

## SUPPLEMENTARY MATERIAL

### New oleanane-type saponins from the leaves of *Derris trifoliata*

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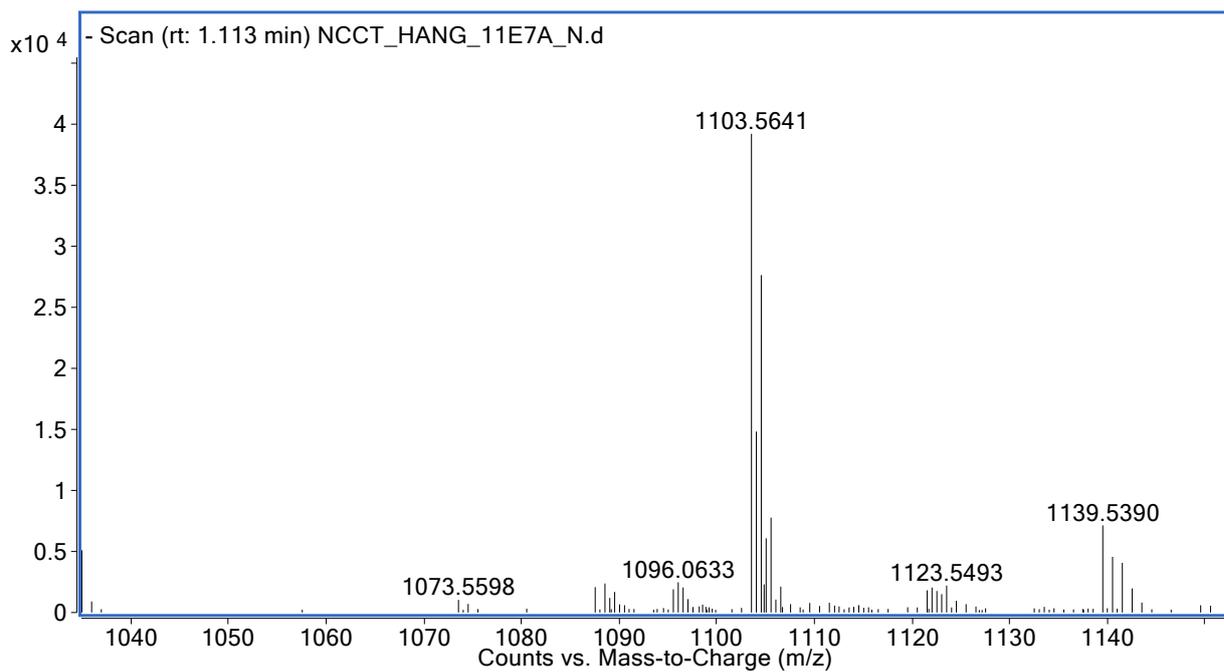


Figure S1. HR-ESI-MS of compound **1**

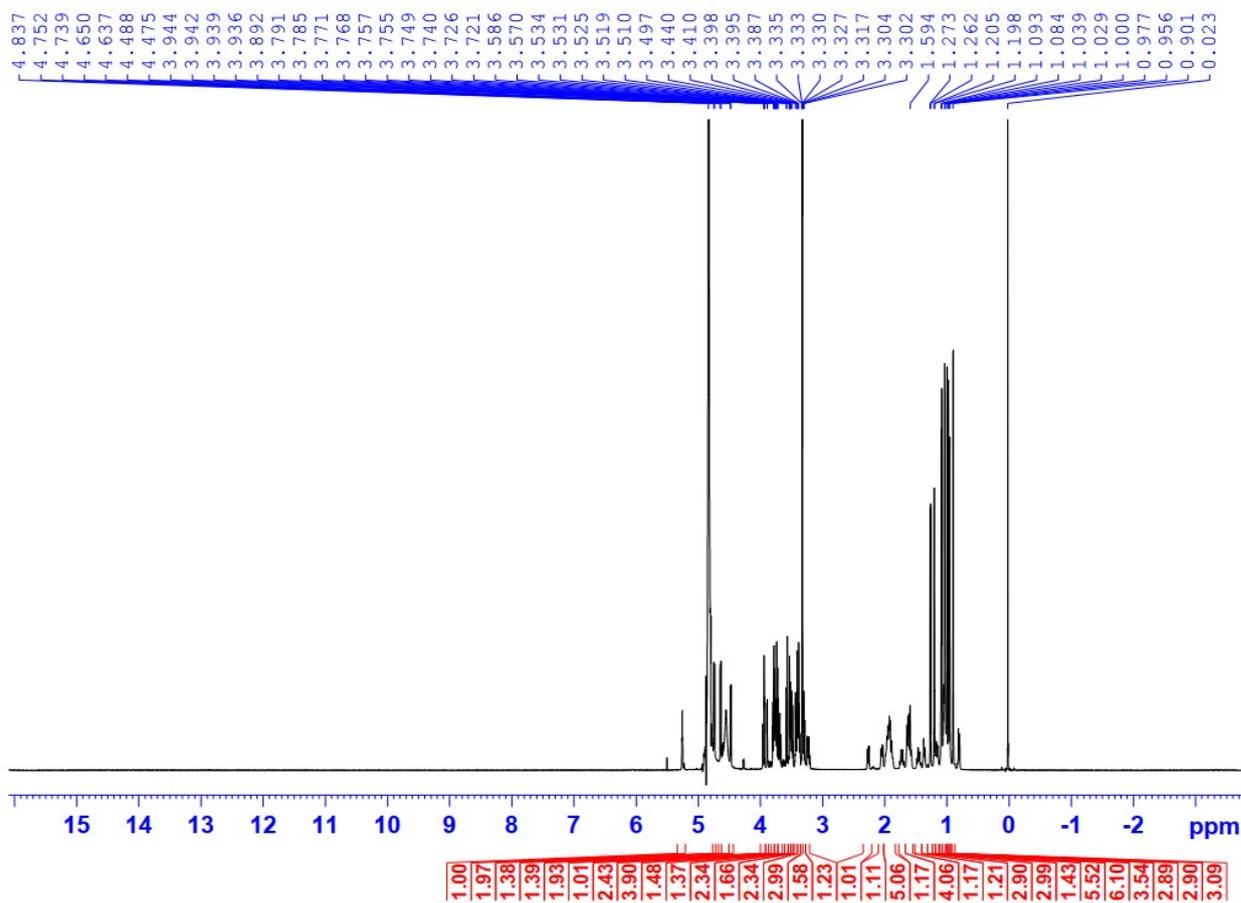


Figure S2.  $^1\text{H-NMR}$  spectrum of compound **1** in  $\text{CD}_3\text{OD}$

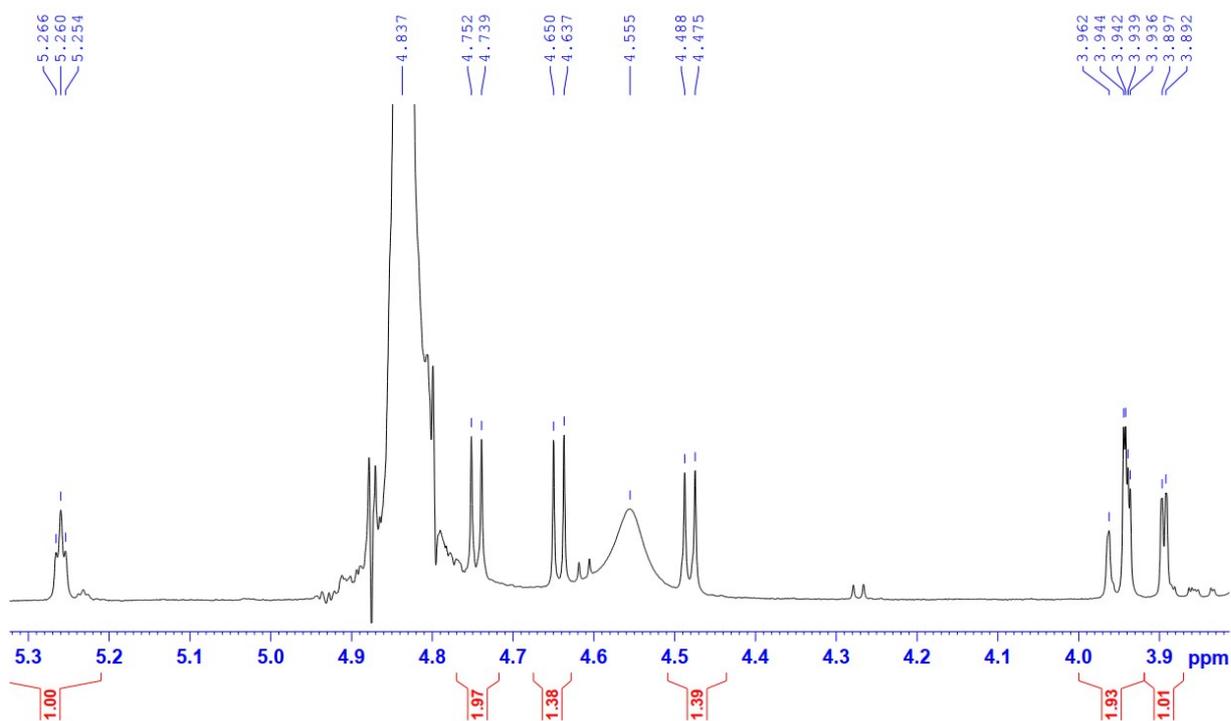


Figure S3. Expanded  $^1\text{H-NMR}$  spectrum of compound **1**

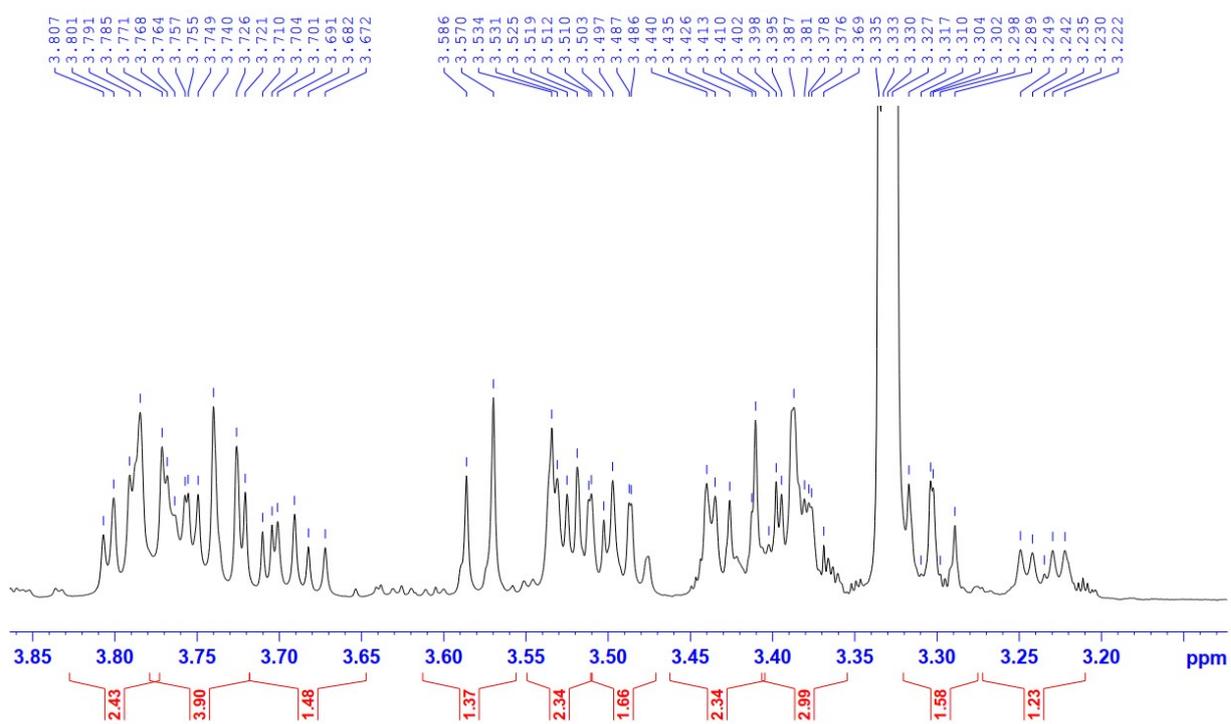


Figure S4. Expanded  $^1\text{H-NMR}$  spectrum of compound **1**

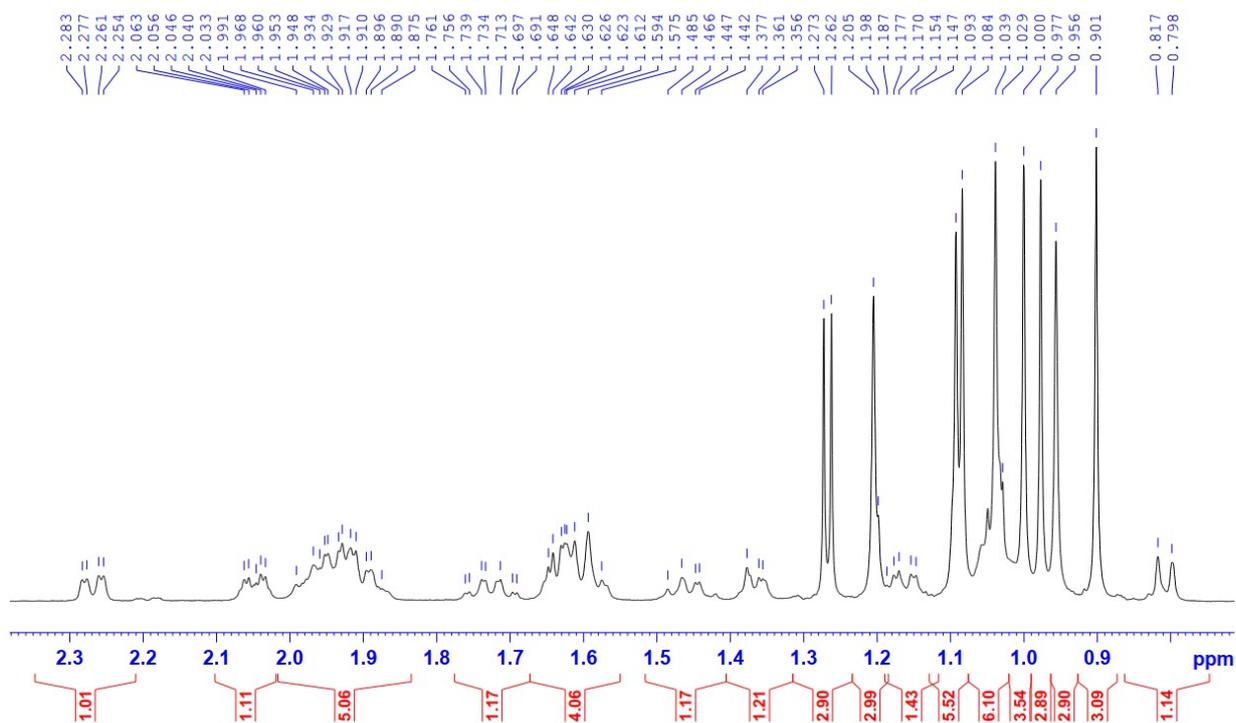


Figure S5. Expanded  $^1\text{H}$ -NMR spectrum of compound **1**

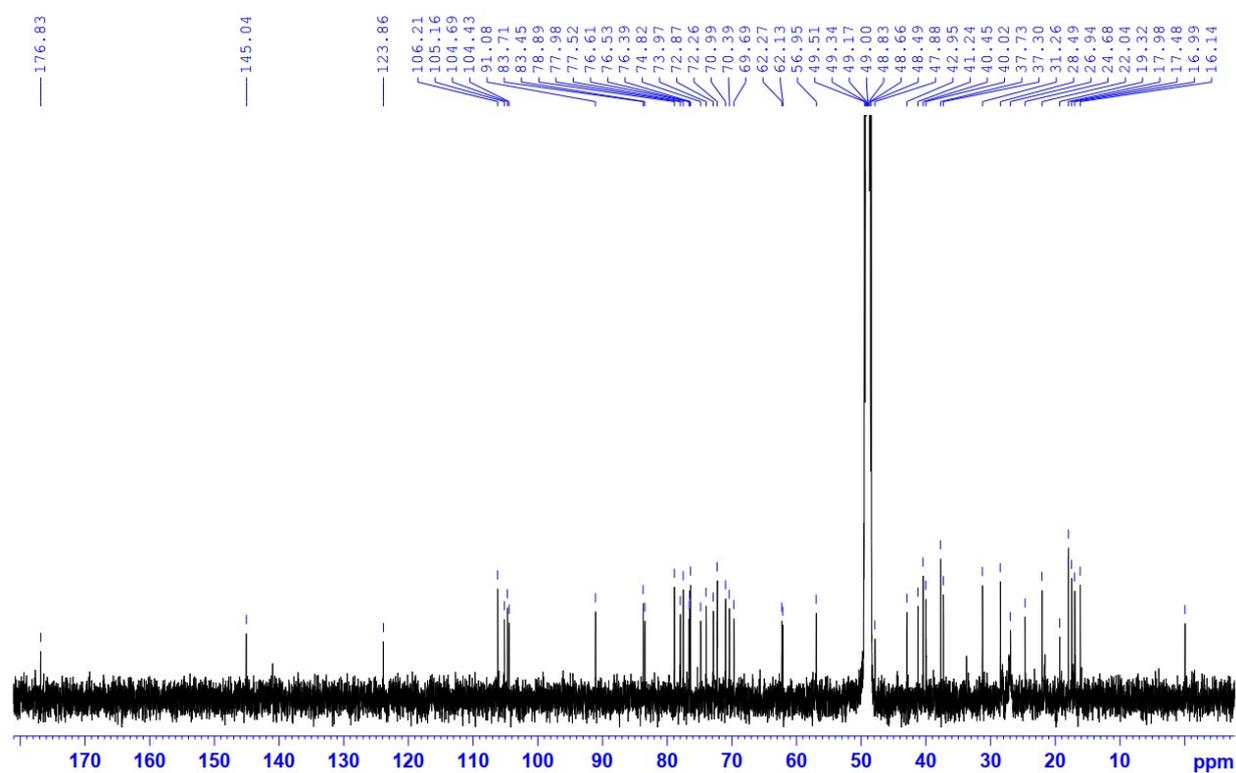


Figure S6.  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{CD}_3\text{OD}$

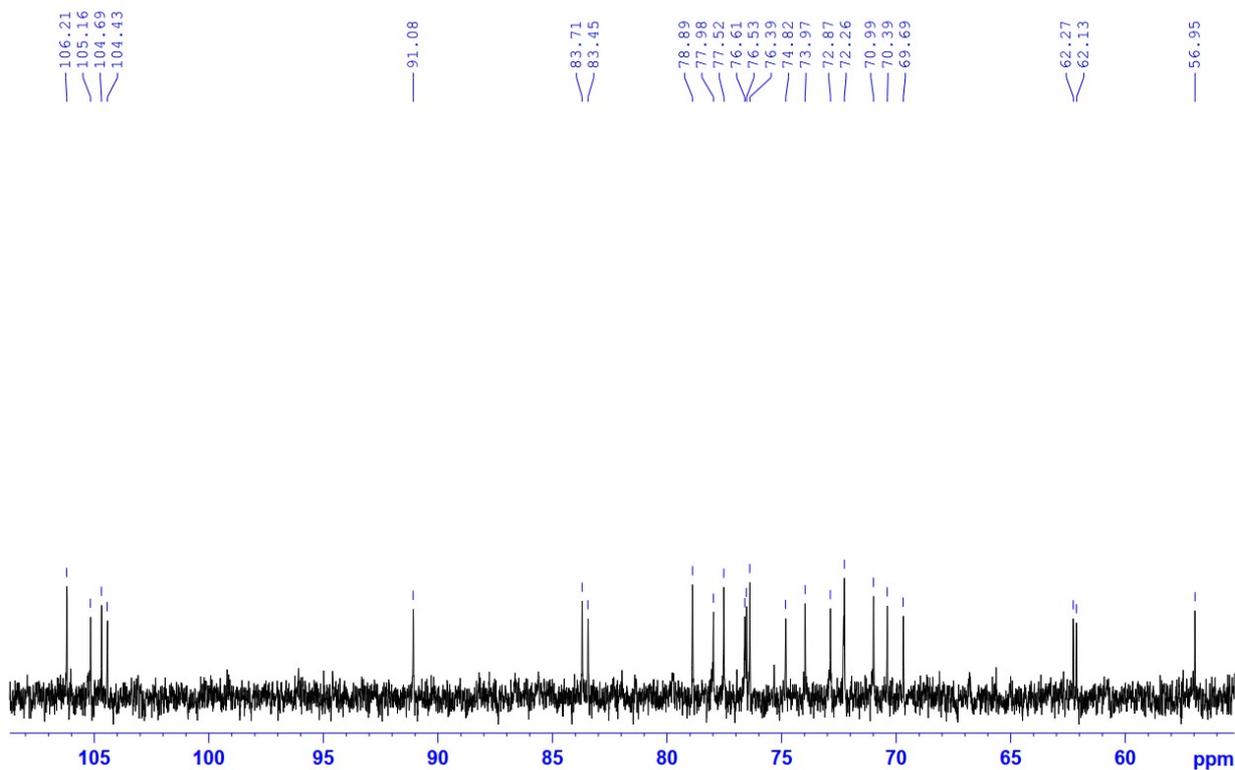


Figure S7. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **1**

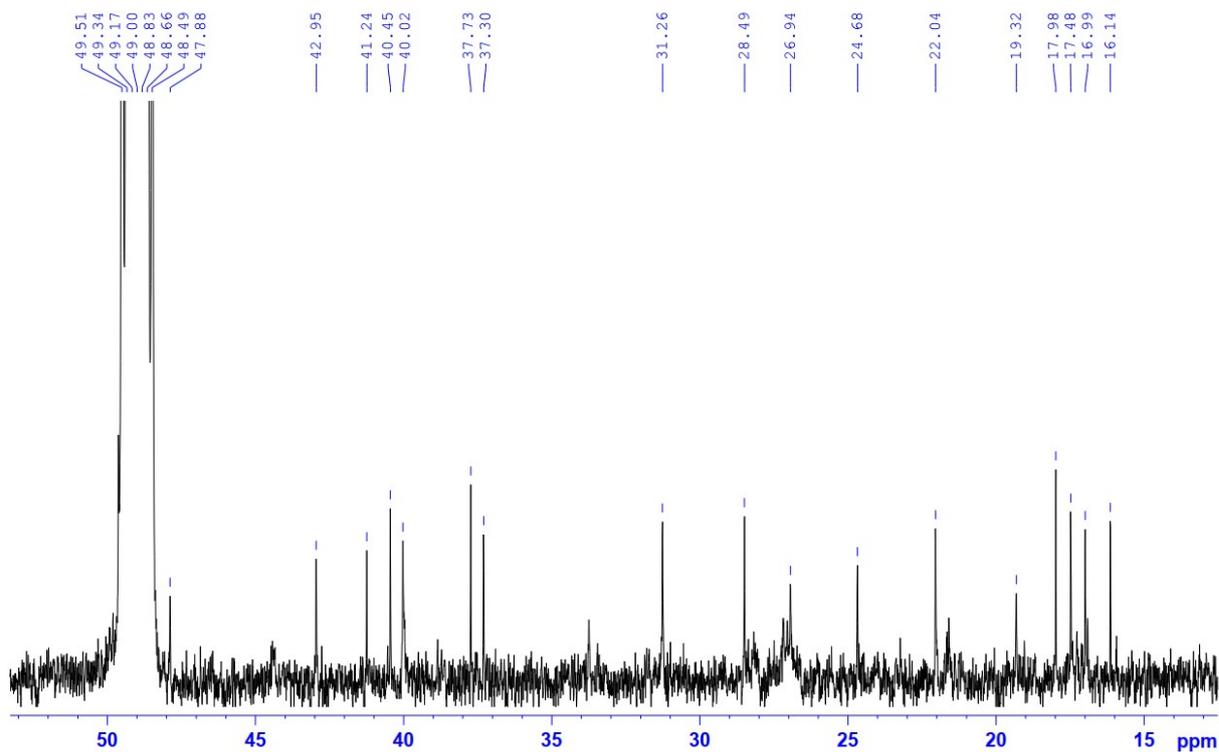


Figure S8. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **1**

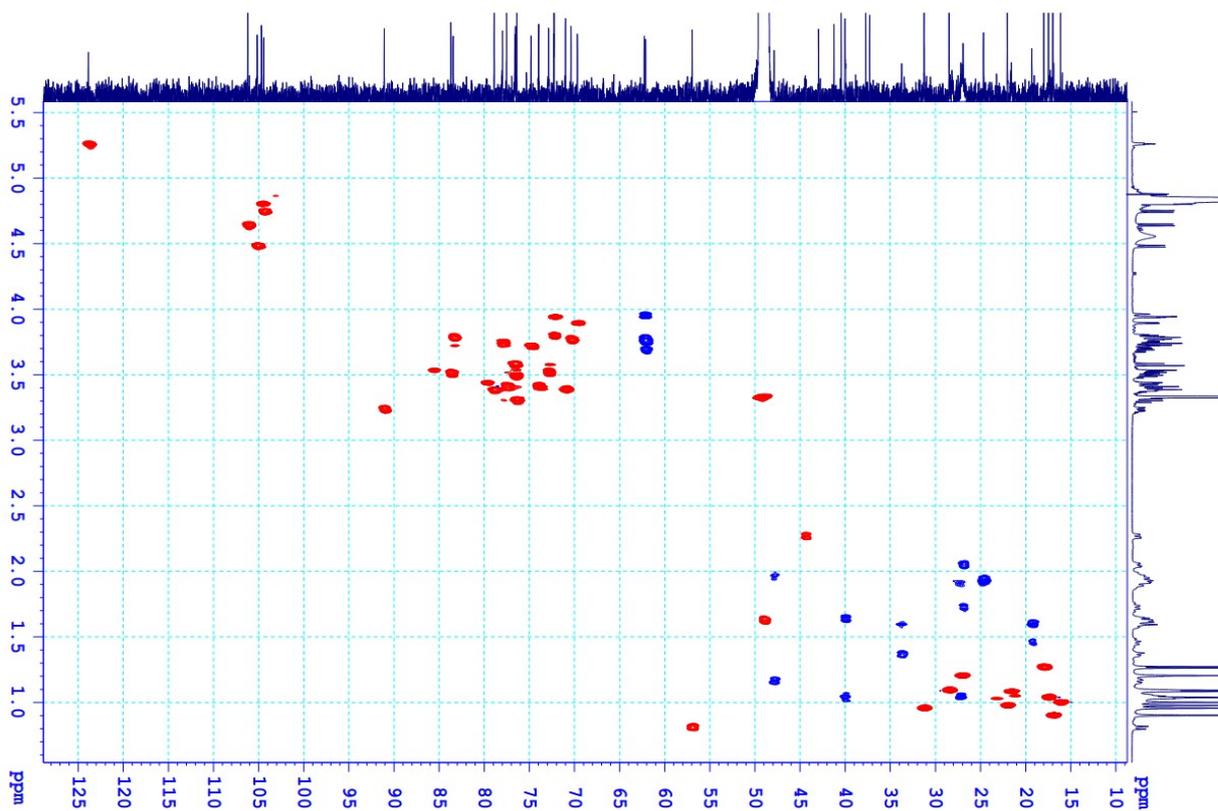


Figure S9. HSQC spectrum of compound **1**

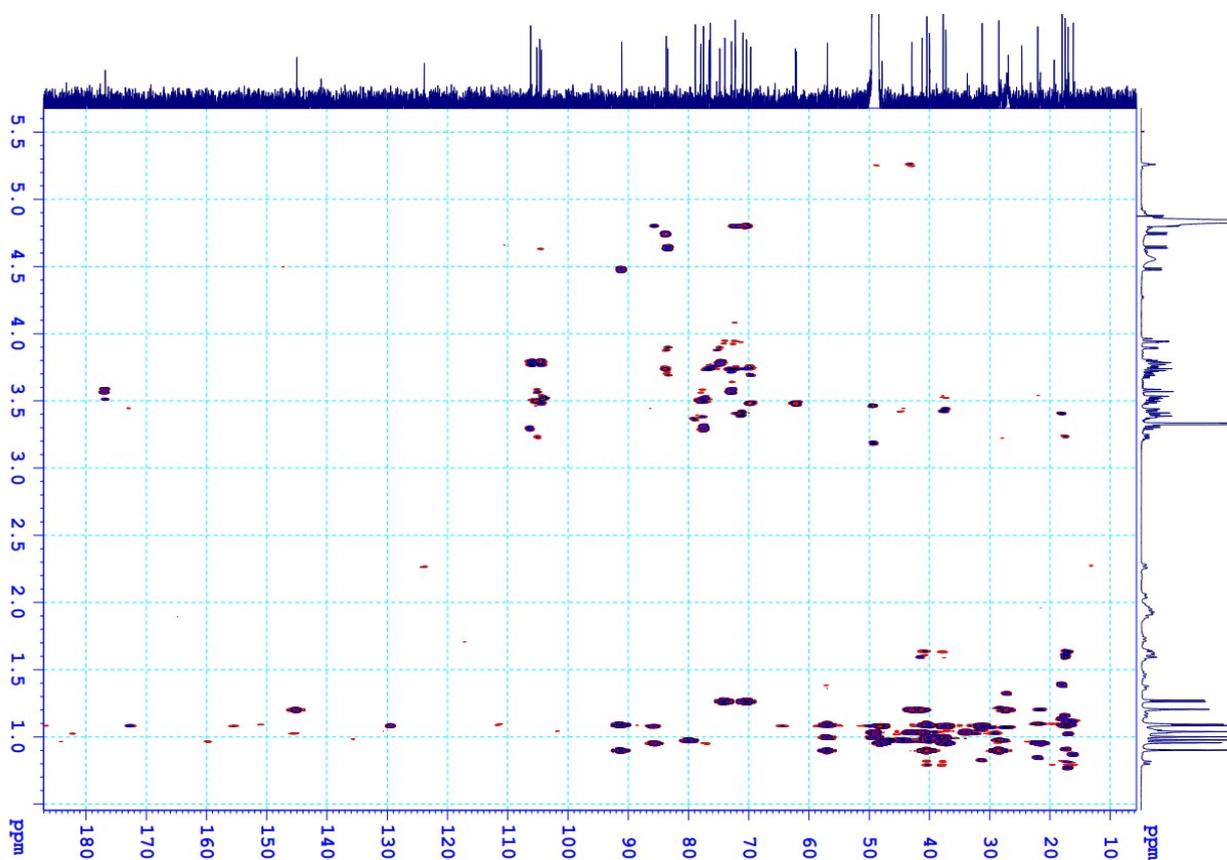


Figure S10. HMBC spectrum of compound **1**

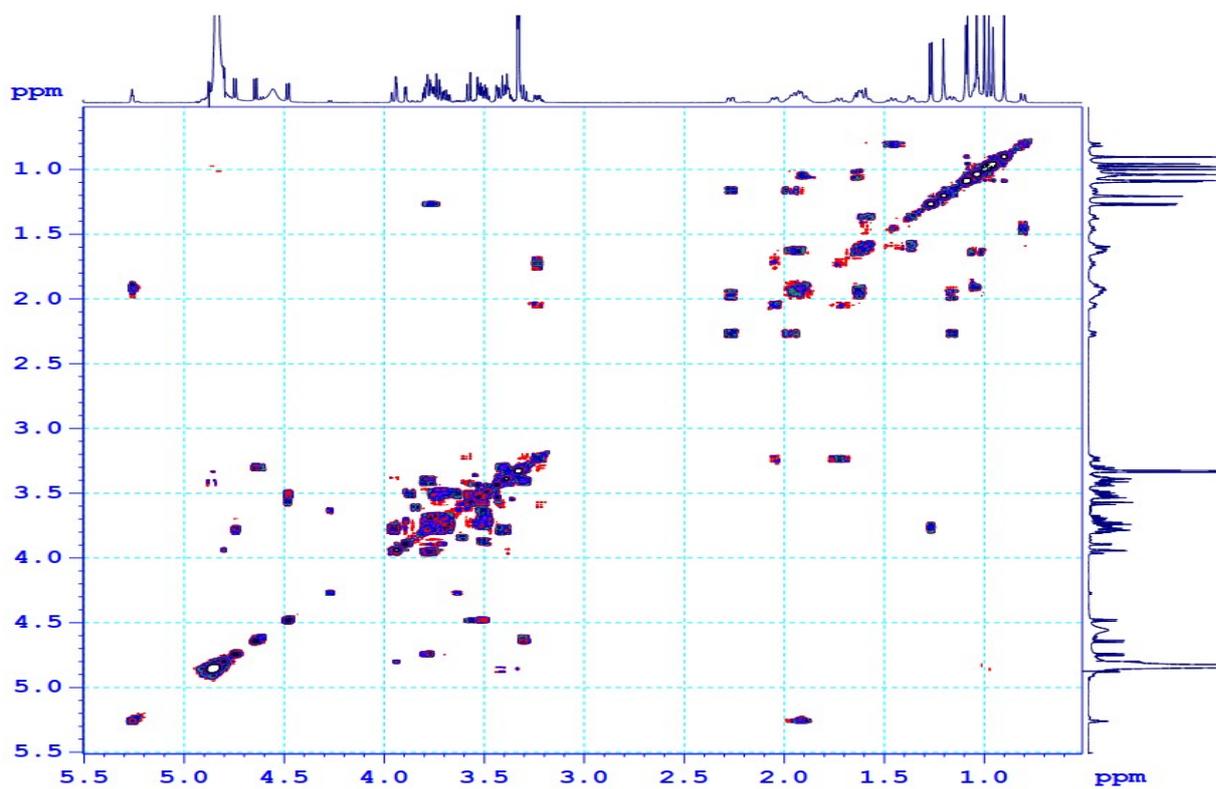


Figure S11. COSY spectrum of compound 1

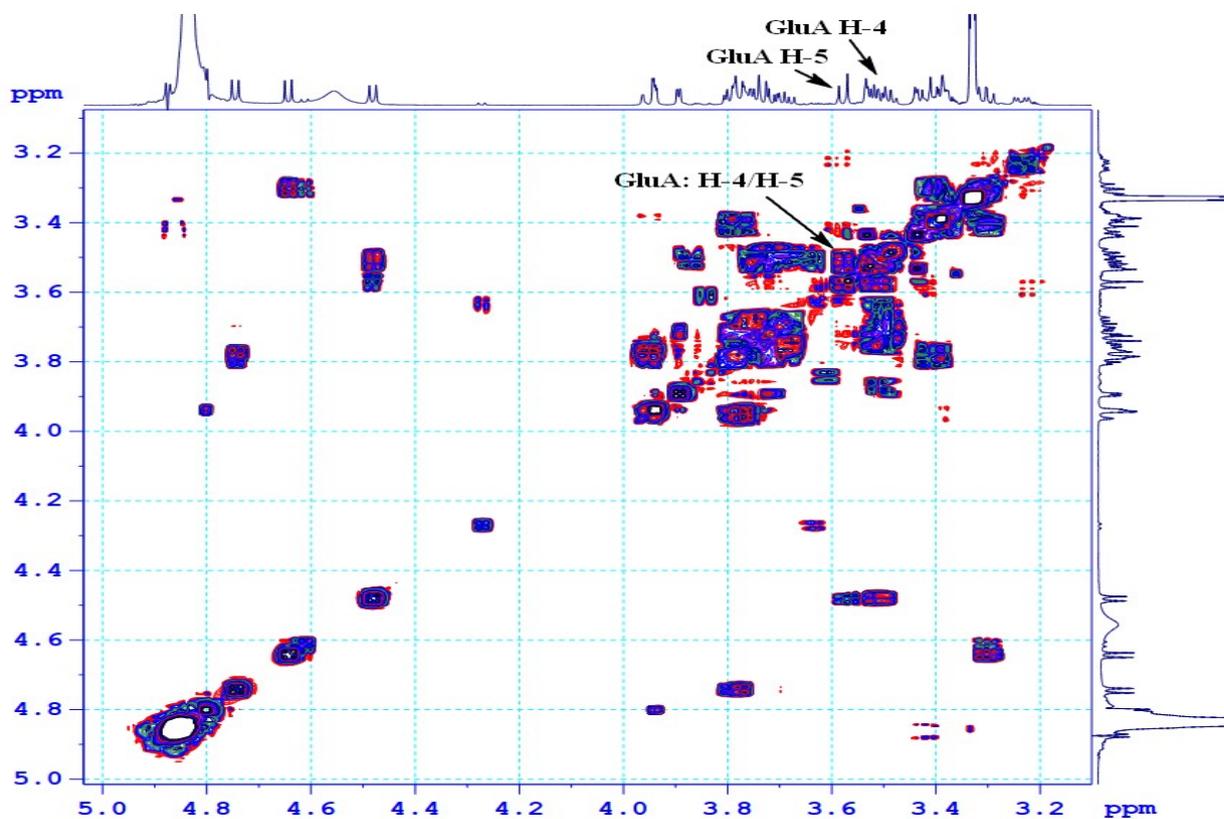


Figure S12. Expanded COSY spectrum of compound 1

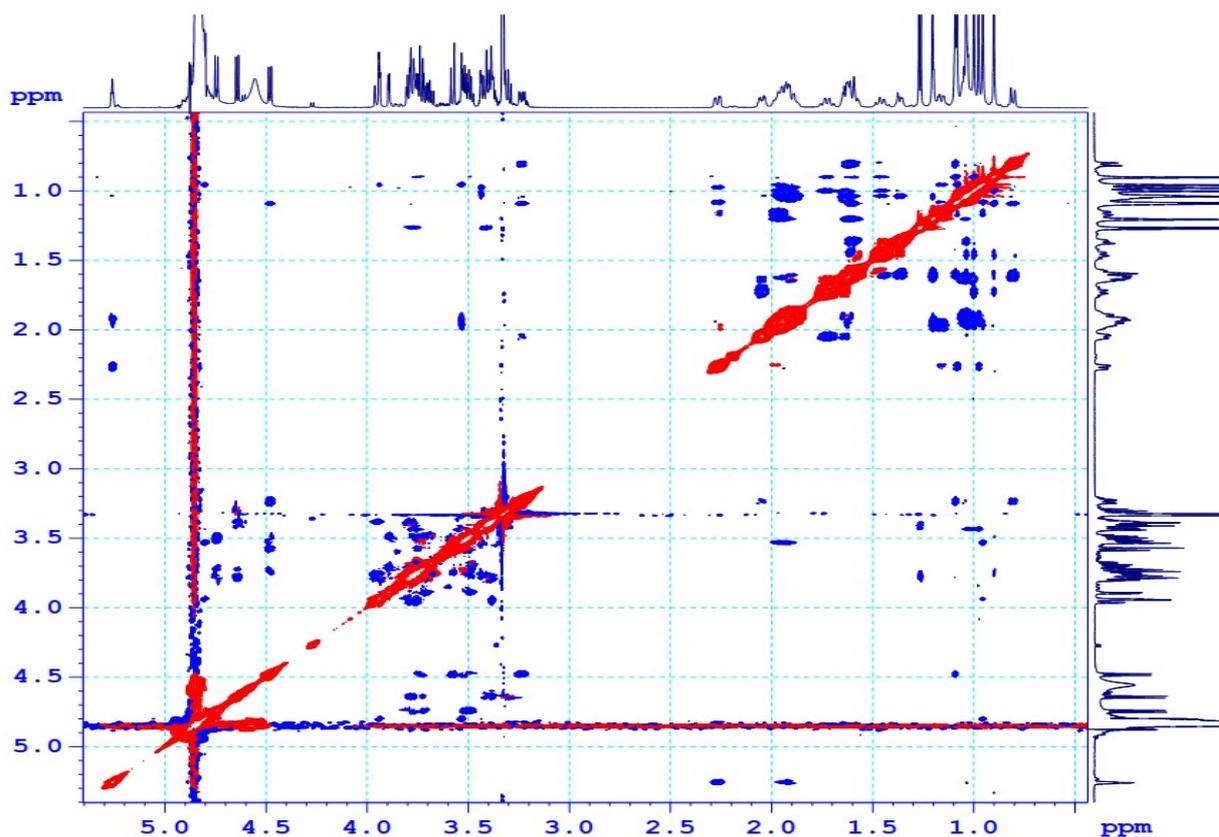


Figure S13. NOESY spectrum of compound 1

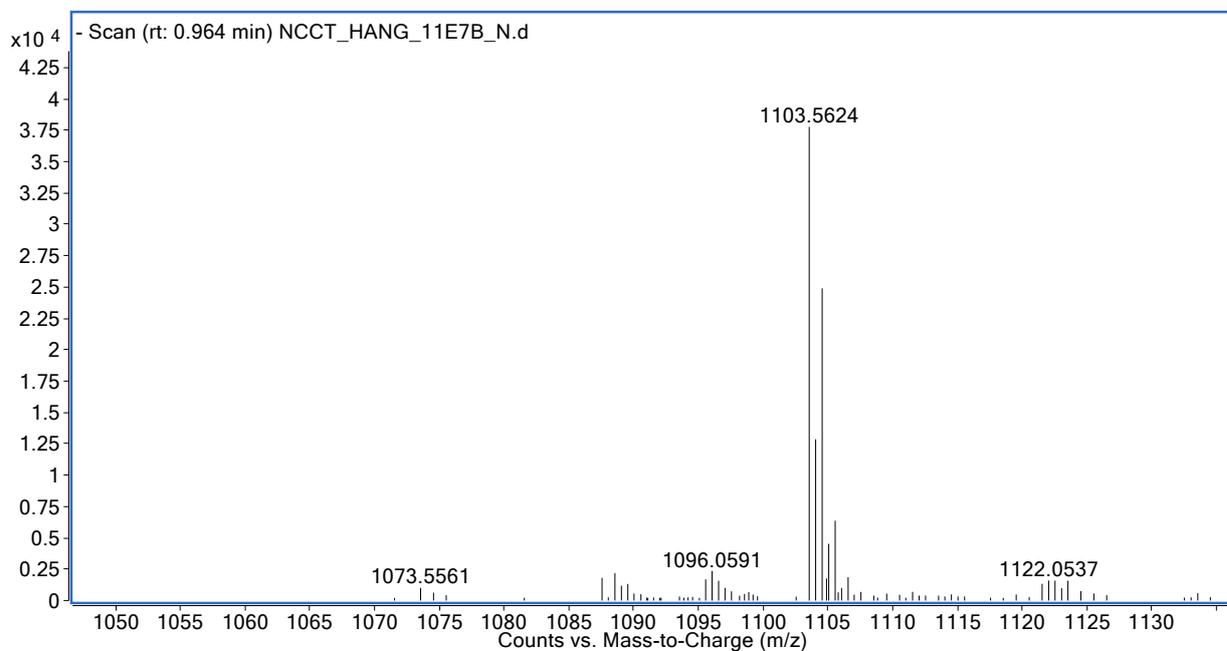


Figure S14. HR-ESI-MS of compound 2

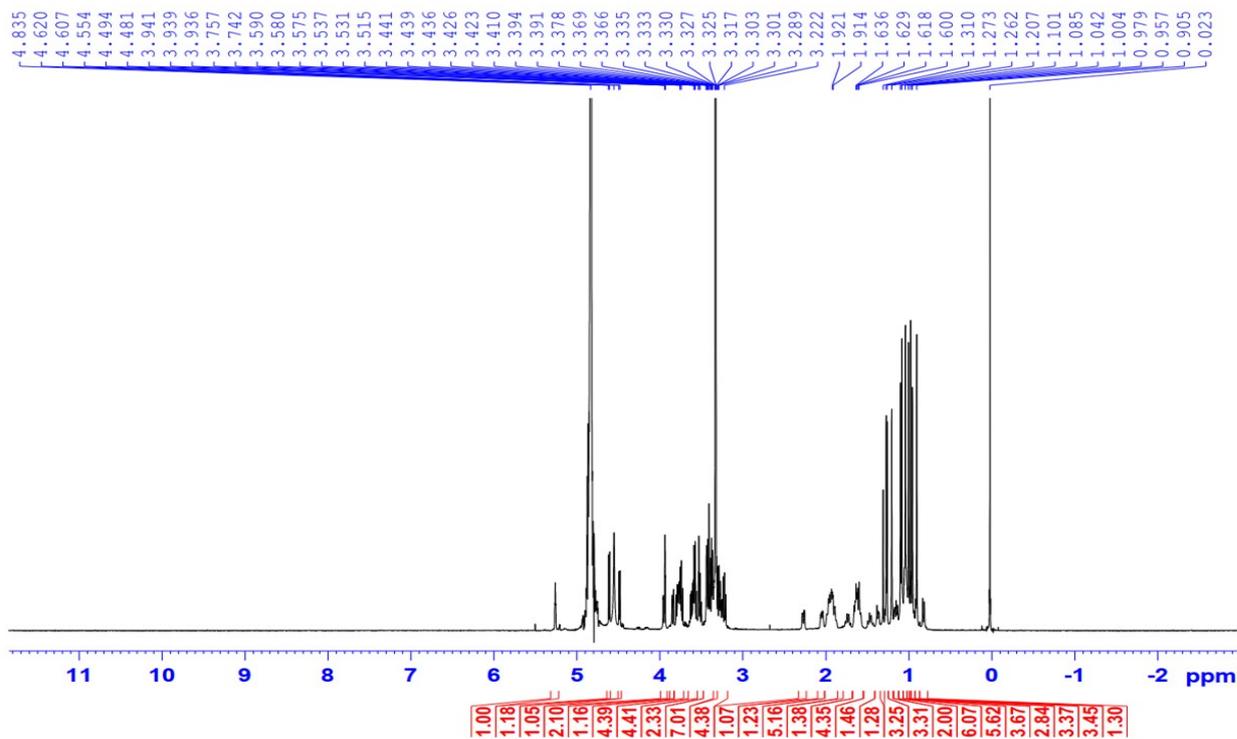


Figure S15.  $^1\text{H-NMR}$  spectrum of compound **2** in  $\text{CD}_3\text{OD}$

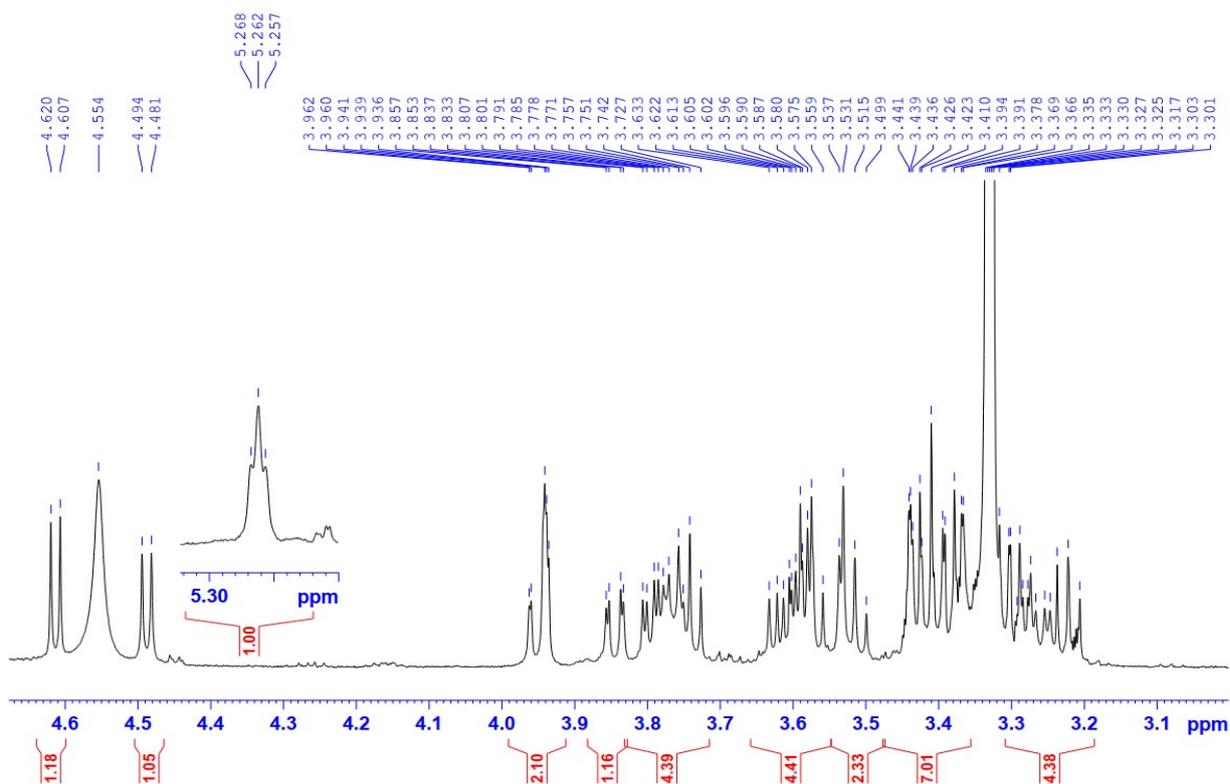


Figure S16. Expanded  $^1\text{H-NMR}$  spectrum of compound **2**

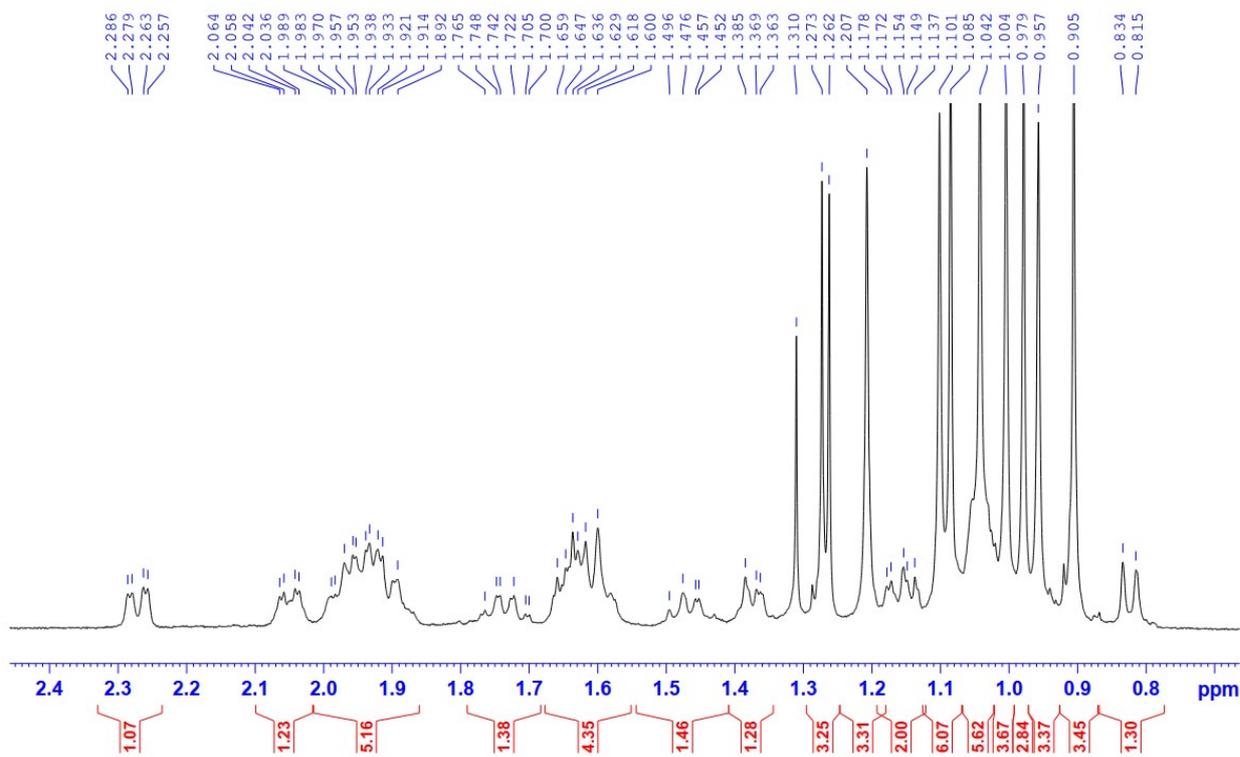


Figure S17. Expanded  $^1\text{H}$ -NMR spectrum of compound **2**

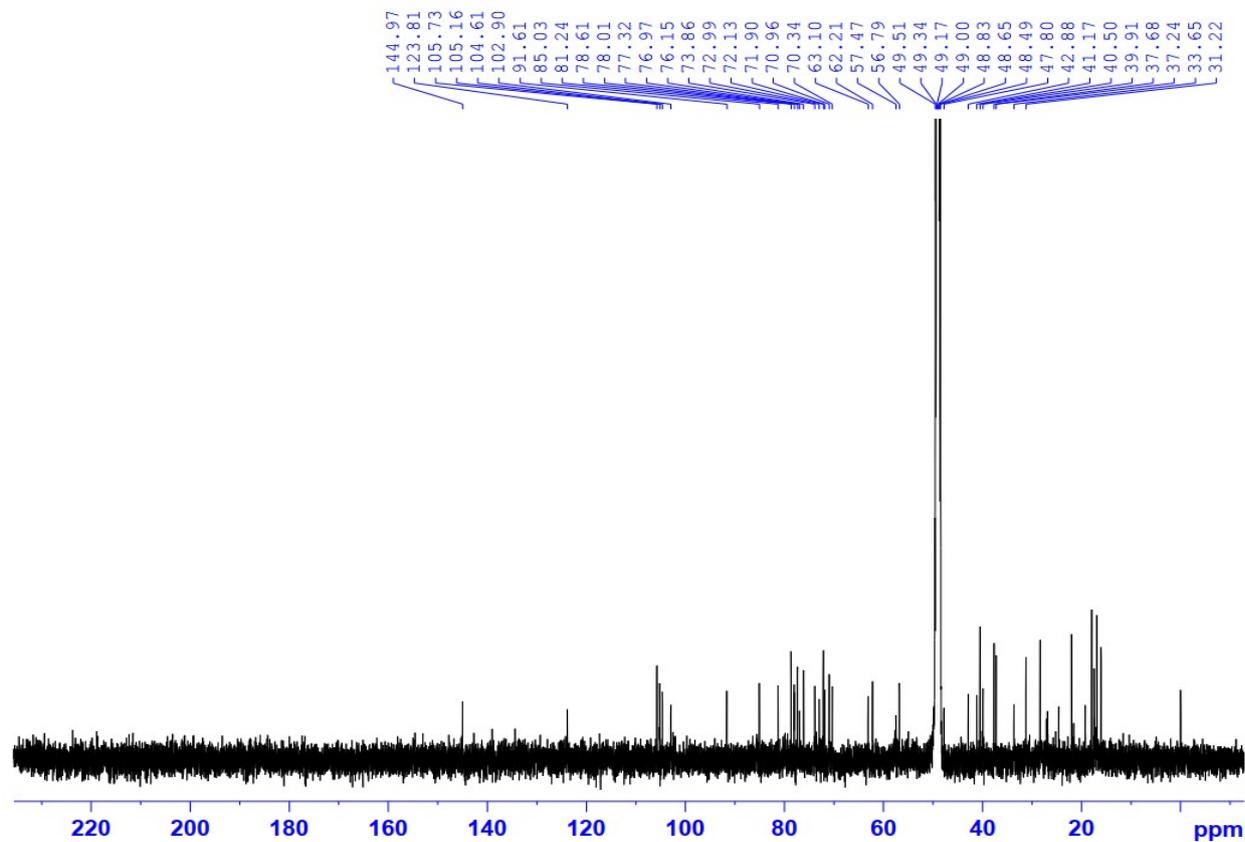


Figure S18.  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{CD}_3\text{OD}$

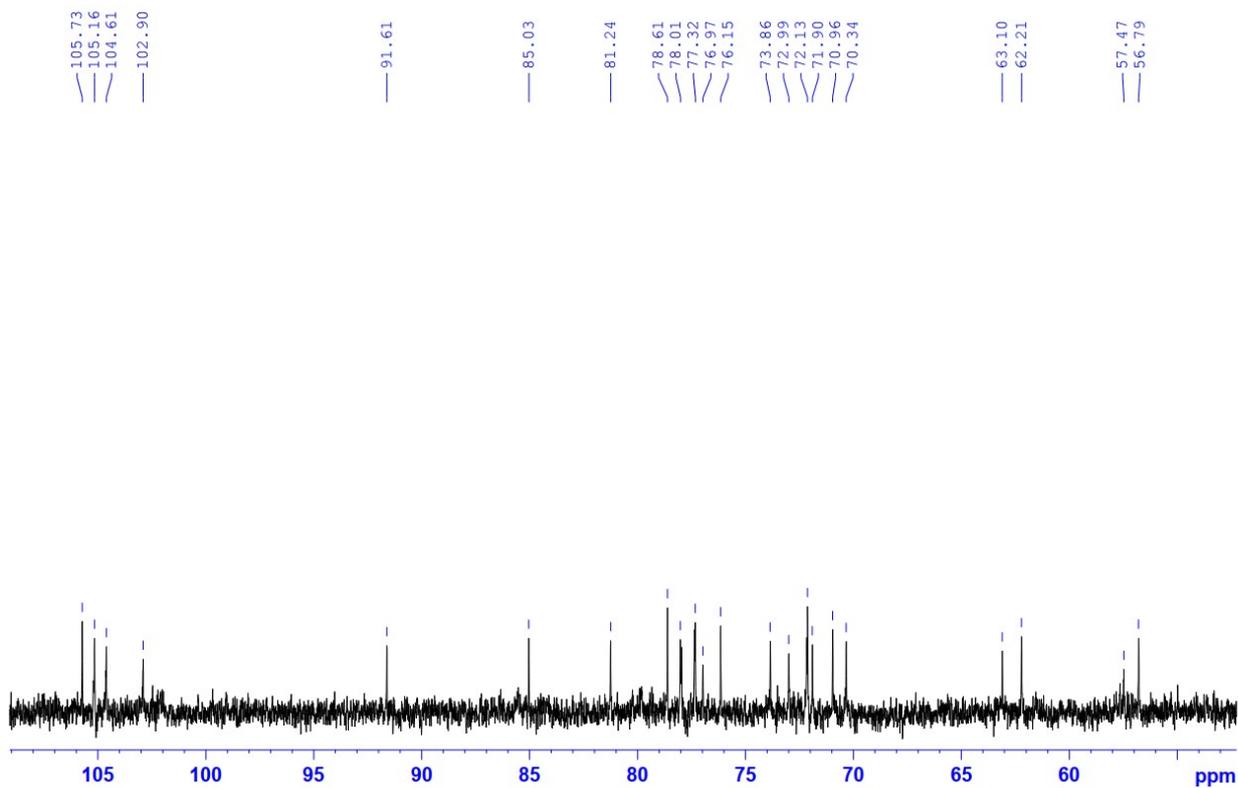


Figure S19. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{CD}_3\text{OD}$

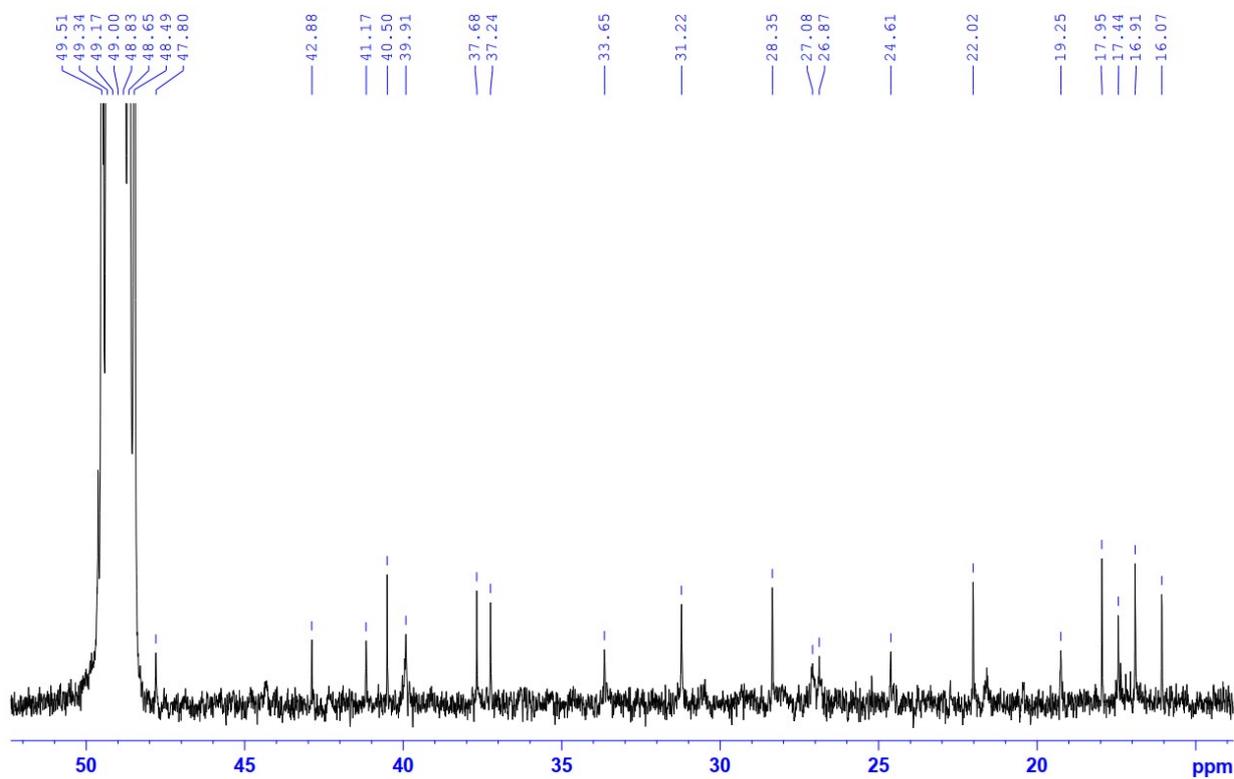


Figure S20. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{CD}_3\text{OD}$

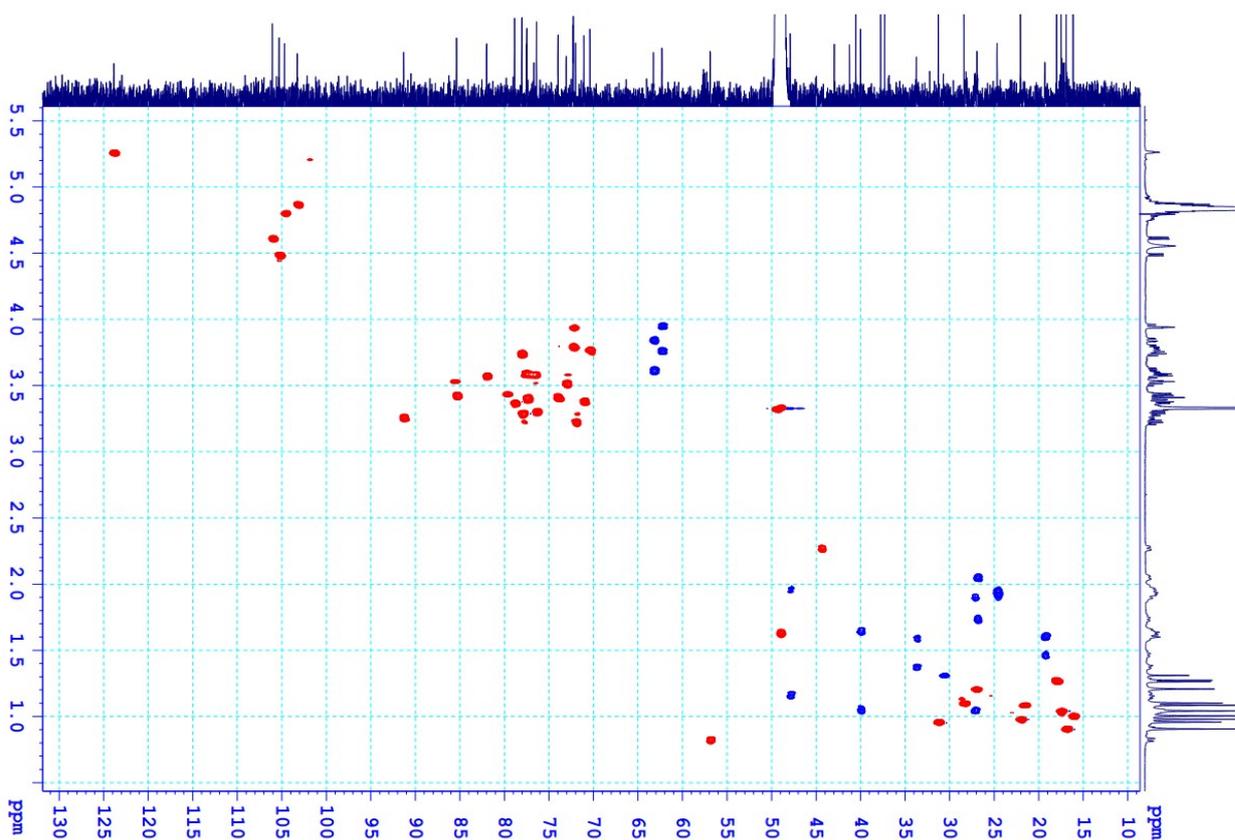


Figure S21. HSQC spectrum of compound 2

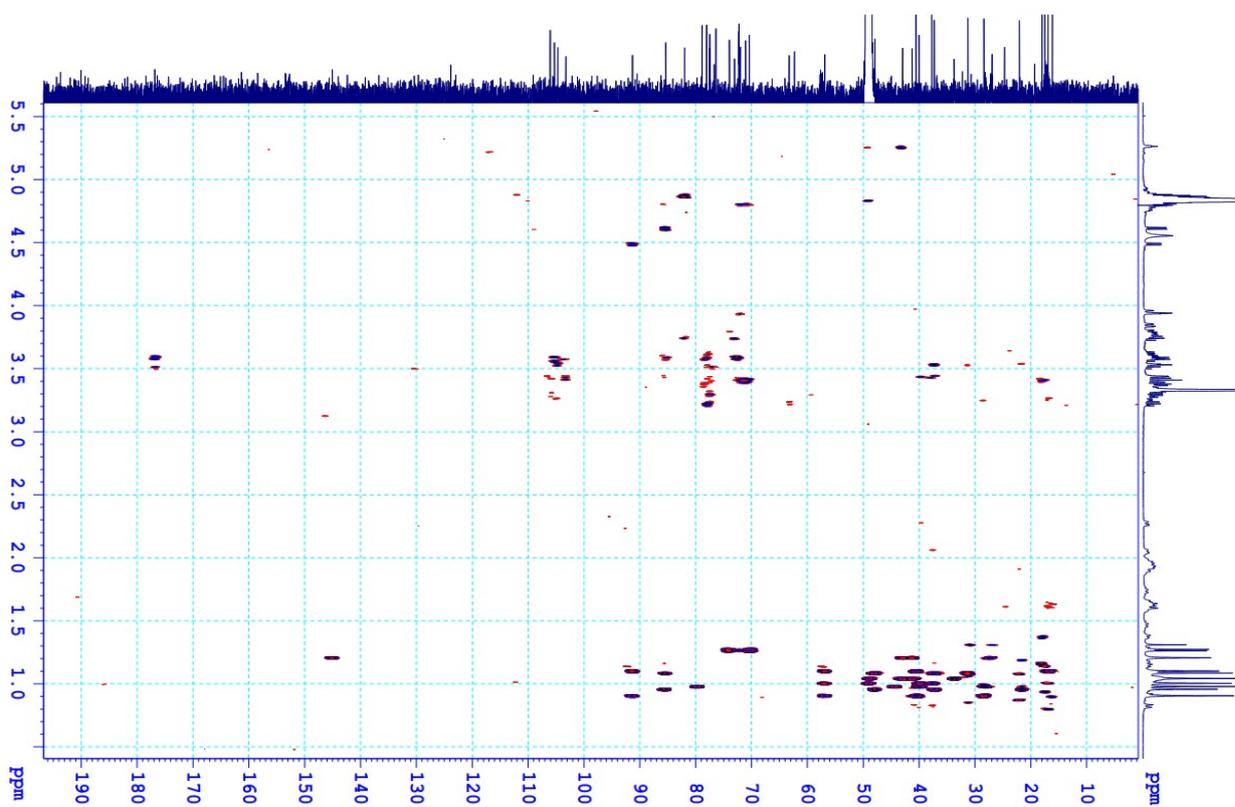


Figure S22. HMBC spectrum of compound 2

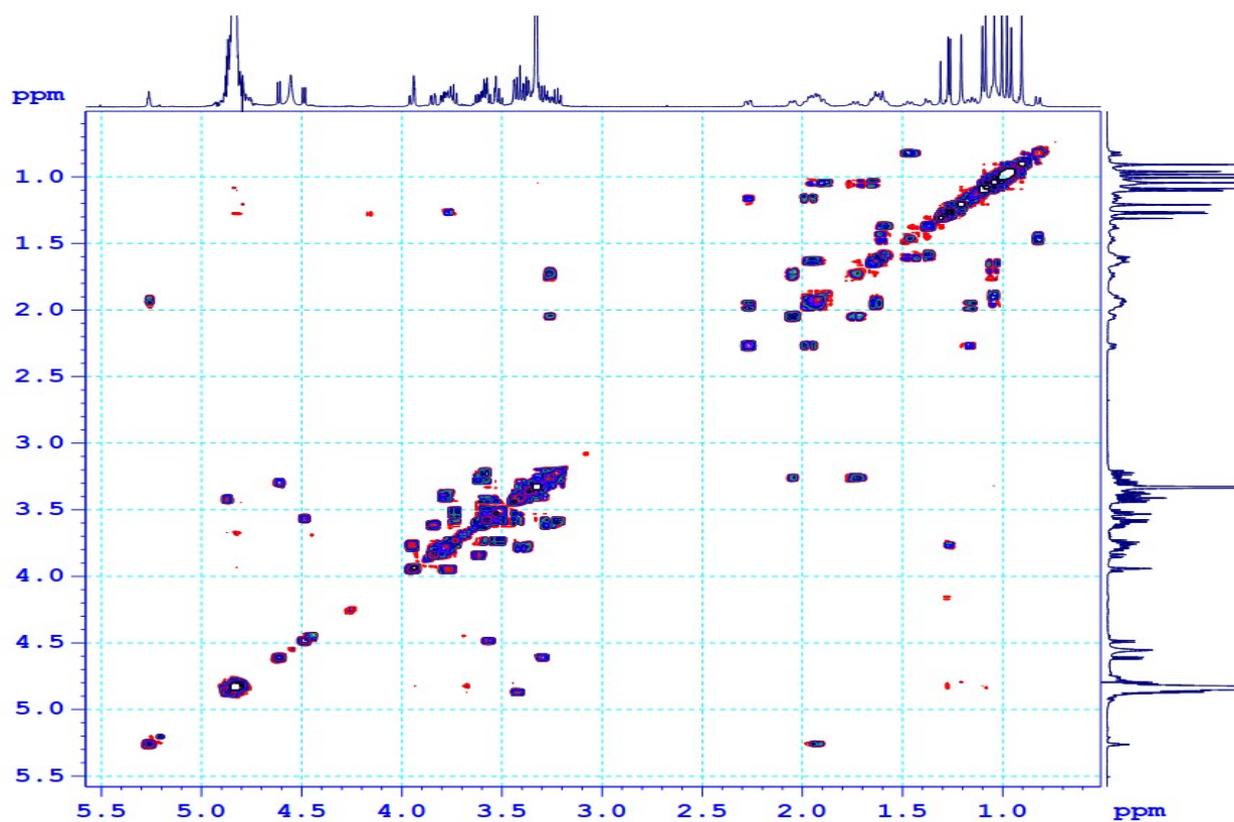


Figure S23. COSY spectrum of compound 2

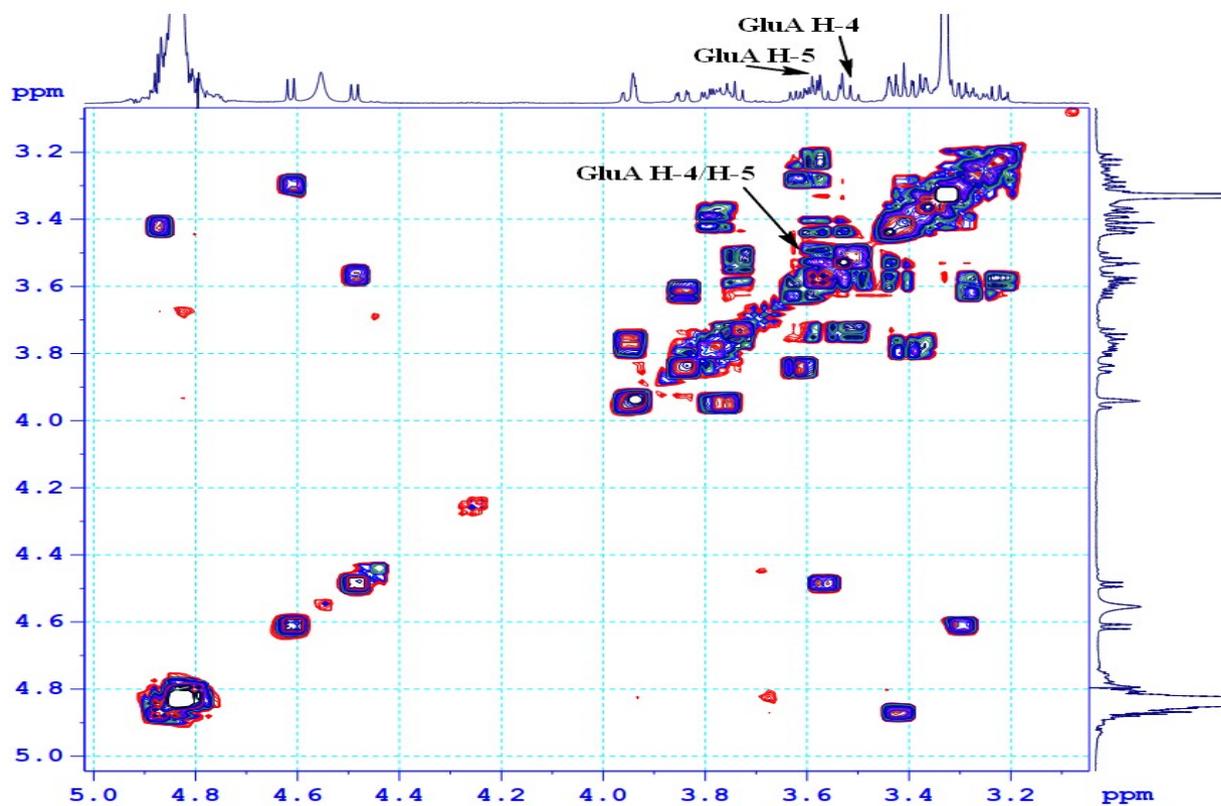


Figure S24. Expanded COSY spectrum of compound 2

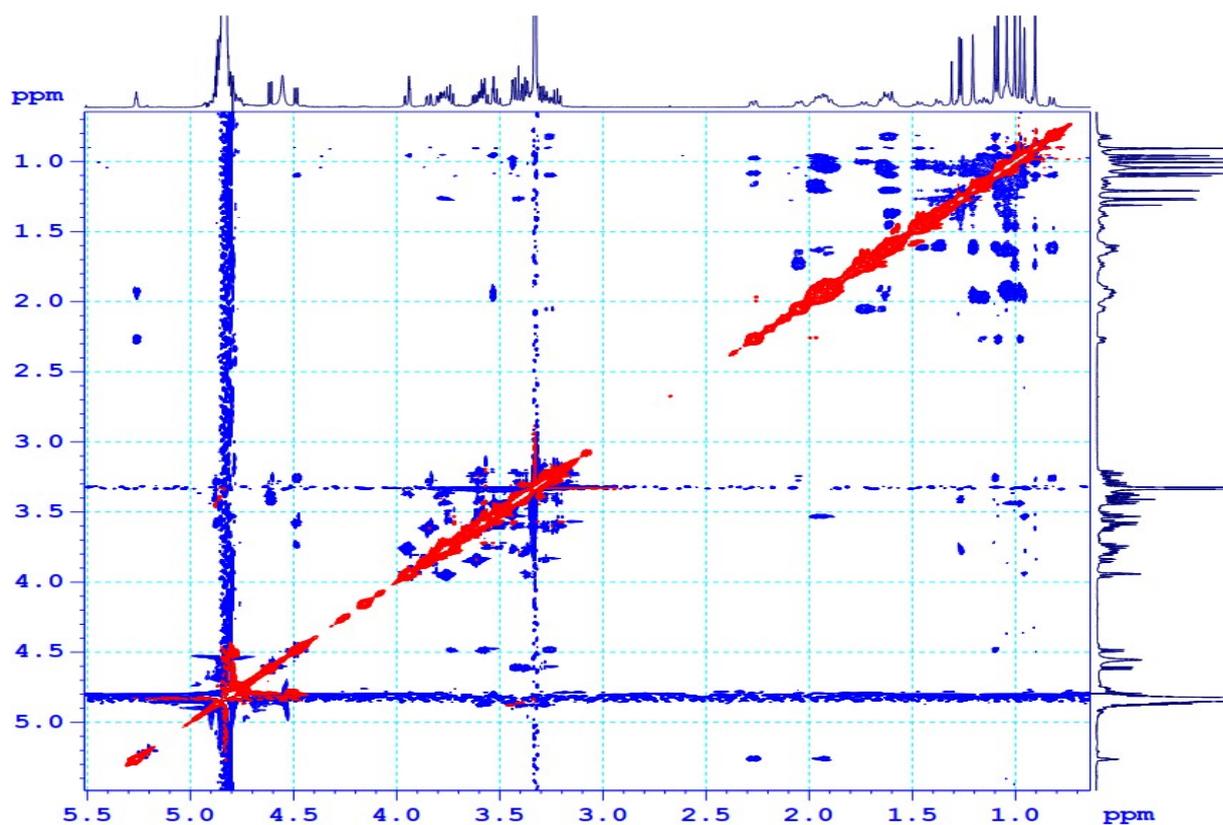


Figure S25. NOESY spectrum of compound 2

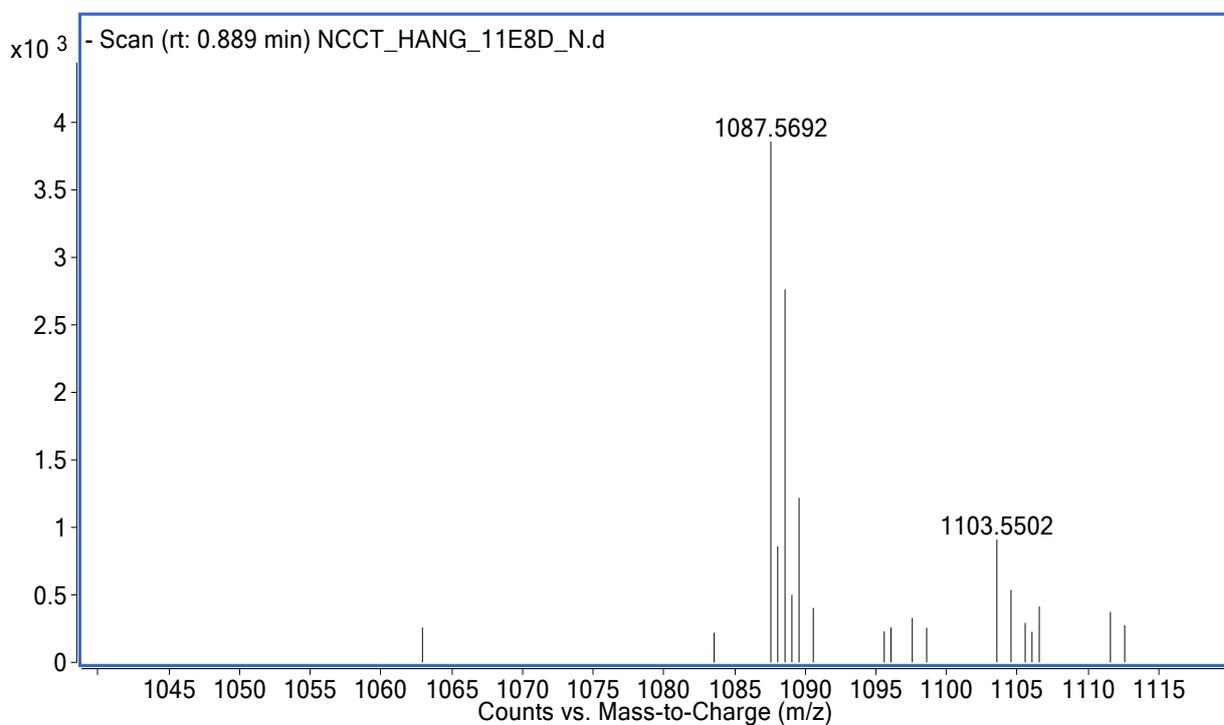


Figure S26. HR-ESI-MS of compound 3

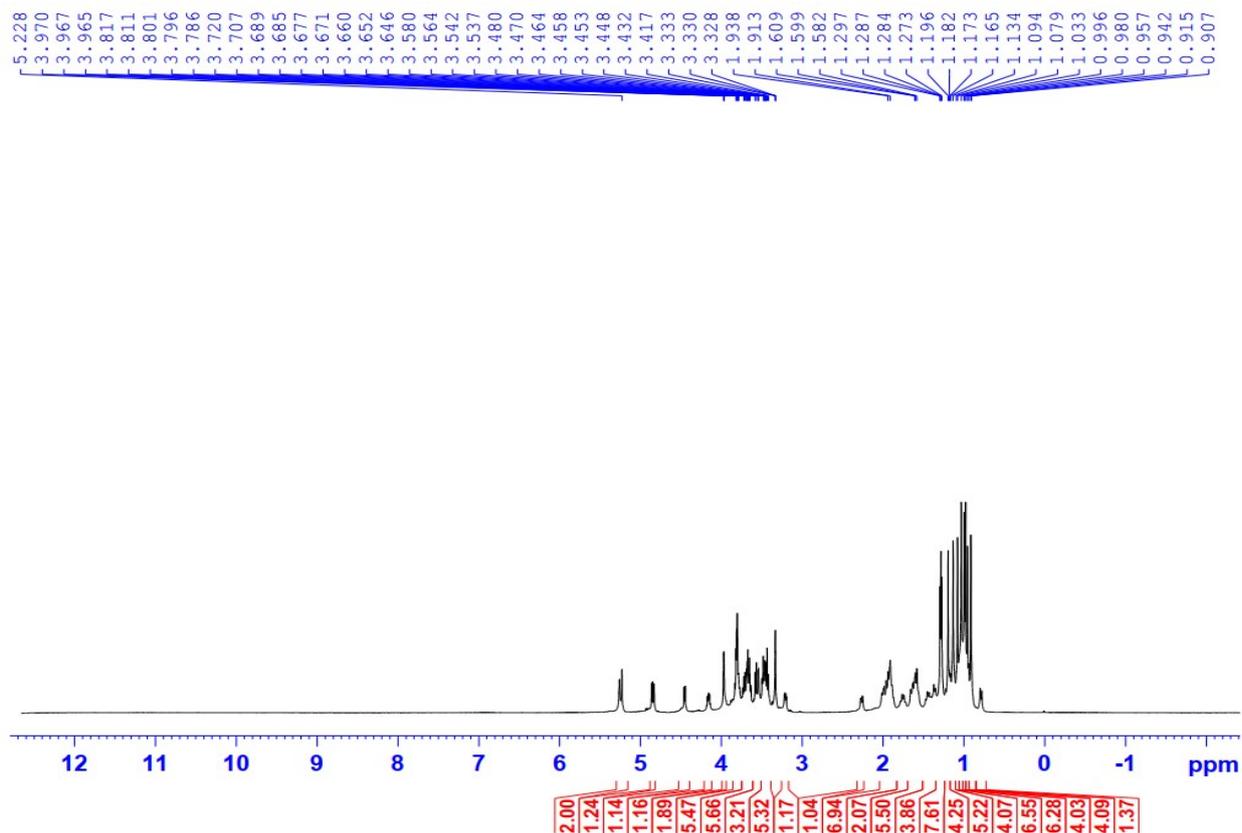


Figure S27.  $^1\text{H-NMR}$  spectrum of compound **3** in  $\text{CD}_3\text{OD}$

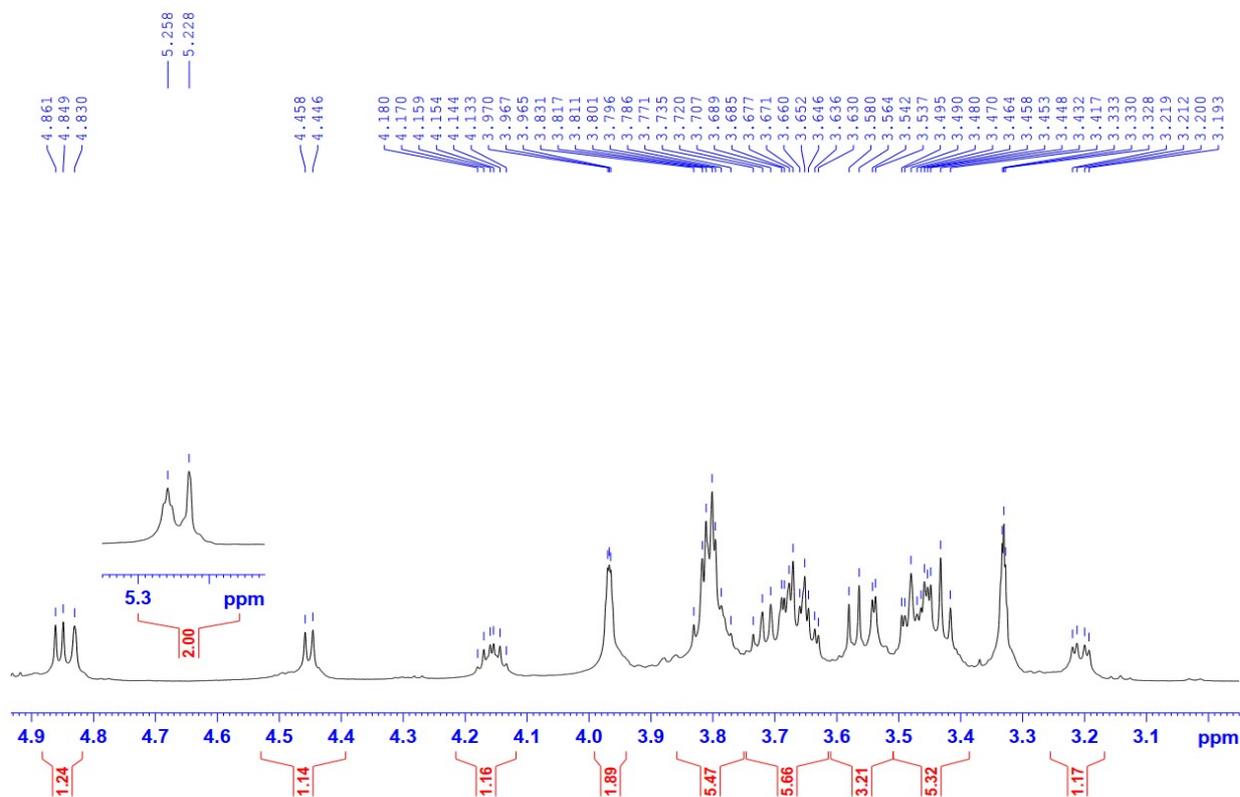


Figure S28. Expanded  $^1\text{H-NMR}$  spectrum of compound **3**

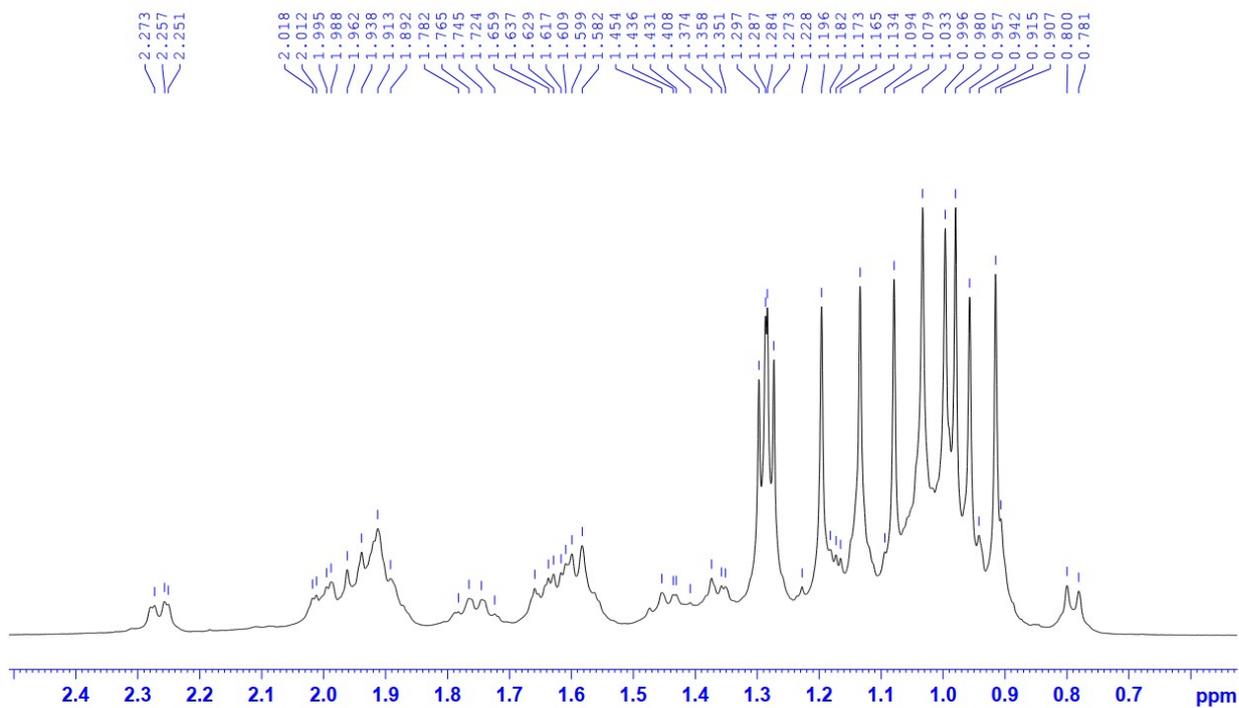


Figure S29. Expanded  $^1\text{H}$ -NMR spectrum of compound **3**

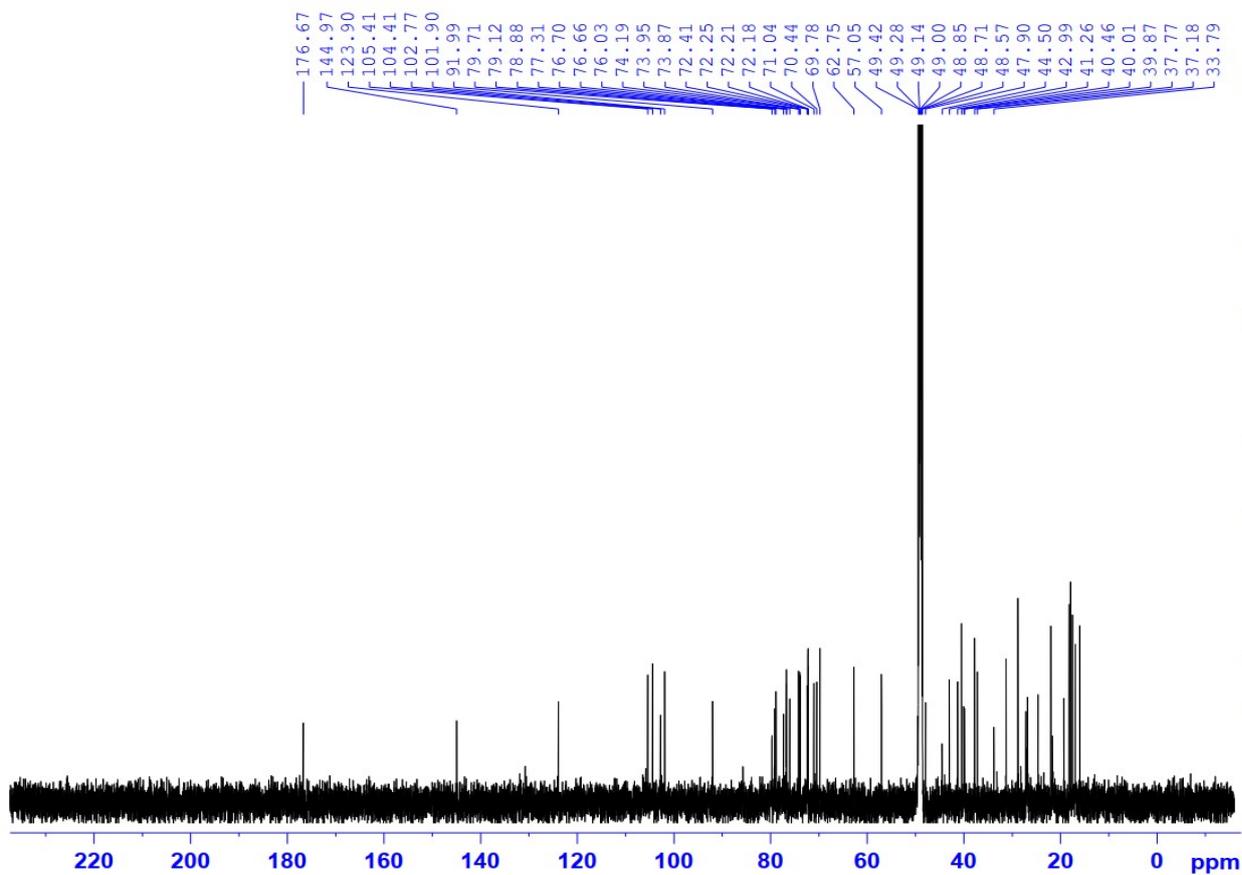


Figure S30.  $^{13}\text{C}$ -NMR spectrum of compound **3** in  $\text{CD}_3\text{OD}$

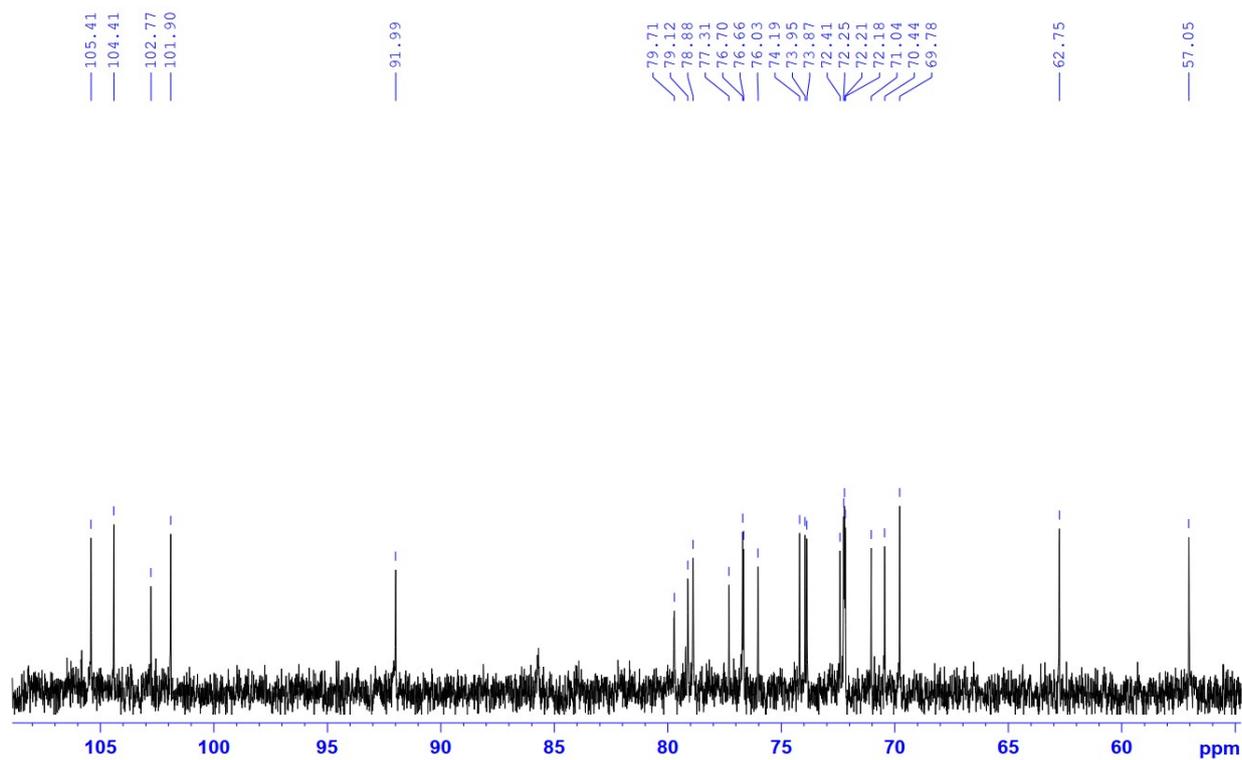


Figure S31. Expande  $^{13}\text{C}$ -NMR spectrum of compound **3** in  $\text{CD}_3\text{OD}$

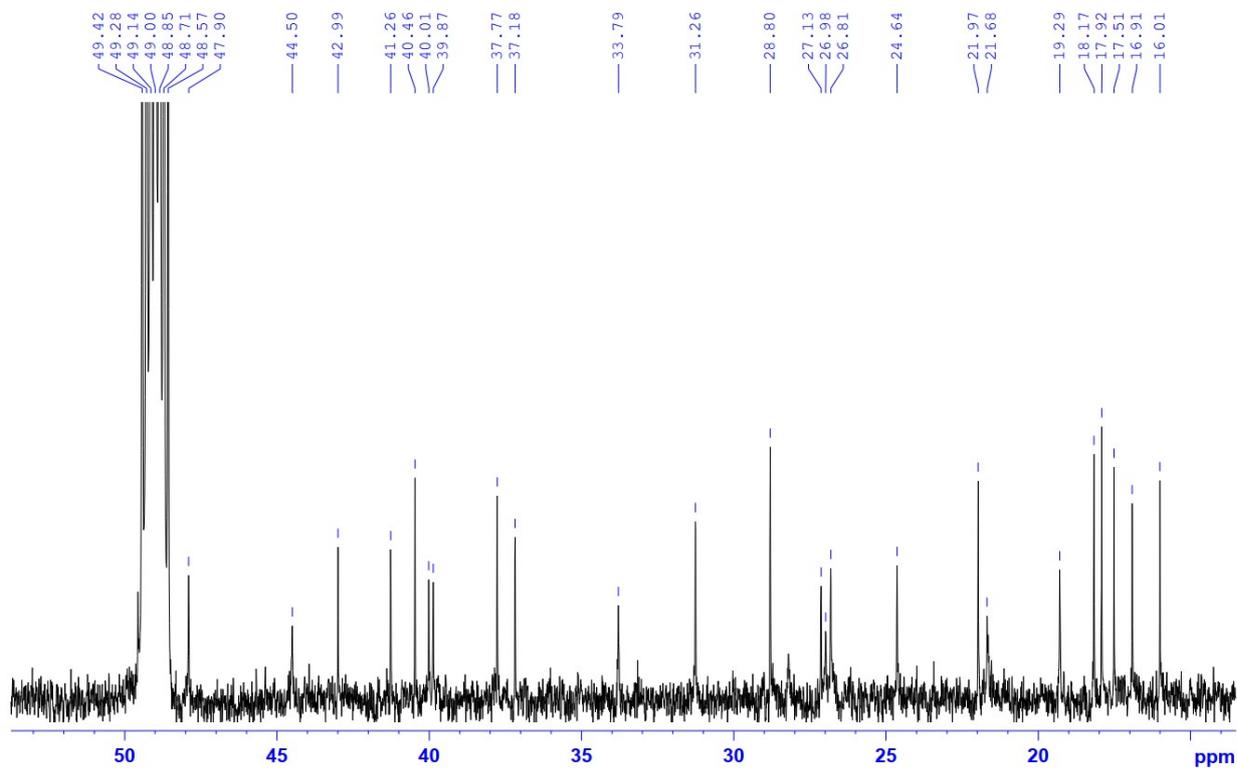


Figure S32. Expande  $^{13}\text{C}$ -NMR spectrum of compound **3** in  $\text{CD}_3\text{OD}$

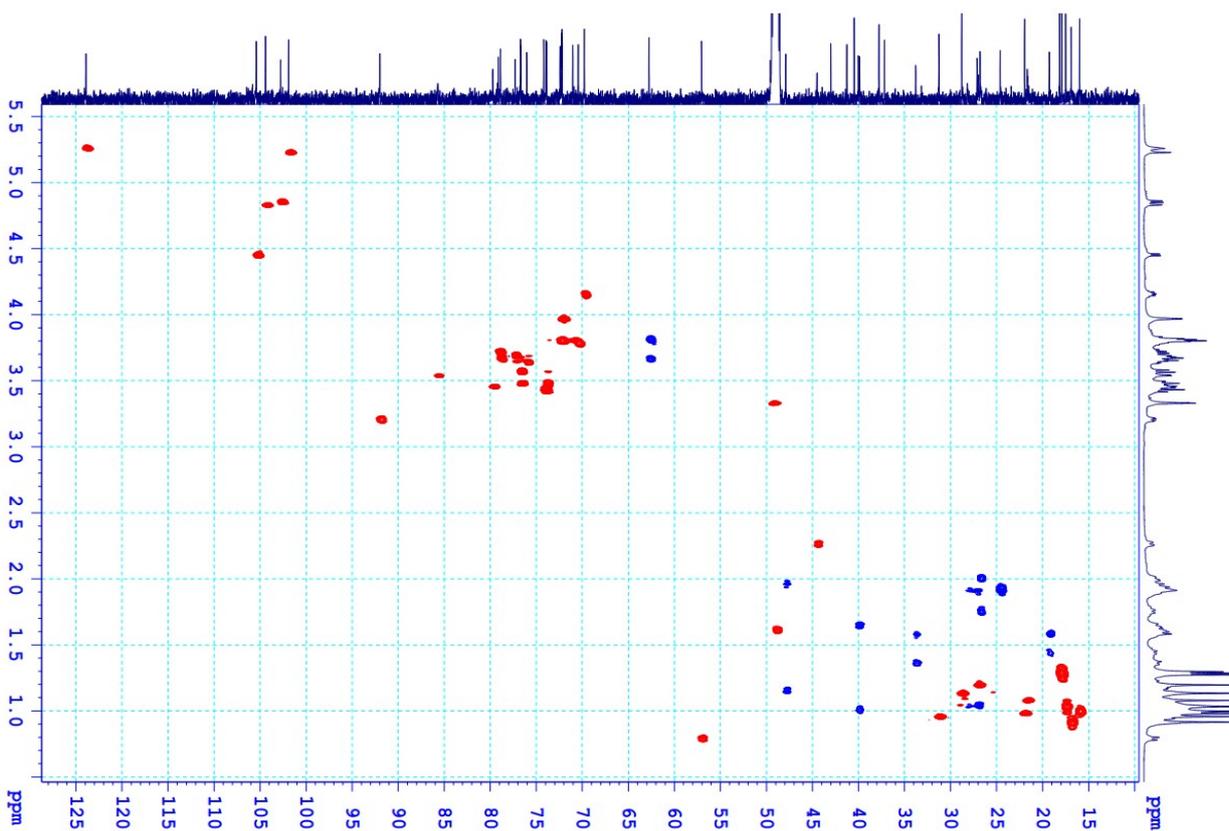


Figure S33. HSQC spectrum of compound 3

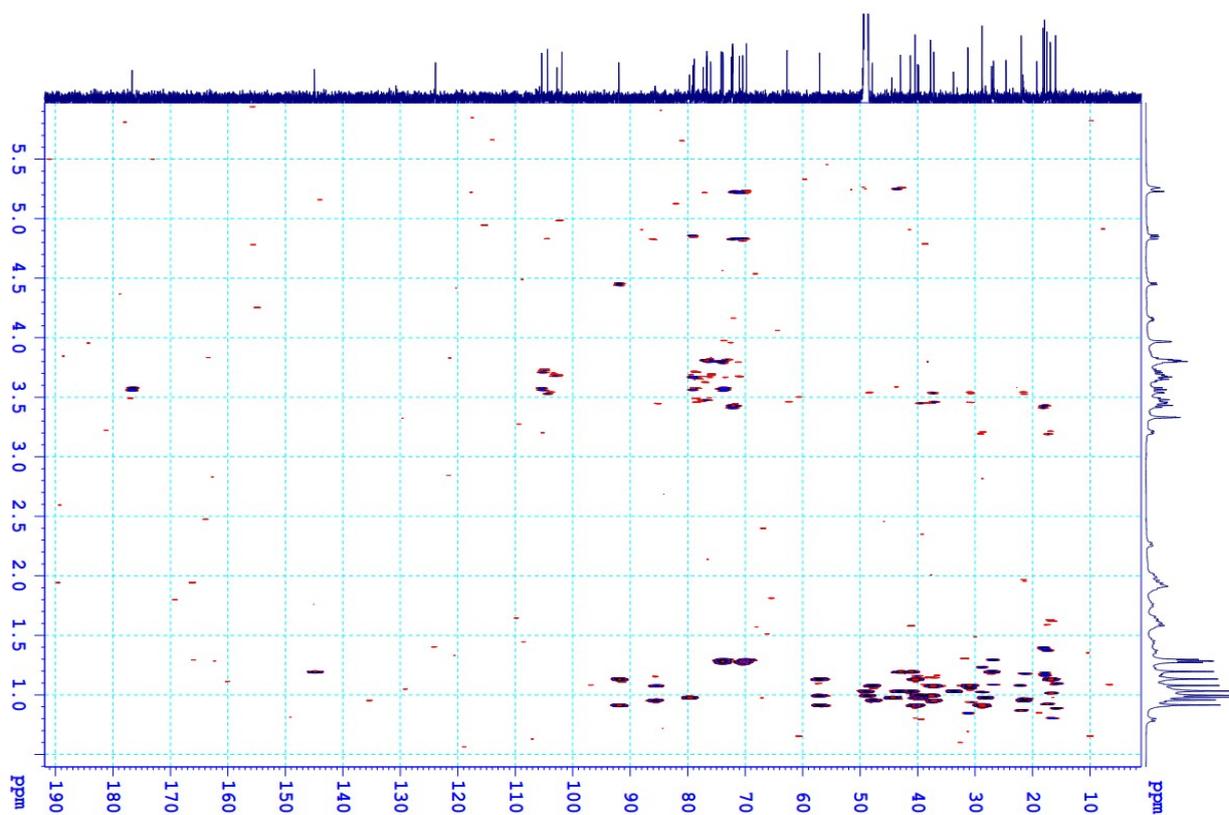


Figure S34. HMBC spectrum of compound 3

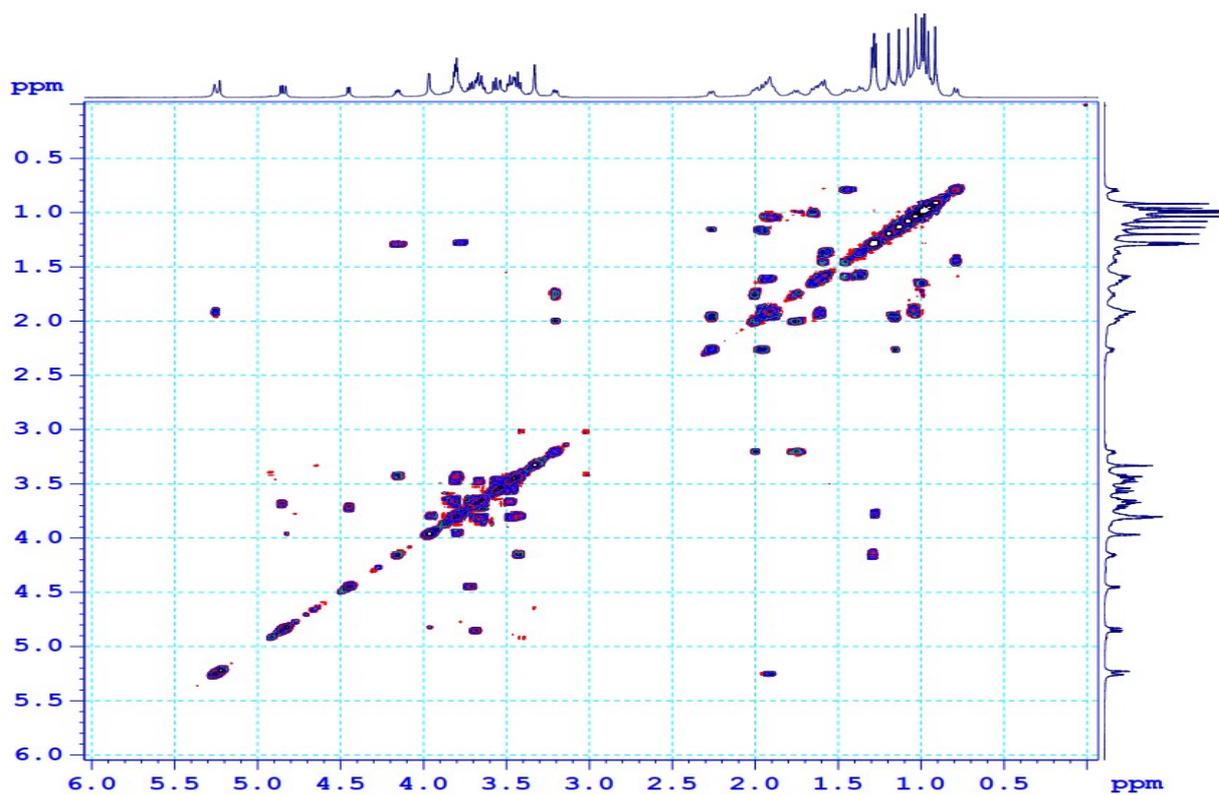


Figure S35. COSY spectrum of compound 3

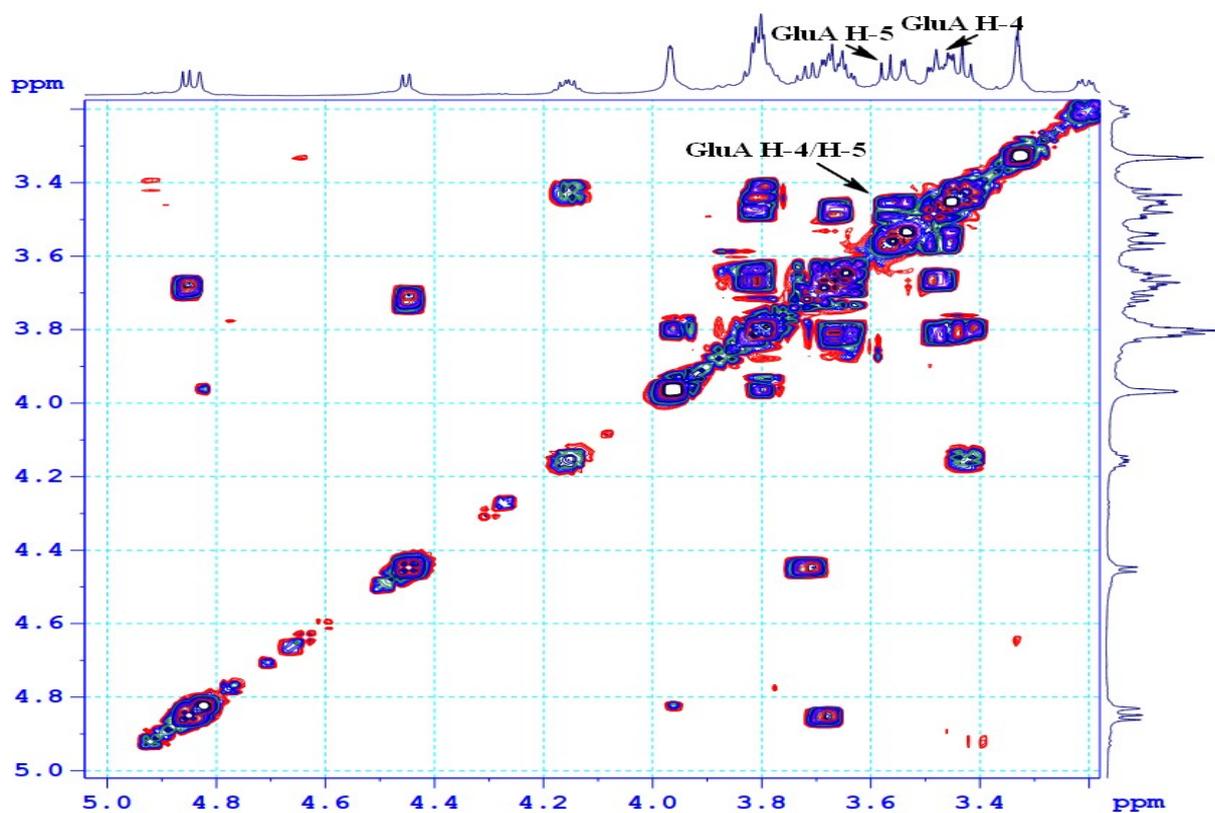


Figure S36. Expanded COSY spectrum of compound 3

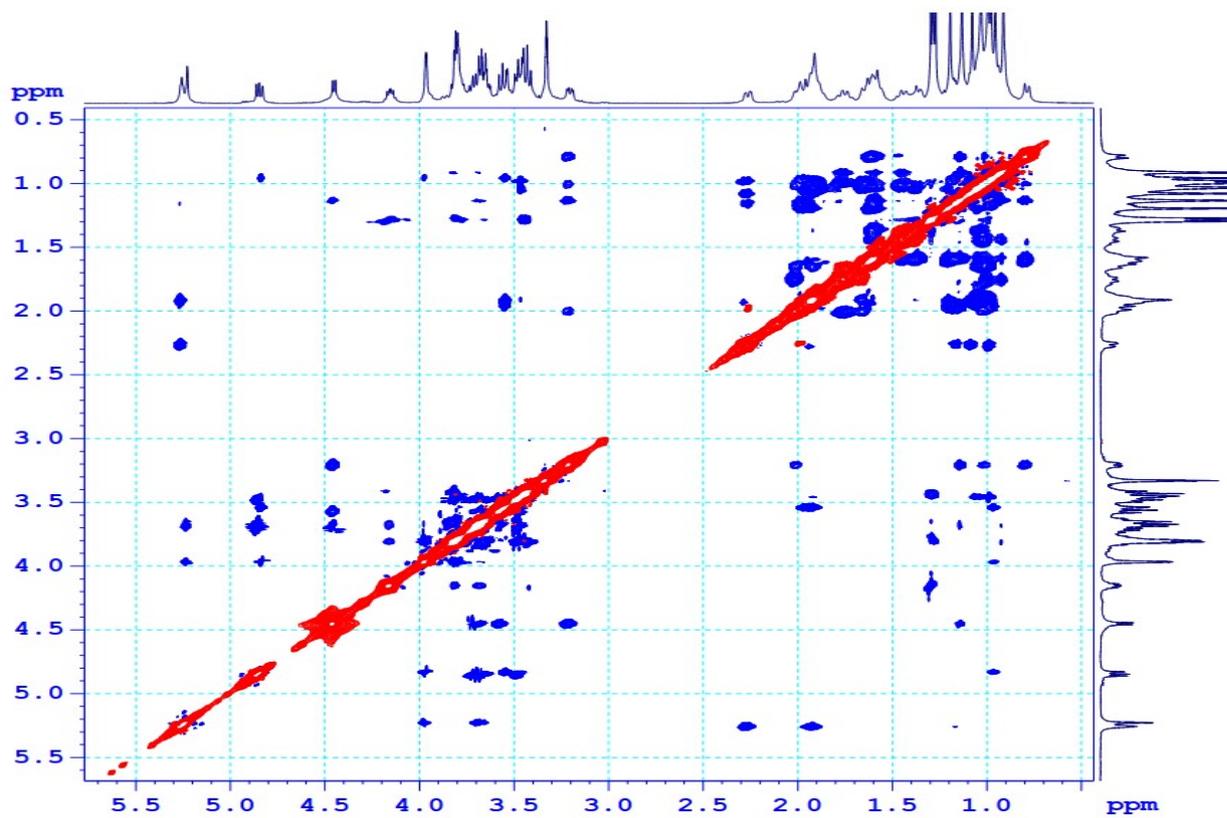


Figure S37. NOESY spectrum of compound 3

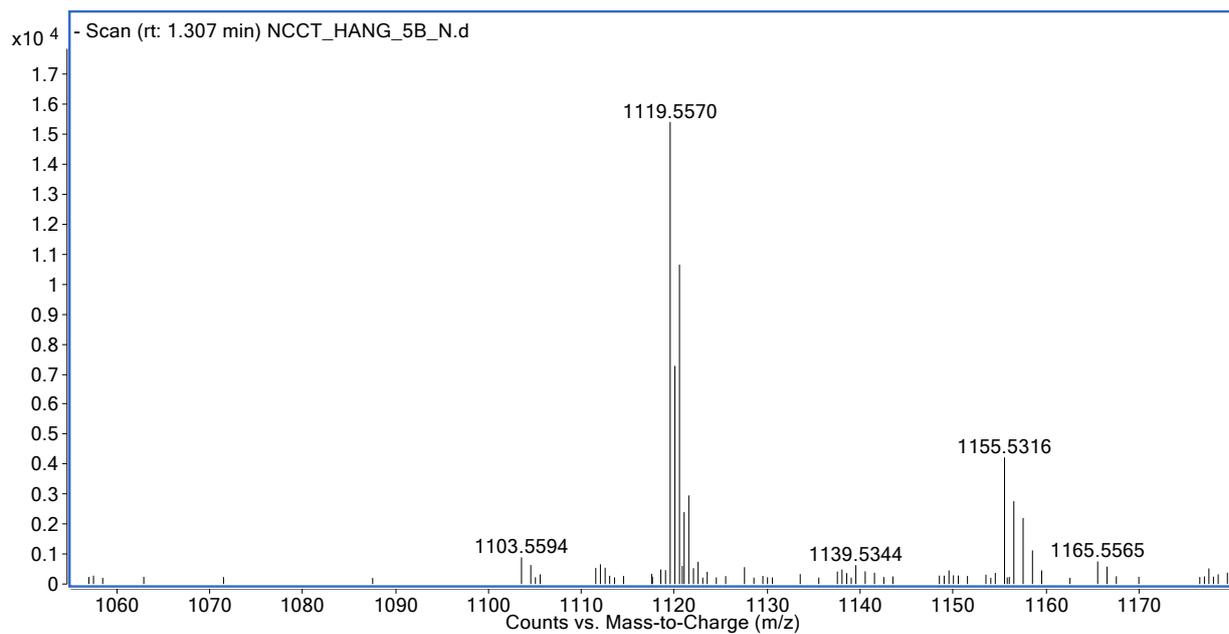


Figure S38. HR-ESI-MS of compound 4

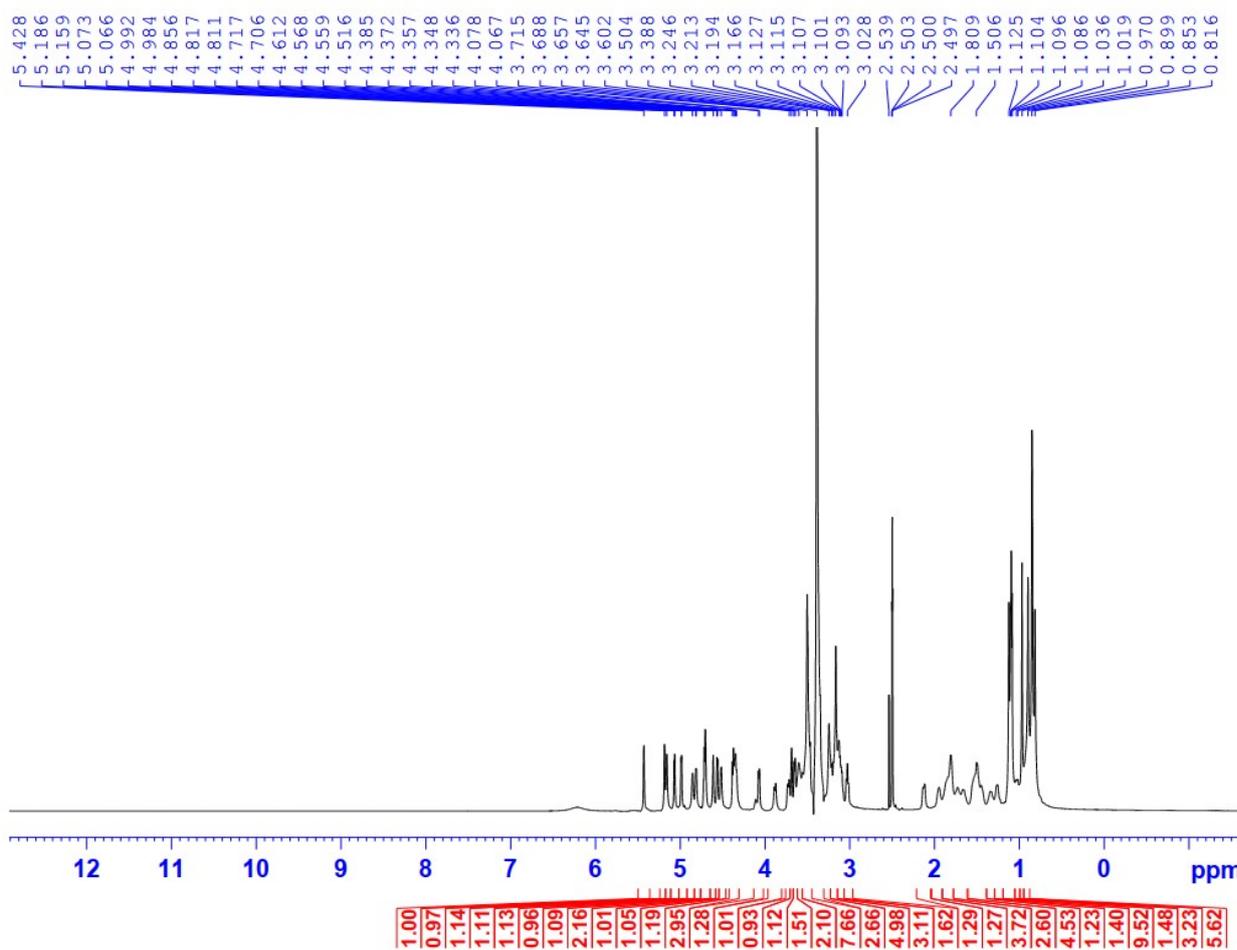


Figure S39. <sup>1</sup>H-NMR spectrum of compound 4 in DMSO-*d*<sub>6</sub>

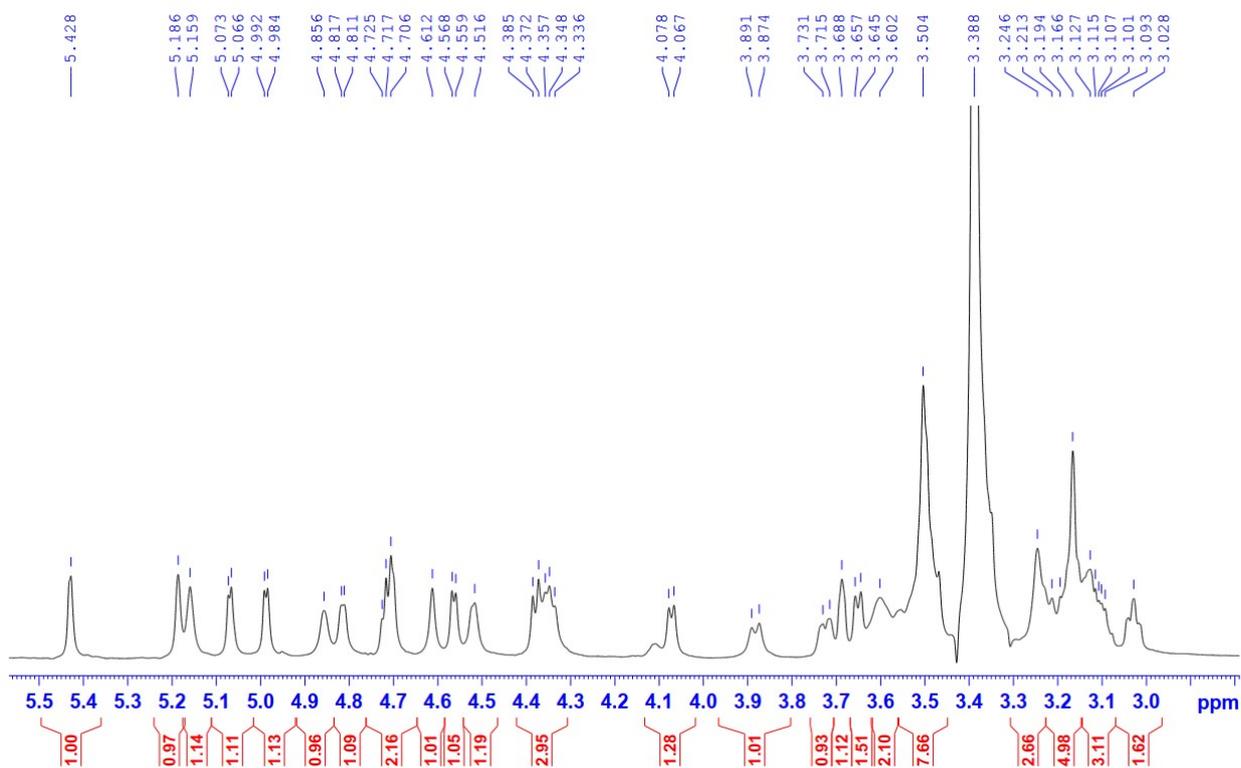


Figure S40. Expanded  $^1\text{H}$ -NMR spectrum of compound 4

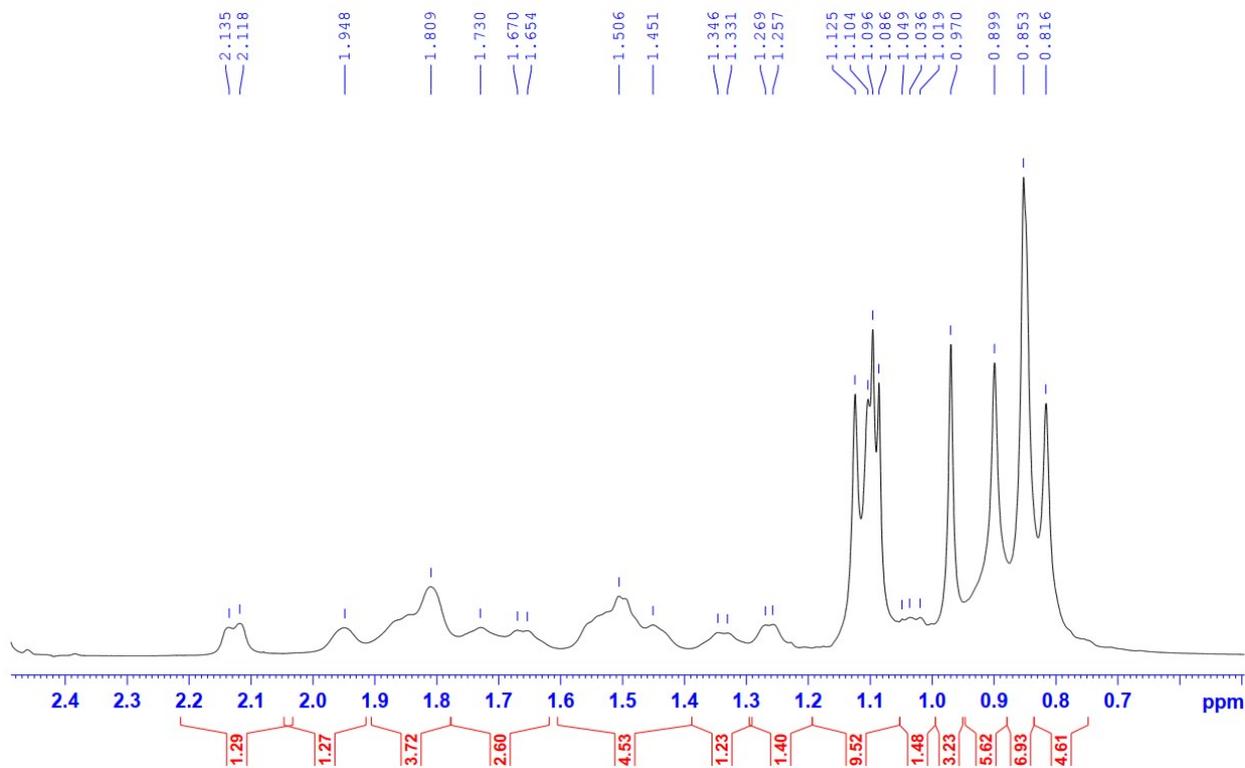


Figure S41. Expanded  $^1\text{H}$ -NMR spectrum of compound 4

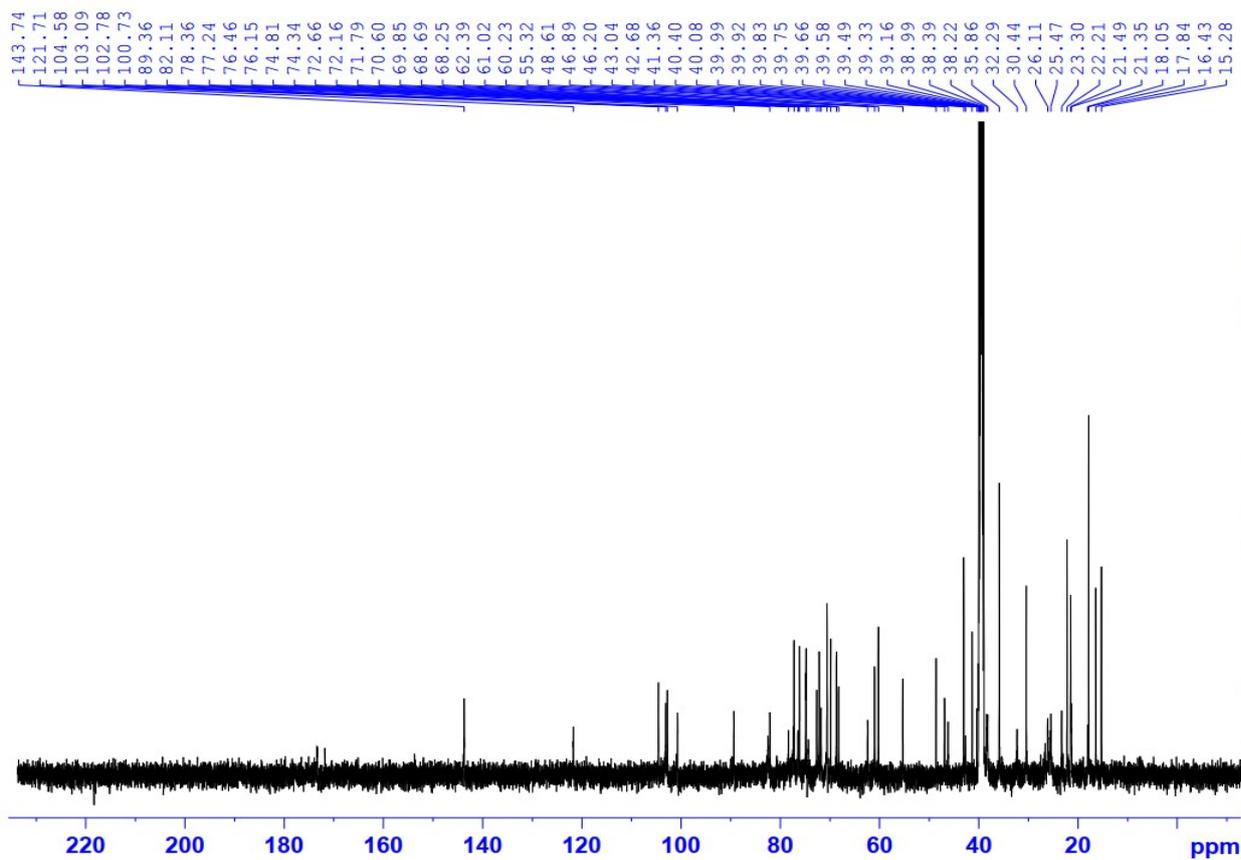


Figure S42.  $^{13}\text{C}$ -NMR spectrum of compound **4** in  $\text{DMSO-}d_6$

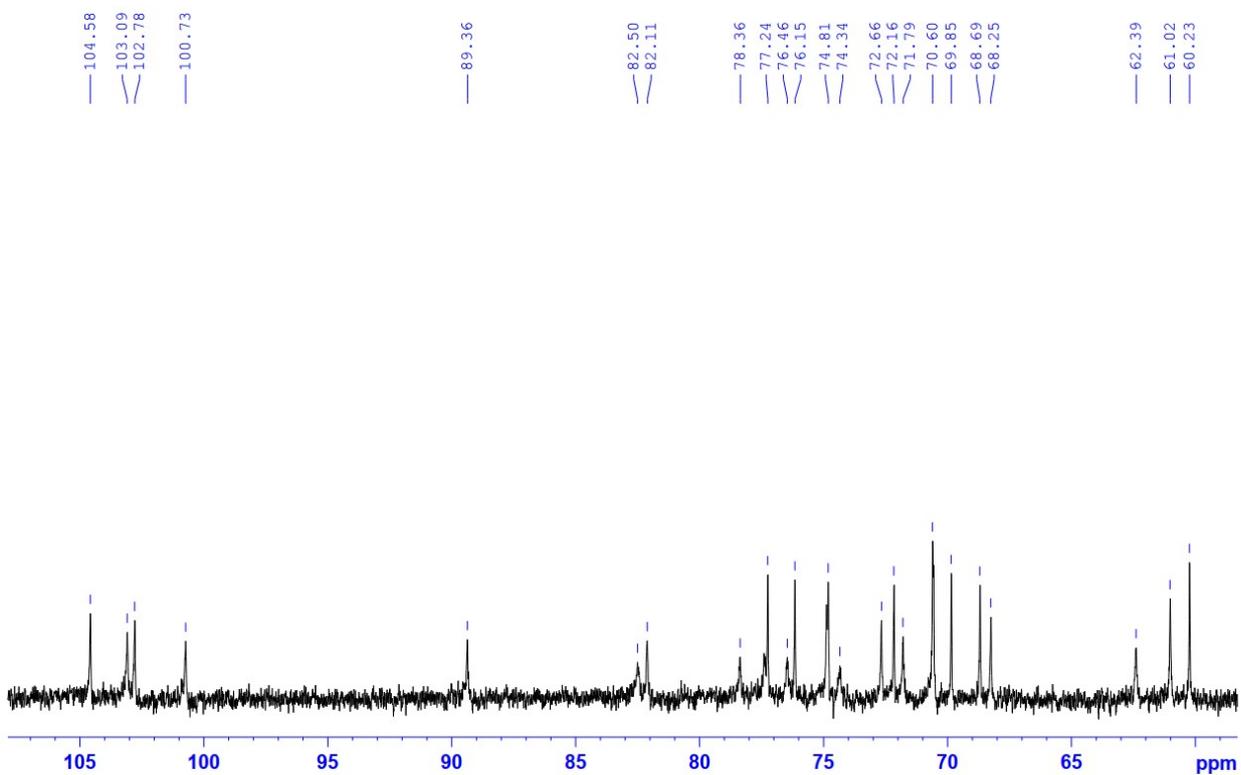


Figure S43. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **4** in  $\text{DMSO-}d_6$

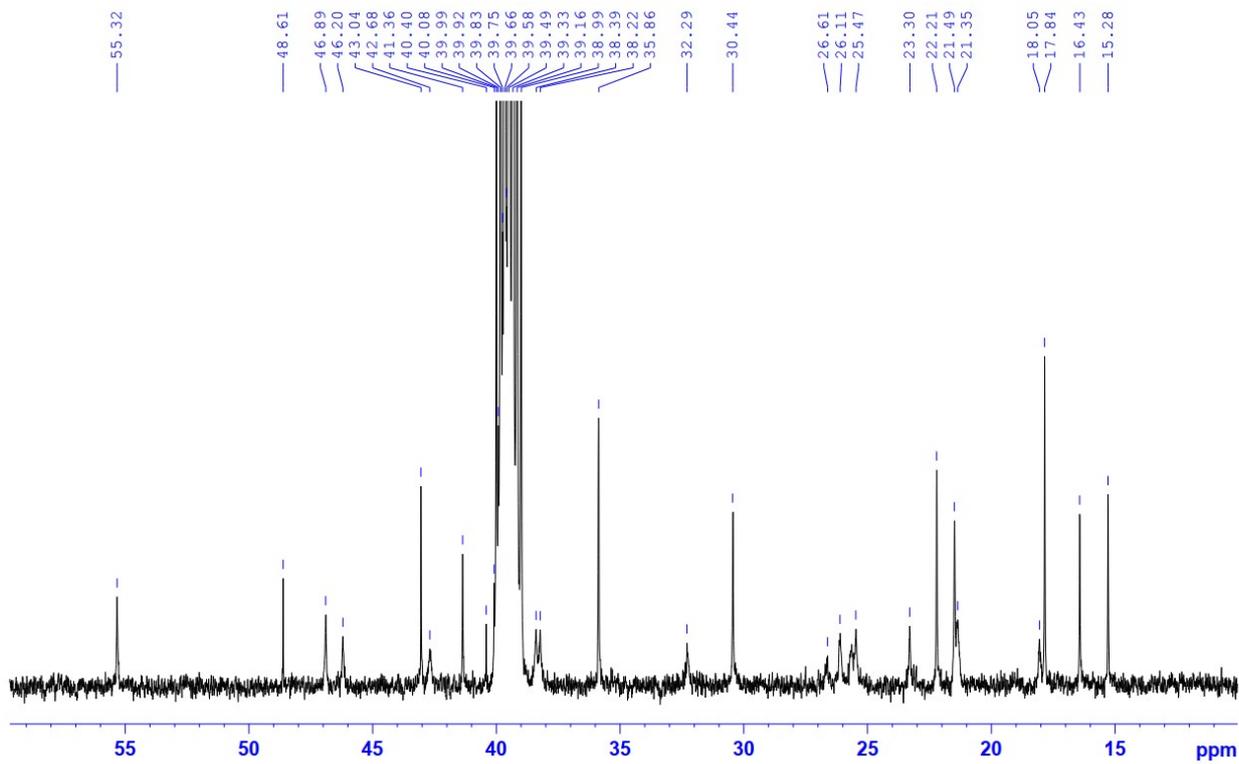


Figure S44. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **4** in  $\text{DMSO-}d_6$

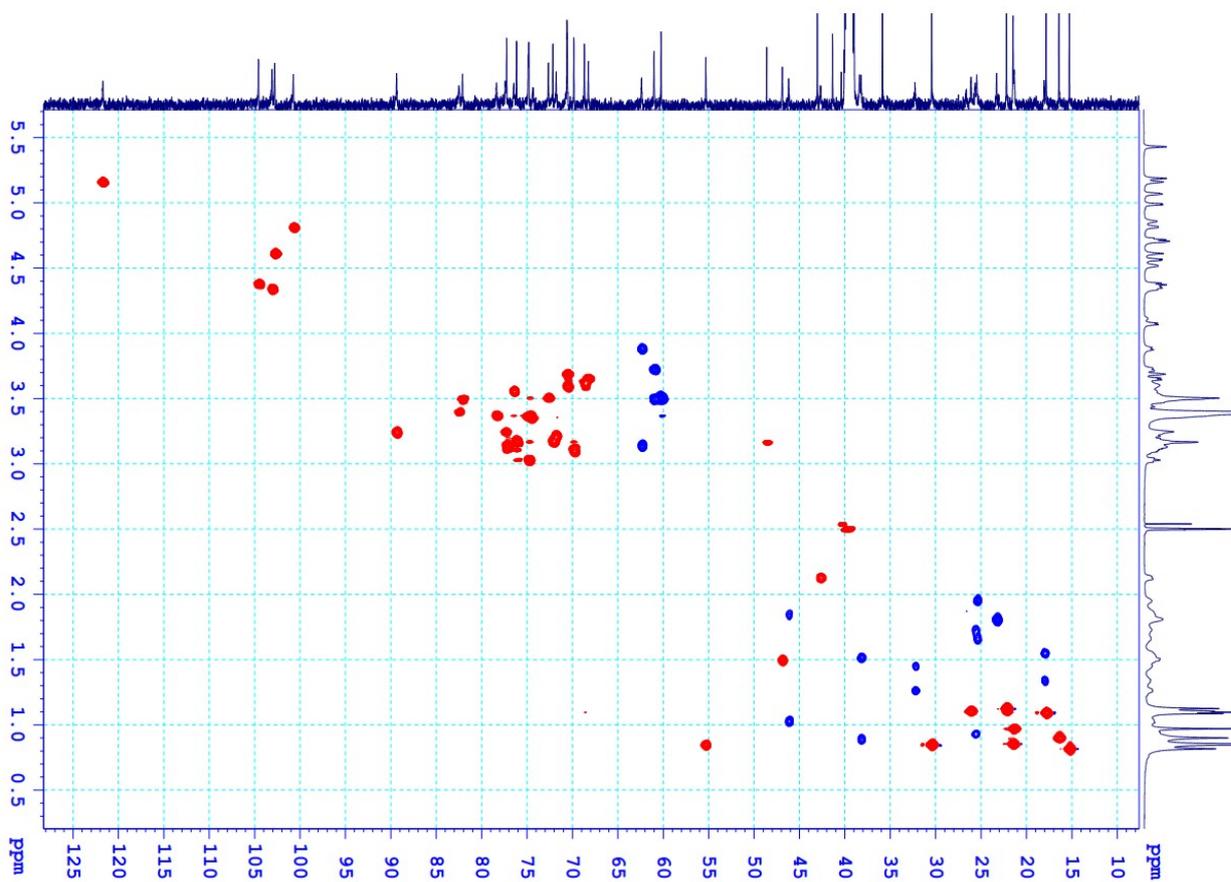


Figure S45. HSQC spectrum of compound **4**

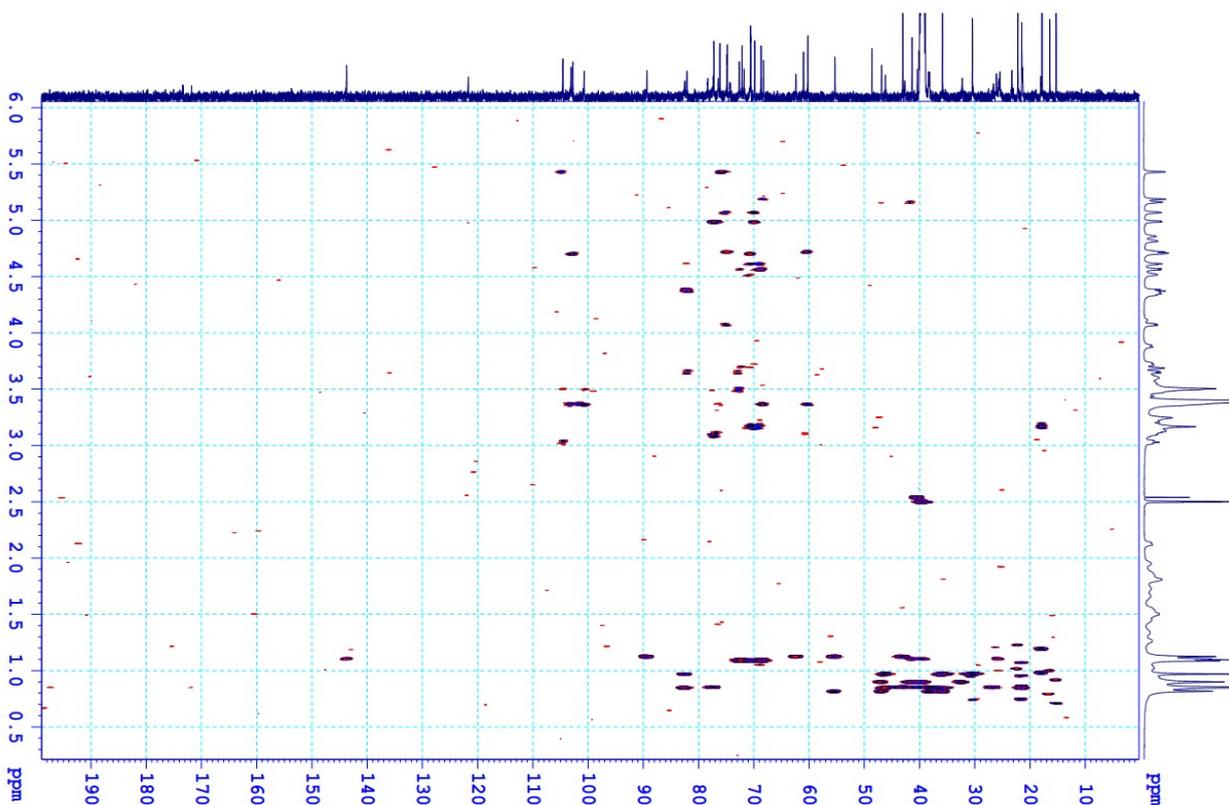


Figure S46. HMBC spectrum of compound 4

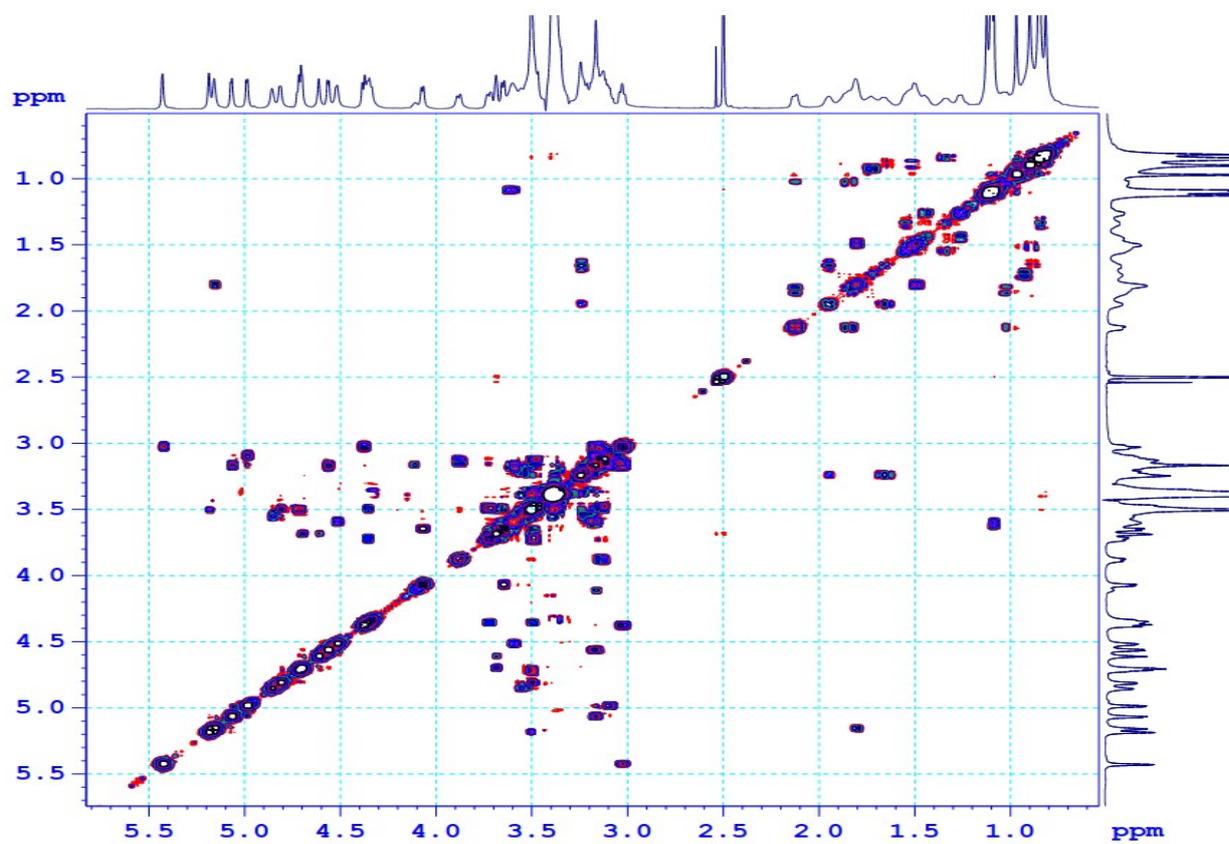


Figure S47. COSY spectrum of compound 4

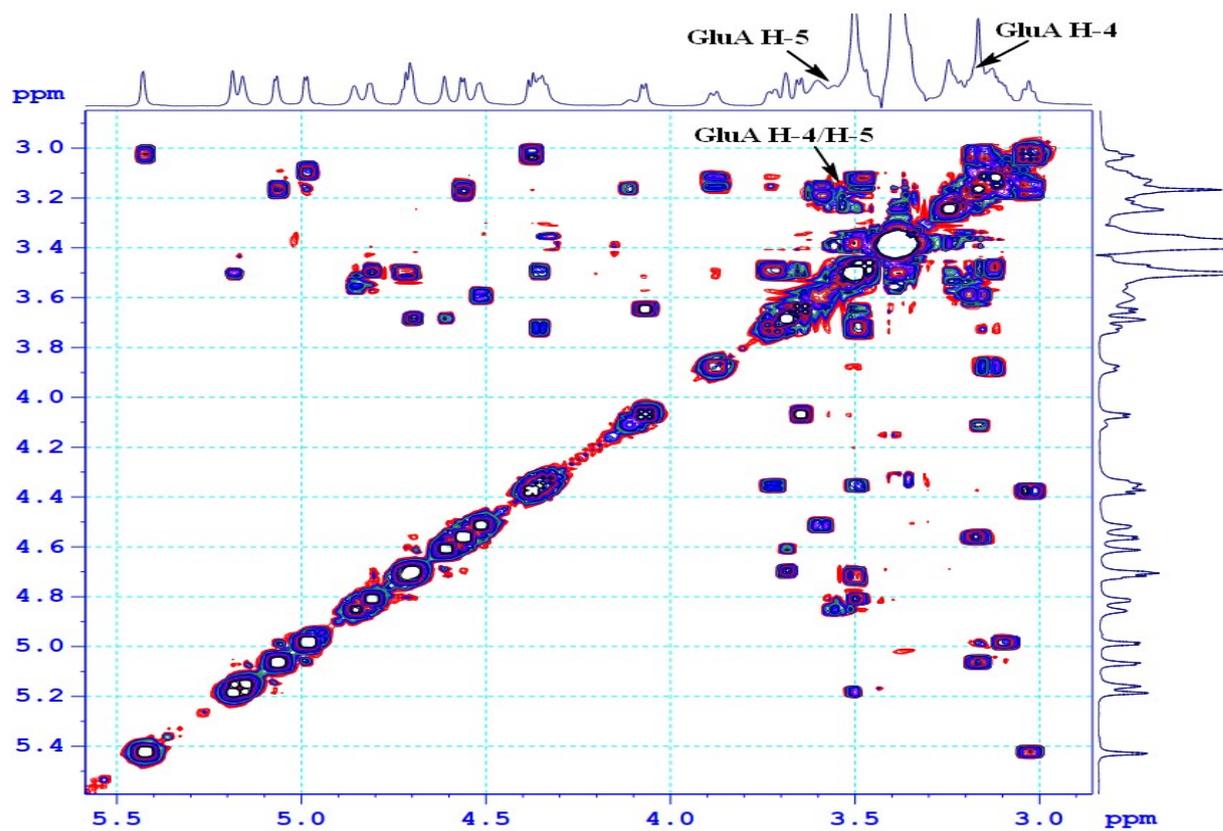


Figure S48. Expanded COSY spectrum of compound 4

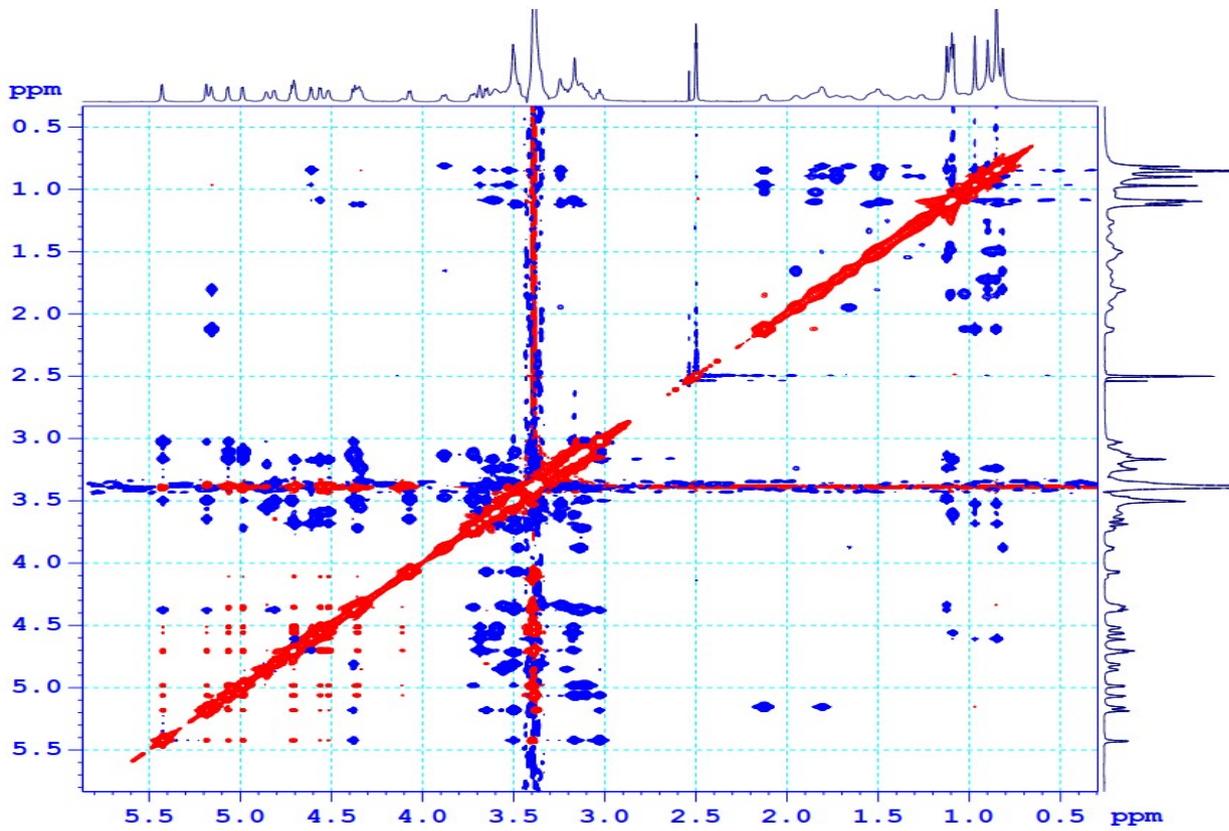


Figure S49. NOESY spectrum of compound 4

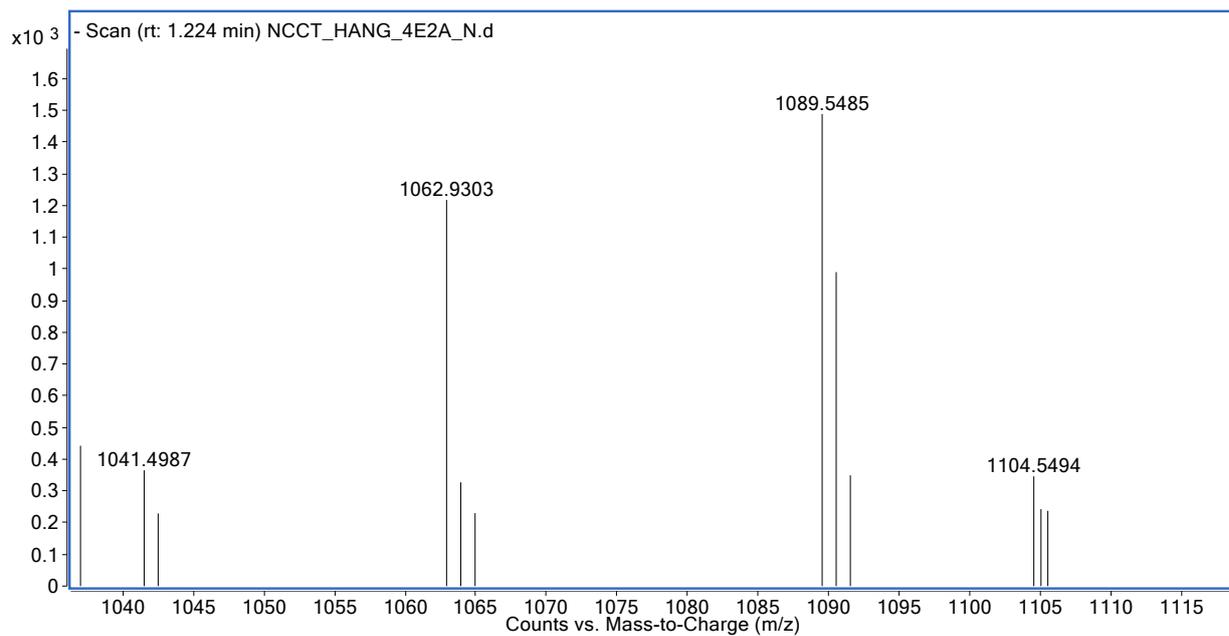


Figure S50. HR-ESI-MS of compound **5**

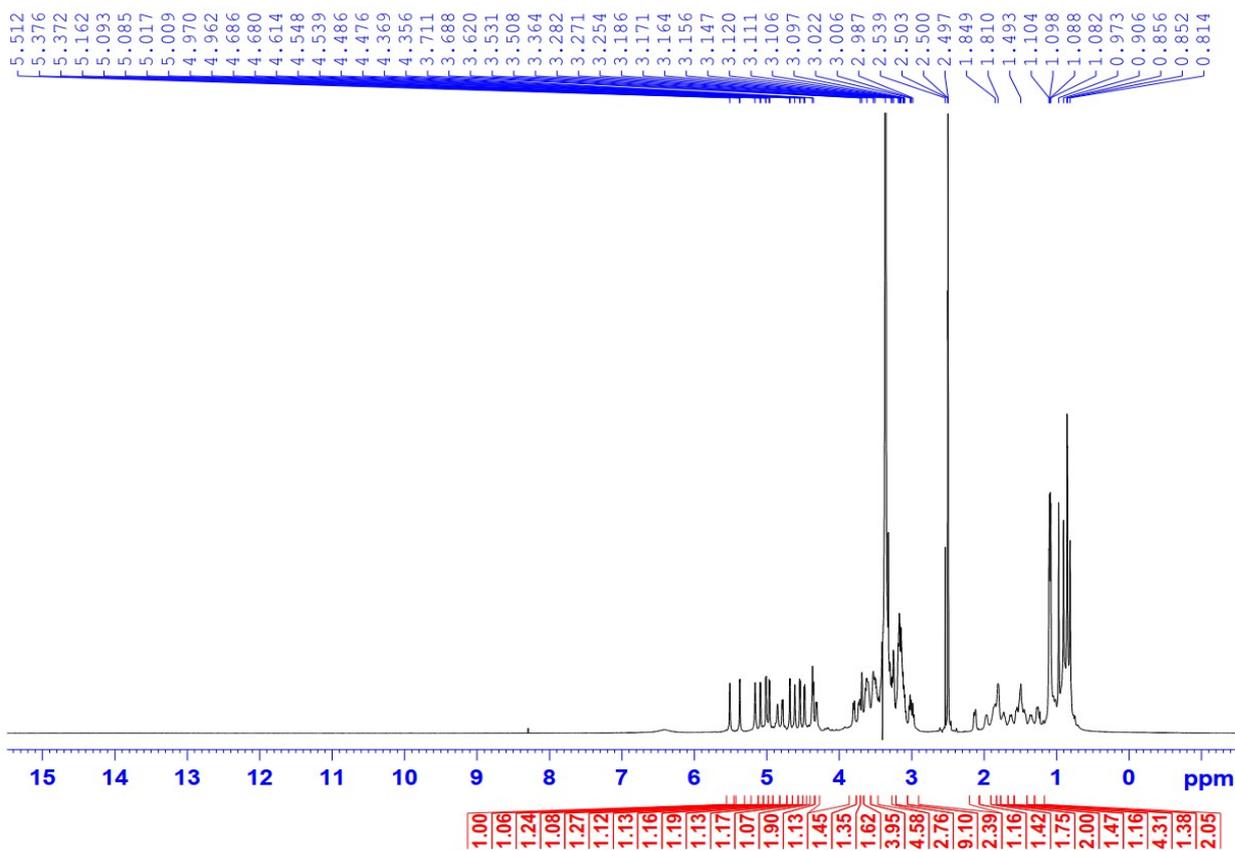


Figure S51. <sup>1</sup>H-NMR spectrum of compound **5** in DMSO-*d*<sub>6</sub>

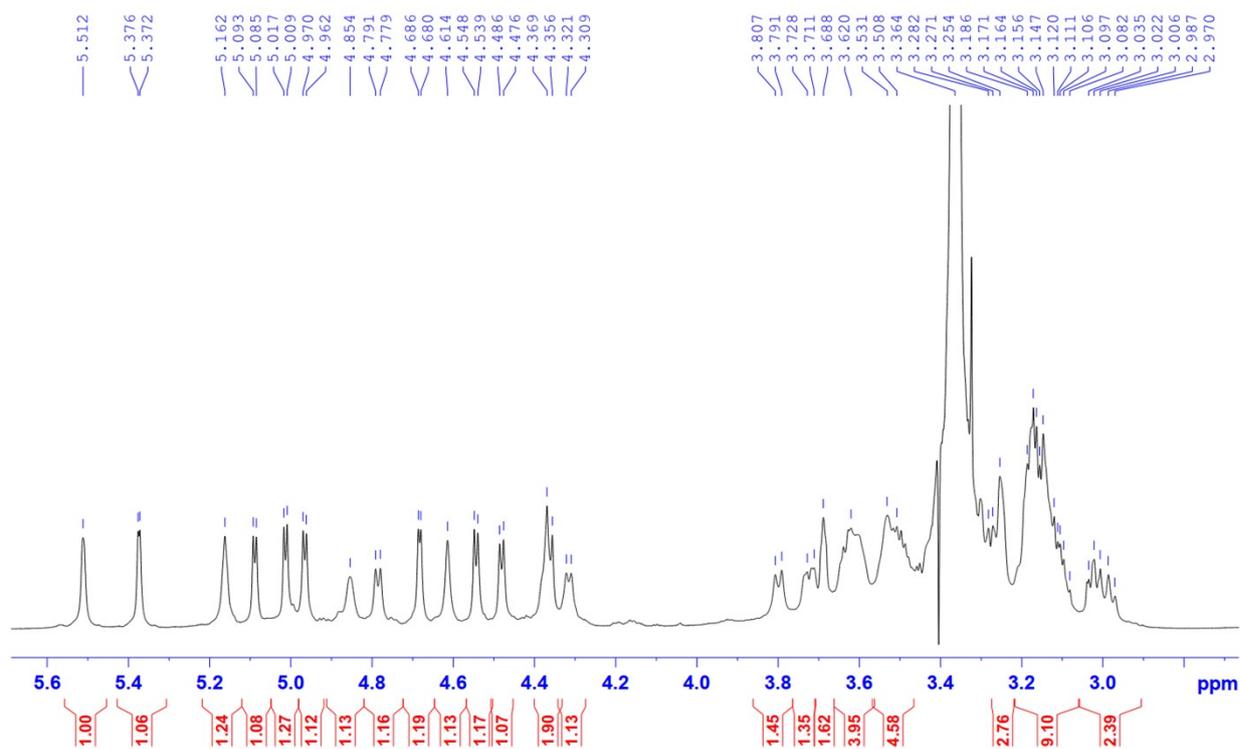


Figure S52. Expanded  $^1\text{H-NMR}$  spectrum of compound **5**

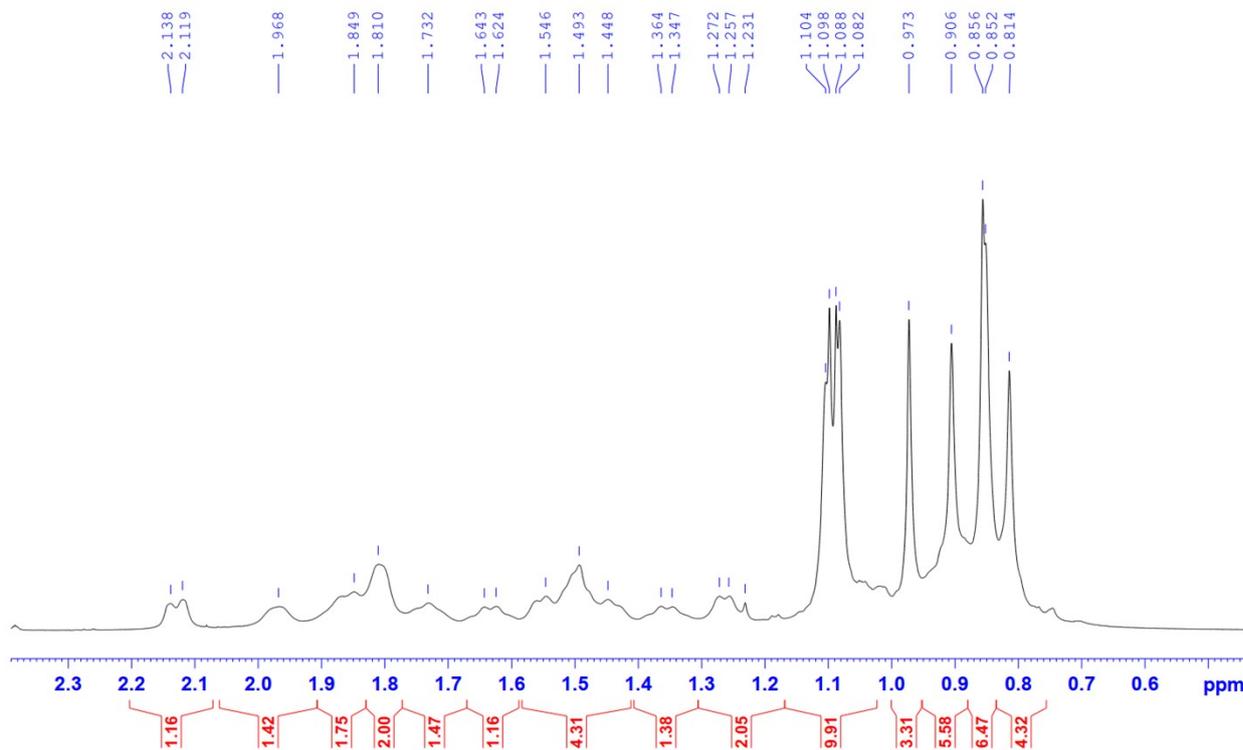


Figure S53. Expanded  $^1\text{H-NMR}$  spectrum of compound **5**

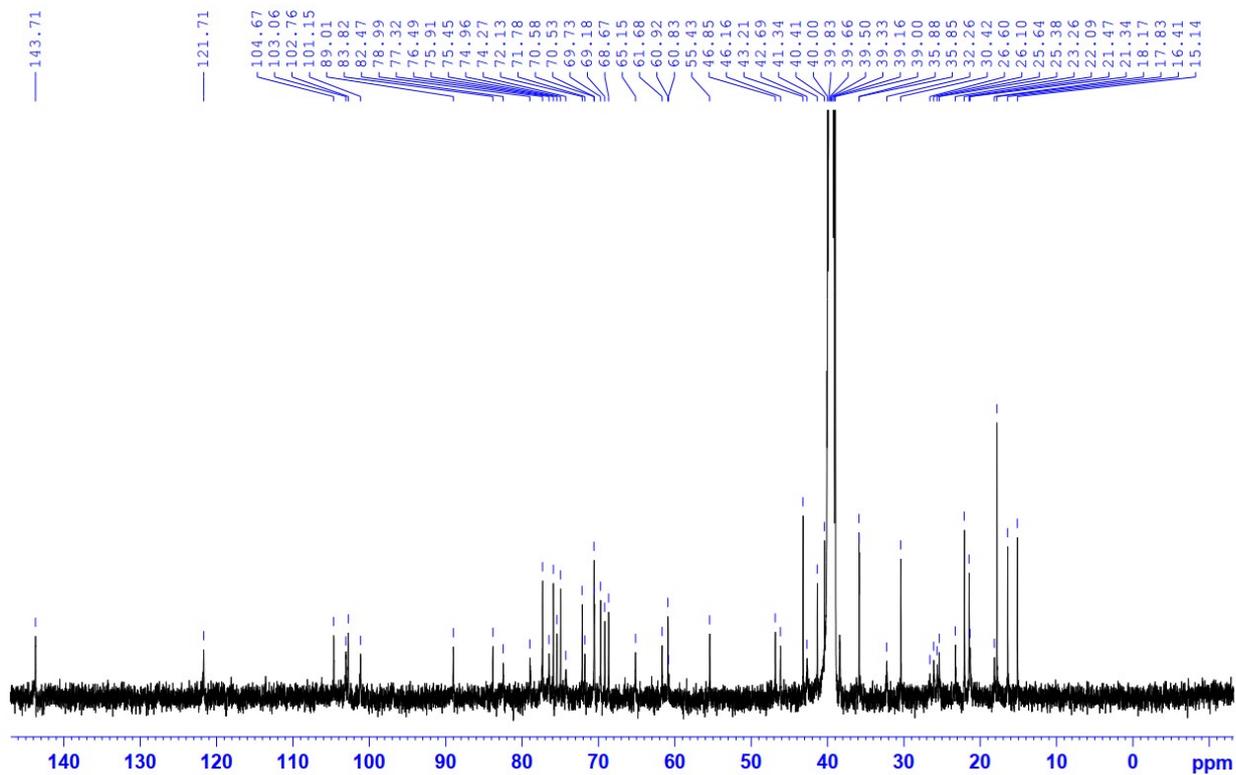


Figure S54.  $^{13}\text{C}$ -NMR spectrum of compound **5** in  $\text{DMSO-}d_6$

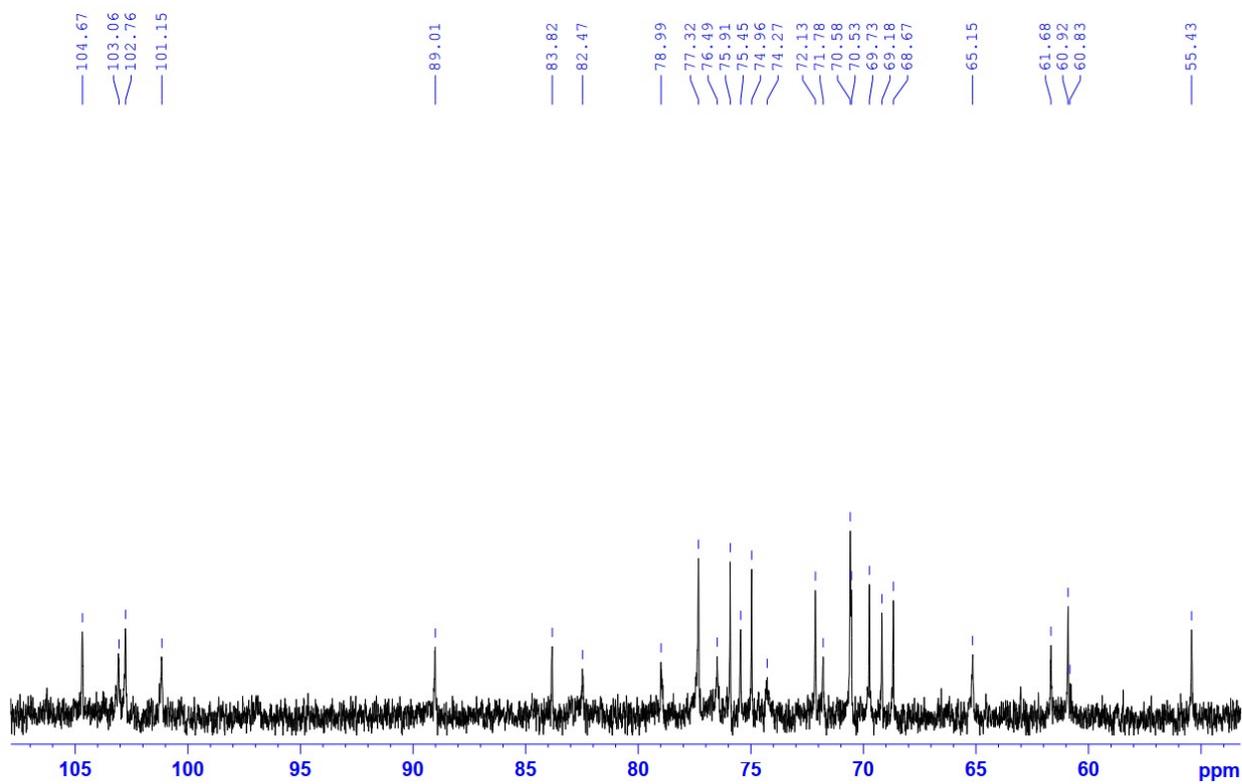


Figure S55. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **5**

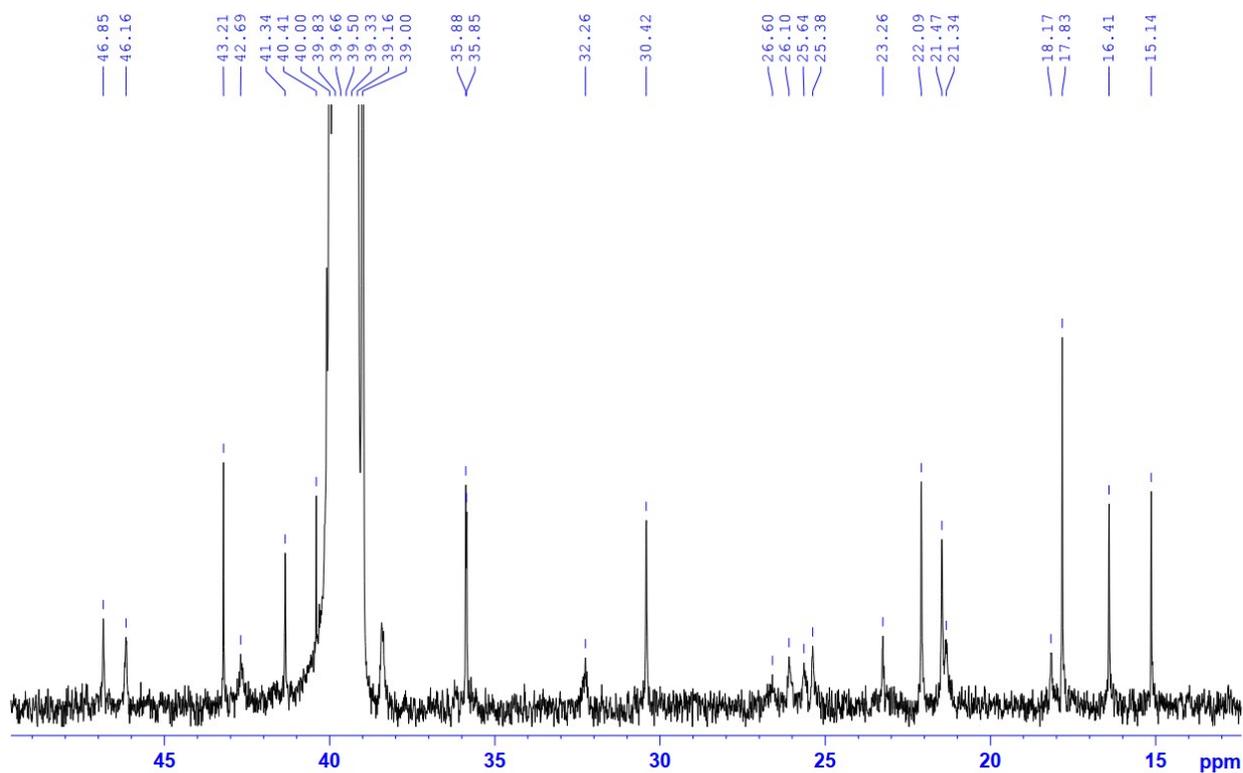


Figure S56. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **5**

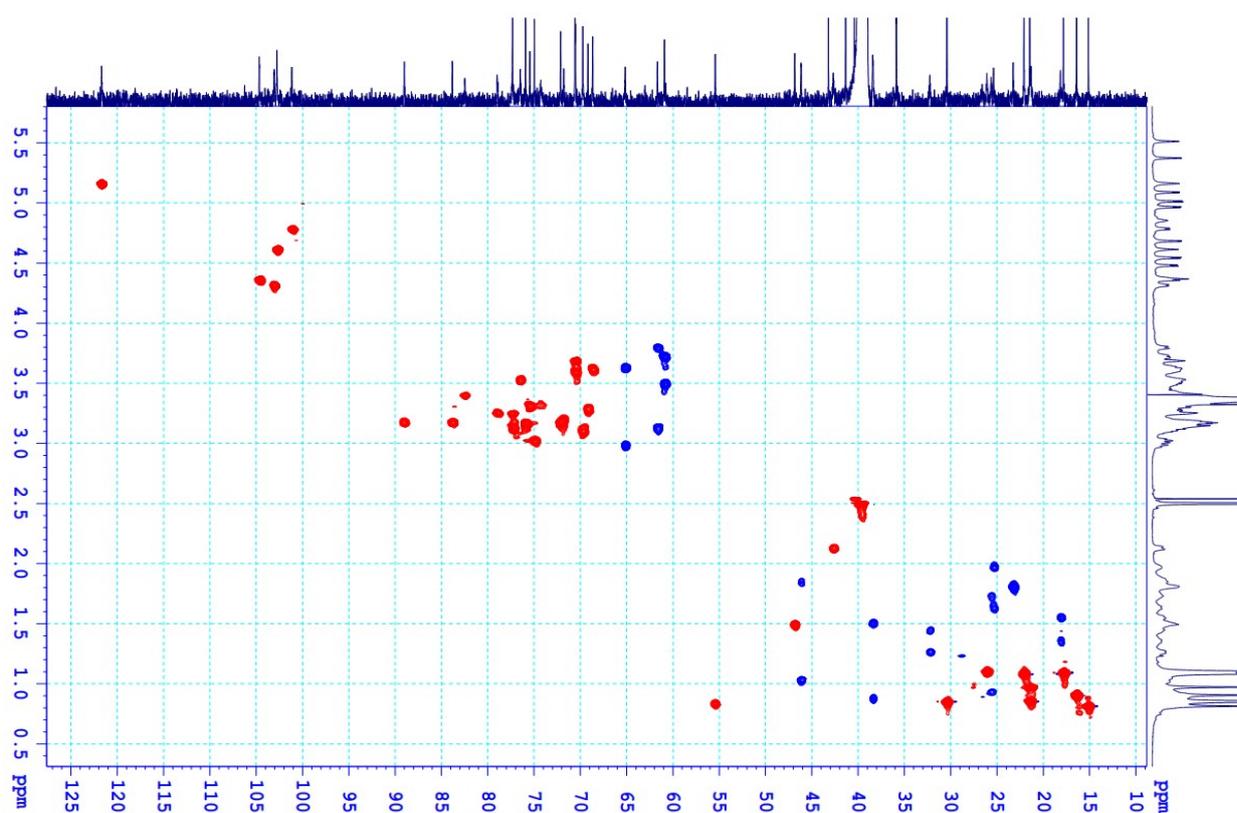


Figure S57. HSQC spectrum of compound **5**

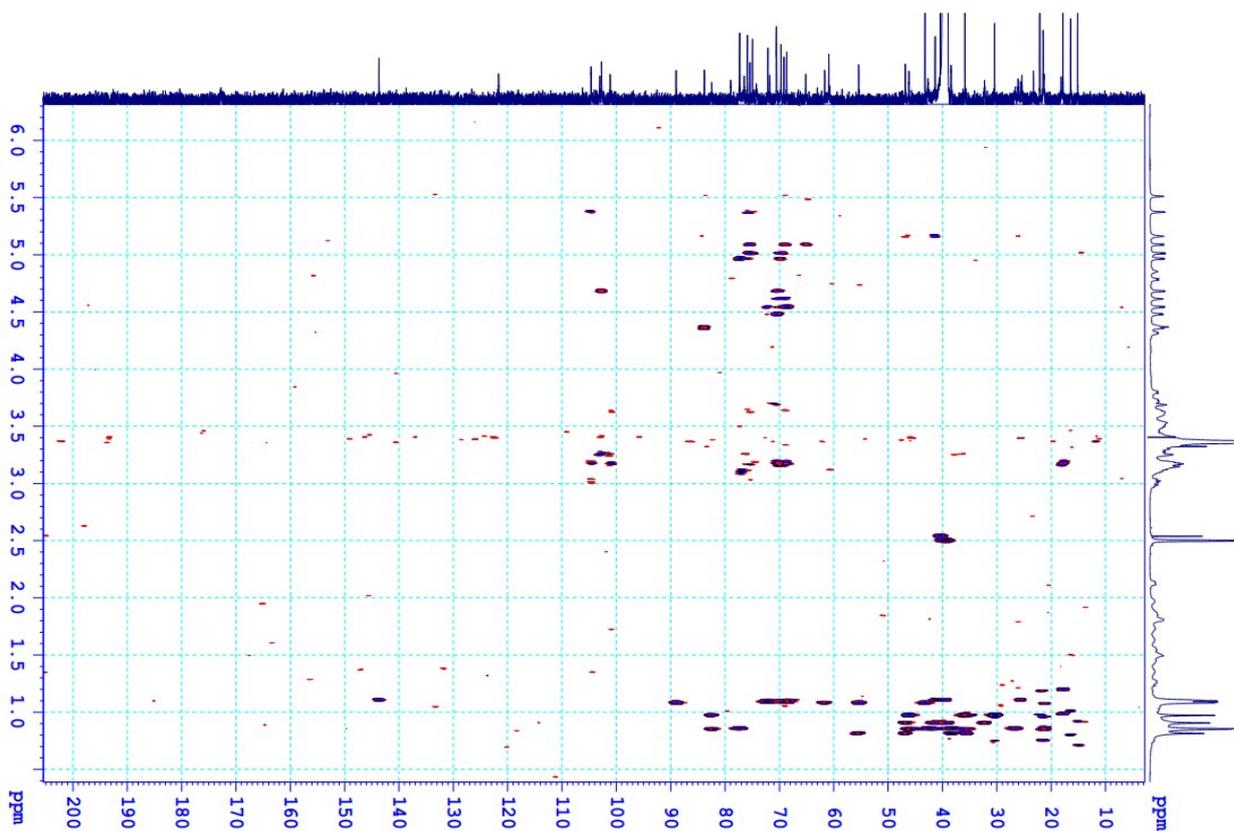


Figure S58. HMBC spectrum of compound 5

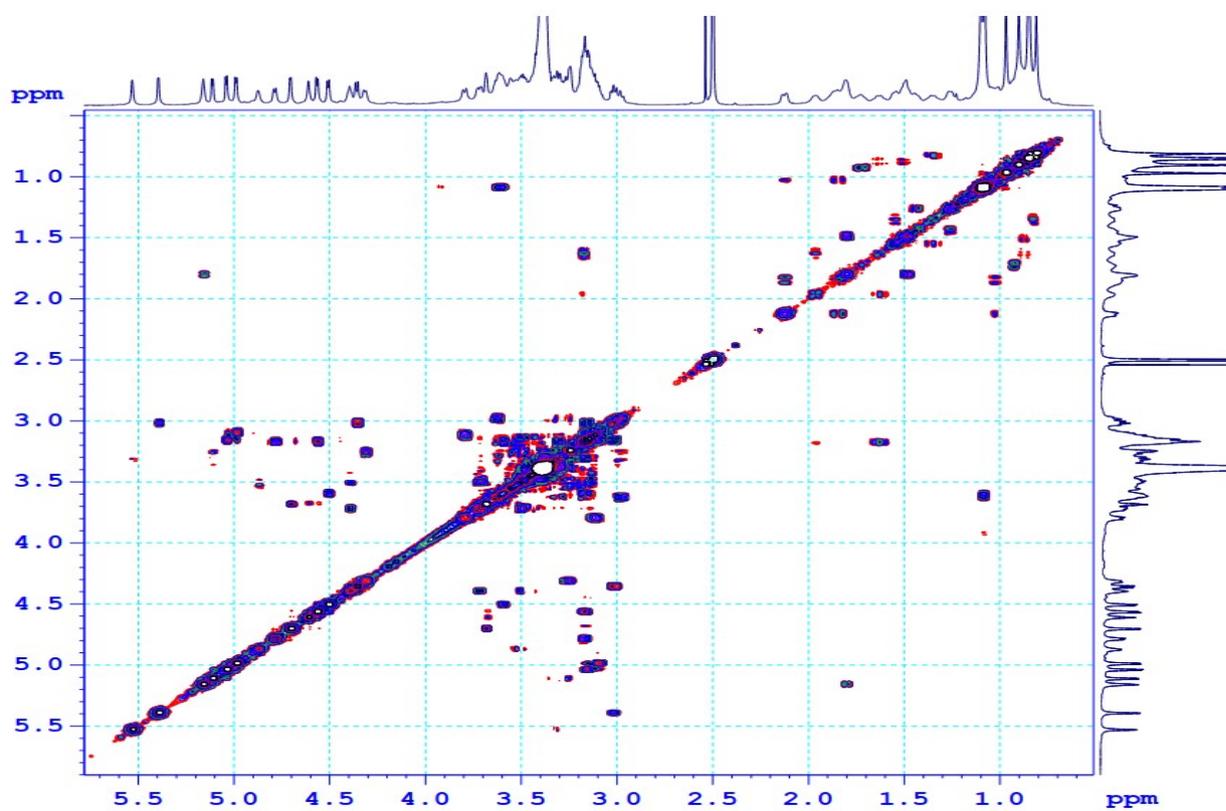


Figure S59. COSY spectrum of compound 5

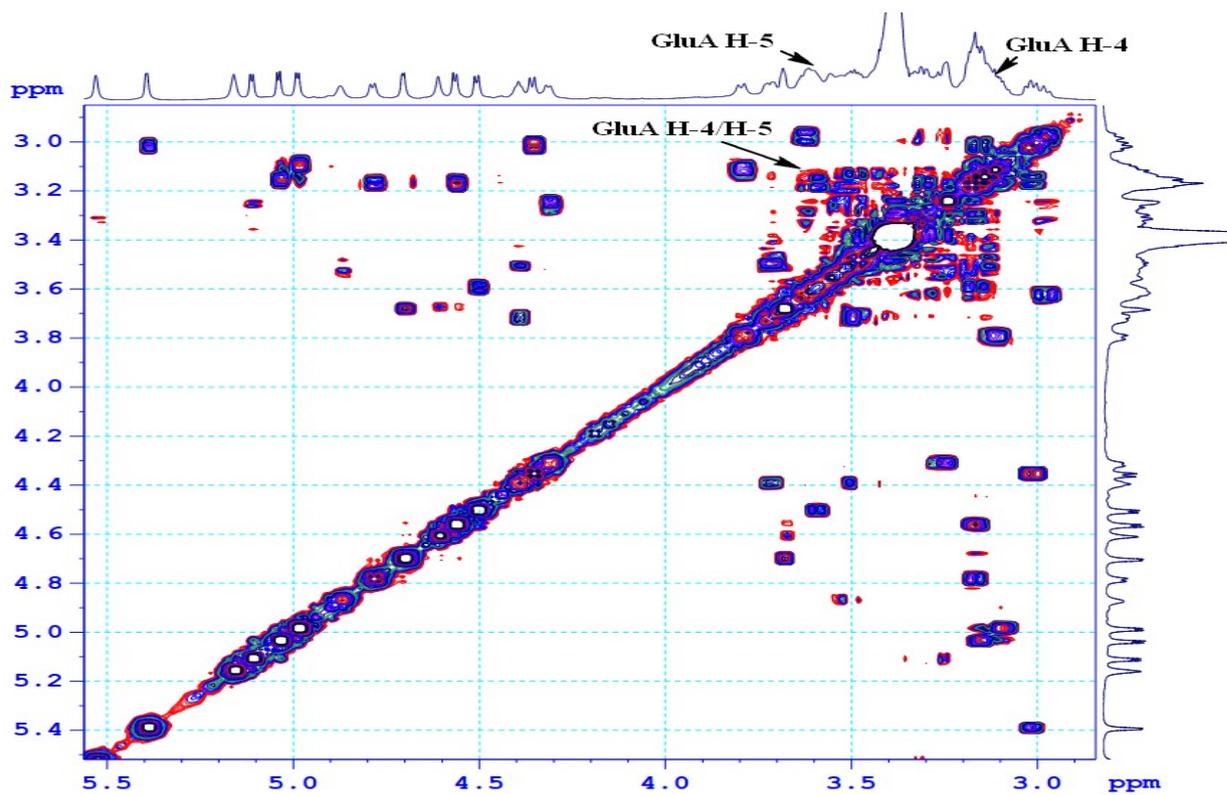


Figure S60. Expanded COSY spectrum of compound 5

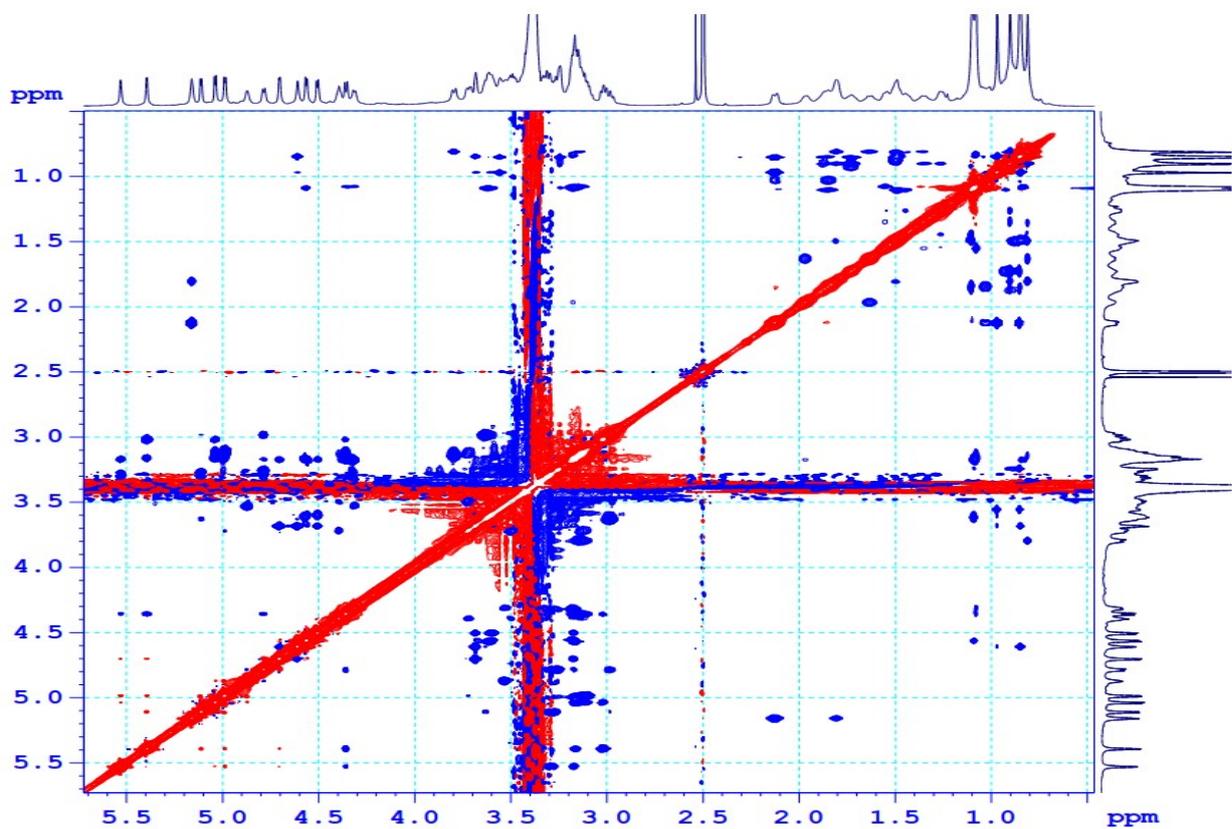


Figure S61. NOESY spectrum of compound 5

### **Acid hydrolysis and confirmation of monosaccharide**

Compounds **1 - 5** (each, 8.0 mg) were separately dissolved in 1.0 M HCl (dioxane–H<sub>2</sub>O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate, the precipitated silver chloride was removed, and the solution was concentrated thoroughly under a nitrogen atmosphere. The residue was re-dissolved in 1.0 mL of water and extracted with chloroform (three times, each 1 mL). The aqueous layer was concentrated to dryness using nitrogen gas and monosaccharides were purified by preparative TLC (pre-coated silica gel 60 F<sub>254</sub>, ethyl acetate/isopropanol/acetone/water (20/10/7/6, v/v/v/v) to obtain rhamnose ( $R_f = 0.65$ ), xylose ( $R_f = 0.59$ ), glucose ( $R_f = 0.41$ ), galactose ( $R_f = 0.33$ ), and glucuronic acid ( $R_f = 0.08$ ) in comparison with that of standard monosaccharide (L-rhamnose, D-xylose, D-glucose, D-galactose, and D-glucuronic acid; purchased from Sigma-Aldrich). The specific rotation of each monosaccharide was determined after dissolving in H<sub>2</sub>O for 1 h to afford L-rhamnose:  $[\alpha]_D^{29} +15.7$  (c 0.08, H<sub>2</sub>O), D-xylose:  $[\alpha]_D^{29} +21.0$  (c 0.05, H<sub>2</sub>O), D-glucose:  $[\alpha]_D^{29} +46.4$  (c 0.08, H<sub>2</sub>O), D-galactose:  $[\alpha]_D^{29} +50.8$  (c 0.06, H<sub>2</sub>O), and D-glucuronic acid:  $[\alpha]_D^{29} +14.4$  (c 0.06, H<sub>2</sub>O).

### **Cytotoxic assay**

SK-LU-1 and HepG2 cell lines were obtained from Milan University, Italy and Long Island University, USA. The cells were maintained and cultured in DMEM supplemented with FBS, trypsin-EDTA, L-glutamine, sodium pyruvate, NaHCO<sub>3</sub>, and penicillin/streptomycin at 37°C in a humidified atmosphere (5% CO<sub>2</sub> and 95% air). Cytotoxic effects of compounds were determined using Sulforhodamine B (SRB) assay. In brief, the cells (1×10<sup>4</sup> cells per well) were incubated with/without compounds for three days in 96-well culture plate. After incubation, cells were stained with sulforhodamine B and measured optical density (OD) at 540 nm. Difference of OD between samples and vehicle well during experiments indicated cells situation induced by compounds. Results are expressed as percentage of cells death in comparison with vehicle well. Ellipticine was used as a positive control throughout experiments.

### **Nitric oxide assay**

The RAW 264.7 cells were received from Perugia University, Italy. The cells were cultured in DMEM containing L-glutamine (2 mM), HEPES (10 mM), sodium pyruvate (1 mM), and fetal bovine serum (10%). The cells (2 × 10<sup>5</sup> cells/well) were incubated in humidified atmosphere (95% air and 5% CO<sub>2</sub>) at 37°C. After 24h incubation, each well was added by compounds (0.4-100 μM) or vehicle and followed by LPS (1μg/mL) in the next 2h. The cells were then incubated for additional 24h. After that, cell viability was then measured by MTT assay and amount of NO production in cell medium was determined by Griess reaction. Cultural medium (100 μL) was

mixed with equal volume of Griess reagent and incubated in room temperature for 10 minutes. Absorbance was measured at 540 nm on a microplate reader. Nitrite concentration as an indicator of NO production was determined using a standard curve which was built by NaNO<sub>2</sub> serial diluted solutions. Experiments were performed in triplicate. IC<sub>50</sub> values were generated by TableCurve 2Dv4 software. Dexamethasone was used as a positive control throughout experiments.

Table S1. Dose-dependent effects of compounds **1-5** on the NO production and viability of RAW 264.7 cells

Comp.	Conc. (μM)	NO inhibition (%)	Cell viability (%)	Comp.	Conc. (μM)	NO inhibition (%)	Cell viability (%)
<b>1</b>	100	23.02 ± 1.68	93.29 ± 1.41	<b>4</b>	100	30.28 ± 1.79	94.92 ± 3.18
	20	12.17 ± 1.22	94.17 ± 2.59		20	16.33 ± 1.03	97.12 ± 0.24
	4	6.08 ± 0.58	NT		4	4.52 ± 0.41	NT
	0.8	0.79 ± 0.09	NT		0.8	1.51 ± 0.15	NT
<b>2</b>	100	25.61 ± 1.45	95.72 ± 2.46	<b>5</b>	100	27.39 ± 0.87	99.75 ± 1.60
	20	15.07 ± 0.84	97.84 ± 1.33		20	17.09 ± 1.16	99.83 ± 1.12
	4	10.13 ± 0.89	NT		4	11.56 ± 0.58	NT
	0.8	2.54 ± 0.26	NT		0.8	5.03 ± 0.41	NT
<b>3</b>	100	84.52 ± 3.52	68.42 ± 5.65	<b>Dex</b>	100	90.58 ± 2.12	91.47 ± 2.44
	20	16.67 ± 0.92	94.40 ± 2.99		20	54.23 ± 1.17	100.04 ± 1.93
	4	10.85 ± 0.68	NT		4	40.64 ± 1.08	NT
	0.8	3.31 ± 0.17	NT		0.8	32.59 ± 1.13	NT

Dex: dexamethasone was used as positive control; <sup>NT</sup>)Not tested.

Table S2. Dose-dependent effects of compounds **1-5** on the cytotoxic activity against SK-LU-1 and Hep-G2 cancer cell lines

Comp.	Conc. (μM)	Cytotoxicity (%)		Comp.	Conc. (μM)	Cytotoxicity (%)	
		SK-LU-1	HepG2			SK-LU-1	HepG2
<b>1</b>	100	37.18 ± 1.40	21.06 ± 1.38	<b>4</b>	100	21.10 ± 2.03	18.73 ± 1.83
	20	21.23 ± 1.91	15.15 ± 1.05		20	12.22 ± 1.02	13.40 ± 1.11
	4	4.85 ± 0.41	7.24 ± 0.35		4	6.79 ± 0.74	8.28 ± 0.65
	0.8	0.47 ± 0.03	4.33 ± 0.25		0.8	2.92 ± 0.23	3.37 ± 0.19
<b>2</b>	100	25.60 ± 1.35	20.52 ± 1.69	<b>5</b>	100	24.92 ± 1.32	20.81 ± 1.80
	20	14.68 ± 1.07	8.02 ± 0.84		20	17.59 ± 0.96	12.17 ± 1.07
	4	6.72 ± 0.78	2.52 ± 0.24		4	13.95 ± 0.79	3.11 ± 0.25
	0.8	2.10 ± 0.12	0.28 ± 0.03		0.8	4.13 ± 0.28	0.96 ± 0.06
<b>3</b>	100	30.72 ± 1.15	23.14 ± 1.72	<b>Ell*</b>	10	94.49 ± 1.25	92.54 ± 1.47
	20	23.31 ± 0.56	17.77 ± 1.15		2	78.62 ± 1.25	79.85 ± 1.78
	4	4.59 ± 0.37	5.40 ± 0.44		0.4	51.70 ± 0.96	52.36 ± 1.35
	0.8	2.31 ± 0.19	1.46 ± 0.13		0.08	22.04 ± 1.05	23.66 ± 0.73

Ell: Ellipticine was used as positive control; \*)concentration unit: μg/mL.