Supporting information for
Synthesis of the 16*S*,17*S*-epoxy-Protectin Intermediate in the Biosynthesis of Protectins by Human Macrophages

Marius Aursnes,[†] Jørn E. Tungen,[†] Romain A. Colas,[‡] Iliyan Vlasakov,[‡] Jesmond Dalli,[‡] Charles N. Serhan,[*,‡] and Trond V. Hansen[*,†]

[†]School of Pharmacy, Department of Pharmaceutical Chemistry, University of Oslo, PO Box 1068 Blindern, N-0316 Oslo, Norway.
*E-mail: t.v.hansen@farmasi.uio.no

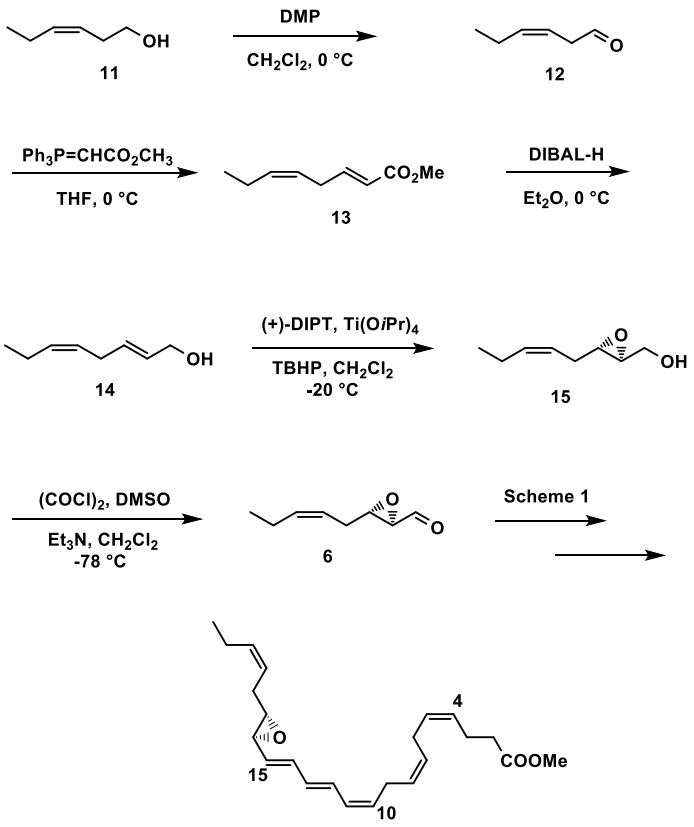
[‡]Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, 02115.
[*]E-mail: cnserhan@zeus.bwh.harvard.edu

Table of Contents:

General Information

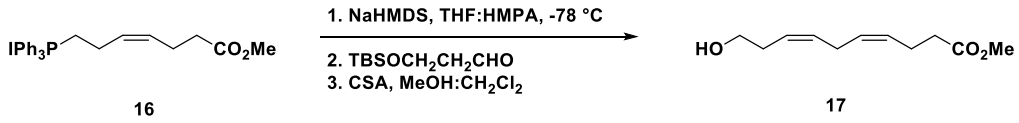
Optical rotations were measured using a 1 mL cell with a 1.0 dm path length on a Perkin Elmer 341 polarimeter. The UV/Vis spectra from 190-900 nm were recorded using a Biochrom Libra S32PC spectrometer using quartz cuvettes. NMR spectra were recorded on a Bruker AVII400 or on a Bruker AVII 600 spectrometer at 400 MHz or at 600 MHz for ^{1}H NMR, and at 101 MHz or at 151 MHz for ^{13}C NMR. Spectra are referenced relative to the central residual protium solvent resonance in ^{1}H NMR ($CDCl_3$ δ = 7.27, C_6D_6 δ = 7.16 ppm and MeOH-d_4 δ = 3.31) and the central carbon solvent resonance in ^{13}C NMR ($CDCl_3$ δ = 77.00 ppm, C_6D_6 δ = 128.06 ppm, MeOH-d_4 = δ 49.00). Mass spectra were recorded at 70 eV on a Waters Prospec Q spectrometer using EI, ES or CI as the method of ionization. High-resolution mass spectra were recorded on a Waters Prospec Q spectrometer using EI or ES as the methods of ionization. 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) produced by Merck. HPLC analyses for chemical purities were performed on an Agilent Technologies 1200 Series instrument with diode array detector set at 254 nm and equipped with a C18 stationary phase (Eclipse XDB-C18 5 μm 4.6 × 150 mm), applying the conditions stated. GLC analyses were performed on an Agilent 7820A with a FID detector, HP-5 capillary column, with helium as the carrier gas and by applying the conditions stated. Unless stated otherwise, all commercially available reagents and solvents were used in the form they were supplied without any further purification. The stated yields are based on isolated material. Diastereomeric ratios reported in this paper have not been validated by calibration, see Wernerova and Hudlicky for discussions and guidelines.[1]

(2*R*,3*S*)-3-((*Z*)-Pent-2-en-1-yl)oxirane-2-carbaldehyde (6)



The epoxy alcohol **15** was prepared from commercially available (*Z*)-hex-3-en-1-ol (**11**) and oxidized to the aldehyde **6** as previously reported.[2] Overall, **6** was obtained in five steps and in 16% overall yield (0.85 g) and 94% *ee*. All spectroscopic and physical data of **6** were in agreement with those reported in the literature.[2,3] [α] = -16.9 (c = 1.27, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (d, *J* = 6.3 Hz, 1H), 5.63 – 5.55 (m, 1H), 5.36 – 5.28 (m, 1H), 3.27 (td, *J* = 5.1, 1.9 Hz, 1H), 3.17 (dd, *J* = 6.3, 1.9 Hz, 1H), 2.54 – 2.37 (m, 2H), 2.05 (p, *J* = 7.4, 6.9 Hz, 2H), 0.98 (t, *J* = 7.5 Hz, 3H), ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.5, 136.3, 120.9, 58.7, 56.1, 28.8, 20.9, 14.2. TLC (hexanes/EtOAc 7:3, $KMnO_4$ stain): R_f = 0.51.

Methyl (4*Z*,7*Z*)-10-hydroxydeca-4,7-dienoate (17)

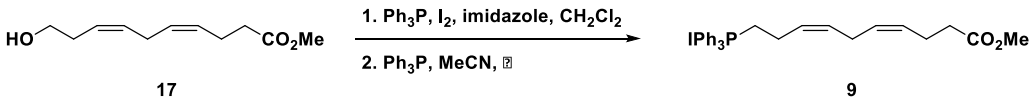


The known Wittig salt (**16**)[4] (800 mg, 1.51 mmol, 1.0 equiv.) was dissolved in THF (20 mL) and HMPA (2 mL) and cooled to -78 °C. NaHMDS (0.6 M in toluene, 2.51 mL, 1.51 mmol, 1.0 equiv.) was added dropwise and the reaction mixture was stirred until essentially homogeneous. TBS-protected 3-hydroxy-propanal (298 mg, 1.58 mmol, 1.05 equiv) dissolved in THF (2 mL) was added dropwise and the reaction mixture was stirred for 1.5 h. The flask was allowed to slowly warm up to 0 °C and then the reaction was quenched with phosphate buffer (10 mL, pH = 7.2), extracted with Et_2O (3 x 15 mL) and the combined organic phases were dried (Na_2SO_4). The suspension was filtrated, concentrated *in vacuo* and the crude material was passed through a short plug of silica gel eluting with 5% EtOAc in heptane (R_f = 0.21). Crude 3-((*tert*-butyldimethylsilyl)oxy)propanal (260 mg, 0.83

mmol, 1.0 equiv.) was taken up in CH_2Cl_2:MeOH (1:2, 10 mL) and cooled to 0 °C. Camphor-10-sulfonic acid (193 mg, 0.83 mmol, 1.0 equiv) was added in one portion and the reaction mixture was stirred for 30 min before it was allowed to warm up to room temperature and stirred for an hour. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (3 x 10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The material thus obtained was purified by flash column chromatography on silica gel eluting with 30% EtOAc in heptane to yield methyl (4*Z*,7*Z*)-10-hydroxydeca-4,7-dienoate (**17**) (140 mg, 0.71 mmol, 47% over two steps) as a colourless oil. ^{1}H NMR (400 MHz, MeOH) δ 5.55 – 5.31 (m, 4H), 3.68 (s, 3H), 3.58 (t, J = 6.9 Hz, 2H), 2.93 – 2.81 (m, 2H), 2.40 (d, J = 2.6 Hz, 4H), 2.37 – 2.29 (m, 2H), ^{13}C NMR (150 MHz, MeOD) δ 173.9, 129.4, 129.0, 128.9, 127.5, 125.7, 61.3, 50.6, 33.4, 33.4, 30.4, 25.1, 22.4, 22.4. HRESTOFMS *m/z* 221.1156 $[M+Na]^+$ (calcd for $C_{11}H_{18}O_3Na$, 221.1153); TLC (heptanes/EtOAc 7:3, $KMnO_4$ stain): R_f = 0.13. The chemical purity (>97%) was determined with GLC analysis: Initial temperature 100 °C, rate 6 °C/min, final temperature 200 °C, retention times: 7.692 min (major), 7.795 min and 7.927 min.

Note: Under the basic reaction conditions, some of the aldehyde undergoes an E1cB elimination to furnish acrolein, which then subsequently reacts with the corresponding ylide of the Wittig salt employed, giving a biproduct with an identical R_f-value to that of the desired product methyl (4*Z*,7*Z*)-10-hydroxydeca-4,7-dienoate. Consequently, this ought to be kept in mind when following the progress of the subsequent TBS-deprotection reaction by TLC analysis.

Methyl (4*Z*,7*Z*)-10-(iodotriphenyl-λ^5-phosphanyl)deca-4,7-dienoate (9)



17 **9**

The hydroxy ester **17** (100 mg, 0.50 mmol, 1.0 equiv.), triphenylphosphine (198 mg, 0.76 mmol, 1.5 equiv.) and imidazole (52 mg, 0.76 mmol, 1.5 equiv.) was dissolved in dichloromethane (5 mL) and cooled to approximately -10 °C using a brine/ice cooling bath. Next, iodine (192 mg, 0.76 mmol, 1.5 equiv.) was added in one portion and the reaction mixture was stirred rapidly until deemed complete by TLC. Next, solid sodium bisulfite (100 mg) was added, followed by a small amount of water. The resulting mixture was stirred for 10 minutes and then dried by the addition of sodium sulfate. Using a Pasteur pipette, the solvent part was added to a short plug of silica and the solid residue was washed several times with 15% Et_2O in heptane with the washings added to the plug. The product was next eluted out of the plug using 15% Et_2O, concentrated *in vacuo* and taken up in acetonitrile (7.4 mL), to which triphenylphosphine (265 mg, 1 mmol, 2 equiv.) was then added. The reaction setup was thoroughly evacuated and re-filled with argon (3x) and thereafter heated to reflux overnight. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The resulting material was purified by flash column chromatography (CH_2Cl_2 until all triphenylphosphine was out of the column followed by 5% MeOH in CH_2Cl_2) to yield a viscous clear oil which was azeotroped three times with 2-methyltetrahydrofuran to provide the Wittig salt **9** in 67% yield (192 mg) over two steps as an oil which solidified in the freezer. ^{1}H NMR (600 MHz, MeOD) δ 7.95 – 7.89 (m, 5H), 7.89 – 7.83 (m, 5H), 7.82 – 7.74 (m, 5H), 5.54 – 5.47 (m, 2H), 5.36 – 5.30 (m, 2H), 3.62 (s, 3H), 3.51 (ddd, J = 16.2, 13.1, 7.8 Hz, 2H), 2.74 – 2.66 (m, 2H), 2.49 (ddd, J = 15.9, 9.8, 5.8 Hz, 2H), 2.32 (t, J = 7.1 Hz, 2H), 2.26 – 2.20 (m, 2H), ^{13}C NMR (151 MHz, MeOD) δ 175.2, 136.4 (d, $^4J_{CP}$ = 2.9 Hz, 6C), 134.9 (d, $^3J_{CP}$ = 10.1 Hz, 6C), 131.8, 131.6 (d, $^2J_{CP}$ = 12.6 Hz, 6C), 129.5 (2C), 127.5 (d, $^3J_{CP}$ = 19.9 Hz), 119.8 (d, $^1J_{CP}$ = 86.3 Hz), 52.1, 34.6, 26.5, 23.8, 22.8 (d, $^2J_{CP}$ = 49.3 Hz), 21.4 (d, $^2J_{CP}$ = 3.3 Hz). HRESTOFMS *m/z* 443.2134 $[M]^+$ (calcd for $C_{29}H_{32}O_2P^+$,443.2139); TLC (CH_2Cl_2/MeOH 95:5, $KMnO_4$ stain): R_f = 0.27.

Hydrolysis of methyl ester 10 prior to incubations and UV measurements with 16*S*,17*S*-epoxy-protectin (5) for half-life determination

A stock solution of 100 μg of **10** in hexane/Et_2O was dried under a gentle stream of nitrogen and then dissolved in 500 μL of THF and cooled to -78 ^oC using a dry ice/acetone cooling bath. Then 100 μL of aqueous 1.0 M LiOH solution was slowly added via a Hamilton syringe at -78 ^oC. Then the vial was covered with aluminium foil and left stirring for 10 hrs. The vial was put on solid dry ice and the solution above the lithium salt was gently removed by using a syrringe, then the solution dried under a gentle stream of nitrogen before quantification using UV (hexane) λ_{max} 272 (log ε 3.61), 280 (log ε 4.05), 291 (log ε 2.79) nm. The THF solution of **5** was suspended in 50 μL $PBS^{+/+}$ pH = 7.45. The PBS solution was kept on solid dry ice in a closed container prior to use, and was used for incubation experiments. The structure of **5** was determined indirectly by using UV and LC/MS-MS experiments, see Figures 2-5 in the article. All attempts to obtain high quality NMR data of isolated 16*S*,17*S*-epoxy-protectin (**5**) in deuterated solvents suchs as C_6D_6, D_8C_7, C_8D_4 failed due to decomposition of **5**. These observations are in accordance with what Brash and co-workers observed when they recently reported the NMR spectra of methyl esters of novel leukotrienes.[5]

UV measurements with 16*S*,17*S*-epoxy-protectin (5) for half-life determination

The PBS solution mentioned above was used for half-life determination of **5**. It is important to keep this solution on solid dry ice in a closed container prior to use. For UV measurements, 2 μLof the PBS solution was added to a quartz cuvette containing 48 μL MeOH. The UV-spectra was recorded over the range of 200 to 400 nm.

Hydrolysis of methyl ester of LTA_4 prior to incubations with LTA_4 and UV measurements

The methyl ester of LTA_4 was purchased from Cayman Chemical Company, product number 20010, chemical purity >97%. Please note the following from the vendor: "LTA_4 as a free acid is highly unstable. The methyl ester is stable and can be readily hydrolyzed to the free acid as needed."

A stock solution of 100 μg of the methyl ester of LTA_4 in hexane containing 1% triethyl amine was dried under a gentle stream of nitrogen and then dissolved in 500 μL of THF and cooled to -78 ^oC using a dry ice/acetone cooling bath. Then 100 μL of aqueous 1.0 M LiOH solution was slowly added via a Hamilton syringe at -78 ^oC. Then the vial was covered with aluminium foil and left stirring for 10 hrs. The vial was put on solid dry ice and the solution above the lithium salt was gently removed, then the solution dried under a gentle stream of nitrogen before quantification using UV (hexane) λ_{max} 269 (log ε 3.55), 278 (log ε 4.01), 287 (log ε 2.70) nm using using 1 μL of the hexane solution and comparing the data with literature.[6] The THF solution of LTA_4 was suspended in 50 μL $PBS^{+/+}$ pH = 7.45. The PBS solution was kept on solid dry ice in a closed container prior to use, and the PBS solution was used for incubation experiments.

UV measurements with LTA_4 for half-life determination and UV measurements

The PBS solution mentioned above was used for half-life determination of LTA_4. It is important to keep this solution on solid dry ice in a closed container prior to use. For UV measurements, 2 μLof the PBS solution was added to a quartz cuvette containing 48 μL MeOH. The UV-spectra was recorded over the range of 200 to 400 nm.

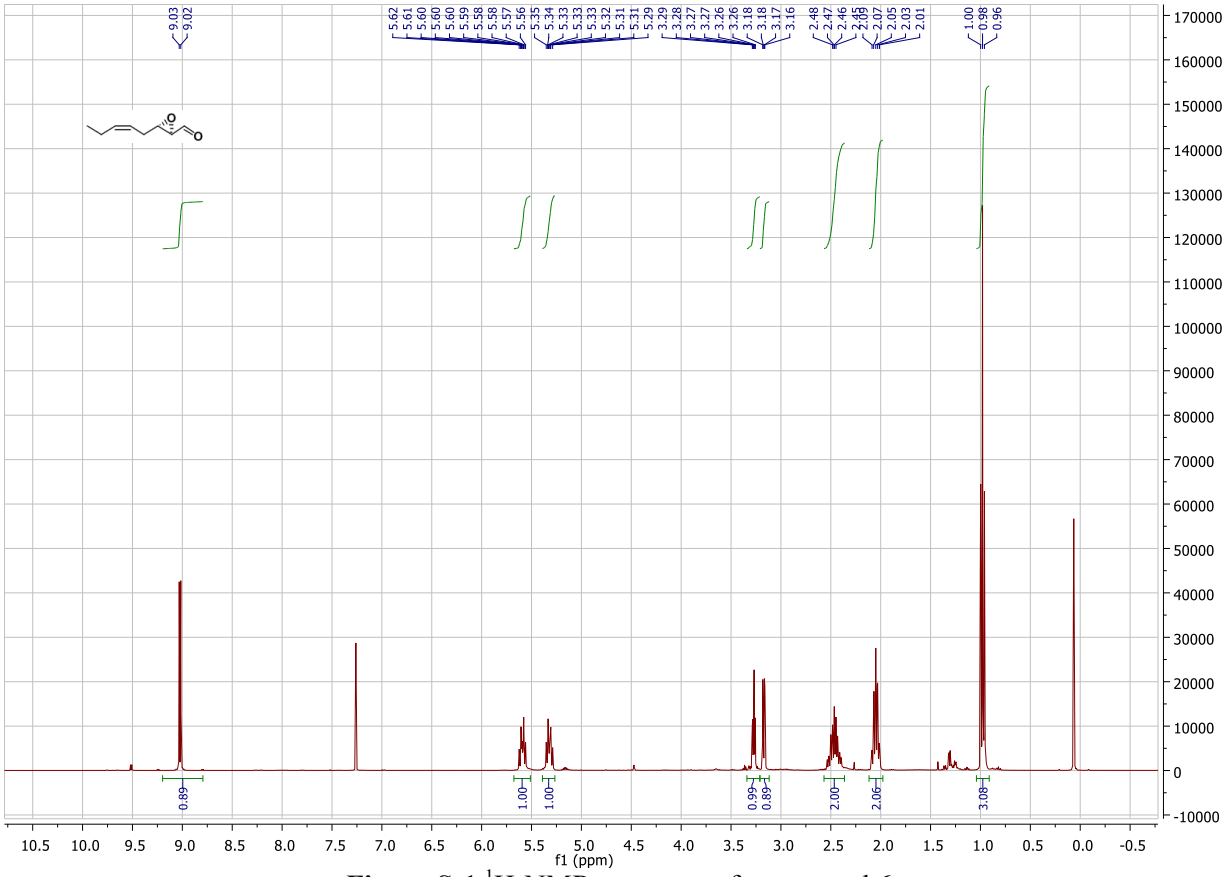


Figure S-1 ^{1}H-NMR spectrum of compound **6**.

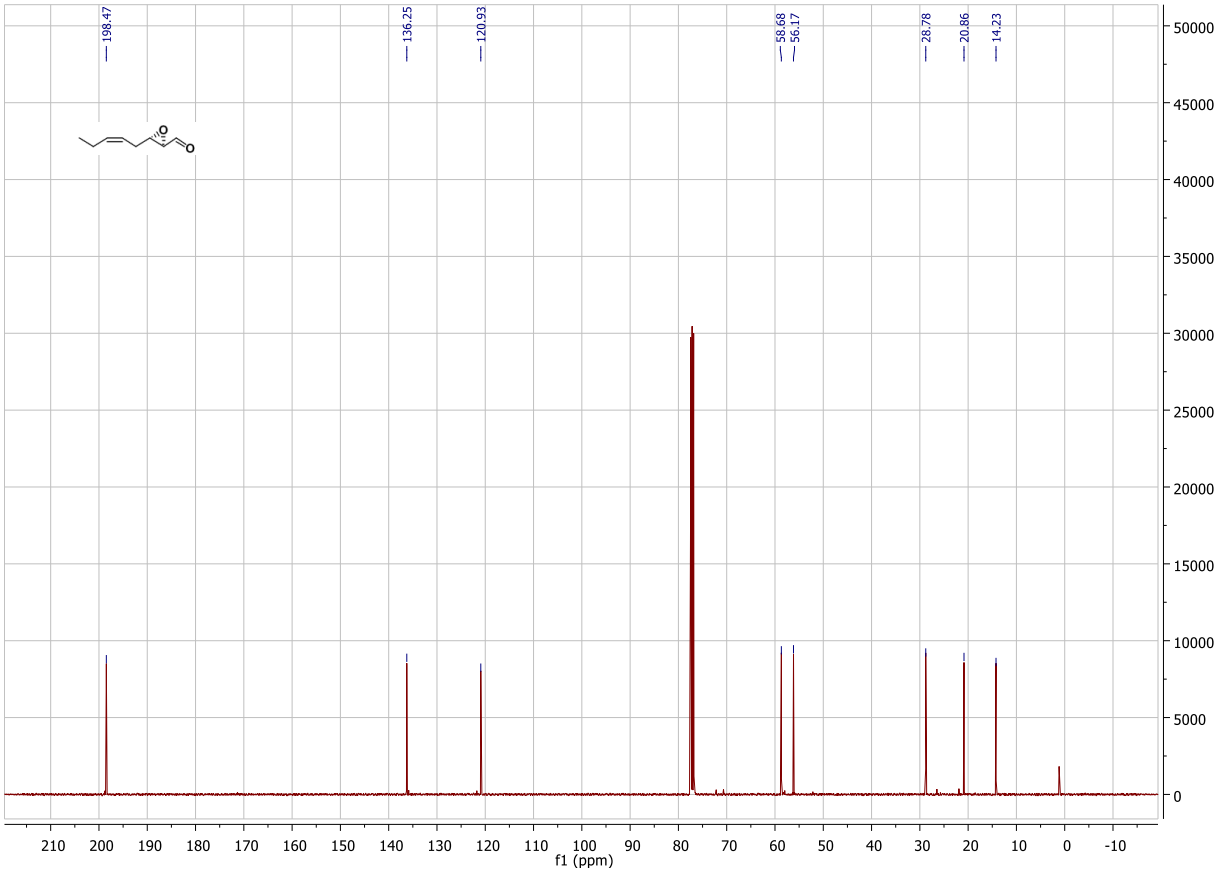


Figure S-2 ^{13}C-NMR spectrum of compound **6**.

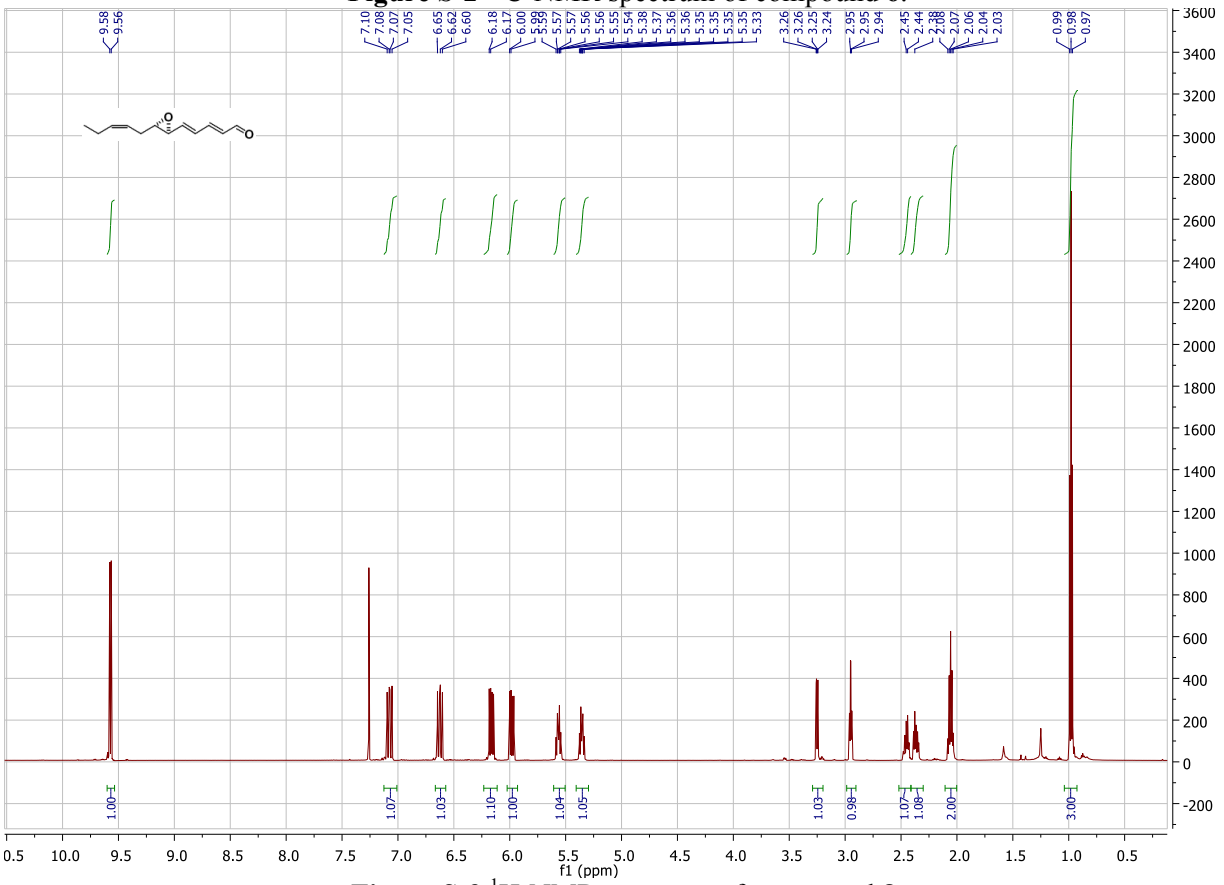


Figure S-3 ^{1}H-NMR spectrum of compound **8**.

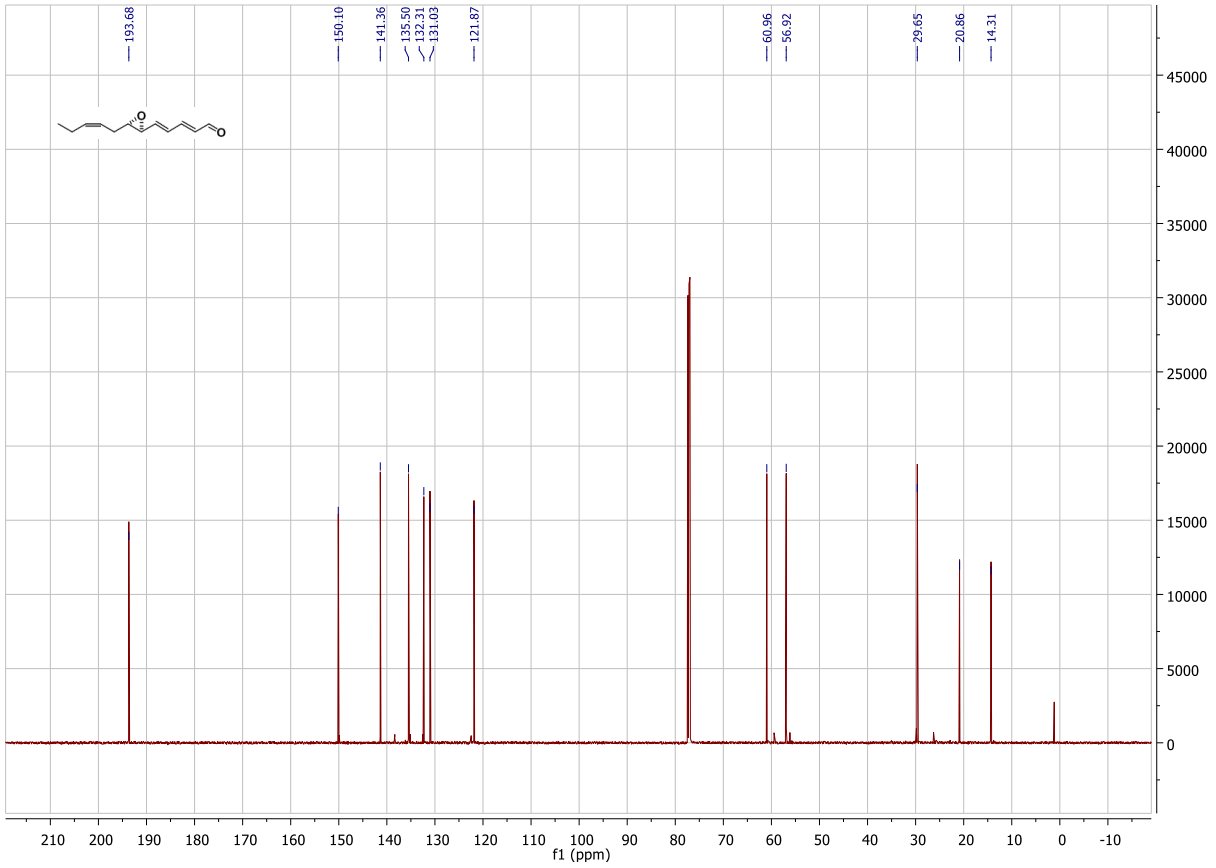


Figure S-4 ^{13}C-NMR spectrum of compound **8**.

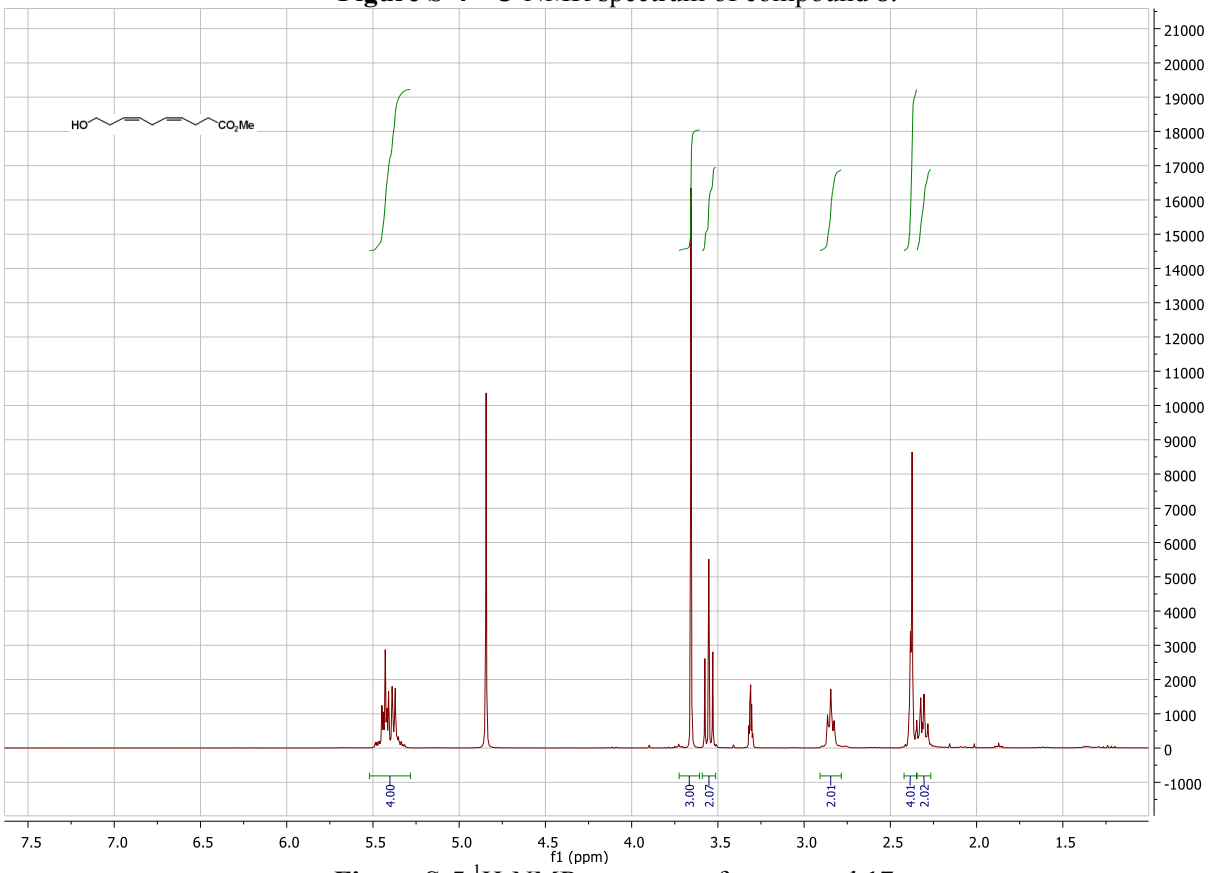
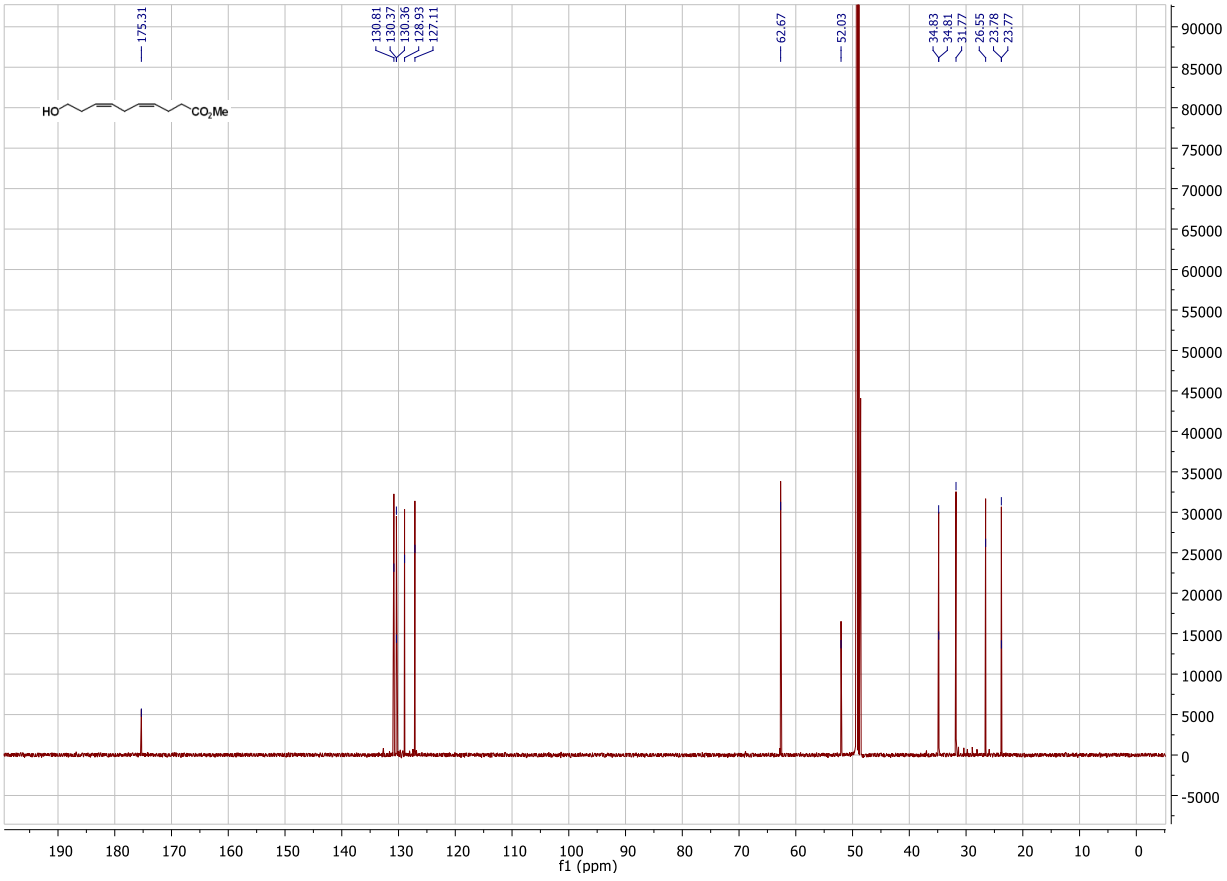


Figure S-5 ^{1}H-NMR spectrum of compound **17**.



Fgure S-6 ^{13}C-NMR spectrum of compound **17**.

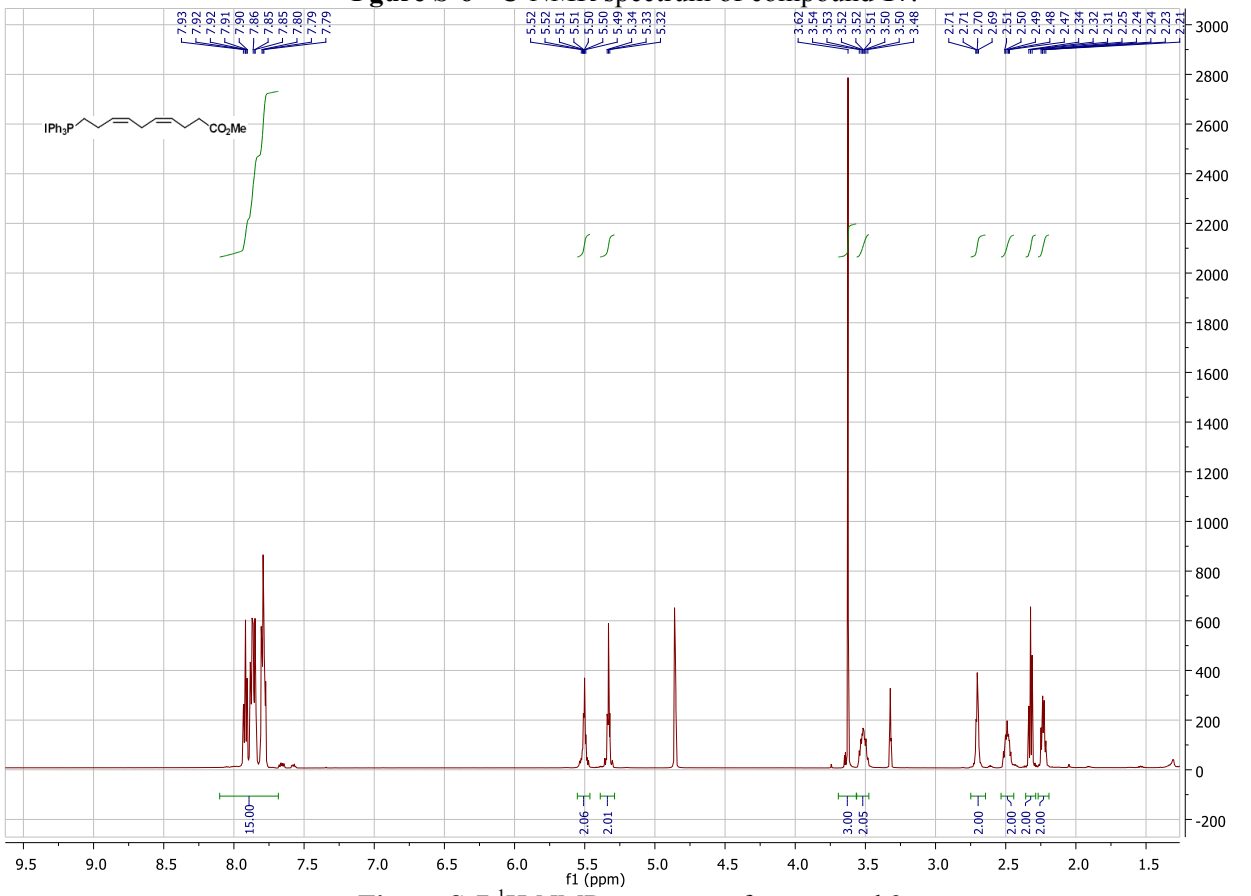


Figure S-7 ^{1}H-NMR spectrum of compound **9**.

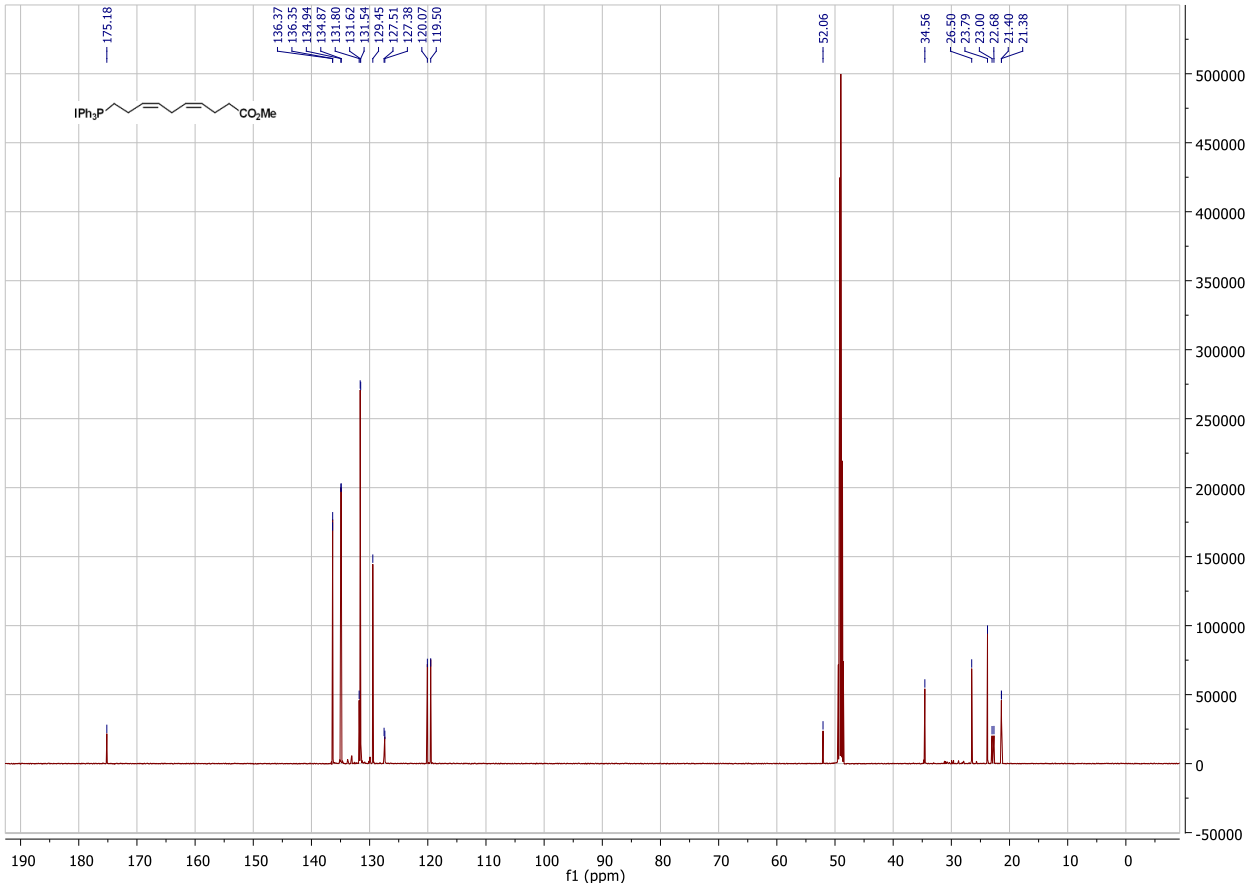


Figure S-8 ^{13}C-NMR spectrum of compound **9**.

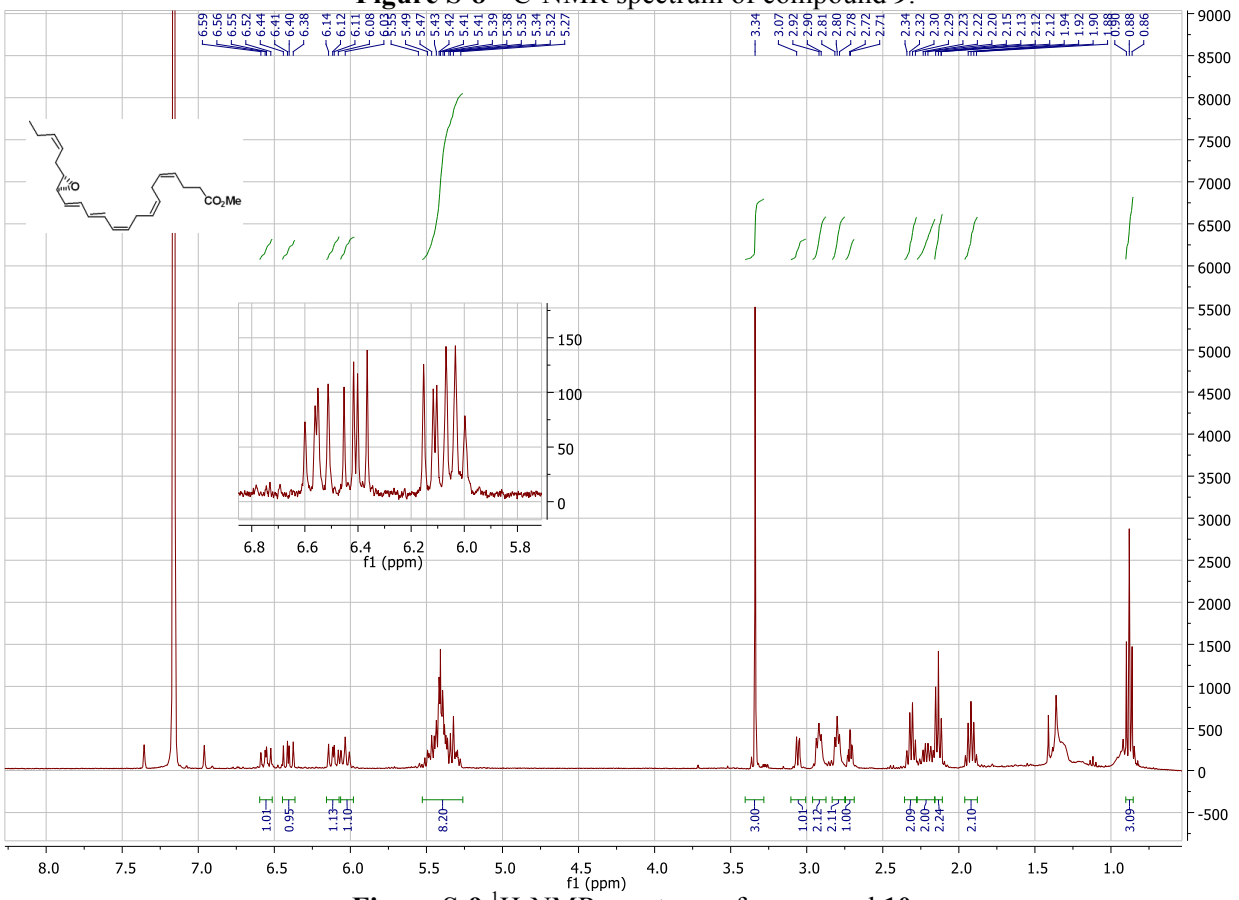


Figure S-9 ^{1}H-NMR spectrum of compound **10**.

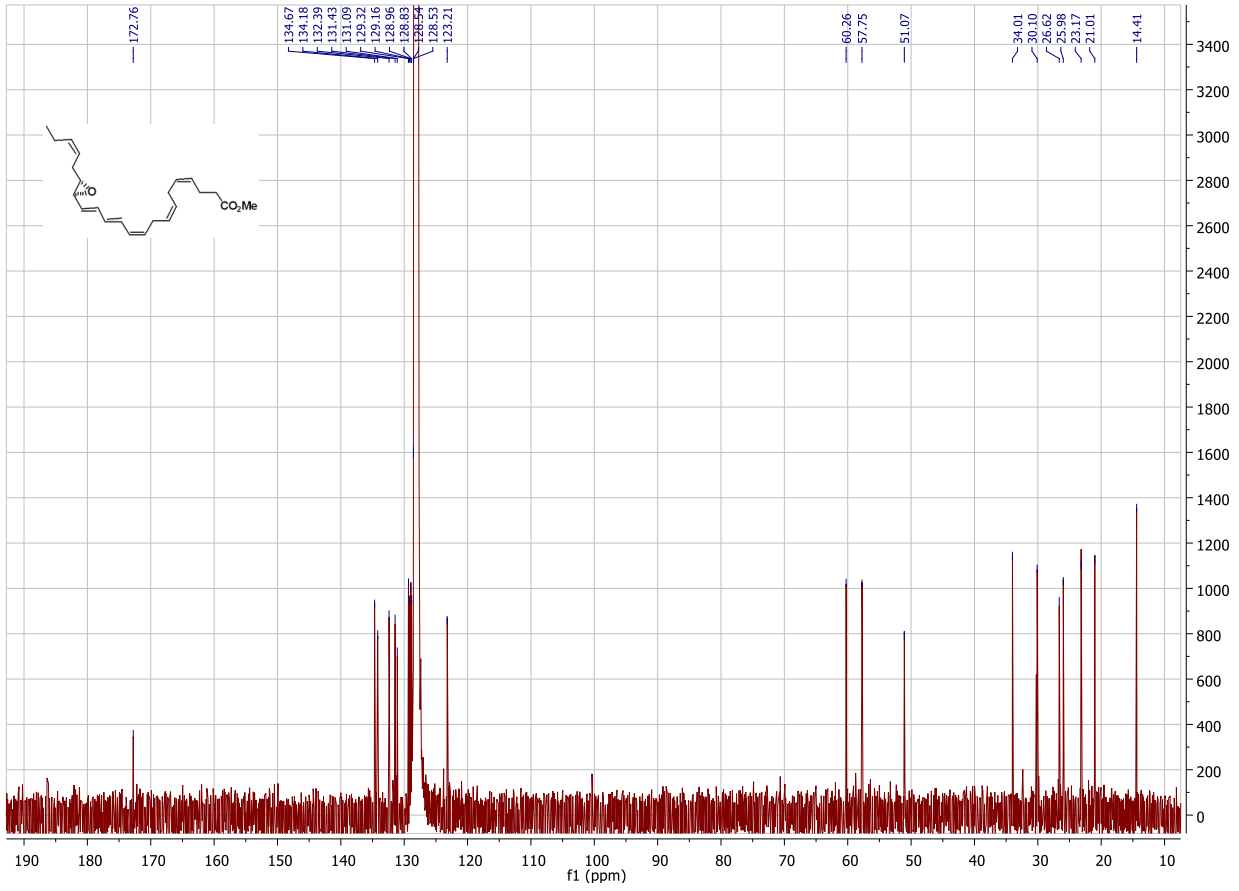


Figure S-10 ^{13}C-NMR spectrum of compound **10**.

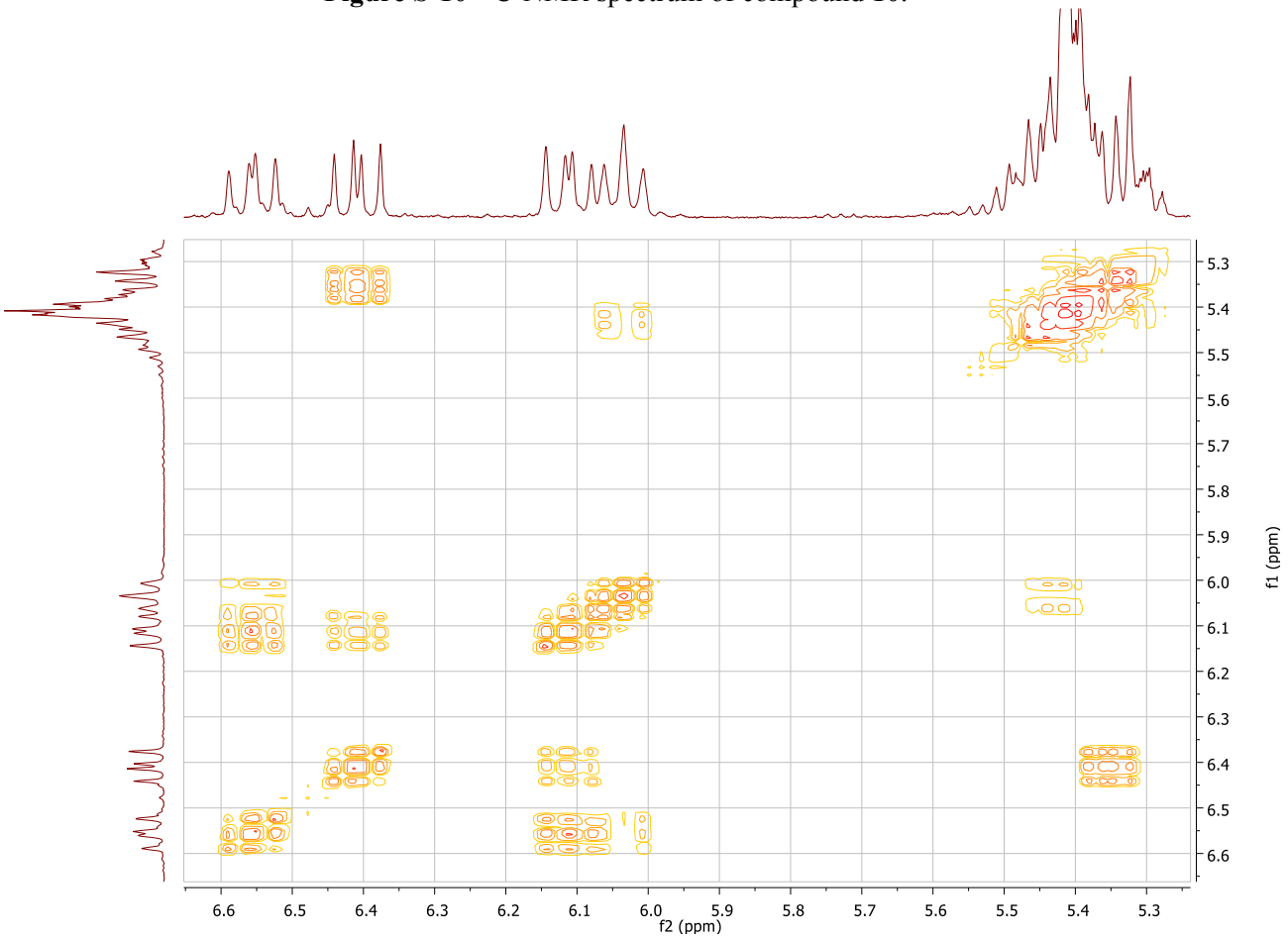


Figure S-11 COSY spectrum of compound **10**.

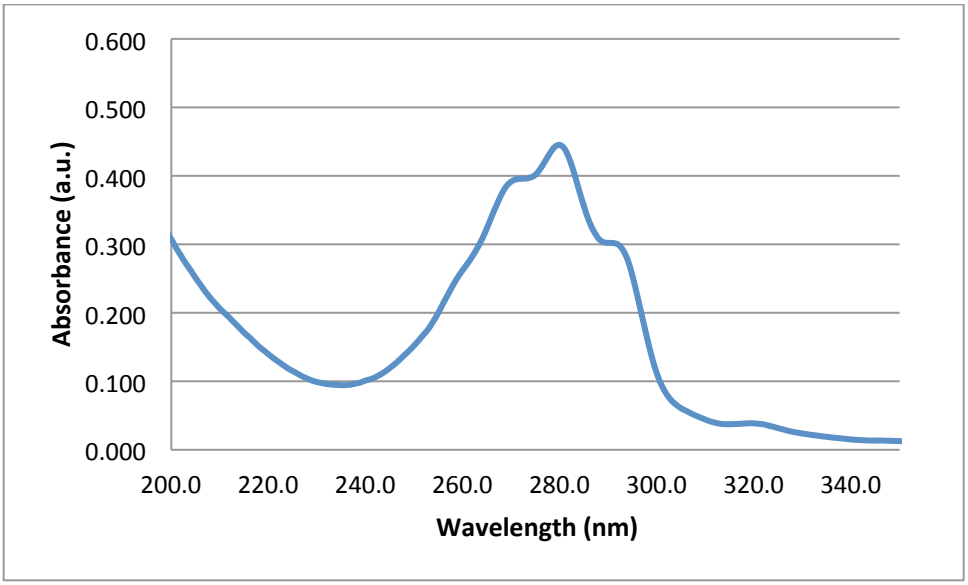
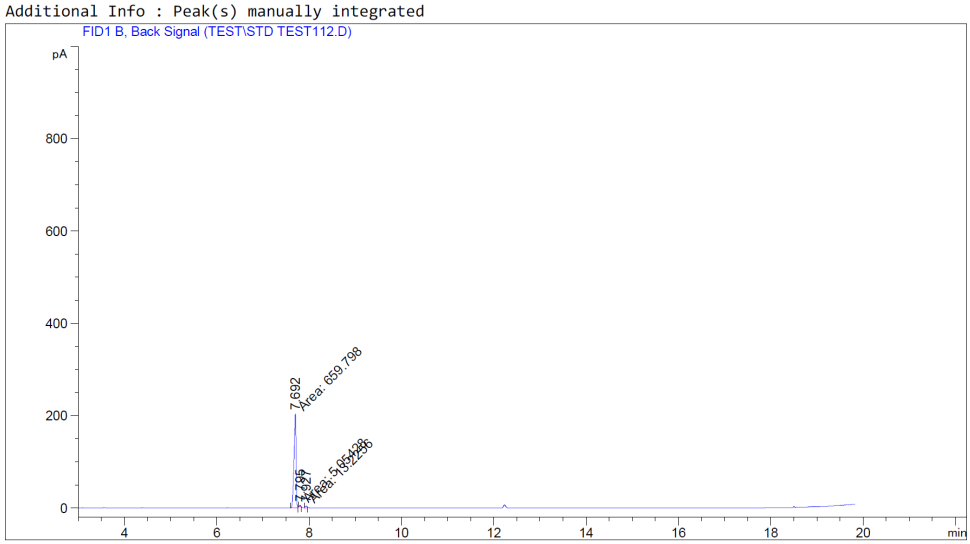


Figure S-12 UV/VIS spectrum of compound **10**.

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 7820 GC                        Location : Vial 1
Injection Date  : 2/3/2015 09:13:21
                                                 Inj Volume : Manually
Method          : C:\CHEM32\1\METHODS\ANDERS METODER\MARIUS WITTIG.M
Last changed    : 2/3/2015 09:03:48 by SYSTEM
Additional Info : Peak(s) manually integrated
```




```
======================================================================
                        External Standard Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 B, Back Signal

======================================================================
======================================================================
                          Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 B, Back Signal

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [pA*s]      [pA]        %
----|-------|----|-------|----------|----------|--------|
   1   7.692 MM    0.0537  659.79785  204.94492 97.30416
   2   7.795 MM    0.0276    5.05428    3.05109  0.74538
   3   7.927 MM    0.0472   13.22564    4.66825  1.95046

Totals :                   678.07777  212.66426


======================================================================
                          *** End of Report ***
```

Figure S-13 GLC chromatogram of compund **17**.

References

1. Wernerova, M.; Hudlicky, T. *Synlett* **2010**, *18*, 2701
2. Kumar, D. A.; Meshram, H. M. *Synth. Commun*. **2013**, *43*, 1145
3. Kang, S-K.; Kim, Y-S.; Lim, J-S-; Kim, K-S.; Kim, S-G.; *Tetrahedron Lett.* **1991**, *32*, 363
4. Aursnes, M.; Tungen, J. E.; Vik, A.; Dalli, J.; Hansen, T. V. *Org. Biomol. Chem.* **2014***, 12*, 432
5. Jin, J.; Zheng, Y.; Boeglin, W. E.; Brash, A. R. *J. Lipid Res.* **2013**, 54, 754
6. (a) Corey, E. J.; Arai, Y.; Mioskowski, C. *J. Am. Chem. Soc.* **1979**, *101*, 6748; (b) Corey, E. J.; Cheng, X.-M. *The Logic of Chemical Synthesis*, Wiley, New York, 1989, p. 314.