Electronic supplementary information (ESI)

Phenanthroindolizidine alkaloids from Tylophora atrofolliculata with hypoxia-inducible factor-1 (HIF-1) inhibitory activity

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Contents

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† zhjiang@must.edu.mo

Electronic Supplementary Information (ESI) available.

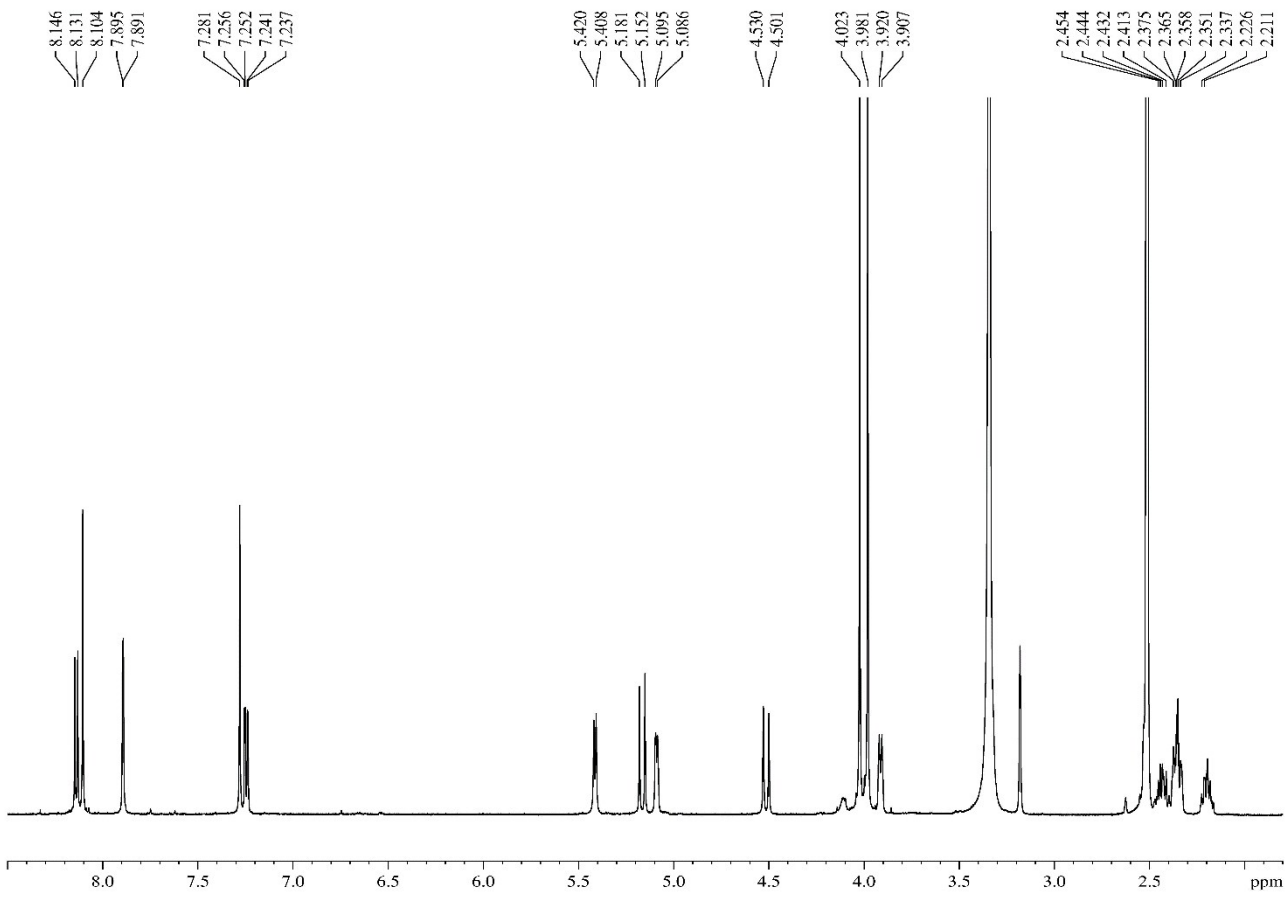


Figure 1 ^{1}H NMR spectrum of compound **1**.

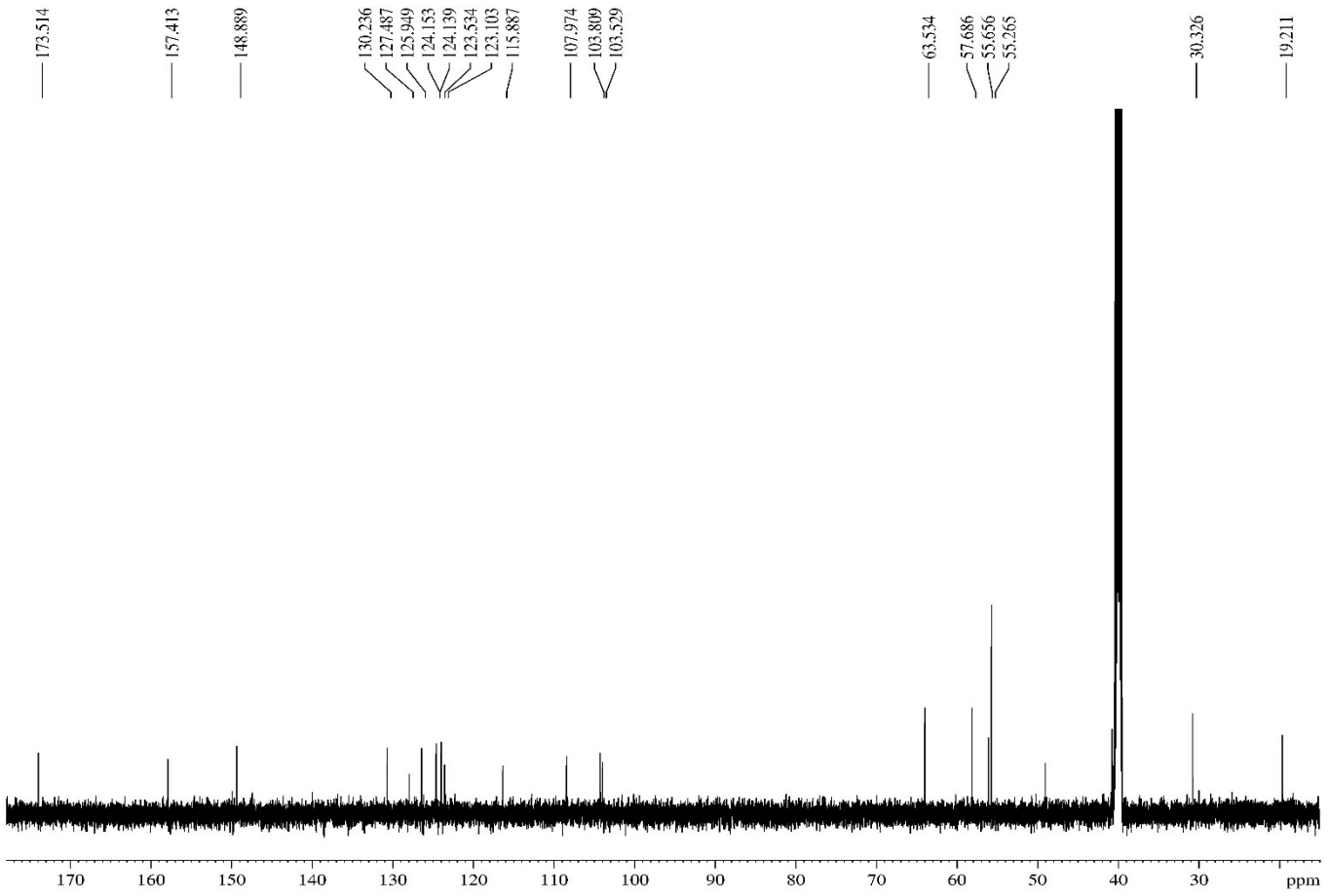


Figure 2 ^{13}C NMR spectrum of compound **1**.

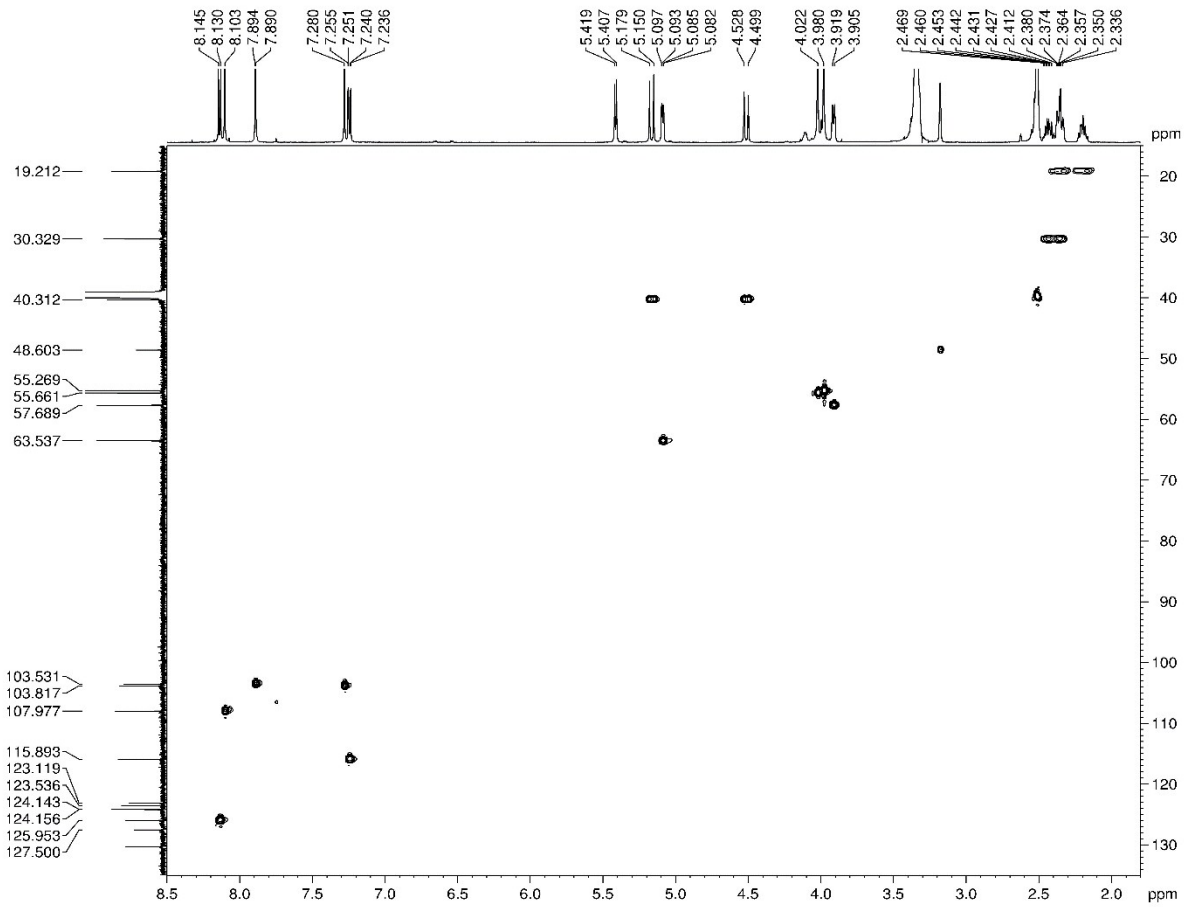


Figure 3 HSQC spectrum of compound **1**.

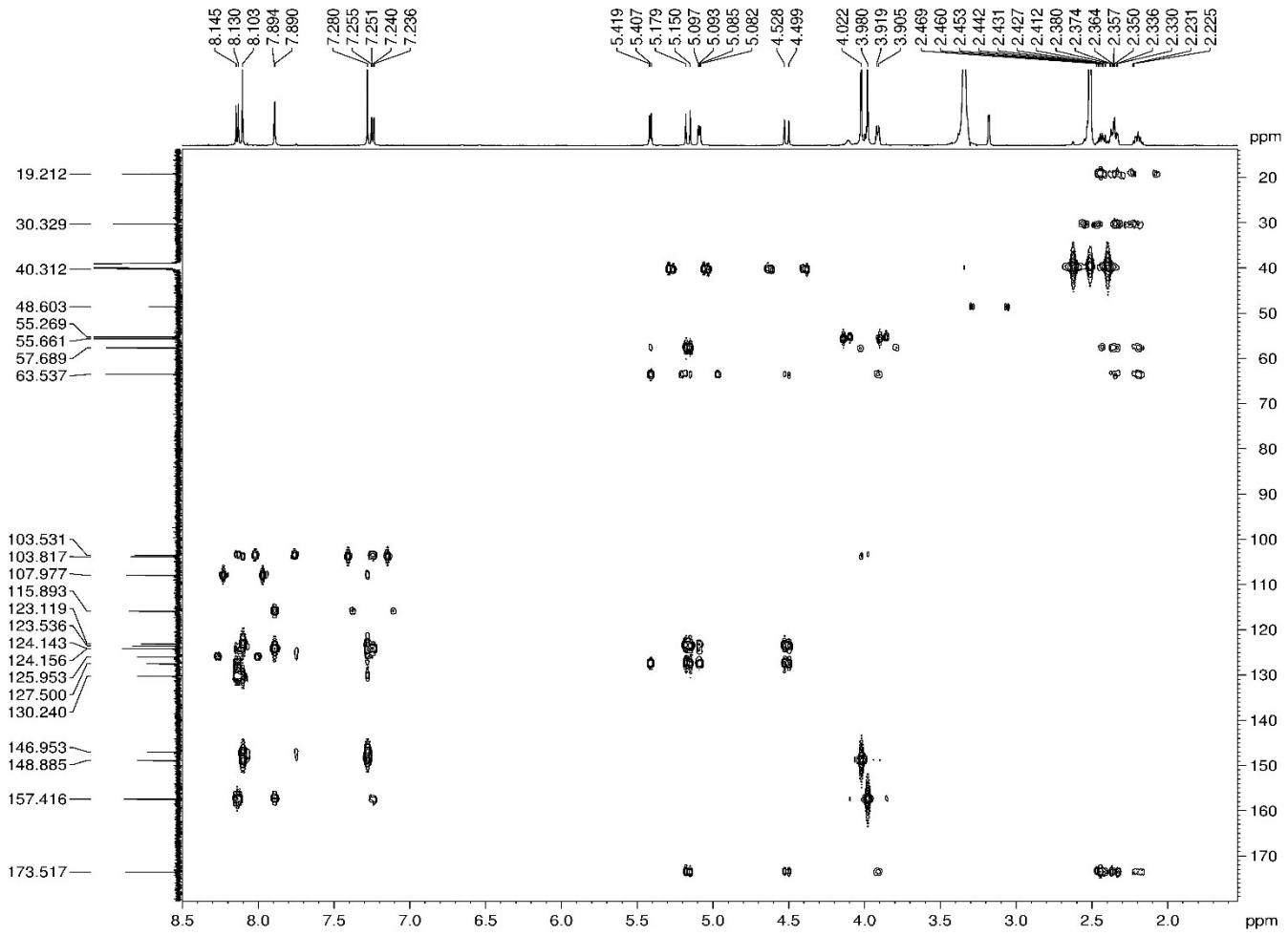


Figure 4 HMBC spectrum of compound **1**.

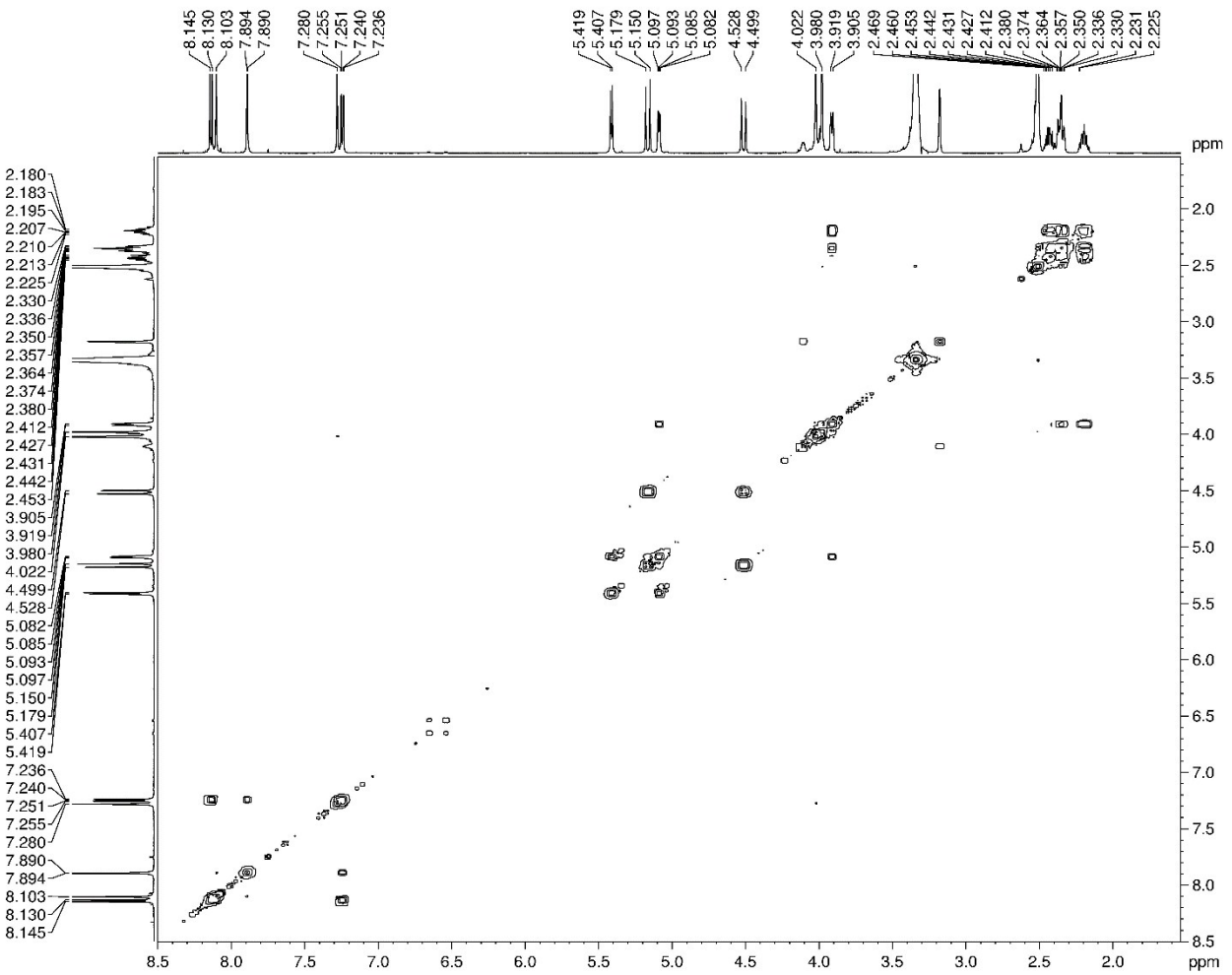


Figure 5 ^{1}H-^{1}H COSY spectrum of compound **1**.

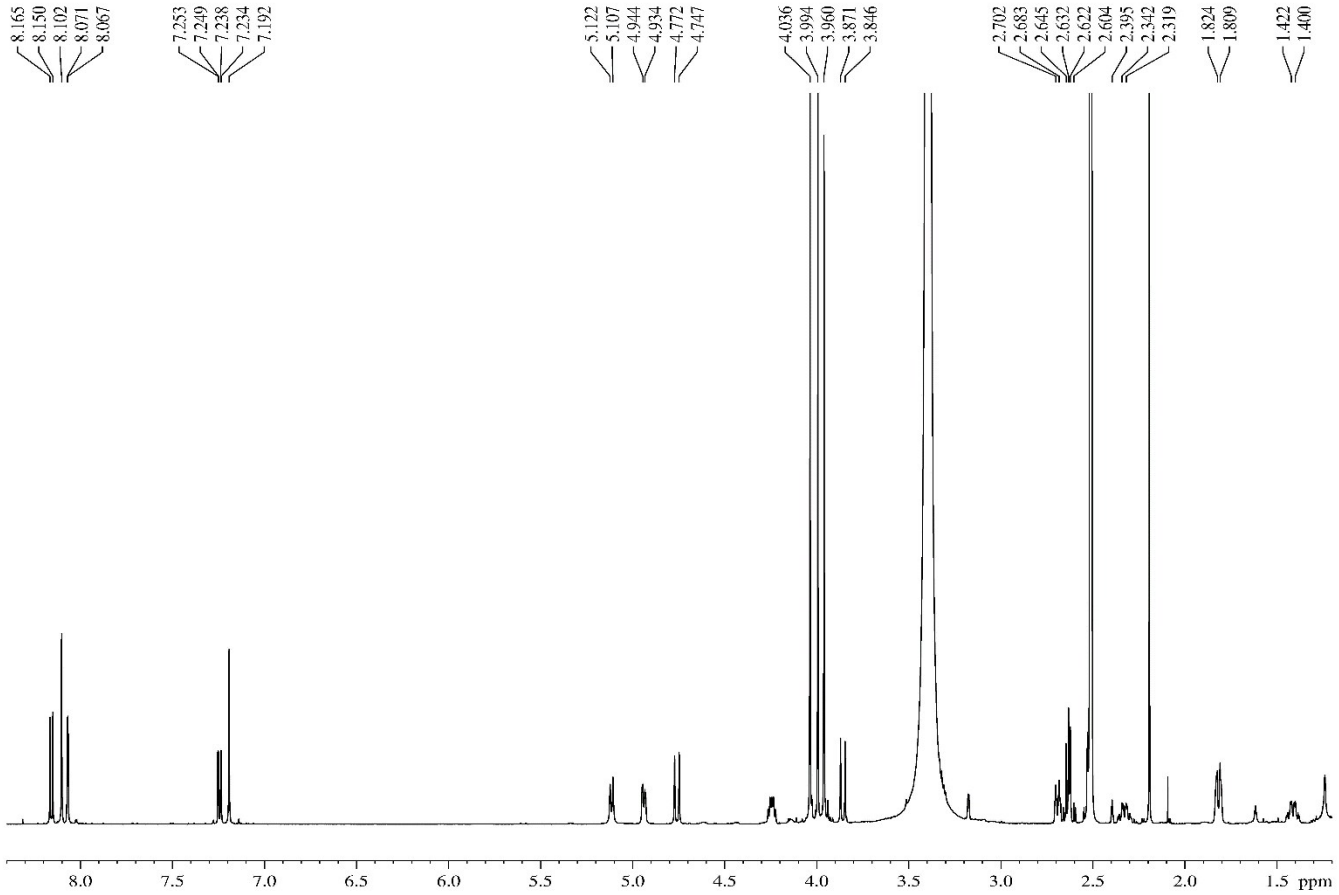


Figure 6 ^{1}H NMR spectrum of compound **2**.

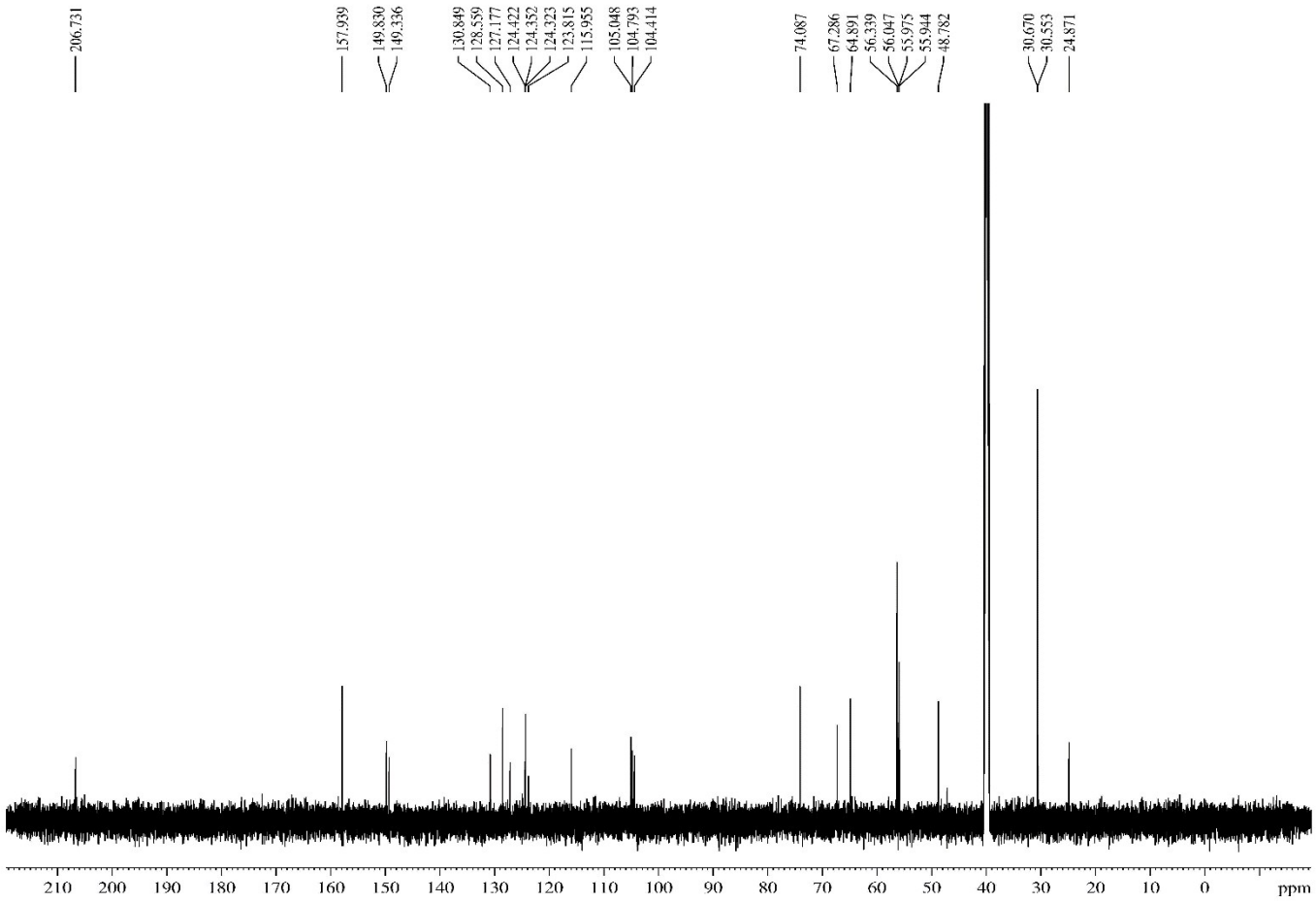


Figure 7 ^{13}C NMR spectrum of compound **2**.

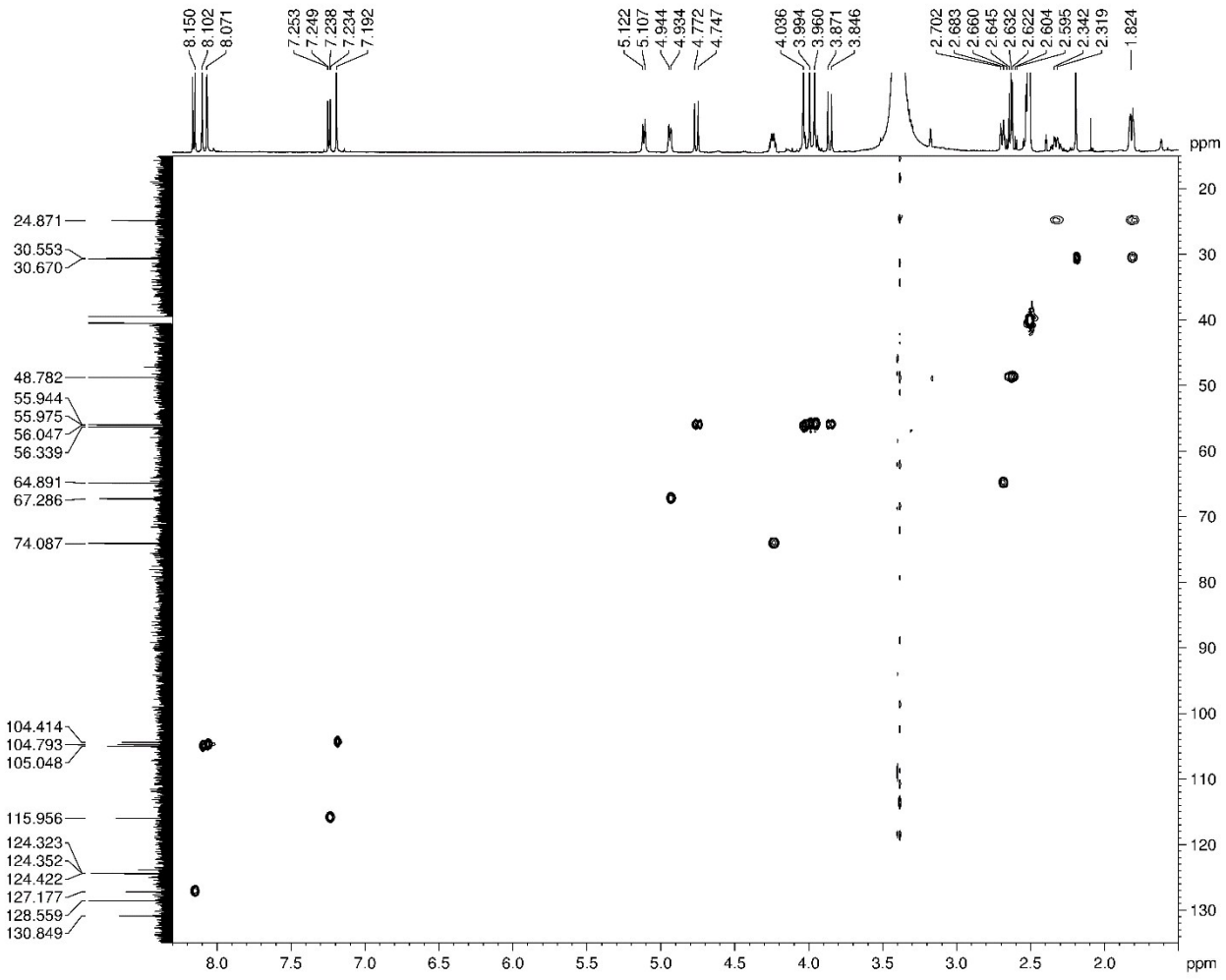


Figure 8 HSQC spectrum of compound **2**.

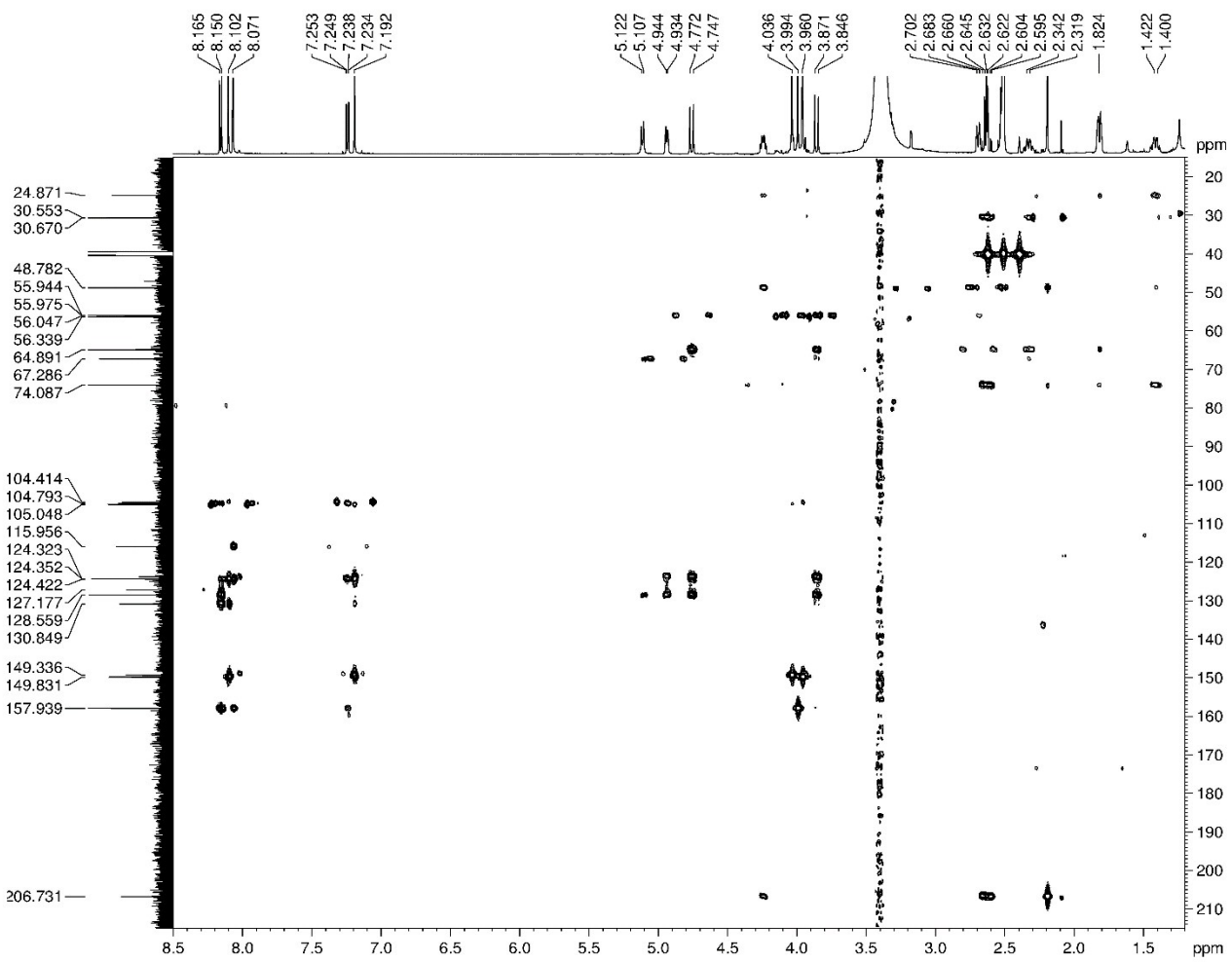


Figure 9 HMBC spectrum of compound **2**.

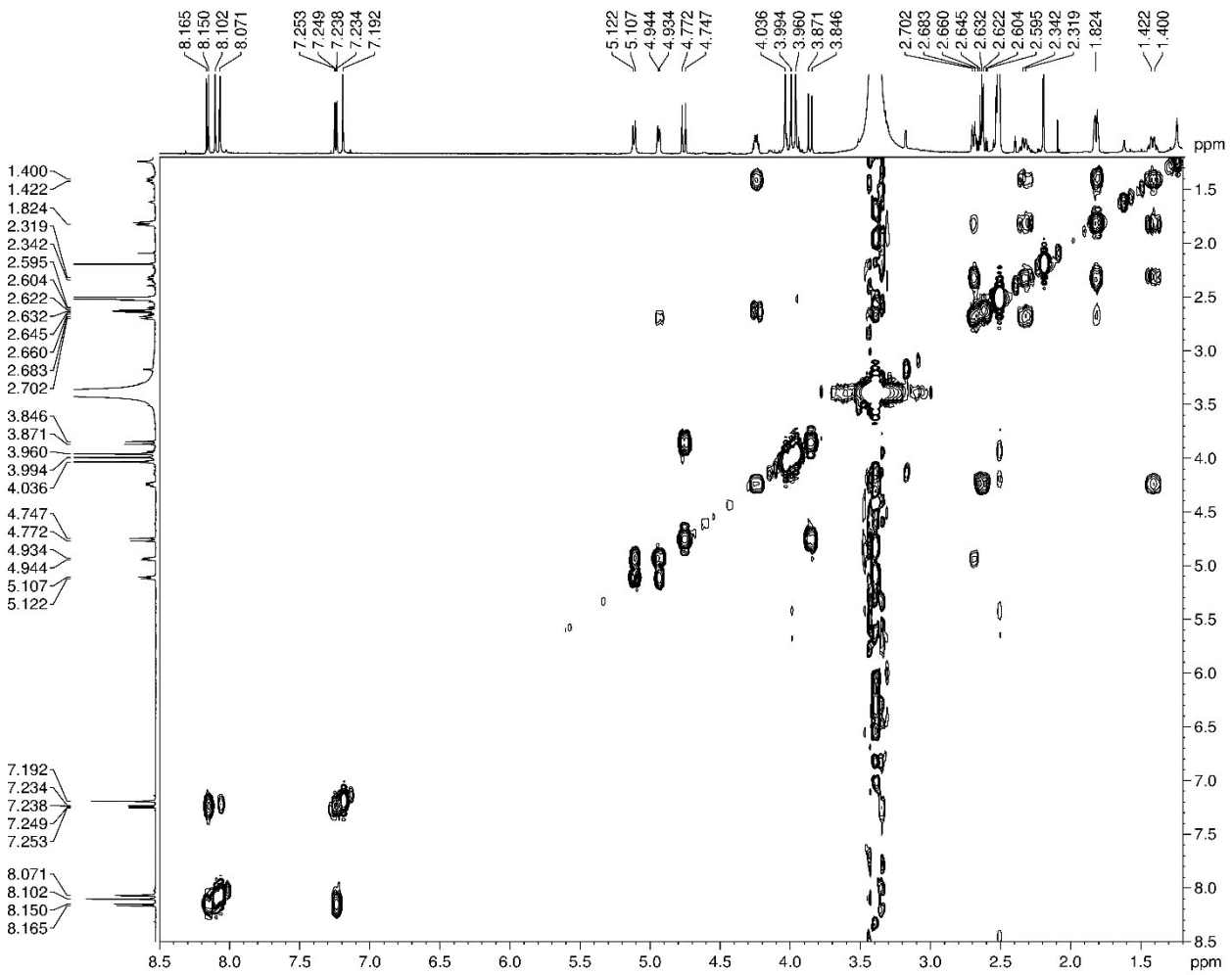


Figure 10 ^{1}H- H COSY spectrum of compound **2**.

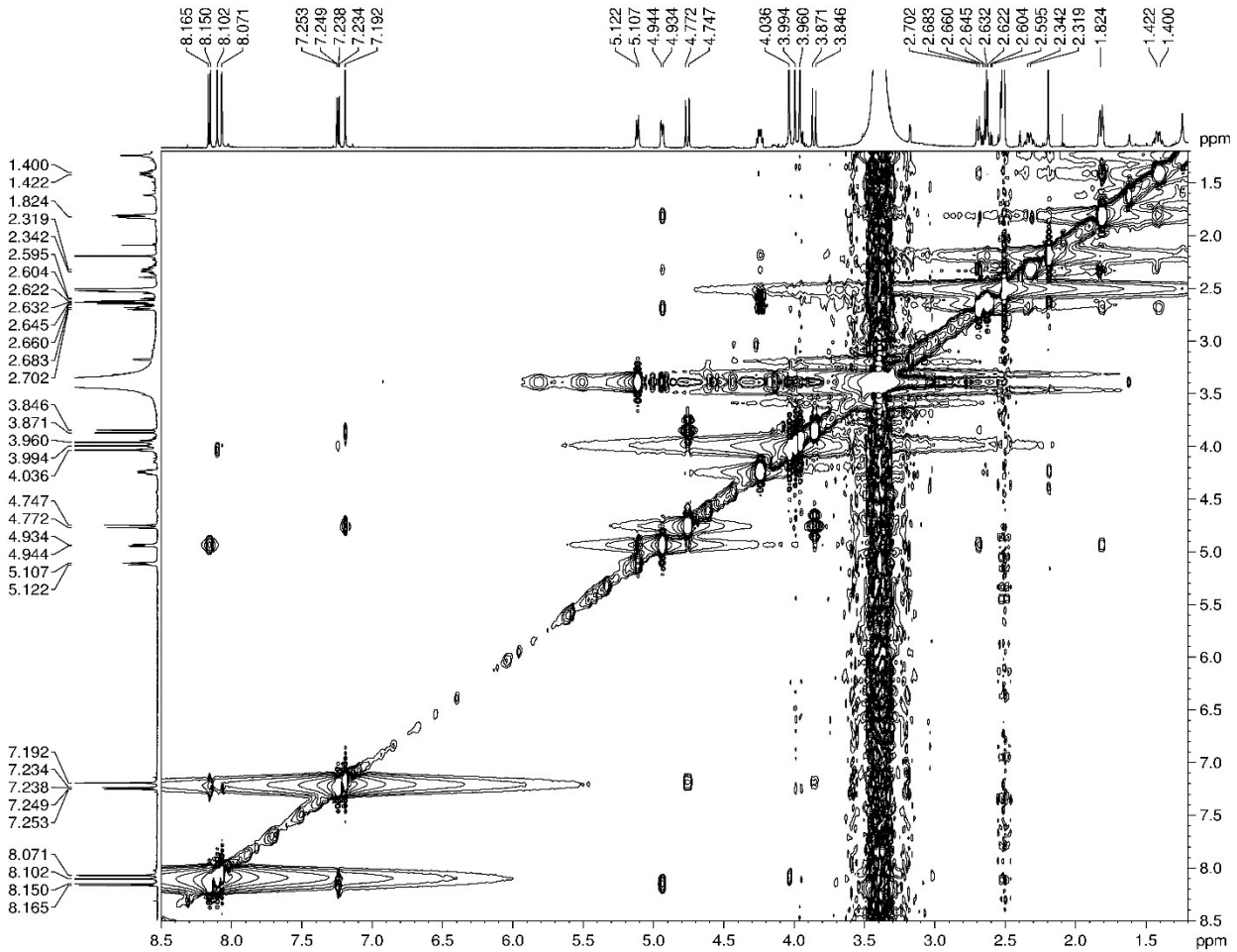


Figure 11 NOESY spectrum of compound **2**.

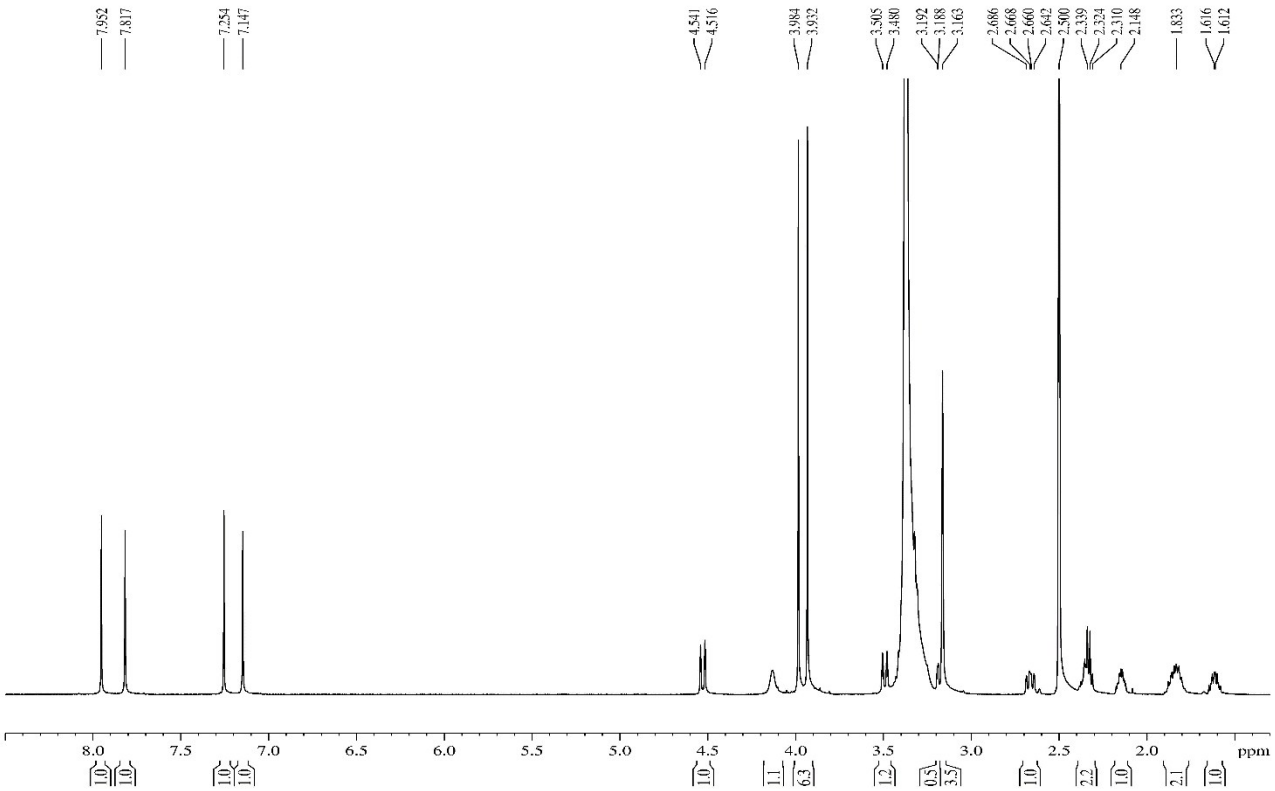


Figure 12 ^{1}H NMR spectrum of compound **3**.

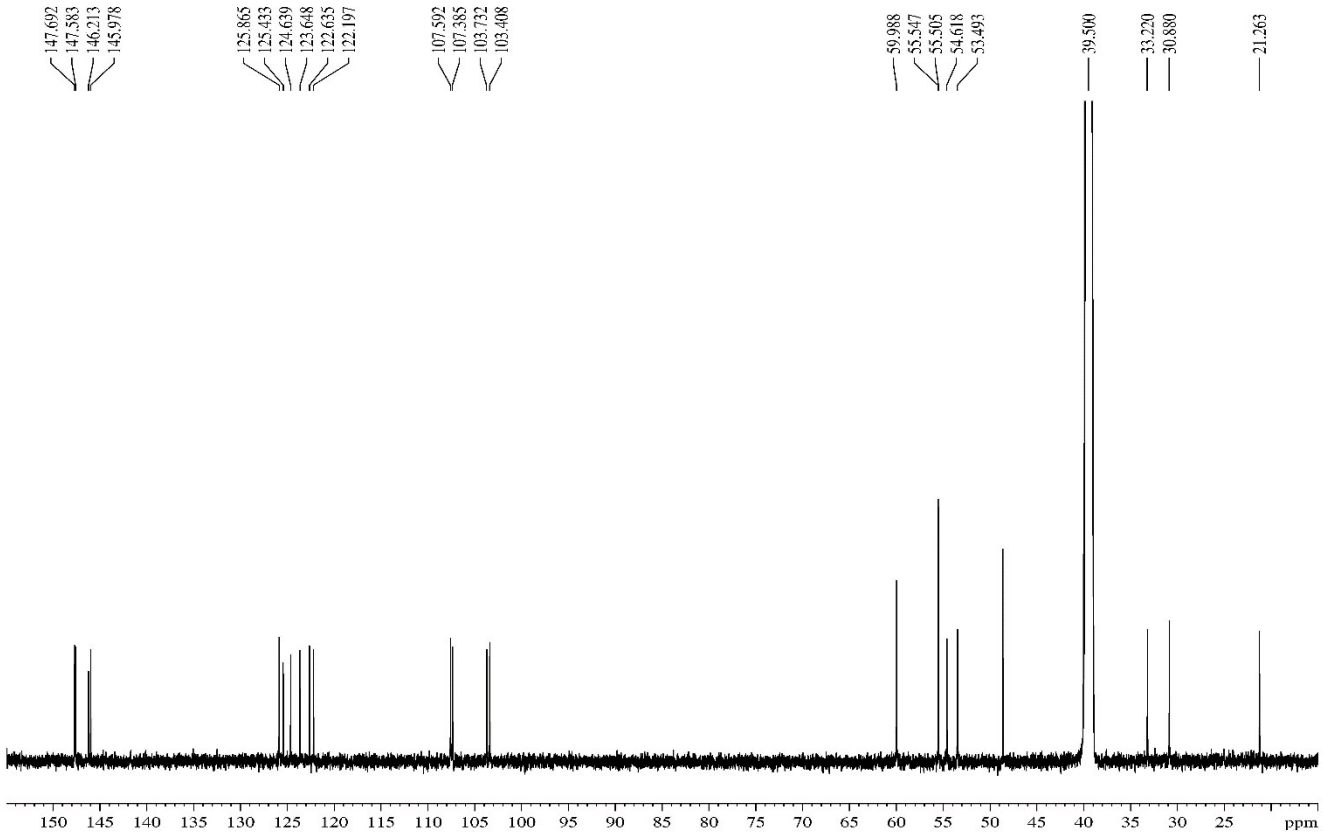
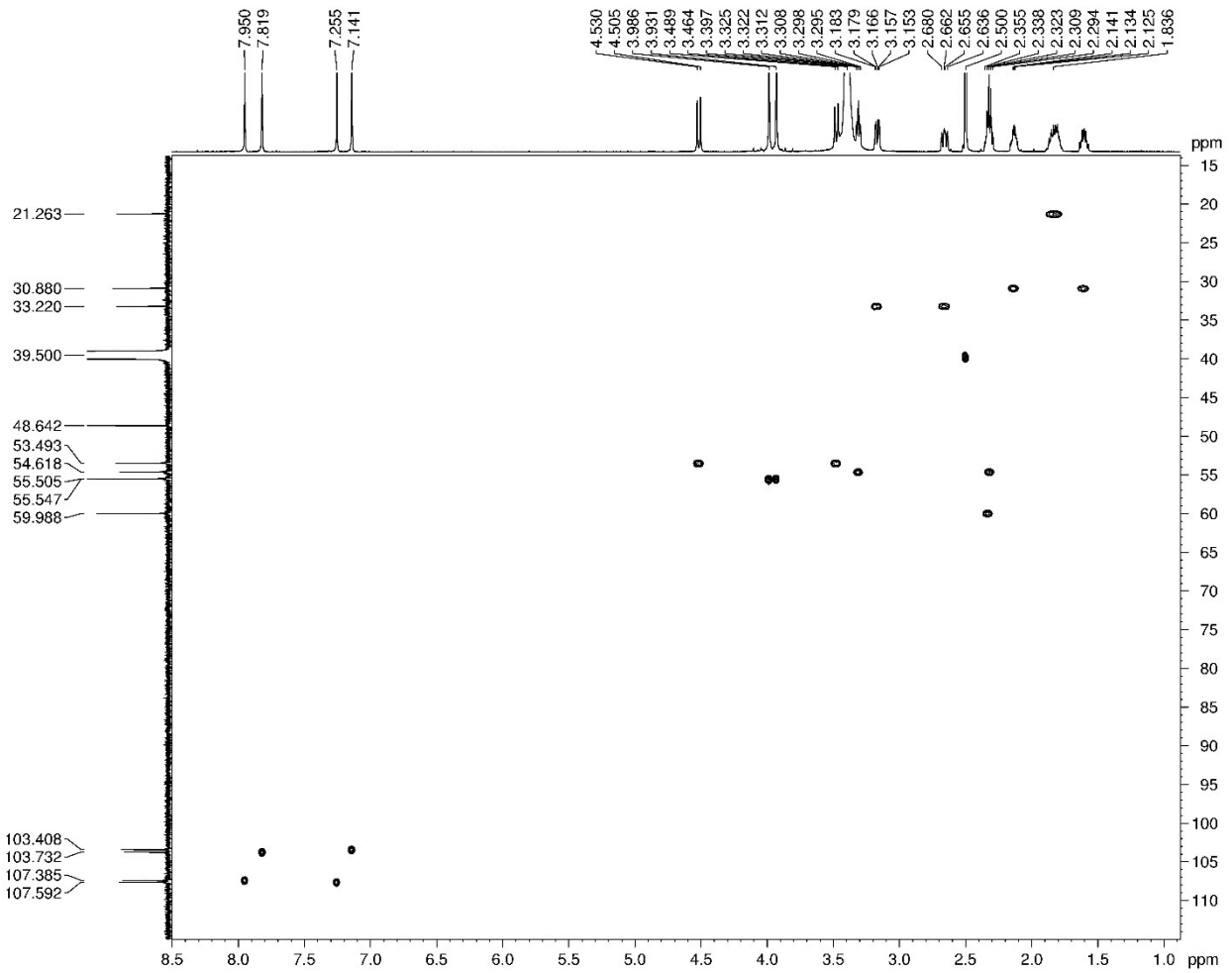


Figure 13 ^{13}C NMR spectrum of compound **3**.



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48 **Figure 14** HSQC spectrum of compound **3**.

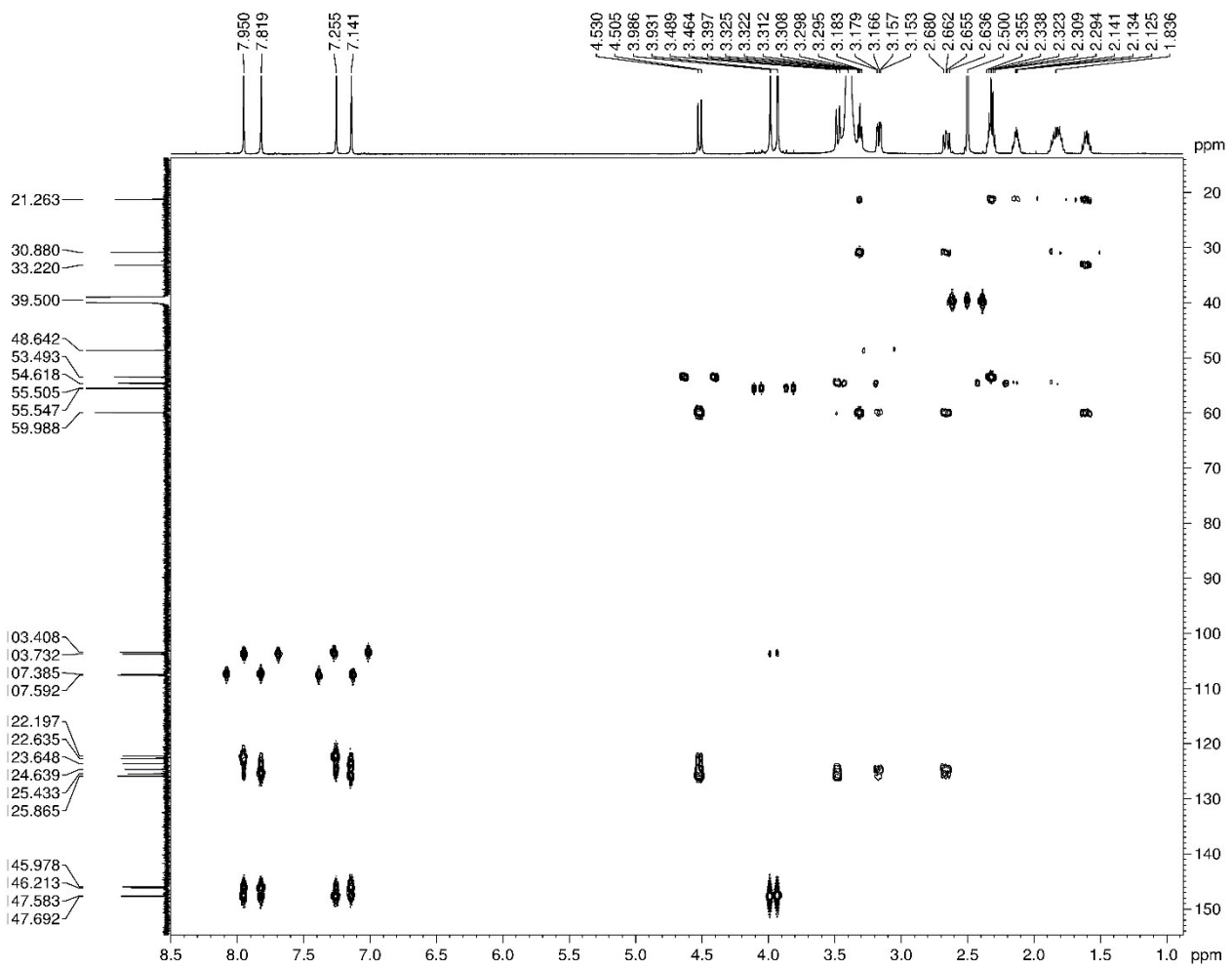
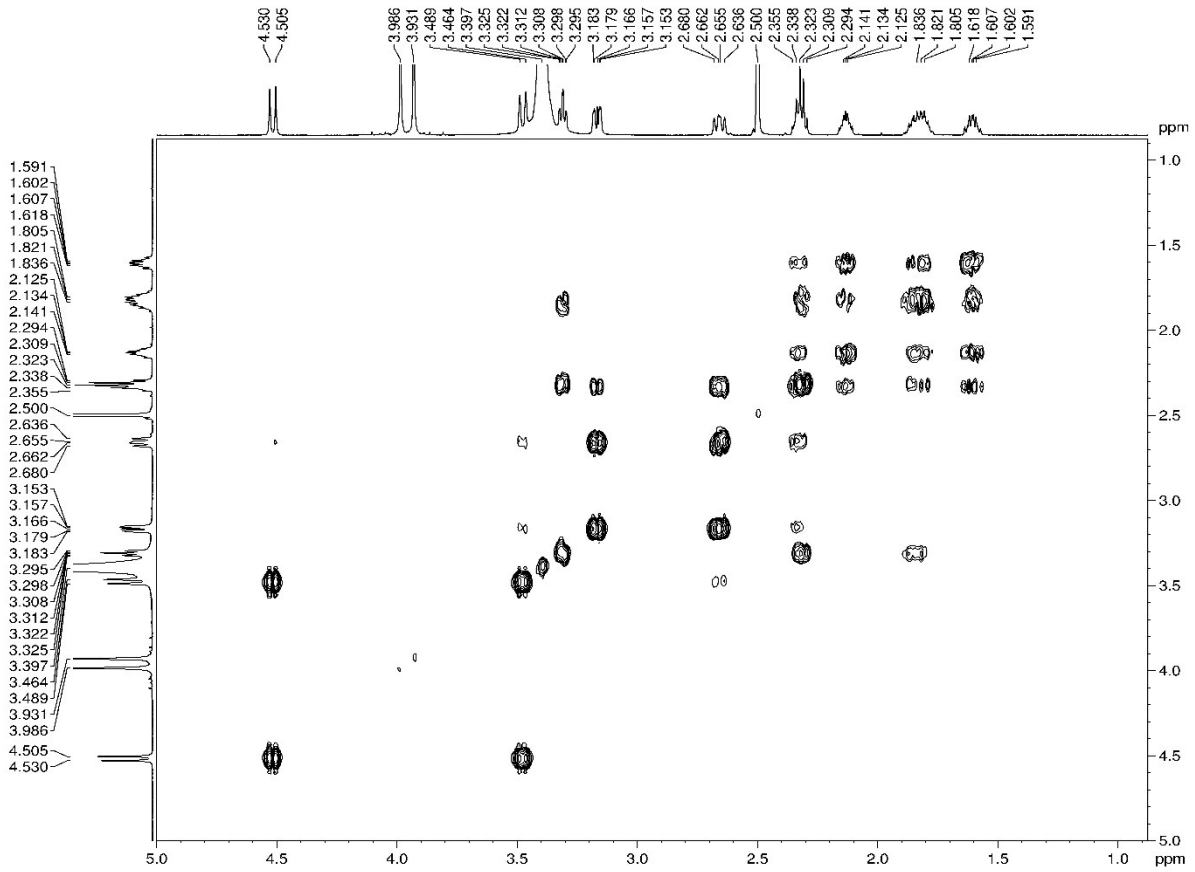


Figure 15 HMBC spectrum of compound **3**.



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52 **Figure 16** ^{1}H-^{1}H COSY spectrum of compound **3**.

53

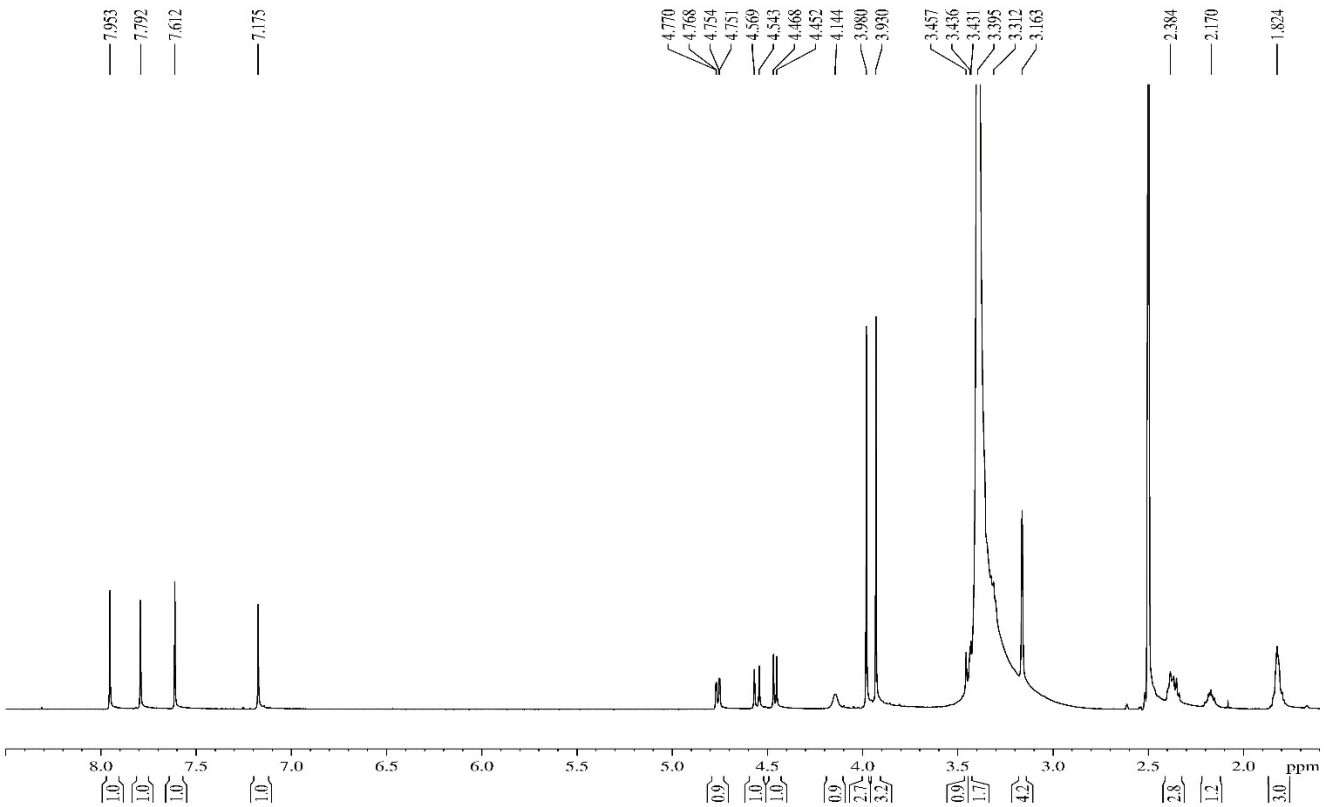


Figure 17 ^{1}H NMR spectrum of compound **4**.

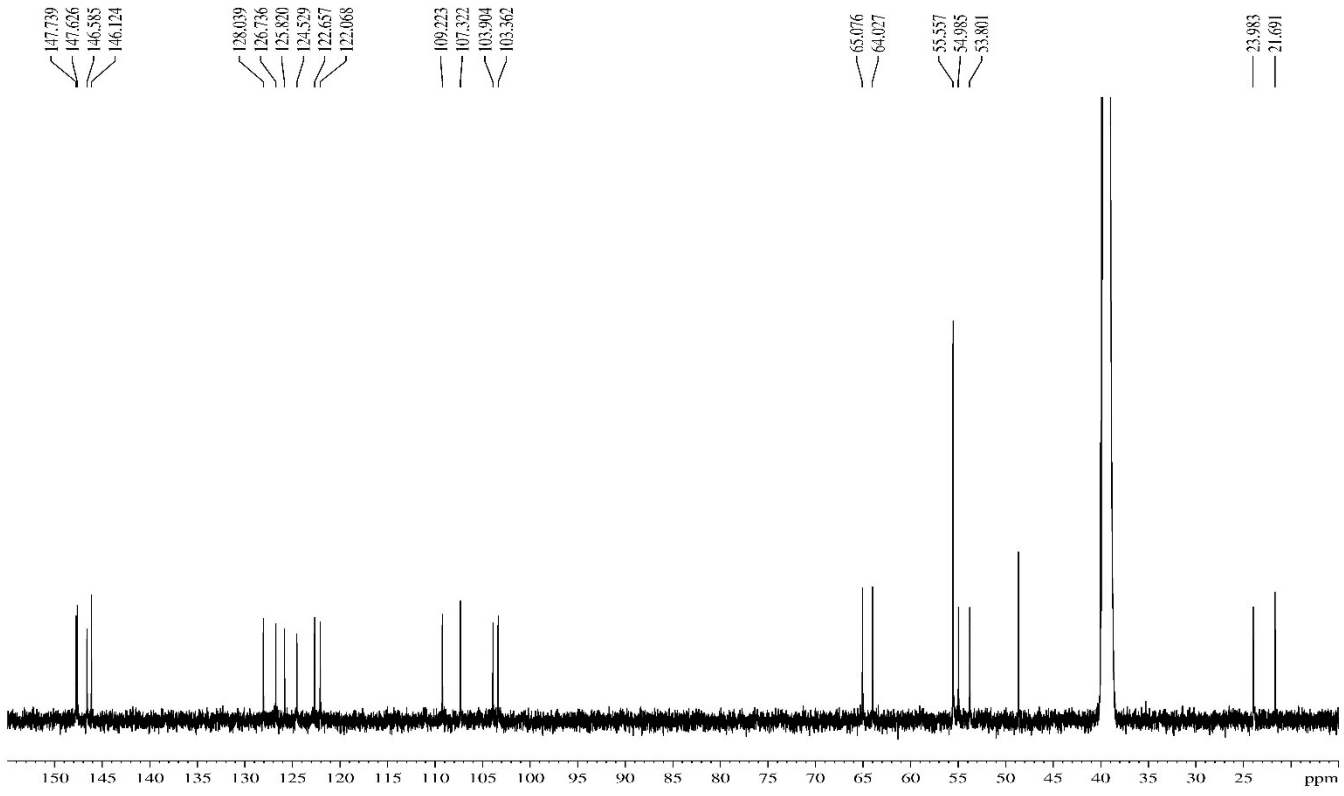


Figure 18 ^{13}C NMR spectrum of compound **4**.

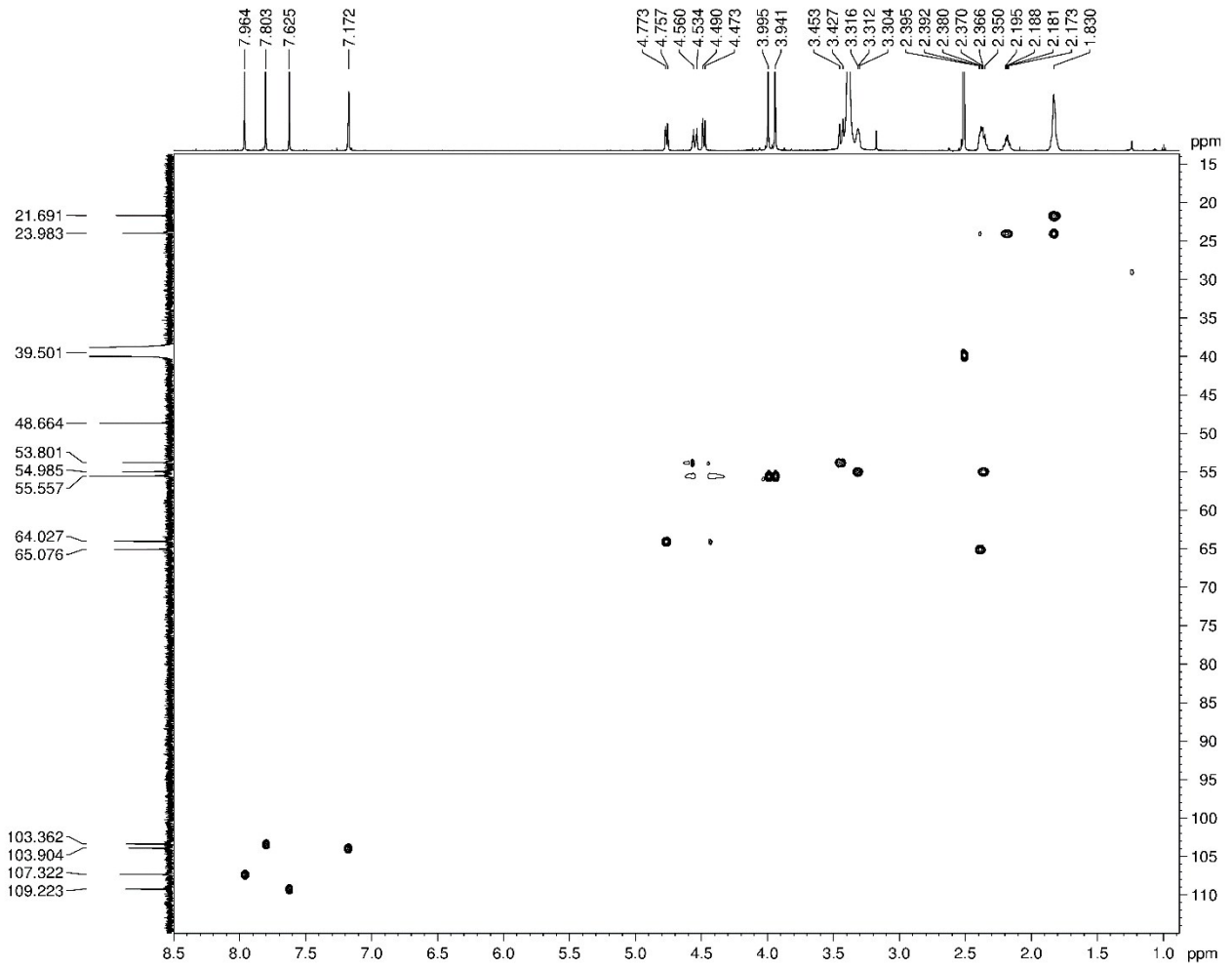
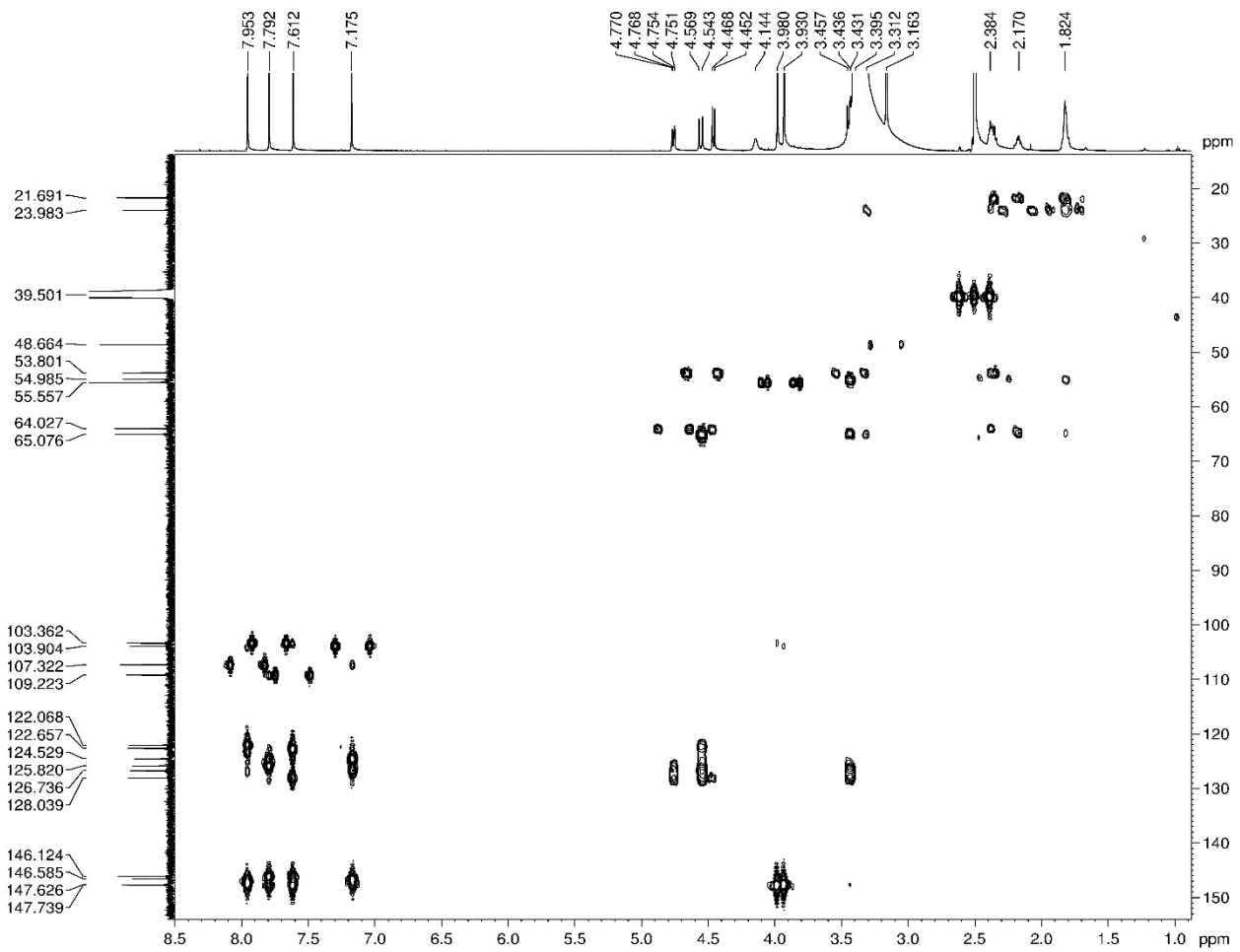


Figure 19 HSQC spectrum of compound **4**.



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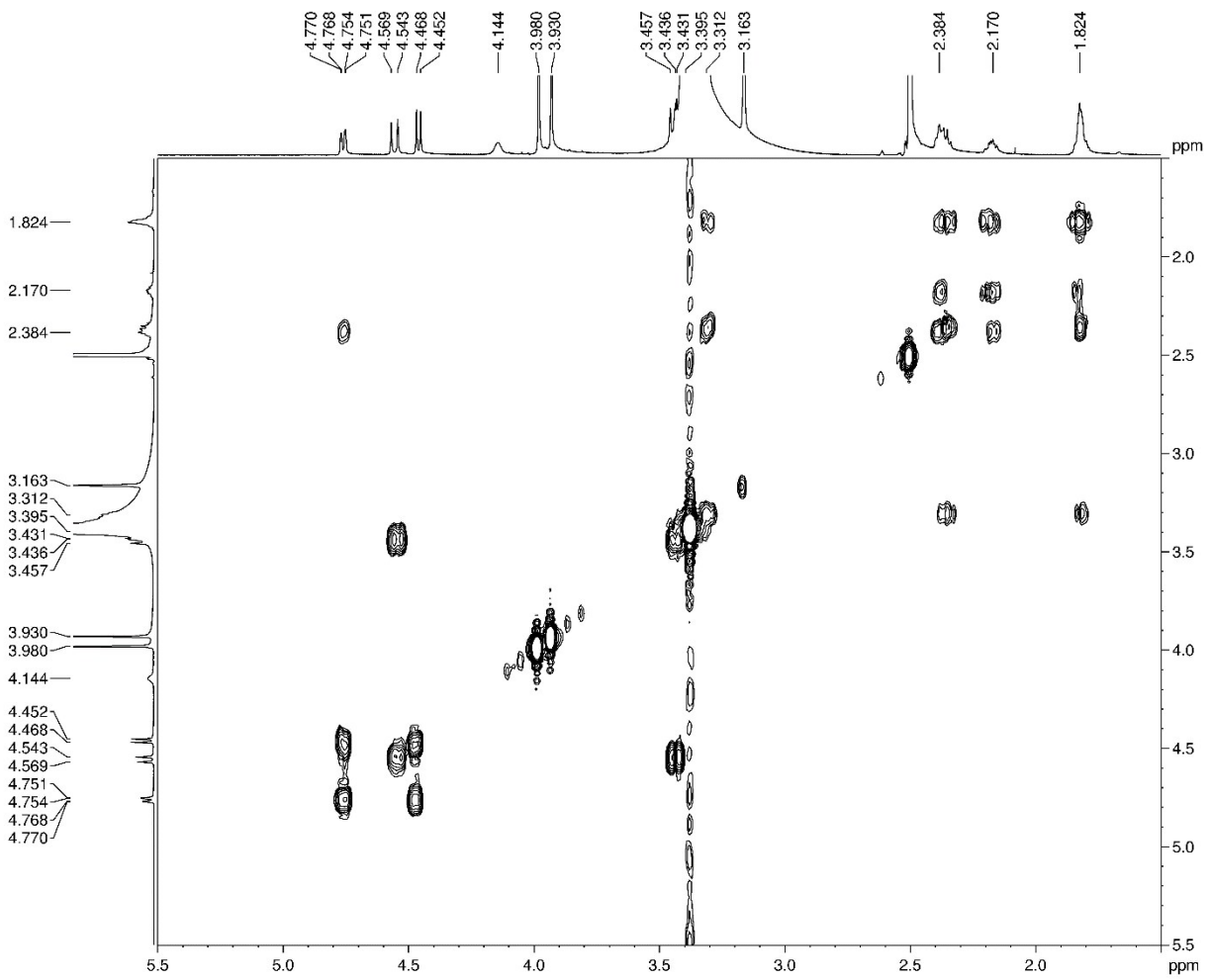
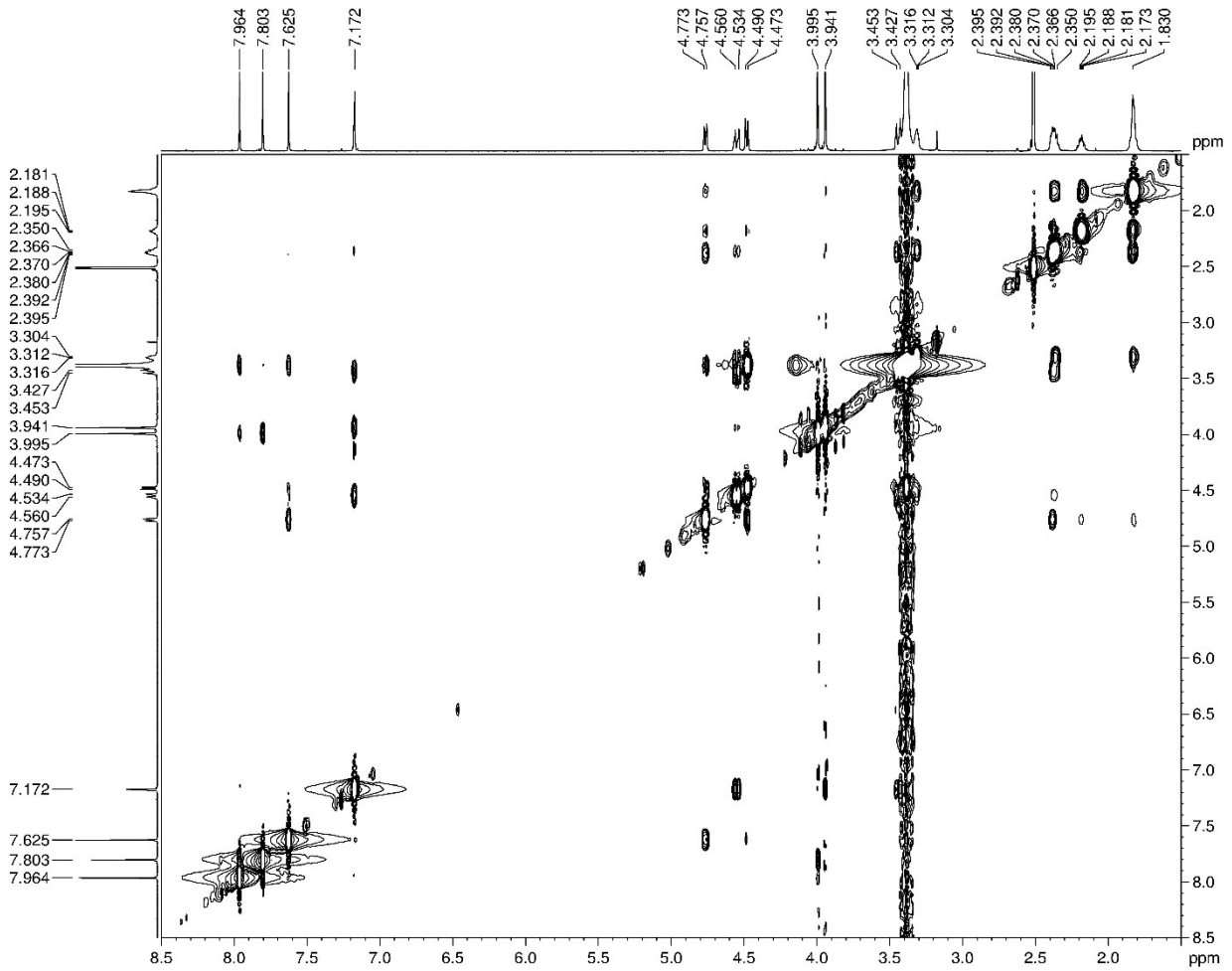


Figure 21 ^{1}H-^{1}H COSY spectrum of compound **4**.



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66 **Figure 22** NOESY spectrum of compound **4**.

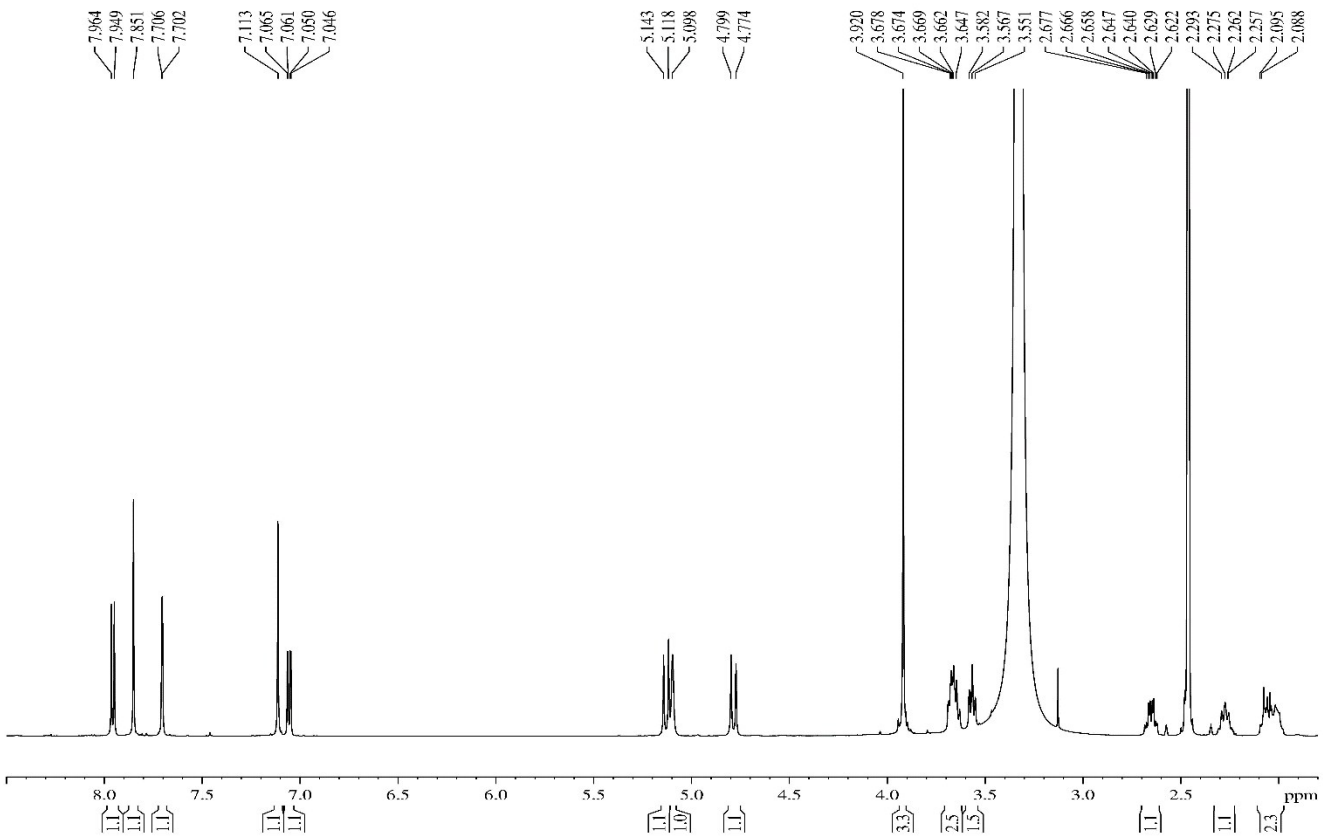
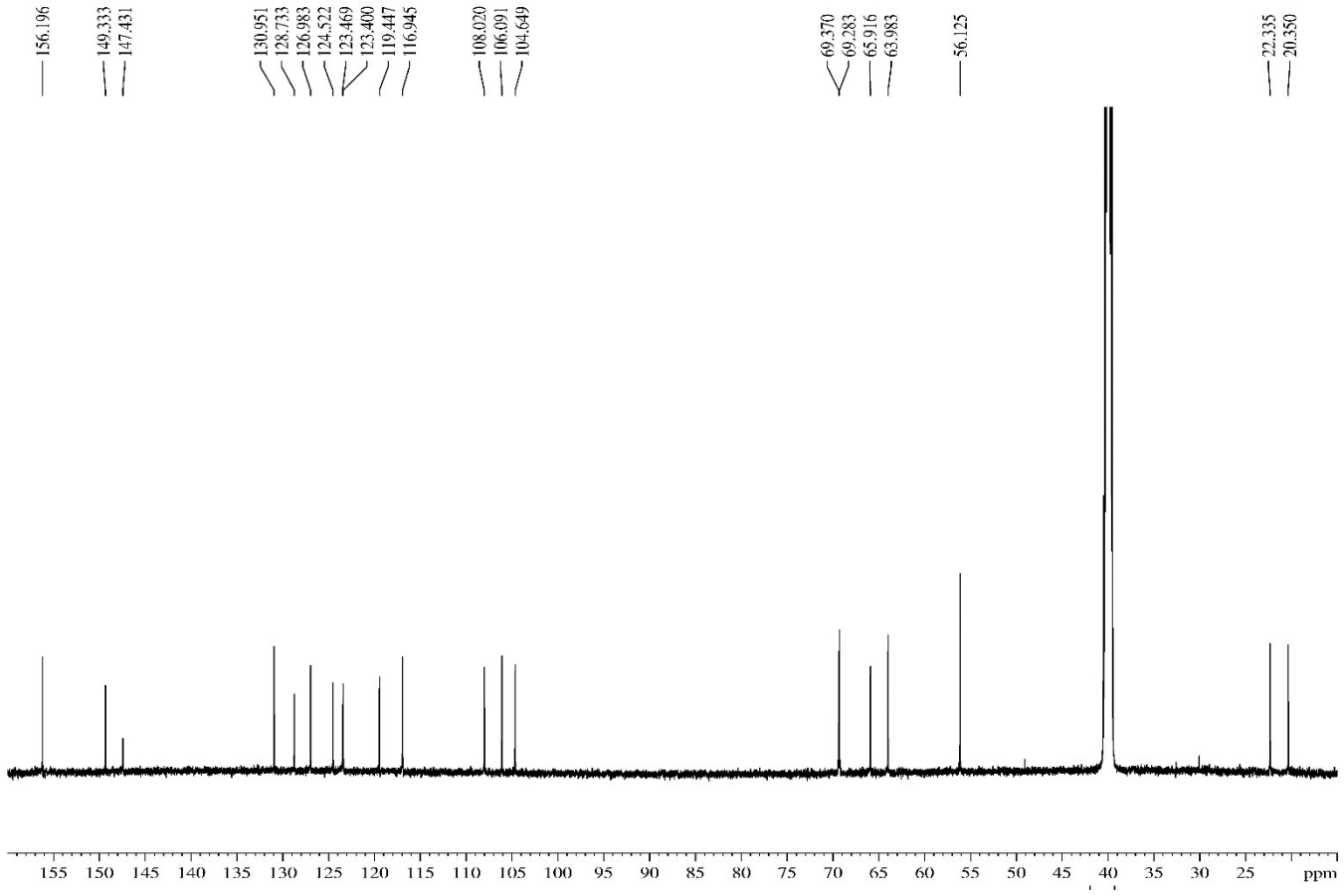


Figure 23 ^{1}H NMR spectrum of compound **5**.



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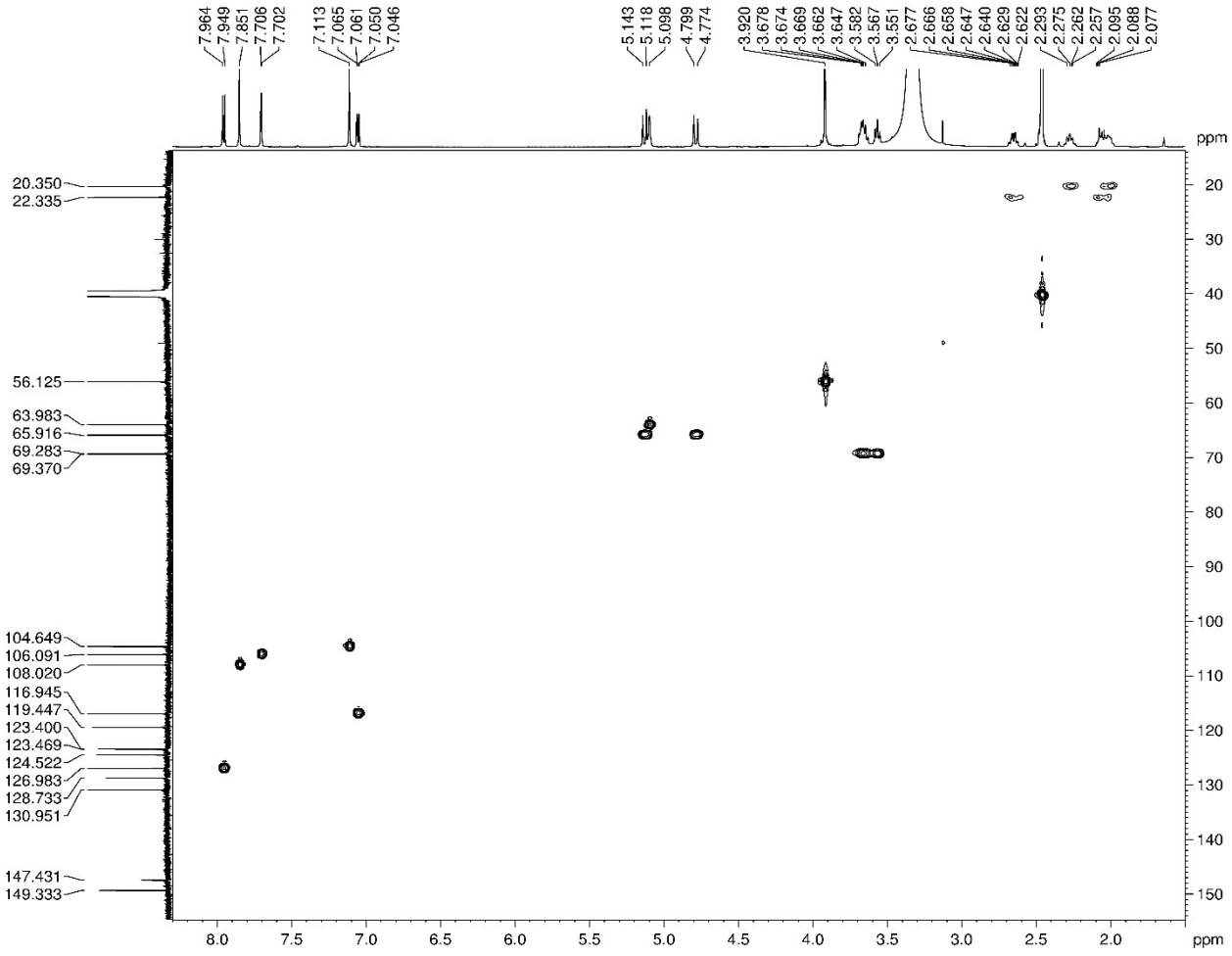


Figure 25 HSQC spectrum of compound **5**.

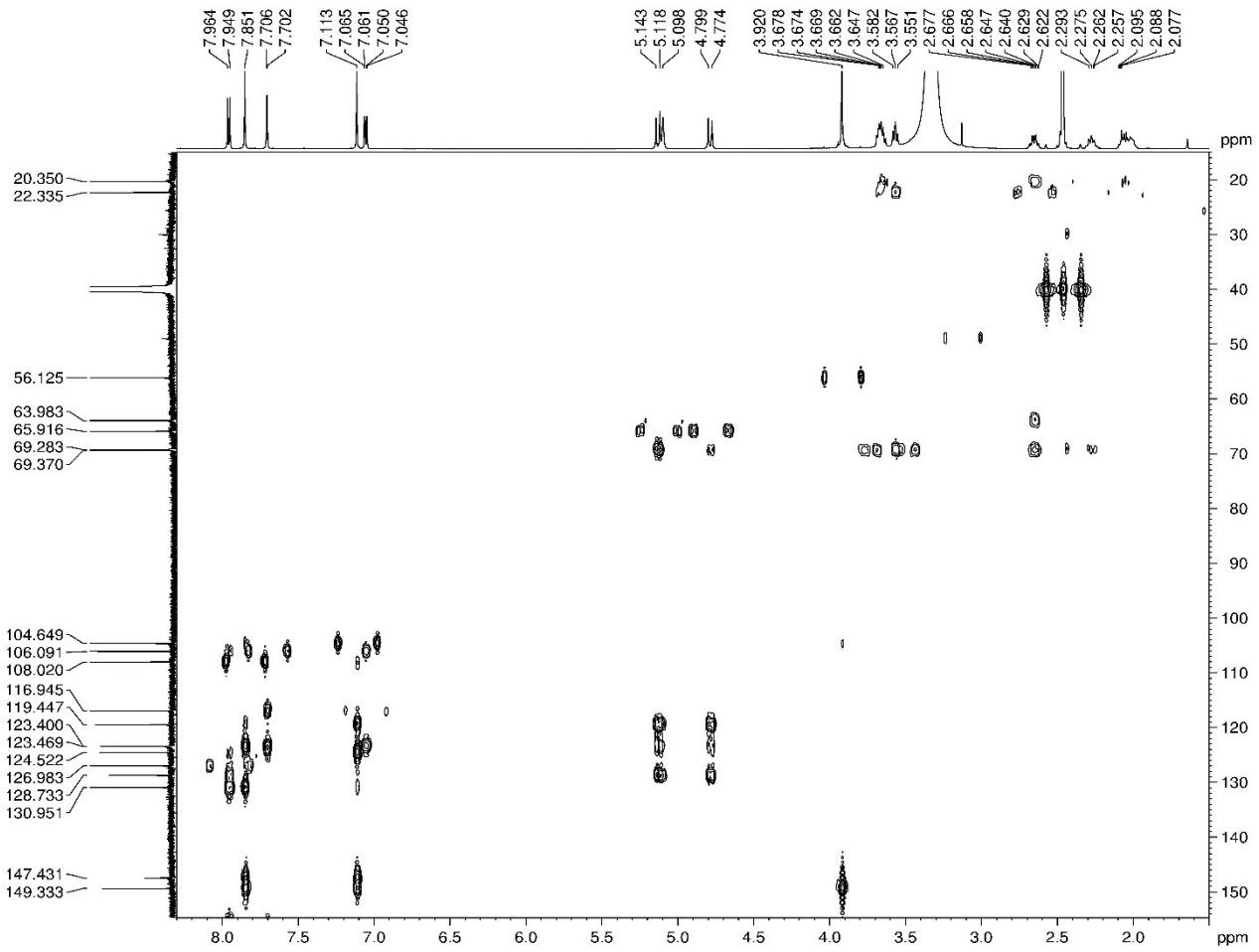
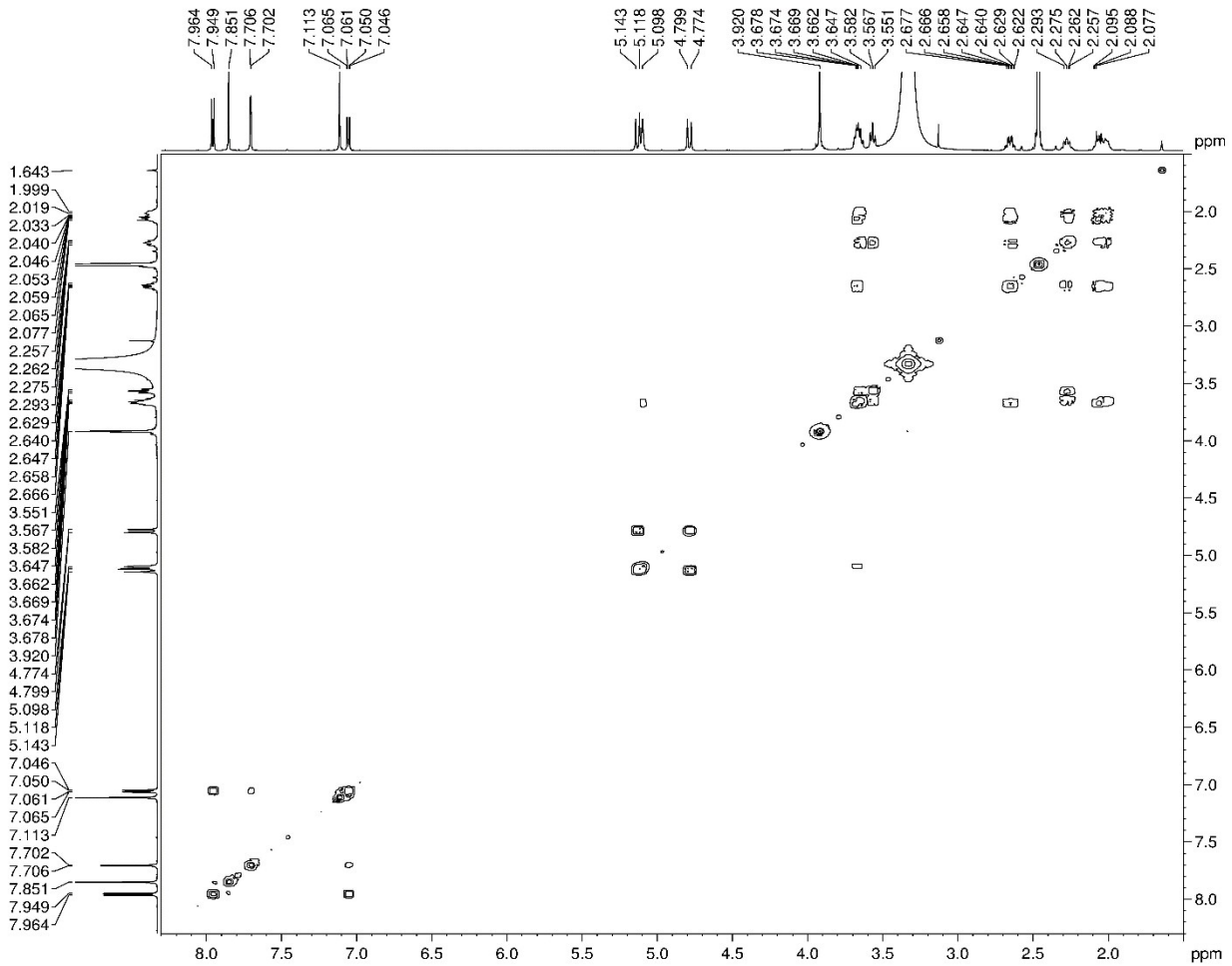


Figure 26 HMBC spectrum of compound **5**.



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79 **Figure 27** ^{1}H-^{1}H COSY spectrum of compound **5**.

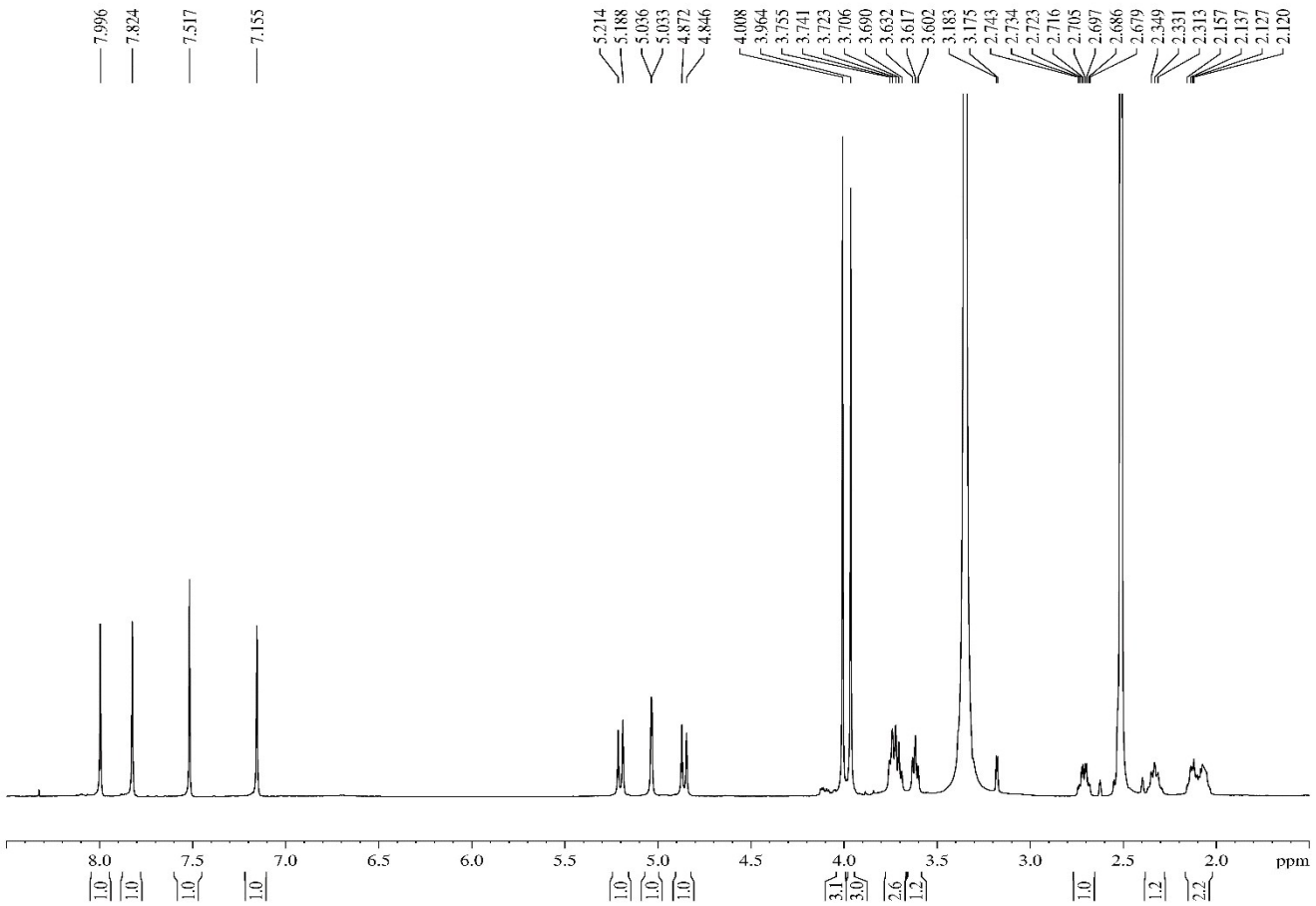


Figure 28 ^{1}H NMR spectrum of compound **6**.

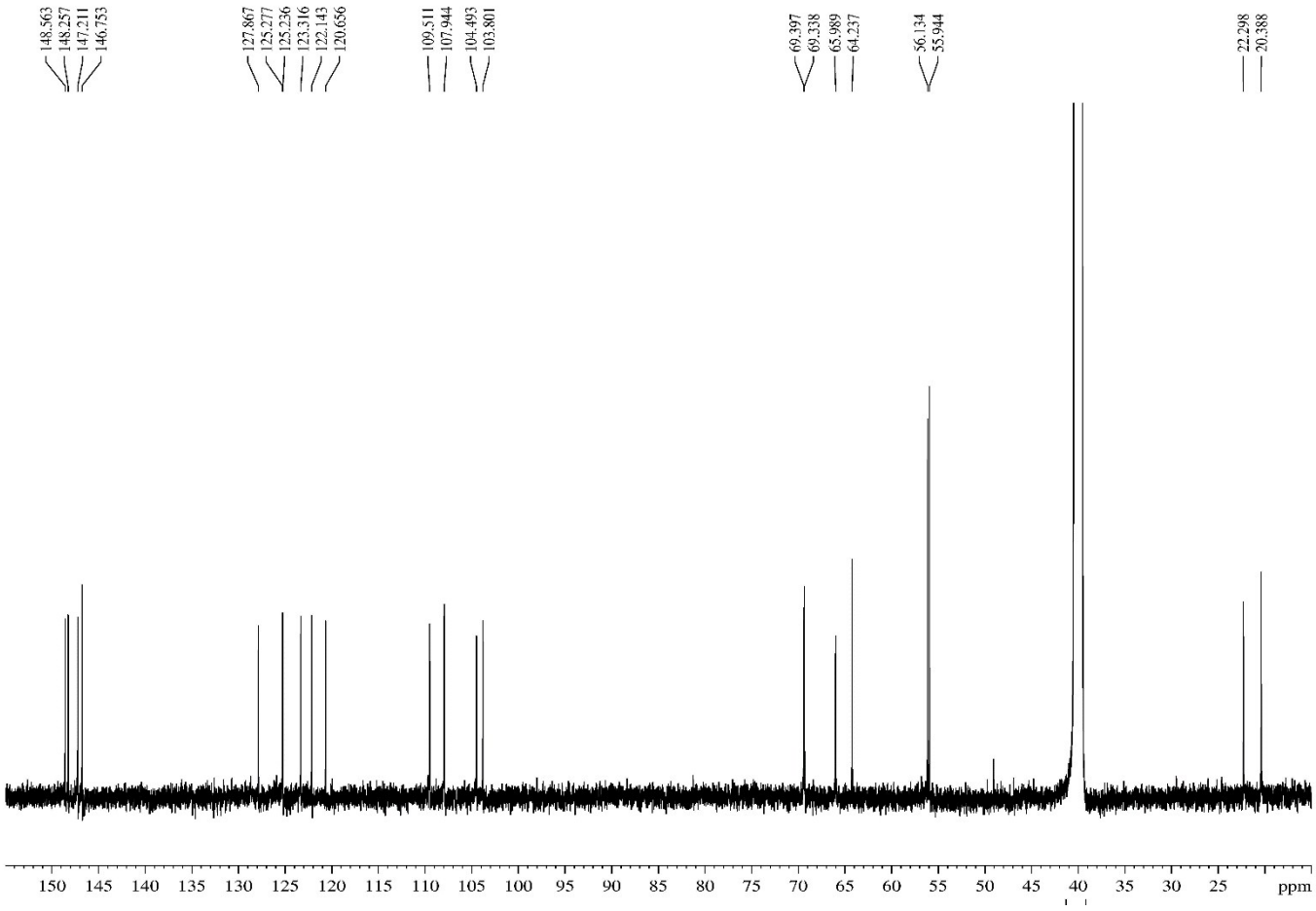


Figure 29 ^{13}C NMR spectrum of compound **6**.

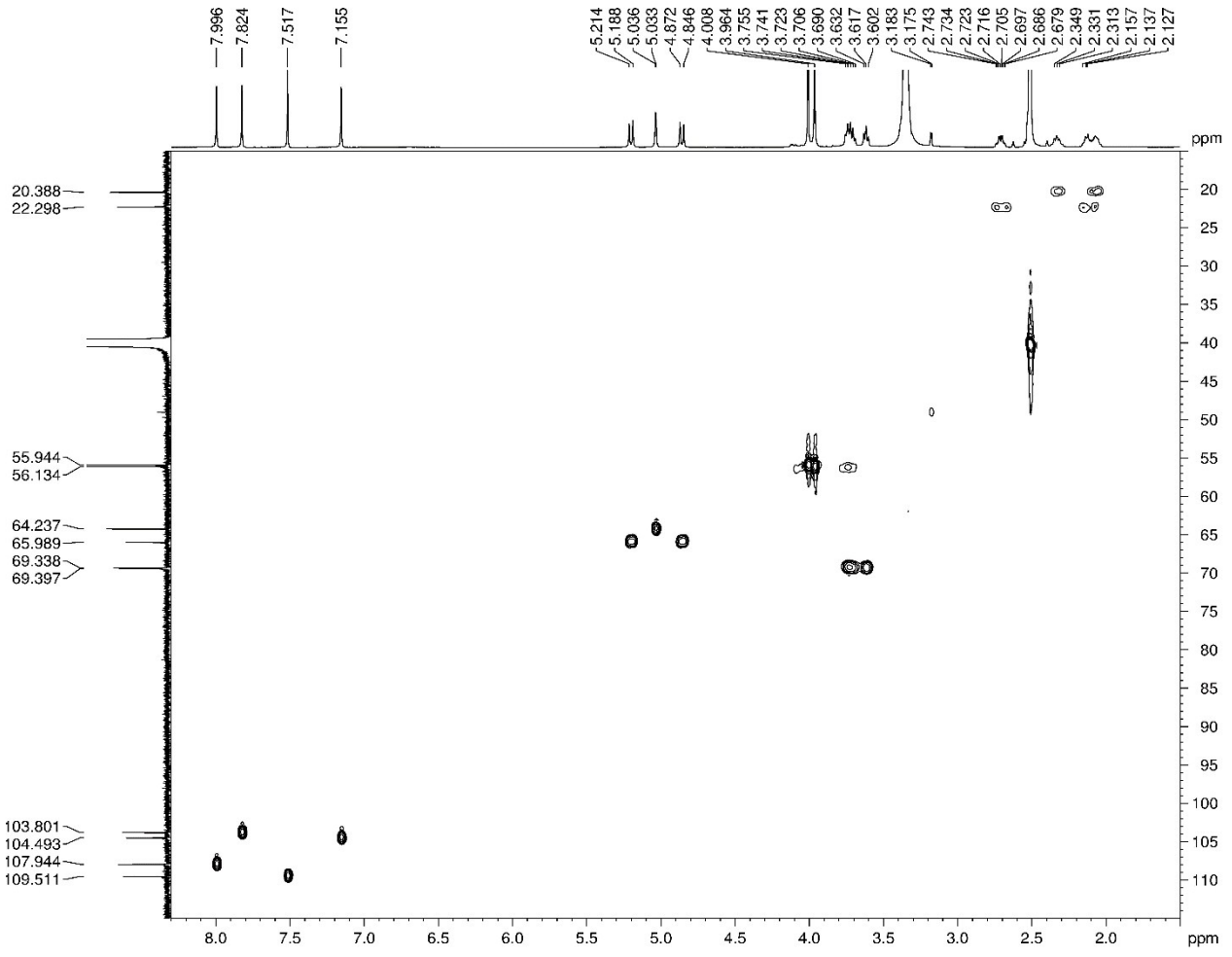


Figure 30 HSQC spectrum of compound **6**.

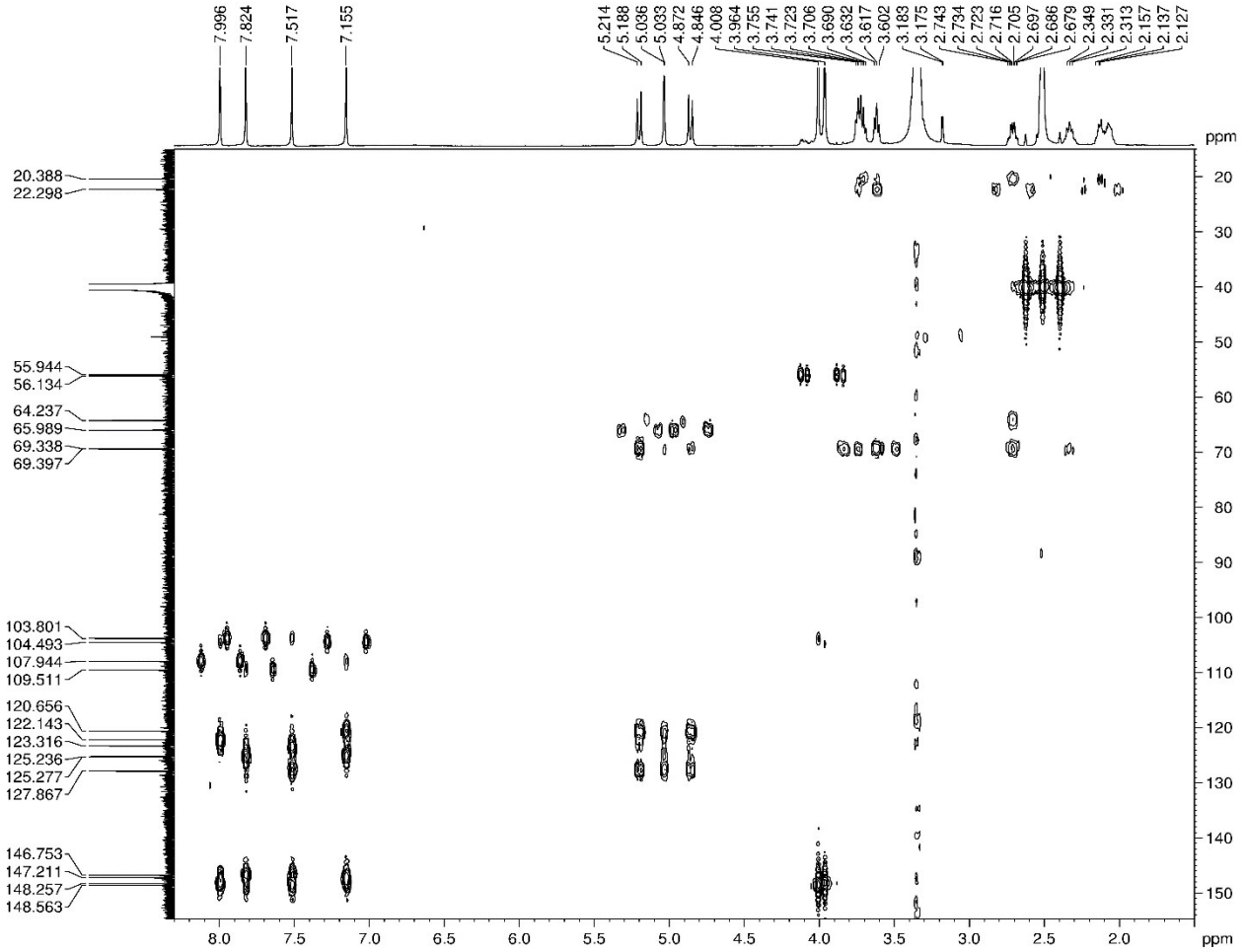


Figure 31 HMBC spectrum of compound **6**.

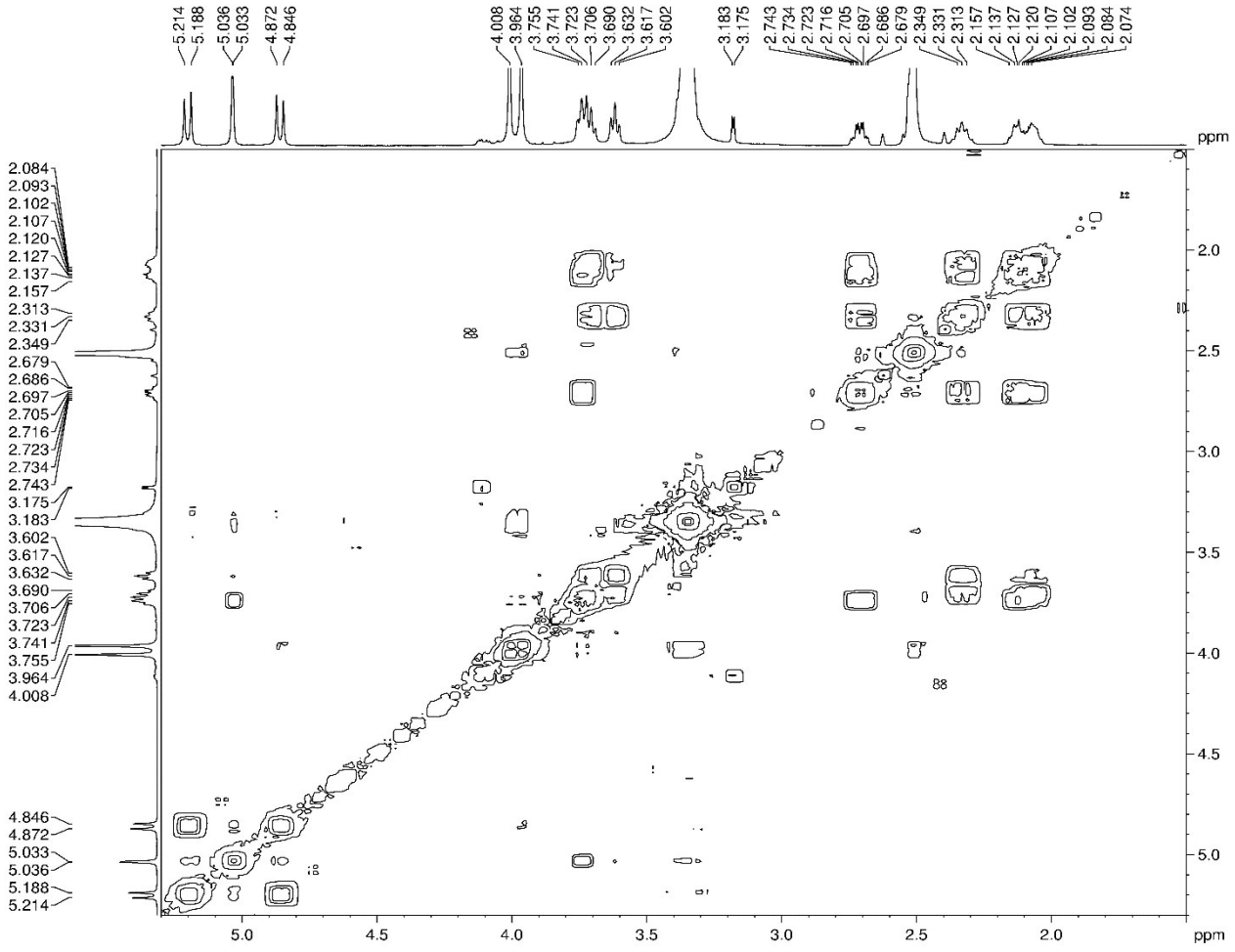
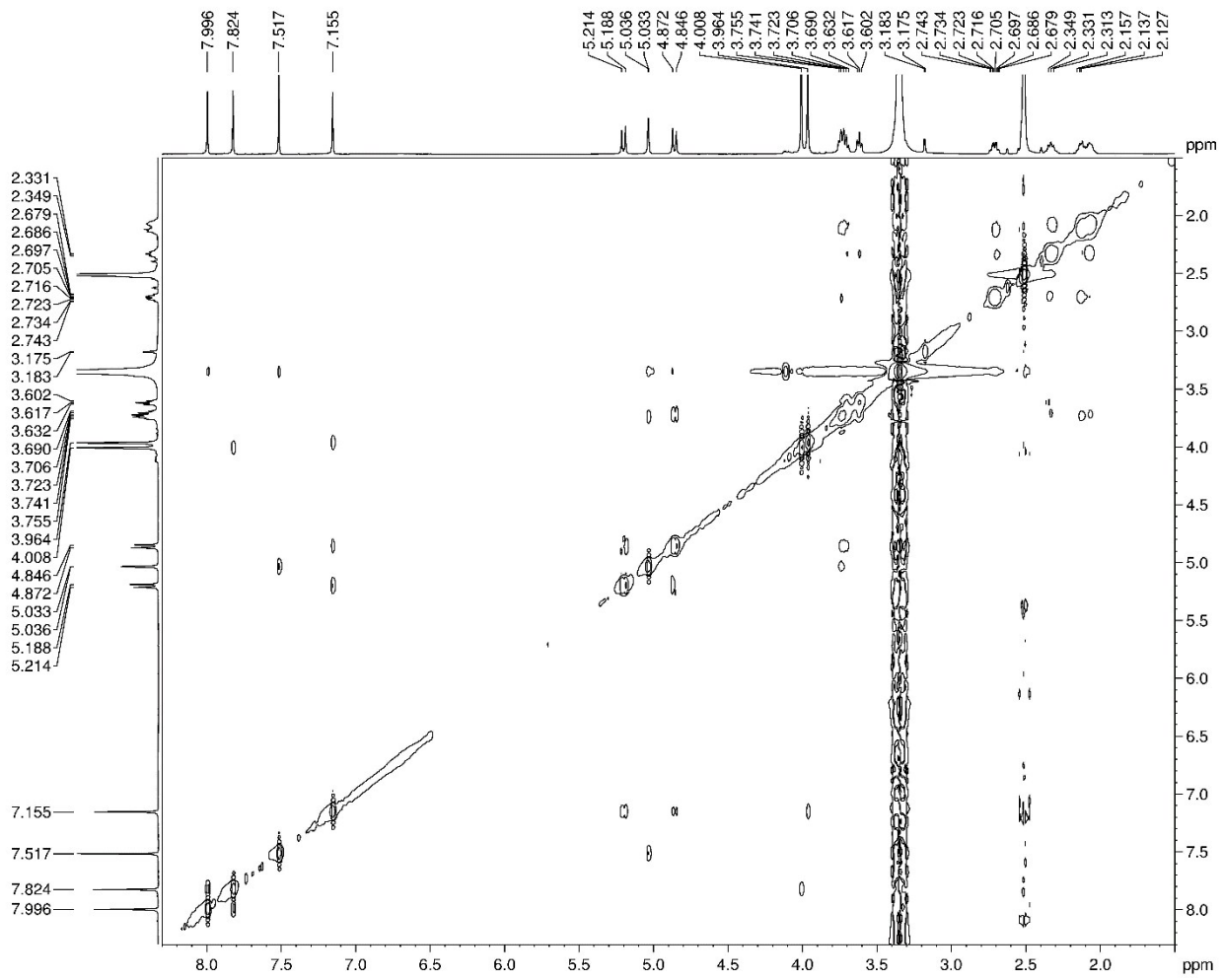


Figure 32 ^{1}H-^{1}H COSY spectrum of compound **6**.



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93 **Figure 33** NOESY spectrum of compound **6**.

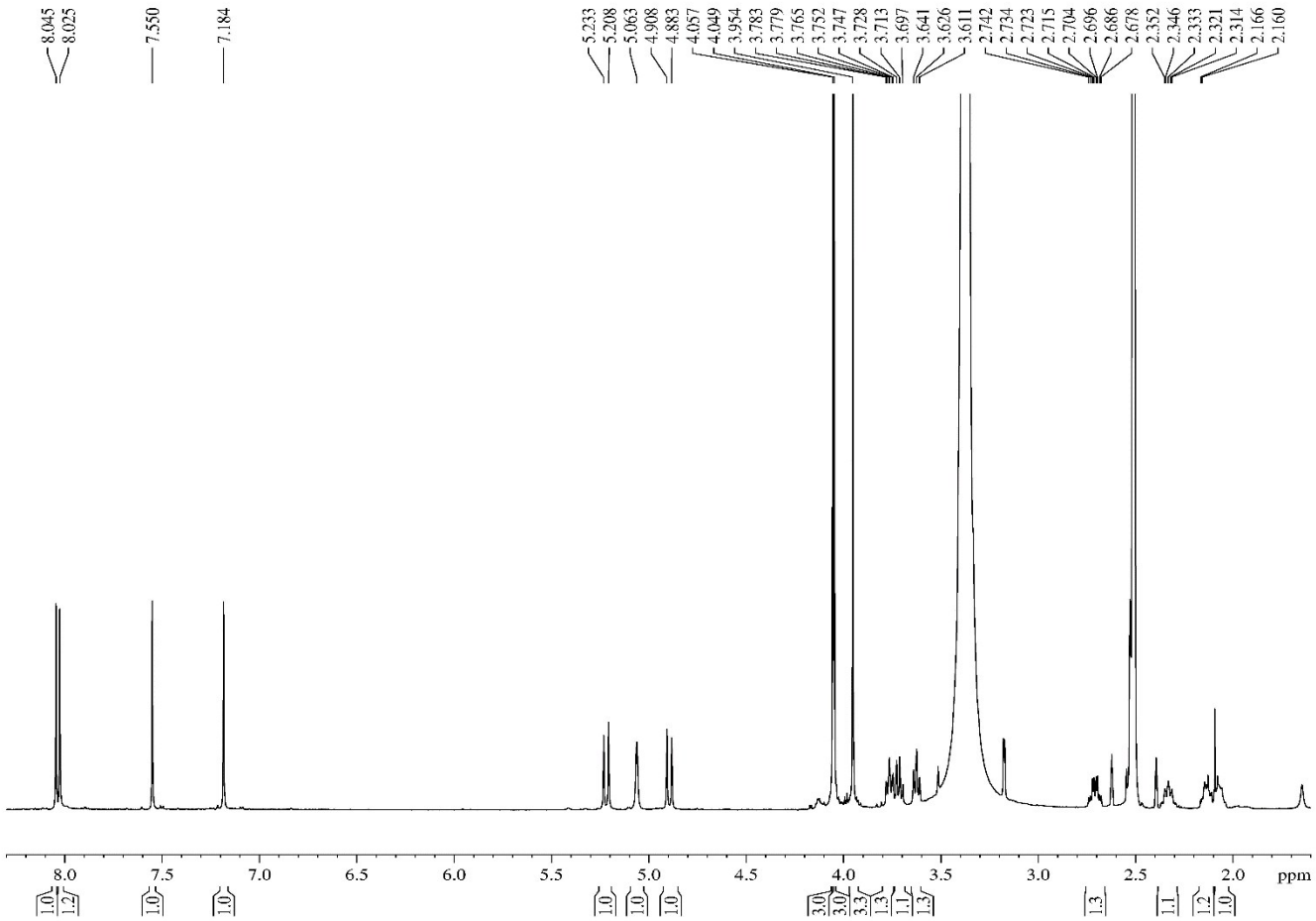


Figure 34 ^{1}H NMR spectrum of compound **7**.

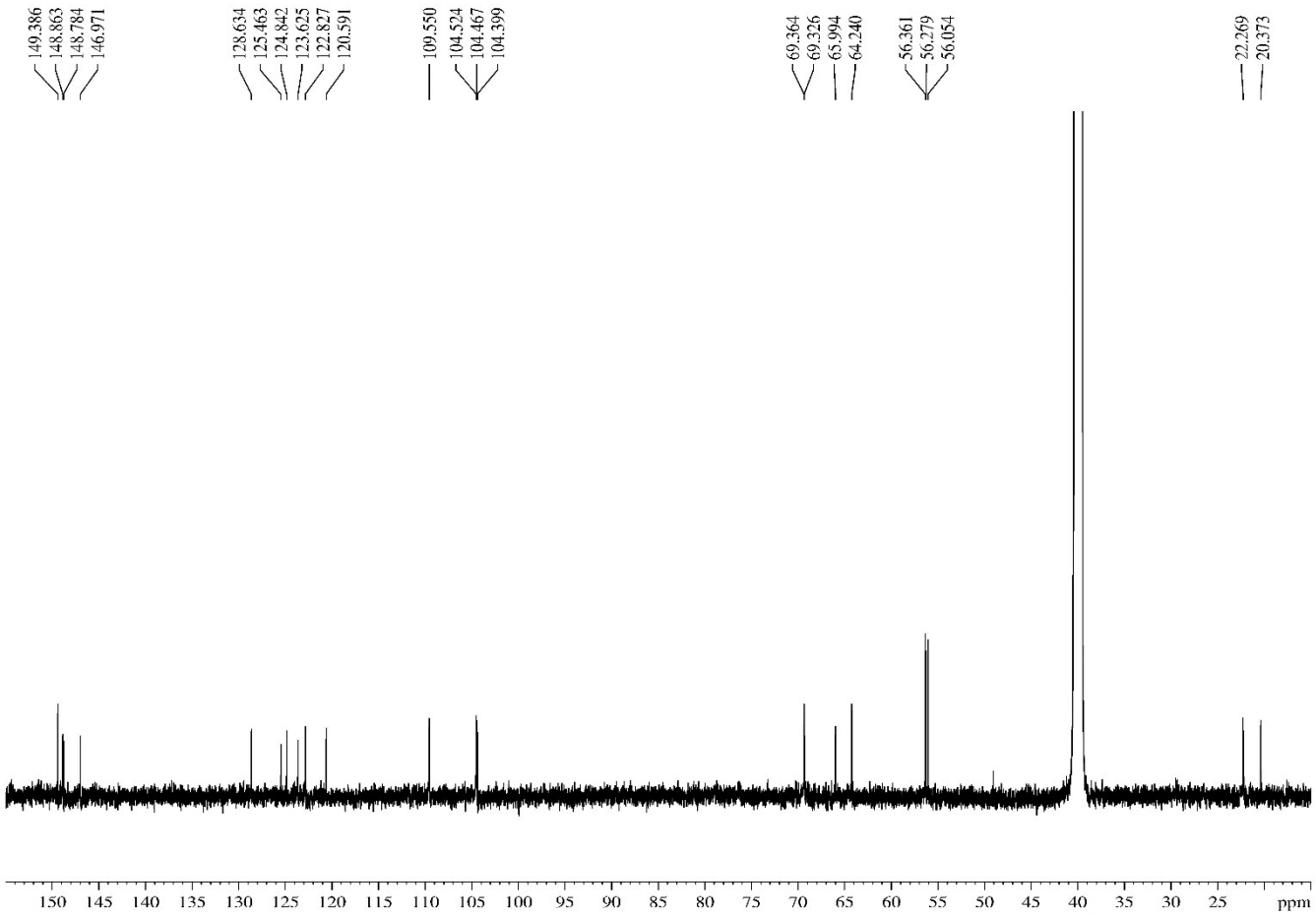
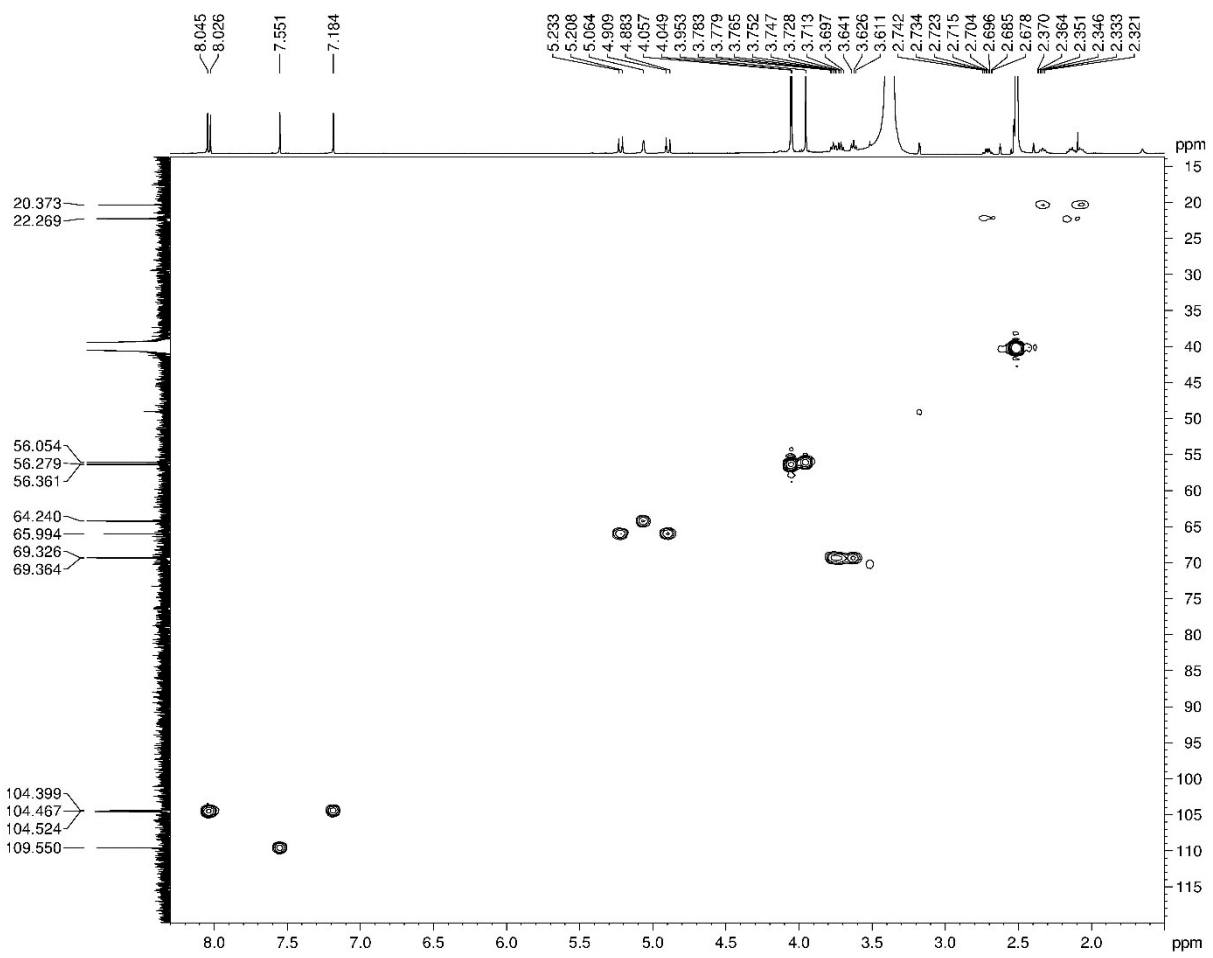


Figure 35 ^{13}C NMR spectrum of compound **7**.



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101 **Figure 36** HSQC spectrum of compound **7**.

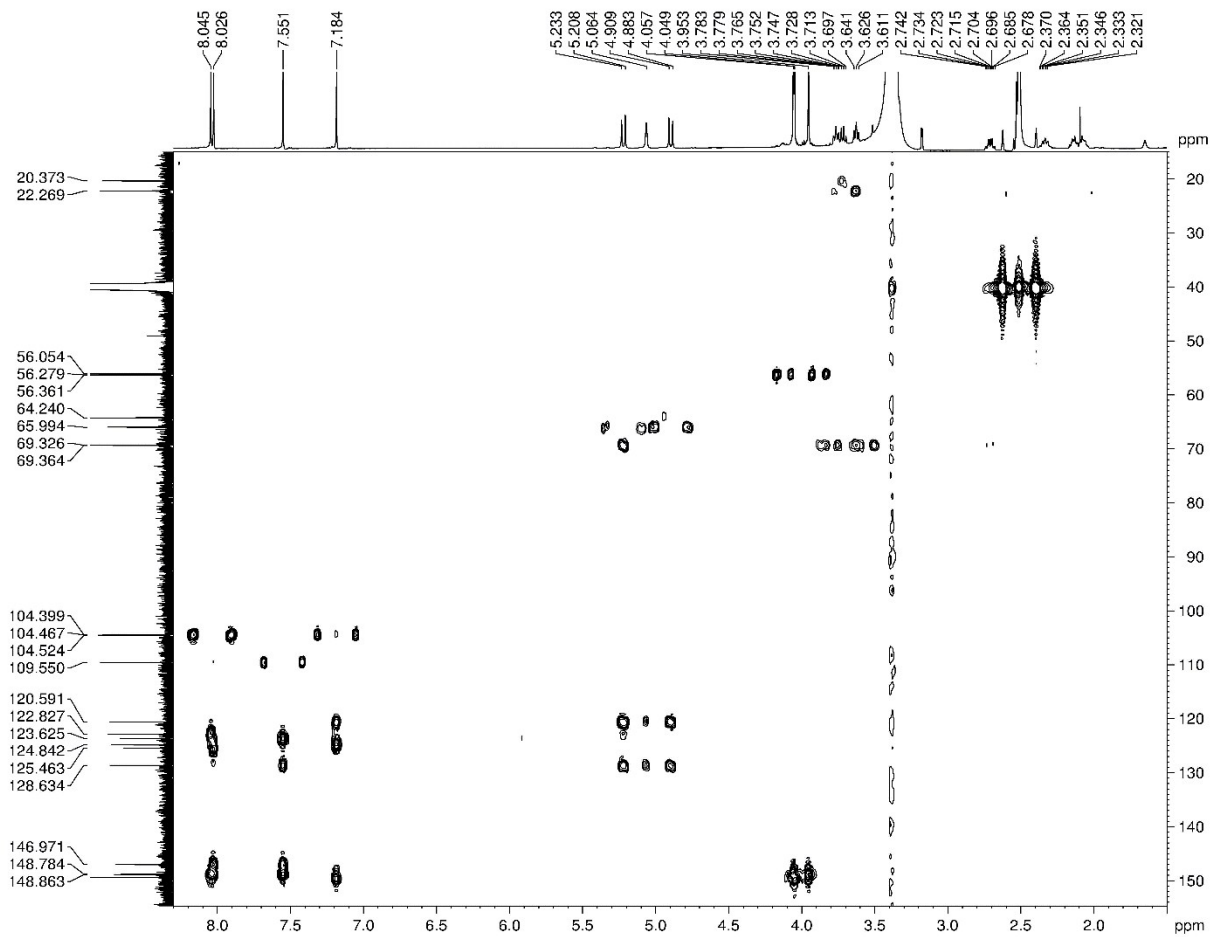
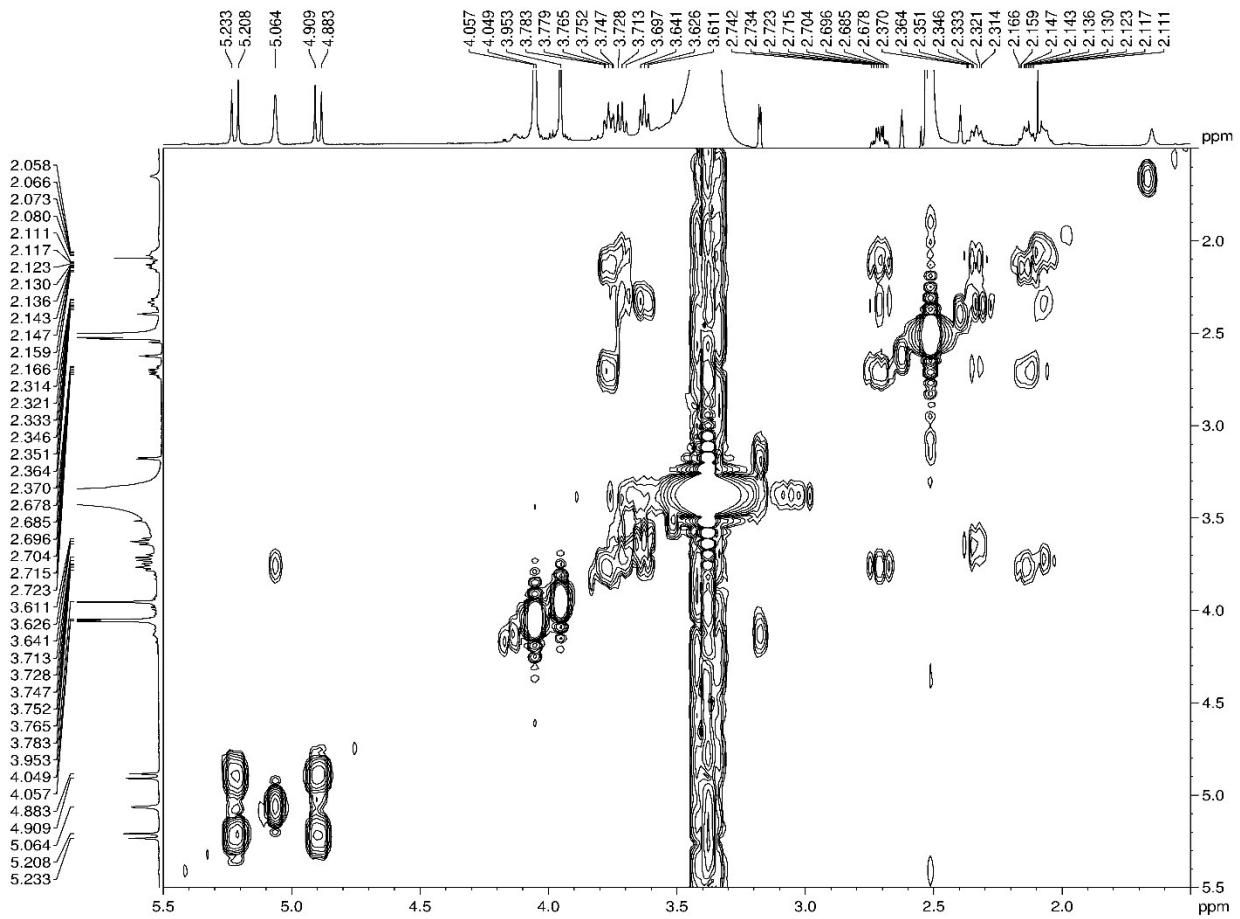


Figure 37 HMBC spectrum of compound **7**.



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105 **Figure 38** ^{1}H-^{1}H COSY spectrum of compound **7**.

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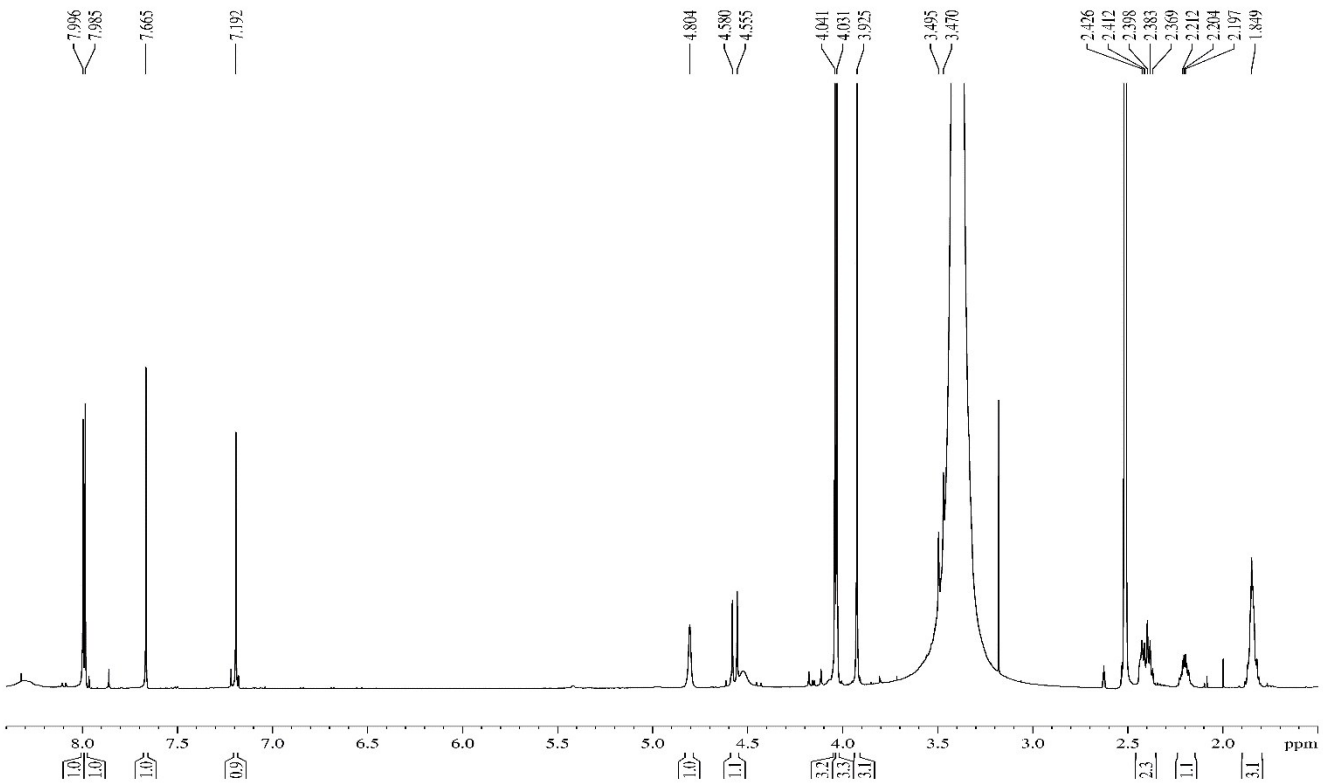


Figure 39 ^{1}H NMR spectrum of compound **8**.

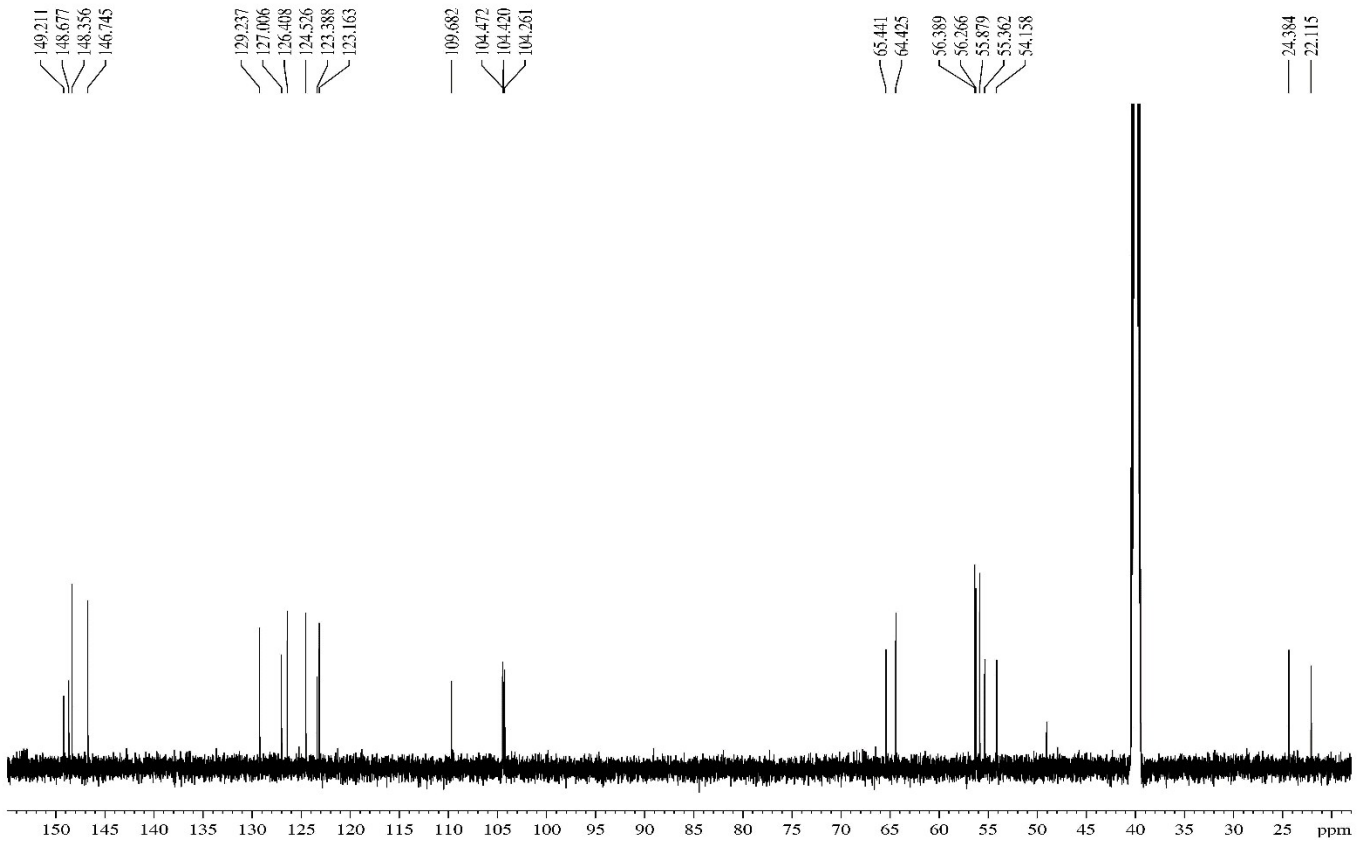
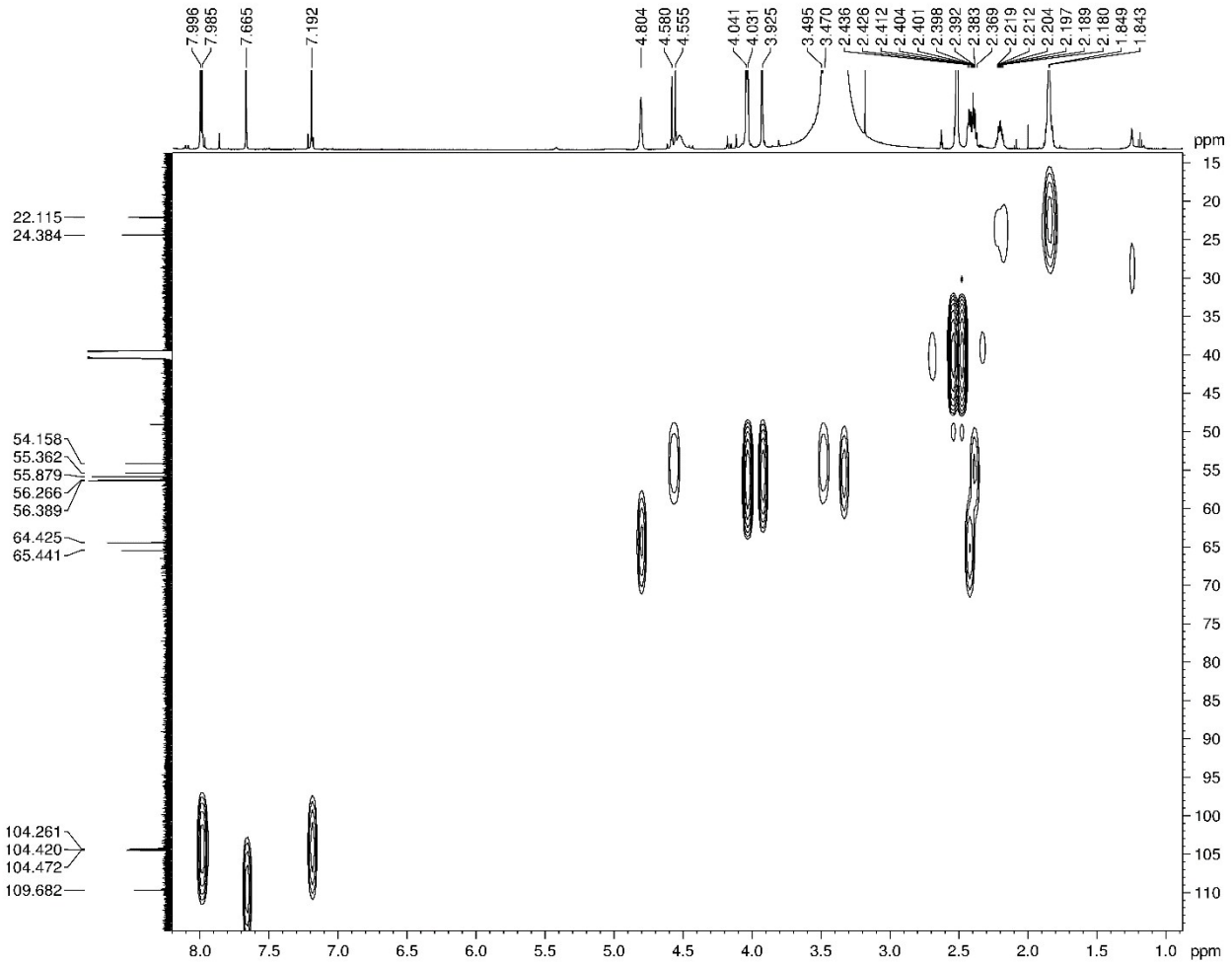


Figure 40 ^{13}C NMR spectrum of compound **8**.



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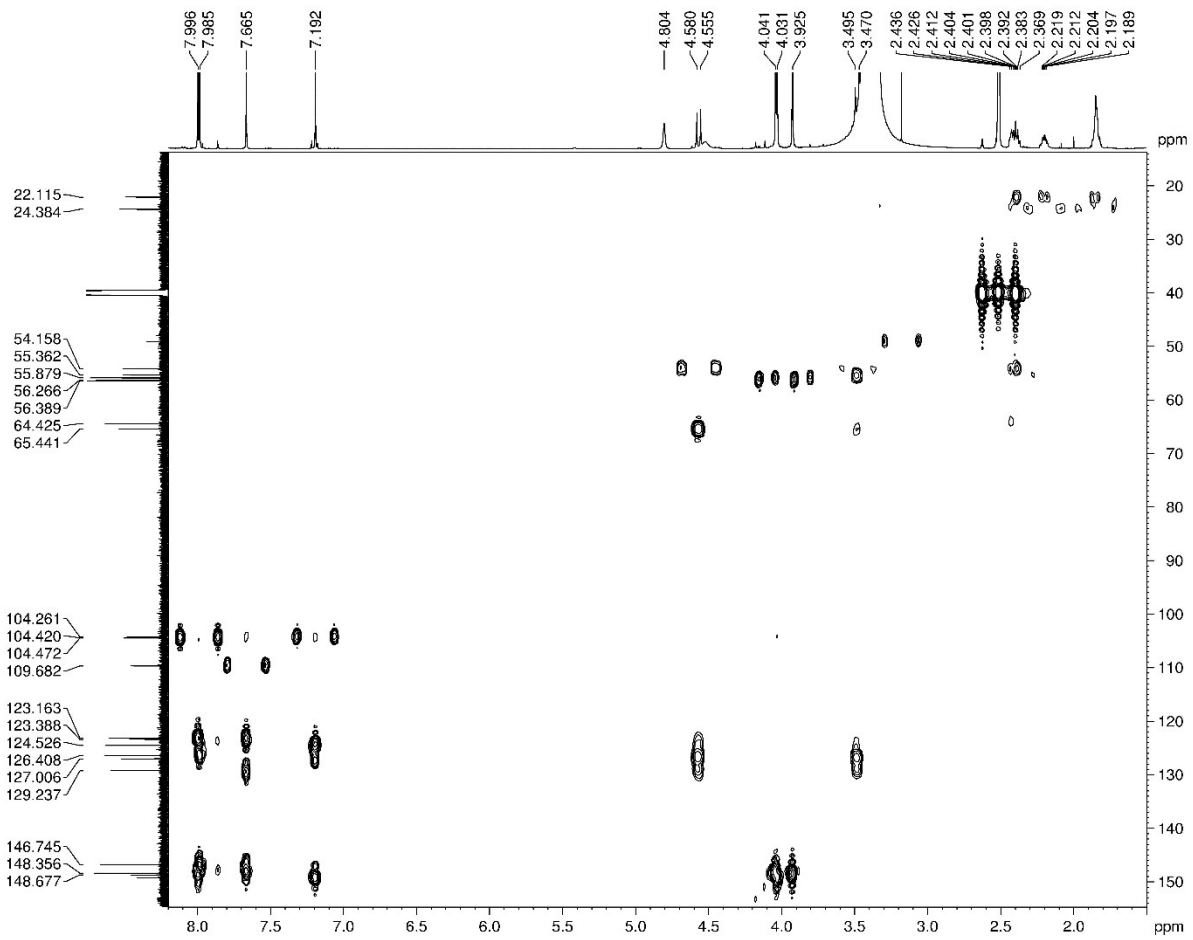
116 **Figure 41** HSQC spectrum of compound **8**.



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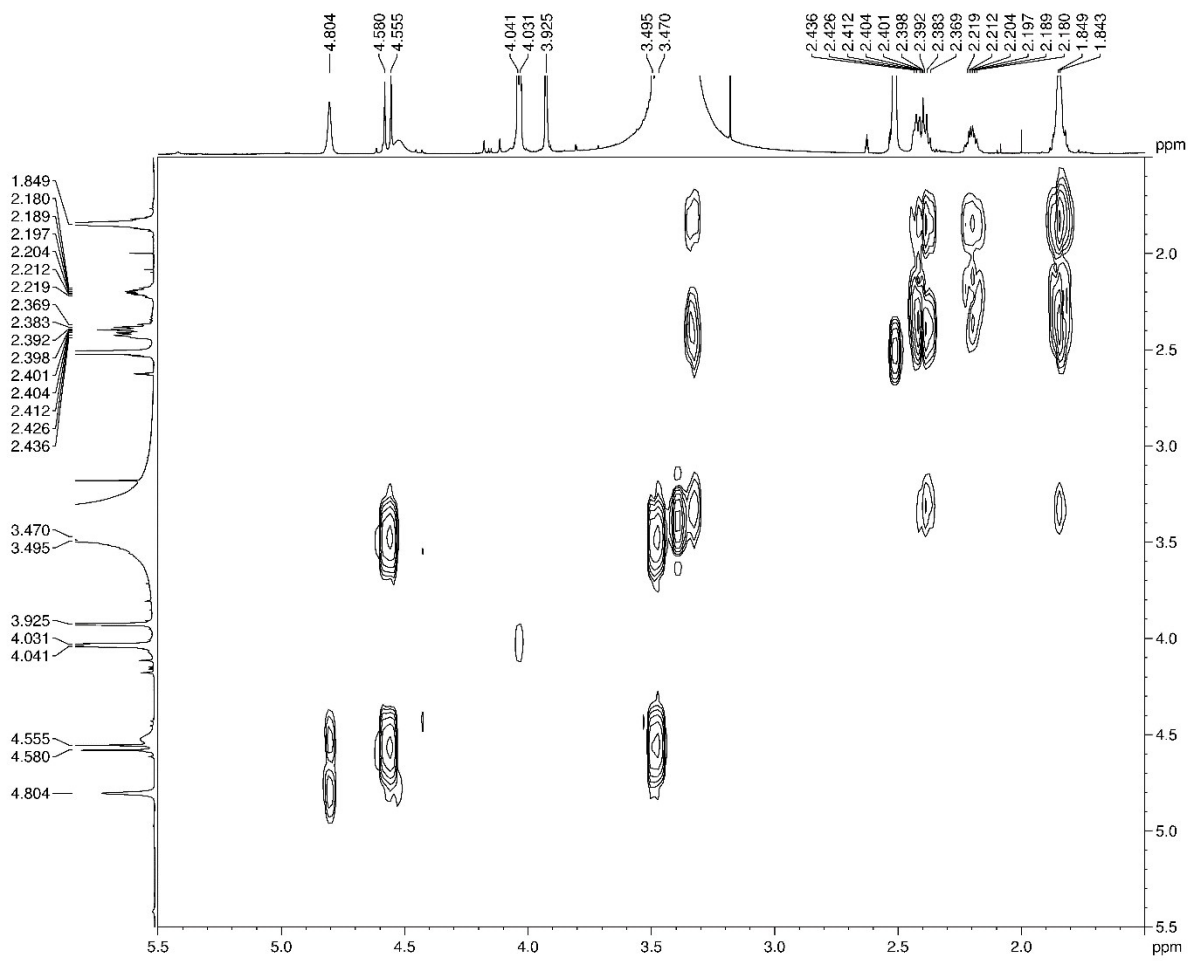
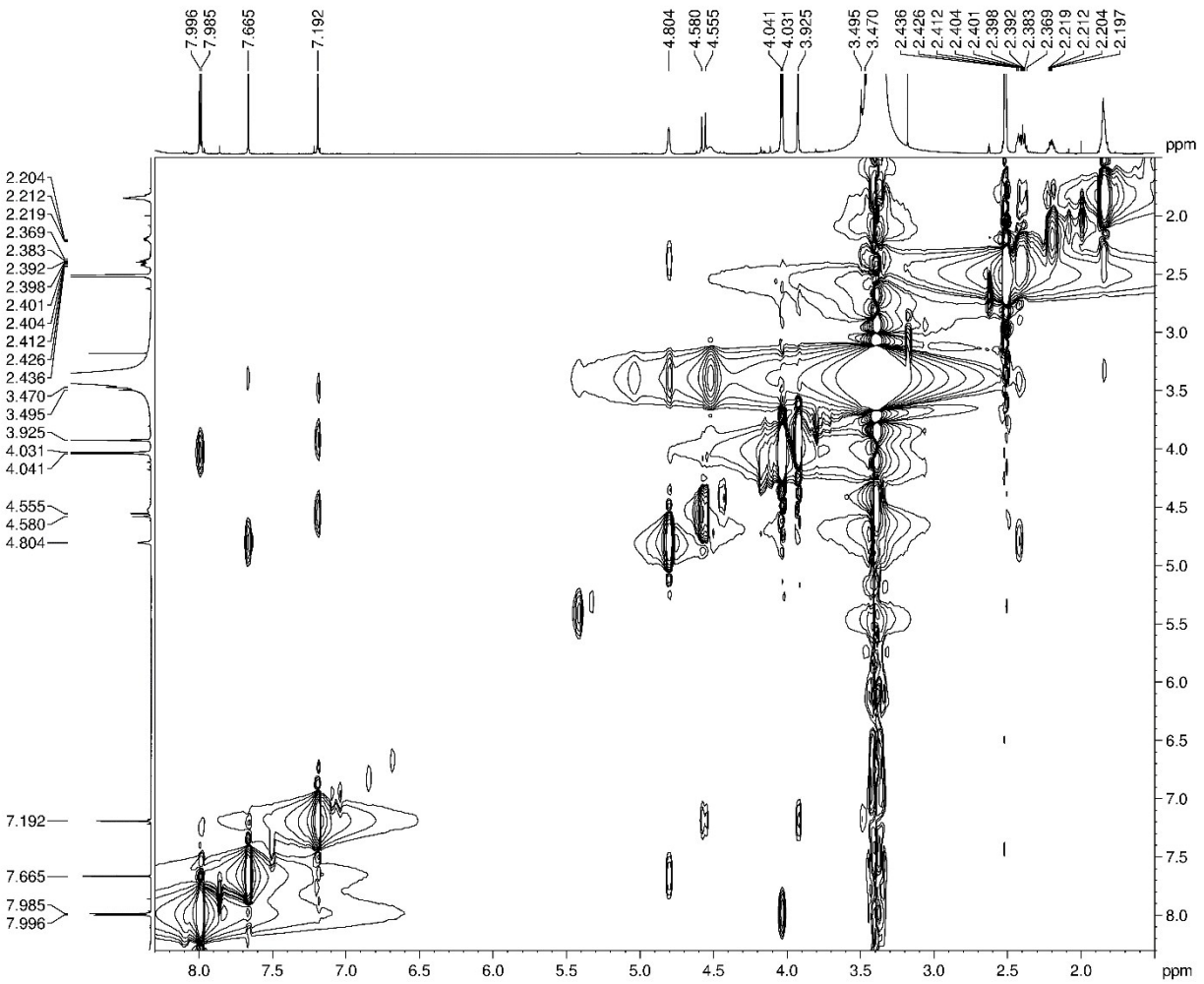
118 **Figure 42** HMBC spectrum of compound **8**.



119

120 **Figure 43** ^{1}H-^{1}H COSY spectrum of compound **8**.



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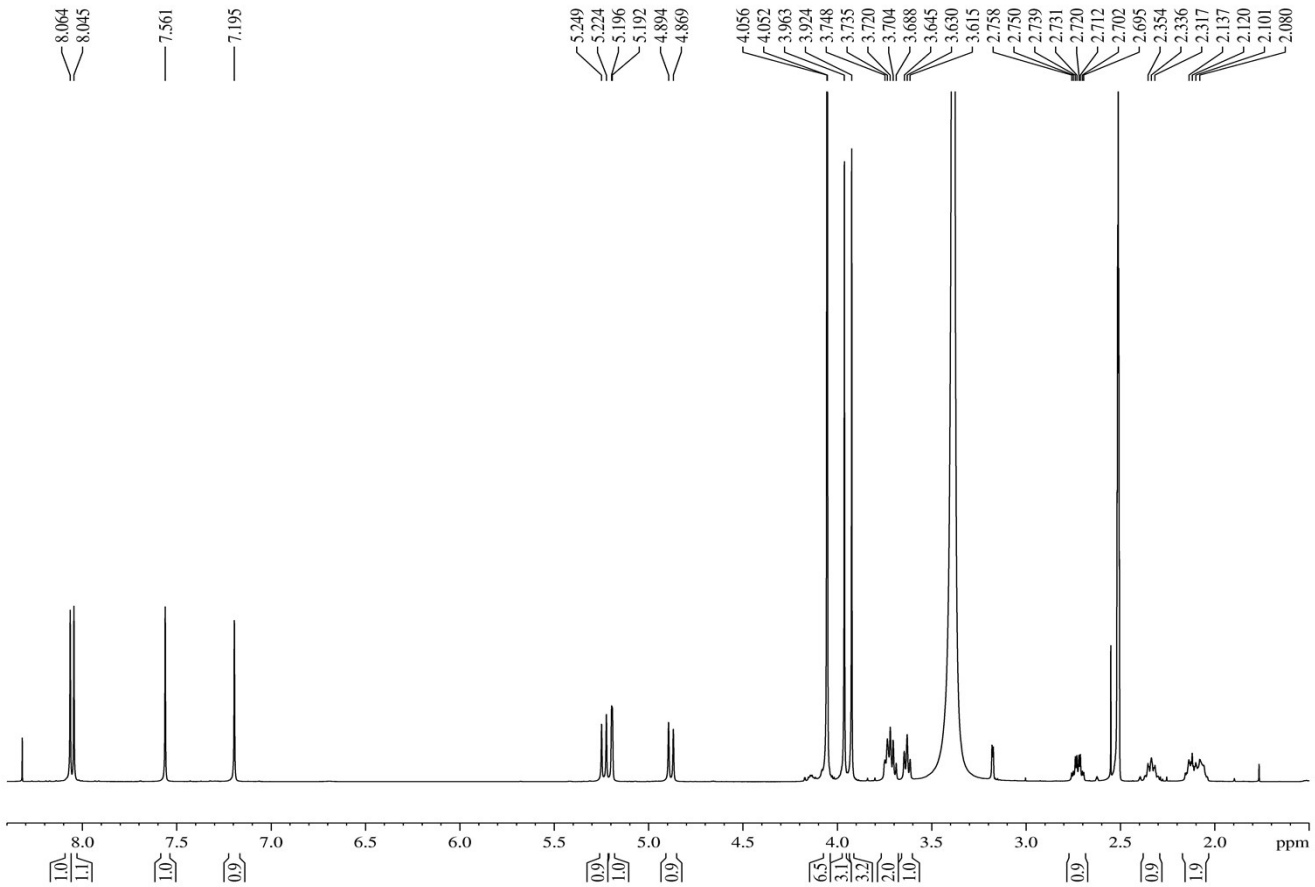
122 **Figure 44** NOESY spectrum of compound **8**.



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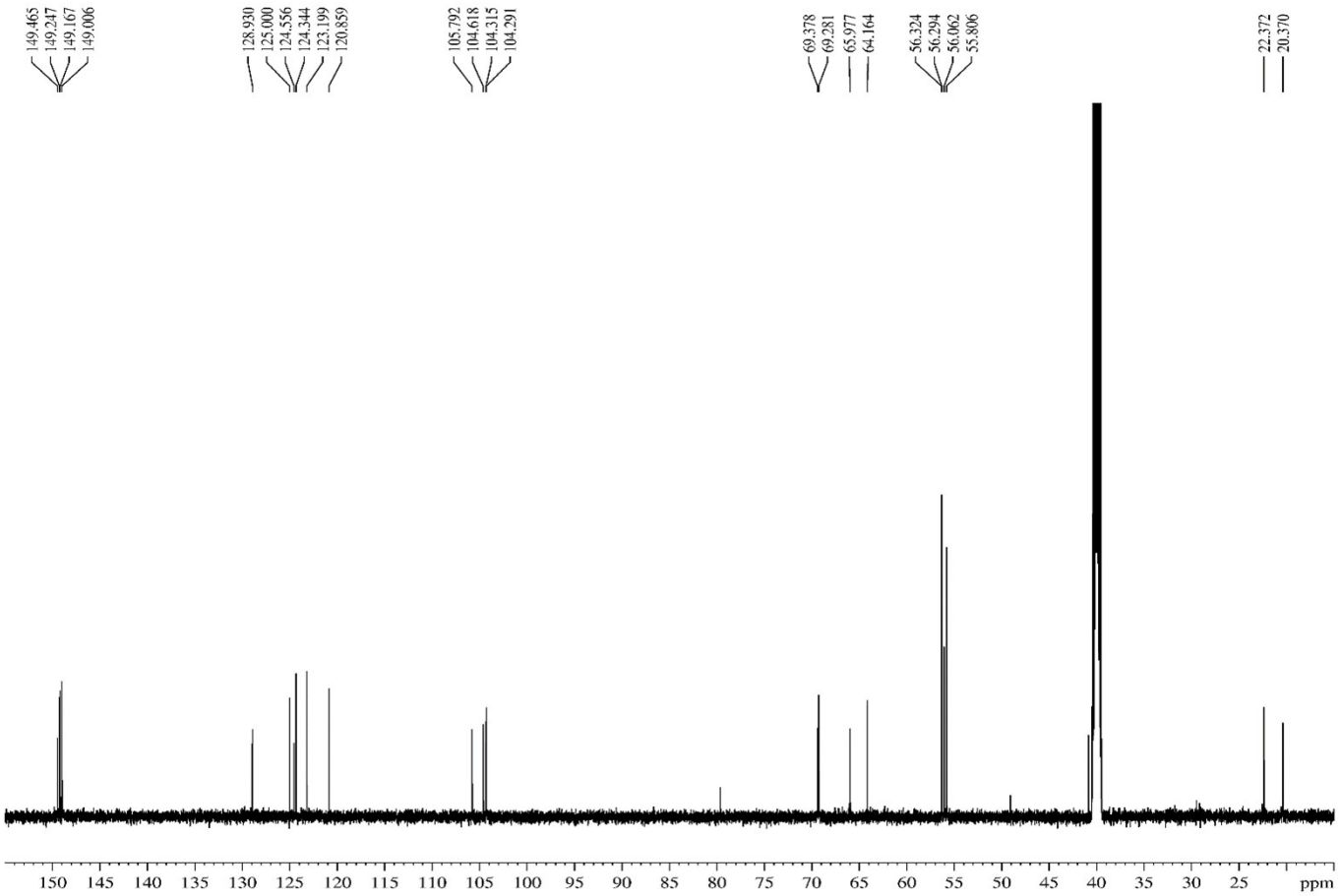
124 **Figure 45** ^{1}H NMR spectrum of compound **9**.

125



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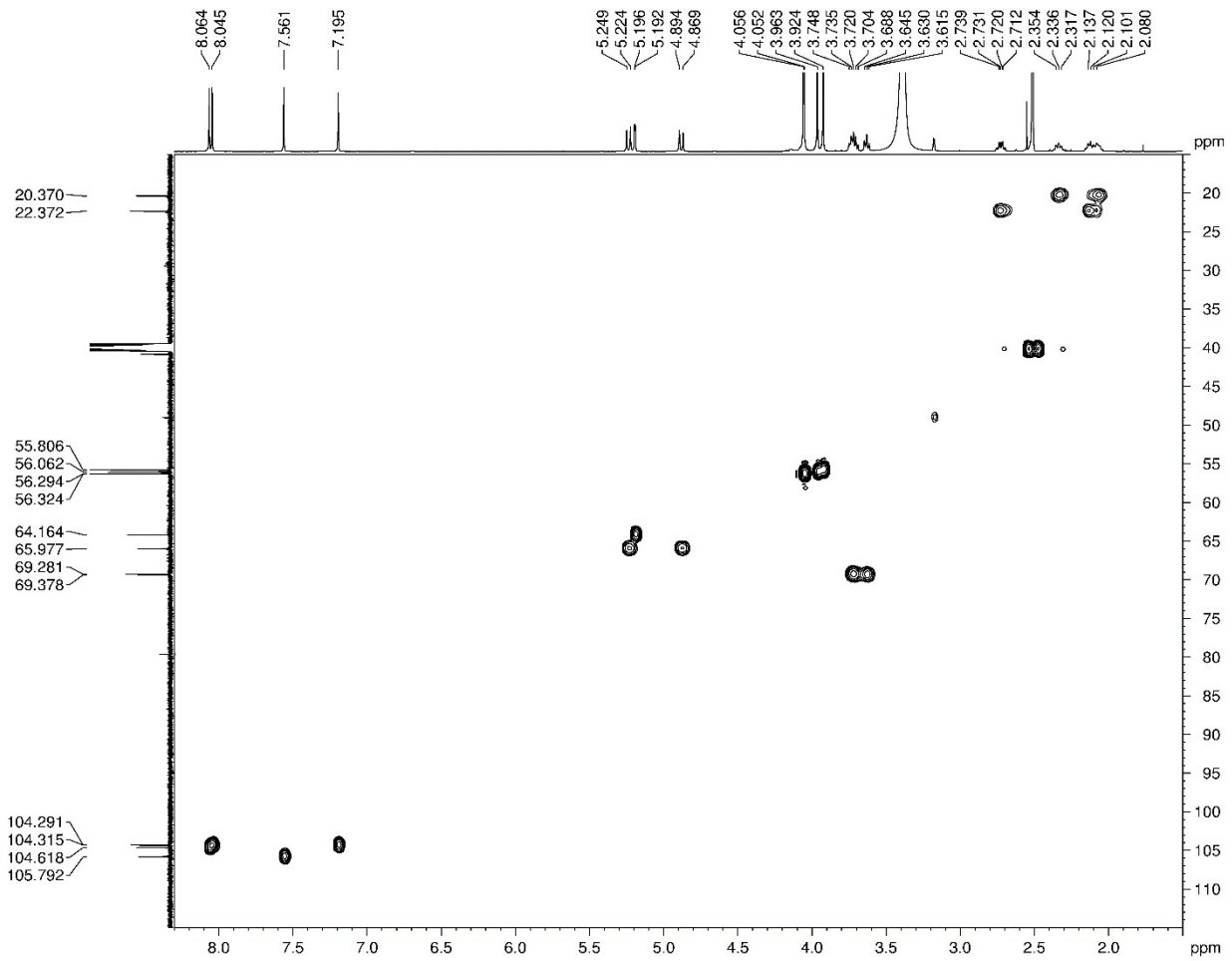
127 **Figure 46** ^{13}C NMR spectrum of compound **9**.



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129 **Figure 47** HSQC spectrum of compound **9**.

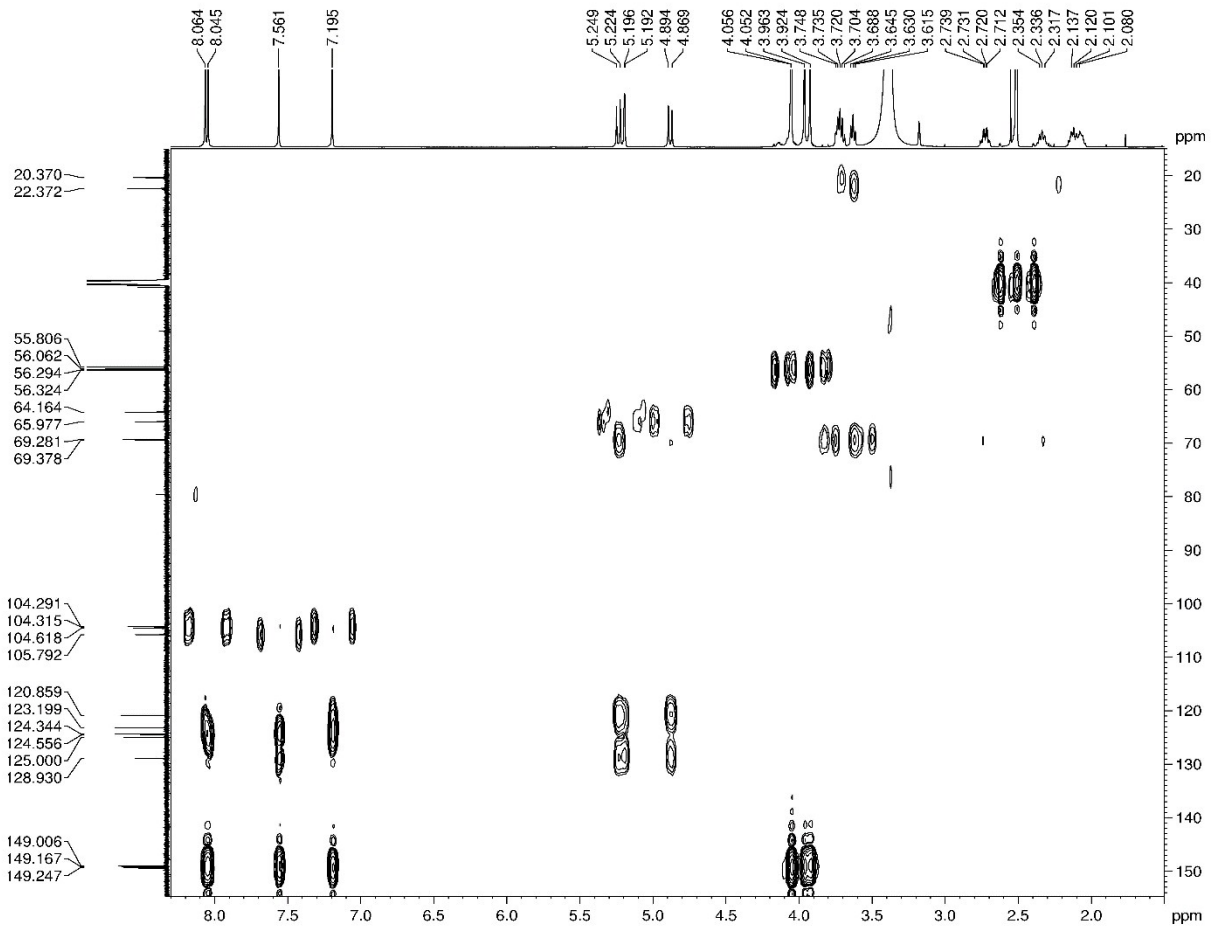
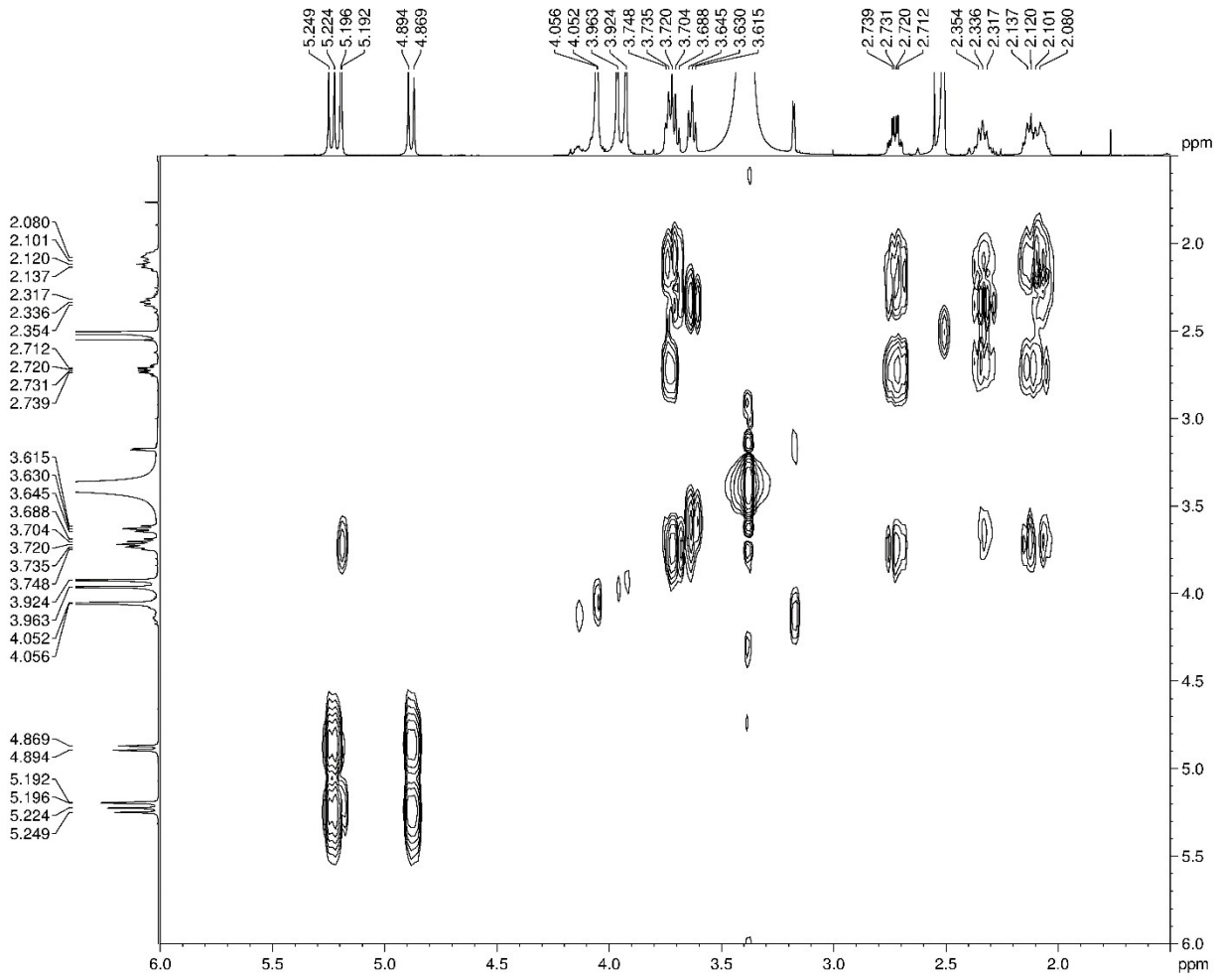


Figure 48 HMBC spectrum of compound **9**.



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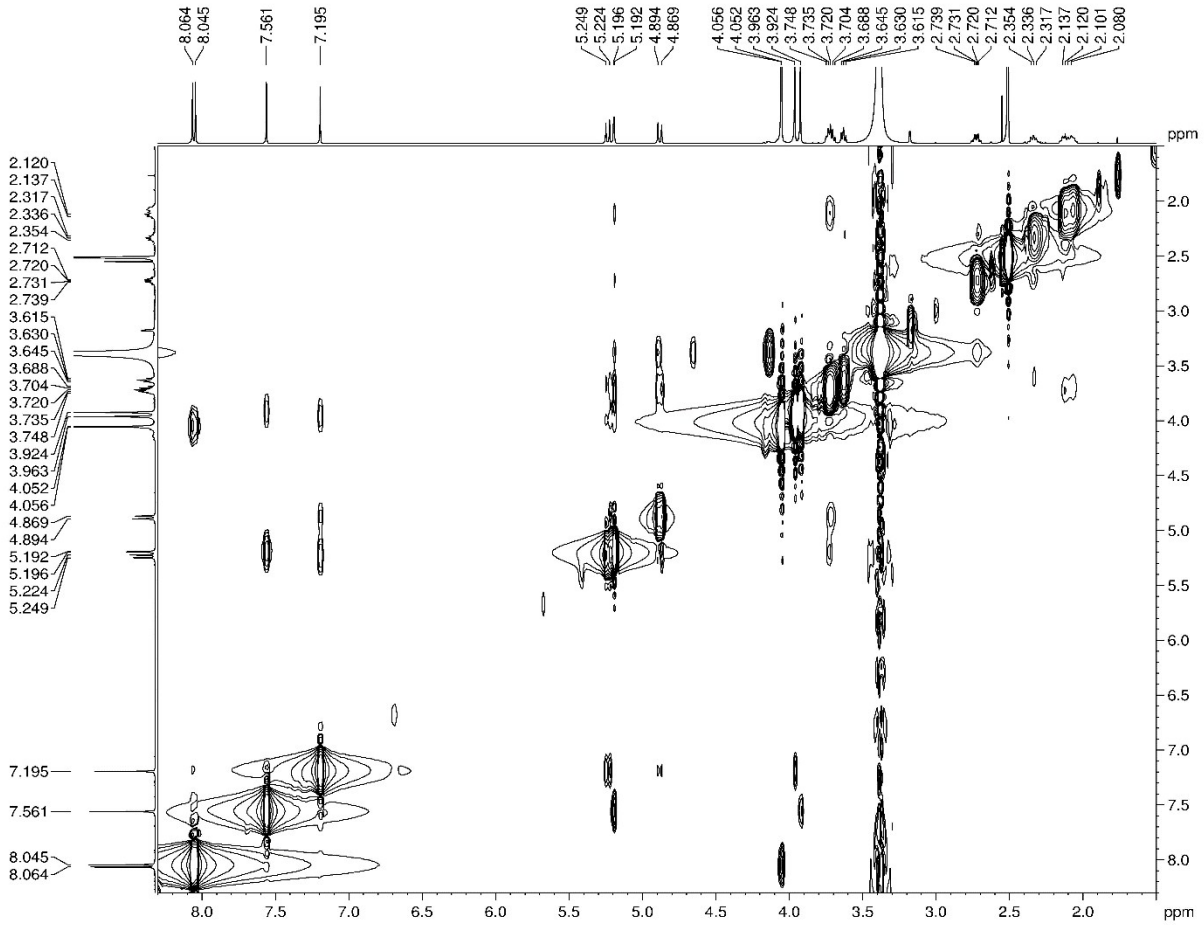
134 **Figure 49** ^{1}H-^{1}H COSY spectrum of compound **9**.



135

136 **Figure 50** NOESY spectrum of compound **9**.

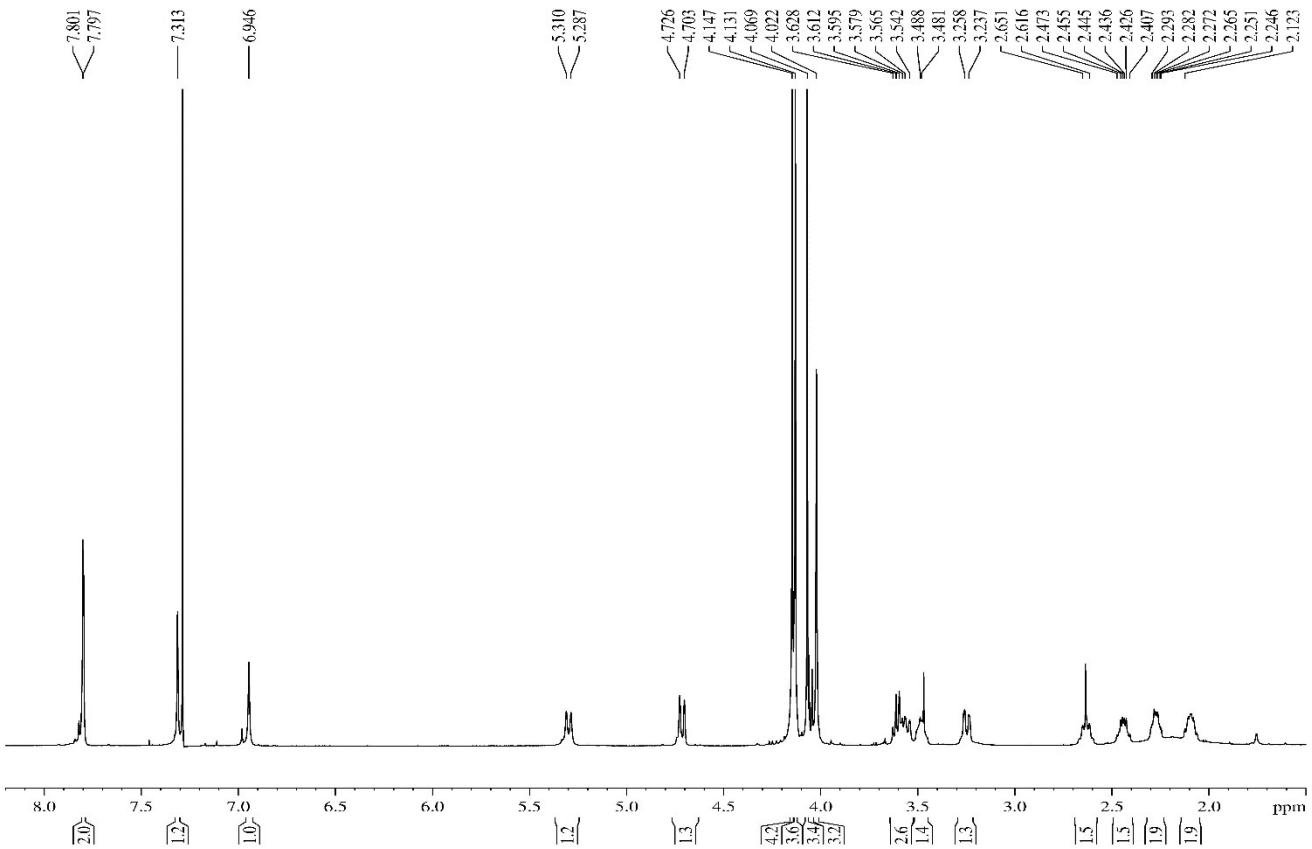
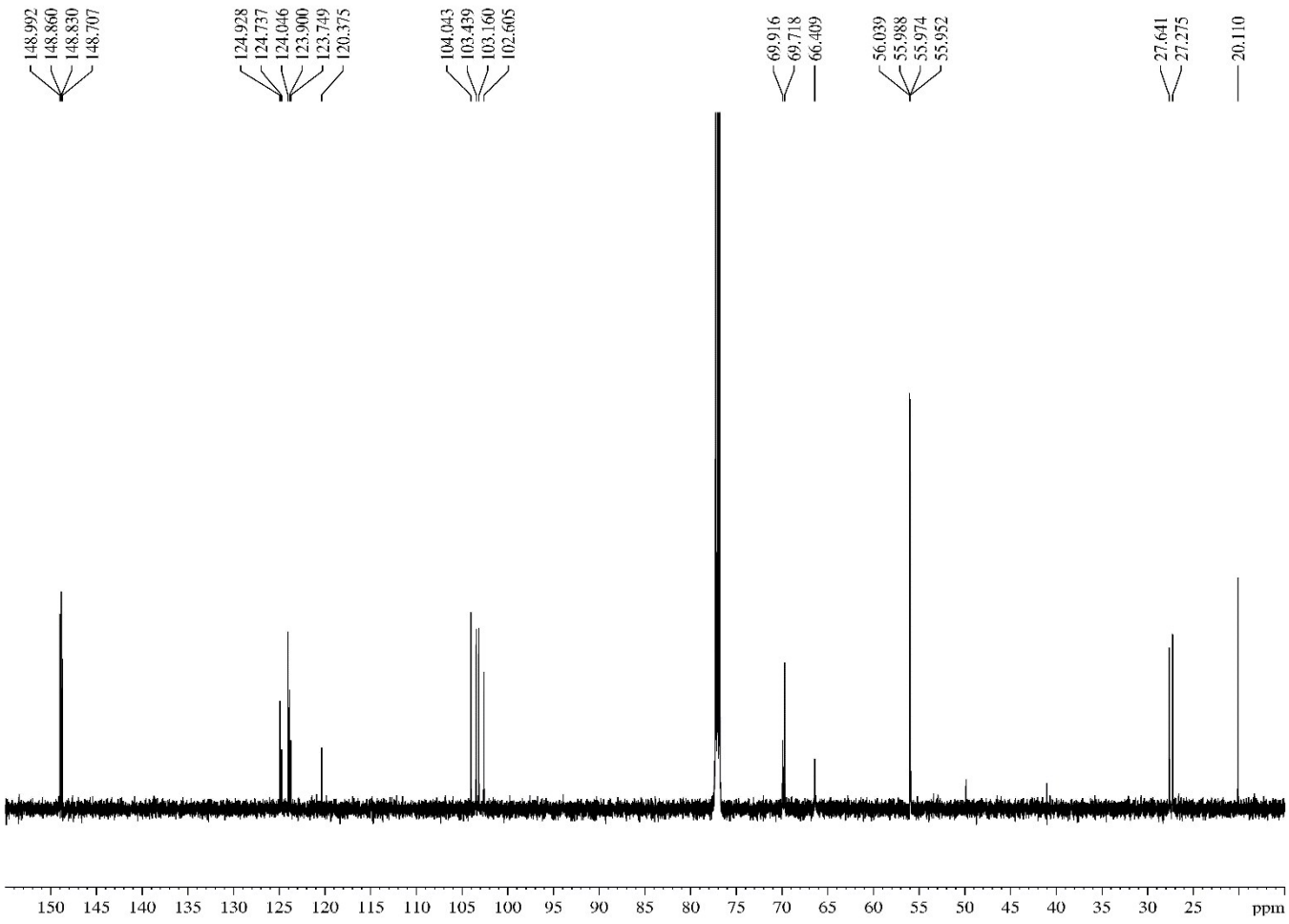


Figure 51 ^{1}H NMR spectrum of compound 1**0**.



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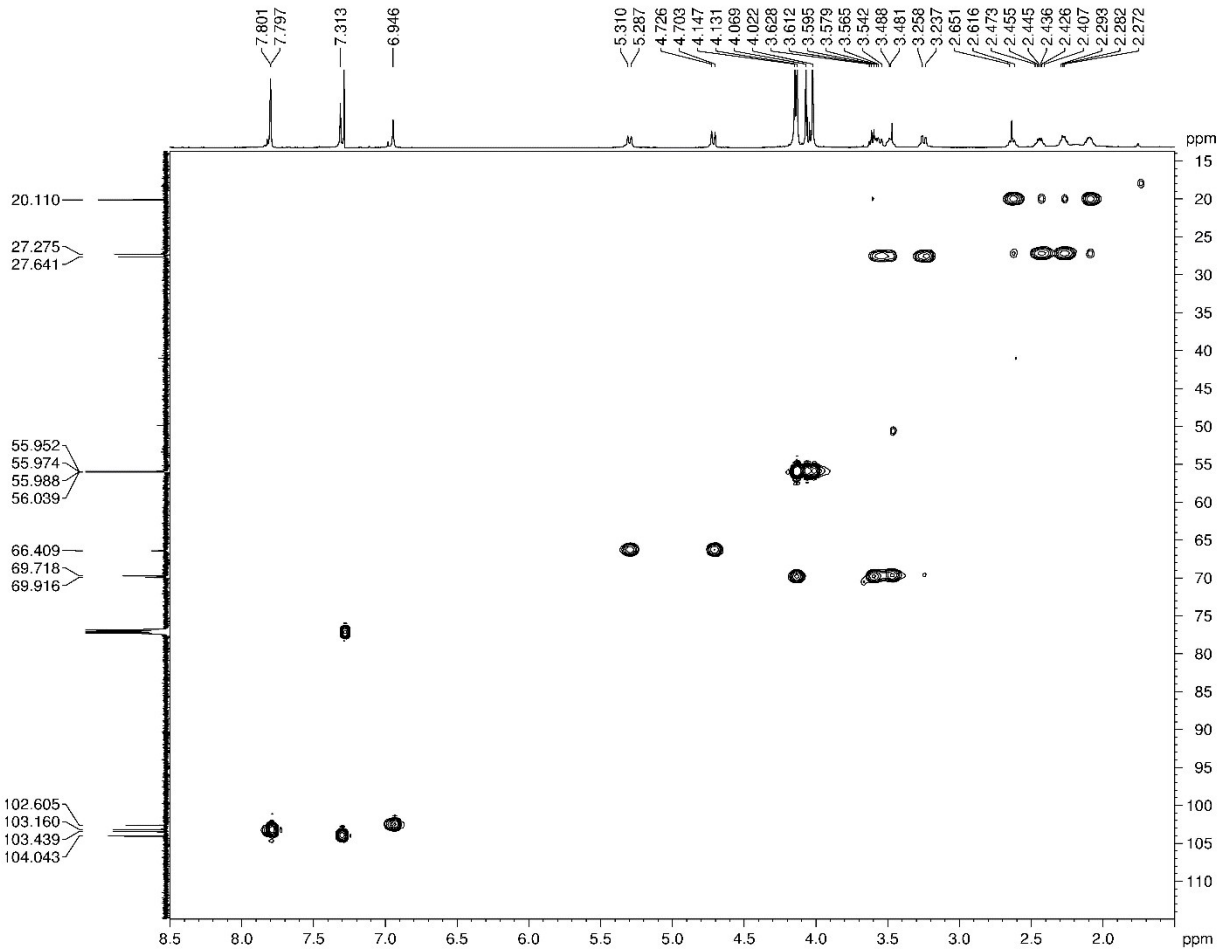
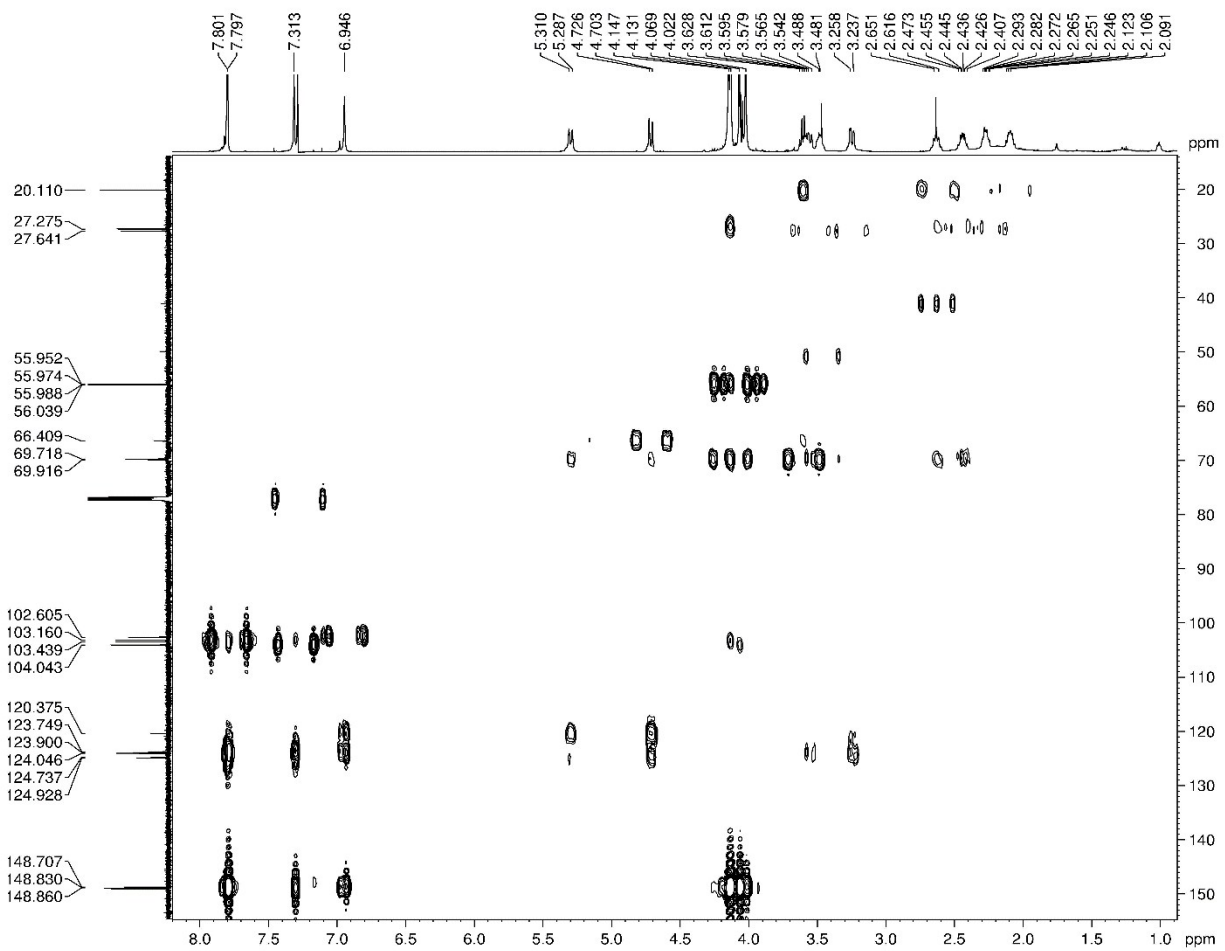
141 **Figure 52** ^{13}C NMR spectrum of compound **10**.



142

143 **Figure 53** HSQC spectrum of compound **10**.



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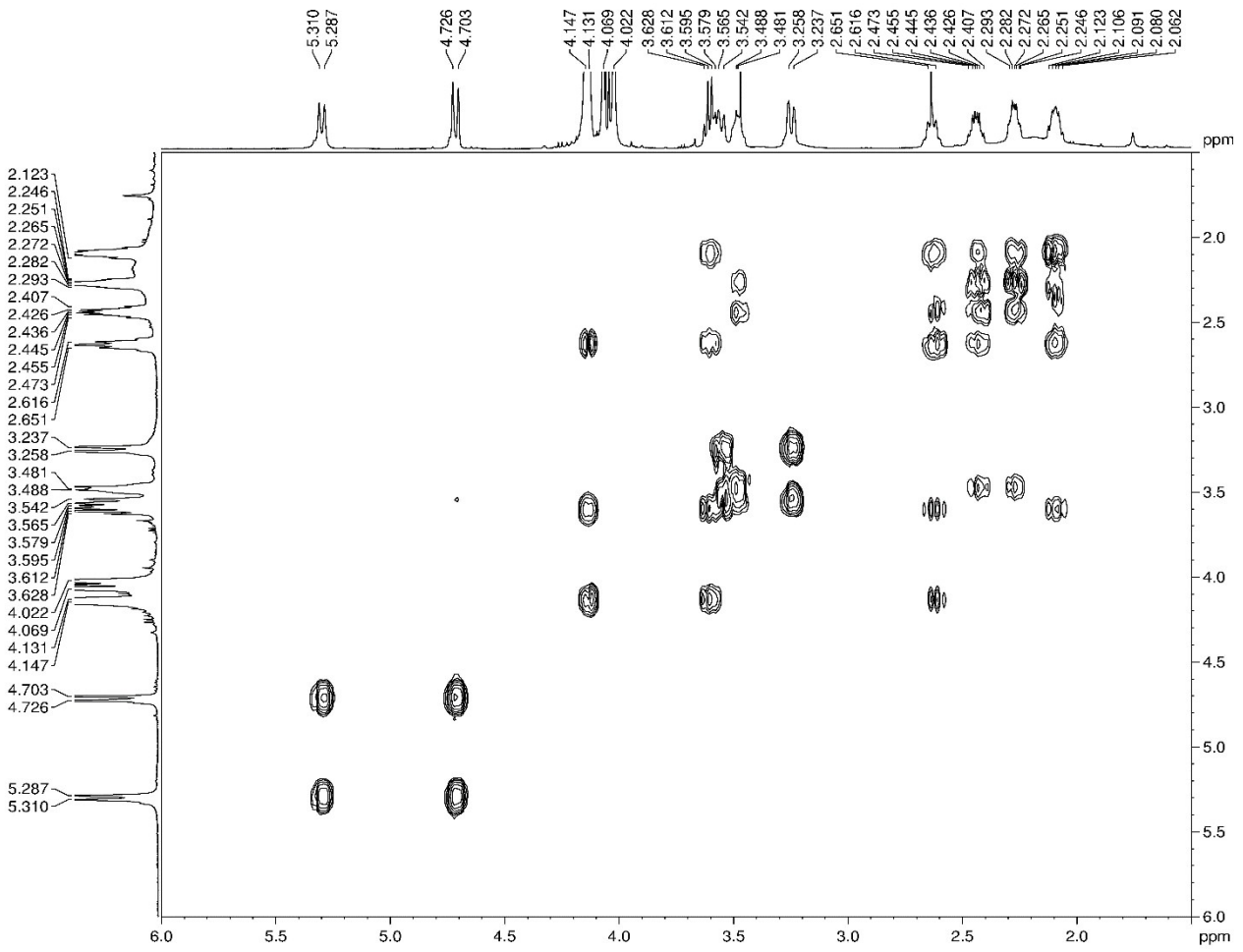
145 **Figure 54** HMBC spectrum of compound **10**.



146

147 **Figure 55** ^{1}H-^{1}H COSY spectrum of compound **10**.

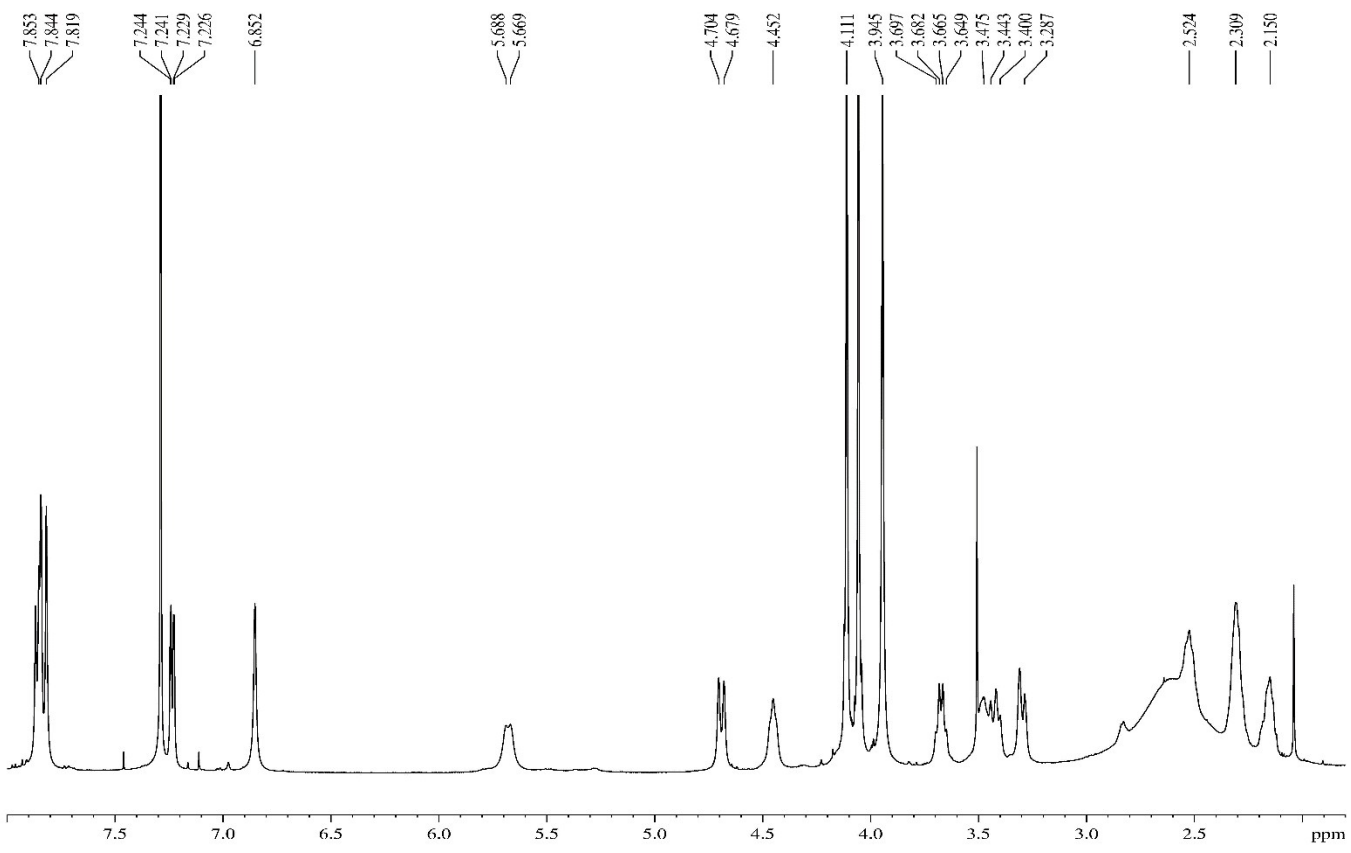
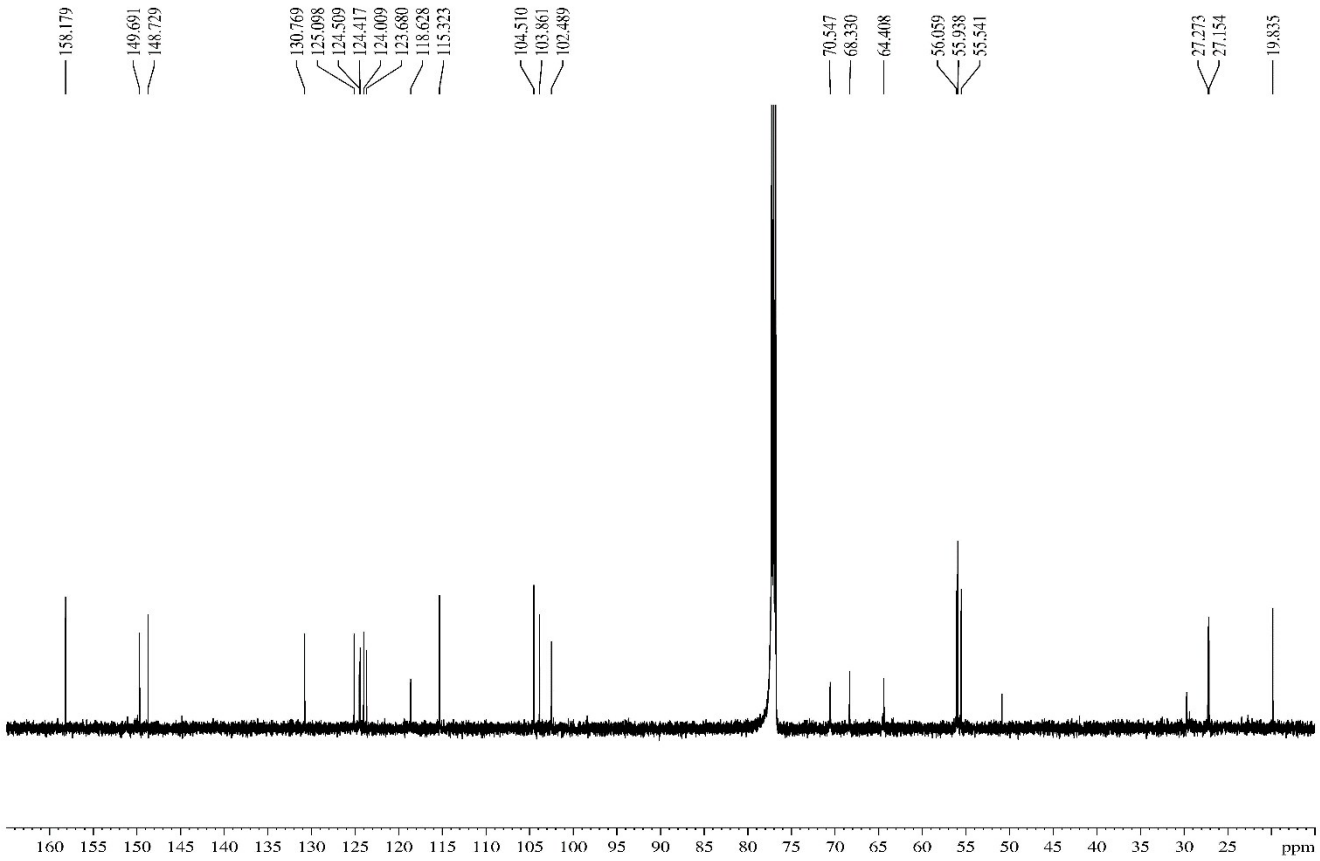
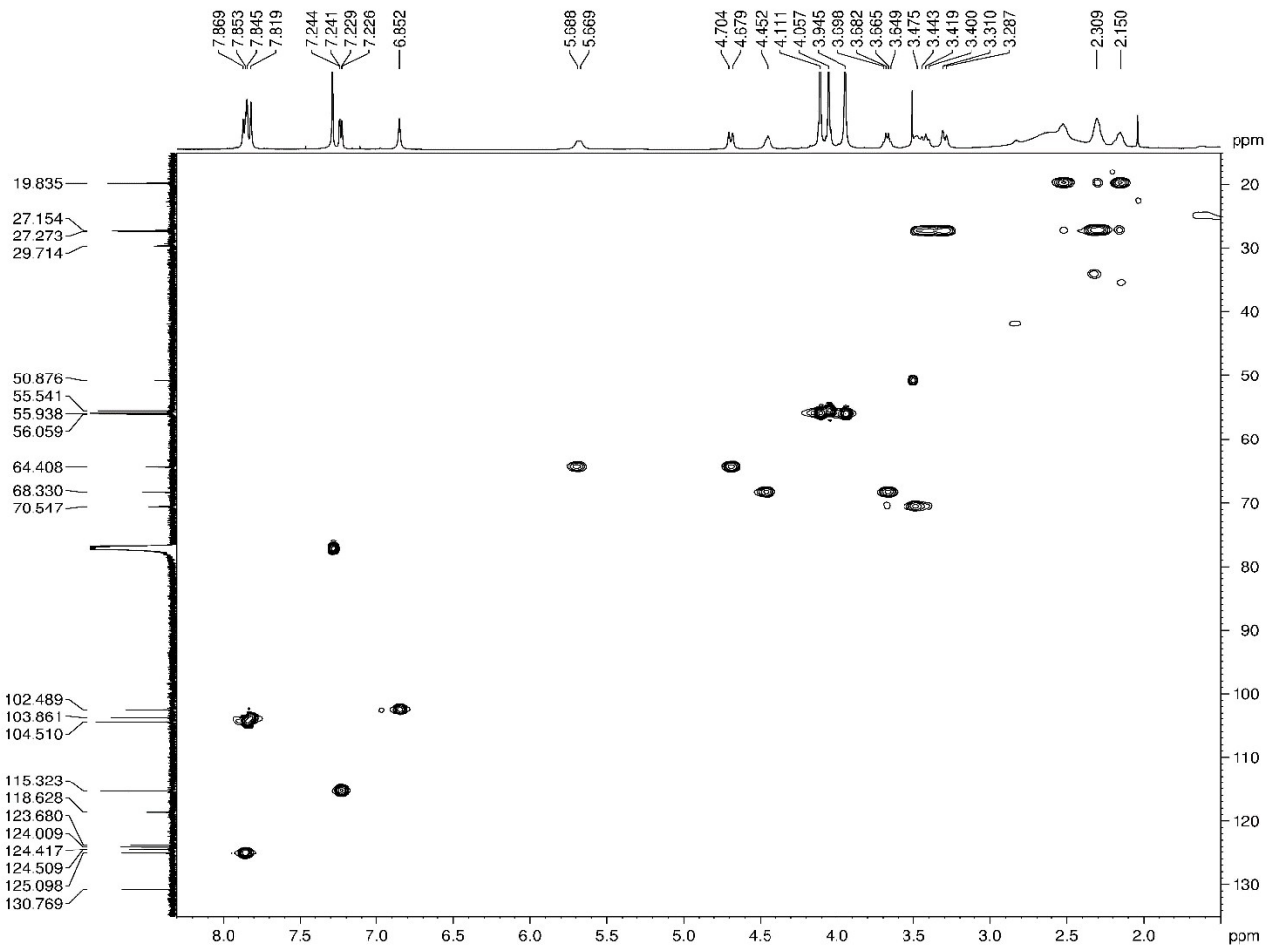


Figure 56 ^{1}H NMR spectrum of compound **11**.



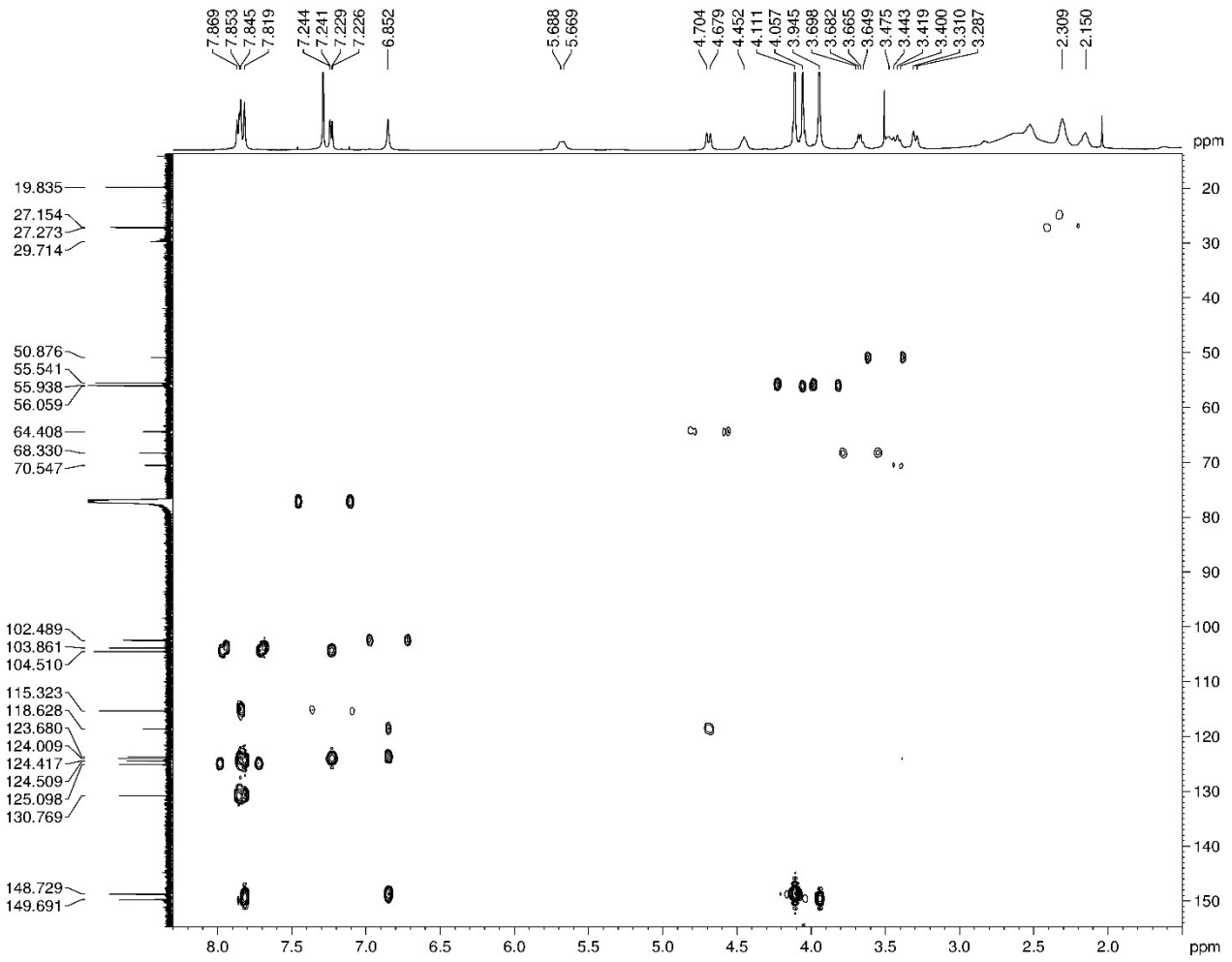
152

153 **Figure 57** ^{13}C NMR spectrum of compound **11**.



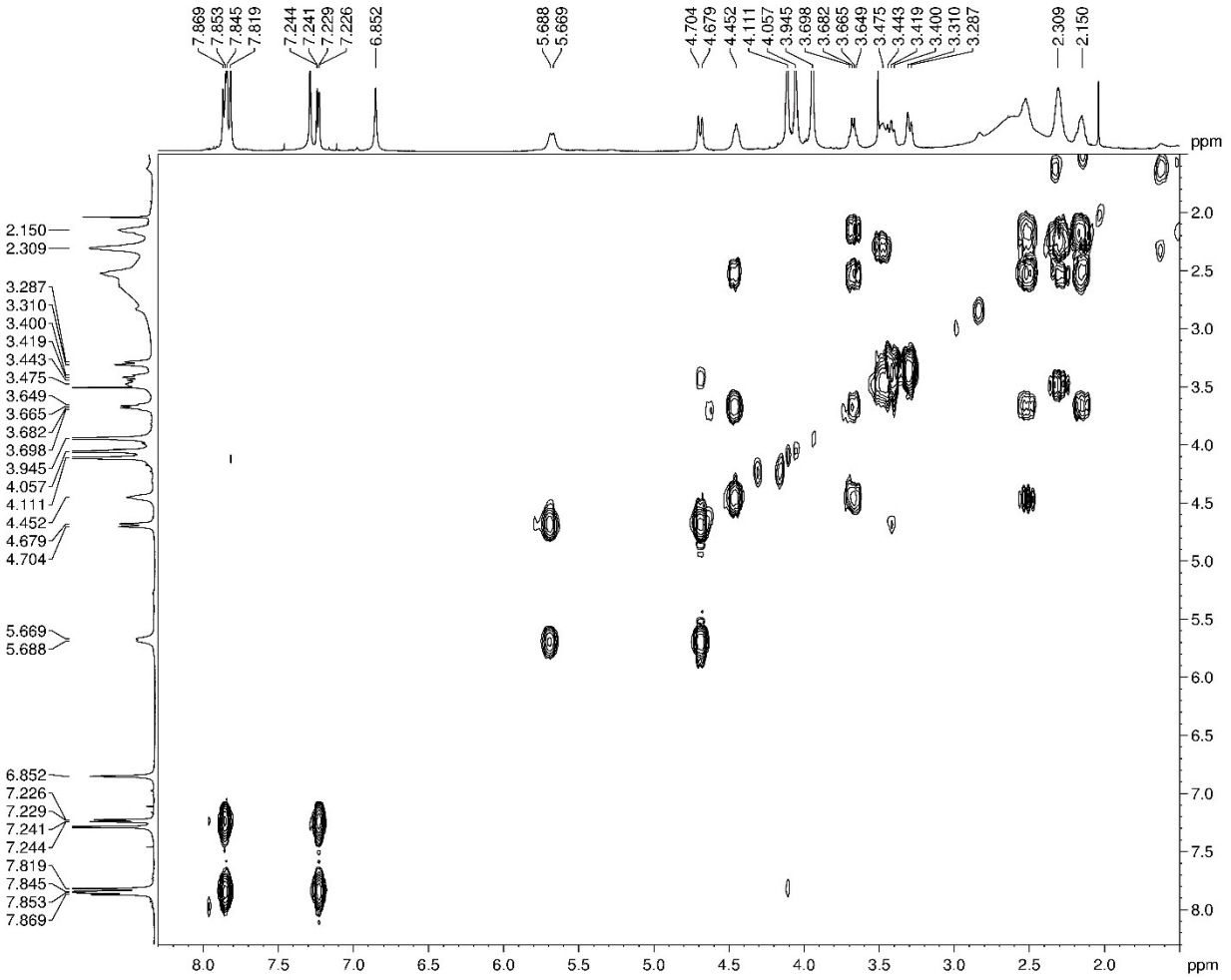
154

155 **Figure 58** HSQC spectrum of compound **11**.



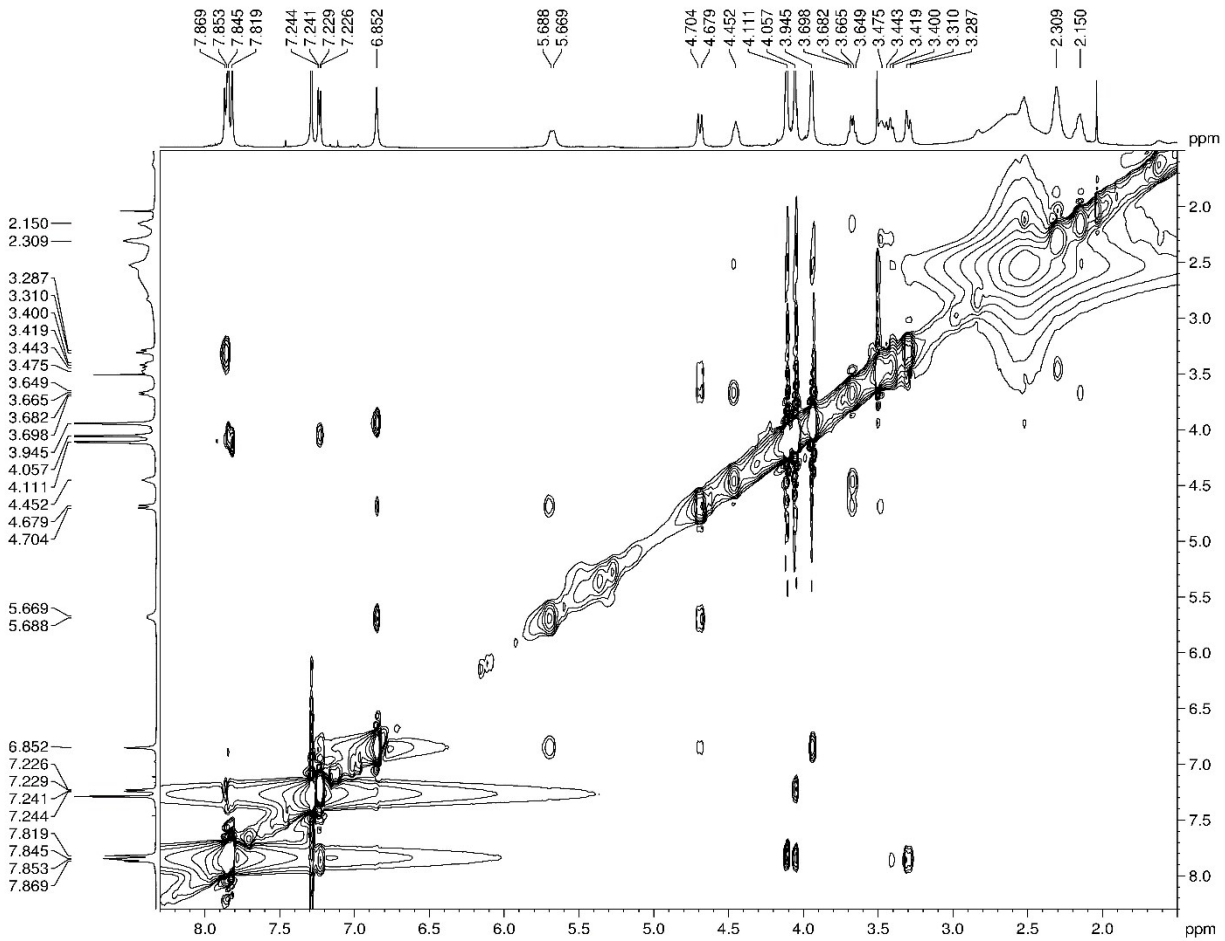
156

157 **Figure 59** HMBC spectrum of compound **11**.



158

159 **Figure 60** ^{1}H-^{1}H COSY spectrum of compound **11**.



160

161 **Figure 61** NOESY spectrum of compound **11**.

Table S$_1$ ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectral data for **12** (δ in ppm, J in Hz, measured in DMSO-d_6).

position	δ_H	δ_C
1	8.17, d, 9	126.5
2	7.28, dd, 2.4, 9	116.2
3		158.0
4	8.10, d, 3	104.9
5	8.13, s	105.0
6		149.5
7		150.0
8	7.29, s	104.1
9	5.18, d, 17.4; 4.53, d, 17.4	40.5
11		174.1
12	2.39, m; 2.36, m	30.8
13	2.35, m; 2.21, m	19.7
13a	3.92, m	58.1
14	5.11, dd, 7.2, 1.8	64.0
4a		131.0
4b		124.2
8a		124.3
8b		123.8
14a		128.8
14b		124.7
OCH_3-3	4.01, s	56.0[a]
OCH_3-6	4.06, s	56.3[a]
OCH_3-7	4.00, s	55.9
OH-14	5.46, d, 7.2	

[a] Overlapped siganl

Cytotoxic Activity

MTT based cytotoxic activity was conducted according to previous protocol with minor modification.[1] T47D cells were cultured in DMEM medium (Invitrogen, American) supplemented with 10% (v/v) fetal serum (FBS) (Invitrogen, American), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, American) in a humidified atmosphere (5% CO_2 and 95% air) at 37 ˚C. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was adopt to assess the cytotoxicity. Briefly, 100 μL cells were cultured in 96-well plated overnight at initial densities of 5 × 10^4/well, then were treated with tested compounds at various concentrations the cells were incubated for 1 h, then were exposed to hypoxic (2% O_2, 5% CO_2 and 93% N_2) or normoxic (5% CO_2 and 95% air) condition at 37 ˚C for 20 h. 10 μL MTT solution (5 mg/mL) was subsequently added into each well and incubated for another 4 h. After the removal of medium, 100 μL DMSO was added to dissolve the formazan. The absorbance was measured at 570 nm by a microplate spectrophotometer (Spectramax X190, Molecular Devices, American). IC_{50} values were determined from the does-response curves using Prism software (American). The data were repeated by three independent experiments.

Table S_2 Cytotoxicity of **2—11** and **13—21** against T47D cells under normoxic and hypoxic conditions.

	IC_{50} (nM)	
compounds	Normoxic	Hypoxic
2	>10000	>20000
3	>500	>500
4	>250	>250
5	>200	>200
6	>1000	>1000
7	>1000	>1000
8	>2000	>2000
9	>2000	>2000
10	>2000	>2000
11	>1000	>2000
13	>250	>250
14	>10	>100
15	>250	>250
16	>100	>100
17	>100	>100
18	>500	>500
19	>1000	>2000
20	>600	>15000
21	>500	>12500
digoxin	>500	>500

References

1. A. K. Lykkeberg, J. Christensen, B. A. Budnik, F. Abe and J. W. Jaroszewski, *J. Nat. Prod.*, 2002, **65**, 1299-1302.