Supporting Information

Chemical Proteomic Identification of Functional Cysteines with Atypical Electrophile Reactivities

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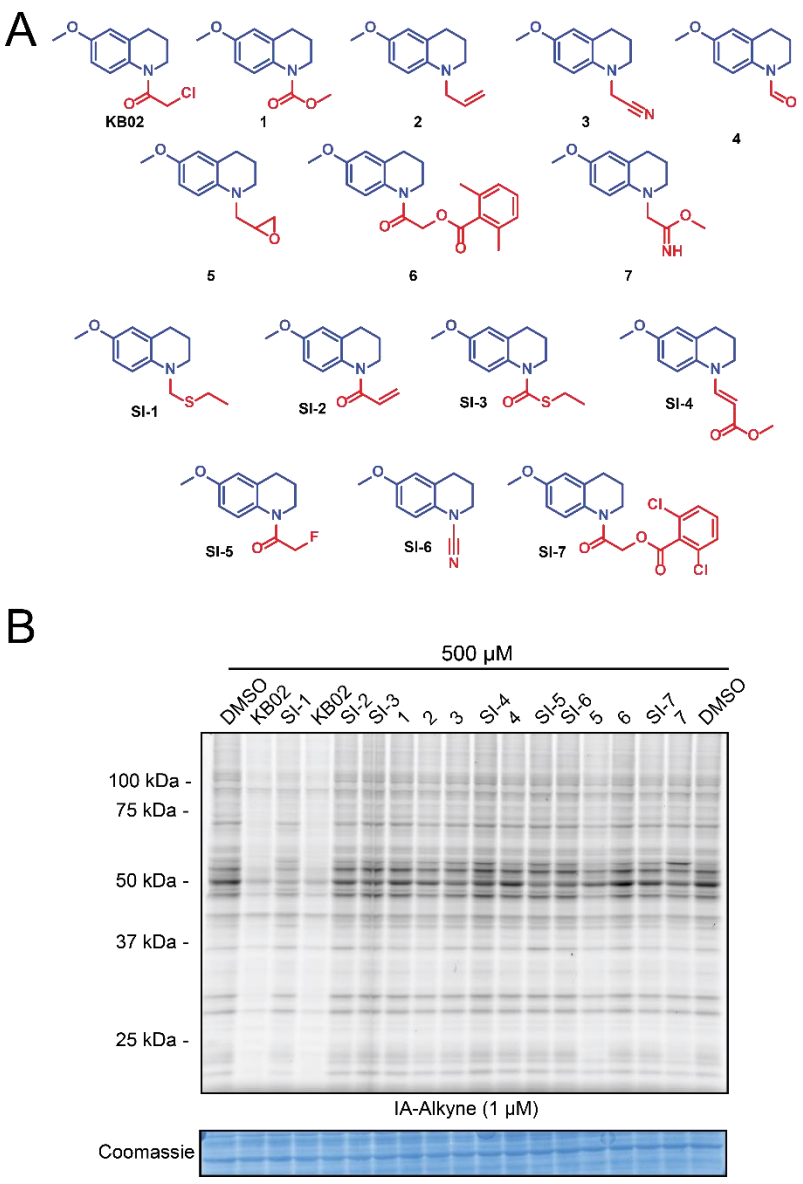
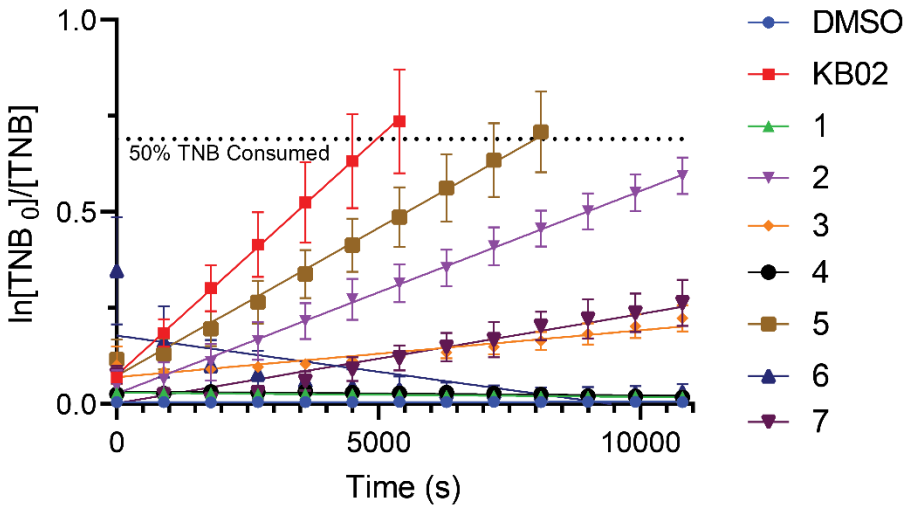
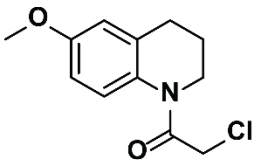


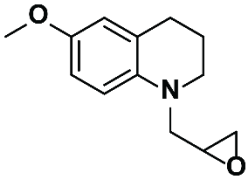
Supplementary Figure S1. Candidate electrophilic compounds and initial gel-ABPP analysis. (**A**) Structures of the α-chloroacetamide (αCA) scout fragment (KB02) and candidate electrophilic compounds **1-7** and **SI-1-7**. The recognition group (blue) remains constant throughout and the reactivity group (red) was varied. (**B**) In-gel fluorescence image of competitive blockade of IA-alkyne (1 µM, 1 h) protein labeling by compounds, KB02, or DMSO (500 µM, 1 h) in Ramos lysates. Coomassie stained gel shown as a loading control.





KB02

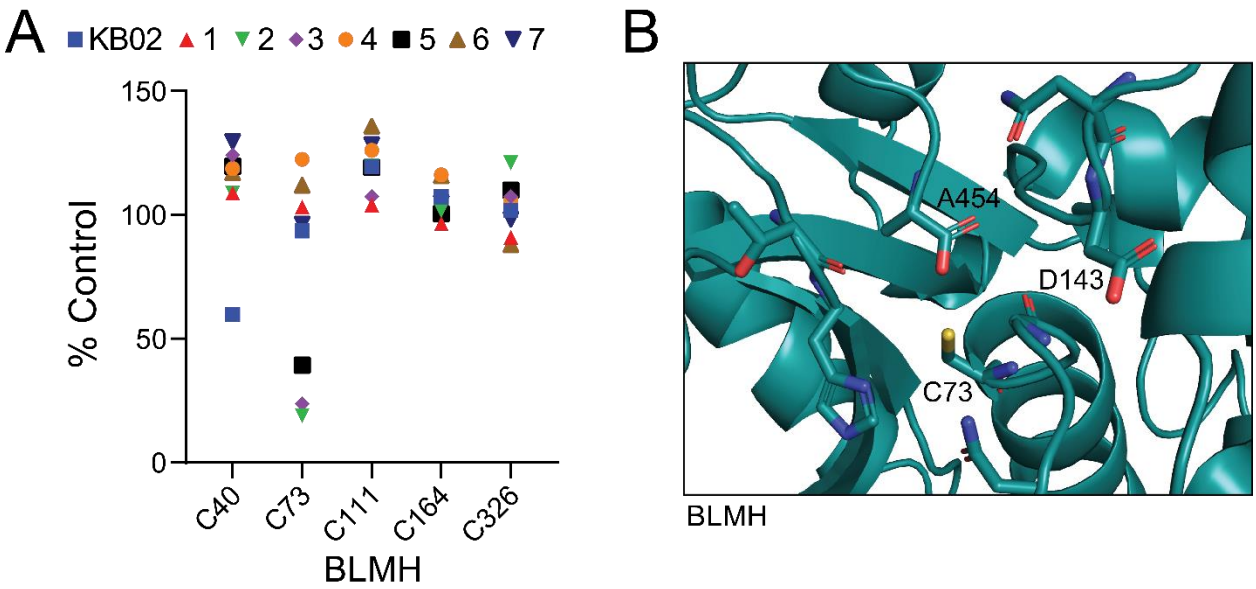
$k = 1.2 \times 10^{-4}\ M^{-1}s^{-1}$



5

$k = 7.7 \times 10^{-5}\ M^{-1}s^{-1}$

Supplementary Figure S2. Kinetic analysis of thiol reactivity for candidate electrophilic compounds (500 µM, up to 3 h) measuring the consumption of 2-nitro-5-thiobenzoic acid (TNB^2, 100 µM) over time. The concentration of compounds was assumed to be insignificantly consumed and therefore would not significantly affect the rate. Data were collected until 50% TNB^{2-} was consumed or for 3 h, whichever occurred first. Second order rate constants are shown for the most reactive compounds. Compounds were tested in triplicate over three replicates.



Supplementary Figure S3. Amine containing candidate electrophilic compounds block IA-DTB labeling of BLMH_C73 potentially through a non-covalent mechanism. (**A**) **2**, **3**, and **5** site-specifically engage the active site nucleophile of BLMH (bleomycin hydrolase). In contrast, (form)amide/carbamate containing compounds, including KB02, did not block IA-DTB reactivity of BLMH_C73. (**B**) Crystal structure of BLMH highlighting the anionic active site (C-terminal A454 and D143) that is favorable for salt bridge formation with the protonated amine group of compounds **2, 3,** and **5**.

Methods

Cell Culture

Ramos cells were obtained from ATCC and cultured in RMPI1840 medium (Corning) with 10% v/v fetal bovine serum (Omega Scientific), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 µg/mL). Cells were incubated at 37 °C with 5% CO_2. The cells tested negative for mycoplasma contamination. Cells were harvested, frozen, and stored at -80 °C until used for in vitro studies.

Competitive Gel-ABPP Assay

Ramos cells were thawed on ice and resuspended in PBS. Cells were lysed by sonication (2 x 7 pulses) and centrifuged (3 min, 1400 *g*). The lysate was diluted to 1 mg/mL with PBS and 50 µg of protein were treated with DMSO or compound (0.5 µL of 50 mM DMSO stock) for 1 h at room temperature. Iodoacetamide-alkyne (IA-alkyne) was then added (0.5 µL of 0.1 mM DMSO stock) and incubated for an additional hour at room temperature. 6 µL of freshly prepared "click" reagent mixture was added to conjugate rhodamine azide to alkyne probe-labeled protein and incubated for 1 h at room temperature. CuAAC reaction mixture contained: 1 µL $CuSO_4$ (50 mM in water, final concentration = 1 mM), 3 µL TBTA (1.7 mM in 4:1 *t*BuOH:DMSO, final concentration = 100 µM), 1 µL TCEP (50 mM in water, final concentration = 1 mM), and 1 µL rhodamine azide (12.5 mM stock in DMSO, final concentration = 250 µM). Reactions were quenched with the addition of 30 µL of loading buffer. Samples were boiled and proteins were resolved by SDS-PAGE (10% acrylamide) and visualized by in-gel fluorescence on a Bio-Rad ChemiDOC MP flatbed fluorescence scanner. Gels were then stained with Coomassie blue as a loading control.

TMT-ABPP Sample Preparation

Ramos cells were thawed on ice and resuspended in PBS. Cells were lysed by sonication (2 x 7 pulses) and centrifuged (10 min, 9,000 *g*). The lysate was diluted to 2 mg/mL with PBS. 0.5 mL of lysate was treated with DMSO or compound (5 µL of 50 mM DMSO stock) and incubated for 1 h at room temperature while rotating. IA-desthiobiotin (5 µL of 10 mM DMSO stock) and incubated for an additional hour at room temperature while rotating. Proteins were pelleted with the addition of 600 µL of a MeOH/$CHCl_3$ (4:1) solution and centrifugation (3 min, 9000 *g*). The top layer was aspirated and the protein disk was washed with 500 µL of the MeOH/$CHCl_3$ solution and aspirated. An additional 300 µL MeOH/$CHCl_3$ was added and then centrifuged (5 min, 17,000 *g*). The protein pellets were air dried for 15 min. The pellet was resuspended by the addition of 90 µL of buffer (9M urea, 10 mM DTT, 50 mM TEAB pH 8.0) and heated for 20 min at 65 °C to resolubilize proteins. Samples were cooled and iodoacetamide (10 µL of 500 mM) was added and then incubated at 37 °C with shaking for 30 min. Sample was then diluted with 350 µL of 50 mM TEAB and 2 µg trypsin (8 µL of 20µg of trypsin was dissolved in 60 µL trypsin buffer and 20µL 100 mM $CaCl_2$) and incubated at 37 °C with shaking overnight. Samples were diluted with 350 µL 50 mM TEAB pH 8 and then streptavidin beads (80 µL of 1:1 slurry with 50 mM TEAB with 0.2% IGEPAL) were added. Probe labeled proteins were enriched for 3 h at room temperature while rotating. Beads were filtered using a BioSpin column and the beads were washed with Wash Buffer (3 x 1 mL, 50 mM TEAB pH 8, 150 mM NaCl, 0.1% IGEPAL), PBS (5 x 1 mL), and water (5 x 1 mL). BioSpin columns were transferred to a clean Eppendorf tube and peptides were eluted with 300 µL of 1:1 Water:MeCN with 0.1% formic acid and then evaporated to dryness in a Speedvac. Samples were resuspended in 100 µL of 200 mM EPPS

pH 8 with 30% MeCN, vortexed, and incubated at 37 °C with shaking for 15 min. Peptides were then labeled with 3 µL of the corresponding TMT-10plex tag solution. Samples were vortexed and incubated for 75 min at room temperature. The reaction was quenched with 5% NH_2OH solution followed by vortexing and a 15 min incubation at room temperature. Samples were then acidified with formic acid (5% final volume), combined, and dried via SpeedVac. Samples were resuspended in 500 µL of Buffer A (95/5 water/MeCN, 0.1% formic acid) and desalted Sep-Pac C18 cartridge, and eluted with 500 µL Buffer B (20/80 water/MeCN, 0.1% formic acid) and evaporated via SpeedVac. The sample was resuspended in buffer A (500 µL) and fractionated into a 96 deep-well plate by HPLC (Agilent). The peptides were separated by a reversed phase chromatography (Zorbax 300 Extended-C18, 3.5 mm) with a flow rate of 0.5 mL/min using the following gradient: 100% 10 mM NH_4HCO_3 from 0-2 min, 0%–13% MeCN from 2-3 min, 13%–42% MeCN from 3-60 min, 42%–100% MeCN from 60-61 min, 100% MeCN from 61-65 min, 100%–0% MeCN from 65-66 min, 100% 10 mM NH_4HCO_3 from 66-75 min, 0%–13% MeCN from 75-78 min, 13%–80% MeCN from 78-80 min, 80% MeCN from 80-85 min, 100% 10 mM NH_4HCO_3 from 86-91 min, 0%–13% MeCN from 91-94 min, 13%–80% MeCN from 94-96 min, 80% MeCN from 96-101 min, and 80%–0% MeCN from 101-102 min. Collection of eluent was started at 5 min and finished at 75 min. Wells were acidified with the addition of 20 µL of 20% formic acid and evaporated to dryness using a SpeedVac. Every 12th fraction was combined by washing wells with 200 µL of Buffer B (3x, 20/80 water/MeCN, 0.1% formic acid) into 12 Eppendorf tubes and then evaporated to dryness by SpeedVac. Samples were stored at -80 °C until further mass spectrometry analysis.

Mass Spectrometry of TMT-ABPP samples

Dried samples were resuspended in Buffer A (95/5 water/MeCN, 0.1% formic acid) and analyzed on an Orbitrap Fusion tribrid mass spectrometer (Thermo Scientific) coupled with a nanopump (Ultimate 3000 HPLC system, Thermo Scientific). Peptides were eluted through a fused silica column packed with C18 (EASY-Spray, Thermo Scientific) under the following conditions: 0.25 µL/min flow rate and a gradient of 5% acetonitrile with 0.1% formic acid from 0-15 min, 5-12.5% acetonitrile with 0.1% formic acid from 15-17.5 min, 12.5-35% acetonitrile with 0.1% formic acid from 17.5-130 min, 35-47.5% acetonitrile with 0.1% formic acid from 130-150 min, 47.5-95% acetonitrile with 0.1% formic acid from 150-160 min, 95% acetonitrile with 0.1% formic acid from 160-169 min, 95-5% acetonitrile with 0.1% formic acid from 169-170 min and 5% acetonitrile with 0.1% formic acid from 170-200 min. Data was acquired using an MS3-based method adapted from Wang, Y *et al.*[1] Briefly, the MS^1 scan parameters were set up as described below: 1) Orbitrap resolution: 120,000, 2) Scan range: m/z 400-1700, 3) RF lens (%): 60, 4) AGC target: 2×10^5, 5) Maximum injection time: 50 ms. The MS^2 data were acquired in the ion trap with the parameter settings as described below: 1) CID collision energy (%): 35, 2) AGC target: 1.8×10^4, 3) Maximum injection time: 120 ms, 4) Activation Q: 0.25. The MS^3 data were acquired in the Orbitrap with the parameter settings as described below: 1) Orbitrap resolution: 50,000, 2) Number of SPS precursors: 10, 3) First mass: m/z 100, 4) HCD collision energy (%): 55, 5) AGC target: 1.5×10^5, 6) maximum injection time: 120 ms. The raw data was acquired in Xcalibur operation software.

Peptide Identification and Quantification

MS^2 and MS^3 spectra were extracted and analyzed in IP2 (Integrated Proteomics Pipeline, http://goldfish.scripps.edu/). MS^2 spectra were searched using the ProLuCID algorithm (http://fields.scripps.edu/downloads.php) and a reverse concatenated, nonredundant variant of

the Human Uniprot database (release-2016_07). Cysteine residues were searched with a static modification for carboxyamidomethylation (+57.02146 m/z) and up to one differential modification for the desthiobiotin (+398.25292 m/z). N-termini and lysine residues were searched with a static modification for the TMT-tag (+229.1629 m/z). An additional differential modification was included for the presence of oxidized methionine (+15.9949 m/z). Peptides were filtered through DTASelect 2.0 to contain unique peptides only. MS^3 quantification was performed using Census 2.52 with 10plex TMT analysis parameters (m/z 126.127726, 127.124761, 127.131081, 128.128116, 128.134436, 129.131471, 129.13779, 130.134825, 130.141145 and 131.13818) and a mass tolerance of 20 ppm.

Proteomics data processing was carried out using custom a custom Python (version 3.8.5) script, making use of the popular data processing libraries: pandas, scipy, and numpy. Peptides were required to have a sum of reporter ion intensities for control channels ≥ 10000, and a coefficient of variation (CV) < 0.5 across control channels (2 channels for each experiment). For each channel, the relative abundance of reporter ion intensities was calculated relative to the mean of the two control (DMSO) channels (i.e. %control). Peptides spanning the same cysteine residues (regardless of charge state, fraction, etc.) were then grouped, and their mean relative abundance was calculated for each experiment, and peptides were required to have been quantified in 2 or more replicates. The mean abundance values were converted to ratios of inhibitor/DMSO, and a maximum ratio of 10 was set. The median across these per-experiment ratios was taken and reported as R, alongside the coefficient of variation (CV) across these values (**Supplementary Dataset 1**). Additionally, for each peptide the following were reported: information about the corresponding protein, the list of unique residues on that peptide that were labeled, the list of unique peptide sequences that were grouped to comprise the peptide entry, the number of experiments the peptide was quantified in, and the maximum CV across R values from different experiments, for any of the inhibitors tested, and the median relative abundance across experiments for each peptide is also reported as “percent of control” (**Supplementary Dataset 1**).

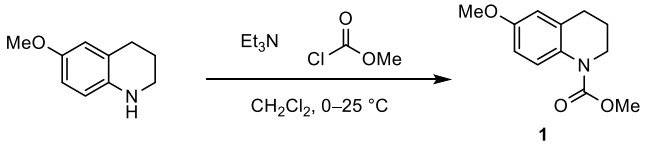
For scatter plots in **Figure 3A**, peptides were required to have a CV ≤ 0.5 across experiments for both groups being plotted.

Kinetic Electrophilicity Assay

This assay was adapted from Resnick et al. Briefly, 50 µM DTNB (5,5,-dithio-bis-(2-nitrobenzoic acid), Ellman’s reagent) was added to reaction buffer (20 mM NaH_2PO_4 pH 7.4 and 150 mM NaCl) containing 200 µM TCEP and incubated for 5 min at room temperature to generate TNB^{2-} anion. This solution was aliquoted into a 96-well microplate. DMSO or compounds (1 µL of 50 mM) was added followed by immediate UV absorption reading at 412 nm at room temperature. The absorbance was read every 15 min for 3 hours on a ClarioStar plate reader. Compounds were measured in triplicate. The raw absorbance data was then fit to a second order rate equation in which k is the slope of $\ln([A][B_0]/ [B][A_0]$. $[A_0]$ and $[B_0]$ are the initial concentration of compound (500 µM) and TNB^{2-} (100 µM) and [A] and [B] are the remaining concentrations as a function of time determined from absorbance readings. The concentration of compound was assumed to be insignificantly consumed throughout the assay and therefore was not included in the final calculations simplifying the equation to $\ln([TNB_0]/[TNB])$. Linear regression was performed using Prism to determine the rate constant. Compounds were tested in triplicate in three replicates.

Chemistry

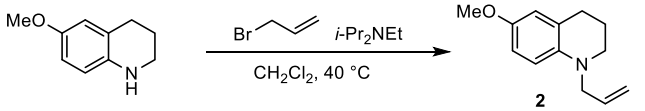
All novel compounds were characterized using ^{1}H, ^{13}C, and HRMS. NMR spectra were recorded on a Bruker 600 MHz instrument. All NMR experiments are reported in δ units (parts per million, ppm) and are listed relative to residual signals for $CHCl_3$ (7.26 ppm/77.2 ppm) or CH_2Cl_2 (5.32 ppm/54.00 ppm) in the deuterated solvent. Reactions were monitored by LCMS and TLC. HRMS analyses were performed using an Agilent ESI-TOF instrument and were found to be within 5 ppm error. **KB02** was synthesized as previously reported.[2]



1

Compound 1, Methyl 6-Methoxy-3,4-dihydroquinoline-1(2*H*)-carboxylate

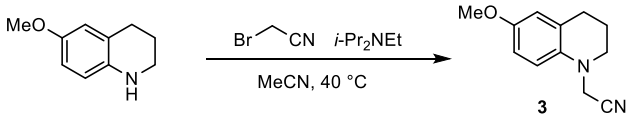
6-Methoxy-1,2,3,4-tetrahydroquinoline (25.0 mg, 0.153 mmol) and Et_3N (23.5 µL, 0.168 mmol) were dissolved in anhydrous CH_2Cl_2 (0.750 mL). The solution was cooled to 0 °C, placed under an atmosphere of Ar, and stirred for 15 min. Methyl chloroformate (23.7 µL, 0.306 mmol) was added dropwise and the mixture was stirred for 3 h, allowing it to slowly warm to room temperature. The reaction was quenched with the addition of cold water and partitioned with CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 10% EtOAc/hexanes). Further purification by PTLC (SiO_2, 25% EtOAc/hexanes) provided **1** (28.9 mg, 80%) as a white oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 (br s, 1H), 6.72 (dd, *J* = 3.0, 6.0 Hz, 1H), 6.62 (d, *J* = 2.9 Hz, 1H), 3.77 (s, 6H), 3.73 (t, *J* = 6.1 Hz, 2H), 2.75 (t, *J* = 6.6 Hz, 2H), 1.93 (quint, *J* = 6.6 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.9, 155.6, 131.6, 131.5, 125.2, 113.4, 111.8, 55.5, 52.9, 44.8, 27.6, 23.6; HRMS (ESI-TOF) *m/z* calculated for $C_{12}H_{15}NO_3$ ($M + H^+$): 222.1130, found: 222.1132.



2

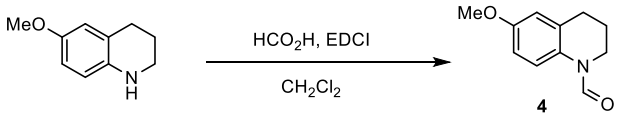
Compound 2, 1-Allyl-6-methoxy-1,2,3,4-tetrahydroquinoline

6-Methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol), allyl bromide (39.8 µL, 0.460 mmol), and *i*-Pr_2NEt (80.0 µL, 0.460 mmol) were dissolved in CH_2Cl_2 (1.75 mL), sealed, and the solution was stirred at 40 °C overnight. The reaction mixture was partitioned between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield **2** (17.9 mg, 52%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.64 (dd, *J* = 3.0, 5.8 Hz, 1H), 6.59 (d, *J* = 3.0 Hz, 1H), 6.53 (d, *J* = 8.8 Hz, 1H), 5.89-5.83 (m, 1H), 5.22-5.14 (m, 2H), 3.83 (d, *J* = 5.2 Hz, 2H), 3.73 (s, 3H), 3.21 (t, *J* = 5.6 Hz, 2H), 2.76 (t, *J* = 6.4 Hz, 2H), 1.96 (quint, *J* = 6.3 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 151.0, 140.1, 134.2, 124.2, 116.2, 115.3, 112.6, 112.5, 55.9, 54.7, 49.3, 28.5, 22.6; HRMS (ESI-TOF) *m/z* calculated for $C_{13}H_{17}NO$ ($M + H^+$): 204.1388, found: 204.1386.



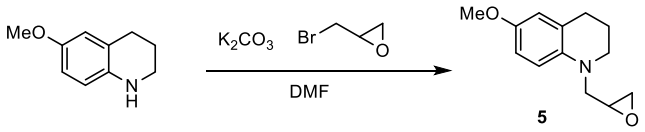
Compound 3, 2-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)acetonitrile

6-Methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol), 2-bromoacetonitrile (213 µL, 3.06 mmol), and *i*-Pr_2NEt (107 µL, 0.613 mmol) were dissolved in acetonitrile (2.50 mL), sealed and the solution was stirred at 40 °C overnight. The reaction mixture was concentrated in vacuo and the crude product was purified by flash chromatography (SiO_2, 50% EtOAc/hexanes) to yield **3** (63.6 mg, 82%) as a beige oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.72 (dd, *J* = 2.9, 5.9 Hz, 1H), 6.64-6.61 (m, 2H), 4.11 (s, 2H), 3.75 (s, 3H), 3.21 (t, *J* = 5.6 Hz, 2H), 2.78 (t, *J* = 5.6 Hz, 2H), 2.03 (quint, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.2, 137.4, 126.5, 116.0, 115.7, 113.5, 112.5, 55.7, 50.4, 41.0, 27.7, 22.5; HRMS (ESI-TOF) *m/z* calculated for $C_{12}H_{14}N_2O$ ($M + H^+$): 203.1184, found: 203.1180.



Compound 4, 6-Methoxy-3,4-dihydroquinoline-1(2*H*)-carbaldehyde

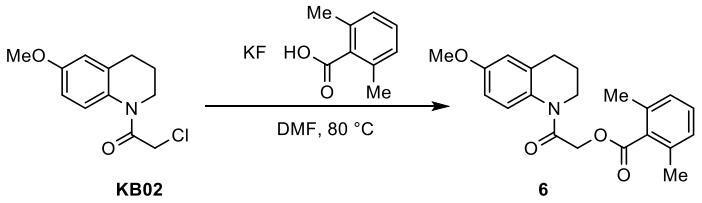
6-Methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol) and formic acid (17.3 µL, 0.460 mmol) were dissolved in CH_2Cl_2 (4.00 mL) and the solution was stirred for 15 min. EDCI (81.3 µL, 0.460 mmol) was added and the mixture was stirred overnight. The reaction mixture was partitioned between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield **4** (40.7 mg, 66%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.77 (dd, *J* = 2.9, 5.9 Hz, 1H), 6.74 (d, *J* = 2.8 Hz, 1H), 3.82-3.80 (m, 5H), 2.81 (t, *J* = 6.5 Hz, 2H), 1.97 (quint, *J* = 6.4 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.1, 156.9, 130.8, 130.6, 118.5, 114.7, 112.7, 55.6, 40.3, 27.5, 22.6; HRMS (ESI-TOF) *m/z* calculated for $C_{11}H_{13}NO_2$ ($M + H^+$): 192.1025, found: 192.1023.



Compound 5, 6-Methoxy-1-(oxiran-2-ylmethyl)-1,2,3,4-tetrahydroquinoline

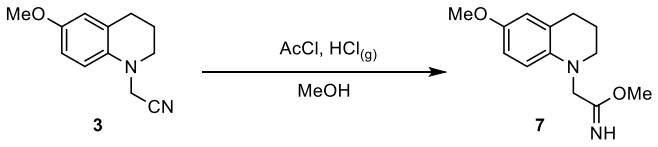
Potassium carbonate (106 mg, 0.766 mmol) and epibromohydrin (52.5 µL, 0.613 mmol) were added to 6-methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol) dissolved in DMF (1.00 mL) and the solution was stirred overnight at room temperature. The reaction mixture was partitioned between CH_2Cl_2 (15 mL) and saturated aqueous $NaHCO_3$ (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield **5** (40.7 mg, 57%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.66 (dd, *J* = 3.0, 5.9 Hz, 1H), 6.60-6.59 (m, 2H), 3.73 (s, 3H), 3.54 (dd, *J* = 3.2, 12.4

Hz, 1H), 3.32-3.25 (m, 3H), 3.15 (quint, *J* = 3.3 Hz, 1H), 2.79 (t, *J* = 4.2 Hz, 1H), 2.77-2.74 (m, 2H), 2.59 (q, *J* = 2.7 Hz, 1H), 1.95 (quint, *J* = 6.1 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 151.3, 140.1, 124.3, 115.5, 112.6, 112.5, 55.9, 53.9, 50.7, 50.6, 45.5, 28.4, 22.5; HRMS (ESI-TOF) *m/z* calculated for $C_{13}H_{17}NO_2$ (M + H^+): 220.1338, found: 220.1340.



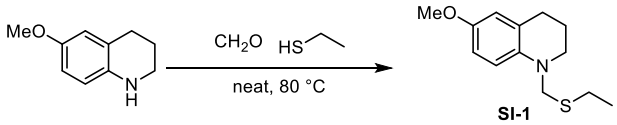
Compound 6, 2-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)-2-oxoethyl 2,6-Dimethylbenzoate

2,6-Dimethylbenzoic acid (45.9 mg, 0.305 mmol) and KF (39.0 mg, 0.672 mmol) were added to a solution of **KB02** (48.8 mg, 0.204 mmol) in DMF (1.50 mL) and stirred overnight at 80 °C. The mixture was cooled to 25 °C and partitioned between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield **6** (51.2 mg, 71%) as a white solid. ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.21 (t, *J* = 7.6 Hz, 1H), 7.12-7.04 (m, 3H), 6.8 (d, *J* = 6.8 Hz, 2H), 4.97 (s, 2H), 3.80-3.78 (m, 5H), 2.74 (s, 2H), 2.37 (s, 6H), 1.99-1.97 (m, 2H); ^{13}C NMR (151 MHz, CD_2Cl_2) δ 172.5, 169.6, 135.9, 135.6, 133.5, 131.3, 129.9, 128.0, 127.9, 125.3, 114.2, 112.0, 62.7, 55.8, 27.4, 24.1, 20.0, 19.9; HRMS (ESI-TOF) *m/z* calculated for $C_{21}H_{23}NO_4$ (M + H^+): 354.1705, found: 354.1704.



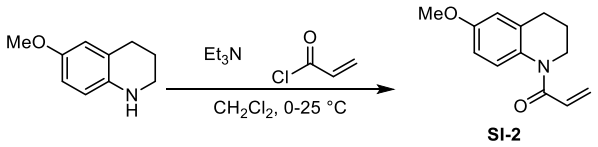
Compound 7, Methyl 2-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)acetimidate

Acetyl chloride (3.00 µL, 0.432 mmol) was added dropwise to a solution of compound **3** (13.9 mg, 0.0690 mmol) dissolved in a minimal amount of anhydrous MeOH. The solution was stirred for 2 min before HCl gas was bubbled through the resulting solution for 2 h. The reaction was quenched with the addition of 1% Et_3N in CH_2Cl_2 and the mixture was passed through a plug of silica gel. The eluent was concentrated in vacuo to provide compound **7** (1.39 mg, 8%) as a clear oil which needed no further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.65-6.62 (m, 2H), 6.36 (d, *J* = 8.6 Hz, 1H), 3.97 (s, 2H), 3.72 (s, 3H), 3.71 (s, 3H), 3.34 (t, *J* = 5.6 Hz, 2H), 2.78 (t, *J* = 6.4 Hz, 2H), 1.99 (quint, *J* = 6.3 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1, 152.0, 139.5, 124.4, 115.4, 112.8, 112.7, 55.9, 55.3, 53.7, 51.2, 28.2, 22.7. MS (ESI) *m/z* calculated for $C_{13}H_{19}N_2O_2$ (M + H^+): 235.1, found: 235.1



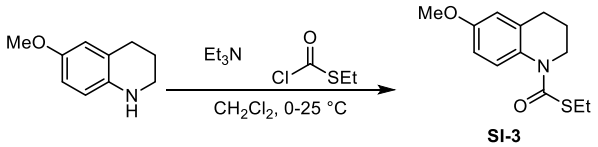
Compound SI-1, 1-((Ethylthio)methyl)-6-methoxy-1,2,3,4-tetrahydroquinoline

6-Methoxy-1,2,3,4-tetrahydroquinoline (25.0 mg, 0.153 mmol), ethanethiol (11.0 µL, 0.153 mmol), and formaldehyde (37 wt% in water, 12.4 µL, 0.153 mmol) were added sequentially to a microwave vial, sealed, and slowly heated to 80 °C over 1 h where stirring continued for 2 h. The mixture was cooled to 25 °C and partitioned between EtOAc (15 mL) and water (15 mL). The aqueous phase was extracted with EtOAc (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 5% EtOAc/hexanes) to yield **SI-1** (33.3 mg, 88%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.70-6.67 (m, 2H), 6.59 (br s, 1H), 4.58 (s, 2H), 3.73 (s, 3H), 3.32 (t, *J* = 5.7 Hz, 2H), 2.75 (t, *J* = 6.5 Hz, 2H), 2.64 (q, *J* = 7.4 Hz, 2H), 1.97 (quint, *J* = 6.4 Hz, 2H), 1.28 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 151.9, 138.1, 125.2, 115.4, 113.5, 112.5, 56.1, 55.8, 49.5, 28.3, 26.3, 22.5, 15.6.



Compound SI-2, 1-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)prop-2-en-1-one

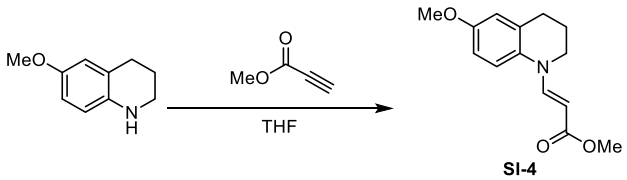
6-Methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol), and Et_3N (47.0 µL, 0.337 mmol) were dissolved in anhydrous CH_2Cl_2 (1.50 mL). The solution was cooled to 0 °C, placed under an atmosphere of Ar, and stirred for 15 min. Acryloyl chloride (49.5 µL, 0.612 mmol) was added dropwise and the solution was stirred for 3 h allowing it to slowly warm to room temperature. The reaction was quenched with the addition of cold water and partitioned with CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield **SI-2** (55.0 mg, 80%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.96 (br s, 1H), 6.72-6.70 (m, 2H), 6.53-6.48 (m, 1H), 6.40 (dd, *J* = 2.1, 14.7 Hz, 1H), 5.63 (dd, *J* = 1.9, 8.2 Hz, 1H), 3.84 (t, *J* = 6.7 Hz, 2H), 3.80 (s, 3H), 2.68 (t, *J* = 6.5 Hz, 2H), 1.96 (quint, *J* = 6.7 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.6, 157.3, 135.0, 131.6, 130.0, 127.4, 126.0, 113.6, 111.5, 55.6, 43.1, 27.4, 24.0; HRMS (ESI-TOF) *m/z* calculated for $C_{13}H_{15}NO_2$ ($M + H^+$): 218.1181, found: 218.1183.



Compound SI-3, *S*-Ethyl 6-Methoxy-3,4-dihydroquinoline-1(2*H*)-carbothioate

6-Methoxy-1,2,3,4-tetrahydroquinoline (25.0 mg, 0.153 mmol), and Et_3N (23.5 µL, 0.168 mmol) were dissolved in anhydrous CH_2Cl_2 (0.750 mL). The solution was cooled to 0 °C, placed under an atmosphere of Ar, and stirred for 15 min. *S*-Ethyl chlorothioformate (31.9 µL, 0.306 mmol)

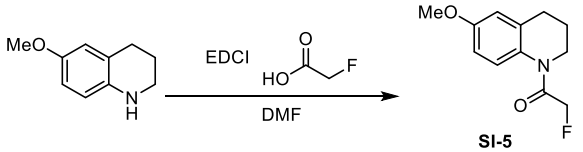
was added dropwise and the solution was stirred for 3 h allowing it to slowly warm to room temperature. The reaction was quenched with the addition of cold water and partitioned with CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 10% EtOAc/hexanes) to yield **SI-3** (32.4 mg, 82%) as a light-yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.6 Hz, 1H), 6.72 (dd, *J* = 2.9, 6.0 Hz, 1H), 6.67 (d, *J* = 2.8 Hz, 1H), 3.78-3.76 (m, 5H), 2.92 (q, *J* = 7.4 Hz, 2H), 2.73 (t, *J* = 6.7 Hz, 2H), 1.97 (quint, *J* = 6.6 Hz, 2H), 1.29 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.6, 157.1, 133.5, 131.4, 126.2, 113.7, 111.4, 55.5, 45.0, 27.2, 25.3, 23.8, 15.3; HRMS (ESI-TOF) *m/z* calculated for $C_{12}H_{14}FNO_2$ (M + H^+): 252.1058, found: 252.1063.



SI-4

Compound SI-4, Methyl (*E*)-3-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)acrylate

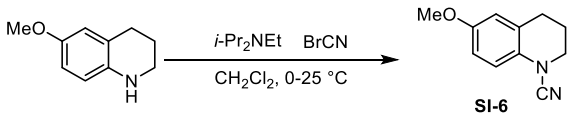
6-Methoxy-1,2,3,4-tetrahydroquinoline (25.0 mg, 0.153 mmol) and methyl propiolate (38.6 µL, 0.460 mmol) were dissolved in THF (1.0 mL) and the solution was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo and the crude product was purified by PTLC (SiO_2, 2.5% MeOH/CH_2Cl_2) to yield **SI-4** (10.9 mg, 29%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (d, *J* = 13.2 Hz, 1H), 7.06 (d, *J* = 8.9 Hz, 1H), 6.75 (dd, *J* = 2.9, 5.9 Hz, 1H), 6.64 (d, *J* = 2.9 Hz, 1H), 4.95 (d, *J* = 13.1 Hz, 1H), 3.77 (s, 3H), 3.71 (s, 3H), 3.43 (t, *J* = 6.4 Hz, 2H), 2.68 (t, *J* = 6.0 Hz, 2H), 2.00 (quint, *J* = 6.3 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.9, 155.3, 146.2, 134.1, 129.8, 117.3, 114.3, 112.9, 89.0, 55.7, 50.9, 45.9, 27.7, 22.7; HRMS (ESI-TOF) *m/z* calculated for $C_{14}H_{17}NO_3$ (M + H^+): 248.1287, found: 248.1291.



SI-5

Compound SI-5, 2-Fluoro-1-(6-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)ethan-1-one

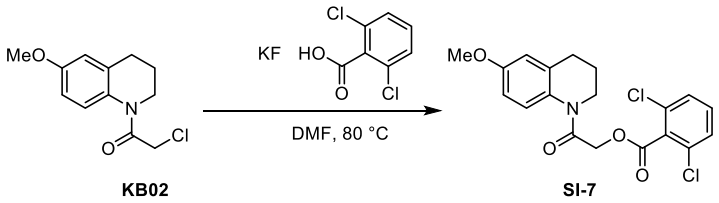
6-Methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol) and 2-fluoroacetic acid (26.2 µL, 0.460 mmol) were dissolved in DMF (2.00 mL) and stirred for 15 min. EDCI (81.3 µL, 0.460 mmol) was added and the solution was stirred overnight. The reaction mixture was partitioned between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and then washed with water (2 x 15 mL). The combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25-50% EtOAc/hexanes gradient) to yield **SI-5** (24.1 mg, 34%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.87 (br s, 1H), 6.73 (d, *J* = 6.9 Hz, 2H), 5.01 (d, *J* = 47.0 Hz, 2H), 3.85-3.77 (m, 5H), 2.70 (s, 2H), 1.99-1.94 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.4, 158.0, 135.9, 130.5, 124.1, 114.1, 111.9, 79.2 (d, CH_2F), 55.6, 42.9, 27.1, 23.7; HRMS (ESI-TOF) *m/z* calculated for $C_{12}H_{14}FNO_2$ (M + H^+): 244.1087, found: 224.1091.



SI-6

Compound SI-6, 6-Methoxy-3,4-dihydroquinoline-1(2*H*)-carbonitrile

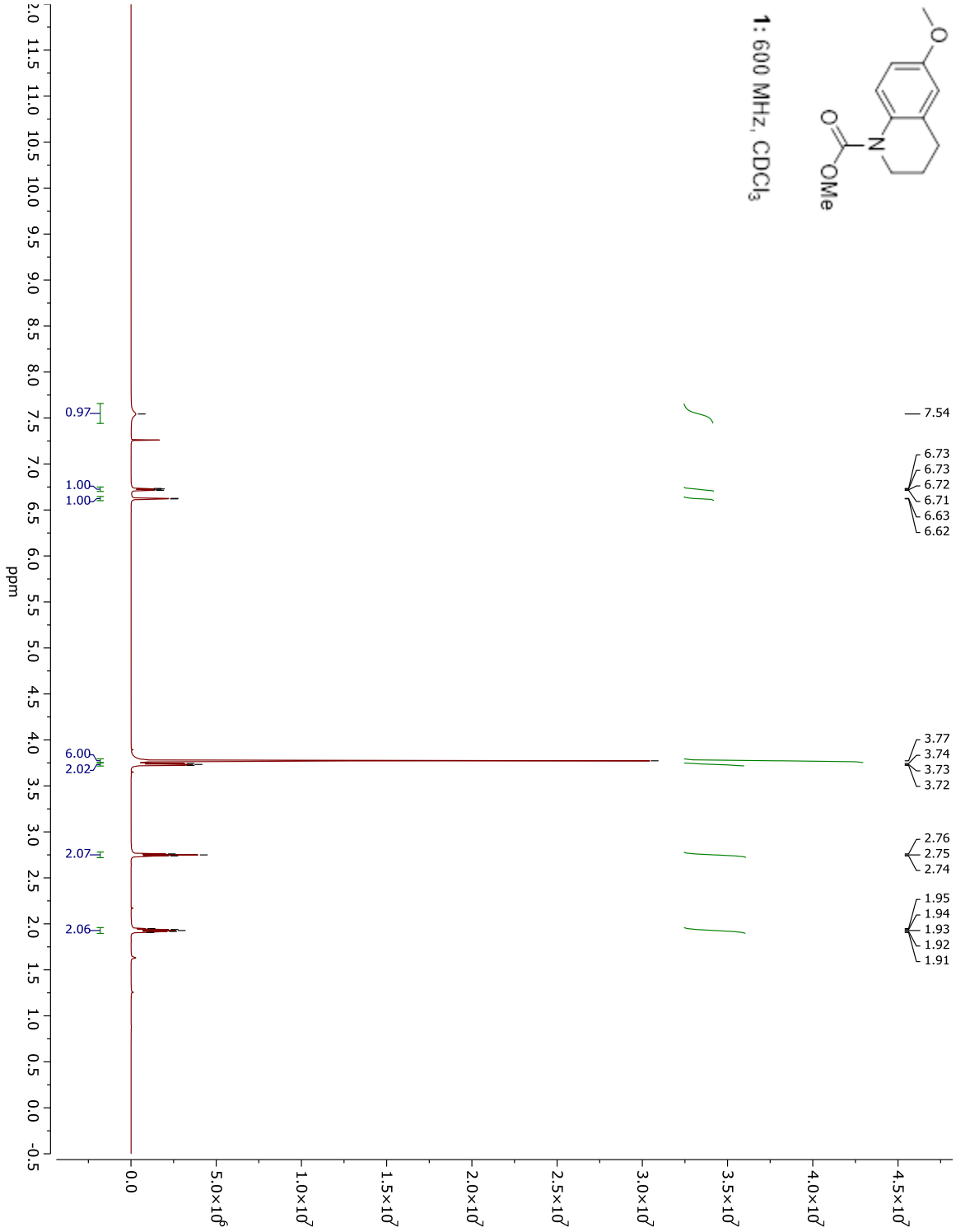
Cyanogen bromide (64.9 mg, 0.613 mmol) dissolved in CH_2Cl_2 (1.00 mL) and *i*-Pr_2NEt (64.0 µL, 0.368 mmol) were added to a solution of 6-methoxy-1,2,3,4-tetrahydroquinoline (50.0 mg, 0.306 mmol) in CH_2Cl_2 (1.00 mL). The solution was stirred at room temperature for 2 h when it was quenched with the addition of 1 M aqueous HCl. The reaction was diluted with CH_2Cl_2 and washed with saturated aqueous NaCl, dried, filtered and concentrated in vacuo to yield **SI-6** (56.7 mg, 72%) as a white solid. Purification was not necessary. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.9 Hz, 1H), 6.76 (dd, *J* = 2.9, 6.0 Hz, 1H), 6.63 (d, *J* = 2.8 Hz, 1H), 3.76 (s, 3H), 3.71 (t, *J* = 5.7 Hz, 2H), 2.77 (t, *J* = 6.3 Hz, 2H), 2.02 (quint, *J* = 6.0 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.4, 129.0, 125.3, 116.8, 115.1, 114.3, 113.2, 55.7, 48.6, 26.6, 21.3; HRMS (ESI-TOF) *m/z* calculated for $C_{11}H_{12}N_2O$ ($M + H^+$): 189.1028, found: 189.1025.



KB02 SI-7

Compound SI-7, 2-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)-2-oxoethyl 2,6-Dichlorobenzoate

2,6-Dichlorobenzoic acid (58.3 mg, 0.305 mmol) and KF (39.0 mg, 0.672 mmol) were added to a solution of **KB02** (48.8 mg, 0.204 mmol) in DMF (1.50 mL) and the reaction was stirred overnight at 80 °C. The mixture was cooled to 25 °C and partitioned between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to yield **SI-7** (54.7 mg, 68%) as a white solid. ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.38-7.32 (m, 3H), 7.09 (br s, 1H), 6.76 (d, *J* = 6.9 Hz, 2H), 5.01 (s, 2H), 3.80-3.77 (m, 5H), 2.73 (s, 2H), 1.97 (s, 2H); ^{13}C NMR (151 MHz, CD_2Cl_2) δ 186.0, 165.3, 164.5, 158.2, 133.2, 132.4, 131.7, 131.2, 128.5, 125.1, 114.2, 112.0, 63.8, 55.8, 43.3, 27.3, 24.1; HRMS (ESI-TOF) *m/z* calculated for $C_{19}H_{17}Cl_2NO_4$ ($M + H^+$): 394.0613, found: 394.0612.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6.73
6.72
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6.62
3.77
3.74
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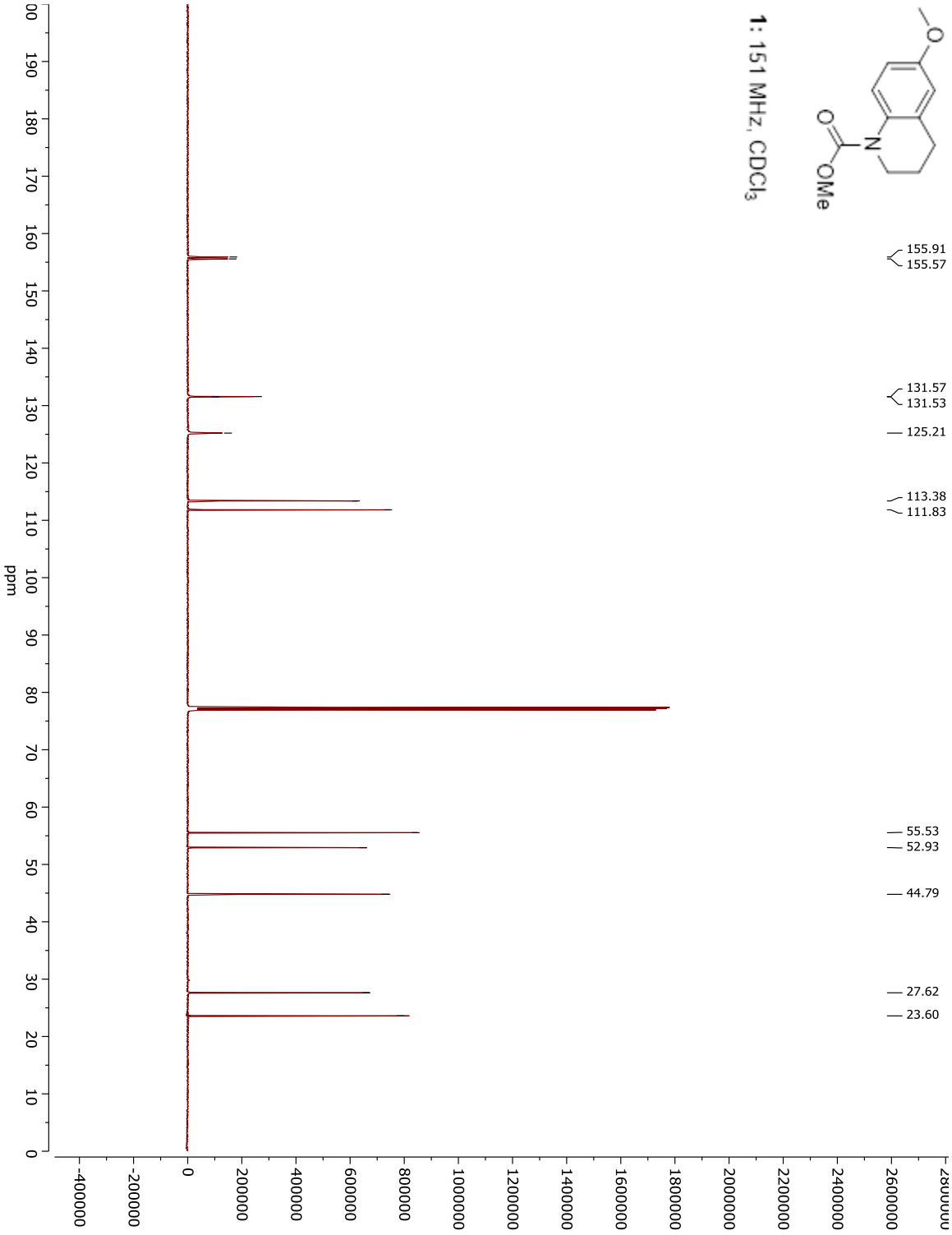
3.72
2.76
2.75
2.74
1.95
1.94
1.93
1.92
1.91
0.97
1.00
1.00
6.00
2.02
2.07
2.06
ppm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OMe
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23.60
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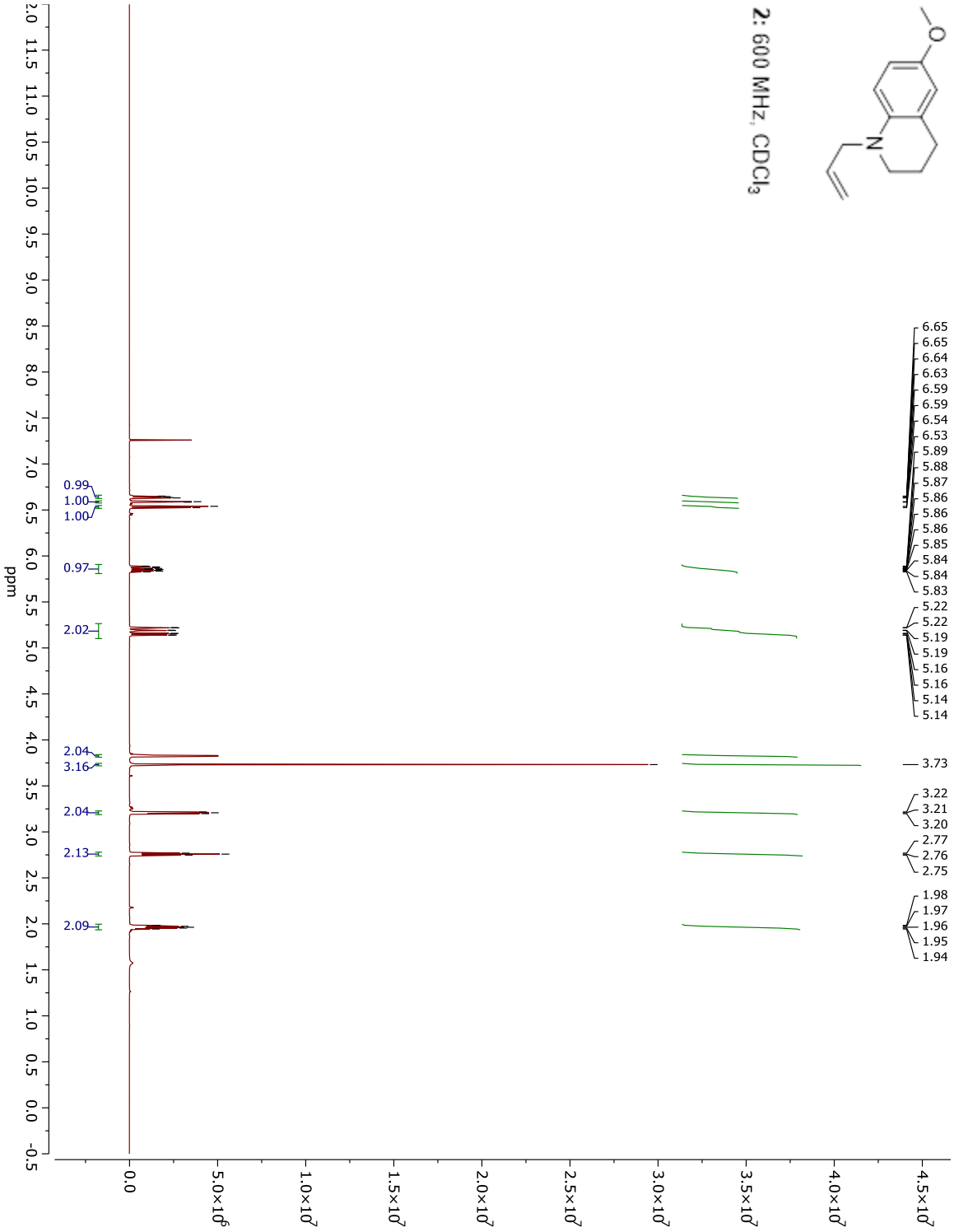
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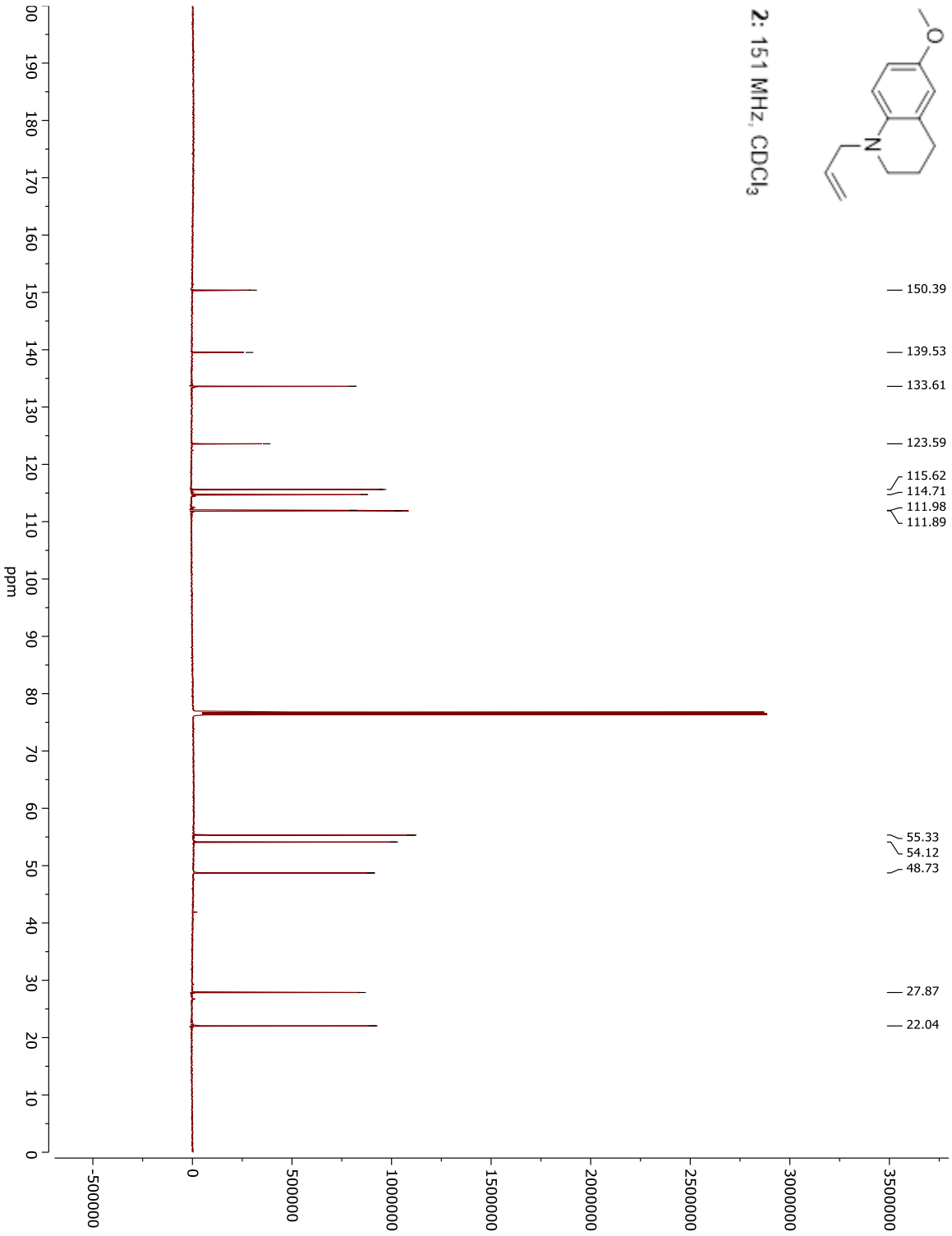
1.96
1.95
1.94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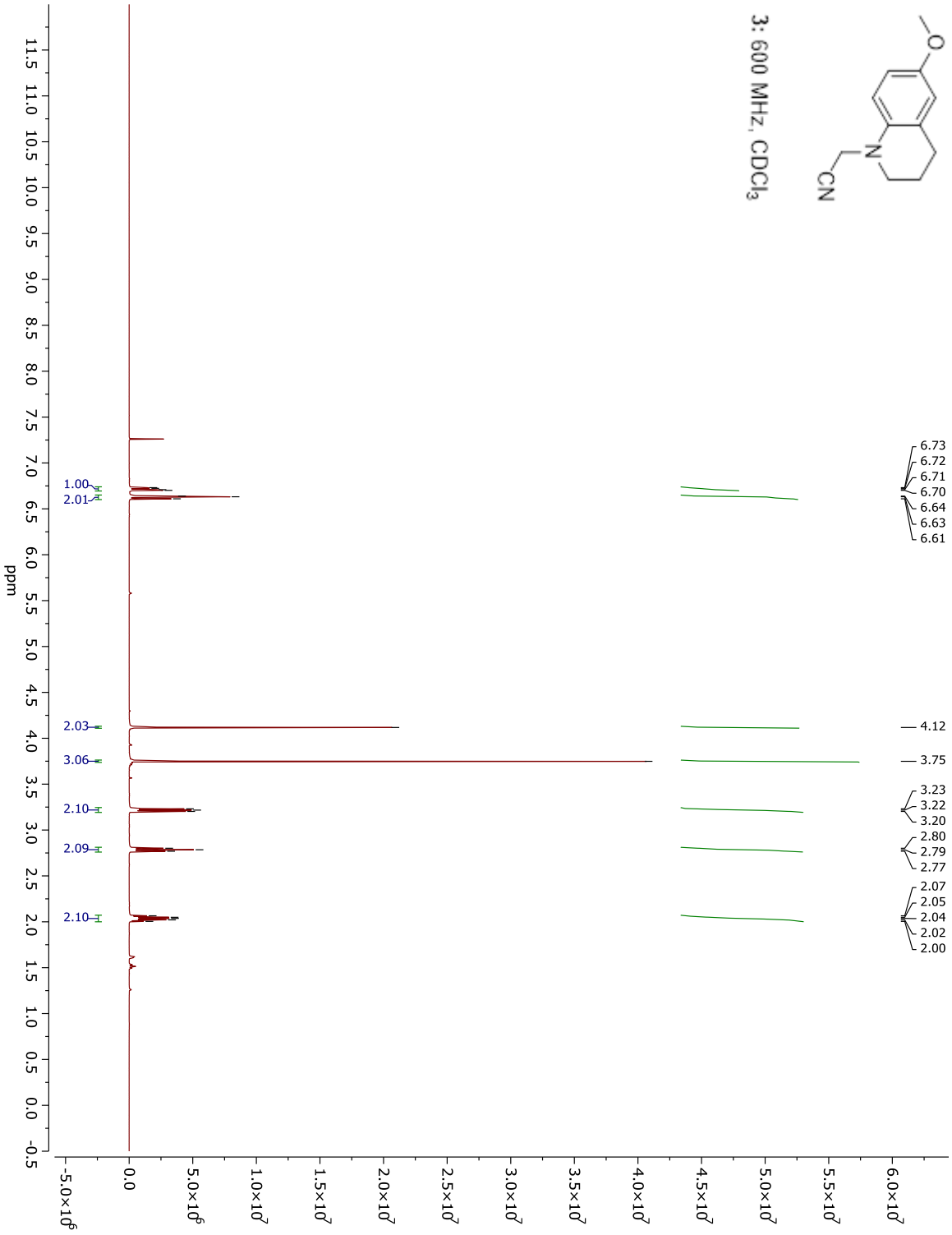
ppm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3.22
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3.06
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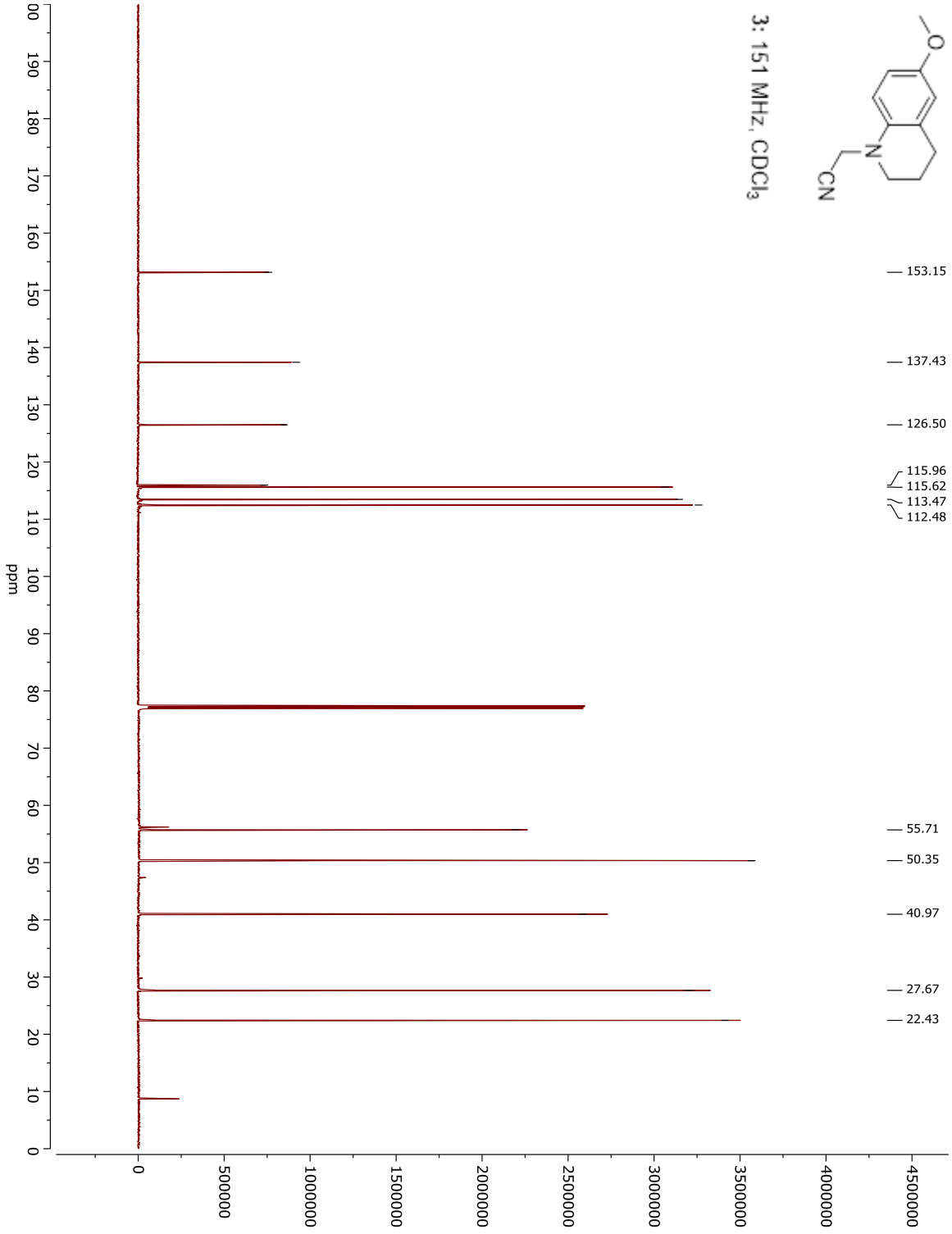
2.09
2.10
ppm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ppm
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70
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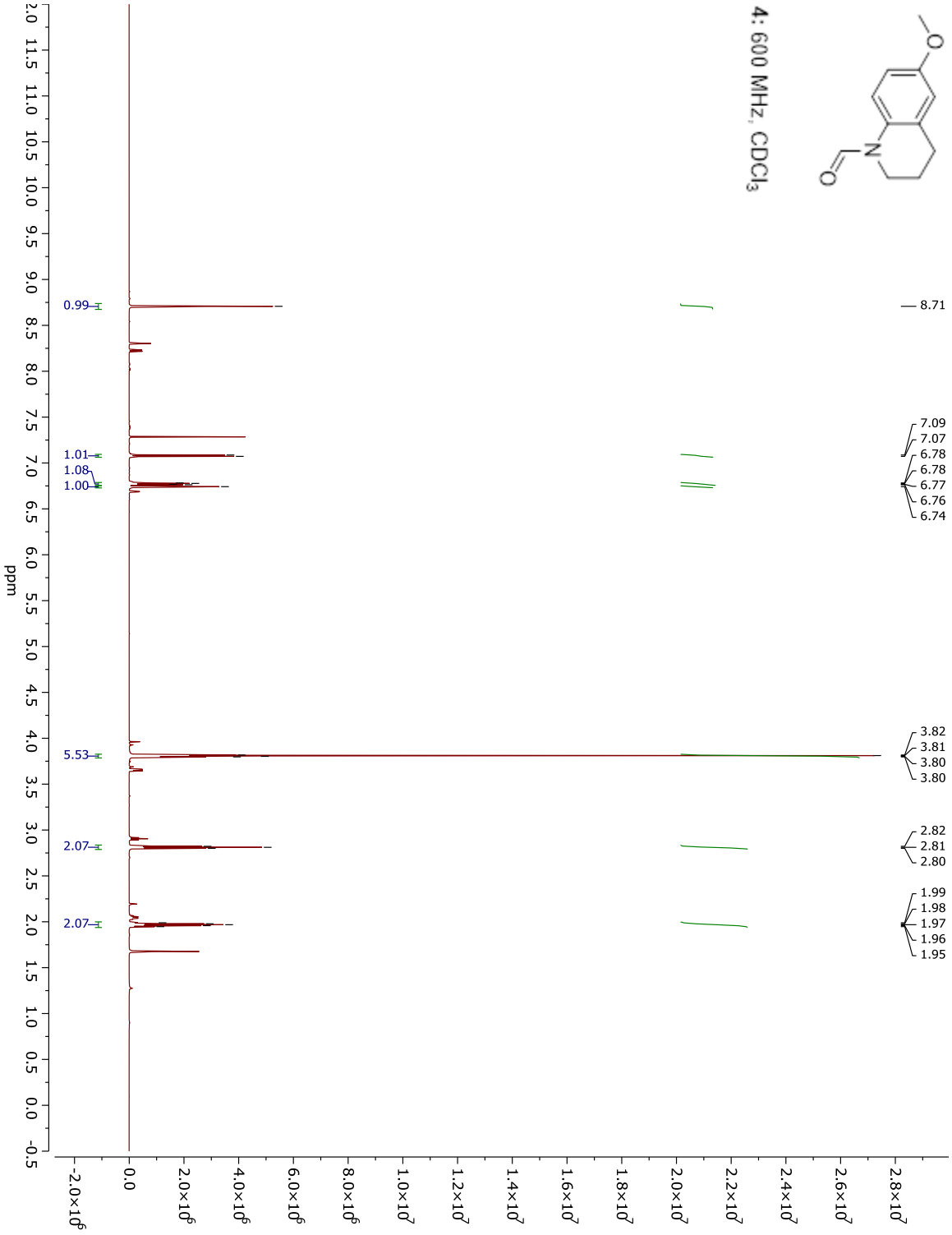
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3.80
3.80
2.82
2.81
2.80
1.99
1.98
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1.96
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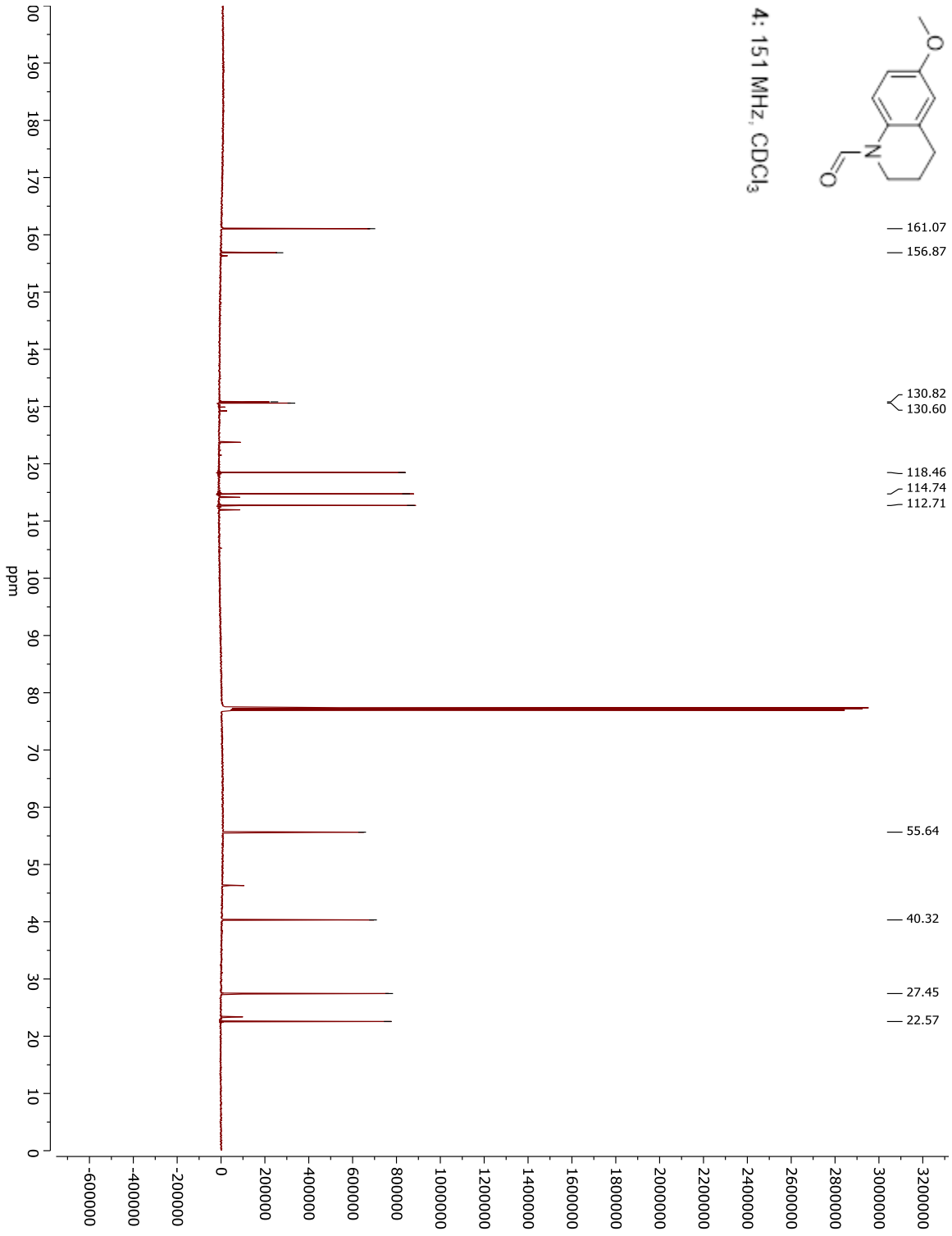
ppm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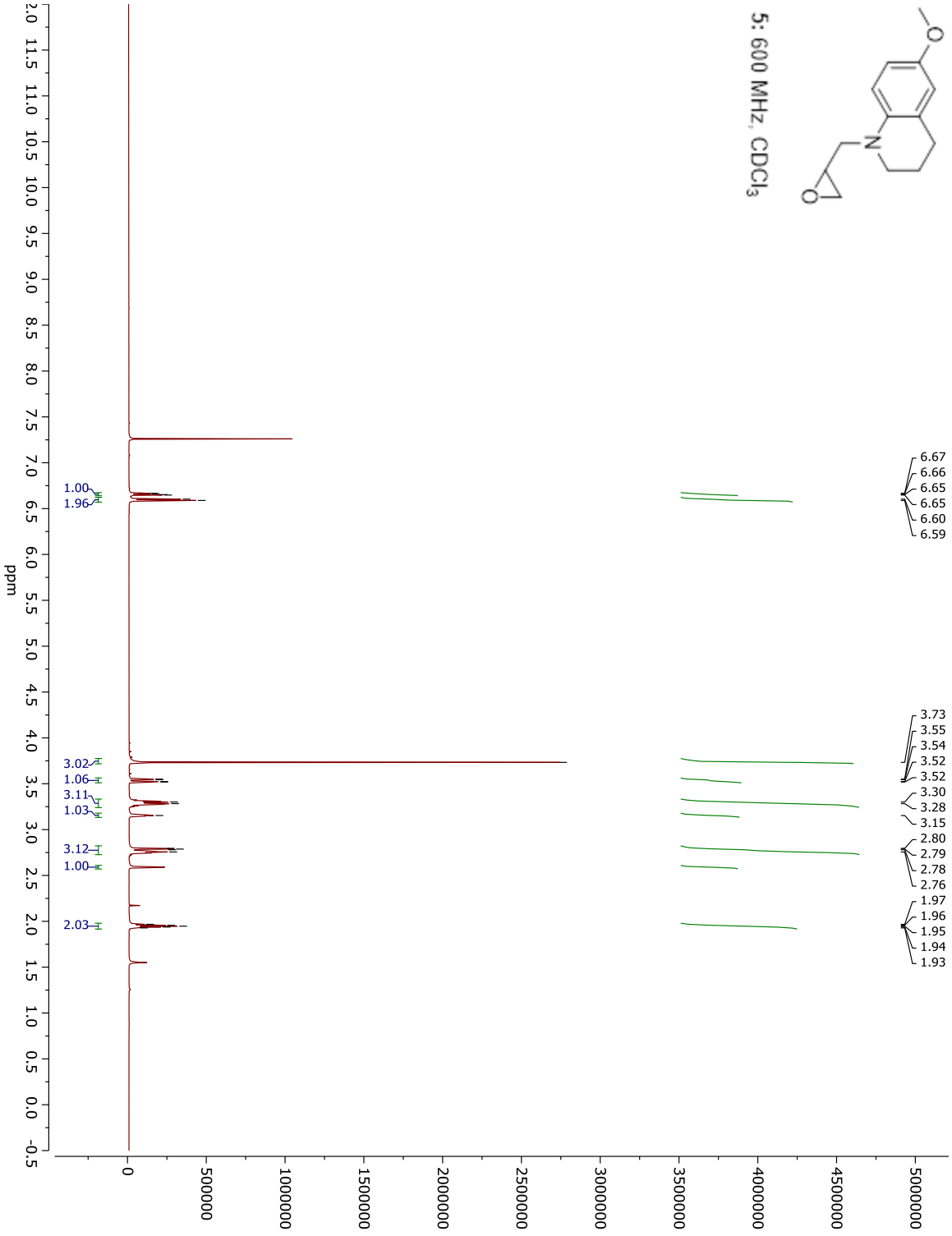
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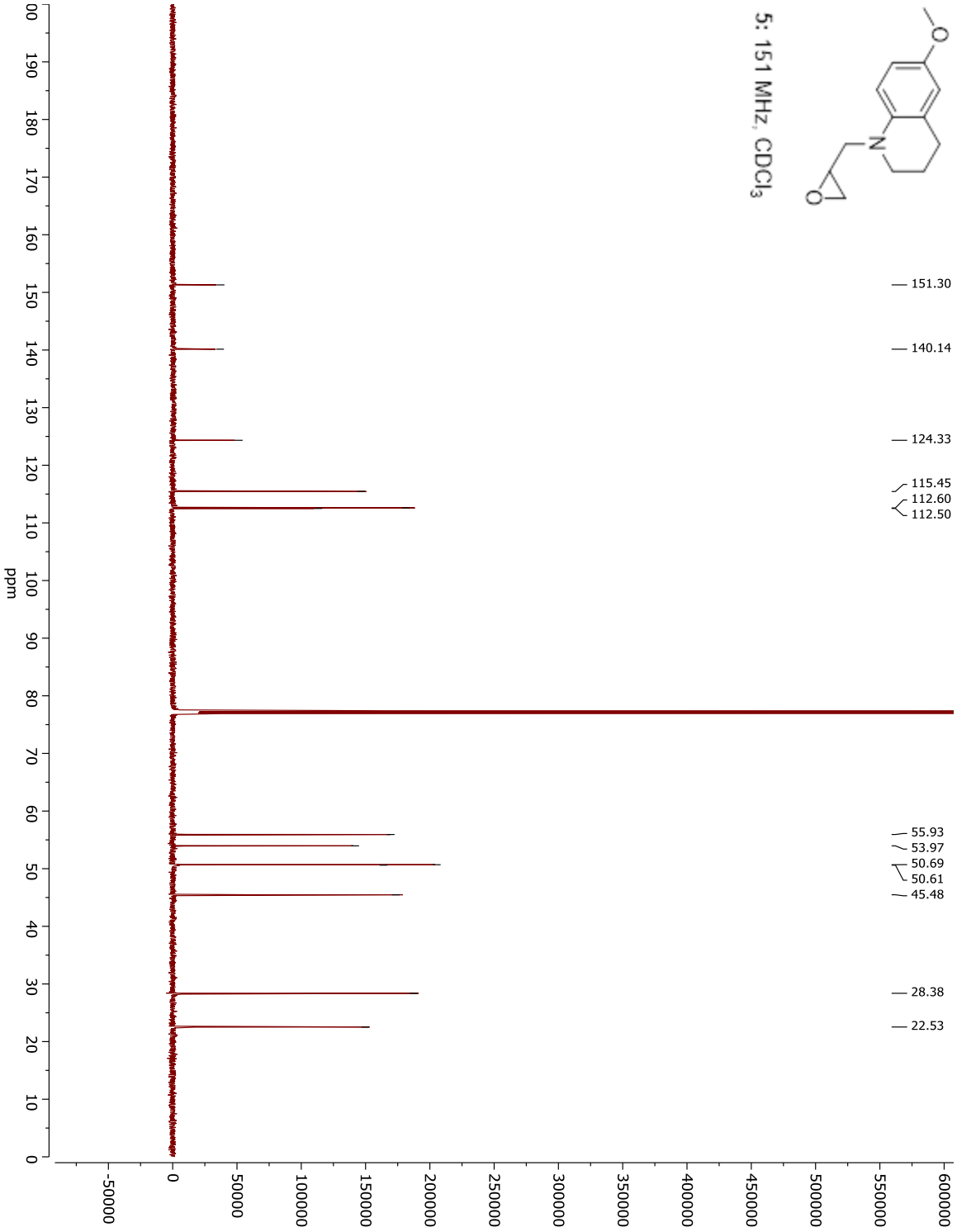
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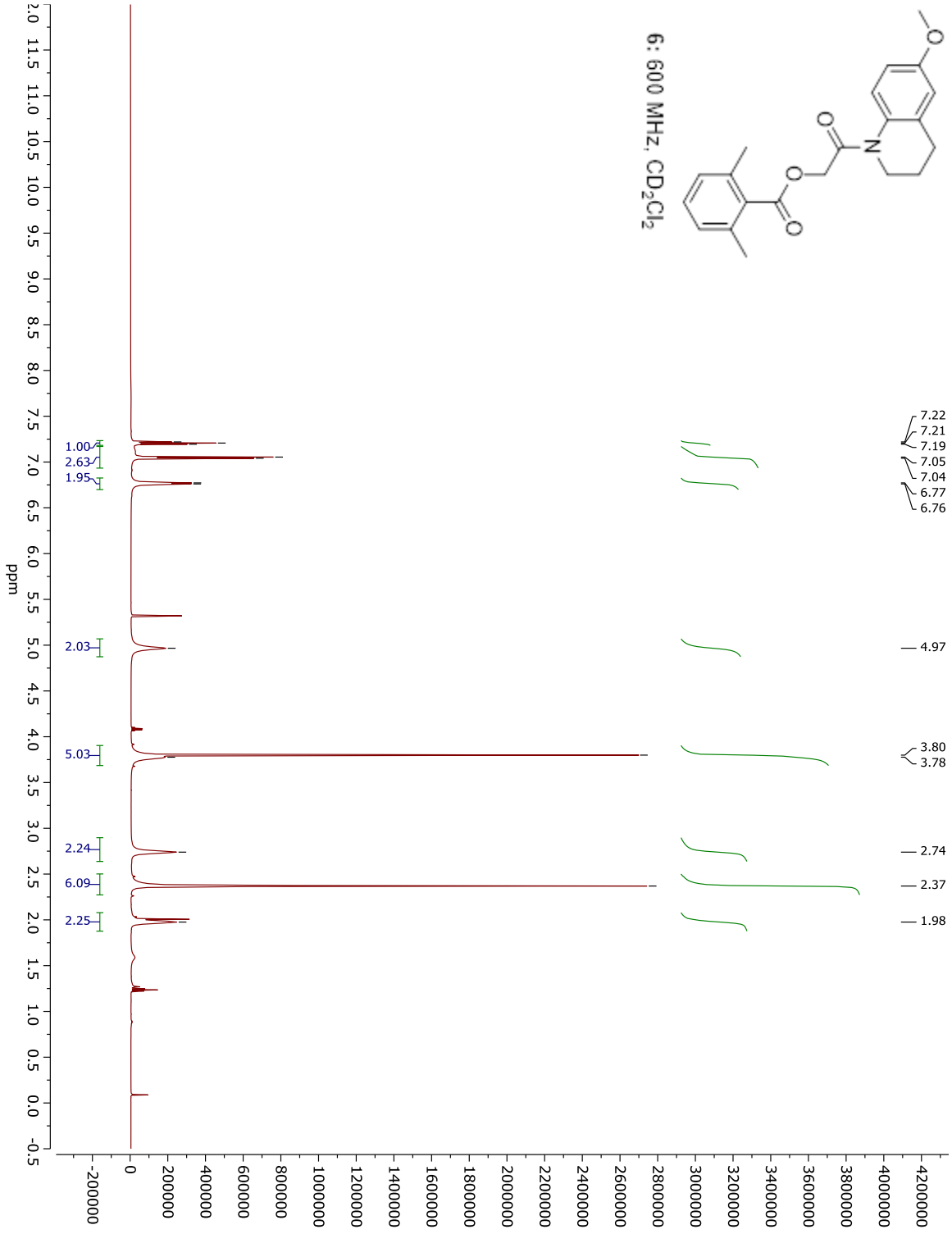
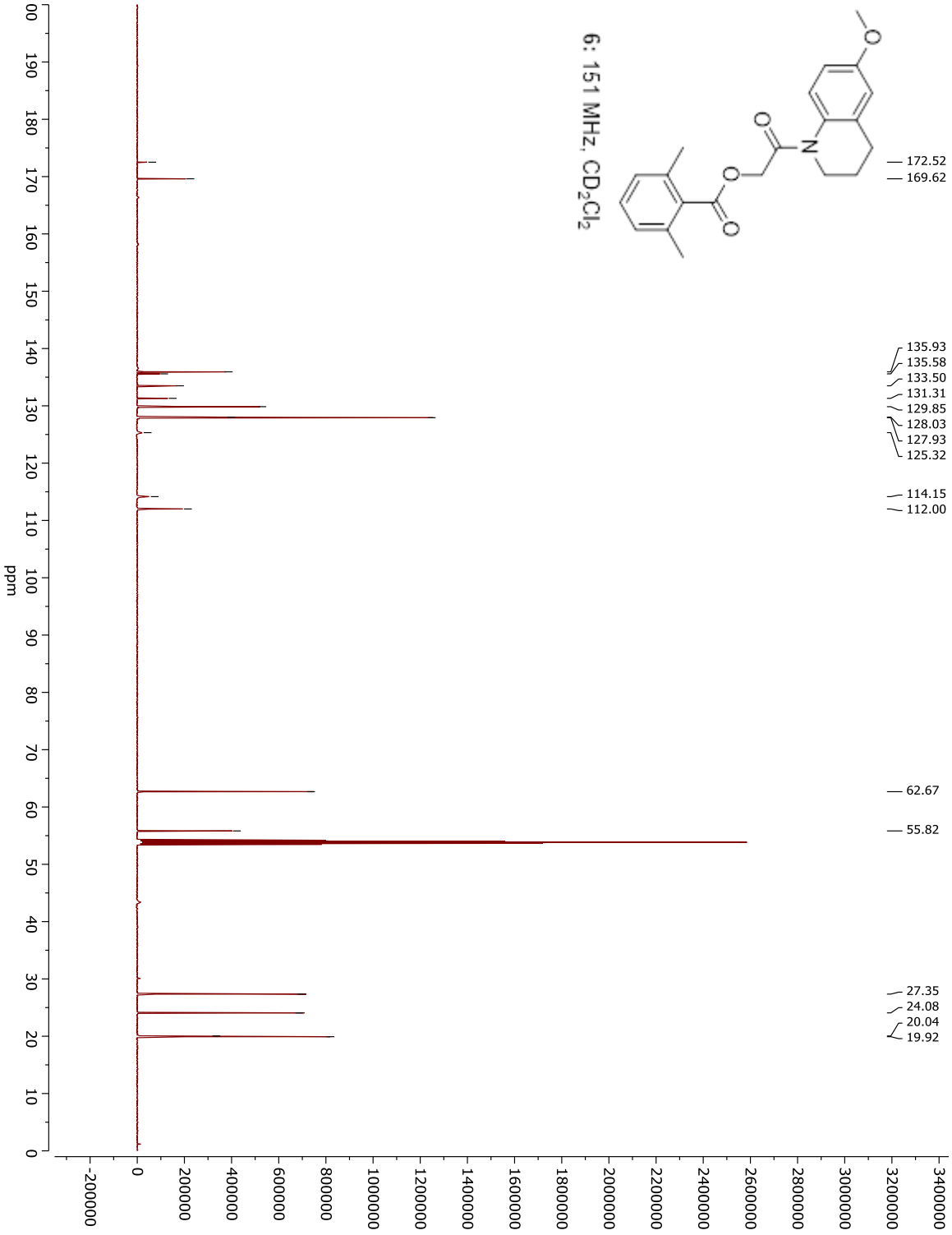
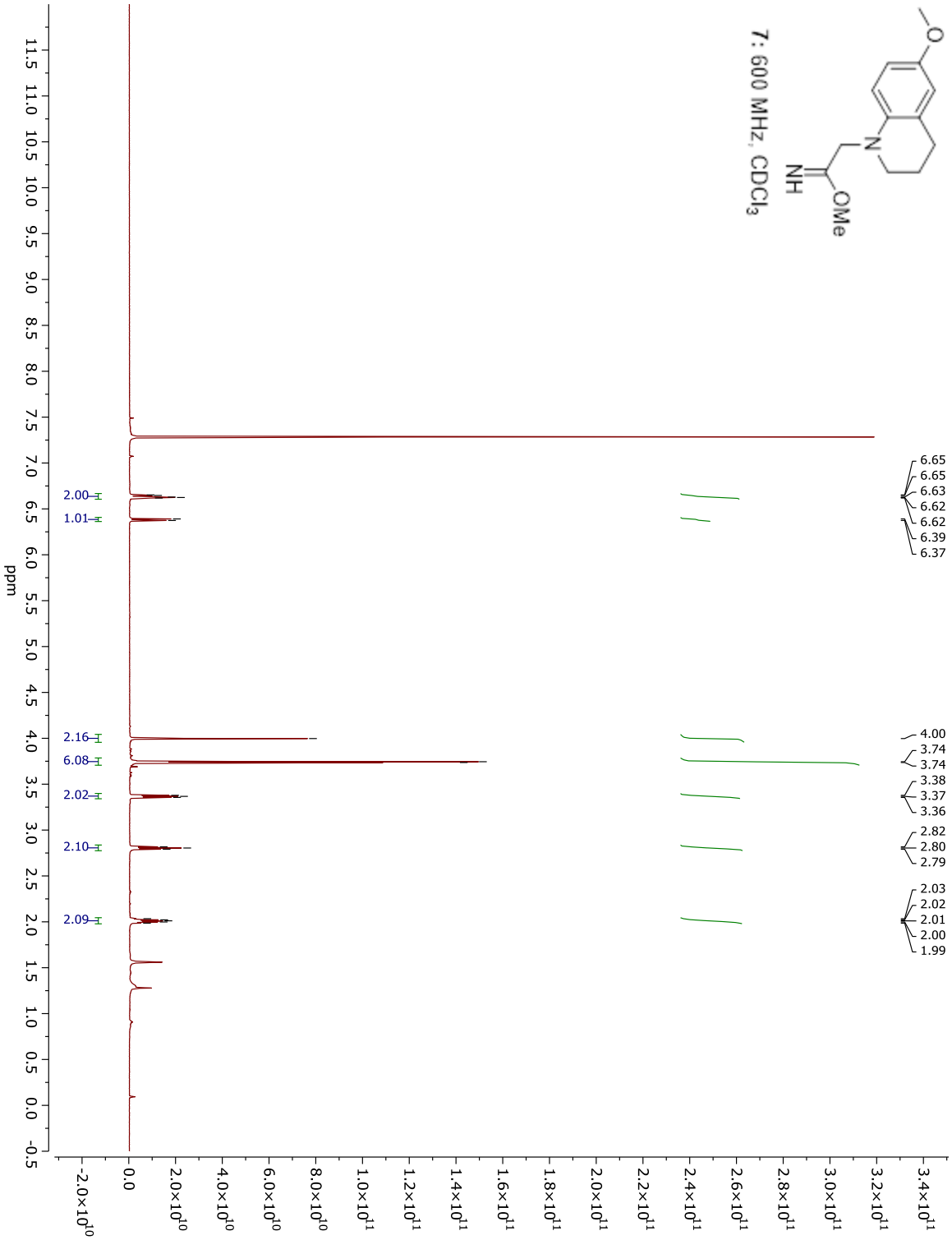
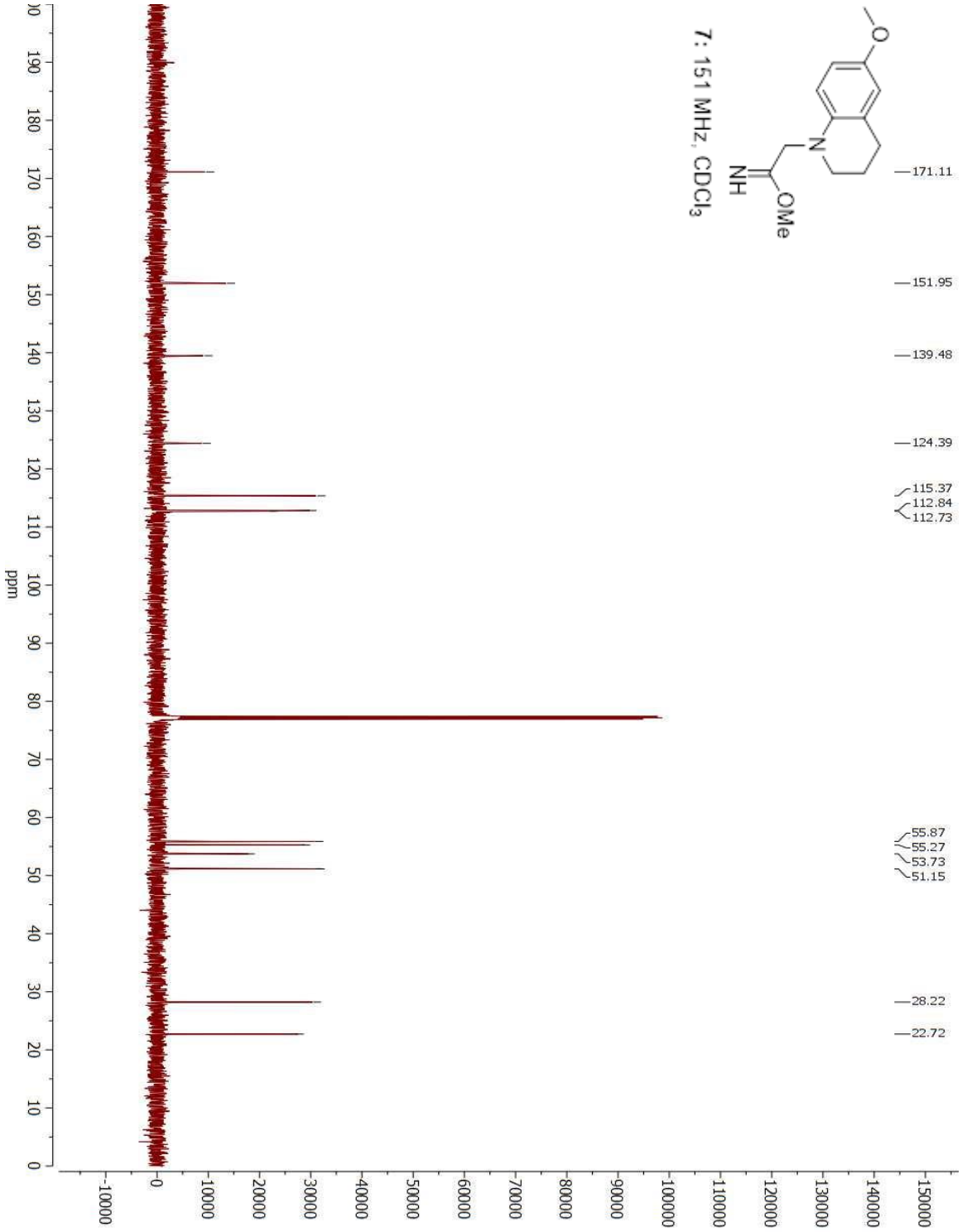
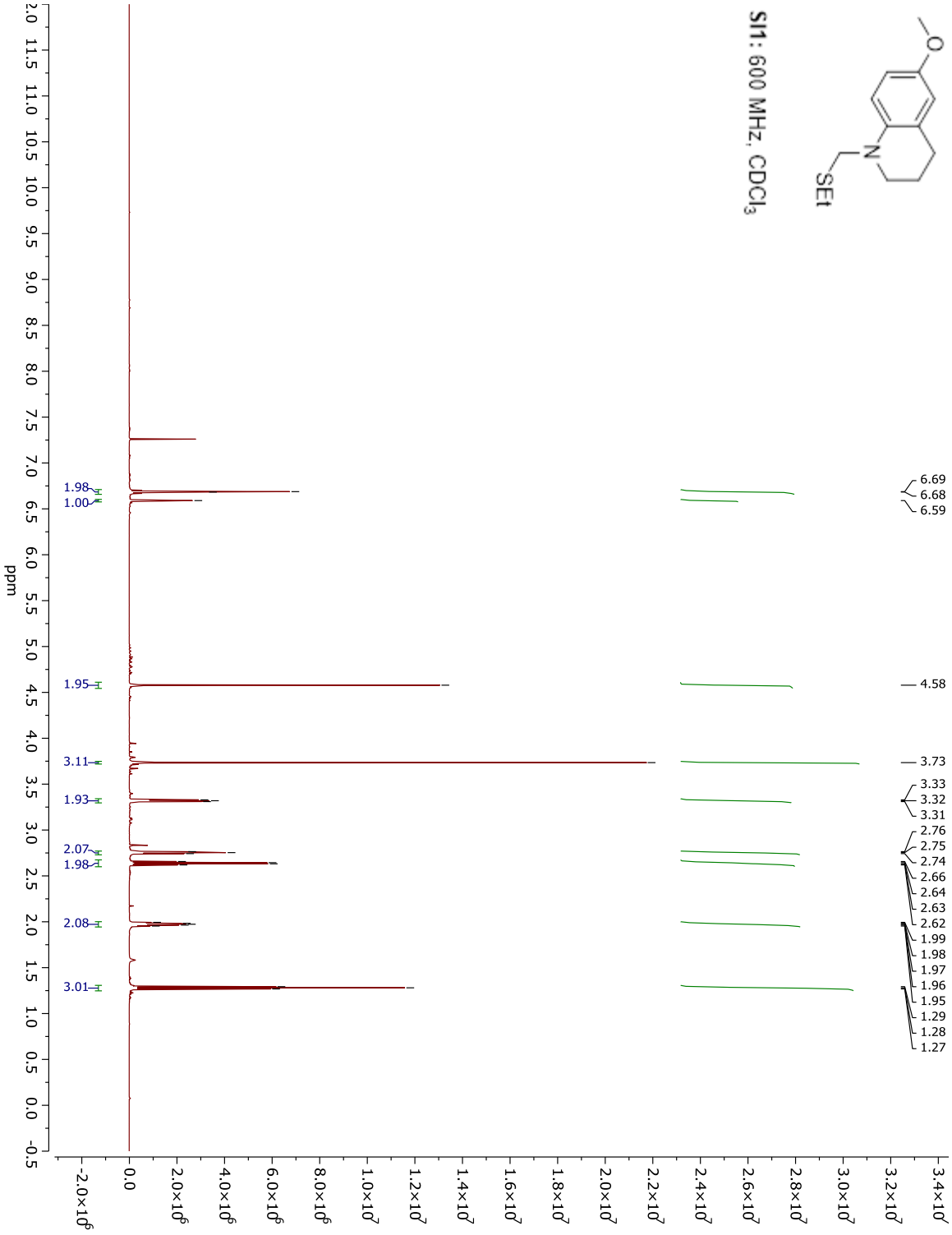
50.61
45.48
28.38
22.53
ppm

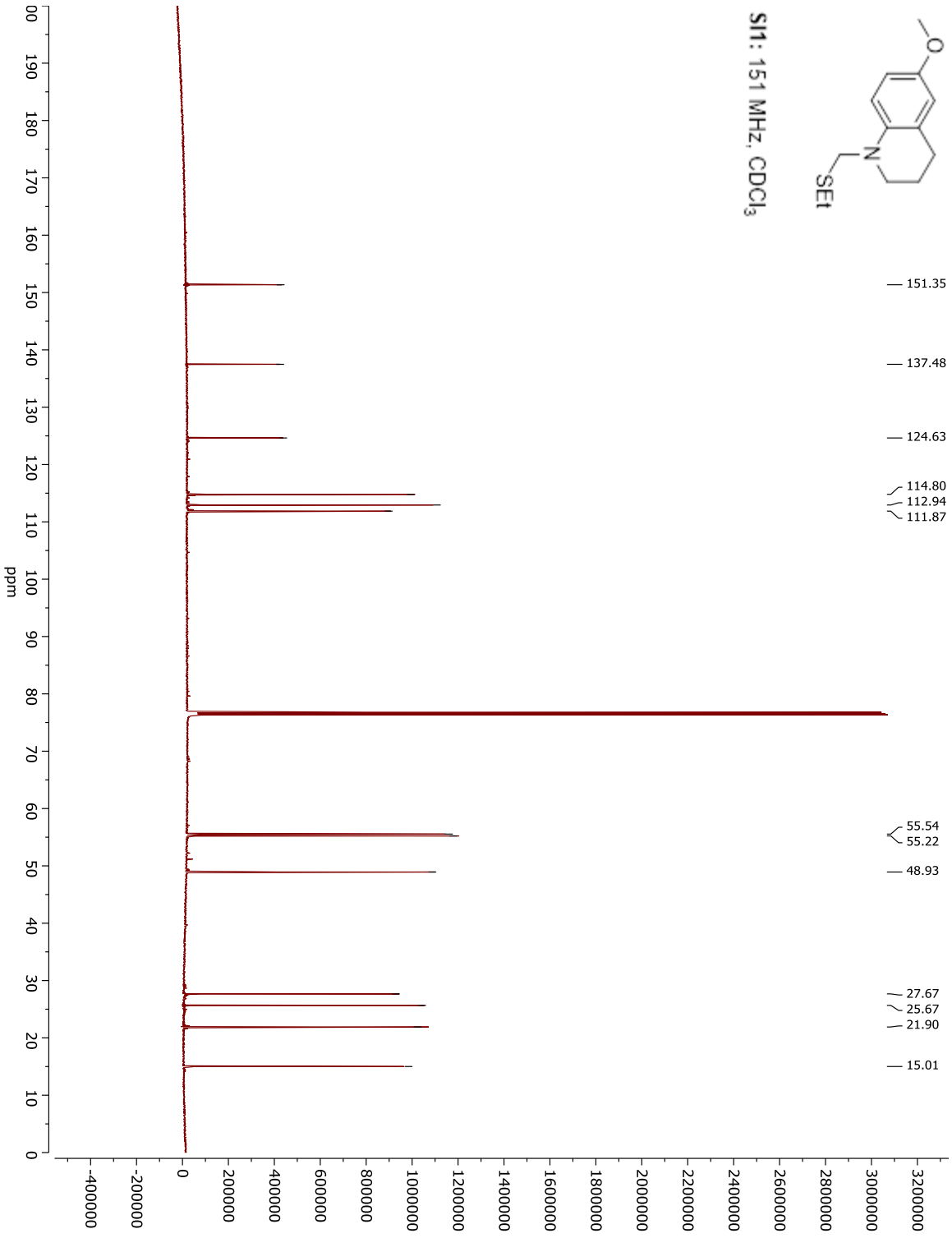

6: 600 MHz, CD_2Cl_2
ppm
1.00
2.63
1.95
2.03
5.03
2.24
6.09
2.25
7.22
7.21
7.19
7.05
7.04
6.77
6.76
4.97
3.80
3.78
2.74
2.37
1.98

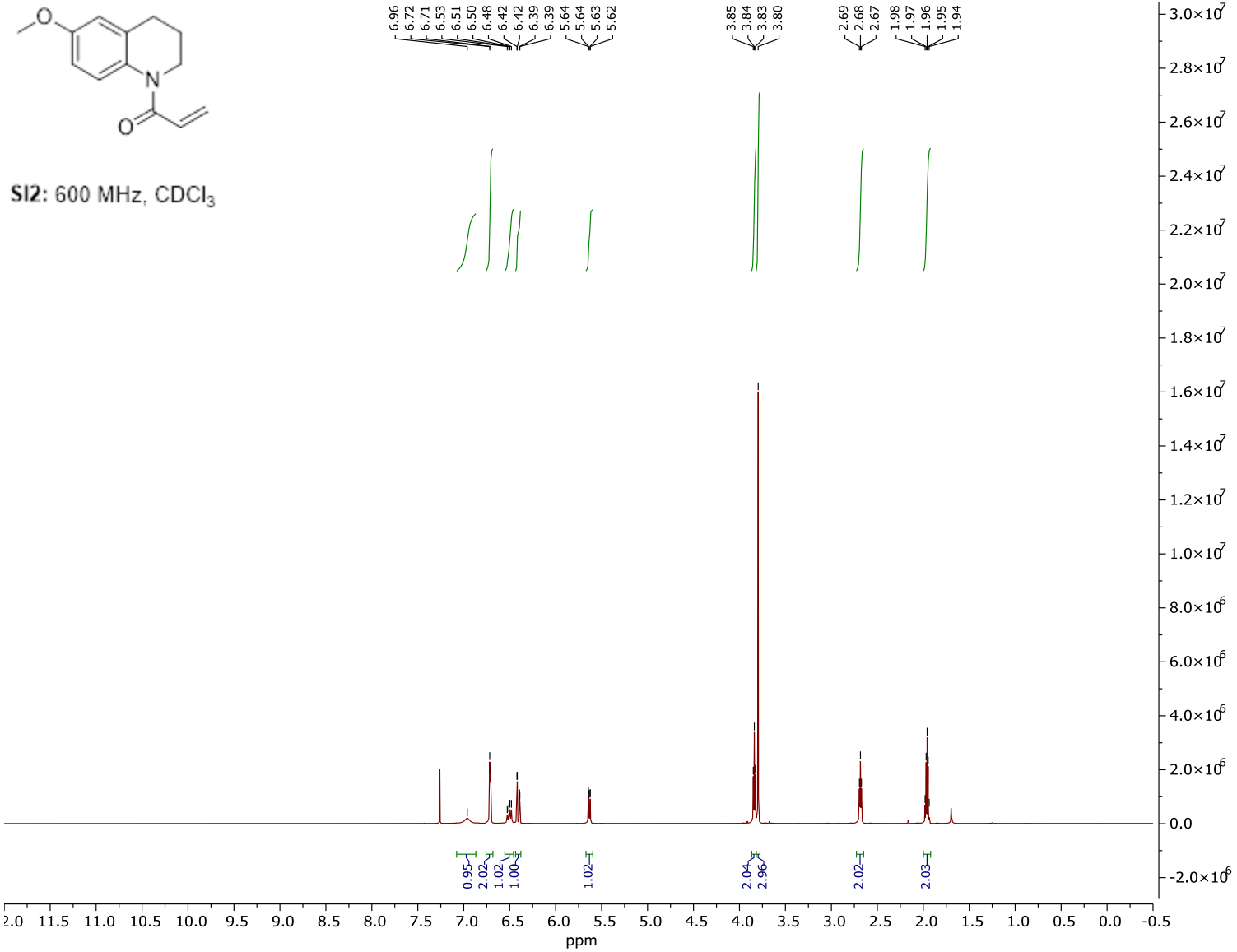

6: 151 MHz, CD_2Cl_2
172.52
169.62
135.93
135.58
133.50
131.31
129.85
128.03
127.93
125.32
114.15
112.00
62.67
55.82
27.35
24.08
20.04
19.92
ppm


7: 600 MHz, CDCl3
NH
OMe
N
O
ppm
2.00
1.01
2.16
6.08
2.02
2.10
2.09
6.65
6.65
6.63
6.62
6.62
6.39
6.37
4.00
3.74
3.74
3.38
3.37
3.36
2.82
2.80
2.79
2.03
2.02
2.01
2.00
1.99

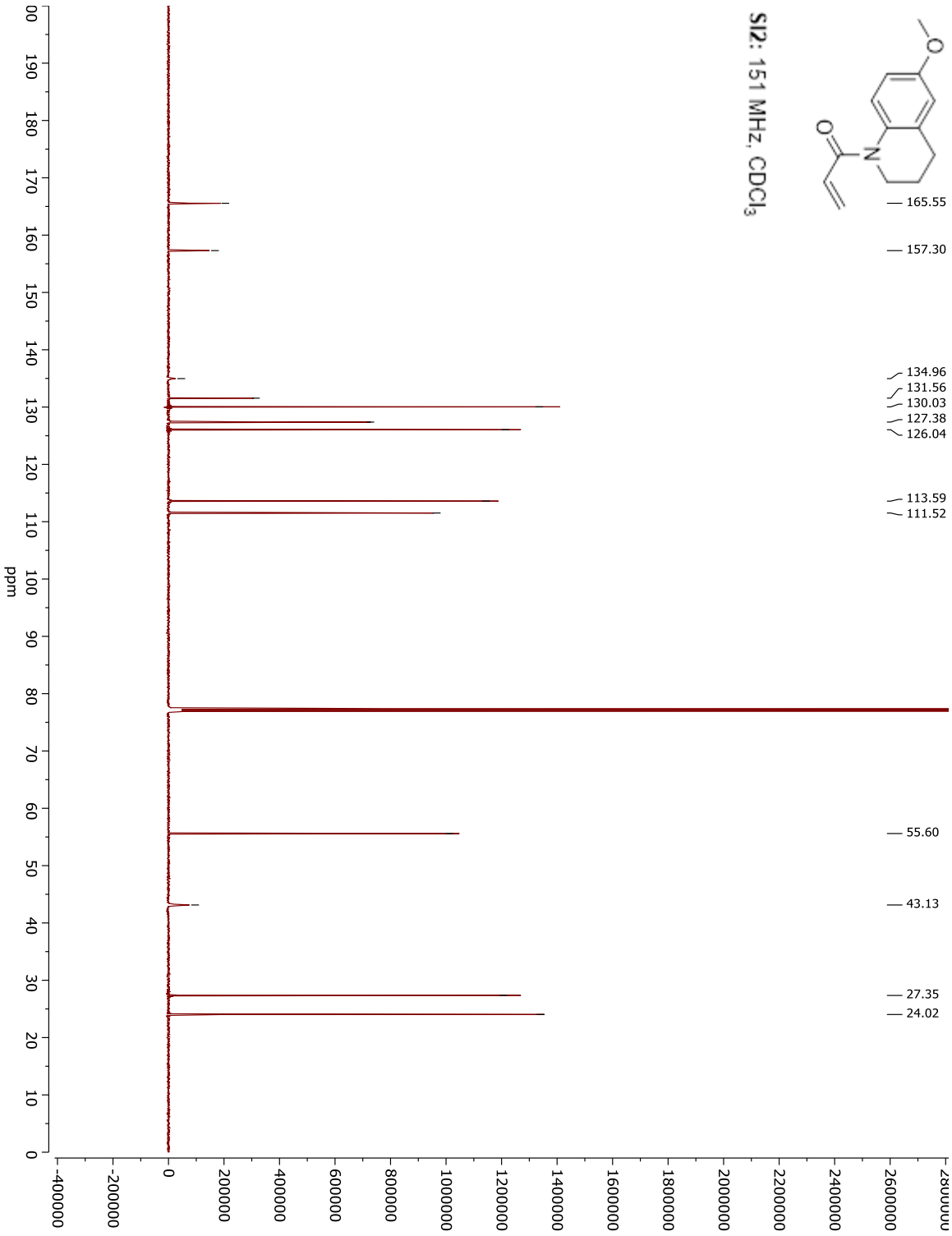

7: 151 MHz, CDCl3
171.11
151.95
139.48
124.39
115.37
112.84
112.73
55.87
55.27
53.73
51.15
28.22
22.72
ppm

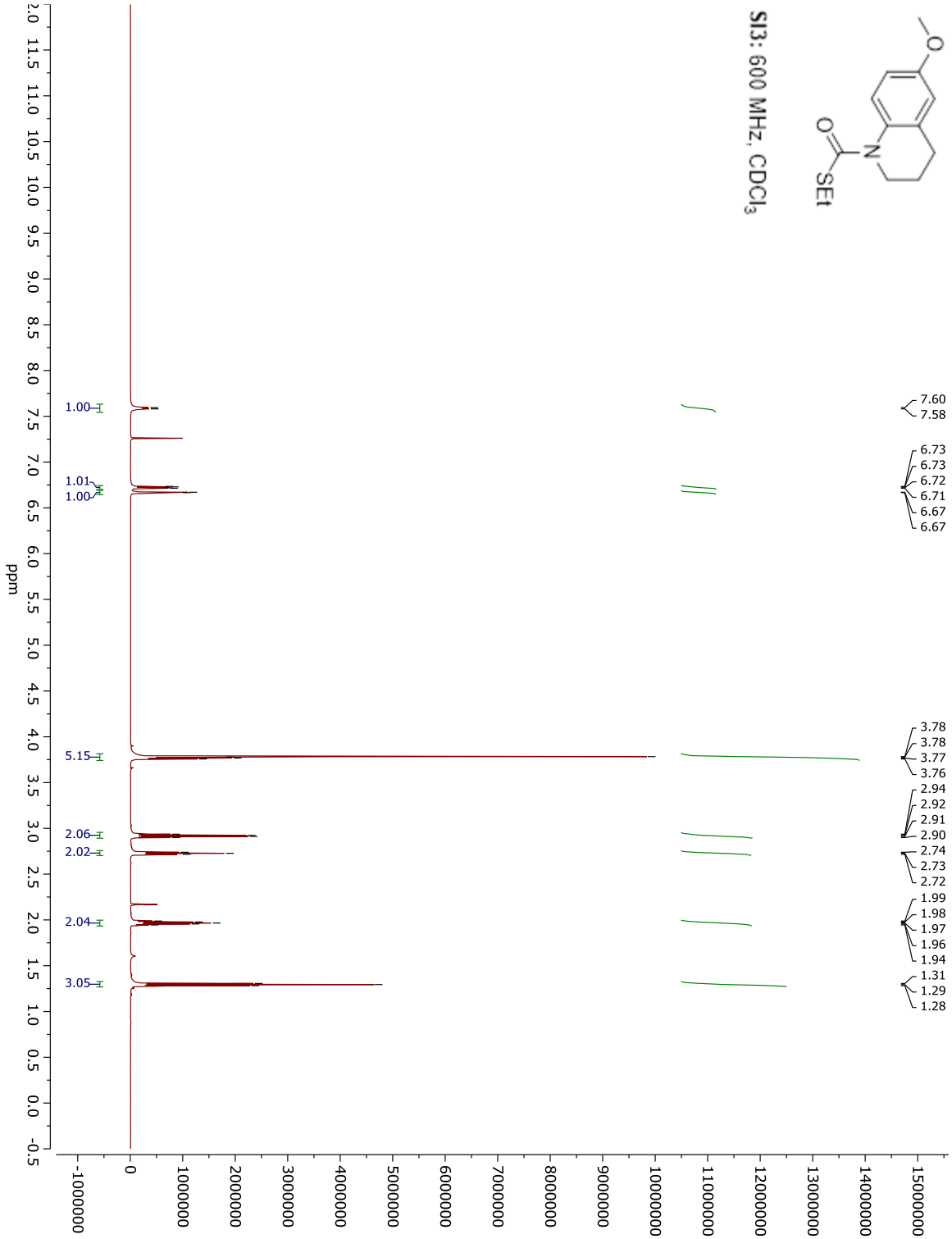

SI1: 600 MHz, CDCl3
SEt
O
N
ppm
1.98
1.00
1.95
3.11
1.93
2.07
1.98
2.08
3.01
6.69
6.68
6.59
4.58
3.73
3.33
3.32
3.31
2.76
2.75
2.74
2.66
2.64
2.63
2.62
1.99
1.98
1.97
1.96
1.95
1.29
1.28
1.27

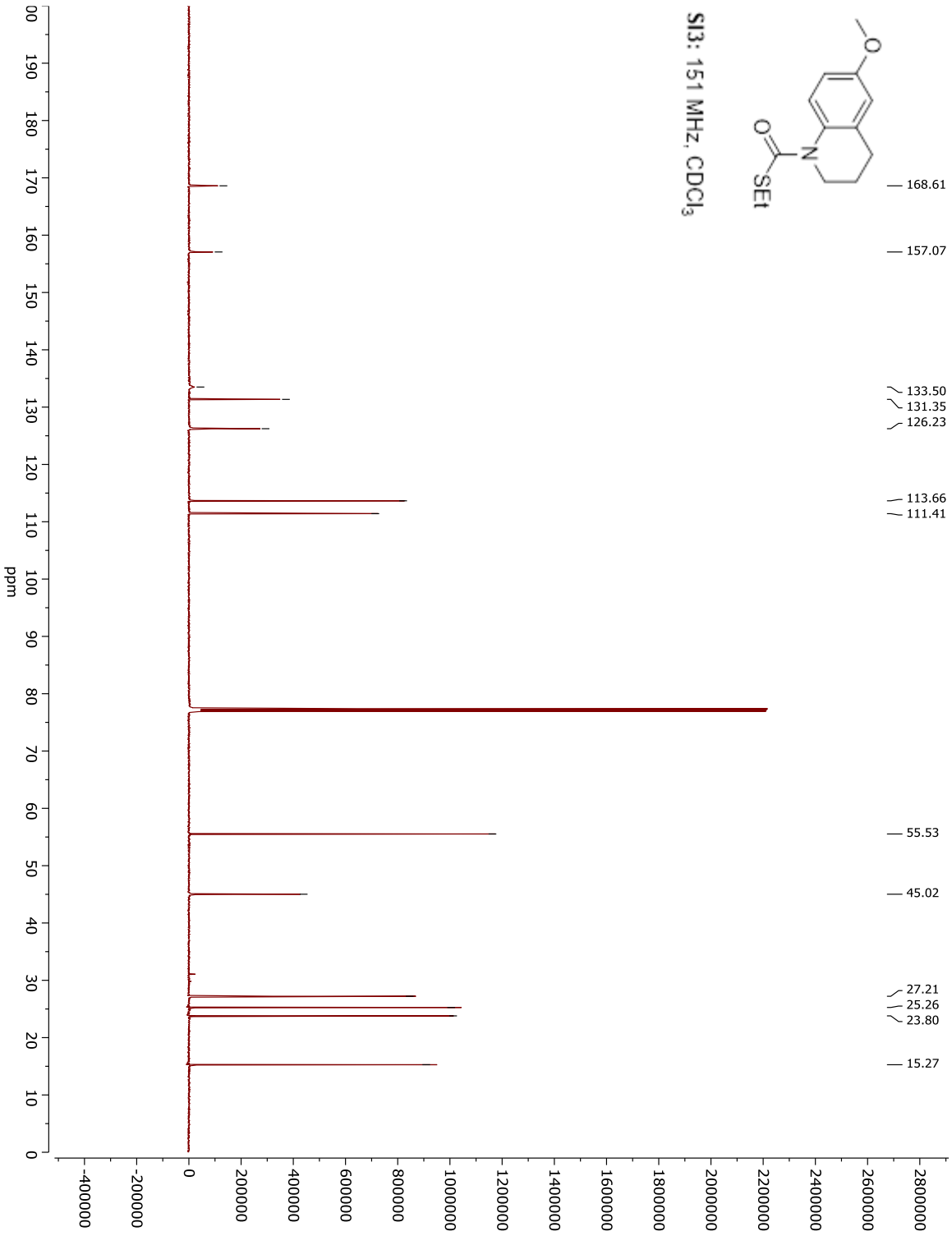

SI1: 151 MHz, CDCl3
SEt
151.35
137.48
124.63
114.80
112.94
111.87
55.54
55.22
48.93
27.67
25.67
21.90
15.01
ppm

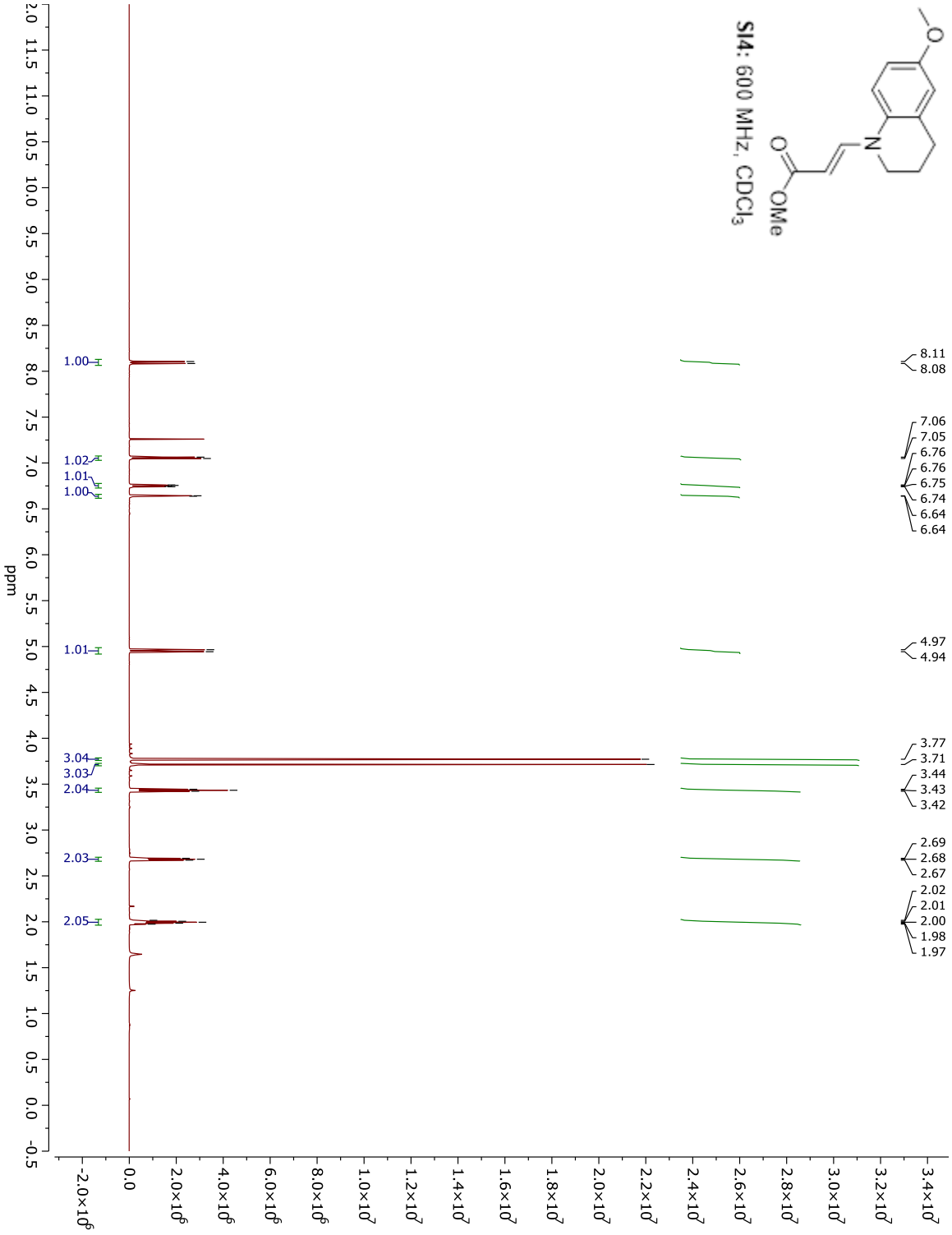


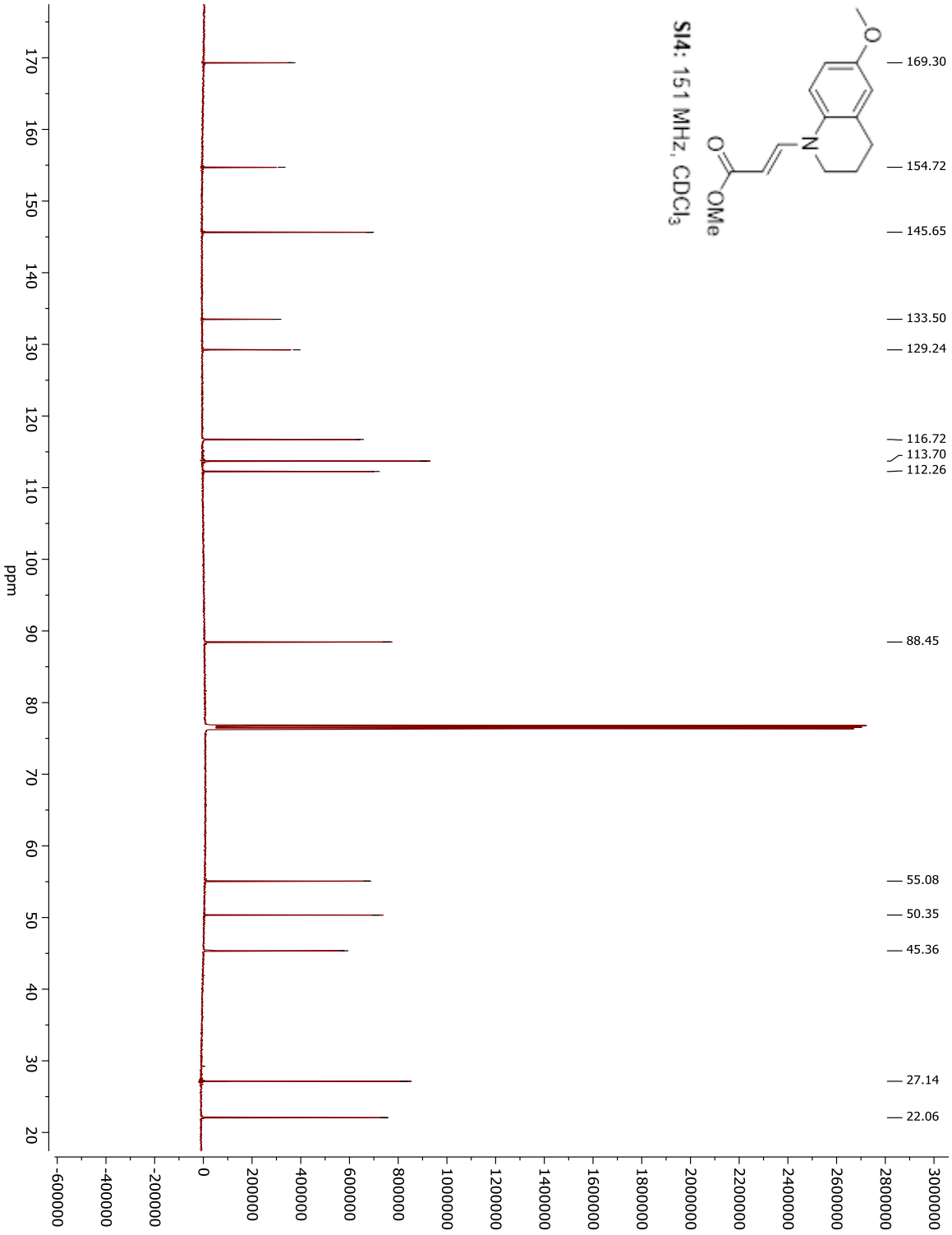
SI2: 600 MHz, $CDCl_3$

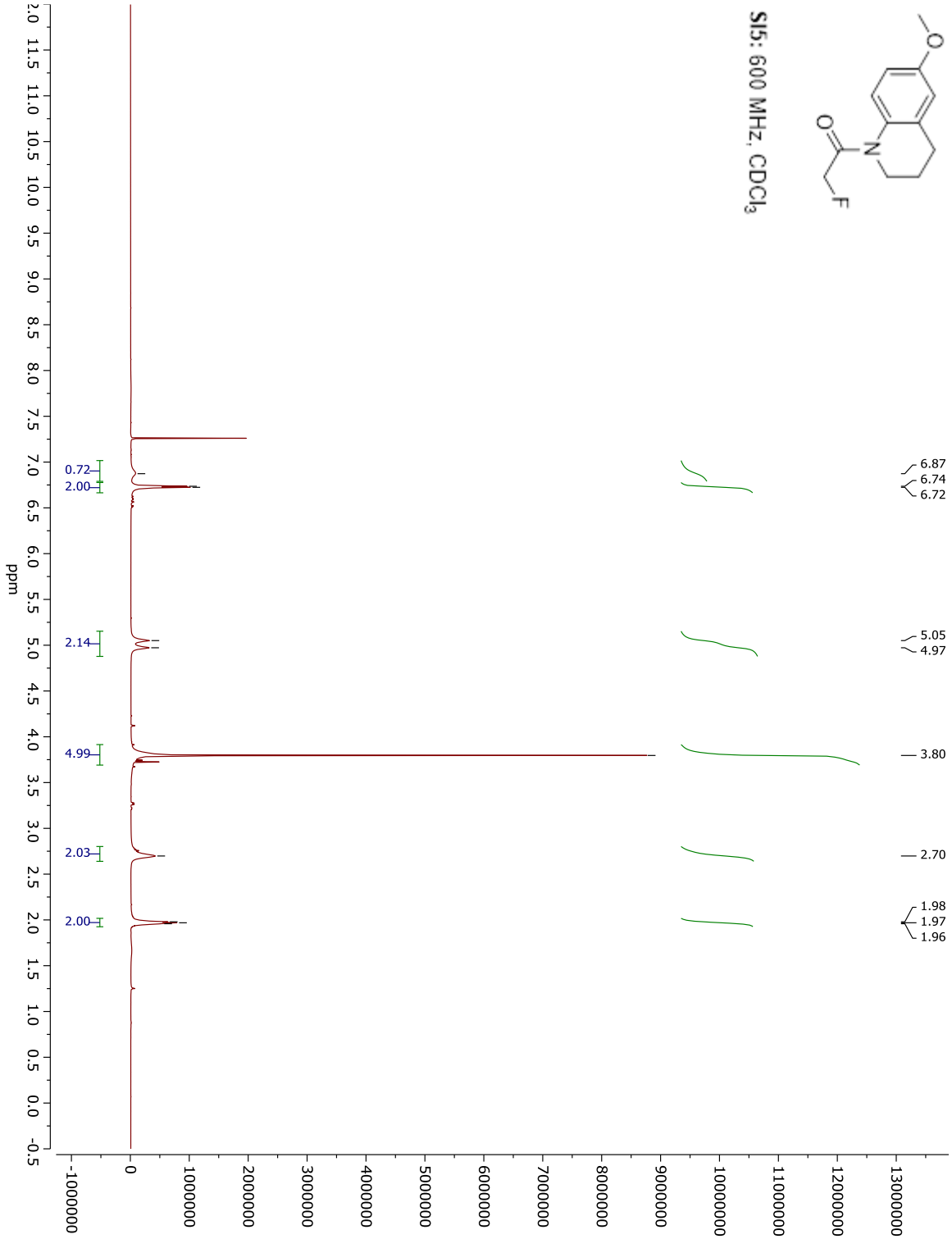

SI2: 151 MHz, CDCl3
165.55
157.30
134.96
131.56
130.03
127.38
126.04
113.59
111.52
55.60
43.13
27.35
24.02
ppm

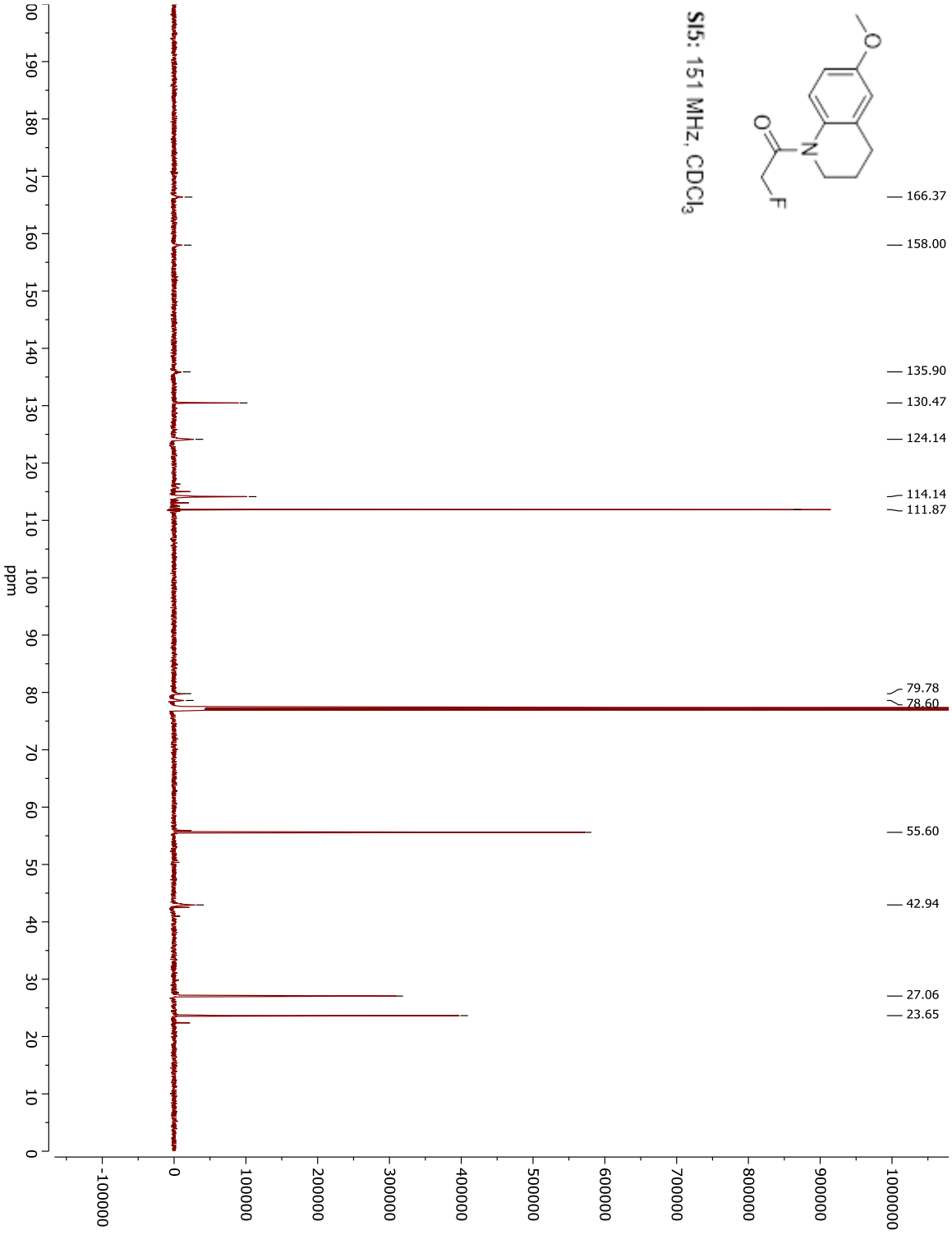

SI3: 600 MHz, CDCl3
O
N
O
SEt
ppm
1.00
1.01
1.00
5.15
2.06
2.02
2.04
3.05
7.60
7.58
6.73
6.73
6.72
6.71
6.67
6.67
3.78
3.78
3.77
3.76
2.94
2.92
2.91
2.90
2.74
2.73
2.72
1.99
1.98
1.97
1.96
1.94
1.31
1.29
1.28

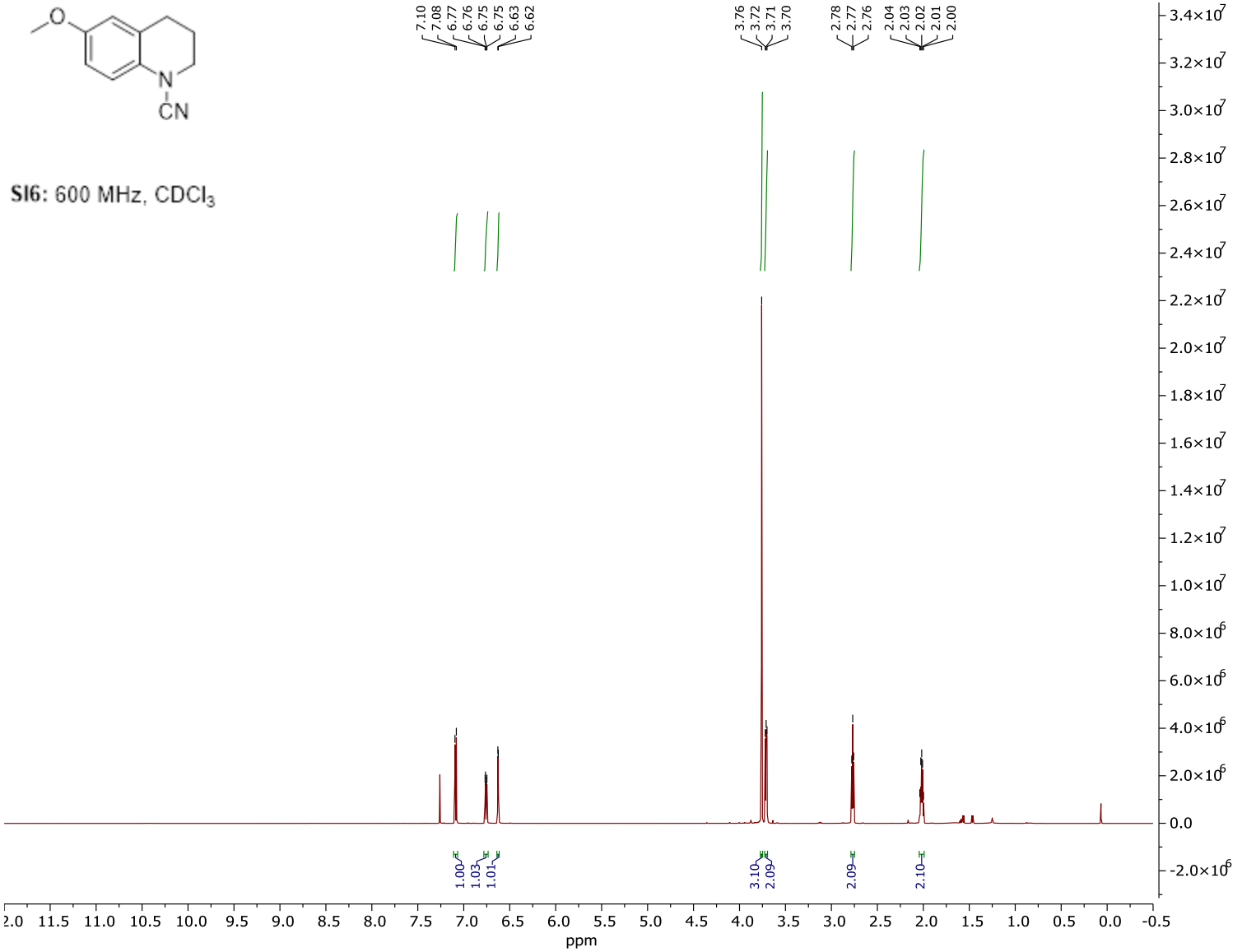

SI3: 151 MHz, CDCl3
SEt
168.61
157.07
133.50
131.35
126.23
113.66
111.41
55.53
45.02
27.21
25.26
23.80
15.27
ppm


SI4: 600 MHz, CDCl3
O
N
O
OMe
ppm
1.00
1.02
1.01
1.00
1.01
3.04
3.03
2.04
2.03
2.05
8.11
8.08
7.06
7.05
6.76
6.76
6.75
6.74
6.64
6.64
4.97
4.94
3.77
3.71
3.44
3.43
3.42
2.69
2.68
2.67
2.02
2.01
2.00
1.98
1.97

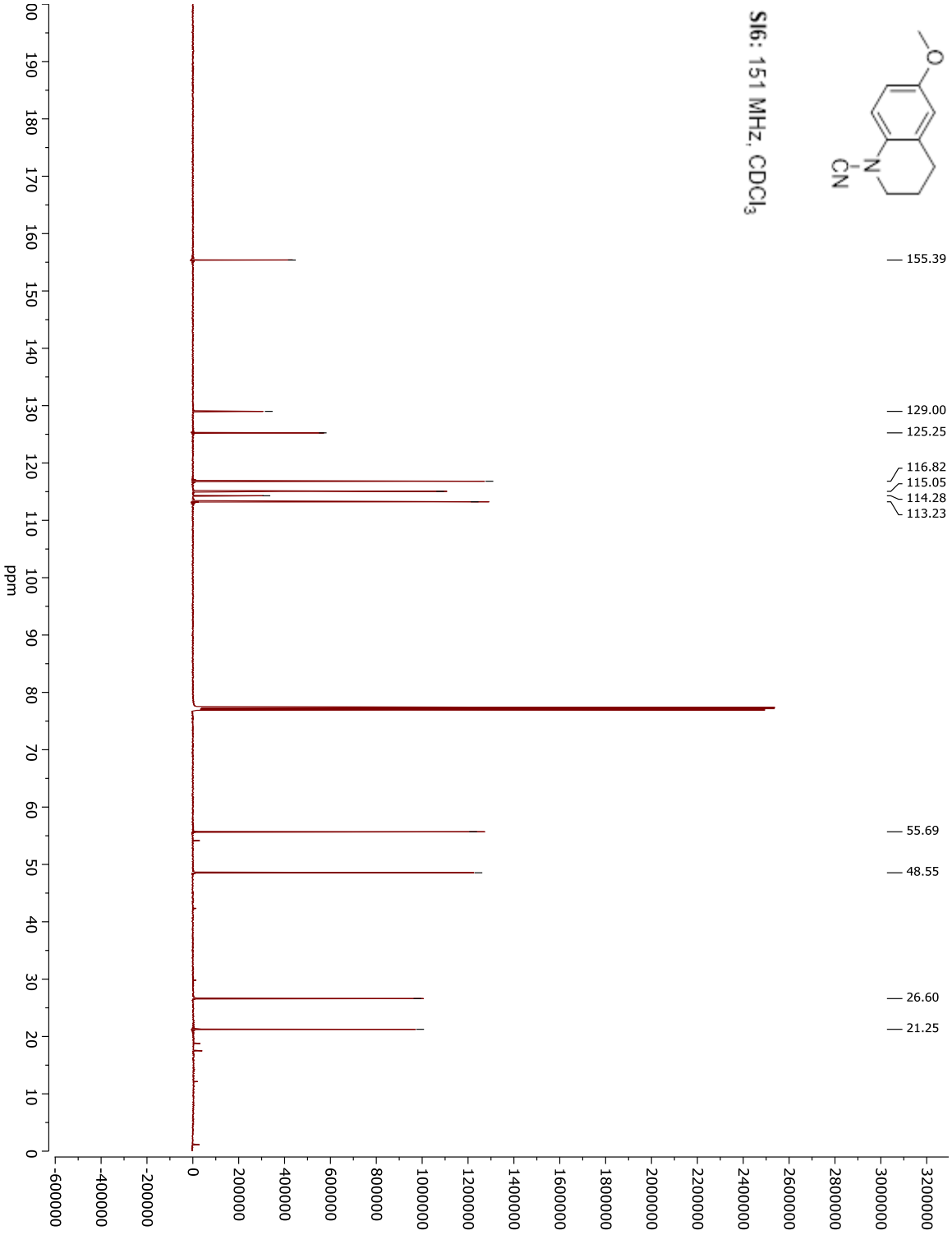

SI4: 151 MHz, CDCl3
O
N
OMe
169.30
154.72
145.65
133.50
129.24
116.72
113.70
112.26
88.45
55.08
50.35
45.36
27.14
22.06
ppm

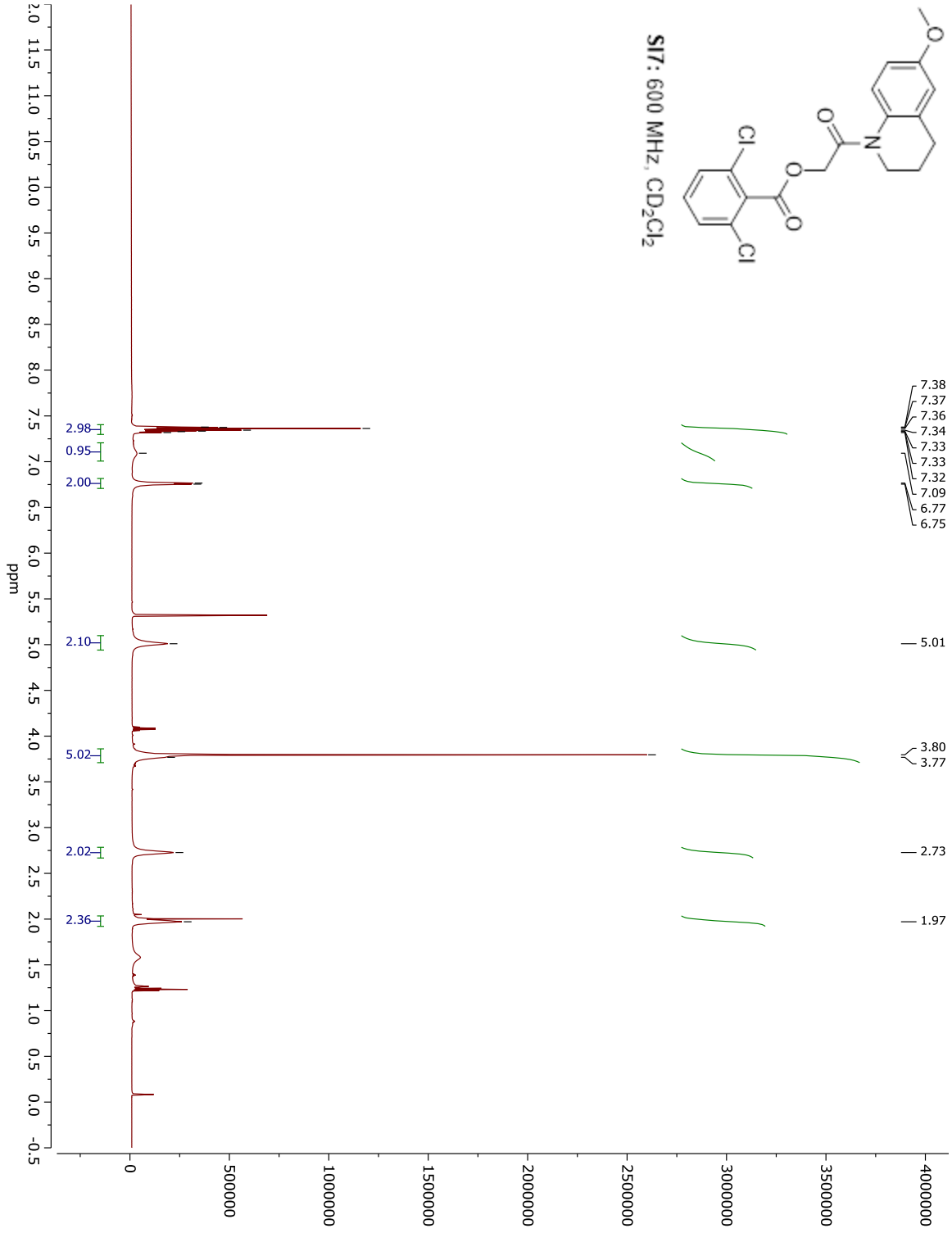

SI5: 600 MHz, CDCl3
ppm
0.72
2.00
2.14
4.99
2.03
2.00
6.87
6.74
6.72
5.05
4.97
3.80
2.70
1.98
1.97
1.96

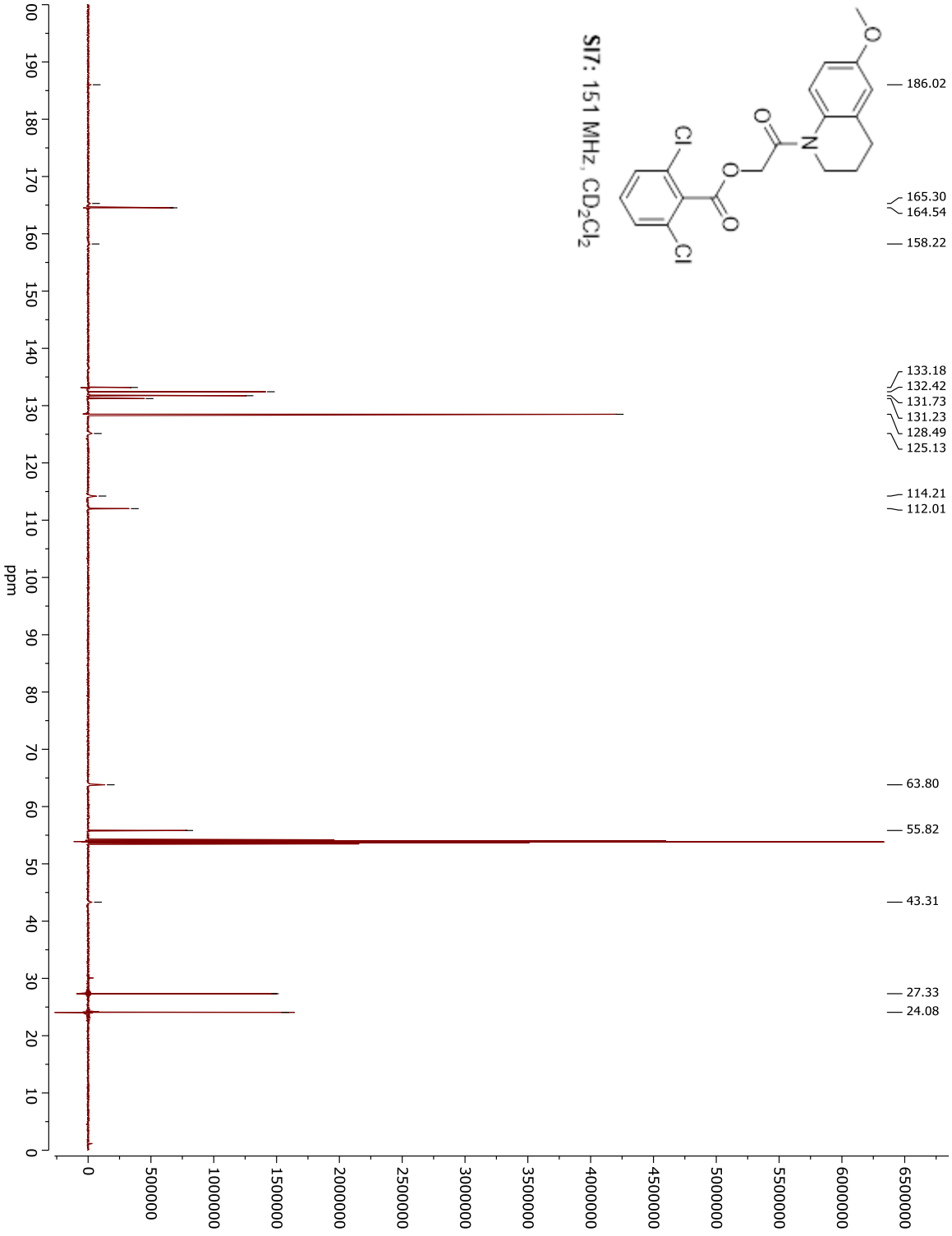

SI5: 151 MHz, CDCl3
166.37
158.00
135.90
130.47
124.14
114.14
111.87
79.78
78.60
55.60
42.94
27.06
23.65
ppm



SI6: 600 MHz, $CDCl_3$


SI6: 151 MHz, CDCl3
155.39
129.00
125.25
116.82
115.05
114.28
113.23
55.69
48.55
26.60
21.25
ppm


SI7: 600 MHz, CD_2Cl_2
Cl
Cl
O
O
O
O
N
O
7.38
7.37
7.36
7.34
7.33
7.33
7.32
7.09
6.77
6.75
5.01
3.80
3.77
2.73
1.97
2.98
0.95
2.00
2.10
5.02
2.02
2.36
ppm
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000


SI7: 151 MHz, CD_2Cl_2
186.02
165.30
164.54
158.22
133.18
132.42
131.73
131.23
128.49
125.13
114.21
112.01
63.80
55.82
43.31
27.33
24.08
ppm

Refrerences

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2. Backus, K. M.; Correia, B. E.; Lum, K. M.; Forli, S.; Horning, B. D.; Gonzalez-Paez, G. E.; Chatterjee, S.; Lanning, B. R.; Teijaro, J. R.; Olson, A. J.; Wolan, D. W.; Cravatt, B. F., Proteome-wide covalent ligand discovery in native biological systems. *Nature* **2016,** *534* (7608), 570-574.