

Supporting Information
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Cytotoxic phloroglucinol glucosides from microalgae
(*Spirulina platensis*)

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1. Materials and Methods

1.1. General Experimental Procedures

Optical rotations were recorded on a Polax-2L polarimeter in MeOH. High resolution mass data were obtained from Korea Basic Science Institute (KBSI, Seoul Center). NMR spectra were performed on Bruker 500 MHz and 600 MHz spectrometers. Melting points were measured in open capillaries on a Buchi melting point B-545 apparatus (Buchi Instrument, Switzerland). TLC silica gel Merck 60 F254 was used as thin layer chromatography. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), RP-18 resins (30–50 μm , Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Sigma-Aldrich, USA). For thin-layer chromatography (TLC), pre-coated silica-gel 60 F254 (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F254S (0.25 mm, Darmstadt, Merck, Germany) plates were used.

1.2. Strain Isolation and Culture Conditions

The *Spirulina platensis* Art.NA12 strain was isolated from aquaculture ponds in Nghe An Province, Vietnam (19.186797°N, 105.707979°E) in November 2024. Individual filaments were separated under an inverted microscope (Olympus CK40) using a sterilized Pasteur pipette with a flame-narrowed, smooth tip. Each filament was rinsed several times in drops of sterile Zarrouk medium to remove contaminating cells and particles and then transferred onto sterile Zarrouk agar (1.5% w/w) plates. Zarrouk's culture medium contained (per liter of distilled water): NaCl: 1.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.2 g, NaHCO_3 : 16.8 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 0.04 g, K_2HPO_4 : 0.5 g, NaNO_3 : 2.5 g, K_2SO_4 : 1.0 g; Na_2EDTA : 0.08 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.01 g, 1ml trace elements solution containing: H_3BO_3 : 2.86 mg, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$: 1.81 mg, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.222 mg, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$: 0.39 mg, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.079 mg, 1 ml B6 solution containing: NH_4VO_3 : 22.9 mg, $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$: 47.8 mg, NaWO_2 : 17.9 mg, $\text{Ti}_2(\text{SO}_4)_3 \cdot 6\text{H}_2\text{O}$, and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$: 4.4mg [1]. The isolated strain was subsequently maintained under LED illumination (2000–2500 lux) at $25 \pm 3^\circ\text{C}$ with a 12:12 h light–dark photoperiod for three weeks. Subsequently, individual filamentous colonies were then transferred into 50 mL of liquid Zarrouk medium for further cultivation.

The morphology of the *S. platensis* Art.NA12 strain was examined using a light microscope with an attached, charge-coupled device camera (Nikon Eclipse 55i). The *P. raciborskii* strain was identified according to their morphological characteristics with standard manual taxonomic references for Cyanobacteria [2].

The isolated *S. platensis* Art.NA12 strain was cultivated in 5 L flasks (20 culture flasks), each containing Zarrouk medium, for a period of 10 days. The cultures were maintained under controlled laboratory conditions with continuous aeration using a sterile air pump and illuminated with LED

light at an intensity of approximately 2000 lux under a 12:12 h light–dark photoperiod. The initial medium pH was adjusted to 9.5 using sterile 0.1 M NaOH. Throughout the cultivation period, pH was actively monitored and regulated as continuous aeration and photosynthetic activity gradually increased the medium pH by adding acid (0.1 M HCl). The temperature was maintained within the range of 25–28 °C throughout the cultivation period. The grow algal culture was then transferred to 200 L photobioreactor (4 culture units) with an inoculum ratio of 10–15% (v/v). Cultivation was carried out under continuous aeration and LED illumination at an intensity of 2000 lux for 18 days. The pH in the photobioreactor was maintained within 9.0–10.0 using the same pH-control approach, and the temperature was maintained within the range of 25–30 °C. The biomass of the *S. platensis* Art.NA12 was collected by centrifugation at 10,000× g for 10 min.

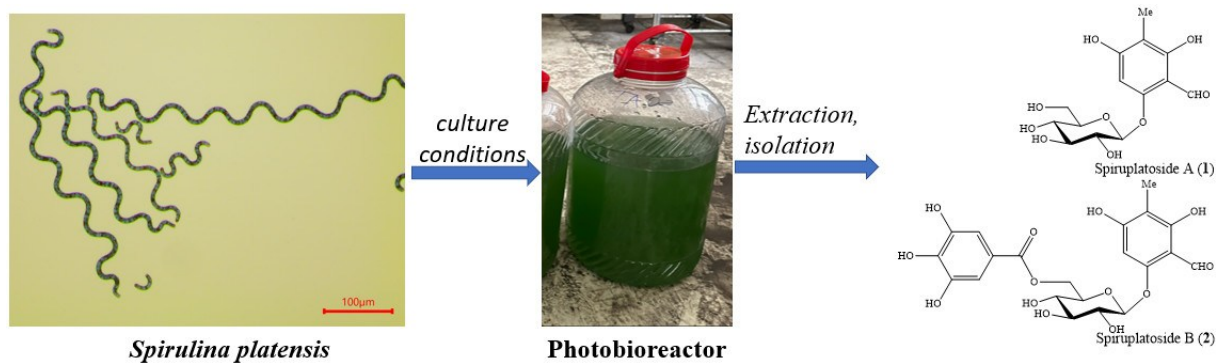


Table S1. NMR data of compounds **3-5**

C	3		4		5	
	$\delta_C^{a,c}$	$\delta_H^{a,d}$ (mult., J = Hz)	$\delta_C^{b,c}$	$\delta_H^{b,d}$ (mult., J = Hz)	$\delta_C^{a,c}$	$\delta_H^{a,d}$ (mult., J = Hz)
2	163.3	-	162.0	-	163.5	-
3	108.9	6.71 (s)	105.7	6.57 (s)	107.3	6.62 (s)
4	180.0	-	178.9	-	180.1	-
5	162.1	-	-	-	162.1	-
6	94.3	6.80 (d, 1.8)	92.3	6.66 (d, 1.8)	94.2	6.54 (d, 1.8)
7	166.6	-	164.8	-	166.5	-
8	96.5	6.54 (d, 1.8)	96.3	6.44 (d, 1.8)	97.4	6.79 (d, 1.8)
9	161.5	-	160.6	-	161.4	-
10	109.5	-	108.0	-	109.3	-
1'	132.4	-	121.9	-	124.5	-
2'	127.2	8.00 (dd, 1.8, 7.8)	128.0	7.80 (d, 8.4)	129.0	7.94 (d, 9.0)
3'	130.2	7.56*	115.9	6.94 (d, 9.0)	115.6	7.10 (d, 9.0)
4'	132.8	7.58*	160.0	-	162.1	-
5'	129.1	7.56*	115.9	7.80 (d, 8.4)	115.6	7.10 (d, 9.0)
6'	125.9	8.00 (dd, 1.8, 7.8)	128.0	6.94 (d, 9.0)	129.0	7.94 (d, 9.0)
5-OMe	56.6	3.93 (s)	-	-	56.6	3.93 (s)
7-OMe	56.6	3.96 (s)	55.7	3.93 (s)	56.6	3.96 (s)
4'-OMe	-	-	55.6	3.93 (s)	56.0	3.94 (s)

^{a)}recorded in CD_3OD , ^{b)}recorded in $CDCl_3+CD_3OD$, ^{c)}150 MHz, ^{d)}600 MHz, *overlapped signals.

Table S2. NMR data of compounds **6** and **7**

C	6		7	
	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (mult., $J = \text{Hz}$)	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (mult., $J = \text{Hz}$)
1	133.9	-	134.2	-
2	129.5	8.01 (s)	129.6	8.05 (m)
3	129.5	8.01 (s)	129.7	8.05 (m)
4	133.9	-	133.7	-
5	129.5	8.01 (s)	129.7	8.05 (m)
6	129.5	8.01 (s)	129.6	8.05 (m)
1-COO	166.2	-	166.2	-
4-COO	166.2	-	166.1	-
1-COOMe	52.4	3.86 (s)	52.5	3.88 (s)
4-COOMe	52.4	3.86 (s)		
1'			67.0	4.42 (t, 4.8)
2'			61.3	3.91 (t, 4.8)

^{a)}recorded in CDCl_3 , ^{b)}150 MHz, ^{c)}600 MHz

Table S3. NMR data of compound **8**

C	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (mult., $J = \text{Hz}$)
1	177.0	-
2	35.1	2.29 (t, 7.2)
3	28.6	1.32 (m)
4	30.3	1.55 (m)
5	30.2	1.41 (m)
6	30.4	1.45 (m)
7	30.5	1.48 (m)
8	32.5	2.21 (q, 7.8))
9	129.3	6.00 (t, 4.8)
10	132.9	5.43 (dt, 4.2, 11.4)
11	126.5	6.51 (dd, 11.4, 15.0)
12	137.3	5.64 (dd, 6.6, 15.0)
13	73.3	4.10 (q, 6.6)
14	38.4	1.56 (m)
15	26.5	1.41 (m)
16	26.1	1.41 (m)
17	23.6	1.32 (m)
18	14.4	0.93 (t, 6.6)

^{a)}recorded in CD_3OD , ^{b)}150 MHz, ^{c)}600 MHz

Figure S1. Graphical procedure

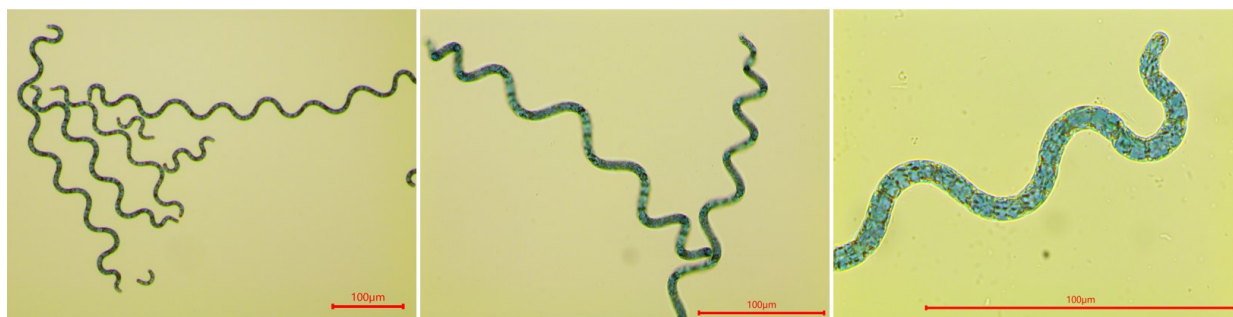


Figure S2. Morphology of *S. platensis* Art.NA12 under the microscope at 100 μ m scale

Structure Match

As Drawn (0)

Substructure (1)

Similarity (96K)

Analyze Structure Precision

Behavior

Filter by Exclude

Search Within Results

Reference Role

Biological Study (1)

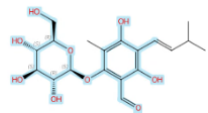
Food or Feed Use (1)

Filtering: Stereochemistry: 2 Selected X

1 Result

1

915773-70-5



Available stereochemistry shown
Double bond geometry unknown

$C_{19}H_{26}O_9$
2-(β-D-Glucopyranosyloxy)-4,6-dihydroxy-3-methyl-5-(3-methyl-1-buten-1-yl)benzal...

1 0 0

exact match (0), Substructure (1)

Search Within Results

Similarity

☒ ≥ 99 (1)

☒ 95-98 (6)

☐ 90-94 (82)

☐ 85-89 (315)

☐ 80-84 (915)

View All

Reaction Role

☐ Product (1)

Reference Role

☐ Preparation (6)

☐ Biological Study (4)

☐ Biological Study, Unclassified (4)

☐ Properties (4)

☐ Purification or Recovery (4)

View All

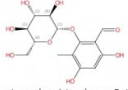
5e Data

New

Commercial Availability

1 100 ...

1207732-16-8



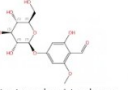
Absolute stereochemistry shown, Rotation (+)

$C_{14}H_{18}O_9$
2-(β-D-Glucopyranosyloxy)-4,6-dihydroxy-3-methylbenzaldehyde

4 0 1

2 96 ...

910884-00-3



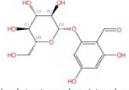
Absolute stereochemistry shown

$C_{14}H_{18}O_9$
Phloroglucinaldehyde, 4-β-glucosidyl-2-O-methyl-

1 0 0

3 96 ...

910884-05-8



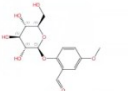
Absolute stereochemistry shown

$C_{13}H_{16}O_9$
Phloroglucinaldehyde, O²-β-glucosidyl-

3 0 0

4 95 ...

38492-94-3



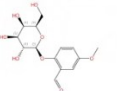
Absolute stereochemistry shown

$C_{14}H_{18}O_8$
2-(β-D-Glucopyranosyloxy)-5-methoxybenzaldehyde

1 1 0

5 95 ...

476611-19-5



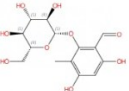
Absolute stereochemistry shown

$C_{14}H_{18}O_8$
2-(β-D-Galactopyranosyloxy)-5-methoxybenzaldehyde

0 0 0

6 100 ...

1207732-16-8



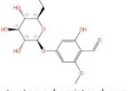
Absolute stereochemistry shown, Rotation (+)

$C_{14}H_{18}O_9$
2-(β-D-Glucopyranosyloxy)-4,6-dihydroxy-3-methylbenzaldehyde

4 0 1

7 96 ...

910884-00-3



Absolute stereochemistry shown

$C_{14}H_{18}O_9$
Phloroglucinaldehyde, 4-β-glucosidyl-2-O-methyl-

1 0 0

Activate Windows
Go to Settings to activate Windows

Figure S3. Illustrate for searching of new compound 1 from SciFinder

Structure Match

As Drawn (0)

Substructure (1)

Similarity (308K)

Analyze Structure Precision

Behavior

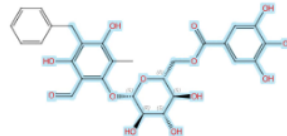
Filter by Exclude

Search Within Results

1 Result

1

1402061-94-2



Absolute stereochemistry shown

$C_{28}H_{28}O_{13}$
2,4-Dihydroxy-5-methyl-3-(phenylmethyl)-6-[[6-O-(3,4,5-trihydroxybenzoyl)-β-D-gl...

2 0 0

exact match (0), Substructure (1)

Search Within Results

Similarity

☒ 95-98 (10)

☐ 90-94 (210)

☐ 85-89 (807)

☐ 80-84 (2,758)

☐ 75-79 (9,788)

View All

Reaction Role

☐ Product (1)

Reference Role

☐ Biological Study (9)

☐ Biological Study, Unclassified (6)

☐ Preparation (6)

☐ Analyte (5)

☐ Analytical Study (5)

View All

Commercial Availability

☒ New **5** **ce Data**

Search Within Results

Similarity

☒ 95-98 (10)

☐ 90-94 (210)

☐ 85-89 (807)

☐ 80-84 (2,758)

☐ 75-79 (9,788)

View All

Reaction Role

☐ Product (1)

Reference Role

☐ Biological Study (9)

☐ Biological Study, Unclassified (6)

☐ Preparation (6)

☐ Analyte (5)

☐ Analytical Study (5)

View All

Commercial Availability

☒ New **5** **ce Data**

1 97 ...

115355-99-2



Absolute stereochemistry shown

$C_{20}H_{20}O_{13}$
3,4-Dihydroxy-5-[[6-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranosyl]oxy]benzaldehyde...

5 0 1

2 97 ...

67771-96-4



Absolute stereochemistry shown

$C_{20}H_{20}O_{13}$
3,5-Dihydroxy-4-[[6-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranosyl]oxy]benzaldehyde...

4 0 1

3 96 ...

1093121-66-4



Absolute stereochemistry shown, Rotation (-)

$C_{20}H_{22}O_{12}$
β-D-Glucopyranoside, 2,4-dihydroxy-6-methylphenyl, 6-(3,4,5-trihydroxybenzoate)

2 0 0

4 96 ...

67771-98-6



Absolute stereochemistry shown

$C_{20}H_{22}O_{13}$
β-D-Glucopyranoside, 2,6-dihydroxy-4-(hydroxymethyl)phenyl, 6-(3,4,5-trihydroxybenzoate)...

2 1 0

5 96 ...

3056020-13-1



Absolute stereochemistry shown

$C_{21}H_{24}O_{11}$

1 0 0

6 96 ...

1415220-21-1



Absolute stereochemistry shown, Rotation (-)

$C_{23}H_{28}O_{11}$
β-D-Glucopyranoside, 4-hydroxy-2,5-dimethylphenyl, 6-(4-hydroxy-3,5-dimethoxybenzoate)...

1 0 0

7 96 ...

1415220-22-2



Absolute stereochemistry shown, Rotation (-)

$C_{22}H_{26}O_{10}$
β-D-Glucopyranoside, 4-hydroxy-2,5-dimethylphenyl, 6-(4-hydroxy-3-methoxybenzoate)...

1 0 0

8 95 ...

203734-36-5



Absolute stereochemistry shown, Rotation (-)

$C_{21}H_{22}O_{13}$
Myricaphenone B

19 0 2

9 95 ...

3034999-20-4



Absolute stereochemistry shown

$C_{21}H_{22}O_{12}$

1 0 0

10 95 ...

756890-20-7



Absolute stereochemistry shown

$C_{21}H_{22}O_{13}$
1-[2,4-Dihydroxy-6-[[6-O-(3,4,5-trihydroxybenzoyl)hexopyranosyl]oxy]phenyl]ethanone...

1 0 1

Activate Windows
Go to Settings to activate Windows

Figure S4. Illustrate for searching of new compound 2 from SciFinder.

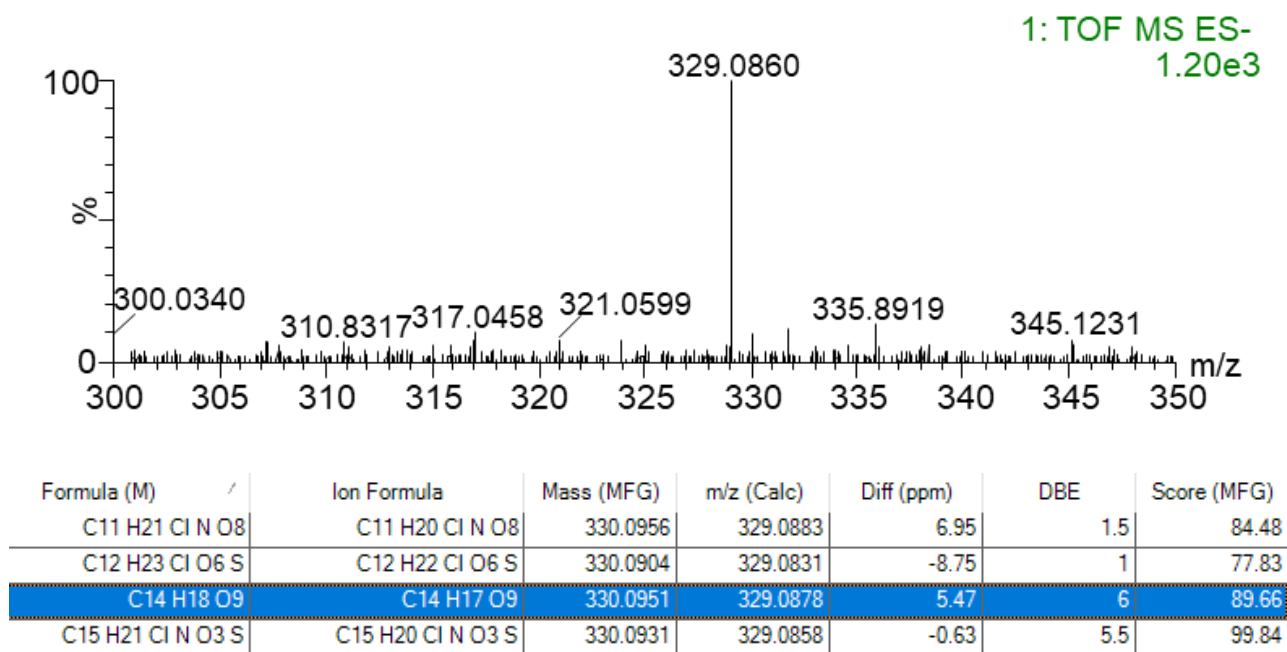


Figure S5. HR-ESI-MS spectrum of compound **1**

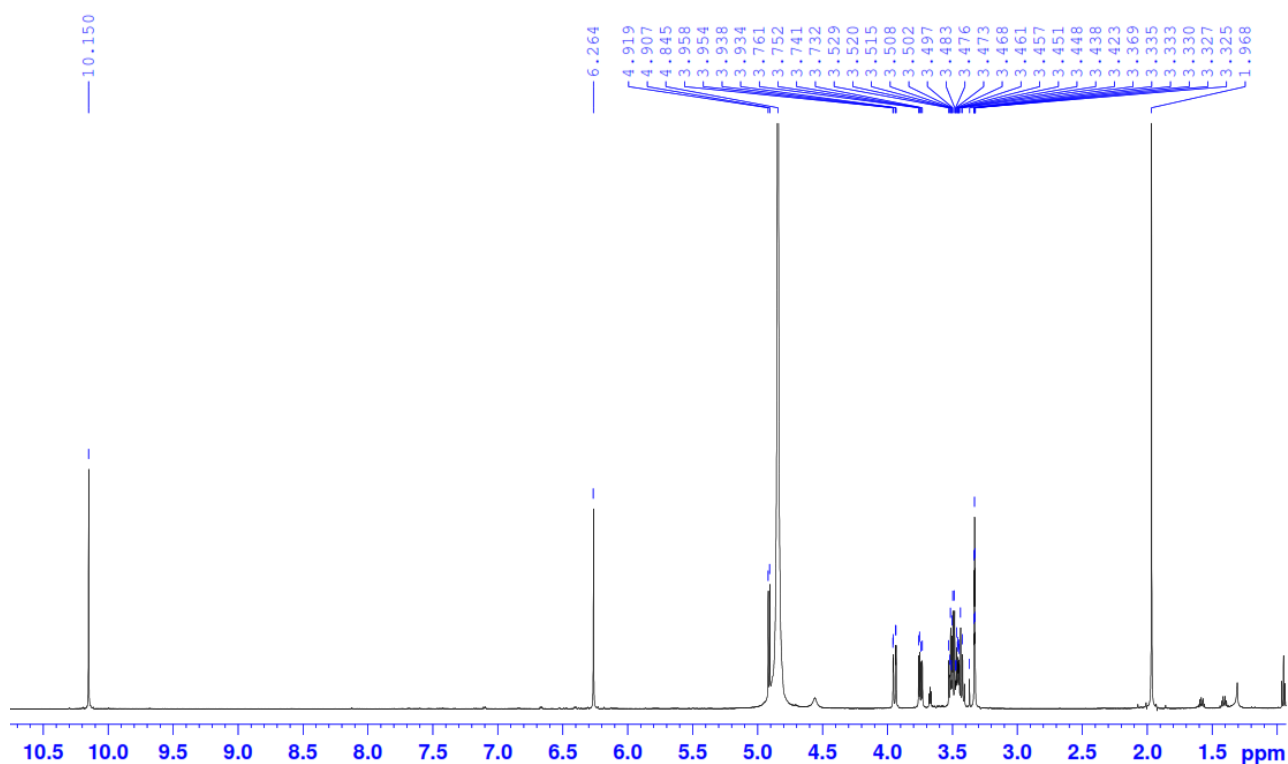


Figure S6. ^1H -NMR spectrum of compound **1**

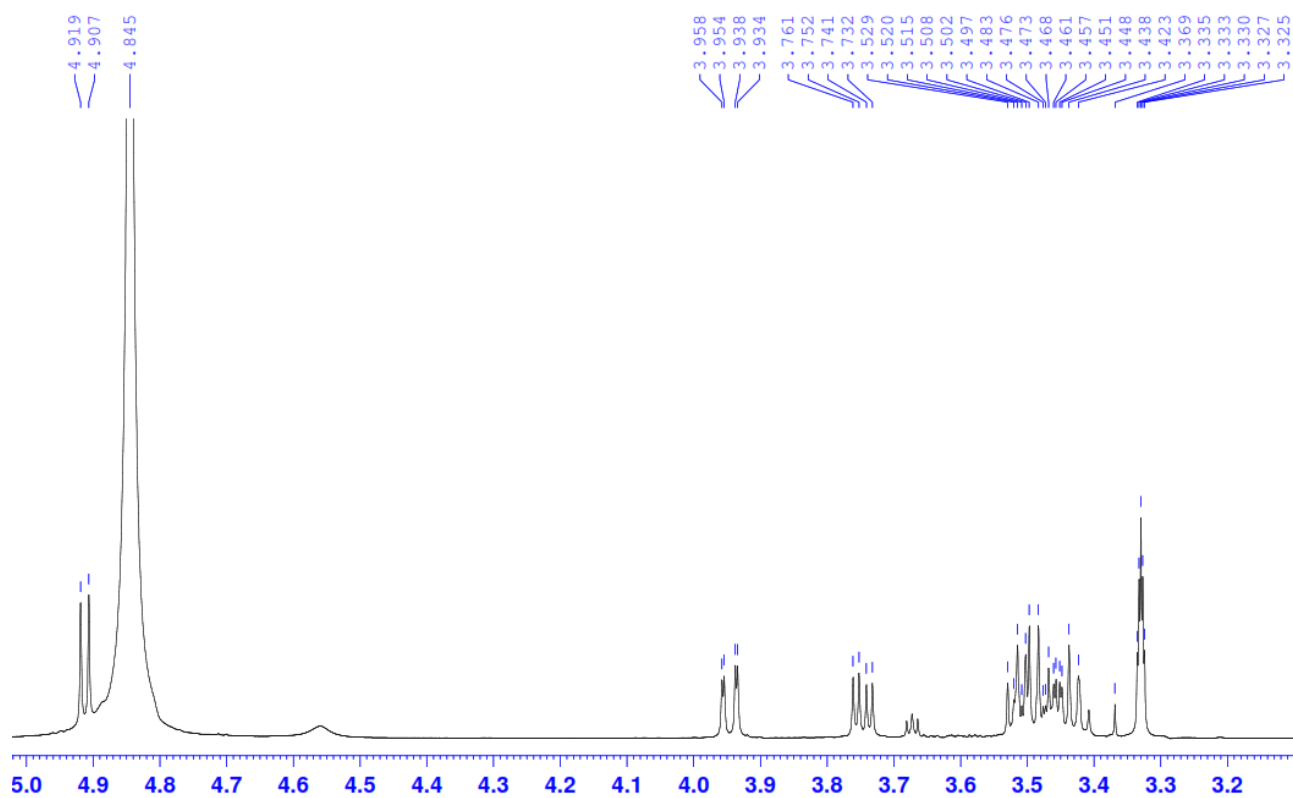


Figure S7. ^1H -NMR spectrum of compound **1** (Expanded)

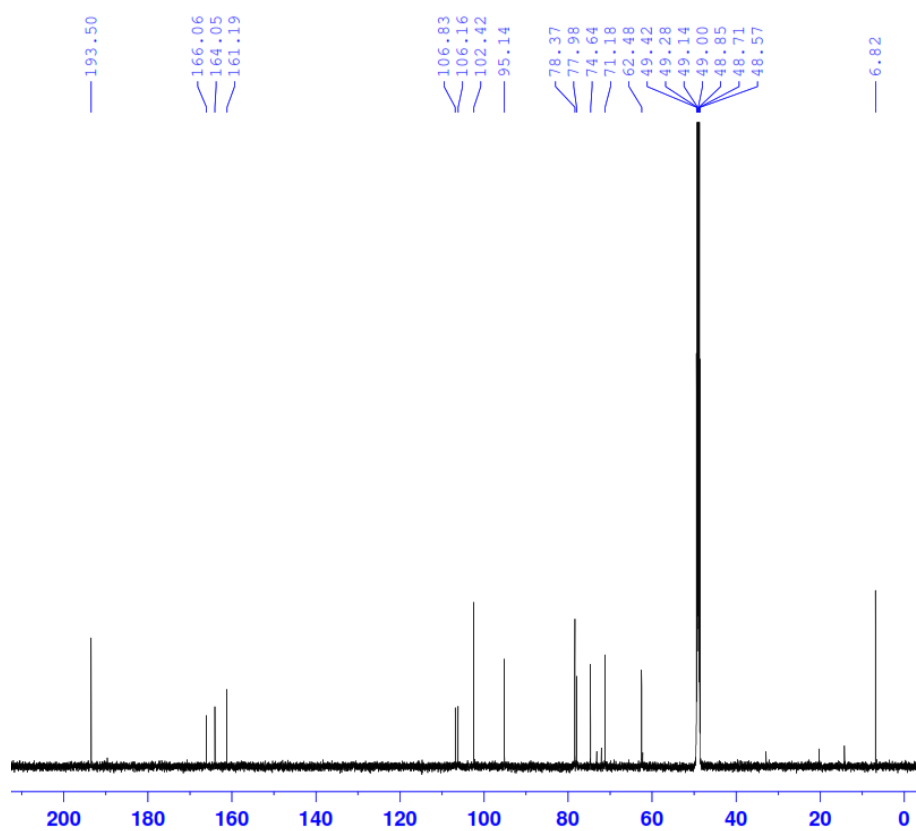


Figure S8. ^{13}C -NMR spectrum of compound **1**

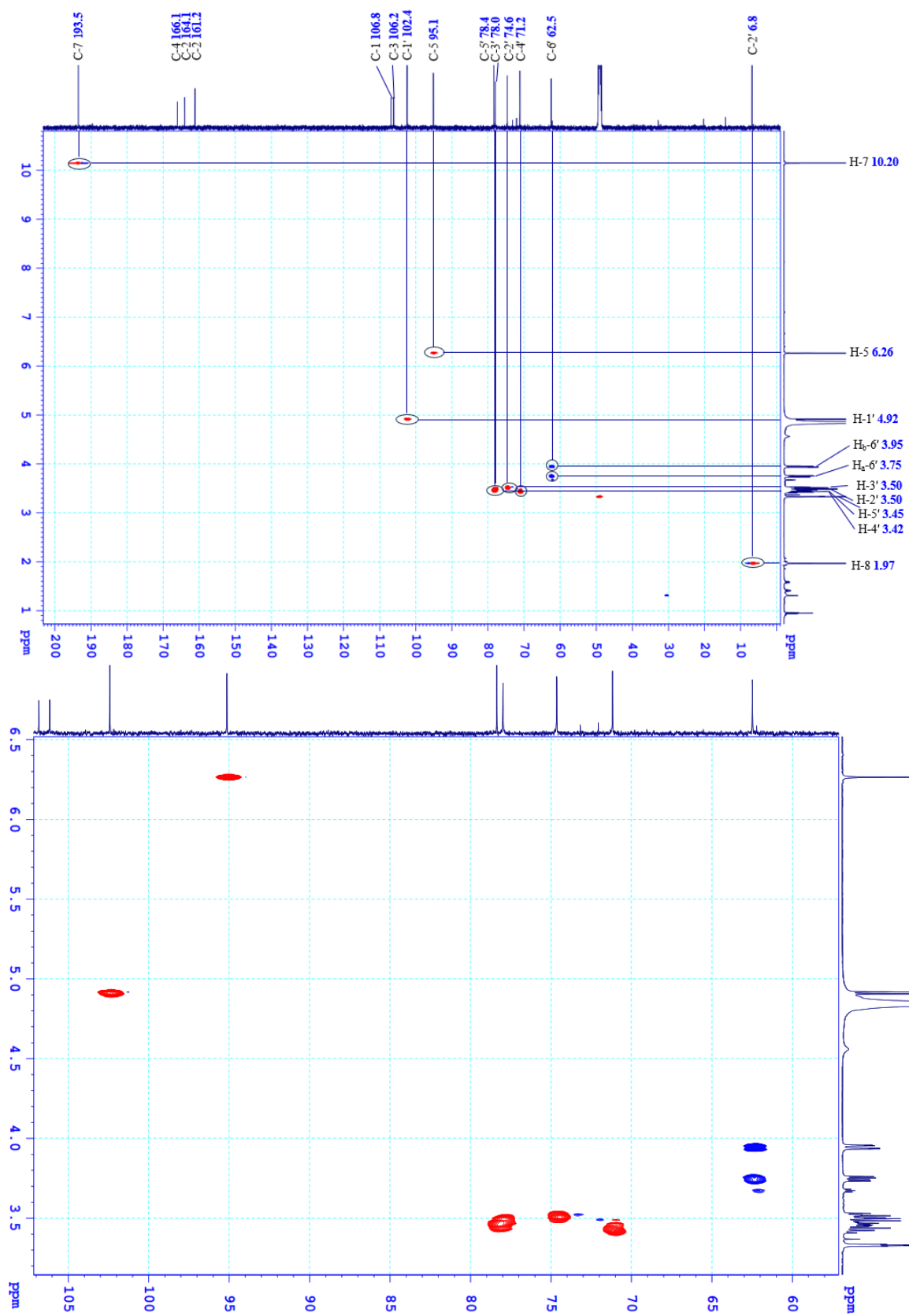


Figure S9. HSQC spectrum of compound **1**

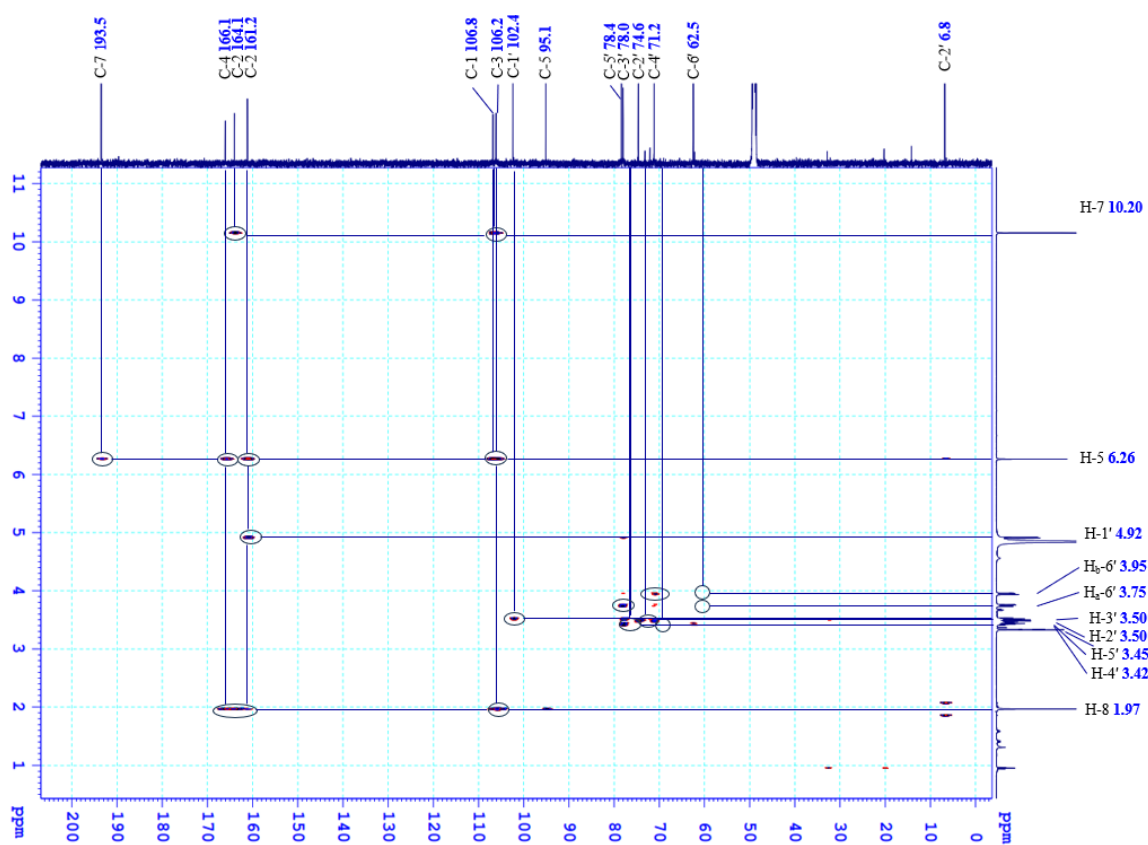
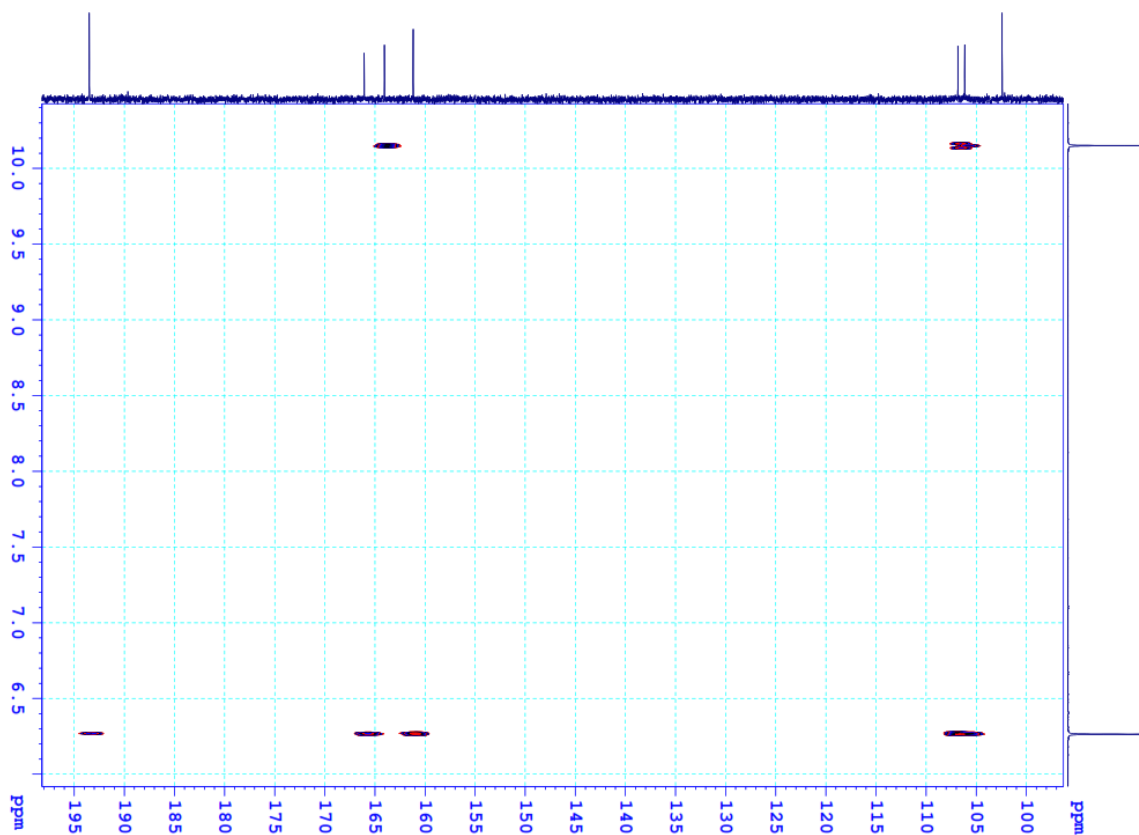


Figure S10. HMBC spectrum of compound 1



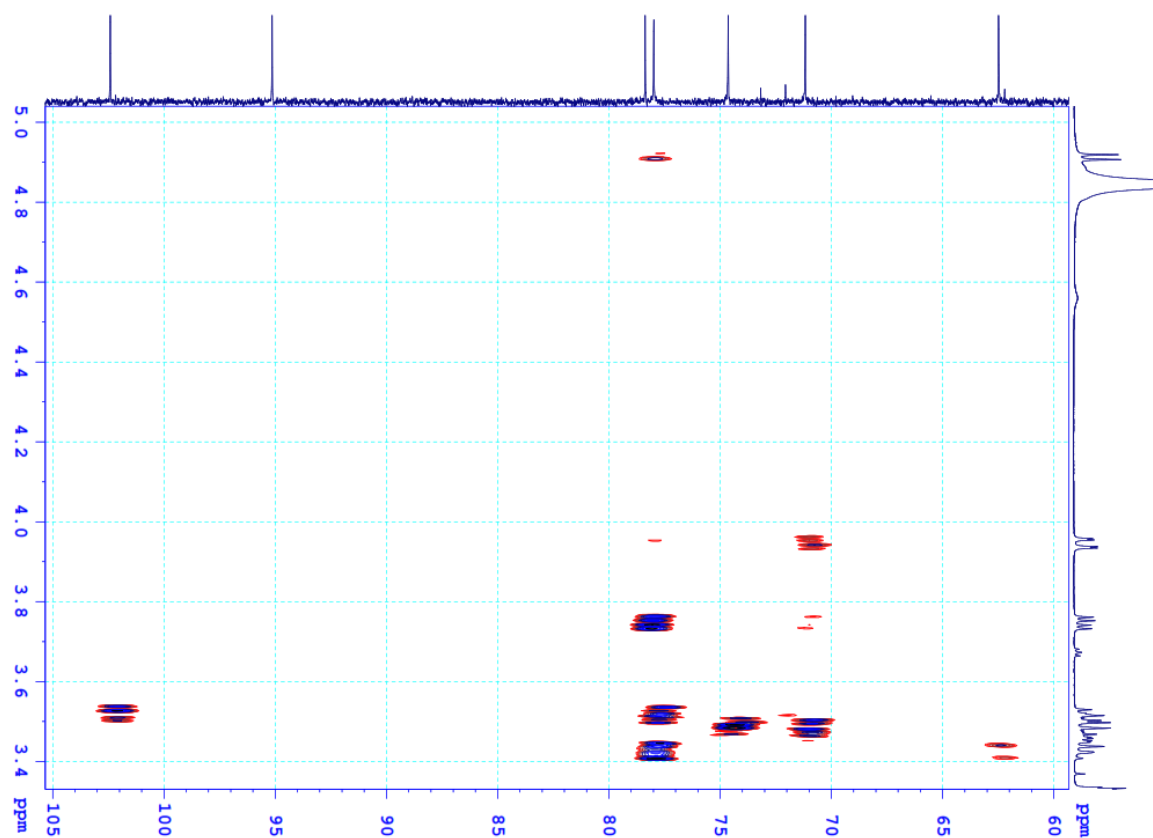
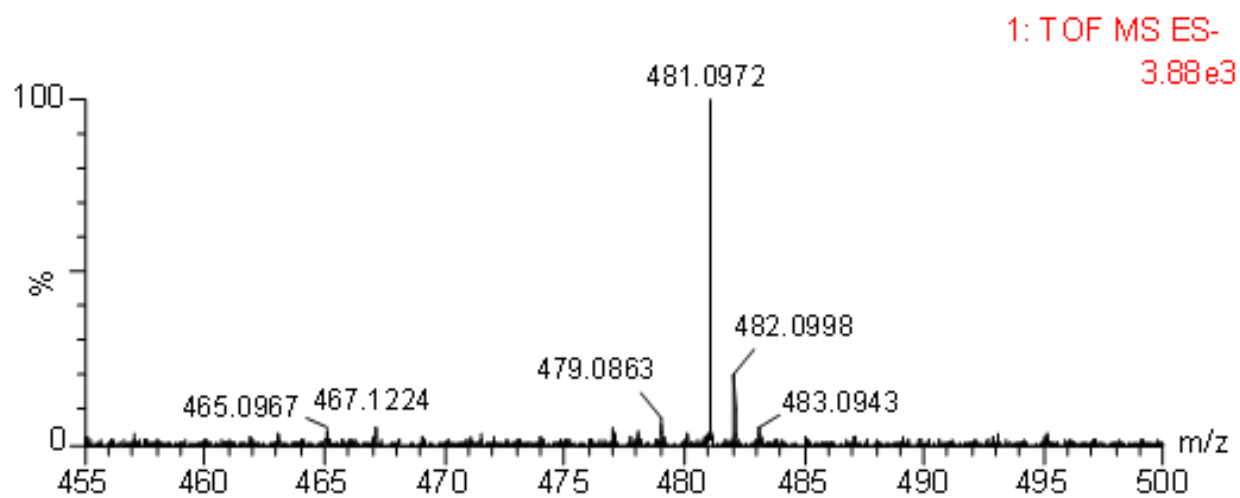


Figure S11. HMBC spectrum of compound **1** (Expanded)



Formula (M)	Ion Formula	Mass (MFG)	m/z (Calc)	Diff (ppm)	DBE	Score (MFG)
C18 H25 Cl N O12	C18 H24 Cl N O12	482.1065	481.0993	4.26	6.5	90.74
C19 H27 Cl O10 S	C19 H26 Cl O10 S	482.1013	481.0941	-6.49	6	81.15
C21 H22 O13	C21 H21 O13	482.106	481.0988	3.24	11	94.37
C22 H25 Cl N O7 S	C22 H24 Cl N O7 S	482.104	481.0967	-0.94	10.5	99.51
C24 H20 N O10	C24 H19 N O10	482.1087	481.1014	8.8	15.5	71.24

Figure S12. HR-ESI-MS spectrum of compound **2**

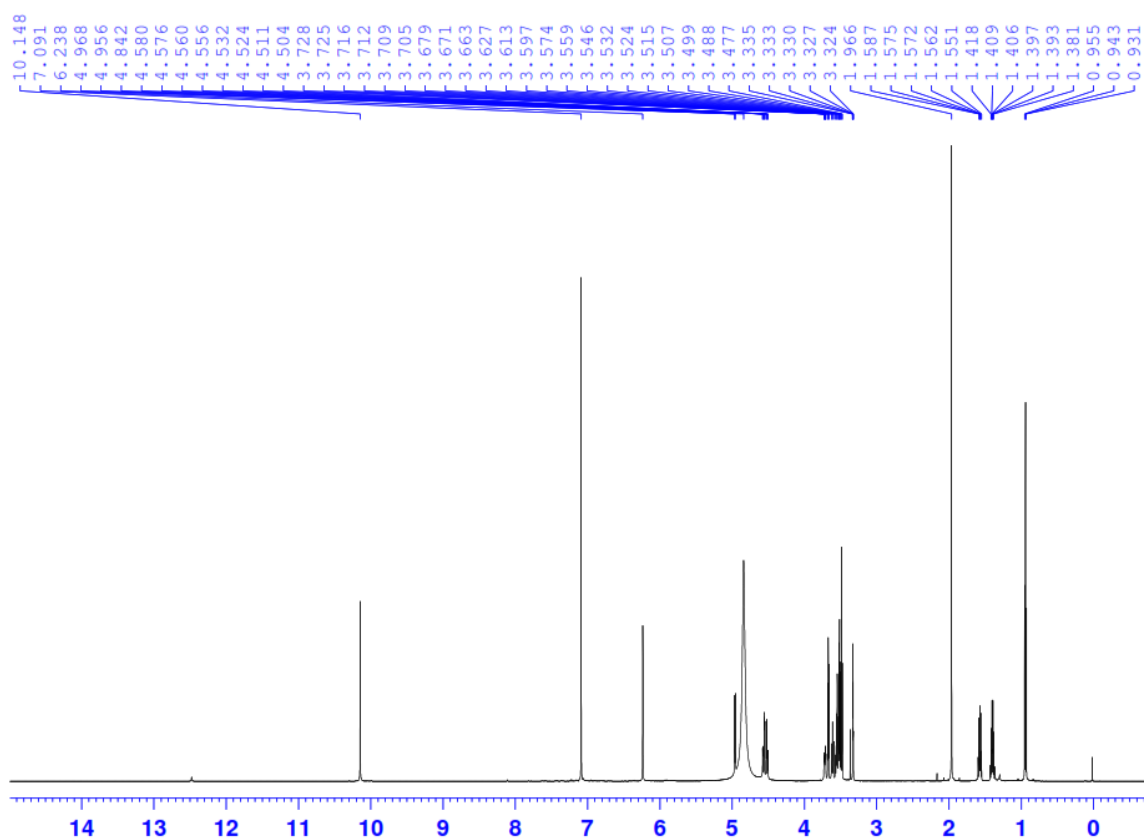


Figure S13. ^1H -NMR spectrum of compound **2**

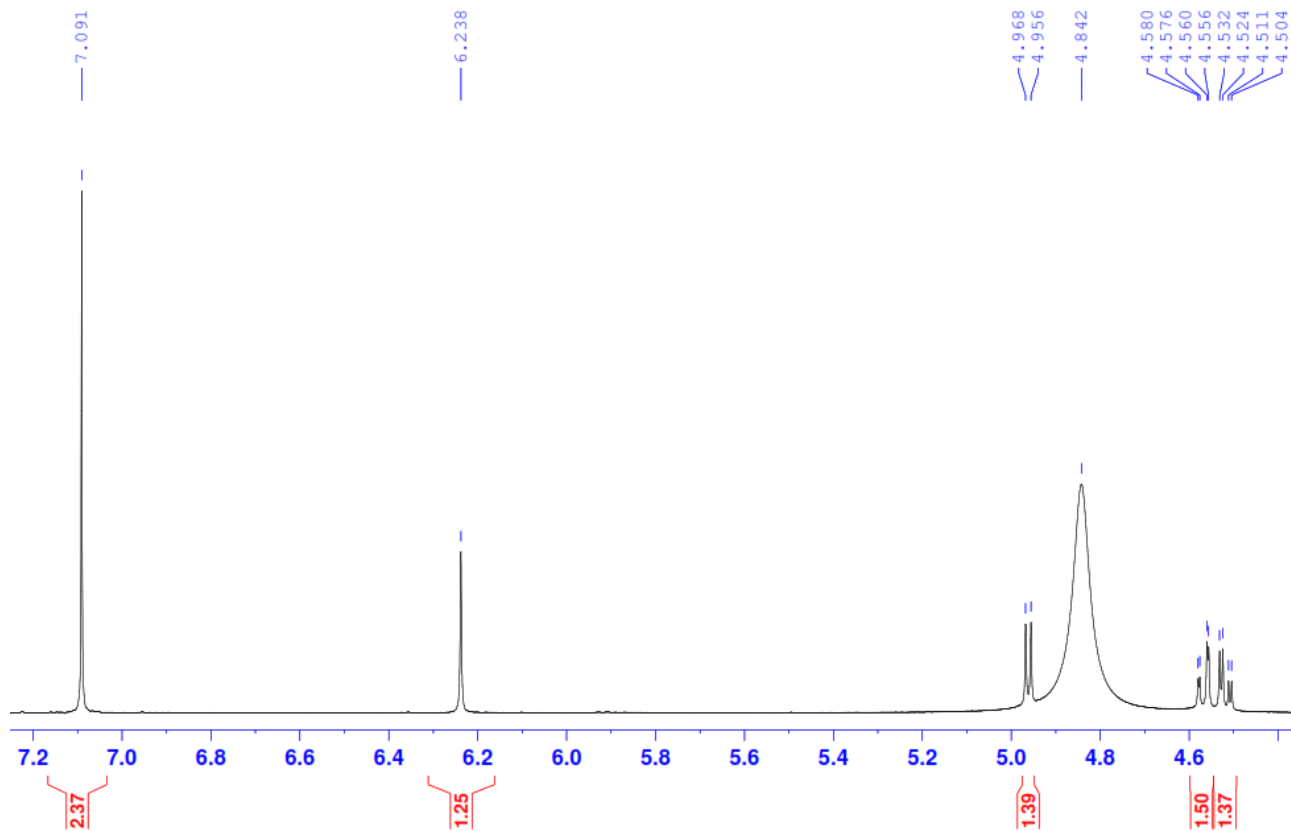


Figure S14. ^1H -NMR spectrum of compound **2** (Expanded)

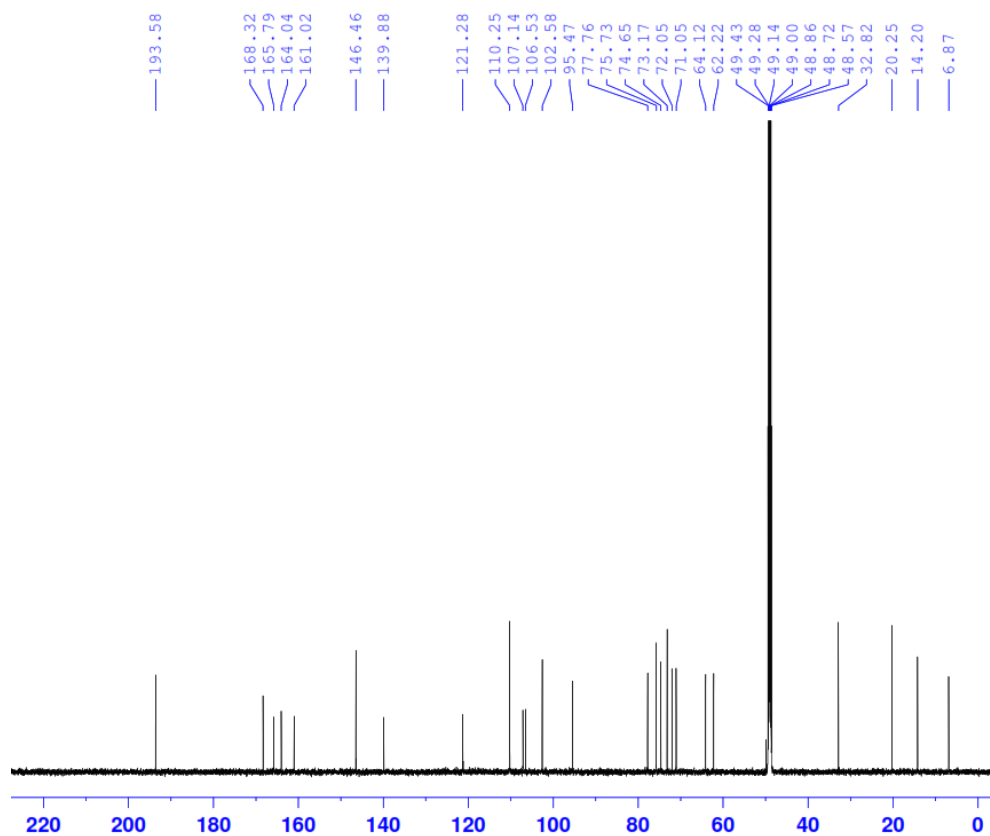
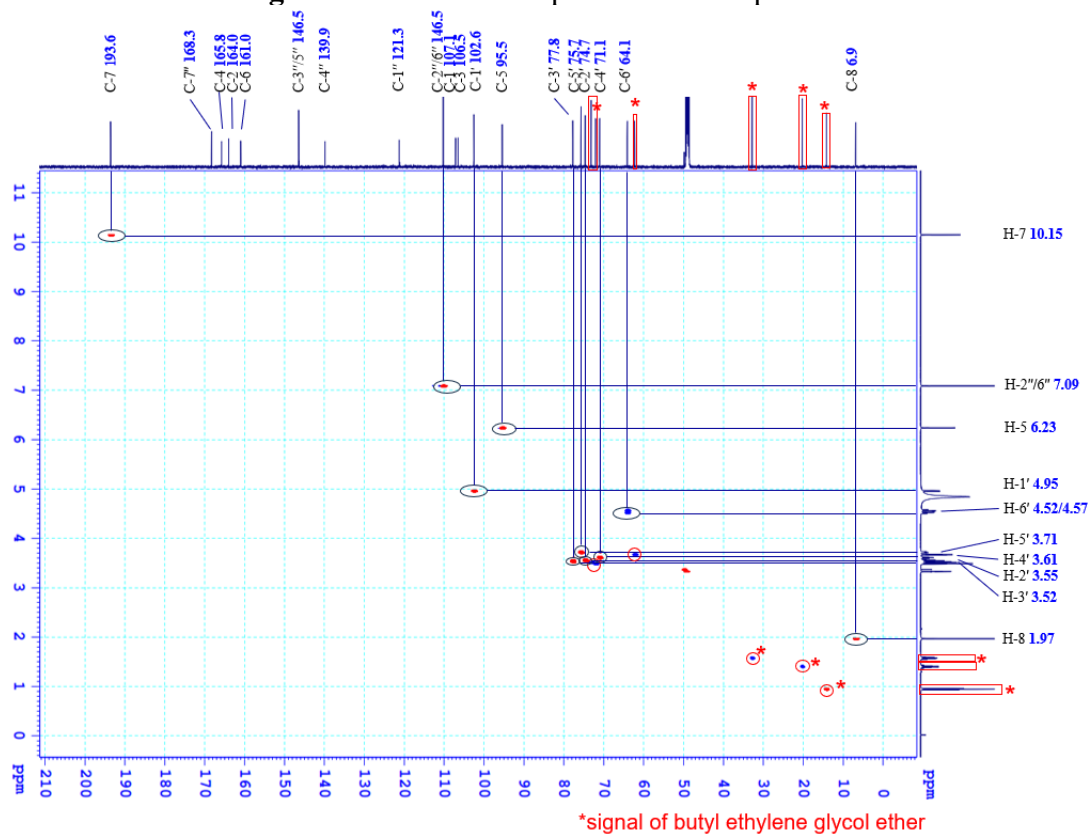


Figure S15. ¹³C-NMR spectrum of compound 2



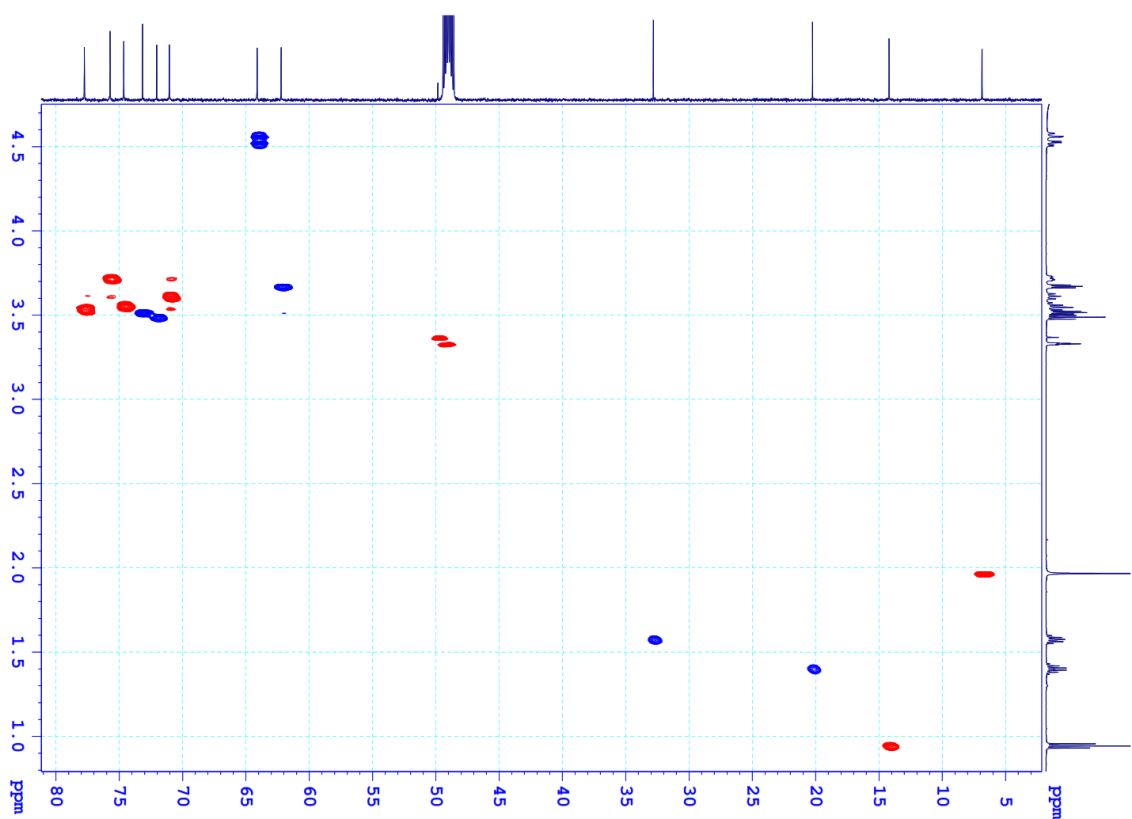
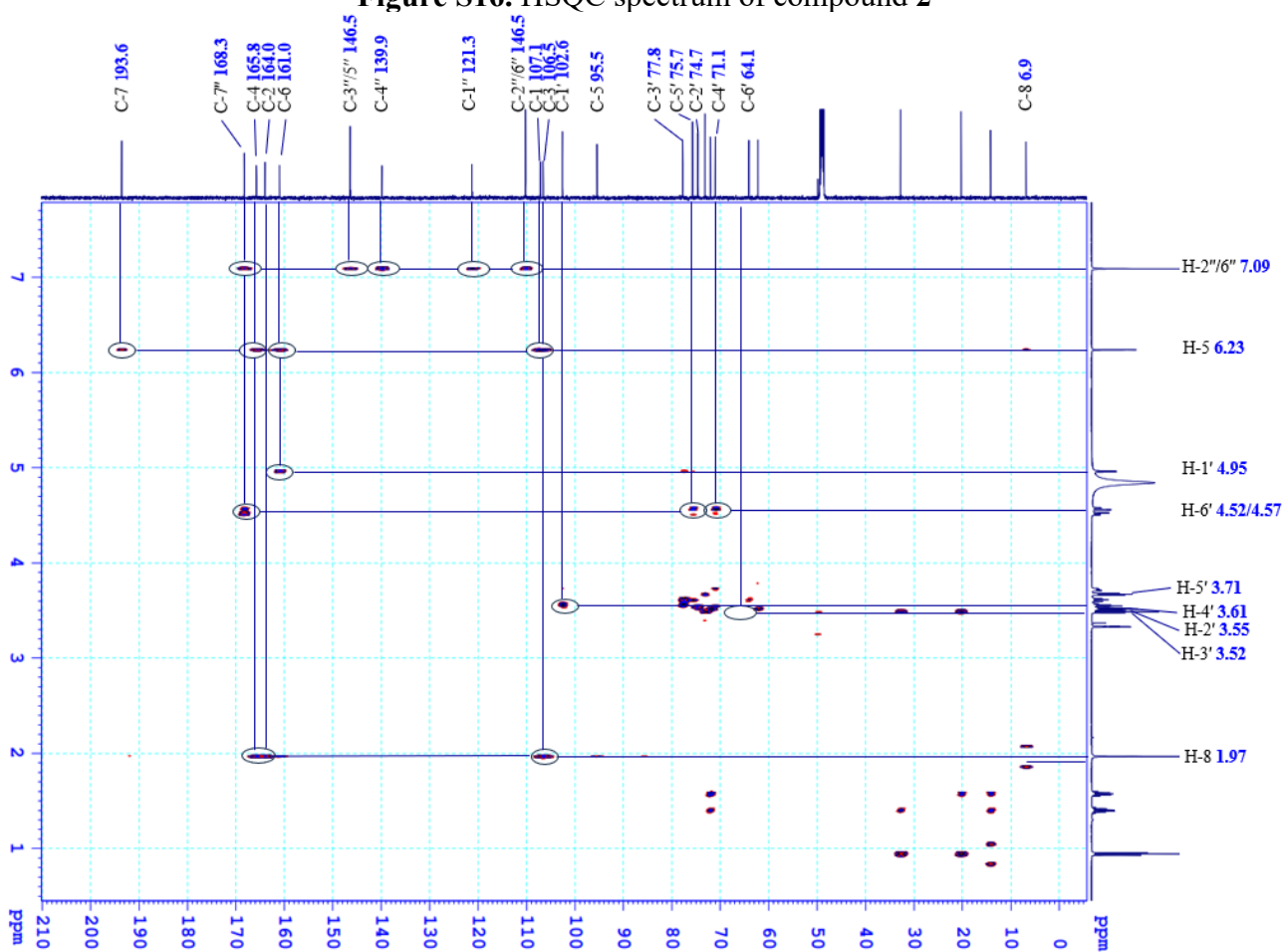


Figure S16. HSQC spectrum of compound **2**



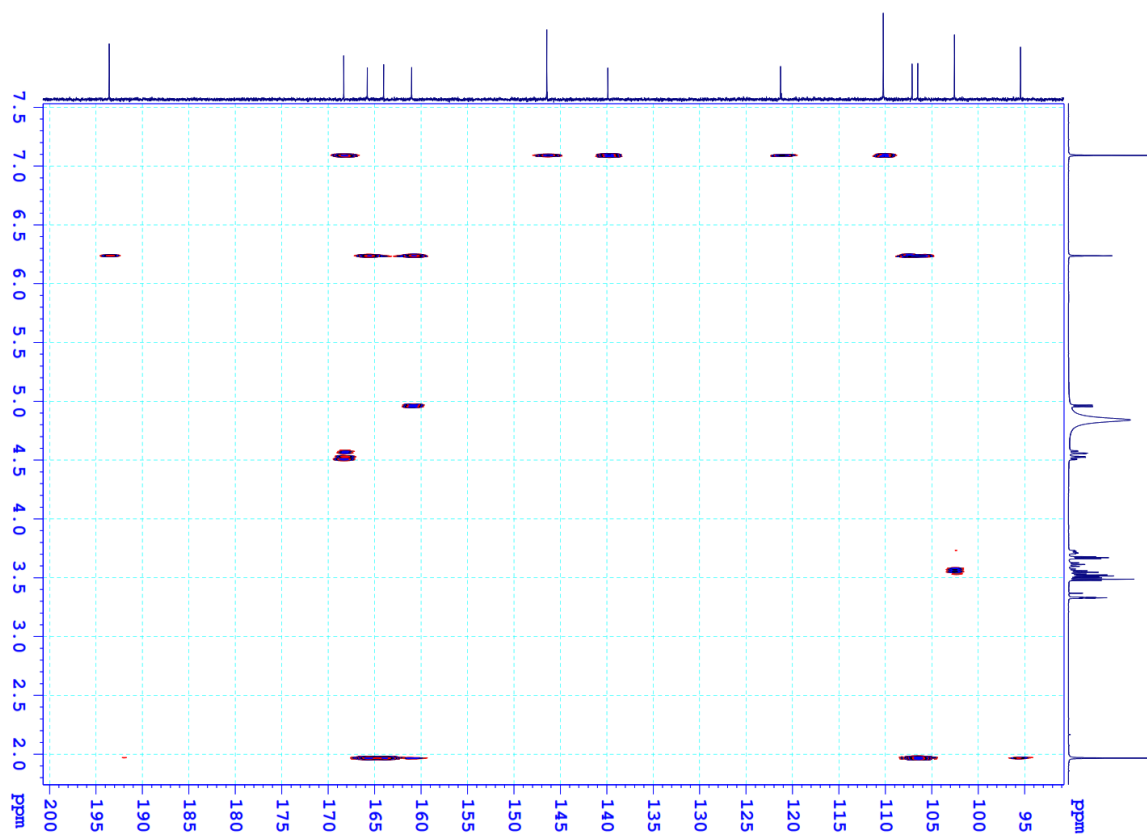


Figure S17. HMBC spectrum of compound **2**

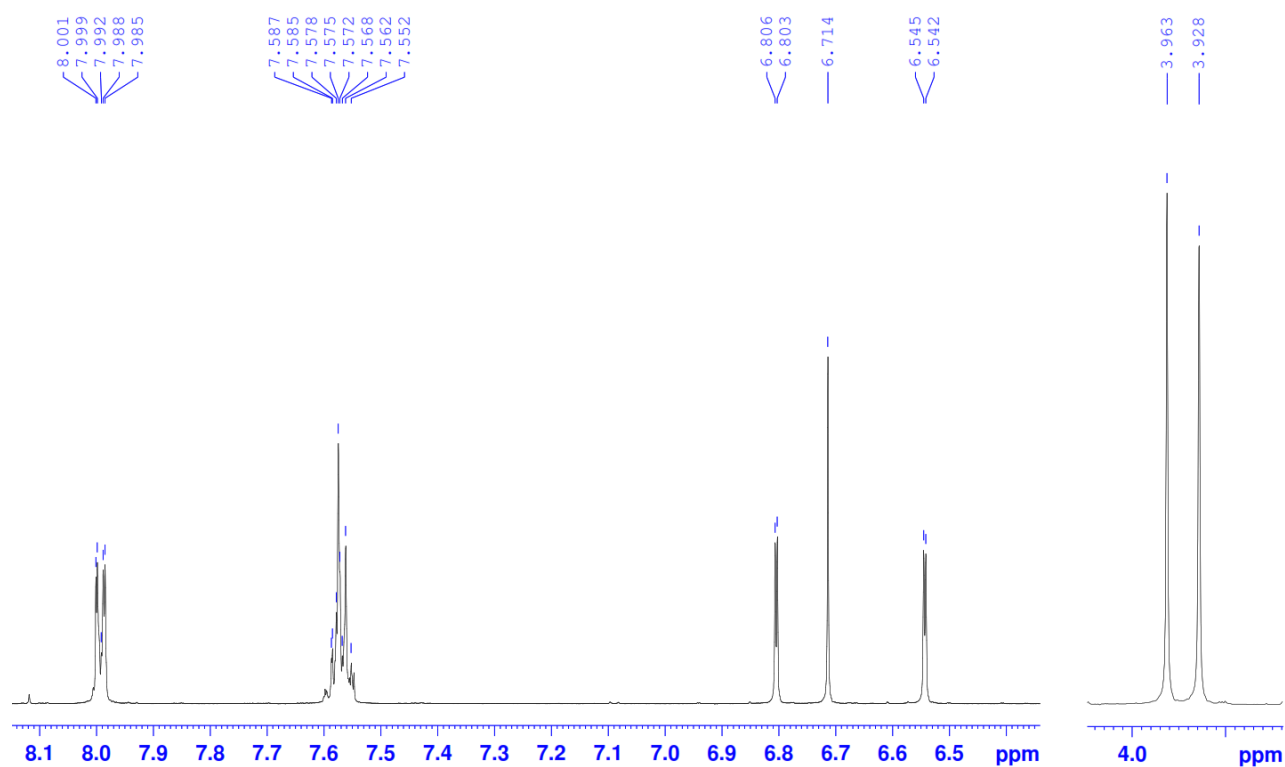


Figure S18. ^1H -NMR spectrum of compound **3**

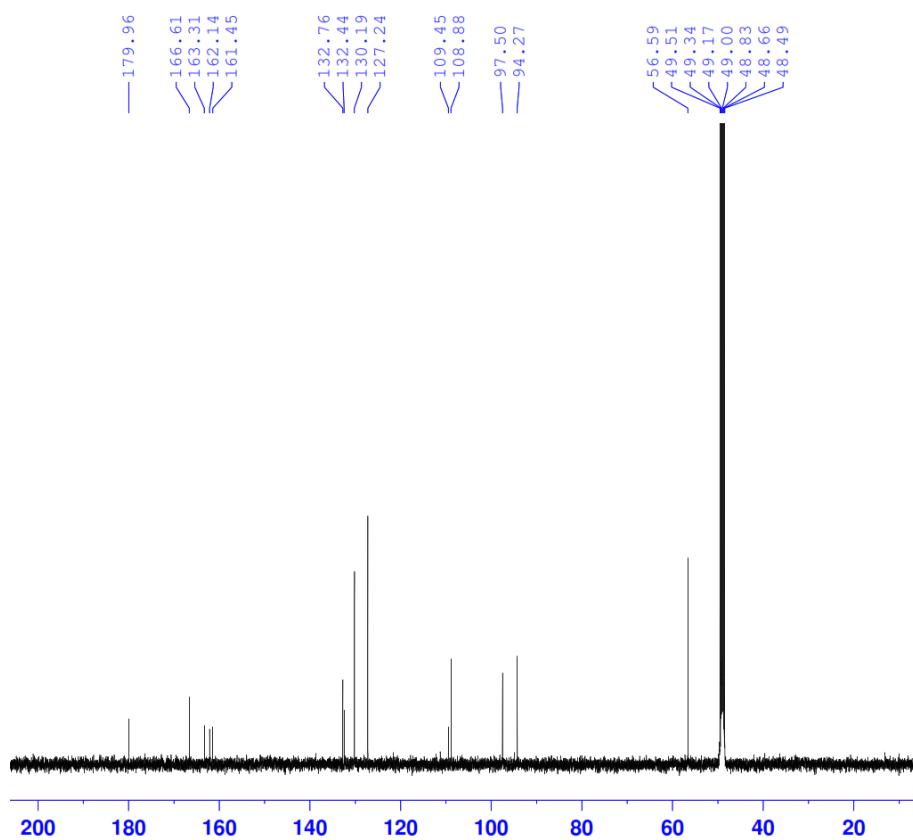


Figure S19. ^{13}C -NMR spectrum of compound **3**

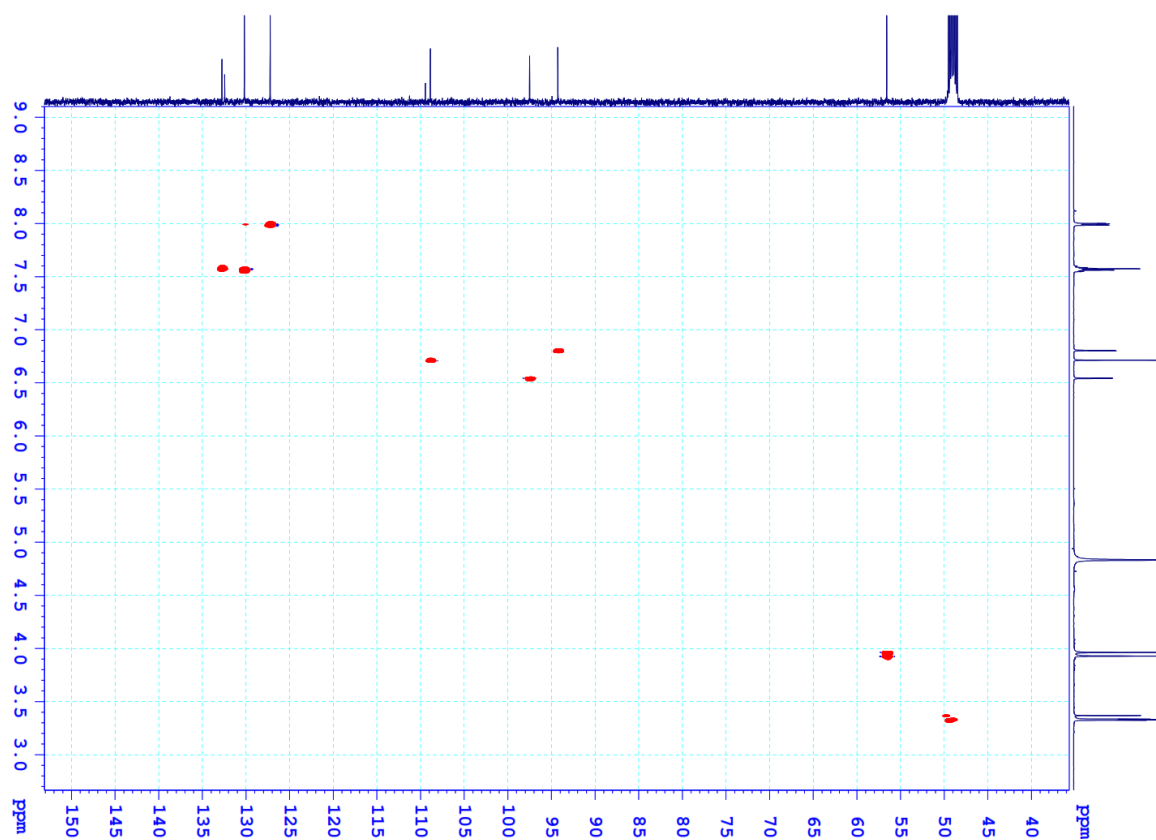


Figure S20. HSQC spectrum of compound **3**

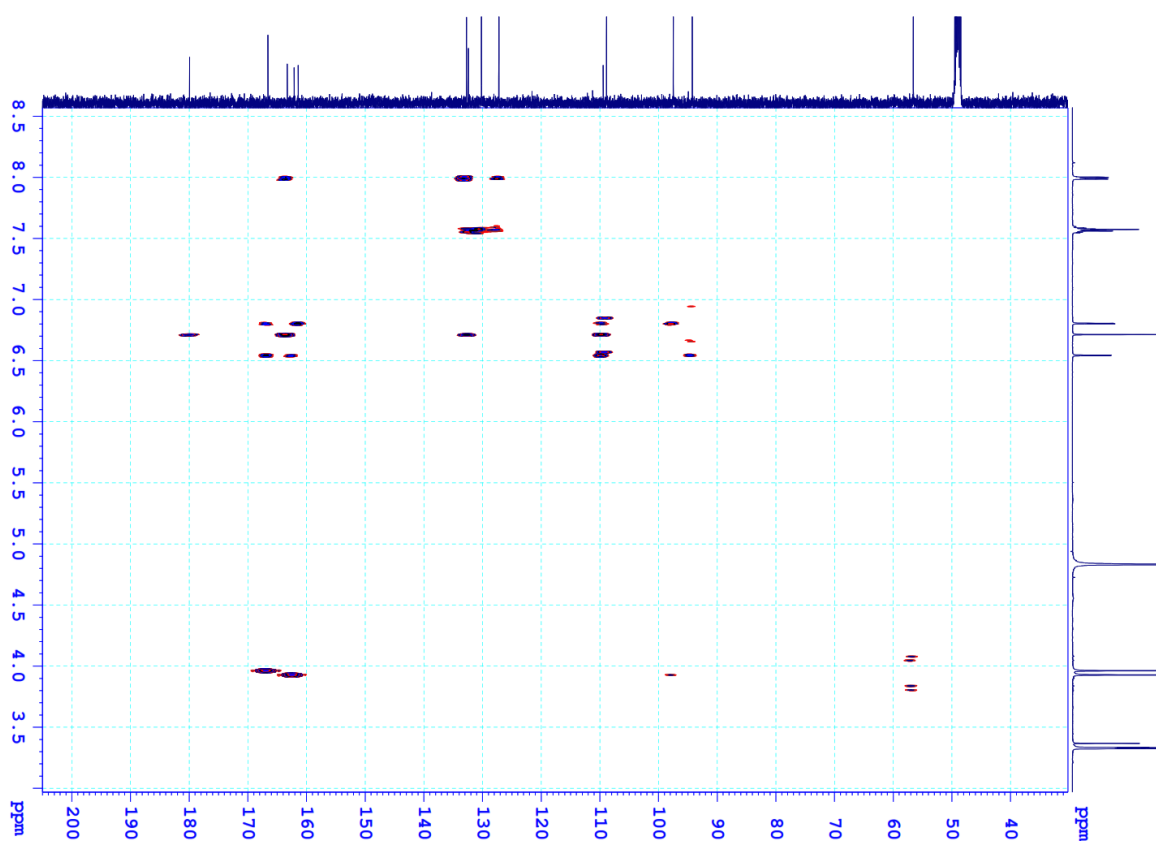


Figure S21. HMBC spectrum of compound **3**

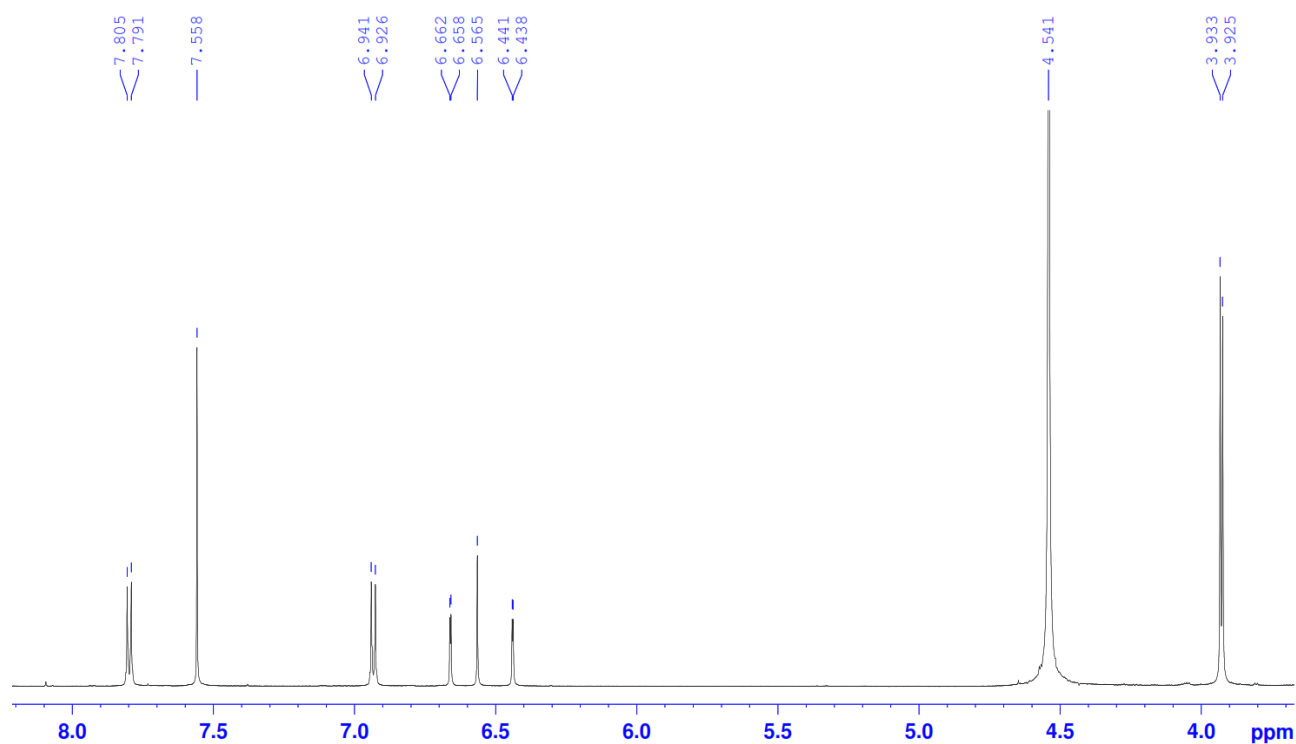


Figure S22. ¹H-NMR spectrum of compound 4

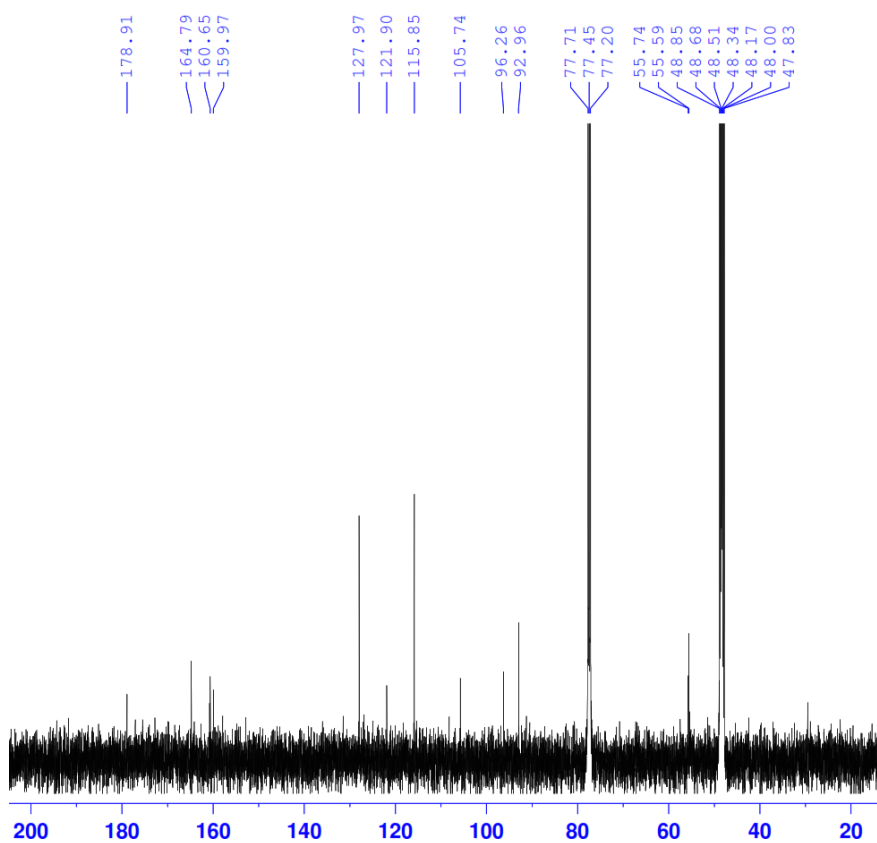


Figure S23. ¹³C-NMR spectrum of compound 4

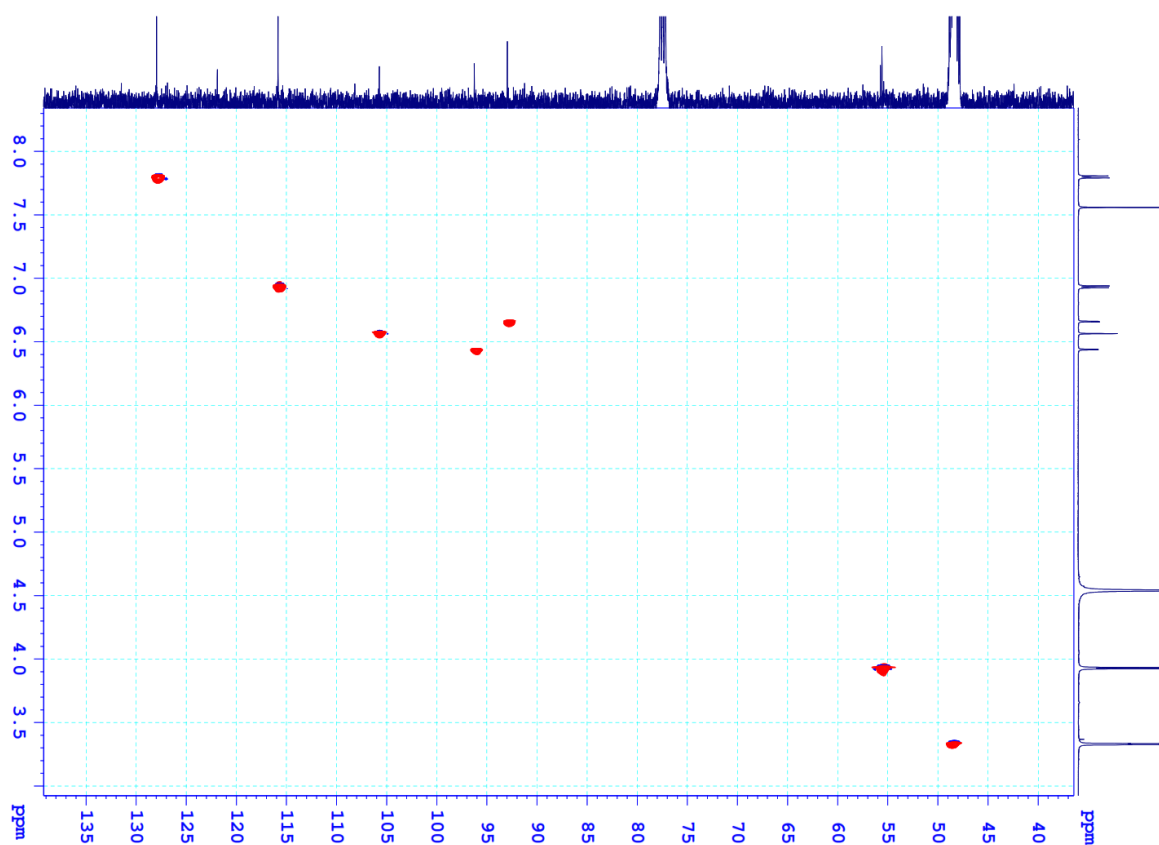


Figure S24. HSQC spectrum of compound 4

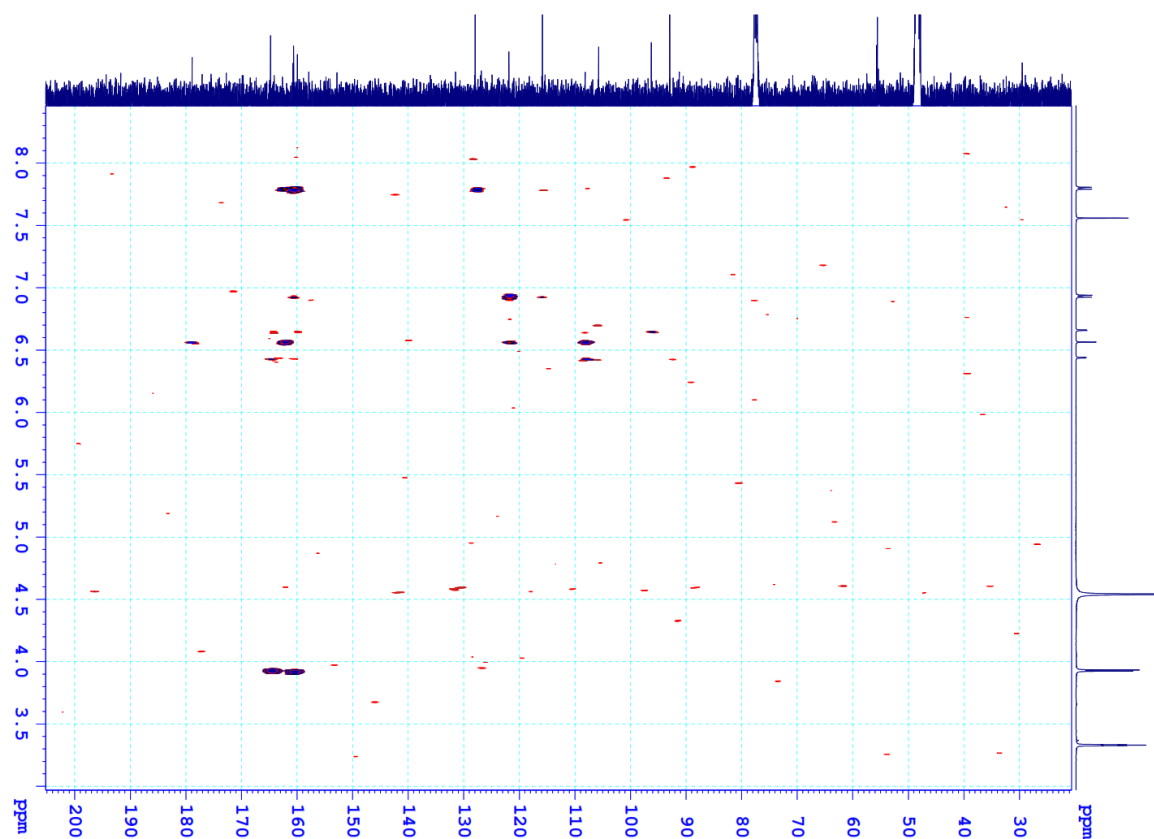


Figure S25. HMBC spectrum of compound 4

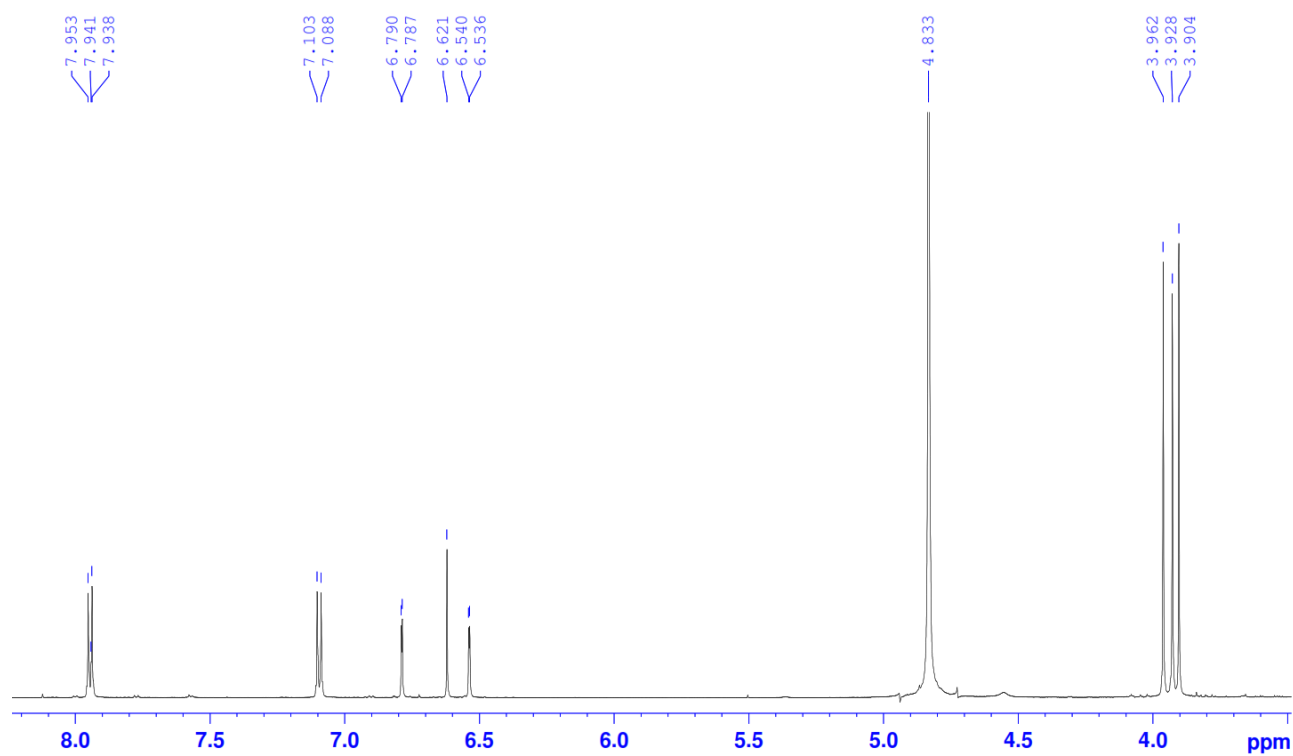


Figure S26. ^1H -NMR spectrum of compound **5**

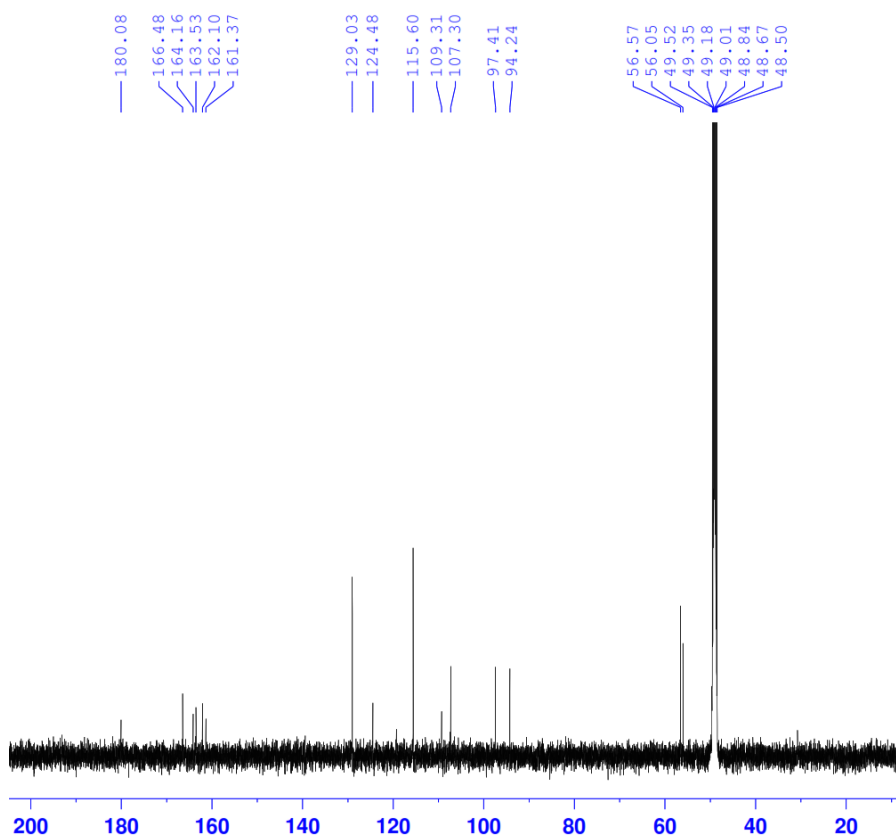


Figure S27. ^{13}C -NMR spectrum of compound **5**

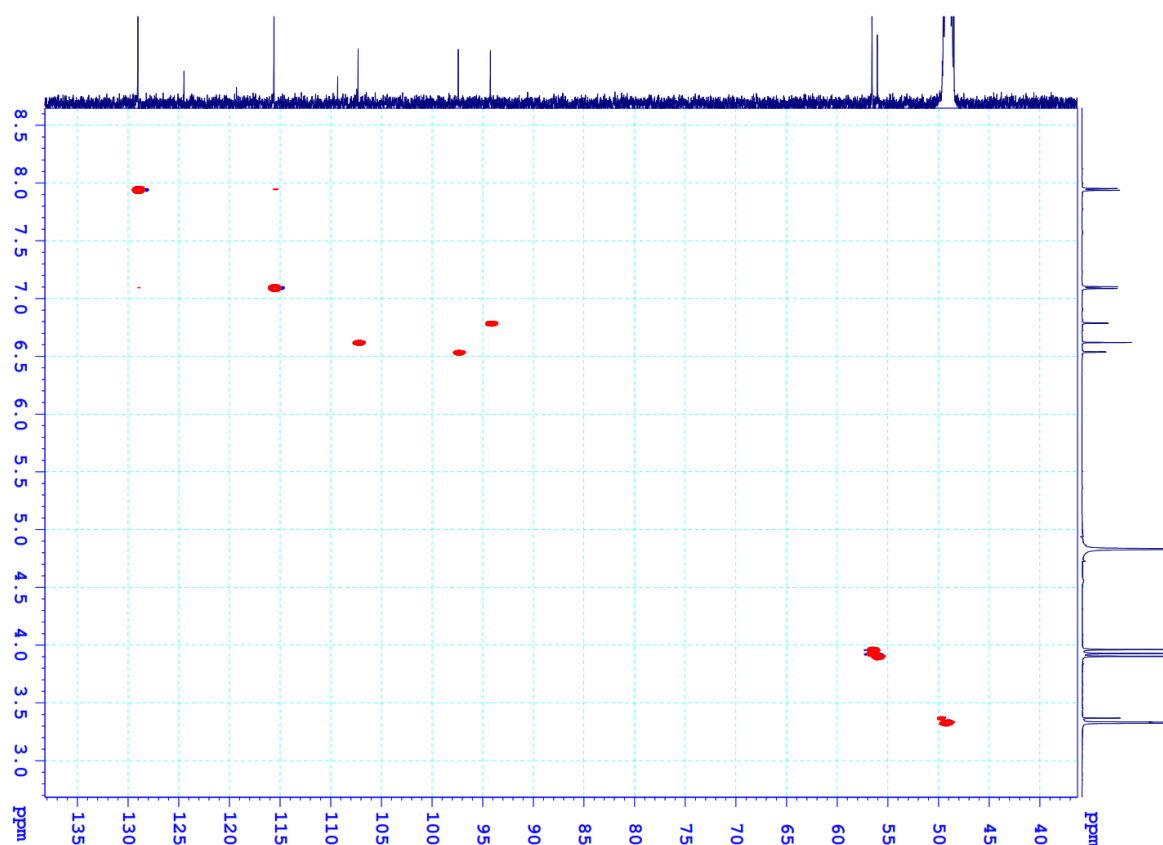


Figure S28. HSQC spectrum of compound **5**

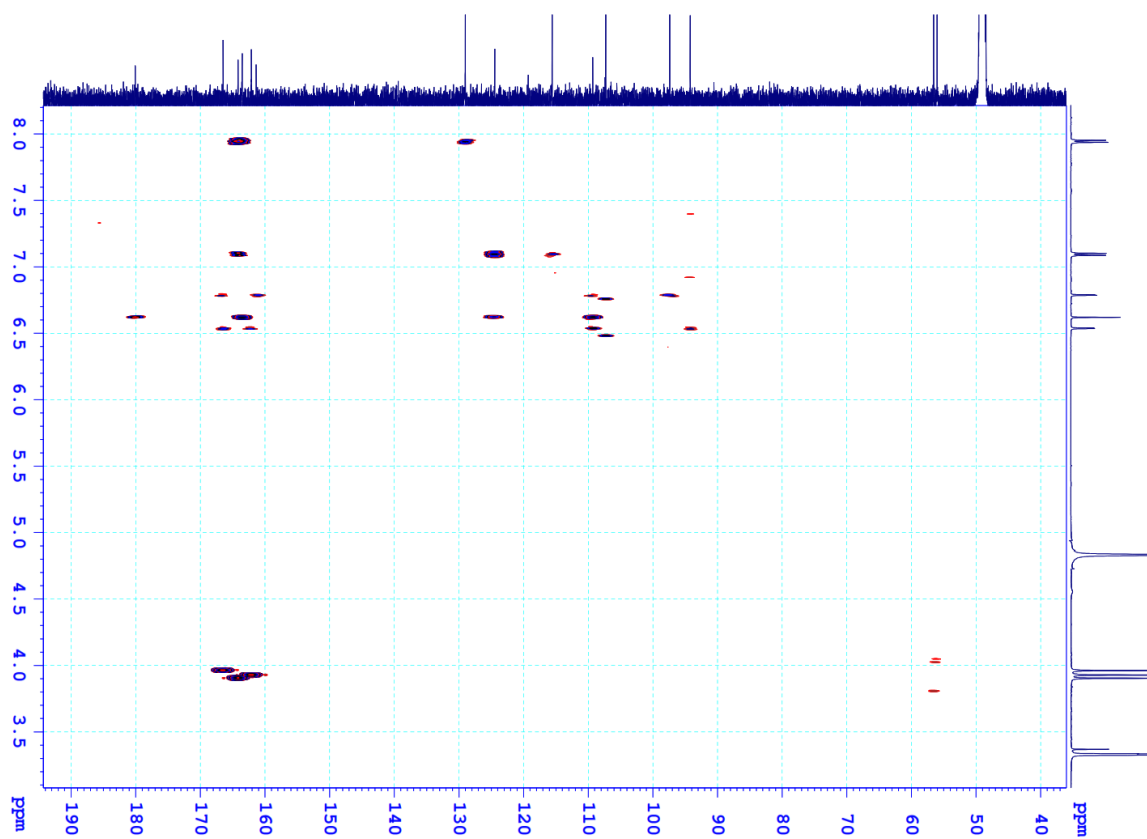


Figure S29. HMBC spectrum of compound **5**

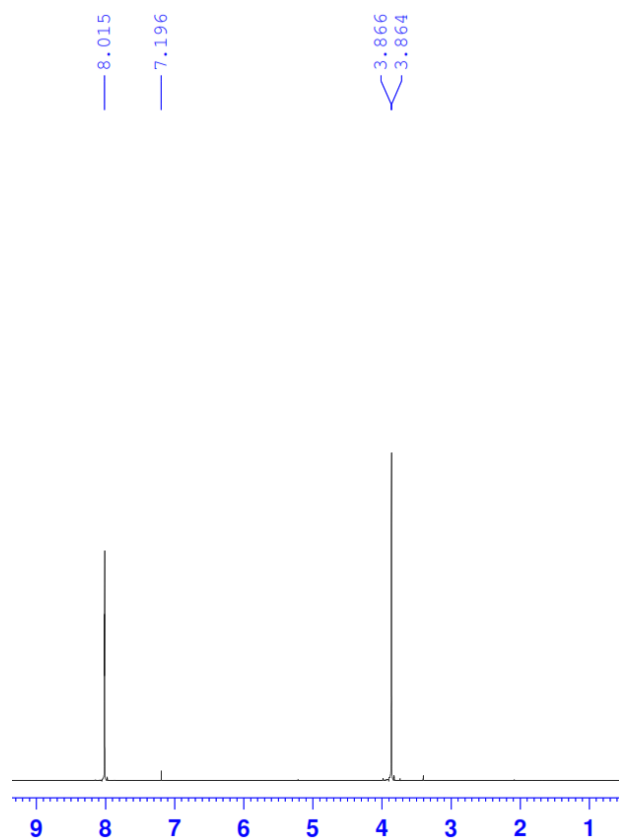


Figure S30. ¹H-NMR spectrum of compound **6**

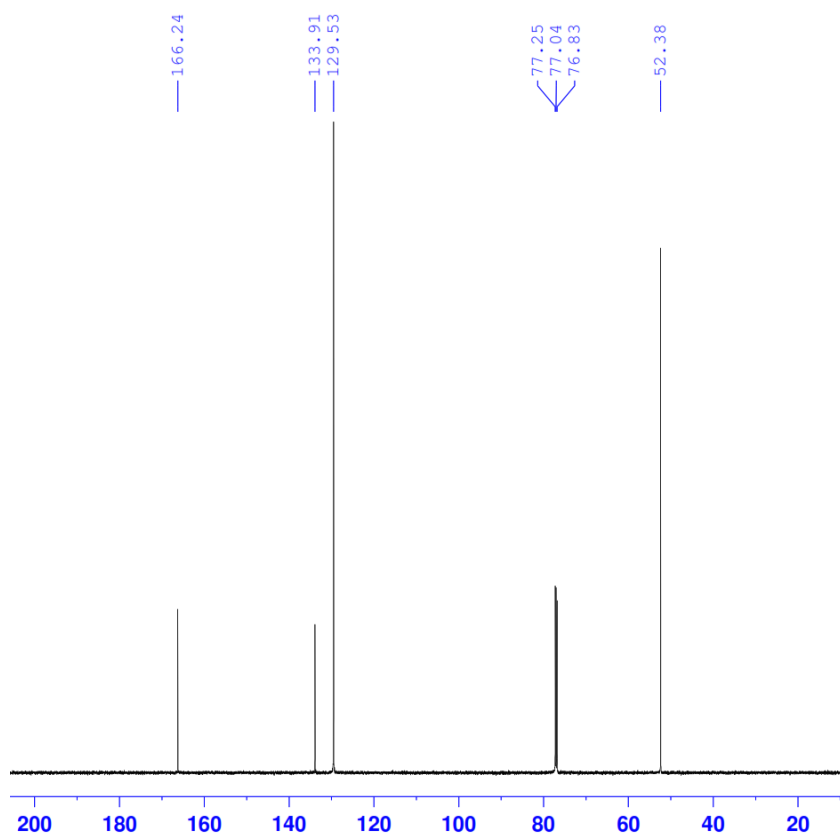


Figure S31. ¹³C-NMR spectrum of compound **6**

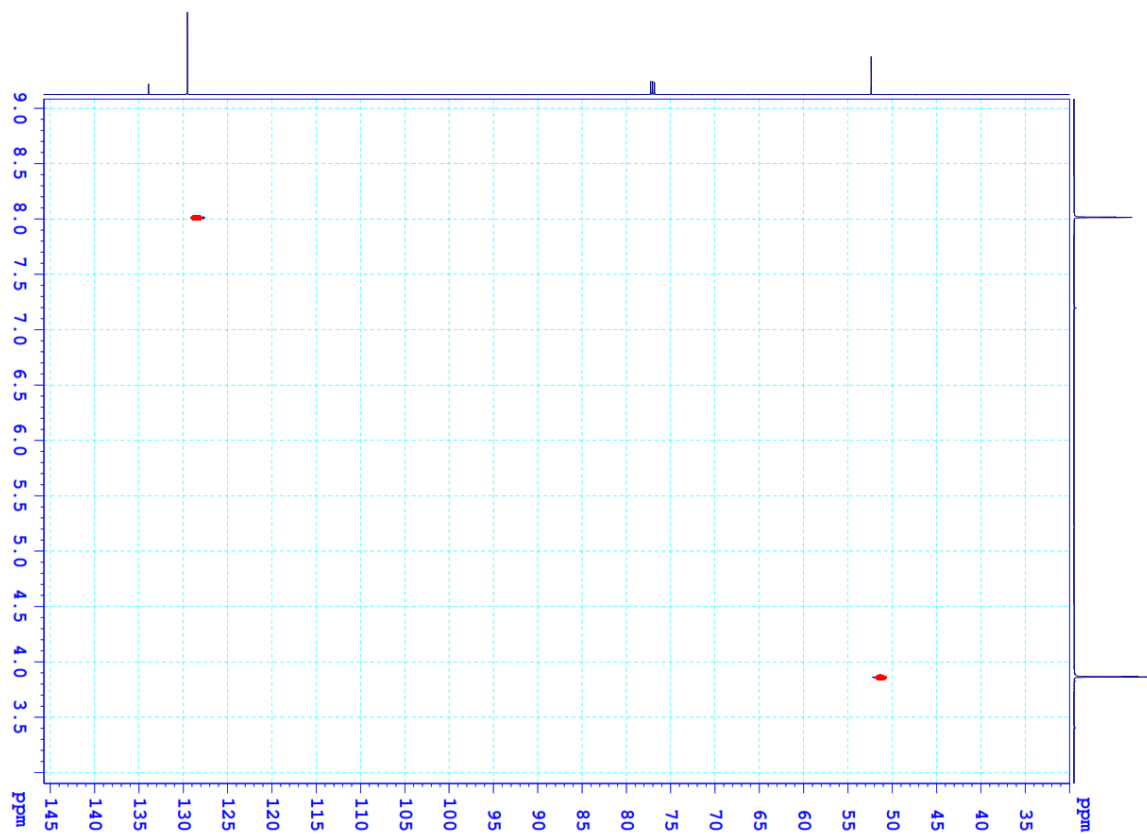


Figure S32. HSQC spectrum of compound 6

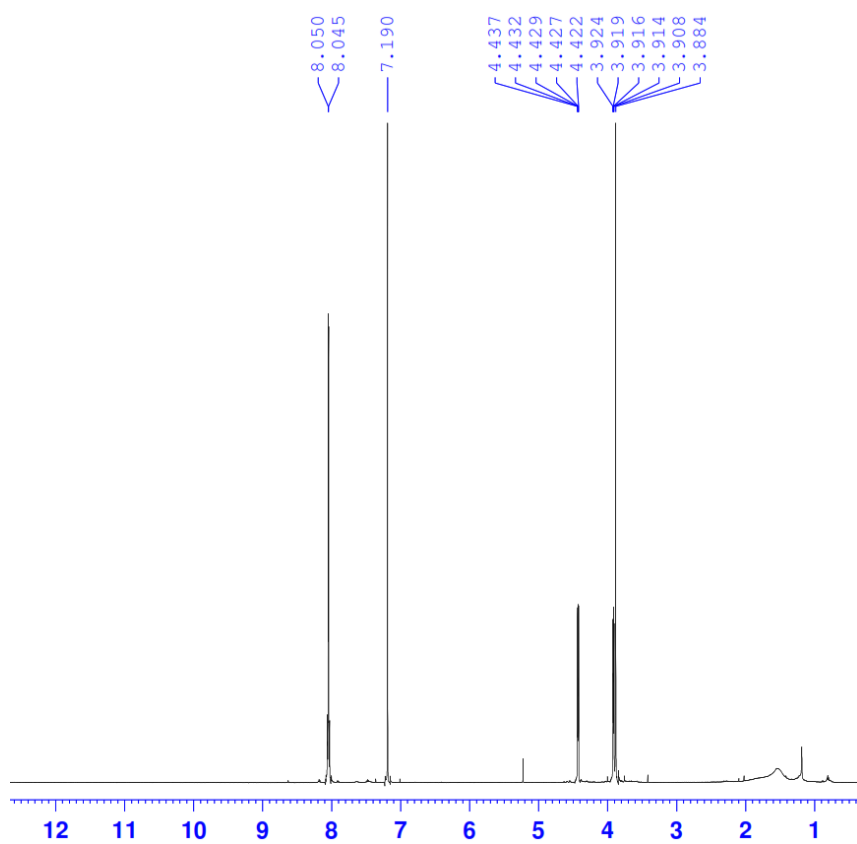


Figure S33. ^1H -NMR spectrum of compound 7

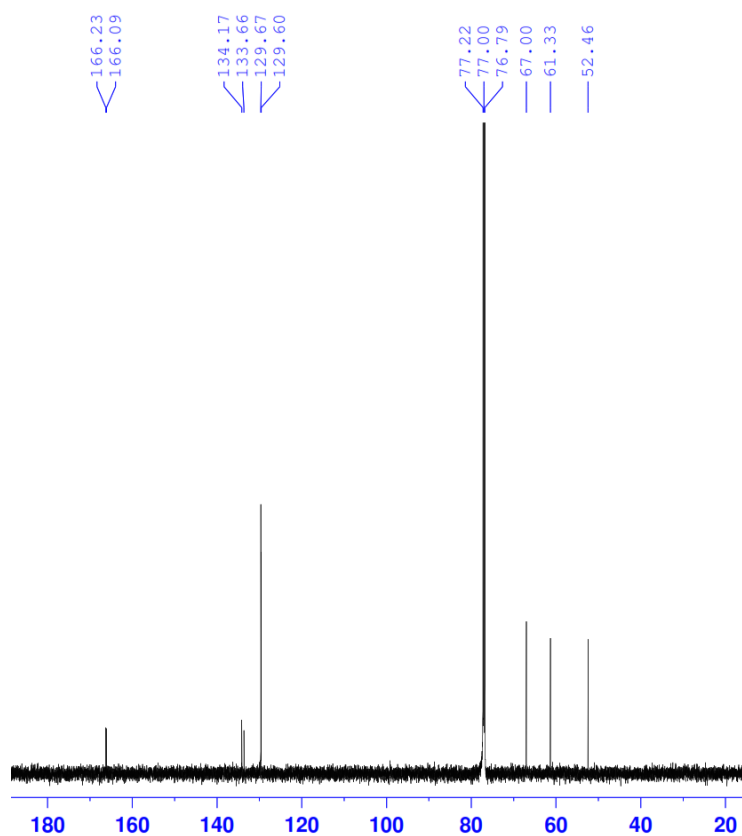


Figure S34. ^{13}C -NMR spectrum of compound **7**

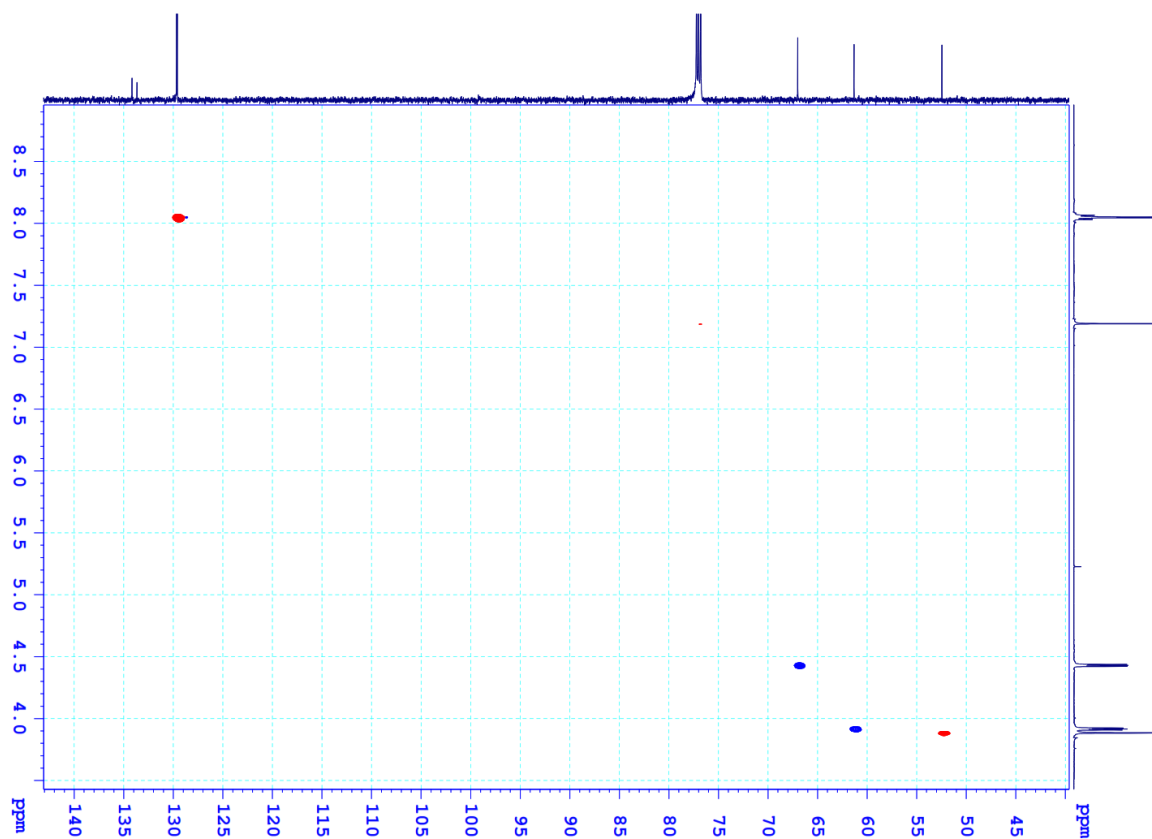


Figure S35. HSQC spectrum of compound **7**

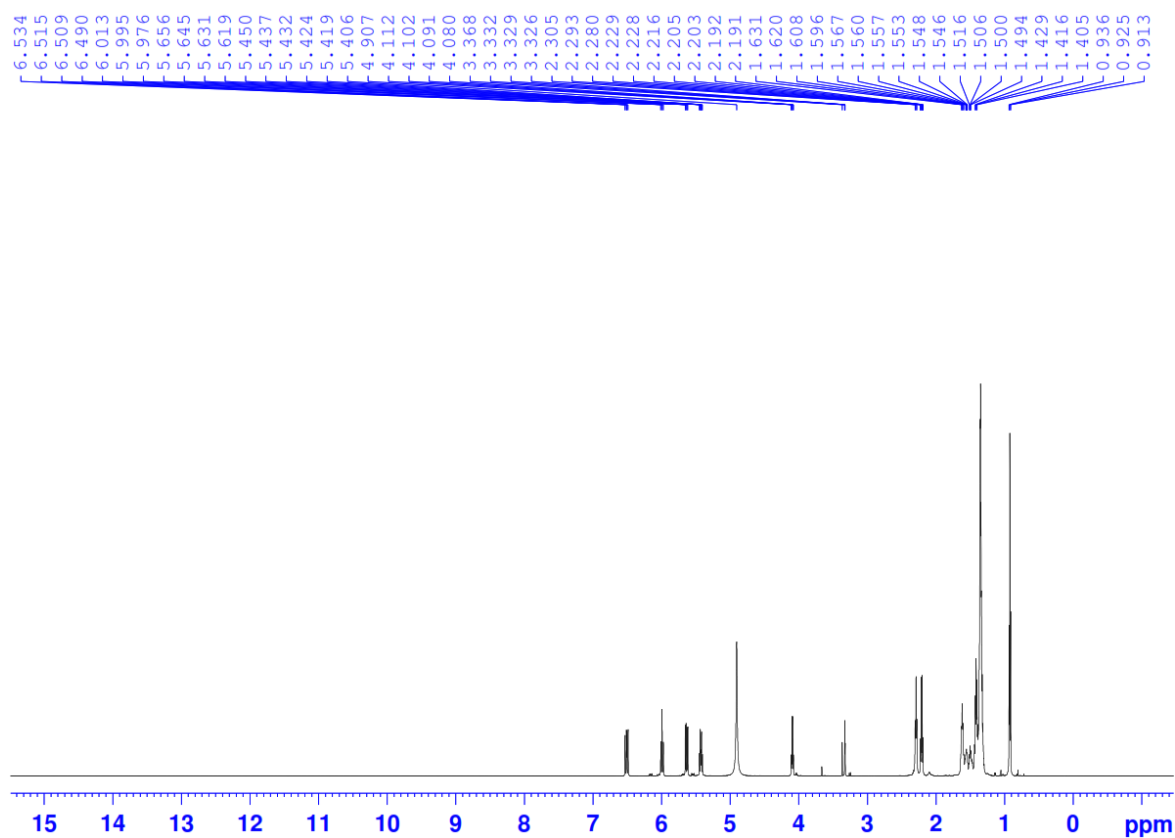


Figure S36. ^1H -NMR spectrum of compound **8**

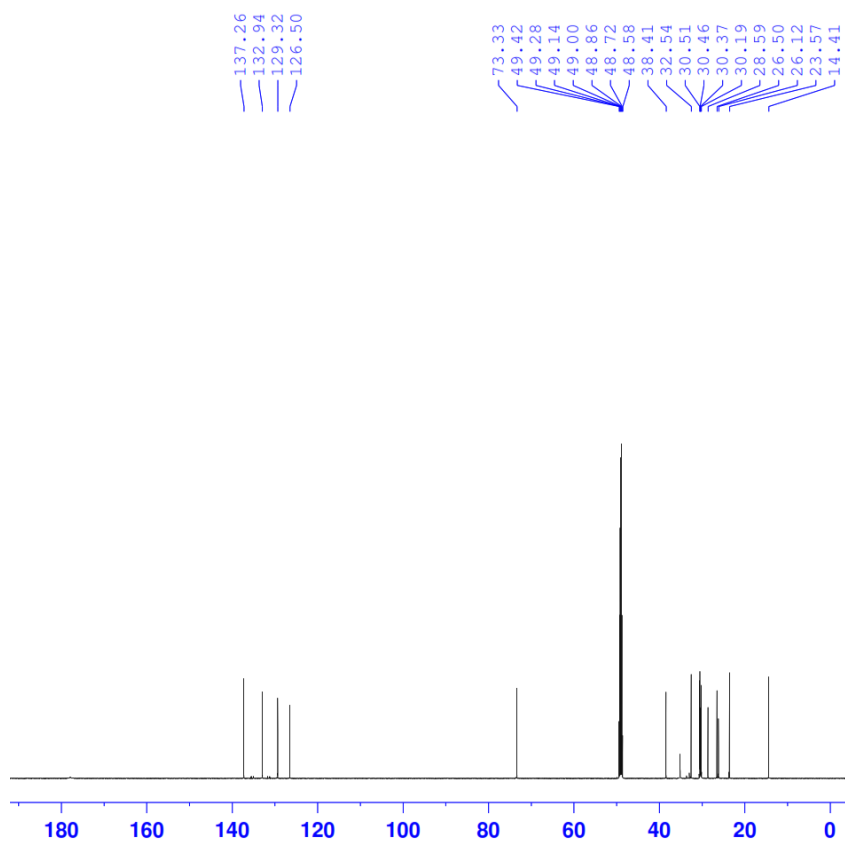


Figure S37. ^{13}C -NMR spectrum of compound **8**

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