Supplementary Information

nor-MC3 and nor-KC2: Ionizable Lipids for the Delivery of Therapeutic Nucleic Acids

Deaglan Arnold, N. D. Prasad Atmuri, Fariba Saadati, Ardalan Nabi, Daniel Kurek, Anthony Tam, Taniya Adak, Dominik Witzigmann, Glenn Sammis, Pieter R. Cullis, Jayesh Kulkarni,* Marco A. Ciufolini*

Table of Contents

A. Preparation of lipid nanoparticles containing siRNA or mRNA

LNPs were prepared by injecting a volume of lipid mixture (ionizable lipids as described, DSPC, cholesterol, and PEG-DMG) at appropriate ratios dissolved in ethanol to a final lipid concentration of 10 mM with an aqueous phase containing RNA through a T-junction at 3:1 aqueous:ethanol (v/v) ratio. The amine-to-phosphate (N/P) ratio was 3 for siRNA and 6 for mRNA-containing LNPs. The flow rates were set to 15 mL/min for the aqueous phase containing RNA dissolved in 25 mM sodium acetate (pH 4) and 5 mL/min for the ethanolic lipid phase, resulting in a total flow rate of 20 mL/min. The resulting formulations were dialyzed against 1000-fold volumes of phosphate-buffered saline (pH 7.4) over a period of 16 hours at room temperature in Spectra/Por 2 12-14 kD MWCO dialysis tubing (Spectrum Labs) in order to remove residual ethanol.

B. Analysis of LNPs

Particle size of the formulations was measured by dynamic light scattering performed on a Malvern Zetasizer NanoZS (Malvern, UK). Entrapment of nucleic acid was quantified using a Quant-iT RiboGreen RNA reagent assay kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Briefly, 50 µL of the LNPs were diluted with 50 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) containing RiboGreen in the presence or absence of 0.5 *w/v%* Triton X-100. The fluorescence (Ex/Em = 485/520 nm) was measured and the encapsulation was calculated using the formula EE% = {1 - (NA-LNPs/NA-LNPs incubated with Triton X-100)} x 100. Apparent pKa of the LNPs was measured using a 6-(p-Toluidino)-2-Naphthalenesulfonic acid (TNS) assay. Briefly, LNPs were diluted to a lipid concentration of 80 µM. 5 µL of the diluted LNPs were then mixed with 195 µL of TNS assay buffer (10 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid, 10 mM 2-(*N*-morpholino)ethansulfonic acid, 10 mM ammonium acetate, 130 mM NaCl, and 6 µM TNS) at pH ranging from 4.0 – 9.0 in a black 96-well plate. Fluorescence was measured (Ex/Em = 321/445 nm) and plotted against

the respective pH for each well. The apparent LNP pKa value was determined as the pH at half-maximum fluorescence.

C. Cell culture and *in vitro* LNP treatments

Luc-expressing 22Rv1 and Huh7 cell lines were cultured in RPMI media supplemented with 10% fetal bovine serum-FBS (ThermoFisher 12483020). For luciferase-knockdown, 22Rv1 cells and Huh7 cells were seeded at 12,000 cells/well and 10,000 cells/well, respectively, in a 96-well tissue culture plate and were allowed to adhere for 24h prior to treatment. For luciferase-knockdown, 22Rv1 cells were treated with LNPs containing a final concentration of 0.01, 0.03, 0.1, 0.3, 1 or 3 µg/ml luc siRNA for 24 h. For luciferase expression, Huh7 cells were treated with LNPs containing a final concentration of 0.01, 0.03, 0.1, 0.3, 1 or 3 µg/ml luc mRNA for 24 h.

D. Luciferase measurements for *in vitro* studies

After removal of cell culture media by inverting the 96-well plate in waste container, dispense 100 µl of GLO lysis buffer per well using a multichannel pipette via reverse pipetting. Incubate the plate in 37°C incubator for 15 min. Afterwards, scrape the bottom of each well using the tips of a multichannel pipette followed by mixing of lysate. Reverse pipette 50 µl of lysate into a white solid 96-well plate. Reverse pipette 50 µl of GLO luciferase assay reagent in each well using a multichannel pipette and measure luminescence immediately. Place the plate in the NEO Gen5 plate reader and select luminescence with a gain of 100. Select the appropriate filter cubes (upper cube: 3, single PMT, LUM) and (lower cube: 61, FP 485/530, LUM). Normalize the luminescence signal to the weight of the tissue sample loaded in the 50 µl of lysate and data were plotted using Prism.

E. Luciferase measurements for *in vivo* studies

Liver (~100 mg), spleen (whole) tissues were collected in FastPrep tubes (2 mL) each with one ceramic bead (MP Biomedicals 1/4in ceramic sphere) and a red cap, and snap frozen in liquid nitrogen. 0.5 mL and 0.7 mL of GLO lysis buffer was added to the above corresponding tissue tubes, respectively, while frozen. After lysis buffer also been added, transfer all samples on ice and proceed with homogenization using the FastPrep Homogenizer. Set the speed to "6" and homogenize for 20 s. Repeat two more rounds for a total of 3 rounds. Liver but not spleen tissue lysates were diluted 1:4 in GLO lysis buffer prior to dispensing 50 µl into a white solid 96-well plate. 50 µl of GLO luciferase assay reagent were added in each well using a multichannel pipette and luminescence were measured immediately. Place the plate in the NEO Gen5 plate reader and select luminescence with a gain of 100. Select the appropriate filter cubes (upper cube: 3, single PMT, LUM) and (lower cube: 61, FP 485/530, LUM). Luminescence signal was normalized to the weight of the tissue sample loaded in the 50 µl of lysate and data were plotted using Prism.

F. Statistical Methods

In vitro and in vivo study data were tested for normality before the selection of a parametric (normal distribution) or Mann–Whitney (non-normal distribution) t test, one-way ANOVA with Dunnett's multiple comparisons test, where appropriate. All data were analyzed using GraphPad Prism (GraphPad Software Inc.) and were expressed as means ± SD. Statistical significance was considered at $P < 0.05$.

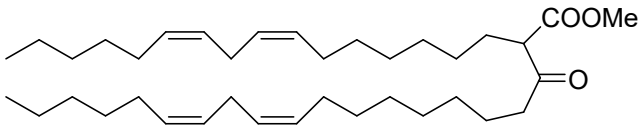
G. Synthesis of *nor*-MC3 and *nor*-KC2

Chemistry Experimental Protocols

Unless otherwise specified, all reagents and solvents were commercial products and were used without further purification. All reactions were performed under an argon atmosphere.

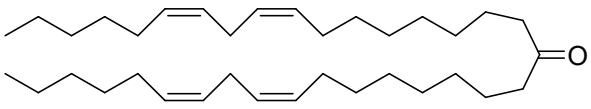
Reaction mixture from aqueous workups were dried by passing over a plug of anhydrous Na_2SO_4 held in a filter tube and rotary-evaporated under reduced pressure. Thin-layer chromatography was performed on silica gel plates coated with silica gel (Merck 60 F254 plates). Visualization of the developed chromatogram was performed by staining with I_2 or potassium permanganate solution. Chromatographic purifications (MPLC) were carried out on a Teledyne ISCO instrument using columns packed with 230−400 mesh silica gel. Nuclear magnetic resonance spectra were recorded at 400 MHz for ^1H / 100 MHz for ^{13}C, at room temperature, in $CDCl_3$ solutions, and referenced to residual $CHCl_3$ both in ^1H (7.26 ppm) and in ^{13}C (central line of the $CDCl_3$ triplet at 77.00 ppm). Chemical shifts are reported in parts per million (ppm) on the δ scale. Multiplicities are reported as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "m" (multiplet), and further qualified as "app" (apparent) and "br" (broad). FT-IR spectra were obtained on PerkinElmer Frontier with attenuated total reflectance (ATR) and reported as wavenumbers (cm^{-1}). Low- and high-resolution mass spectra (m/z) were obtained in the electrospray (ESI) or field desorption/field ionisation (FD/FI) mode.

Methyl (11Z,14Z)-2-((7Z,10Z)-hexadeca-7,10-dien-1-yl)-3-oxoicosa-11,14-dienoate (6). A solution of $TiCl_4$ (29.0 g, 16.8 mL, 153.0 mmol) in toluene (30.0 mL) was added dropwise to a cold (0 °C, ice bath), stirred solution of methyl linoleate (30.0 g, 102.0 mmol) and tributylamine (Bu_3N) (34.5 g, 44.4 mL, 183.0 mmol) in toluene (170.0 mL). After stirring at 0°C for 1.5 h, the reaction was complete as determined by TLC and ^1H NMR. The reaction solution was diluted with hexanes (200 mL) and water (200 mL) was cautiously added. Addition of water caused evolution of heat, so the temperature of the mixture was controlled by thorough stirring and cooling in an ice bath. The organic phase was separated, and the aqueous phase was extracted with hexanes (3×100



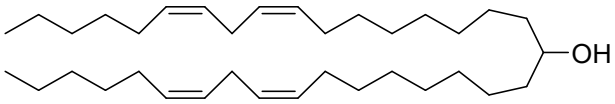
mL). The combined organic extracts were washed with water, dried (Na_2SO_4) and concentrated under vacuum. ^{1}HNMR analysis of the residual crude product (brown oil) indicated that the product existed as a mixture of keto (major) and enol derivatives, typically in a 2:1 ratio. Crude **6** may be purified by MPLC (1% EtOAc in hexanes). This produces some fractions highly enriched in the enol form, while the bulk of material is obtained as the keto tautomer (92% combined yield). However, it is expedient to advance crude **6** directly to the next step. **^{1}H NMR [keto form] (**400 MHz, $CDCl_3$**)** δ 5.35 (m, 8H), 3.71 (s, 3H), 3.43 (t, *J* 7.4 Hz, 1 H), 2.77 (t, *J* 6.3 Hz, 4H), 2.49 (m, 2H), 2.04 (m, 8H), 1.83 (m, 4H), 1.30 (m, 28H), 0.89 (t, *J* 6.8 Hz, 6H). **^{13}C NMR [keto form]** (100 MHz, $CDCl_3$) δ 205.5, 170.5, 130.25, 130.24, 130.04, 129.97, 128.12, 128.07, 127.91, 127.89, 59.0, 52.3, 41.9, 31.5, 29.6, 29.5, 29.4, 29.30, 29.28, 29.1, 29.0, 28.99, 28.3, 27.5, 27.21, 27.16, 25.6, 23.5, 22.6, 14.1 (some peaks are doubled). **IR** (neat) 3010, 2924, 2855, 1745, 1717, 1459, 1435, 1240, 1197, 1168, 722 cm^{-1}. **LRMS** m/z 557 $[M+H]^+$, 579 $[M+Na]^+$. **HRMS** Calcd for $C_{37}H_{65}O_3^+$ $[M+H]^+$ 557.4934, found 557.4957.

(6Z,9Z,26Z,29Z)-Pentatriaconta-6,9,26,29-tetraen-18-one (7). Aqueous NaOH (10% w/vol, 72.0 mL) was added to a solution of the crude **6** (50.0 g, 90.0 mmol) in 95% ethanol (150.0 mL). The mixture was stirred at room temperature for 4 h. During this time, the reaction was periodically monitored for completion by adding 3-4 drops of the reaction mixture to 3.0 N aqueous HCl solution (0.5 mL), extracting the mixture with hexanes, evaporating the combined extracts to dryness, and checking the residue by ^{1}H NMR. The disappearance of the OCH_3 signal indicated that the reaction was complete. The reaction mixture was concentrated on a rotary evaporator to remove ethanol. The aqueous residue was cooled in an ice bath, diluted with hexanes (200 mL), and vigorously stirred during careful dropwise addition of conc. aqueous HCl solution.



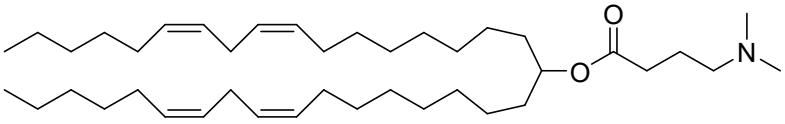
When the mixture attained pH ~ 2, the phases were separated, and the aqueous layer was extracted with more hexanes (3 x 100 mL). The combined organic extracts were washed with DI water and dried (Na_2SO_4). A small volume of solution was concentrated on the rotary evaporator at a bath temperature of 25 °C and an NMR spectrum of the crude product was recorded to ascertain the presence of the desired ketoacid. The bulk of the solution was concentrated on the rotary evaporator at a bath temperature of 60 °C to induce decarboxylation. After approximately 1 h, analysis of the residue by ^{1}H NMR revealed it to be nearly pure ketone. If desired, the brown-colored crude ketone may be purified by MPLC (gradient 1 → 3% v/v ether in hexanes; clear, pale yellowish oil). However, the crude ketone (40.4 g, 90% from methyl linoleate, **5**) is most advantageously utilized directly for the next steps. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.35 (m, 8H), 2.77 (t, *J* 6.4 Hz, 4H), 2.38 (t, *J* 7.4 Hz, 4H), 2.04 (m, 8H), 1.54 (m, 4H), 1.32 (m, 28H), 0.89 (t, *J* 7.0 Hz, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) 211.8, 130.4, 130.2, 128.2, 128.1, 43.0, 31.7, 29.8, 29.5, 29.48, 29.4 29.3, 27.4 (2 overlapping peaks), 25.8, 24.0, 22.7, 14.2. **IR** (neat) 3010, 2924, 2854, 1716, 1464, 1400, 1376, 1047, 913, 721 cm^{-1}. **LRMS** m/z 499 $[M+H]^+$, 521 $[M+Na]^+$. **HRMS** (FD) Calcd for $C_{35}H_{62}O^+$ $[M]^{\cdot+}$ 498.4801, found 498.4811.

(6Z,9Z,26Z,29Z)-Pentatriaconta-6,9,26,29-tetraen-18-ol (8). Solid $NaBH_4$ (0.76 g, 20.0 mmol) was added in portions to a cold (0 °C, ice bath), stirred solution of ketone **7** (10.0 g, 20.0 mmol) in 95% ethanol (50.0 mL). After stirring at room temperature for 2 h, the reaction was checked for completion, either by TLC (5% ether in hexanes) or, more reliably, by adding 3-4 drops of the reaction mixture to saturated aqueous NH_4Cl solution (0.5 mL), extracting with hexanes, evaporating the combined extracts to dryness, and checking the residue by ^{1}H NMR. Either method



indicated that the reaction was complete. The reaction was quenched by careful addition of aqueous saturated NH_4Cl solution (**CAUTION**: H_2 evolution and foaming) and concentrated on the rotary evaporator to remove the ethanol. The aqueous residue was extracted with hexanes (3 x 50.0 mL). The combined extracts dried (Na_2SO_4) and concentrated to afford crude alcohol, which was purified by MPLC with 5 → -10% v/v ethyl acetate in hexanes (8.7 g, 87% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.36 (m, 8H), 3.57 (p, *J* 4.5 Hz, 1H), 2.77 (t, *J* 6.5 Hz, 4H), 2.05 (m, 8H), 1.60 (s, 1H), 1.34 (m, 36H), 0.89 (t, *J* 7.0 Hz, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 130.2, 130.1, 128.0, 127.9, 72.0, 37.5, 31.6, 29.71, 29.68, 29.5, 29.4, 29.3, 27.24, 24.22, 25.7, 25.6, 22.6, 14.1. **IR** (neat) 3352, 3010, 2924, 2854, 1464, 1398, 1378, 1378, 1106, 1024, 911, 722 cm^{-1}. **LRMS** m/z 501 $[M+H]^+$, 523 $[M+Na]^+$ **HRMS** (FD) Calcd for $C_{35}H_{64}O^+$ $[M]^{\cdot+}$ 500.4957, found 500.4968.

Pentatriaconta-6,9,26,29-tetraen-18-yl 4-(dimethylamino)butanoate (*nor*-MC3, 1). A



solution of alcohol **8** (8.0 g, 16.0 mmol), 4-dimethylaminobutyric acid hydrochloride (4.35 g, 24.0 mmol), and DMAP (1.95g, 16.0 mmol) in dry CH_2Cl_2 (25.0 mL) was stirred at room temperature for 5 minutes prior to the addition of EDCI (4.59 g, 24.0 mmol). The mixture was stirred overnight at room temperature, under argon, whereupon TLC (5% MeOH in CH_2Cl_2) and ^{1}H NMR indicated that the reaction had completed. The solution was diluted with more CH_2Cl_2 (10 mL) and sequentially washed with aqueous saturated $NaHCO_3$ (3 x 25.0 mL) and water (25.0 mL). The organic phase was passed over a plug of anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography with 3% v/v MeOH in CH_2Cl_2 containing to afford (6.40 g, 65% yield) of pure **1** as a clear, colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.35 (m, 8H), 4.86 (p, *J* 6.3 Hz, 1H), 2.77 (t, *J*

6.5 Hz, 4H), 2.30 (dt, *J* 15.1, 7.5 Hz, 4H), 2.22 (s, 6H), 2.04 (m, 8H), 1.79 (m, 2H), 1.50 (m, 4H), 1.41 (m, 32H), 0.89 (t, *J* 6.8 Hz, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.4, 130.2, 130.1, 128.0, 127.9, 74.2, 59.0, 45.5, 34.2, 32.5, 31.5, 29.7, 29.6, 29.5, 29.4, 29.3, 27.23, 27.21, 25.6, 25.4, 23.2, 22.6, 14.1. **IR** (neat) 3010, 2924, 2855, 2766, 1733, 1460, 1377, 1256, 1190, 1137, 1042, 1100, 963, 912, 842, 722 cm^{-1}. **LRMS** m/z 614 $[M+H]^+$. **HRMS** (FD) Calcd for $C_{41}H_{75}NO_2^+$ $[M]^{\cdot+}$ 613.5798, found 613.5802. The purity of the chromatographed lipid was estimated to be greater that 98% by LC-MS (**B%** 0→0.5 min [50%→53%], 0.5→4 min [53%→55%], 4 →7 min [55%→65%], 7→7.5 min [65%→80%], 7.5 →17 min [80%→99%], 17→19 min [99%→55%], column: Agilent Peptide 2.7μm, 4.6 × 150 mm). Eluent **A**: (600 mL MeCN, 390 mL H_2O, 10 mL 1M NH_4HCO_2, 1 mL HCO_2H); Eluent **B**: (900 ^{i}PrOH, 90 mL MeCN, 10 mL 1M NH_4HCO_2, 1 mL HCO_2H).

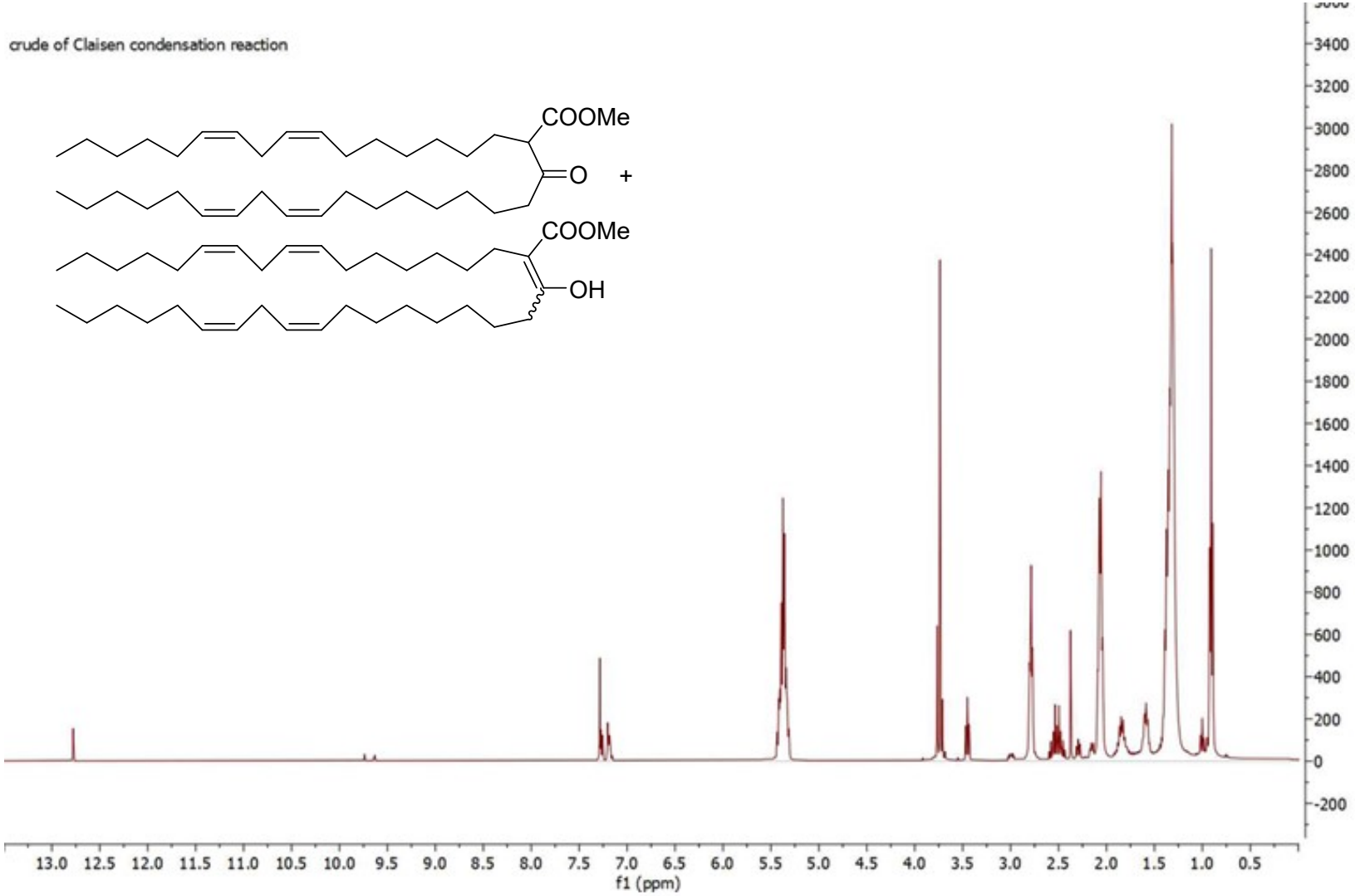
4-(2-chloroethyl)-2,2-di((8Z,11Z)-heptadeca-8,11-dien-1-yl)-1,3-dioxolane (10). A solution of ketone **7** (540 mg, 1.0 mmol), 4-chlorobutane-1,2-diol[11] (405 mg, 3.25 mmol) and pyridinium *p*-toluenesulfonate (27.0 mg, 0.11 mmol) in 35.0 mL of toluene was refluxed under nitrogen for 12 h with continuous removal of water (Dean-Stark trap), whereupon the reaction was complete (checked by NMR), the mixture was cooled to room temperature, treated with solid $NaHCO_3$ (100 mg), and evaporated to dryness *in vacuo*. The residue was dissolved in hexanes (100 mL) and the resulting solution was sequentially washed with aqueous saturated $NaHCO_3$ solution, water, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by MPLC with EtOAc-hexanes (1:99) to afford **10** (580 mg, 89%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.36 (m, 8H), 4.24 (m, 1H), 4.09 (dd, *J* 7.9, 6.1 Hz, 1H), 3.65 (m, 2H), 3.52 (dd, *J* 7.7, 7.7 Hz, 1H), 2.77 (t, *J* 6.8

Hz, 4H), 1.99 (m, 10H), 1.58 (m, 4H), 1.33 (m, 32 H), 0.89 (t, 6H, *J* 6.7 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ130.2, 130.1, 128. 0, 127.9, 112.5, 73.3, 69.5, 41.5, 37.8, 37.3, 36.7, 31.5, 29.92, 29.89, 29.7, 29.5, 29.4, 29.29, 29.28, 27.24, 27.21, 25.7, 24.0, 23.7, 22.6, 14.1. **FTIR** 3009, 2924, 2855, 1651, 1464, 1397, 1377, 1349, 1291, 1081, 947, 913, 722, 663 cm^{-1}. **LRMS** (ES-API) 605.5 (^{35}Cl, $[M+H]^+$) & 607.5 (^{37}Cl, $[M+H]^+$). **HRMS** (ESI) calcd for $C_{39}H_{69}{}^{35}ClO_2$ $[M+H]^+$ 605.5059, found:605.5053.

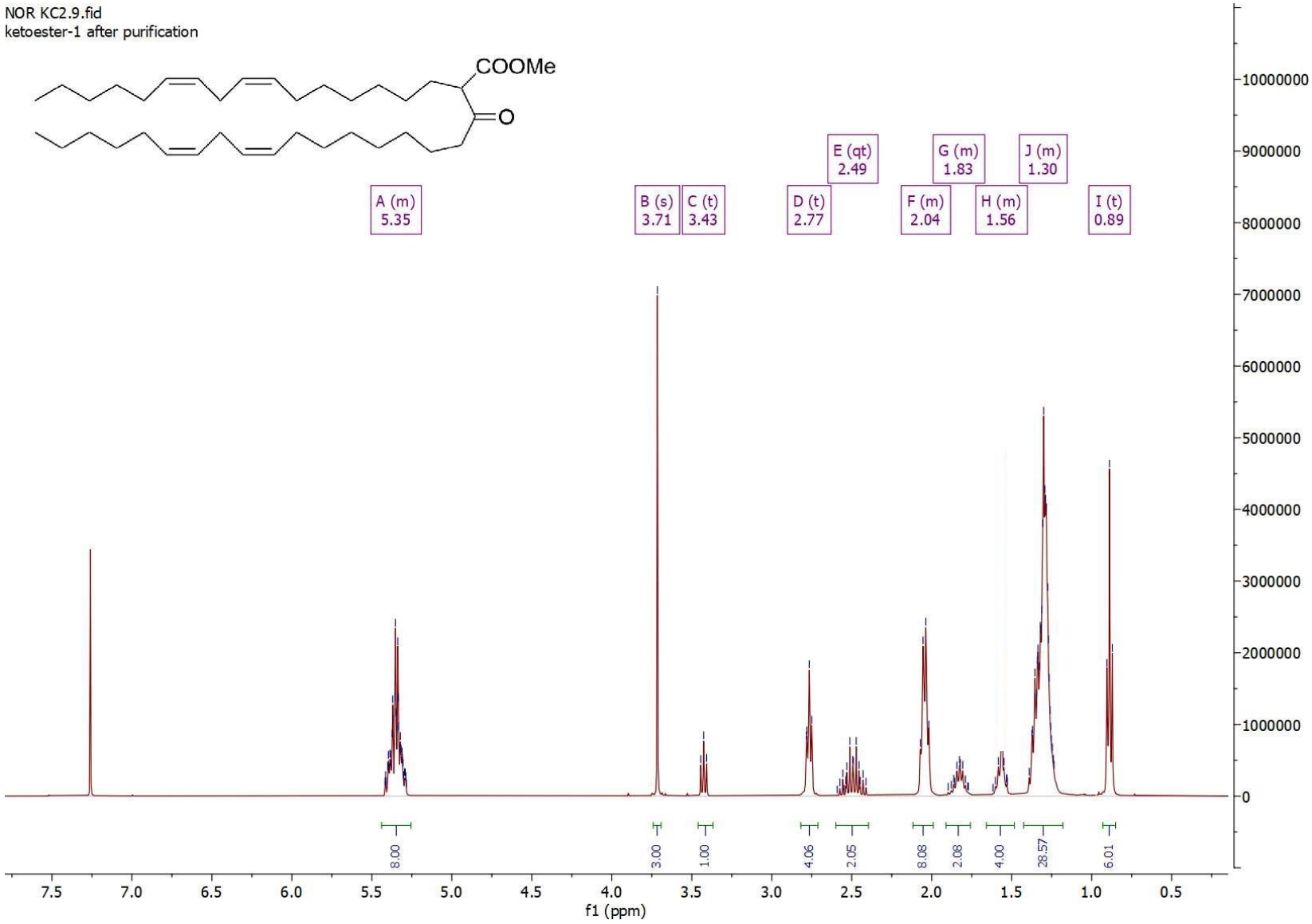
2-(2,2-Di((8Z,11Z)-heptadeca-8,11-dien-1-yl)-1,3-dioxolan-4-yl)-N,N-dimethylethan-1-amine (*nor*-KC2, 2). To a cold (0 °C) solution of chloroketal **10** (440 mg, 0.73 mmol) in dry THF (2.0 mL) in a heavy walled sealable glass tube (Teflon screwcap) was added dimethylamine (2.0 M in THF, 1.1 mL, 2.2 mmol). The resulting mixture was degassed (N_2), the tube was sealed, and the solution was heated at 110 °C. After completion of the reaction (ca. 48 h), the volatiles were removed *in vacuo*. The residue was dissolved in diethyl ether (75 mL), sequentially washed with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by MPLC by eluting with 5 → 10% v/v ethyl acetate in methylene chloride to afford **2** (405 mg, 91%) as a colorless oil. **NMR** (400 MHz, $CDCl_3$) δ 5.35 (m, 8H), 4.07 (m, 2H), 3.48 (td, *J* 7.3, 1.7 Hz, 1H), 2.77 (t, *J* 6.5 Hz, 4H), 2.41 (ddd, *J* 12.0, 9.6, 5.6 Hz, 1H), 2.30 (ddd, *J* 12.0, 9.4, 5.8 Hz, 1H), 2.23 (s, 6H), 2.04 (m, 8H), 1.81 (m, 1H), 1.57 (m, 5H), 1.38 (m, 32H), 0.89 (t, *J* = 6.9 Hz, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 130.21, 130.15, 127.98, 127.95, 112.1, 74.7, 70.0, 56.3, 45.5, 37.8, 37.5, 31.8, 31.5, 27.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.31, 29.29, 27.3, 27.2, 25.6, 24.0, 23.7, 22.6, 14.1. **IR** (neat) 3010, 2924, 2854, 2817, 2765, 1650, 1461, 1397, 1377, 1308, 1268, 1156, 1086, 1042, 944, 912, 846, 722 cm^{-1}. **LRMS**: m/z 614 $[M+H]^+$; **HRMS** (FD) Calcd for $C_{41}H_{75}NO_2^+$ 613.5798, found 613.5802. The purity of the chromatographed lipid was estimated to be greater that 97% by LC-MS (**B%** 0⟶0.5 min

[50%→99%], 0.5→4 min [99%], 4.0 →4.5 min [99%→50%], column: Agilent PheHex 2.7μm, 4.6 × 50 mm). Eluent **A**: (600 mL MeCN, 390 mL H_2O, 10 mL 1M NH_4HCO_2, 1 mL HCO_2H); Eluent **B**: (900 ^{i}PrOH, 90 mL MeCN, 10 mL 1M NH_4HCO_2, 1 mL HCO_2H).

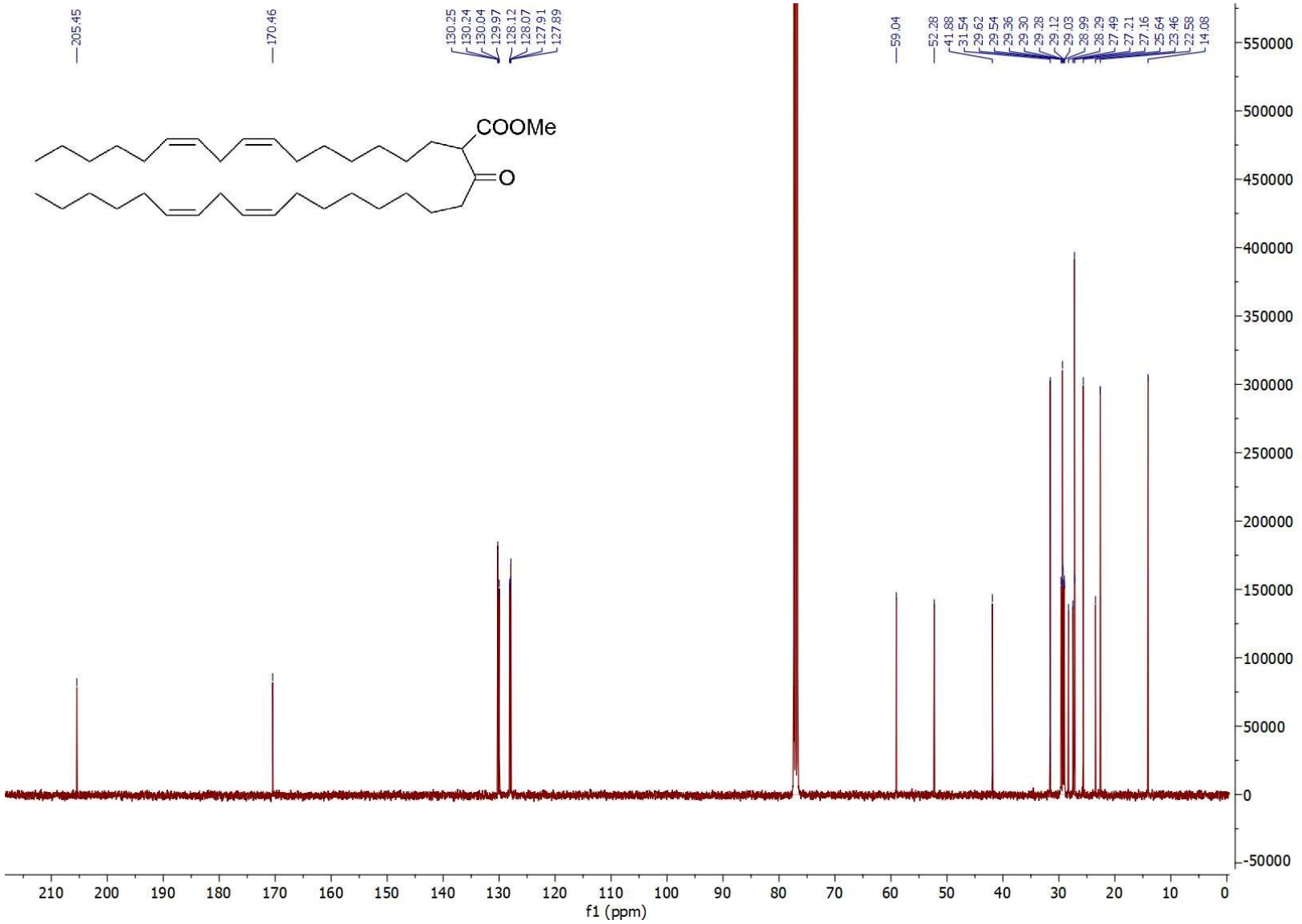
H. Proton and ^{13}C NMR Spectra



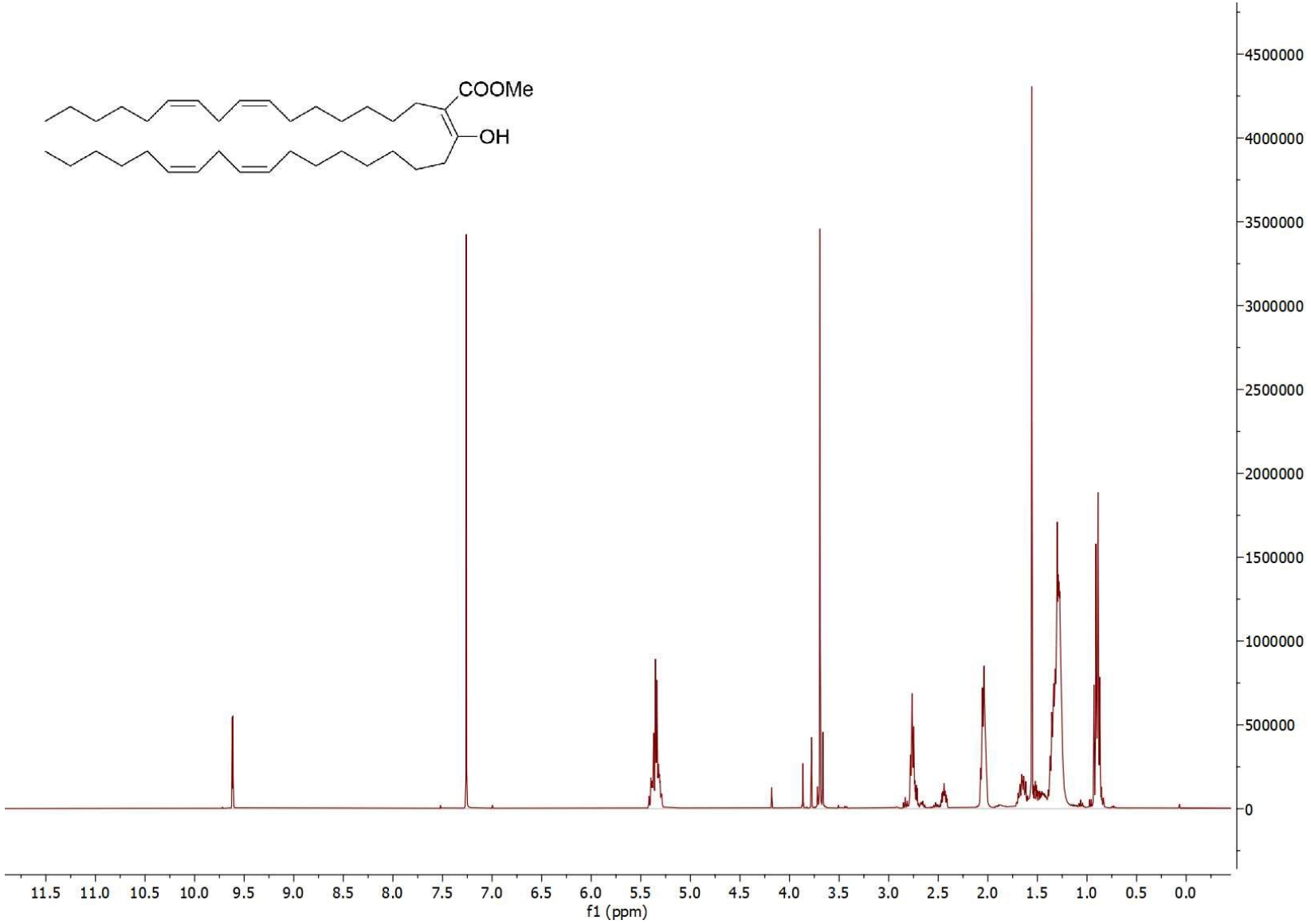
400 MHz ^{1}H NMR spectrum of crude 6 [mixture of keto- (major) and enol tautomers] in $CDCl_3$



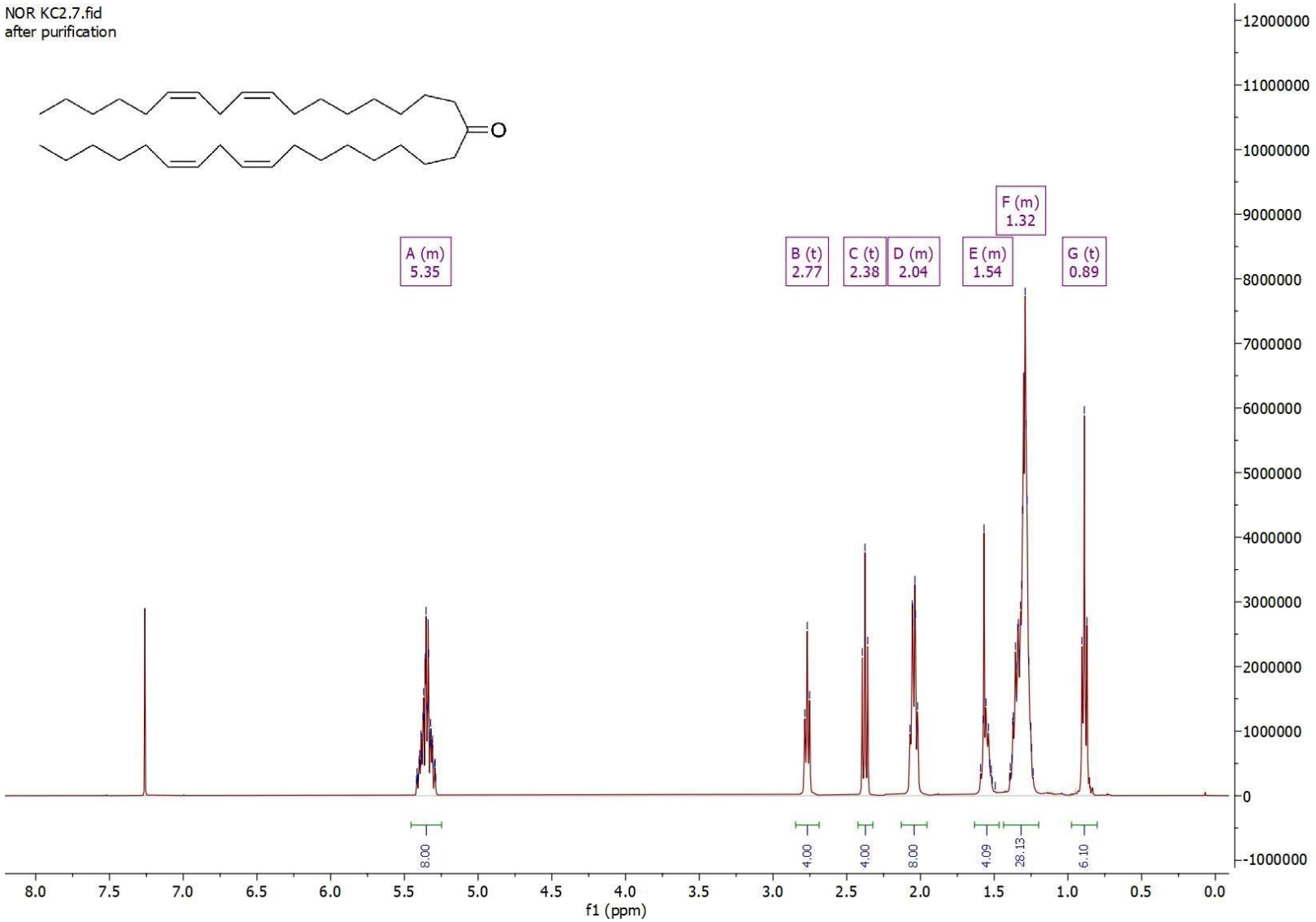
400 MHz ^{1}H NMR spectrum of the purified keto tautomer of 6 in $CDCl_3$



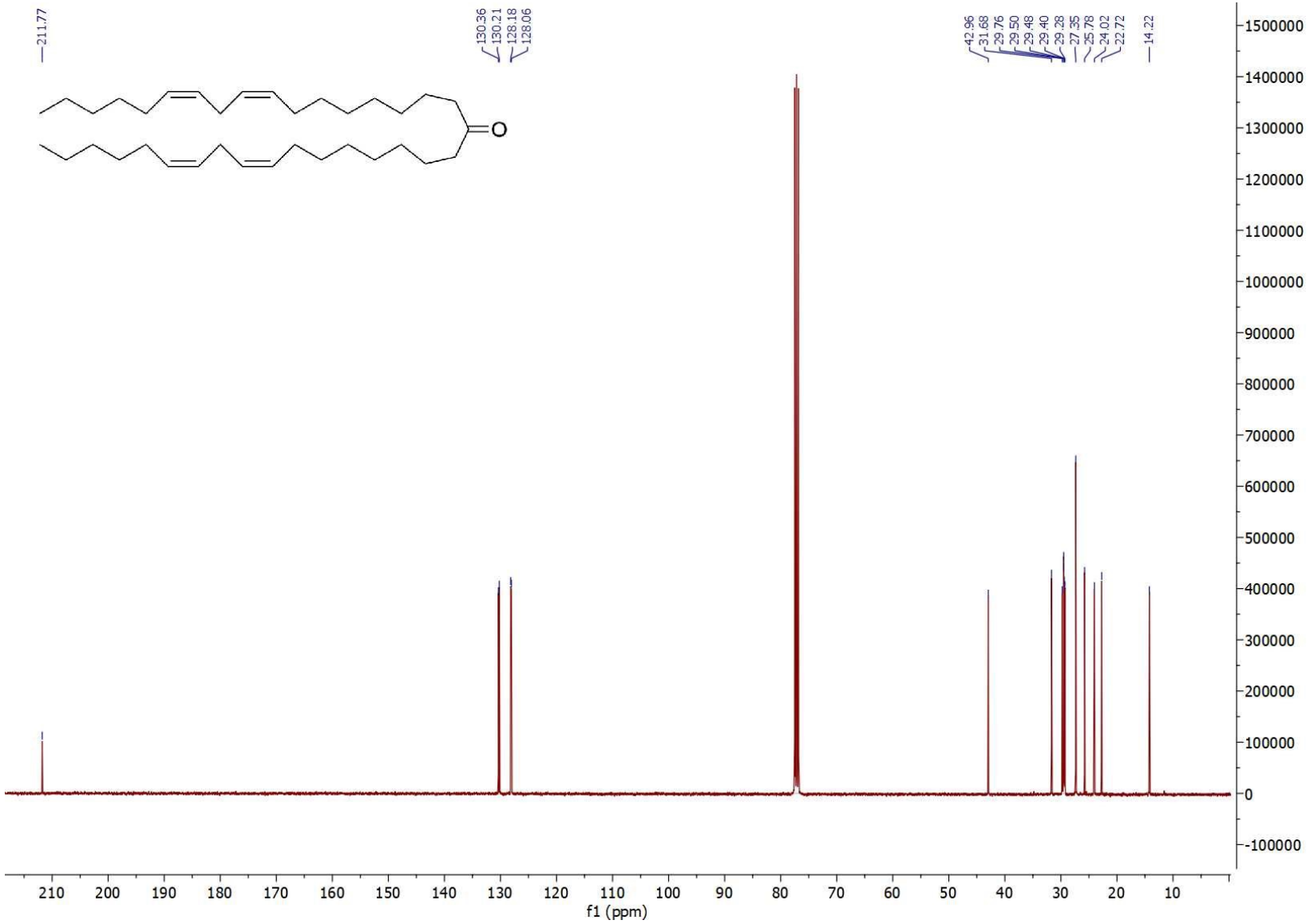
100 MHz ^{13}C {H} NMR spectrum of the purified keto tautomer of 6 in $CDCl_3$



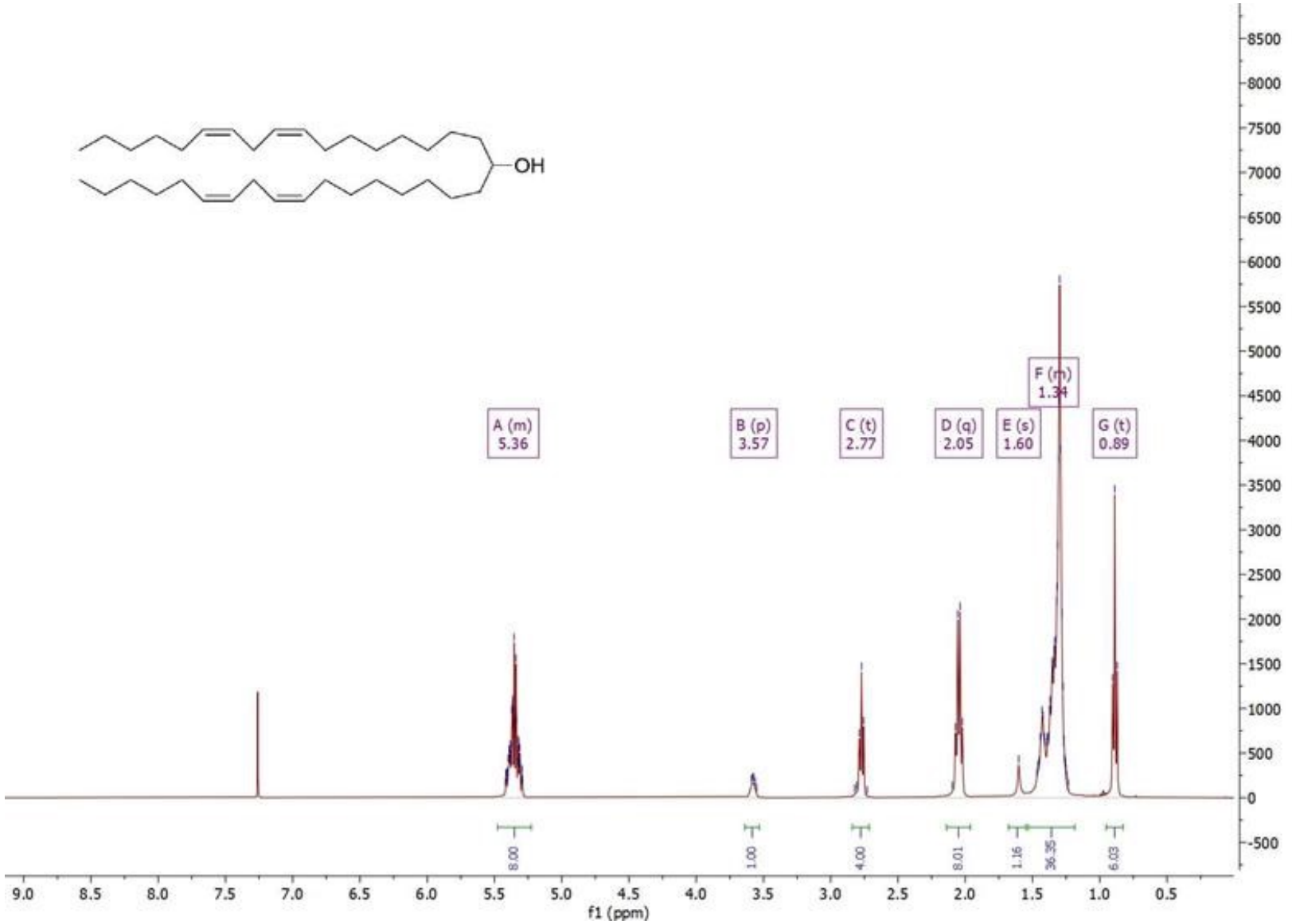
400 MHz ^{1}H NMR spectrum of mostly enol tautomer of 6 in $CDCl_3$



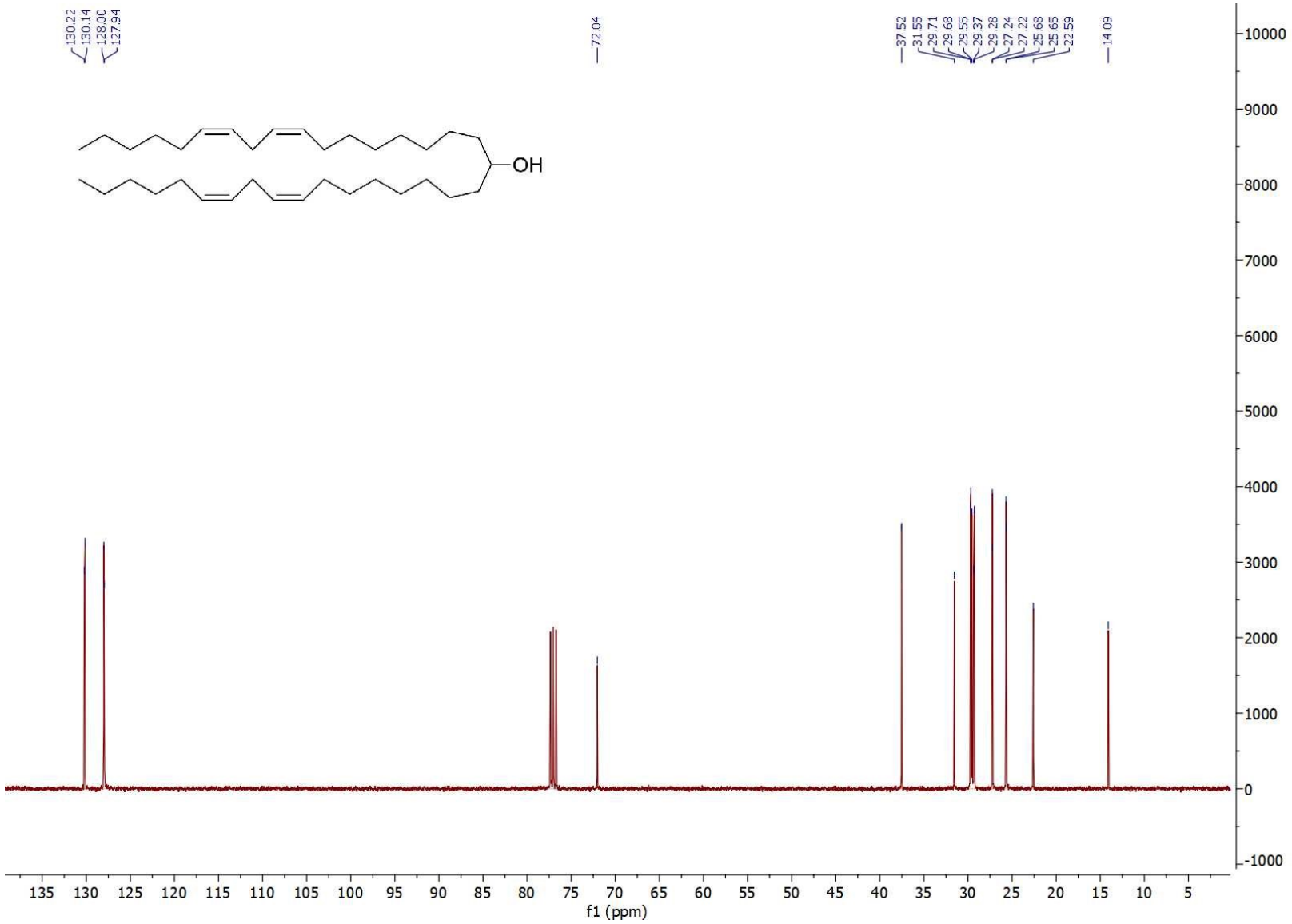
400 MHz ^{1}H NMR spectrum of ketone 7 in $CDCl_3$



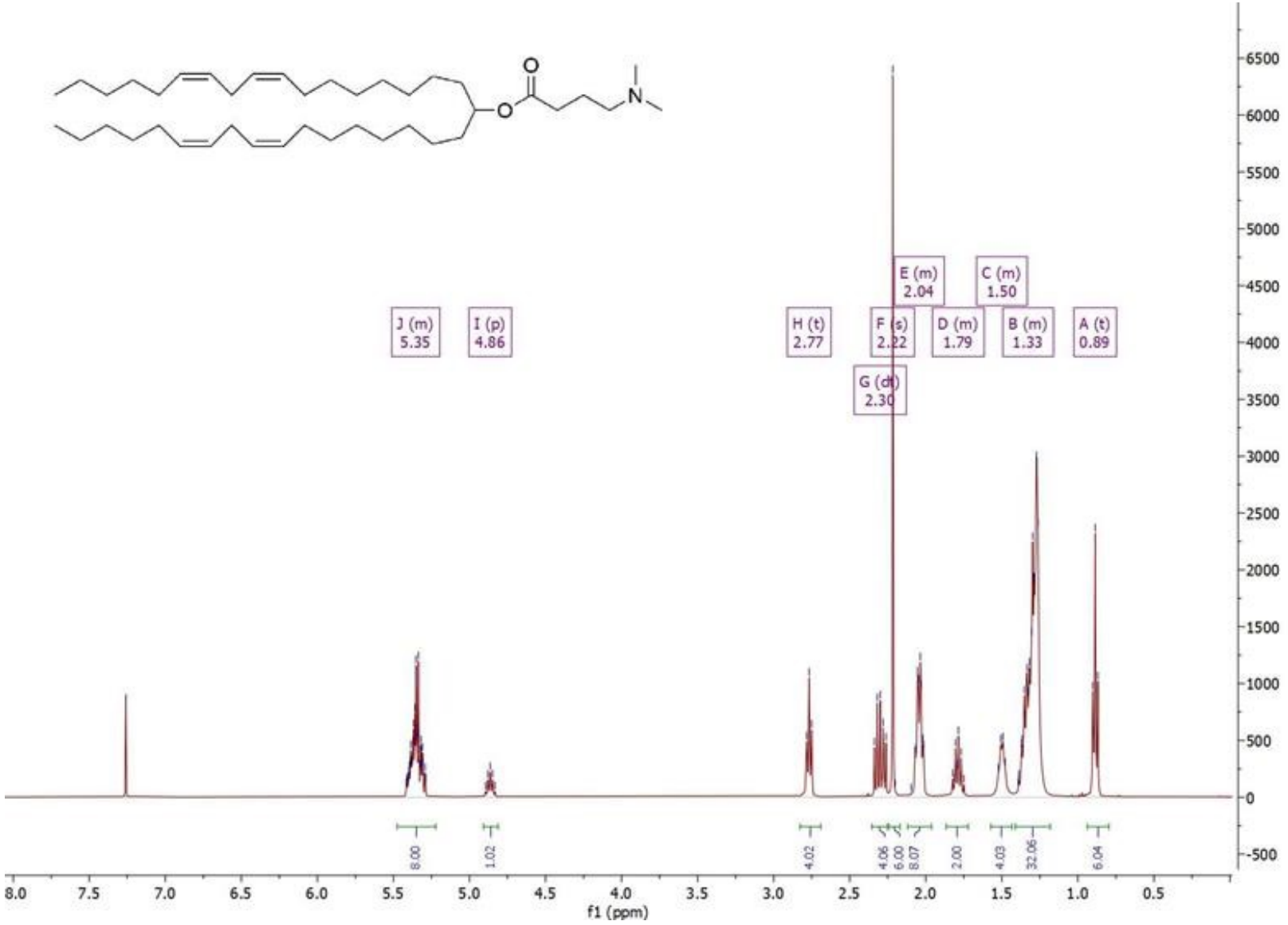
100 MHz ^{13}C {H} NMR spectrum of ketone 7 in $CDCl_3$



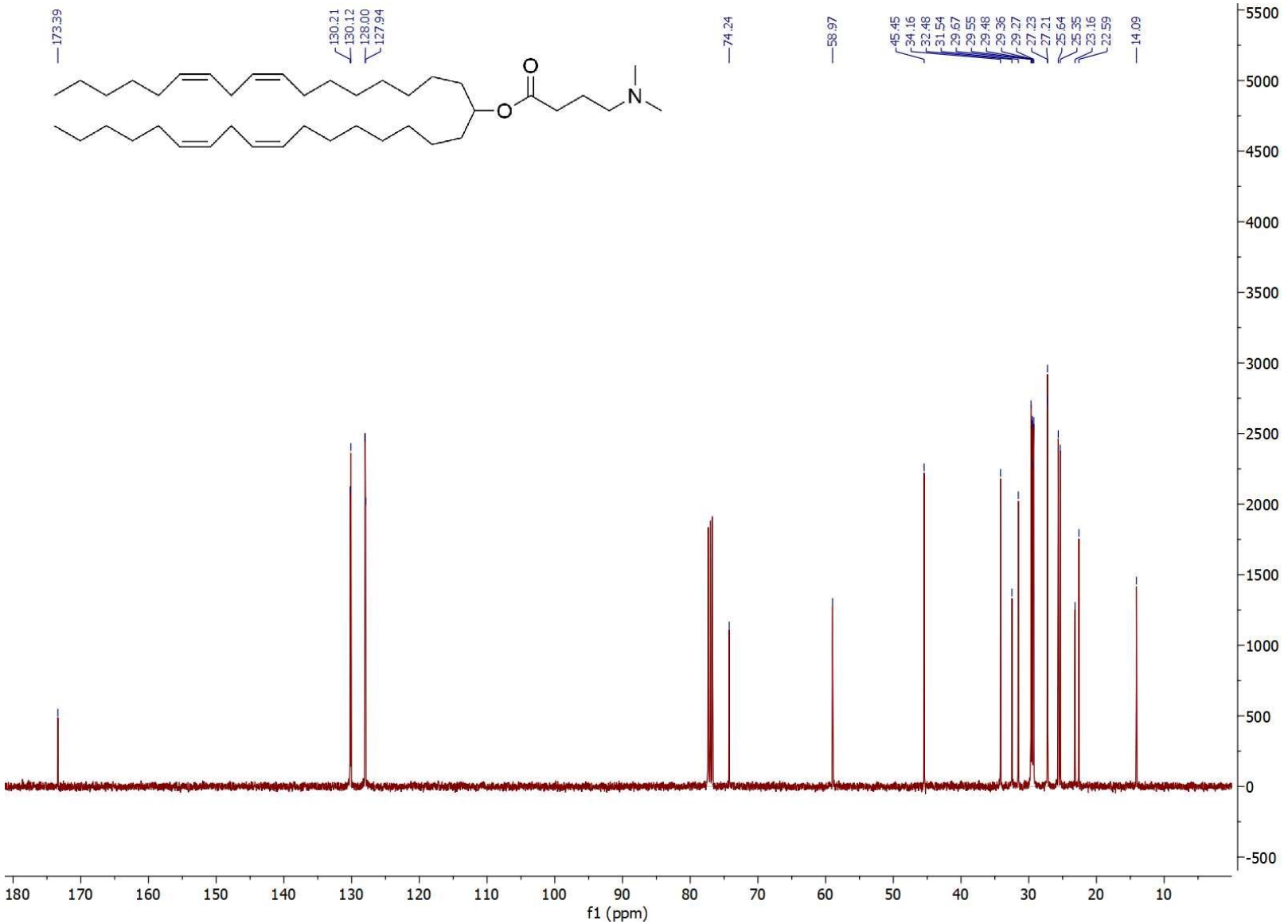
400 MHz ^{1}H NMR spectrum of alcohol 8 in $CDCl_3$



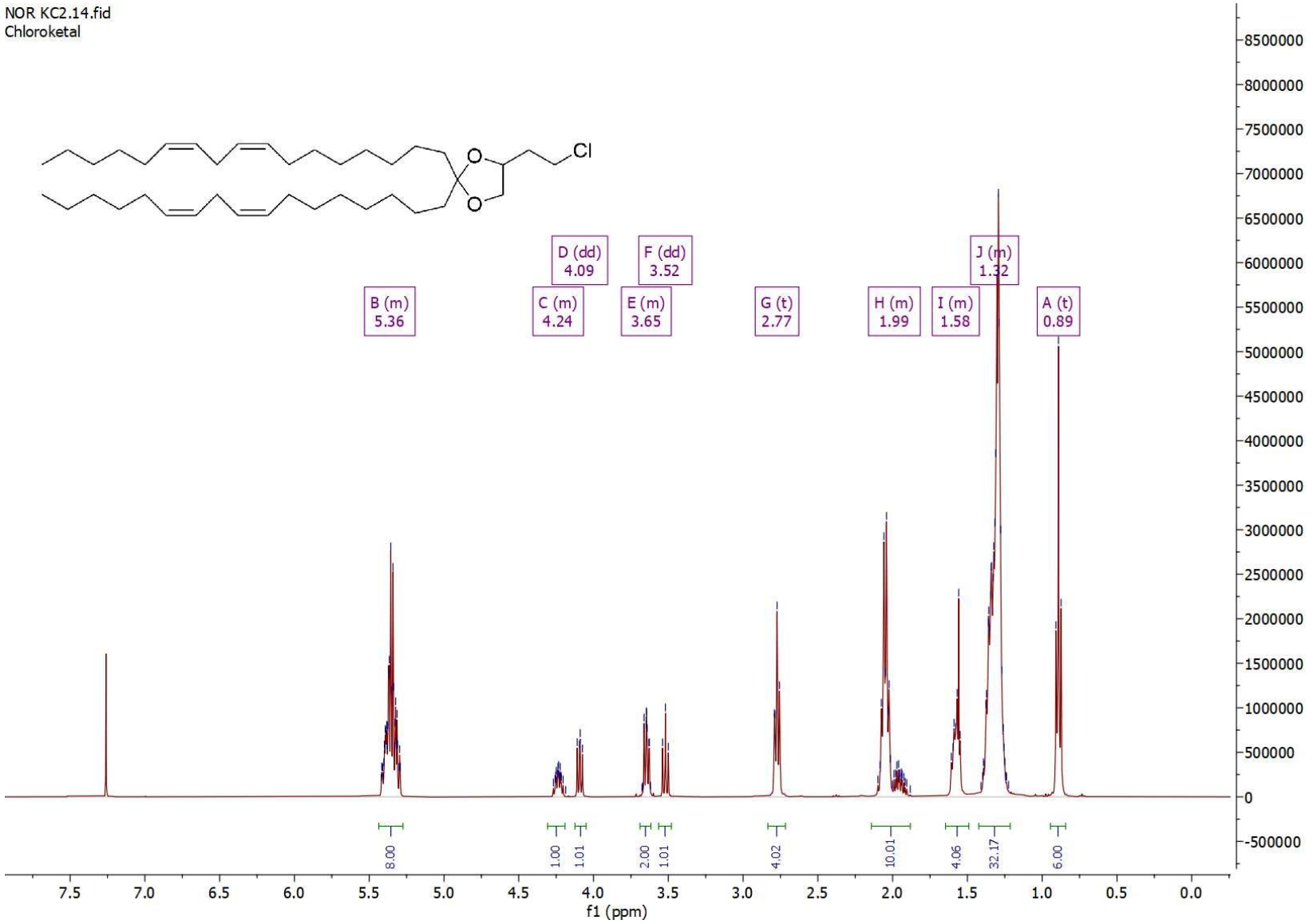
100 MHz ^{13}C {H} NMR spectrum of alcohol 8 in $CDCl_3$



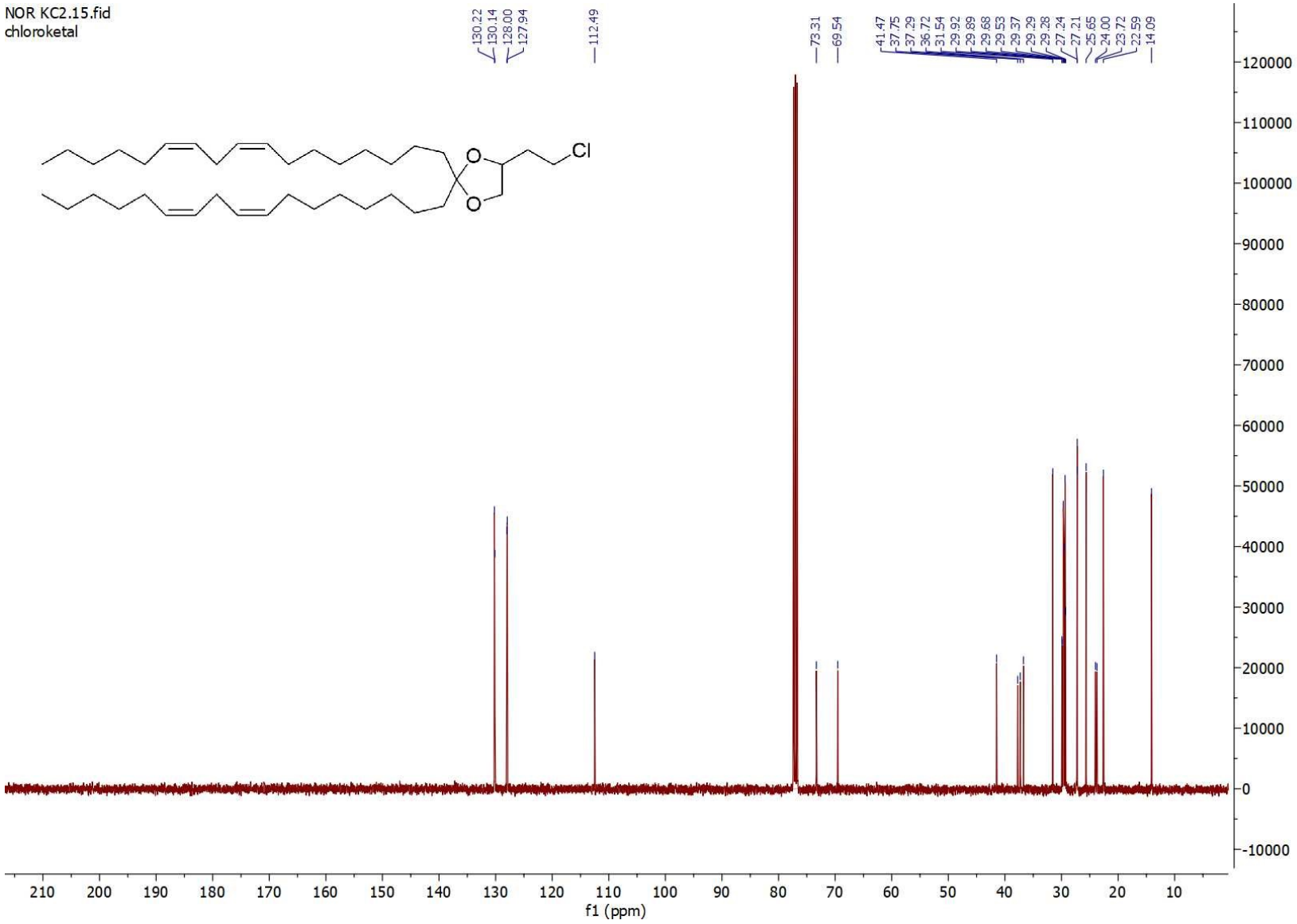
300 MHz ^{1}H NMR spectrum of *nor*-MC3, 1, in $CDCl_3$



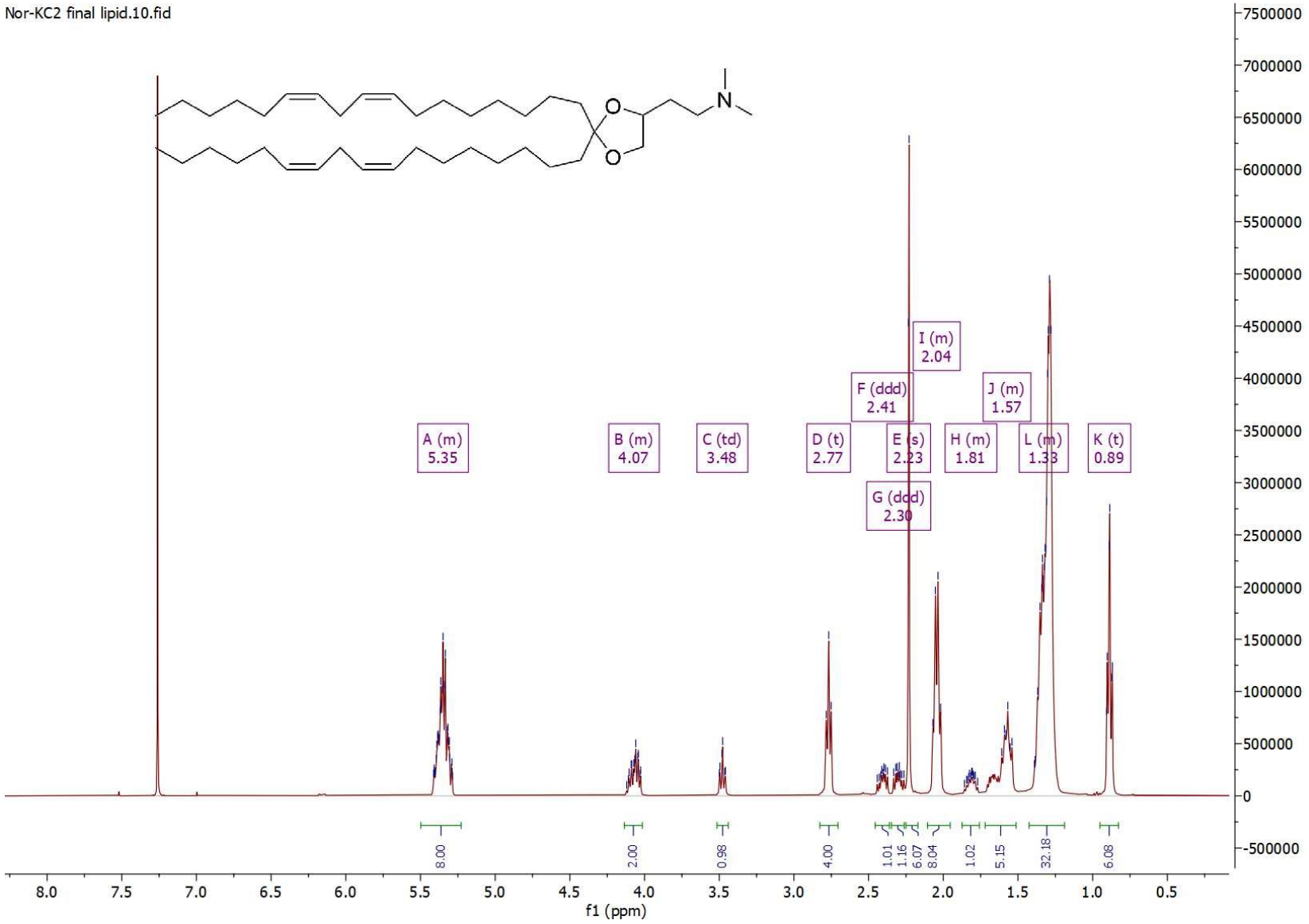
100 MHz ^{13}C {H} NMR spectrum of *nor*-MC3, 1, in $CDCl_3$



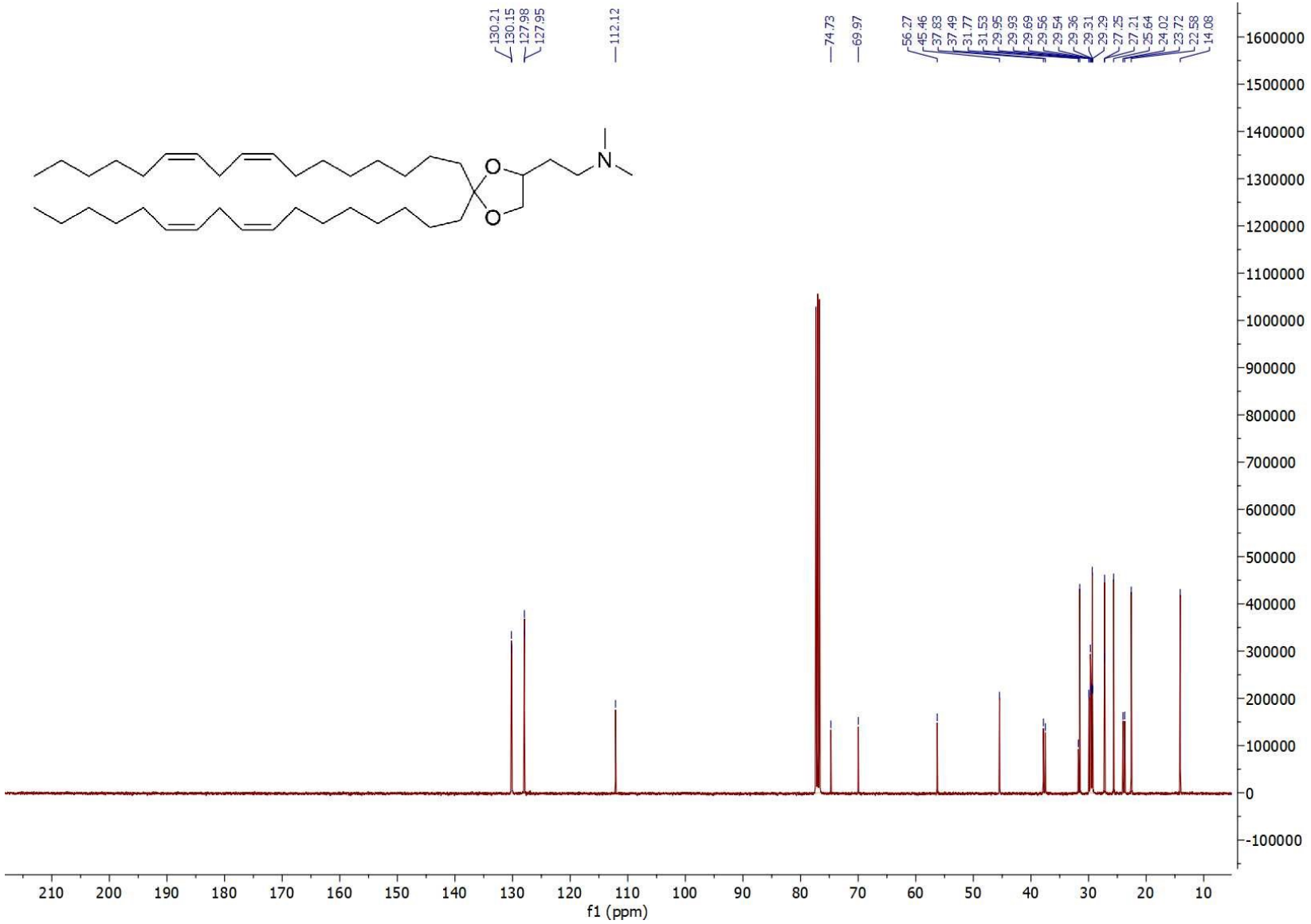
400 MHz ^{1}H NMR spectrum of ketal 10 in $CDCl_3$



100 MHz ^{13}C {H} NMR spectrum of ketal 10 in $CDCl_3$



400 MHz ^{1}H NMR spectrum of *nor*-KC2, 2, in $CDCl_3$



100 MHz ^{13}C {H} NMR spectrum of *nor*-KC2, 2, in $CDCl_3$

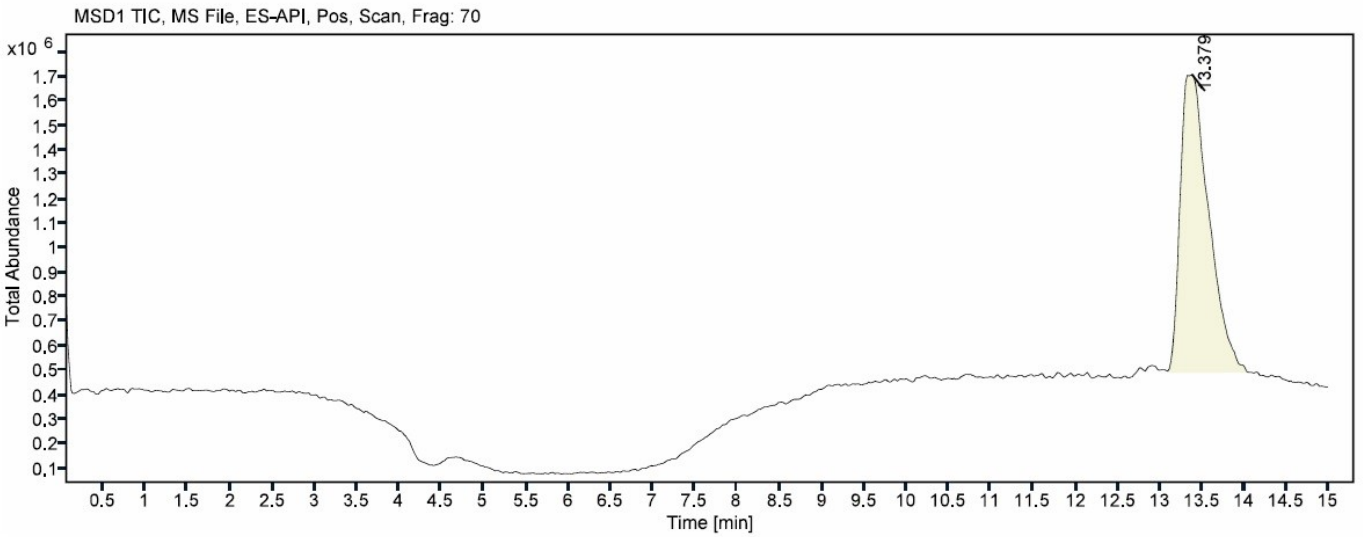
I. HPLC Traces and mass spectra of 1 and 2

Preparative LC - With MS Spectra

Data Folder: 2025-03-27

Sample: n-MC3

Signal details: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70



RT [min]	Signal Description	Symmetry	Resolution	Height [count]	Area [count*min]	Rel. Area [%]
13.379	MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70	0.65185	1.12813	1221125	28698554	98.7

Filtered on peak height > 100000 counts

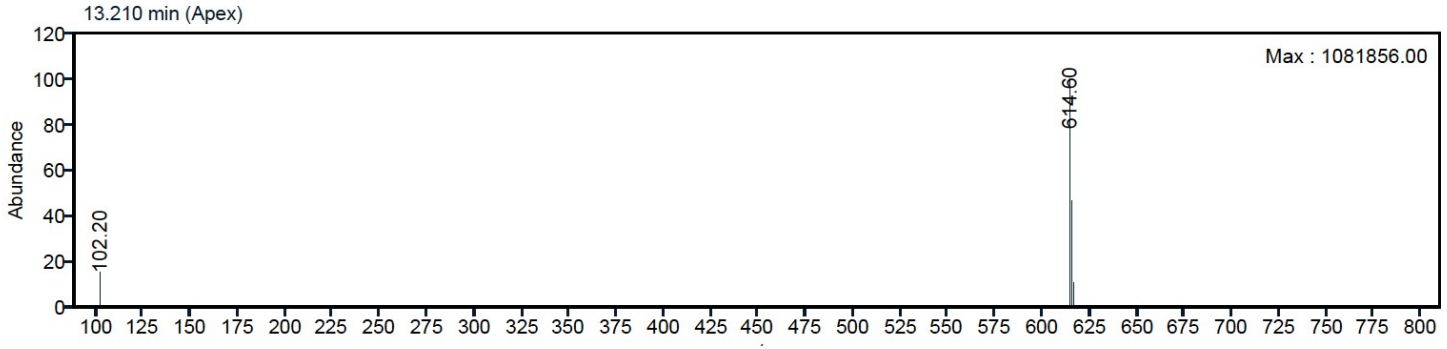
HPLC-MS Trace of *nor*-MC3, 1.

Preparative LC - With MS Spectra

Data Folder: 2025-03-27

Sample: n-MC3

Retention time: 13.21 min **Area Percent:** 88%



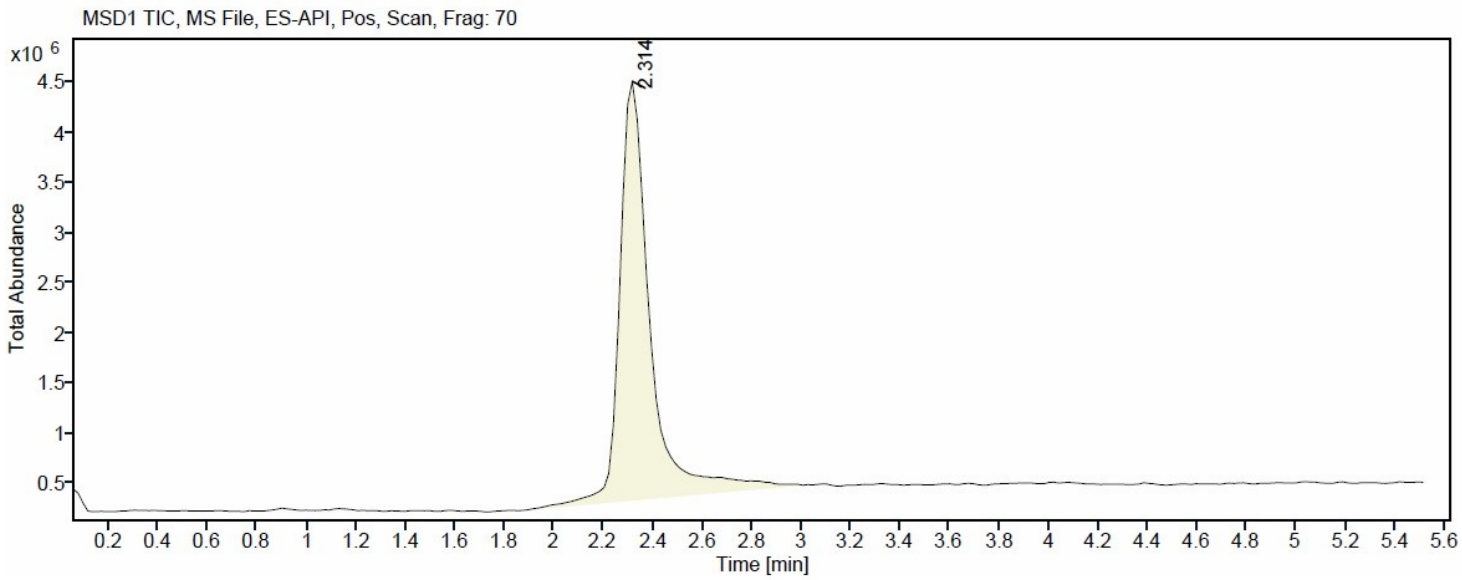
MS Spectrum of *nor*-MC3, 1.

Preparative LC - With MS Spectra

Data Folder: 2025-08-18

Sample: nor-KC2-F

Signal details: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70



RT [min]	Signal Description	Symmetry	Resolution	Height [count]	Area [count*min]	Rel. Area [%]
2.314	MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70	0.59265	5.38803	4183478	36082252	97.7

Filtered on peak height > 100000 counts

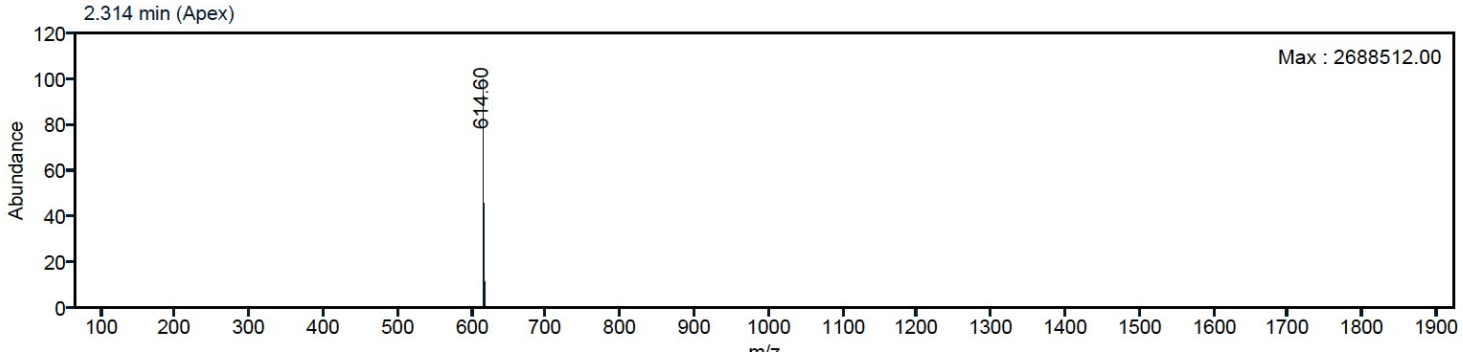
HPLC-MS Trace of *nor*-KC2, 2.

Preparative LC - With MS Spectra

Data Folder: 2025-08-18

Sample: nor-KC2-F

Retention time: 2.314 min **Area Percent:** 98%



MS Spectrum of *nor*-KC2, 2.