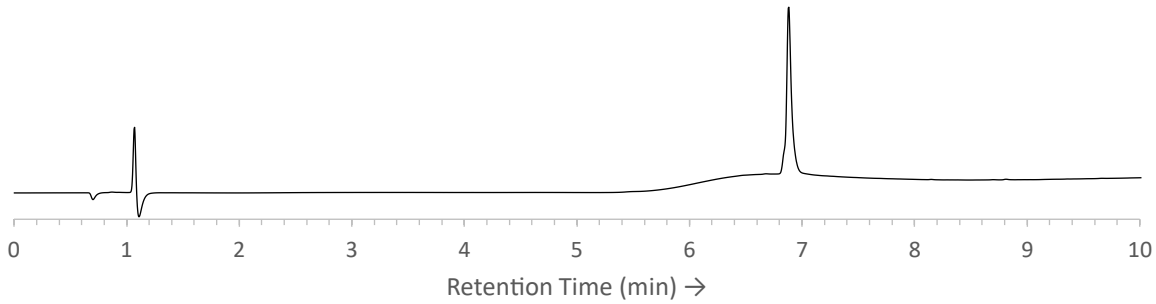
6. Synthesis of Cyclic Peptides

(5S,8S,11S,14S)-14-amino-8,11-dimethyl-7,10,13,16-tetraoxo-1-thia-6,9,12-triazacyclohexadecane-5-carboxamide (7)

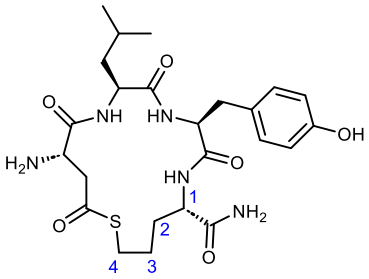


Prepared following the general cyclisation procedure from linear peptide **6** (10 mg). Purified by semi-preparative HPLC (5 – 15% MeCN in H_2O with 0.1% TFA over 60 min). After lyophilisation, the desired product was isolated as a fluffy white powder (5.4 mg, 54%). Purity: >95%.

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 6.89 min.
^{1}H-NMR (600 MHz, DMSO-d6): δ 8.58 (d, *J* = 8.1 Hz, 1H, Ala NH), 8.27 (bs, 2H, amino NH_2), 7.87 (d, *J* = 9.2 Hz, 1H, NH), 7.68 (d, *J* = 6.2 Hz, 1H, Ala NH), 7.30 (s, 1H, amide NH_2), 7.03 (s, 1H, amide NH_2), 4.40 – 4.33 (m, 1H, H1), 4.26 – 4.21 (m, 1H, Ala αCH), 4.15 – 4.09 (m, 1H, Ala αCH), 4.09 – 4.04 (m, 1H, Asp αCH), 3.17 (dd, J =16.3, 3.9 Hz, 1H, Asp βCH_2), 3.14 – 3.04 (m, 2H, Asp βCH_2, H4), 2.58 – 2.51 (m, 1H, H4), 1.75 – 1.68 (m, 1H, H3), 1.59 – 1.45 (m, 2H, H2, H3), 1.45 – 1.37 (m, 1H, H2), 1.28 (d, *J* = 7.3 Hz, 3H, Ala CH_3), 1.23 (d, *J* = 6.6 Hz, 3H, Ala CH_3).
^{13}C-NMR (151 MHz, DMSO-d6): δ 194.8 (SC=O), 173.6 (C=O), 171.1 (C=O), 170.8 (C=O), 166.6 (C=O), 50.5 (C1) ,49.3 (Ala αC), 48.9 (Asp αC), 43.5 (Asp βC), 30.7 (C2), 27.6 (C4), 25.9 (C3), 18.4 (Ala CH_3), 16.9 (Ala CH_3).
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{15}H_{26}N_5O_5S$ 388.1649, found 388.1653.

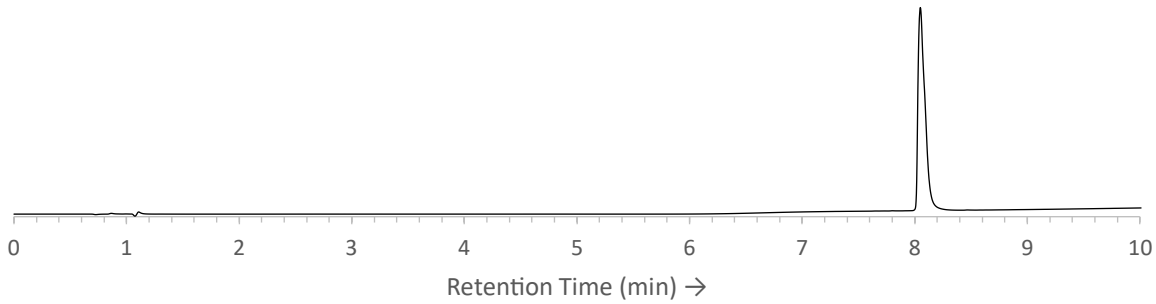


(5S,8S,11S,14S)-14-amino-8-(4-hydroxybenzyl)-11-isobutyl-7,10,13,16-tetraoxo-1-thia-6,9,12-triazacyclohexadecane-5-carboxamide (8)

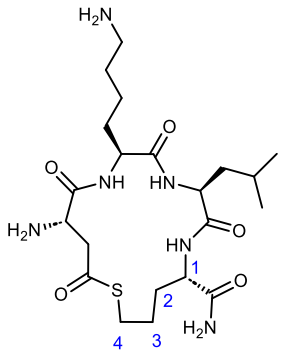


Prepared following the general cyclisation procedure from linear peptide **S1** (10 mg). Purified by semi-preparative HPLC (5 – 25% MeCN in H_2O with 0.1% TFA over 60 min). After lyophilisation, the desired product was isolated as a fluffy white powder (4.4 mg, 44%). Purity: >90%.

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 8.05 min.
^{1}H-NMR (600 MHz, DMSO-d6): δ 9.13 (s, 1H, OH), 8.46 (d, *J* = 8.8 Hz, 1H, Leu NH), 8.26 (bs, 2H, amino NH_2), 7.80 (d, *J* = 8.7 Hz, 1H, NH), 7.63 (d, *J* = 7.1 Hz, 1H, Tyr NH), 7.04 (s, 1H, amide NH_2), 6.99 (s, 1H, amide NH_2), 6.93 (d, *J* = 8.1 Hz, 2H, Tyr Ar H), 6.62 (d, *J* = 8.1 Hz, 2H, Tyr Ar H), 4.38 – 4.32 (m, 1H, H1), 4.32 – 4.27 (m, 1H, Tyr αCH), 4.26 – 4.20 (m, 1H, Leu αCH), 4.10 – 4.00 (m, 1H, Asp αCH), 3.18 (dd, *J* = 16.6, 3.8 Hz, 1H, Asp βCH_2), 3.10 – 3.00 (m, 2H, Asp βCH_2, H4), 2.94 (dd, *J* = 13.7, 6.6 Hz, 1H, Tyr βCH_2), 2.83 (dd, *J* = 13.7, 7.7 Hz, 1H, Tyr βCH_2), 2.64 – 2.55 (m, 1H, H4), 1.73 – 1.66 (m, 1H, H2), 1.58 – 1.31 (m, 5H, Leu γCH, H2, H3, Leu βCH_2), 1.28 – 1.21 (m, 1H, Leu βCH_2), 0.84 (d, *J* = 6.4 Hz, 3H, Leu CH_3), 0.79 (d, *J* = 6.4 Hz, 3H, Leu CH_3).
^{13}C-NMR (151 MHz, DMSO-d6): δ 194.7 (SC=O), 173.4 (C=O), 171.0 (C=O), 170.1 (C=O), 166.8 (C=O), 156.0 (Ar qC), 130.3 (Ar C), 127.0 (Ar C), 115.0 (Ar qC), 54.9 (Tyr αC), 52.4 (Leu αC), 50.8 (C1), 49.2 (Asp αC), 43.7 (Asp βC), 42.1 (Leu βC), 35.7 (Tyr βC), 30.9 (C2), 27.8 (C4), 25.9 (C3), 23.8 (Leu γC), 23.0 (Leu δC), 21.7 (Leu δC).
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{24}H_{36}N_5O_6S$ 522.2381, found 522.2387.

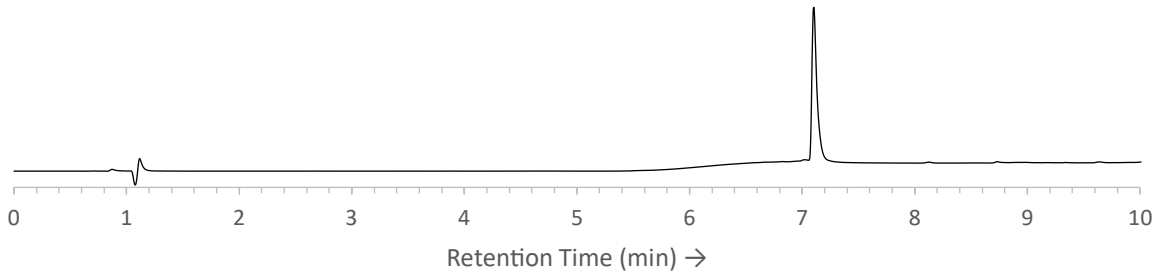


(5S,8S,11S,14S)-14-amino-11-(4-aminobutyl)-8-isobutyl-7,10,13,16-tetraoxo-1-thia-6,9,12-triazacyclohexadecane-5-carboxamide (9)

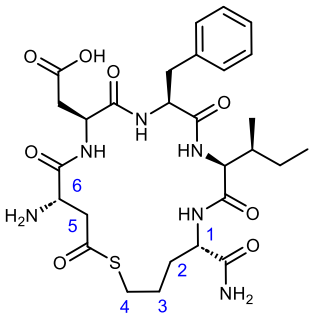


Prepared following the general cyclisation procedure from linear peptide **S2** (10 mg). Purified by semi-preparative HPLC (5 – 25% MeCN in H_2O with 0.1% TFA over 60 min). After lyophilisation, the desired product was isolated as a fluffy white powder (3.6 mg, 36%). Purity: >90%.

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 7.10 min.
^{1}H-NMR (600 MHz, DMSO-d6): δ 8.47 (dd, *J* = 8.5 Hz, 1H, Leu NH), 8.33 (bs, 1H, amino NH_2), 7.95 (dd, *J* = 9.0 Hz, 1H, NH), 7.73 – 7.60 (m, 3H, Lys NH, Lys NH_2), 7.26 (s, 1H, amide NH_2), 7.02 (s, 1H, amide NH_2), 4.42 – 4.27 (m, 1H, H1), 4.25 – 4.19 (m, 1H, Lys αCH), 4.18 – 4.13 (m, 1H, Leu αCH), 4.09 – 4.03 (m, 1H Asp αCH), 3.21 – 3.15 (m, 1H, Asp βCH_2), 3.13 – 3.05 (m, 2H, Asp βCH_2, H4), 2.78 (m, 2H, Lys εCH_2), 2.58 – 2.52 (m, 1H, H4), 1.75 – 1.65 (m, 1H, H2), 1.62 – 1.27 (m, 12H, H2, H3, Leu βCH_2, Leu γCH, Lys βCH_2, Lys γCH_2, LysδCH_2), 0.91 (d, *J* = 6.5 Hz, 3H, Leu CH_3), 0.84 (d, *J* = 6.5 Hz, 3H, Leu CH_3).
^{13}C-NMR (151 MHz, DMSO-d6): δ 194.8 (SC=O), 173.6 (C=O), 170.5 (C=O), 170.3 (C=O), 53.6 (Lys αC), 52.1 (Leu αC), 50.6 (C1), 49.2 (Asp αC), 43.6 (Asp βC), 38.8 (Lys εC), 31.9 (Lys βC), 30.8 (C2), 27.7 (C4), 26.7 (Lys δC), 26.0 (C3), 24.5 (Leu γC), 22.8 (Leu δC), 22.6 (Leu δC), 22.0 (Lys γC).
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{21}H_{39}N_6O_5S$ 487.2697, found 487.2699.

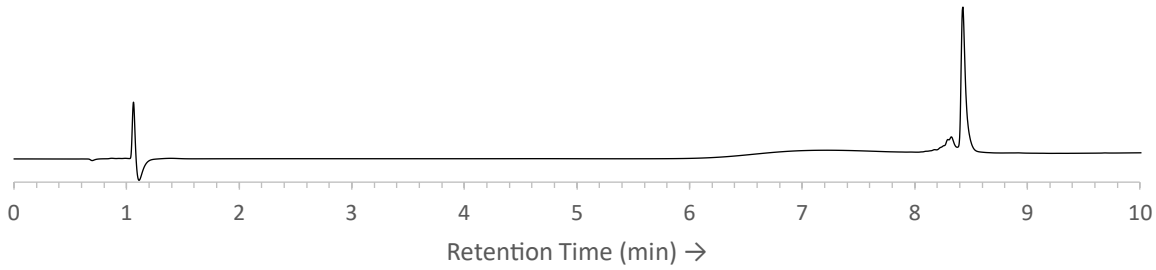


2-((4S,7S,10S,13S,16S)-4-amino-10-benzyl-13-((S)-sec-butyl)-16-carbamoyl-2,5,8,11,14-pentaoxo-1-thia-6,9,12,15-tetraazacyclononadecan-7-yl)acetic acid (10)

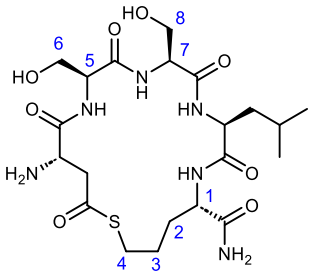


Prepared following the general cyclisation procedure from linear peptide **S3** (10 mg). Purified by semi-preparative HPLC (5 – 95% MeCN in H_2O with 0.1% TFA over 60 min). After lyophilisation, the desired product was isolated as a fluffy white powder (5.2 mg, 52%). Purity: >95%.

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 8.43 min.
^{1}H NMR (600 MHz, DMSO-d6): δ 12.41 (bs, 1H, COOH), 8.94 (d, *J* = 7.6 Hz, 1H, Asp NH), 8.33 (m, 3H, NH_2, Ile NH), 7.71 (d, *J* = 9.2 Hz, 1H, NH), 7.35 (d, *J* = 9.1 Hz, 1H, Phe NH), 7.32 – 7.17 (m, 5H, Phe Ar H), 7.14 (s, 1H, amide NH_2), 7.09 (s, 1H, amide NH_2), 4.61 (td, *J* = 9.4, 9.1, 3.5 Hz, 1H, Phe αCH), 4.49 (td, *J* = 8.1, 7.6, 4.3 Hz, 1H, Asp αCH), 4.25 (td, *J* = 9.2, 9.1, 3.4 Hz, 1H, H1), 4.11- 4.07 (m, 1H, H6), 3.91 (dd, *J* = 10.2, 7.7 Hz, 1H, Ile αCH), 3.25 – 3.12 (m, 3H, H5, H4), 3.0 (dd, *J* = 13.9, 3.5 Hz, 1H, Phe βCH_2), 2.73 – 2.65 (m, 1H, Phe βCH_2), 2.59 – 2.52 (m, 1H, H4), 2.44 (dd, *J* = 16.9, 4.3 Hz, Asp βCH_2), 2.33 (dd, *J* = 16.9, 8.1 Hz, 1H, Asp βCH_2), 1.82 – 1.74 (m, 1H, Ile βCH), 1.67 – 1.49 (m, 4H, Ile γCH_2, H2, H3), 1.42 – 1.43 (m, 1H, H3), 1.18 – 1.04 (Ile γCH_2), 0.85 (t, *J* = 7.5 Hz, 3H, Ile δCH_3), 0.81 (d, *J* = 6.7 Hz, 3H, Ile γCH_3).
^{13}C NMR (151 MHz, DMSO-d6): δ 196.1 (SC=O), 173.4 (C=O), 171.6 (COOH), 170.9 (C=O), 169.2 (C=O), 169.0 (C=O), 168.1 (C=O), 137.5 (Phe qC), 129.6 (Phe ortho Ar C), 128.2 (Phe meta Ar C), 126.4 (Phe para Ar C), 58.4 (Ile αC), 52.4 (Phe αC), 52.2 (C1), 50.4 (Asp αC), 48.5 (C6), 43.5 (C5), 38.3 (Phe βC), 35.8 (Asp βC), 33.9 (Ile βC), 31.8 (C2), 28.7 (C4), 25.9 (C3), 24.9 (Ile γCH_2), 15.3 (Ile γCH_3), 10.5 (Ile δCH_3).
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{28}H_{41}N_6O_8S$ 621.2701, found 621.2704.

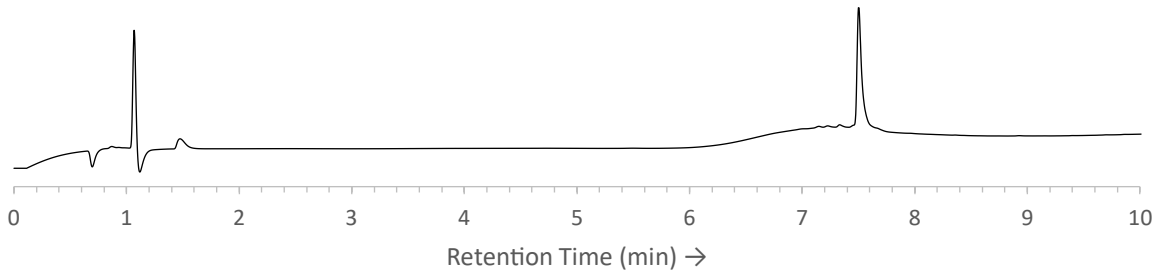


(5S,8S,11S,14S,17S)-17-amino-11,14-bis(hydroxymethyl)-8-isobutyl-7,10,13,16,19-pentaoxo-1-thia-6,9,12,15-tetraazacyclononadecane-5-carboxamide (11)

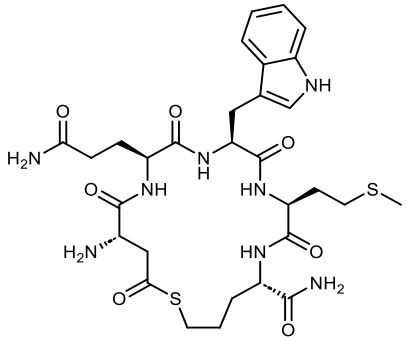


Linear peptide **S4** (10 mg) and DPAP (1 equiv.) was dissolved in 1:1 H_2O:MeCN containing 0.1% TFA and 6 M guanidinium hydrochloride at 5 mM concentration in a vial. The resulting solution was stirred at rt under UV light illumination for 15 min. The reaction mixture was purified by semi-preparative HPLC (5 – 95% MeCN in H_2O with 0.1% TFA over 60 min). After lyophilisation, the desired product was isolated as a fluffy white powder (6.1 mg, 61%). Purity: >95%.

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 7.50 min.
^{1}H NMR (600 MHz, DMSO-d6): δ 8.28 (bs, 2H, amino NH_2), 8.23 (d, *J* = 7.1 Hz, 1H, Ser NH), 8.16 (d, *J* = 6.2 Hz, 1H, Ser NH), 7.84 (d, *J* = 6.9 Hz, 1H, Leu NH), 7.45 (d, *J* = 7.8 Hz, 1H, NH), 7.06 (s, 1H, amide NH_2), 6.99 (s, 1H, Amide NH_2), 5.46 (t, *J* = 5.5 Hz, 1H, Ser OH), 4.98 (m, 1H, Ser OH), 4.38 (dt, *J* = 7.1, 5.0 Hz, 1H, H5), 4.27 – 4.21 (m, 1H, Asp αCH), 4.17 (dt, *J* = 6.2, 4.0 Hz, 1H, H7), 4.11 – 4.01 (m, 2H, H1, Leu αCH), 3.98 – 3.92 (m, 1H, Ser H6), 3.72 – 3.61 (m, 2H, Ser H8), 3.61 – 3.55 (m, 1H, H6), 3.28 (dd, *J* = 17.2, 3.0, 1H, Asp βCH_2), 3.17 – 3.07 (m, 2H, Asp βCHb, H2), 2.75 – 2.69 (m, 1H, H2), 1.68 – 1.55 (m, 4H, H3, Leu γCH, Leu βCH_2), 1.54 – 1.41 (m, 3H, H4, Leu βCH_2), 0.88 (d, *J* = 6.6 Hz, 3H, Leu CH_3), 0.82 (d, *J* = 6.5 Hz, 3H, Leu CH_3).
^{13}C NMR (151 MHz, DMSO-d6): δ 195.5 (SC=O), 173.2 (C=O), 171.8 (C=O), 170.4 (C=O), 170.2 (C=O), 167.4 (C=O), 61.6 (C6), 61.1 (C8), 56.2 (C7), 55.1 (C5), 52.9 (C1), 52.2 (Leu αC), 48.3 (Asp αC), 43.6 (Asp βC), 38.8 (C4), 30.6 (C3), 28.5 (C2), 25.5 (Leu βC), 24.3 (Leu γC), 22.8 (Leu δC), 21.4 (Leu δC).
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{21}H_{37}N_6O_8S$ 533.2388, found 533.2390.

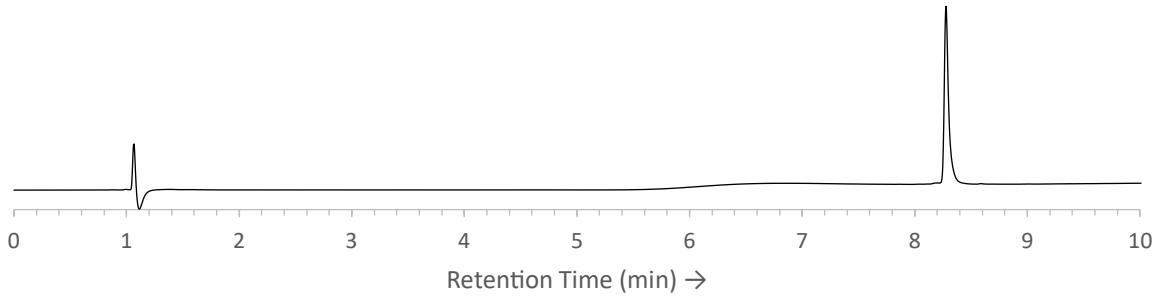


(5S,8S,11S,14S,17S)-11-((1H-indol-3-yl)methyl)-17-amino-14-(3-amino-3-oxopropyl)-8-(2-(methylthio)ethyl)-7,10,13,16,19-pentaoxo-1-thia-6,9,12,15-tetraazacyclononadecane-5-carboxamide (S7)

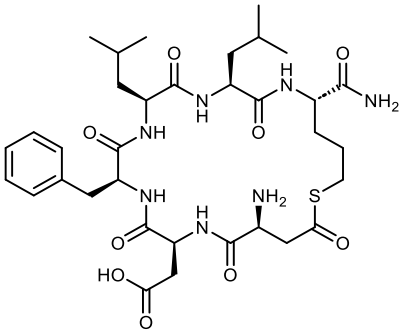


Prepared following the general cyclisation procedure from linear peptide **S5** (10 mg). Purified by semi-preparative HPLC (5 – 15% MeCN in H_2O with 0.1% TFA over 60 min). After lyophilisation, the desired product was isolated as a fluffy white powder (2.2 mg, 22%). Purity: >75%.

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 10 min, 220 nm): 8.28 min.
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{30}H_{43}N_8O_7S_2$ 691.2691, found 691.2688.

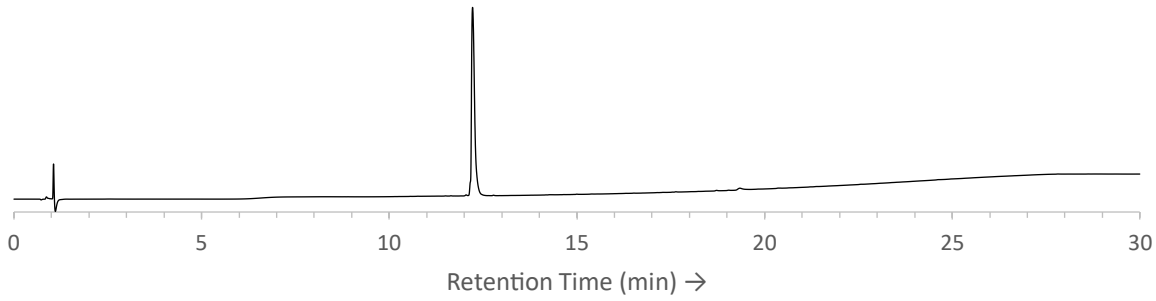


2-((4S,7S,10S,13S,16S,19S)-4-amino-10-benzyl-19-carbamoyl-13,16-diisobutyl-2,5,8,11,14,17-hexaoxo-1-thia-6,9,12,15,18-pentaazacyclodocosan-7-yl)acetic acid (S8)



Prepared following the general cyclisation procedure from linear peptide **S6** (10 mg). Purified by semi-preparative HPLC (5 – 15% MeCN in H_2O with 0.1% TFA over 80 min). After lyophilisation, the desired product was isolated as a fluffy white powder (3.1 mg, 31%). Purity: >75%

HPLC t_R (5 – 95% MeCN in H_2O with 0.1% TFA over 30 min, 220 nm): 12.22 min.
HRMS ESI m/z: $[M+H]^+$ calculated for $C_{34}H_{53}N_7O_9S$ 734.3454, found 734.3546.



7. References and Notes

1. A. Kapurniotu and J. W. Taylor, *Tetrahedron Lett.*, 1993, **34**, 7031-7034.
2. T. Nuijens, C. Cusan, J. A. W. Kruijtzer, D. T. S. Rijkers, R. M. J. Liskamp and P. J. L. M. Quaedflieg, *Synthesis*, 2009, **5**, 809-814.
3. The compound aggregates in $CDCl_3$, acetone-d_6 and toluene-d_8 resulting in very broad peaks. In solvents such as DMSO-d_6 and CD_3OD, the compound readily decomposes via the elimination of triphenylmethanethiol. Thus, the NMR was conducted in $CDCl_3$ with 2% v/v CD_3OD to prevent aggregation.

Figure S6: ^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$)

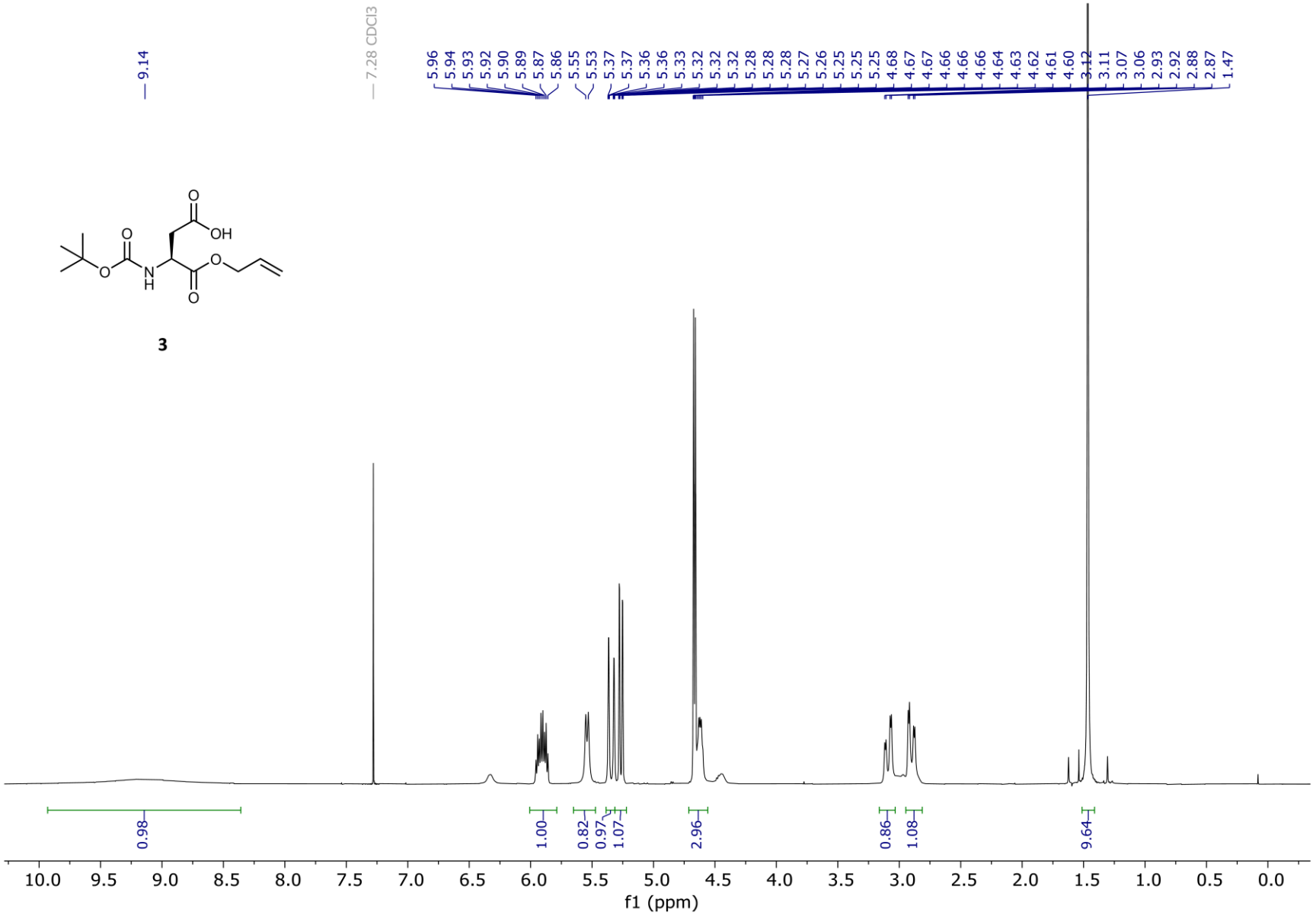


Figure S7: ^{13}C NMR spectrum of **3** (101 MHz, $CDCl_3$)

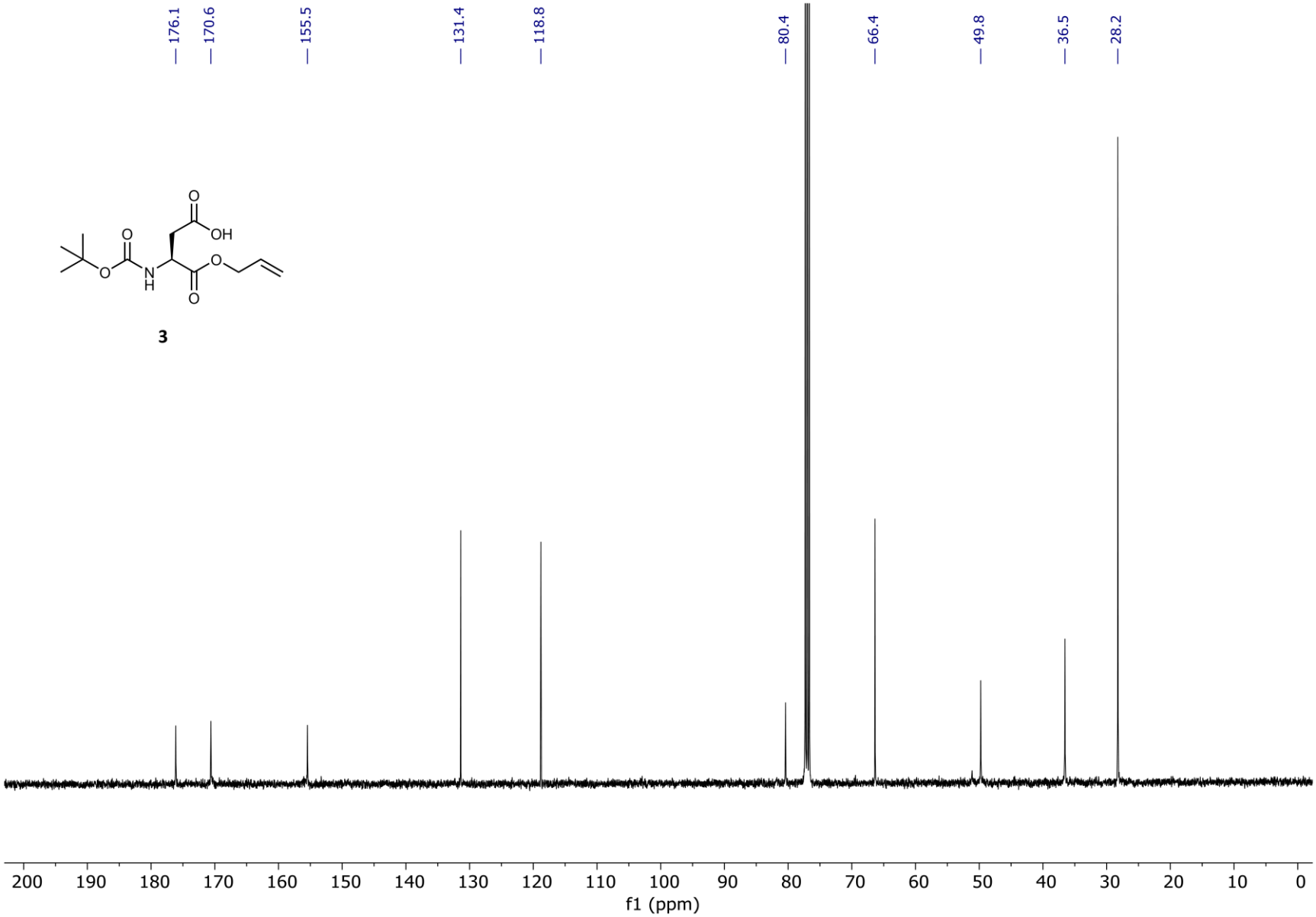
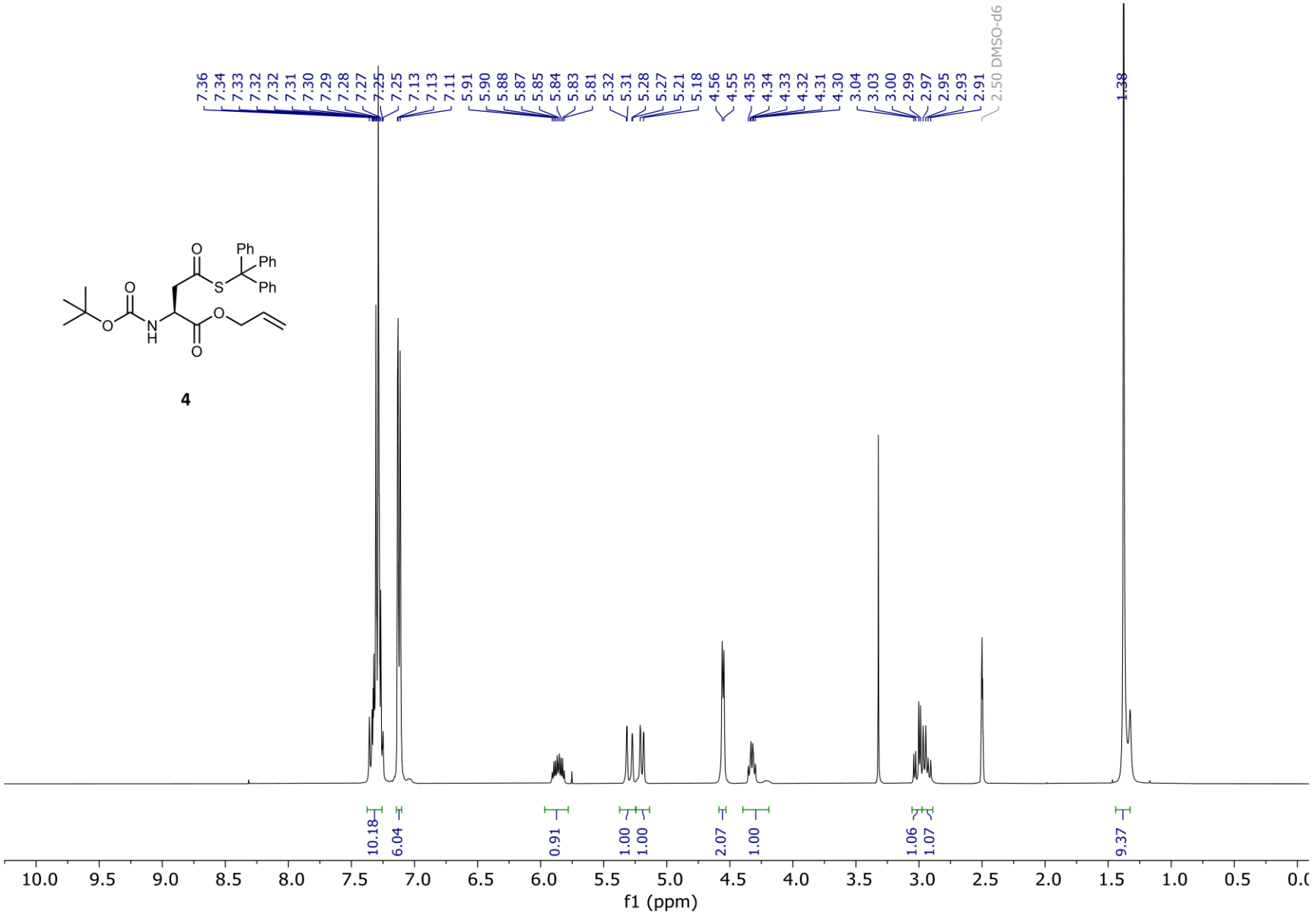


Figure S8: ^{13}H NMR spectrum of **4** (400 MHz, DMSO-d_6)



4

Figure S9: ^{13}C NMR spectrum of **4** (101 MHz, DMSO-d_6)

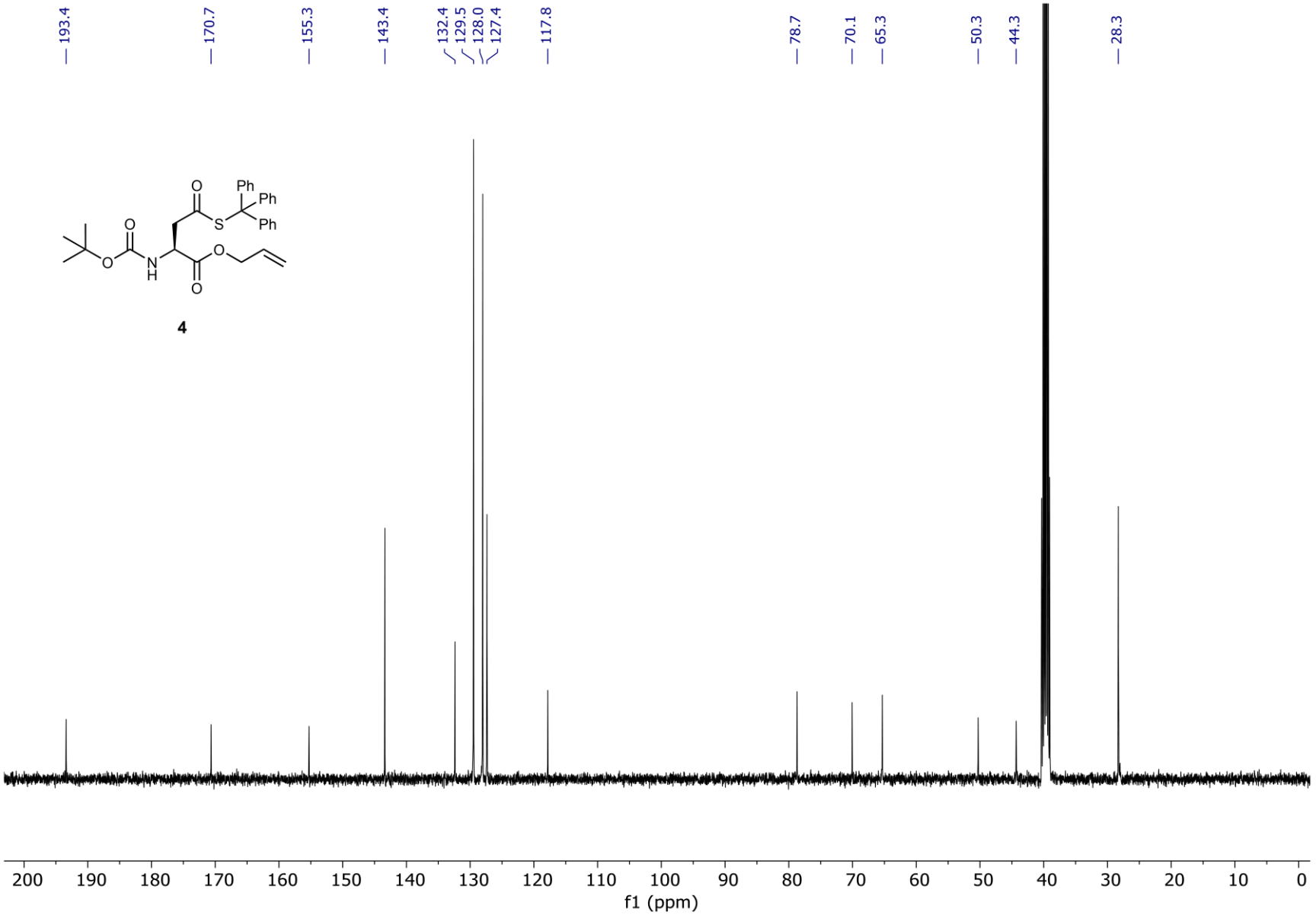
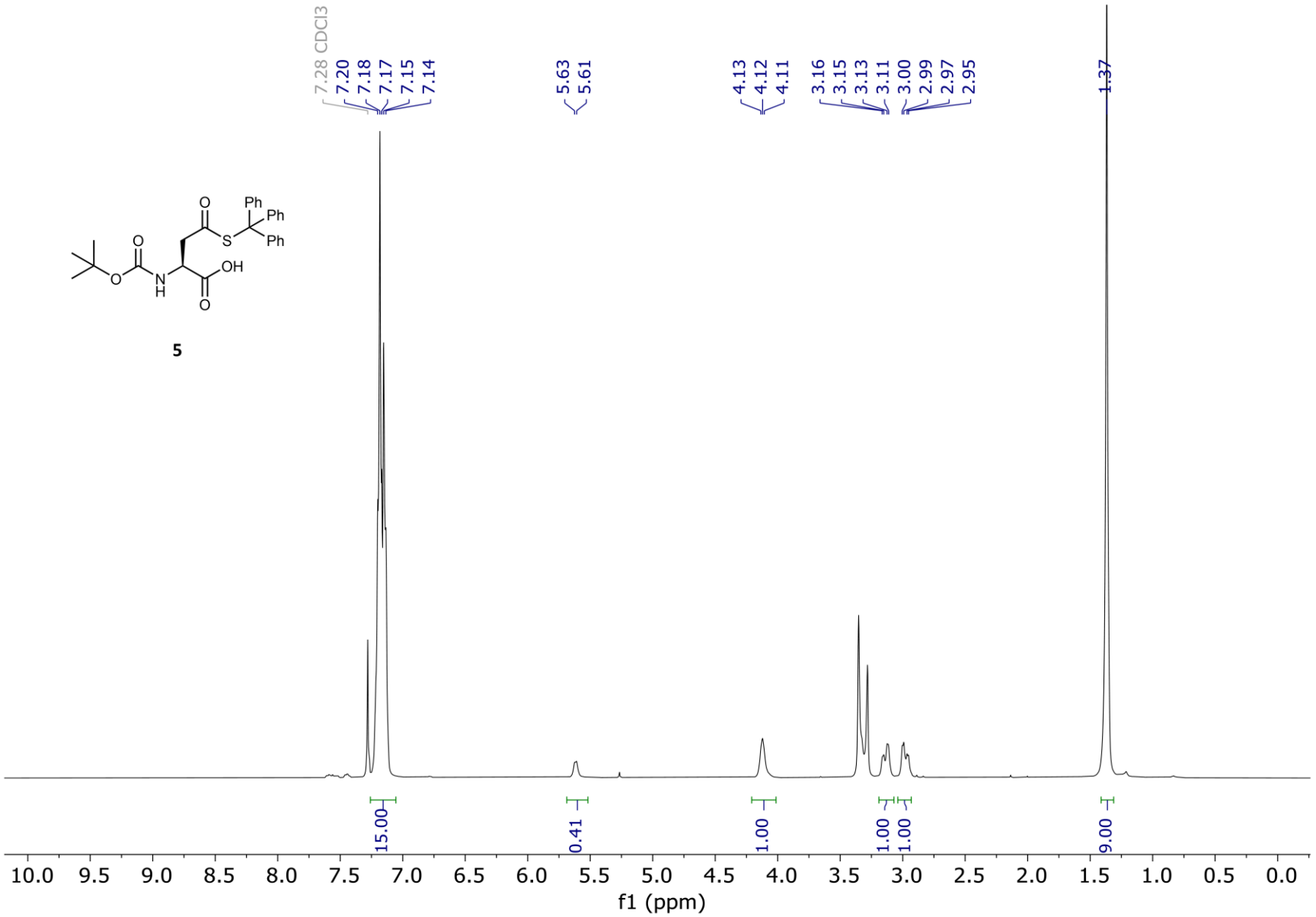


Figure S10: ^{1}H NMR spectrum of **5** (400 MHz, $CDCl_3/CD_3OD$ 98:2)



5

Figure S11: ^{13}C NMR spectrum of **5** (101 MHz, $CDCl_3/CD_3OD$ 98:2)

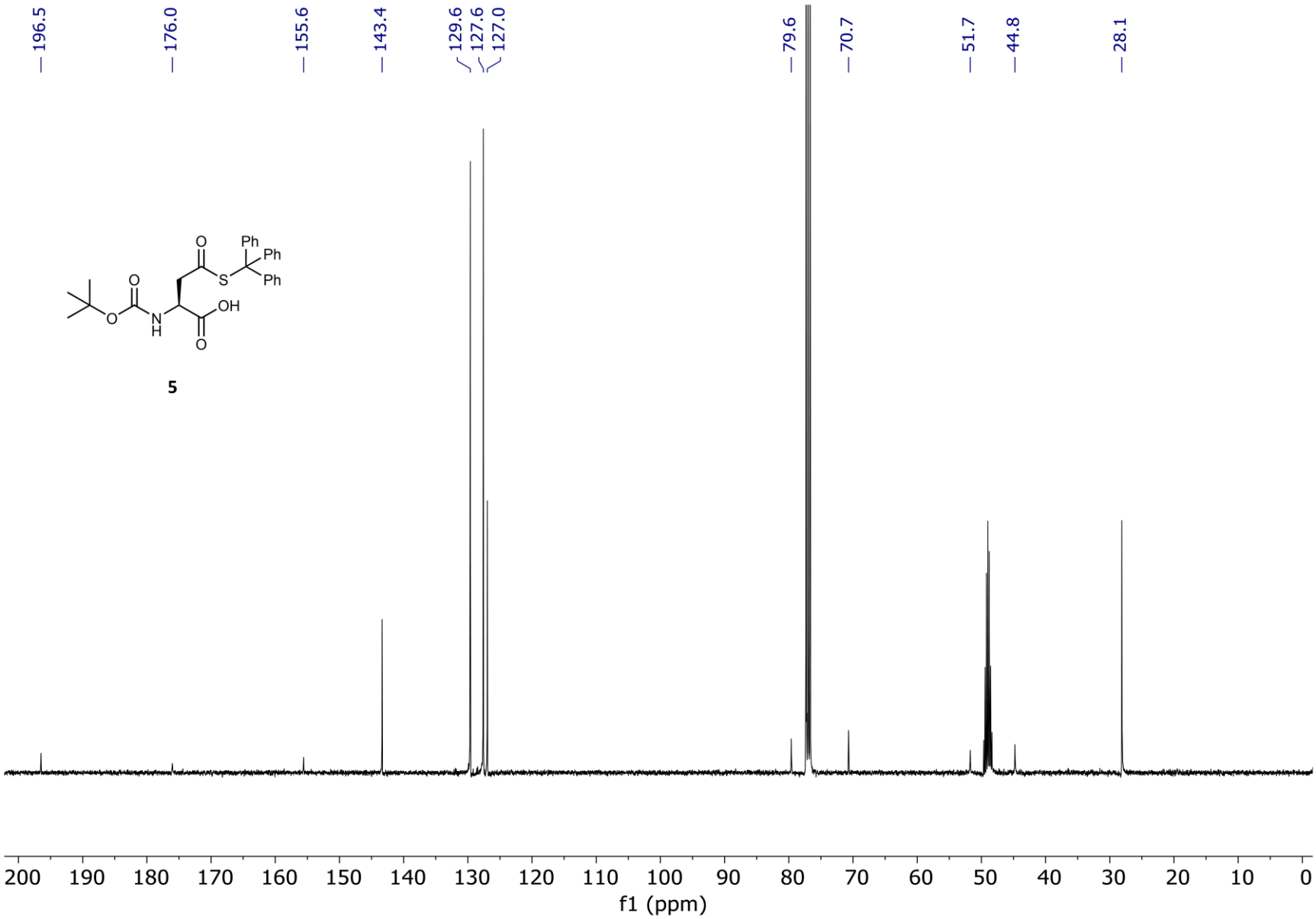


Figure S12: ^{1}H NMR spectrum of **1** (400 MHz, $CDCl_3$)

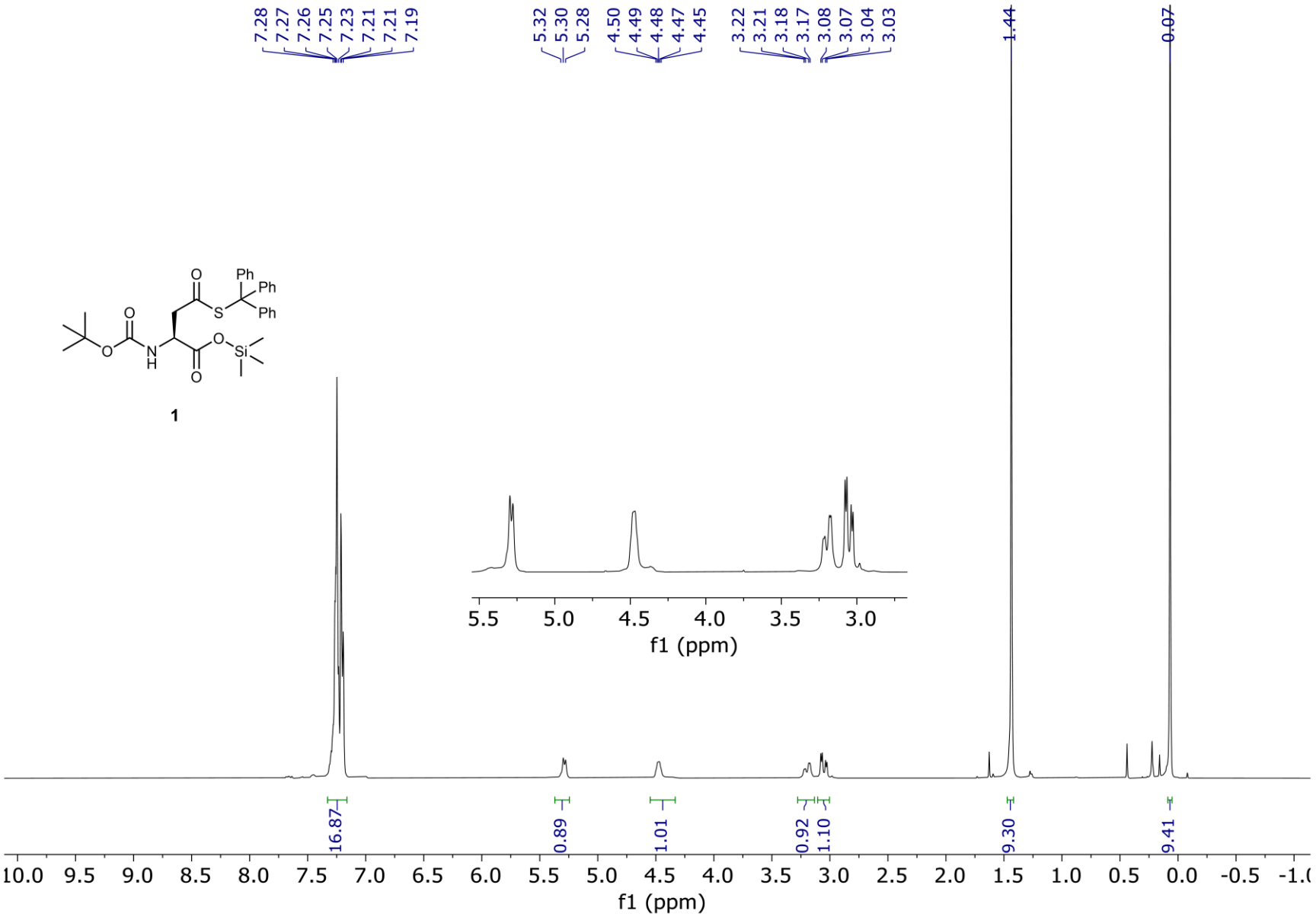


Figure S13: ^{13}C NMR spectrum of **1** (101 MHz, $CDCl_3$)

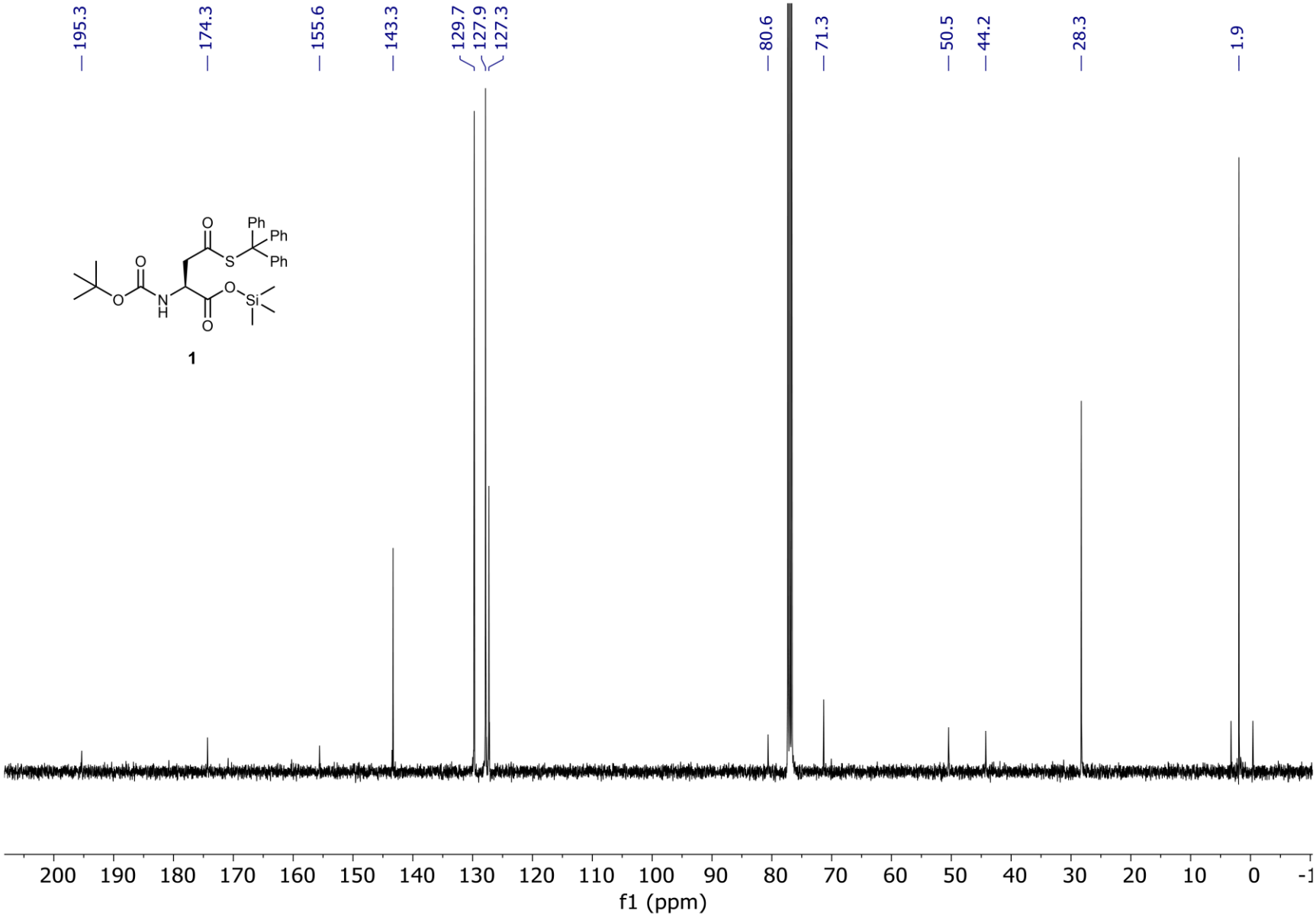


Figure S14: ^{1}H NMR spectrum of **7** (600 MHz, DMSO-d_6)

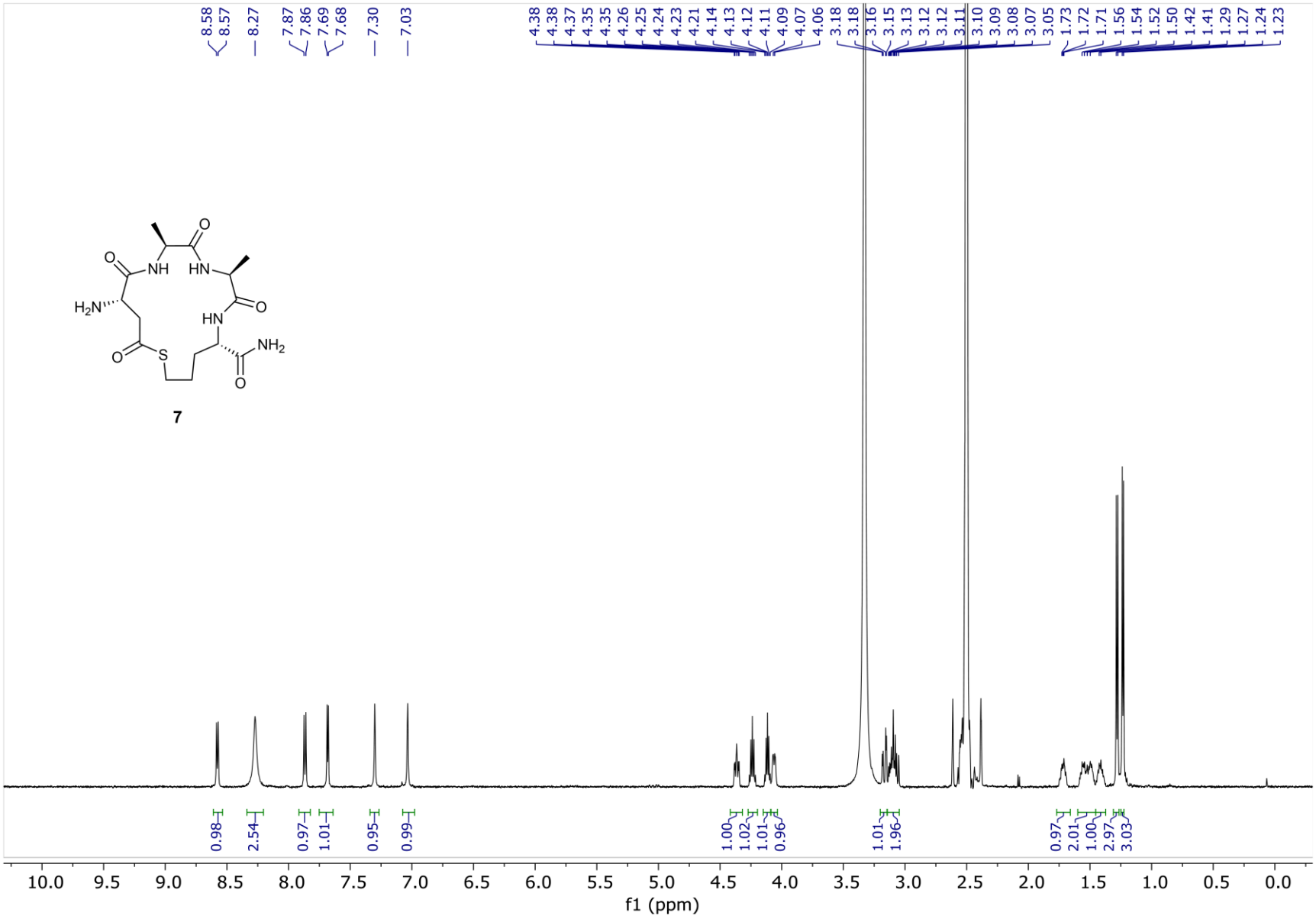


Figure S15: ^{13}C NMR spectrum of **7** (151 MHz, DMSO-d_6)

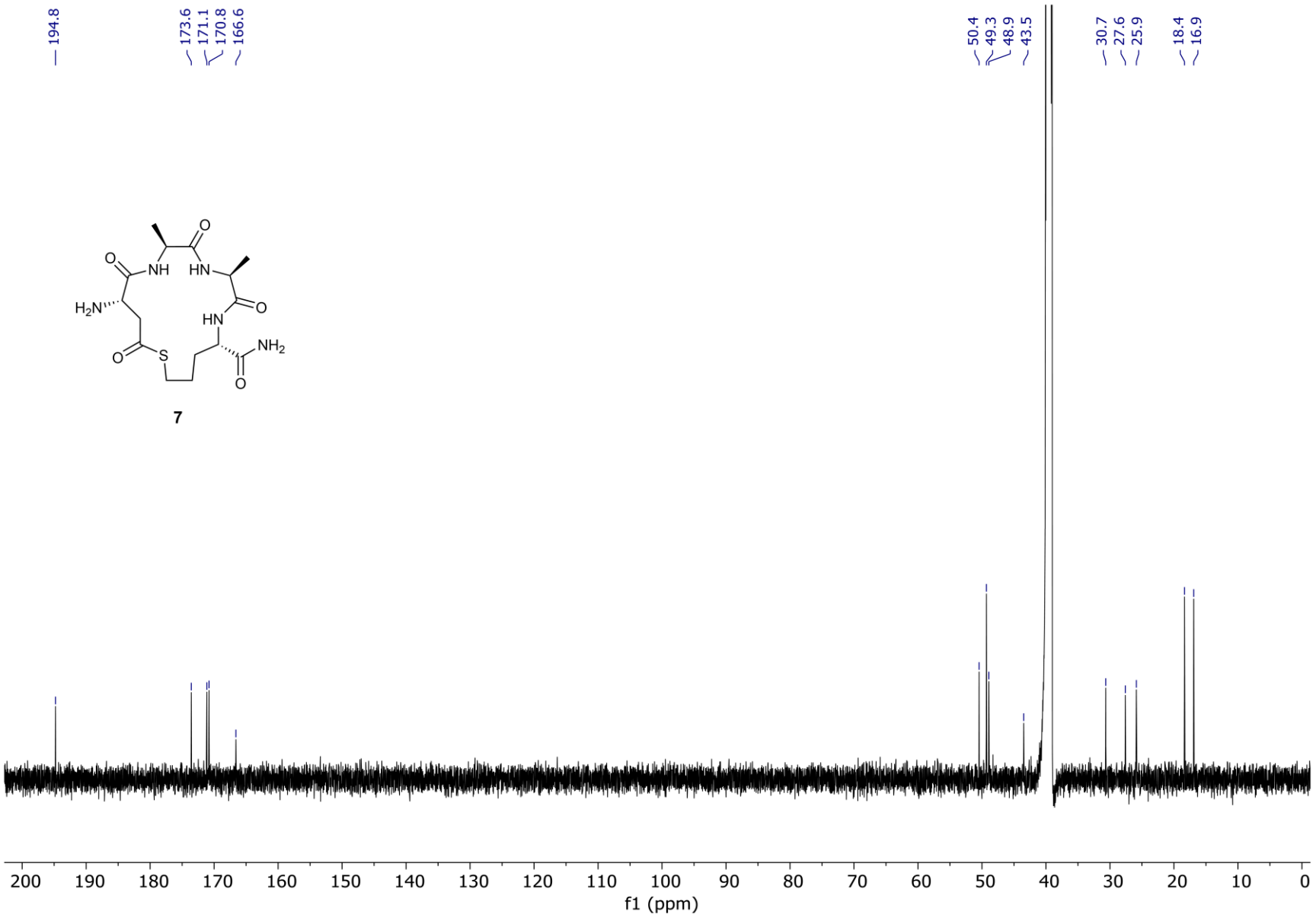


Figure S16: HSQC spectrum of **7**

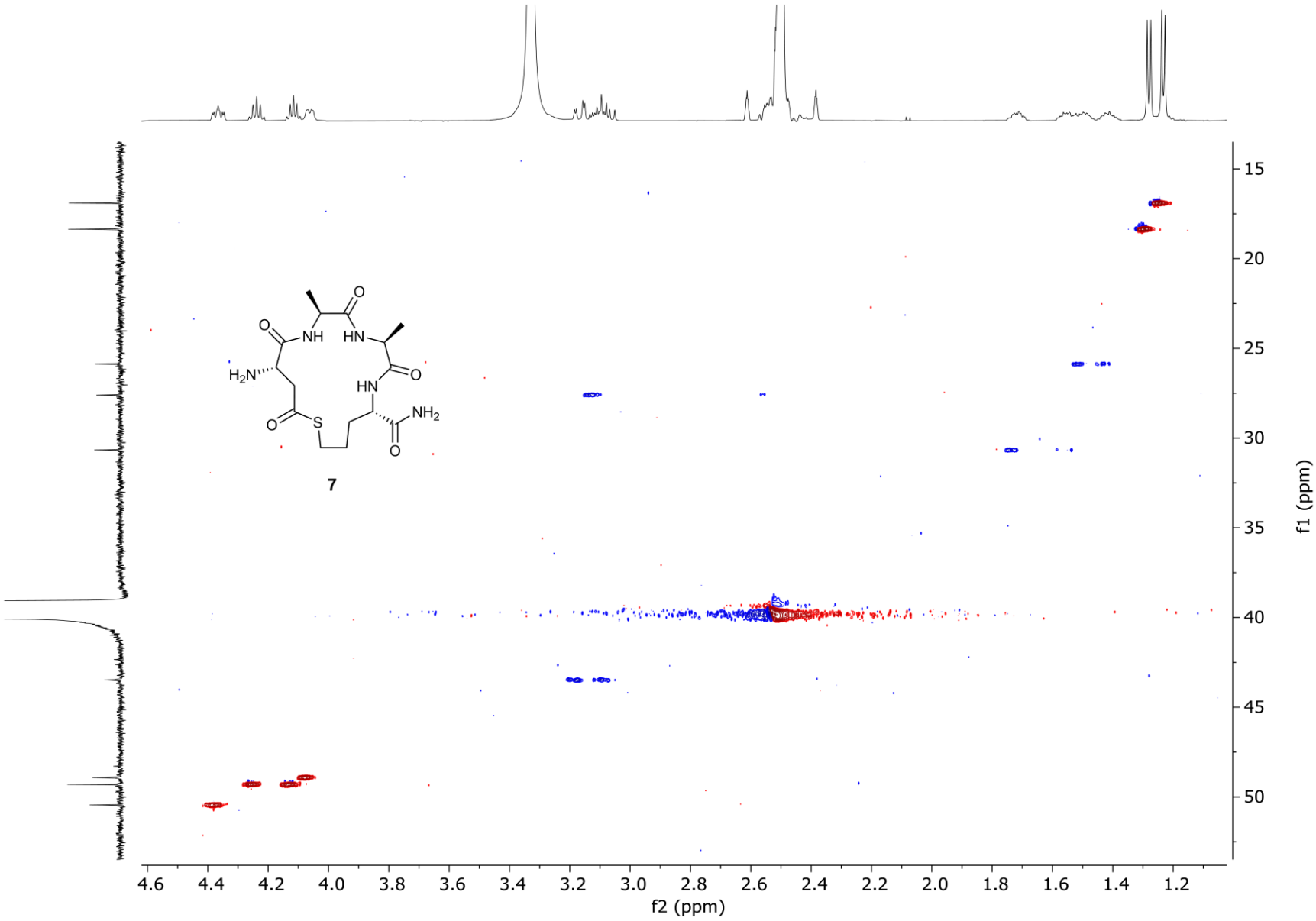


Figure S17: HMBC spectrum of **7**

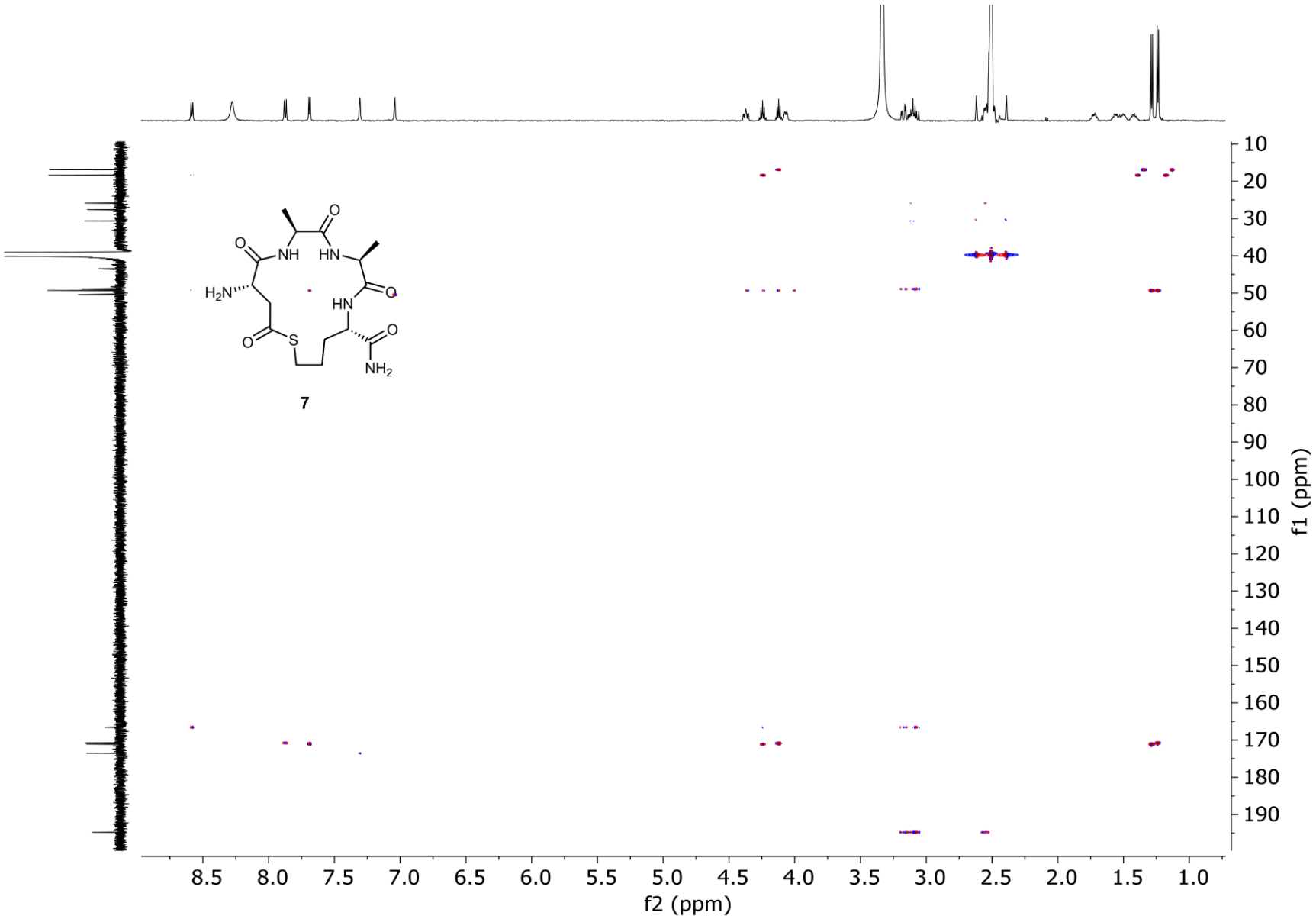
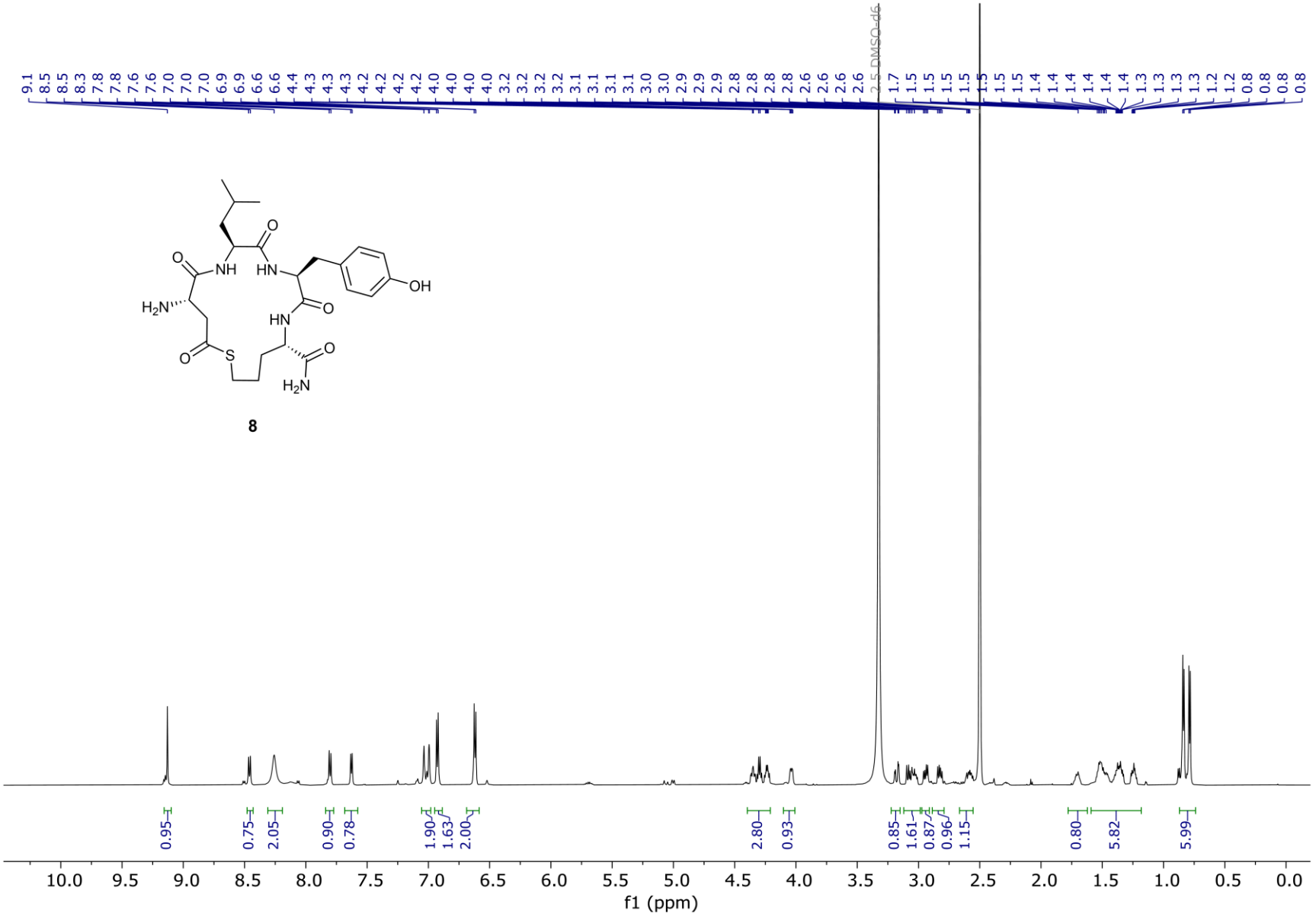


Figure S18: ^{1}H NMR spectrum of **8** (600 MHz, DMSO-d_6)



8

Figure S19: ^{13}C NMR spectrum of **8** (151 MHz, DMSO-d_6)

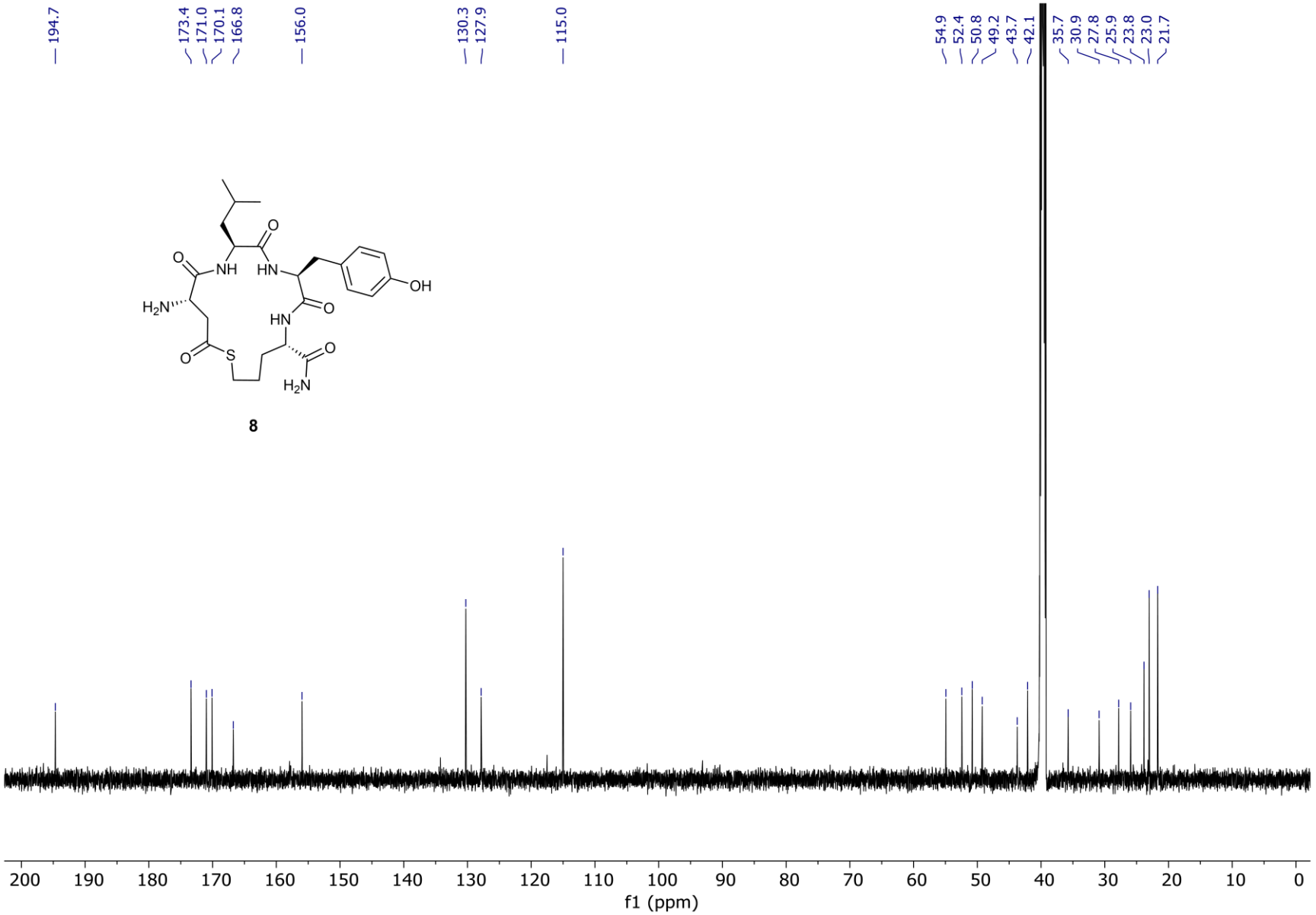


Figure S20: ^{1}H NMR spectrum of **9** (600 MHz, DMSO-d_6)

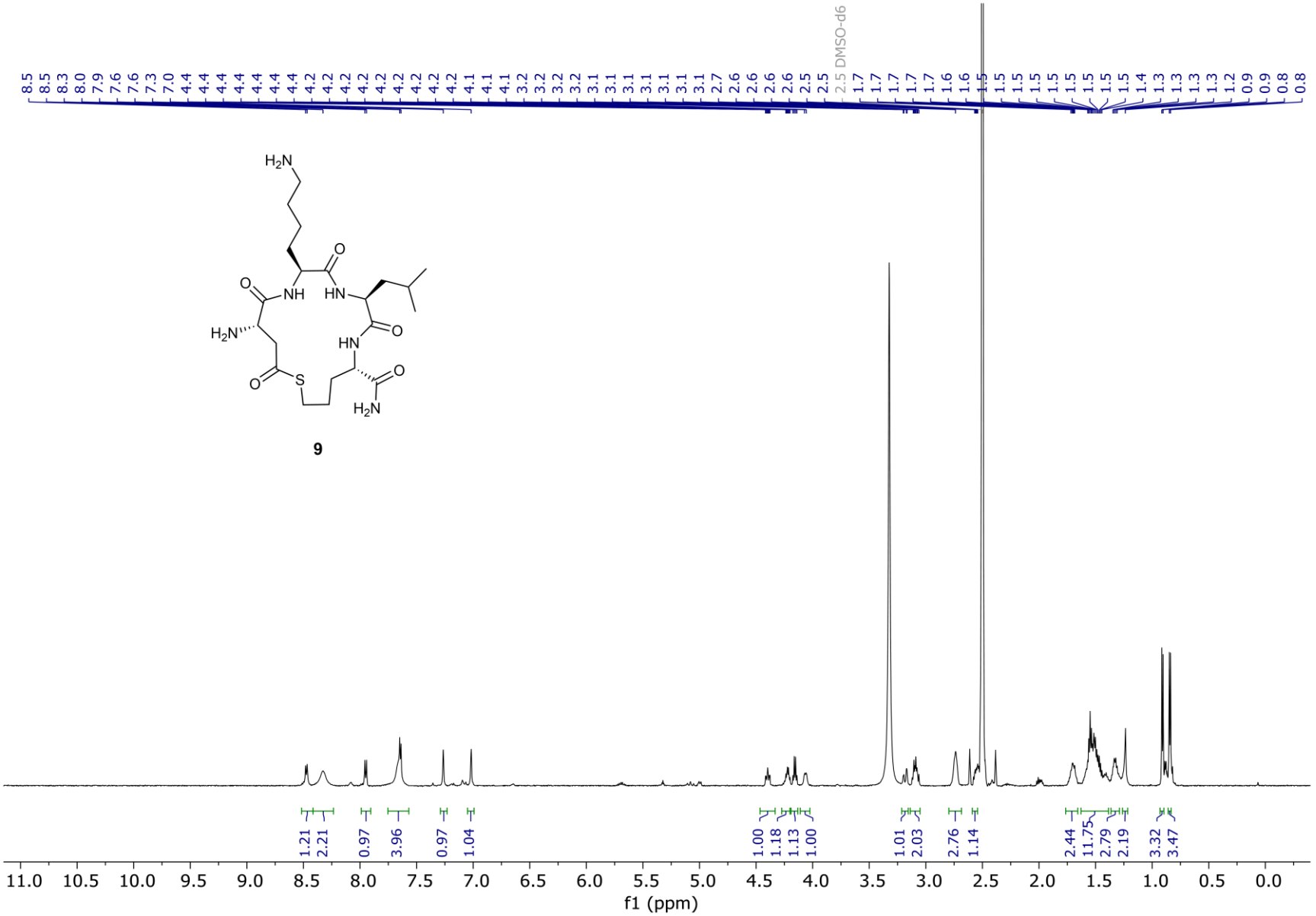


Figure S21: ^{13}C NMR spectrum of **9** (151 MHz, DMSO-d_6)

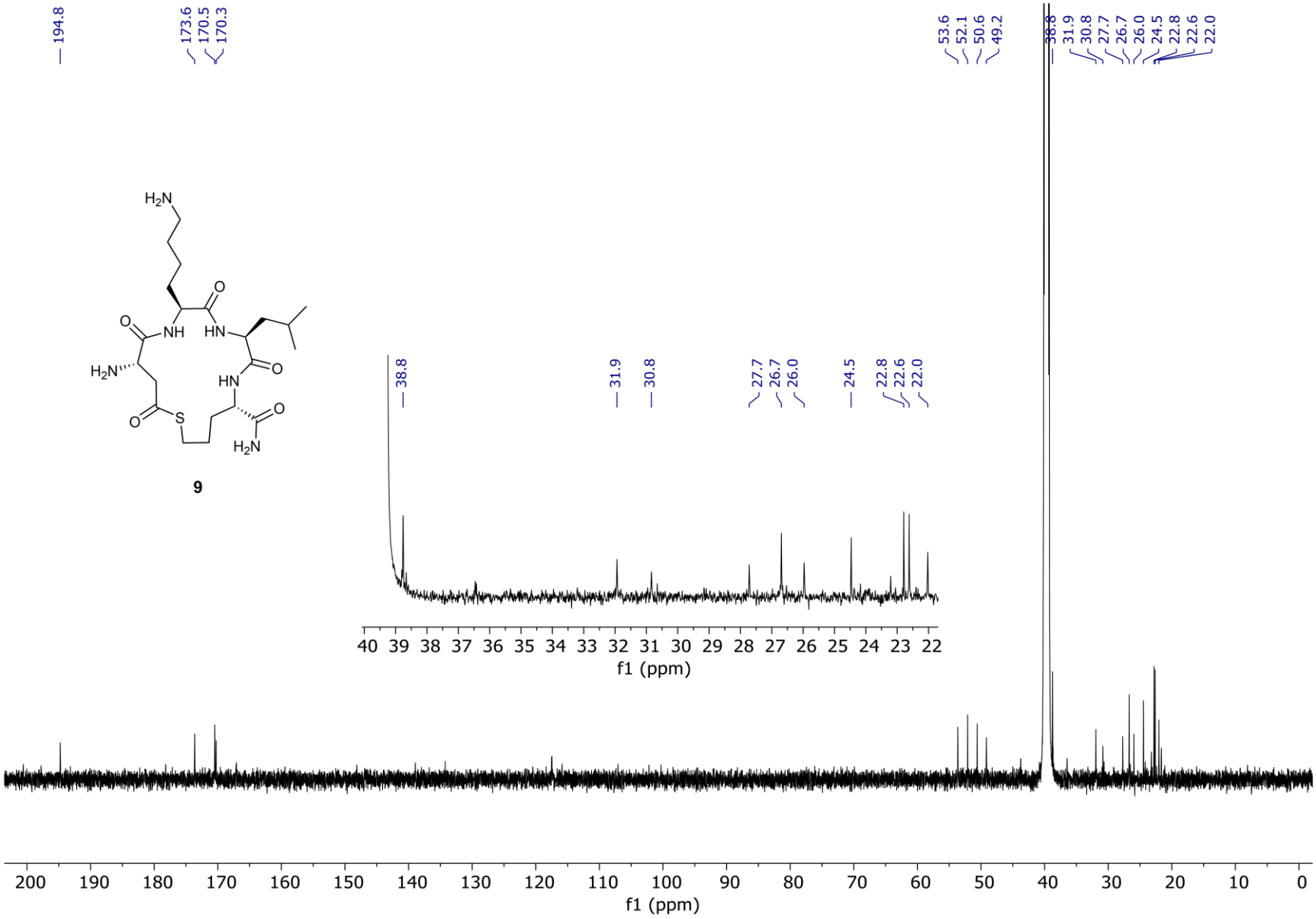


Figure S22: ^{1}H NMR spectrum of **10** (600 MHz, DMSO-d_6)

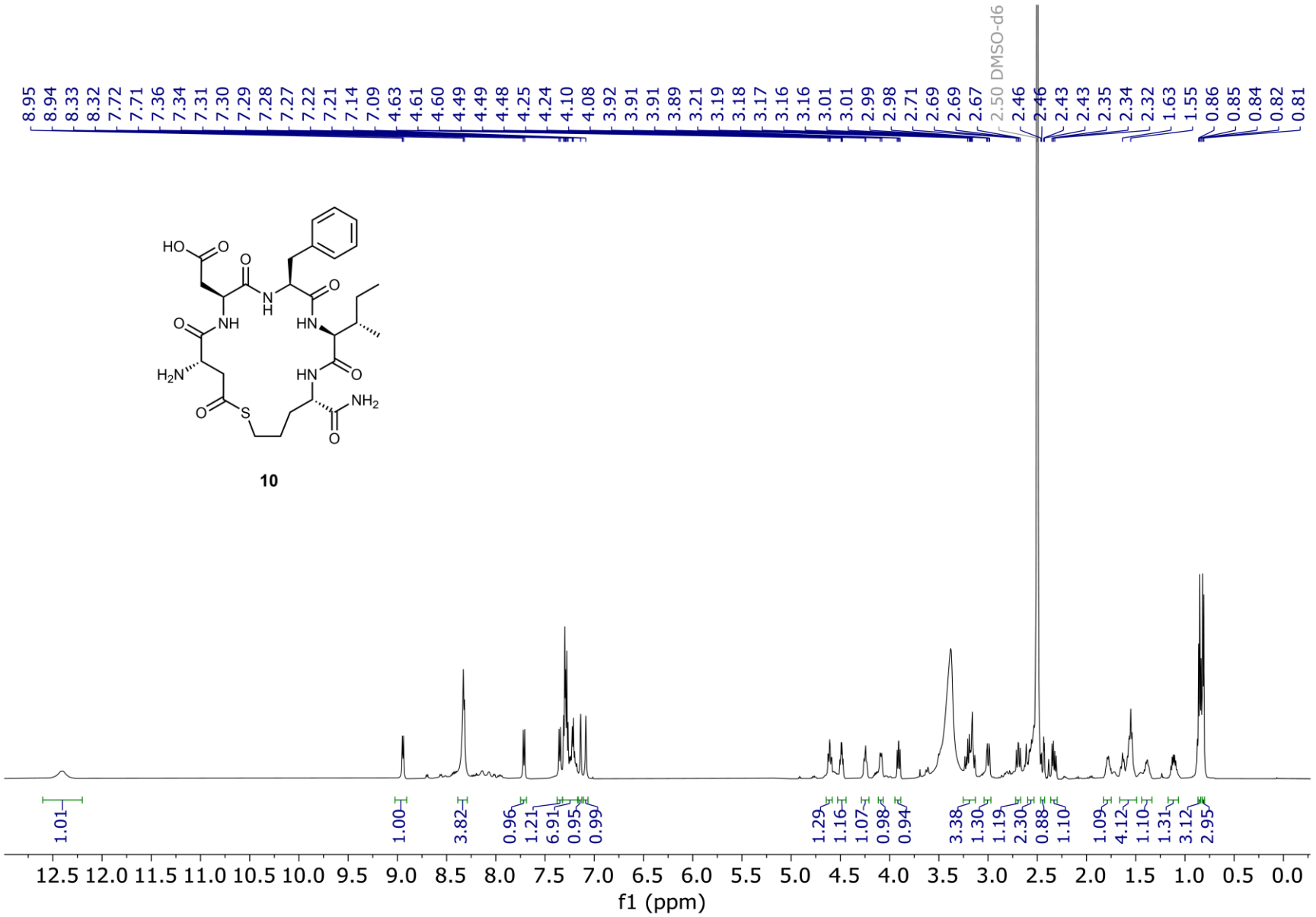
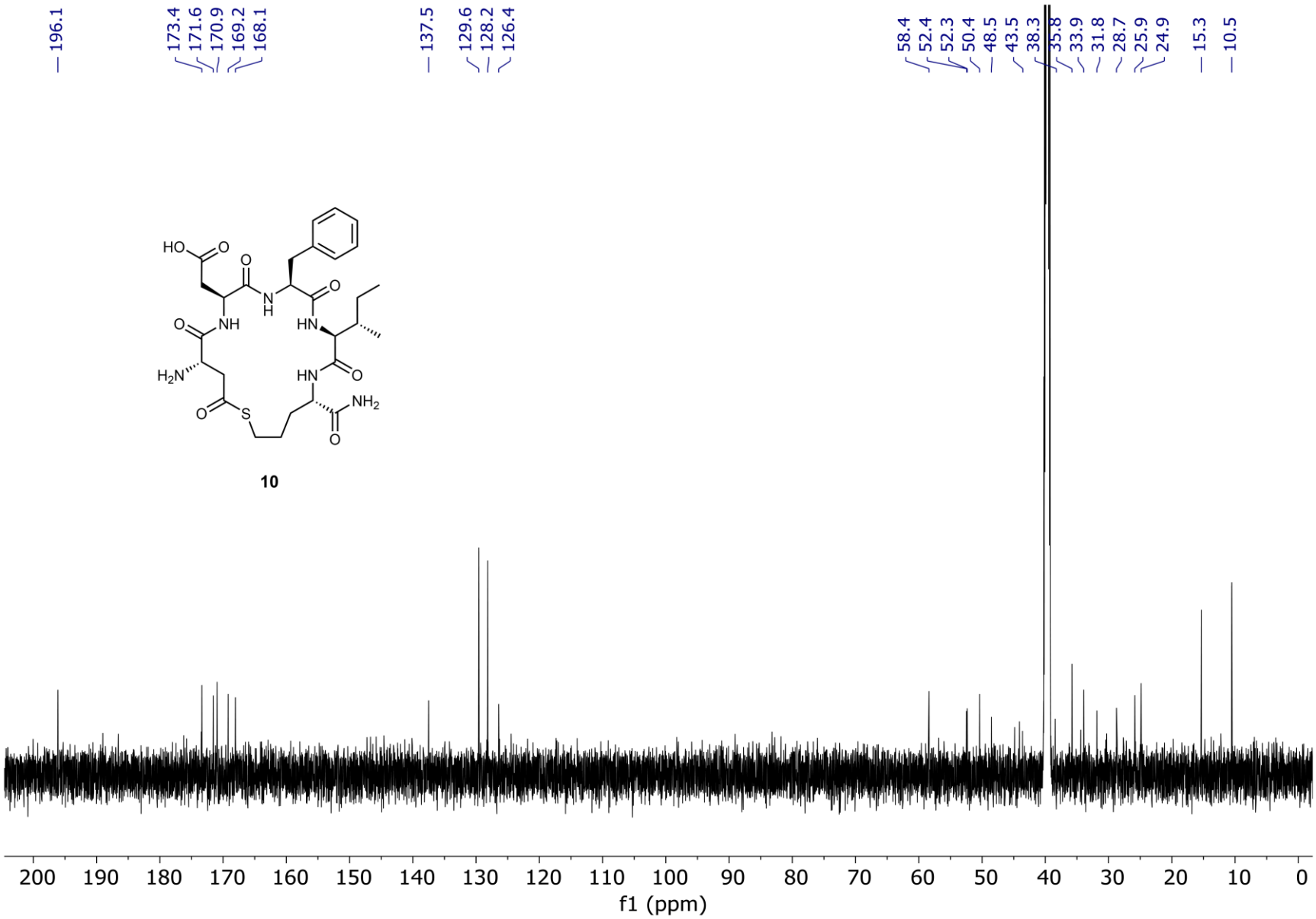


Figure S23: ^{13}C NMR spectrum of **10** (600 MHz, DMSO-d_6)



10

Figure S24: HSQC spectrum of **10**

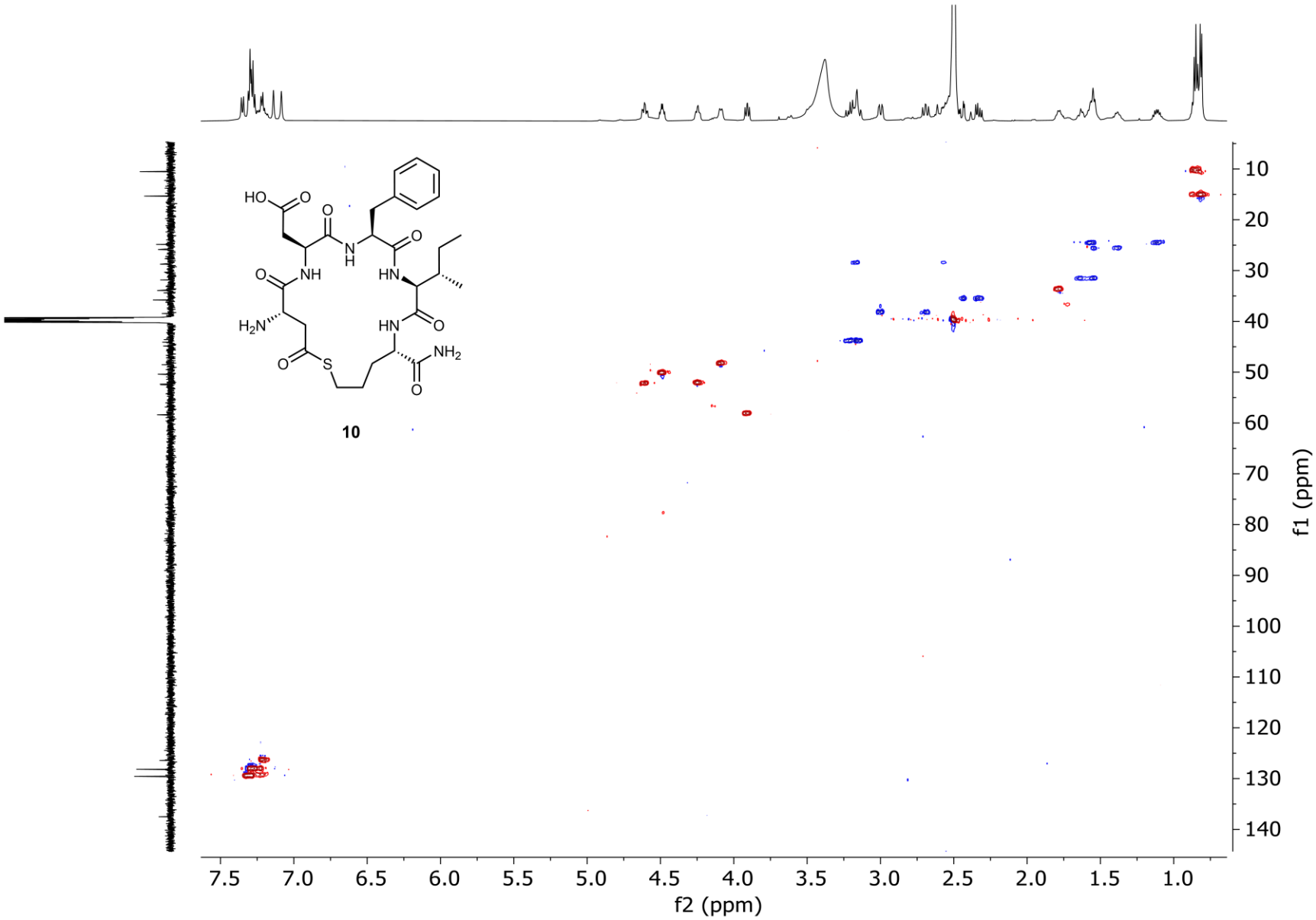


Figure S25: ^{1}H NMR spectrum of **11** (600 MHz, DMSO-d_6)

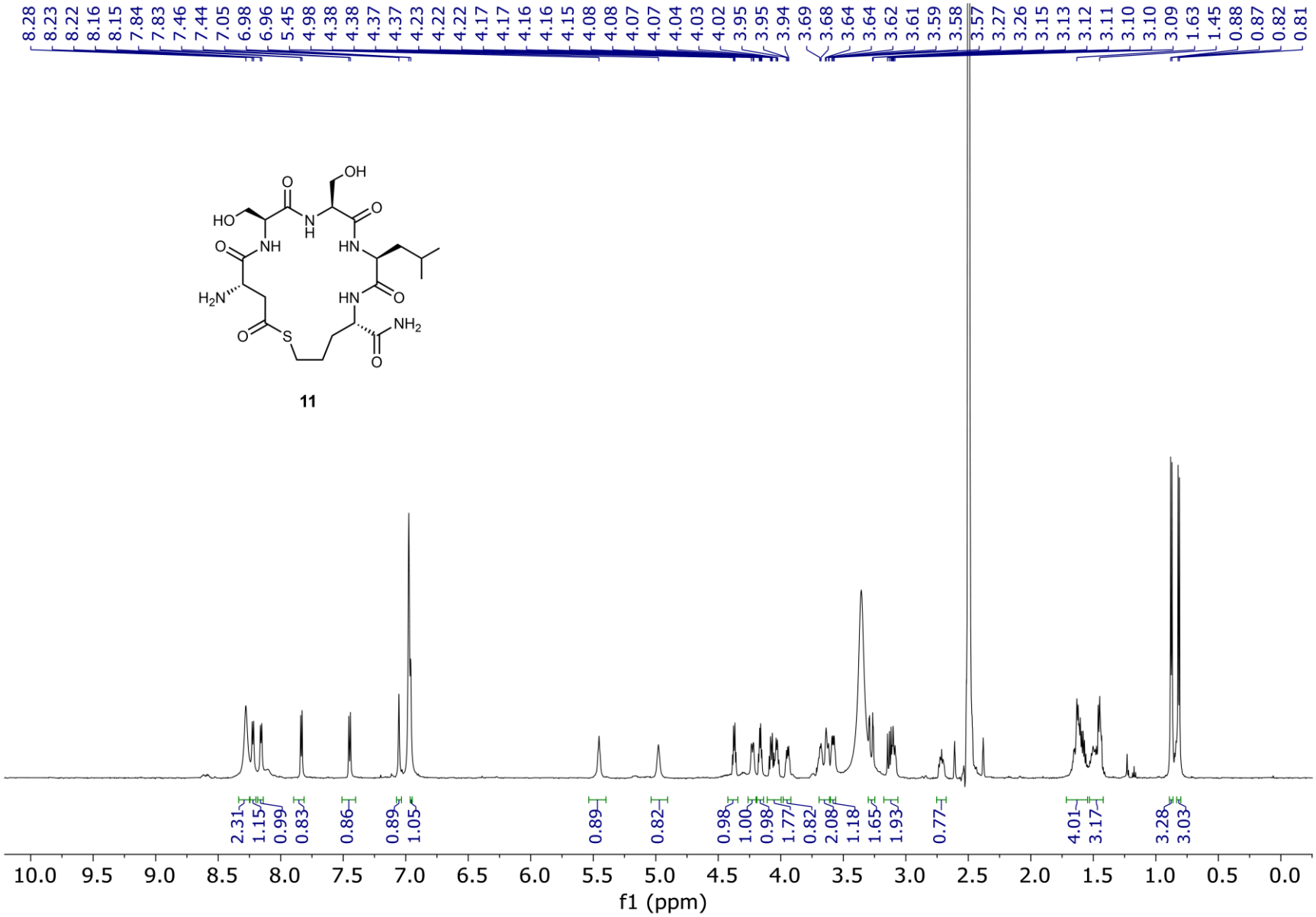


Figure S26: ^{13}C NMR spectrum of **11** (151 MHz, DMSO-d_6)

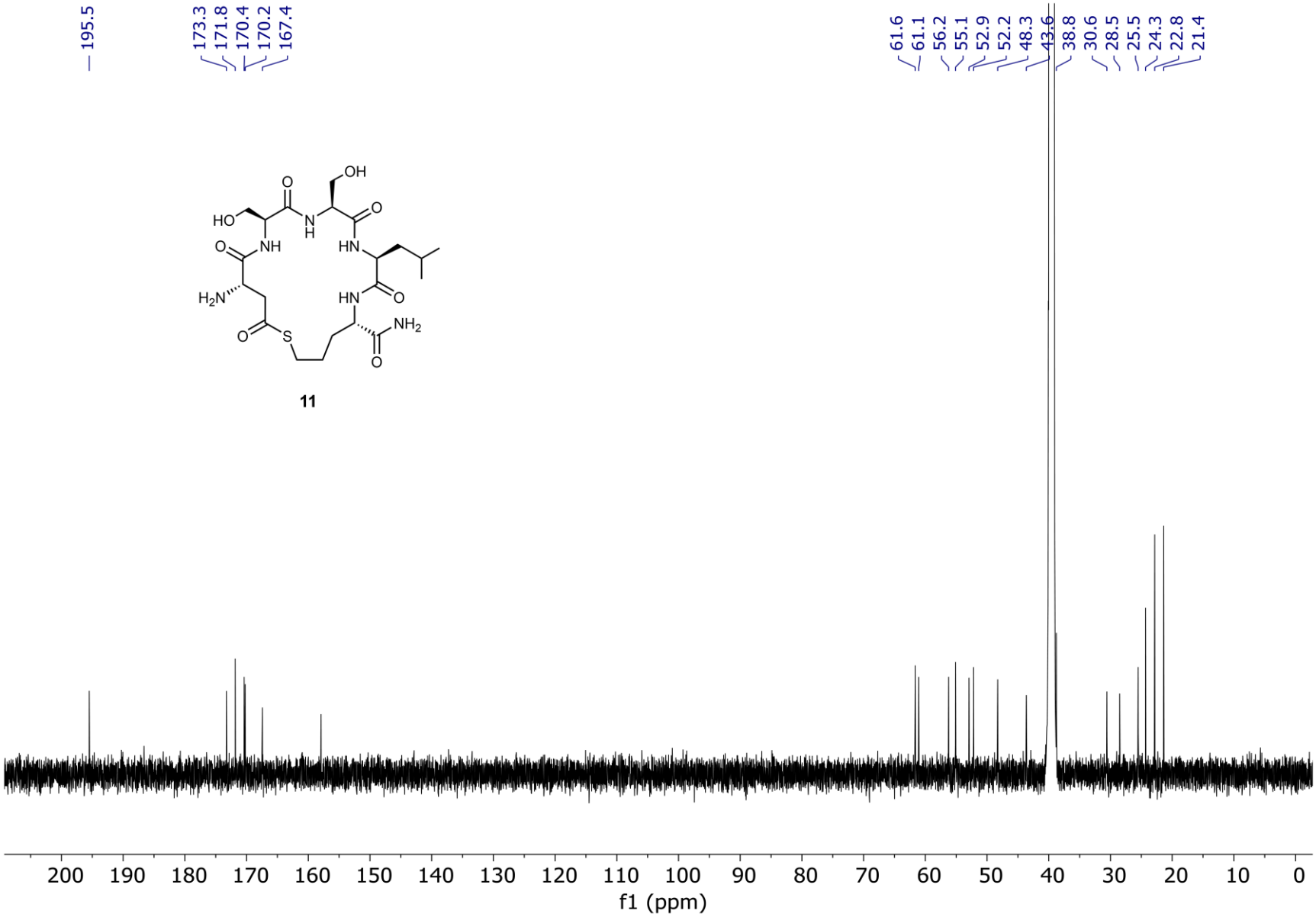


Figure S27: HSQC NMR spectrum of **11**

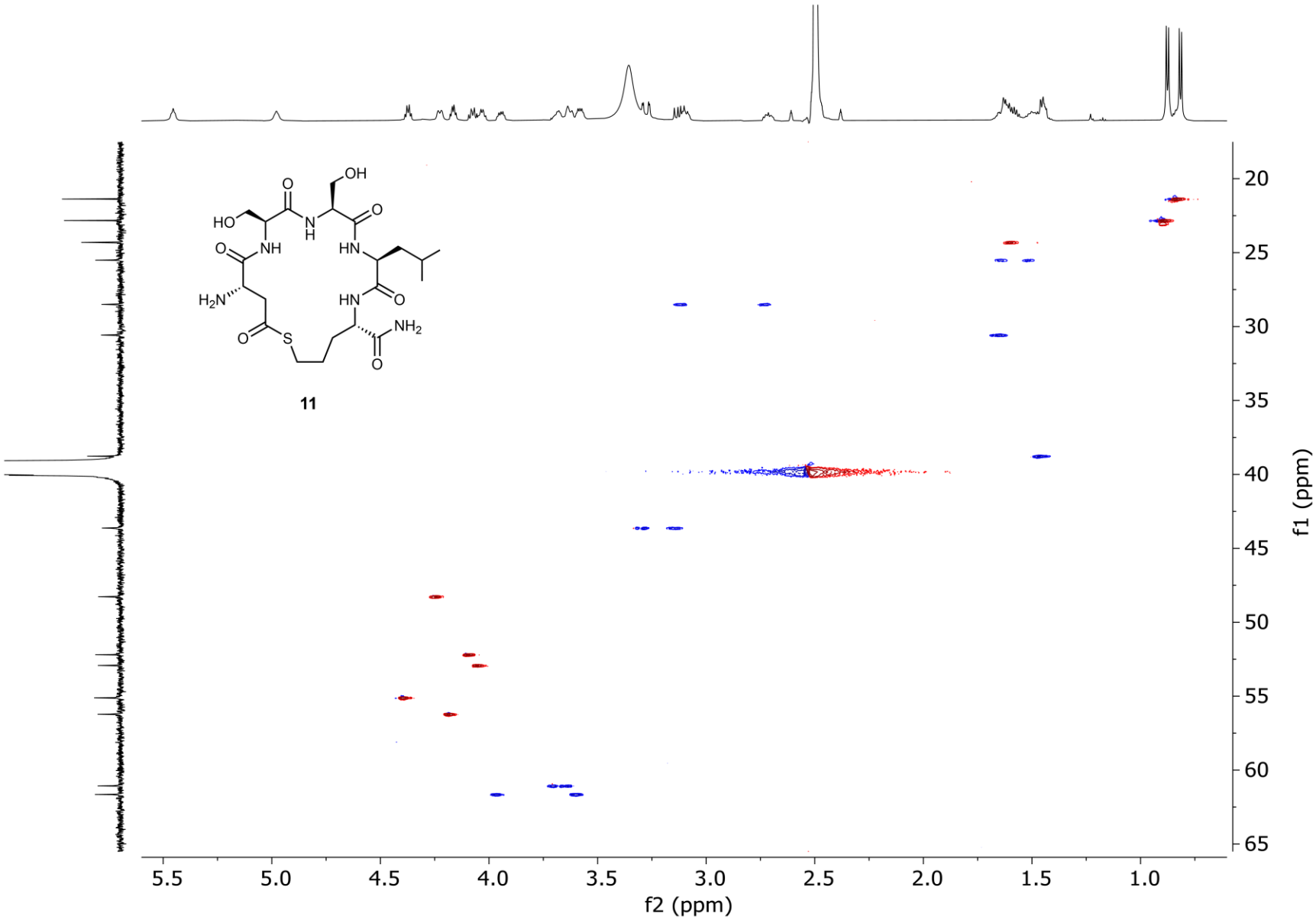
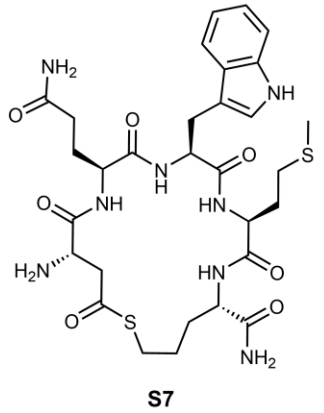


Figure S28: ^{1}H NMR spectrum of **S7** (600 MHz, DMSO-d_6)



S7

Figure S29: ^{1}H NMR spectrum of **S8** (400 MHz, DMSO-d_6)