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ANTIOXIDANT CAPACITY ASSESSMENT BY ABTS OF 9'*Z*-BIXIN, 9'*Z*-NORBIXIN, 9'*Z*-METHYLBIXIN AND ALL *E*-METHYLBIXIN FROM *BIXA ORELLANA* SEEDS

SUPPLEMENTARY DATA

Full original article *Peer-reviewed*

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Keywords: *9′Z-Bixin, 9´Z-Norbixin, 9´Z-Methylbixin, All E-methylbixin, Food colorant, Antioxidant activity, structure–activity relationship*

Palabras clave: *9′Z-Bixina, 9´Z-Norbixina, 9´Z-Methylbixina, All E-methylbixina, Colorante alimentario, Actividad antioxidante, Relación estructura-actividad*

ABSTRACT

The 9´Z-bixin is the major pigment present in the annatto (*Bixa orellana L.*) seeds. Other carotenoids present in the seeds of annatto are 9´Z-norbixin and 9´Z-methylbixin. Annatto seeds extract is used as a food colorant. In this study, we extracted and isolated 9´Z-bixin from annatto seeds. 9’Z-norbixin was obtained and purified by saponification of annatto seeds extract. Then, 9’Z-methylbixin was obtained by Steglich esterification of 9´Z-bixin with EDC/DMAP. Also, all *E*-methylbixin was obtained by esterification of 9’*Z*-bixin in acid conditions with hydrochloric acid in methanol. The antioxidant capacity of 9´Z-bixin and bixin derivatives was measured in ABTS assay to determine their structure–activity relationship. We observed that the esterification of 9´Z-bixin decreases its antioxidant capacity and that the presence of the free carboxyl group is important in the antioxidant capacity of bixin derivatives.

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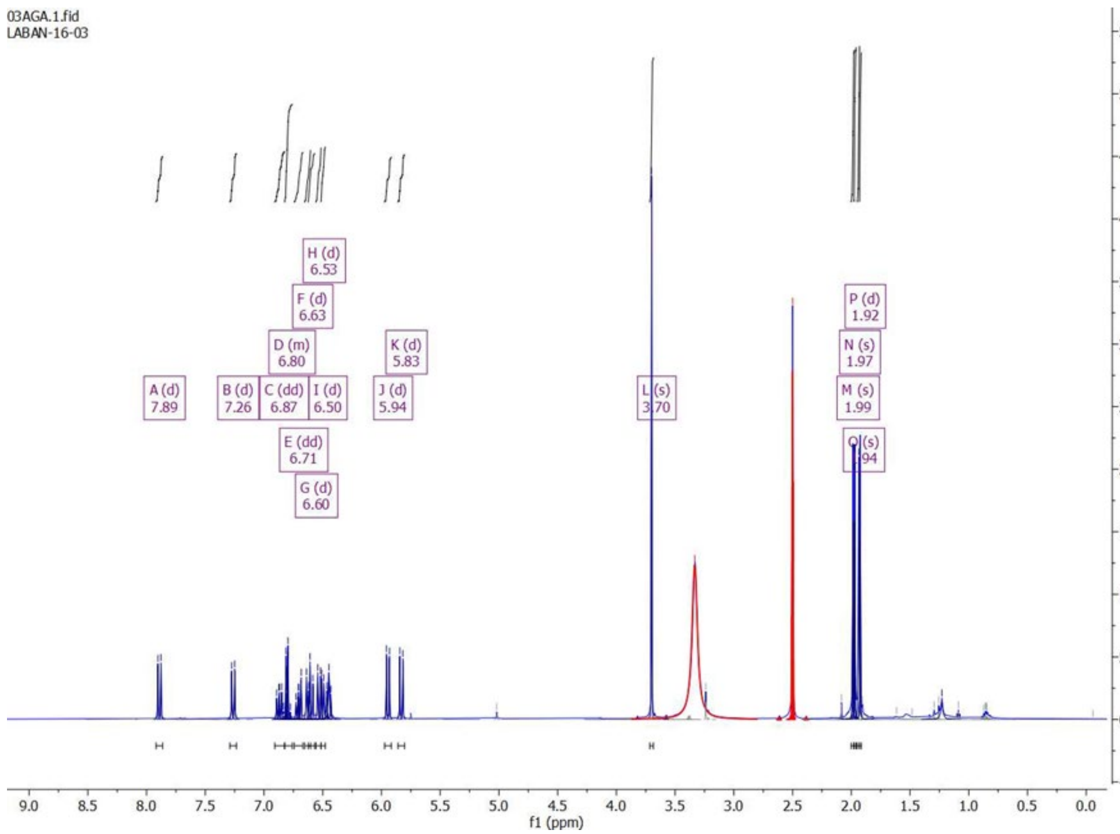
RESUMEN

La 9´Z-bixina es el principal pigmento presente en las semillas de achiote (*Bixa orellana L.*). Otros carotenoides presentes en las semillas de achiote son la 9´Z-norbixina y la 9´Z-metilbixina. El extracto de semillas de achiote se utiliza como colorante alimentario. En este estudio, se extrajo y se aisló 9´Z-bixina de semillas de achiote. Se obtuvo y se purificó 9'Z-norbixina a partir de la saponificación de extracto de semillas de achiote. Luego, se obtuvo 9'Z-metilbixina por esterificación de Steglich con EDC/DMAP. También se obtuvo E-metilbixina por esterificación en medio ácido. La capacidad antioxidante de 9´Z-bixina y de los derivados de bixina se midió con el ensayo ABTS para determinar su relación estructura-actividad. Observamos que la esterificación de la 9´Z-bixina disminuye su capacidad antioxidante y que la presencia del grupo carboxilo libre es importante en la capacidad antioxidante de los derivados de bixina.

SUPPLEMENTARY DATA

a)



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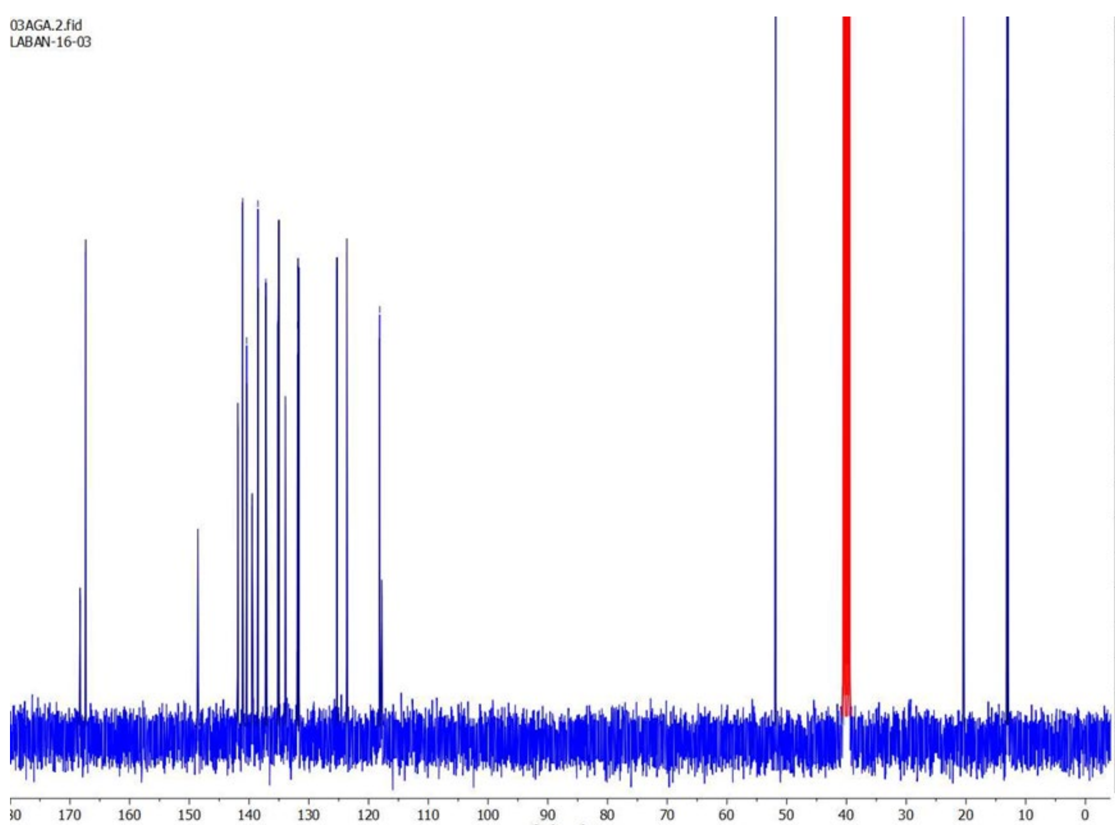

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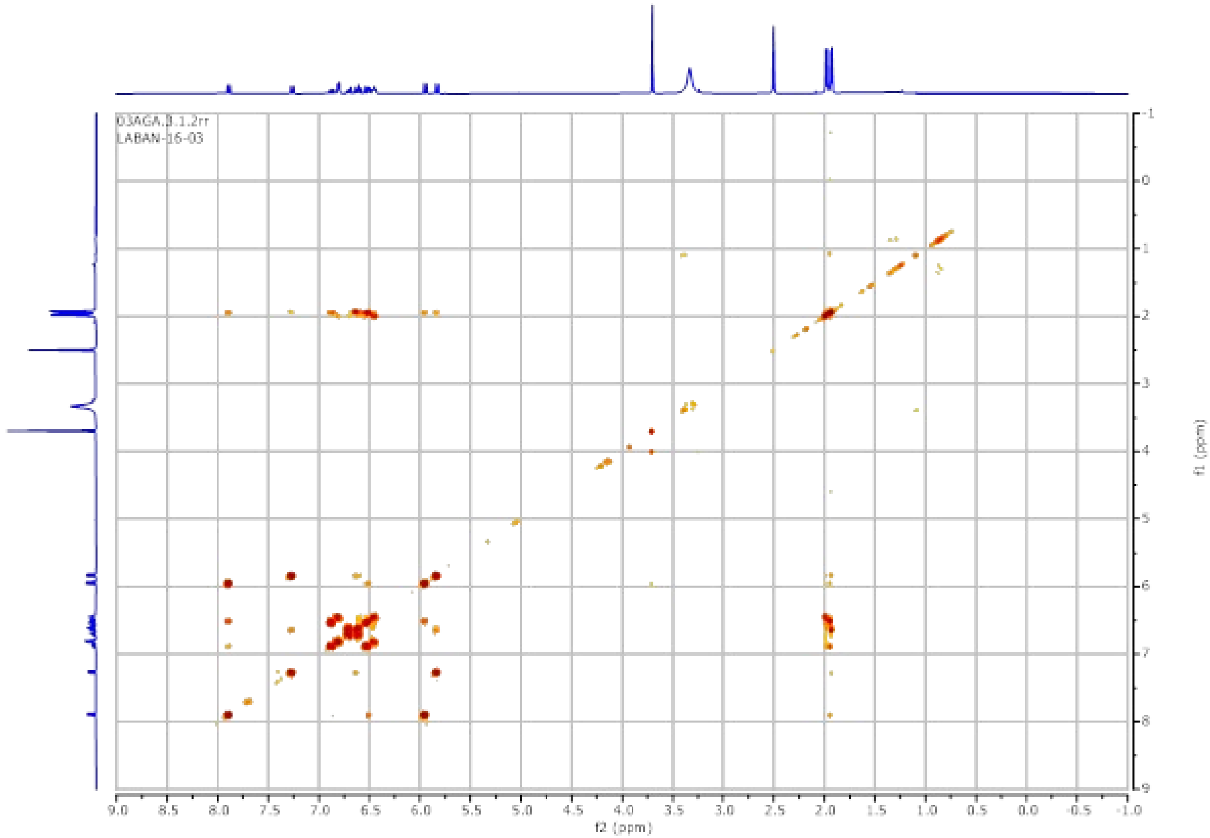

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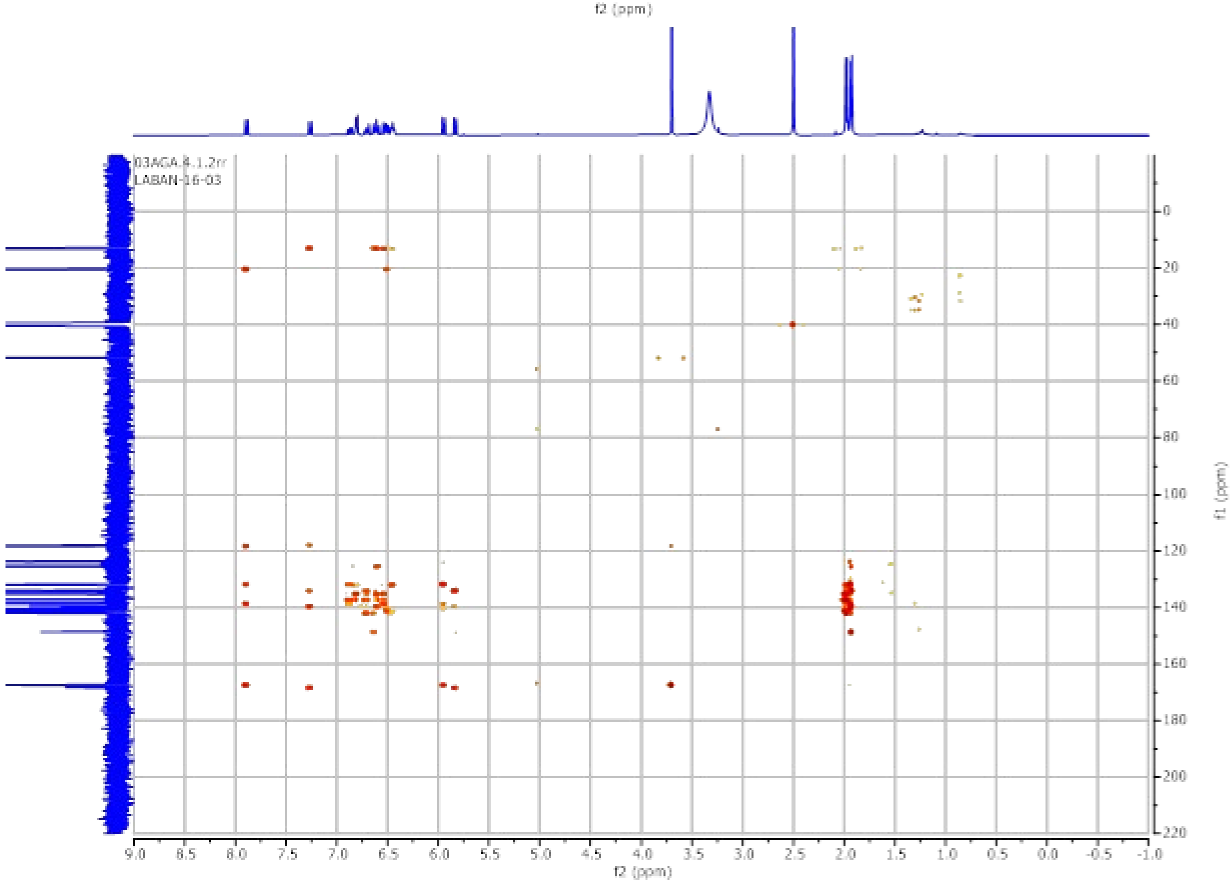


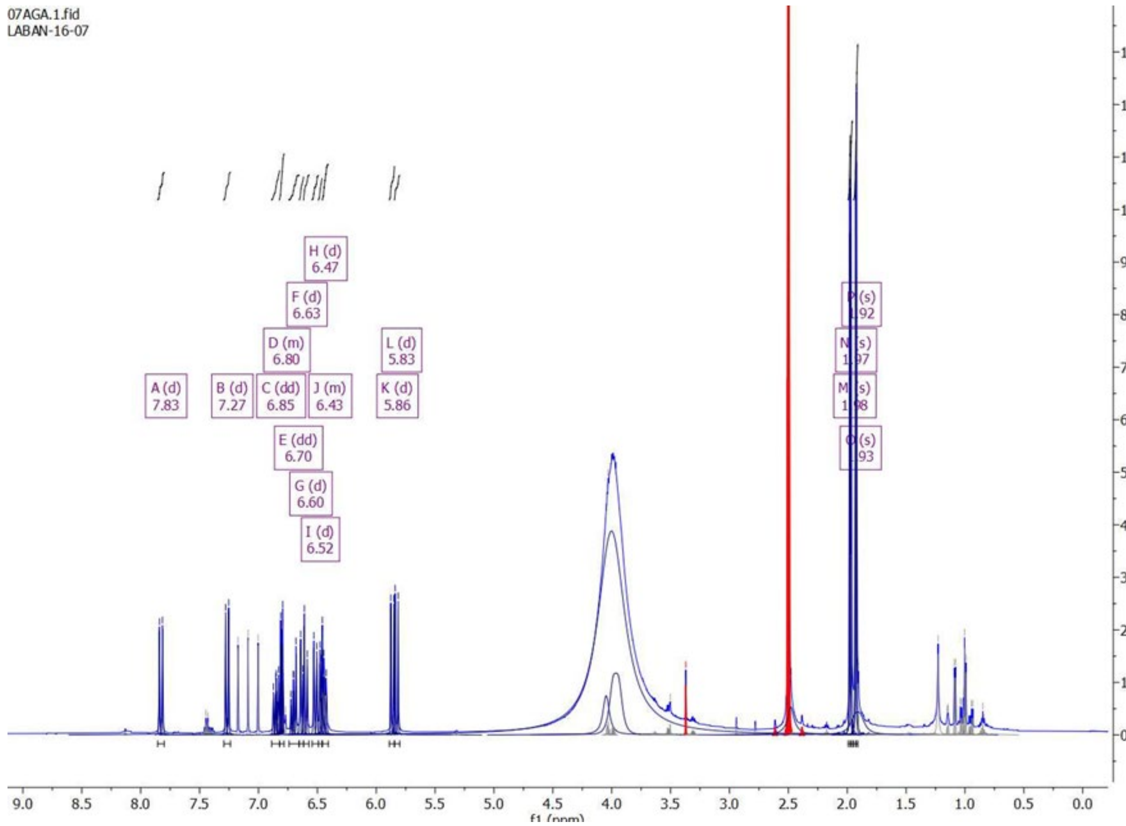
Figure S1. *NMR spectra of 1. a)* ^{1}H*, b)* ^{13}C*, c) COSY, d) HSQC.*

Table S1. ^{1}H-NMR (600 MHz, D_6-DMSO) and ^{13}C-NMR (121 MHz, D_6-DMSO) Data of 1. Chemical shifts δ in ppm and coupling constants *J* in Hz.

H atom	**^{1}H δ (ppm)**	**C atom**	**^{13}C δ (ppm)**
MeO (1'')	3.70 *(s)*	MeO (1'')	51.39
		C(6')	166.86
H-C(7')	5.94 (*d*, *J* = 15.5 Hz, 1H)	C(7')	117.67
H-C(8')	7.89 (*d, J* = 15.5 Hz, 1H)	C(8')	139.94
		C(9')	131.18
H-C(10')	6.50 (*d*, *J* = 11.8 Hz, 1H)	C(10')	138.04
H-C(11')	6.87 (*dd, J* = 14.7, 11.8 Hz, 1H)	C(11')	123.15
H-C(12')	6.53 (*d*, *J* = 14.7 Hz, 1H)	C(12')	140.60
		C(13')	136.72
H-C(14')	6.45(*m*)	C(14')	134.68
H-C(15')	6.80 (*m*)	C(15')	131.32
Me(19')	1.97 (*s*)	C(19')	12.67
Me(20')	1.94 (*s*)	C(20')	19.92
		C(6)	167.83
H-C(7)	5.83 (*d*, *J* = 15.5 Hz, 1H)	C(7)	117.32
H-C(8)	7.26 (d, J = 15.5 Hz, 1H)	C(8)	148.11
		C(9)	133.45
H-C(10)	6.60 (d, J = 14.8 Hz, 1H)	C(10)	141.42
H-C(11)	6.71 (dd, *J* = 14.8, 11.3 Hz, 1H)	C(11)	124.81
H-C(12)	6.63 (d, J = 11.3 Hz, 1H)	C(12)	139.00
		C(13)	136.67
H-C(14)	6.45 (m)	C(14)	134.54
H-C(15)	6.80 (m)	C(15)	131.43
Me(19)	1.92 (s)	C(19)	12.48
Me(20)	1.96 (s)	C(20')	12.57

^{1}H NMR (600 MHz, DMSO) δ 12.07 (s, 1H), 7.89 (d, *J* = 15.5 Hz, 1H), 7.26 (d, *J* = 15.5 Hz, 1H), 6.86 (dd, *J* = 14.7, 11.8 Hz, 1H), 6.82 – 6.76 (m, 2H), 6.71 (dd, *J* = 14.8, 11.3 Hz, 1H), 6.63 (d, *J* = 11.3 Hz, 1H), 6.60 (d, *J* = 14.8 Hz, 1H), 6.53 (d, *J* = 14.7 Hz, 1H), 6.50 (d, *J* = 11.8 Hz, 1H), 6.48 – 6.42 (m, 2H), 5.94 (d, *J* = 15.4 Hz, 1H), 5.83 (d, *J* = 15.5 Hz, 1H), 3.70 (s, 3H), 2.00 – 1.95 (m, 6H), 1.95 – 1.91 (m, 6H), 1.23 (s, 1H).^{13}C NMR (151 MHz, DMSO) δ 167.83, 166.86, 148.11, 141.42, 140.60, 139.94, 139.00, 138.04, 136.72, 136.67, 134.68, 134.54, 133.45, 131.43, 131.32, 131.18, 124.81, 123.15, 117.67, 117.32, 51.39, 19.92, 12.67, 12.57, 12.48.

a)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D (m) 6.80
L (d) 5.83
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B (d) 7.27
C (dd) 6.85
J (m) 6.43
K (d) 5.86
E (dd) 6.70
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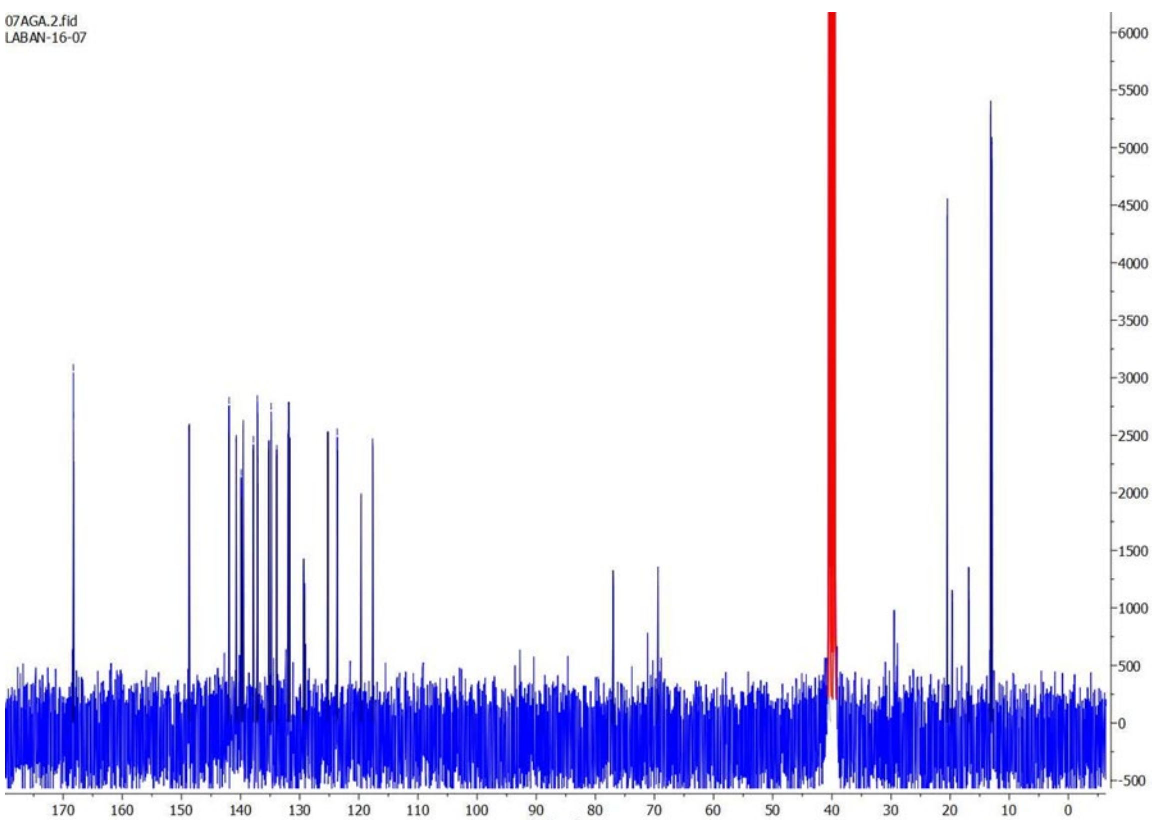
I (d) 6.52
f1 (ppm)

b)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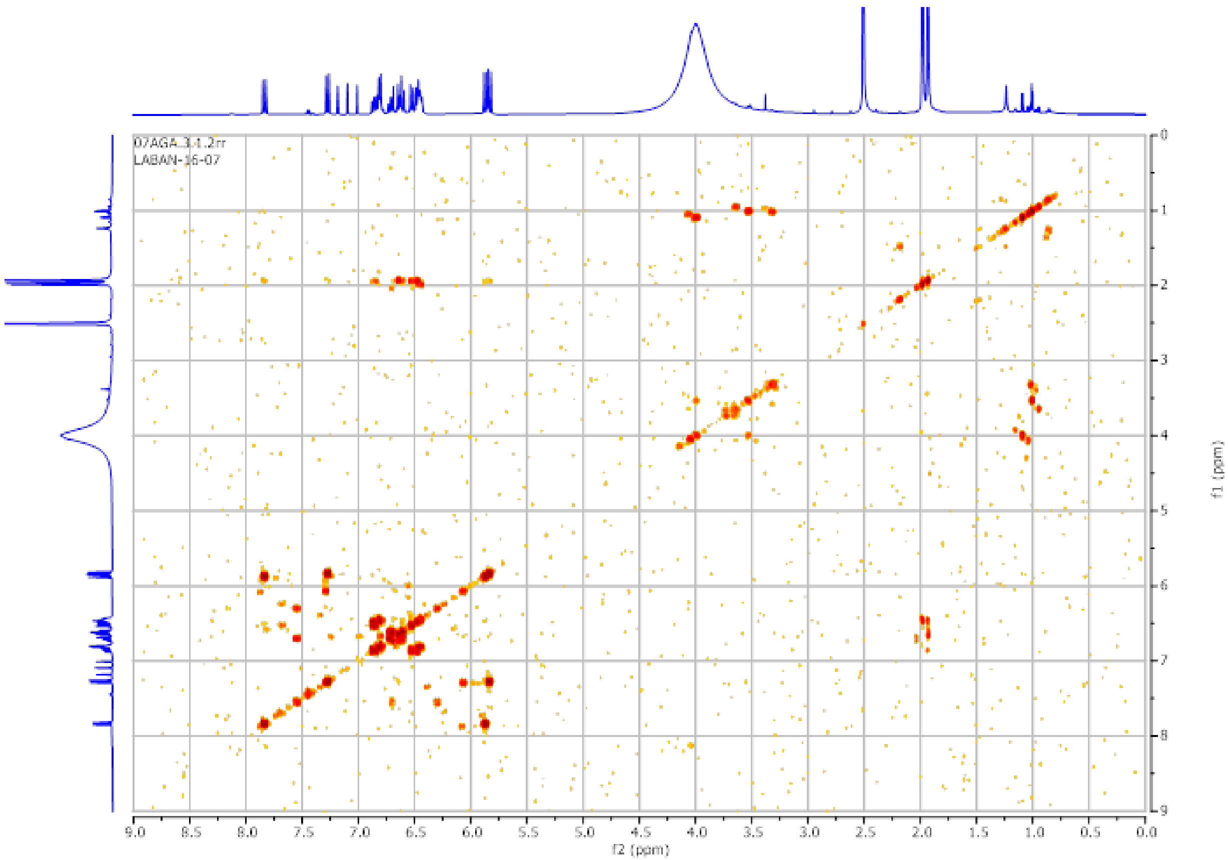
f1 (ppm)

c)



d)

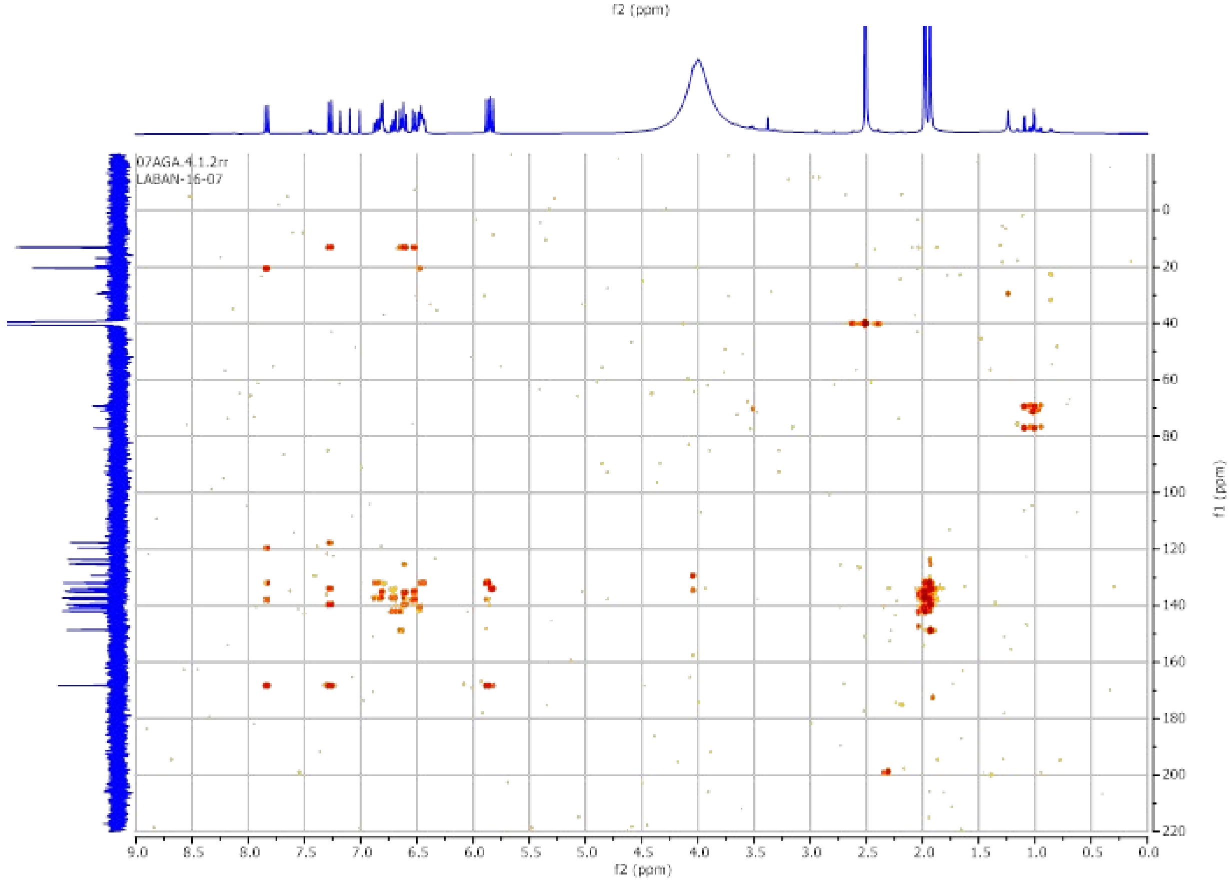


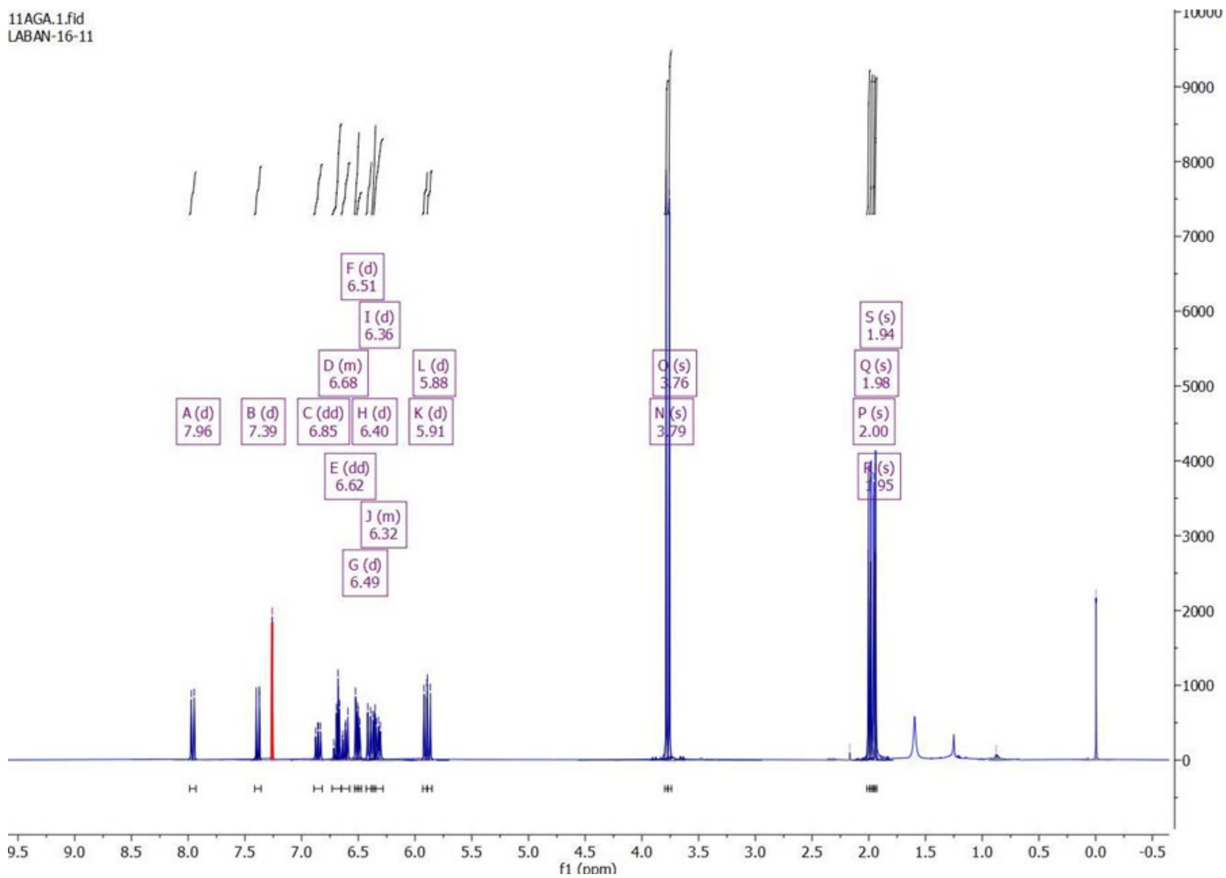
Figure S2. *NMR spectra of 2. a)* ^{1}H*, b)* ^{13}C*, c) COSY, d) HSQC.*

Table S2. ^{1}H-NMR (600 MHz, D_6-DMSO) and ^{13}C-NMR (121 MHz, D_6-DMSO) Data of 1. Chemical shifts δ in ppm and coupling constants *J* in Hz.

H atom	**^{1}H δ (ppm)**	**C atom**	**^{13}C δ (ppm)**
		C(6')	168.25
H-C(7')	5.86 (d, *J* = 15.4 Hz, 1H)	C(7')	119.6
H-C(8')	7.83 (d, *J* = 15.4 Hz, 1H)	C(8')	139.55
		C(9')	129.14
H-C(10')	6.48 (d, J = 11.7 Hz, 1H)	C(10')	137.12
H-C(11')	6.85 (dd, J = 14.8, 11.7 Hz, 1H)	C(11')	126.65
H-C(12')	6.52 (d, J = 14.8 Hz, 1H)	C(12')	140.72
		C(13')	135.2
H-C(14')	6.46 – 6.42 (m)	C(14')	133.88
H-C(15')	6.82 – 6.78 (m)	C(15')	129.31
Me(19')	1.97 (s)	C(19')	12.94
Me(20')	1.93 (s)	C(20')	13.04
		C(6)	168.27
H-C(7)	5.83 (d, J = 15.5 Hz, 1H)	C(7)	117.67
H-C(8)	7.27 (d, *J* = 15.5 Hz, 1H)	C(8)	148.67
		C(9)	131.85
H-C(10)	6.60 (d, J = 14.8 Hz, 1H)	C(10)	141.95
H-C(11)	6.70 (dd, J = 14.8, 11.3 Hz, 1H)	C(11)	125.24
H-C(12)	6.63 (d, J = 11.3 Hz, 1H)	C(12)	137.16
		C(13)	134.83
H-C(14)	6.46 – 6.42 (m)	C(14)	131.94
H-C(15)	6.82 – 6.78 (m)	C(15)	131.69
Me(19)	1.92(s)	C(19)	12.94
Me(20)	1.97(s)	C(20')	13.04

^{1}H NMR (600 MHz, DMSO) δ 7.83 (d, *J* = 15.4 Hz, 1H), 7.27 (d, *J* = 15.5 Hz, 1H), 6.85 (dd, *J* = 14.8, 11.7 Hz, 1H), 6.82 – 6.78 (m, 2H), 6.70 (dd, *J* = 14.8, 11.3 Hz, 1H), 6.63 (d, *J* = 11.3 Hz, 1H), 6.60 (d, *J* = 14.8 Hz, 1H), 6.52 (d, *J* = 14.8 Hz, 1H), 6.48 (d, *J* = 11.7 Hz, 1H), 6.46 – 6.41 (m, 2H), 5.86 (d, *J* = 15.4 Hz, 1H), 5.83 (d, *J* = 15.5 Hz, 1H), 1.98 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 168.27, 168.25, 148.67, 141.95, 140.72, 139.55, 137.16, 137.12, 135.20, 134.83, 133.88, 131.94, 131.85, 131.69, 129.31, 129.14, 125.24, 123.65, 119.60, 117.67, 20.46, 19.66, 16.86, 13.13, 13.04, 12.94.

a)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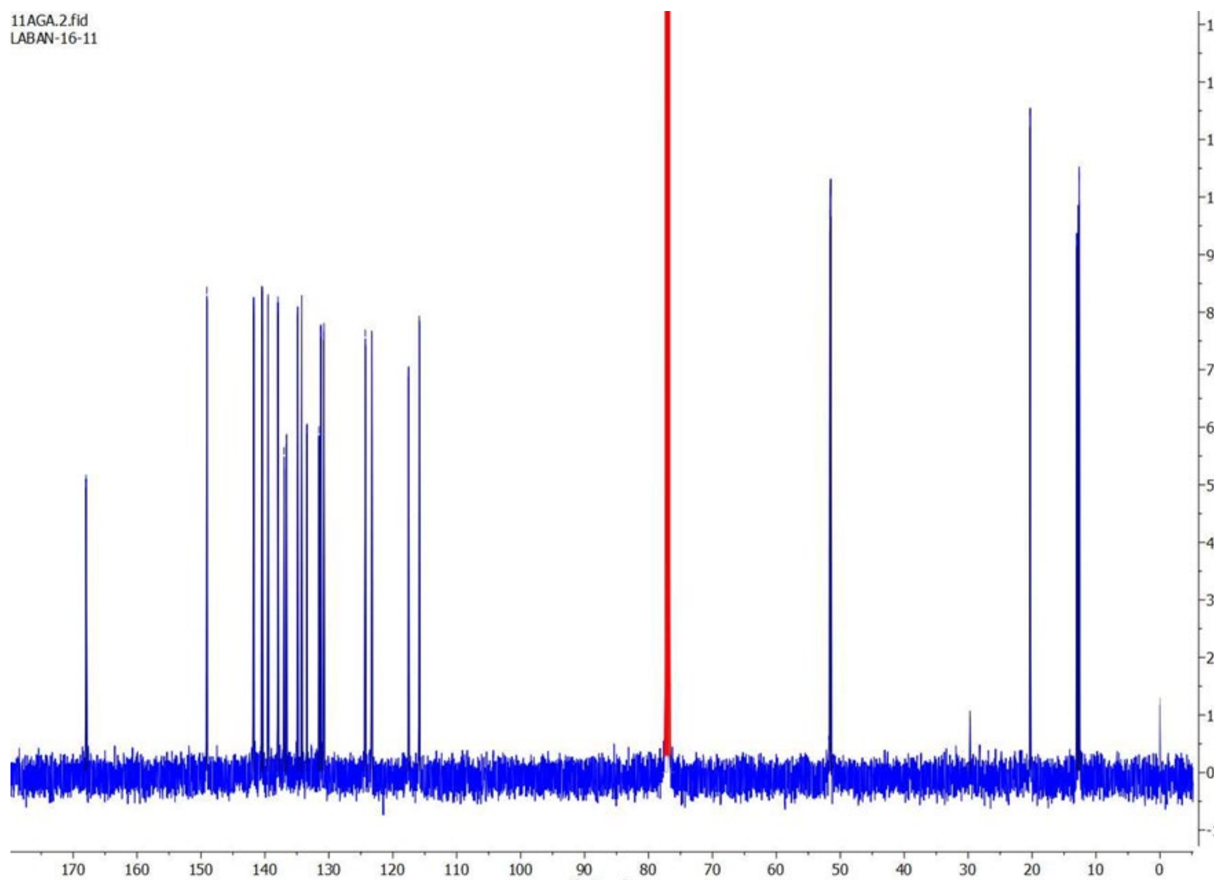
D (m) 6.68
L (d) 5.88
A (d) 7.96
B (d) 7.39
C (dd) 6.85
H (d) 6.40
K (d) 5.91
E (dd) 6.62
J (m) 6.32
G (d) 6.49
O (s) 3.76
N (s) 3.79
S (s) 1.94
Q (s) 1.98
P (s) 2.00
R (s) 1.95
f1 (ppm)

b)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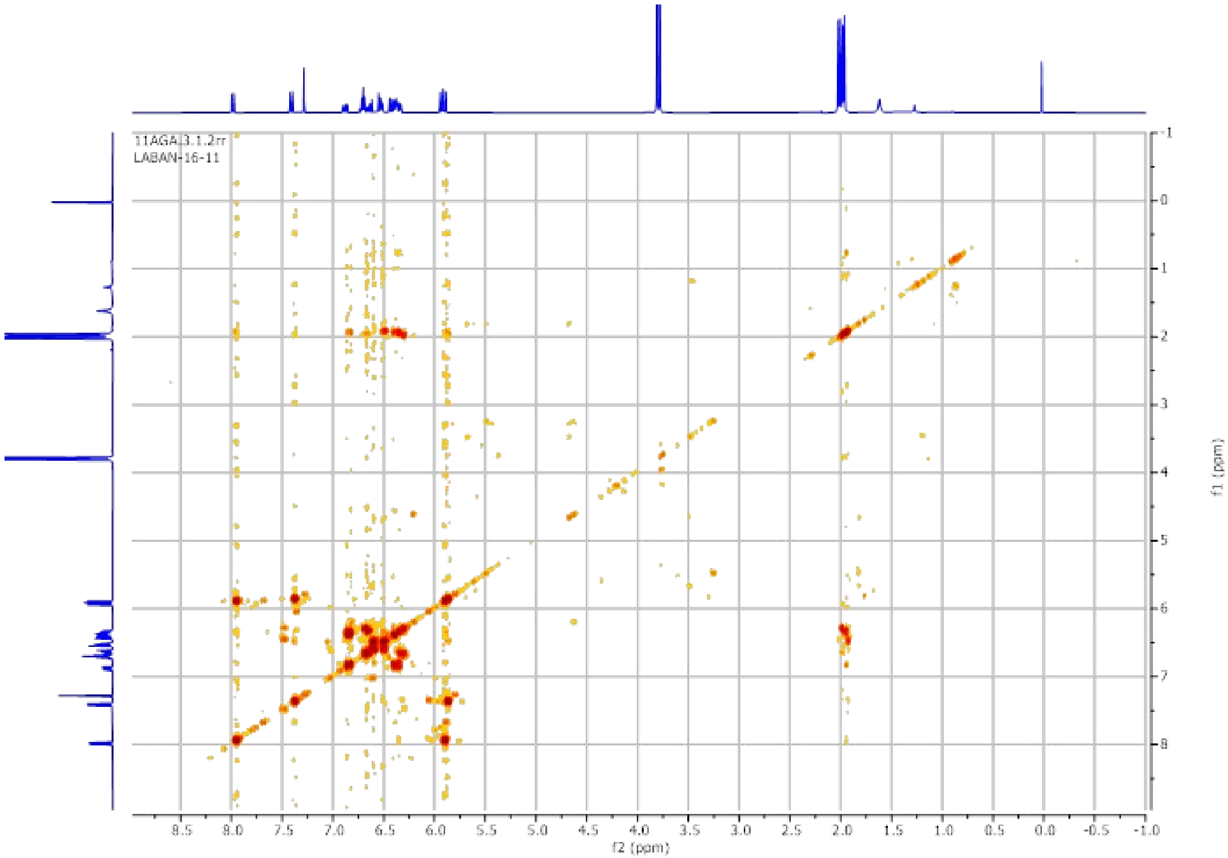
f1 (ppm)

c)



d)

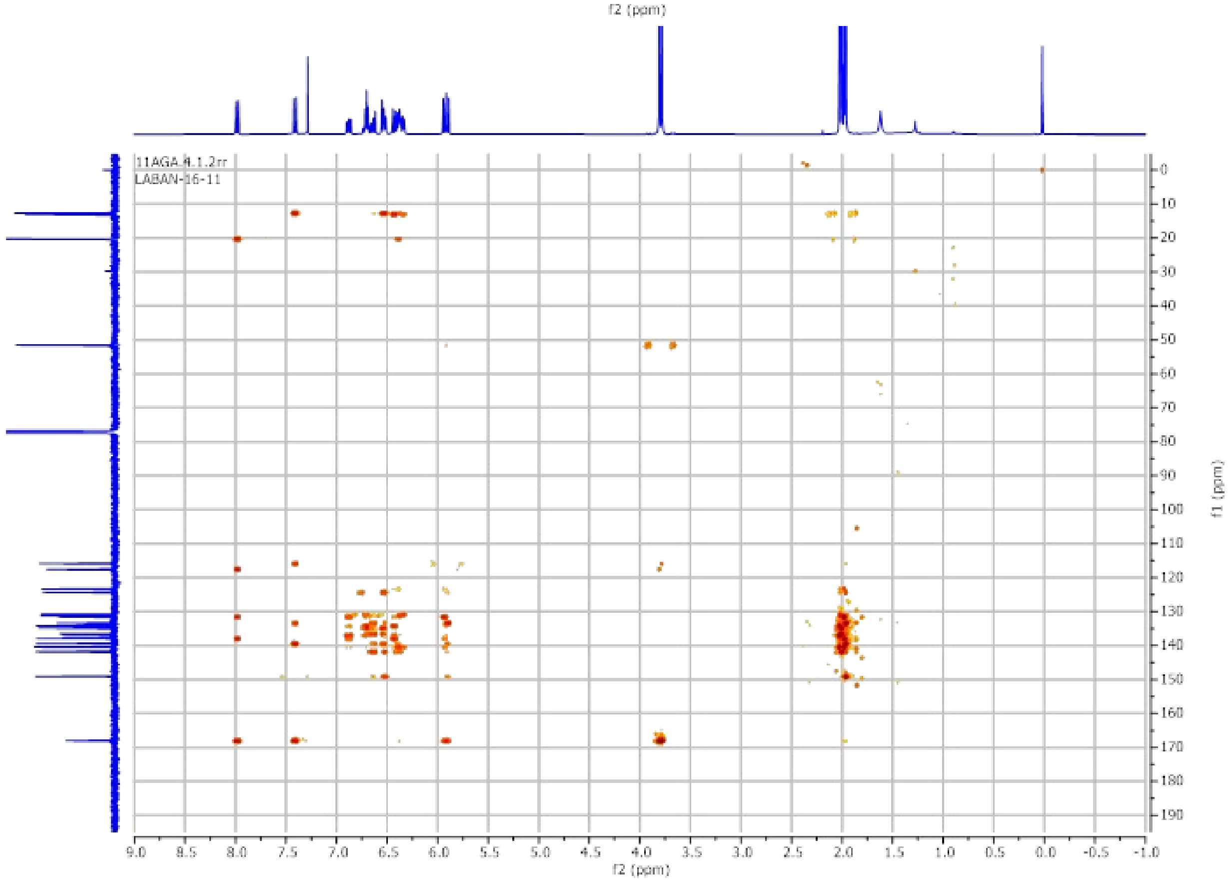
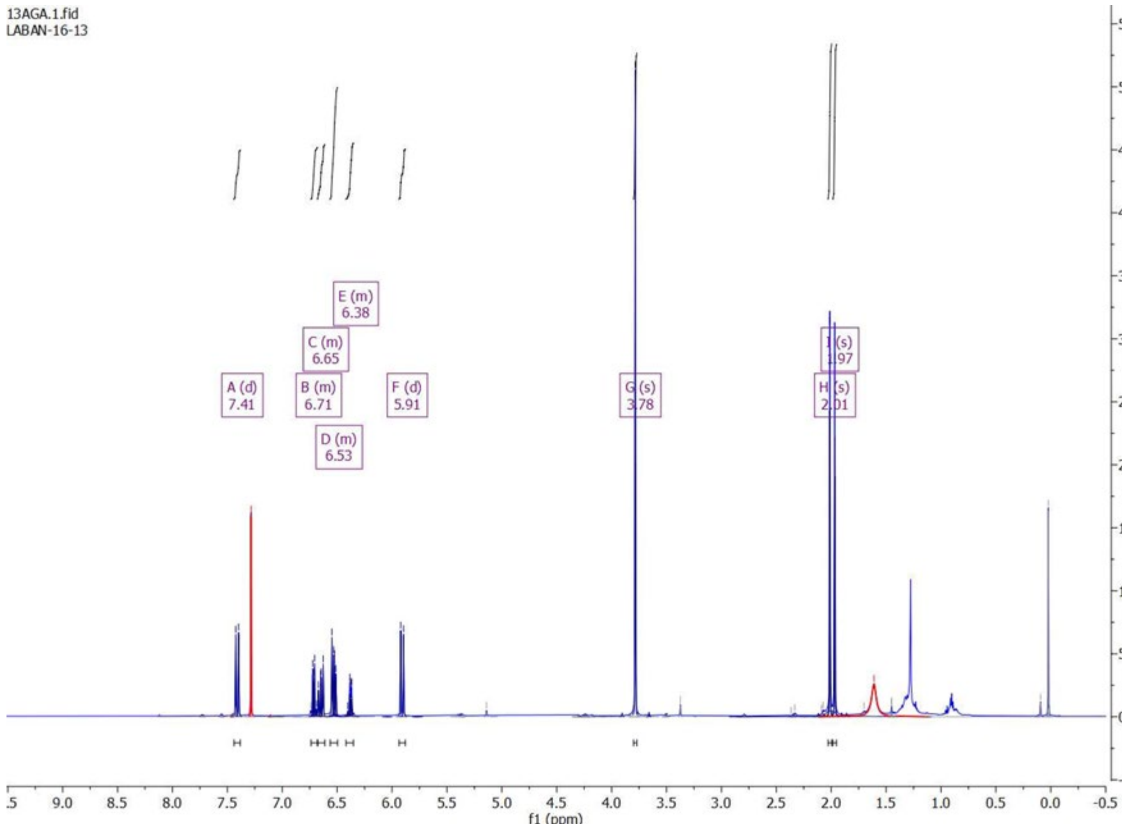


Figure S3. *NMR spectra of 3 a) ^{1}H, b) ^{13}C, c) COSY, d) HSQC.*

Table S3. ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (121 MHz, $CDCl_3$). Data of 3. Chemical shifts δ in ppm and coupling constants *J* in Hz.

H atom	^{1}H δ (ppm)	C atom	^{13}C δ (ppm)
MeO (1'')	3.76 (s, 3H)	C(1'')	51.48
		C(6')	167.94
H-C(7')	5.91 (d, *J* = 15.4 Hz, 1H)	C(7')	117.51
H-C(8')	7.96 (d, *J* = 15.4 Hz, 1H)	C(8')	140.46
		C(9')	133.41
H-C(10')	6.36 (d, *J* = 11.6 Hz, 1H)	C(10')	137.95
H-C(11')	6.85 (dd, *J* = 14.8, 11.6 Hz, 1H)	C(11')	123.30
H-C(12')	6.40 (d, *J* = 14.8 Hz, 1H)	C(12')	140.41
		C(13')	136.99
H-C(14')	6.35 – 6.29 (m)	C(14')	134.86
H-C(15')	6.73 – 6.65 (m)	C(15')	131.24
Me(19')	1.98 (s, 3H)	C(19')	13.01
Me(20')	1.95 (s, 3H)	C(20')	20.29
MeO (1''')	3.79 (s, 3H)	C(1''')	51.60
		C(6)	167.98
H-C(7)	5.88 (d, *J* = 15.5 Hz, 1H)	C(7)	115.81
H-C(8)	7.39 (d, J = 15.5 Hz, 1H)	C(8)	149.08
		C(9)	131.54
H-C(10)	6.49 (d, *J* = 11.5 Hz, 1H)	C(10)	139.50
H-C(11)	6.62 (dd, *J* = 14.6, 11.5 Hz, 1H)	C(11)	124.29
H-C(12)	6.51 (d, *J* = 14.6 Hz, 1H)	C(12)	141.76
		C(13)	136.60
H-C(14)	6.36 – 6.28 (m)	C(14)	134.24
H-C(15)	6.73 – 6.65 (m)	C(15)	130.77
Me(19)	1.94 (s, 3H)	C(19)	12.65
Me(20)	2.00 (s, 3H)	C(20')	12.78

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (d, *J* = 15.4 Hz, 1H), 7.39 (d, *J* = 15.5 Hz, 1H), 6.85 (dd, *J* = 14.8, 11.6 Hz, 1H), 6.73 – 6.65 (m, 2H), 6.62 (dd, *J* = 14.6, 11.5 Hz, 1H), 6.51 (d, *J* = 14.6 Hz, 2H), 6.49 (d, *J* = 11.5 Hz, 0H), 6.40 (d, *J* = 14.8 Hz, 1H), 6.36 (d, *J* = 11.5 Hz, 1H), 6.36 – 6.28 (m, 2H), 5.91 (d, *J* = 15.4 Hz, 1H), 5.88 (d, *J* = 15.5 Hz, 1H), 3.79 (s, 3H), 3.76 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.98, 167.94, 149.08, 141.76, 140.46, 140.41, 139.50, 137.95, 136.99, 136.60, 134.86, 134.24, 133.41, 131.54, 131.24, 130.77, 124.29, 123.30, 117.51, 115.85, 51.60, 51.48, 20.29, 13.01, 12.78, 12.65.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B (m) 6.71
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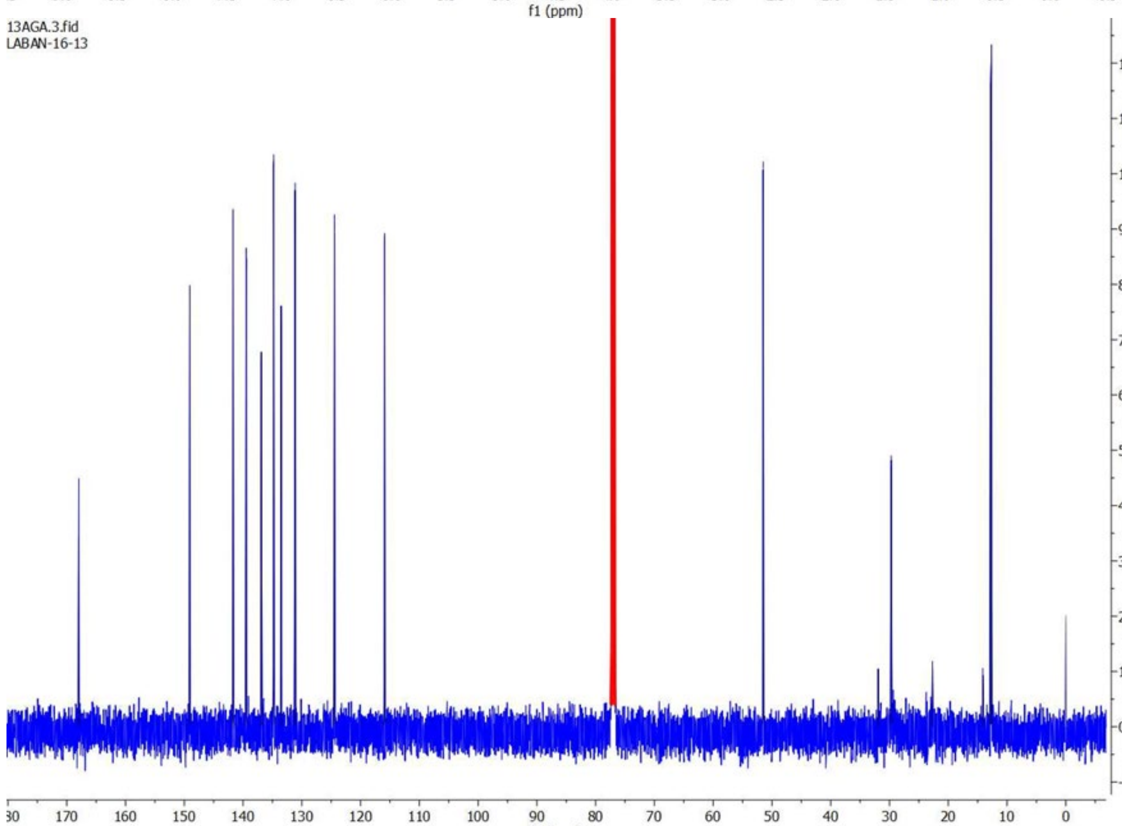
D (m) 6.53
G (s) 3.78
I (s) 1.97
H (s) 2.01
f1 (ppm)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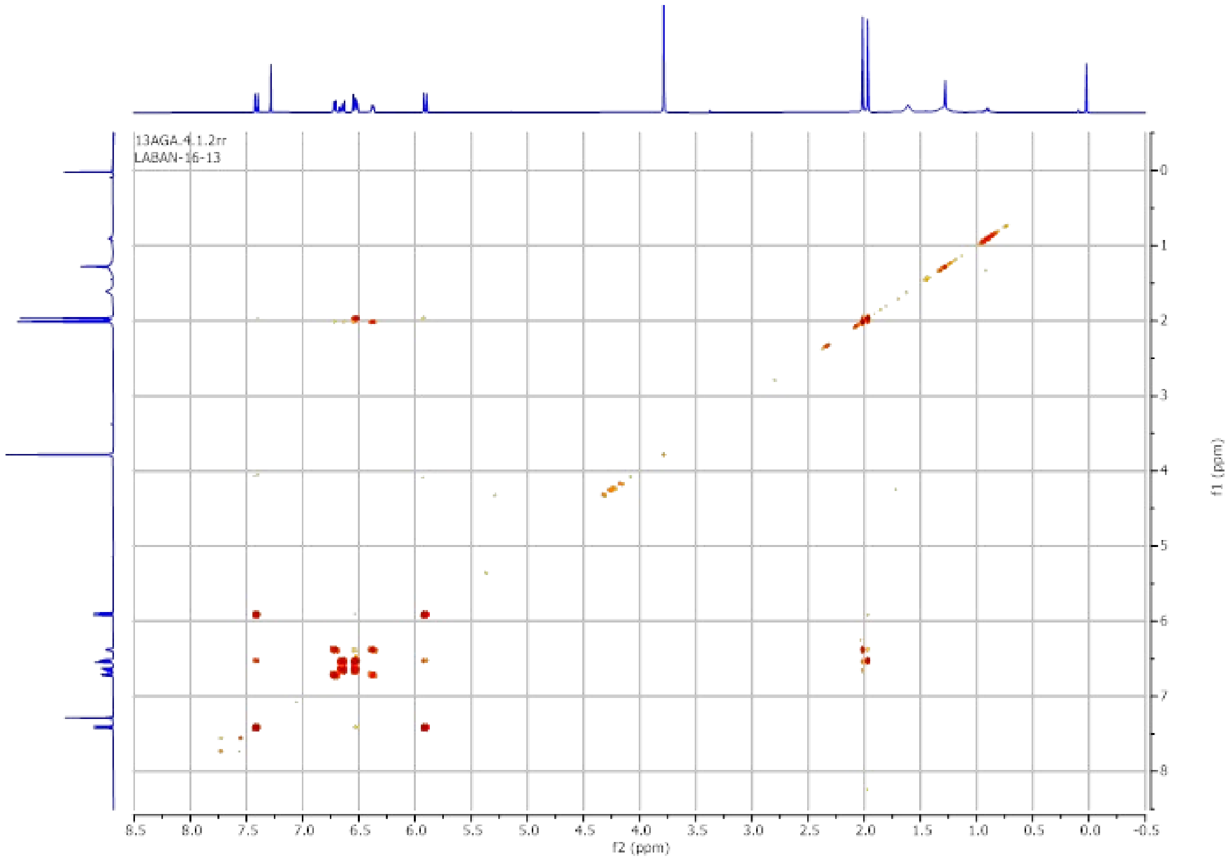


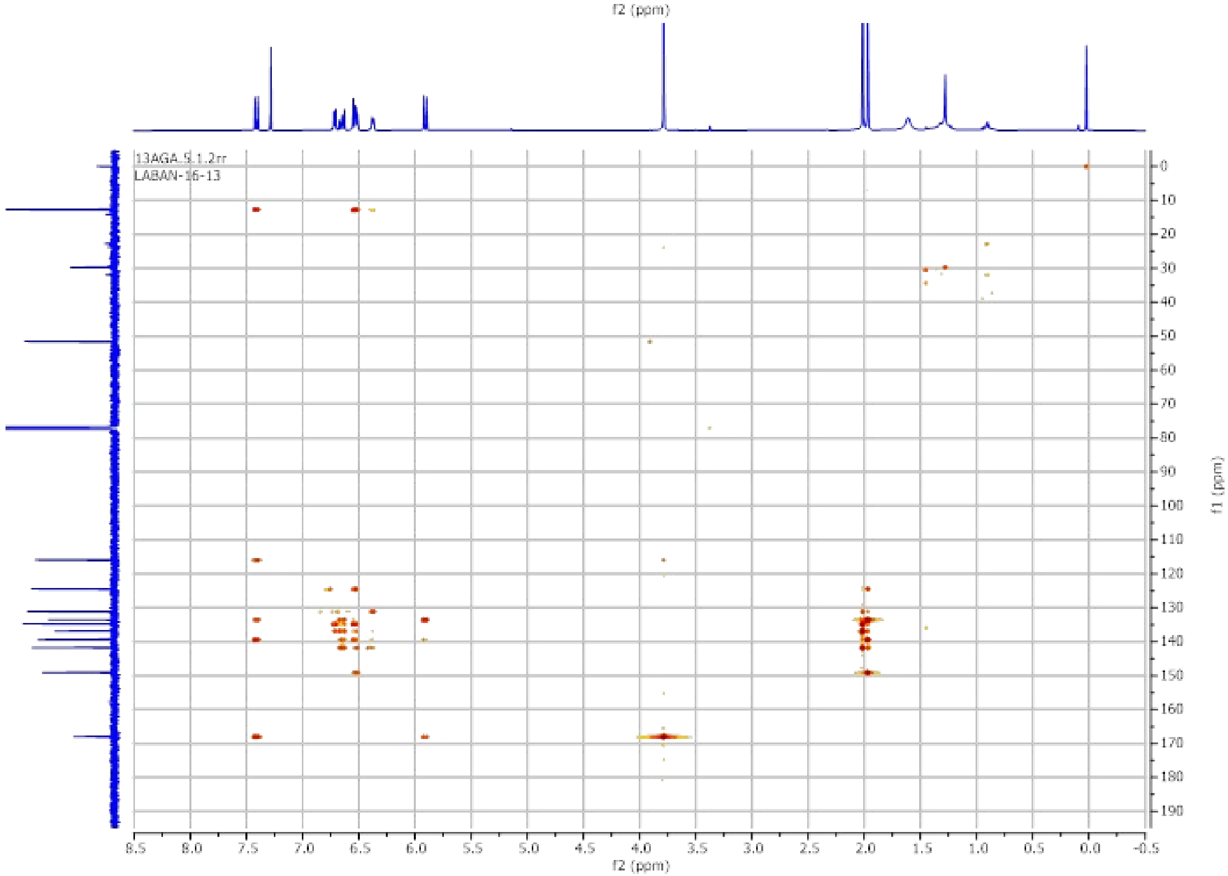


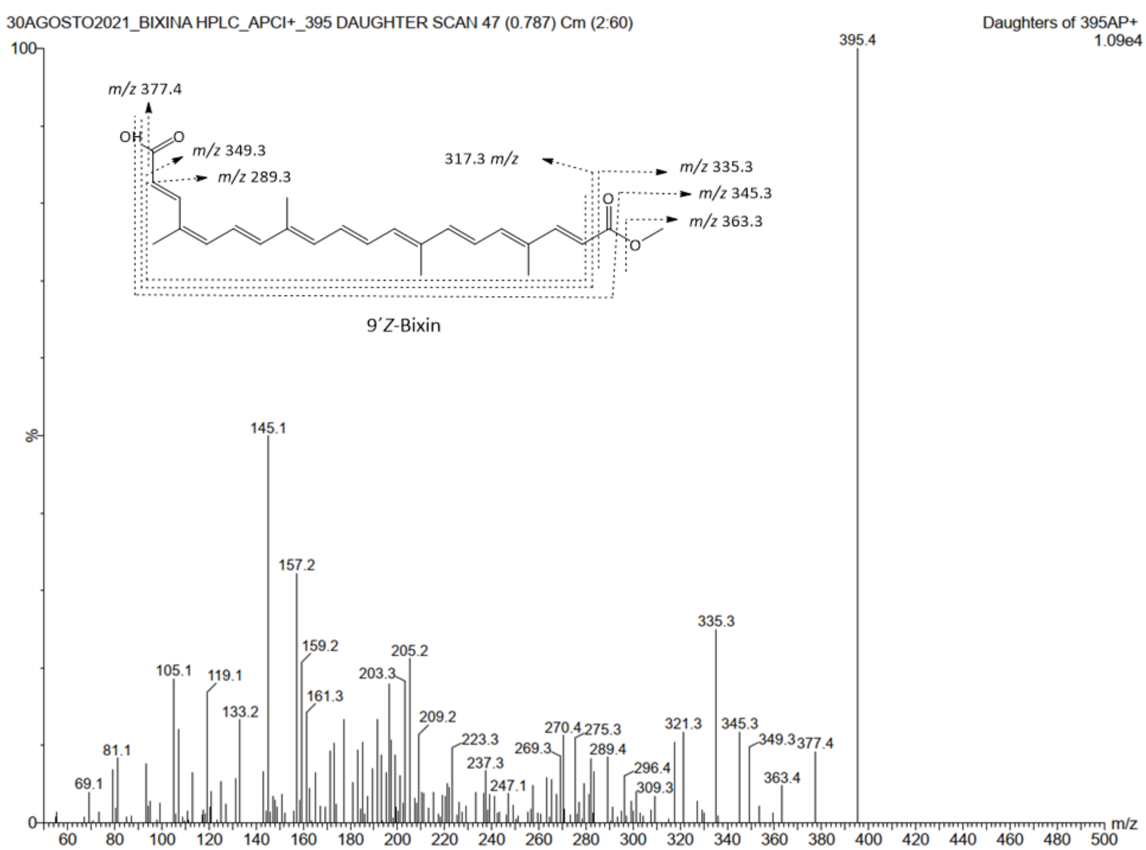
Figure S4. *NMR spectra of 4 a) ^{1}H, b) ^{13}C, c) COSY, d) HSQC.*

Table S4. ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (121 MHz, $CDCl_3$). Data of 4. Chemical shifts δ in ppm and coupling constants *J* in Hz.

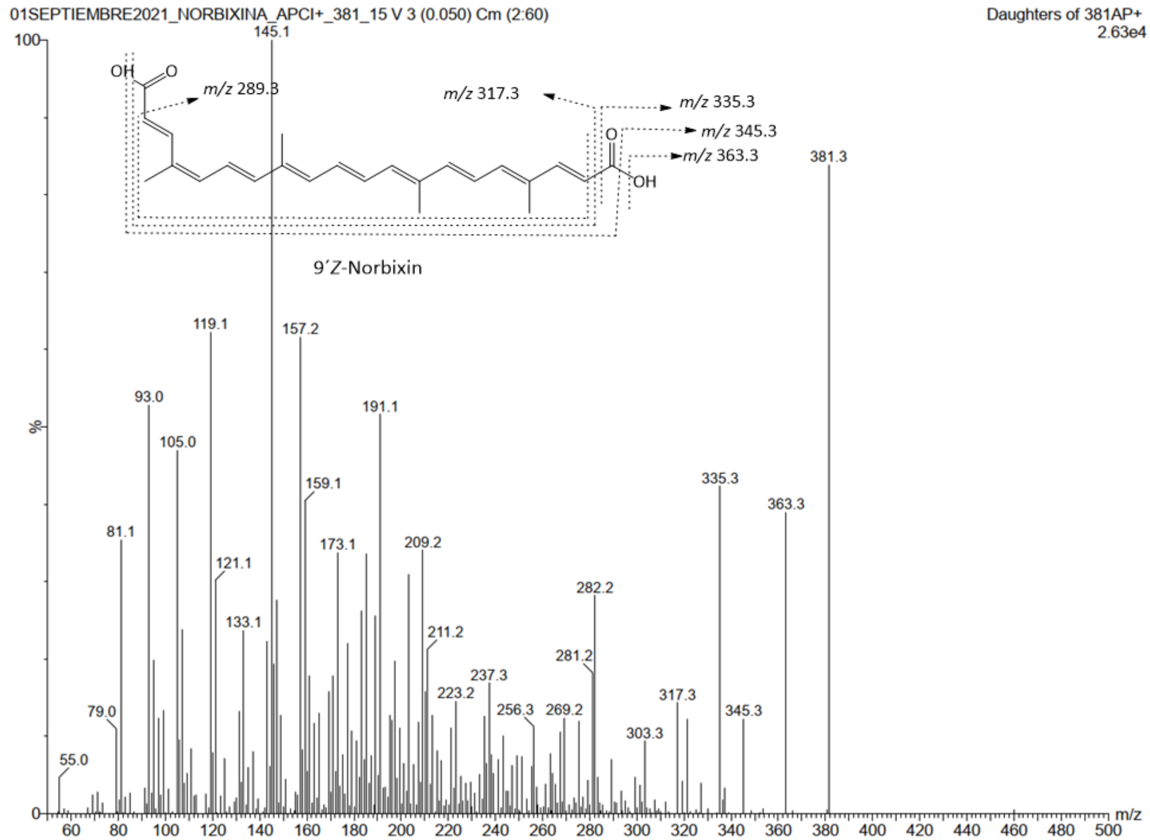
H atom	^{1}H δ (ppm)	C atom	^{13}C δ (ppm)
MeO (1'')	3.76 (s, 6H)	C(1'')	51.49
		C(6')	167.94
H-C(7')	5.88 (d, *J* = 15.5 Hz, 2H)	C(7')	117.51
H-C(8')	7.39 (d, *J* = 15.5 Hz, 2H)	C(8')	149.06
		C(9')	133.52
H-C(10')	6.39 – 6.33 (m, 2H)	C(10')	139.46
H-C(11')	6.65 – 6.59 (m, 2H)	C(11')	124.43
H-C(12')	6.54 – 6.47 (m, 2H)	C(12')	141.70
		C(13')	136.87
H-C(14')	6.35 – 6.29 (m)	C(14')	134.79
H-C(15')	6.71 – 6.65 (m, 2H)	C(15')	131.14
Me(19')	1.99 (s, 6H)	C(19')	12.80
Me(20')	1.94 (s, 6H).	C(20')	12.66
MeO (1')	3.76 (s, 6H)	C(1')	51.49
		C(6)	167.94
H-C(7)	5.88 (d, *J* = 15.5 Hz, 2H)	C(7)	115.81
H-C(8)	7.39 (d, *J* = 15.5 Hz, 2H)	C(8)	149.06
		C(9)	133.52
H-C(10)	6.39 – 6.33 (m, 2H)	C(10)	139.46
H-C(11)	6.65 – 6.59 (m, 2H)	C(11)	124.43
H-C(12)	6.54 – 6.47 (m, 2H)	C(12)	141.70
		C(13)	136.87
H-C(14)	6.36 – 6.28 (m)	C(14)	134.79
H-C(15)	6.71 – 6.65 (m, 2H)	C(15)	131.14
Me(19)	1.99(s, 6H)	C(19)	12.80
Me(20)	1.94 (s, 6H).	C(20')	12.66

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, *J* = 15.5 Hz, 2H), 6.71 – 6.65 (m, 2H), 6.65 – 6.59 (m, 2H), 6.54 – 6.47 (m, 4H), 6.39 – 6.33 (m, 2H), 5.88 (d, *J* = 15.5 Hz, 2H), 3.76 (s, 6H), 1.99 (s, 6H), 1.94 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.94, 149.06, 141.70, 139.46, 136.87, 134.79, 133.52, 131.14, 124.43, 115.91, 12.80, 12.66.

a)

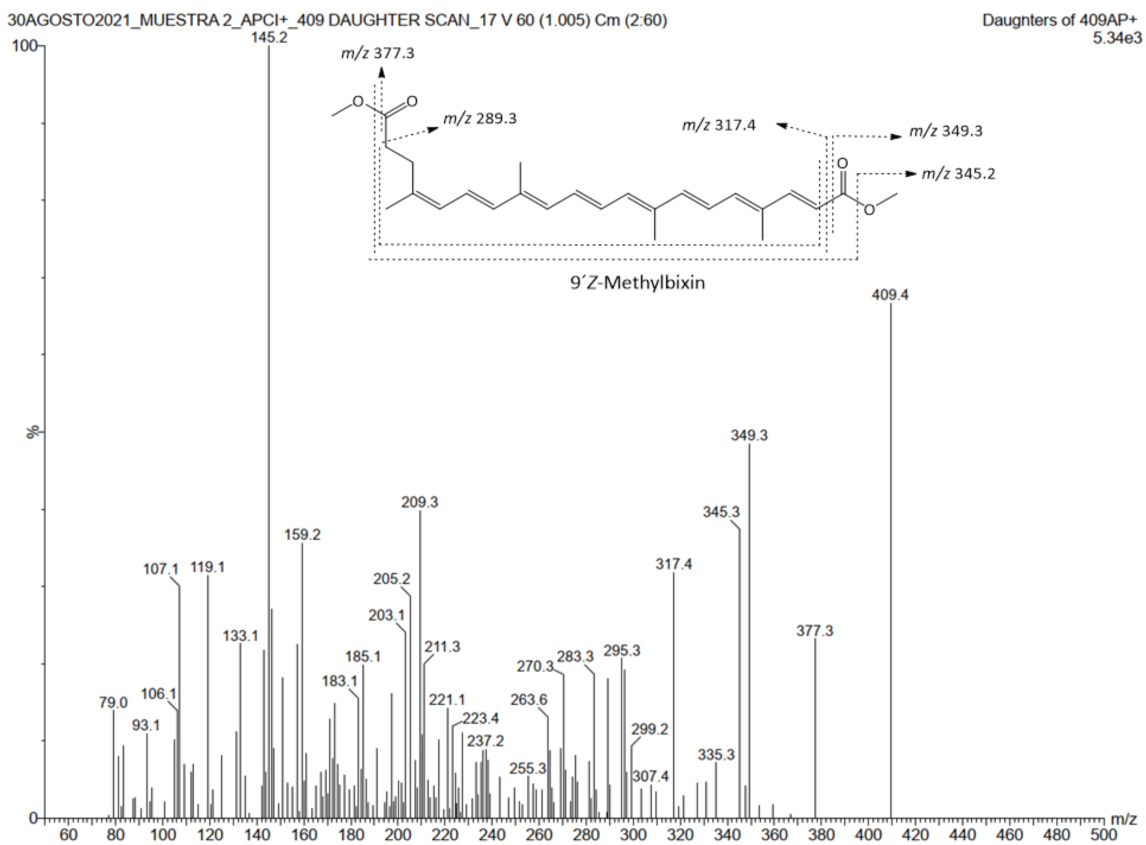


b)



01SEPTIEMBRE2021_NORBIXINA_APCI+_381_15 V 3 (0.050) Cm (2:60)
Daughters of 381AP+
2.63e4
m/z 289.3
m/z 317.3
m/z 335.3
m/z 345.3
m/z 363.3
9´Z-Norbixin
145.1
381.3
119.1
157.2
93.0
191.1
105.0
159.1
335.3
363.3
81.1
173.1
209.2
121.1
282.2
133.1
211.2
281.2
237.3
223.2
317.3
79.0
256.3
269.2
345.3
303.3
55.0
%
m/z

c)



30AGOSTO2021_MUESTRA 2_APCI+_409 DAUGHTER SCAN_17 V 60 (1.005) Cm (2:60)
Daughters of 409AP+
5.34e3
145.2
m/z 377.3
m/z 289.3
m/z 317.4
m/z 349.3
m/z 345.2
9´Z-Methylbixin
409.4
349.3
209.3
345.3
159.2
317.4
107.1
119.1
205.2
203.1
377.3
133.1
211.3
185.1
295.3
270.3
283.3
183.1
106.1
221.1
263.6
79.0
223.4
299.2
93.1
237.2
335.3
255.3
307.4
%
m/z

d)

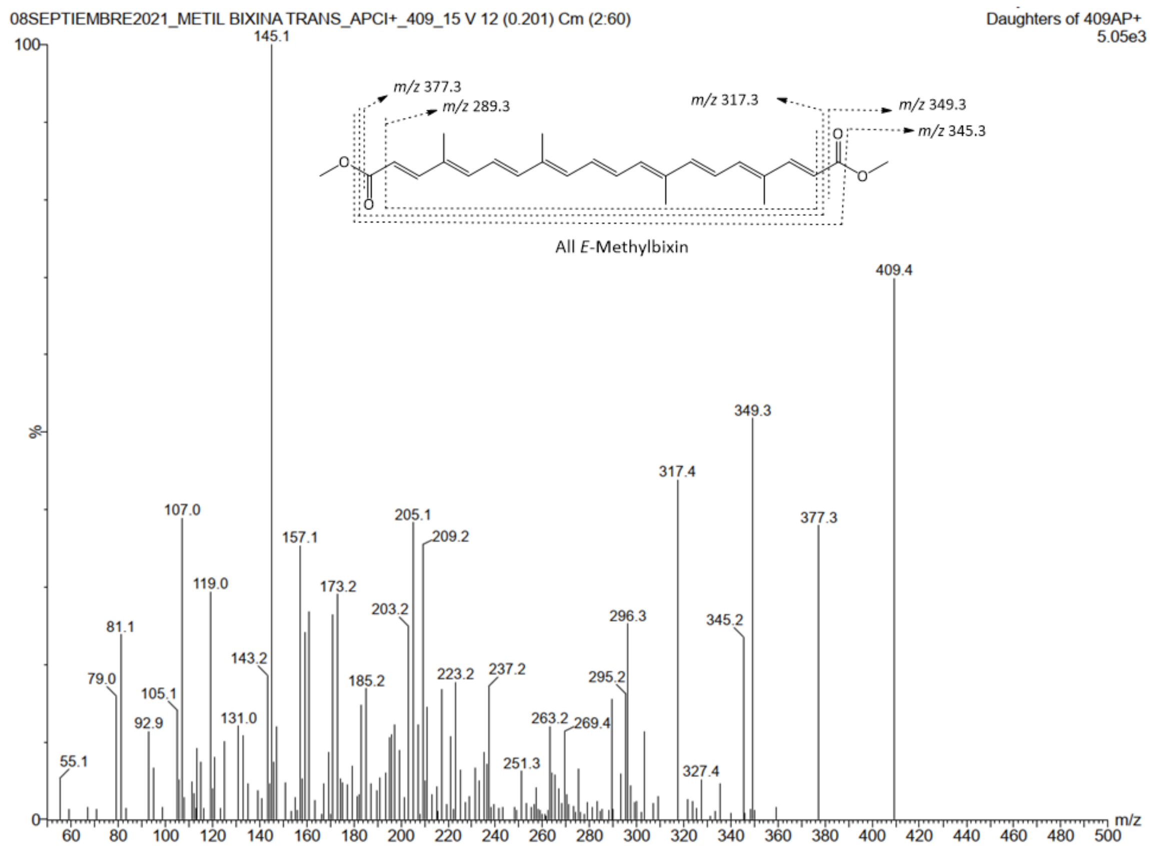
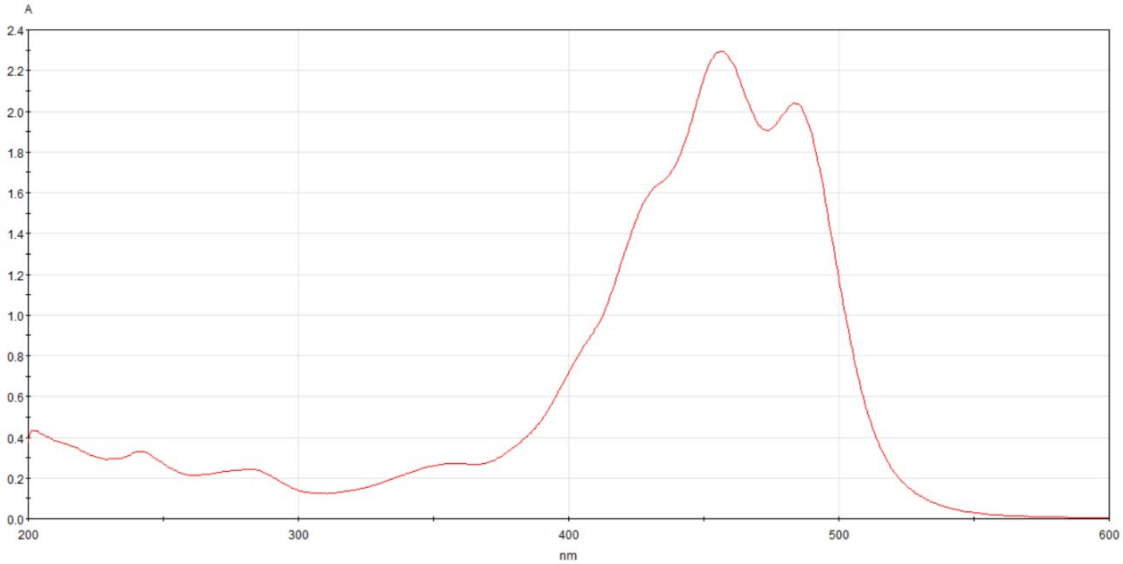


Figure S5. *Mass spectrum of a) 1, b) 2, c) 3 and 4).*

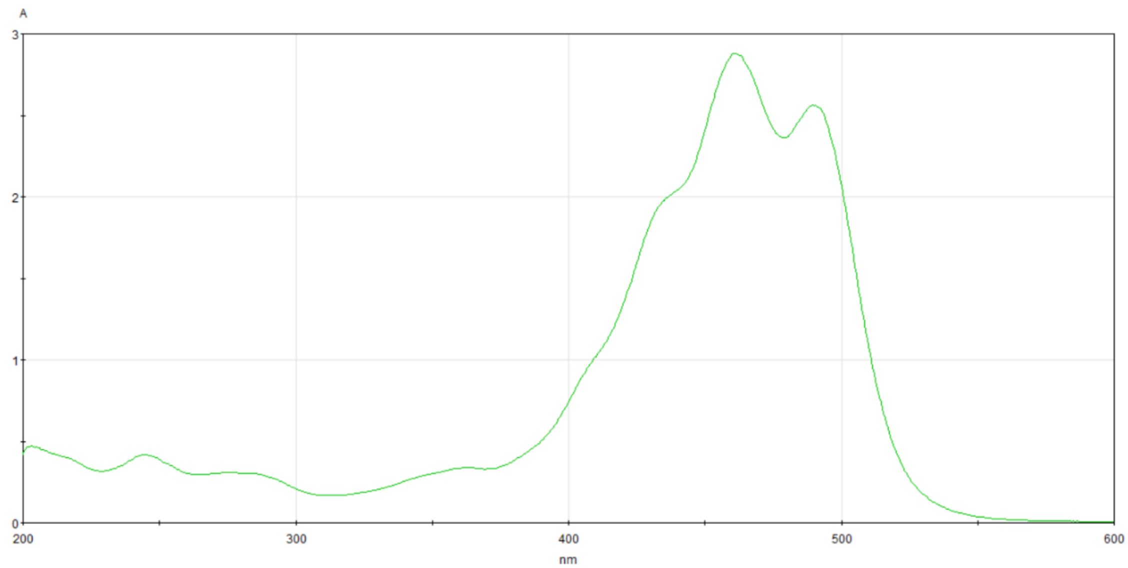
Table 5. Mass spectrometric information of bixin (1) and bixin derivatives (2, 3 and 4)

Compound	Molecular formula	Experimental m/z		Fragmentation MS^2 *m/z*
		$M^{\cdot+}$	$[M+H]^+$	
9´Z-bixin (1)	$C_{25}H_{30}O_4$	394.4	395.4	377.4, 363.4, 349.3, 345.3, 335.3, 317.3, 289.3, 209.2, 197.2, 157.2, 145.2
9´Z-norbixin (2)	$C_{24}H_{28}O_4$	380.2	381.2	363.3, 345.3, 335.3 317.3, 282.2, 289.3, 209.2, 191.1, 145.1
9´Z-methylbixin (3)	$C_{26}H_{32}O_4$	408.2	409.2	377.3, 349.3, 345.3, 317.4, 295.3, 289.3, 283.3, 209.3, 145.2
All E- methylbixin (4)	$C_{26}H_{32}O_4$	408.2	409.2	377.3, 349.3, 345.2 317.4, 296.3, 289.3, 205.1, 145.1

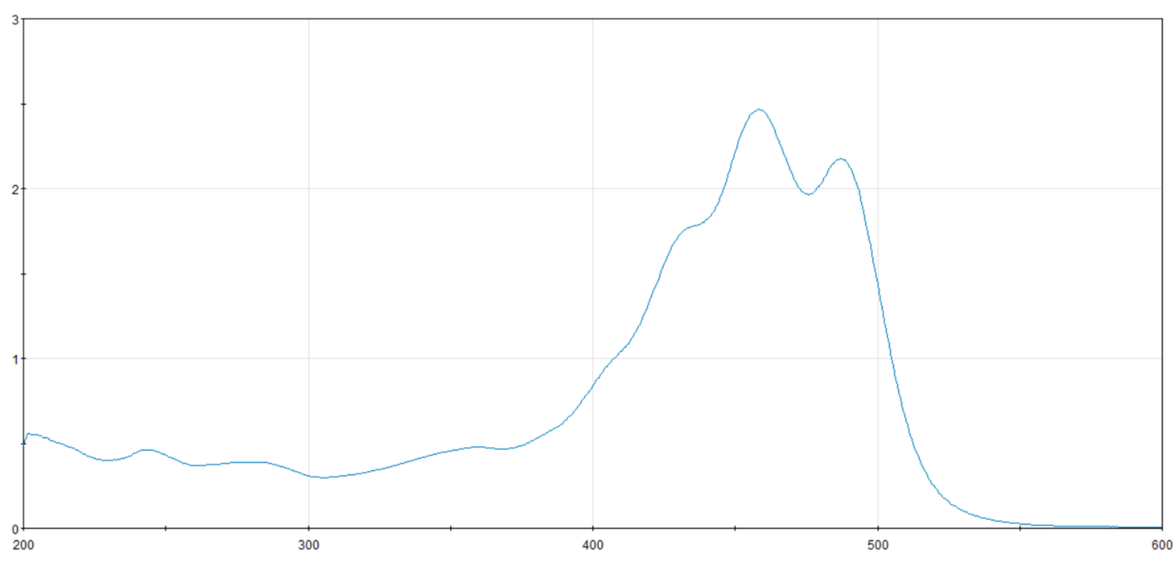
a)


A
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
nm

b)


A
3
2
1
0
200
300
400
500
600
nm

c)


3
2
1
0
200
300
400
500
600
nm

d)

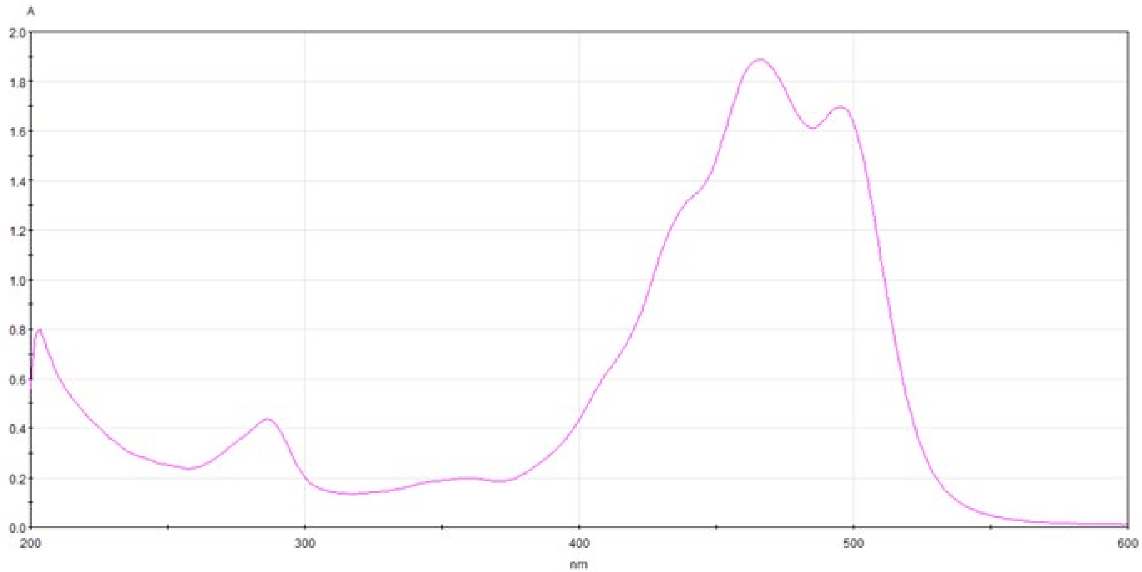


Figure S6. *UV-Vis spectrum of a) 1, b) 2, c) 3 and d) 4.*