# Supporting information for Synthesis of methyl ester of 17(*R*/*S*)-Me-RvD5$_{n\text{-}3\ DPA}$ and relief of postoperative pain in male mice

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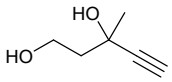
## General Information

Unless otherwise stated, all commercially available reagents and solvents were used in the form they were supplied without any further purification. The stated yields are based on the isolated material. All sensitive reactions were performed under an argon atmosphere using Schlenk techniques. Reaction flasks were covered with aluminum foil during sensitive reactions and storage to minimize exposure to light. Thin layer chromatography was performed on silica gel 60 F254 aluminum-backed plates fabricated by Merck. Flash column chromatography was performed on silica gel 60 (40 - 63 µm) fabricated by Merck. NMR spectra were recorded on a Bruker AVII 400, AVII 600 or AVIII 800 spectrometer at 400 MHz/600 MHz/800 MHz for $^{1}$H NMR and at 101 MHz/151 MHz/201 MHz for $^{13}$C NMR. Coupling constants (*J*) are reported in hertz and chemical shifts are reported in parts per million (δ) relative to the central residual protium solvent resonance in $^{1}$H NMR ($CDCl_3$ = δ 7.26 and $CD_3OD$ = δ 3.31) and the central carbon solvent resonance in $^{13}$C NMR ($CDCl_3$ = δ 77.16 ppm and $CD_3OD$ = δ 49.0). High-resolution mass spectra were recorded at 70 eV on a Micromass Prospec Q or Micromass QTOF 2W spectrometer using ESI as the method of ionization. Optical rotations were measured using a 0.2 mL cell with a 0.1 dm path length on a PerkinElmer 341 polarimeter. HPLC-analyses were performed using a C18 stationary phase (Eclipse XDBC18, 4.6 x 250 mm, particle size 5 µm, from Agilent Technologies), applying the conditions stated. The UV-Vis spectrum was recorded using an Agilent Technologies Cary 8485 UV-Vis spectrophotometer using quartz cuvettes.

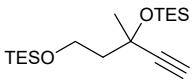
## Synthetic protocols

### (±)-3-Methylpent-4-yne-1,3-diol (6)



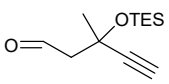

4-Hydroxybutan-2-one **5** (2.00 g, 1.96 mL, 22.7 mmol, 1.00 equiv.) was dissolved in dry THF (100 mL) and cooled to 0 °C. Ethynylmagnesium bromide (0.5 M in THF, 91.0 mL, 45.5 mmol, 2.00 equiv.) was added in a dropwise manner. The reaction mixture was allowed to attain ambient temperature overnight. Then, the reaction mixture was re-cooled to 0 °C and carefully treated with saturated aqueous $NaH_2PO_4$. The phases were separated and the aqueous phase was extracted with $Et_2O$ (3 × 30 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (heptane, followed by 50% EtOAc in heptane). This afforded the title compound as a colorless liquid in 48% yield (1.233 g, 10.90 mmol). Spectroscopic data were in agreement with those reported in the literature.[1] $R_f$ (50% EtOAc in heptane, visualized by $KMnO_4$ stain) = 0.17; $^1$H NMR (400 MHz, $CDCl_3$) δ 4.20 – 4.12 (m, 1H), 3.94 – 3.87 (m, 1H), 3.49 – 3.30 (br, 2H), 2.49 (s, 1H), 2.03 – 1.94 (m, 1H), 1.85 – 1.78 (m, 1H), 1.53 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 87.08, 72.09, 68.76, 60.68, 43.40, 30.70.

### (±)-1,3-Bis(triethylsilyl(oxy))3-methylpent-4-yn (7)




(±)-3-Methylpent-4-yne-1,3-diol **6** (250 mg, 2.19 mmol, 1.00 equiv.) was dissolved in $CH_2Cl_2$ (25 mL) and cooled to -78 °C. 2,6-Lutidine (1.41 g, 1.52 mL, 13.1 mmol, 6.00 equiv.) was added, followed by TESOTf (1.27 g, 1.09 mL, 4.82 mmol, 2.20 equiv.). The reaction mixture was allowed to attain ambient temperature overnight. After 24h *in toto*, the reaction mixture was treated with saturated aqueous $NH_4Cl$ (25 mL) and $CH_2Cl_2$ (25 mL). The phases were separated and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and the solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (heptane, followed by 5% $Et_2O$ in heptane). This afforded the title compound as colorless liquid in 88% yield (660 mg, 1.93 mmol). $R_f$ (5% $Et_2O$ in heptane, visualized by CAM stain) = 0.56; $^1$H NMR (400 MHz, $CDCl_3$) δ 3.88 – 3.80 (m, 2H), 2.40 (s, 1H), 2.00 – 1.86 (m, 2H), 1.47 (s, 3H), 0.99 – 0.93 (m, 18H), 0.69 – 0.57 (m, 12H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 87.96, 77.37, 77.16, 76.95, 72.05, 67.61, 59.70, 47.77, 31.56, 7.09 (3C), 6.91 (3C), 6.23 (3C), 4.62 (3C).

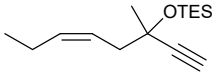
### 3-Methyl-3-((triethylsilyl)oxy)pent-4-ynal (8)




Oxalyl chloride (2 M in $CH_2Cl_2$, 2.79 mL, 5.57 mmol, 4.40 equiv.) was added dropwise to a solution of DMSO (0.78 mL, 11.0 mmol, 8.70 equiv.) in dry $CH_2Cl_2$ (5 mL) at -78 °C. The solution was stirred for 20 min before (±)-1,3-bis(triethylsilyl(oxy))3-methylpent-4-yn **7** (433 mg, 1.27 mmol, 1.00 equiv.) dissolved in $CH_2Cl_2$ (4 mL) was added in a dropwise manner. The reaction was stirred for 45 min at -78 °C, thereafter 55 min at -40 °C before it was re-cooled to -78 °C. Triethylamine (2.64 mL, 19.1 mmol,

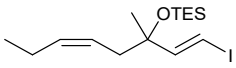
15.0 equiv.) was added, and the reaction mixture stirred for additional 10 min before it was allowed to reach ambient temperature. $H_2O$ (10 mL) was added before the mixture was diluted with $CH_2Cl_2$ (15 mL). The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 10 mL). The organic phases were combined, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (5% $Et_2O$ in heptane) to afford aldehyde **8** as a colorless oil in 86% yield (246 mg, 1.09 mmol). The aldehyde was used immediately in the next reaction. $R_f$ (10% EtOAc in heptane, visualized by CAM and $KMnO_4$ stain) = 0.29; $^1$H NMR (400 MHz, $CDCl_3$) δ 9.88 (t, *J* = 2.8 Hz, 1H), 2.62 (d, *J* = 2.8 Hz, 2H), 2.57 (s, 1H), 1.58 (s, 3H), 0.96 (t, *J* = 7.9 Hz, 9H), 0.76 – 0.62 (m, 6H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 201.69, 86.59, 73.87, 66.40, 57.28, 31.55, 7.01 (3C), 6.12 (3C).

**(*Z*)-Triethyl((3-methyloct-5-en-1-yn-3-yl)oxy)silane (9)**




Propyltriphenylphosphonuim bromide (336 mg, 0.87 mmol, 1.20 equiv.) was suspended in dry THF (7.0 mL) and HMPA (1.5 mL). The suspension was degassed twice (switching from vacuum and Ar-atmosphere) before cooled to -78 °C. NaHMDS (0.6 M in toluene, 1.45 mL, 0.87 mmol, 1.20 equiv.) was added dropwise, giving the suspension a slightly yellow color. The cooling bath was removed, allowing the suspension to reach rt. When the color changed to red/orange (ca. 30 min), it was re-cooled to -78 °C. Aldehyde **8** (165 mg, 0.73 mmol, 1.00 equiv.) was azeotroped with 2-MeTHF (2 × 1 mL) before dissolved in dry THF (2.0 mL) and added dropwise at -78 °C. The reaction was allowed to reach ambient temperature, and stirred until deemed completed by TLC (~2 hours). The reaction was quenched with phosphate buffer (pH = 7, 4 mL) and $H_2O$ (7 mL), and diluted with $Et_2O$ (7 mL). The aqueous phase was extracted with $Et_2O$ (2 × 10 mL) before the organic phases were combined, dried over $Na_2SO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (2.5% $Et_2O$ in heptane) to afford the product **9** (142 mg, 0.56 mmol) as a colorless oil in 77% yield. $R_f$ (5% EtOAc in heptane, visualized by $KMnO_4$ stain) = 0.65; $^1$H NMR (400 MHz, $CDCl_3$) δ 5.57 – 5.44 (m, 2H), 2.48 – 2.34 (m, 3H), 2.11 – 2.02 (m, 2H), 1.43 (s, 3H), 0.97 (t, *J* = 7.8 Hz, 12H), 0.73 – 0.63 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 134.36, 124.06, 88.44, 71.96, 68.94, 42.83, 30.54, 20.98, 14.35, 7.15 (3C), 6.24 (3C); HRESIMS *m/z*: 275.1802 $[M + Na]^+$ (calcd for $C_{15}H_{28}OSiNa$, 275.1802).

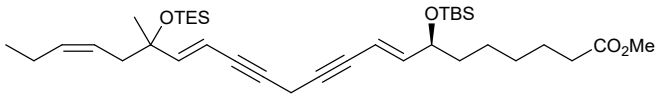
**Triethyl(((1*E*,5*Z*)-1-iodo-3-methylocta-1,5-dien-3-yl)oxy)silane (3)**




To a suspension of bis(cyclopentadienyl)zirconium(IV) dichloride (643 mg, 2.12 mmol, 2.00 equiv.) in THF (2.5 mL) was added DiBAl-H (1.0 M in THF, 1.90 mL, 1.90 mmol, 1.80 equiv.) at 0 °C. After stirring for 30 min, a solution of alkyne **9** (269 mg, 1.06 mmol, 1.00 equiv.) in THF (0.5 mL) was added, and the suspension stirred for an additional 60 min, allowing to reach ambient temperature. A solution of iodine (350 mg, 1.38 mmol, 1.30 equiv.) in THF (0.5 mL) was added at rt to the yellow solution and stirring was continued for further 40 min. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (1.0 mL), $H_2O$ (0.8 mL) and sat. aq. $NaHCO_3$ (1.0 mL). The mixture was stirred for 5 min before the phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 5 mL). The combined organic phases were dried ($MgSO_4$), filtrated, and concentrated *in vacuo*. The crude product thus

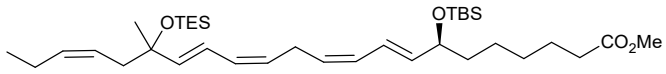
obtained was purified by column chromatography ($SiO_2$, heptane) to obtain the vinyl iodide **3** (213 mg, 0.56 mmol) as a colorless oil in 53% yield. $R_f$ (heptane, visualized by UV and $KMnO_4$ stain) = 0.22; $^1$H NMR (400 MHz, $CDCl_3$) δ 6.56 (d, *J* = 14.4 Hz, 1H), 6.21 (d, *J* = 14.4 Hz, 1H), 5.52 – 5.42 (m, 1H), 5.39 – 5.30 (m, 1H), 2.24 (d, *J* = 7.3 Hz, 2H), 2.07 – 1.96 (m, 2H), 1.28 (s, 3H), 0.98 – 0.92 (m, 12H), 0.62 – 0.55 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 152.79, 134.27, 123.82, 78.18, 75.25, 41.28, 26.72, 20.92, 14.31, 7.18 (3C), 6.75 (3C); HRESIMS *m*/*z*: 403.0924 $[M + Na]^+$ (calcd for $C_{15}H_{29}IOSiNa$, 403.0925).

**Methyl (7*S*,8*E*,15*E*,19*Z*)-7-((*tert*-butyldimethylsilyl)oxy)-17-methyl-17-((triethylsilyl)oxy)docosa-8,15,19-trien-10,13-diynoate (10)**




Vinyl iodide **3** (25 mg, 0.07 mmol, 1.00 equiv.) was dissolved in THF (0.8 mL), and the solution was cooled to 0 °C before $Pd(PPh_3)_2Cl_2$ (2.5 mg, 3.5 µmol, 5.0 mol %), CuI (1.5 mg, 0.8 µmol, 11 mol %), and $Et_3N$ (13 mg, 18 µL, 0.13 mmol, 2.0 equiv.) were added. Alkyne **4** (42 mg, 0.13 mmol, 2.0 equiv.) was dissolved in THF (0.3 mL) and added dropwise. The reaction mixture was allowed to slowly warm up to rt and stirred in the dark for 16 h. After completion, the reaction mixture was filtrated through a plug of silica gel (15% EtOAc in heptane) and concentrated *in vacuo*. The crude product thus obtained was purified by column chromatography ($SiO_2$, 5% EtOAc in heptane) to obtain product **10** (27 mg, 0.04 mmol) as a pale yellow oil in 63% yield. $R_f$ (10% EtOAc in heptane, visualized by UV and $KMnO_4$ stain) = 0.32; $[\alpha]_D^{20}$ = -5.00 (c 0.2, $CH_2Cl_2$); $^1$H NMR (400 MHz, $CDCl_3$) δ 6.16 – 6.03 (m, 2H), 5.69 – 5.57 (m, 2H), 5.49 – 5.40 (m, 1H), 5.38 – 5.29 (m, 1H), 4.16 – 4.09 (m, 1H), 3.66 (s, 3H), 3.42 (s, 2H), 2.33 – 2.22 (m, 4H), 2.05 – 1.96 (m, 2H), 1.66 – 1.57 (m, 2H), 1.50 – 1.43 (m, 2H), 1.32 – 1.26 (m, 7H), 0.95 (td, *J* = 7.7, 2.1 Hz, 12H), 0.89 (s, 9H), 0.58 (q, *J* = 7.9 Hz, 6H), 0.03 (s, 3H), 0.02 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.35, 150.31, 146.51, 133.93, 124.18, 108.57, 107.23, 83.67, 83.50, 79.35, 79.10, 77.48, 77.16, 76.84, 75.43, 72.52, 51.61, 41.50, 37.80, 34.17, 29.29, 27.28, 25.99 (3C), 25.04, 24.69, 20.94, 18.34, 14.22, 11.29, 7.23 (3C), 6.87 (3C), -4.33, -4.72; HRESIMS *m*/*z*: 637.4081 $[M + Na]^+$ (calcd for $C_{36}H_{62}O_4Si_2Na$, 637.4079).

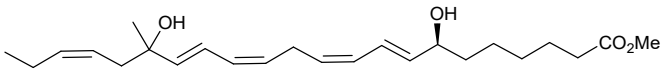
**Methyl (7*S*,8*E*,10*Z*,13*Z*,15*E*,19*Z*)-7-((*tert*-butyldimethylsilyl)oxy)-17-methyl-17-((triethylsilyl)oxy)docosa-8,10,13,15,19-pentaenoate (11)**




Diyne **10** (20 mg, 33 µmol, 1.0 equiv.) was dissolved in EtOAc (2.0 mL) under argon. Quinoline (20 µL, 0.24 mmol) and 5% $Pd/BaSO_4$ (20 mg) was added before the flask was evacuated and refilled with hydrogen gas twice. The reaction was stirred for 1 h before it was filtrated through a short plug of silica gel (15% EtOAc in heptane) and concentrated *in vacuo*. The crude product thus obtained was purified by column chromatography ($SiO_2$, 5% EtOAc in heptane) to obtain product **11** (14 mg, 23 µmol) as a colorless oil in 70% yield. $R_f$ (10% EtOAc in heptane, visualized by UV and $KMnO_4$ stain) = 0.37; $[\alpha]_D^{20}$ = +5.20 (c 0.2, $CH_2Cl_2$); $^1$H NMR (400 MHz, $CDCl_3$) δ 6.52 – 6.36 (m, 2H), 6.08 – 5.91 (m, 2H), 5.74 (d, *J* = 15.3 Hz, 1H), 5.65 (dd, *J* = 15.1, 6.3 Hz, 1H), 5.48 – 5.30 (m, 4H), 4.15 (q, *J* = 5.9 Hz, 1H), 3.66 (s, 3H), 3.06 (t, *J* = 7.5 Hz, 2H), 2.33 – 2.22 (m, 4H), 2.07 – 1.97 (m, 2H), 1.67 – 1.58 (m, 2H), 1.52 – 1.42 (m, 2H), 1.32 – 1.26 (m, 7H), 0.98 – 0.91 (m, 12H), 0.90 – 0.88 (m, 9H), 0.58 (q, *J* = 7.9 Hz, 6H), 0.05 (s, 3H), 0.03

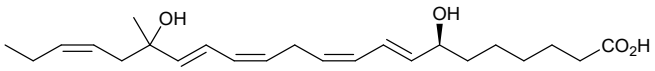
(s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.39, 141.56, 137.72, 133.58, 129.15, 129.06, 128.95, 128.67, 124.76, 124.25, 122.51, 77.48, 77.16, 76.84, 75.34, 73.25, 51.60, 41.95, 38.34, 34.21, 32.03, 29.32, 27.28, 26.60, 26.04 (3C), 25.09, 20.93, 18.40, 14.27, 7.26 (3C), 6.91 (3C), -4.12, -4.62; HRESIMS *m*/*z*: 641.4390 $[M + Na]^+$ (calcd for $C_{36}H_{66}O_4Si_2Na$, 641.4392).

**Methyl (7*S*,8*E*,10*Z*,13*Z*,15*E*,19*Z*)-7,17-dihydroxy-17-methyldocosa-8,10,13,15,19-pentaenoate (12)**




The bis-silyl protected compound **11** (5.00 mg, 0.003 mmol, 1.00 equiv.) was dissolved in 0.2 mL dry THF and cooled to 0 °C. TBAF (1 M in THF, 0.01 mL, 0.01 mmol, 5.00 equiv.), was added and the reaction mixture stirred overnight, allowing to reach rt. The reaction was quenched with phosphate buffer (pH = 7, 0.1 mL), brine (0.2 mL) and diluted with $CH_2Cl_2$ (0.5 mL). The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (2 × 0.5 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (2.5% MeOH in $CH_2Cl_2$), thereafter on preparative TLC (2.5% MeOH in $CH_2Cl_2$) to afford the 17(*R*/*S*)-Me-RvD5$_{n\text{-}3\ DPA}$ methyl ester (0.5 mg, 0.39 µmol, 13%) as a colorless oil. The chemical purity (96%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/$H_2O$ 76:24, 1.0 mL/min): $t_r$ (minor) = 20.09 min, $t_r$ (major) = 22.89 min. UV-Vis (MeOH): $\lambda_{max}$ 243 nm (log ε = 4.64); $R_f$ (40% EtOAc in heptane, visualized by UV and $KMnO_4$ stain) = 0.18; $[\alpha]_D^{20}$ = +4.10 (c 0.2, MeOH); $^1$H NMR (600 MHz, MeOD-higher-lock-power) δ 6.61 – 6.53 (m, 2H), 6.00 (q, *J* = 10.9 Hz, 2H), 5.77 (d, *J* = 15.3 Hz, 1H), 5.67 (dd, *J* = 15.1, 6.7 Hz, 1H), 5.44 – 5.30 (m, 4H), 4.55 (s, 2H), 4.12 – 4.08 (m, 1H), 3.65 (s, 3H), 3.13 – 3.07 (m, 2H), 2.34 – 2.29 (m, 4H), 2.07 – 2.04 (m, 2H), 1.63 – 1.60 (m, 2H), 1.54 – 1.48 (m, 2H), 1.35 – 1.32 (m, 4H), 1.27 (s, 3H), 0.95 (t, *J* = 7.5 Hz, 3H). $^{13}$C NMR (201 MHz, MeOD) δ 175.99, 141.95, 138.05, 134.92, 130.27, 129.82, 129.71, 129.59, 126.24, 125.13, 123.62, 73.72, 73.20, 51.95, 41.33, 38.22, 34.75, 33.07, 30.12, 27.40, 26.22, 25.98, 21.66, 14.48. HRESIMS *m*/*z*: 413.2662 $[M + Na]^+$ (calcd for $C_{24}H_{38}O_4Na$, 413.2662).

**(7*S*,8*E*,10*Z*,13*Z*,15*E*,19*Z*)-7,17-Dihydroxy-17-methyldocosa-8,10,13,15,19-pentaenoic acid (2)**




The methyl ester **12** (210 µg, 0.54 µmol, 1.00 equiv.) was dissolved in a solution of MeOH:THF:$H_2O$ (2:2:1, 0.2 mL) and cooled to 0 °C before LiOH (1.0 mg, 0.04 mmol, 50 equiv.) was added in one portion. The reaction mixture was stirred for 4 h, allowing to reach ambient temperature before it was acidified by addition of sat. aq. $NaH_2PO_4$ (0.3 mL) and diluted with EtOAc (0.3 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 0.5 mL). The combined organic phases were dried over $MgSO_4$ and directly filtered through a short plug of silica gel to yield the title compound **2** in 83 % (168 µg, 0.45 µmol) as a colorless oil. UV-Vis (EtOH): $\lambda_{max}$ 243 nm (log ε = 4.64); $R_f$ (5% MeOH in $CH_2Cl_2$, visualized by UV and $KMnO_4$ stain) = 0.19.

## Biological experiments in animals

Animal protocols were approved by the Animal Care Committee of Duke University and we followed the ethics guidelines of the International Association for the Study of Pain. Adult CD1 mice (male and female, 25-35 grams) were obtained from Charles River Laboratories and housed in a light and humidity-controlled facility. Animals were habituated in the behavioural testing room for at least two days prior to baseline testing. Animals were randomly assigned to each experimental group. The mice were anesthetized with isoflurane prior to the procedure. The tibial fracture was performed as previously reported.[2] An incision was made on the left knee and a stainless-steel pin (0.38 mm) was inserted into the intramedullary canal, followed by osteotomy. The incision was closed with 4-0 Prolene sutures. Mice were anesthetized with isoflurane while receiving intravenous injection. For intravenous (i.v.) treatments, drugs were dissolved in PBS as vehicle (100 µL injection volume, 300 ng/mice) and injected into the tail vein. Mechanical pain was assessed in von Frey test conducted in a blinded manner, as previously reported. For each test, mice were placed in individual plastic boxes (5 cm × 5 cm) on an elevated mesh floor and habituated for at least 30 min. The paw withdrawal threshold (PWT) was determined via Dixon's up-down method using von Frey filaments with logarithmically increasing stiffnesses (0.02-2.56 g, Stoelting).[3, 4] The filaments were applied perpendicularly to the central plantar surface of the hind paw.

## NMR Spectra

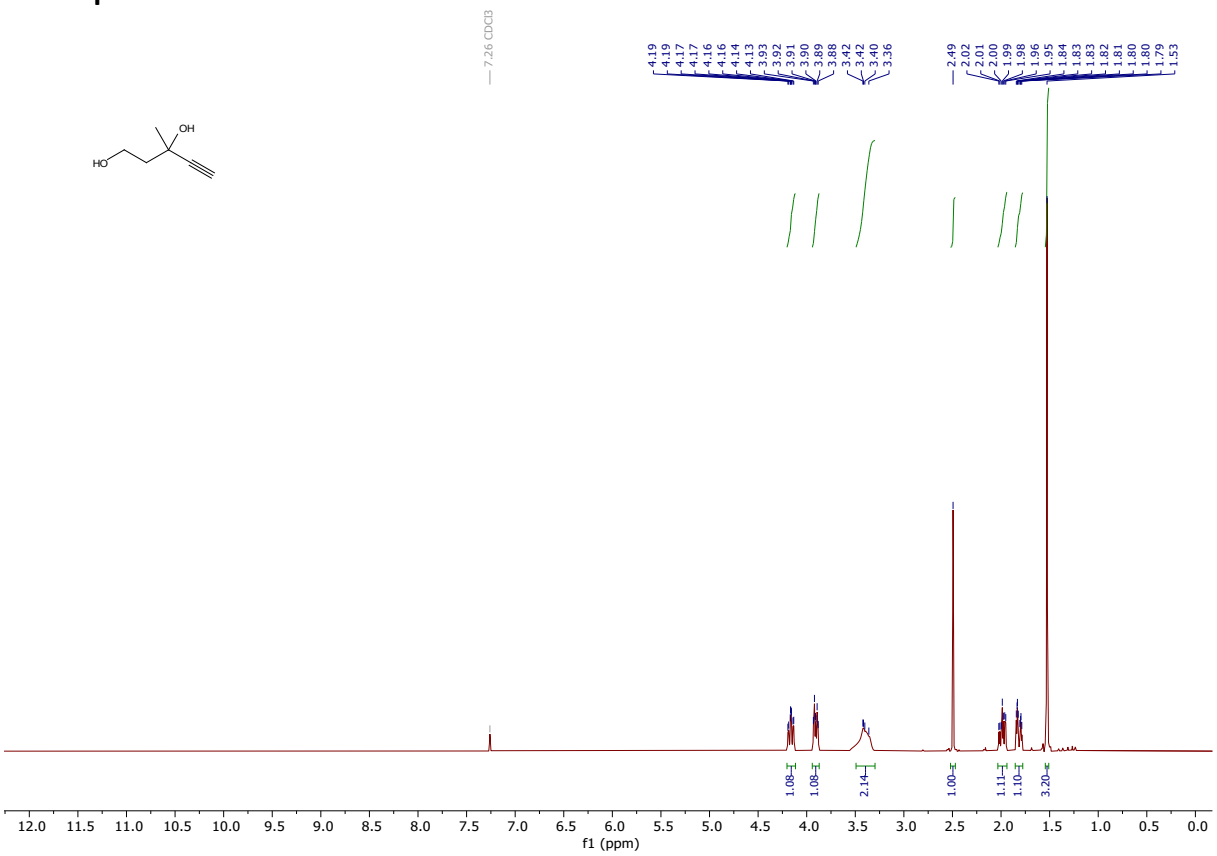



**Figure 1**: $^{1}H$ NMR spectrum of compound **6**.

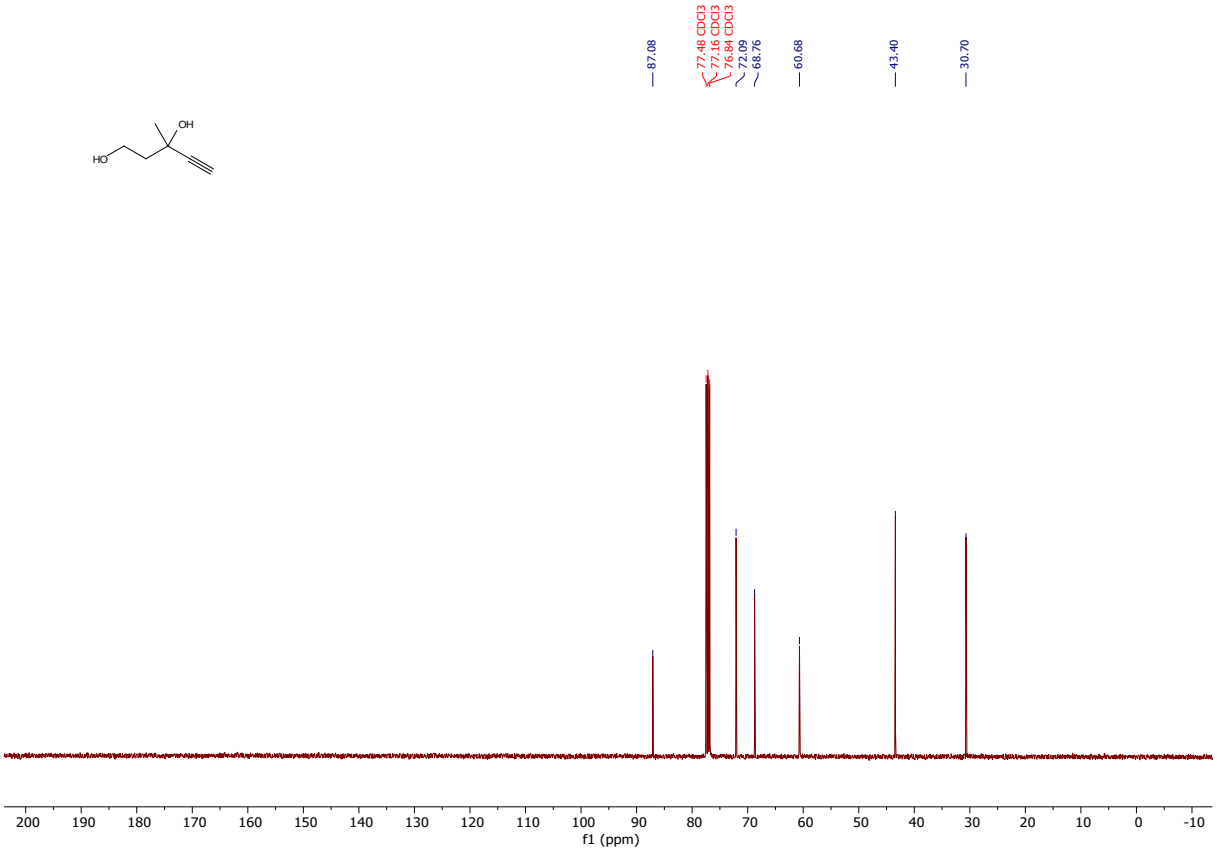



**Figure 2**: $^{13}C$ NMR spectrum of compound **6**.

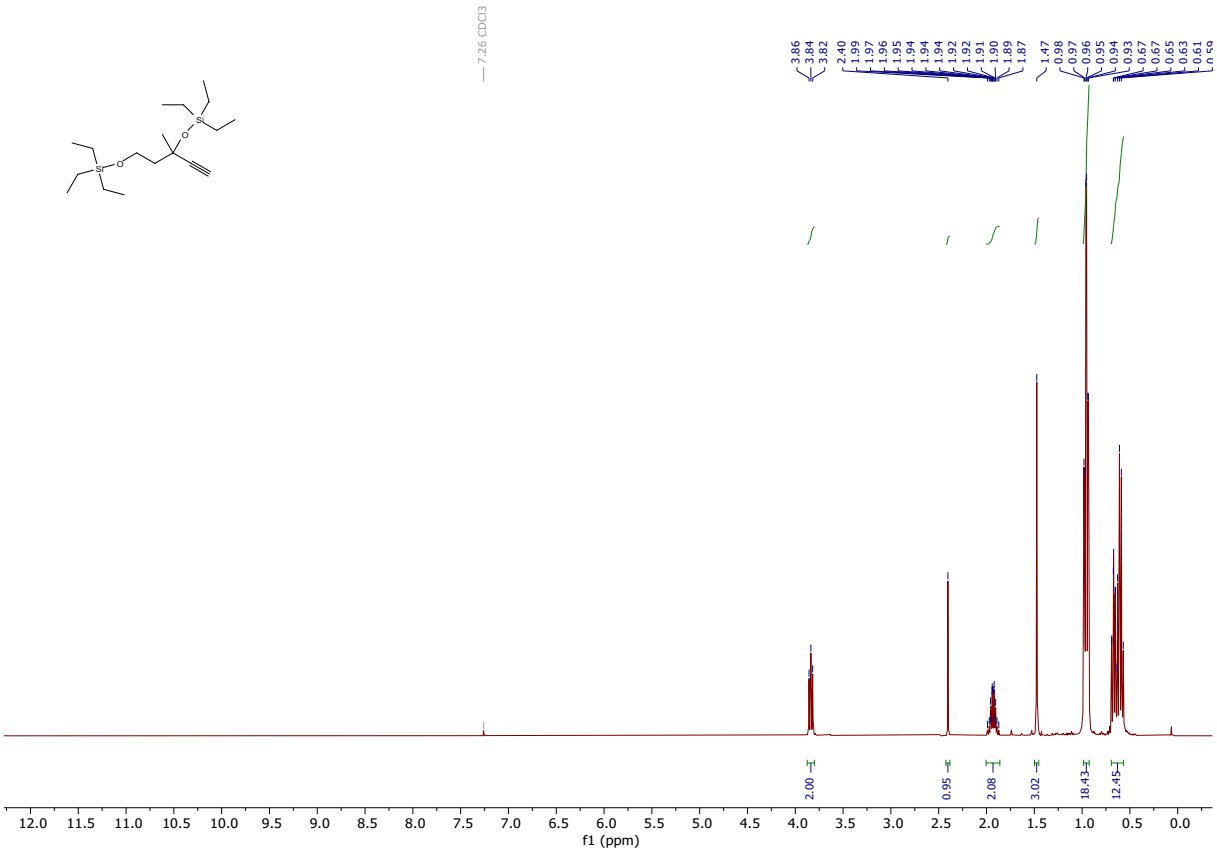



**Figure 3**: $^{1}$H NMR spectrum of compound **7**.

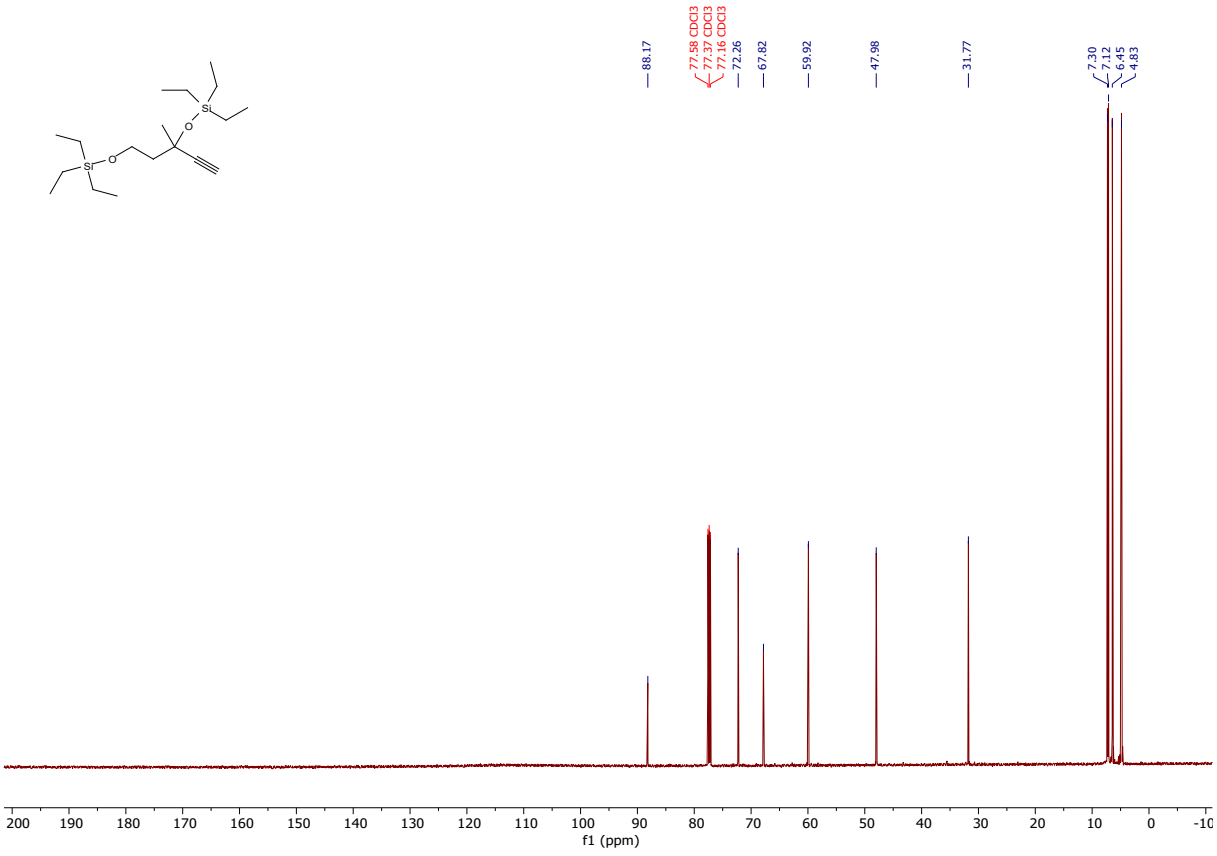



**Figure 4**: $^{13}$C NMR spectrum of compound **7**.

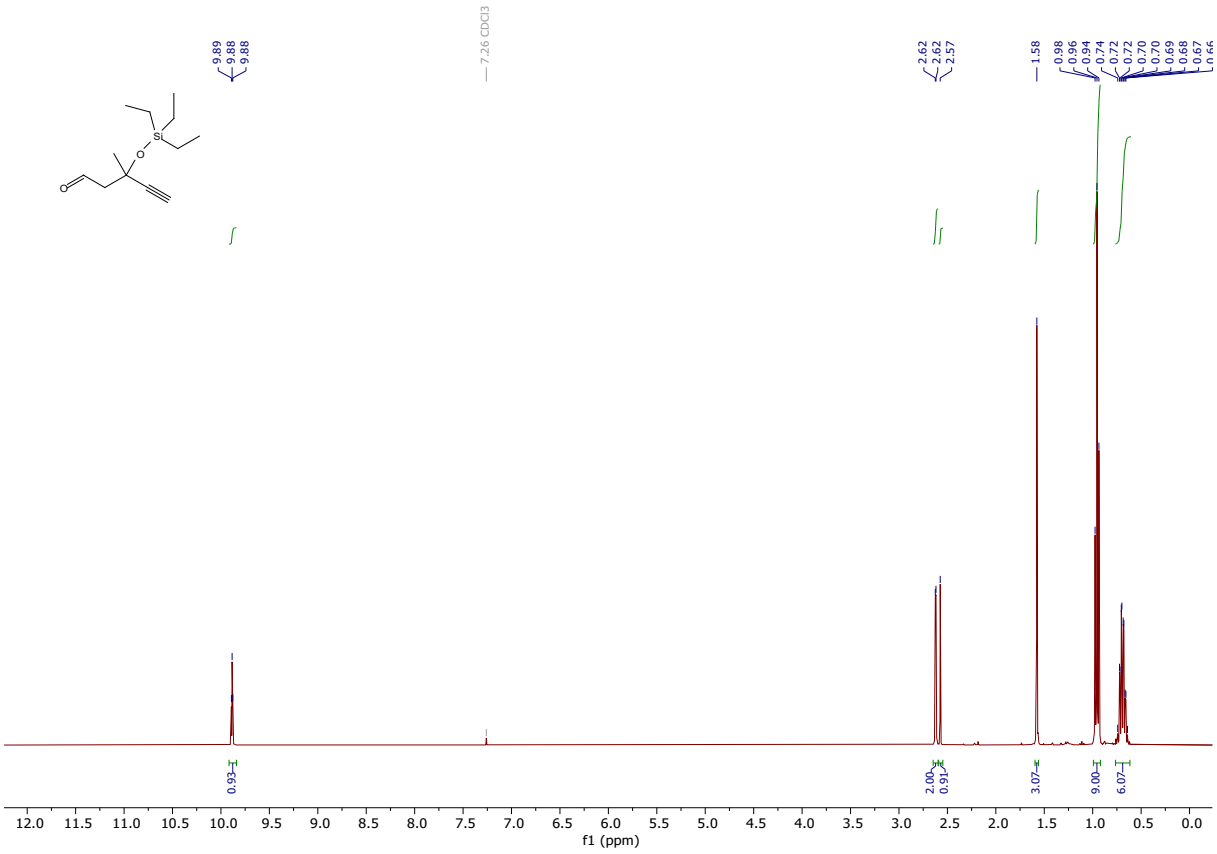



**Figure 5**: $^{1}$H NMR spectrum of compound **8**.

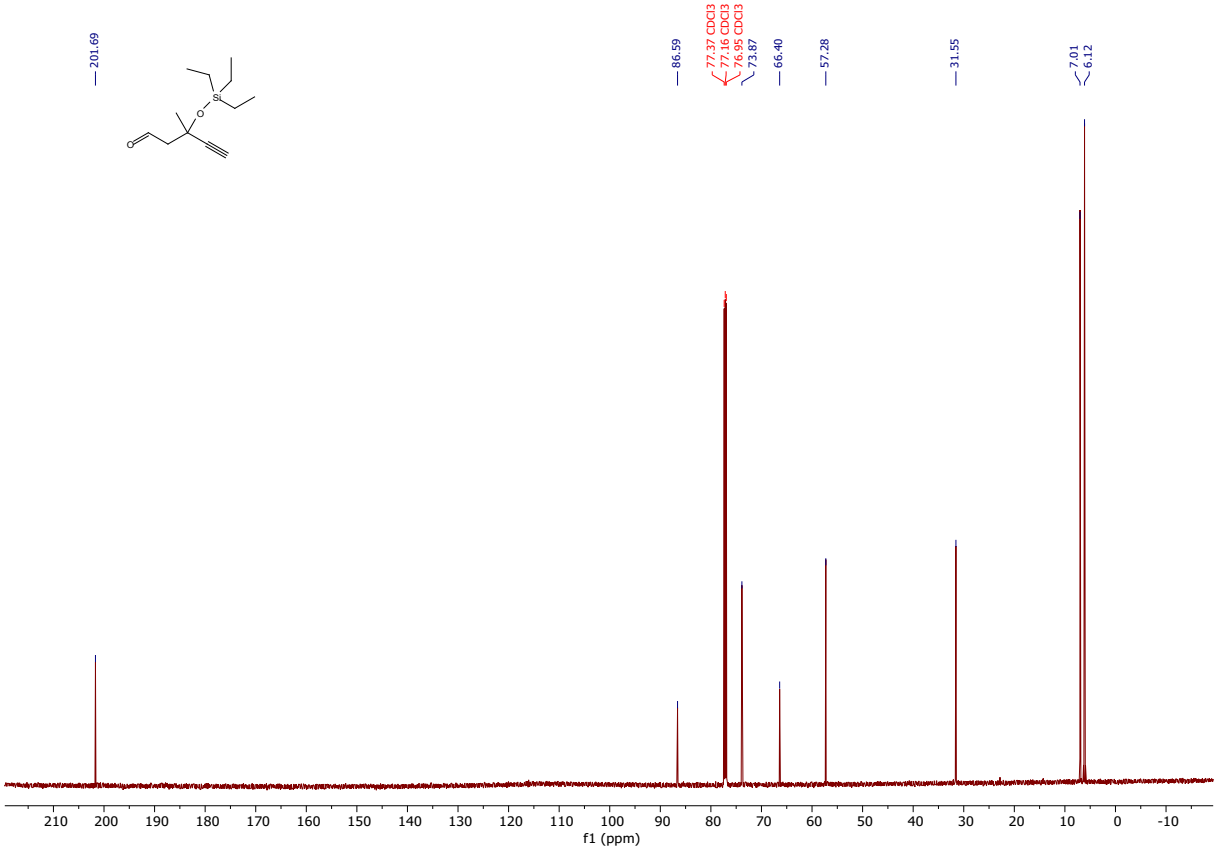



**Figure 6**: $^{13}$C NMR spectrum of compound **8**.

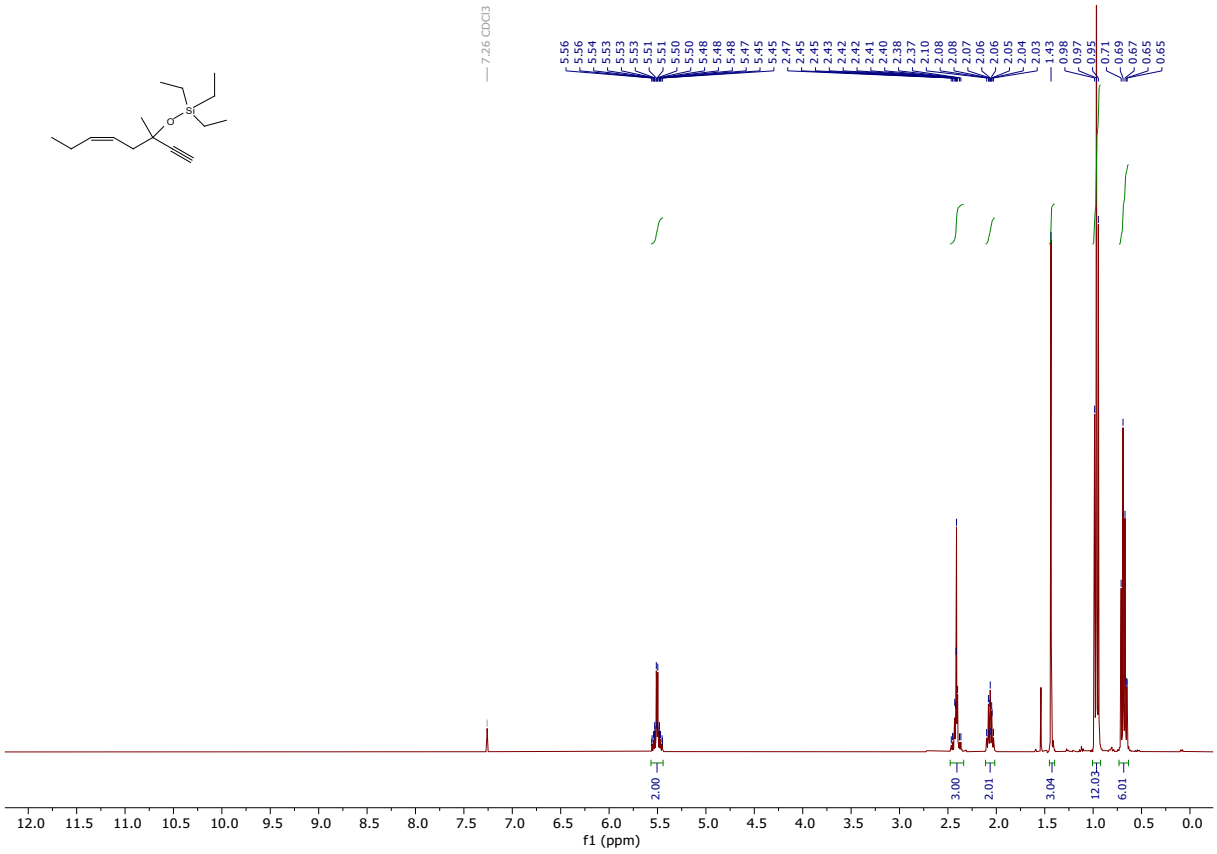



**Figure 7**: $^{1}$H NMR spectrum of compound **9**.

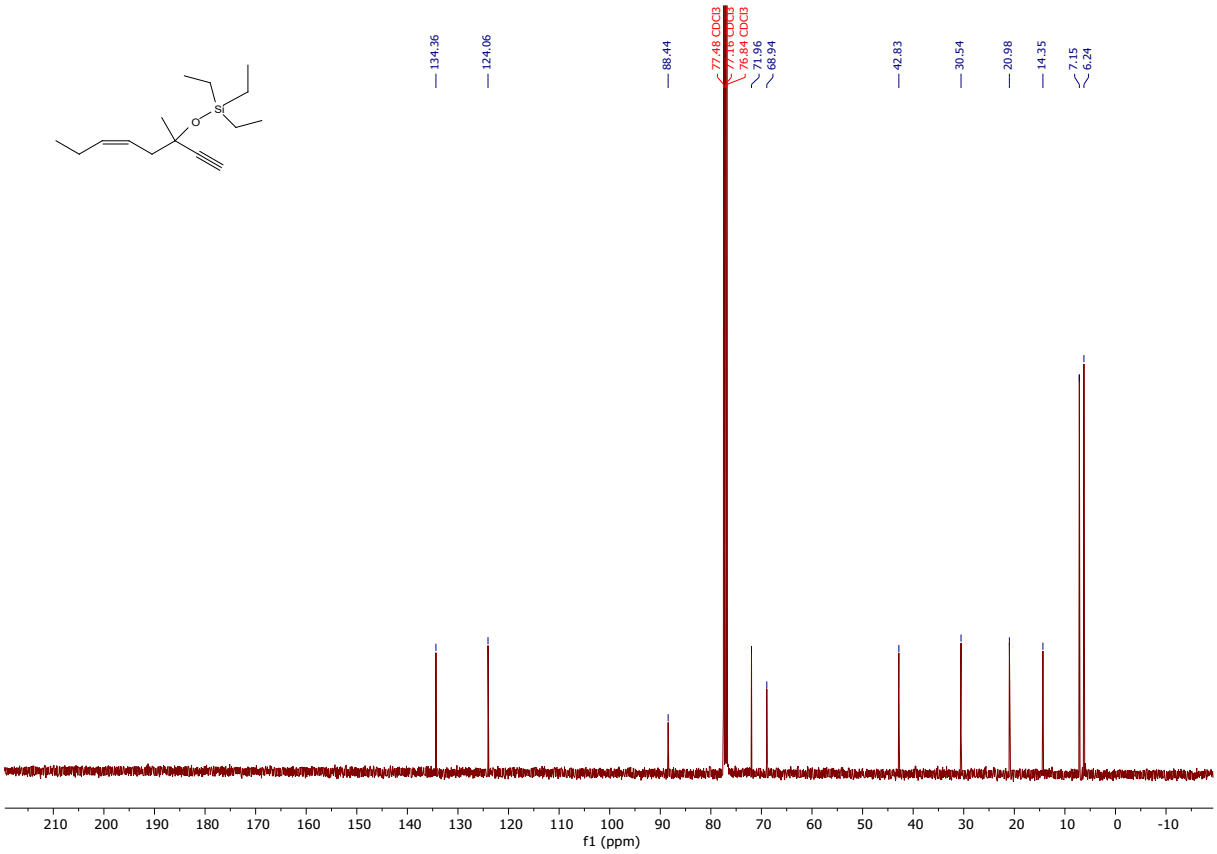



**Figure 8**: $^{13}$C NMR spectrum of compound **9**.

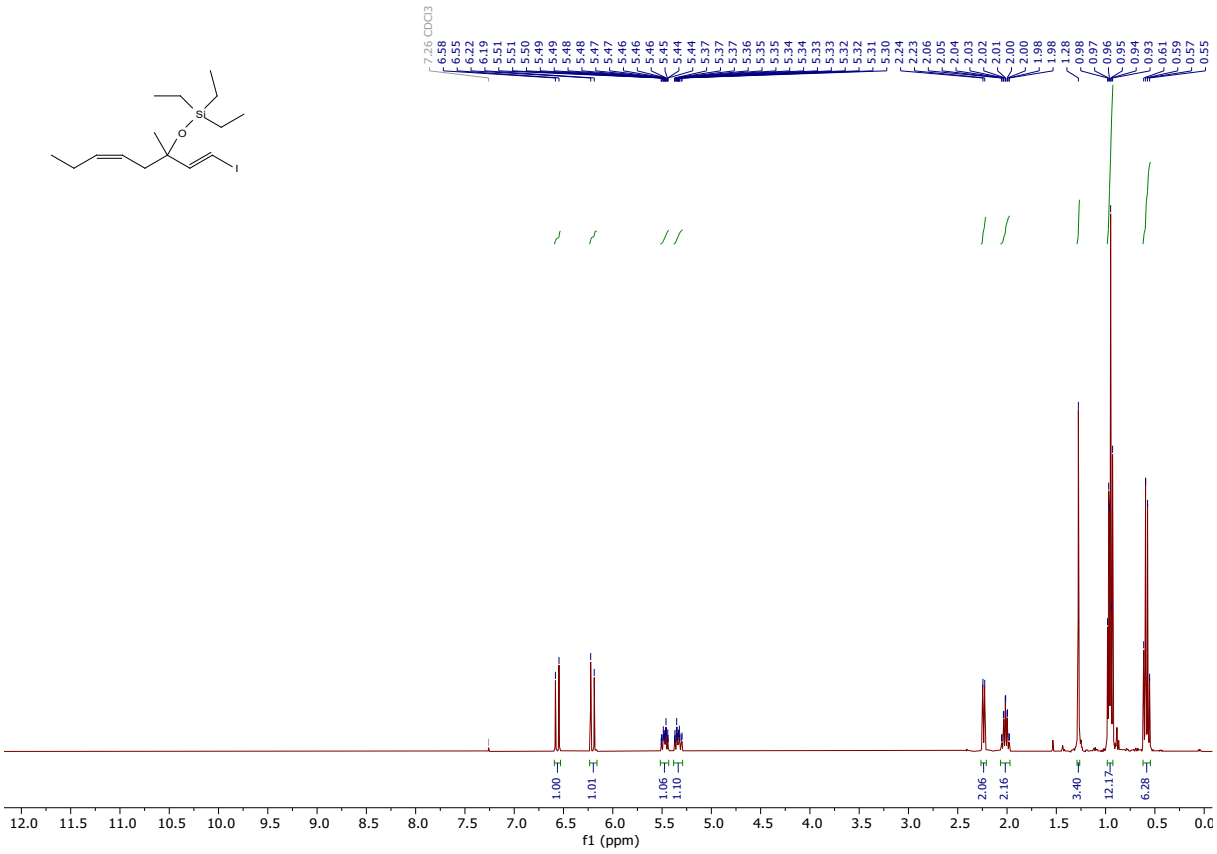



**Figure 9**: $^{1}$H NMR spectrum of compound **3**.

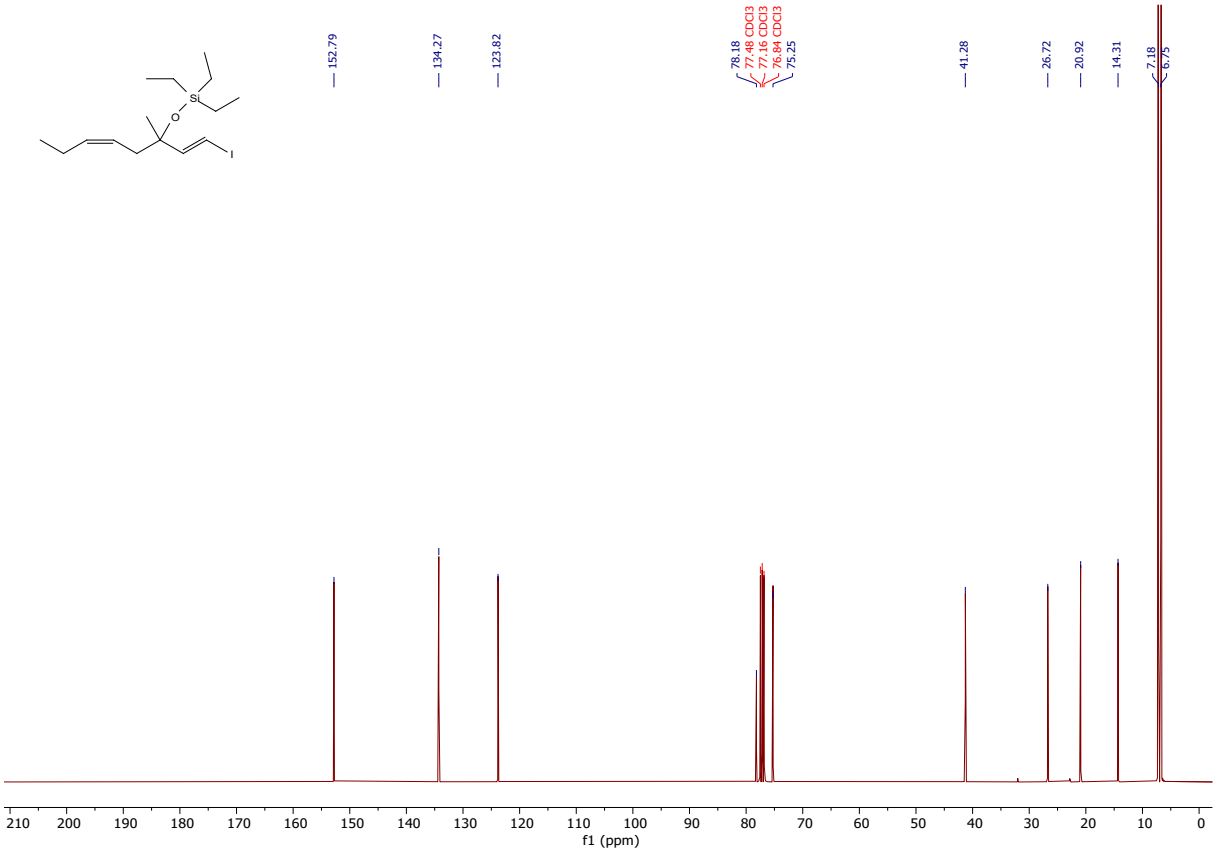



**Figure 10**: $^{13}$C NMR spectrum of compound **3**.

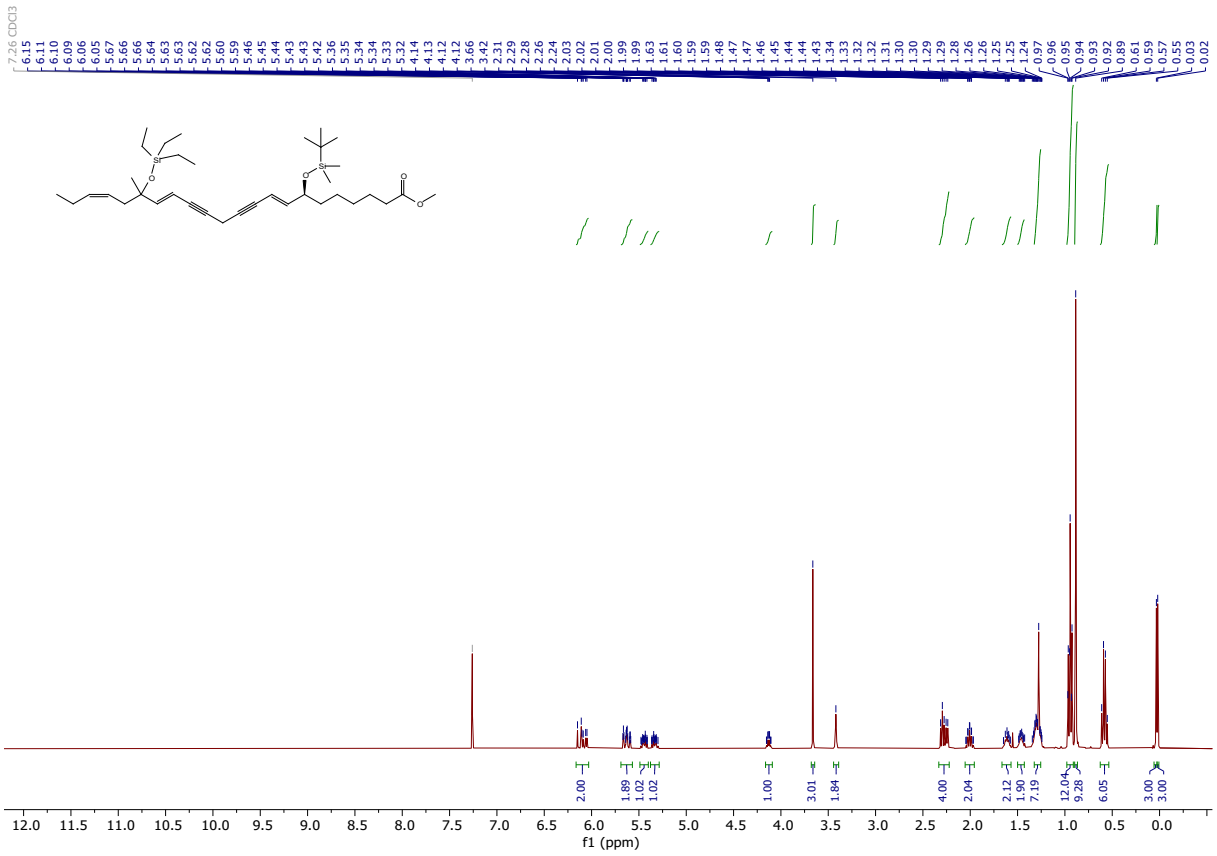


**Figure 11**: $^{1}$H NMR spectrum of compound **10**.

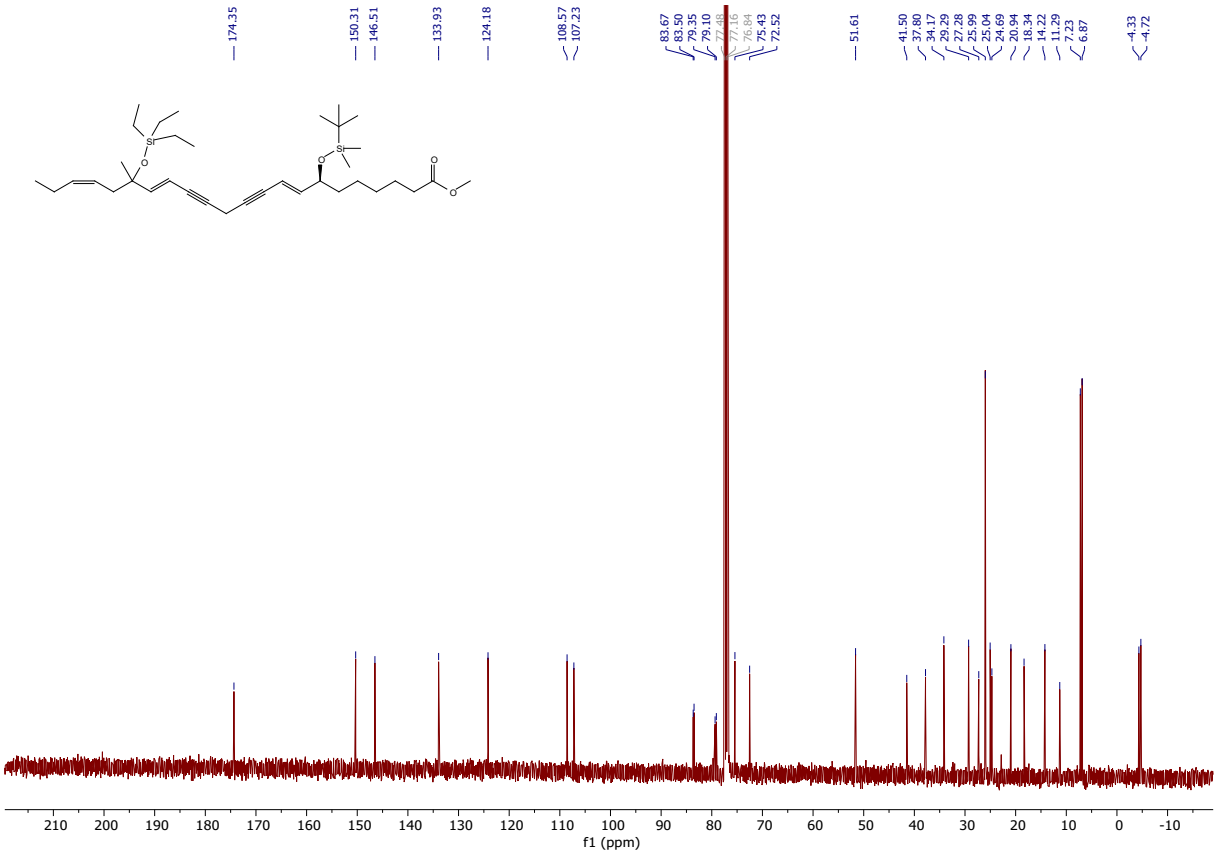


**Figure 12**: $^{13}$C NMR spectrum of compound **10**.

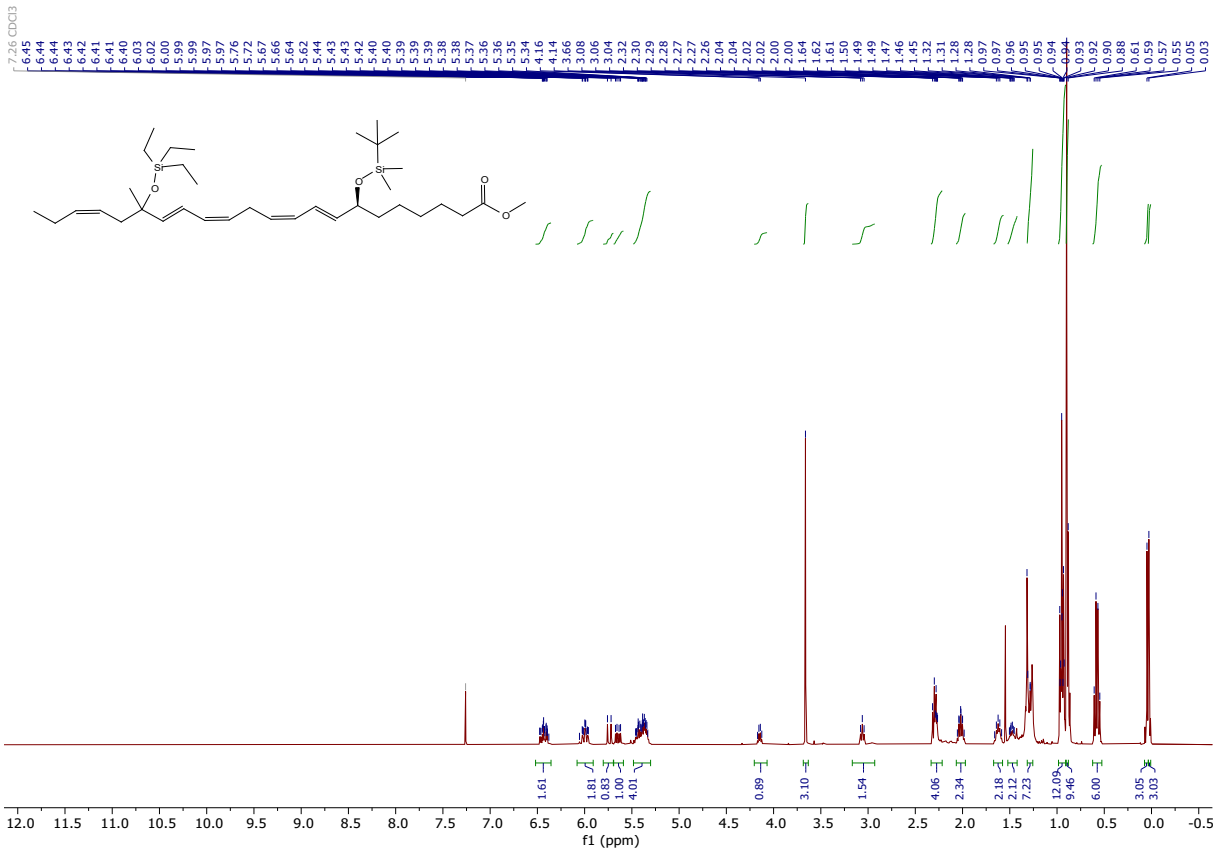



**Figure 13**: $^{1}$H NMR spectrum of compound **11**.

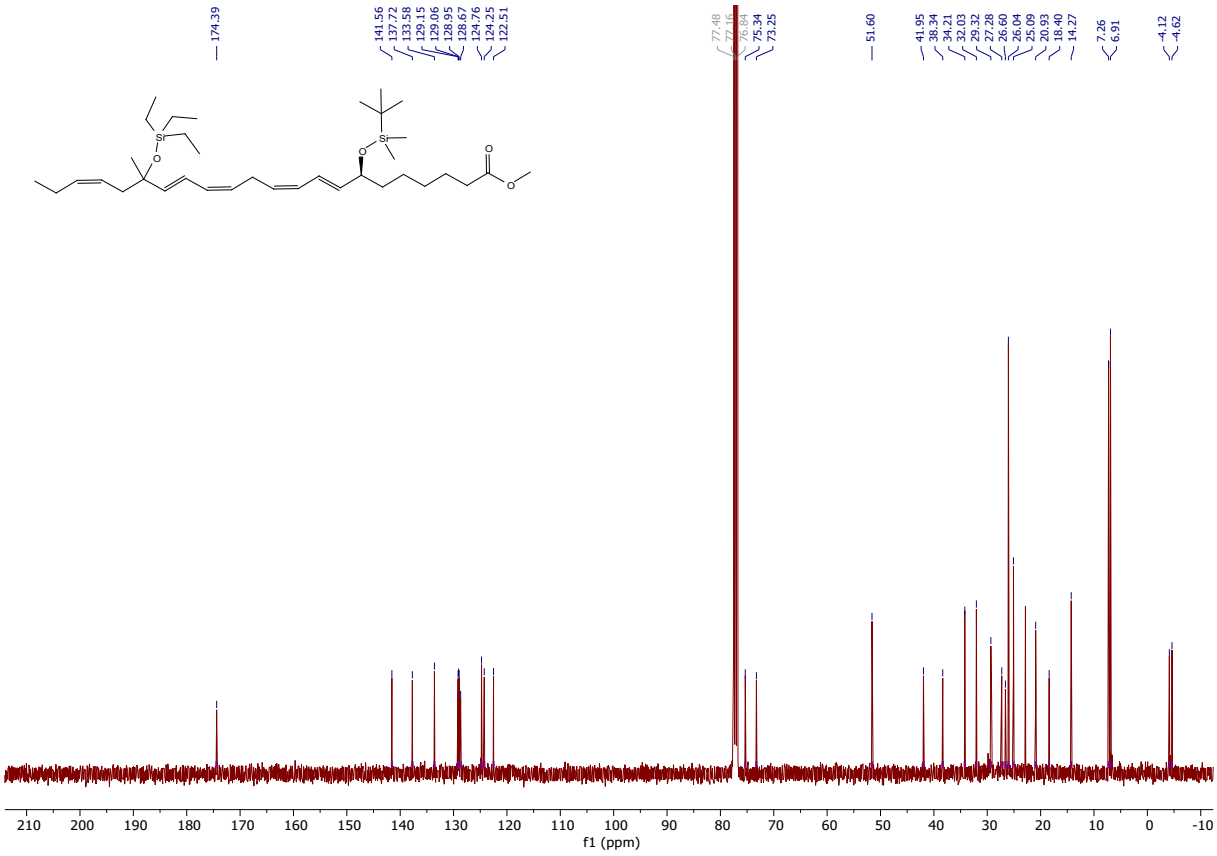



**Figure 14**: $^{13}$C NMR spectrum of compound **11**.

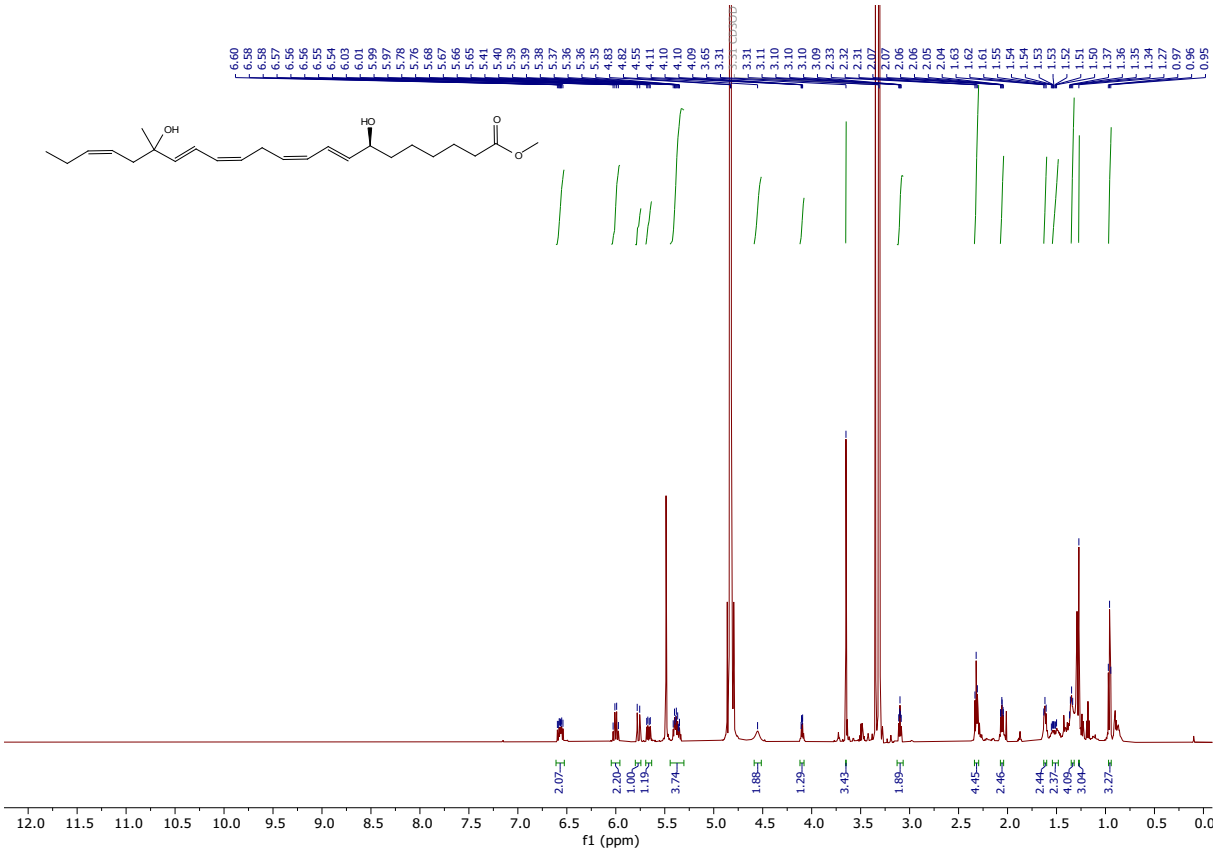



**Figure 15**: $^{1}H$ NMR spectrum of compound **12**.

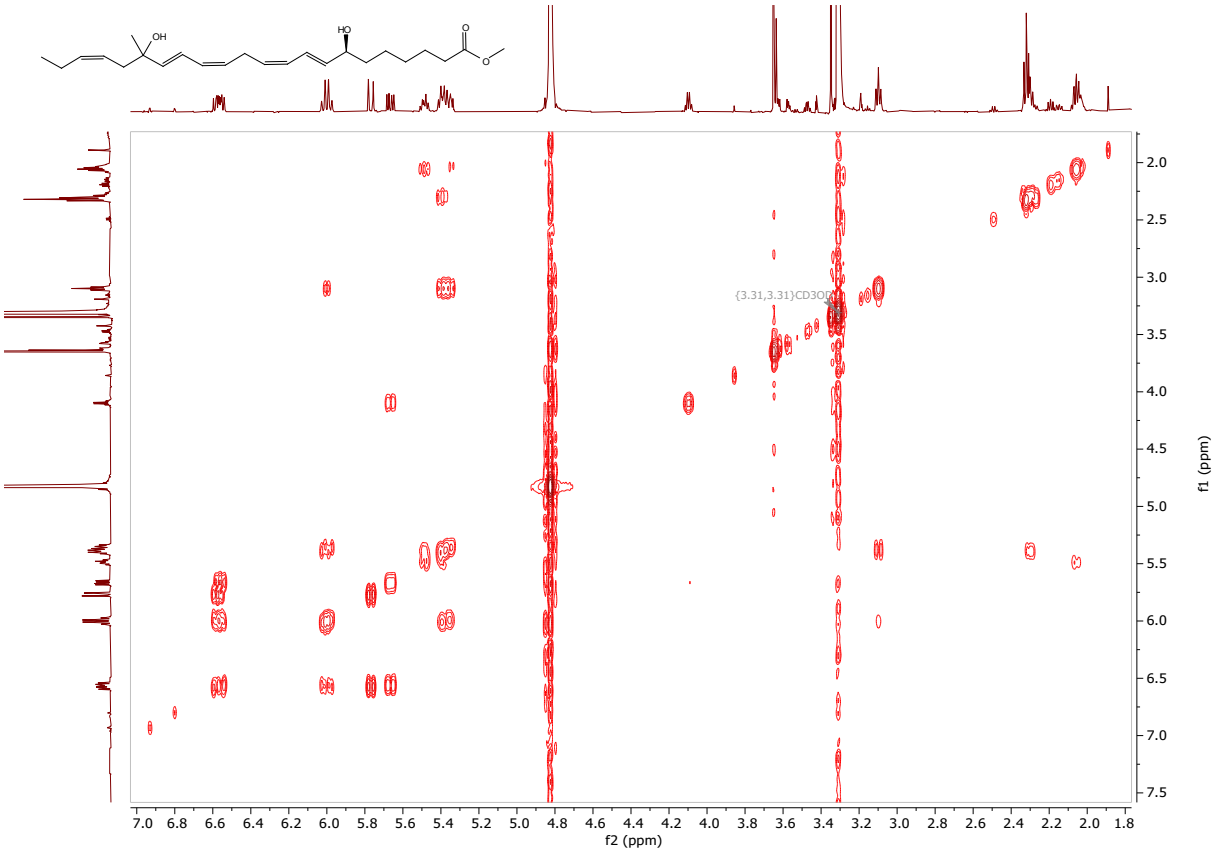



**Figure 16**: COSY NMR spectrum of compound **12**.

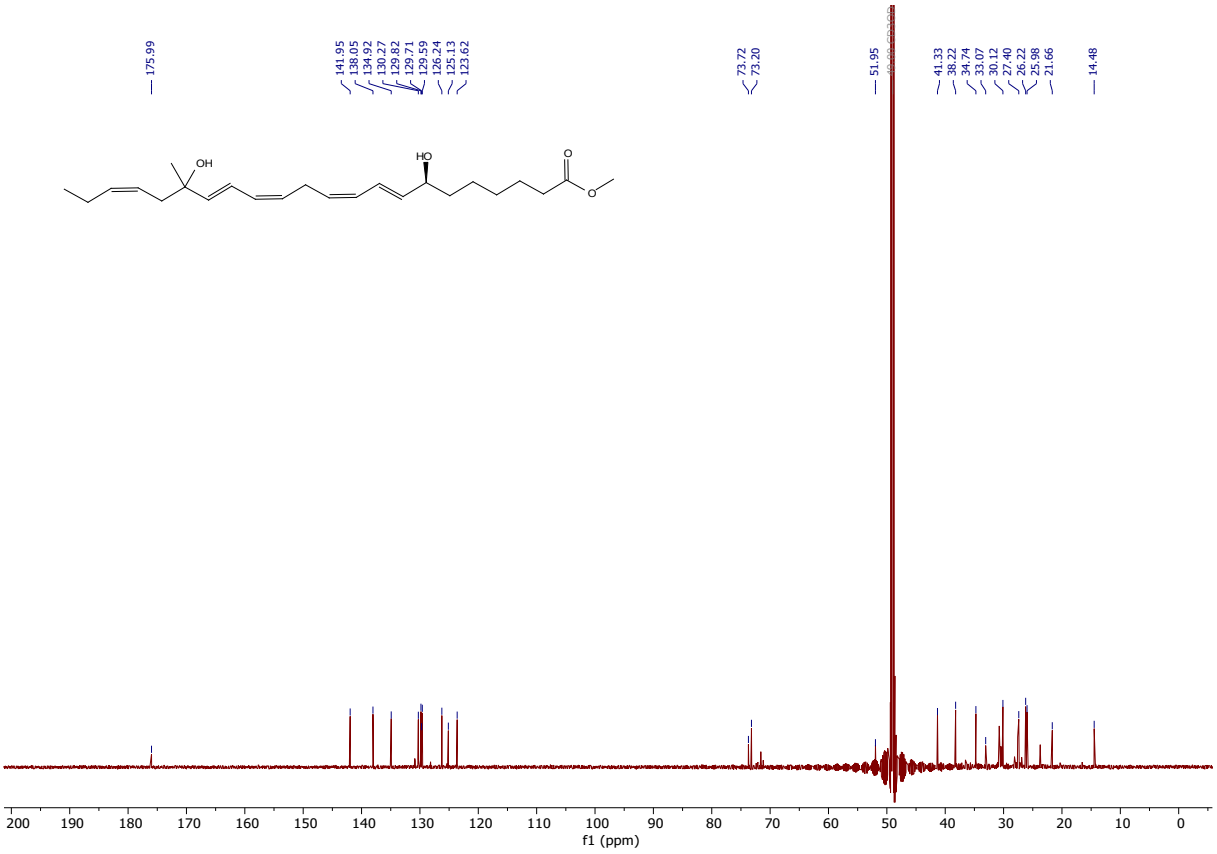



**Figure 17**: $^{13}C$ NMR spectrum of compound **12**.

**HRMS Spectra**

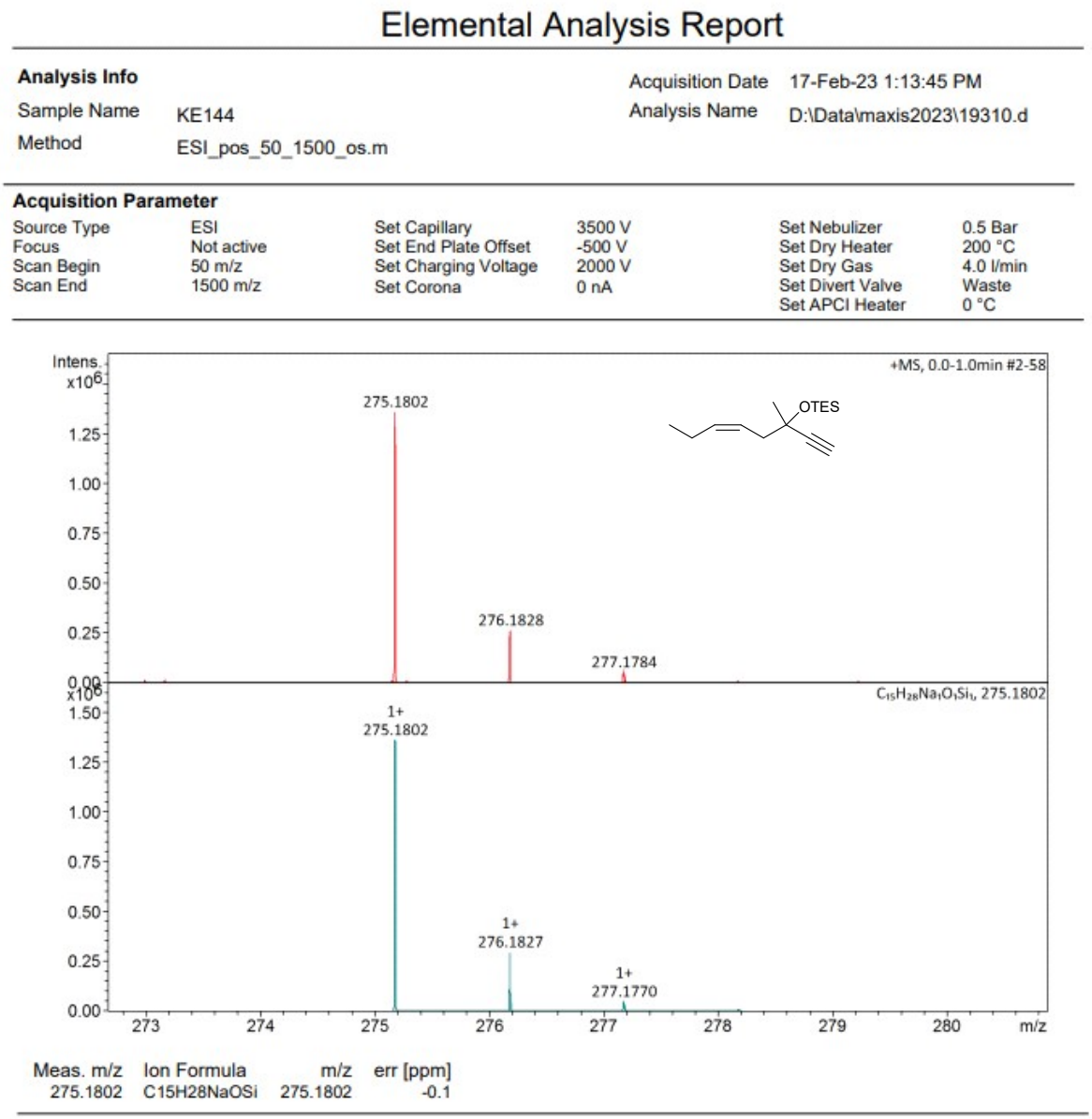



**Figure 18**: HRMS spectrum of compound **9**.

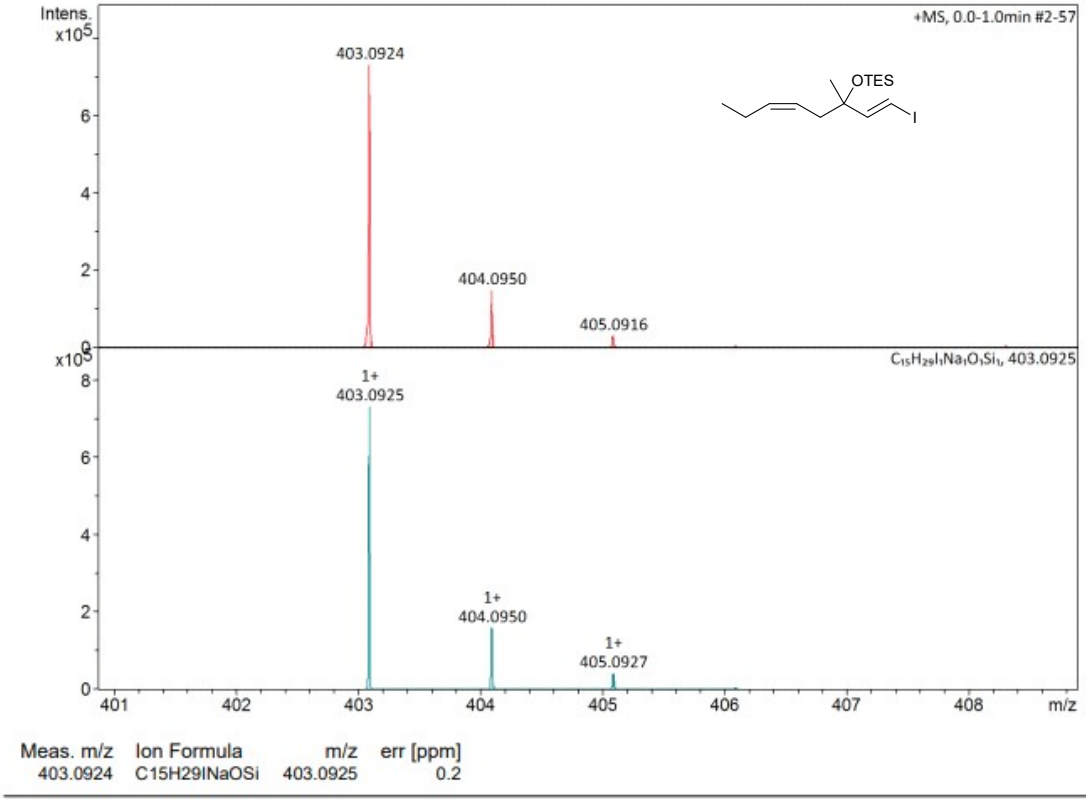
# Elemental Analysis Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Sample Name | KE131 | Acquisition Date | 15-Dec-22 3:05:43 PM |
| Method | ESI_pos_50_1500_os.m | Analysis Name | D:\Data\maxis2022\19198.d |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Set Capillary | 3500 V | Set Nebulizer | 0.5 Bar |
| Focus | Not active | Set End Plate Offset | -500 V | Set Dry Heater | 200 °C |
| Scan Begin | 50 m/z | Set Charging Voltage | 2000 V | Set Dry Gas | 4.0 l/min |
| Scan End | 1500 m/z | Set Corona | 0 nA | Set Divert Valve | Waste |
| | | | | Set APCI Heater | 0 °C |




| Meas. m/z | Ion Formula | m/z | err [ppm] |
|---|---|---|---|
| 403.0924 | C15H29INaOSi | 403.0925 | 0.2 |

**Figure 19**: HRMS spectrum of compound **3**.

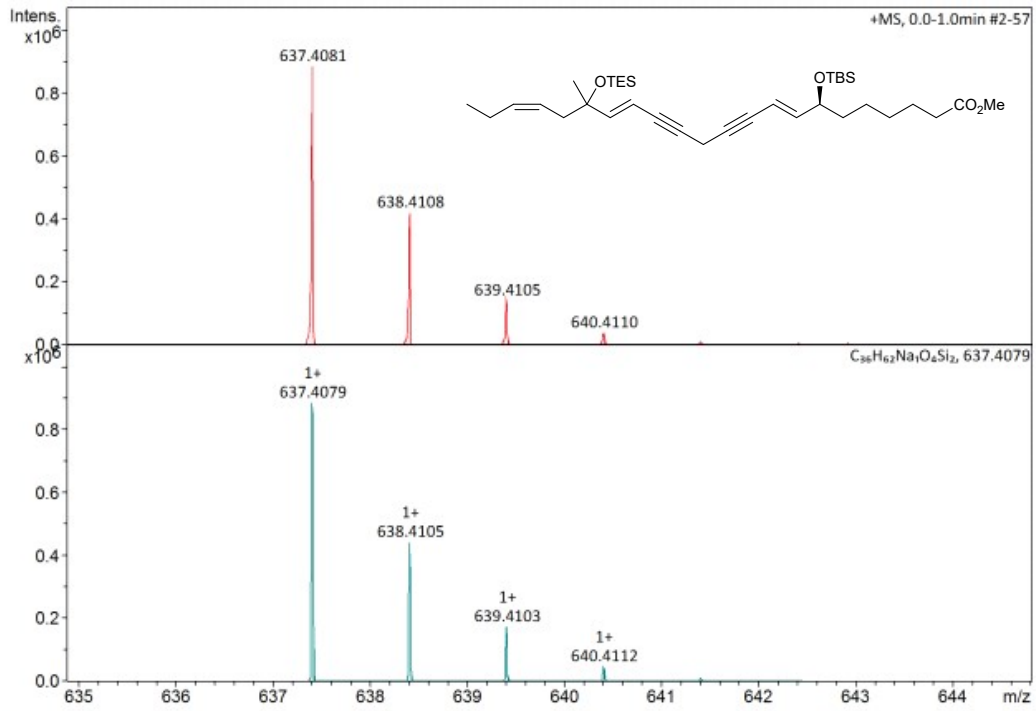
# Elemental Analysis Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Sample Name | KE135 | Acquisition Date | 15-Dec-22 3:25:11 PM |
| Method | ESI_pos_50_1500_os.m | Analysis Name | D:\Data\maxis2022\19199.d |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Set Capillary | 3500 V | Set Nebulizer | 0.5 Bar |
| Focus | Not active | Set End Plate Offset | -500 V | Set Dry Heater | 200 °C |
| Scan Begin | 50 m/z | Set Charging Voltage | 2000 V | Set Dry Gas | 4.0 l/min |
| Scan End | 1500 m/z | Set Corona | 0 nA | Set Divert Valve | Waste |
| | | | | Set APCI Heater | 0 °C |




| Meas. m/z | Ion Formula | m/z | err [ppm] |
|---|---|---|---|
| 637.4081 | C36H62NaO4Si2 | 637.4079 | -0.4 |

**Figure 20**: HRMS spectrum of compound **10**.

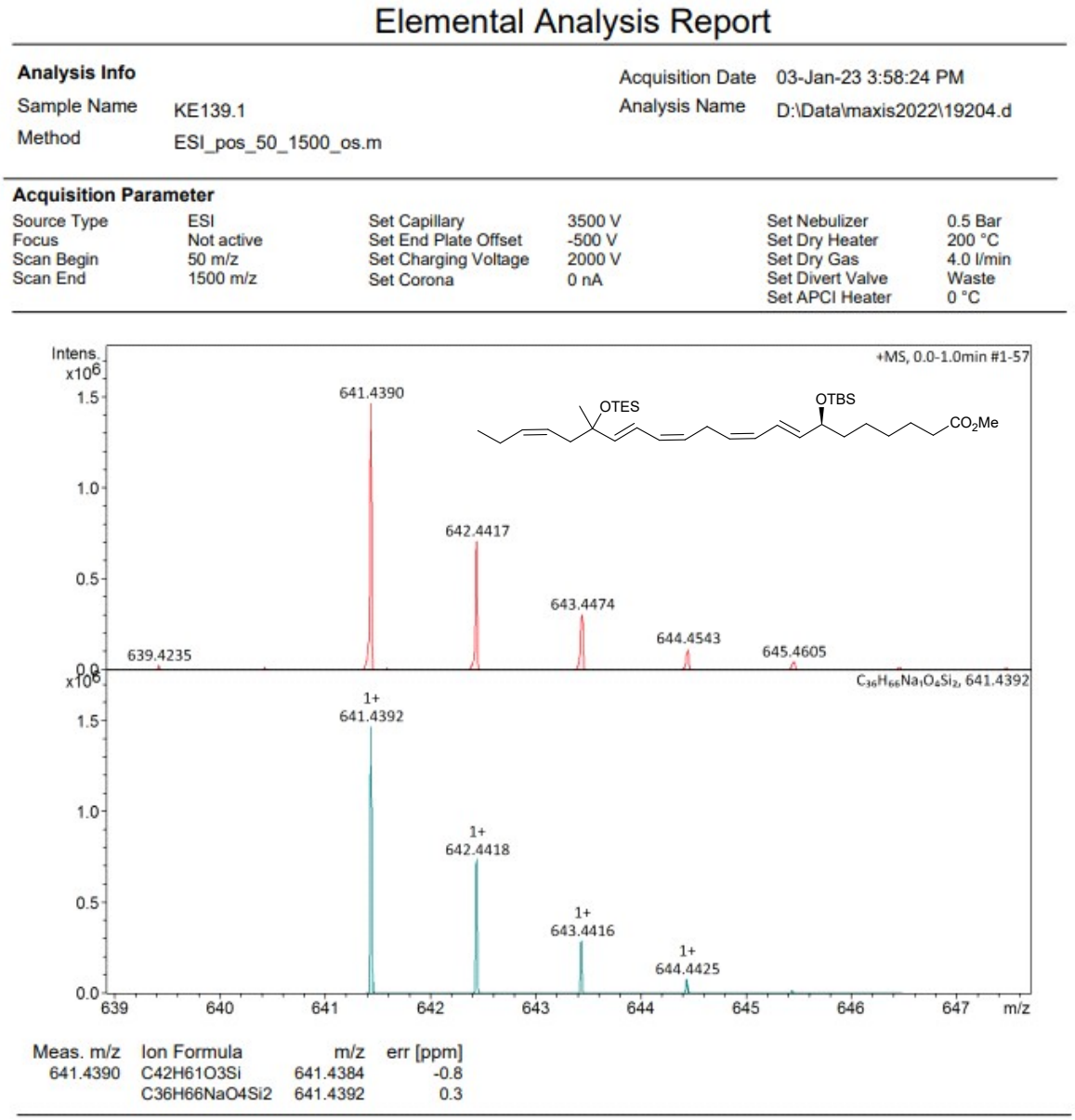



**Figure 21**: HRMS spectrum of compound **11**.

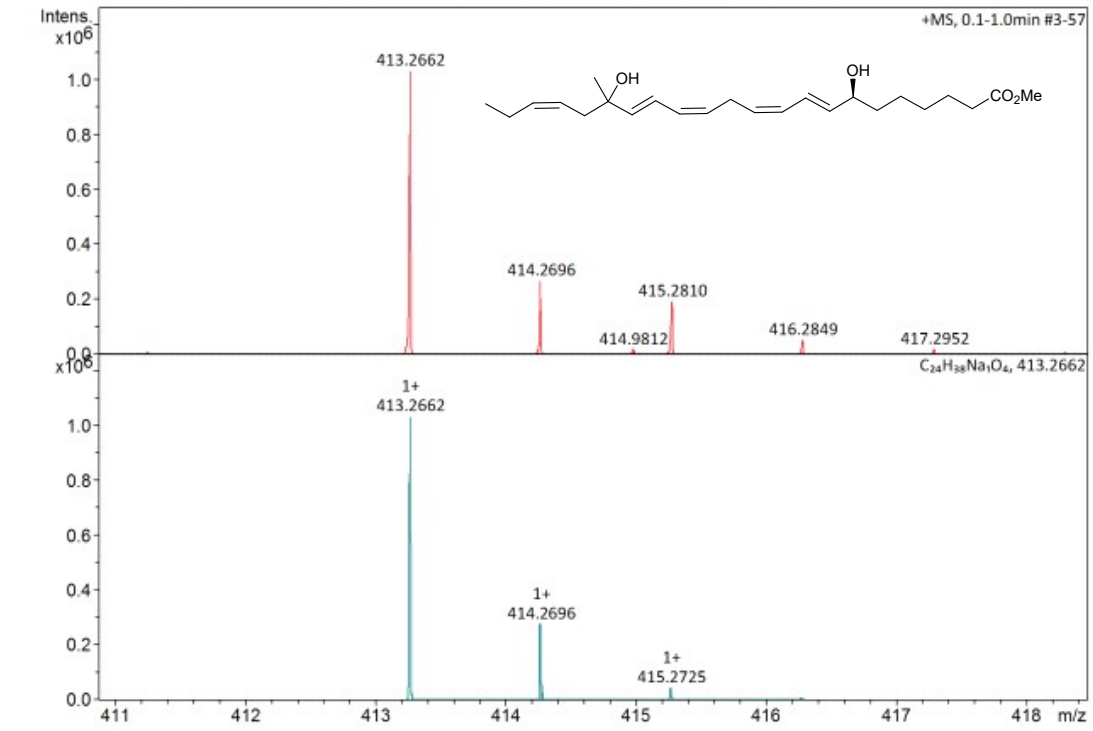
# Elemental Analysis Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Sample Name | KE142 | Acquisition Date | 20-Jan-23 1:54:36 PM |
| Method | ESI_pos_50_1500_os.m | Analysis Name | D:\Data\maxis2023\19234.d |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Set Capillary | 3500 V | Set Nebulizer | 1.0 Bar |
| Focus | Not active | Set End Plate Offset | -500 V | Set Dry Heater | 200 °C |
| Scan Begin | 50 m/z | Set Charging Voltage | 2000 V | Set Dry Gas | 4.0 l/min |
| Scan End | 1500 m/z | Set Corona | 0 nA | Set Divert Valve | Waste |
| | | | | Set APCI Heater | 0 °C |




| Meas. m/z | Ion Formula | m/z | err [ppm] |
|---|---|---|---|
| 413.2662 | C22H33N6O2 | 413.2660 | -0.5 |
| | C24H38NaO4 | 413.2662 | 0.2 |

**Figure 22**: HRMS spectrum of compound **12**.

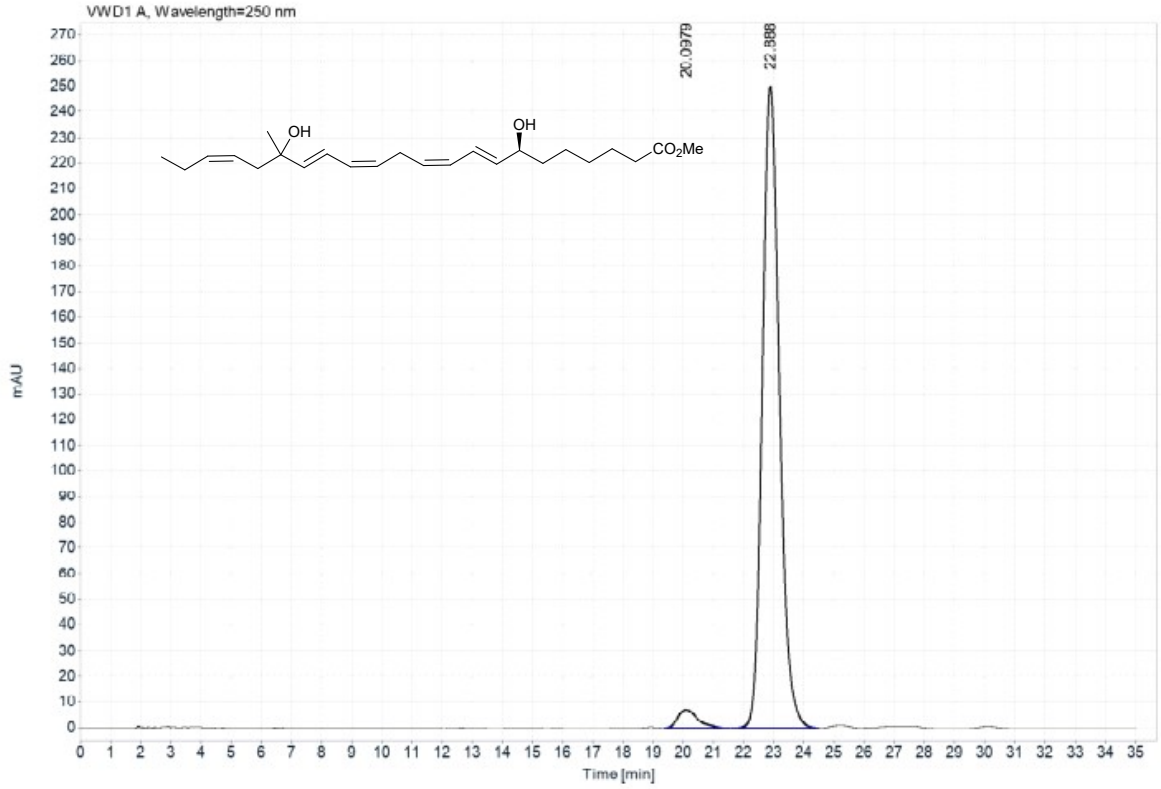
## HPLC chromatogram

**Area Percent Report**

Agilent Technologies

**Data file:** C:\Chem32\2\Data\Karina\KE142.3 2023-01-25 13-53-08.D
**Instrument:** LC1260-1
**Description:** 1 mL/min, C-18 MeOH/H2O 76/24, 250 nm, 10 uL injeksjonsvolum

| | | | |
|---|---|---|---|
| **Sample name:** | KE142.3 | **Injection date:** | 1/25/2023 1:53:51 PM |
| **Sample type:** | Sample | **Acq. method:** | C-18SPM-Methylester.M |
| **Location:** | P1-A-01 | **Analysis method:** | C-18SPM-Methylester.M |
| **Injection:** | 1 of 1 | | |
| **Injection volume:** | 10.000 | | |




**Signal:** VWD1 A, Wavelength=250 nm

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 20.098 | BB | 0.7200 | 316.4789 | 6.7751 | 3.1313 | |
| 22.888 | BB | 0.6080 | 9790.4648 | 249.9353 | 96.8687 | |
| | | Sum | 10106.9437 | | | |

**Figure 23**: HPLC chromatogram of compound **12**.

## UV-Vis spectra

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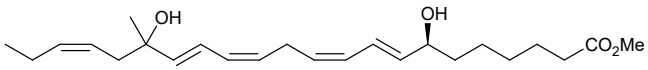
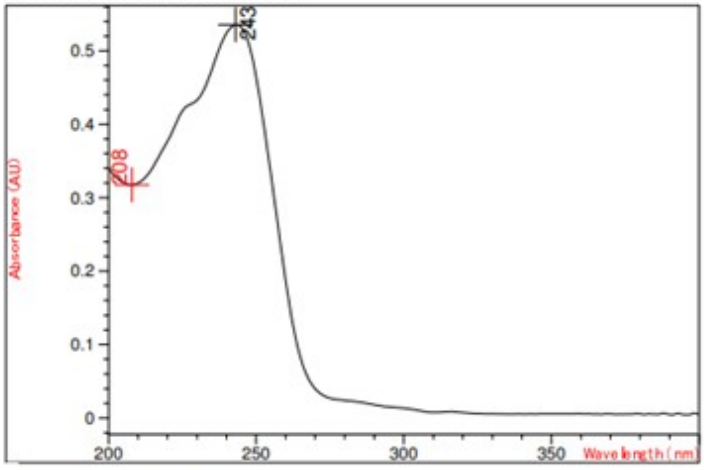



Overlaid Sample Spectra

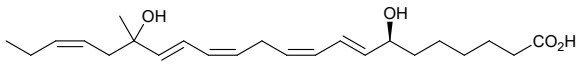



Sample/Result Table

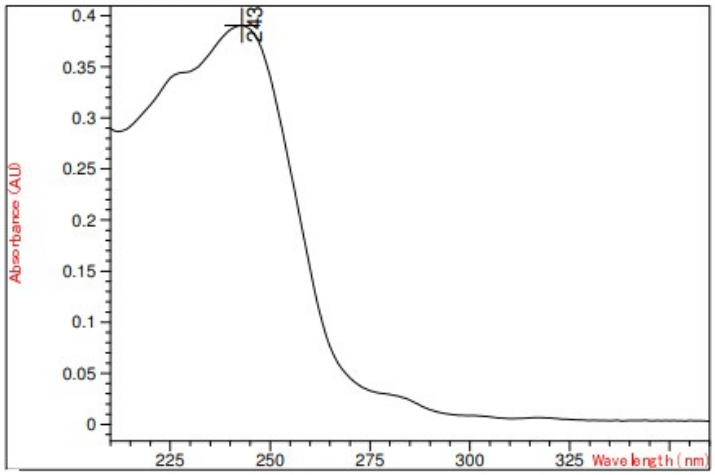
| # | Name | Peaks(nm) | Abs(AU) | Valleys(nm) | Abs(AU) |
|---|---|---|---|---|---|
| 1 | | 243.0 | 0.53536 | 208.0 | 0.31741 |
| 1 | | *** | *** | *** | *** |
| 1 | | *** | *** | *** | *** |

**Figure 24**: UV-Vis spectrum of compound **12**.

Hardcopy view Date 3/13/2024 Time 14:25:32 Page 1 of 1




Last Sample Spectrum




Sample/Result Table

| # | Name | Peaks(nm) | Abs(AU) |
|---|---|---|---|
| 1 | | 243.0 | 0.39041 |

*** End Hardcopy view ***

**Figure 25**. UV-Vis spectrum of compound **2**.

## References


1. J. Sueiras and W. H. Okamura, *J. Am. Chem. Soc.*, 1980, **102**, 6255-6259.
2. L. Zhang, N. Terrando, Z. Z. Xu, S. Bang, S. E. Jordt, W. Maixner, C. N. Serhan and R. R. Ji, *Front. Pharmacol.*, 2018, **9**, 412.
3. G. Chen, C. K. Park, R. G. Xie and R. R. Ji, *J. Clin. Invest.*, 2015, **125**, 3226-3240.
4. J. R. Deuis, L. S. Dvorakova and I. Vetter, *Front. Mol. Neurosci.*, 2017, **10**, 284.