Supporting Information

Macrophilone A: Structure Elucidation, Total Synthesis, and Functional Evaluation of a Biologically Active Iminoquinone from the Marine Hydroid *Macrorhynchia philippina*

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Isolation Experimental Procedures

General Remarks

NMR spectra were obtained with a Bruker Avance III NMR spectrometer equipped with a 3 mm cryogenic probe and operated at 600 MHz for ^{1}H and 150 MHz for ^{13}C. (+)HRESIMS data were acquired on an Agilent Technology 6530 Accurate-mass Q-TOF LC/MS. UV spectra were measured with a Varian Cary 50 UV/Vis spectrophotometer. IR spectra were recorded using a Perkin Elmer Spectrum 2000 FT-IR spectrometer. Preparative reversed-phase HPLC was run on a Varian PrepStar preparative HPLC system using a Phenomenex Jupiter C_{18} (5μ, 300Å, 250 × 10 mm) column with the indicated gradient.

Animal Material

Specimens of the hydroid *Macrorhynchia philippina* were collected in Northwestern Australia in August 1988, under contract through the Coral Reef Research Foundation for the Natural Products Branch, National Center Institute. Taxonomic identification of the hydroid was done by Jeanette E. Watson, Museum of Victoria, Melbourne, Australia and a voucher specimen (voucher ID # Q66C1539; NSC # C004385) was deposited at the Smithsonian Institute, Washington, D.C.

Extraction and Isolation

The hydroid specimen (165 g, dry weight) was extracted according to the procedures detailed by McCloud to give 3.75 g of organic solvent (CH_2Cl_2-MeOH, 1:1) extract.[1] A portion of the organic extract (2.01 g) was fractionated on diol SPE cartridges (2 g) eluted with 9:1 hexane-CH_2Cl_2, 5:1 CH_2Cl_2-EtOAc, 100% EtOAc, 5:1 EtOAc-MeOH, and 100% MeOH in a stepwise

manner. The latter two fractions were combined and chromatographed on Sephadex LH-20, using 1:1 CH_2Cl_2-MeOH as eluent, followed by reversed-phase C_{18} HPLC using a linear gradient elution of MeCN-H_2O (10:90–50:50, containing 0.2% formic acid) over 30 min to afford 7.2 mg of macrophilone A (**1**). *Macrophilone A (1):* brown solid; UV (MeOH) λ_{max} (log ε) 238 (3.63), 324 (3.46), 380 (sh); IR (KBr) ν_{max} 3240 (br), 2935, 2815, 1667, 1614, 1589, 1352 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 (main text); HRESIMS *m/z* 238.0645 $[M+H]^+$ (calculated for $C_{10}H_{12}N_3O_2S$, 238.0650).

Quantum Calculations

Structures for the four geometric isomers were optimized in the gas phase using the M06-2X functional and the 6-31+G(d,p) basis set.[2] NMR shielding constants were calculated using gauge-including atomic orbitals (GIAO) at the mPW1PW91/6-311+G(2d,p) level, and were calculated both in gas phase and in methanol solvent with a polarizable continuum model using the integral equation formalism variant (IEF-PCM).[3-4] Calculated shielding constants were converted to chemical shifts using the multi-standard method with benzene as the reference standard for sp^2 carbons and methanol for sp^3 carbons.[5] Values for the experimental ^{13}C chemical shifts of benzene and methanol in CD_3OD have been previously described.[6] All calculations used Gaussian 09 version D.01 (Gaussian, Inc., Wallingford, CT).

Synthetic Experimental Procedures

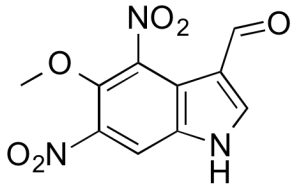
General Remarks

Flash column chromatography was performed using a Teledyne ISCO CombiFlash Rf automated chromatography system. HPLC purifications were performed on a Waters HPLC with a 2545 model pump and a Phenomenex Luna 10 micron C18 column (75 x 30 mm), using an acetonitrile/water gradient with 0.1% trifluoroacetic acid. Thin layer chromatography (TLC) was carried out on silica gel plates with UV detection. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra where obtained in DMSO-d_6 at 400 MHz and 101 MHz, respectively, unless otherwise noted. The following abbreviations were utilized to describe peak patterns: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, app = apparent, and m = multiplet.

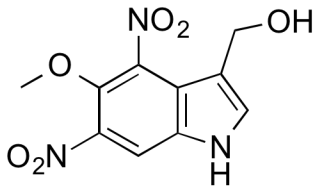
High resolution mass spectrometry data were acquired on an Agilent 6520 Accurate-Mass Q-TOF LC/MS System (Agilent Technologies, Inc.), equipped with a dual electro-spray source and operated in the positive-ion mode. Separation was performed on a Zorbax 300SB-C18 Poroshell column (2.1 mm x 150 mm; particle size 5 μm). The analytes were eluted using a water/acetonitrile gradient with 0.1% formic acid. Data were acquired at high resolution (1,700 *m/z*), 4 GHz. To maintain mass accuracy during the run time, an internal mass calibration sample was infused continuously throughout the LC/MS runs. Data acquisition and analysis were performed using MassHunter Workstation Data Software, LCMS Data Acquisition (version B.06.01), and Qualitative Analysis (version B.07.00).

Unless otherwise noted, all chemicals were obtained from commercial suppliers and used without further purification. THF was obtained from GlassContour Solvent Systems and was dried

over alumina under an argon atmosphere. Compounds **2** and **5** were purchased from Combi-Blocks. All other chemical reagents were purchased from Sigma Aldrich.

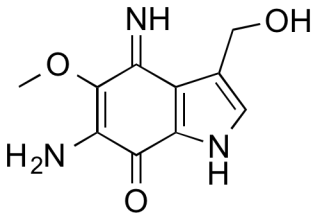


5-methoxy-4,6-dinitro-1H-indole-3-carbaldehyde (S1). To a solution of 3-formyl-5-methoxyindole (**2**, 175 mg, 1.00 mmol) in H_2SO_4 (12 mL) at 0°C was added $NaNO_3$ (187 mg, 2.20 mmol) in one portion with stirring. The solution was stirred for 30 min, after which it was poured into H_2O on ice (50 mL). The aqueous solution was extracted with EtOAc (3 x 30 mL) and the combined extracts were washed with sat. aq. $NaHCO_3$ (2 x 20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated to afford the desired product (226 mg, 0.852 mmol, 85%) which was used without further purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.16 (s, 1H), 9.86 (s, 1H), 8.82 (d, J = 2.9 Hz, 1H), 8.51 (s, 1H), 3.91 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 184.0, 145.4, 140.8, 138.6, 138.5, 133.0, 118.3, 116.5, 113.4, 65.0. HRMS for $C_{10}H_8N_3O_6^+$ [M+H] calc. 266.04076, found 266.04097 (error = -0.58).



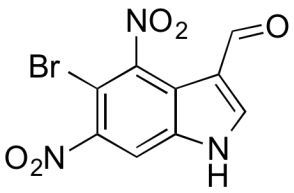
(5-methoxy-4,6-dinitro-1H-indol-3-yl)methanol (3). To a suspension of **S1** (776 mg, 2.93 mmol) in THF (21 mL) at 0°C was added DIBAL-H (1 M solution in THF, 8.78 mL, 8.78 mmol). The solution was stirred for 1 h, then diluted in diethyl ether (200 mL). The solution was quenched with sequential addition of H_2O (351 µL), 15% aq. NaOH (351 µL), and H_2O (878 µL), after which the solution was warmed to room temperature and stirred an

additional 1 h. The solution was dried (Na_2SO_4), filtered through celite, the filter cake rinsed with EtOAc, and the filtrate concentrated to obtain the desired product (481 mg, 1.80 mmol, 61%) as an orange solid which was used without further purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.18 (s, 0H), 8.42 (s, 1H), 7.90 (d, J = 2.6 Hz, 1H), 4.89 (t, J = 4.9 Hz, 1H), 4.46 (d, J = 4.4 Hz, 2H), 3.91 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 139.4, 137.3, 136.5, 133.9, 132.3, 120.9, 115.4, 112.9, 64.8, 55.0. HRMS for $C_{10}H_8N_3O_6^-$ [M-H] calc. 266.04186, found 266.04129 (error = 2.33).

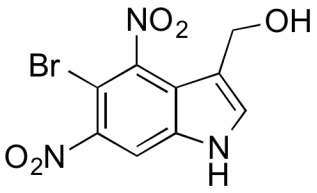


6-amino-3-(hydroxymethyl)-4-imino-5-methoxy-1,4-dihydro-7H-indol-7-one (4). A roundbottom flask was sequentially charged with **3** (450 mg, 1.68 mmol), methanol (17 mL, 0.1 M), charcoal (286 mg), iron (III) chloride hexahydrate (46 mg, 0.168 mmol, 0.1 equiv.), and hydrazine monohydrate (408 μL, 8.42 mmol, 5 equiv.). The mixture was refluxed with stirring for 18 h, after which additional hydrazine hydrate was added (816 μL, 16.8 mmol, 10 equiv.) and the reaction was refluxed for an additional 4h. The reaction mixture was concentrated under a stream of N_2, resuspended in EtOAc, and filtered through celite, rinsing with EtOAc (2 x 50 mL). The combined filtrates were concentrated, yielding 123 mg of a brown solid. A portion of this residue (25 mg, 0.120 mmol) was dissolved in acetone (1 mL). To this was added a solution of potassium nitrosodisulfonate (64 mg, 0.239 mmol, 2 equiv.) in phosphate buffer (1 mL, 200 mM, pH = 6). The reaction mixture was stirred for 10 min, after which the mixture was diluted with methanol (2 mL) then filtered through celite, the filtrate purified directly by HPLC (10% to 20% CH_3CN/H_2O gradient, 0.1% TFA additive), and the fractions lyophilized to afford compound **4** (8.51 mg, 0.0254 mmol, 7% over 2 steps) as a green solid. ^{1}H NMR (500 MHz, Methanol-d_4) δ

7.18 (s, 1H), 4.71 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 171.3, 158.8, 145.3, 129.7, 128.1, 127.5, 127.3, 119.7, 60.3, 57.2. HRMS for $C_{10}H_{12}N_3O_3^+$ [M+H] calc. 222.08732, found 222.08774 (error = -1.92).

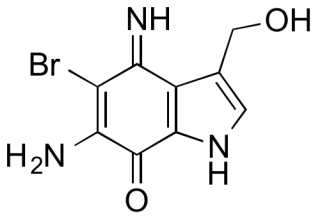


5-bromo-4,6-dinitro-1*H*-indole-3-carbaldehyde (S2). To a solution of 5-bromo-1H-indole-3-carbaldehyde (**5**, 1.12 g, 5.00 mmol) in H_2SO_4 (10 mL) at 0°C was added dropwise a solution of $NaNO_3$ (1.27g, 15.00 mmol) in H_2SO_4. The solution was stirred at 0°C for an additional 2h, after which the reaction mixture was poured into ice water (250 mL) and the precipitate collected by filtration. The filtrate was dried under vacuum to afford the desired product (1.38 g, 4.41 mmol, 88%) as a brown solid. The crude product was used in the next reaction without any additional purification. A small sample of this product was purified by reverse-phase HPLC for analytical characterization. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.41 (s, 1H), 9.87 (s, 1H), 8.85 (s, 1H), 8.53 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 184.0, 145.5, 145.0, 143.9, 136.7, 117.6, 115.7, 112.9, 99.0. HRMS for $C_9H_5BrN_3O_5^+$ [M+H] calc. 313.94071, found 313.94156 (error = -1.53).



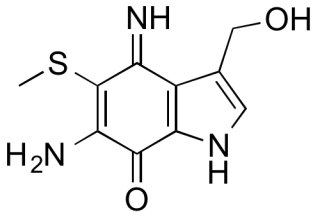
(5-bromo-4,6-dinitro-1*H*-indol-3-yl)methanol (6). To a roundbottom flask charged with **S2** (1.266 g, 4.031 mmol) was added THF (28 mL). The resulting suspension was cooled to 0°C, after which a solution of diisobutylaluminum hydride (1M in THF, 12.09 mL,

12.09 mmol, 3 equiv.) was added dropwise as the solution turned deep red. The solution was stirred for 1.5 h, after which it was poured slowly into EtOAc at 0°C. The suspension was stirred with a saturated solution of sodium potassium tartarate (250 mL) overnight. The bilayer was transferred to a separatory funnel, the aqueous layer removed, and the organic layer washed again with a saturated solution of sodium potassium tartrate (150 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to afford the desired product (945 mg, 2.99 mmol, 74%) as a dark brown solid. The crude product was used in the next reaction without any additional purification. A small sample of this product was purified by reverse-phase HPLC for analytical characterization. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.42 (s, 1H), 8.42 (s, 1H), 7.90 (d, J = 2.6 Hz, 1H), 4.96 (t, J = 5.1 Hz, 1H), 4.45 (d, J = 3.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 143.1, 142.9, 135.6, 133.2, 120.3, 114.8, 112.4, 95.7, 54.8. HRMS for $C_9H_5BrN_3O_5^-$ [M-H] calc. 313.94181, found 313.94210 (error = -0.92).



6-amino-5-bromo-3-(hydroxymethyl)-4-imino-1,4-dihydro-7*H*-indol-7-one (7). To a roundbottom flask under argon was added **6** (63 mg, 0.200 mmol), charcoal (34 mg), iron (III) chloride hexahydrate (5 mg, 0.020 mmol, 0.1 equiv.), methanol (6.3 mL, 0.1 M), and hydrazine hydrate (152 μL, 3.13 mmol, 5 equiv.). The resulting suspension was refluxed for 3 h, with additional hydrazine hydrate additions after each hour. The reaction mixture was then cooled to room temperature and filtered through celite, rinsing the celite plug with additional methanol. The combined filtrates were concentrated to give a brown solid (50 mg, 0.197 mmol, 98%). This solid was redissolved in acetone (2 mL), and to the resulting dark solution was added

a solution of potassium nitrosodisulfonate (111 mg, 0.413 mmol, 2.1 equiv.) in aqueous phosphate buffer (2 mL, 200 mM, pH = 6). The reaction was stirred for 15 min then diluted with methanol (2 mL), filtered through celite, and the filtrate filtered again through a cotton plug. The resulting solution was purified directly by HPLC (10% to 20% CH_3CN/H_2O gradient, 0.1% TFA additive), and the fractions lyophilized to afford the desired product (5.81 mg, 0.0151 mmol, 7.5% over 2 steps) as a green solid. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.27 (s, 1H), 4.74 (s, 2H). ^{13}C NMR (126 MHz, MeOD) δ 167.9, 161.1, 153.2, 129.6, 128.2, 127.4, 121.9, 86.1, 57.1. HRMS for $C_9H_9BrN_3O_2^+$ [M+H] calc. 269.98727, found 269.98746 (error = -0.35).



6-amino-3-(hydroxymethyl)-4-imino-5-(methylthio)-1,4-dihydro-7*H*-indol-7-one (1). To a vial charged with bromoiminoquinone **7** (2.59 mg, 6.74 μmol) was added a freshly prepared 1 M solution of sodium methanethiolate in methanol (400 μL). The solution was stirred for 30 min, after which the reaction was quenched with formic acid (40 μL), concentrated, reconstituted in methanol (2 mL), and purified by reverse-phase HPLC (10% to 20% CH_3CN/H_2O gradient). Fractions containing product were combined and concentrated to yield the desired compound **1** (1.06 mg, 3.02 μmol, 45% yield). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.30 (s, 1H), 4.75 (s, 2H), 2.22 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 168.1, 162.4, 155.5, 129.1, 127.0, 125.9, 121.1, 93.8, 55.7, 15.7. HRMS for $C_{10}H_{12}N_3O_2S^+$ [M+H] calc. 238.06447, found 238.06477 (error = -1.38).

Biochemical and Biological Experimental Procedures

General Remarks

N-acetyl-L-cysteine and β-lapachone were purchased from VWR. All other compounds were purchased from Sigma-Aldrich. The following recombinant proteins were purchased and used without further purification in biochemical assays: SUMO E1 (E-315, Boston Biochem), Ubc9 (BML-UW9320, Enzo Life Sciences), and SUMO-1 (His-tag, UL-715, Boston Biochem).

Sumoylation Electrophoretic Mobility Shift Assay

The sumoylation assay was performed as previously described.[7-8] Briefly, the assay was conducted in 384-well plate format with 20 μL Tris buffer (50 mM Tris pH 9, 5 mM $MgCl_2$, and 1 mM DTT), SUMO E1 (0.1 μM), SUMO-1 (1.4 μM), Ubc9 (0.15 μM), fluorescent peptide FL-AR (1 μM), and small molecule (4% DMSO final). The reactions were initiated by the addition of ATP (2 mM final concentration). After 90 minutes, EDTA (0.25 M, 8 μL) was added to each well to quench the reactions. Samples were analyzed using a LabChip EZ Reader II (Caliper Life Sciences) with the following run conditions: downstream voltage of -500 V, upstream voltage of -2500 V, and pressure of -1.0 psi. The sumoylation reactions were analyzed by quantifying percent conversion, defined as 100 × P/(P+S), where P is peak height of sumoylated product SUMO-1-FL-AR and S is peak height of peptide substrate FL-AR. Data were normalized to positive and negative controls (ginkgolic acid and DMSO, respectively).

Gel-Based Sumoylation Assay

The sumoylation assay was performed in 20 μL reaction buffer (50 mM Tris pH 9, 5 mM $MgCl_2$, 1 mM DTT) with or without 5 mM *N*-acetyl-L-cysteine (NAC). SUMO E1 (0.1 μM), Ubc9

(0.15 μM), SUMO-1 (1.4 μM), and a fluorescent peptide (FL-AR) substrate (1 μM) as well as various concentrations of small molecule in DMSO or DMSO alone (4% final concentration) were added. Ginkgolic acid (30 μM) was used as a positive control. The reactions were then initiated by the addition of ATP (2 mM final concentration) and incubated at room temperature for 90 minutes. Following incubation, samples were quenched by the addition of NuPAGE 4X LDS sample buffer (ThermoFisher), heated at 95 °C for 5 minutes, loaded onto NuPAGE Novex 4-12% Bis-Tris Protein Gels (ThermoFisher), resolved by electrophoresis, and visualized by in-gel fluorescence method using an ImageQuant LAS 4000 (GE Life Sciences) imaging system at an appropriate wavelength (488 nm excitation with blue Epi-RGB light, Y515 Di emission filter, and F0.85 iris).

E1-E2 Crosslinking Assay

SUMO E1 (0.1 μM) and Ubc9 (1 μM) were incubated with small molecules or DMSO (4% final) in 20 μL reaction buffer (50 mM Tris pH 9, 5 mM $MgCl_2$, 1 mM DTT) at 37 °C for 30 minutes. Following incubation, reactions were quenched by the addition of 4X LDS sample buffer, heated at 95 °C for 5 minutes, resolved by electrophoresis using 4-12% Bis-Tris gels, and visualized by silver stain using SilverQuest™ Staining Kit (ThermoFisher).

Cell Culture

A549 cells were purchased from ATCC and were maintained at 37 °C with an atmosphere of 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin. For confluence measurements, A549 cells were passaged into 96-well plates at 1,000 cells/well and allowed to attach overnight. The

following day media was removed and fresh media (100 μL) containing compound or DMSO (0.1% final) added to each well. Cells were incubated at 37 °C and monitored every hour for at least 24 hours by IncuCyte® ZOOM Live-Cell Analysis System (Essen BioScience).

Reactive Oxygen Species Detection

ROS generation was determined using the dye CM-H2DCFDA (ThermoFisher). A549 cells were passaged into 96-well black wall, clear bottom plates at 5,000 cells/well and allowed to attach overnight. The following day media was removed and cells were incubated with small molecules or DMSO (0.2% final) and the dye (5 μM) in PBS (100 μL) with or without NAC (5 mM) at 37 °C for 30 minutes. Fluorescence intensity was measured directly at the desired timepoint using a BioTek Synergy 2 Microplate Reader with excitation and emission wavelengths of 485 nm and 528 nm, respectively. Data was normalized to wells with identical exposure conditions but lacking cells and expressed relative to DMSO controls.

Sulfenic Acid Detection

A549 cells were passaged into 6-well plates at 100,000 cells/well and allowed to grow for 24 hours. Day-old media was removed and cells were incubated with compounds or DMSO (0.1% final) in fresh media for 2 hours. Following incubation, cells were washed with PBS (3 x 1 mL) then lysed by the addition of cold RIPA buffer (200 μL) supplemented with 6 M urea, 10 mM iodoacetamide, 10 mM *N*-ethylmaleimide, 200 U/mL catalase, 1X Halt™ protease and phosphatase inhibitor cocktail with EDTA (ThermoFisher), and 1 mM DCP-Bio1 (Kerafast). Wells were scraped and lysates were transferred to microcentrifuge tubes where they were incubated on ice for 1 hour. Protein concentration was determined by Qubit™ Assay

(ThermoFisher). Equal amounts of protein were loaded onto 4-12% Bis-Tris protein gels, resolved by electrophoresis, transferred to nitrocellulose membranes for Western blot analysis with anti-biotin-HRP antibody (sc-53179, Santa Cruz Biotechnology), and visualized by chemiluminescence with LumiGLO® Reagent (Cell Signaling Technology).

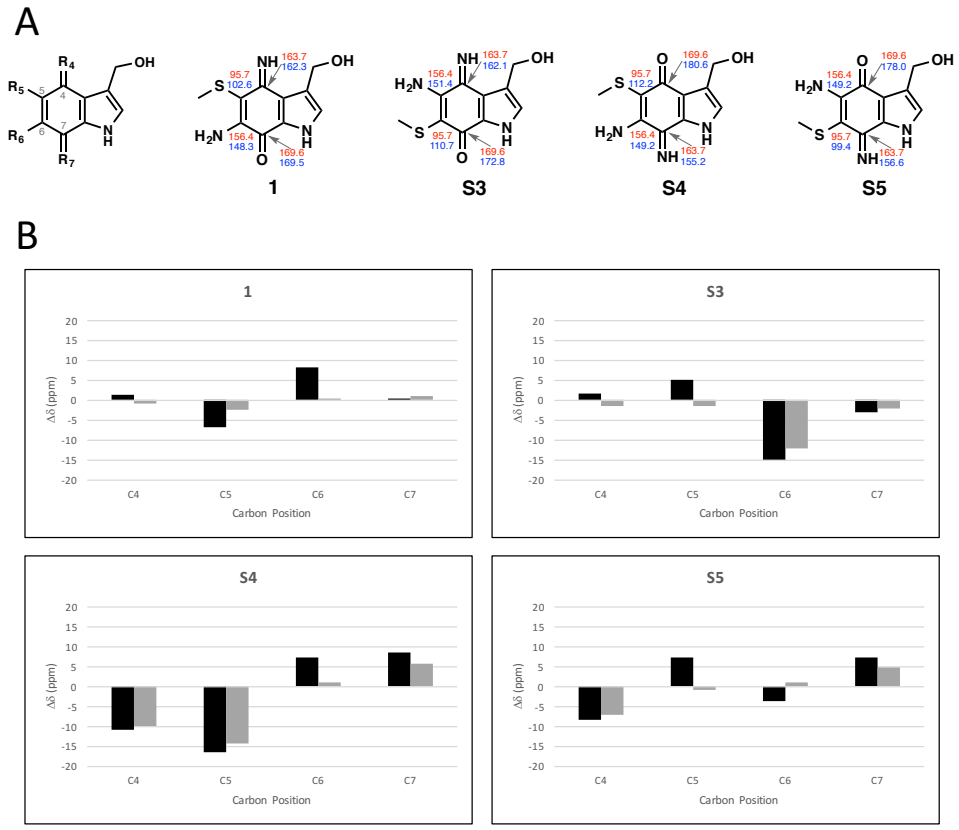


Figure S1. (A) Comparison of experimentally observed (red) and DFT calculated (blue) ^{13}C NMR chemical shifts in CD_3OD for the possible isomers of the natural product macrophilone A. (B) Differences between the CD_3OD calculated/experimental ^{13}C NMR chemical shifts (ppm) for C-4, C-5, C-6, and C-7 of the four iminoquinone rings are shown in graphical representation (black). Differences between the respective chemical shifts for experimentally observed DMSO-d_6 and DFT calculated gas phase are also graphed (grey, see Table 1 in main text for these chemical shift values). The GIAO calculations of the predicted chemical shifts including implicit solvent appear to have lower accuracy than in vacuum, likely due to the limitations of PCM solvent modeling in which the solvent is described as a homogeneous reaction field with a given dielectric constant. Improvements in calculated shielding constants with solvent may only apply to aprotic or nonpolar solvents such as chloroform, and not to methanol or other solvents which can hydrogen bond.

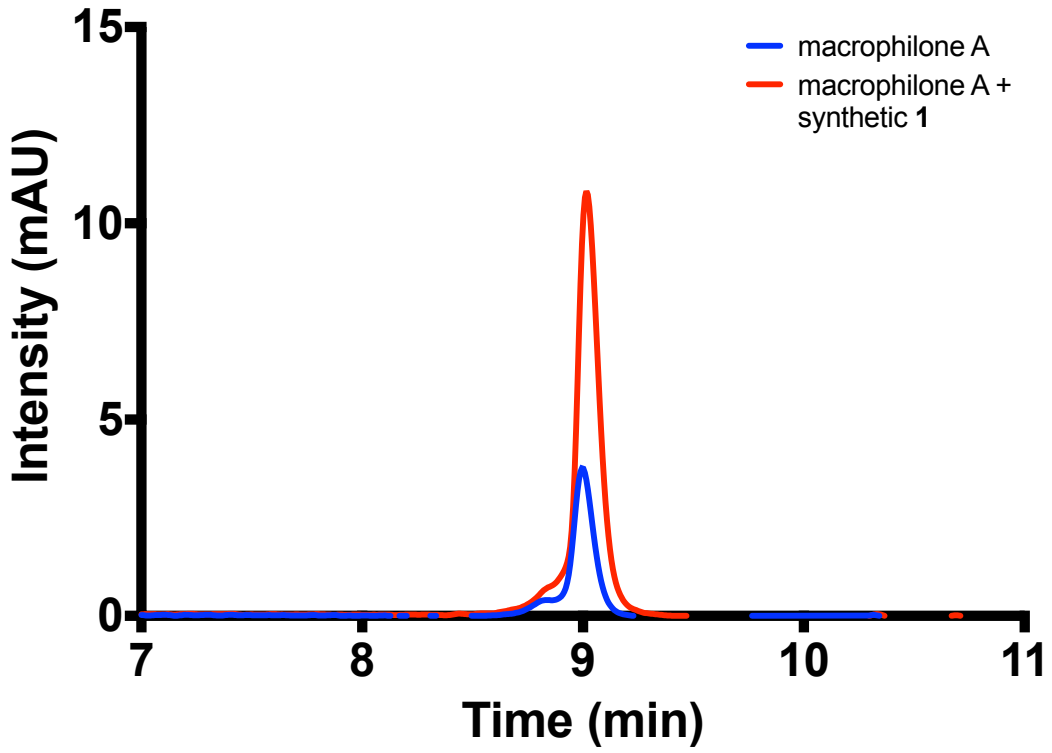


Figure S2. HPLC chromatogram of the co-injection of isolated and synthetic macrophilone A (red), eluting as a single peak at the same timepoint as the isolated natural product (blue).

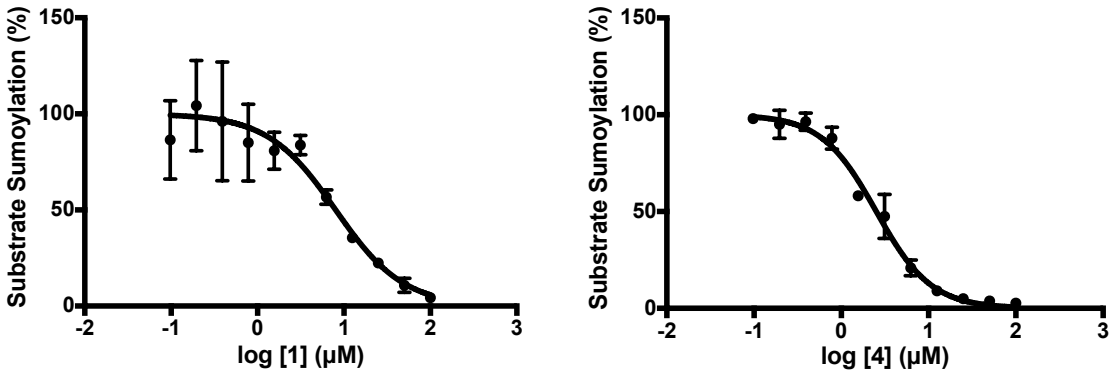


Figure S3. Inhibitory activity of macrophilone A (**1**) (left) and analog **4** (right) in a biochemical sumoylation assay.

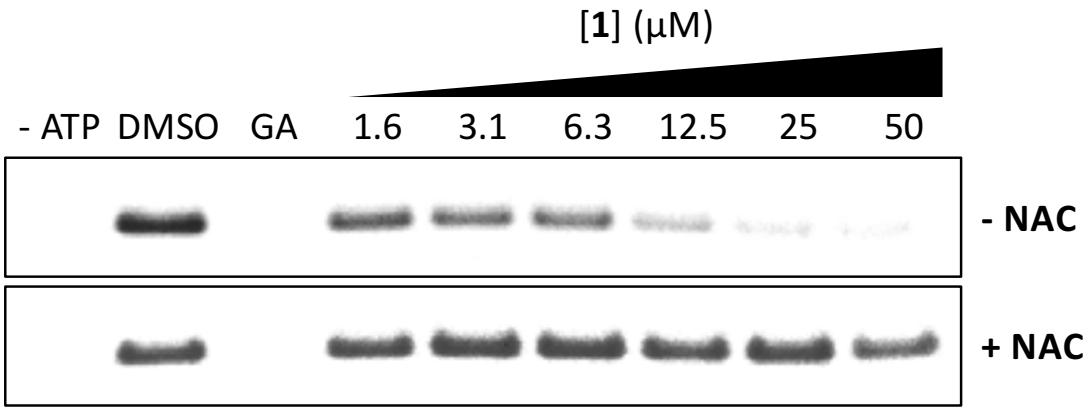


Figure S4. Inhibition of SUMO conjugation to a fluorescent peptide substrate by macrophilone A (**1**) in a reconstituted biochemical assay. Inhibitory activity of macrophilone A is abolished by the addition of the antioxidant NAC. GA = ginkgolic acid, 30 μM.

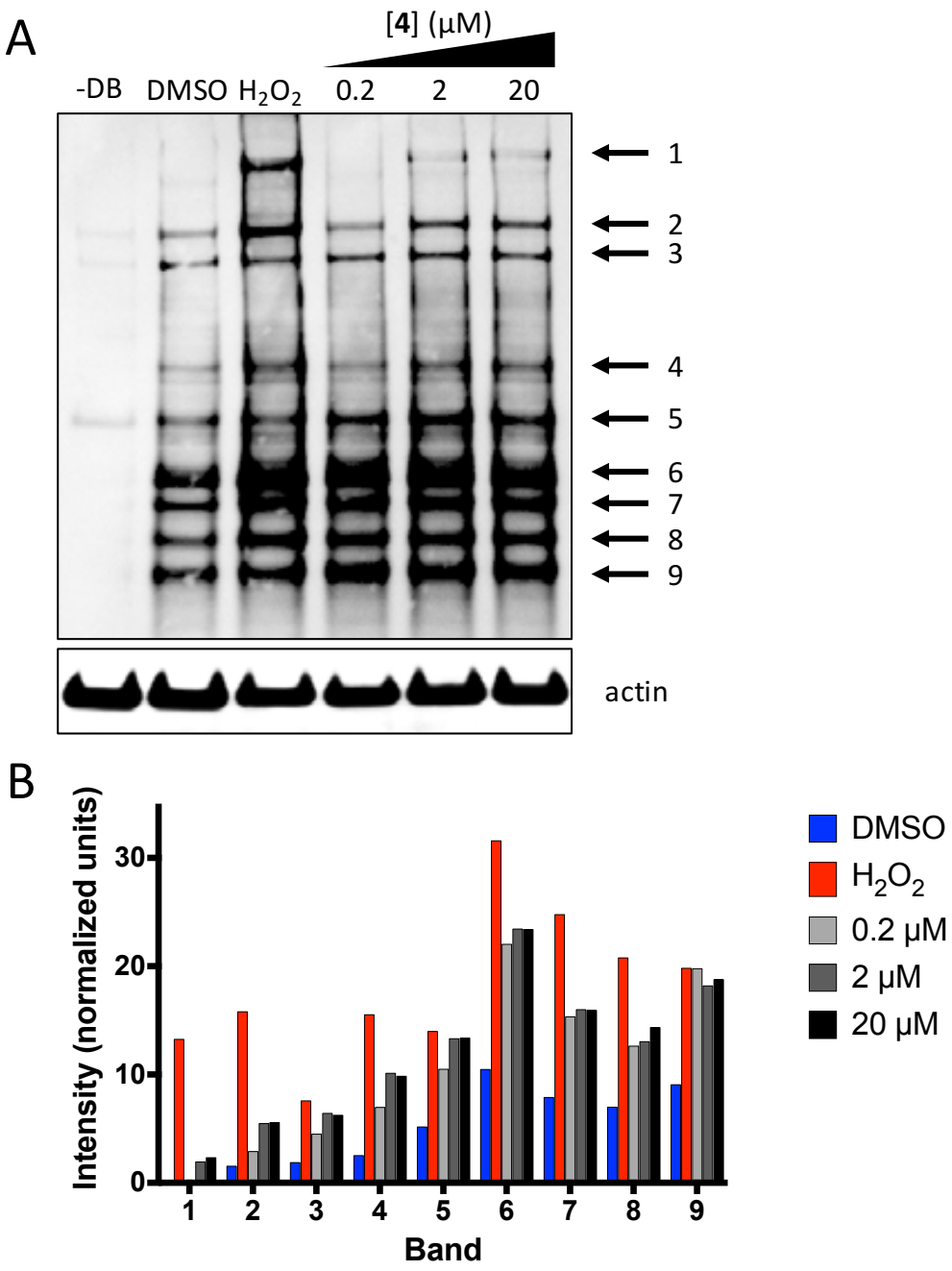


Figure S5. (A) Detection of sulfenic acids in A549 cells by Western blot utilizing the probe DCP-Bio1 (DB) and an anti-biotin antibody, indicating proteome-wide oxidation by **4**. Note the appearance of new bands or increase in band intensities with addition of **4**. H_2O_2 = 5 mM. (B) Quantification of Western blot shown in (A), normalized to actin.

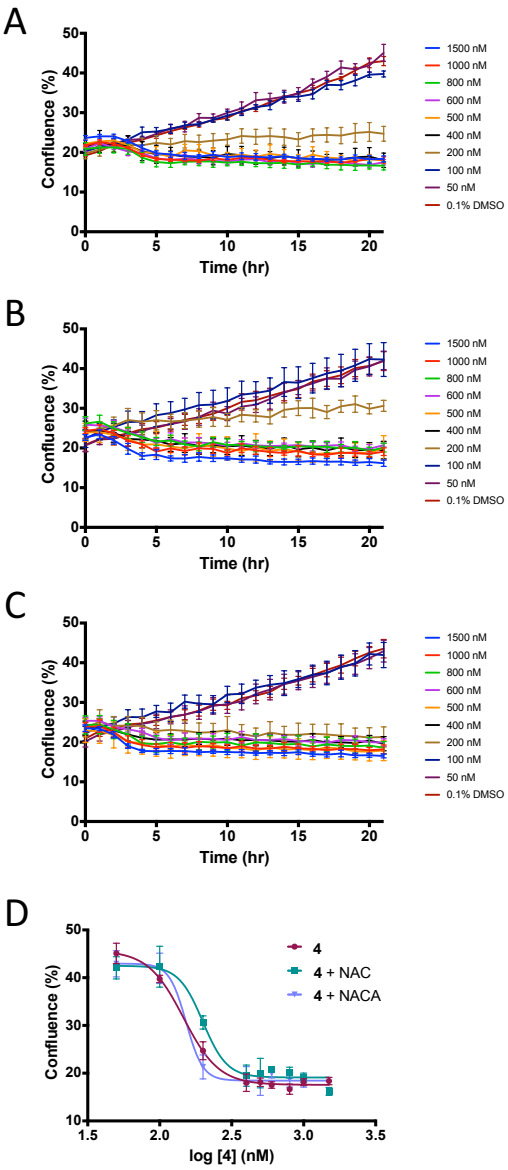


Figure S6. Toxicity of analog **4** in A549 cells after 21 hour exposure (A) alone as well as in combination with the antioxidants (B) NAC or (C) NACA. Cells were pretreated with 2 mM NAC or NACA for 2 hours followed by co-incubation with **4** and 1 mM of the respective antioxidant for the remainder of the experiment. Growth was measured by percent confluence over time after treatment. (D) EC_{50} curves derived from this data.

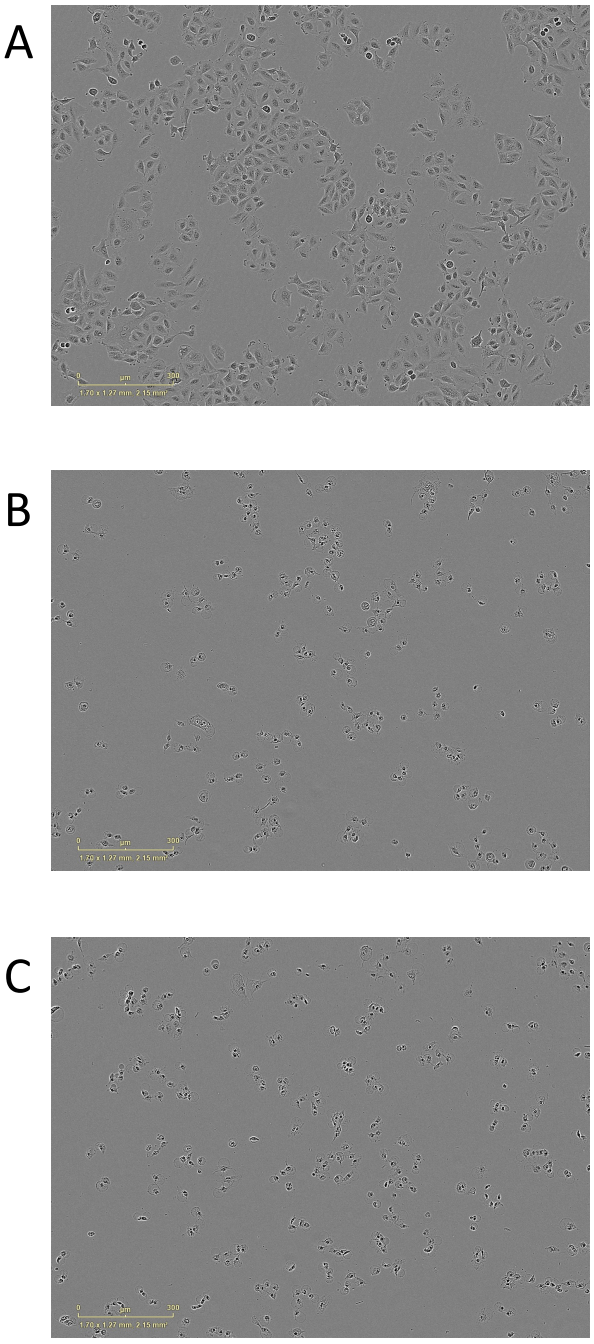


Figure S7. Images of A549 cells after 24 hour treatment with (A) 0.1% DMSO, (B) 500 nM **4**, or (C) 500 nM **4** with 1 mM NAC.

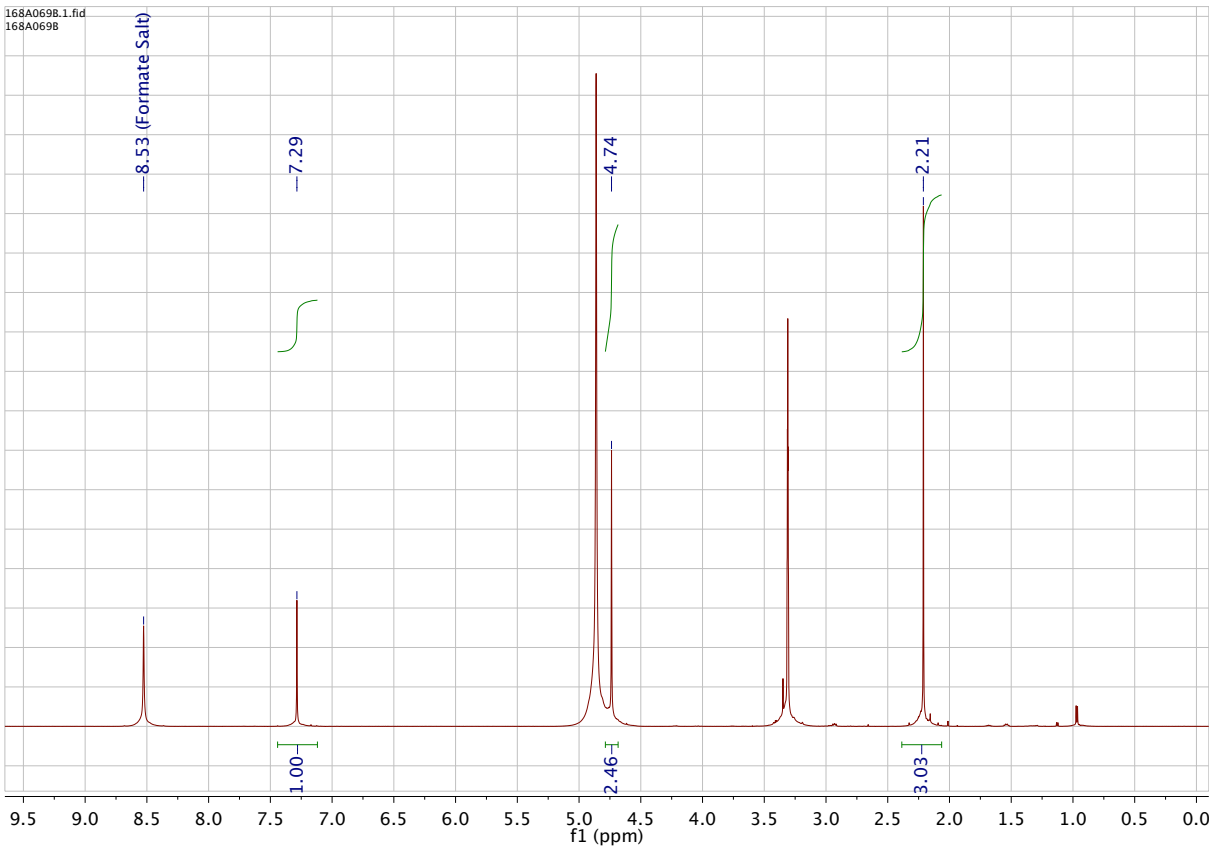


Figure S8. ^{1}H NMR spectrum (600 MHz) of isolated macrophilone A in CD_3OD.

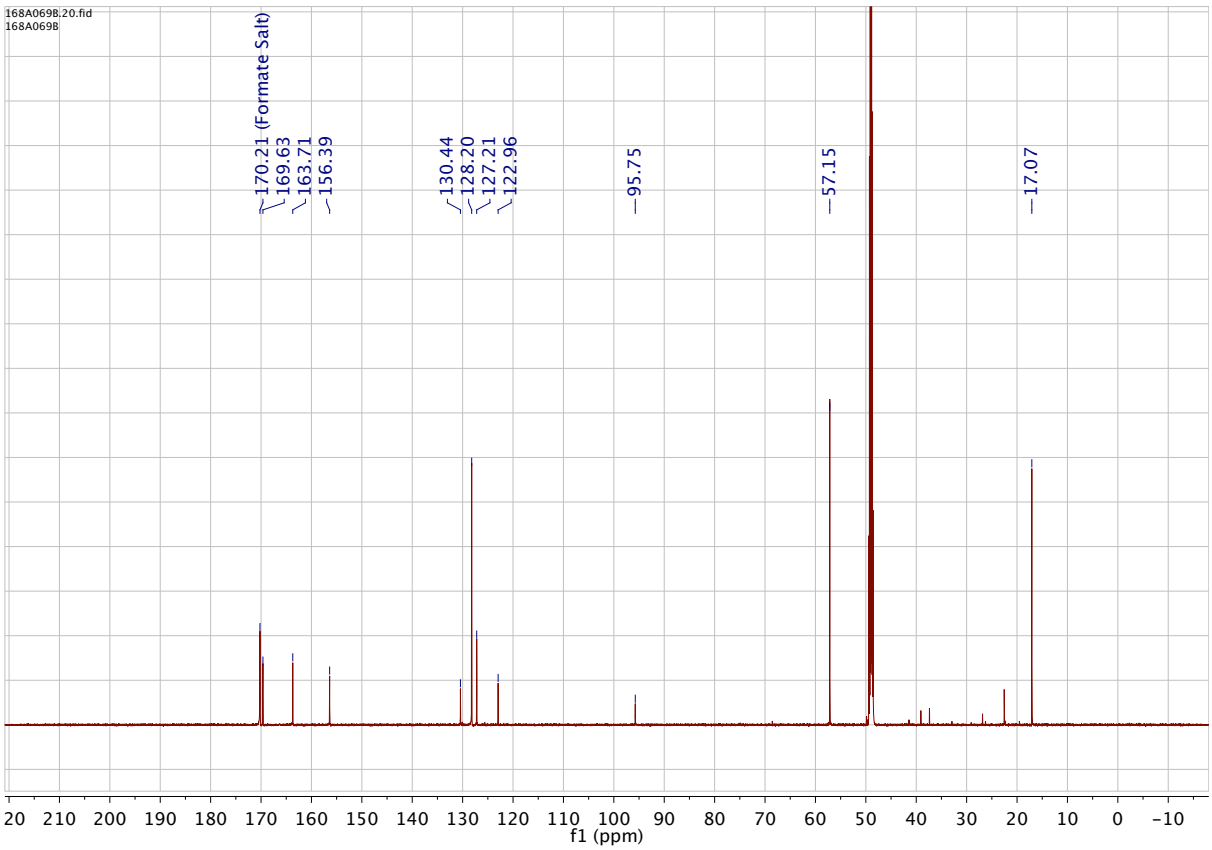


Figure S9. ^{13}C NMR spectrum (150 MHz) of isolated macrophilone A in CD_3OD.

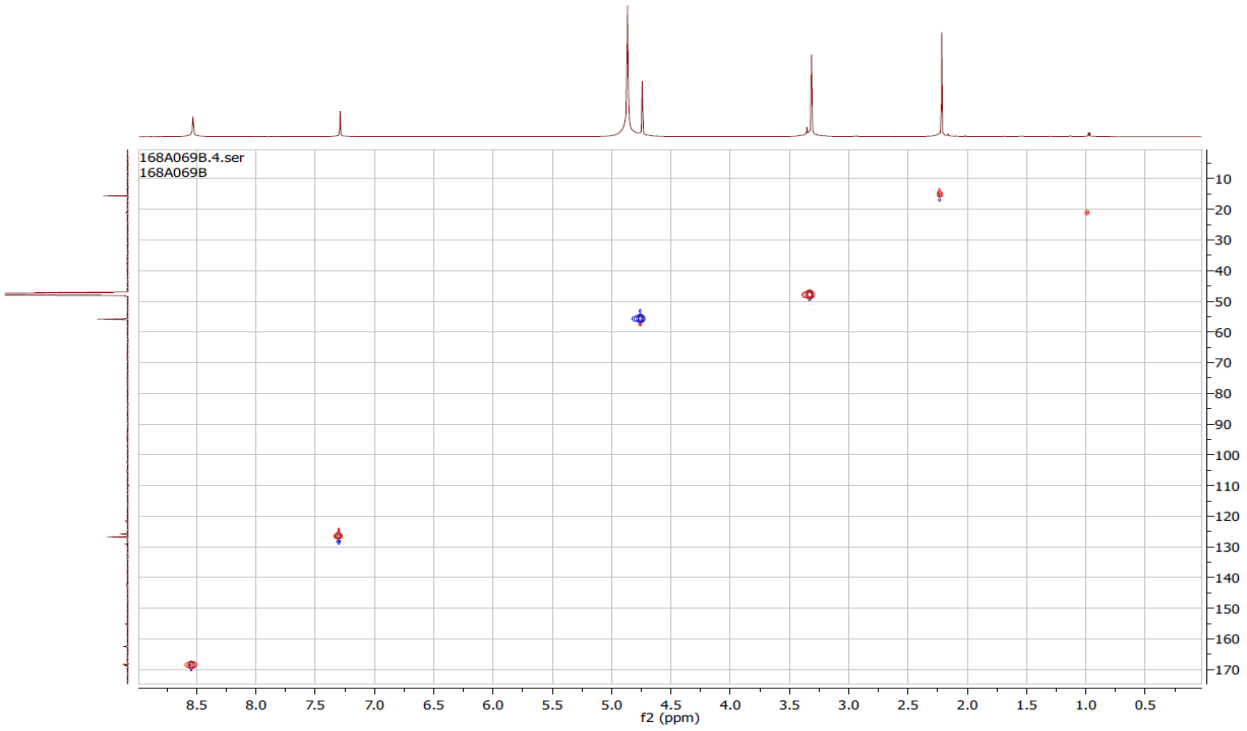


Figure S10. HSQC spectrum of isolated macrophilone A in CD_3OD.

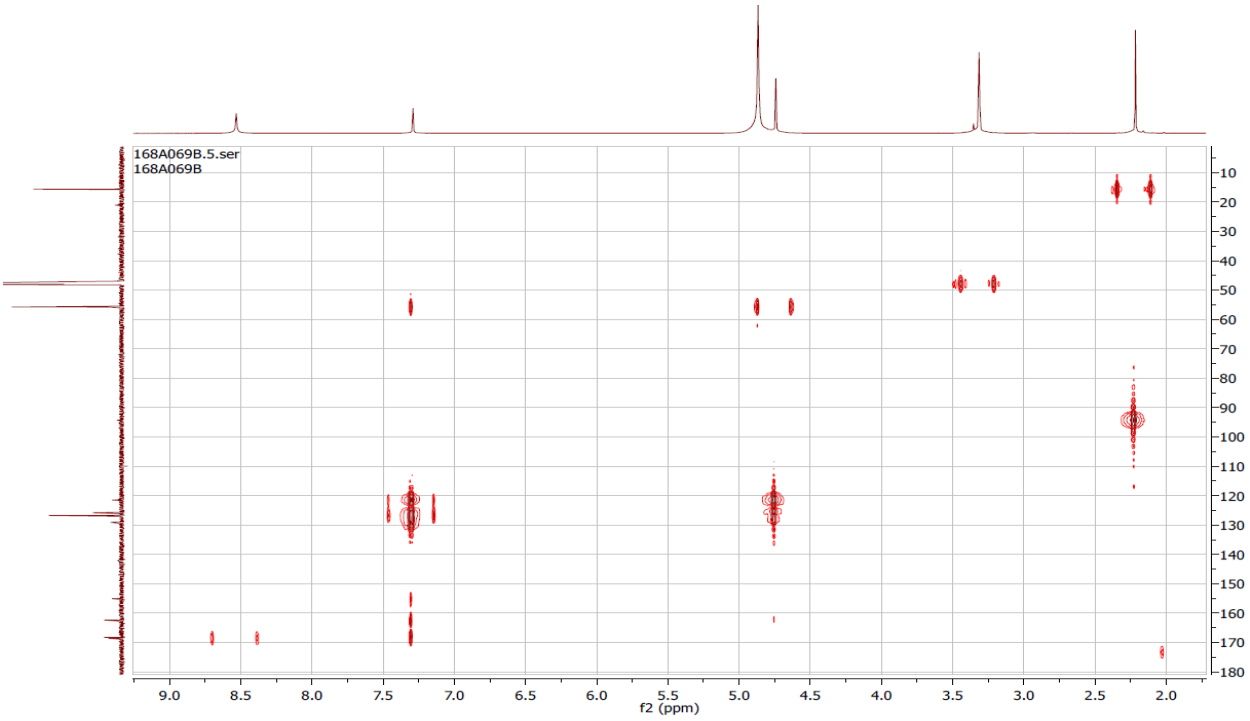


Figure S11. HMBC spectrum of isolated macrophilone A in CD_3OD.

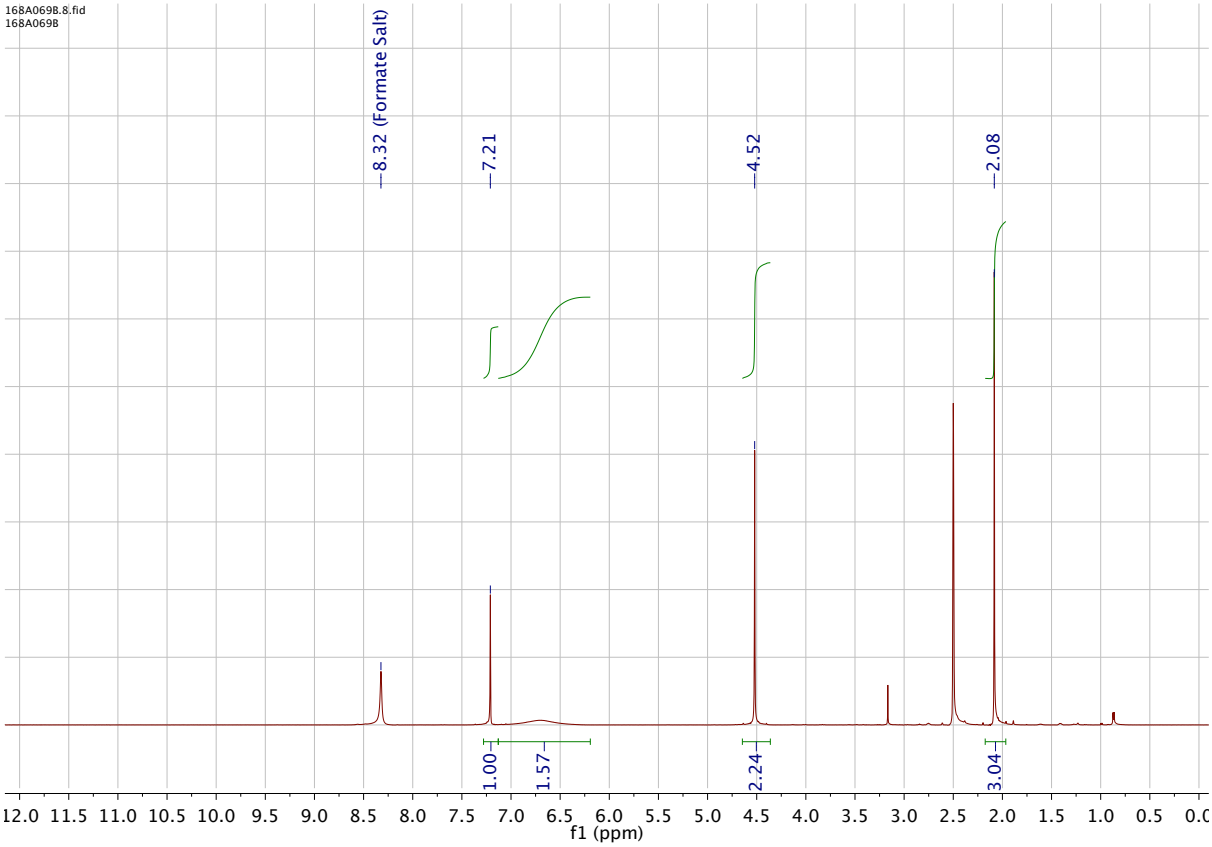


Figure S12. ^{1}H NMR spectrum (600 MHz) of isolated macrophilone A in DMSO-d_6.

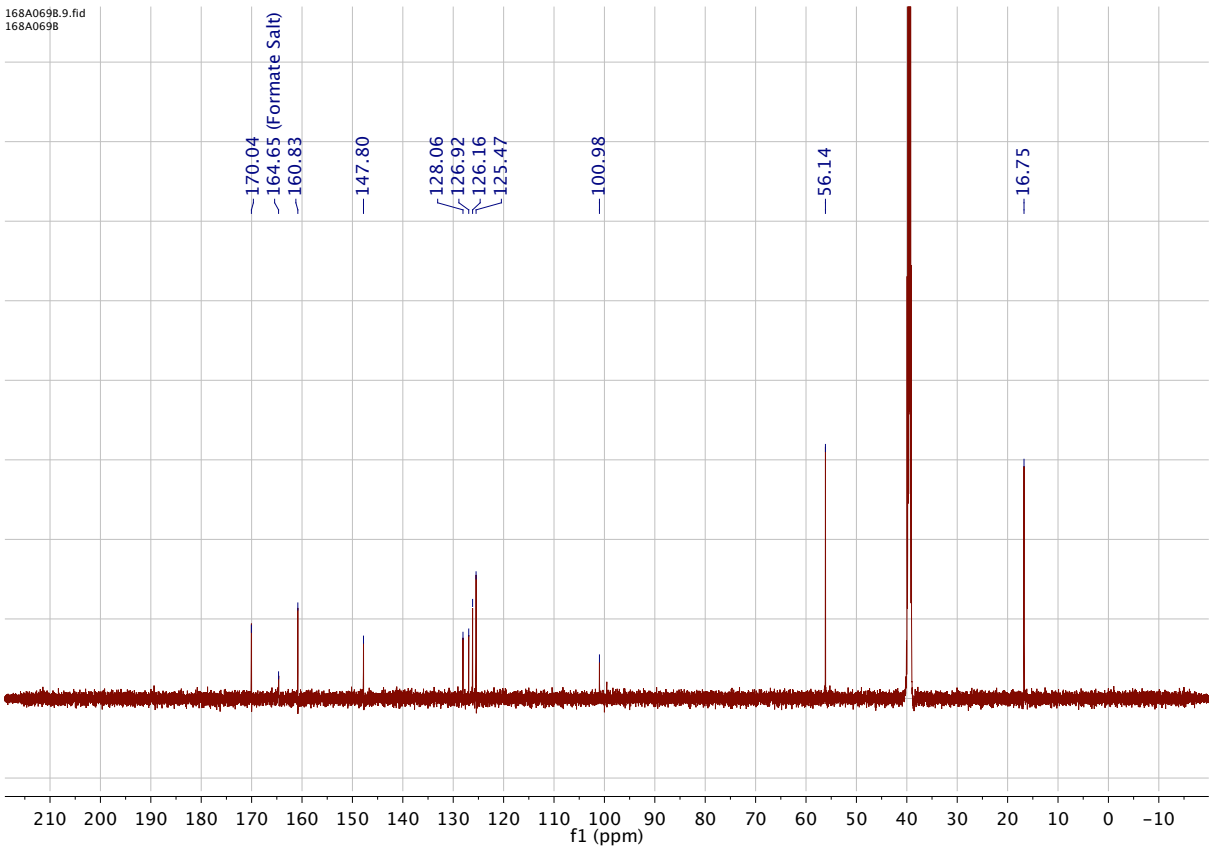


Figure S13. ^{13}C NMR spectrum (150 MHz) of isolated macrophilone A in DMSO-d_6.

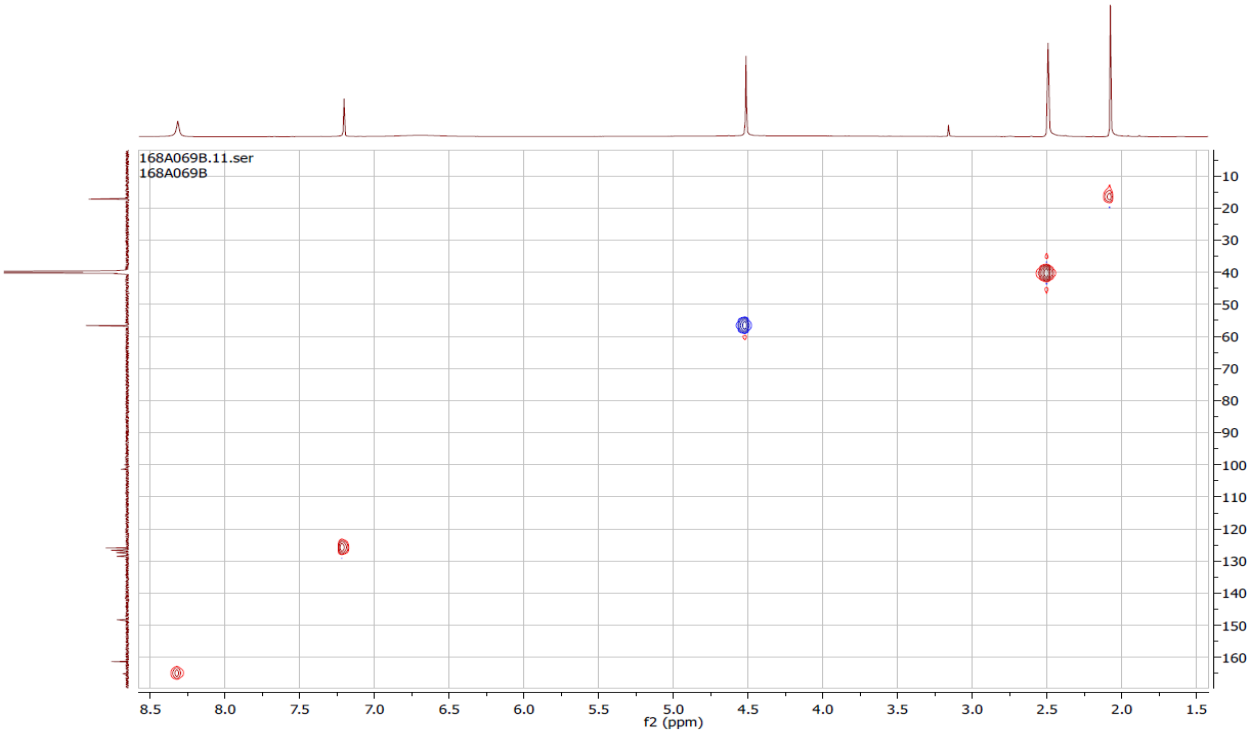


Figure S14. HSQC spectrum of isolated macrophilone A in DMSO-d_6.

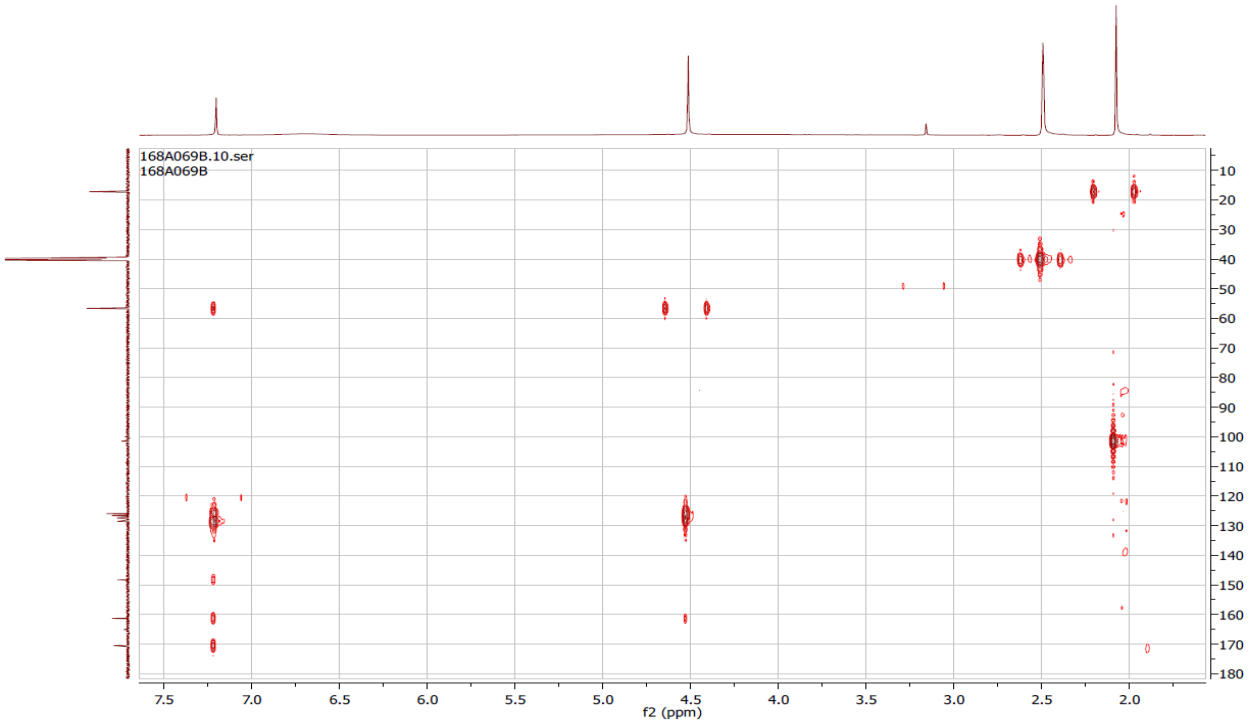


Figure S15. HMBC spectrum of isolated macrophilone A in DMSO-d_6.

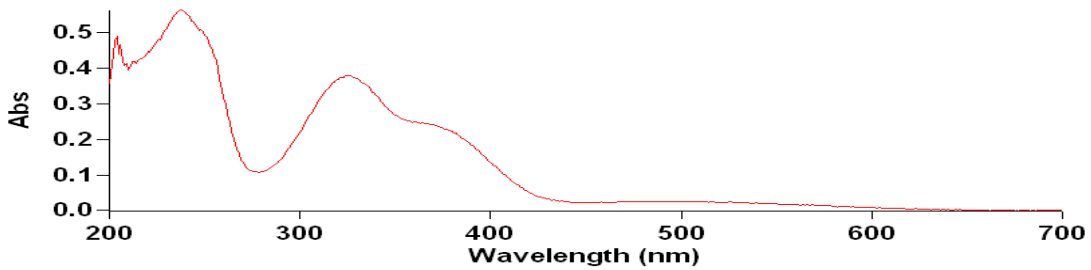


Figure S16. UV spectrum of isolated macrophilone A in CH_3OH.

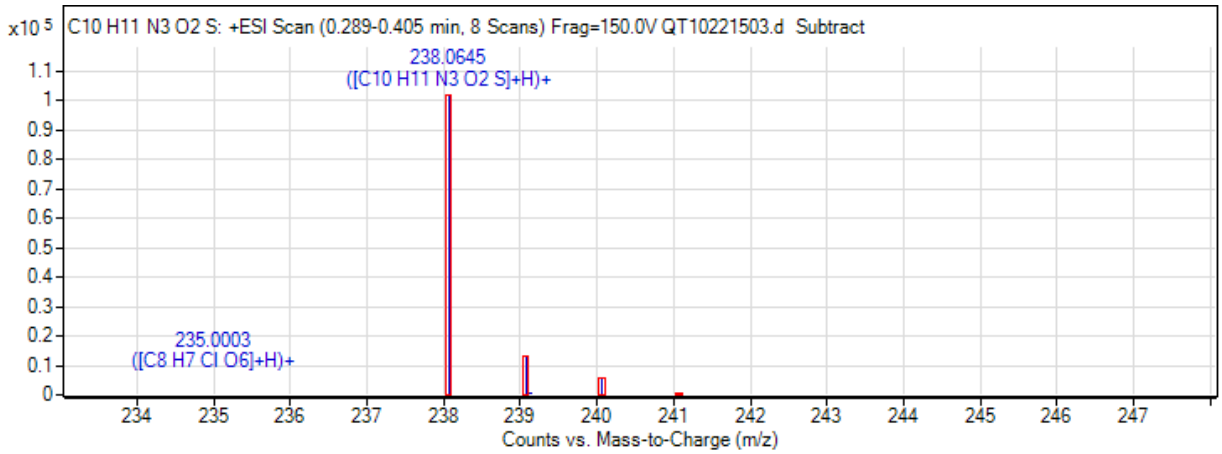
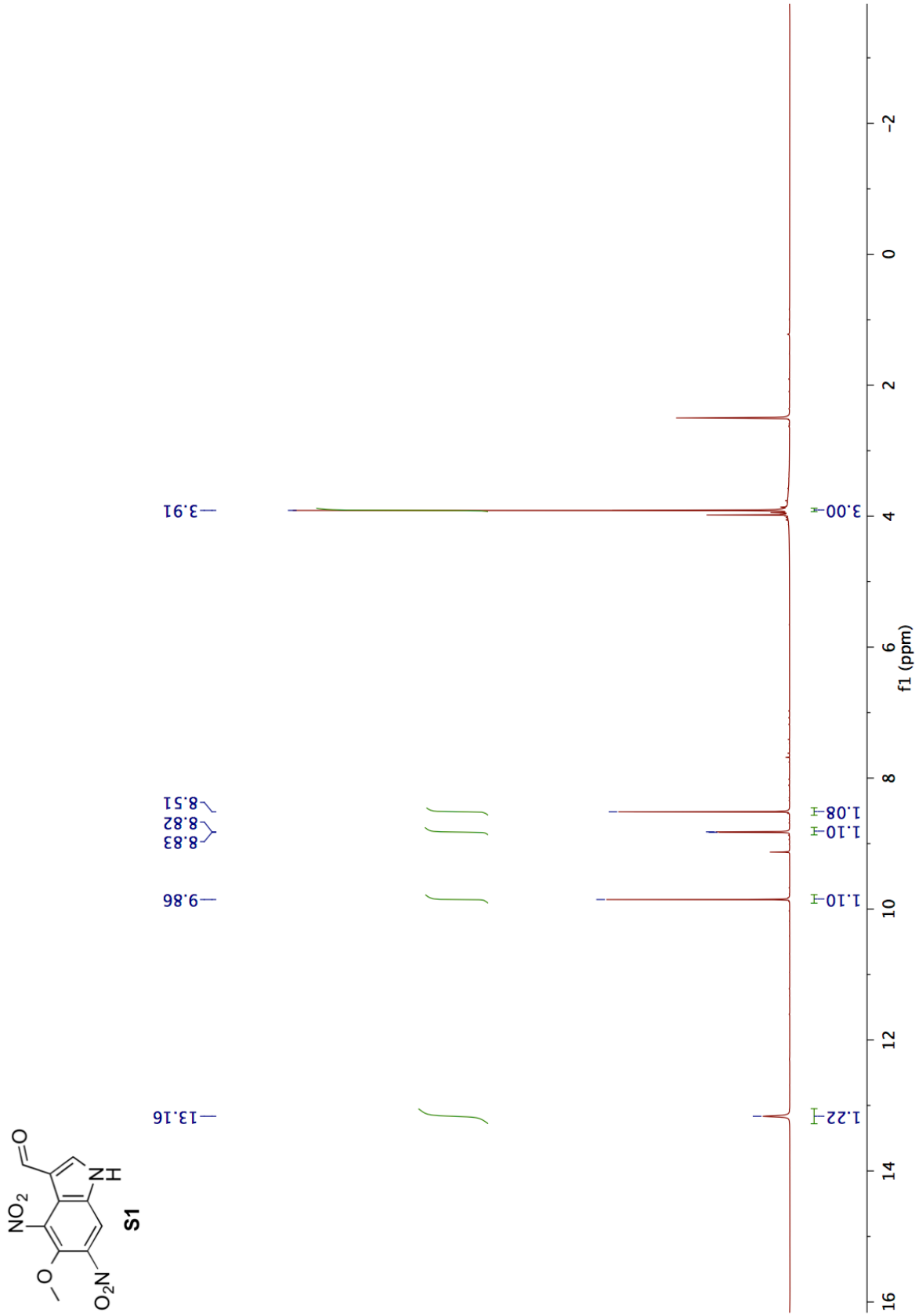
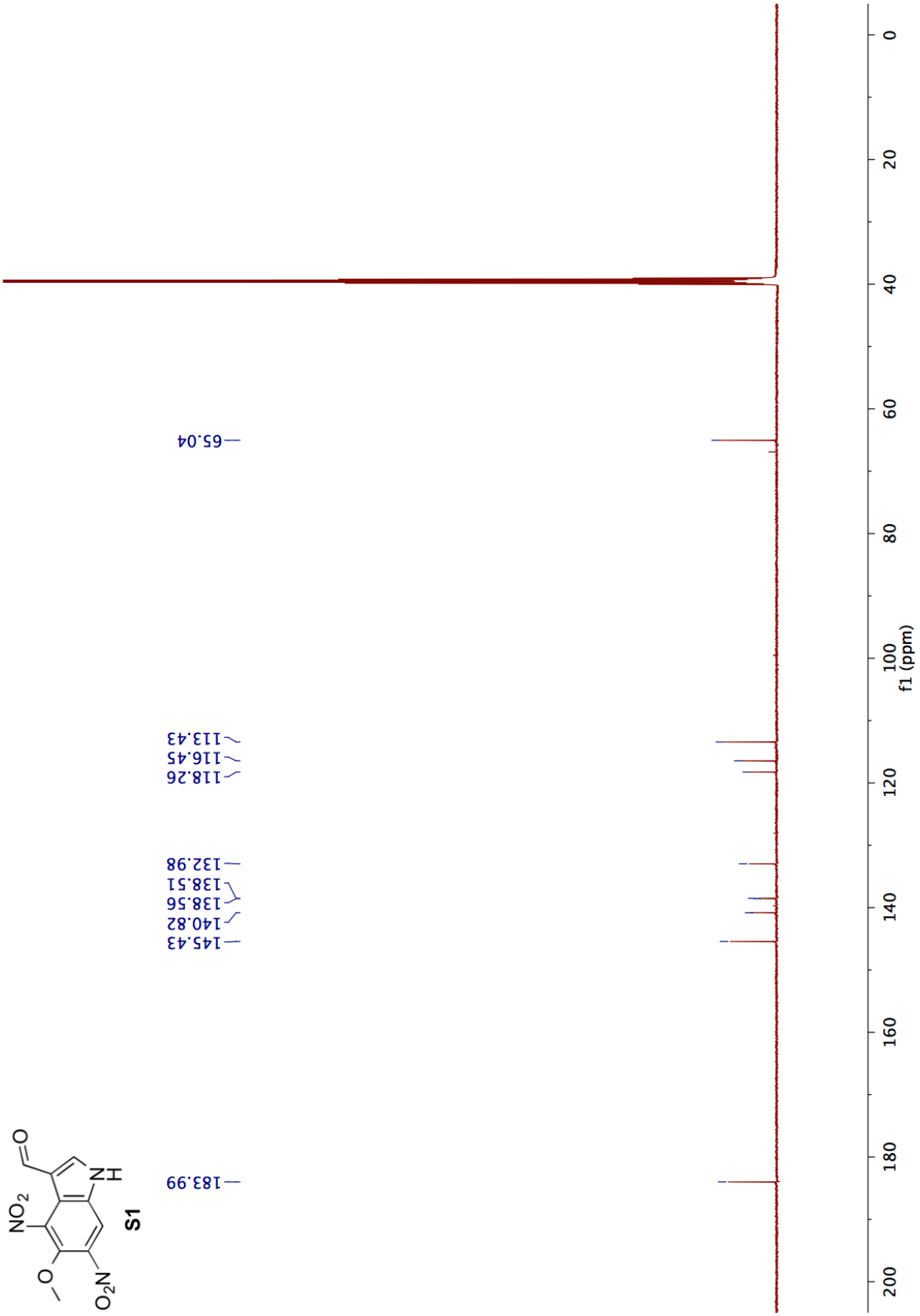
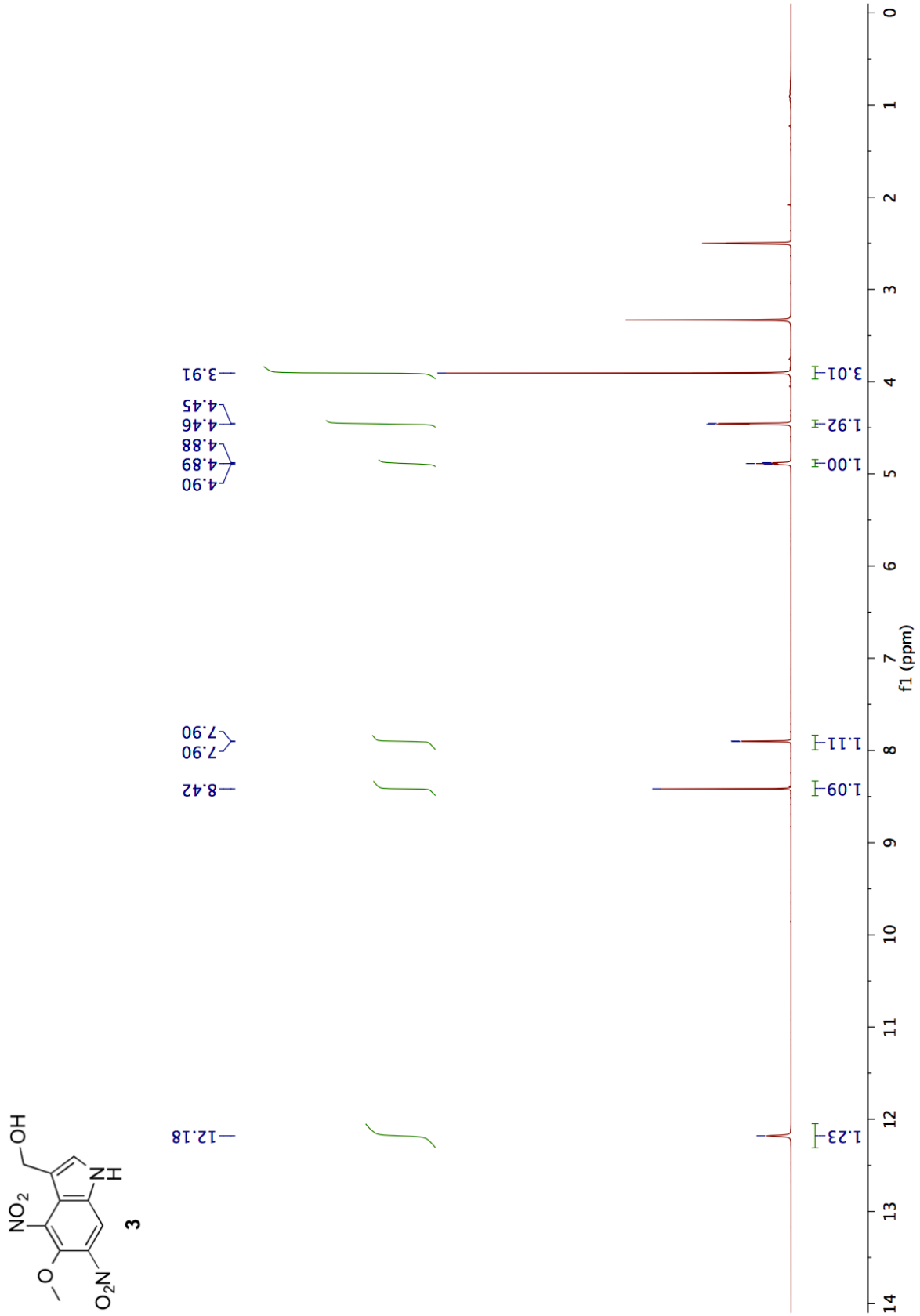
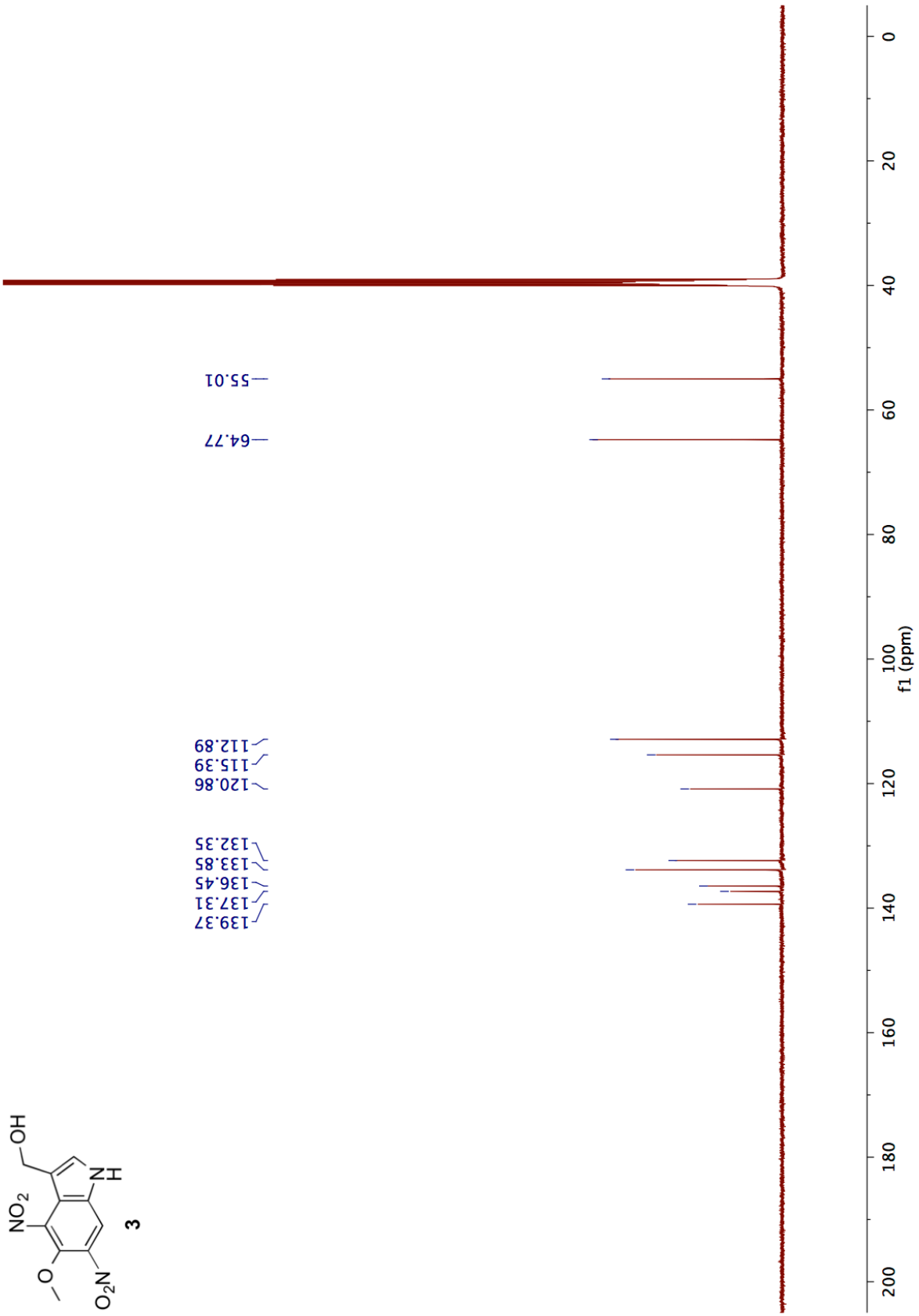


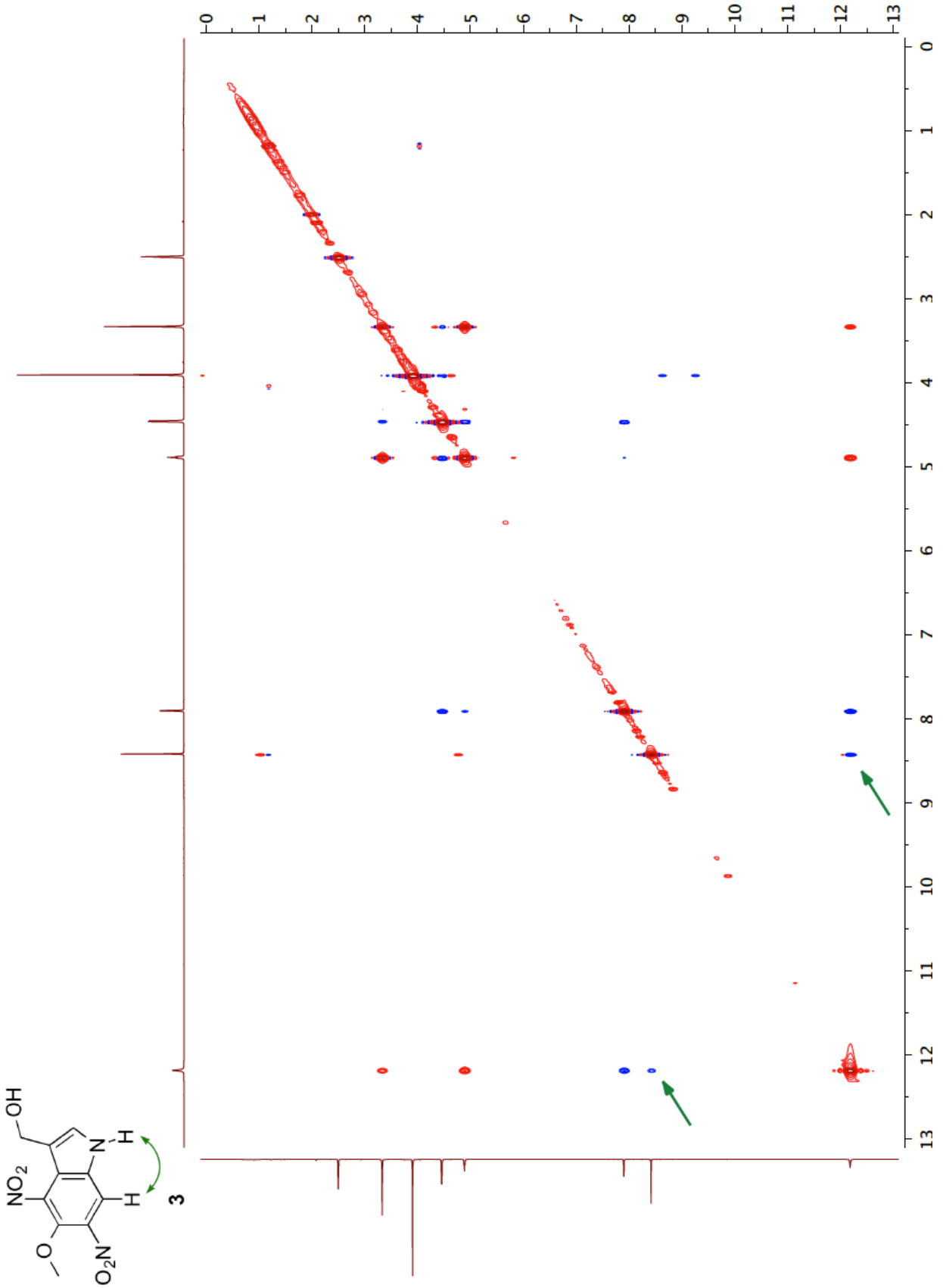
Figure S17. HRESIMS spectrum of isolated macrophilone A.

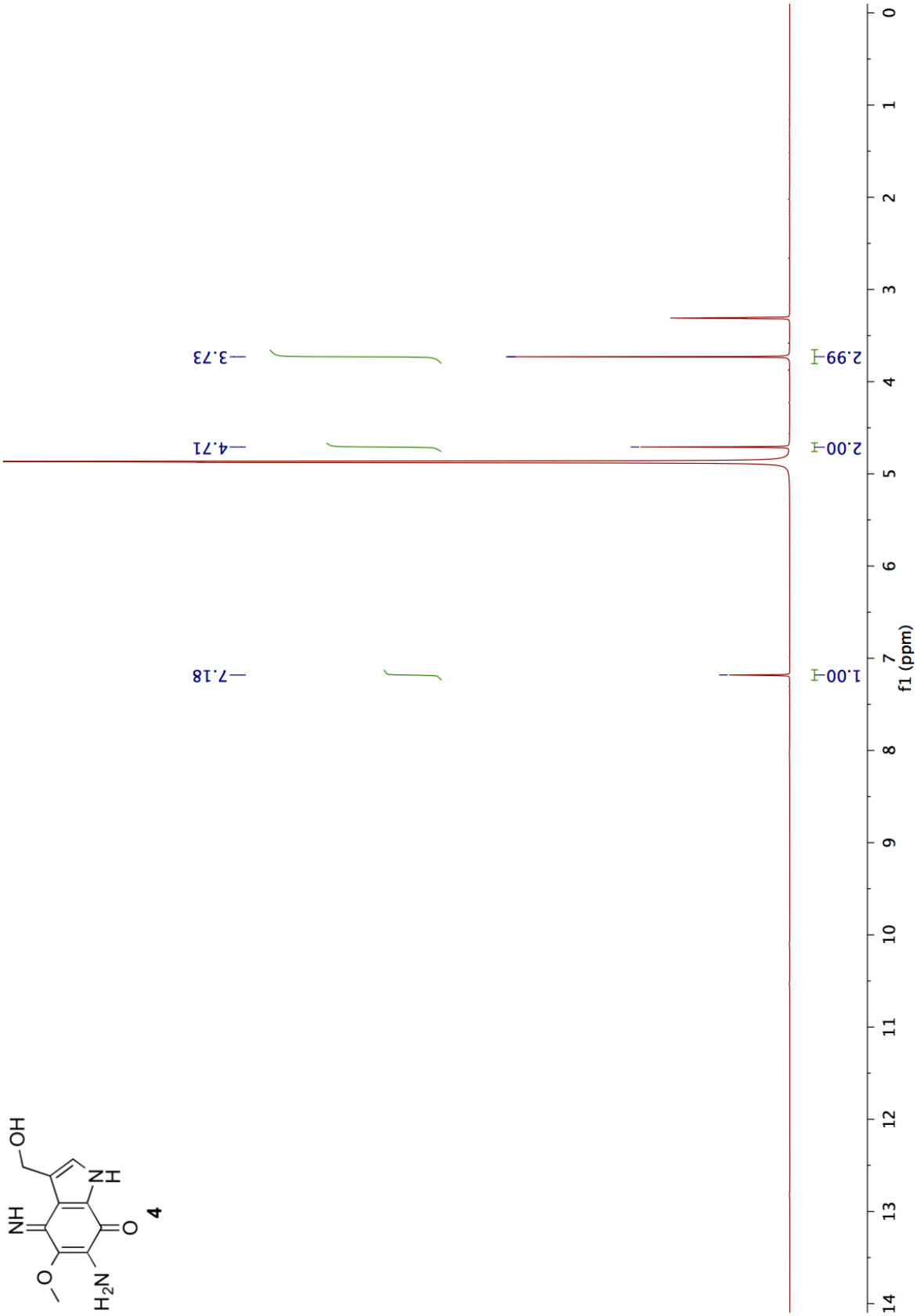

S1
13.16
9.86
8.83
8.82
8.51
3.91
1.22
1.10
1.08
1.10
3.00
f1 (ppm)

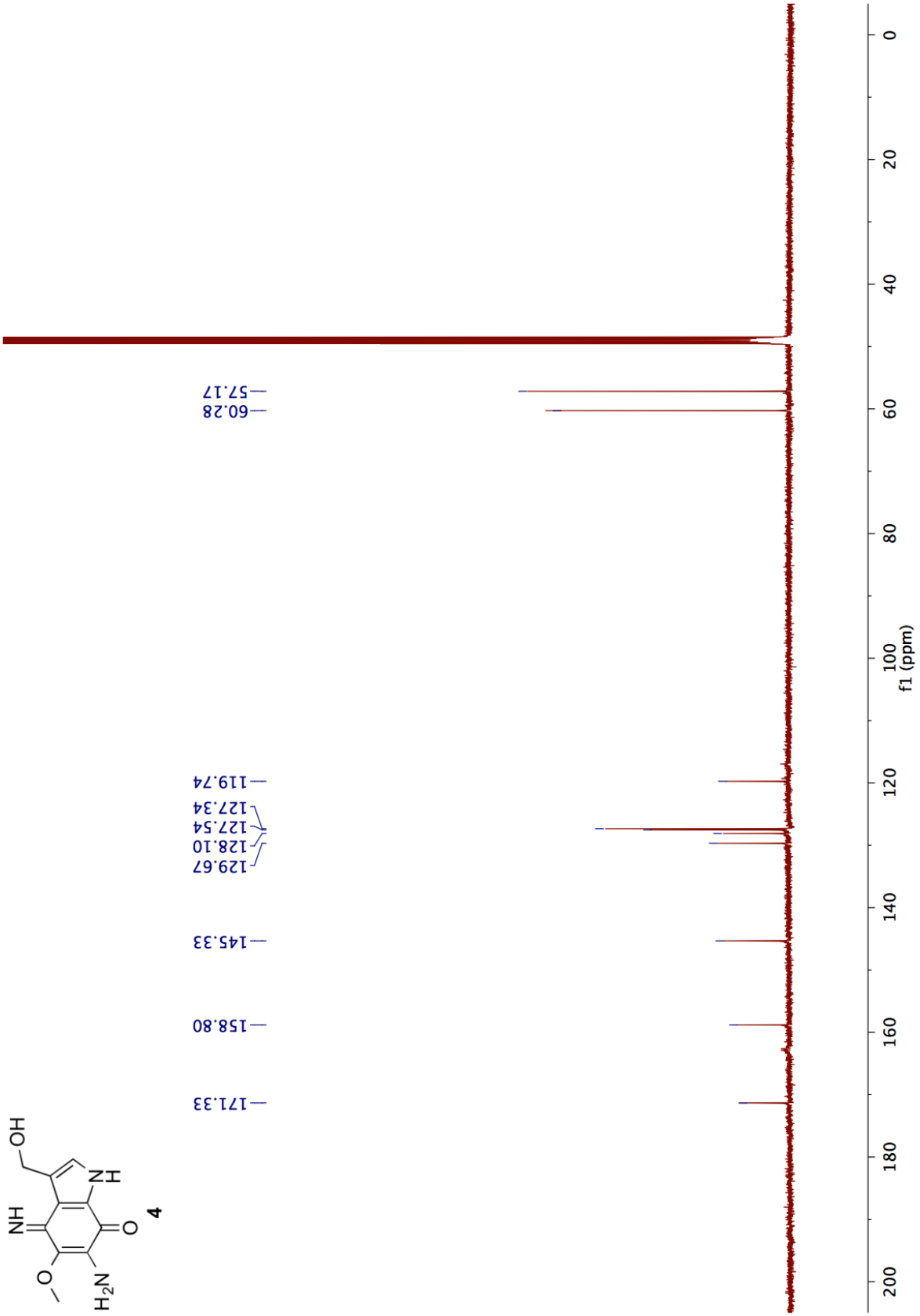

183.99
145.43
140.82
138.56
138.51
132.98
118.26
116.45
113.43
65.04
f1 (ppm)
S1

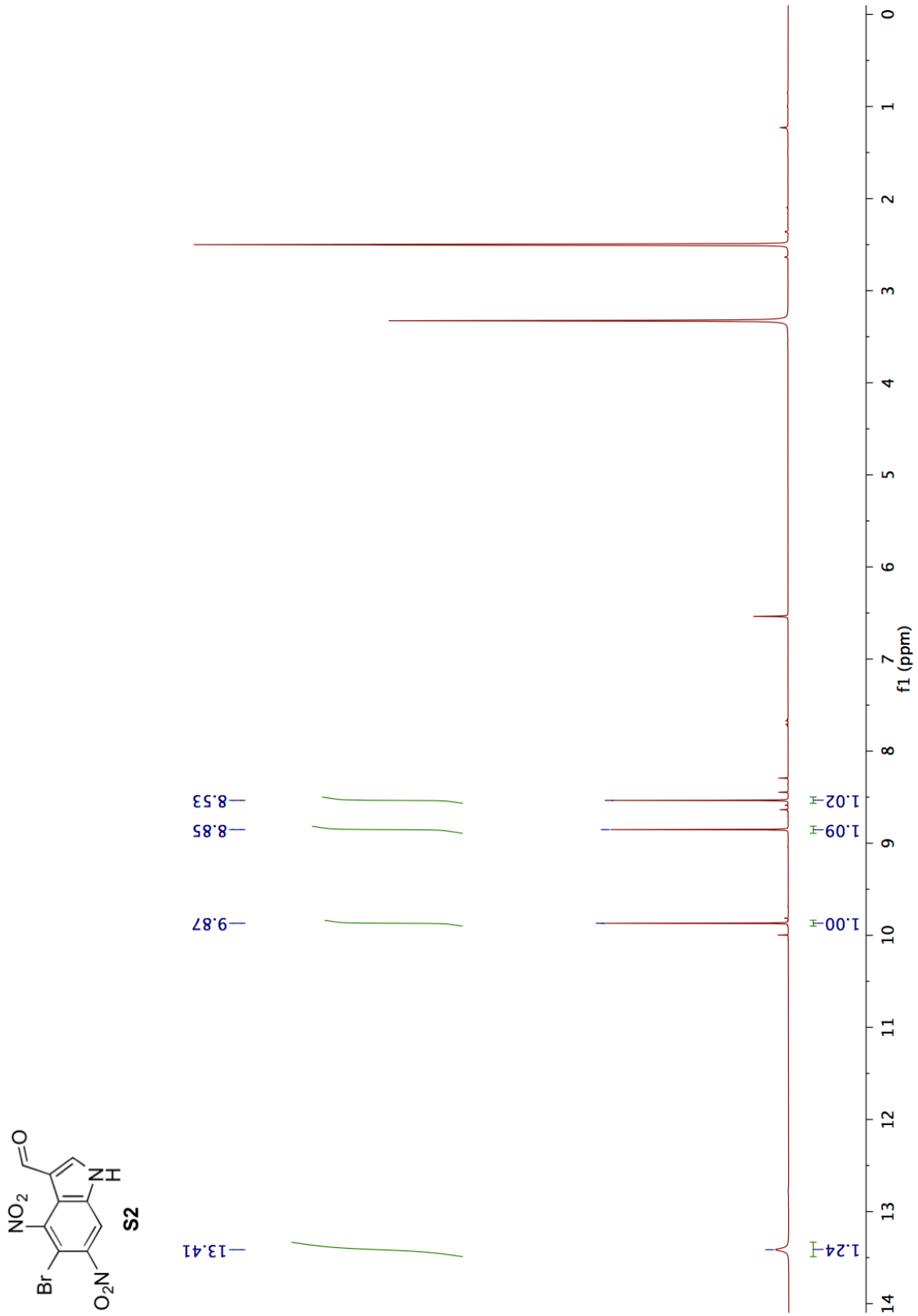

OH
NO2
N
H
O
O2N
3
12.18
8.42
7.90
7.90
4.90
4.89
4.88
4.46
4.45
3.91
1.23
1.09
1.11
1.00
1.92
3.01
f1 (ppm)

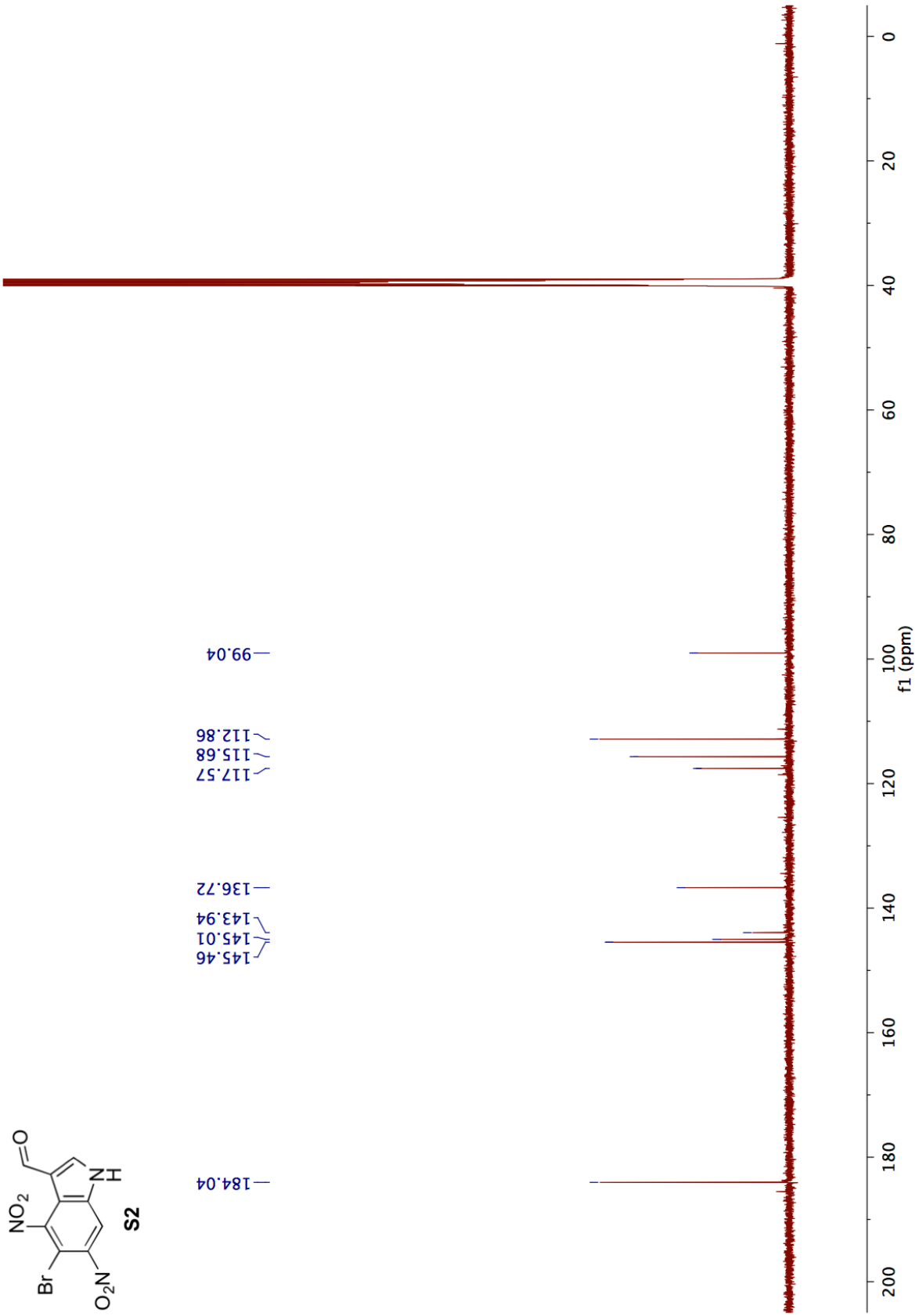

55.01
64.77
112.89
115.39
120.86
132.35
133.85
136.45
137.31
139.37
f1 (ppm)
0
20
40
60
80
100
120
140
160
180
200
OH
NO2
O
O2N
H
N
3

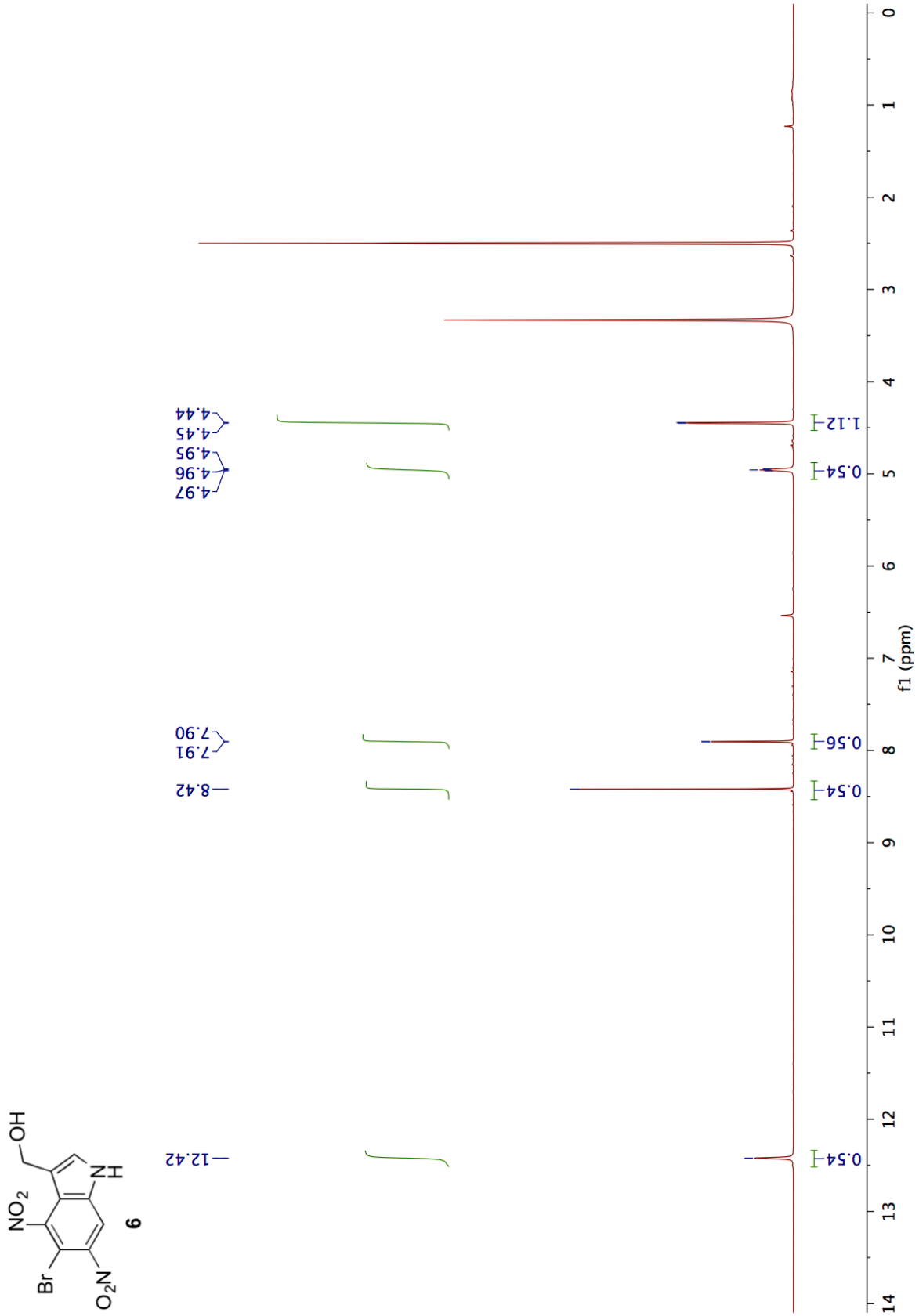

OH
NO2
O
O2N
H
N
H
3

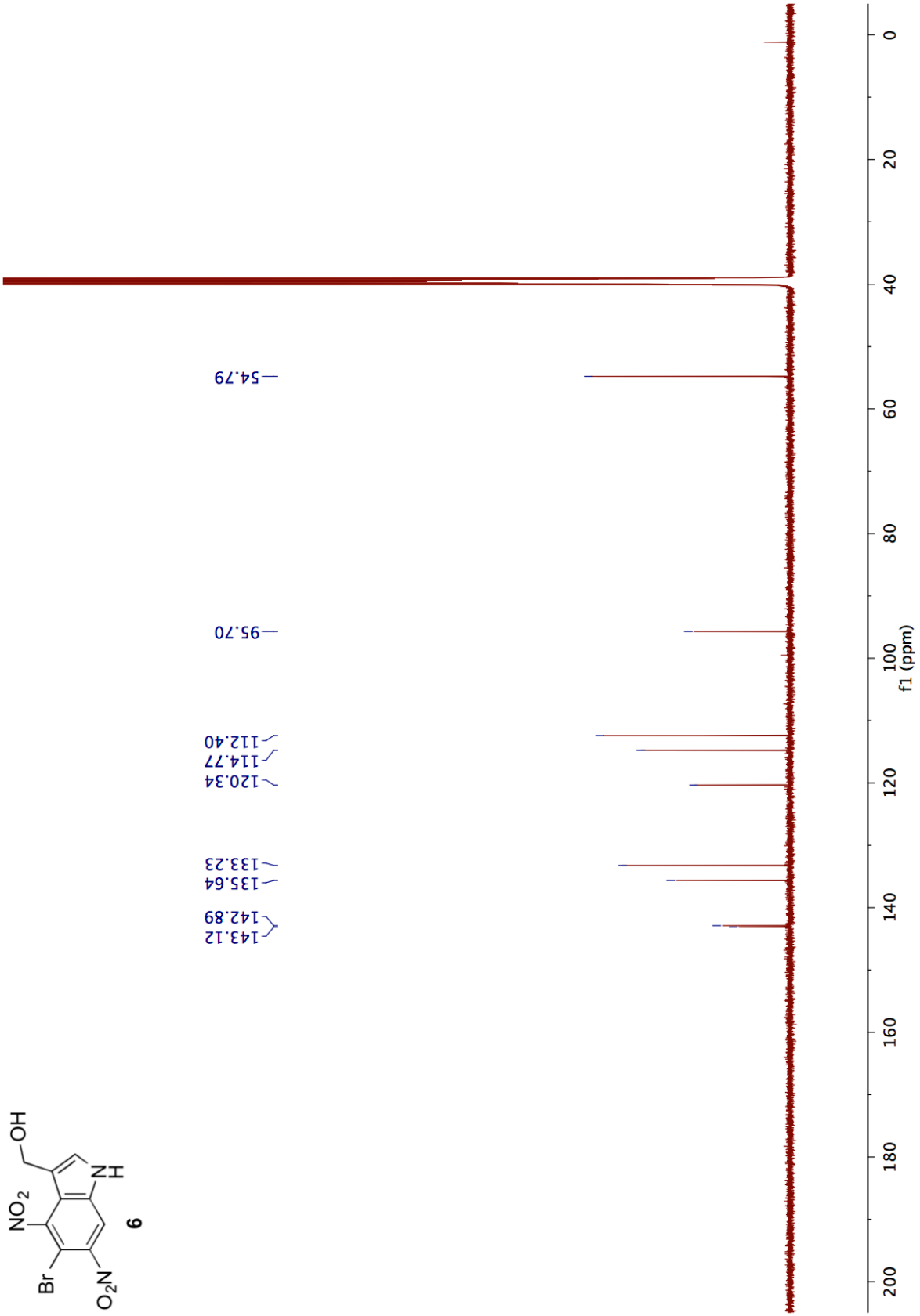

7.18
4.71
3.73
1.00
2.00
2.99
f1 (ppm)
4

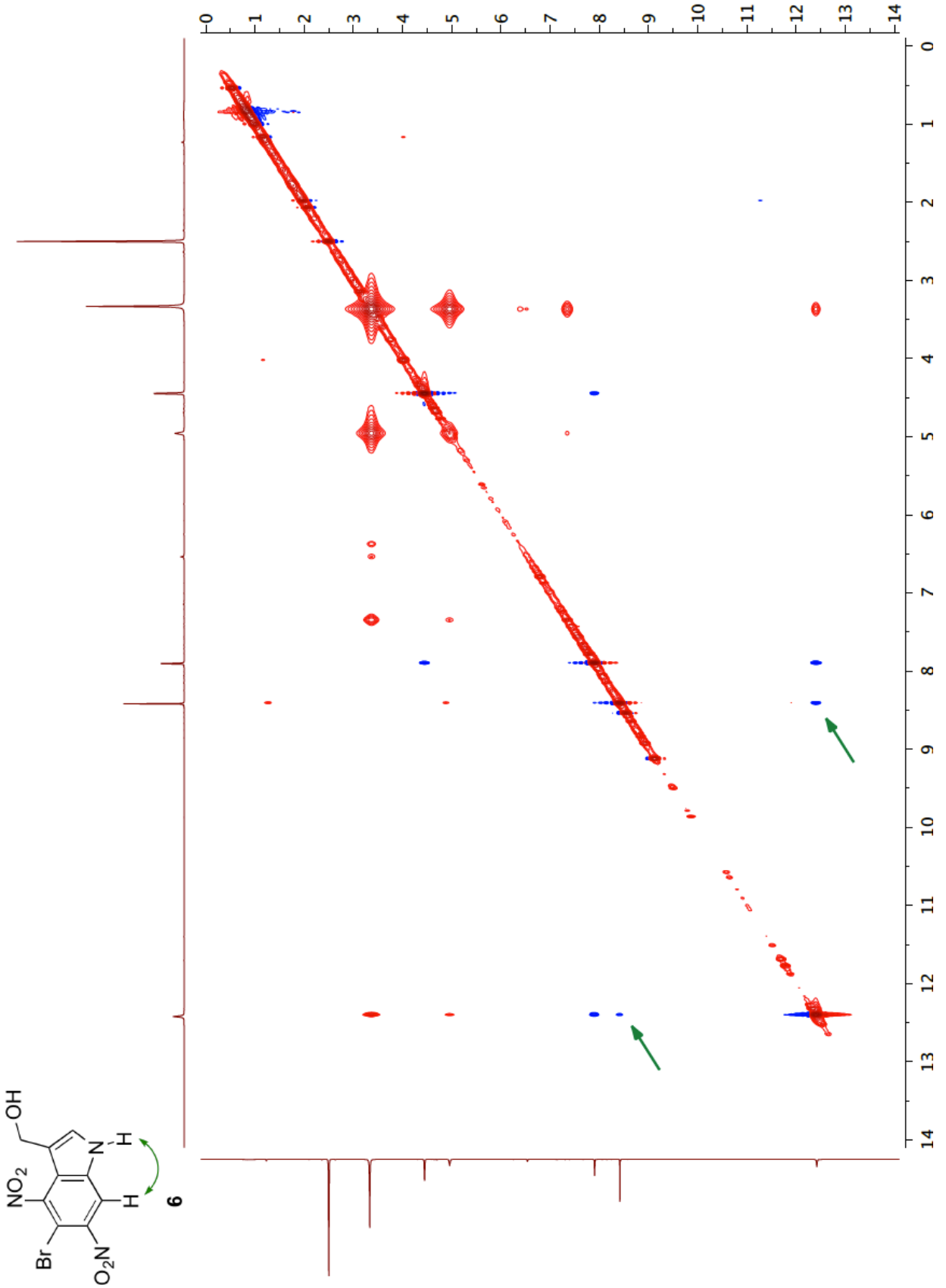

171.33
158.80
145.33
129.67
128.10
127.54
127.34
119.74
60.28
57.17
f1 (ppm)
4

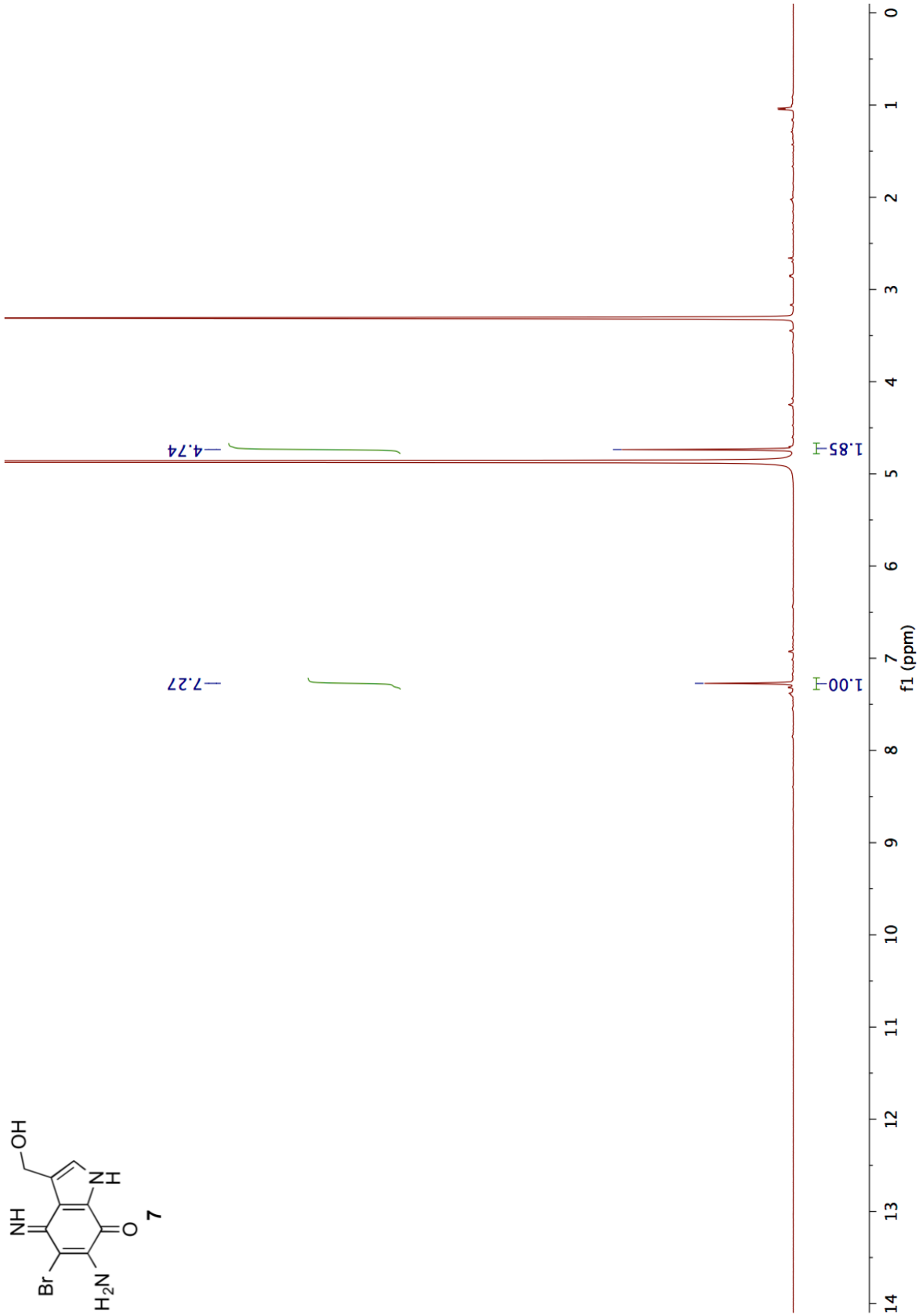

O
NO2
N
H
Br
O2N
S2
13.41
8.53
8.85
9.87
1.24
1.02
1.09
1.00
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
f1 (ppm)

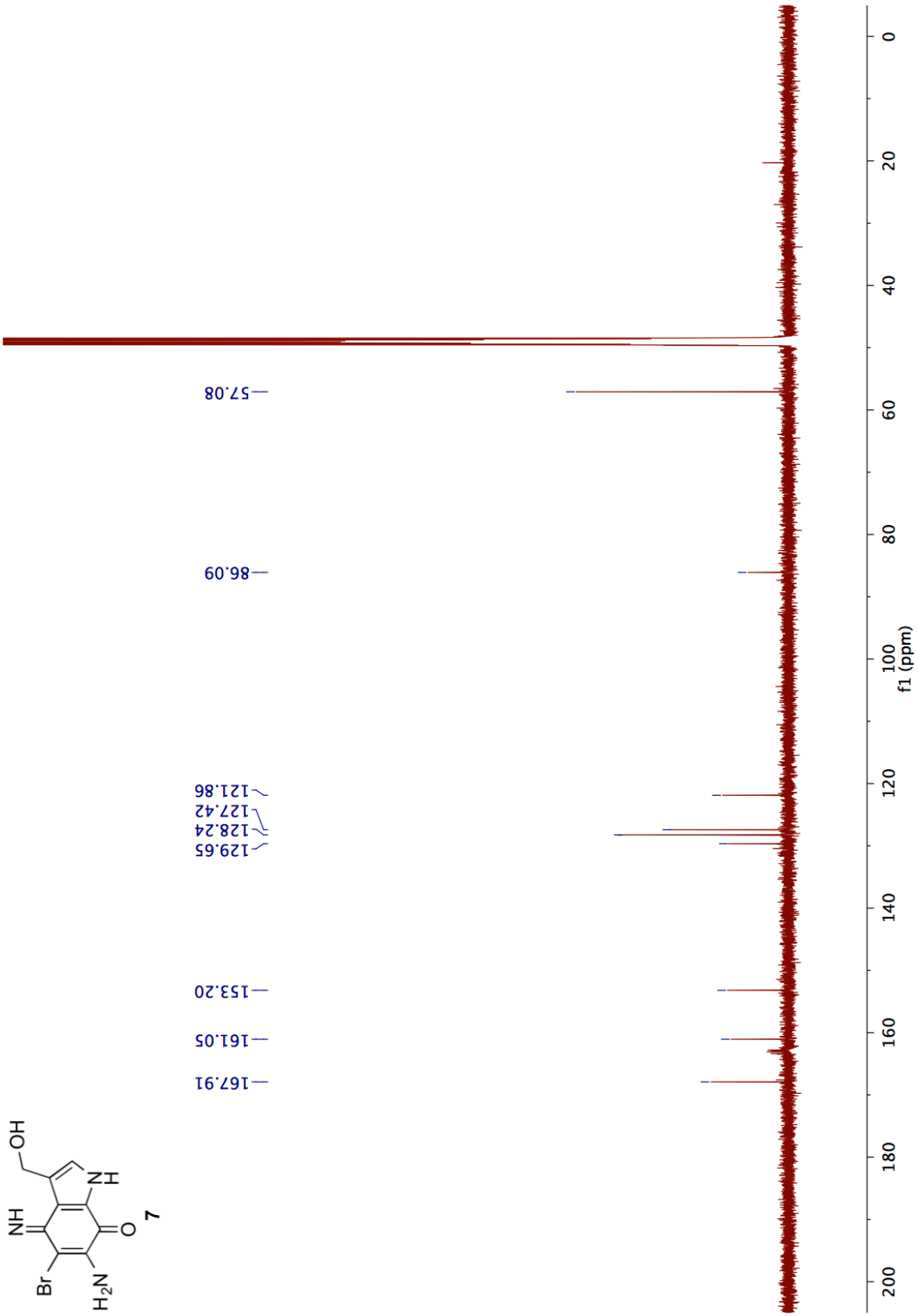

184.04
145.46
145.01
143.94
136.72
117.57
115.68
112.86
99.04
f1 (ppm)
S2

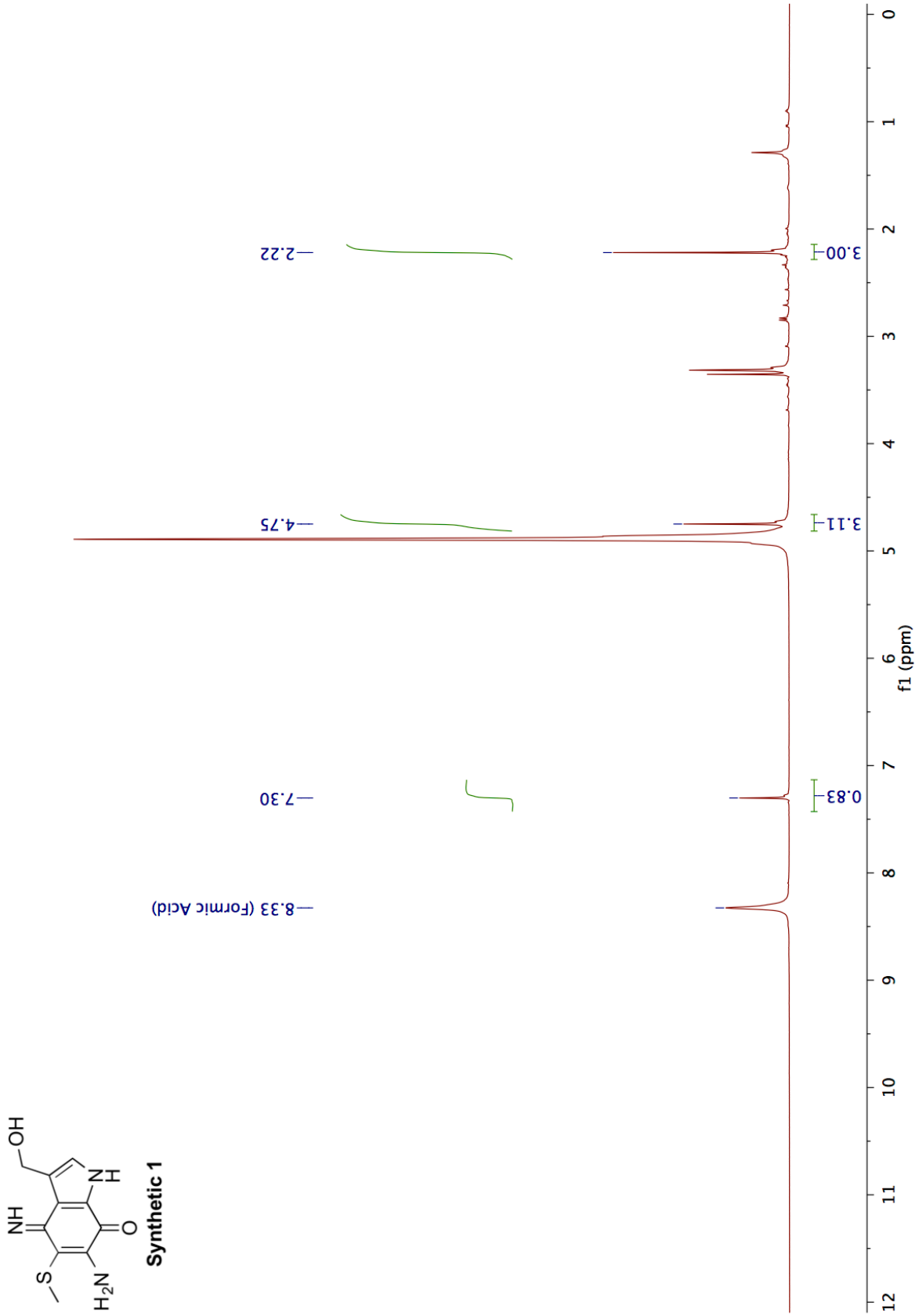

12.42
8.42
7.91
7.90
4.97
4.96
4.95
4.45
4.44
0.54
0.56
0.54
1.12
0.54
f1 (ppm)
6

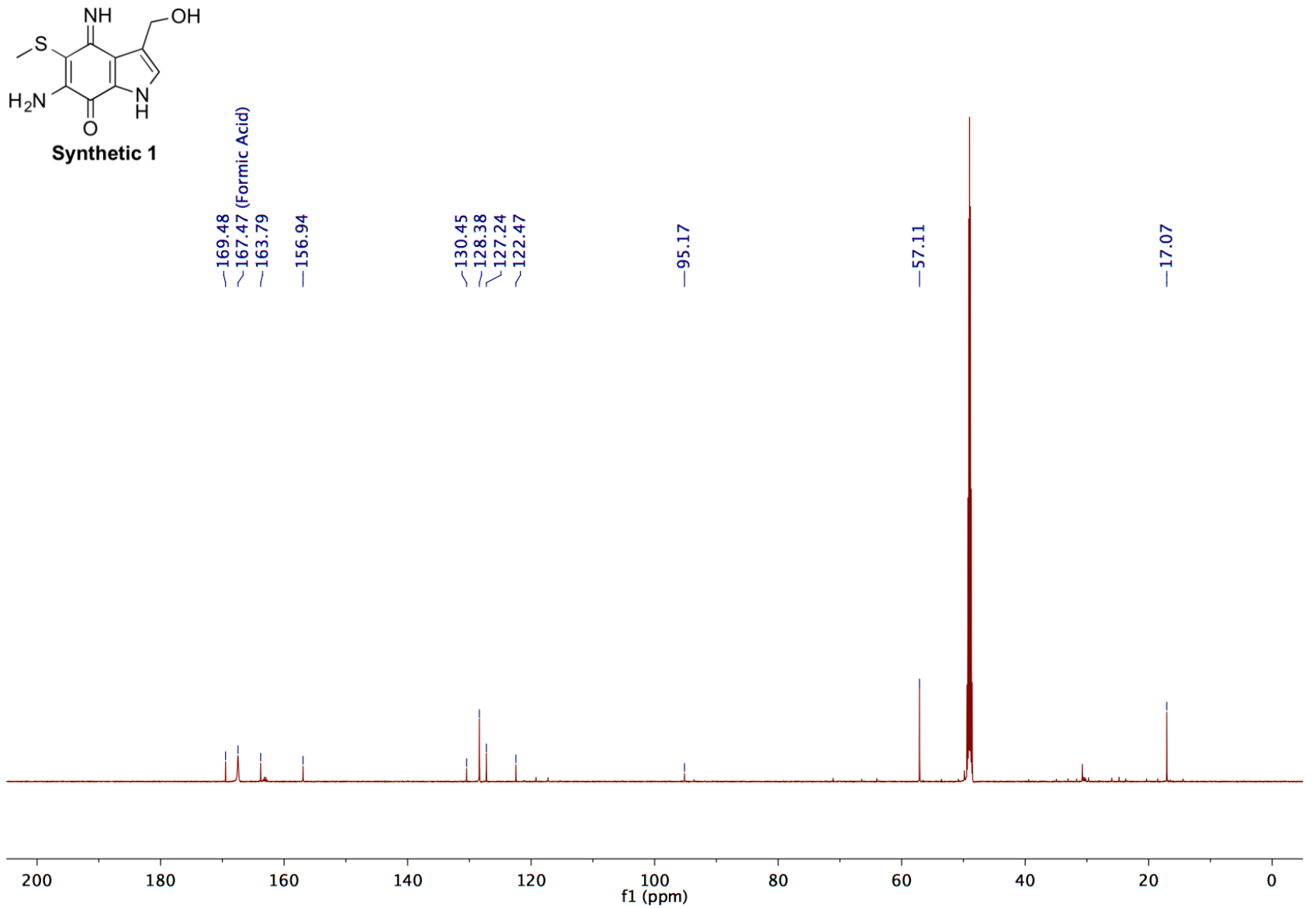

OH
NO2
Br
O2N
H
N
6
54.79
95.70
112.40
114.77
120.34
133.23
135.64
142.89
143.12
0
20
40
60
80
100
120
140
160
180
200
f1 (ppm)


OH
NO2
Br
O2N
H
H
N
6


OH
NH
NH
O
Br
H_2N
7
7.27
4.74
1.00
1.85
f1 (ppm)


167.91
161.05
153.20
129.65
128.24
127.42
121.86
86.09
57.08
f1 (ppm)
7


Synthetic 1
8.33 (Formic Acid)
7.30
4.75
2.22
0.83
3.11
3.00
f1 (ppm)


NH
OH
S
H2N
N
H
O
Synthetic 1
169.48
167.47 (Formic Acid)
163.79
156.94
130.45
128.38
127.24
122.47
95.17
57.11
17.07
200
180
160
140
120
100
80
60
40
20
0
f1 (ppm)

References

1. McCloud, T. G., High Throughput Extraction of Plant, Marine and Fungal Specimens for Preservation of Biologically Active Molecules. *Molecules* **2010,** *15* (7), 4526-4563.
2. Zhao, Y.; Truhlar, D. G., The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **2008,** *120* (1-3), 215-241.
3. Tomasi, J.; Mennucci, B.; Cammi, R., Quantum mechanical continuum solvation models. *Chem Rev* **2005,** *105* (8), 2999-3093.
4. Wolinski, K.; Hinton, J. F.; Pulay, P., EFFICIENT IMPLEMENTATION OF THE GAUGE-INDEPENDENT ATOMIC ORBITAL METHOD FOR NMR CHEMICAL-SHIFT CALCULATIONS. *J Am Chem Soc* **1990,** *112* (23), 8251-8260.
5. Sarotti, A. M.; Pellegrinet, S. C., A Multi-standard Approach for GIAO C-13 NMR Calculations. *J Org Chem* **2009,** *74* (19), 7254-7260.
6. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A., NMR chemical shifts of common laboratory solvents as trace impurities. *J Org Chem* **1997,** *62* (21), 7512-7515.
7. Kim, Y. S.; Nagy, K.; Keyser, S.; Schneekloth, J. S., An Electrophoretic Mobility Shift Assay Identifies a Mechanistically Unique Inhibitor of Protein Sumoylation. *Chem Biol* **2013,** *20* (4), 604-613.
8. Hewitt, W. M.; Lountos, G. T.; Zlotkowski, K.; Dahlhauser, S. D.; Saunders, L. B.; Needle, D.; Tropea, J. E.; Zhan, C.; Wei, G.; Ma, B.; Nussinov, R.; Waugh, D. S.; Schneekloth, J. S., Jr., Insights Into the Allosteric Inhibition of the SUMO E2 Enzyme Ubc9. *Angew Chem Int Ed Engl* **2016,** *55* (19), 5703-7.