Supporting Information

Radical Termination via β-Scission Enables a Photoenzymatic Allylic Alkylation Using 'Ene'-Reductases

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Experimental Procedures

General. Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers and used as received (Sigma-Aldrich, Oakwood Chemical, Combi-Blocks, TCI, Acros, and VWR). GDH-105 was purchased as cell-free lysate from Codexis and was used as received. Silica gel chromatography purifications were carried out using AMD Silica Gel 60 on a Biotage Isolera One automated flash chromatography system. ^{1}H and ^{13}C NMR spectra were recorded on Bruker UltraShield Plus 500 MHz, Bruker 400 MHz, Bruker 500 MHz, or a Varian 600 MHz instruments, and are internally referenced to residual proton and carbon signals in $CDCl_3$ (7.26 ppm and 77.16 ppm, respectively). ^{1}H NMR data are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift. High resolution mass spectra (HRMS) were obtained on a Thermo Fisher Scientific Exactive series DART Mass Spectrometer. IR spectra were recorded on a Thermo Scientific Nicolet iS50 FTIR spectrometer and peaks are reported in terms of frequency of absorption (cm^{-1}). Chromatography. Analytical high performance liquid chromatography (HPLC) was carried out using an Agilent 1260 Infinity HPLC System. Enantiomeric ratios (e.r.) were determined by chiral GC analysis on a Hewlett Packard 6890 gas chromatograph equipped with an Astec CHIRALDEX A-TA column.

Light Sources. The cyan LEDs lamps were constructed in-house from Chanzon High Power 50W Cyan LED Chips (497nm / 1500mA / DC30-34V / 50W) (Amazon 1DGL-JC-50W-490) powered by Mean Well HLG-320H-C1750A power supplies (320W / 183V / 1750 mA) by wiring four LED Chips in series. Each LED chip was secured to a Nagulagu cooling aluminum LED heatsink equipped with a 12V fan (Amazon B01K1Z6VP6). Blue (456 nm; PR160L-456) and violet (390 nm; PR160L-390) Kessil lamps were used for blue and violet light irradiation in evaluating different color light sources. Violet irradiation for racemic standards was achieved using an air-cooled 96-LED lumidox II array (415 nm; LUM96-415UV) equipped with a 24-vial metal block.

Protein Expression and purification. Enzymes were produced in BL21(DE3) E. coli transformed with plasmids encoding each of the 'ene'-reductase variants from pre-existing glycerol stocks in our laboratory. Mutants were expressed identically to *wild-type*. Glycerol stocks of BL21(DE3) *E. coli* transformed with plasmids encoding ERED enzymes were used to initiate a 10 mL overnight culture in LB media with 100 µg/mL ampicillin (37 °C, 250 rpm). Auto-Inducing Turbo Broth™ media (500 mL in 2 L flask) containing 100 µg/mL ampicillin was inoculated with 1 mL of the overnight culture and grown for 24 hours (30 °C, 250 rpm). After expression cells were harvested by centrifugation (4000 x g, 20 min, 4 °C) and frozen at -20 ºC for later purification. Frozen cells were thawed on ice and resuspended in buffer A (50 mM TEOA pH 7, 25 mM imidazole) to a final concentration of 2 mL/g of cells. The resuspended cells were treated with lysozyme (1mg/mL), FMN (1mg/mL), DNAse (0.1 mg/mL), PMSF (1mM) and allowed to shake for 1 hour at 25 °C. The cells were further disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle; Sonicator QSonica Q500 Ultra Sonicator). To pellet insoluble material, lysates were centrifuged (20,000 x g, 1.5 hours, 4 °C). Proteins were purified using a nickel-NTA column (5 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTAStart purifier FPLC system (GE Healthcare). Enzymes were eluted with 100 % buffer B (50 mM TEOA pH 7, 250 mM imidazole. Storage buffer 50 mM TEOA pH 7) over five

column volumes. Yellow fractions containing ‘ene’-reductase enzymes were pooled, concentrated, and subjected to three buffer exchanges into an imidazole free storage buffer. Concentrated enzymes (1-4 mM measured by UV-vis spectroscopy at 464 nm; extinction coefficient = ε_{464} = 11.4 x 10^{-3} M^{-1} cm^{-1}) were portioned into 100 or 500 nmol aliquots, flash frozen in liquid nitrogen, and stored at -20 °C until later use.

Sequence Information

GluER-wild type ("WT")

DNA Sequence:
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Amino Acid Sequence:
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GluER-T36A-K317M-Y343F (“G6”)

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Reaction Optimization

General Procedure for reaction optimization experiments: For each reaction, 3.1 mg of substrate **1** (10 μmol, 1 equiv.) was weighed into a shell vial and imported into a Coy anaerobic chamber. Inside the anaerobic chamber, a 10x stock solution of "turnover mix" was prepared by dissolving GDH-105 (3 mg, 10 wt%/rxn), $NADP^+$ (2 mg, 2 mol%/rxn) and glucose (108 mg, 6 equiv./rxn) in 1 mL of 100 mM buffer. 100 μL of this solution was transferred to a shell vial equipped with a cross stir bar and diluted with 100 mM buffer (365 μL). A 100 nmol aliquot of GluER enzyme (33 μL*) was added and the mixture was gently vortexed until the yellow color faded and the mixture became clear. Substrate **1** was dissolved in co-solvent (30 μL) and transferred via pipette to the reaction mixture. The substrate vial was rinsed with an additional 30 μL of co-solvent that was then transferred to the reaction mixture. The vial was capped with a septum, removed from the anaerobic chamber, and irradiated with cyan LEDs at room temperature for 48 hours. At this time, the reaction mixture was removed from the light source and diluted with 2 mL of MeCN containing 0.5 mg/mL 1,3,5-trimethoxybenzene (TMB) and stirred 30-60 minutes at room temperature. The reaction was further diluted with 1.44 mL of water to a final volume of 4 mL. Insoluble protein components were removed by centrifuging the vials in a Genevac and filtering a portion of the mixture through a cotton plug directly into an HPLC vial. Yields were calculated based on the ratio of product to TMB. The contents of the HPLC vial were then combined into the total reaction mixture and the mixtures were extracted three times with EtOAc. The combined organic layers were concentrated by Genevac and redissolved in 20% isopropanol in hexane for analysis by chiral HPLC.

*If a larger volume aliquot of enzyme was used, the amount of buffer used to dilute the turnover mix was decreased by a corresponding amount. The total reaction volume adds to 558 μL.

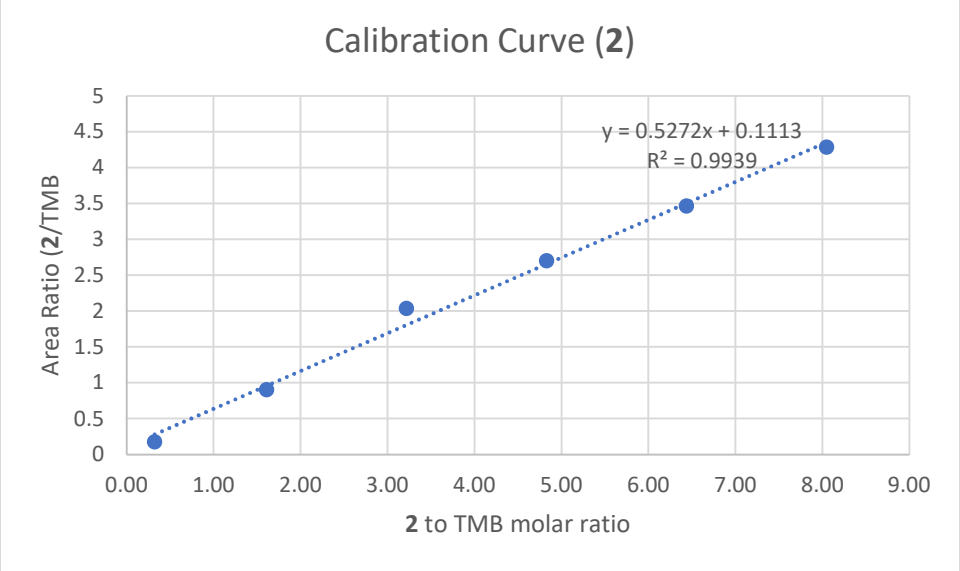
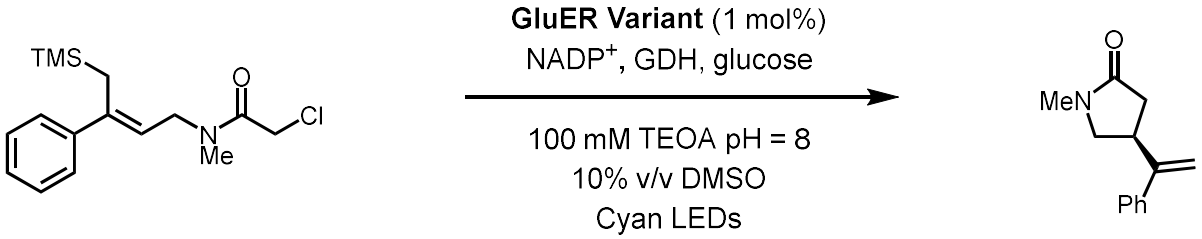


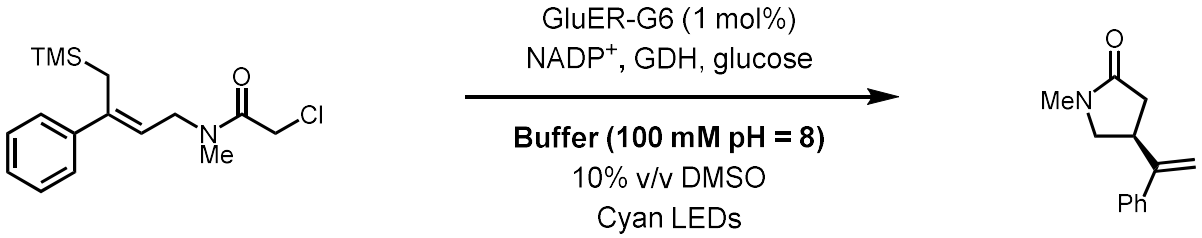
Figure SI-1. Calibration curve for product **2**.

Table SI-1: Evaluation of GluER variants.



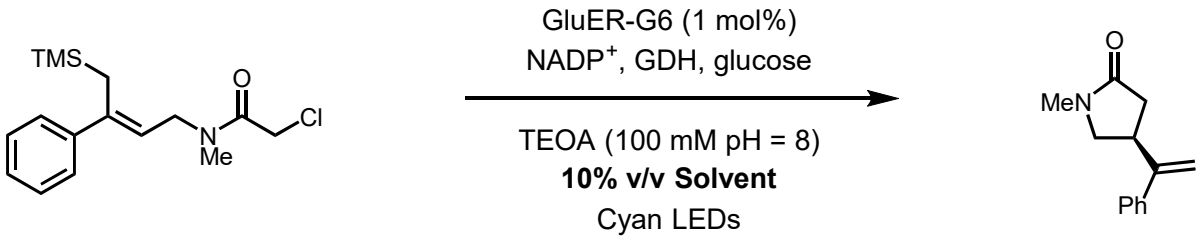
GluER Variant	% yield (HPLC)	e.r.
GluER-WT	77	>99:1
GluER-T36A	64	>99:1
GluER-T36A-K317M-Y343F (G6)	79	>99:1

Table SI-2: Evaluation of reaction buffers.



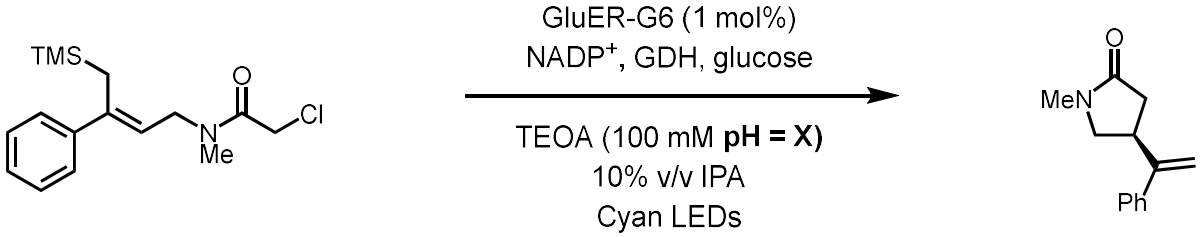
Buffer	% yield (HPLC)	e.r.
KP_i	56	>99:1
Tricine	78	>99:1
TRIS	69	>99:1
TEOA	79	>99:1

Table SI-3: Evaluation of organic co-solvents.



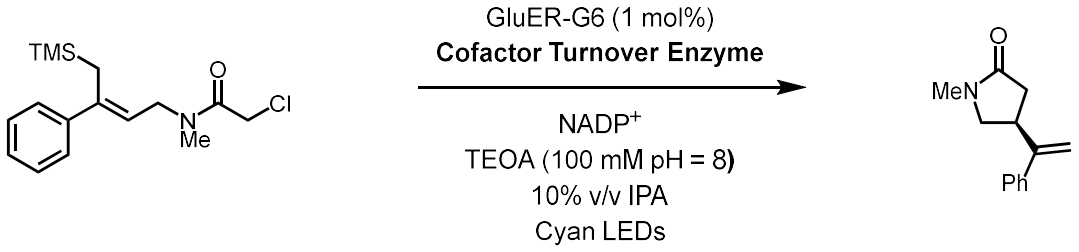
Solvent	% yield (HPLC)	e.r.
DMSO	79	>99:1
MeCN	63	>99:1
THF	71	>99:1
EtOH	76	>99:1
iPrOH	92	>99:1
Acetone	80	>99:1
DMF	72	>99:1

Table SI-4: Evaluation of buffer pH.



pH	% yield (HPLC)	e.r.
7	72	>99:1
7.5	78	>99:1
8	92	>99:1
8.5	83	>99:1

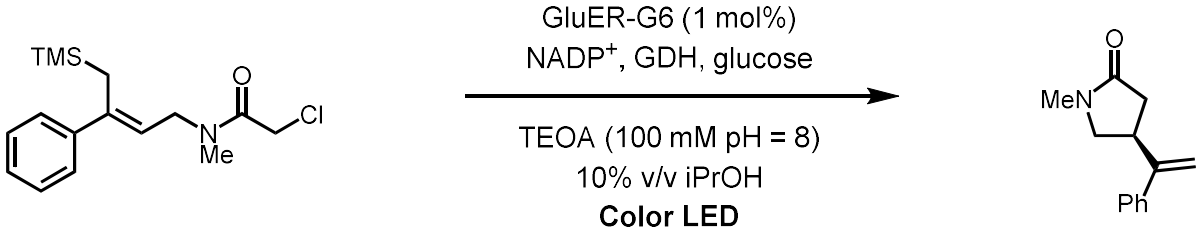
Table SI-5: Evaluation of flavin recycling systems.



substrate concentration	KRED* Turnover (% yield)	GDH Turnover (% yield)	dithionite (% yield)
18	84	86	25
24	63	64	n.d.
36	62	38	n.d.

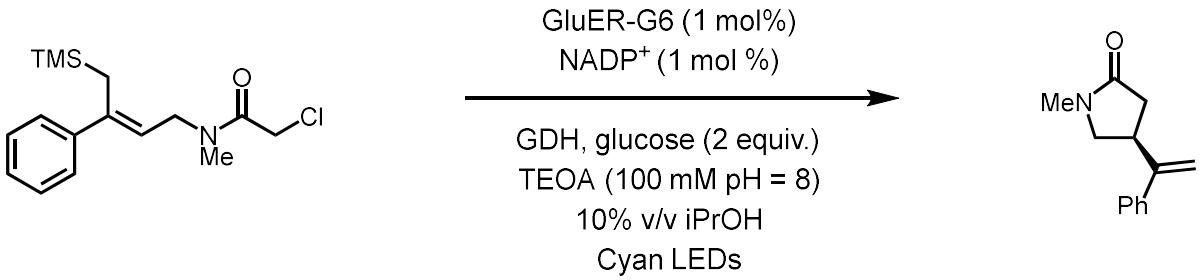
*Codexis KRED P103

Table SI-6: Evaluation of different wavelengths of light.



LED Color	% yield (HPLC)	e.r.
Cyan (λ_{max} = 497 nm)	92	>99:1
Blue (λ_{max} = 465 nm)	72	>99:1
Violet (λ_{max} = 395 nm)	19	>99:1
No Light	0	>99:1

Table SI-7: Various control experiments



Control Experiments	% yield (HPLC)	e.r.
Standard Conditions	92	>99:1
1 equiv. of Glucose	79	>99:1
GluER-T36A (0.5 mol %)	67	>99:1
GluER-G6 (0.5 mol %)	93	>99:1
no turnover mix	72	>99:1
no GluER-G6	0	>99:1

Mechanistic Investigation

Transient Absorption Studies

All samples were prepared in an Mbraun oxygen free (<0.5ppm) glovebox. Solvents were freeze pump thawed and allowed to equilibrate in inert atmosphere for 24 hours. The buffer solution of 100mM TEOA pH 8 with 5% isopropyl alcohol. Each sample was prepared with 200nmoles of protein, and 1mg of sodium dithionate was used as the reducing agent to convert FMN_{OX} to FMN_{HQ}. Substrate **1** was added in 5mg quantity and the total volume of the solution was kept at 1mL. Transient absorption spectroscopy using the Ultrafast systems EOS or Helios and was conducted in sealed 2mm quartz cuvettes that were stirred and translated continually during the measurement.

Samples were excited at 370nm (Figure **SI-3**) with 300nJ of energy and the spot size was characterized in Figure **SI-2**. No appreciable aggregation or sample degradation was observed over the course of the experiment. Using the same methodology as in our previous studies[1] a quantum yield of 0.76% is observed for this reaction. To test the validity of the laser experiments LCMS was performed post experiment for both 370nm and 490nm samples and product formation comparted. Product was formed under both conditions (Table SI-6).

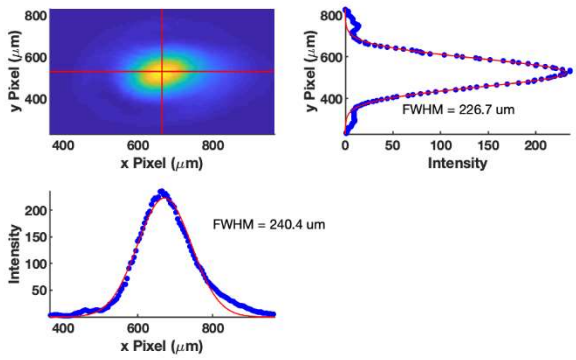


Figure SI-2: Gaussian fits to the excitation pulse size as measured on a CCD camera at sample position

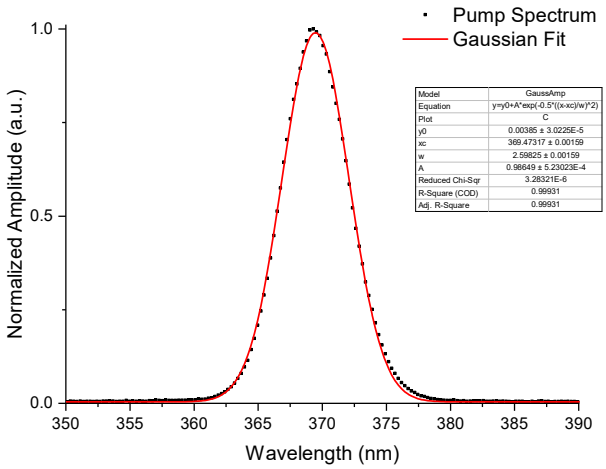


Figure SI-3: Gaussian fit to excitation pulse

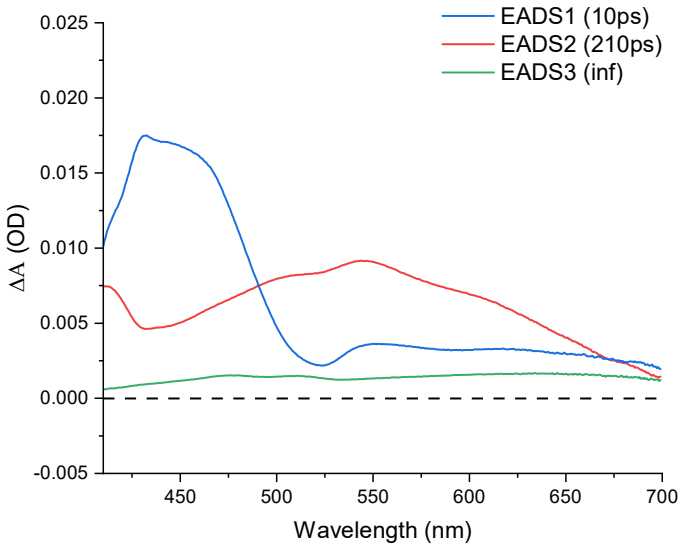


Figure SI-4: Evolutionary associated difference spectra for G6 and model substrate **1** in 100mM TEOA buffer pH 8 from 0 to 8ns.

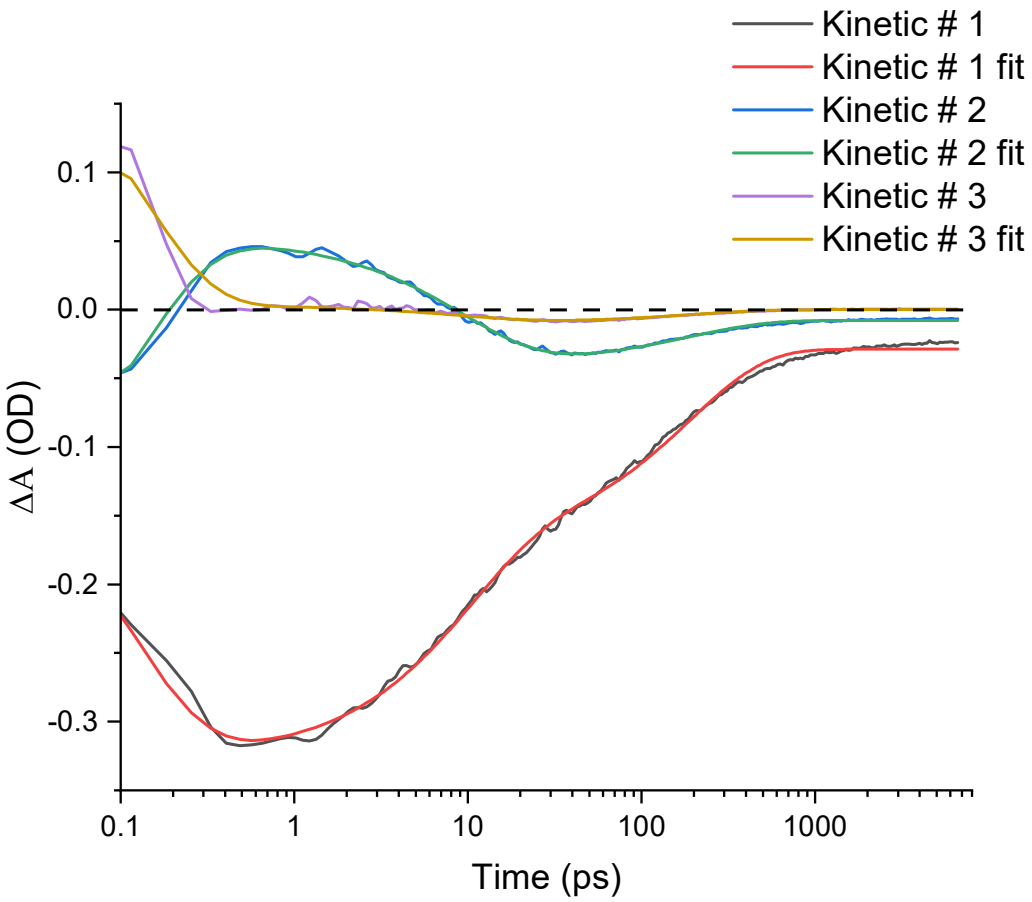


Figure SI-5: Principle kinetic fits for G6 and model substrate **1** in 100mM TEOA buffer pH 8 from 0 to 8ns.

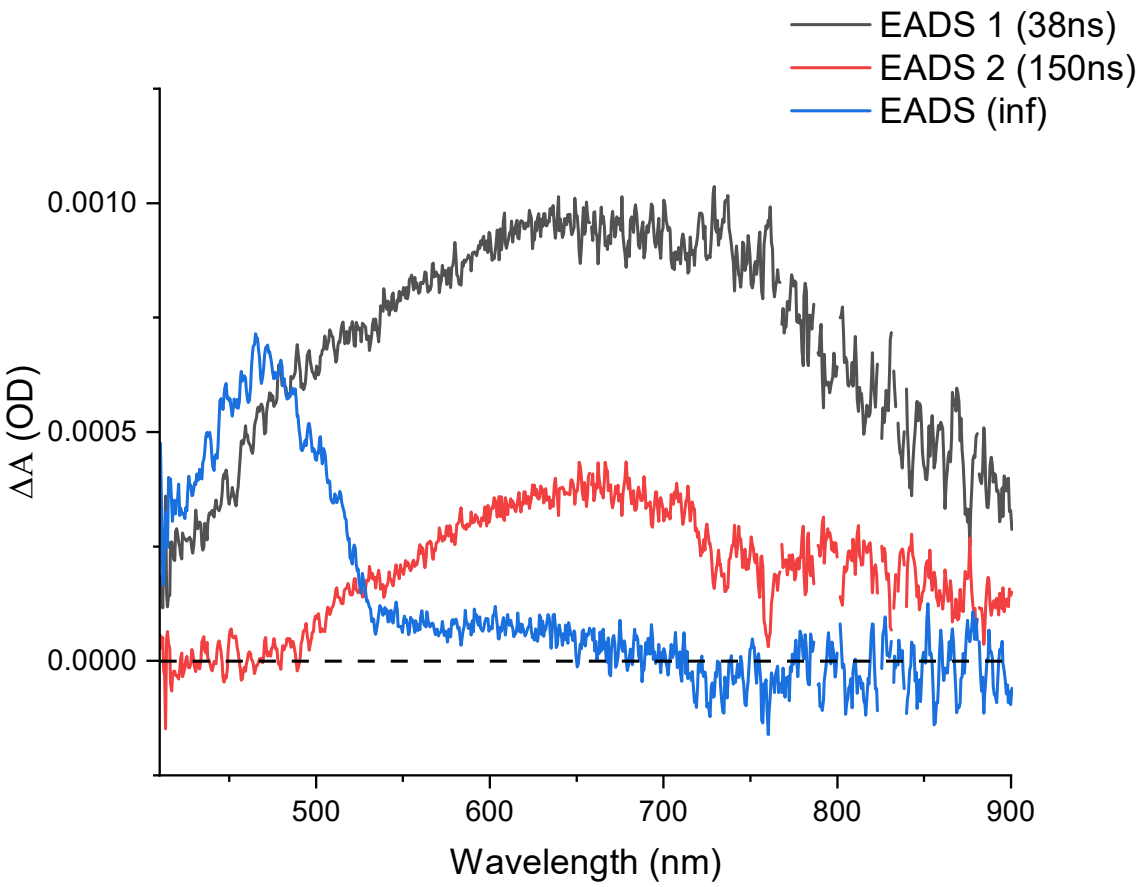


Figure SI-6: Evolutionary associated difference spectra for G6 and model substrate **1** in 100mM TEOA buffer pH 8 from 0 to 1 µs.

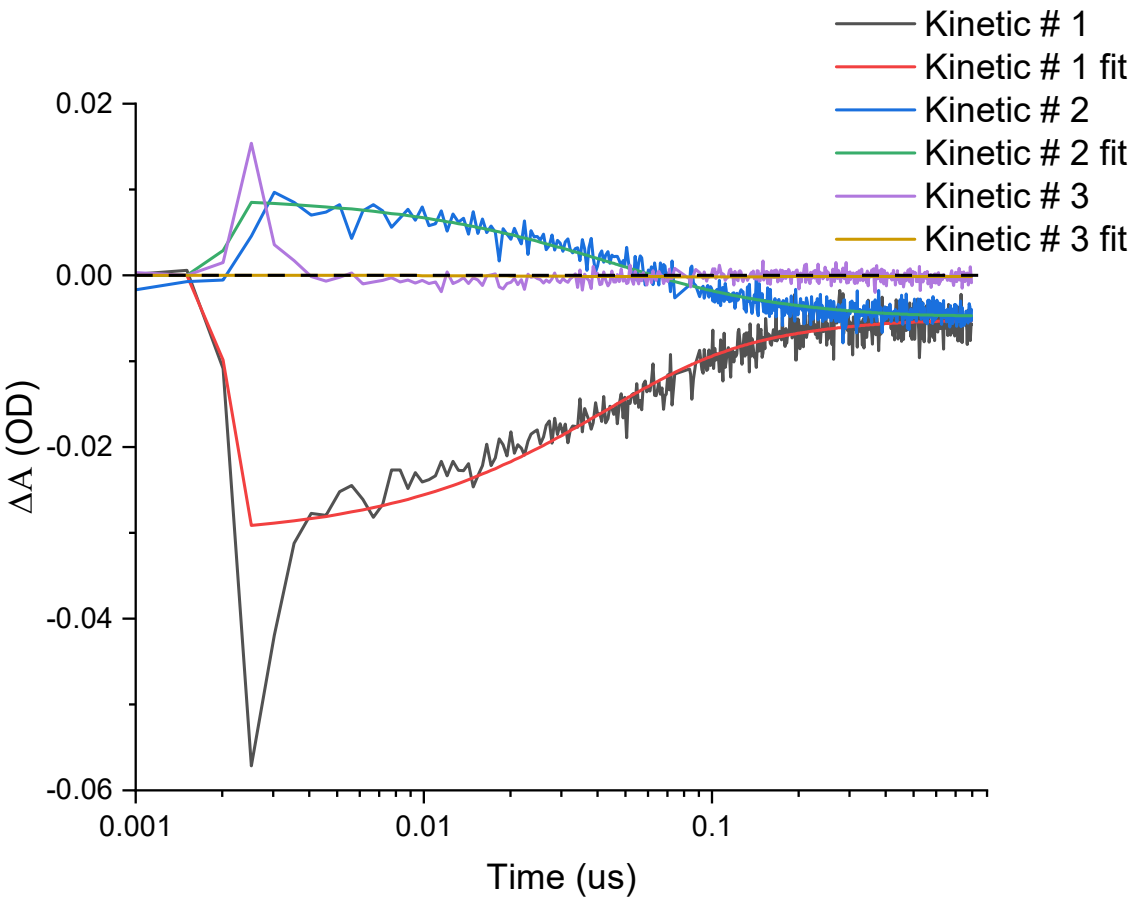
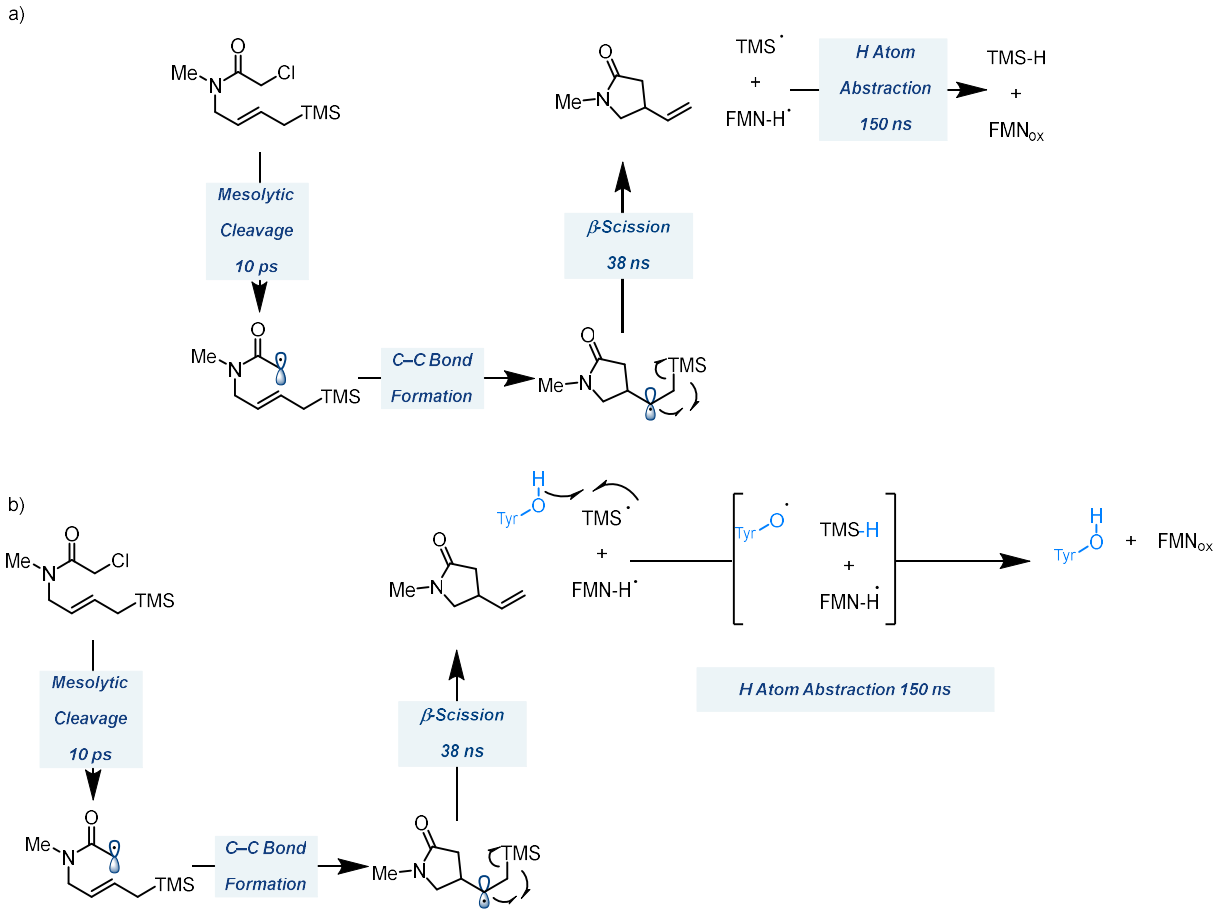


Figure SI-7: Principle kinetic fits for G6 and model substrate in 100mM TEOA buffer pH 8 from 0 to 1 μs.

Description

Description

Performing global analysis on the transient absorption results and comparing them to data obtained previously[2]. The short time transient absorption results show initial mesolytic cleavage step after electron injection occurs within 10 ps as previously observed[1,2]. (Figure SI-7) Two other features are observed new broadly absorbing component is fit with infinite lifetime as it does not decay on this timescale and a 200 ps component is also observed and attributed to excited uncomplexed G6 hydroquinone. To elucidate the infinite time component, long-time transition absorption spectroscopy was conducted, and global analysis was again applied (Figure SI-6). The first 1 ns is fit with an instrument response function, so the short-time components are removed. The broadly absorbing feature can be seen to decay with a lifetime of 38 ns which is attributed to the beta-scission. An absorption spectrum of the neutral semiquinone then persists with a lifetime of 150 ns which decays to the oxidized flavin. As formation of oxidized flavin is observed the silyl radical abstracts the H-atom from the neutral semiquinone.



Scheme SI-1: Transient absorption mechanism with two possibilities for TMS radical termination. A) HAT from flavin semiquinone to TMS radical. B) HAT from an enzymatic tyrosine to TMS radical, followed by reaction between tyrosyl radical and FMN semiquinone to form the flavin quinone.

Charge-Transfer Complex

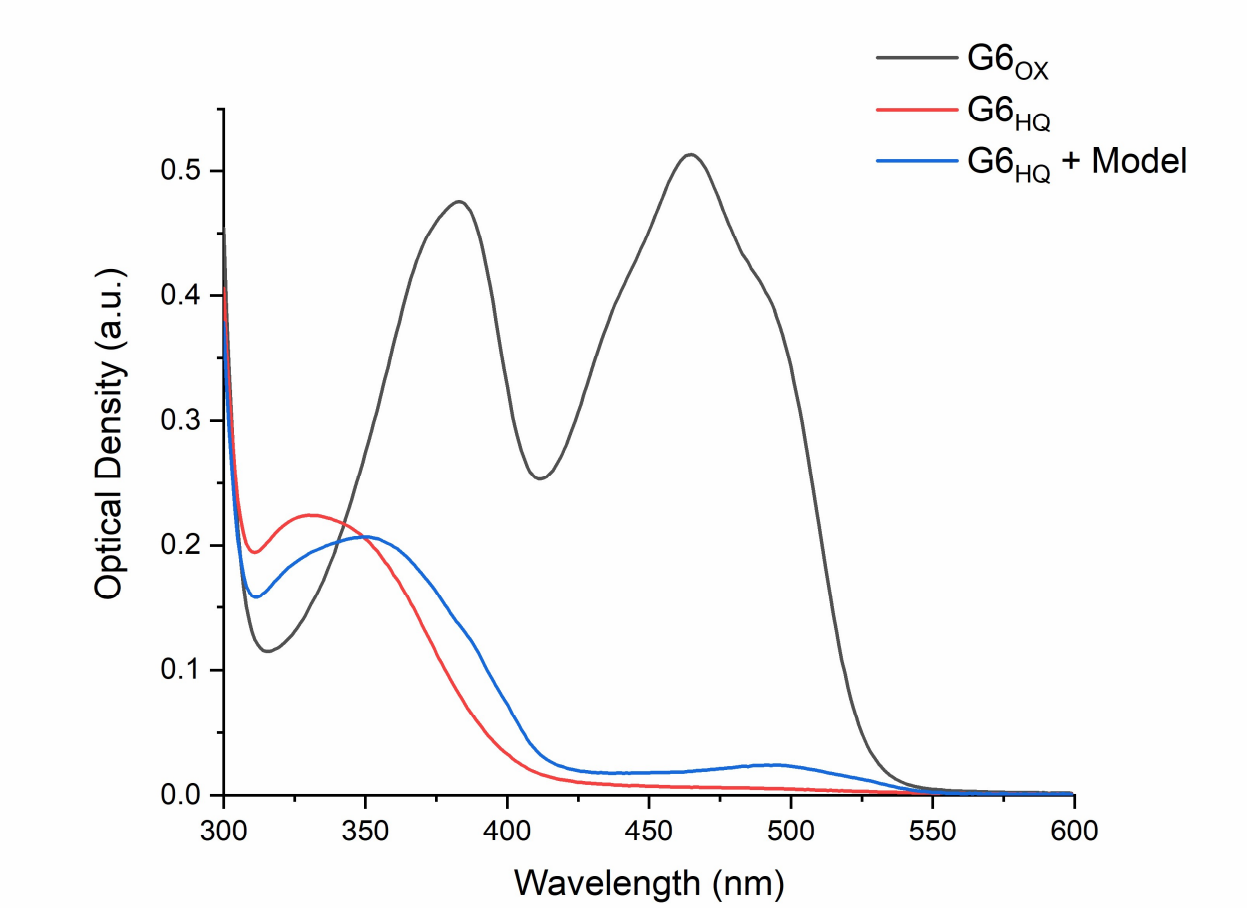


Figure SI-8: UV-vis spectrum of G6 enzyme with FMN in the oxidized state (gray), reduced G6 (FMN hydroquinone, red), and reduced G6 with an excess of model substrate **1** (blue). The spectral feature at 490 nm indicates the presence of a charge-transfer complex.

The charge transfer state was characterized in 2mm quartz cuvette prepared in the same manner as for transient absorption studies. The UV-Vis spectrum of G6 in the oxidized form was taken with baseline subtraction followed by the additional of 0.05mg of sodium dithionite in 10uL of buffer solution to generate the reduced form of the flavin and again verified by UV-Vis. Finally, addition of 5mg of **1** was added in 50uL of isopropyl alcohol and the UV-Vis spectrum was taken showing a growth at 490nm.

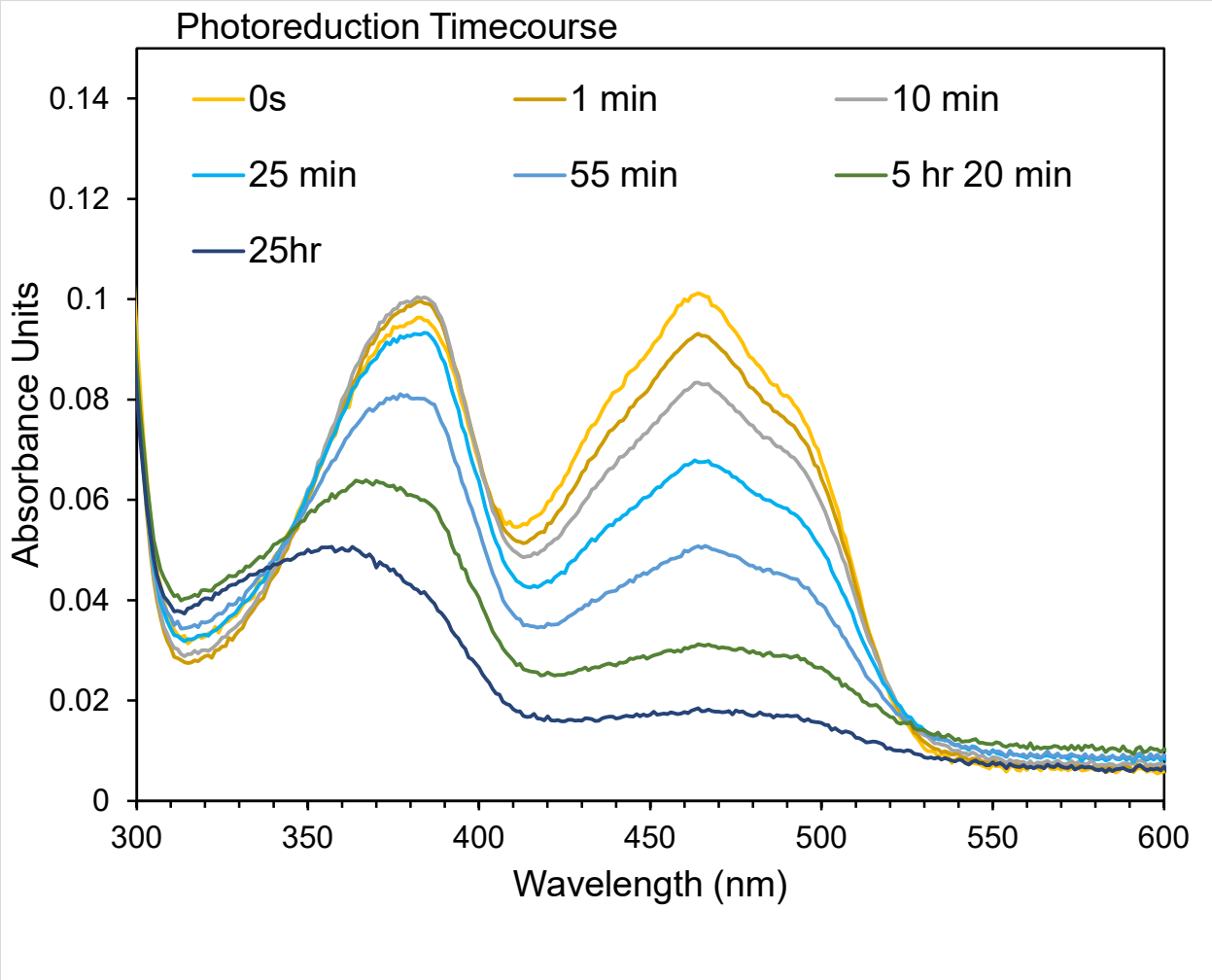


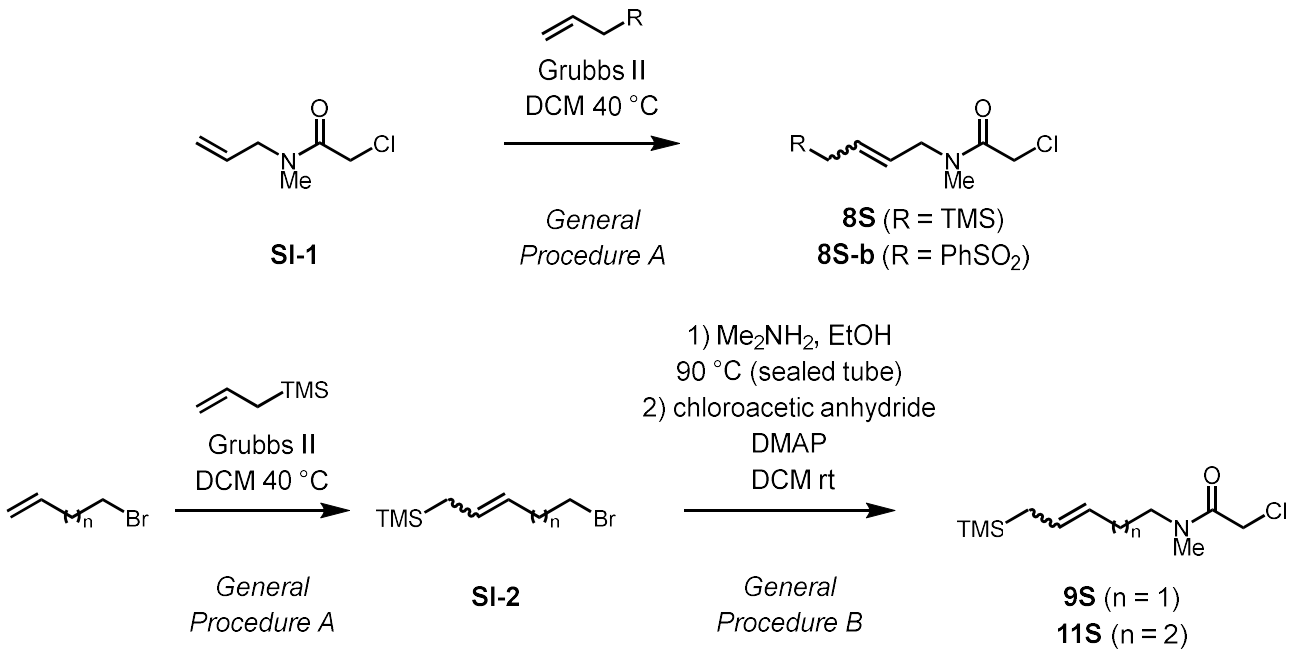
Figure SI-9: UV-vis spectrum of G6 enzyme in TEOA buffer (pH = 8) with FMN in the oxidized state (yellow, 0 s), irradiated with cyan light over the course of 25 hours, at which point the primary species was the fully reduced flavin hydroquinone (dark blue, 25 hr).

A thoroughly cleansed 1mL quartz cuvette with a Schleck adaptor was filled with approximately 2 mL of buffer (100 mM TEOA, pH = 8). The volume of buffer then thoroughly freeze-pump-thawed for a total of 4 cycles. Each cycle entailed the following: first freezing the buffer/enzymatic mixture for 3 minutes using an acetone and dry ice bath. Then once the mixture was completely frozen, it was opened to vacuum for another 5 minutes while in the dry ice/IPA bath. The vacuum was then closed and the mixture was allowed to thaw under static vacuum in room temperature water, and then repeated for another 2 cycles. The buffer vessel was then placed under static vacuum and taken into an oxygen free mBraun glove box. Once inside the glove box, the static vacuum was released. 100 nanomoles/95 microliters of G6 GluER aliquots were added to the freeze pump thawed buffer mixture and the schlenck cuvette was resealed inside this oxygen free environment(<4ppm O_2). This schlenck cuvetted of the GluER enzymatic buffer mixture was then sealed tightly, removed from the glovebox and exposed to cyan irradiation for over a course of 26 hour. At several time increments, the enzymatic buffer filled

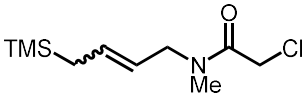
cuvette was briefly taken to a UV spectrometer to observe the photoreduction of Flavin cofactor inside the GluER ene-reductase, before returing to being cyran irradiated. At the beginning of the experiment (0 s of photoreduction), most of the flavin species present in the mixture is currently residing at its fully oxidized state, as indicated by large abosorbance bands at approximately 380 nm and 460 nm. However, over the course of a few houres, it can be seen that the primary Flavin redox species is that of its hydroquinone, by a significant band feature growth at 360 nm.

General Procedures for Substrate Synthesis

Synthesis of Unsubstituted Allylsilane Substrates

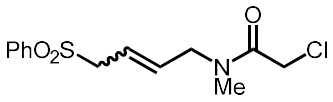


General Procedure A: Terminal olefin (1 equiv.) and allyltrimethylsilane (3 equiv.) were dissolved in DCM (0.1 M) and Grubbs second generation catalyst (3 mol%) was added according to a procedure adapted from Rodriguez and coworkers[3]. The reaction mixture was sparged for 15 minutes with N_2 and heated to reflux overnight. The crude reaction mixture was concentrated by rotary evaporator and purified by flash column chromatography eluting with hexanes/EtOAc to afford the title compound as a clear or brown oil. For mixtures of *E* and *Z* isomers, the ^{1}H and ^{13}C NMR data is reported for the mixture. Note: amide rotamers lead to the doubling of many peaks in the ^{13}C NMR spectra. When non-overlapping peaks chemical shift round to the same value, they are reported followed by "(2)".



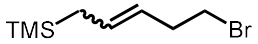
16

(*E*/*Z*)-2-chloro-N-methyl-N-(4-(trimethylsilyl)but-2-en-1-yl)acetamide (8S): Prepared according to General Procedure A using346 mg (2.4 mmol) **SI-1**[4] to afford 67 mg (11% yield) of **8S** as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.62 (ddddd, *J* = 11.6, 9.6, 8.2, 5.1, 3.5 Hz, 1H), 5.23 (dddd, *J* = 12.0, 10.6, 6.4, 3.0 Hz, 1H), 4.07 (s, 2H), 3.93 (d, *J* = 6.7 Hz, 1H), 3.88 (dd, *J* = 6.0, 1.4 Hz, 1H), 2.96 (d, *J* = 27.1 Hz, 3H), 1.55 – 1.44 (m, 2H), 0.00 (d, *J* = 2.9 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.7, 166.3, 132.2, 131.7, 122.1, 122.0, 52.5, 50.2, 41.7, 41.3, 34.7, 33.7, 23.1, -1.7, -1.8. **IR** (cm^{-1}): 2953, 1734, 1616, 1406, 1248, 1192, 849. **HRMS**: calculated for $C_{10}H_{21}ClNOSi$ $[M+H^+]^+$: 234.1075. Found $[M+H^+]^+$: 234.1033.



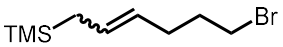
16b

(*E*/*Z*)-2-chloro-N-methyl-N-(4-(phenylsulfonyl)but-2-en-1-yl)acetamide (8S-b): Prepared according to General Procedure A using **SI-1** using 100 mg **SI-1** (0.68 mmol) to afford 56 mg (27% yield) of **8S-b** as a slightly brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dt, *J* = 7.2, 1.4 Hz, 2H), 7.70 – 7.62 (m, 1H), 7.60 – 7.52 (m, 2H), 5.71 – 5.36 (m, 2H), 4.04 (s, 1H), 3.97 – 3.91 (m, 3H), 3.81 (dd, *J* = 13.9, 6.1 Hz, 2H), 2.89 (d, *J* = 51.5 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.5, 138.5, 134.6, 134.1, 129.4, 128.5, 119.7, 59.7, 49.5, 41.4, 35.4. **IR** (cm^{-1}): 2968, 1645, 1447, 1404, 1378, 1305, 1138, 1085, 974, 951. **HRMS**: calculated for $C_{13}H_{17}ClNO_3S$ $[M+H^+]^+$: 302.06122. Found $[M+H^+]^+$: 302.0624.



SI-2a

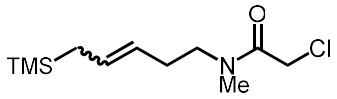
(*E*/*Z*)-(5-bromopent-2-en-1-yl)trimethylsilane (SI-2a): Prepared according to General Procedure A using 1.0 g (7.4 mmol) of 4-bromobut-1-ene to afford 1.4 g (85% yield) of **SI-2a** as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.52 (dddd, *J* = 16.2, 9.4, 6.1, 2.0 Hz, 1H), 5.25 – 5.17 (m, 1H), 3.35 (td, *J* = 7.3, 1.7 Hz, 2H), 2.65 – 2.48 (m, 2H), 1.40 (dt, *J* = 4.3, 2.6 Hz, 2H), 0.01 – -0.01 (m, 9H).



SI-2b

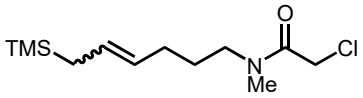
(*E*/*Z*)-(6-bromohex-2-en-1-yl)trimethylsilane (SI-2b): Prepared according to General Procedure A using 1.0 g (6.7 mmol) of 5-bromopent-1-ene to afford 1.0 g (63% yield) of **SI-2b** as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.59 – 5.38 (m, 1H), 5.20 (tdd, *J* = 9.5, 6.5, 4.5 Hz, 1H), 3.46 – 3.29 (m, 2H), 2.31 – 2.09 (m, 2H), 1.90 (q, *J* = 7.0 Hz, 2H), 1.45 – 1.36 (m, 2H), -0.01 (d, *J* = 2.4 Hz, 9H).

General Procedure B: Alkyl bromide (1 equiv.) was dissolved in a solution of $MeNH_2$ (33% in EtOH, 0.3 M) in a sealed tube and heated to 90 °C overnight. The reaction mixture was concentrated by rotary evaporator and the crude product was diluted with diethyl ether and 1M HCl. The layers were separated and the aqueous layer was adjusted to pH >12 by addition of 1M NaOH and extracted with three portions of diethyl ether. The combined organic layers were dried over $MgSO_4$ and concentrated by rotary evaporator. The resulting crude amine was dissolved in DCM (0.1 M) and treated with chloroacetic anhydride (1.2 equiv.) and DMAP (0.1 equiv.) at room temperature. When judged complete by TLC analysis, the reaction mixture was poured onto 1M HCl and diluted with DCM. The layers were separated and the aqueous layer was extracted with two additional portions of DCM. The combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporator before purification by flash column chromatography to afford the title α-chloroamide as a clear oil. For mixtures of *E* and *Z* isomers, the ^{1}H and ^{13}C NMR data is reported for the mixture.



9S

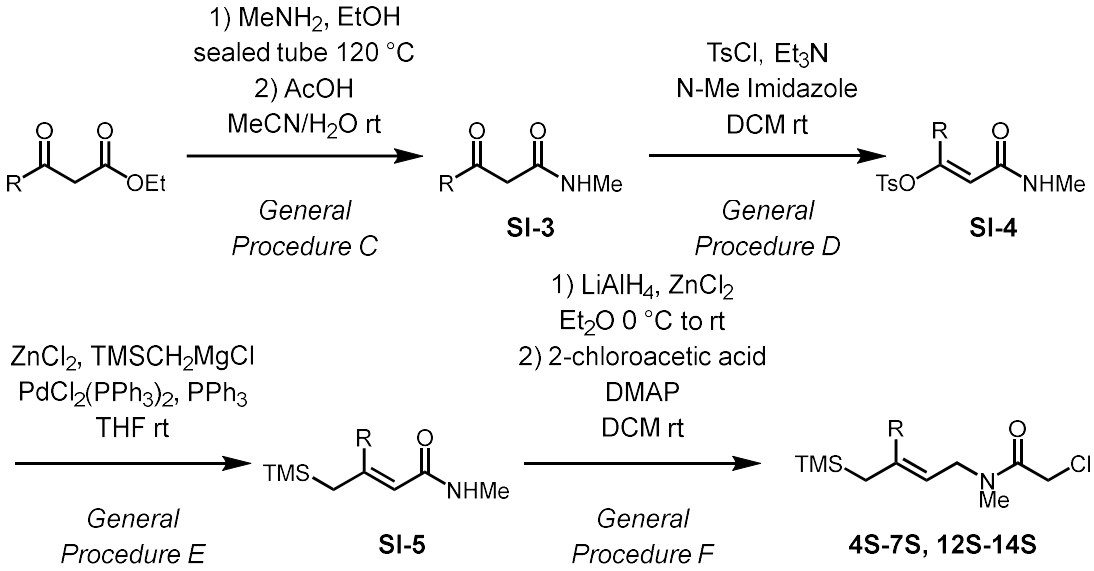
(*E*/*Z*)-2-chloro-N-methyl-N-(5-(trimethylsilyl)pent-3-en-1-yl)acetamide (9S): Prepared according to General Procedure B using **SI-2a** (451 mg 2.0 mmol) to afford 188 mg (37% yield) of **9S** as a clear oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.59 – 5.41 (m, 1H), 5.26 – 5.11 (m, 1H), 4.07 (dd, *J* = 12.6, 7.4 Hz, 2H), 3.43 – 3.30 (m, 2H), 3.01 (dd, *J* = 52.5, 11.1 Hz, 3H), 2.28 (ddd, *J* = 25.8, 10.7, 5.1 Hz, 2H), 1.52 – 1.38 (m, 2H), -0.01 (dd, *J* = 14.0, 6.0 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.5, 130.8, 129.5, 129.3, 128.4, 124.6, 123.3, 123.0, 121.8, 50.9, 50.3, 48.8, 48.6, 41.7, 41.2, 41.1, 36.2, 36.0, 33.9, 33.8, 32.0, 30.7, 26.2, 25.0, 23.1, 22.9, 19.0, 18.8, -1.8. **IR** (cm^{-1}): 2959, 1652, 1404, 1247, 1152, 849. **HRMS**: calculated for $C_{11}H_{23}ClNO_3Si$ $[M+H^+]^+$: 248.1232. Found $[M+H^+]^+$: 248.1245.



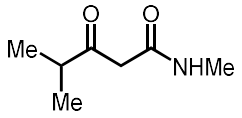
11S

(*E*/*Z*)-2-chloro-N-methyl-N-(6-(trimethylsilyl)hex-4-en-1-yl)acetamide (11S): Prepared according to General Procedure B using **SI-2b** (265 mg, 1.1 mmol) to afford 96 mg (33% yield) of **11S** as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.55 – 5.34 (m, 1H), 5.23 (dt, *J* = 14.8, 7.0 Hz, 1H), 4.11 (d, *J* = 16.8 Hz, 2H), 3.44 – 3.26 (m, 2H), 3.01 (dd, *J* = 45.0, 1.9 Hz, 3H), 2.02 (dt, *J* = 12.5, 5.8 Hz, 2H), 1.63 (dt, *J* = 32.5, 7.7 Hz, 2H), 1.49 – 1.36 (m, 2H), -0.01 (dd, *J* = 5.7, 4.3 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.2, 166.9, 166.8, 128.3, 127.7, 127.5, 127.3, 126.7, 126.7, 126.2, 125.4, 50.1, 48.4, 48.3, 41.5, 40.9, 40.8, 40.8, 35.9 (2), 34.0, 30.1, 29.9, 28.6, 28.5, 27.3, 27.1, 24.3, 24.0, 22.9, 22.8, 18.9, 18.7, -1.8 (2). **IR** (cm^{-1}): 2952, 1652, 1405, 1247, 1152, 841.**HRMS**: calculated for $C_{12}H_{25}ClNOSi$ $[M+H^+]^+$: 262.1388. Found $[M+H^+]^+$: 262.1401.

Synthesis of Trisubstituted Substrates

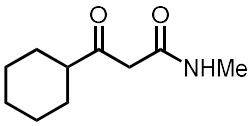


General procedure C: β-keto ester (1 equiv.) was added to a solution of $MeNH_2$ (33% in EtOH, 0.3 M) in a sealed tube and heated to 120 °C for 24 hours. After cooling to room temperature, the reaction mixture was concentrated by rotary evaporator and the crude reaction mixture was redissolved in MeCN/H_2O (9:1, 0.25 M) and AcOH (1% v/v of MeCN/H_2O mix) was added. The reaction mixture was allowed to stir for 24 hours at room temperature before diluting with sodium bicarbonate solution and extracting with three portions of EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated by rotary evaporator and purified by flash column chromatography eluting with hexane/EtOAc to afford the β-keto amides **SI-3**.



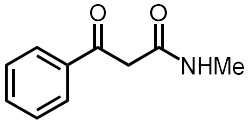
SI-3a

***N*,4-dimethyl-3-oxopentanamide (SI-3a)**: Prepared according to General Procedure C using ethyl 4-methyl-3-oxopentanoate (3.0 g, 19.0 mmol) to afford 1.03g (38% yield) of **SI-3a**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.09 (s, 1H), 3.44 (s, 2H), 2.83 (d, *J* = 4.9 Hz, 3H), 2.68 (p, *J* = 6.9 Hz, 1H), 1.13 (d, *J* = 7.0 Hz, 6H).



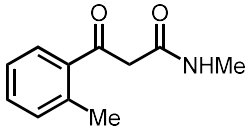
SI-3b

3-cyclohexyl-N-methyl-3-oxopropanamide (SI-3b): Prepared according to General Procedure C using methyl 3-cyclohexyl-3-oxopropanoate[5] (10.0 g, 54.2 mmol) split across two sealed tubes (5 g each) to afford 3.0 g (30% yield) of **SI-3b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45 (s, 2H), 2.85 (d, *J* = 4.9 Hz, 3H), 2.44 (tt, *J* = 11.1, 3.5 Hz, 1H), 1.93 – 1.06 (m, 10H).



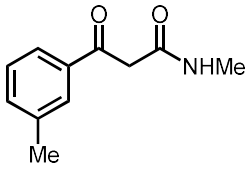
SI-3c

***N*-methyl-3-oxo-3-phenylpropanamide (SI-3c)**: Prepared according to General Procedure C using methyl 3-oxo-3-phenylpropanoate (5.0 g, 26.0 mmol) to afford 2.05 g (44% yield) of **SI-3c**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.05 – 7.90 (m, 2H), 7.66 – 7.55 (m, 1H), 7.49 (dd, *J* = 8.4, 7.1 Hz, 2H), 3.95 (s, 2H), 2.86 (d, *J* = 4.9 Hz, 3H).



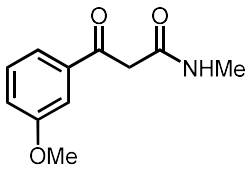
SI-3d

***N*-methyl-3-oxo-3-(*o*-tolyl)propenamide (SI-3d)**: Prepared according to General Procedure C using methyl 3-oxo-3-(*o*-tolyl)propanoate[6] (4.0 g, 20.8 mmol) to afford 1.4 g (35% yield) of **SI-3d**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.7 Hz, 1H), 7.48 – 7.28 (m, 2H), 3.91 (s, 2H), 2.88 (d, *J* = 4.6 Hz, 3H), 2.52 (s, 3H).



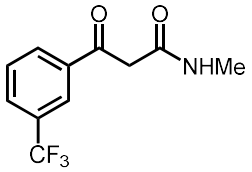
SI-3e

***N*-methyl-3-oxo-3-(*m*-tolyl)propenamide (SI-3e)**: Prepared according to General Procedure C using methyl 3-oxo-3-(*m*-tolyl)propanoate[4] (2.6g, 13.5 mmol) to afford 1.65 g (64% yield) of **SI-3e**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.46 – 7.35 (m, 2H), 7.29 (s, 1H), 3.94 (s, 2H), 2.87 (d, *J* = 4.9 Hz, 3H), 2.42 (s, 3H).



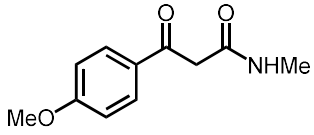
SI-3f

3-(3-methoxyphenyl)-*N*-methyl-3-oxopropanamide (SI-3f): Prepared according to General Procedure C using methyl 3-(3-methoxyphenyl)-3-oxopropanoate[7] (3.4 g, 16.3 mmol) to afford 2.06 g (61% yield) of **SI-3f**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.58 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.19 – 7.13 (m, 1H), 3.94 (s, 2H), 3.86 (s, 3H), 2.87 (d, *J* = 4.8 Hz, 3H).



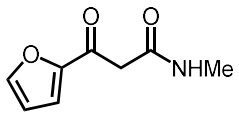
SI-3g

***N*-methyl-3-oxo-3-(3-(trifluoromethyl)phenyl)propenamide (SI-3g)**: Prepared according to General Procedure C using methyl 3-oxo-3-(3-(trifluoromethyl)phenyl)propanoate[8] (5.89 g, 23.9 mmol) to afford 2.6g (44% yield) of **SI-3g**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.29 – 8.16 (m, 2H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 3.99 (s, 2H), 2.88 (d, *J* = 4.6 Hz, 3H).



SI-3h

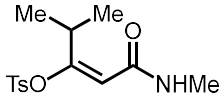
3-(4-methoxyphenyl)-*N*-methyl-3-oxopropanamide (SI-3h): Prepared according to General Procedure C using methyl 3-(4-methoxyphenyl)-3-oxopropanoate[4] (2.5 g, 12 mmol) to afford 2.13 g (86% yield) of **SI-3h**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.03 – 7.91 (m, 2H), 7.18 (s, 1H), 6.99 – 6.88 (m, 2H), 3.89 (s, 2H), 3.88 (s, 3H), 2.85 (d, *J* = 4.8 Hz, 3H).



SI-3i

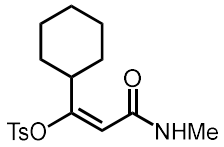
3-(furan-2-yl)-N-methyl-3-oxopropanamide (SI-3i): Prepared according to General Procedure C using methyl 3-(furan-2-yl)-3-oxopropanoate[9] (5.7 g, 34 mmol) to afford 580 mg (10% yield) of **SI-3i**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.30 (s, 1H), 7.11 (s, 1H), 6.54 (s, 1H), 3.77 (d, *J* = 1.8 Hz, 2H), 2.81 (d, *J* = 4.5 Hz, 3H).

General procedure D: Following a modified procedure to that reported by Mazet and coworkers[10] (The *E* enol tosylate stereochemistry was assigned by analogy to the esters prepared therein): β-keto amide **SI-3** (1 equiv.) was dissolved in DCM (0.13 M) and treated with triethylamine (3 equiv.), N-methylimidazole (2 equiv.), and tosyl chloride (2 equiv.) and allowed to stir at room temperature overnight. The mixture was diluted with DCM and washed with 1M HCl. The aqueous layer was extracted with two portions of EtOAc and the combined organic layers were dried over Na_2SO_4, concentrated by rotary evaporator and purified by flash column chromatography eluting with hexanes/EtOAc to afford the tosylates **SI-4**.



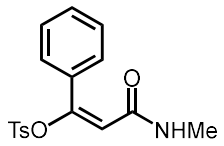
SI-4a

(*E*)-4-methyl-1-(methylamino)-1-oxopent-2-en-3-yl 4-methylbenzenesulfonate (SI-4a): Prepared according to General Procedure D using **SI-3a** (2.0 g, 14.0 mmol) to afford 1.16 g (28% yield) of **SI-4a**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.89 – 7.75 (m, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 6.29 (s, 1H), 5.50 (d, *J* = 1.2 Hz, 1H), 2.80 – 2.71 (m, 1H), 2.68 (d, *J* = 4.9 Hz, 3H), 2.46 (s, 3H), 1.08 (d, *J* = 6.8 Hz, 6H).



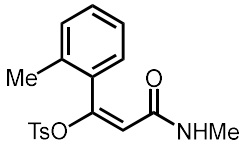
SI-4b

(*E*)-1-cyclohexyl-3-(methylamino)-3-oxoprop-1-en-1-yl 4-methylbenzenesulfonate (SI-4b): Prepared according to General Procedure D using **SI-3b** (3.0 g, 16.4 mmol) to afford 2.3 g (42% yield) of **SI-4b**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.86 – 7.77 (m, 2H), 7.44 – 7.35 (m, 2H), 6.28 (s, 1H), 5.45 (d, *J* = 1.2 Hz, 1H), 2.69 (d, *J* = 4.9 Hz, 3H), 2.47 (s, 3H), 2.42 – 2.34 (m, 1H), 1.93 (d, *J* = 12.3 Hz, 2H), 1.75 (d, *J* = 12.1 Hz, 2H), 1.25 – 1.00 (m, 6H).



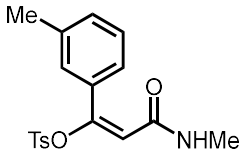
SI-4c

(*E*)-3-(methylamino)-3-oxo-1-phenylprop-1-en-1-yl 4-methylbenzenesulfonate (SI-4c): Prepared according to General Procedure D using **SI-3c** (1.6 g, 9.0 mmol) to afford 1.9 g (64% yield) of **SI-4c**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.0 Hz, 2H), 7.34 (t, *J* = 9.1 Hz, 3H), 7.25 (dd, *J* = 11.6, 7.6 Hz, 4H), 6.61 – 6.48 (m, 1H), 6.01 (s, 1H), 2.80 (d, *J* = 4.6 Hz, 3H), 2.42 (s, 3H).



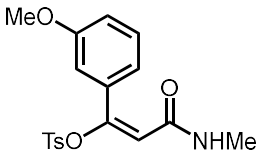
SI-4d

(*E*)-3-(methylamino)-3-oxo-1-(*o*-tolyl)prop-1-en-1-yl 4-methylbenzenesulfonate (SI-4d): Prepared according to General Procedure D using **SI-3d** (1.4 g, 7.28 mmol) to afford 1.16 g (46% yield) of **SI-4d**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.45 – 7.34 (m, 2H), 7.21 – 7.06 (m, 5H), 6.96 (d, *J* = 7.5 Hz, 1H), 6.79 (s, 1H), 5.72 (s, 1H), 2.92 (d, *J* = 4.9 Hz, 3H), 2.38 (s, 3H), 2.16 (s, 3H).



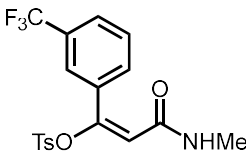
SI-4e

(*E*)-3-(methylamino)-3-oxo-1-(*m*-tolyl)prop-1-en-1-yl 4-methylbenzenesulfonate (SI-4e): Prepared according to General Procedure D using **SI-3e** (1.53 g, 8.0 mmol) to afford 1.75 g (63% yield) of **SI-4e**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.77 – 7.68 (m, 2H), 7.41 – 7.22 (m, 6H), 6.67 (s, 1H), 6.11 (d, *J* = 1.3 Hz, 1H), 2.93 (dd, *J* = 4.9, 1.3 Hz, 3H), 2.53 (s, 3H), 2.34 (s, 3H).



SI-4f

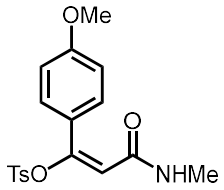
(*E*)-1-(3-methoxyphenyl)-3-(methylamino)-3-oxoprop-1-en-1-yl 4-methylbenzenesulfonate (SI-4f): Prepared according to General Procedure D using **SI-3f** (1.79 g, 8.6 mmol) to afford 3.06 g (98% yield) of **SI-4f**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.73 – 7.66 (m, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 1H), 7.05 – 6.81 (m, 3H), 6.61 (s, 1H), 6.09 (s, 1H), 3.79 (s, 3H), 2.87 (d, *J* = 4.9 Hz, 3H), 2.49 (s, 3H).



SI-4g

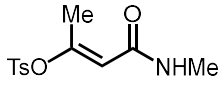
(*E*)-3-(methylamino)-3-oxo-1-(3-(trifluoromethyl)phenyl)prop-1-en-1-yl 4-methylbenzenesulfonate (SI-4g): Prepared according to General Procedure D using **SI-3g** (2.6

g, 10.5 mmol) to afford 2.9 g (70% yield) of **SI-4g**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.63 – 7.54 (m, 4H), 7.46 – 7.40 (m, 2H), 7.24 – 7.19 (m, 2H), 6.58 (s, 1H), 6.09 (s, 1H), 2.86 (d, *J* = 4.9 Hz, 3H), 2.40 (s, 3H).



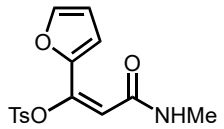
SI-4h

(*E*)-1-(4-methoxyphenyl)-3-(methylamino)-3-oxoprop-1-en-1-yl 4-methylbenzenesulfonate (SI-4h): Prepared according to General Procedure D using **SI-3h** (2.5 g, 12 mmol) to afford 2.2 g (60% yield) of **SI-4h**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.67 – 7.56 (m, 2H), 7.33 – 7.18 (m, 4H), 6.84 – 6.70 (m, 2H), 6.48 (s, 1H), 5.89 (s, 1H), 3.80 (s, 3H), 2.76 (d, *J* = 4.8 Hz, 3H), 2.42 (s, 3H).



SI-4i

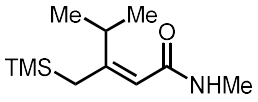
(*E*)-4-(methylamino)-4-oxobut-2-en-2-yl 4-methylbenzenesulfonate (SI-4i): Prepared according to General Procedure D using N-methyl-3-oxobutanamide (3 g, 26.1 mmol, ~70% in water) to afford 700 mg (10% yield) of **SI-4i**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.87 – 7.72 (m, 2H), 7.37 (d, *J* = 8.2 Hz, 2H), 6.28 (s, 1H), 5.43 (d, *J* = 1.3 Hz, 1H), 2.69 (d, *J* = 5.0 Hz, 3H), 2.46 (s, 3H), 2.13 – 2.03 (m, 3H).



SI-4j

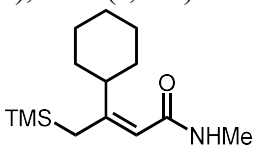
(*E*)-1-(furan-2-yl)-3-(methylamino)-3-oxoprop-1-en-1-yl 4-methylbenzenesulfonate (SI-4j): Prepared according to General Procedure D using **SI-3i** (580 mg, 3.5 mmol) to afford 388 mg (34% yield) of **SI-4j**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.2 Hz, 2H), 7.35 (dd, *J* = 18.6, 10.4 Hz, 3H), 6.48 (d, *J* = 3.4 Hz, 1H), 6.38 (s, 1H), 6.13 (s, 2H), 2.71 (d, *J* = 4.9 Hz, 3H), 2.46 (s, 3H).

General procedure E: $ZnCl_2$ (2 equiv.) was dissolved in diethyl ether (1M) and cooled on an ice bath. $TMSCH_2MgCl$ (1M in diethyl ether, 2.3 equiv.) was added as a slow stream via syringe and the ice bath was removed and stirring was continued at room temperature for one hour. Tosylate **SI-4** (1 equiv.), $PdCl_2(PPh_3)_2$ (2 mol%) and PPh_3 (1 mol%) were combined in a separate vessel and dissolved in THF (0.1 M), then transferred via syringe to the freshly formed organozinc reagent. The reaction mixture was stirred at room temperature until judged complete by TLC (~5-18 hours), at which time it was diluted with EtOAc and poured onto saturated ammonium chloride solution. The layers were separated and the aqueous layer was washed with two additional portions of EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography eluting with hexanes/EtOAc.



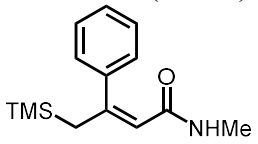
SI-5a

(*E*)-N,4-dimethyl-3-((trimethylsilyl)methyl)pent-2-enamide (SI-5a): Prepared according to General Procedure E using **SI-4a** (565 mg, 1.9 mmol) to afford 272 mg (67% yield) of **SI-5a.** **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.36 (s, 1H), 5.27 (s, 1H), 2.85 – 2.75 (m, 3H), 2.47 (s, 2H), 2.15 (p, *J* = 6.8 Hz, 1H), 1.05 (d, *J* = 6.8 Hz, 6H), 0.04 (s, 9H).



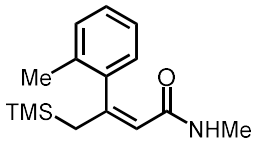
SI-5b

(*E*)-3-cyclohexyl-N-methyl-4-(trimethylsilyl)but-2-enamide (SI-5b): Prepared according to General Procedure E using **SI-4b** (800 mg, 2.4 mmol) to afford 480 mg (80% yield) of **SI-5b**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.33 (s, 1H), 5.23 (s, 1H), 2.81 (d, *J* = 4.1 Hz, 3H), 2.51 – 2.41 (m, 2H), 1.76 (dd, *J* = 24.7, 11.4 Hz, 6H), 1.32 – 1.04 (m, 5H), 0.06 (d, *J* = 3.2 Hz, 9H).



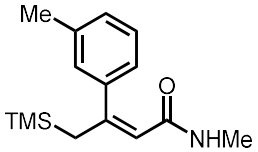
SI-5c

(*E*)-N-methyl-3-phenyl-4-(trimethylsilyl)but-2-enamide (SI-5c): Prepared according to General Procedure E using **SI-4c** (1.9 g, 9.0 mmol) to afford 850 mg (60% yield) of **SI-5c**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.38 – 7.31 (m, 5H), 5.71 (s, 1H), 5.41 (s, 1H), 2.92 – 2.84 (m, 5H), -0.12 (d, *J* = 1.6 Hz, 9H).



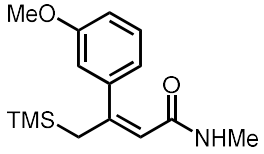
SI-5d

(*E*)-N-methyl-3-(*o*-tolyl)-4-(trimethylsilyl)but-2-enamide (SI-5d): Prepared according to General Procedure E using **SI-4d** (750 mg, 2.2 mmol) to afford 254 mg (45% yield) of **SI-5d**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.21 – 7.10 (m, 3H), 7.05 (d, *J* = 7.3 Hz, 1H), 5.45 (s, 1H), 5.34 (s, 1H), 2.86 (d, *J* = 4.4 Hz, 3H), 2.77 (s, 2H), 2.32 (s, 3H), -0.07 (s, 9H).



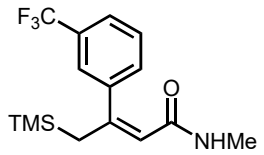
SI-5e

(*E*)-N-methyl-3-(*m*-tolyl)-4-(trimethylsilyl)but-2-enamide (SI-5e): Prepared according to General Procedure E using **SI-4e** (1.0 g, 2.9 mmol) to afford 490 mg (65% yield) of **SI-5e**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.23 – 7.09 (m, 4H), 5.70 (s, 1H), 5.38 (s, 1H), 2.91 – 2.83 (m, 5H), 2.36 (s, 3H), -0.11 (s, 9H).



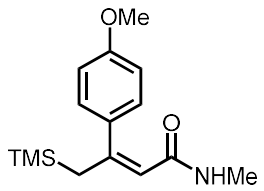
SI-5f

(*E*)-3-(3-methoxyphenyl)-N-methyl-4-(trimethylsilyl)but-2-enamide (SI-5f): Prepared according to General Procedure E using **SI-4f** (1.05 g, 2.9 mmol) to afford 570 mg (71% yield) of **SI-5f**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.23 (d, J = 7.9 Hz, 1H), 6.98 – 6.83 (m, 3H), 5.72 (s, 1H), 5.39 (s, 1H), 3.82 (s, 3H), 2.87 (d, J = 5.1 Hz, 5H), -0.10 (s, 9H).



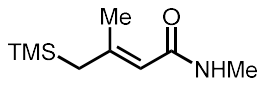
SI-5g

(*E*)-N-methyl-3-(3-(trifluoromethyl)phenyl)-4-(trimethylsilyl)but-2-enamide (SI-5g): Prepared according to General Procedure E using **SI-4g** (1.7 g, 4.3 mmol) to afford 988 mg (72% yield) of **SI-5g**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.68 – 7.36 (m, 4H), 5.74 (s, 1H), 5.44 (s, 1H), 3.01 – 2.80 (m, 5H), -0.11 (d, J = 2.0 Hz, 9H).



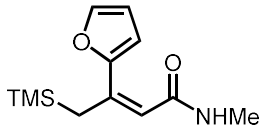
SI-5h

(*E*)-3-(4-methoxyphenyl)-N-methyl-4-(trimethylsilyl)but-2-enamide (SI-5h): Prepared according to General Procedure E using **SI-4h** (2.1 g, 5.8 mmol) to afford 776 mg (48% yield) of **SI-5h**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 5.68 (s, 1H), 5.36 (s, 1H), 3.82 (s, 3H), 2.93 – 2.82 (m, 5H), -0.11 (s, 9H).



SI-5i

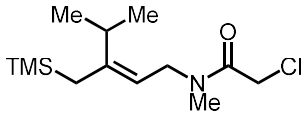
(*E*)-N,3-dimethyl-4-(trimethylsilyl)but-2-enamide (SI-5i): Prepared according to General Procedure E using **SI-4i** (700 mg, 2.6 mmol) to afford 403 mg (85% yield) of **SI-5i. ^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.38 (s, 1H), 5.19 (s, 1H), 2.80 (d, J = 4.1 Hz, 3H), 2.45 (s, 2H), 1.80 (s, 3H), 0.06 (s, 9H).



SI-5j

(*E*)-3-(furan-2-yl)-N-methyl-4-(trimethylsilyl)but-2-enamide (SI-5j): Prepared according to General Procedure E using **SI-4j** (388 mg, 1.2 mmol) to afford 204 mg (70% yield) of **SI-5j.** **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 1.4 Hz, 1H), 6.49 (d, *J* = 3.4 Hz, 1H), 6.42 (dd, *J* = 3.4, 1.8 Hz, 1H), 6.11 (s, 1H), 5.45 (br s, 1H), 2.86 (d, *J* = 4.9 Hz, 3H), 2.75 (s, 2H), 0.06 – -0.01 (m, 9H).

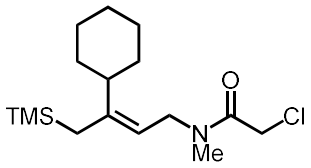
General procedure F: $ZnCl_2$ (5 equiv.) and $LiAlH_4$ (10 equiv.) were dissolved in diethyl ether (0.2 M) and stirred at room temperature for 3 hours, at which point the slurry was filtered through an oven-dried fritted funnel into a dry round-bottom flask. The filter cake was washed with additional diethyl ether (equal volume to the initial portion, such that the final concentration of the substrate is 0.1 M). *Safety note*: the resulting filter cake can readily ignite and should be quenched with caution. The filtrate was cooled to 0 °C and amide **SI-5** (1 equiv.) was added as a solid and the reaction mixture was allowed to warm to room temperature and stirred for 48 hours. The mixture was quenched by the Fieser workup (sequential addition of 1 mL H_2O/g $LiAlH_4$, 1 mL 15% aqueous NaOH/g $LiAlH_4$ and 3 mL H_2O/g $LiAlH_4$). The mixture was stirred for 30 minutes, then dried with excess $MgSO_4$ and stirred another 30-90 minutes. The solids were removed by filtration and the filter cake washed with diethyl ether. The solvent was removed by rotary evaporator. The crude material was taken up in DCM (0.3 M) and 2-chloroacetic acid (1 equiv.) and DMAP (0.1 equiv.) were added and stirred at room temperature until judged complete by TLC analysis (<1 hour). The reaction mixture was diluted with DCM and 1M HCl and the layers were separated. The aqueous layer was extracted with two additional portions of DCM and the combined organic layers were dried over Na_2SO_4, concentrated by rotary evaporator, and purified by flash column chromatography eluting with hexanes/EtOAc. The yields of the products reported here are of the material isolated from column chromatography and contained minor unidentified impurities. The materials were further purified by reverse phase chromatography on a semi-preparative HPLC column and the characterization data is reported based on this further purified material. Note: amide rotamers lead to the doubling of many peaks in the ^{13}C NMR spectra and the full list of peaks is reported. When non-overlapping peaks chemical shift round to the same value, they are reported followed by "(2)".



13S

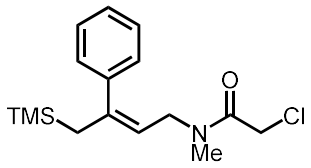
(*E*)-2-chloro-N-methyl-N-(4-methyl-3-((trimethylsilyl)methyl)pent-2-en-1-yl)acetamide (13S): Prepared according to General Procedure F using **SI-5a** (163 mg, 0.76 mmol) to afford 86 mg (40% yield) of **13S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.04 – 4.92 (m, 1H), 4.12 – 4.01 (m, 2H), 3.97 (d, *J* = 6.9 Hz, 1H), 3.87 (d, *J* = 6.3 Hz, 1H), 2.94 (dd, *J* = 19.8, 1.3 Hz, 3H), 2.06 (h, *J* = 6.9 Hz, 1H), 1.62 (d, *J* = 13.2 Hz, 2H), 1.02 (ddd, *J* = 6.9, 3.2, 1.3 Hz, 6H), 0.03 (dd, *J* = 9.9, 1.3 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.5, 166.1, 149.8, 149.7, 112.7, 112.4, 48.7, 45.6,

41.7, 41.3, 35.6 (2), 34.5, 33.8, 22.5, 22.2, 22.1 (2), -0.6 (2). **IR** (cm^{-1}): 2957, 1648, 1461, 1403, 1248, 1155, 1105, 893, 854, 837. **HRMS**: Calculated for $C_{13}H_{27}ClNOSi$ $[M+H^+]^+$: 276.1545. Found $[M+H^+]^+$: 276.1541.



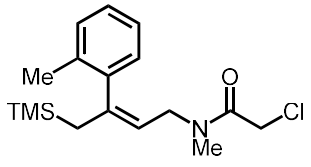
14S

(*E*)-2-chloro-N-(3-cyclohexyl-4-(trimethylsilyl)but-2-en-1-yl)-N-methylacetamide (14S): Prepared according to General Procedure F using **SI-5b** (90 mg, 0.36 mmol) to afford 30 mg (27% yield) of **14S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.89 (dt, *J* = 11.5, 6.7 Hz, 1H), 4.00 (d, *J* = 14.4 Hz, 2H), 3.91 (d, *J* = 6.9 Hz, 1H), 3.82 (d, *J* = 6.3 Hz, 1H), 2.88 (d, *J* = 22.9 Hz, 3H), 1.80 – 1.46 (m, 8H), 1.32 – 0.95 (m, 5H), -0.03 (d, *J* = 10.4 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.3, 165.9, 148.7 (2), 113.3, 112.9, 48.5, 46.4, 46.3, 45.4, 41.6, 41.2, 34.3, 33.5, 32.8, 32.7, 27.0, 26.9, 26.4, 26.3, 22.6, 22.2, -0.8 (2). **IR** (cm^{-1}): 2926, 2852, 1661, 1449, 1328, 854, 839. **HRMS**: Calculated for $C_{16}H_{31}ClNOSi$ $[M+H^+]^+$: 316.1858. Found $[M+H^+]^+$: 316.1837.



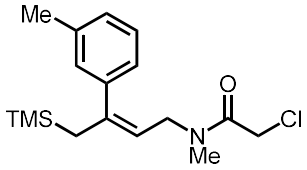
1

(*E*)-2-chloro-N-methyl-N-(3-phenyl-4-(trimethylsilyl)but-2-en-1-yl)acetamide (1): Prepared according to General Procedure F using **SI-5c** (500 mg, 2.0 mmol) to afford 312 mg (50% yield) of **1**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 – 7.15 (m, 5H), 5.43 – 5.33 (m, 1H), 4.09 (d, *J* = 7.0 Hz, 1H), 4.05 – 3.99 (m, 3H), 2.96 (d, *J* = 22.9 Hz, 3H), 2.00 (d, *J* = 21.2 Hz, 2H), -0.19 (d, *J* = 9.4 Hz, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.5, 166.2, 144.0, 143.4, 143.3, 143.2, 128.4, 128.3, 127.7, 127.4, 126.7, 126.6, 119.5, 119.3, 49.3, 46.5, 41.6, 41.3, 35.0, 34.0, 22.0, 21.7, -0.9, -1.0. **IR** (cm^{-1}): 2359, 1659, 1491, 1443, 1249, 909, 856, 841. **HRMS**: Calculated for $C_{16}H_{25}ClNOSi$ $[M+H^+]^+$:310.1388. Found $[M+H^+]^+$:310.1400.



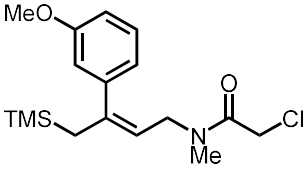
5S

(*E*)-2-chloro-N-methyl-N-(3-(*o*-tolyl)-4-(trimethylsilyl)but-2-en-1-yl)acetamide (5S): Prepared according to General Procedure F using **SI-5d** (700 mg, 2.6 mmol) to afford 47 mg (6% yield) of **5S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.21 – 7.04 (m, 4H), 5.43 (d, *J* = 6.7 Hz, 1H), 4.20 – 4.04 (m, 4H), 3.02 (d, *J* = 27.5 Hz, 3H), 2.35 (d, *J* = 6.7 Hz, 3H), 2.04 (d, *J* = 26.4 Hz, 2H), -0.13 (d, *J* = 12.2 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.6, 166.3, 144.0, 143.4, 143.3, 138.0, 137.8, 128.5, 128.3, 128.2 (2), 127.5, 127.3, 123.8, 123.7, 119.3, 119.1, 49.3, 46.5, 41.6, 41.3, 35.0, 34.0, 22.0, 21.7, 21.6, -0.9 (2). **IR** (cm^{-1}): 2952, 1733, 1651, 1403, 1248, 840. **HRMS**: Calculated for $C_{17}H_{27}ClNOSi$ $[M+H^+]^+$:324.1545. Found $[M+H^+]^+$: 324.1560.



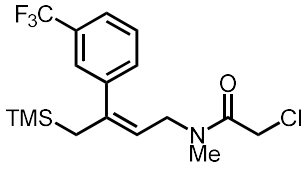
7S

(*E*)-2-chloro-N-methyl-N-(3-(*m*-tolyl)-4-(trimethylsilyl)but-2-en-1-yl)acetamide (7S): Prepared according to General Procedure F using **SI-5e** (200 mg, 0.77 mmol) to afford 60 mg (24% yield) of **7S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.25 – 7.03 (m, 4H), 5.43 (t, *J* = 6.9 Hz, 1H), 4.15 (d, *J* = 7.0 Hz, 1H), 4.12 – 4.04 (m, 3H), 3.02 (d, *J* = 27.4 Hz, 3H), 2.35 (d, *J* = 6.7 Hz, 3H), 2.04 (d, *J* = 26.2 Hz, 2H), -0.12 (d, *J* = 12.0 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.3, 144.0, 143.4, 143.3, 138.0, 137.8, 128.5, 128.3, 128.2, 128.1, 127.5, 127.3, 123.8, 123.7, 119.3, 119.1, 49.3, 46.5, 41.6, 41.3, 35.0, 34.0, 22.0, 21.7, 21.6, -0.9 (2). **IR** (cm^{-1}): 2952, 1733, 1614, 1408, 1247, 839. **HRMS**: Calculated for $C_{17}H_{27}ClNOSi$ $[M+H^+]^+$: 324.1545. Found $[M+H^+]^+$: 324.1560.



6S

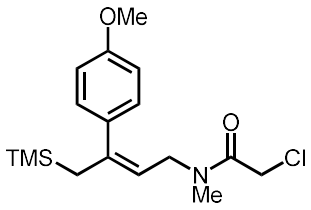
(*E*)-2-chloro-N-(3-(3-methoxyphenyl)-4-(trimethylsilyl)but-2-en-1-yl)-N-methylacetamide (6S): Prepared according to General Procedure F using **SI-5f** (485 mg, 1.8 mmol) to afford 40 mg (7% yield) of **6S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.24 – 7.17 (m, 1H), 6.93 (dt, *J* = 7.8, 1.2 Hz, 1H), 6.86 (dt, *J* = 9.1, 2.2 Hz, 1H), 6.80 (d, *J* = 2.8 Hz, 1H), 5.50 – 5.38 (m, 1H), 4.14 (d, *J* = 6.9 Hz, 1H), 4.08 (t, *J* = 7.7 Hz, 3H), 3.81 (d, *J* = 4.0 Hz, 3H), 3.01 (d, *J* = 29.3 Hz, 3H), 2.03 (d, *J* = 27.4 Hz, 2H), -0.12 (d, *J* = 12.0 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.5, 166.3, 159.7, 159.6, 145.5, 144.9, 143.1, 143.0, 129.4, 129.2, 119.6, 119.3, 119.3, 119.2, 112.8, 112.7, 112.6 (2z), 55.4, 49.3, 46.4, 41.5, 41.3, 35.0, 34.0, 22.1, 21.8, -0.9, -1.0. **IR** (cm^{-1}): 2952, 1750, 1652, 1597, 1464, 1285, 1247, 1043, 850. **HRMS**: Calculated for $C_{17}H_{27}ClNO_2Si$ $[M+H^+]^+$: 340.1494. Found $[M+H^+]^+$: 340.1509.



15

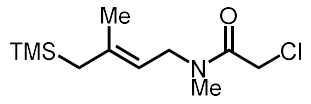
(*E*)-2-chloro-N-methyl-N-(3-(3-(trifluoromethyl)phenyl)-4-(trimethylsilyl)but-2-en-1-yl)acetamide (15): Prepared according to General Procedure F using **SI-5g** (300 mg, 0.95 mmol) to afford 130 mg (36% yield) of **15S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.2 Hz, 1H), 7.52 (q, *J* = 7.9 Hz, 2H), 7.43 (dt, *J* = 15.2, 7.7 Hz, 1H), 5.49 (q, *J* = 6.8 Hz, 1H), 4.16 (d, *J* = 6.9 Hz, 1H), 4.09 (d, *J* = 3.8 Hz, 3H), 3.03 (d, *J* = 38.2 Hz, 3H), 2.07 (d, *J* = 26.2 Hz, 2H), -0.12 (d, *J* = 12.1 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.4, 166.3, 144.6, 144.0, 141.9 (2), 130.9,

130.7 (2), 130.5, 129.9, 129.8, 128.9, 128.7, 125.2, 124.3 (2), 124.0 (2), 123.9, 123.3 (3), 123.2 (3), 123.1, 121.0, 120.8, 49.1, 46.5, 41.4, 41.1, 35.1, 33.9, 22.0, 21.6, -1.0, -1.1. **^{19}F NMR** (470 MHz, $CDCl_3$) δ -62.6 (2; rotamers). **IR** (cm^{-1}): 2954, 1654, 1488, 1434, 1334, 1249, 1165, 1123, 1073, 841. **HRMS**: Calculated for $C_{17}H_{24}ClF_3NO_2Si$ $[M+H^+]^+$: 378.1262 Found $[M+H^+]^+$: 378.1252.



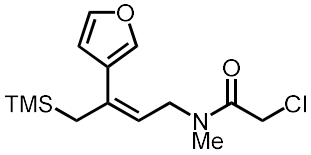
4S

(*E*)-2-chloro-N-(3-(4-methoxyphenyl)-4-(trimethylsilyl)but-2-en-1-yl)-N-methylacetamide (4S): Prepared according to General Procedure F using **SI-5h** (700 mg, 2.5 mmol) to afford 315 mg (37% yield) of **4S**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 – 7.24 (m, 2H), 6.89 – 6.79 (m, 2H), 5.38 (t, *J* = 6.7 Hz, 1H), 4.18 – 4.05 (m, 4H), 3.81 (d, *J* = 2.9 Hz, 3H), 3.02 (d, *J* = 23.0 Hz, 3H), 2.07 – 1.95 (m, 2H), -0.12 (d, *J* = 10.0 Hz, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.5, 166.2, 159.3, 159.1, 142.6, 142.5, 136.4, 135.7, 127.7, 127.6, 118.2, 117.9, 113.8, 113.6, 55.4 (2), 49.4, 46.5, 41.6, 41.3, 34.9, 34.0, 21.9, 21.6, -0.9 (2). **IR** (cm^{-1}): 2951, 1652, 1606, 1509, 1243, 1178, 1033, 830, 802. **HRMS**: Calculated for $C_{17}H_{27}ClNO_2Si$ $[M+H^+]^+$: 340.1494. Found $[M+H^+]^+$: 340.1485.



12S

(*E*)-2-chloro-N-methyl-N-(3-methyl-4-(trimethylsilyl)but-2-en-1-yl)acetamide (12S): Prepared according to General Procedure F using **SI-5i** (400 mg, 2.2 mmol) to afford 144 mg (27% yield) of **12S**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.05 – 4.96 (m, 1H), 4.06 (d, *J* = 5.6 Hz, 2H), 3.93 (d, *J* = 7.0 Hz, 1H), 3.86 (d, *J* = 6.4 Hz, 1H), 2.96 (d, *J* = 28.0 Hz, 3H), 1.79 – 1.70 (m, 3H), 1.59 (d, *J* = 18.7 Hz, 2H), 0.05 (d, *J* = 8.9 Hz, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.5, 166.1, 139.3, 139.2, 116.5, 116.1, 48.6, 45.8, 41.6, 41.3, 34.8, 33.8, 26.4 (2), 24.0, 23.8, -0.7 (2). **IR** (cm^{-1}): 2953, 1647, 1403, 1247, 836. **HRMS**: Calculated for $C_{11}H_{23}ClNOSi$ $[M+H^+]^+$: 248.1232. Found $[M+H^+]^+$: 248.1222.

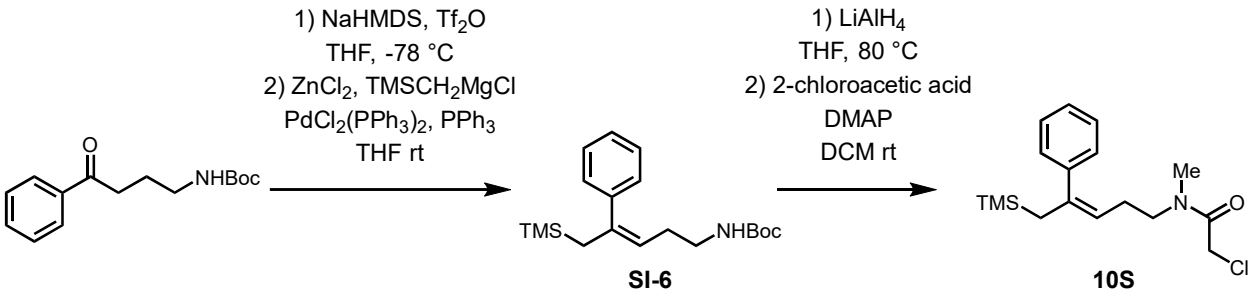


15S

(*E*)-2-chloro-N-(3-(furan-3-yl)-4-(trimethylsilyl)but-2-en-1-yl)-N-methylacetamide (15S): Prepared according to General Procedure F using **SI-5j** (204 mg, 0.84 mmol) to afford 40 mg (16% yield) of **15S**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 (d, *J* = 10.8 Hz, 1H), 6.36 (ddd, *J* = 9.9, 3.1, 1.8 Hz, 1H), 6.25 (dd, *J* = 10.5, 3.3 Hz, 1H), 5.83 (t, *J* = 6.9 Hz, 1H), 4.14 (d, *J* = 7.2 Hz,

1H), 4.10 – 4.03 (m, 3H), 3.02 (d, J = 36.1 Hz, 3H), 1.92 (d, J = 30.9 Hz, 2H), -0.00 (d, J = 12.7 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.5, 166.3, 155.4, 154.8, 142.0, 141.6, 131.7, 131.4, 116.0, 115.8, 111.3, 111.2, 106.7, 106.3, 48.5, 45.8, 41.6, 41.3, 35.1, 34.0, 19.0, 18.8, -0.8, -0.9. **IR** (cm^{-1}):2953, 1654, 1488, 1404, 1165, 1111, 855, 841. **HRMS**: Calculated for $C_{14}H_{23}ClNO_2Si$ $[M+H^+]^+$: 300.1187 Found $[M+H^+]^+$: 300.1183.

Synthesis of Aryl-Substituted 6-memebered substrate

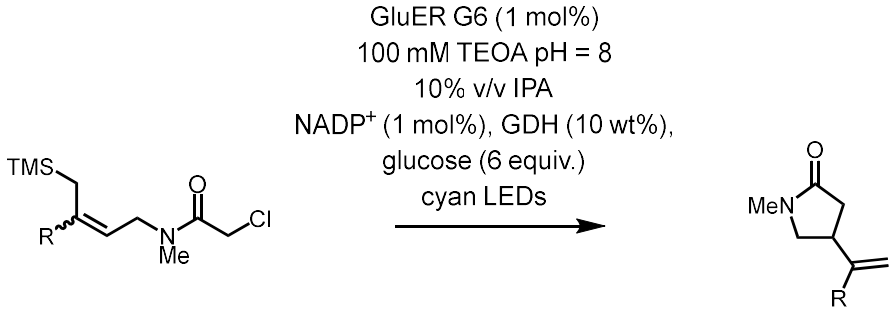


***tert*-butyl (*E*)-(4-phenyl-5-(trimethylsilyl)pent-3-en-1-yl)carbamate (SI-6):** *tert*-butyl (4-oxo-4-phenylbutyl)carbamate[11] (500 mg, 1.9 mmol) and $PhNTf_2$ were dissolved in THF (10 mL) and cooled to -78 °C and NaHMDS (2M in THF, 2 mL, 4.0 mmol) was added slowly dropwise. The resulting mixture was stirred for 30 minutes and quenched with saturated aqueous ammonium chloride and extracted with two portions of EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated. The triflate was crudely purified by rapid flash column chromatography eluting with hexanes/EtOAc to afford 250 mg (33% yield) of material that was not analyzed further. Anydrous ZnCl2 (172 mg, 1.3 mmol) was dissolved in THF (1.3 mL) and cooled to 0 °C on an ice bath before addition of trimethylsilylmethylmagnesium chloride (1 M, 1.5 mL, 1.5 mmol). After stirring for 30 minutes, $PdCl_2(PPh_3)_2$ and (22 mg, 5 mol%), PPh_3 (4 mg, 2.5 mol%) were added, followed by a solution of the triflate in THF (6 mL). The reaction mixture was stirred overnight with warming to room temperature and quenched with saturated ammonium chloride solution. The aqueous layer was extracted with 3 portions of EtOAc, dried over Na_2SO_4 and concentrated by rotary evaporator. Flash column chromatography eluting with hexanes/EtOAc afforded 79 mg (37% yield from the triflate) of **SI-6**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 – 7.27 (m, 4H), 7.25 – 7.19 (m, 1H), 5.43 (t, J = 7.1 Hz, 1H), 3.28 – 3.17 (m, 2H), 2.32 (q, J = 6.9 Hz, 2H), 2.00 (s, 2H), 1.45 (s, 9H), -0.15 (s, 10H).

(*E*)-2-chloro-N-methyl-N-(4-phenyl-5-(trimethylsilyl)pent-3-en-1-yl)acetamide (10S): **SI-6** (79 mg, 0.24 mmol) was dissolved in THF (10 mL) and $LiAlH_4$ (36 mg, 0.95 mmol) were added at 0 °C. The reaction mixture was heated to reflux overnight. After cooling on an ice bath, the reaction was diluted with diethyl ether and quenched with sodium sulfate decahydrate and filtered, washing the filter cake with ether. The solvent was removed by rotary evaporator and the crude material was redissolved in DCM (10 mL). 2-chloroacetic anhydride (49 mg, 0.28 mmol) and DMAP (6 mg, 0.47 mmol) were added sequentially and the reaction mixture was stirred until judged complete by TLC (approximately 30 minutes). The mixture was poured onto 1 M HCl solution and diluted with additional DCM. The aqueous layer was washed with 2 additional portions of DCM and the combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporator. Flash column chromatography afforded 23 mg (29% yield) of **10S**. Further purification by preparative HPLC afforded an analytically pure sample and material that was used for the biocatalytic cyclization. Note: various impurities are present and unable to be separated by flash chromatography. One decomposition pathway was identified: a Peterson-

type elimination to form a diene[9]. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 4H), 7.25 – 7.20 (m, 1H), 5.40 (dt, *J* = 21.6, 7.2 Hz, 1H), 4.09 (d, *J* = 3.9 Hz, 2H), 3.53 – 3.38 (m, 2H), 3.06 (d, *J* = 37.0 Hz, 3H), 2.50 – 2.34 (m, 2H), 2.00 (d, *J* = 3.1 Hz, 2H), -0.15 (d, *J* = 2.8 Hz, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.8, 166.6, 144.6, 144.2, 142.0, 140.8, 128.5, 128.3, 128.2, 127.7, 127.2, 126.9, 126.7, 126.6, 121.2, 119.8, 50.3, 48.7, 41.5, 40.9, 36.3, 34.1, 28.3, 27.1, 21.7, 21.4, -0.9, -1.0. **IR** (cm^{-1}): 2951, 1650, 1492, 1405, 1247, 1154, 1028, 978. **HRMS**: Calculated for $C_{17}H_{27}ClNOSi$ $[M+H^+]^+$: 324.1545. Found $[M+H^+]^+$: 325.1537.

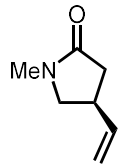
Biocatalytic Reaction



General procedure for biocatalytic cyclization: Allylsilane substrate (20 μmol, 1 equiv.) was weighed into a shell vial and imported into a Coy anaerobic chamber. Inside the anaerobic chamber, a 10x stock solution of "turnover mix" was prepared by dissolving GDH-105 (10 wt%/rxn), $NADP^+$ (2 mol%/rxn) and glucose (2 equiv./reaction) in 1 mL of 100 mM buffer. 100 μL of this solution was transferred to a shell vial equipped with a cross stir bar and diluted with 100 mM buffer (836 μL). Two 100 nmol aliquots of GluER enzyme (33 μL each) was added and the mixture was gently vortexed until the yellow color faded and the mixture became clear. Substrate was dissolved in isopropanol (55 μL) and transferred via pipette to the reaction mixture. The substrate vial was rinsed with an additional 55 μL of isopropanol that was then transferred to the reaction mixture. The vial was capped with a septum, removed from the anaerobic chamber, and irradiated with cyan LEDs at room temperature (with fan cooling to offset the heat from the LEDs) for 48 hours. At this time, the reaction mixture was removed from the light source and 100 μL of a 10 mg/mL 1,3,5-trimethoxybenzene (TMB) stock solution (total 1 mg TMB/reaction) was added. The reaction mixture was diluted with approximately 1 mL of EtOAc and stirred 30-60 minutes at room temperature. The reaction mixture was centrifuged (Genevac) to facilitate separation of the layers. The organic layer was removed and the aqueous layer was extracted with five additional portions of EtOAc, centrifuging as needed if an emulsion was formed. The combined organic layers were concentrated by rotary evaporator or Genevac and yields were calculated based on the ratio of product to TMB. Each reaction was performed in duplicate and the reported yield is the average of the two replicates. The crude reaction products were analyzed by chiral HPLC to determine e.r. The absolute configuration (*S*) was assigned by analogy to the reductive cyclization product previously studied in our laboratories using this enzyme[1,2].

Note: 33 μL aliquots 100 nmol aliquots were commonly used in this study but not exclusively. For different concentrations, an aliquot equivalent to 100 nmol protein was used and the volume of buffer (836 μL in the above procedure) was increased or decreased accordingly.

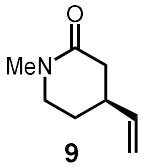
For unsubstituted and alkyl-substituted products (**8-14**), multiple biocatalytic reactions were performed in tandem and combined for HPLC purification to isolate an analytically pure sample. The characterization data from these samples is reported here. For aryl-substituted products (**2, 4-7**), characterization data and HPLC conditions are reported under the racemic synthesis section below ("Synthesis of Aryl-Substituted Product Standards").



8

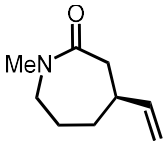
1-methyl-4-vinylpyrrolidin-2-one (8): 56% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization with 4.6 mg of **8S**). 70:30 e.r. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.82 (ddd, *J* = 17.4, 10.2, 7.7 Hz, 1H), 5.17 – 5.03 (m, 2H), 3.50 (dd, *J* = 9.6, 8.2 Hz, 1H), 3.16 (dd, *J* = 9.7, 6.9 Hz, 1H), 3.01 (q, *J* = 8.0 Hz, 1H), 2.84 (s, 3H), 2.56 (dd, *J* = 16.8, 8.9 Hz, 1H), 2.26 (dd, *J* = 16.8, 8.1 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.1, 138.9, 115.8, 54.8, 37.2, 36.0, 29.7. **IR** (cm^{-1}): 2954, 2922, 2852, 1733, 1683, 1463, 1264, 915, 812. **HRMS**: Calculated for $C_7H_{12}NO$ $[M+H^+]^+$: 126.0913. Found $[M+H^+]^+$: 126.0913. **HPLC**: AS-H, 20% isopropanol in hexanes, 45 minute method.

When using allylsulfone starting material **8S-b** (6.0 mg) as a substrate in the general procedure for biocatalytic cyclization, 25% yield is observed (68:32 e.r.).



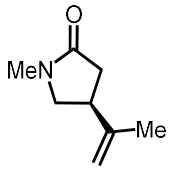
9

1-methyl-4-vinylpiperidin-2-one (9): 75% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 5 mg of **9S**). 57:43 e.r. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.85 – 5.71 (m, 1H), 5.11 – 4.99 (m, 2H), 3.36 – 3.26 (m, 2H), 2.94 (s, 3H), 2.52 (dtd, *J* = 13.8, 4.5, 2.1 Hz, 2H), 2.20 (dd, *J* = 18.5, 11.5 Hz, 1H), 1.95 (dtt, *J* = 13.6, 4.6, 1.8 Hz, 1H), 1.70 – 1.58 (m, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.3, 140.4, 114.4, 48.8, 37.5, 36.7, 34.6, 28.8. **IR** (cm^{-1}): 3079, 2933, 1635, 1504, 1447, 1700, 1337, 1252, 913. **HRMS**: Calculated for $C_8H_{14}NO$ $[M+H^+]^+$: 140.1070. Found $[M+H^+]^+$: 140.1070. **HPLC**: AS-H, 20% isopropanol in hexanes, 70 minute method.



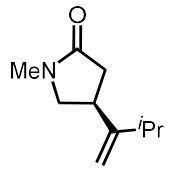
11

1-methyl-4-vinylazepan-2-one (11): 49% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization with 5.2 mg **11S**). 61:39 e.r. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.79 (ddd, *J* = 17.2, 10.4, 6.8 Hz, 1H), 5.09 – 4.92 (m, 2H), 3.48 (ddd, *J* = 15.1, 10.3, 1.2 Hz, 1H), 3.28 – 3.18 (m, 1H), 2.99 (s, 3H), 2.58 – 2.55 (m, 2H), 2.40 – 2.26 (m, 1H), 2.01 – 1.91 (m, 1H), 1.90 – 1.79 (m, 1H), 1.62 – 1.55 (m, 1H), 1.52 – 1.41 (m, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.3, 142.5, 113.4, 51.3, 42.4, 38.0, 36.0 (2), 26.9. **IR** (cm^{-1}): 2926, 1642, 1491, 1396, 1220, 1061, 912. **HRMS**: Calculated for $C_9H_{16}NO$ $[M+H^+]^+$: 154.1226. Found $[M+H^+]^+$: 154.1227. **HPLC**: AS-H, 20% isopropanol in hexanes, 45 minute method.



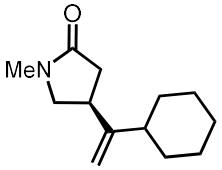
12

1-methyl-4-(prop-1-en-2-yl)pyrrolidin-2-one (12): 50% yield (Average of two reactions performed with 4.6 mg **12S** according to general procedure for biocatalytic cyclization with 5.0 mg of **12S**). 76:24 e.r. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.86 – 4.74 (m, 2H), 3.49 (dd, *J* = 9.7, 8.3 Hz, 1H), 3.23 (dd, *J* = 9.7, 7.2 Hz, 1H), 3.06 – 2.96 (m, 1H), 2.86 (s, 3H), 2.53 (dd, *J* = 16.8, 9.0 Hz, 1H), 2.34 (dd, *J* = 16.8, 8.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.3, 144.7, 111.2, 53.8, 38.7, 35.9, 29.7, 20.2. **IR** (cm^{-1}): 3080, 2925, 2857, 1694, 1500, 1436, 1401, 1235, 1131, 995. **HRMS**: Calculated for $C_8H_{14}NO$ $[M+H^+]^+$: 140.1070. Found $[M+H^+]^+$: 140.1061. **HPLC**: AS-H, 20% isopropanol in hexanes, 45 minute method.



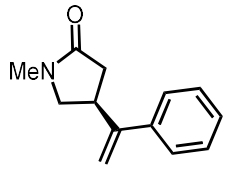
13

1-methyl-4-(3-methylbut-1-en-2-yl)pyrrolidin-2-one (13): 69% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 5.5 mg of **13S**). 91:9 e.r. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.89 (d, *J* = 1.0 Hz, 1H), 4.81 (d, *J* = 1.2 Hz, 1H), 3.51 (dd, *J* = 9.5, 8.2 Hz, 1H), 3.23 (dd, *J* = 9.5, 7.4 Hz, 1H), 3.07 – 2.94 (m, 1H), 2.85 (s, 3H), 2.55 (dd, *J* = 16.6, 8.8 Hz, 1H), 2.34 (dd, *J* = 16.7, 8.8 Hz, 1H), 2.28 – 2.14 (m, 1H), 1.06 (d, *J* = 6.8 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.2, 156.4, 107.1, 55.3, 37.6, 36.2, 33.5, 29.7, 22.4, 22.3. **IR** (cm^{-1}): 2961, 2873, 1693, 1501, 1434, 1401, 1381,1266, 1130, 1101, 992, 893. **HRMS**: Calculated for $C_{10}H_{18}NO$ $[M+H^+]^+$: 168.1383. Found $[M+H^+]^+$: 168.1373. **HPLC**: AS-H, 20% isopropanol in hexanes, 60 minute method.



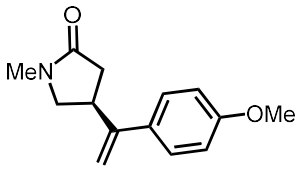
14

4-(1-cyclohexylvinyl)-1-methylpyrrolidin-2-one (14): 41% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization with 6.3 mg **14S**). 97:3 e.r. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.86 (s, 1H), 4.81 (d, *J* = 1.0 Hz, 1H), 3.49 (dd, *J* = 9.5, 8.2 Hz, 1H), 3.21 (dd, *J* = 9.5, 7.5 Hz, 1H), 3.05 – 2.95 (m, 1H), 2.85 (s, 3H), 2.53 (dd, *J* = 16.6, 8.8 Hz, 1H), 2.33 (dd, *J* = 16.6, 8.8 Hz, 1H), 1.85 – 1.67 (m, 6H), 1.21 (ttd, *J* = 25.9, 12.7, 3.8 Hz, 5H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.3, 155.6, 107.7, 55.2, 43.8, 37.4, 36.6, 33.2, 33.1, 29.7, 26.9, 26.9, 26.4. **IR** (cm^{-1}): 2925, 2852, 1697, 1499, 1448, 1400, 1268. **HRMS**: Calculated for $C_{13}H_{22}NO$ $[M+H^+]^+$: 208.1696. Found $[M+H^+]^+$: 208.1683. **HPLC**: IB, 1% isopropanol in hexanes, 30 minute method.



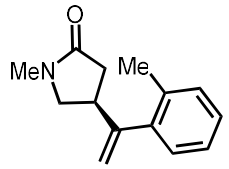
2

1-methyl-4-(1-phenylvinyl)pyrrolidin-2-one (2): 92% yield (3.1 mg, 10 μmol scale; yield measured by HPLC analysis vs. TMB). >99:1 e.r.
This reaction was also performed on isolation scale with 75mg (0.24 mmol) of starting material **1** according to the following procedure, adapted from previous work in our group[2]: Inside of an oxygen-free glove box and using deoxygenated buffer and solvents, a stock solution containing the following solids was prepared in 2 mL 100 mM TEOA buffer (pH = 8): 12.4 mg of KRED, 6 mg $NADP^+$ and 144 mg of glucose. 1.75 mL of this turnover system stock solution was then added to a 50 mL round-bottomed flask equipped with a large cross stir bar (3 mol% NADP+, 14.5 wt% KRED, 2.9 eq glucose). Next, eighteen 100 nanomole aliquots of GluER-G6 (0.75 mol %) were added and the mixture was diluted to a total volume of 5.5 mL with 100 mM TEOA buffer (pH = 8). 75 mg (0.24 micromole) of **1** was dissolved in a total of 200 microliters of isopropanol in a 1 mL vessel, and added to the reaction mixture. The flask was sealed with a septum, parafilm and electrical tape and then removed from the glove box to a 3 °C cold room and stirred on an aluminum-foil wrapped stir plate under cyan light irradiation (2 lamps) with fan cooling. After 96 hours, the reaction mixture was removed from irradiation and quenched with 30 mL EtOAc and solid NaCl (until supersaturation). 5 mL of 15 wt % KOH was added and the mixture was stirred for 30 minutes, at which time the mixture was transferred into four 20 mL scintillation vials and extracted 6 times with EtOAc. Emulsions were resolved by centrifugation. The organic phases were combined and the combined aqueous phases were further extracted with three portions of DCM, which was added to the EtOAc extract and dried over Na2SO4, concentrated and purified by flash column chromatography (biotage, 25 g cartridge) eluting with hexanes/EtOAc to afford 22 mg (46% yield) of **2**.



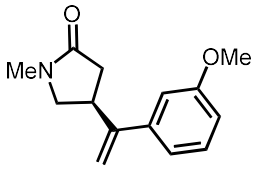
4

4-(1-(4-methoxyphenyl)vinyl)-1-methylpyrrolidin-2-one (4): 82% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 6.8 mg of **4S**). >99:1 e.r.



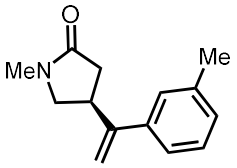
5

1-methyl-4-(1-(o-tolyl)vinyl)pyrrolidin-2-one (5): 87% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 6.5 mg of **5S**). 97:3 e.r.



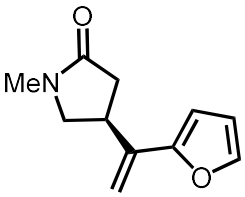
6

4-(1-(3-methoxyphenyl)vinyl)-1-methylpyrrolidin-2-one (6): 63% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 6.8 mg of **6S**). >99:1 e.r.



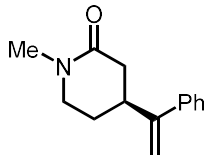
7

1-methyl-4-(1-(m-tolyl)vinyl)pyrrolidin-2-one (7): 51% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 6.5 mg of **S7**). 99:1 e.r.



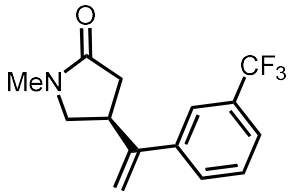
15

4-(1-(furan-2-yl)vinyl)-1-methylpyrrolidin-2-one (15): 38% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 6.5 mg of **S15**). 99:1 e.r.



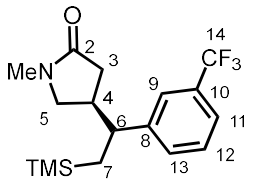
10

(*S*)-1-methyl-4-(1-phenylvinyl)piperidin-2-one (10): 53% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 6.5 mg of **10S**). 65:35 e.r. An authentic sample was isolated by silica gel column chromatography. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 (d, *J* = 6.7 Hz, 5H), 5.27 (s, 1H), 5.04 (s, 1H), 3.34 – 3.26 (m, 2H), 3.13 – 2.99 (m, 1H), 2.95 (s, 3H), 2.64 (dd, *J* = 17.5, 4.8 Hz, 1H), 2.32 (dd, *J* = 17.3, 10.2 Hz, 1H), 2.03 (dd, *J* = 11.7, 6.8 Hz, 1H), 1.69 (dt, *J* = 13.6, 4.6 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.5, 150.9, 141.3, 128.6 (2), 127.8, 126.7 (2), 112.2, 48.9, 37.7, 37.1, 34.5, 28.1. **IR** (cm^{-1}): 2925, 2855, 1643, 1504, 1338, 1251, 902. **HRMS**: Calculated for $C_{14}H_{18}NO$ $[M+H^+]^+$:216.1383. Found $[M+H^+]^+$: 216.1020. **HPLC**: AS-H, 20% isopropanol in hexanes, 60 minute method.



17

1-methyl-4-(1-(3-(trifluoromethyl)phenyl)vinyl)pyrrolidin-2-one (15): 3% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 7.5 mg of **15S**. e.r not determined.



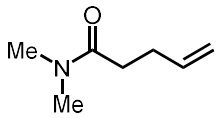
18

1-methyl-4-(1-(3-(trifluoromethyl)phenyl)-2-(trimethylsilyl)ethyl)pyrrolidin-2-one (18): 53% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization using 7.5 mg of **15**). e.r.not determined. An analytical sample was obtained by pr**^{1}H NMR** (599 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.40 (m, 1H), 7.39 (s, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 3.56 (dd, *J* = 9.7, 8.2 Hz, 1H), 3.17 (dd, *J* = 9.5, 7.9 Hz, 1H), 2.82 (s, 3H), 2.68 – 2.60 (m, 1H), 2.56 (app. p, *J* = 8.4 Hz, 1H), 2.03 (dd, *J* = 17.0, 9.0 Hz, 1H), 1.95 (dd, *J* = 17.2, 9.0 Hz, 1H), 1.00 – 0.93 (m, 2H), -0.26 (s, 9H). ^{13}C NMR signals were not detectable on the small sample isolated and most of the peaks could be extracted from NOESY and HMBC analysis. See "1H and 13C NMR Spectra of Substrates and Products" for additional details.

Atom #	δ ^{13}C (ppm)[a]	δ ^{1}H (ppm)	Multiplicity	NOESY	HMBC
2	173.93				

3	36.17	1.95 2.03	dd, J = 17.2, 9.0 Hz dd, J = 17.0, 9.0 Hz	 H-4	
4	40.81	2.55	app. p, J = 8.4 Hz	H-3'', H-5''	
5	54.09	3.17 3.56	dd, J = 9.5, 7.9 Hz dd, J = 9.7, 8.2 Hz	H-5'', H-7, N-Me H-4, H-5', H-7, N-Me	
6	46.62	2.60-2.68	m	H-7, H-13, TMS	
7	21.67	0.93-1.00	m	H-5',H-5'', H-6, TMS	
8	n/d[b]				
9	124.34	7.39	s		
10	n/d[b]				
11	123.70	7.49	d, J = 8.0 Hz		
12	129.16	7.43	t, J = 8.0 Hz[c]		
13	131.22	7.33	d, J = 8.0 Hz	H-6	
TMS	-1.23	-0.26	s	H-6, H-7	C-7, TMS
N-Me	29.53	2.82	S	H-5', H-5''	C-2, C-5

[a] ^{13}C chemical shifts were determined from 2D HSQC and HMBC experiments
[b] Not detected
[c] Multiplicity determined from HSQC crosspeak



23

N,N-dimethylpent-4-enamide (23): 61% yield (10 µmol scale; yield measured by HPLC analysis vs. TMB). When using allyl phenyl sulfone instead of trimethylallylsulfone: 74% yield. Spectral data matches the literature[12]. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.97 – 5.77 (m, 1H), 5.13 – 4.90 (m, 2H), 3.00 (s, 3H), 2.94 (s, 3H), 2.39 (d, J = 3.0 Hz, 4H).

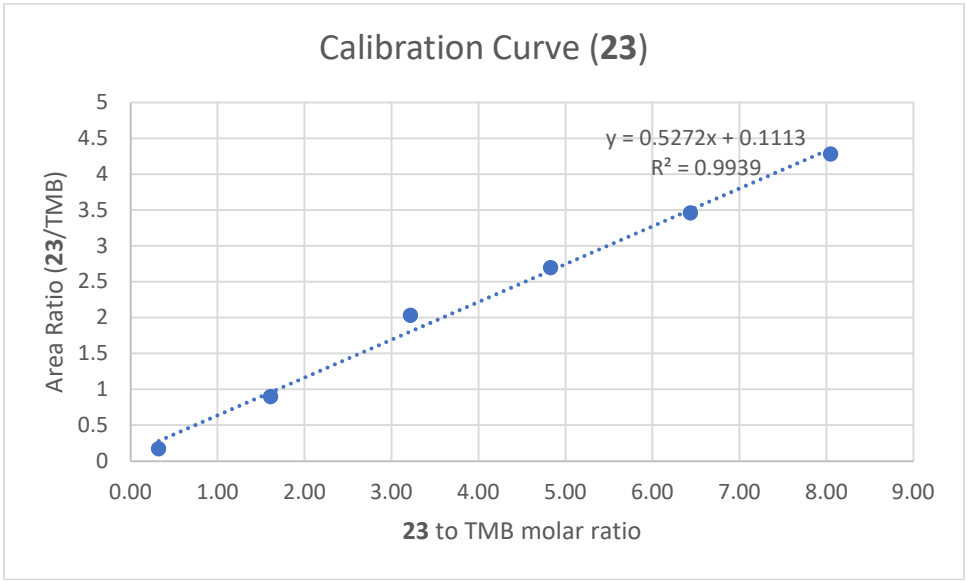
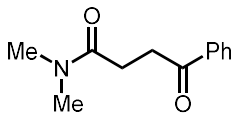


Figure SI-10: Calibration curve for product **23**.



24

N,N-dimethyl-4-oxo-4-phenylbutanamide (24): 99% yield (Average of two reactions performed according to general procedure for biocatalytic cyclization 10 mmol, yield measured by HPLC vs. TMB). Spectral data matches the literature[13].

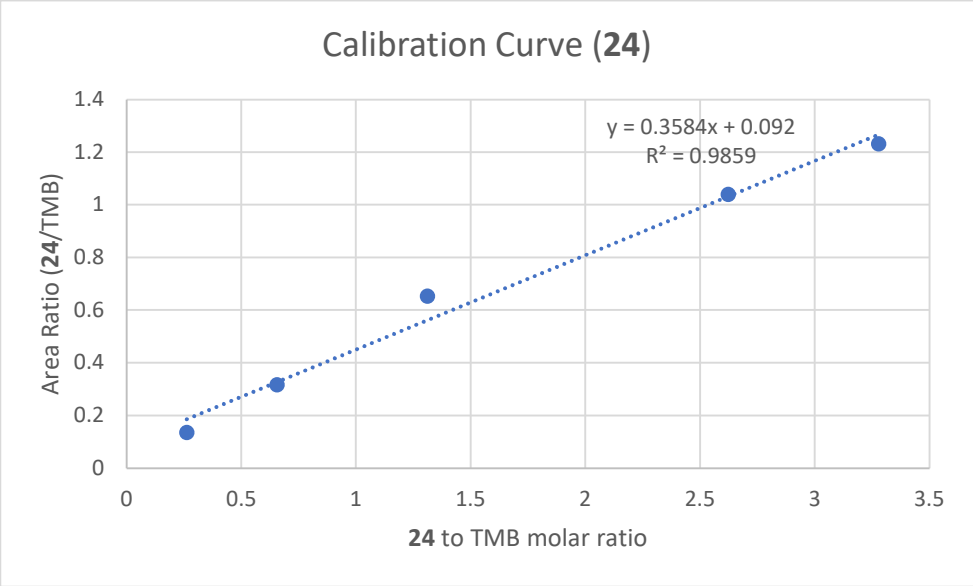
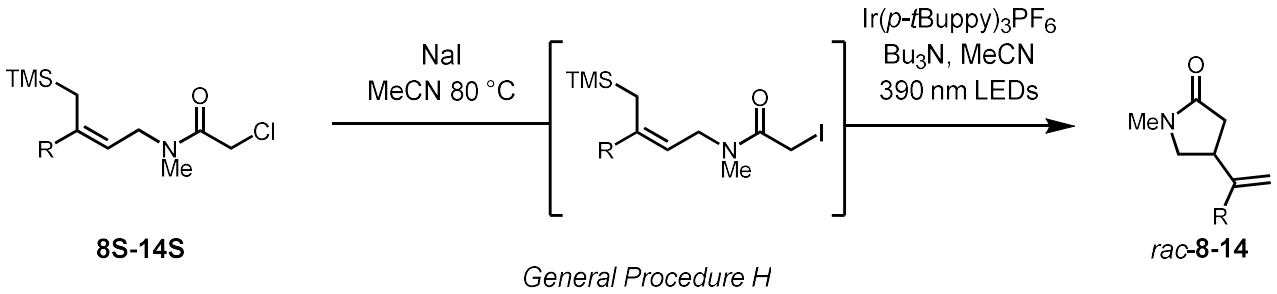


Figure SI-11: Calibration curve for product **24**.

Preparation of Racemic Product Standards

Synthesis of Unsubstituted and Alkyl-Substituted Racemic Standards



General Procedure H: α-chloroamide substrates (1 equiv.) was dissolved in MeCN (0.1 M) and sodium iodide (3 equiv.) was added. The reaction mixture was heated at 80 °C overnight. After cooling to room temperature the mixture was filtered though a pad of Celite and concentrated. The crude material was taken up in DCM and additional insoluble salts were removed by again filtering through Celite. The mixture was concentrated and the resulting yellow/brown oil in a 20 mL scintillation vial was imported into a Coy anaerobic chamber along with Ir(*p*-*t*Bu-ppy)$_3$ (1 mol%) and both reagents were dissolved in MeCN (0.04 M) and tributylamine (2 equiv.) was added. The reaction mixture was vortexed until homogeneous and portioned into 4 mL shell vials (max 3.5 mL per vial) and capped with septa. The solutions were removed from the anaerobic chamber and irradiated with 395 nm violet light on a 96-well lumidox II LED array mounted on a tumble stirrer and cooled by a stream of air. Yields are less than 20% and not reported. The desired product is a minor component of the reaction mixture and an analytical sample of sufficient purity to analyze by chiral HPLC as a racemic standard. Figure SI-12 shows the compounds prepared according to this procedure. The optimal chiral HPLC separation conditions are reported with the biocatalytic cyclization product data under "Biocatalytic Reaction". The HPLC traces are reported in the section "Chiral HPLC Traces of Products".

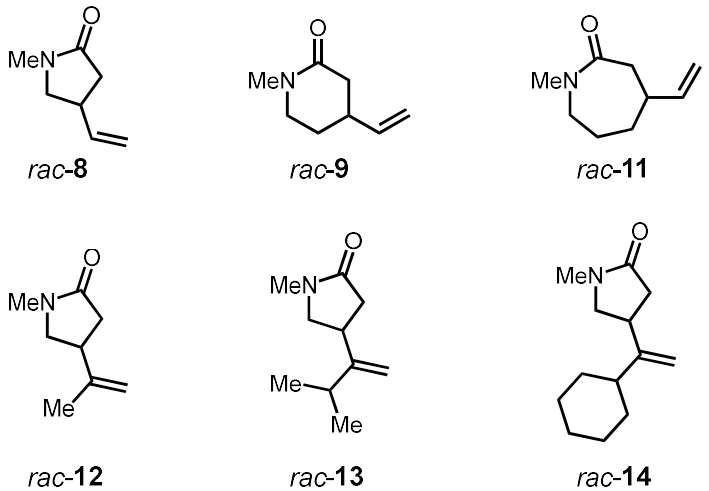


Figure SI-12: Racemic standards prepared by General Procedure H.

Synthesis of Aryl-Substituted Product Standards

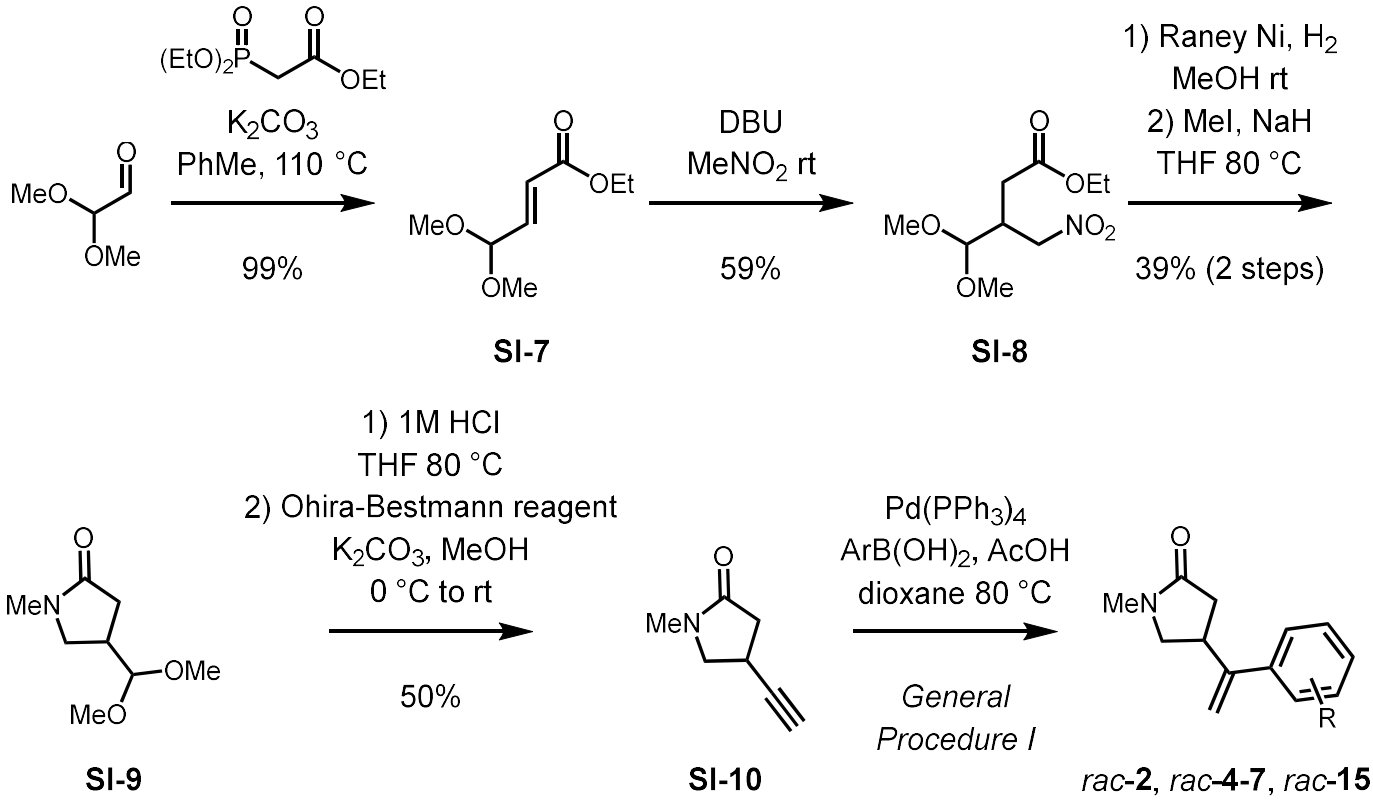


Figure SI-13: Synthetic approach to aryl-substituted racemic standards

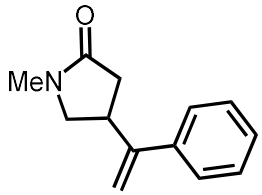
Ethyl (*E*)-4,4-dimethoxybut-2-enoate (SI-7): Prepared according to the procedure reported by Hong and coworkers[14] as follows: Triethylphosphonoacetate (20 g, 89 mmol, 1 equiv.) and dimethyl glyoxal (60% solution in water, 17 g, 98 mmol, 1.1 equiv.) were dissolved in toluene (300 mL) and potassium carbonate (14.8 g, 107 mmol, 1.2 equiv.) was added before the mixture was heated to reflux for 2 hours. After cooling to room temperature, the reaction mixture was diluted with water and EtOAc and the layers were separated. The aqueous layer was washed with two additional portions of EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporator to afford the title compound (15.5 g, 99% yield) as a clear oil that was used without further purification or analysis.

Ethyl 4,4-dimethoxy-3-(nitromethyl)butanoate (SI-8): Adapted from the procedure reported by Kenda and coworkers[15] as follows: **SI-7** (15.5 g, 89 mmol, 1 equiv.) was dissolved in nitromethane (48 mL, 890 mmol, 10 equiv.) and DBU (14.6 mL, 98 mmol, 1.1 equiv.) was added dropwise, leading to a yellow-red solution. When the reaction was judged complete by TLC, the reaction mixture was concentrated by rotary evaporator and the crude material was purified by flash column chromatography eluting with hexanes/EtOAc to afford the title compound (17.4 g, 83% yield) as a pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.60 (dd, *J* = 13.3, 5.7 Hz, 1H), 4.45 (dd, *J* = 13.2, 6.8 Hz, 1H), 4.39 (d, *J* = 5.1 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.40 (s, 6H), 2.99 (ttd, *J* = 6.9, 5.9, 5.0 Hz, 1H), 2.59 (dd, *J* = 16.8, 6.2 Hz, 1H), 2.42 (dd, *J* = 16.8, 7.1 Hz, 1H), 1.26 (t, *J* = 7.1 Hz, 4H).

4-(dimethoxymethyl)-1-methylpyrrolidin-2-one (SI-9): Adapted from a protocol reported by Domingos and coworkers[16]: Raney nickel (3 g wet) was washed with three portions of methanol and added to a flask containing **SI-8** (5 g, 21 mmol, 1 equiv.) and methanol (70 mL) was added. The reaction mixture was evacuated under vacuum and backfilled with H_2. After three additional evacuation/backfill cycles, the reaction mixture was allowed to vigorously stir under an atmosphere of H_2 (balloon) overnight. The reaction mixture was filtered through a pad of Celite and concentrated by rotary evaporator to afford crude 4-(dimethoxymethyl)pyrrolidin-2-one as a yellow-orange solid (2.83 g, 84% yield) that was not purified further. 1.83 g (11.5 mmol, 1 equiv.) of this material was dissolved in THF (38 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 690 mg, 1.5 equiv.) was added and stirred for 30 minutes before iodomethane (1.4 mL, 23 mmol) was added and the reaction mixture was heated to reflux for six hours. The reaction mixture was poured onto saturated ammonium chloride solution and diluted with EtOAc. The layers were separated and the aqueous layer was washed with four additional portions of EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporator, then purified by flash column chromatography eluting with hexanes/EtOAc to afford the title compound (911 mg, 46% yield; 39% calculated over 2 steps) as a yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.25 (d, *J* = 7.2 Hz, 1H), 3.43 – 3.32 (m, 7H), 3.26 (dd, *J* = 10.1, 5.8 Hz, 1H), 2.82 (d, *J* = 1.0 Hz, 3H), 2.74 – 2.60 (m, 1H), 2.46 (dd, *J* = 17.2, 9.6 Hz, 1H), 2.30 (dd, *J* = 17.2, 6.9 Hz, 1H).

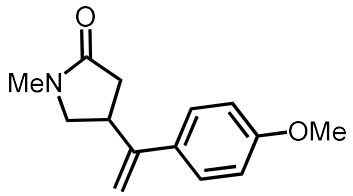
4-ethynyl-1-methylpyrrolidin-2-one (SI-10): **SI-10** (1 g, 5.8 mmol, 1 equiv.) was dissolved in THF (19 mL) and HCl (1 M, 5.8mmol, 5.8 mL) was added. The mixture was heated to reflux until judged complete by TLC. Upon cooling to room temperature, the reaction mixture was diluted with EtOAc and dried over Na_2SO_4 before being concentrated by rotary evaporator to afford a crude red-orange oil. This crude material was directly taken up in MeOH (10 mL) and cooled to 0 °C before dimethyldiazomethylphosphonate (1.2 g, 5.8 mmol, 1 equiv.) was added, followed by solid K_2CO_3 (1.2 g, 8.7 mmol, 1.5 equiv.). The reaction mixture was allowed to warm to room temperature overnight and then poured to onto saturated aqueous ammonium chloride and extracted with three portions of EtOAc. The combined organic layers were dried over Na_2SO_4 and purified by flash column chromatography eluting with hexanes/EtOAc to afford the title compound (358 mg, 50% yield) as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.25 (d, *J* = 7.2 Hz, 1H), 3.43 – 3.32 (m, 7H), 3.26 (dd, *J* = 10.1, 5.8 Hz, 1H), 2.82 (d, *J* = 1.0 Hz, 3H), 2.74 – 2.60 (m, 1H), 2.46 (dd, *J* = 17.2, 9.6 Hz, 1H), 2.30 (dd, *J* = 17.2, 6.9 Hz, 1H).

General Procedure I: In a procedure adapted from Schindler and coworkers[17], alkyne **SI-10** (1 equiv.) was dissolved in degassed (sparged with N_2, ~20 minutes) dioxane (0.3 M) and aryl boronic acid (1.2 equiv.) was added, followed by $Pd(PPh_3)_4$ (3 mol%). Acetic acid (10 mol%) was added last and the reaction mixture was heated to 80 °C overnight. After cooling to room temperature, the reaction mixture was filtered through a plug of silica gel eluting with EtOAc. The solvent was removed by rotary evaporator and an analytical sample of the product was isolated by preparative HPLC to obtain characterization data and racemic HPLC traces for analysis of the biocatalytic reaction products.



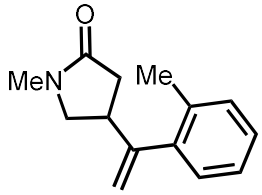
1

1-methyl-4-(1-phenylvinyl)pyrrolidin-2-one (1): Prepared according to General Procedure I using 20 mg (.16 mmol) of **SI-10** to afford 12 mg (37% yield) of **1**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 5.32 (s, 1H), 5.12 (d, *J* = 1.3 Hz, 1H), 3.61 – 3.50 (m, 2H), 3.25 – 3.16 (m, 1H), 2.83 (s, 3H), 2.65 (dd, *J* = 16.6, 8.3 Hz, 1H), 2.52 – 2.44 (m, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.9, 149.1, 141.0, 128.7, 128.0, 126.6, 112.5, 54.7, 36.7, 36.2, 29.7. **IR** (cm^{-1}): 2954, 1691, 1497, 1435, 1401, 1300, 1259, 992, 910. **HRMS**: Calculated for $C_{13}H_{16}NO$ $[M+H^+]^+$: 202.1226. Found $[M+H^+]^+$: 202.1236. **HPLC**: IB, 2% isopropanol in hexanes, 60 minute method.



4

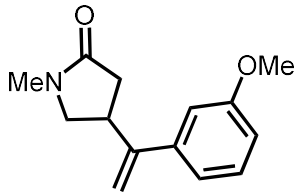
4-(1-(4-methoxyphenyl)vinyl)-1-methylpyrrolidin-2-one (4): Prepared according to General Procedure I using 30 mg (0.24 mmol) of **SI-10** to afford 8 mg (17% yield) of **4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31 – 7.26 (m, 2H), 6.95 – 6.77 (m, 2H), 5.26 (s, 1H), 5.04 (d, *J* = 1.3 Hz, 1H), 3.82 (s, 3H), 3.62 – 3.44 (m, 2H), 3.29 – 3.13 (m, 1H), 2.84 (s, 3H), 2.65 (dd, *J* = 16.6, 8.0 Hz, 1H), 2.55 – 2.38 (m, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.0, 159.5, 148.4, 133.3, 127.6, 114.0, 111.0, 55.4, 54.8, 36.7, 36.2, 29.7. **IR** (cm^{-1}):2936, 1689, 1625, 1511, 1296, 1247, 1182, 1036, 913. **HRMS**: Calculated for $C_{14}H_{18}NO_2$ $[M+H^+]^+$: 232.1332. Found $[M+H^+]^+$: 232.1329. **HPLC**: IC, 20% isopropanol in hexanes, 55 minute method.



5

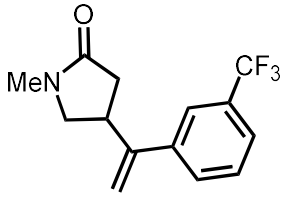
1-methyl-4-(1-(o-tolyl)vinyl)pyrrolidin-2-one (5): Prepared according to General Procedure I using 30 mg (0.24 mmol) of **SI-10** to afford 7.2 mg (14% yield) of **5**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.23 – 7.11 (m, 3H), 7.08 – 6.98 (m, 1H), 5.28 (t, *J* = 1.2 Hz, 1H), 5.02 (t, *J* = 1.0 Hz, 1H), 3.46 – 3.28 (m, 2H), 3.23 (dd, *J* = 8.3, 6.9 Hz, 1H), 2.81 (s, 3H), 2.62 – 2.40 (m, 2H), 2.27 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.0, 149.1, 141.3, 135.0, 130.5, 128.6, 127.5, 125.8,

114.5, 54.0, 38.6, 36.3, 29.7, 20.0. **IR** (cm^{-1}): 2923, 2859, 1693, 1498, 1428, 1401, 1256, 913. **HRMS**: Calculated for $C_{14}H_{18}NO$ $[M+H^+]^+$: 216.1383. Found $[M+H^+]^+$: 216.1376. **HPLC**: OJ-H, 5% isopropanol in hexanes, 50 minute method.



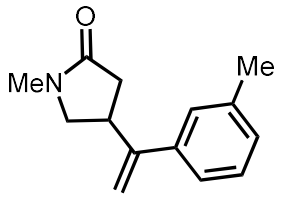
6

4-(1-(3-methoxyphenyl)vinyl)-1-methylpyrrolidin-2-one (6): Prepared according to General Procedure I using 30 mg (0.24 mmol) of **SI-10** to afford 2 mg (4% yield) of **6**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.94 – 6.89 (m, 1H), 6.88 – 6.81 (m, 2H), 5.33 (d, *J* = 1.9 Hz, 1H), 5.11 (d, *J* = 2.0 Hz, 1H), 3.89 – 3.76 (m, 3H), 3.60 – 3.47 (m, 2H), 3.20 (q, *J* = 2.8 Hz, 1H), 2.83 (d, *J* = 2.0 Hz, 3H), 2.65 (dd, *J* = 17.0, 7.8 Hz, 1H), 2.47 (dd, *J* = 16.6, 7.3 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.9, 159.8, 149.0, 142.5, 129.6, 119.0, 113.0, 112.7, 112.6, 55.4, 54.7, 36.6, 36.3, 29.7. **IR** (cm^{-1}): 2999, 2362, 1609, 1576, 1488, 1289, 1225, 1046, 910. **HRMS**: Calculated for $C_{14}H_{18}NO_2$ $[M+H^+]^+$: 232.1332. Found $[M+H^+]^+$: 232.1321. **HPLC**: AS-H, 20% isopropanol in hexanes, 60 minute method.



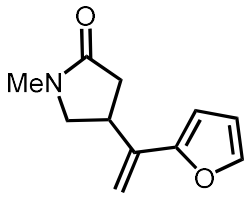
17

1-methyl-4-(1-(3-(trifluoromethyl)phenyl)vinyl)pyrrolidin-2-one **(16)**: Prepared according to General Procedure I using 30 mg (0.24 mmol) of **SI-10** to afford 3 mg (5% yield) of **15**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 4.3, 2.2 Hz, 2H), 7.54 – 7.46 (m, 2H), 5.39 (s, 1H), 5.22 (d, *J* = 1.5 Hz, 1H), 3.61 – 3.51 (m, 2H), 3.27 – 3.18 (m, 1H), 2.85 (s, 3H), 2.71 – 2.63 (m, 1H), 2.46 (dd, *J* = 16.6, 7.7 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.5, 148.1, 141.8, 131.2(q), 129.8, 129.2, 124.7(q), 124.1(q, CF_3) 123.3 (q), 114.0, 54.5, 36.6, 36.1, 29.7. **^{19}F NMR** (470 MHz, $CDCl_3$) δ -62.7. **IR** (cm^{-1}): 2927, 1692, 1500, 1434, 1402, 1330, 1165, 1123, 903. **HRMS**: Calculated for $C_{14}H_{15}F_3NO$ $[M+H^+]^+$: 270.1100. Found $[M+H^+]^+$: 270.1104.



7

1-methyl-4-(1-(m-tolyl)vinyl)pyrrolidin-2-one (7): Prepared according to General Procedure I using 30 mg (0.24 mmol) of **SI-10** to afford 4 mg (8% yield) of **7**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 3H), 5.30 (s, 1H), 5.10 (d, *J* = 1.2 Hz, 1H), 3.62 – 3.48 (m, 2H), 3.21 (dd, *J* = 10.0, 3.5 Hz, 1H), 2.84 (s, 3H), 2.65 (dd, *J* = 16.6, 7.8 Hz, 1H), 2.47 (dd, *J* = 16.5, 7.7 Hz, 1H), 2.37 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.9, 149.3, 141.0, 138.3, 128.7, 128.5, 127.4, 123.6, 112.2, 54.8, 36.7, 36.3, 29.7, 21.6. **IR** (cm^{-1}): 2925, 2857, 1698, 1457, 1400, 1266. **HRMS**: Calculated for $C_{14}H_{18}NO$ $[M+H^+]^+$: 216.1383. Found $[M+H^+]^+$: 216.1380. **HPLC**: AS-H, 20% isopropanol in hexanes, 45 minute method.



15

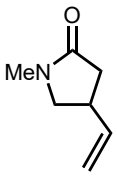
4-(1-(furan-2-yl)vinyl)-1-methylpyrrolidin-2-one (15): Prepared according to General Procedure I using 45 mg (0.36 mmol) of **SI-10** to afford 8 mg (12% yield) of **15**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.42 (d, *J* = 1.8 Hz, 1H), 6.34 (d, *J* = 3.3 Hz, 1H), 5.62 (s, 1H), 5.06 (s, 1H), 3.71 – 3.62 (m, 1H), 3.46 – 3.29 (m, 2H), 2.87 (d, *J* = 5.0 Hz, 3H), 2.68 (dd, *J* = 17.0, 9.0 Hz, 1H), 2.53 (q, *J* = 7.5 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.8, 153.5, 142.4, 138.2, 111.3, 109.1, 106.6, 54.7, 36.5, 34.2, 29.7. **IR** (cm^{-1}):2941, 1604, 1505, 1434, 1403, 1301, 1264, 1163, 1018, 913. **HRMS**: Calculated for $C_{11}H_{14}NO_2$ $[M+H^+]^+$: 192.1025. Found $[M+H^+]^+$: 192.1376. **HPLC**: OJ-H, 5% isopropanol in hexanes, 50 minute method.

REFERENCE LIST

1) Biegasiewicz, K. F.; Cooper, S. J.; Gao, X.; Oblinsky, D. G.; Kim, J. H.; Garfinkle, S. E.; Joyce, L. A.; Sandoval, B. A.; Scholes, G. D.; Hyster, T. K. *Science* **2019**, *364*, 1166–1169.
2) Nicholls, B. T.; Oblinsky, D. G.; Kurtoic, S. I.; Grosheva, D.; Ye, Y.; Scholes, G. D.; Hyster, T. K. *Angewandte Chemie International Edition* **2022**, *61*, e202113842.
3) Michaut, A.; Boddaert, T.; Coquerel, Y.; Rodriguez, J. *Synthesis* **2007**, *2007*, 2867–2871.
4) Sato, T.; Wada, Y.; Nishimoto, M.; Ishibashi, H.; Ikeda, M. *J. Chem. Soc., Perkin Trans. 1* **1989**, 5, 879–886.
5) Zheng, H.-J.; Chen, W.-B.; Wu, Z.-J.; Deng, J.-G.; Lin, W.-Q.; Yuan, W.-C.; Zhang, X.-M. *Chemistry – A European Journal* **2008**, *14*, 9864–9867.
6) Zhang, C.-C.; Chen, L.-J.; Shen, B.-C.; Xie, H.-D.; Li, W.; Sun, Z.-W. *Org. Biomol. Chem.* **2021**, *19*, 8607–8612.
7) Takeda, T.; Harada, S.; Nishida, A. *Org. Lett.* **2015**, *17*, 5184–5187.
8) Zhang, G.; Wang, Y.; Xu, J.; Sun, J.; Sun, F.; Zhang, Y.; Zhang, C.; Du, Y. *Chem. Sci.* **2020**, *11*, 947–953.
9) Yang, P.; Xu, H.; Zhou, J. *Angew. Chem. Int. Ed*. **2014**, *53*, 12210-12213.
10) Li, H.; Fiorito, D.; Mazet, C. *ACS Catal.* **2017**, *7*, 1554–1562.
11) Chen, Z.-D.; Xu, W.-K.; Guo, J.-M.; Chen, L.; Wei, B.-G.; Si, C.-M.; Lin, G.-Q. *J. Org. Chem.* **2021**, *86* , 11442–11455.

12) Lu, Y.-S.; Yu, W.-Y. *Org. Lett.* **2016**, *18*, 1350–1353.
13) Tanaka, K.; Shibata, Y.; Suda, T.; Hagiwara, Y.; Hirano, M., *Org. Lett.* **2007**, *9*, 1215-1218.
14) Hong, B.-C.; Chen, P.-Y.; Kotame, P.; Lu, P.-Y.; Lee, G.-H.; Liao, J.-H. *Chem. Commun.* **2012**, *48*, 7790–7792.
15) Kenda, B. M.; Matagne, A. C.; Talaga, P. E.; Pasau, P. M.; Differding, E.; Lallemand, B. I.; Frycia, A. M.; Moureau, F. G.; Klitgaard, H. V.; Gillard, M. R.; Fuks, B.; Michel, P. *J. Med. Chem.* **2004**, *47*, 530–549.
16) Domingos, J. L. O.; Lima, E. C.; Dias, A. G.; Costa, P. R. R. *Tetrahedron: Asymmetry* **2004**, *15*, 2313–2314.
17) Ludwig, J. R.; Watson, R. B.; Nasrallah, D. J. Gianiono, J. B.; Zimmerman, P. M.; Wiscons, R. A.; Schindler C. S. *Science*, **2018**, *361*, 1363-1369.

Chiral HPLC Traces of Products



8

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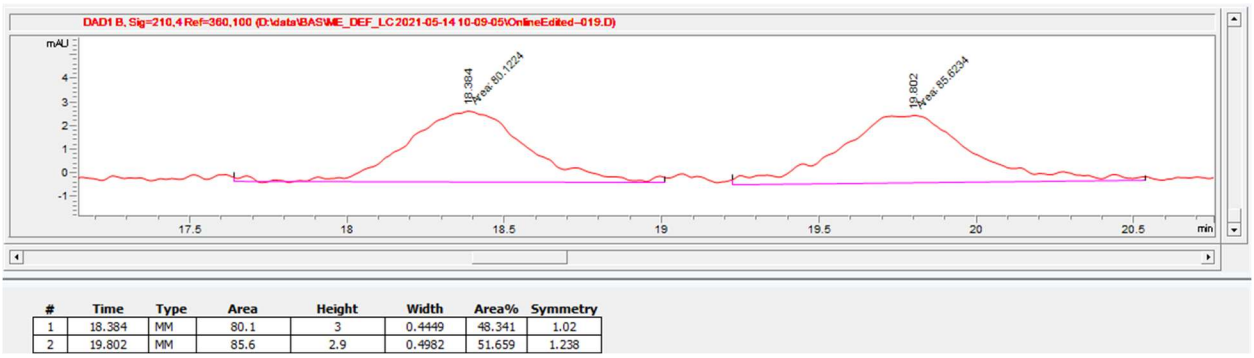

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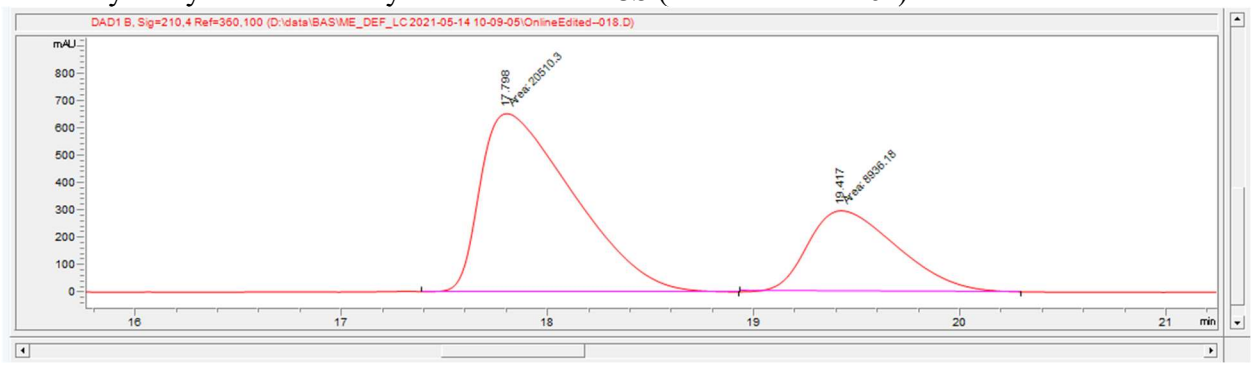
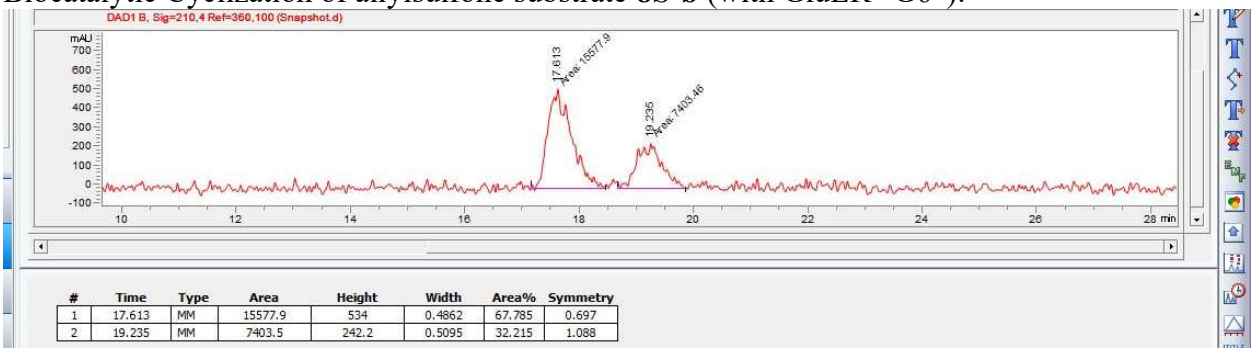
Biocatalytic Cyclization of allylsilane substrate **8S** (with GluER "G6"):



Biocatalytic Cyclization of allylsulfone substrate **8S-b** (with GluER "G6"):



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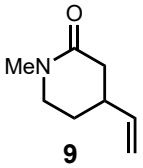

9

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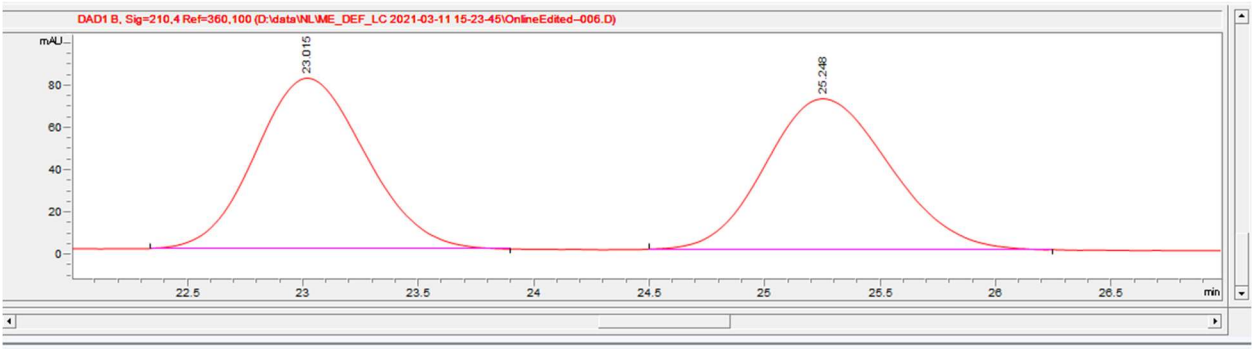

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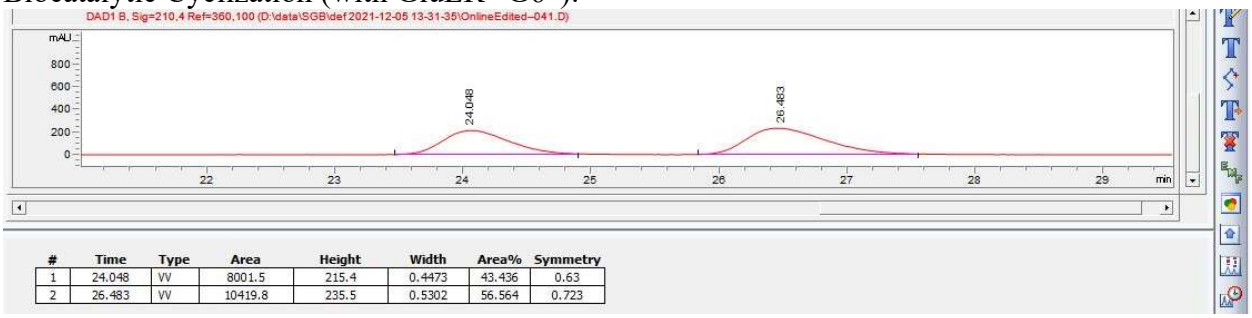
Biocatalytic Cyclization (with GluER “G6”):



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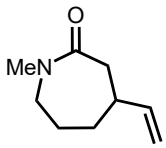
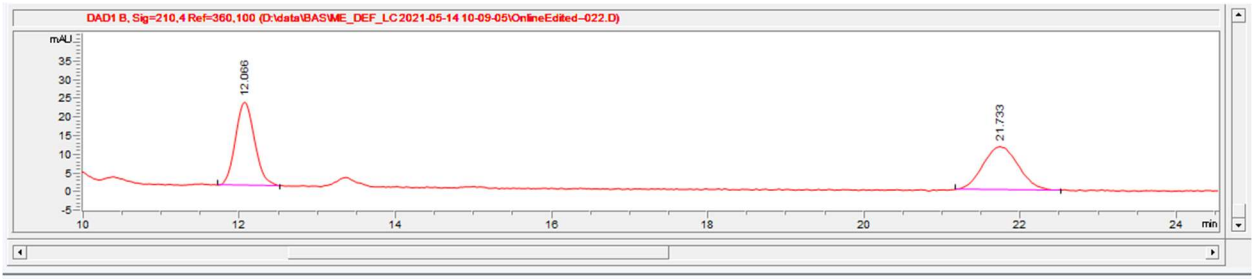


11

Racemic:



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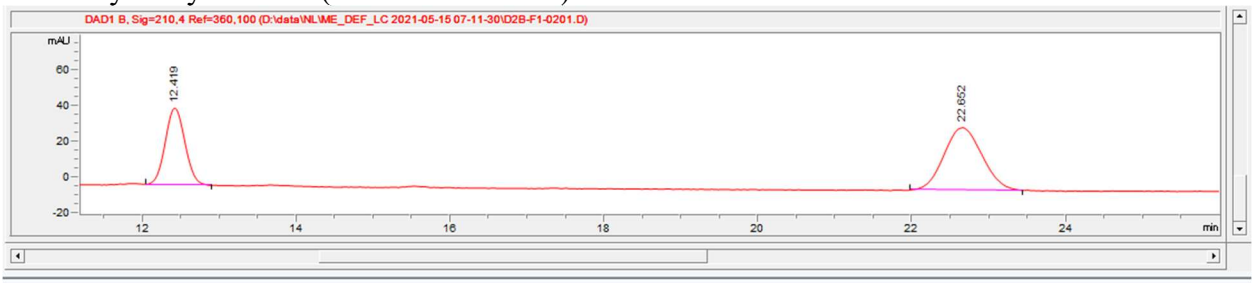
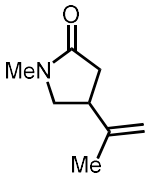
2	21.733	BV	375.8	11.7	0.3881	49.284	0.896

Biocatalytic Cyclization (with GluER “G6”):

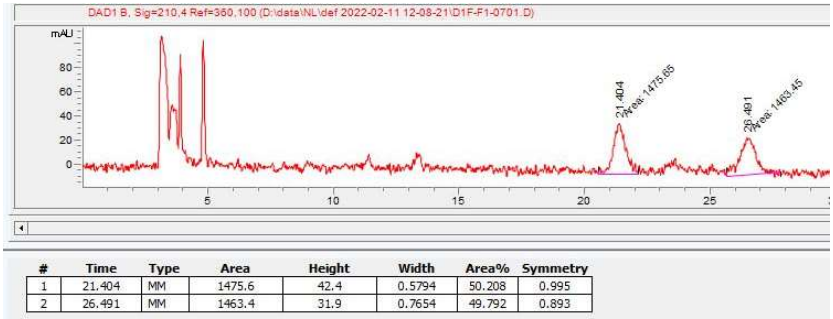


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	12.419	VB	775.7	43.2	0.2797	38.888	0.915
2	22.652	BV	1218.9	35.1	0.5146	61.112	0.94



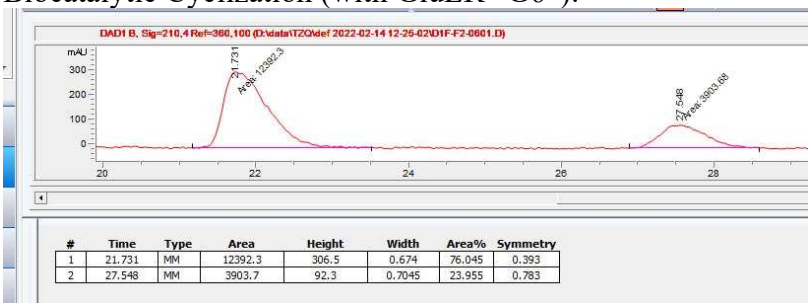
12

Racemic:

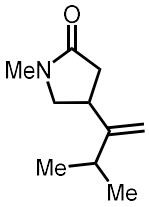


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.404	MM	1475.6	42.4	0.5794	50.208	0.995
2	26.491	MM	1463.4	31.9	0.7654	49.792	0.893

Biocatalytic Cyclization (with GluER “G6”):

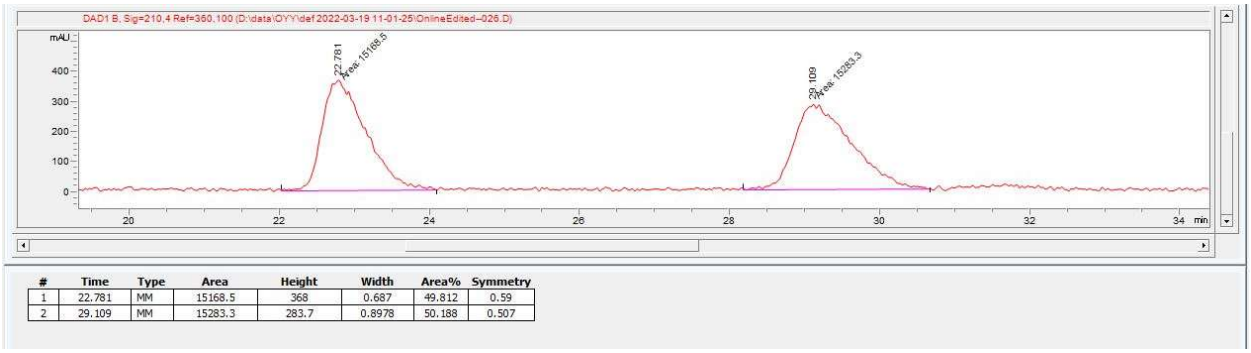


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.731	MM	12392.3	306.5	0.674	76.045	0.393
2	27.548	MM	3903.7	92.3	0.7045	23.955	0.783



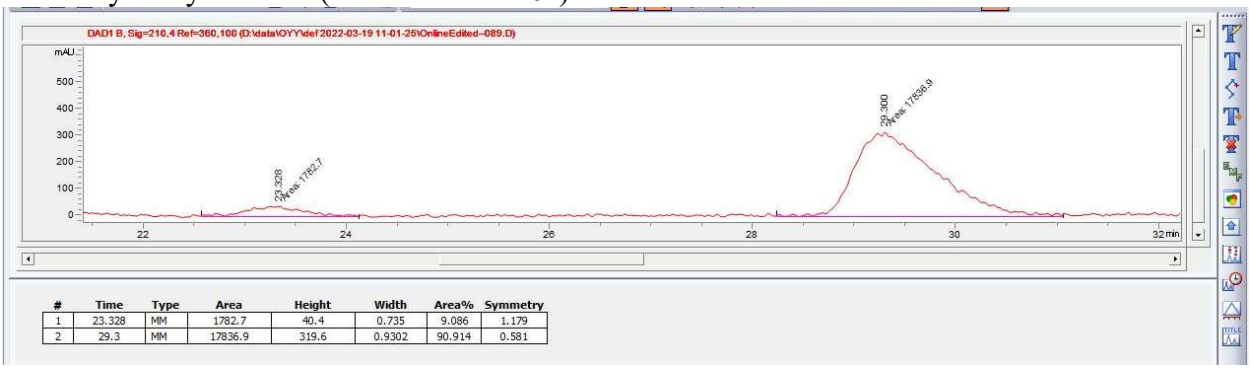
13

Racemic:

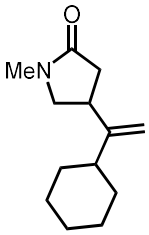


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	22.781	MM	15168.5	368	0.687	49.812	0.59
2	29.109	MM	15283.3	283.7	0.8978	50.188	0.507

Biocatalytic Cyclization (with GluER “G6”):

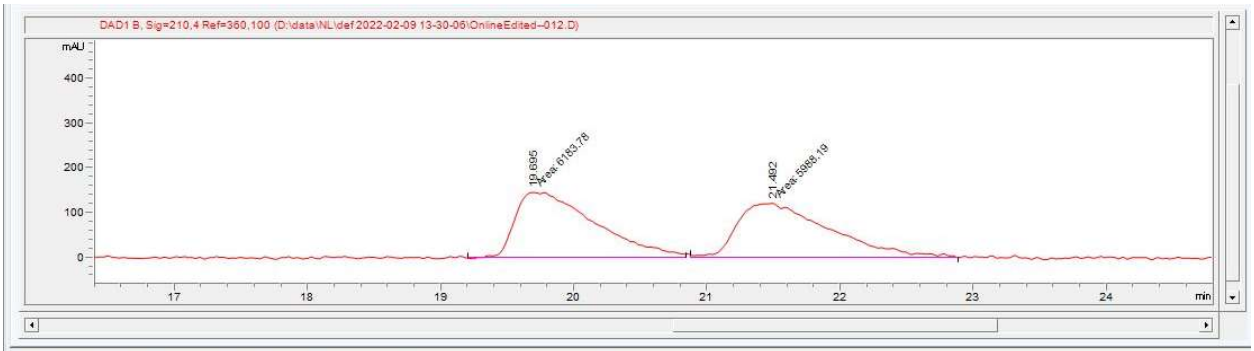


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	23.328	MM	1782.7	40.4	0.735	9.086	1.179
2	29.3	MM	17836.9	319.6	0.9302	90.914	0.581



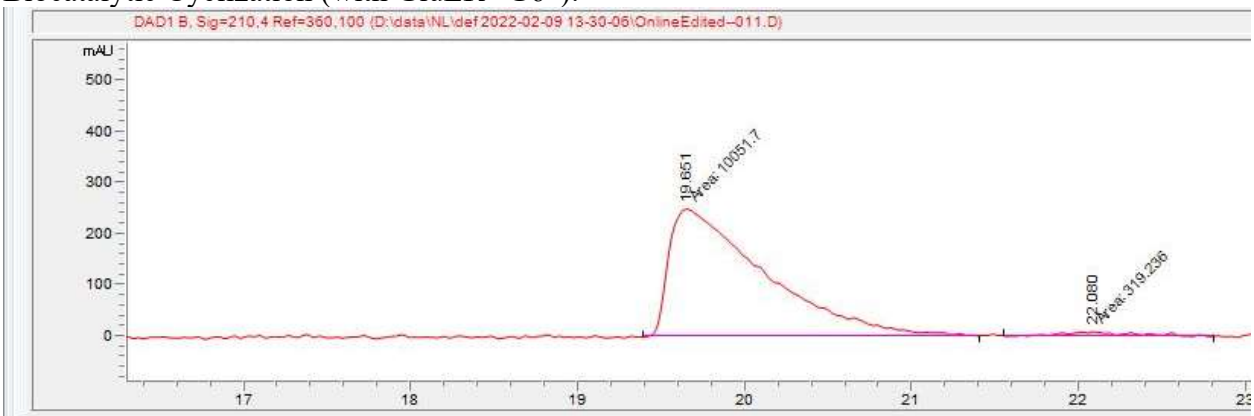
14

Racemic:

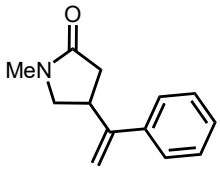


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.695	MM	6183.8	146.9	0.7016	50.803	0.302
2	21.492	MM	5988.2	122.2	0.8169	49.197	0.589

Biocatalytic Cyclization (with GluER “G6”):

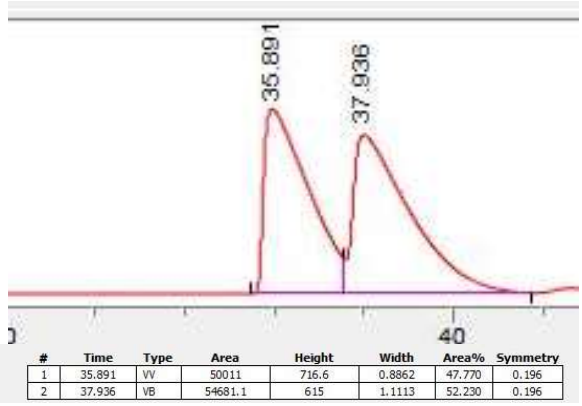


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.651	MM	10051.7	250	0.67	96.922	0.228
2	22.08	MM	319.2	9.3	0.571	3.078	0.717



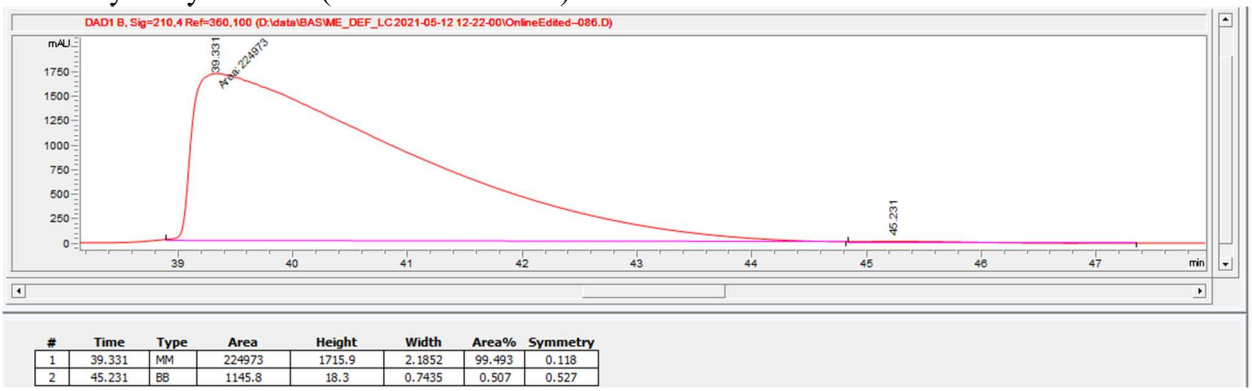
1

Racemic:

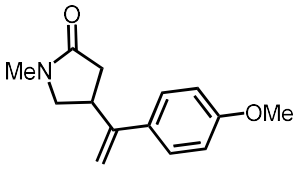


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	35.891	VV	50011	716.6	0.8862	47.770	0.196
2	37.936	VB	54681.1	615	1.1113	52.230	0.196

Biocatalytic Cyclization (with GluER “G6”):

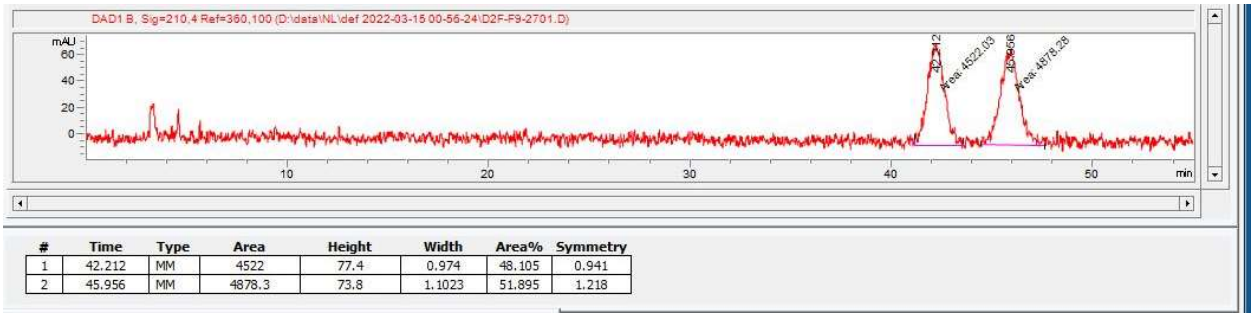


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	39.331	MM	224973	1715.9	2.1852	99.493	0.118
2	45.231	BB	1145.8	18.3	0.7435	0.507	0.527



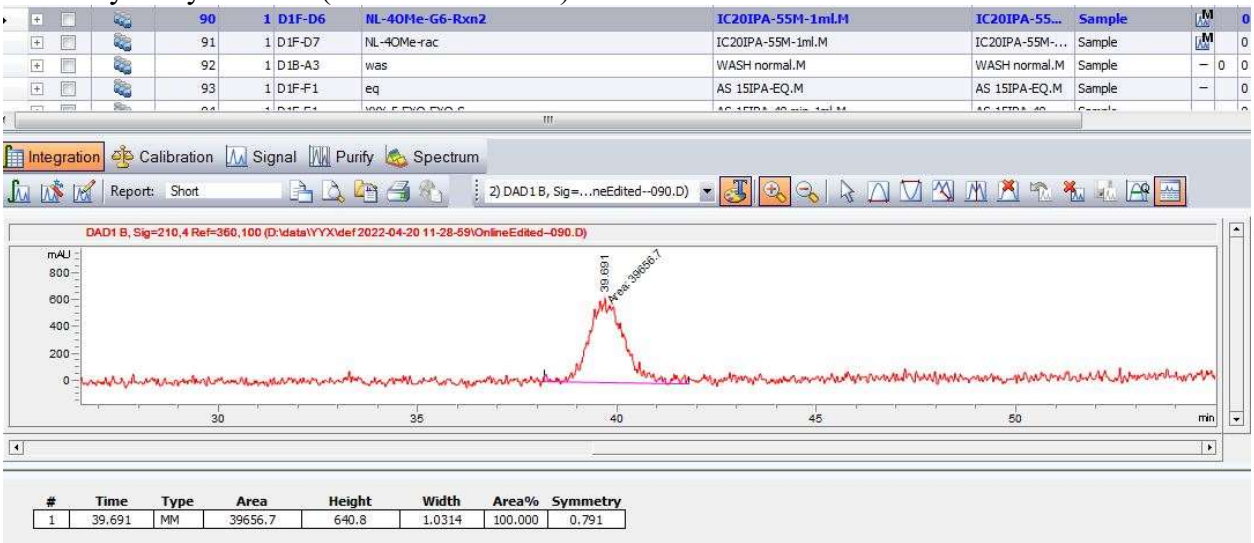
4

Racemic:

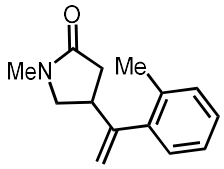


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	42.212	MM	4522	77.4	0.974	48.105	0.941
2	45.956	MM	4878.3	73.8	1.1023	51.895	1.218

Biocatalytic Cyclization (with GluER “G6”):

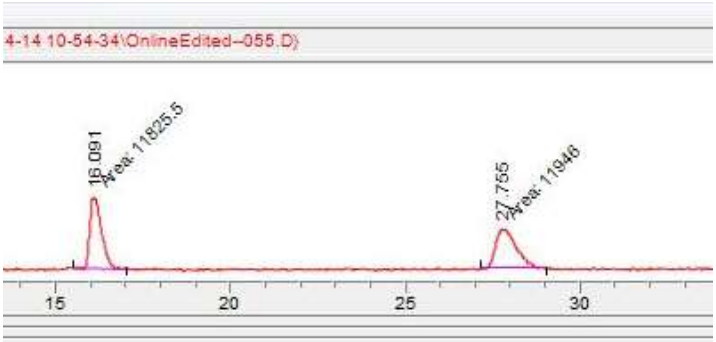


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	39.691	MM	39656.7	640.8	1.0314	100.000	0.791



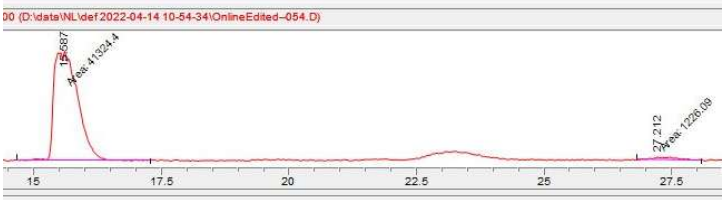
5

Racemic:

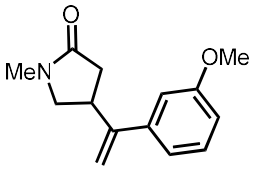


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	16.091	MM	11825.5	499.7	0.3945	49.747	0.631
2	27.755	MM	11946	283.7	0.7019	50.253	0.449

Biocatalytic Cyclization (with GluER “G6”):

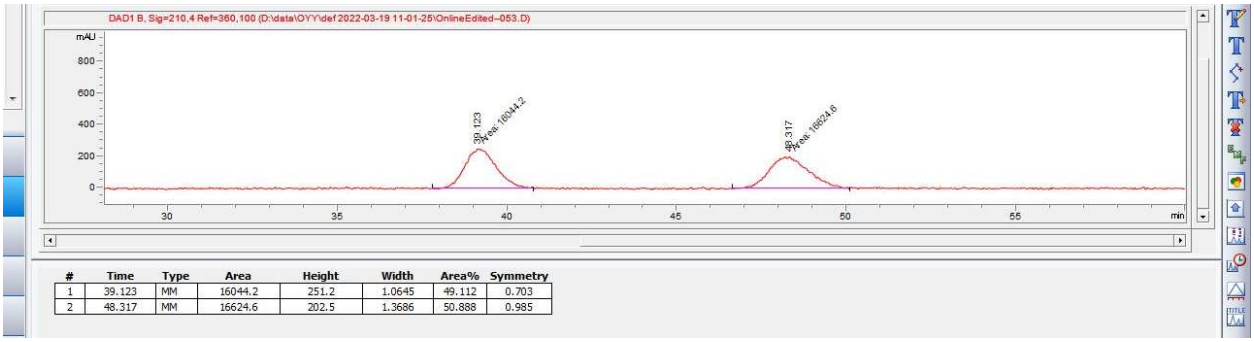


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.587	MM	41324.4	1255.2	0.5487	97.119	0.768
2	27.212	MM	1226.1	34.2	0.5981	2.881	0.379



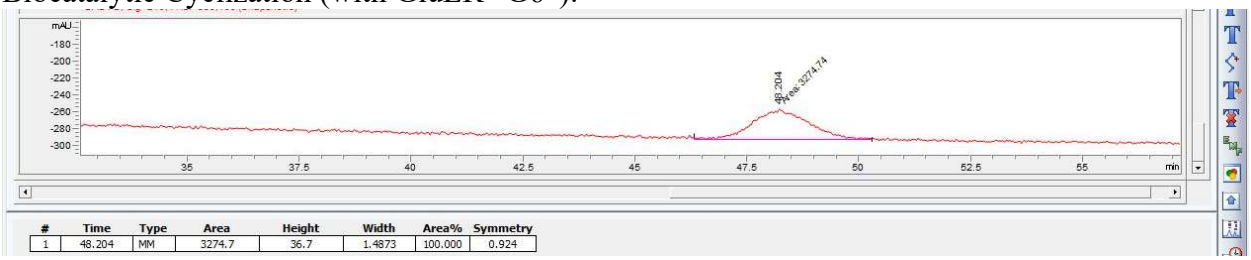
6

Racemic:

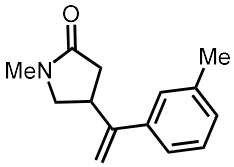


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	39.123	MM	16044.2	251.2	1.0645	49.112	0.703
2	48.317	MM	16624.6	202.5	1.3686	50.888	0.985

Biocatalytic Cyclization (with GluER “G6”):

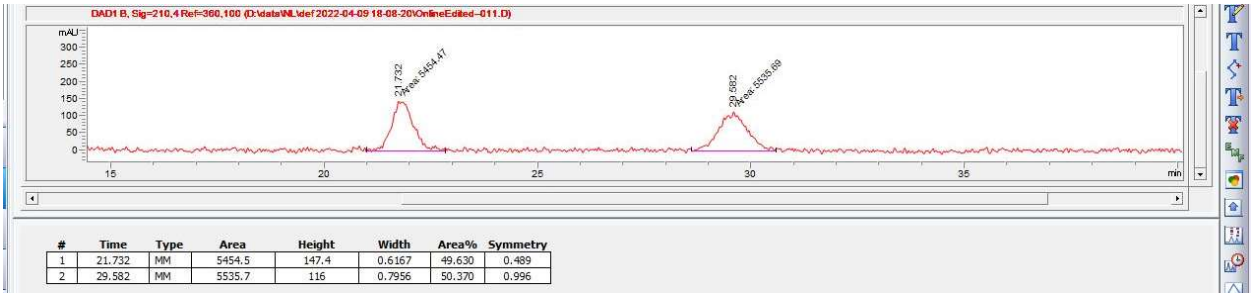


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	48.204	MM	3274.7	36.7	1.4873	100.000	0.924



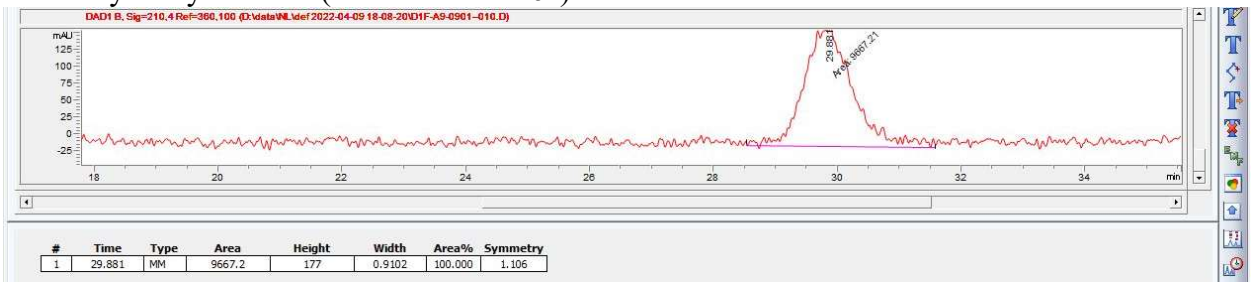
7

Racemic:

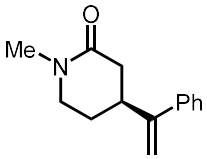


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.732	MM	5454.5	147.4	0.6167	49.630	0.489
2	29.582	MM	5535.7	116	0.7956	50.370	0.996

Biocatalytic Cyclization (with GluER “G6”):

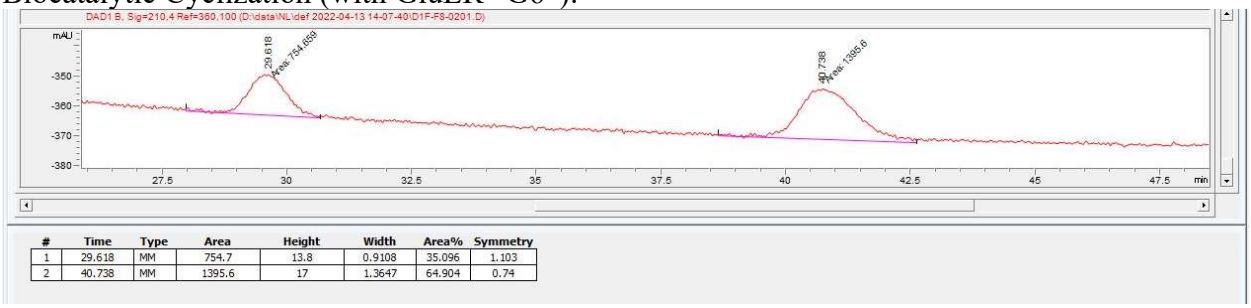


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	29.881	MM	9667.2	177	0.9102	100.000	1.106

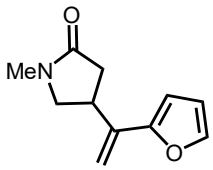


10

Biocatalytic Cyclization (with GluER “G6”):



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	29.618	MM	754.7	13.8	0.9108	35.096	1.103
2	40.738	MM	1395.6	17	1.3647	64.904	0.74

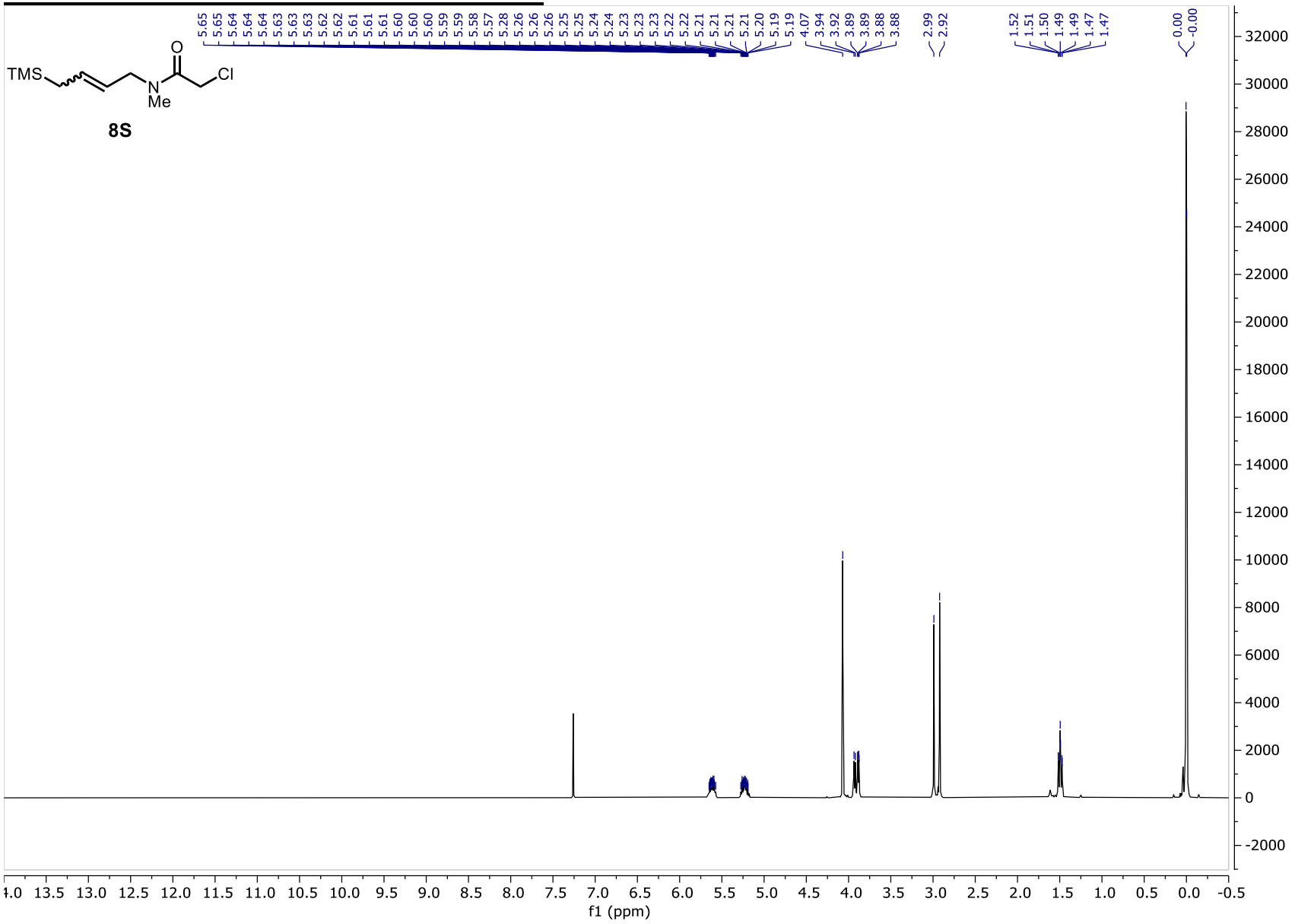


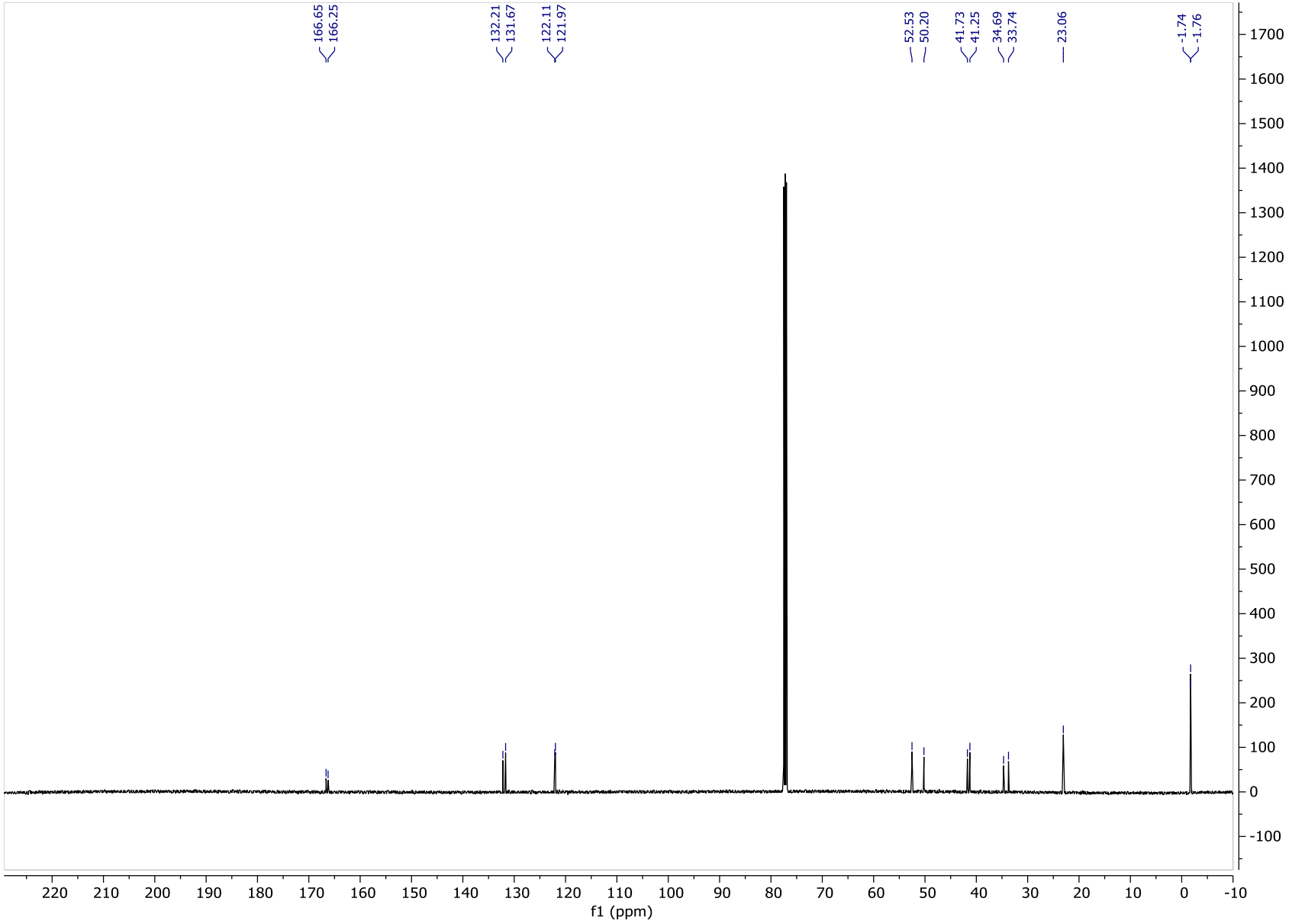
15

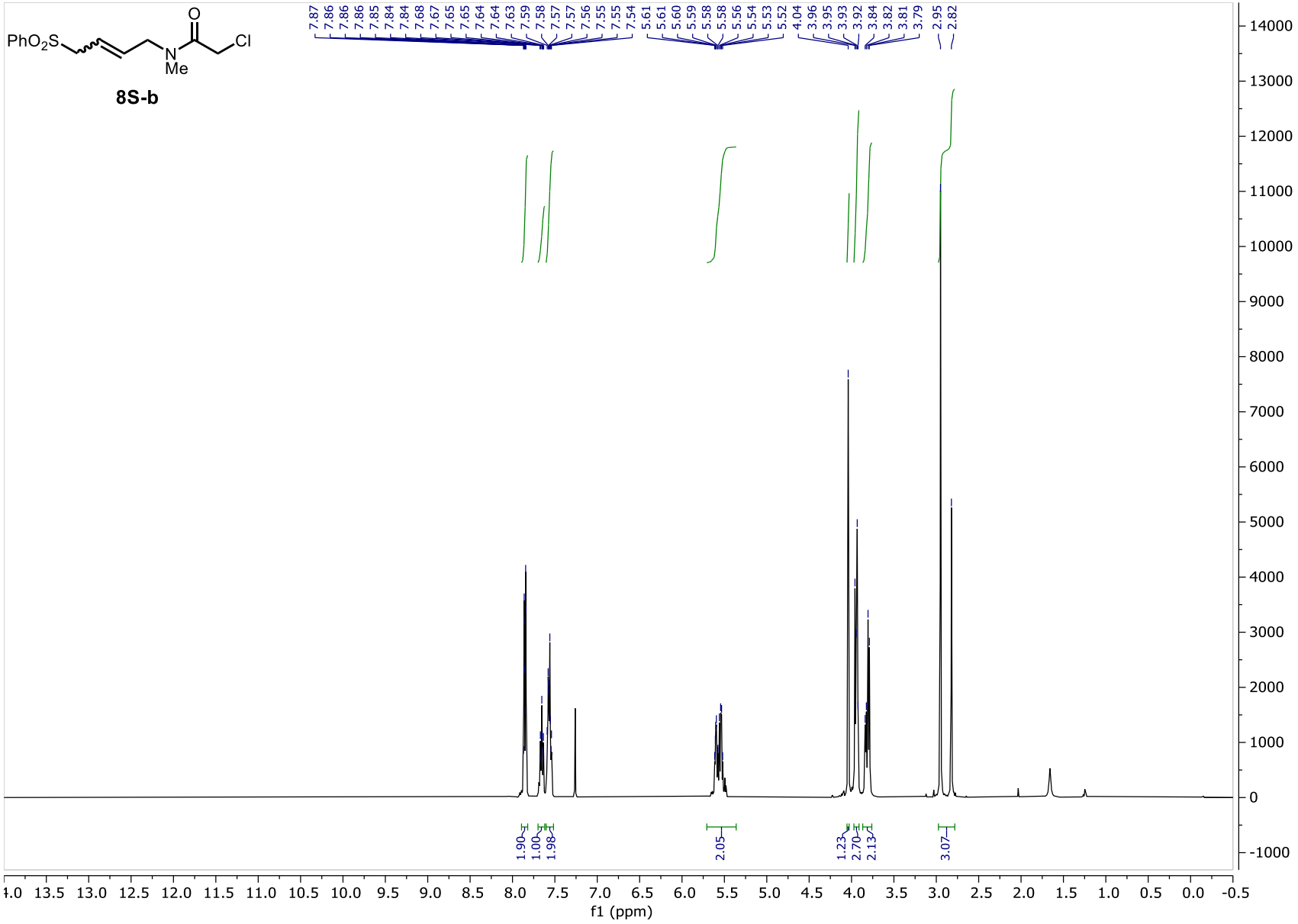
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	26.654	MM	726.9	20.9	0.579	51.479	0.761
2	28.905	MM	685.2	18.6	0.6138	48.521	0.775

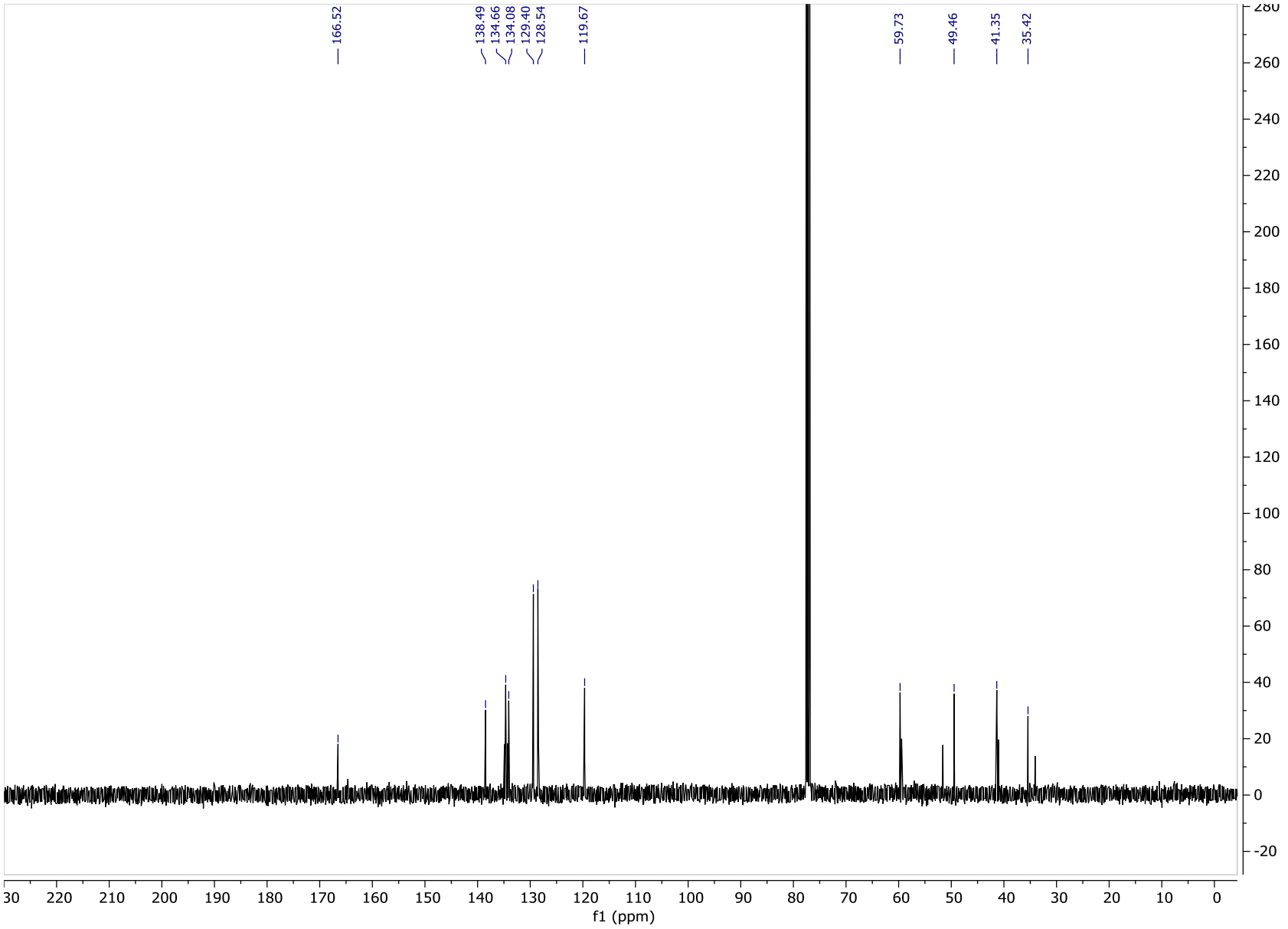
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	26.024	VB	20735.6	448.8	0.679	98.782	0.401
2	28.932	MM	255.7	6.9	0.6138	1.218	0.763

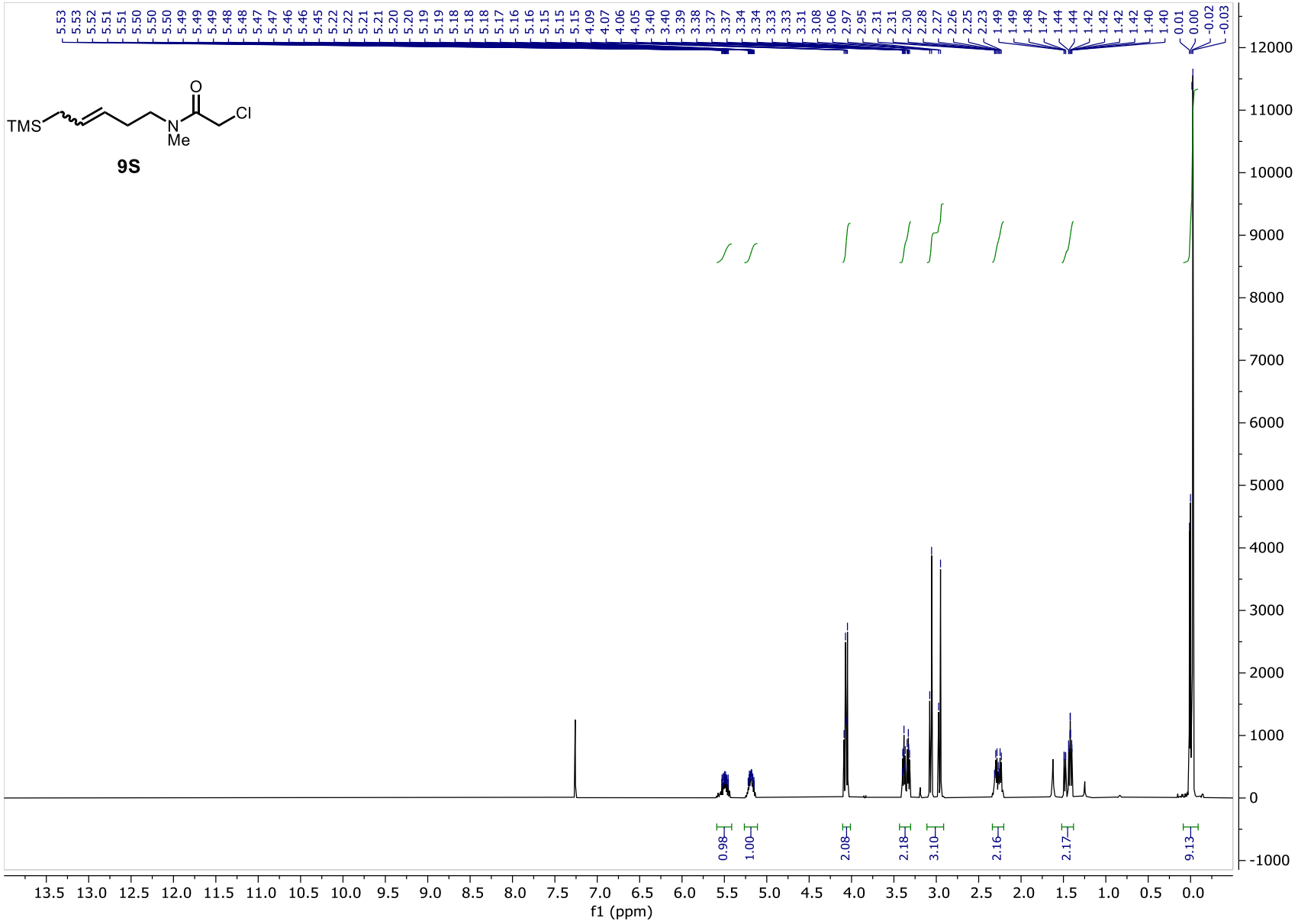
^{1}H and ^{13}C NMR of Substrates and Products

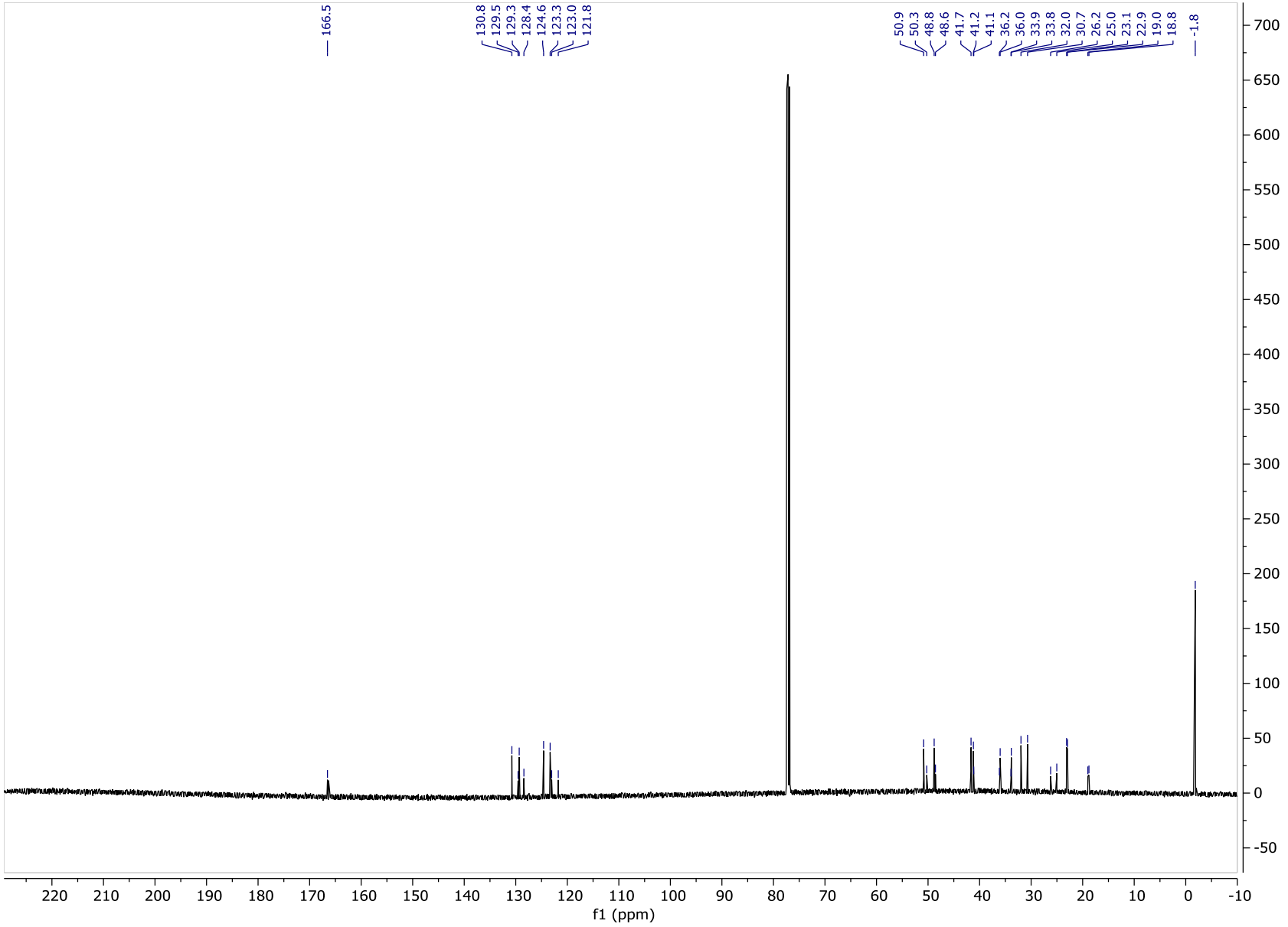


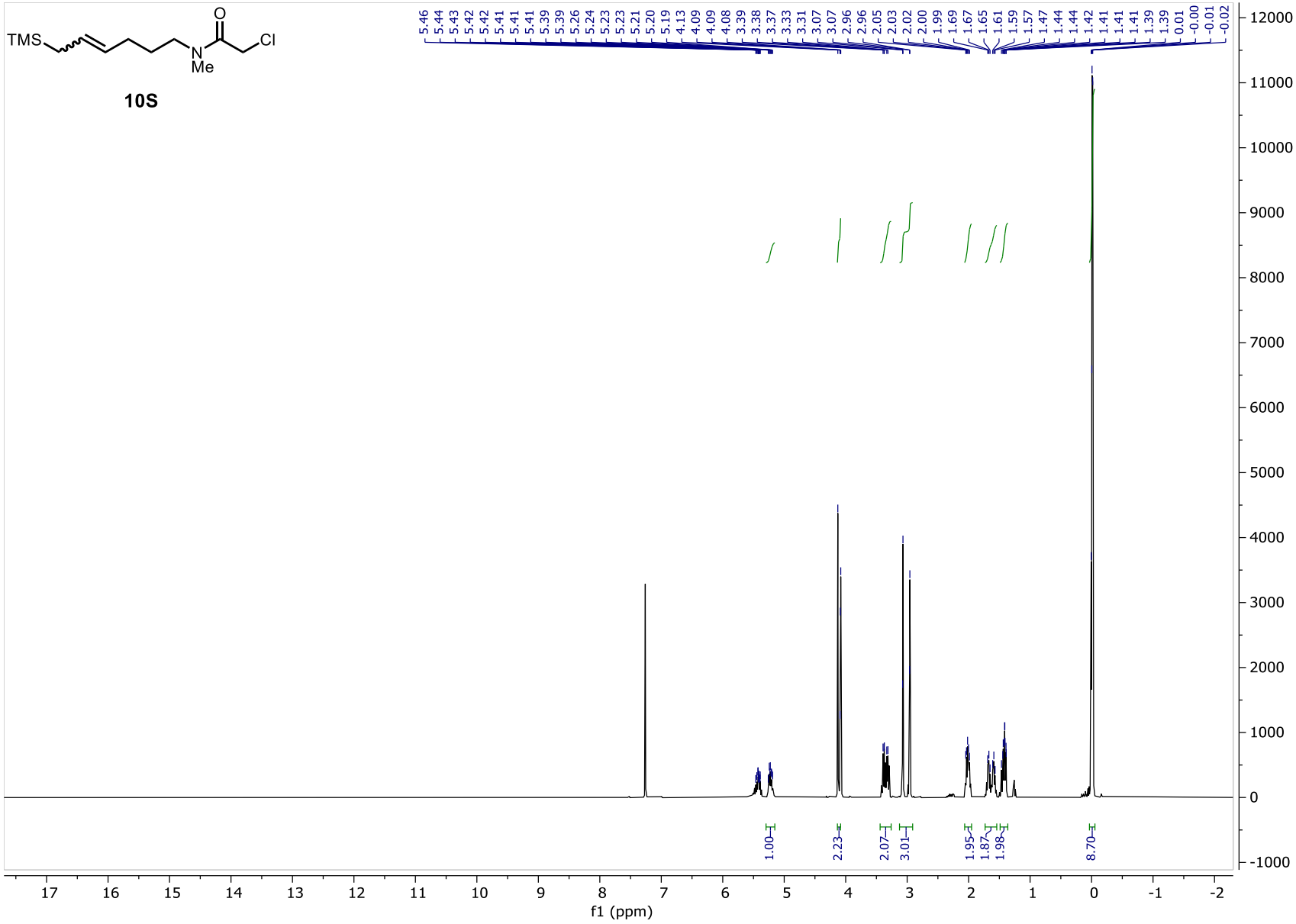

166.65
166.25
132.21
131.67
122.11
121.97
52.53
50.20
41.73
41.25
34.69
33.74
23.06
-1.74
-1.76
f1 (ppm)

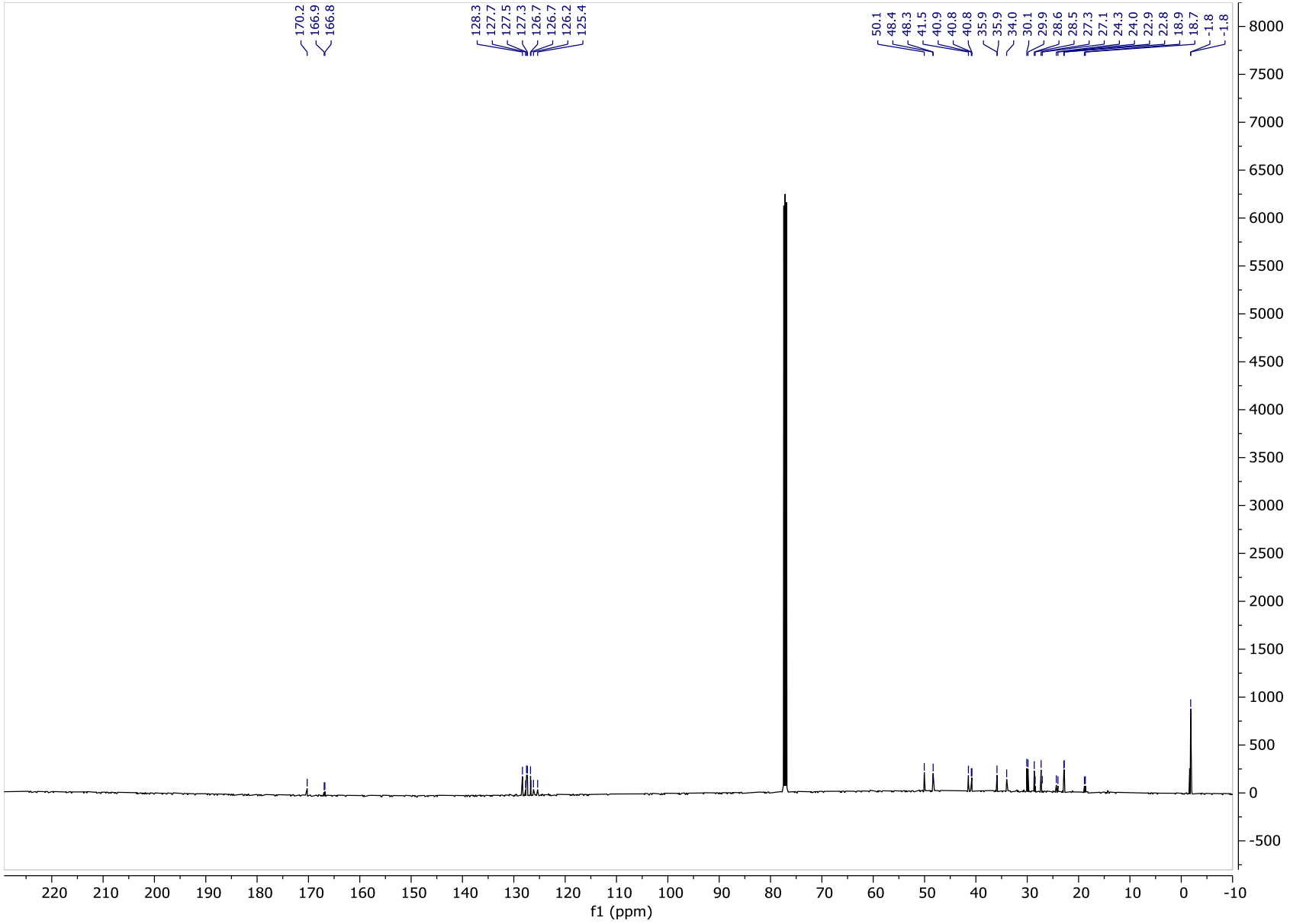

PhO2S
O
N
Me
Cl
8S-b
7.87 7.86 7.86 7.86 7.85 7.84 7.84 7.68 7.67 7.65 7.65 7.64 7.64 7.63 7.59 7.58 7.57 7.57 7.56 7.55 7.55 7.54 5.61 5.61 5.60 5.59 5.58 5.58 5.56 5.54 5.53 5.52 4.04 3.96 3.95 3.93 3.92 3.84 3.82 3.81 3.79 2.95 2.82
1.90 1.00 1.98 2.05 1.23 2.70 2.13 3.07
f1 (ppm)

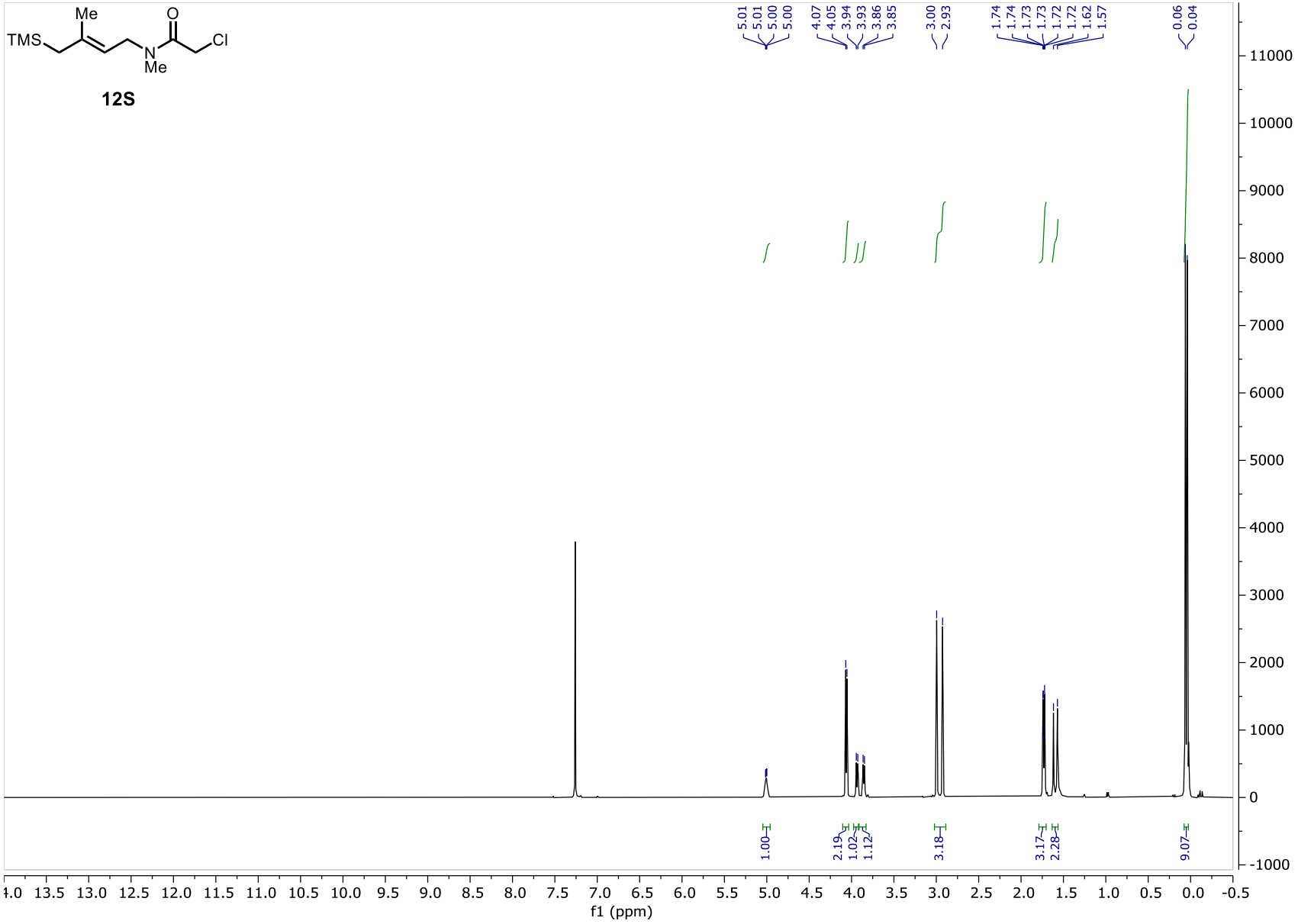

166.52
138.49
134.66
134.08
129.40
128.54
119.67
59.73
49.46
41.35
35.42
f1 (ppm)

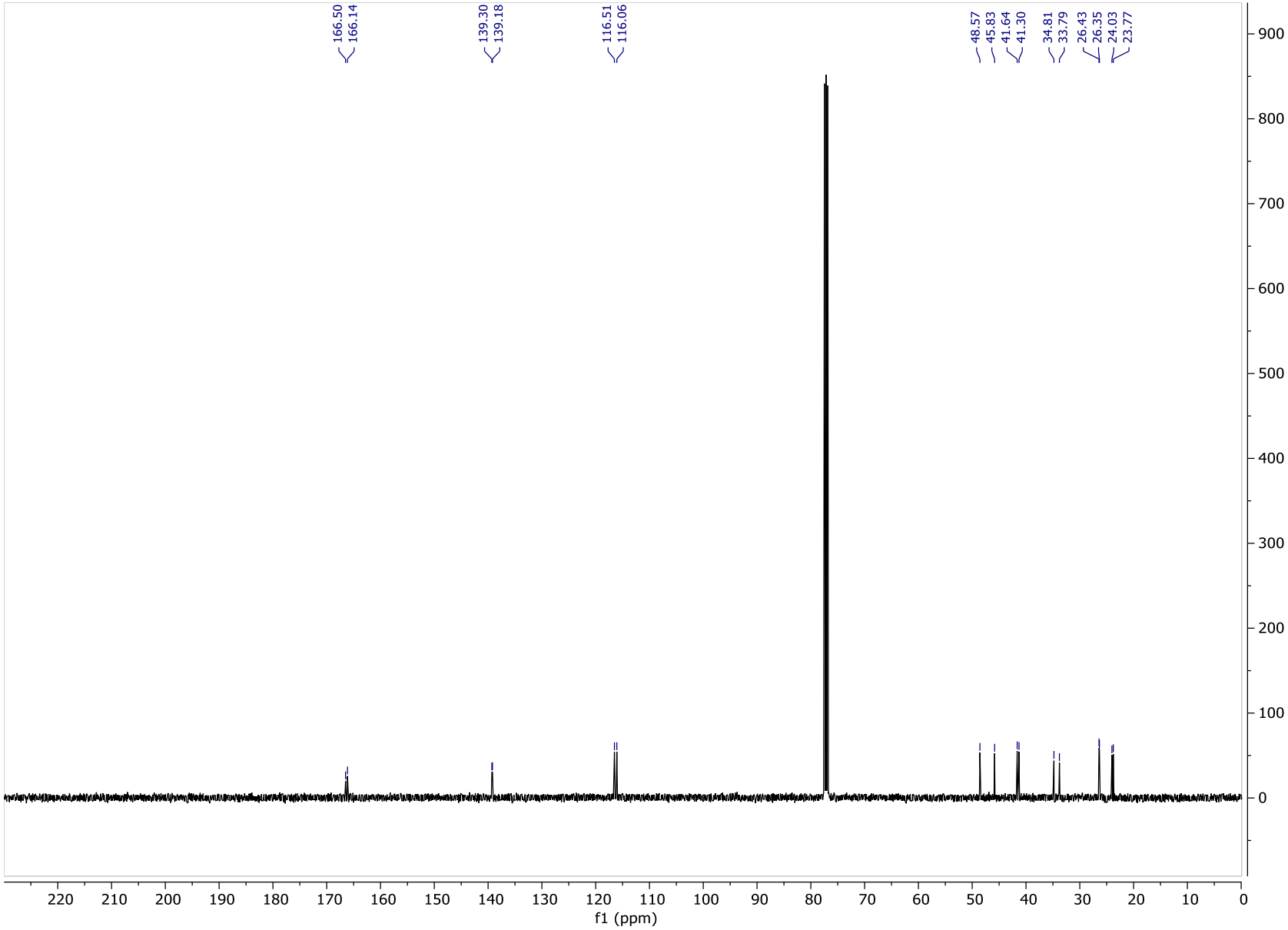

TMS
N
Me
O
Cl
9S
f1 (ppm)

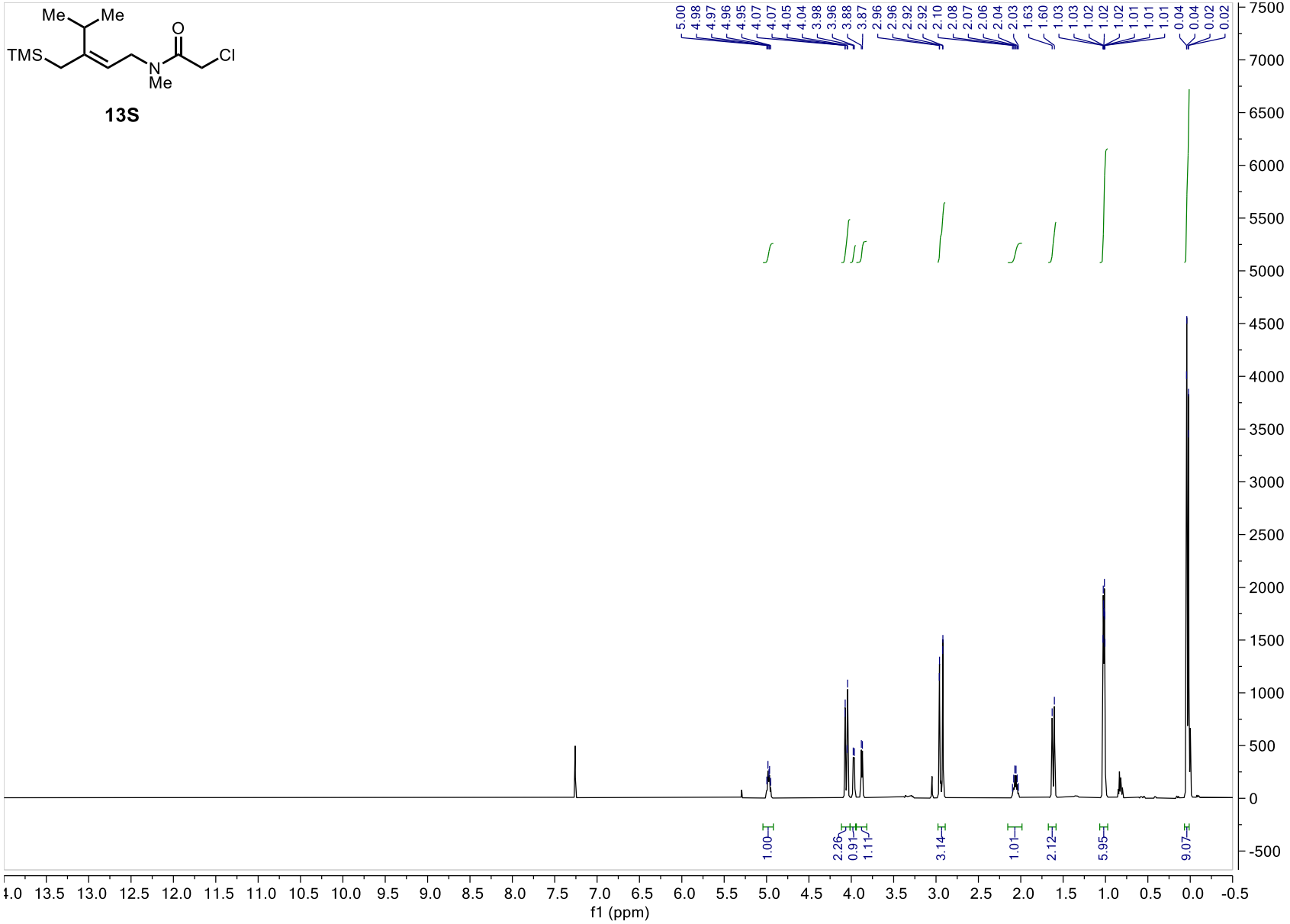

166.5
130.8
129.5
129.3
128.4
124.6
123.3
123.0
121.8
50.9
50.3
48.8
48.6
41.7
41.2
41.1
36.2
36.0
33.9
33.8
32.0
30.7
26.2
25.0
23.1
22.9
19.0
18.8
-1.8
f1 (ppm)

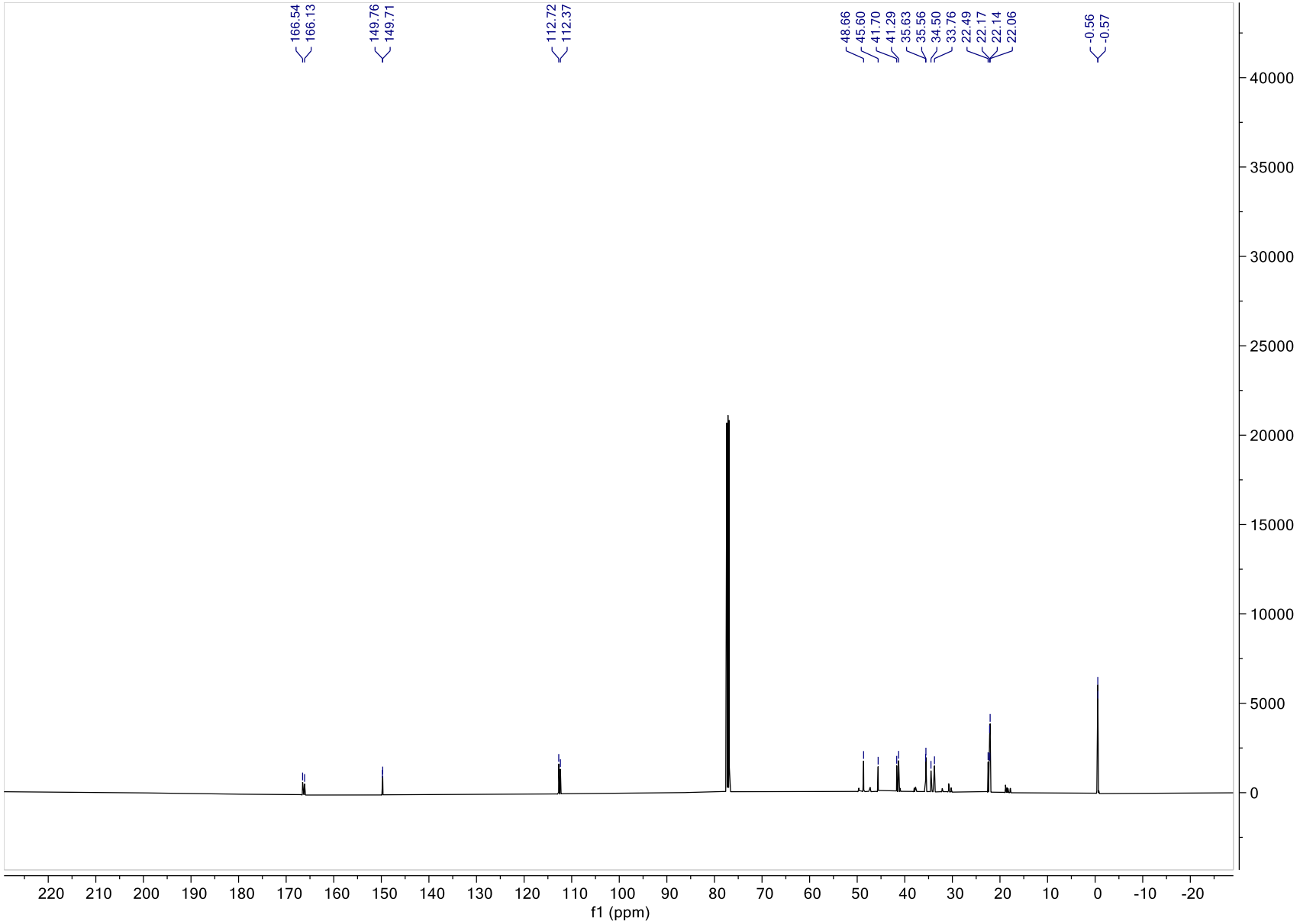

TMS
O
N
Me
Cl
10S
5.46 5.44 5.43 5.42 5.42 5.41 5.41 5.41 5.39 5.39 5.26 5.24 5.23 5.23 5.21 5.20 5.19 4.13 4.09 4.09 4.08 3.39 3.38 3.37 3.33 3.31 3.07 3.07 2.96 2.96 2.05 2.03 2.02 2.00 1.99 1.69 1.67 1.65 1.61 1.59 1.57 1.47 1.44 1.44 1.42 1.41 1.41 1.41 1.39 1.39 0.01 -0.00 -0.01 -0.02
1.00 2.23 2.07 3.01 1.95 1.87 1.98 8.70
f1 (ppm)


170.2
166.9
166.8
128.3
127.7
127.5
127.3
126.7
126.7
126.2
125.4
50.1
48.4
48.3
41.5
40.9
40.8
40.8
35.9
35.9
34.0
30.1
29.9
28.6
28.5
27.3
27.1
24.3
24.0
22.9
22.8
18.9
18.7
-1.8
-1.8
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


Me
O
TMS
Cl
N
Me
12S
5.01
5.01
5.00
5.00
4.07
4.05
3.94
3.93
3.86
3.85
3.00
2.93
1.74
1.74
1.73
1.73
1.72
1.72
1.62
1.57
0.06
0.04
1.00
2.19
1.02
1.12
3.18
3.17
2.28
9.07
f1 (ppm)


166.50
166.14
139.30
139.18
116.51
116.06
48.57
45.83
41.64
41.30
34.81
33.79
26.43
26.35
24.03
23.77
900
800
700
600
500
400
300
200
100
0
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Me
Me
TMS
O
Cl
N
Me
13S
f1 (ppm)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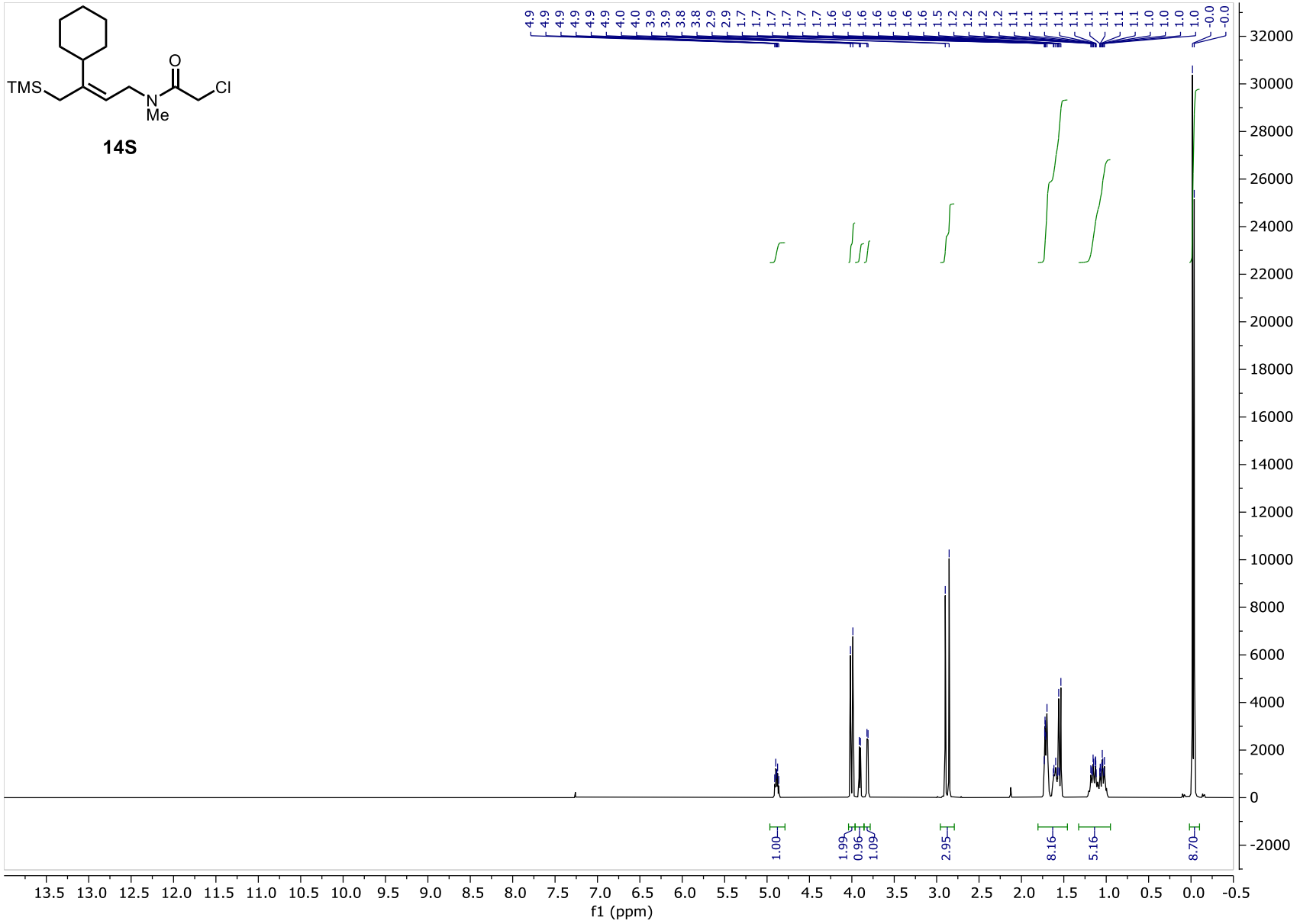
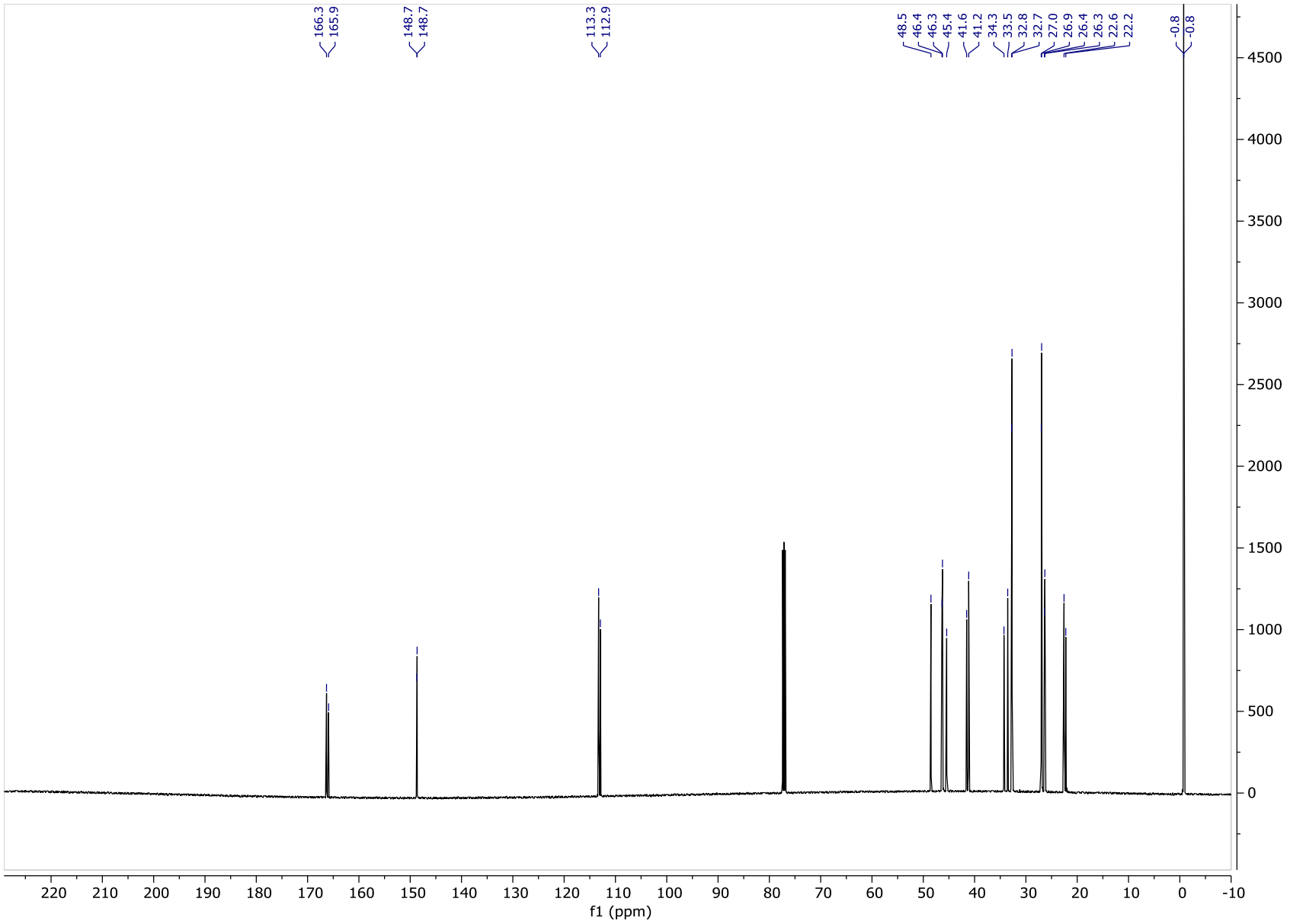
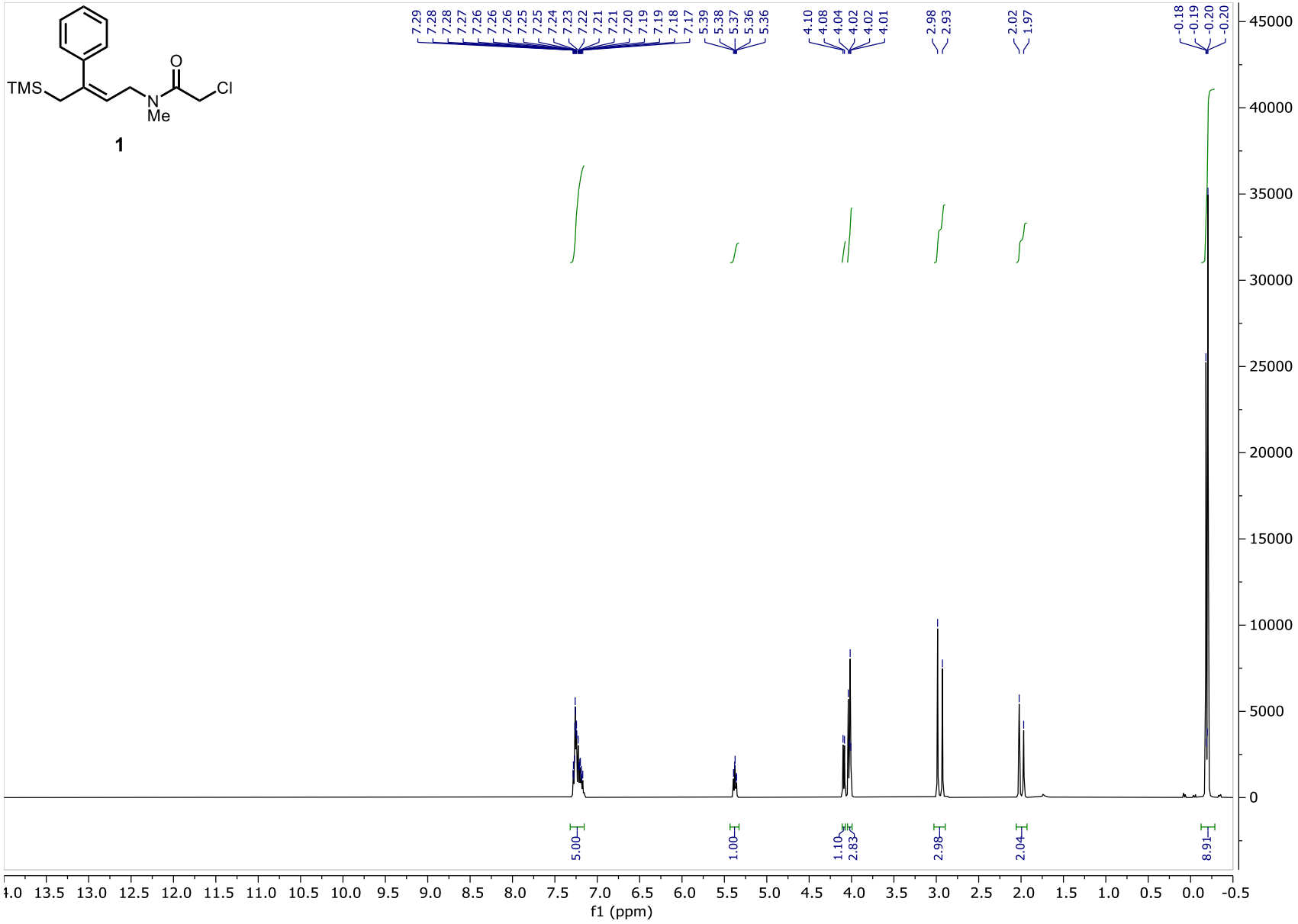
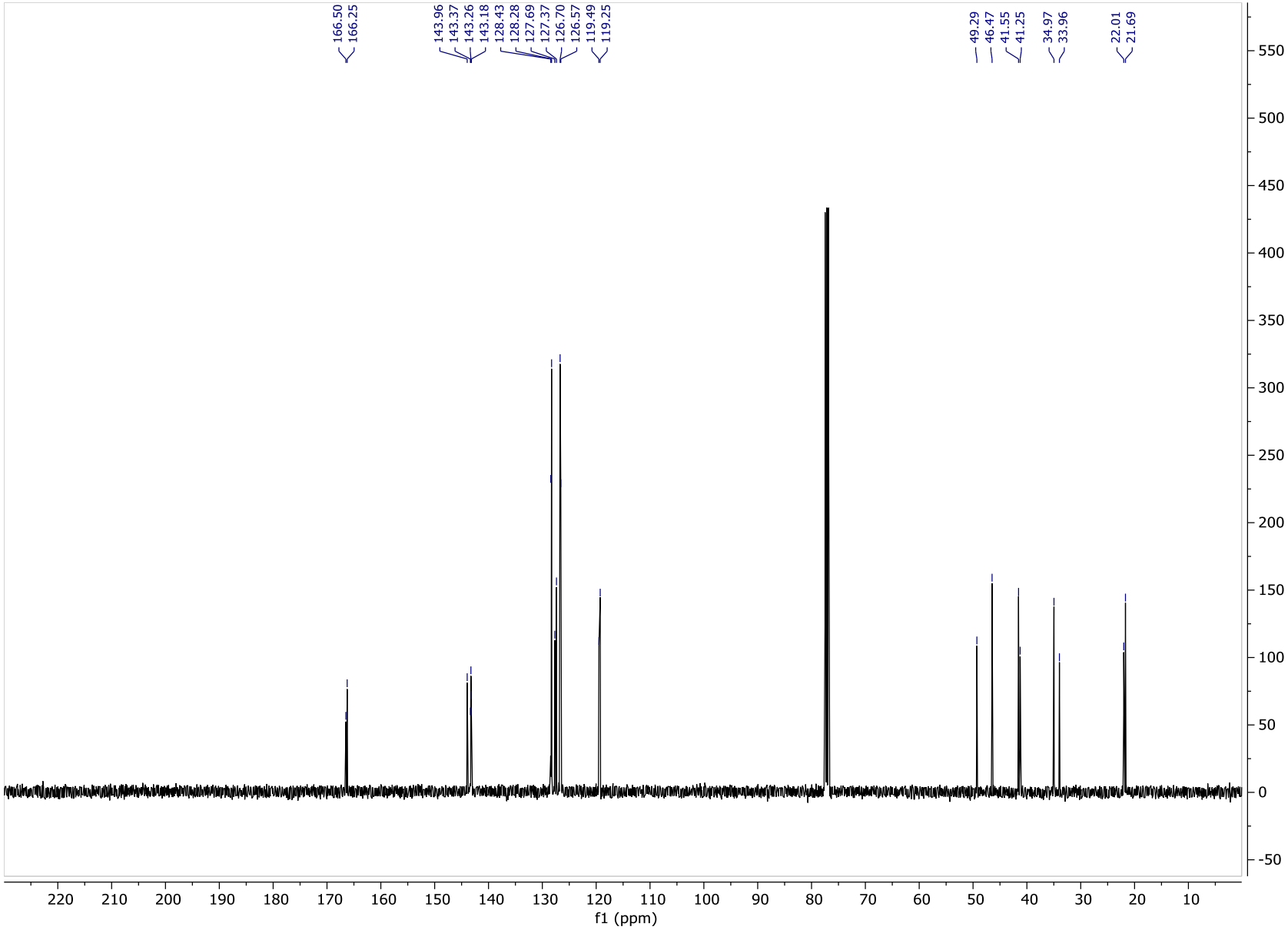
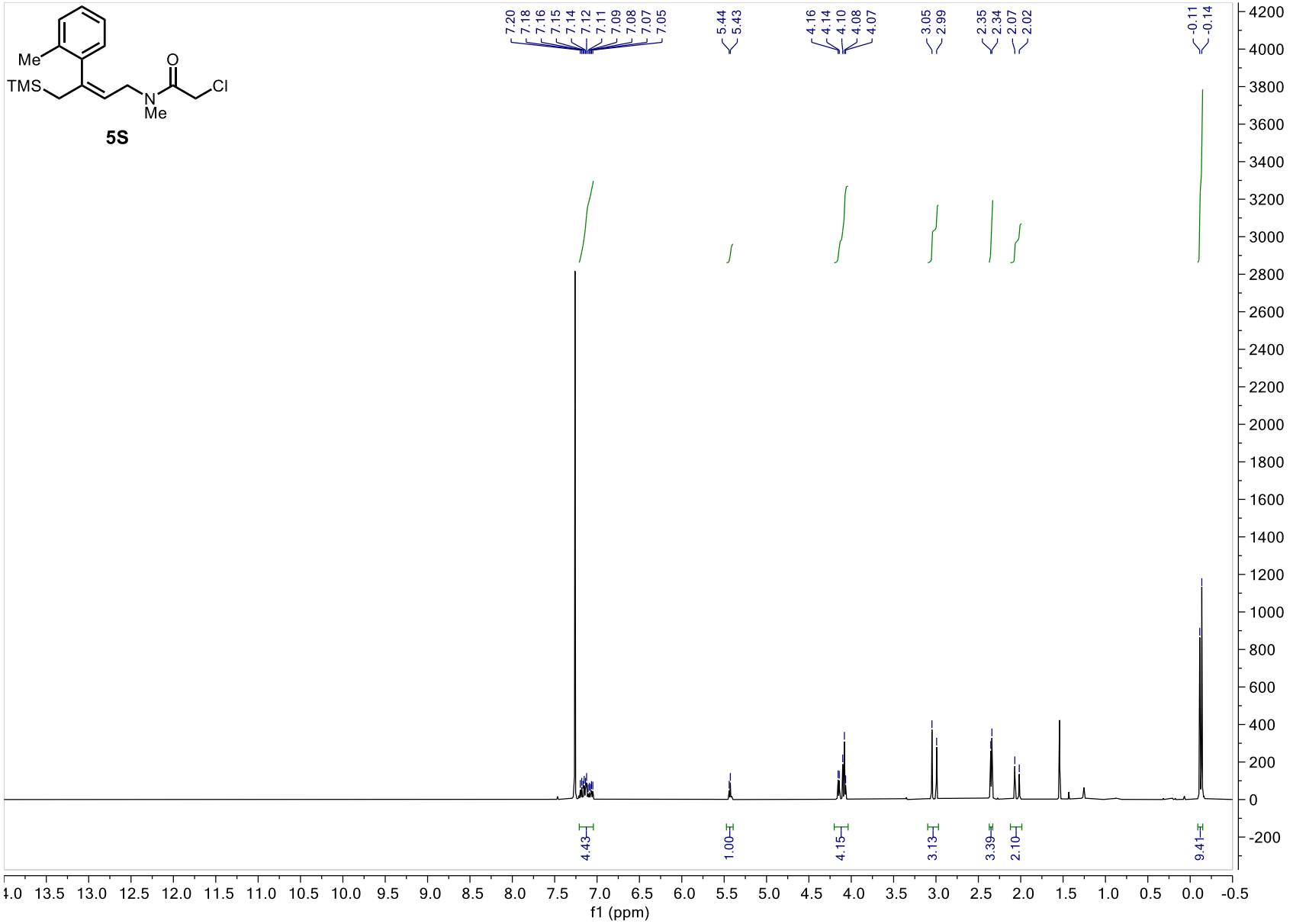
166.13
149.76
149.71
112.72
112.37
48.66
45.60
41.70
41.29
35.63
35.56
34.50
33.76
22.49
22.17
22.14
22.06
-0.56
-0.57
40000
35000
30000
25000
20000
15000
10000
5000
0
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

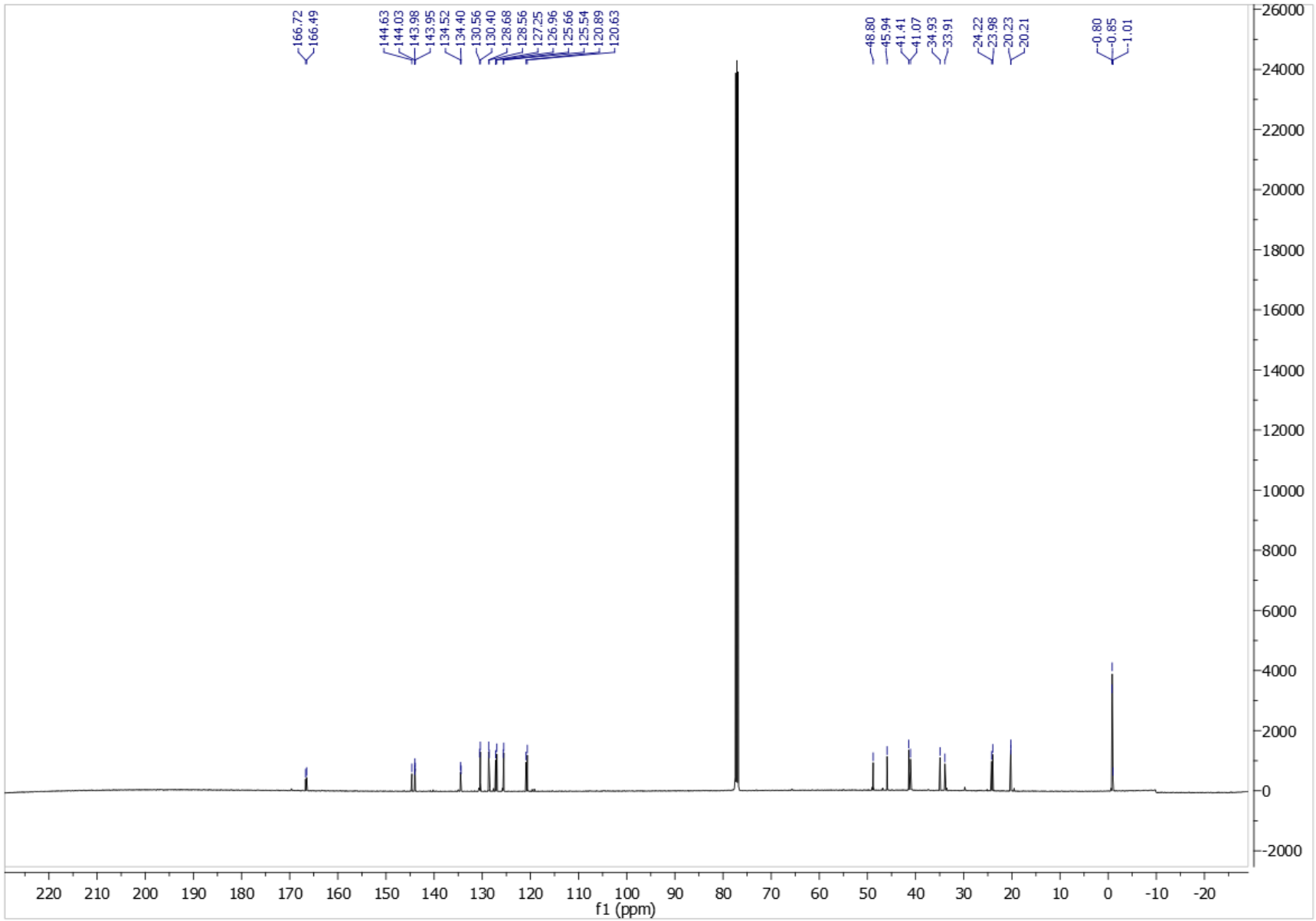

TMS
O
N
Me
Cl
14S
f1 (ppm)

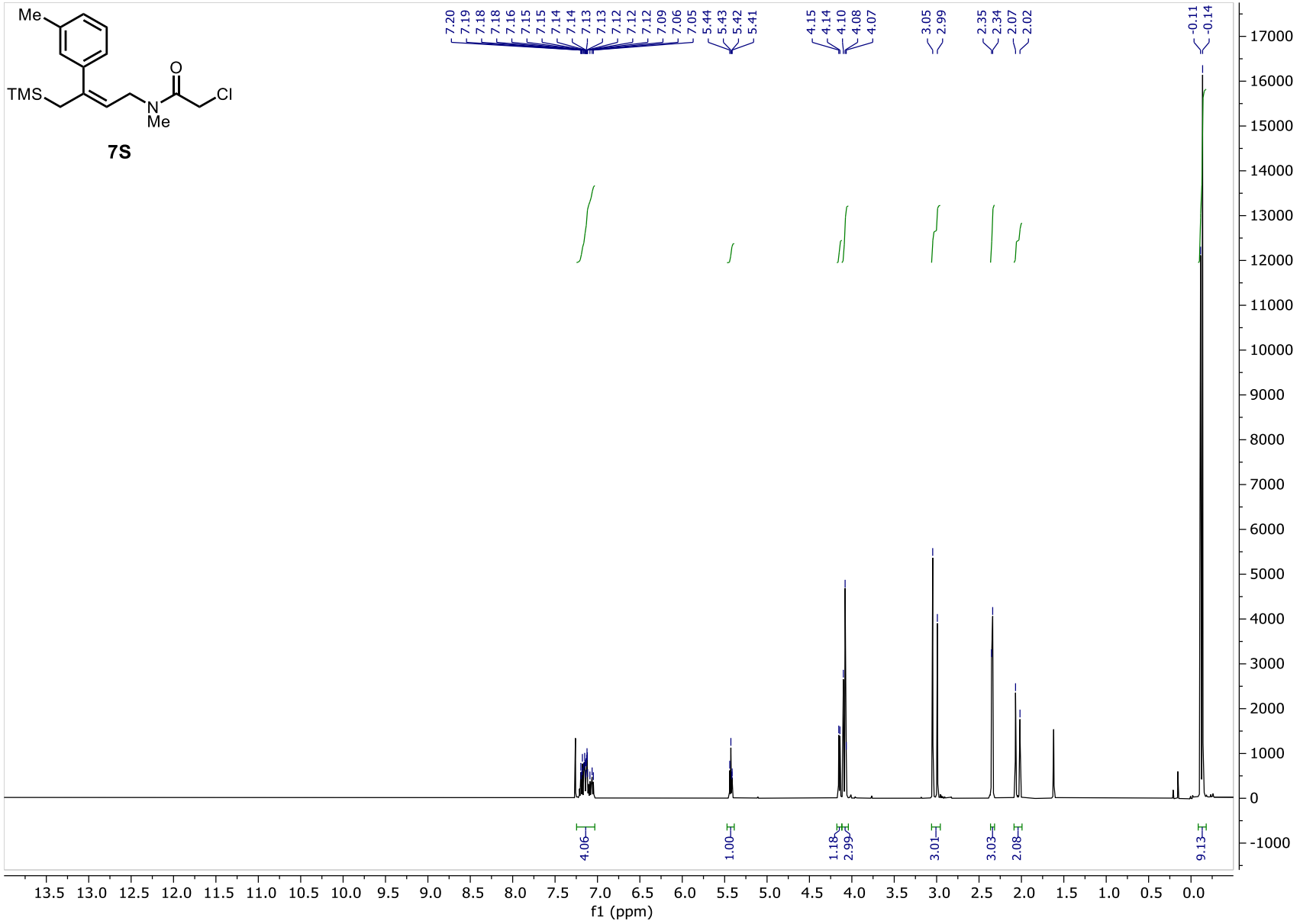

166.3
165.9
148.7
148.7
113.3
112.9
48.5
46.4
46.3
45.4
41.6
41.2
34.3
33.5
32.8
32.7
27.0
26.9
26.4
26.3
22.6
22.2
-0.8
-0.8
4500
4000
3500
3000
2500
2000
1500
1000
500
0
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

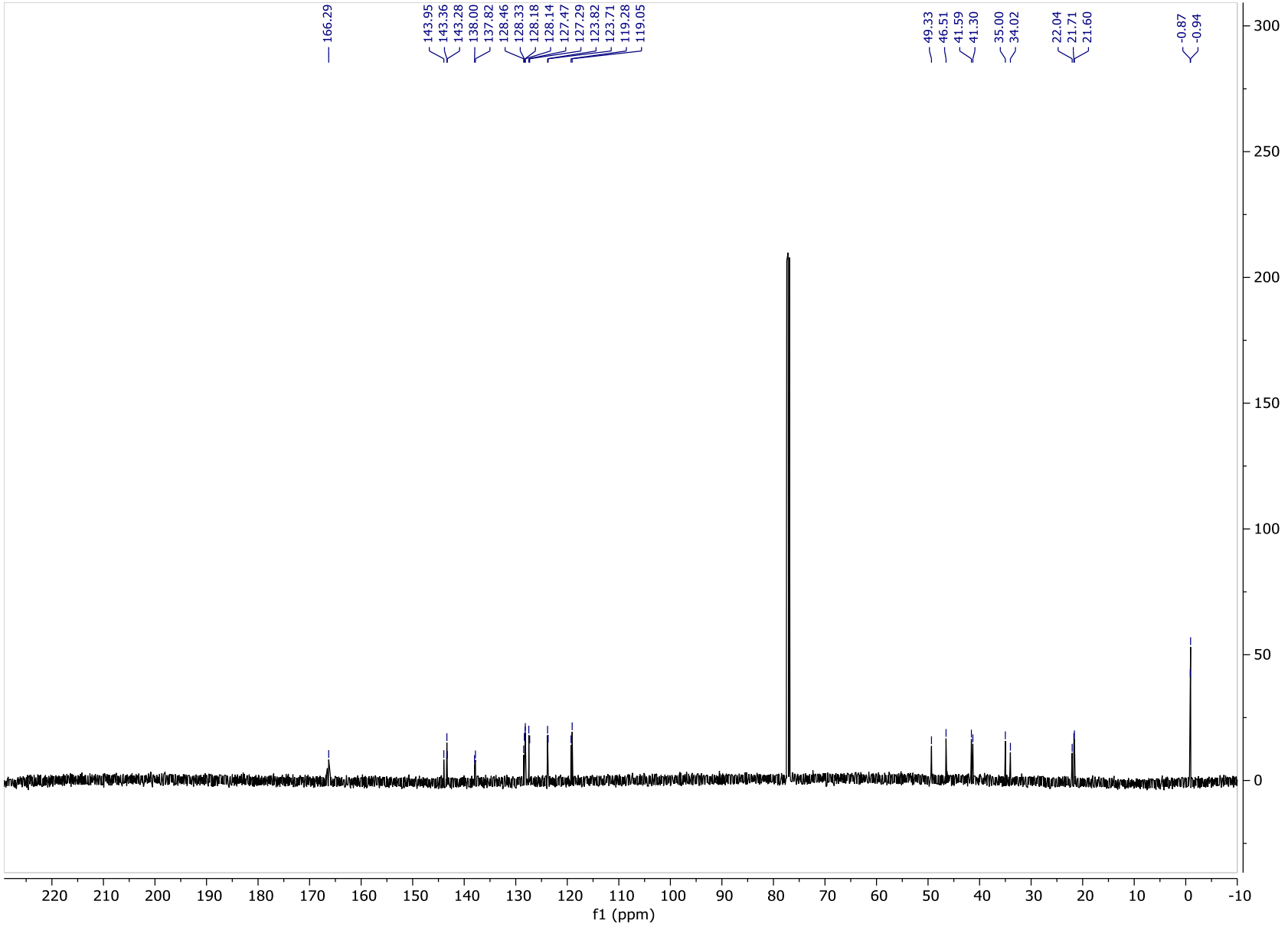

TMS
O
N
Me
Cl
1
7.29 7.28 7.28 7.27 7.26 7.26 7.26 7.25 7.25 7.24 7.23 7.22 7.21 7.21 7.20 7.19 7.19 7.18 7.17
5.39 5.38 5.37 5.36 5.36
4.10 4.08 4.04 4.02 4.02 4.01
2.98 2.93
2.02 1.97
-0.18 -0.19 -0.20 -0.20
5.00 1.00 1.10 2.83 2.98 2.04 8.91
45000 40000 35000 30000 25000 20000 15000 10000 5000 0
f1 (ppm)


166.50
166.25
143.96
143.37
143.26
143.18
128.43
128.28
127.69
127.37
126.70
126.57
119.49
119.25
49.29
46.47
41.55
41.25
34.97
33.96
22.01
21.69
550
500
450
400
350
300
250
200
150
100
50
0
-50
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


Me
TMS
O
N
Me
Cl
5S
7.20
7.18
7.16
7.15
7.14
7.12
7.11
7.09
7.08
7.07
7.05
5.44
5.43
4.16
4.14
4.10
4.08
4.07
3.05
2.99
2.35
2.34
2.07
2.02
-0.11
-0.14
4.43
1.00
4.15
3.13
3.39
2.10
9.41
f1 (ppm)


166.72
166.49
144.63
144.03
143.98
143.95
134.52
134.40
130.56
130.40
128.68
128.56
127.25
126.96
125.66
125.54
120.89
120.63
48.80
45.94
41.41
41.07
34.93
33.91
24.22
23.98
20.23
20.21
0.80
0.85
-1.01
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


Me
TMS
O
N
Me
Cl
7S
7.20
7.19
7.18
7.18
7.16
7.15
7.15
7.14
7.14
7.13
7.13
7.12
7.12
7.09
7.06
7.05
5.44
5.43
5.42
5.41
4.15
4.14
4.10
4.08
4.07
3.05
2.99
2.35
2.34
2.07
2.02
-0.11
-0.14
4.06
1.00
1.18
2.99
3.01
3.03
2.08
9.13
f1 (ppm)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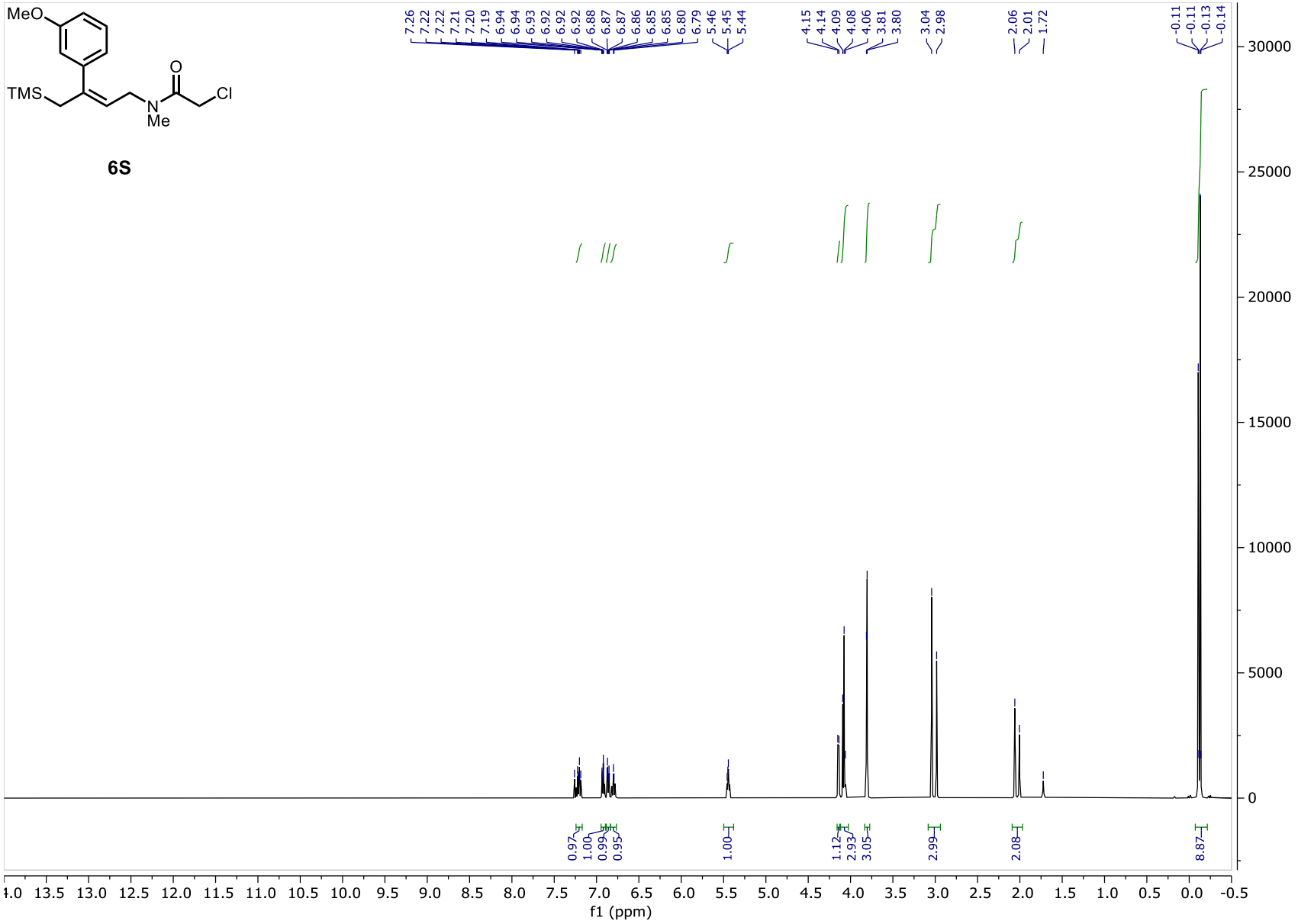
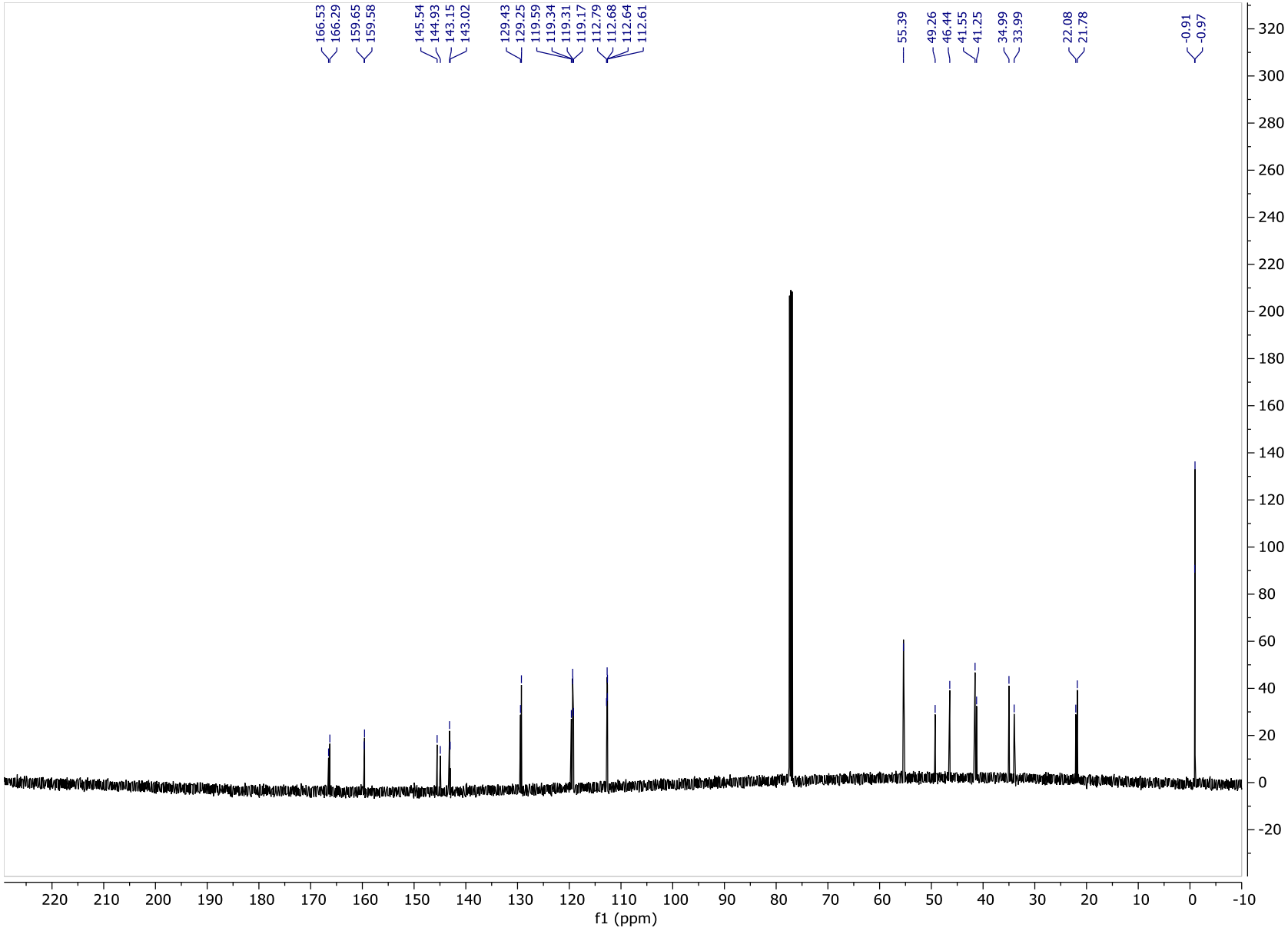
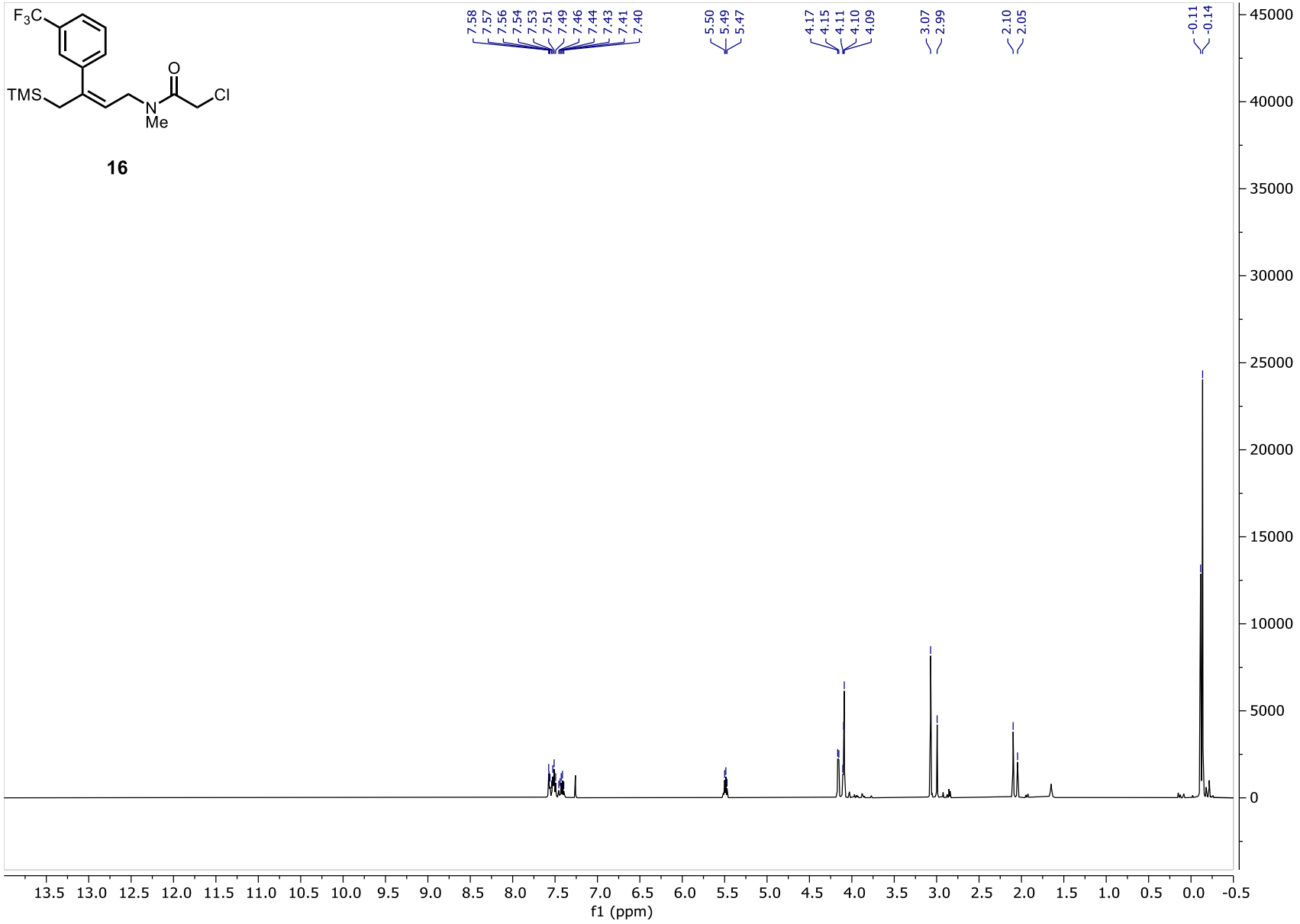
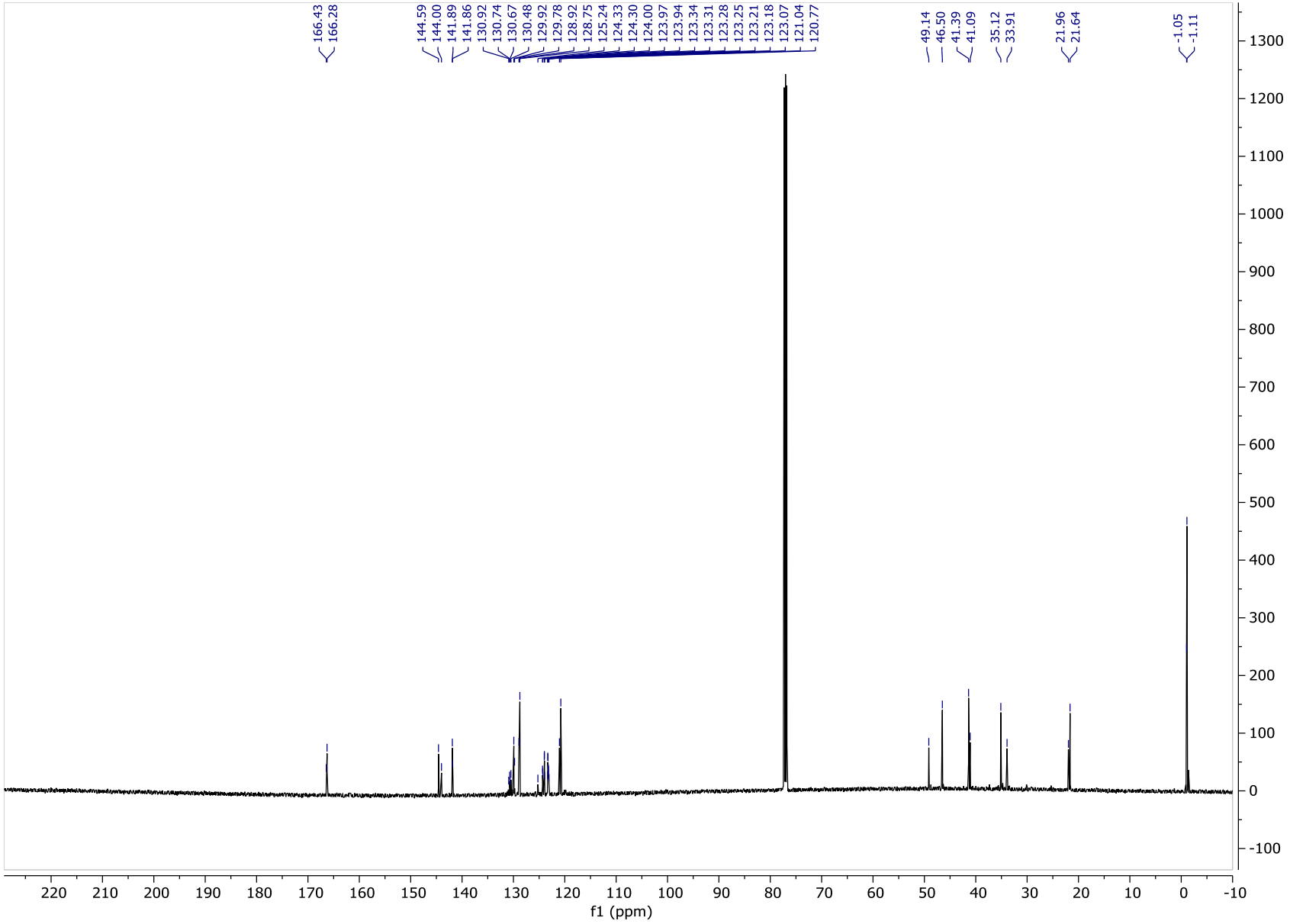
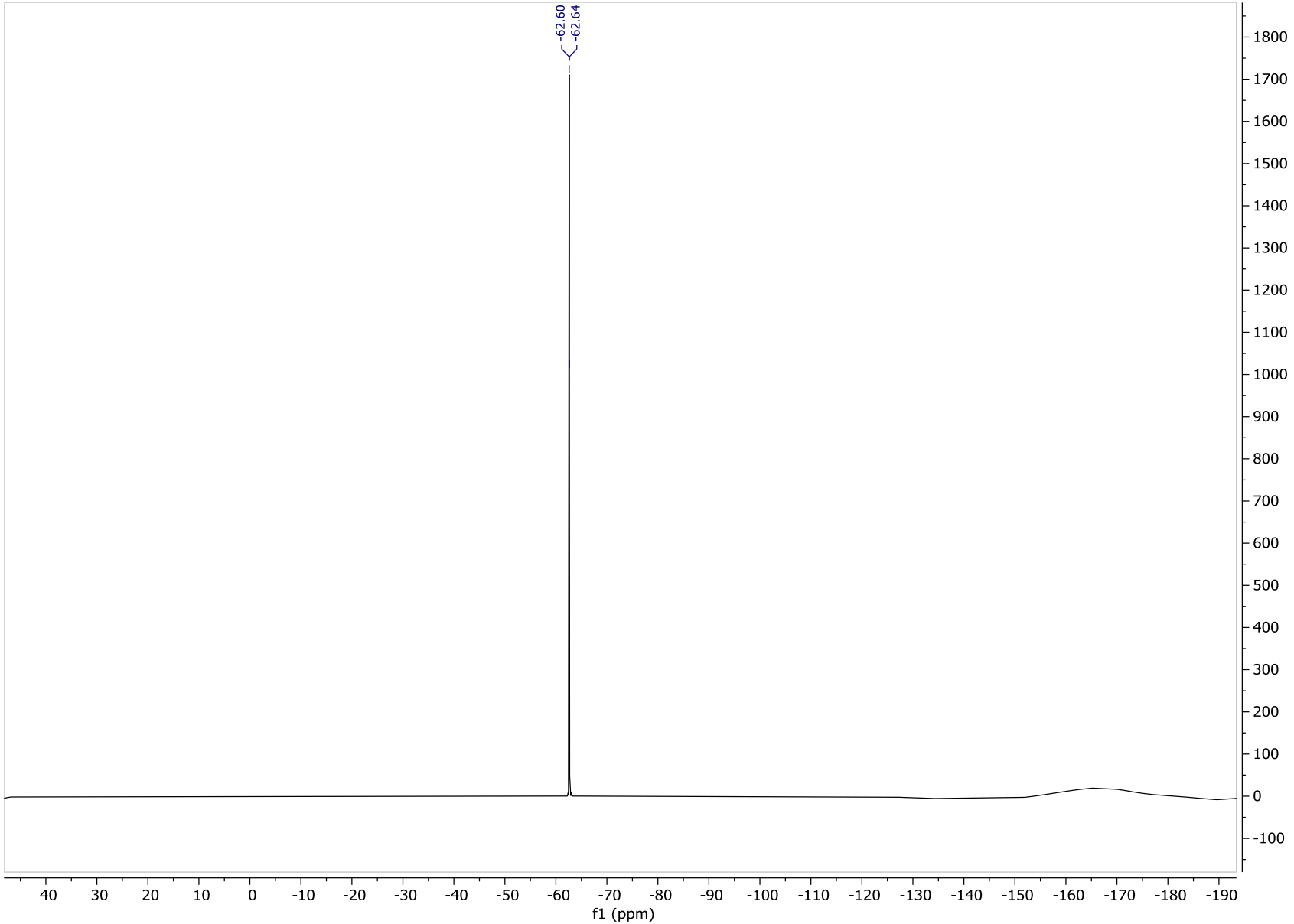
143.36
143.28
138.00
137.82
128.46
128.33
128.18
128.14
127.47
127.29
123.82
123.71
119.28
119.05
49.33
46.51
41.59
41.30
35.00
34.02
22.04
21.71
21.60
-0.87
-0.94
300
250
200
150
100
50
0
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

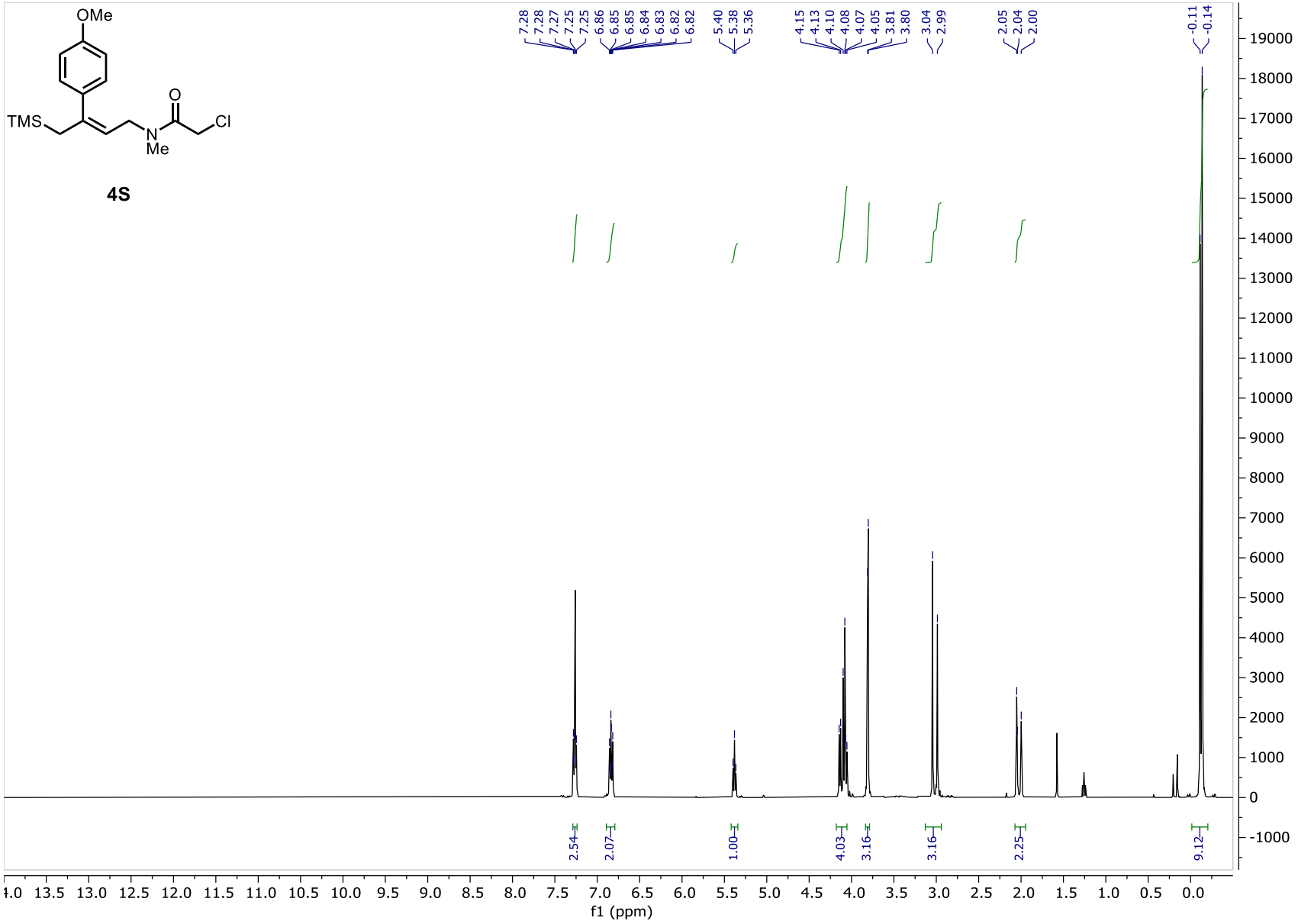

MeO
TMS
O
N
Me
Cl
6S
7.26
7.22
7.22
7.21
7.20
7.19
6.94
6.94
6.93
6.92
6.92
6.92
6.88
6.87
6.87
6.86
6.85
6.85
6.80
6.79
5.46
5.45
5.44
4.15
4.14
4.09
4.08
4.06
3.81
3.80
3.04
2.98
2.06
2.01
1.72
-0.11
-0.11
-0.13
-0.14
30000
25000
20000
15000
10000
5000
0
0.97
1.00
0.99
0.95
1.00
1.12
2.93
3.05
2.99
2.08
8.87
f1 (ppm)

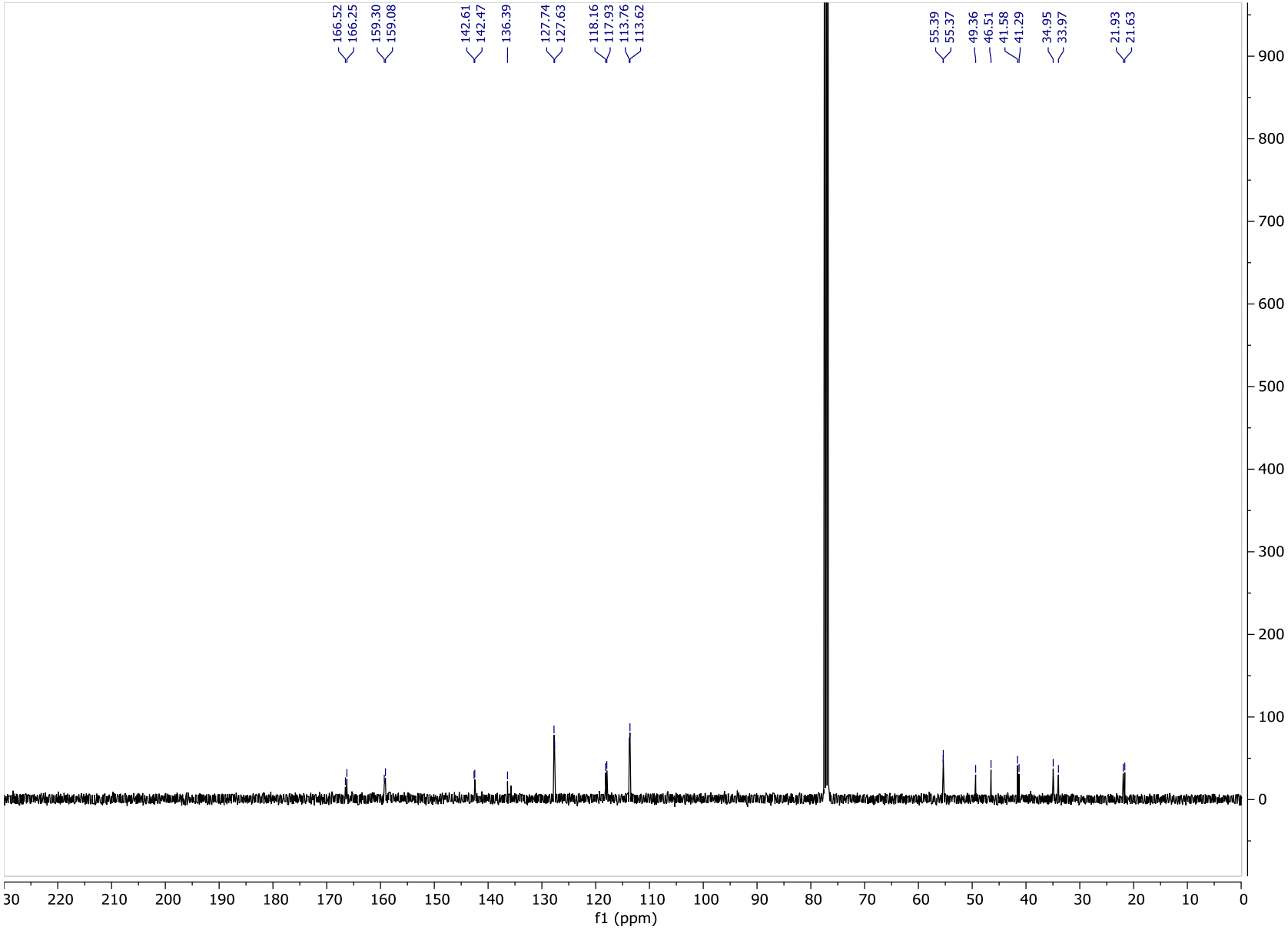

166.53
166.29
159.65
159.58
145.54
144.93
143.15
143.02
129.43
129.25
119.59
119.34
119.31
119.17
112.79
112.68
112.64
112.61
55.39
49.26
46.44
41.55
41.25
34.99
33.99
22.08
21.78
-0.91
-0.97
f1 (ppm)


F3C
TMS
N
Me
O
Cl
16
7.58
7.57
7.56
7.54
7.53
7.51
7.49
7.46
7.44
7.43
7.41
7.40
5.50
5.49
5.47
4.17
4.15
4.11
4.10
4.09
3.07
2.99
2.10
2.05
-0.11
-0.14
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
13.5 13.0 12.5 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
f1 (ppm)


166.43
166.28
144.59
144.00
141.89
141.86
130.92
130.74
130.67
130.48
129.92
129.78
128.92
128.75
125.24
124.33
124.30
124.00
123.97
123.94
123.34
123.31
123.28
123.25
123.21
123.18
123.07
121.04
120.77
49.14
46.50
41.39
41.09
35.12
33.91
21.96
21.64
1.05
-1.11
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


-62.60
-62.64
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190
f1 (ppm)


OMe
TMS
O
N
Me
Cl
4S
7.28
7.28
7.27
7.25
7.25
6.86
6.85
6.85
6.84
6.83
6.82
6.82
5.40
5.38
5.36
4.15
4.13
4.10
4.08
4.07
4.05
3.81
3.80
3.04
2.99
2.05
2.04
2.00
-0.11
-0.14
2.54
2.07
1.00
4.03
3.16
3.16
2.25
9.12
f1 (ppm)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166.25
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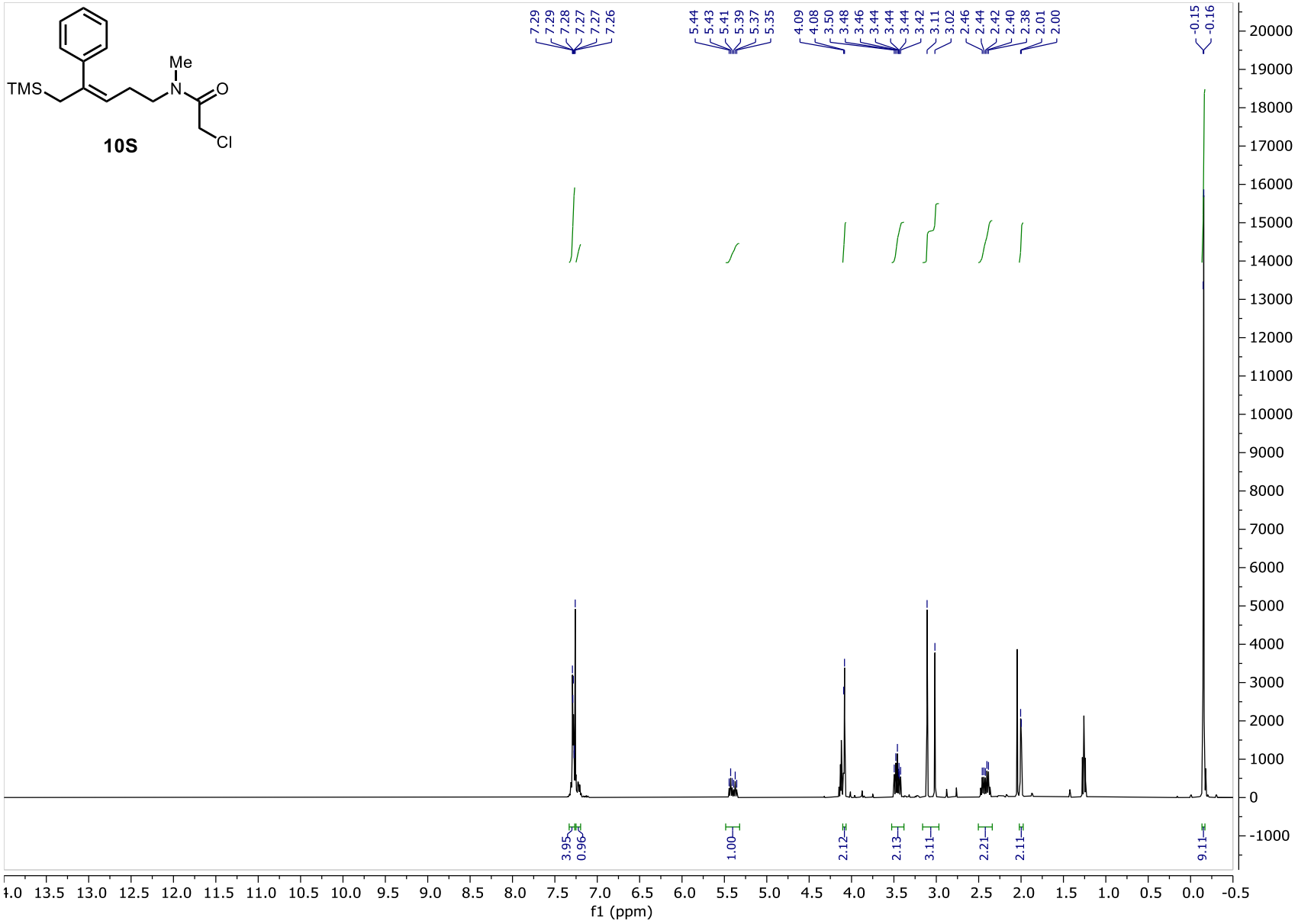
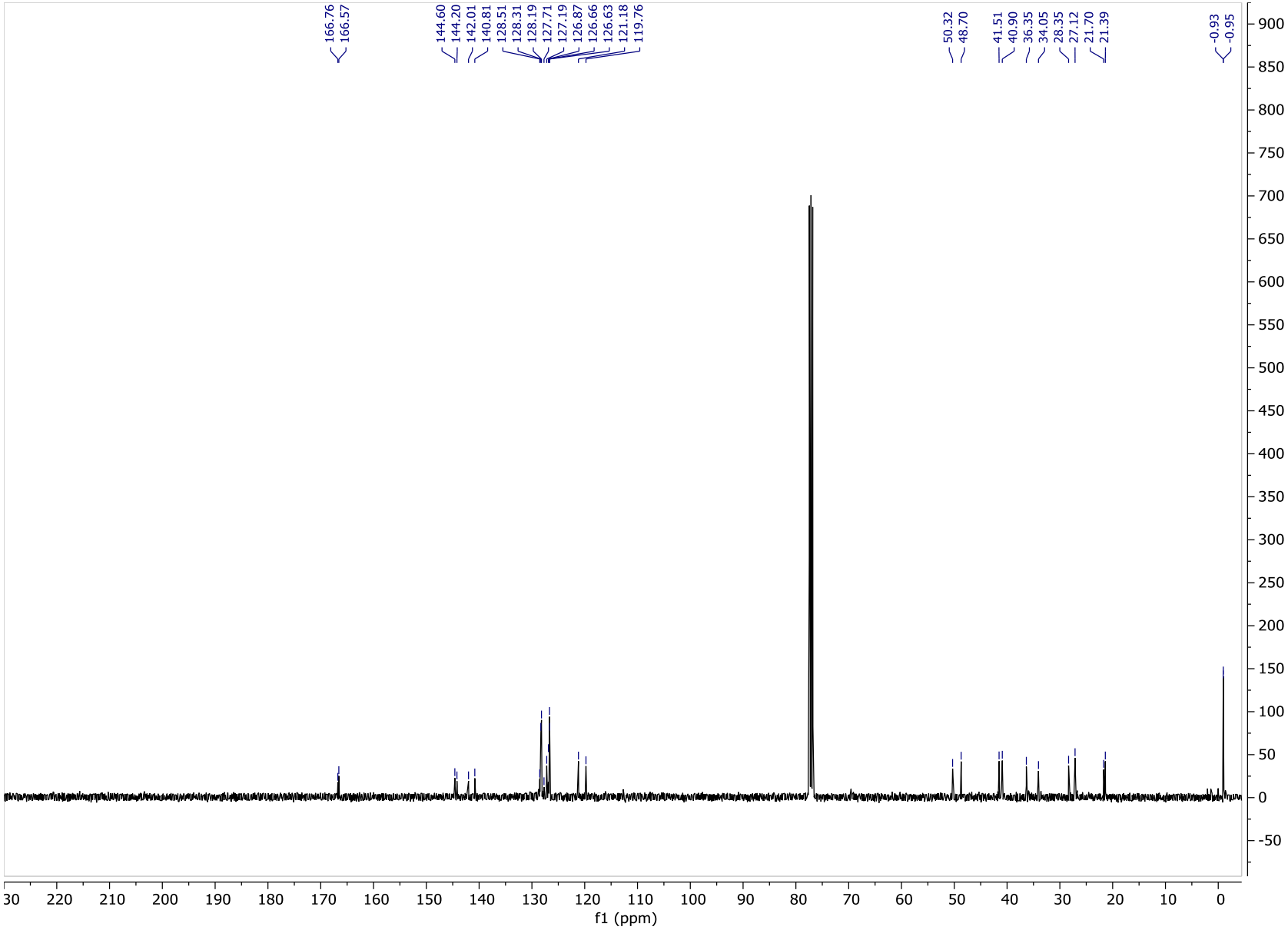
159.08
142.61
142.47
136.39
127.74
127.63
118.16
117.93
113.76
113.62
55.39
55.37
49.36
46.51
41.58
41.29
34.95
33.97
21.93
21.63
900
800
700
600
500
400
300
200
100
0
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


TMS
Me
N
O
Cl
10S
7.29
7.29
7.28
7.27
7.27
7.26
5.44
5.43
5.41
5.39
5.37
5.35
4.09
4.08
3.50
3.48
3.46
3.44
3.44
3.44
3.42
3.11
3.02
2.46
2.44
2.42
2.40
2.38
2.01
2.00
-0.15
-0.16
3.95
0.96
1.00
2.12
2.13
3.11
2.21
2.11
9.11
f1 (ppm)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128.31
128.19
127.71
127.19
126.87
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126.63
121.18
119.76
50.32
48.70
41.51
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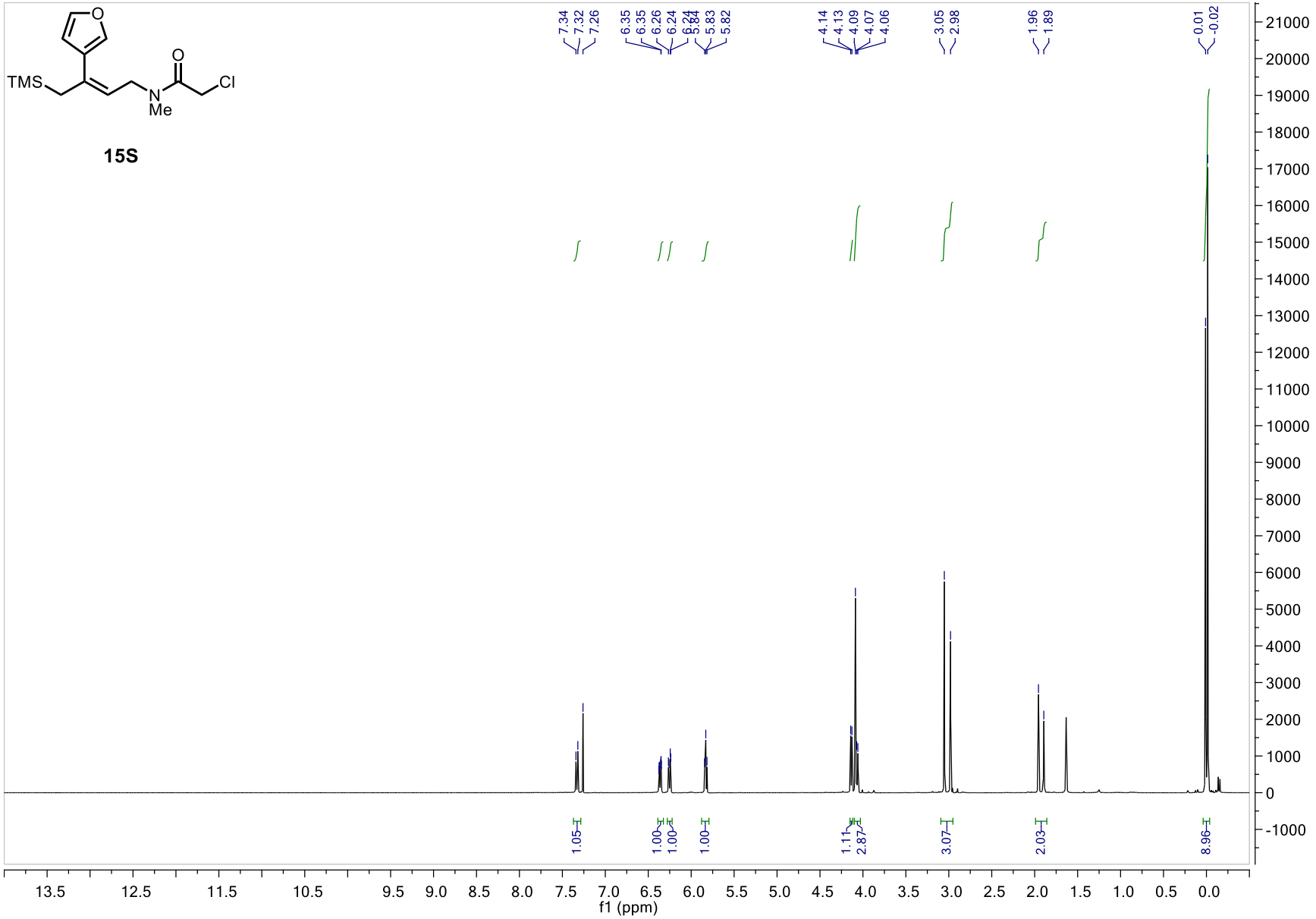
36.35
34.05
28.35
27.12
21.70
21.39
-0.93
-0.95
f1 (ppm)


TMS
O
N
Me
O
Cl
15S
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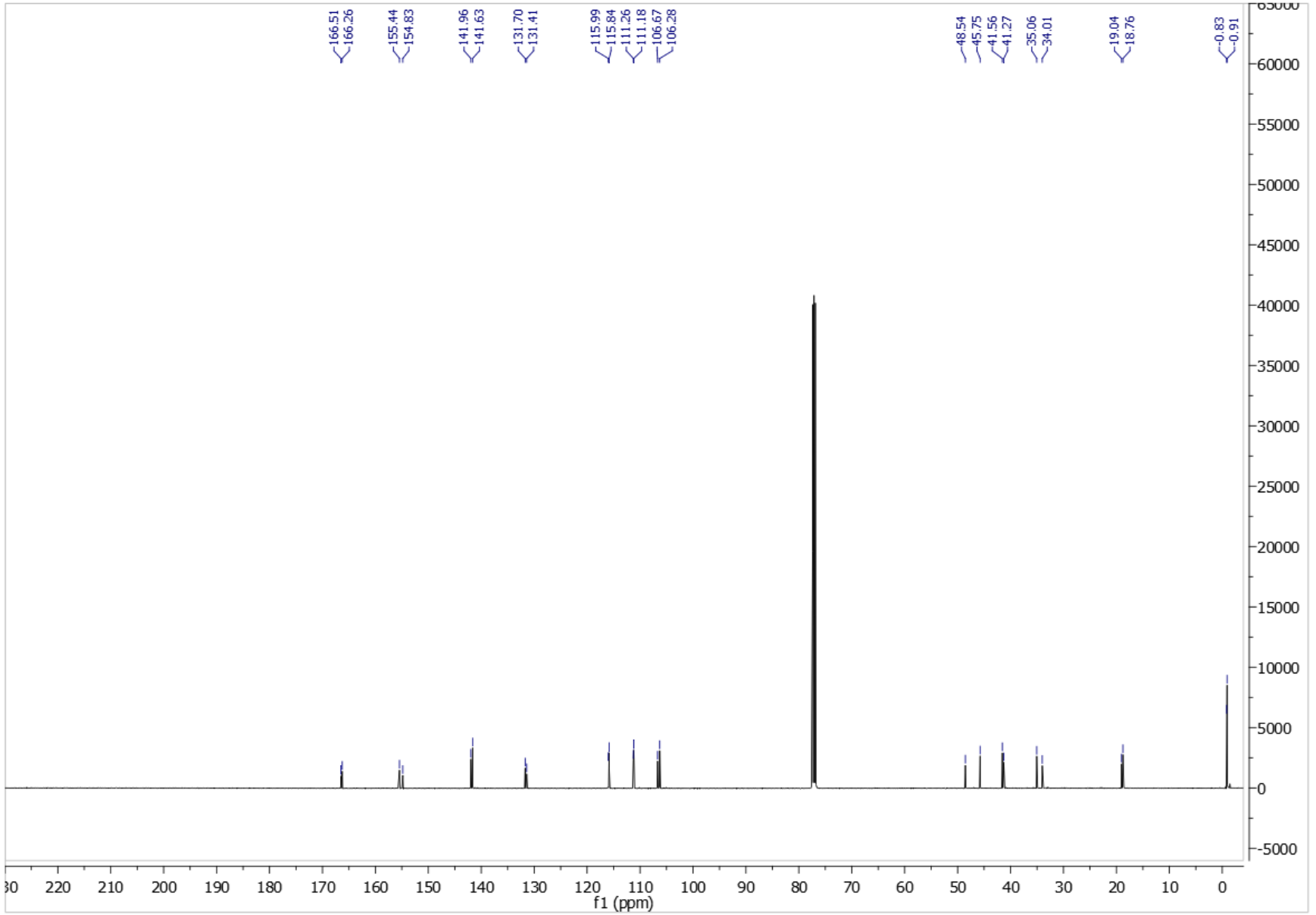
7.32
7.26
6.35
6.35
6.26
6.24
6.24
5.84
5.83
5.82
4.14
4.13
4.09
4.07
4.06
3.05
2.98
1.96
1.89
0.01
-0.02
1.05
1.00
1.00
1.00
1.11
2.87
3.07
2.03
8.96
f1 (ppm)


166.51
166.26
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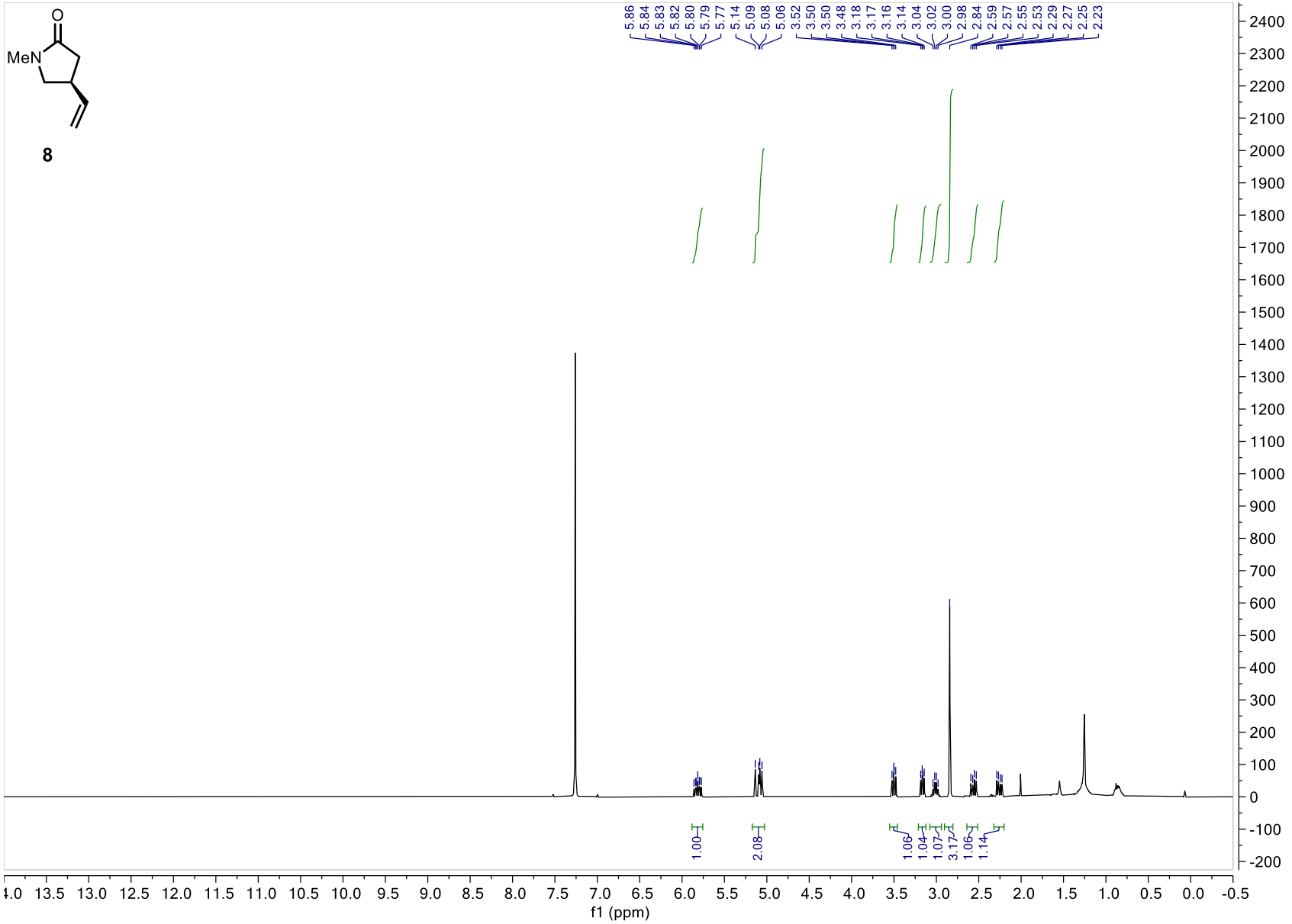
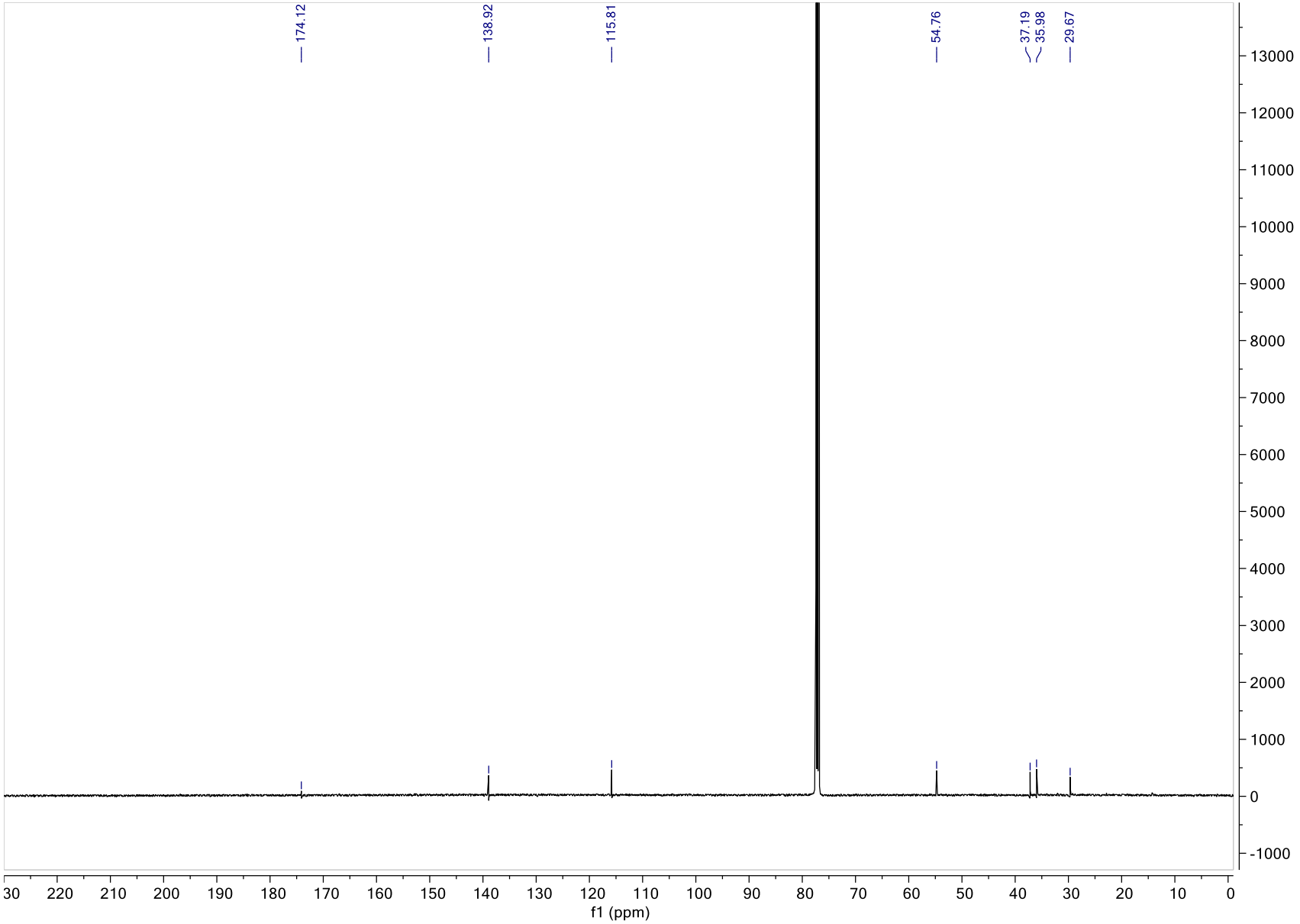
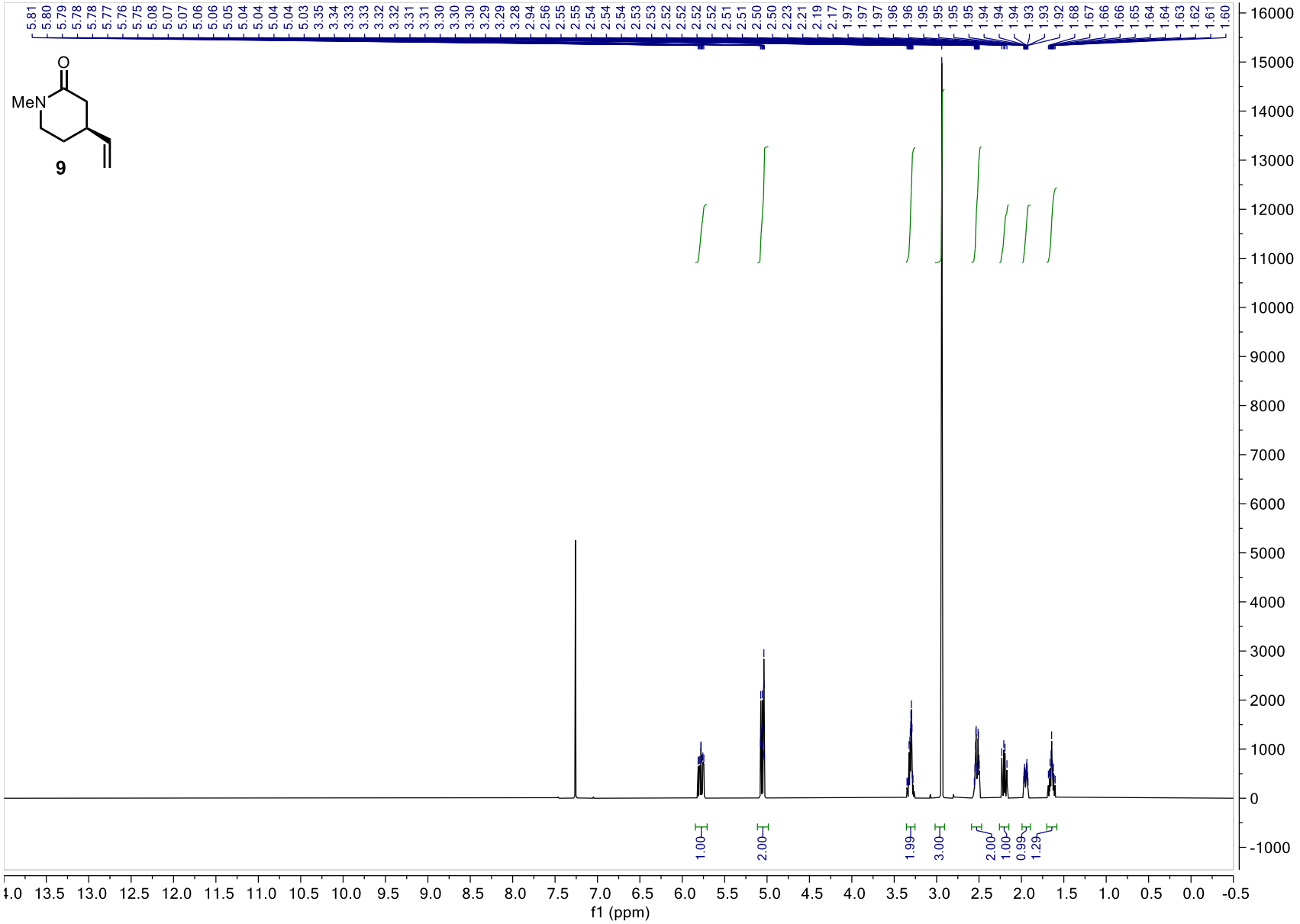
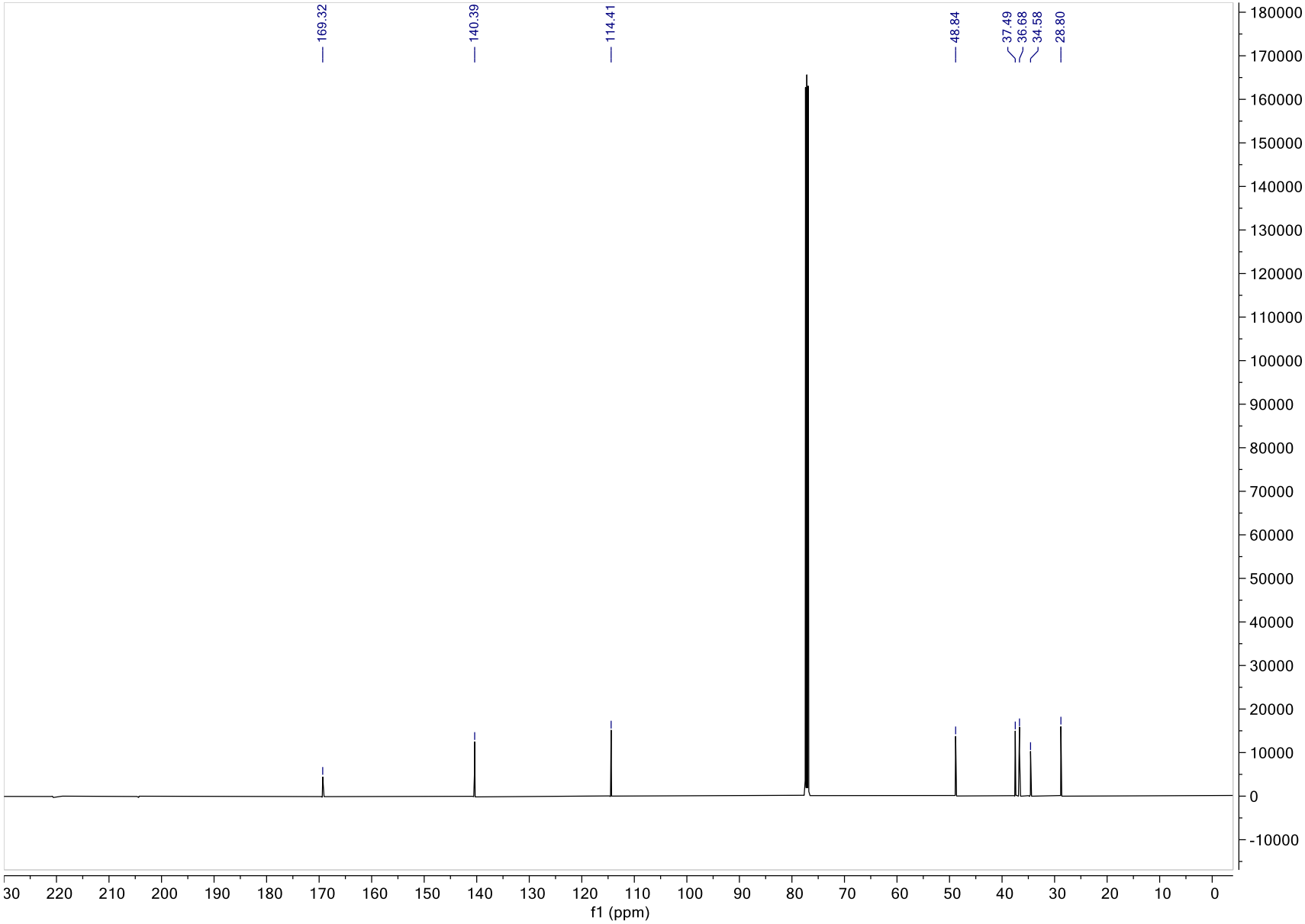
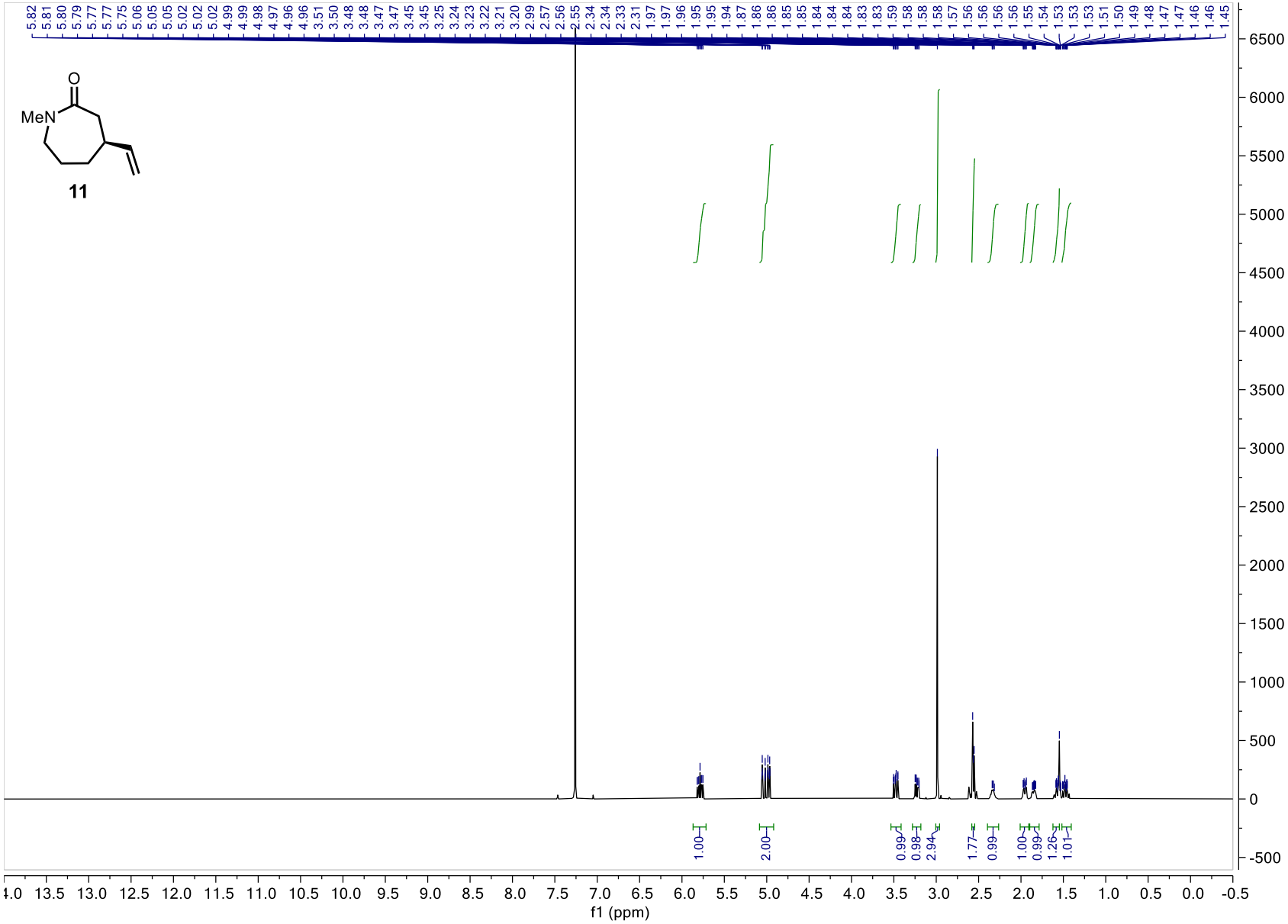
154.83
141.96
141.63
131.70
131.41
115.99
115.84
111.26
111.18
106.67
106.28
48.54
45.75
41.56
41.27
35.06
34.01
19.04
18.76
-0.83
-0.91
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

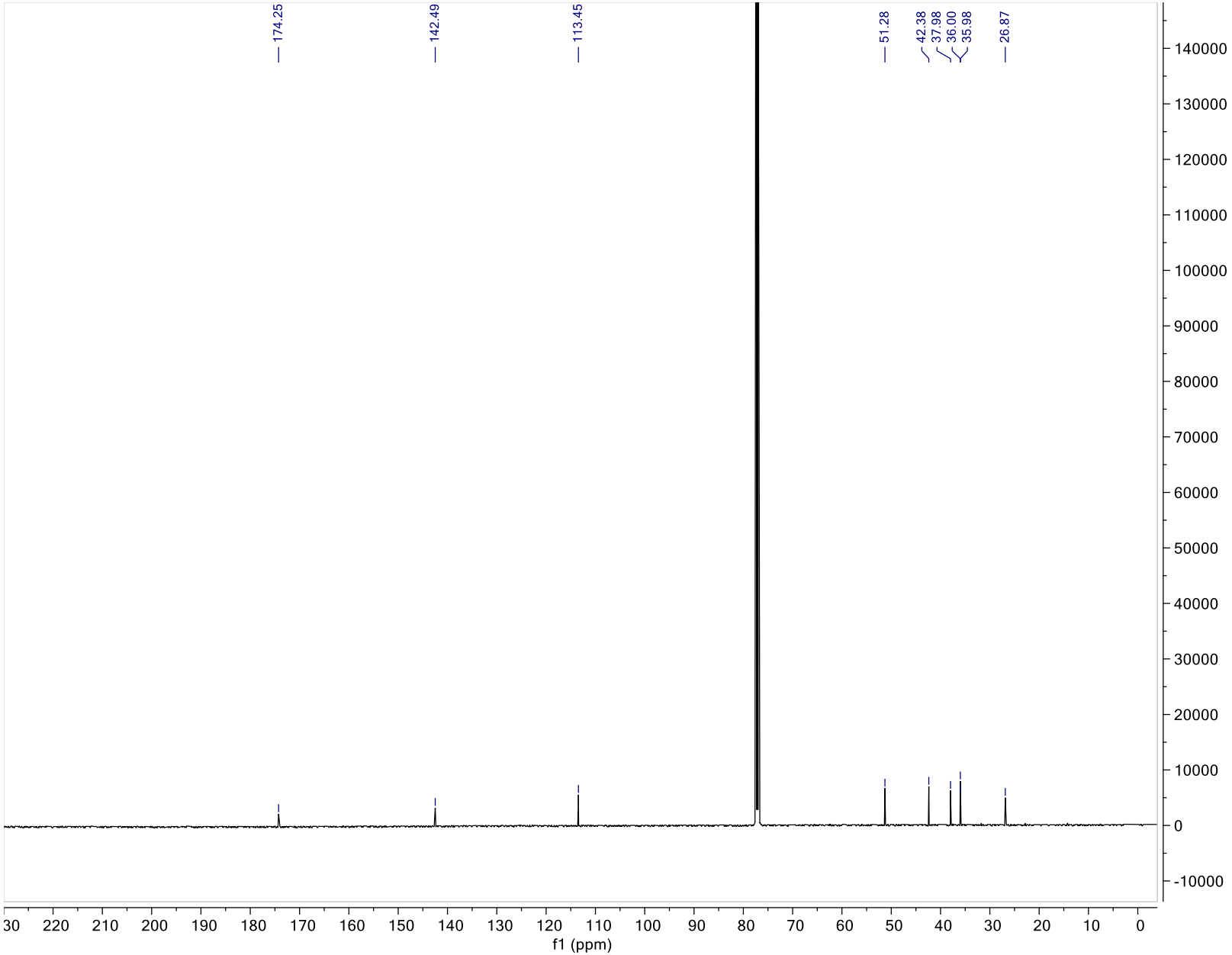

O
MeN
8
5.86
5.84
5.83
5.82
5.80
5.79
5.77
5.14
5.09
5.08
5.06
3.52
3.50
3.50
3.48
3.18
3.17
3.16
3.14
3.04
3.02
3.00
2.98
2.84
2.59
2.57
2.55
2.53
2.29
2.27
2.25
2.23
1.00
2.08
1.06
1.04
1.07
3.17
1.06
1.14
f1 (ppm)


174.12
138.92
115.81
54.76
37.19
35.98
29.67
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
MeN
9
f1 (ppm)


169.32
140.39
114.41
48.84
37.49
36.68
34.58
28.80
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeN
O
11
f1 (ppm)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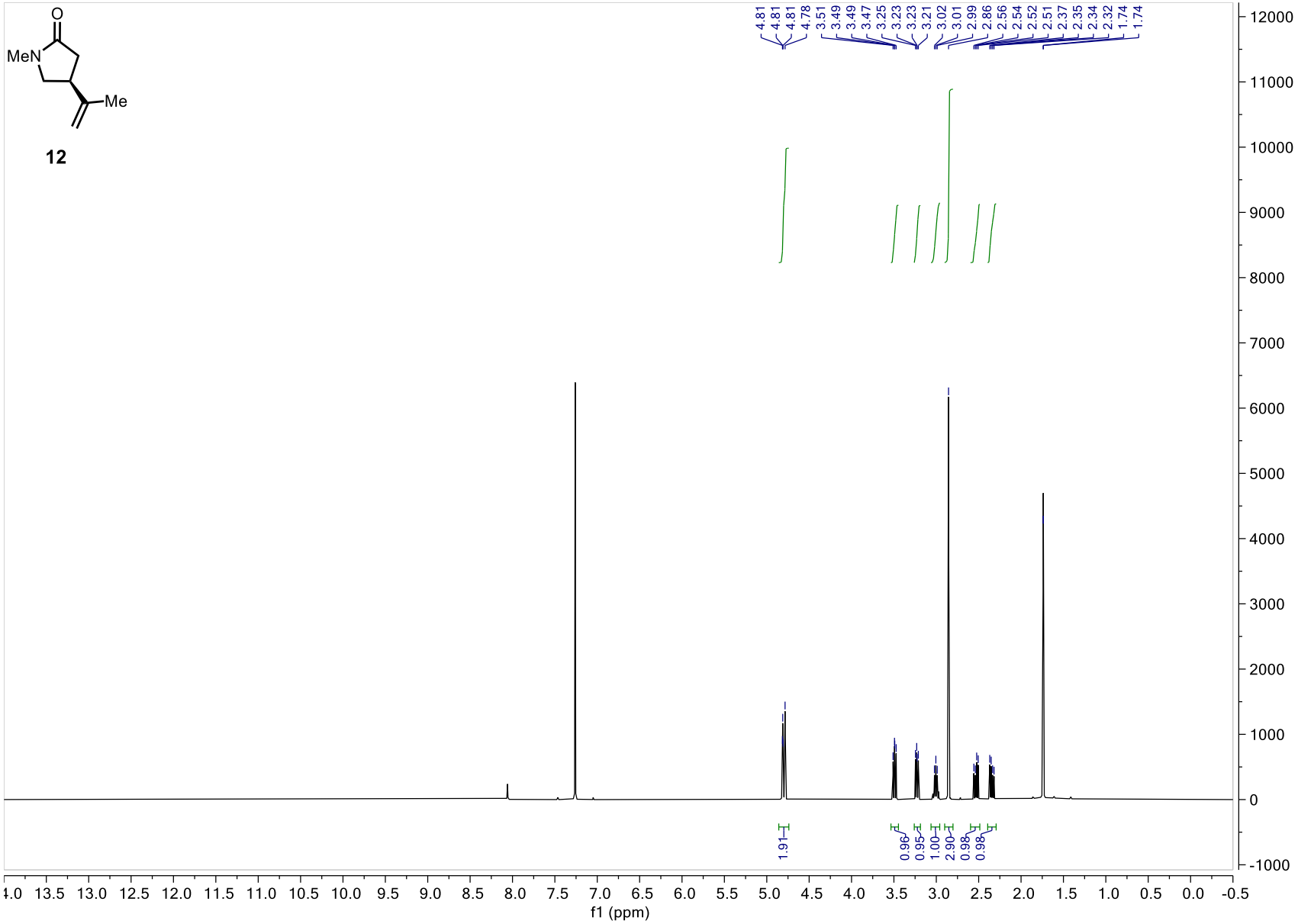
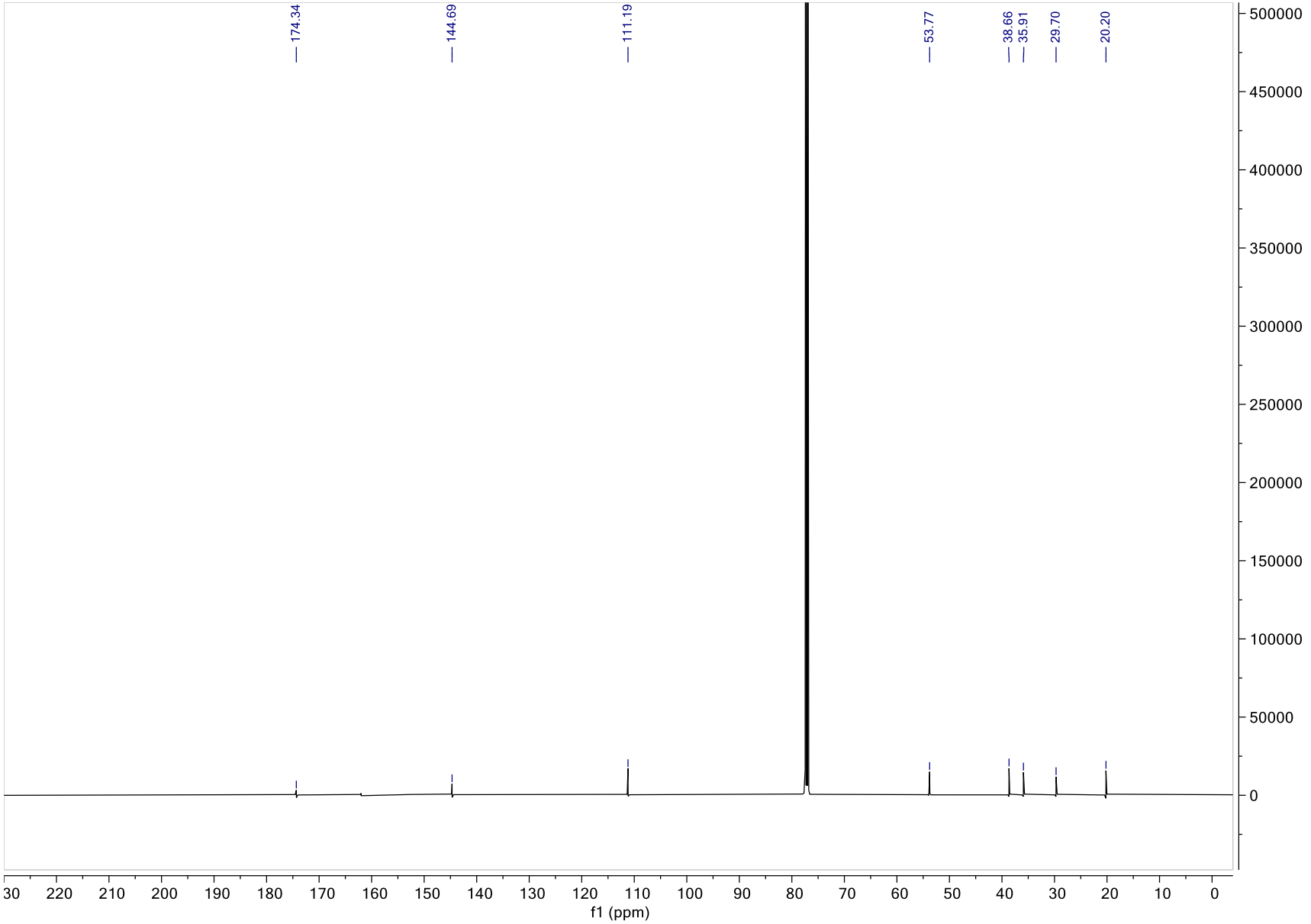
142.49
113.45
51.28
42.38
37.98
36.00
35.98
26.87
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
MeN
Me
12
4.81
4.81
4.81
4.78
3.51
3.49
3.49
3.47
3.25
3.23
3.23
3.21
3.02
3.01
2.99
2.86
2.56
2.54
2.52
2.51
2.37
2.35
2.34
2.32
1.74
1.74
1.91
0.96
0.95
1.00
2.90
0.98
0.98
f1 (ppm)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144.69
111.19
53.77
38.66
35.91
29.70
20.20
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
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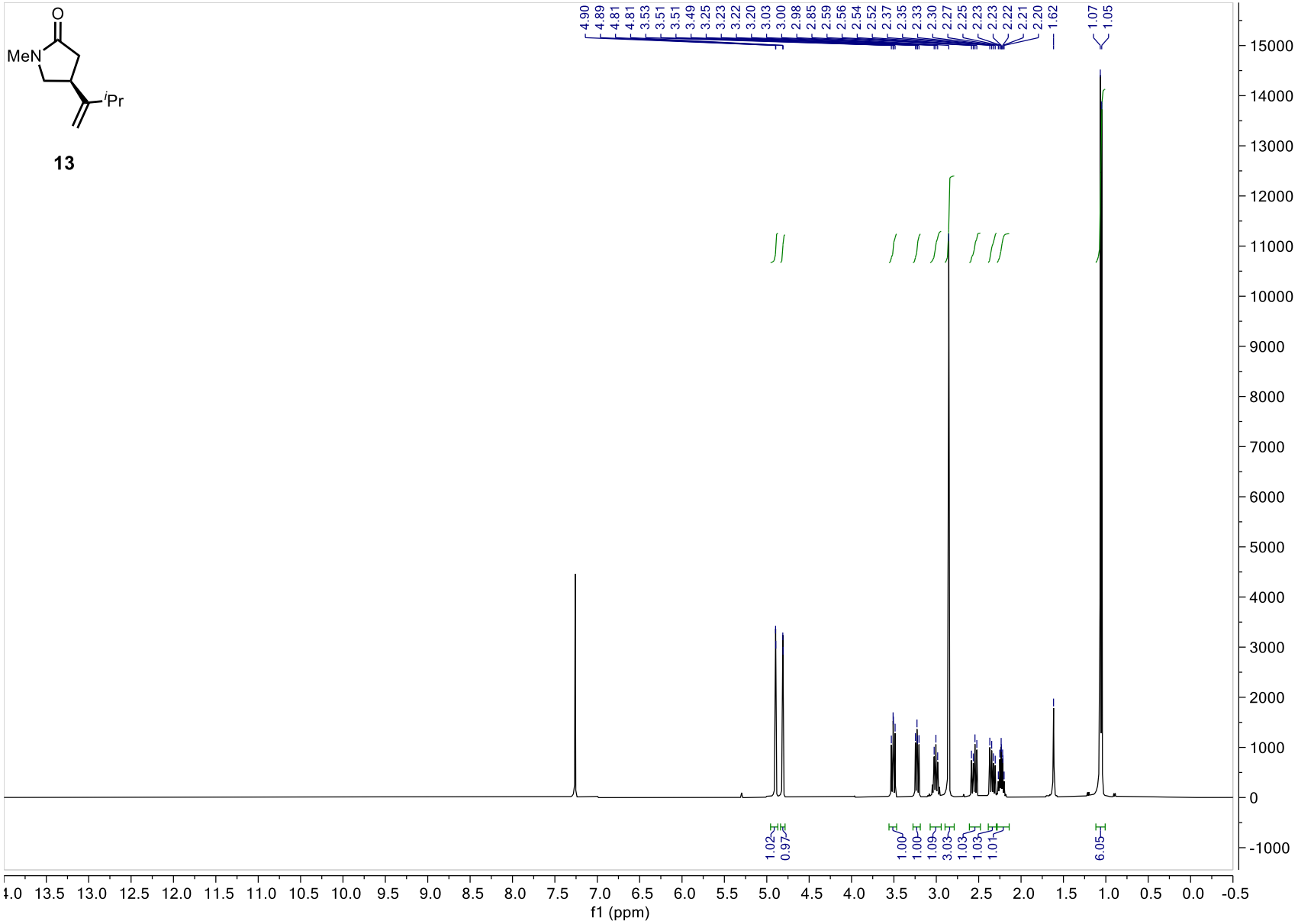
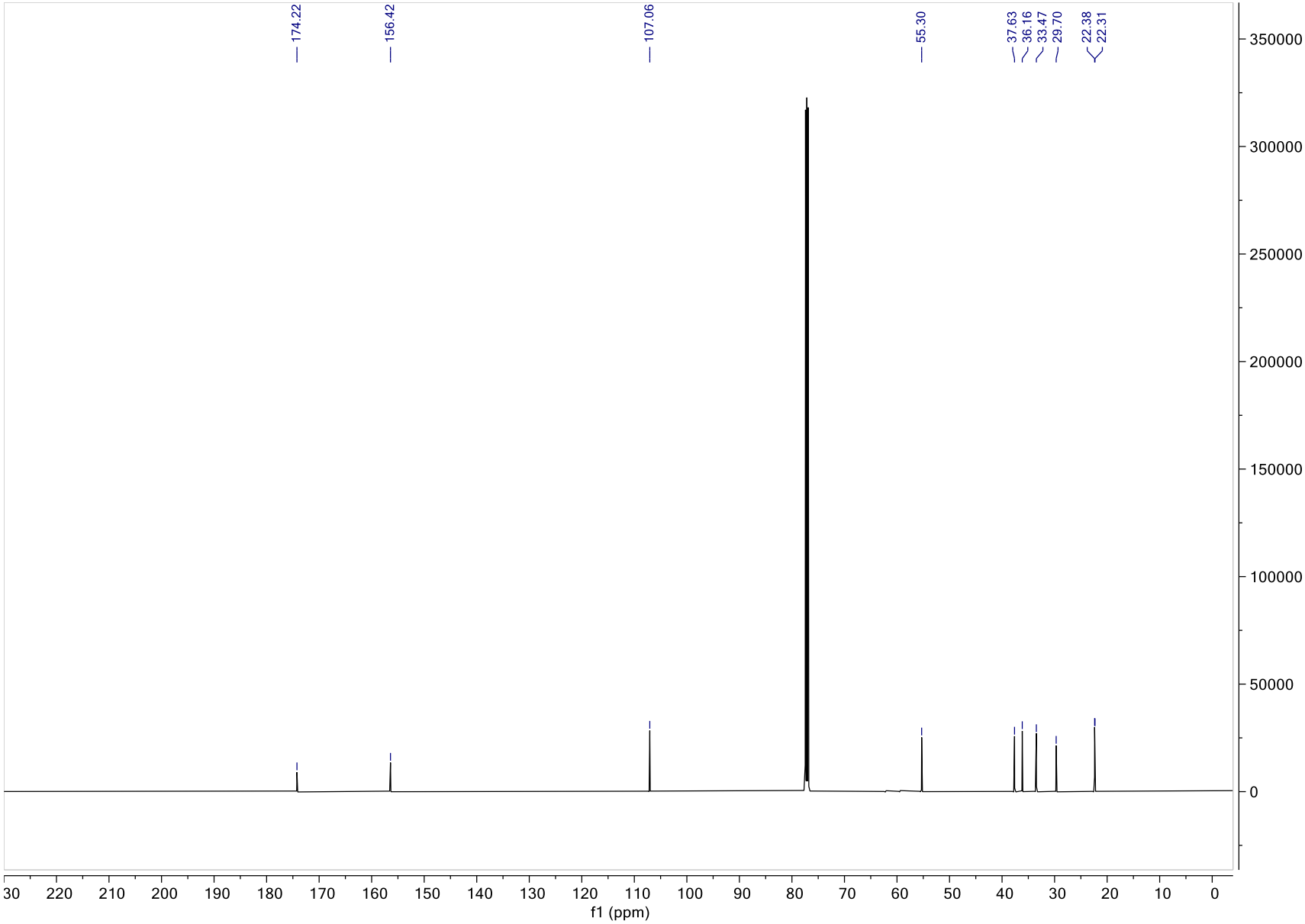
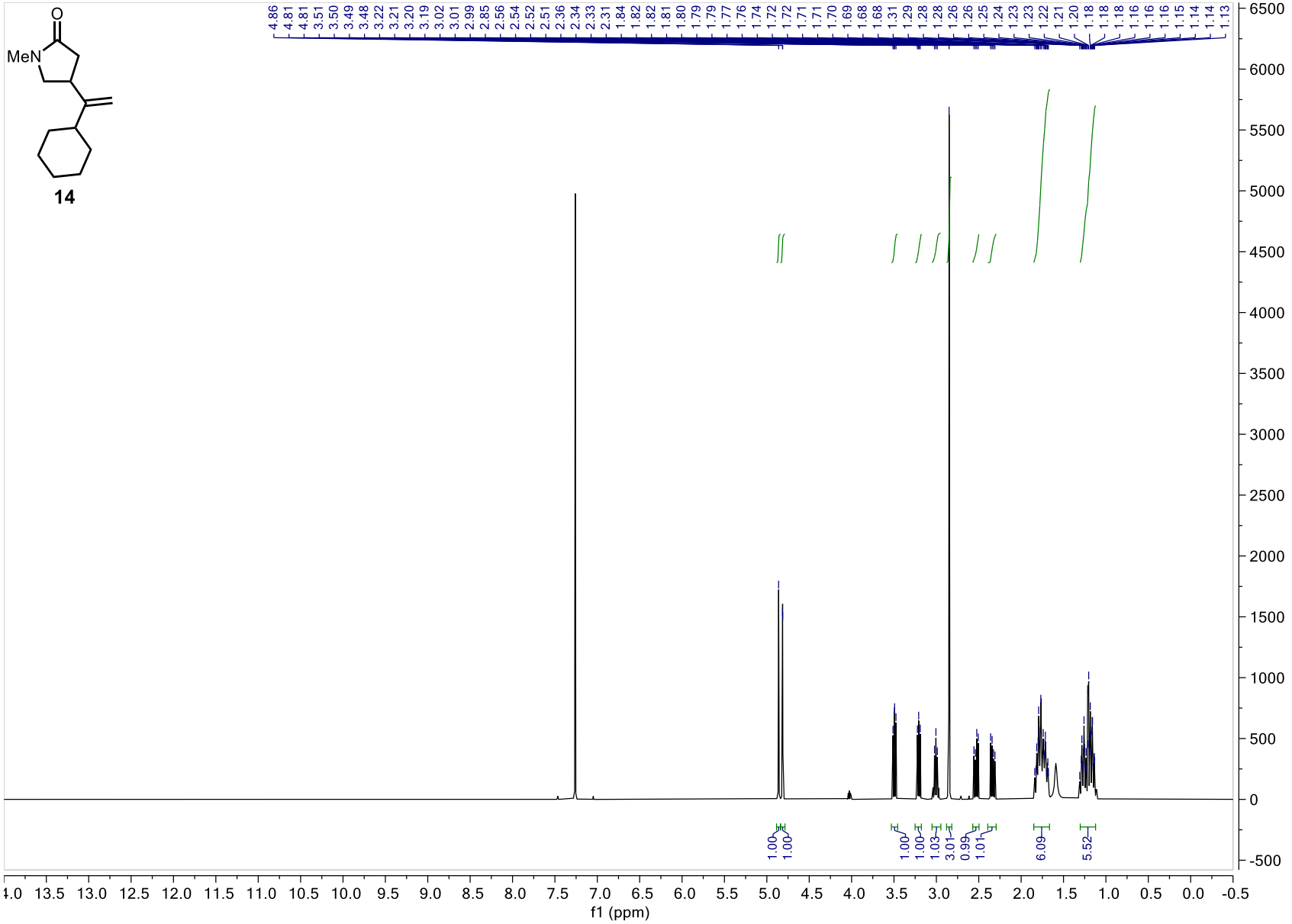
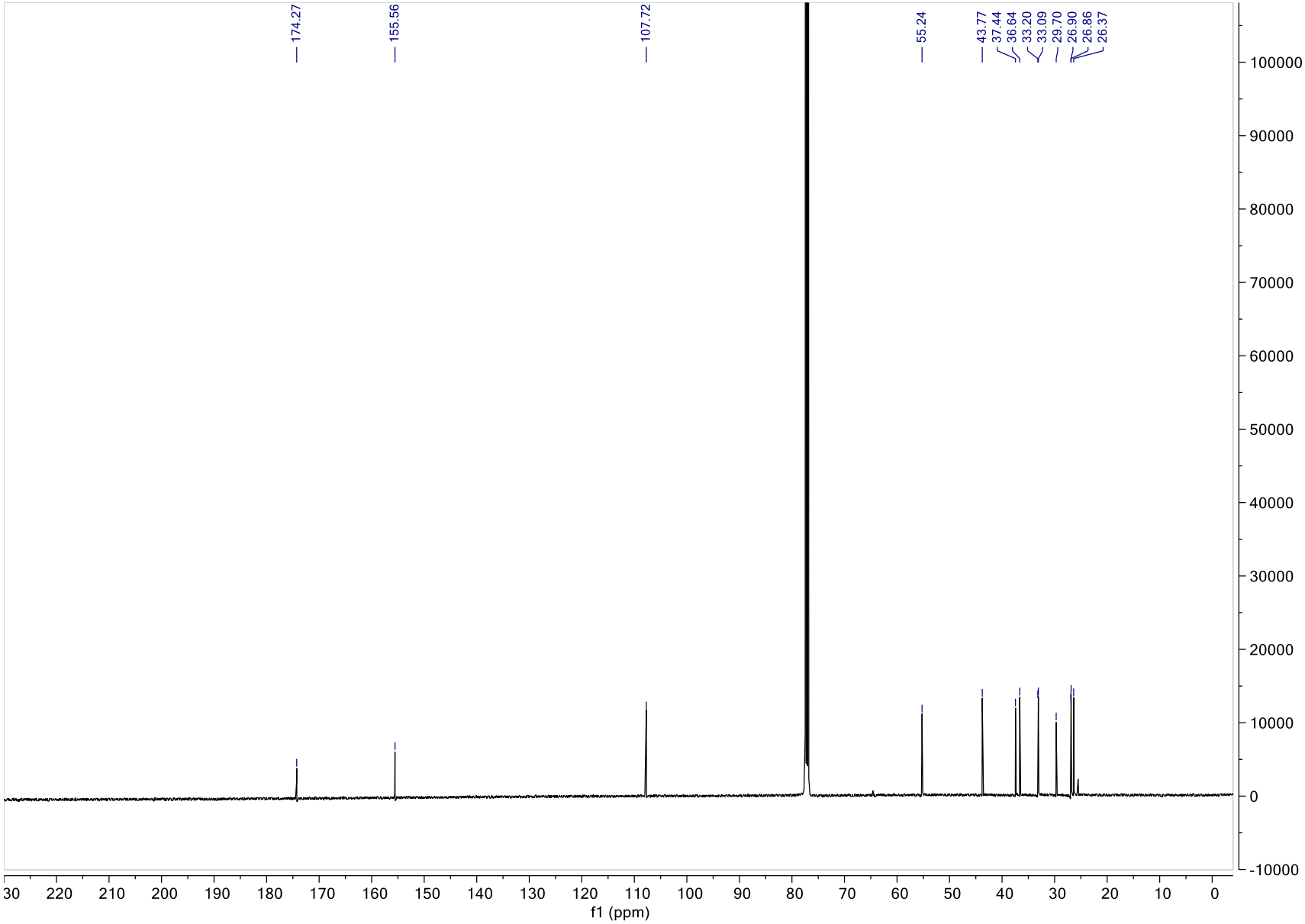
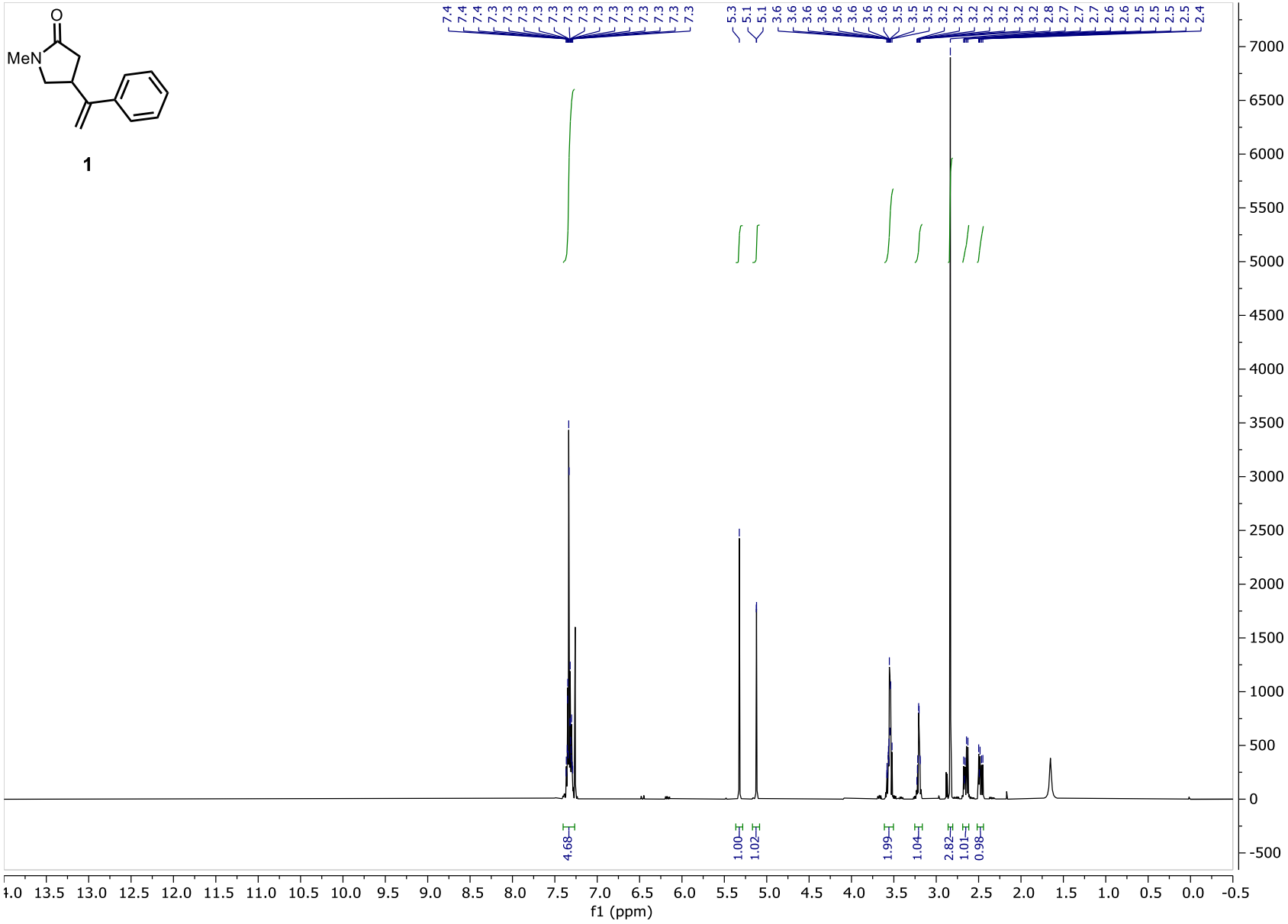
f1 (ppm)

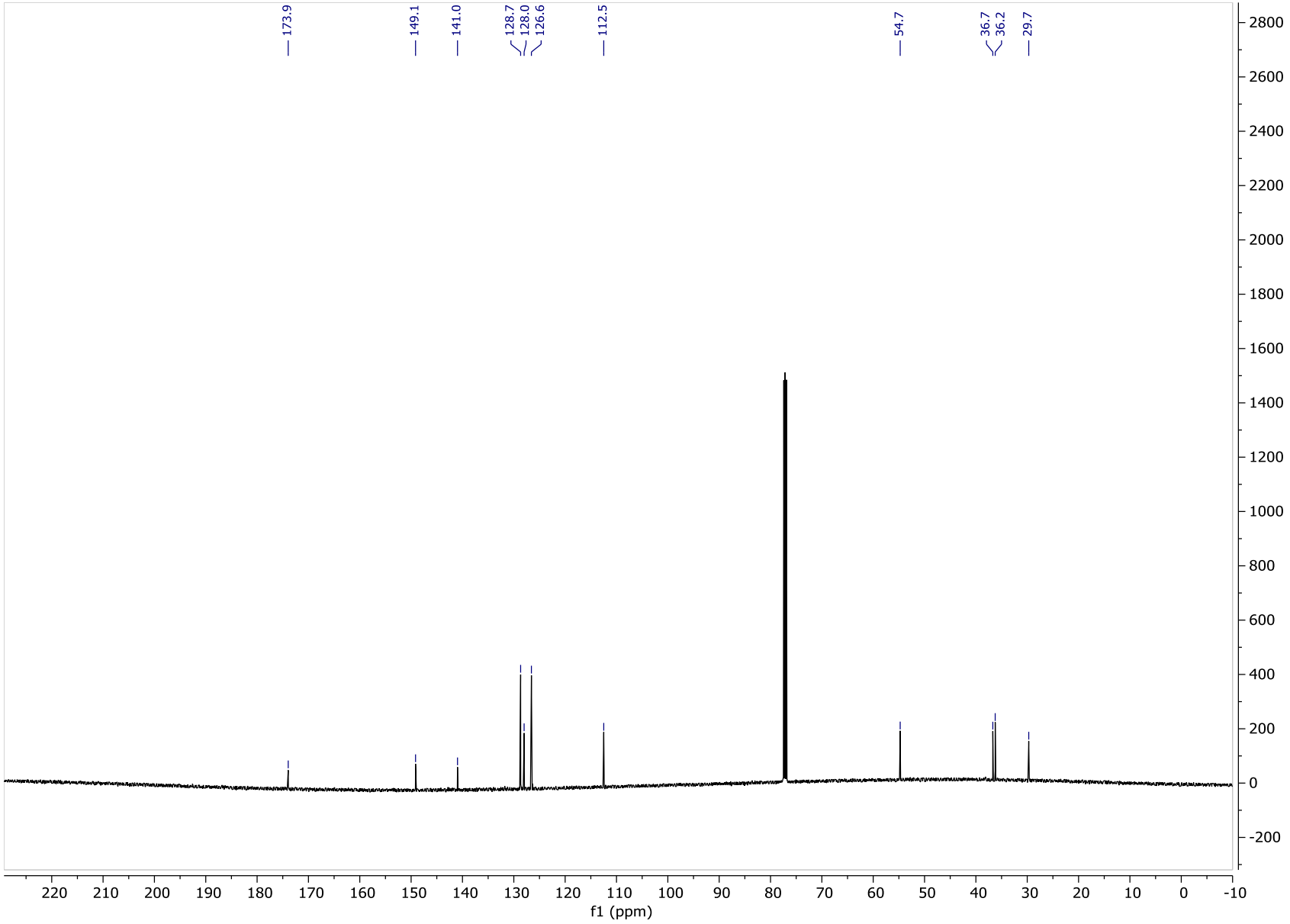

MeN
O
iPr
13
4.90
4.89
4.81
4.81
3.53
3.51
3.51
3.49
3.25
3.23
3.22
3.20
3.03
3.00
2.98
2.85
2.59
2.56
2.54
2.52
2.37
2.35
2.33
2.30
2.27
2.25
2.23
2.23
2.22
2.21
2.20
1.62
1.07
1.05
1.02
0.97
1.00
1.00
1.09
3.03
1.03
1.03
1.01
6.05
f1 (ppm)

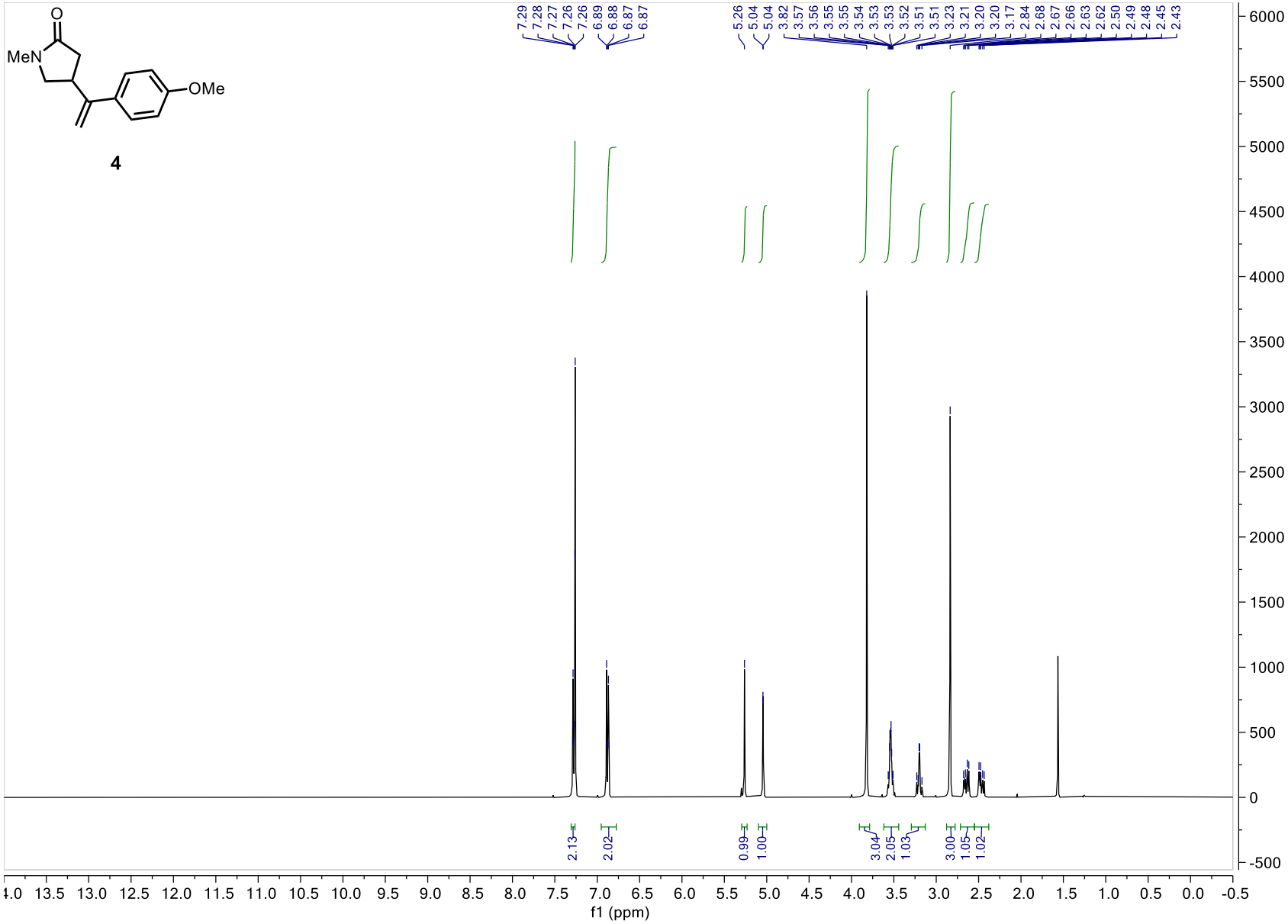

174.22
156.42
107.06
55.30
37.63
36.16
33.47
29.70
22.38
22.31
350000
300000
250000
200000
150000
100000
50000
0
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

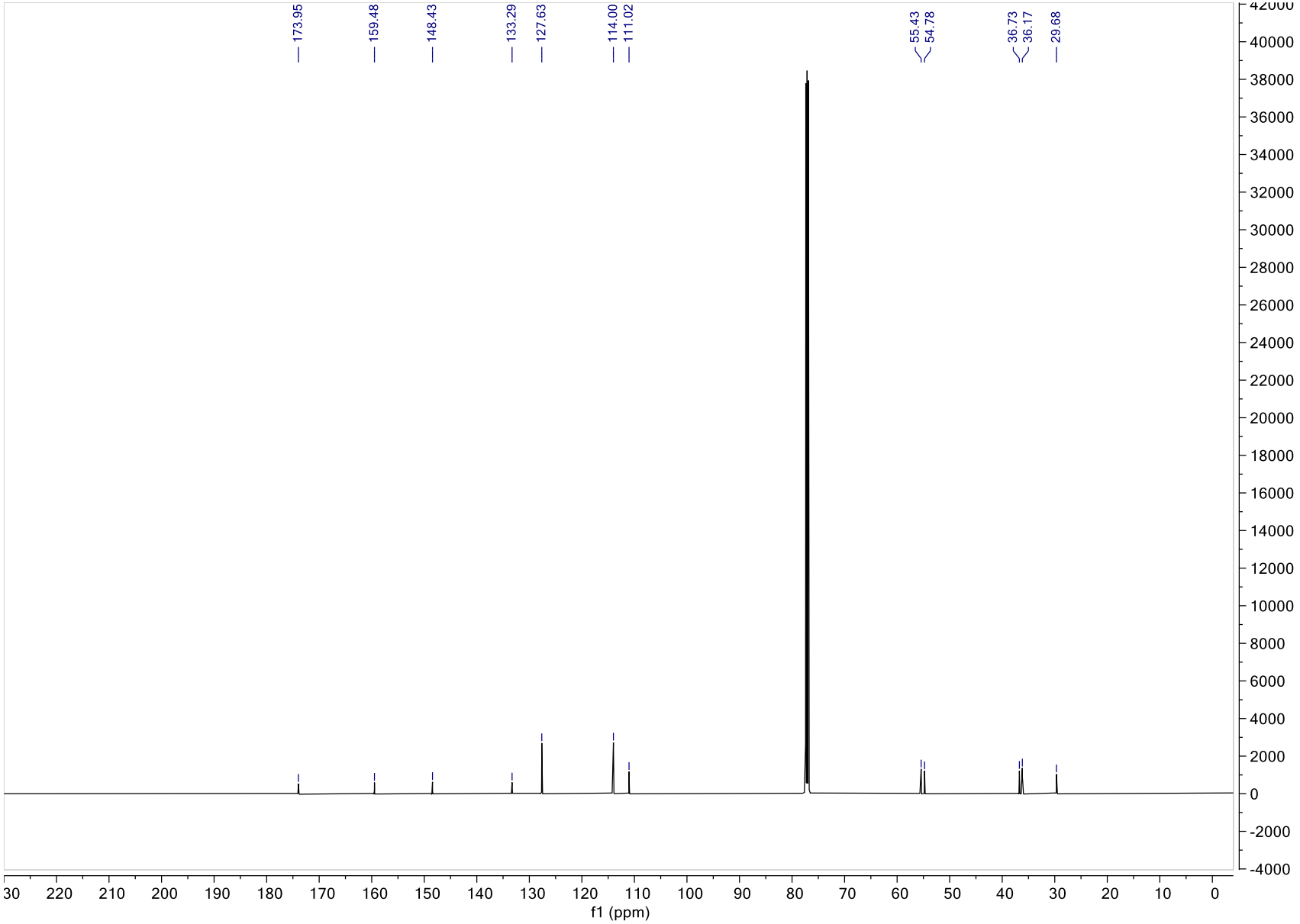

MeN
O
14
4.86 4.81 4.81 3.51 3.50 3.49 3.48 3.22 3.21 3.20 3.19 3.02 3.01 2.99 2.85 2.56 2.54 2.52 2.51 2.36 2.34 2.33 2.31 1.84 1.82 1.82 1.81 1.80 1.79 1.79 1.77 1.76 1.74 1.72 1.72 1.71 1.71 1.70 1.69 1.68 1.68 1.31 1.29 1.28 1.28 1.26 1.26 1.25 1.24 1.23 1.23 1.22 1.21 1.20 1.18 1.18 1.18 1.16 1.16 1.16 1.15 1.14 1.14 1.13
1.00 1.00 1.00 1.00 1.03 3.01 0.99 1.01 6.09 5.52
6500 6000 5500 5000 4500 4000 3500 3000 2500 2000 1500 1000 500 0 -500
4.0 13.5 13.0 12.5 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
f1 (ppm)

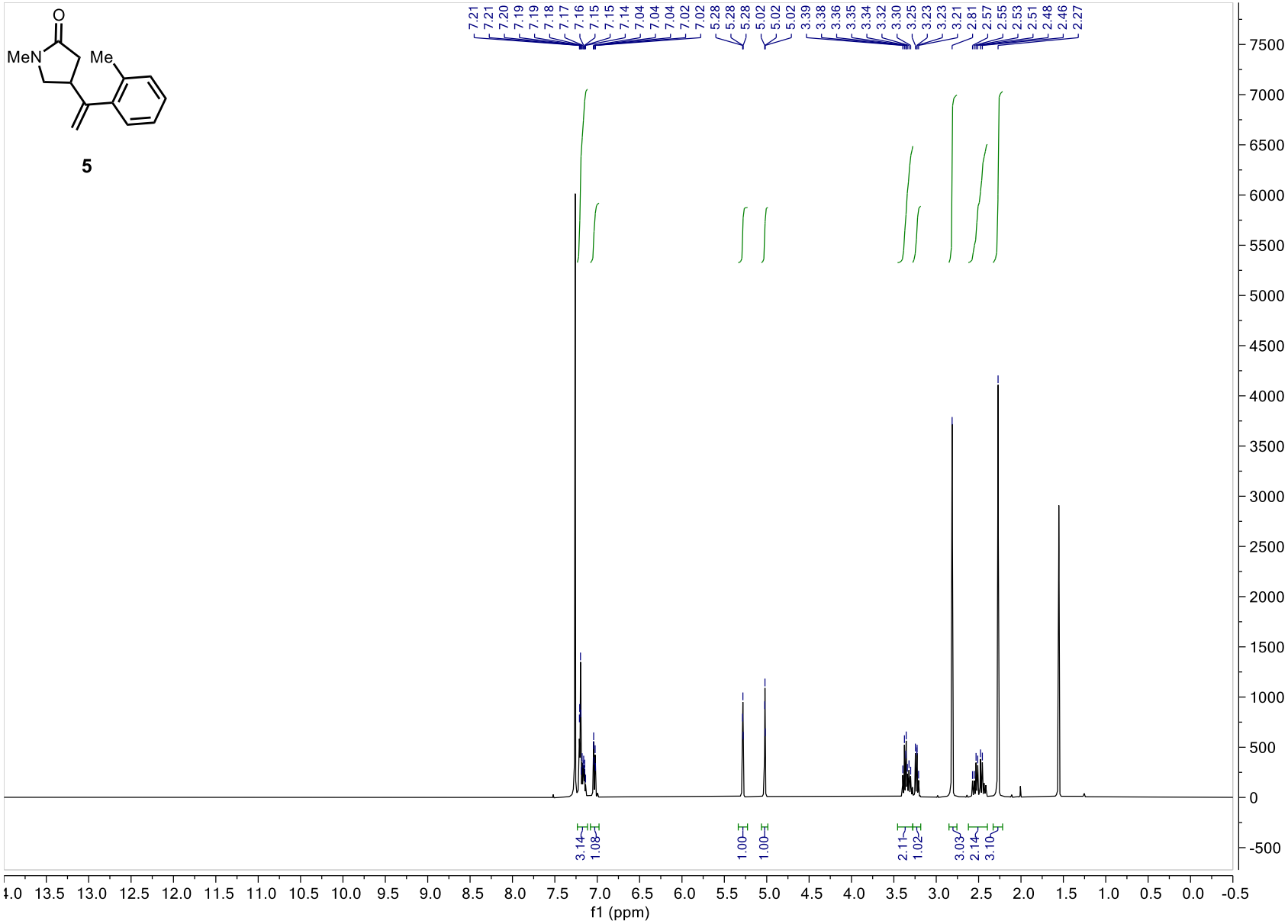

174.27
155.56
107.72
55.24
43.77
37.44
36.64
33.20
33.09
29.70
26.90
26.86
26.37
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

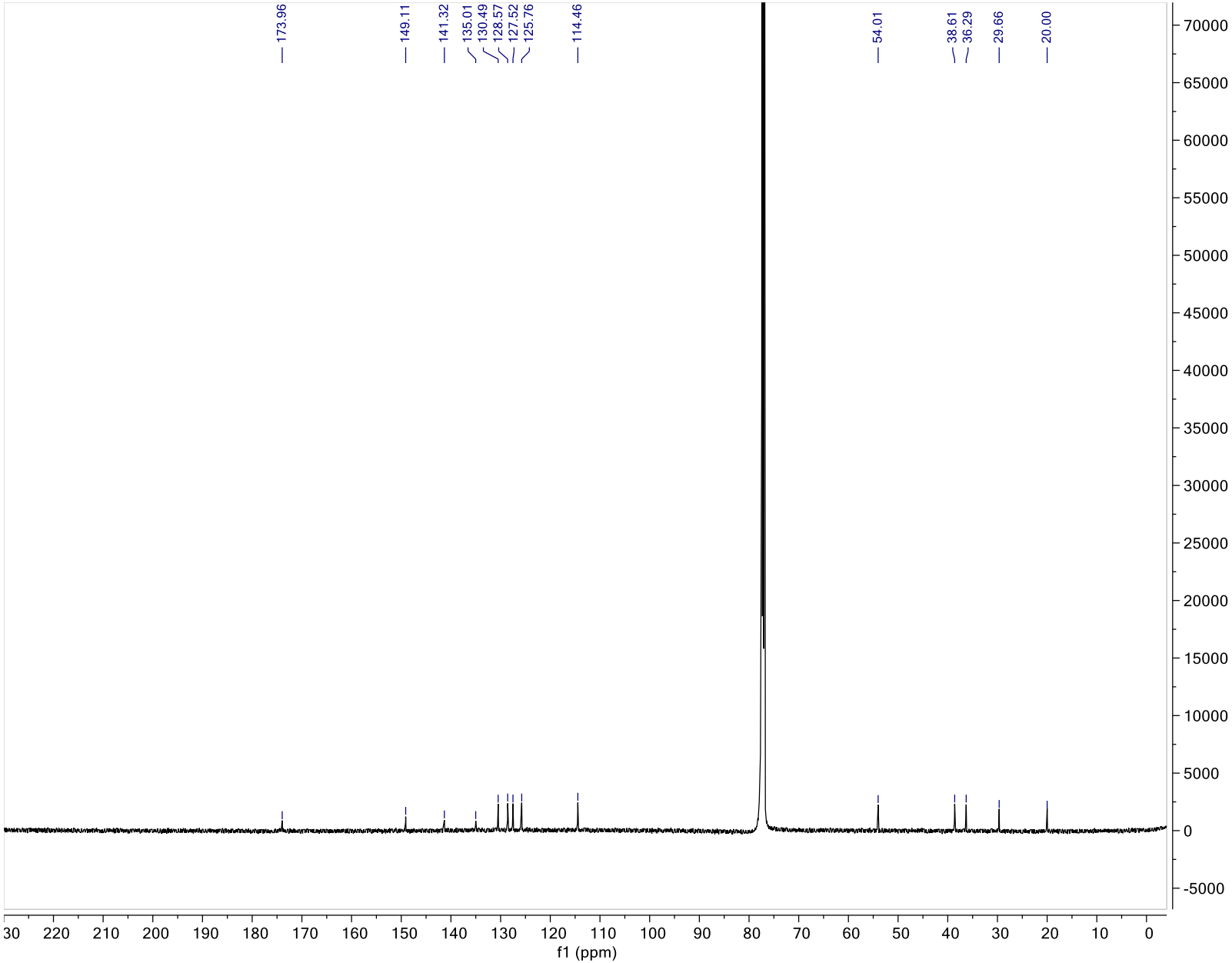

MeN
O
1
f1 (ppm)

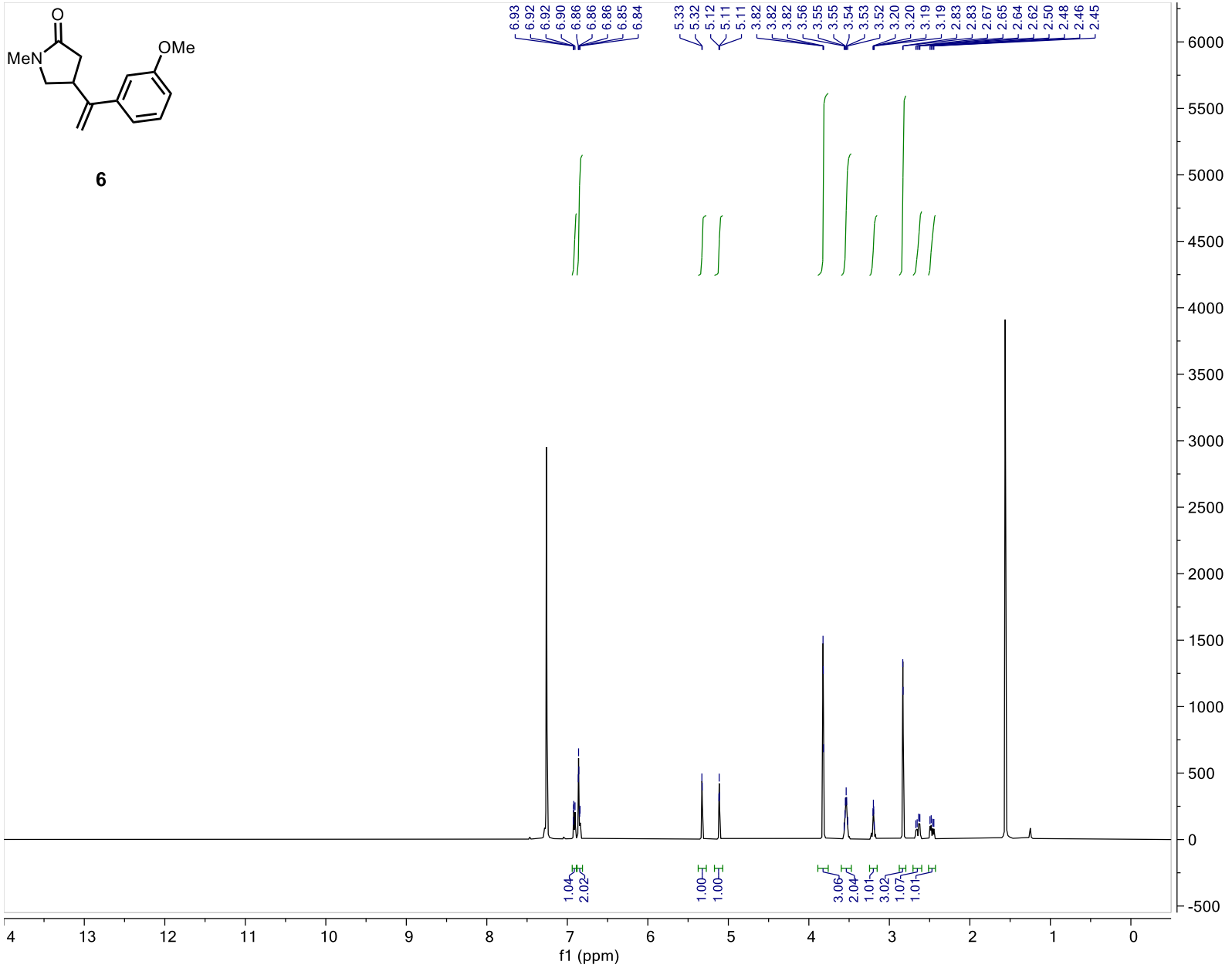

173.9
149.1
141.0
128.7
128.0
126.6
112.5
54.7
36.7
36.2
29.7
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

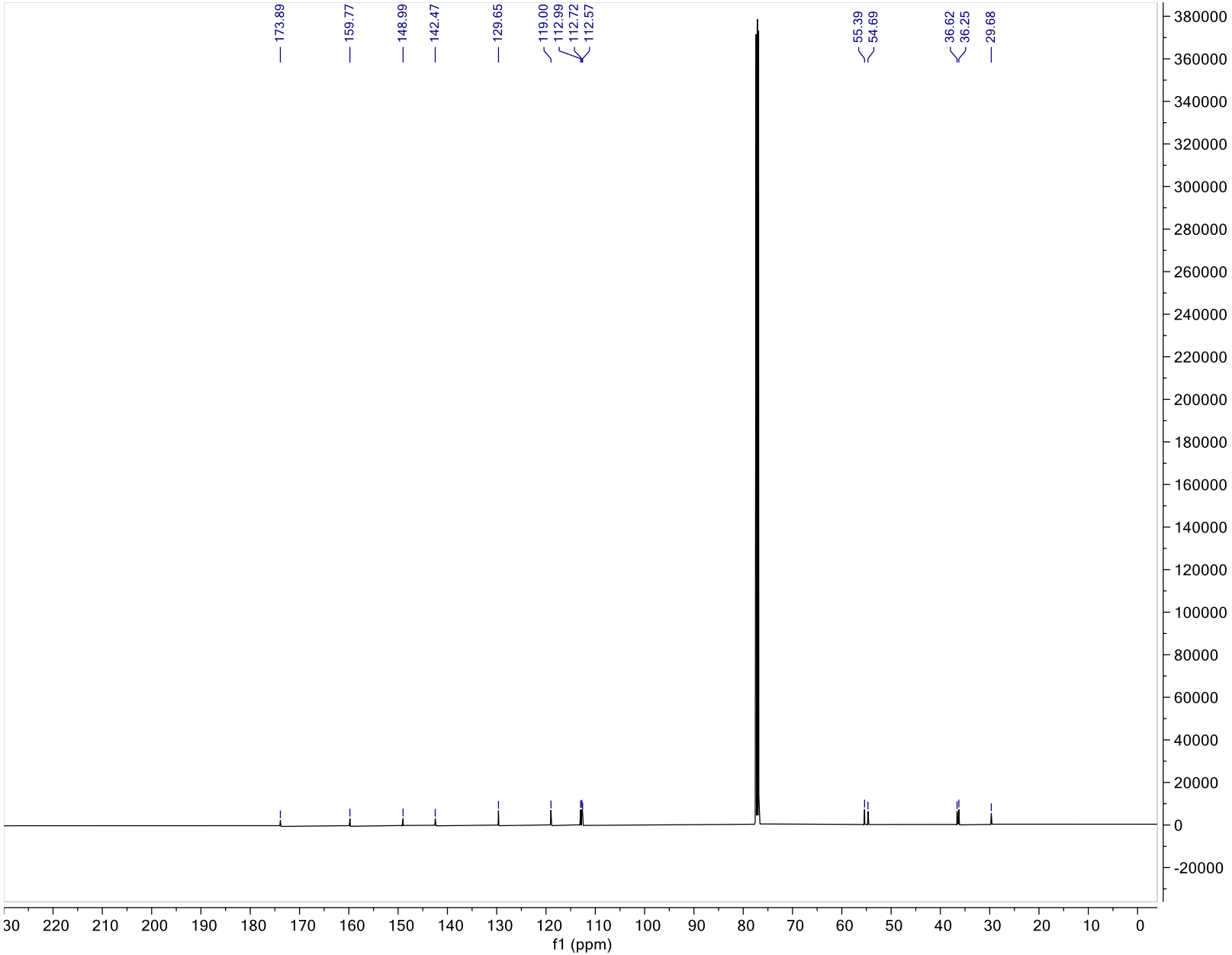

MeN
O
OMe
4
7.29
7.28
7.27
7.26
7.26
6.89
6.88
6.87
6.87
5.26
5.04
5.04
3.82
3.57
3.56
3.55
3.55
3.54
3.53
3.53
3.52
3.51
3.51
3.23
3.21
3.20
3.20
3.17
2.84
2.68
2.67
2.66
2.63
2.62
2.50
2.49
2.48
2.45
2.43
2.13
2.02
0.99
1.00
3.04
2.05
1.03
3.00
1.05
1.02
f1 (ppm)


173.95
159.48
148.43
133.29
127.63
114.00
111.02
55.43
54.78
36.73
36.17
29.68
f1 (ppm)


O
MeN
Me
5
7.21 7.21 7.20 7.19 7.19 7.18 7.17 7.16 7.15 7.15 7.14 7.04 7.04 7.04 7.02 7.02
5.28 5.28 5.02 5.02 5.02
3.39 3.38 3.36 3.35 3.34 3.32 3.30 3.25 3.23 3.23 3.21
2.81 2.57 2.55 2.53 2.51 2.48 2.46 2.27
3.14 1.08 1.00 1.00 2.11 1.02 3.03 2.14 3.10
f1 (ppm)


173.96
149.11
141.32
135.01
130.49
128.57
127.52
125.76
114.46
54.01
38.61
36.29
29.66
20.00
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
MeN
OMe
6
6.93
6.92
6.92
6.90
6.86
6.86
6.86
6.85
6.84
5.33
5.32
5.12
5.11
3.82
3.82
3.82
3.56
3.55
3.54
3.53
3.52
3.20
3.20
3.19
3.19
2.83
2.83
2.67
2.65
2.64
2.62
2.50
2.48
2.46
2.45
1.04
2.02
1.00
1.00
3.06
2.04
1.01
3.02
1.07
1.01
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)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142.47
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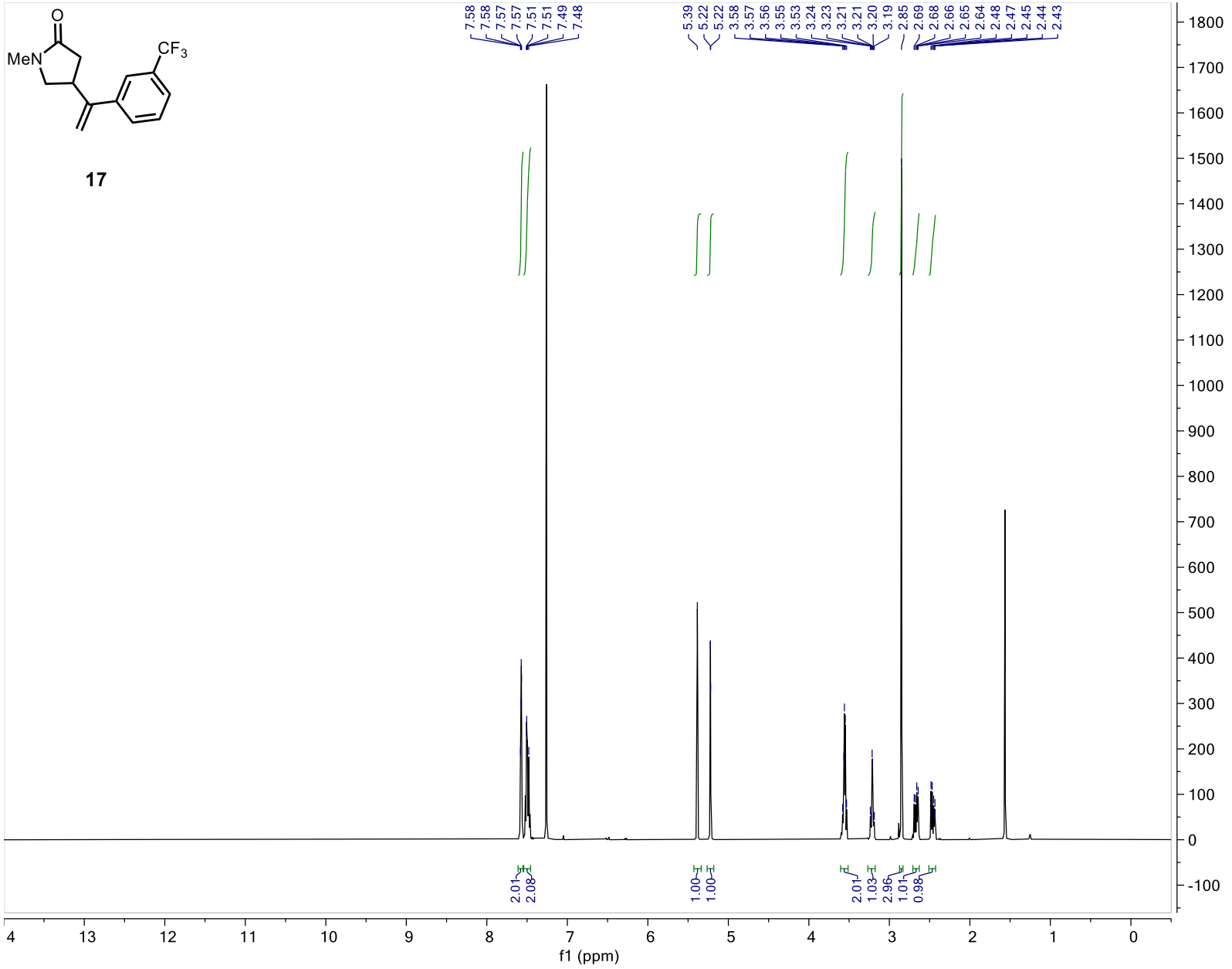
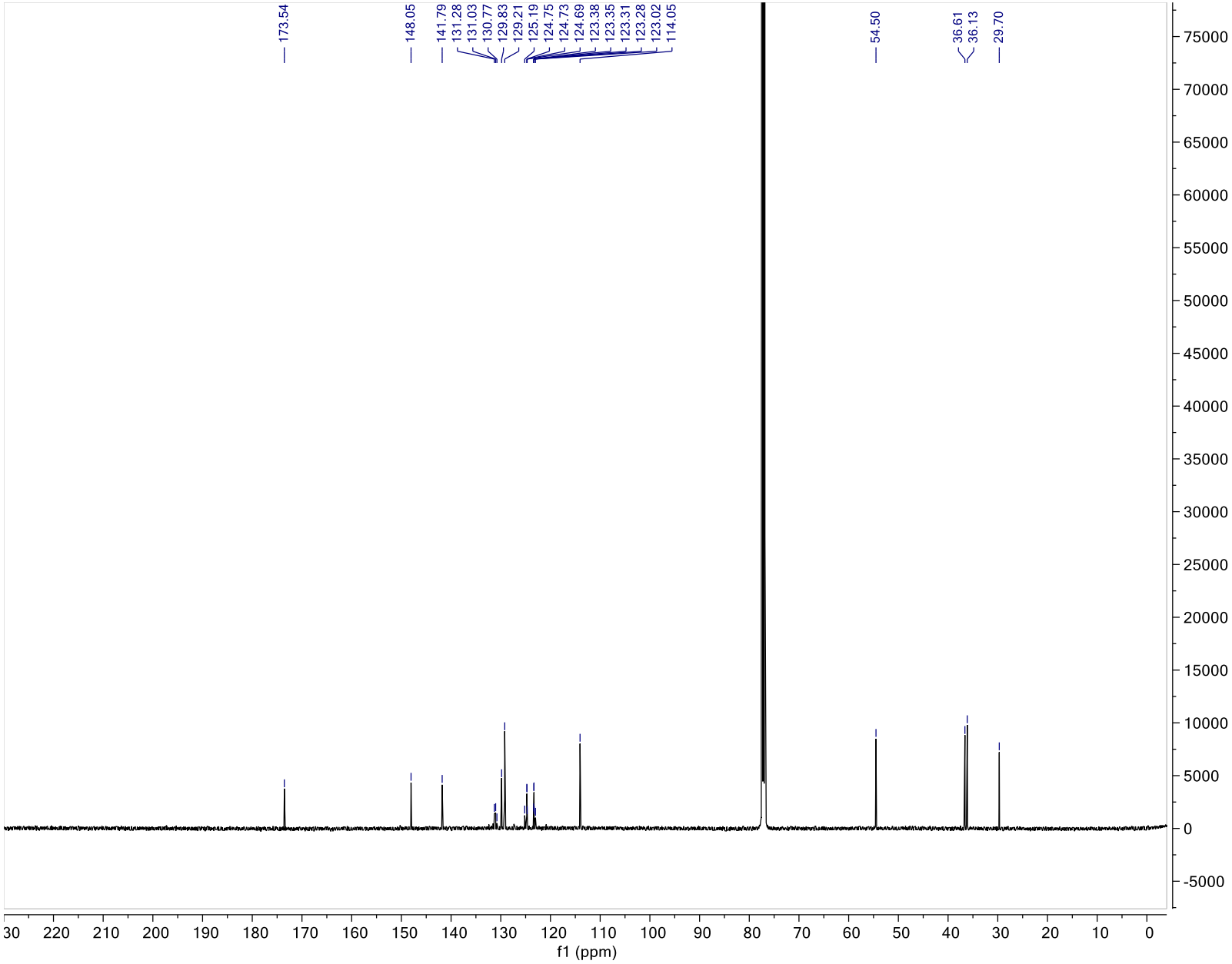
112.99
112.72
112.57
55.39
54.69
36.62
36.25
29.68
380000
360000
340000
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeN
O
CF3
17
7.58
7.58
7.57
7.57
7.51
7.51
7.49
7.48
5.39
5.22
5.22
3.58
3.57
3.56
3.55
3.53
3.24
3.23
3.21
3.21
3.20
3.19
2.85
2.69
2.68
2.66
2.65
2.64
2.48
2.47
2.45
2.44
2.43
2.01
2.08
1.00
1.00
2.01
1.03
2.96
1.01
0.98
f1 (ppm)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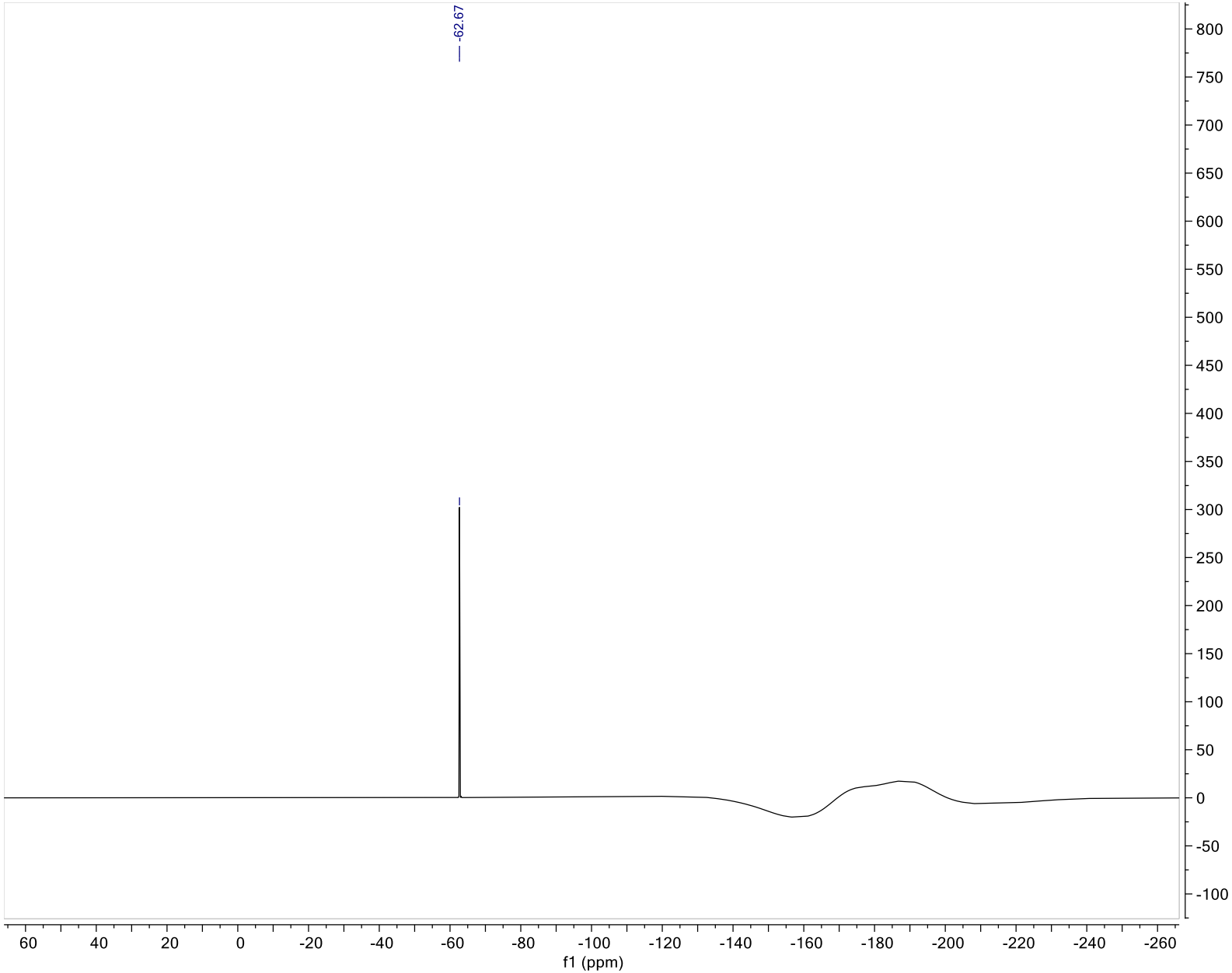
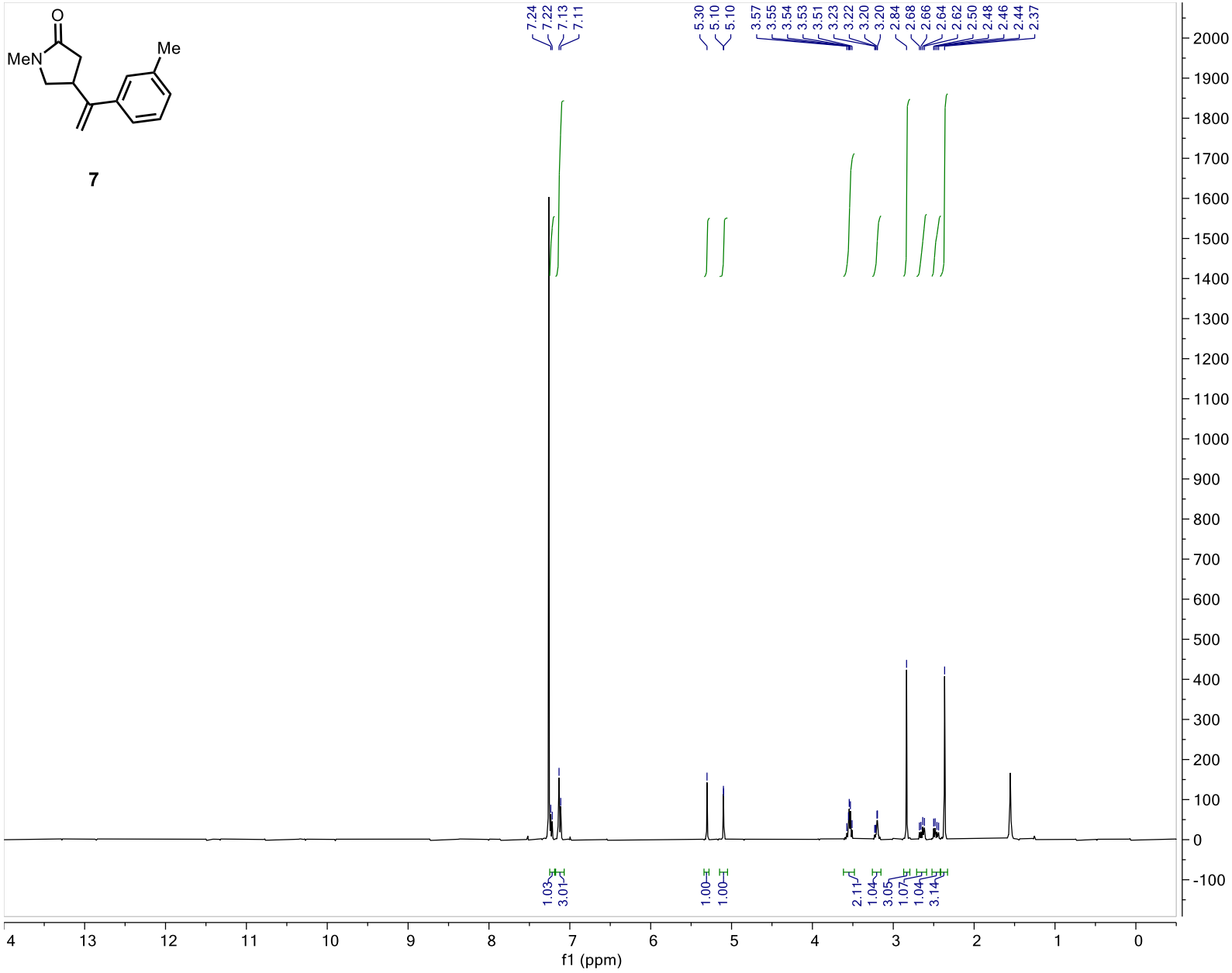
148.05
141.79
131.28
131.03
130.77
129.83
129.21
125.19
124.75
124.73
124.69
123.38
123.35
123.31
123.28
123.02
114.05
54.50
36.61
36.13
29.70
75000
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


-62.67
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
f1 (ppm)


O
MeN
Me
7
7.24
7.22
7.13
7.11
5.30
5.10
5.10
3.57
3.55
3.54
3.53
3.51
3.23
3.22
3.20
3.20
2.84
2.68
2.66
2.64
2.62
2.50
2.48
2.46
2.44
2.37
1.03
3.01
1.00
1.00
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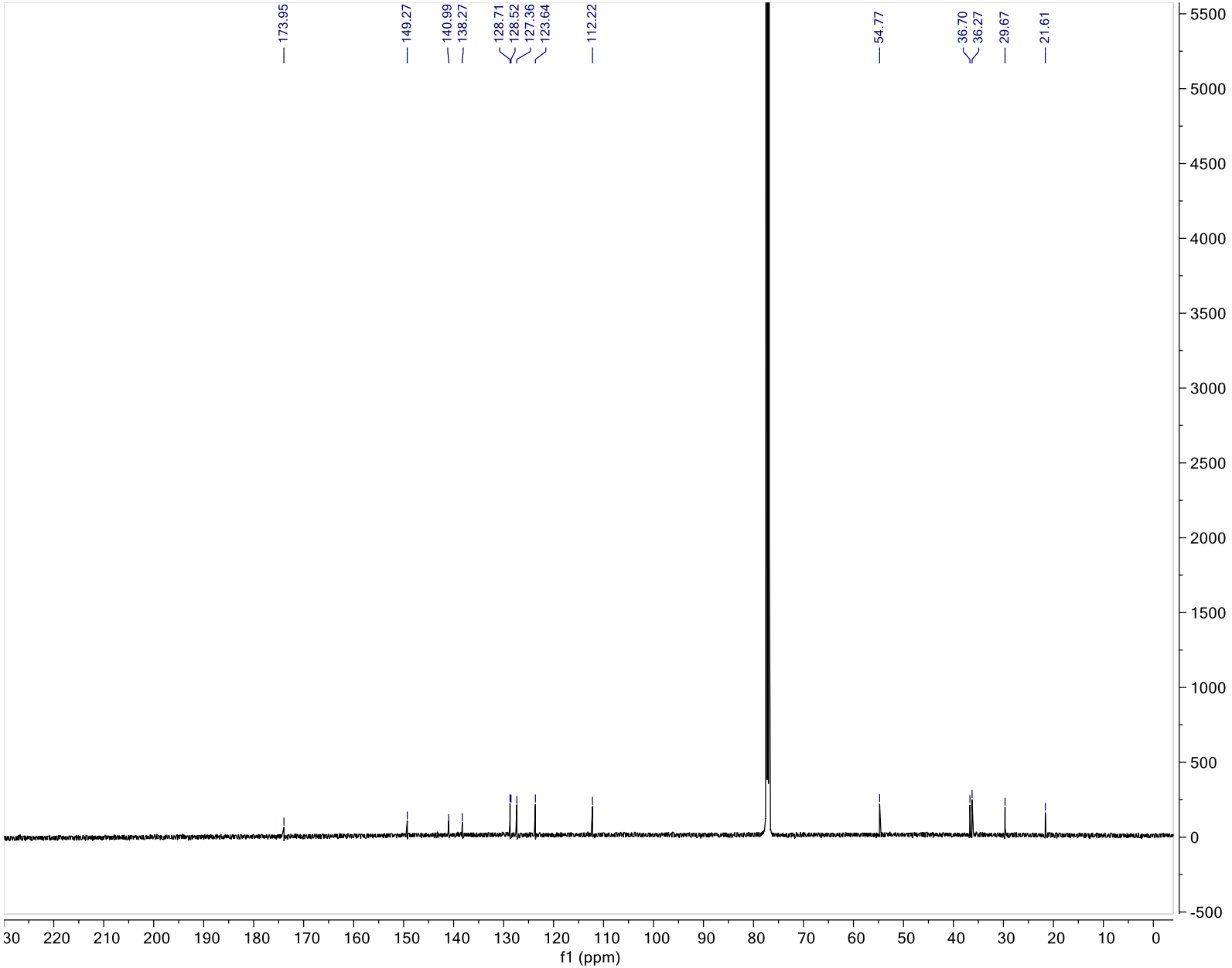
3.05
1.07
1.04
3.14
f1 (ppm)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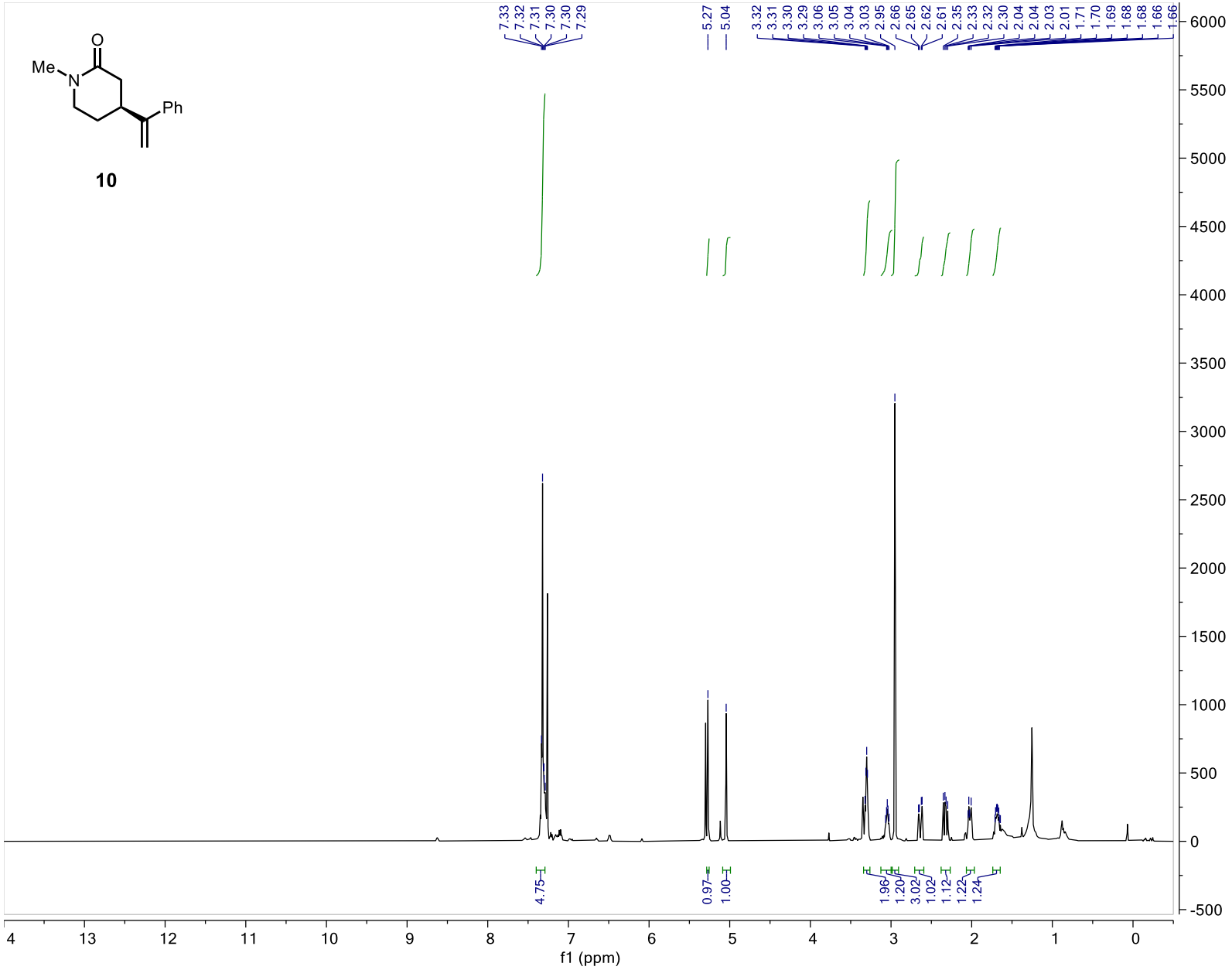
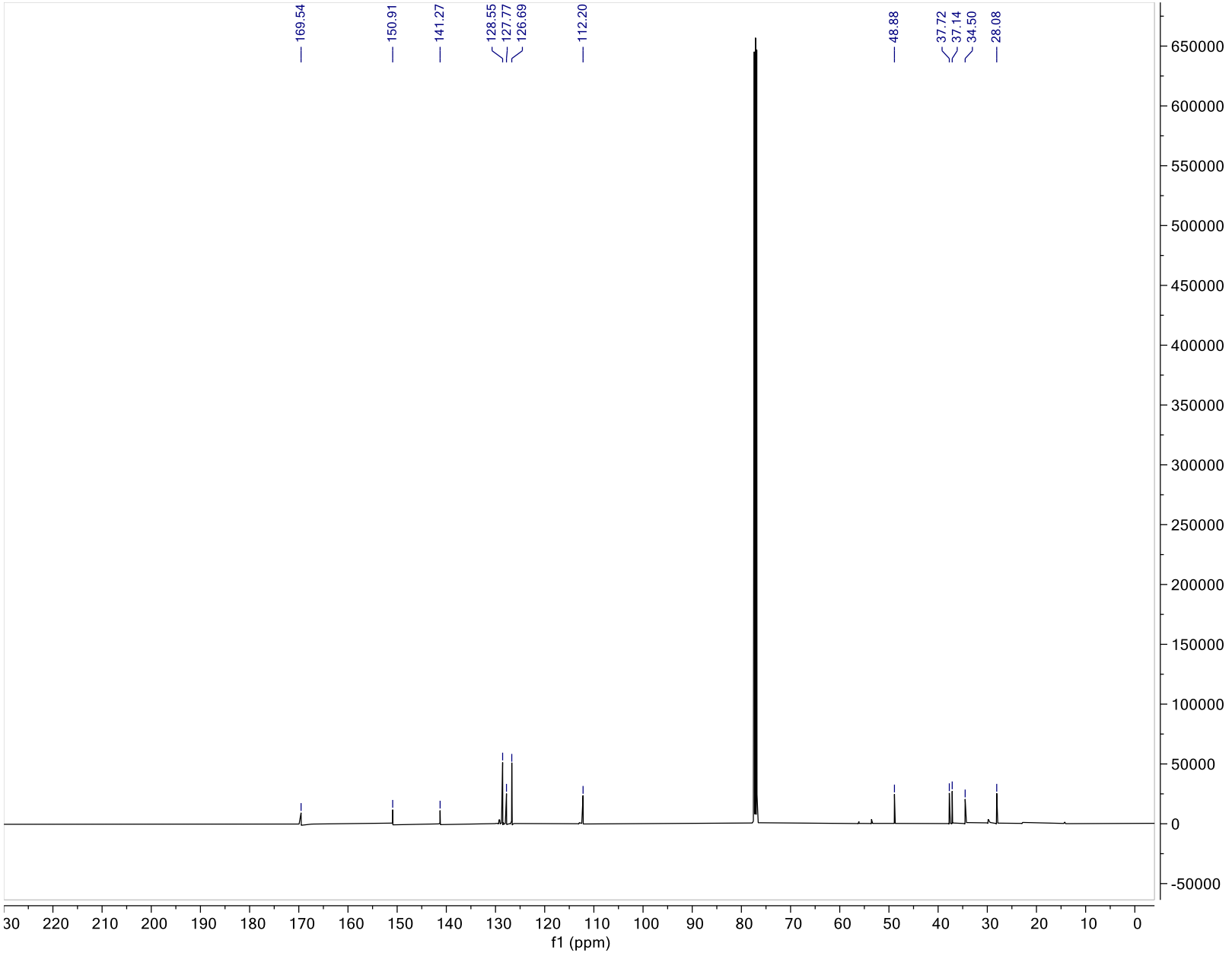
138.27
128.71
128.52
127.36
123.64
112.22
54.77
36.70
36.27
29.67
21.61
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Me
N
O
Ph
10
7.33
7.32
7.31
7.30
7.30
7.29
5.27
5.04
3.32
3.31
3.30
3.29
3.06
3.05
3.04
3.03
2.95
2.66
2.65
2.62
2.61
2.35
2.33
2.32
2.30
2.04
2.04
2.03
2.01
1.71
1.70
1.69
1.68
1.68
1.66
1.66
4.75
0.97
1.00
1.96
1.20
3.02
1.02
1.12
1.22
1.24
f1 (ppm)


169.54
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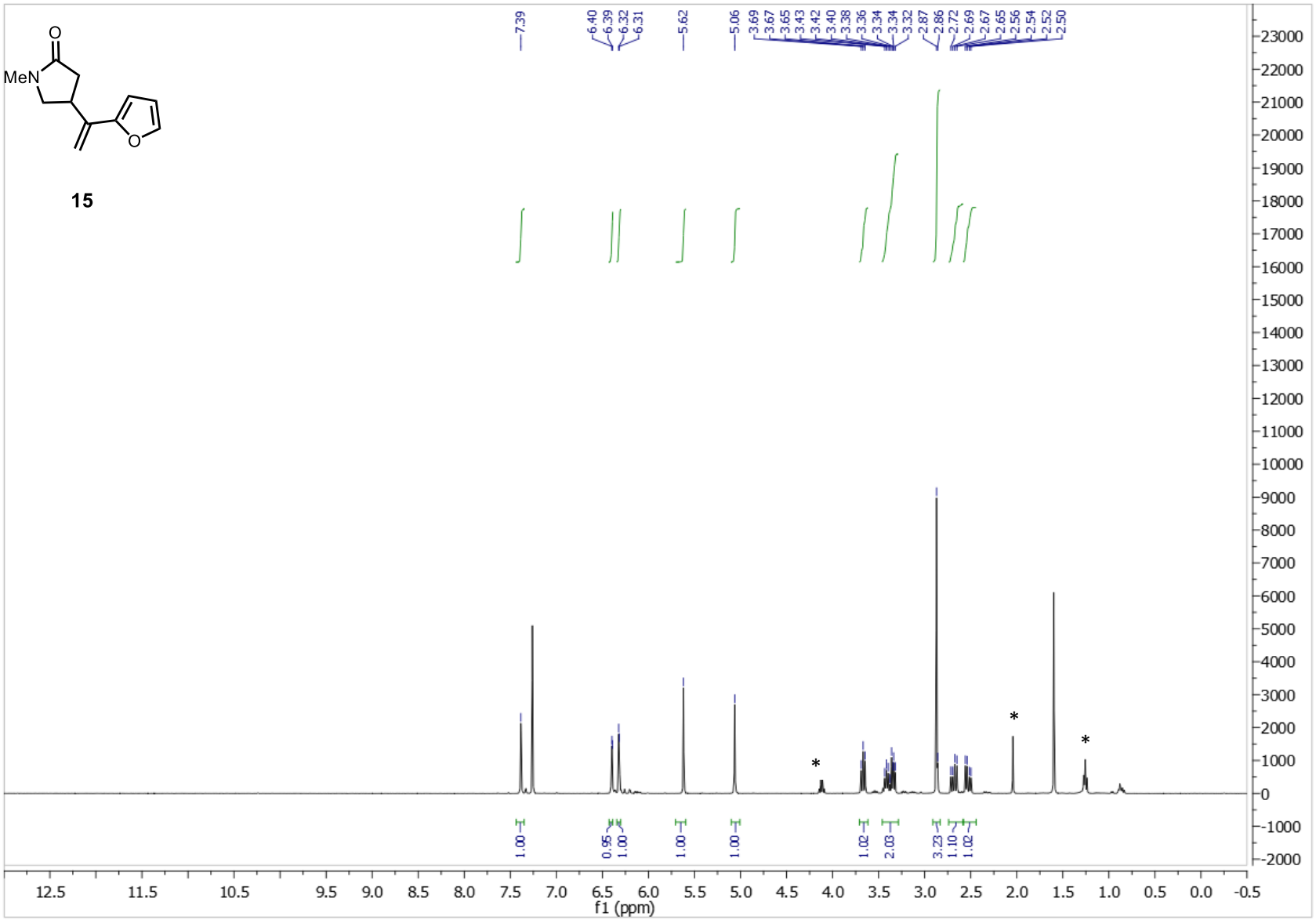
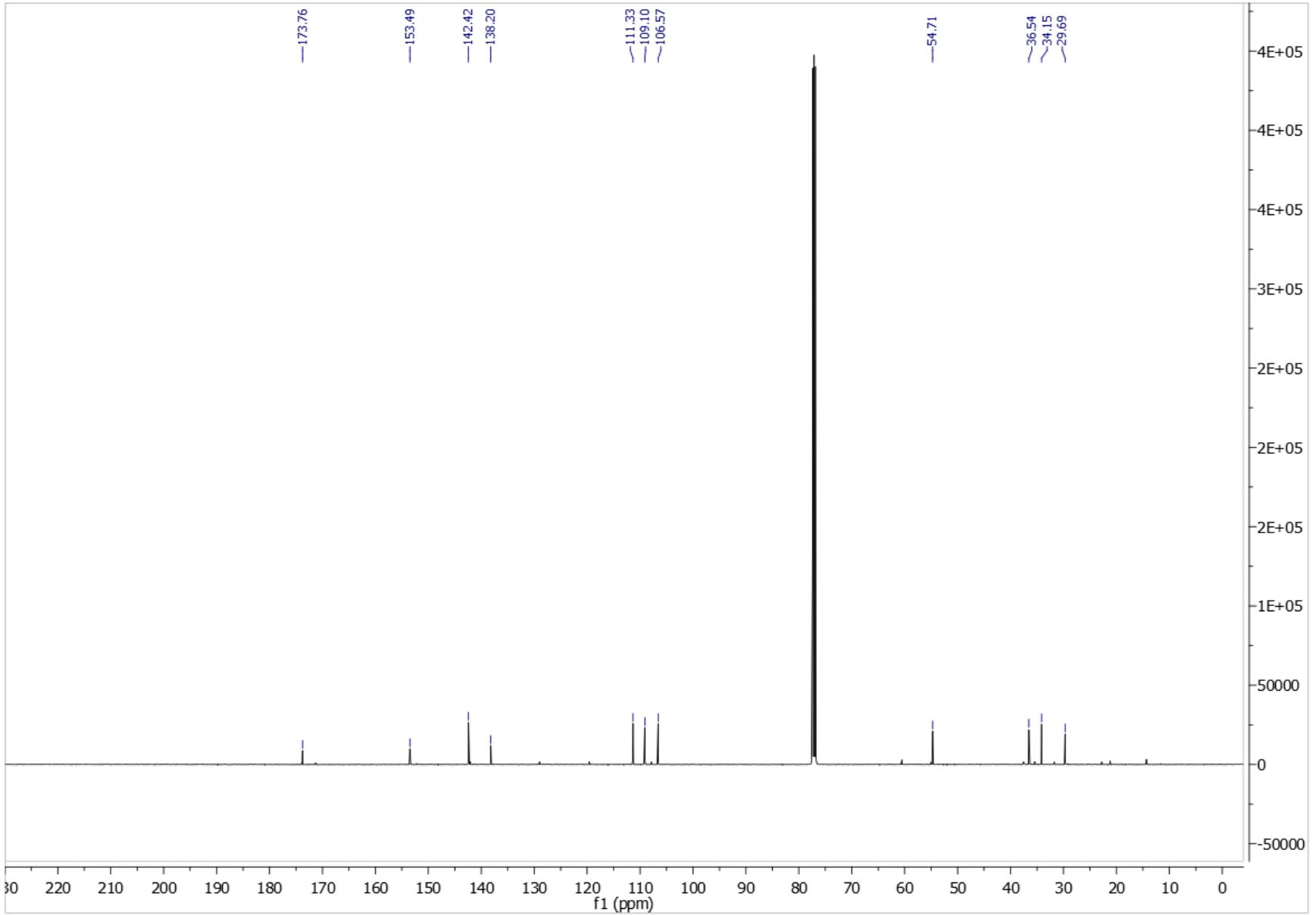
127.77
126.69
112.20
48.88
37.72
37.14
34.50
28.08
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeN
O
O
15
7.39
6.40
6.39
6.32
6.31
5.62
5.06
3.69
3.67
3.65
3.43
3.42
3.40
3.38
3.36
3.34
3.34
3.32
2.87
2.86
2.72
2.69
2.67
2.65
2.56
2.54
2.52
2.50
1.00
0.95
1.00
1.00
1.00
1.02
2.03
3.23
1.10
1.02
*
*
*
f1 (ppm)

*EtOAc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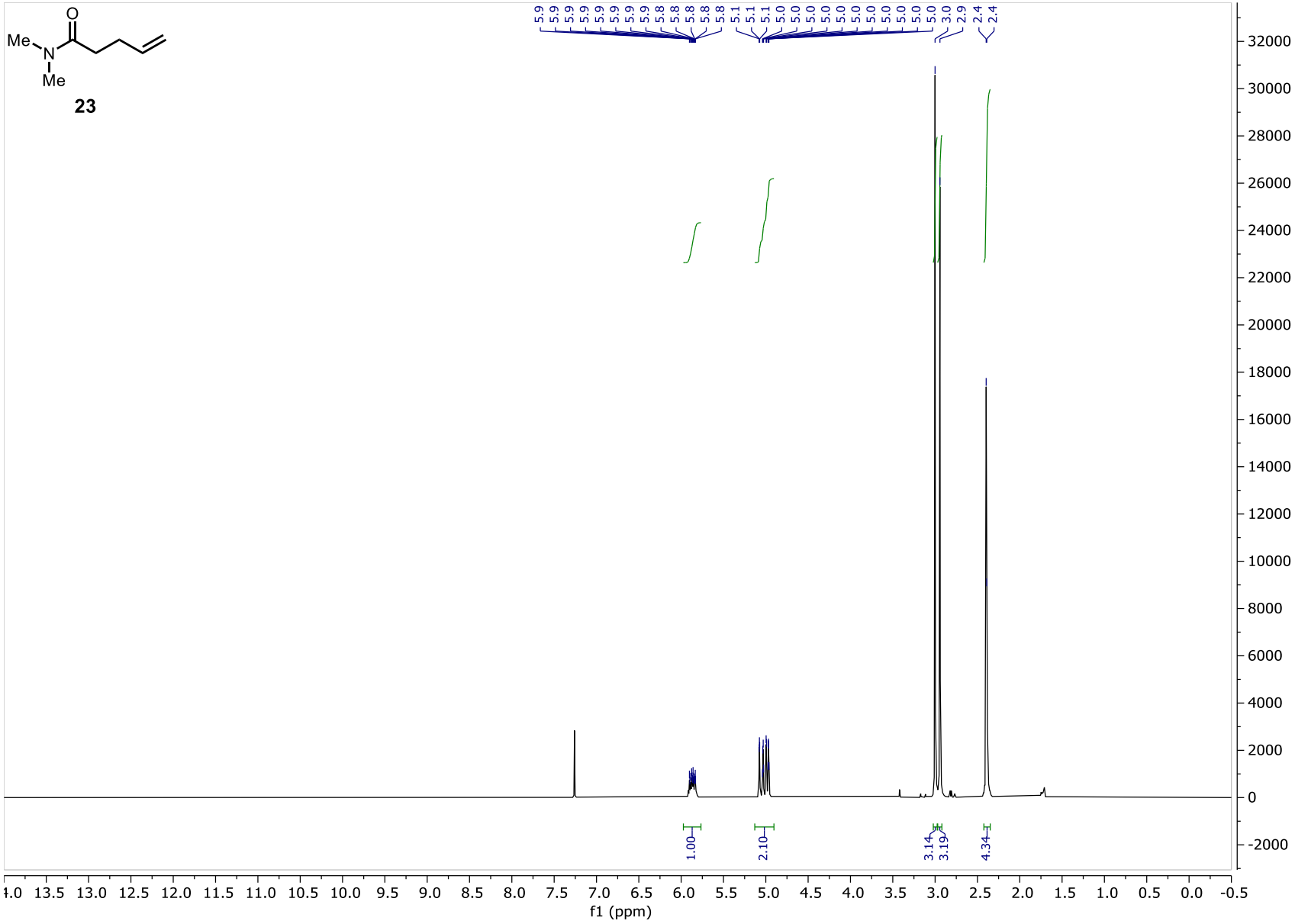
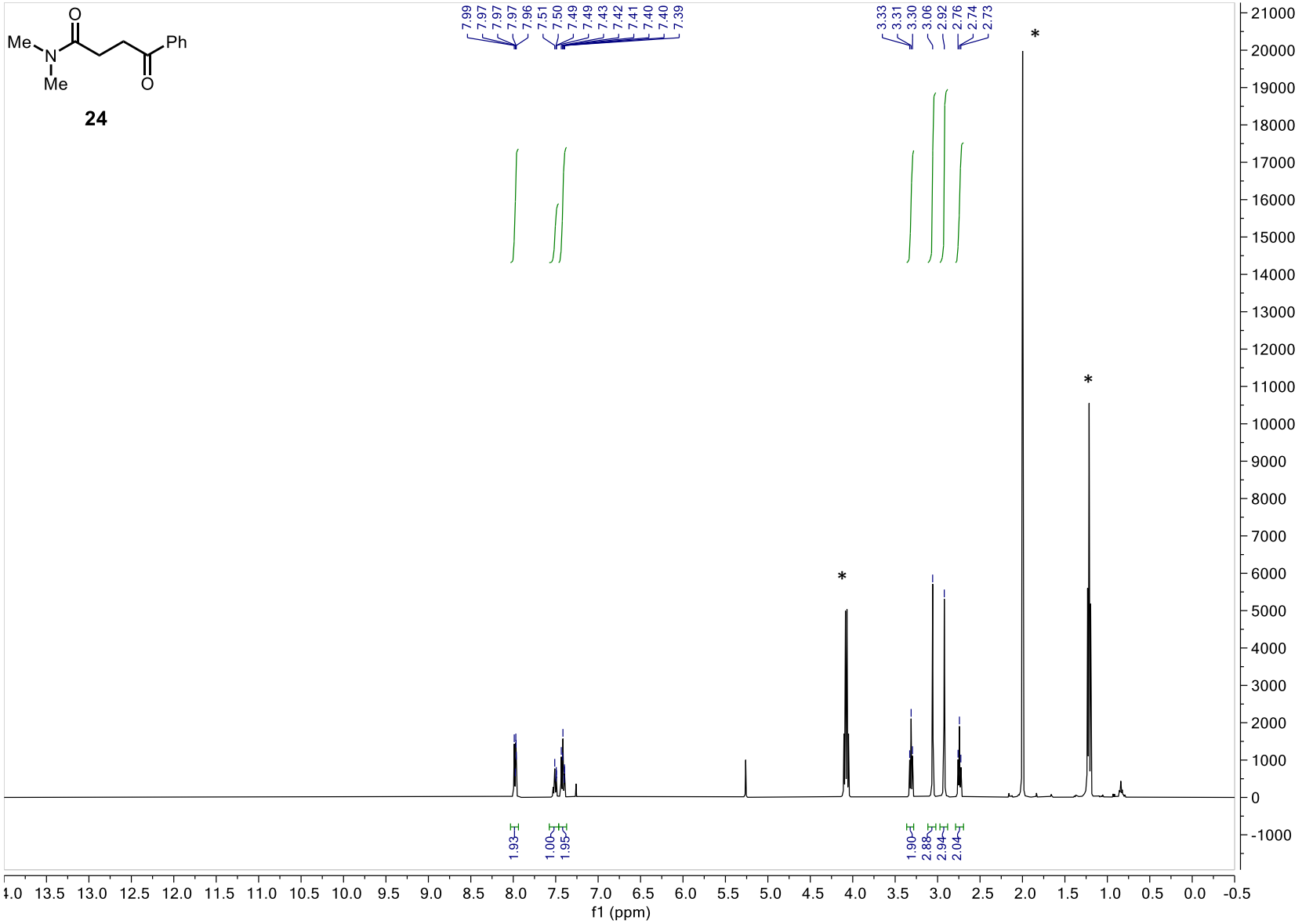
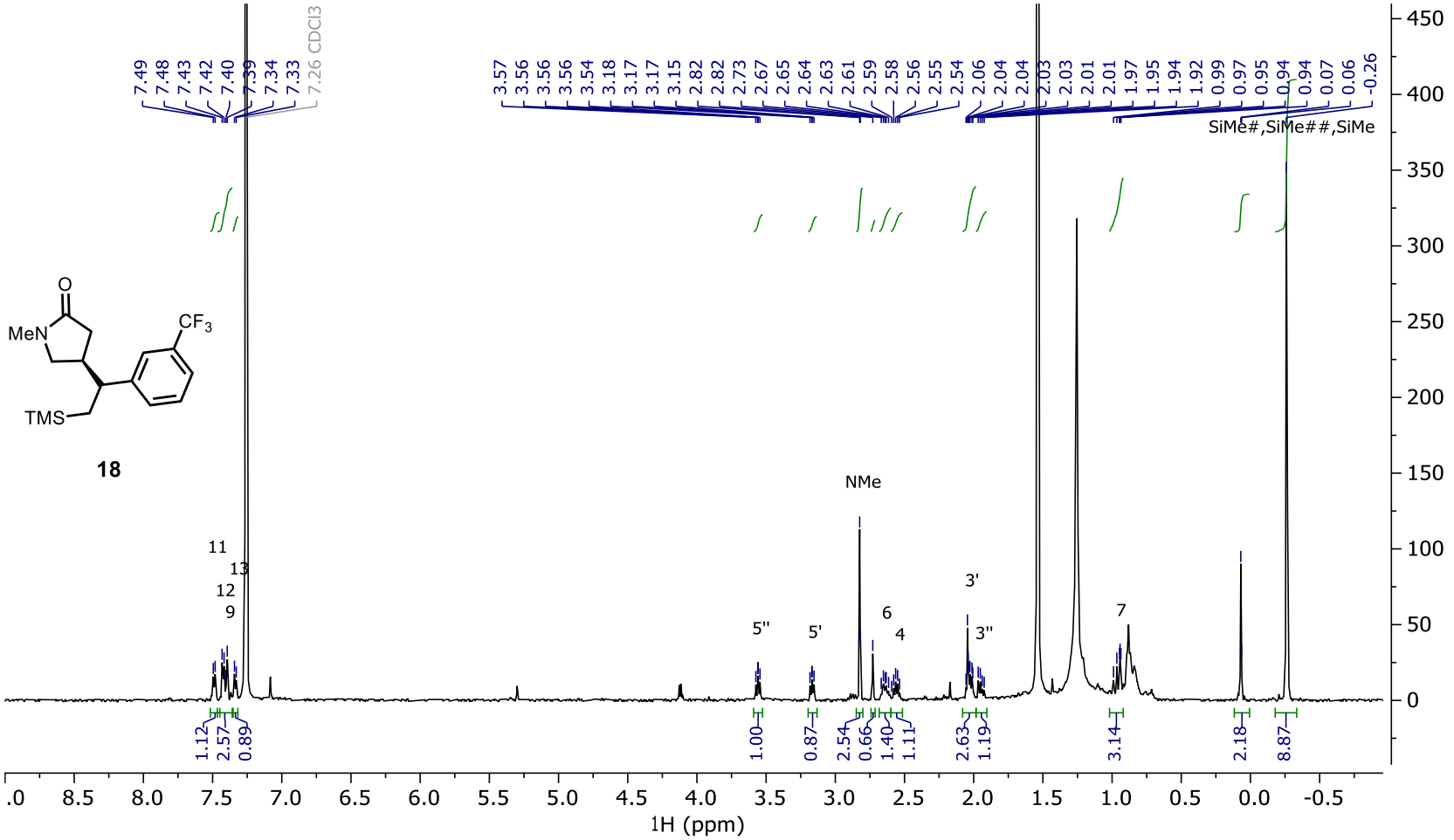
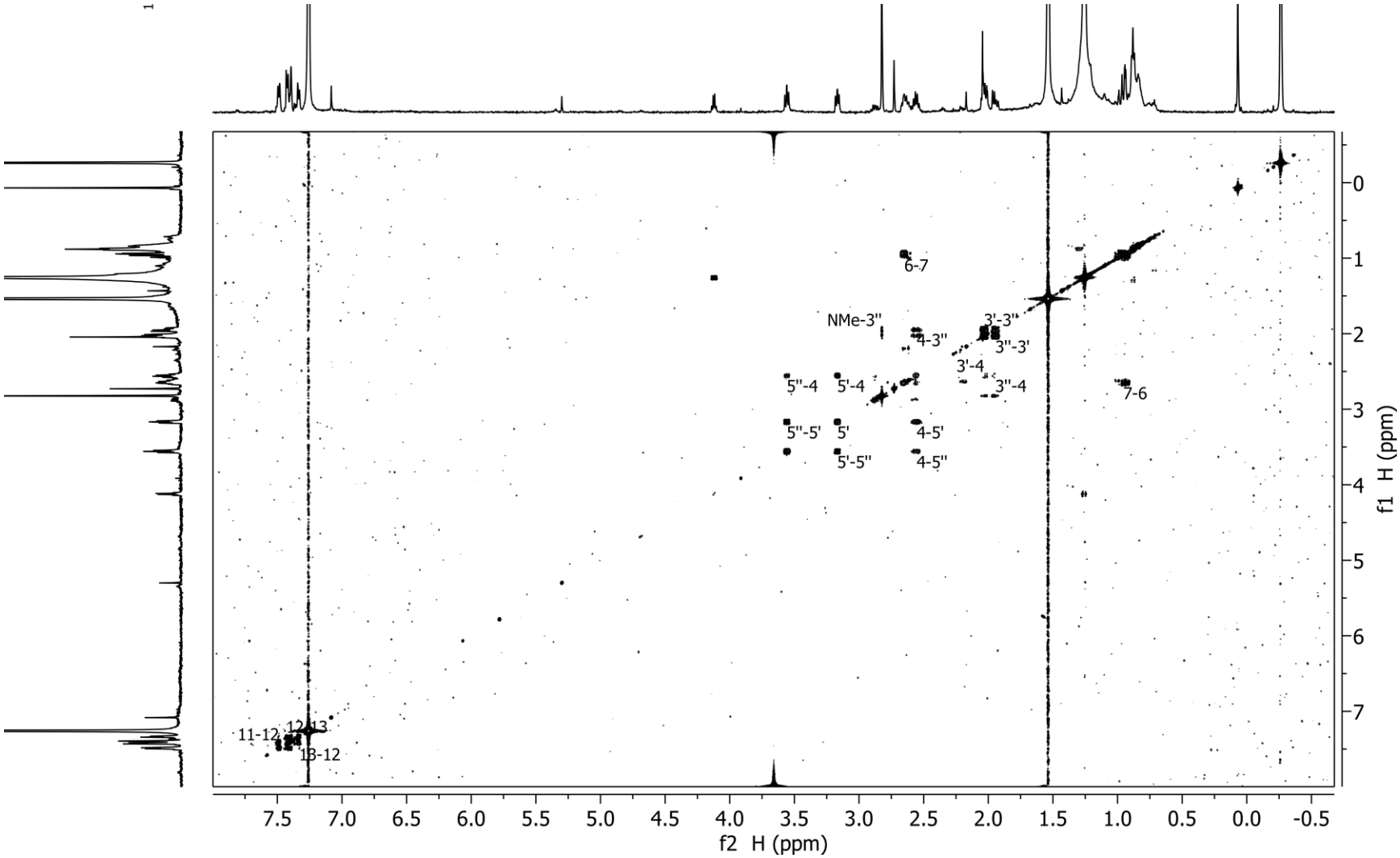
109.10
106.57
54.71
36.54
34.15
29.69
4E+05
4E+05
4E+05
3E+05
2E+05
2E+05
2E+05
1E+05
50000
0
-50000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Me
N
Me
O
23
5.9 5.9 5.9 5.9 5.9 5.9 5.9 5.8 5.8 5.8 5.8 5.1 5.1 5.1 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 3.0 2.9 2.4 2.4
32000 30000 28000 26000 24000 22000 20000 18000 16000 14000 12000 10000 8000 6000 4000 2000 0 -2000
1.00 2.10 3.14 3.19 4.34
4.0 13.5 13.0 12.5 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
f1 (ppm)

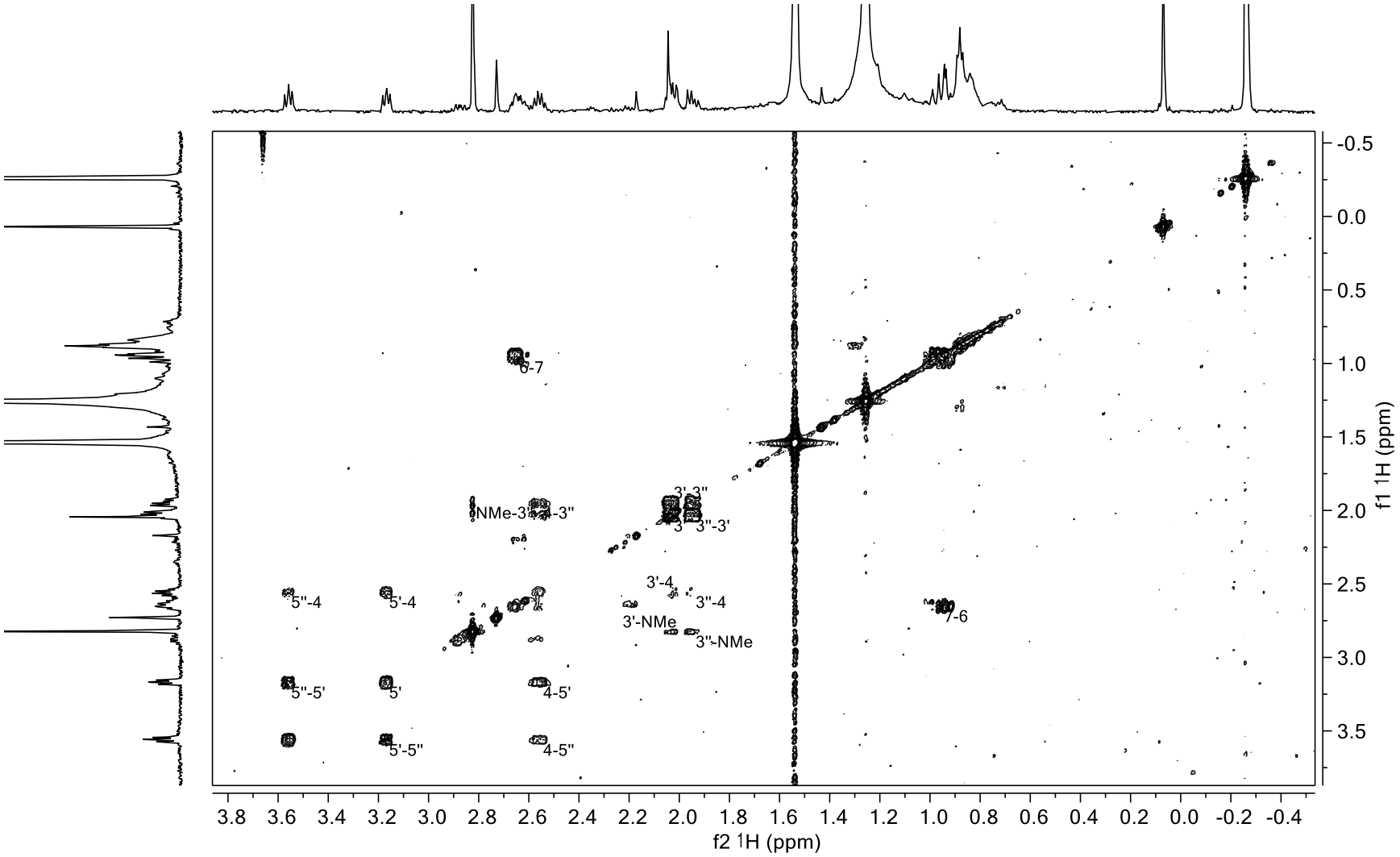

Me
N
Me
O
O
Ph
24
7.99
7.97
7.97
7.97
7.96
7.51
7.50
7.49
7.49
7.43
7.42
7.41
7.40
7.40
7.39
3.33
3.31
3.30
3.06
2.92
2.76
2.74
2.73
*
*
*
1.93
1.00
1.95
1.90
2.88
2.94
2.04
f1 (ppm)

*EtOAc

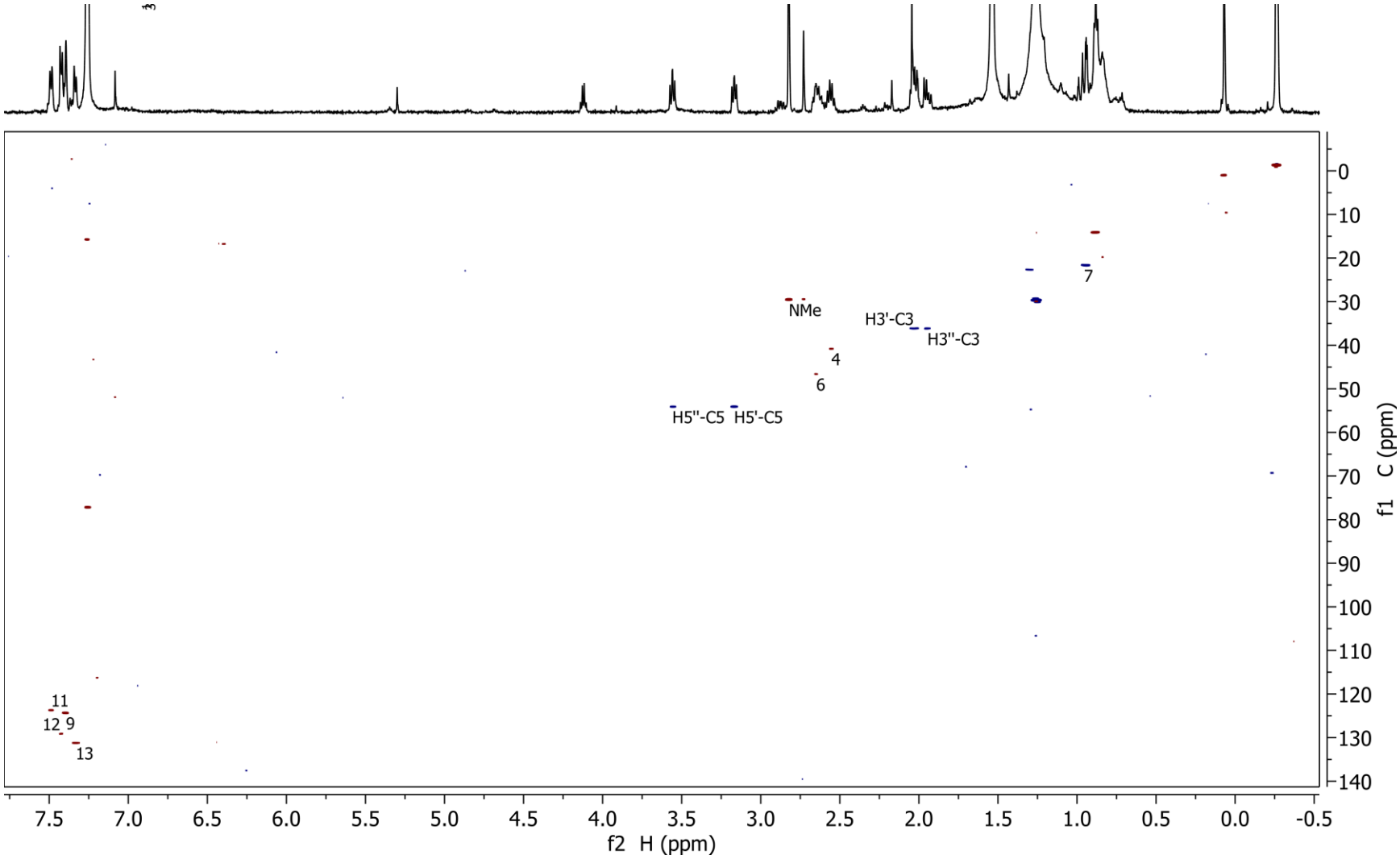

7.49
7.48
7.43
7.42
7.40
7.39
7.34
7.33
7.26 CDCl3
3.57
3.56
3.56
3.56
3.54
3.18
3.17
3.17
3.15
2.82
2.82
2.73
2.67
2.65
2.64
2.63
2.61
2.59
2.58
2.56
2.55
2.54
2.06
2.04
2.04
2.03
2.03
2.01
2.01
1.97
1.95
1.94
1.92
0.99
0.97
0.95
0.94
0.94
0.07
0.06
-0.26
SiMe#,SiMe##,SiMe
MeN
O
CF3
TMS
18
NMe
11
13
12
9
5''
5'
6
4
3'
3''
7
1.12
2.57
0.89
1.00
0.87
2.54
0.66
1.40
1.11
2.63
1.19
3.14
2.18
8.87
450
400
350
300
250
200
150
100
50
0
.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
1H (ppm)



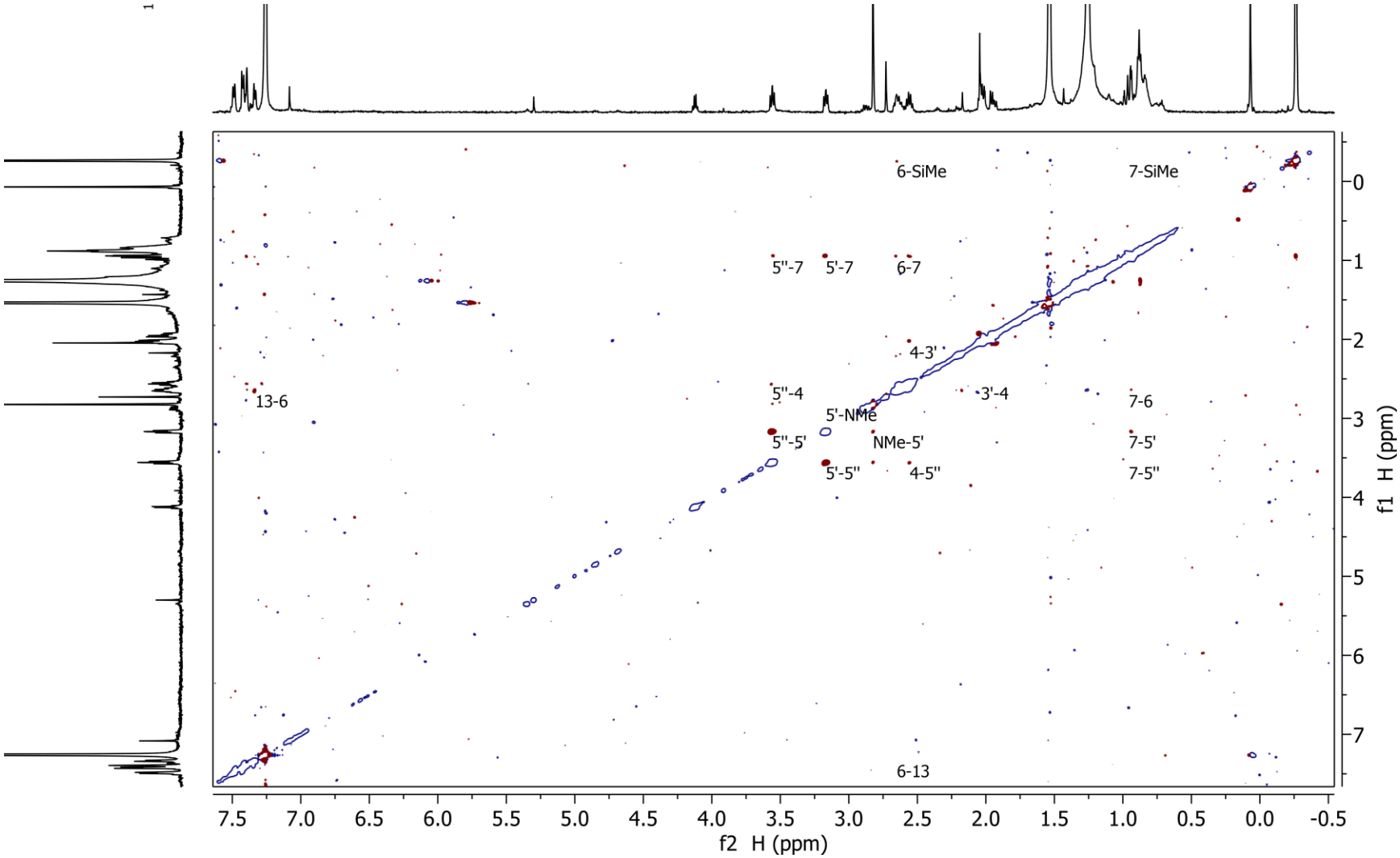
2D Gradient-selected COSY spectrum of XX acquired at 600 MHz at 25C in $CDCl_3$. Correlations are labelled with assignments.



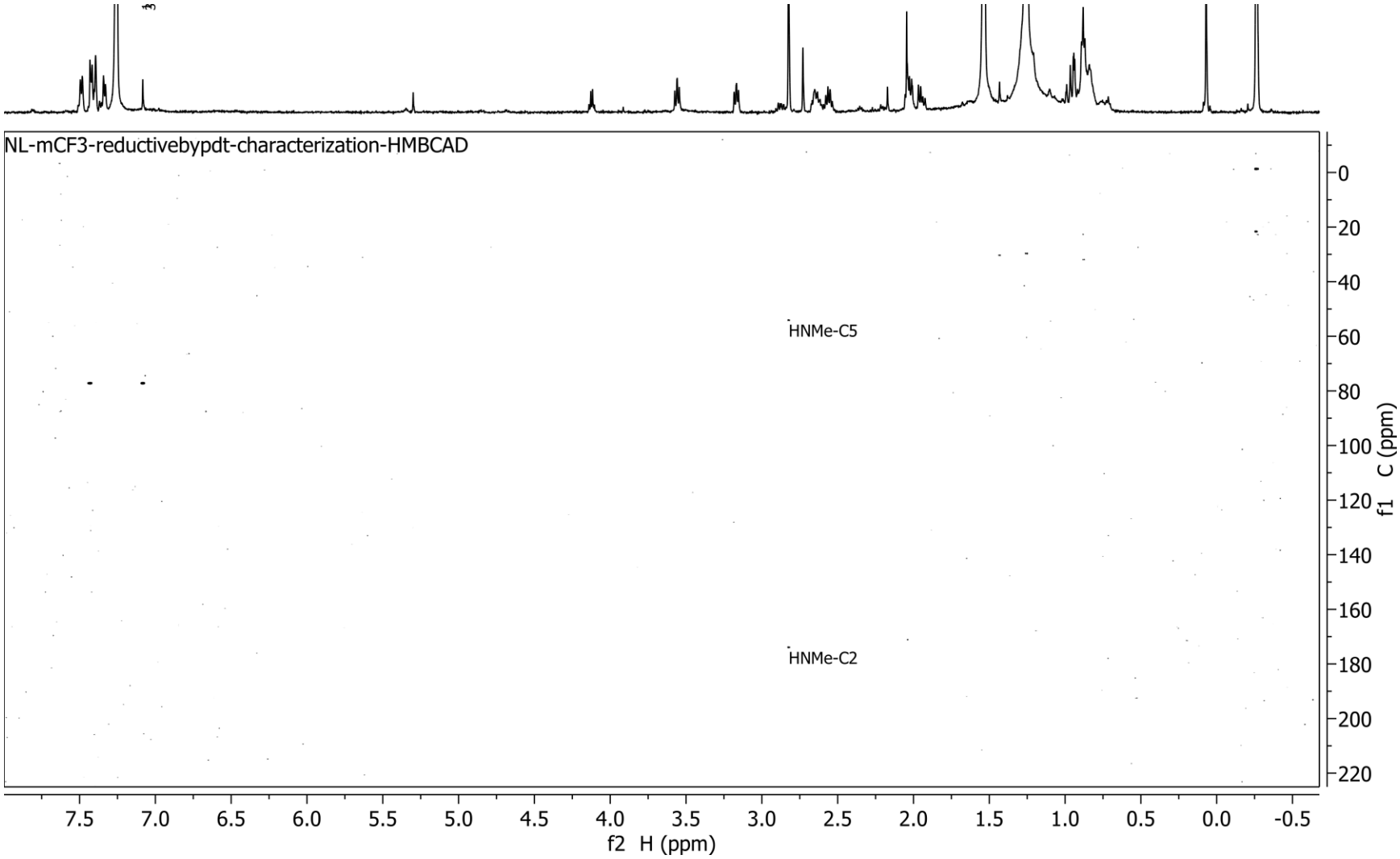
Aliphatic expansion of a 2D gradient-selected COSY spectrum of XX acquired at 600 MHz at 25C in $CDCl_3$. Correlations are labelled with assignments.



2D Multiplicity-edited $^{1}H\{^{13}C\}$ HSQC spectrum of XX acquired at 600 MHz at 25C in $CDCl_3$. Red contours represent CH and CH_3 and blue contours represent CH2's. Numbers indicate assignments.



2D NOESY spectrum of XX acquired at 600 MHz at 25C in $CDCl_3$ with 800 ms mixing time. The diagonal is phased down and displayed with a single blue contour level for clarity. Correlations are labelled with assignments.



2D phase-sensitive $^{1}H\{^{13}C\}$ HMBC spectrum of XX acquired at 600 MHz at 25C in $CDCl_3$. Labels indicate assignments.