Supporting Information

Spithioneines A and B, Two New Bohemamine Derivatives Possessing Ergothioneine Moiety from a Marine-derived *Streptomyces spinoverrucosus*

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List of Supporting Information

Experimental details

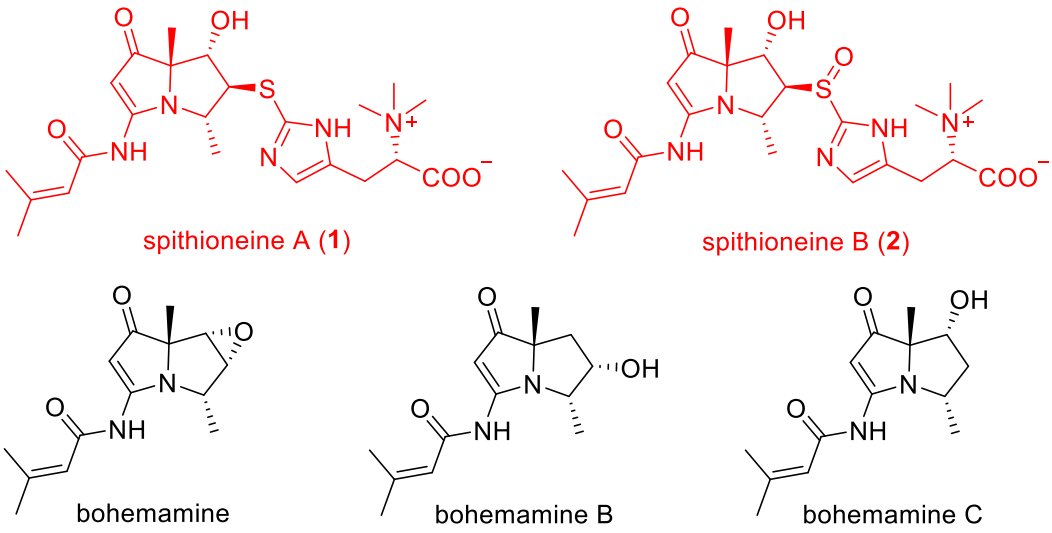
General Procedures. Specific rotations were recorded with an AUTOPOL AP IV-6W polarimeter equipped with a halogen lamp (589 nm). UV spectra were recorded on a Shimadzu UV-1601 UV–VIS spectrophotometer. CD spectra were measured on JASCO J-815 spectrometer. ^{1}H and 2D NMR spectral data were recorded at 600 MHz in CD_3OD and DMSO-d_6 solution on Varian System spectrometer, and chemical shifts were referenced to the corresponding residual solvent signal (δ_H 3.31/δ_C 49.00 for CD_3OD, and δ_H 2.50/δ_C 39.52 for DMSO-d_6.). ^{13}C NMR spectra were acquired at 100 MHz on a Varian System spectrometer. High resolution ESI-TOF mass spectra were provided by The Scripps Research Institute, La Jolla, CA. Low-resolution LC/ESI-MS data were measured using an Agilent 1200 series LC/MS system with a reversed-phase C_{18} column (Phenomenex Luna, 150 mm × 4.6 mm, 5 μm) at a flow rate of 0.7 mL/min. Preparative HPLC was performed on an Agilent 1200 series instrument with a DAD detector, using a reversed-phase C_{18} column (Phenomenex Luna, 250 × 10.0 mm, 5 μm). Sephadex LH-20 (GE Healthcare, Sweden) and ODS (50 mm, Merck) were used for column chromatography. Artificial seawater was used in microbial fermentations as described in a previous reference.[S1]

Collection and phylogenetic analysis of strain SNB-048. *Streptomyces* sp. strain SNB-048 was isolated from a sediment sample collected from a Bahamian tidal flat. The sediment was desiccated and stamped (sediment grains transferred) onto agar plates using gauze 1 acidic media (10 g starch, 1 g $NaNO_3$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 0.01 g $FeSO_4$, 1 L seawater, 15 g agar, adjust pH to 5.3 with phosphate buffer). Bacterial colonies were selected and streaked to purity using the same agar media. Analysis of the strain by 16S rRNA revealed 99.9% identity to *Streptomyces spinoverrucosus*. The sequence is deposited in GenBank under accession no. KR091963.

Cultivation and extraction. Bacterium SNB-048 was cultured in 20 × 2.8 L Fernbach flasks each containing 1 L of a seawater-based medium (10 g starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40mg $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg KBr) and shaken at 200 rpm at 27 °C. After seven days of cultivation, sterilized XAD-7-HP resin (20 g/L) was added to adsorb the organic products, and the culture and resin were shaken at 200 rpm for 2 h. The resin was filtered through cheesecloth, washed with deionized water, and eluted with acetone. The acetone-soluble fraction was dried *in vacuo* to yield 1.2 g of extract.

Purification. The extract (1.2 g) was fractionated by flash column chromatography on ODS (50 μm, 30 g), eluting with a step gradient of MeOH and H_2O (10:90–100:0), and 12 fractions (Fr.1–Fr.12) were collected. Fractions 7 and 8 (403.7 mg) were combined and separated into six fractions (Fr.7.1–Fr.7.6) on Sephadex LH-20, eluting with MeOH. Fr.7.2 (24.7 mg) was purified by reversed-phase HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 5 μm, 2.5

mL/min) using a gradient solvent system from 20% to 50% CH_3CN (0.1% formic acid) over 10 min to afford compounds **1** (13.6 mg, t_R = 6.9 min) and **2** (1.1 mg, t_R = 8.1 min). Fr.7.3 (151.2 mg) was further separated into 16 fractions (Fr.7.3.1–Fr.7.3.16) by flash column chromatography on ODS (50 μm, 30 g), eluting with a step gradient of MeOH and H_2O (10:90–100:0). Fr.7.3.7 (37.5 mg) was purified by reversed-phase HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 5 μm, 2.5 mL/min) using a gradient solvent system from 20% to 80% CH_3CN (0.1% formic acid) over 20 min to afford two known compounds, bohemamine B (10.3 mg, t_R = 11.2 min) and bohemamine (15.8 mg, t_R = 12.4 min).



Spithioneine A (1): colorless oil, $[\alpha]_D^{23}$ +9.2 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε): 250 (4.05), 278 (3.77), 330 (3.62) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 337 (+48.5), 286 (–107.3), 248 (+32.5), 210 (–5.6) nm; IR (NaCl disk) ν_{max}: 3409, 1649, 1025, 996 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and 2; HRESIMS *m/z* 492.2274 $[M + H]^+$ (calcd for $C_{23}H_{34}N_5O_5S$, 492.2275).

Spithioneine B (2): colorless oil, $[\alpha]_D^{23}$ +29.6 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε): 254 (4.11), 280 (3.86), 336 (3.74) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 337 (+39.1), 286 (–82.2), 252 (+38.6), 208 (–17.9) nm; IR (NaCl disk) ν_{max}: 3428, 1643, 672 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and 2; HRESIMS *m/z* 508.2224 $[M + H]^+$ (calcd for $C_{23}H_{34}N_5O_6S$, 508.2224).

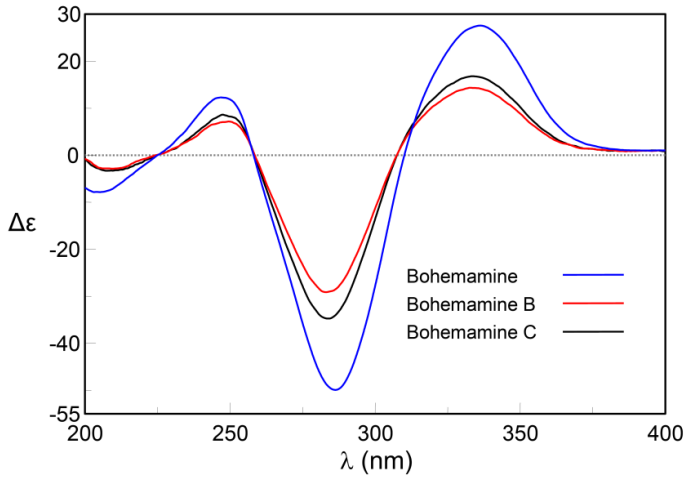


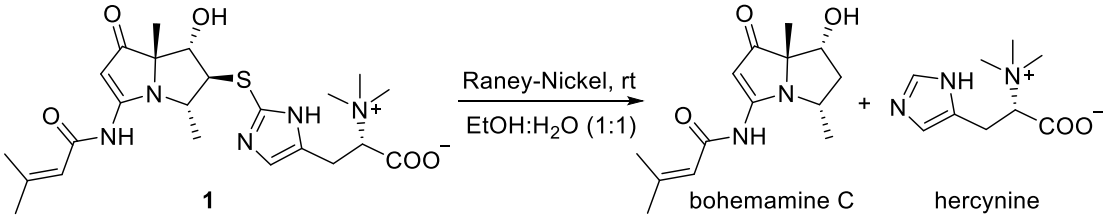
Figure S1. CD spectra of bohemamine, bohemamine B and bohemamine C.

Table S1. ^1H (600 MHz) and ^{13}C (100 MHz) NMR data for compounds **1** and **2** in CD_3OD

no.	1		2	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	204.8, C		204.0, C	
2	ND[a]	ND	ND	5.69, s
3	169.6, C		169.2, C	
4	62.4, CH	3.91, qd (6.7, 3.4)	56.0, CH	4.54, qd (6.7, 2.0)
5	65.3, CH	3.68, dd (3.4, 2.0)	82.9, CH	4.03, brs
6	78.8, CH	4.03, d (2.0)	74.8, CH	3.86, brs
7	80.1, C		80.5, C	
8	24.7, CH_3	1.56, s	23.7, CH_3	1.39, s
9	19.2, CH_3	1.44, d (6.7)	20.2, CH_3	1.49, d (6.7)
1'	165.8, C		165.7, C	
2'	118.2, CH	5.93, m	118.1, CH	5.94, m
3'	159.9, C		160.2, C	
4'	27.8, CH_3	1.96, d (1.3)	27.8, CH_3	1.97, d (1.3)
5'	20.6, CH_3	2.21, d (1.3)	20.6, CH_3	2.23, d (1.2)
1"	138.7, C		145.4, C	
2"	121.0, CH	7.05, s	ND	7.28, s
3"	136.6, C		135.8, C	
4"	27.0, CH_2	3.23, m	27.3, CH_2	3.30, overlap
5"	80.0, CH	3.89, dd (9.2, 5.4)	79.8, CH	3.95, dd (8.6, 5.9)
6"	170.8, C		170.8, C	
5"-NCH_3	52.9, CH_3	3.28, s	52.9, CH_3	3.29, s

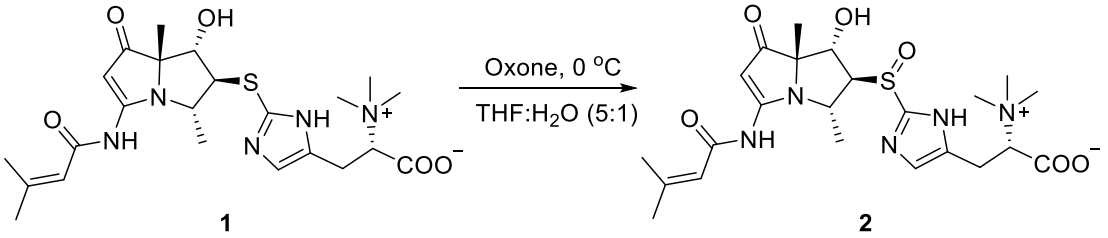
[a] ND: not detected.

Reduction of 1 with Raney-Nickel.



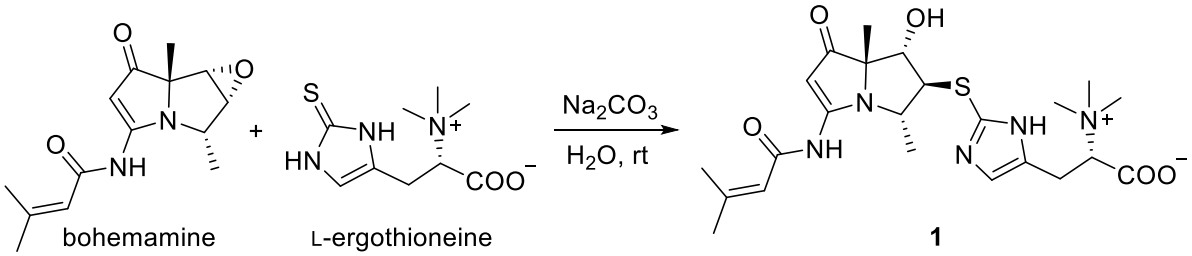
A mixture of Raney-Nickel (0.2 mL of suspension) and compound **1** (2.5 mg) in EtOH (0.5 mL) and water (0.5 mL) was stirred at room temperature. After 2 h, the mixture was filtered through Celite and the solvent was evaporated. The crude product was purified by preparative HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 5 μm, 2.5 mL/min) using a gradient solvent system from 20% to 70% CH_3CN (0.1% formic acid) over 15 min to give hercynine (0.7 mg, t_R = 3.3 min) and bohemamine C (0.8 mg, t_R = 10.9 min).

Sulfoxidation of 1 with Oxone.



Compound **1** (2.0 mg) was dissolved into 0.5 mL of THF and 0.1 mL of water. The mixture was cooled in an ice bath, and 25 μL of aqueous solution of potassium peroxomonosulfate (Oxone) (0.1 g/mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2.5 h. After reaction completion, the solution was allowed to warm to room temperature and 0.2 mL of aqueous solution of $NaHCO_3$ (pH 8) was added. The reaction product was purified by HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 5 μm, 2.5 mL/min) using a gradient solvent system from 20% to 50% CH_3CN (0.1% formic acid) over 10 min to give the semisynthetic product **2** (1.8 mg, t_R = 8.1 min). LC/MS and ^{1}H NMR analysis confirmed the identity of this semisynthetic product and spithioneine B (**2**).

Synthesis of 1 from bohemamine and L-ergothioneine.



A mixture of bohemamine (1.0 mg), L-ergothioneine (2.0 mg), and Na_2CO_3 (20 mg) in water (0.6 mL) was stirred at room temperature for 48 h. The reaction mixture was analyzed by LC-MS (Phenomenex Luna, C_{18}, 150 mm × 4.6 mm, 5 μm, 0.7 mL/min) using a gradient solvent system from 10% to 99% CH_3CN (0.1% formic acid) over 17 min. In this reaction, compound **1** was produced (Figure S2).

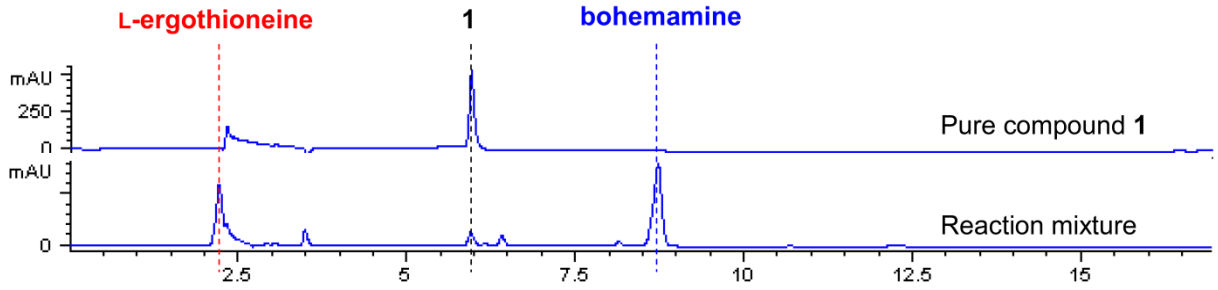


Figure S2. LC/MS profile (280 nm) of the reaction mixture of bohemamine and L-ergothioneine.

Antibiotic assays. The antibiotic activities against *Pseudomonas aeruginosa* and *Bacillus subtilis* were evaluated by an agar dilution method. The tested strains were cultivated in LB agar plates at 37 °C. Compounds **1** and **2**, and positive control (erythromycin) were dissolved in MeOH at different concentrations from 100 to 0.1 μg/mL by the continuous 10-fold dilution methods. A 10 μL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 24 h incubation, zones of inhibition (mm in diameter) were recorded.

Cytotoxicity assays. Cell lines were cultivated in 10 cm dishes (Corning, Inc.) in NSCLC cell-culture medium: RPMI/L-glutamine medium (Invitrogen, Inc.), 1000 U/mL penicillin (Invitrogen, Inc.), 1 mg/mL streptomycin (Invitrogen, Inc.), and 5% fetal bovine serum (Atlanta Biologicals, Inc.). Cell lines were grown in a humidified environment in the presence of 5% CO_2 at 37 °C. For cell viability assays, HCC366, A549, HCC44 and HCC1171 cells (60 μL) were plated individually at a density of 1200, 750 and 500 cells/well, respectively, in 384-well microtiter assay plates (Bio-one; Greiner, Inc.). After incubating the assay plates overnight under the growth conditions described above, purified compounds were dissolved and diluted in DMSO and subsequently added to each plate with final compound concentrations ranging from 50 μM to 1 nM and a final DMSO concentration of 0.5%. After an incubation of 96 h under growth conditions, Cell Titer Glo reagent (Promega, Inc.) was added to each well (10 mL of a 1:2 dilution in NSCLC culture medium) and mixed. Plates were incubated for 10 min at room temperature, and luminescence was determined for each well using an Envision multimodal plate reader (Perkin-Elmer, Inc.). Relative luminescence units were normalized to the untreated control wells (cells plus DMSO only). Data were analyzed using the Assay Analyzer and Condoseo modules of the Screener Software Suite (GeneData, Inc.) as described previously.[S2]

(S1) Keller, M. D.; Selvin, R. C.; Claus, W.; Guillard, R. R. L. *J. Phycol.* **1987**, *23*, 633−638.

(S2) Kim, H. S.; Mendiratta, S.; Kim, J.; Pecot, C. V.; Larsen, J. E.; Zubovych, I.; Seo, B. Y.; Kim, J.; Eskiocak, B.; Chung, H.; McMillan, E.; Wu, S.; De Brabander J; Komurov, K.; Toombs, J. E.; Wei, S.; Peyton, M.; Williams, N.; Gazdar, A. F.; Posner, B. A.; Brekken, R. A.; Sood, A. K.; Deberardinis, R. J.; Roth, M. G.; Minna, J. D.; White, M. A. *Cell* **2013**, *155*, 552–566.

Figure S3. HRESIMS spectrum of spithioneine A (**1**)

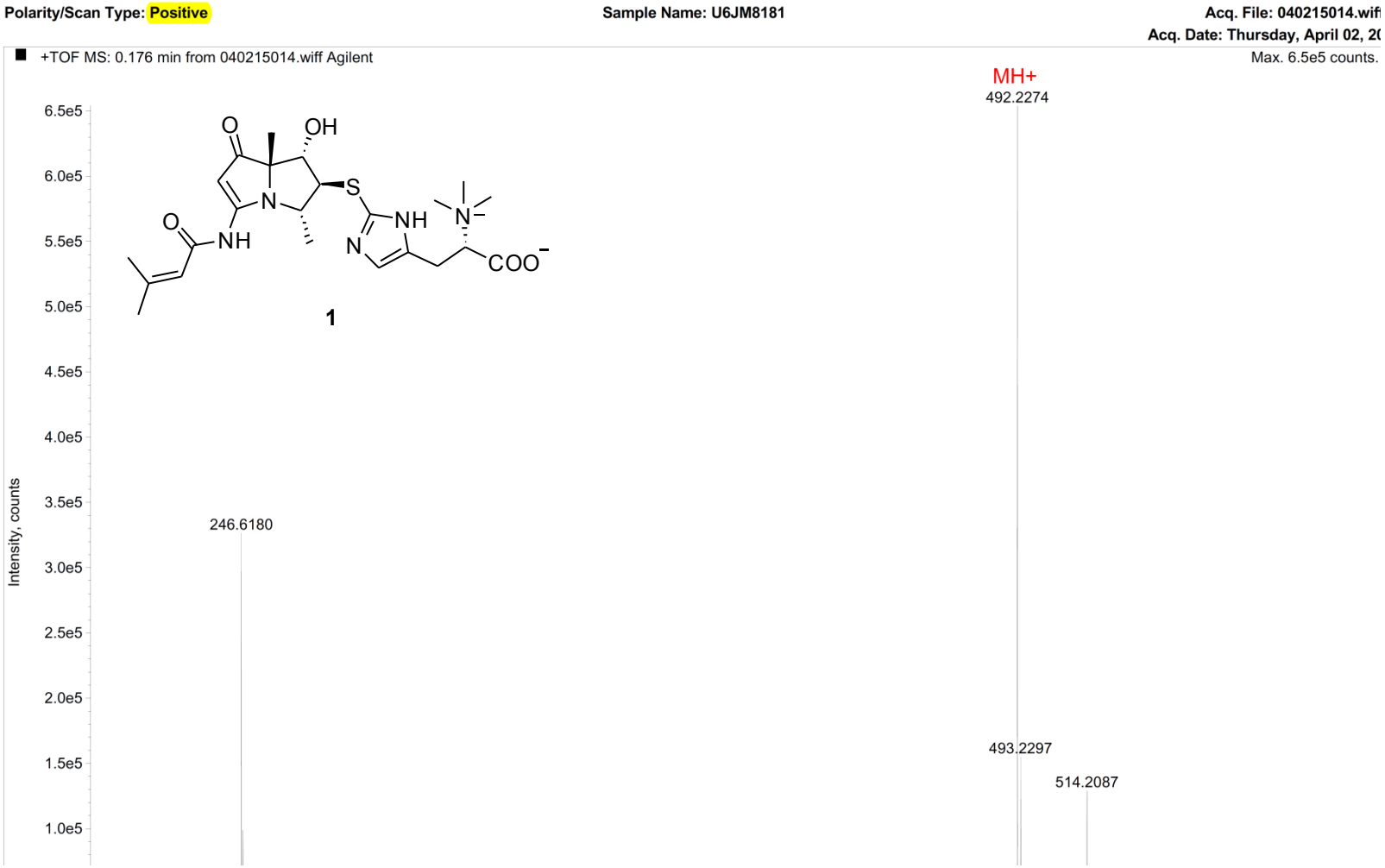
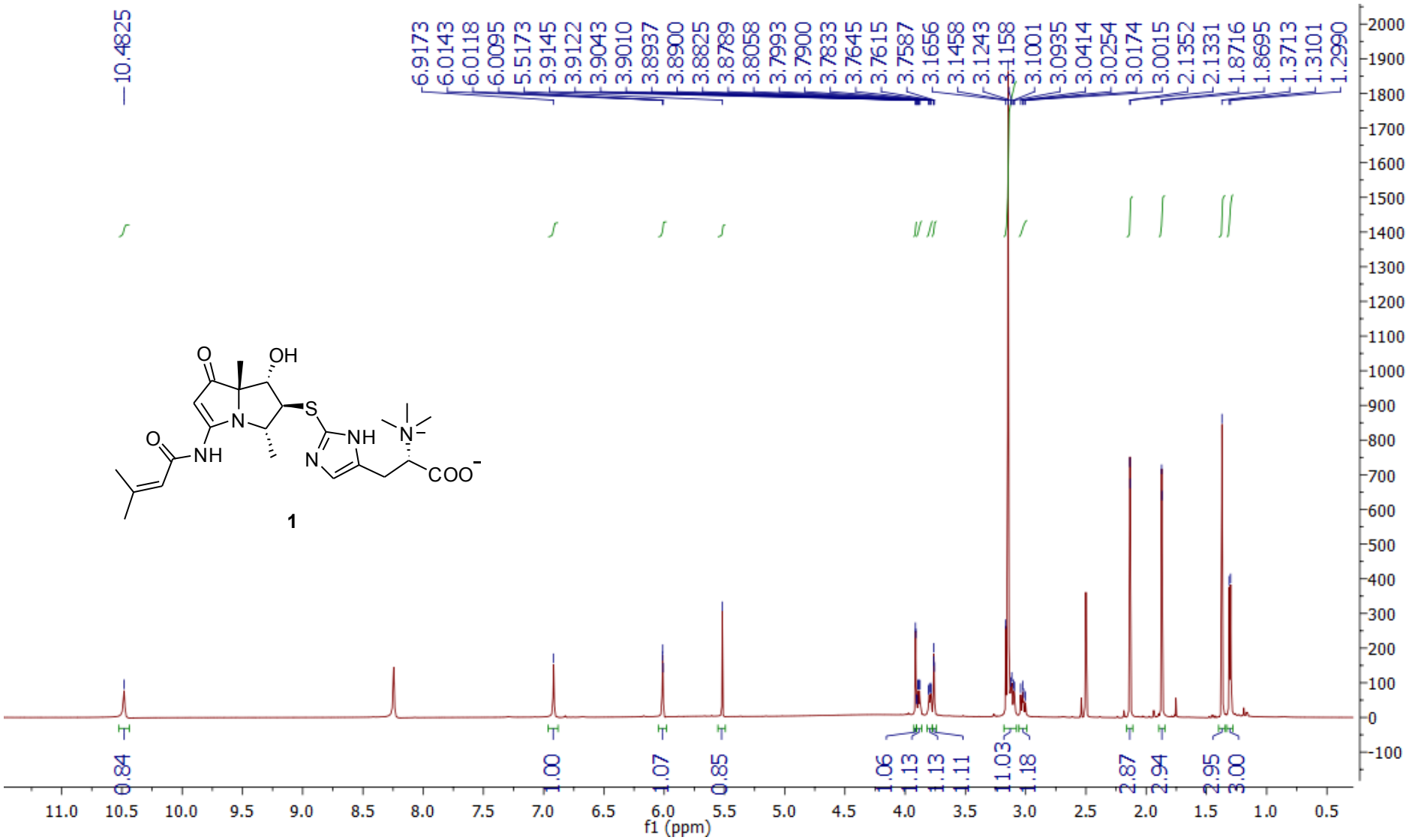


Figure S4. The ^{1}H-NMR spectrum of spithioneine A (**1**) in DMSO-d_6



1

Figure S5. The ^{13}C-NMR spectrum of spithioneine A (**1**) in DMSO-d_6

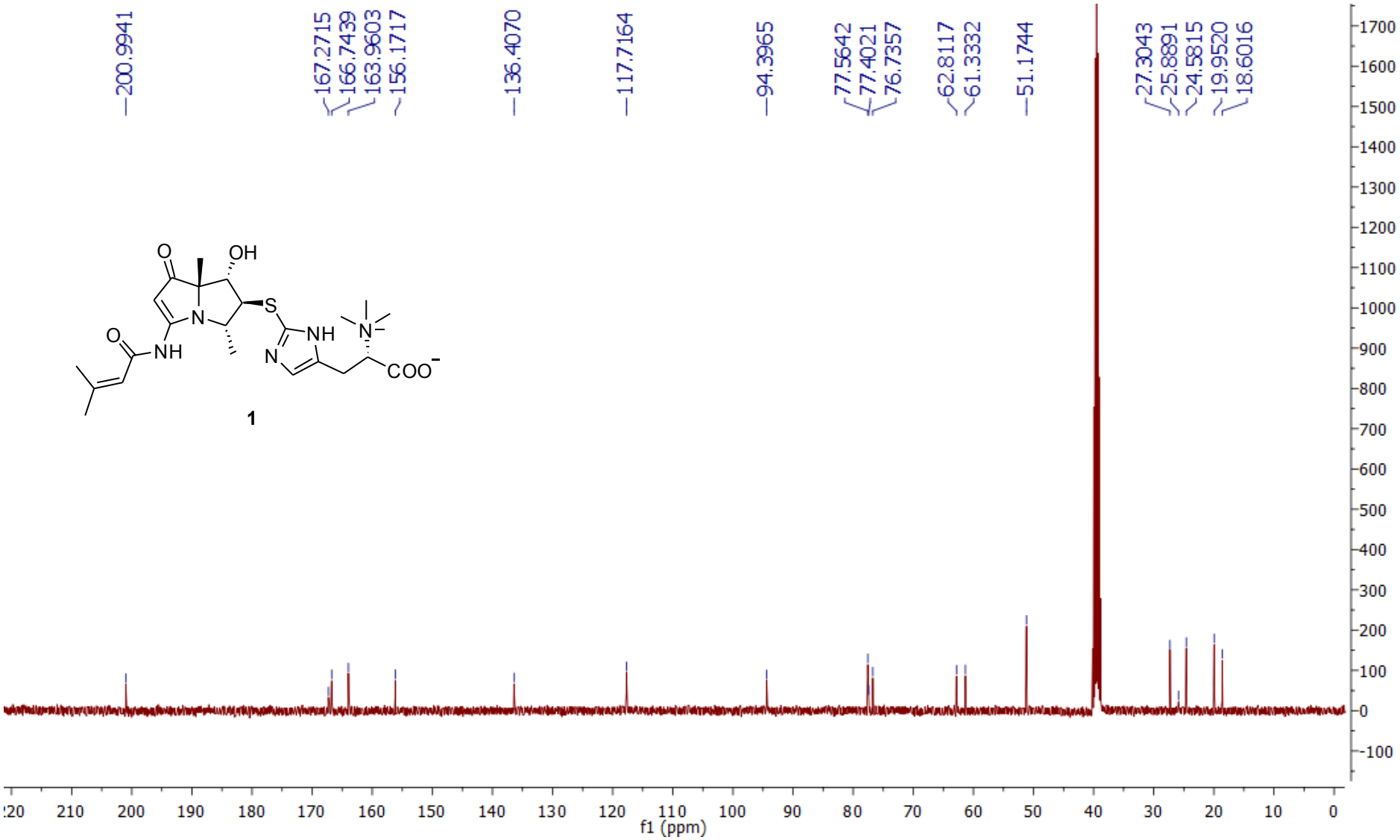


Figure S6. The HSQC spectrum of spithioneine A (**1**) in DMSO-d_6

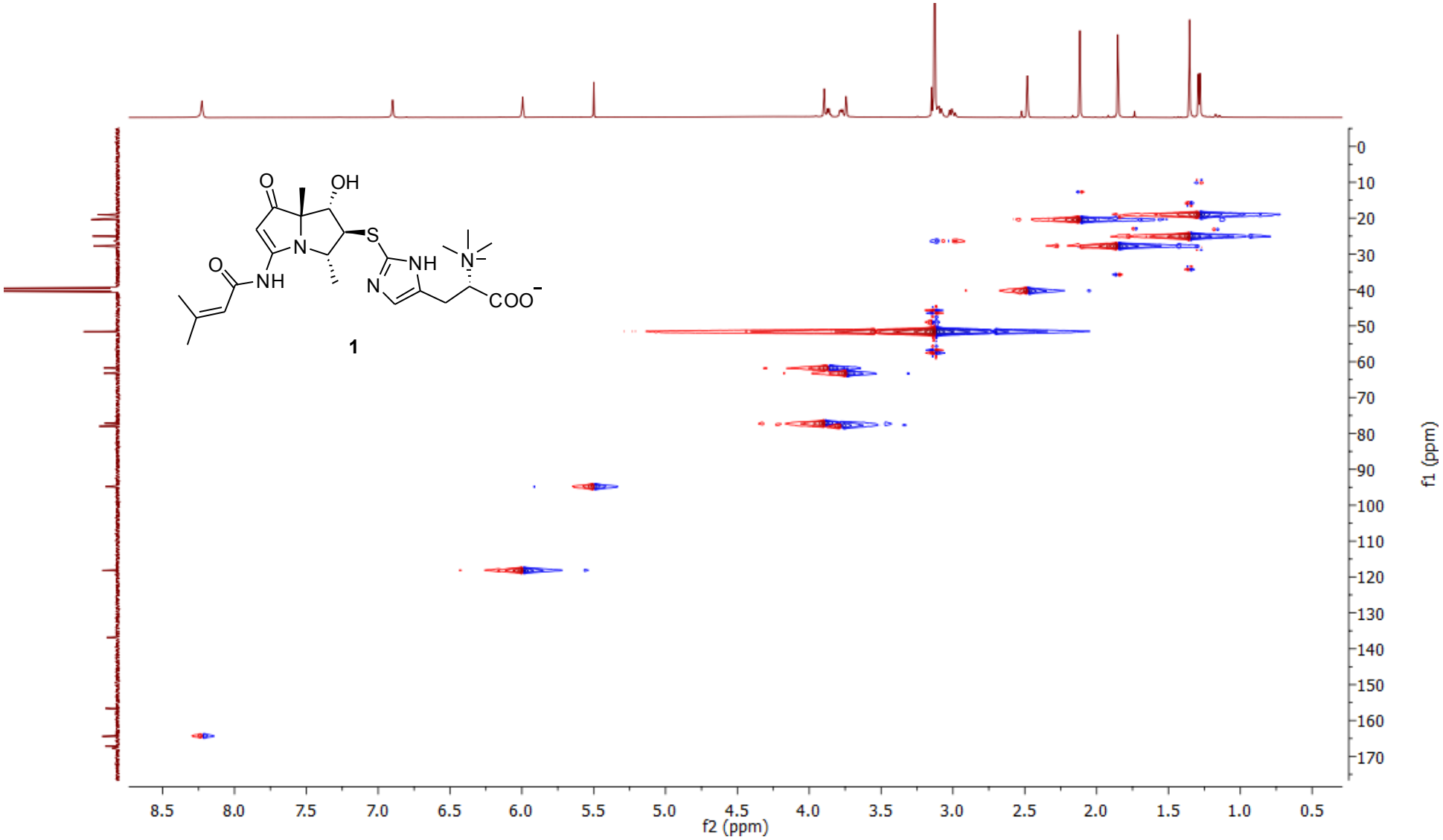


Figure S7. The ^{1}H-^{1}H COSY spectrum of spithioneine A (**1**) in DMSO-d_6

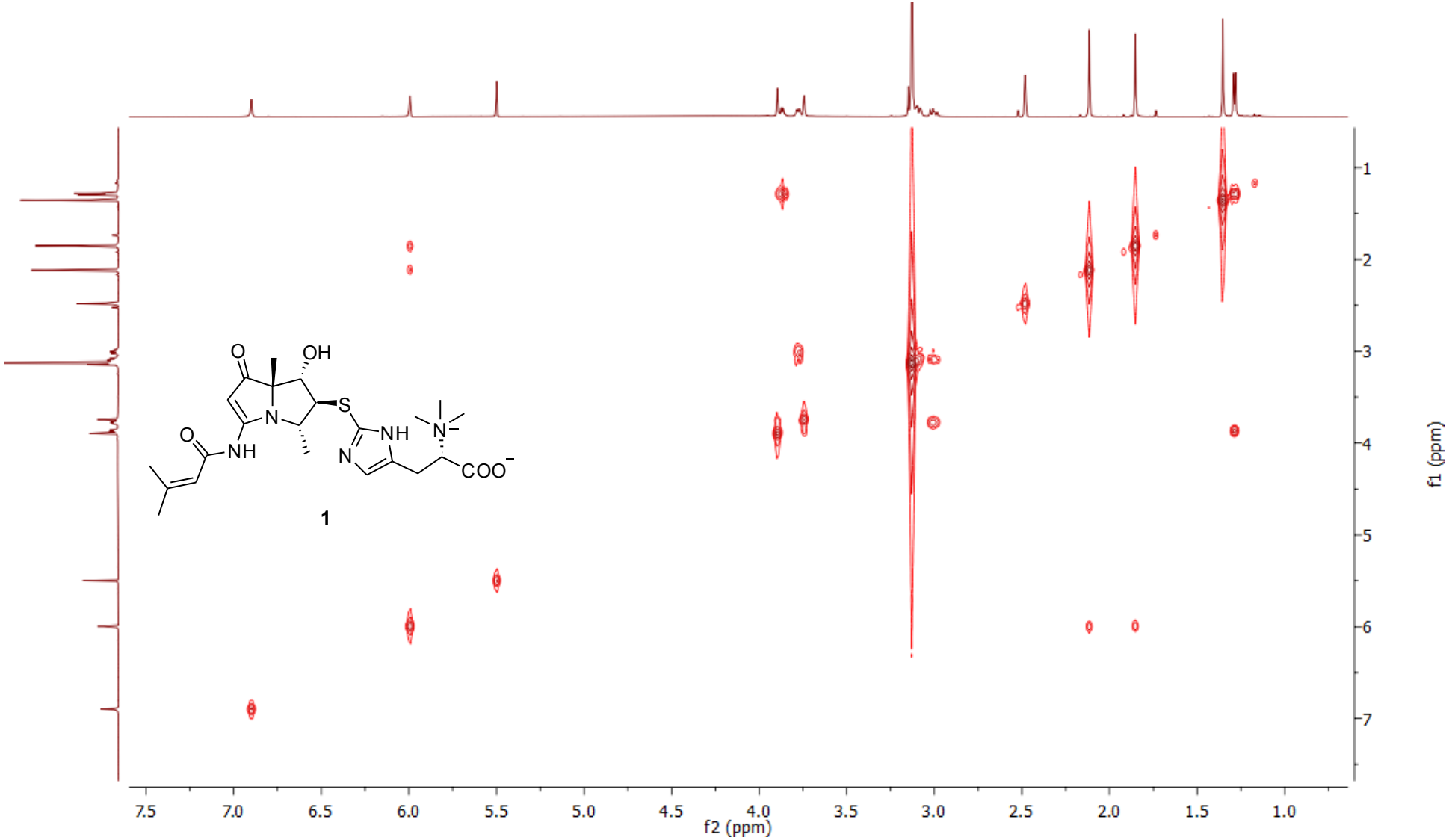


Figure S8. The HMBC spectrum of spithioneine A (**1**) in DMSO-d_6

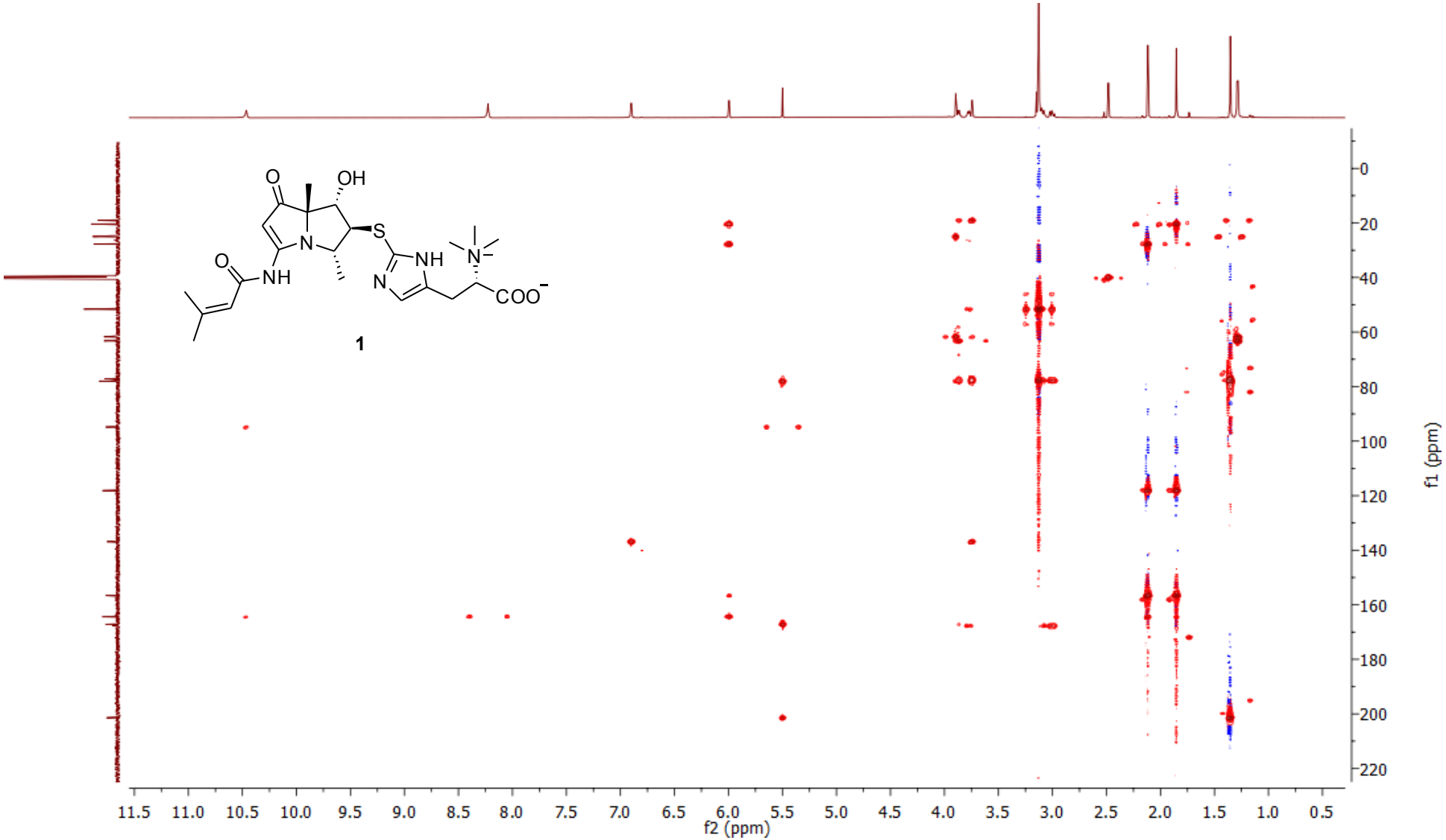


Figure S9. The ^{1}H-NMR spectrum of spithioneine A (**1**) in CD_3OD

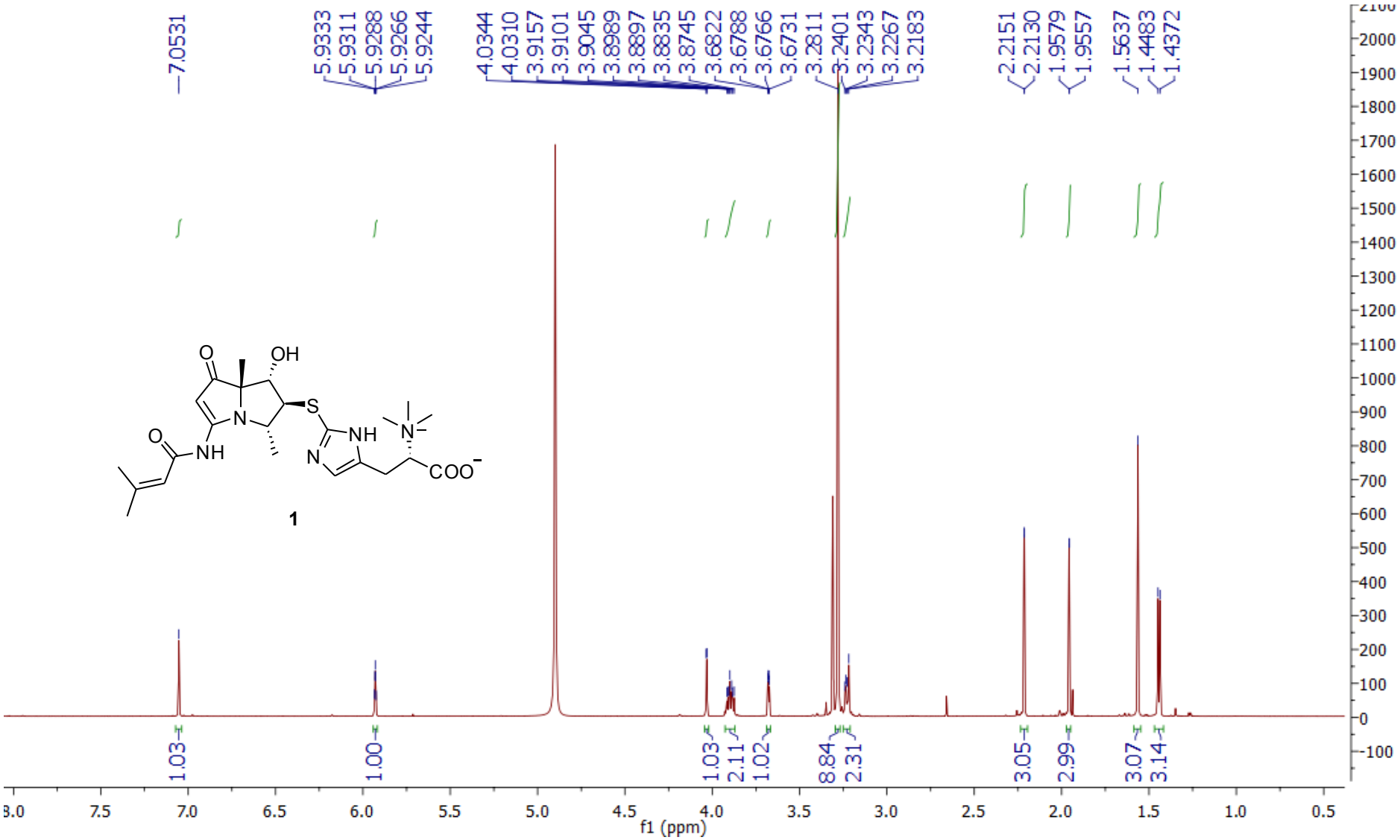


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Figure S10. The ^{13}C-NMR spectrum of spithioneine A (**1**) in CD_3OD

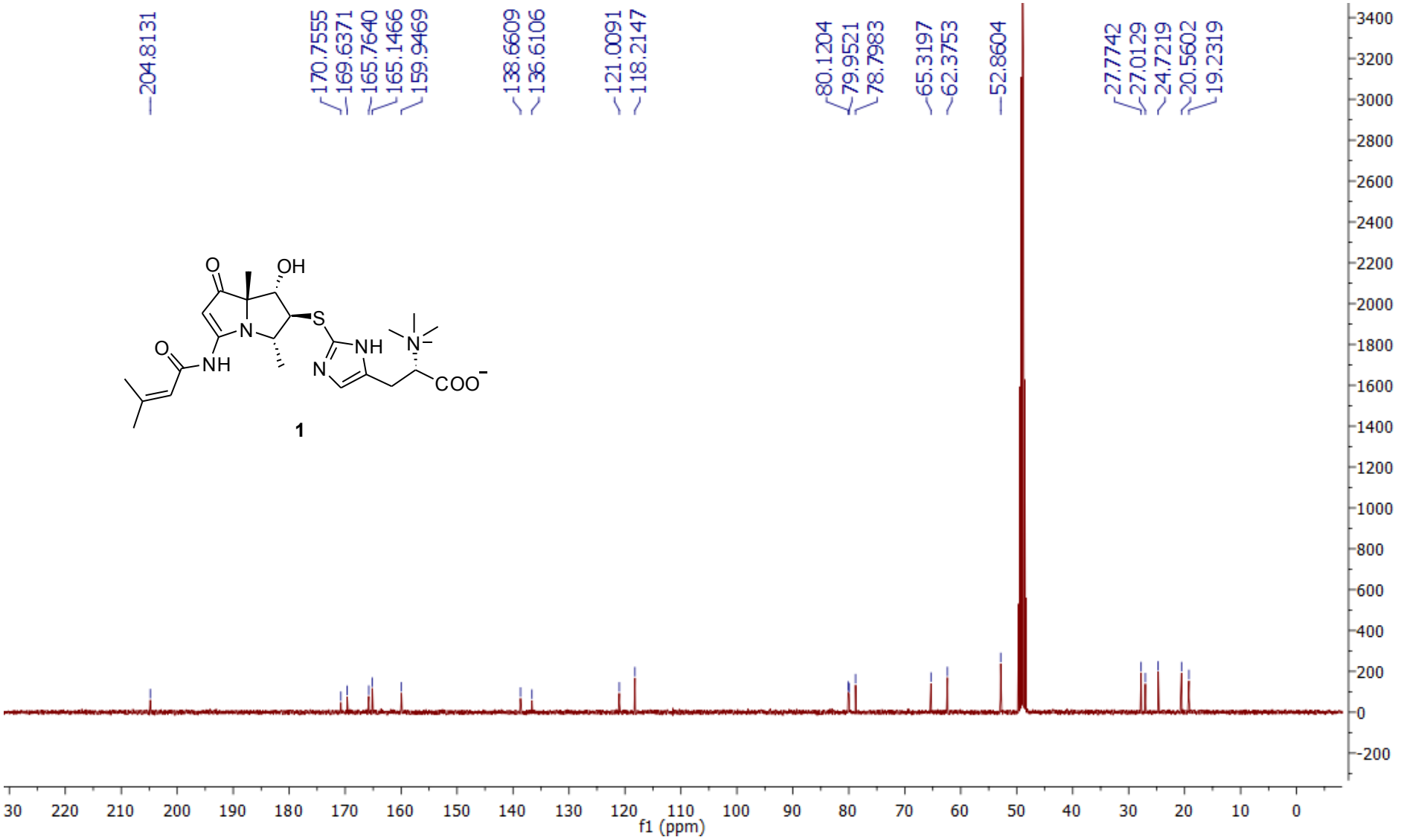

Figure S11. The HSQC spectrum of spithioneine A (**1**) in CD_3OD

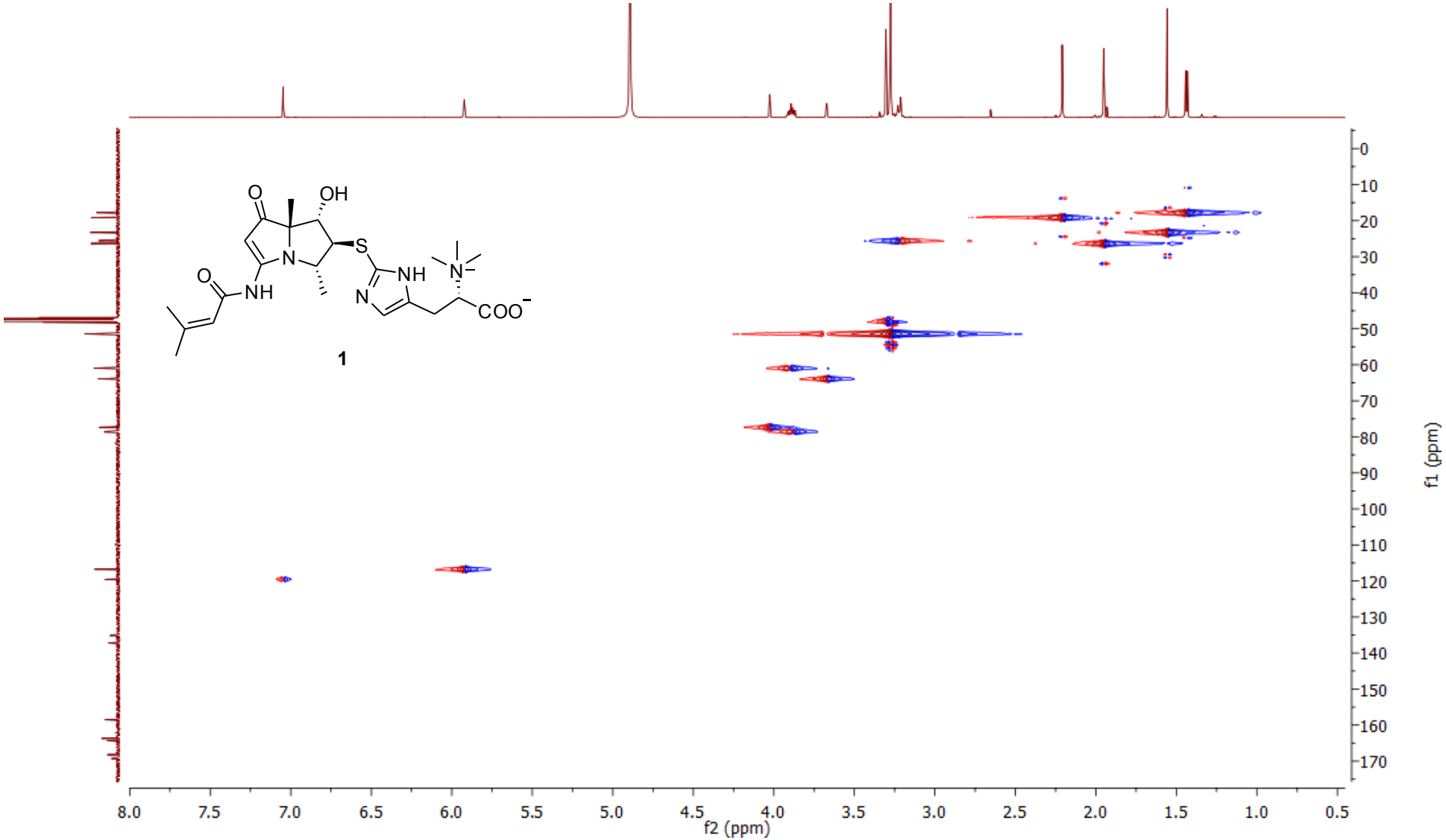

Figure S12. The ^{1}H-^{1}H COSY spectrum of spithioneine A (**1**) in CD_3OD

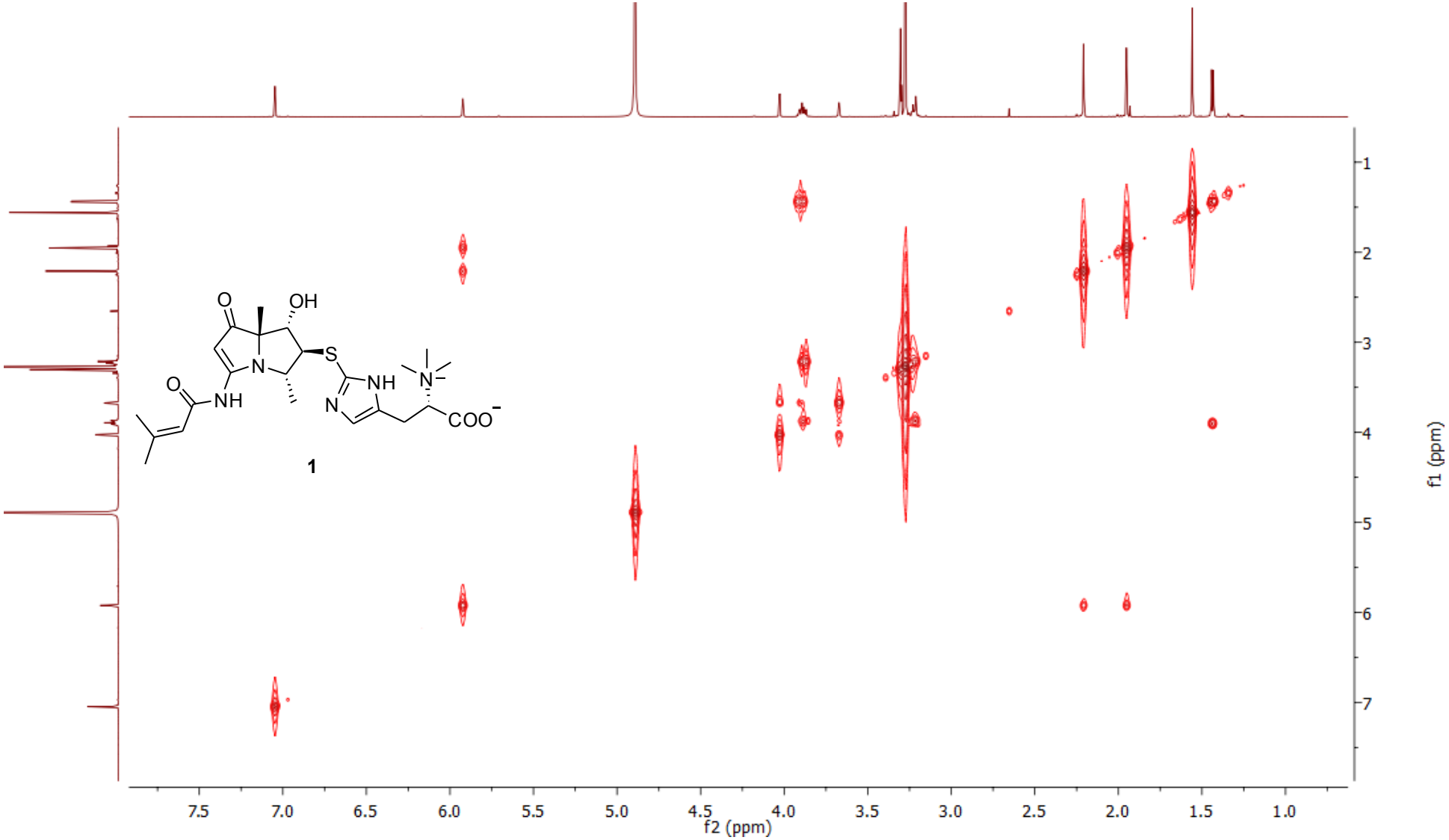

Figure S13. The HMBC spectrum of spithioneine A (**1**) in CD_3OD

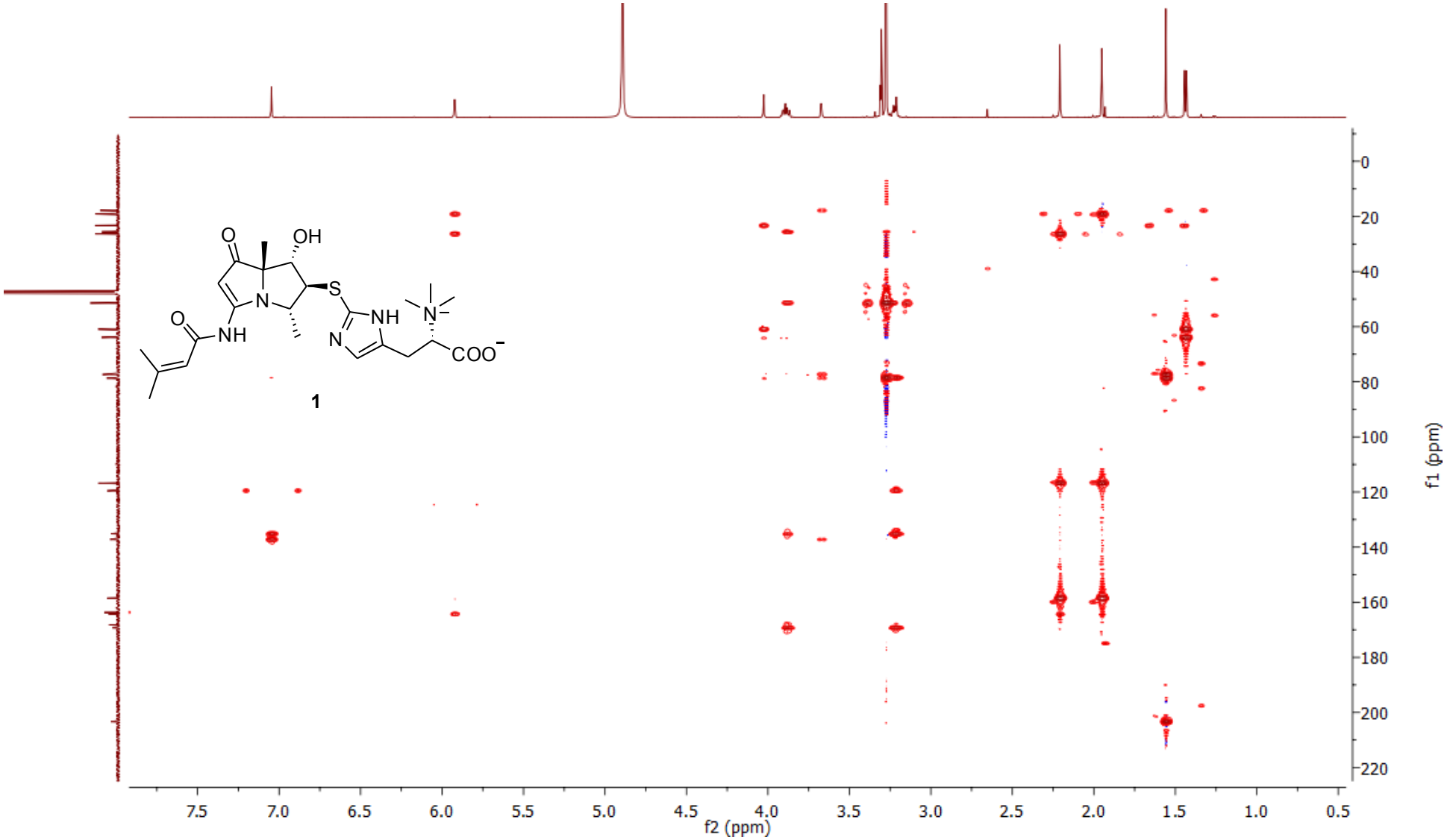

Figure S14. The NOESY spectrum of spithioneine A (**1**) in CD_3OD

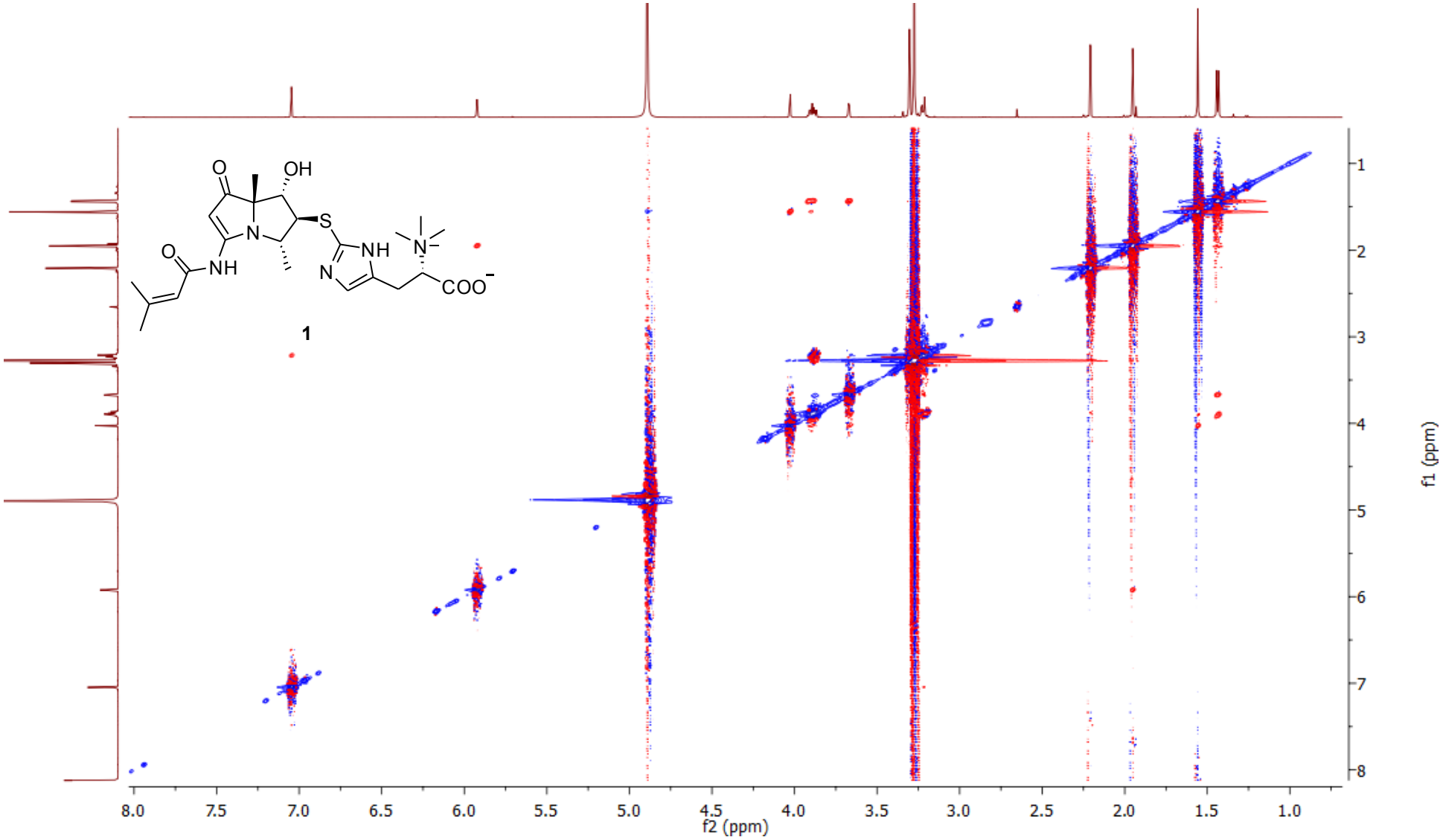

Figure S15. HRESIMS spectrum of spithioneine B (**2**)

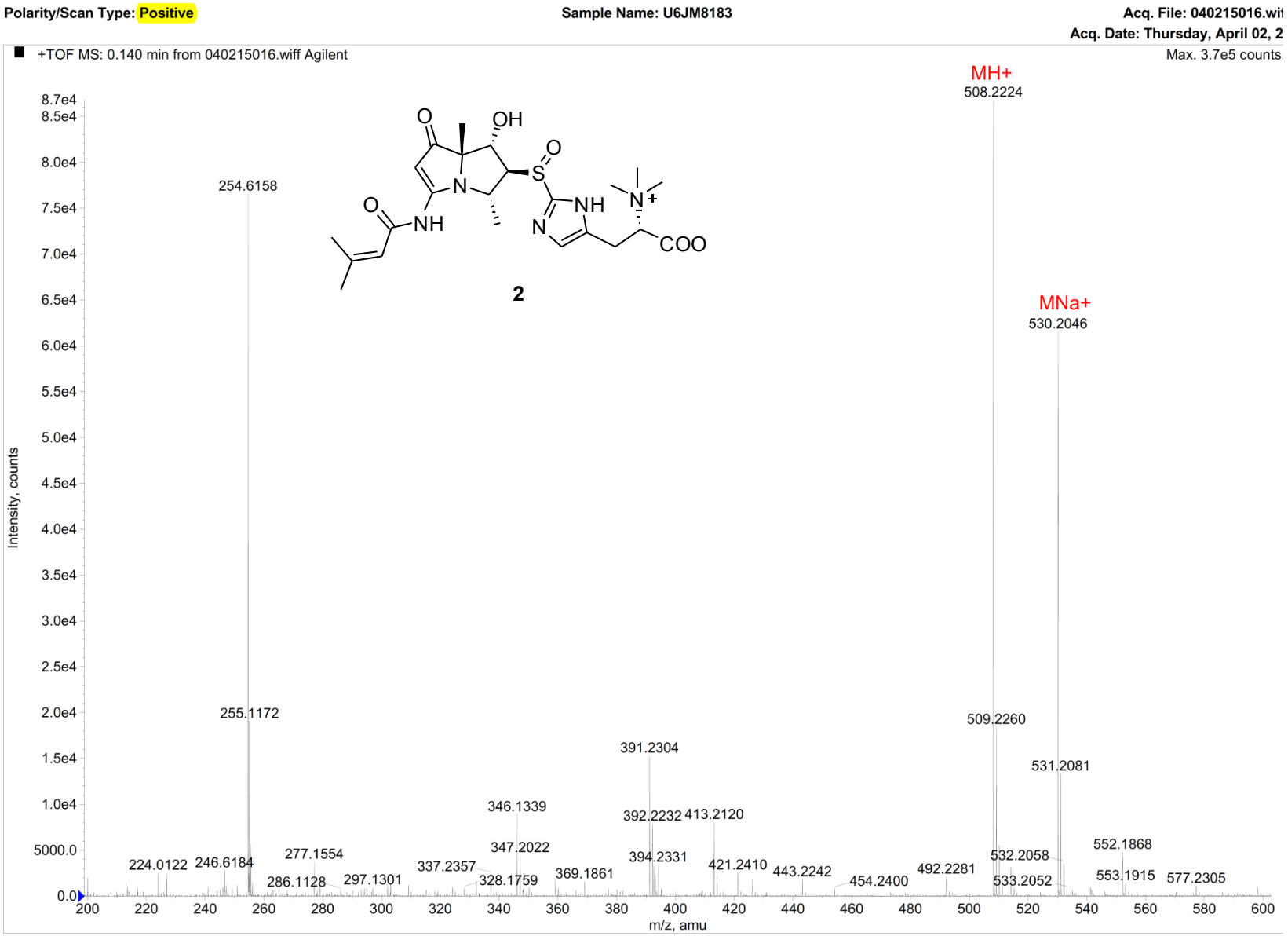

Figure S16. The ^{1}H-NMR spectrum of spithioneine B (**2**) in DMSO-d_6

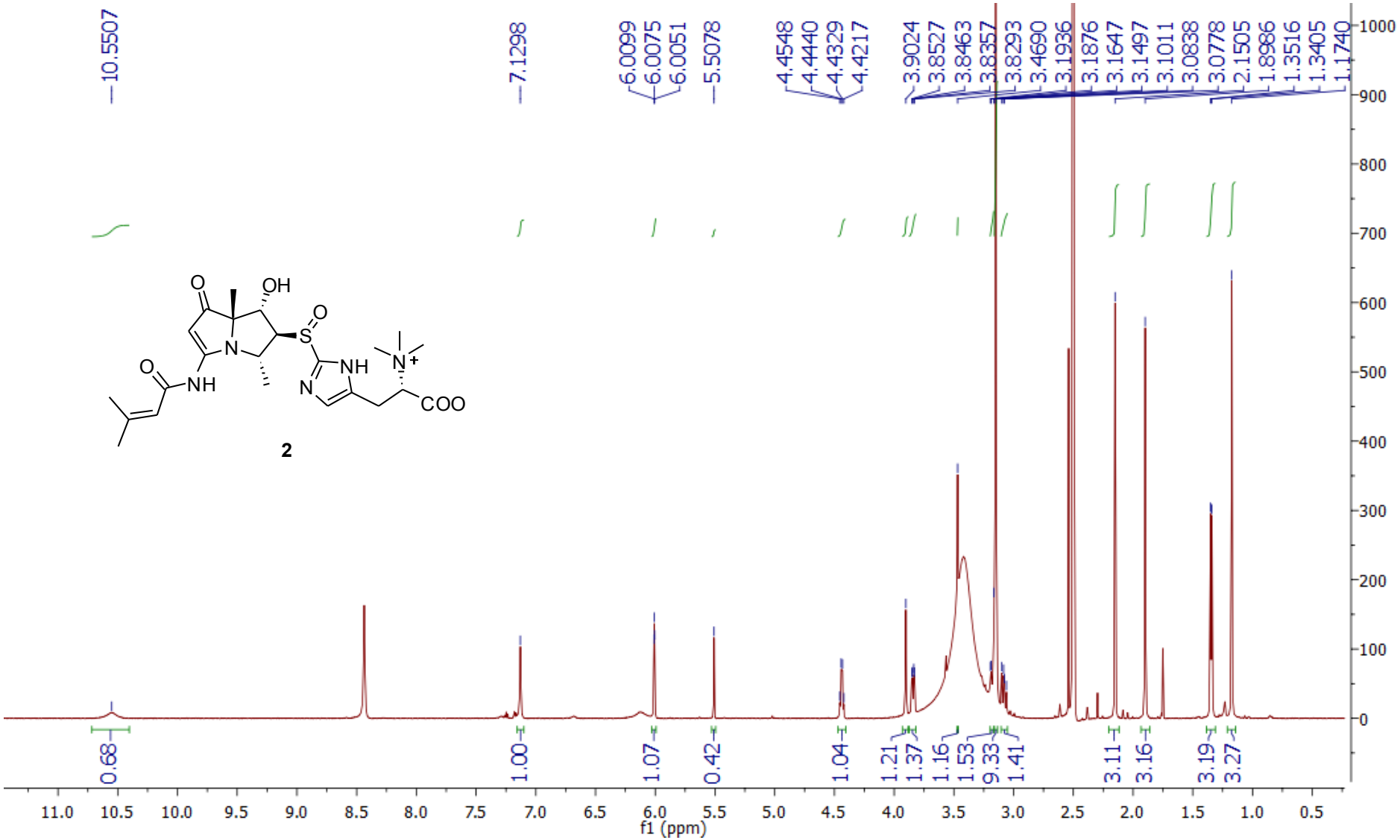

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Figure S17. The ^{13}C-NMR spectrum of spithioneine B (**2**) in DMSO-d_6

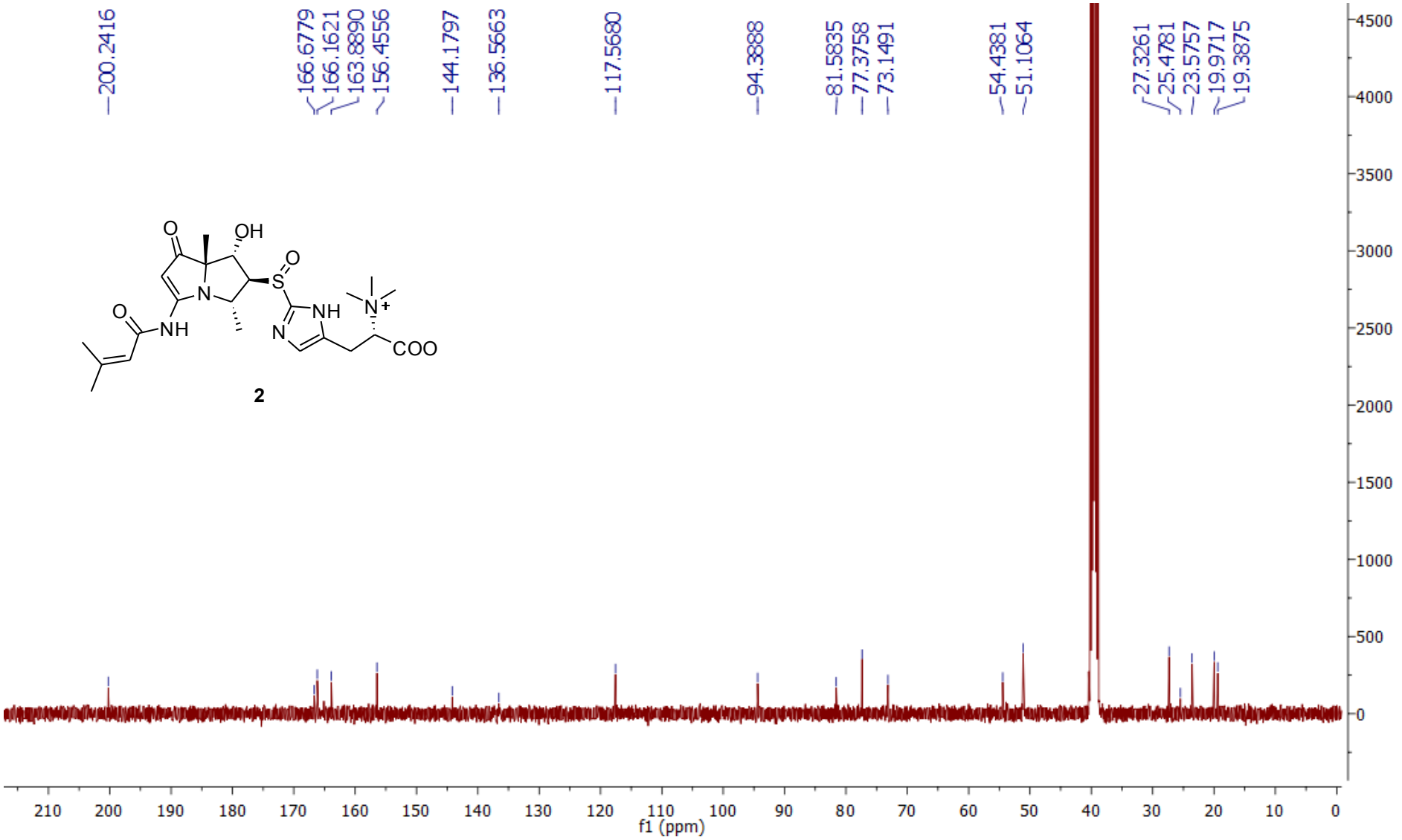

Figure S18. The HSQC spectrum of spithioneine B (**2**) in DMSO-d_6

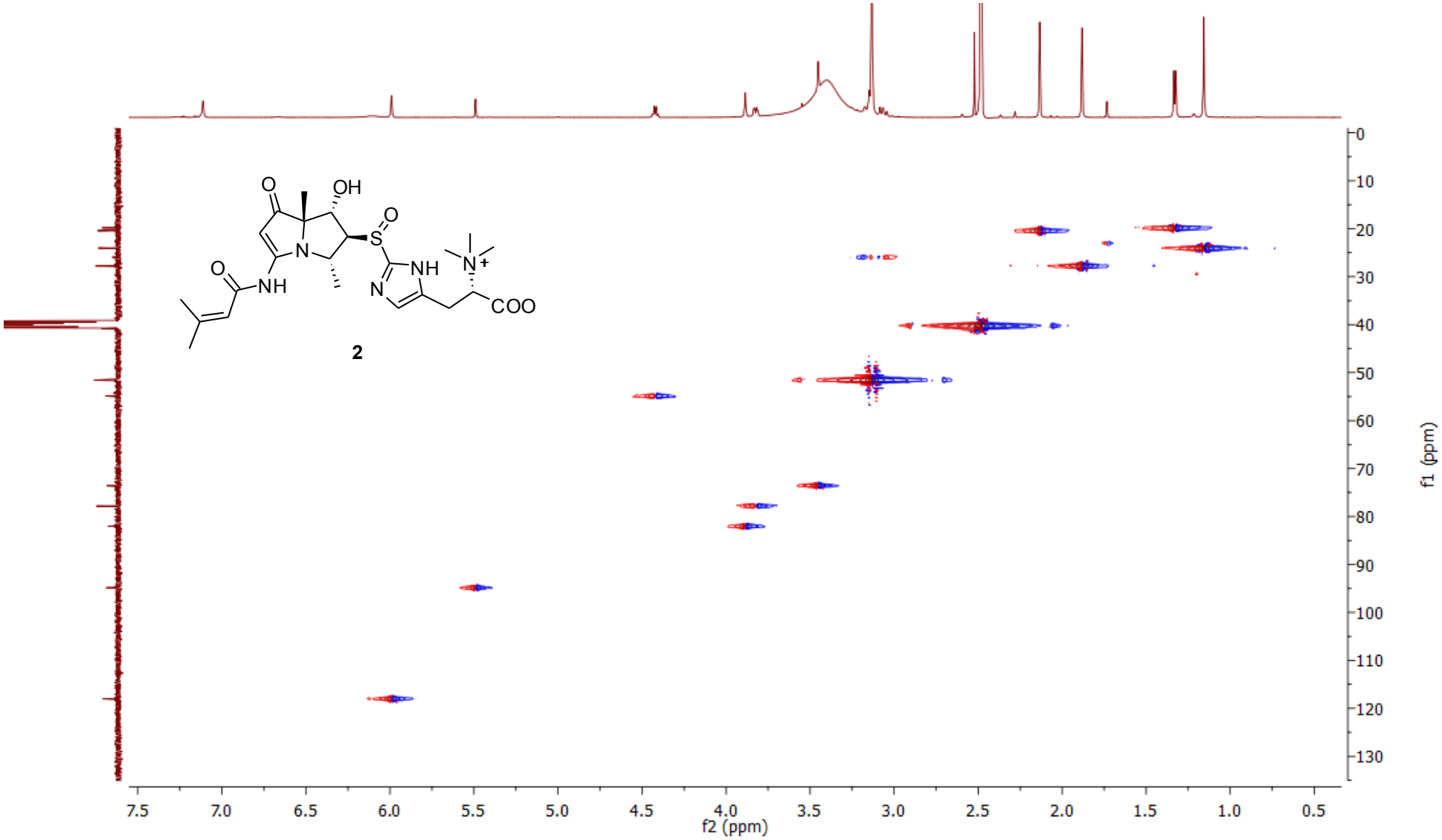

Figure S19. The ^{1}H-^{1}H COSY spectrum of spithioneine B (**2**) in DMSO-d_6

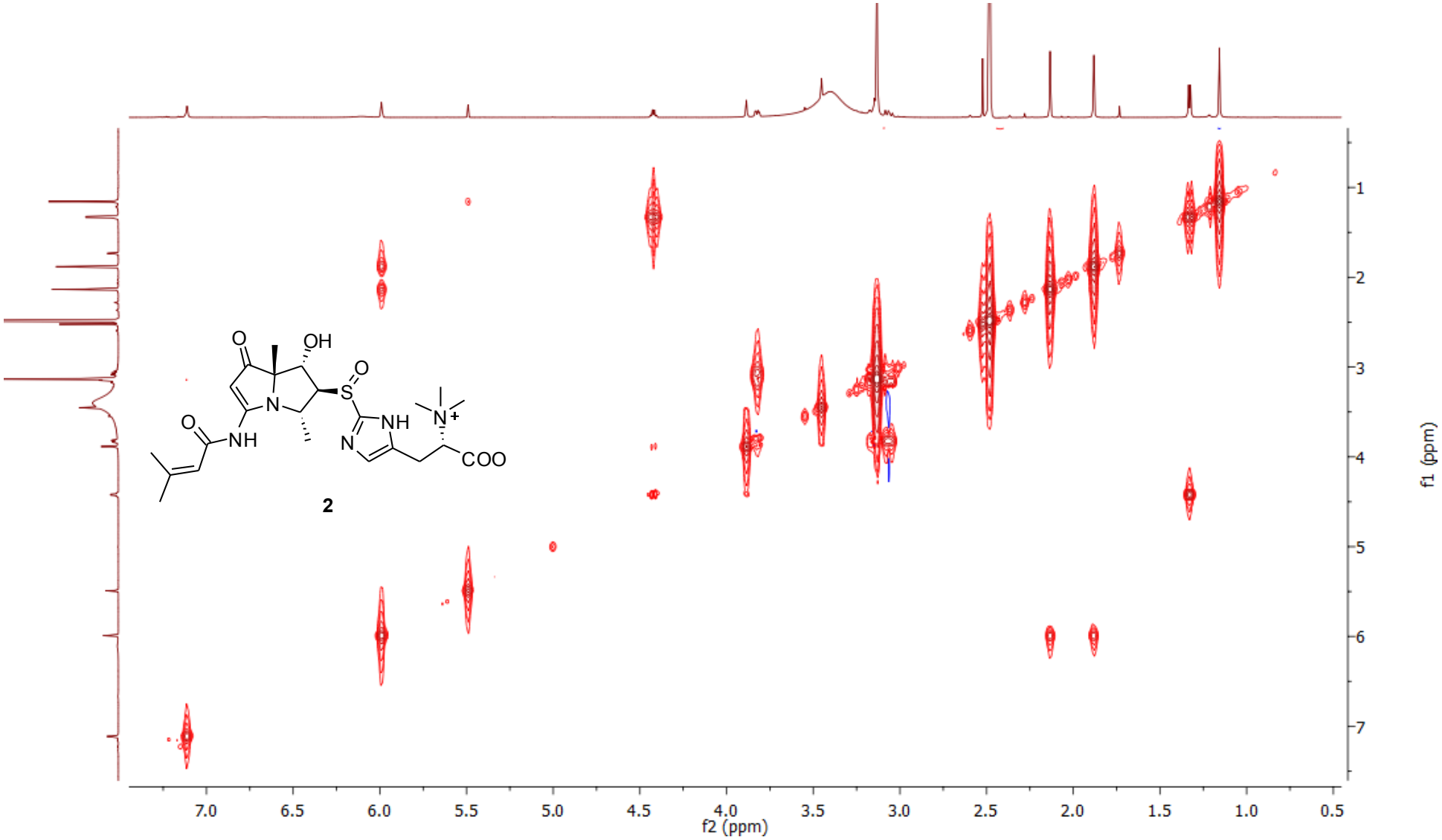

Figure S20. The HMBC spectrum of spithioneine B (**2**) in DMSO-d_6

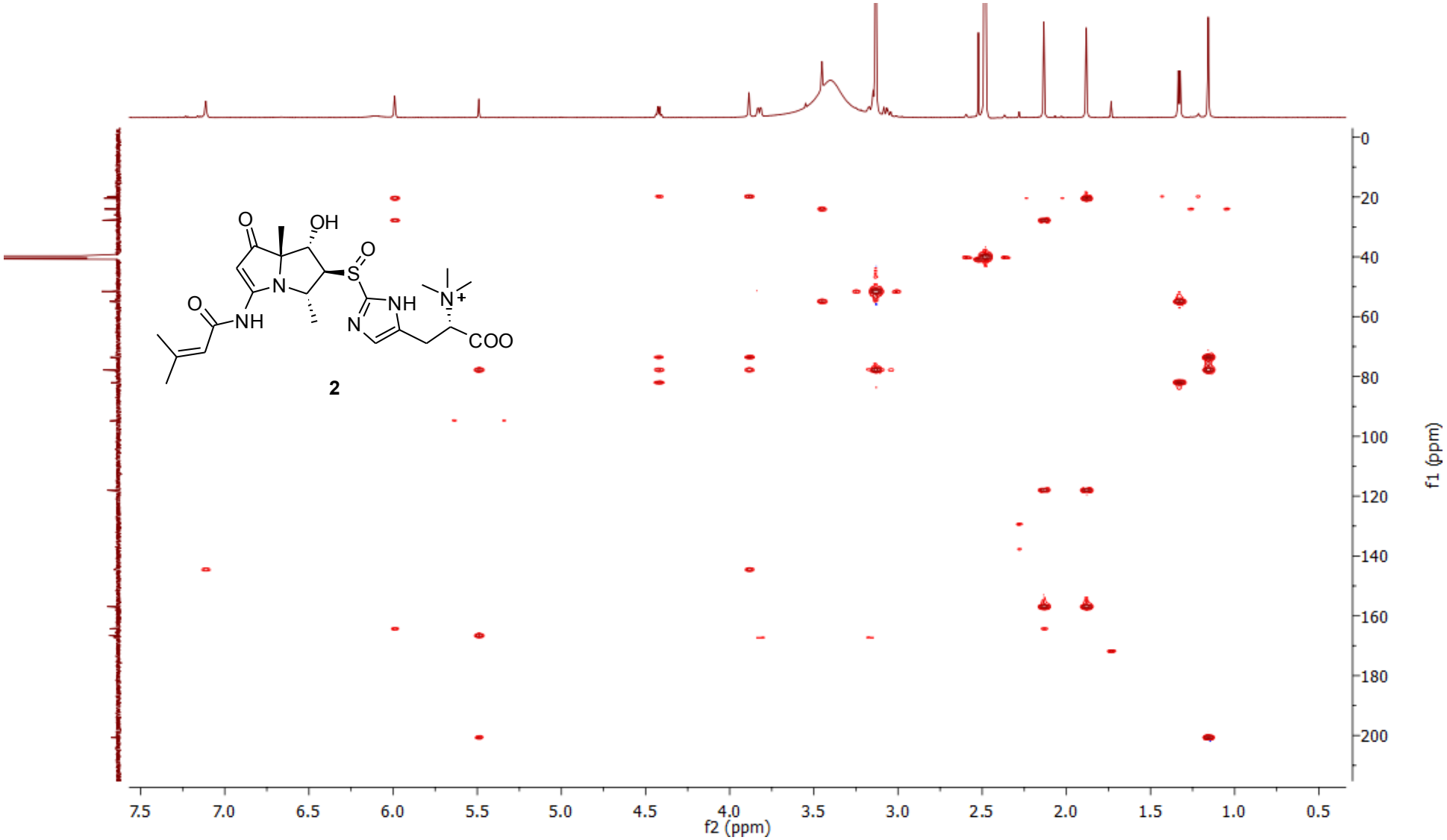

Figure S21. The ^{1}H-NMR spectrum of spithioneine B (**2**) in CD_3OD

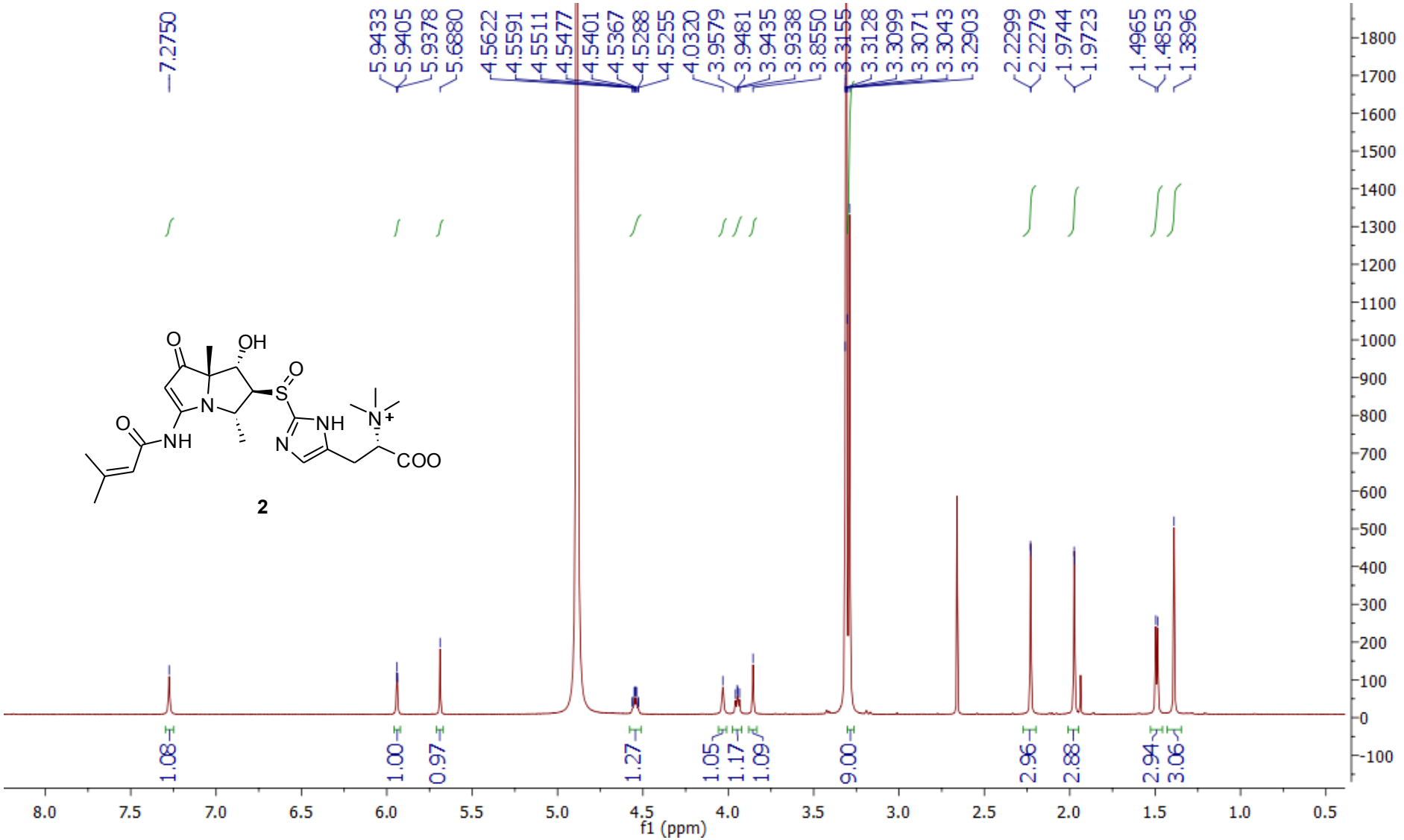

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Figure S22. The ^{13}C-NMR spectrum of spithioneine B (**2**) in CD_3OD

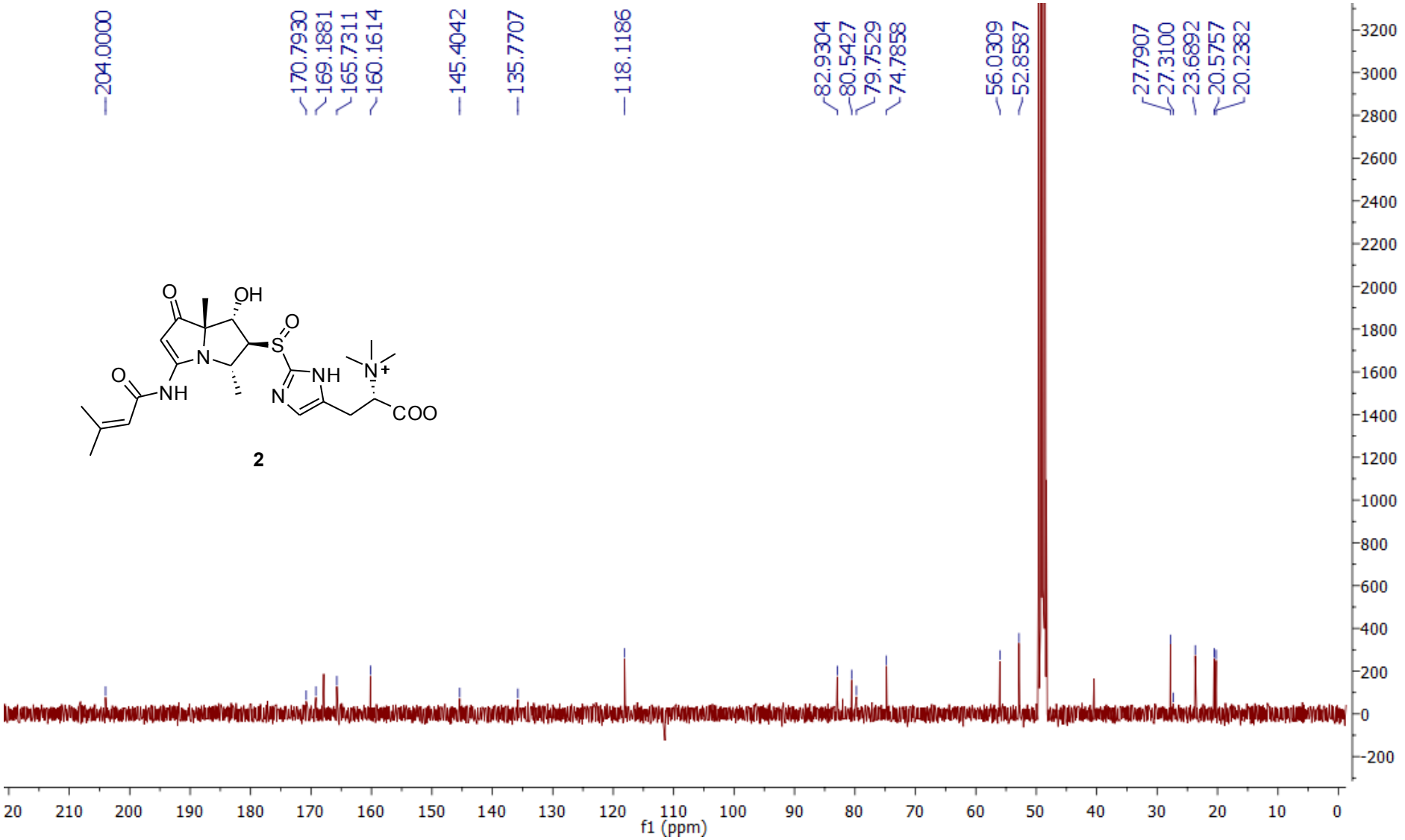

Figure S23. The HSQC spectrum of spithioneine B (**2**) in CD_3OD

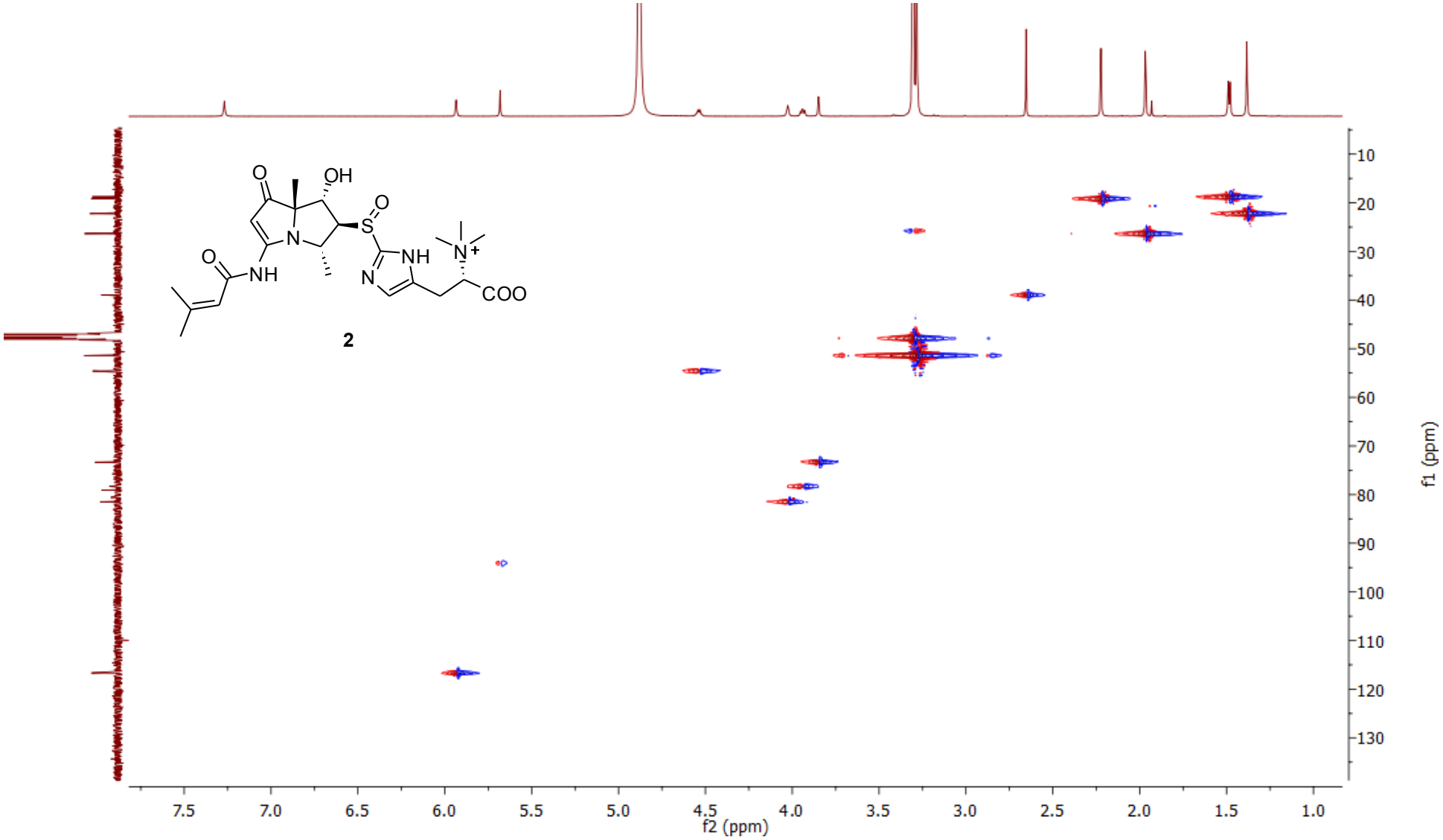

Figure S24. The ^{1}H-^{1}H COSY spectrum of spithioneine B (**2**) in CD_3OD

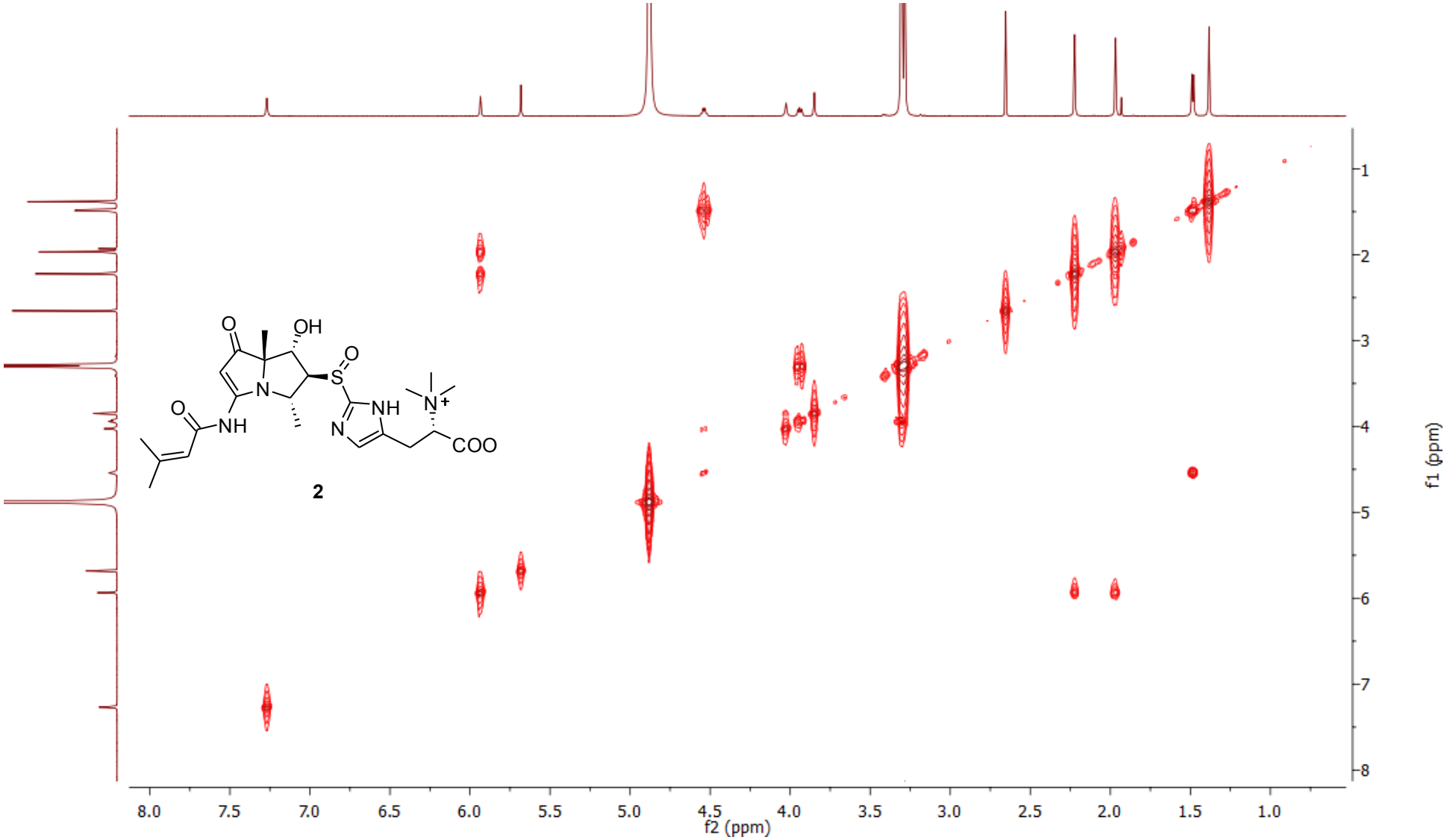

Figure S25. The HMBC spectrum of spithioneine B (**2**) in CD_3OD

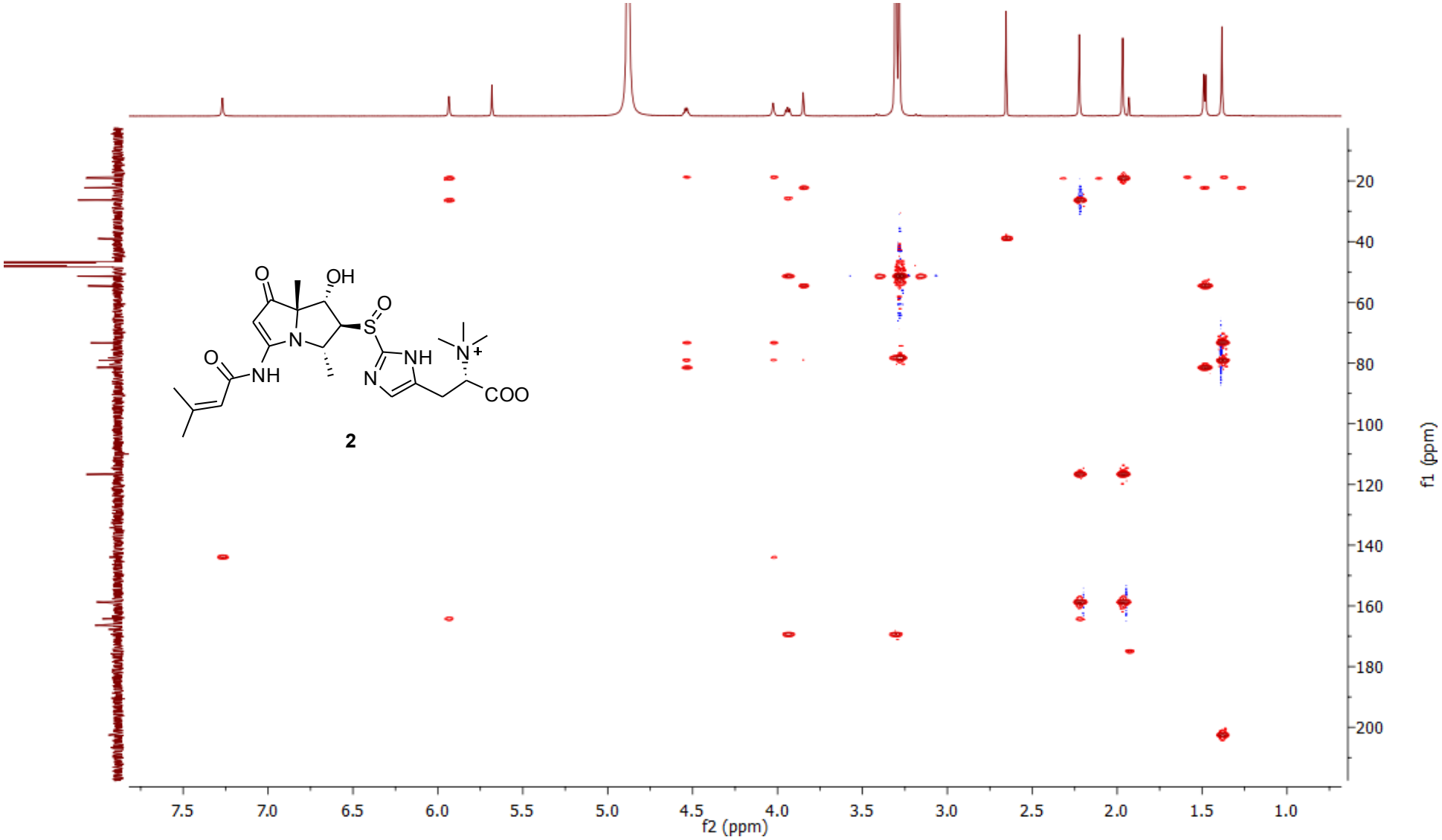

Figure S26. The NOESY spectrum of spithioneine B (**2**) in CD_3OD