Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

P450-catalyzed asymmetric cyclopropanation of electron-deficient olefins under aerobic conditions

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SUPPLEMENTARY MATERIAL

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Materials and Methods

Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers (Sigma-Aldrich, Acros) and used without further purification. Silica gel chromatography purifications were carried out using AMD Silica Gel 60, 230-400 mesh. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Mercury 300 spectrometer (300 MHz and 75 MHz, respectively) or a Varian Inova 500 MHz (500 MHz and 126 MHz, respectively) and are internally referenced to residual solvent peak for chloroform. Data for ^{1}H NMR are reported in the conventional form: chemical shift (δ ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad), coupling constant (Hz), integration. High-resolution mass spectra were obtained with a JEOL JMS-600H High Resolution Mass Spectrometer at the California Institute of Technology Mass Spectral Facility. Reactions were monitored using thin layer chromatography (Merck 60 silica gel plates) using an UV light for visualization and an acidic mixture of anisaldehyde, phosphomolybdic acid, or ceric ammonium molybdate, or basic aqueous $KMnO_4$ as developing agents. Gas chromatography (GC) analyses were carried out using a Shimadzu GC-17A gas chromatograph with FID detector and J&W HP-5 column (30 m x 0.32 mm, 0.25 μm film) and 2-phenylethanol as an internal standard. Gas chromatography mass spectrometry (GC-MS) analyses were carried out using a Shimadzu TQ8030 GC-MS with ion count detector and J&W HP-5 column (30 m x 0.32 mm, 0.25 μM film). Analytical SFC was performed with a Mettler SFC supercritical CO_2 analytical chromatography system utilizing Chiralpak AS column or OD column (4.6 mm x 25 cm) obtained from Daicel Chemical Industries, Ltd. Semi-preparative HPLC was performed using an Agilent 1200 series, a UV detector, and an Agilent XDB-C18 column (9.4 mm x 250 mm, 5 μm).

Plasmids pCWori[BM3] and pET22 were used as cloning vectors. Site-directed mutagenesis was accomplished by a modified Quikchange protocol using primers bearing desired mutations (IDT, San Diego, CA). Restriction enzymes BamHI, EcoRI, XhoI, Phusion polymerase, and T4 ligase were purchased from New England Biolabs (NEB, Ipswich, MA).

General Procedures

CO binding assay. CO assay was used to determine the concentration in crude lysate. Cells were lysed by sonication and two cuvettes containing crude lysate with hemoprotein of unknown concentration were prepared. Carbon monoxide was gently bubbled through one solution for 30 seconds and $Na_2S_2O_4$ (<2 mg) was added immediately. $Na_2S_2O_4$ (2 mg) was added to the other cuvette as well and both were sealed with parafilm. Hemoprotein concentration was determined from ferrous CO binding difference spectrum between the two samples using extinction coefficients of $\varepsilon_{422\text{-}490}$ = 180 mM^{-1} cm^{-1} for histidine-ligated BM3.[1]

Small scale whole cell reactions. *E. coli* (BL21) cells coding for appropriate enzyme variant were grown from glycerol stock overnight (37 °C, 250 rpm) in 5 ml TB_{amp}. The pre-culture was used to inoculate 45 mL of Hyperbroth medium (1 L Hyperbroth prepared from powder from AthenaES©, 0.1 mg mL^{-1} ampicillin) in a 125 mL Erlenmeyer flask and this culture was incubated at 37 °C, 200 rpm for approximately 3 h. At OD_{600} = 1.8, the cultures were cooled to 22 °C and the shaking was reduced to 140 rpm before inducing with IPTG (0.25 mM) and δ-aminolevulinic acid (0.50 mM). Cultures were harvested after 20 h and resuspended in nitrogen-free M9-N medium (1 L: 31 g Na_2HPO_4, 15 g KH_2PO_4, 2.5 g NaCl, 0.24 g $MgSO_4$, 0.010 g $CaCl_2$) until the indicated OD_{600} (usually OD_{600} = 60) is obtained. Aliquots of the cell suspension were used for determination of the enzyme expression level (2–3 mL) after lysis.

Anaerobic conditions: E. coli cells (OD_{600} = 60) were transferred to a crimped 6 mL vial and made anaerobic by degassing with argon for 5-10 min. In parallel, glucose (50 µL, 250 mM) was added to 2 mL crimp vials that are sealed. The headspaces of these vials were purged with argon for 5-10 min. If multiple reactions were being carried out in parallel, a maximum of 8 vials were connected via cannulae and degassed in series. Cells (425 µL) were transferred to each vial via syringe and the olefin substrate was added (12.5 µL of a 800 mM solution of styrene in EtOH or a 400 mM solution of acrylamide **1** in EtOH), followed by EDA (12.5 µL of a 350 mM or 400 mM solution in EtOH). The reactions were shaken on a table-top shake plate at room temperature for 5 h. The reactions were quenched by addition of 25 µL of 3 M HCl, followed by 20 µL of the internal standard (20 mM 2-phenylethanol solution in cyclohexane) and 1 mL cyclohexane. The mixture was transferred to a 2 mL Eppendorf tube, vortexed and then centrifuged (10,000x rcf, 30 s). The organic layer was removed and analyzed by GC to determine yield and chiral SFC to determine enantioselectivity.

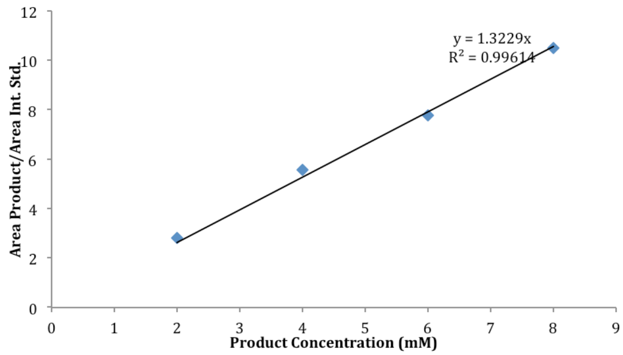
Aerobic conditions: Cell suspension was used without sparging with argon. Cells (425 µL, OD_{600} = 60) and glucose (50 µL, 250 mM) were combined in an unsealed 2 mL glass vial. The olefin substrate was added (12.5 µL, 400 mM in EtOH), followed by EDA (12.5 µL, 400 mM in EtOH). The vial was covered with foil then shaken at 35 rpm for 5 h. The reactions were quenched by addition of 25 µL of 3 M HCl, followed by 20 µL of the internal standard (20 mM 2-phenylethanol solution in cyclohexane) and 1 mL cyclohexane. The mixture was transferred to a 2 mL Eppendorf tube, vortexed and then centrifuged (10,000x rcf, 30 s). The organic layer was removed and analyzed by GC to determine yield and chiral SFC to determine enantioselectivity.

Analysis of crude reaction mixture: GC analysis of product was performed using J&W HP-5 column (30 m x 0.32 mm, 0.25 µM film) with the method 90 °C hold 2 min, 90–110 at 6 °C/min, 110–190 at 40 °C/min, 190–300 at 20 °C/min, 300 °C hold 1 min, 12.8 min total): internal standard (3.55 min), retention times for the *cis* and *trans* products are listed in the characterization section below. Analytical SFC of product was performed on either Chiralpak AS column or OD column, eluting with iPrOH at 2.5 mL/min and detecting at 210 nm. Semi-preparative HPLC for all products was performed on 9.4 mm x 250 mm, 5 µm Agilent XDB-C18 column, detection at 210 nm, flow rate 3.0 mL/min, H_2O/MeCN, gradient: 0 min 10% MeCN, 30 min 70% MeCN, hold 5 min, 40 min 95% MeCN

Calibration of Cyclopropanation Products

Yields of cyclopropanation products were determined using calibration curves made with independently synthesized standards. Stock solutions of product were made at 120 or 160 mM in DMSO. To 4 samples containing cells at OD_{600} = 60, product was added from either of the stock solutions such that a final concentration of 1.5-6.0 or 2.0-8.0 mM product was obtained. Additional DMSO was added such that the total volume of organics added to each tube was 25 µL. Next, 20 µL of a 20 mM stock solution of internal standard in cyclohexane was added to each Eppendorf tube, followed by 1 mL of cyclohexane. The Eppendorf tubes were vortexed and centrifuged (13,000 x rcm, 30 seconds). The organic layer was then analyzed by GC using J&W HP-5 column (30 m x 0.32 mm, 0.25 µM film: 90 °C hold 2 min, 90-110 at 6 °C/min, 110-190 at 40 °C/min, 190–300 at 20 °C/min, 300 °C hold 1 min, 12.8 min total). The ratio of the areas under the internal standard and product peaks was plotted against the concentration for each solution (1.5 to 6.0 mM or 2.0 to 8.0 mM).

6a



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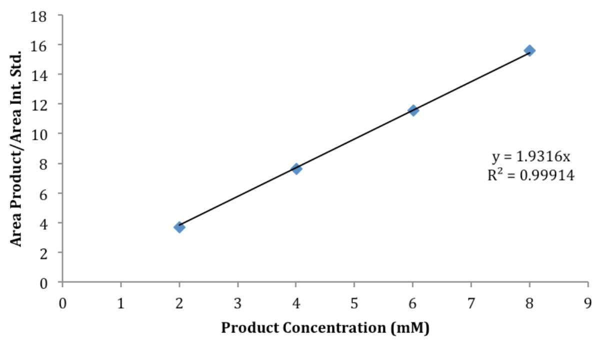

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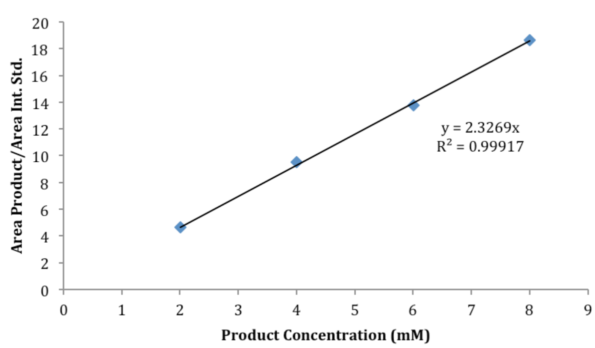

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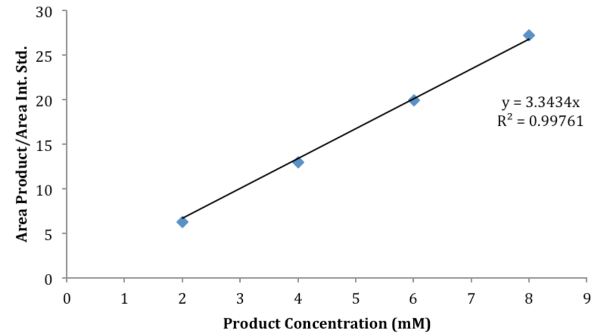

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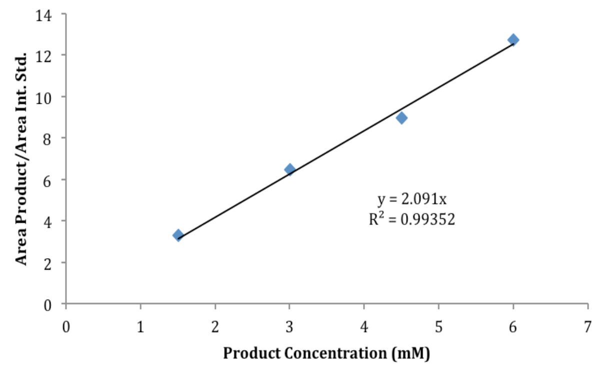

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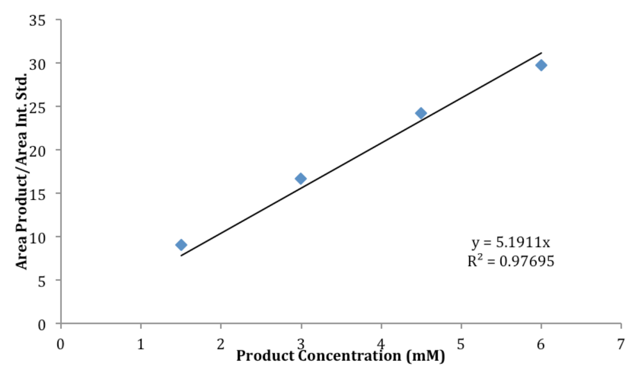

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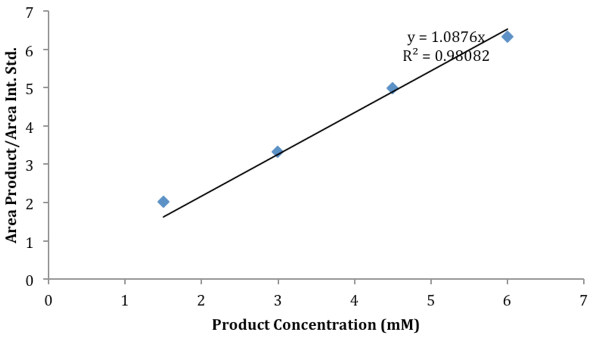

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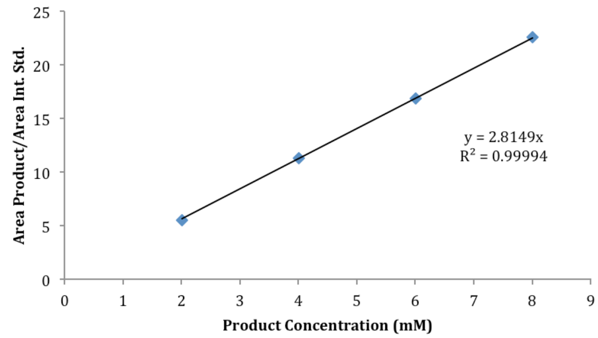

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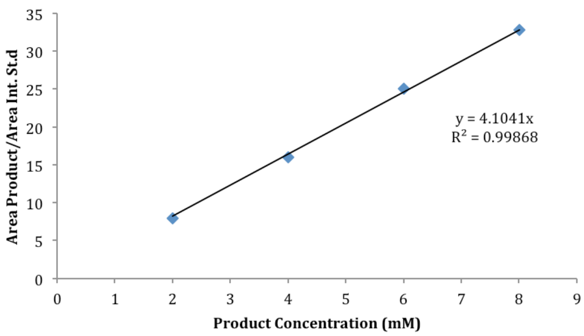

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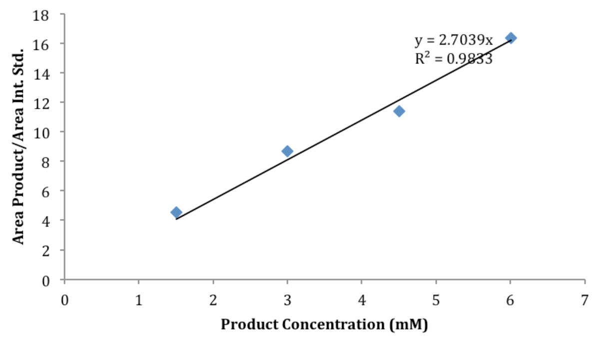

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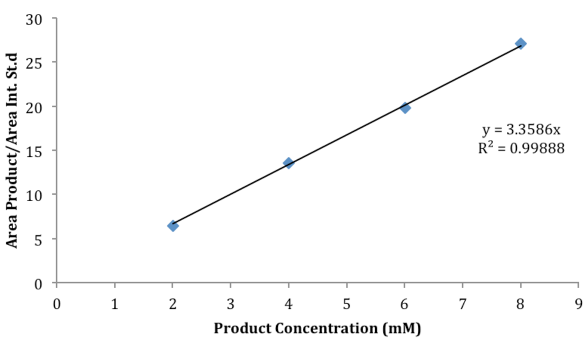

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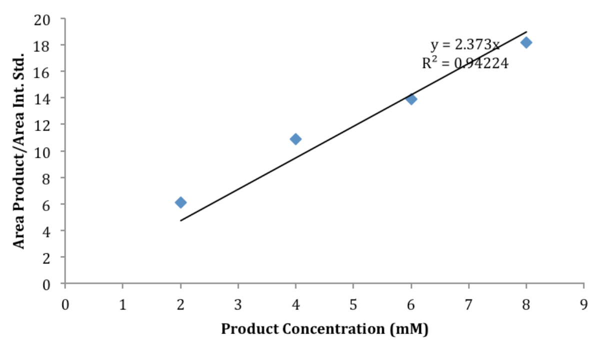

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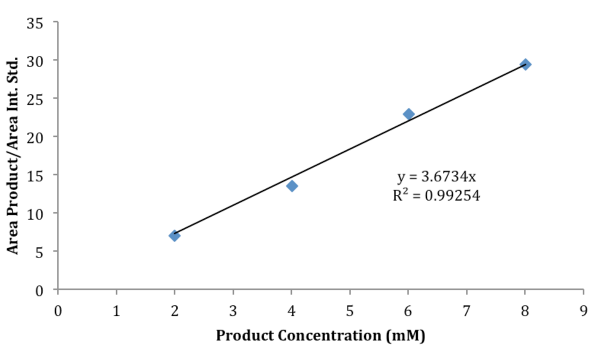

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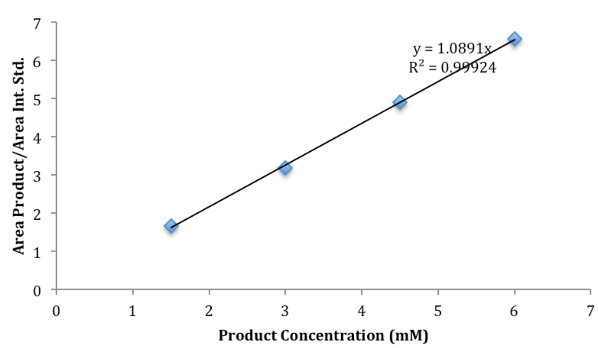

Amino Acid Sequences

Table S1. List of mutations in enzyme variants, relative to wild type BM3 holoprotein (WT). All mutations listed below are for the heme domain. There are no mutations present in the reductase domain relative to wild type.

Enzyme	Amino acid substitution with respect to WT
T268A-AxH	T268A, C400H
BM3-HStar	V78M, L181V, T268A, C400H, L437W

The amino acid sequence for WT (holoprotein) is as follows:

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>SEQ ID NO:1: gi|142798|gb|AAA87602.1| cytochrome P-450:NADPH-P-450 reductase
precursor [Bacillus megaterium]

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDESRFDKNLSQALK
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VKQLKYVGMVLNEALRLWPTAPAFSLYAKEDTVLGGEYPLEKGDELMVLIPQLHRDKTIWGDDVEEFRPERFENPSA
IPQHAFKPFGNGQRACIGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKETLTLKPEGFVVKAKSKKIPLGGIPSP
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GQSLGEAHLYFGCRSPHEDYLYQEELENAQSEGIITLHTAFSRMPNQPKTYVQHVMEQDGKKLIELLDQGAHFYICG
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The nucleotide sequence for WT (holoprotein) is as follows:

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General Procedures for Synthesis of Acrylamides 5a–5g

To a solution of atropic acid (444 mg, 3.0 mmol) in DCM (6 mL, 0.5 M) at 0 ºC was added oxalyl chloride (0.31 mL, 3.6 mmol) dropwise, followed by catalytic amount of DMF. The resulting colorless solution was then warmed to room temperature and stirred for 2 h, upon which it turned slightly yellow. After cooling to 0 ºC, saturated $NaHCO_3$ (6 mL) was added, followed by the appropriate amine (4.5 mmol, 1.5 eq) dropwise. The resulting solution was stirred vigorously until a uniform emulsion was obtained at room temperature overnight. The organic and aqueous layers were separated and the aqueous layer was extracted with Et_2O (2 x 5 mL). The combined organic layer was washed with H_2O (5 mL), and brine (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. When necessary, purification was performed with silica gel chromatography. Spectral data for **5a–5f** are in agreement with those reported in the literature.[2,3,4,5,6]

5g

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46–7.38 (m, 1H), 7.34–7.23 (m, 2H), 7.09–6.98 (m, 1H), 5.70 (s, 1H), 5.47 (s, 1H), 3.81 (s, 2H), 2.74–2.66 (m, 2H), 2.01–1.86 (m, 2H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.0, 146.2, 138.5, 136.4, 128.6, 128.4, 126.2, 126.0, 125.0, 124.6, 116.9, 45.8, 26.9, 24.0.

General Procedures for Synthesis of Acrylamides 7a–7e

To a solution of the appropriately-substituted phenylacetic acid (3.0 mmol) in DCM (6 mL) was added oxalyl chloride (0.31 mL, 3.6 mmol) dropwise, followed by catalytic amount of DMF. The resulting colorless solution was then stirred at room temperature for 2 h, upon which it turned slightly yellow. Saturated $NaHCO_3$ (6 mL) was added, followed by Et_2NH (4.5 mmol, 1.5 eq) dropwise. The resulting solution was stirred vigorously (uniform emulsion) at room temperature overnight. The organic and aqueous layers were separate and the aqueous layer was extracted with Et_2O (2 x 5 mL). The combined organic layer was washed with H_2O (5 mL), and brined (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The resulting oil was used for the next step without further purification.

To a solution of the crude diethylacetamide (1 mmol) in DMF (4 mL) was added Cs_2CO_3 (977 mg, 3 mmol, 3 eq), HCHO (60 mg, 2 mmol, 2 eq), and tetrabutylammonium bromide (81 mg, 0.25 mmol, 0.25 eq). The resulting suspension was stirred vigorously and heated to 80 ºC for 24 h. After cooling to room temperature, the reaction was quenched with H_2O (5 mL). The organic and aqueous layers were separated and the aqueous layer was extracted with Et_2O (2 x 5 mL). The combined organic layer was washed with H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. After a short silica plug eluting with 2:1 hexanes:EtOAc, a pale yellow oil was obtained which was pure enough to be used for the next step without purification. Spectral data for **7c** and **7d** are in agreement with those reported in the literature.[7]

7a

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27–7.23 (m, 4H), 5.68 (s, 1H), 5.31 (s, 1H), 3.52 (q, J = 7.1 Hz, 2H), 3.23 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H), 1.02 (t, J = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.4, 145.9, 138.4, 135.9, 129.4, 129.32, 128.7, 126.4, 122.9, 112.9, 42.9, 38.9, 21.5, 14.1, 12.9.

7b

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 8.0, 2H), 5.65 (s, 1H), 5.26 (s, 1H), 3.49 (dt, *J* = 7.7, 6.7 Hz, 2H), 3.22 (dt, *J* = 7.7, 6.7 Hz, 2H), 1.21 (td, *J* = 7.2, 0.9 Hz, 3H), 1.00 (td, *J* = 7.1, 0.8 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.5, 145.7, 138.5, 133.2, 129.6, 125.7, 112.13, 42.9, 38.9, 21.3, 14.1, 12.9.

7e

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64–7.61 (m, 2H), 7.59–7.55 (m, 2H), 5.79 (s, 1H), 5.46 (s, 1H), 3.53 (q, *J* = 7.1 Hz, 2H), 3.24 (q, *J* = 7.1 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 169.5, 144.6, 139.5, 130.4 (q, *J* = 32.2 Hz), 126.1, 125.9 (q, *J* = 3.8 Hz), 124.1 (q, *J* = 272.8 Hz), 115.3, 43.0, 39.1, 14.2, 12.9.

7f

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91–7.77 (m, 4H), 7.64 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.55–7.44 (m, 2H), 5.84 (s, 1H), 5.43 (s, 1H), 3.57 (q, *J* = 7.1 Hz, 2H), 3.26 (q, *J* = 7.1 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.4, 145.7, 133.5, 133.4, 133.2, 128.6, 128.5, 127.7, 126.5, 125.3, 123.2, 123.2, 113.5, 42.9, 39.0, 14.2, 12.94.

Synthesis of acrylate 9

To a solution of the corresponding ethyl ester (0.5 mmol) in DMF (3 mL) was added paraformaldehyde (1.0 mmol, 2 eq), followed by K_2CO_3 (0.5 mmol). The resulting suspension was stirred vigorously and heated to 70 ºC overnight. The organic and aqueous layers were separated and the aqueous layer was extracted with Et_2O (2 x 5 mL). The combined organic layer was washed with H_2O (5 mL), and brine (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. After a short silica plug (washing with 2:1 hexanes:EtOAc), a pale yellow oil was obtained which was pure enough to be used for the next step without further purification.

Preparative Scale Whole Cell Aerobic Reactions

For characterization purposes, the aerobic reactions were scaled up as follows: Cells (8.5 mL, OD_{600} = 60) and glucose (1.0 mL, 250 mM) were combined in an unsealed scintillation vial. The olefin substrate was added (0.25 mL, 400 mM in EtOH), followed by EDA (0.25 mM, 400 mM in EtOH). The vial was capped and then shaken at 35 rpm for 5 h. The reactions were quenched by addition of 0.25 mL of 3 M HCl, poured into a Falcon tube, extracted with 1:1 EtOAc:hexanes (7.5 mL), and centrifuged (5,000 rpm, 5 min). The organics were collected, and this extraction sequence was repeated once. The organics were combined, dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified via semi-preparative HPLC, with the exception of **10**, which was purified by silica gel chromatography (1;1 Hexanes:DCM to 100% DCM).

Characterization Data for Cyclopropanes

6a

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H), 4.19 (ddt, *J* = 7.2, 5.4, 2.6 Hz, 2H), 2.94 (s, 3H), 2.90 (s, 3H), 2.43 (dd, *J* = 8.3, 6.3, 1H), 2.14 (dd, *J* = 6.4, 4.9 Hz, 1H), 1.51 (dd, *J* = 8.4, 5.1 Hz, 1H), 1.28 (td, *J* = 7.2, 1.8 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.9, 168.5, 138.8, 129.0, 127.4, 126.3, 61.1, 38.8, 37.3, 35.8, 28.9, 21.8, 14.4.

HRMS (*m/z*): calcd for $C_{15}H_{19}O_3N$, $[M+H]^+$, 262.1443; found, 262.1446;

GC: Using method described on page SI-4, t_R (min): *cis* = 9.25, *trans* = 9.42.

HPLC: Using method described on page SI-4, t_R (min) = 21.7.

SFC: AS column, 4% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 6.45, minor = 7.53.

6b

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H), 4.39–3.98 (m, 2H), 3.23 (s, 2H), 3.11 (s, 3H), 2.51 (dd, *J* = 8.6, 6.3 Hz, 1H), 2.09 (t, *J* = 5.6 Hz, 1H), 1.58 (d, *J* = 4.4 Hz, 1H), 1.28 (dd, *J* = 7.8, 6.5 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.4, 138.8, 128.7, 127.8, 127.5, 110.2, 61.1, 60.8, 38.9, 33.6, 26.4, 20.8, 14.3.

HRMS (*m/z*): calcd for $C_{15}H_{19}O_4N$, $[M+H]^+$, 278.1392; found, 278.1398;

GC: Using method described on page SI-4, t_R (min): *cis* = 9.22, *trans* = 9.40.

HPLC: Using method described on page SI-4, t_R = 23.9 min.

SFC: OD column, 10% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 3.88, minor = 4.40.

6c

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H), 4.19 (qd, *J* = 7.1, 1.9 Hz, 2H), 3.54 –3.43 (m, 2H), 3.39–3.28 (m, 1H), 3.29–3.16 (m, 1H), 2.41 (dd, *J* = 8.4, 6.2 Hz, 1H), 2.18 (dd, *J* = 6.2, 4.9 Hz, 1H), 1.86–1.68 (m, 4H), 1.49 (dd, *J* = 8.4, 4.9 Hz, 1H), 1.29 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.0, 166.8, 138.6, 128.9, 127.4, 126.7, 61.1, 46.6, 46.4, 40.1, 28.3, 26.2, 24.2, 21.2, 14.4.

HRMS (*m/z*): calcd for $C_{17}H_{21}O_3N$, $[M+H]^+$, 288.1600; found, 288.1591;

GC: Using method described on page SI-4, t_R (min): *cis* = 10.51, *trans* = 10.60.

HPLC: Using method described on page SI-4, t_R = 23.5 min.

SFC: AS column, 10% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 5.12, minor = 6.54.

6d

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H), 3.83–3.66 (m, 1H), 3.51 (ddd, *J* = 13.3, 6.7, 3.8 Hz, 1H), 3.45–3.32 (m, 1H), 3.25 (ddd, *J* = 13.3, 8.2, 3.6 Hz, 1H), 2.46 (dd, *J* = 8.4, 6.2 Hz, 1H), 2.16 (dd, *J* = 6.2, 4.9 Hz, 1H), 1.59–1.45 (m, 4H) 1.50 (dd, *J* = 8.3, 5.0 Hz, 1H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.26–1.09 (m, 2H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.9, 166.9, 139.1, 128.9, 128.9, 127.3, 126.4, 61.1, 46.7, 43.3, 38.8, 28.5, 25.7, 25.6, 25.5, 24.6, 21.7, 14.4.

HRMS (*m/z*): calcd for $C_{18}H_{23}O_3N$, $[M+H]^+$, 302.1756; found, 302.1770;

GC: Using method described on page SI-4, t_R (min): *cis* = 10.68, *trans* = 10.81.

HPLC: Using method described on page SI-4, t_R = 27.4 min.

SFC: AS column, 10% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 4.06, minor = 4.36.

6e

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H), 4.45–4.05 (m, 2H), 3.71–3.65 (m, 1H), 3.64–3.55 (m, 3H), 3.51–3.43 (m, 1H), 3.42–3.20 (m, 3H), 2.49 (dd, *J* = 8.4, 6.2 Hz, 1H), 2.16 (dd, *J* = 6.2, 4.9 Hz, 1H), 1.50 (dd, *J* = 8.5, 4.9 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.7, 167.4, 138.5, 129.1, 129.1, 127.6, 126.2, 66.8, 66.3, 61.3, 46.3, 42.7, 38.4, 28.3, 21.7, 14.4.

HRMS (*m/z*): calcd for $C_{17}H_{21}O_4N$, $[M+H]^+$, 304.1549; found, 304.1538;

GC: Using method described on page SI-4, t_R (min): *cis* = 10.60, *trans* = 10.77.

HPLC: Using method described on page SI-4, t_R (min) = 21.0.

SFC: AS column, 5% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 5.98, minor = 6.47.

6f

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.18 (m, *6*H), 7.14–7.09 (m, 2H), 6.94–6.82 (m, 2H), 4.23 (q, *J* = 7.2 Hz, 2H), 3.27 (s, 3H), 1.97 (t, *J* = 7.3 Hz, 1H), 1.73 (t, *J* = 5.9 Hz, 1H), 1.37–1.25 (m, 4H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.5, 168.2, 143.7, 139.7, 129.1, 128.5, 127.9, 127.5, 127.2, 61.1, 39.8, 38.7, 30.1, 21.2, 14.4.

HRMS (*m/z*): calcd for $C_{20}H_{21}O_3N$, $[M+H]^+$, 324.1600; found, 324.1596;

GC: Using method described on page SI-4, t_R (min): *cis* = 10.94, *trans* = 11.11.

HPLC: Using method described on page SI-4, t_R (min) = 30.8.

SFC: OD column, 10% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 6.27, minor = 7.09.

6g

^{1}H NMR (500 MHz, C_6D_6, 25 ºC): δ 8.49 (brs, 1H), 7.59–6.70 (m, 8H), 4.14–3.89 (m, 2H), 3.20 (brs, 1H), 2.70–1.83 (brm, 3H), 2.24 (dt, *J* = 15.8, 6.6 Hz, 1H), 1.41–1.06 (brm, 1H), 1.24 (dd, *J* = 8.2, 5.4 Hz, 1H), 0.97 (t, *J* = 7.1 Hz, 3H).

^{1}H NMR (500 MHz, C_6D_6, 65 ºC): δ 7.68 (brs, 1H), 7.36 (d, *J* = 7.7 Hz, 2H), 7.12–6.81 (m, 6H), 4.15–3.92 (m, 3H), 3.29 (dt, *J* = 12.6, 5.8 Hz, 1H), 2.47 (dd, *J* = 15.2, 7.6 Hz, 1H), 2.31 (dt, *J* = 15.8, 6.6 Hz, 1H), 1.95 (brs, 1H), 1.67 (brs, 1H), 1.37–1.31 (m, 1H), 1.29 (dd, *J* = 8.2, 5.4 Hz, 1H), 1.04 (t, *J* = 7.1, 3H).

^{13}C NMR (C_6D_6, 126 MHz): δ 170.8, 167.7, 146.9, 139.9, 129.0, 128.6, 127.3, 126.9, 126.0, 125.4, 124.9, 61.0, 44.4, 39.7, 26.9, 23.8, 21.6, 14.3.

HRMS (*m/z*): calcd for $C_{22}H_{23}O_3N$, $[M+H]^+$, 350.1756; found, 350.1760;

GC: Using method described on page SI-4, t_R (min): *cis* = 12.30, *trans* = 12.38.

HPLC: Using method described on page SI-4, t_R (min) = 35.6.

SFC: OD column, 10% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 10.17, minor = 11.21.

8a

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (t, J = 7.6 Hz, 1H), 7.14 (td, J = 1.6, 0.7 Hz, 1H), 7.07 (dddt, J = 12.6, 7.5, 1.8, 1.0 Hz, 2H), 4.18 (qd, J = 7.2, 1.1 Hz, 2H), 3.59–3.41 (m, 2H), 3.19 (ddq, J = 38.7, 14.2, 7.1 Hz, 2H), 2.43 (dd, J = 8.4, 6.2 Hz, 1H), 2.33 (s, 3H), 2.17 (dd, J = 6.2, 4.9 Hz, 1H), 1.48 (dd, J = 8.4, 4.9 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.10 (t, J = 7.1 Hz, 3H), 0.78 (t, J = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.8, 167.9, 139.1, 138.6, 128.8, 128.1, 127.3, 123.4, 61.1, 41.5, 39.4, 39.1, 28.3, 21.5, 21.3, 14.4, 13.2, 12.4.

HRMS (*m*/*z*): calcd for $C_{18}H_{25}O_3N$, $[M+H]^+$, 304.1913; found, 304.1917;

GC: Using method described on page SI-4, t_R (min): *cis* = 9.80, *trans* = 10.09.

HPLC: Using method described on page SI-4, t_R (min) = 29.8.

SFC: AS column, 2% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 7.66, minor = 9.24.

8b

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.23–7.17 (m, 2H), 7.15–7.08 (m, 2H), 4.18 (qd, J = 7.1, 1.6 Hz, 2H), 3.60–3.36 (m, 2H), 3.30–3.09 (m, 2H), 2.41 (dd, J = 8.3, 6.2 Hz, 1H), 2.33 (s, 1H), 2.16 (dd, J = 6.2, 4.9 Hz, 1H), 1.46 (dd, J = 8.4, 4.8 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.09 (t, J = 7.1 Hz, 3H), 0.78 (t, J = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.9, 167.9, 137.0, 136.2, 129.6, 129.0, 128.8, 126.4, 61.0, 41.5, 39.4, 38.9, 28.3, 21.2, 21.1, 14.4, 13.2, 12.5.

HRMS (*m*/*z*): calcd for $C_{18}H_{25}O_3N$, $[M+H]^+$, 340.1913; found, 340.1917;

GC: Using method described on page SI-4, t_R (min): *cis* = 9.93, *trans* = 10.19.

HPLC: Using method described on page SI-4, t_R (min) = 29.9.

SFC: AS column, 2% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 8.85, minor = 10.47.

8c

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25 (d, J = 8.9 Hz, 2H), 6.85 (d, J = 8.9, 2H), 4.17 (qd, J = 6.3, 5.5, 3.4 Hz, 2H), 3.80 (s, 3H), 3.54 (dq, J = 15.0, 7.5 Hz, 1H), 3.45 (dq, J = 14.1, 7.1 Hz, 1H), 3.20 (ddt, J = 28.0, 14.2, 7.1 Hz, 2H), 2.37 (dd, J = 8.2, 6.3 Hz, 1H), 2.14 (dd, J = 6.3, 4.7 Hz, 1H), 1.45 (dd, J = 8.2, 4.7 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.09 (t, J = 7.1 Hz, 3H), 0.79 (t, J = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.9, 168.0, 159.0, 131.3, 130.0, 127.9, 114.3, 61.0, 55.5, 41.5, 39.4, 38.6, 28.3, 21.0, 14.4, 13.3, 12.5.

HRMS (*m/z*): calcd for $C_{18}H_{25}O_4N$, $[M+H]^+$, 320.1862; found, 320.1866;

GC: Using method described on page SI-4, t_R (min): *cis* = 10.56, *trans* = 10.84.

HPLC: Using method described on page SI-4, t_R (min) = 26.6.

SFC: AS column, 4% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 6.49, minor = 7.50.

8d

^{1}H NMR (500 MHz, CDCl$_3$): δ 7.35–7.21 (m, 4H), 4.18 (qd, *J* = 7.1, 1.6 Hz, 2H), 3.58–3.36 (m, 2H), 3.21 (ddq, *J* = 37.3, 14.2, 7.1 Hz, 2H), 2.38 (dd, *J* = 8.4, 6.2 Hz, 1H), 2.18 (dd, *J* = 6.3, 5.0 Hz, 1H), 1.47 (dd, *J* = 8.4, 5.0 Hz, 1H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.1 Hz, 3H), 0.82 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (CDCl$_3$, 126 MHz): δ 170.5, 167.4, 137.8, 133.3, 130.2, 129.1, 128.6, 128.0, 61.2, 41.5, 39.5, 38.5, 28.5, 21.2, 14.4, 13.3, 12.4.

HRMS (*m/z*): calcd for $C_{17}H_{22}O_3ClN$, $[M+H]^+$, 324.1366; found, 324.1368;

GC: Using method described on page SI-4, t_R (min): *cis* = 10.29, *trans* = 10.56.

HPLC: Using method described on page SI-4, t_R (min) = 31.1.

SFC: AS column, 4% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 5.47, minor = 5.91.

8e

^{1}H NMR (500 MHz, CDCl$_3$): δ 7.62–7.56 (m, 2H), 7.47–7.36 (m, 2H), 4.20 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.60–3.42 (m, 2H), 3.22 (ddq, *J* = 43.6, 14.2, 7.1 Hz, 2H), 2.45 (dd, *J* = 8.4, 6.3 Hz, 1H), 2.24 (dd, *J* = 6.3, 5.1 Hz, 1H), 1.54 (dd, *J* = 8.5, 5.1 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H), 1.11 (t, *J* = 7.1 Hz, 3H), 0.82 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (CDCl$_3$, 126 MHz): δ 170.3, 167.1, 143.4, 129.8 (q, *J* = 32.7 Hz), 126.9, 125.9 (q, *J* = 3.7 Hz), 124.1 (q, *J* = 271.9 Hz), 61.3, 41.5, 39.6, 38.7, 28.8, 21.4, 14.3, 13.3, 12.4.

HRMS (*m/z*): calcd for $C_{18}H_{22}F_3O_3N$, $[M+H]^+$, 358.1630; found, 358.1635;

GC: Using method described on page SI-4, t_R (min): *cis* = 9.28, *trans* = 9.54.

HPLC: Using method described on page SI-4, t_R (min) = 31.8.

SFC: AS column, 1% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 6.82, minor = 7.41.

8f

^{1}H NMR (500 MHz, CDCl$_3$): δ 7.85–7.78 (m, 3H), 7.74 (dt, *J* = 1.4, 0.7 Hz, 1H), 7.53–7.43 (m, 3H), 4.22 (qd, *J* = 7.2, 1.0 Hz, 2H), 3.65–3.43 (m, 2H), 3.23 (ddq, *J* = 28.4, 14.1, 7.0 Hz, 2H), 2.57 (dd, *J* =

8.4, 6.2 Hz, 1H), 2.26 (dd, *J* = 6.2, 4.9 Hz, 1H), 1.60 (dd, *J* = 8.4, 4.9 Hz, 1H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.12 (t, *J* = 7.1 Hz, 3H), 0.74 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.8, 167.7, 136.6, 133.5, 132.7, 128.8, 127.9, 127.7, 126.6, 126.2, 125.1, 124.8, 61.2, 41.5, 39.5, 39.3, 28.4, 21.3, 14.4, 13.3, 12.5.

HRMS (*m/z*): calcd for $C_{21}H_{25}O_3N$, $[M+H]^+$, 340.1913; found, 340.1917;

GC: Using method described on page SI-4, t_R (min): *cis* = 11.63, *trans* = 12.05.

HPLC: Using method described on page SI-4, t_R (min) = 32.0.

SFC: AS column, 7% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 6.02, minor = 6.80.

10

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 4.19 (qd, *J* = 7.1, 2.8 Hz, 2H), 4.15–4.02 (m, 2H), 2.33 (s, 3H), 2.20 (dd, *J* = 8.5, 6.3 Hz, 1H), 2.08 (dd, *J* = 6.3, 4.9 Hz, 1H), 1.48 (dd, *J* = 8.5, 4.9 Hz, 1H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.89, 169.9, 137.8, 135.6, 130.6, 129.9, 129.4, 129.2, 61.5, 61.1, 38.9, 28.1, , 19.0, 14.39, 14.2.

R_f = 0.23 (silica gel, DCM)

HRMS (*m/z*): calcd for $C_{16}H_{20}O_4$, $[M+H]^+$, 277.1440; found, 277.1442;

GC: Using method described on page SI-4, t_R (min): *cis* = 8.77, *trans* = 9.00.

SFC: AS column, 1% IPA, 2.5 mL/min: λ = 210 nm, t_R (min): major = 5.79, minor = 7.63.

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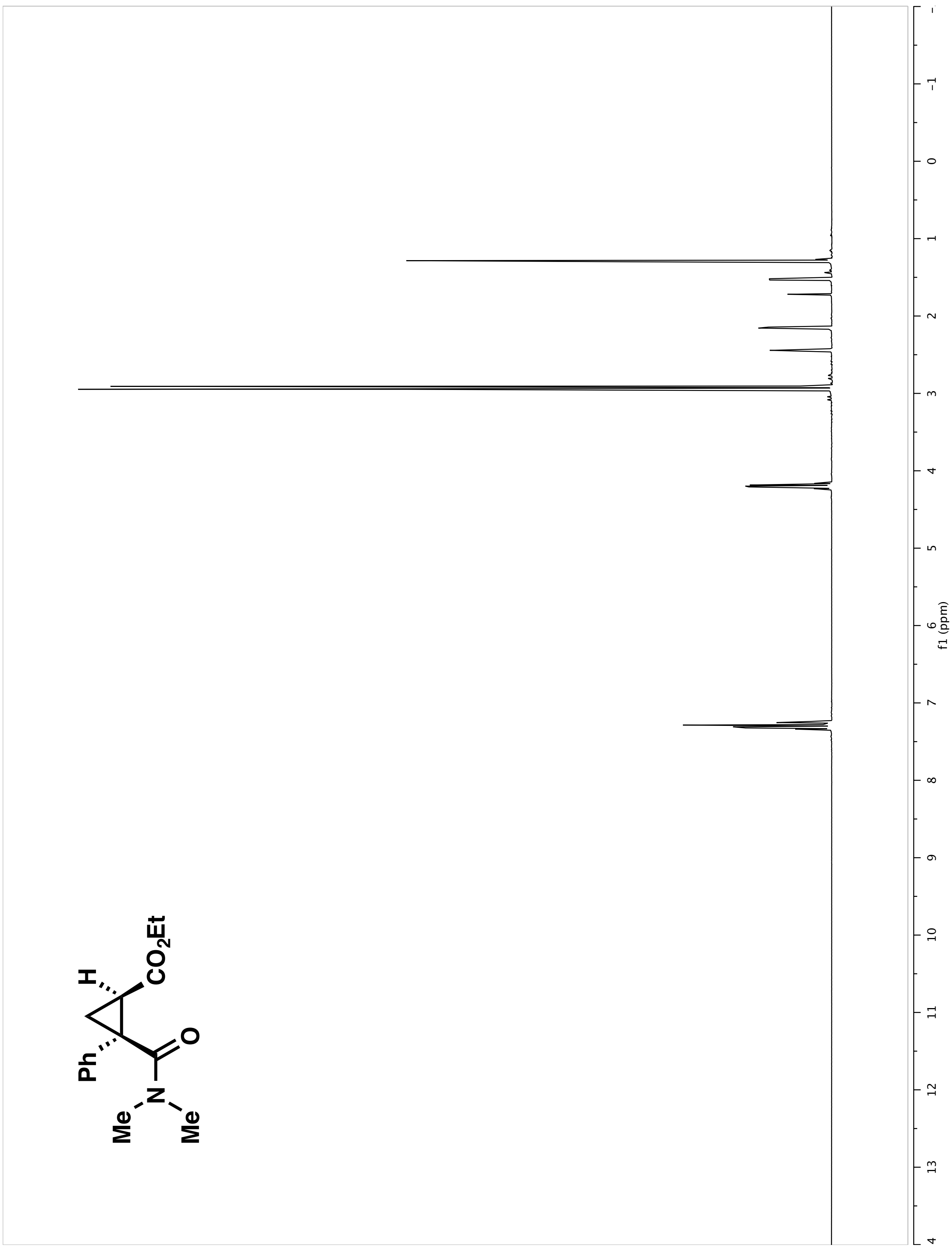
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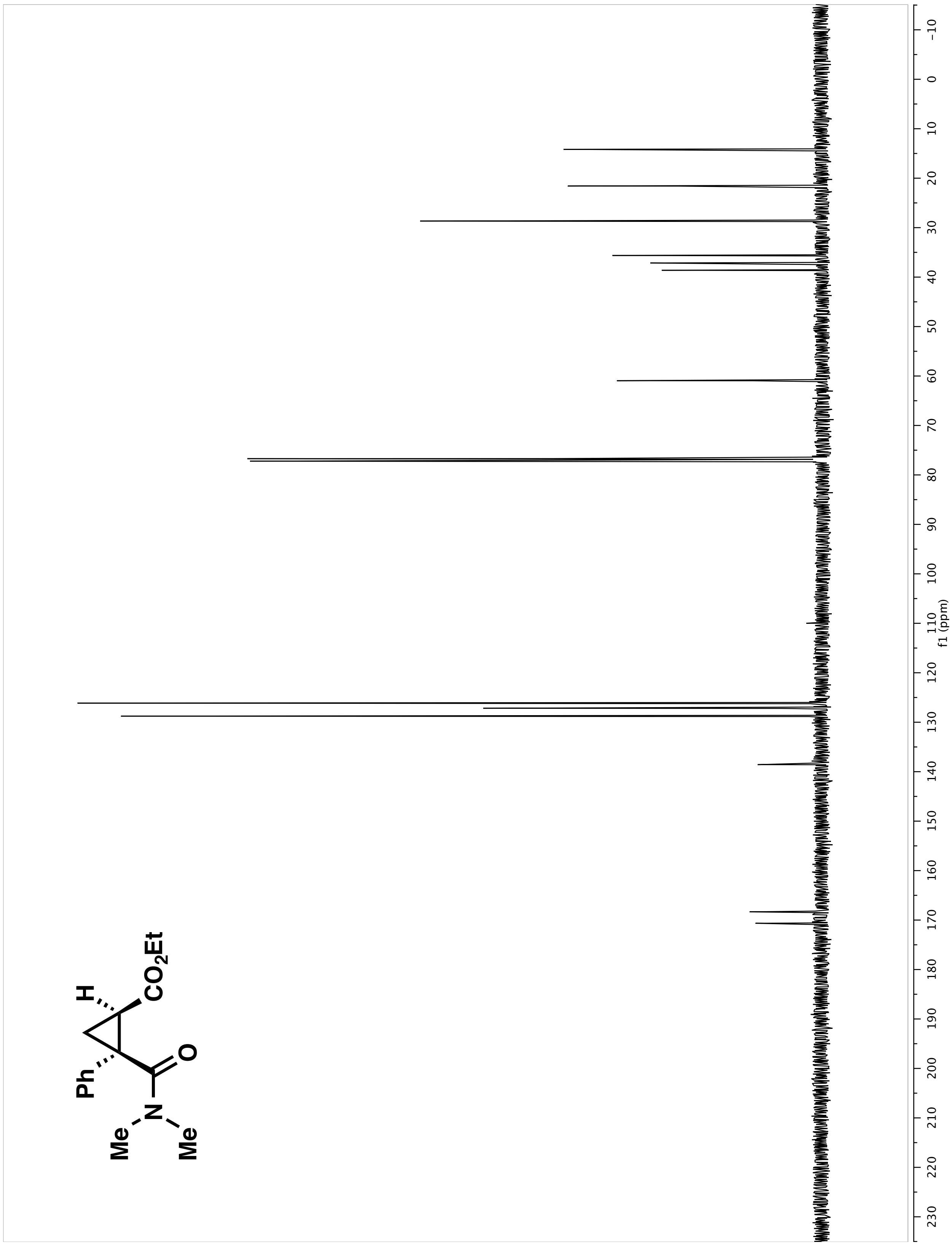
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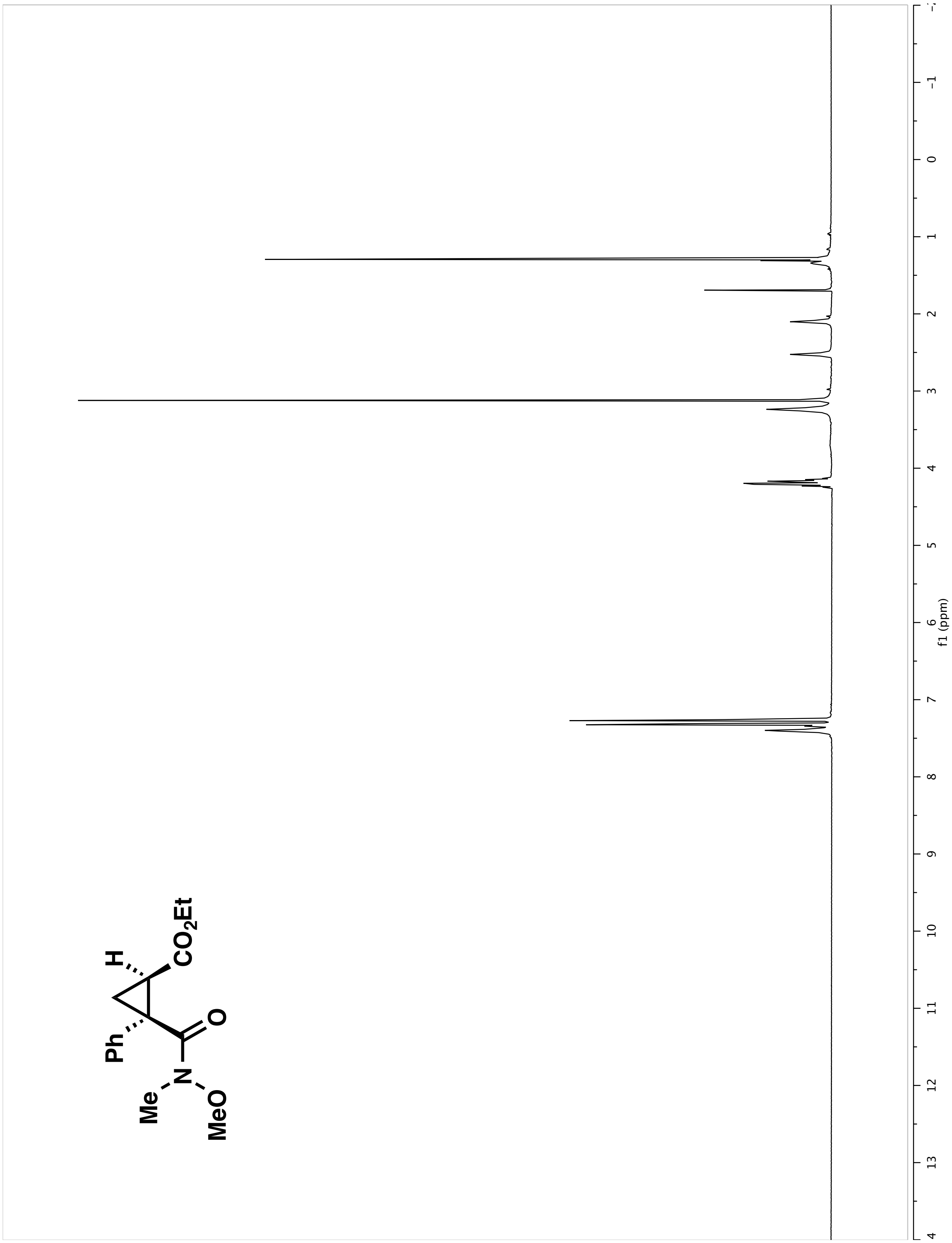

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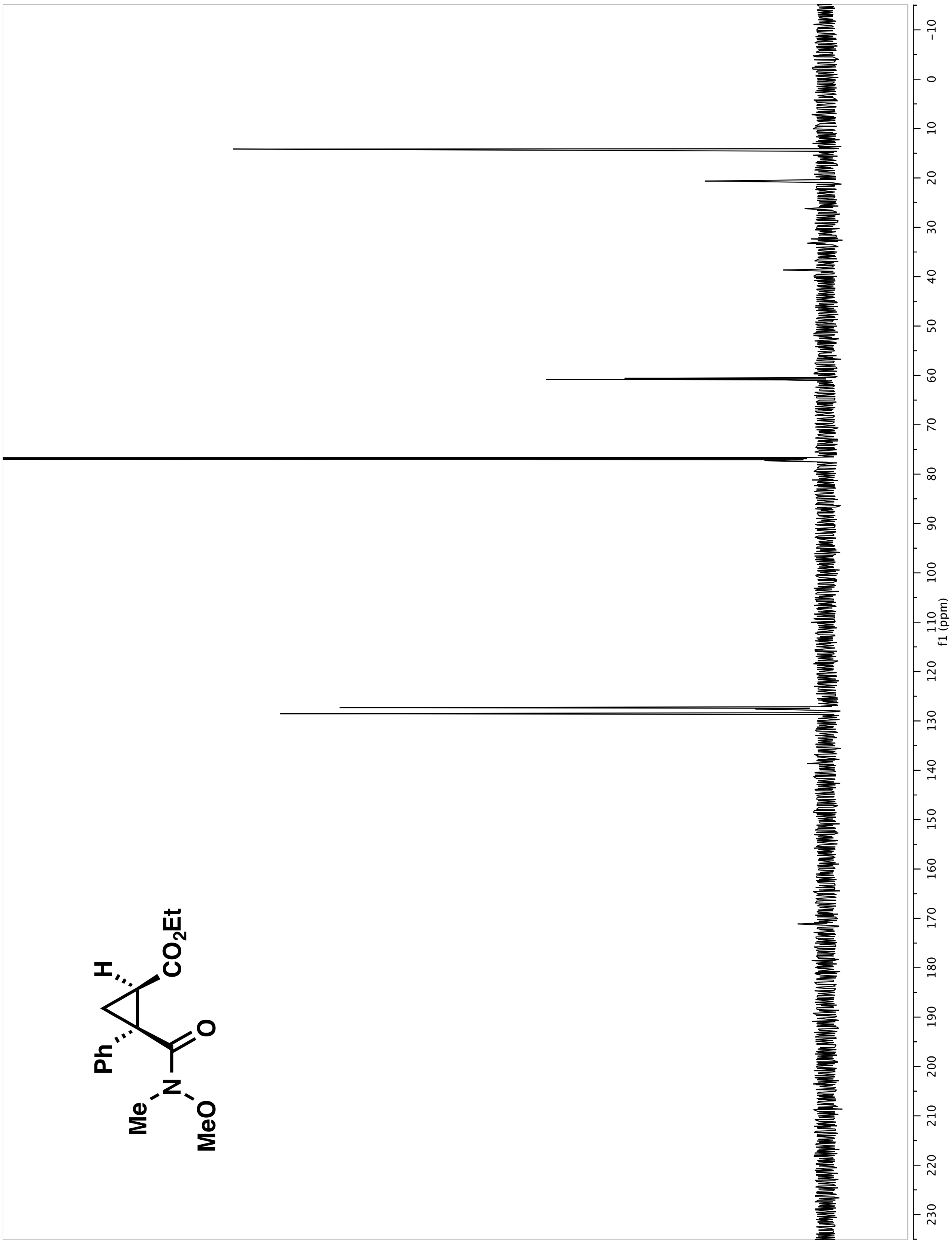
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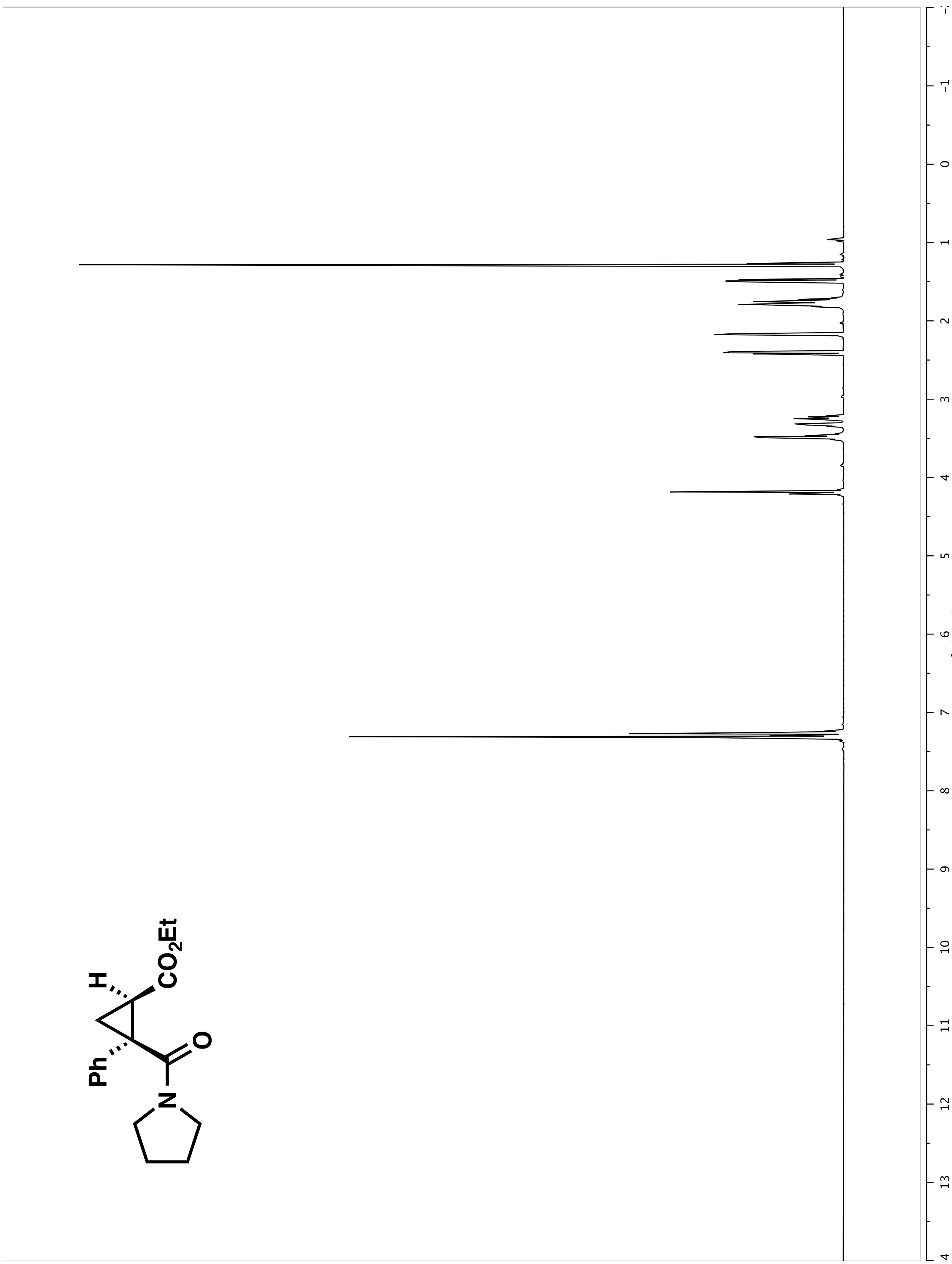
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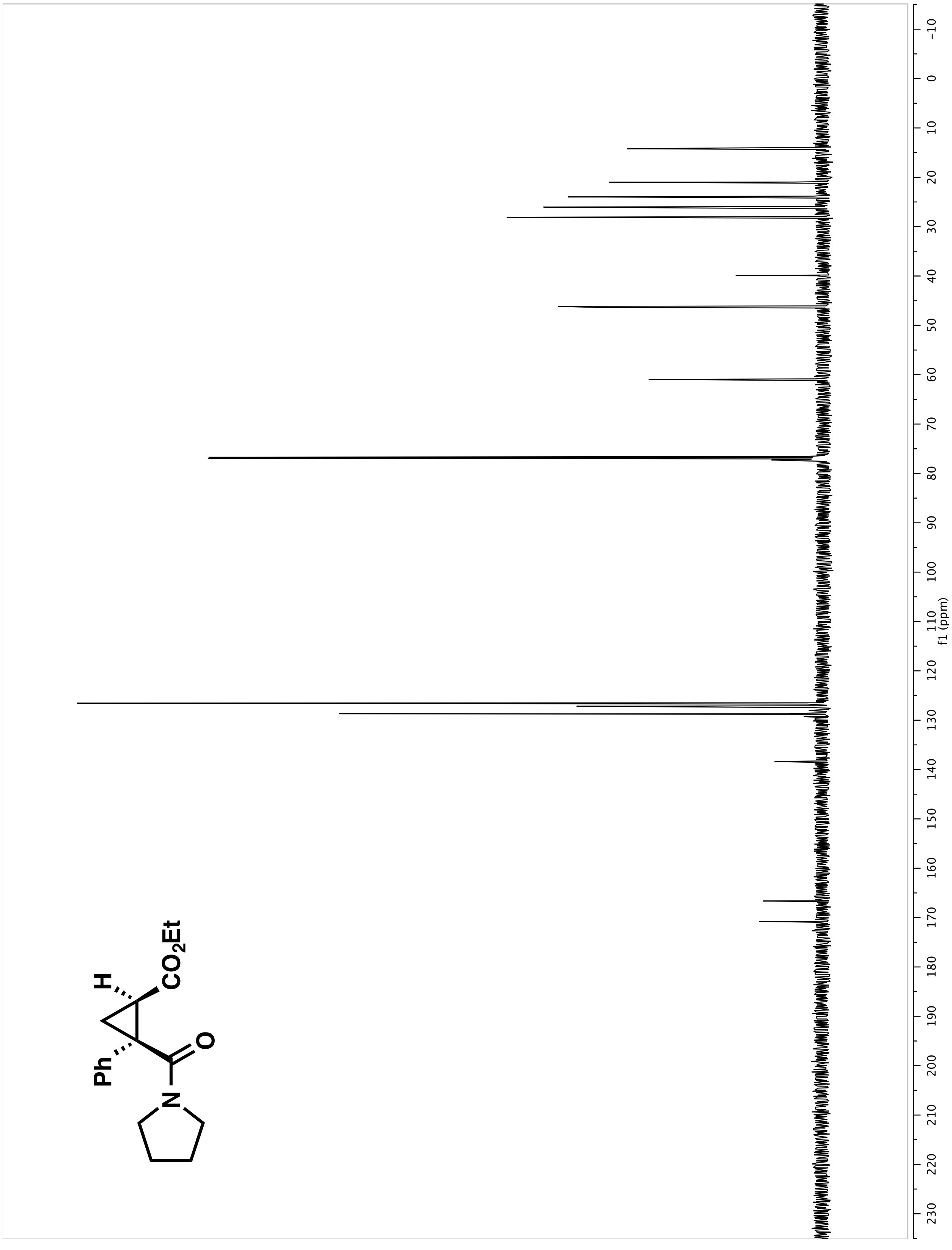
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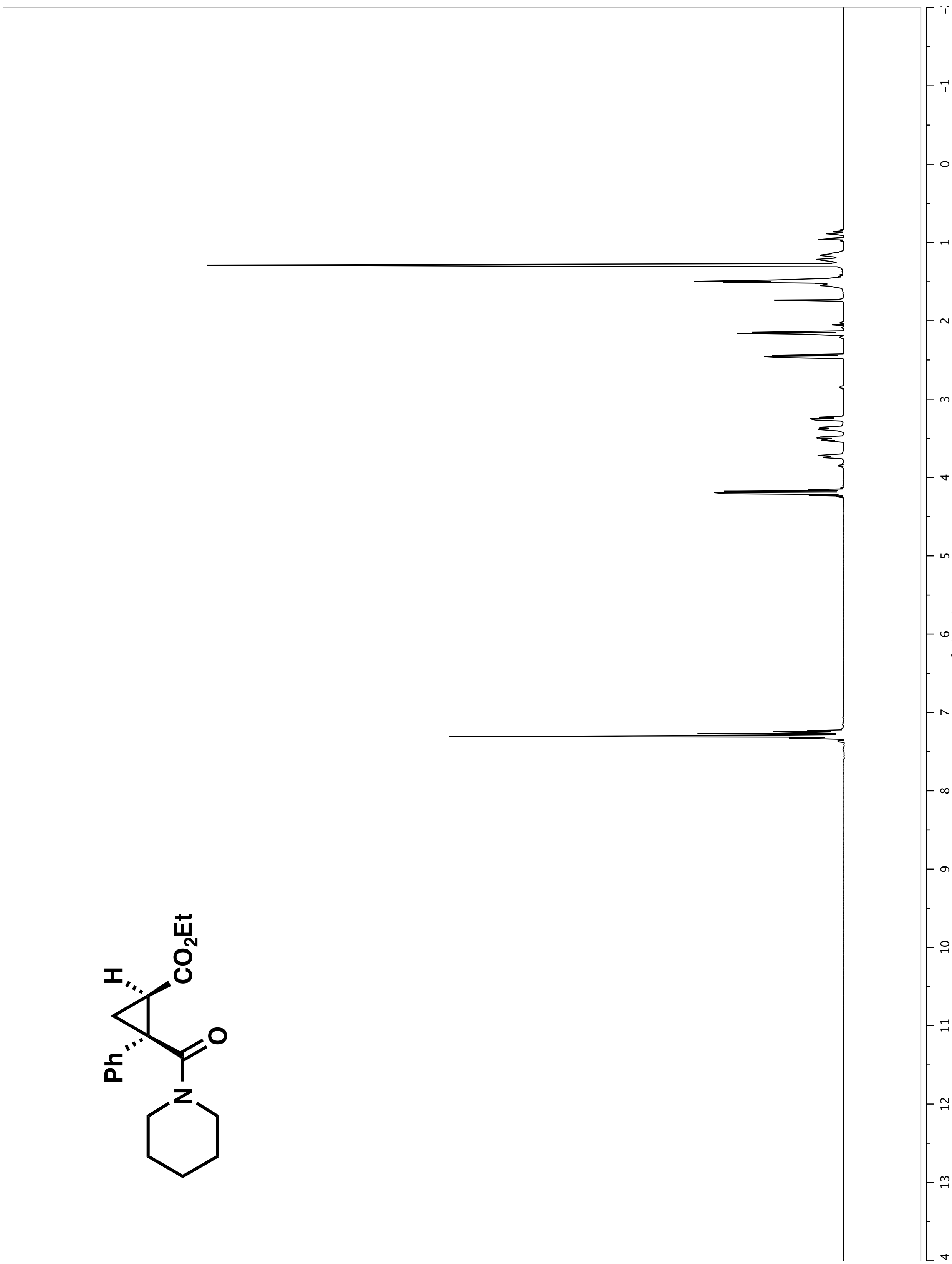
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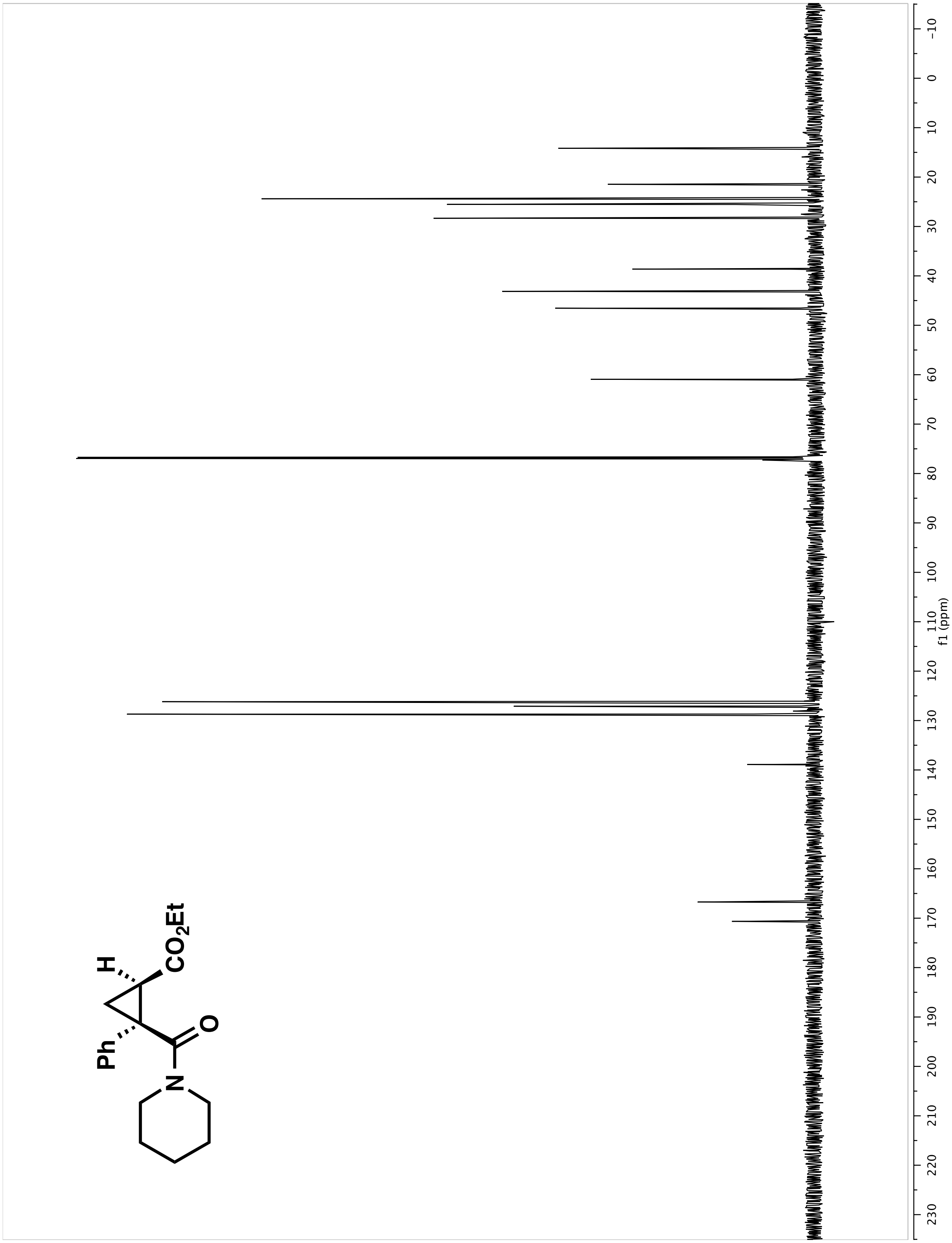
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f1 (ppm)
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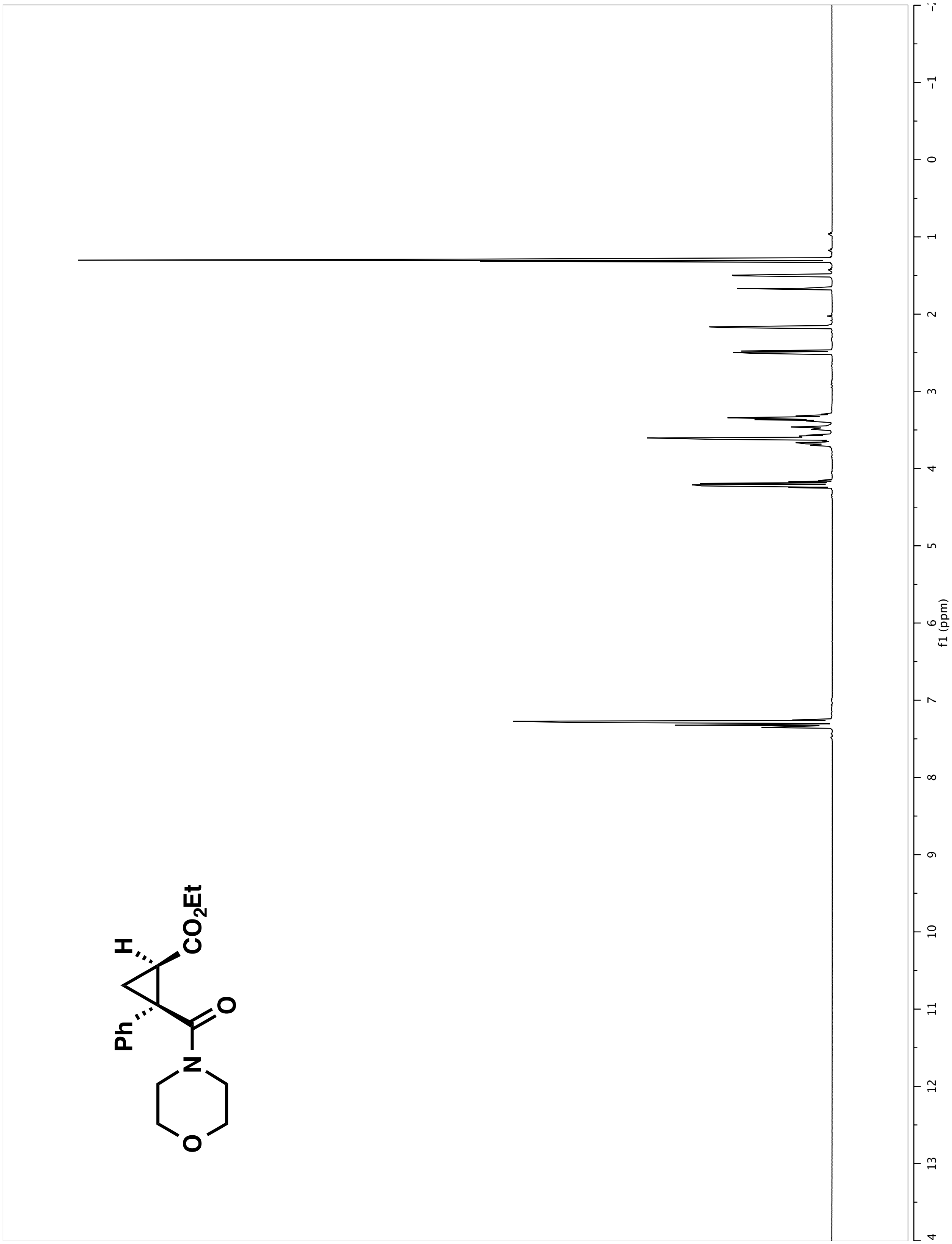
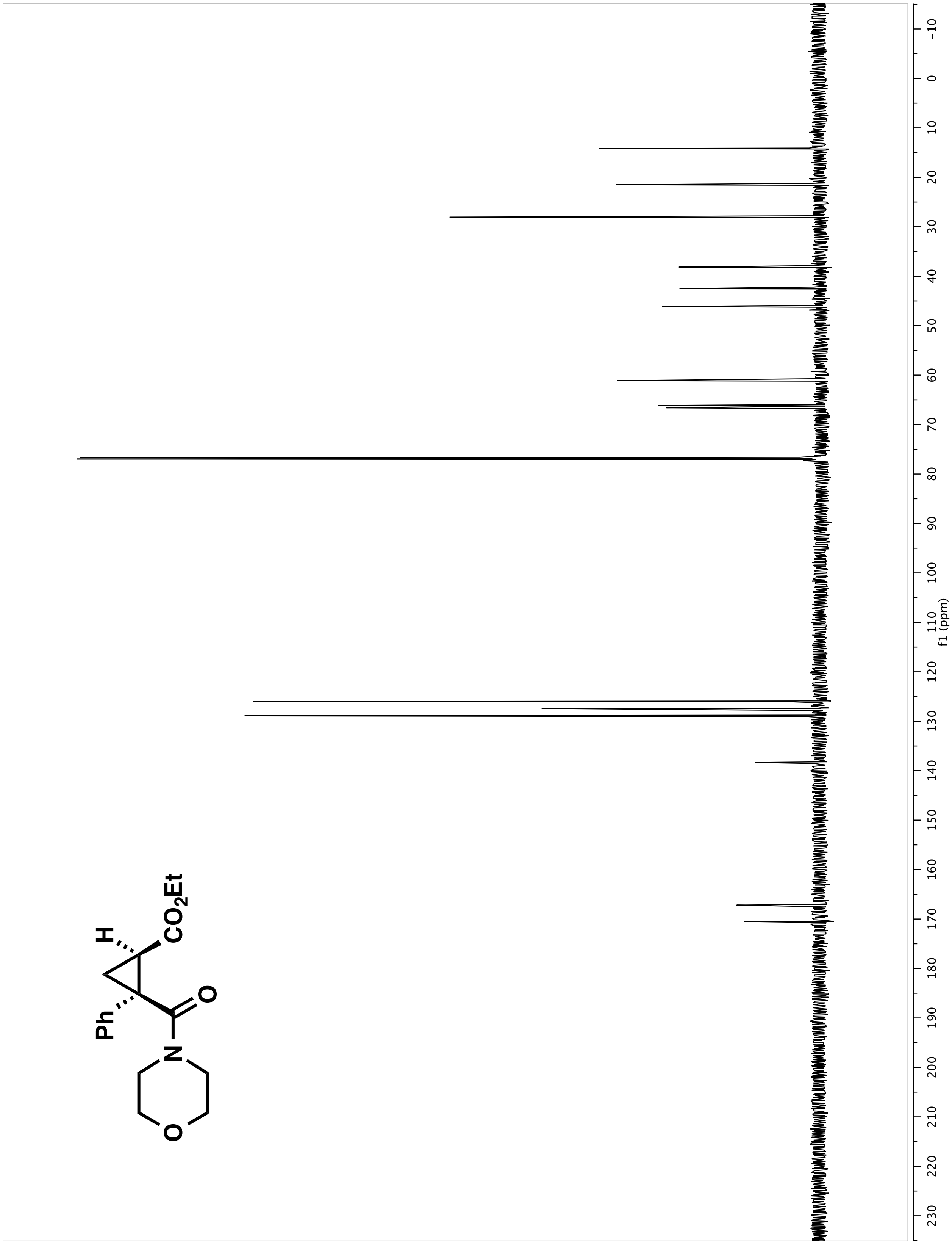
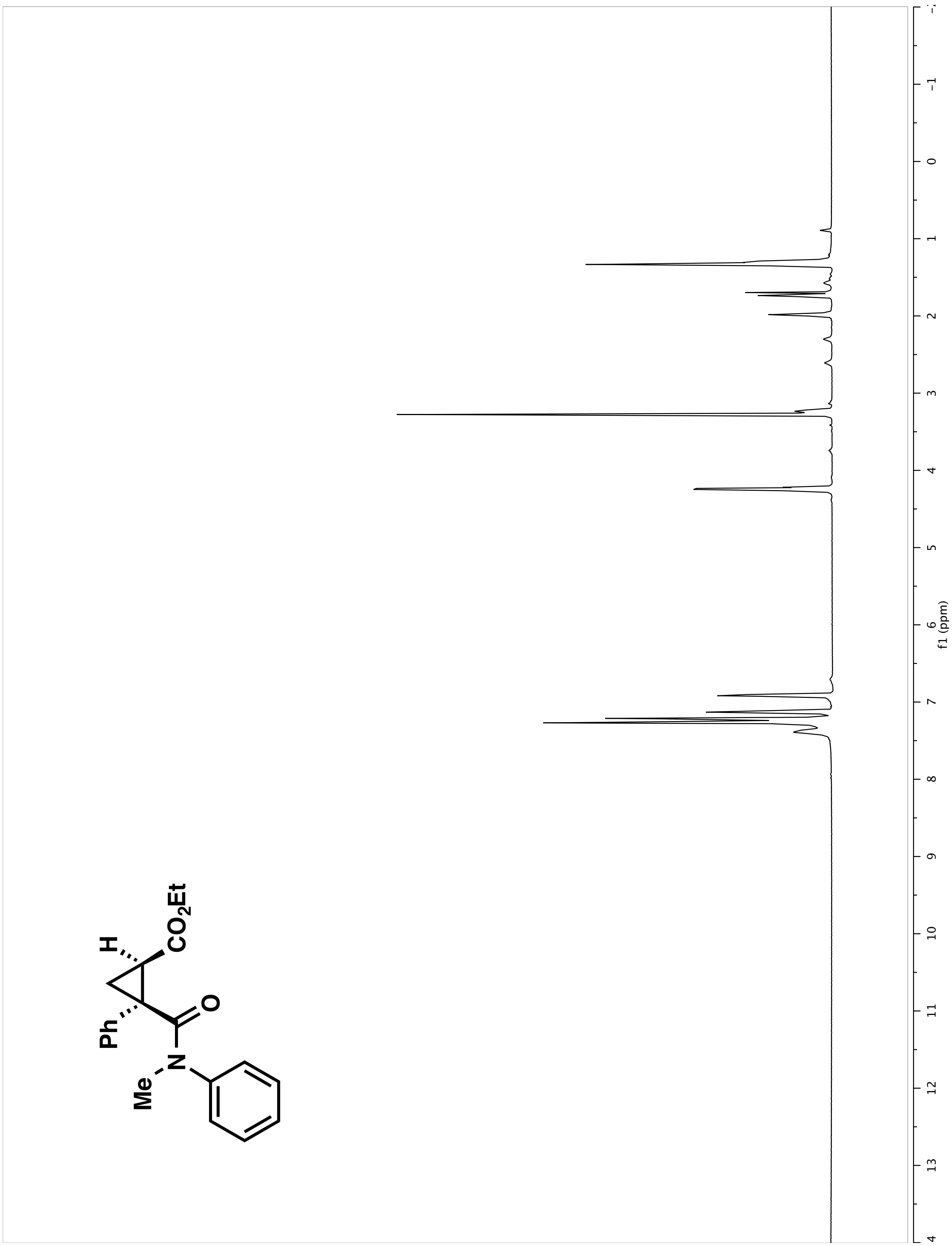
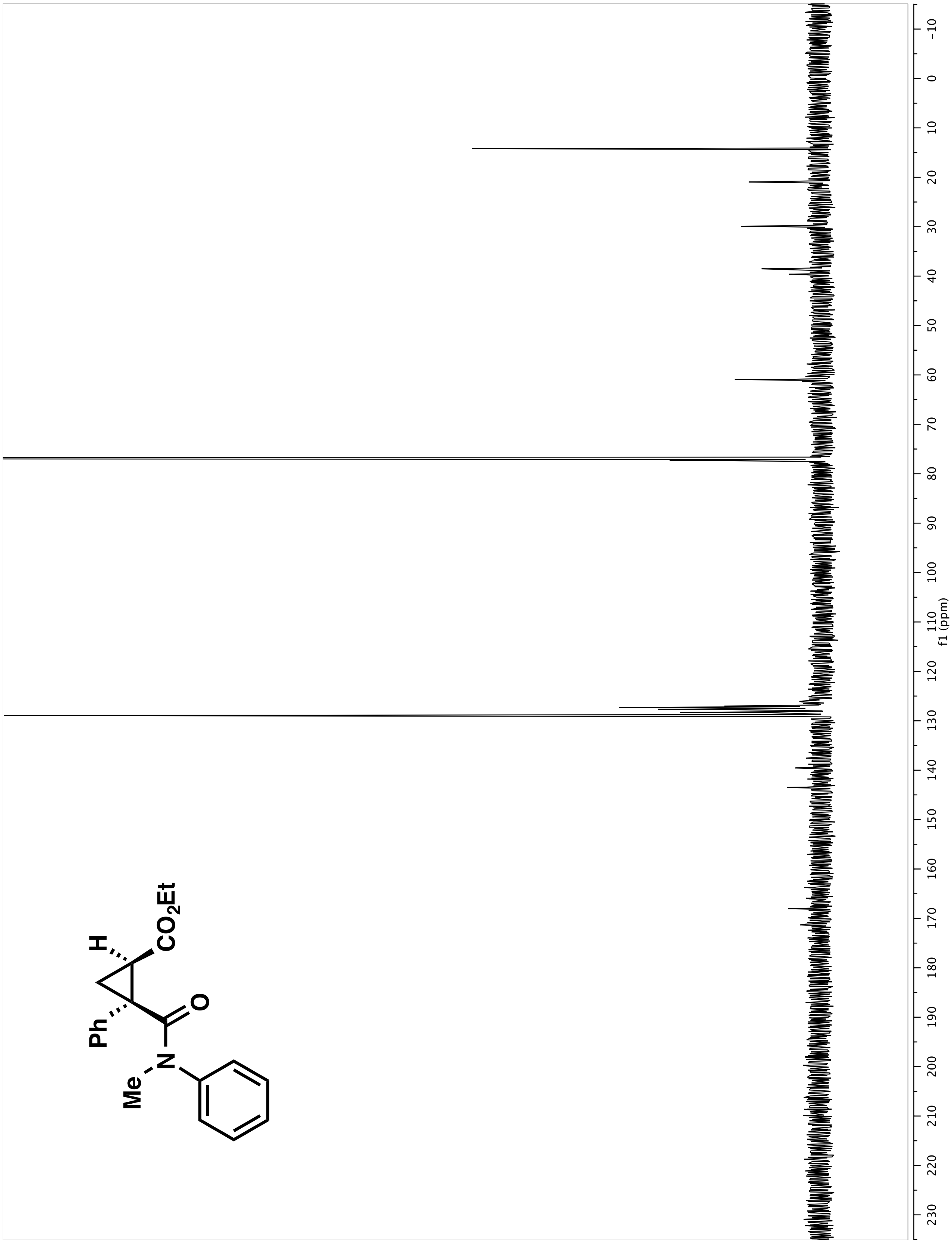
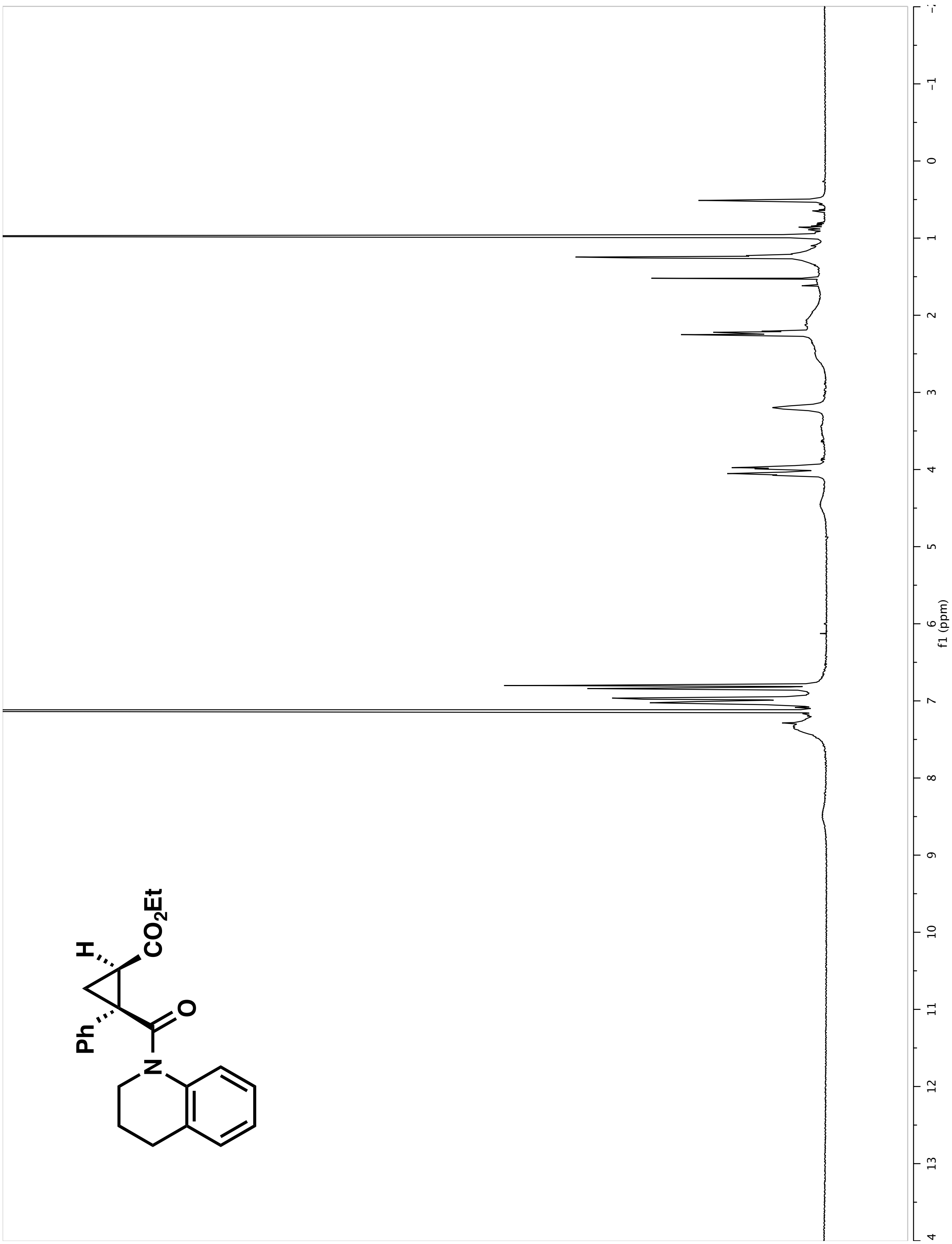

f1 (ppm)
Ph
H
CO_2Et
N
O

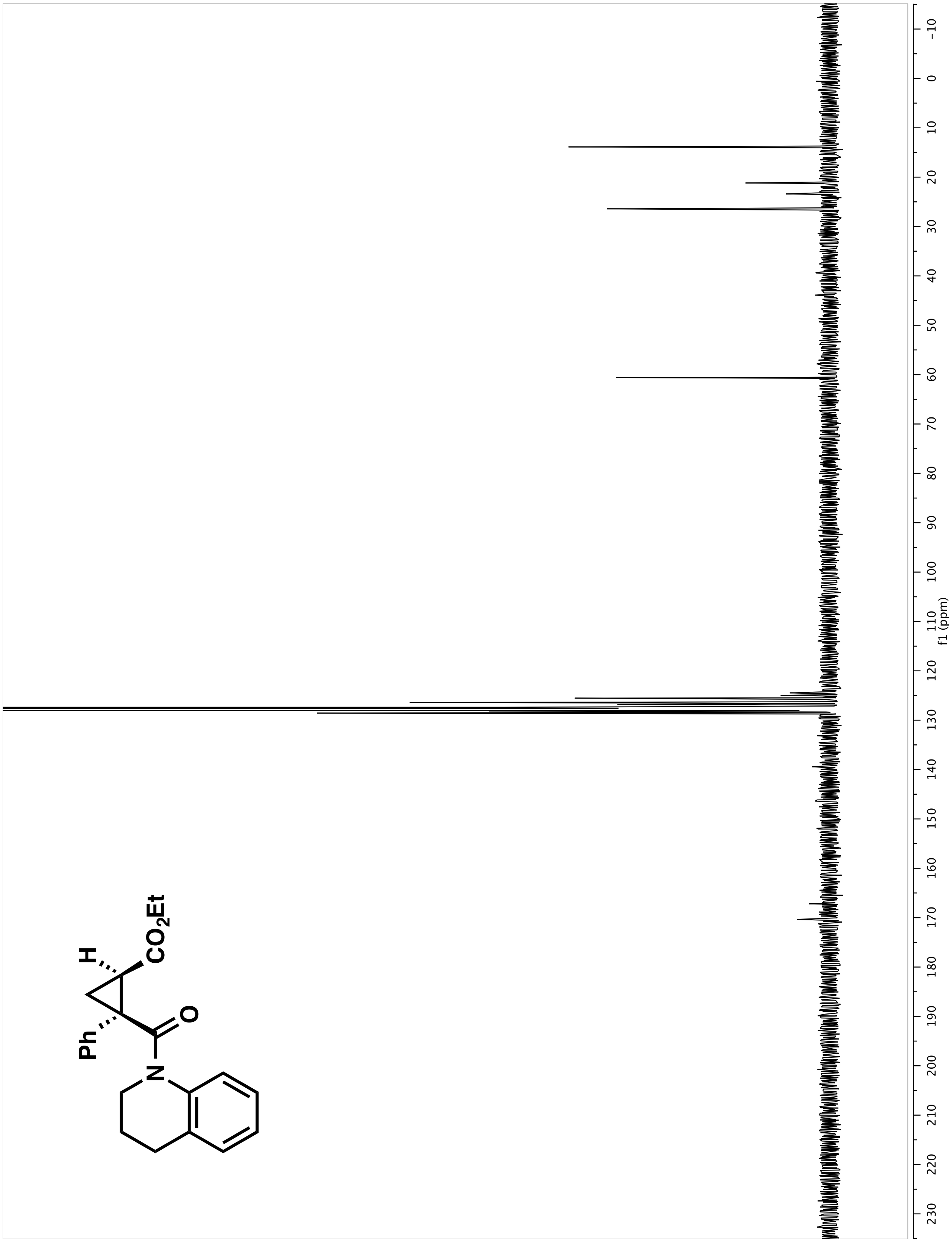

H
CO2Et
Ph
N
O
O
f1 (ppm)


f1 (ppm)
Ph
H
CO2Et
O
N
Me

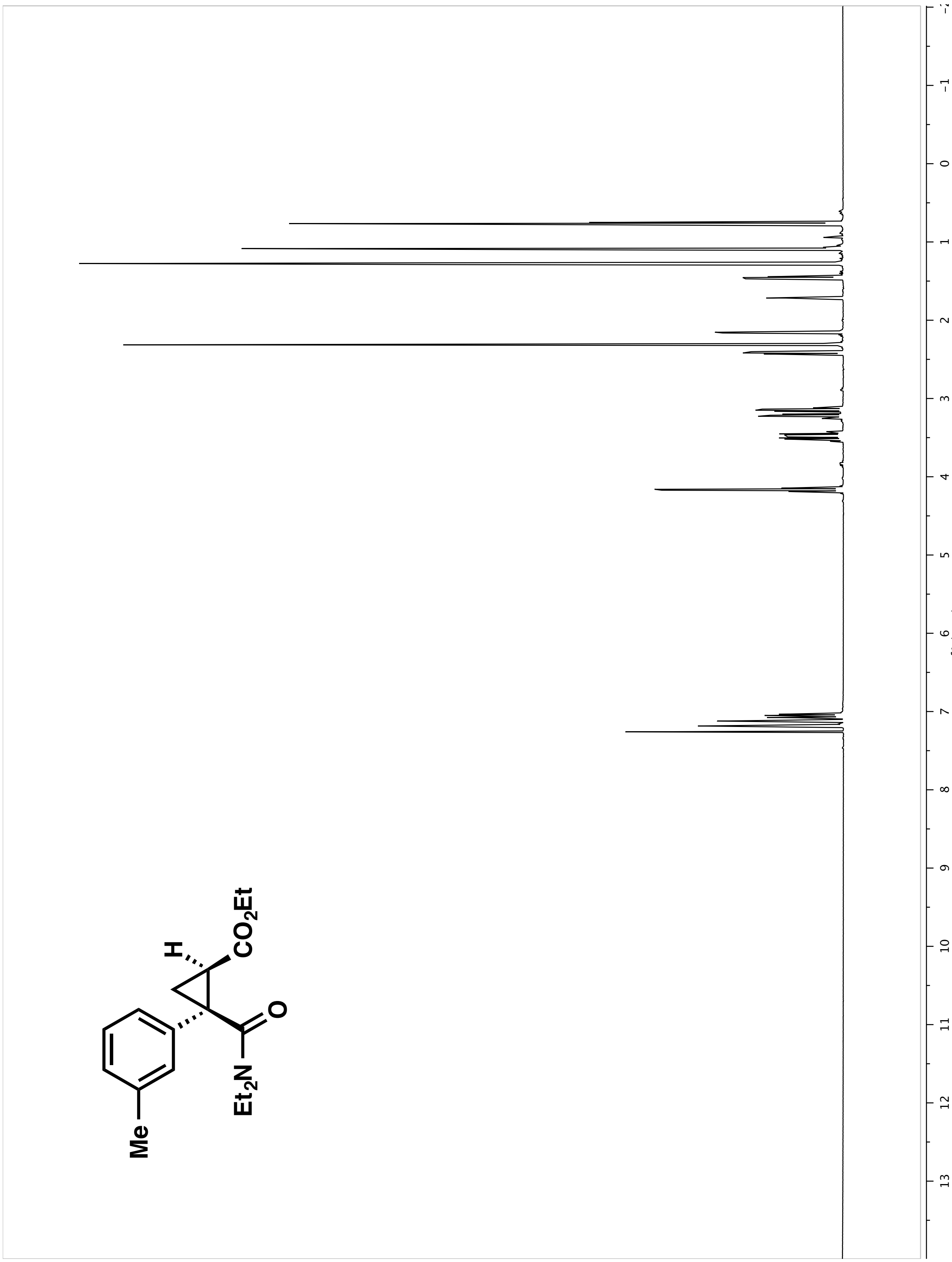


H
CO2Et
Ph
O
N
Me
f1 (ppm)

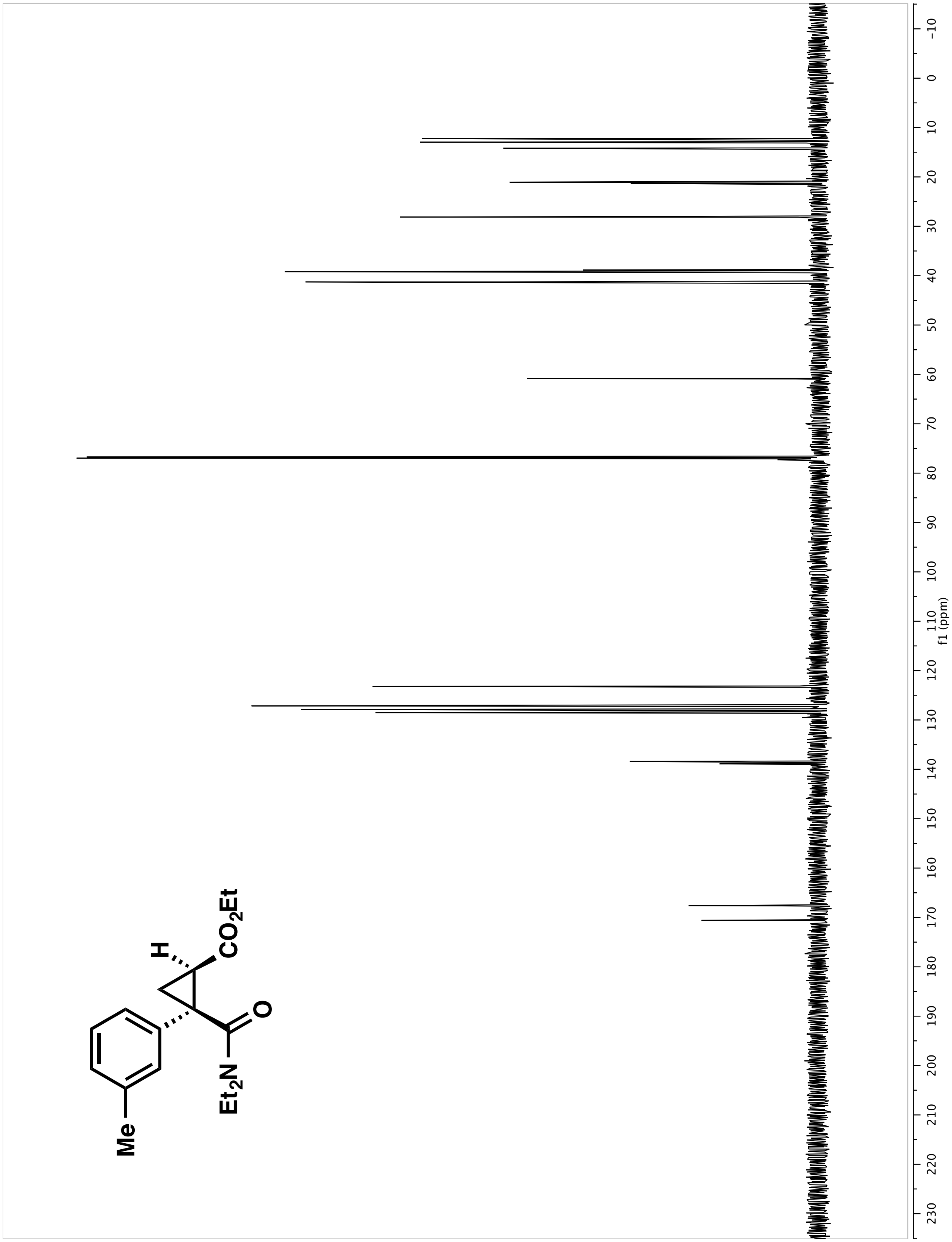

f1 (ppm)
Ph
H
CO2Et
O
N

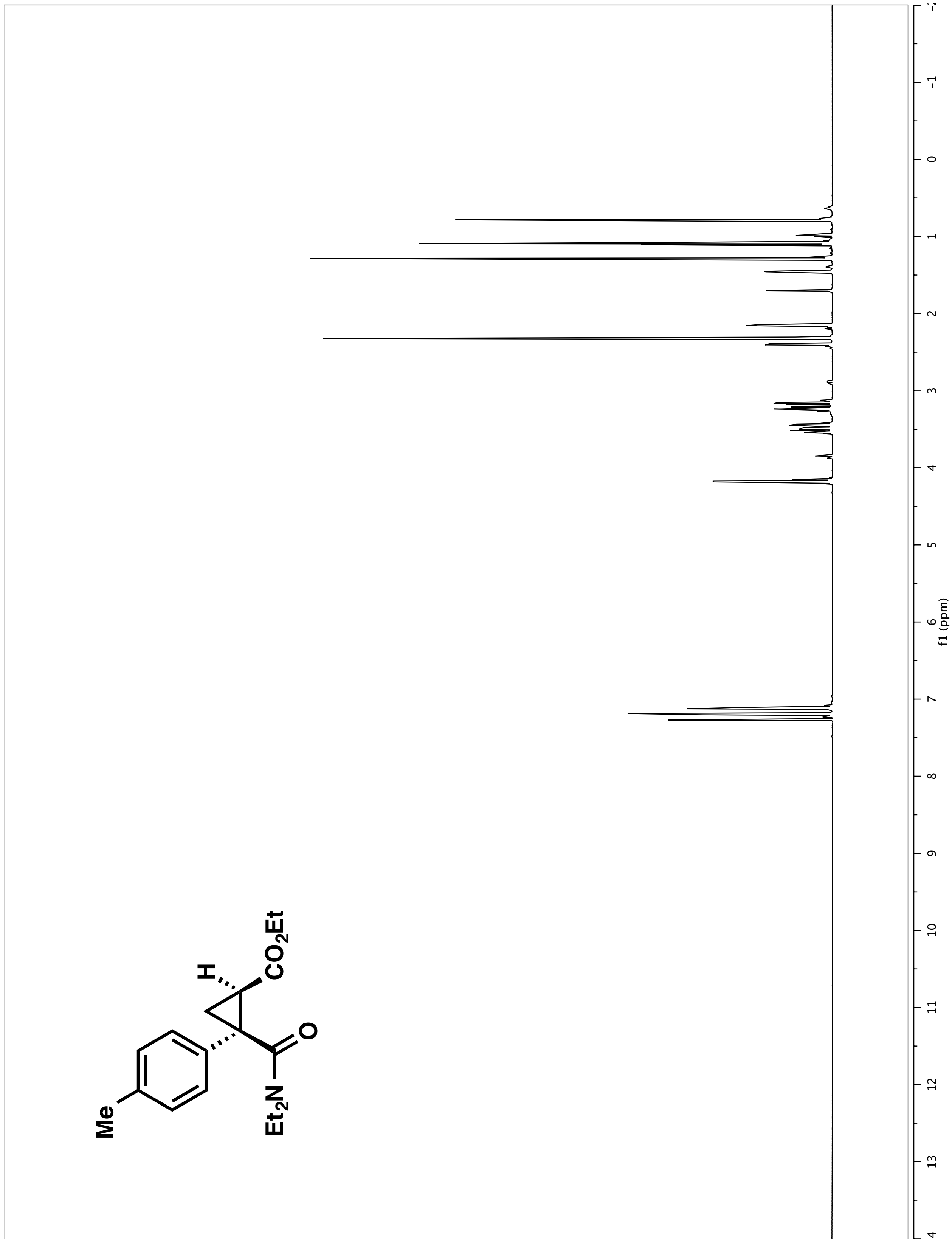


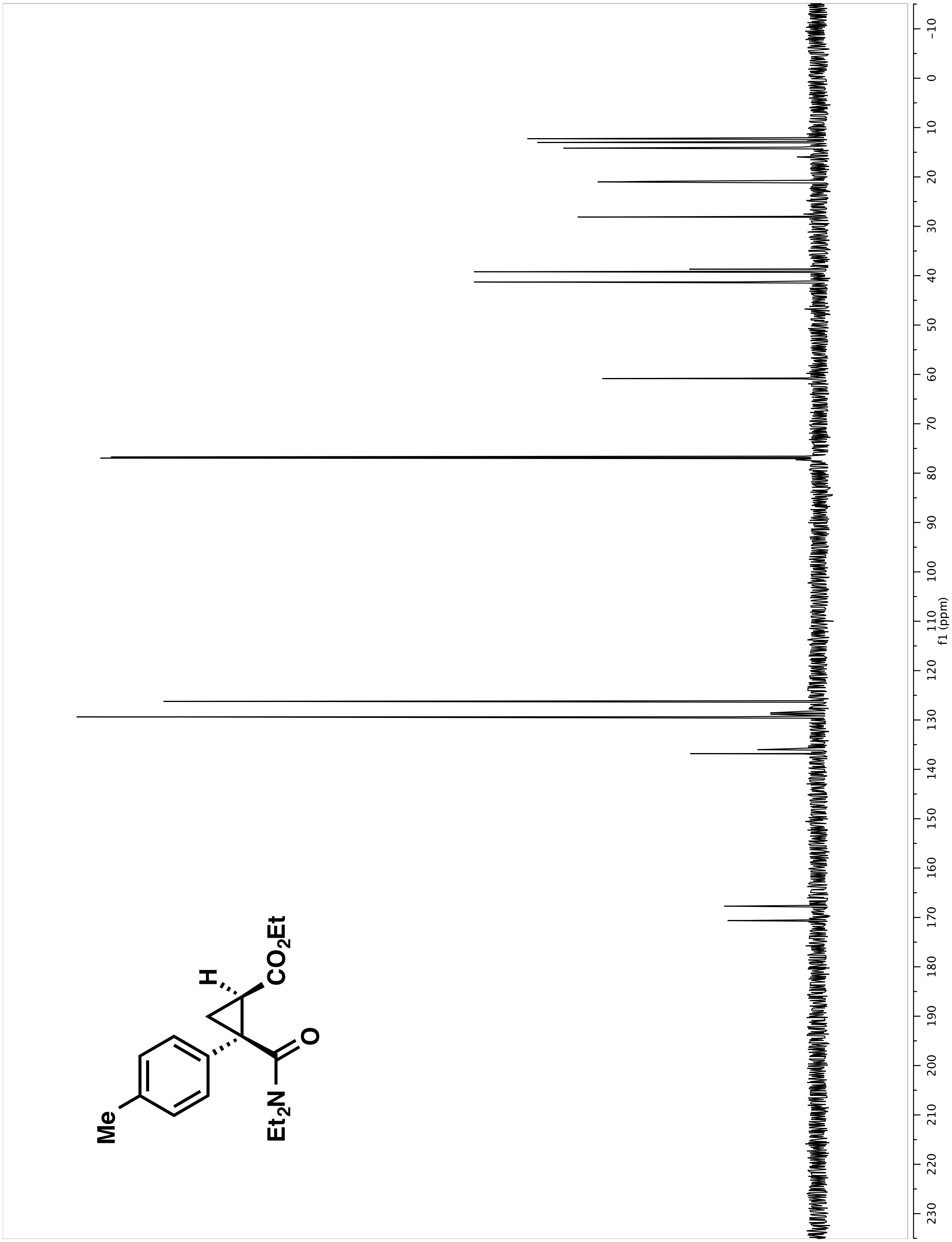
f1 (ppm)
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
Ph
H
CO2Et
O
N

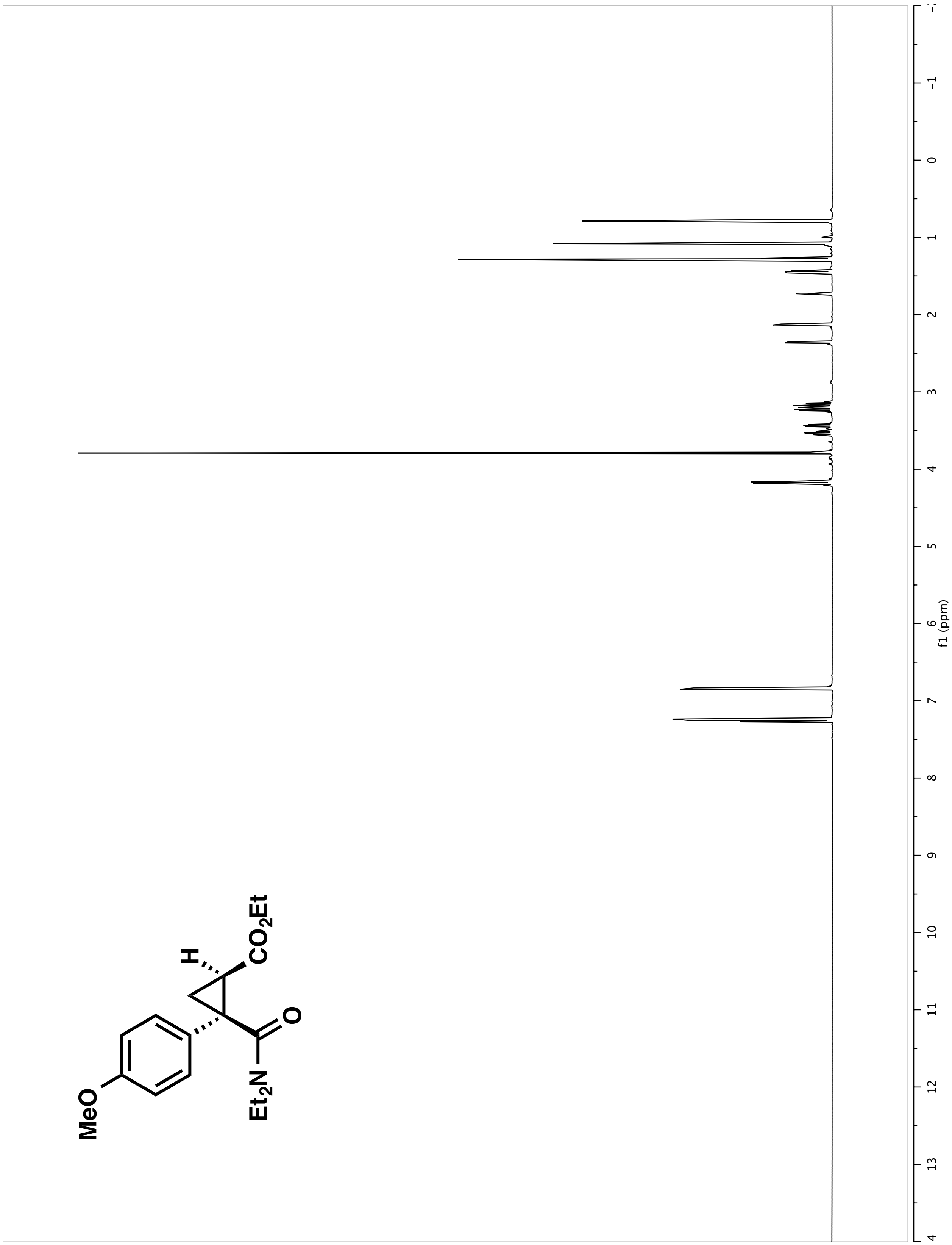


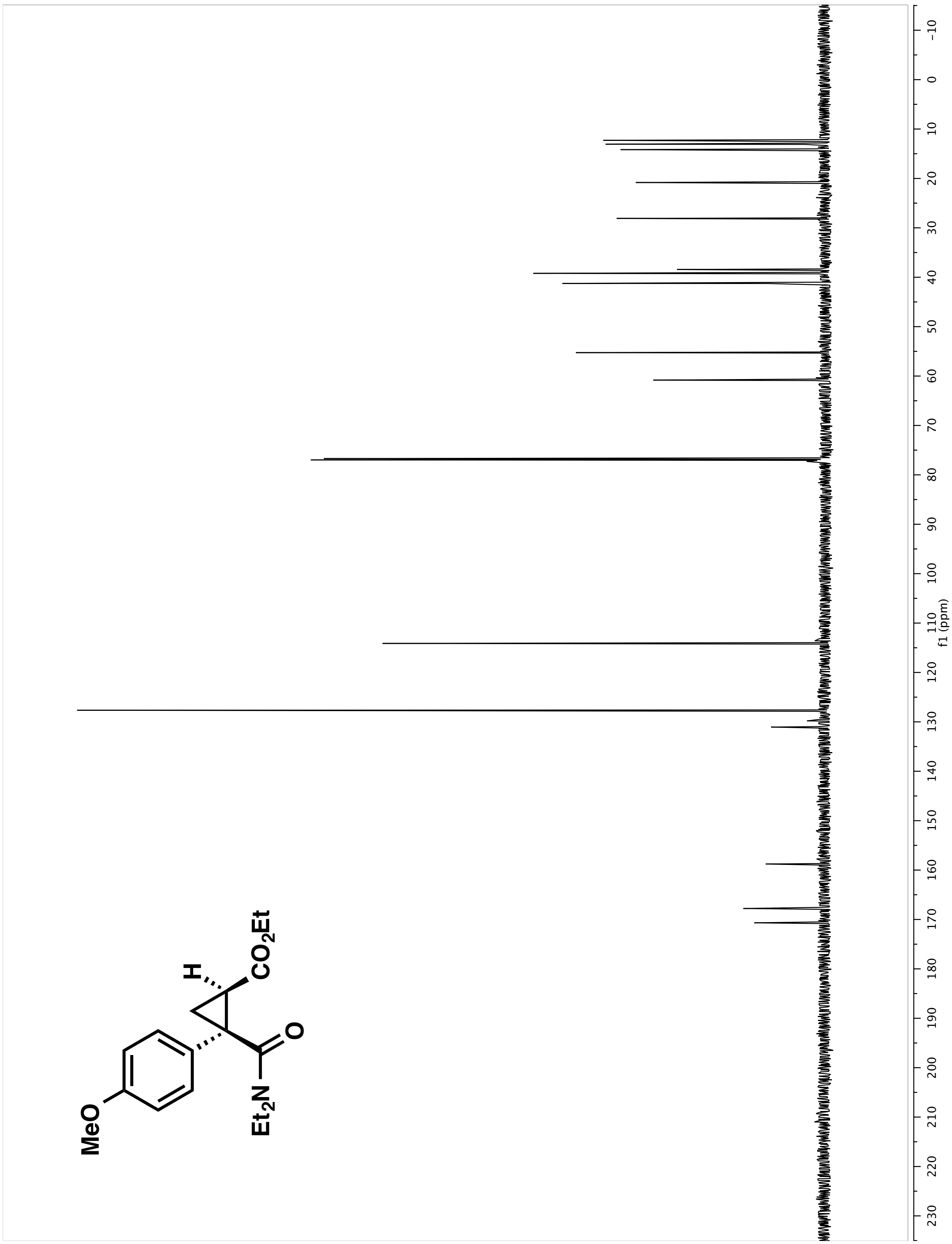
Me
Et2N
O
H
CO2Et
f1 (ppm)

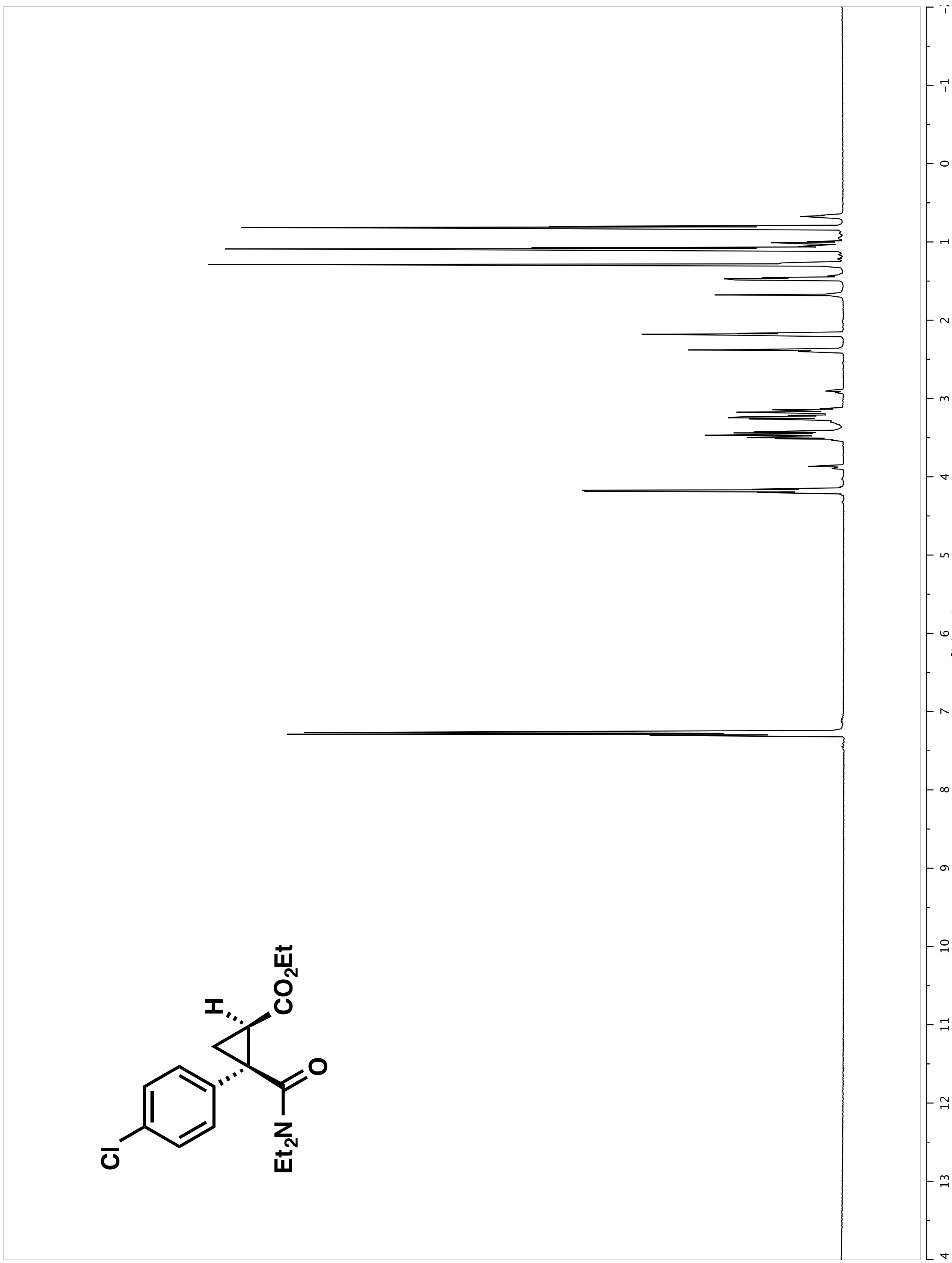

f1 (ppm)
Me
Et2N
O
H
CO2Et

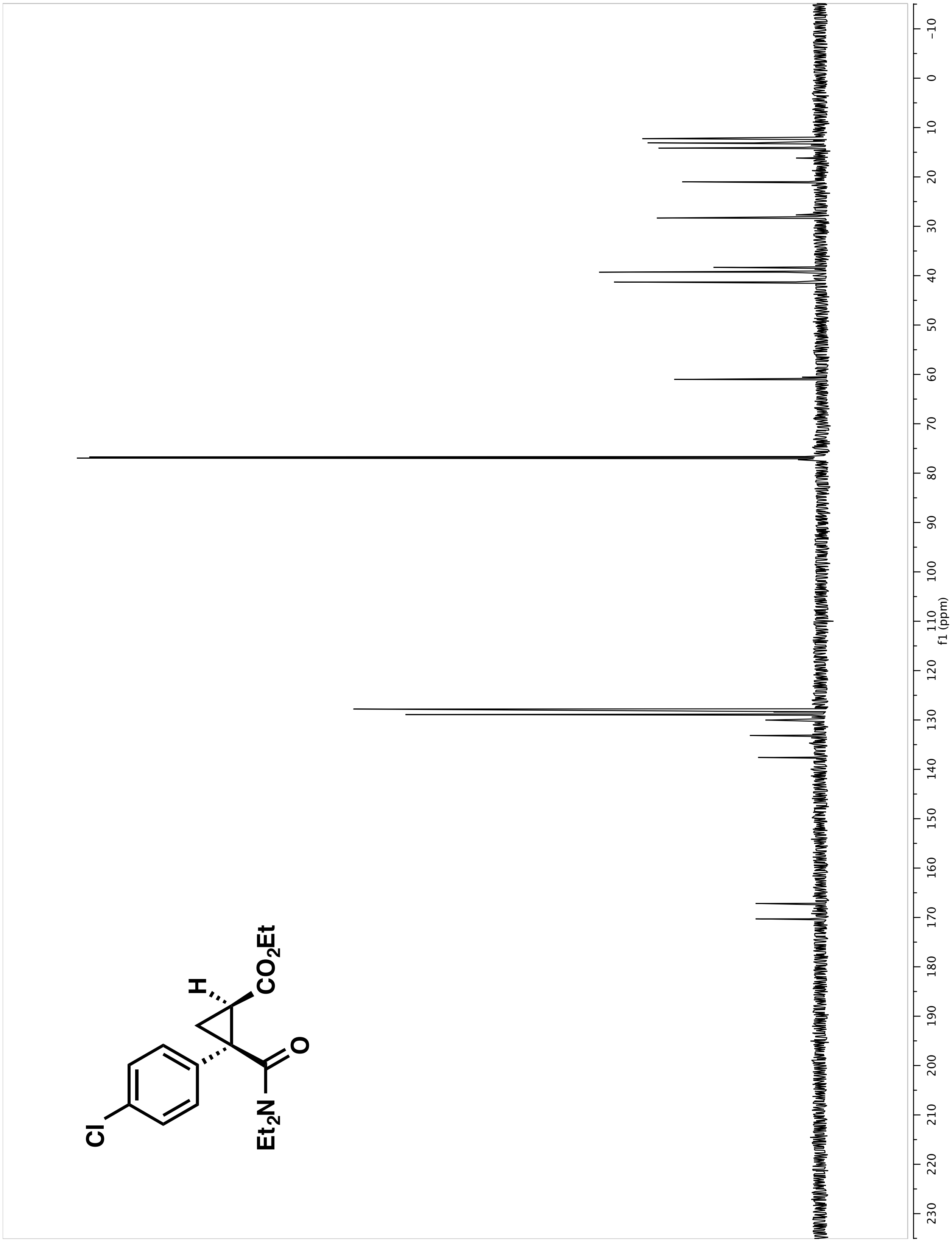

Me
H
CO2Et
O
Et2N
f1 (ppm)
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

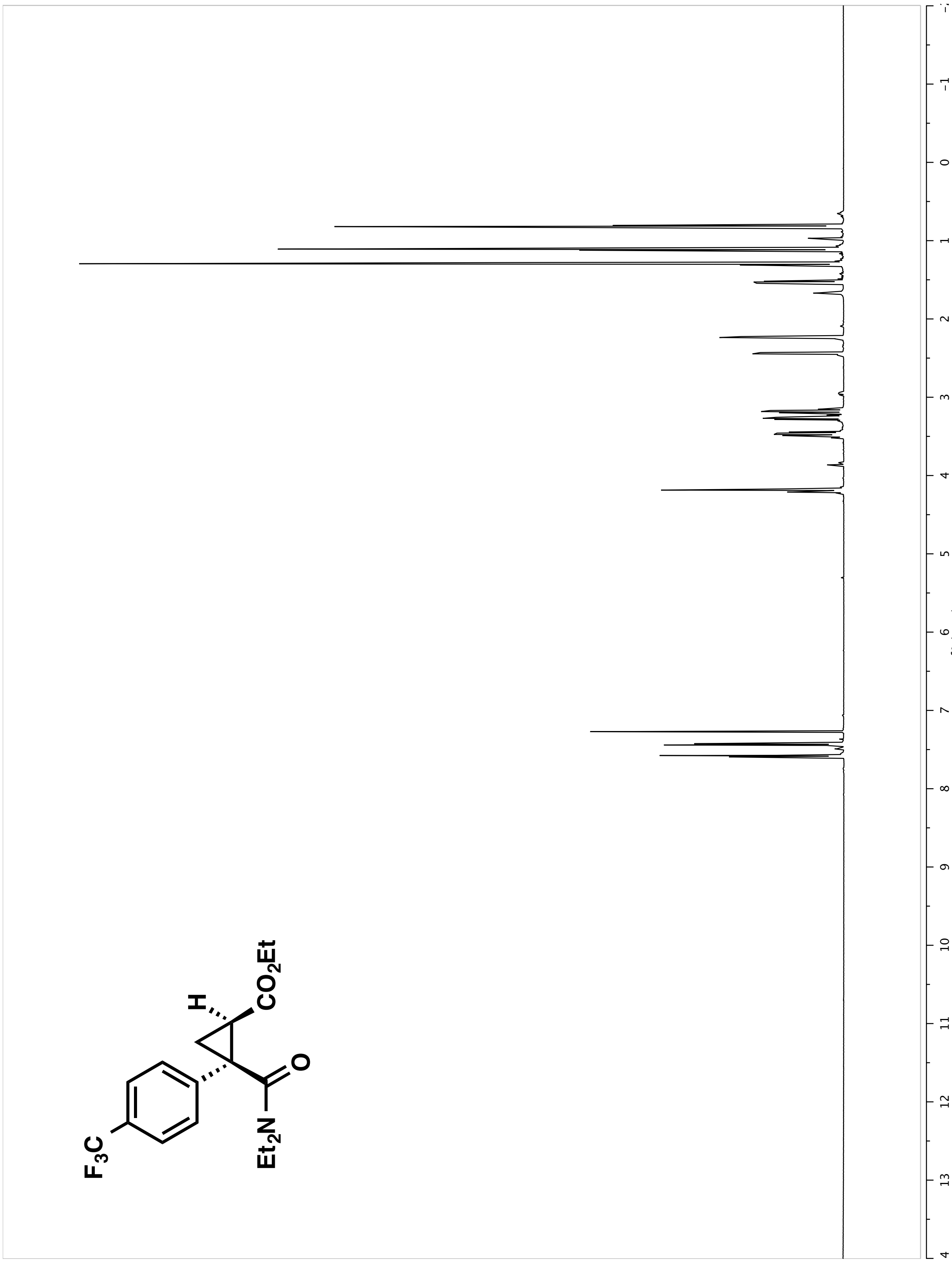

Me
Et2N
O
H
CO2Et
f1 (ppm)

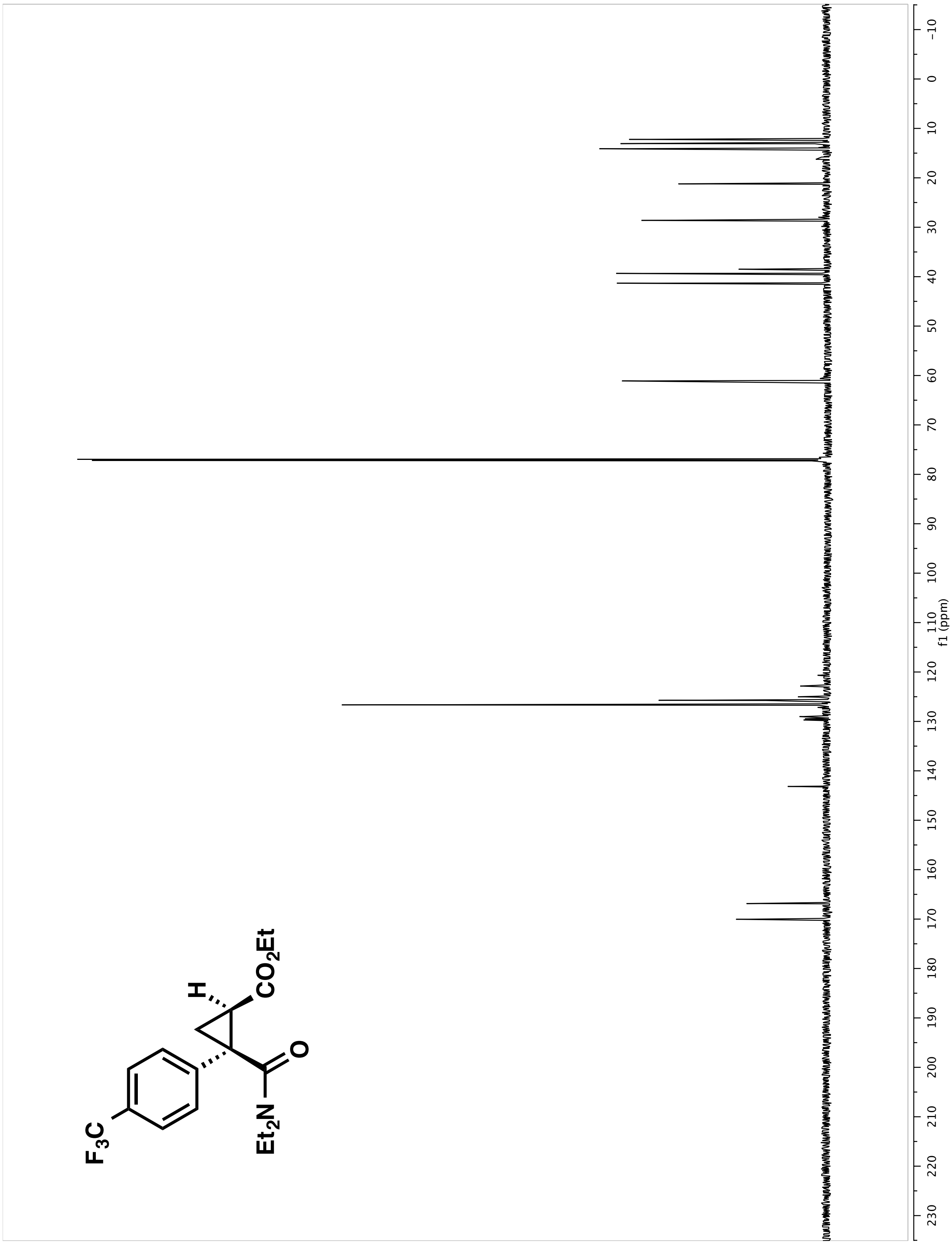

MeO
H
CO_2Et
O
Et_2N
f1 (ppm)

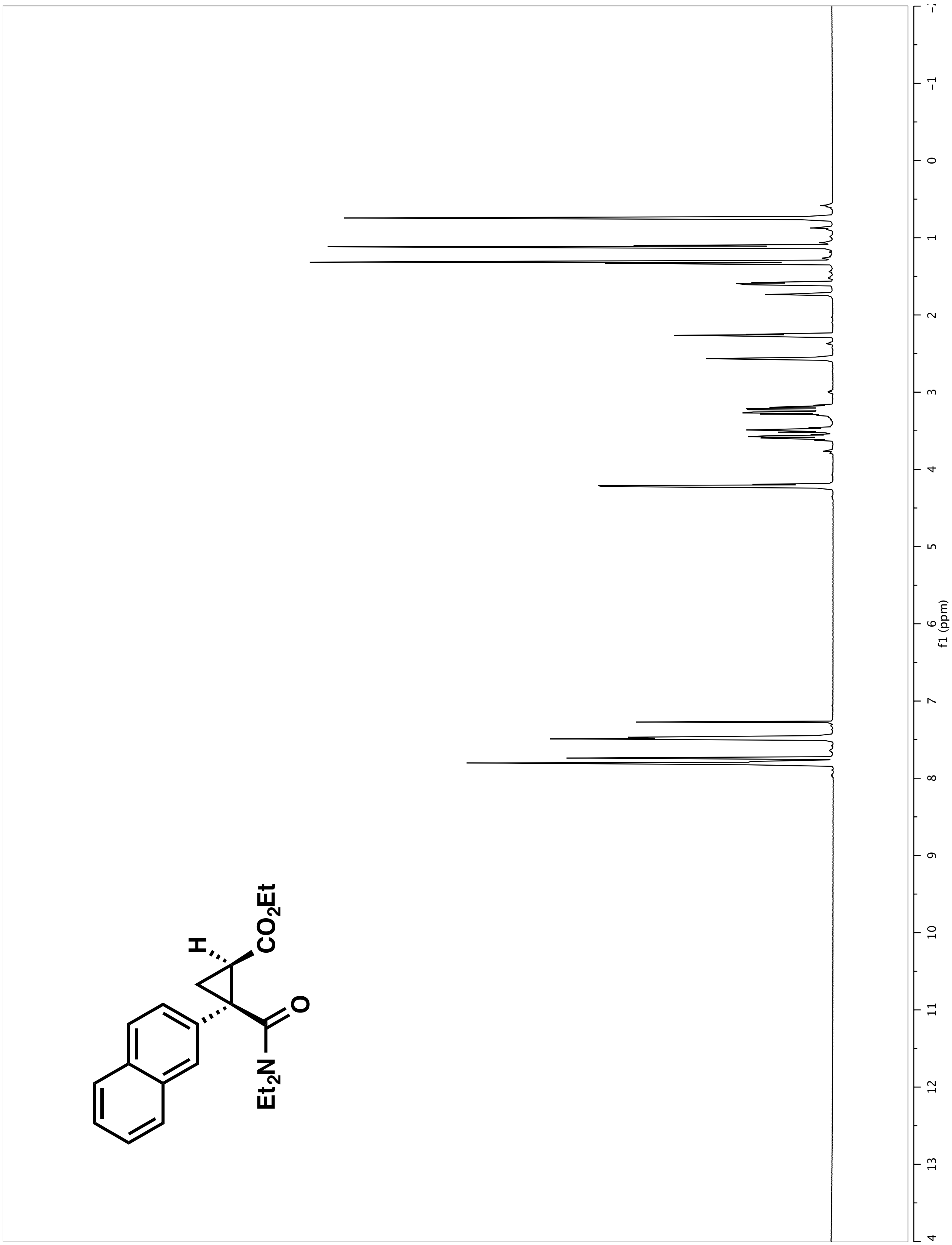

MeO
Et2N
O
H
CO2Et
f1 (ppm)

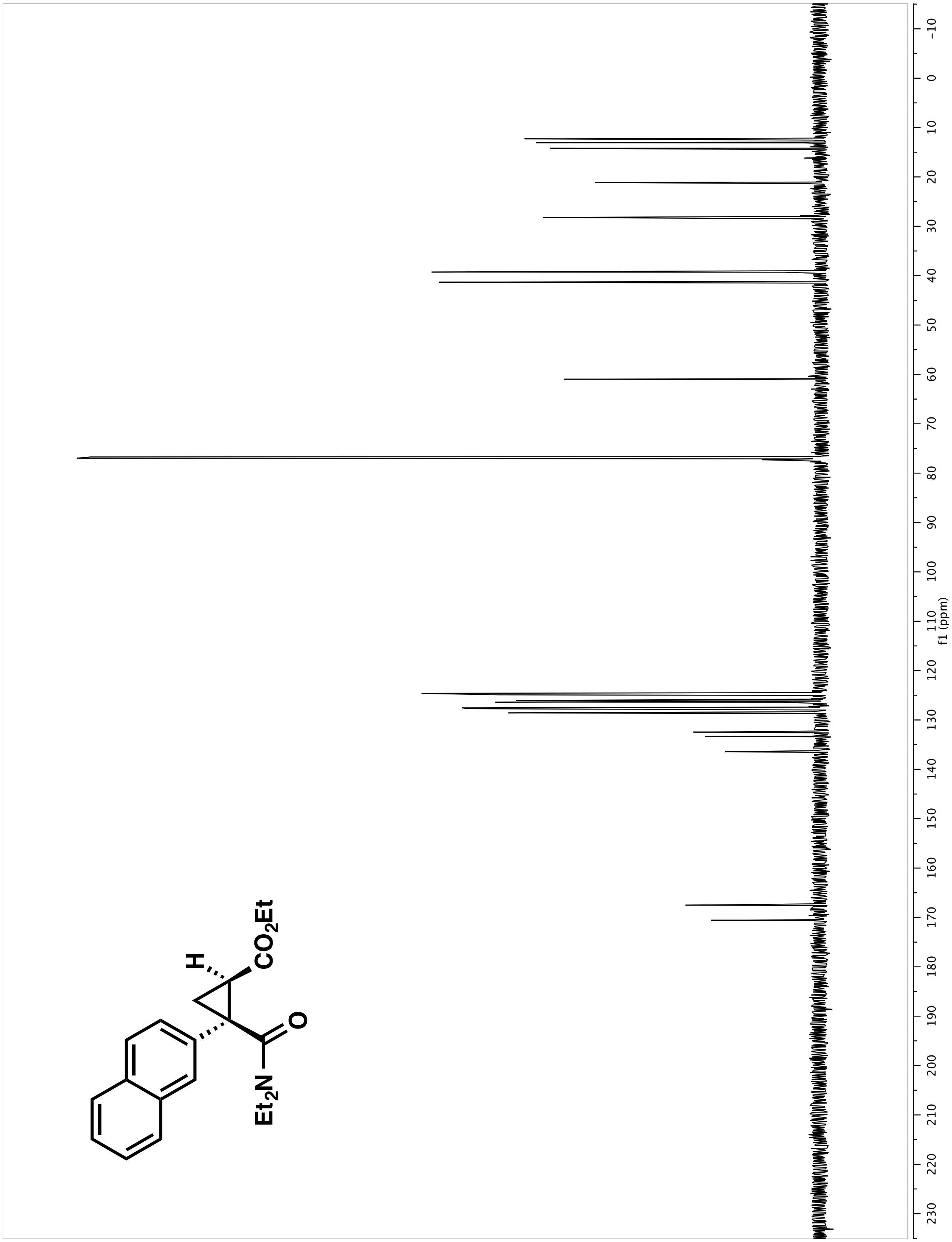


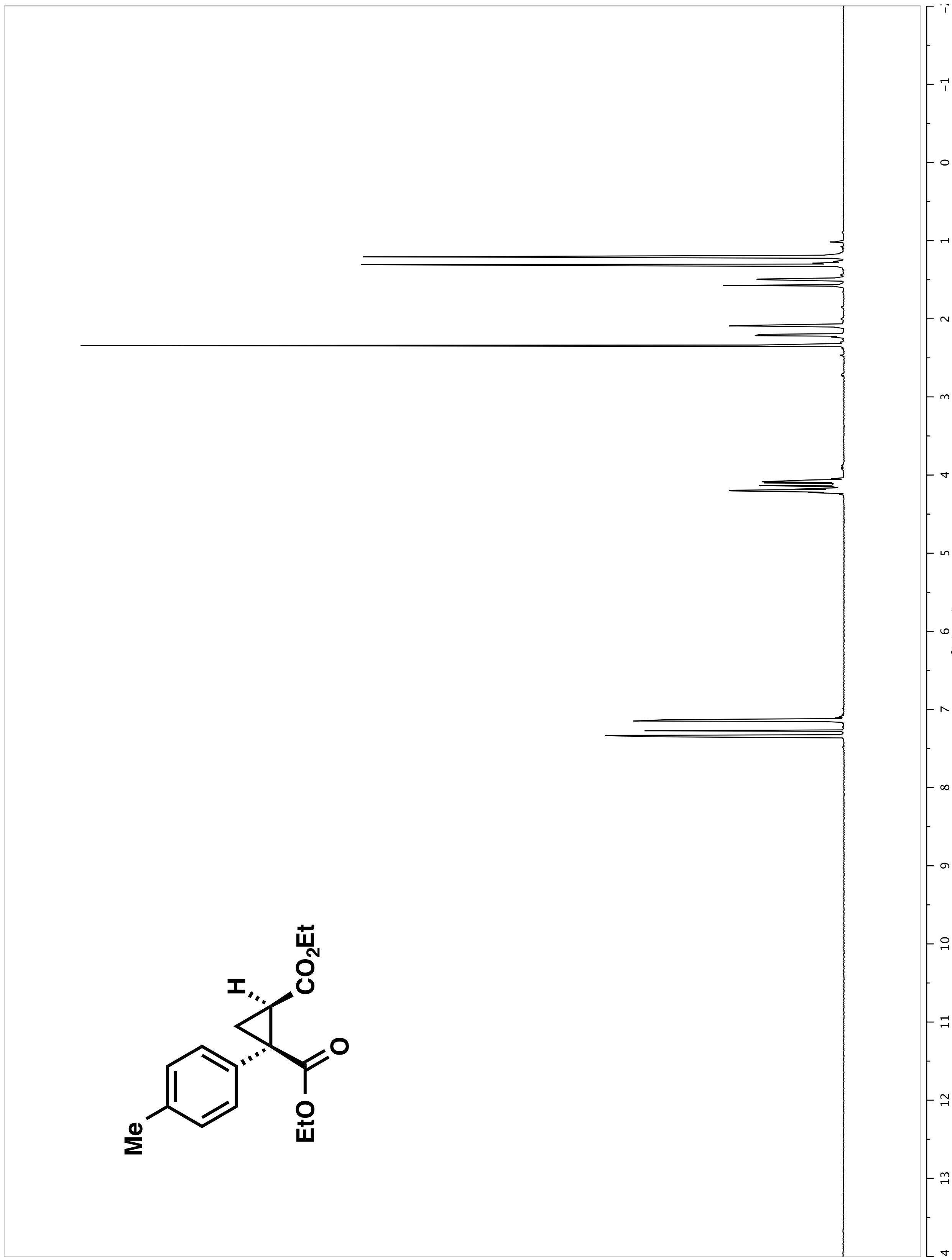

Cl
H
CO2Et
O
Et2N
f1 (ppm)

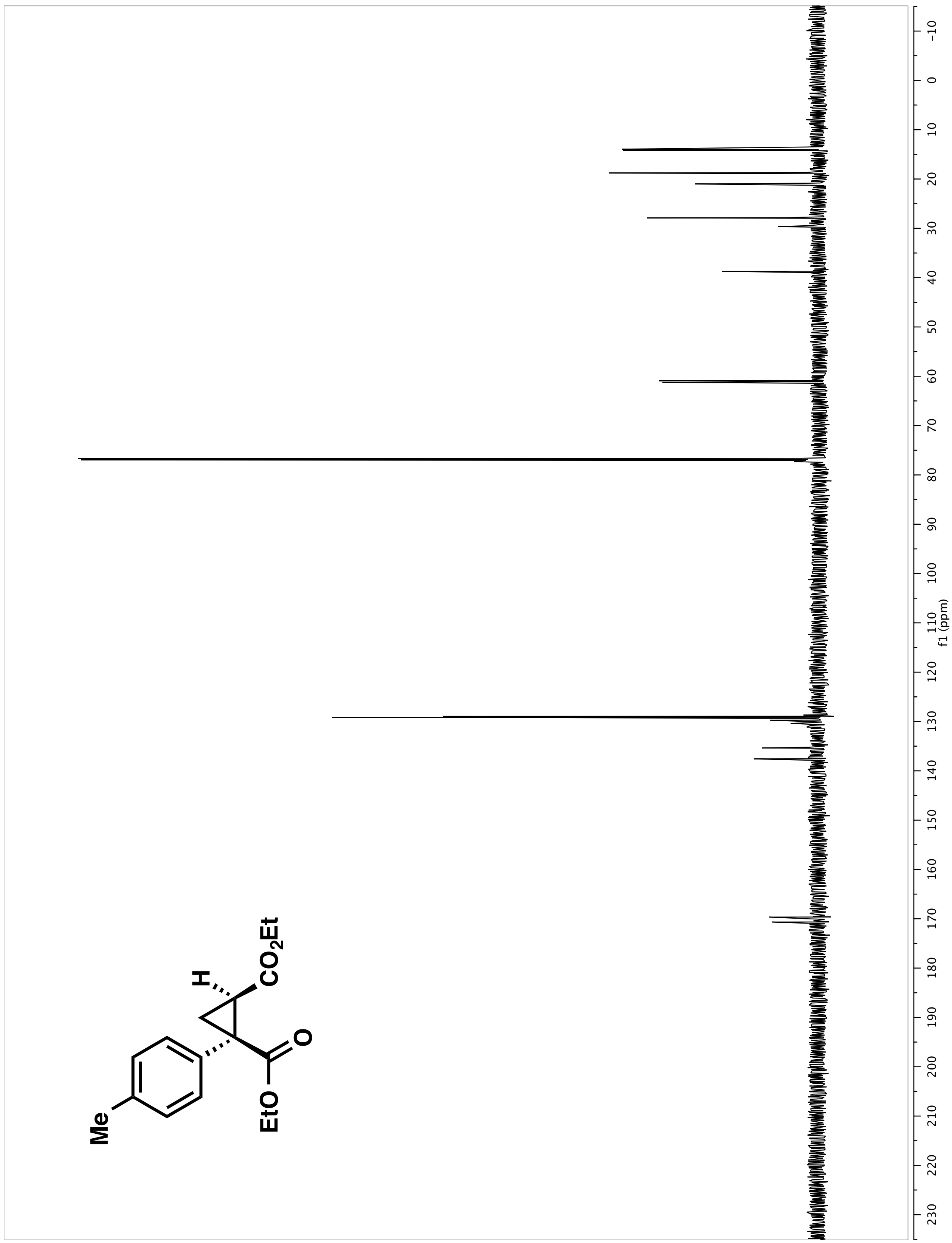

f1 (ppm)

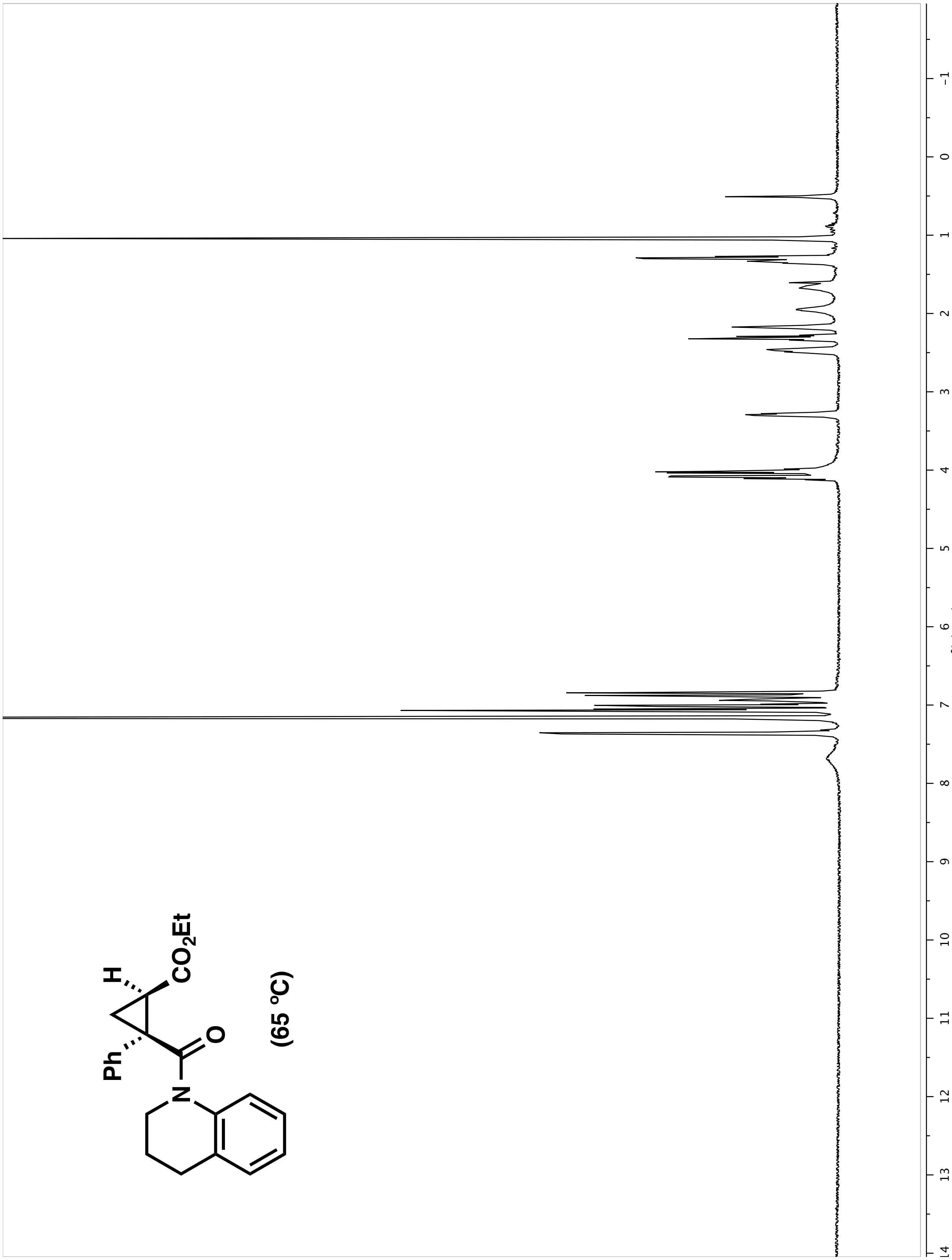

f1 (ppm)
F3C
H
CO2Et
O
Et2N


CO2Et
H
O
Et2N
f1 (ppm)


f1 (ppm)
H
CO2Et
O
Et2N


Me
EtO
O
H
CO2Et
f1 (ppm)
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1


Me
EtO
O
H
CO_2Et
f1 (ppm)


f1 (ppm)
(65 °C)