Supporting information for "DHA-Like Polyunsaturated Ether Lipid: Modular Semi-Synthetic Approach and 15-Lipoxygenase-1 Substrate Activity"

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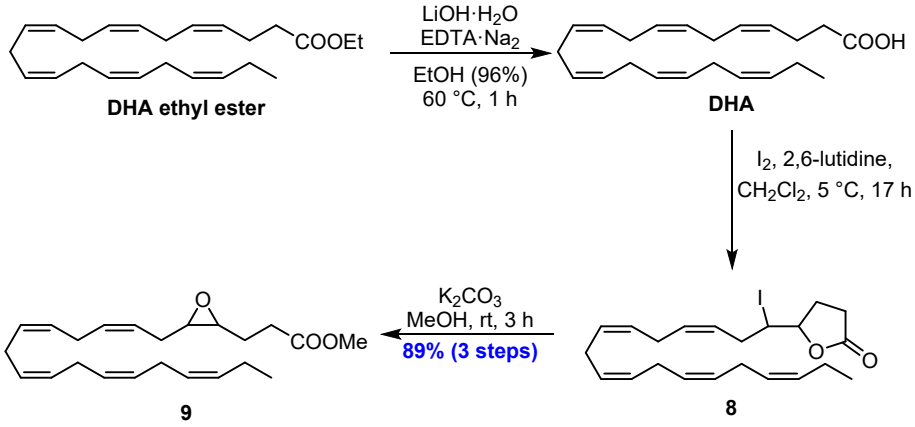
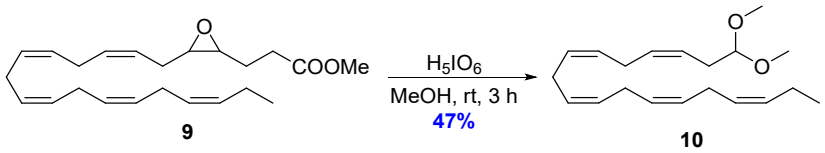
Synthetic Protocols:

Synthesis of methyl 3-(3-((2*Z*,5*Z*,8*Z*,11Z,14*Z*)-heptadeca-2,5,8,11,14-pentaen-1-yl)oxiran-2-yl)propanoate (9)



$LiOH{\cdot}H_2O$ (4.58 g, 110 mmol, 6.5 eq.) was added to water (45 mL) along with $EDTA{\cdot}Na_2$ (31.2 mg), and the resulting mixture was heated to 60 °C under an argon atmosphere. Then, a solution of DHA-ethyl ester (6.03 g, 16.9 mmol, 1,0 eq.) in ethanol (45 mL, 96%) was added and the mixture was stirred under argon at 60 °C for 1 h. The mixture was cooled down and quenched by the addition of 10% HCl (aq., 60 mL) to acidic solution (pH < 3). It was thereafter extracted with ethyl acetate/hexane (2:1, 5 x 15 mL) and the combined organic layers were washed with water (15 mL) and brine (15 mL), and dried over anhydrous $MgSO_4$. After filtration the solvent was removed *in vacuo* to give DHA in quantitative yields. The product was used immediately without any further purification. Iodolactone **8** was prepared according to a procedure by Ulven and co-workers, slightly modified by Primdahl *et al*.[1,2] 2,6-Lutidine (3.62 g, 33.8 mmol, 2 eq.) was dissolved in dichloromethane (70 mL) and added dropwise to DHA (5.55 g, 16.9 mmol, 1 eq.). The reaction mixture was cooled down to 0 °C under an argon atmosphere and an ice-cold solution of I_2 (8.68 g, 33.8 mmol, 2 eq.) in dichloromethane (200 mL) added. It was then stirred under argon at 5 °C for 17 h before it was quenched with $Na_2S_2O_3$ (10%, 250 mL). The phases were separated, and the water phase was extracted with dichloromethane (2 x 50 mL), before the combined organic phases were washed with sat. monosodium phosphate (aq., 2 x 50 mL) and then dried with brine (2 x 50 mL) and $MgSO_4$. The solvent was removed *in vacuo* and iodolactone **8** was used immediately without any further purification. For the formation of epoxide **9** a procedure by Flock *et al.* was utilised.[3] Iodolactone **8** was dissolved in methanol (100 mL) and added potassium carbonate (7.48 g, 54.1 mmol, 3.2 eq.). The mixture was stirred under argon at room temperature for 3 h and then filtered through celite. Diethyl ether (80 mL) was then used to wash the filter. Water (100 mL) and brine (100 mL) were added to the filtrate, before the mixture was extracted with diethyl ether (5 x 50 mL). The combined organic phases were washed with water (2 x 30 mL) and brine (30 mL) and dried over anhydrous $MgSO_4$. After filtration the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate, 5:1) to afford 5.41 g (15.1 mmol, 89%) of **9** as a pale-yellow oil. All spectroscopic data were in agreement with those reported in literature.[4, 5] ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.58 – 5.29 (m, 10H), 3.71 (s, 3H), 3.02 – 2.97 (m, 2H), 2.90 – 2.81 (m, 8H), 2.59 – 2.48 (m, 2H), 2.46 – 2.39 (m, 1H), 2.30 – 2.23 (m, 1H), 2.09 (q, *J* = 7.3 Hz, 2H), 2.00 – 1.91 (m, 1H), 1.87 – 1.78 (m, 1H), 0.99 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 132.2, 130.8, 128.7, 128.6, 128.5, 128.1, 128.0, 127.8, 127.1, 124.3, 56.7, 56.1, 51.9, 31.1, 26.3, 25.9, 25.8, 25.74, 25.65, 23.5, 20.7, 14.4.

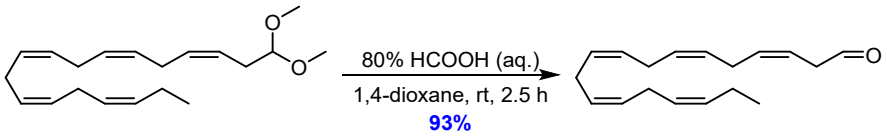
Synthesis of (3Z,6Z,9Z,12Z,15Z)-1,1-dimethoxyoctadeca-3,6,9,12,15-pentaene (10)



9 **10**

For the synthesis of acetal **10** a procedure by Flock *et al.*[3] was followed. Epoxide **9** (10.4 g, 29.0 mmol, 1,0 eq.) was dissolved in dry methanol (150 mL), before periodic acid (7.96 g, 34.9 mmol, 1.2 eq.) was added. The reaction mixture was stirred at room temperature under an argon atmosphere for 3 h. The reaction was then quenched with water (100 mL) and extracted with heptane (5 x 50 mL). The combined organic phases were washed with water (25 mL) and brine (25 mL) and dried over $MgSO_4$. After filtration the solvent was evaporated *in vacuo*. Purification with flash chromatography (SiO_2, hexane/ethyl acetate, 95:5) afforded the acetal **10** as 4.15 g (13.6 mmol, 47%) of a pale-yellow oil. All spectroscopic data were in agreement with those reported in literature.[6] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 – 5.27 (m, 10H), 4.38 (t, *J* = 5.8 Hz, 1H), 3.33 (s, 6H), 2.88 – 2.79 (m, 8H), 2.42 – 2.39 (m, 2H), 2.11 – 2.03 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.1, 130.4, 128.7, 128.4, 128.2, 128.1, 128.0, 127.1, 124.1, 104.2, 53.0, 31.1, 26.0, 25.8, 25.7, 25.74, 20.66, 14.4.

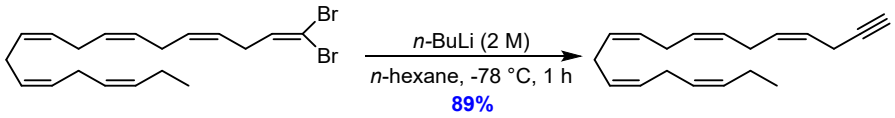
Synthesis of (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-octadeca-3,6,9,12,15-pentaenal (7)



10 **7**

For the synthesis of aldehyde **7** a procedure by Flock *et. al*[3] was followed. Acetal **10** (1.02 g, 3.35 mmol, 1,0 eq.) was dissolved in 1,4-dioxane (9 mL) and a solution of formic acid (11 mL, aq., 80% w:w) added. The reaction was stirred at room temperature for 2.5 h, before water (40 mL) was added. The reaction mixture was extracted with diethyl ether (4 x 10 mL) and the combined organic phases were neutralized with sat. sodium bicarbonate (aq., 2 x 10 mL), washed with brine (10 mL) and dried over $MgSO_4$. The solvents were removed under reduced pressure to afford 804 mg (3.11 mmol, 93%) of aldehyde **7** as a yellow oil. The aldehyde was used further without purification to avoid isomerization. All spectroscopic data were in agreement with those reported in literature.[4, 6] ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (t, *J* = 1.9 Hz, 1H), 5.73 – 5.67 (m, 1H), 5.63 – 5.56 (m, 1H), 5.45 – 5.28 (m, 8H), 3.24 – 3.21 (m, 2H), 2.86 – 2.80 (m, 8H), 2.08 (p, *J* = 7.4 Hz, 2H), 0.98 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.5, 133.3, 132.2, 129.0, 128.8, 128.6, 127.9, 127.9, 127.2, 127.1, 118.8, 42.6, 26.1, 25.8, 25.8, 25.7, 20.7, 14.4.

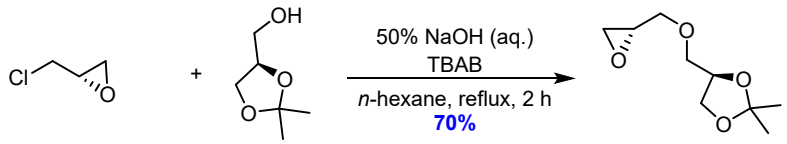
Synthesis of (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*)-nonadeca-4,7,10,13,16-pentaen-1-yne (6)



11 **5**

Following a procedure described by Flock et al.[7] dibromide **11** (143 mg, 0.345 mmol, 1,0 eq.) was dissolved in dry hexane and cooled to -78 °C. A solution of *n*-butyllithium in cyclohexane (2 M, 0.8 mL, 2.3 eq.) was added dropwise. The reaction was stirred at -78 °C for 1 h and then quenched by the addition of sat. ammonium chloride (aq., 1.5 mL). The rection mixture was warmed to room temperature and extracted with diethyl ether (4 x 2 mL). After washing with brine (2 mL) and drying over $MgSO_4$, the solvent was evaporated. The resulting crude oil was purified by flash chromatography (SiO_2, petroleum ether) producing 78 mg of alkyne **5** as a light-yellow oil (0.307 mmol, 89%). All spectroscopic data were in agreement with those reported in literature.[7] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 – 5.28 (m, 10H), 2.99 – 2.96 (m, 2H), 2.89 – 2.78 (m, 8H), 2.12 – 2.04 (m, 2H), 1.98 (t, *J* = 2.7 Hz, 1H), 0.98 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.2, 130.2, 128.8, 128.7, 128.5, 128.1, 128.0, 127.5, 127.1, 124.2, 82.7, 68.3, 25.8 (2C), 25.7 (2C), 20.7, 17.0, 14.4.

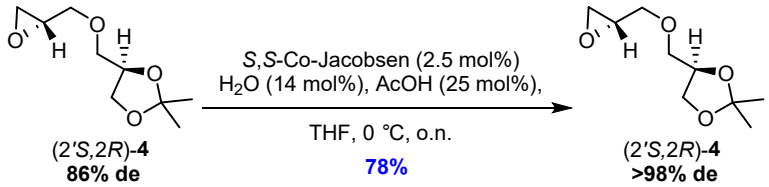
Synthesis of (2'*S*)-1-*O*-(Oxiran-2-ylmethyl)-2,3-*O*-isopropylidene-*sn*-glycerol ((2*R*,2'*S*)-4)



(2'*S*)-**epichlorohydrin** (2*R*)-**solketal** **4**

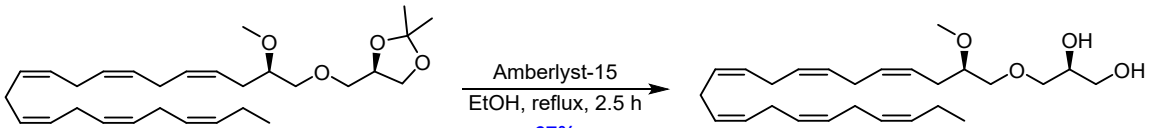
(2*R*)-Solketal (0.47 mL, 0.500 g, 3.78 mmol, 1 eq.) was dissolved in hexane (1.75 mL) before TBAB (121.2 mg, 0.376 mmol, 0.1 eq.) and sodium hydroxide solution (50% aq., 602.4 mg, 7.53 mmol, 2 eq.) was added. (2'*S*)-Epichlorohydrin (0.61 mL, 0.720 g, 7.80 mmol, 2.1 eq.) was dissolved in hexane (3.5 mL) and added dropwise to the reaction mixture. The reaction was then stirred at reflux for 2 h. After cooling to room temperature, the reaction was diluted with water (10 mL) and ethyl acetate (10 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (3 x 10 mL). After washing with brine (10 mL) and drying over $MgSO_4$ the solvent was removed *in vacuo*. The resulting oil was purified by flash chromatography (SiO_2, petroleum ether:ethyl acetate, 3: 1) to yield the desired (2*R*,2'*S*)-**4** product as a slightly yellow oil in 70% yield (501.3 mg, 2.66 mmol). The ^{1}H-NMR spectra indicated the presence of its (2*R*,2'*R*)-**4** diastereomer (6-7%). The spectroscopic data were in agreement with that previously reported.[8] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 – 4.24 (m, 1H), 4.04 (dd, *J* = 8.3, 6.5 Hz, 1H), 3.79 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.73 (dd, *J* = 8.3, 6.4 Hz, 1H), 3.62 (dd, *J* = 10.0, 5.8 Hz, 1H), 3.50 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.44 (dd, *J* = 11.7, 5.9 Hz, 1H), 3.16 – 3.12 (m, 1H), 2.78 (dd, *J* = 5.0, 4.1 Hz, 1H), 2.59 (dd, *J* = 5.0, 2.7 Hz, 1H), 1.41 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.6, 74.8, 72.5, 72.3, 66.7, 50.9, 44.2, 26.9, 25.5.

Hydrolytic Kinetic Resolution by the (*S*,*S*)-Co-Jacobsen Catalyst ((2*R*,2'*S*)-4)



The (*S*,*S*)-Co-Jacobsen catalyst (38.0 mg, 0.0.0629 mmol, 2.5 mol%) was dissolved in dry toluene (3 mL) before acetic acid (37.0 µL, 38.6 mg, 0.643 mmol, 25 mol%.) was added via syringe. The bright red solution was stirred for 2 h exposed to air, before the solvent was evaporated under reduced pressure. The resulting brown sludge was kept in vacuum for 30 minutes. The atmosphere was then exchanged for argon, dry THF (2 mL) was added, and the mixture cooled to 0 °C. (2*R*,2*'S*)-**4** (484 mg, 2.57 mmol, 1 eq., 86% de) dissolved in dry THF (1 mL) was added followed by water (6.5 mg, 0.359 mmol, 14 mol%). The reaction was stirred overnight, slowly reaching room temperature. The solvent was evaporated, and the resulting crude product purified by flash chromatography (SiO_2, heptane:ethyl acetate, 1:1). This gave 379 mg (2.02 mmol, 78% yield, >98% de) of (2*R*,2*'S*)-**4** as a light brown oil. All spectroscopic data were in accordance with that previously published.[8] $[\alpha]_D^{25}$ = -18.8 (c = 1.38, methanol). [Lit.[8] $[\alpha]_D^{20}$ = −24.3 (c = 1.00, ethanol)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 – 4.24 (m, 1H), 4.05 (dd, *J* = 8.3, 6.4 Hz, 1H), 3.80 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.73 (dd, *J* = 8.3, 6.4 Hz, 1H), 3.62 (dd, *J* = 10.0, 5.8 Hz, 1H), 3.50 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.44 (dd, *J* = 11.7, 5.9 Hz, 1H), 3.17 – 3.13 (m, 1H), 2.79 (dd, *J* = 5.0, 4.1 Hz, 1H), 2.60 (dd, *J* = 5.0, 2.7 Hz, 1H), 1.42 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.6, 74.8, 72.5, 72.3, 66.8, 50.9, 44.3, 26.9, 25.5.

Synthesis of ((2'*R*)-1-*O*-[(4'*Z*,7'*Z*,10'*Z*,13'*Z*,16'*Z*,19'*Z*)-2'-Methoxydocosa-4',7',10',13',16',19'-hexaenyl]-*sn*-glycerol ((2*R*,2'*R*)-3)



13 **3**

The methoxylated acetonide **13** (10 mg, 0.021 mmol, 1,0 eq.) was dissolved in ethanol (96%, 2 mL) under a nitrogen atmosphere, and wet, acidic Amberlyst-15 (6 mg, 60 wt.%) was added. The solution was refluxed for 2.5 h, cooled to room temperature and filtered to remove the catalyst. After concentration *in vacuo* purification with flash chromatography (hexane/ethyl acetate, 1:1 to 0:1) gave the desired product as a pale-yellow oil (6 mg, 67%). All spectroscopic data were in accordance with that previously published.[9] $[\alpha]_D^{23}$ = -3.98 (c = 0.460, ethanol) [Lit.[9] $[\alpha]_D^{20}$ = -3.68 (c = 0.125, ethanol)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51 – 5.28 (m, 12H), 3.89 – 3.84 (m, 1H), 3.70 (dd, *J* = 11.4, 3.9 Hz, 1H), 3.65 – 3.52 (m, 4H), 3.48 (dd, *J* = 10.5, 6.0 Hz, 1H), 3.42 (s, 4H), 2.95 (s, 1H), 2.88 – 2.68 (m, 10H), 2.38 – 2.17 (m, 3H), 2.11 – 2.04 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.2, 130.5, 128.7, 128.5, 128.5, 128.4, 128.2, 128.2, 128.0, 128.0, 127.1, 125.1, 80.2, 73.6, 73.3, 70.7, 64.2, 57.5, 28.7, 25.9, 25.8, 25.8, 25.8, 25.7, 20.7, 14.4. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{26}H_{42}O_4Na$ 441.2975; Found 441.2974.

NMR and HRMS spectra:
Compound 11

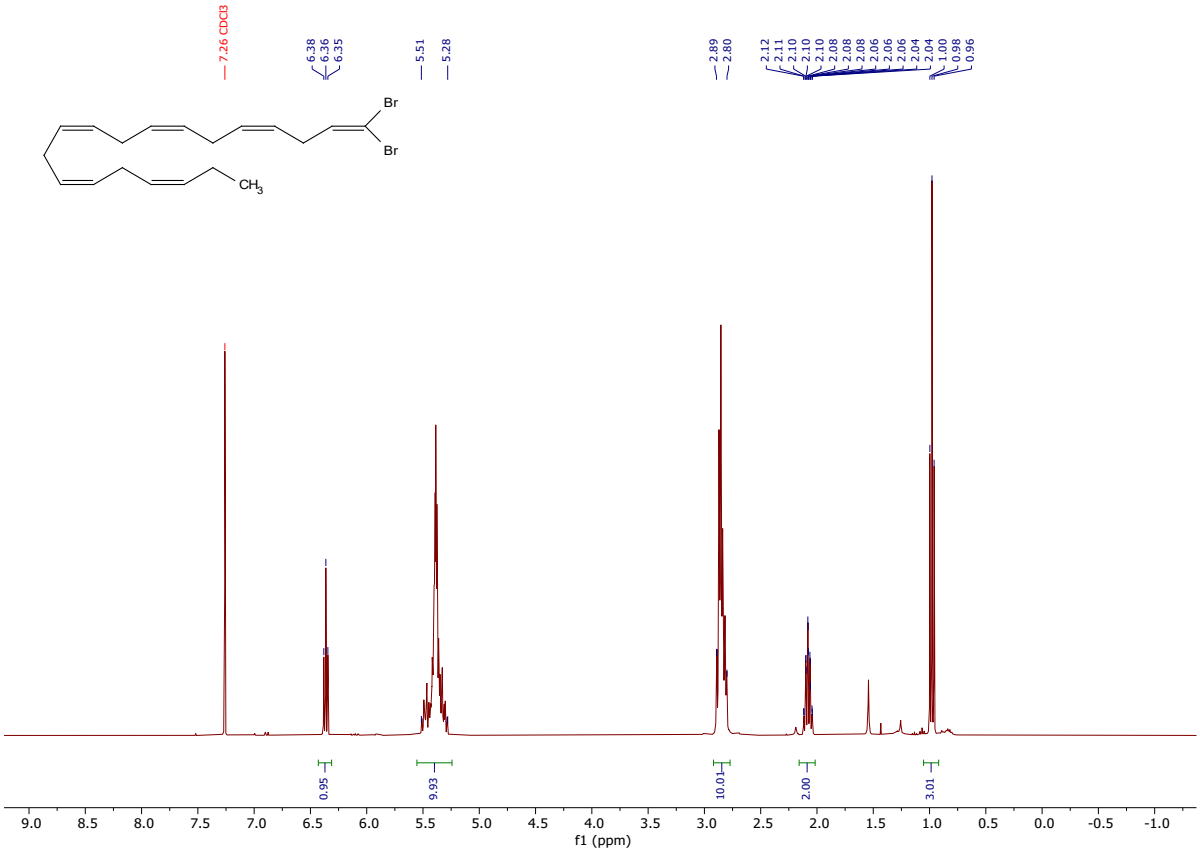


Figure 1 ^{1}H NMR (400 MHz, $CDCl_3$) of compound **11.**

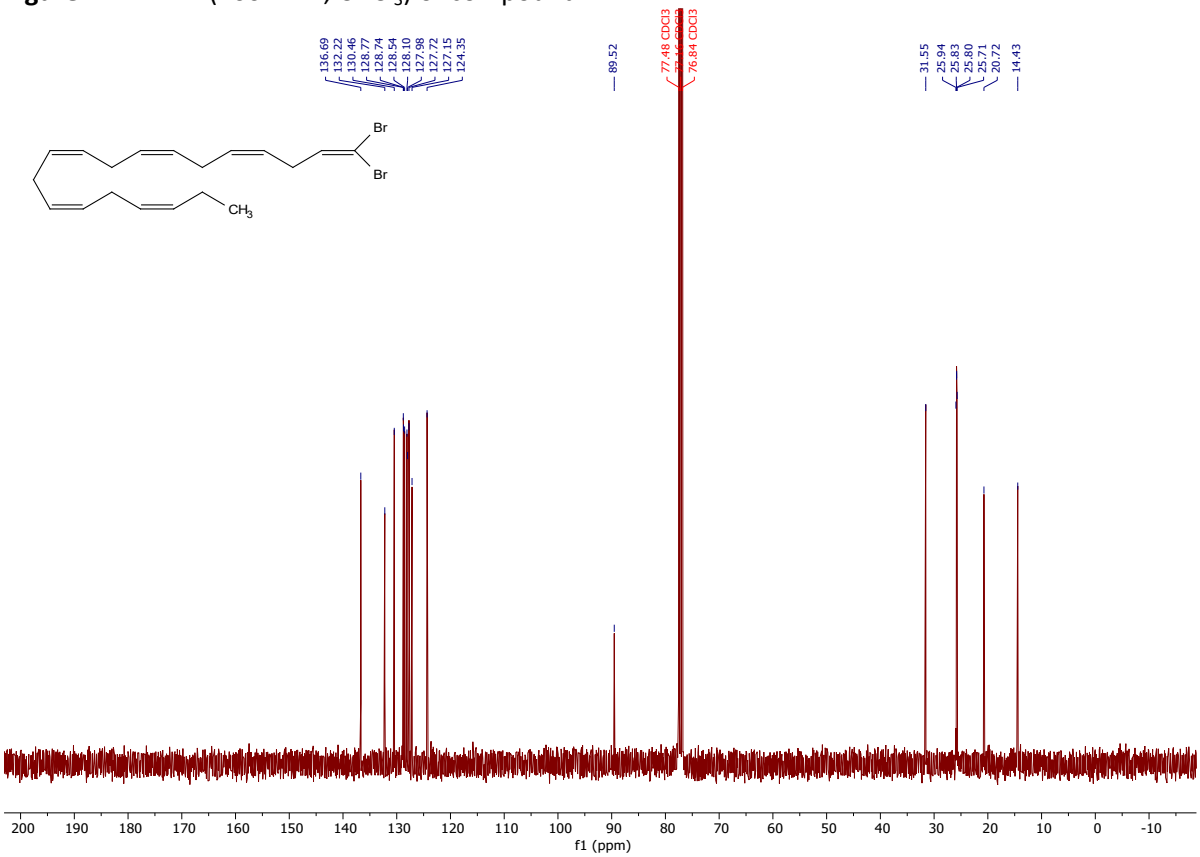


Figure 2 ^{13}C NMR (101 MHz, $CDCl_3$) of compound **11.**

Compound 6:

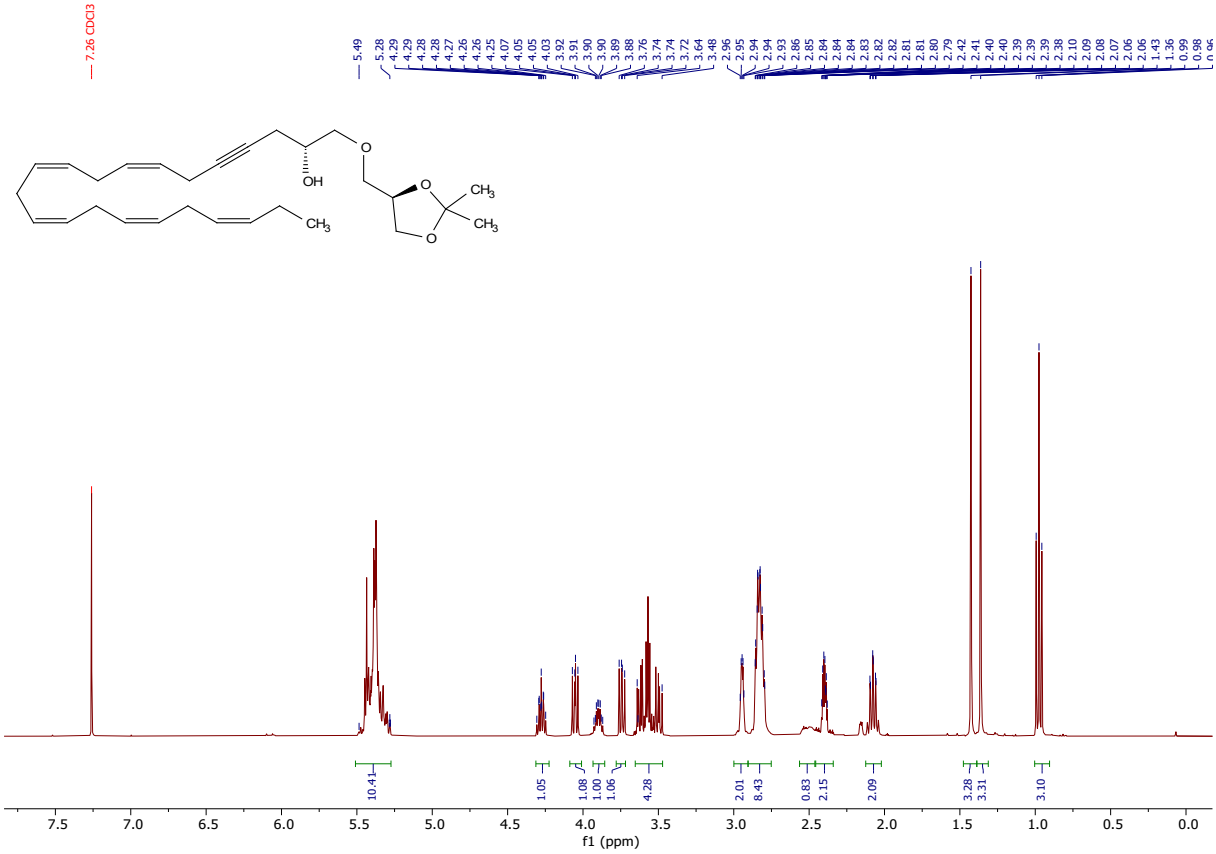


Figure 3 ^{1}H NMR (400 MHz, $CDCl_3$) of compound **6**.

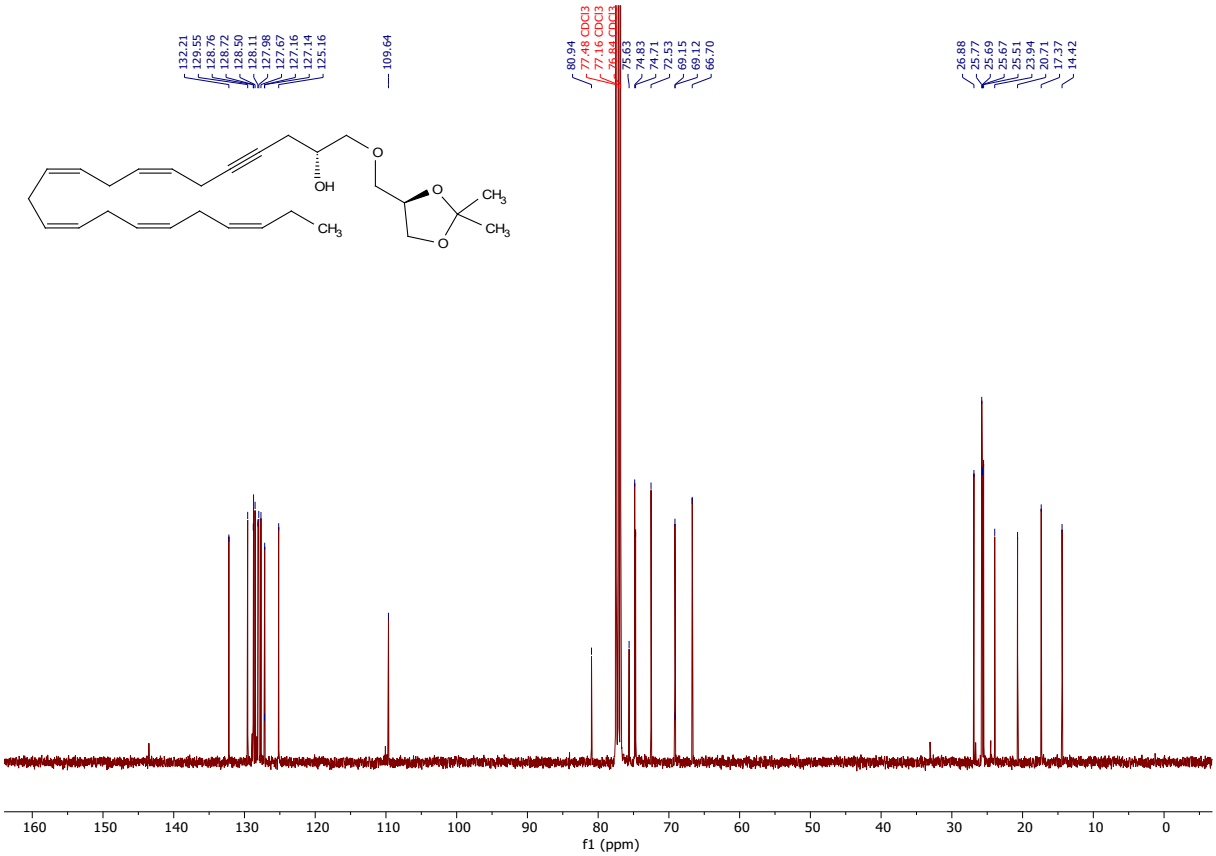


Figure 4 ^{13}C NMR (101 MHz, $CDCl_3$) of compound **6.**

Compound 12:

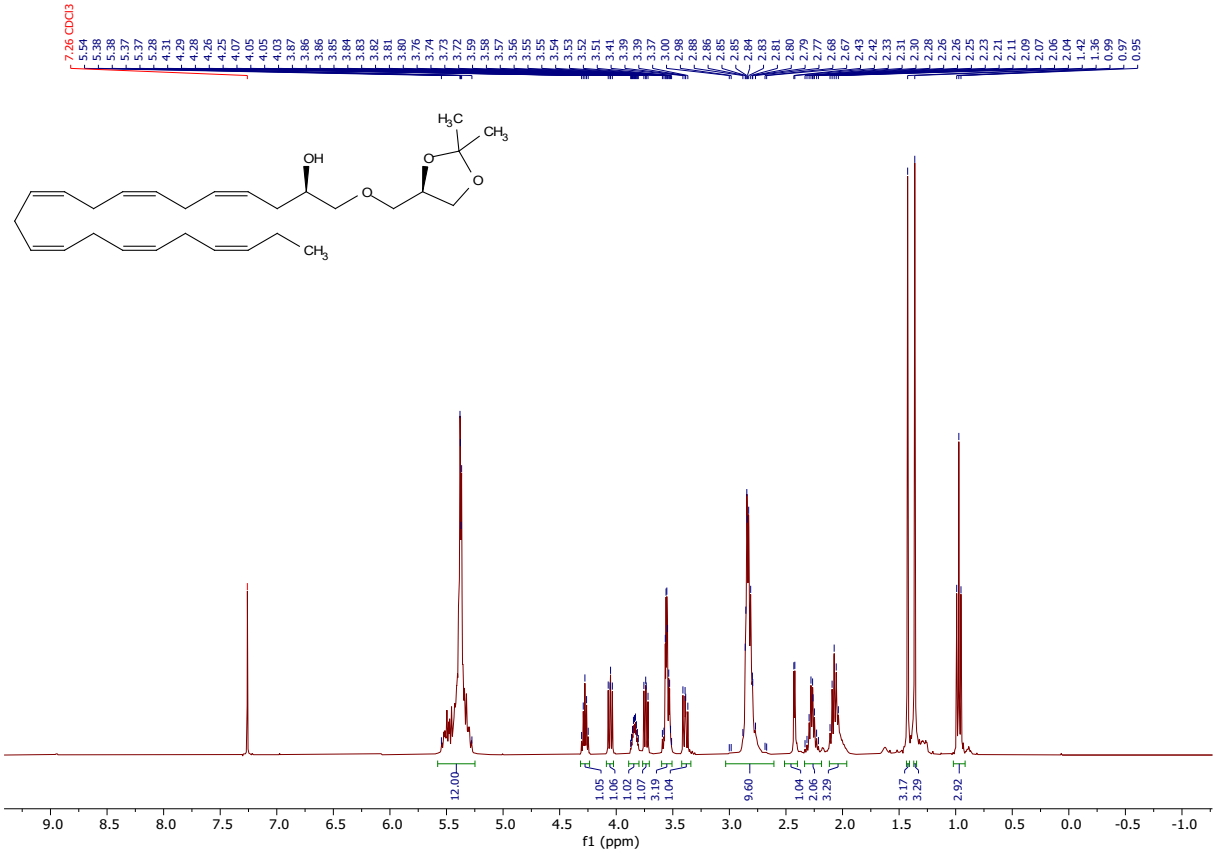


Figure 5 ^{1}H NMR (400 MHz, $CDCl_3$) of compound **12.**

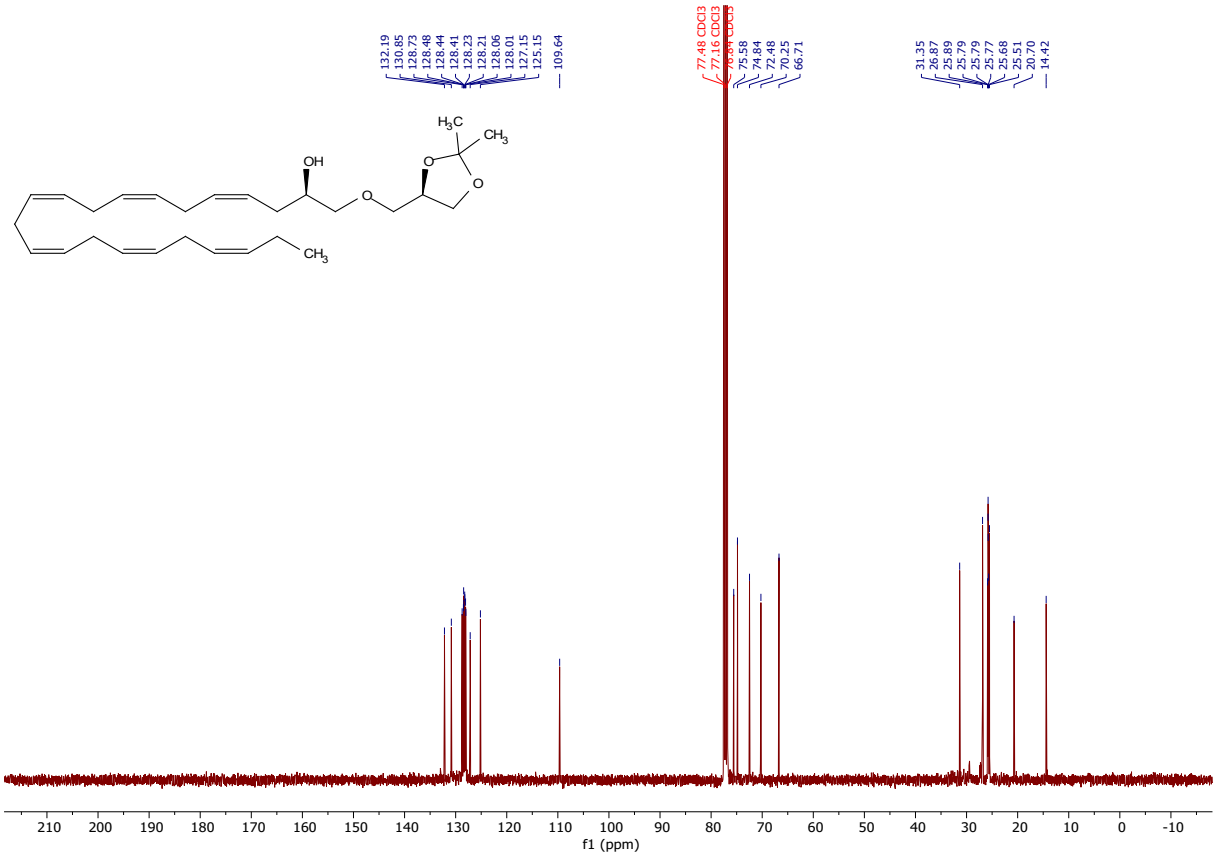


Figure 6 ^{13}C NMR (101 MHz, $CDCl_3$) of compound **12.**

Compound 13:

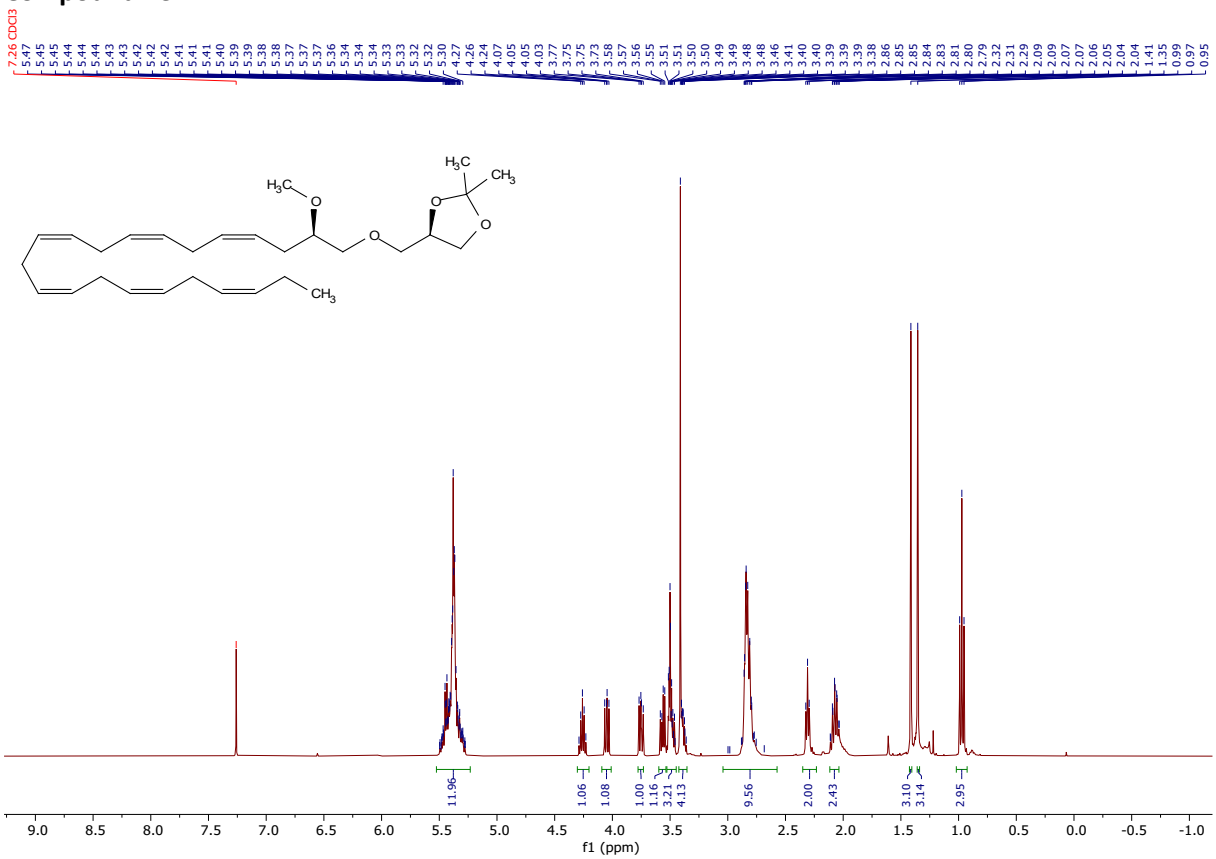


Figure 7 ^{1}H NMR (400 MHz, $CDCl_3$) of compound **13.**

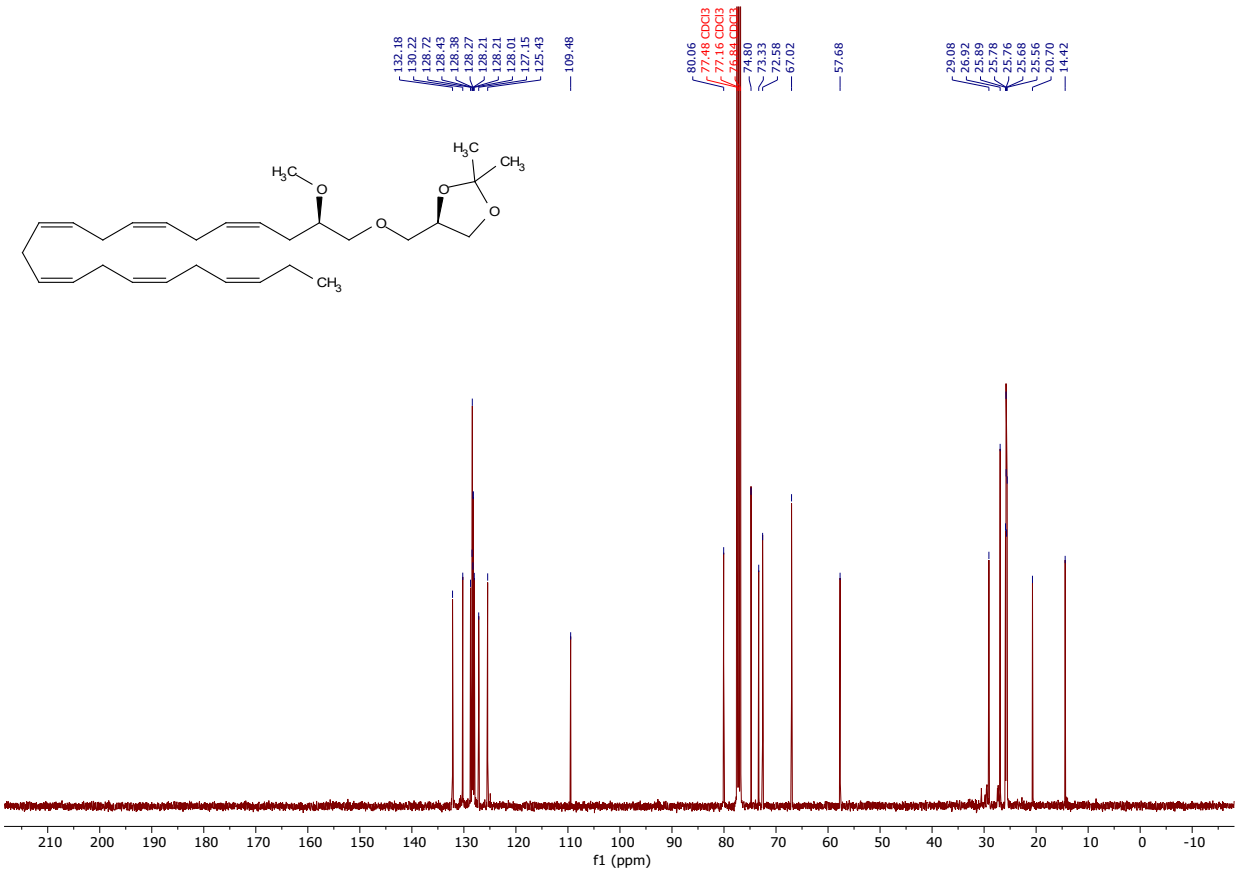


Figure 8 ^{13}C NMR (101 MHz, $CDCl_3$) of compound **13.**

Compound 3

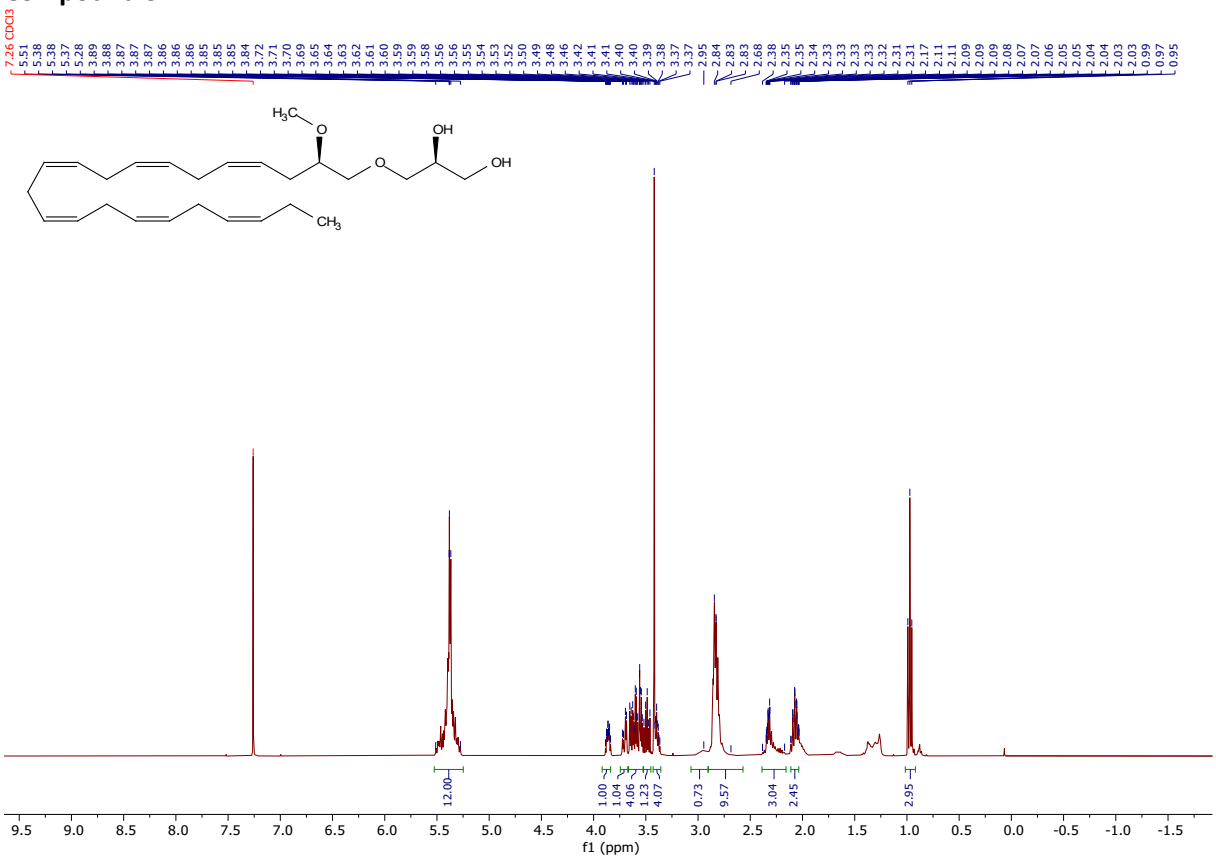


Figure 9 ^{1}H NMR (400 MHz, $CDCl_3$) of compound **3**.

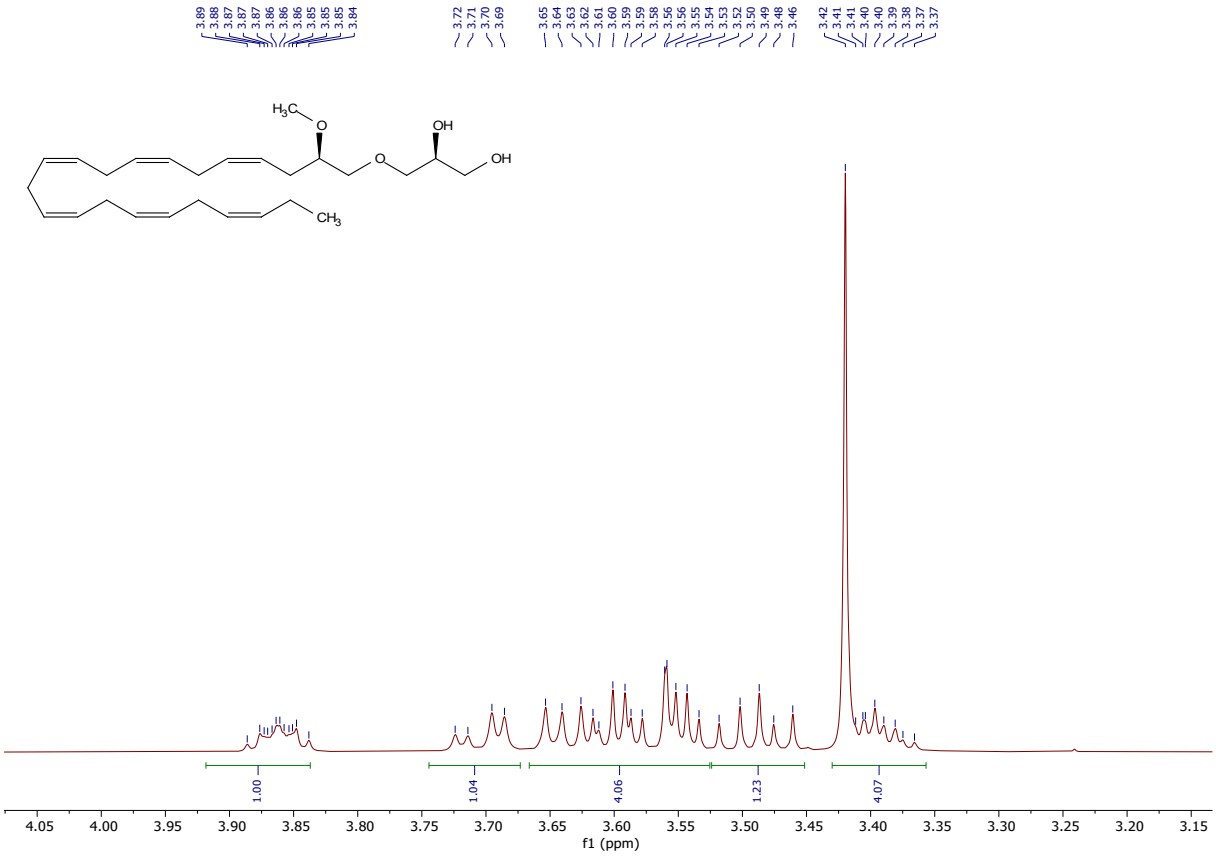


Figure 10 Close-up of ^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.**

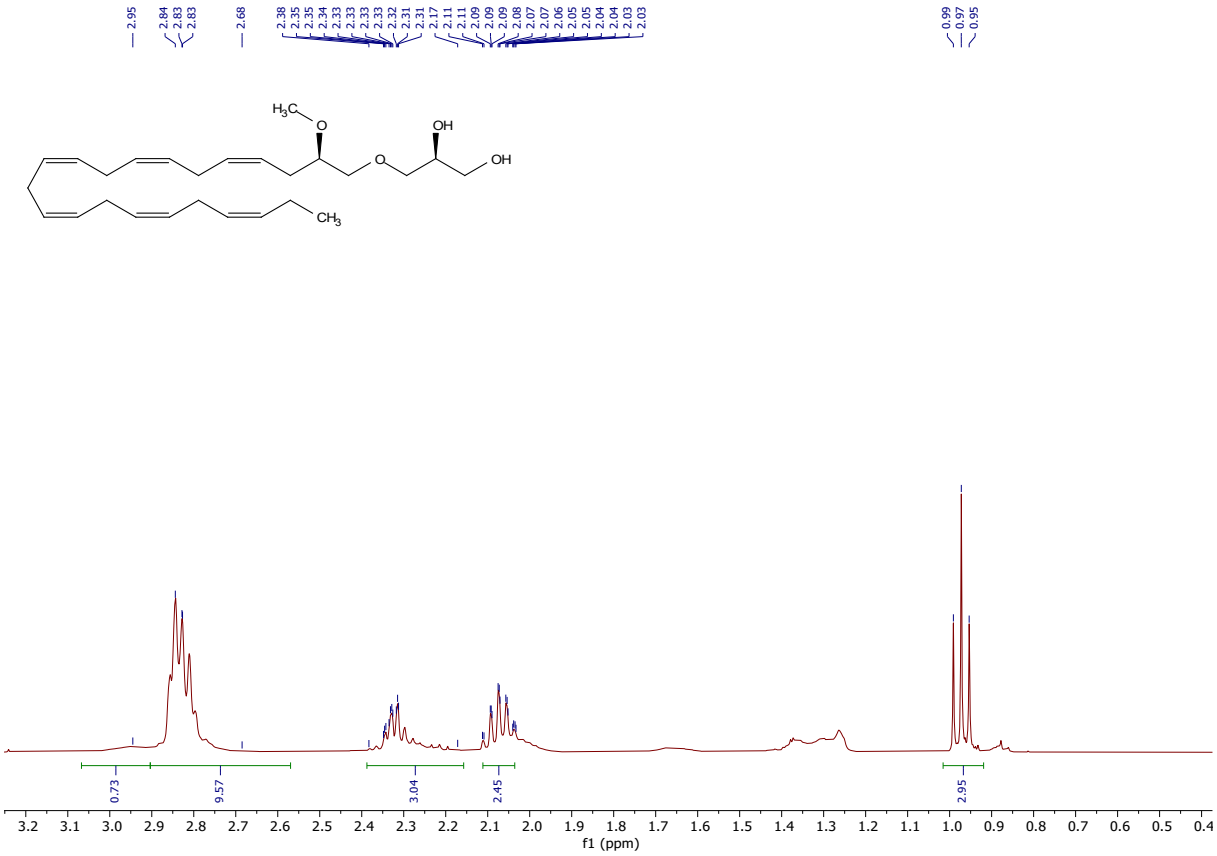


Figure 11 Close-up of ^{1}H NMR (400 MHz, $CDCl_3$) of compound **3**

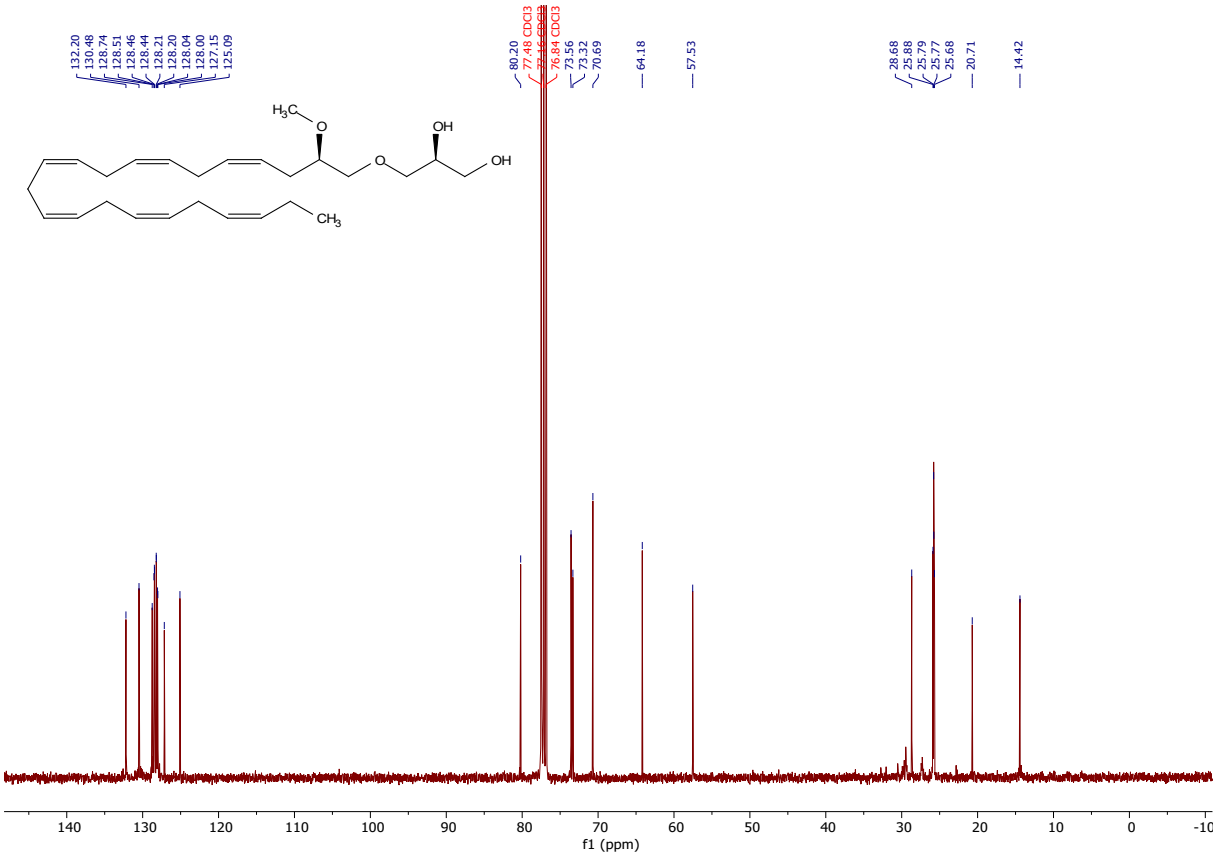


Figure 12 ^{13}C NMR (101 MHz, $CDCl_3$) of compound **3**

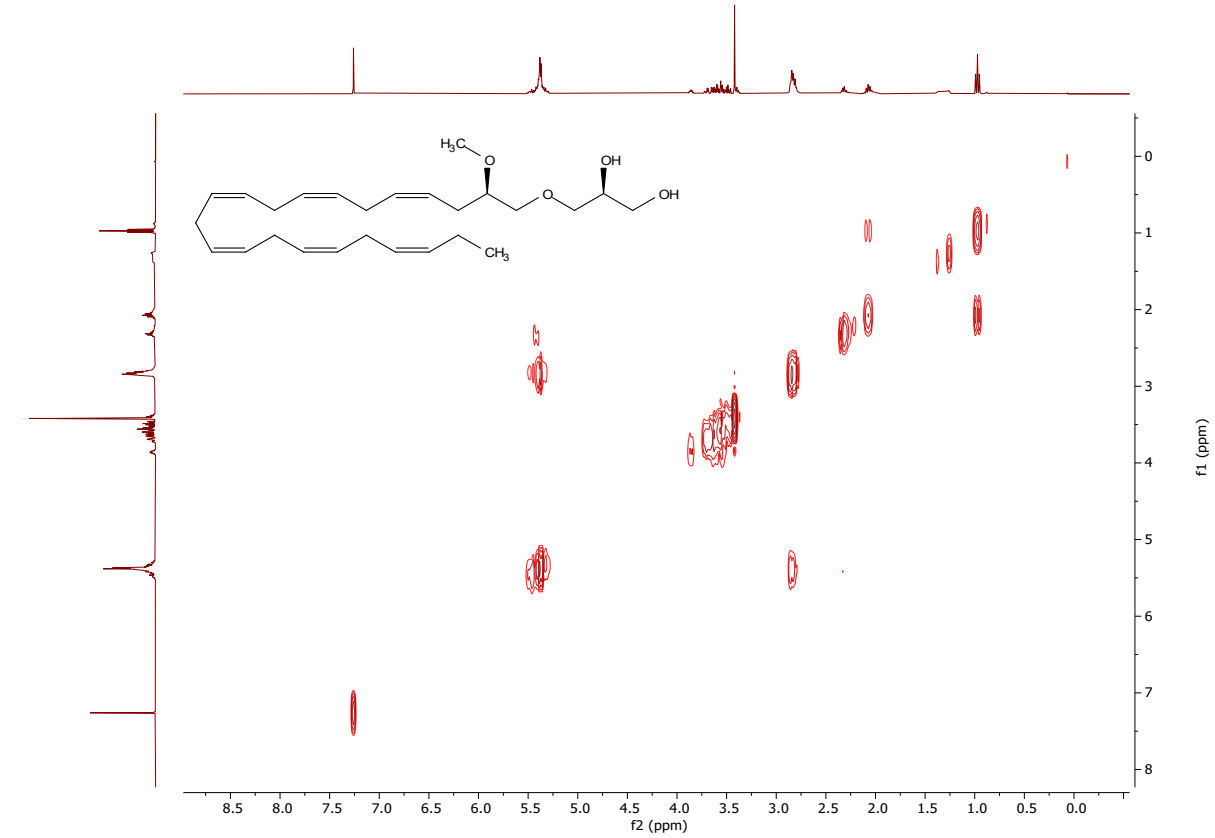


Figure 13 COSY (400 MHz, $CDCl_3$) of compound **3**

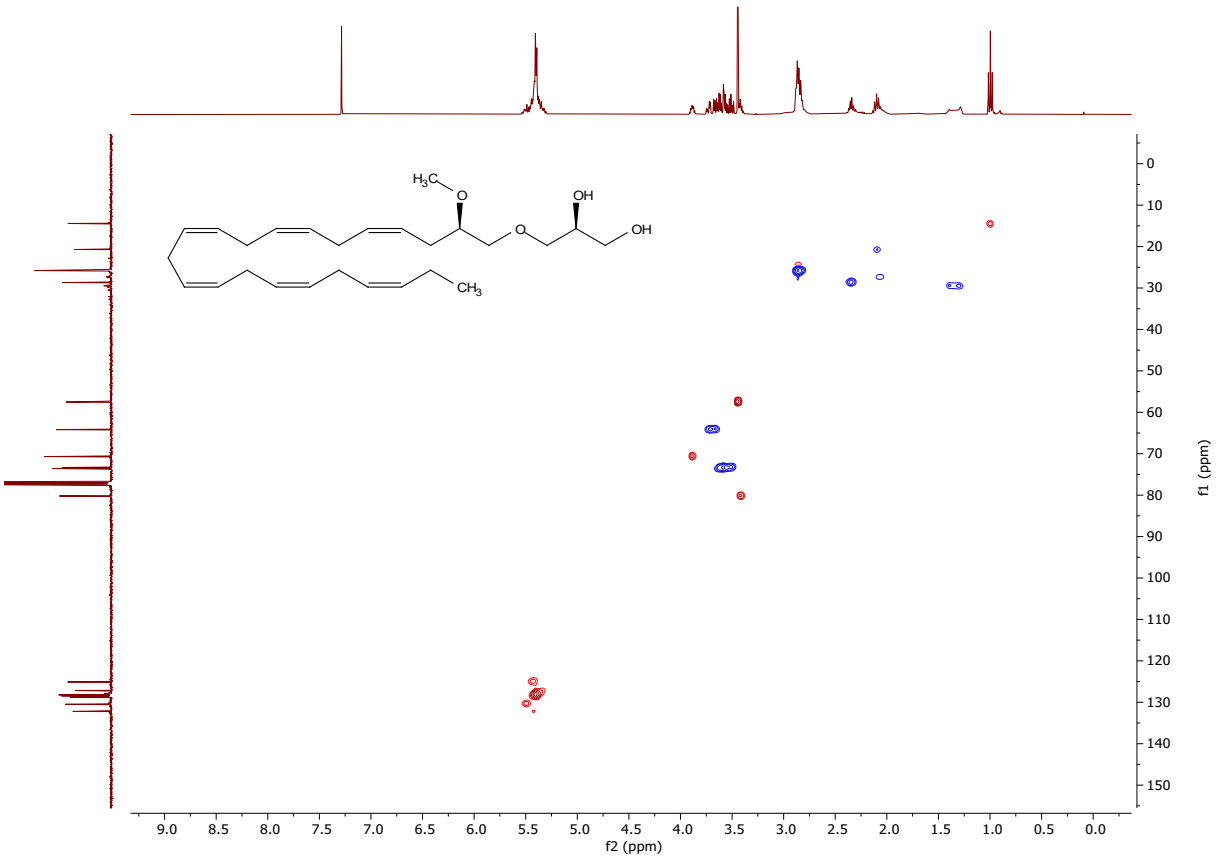


Figure 14 HSQC (400 MHz, $CDCl_3$) of compound **3**

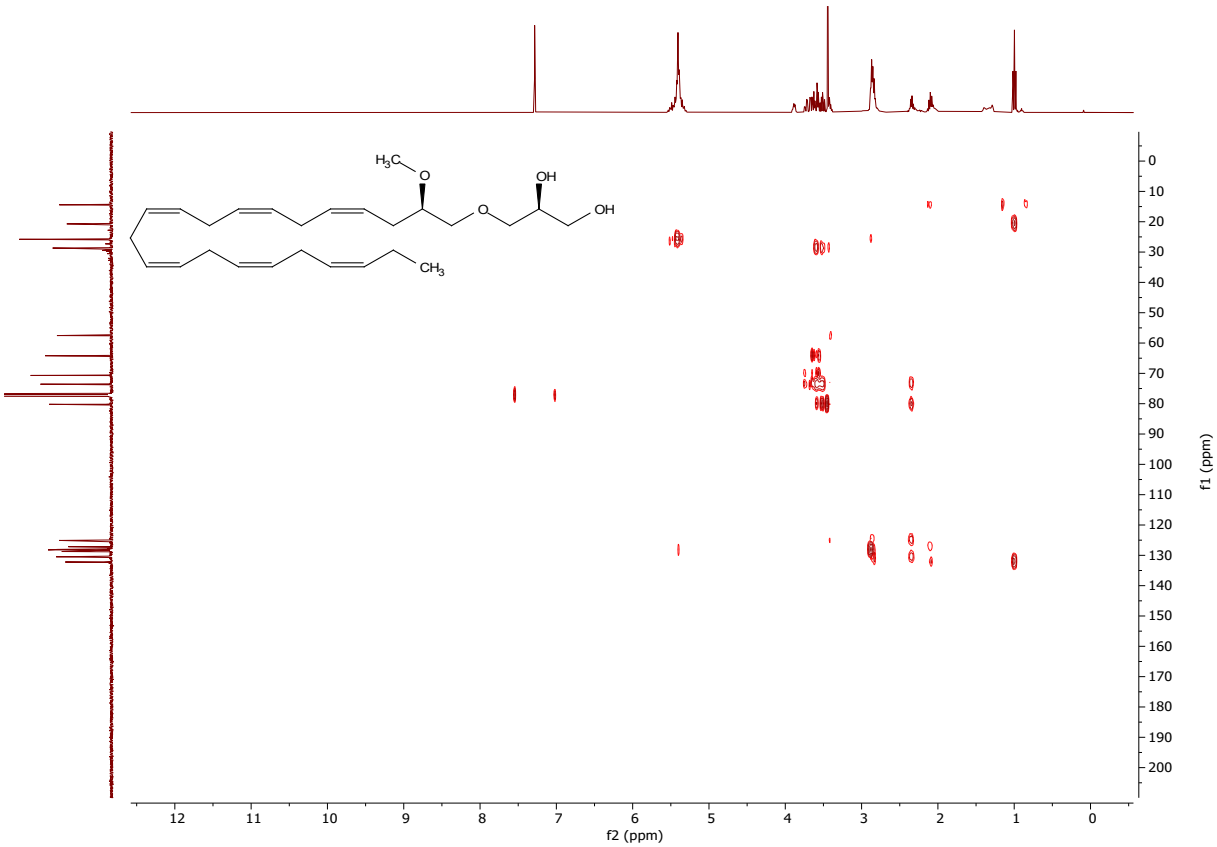


Figure 15 HMBC (400 MHz, $CDCl_3$) of compound **3**

Mass Spectrum List Report

Analysis Info

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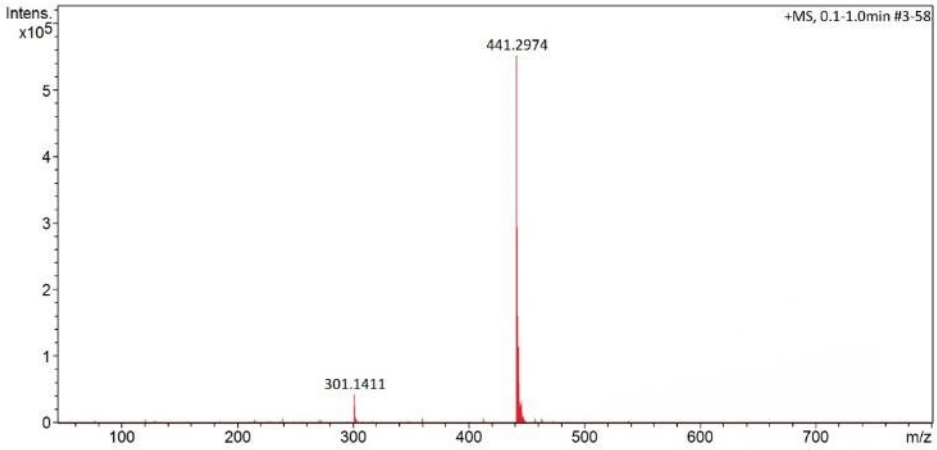
Method	ESI_pos_50_1500_os.m	Operator	Erlend
Sample Name	MB-3-017-A	Instrument	maXis II ETD 1823391.22318
Comment			

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	Source
				Set APCI Heater	0 °C



#	m/z	Res.	S/N	I	I %	FWHM
1	120.9871	29888	929.2	4213	0.8	0.0040
2	129.0522	28539	520.8	2356	0.4	0.0045
3	157.0834	30209	553.3	2471	0.4	0.0052
4	185.1148	33250	580.3	2576	0.5	0.0056
5	215.1254	35545	646.1	2952	0.5	0.0061
6	217.1047	33126	541.8	2483	0.4	0.0066
7	227.1253	35895	505.4	2356	0.4	0.0063
8	229.1408	32073	570.3	2682	0.5	0.0071
9	239.0890	33576	490.6	2359	0.4	0.0071
10	239.1617	37166	1049.5	5046	0.9	0.0064
11	249.1826	37181	497.9	2439	0.4	0.0067
12	257.1360	36944	552.5	2815	0.5	0.0070
13	271.1879	36979	537.4	2954	0.5	0.0073
14	273.1673	37094	603.3	3344	0.6	0.0074
15	301.1411	35769	6666.9	43037	7.8	0.0084
16	302.1444	39272	1242.6	8113	1.5	0.0077
17	304.2610	39618	636.6	4244	0.8	0.0077
18	360.3238	40212	493.9	4737	0.9	0.0090
19	413.2663	39489	381.8	4502	0.8	0.0105
20	441.2974	38725	42748.5	552855	100.0	0.0114
21	442.3008	40180	12400.5	160683	29.1	0.0110
22	443.3122	34468	8761.7	113685	20.6	0.0129
23	444.3163	40131	2432.6	31587	5.7	0.0111
24	445.3281	36453	2111.4	27443	5.0	0.0122
25	446.3321	36880	566.7	7370	1.3	0.0121
26	447.3441	37799	731.9	9532	1.7	0.0118
27	448.3478	42231	215.2	2806	0.5	0.0106
28	449.3598	37106	225.4	2947	0.5	0.0121
29	457.2715	41888	382.3	5071	0.9	0.0109
30	463.2795	38868	396.8	5330	1.0	0.0119

Figure 16 MS spectra of compound **3**

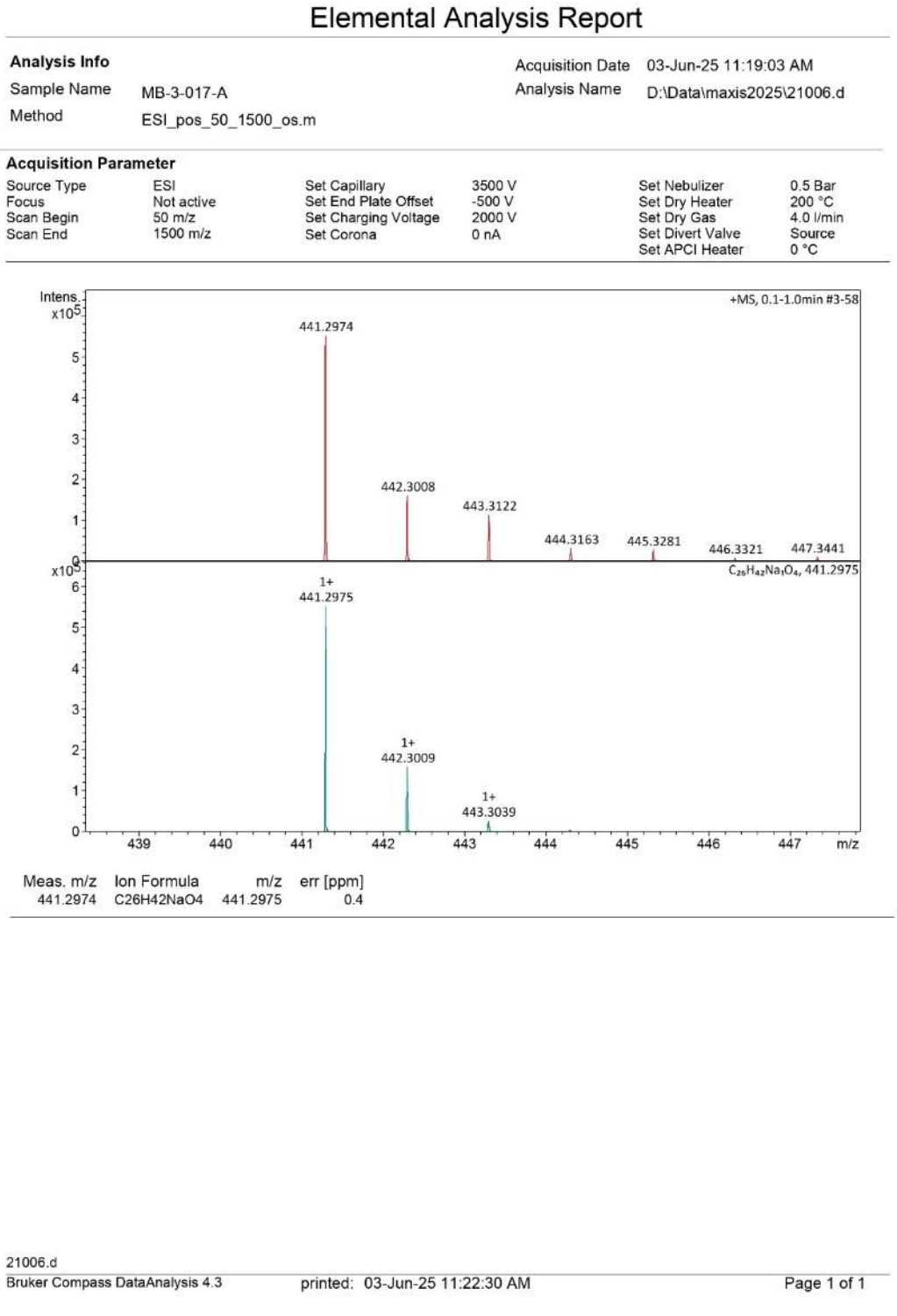


Figure 17 HRMS spectra of compound **3**

Methods for molecular dynamic simulations
Protein-ligand complex prediction

The FASTA sequence of soybean 15-LOX-1 was retrieved from UniProt (ID: P08170). The sequence was submitted to Boltz-2[10] cofolding and affinity prediction on the Rowan server (Rowan Scientific. https://www.rowansci.com (accessed 2025-10-20), using linoleic acid, one water molecule and Fe^{3+} as binding partners. The resulting protein-ligand complex was further modelled in Maestro (Schrödinger Release 2025-1). The protein structure was prepared using the Protein Preparation Workflow[11] at pH 7.4 with default parameters. Ligands were prepared using the LigPrep module (**Schrödinger Release 2025-1;** LigPrep, Schrödinger, LLC, New York, NY, 2025). For carboxylic acid–containing ligands, only the carboxylate ionization state was retained for further modelling.

Molecular dynamics simulations and MM/GBSA scoring

The Orientation of Proteins in Membranes server[12] (PPM 2.0) was used to position the protein–ligand complex relative to the lipid bilayer. The docked complex of linoleic acid was prepared for molecular dynamics (MD) simulations using Desmond (Schrödinger Release 2025-1).[13] The system was embedded in a POPC bilayer, solvated with the TIP3P water model, and neutralized with NaCl. Additional KCl was added to a final concentration of 0.15 M. All components were parameterized using the OPLS4 force field.[14] Following the standard Desmond relaxation protocol, all-atom MD simulations were performed in the NPT ensemble at 310 K using the Nosé–Hoover chain thermostat and at 1 atm pressure using the Martyna–Tobias–Klein barostat. Production runs of 200–400 ns were carried out, with trajectory frames saved every 100 ps.

Trajectory snapshots (extracted every 1 ns; 200 per system) from the 200 ns production runs of the linoleic acid and DHA–MEL complexes were evaluated with Prime MM-GBSA (**Schrödinger Release 2025-1**; Schrödinger, LLC, New York, NY, 2025) to estimate binding free energies.

Induced Fit Docking and MM/GBSA rescoring

A representative frame from the 400 ns MD simulation of the linoleic acid complex was extracted using k-means clustering of the simulation trajectory. All water molecules, lipids, sodium, and potassium ions were removed. Induced Fit Docking (IFD)[15-17] with extended sampling was then used to generate optimized binding poses for all four ligands. The resulting poses were rescored using Prime MM/GBSA to assess binding affinities.

Induced-Fit–refined complexes with 15-LOX-1

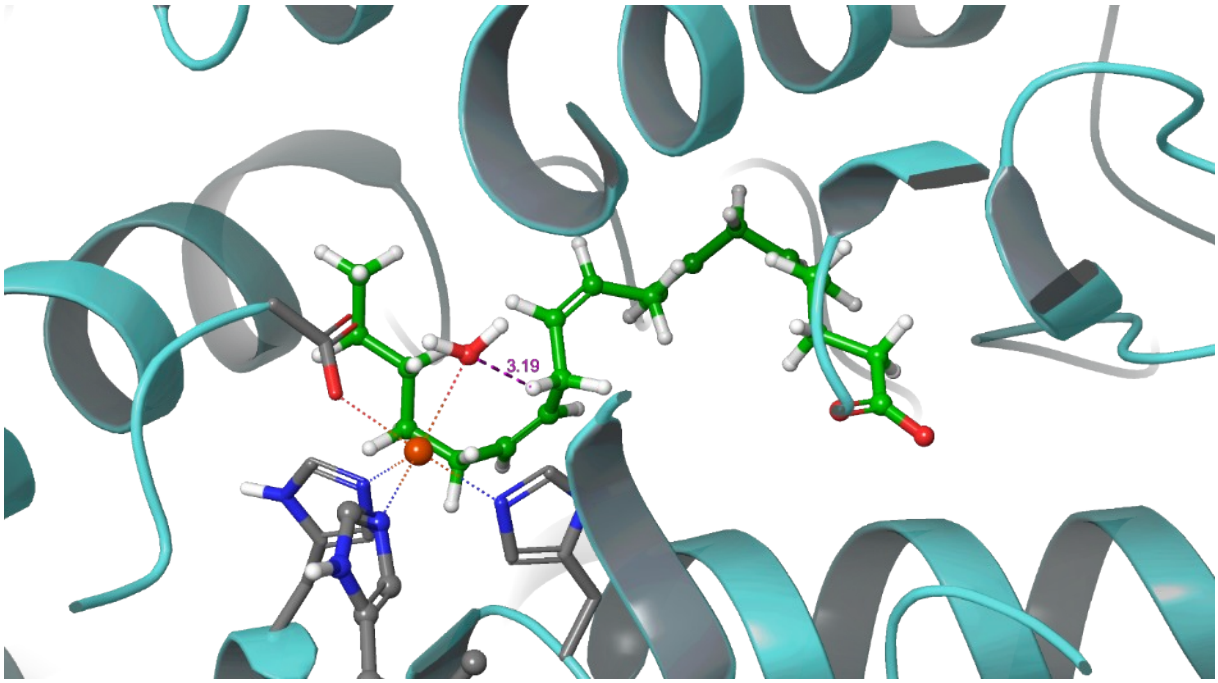


Figure 18 Arachidonic acid (green) positioned in the active site of the soybean 15-LOX-1 model. The catalytic Fe^{3+} is coordinated by three histidines, one isoleucine, and a water molecule. The distance between the coordinating water molecule and the bis-allylic hydrogen of arachidonic acid is indicated by the dotted purple line.

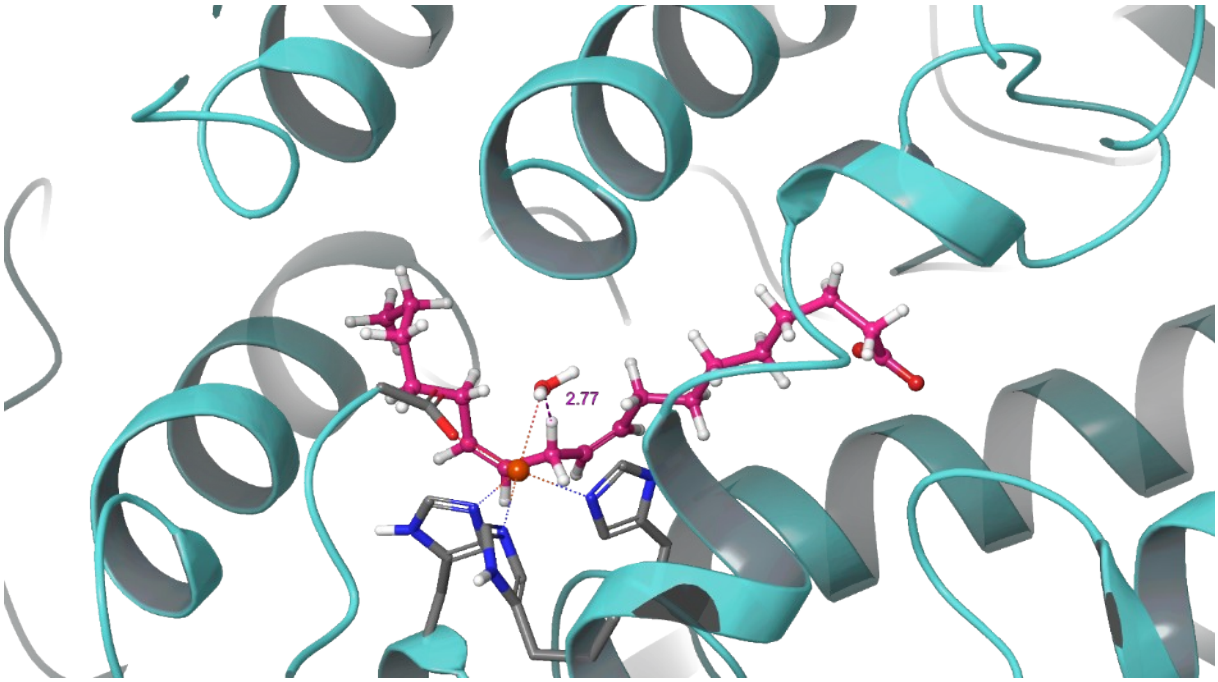


Figure 19 Linoleic acid (pink) positioned in the active site of the soybean 15-LOX-1 model. The catalytic Fe^{3+} is coordinated by three histidines, one isoleucine, and a water molecule. The distance between the coordinating water molecule and the bis-allylic hydrogen of linoleic acid is indicated by the dotted purple line.

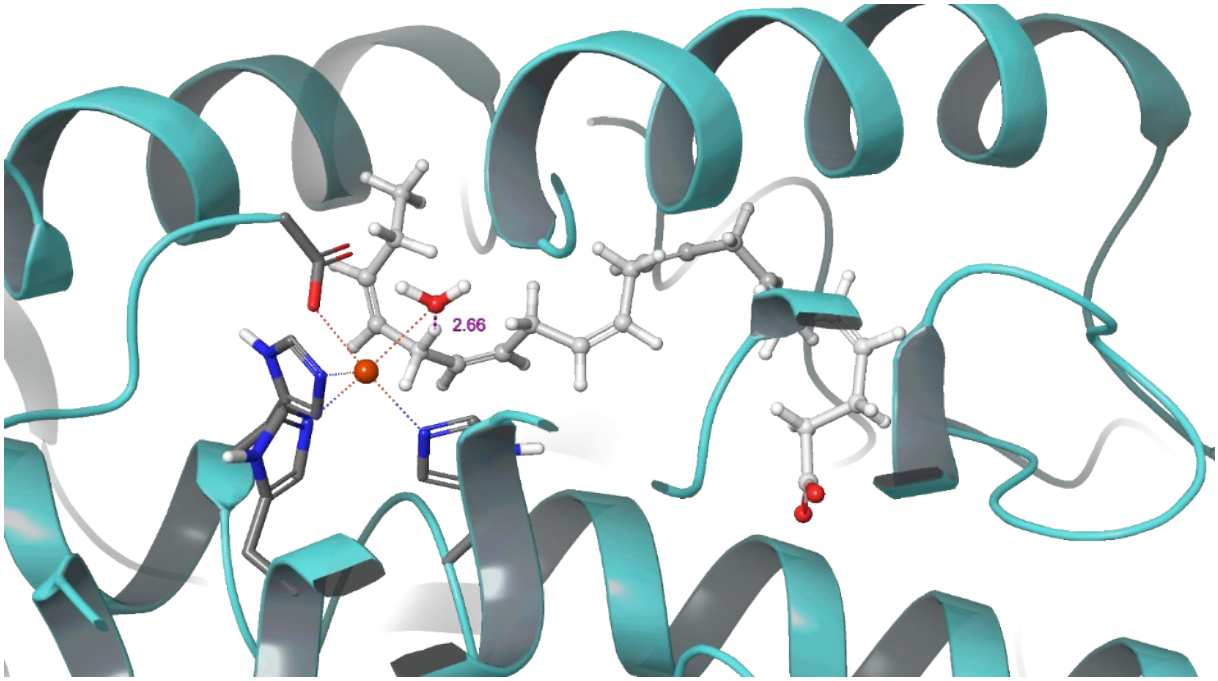


Figure 20 DHA (white) positioned in the active site of the soybean 15-LOX-1 model. The catalytic Fe^{3+} is coordinated by three histidines, one isoleucine, and a water molecule. The distance between the coordinating water molecule and the bis-allylic hydrogen of DHA is indicated by the dotted purple line.

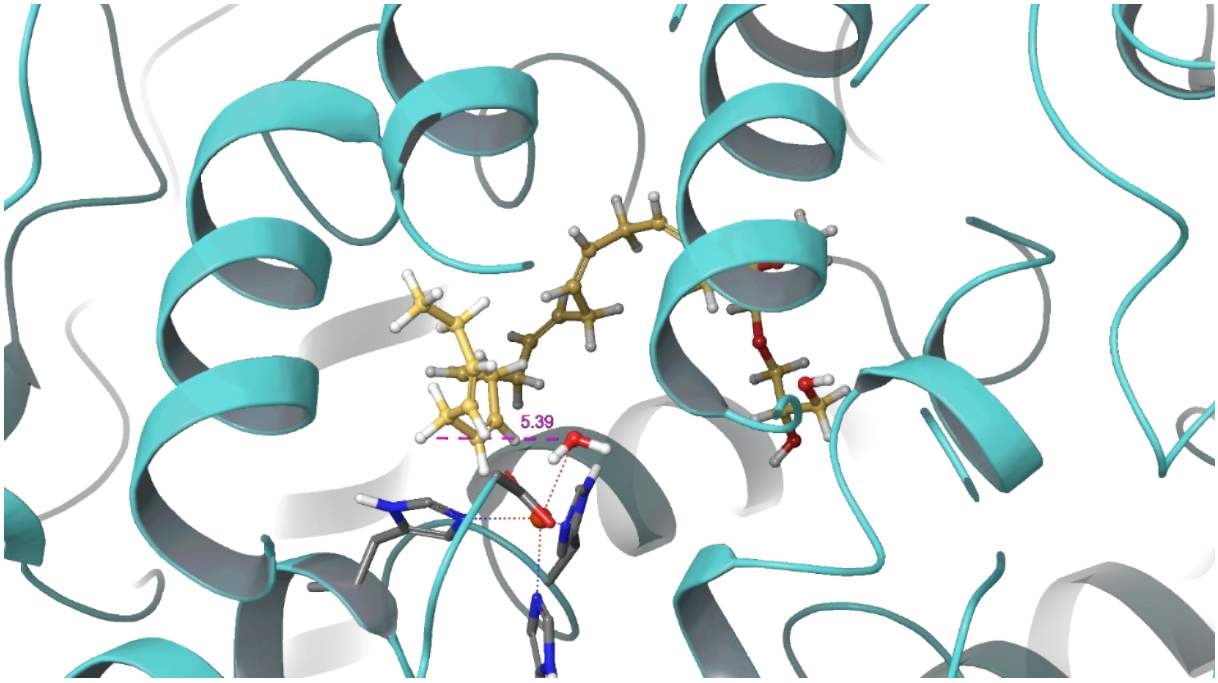


Figure 21 DHA-like MEL **3** (yellow) positioned in the active site of the soybean 15-LOX-1 model. The catalytic Fe^{3+} is coordinated by three histidines, one isoleucine, and a water molecule. The distance between the coordinating water molecule and the bis-allylic hydrogen of DHA-like MEL **3** is indicated by the dotted purple line.

Docking poses with coordinating amino acids:

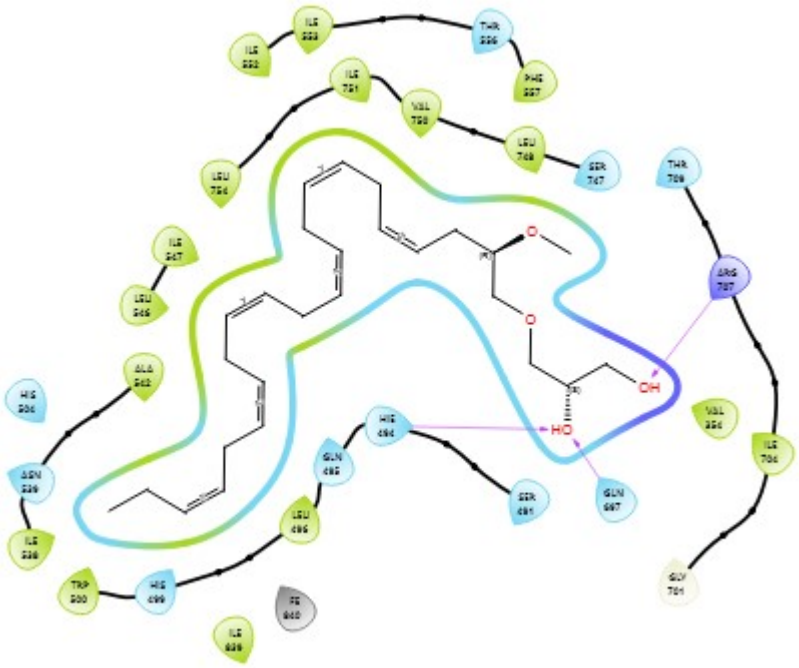


Figure 22 2D interaction diagram of DHA-like MEL **3** in the 15-LOX-1 model. The hydrophobic residues are shown in green around the hydrocarbon chain, while polar residues appear in blue and the charged arginine in purple near the headgroup. Pink lines indicate hydrogen bonds with nearby polar and charged residues, and the catalytic iron is shown in gray.

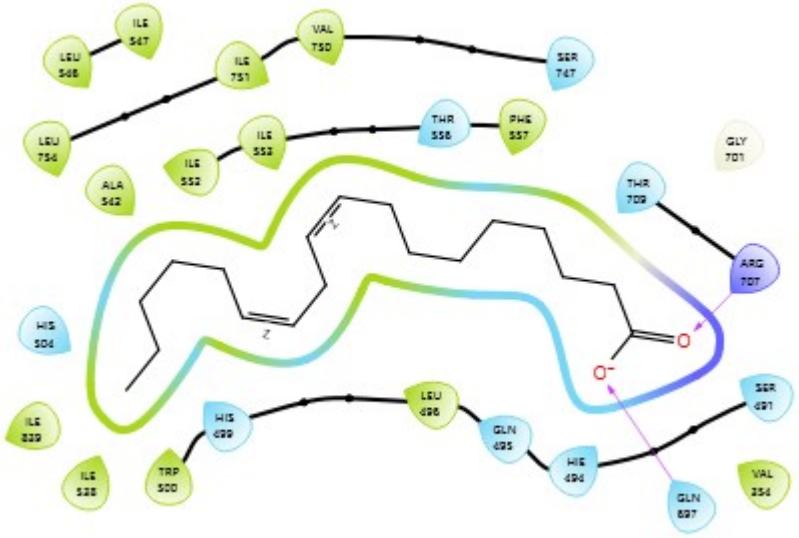


Figure 23 2D interaction diagram of linoleic acid in the 15-LOX-1 model. The hydrophobic residues are shown in green around the hydrocarbon chain, while polar residues appear in blue and the charged arginine in purple near the headgroup. Pink lines indicate hydrogen bonds and electrostatic interactions with nearby polar and charged residues.

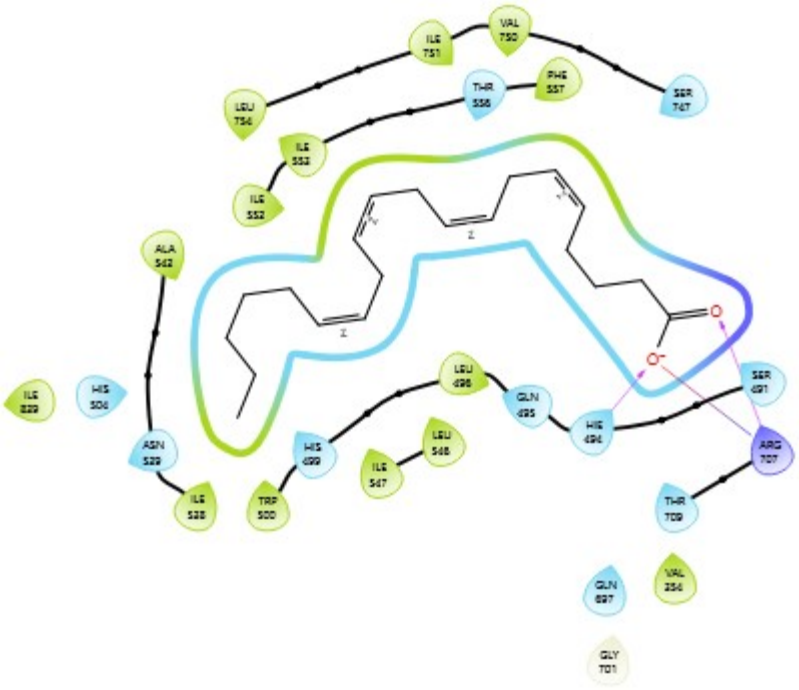


Figure 24 2D interaction diagram of arachidonic acid in the 15-LOX-1 model. The hydrophobic residues are shown in green around the hydrocarbon chain, while polar residues appear in blue and the charged arginine in purple near the headgroup. Pink lines indicate hydrogen bonds and electrostatic interactions with nearby polar and charged residues

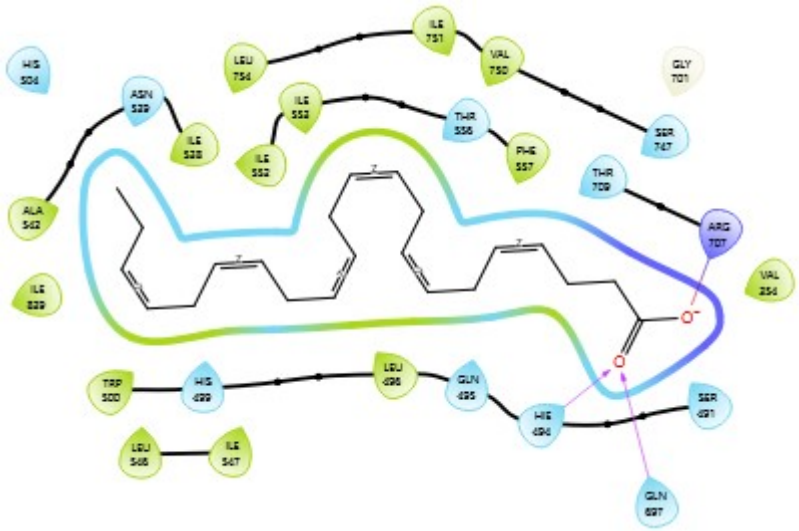
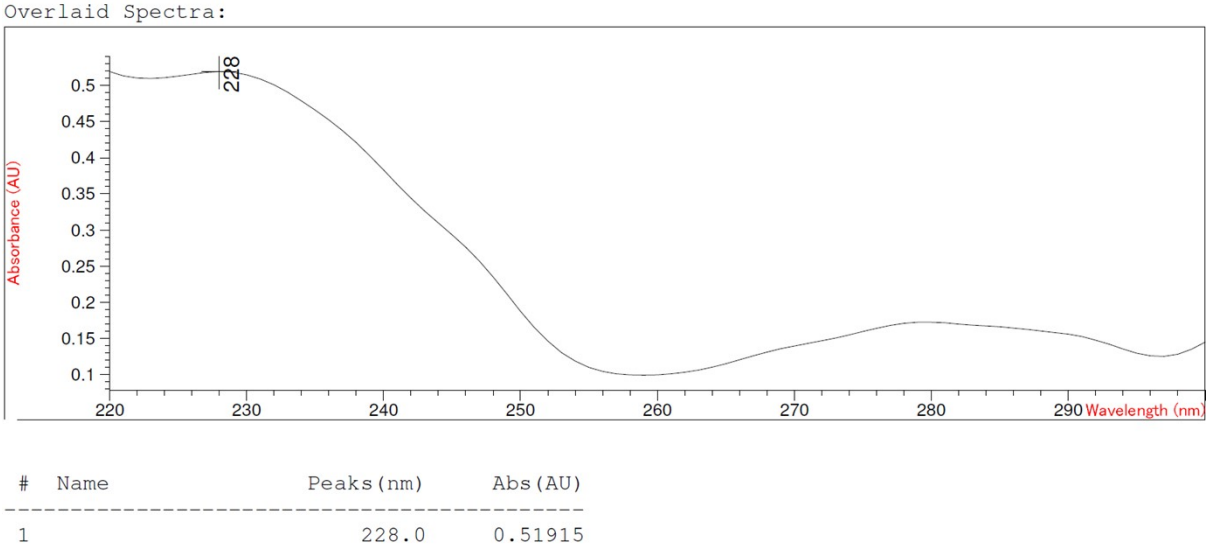


Figure 25 2D interaction diagram of docosahexaenoic acid in the 15-LOX-1 model. The hydrophobic residues are shown in green around the hydrocarbon chain, while polar residues appear in blue and the charged arginine in purple near the headgroup. Pink lines indicate hydrogen bonds and electrostatic interactions with nearby polar and charged residues

Incubation studies with DHA-like MEL 3

In six parallels, DHA-like MEL **3** (168 µg), was incubated with soybean 15-LOX-1 (borate buffer, pH 9.0, 10 000 U/mL) for 45 minutes. The reactions were then combined and quenched by addition of sodium borohydride in methanol (0.1 M, 20 mL) at 0 °C. Excess sodium borohydride was neutralised by dropwise addition of acetic acid. The aqueous phase was then extracted with diethyl ether (4 x 10 mL), dried over $MgSO_4$ and concentrated *in vacuo.* The resulting product mixture was dissolved in methanol and subjected to UV-Vis and MS analyses. The major peak in the MS spectra (*m/z* 441.2973) corresponds to the Na-adduct of DHA-like MEL **3**. The peak at *m/z* 457.2923 corresponds to the Na-adduct of $C_{26}H_{42}O_5$, which could indicate the introduction of a hydroxyl group on DHA-like MEL **3**. The product mixture was also analysed by MS/MS of the peak at *m/z* 457.2923 and HPLC-MS. UV (MeOH) λ_{max} 228 nm; HRMS (ESI) *m/z*: $[M+Na]^+$ CacId for $C_{26}H_{42}O_5Na$ 457.2924, Found 457.2923.



#	Name	Peaks(nm)	Abs(AU)
1		228.0	0.51915

Figure 26 UV-Vis chromatogram of the resulting product mixture after incubation of DHA-like MEL **3** with 15-LOX-1.

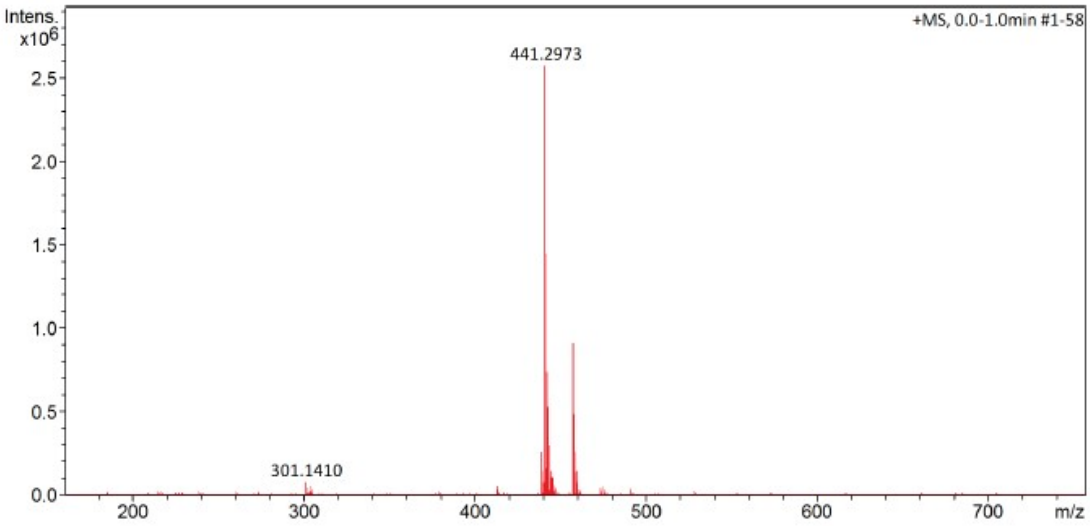
Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\maxis2025\21276.d	Acquisition Date	17-Nov-25 9:02:29 AM
Method	ESI_pos_50_1500_os.m	Operator	n/a
Sample Name	MB-3-055-A	Instrument	maXis II ETD 1823391.22318
Comment			

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	Source
				Set APCI Heater	0 °C



#	m/z	Res.	S/N	I	I %	FWHM
1	215.1254	31616	2427.0	20262	0.8	0.0068
2	239.1618	32210	1878.1	18043	0.7	0.0074
3	261.1309	33484	1391.3	15941	0.6	0.0078
4	273.1673	34016	1378.6	18075	0.7	0.0080
5	301.1410	35633	4652.0	79610	3.1	0.0085
6	303.2294	35309	1004.8	17943	0.7	0.0086
7	304.2611	34852	1988.9	36165	1.4	0.0087
8	379.3183	35238	499.1	18545	0.7	0.0108
9	413.2662	36062	1287.4	57439	2.2	0.0115
10	414.2697	37105	348.2	15608	0.6	0.0112
11	439.2818	36346	5104.2	260467	10.1	0.0121
12	440.2853	37042	1474.9	75686	2.9	0.0119
13	441.2973	35925	49891.7	2574060	100.0	0.0123
14	441.4225	65332	780.6	40293	1.6	0.0068
15	442.3008	35829	14337.3	742140	28.8	0.0123
16	443.3120	30803	10330.2	535598	20.8	0.0144
17	444.3162	34391	2842.7	147555	5.7	0.0129
18	445.3279	31485	2086.9	108496	4.2	0.0141
19	446.3319	34200	564.2	29395	1.1	0.0131
20	447.3438	31800	548.0	28578	1.1	0.0141
21	455.2767	35449	348.6	18609	0.7	0.0128
22	457.2923	36070	17095.4	916725	35.6	0.0127
23	458.2957	36396	4867.9	261528	10.2	0.0126
24	459.3067	28837	2757.5	148377	5.8	0.0159
25	460.3110	33306	741.2	39964	1.6	0.0138
26	461.3226	30490	488.8	26414	1.0	0.0151
27	473.2873	36866	793.4	43638	1.7	0.0128
28	475.3028	35293	698.4	38480	1.5	0.0135
29	477.3181	32354	279.9	15444	0.6	0.0148
30	491.2979	37431	605.9	33735	1.3	0.0131

Figure 27 MS spectra of the product mixture after incubation of DHA-like MEL **3** with 15-LOX-1.

Elemental Analysis Report

Analysis Info

Sample Name	MB-3-055-A	Acquisition Date	17-Nov-25 9:02:29 AM
Method	ESI_pos_50_1500_os.m	Analysis Name	D:\Data\maxis2025\21276.d

Acquisition Parameter

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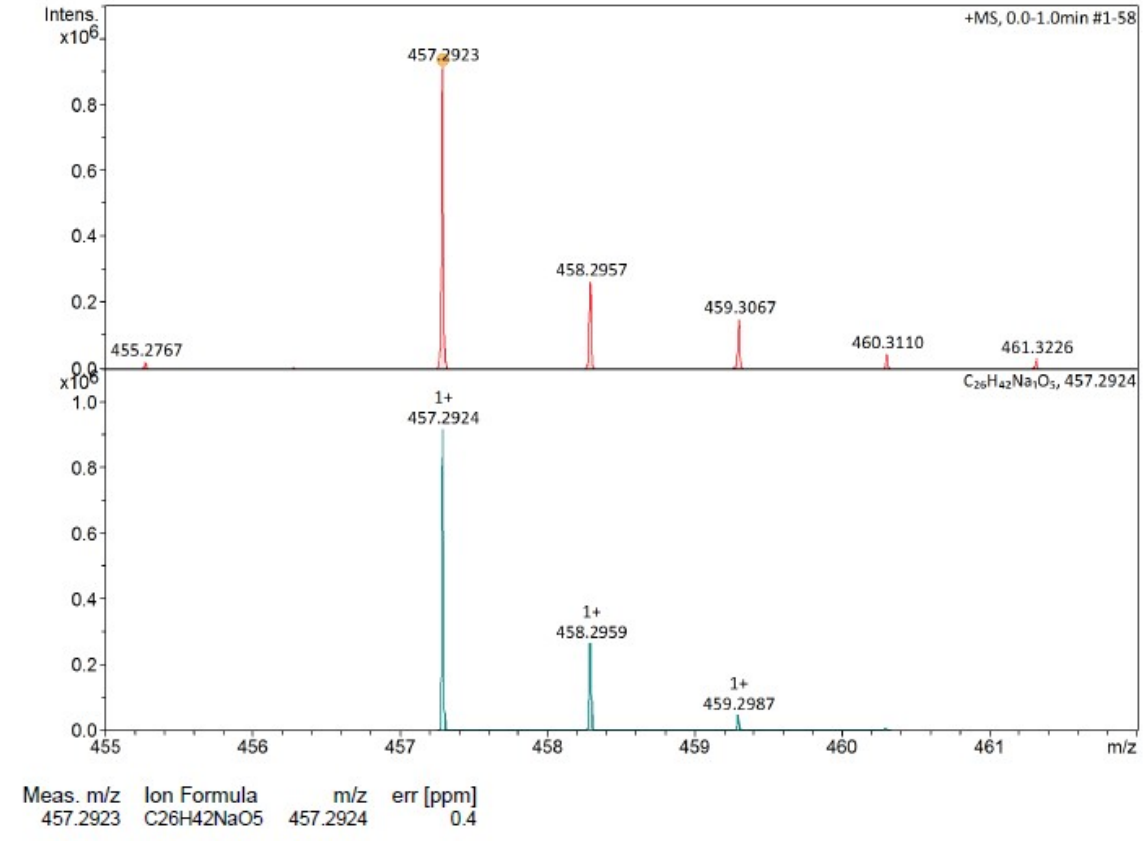
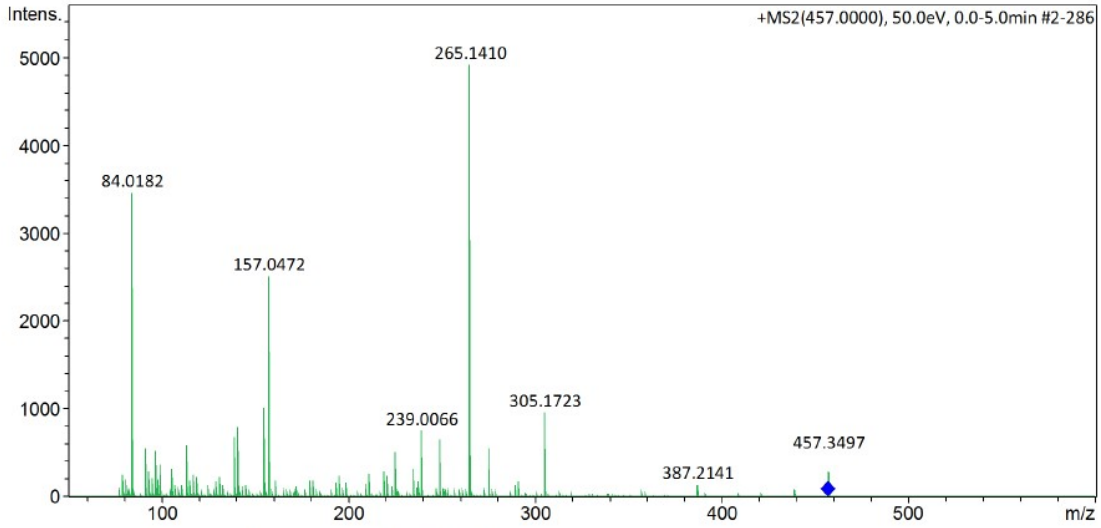
Scan Begin	50 m/z	Set Charging Voltage	2000 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Corona	0 nA	Set Divert Valve	Source
				Set APCI Heater	0 °C



Meas. m/z	Ion Formula	m/z	err [ppm]
457.2923	C26H42NaO5	457.2924	0.4

Figure 28 HRMS spectra of the formed product after incubation of DHA-like MEL **3** with 15-LOX-1.

Mass Spectrum List Report



#	m/z	Res.	S/N	I	I %	FWHM
1	79.0543	23123	12.9	253	5.1	0.0034
2	84.0182	23887	177.0	3461	70.3	0.0035
3	91.0543	25413	28.4	553	11.2	0.0036
4	93.0700	24533	15.2	297	6.0	0.0038
5	97.0260	26130	15.9	308	6.3	0.0037
6	97.0472	25621	26.8	520	10.6	0.0038
7	99.0629	26066	18.9	365	7.4	0.0038
8	105.0699	26329	16.5	318	6.5	0.0040
9	113.0210	27372	30.7	590	12.0	0.0041
10	117.0699	28315	13.2	253	5.1	0.0041
11	119.0856	28750	11.9	228	4.6	0.0041
12	139.0941	30311	36.4	682	13.9	0.0046
13	141.0733	28156	42.5	797	16.2	0.0050
14	155.0679	30462	55.0	1019	20.7	0.0051
15	157.0472	30269	136.3	2516	51.1	0.0052
16	195.0993	33467	13.5	240	4.9	0.0058
17	211.0941	34137	15.6	271	5.5	0.0062
18	219.1567	32151	17.2	295	6.0	0.0068
19	221.1360	32902	14.0	240	4.9	0.0067
20	225.1098	33017	30.2	516	10.5	0.0068
21	235.1305	33595	19.1	322	6.5	0.0070
22	239.0066	34721	44.8	753	15.3	0.0069
23	239.1254	34584	21.1	354	7.2	0.0069
24	249.0021	35759	39.1	649	13.2	0.0070
25	249.1672	34347	30.7	508	10.3	0.0073
26	265.1410	35738	303.3	4924	100.0	0.0074
27	275.1618	35834	33.9	546	11.1	0.0077
28	305.1723	37423	61.0	963	19.6	0.0082
29	457.2922	37593	17.3	272	5.5	0.0122
30	457.3497	39213	18.9	298	6.1	0.0117

Figure 29 MS/MS spectra of the peak at 457.2923 m/z.

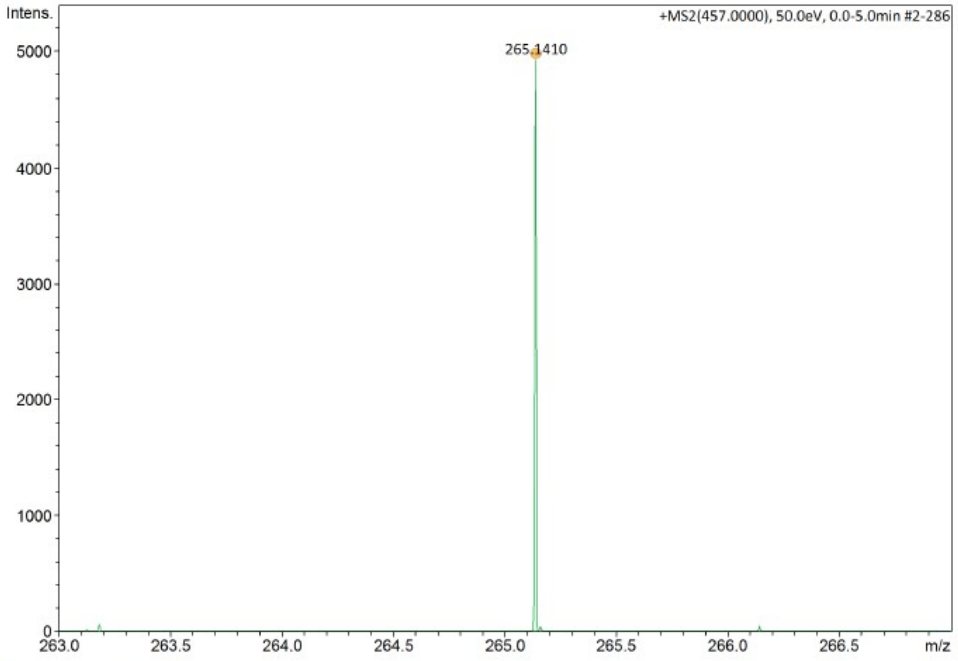
Elemental Analysis Report

Analysis Info

Sample Name	MB-3-055-A_1	Acquisition Date	17-Nov-25 9:51:35 AM
Method	ESI_pos_50_1500_os.m	Analysis Name	D:\Data\maxis2025\21276_1.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	Source
				Set APCI Heater	0 °C



Meas. m/z	Ion Formula	m/z	err [ppm]
84.0182	C2H2N3O	84.0192	11.8
157.0472	C3H5N6O2	157.0468	-2.1
	C5H7N3O3	157.0482	6.4
	C5H10NaO4	157.0471	-0.3
265.1410	C11H17N6O2	265.1408	-0.8
	C13H22NaO4	265.1410	0.2
457.3497	C22H45N6O4	457.3497	-0.1
	C23H44N7NaO	457.3500	0.5
	C24H50NaO6	457.3500	0.6

21276_1.d

Bruker Compass DataAnalysis 4.3 printed: 17-Nov-25 2:20:49 PM Page 1 of 1

Figure 30 HRMS analysis of the MS/MS spectra.

Acquisition Parameter

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

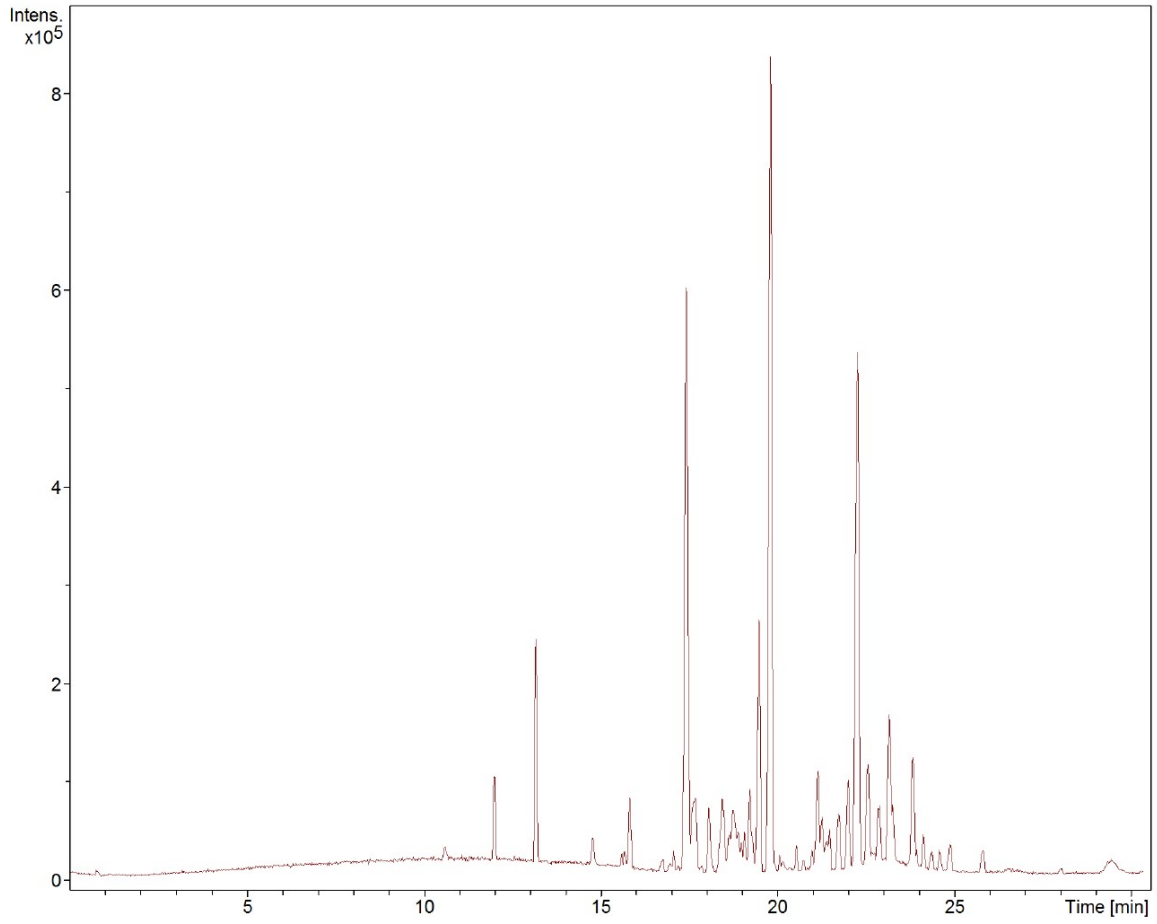


Figure 31 Base Peak Chromatogram after HPLC-MS.

Acquisition Parameter

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

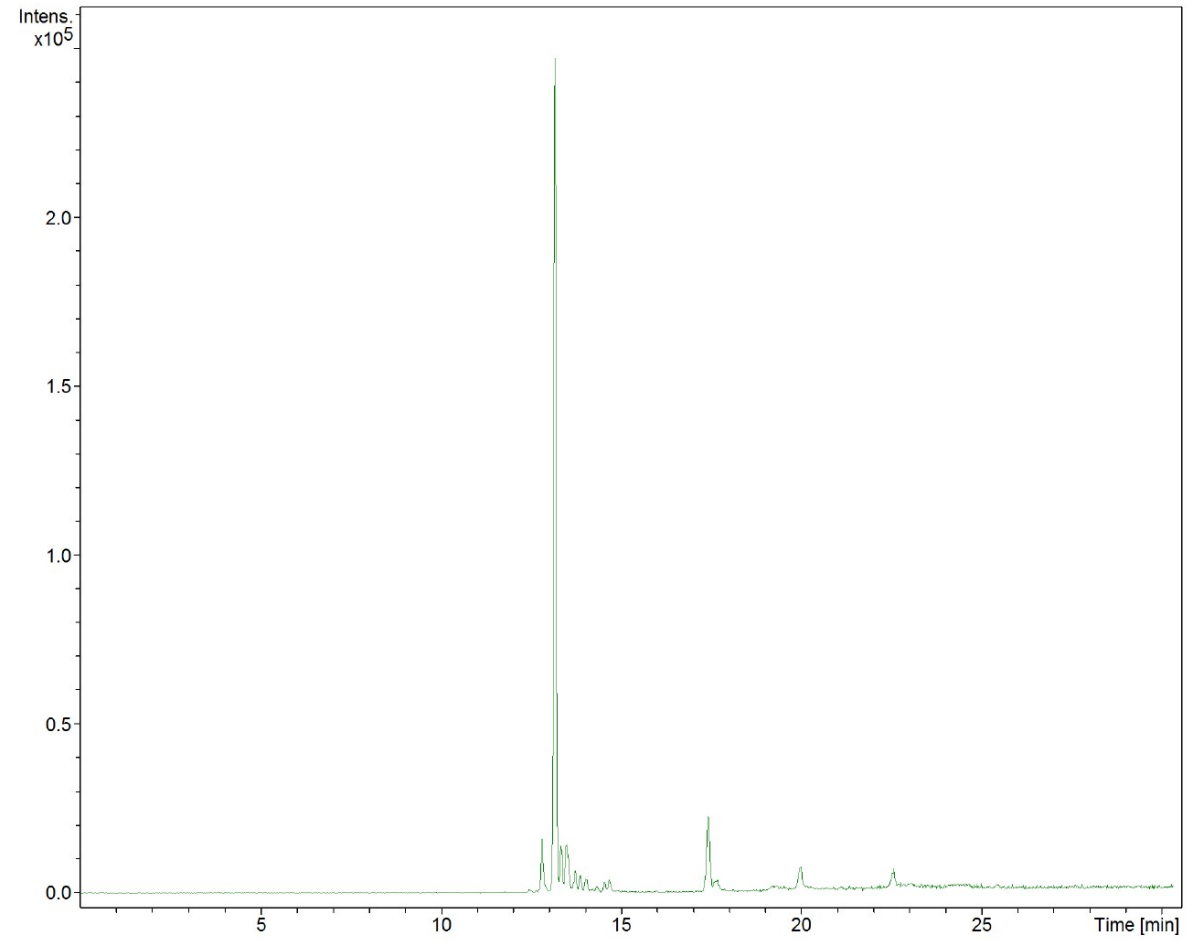


Figure 32 Extracted Ion Chromatogram for *m/z* 457.2944.

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

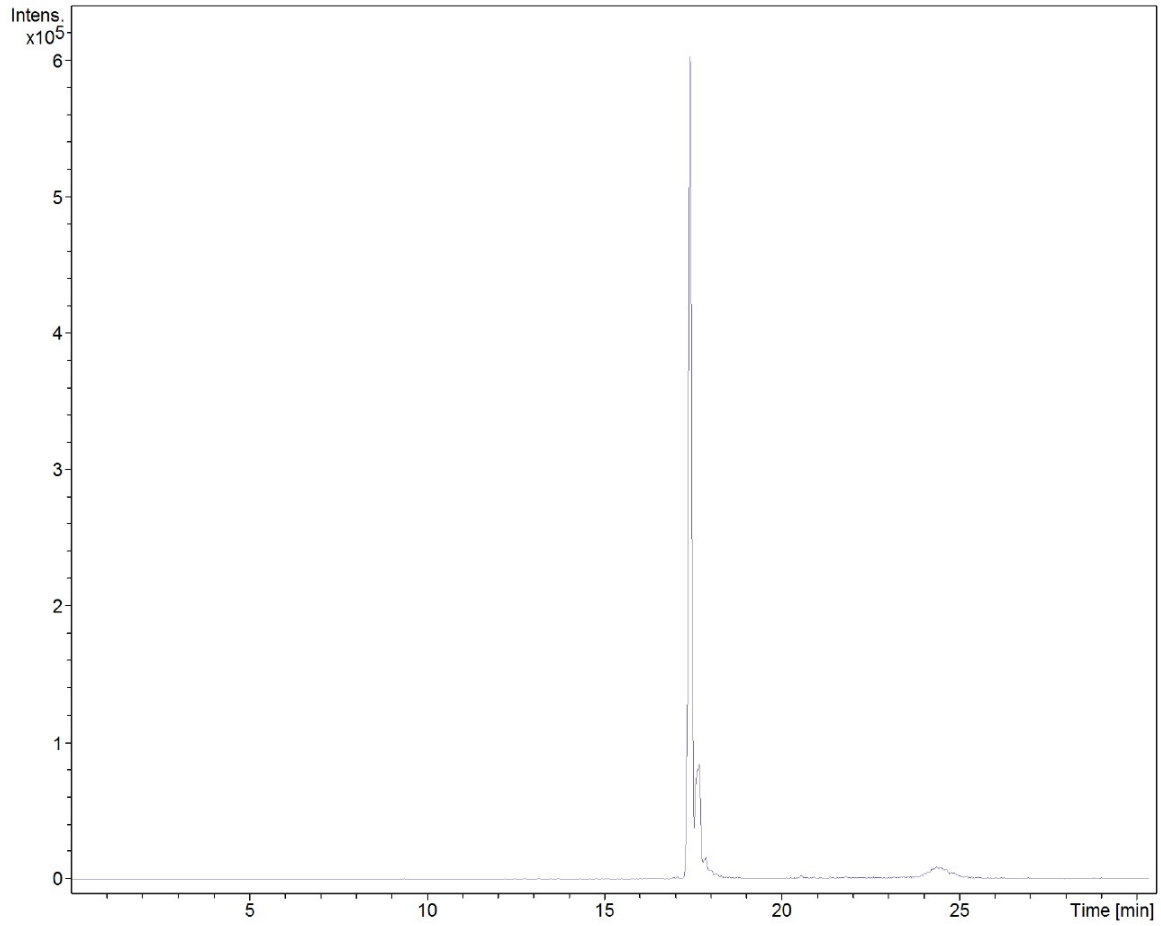


Figure 33 Extracted Ion Chromatogram for *m/z* 441.2991.

References:

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