

Anthelmintic activity and cytotoxic effects of compounds isolated from the fruits of *Ozoroa insignis* Del. (Anacardiaceae)

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Figures S1 - S9: HR-ESI-MS, 1D and 2D NMR of 6-[8(Z)-pentadecenyl] anacardic acid (1)

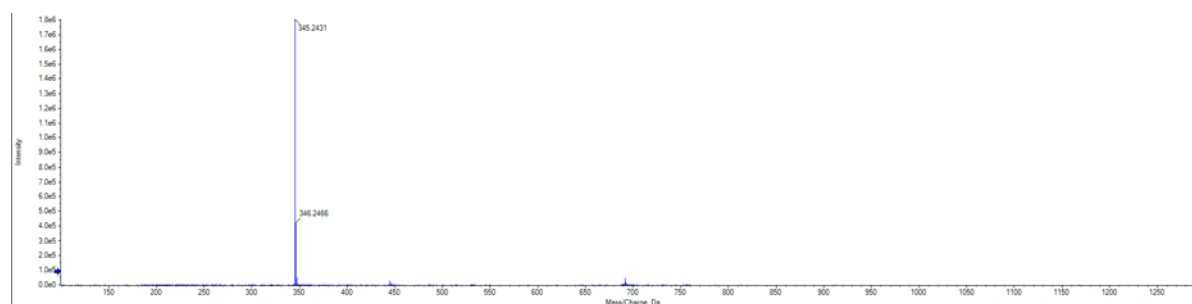


Figure S1: HR-ESI-MS spectrum (neg. mode) of 6-[8(Z)-pentadecenyl] anacardic acid (1).

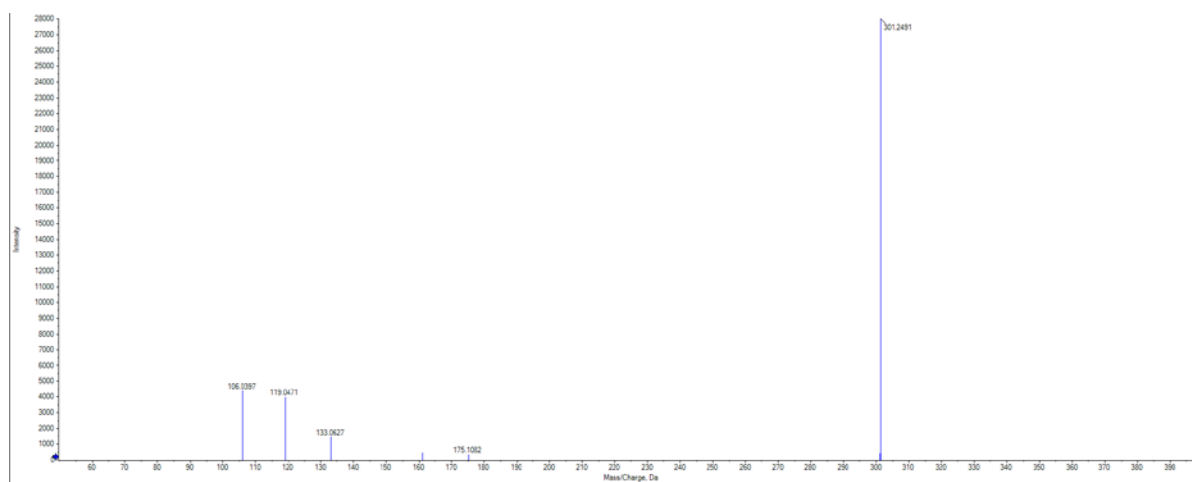


Figure S2: TOF MS² (neg. mode) of 6-[8(Z)-pentadecenyl] anacardic acid (**1**).

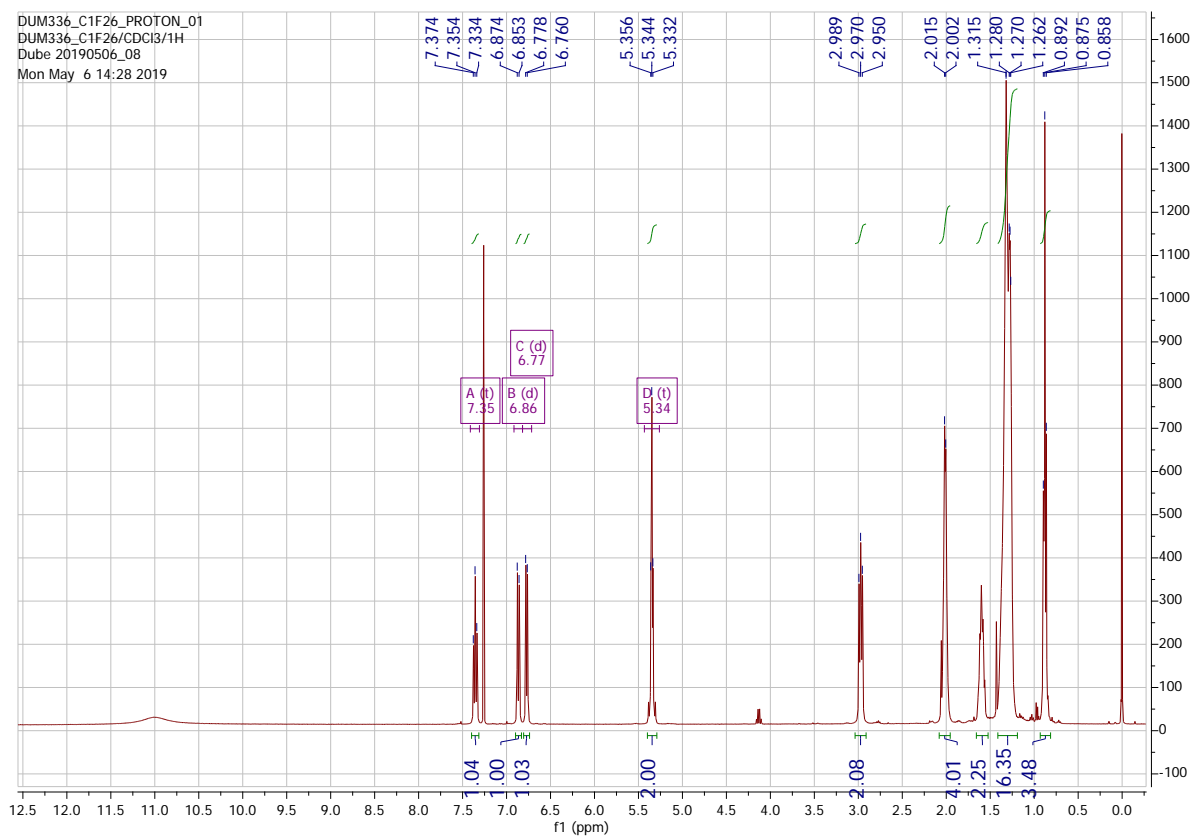


Figure S3: ^1H NMR spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl_3 (400 MHz).

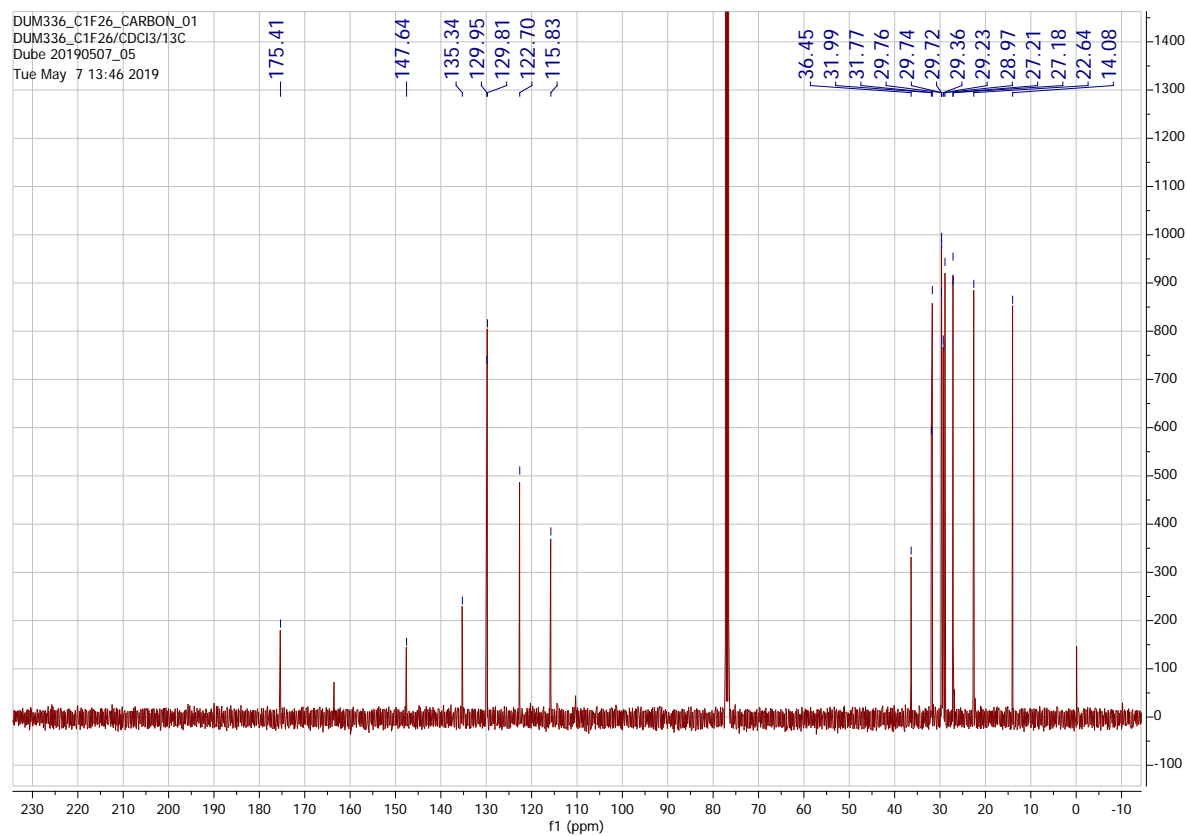


Figure S4: ^{13}C NMR spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl_3 (400 MHz).

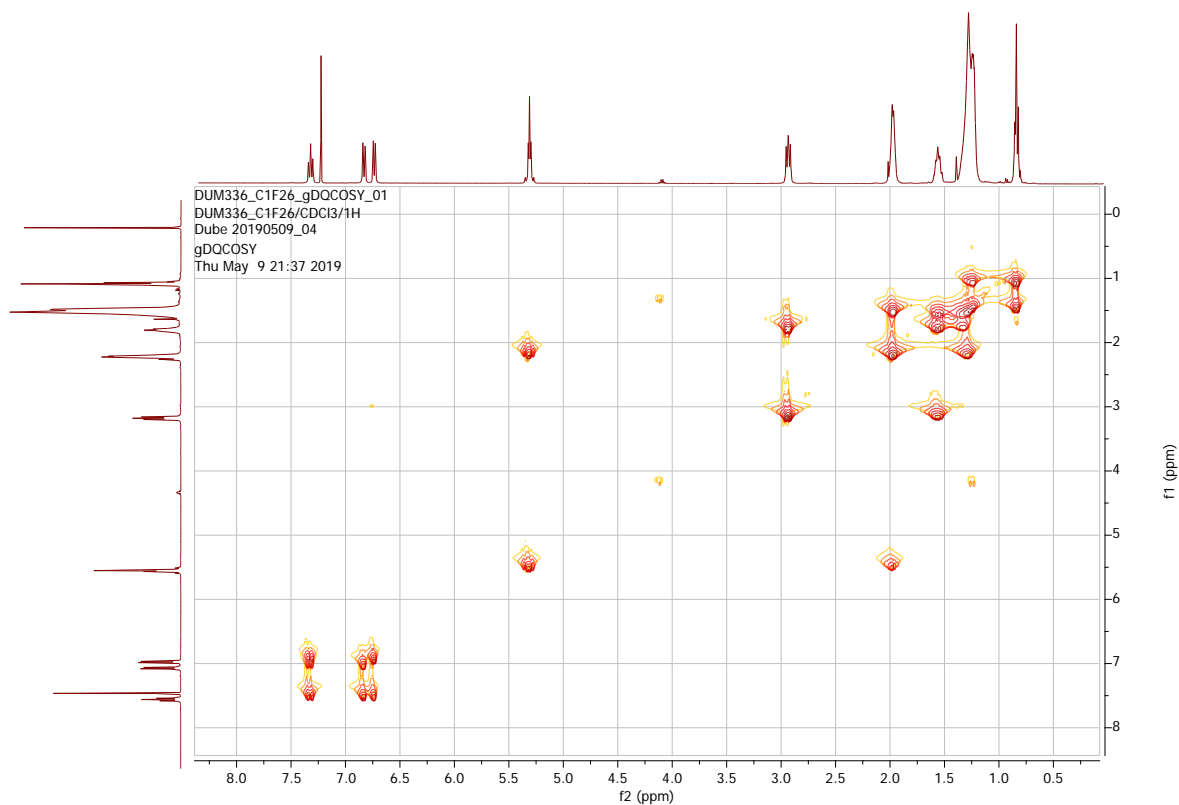


Figure S5: gDQCOSY spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl₃ (400 MHz).

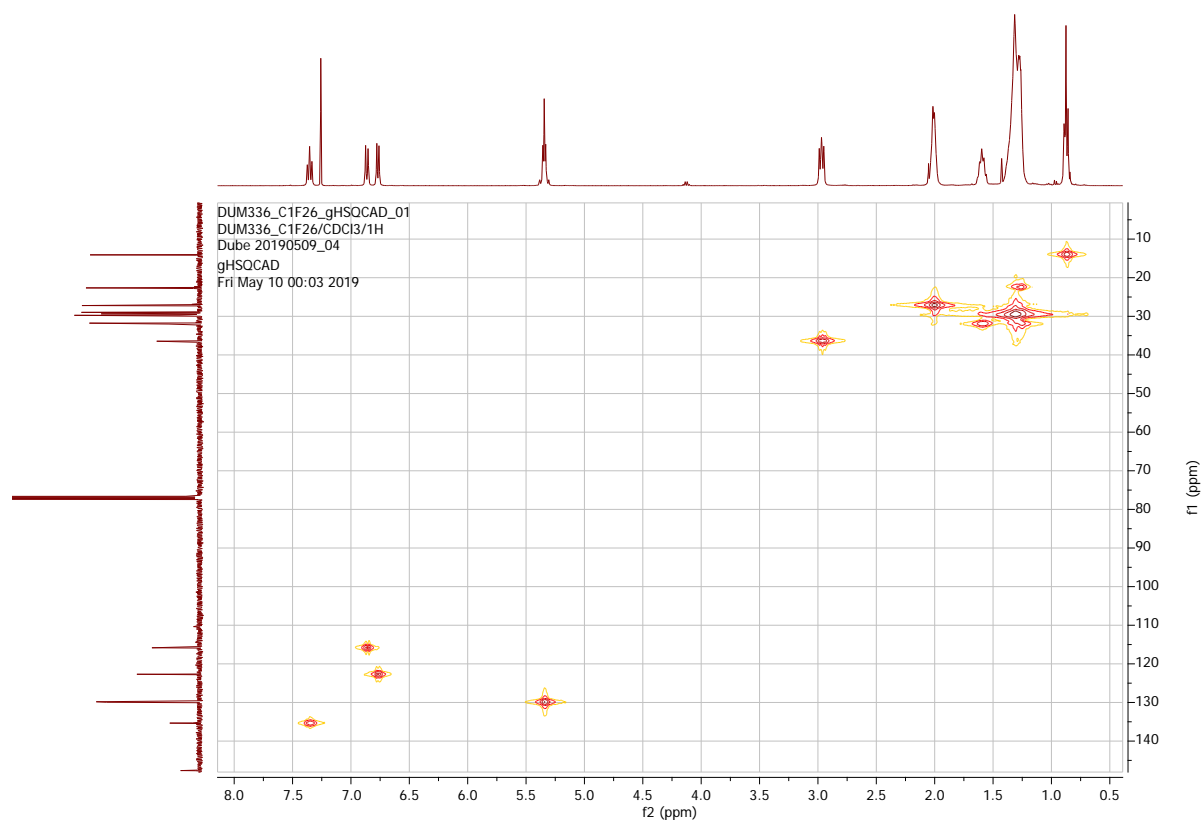


Figure S6: gHSQC spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl₃ (400 MHz).

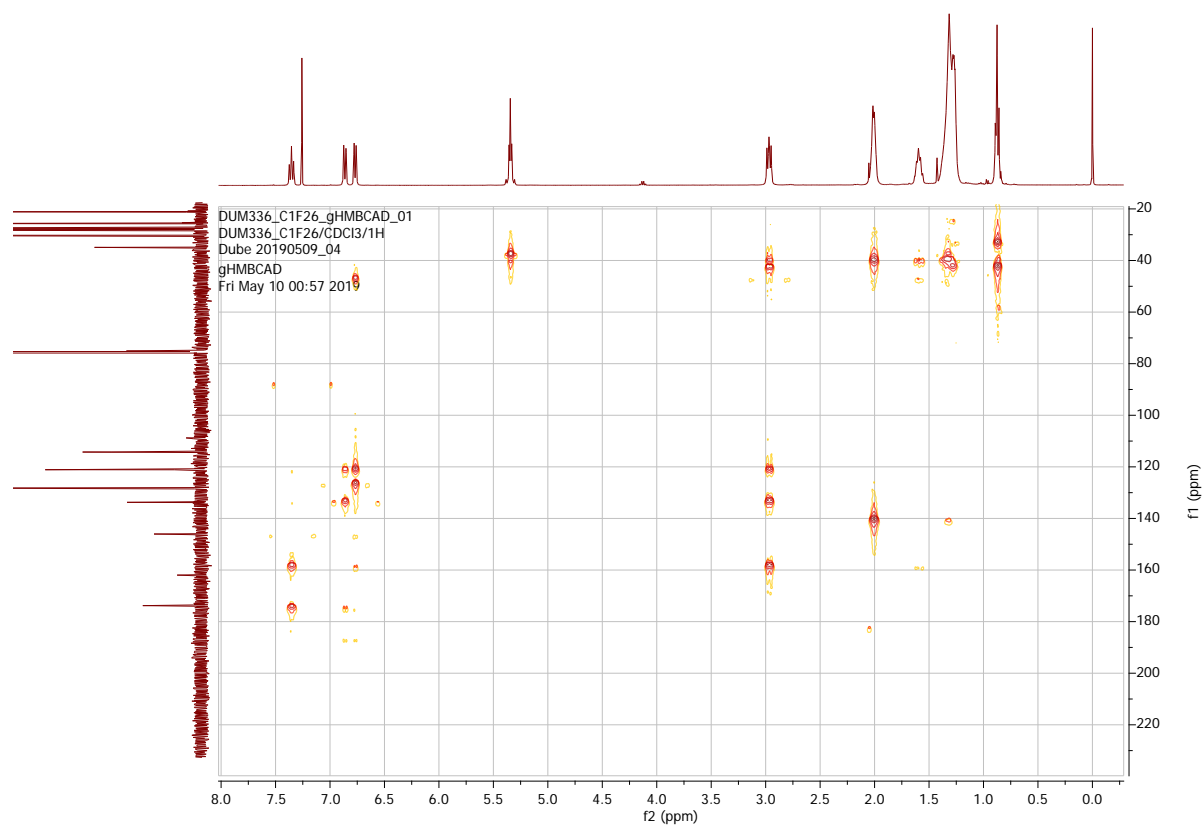


Figure S7: gHMBC spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl₃ (400 MHz).

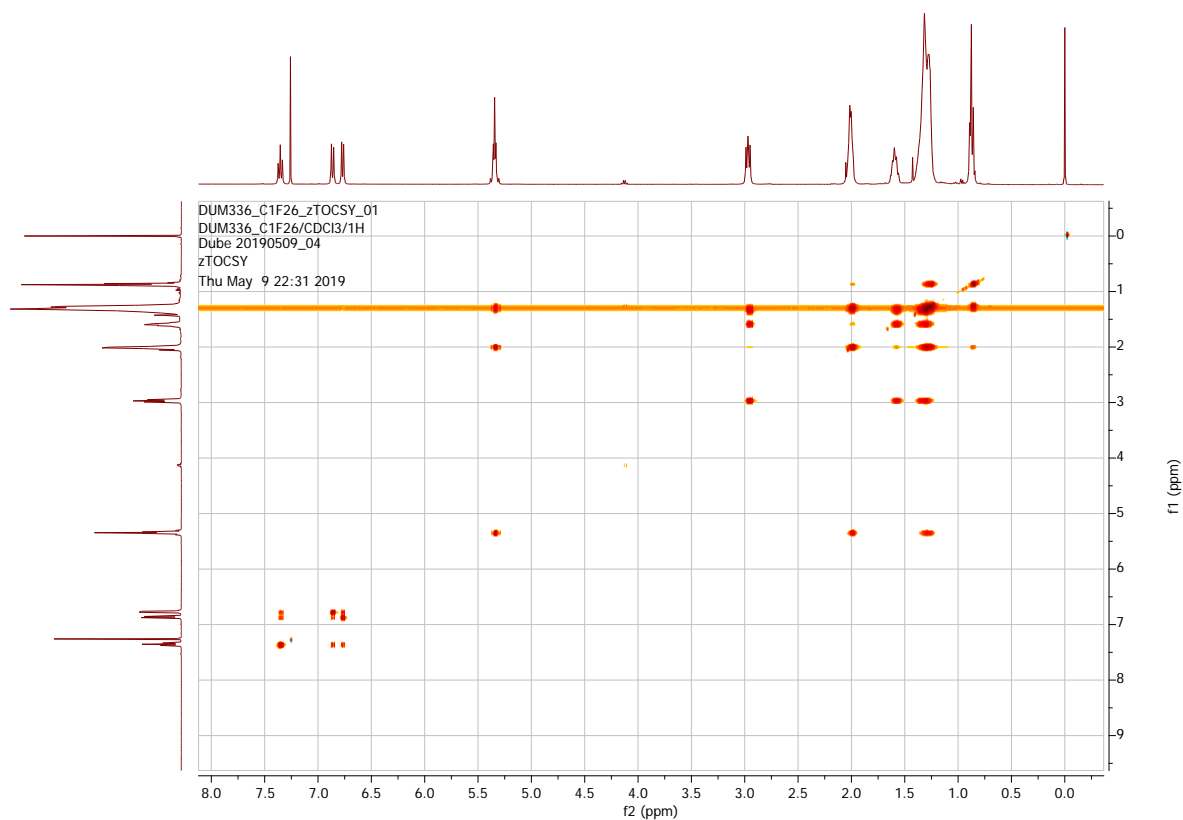


Figure S8: gTOCSY spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl₃ (400 MHz).

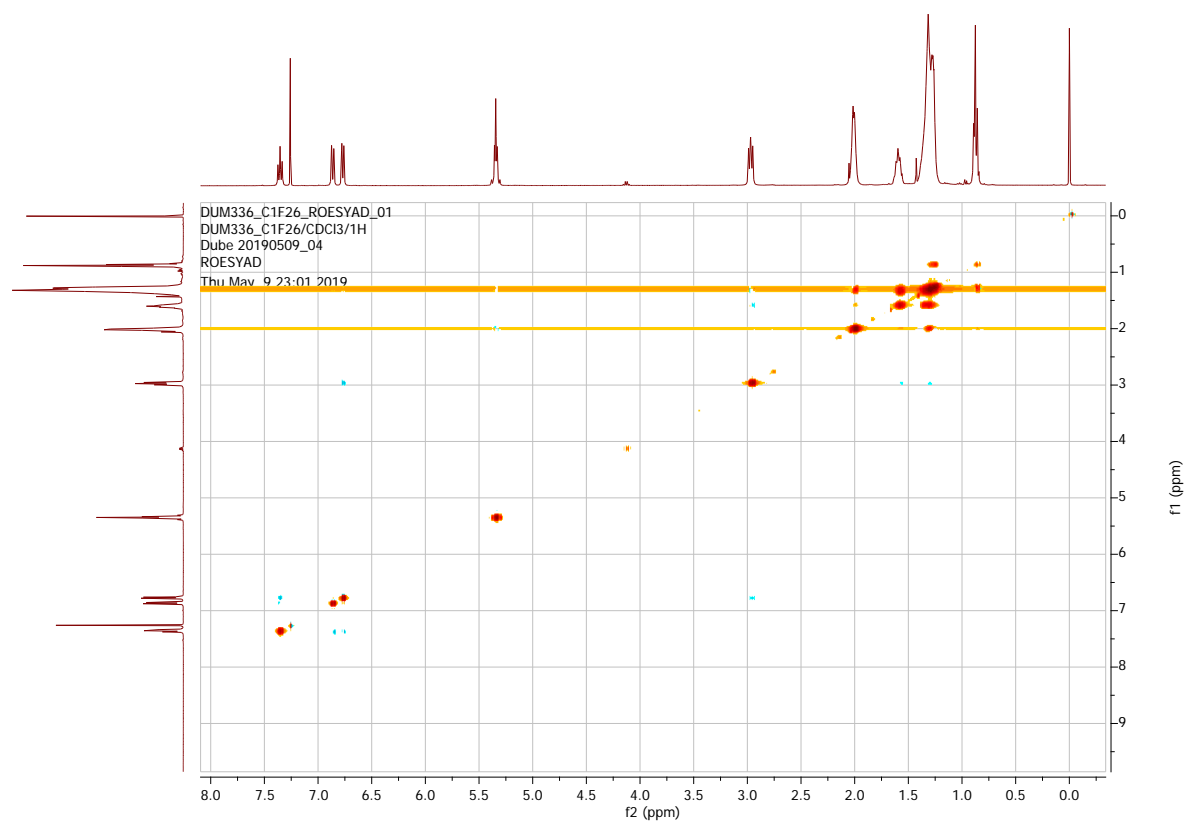


Figure S9: gROESY spectrum of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) in CDCl₃ (400 MHz).

Figures S10-S18: HR-ESI-MS, 1D and 2D NMR of 6-[10(Z)-heptadecenyl] anacardic acid (2**)**

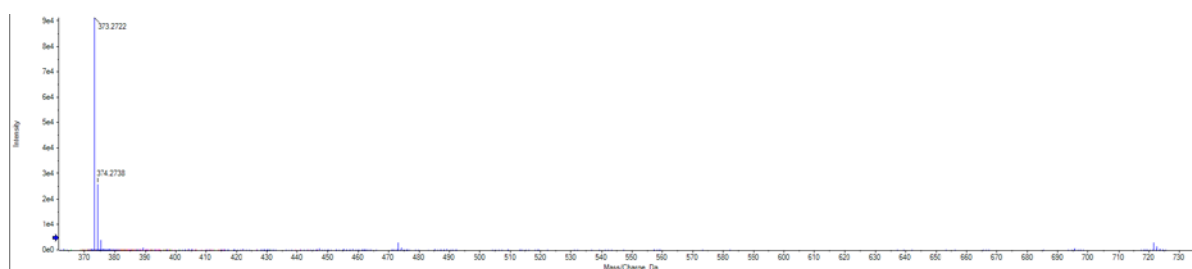


Figure S10: HR-ESI-MS spectrum (neg. mode) of 6-[10(Z)-heptadecenyl] anacardic acid (**2**).

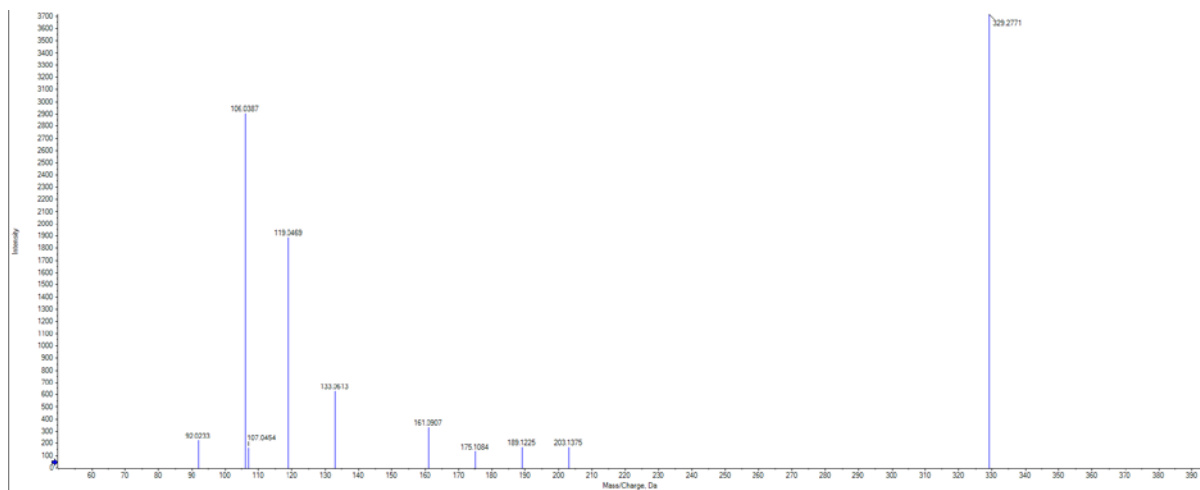


Figure S11: TOF MS² spectrum (neg. mode) of 6-[10(Z)-heptadecenyl] anacardic acid (2).

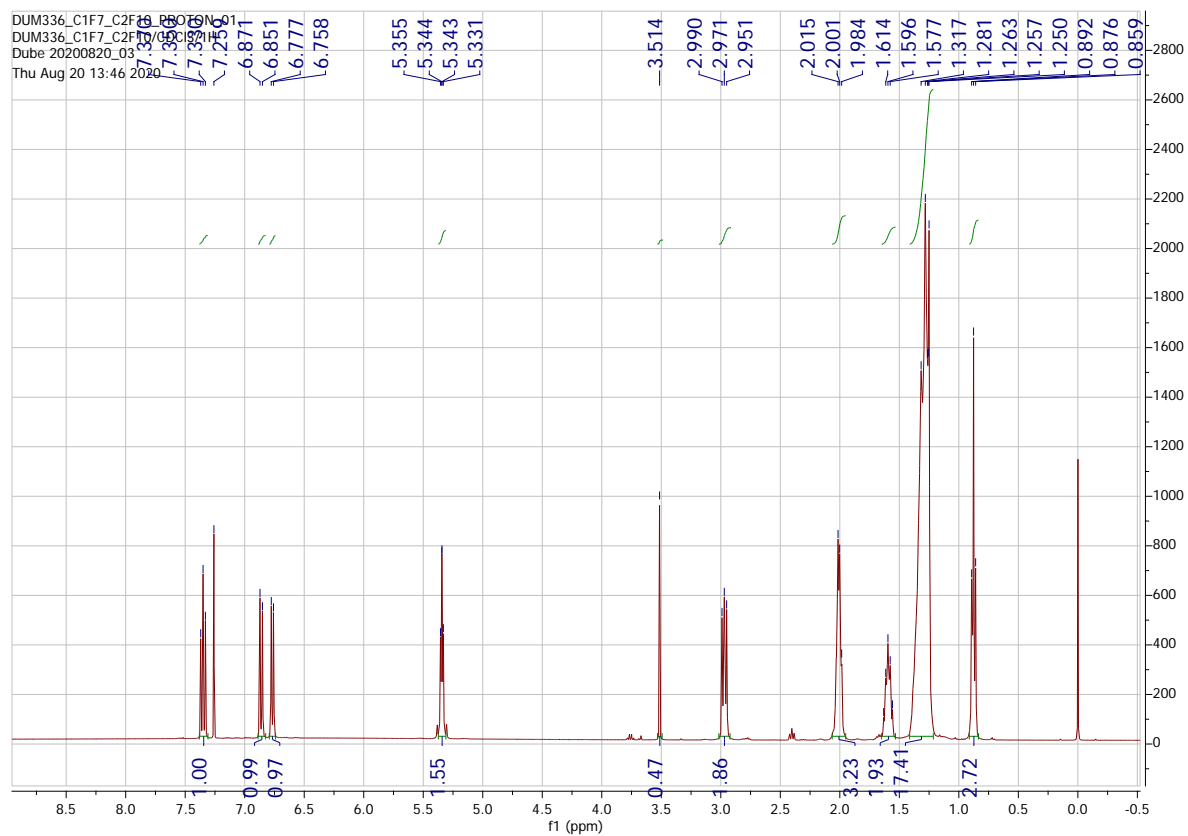


Figure S12: ¹H NMR spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (2) in CDCl₃ (400 MHz).

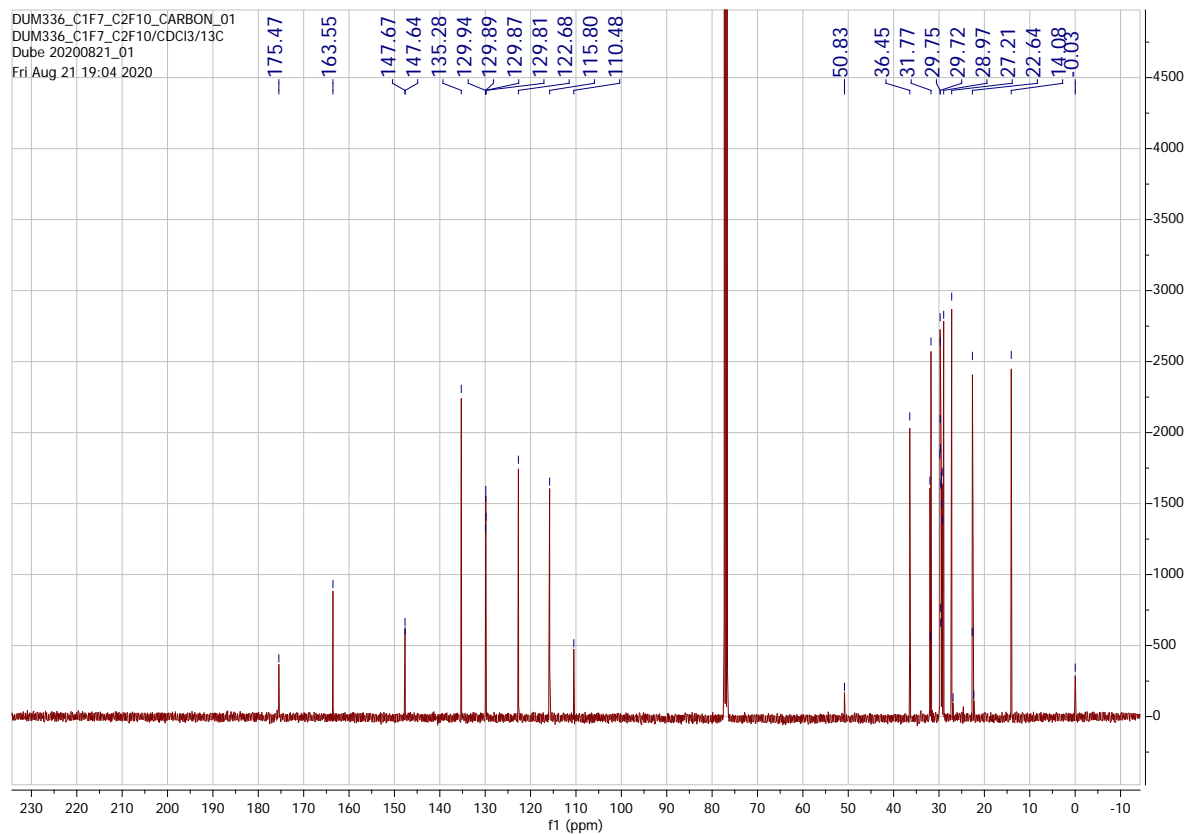


Figure S13: ^{13}C NMR spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (**2**) in CDCl_3 (400 MHz).

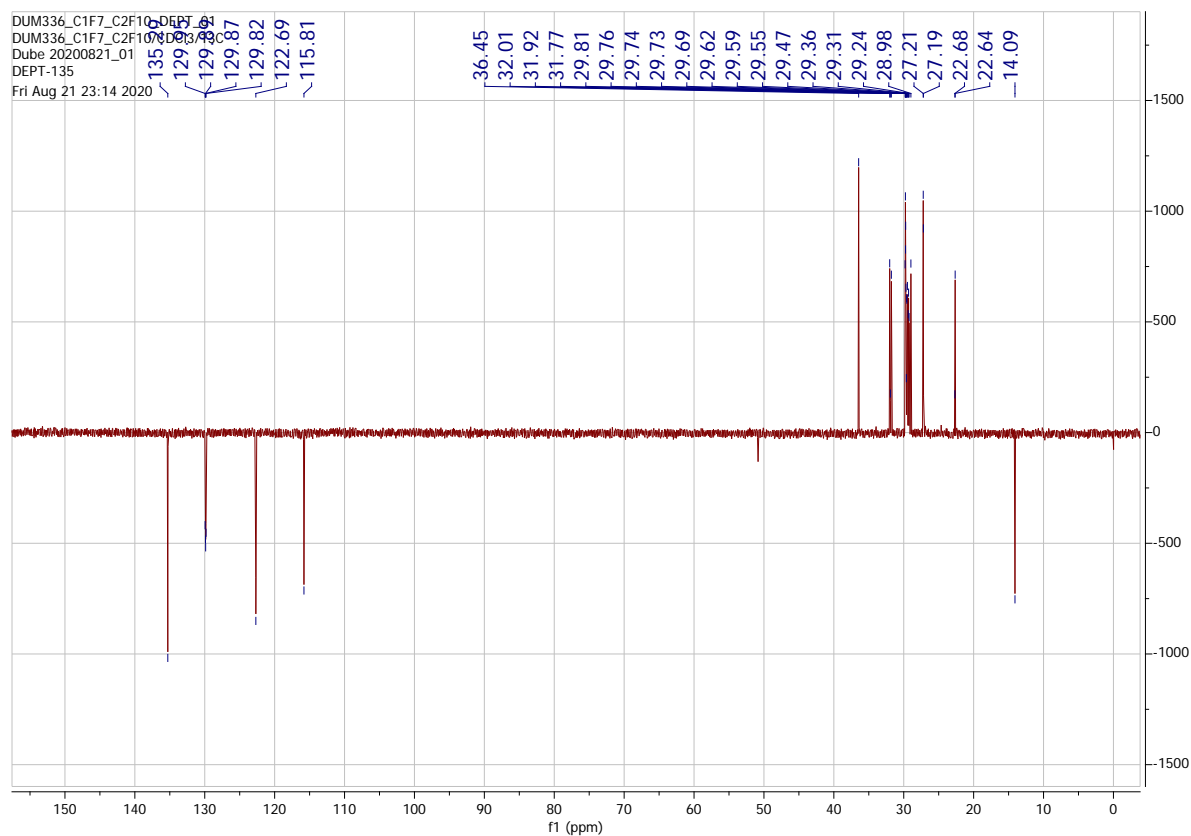


Figure S14: DEPT 135 spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (**2**) in CDCl_3 (400 MHz).

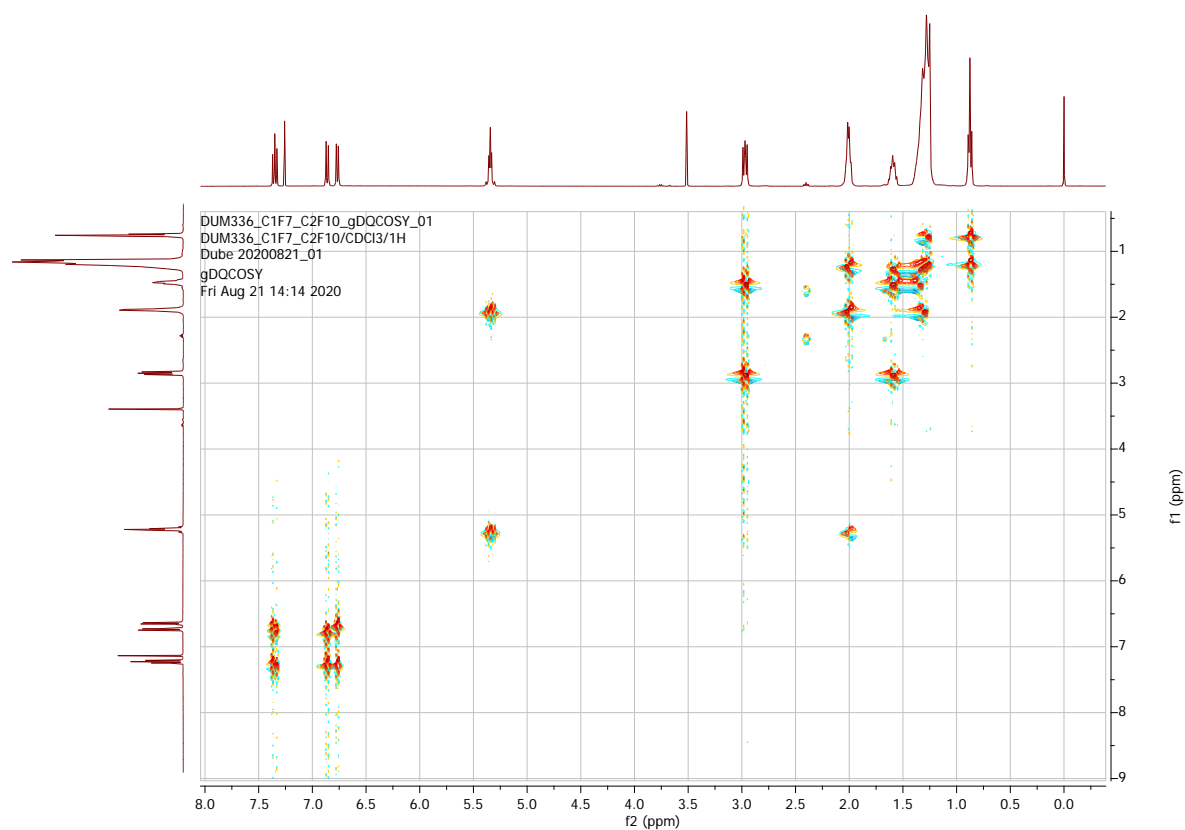


Figure S15: gDQCOSY spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (**2**) in CDCl₃ (400 MHz).

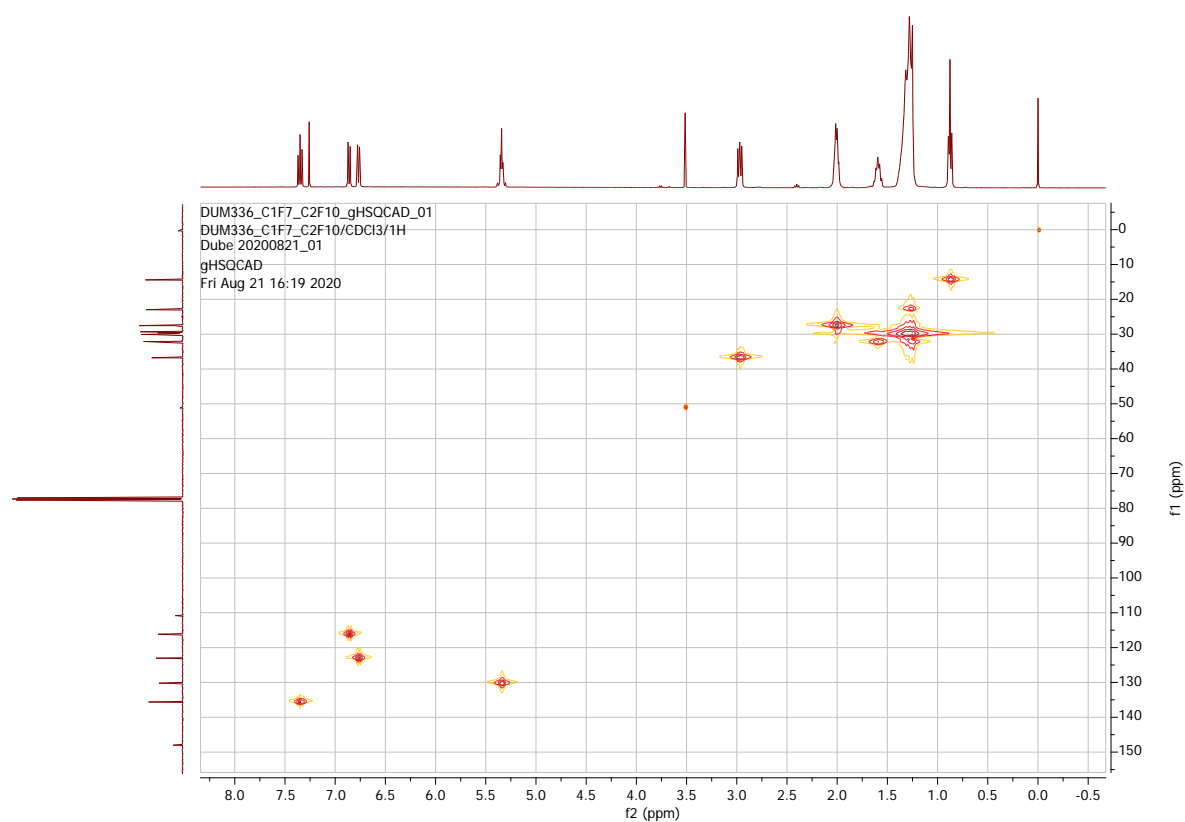


Figure S16: gHSQC spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (**2**) in CDCl₃ (400 MHz).

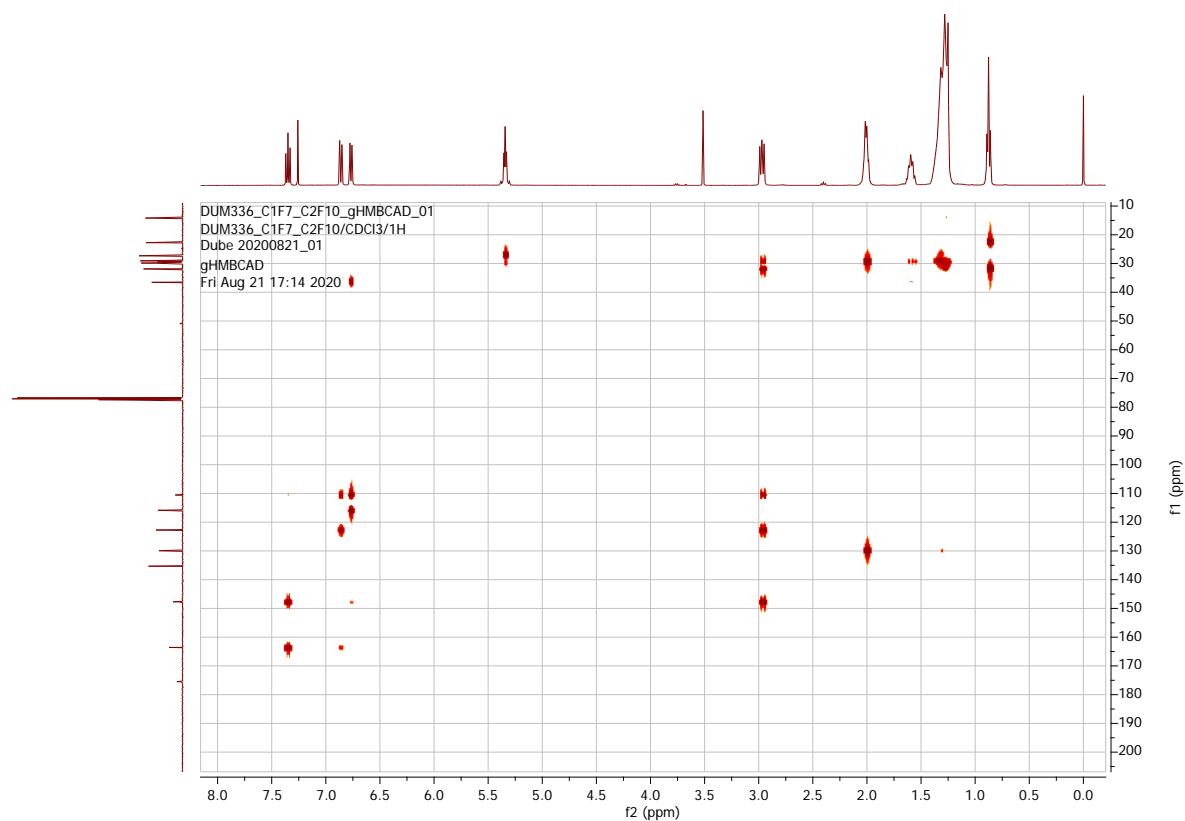


Figure S17: gHMBC spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (2) in CDCl₃ (400 MHz).

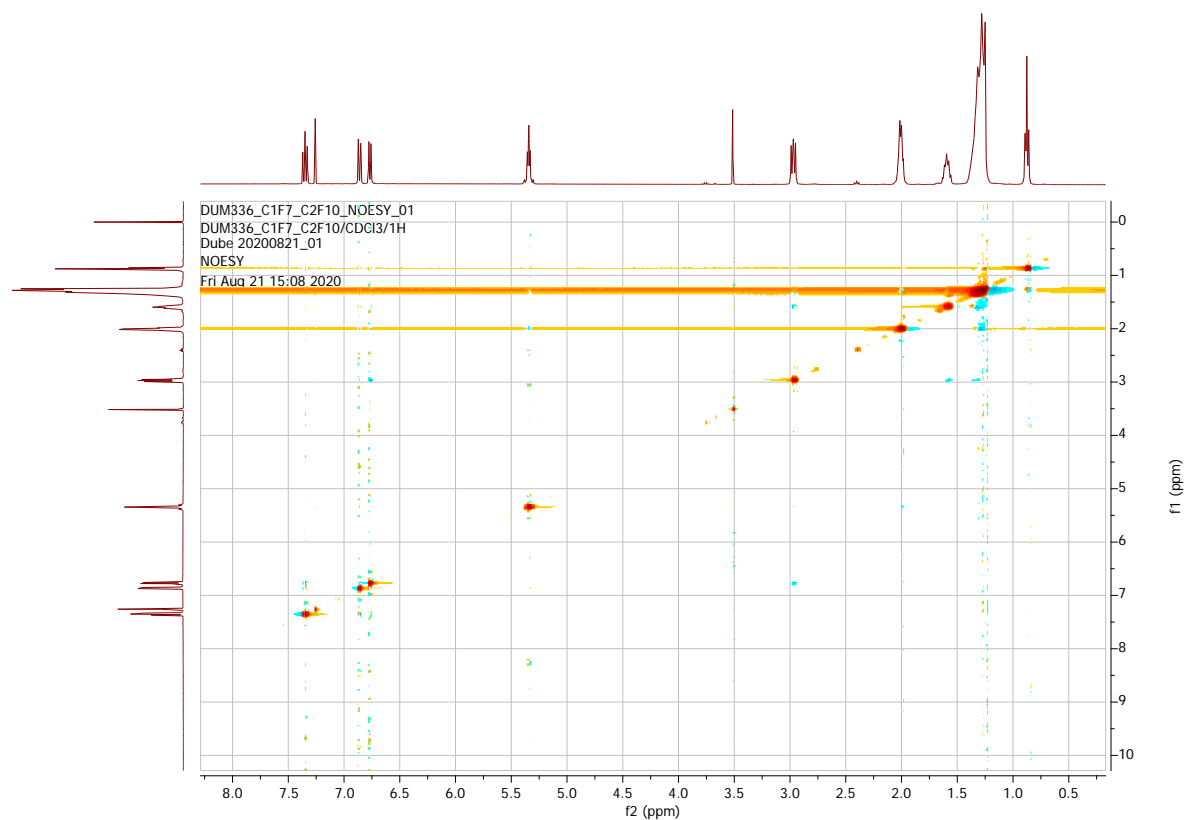


Figure S18: NOESY spectrum of 6-[10(Z)-heptadecenyl] anacardic acid (2) in CDCl₃ (400 MHz).

Figures S19 - S27: HR-MS, 1D and 2D NMR of 3-[7(Z)-pentadecenyl] phenol (3)

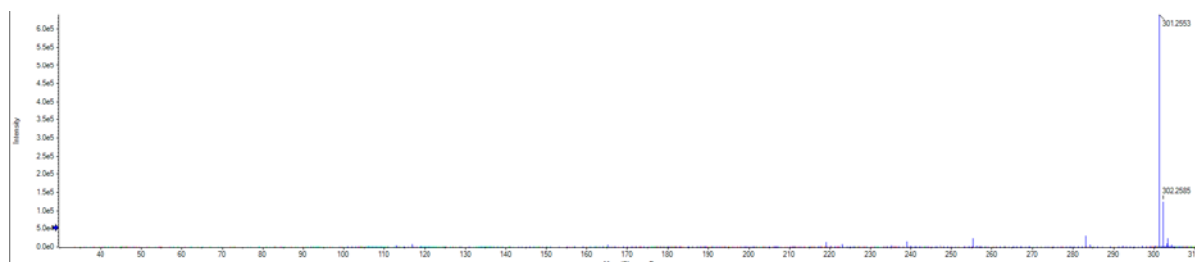


Figure S19: HR-ESI-MS spectrum (neg. mode) of 3-[7(Z)-pentadecenyl] phenol (3).

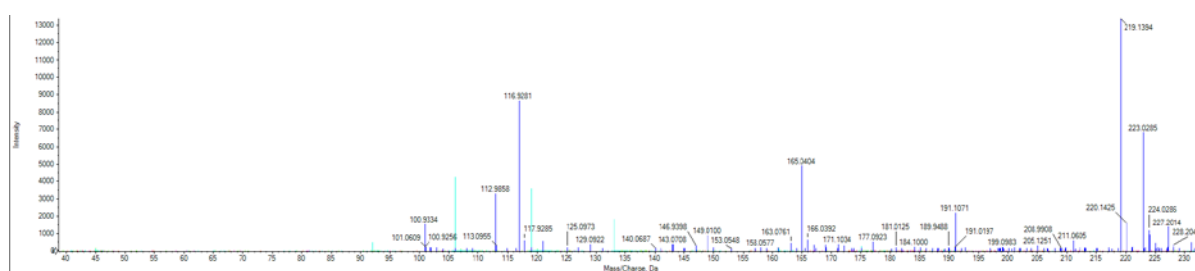


Figure S20: TOF MS² spectrum (neg. mode) of 3-[7(Z)-pentadecenyl] phenol (3).

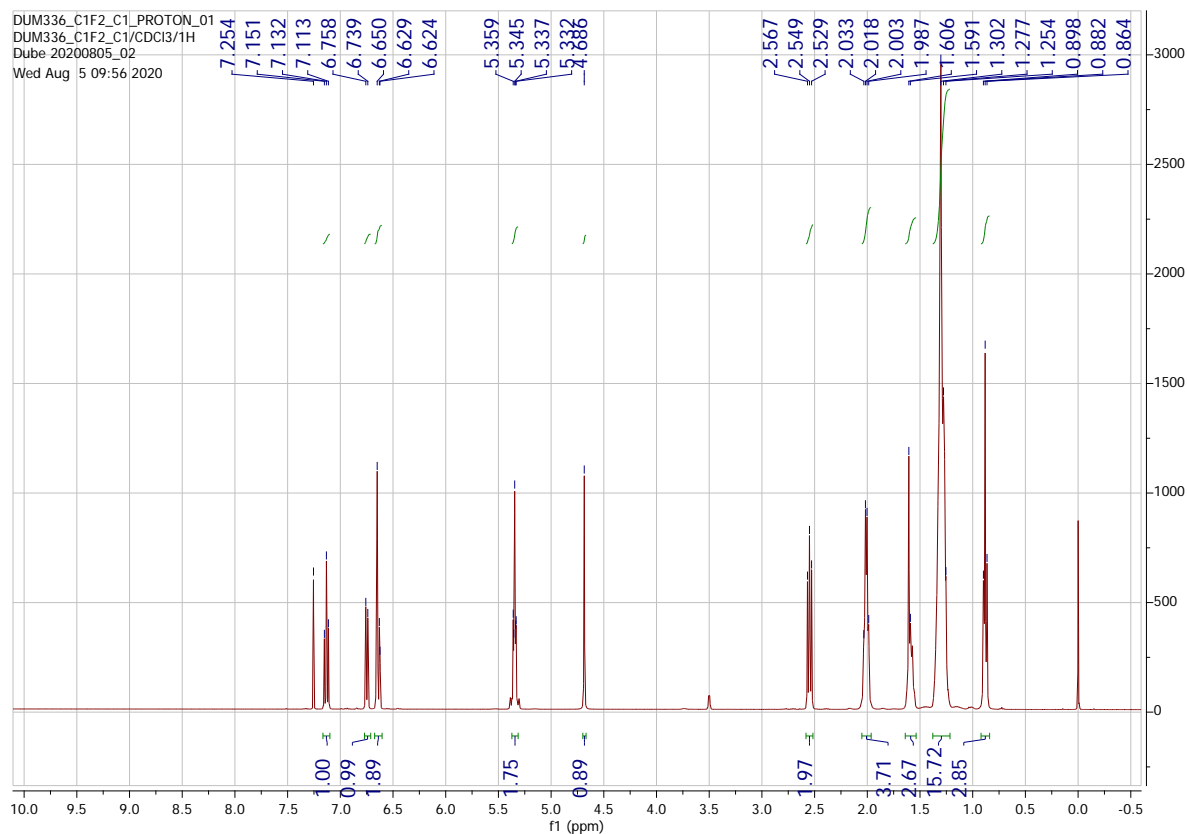


Figure S21: ¹H NMR spectrum of 3-[7(Z)-pentadecenyl] phenol (3) in CDCl₃ (400 MHz).

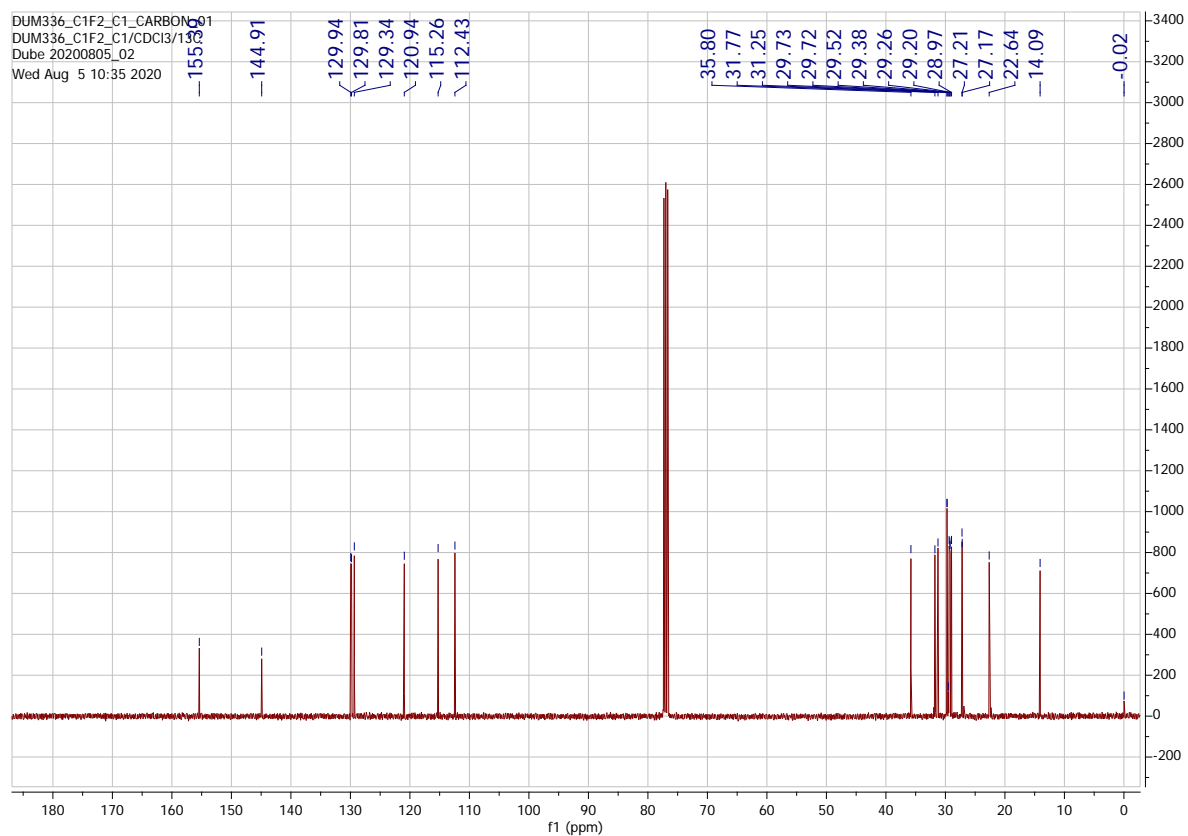


Figure S22: ^{13}C NMR spectrum of 3-[7(Z)-pentadecenyl] phenol (**3**) in CDCl_3 (400 MHz).

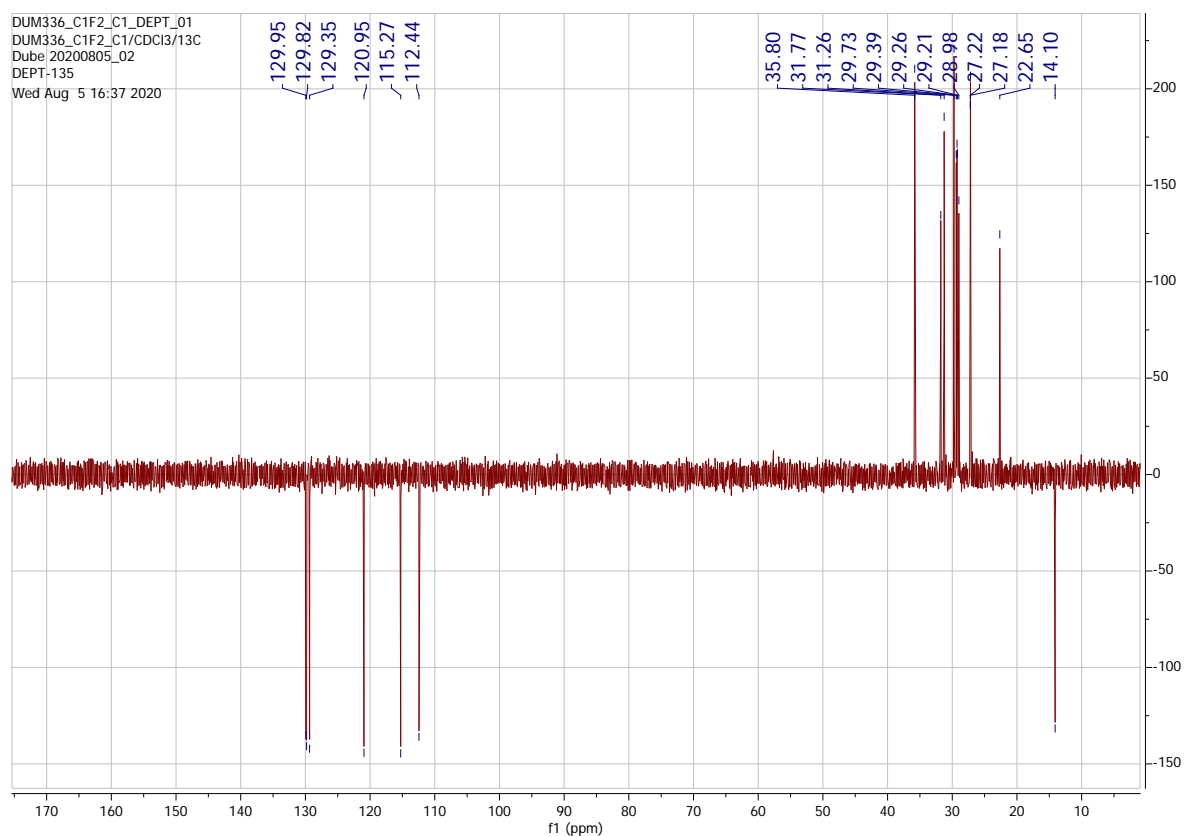


Figure S23: DEPT 135 spectrum of 3-[7(Z)-pentadecenyl] phenol (**3**) in CDCl_3 (400 MHz).

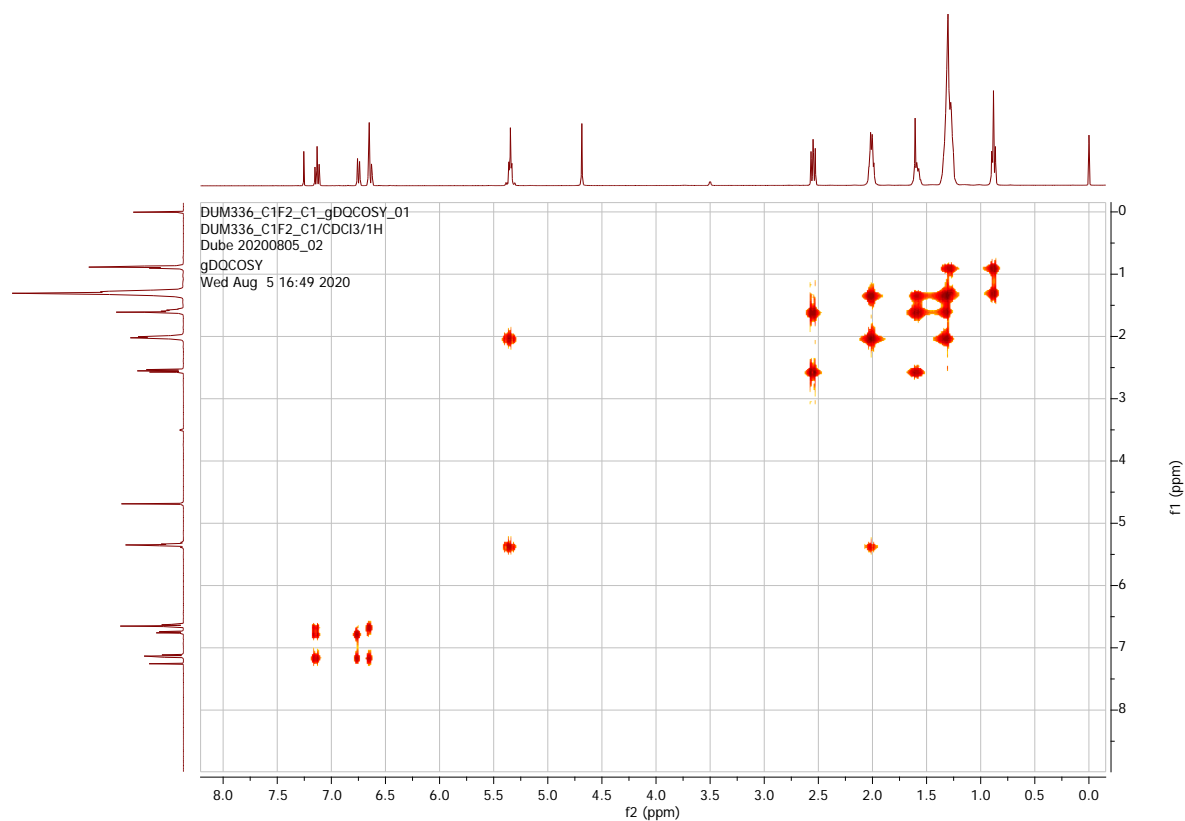


Figure S24: gDQCOSY spectrum of 3-[7(Z)-pentadecenyl] phenol (**3**) in CDCl₃ (400 MHz).

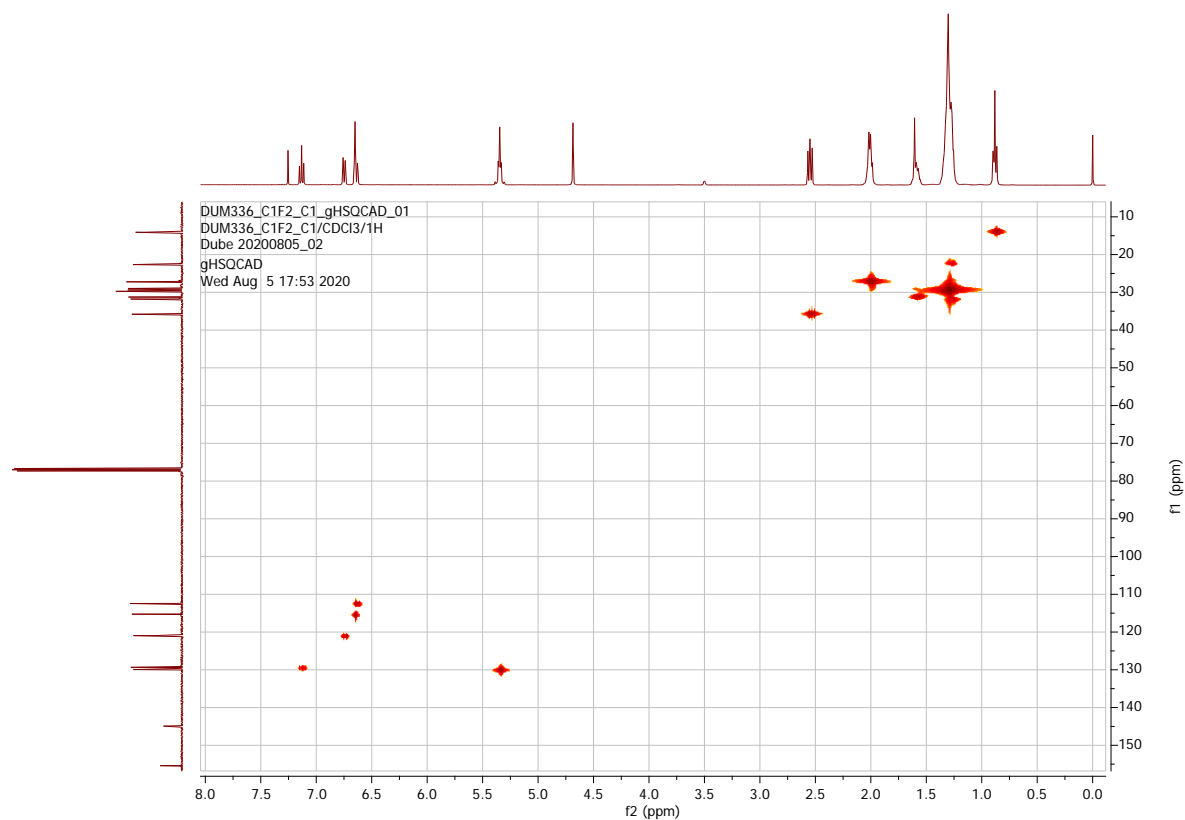


Figure S25: gHSQC spectrum of 3-[7(Z)-pentadecenyl] phenol (**3**) in CDCl₃ (400 MHz).

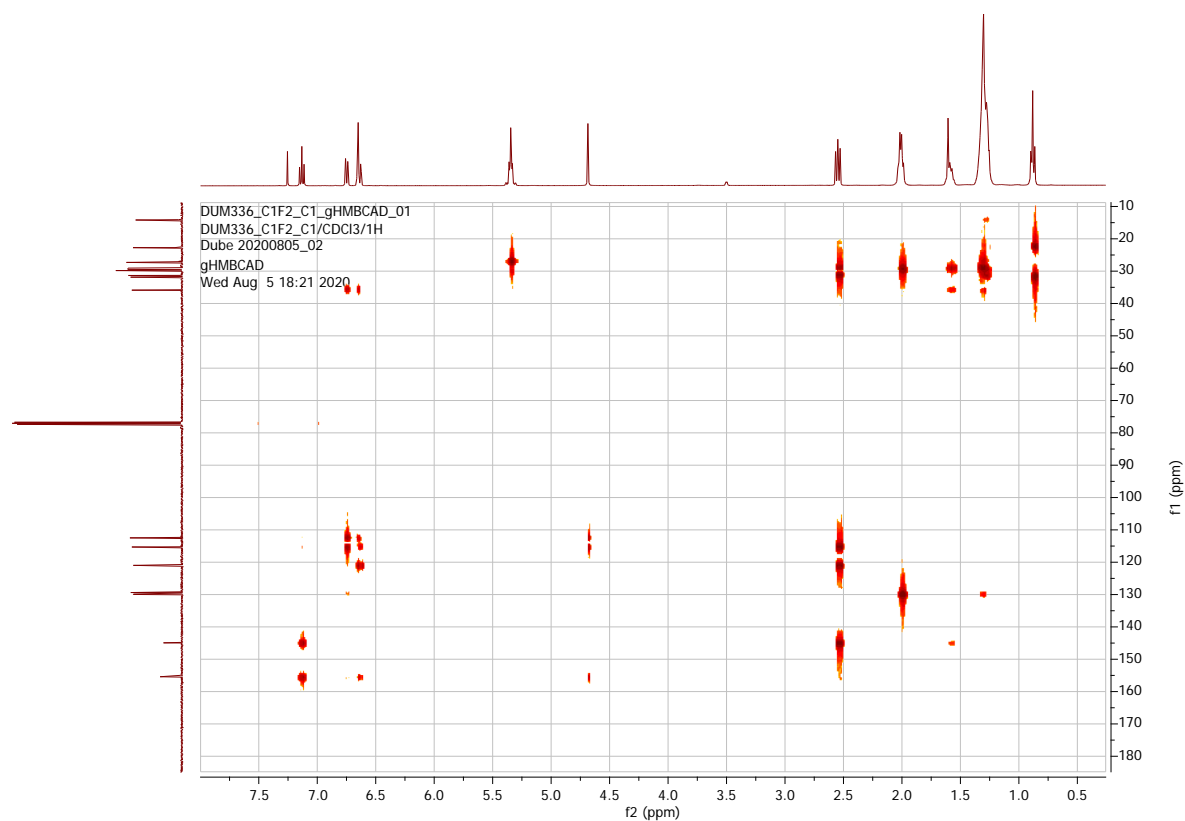


Figure S26: gHMBC spectrum of 3-[7(Z)-pentadecenyl] phenol (**3**) in CDCl₃ (400 MHz).

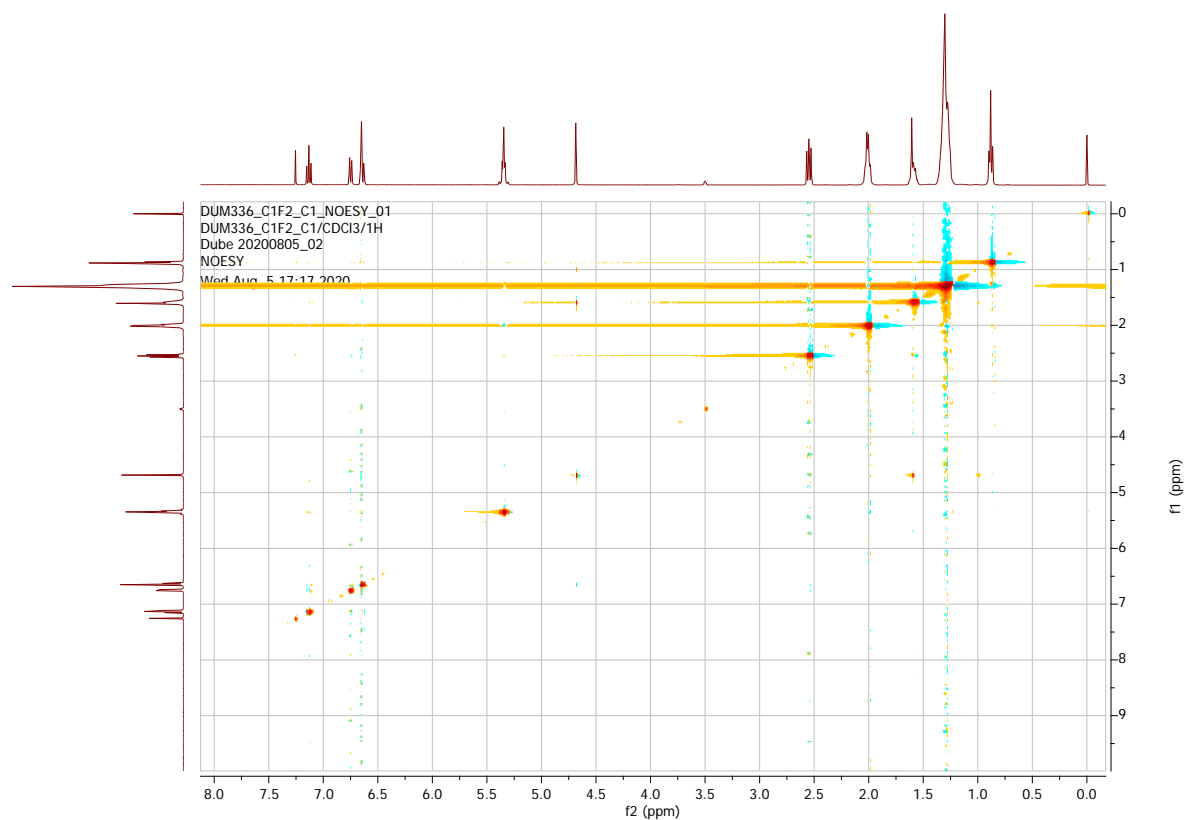


Figure S27: NOESY spectrum of 3-[7(Z)-pentadecenyl] phenol (**3**) in CDCl₃ (400 MHz).

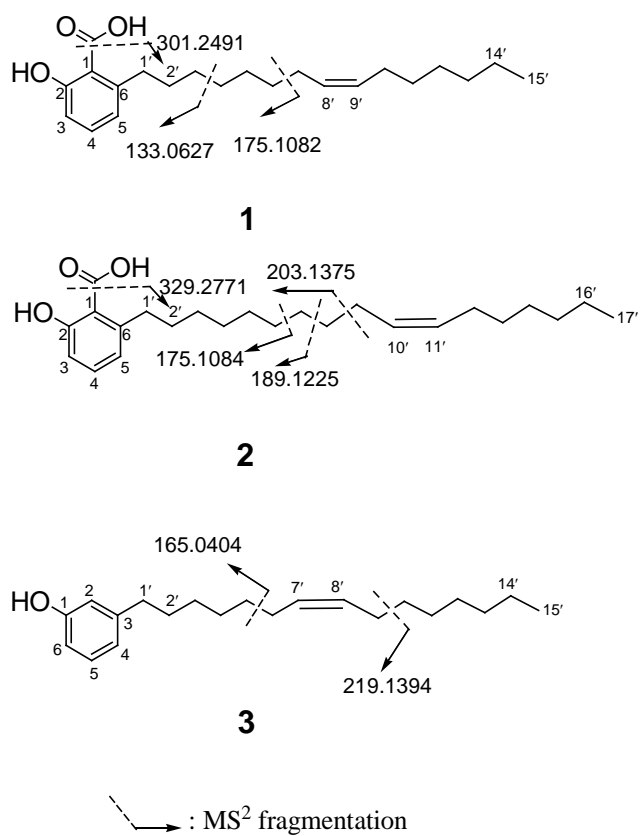


Figure S28: Important fragment ions on the TOF MS² spectra of compounds 1-3.

Table S1: Anthelmintic activity using *C. elegans*: *Ozoroa insignis* plant parts (80 % methanol extract; test concentration 500 µg/mL; positive control ivermectin 10µg/mL).

Phase	Activity % *
Fruit extract	91.73 ± 6.05
Root extract	35.11 ± 2.91
Leaves extract	17.58 ± 3.28
Stem extract	16.42 ± 7.86
Ivermectin	98.77 ± 1.74
2% DMSO	1.91 ± 1.38

*Activity % based on three replicates.

Table S2: Anthelmintic activity using *C. elegans*: fractions from partition of *Ozoroa insignis* fruit extract (80 % methanol); test concentration 500 µg/mL; positive control ivermectin 10µg/mL).

Phase	Activity %*
<i>n</i> -Hexane	92.54 ± 0.81
Ethyl acetate	4.54 ± 4.01
<i>n</i> -Butanol	4.28 ± 1.07
Aqu. residue	4.90 ± 1.70
Ivermectin	100 ± 0
2% DMSO	3.45 ± 1.48

*mortality % based on three replicates.

Table S3: ¹H NMR data (400 MHz, CDCl₃, δ in ppm) of 6-[8(Z)-pentadecenyl] anacardic acid (**1**), 6-[10(Z)-heptadecenyl] anacardic acid (**2**) and 3-[7(Z)-pentadecenyl] phenol (**3**) including HMBC.

Position 1			Position 2			Position 3		
	δ _H (m, J in Hz)	Key HMBC		δ _H (m, J in Hz)	Key HMBC		δ _H (m, J in Hz)	Key HMBC
2	–	–	2	–	–	2	6.65 (1H, brs)	1, 1', 3
3	6.87 (1H, d, 8.4)	1, 5	3	6.86 (1H, d, 8.3)	1, 5	3	–	–
4	7.36 (1H, t, 8.0)	2, 6	4	7.35 (1H, t, 8.0)	2, 6	4	6.75 (1H, d, 7.6)	2, 6
5	6.77 (1H, d, 7.6)	1, 1', 3	5	6.77 (1H, d, 7.4)	1, 1', 3	5	7.13 (1H, t, 7.7)	1, 3
6	–	–	6	–	–	6	6.64 (1H, d, 8.3)	2, 4
1'	2.97 (2H, t, 7.6)	1, 5, 6	1'	2.97 (2H, t, 7.6)	1, 5, 6	1'	2.55 (2H, t, 7.2)	2, 3, 4, 2', 3'
2'	1.61 (1H, m)	–	2'	1.60 (1H, m)	–	2'	1.61 (1H, m)	1', 2', 3'
3'–6'	1.29 (8H, brs)	–	3'–8'	1.29 (8H, brs)	–	3'–5'	1.30 (6H, brs)	–
7'	2.04 (2H, m)	8'	9'	2.00 (2H, m)	8'	6'	2.00 (2H, m)	8', 4'
8'	5.35 (1H, m)	10'	10'	5.34 (1H, m)	10'	7'	5.34 (1H, m)	5', 6'
9'	5.35 (1H, m)	7'	11'	5.34 (1H, m)	7'	8'	5.34 (1H, m)	9', 10'
10'	2.04 (2H, m)	9'	12'	2.00 (2H, m)	9'	9'	2.00 (2H, m)	7, 11'
11'–13'	1.29 (8H, brs)	–	13'–14'	1.27 (6H, brs)	–	10'–12'	1.30 (6H, brs)	–
14'	1.29 (2H, brs)	–	15'	1.27 (2H, brs)	–	13'	1.28 (2H, brs)	–
15'	0.88 (3H, t, 7.0)	13'	16'	1.27 (2H, brs)	13'	14'	1.27 (2H, m)	–
-COOH	11.04 (1H, s)	–	17'	0.88 (3H, t, 6.7)	–	15'	0.88 (3H, t, 6.8)	13', 14'
	–	–	-COOH	–	–			

Table S4: ¹³C NMR data (100 MHz, CDCl₃, δ in ppm) of 6-[8(Z)-pentadecenyl] anacardic acid (**1**), 6-[10(Z)-heptadecenyl] anacardic acid (**2**) and 3-[7(Z)-pentadecenyl] phenol (**3**).

Position	1	Position	2	Position	3
	δ _c (m)		δ _c (m)		δ _c (m)
1	107.5 (s)	1	110.5 (s)	1	155.4 (s)
2	163.6 (s)	2	163.6 (s)	2	115.3 (d)
3	115.8 (d)	3	115.8 (d)	3	144.9 (d)
4	135.3 (d)	4	135.3 (d)	4	120.9 (d)
5	122.7 (d)	5	122.7 (d)	5	129.3 (d)
6	147.6 (s)	6	147.6 (s)	6	112.4 (d)
1'	36.5 (t)	1'	36.5 (t)	1'	35.8 (t)
2'	32.0 (t)	2'	32.0 (t)	2'	31.2 (t)
3'–6'	29.0 – 29.8 (t)	3'–8'	29.0 – 29.8 (t)	3'–5'	29.0 – 29.8 (t)
7'	27.2 (t)	9'	27.2 (t)	6'	27.2 (t)
8'	129.9 (d)	10'	129.9 (d)	7'	129.9 (d)
9'	129.9 (d)	11'	129.8 (d)	8'	129.8 (d)
10'	27.2 (t)	12'	27.2 (t)	9'	27.2 (t)
11'–12'	29.0 – 29.8 (t)	13'–14'	29.0 – 29.8 (t)	10'–12'	29.0 – 29.8 (t)
13'	31.8 (t)	15'	31.8 (t)	13'	31.8 (t)
14'	22.6 (t)	16'	22.6 (t)	14'	22.5 (t)
15'	14.1 (q)	17'	14.1 (q)	15'	14.1 (q)
-COOH	175.4 (s)	-COOH	175.5 (s)		

Table S5: Anthelmintic activity using *C. elegans*: anthelmintic activity of 6-[8(Z)-pentadecenyl] anacardic acid (**1**), 6-[10(Z)-heptadecenyl] anacardic acid (**2**) and 3-[7(Z)-pentadecenyl] phenol (**3**).

conc. ($\mu\text{g/mL}$)	Activity %* (1)	μM (1)	Activity %* (2)	μM (2)	Activity %* (3)
500	100 \pm 0	1440.00	100 \pm 0	1334.80	0.79 \pm 1.12
250	100 \pm 0	721.40	97.73 \pm 1.72	667.40	n. t.
125	97.01 \pm 0.22	360.70	94.39 \pm 5.36	333.70	n. t.
62.5	78.19 \pm 9.99	180.30	73.98 \pm 3.12	166.80	n. t.
31.25	63.42 \pm 7.29	90.18	47.41 \pm 3.37	83.43	n. t.
15.625	46.40 \pm 10.02	45.09	24.78 \pm 2.28	41.71	n. t.
Ivermectin (10 $\mu\text{g/mL}$)	99.07 \pm 1.31		95.59 \pm 0.71		98.45 \pm 1.26
2% DMSO	0.95 \pm 1.35		0		1.36 \pm 0.97

*Activity % based on three replicates.

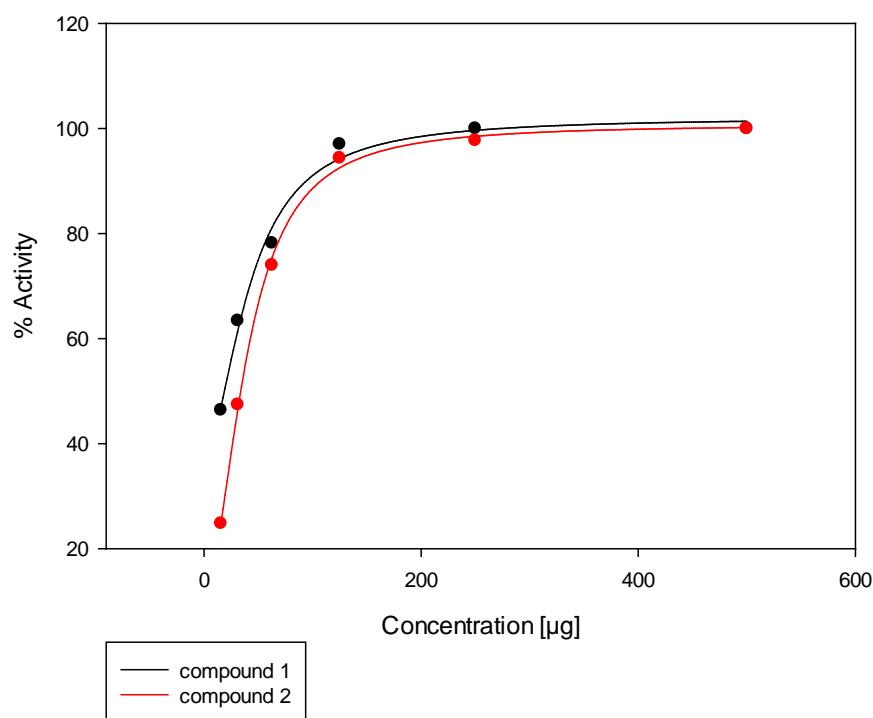


Figure S29: Graph for LC_{50} of 6-[8(Z)-pentadecenyl] anacardic acid (**1**) and 6-[10(Z)-heptadecenyl] anacardic acid (**2**) (testorganism: *C. elegans*).

Table S6: LC_{50} values of 6-[8(Z)-pentadecenyl] anacardic acid (**1**), 6-[10(Z)-heptadecenyl] anacardic acid (**2**) (testorganism: *C. elegans*).

Compound	LC_{50} (μM)
1	51,9 \pm 10,33
2	93,4 \pm 3,53