SUPPLEMENTARY MATERIAL

Three new labdane-type diterpenoids from *Callicarpa macrophylla* Vahl.

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Abstract

Three new labdane-type diterpenoids, callicapene M3 (**1**), callicapene M4 (**2**) and callicapene M5 (**3**) were isolated from the *Callicarpa macrophylla* Vahl.. Their structures were identified by spectral methods including 2D NMR. All the isolated three compounds were evaluated for inhibitory activity on NO production in LPS-activated RAW 264.7 macrophage cells by using MTT assays. Compounds **1**, **2** and **3** showed potent inhibitory activity, with IC_{50} value of 48.15, 46.31 and 38.72μM respectively.

Key words: *Callicarpa macrophylla* Vahl., Labdane-type diterpenoids, NMR, NO inhibition.

List of contents

Table S1 The ^{1}H and ^{13}C NMR data for compound **1-3** ($CDCl_3$, ^{1}H NMR 600 MHZ, ^{13}C NMR 150 MHZ)

	1		**2**		**3**	
No.	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1*α*	1.18 (td, 13.2, 3.7)	37.3	1.13 (td, 13.1, 4.1)	37.6	1.60 (m)	37.6
1*β*	1.78 (m)		1.89 (td, 13.1, 3.5)		2.01 (m)	
2*α*	1.62 (m)	27.9	1.61 (m)	27.4	2.38 (m)	34.9
2*β*	1.73 (m)		1.65 (m)		2.60 (ddd, 14.8, 12.5, 6.4)	
3*α*	3.26 (dd, 11.8, 4.3)	78.7	3.25 (dd, 11.4, 4.4)	79.0		217.6
4		39.3		36.8		47.9
5	1.09 (dd, 12.5, 2.7)	54.7	1.20 (dd, 11.5, 5.5)	49.6	1.61 (dd, 12.8, 2.7)	55.2
6*α*	1.41 (ddd, 17.2, 13.0, 4.2)	24.1	1.97 (m)	23.5	1.50 (m)	25.2
6*β*	1.77 (m)		2.00 (m)		1.69 (m)	
7*α*	1.96 (td, 13.0, 5.0)	38.2	5.47 (d, 1.4)	123.6	2.02 (m)	37.9
7*β*	2.43 (ddd, 6.5, 4.0, 2.5)				2.45 (ddd, 12.8, 3.2, 3.2)	
8		147.4		133.6		147.1
9	1.61 (1H, m)	56.0	1.67 (m)	54.5	1.72 (m)	56.4
10		39.6		38.8		39.6
11	1.67 (m)	21.5	1.50 (m)	25.0	1.61 (m)	23.9
	1.76 (m)		1.73 (m)		1.66 (m)	
12	2.26 (ddd, 16.4, 8.1, 3.7)	27.6	2.37 (ddd, 16.8, 10.8, 6.0)	30.7	2.45 (m)	29.2
	2.55 (ddd, 15.0, 9.3, 3.8)		2.60 (ddd, 16.0, 11.4, 4.5)		2.49 (m)	
13		171.0		171.4		164.9
14	5.84 (t, 1.5)	115.3	5.86 (s)	115.6	6.00 (s)	113.1
15		174.2		174.1		171.3
16*a*	4.69 (dd, 17.3, 1.5)	73.2	4.73 (dd, 17.4, 1.4)	73.1	4.18 (d, 16.9)	66.0

16*b*	4.74 (dd, 17.3, 1.5)		4.77 (dd, 17.4, 1.4)		4.24 (d, 16.9)	
17*a*	4.47 (d, 0.8)	107.0	1.69 (s)	22.1	4.73 (s)	107.8
17*b*	4.90 (d, 0.8)				4.94 (s)	
18	1.00 (s)	28.4	0.98 (s)	28.0	1.09 (s)	26.1
19	0.78 (s)	15.5	0.86 (s)	15.2	1.01 (s)	21.8
20	0.71 (s)	14.6	0.78 (s)	13.8	0.83 (s)	14.2

Table S2. Inhibitory effects of compounds **1-3** against LPS-induced NO production in RAW264.7 macrophages.

Compound	IC_{50} value (μM)	Cell Viability (%)[a]
1	48.15	99.69±3.14
2	46.31	100.23±3.17
3	38.72	98.83±2.38
MINO [b]	35.13	100.19±3.29

[a] The cell viability of RAW 264.7 cells in the presence of derivatives at a dose of 50μM after a period of 24 h.

[b] Minocycline (MINO) was used as the positive control for NO production.

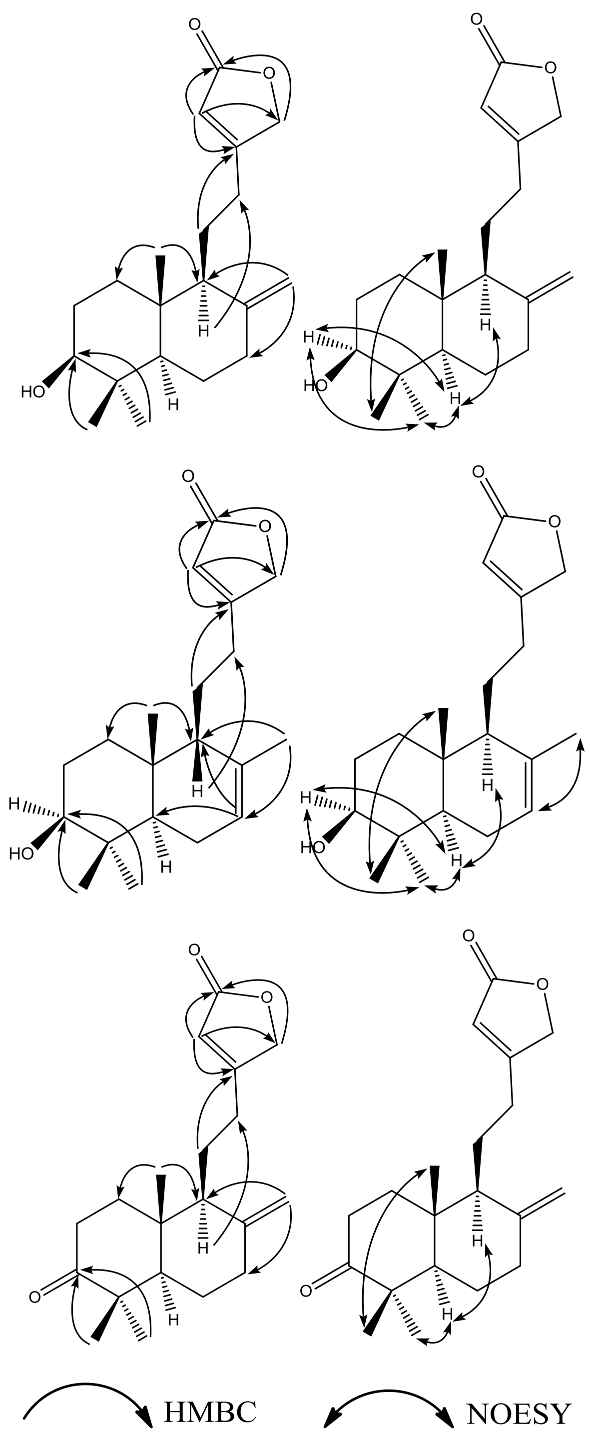


Figure S1. Key HMBC and NOESY correlations for Compounds **1** to **3**

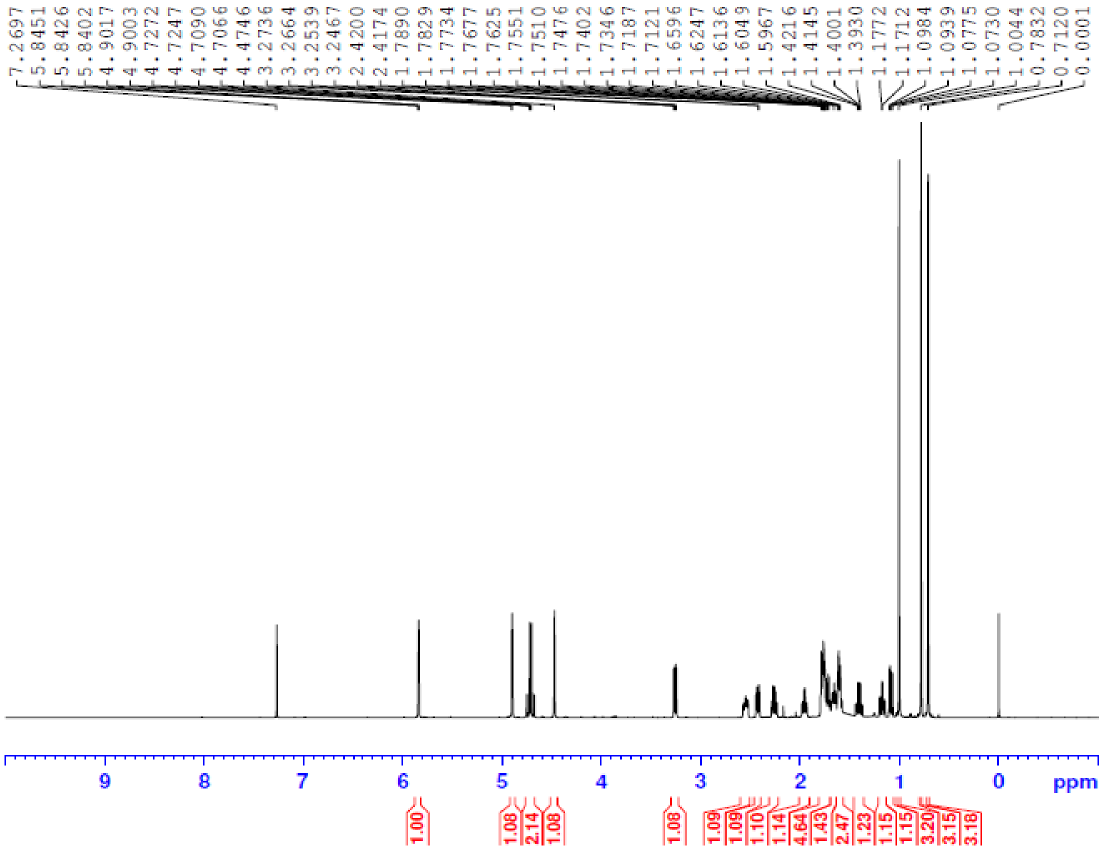


Figure S2. ^{1}H-NMR spectrum of **1** (600 MHz, $CDCl_3$)

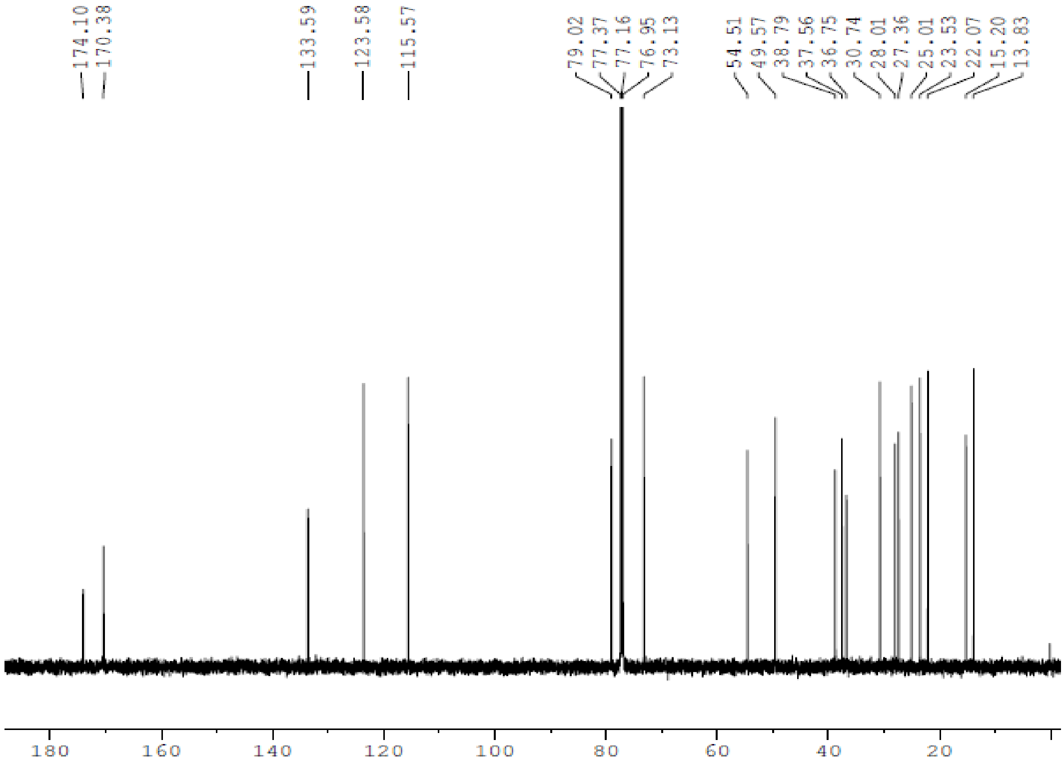


Figure S3. ^{13}C-NMR spectrum of **1** (150 MHz, $CDCl_3$)

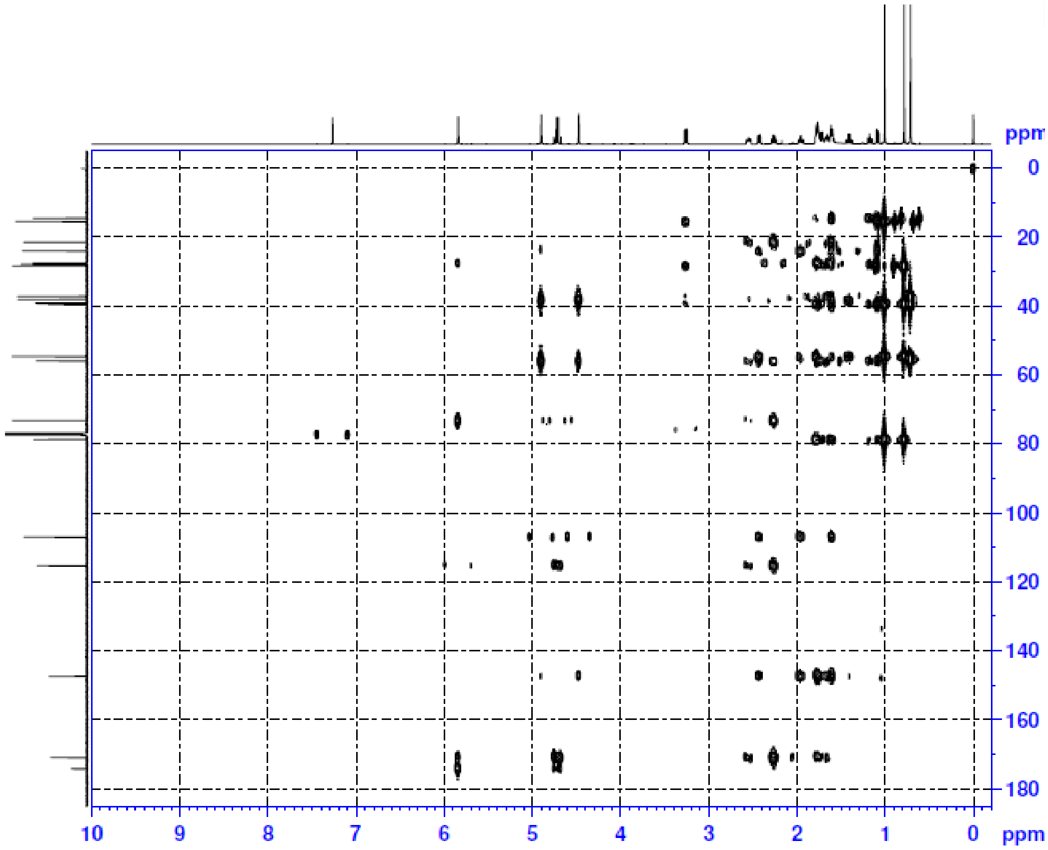


Figure S4. HMBC spectrum of **1** ($CDCl_3$)

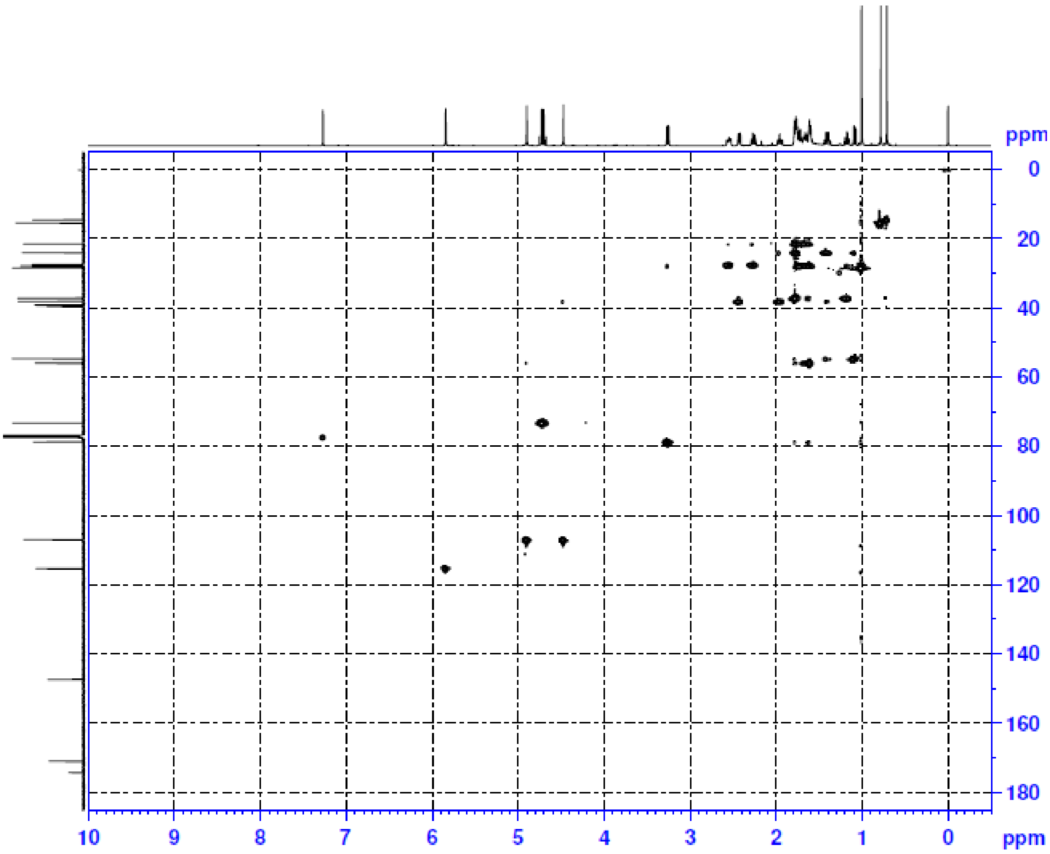


Figure S5. HSQC spectrum of **1** ($CDCl_3$)

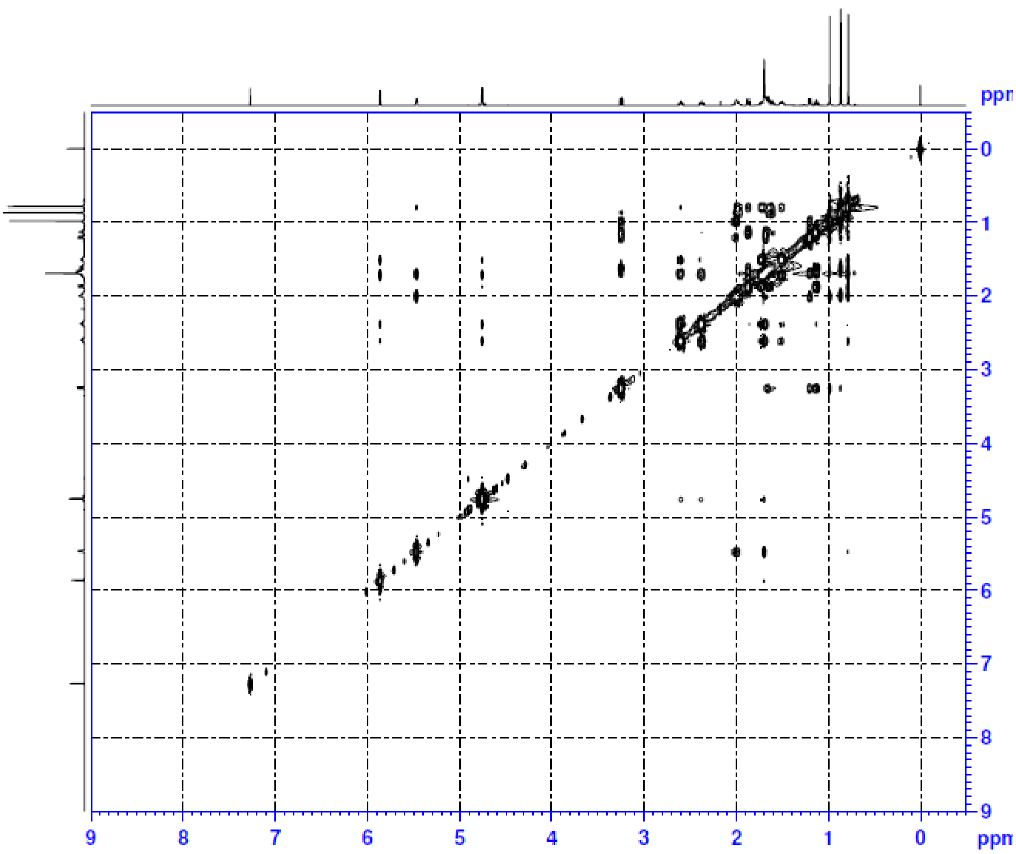


Figure S6. NOESY spectrum of **1** ($CDCl_3$)

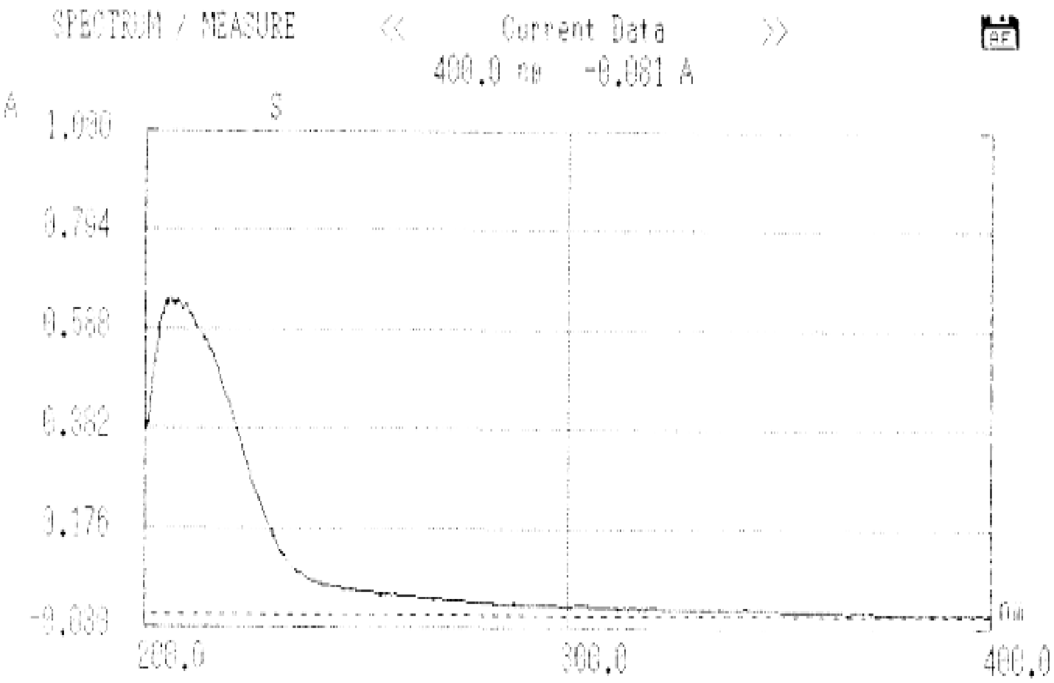


Figure S7. UV spectrum of **1**

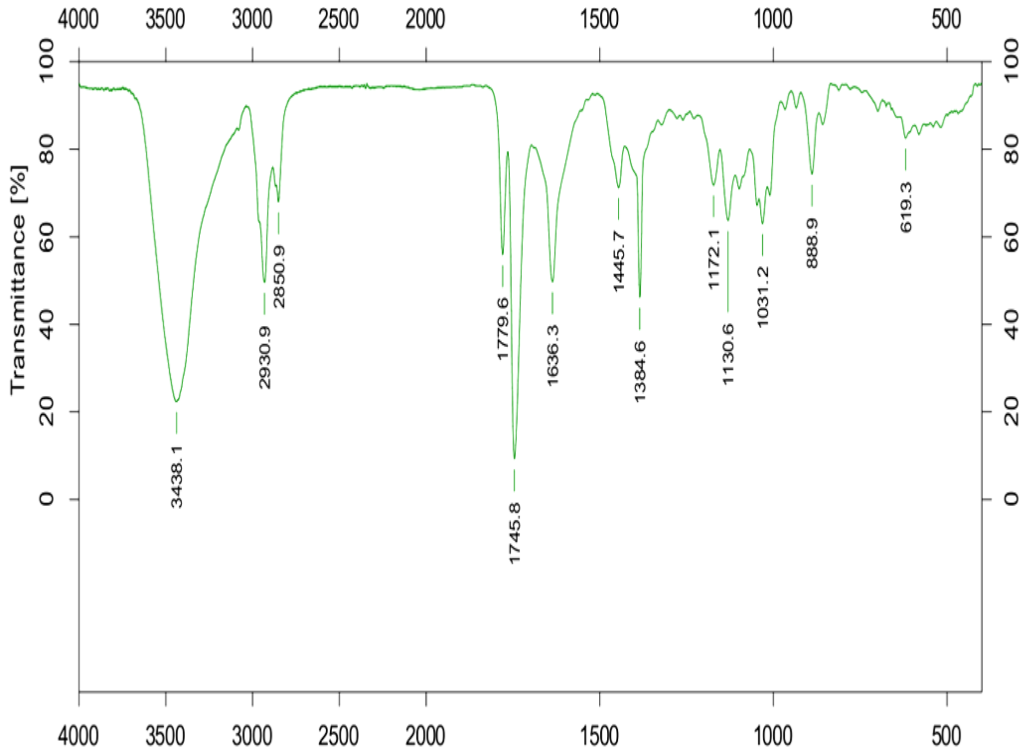
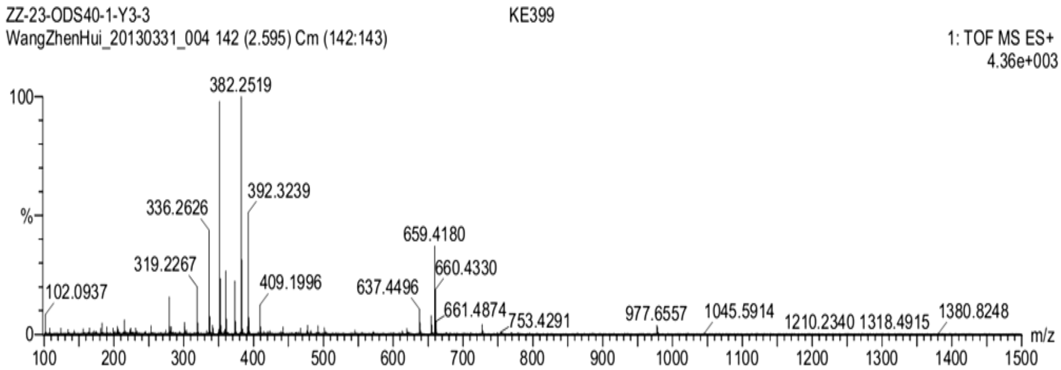


Figure S8. IR spectrum of **1**

Monoisotopic Mass, Even Electron Ions
62 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 O: 0-30



Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
319.2267	319.2273	-0.6	-1.9	5.5	0.9	C20 H31 O3
	319.2121	14.6	45.7	1.5	12.9	C16 H31 O6
	319.2426	-15.9	-49.8	9.5	8.8	C24 H31

Figure S9. HR-MS spectrum of **1**

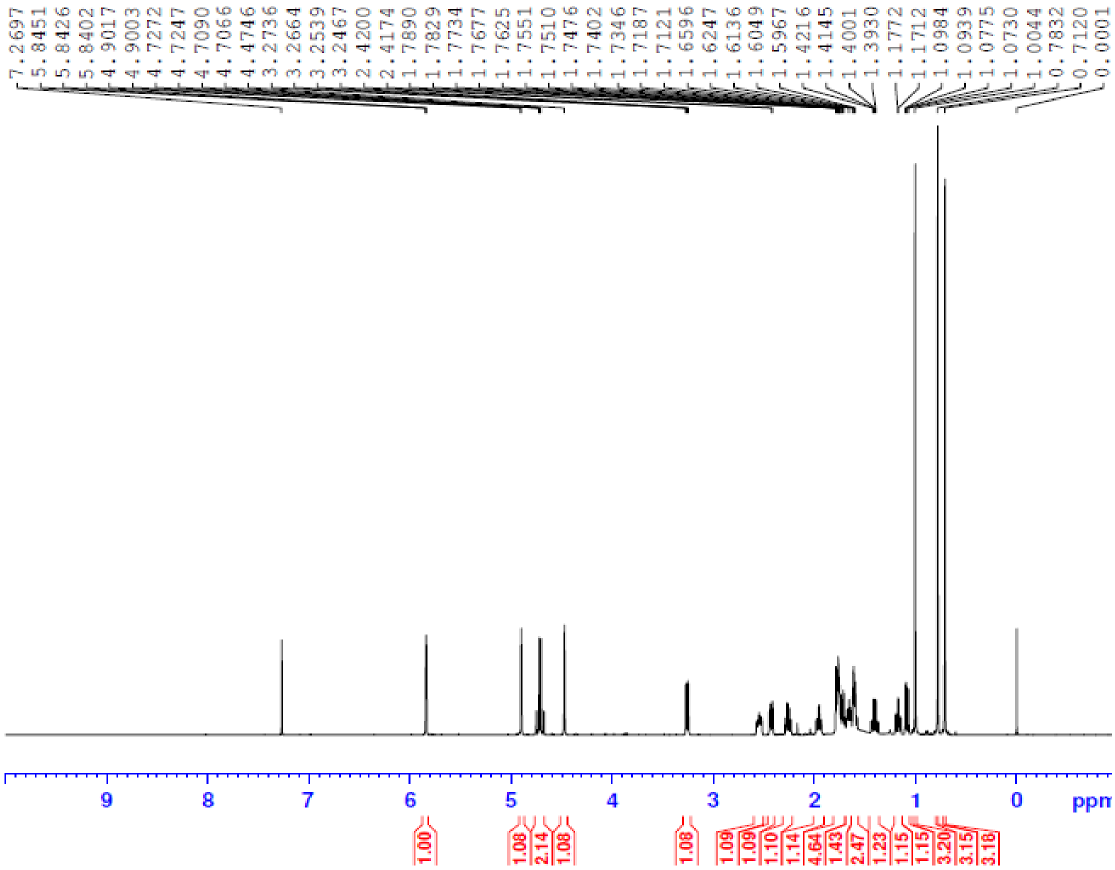


Figure S10. ^{1}H-NMR spectrum of **2** (600 MHz, $CDCl_3$)

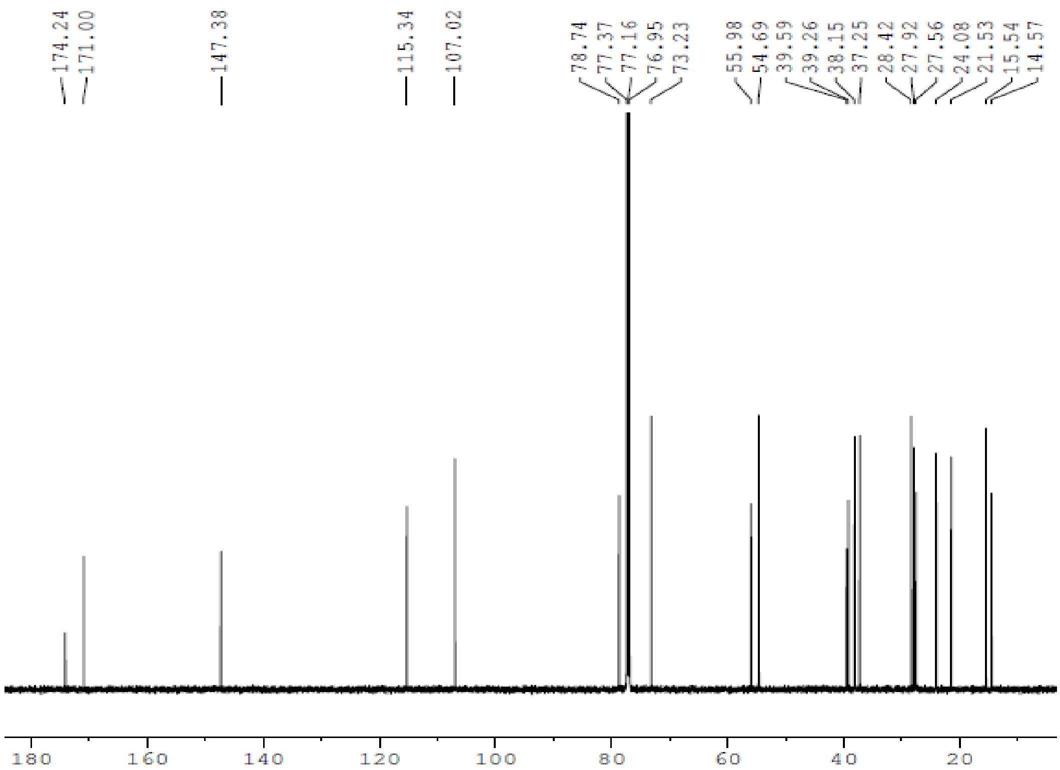


Figure S11. ^{13}C-NMR spectrum of **2** (150 MHz, $CDCl_3$)

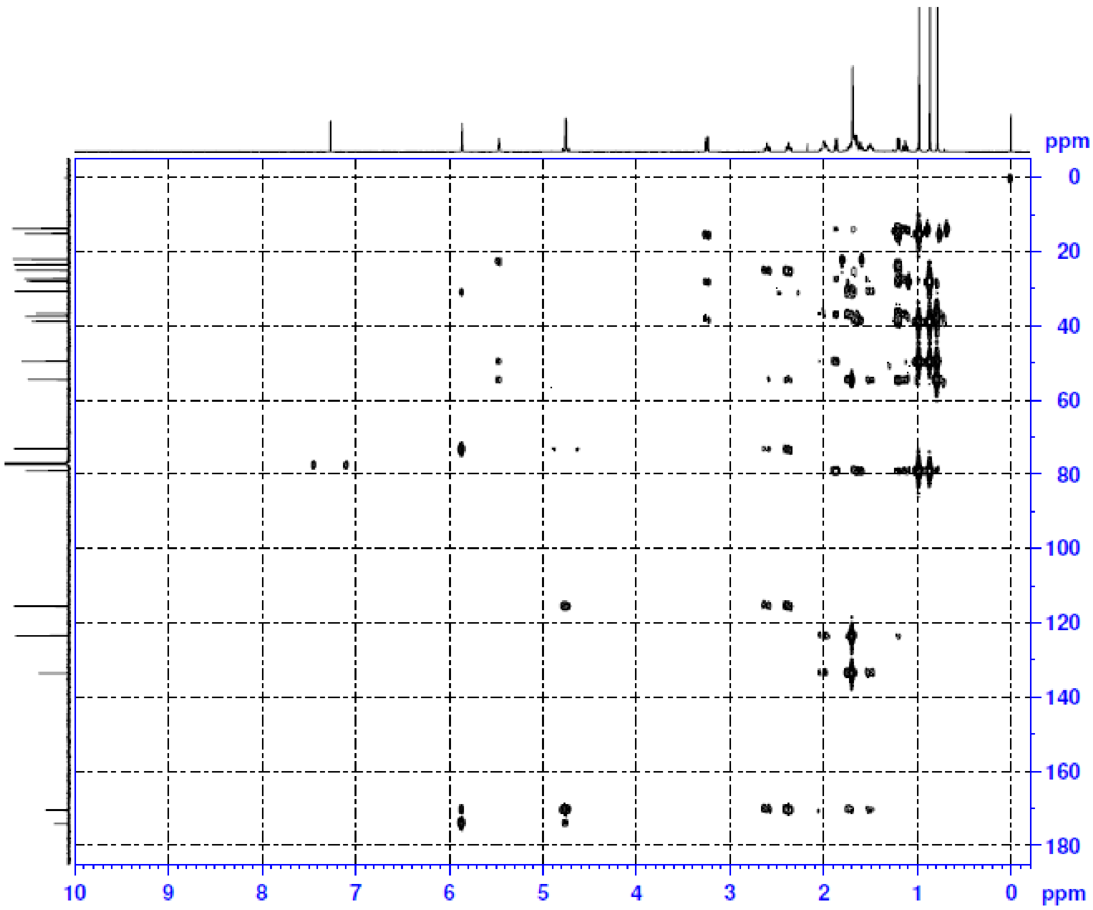


Figure S12. HMBC spectrum of **2** ($CDCl_3$)

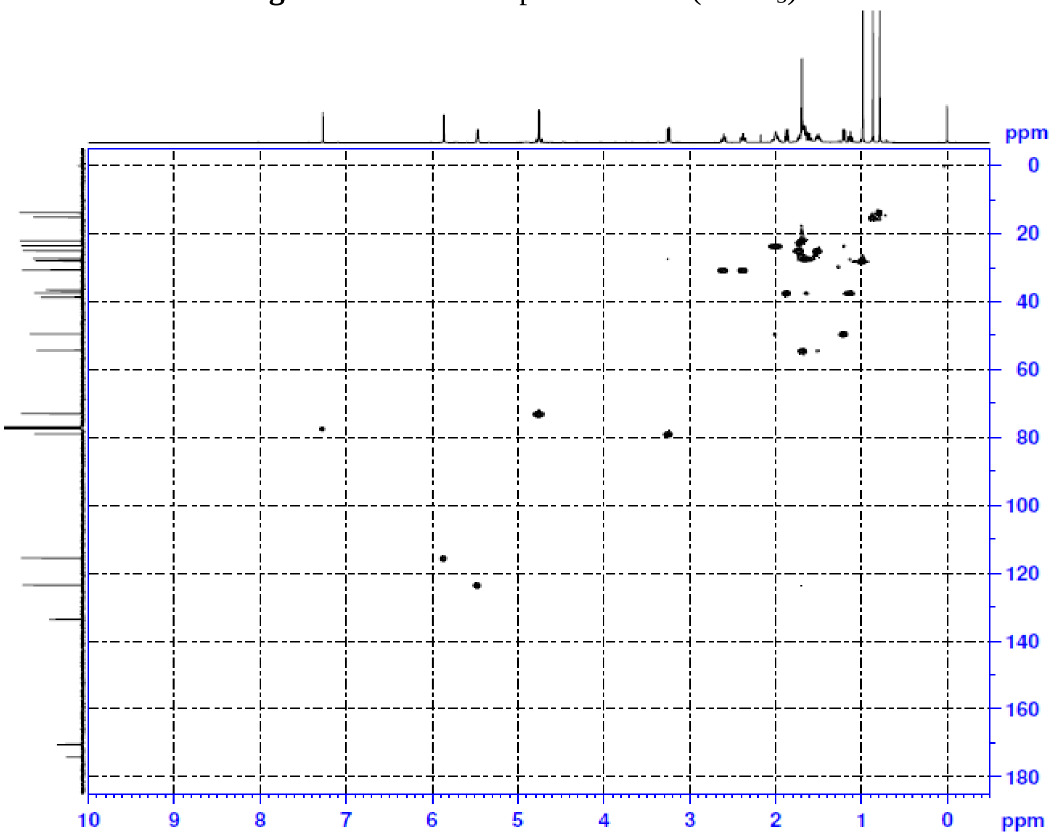


Figure S13. HSQC spectrum of **2** ($CDCl_3$)

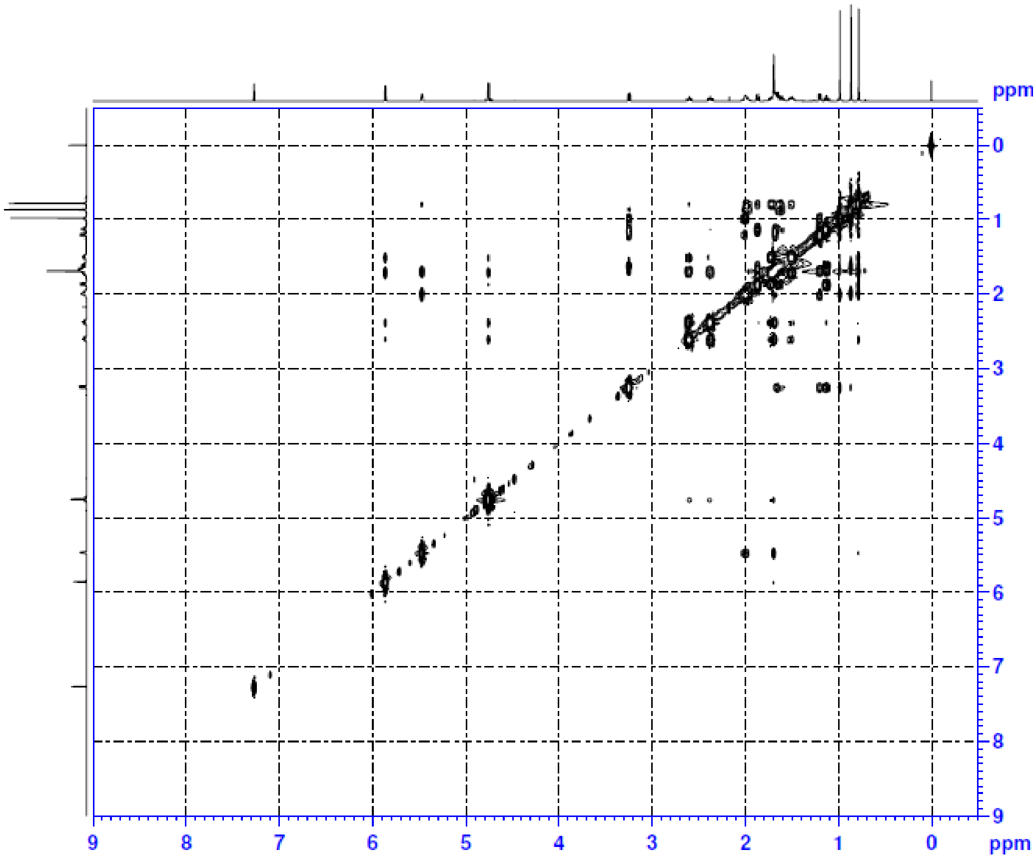


Figure S14. NOESY spectrum of **2** ($CDCl_3$)

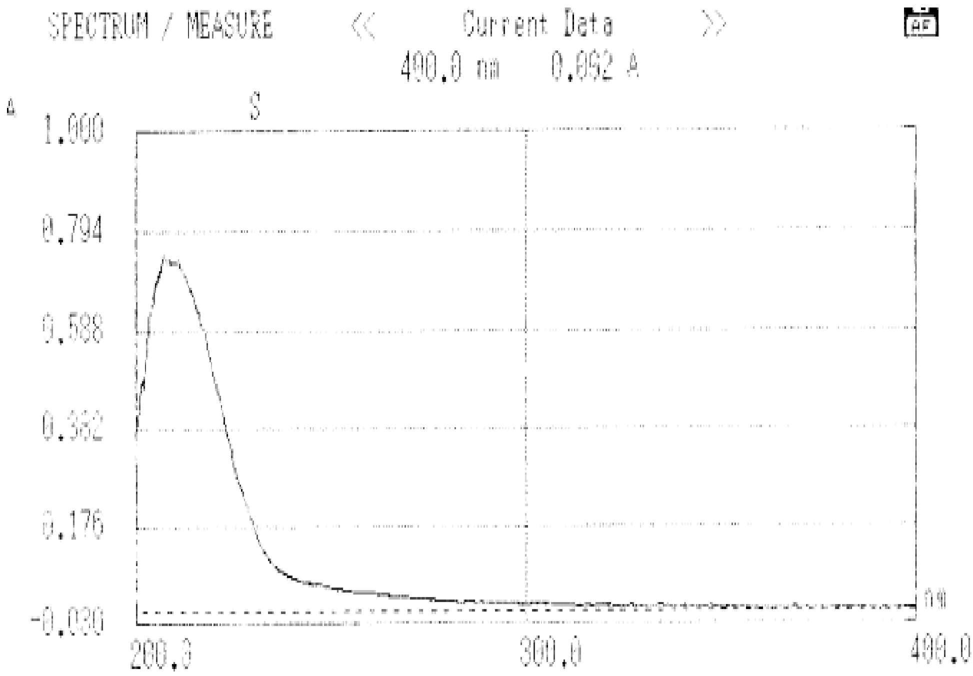


Figure S15. UV spectrum of **2**

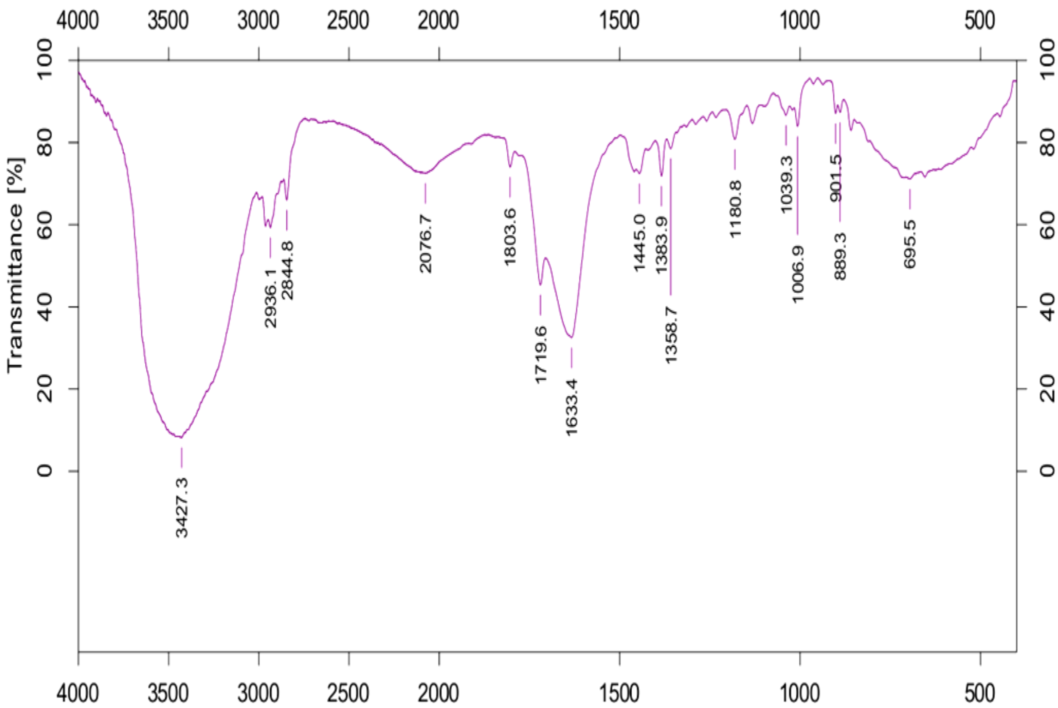
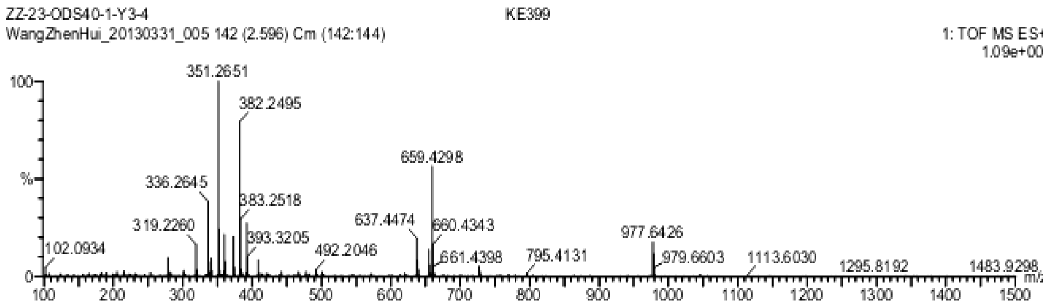


Figure S16. IR spectrum of **2**

Single Mass Analysis
Tolerance = 20.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
365 formula(e) evaluated with 12 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 O: 0-30 Na: 0-1



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
659.4298	659.4288	1.0	1.5	10.5	179.6	C40 H60 O6 Na
	659.4312	-1.4	-2.1	13.5	223.0	C42 H59 O6
	659.4253	4.5	6.8	22.5	387.3	C49 H55 O
	659.4346	-4.8	-7.3	1.5	65.7	C33 H64 O11 Na
	659.4229	6.9	10.5	19.5	336.2	C47 H56 O Na

Figure S17. HR-MS spectrum of **2**

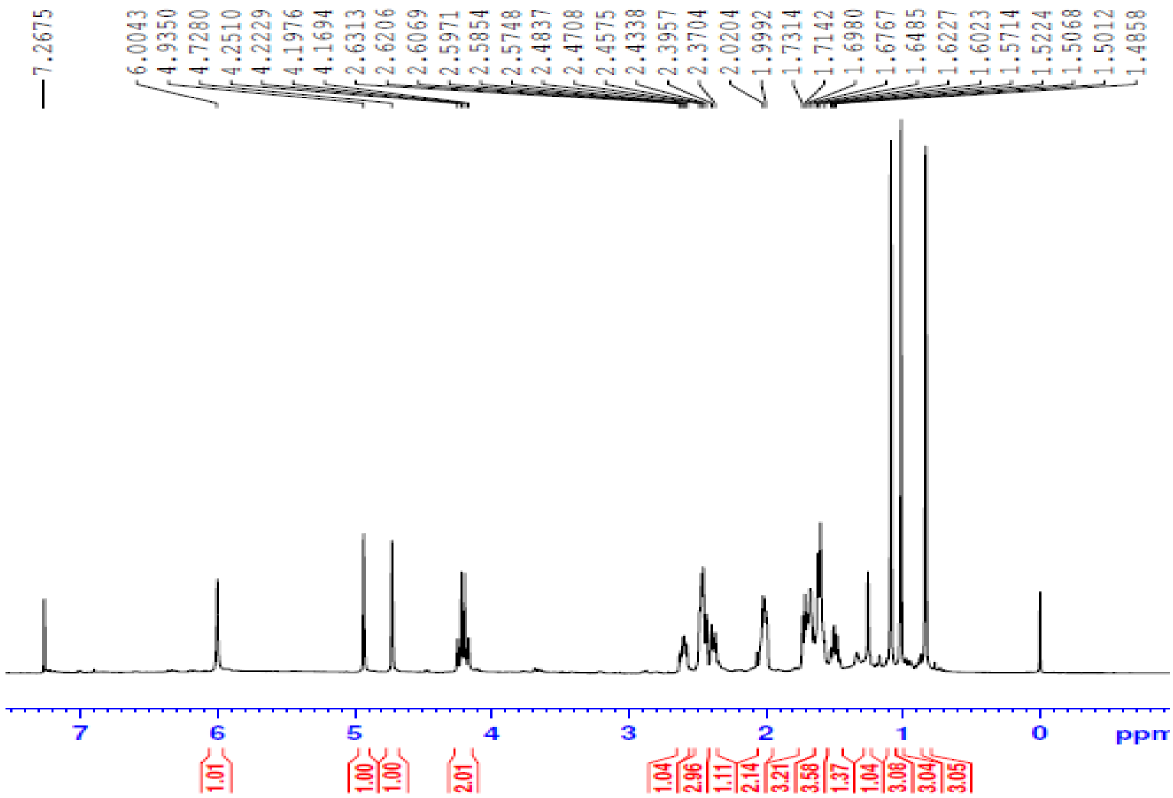


Figure S18. ^{1}H-NMR spectrum of **3** (600 MHz, $CDCl_3$)

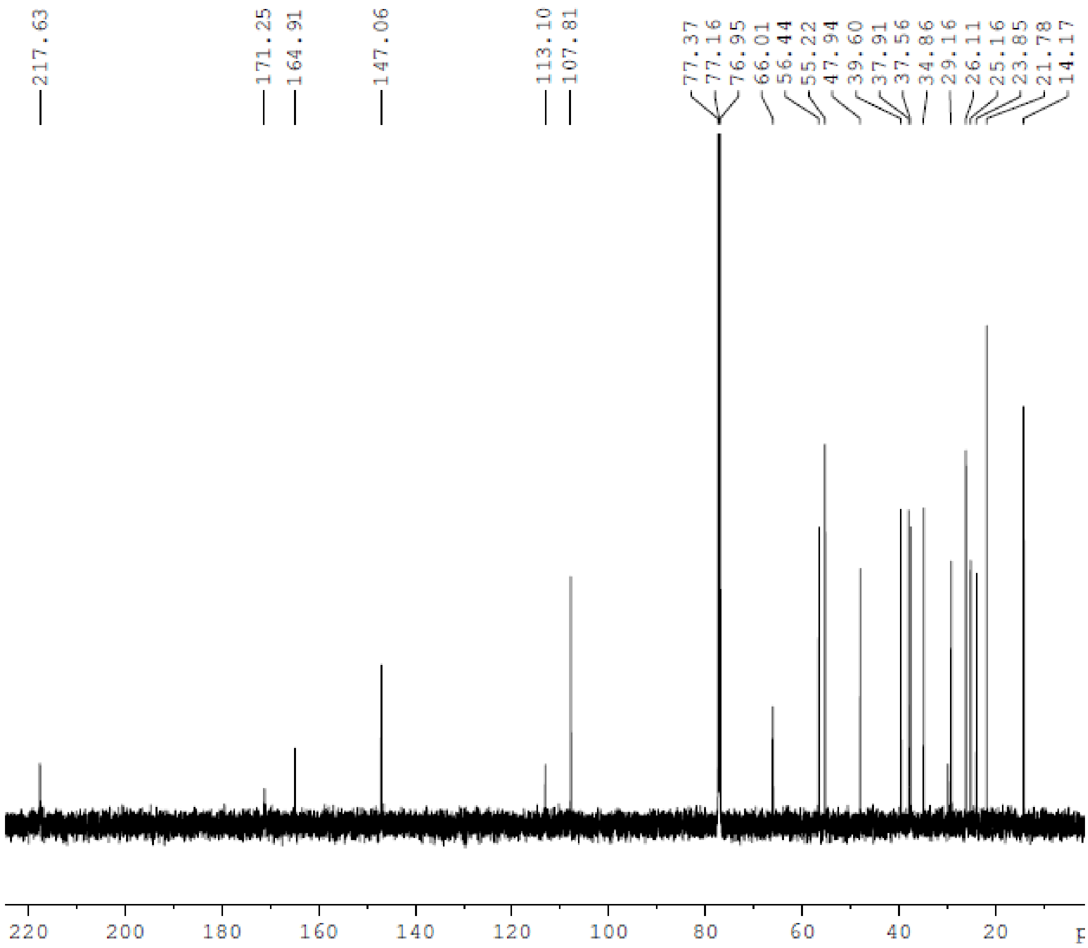


Figure S19. ^{13}C-NMR spectrum of **3** (150 MHz, $CDCl_3$)

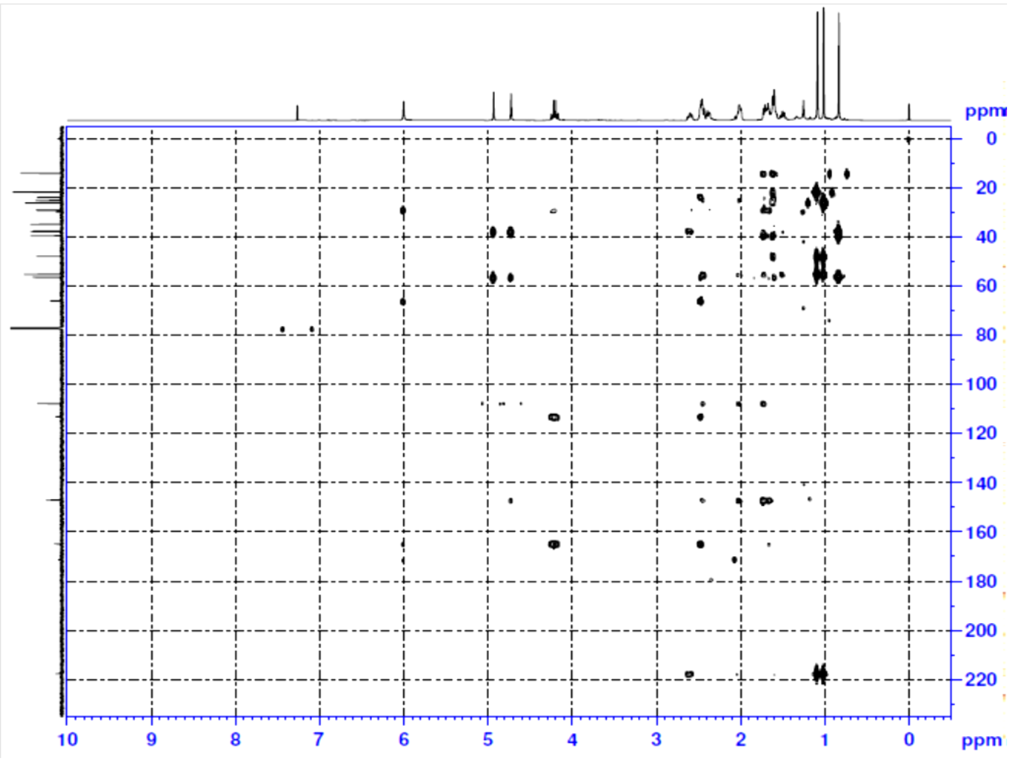


Figure S20. HMBC spectrum of **3** ($CDCl_3$)

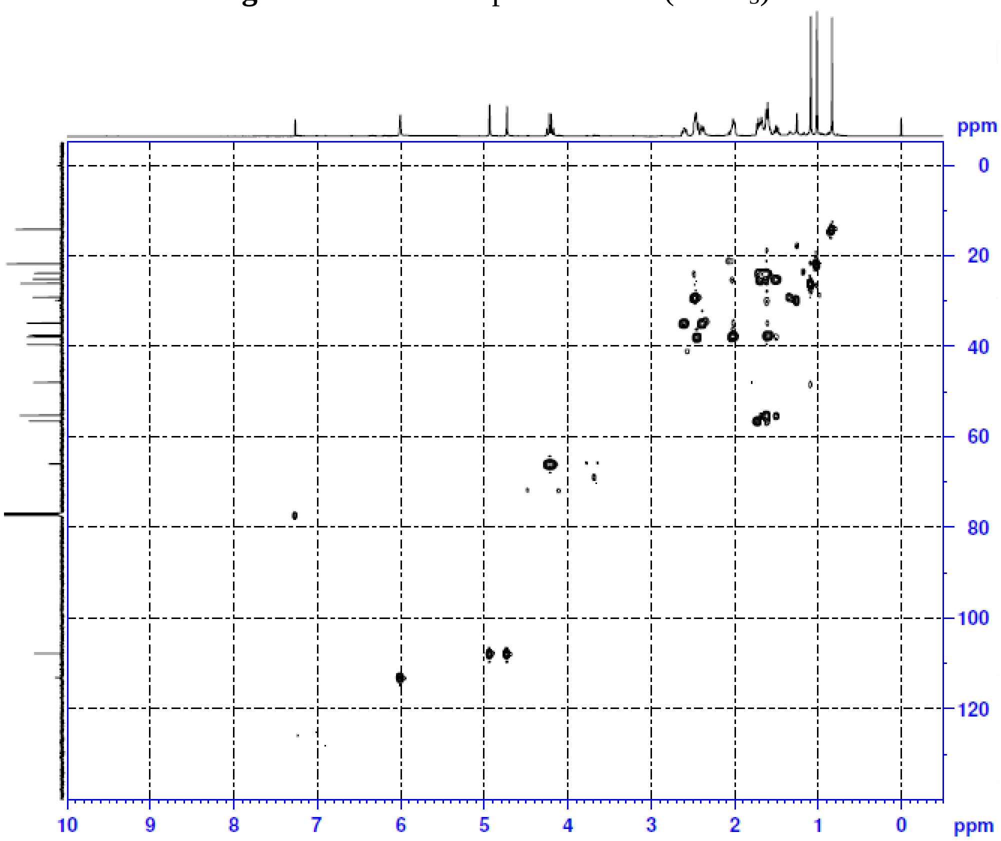


Figure S21. HSQC spectrum of **3** ($CDCl_3$)

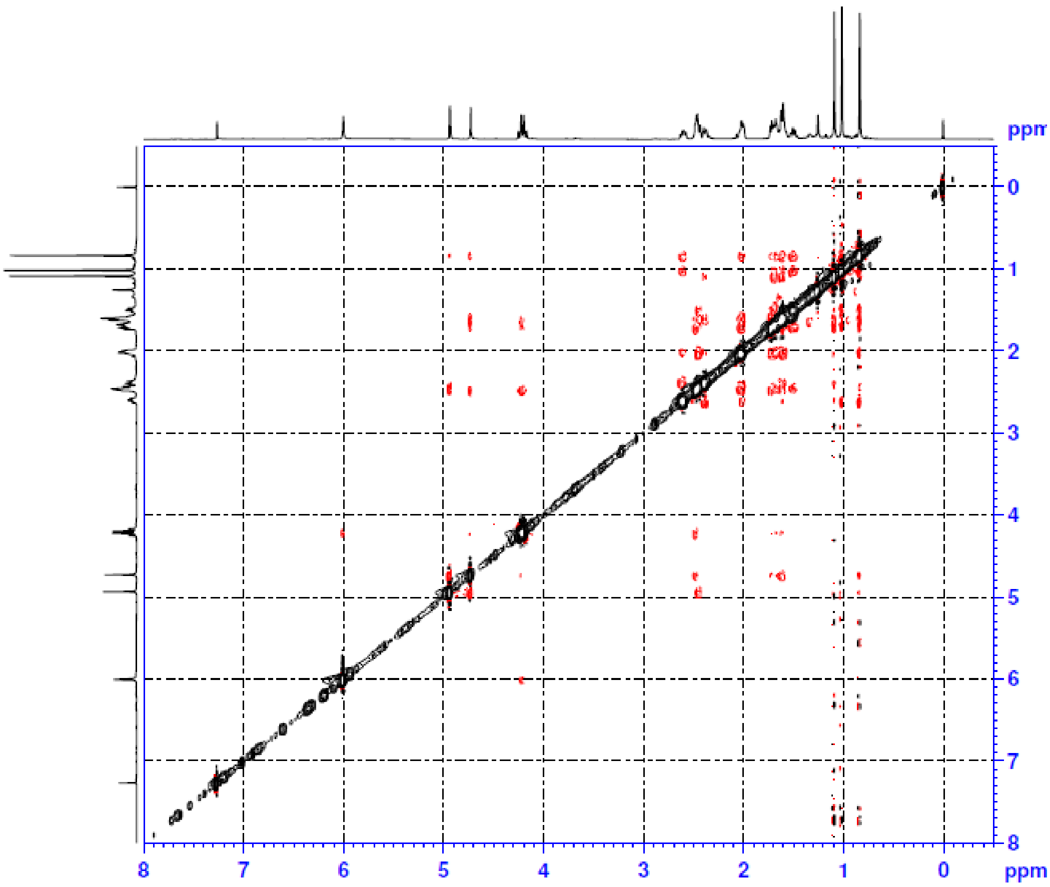


Figure S22. NOESY spectrum of **3** ($CDCl_3$)

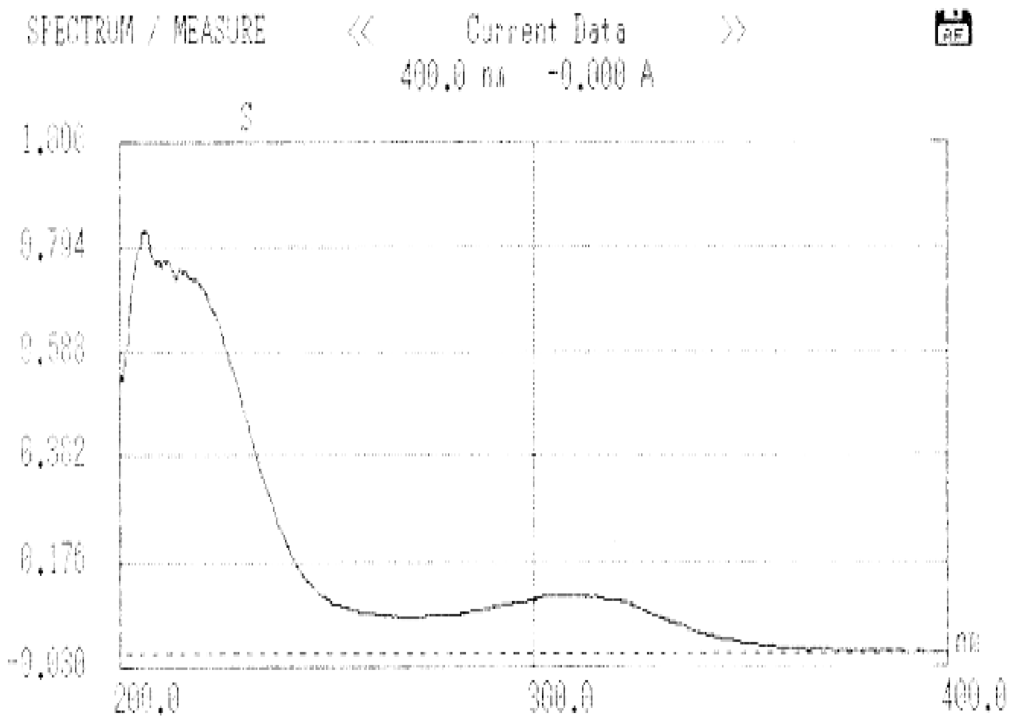


Figure S23. UV spectrum of **3**

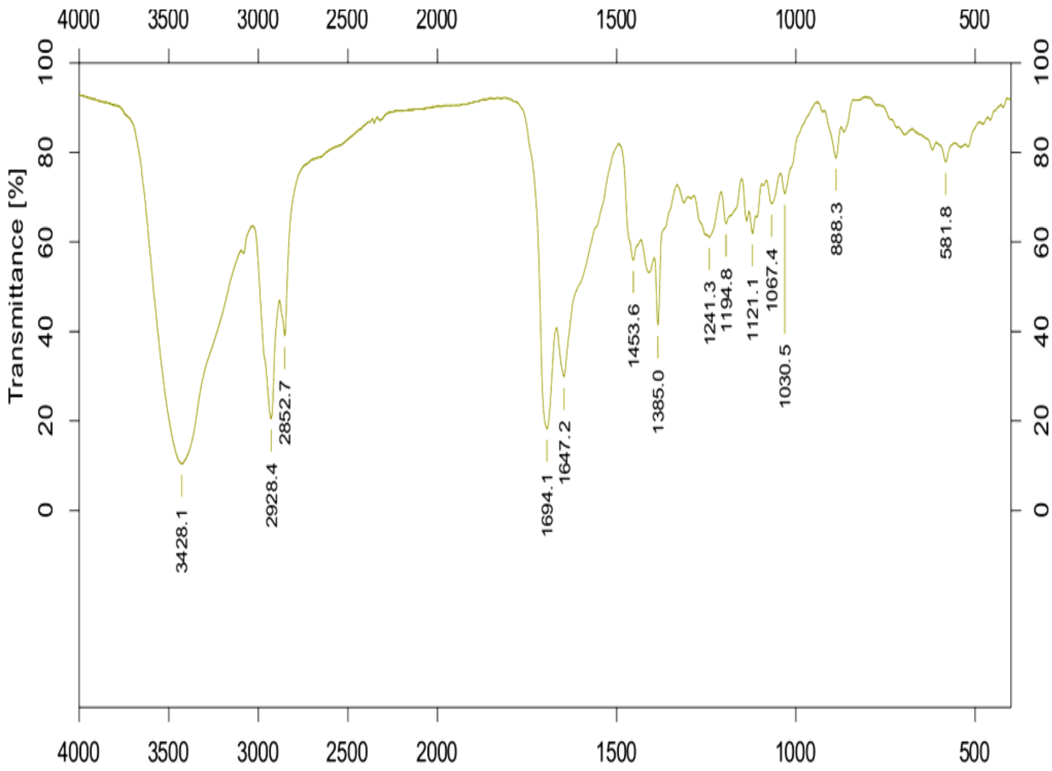


Figure S24. IR spectrum of **3**