

**Fast, Irreversible Modification of Cysteines through Strain Releasing Conjugate Additions
of Cyclopropenyl Ketones**

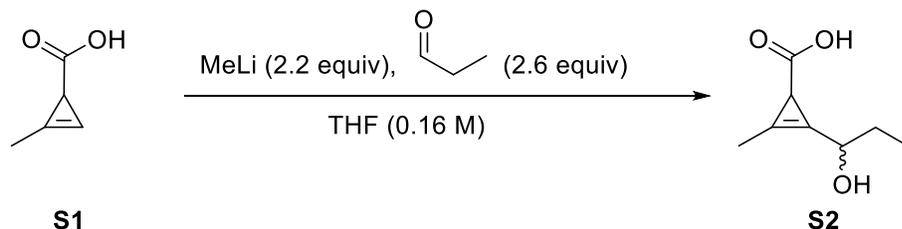
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General Considerations. For ^{13}C NMR, when an APT pulse sequence was used to distinguish multiplicities, quaternary and methylene carbons appear ‘up’ (C or CH_2) and methane and methyl carbons appear ‘down’ (CH or CH_3). Mass spectrometry was carried out on a Waters GCT Premier and Thermo Q-Exactive Orbitrap. Thin layer chromatography was performed on Merck/Millipore Silica Gel 60, F₂₅₄. Normal phase flash chromatography was performed on Silicycle 40-63D, 60Å. Stopped-Flow kinetics experiments were performed using an Applied Photophysics SX18MV-R stopped-flow spectrophotometer with temperature control (25 °C). Anhydrous dichloromethane was dried through an alumina column solvent purification system. Anhydrous tetrahydrofuran was freshly distilled from sodium/benzophenone. Cycloprop-2-ene carboxylic acid was prepared by a literature procedure.¹ All other solvents and chemical reagents were purchased from commercial suppliers and used without further purification. All non-aqueous reactions were carried out in flame-dried glassware under an inert atmosphere of nitrogen.

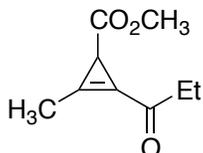
2-(1-Hydroxypropyl)-3-methylcycloprop-2-ene carboxylic acid (S2)



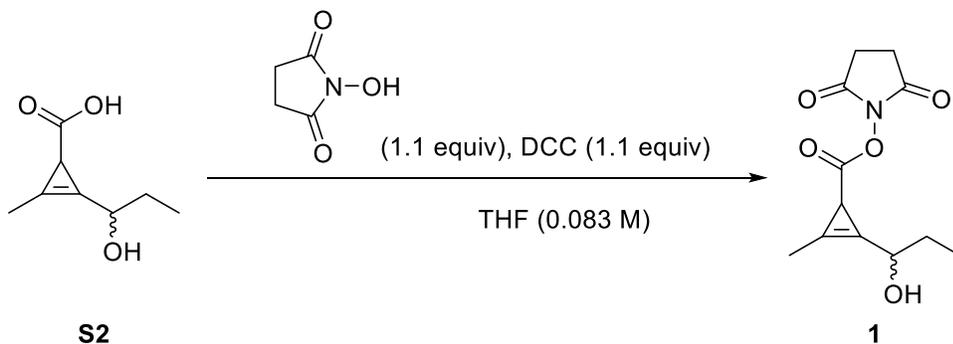
The following is a slight modification of a literature protocol.²

To a dry 250 mL round bottom flask was added Cycloprop-2-ene carboxylic acid (**S1**)¹ (2.0 g, 20 mmol) dissolved in dry THF (128 mL) and the resulting solution was cooled to $-78\text{ }^{\circ}\text{C}$. MeLi (28 mL of 1.6 M solution in Et₂O, 45 mmol) was added dropwise via syringe. After 10 minutes, the dry ice bath was removed and stirring was continued for 15 min until an internal temperature of $\sim 0\text{-}5\text{ }^{\circ}\text{C}$ was reached. *The reaction mixture should not be permitted to reach room temperature due to the thermal instability of the dianion.* To the bright yellow/orange solution was added propionaldehyde (3.8 mL, 53 mmol) via syringe. After being stirred at rt for 10 min, the reaction mixture was quenched with water (100 mL) and the solution acidified to pH 1-3 using 3 M HCl. NaCl was added to saturate the aqueous layer. The aqueous phase was extracted with EtOAc (4 x 50 mL). The combined organics were dried (MgSO₄) and filtered. Silica gel (5 g) was added to the filtrate, which was then concentrated to dryness on the rotary evaporator. Column chromatography (gradient from 0-40% ethyl acetate in hexanes) furnished alkenol **S2** (2.7 g, 17 mmol, 85%) as a pale yellow solid. The diastereomer ratio was 1.1:1 by ¹H NMR analysis. The spectra agreed with those previously reported.² ¹H NMR (600 MHz, CDCl₃) δ : 5.55 (bs, OH), 4.54-4.51 (td, J = 6.6, 1.2 Hz, 0.5H), 4.46-4.43 (td, J = 6.6, 1.5 Hz, 0.5H), 2.11-2.10 (d, J = 2.2 Hz, 1H), 2.06 (s, 3H), 1.74 – 1.61 (m, 2H), 0.93-0.92 (t, J = 7.4 Hz, 1.5H), 0.90-0.86 (t, J = 7.4 Hz, 1.5H).; ¹³C NMR (CDCl₃, 150 MHz, δ): 182.5 (C), 182.4 (C), 107.1 (C), 106.8 (C), 104.7 (C), 104.6 (C), 68.2 (CH), 67.4 (CH), 28.8 (CH₂), 28.2 (CH₂), 22.9 (CH), 22.2 (CH), 9.85 (CH₃, 2 carbons), 9.52 (CH₃), 9.49 (CH₃).

Methyl 2-methyl-3-propionylcycloprop-2-ene-1-carboxylate (4) was prepared from **S2** by following the published procedure².



2,5-dioxopyrrolidin-1-yl 2-(1-hydroxypropyl)-3-methylcycloprop-2-ene-1-carboxylate (1)

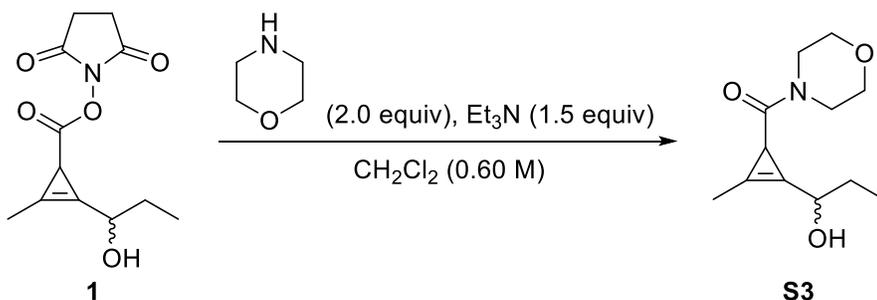


To a solution of 2-(1-hydroxypropyl)-3-methylcycloprop-2-ene-1-carboxylic acid (1.0 g, 6.4 mmol) in anhydrous THF (77 mL, 0.083M), *N,N*-dicyclohexylcarbodiimide (1.4 g, 7.0 mmol) and *N*-hydroxysuccinimide (810 mg, 7.0 mmol) were added. The mixture was allowed to stir at r.t. for 18 h and then filtered through celite. The celite was rinsed with THF and silica gel (5 g) was added to the filtrate, which was then concentrated to dryness on the rotary evaporator.

Column chromatography (gradient from 0 - 50 % ethyl acetate in hexanes) furnished the product **1** (1.1 g, 4.4 mmol, 68%) as a yellow oil. The diastereomer ratio was 1.1:1 by ¹H NMR analysis. ¹H NMR (CDCl₃, 600 MHz, δ): 4.59 (t, *J* = 6.4 Hz, 0.5H), 4.54 (t, *J* = 6.4 Hz, 0.5H), 3.06 (bs, OH), 2.80 (bs, 4H), 2.38 (d, *J* = 4.2 Hz, 1H), 2.17 (s, 3H), 1.81-1.73 (m, 2H), 1.00-0.95 (m, 3H); ¹³C NMR (CDCl₃, 100 MHz, δ): 170.9 (C), 170.8 (C), 169.8 (broad, C, 2 carbons), 106.8 (C),

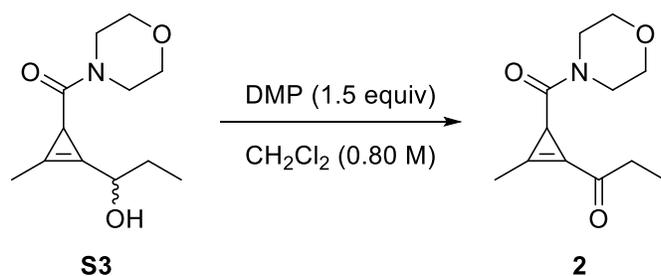
106.6 (C), 103.1 (C), 67.9 (CH), 67.3 (CH), 29.0 (CH₂), 28.2 (CH₂), 25.6 (CH₂, 2 carbons), 20.4 (CH), 20.0 (CH), 9.82 (CH₃), 9.80 (CH₃), 9.50 (CH₃), 9.46 (CH₃); FT-IR (NaCl, thin film, cm⁻¹): 3467, 2970, 2879, 1770, 1736, 1431, 1372, 1210, 1067; LIFDI MS *m/z* [M +] Calcd for C₁₂H₁₅NO₅ 253.0950, Found 253.0924.

(2-(1-hydroxypropyl)-3-methylcycloprop-2-en-1-yl)(morpholino)methanone (S3)



To a dry round bottom flask charged with a solution of **1** (700 mg, 2.76 mmol) in CH₂Cl₂ (2.3 mL) was added a solution of morpholine (481 mg, 5.53 mmol) in CH₂Cl₂ (2.2 mL). Et₃N was added via syringe (0.60 mL, 4.2 mmol) at r.t. The reaction turned cloudy after 5 minutes and was complete after 15 minutes by TLC monitoring. Silica gel (3 g) was then added, and the mixture concentrated to dryness using a rotary evaporator. Column chromatography (gradient from 10-100% ethyl acetate in hexanes) furnished **S3** (500 mg, 81% yield) as a pale orange oil. The diastereomer ratio was 1.1:1 by ¹H NMR analysis. ¹H NMR (CDCl₃, 600 MHz, δ): 4.71 (t, *J* = 6.7 Hz, 0.5H), 4.42 (t, *J* = 6.7 Hz, 0.5H), 4.23 (bs, OH), 3.70 (bs, 7H), 3.57 (bs, 1H), 2.35 (s, 1H), 2.12 (s, 3H), 1.88-1.78 (m, 1H), 1.77-1.73 (m, 0.5H), 1.64-1.58 (m, 0.5H), 1.04 (t, *J* = 7.6 Hz, 1.4H), 0.90 (t, *J* = 7.6 Hz, 1.6H); ¹³C NMR (CDCl₃, 100 MHz, δ): 175.0 (C), 174.9 (C), 108.7 (C), 107.9 (C), 104.8 (C), 104.4 (C), 67.9 (CH), 66.9 (CH₂, 2 carbons), 66.3 (CH), 46.1 (CH₂), 42.5 (CH₂), 29.8 (CH₂), 28.3 (CH₂), 22.9 (CH), 21.8 (CH), 10.5 (CH₃), 10.3 (CH₃), 9.6 (CH₃), 9.5 (CH₃); FT-IR (NaCl, thin film, cm⁻¹): 3382, 2964, 2921, 2855, 1894, 1613, 1437, 1236, 1115, 1046, 1019, 974, 853, 573; LIFDI MS *m/z* [M +] calcd for C₁₂H₁₉NO₃ 225.1365, found 225.1353.

1-(2-methyl-3-(morpholine-4-carbonyl)cycloprop-1-en-1-yl)propan-1-one (**2**)



To a solution of **S3** (300 mg, 1.30 mmol) in CH₂Cl₂ (1.7 mL, 0.80 M) was added Dess Martin Periodinane, DMP, (850 mg, 2.00 mmol) in one portion. After 30 minutes, the reaction was filtered through celite and the filtrate concentrated onto C-2 silica gel prepared as described below. Column chromatography (slow gradient from 0-80% ethyl acetate in toluene) furnished **2** (214 mg, 72%) as a pale yellow oil. Residual toluene peaks are observable in the ¹H NMR spectrum at 7.20 and 2.37 ppm. ¹H NMR (CDCl₃, 400 MHz, δ): 3.92- 3.89 (m, 1H), 3.80-3.62 (m, 6H) 3.50-3.44 (m, 1H), 2.81-2.74 (m, 2H), 2.58 (s, 1H), 2.44 (d, *J* = 2.1 Hz, 3H), 1.19 (td, *J* = 7.4, 2.3 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz, δ): 192.1 (C), 171.5 (C), 122.4 (C), 105.2 (C), 66.90 (CH₂), 66.86 (CH₂), 46.1 (CH₂), 42.3 (CH₂), 36.6 (CH₂), 22.6 (CH), 11.9 (CH₃), 7.9 (CH₃); FT-IR (NaCl, thin film, cm⁻¹): 2973, 2856, 1849, 1679, 1637, 1457, 1436, 1270, 1236, 1115, 1036. LIFDI MS *m/z* [M +] calcd for C₁₂H₁₇NO₃ 223.1208, found 223.1196.

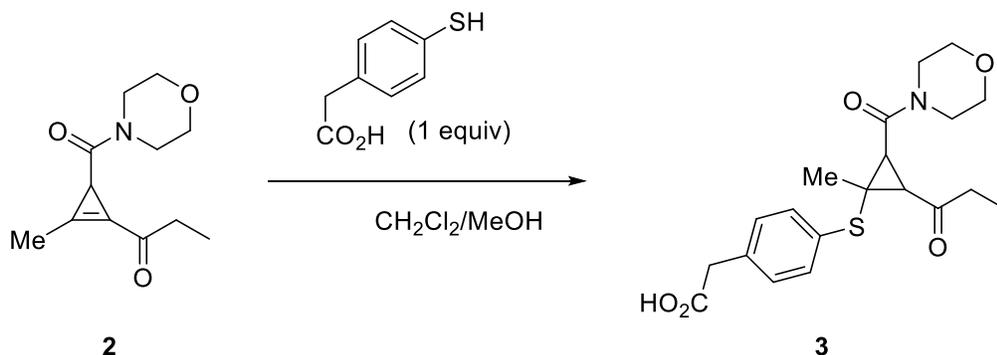
(NB. In some cases after column chromatography, solid DMP byproduct was observed. This could be removed by re-suspending the material in toluene, filtering through celite, rinsing the filtered solid with ethyl acetate and concentrating the filtrate to furnish pure material.)

C-2 Silica Gel Preparation³

Flash silica gel (100 g, SiliaFlash ® F60, 40-63 μm (230-400 mesh) was suspended in 200 mL of dry chloroform in a round bottomed flask under a N₂ atmosphere. The flask was

chilled by an ice bath. Ethyltrichlorosilane (5.1 g, 31 mmol) was added via syringe. After the addition was completed, the flask was closed and shaken vigorously to mix (HCl is formed). The mixture was allowed to sit at rt with occasional shaking until the next day (suspension becomes yellow). The silica gel was filtered on a Buchner funnel and washed twice with 200 mL portions of chloroform and three times with 200 mL portions of methanol. The silica gel was transferred to a round bottomed flask, and was dried by heating (40°C oil bath) under vacuum.

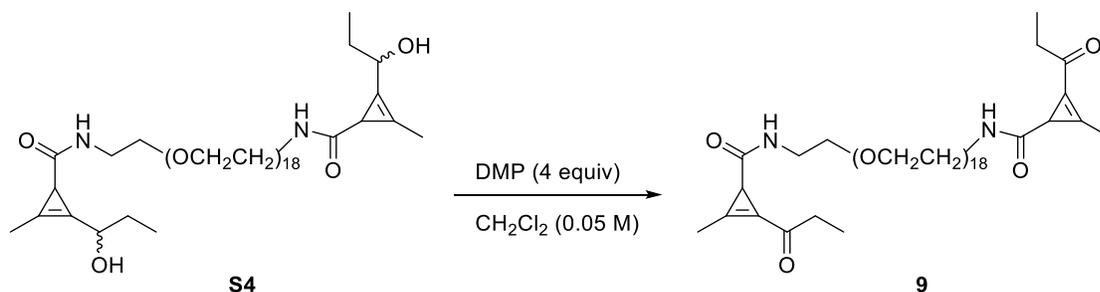
2-(4-((1-methyl-2-(morpholine-4-carbonyl)-3-propionylcyclopropyl)thio)phenyl)acetic acid (3)



To a solution of **2** (100 mg, 0.45 mmol) in CH₂Cl₂ (0.5 mL, 0.9 M) was added a solution of 4-mercaptophenylacetic acid (75 mg, 0.45 mmol) in MeOH (0.5 mL, 0.9 M). After 15 minutes, silica gel (0.5 g) was added, and the mixture concentrated to dryness using a rotary evaporator. Column chromatography (gradient from 10-100% ethyl acetate in hexanes) furnished **3** as a pale yellow oil (68 mg, 0.17 mmol, 40% isolated yield, quantitative ¹H NMR yield) as a mixture of diastereomers. ¹H NMR (CDCl₃, 400 MHz, δ): 7.33-7.32 (d, *J*=8.0 Hz, 0.9H), 7.29 (d, *J*= 8.0 Hz, 1H), 7.20-7.17 (m, 2H), 3.69-3.59 (m, 2H), 3.55 (s, 2H), 3.53-3.40 (m, 2H), 3.37-3.31 (m, 2H), 3.29-3.25 (m, 1H), 3.18-3.14 (m, 1H), 2.83 (d, *J* = 6.1 Hz, 0.5H), 2.71 (d, *J* = 6.1Hz, 0.6H), 2.64-2.57 (m, 1H), 2.51-2.44 (m, 1.4H), 2.35 (d, *J*=9.7Hz, 0.5H), 1.6 (s, 1H), 1.49 (s, 1.6H), 1.03-0.99 (m, 3H); ¹³C NMR (CDCl₃, 100 MHz, δ): 205.8 (C), 204.3 (C), 176.4 (C), 176.0 (C), 165.9 (C), 165.4 (C), 133.7 (C), 133.4 (CH), 133.0 (C), 132.0 (C), 130.3 (CH), 130.2 (CH),

1539, 1457, 1350, 1300, 1250, 1110; LIFDI MS m/z $[M+Na]$ calcd for $C_{56}H_{104}N_2O_{23}$ 1195.6928
found 1195.6914

***N,N'*-(3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57-nonadecaoxanonapentacontane-1,59-diyl)bis(2-methyl-3-propionylcycloprop-2-ene-1-carboxamide) (**9**)**



To a solution of **S4** (55 mg, 0.05 mmol) in CH_2Cl_2 (1 mL, 0.05M) was added Dess Martin Periodinane, DMP, (50 mg, 0.12 mmol) in one portion. After 30 minutes, the reaction was diluted with 0.5 mL hexanes, filtered through celite and the filtrate was concentrated onto C-2 silica gel prepared as described above. Column chromatography (gradient from 0-8% methanol in dichloromethane) furnished **9** (35 mg, 0.03 mmol, 60%) as a colorless oil. 1H NMR ($CDCl_3$, 400 MHz, δ): 6.31(s, NH), 3.66-3.43 (m, 41H), 2.83-2.71 (m, 2H), 2.37 (s, 2H), 2.34 (s, 1H), 1.18-1.14 (t, $J = 7.3$ Hz, 3H; ^{13}C NMR (CD_2Cl_2 , 90 MHz, δ): 192.1 (C), 172.1 (C), 123.1 (C), 105.8 (C), 70.5 (CH_2), 70.4 (CH_2), 70.2 (CH_2), 69.8 (CH_2), 39.3 (CH_2), 36.7 (CH_2), 26.1 (CH), 11.5 (CH_3), 7.6 (CH_3); FT-IR (neat, cm^{-1}): 2870, 1625, 1539, 1457, 1350, 1300, 1250, 1110; LIFDI MS m/z $[M + Na]$ calcd for $C_{56}H_{100}N_2O_{23}$ 1192.6615, found 1192.6620

Protein labeling experiment

To Thioredoxin (Trx, 5 μL of a 1 mM stock solution in H_2O) in 420 μL pH 6 acetate buffer was added aqueous tris(hydroxypropyl)phosphine (THP, 25 μL of a 1 mM stock solution in H_2O). After 12 h, **4** (50 μL of a 5 mM stock solution in methanol) was added. The final concentrations were Trx (10 μM), THP (50 μM) and **4** (500 μM). Within 10 minutes of addition, ESI-MS

analysis in the positive mode (Shimadzu LCMS-2020) indicated double alkylation of Trx (deconvoluted m/z 11998).

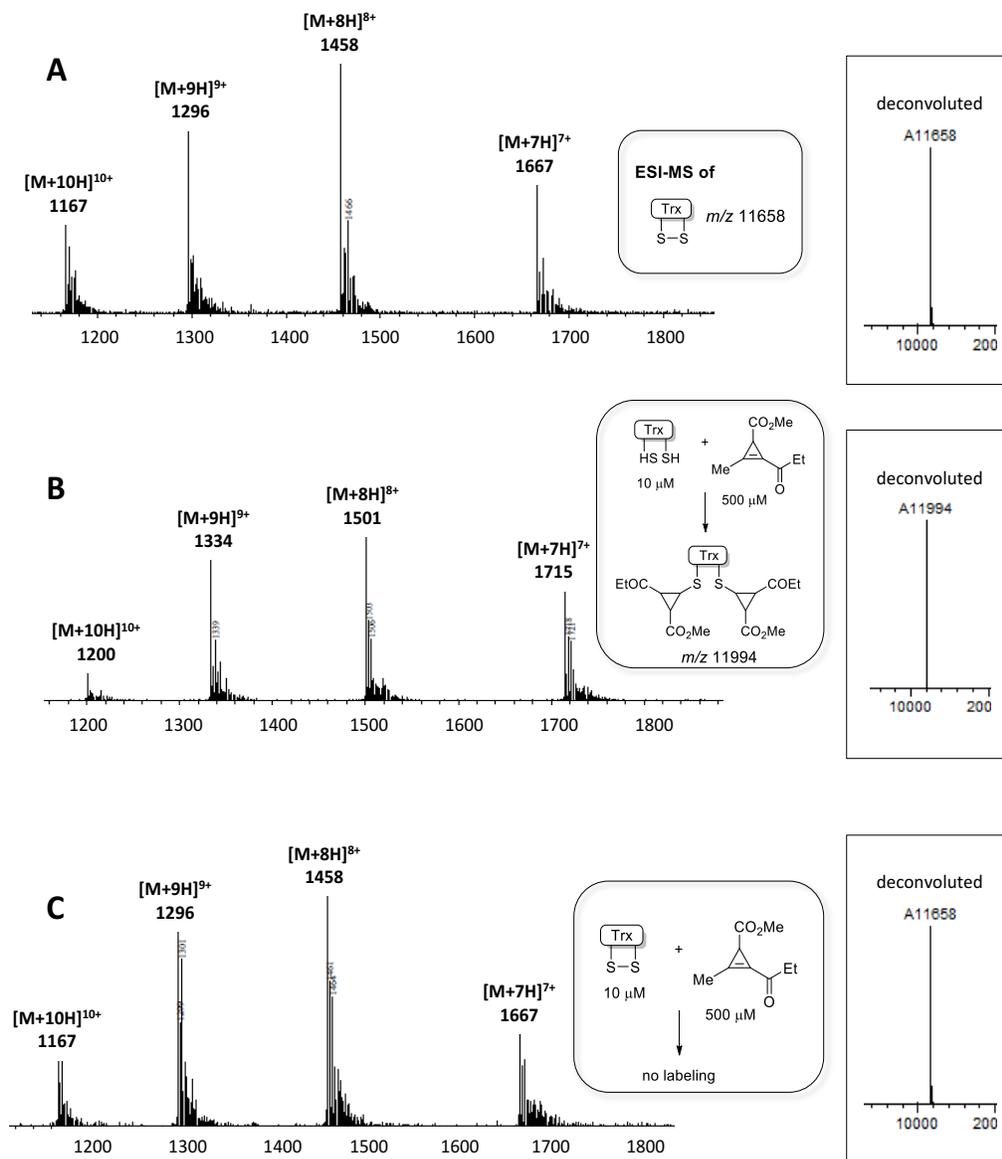


Figure S1: Protein labeling by cyclopropenyl ketone **4** is selective for cysteine modification (a) ESI-MS of Trx. The raw mass spectrum was deconvoluted using the MagTran software package. (b) Double labeling is observed by ESI-MS for Trx (10 μ M) that had been reduced by THP (50 μ M) and subsequently reacted with **4** (500 μ M) for 10 min. (c) No labeling is observed by ESI-MS when Trx (10 μ M) is directly incubated with **4** (500 μ M) for 10 min.

Stability of **2** in 9:1 D₂O/CD₃OD determined by ¹H NMR

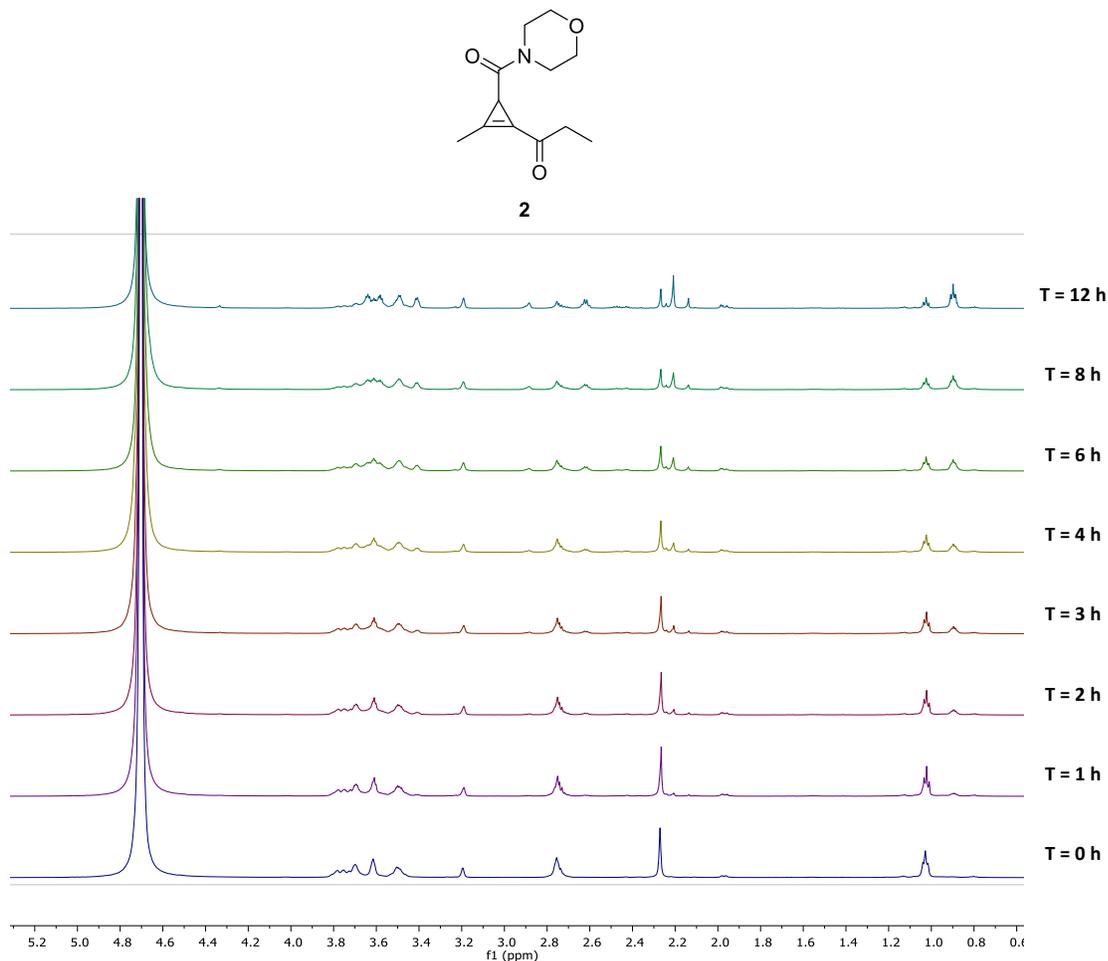


Figure S2: 20 mM solution of **2** in 9:1 D₂O/CD₃OD was prepared and followed over the course of 12 hours

Kinetic analysis of the reaction between **4 and glutathione under pseudo first order conditions**

The reaction between **4** and glutathione (GSH) was measured under pseudo-first order conditions with 10, 20 and 40 equivalents of GSH by following the exponential decay of **4** at 252 nm over time using an SX 18MV-R stopped-flow spectrophotometer (Applied Photophysics Ltd.).

Solutions were prepared in pH 7.4, 50 mM phosphate buffer with 1mM EDTA for **4** (0.5 mM, 0.5% MeOH) and GSH (5 mM, 10 mM and 20 mM) and thermostated in the syringes of the spectrophotometer before measuring. The pH values of the GSH samples were adjusted to 7.4 before kinetics experiments were run. An equal volume of each was mixed by the stopped-flow device, resulting in a final concentration of 0.25 mM for **4** and 2.5 mM, 5 mM and 10 mM for GSH. Data was recorded for 0.1-5 seconds and performed in triplicate at 298 K. The averages of the observed rates (k_{obs}), determined by nonlinear regression analysis of the data points using Prism software (v. 6.00, GraphPad Software Inc.) were plotted against the concentration of GSH and the bimolecular rate constant k_2 , obtained from the slope of the plot was found to be $595 \pm 30 \text{ M}^{-1}\text{s}^{-1}$.

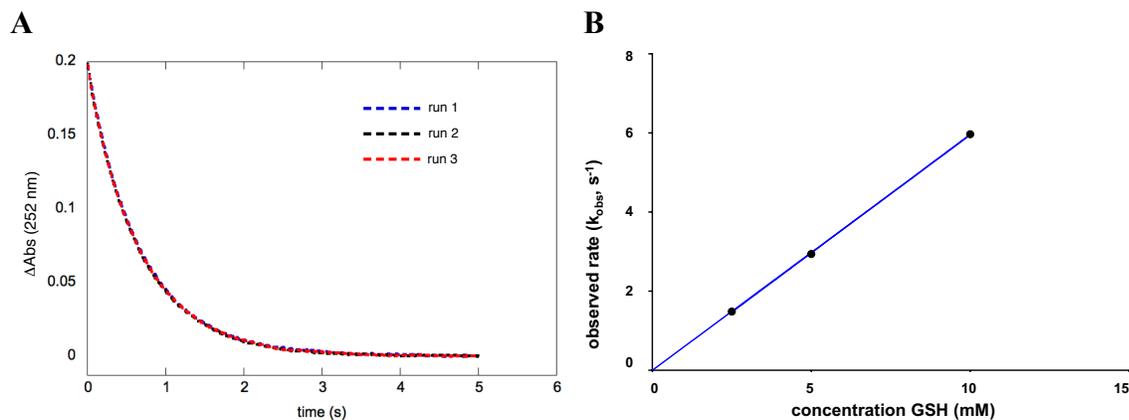


Figure S3: Stopped flow kinetics experiments were used to measure the rate of reaction between **4** (0.25 mM) and GSH (2.5, 5, 10 mM) at 25 °C in pH 7.5, 50 mM phosphate buffer with 1 mM EDTA containing 0.5 % MeOH. (A) triplicate kinetic data with 2.5 mM GSH. (B) Based on rates from triplicate runs at three different GSH concentrations, the second order rate constant was calculated to be $595 \pm 30 \text{ M}^{-1}\text{s}^{-1}$

LCMS Competition Experiment

To GSH, (25 μL of a 1mM stock solution in pH 6 acetate buffer) in 450 μL pH 6 acetate buffer was added a freshly made 1:1 mixture of **4** and *N*-ethyl maleimide, NEM, (25 μL of a 10 mM stock solution in pH 6 acetate buffer). Final concentrations were GSH (50 μM) and 1:1 mixture of **4**/NEM (500 μM). Within 10 minutes of the addition, mass spectrometry analysis using a

Shimadzu LCMS-2020, operating under negative electrospray ionization (ESI) mode identified adducts of both NEM and **4** as present in the crude mixture (m/z [M-H] 431 and 474 respectively).

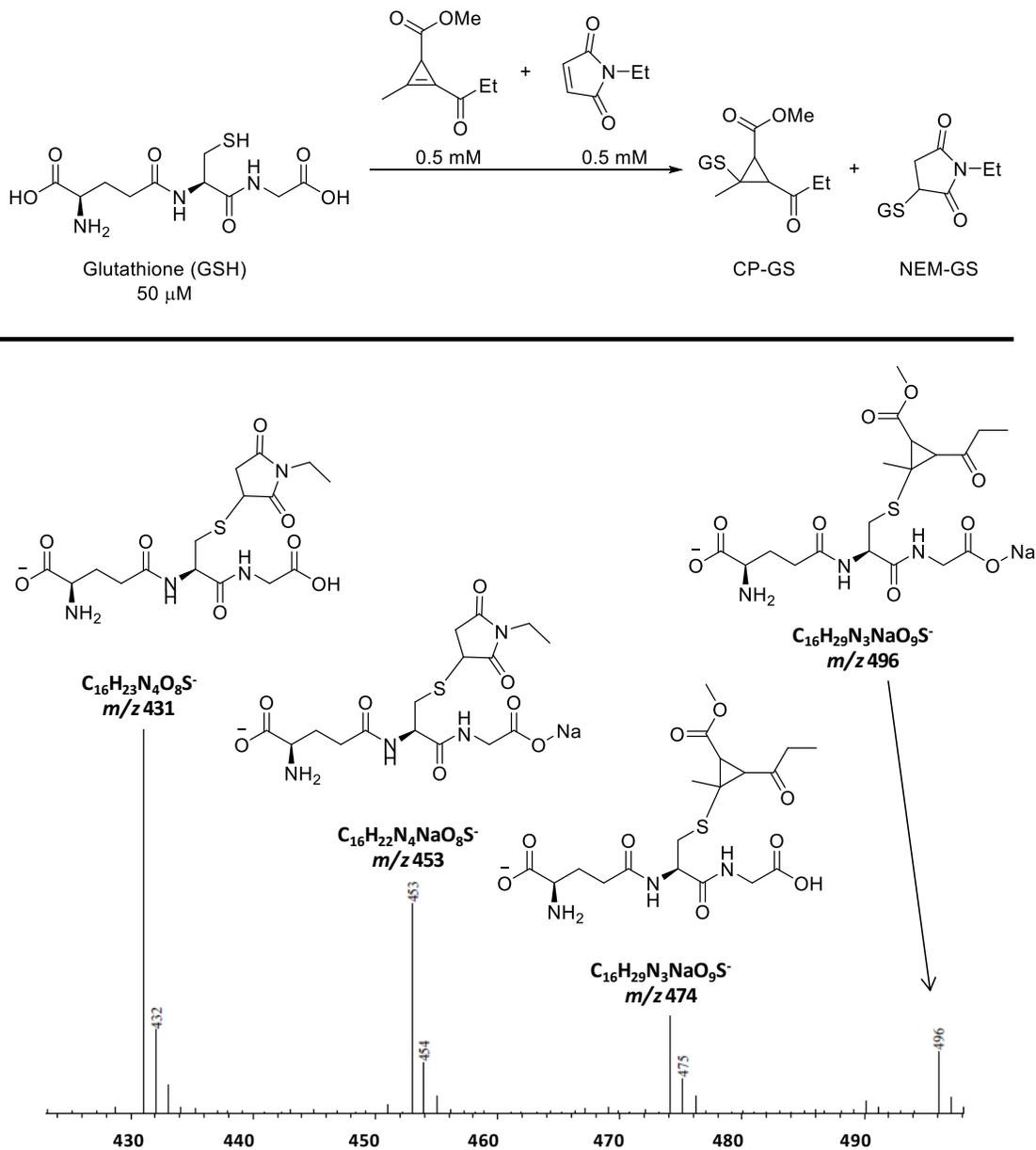


Figure S4: Competition experiment between NEM and **4** for GSH. ESI-MS in positive mode shows that alkylation by **4** competes with NEM.

HPLC Stability studies

Evaluating the stability of cyclopropenyl ketone thiol adducts in reducing conditions and in human plasma

Control experiment: 4 (30 μ L of a 10 mM stock solution in methanol) was added to 120 μ L of a 10:1 mixture of GSH (20 mM) and MPA (2 mM) in pH 6 acetate buffer. Within 10 minutes of the addition, mass spectrometry analysis using a Shimadzu LCMS-2020, operating under positive electrospray ionization (+ESI) mode was conducted. Masses corresponding to adducts of both GSH and MPA were observed (m/z [M+H] 531 and 392 respectively).

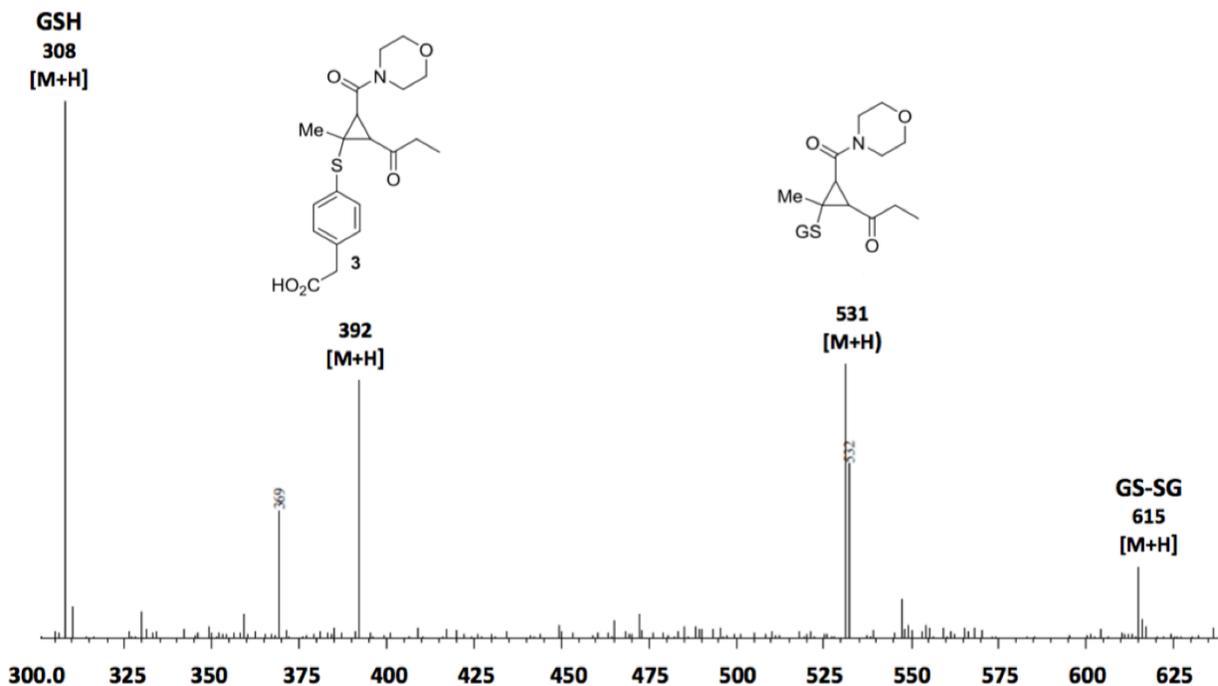


Figure S5: Reacting **2** with a 10:1 mixture of GSH:MPA demonstrates that should **2** be liberated during the course of the incubation study **3** should be observed.

Cyclopropenyl ketone thiol adduct stability in reducing conditions study

To a 245 μL solution of **3** (5 μL of a 10 mM stock solution in MeOH in 240 μL of pH 7.4 50 mM phosphate buffer with 1 mM EDTA), was added GSH (5 μL of a 100 mM stock solution in pH 7.4 50 mM phosphate buffer with 1 mM EDTA). Final concentrations were **3** (0.2 mM) and GSH (2 mM). Samples were incubated at 37°C and removed periodically for reverse phase HPLC analysis (254 nm) on a HP 1090 Series .system equipped with an analytical Halo C18 (7.5 x 3.0 mm, 2.7 μL) column. A linear gradient from 10 % to 100 % solvent B was run over 32 min at 0.2 mL/min, where solvent A is 0.1 % formic acid in water and solvent B is 0.1 % formic acid in acetonitrile . Areas of the peaks attributed to **3** were integrated in each sample taken to calculate stability. The identities of the compounds present in each peak were determined by LC-MS analysis using a Shimadzu LCMS-2020, operating under positive electrospray ionization (+ESI) mode, connected to an LC-20AD (Shimadzu, Kyoto, Japan).

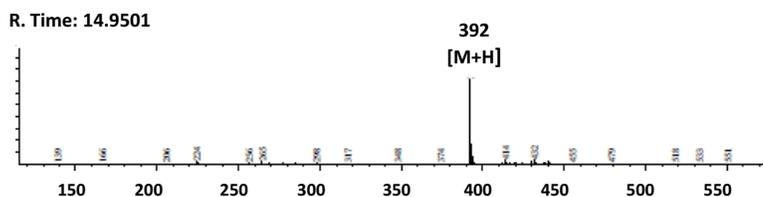
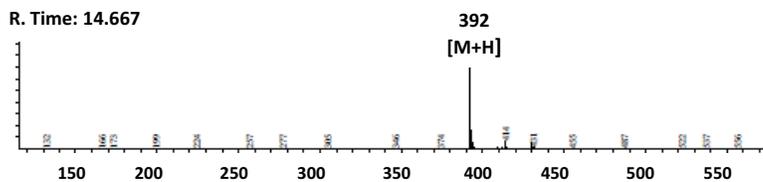
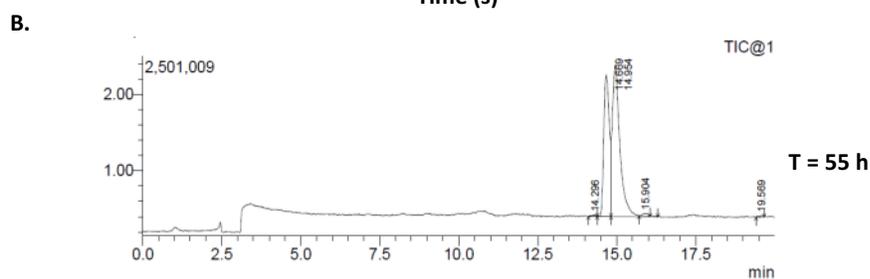
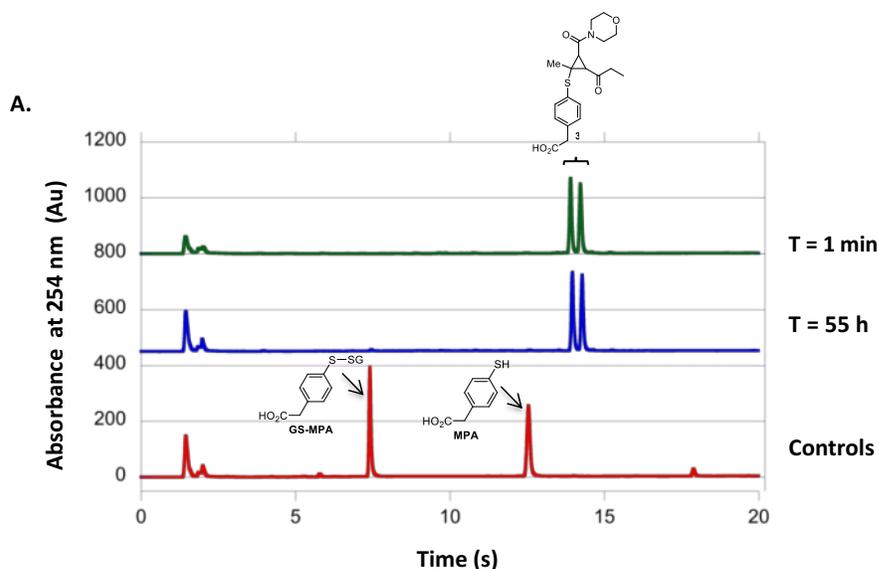


Figure S6: (a) Shown are HPLC traces of stability of **3** (0.2 mM) in pH 7.4, 50 mM phosphate buffer with 1 mM EDTA incubated with GSH (2 mM) at 37°C over a period of 55h. The peak area for the diastereomers of **3** remains the same over time. (b) LCMS spectrum of T = 55 h confirms that **3** remains intact under these conditions.

Cyclopropenyl ketone thiol adduct stability in human serum study

3 (40 μ L of a 10 mM stock solution in methanol) was incubated in human serum (from human male AB plasma, USA origin, sterile-filtered, procured from Sigma-Aldrich) (1960 μ L) at 37°C over a period of 8 days. Periodically, 250 μ L aliquots were removed and subjected to centrifugal filtration using an Amicon Ultra – 0.5 Centrifugal Filter Unit with Ultracel – 10 membrane to allow for large protein removal, and the filtrate obtained assayed by HPLC using the same conditions as described in the previous stability study. The peaks corresponding to **3** were integrated to determine the stability of the adduct over time.

Hydrogel formation and rheological characterization

Four-arm thiol-functionalized poly(ethylene glycol) (PEG-4-SH, $M_n \sim 20,000$ g/mol) was synthesized as previously described.^{4,5} PEG-4-SH was dissolved in phosphate buffered saline (PBS, pH ~ 7), and biscyclopropenyl ketone **9** was dissolved in PBS on the day of gel formation (< 2 hours prior to polymerization) and stored on ice. Monomer solutions of PEG-4-SH and biscyclopropenyl ketone **9** subsequently were mixed at 4 °C with a 1:1 thiol:ene molar ratio to form 10-wt% hydrogels (w/w with respect to PEG-4-SH). For rheological studies, hydrogels were formed directly on the rheometer (AR-G2, TA Instruments, USA): the precursor solution was mixed and added directly onto a Peltier plate, and a 8-mm parallel plate geometry immediately was lowered (200- μ m gap). Time sweep measurements were carried out within the linear viscoelastic regime (2% constant strain mode at a frequency of 6 rad s⁻¹) at 37 °C, and three independent samples were analyzed.

Hydrogel degradation

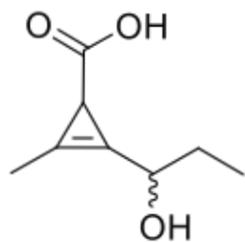
Hydrogels were formed by mixing a 10-wt% hydrogel precursor solution, as described above, in a cylindrical mold (diameter = 4.6 mm, thickness = 1.8 mm, volume = 20 μ L; e.g., 1-mL syringe with the end removed) and incubated overnight at 37 °C with parafilm to cover the top and prevent drying. The resulting hydrogels were washed with PBS (2 mL) and incubated in

PBS (control) or a reducing microenvironment (10 mM glutathione [GSH] dissolved in PBS) at room temperature over the experimental time frame. At predefined time points, the mechanical properties of the hydrogels were assessed using oscillatory rheometry within the linear viscoelastic regime (2 rad/s, 2% strain, 0.25 N force to avoid hydrogel slip, 25 °C maintained with Peltier plate), and three independent samples per time point were analyzed.

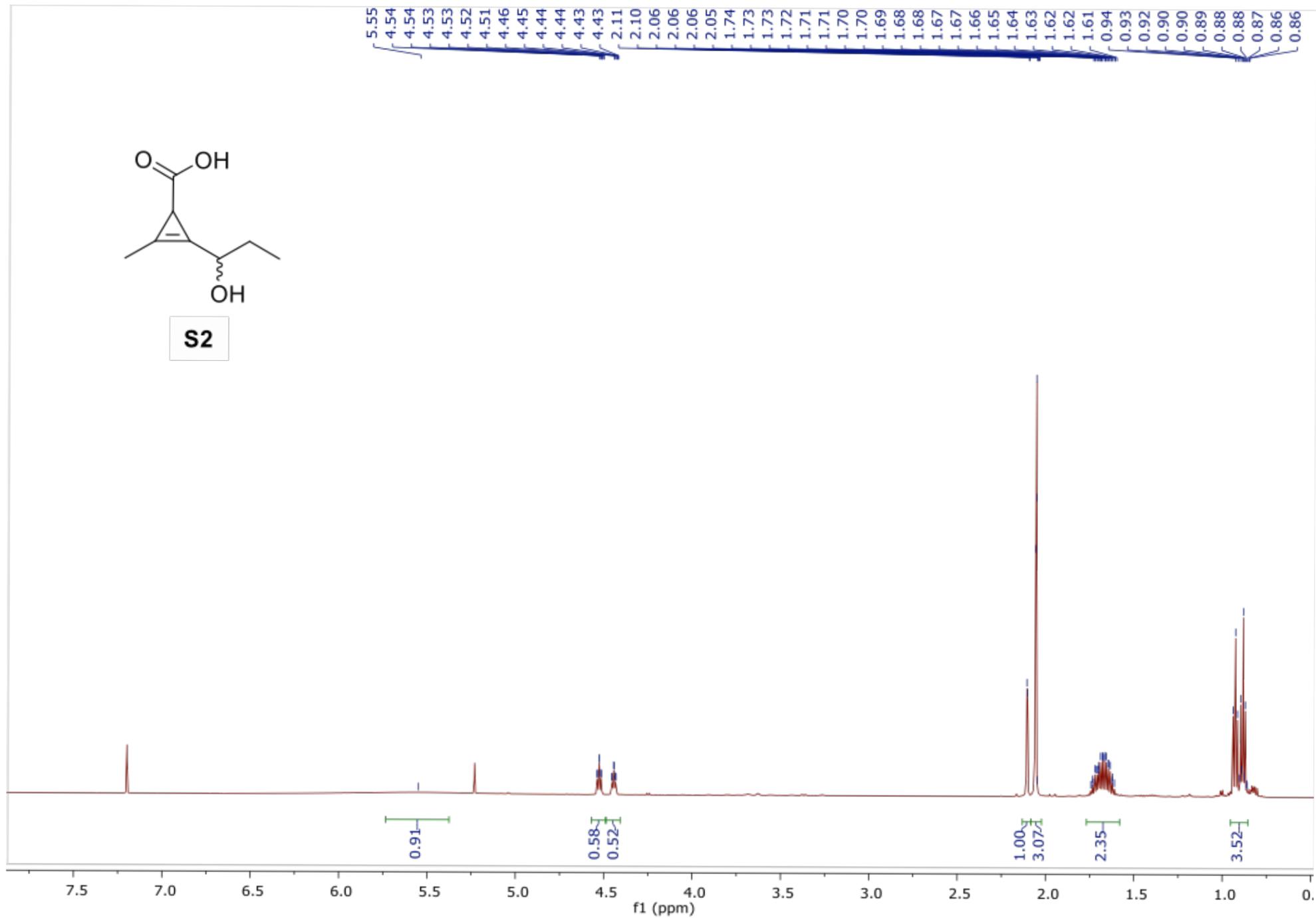
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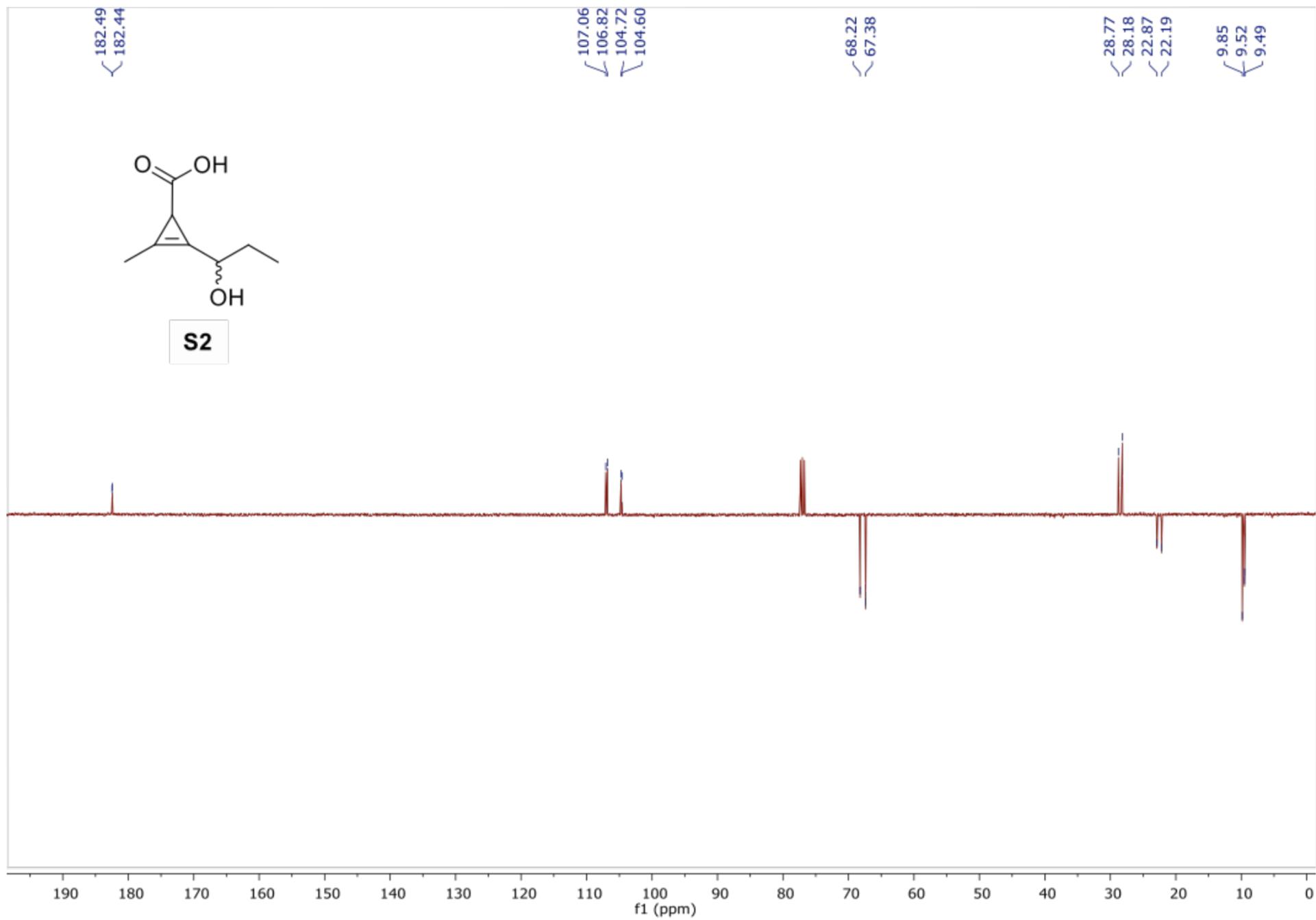
¹H NMR (CDCl₃, 600 MHz) of **S2**



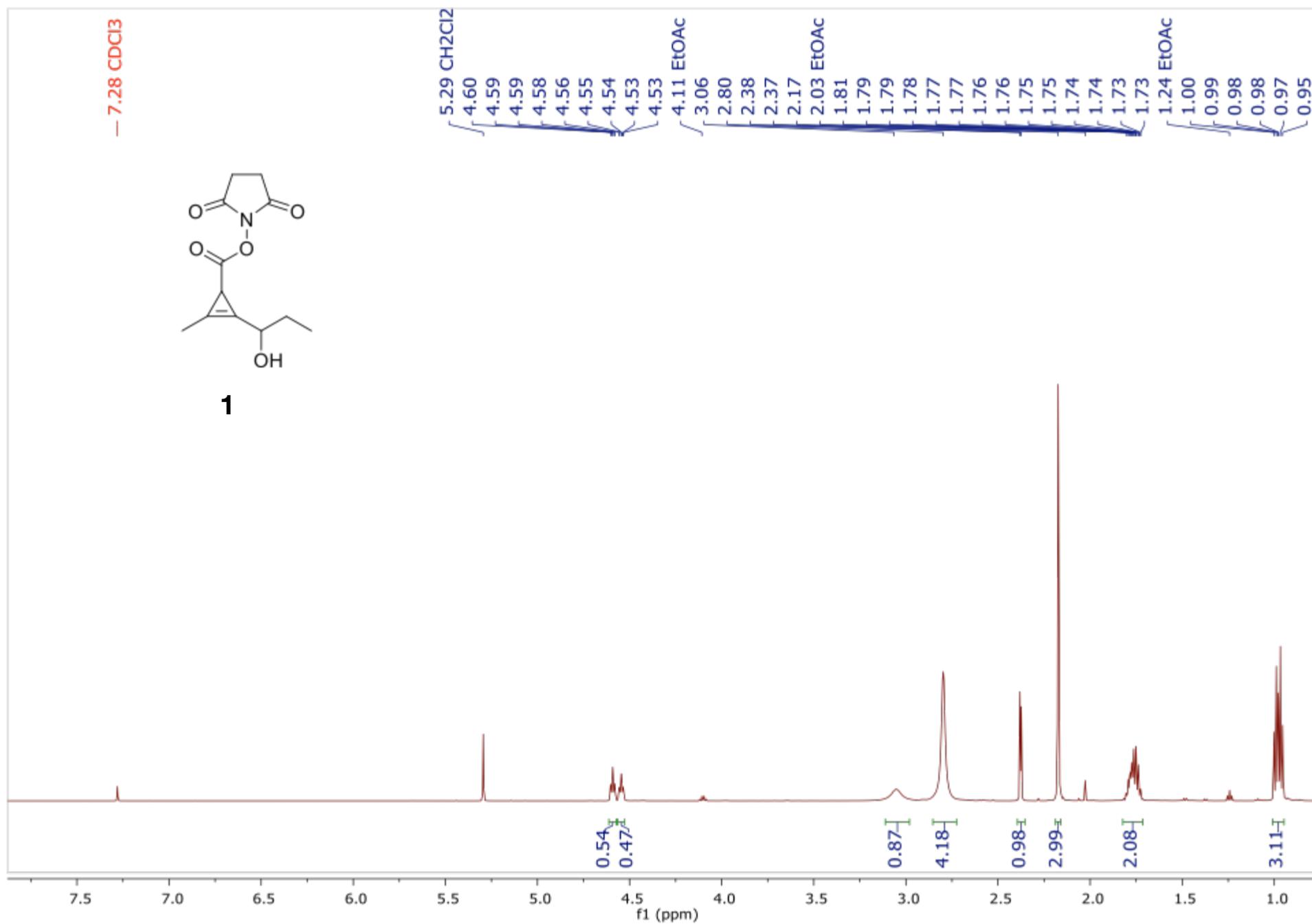
S2



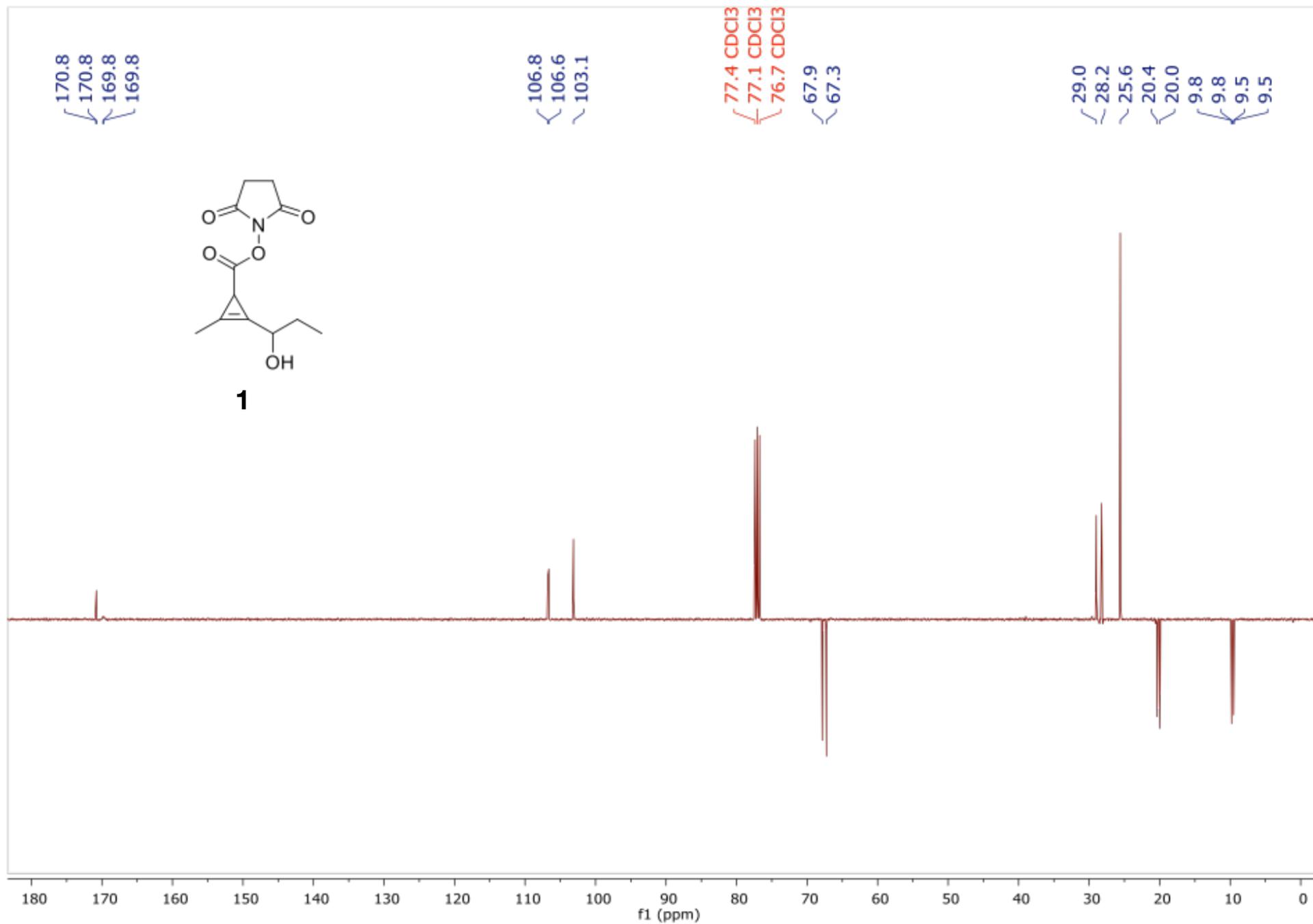
^{13}C NMR (CDCl_3 , 150 MHz) of **S2**



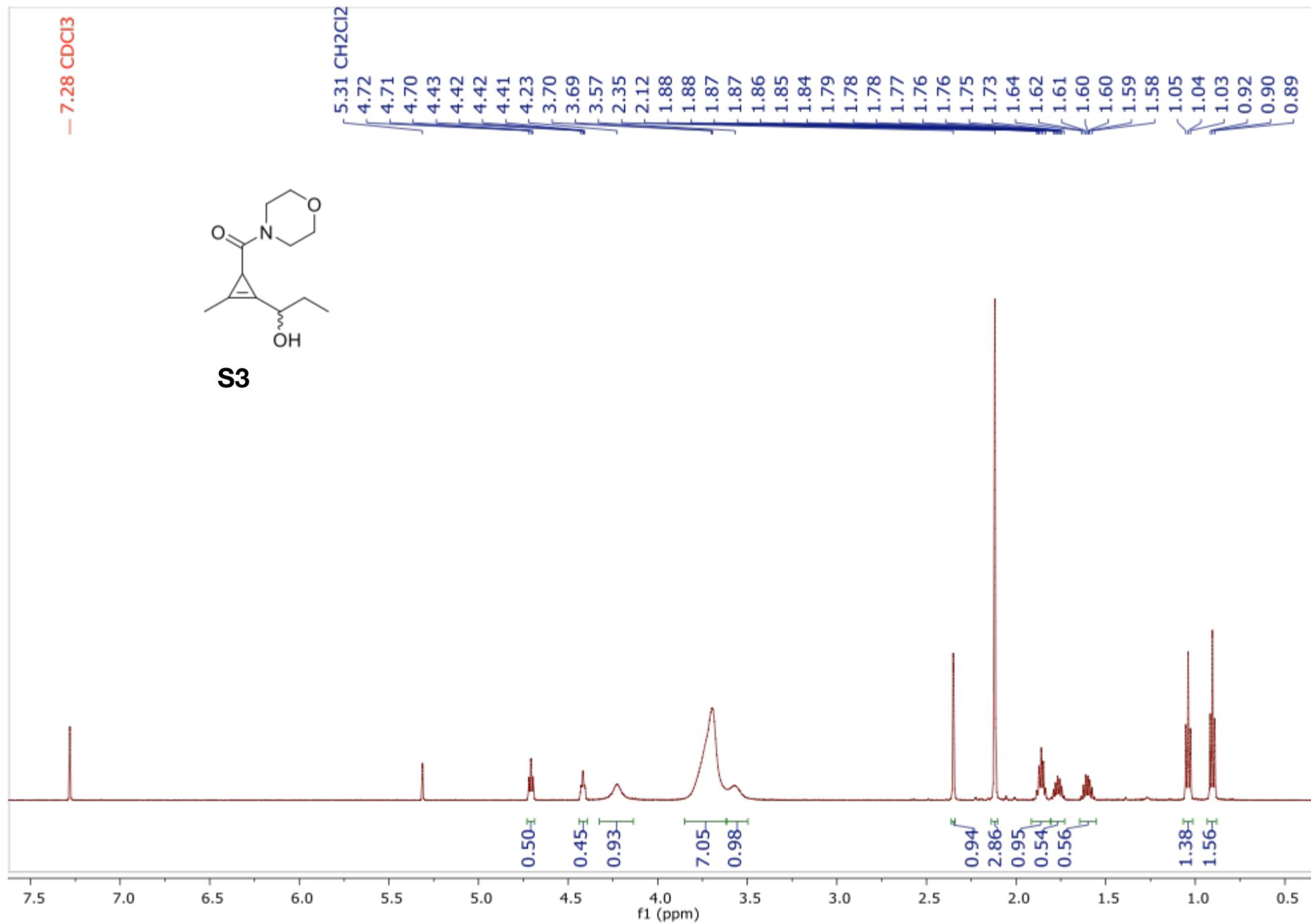
^1H NMR (CDCl_3 , 600 MHz) of **1**



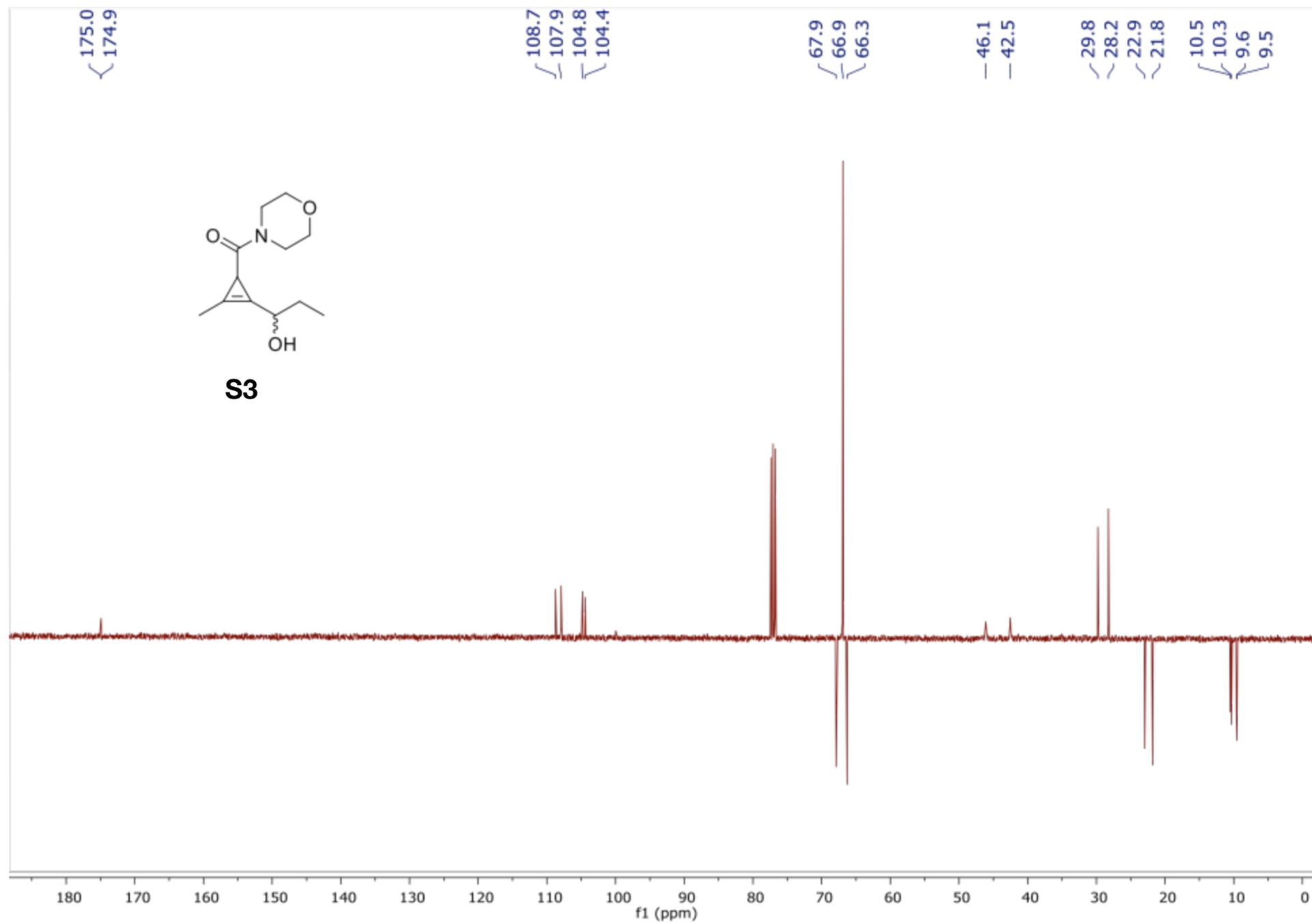
¹³C NMR (CDCl₃, 150 MHz) of **1**



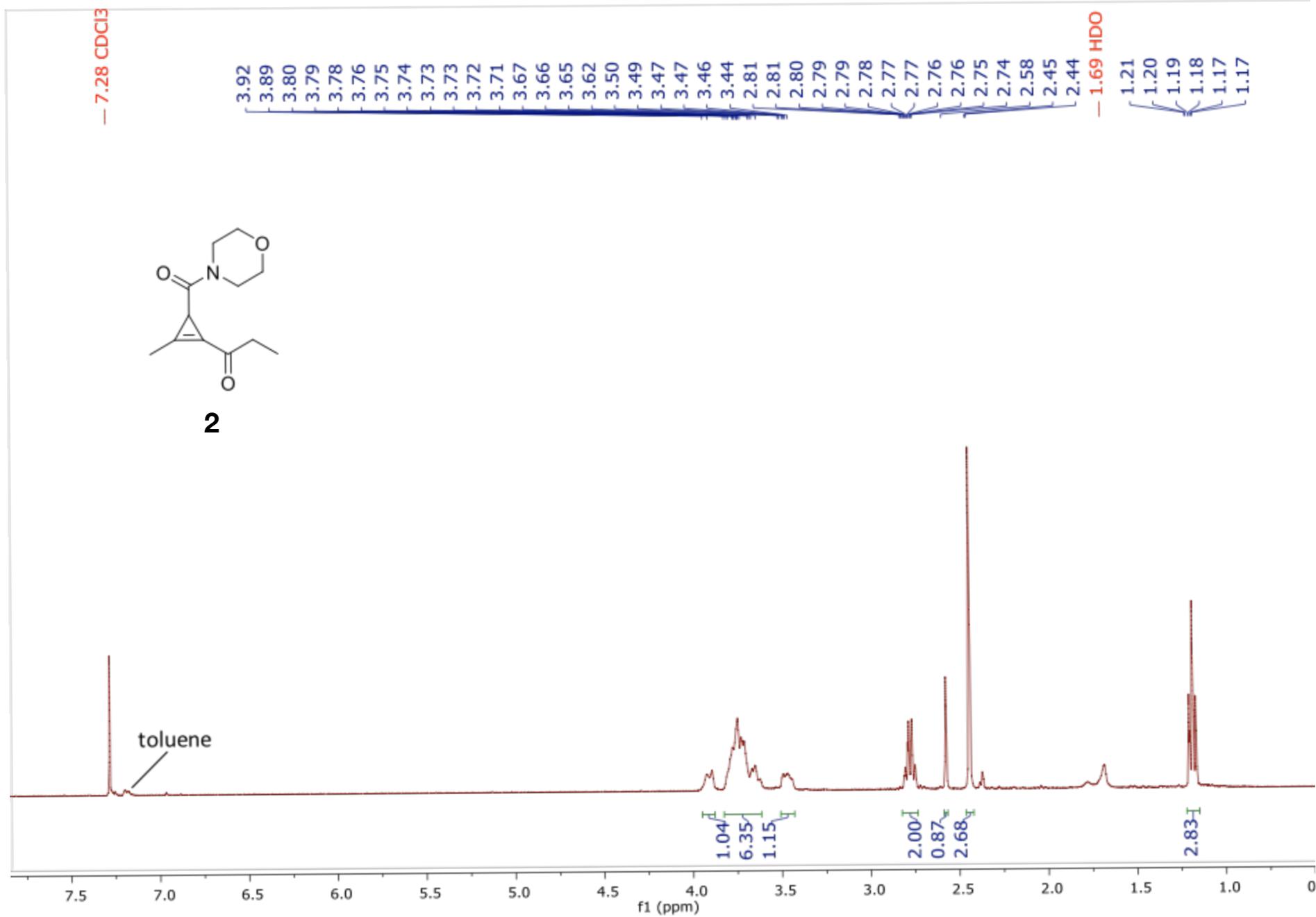
^1H NMR (CDCl_3 , 600 MHz) of **S3**



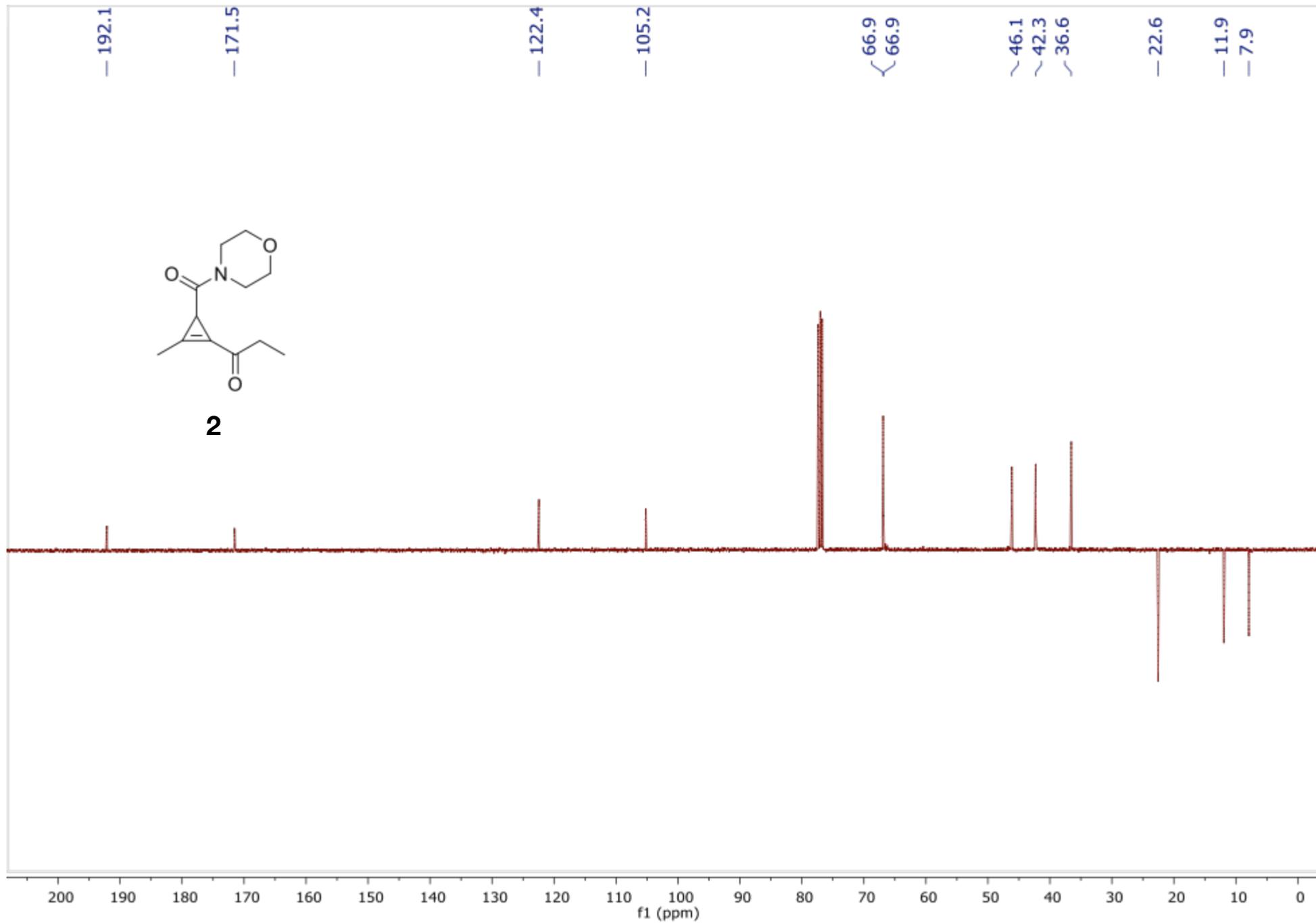
^{13}C NMR (CDCl_3 , 150 MHz) of **S3**



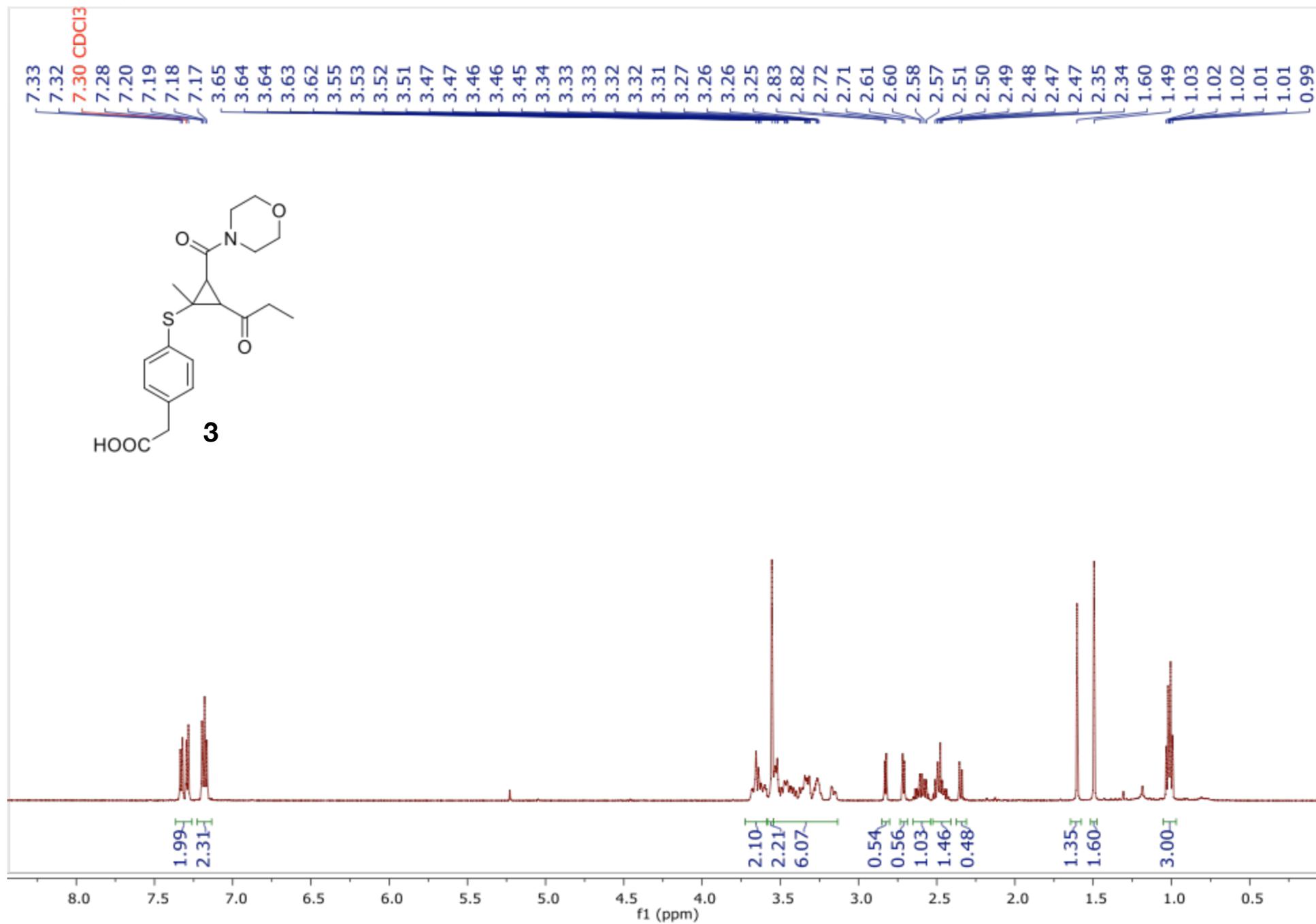
^1H NMR (CDCl_3 , 600 MHz) of **2**



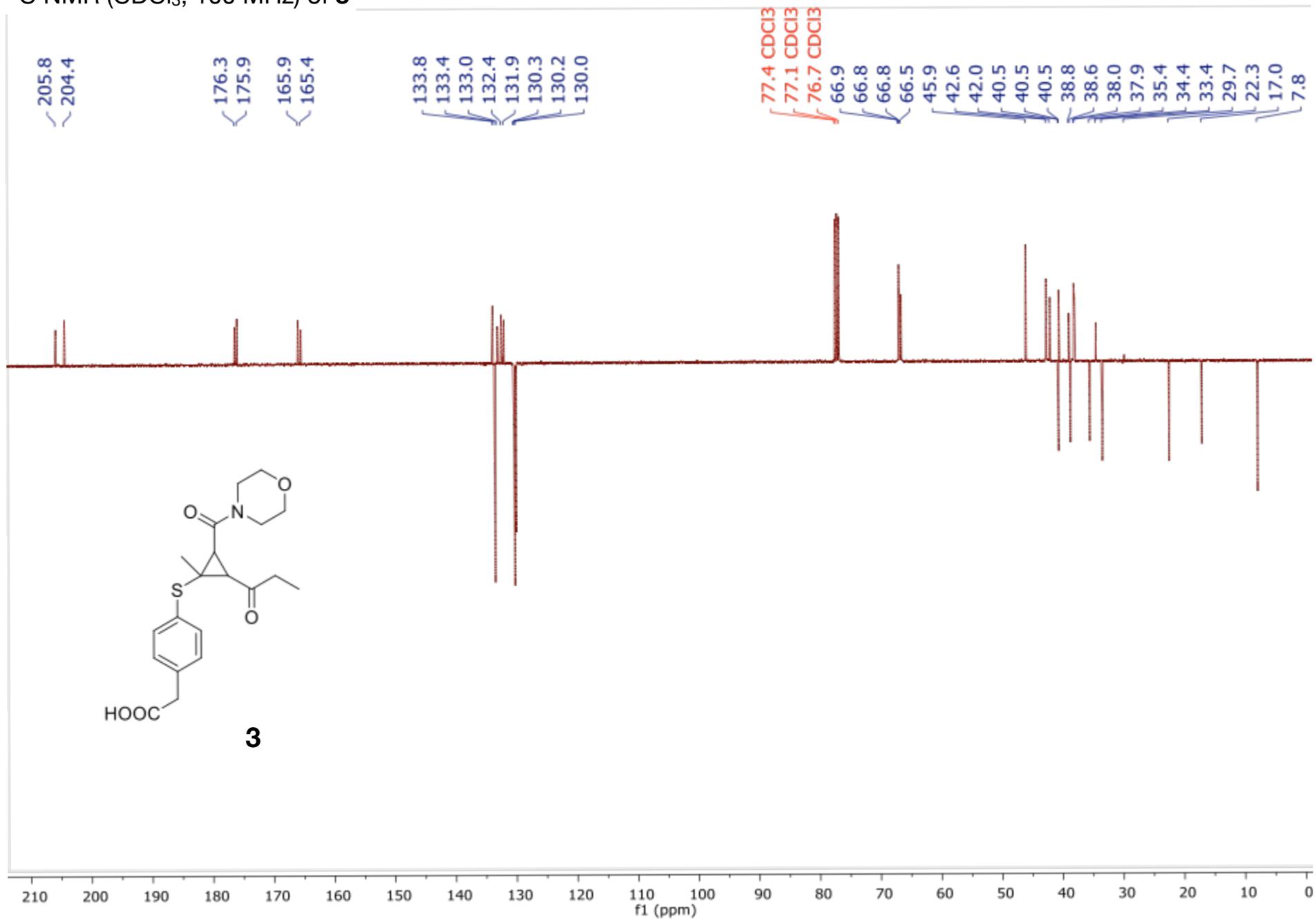
^{13}C NMR (CDCl_3 , 150 MHz) of **2**



^1H NMR (CDCl_3 , 400 MHz) of **3**

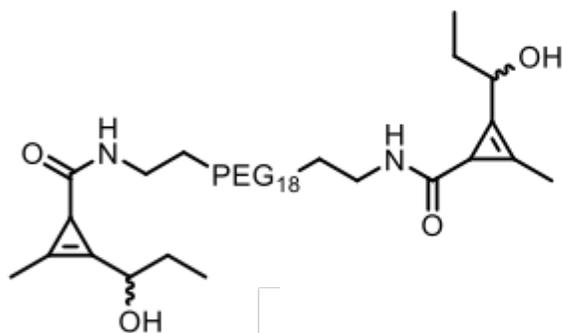


^{13}C NMR (CDCl_3 , 100 MHz) of **3**

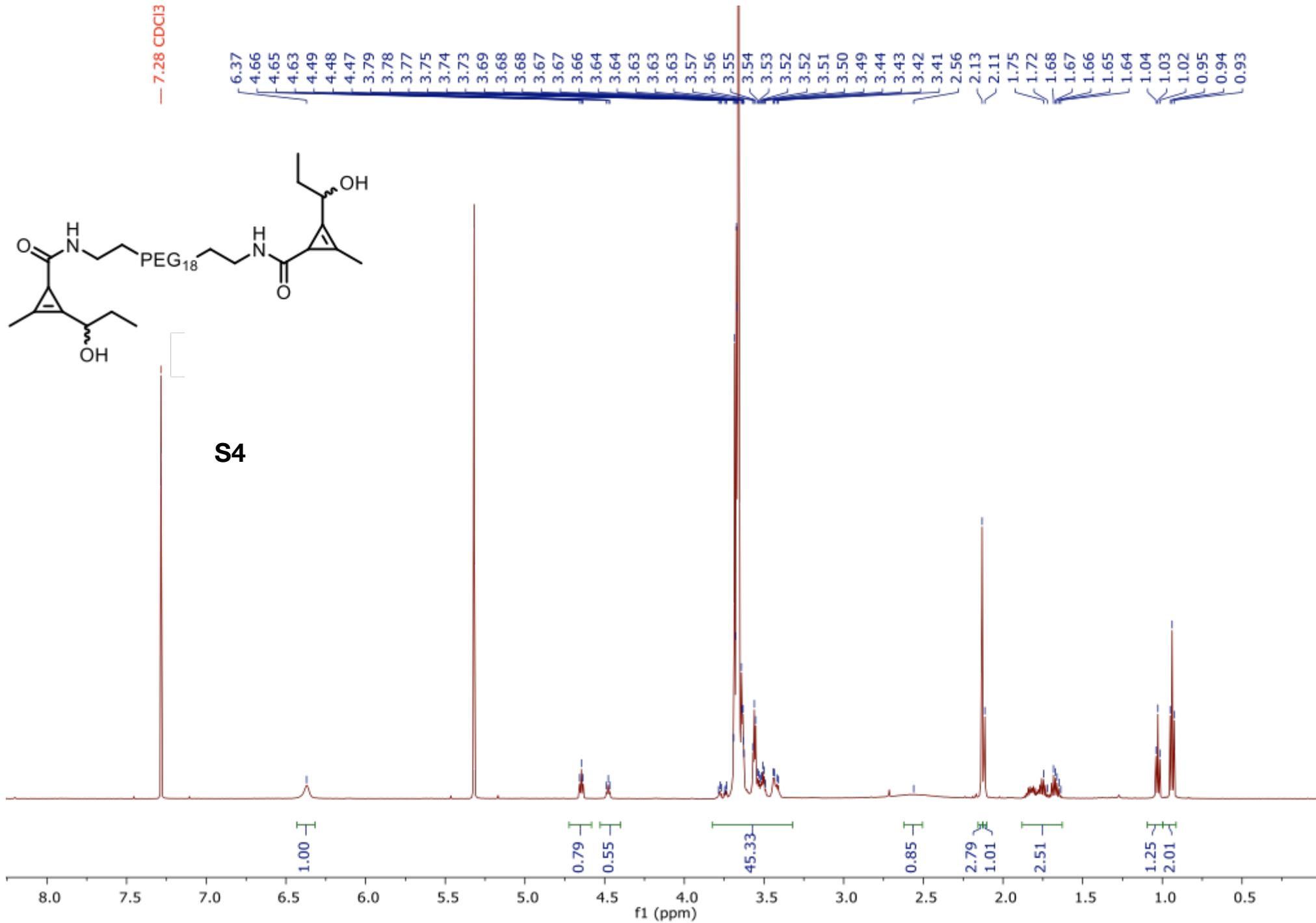


^1H NMR (CDCl_3 , 400 MHz) of **S4**

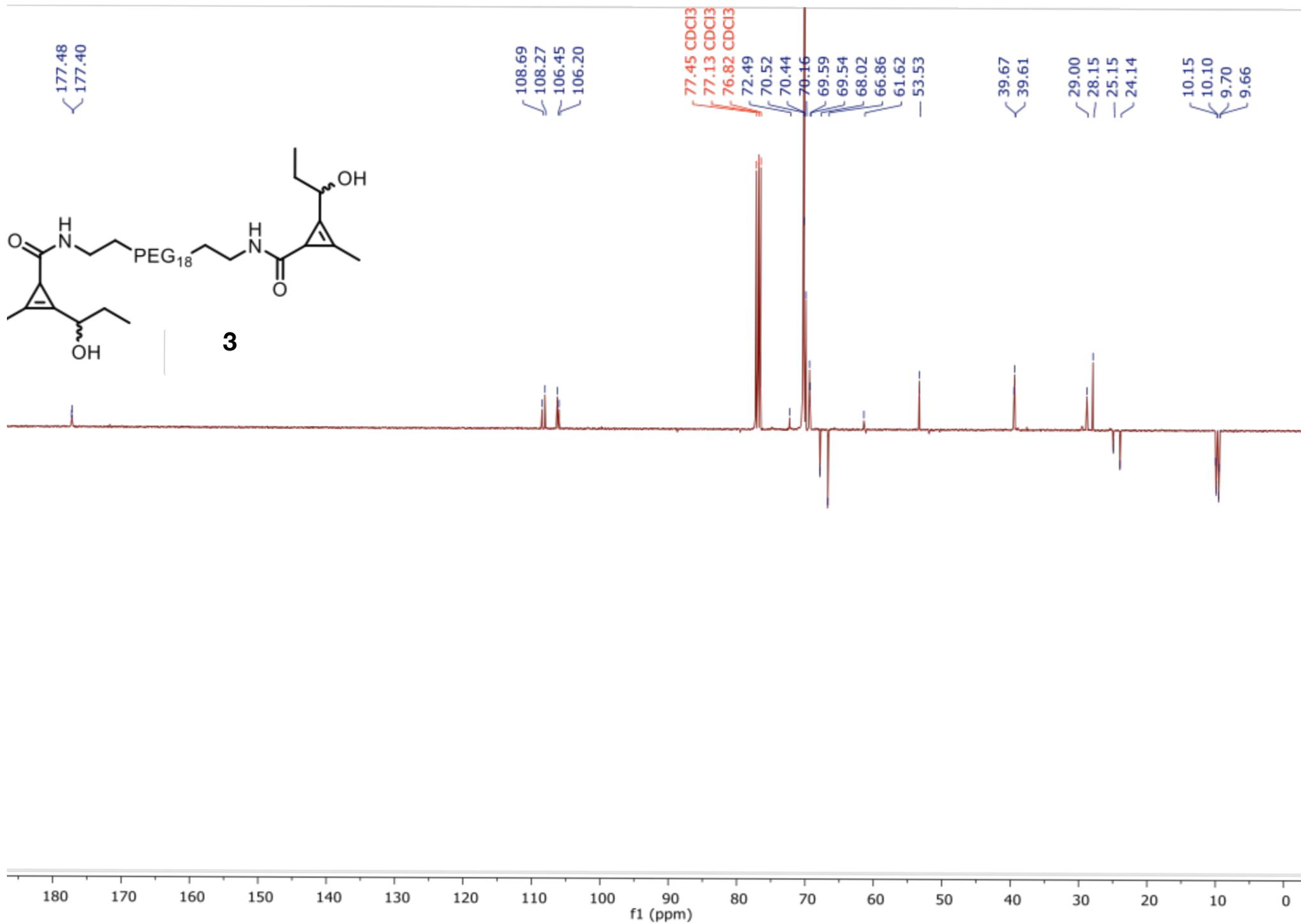
— 7.28 CDCl_3



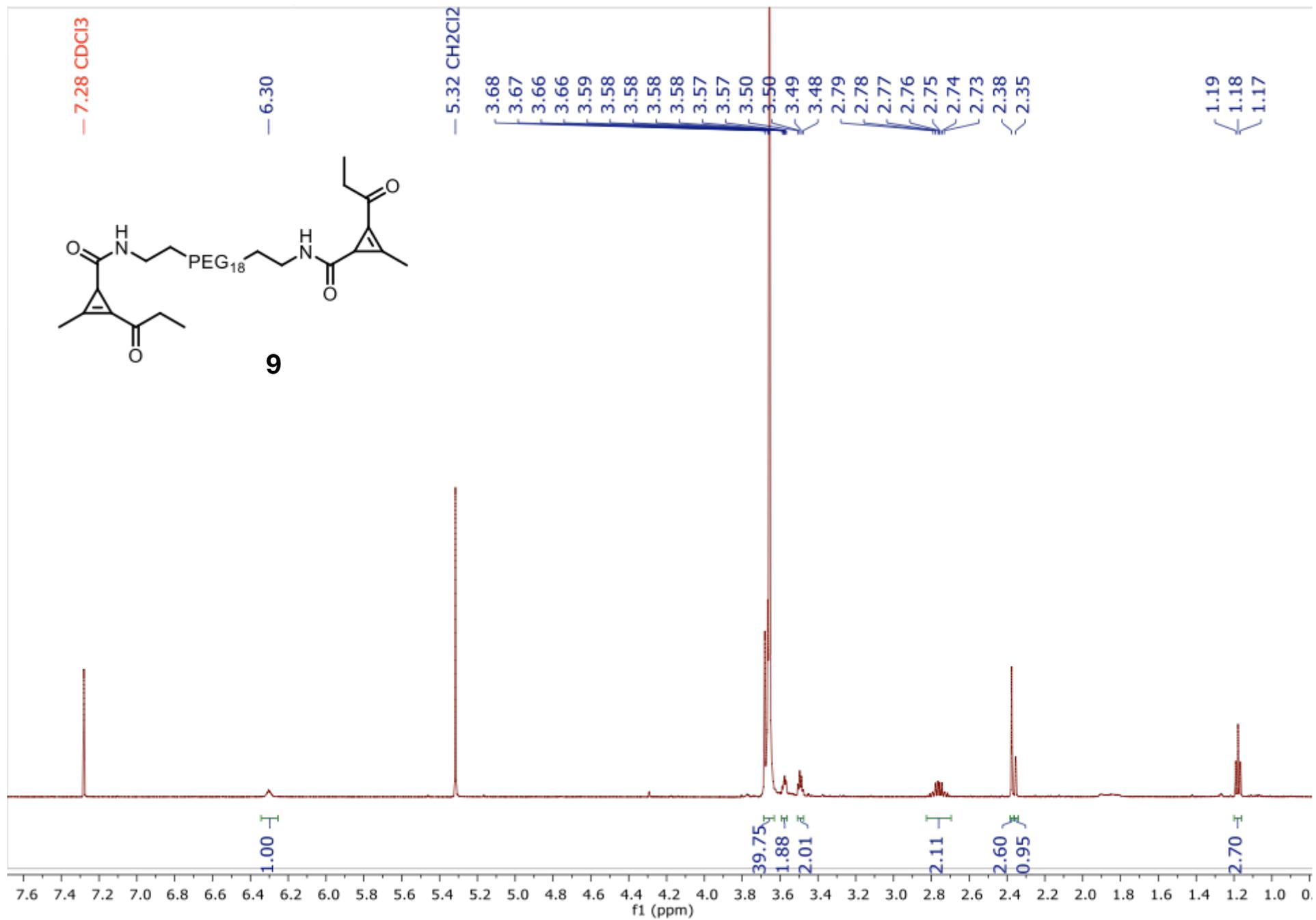
S4



^{13}C NMR (CDCl_3 , 100 MHz) of **S4**



^1H NMR (CDCl_3 , 400 MHz) of **9**



^{13}C NMR (CDCl_3 , 100 MHz) of **9**

