Supporting Information

Understanding Desaturation/Hydroxylation Activity of Castor Stearoyl Δ^9-Desaturase through Rational Mutagenesis

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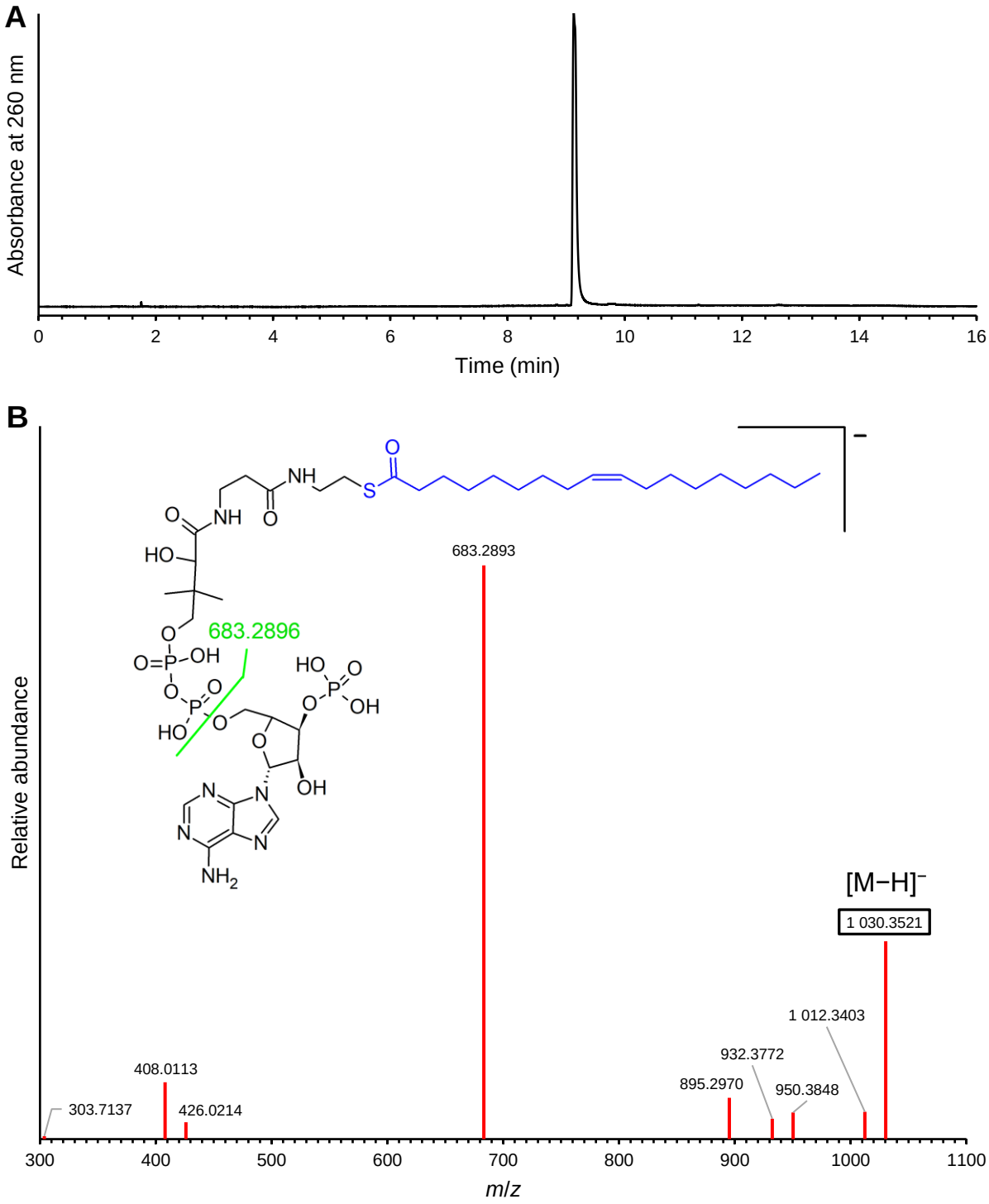


Figure S1. Enzymatic preparation of 9*Z*-18:1CoA. (**A**) Chromatogram after preparative HPLC. (**B**) MS/MS spectrum with the most prominent fragmentation highlighted in green.

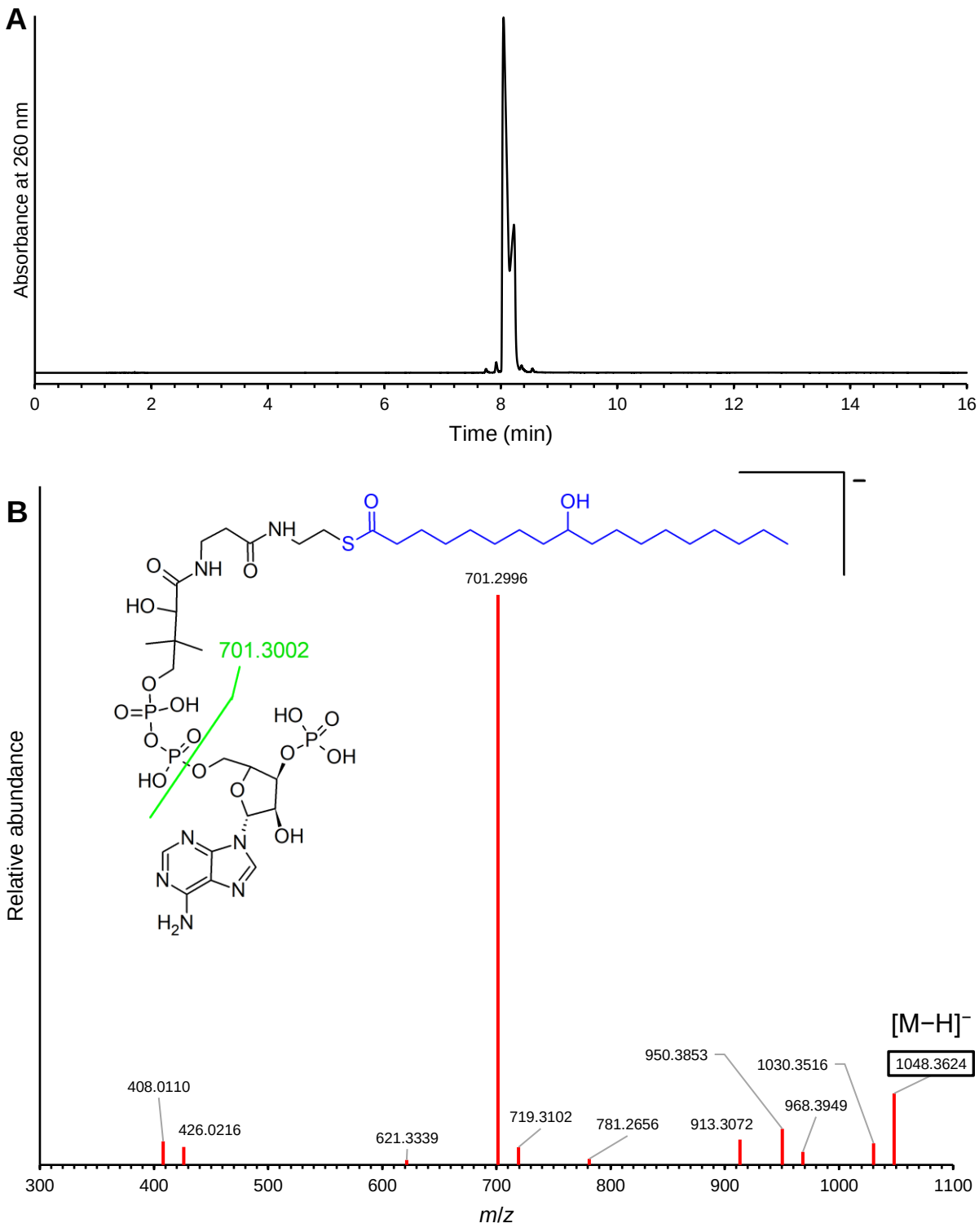


Figure S2. Enzymatic preparation of 9-OH-18:CoA. (**A**) Chromatogram after preparative HPLC. (**B**) MS/MS spectrum with the most abundant fragmentation highlighted in green.

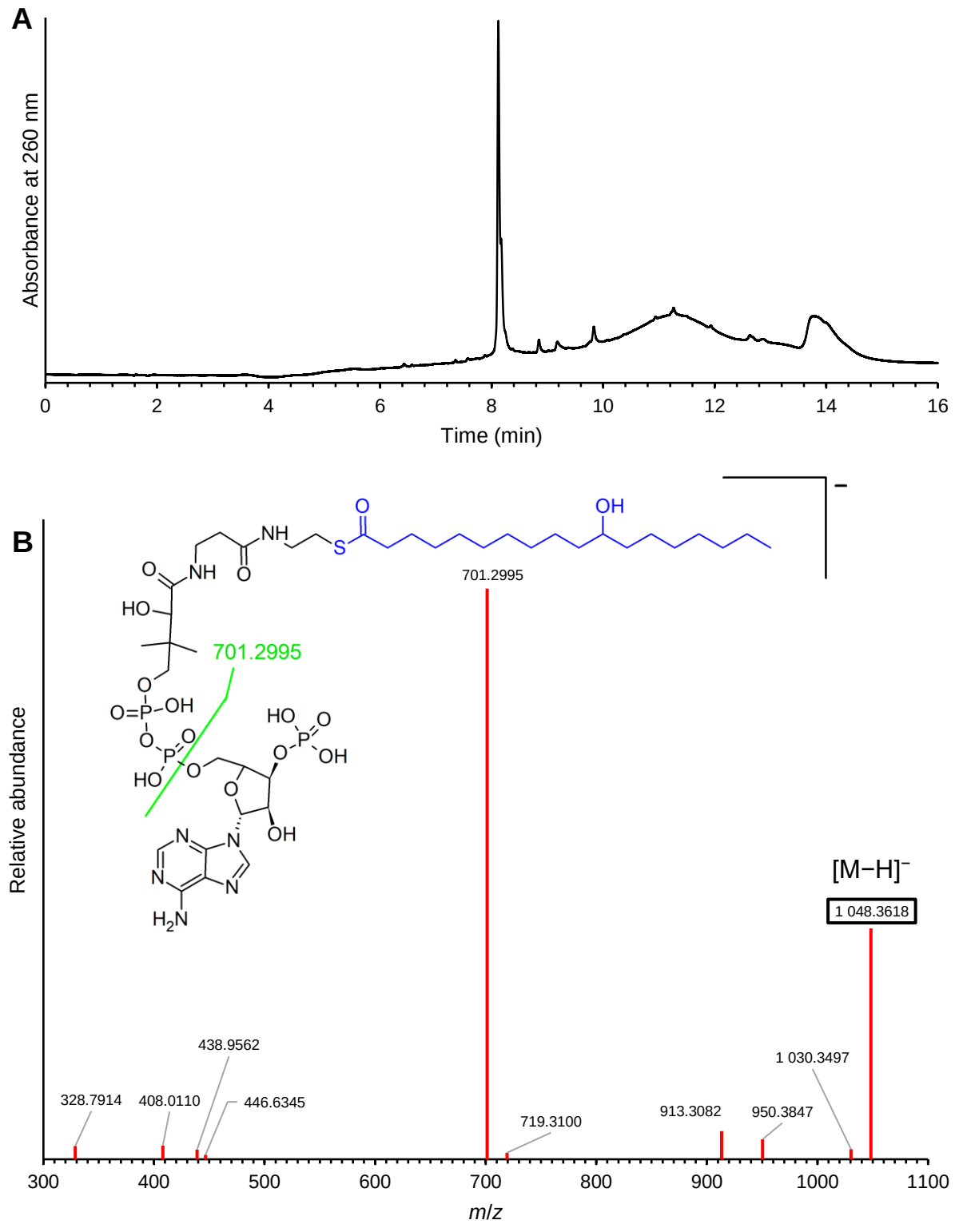


Figure S3. Enzymatic preparation of 11-OH-18:1CoA. (**A**) Chromatogram after preparative HPLC. (**B**) MS/MS spectrum with the most abundant fragmentation highlighted in green.

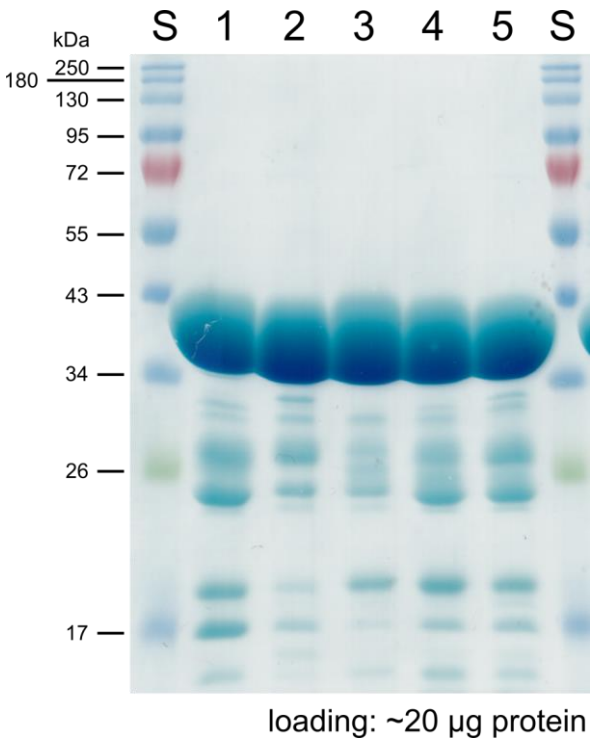


Figure S4. Analysis of the prepared enzymes by SDS-PAGE. Photograph of a Coomassie brilliant blue-stained gel. *Lanes*: Δ^9D (**1**), Δ^9D^{D101I} (**2**), Δ^9D^{H203I} (**3**), $\Delta^9D^{D101I/H203I}$ (**4**), $\Delta^9D^{D101I/H203I/T206V/C222F}$ (**5**), colored standard (**S**; New England Biolabs).

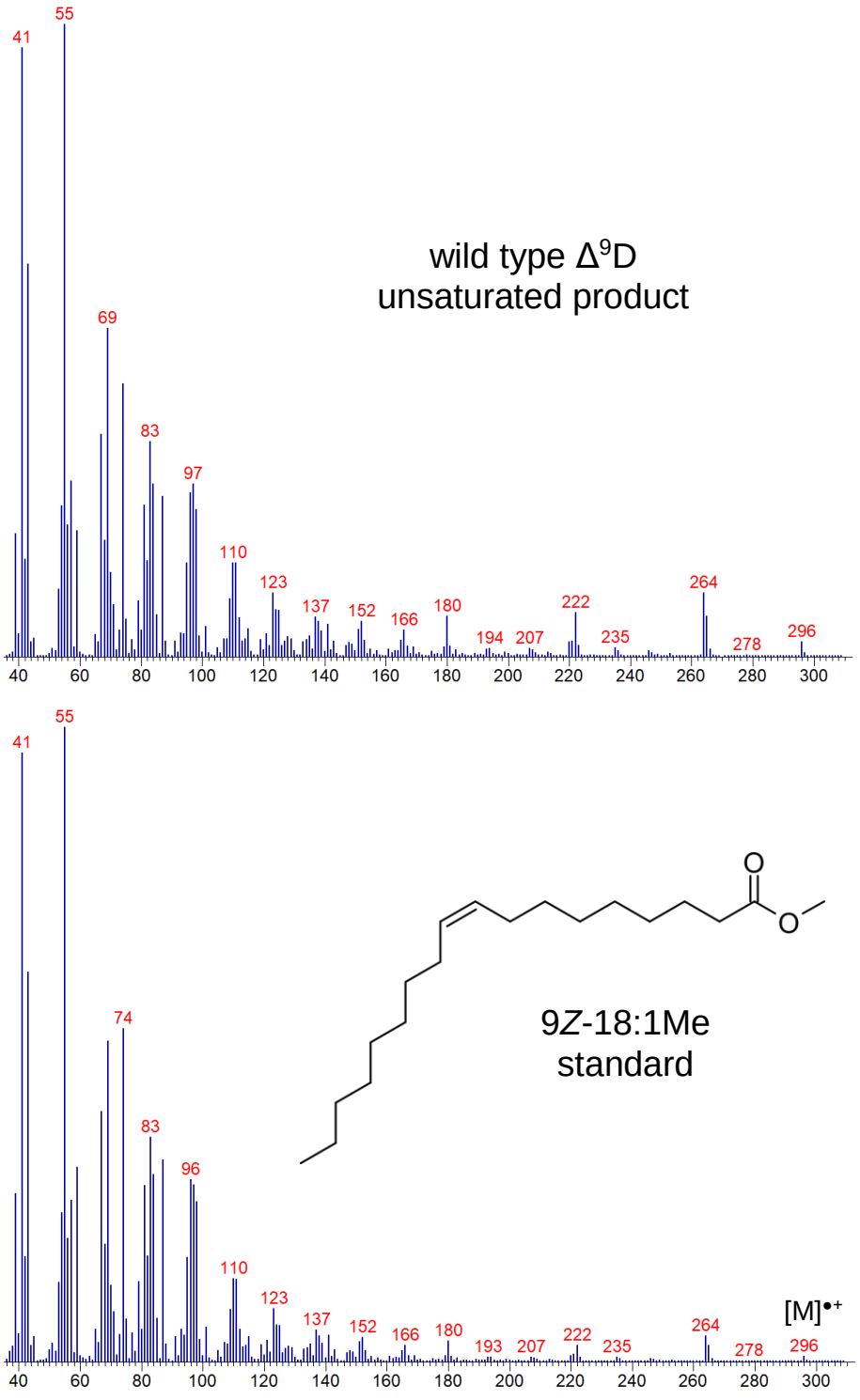


Figure S5. Mass spectrometric confirmation of desaturase products. Spectra of a derivatized unsaturated enzymatic product and a standard.

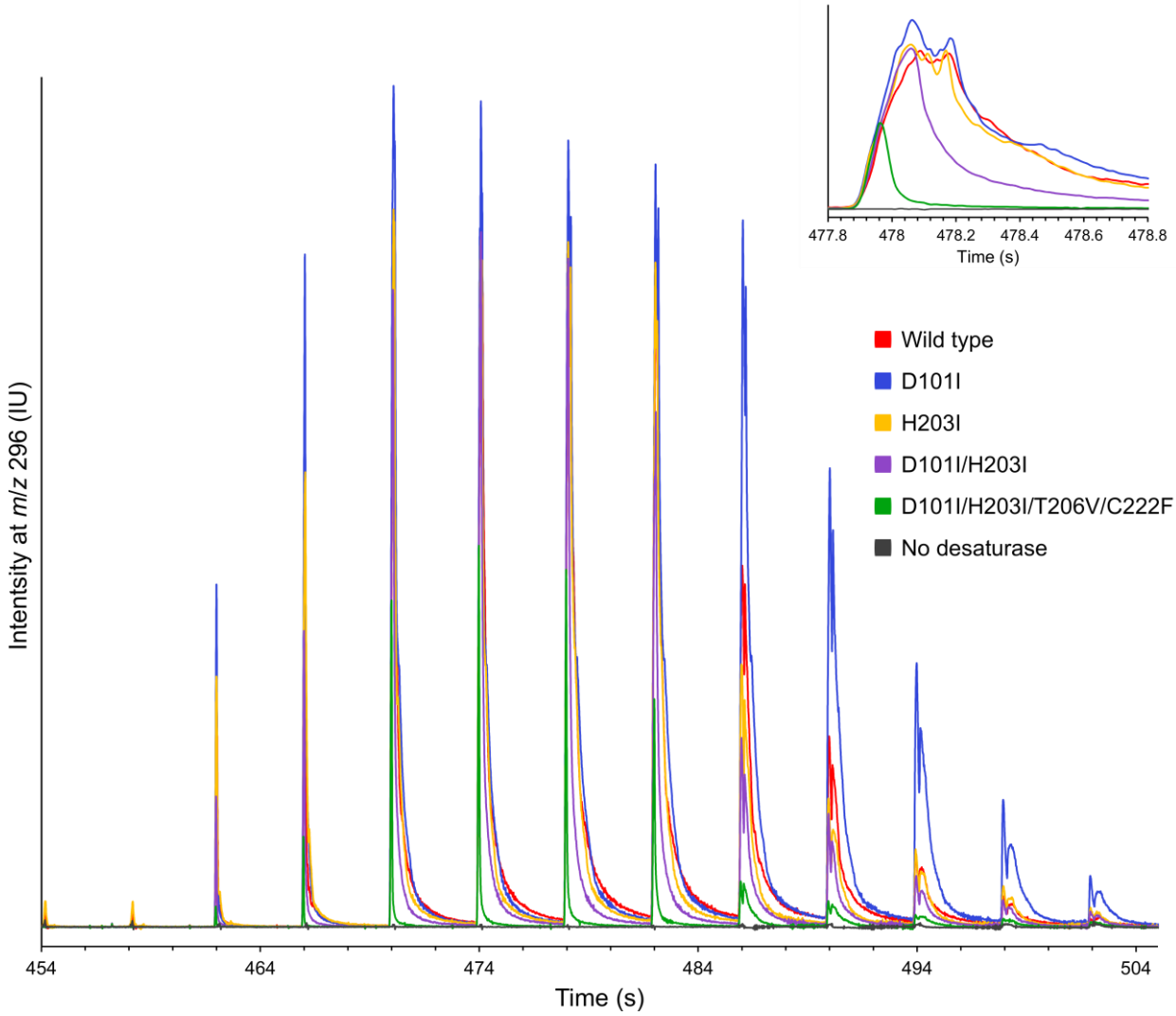


Figure S6. Analysis of the unsaturated desaturase product from reactions with 18:CoA substrate. Portion of GC×GC-MS chromatograms of derivatized reaction mixtures extracted at *m/z* 296 (i.e. specific for 18:1Me).

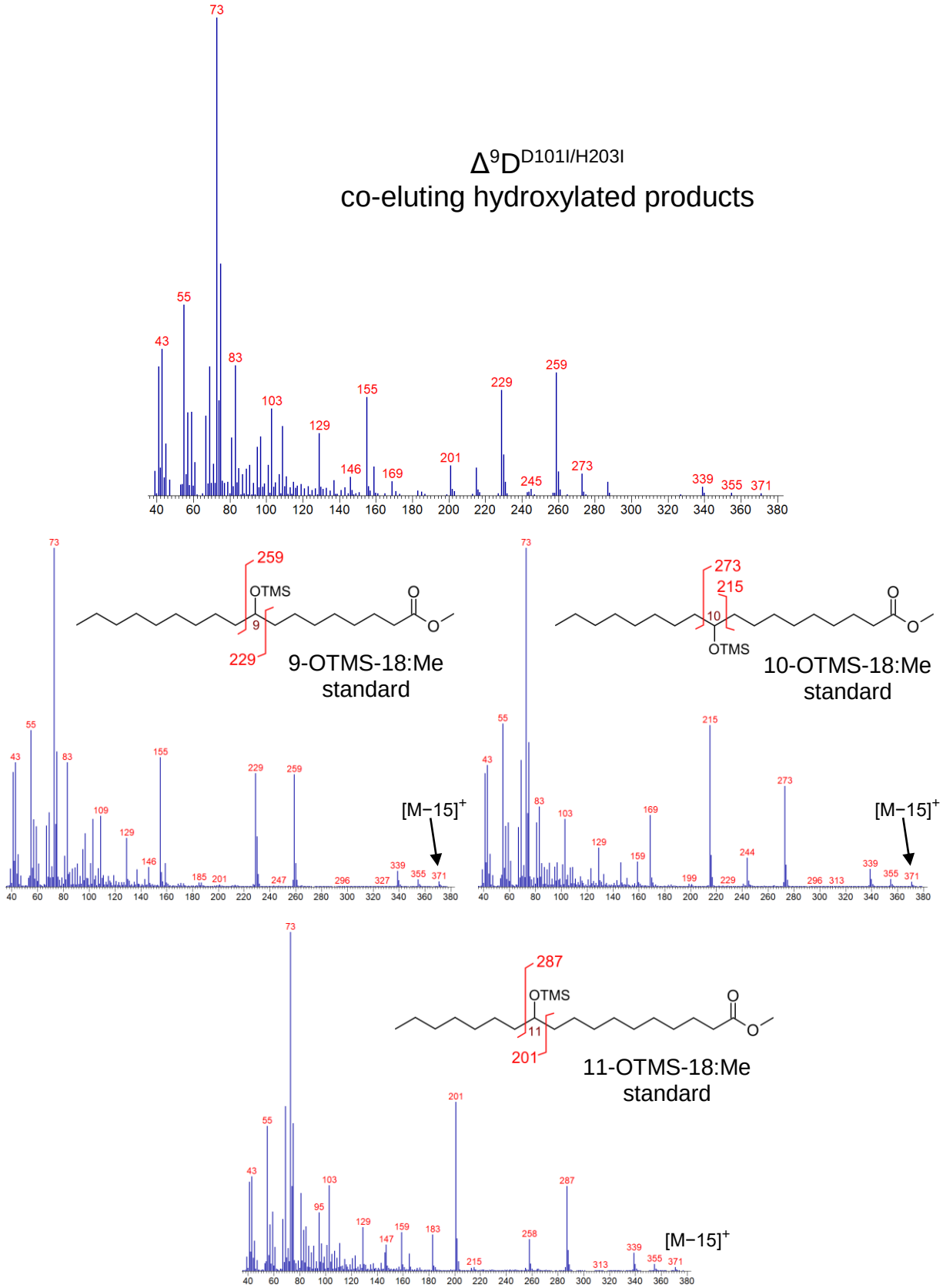


Figure S7. Mass spectrometric confirmation of hydroxylated desaturase products. Spectra of derivatized hydroxylated enzymatic products and standards, the latter with diagnostic fragmentations indicated.

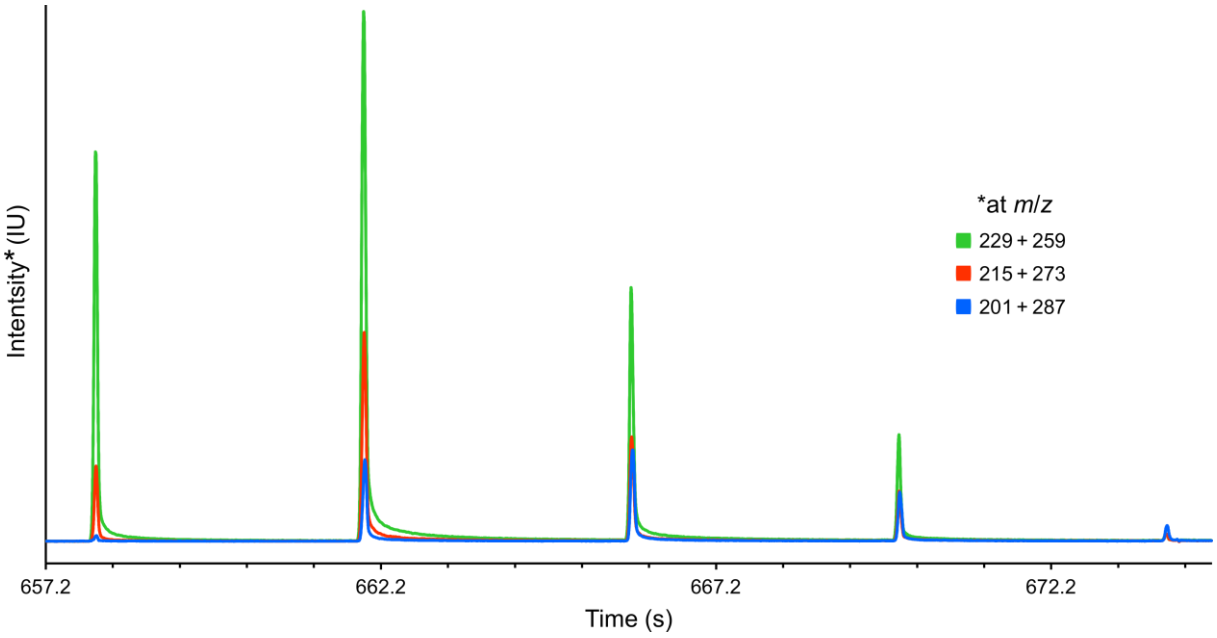


Figure S8. Composition of the major peaks of co-eluting monohydroxylated products. Portion of GC×GC-MS chromatograms from a reaction of $\Delta^9D^{D101I/H203I}$ extracted at *m*/*z* 229 + 259 (i.e. specific for 9-OTMS-18:Me), 215 + 273 (10-OTMS-18:Me) and 201 + 287 (11-OTMS-18:Me).

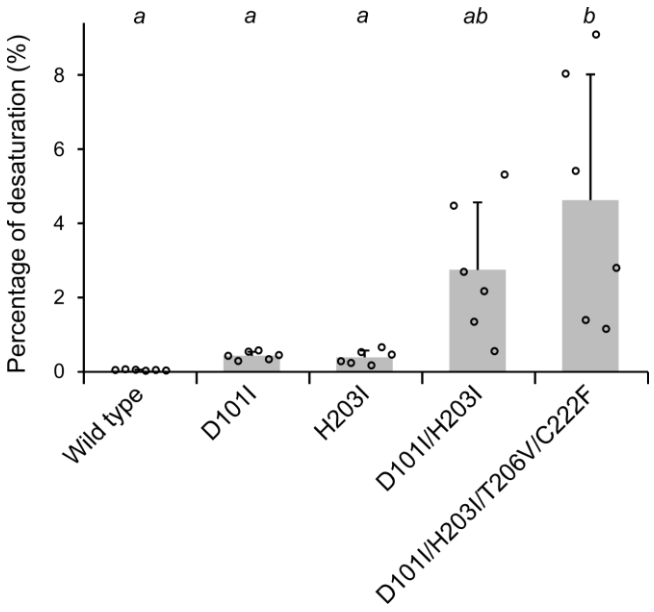


Figure S9. Ratio of hydroxylation to desaturation. The means sharing an identical italicized letter are not significantly different from each other (see section ***2.10.***).

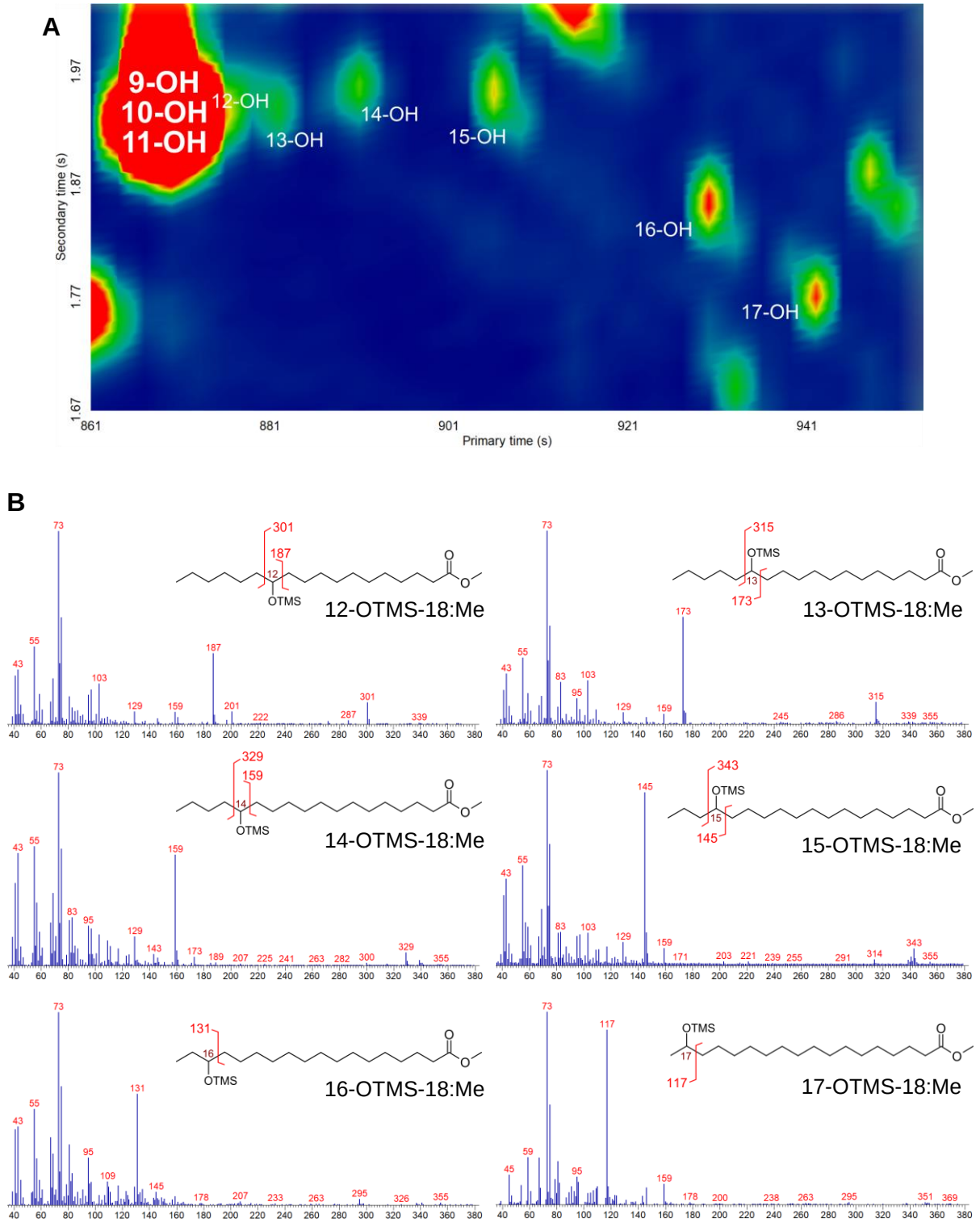


Figure S10. Additional trace hydroxylation products. (**A**) Portion of GC×GC-MS chromatogram (extracted at *m/z* 73) of a derivatized reaction mixture of $\Delta^9D^{D101I/H203I}$ with the assigned hydroxylated product peaks. (**B**) Spectra and proposed diagnostic fragmentations of all tentatively characterized peaks.

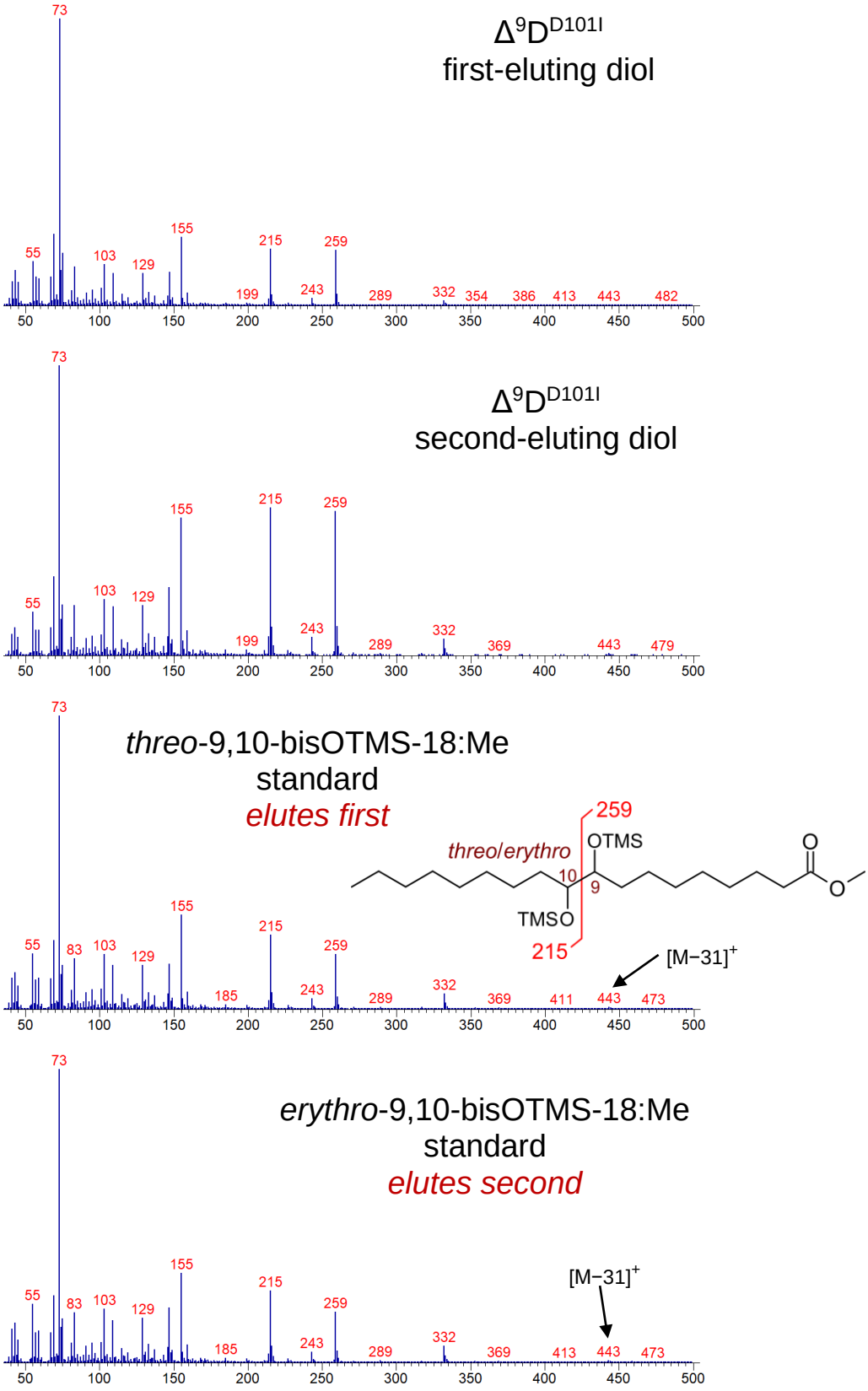


Figure S11. Mass spectrometric confirmation of diol products in reactions with oleoyl-CoA. Spectra of identified peaks from a derivatized Δ^9D^{D101I} reaction mixture and of synthetic standards, the latter with diagnostic fragmentations indicated.

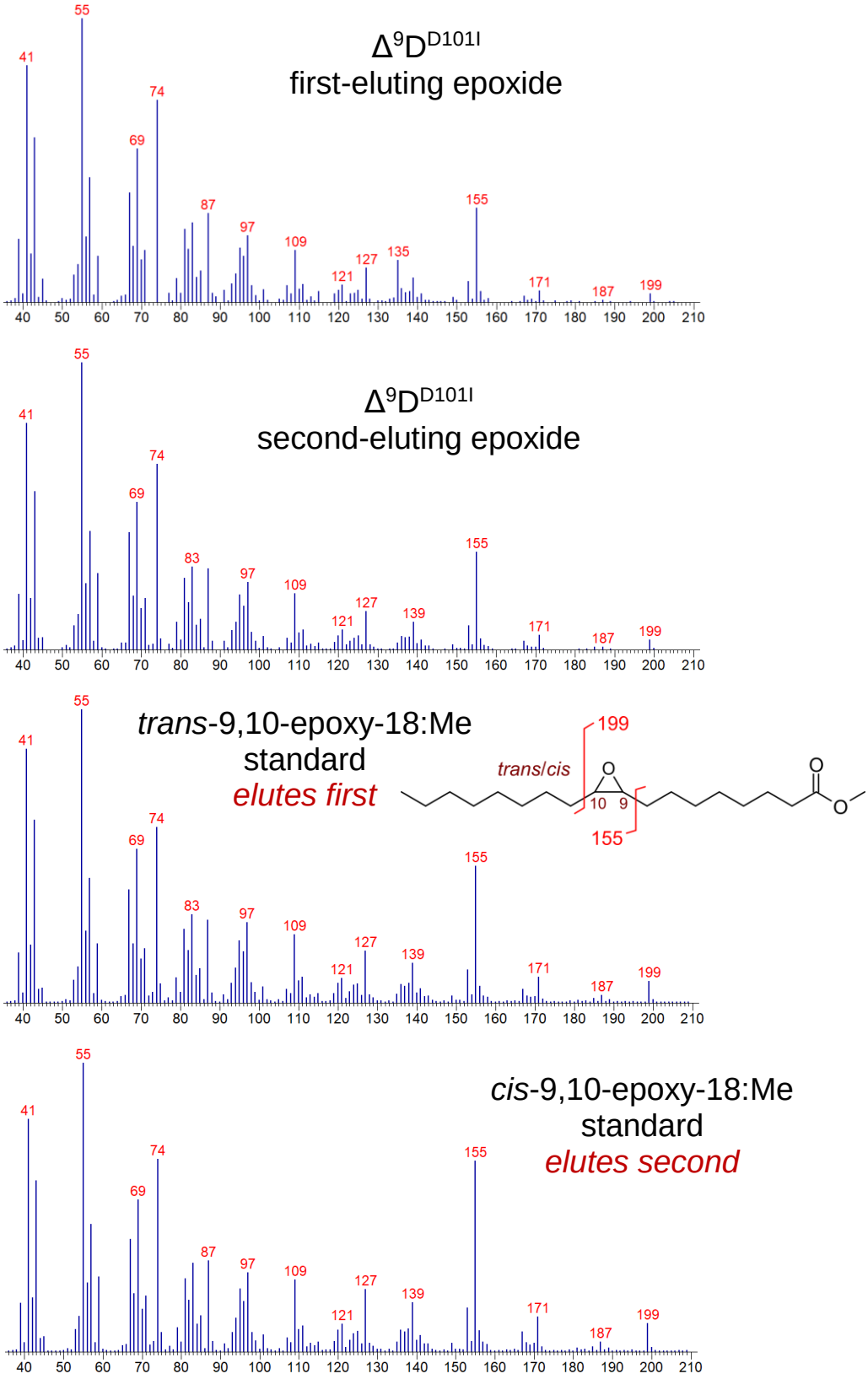


Figure S12. Mass spectrometric confirmation of epoxide products in reactions with oleoyl-CoA. Spectra of identified peaks from a derivatized Δ^9D^{D101I} reaction mixture and of synthetic standards, the latter with diagnostic fragmentations indicated.

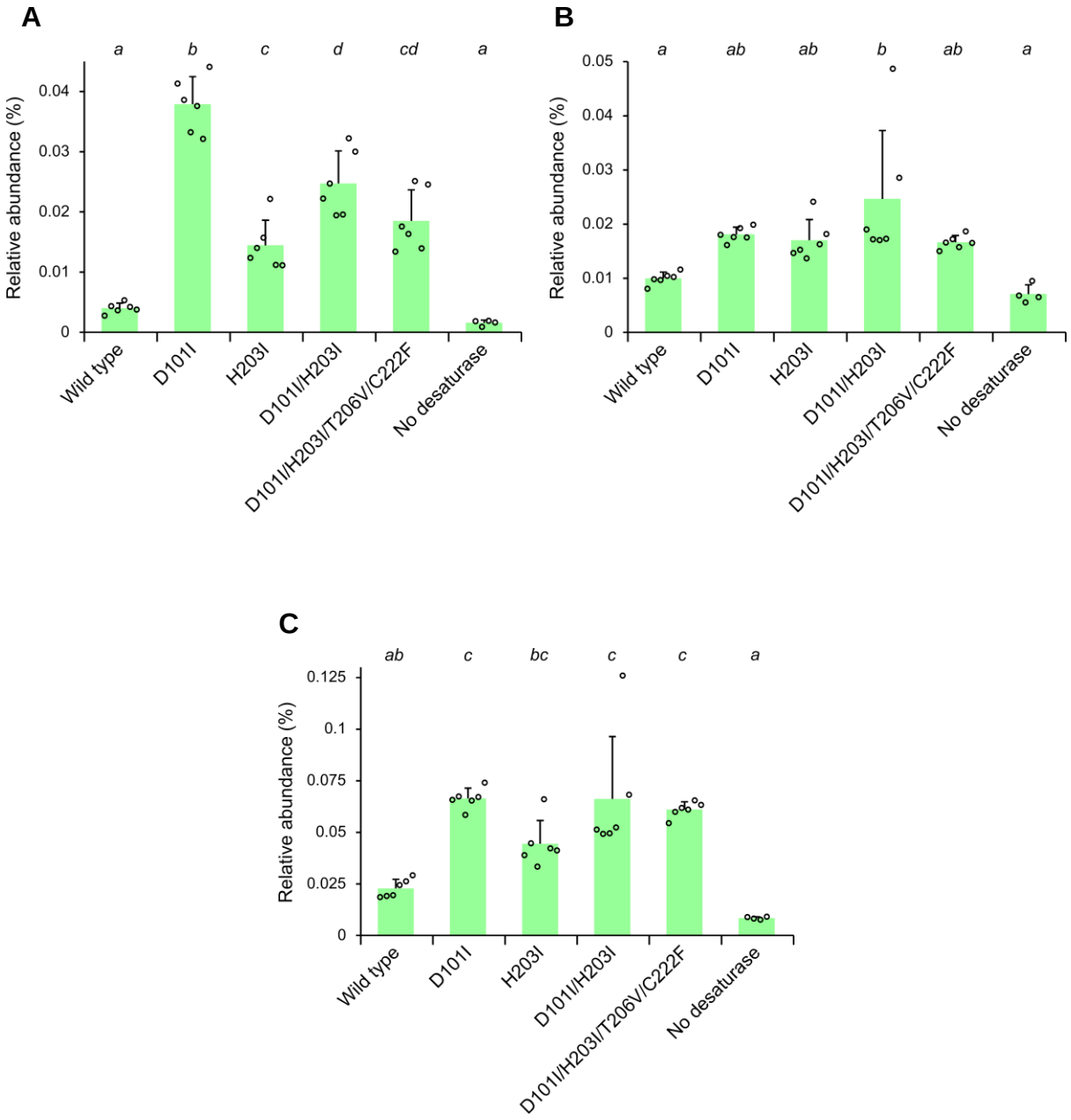


Figure S13. GC×GC-MS analysis of desaturase reactions with oleoyl-CoA substrate after derivatization. The relative abundances (see section ***2.10.***) of *threo*-9,10-dihydroxystearate (**A**), *trans*-9,10-epoxystearate (**B**) and a mixture of *Z*-allylic hydroxyoctadecenoates (**C**). The means sharing an identical italicized letter are not significantly different from each other (see section ***2.10.***, $N = 6$, $N = 4$ for control; $p < 0.01$).

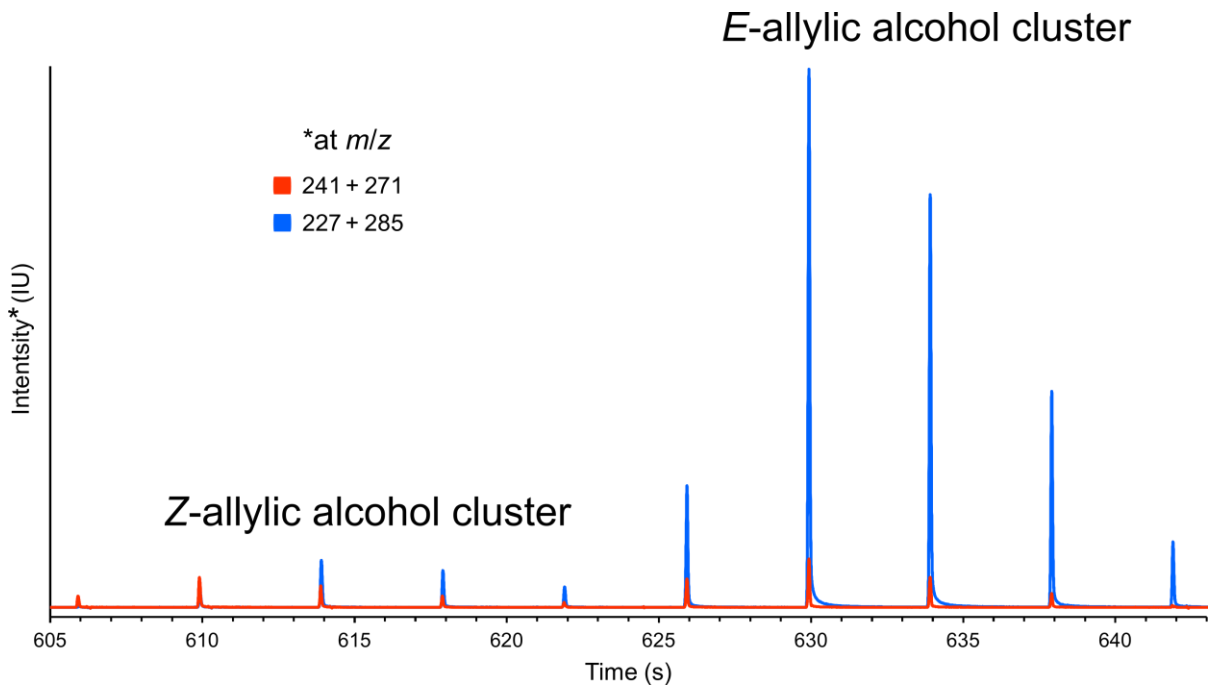


Figure S14. Composition of the peaks of co-eluting allylic products. Portion of GC×GC-MS chromatograms from a reaction of Δ^9D^{D101I} extracted at *m*/*z* 241 + 271 (i.e. specific for 8–10 allylic system) and 227 + 285 (9–11 allylic system).

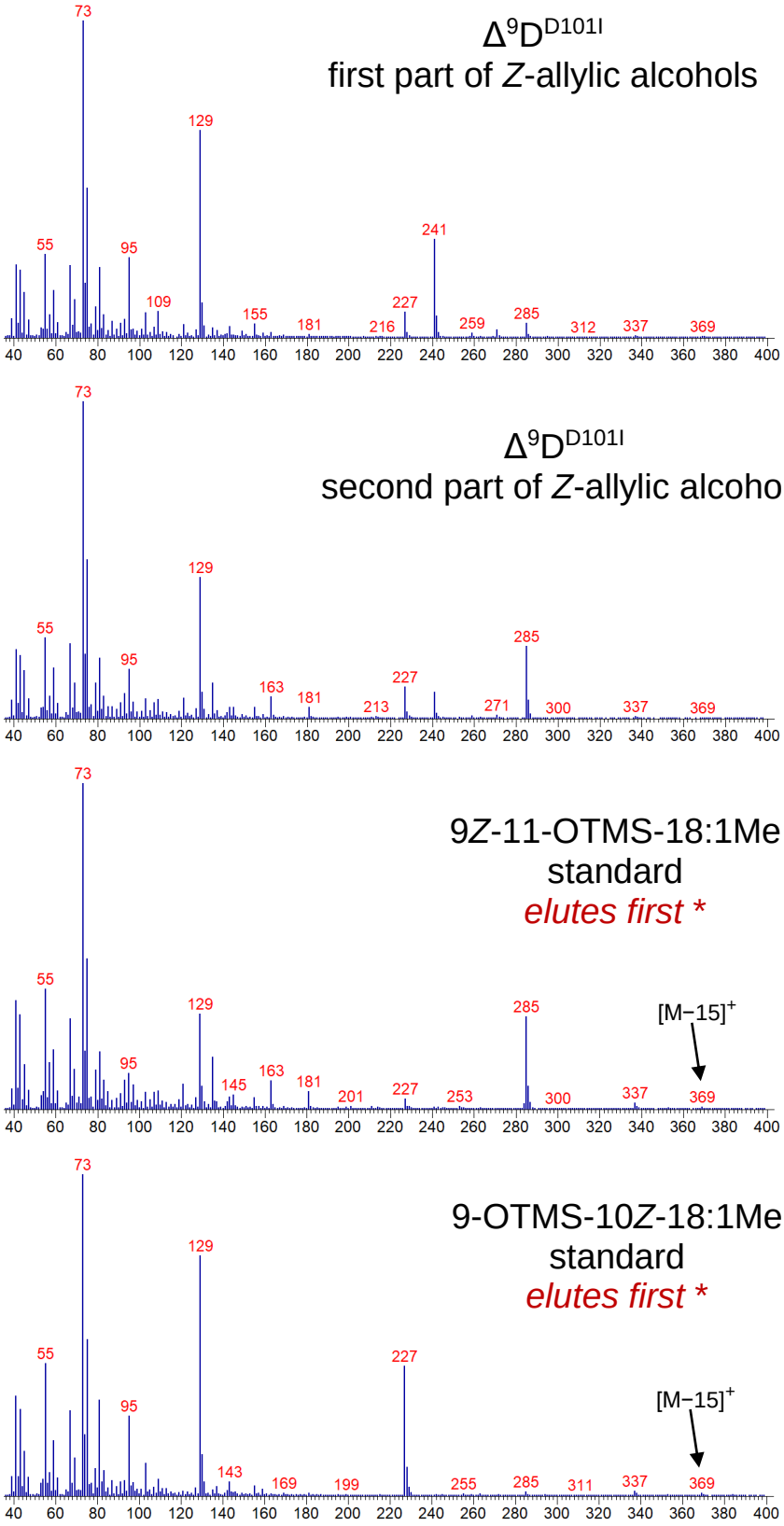


Figure S15. Mass spectrometric analysis of *Z*-allylic alcohol products in reactions with oleoyl-CoA as a substrate. Spectra of peaks from a derivatized Δ^9D^{D101I} reaction mixture and of synthetic standards. The order of elution relative to the opposite isomer (*E*) is indicated by an asterisk.

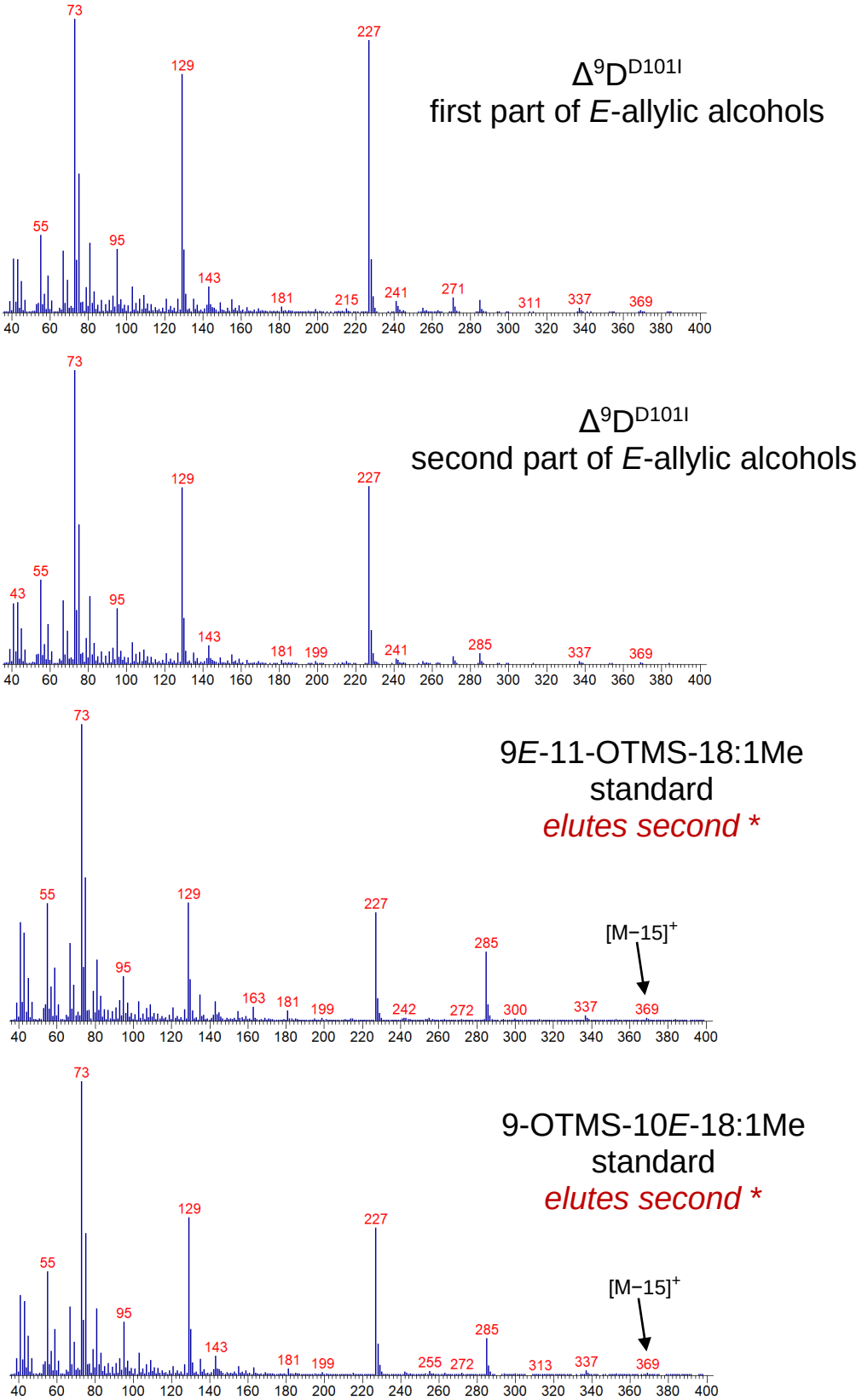


Figure S16. Mass spectrometric analysis of *E*-allylic alcohol products in reactions with oleoyl-CoA as a substrate. Spectra of peaks from a derivatized Δ^9D^{D101I} reaction mixture and of synthetic standards. The order of elution relative to the opposite isomer (*Z*) is indicated by an asterisk.

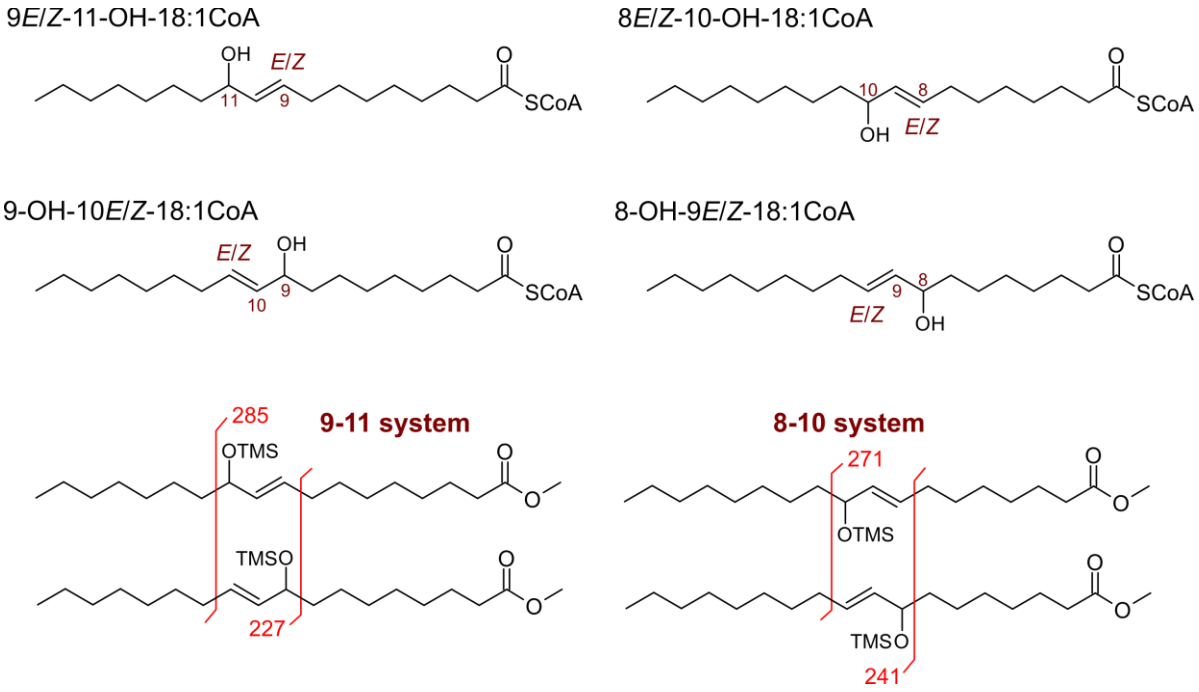


Figure S17. Possible 9–11 and 8–10 allylic products arising from 9*Z* unsaturated substrate with indicated diagnostic fragmentations of the allylic systems after derivatization.

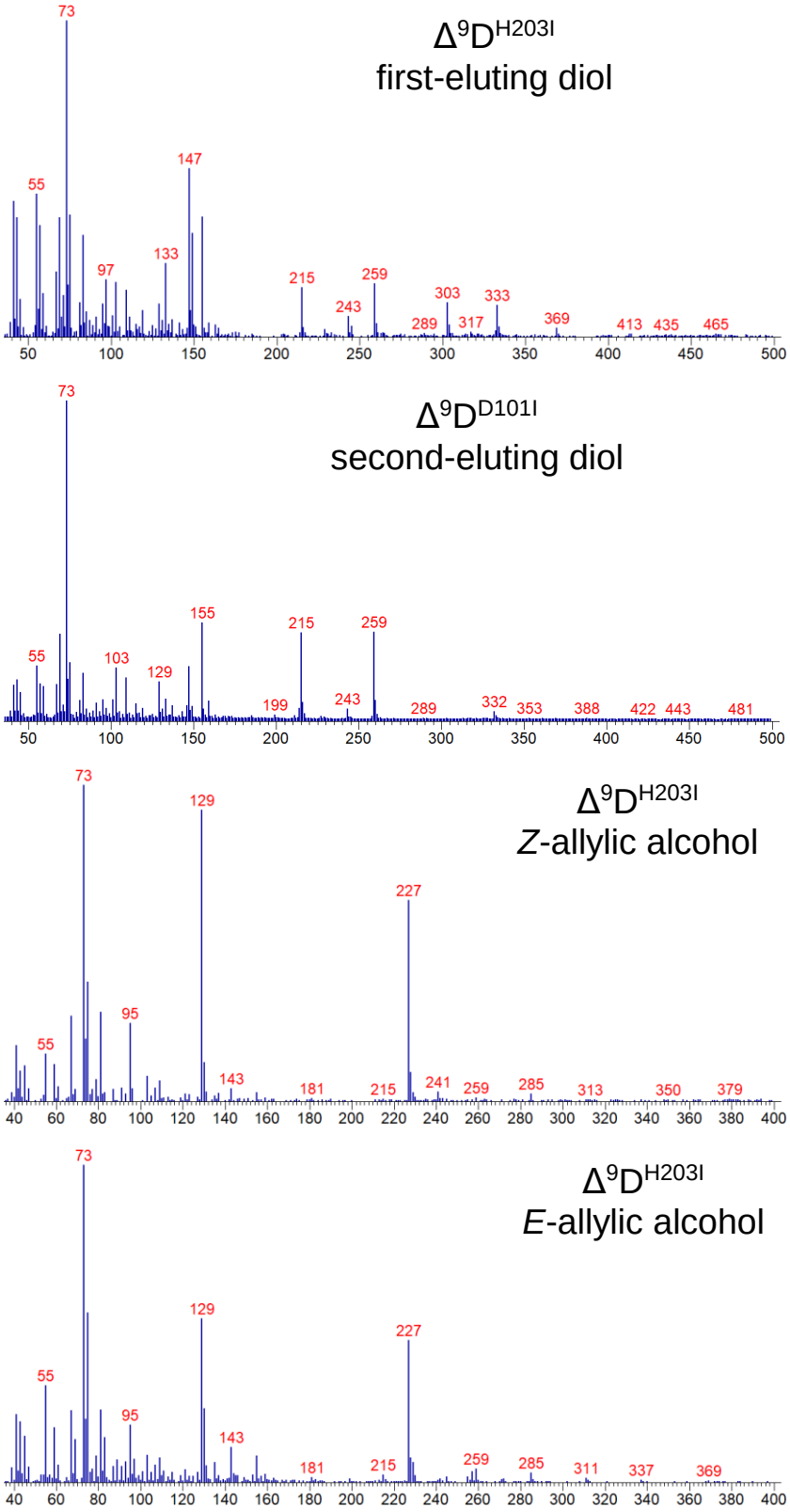


Figure S18. Mass spectrometric analysis of diol and allylic alcohol products in reactions with 9-hydroxystearoyl-CoA. Spectra of peaks from a derivatized Δ^9D^{D101I} and Δ^9D^{H203I} reaction mixtures.

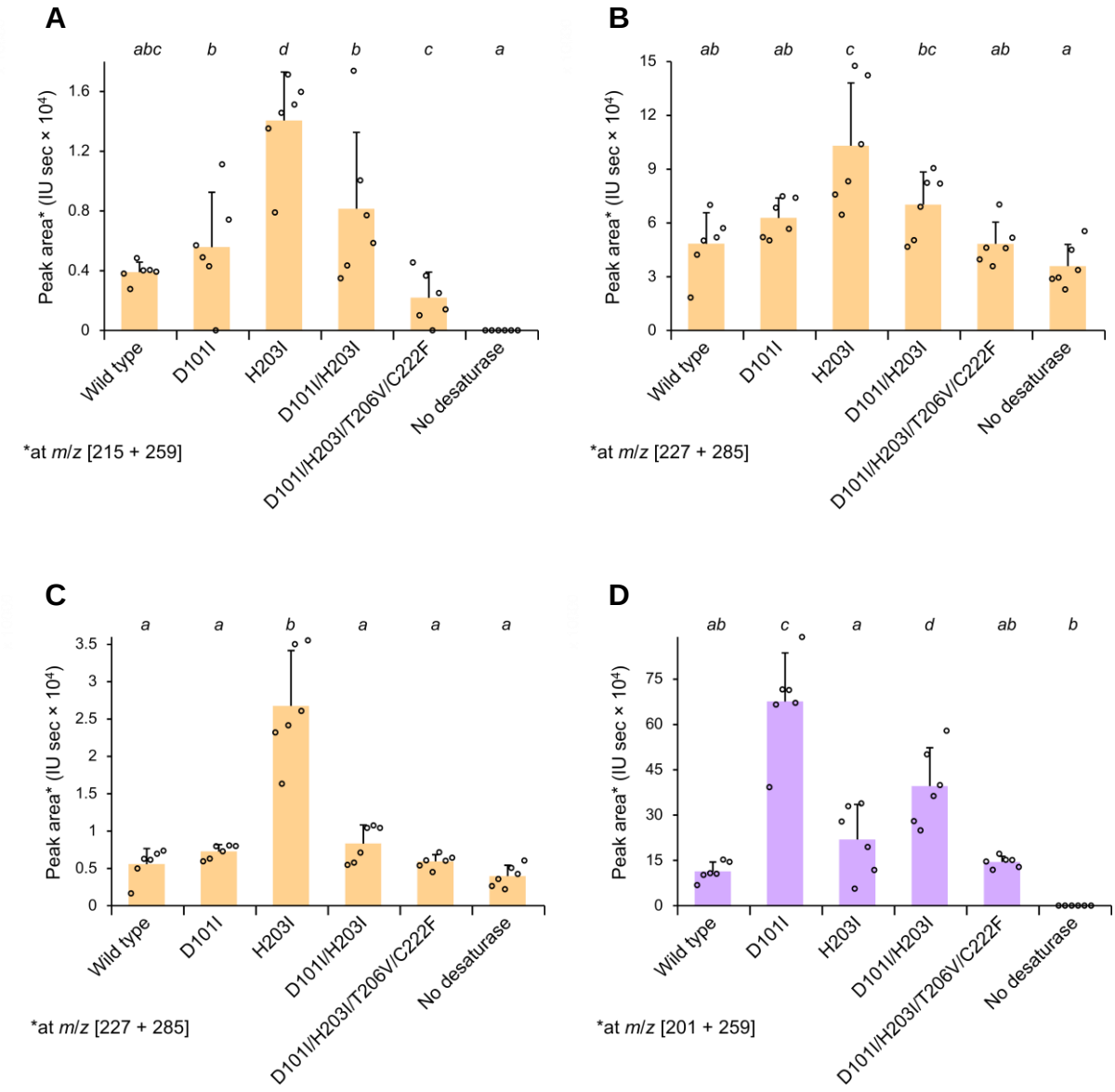


Figure S19. GC×GC-MS analysis of desaturase reactions with 9- (*yellow*) and 11-hydroxystearoyl-CoA (*lila*) substrate after derivatization. The relative abundances (see section ***2.10.***) of *threo*-9,10-dihydroxystearate (**A**), 10*E*-9-hydroxyoctadec-10-enoate (**B**), 10*Z*-9-hydroxyoctadec-10-enoate (**C**) and diastereomer 2 of 9,11-dihydroxystearate (**D**). The means sharing an identical italicized letter are not significantly different from each other (see section ***2.10.***, $N = 6$; **A**: $p < 0.05$, **B**: $p < 0.05$, **C**: $p < 0.01$, **D**: $p < 0.05$).

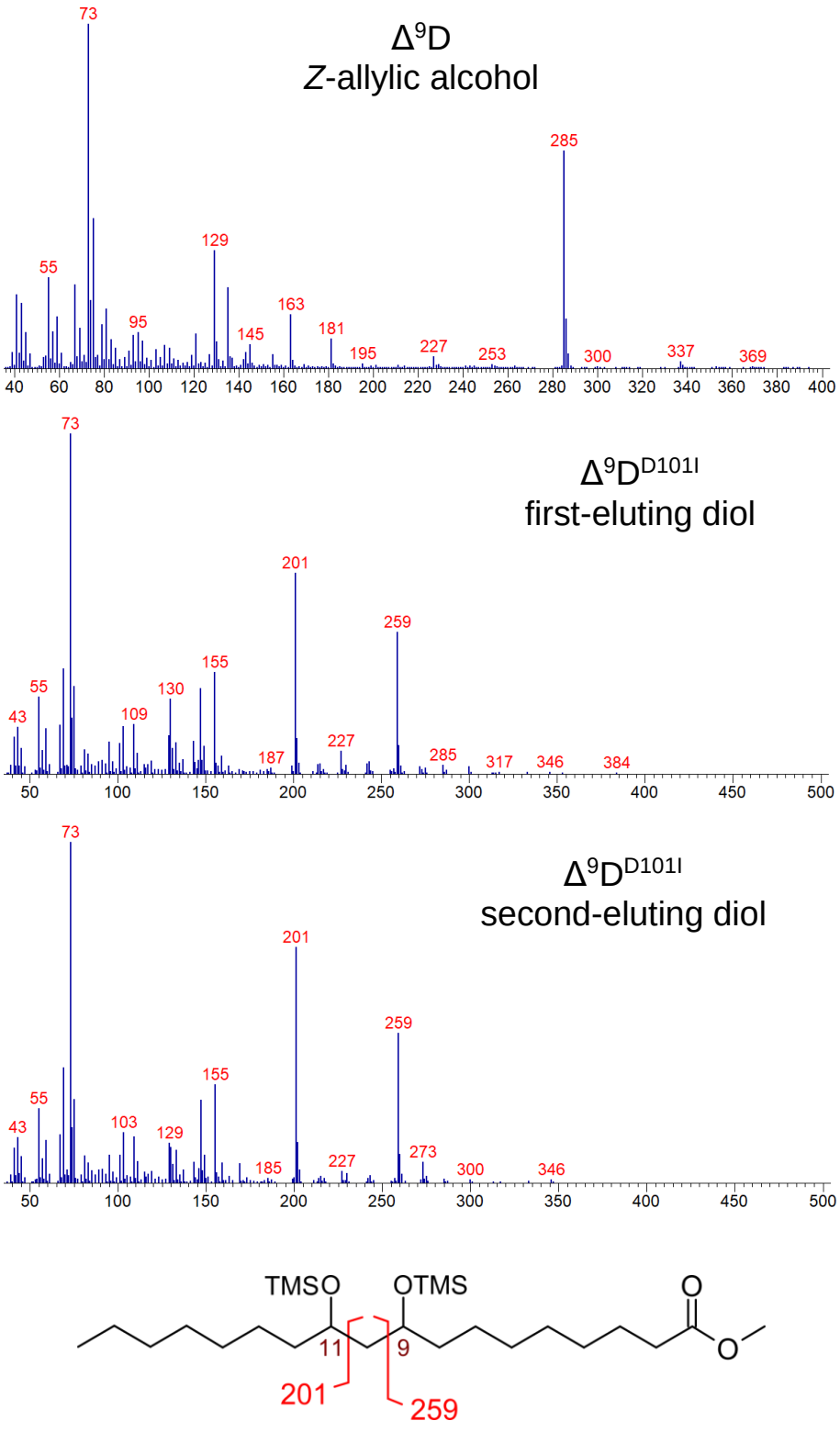


Figure S20. Mass spectrometric analysis of allylic alcohol and diol products in reactions with 11-hydroxystearoyl-CoA. Spectra of peaks from a derivatized wild type and Δ^9D^{D101I} reaction mixtures, and proposed diagnostic fragmentations of both diastereomeric 9,11-dihydroxystearates.

Supplementary Tables

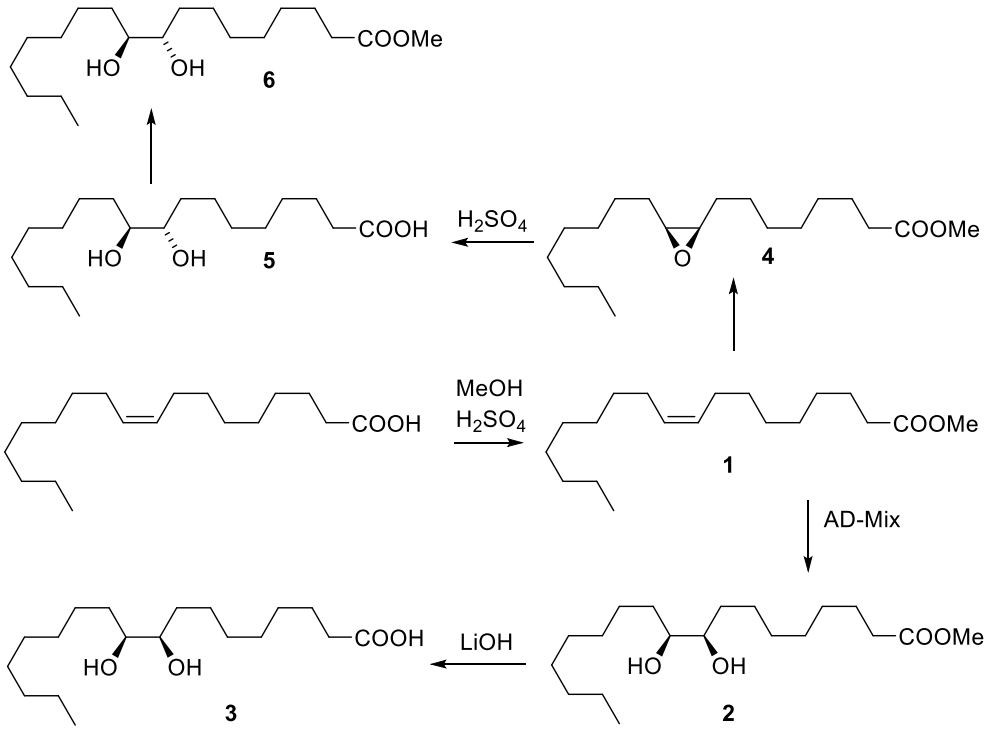
Table S1. Primers used for cloning. Endonuclease restriction sites are underlined.

Name	Sequence (5'–3')	Purpose
RcoD_NdeI_F	TTAGTTATACATATGCCACCACGCGAAGTTC	Restriction cloning
RcoD_BamHI_R	ATAGGATCCTTACAGTTTCACCTGGCGG	—
RcoD-101I_F	ATTATGATTACCGAAGAAGCGCTG	Mutagenesis
RcoD-101I_R	CTTCGGTAATCATAATACCAACCAGAA	—
RcoD-203I_F	ATTGGTAACACCGCACGTCAAG	—
RcoD-203I_R	CGGTGTTACCAATGCTAATAAAGG	—
EcoFadD_NdeI_F	TAGTAAATACATATGAAAAAGGTTTGGCTGAACC	Restriction cloning
EcoFadD_BamHI_R	ATAAGGATCCTTACGCCTTGTTGTCCAC	—
T7_seq_F	TAATACGACTCACTATAGGG	Sequencing
T7_seq_R	GCTAGTTATTGCTCAGCG	—

Supplementary Methods

Organic synthesis. Unless otherwise noted, all reactions were carried out under argon in oven-dried glassware. Solvents were distilled from drying agents as indicated and transferred under argon: THF (Na/benzophenone), toluene (Na/benzophenone) and DCM (CaH_2). Chromatography was performed using Fluka silica gel 60 (0.040 - 0.063 mm) or Merck silica gel 60. Spots were detected both by UV light and a solution of $Ce(SO_4)_2 \cdot 4H_2O$ (1%) and $H_3P(Mo_3O_{10})_4$ (2%) in 10% sulfuric acid. All starting materials were used as purchased (Sigma Aldrich, TCI Chemicals), unless otherwise indicated. Grignard reagents were purchased from Sigma Aldrich. ^{1}H-NMR spectra were recorded on Bruker instrument at 400 MHz; ^{13}C-NMR spectra at were recorded at 100 MHz. Chemical shifts are provided in δ-scale; coupling constants J are given in Hz. The EI mass spectra were determined at an ionizing voltage of 70 eV; m/z values are given alone with their relative intensities (%). ESI mass spectra were recorded using a ZQ micromass mass spectrometer (Waters) equipped with an ESCi multimode ion source and controlled by MassLynx software. Methanol was used as the solvent.

Scheme S1. Preparation of *erythro* and *threo* diols and *cis*-epoxystearate.



Methyl oleate (1)

Oleic acid (1.0 g; 3.54 mmol) was dissolved in methanol (10 mL) and *conc*. sulfuric acid (0.5 mL) was added. The reaction mixture was stirred at 80 °C for 8 hours. Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:9) affording 0.92 g (87 %) of **1** as a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.52 – 5.14 (m, 2H), 3.69 (s, 3H), 2.33 (t, J = 7.6 Hz, 2H), 2.03 (dd, J = 5.2, 2.3 Hz, 4H), 1.64 (dd, J = 9.3, 5.4 Hz, 2H), 1.55 – 1.20 (m, 20H), 1.13 – 0.76 (m, 3H).

Methyl *erythro*-9,10-dihydroxyoctadecanoate (2)

A mixture of *t*-BuOH (5 mL), water (5 mL), methanesulfonamide (0.10 g; 1.0 mmol) and AD-Mix-α (1.4 g) was stirred for 1 hour at room temperature and then was placed into an ice bath. Then methyl oleate (0.29 g; 1.0 mmol) was added dropwise and the mixture was stirred overnight in the ice bath. The reaction mixture was quenched with addition of Na_2SO_3 (1.5 g; 12.0 mmol) and then was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water and brine. The solvent was evaporated and the crude product was purified by column chromatography (silica gel, eluent EtOAc:cyclohexane/2:1) affording 0.25 g (75 %) of diol **2** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{38}O_4Na$ $[M+Na]^+$ 353.2662, found 353.2660.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.69 (s, 3H), 3.66 – 3.55 (m, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.64 (m, 4 H), 1.56 – 1.39 (m, 6H), 1.38 – 1.20 (m, 18H), 1.03 – 0.82 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 74.7, 74.6, 51.5, 34.1, 31.9, 31.2, 31.1, 29.7, 29.6, 29.4, 29.3, 29.1, 29.0, 26.0, 25.9, 24.9, 22.7, 14.1.

***erythro*-9,10-Dihydroxyoctadecanoic acid (3)**

A mixture of ester **2** (0.23 g; 0.7 mmol), lithium hydroxide (0.05 g; 2.1 mmol), THF (4 mL), methanol (4 mL) and water (4 mL) was stirred overnight at room temperature. Then the reaction was quenched with aqueous citric acid and product was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine and evaporated. The crude product was purified by column chromatography (silica gel, eluent DCM:MeOH:AcOH/46:2:1) affording 0.15 g (67 %) of diol **3** as a colorless oil.

HR-ESI-MS calculated for $C_{18}H_{36}O_4Na$ $[M+Na]^+$ 339.2506, found 339.2505.

^{1}H NMR (400 MHz, Methanol-d_4) δ 3.37 (m, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.61 (m, 6H), 1.50 – 1.26 (m, 20H), 1.00 – 0.92 (m, 3H).

^{13}C NMR (100 MHz, Methanol-d_4) δ 176.3, 74.56, 74.54, 33.6, 32.2, 32.2, 31.7, 29.5, 29.3, 29.3, 29.05, 29.02, 28.8, 25.63, 25.56, 24.7, 22.3, 13.0.

Methyl *cis*-9,10-epoxyoctadecanoate (4)

Methyl oleate **1** (1.0 g; 3.35 mmol) was dissolved in DCM (20 mL) and the reaction mixture was placed into an ice bath. Then MCPBA (80% w/w; 0.91 g; 4.25 mmol) was added at once. The reaction mixture was stirred in an ice bath for 2 hours. Then the mixture was partitioned between water and DCM. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by chromatography (silica gel, eluent EtOAc:cyclohexane/1:9) affording 0.99 g (94 %) of **4** as a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.69 (s, 3H), 2.92 (bs, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.65 (m, 2H), 1.56– 1.23 (m, 24H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 57.25, 57.20, 51.5, 34.1, 31.9, 29.57, 29.54, 29.35, 29.23, 29.2, 29.1, 27.84, 27.81, 26.61, 26.57, 24.9, 22.7, 14.1.

threo-9,10-Dihydroxyoctadecanoic acid (5)

Epoxy acid **4** (0.1 g; 0.32 mmol) was dissolved in THF (3 mL), water (2 mL) and one drop of *conc*. sulfuric acid was added. The reaction mixture was stirred at room temperature for 5 days. Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silica gel, eluent DCM:MeOH:AcOH/46:2:1) affording 0.08 g (79 %) of **5** as a colorless oil.

HR-ESI-MS calculated for $C_{18}H_{36}O_4Na$ $[M+Na]^+$ 339.2506, found 339.2506.

^{1}H NMR (400 MHz, Methanol-d_4) δ 3.44 – 3.37 (m, 2H), 2.30 (t, J = 7.4 Hz, 2H), 1.71 – 1.58 (m, 2H), 1.55 – 1.19 (m, 20H), 0.92 (m, 3H).

^{13}C NMR (100 MHz, Methanol-d_4) δ 73.90, 73.87, 33.6, 32.5, 32.5, 31.7, 29.46, 29.34, 29.26, 29.04, 29.01, 28.8, 25.68, 25.61, 24.7, 22.3, 13.0.

Methyl *threo*-9,10-dihydroxyoctadecanoate (6)

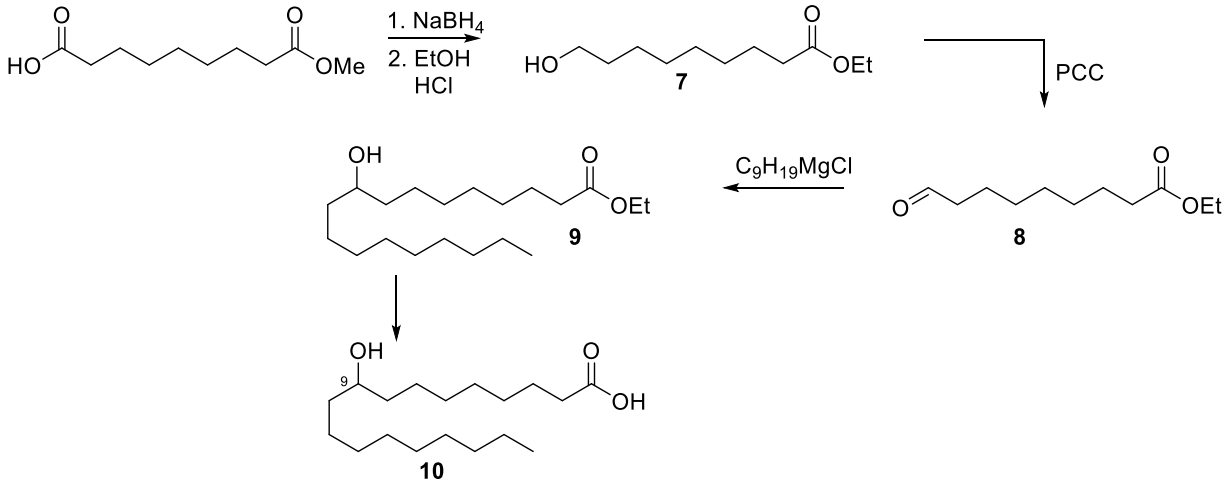
To a mixture of acid **5** (0.055 g; 0.174 mmol) in dry THF (2 mL) was added solution of diazomethane (0.3 M in Et_2O; 0.6 mL) was added. The reaction mixture was stirred at room temperature for 1 hours. Then the mixture was evaporated to dryness and the crude product was purified by column chromatography (silica gel, eluent EtOAc:cyclohexane/2:1) affording 0.04 g (69 %) of **6** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{38}O_4Na$ $[M+Na]^+$ 353.2662, found 353.2660.

^{1}H NMR (400 MHz, Chloroform-d) δ 3.69 (s, 3H), 3.51 – 3.29 (m, 2H), 2.33 (t, J = 7.5 Hz, 2H), 1.64 (m, 6H), 1.57 – 1.23 (m, 20H), 0.98 – 0.79 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-d) δ 174.3, 74.55, 74.49, 51.5, 34.1, 33.65, 33.59, 31.9, 29.69, 29.56, 29.42, 29.28, 29.16, 29.0, 25.67, 25.56, 24.9, 22.7, 14.1.

Scheme S2. Preparation of 9-hydroxystearic acid.



Ethyl 9-hydroxynonanoate (7)

To a mixture of monomethyl azelate (2.0 g; 9.89 mmol) in dioxane (25 mL) and water (25 mL) was added sodium borohydride (2.6 g; 70.27 mmol) portion wise. The reaction mixture was stirred at room temperature overnight. Then the reaction was quenched with 1 M aqueous HCl and product was extracted with DCM (4 × 10 mL). The combined organic layers were washed with brine and were dried over $MgSO_4$. The desiccant was filtered off and the filtrate was evaporated. The residue was dissolved in dry ethanol (30 mL) and *conc*. sulfuric acid (1 mL) was added. The reaction mixture was stirred at 80 °C for 8 hours, cooled down and partitioned between water and EtOAc. Organic layer was separated and washed with brine and evaporated to dryness. Column chromatography of the residue (silica gel, eluent EtOAc:cyclohexane/1:2) afforded 1.06 g (53 %) of ester **7** as a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.15 (q, *J* = 7.1 Hz, 2H), 3.66 (t, *J* = 6.6 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.70 – 1.53 (m, 4H), 1.34 (m, 8H), 1.28 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.9, 63.0, 60.2, 34.4, 32.8, 29.2, 29.0, 25.6, 24.9, 14.3.

Ethyl 9-oxononanoate (8)

Solid pyridinium chlorochromate (PCC, 1.06 g; 4.94 mmol) was added to a stirred suspension of ester **7** (0.5 g; 2.47 mmol) and Celite (2.0 g) in DCM (20 mL). After stirring at room temperature for 4 h the reaction mixture was evaporated and the solid material was loaded on a column of silica gel. Column chromatography (eluent EtOAc:cyclohexane/1:2) afforded 0.35 g (70 %) of aldehyde **8** a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.79 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 2.44 (dd, *J* = 7.3, 1.8 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.81 – 1.62 (m, 4H), 1.35 (m, 6H), 1.28 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 202.8, 173.8, 60.2, 43.9, 34.3, 29.0, 28.96, 28.90, 24.9, 21.9, 14.3.

Ethyl 9-hydroxystearate (9)

Nonylmagnesium chloride (1.90 ml; 1.90 mmol, 1 M in diethyl ether) was added dropwise to a solution of 9-oxononanoate **8** (0.35 g; 1.73 mmol) in THF (10 mL) at -78 °C. The reaction mixture was then removed from a cooling bath and was stirred at room temperature for 3 h. The reaction was quenched with addition of water (2 mL) and saturated solution of ammonium chloride (2 mL). Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:2) affording 0.24 g (42 %) of alcohol **9** as a colorless oil.

HR-ESI-MS calculated for $C_{20}H_{40}O_3Na$ $[M+Na]^+$ 351.2870, found 351.2870.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.15 (q, *J* = 7.2 Hz, 2H), 3.74 – 3.49 (m, 1H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.64 (t, *J* = 7.4 Hz, 2H), 1.52 – 1.20 (m, 30H), 0.89 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.9, 71.9, 60.2, 37.5, 37.4, 34.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 25.7, 25.6, 24.9, 22.7, 14.3, 14.1.

9-Hydroxystearic acid (10)

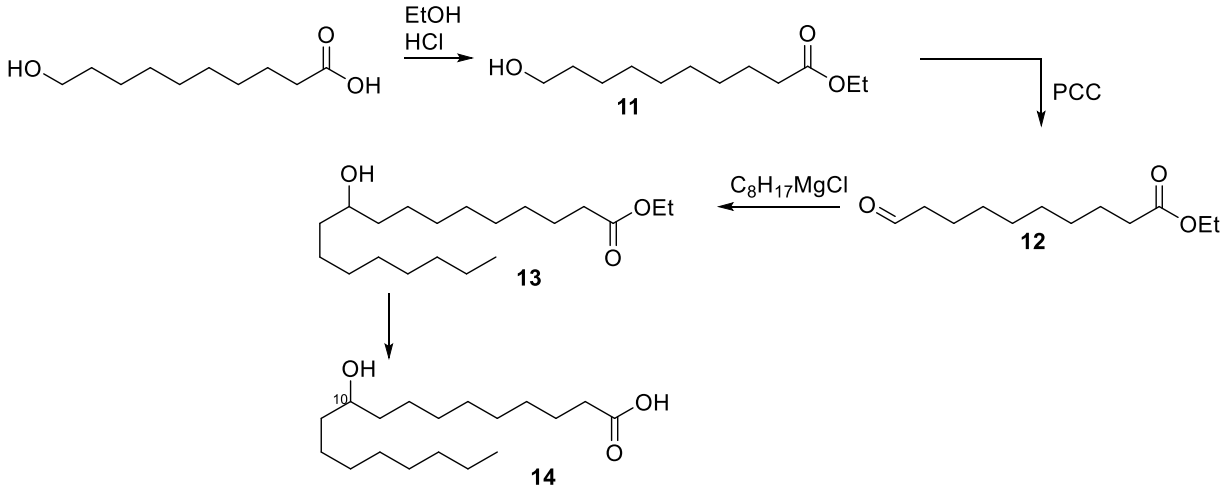
A mixture of ethyl ester **9** (0.23 g; 0.7 mmol), sodium hydroxide (0.08 g; 2.1 mmol), THF (2 mL), methanol (2 mL) and water (2 mL) was stirred overnight at room temperature. Then the reaction was quenched with aqueous HCl (5% solution) and product was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine and evaporated. The crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:1) affording 0.17 g (80 %) of acid **10** as a colorless oil.

HR-ESI-MS calculated for $C_{18}H_{35}O_3$ $[M-H]^-$ 299.2592, found 299.2586.

^{1}H NMR (400 MHz, Chloroform-*d*) δ. 5.03 (bs, 2H), 3.88 – 3.37 (m, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.65 (m, 2H), 1.54 – 1.21 (m, 28H), 0.9 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 179.1, 72.1, 37.5, 37.4, 33.9, 31.9, 29.7, 29.65, 29.59, 29.4, 29.3, 29.2, 28.9, 25.6, 25.5, 24.6, 22.7, 14.1.

Scheme S3. Preparation of 10-hydroxystearic acid.



Ethyl 10-hydroxydecanoate (11)

10-Hydroxydecanoic (1.0 g; 5.31 mmol) was dissolved in dry ethanol (15 mL) and HCl (4 M solution in dioxane, 0.5 mL) was added. The reaction mixture was stirred at room temperature for 2 days. Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:2) affording 1.08 g (94 %) of **11** as a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.15 (q, *J* = 7.1 Hz, 2H), 3.66 (t, *J* = 6.6 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.84 – 1.55 (m, 4H), 1.32 (m, 10H), 1.28 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.9, 63.1, 60.2, 34.4, 32.8, 29.4, 29.3, 29.2, 29.1, 25.7, 24.9, 14.3.

Ethyl 10-oxodecanoate (12)

Solid pyridinium chlorochromate (PCC, 2.16 g; 10.0 mmol) was added to a stirred suspension of ester **11** (1.08 g; 5.0 mmol) and Celite (3.0 g) in DCM (20 mL). After stirring at room temperature overnight the reaction mixture was filtered through a short pad of Celite. The filtrate was evaporated and loaded on a column of silica gel. Column chromatography (silicagel, eluent EtOAc:cyclohexane/1:2) afforded 0.57 g (52 %) of aldehyde **12** a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.78 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 2.44 (td, *J* = 7.4, 1.9 Hz, 2H), 2.38 – 2.14 (m, 2H), 1.88 – 1.57 (m, 6H), 1.36 – 1.31 (m, 6H), 1.28 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 202.9, 173.9, 60.2, 43.9, 34.4, 29.2, 29.1, 29.0, 24.9, 22.0, 14.3.

Ethyl 10-hydroxystearate (13)

Octylmagnesium chloride (1.60 ml; 3.20 mmol, 2 M in diethyl ether) was added dropwise to a solution of 10-oxodecanoate **12** (0.57 g; 2.66 mmol) in THF (10 mL) at -78 °C. The reaction mixture was then removed from a cooling bath and was stirred at room temperature for 4 h. The reaction was quenched with addition of water (2 mL) and saturated solution of ammonium chloride (2 mL). Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:4) affording 0.60 g (68 %) of alcohol **13** as a colorless oil.

HR-ESI-MS calculated for $C_{20}H_{40}O_3Na$ $[M+Na]^+$ 351.2870, found 351.2869.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.14 (q, *J* = 7.1 Hz, 2H), 3.85 – 3.45 (m, 1H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.62 (m, 2H), 1.55 – 1.20 (m, 28H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.9, 72.0, 60.2, 37.5, 37.5, 34.4, 31.9, 29.7, 29.63, 29.61, 29.4, 29.3, 29.2, 29.1, 25.7, 25.6, 24.9, 22.7, 14.3, 14.1.

10-Hydroxystearic acid (14)

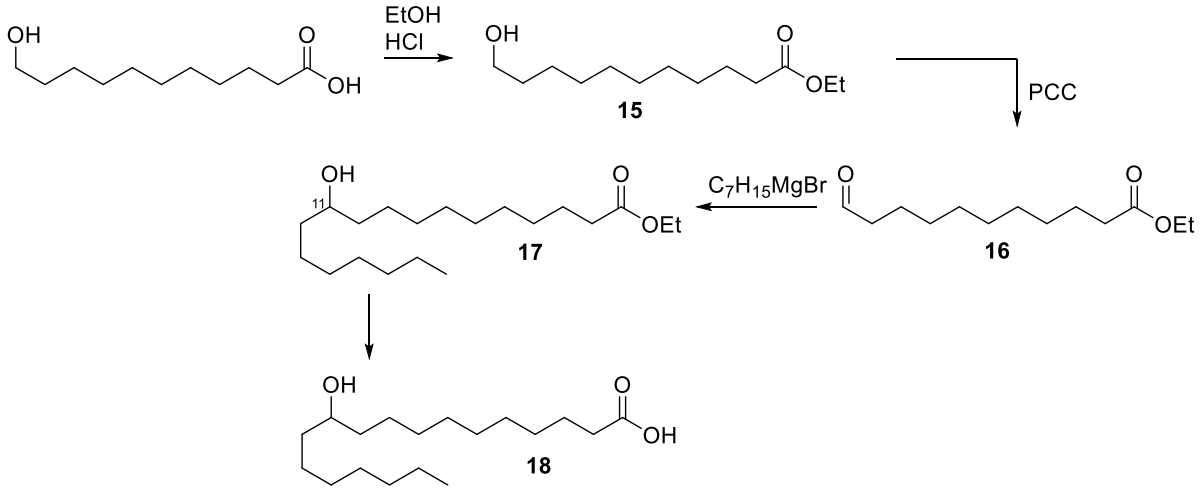
A mixture of ethyl ester **13** (0.51 g; 1.56 mmol), sodium hydroxide (0.19 g; 4.7 mmol), THF (3 mL), methanol (3 mL) and water (3 mL) was stirred overnight at room temperature. Then the reaction was quenched with aqueous HCl (5% solution) and product was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine and evaporated. The crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:1) affording 0.34 g (68 %) of acid **14** as a colorless oil.

HR-ESI-MS calculated for $C_{18}H_{35}O_3$ $[M-H]^-$ 299.2592, found 299.2587.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.78 – 3.47 (m, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.65 (d, *J* = 7.2 Hz, 2H), 1.56 – 1.21 (m, 26H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 178.7, 72.1, 37.5, 37.4, 33.8, 31.9, 29.7, 29.6, 29.6, 29.3, 29.3, 29.1, 29.0, 25.66, 25.58, 24.6, 22.7, 14.1.

Scheme S4. Preparation of 11-hydroxystearic acid.



Ethyl 11-hydroxyundecanoate (15)

11-Hydroxyundecanoic (1.0 g; 4.94 mmol) was dissolved in dry ethanol (20 mL) and HCl (4 M solution in dioxane, 0.5 mL) was added. The reaction mixture was stirred at room temperature for 3 days. Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:2) affording 1.10 g (96 %) of **15** as a colorless oil.

HR-ESI-MS calculated for $C_{13}H_{23}O_3$ $[M-H]^-$ 227.1641, found 227.1641.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.15 (q, *J* = 7.1 Hz, 2H), 3.66 (td, *J* = 6.6, 5.4 Hz, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.61 (m, 4H), 1.41 – 1.29 (m, 12H), 1.32 – 1.23 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.9, 63.1, 60.2, 34.4, 32.8, 29.5, 29.4, 29.4, 29.2, 29.1, 25.7, 24.9, 14.3.

Ethyl 11-oxoundecanoate (16)

Solid pyridinium chlorochromate (PCC, 2.23 g; 10.34 mmol) was added to a stirred suspension of ester **15** (1.1 g; 5.0 mmol) and Celite (3.0 g) in DCM (20 mL). After stirring at room temperature overnight the reaction mixture was filtered through a short pad of Celite. The filtrate was evaporated and loaded on a column of silica gel. Column chromatography (silicagel, eluent EtOAc:cyclohexane/1:2) afforded 0.66 g (60 %) of aldehyde **16** a colorless oil.

HR-ESI-MS calculated for $C_{13}H_{23}O_3$ $[M-H]^-$ 227.1641, found 227.1641.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.79 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 2.44 (td, *J* = 7.3, 1.9 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.64 (m, 4H), 1.34 – 1.30 (m, 10H), 1.28 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 202.9, 173.9, 60.2, 43.9, 34.4, 29.3, 29.2, 29.2, 29.1, 29.1, 24.9, 22.1, 14.3.

Ethyl 11-hydroxystearate (17)

Heptylmagnesium chloride (3.42 ml; 3.42 mmol, 1 M in diethyl ether) was added dropwise to a solution of 11-oxoundecanoate **16** (0.65 g; 2.85 mmol) in THF (15 mL) at -78 °C. The reaction mixture was then removed from a cooling bath and was stirred at room temperature for 4 h. The reaction was quenched with addition of water (2 mL) and 10% aqueous solution of citric acid (5 mL). Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:4) affording 0.65 g (69 %) of alcohol **17** as a colorless oil.

HR-ESI-MS calculated for $C_{20}H_{40}O_3Na$ $[M+Na]^+$ 351.2870, found 351.2868.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.14 (q, *J* = 7.1 Hz, 2H), 3.60 (m, 1H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.79 – 1.56 (m, 2H), 1.54 – 1.18 (m, 28H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.9, 72.0, 60.2, 37.5, 37.5, 34.4, 31.8, 29.7, 29.7, 29.5, 29.4, 29.3, 29.2, 29.1, 25.7, 25.6, 24.9, 22.7, 14.3, 14.1.

11-Hydroxystearic acid (18)

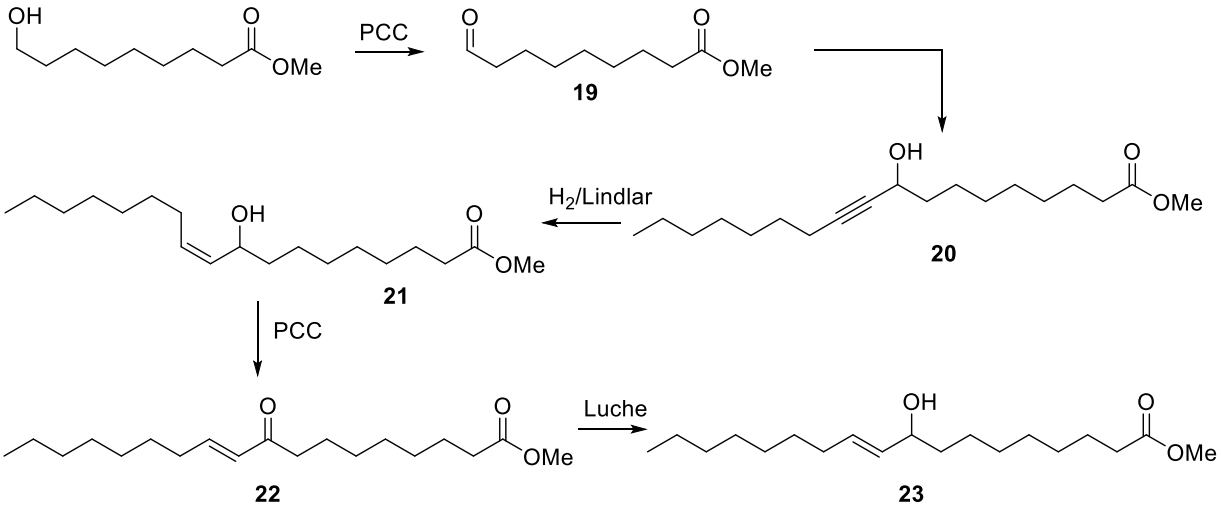
A mixture of ethyl ester **17** (0.13 g; 0.39 mmol), sodium hydroxide (0.05 g; 1.2 mmol), THF (3 mL), methanol (3 mL) and water (3 mL) was stirred overnight at room temperature. Then the reaction was quenched with aqueous HCl (5% solution) and product was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine and evaporated. The crude product was purified by column chromatography (silicagel, eluent EtOAc:cyclohexane/1:1) affording 0.06 g (51 %) of acid **18** as a colorless oil.

HR-ESI-MS calculated for $C_{18}H_{35}O_3$ $[M-H]^-$ 299.2592, found 299.2591.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.87 – 3.46 (m, 1H), 2.36 (t, *J* = 7.5 Hz, 2H), 1.64 (m, 2H), 1.55 – 1.17 (m, 26H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 179.4, 72.1, 37.4, 37.4, 34.0, 31.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.2, 29.0, 25.6, 25.6, 24.7, 22.7, 14.1.

Scheme S5. Preparation of 10-(*Z*)-9-OH-18:1 and 10-(*E*)-9-OH-18:1.



Methyl 9-oxononanoate (19)

Solid pyridinium chlorochromate (PCC, 2.30 g; 10.62 mmol) was added to a stirred suspension of methyl 9-hydroxynonanoate (1.0 g; 5.32 mmol) and Celite (3.0 g) in DCM (30 mL). After stirring at room temperature for 3 hours the reaction mixture was filtered through a short pad of Celite. The filtrate was evaporated and loaded on a column of silica gel. Column chromatography (silica gel, eluent EtOAc:cyclohexane/1:2) afforded 0.48 g (48 %) of aldehyde **19** a colorless oil.

HR-ESI-MS calculated for $C_{10}H_{18}O_3Na$ $[M+Na]^+$ 209.1148, found 209.1148.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.78 (s, 1H), 3.69 (s, 3H), 2.44 (td, *J* = 7.3, 1.8 Hz, 2H), 2.32 (t, *J* = 7.5 Hz, 2H), 1.74 – 1.50 (m, 4H), 1.43 – 1.26 (m, 6H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 202.8, 174.2, 51.5, 43.8, 34.0, 29.0, 28.94, 28.90, 24.8, 21.9.

Methyl 9-hydroxyoctadec-10-ynoate (20)

n-BuLi (1.11 ml; 2.77 mmol, 2.5 M in hexane) was added dropwise to a stirred solution of 1-nonyne (0.35 g; 2.77 mmol) in THF (10 mL) and dry HMPA (1 mL) at -78 °C. The reaction mixture was stirred for 30 min and then aldehyde **19** (0.47 g; 2.52 mmol) was added dropwise. The reaction mixture was stirred for 30 min, then the cooling bath was removed, and the mixture was allowed to come to room temperature. The reaction was stirred at room temperature for 3 hours and then was quenched with addition of water (2 mL) and 10% aqueous solution of citric acid (5 mL). Then the mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silica gel, eluent EtOAc:cyclohexane/1:4) affording 0.16 g (20 %) of alcohol **20** as a colorless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.36 (m, 1H), 3.69 (s, 3H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.22 (td, *J* = 7.1, 2.0 Hz, 2H), 1.75 – 1.62 (m, 4H), 1.57 – 1.48 (m, 2H), 1.37 – 1.19 (m, 16H), 0.91 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 85.6, 81.3, 62.7, 51.5, 38.2, 34.01 31.7, 29.2, 29.1, 29.1, 28.8, 28.8, 28.7, 26.9, 25.1, 24.9, 22.6, 18.7, 14.1.

Methyl (*Z*)-9-hydroxyoctadec-10-enoate (21)

Ester **20** (0.10 g; 0.32 mmol) was hydrogenated over Lindlar catalyst (from Johnson-Matthey, product code A305060-5; 0.01 g) in a mixture of EtOAc (10 mL) and quinoline (0.01 g) for 3 hours. The mixture was filtered through a Celite plug and the filtrate was collected. The solvent was evaporated and column chromatography (silica gel, eluent EtOAc:cyclohexane/1:4) of the residue afforded 0.09 g (90 %) of allylalcohol **21** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{36}O_3Na$ $[M+Na]^+$ 335.2557, found 335.2554.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.50 (dt, *J* = 11.0, 7.4 Hz, 1H), 5.45 – 5.22 (m, 1H), 4.52 – 4.30 (m, 1H), 3.69 (s, 3H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.22 – 1.96 (m, 2H), 1.68 – 1.26 (m, 22H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 132.5, 132.4, 67.7, 51.5, 37.5, 34.1, 31.8, 29.7, 29.4, 29.3, 29.2, 29.17, 29.08, 27.7, 25.3, 24.9, 22.7, 14.1.

Methyl (*E*)-9-oxooctadec-10-enoate (22)

Solid pyridinium chlorochromate (PCC, 0.12 g; 0.58 mmol) was added to a stirred suspension of ester **21** (0.09 g; 0.29 mmol) and silica gel (0.5 g) in DCM (20 mL). After stirring at room temperature overnight the reaction mixture was evaporated and loaded on a column of silica gel. Column chromatography (silicagel, eluent EtOAc:cyclohexane/1:4) afforded 0.05 g (55 %) of enone **22** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{34}O_3Na$ $[M+Na]^+$ 333.2400, found 333.2398.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.96 – 6.74 (m, 1H), 6.17 – 5.99 (m, 1H), 3.68 (s, 3H), 2.53 (t, *J* = 7.5 Hz, 2H), 2.32 (td, *J* = 7.6, 2.4 Hz, 2H), 2.29 – 2.13 (m, 2H), 1.62 (m, 4H), 1.37 – 1.21 (m, 16H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 200.9, 174.2, 147.4, 130.3, 51.5, 40.0, 34.0, 32.5, 31.7, 29.16, 29.11, 29.06, 29.02, 29.00, 28.9, 26.9, 24.9, 22.6, 14.1.

Methyl (*E*)-9-hydroxyoctadec-10-enoate (23)

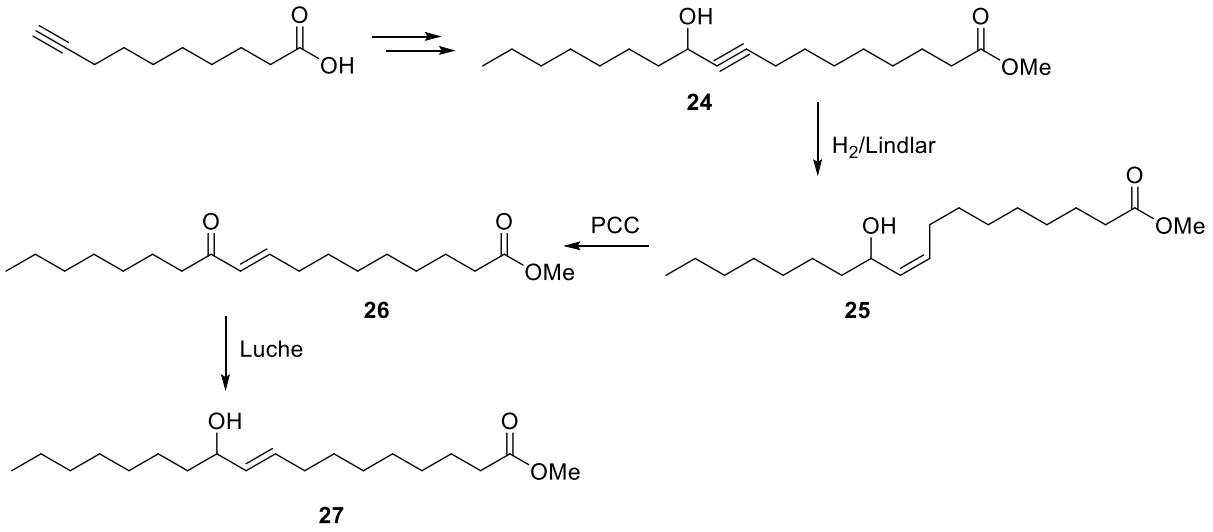
Sodium borohydride (0.007 g; 0.19 mmol) in methanol (1 mL) was added dropwise to a stirred mixture of enone **22** (0.04 g; 0.13 mmol) and cerium(III) chloride heptahydrate (0.095 g; 0.38 mmol) in methanol (5 mL) at -78 °C. The reaction mixture was stirred for 30 min, then the cooling bath was removed, and the mixture was allowed to come to room temperature then was quenched with saturated solution of ammonium chloride (2 mL). The mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silica gel, eluent EtOAc:cyclohexane/1:4) affording 0.02 g (50 %) of alcohol **23** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{36}O_3Na$ $[M+Na]^+$ 335.2557, found 335.2554.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.76 – 5.56 (m, 1H), 5.46 (ddd, *J* = 15.4, 7.1, 1.3 Hz, 1H), 4.08 – 3.97 (m, 1H), 3.69 (s, 3H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.07 (s, 2H), 1.62 (m, 2H), 1.53 – 1.25 (m, 20H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 132.9, 132.3, 73.2, 60.4, 51.4, 37.3, 34.1, 32.2, 31.8, 31.6, 29.5, 29.2, 29.1, 25.5, 25.4, 24.9, 22.6, 14.1.

Scheme S6. Preparation of 9-(*Z*)-11-OH-18:1 and 9-(*E*)-11-OH-18:1.



Methyl 11-hydroxyoctadec-9-ynoate (24)

To 9-decynoic acid (1.24 g; 7.37 mmol) in THF (10 mL) at -78 °C was added *n*-BuLi (6.2 mL; 15.49 mmol, 2.5 M solution in hexane) and the resulting mixture was stirred for 30 min. Then solution of octanal (1.13 g; 8.85 mmol) in THF (2 mL) was added and the mixture was stirred for 30 min at -78 °C. After this period of time the mixture was allowed to slowly reach room temperature. The reaction was quenched with saturated solution of ammonium chloride (10 mL). The mixture was partitioned between water and EtOAc. The organic phase was removed, and the aqueous phase was extracted with EtOAc (2 × 10 mL). The organic phases were combined and washed with brine. The solvent was evaporated, and the residue was dissolved in Et_2O (10 mL) and solution od diazomethane in Et_2O was added (15 mL). After one hour the reaction mixture was evaporated and product was purified by column chromatography (silica gel, eluent EtOAc:cyclohexane/1:6) affording 0.74 g (32 %) of alcohol **24** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{34}O_3Na$ $[M+Na]^+$ 333.2400, found 333.2400.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.36 (m, 1H), 3.69 (s, 3H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.22 (td, *J* = 7.0, 2.0 Hz, 2H), 1.66 (m, 2H), 1.53 – 1.17 (m, 20H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 85.4, 81.5, 62.8, 51.5, 38.2, 34.0, 31.8, 29.3, 29.2, 28.9, 28.7, 28.54, 28.52, 25.2, 24.8, 22.7, 18.6, 14.1.

Methyl (*Z*)-11-hydroxyoctadec-9-enoate (25)

Ester **24** (0.23 g; 0.74 mmol) was hydrogenated over Lindlar catalyst (from Johnson-Matthey, product code A305060-5; 0.02 g) in a mixture of EtOAc (10 mL) and quinoline (0.02 g) for 3 hours. The mixture was filtered through a Celite plug and the filtrate was collected. The solvent was evaporated and column chromatography (silica gel, eluent EtOAc:cyclohexane/1:4) of the residue afforded 0.20 g (86 %) of allylalcohol **25** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{36}O_3Na$ $[M+Na]^+$ 335.2557, found 335.2554.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.49 (dtd, *J* = 10.9, 7.3, 0.9 Hz, 1H), 5.44 – 5.21 (m, 1H), 4.59 – 4.27 (m, 1H), 3.69 (s, 3H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.19 – 1.97 (m, 2H), 1.77 – 1.56 (m, 2H), 1.47 – 1.20 (m, 20H), 0.90 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 132.7, 132.2, 67.7, 51.5, 37.5, 34.1, 31.8, 29.6, 29.5, 29.3, 29.1, 29.0, 27.7, 25.4, 24.9, 22.7, 14.1.

Methyl (*E*)-11-oxooctadec-9-enoate (26)

Solid pyridinium chlorochromate (PCC, 0.90 g; 4.16 mmol) was added to a stirred suspension of ester **25** (0.65 g; 2.08 mmol) and silica gel (3.0 g) in DCM (20 mL). After stirring at room temperature overnight the reaction mixture was evaporated and loaded on a column of silica gel. Column chromatography (silicagel, eluent EtOAc:cyclohexane/1:4) afforded 0.45 g (69 %) of enone **26** as a colorless oil.

HR-ESI-MS calculated for $C_{19}H_{34}O_3$ $[M+H]^+$ 311.2581, found 311.2579.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.83 (dd, *J* = 15.8, 3.5 Hz, 1H), 6.10 (dd, *J* = 15.9, 0.6 Hz, 1H), 3.68 (d, *J* = 1.5 Hz, 3H), 2.54 (t, *J* = 7.5 Hz, 2H), 2.32 (td, *J* = 7.5, 2.5 Hz, 2H), 2.26 – 2.15 (m, 2H), 1.63 (s, 2H), 1.46 (d, *J* = 12.9 Hz, 2H), 1.39 – 1.26 (m, 16H), 1.00 – 0.78 (m, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 200.9, 174.2, 147.5, 130.3, 51.5, 40.1, 34.1, 32.4, 31.7, 29.3, 29.1, 29.1, 29.0, 28.9, 28.9, 28.1, 26.9, 24.9, 24.4, 24.2, 22.6, 14.1.

Methyl (*E*)-11-hydroxyoctadec-9-enoate (27)

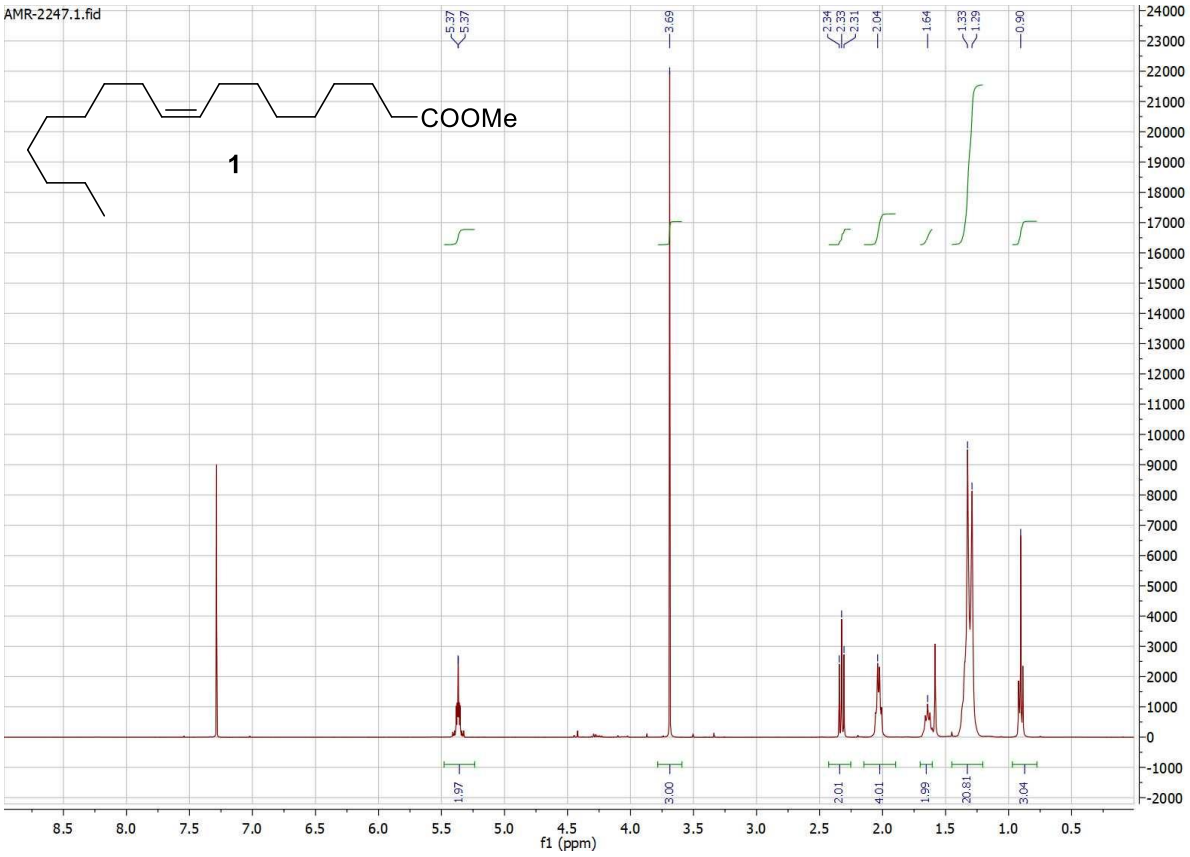
Sodium borohydride (0.08 g; 2.13 mmol) in methanol (2 mL) was added dropwise to a stirred mixture of enone **26** (0.44 g; 1.42 mmol) and cerium(III) chloride heptahydrate (1.04 g; 4.25 mmol) in methanol (10 mL) at -78 °C. The reaction mixture was stirred for 30 min, then the cooling bath was removed, and the mixture was allowed to come to room temperature then was quenched with saturated solution of ammonium chloride (5 mL). The mixture was partitioned between water and EtOAc. After the organic layer was washed with brine and the solvent was evaporated, the crude product was purified by column chromatography (silica gel, eluent EtOAc:cyclohexane/1:4) affording 0.36 g (81 %) of alcohol **27** as a colorless oil.

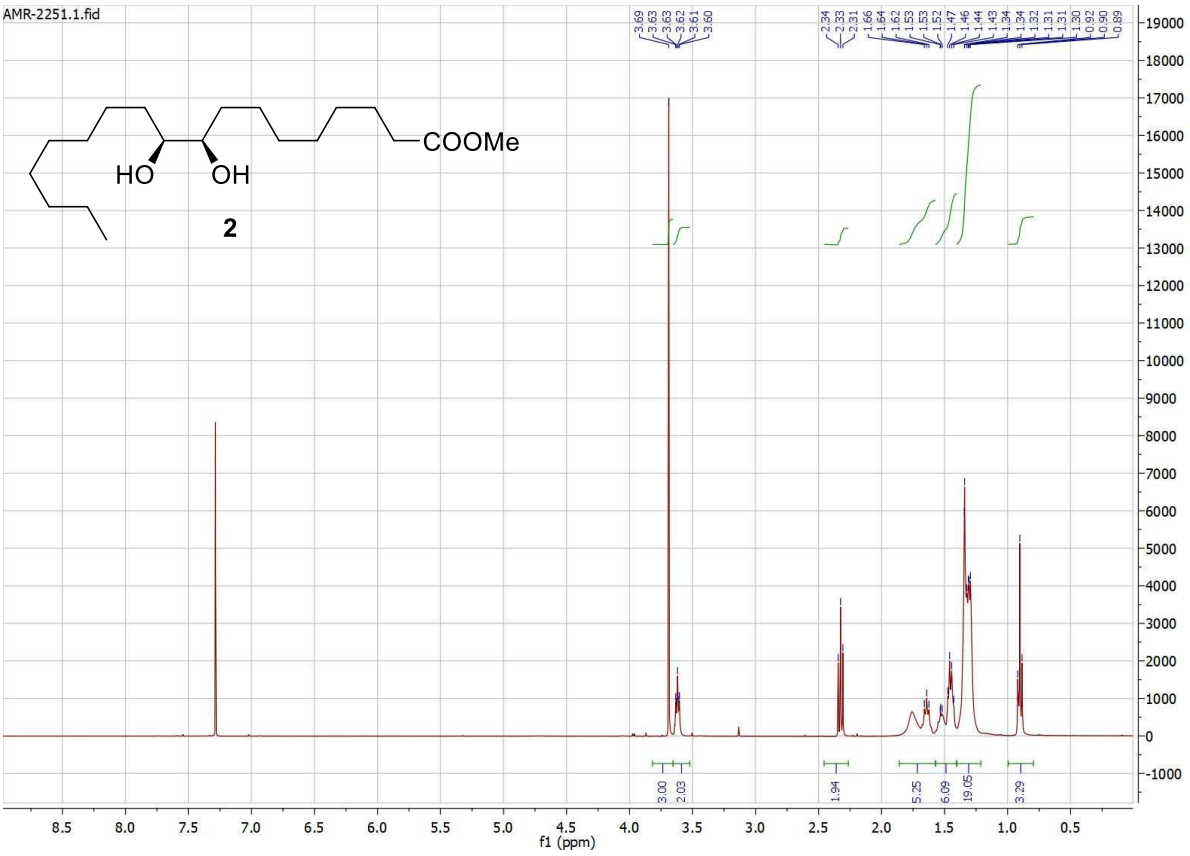
HR-ESI-MS calculated for $C_{19}H_{36}O_3Na$ $[M+Na]^+$ 335.2557, found 335.2555.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.64 (dddd, *J* = 11.9, 6.8, 3.4, 0.9 Hz, 1H), 5.46 (ddd, *J* = 15.3, 7.2, 1.5 Hz, 1H), 4.05 (m, 1H), 3.69 (s, 3H), 2.32 (td, *J* = 7.5, 0.9 Hz, 2H), 2.09 – 1.87 (m, 2H), 1.71 – 1.17 (m, 22H), 0.90 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.3, 133.1, 132.0, 73.2, 51.5, 37.3, 37.3, 34.1, 32.2, 32.1, 31.8, 29.5, 29.3, 29.2, 29.13, 29.09, 29.07, 25.5, 25.4, 24.9, 22.7, 14.1.

^{1}H-NMR and ^{13}C-NMR spectra

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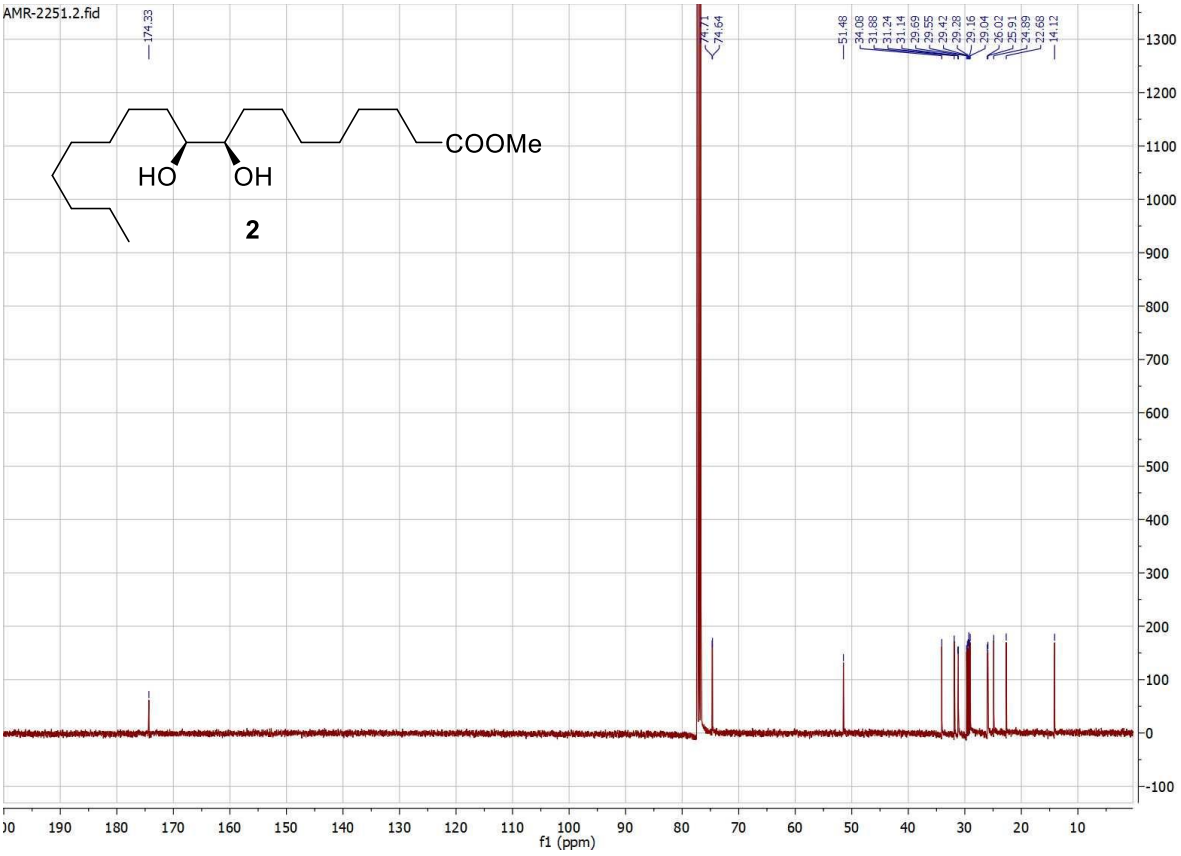
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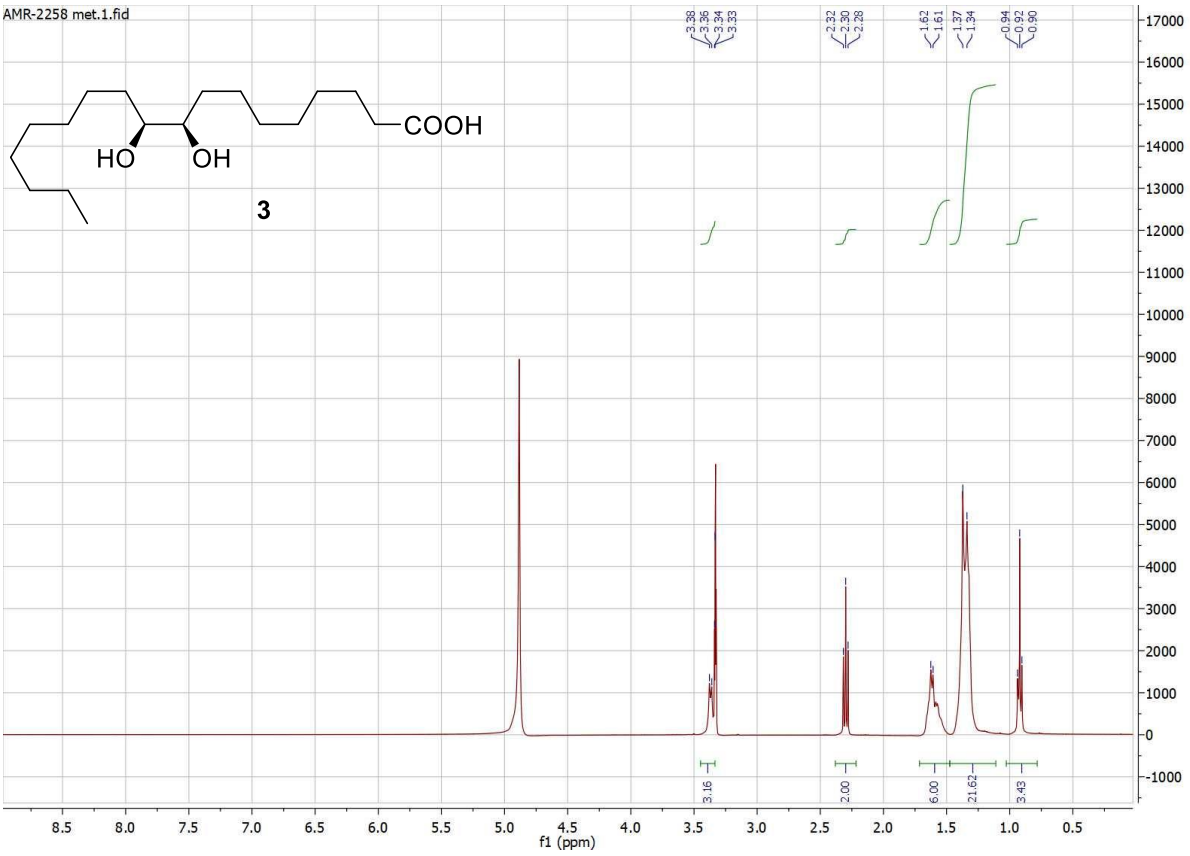
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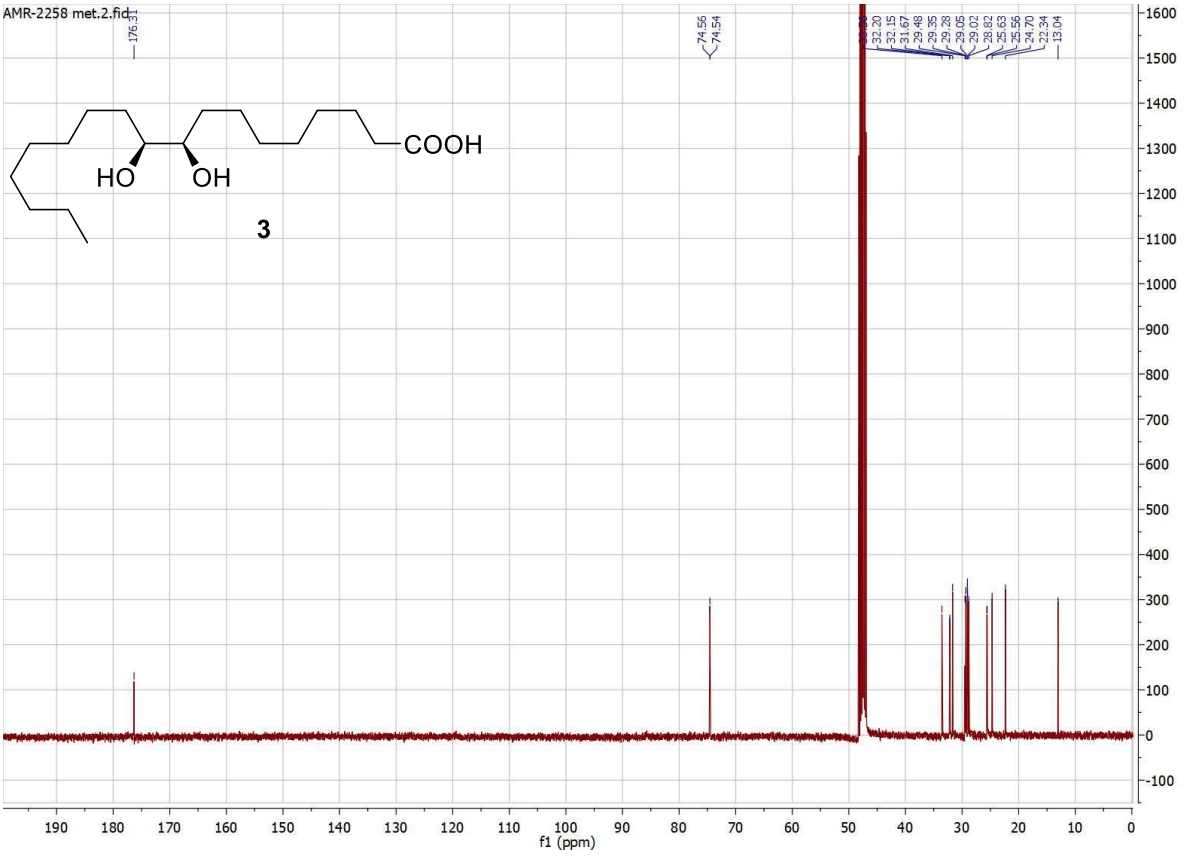
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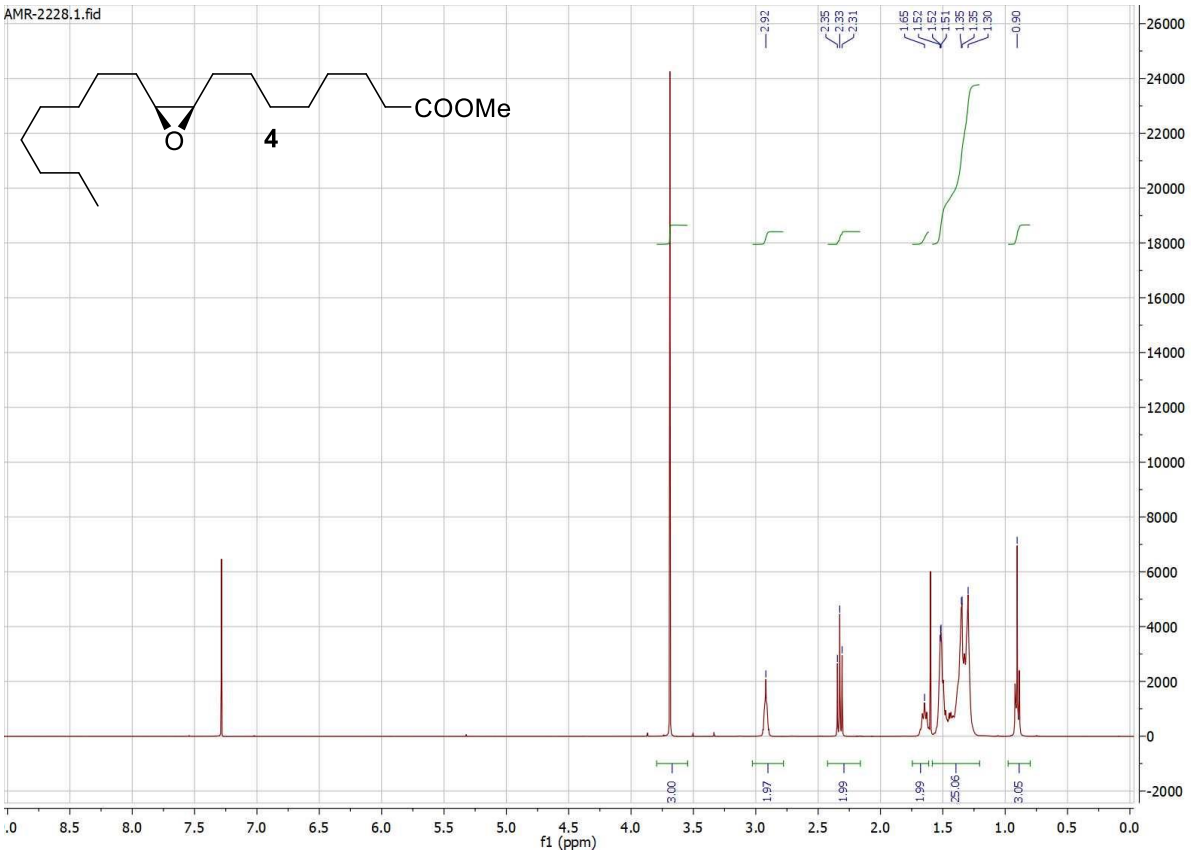
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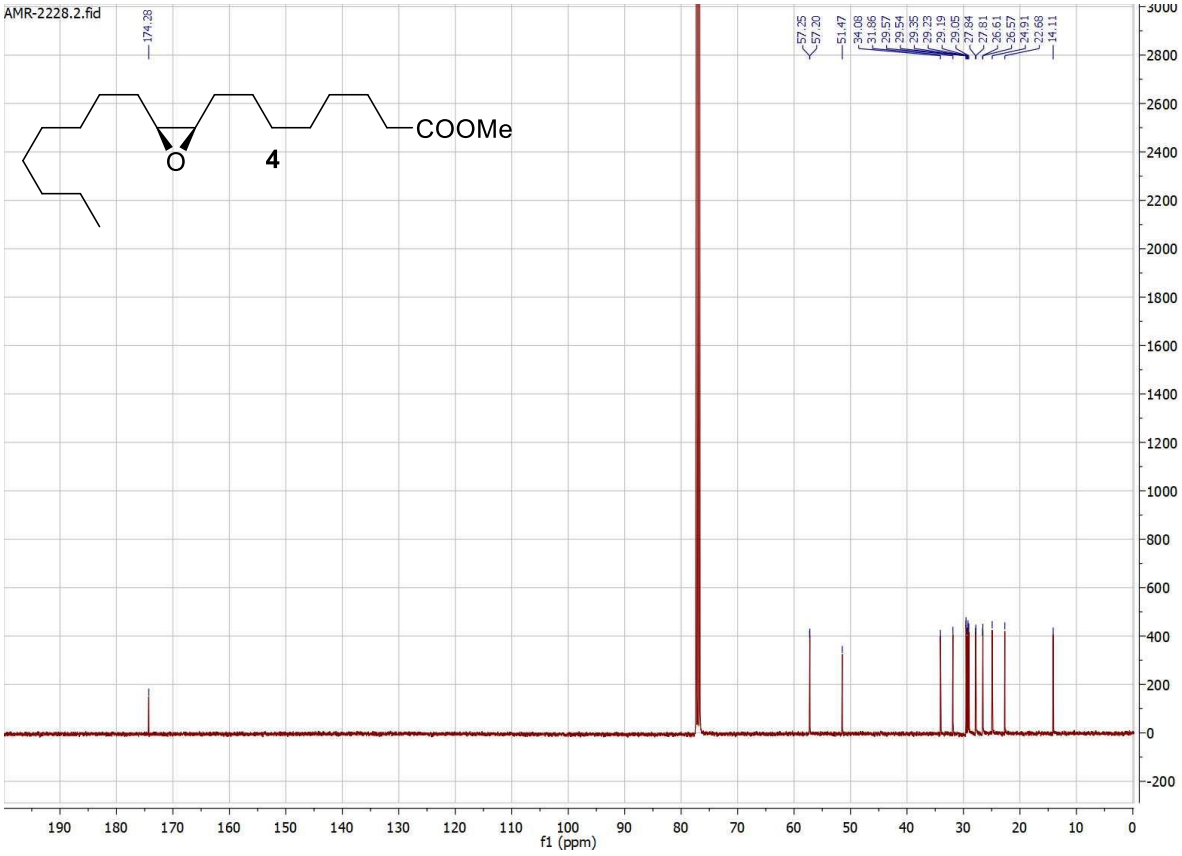
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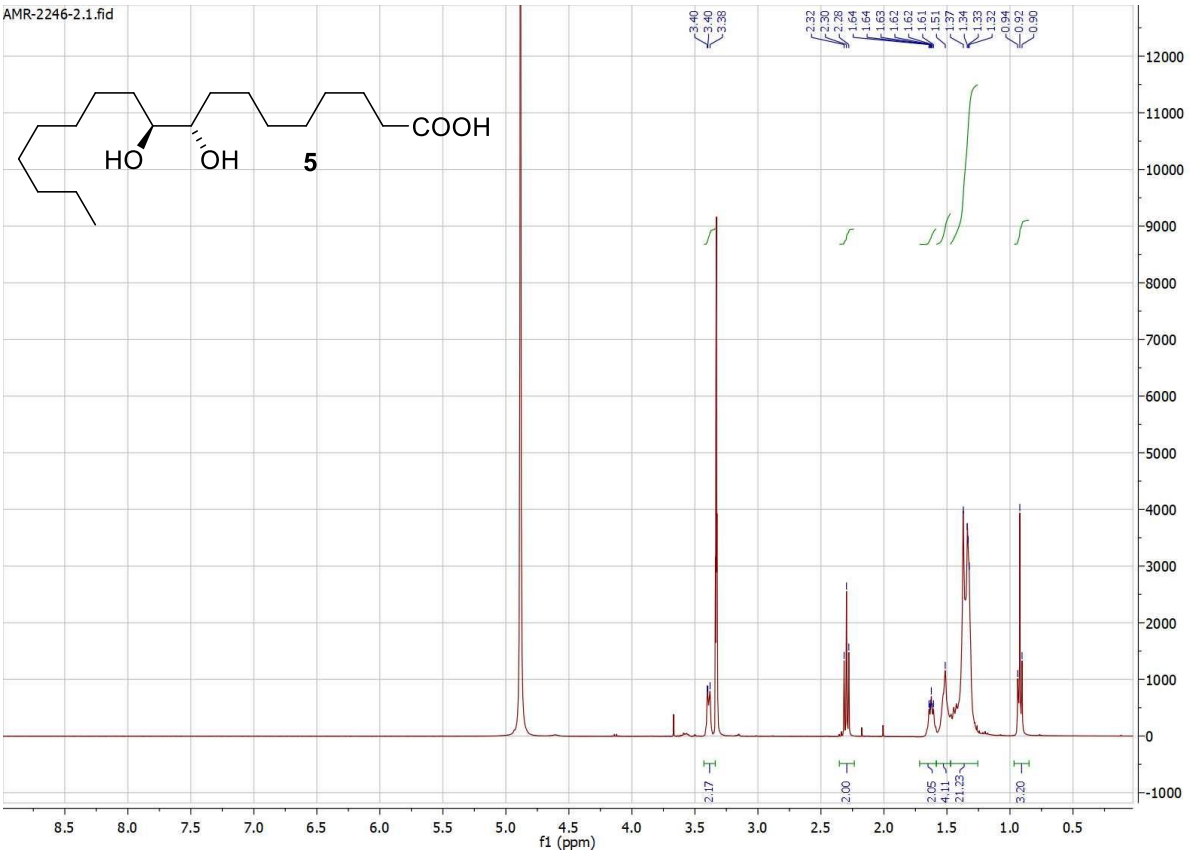
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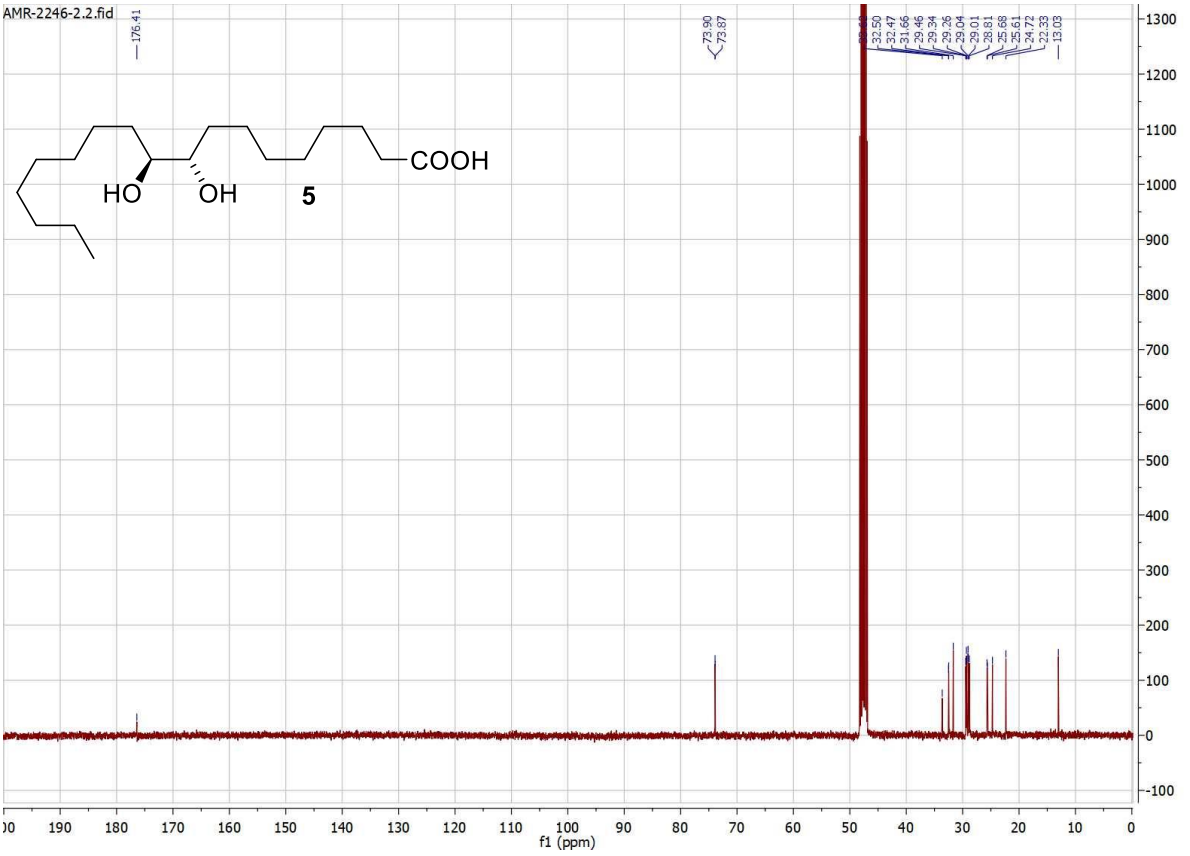
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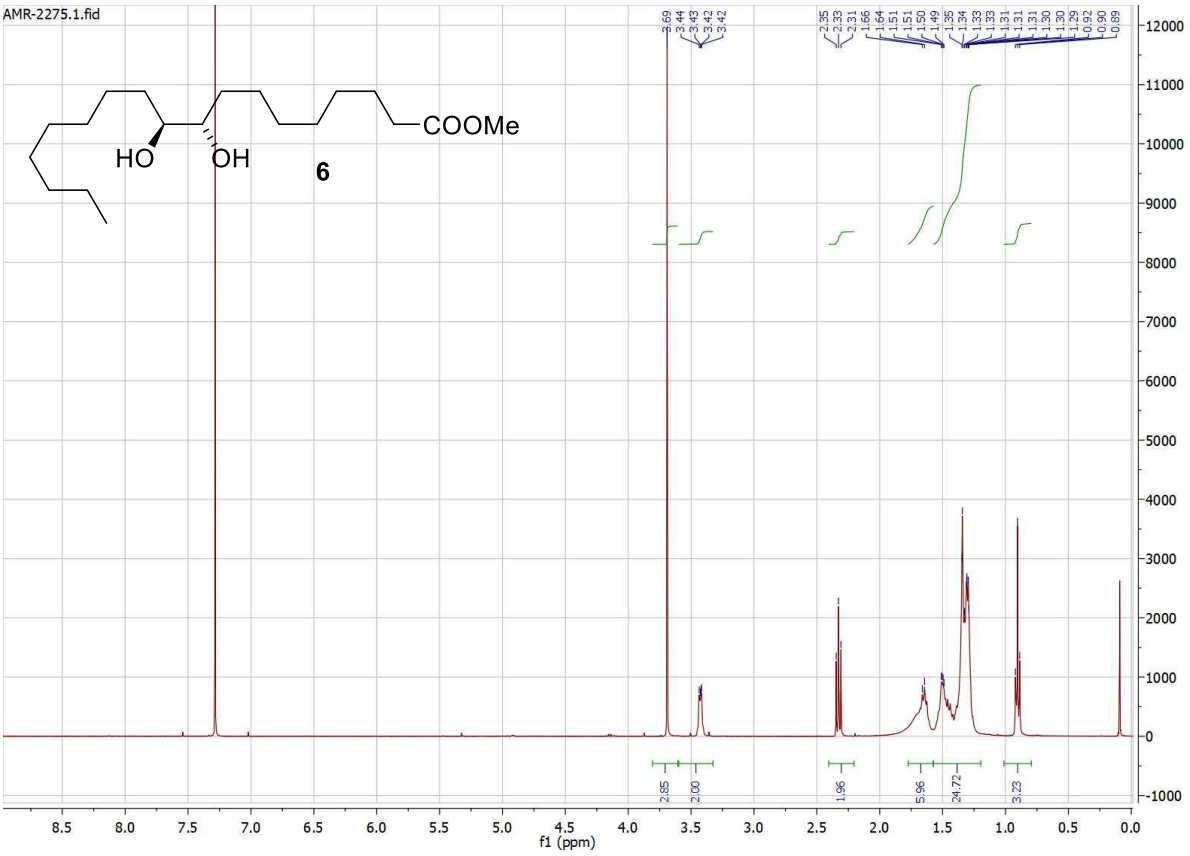
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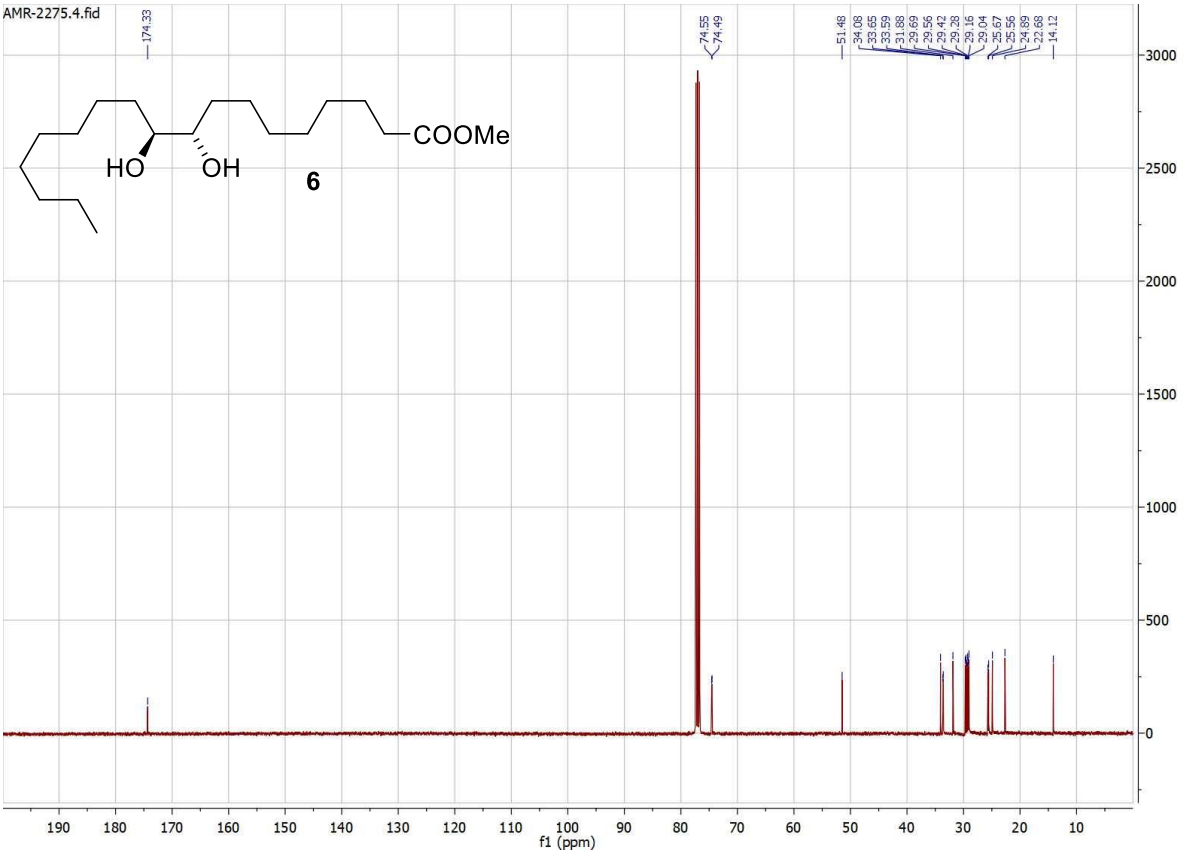
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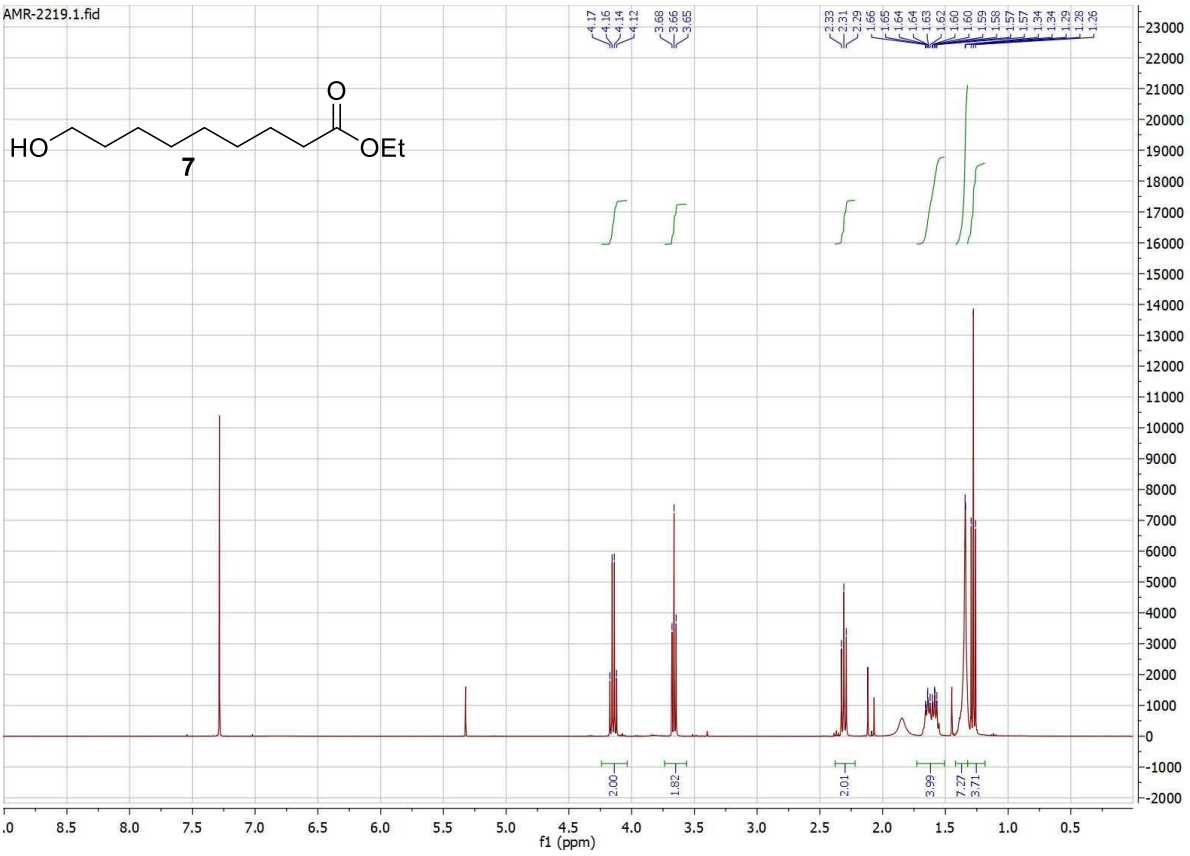
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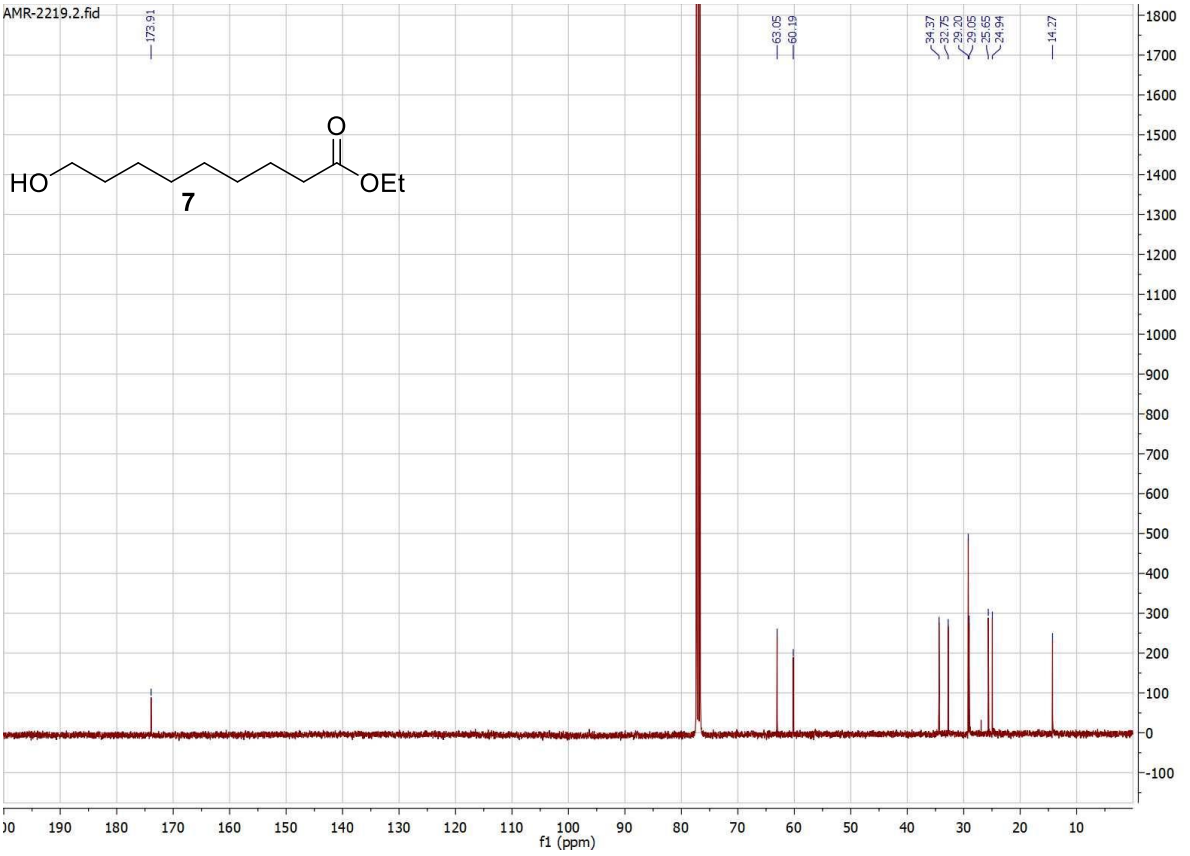
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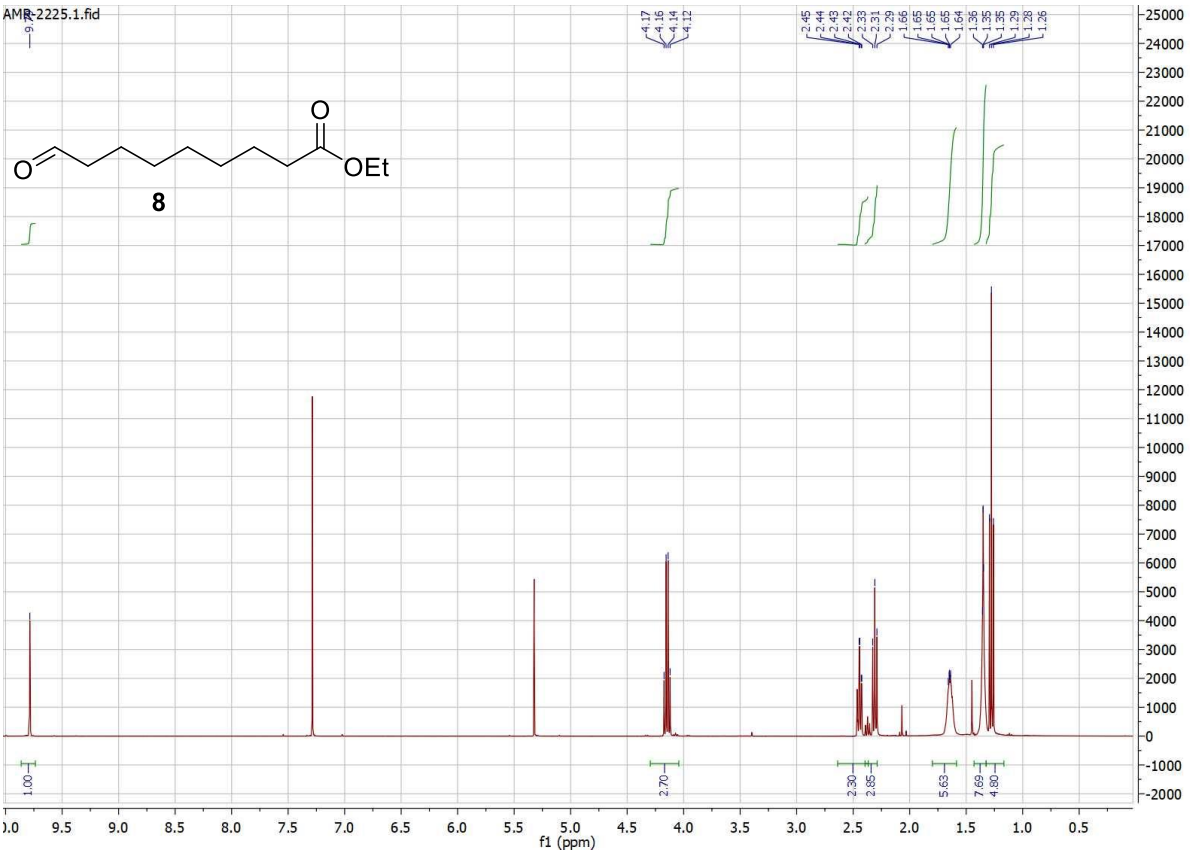
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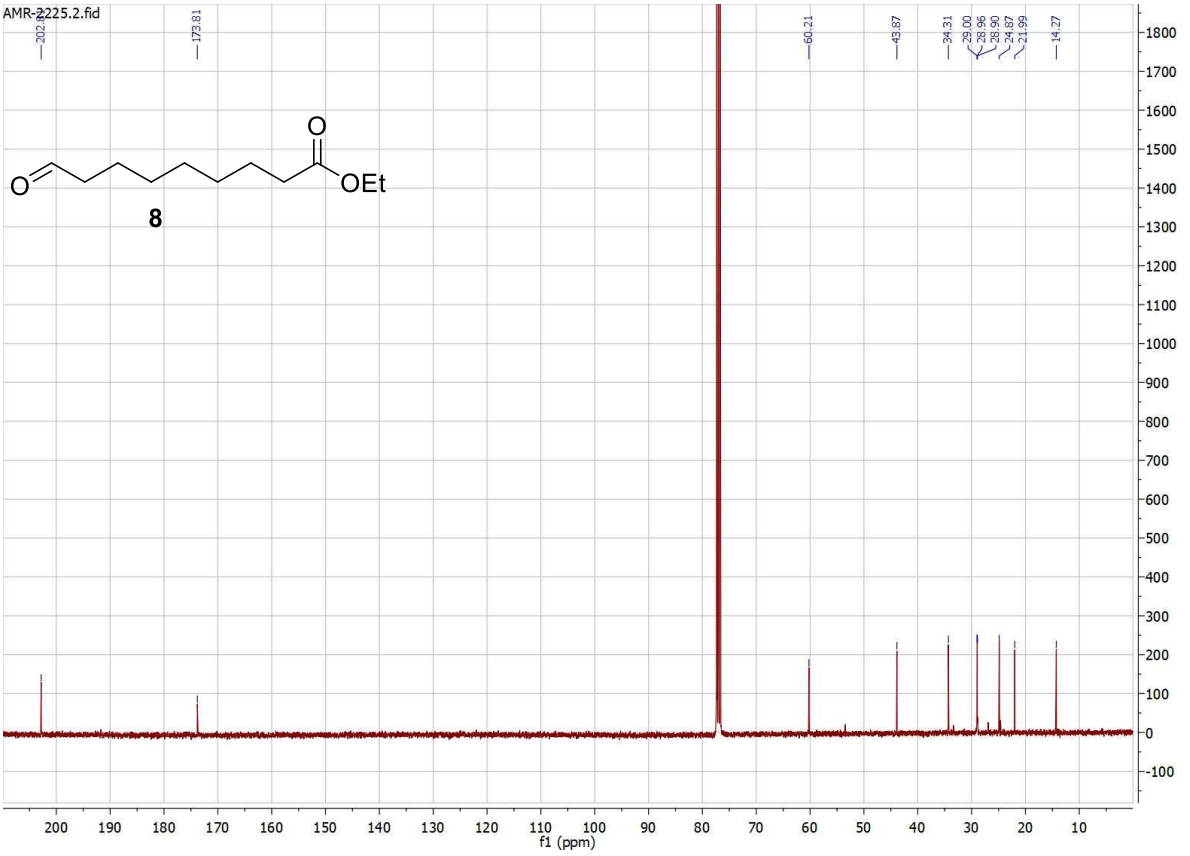
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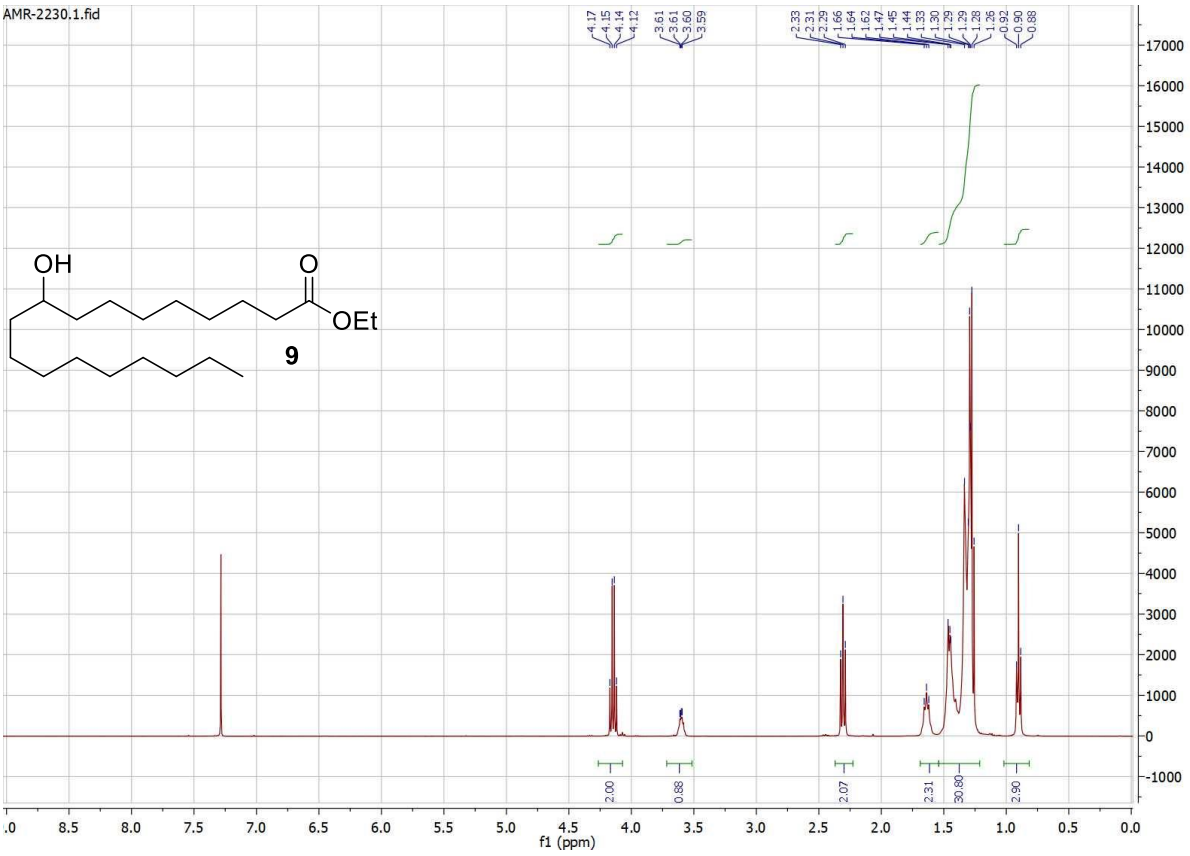
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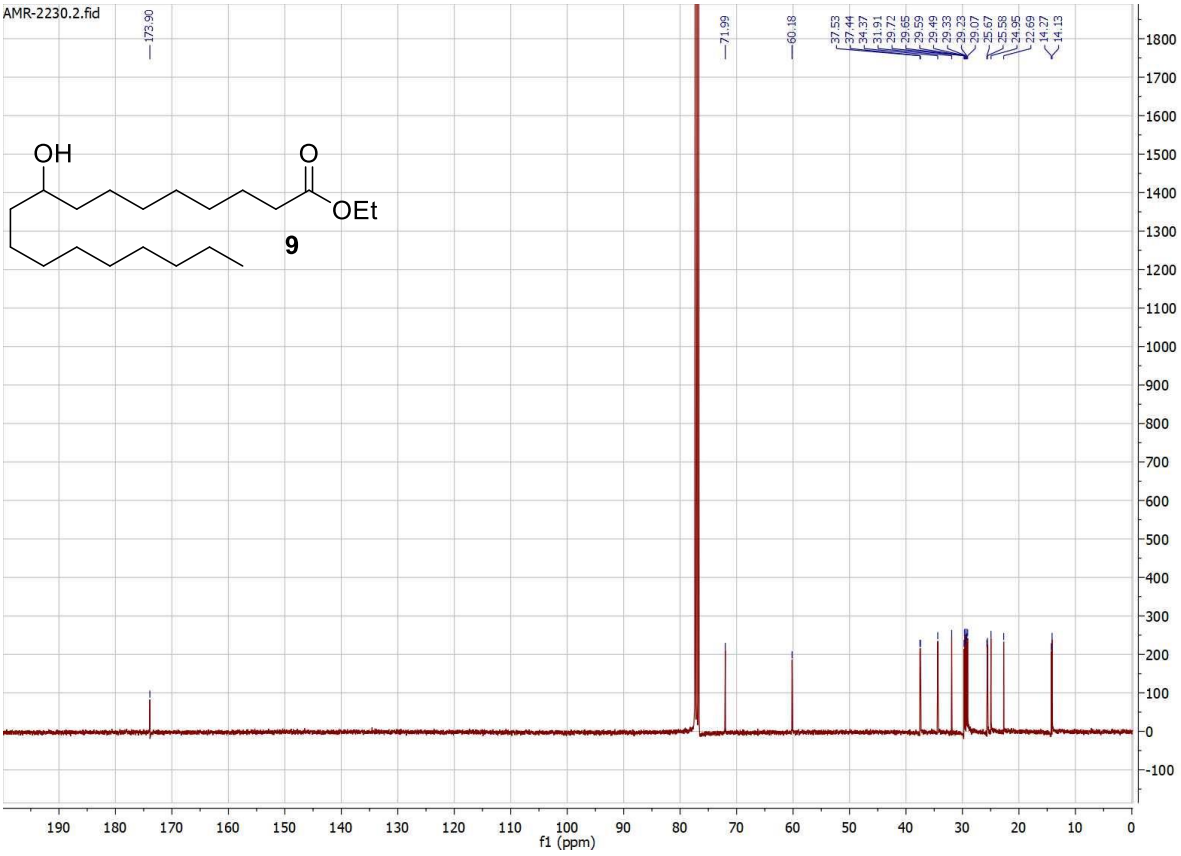
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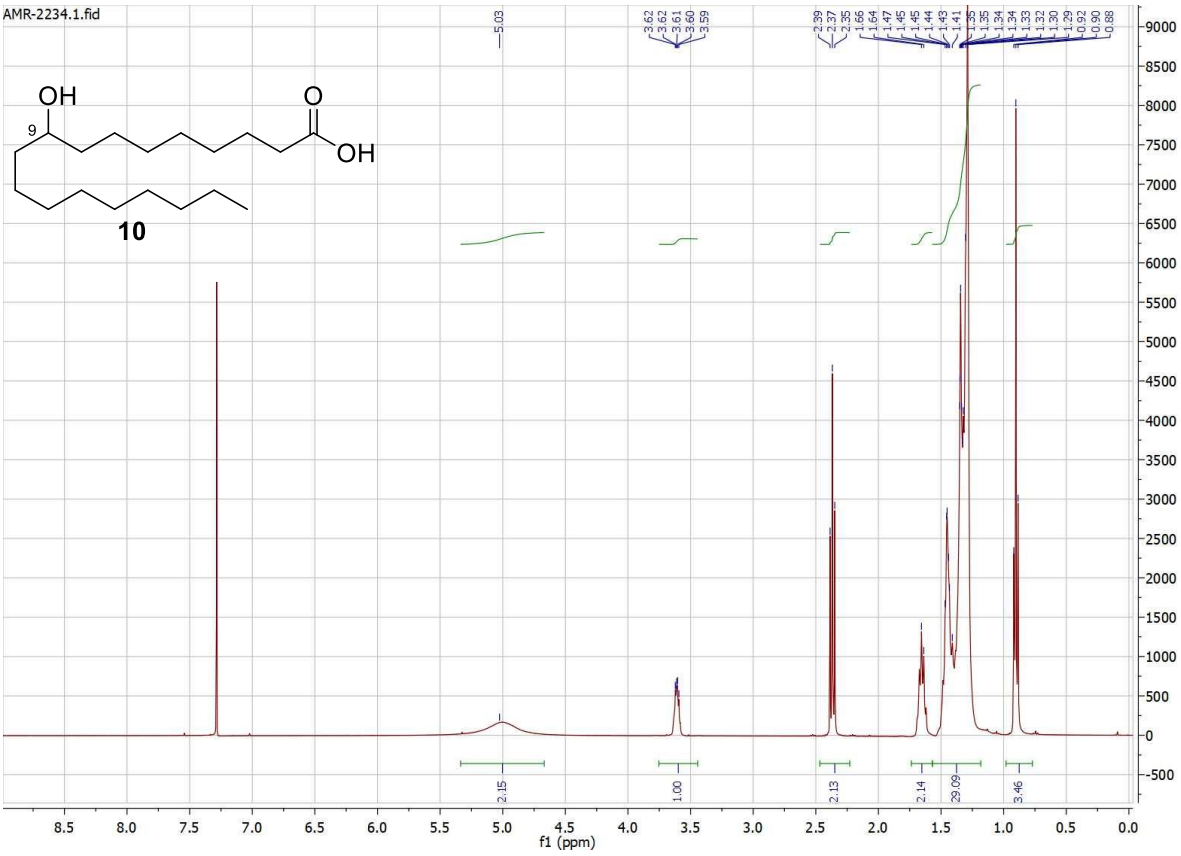
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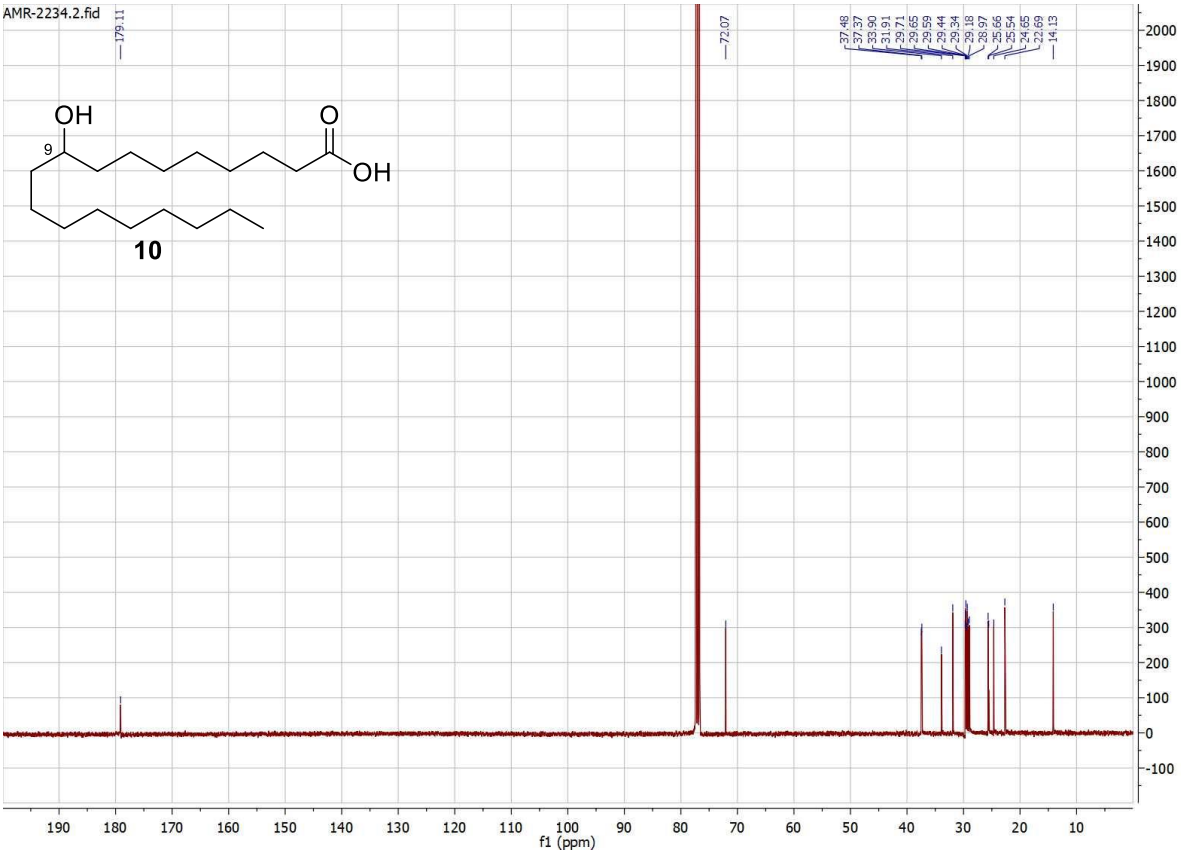
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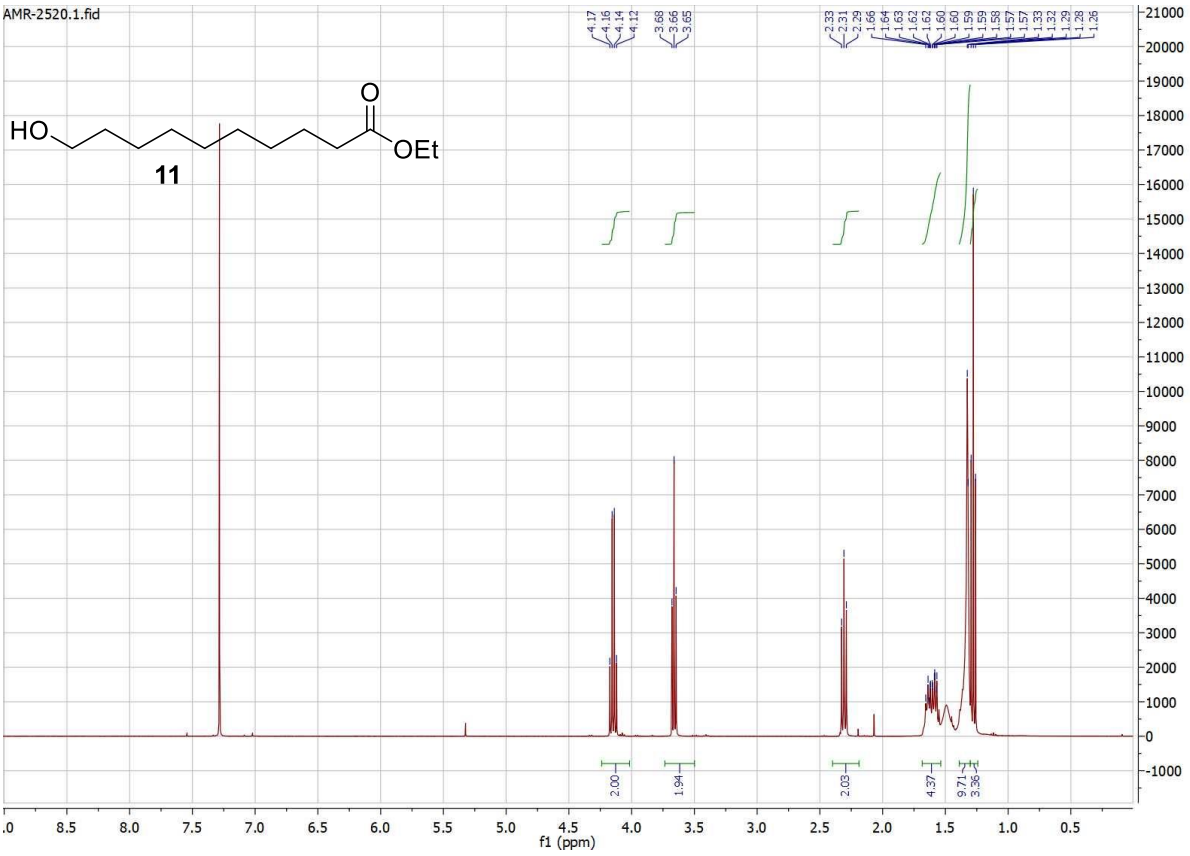
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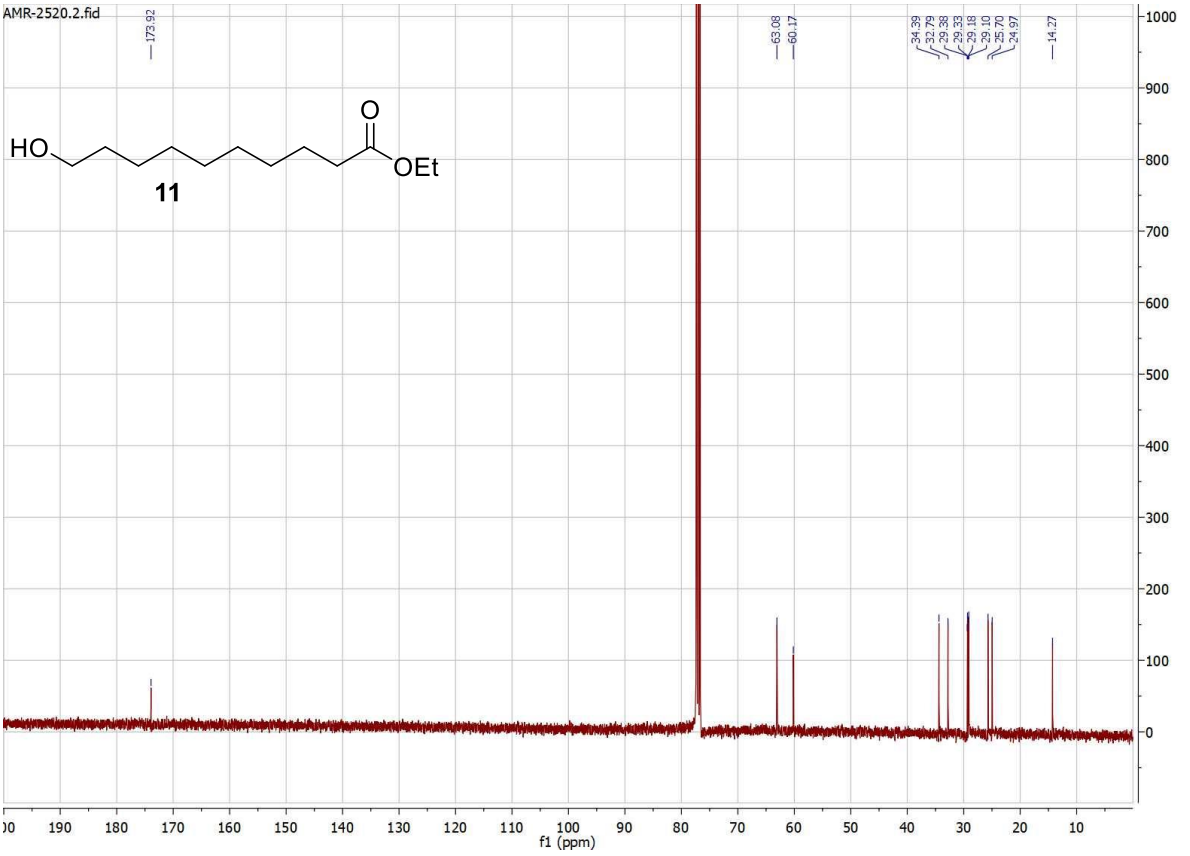
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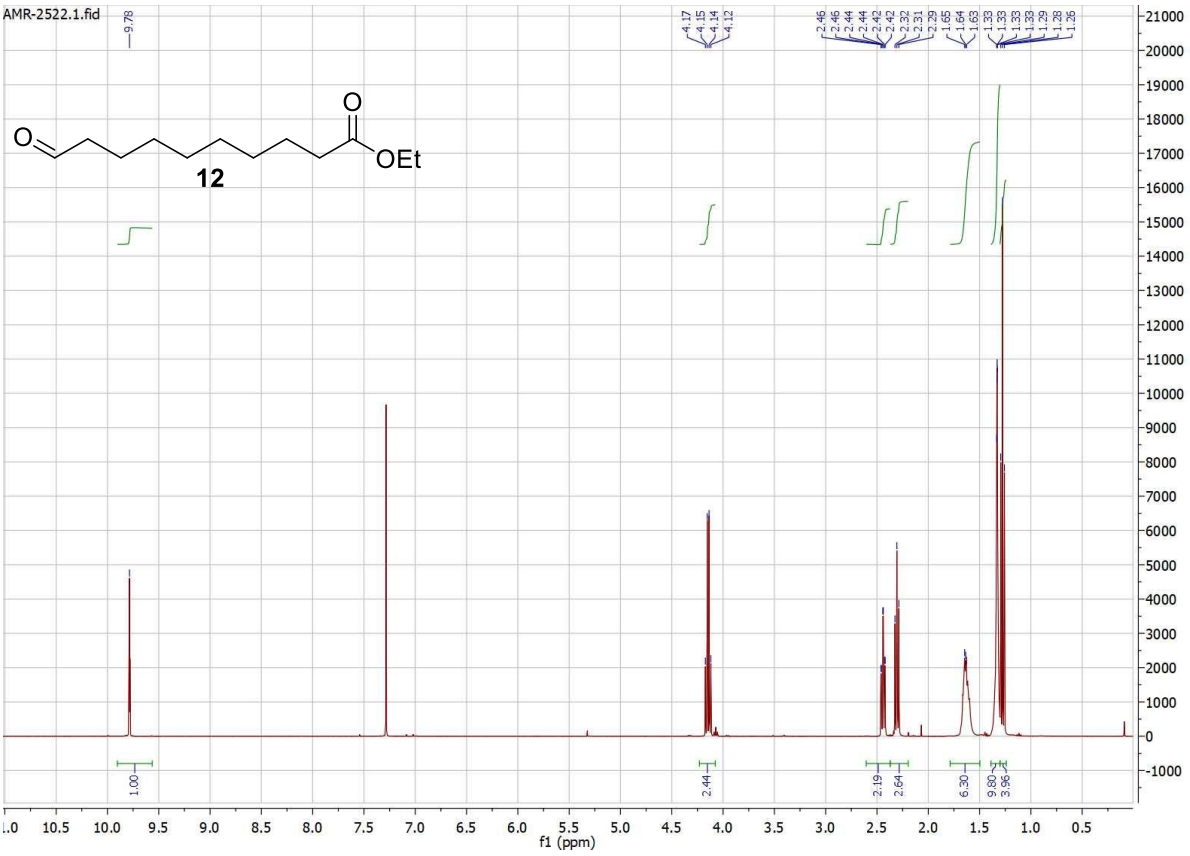
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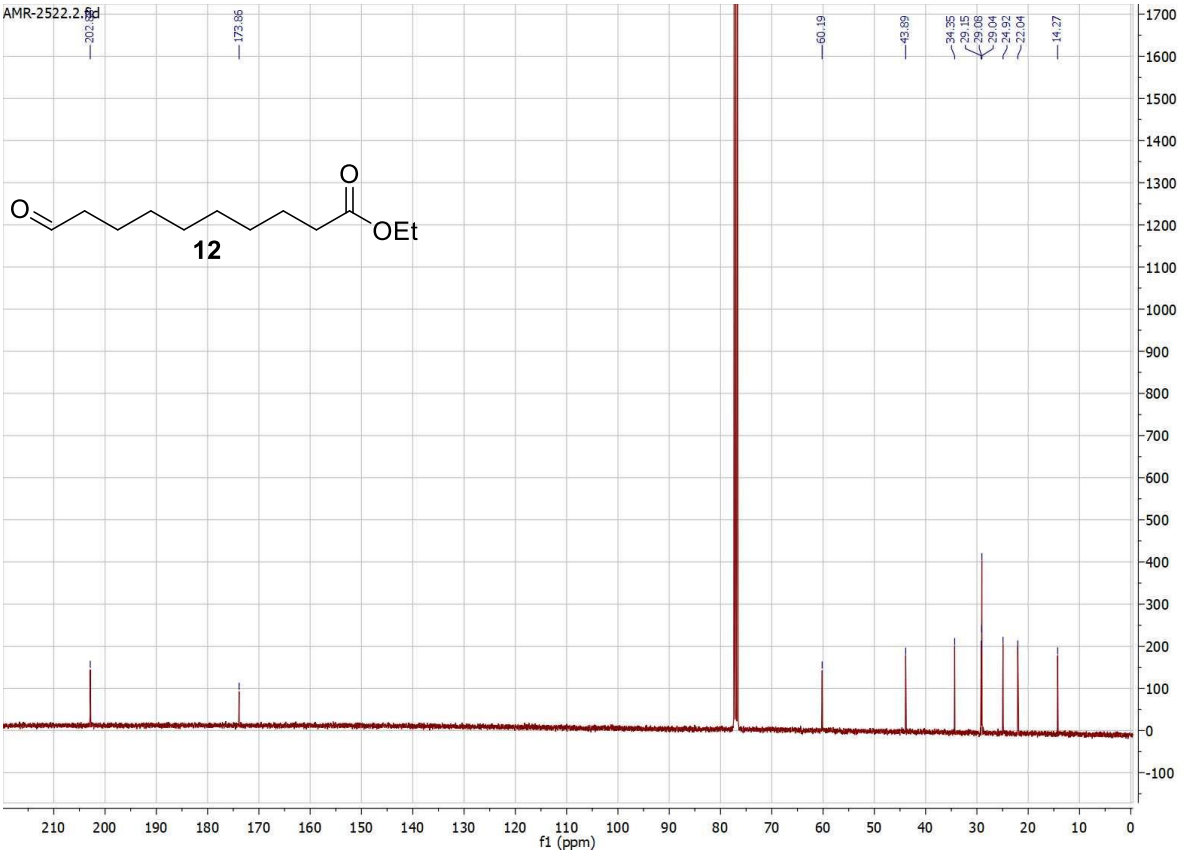
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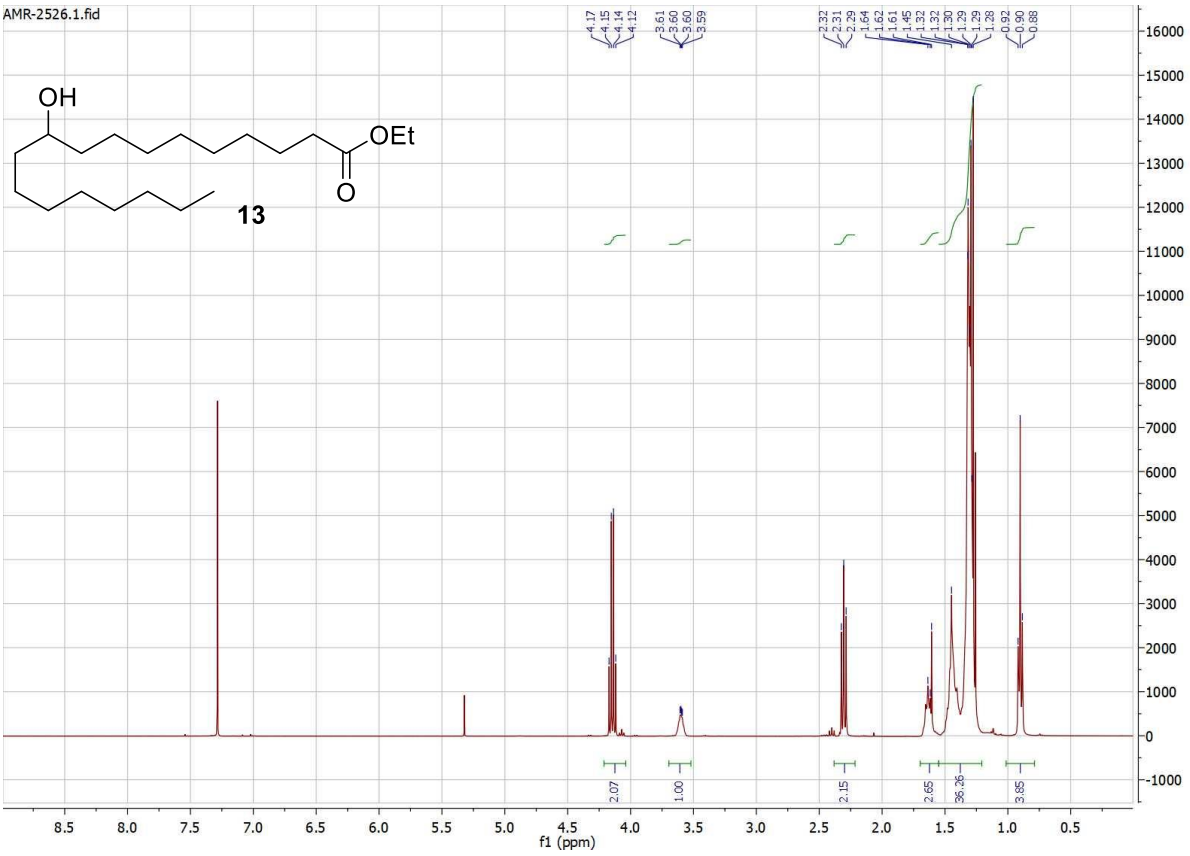
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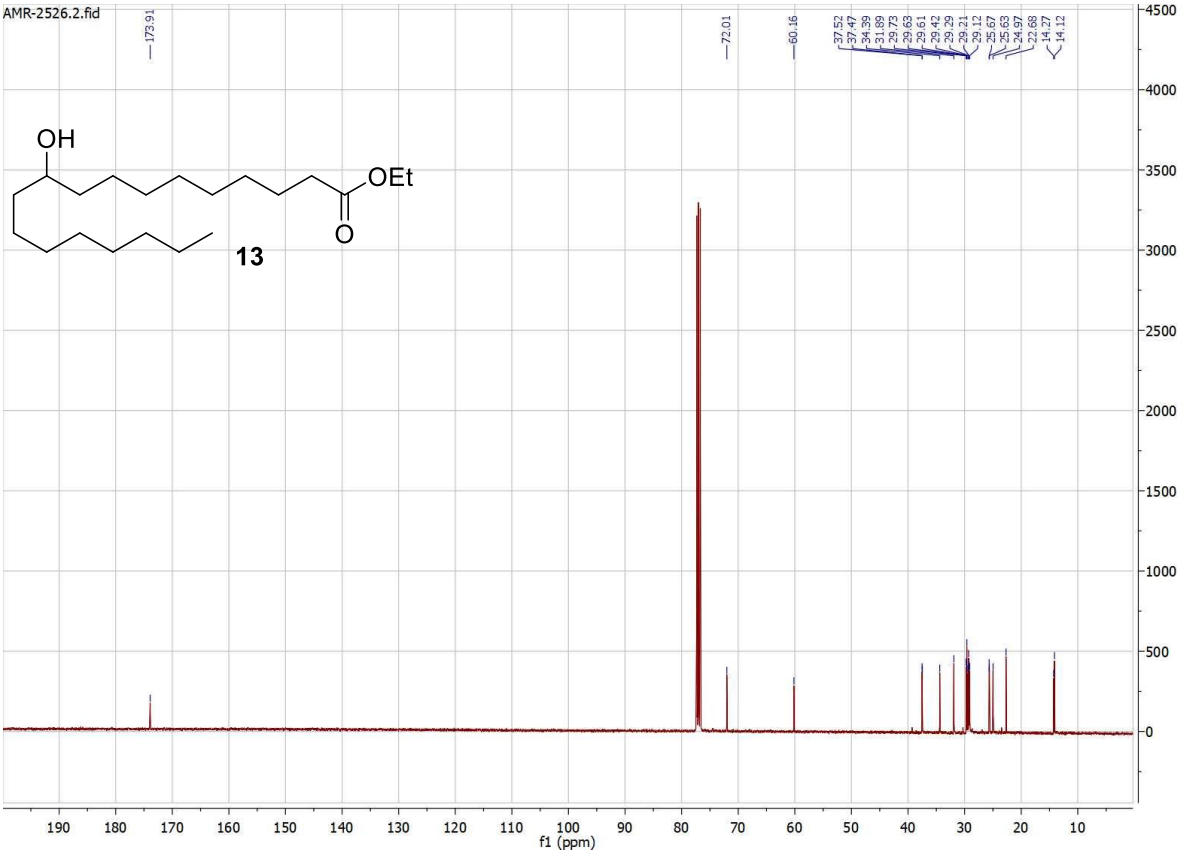
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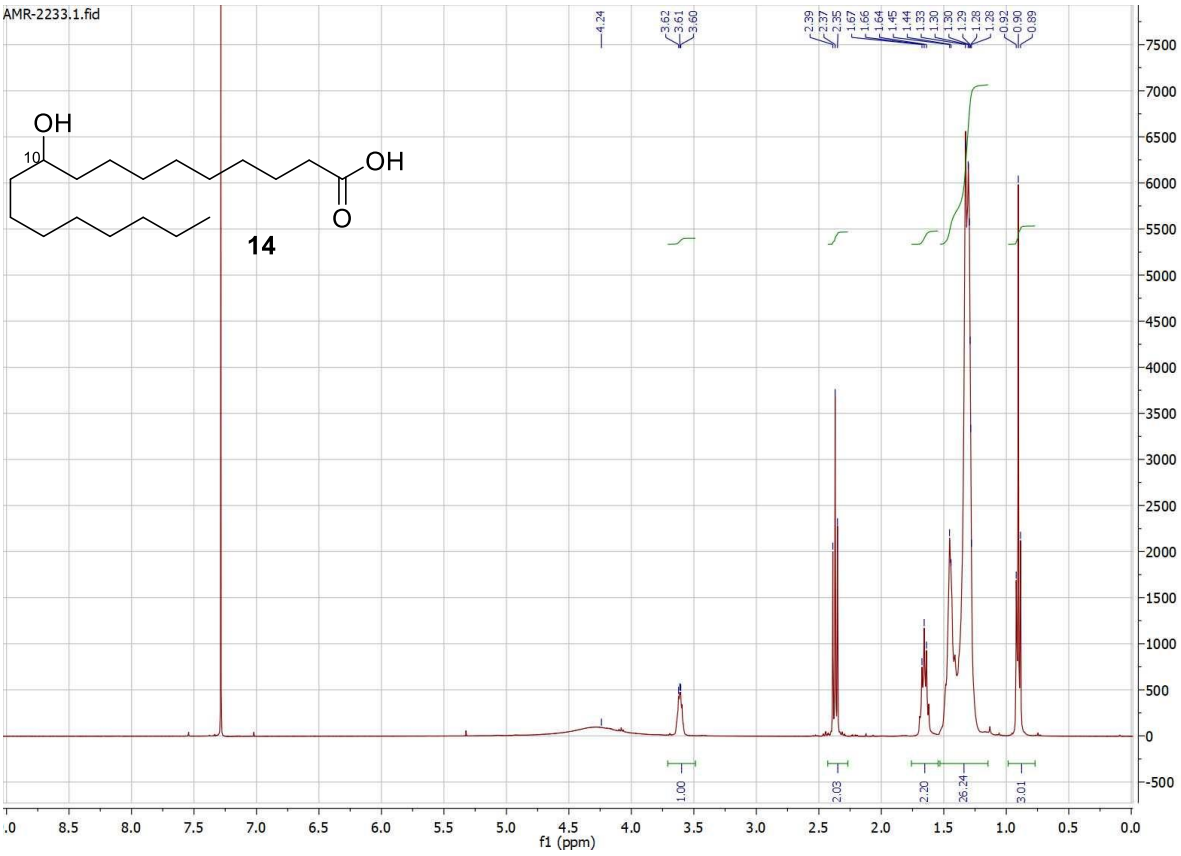
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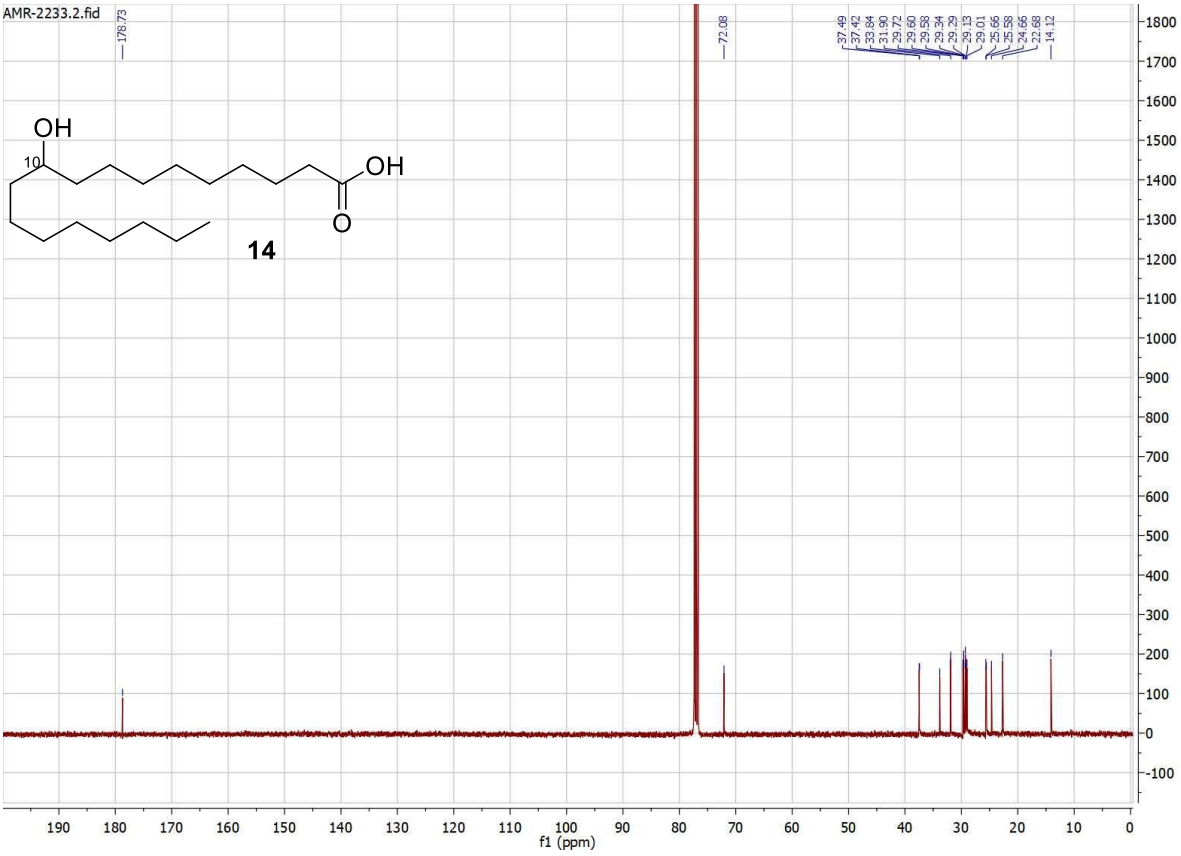
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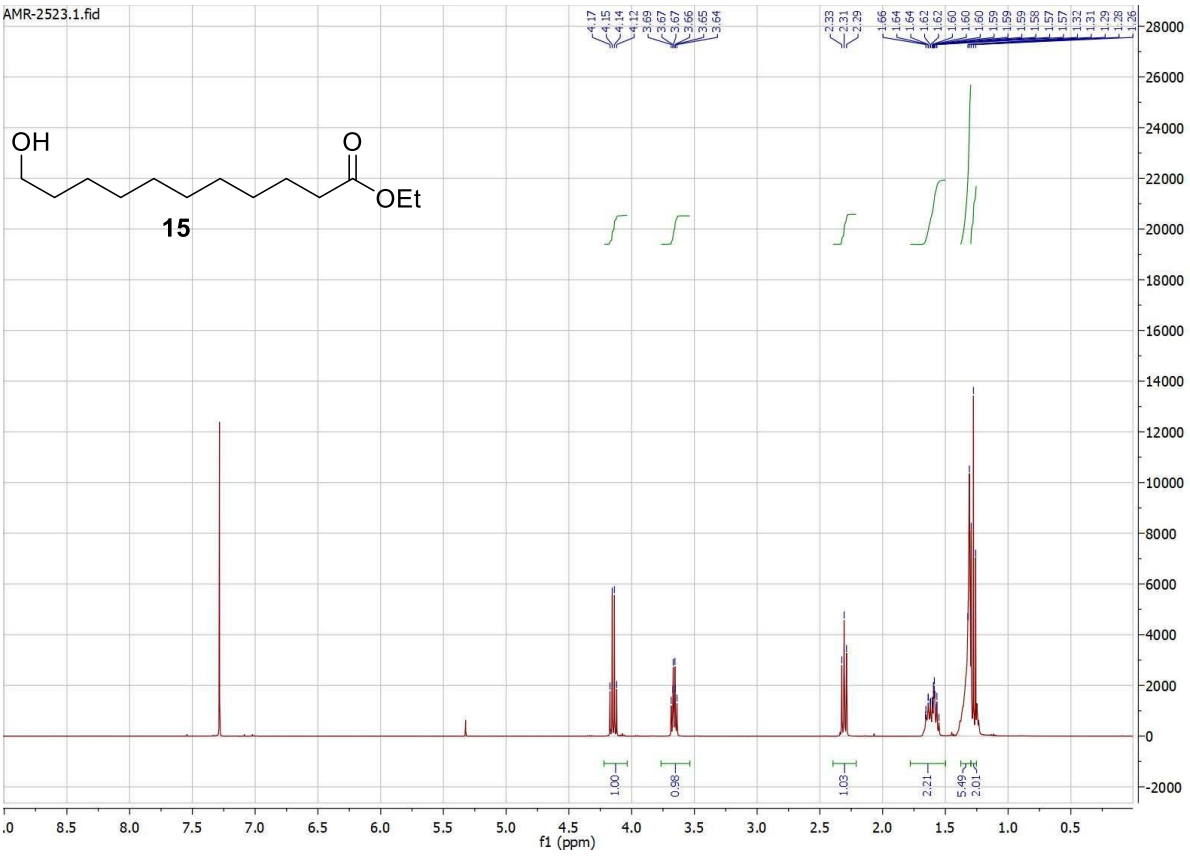
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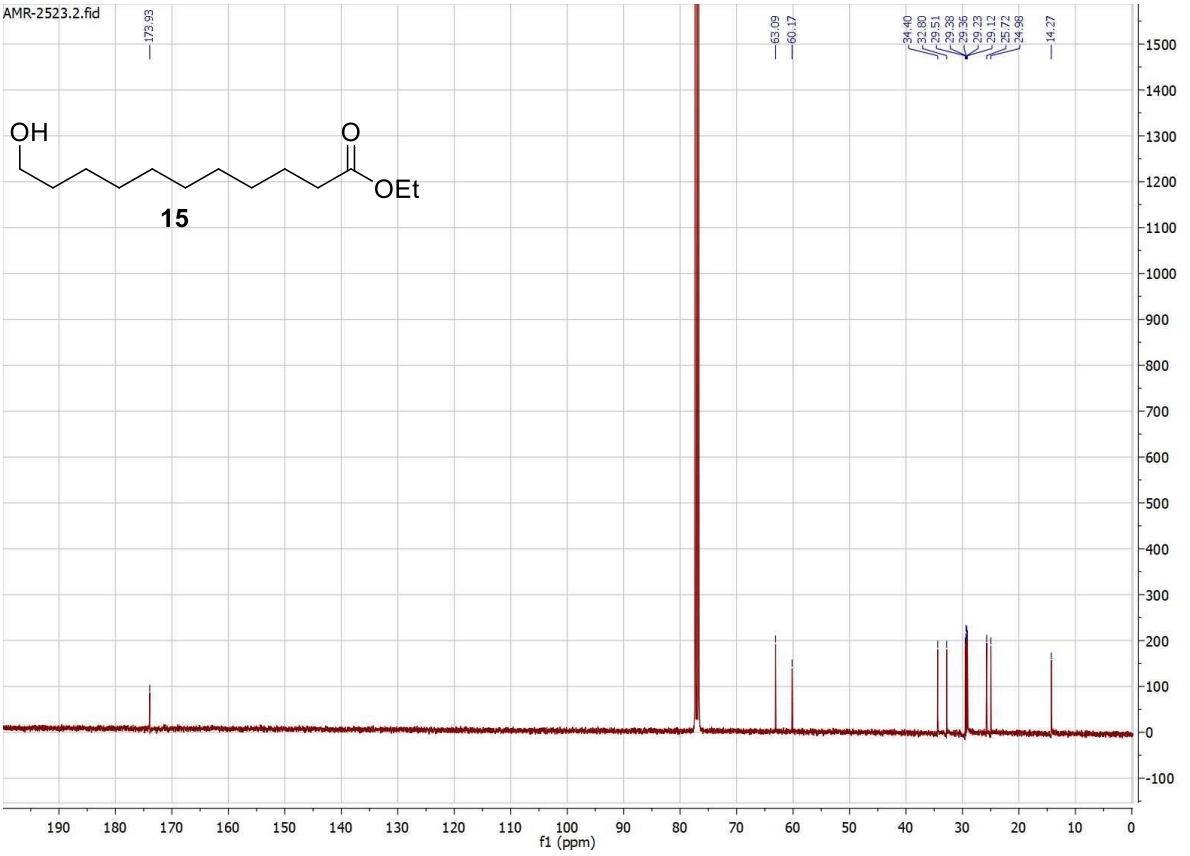
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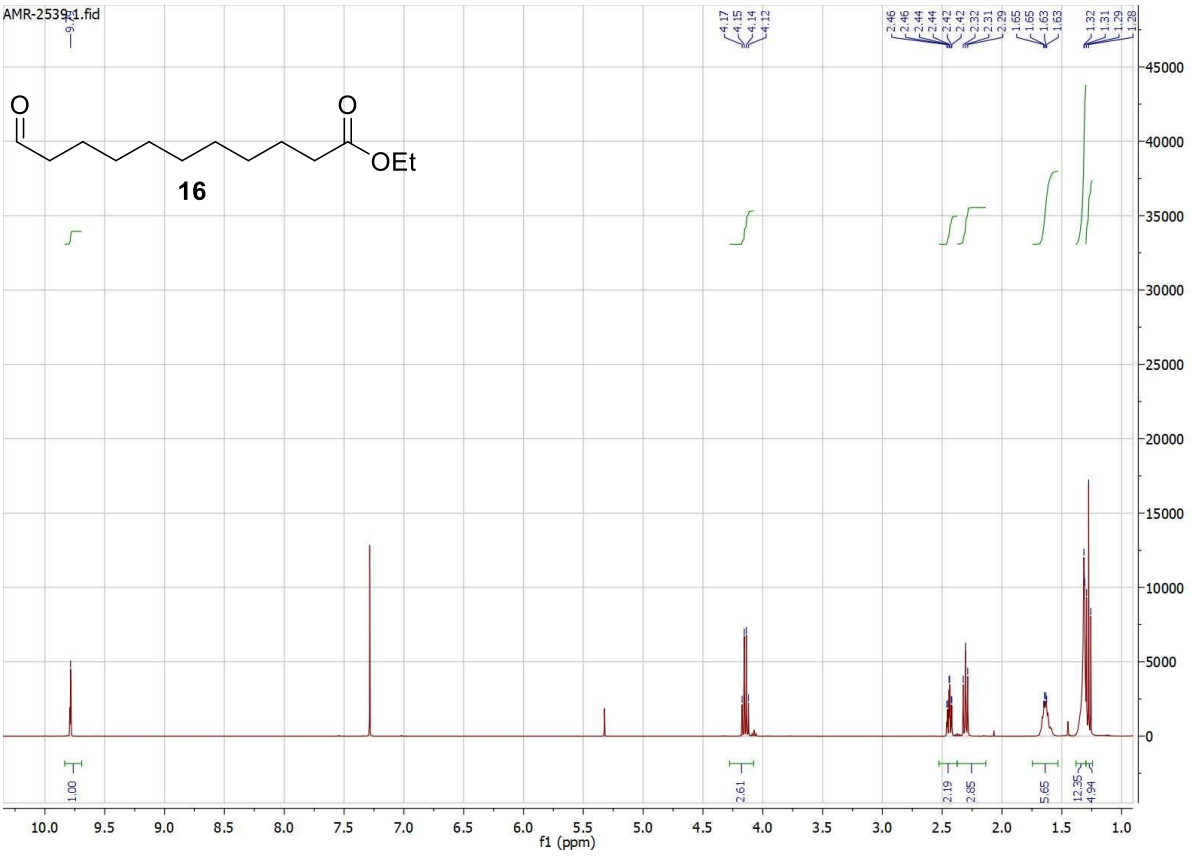
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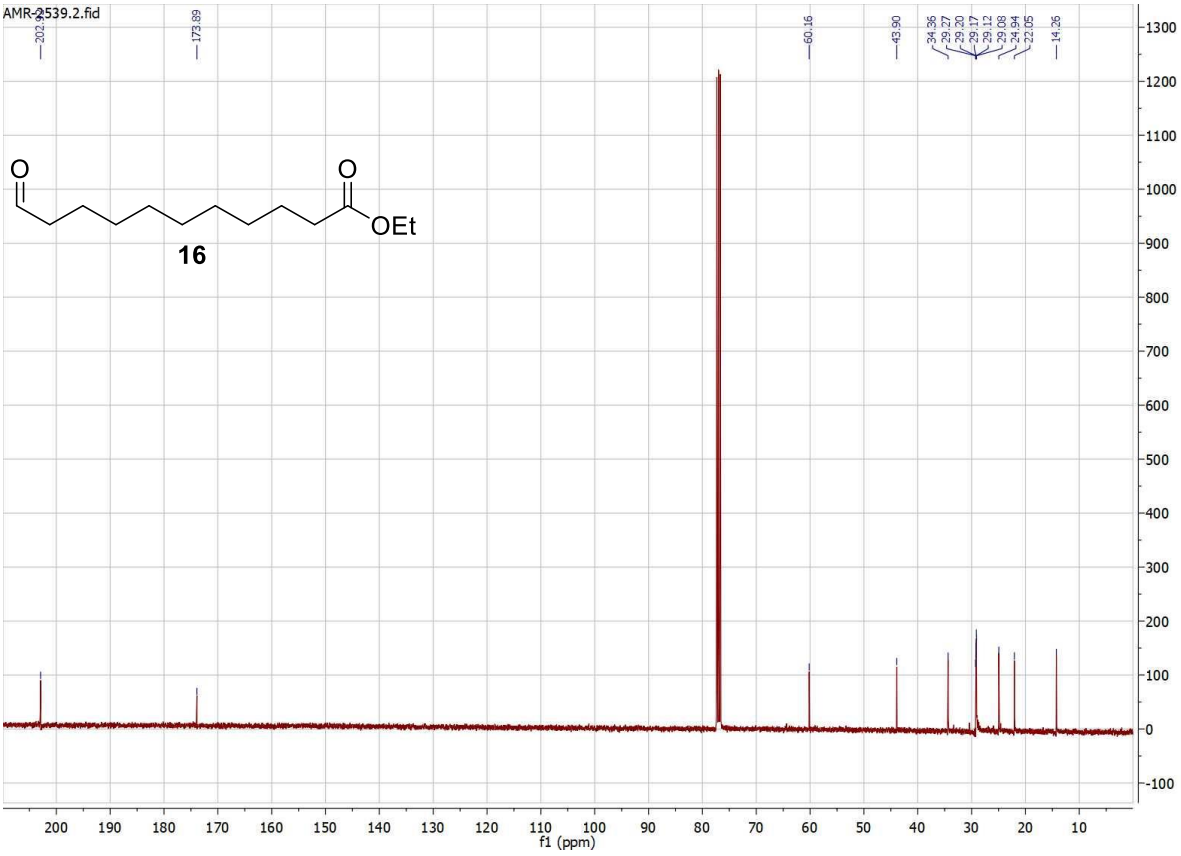
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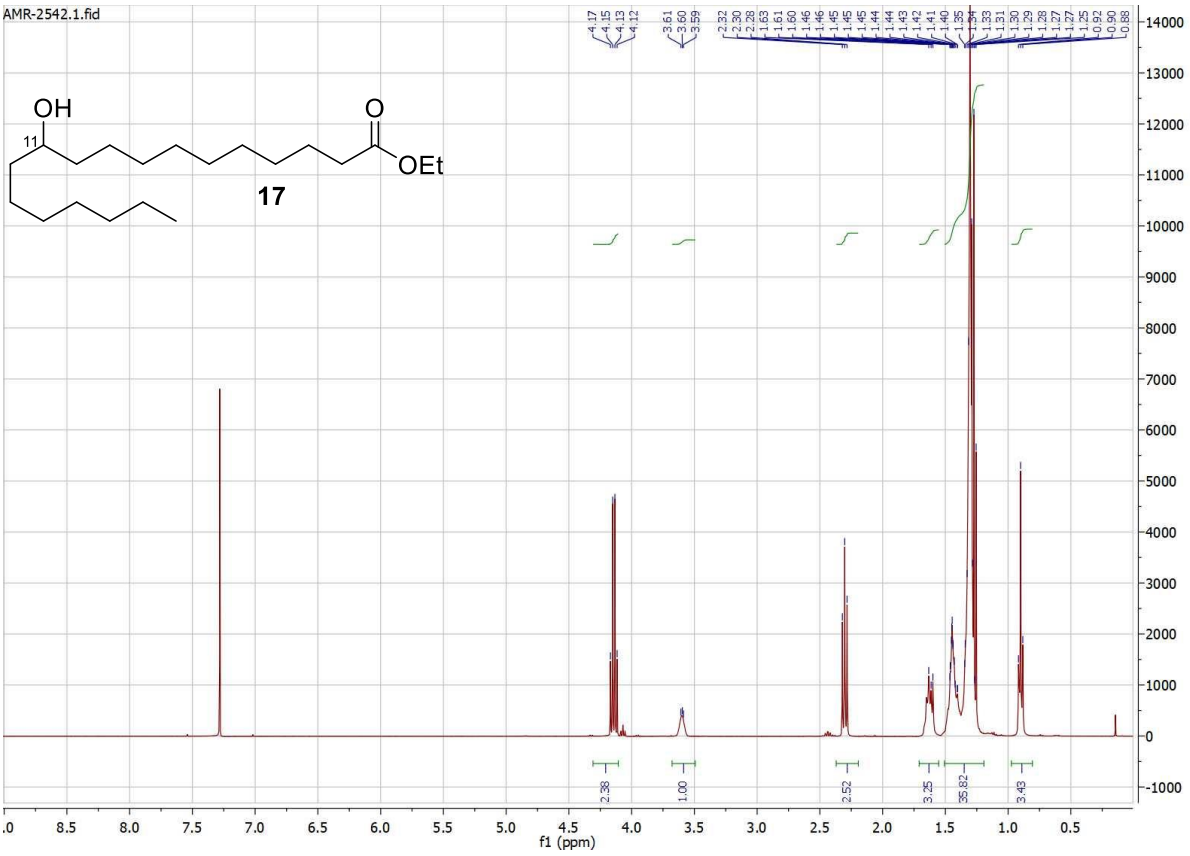
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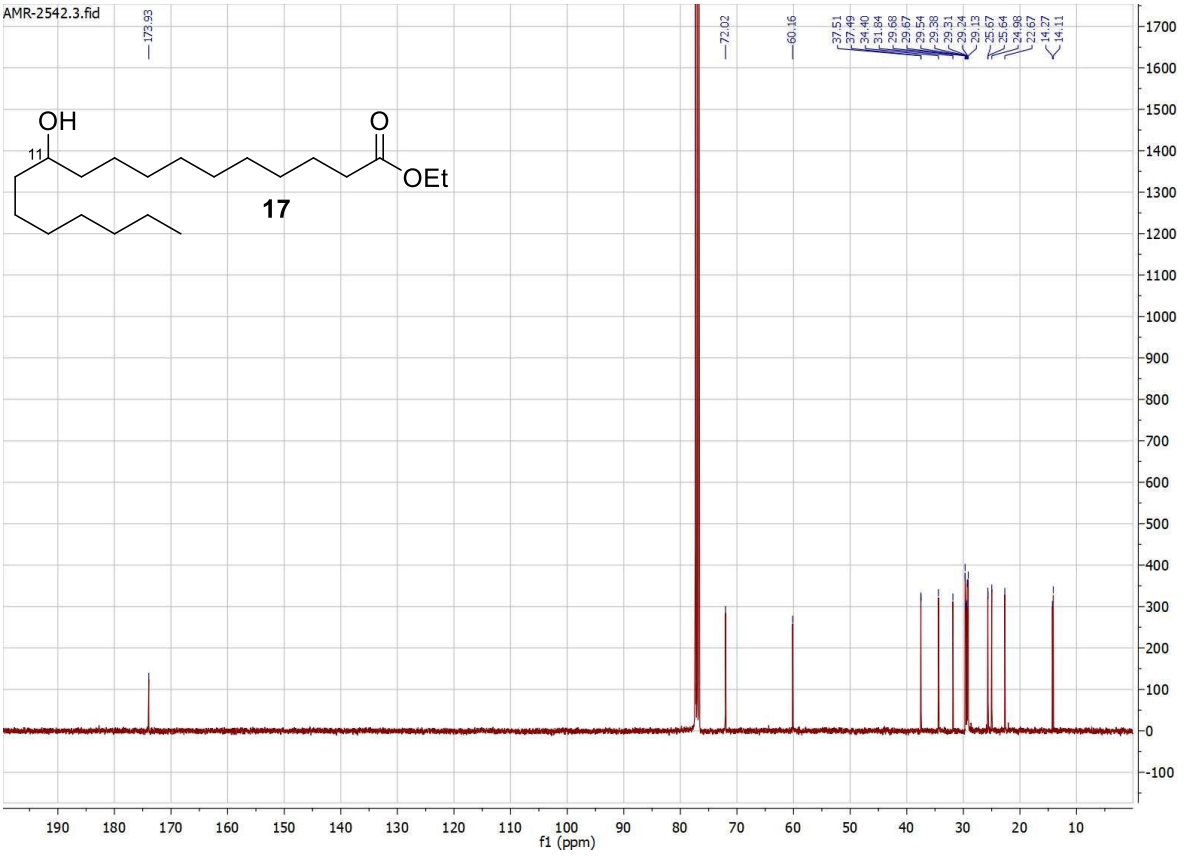
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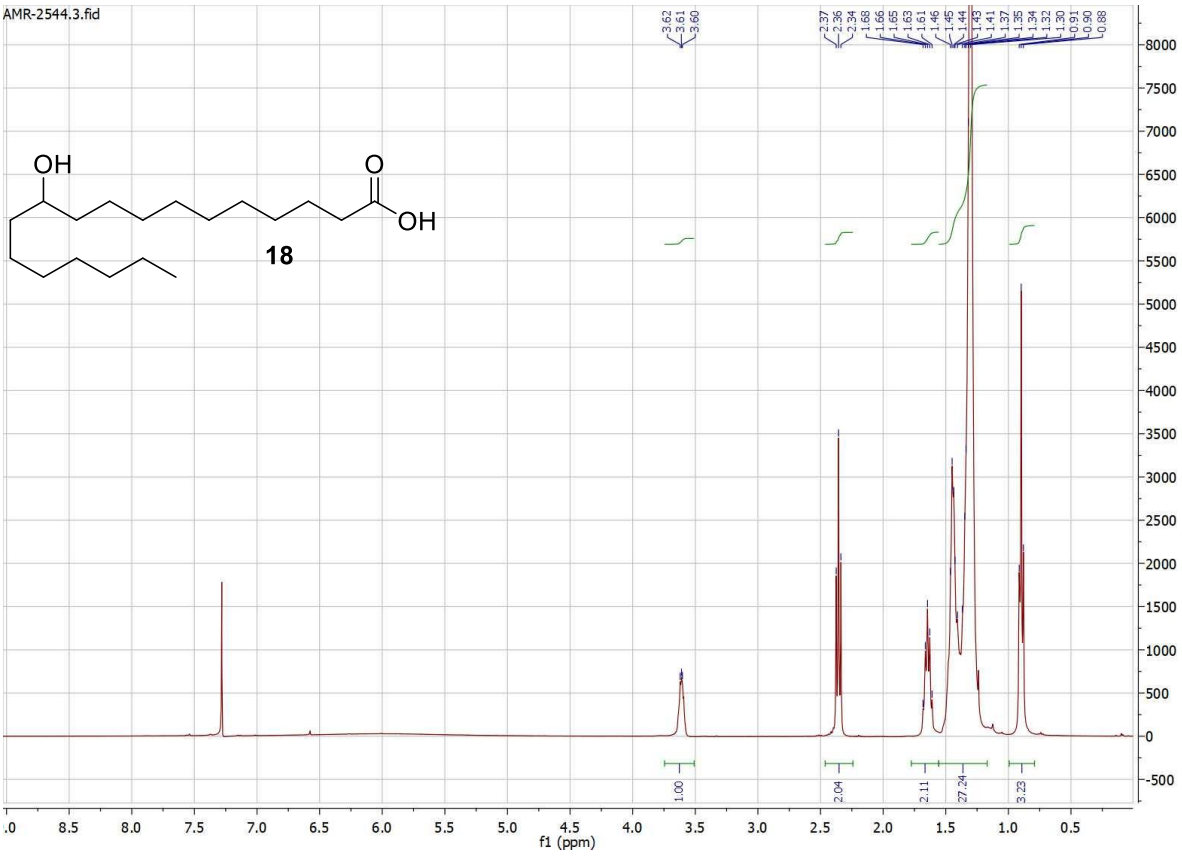
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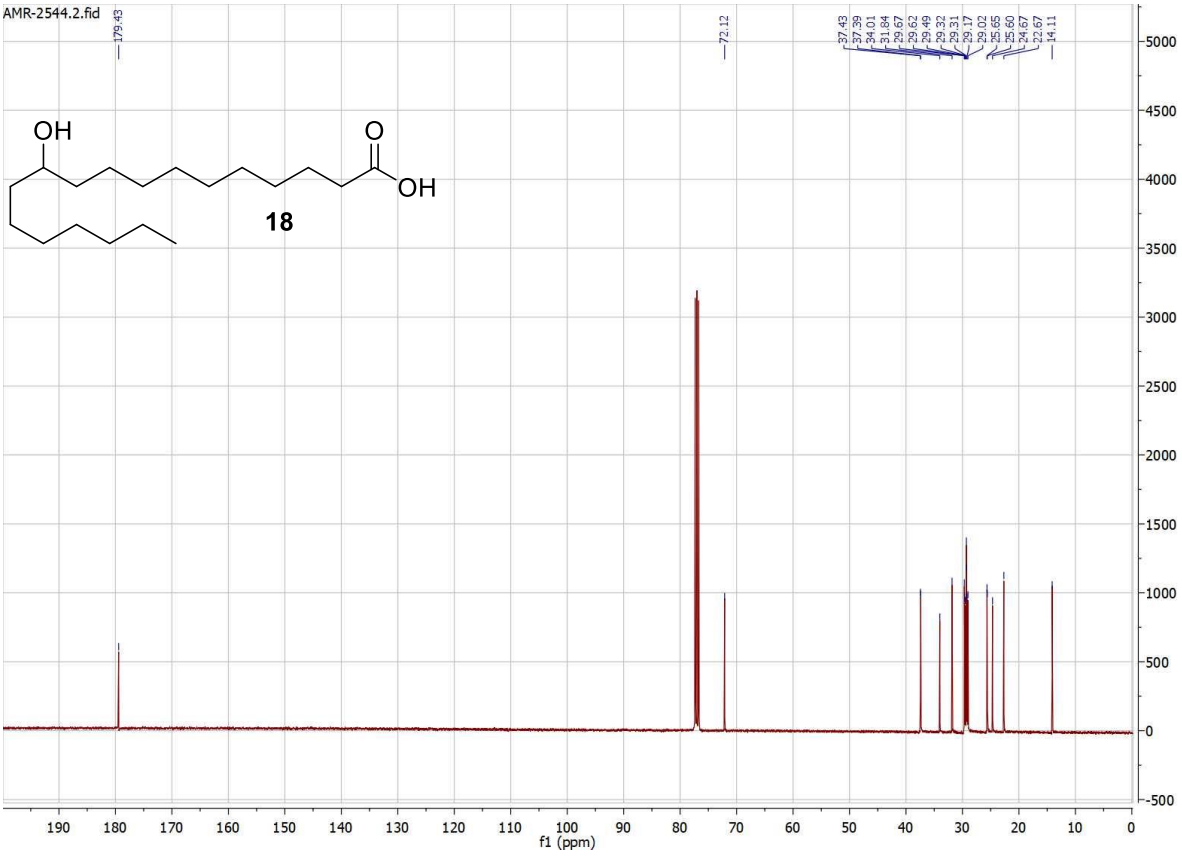
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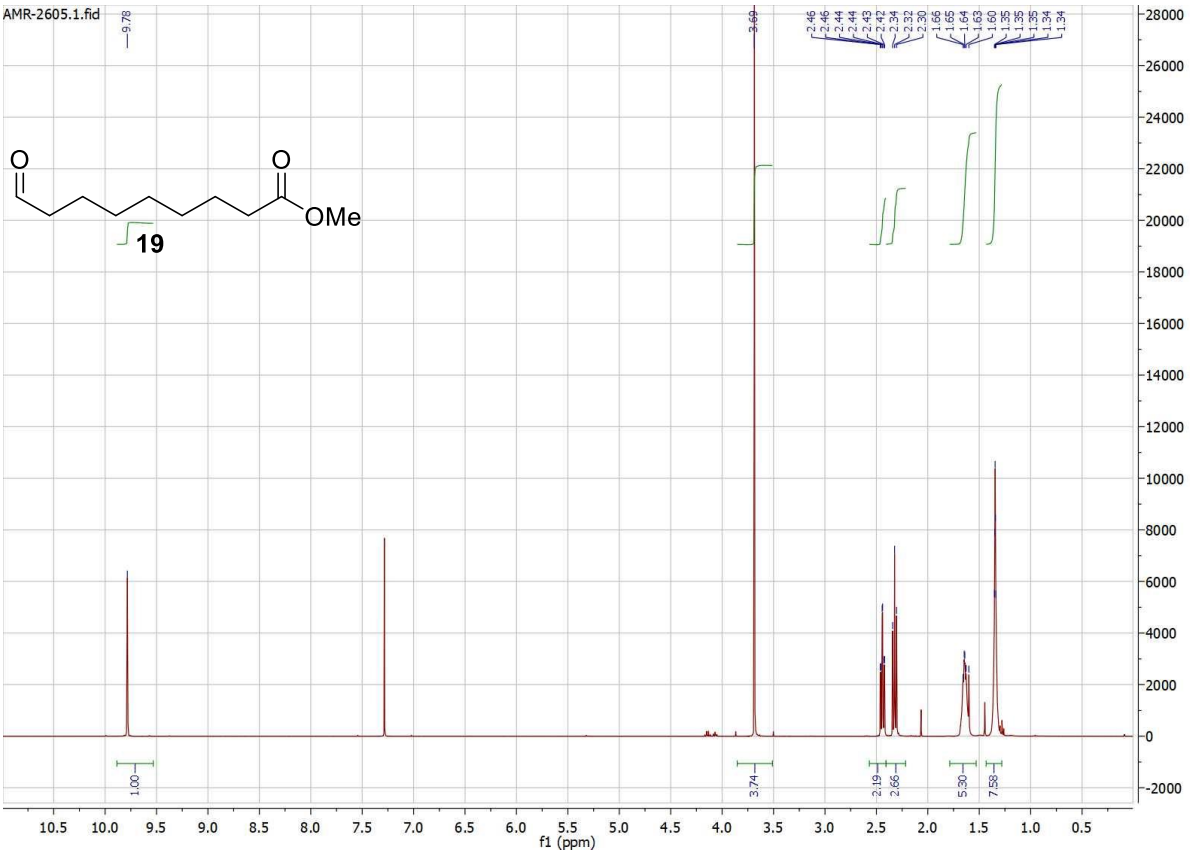
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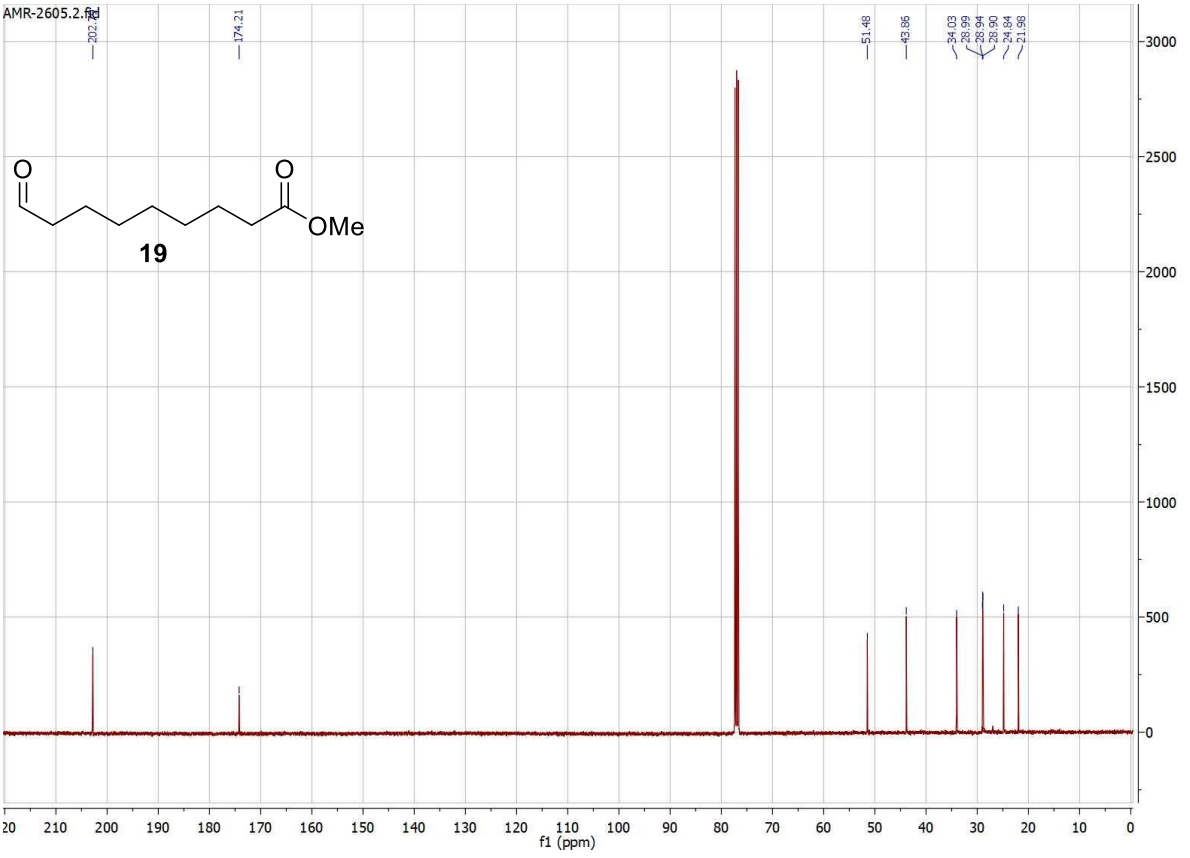
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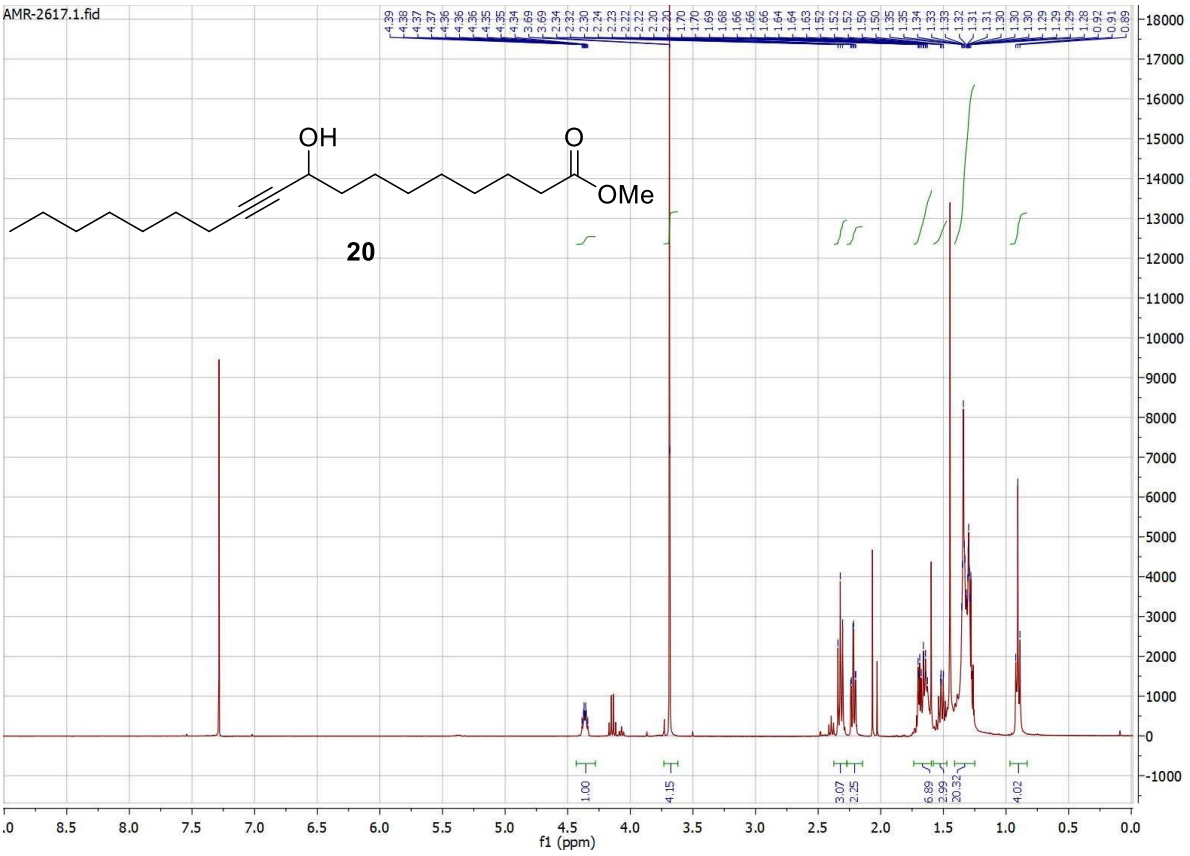
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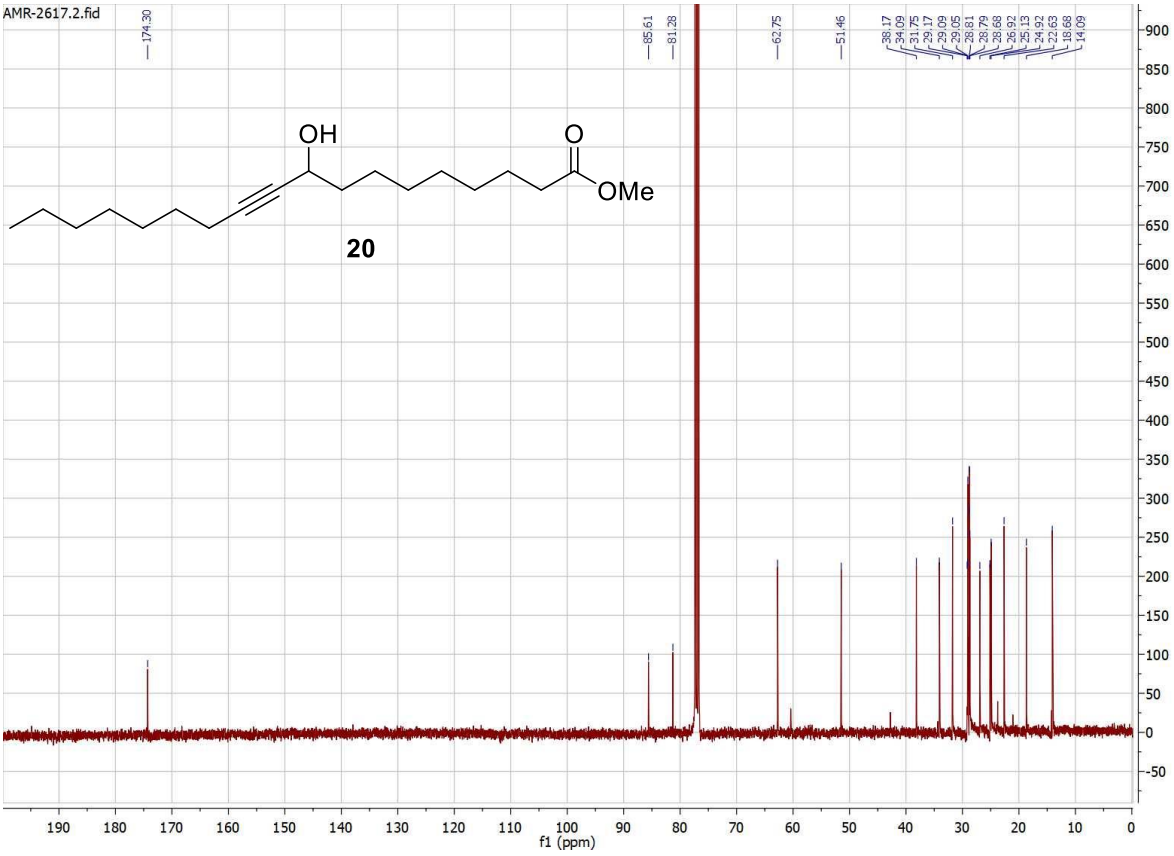
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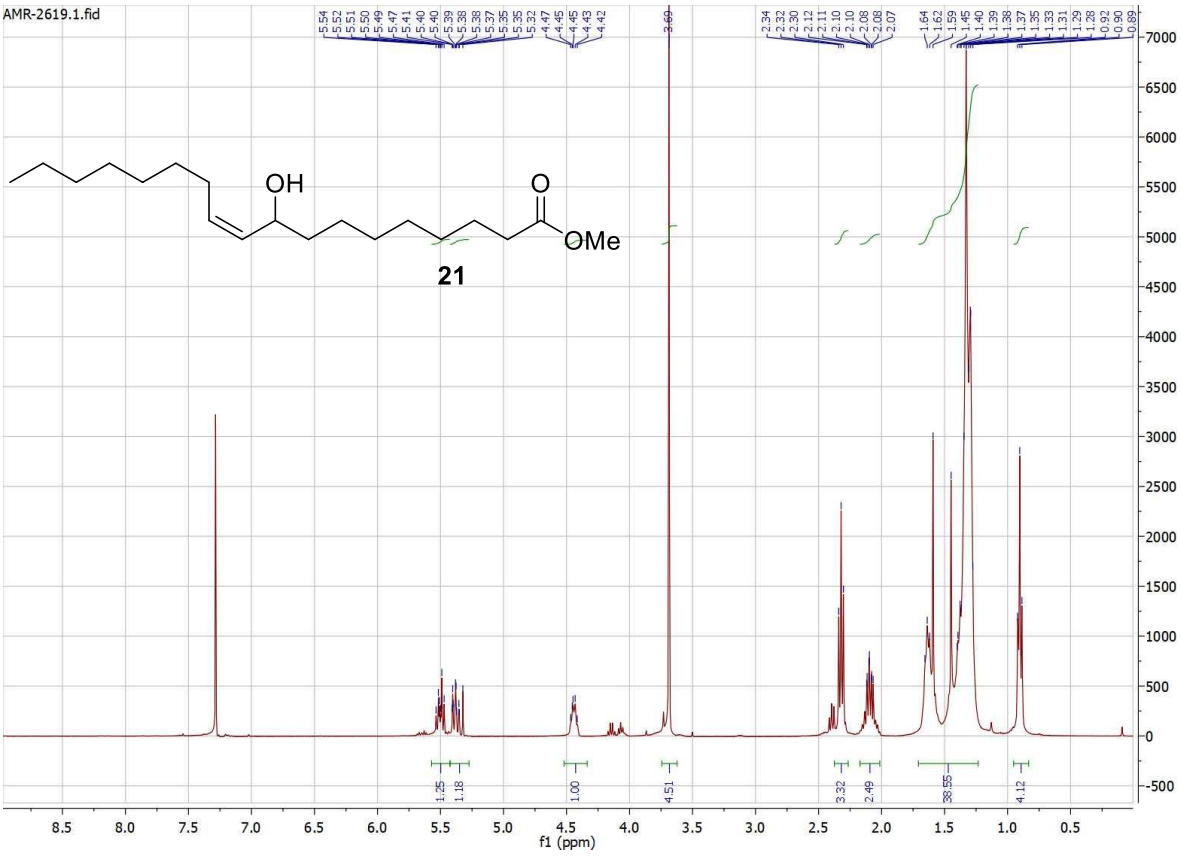
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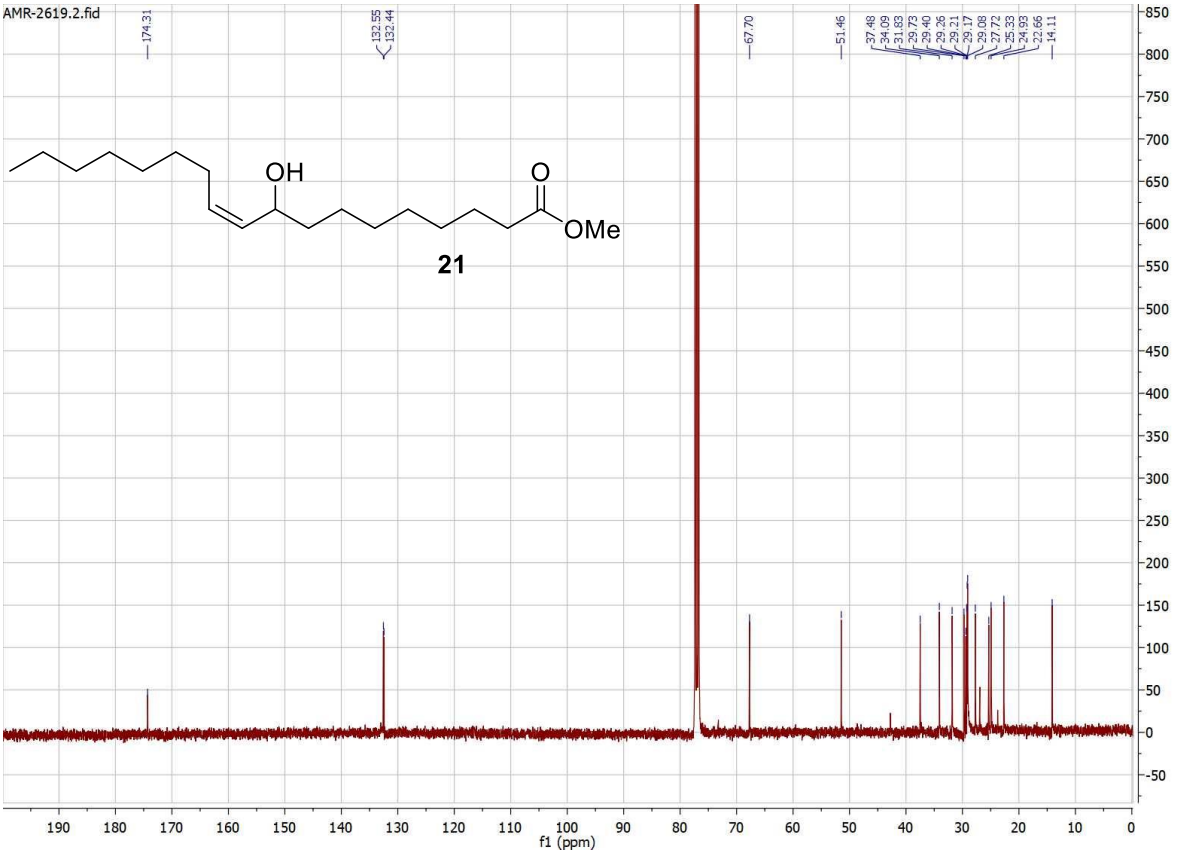
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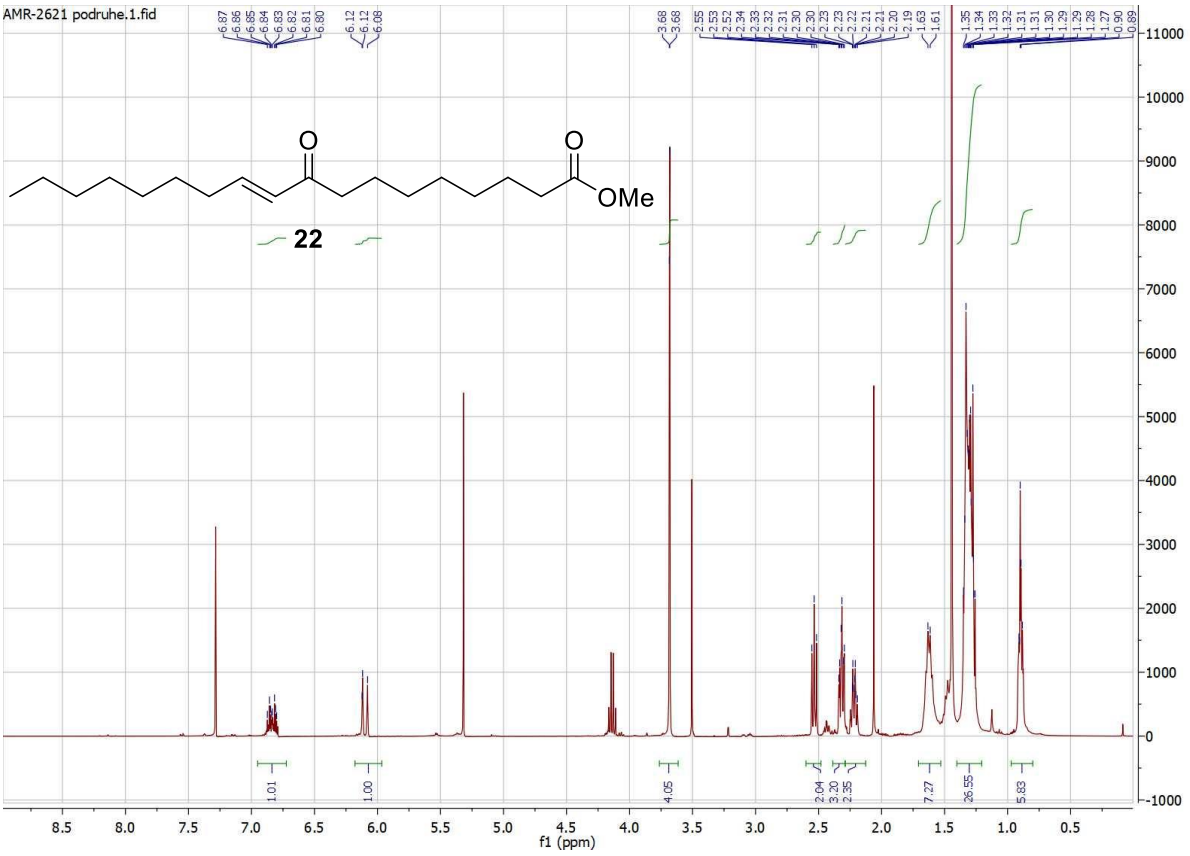
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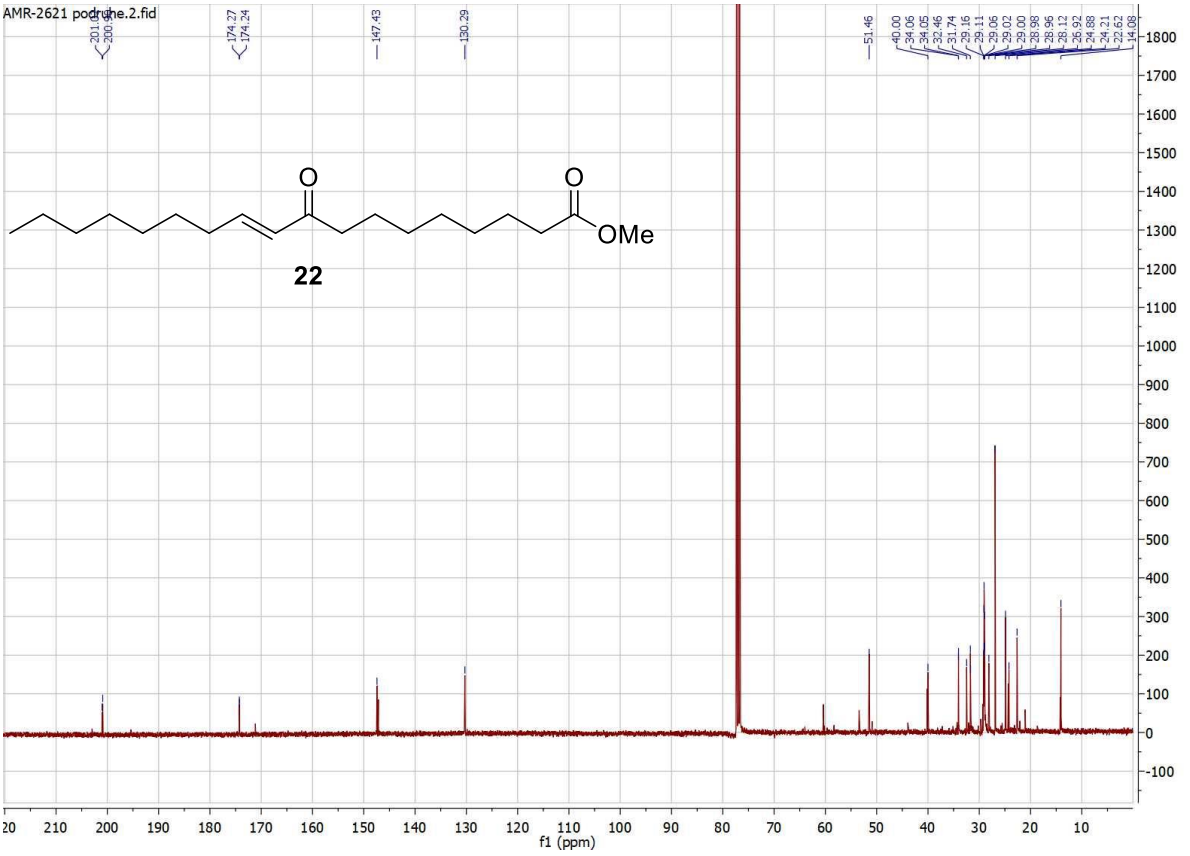
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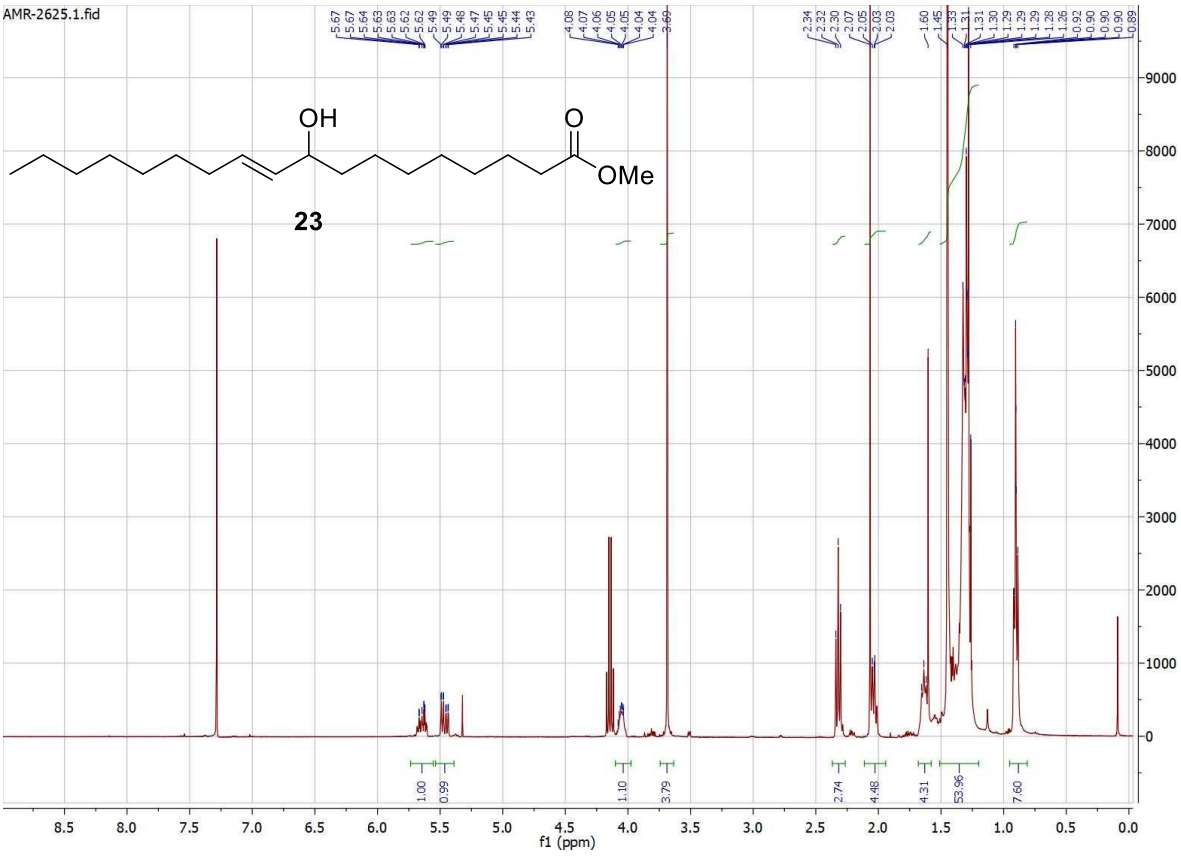
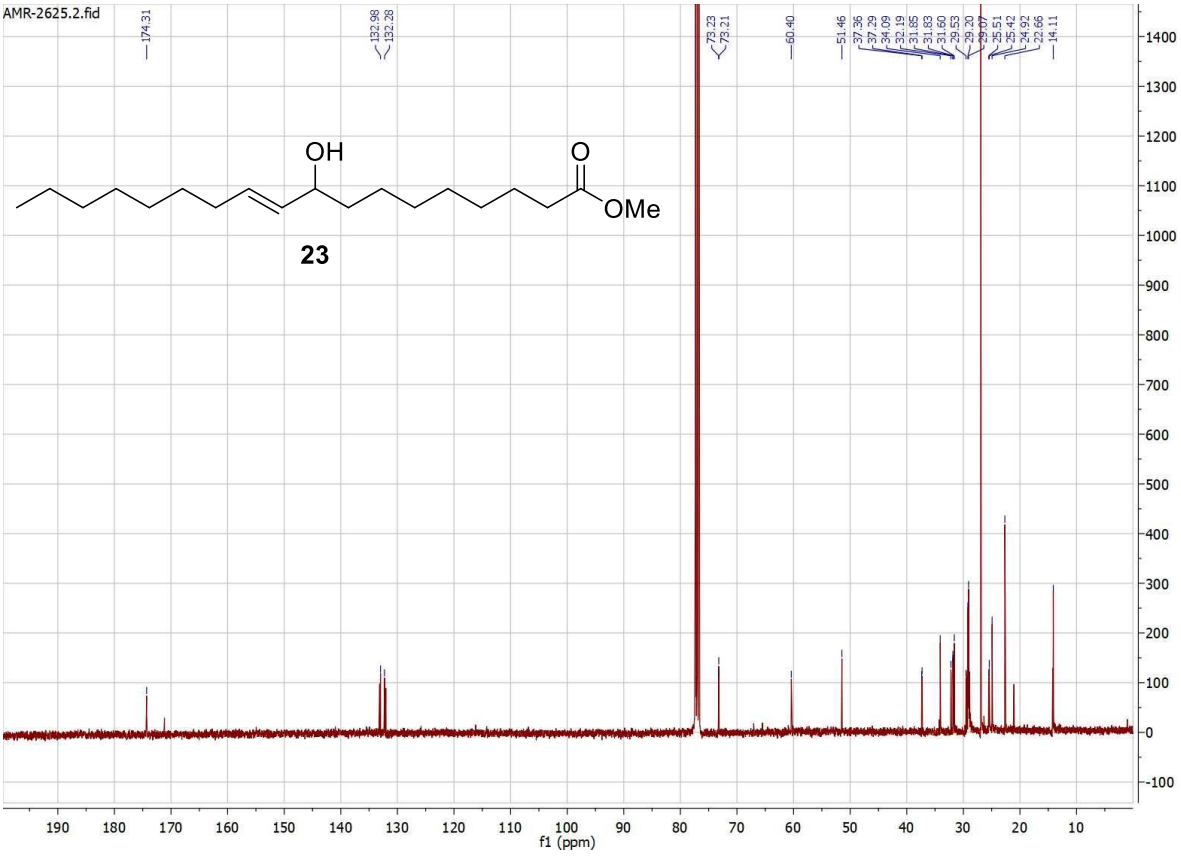
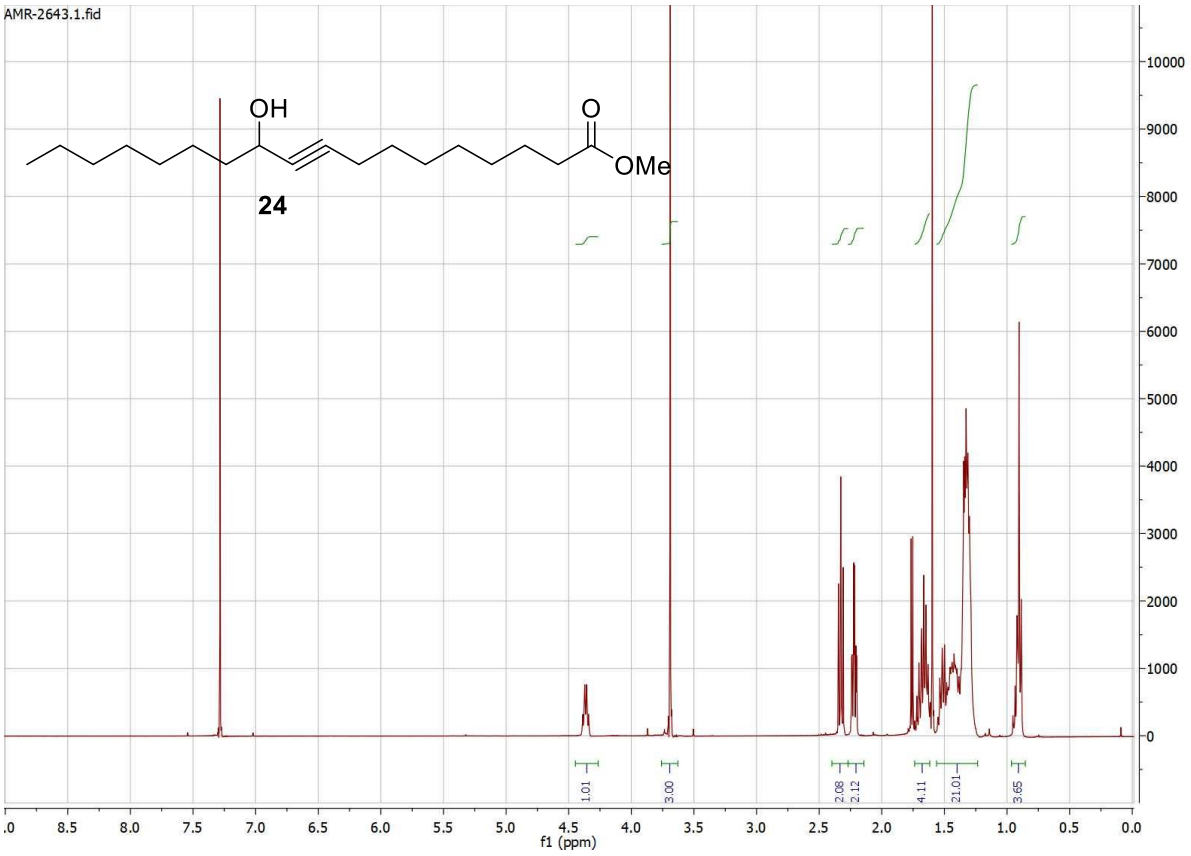
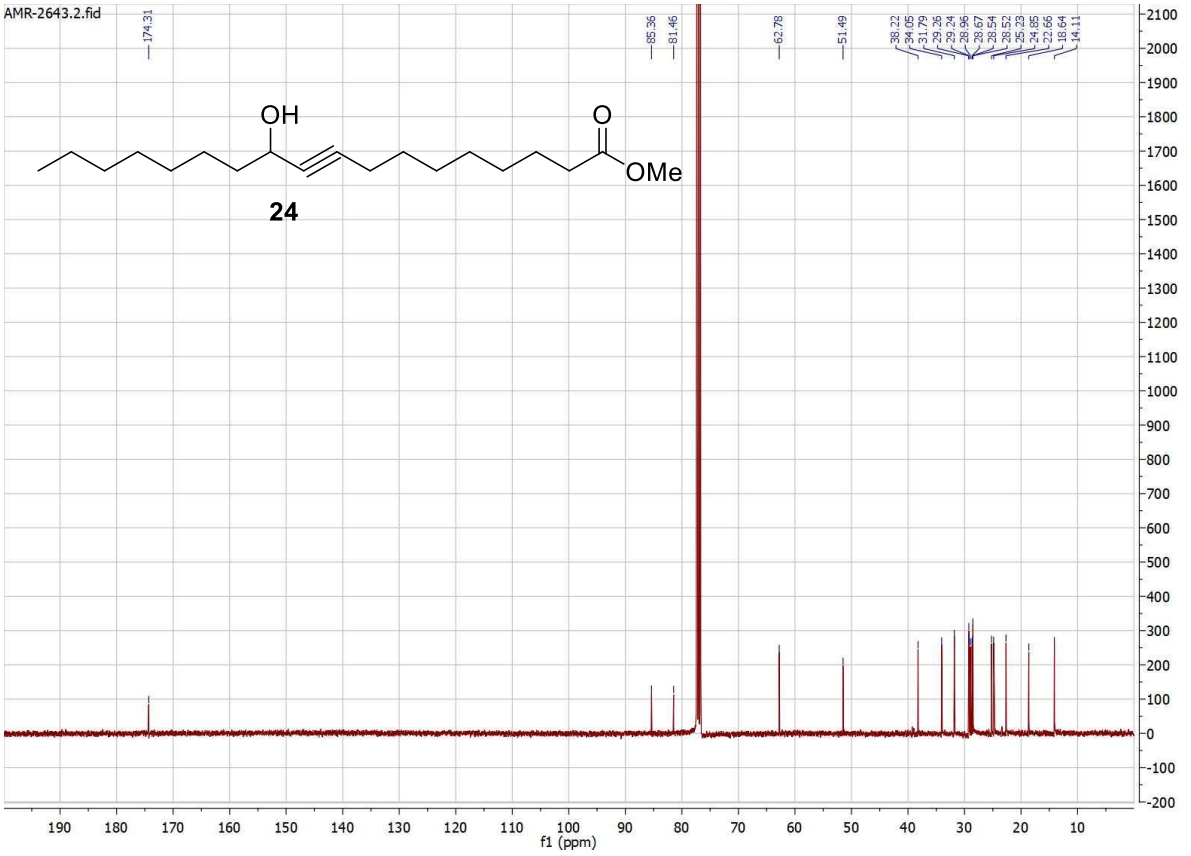
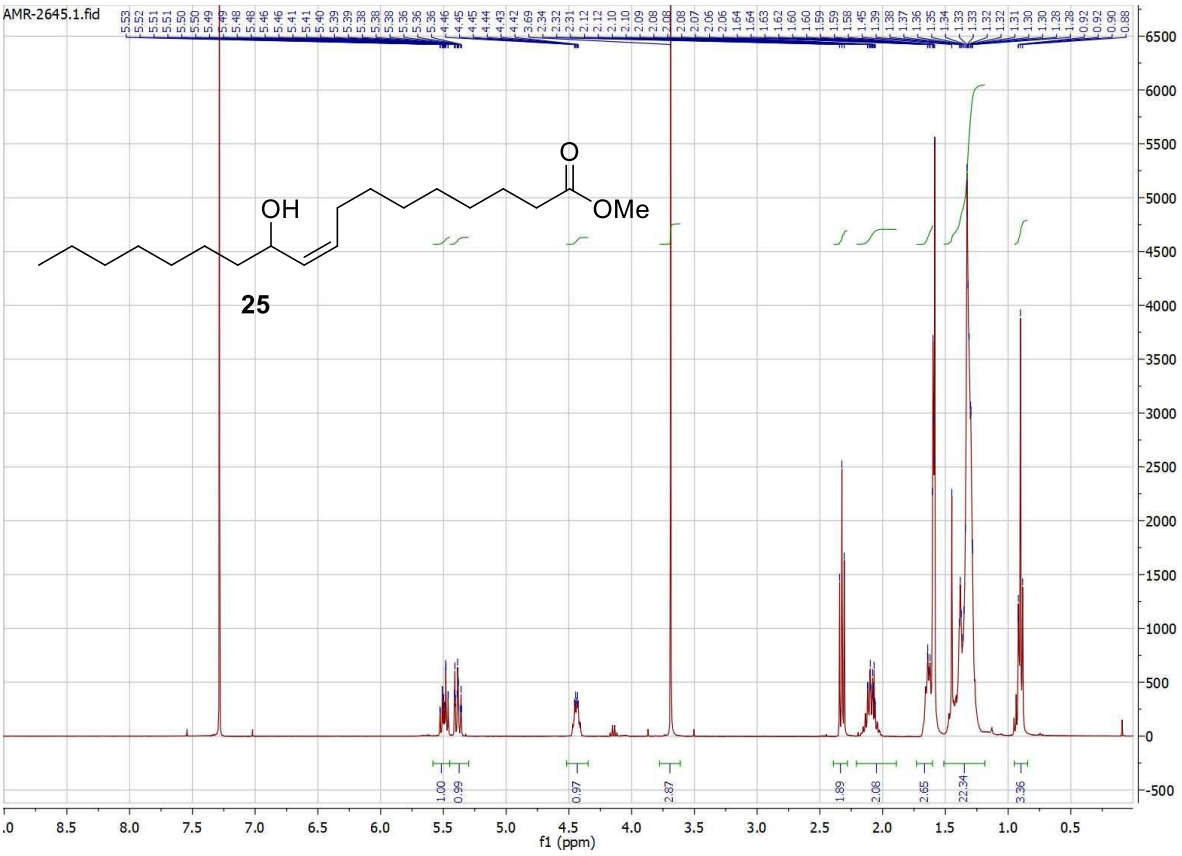
22
f1 (ppm)

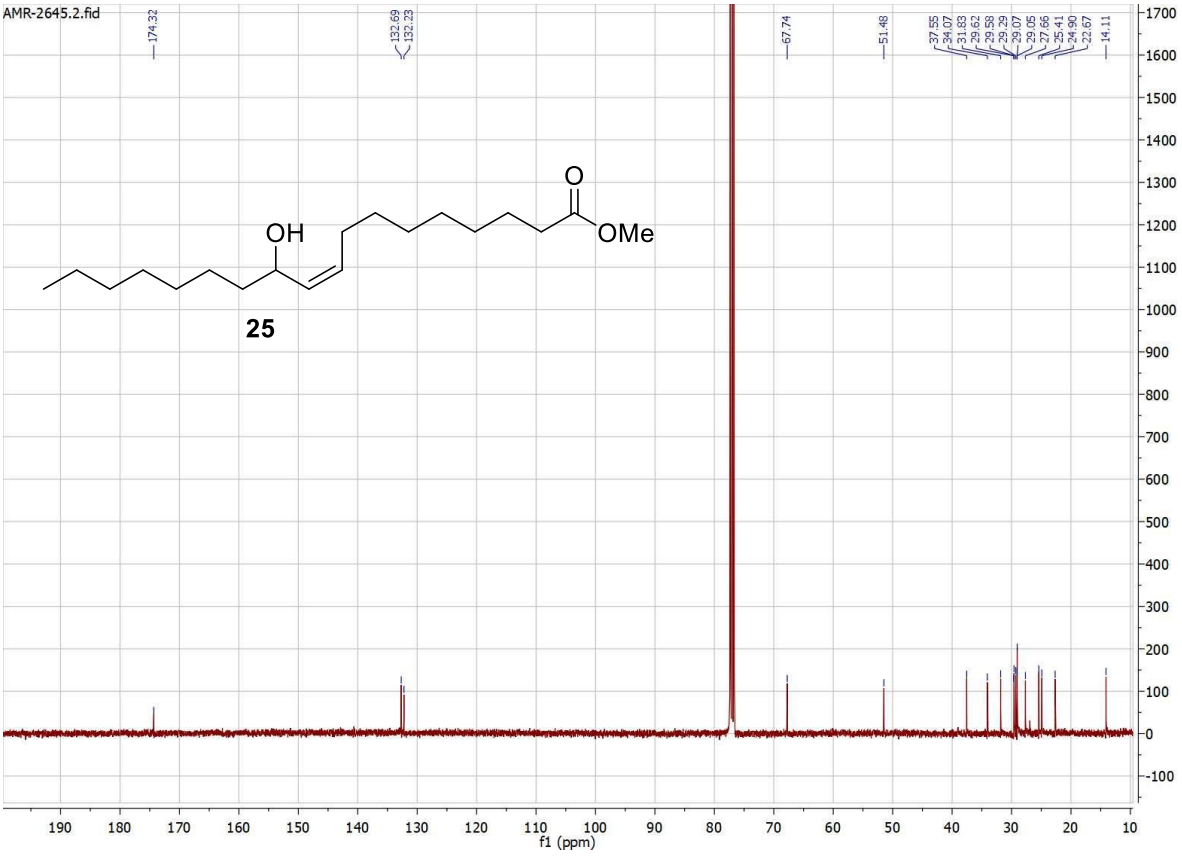

AMR-2625.1.fid
OH
O
OMe
23
f1 (ppm)

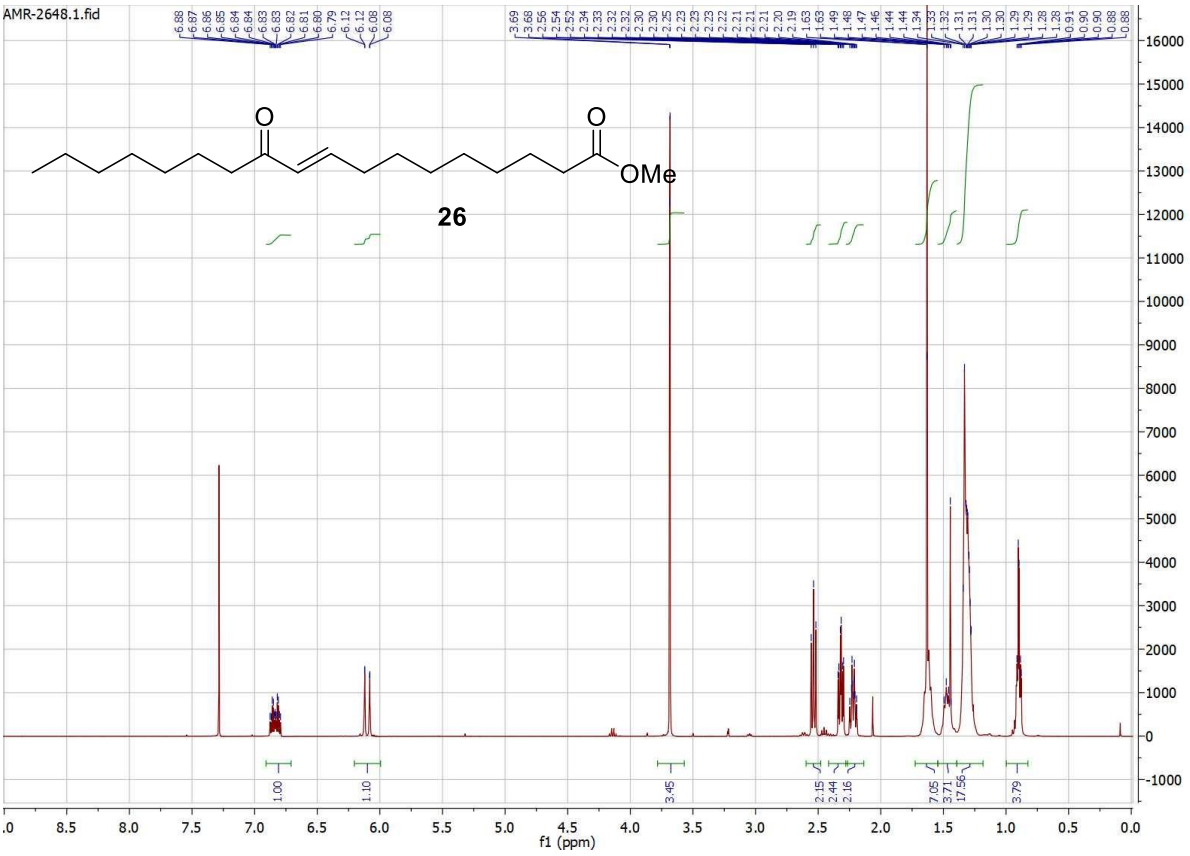

AMR-2625.2.fid
OH
O
OMe
23
f1 (ppm)

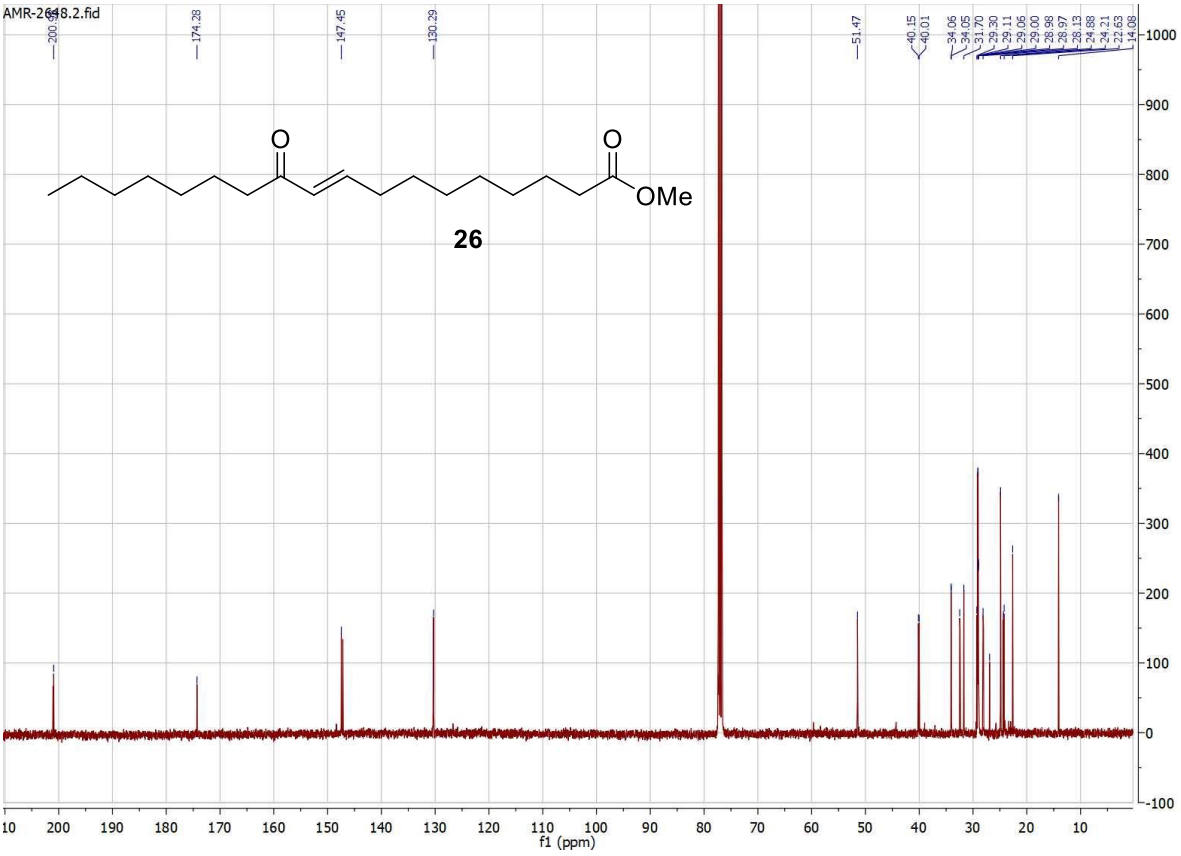

AMR-2643.1.fid
OH
O
OMe
24
1.01
3.00
2.08
2.12
4.11
21.01
3.65
f1 (ppm)

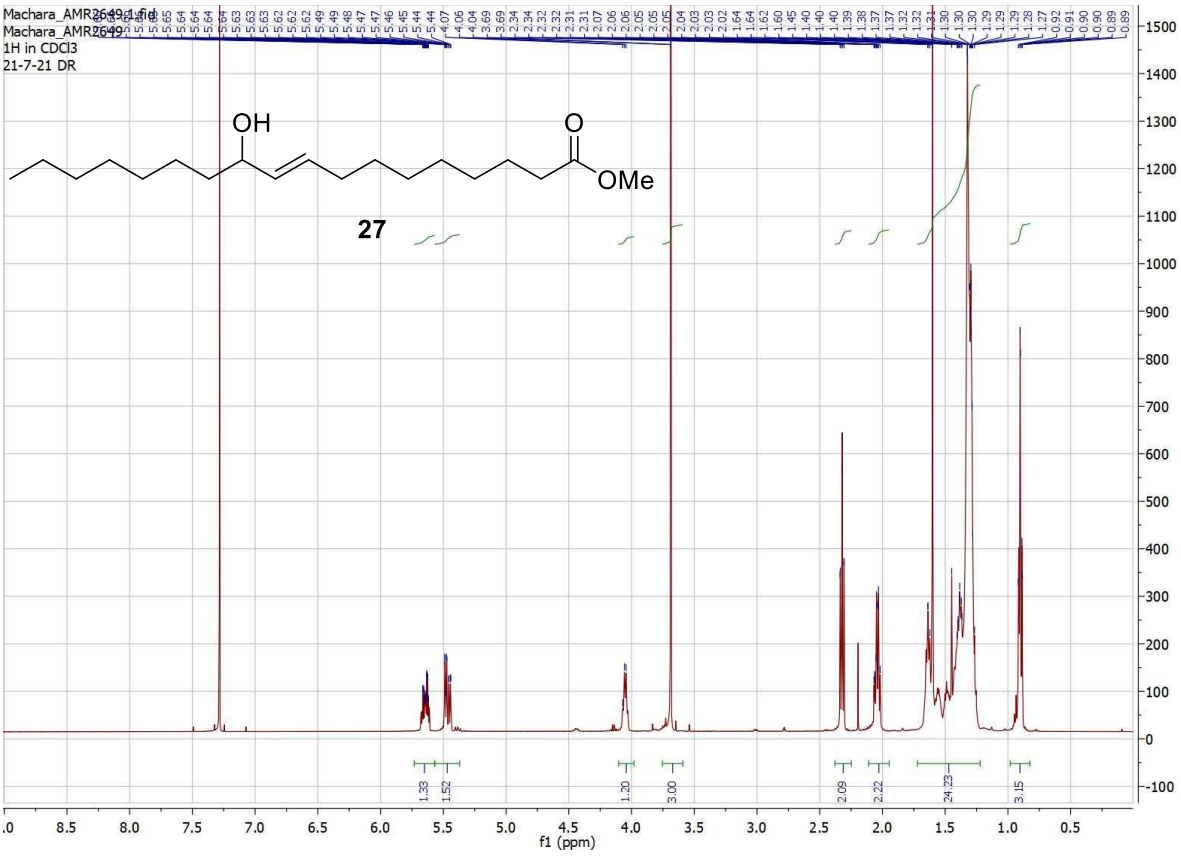

AMR-2643.2.fid
174.31
85.36
81.46
62.78
51.49
38.22
34.05
31.79
29.26
29.24
28.96
28.67
28.54
28.52
25.23
24.85
22.66
18.64
14.11
OH
O
OMe
24
f1 (ppm)

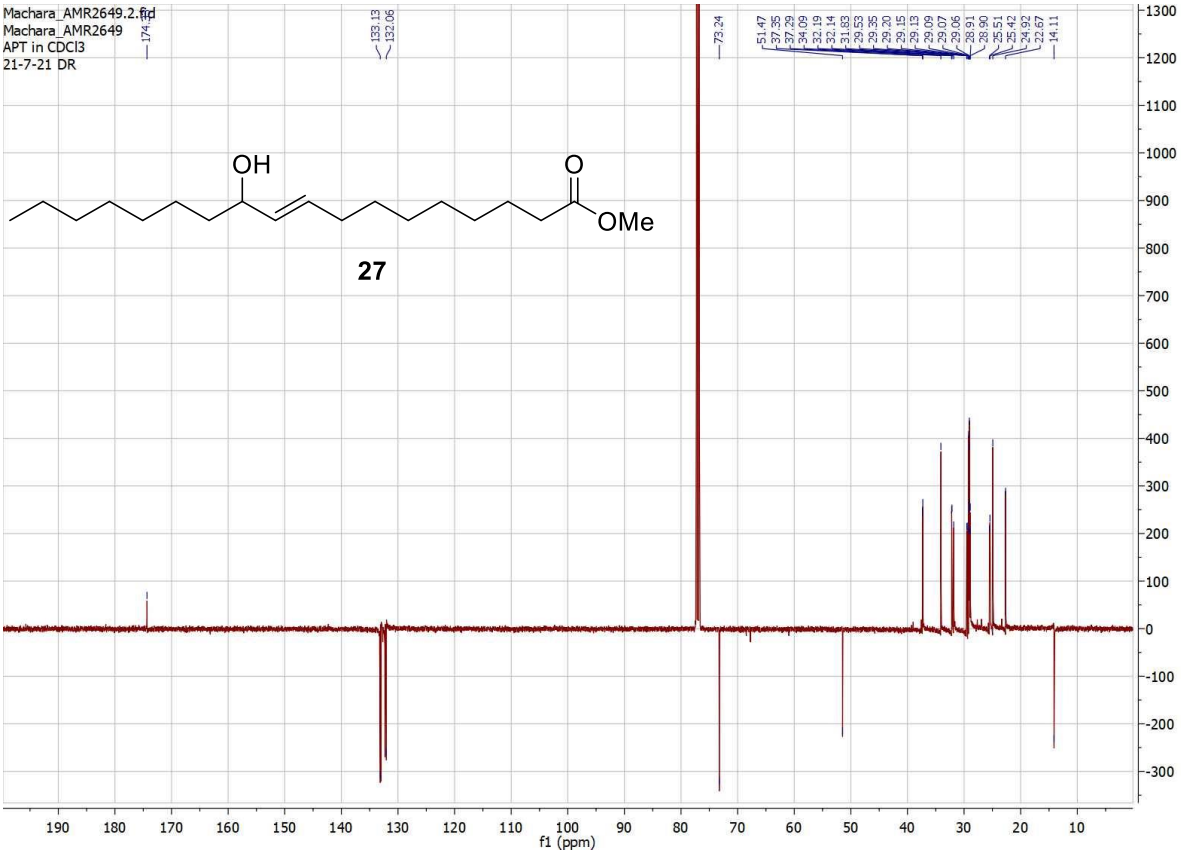

AMR-2645.1.fid
OH
O
OMe
25
f1 (ppm)


AMR-2645.2.fid
174.32
132.69
132.23
67.74
51.48
37.55
34.07
31.83
29.62
29.58
29.29
29.07
29.05
27.66
25.41
24.90
22.67
14.11
OH
O
OMe
25
f1 (ppm)


AMR-2648.1.fid
O
O
OMe
26
f1 (ppm)


AMR-2648.2.fid
O
O
OMe
26
f1 (ppm)


Machara_AMR2649.1.fid
Machara_AMR2649
1H in CDCl3
21-7-21 DR
OH
O
OMe
27
f1 (ppm)


Machara_AMR2649.2.fid
Machara_AMR2649
APT in CDCl3
21-7-21 DR
OH
O
OMe
27
f1 (ppm)