

Supporting Information

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A New Short Chain Acetamide from the Biosphere and Bioactive Glycerolipids Extracted from the Marine Bivalve *Codakia orbicularis* (Lucinidae).

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S1: General Experimental Procedures.

Gills of bivalve individuals freshly collected from the seagrass beds were extracted with ethyl acetate (EtOAc) (VWR Chemicals, CAS number 141-78-6). Column chromatography (63*3 cm) was performed on silica gel (MERCK silica gel, 70-230 mesh ASTM) and flash column was performed on Redisep®Rf (220-gram, 69-2203-422 TELEDYNE Isco). These were carried out using hexane (CAS number 110-54-3), ethyl acetate (CAS number 141-78-6) and methanol (CAS number 67-56-1). Thin layer chromatography (TLC) was performed on commercial TLC plates (pre-coated Kiesegel 60 F254 TLC, 20 x 20 mm, thickness 0.25 mm, Merck) and was visualized under UV (254nm and 366nm).

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Solvents used for chromatography (column, thin layer and HPLC) were purchased from MERCK and CARLO ERBA. HPLC-grade acetonitrile (MeCN, CAS number 75-05-8) and milliQ water, both acidified by 0.1% formic acid (noted HCOOH,) were used for analytical and semi-preparative HPLC analysis. LC/MS analyses were performed using Waters Alliance 2695 separation module equipped with mass spectrometer (Waters ZQ 2000 with a single-quadrupole and electrospray ionization source), ELS detector (Waters 2420) and photodiode array detector (Waters 996). Semi-preparative HPLC was performed using an Auto Prep system (Waters 600 controller and Waters 600 pump, equipped with a Waters 996 Photodiode Array Detector). Data acquisitions were dealt with two softwares from Waters company: Empower (analytical analysis) and Masslynx (semi-preparative analysis). High-resolution mass spectra were obtained on a Waters LCT Premier XE in electrospray ionization mode by direct infusion of the purified compounds. NMR experiences (1D and 2D) were operated on a Bruker 500 AVANCE II or on a Bruker AVANCE II 600 MHz spectrometer (TXI 1.7 mm probe) (CNRS-ICSN). Chemical shift values are given in δ (ppm) employing residual CDCl_3 signal as reference ($\delta_{\text{H}} = 7.26$ and $\delta_{\text{C}} = 77.00$). All measurements were analyzed with the software NMRnotebook.

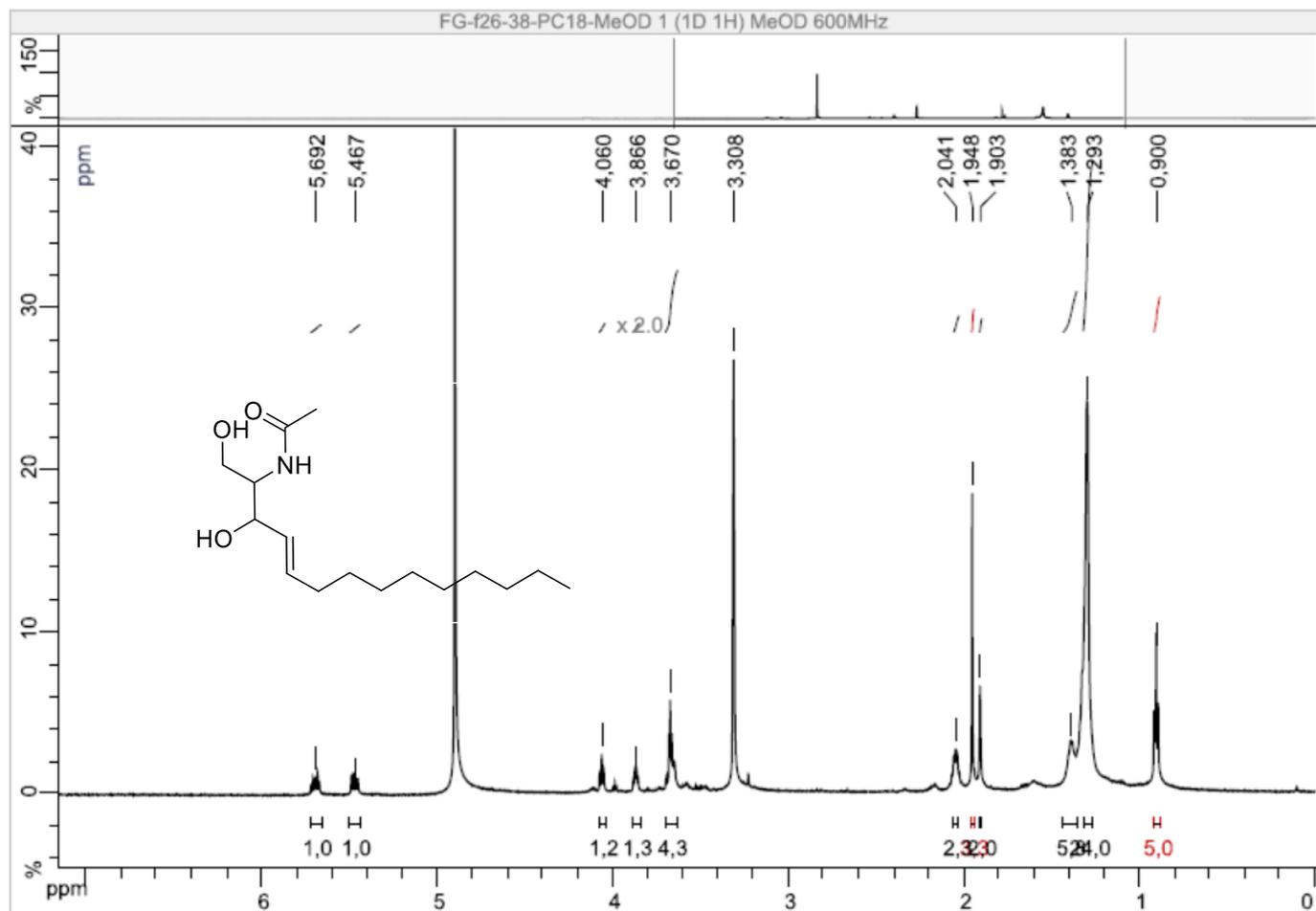


Figure S1: ¹H NMR spectrum of compound 1 (MeOD, 600 MHz).

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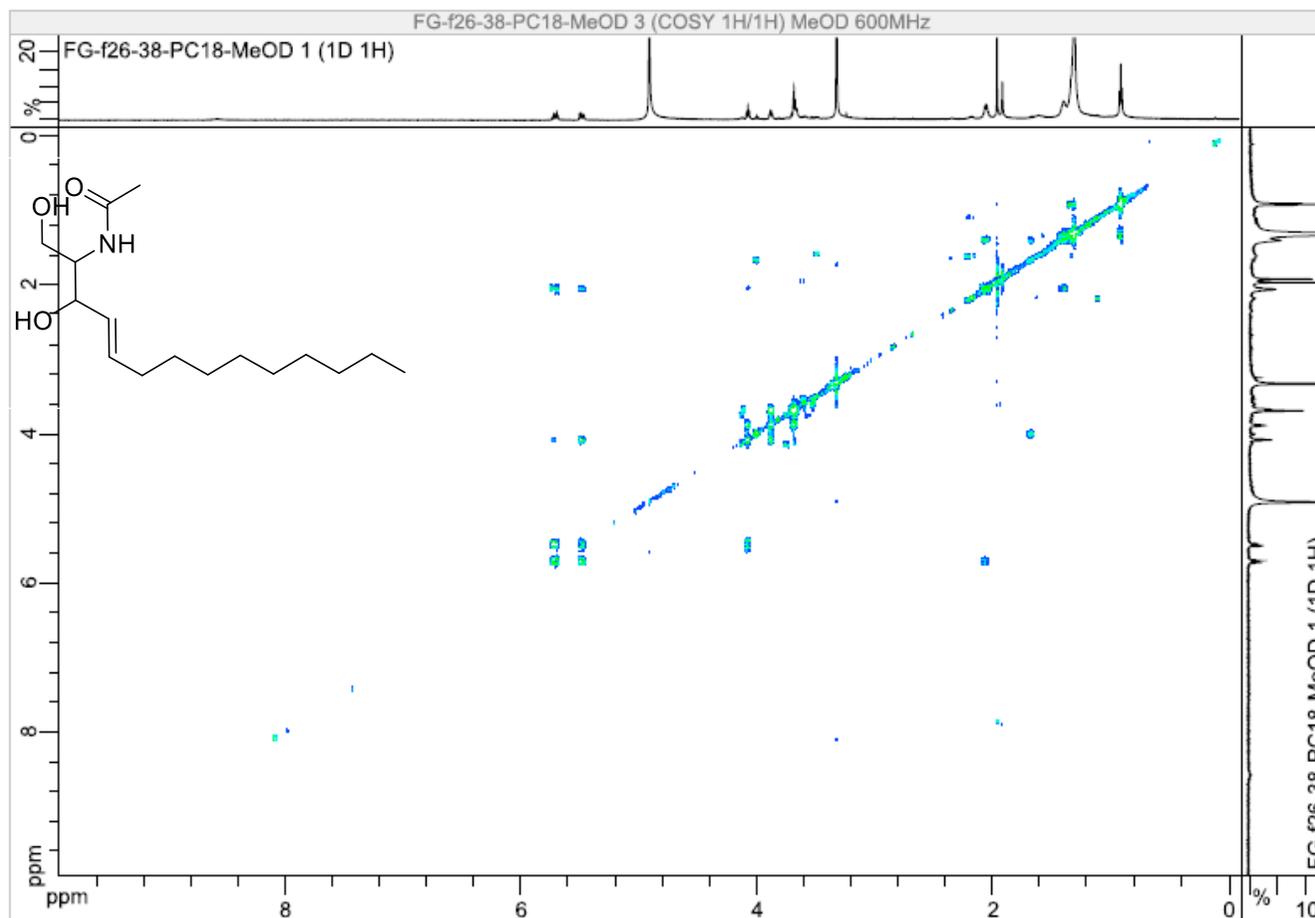


Figure S2: ^1H - ^1H COSY spectrum of compound **1** (MeOD, 600 MHz).

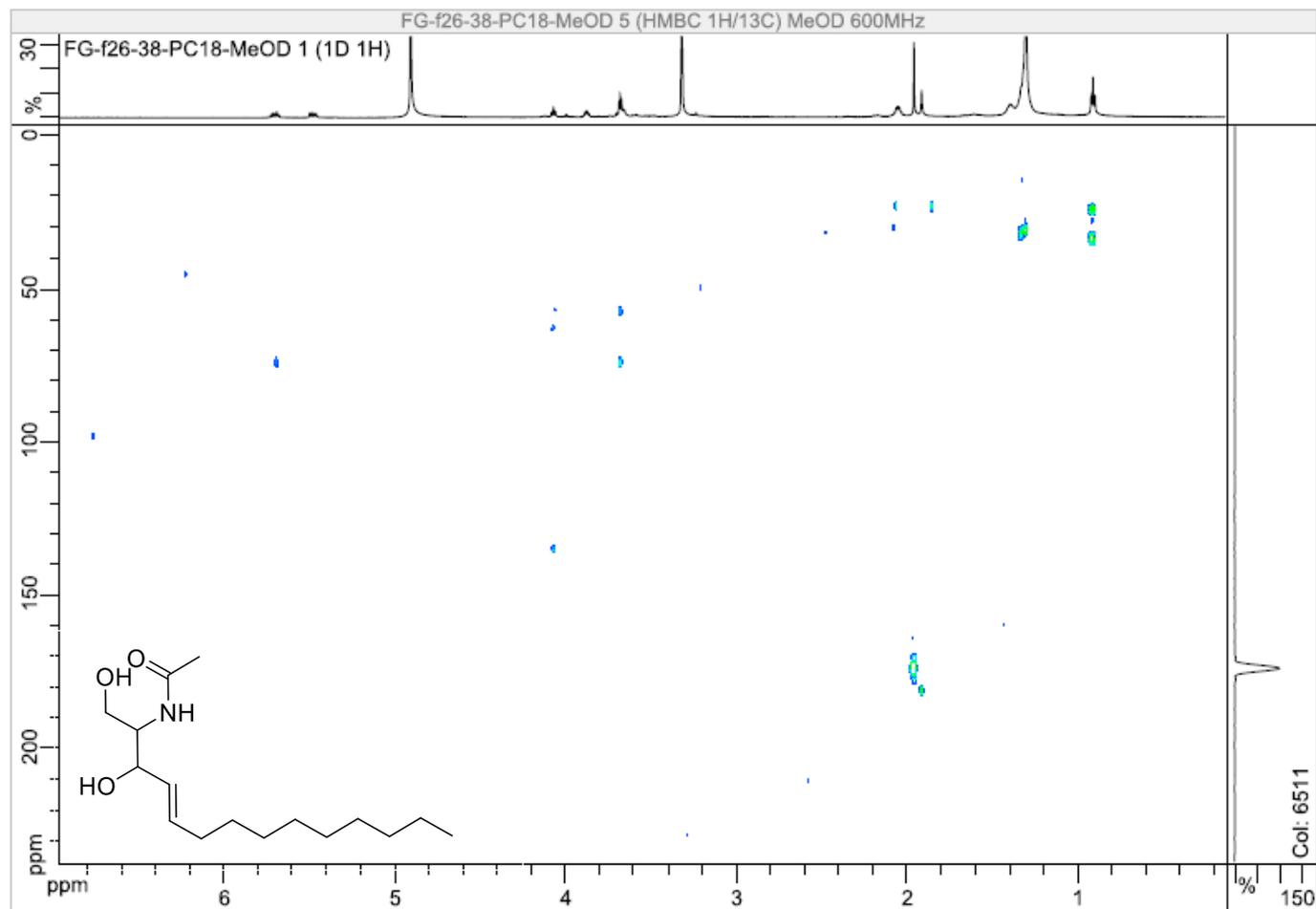


Figure S3: ^1H - ^{13}C HSQC spectrum of compound 1 (MeOD, 600 MHz).

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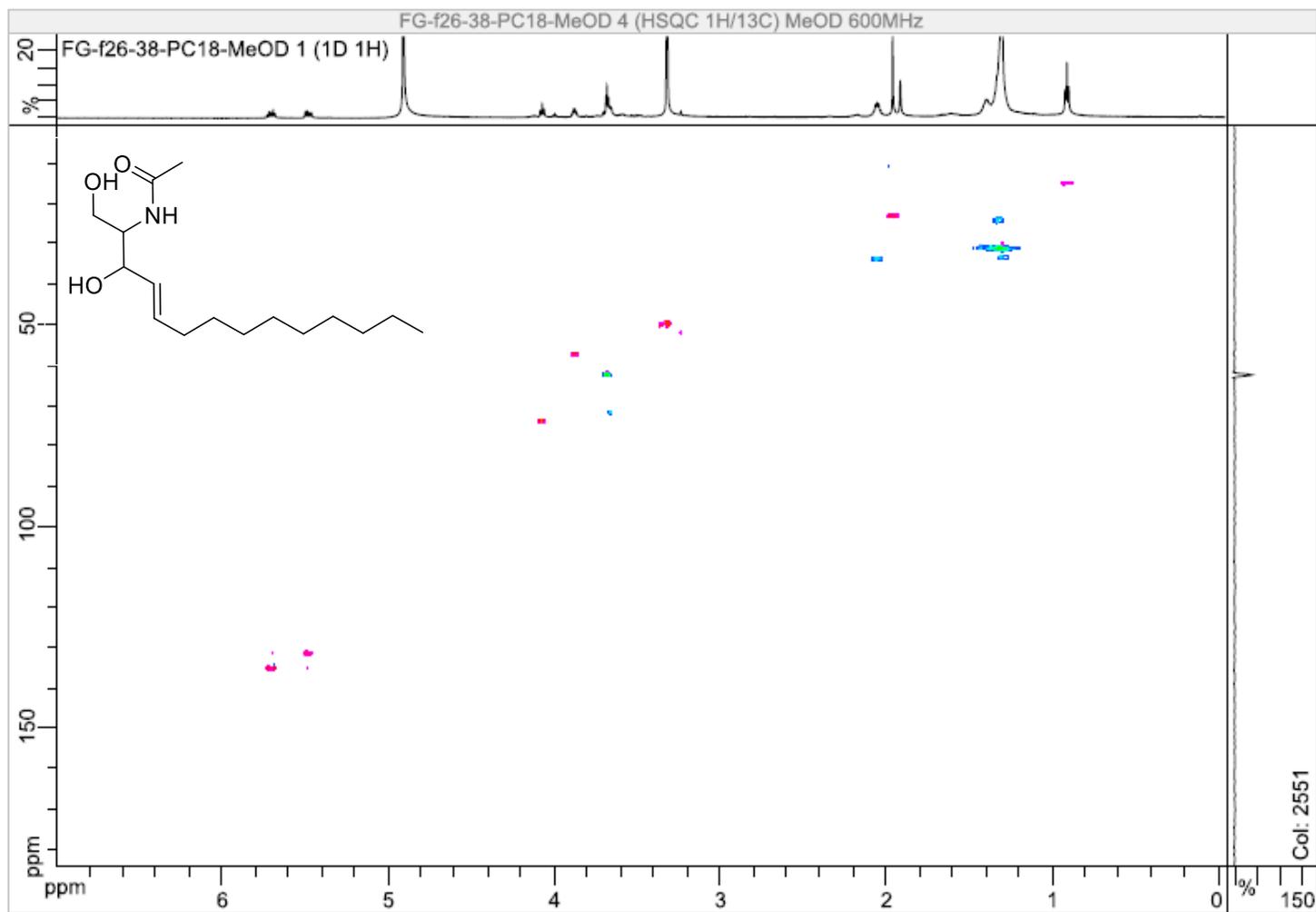


Figure S4: ^1H - ^{13}C HMBC spectrum of compound **1** (MeOD, 600 MHz).

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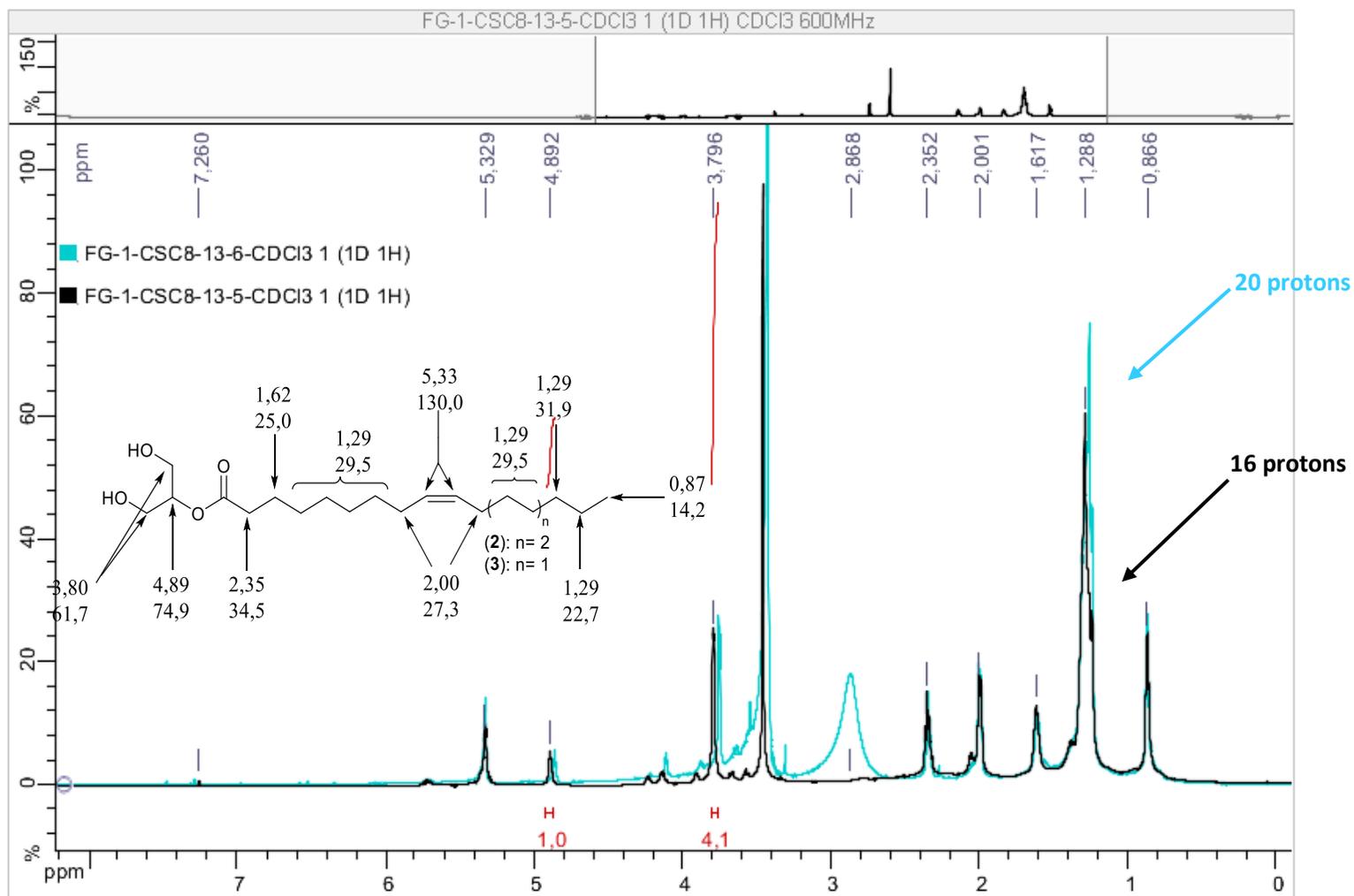


Figure S5: Overlays of ^1H NMR spectra of compounds **2** and **3** (CDCl_3 , 600 MHz).

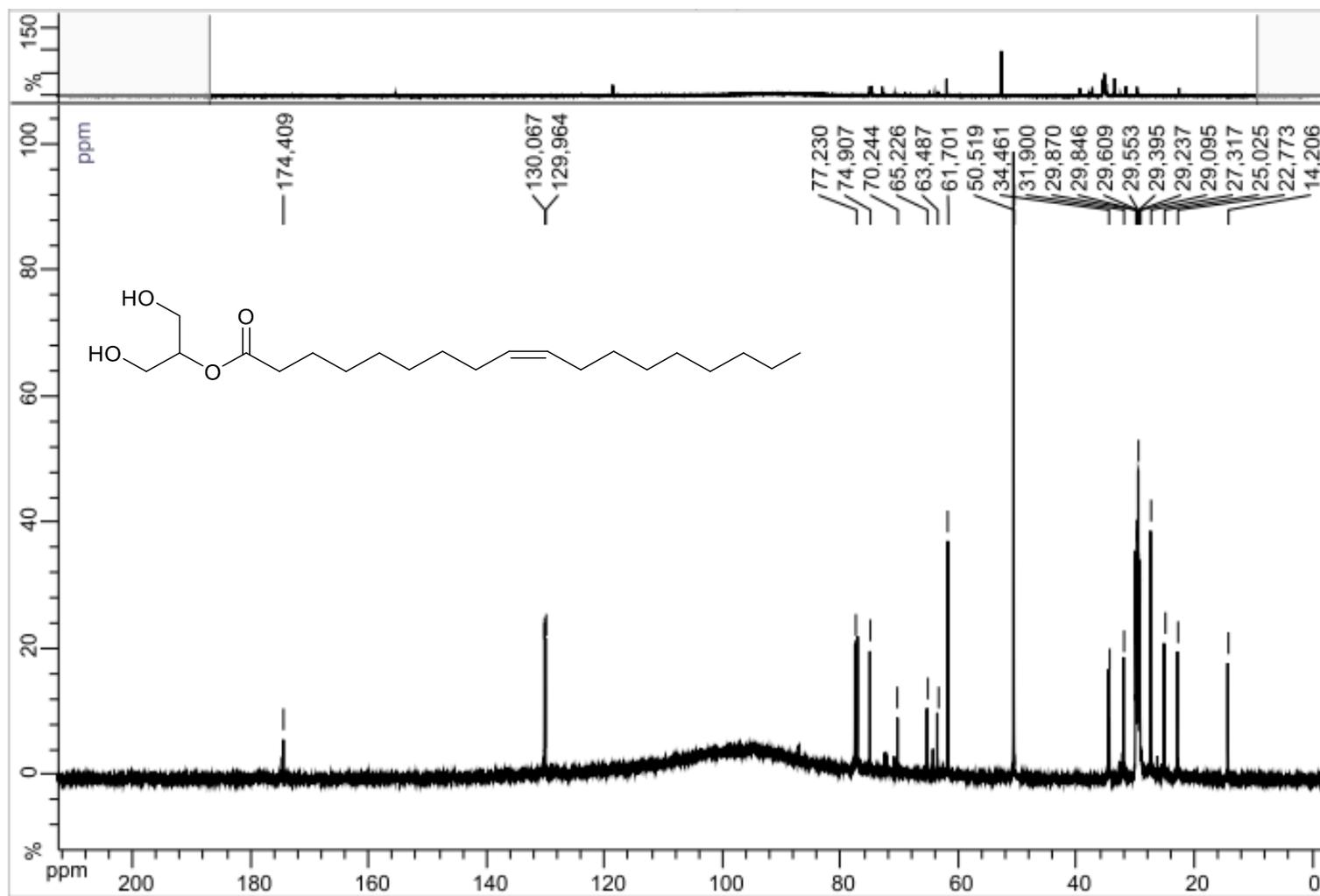


Figure S6: ^{13}C Spectrum of compound **2** (CDCl_3 , 150 MHz).

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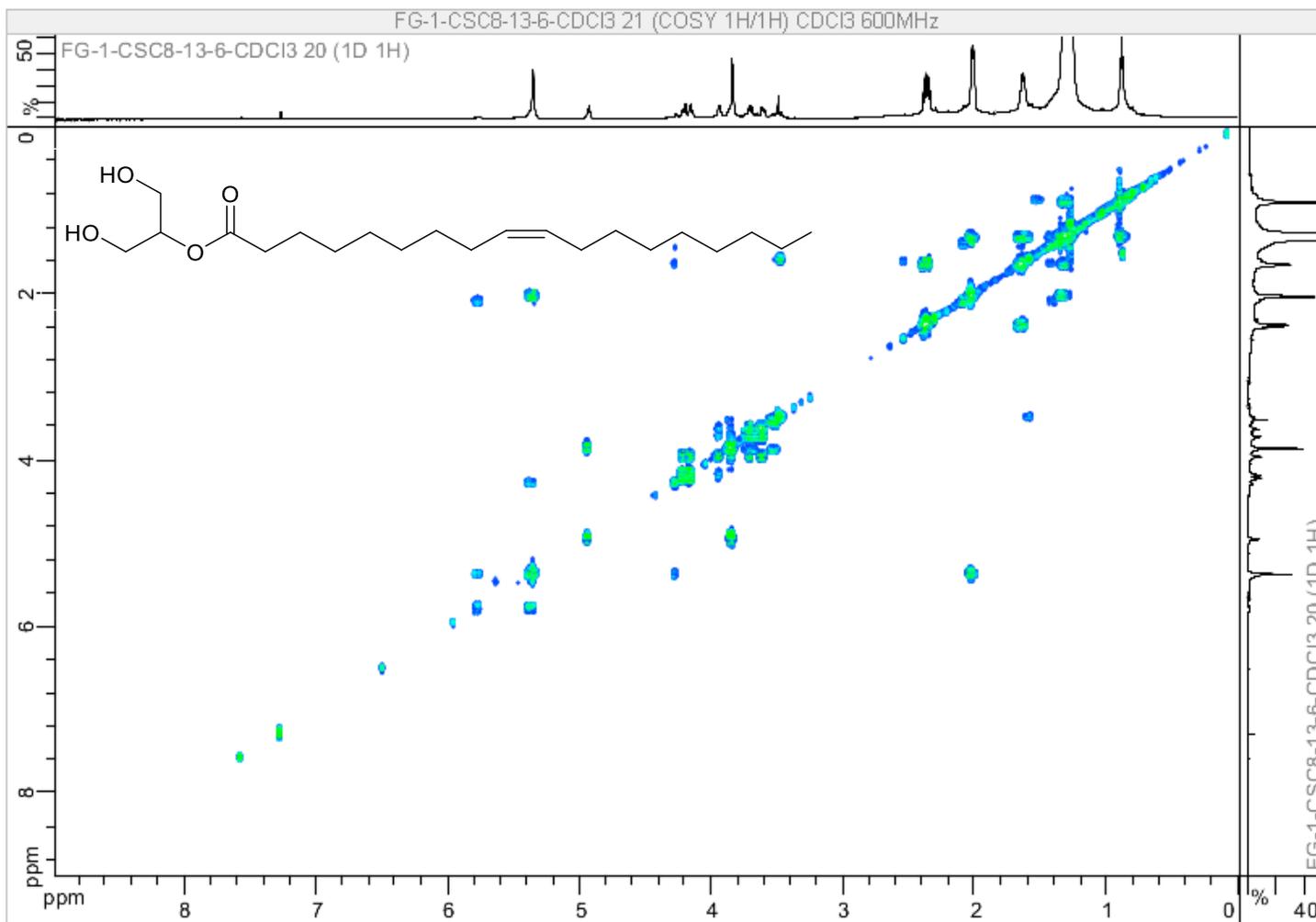


Figure S7: ^1H - ^1H COSY spectrum of compound **2** (CDCl_3 , 600 MHz).

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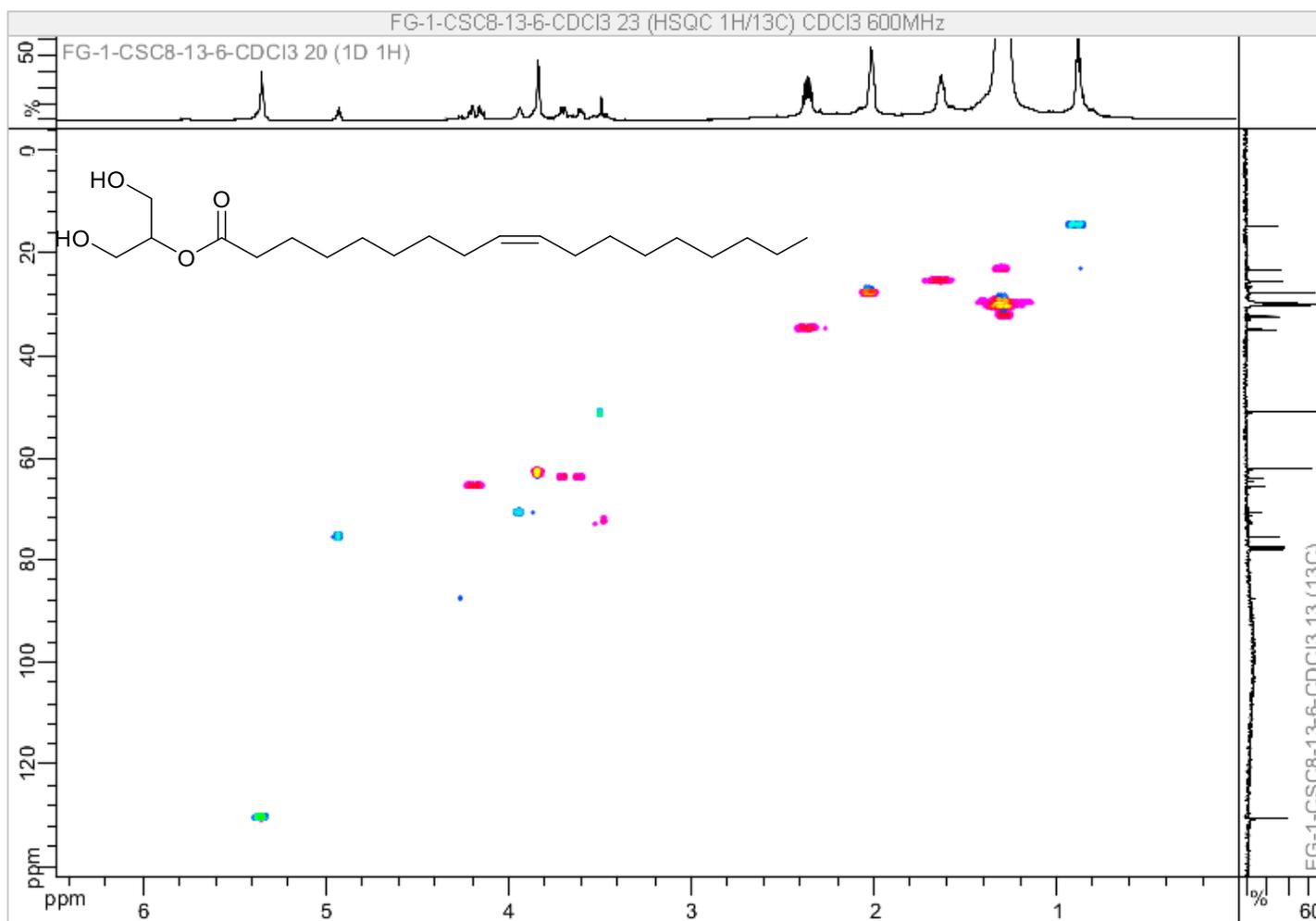


Figure S8: ^1H - ^{13}C HSQC spectrum of compound **2** (CDCl_3 , 600 MHz).

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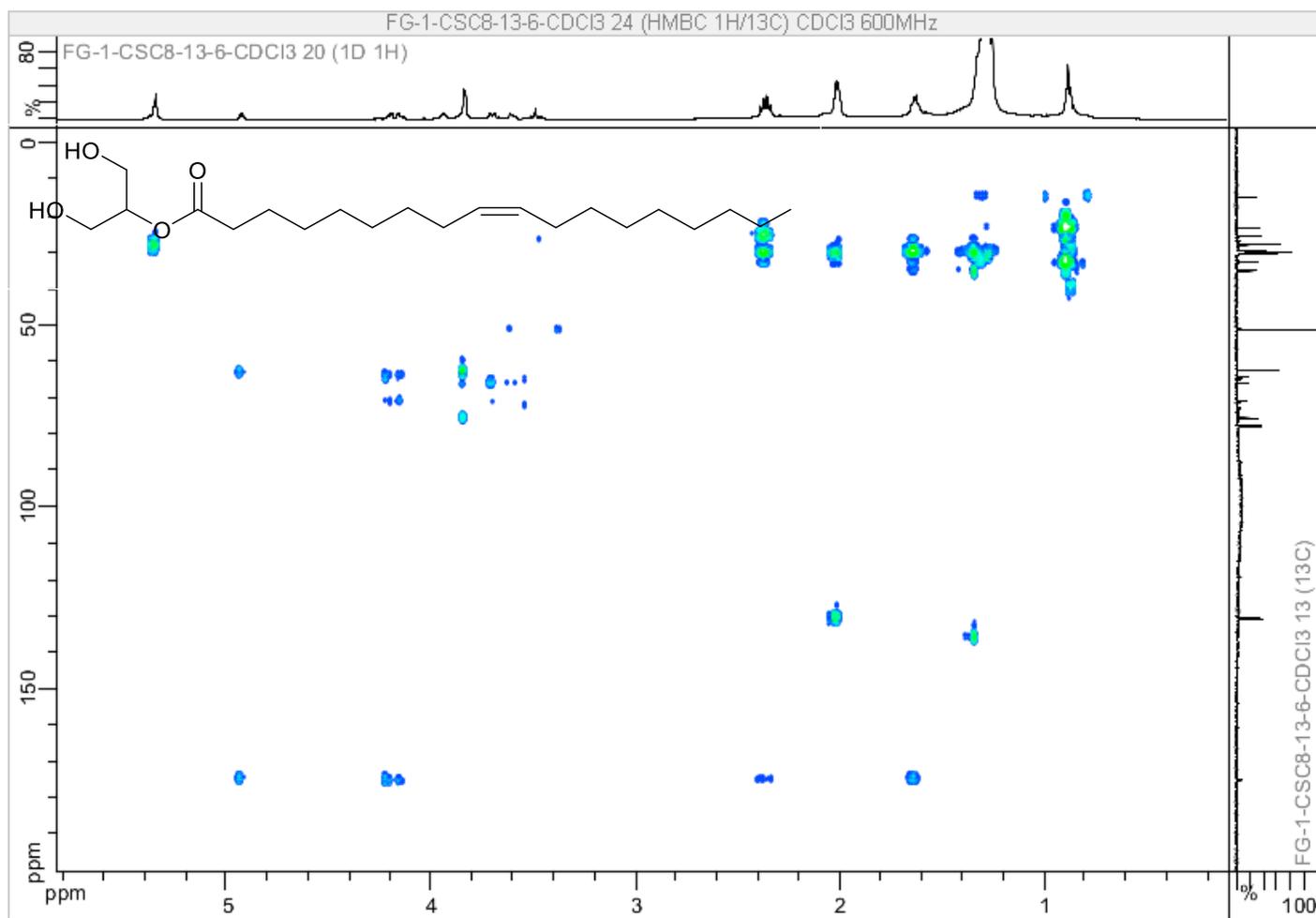


Figure S9: ^1H - ^{13}C HMBC spectrum of compound **2** (CDCl_3 , 600 MHz).

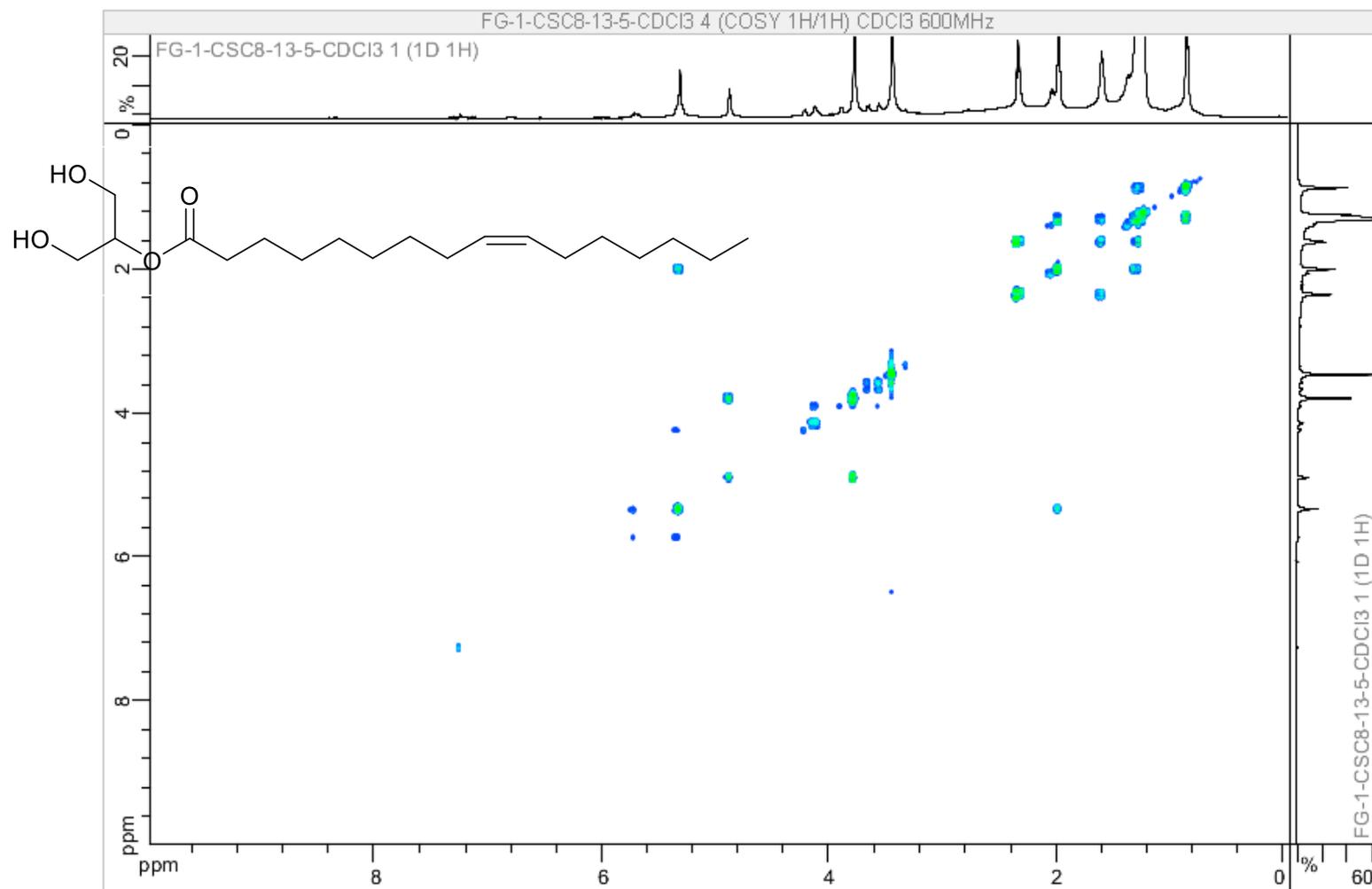


Figure S10: ^1H - ^1H COSY spectrum of compound **3** (CDCl_3 , 600 MHz).

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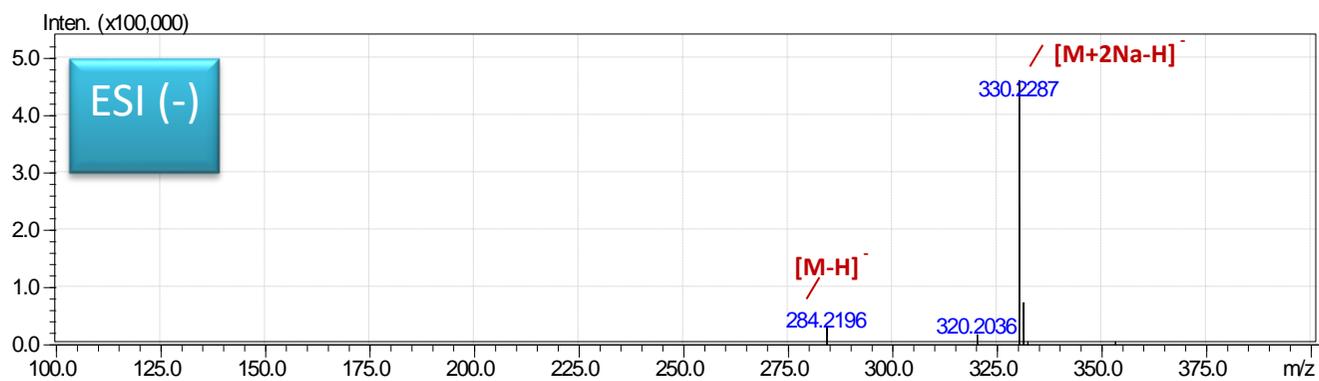
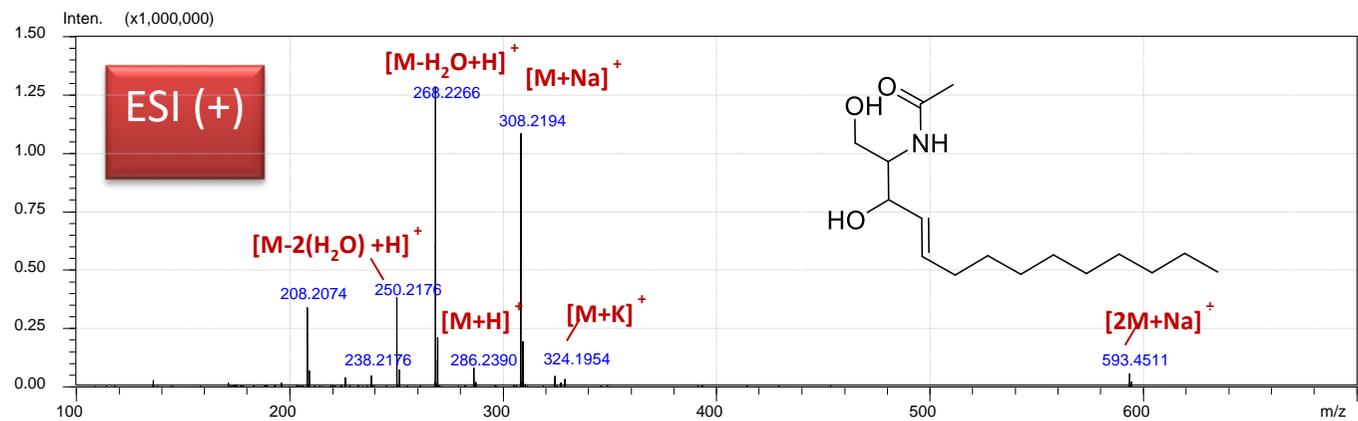


Figure S11: UHPLC – HRESIMS/SM spectra of compound 1.

ESI (-)

m/z 284.2187

[M-H]⁻



Formula Predictor Summary Report - F-26-38_2017-09-07.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Data for all - MMS\Olivier\Data\2017-09-07\F-26-38_2017-09-07.lcd

Elmt	Val.	Min	Max	Use Adduct												
H	1	0	250	F	1	0	0	S	2	0	2	Br	1	0	0	H
2H	1	0	0	Na	1	0	2	Cl	1	0	2	I	3	0	0	
C	4	1	75	Mg	2	0	0	Ca	2	0	0					
N	3	0	5	Si	4	0	0	Ti	2	0	0					
O	2	0	50	P	3	0	0	Fe	2	0	0					

Error Margin (ppm): 30 DBE Range: 0.0 - 50.0 Electron Ions: both
HC Ratio: 0.0 - 5.0 Apply N Rule: yes Use MSn Info: yes
Max Isotopes: all Isotope RI (%): 1.00 Isotope Res: 8000
MSn Iso RI (%): 5.00 MSn Logic Mode: AND Max Results: 50

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C16 H31 N O3	[M-H] ⁻	284.2213	284.2231	-1.8	-6.33	0.00	2.0
2	0.00	C15 H31 N3 S	[M-H] ⁻	284.2213	284.2166	4.7	16.54	0.00	2.0
3	0.00	C17 H32 N Cl	[M-H] ⁻	284.2213	284.2151	6.2	21.81	0.00	2.0
4	0.00	C18 H27 N3	[M-H] ⁻	284.2213	284.2132	8.1	28.50	0.00	7.0

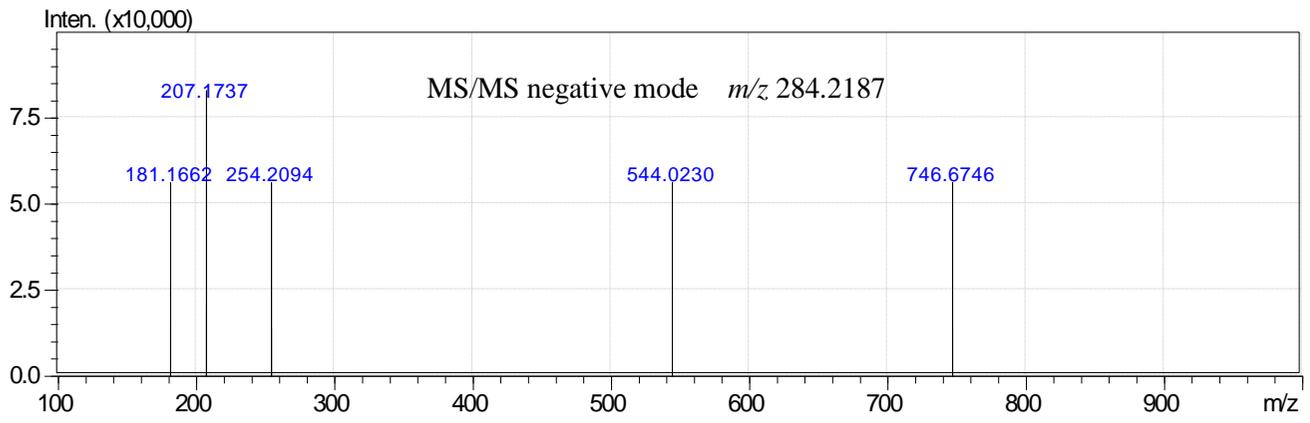


Figure S12: Molecular formula propositions for ion [M-H]⁻ m/z 284.2187 and SM/SM fragmentations.

ESI (+)

Ion m/z 286.2390 [M+H]⁺

Formula Predictor Summary Report - F-26-38_2017-09-07.lcd Page 1 of 1

Data File: C:\LabSolutions\Data\Data for all - MMS\Olivier\Data\2017-09-07\F-26-38_2017-09-07.lcd

Elmt	Val.	Min	Max	Use Adduct												
H	1	0	250	F	1	0	0	S	2	0	2	Br	1	0	0	H
2H	1	0	0	Na	1	0	0	Cl	1	0	2	I	3	0	0	Na
C	4	1	75	Mg	2	0	0	Ca	2	0	0					
N	3	0	5	Si	4	0	0	Ti	2	0	0					
O	2	0	50	P	3	0	0	Fe	2	0	0					

Error Margin (ppm): 30 DBE Range: 0.0 - 50.0 Electron Ions: both
 HC Ratio: 0.0 - 5.0 Apply N Rule: yes Use MSn Info: yes
 Max Isotopes: all Isotope RI (%): 1.00 Isotope Res: 8000
 MSn Iso RI (%): 5.00 MSn Logic Mode: AND Max Results: 50

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C16 H31 N O3	[M+H] ⁺	286.2386	286.2377	0.9	3.14	0.00	2.0
2	0.00	C15 H31 N3 S	[M+H] ⁺	286.2386	286.2311	7.5	26.20	0.00	2.0

Figure S13: Molecular formula propositions for ion [M+H]⁺ m/z 286.2390.

ESI (+)

m/z 268.2176

$[M-H_2O+H]^+$

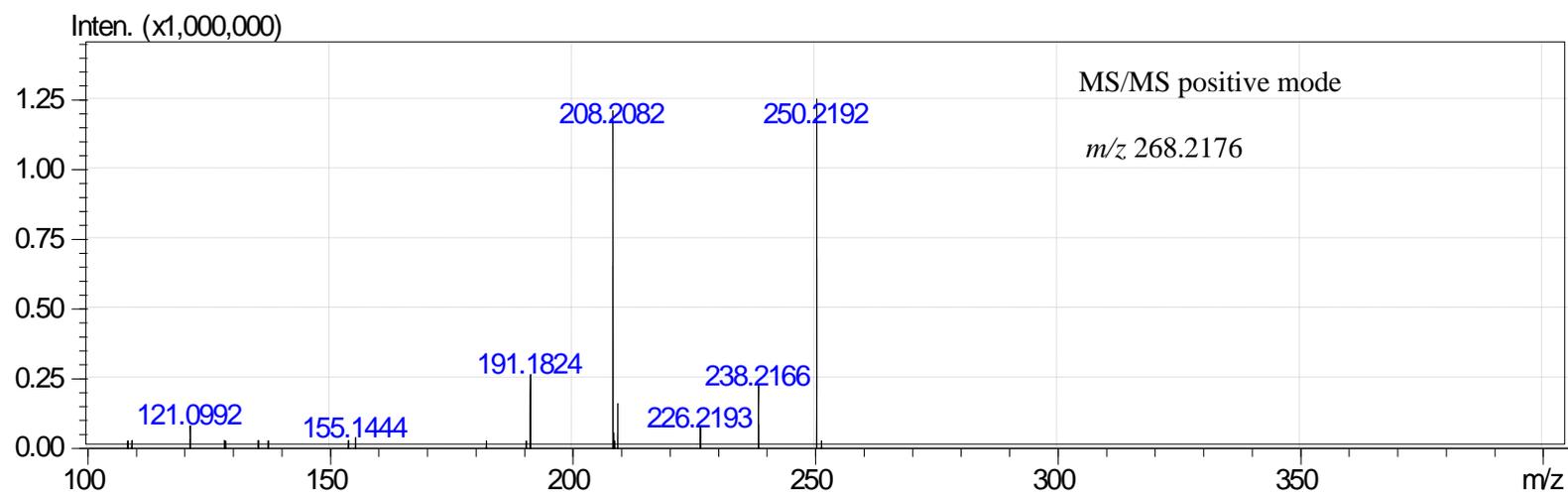


Figure S14: Molecular formula proposition for ion $[M-H_2O + H]^+$ m/z 268.2176 and SM/SM fragmentations.

ESI (+)

m/z 250.2078

$[M-2(H_2O) + H]^+$

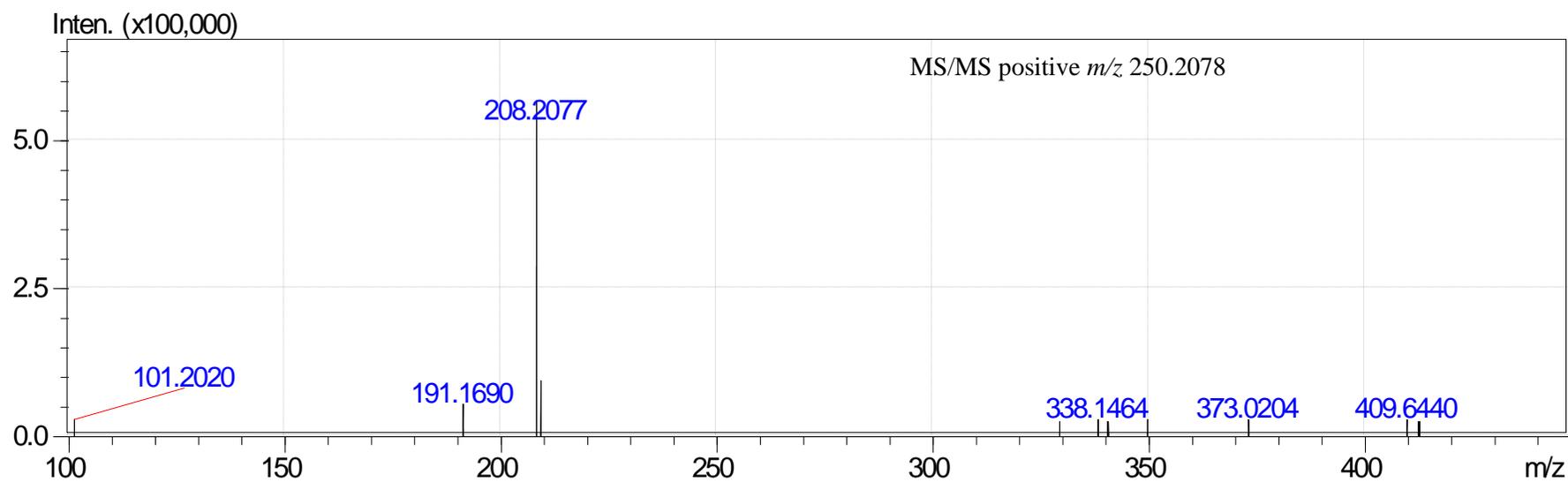


Figure S15: Molecular formula proposition for ion $[M-2(H_2O) + H]^+$ *m/z* 250.2078 and SM/SM fragmentations.

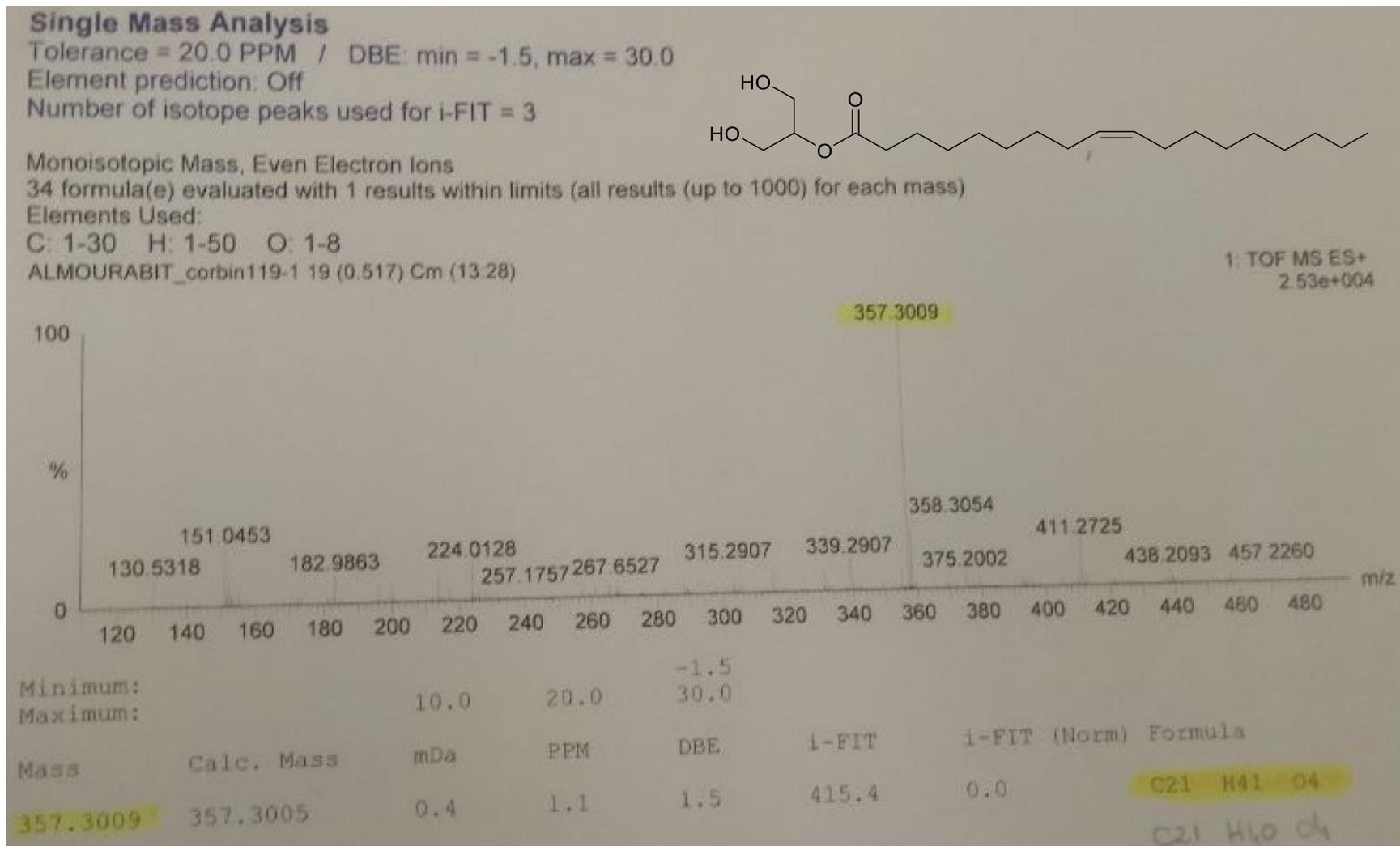


Figure S16: HRESIMS spectrum of compound **2** (positive mode).

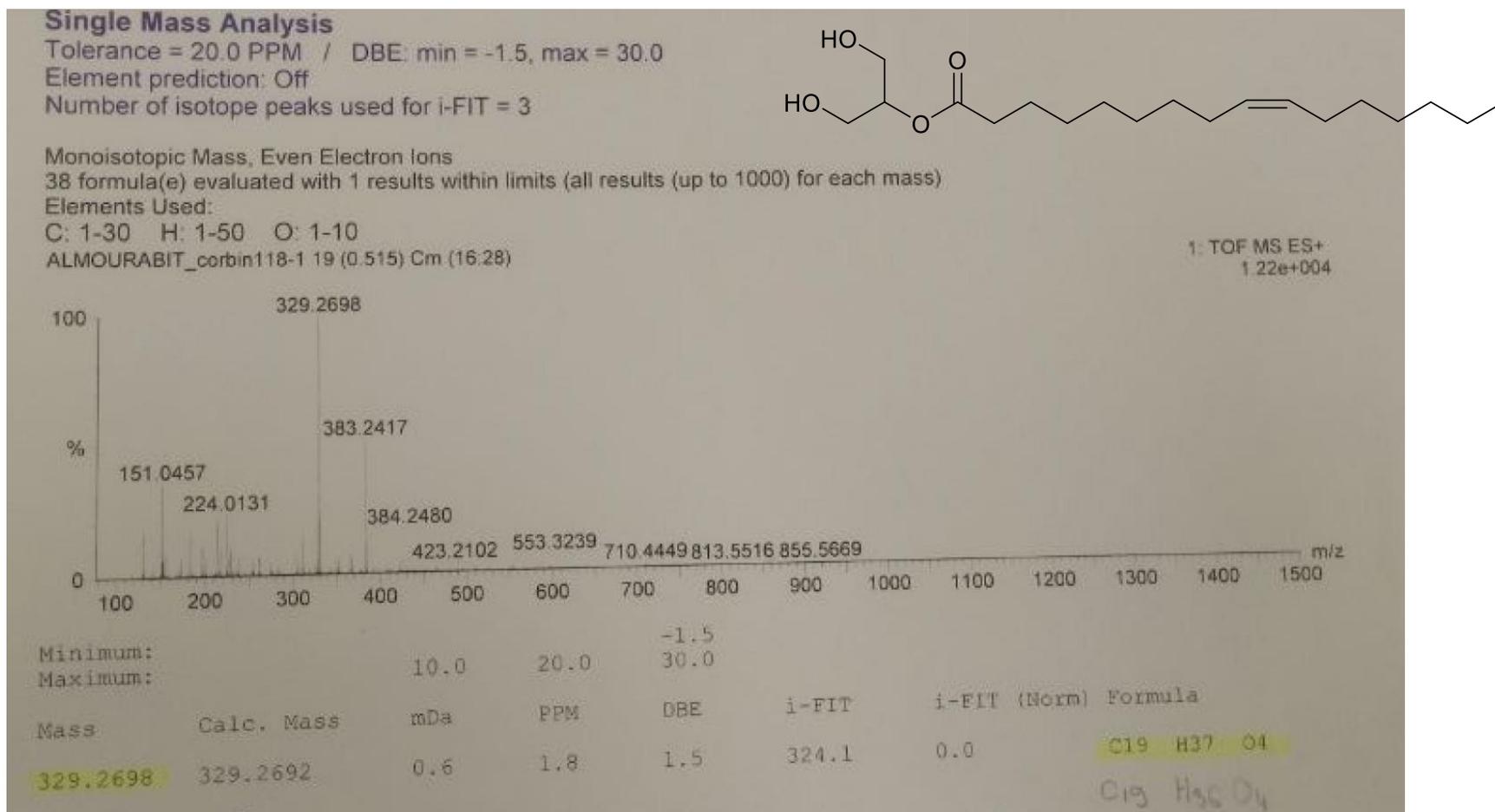
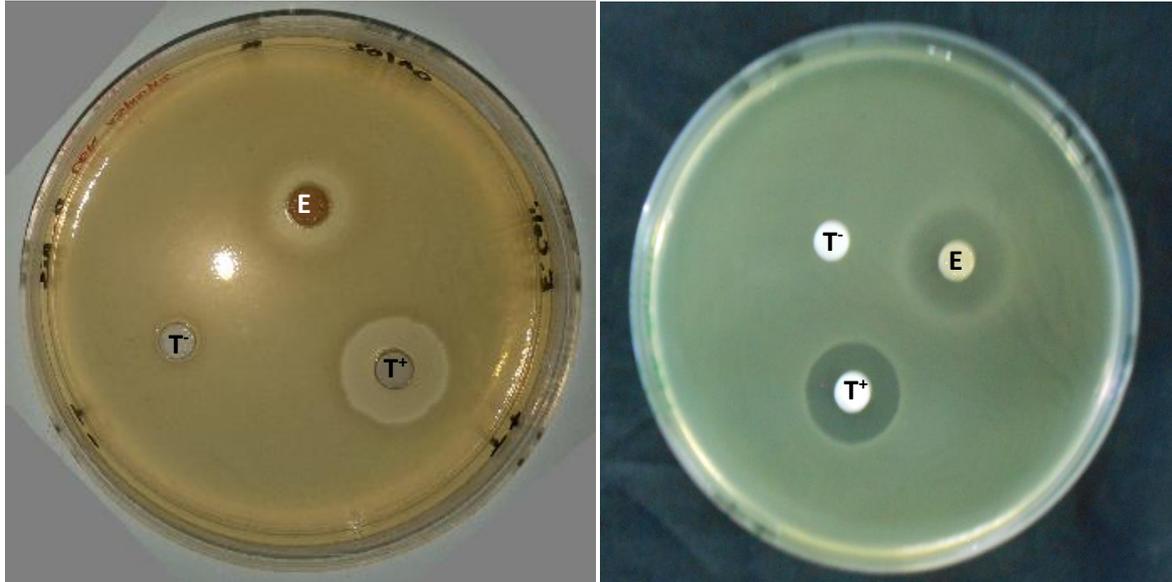


Figure S17: HRESIMS spectrum of compound 3 (positive mode).



Bioassays. A: Bioassay on *Micrococcus luteus* ATCC 10240 (Gram⁺).

E: cellulose disc on which 20 μ L of the crude extract of *Codakia orbicularis* (diameter of inhibition de 10 mm). **T⁺:** Antibiogram of colistin (50 μ g; positive control). **T:** cellulose disc containing 20 μ L ethyl acetate (negative control).

B: Bioassay on *Escherichia coli* ATCC 35218 (Gram⁻). **E:** cellulose disc on which 20 μ L of the crude extract of *Codakia orbicularis* (diameter of inhibition de 15 mm). **T⁺:** Antibiogram of vancomycin (50 μ g; positive control). **T:** cellulose disc containing 20 μ L ethyl acetate (negative control).

Figure S18: Bioassays on *Escherichia coli* ATCC 35218 and *Micrococcus luteus* ATCC 10240 with the crude extract of endosymbiotic gills of *Codakia orbicularis*.