

## SUPPLEMENTARY MATERIAL

### **New diterpene lactone derivatives from *Aphanamixis polystachya* leaves inhibit nitric oxide production in RAW 264.7 cells**

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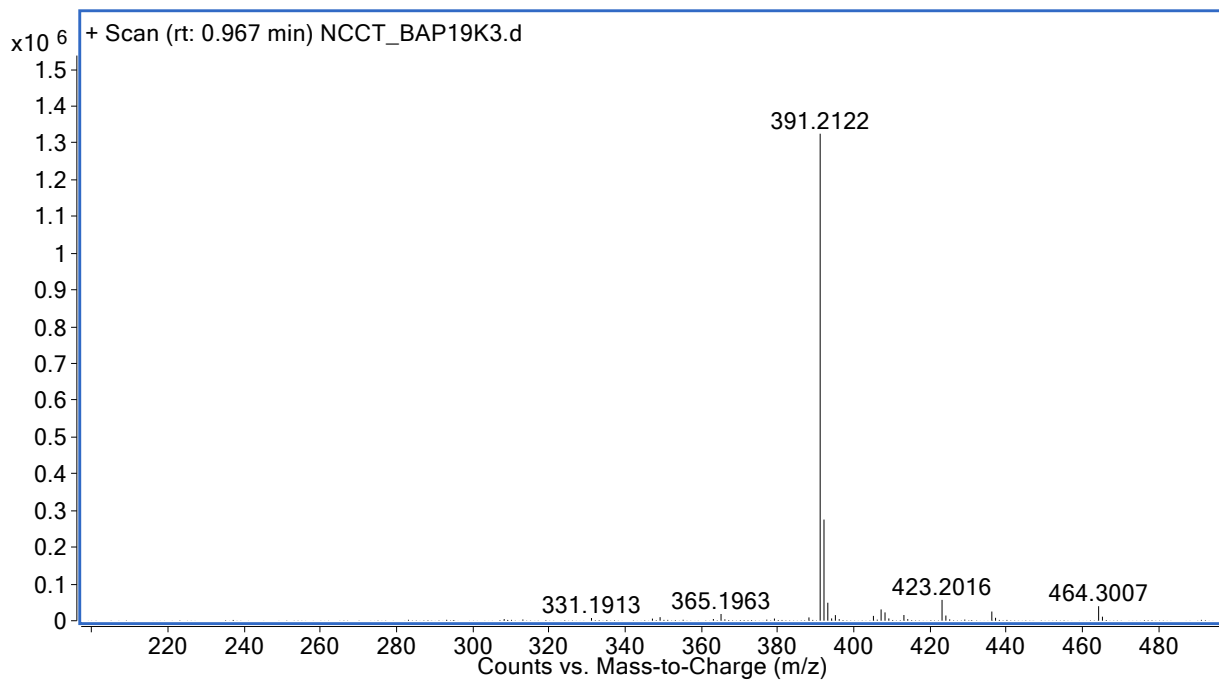


Figure S1. HR-ESI-MS of compound **1**

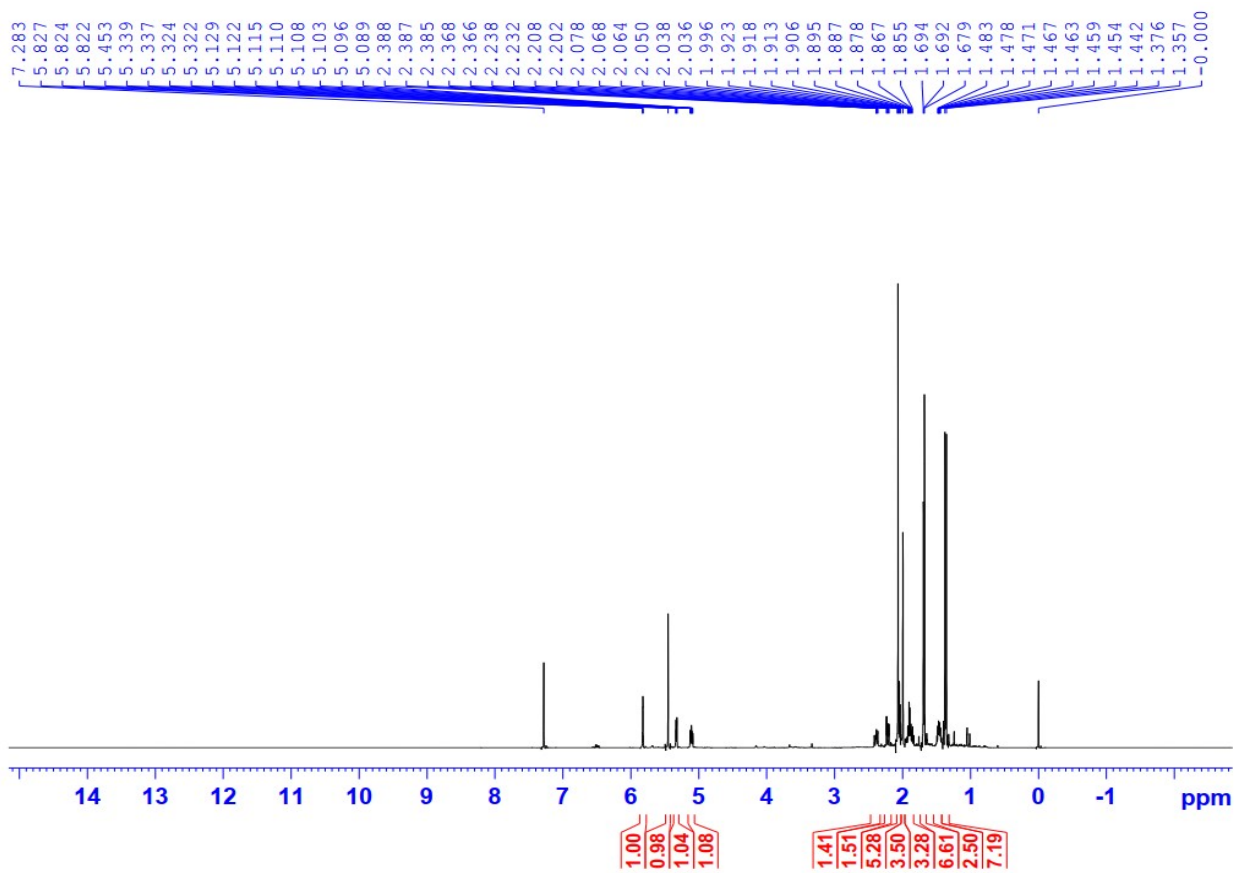


Figure S2. <sup>1</sup>H-NMR spectrum of compound **1** in CDCl<sub>3</sub>

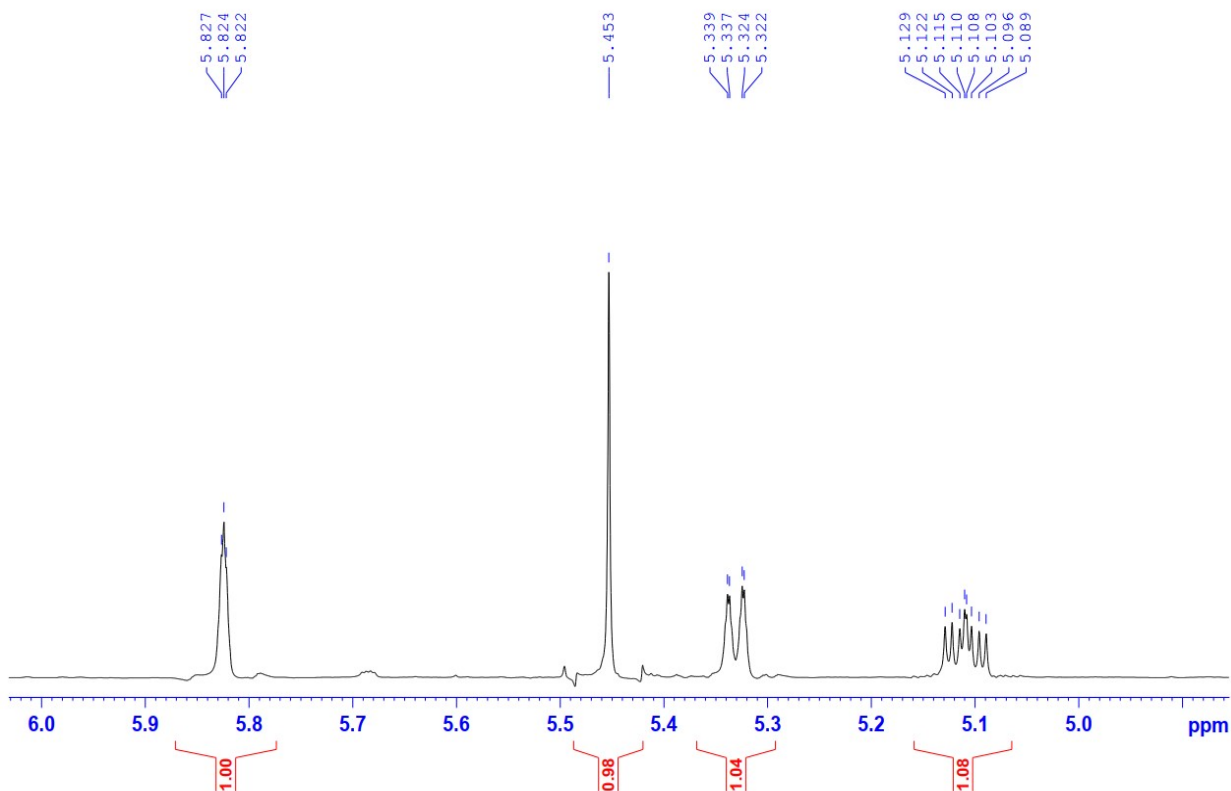


Figure S3. Expanded  $^1\text{H-NMR}$  spectrum of compound **1**

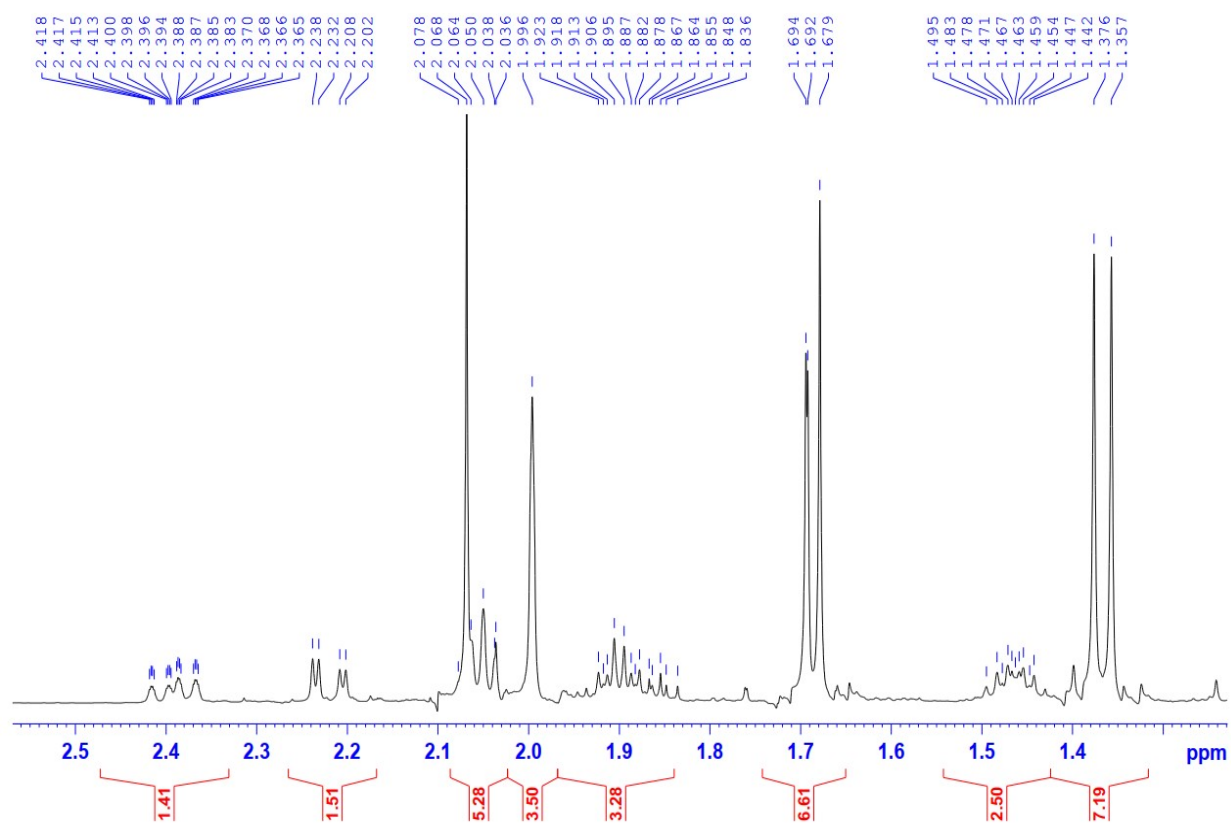


Figure S4. Expanded  $^1\text{H-NMR}$  spectrum of compound **1**

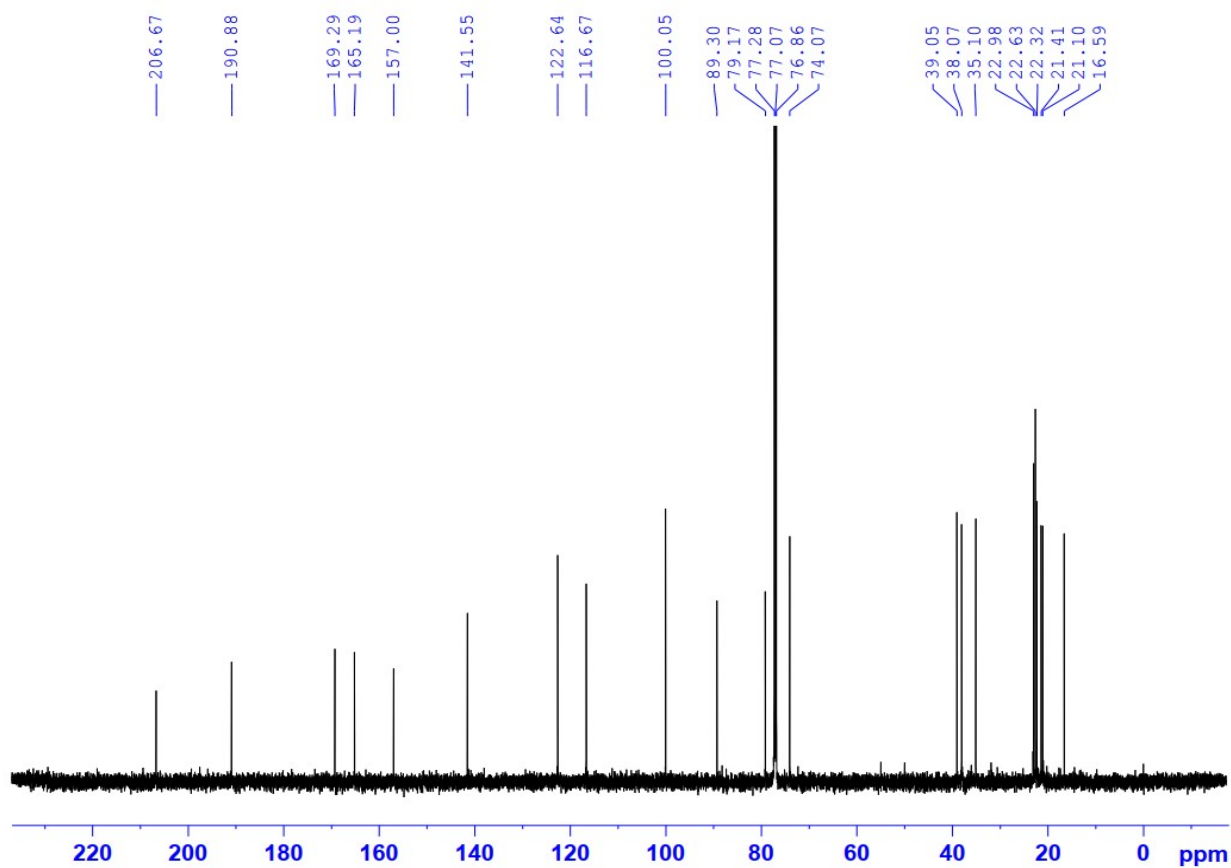


Figure S5.  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{CDCl}_3$

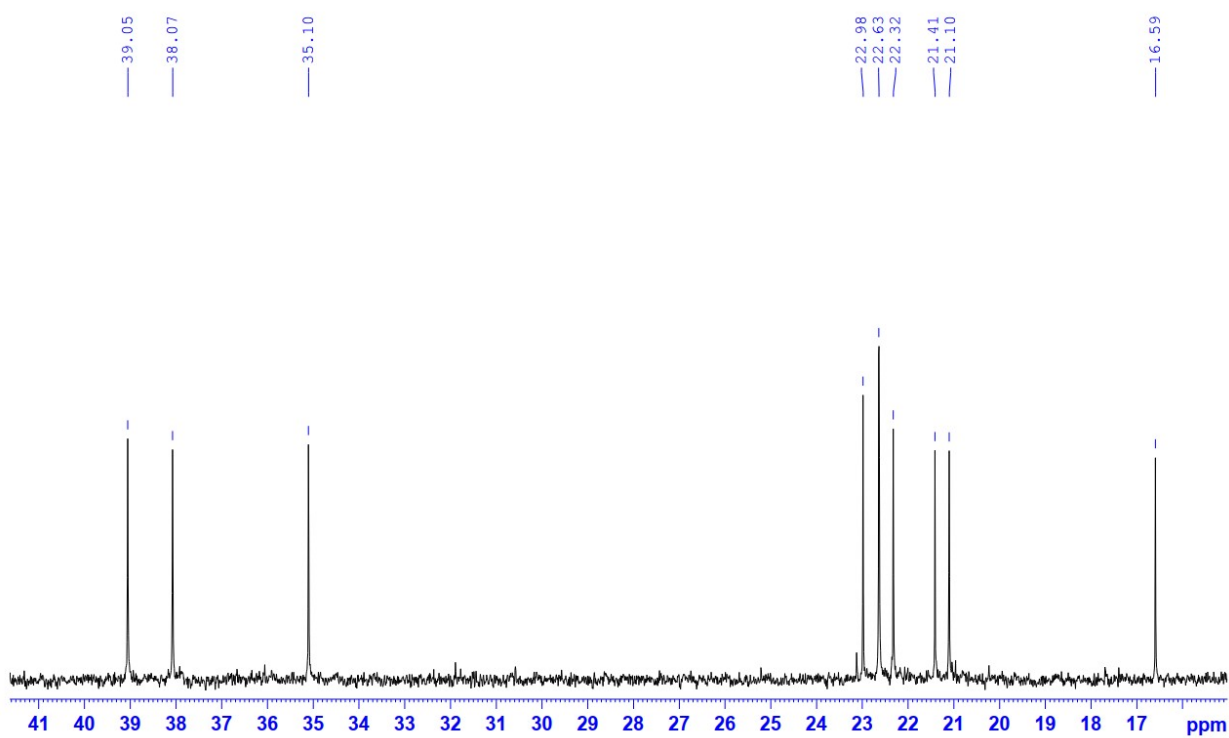


Figure S6. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{CDCl}_3$

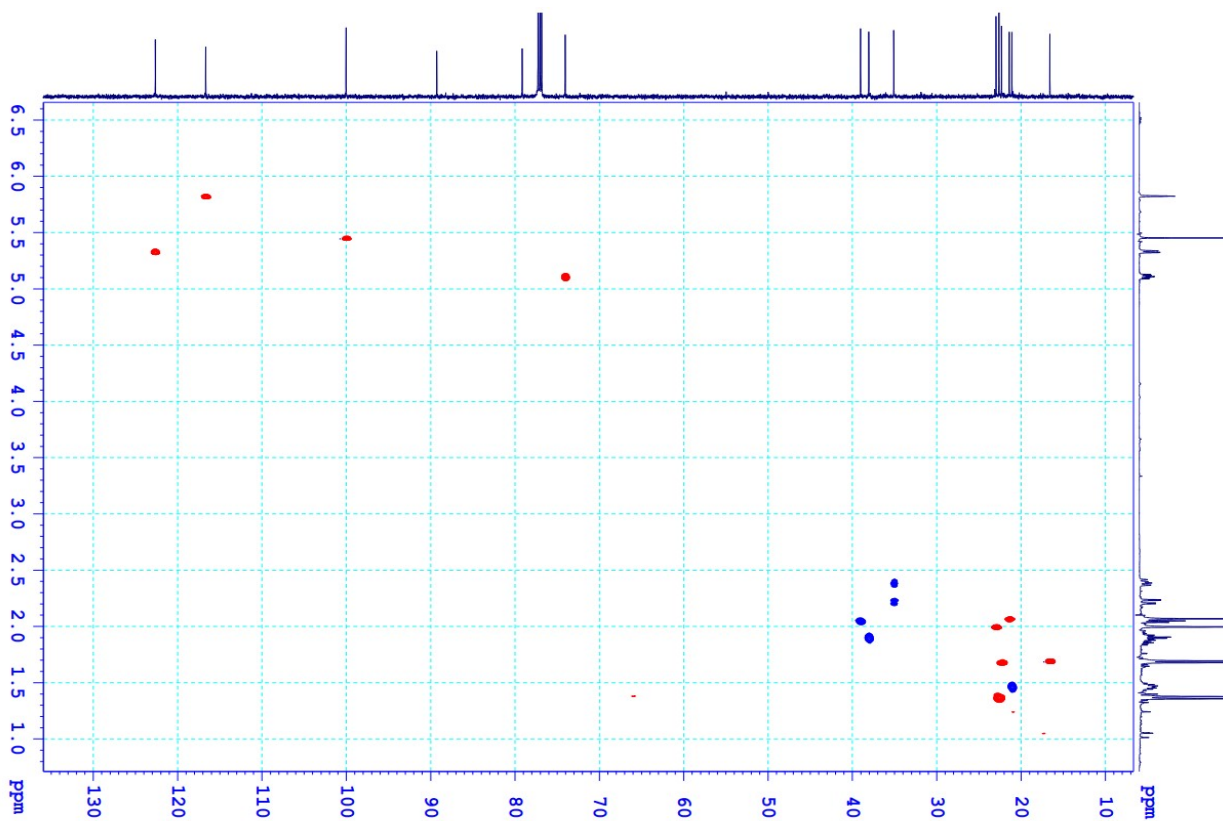


Figure S7. HSQC spectrum of compound 1

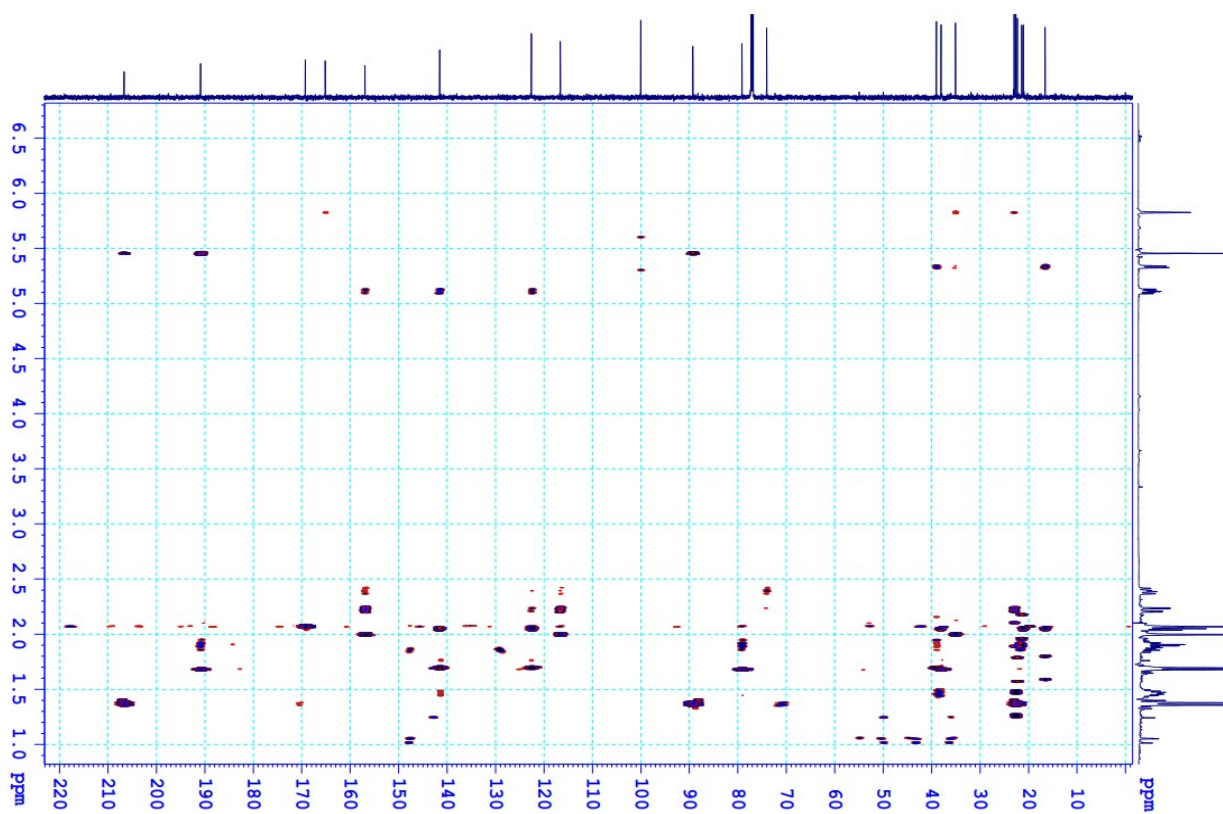


Figure S8. HMBC spectrum of compound 1

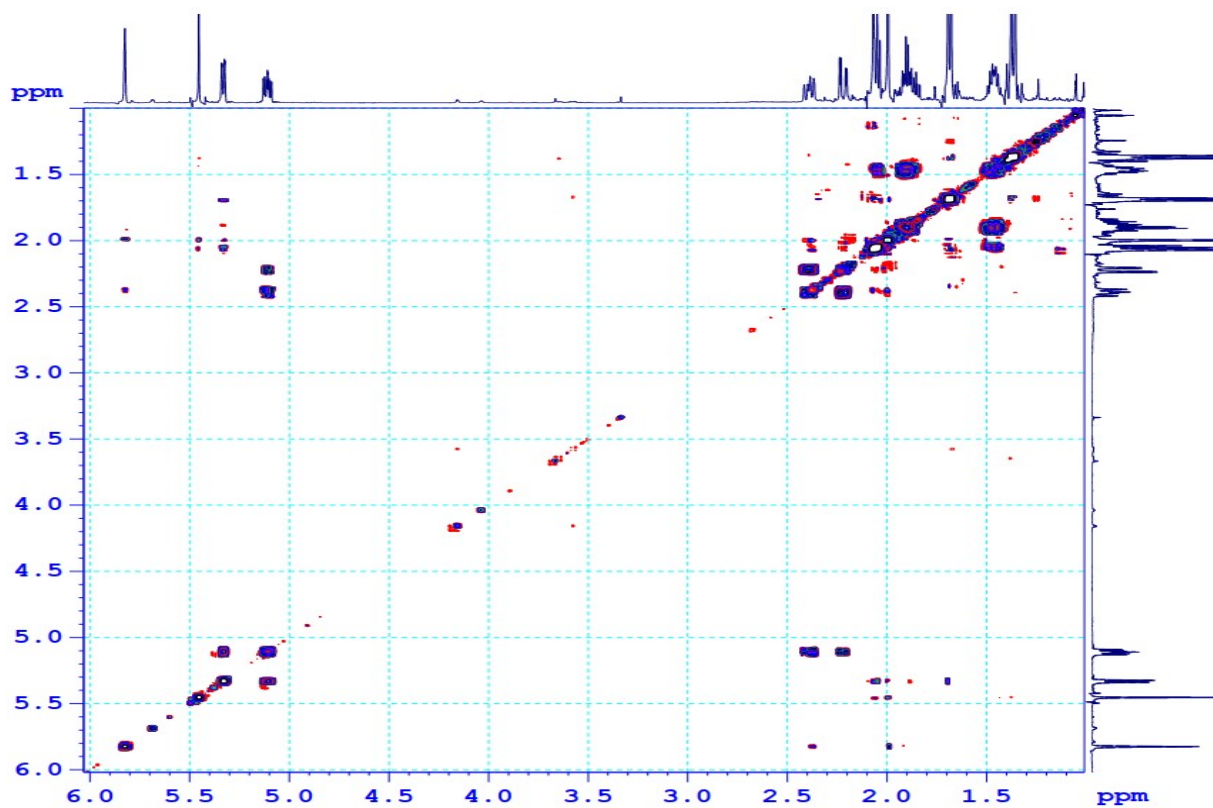


Figure S9. COSY spectrum of compound 1

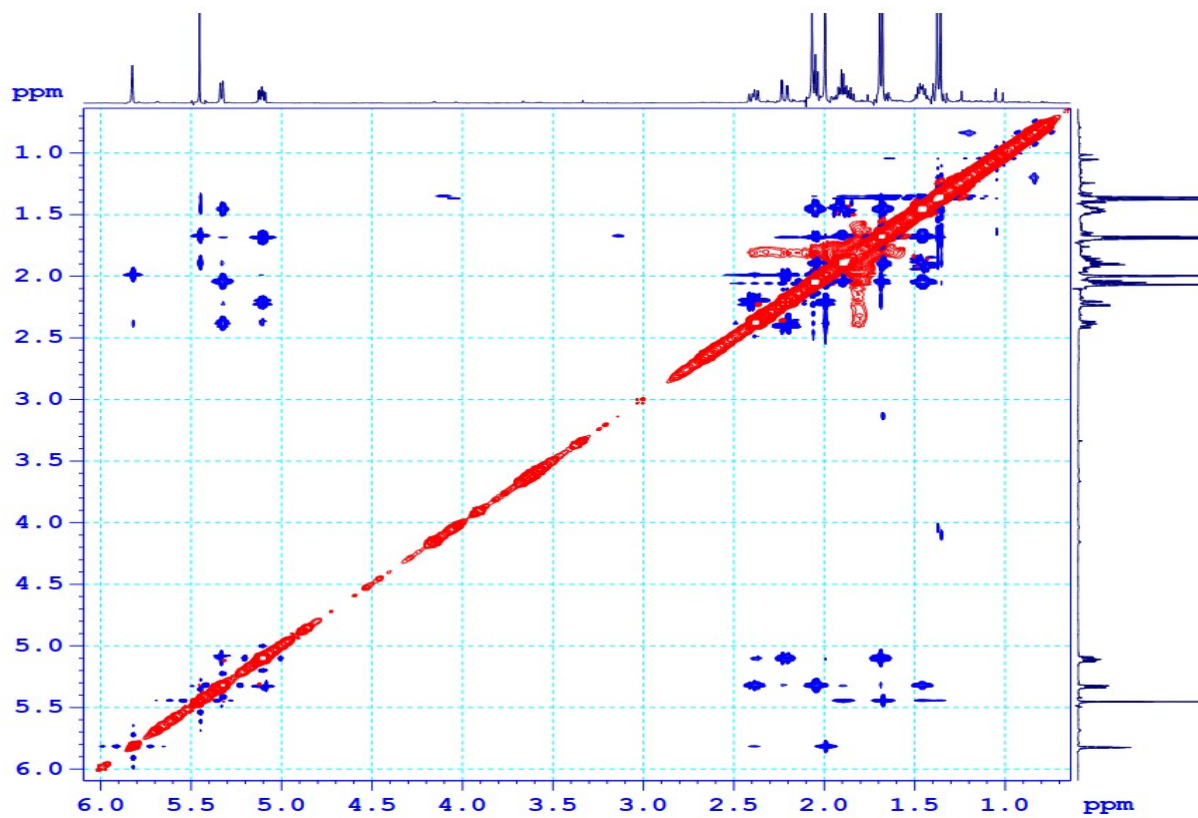


Figure S10. NOESY spectrum of compound 1



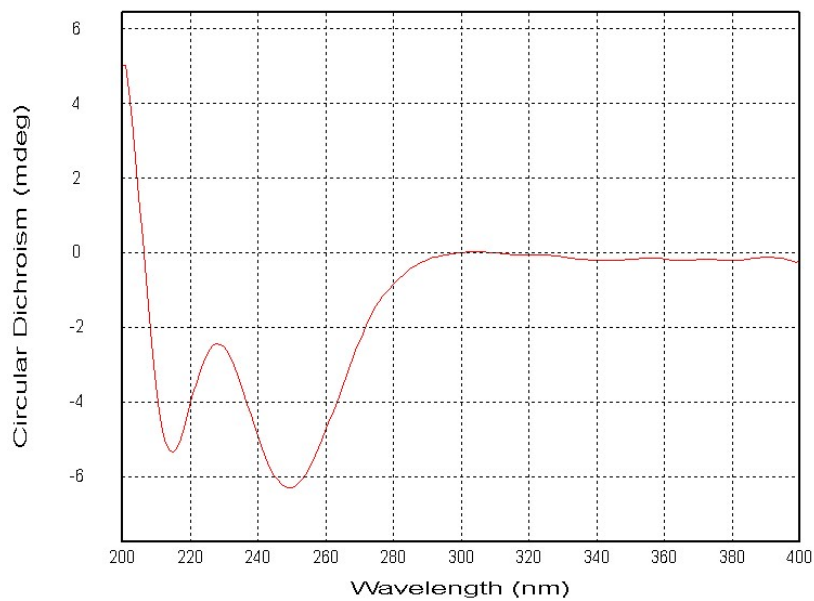


Figure S11. ECD spectrum of compound **1**

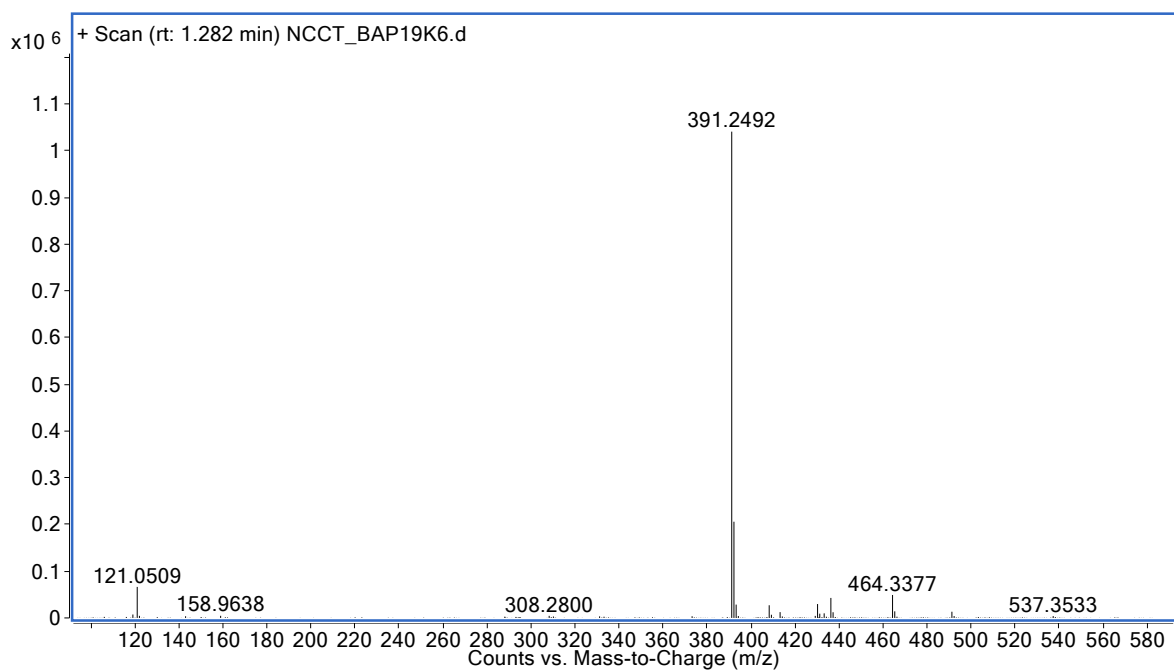


Figure S12. HR-ESI-MS of compound **2**

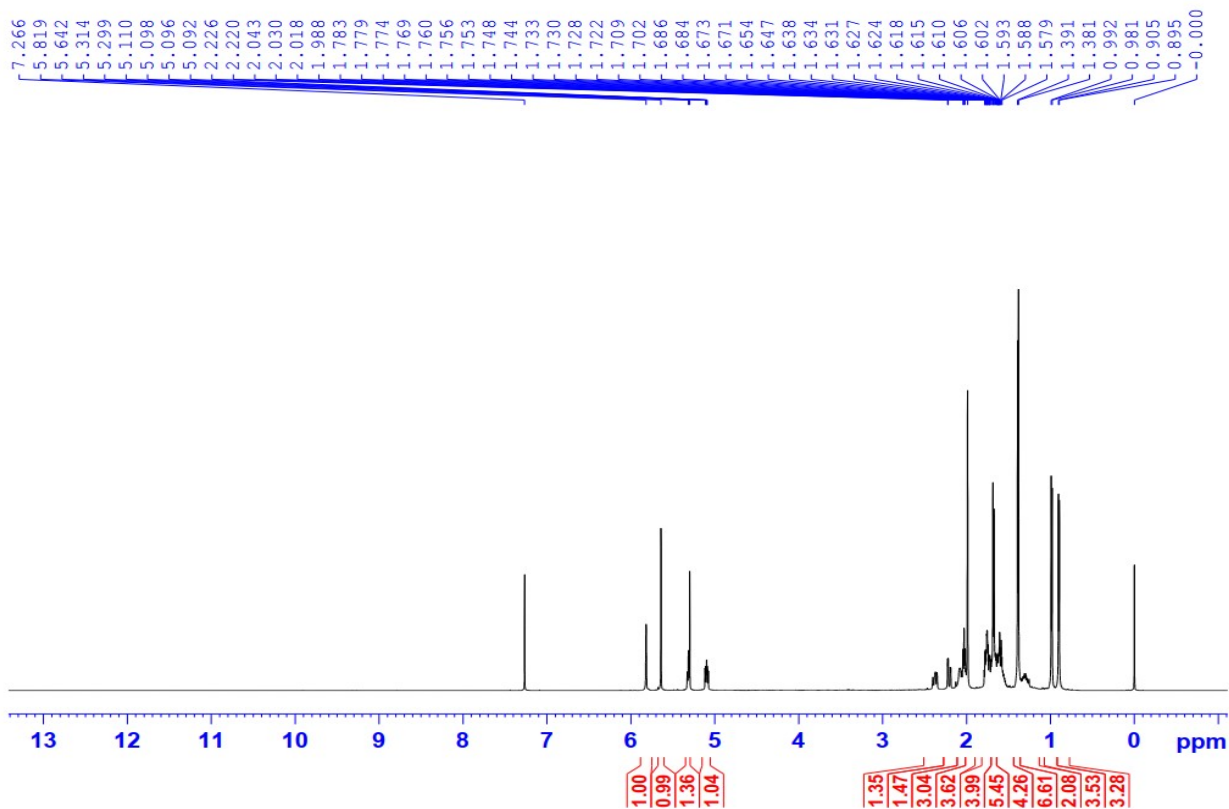


Figure S13.  $^1\text{H-NMR}$  spectrum of compound **2** in  $\text{CDCl}_3$

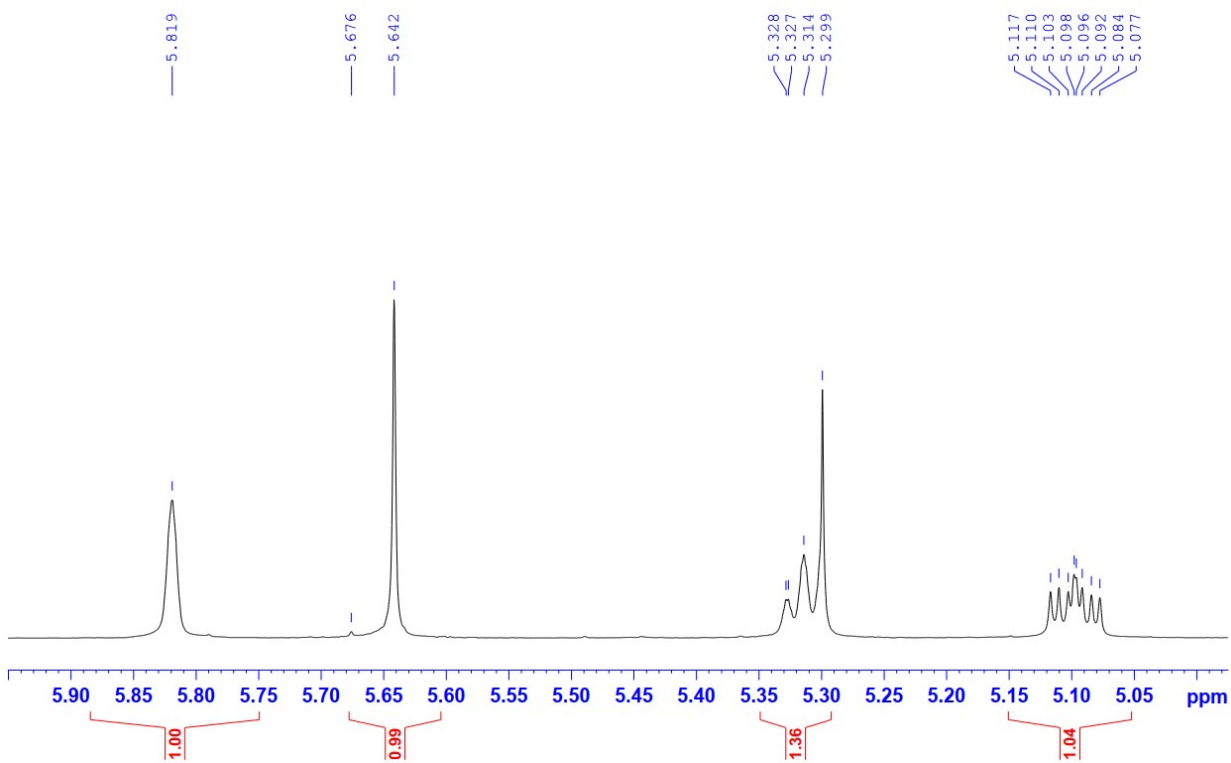


Figure S14. Expanded  $^1\text{H-NMR}$  spectrum of compound **2**

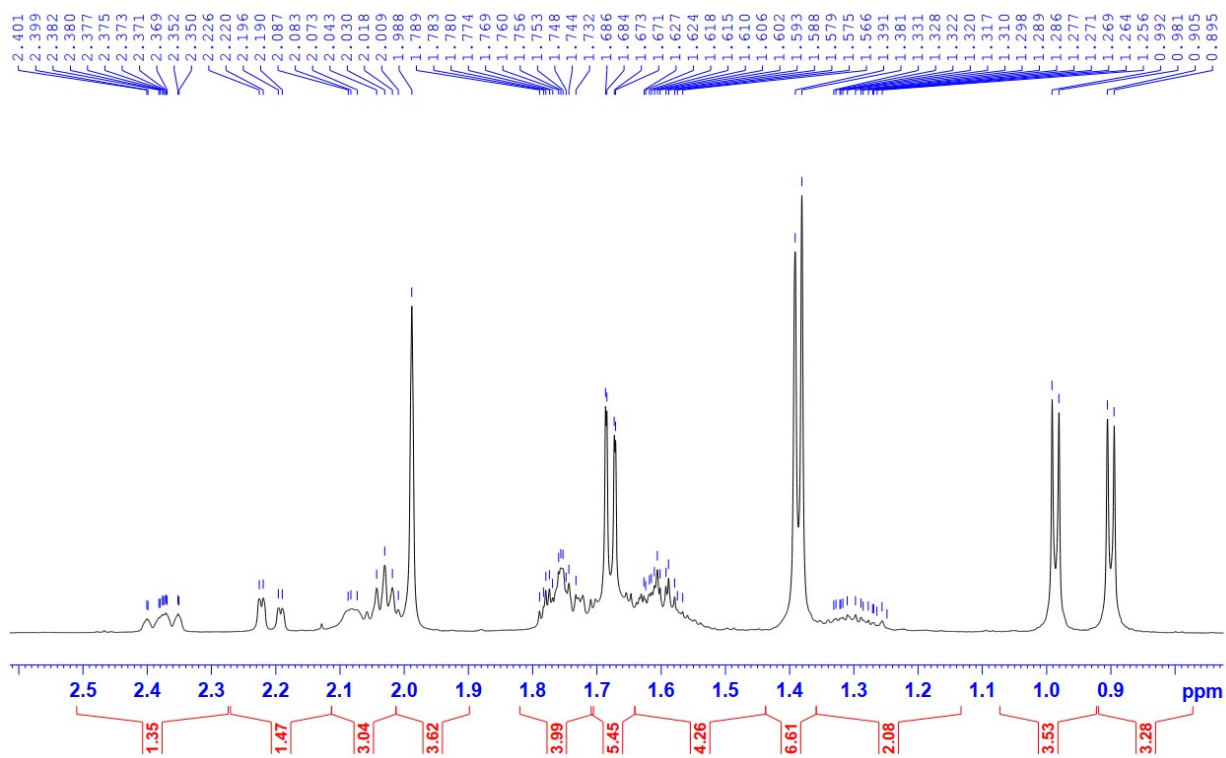


Figure S15. Expanded  $^1\text{H}$ -NMR spectrum of compound **2**

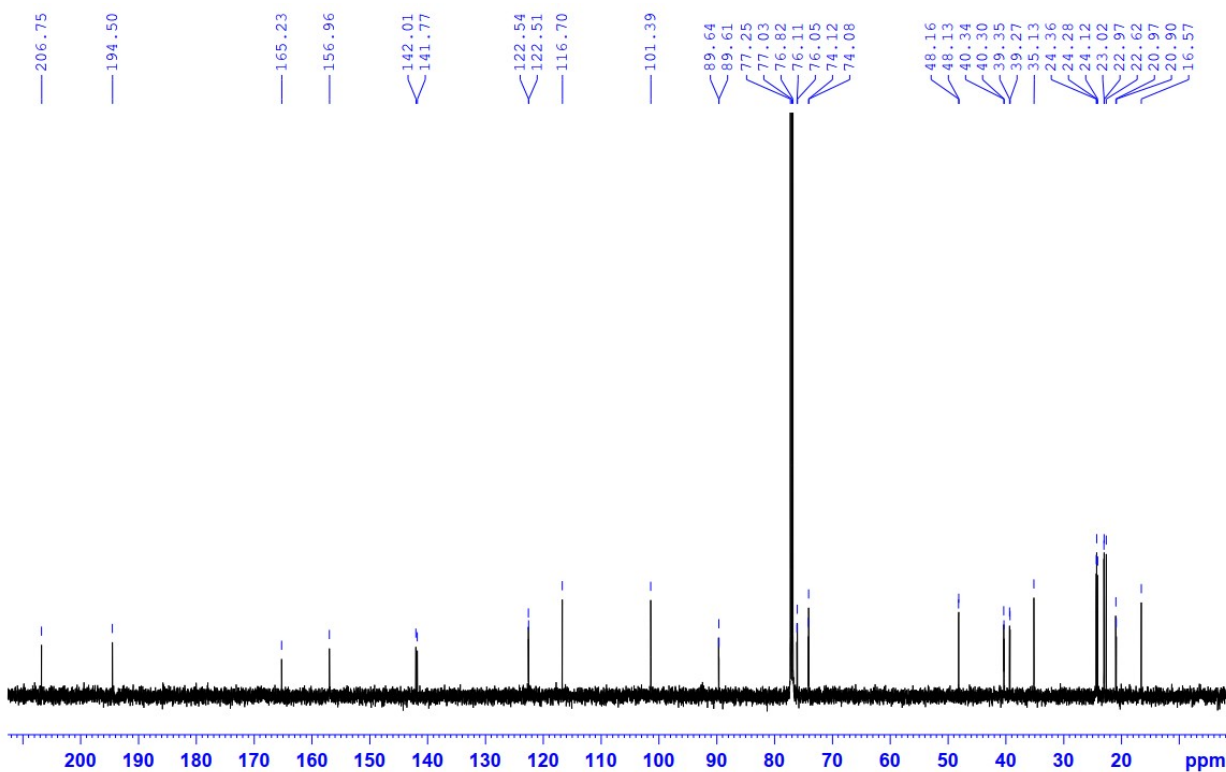


Figure S16.  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{CDCl}_3$

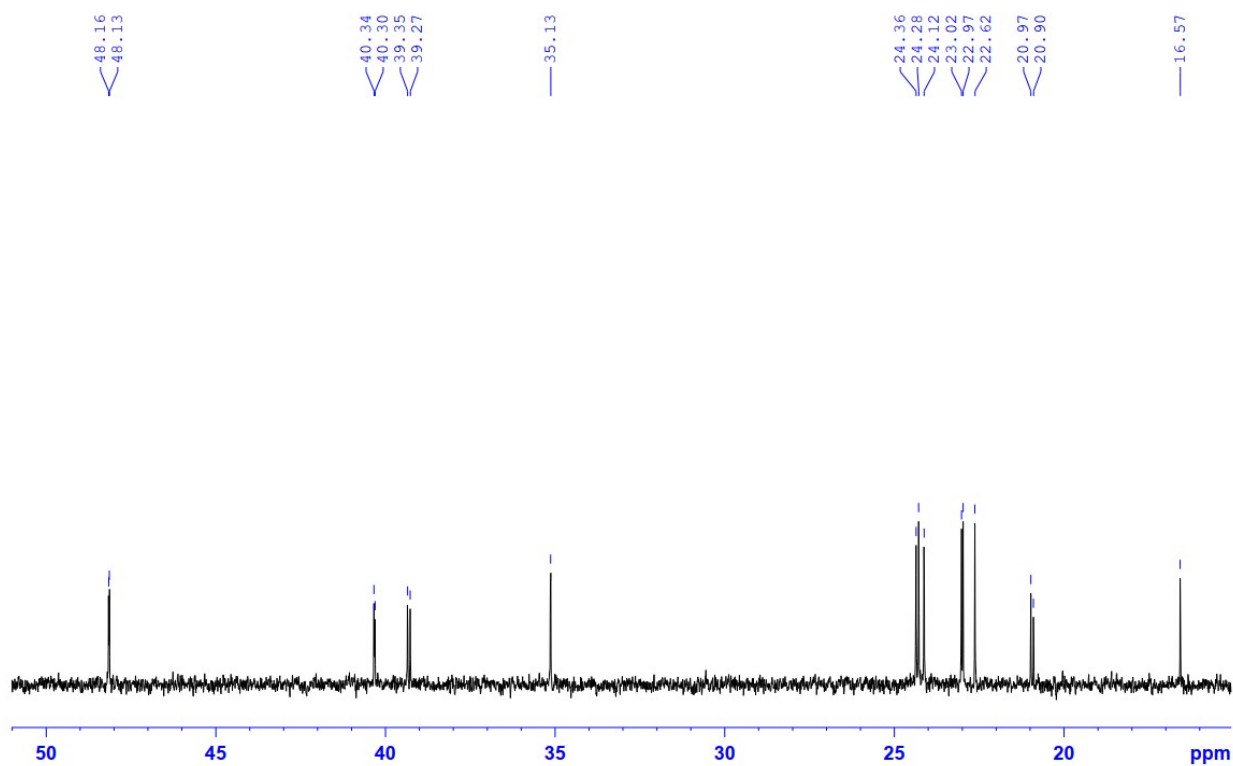


Figure S17. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{CDCl}_3$

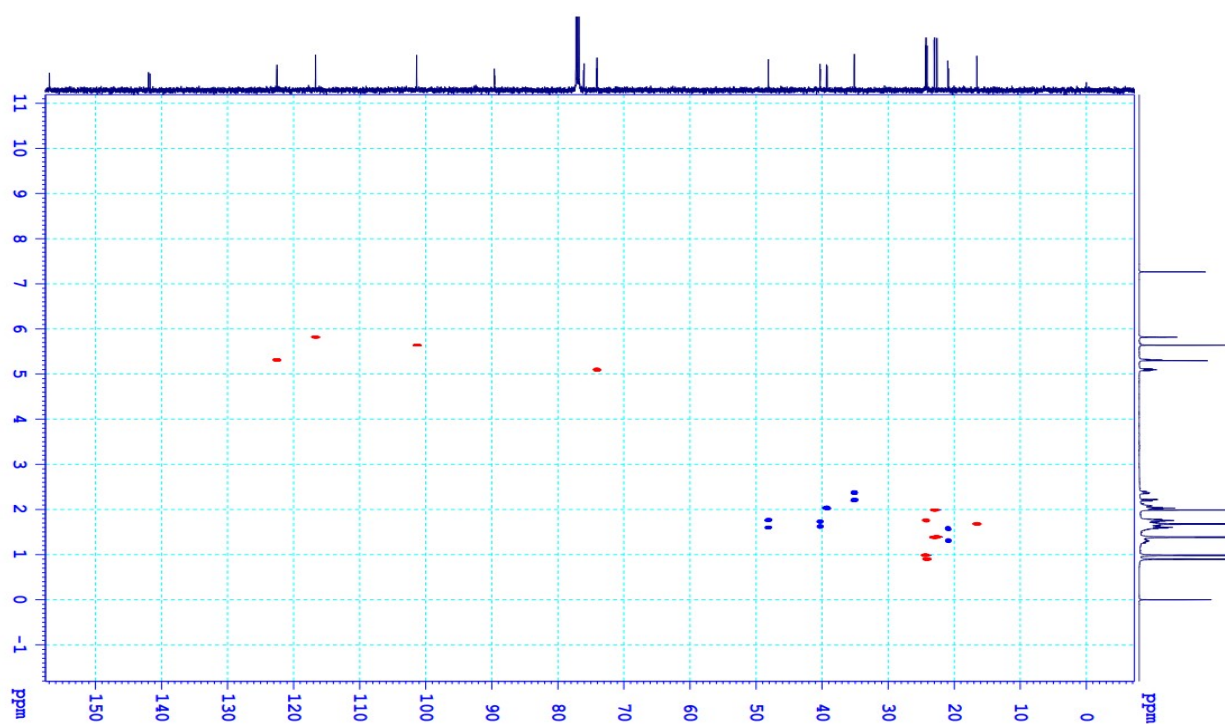


Figure S18. HSQC spectrum of compound **2**

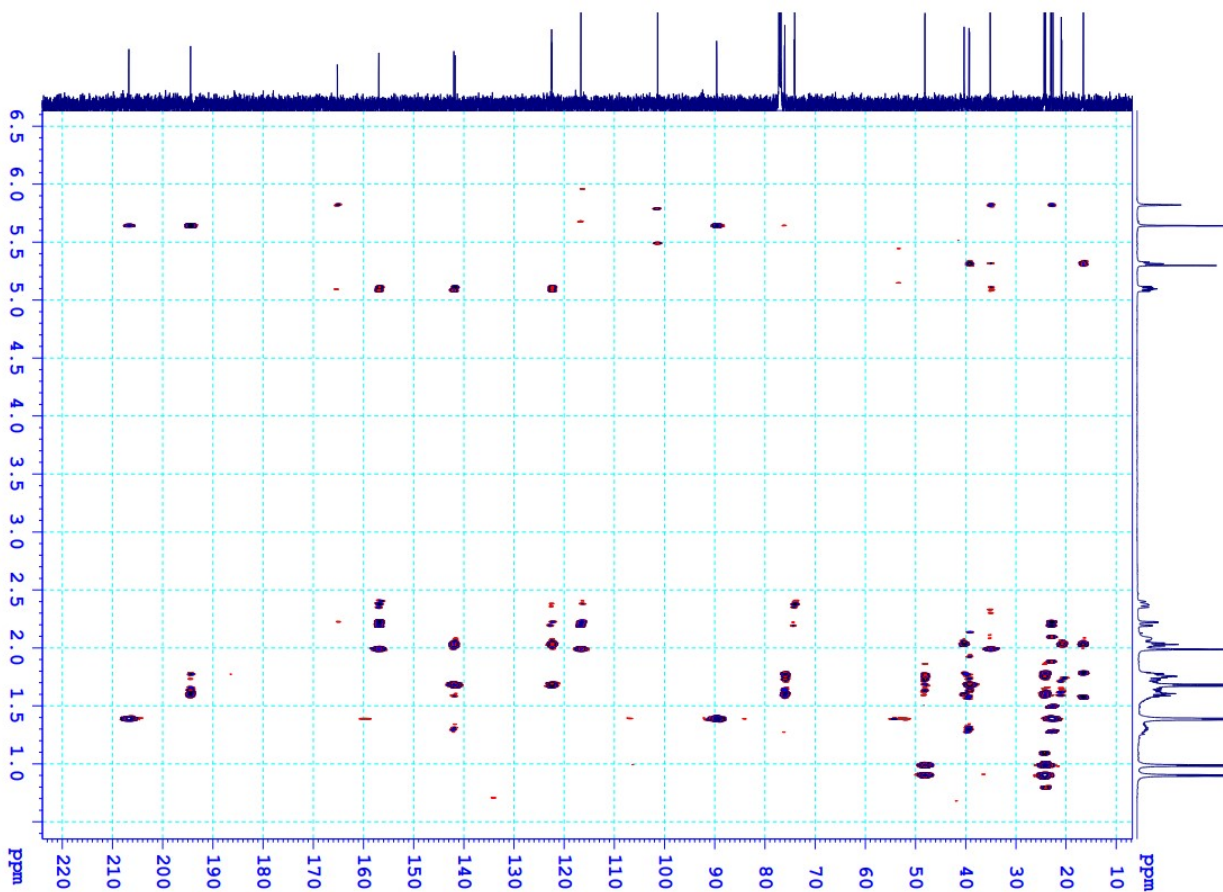


Figure S19. HMBC spectrum of compound 2

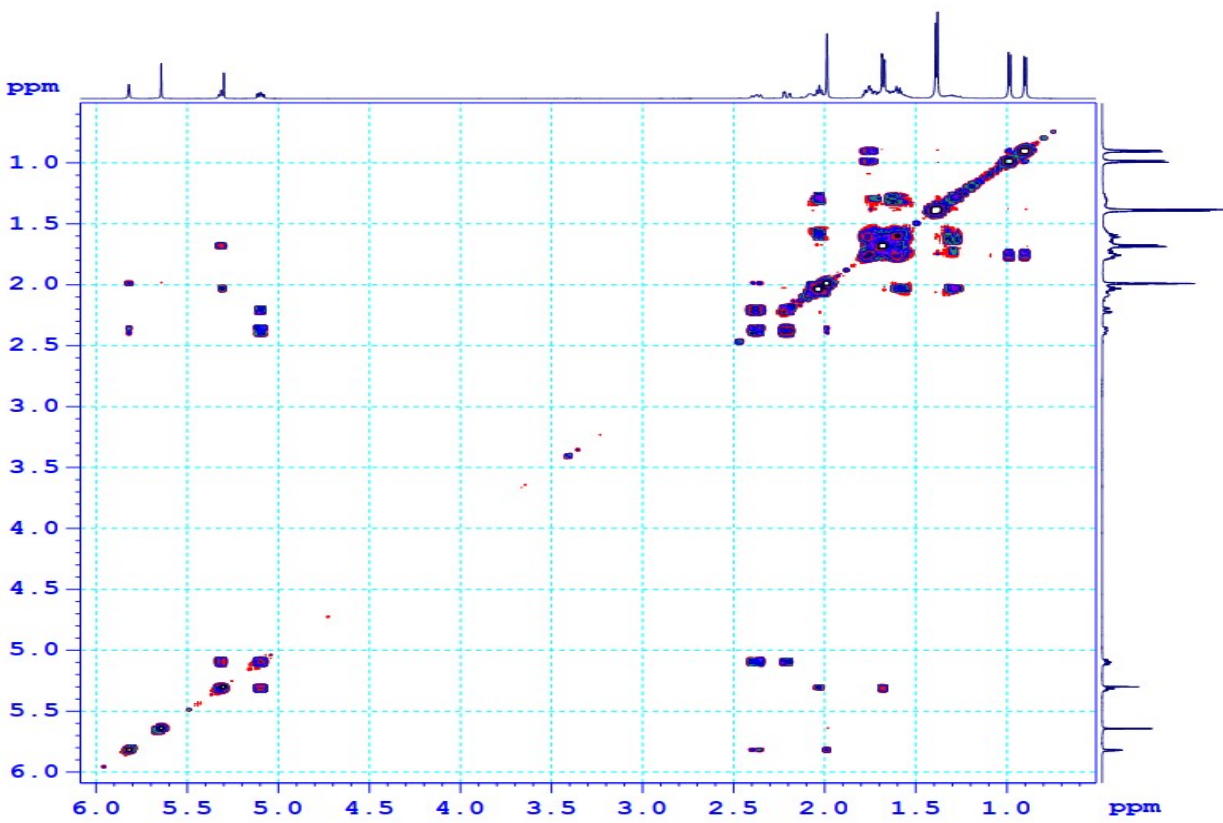


Figure S20. COSY spectrum of compound 2

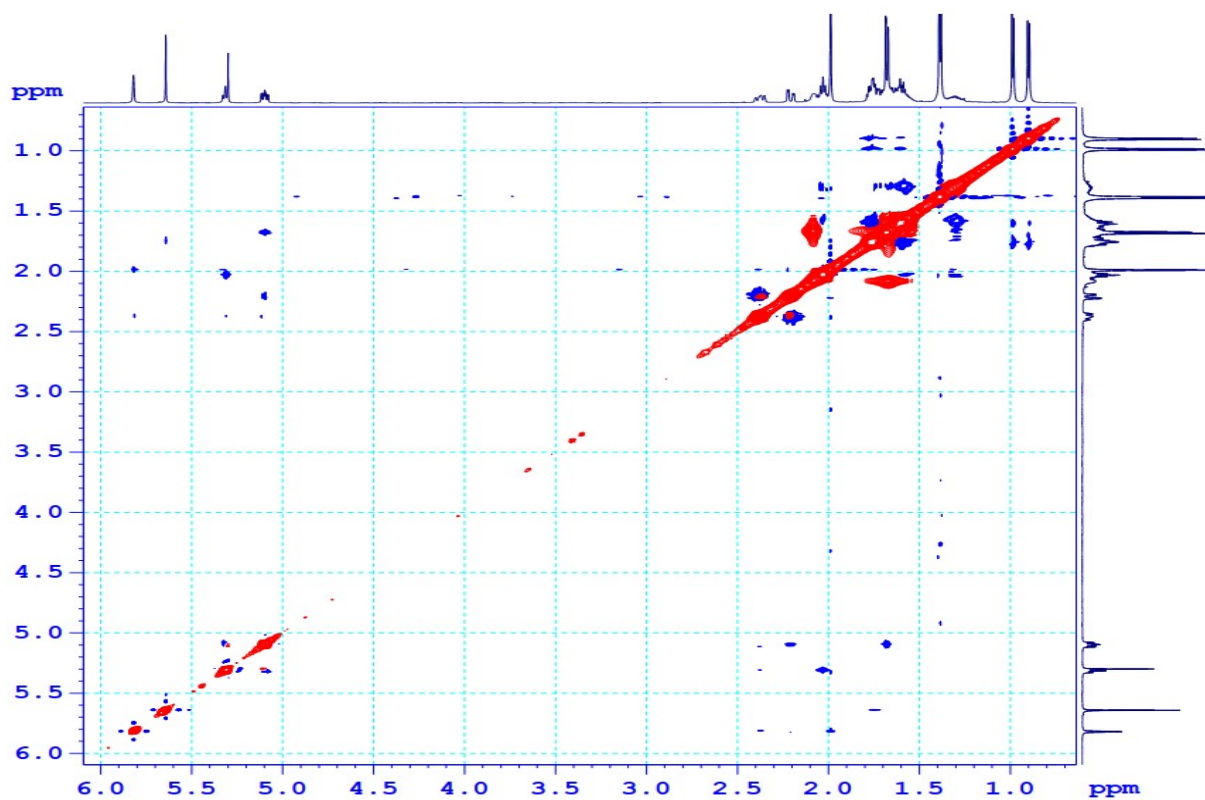


Figure S21. NOESY spectrum of compound 2

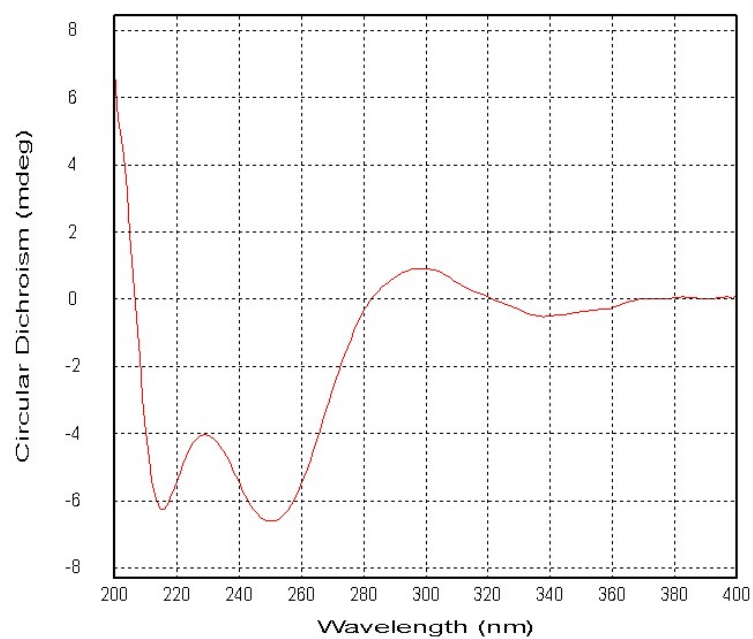


Figure S22. ECD spectrum of compound 2

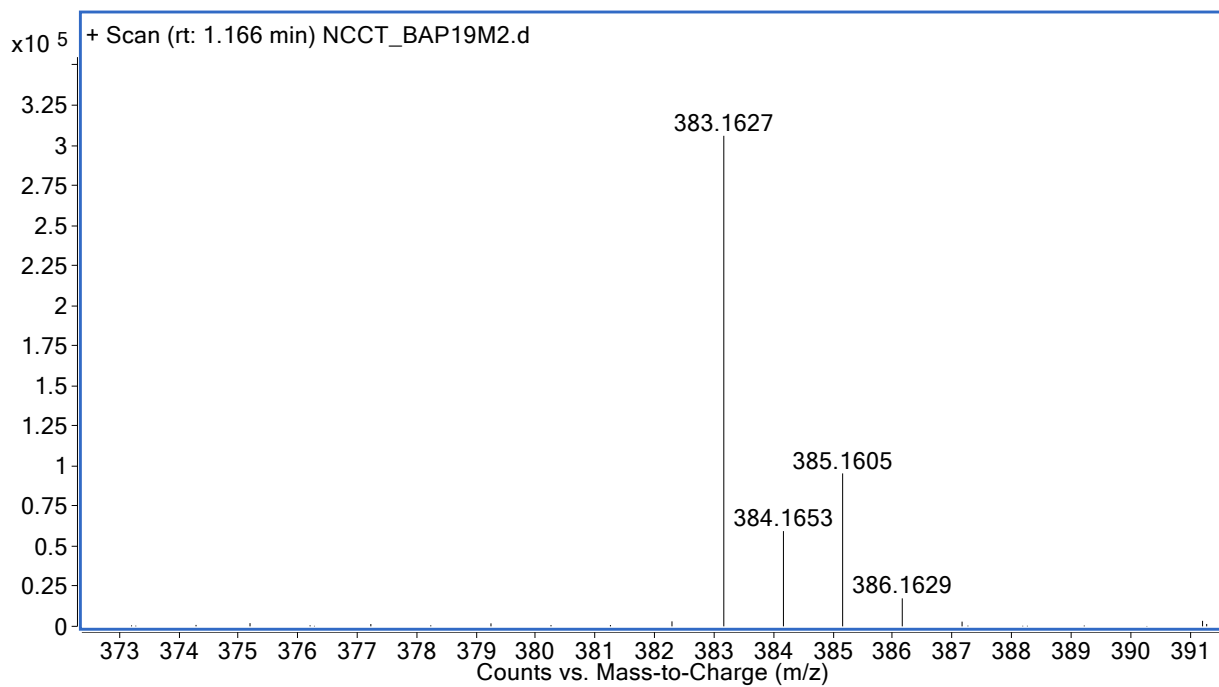
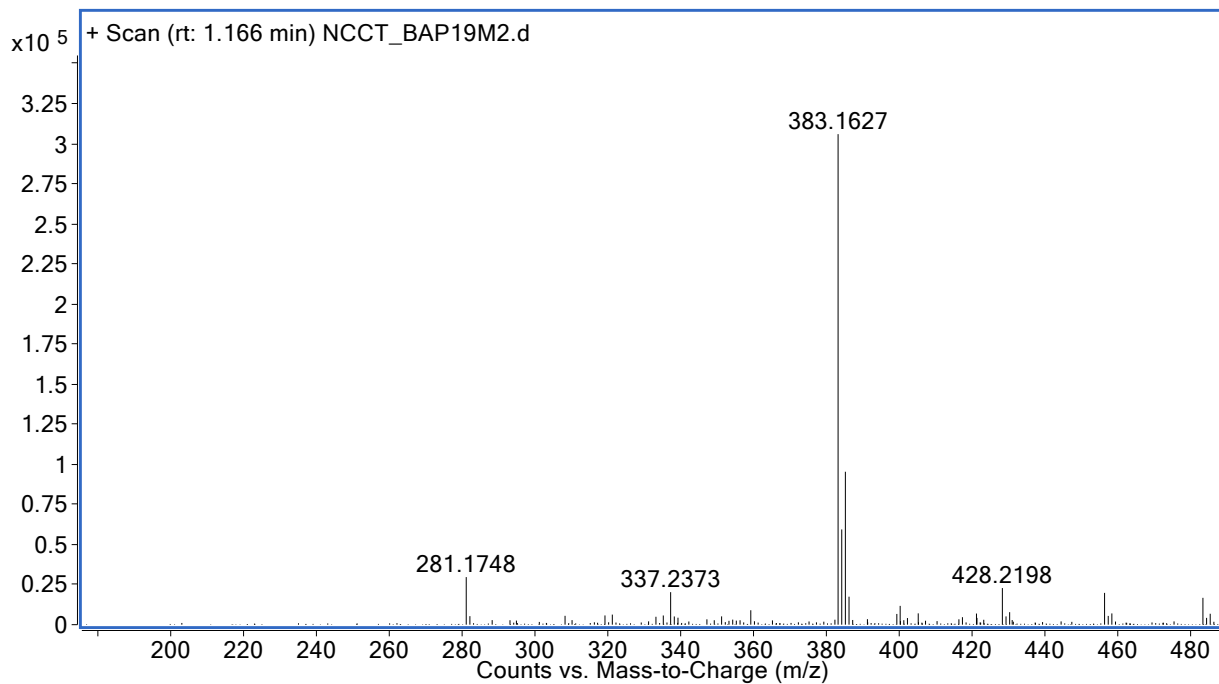


Figure S23. HR-ESI-MS of compound **3**

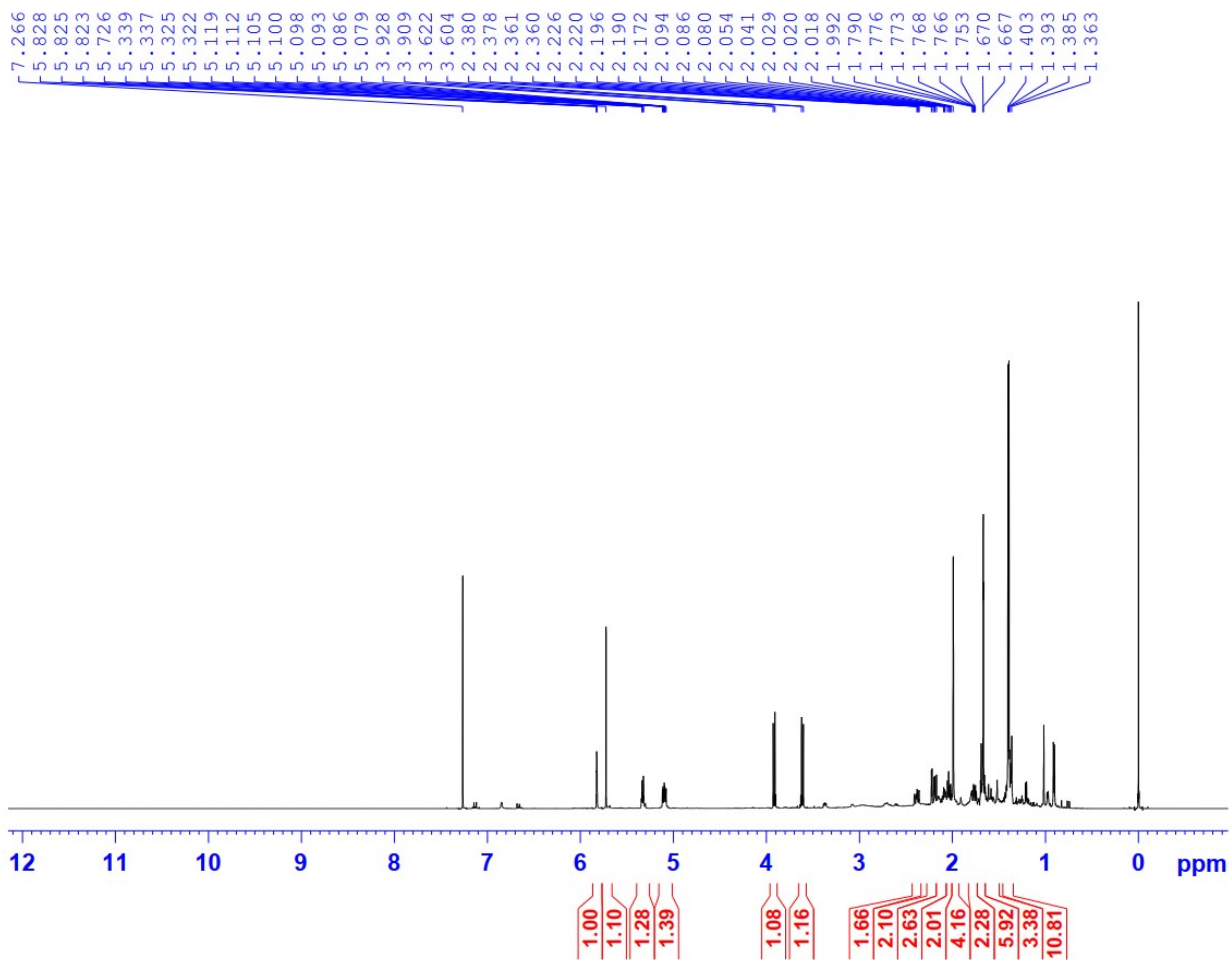


Figure S24.  $^1\text{H-NMR}$  spectrum of compound **3** in  $\text{CDCl}_3$

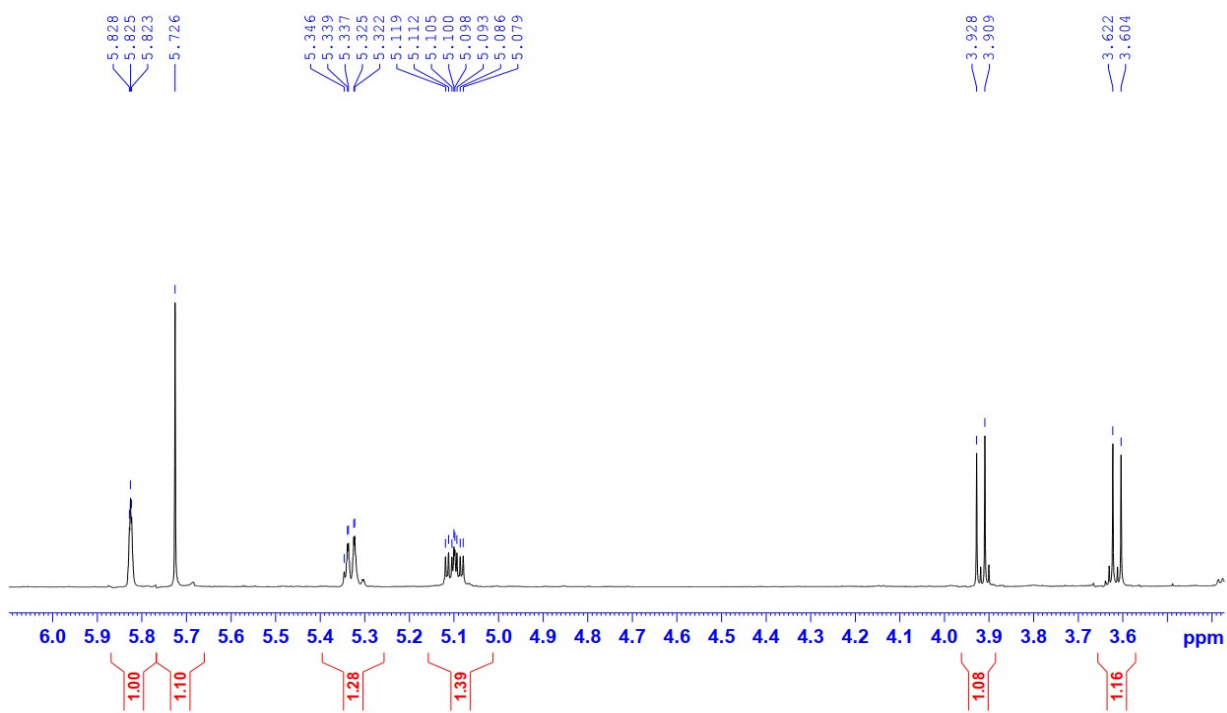




Figure S25. Expanded  $^1\text{H-NMR}$  spectrum of compound **3**

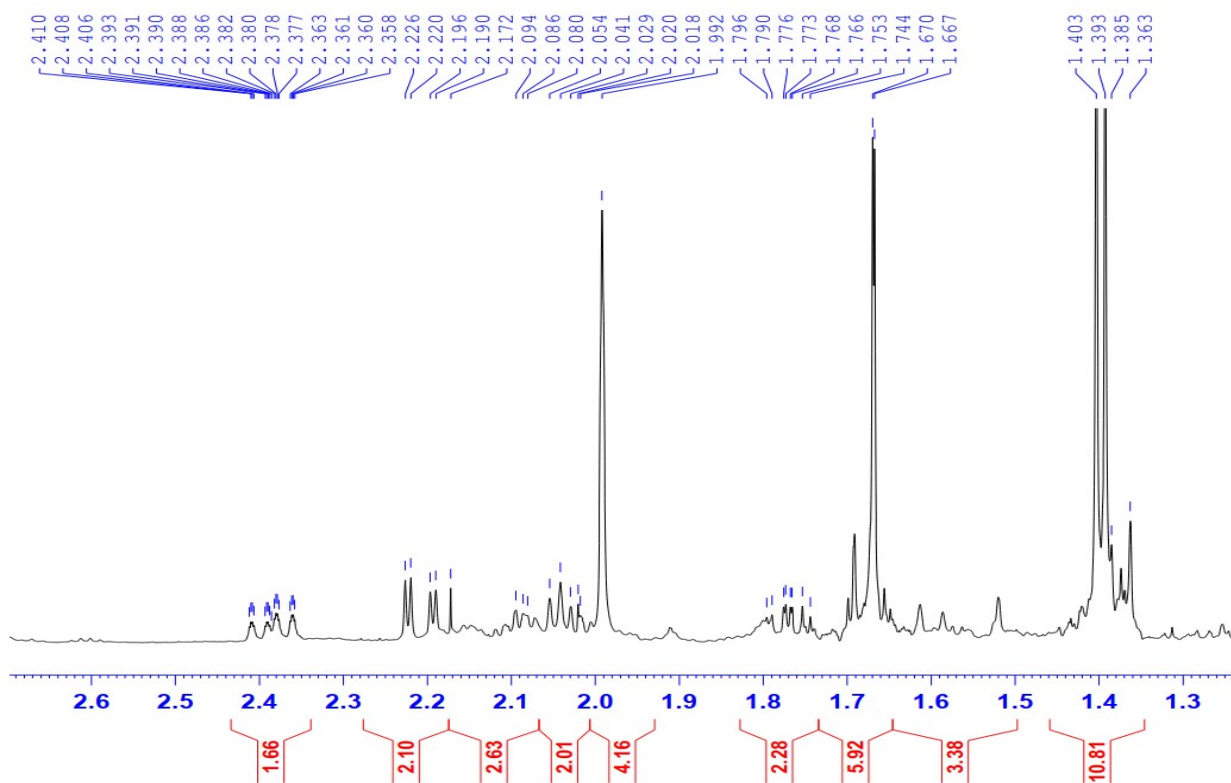


Figure S26. Expanded  $^1\text{H-NMR}$  spectrum of compound **3**

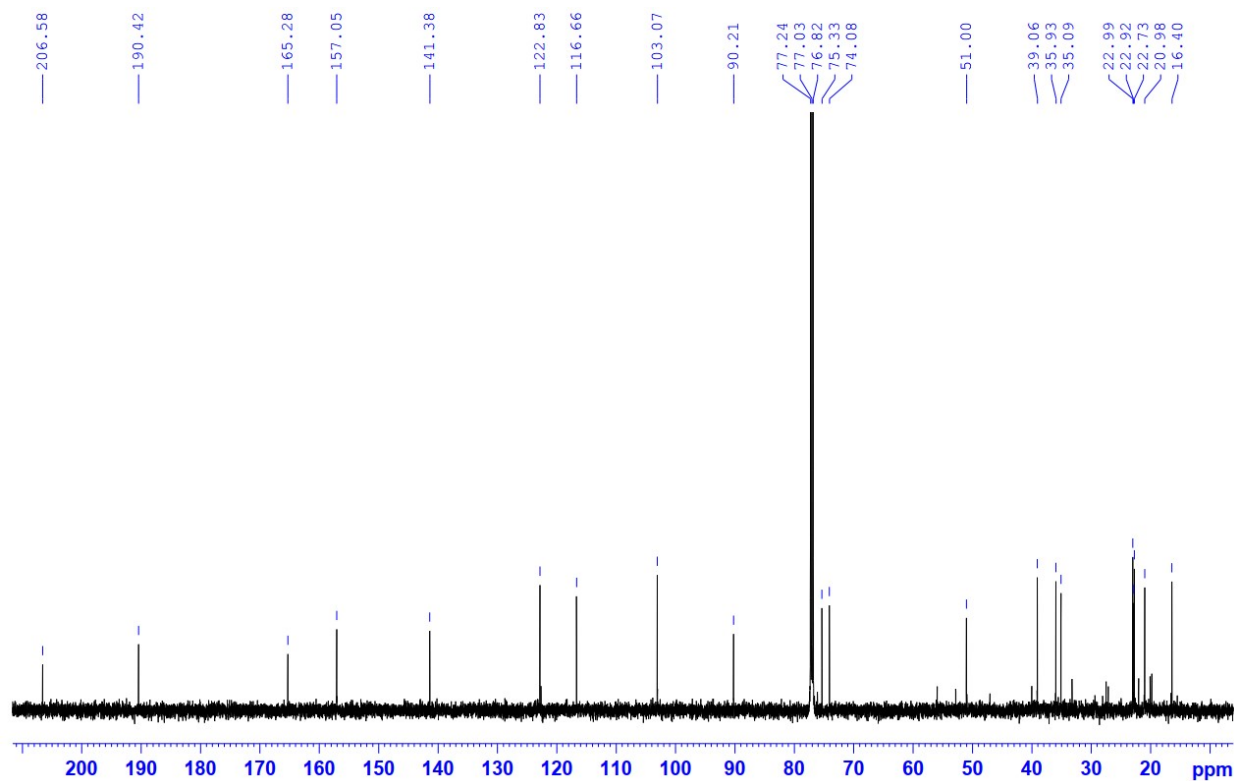


Figure S27.  $^{13}\text{C}$ -NMR spectrum of compound **3** in  $\text{CDCl}_3$

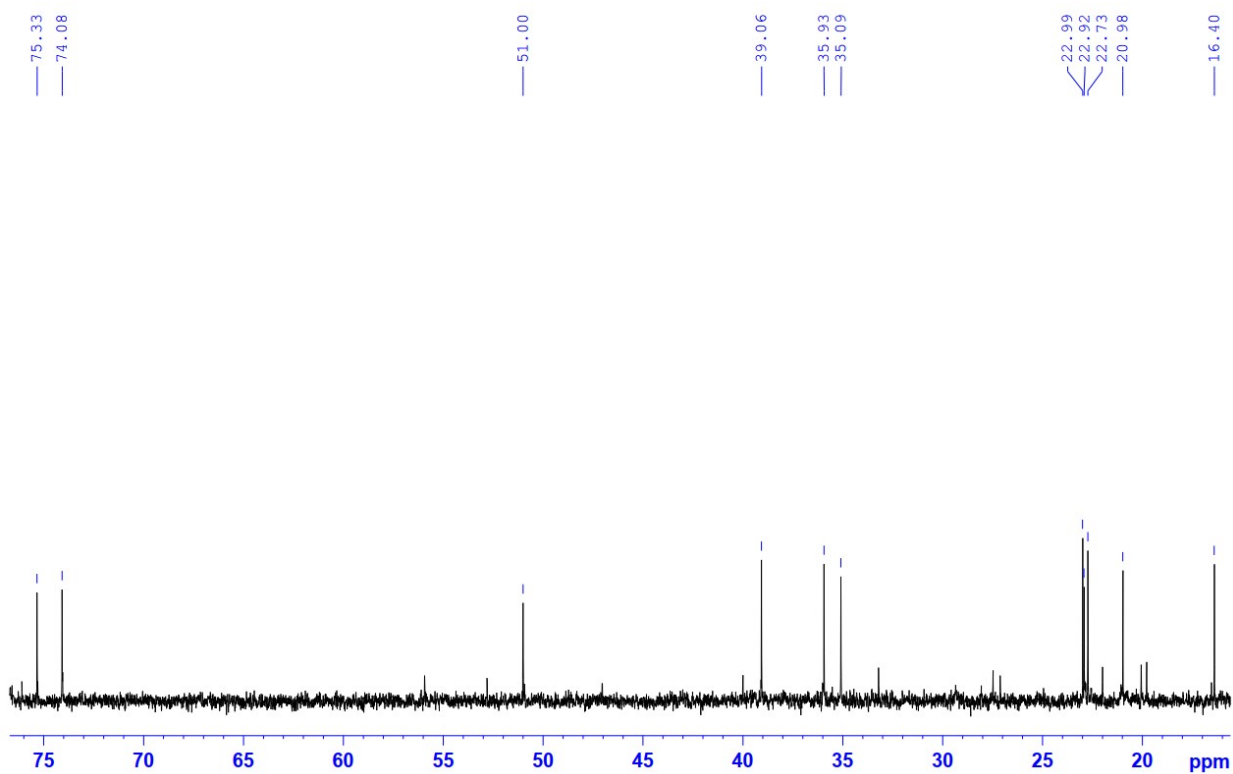


Figure S28. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **3**

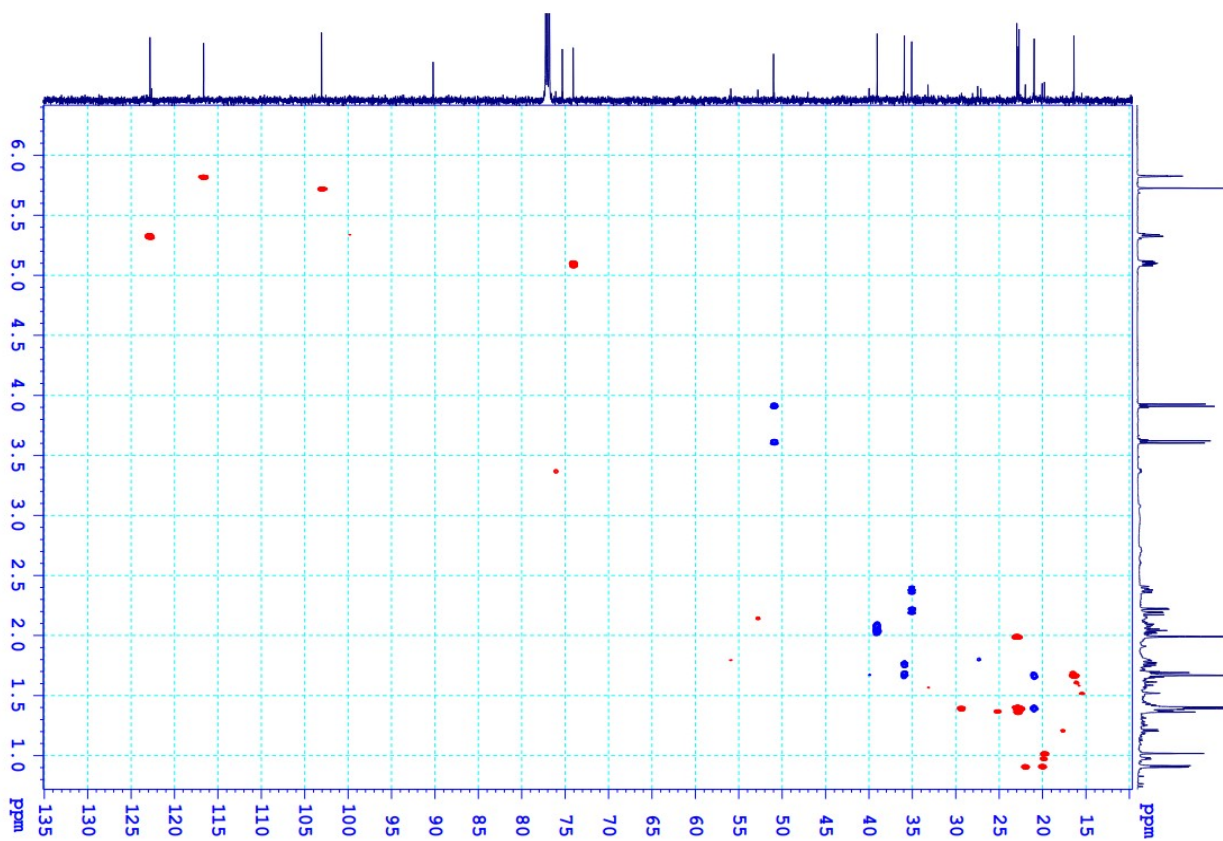


Figure S29. HSQC spectrum of compound 3

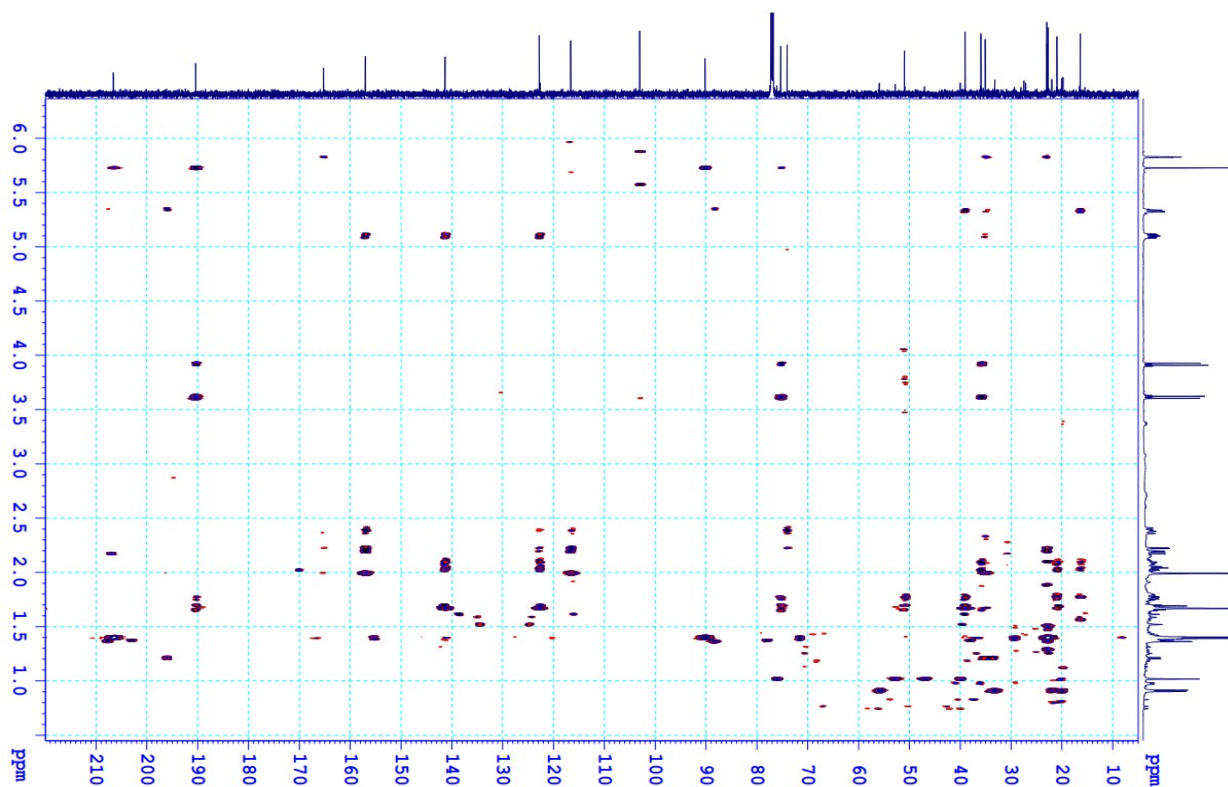


Figure S30. HMBC spectrum of compound 3

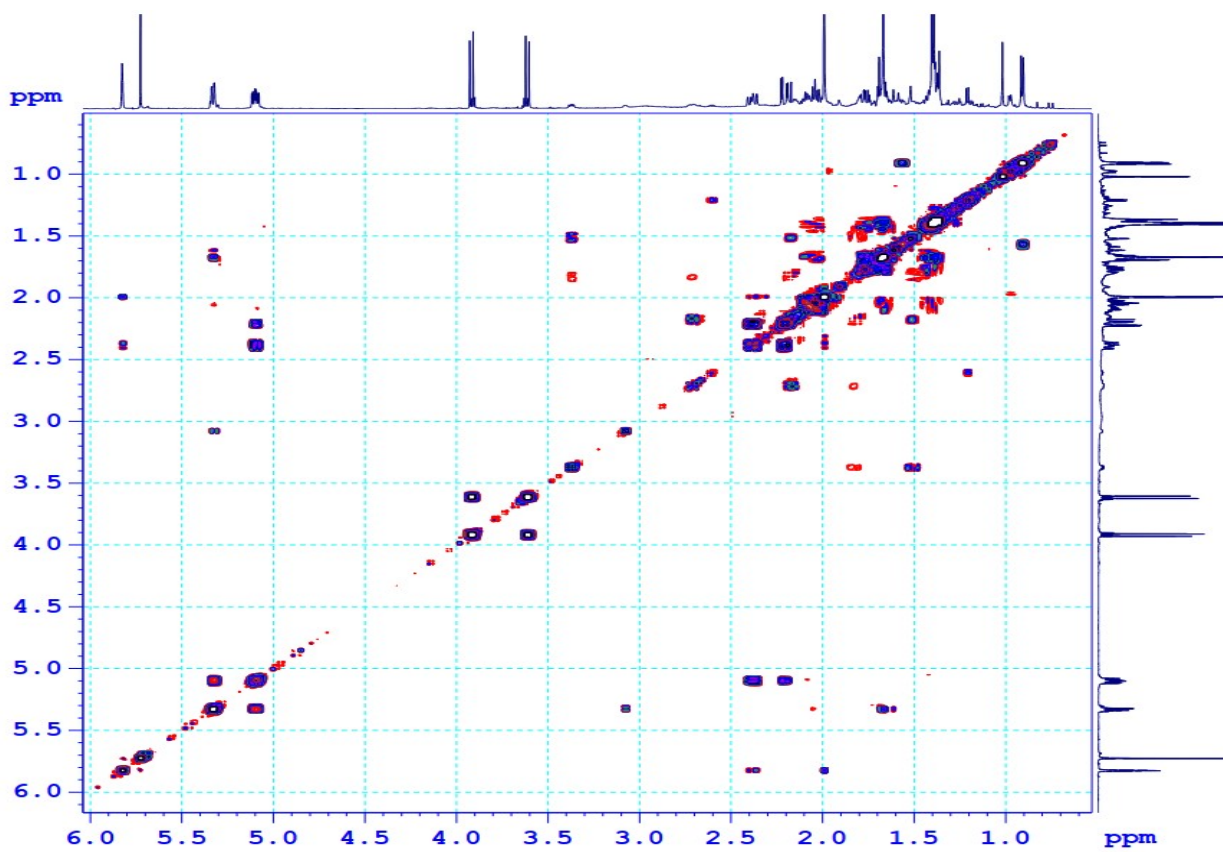


Figure S31. COSY spectrum of compound 3

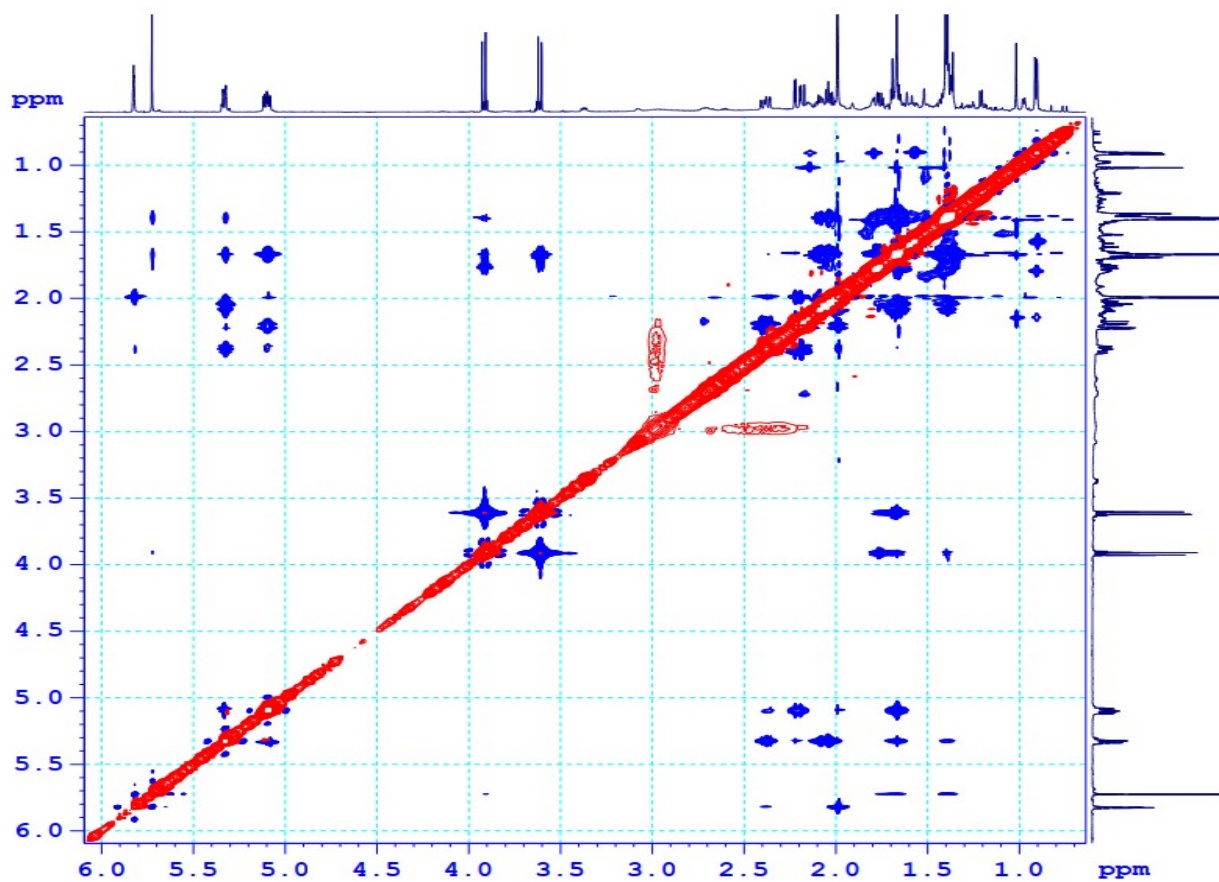


Figure S32. NOESY spectrum of compound 3

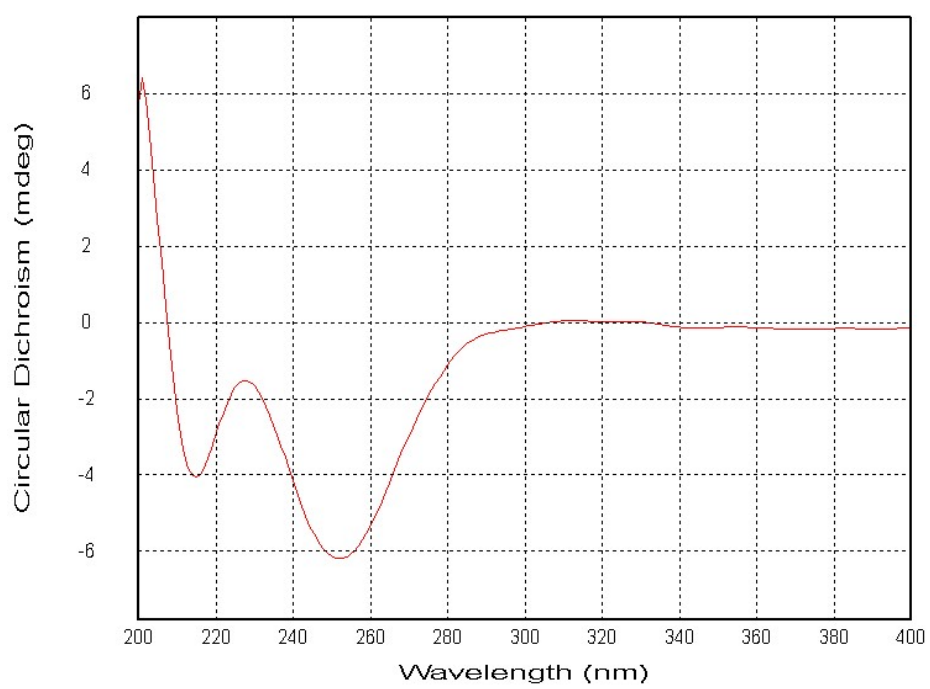


Figure S33. ECD spectrum of compound **3**

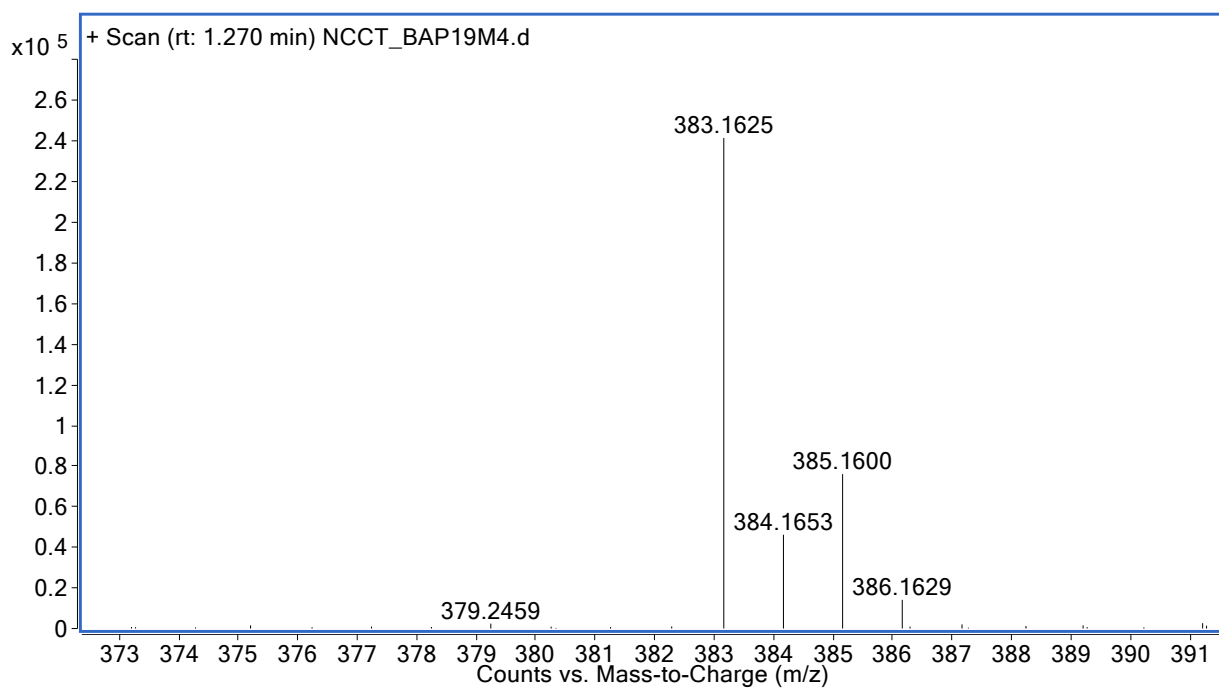
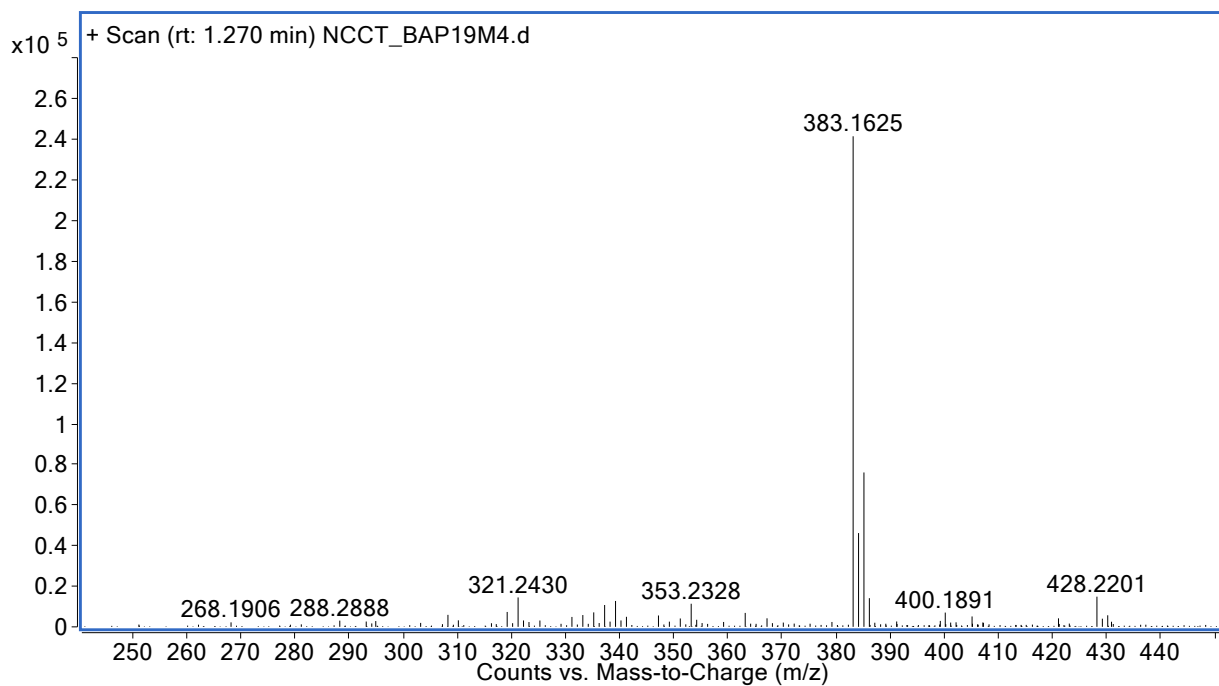


Figure S34. HR-ESI-MS of compound 4

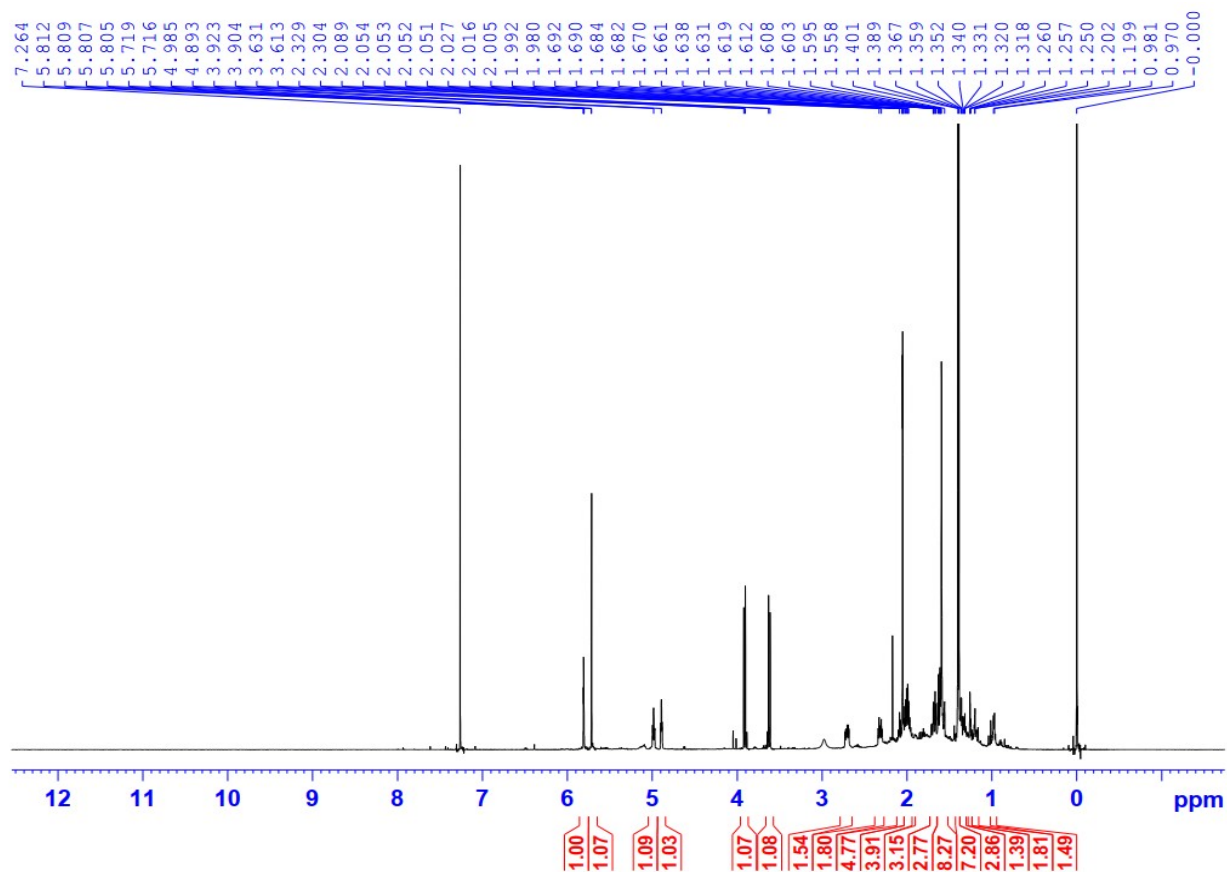


Figure S35.  $^1\text{H-NMR}$  spectrum of compound **4** in  $\text{CDCl}_3$

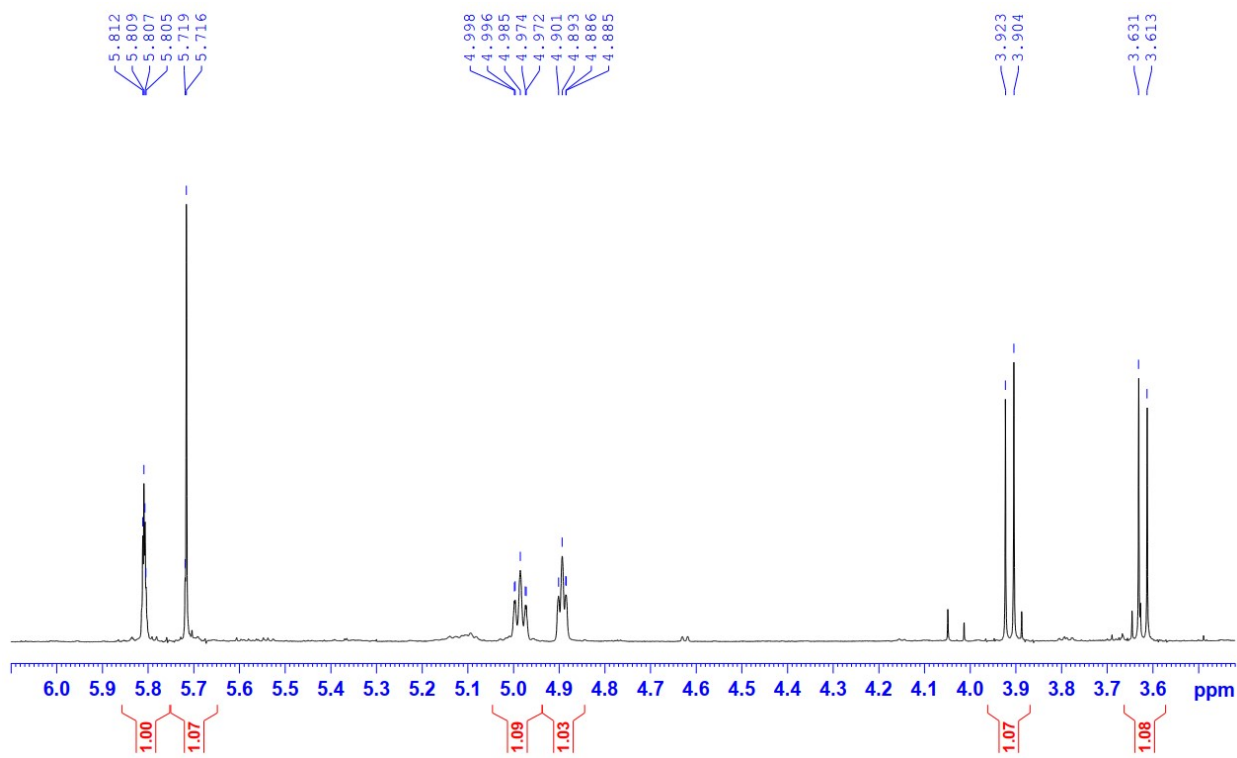


Figure S36. Expanded  $^1\text{H-NMR}$  spectrum of compound 4

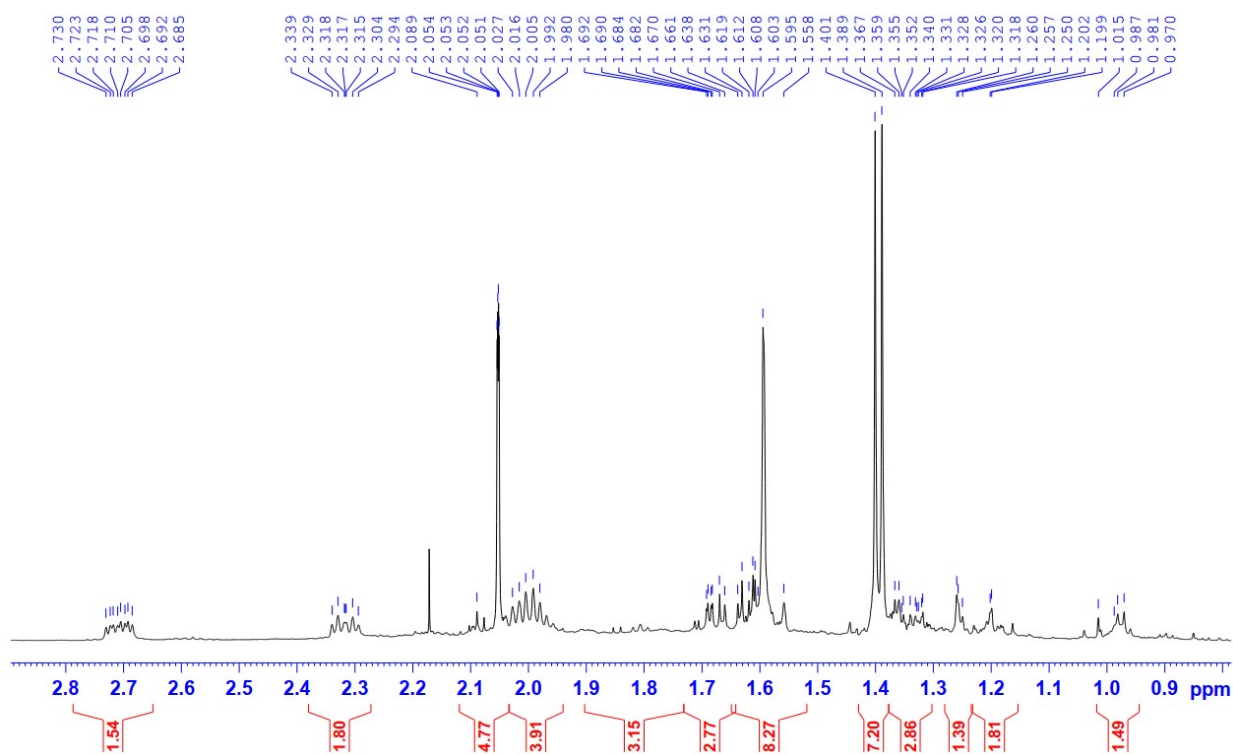


Figure S37. Expanded  $^1\text{H-NMR}$  spectrum of compound 4

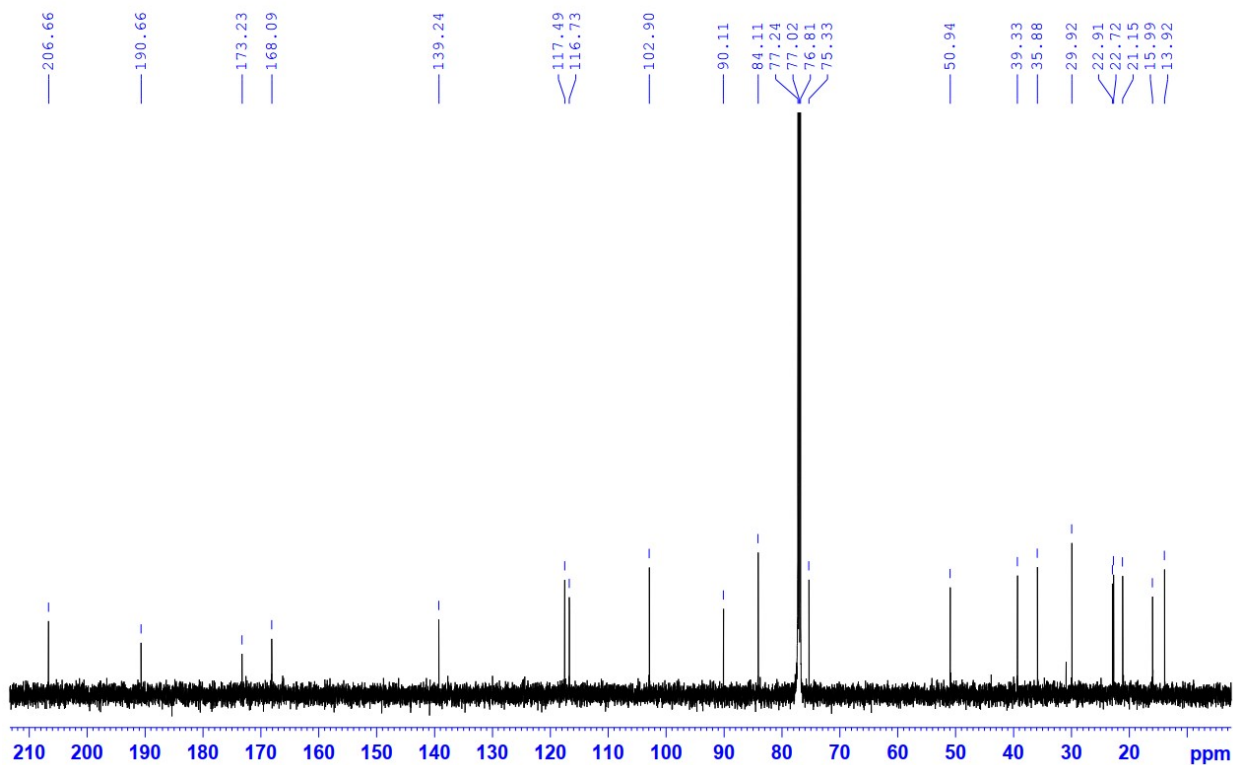




Figure S38.  $^{13}\text{C}$ -NMR spectrum of compound **4** in  $\text{CDCl}_3$

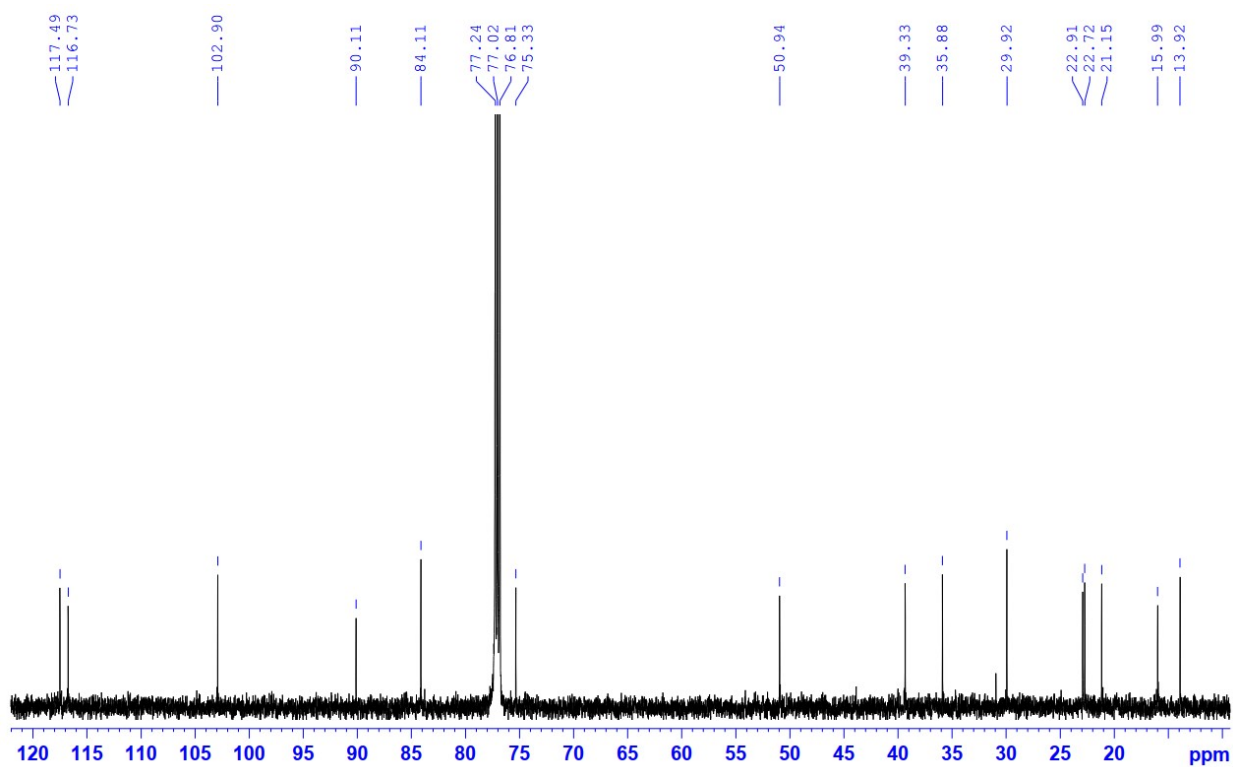


Figure S39. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **4**

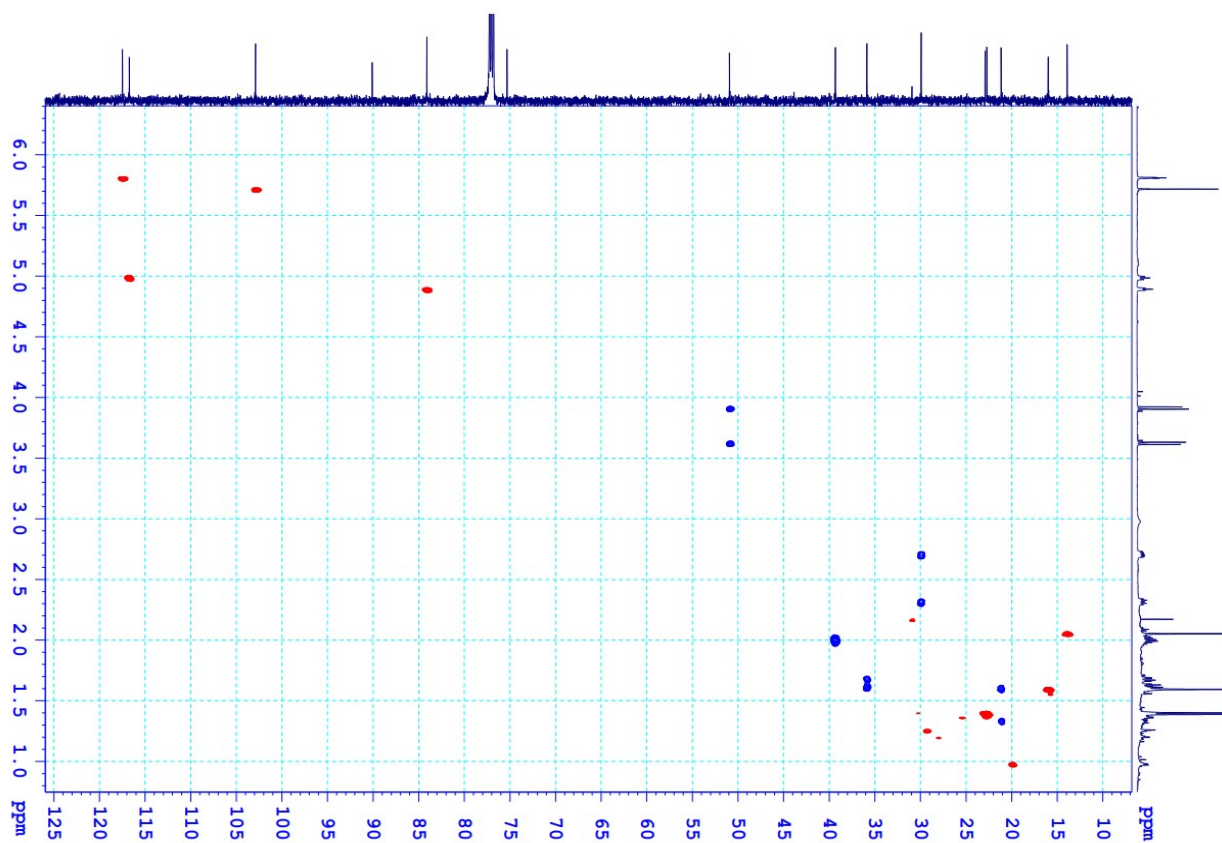


Figure S40. HSQC spectrum of compound 4

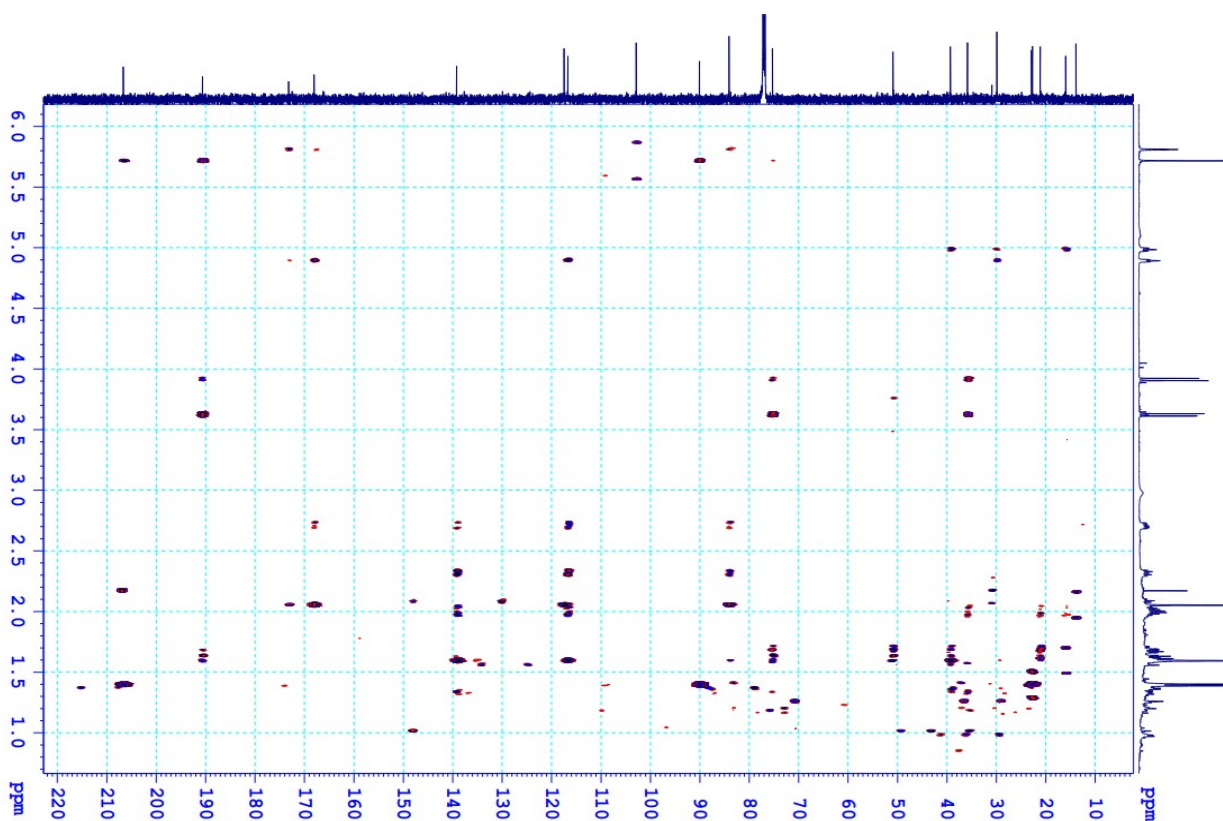


Figure S41. HMBC spectrum of compound 4

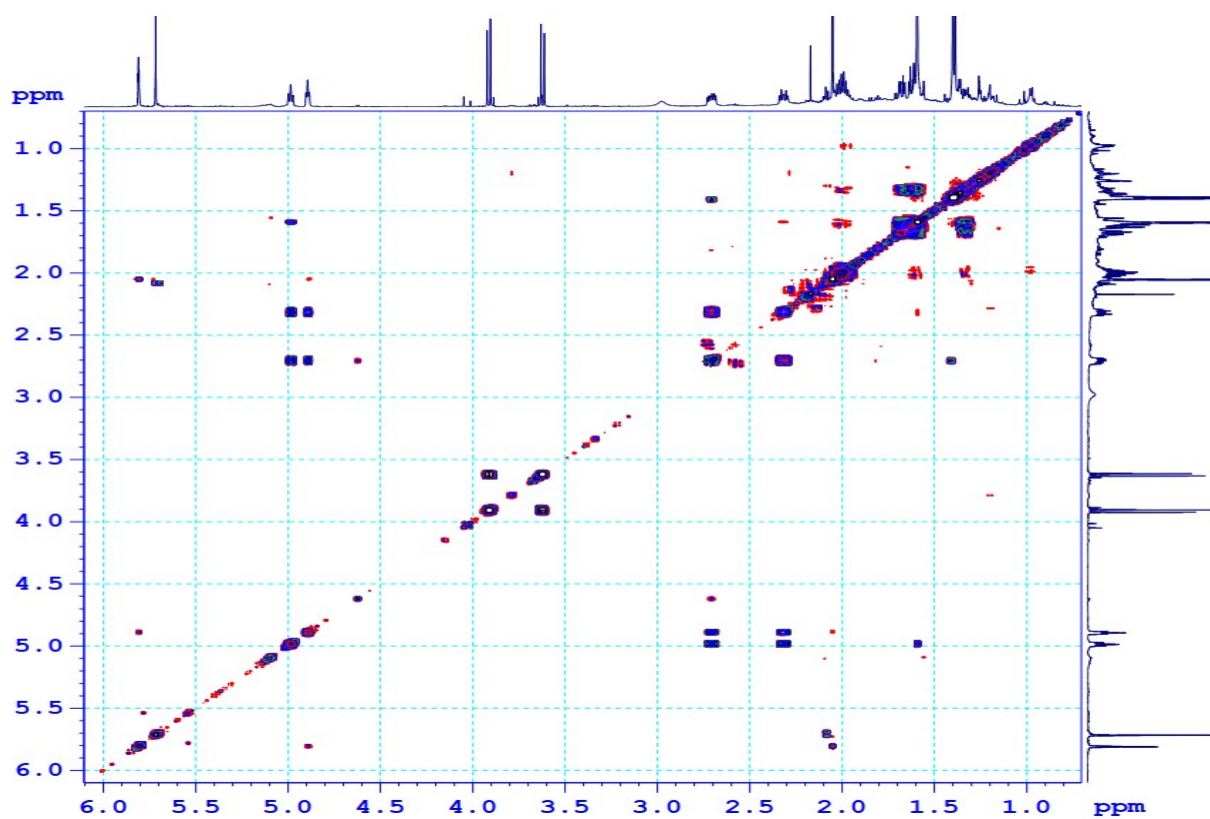


Figure S42. COSY spectrum of compound 4

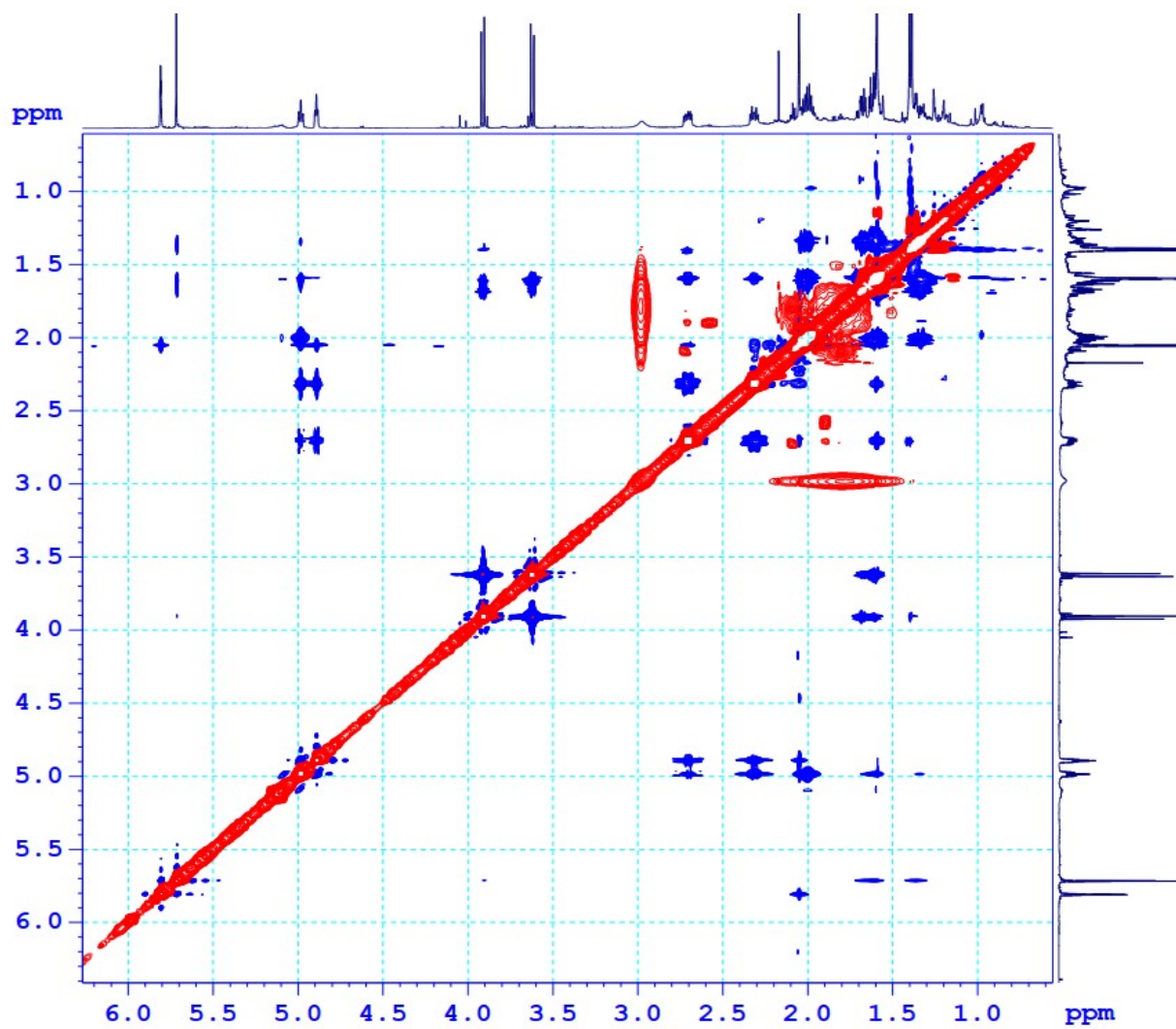


Figure S43. NOESY spectrum of compound 4

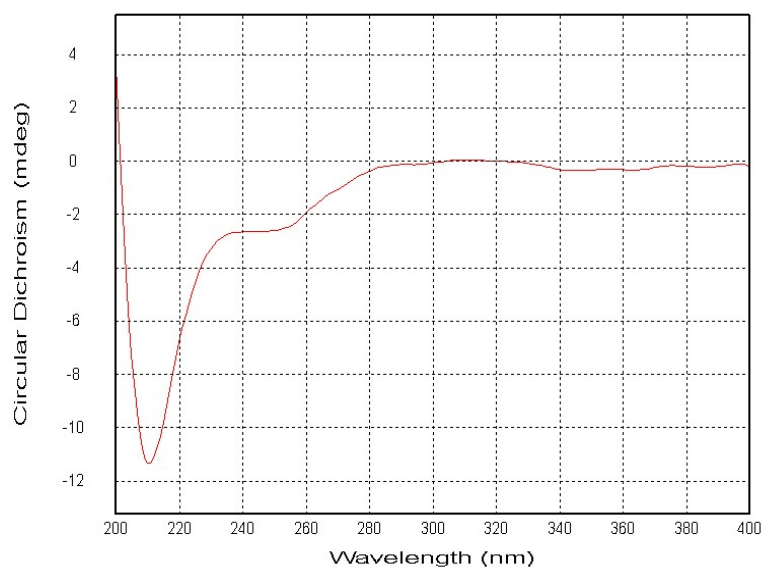


Figure S44. ECD spectrum of compound 4

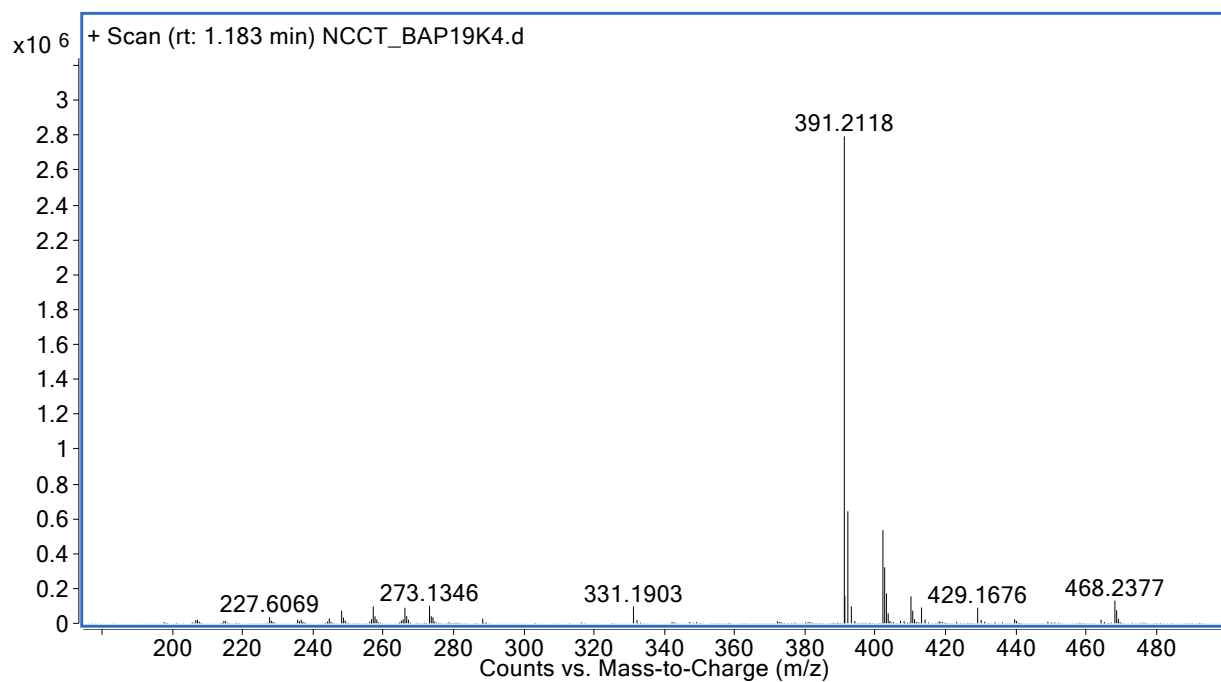


Figure S45. HR-ESI-MS of compound 5

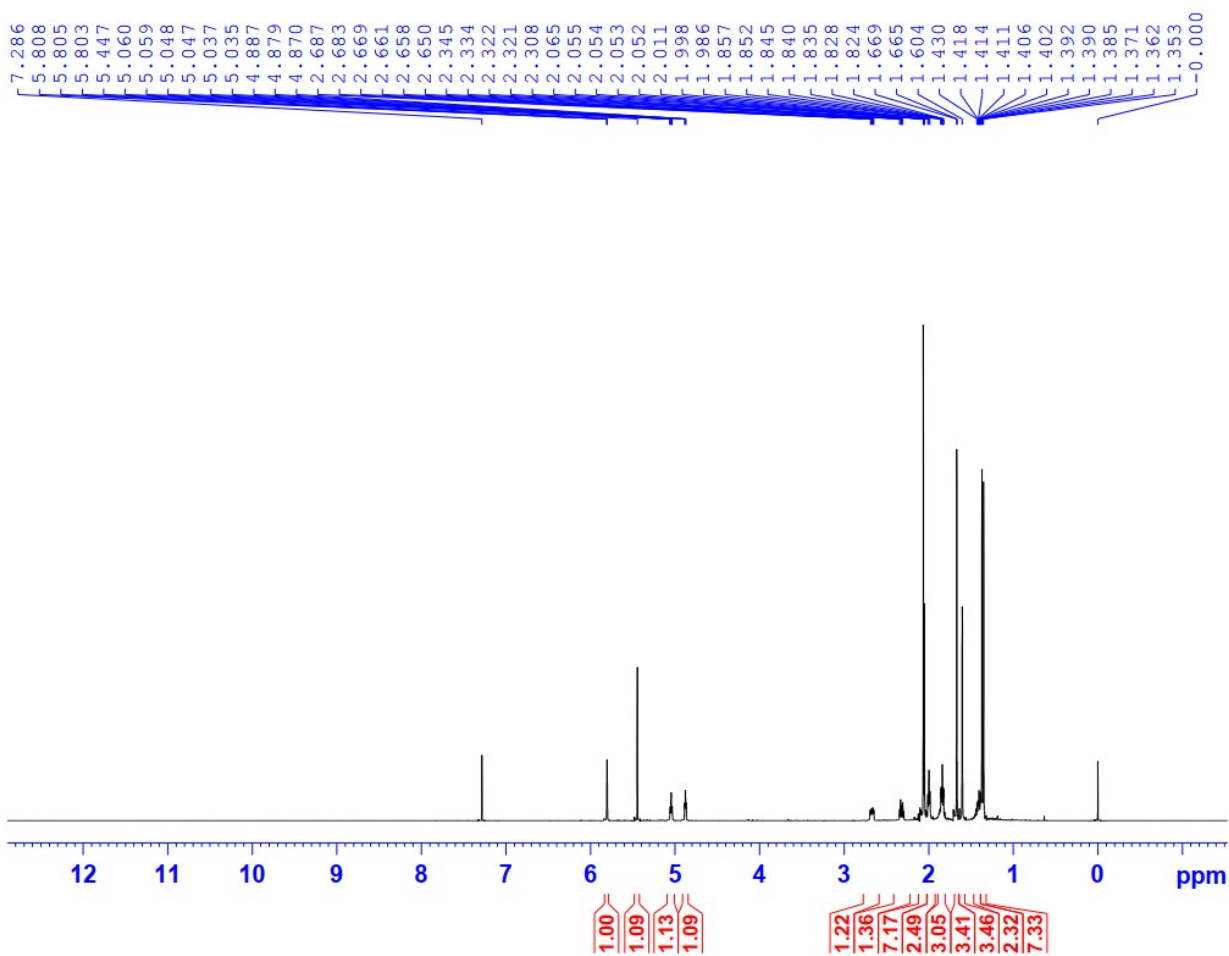


Figure S46.  $^1\text{H-NMR}$  spectrum of compound **5** in  $\text{CDCl}_3$

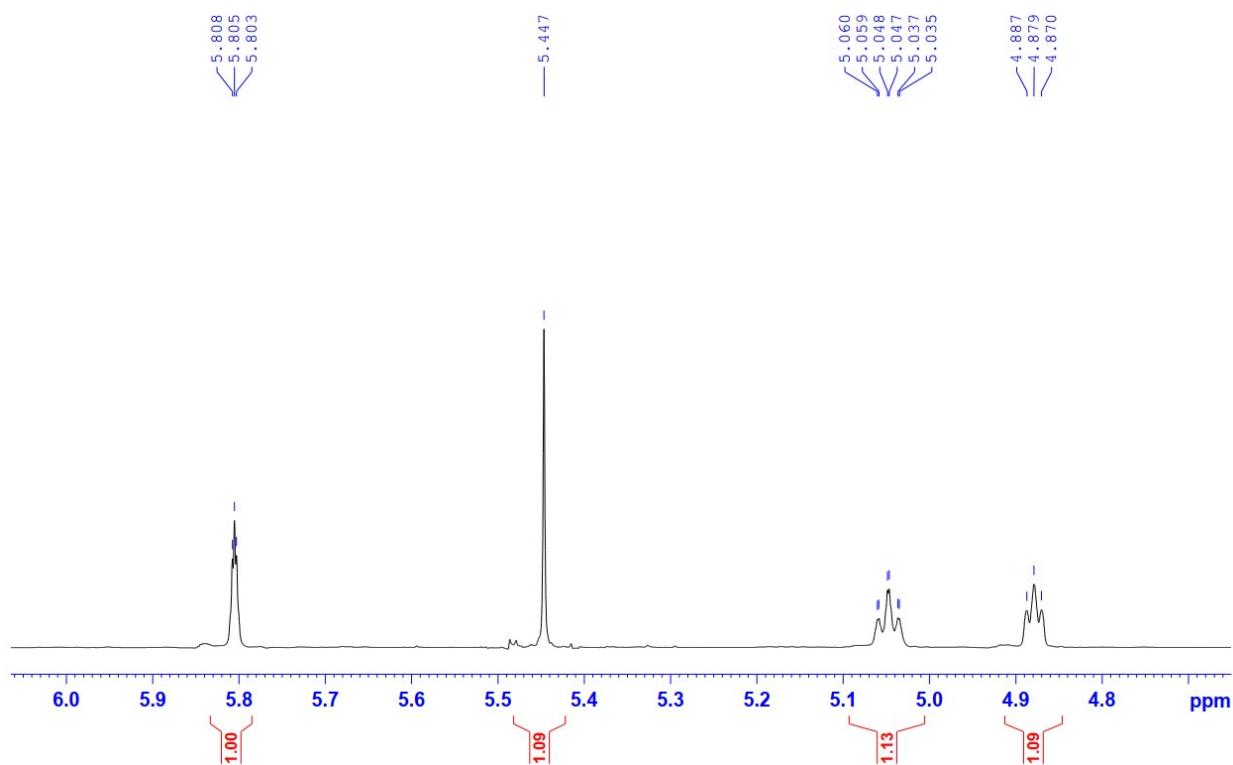


Figure S47. Expanded  $^1\text{H-NMR}$  spectrum of compound **5**

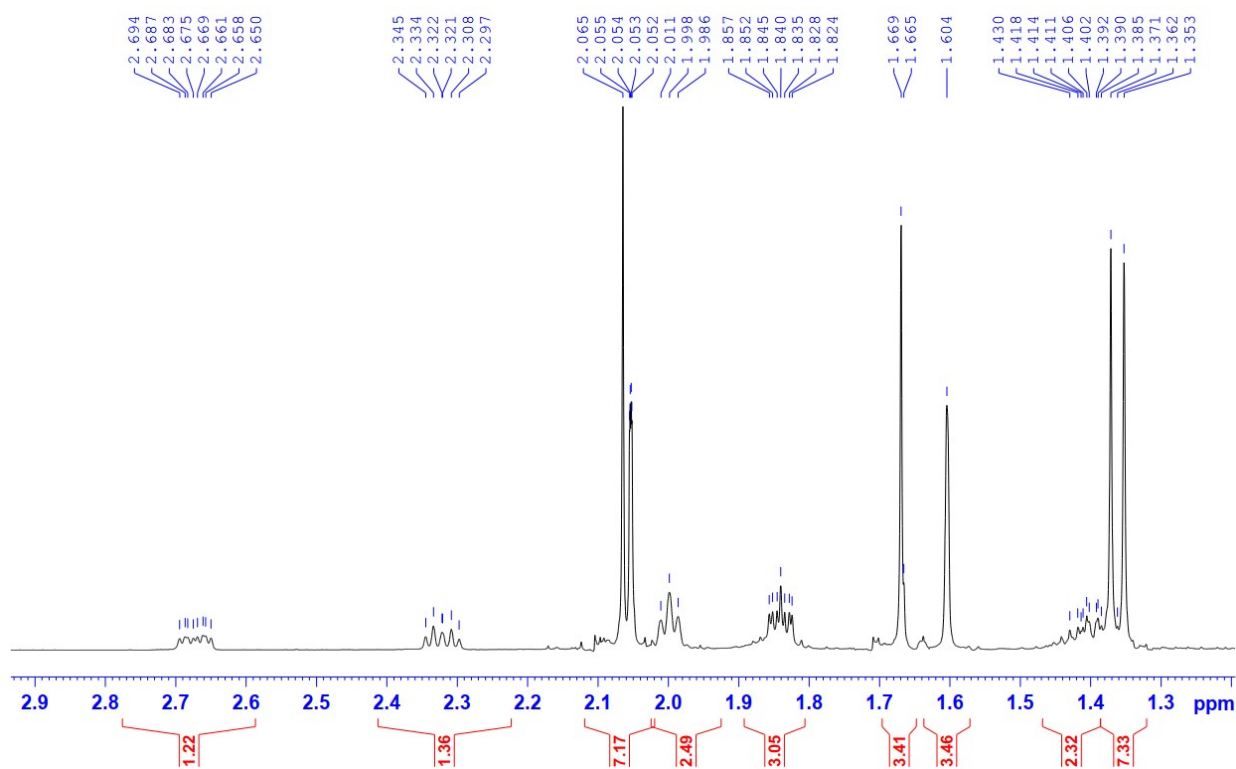


Figure S48. Expanded  $^1\text{H-NMR}$  spectrum of compound **5**

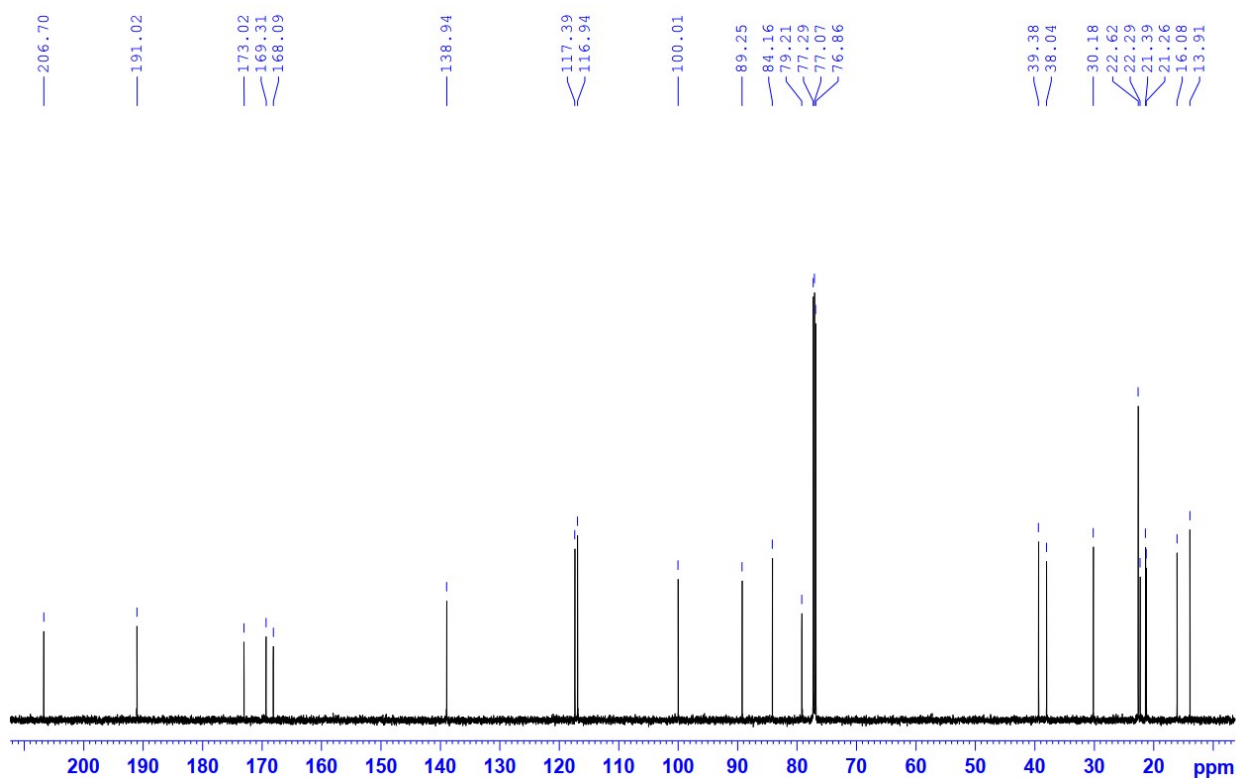


Figure S49.  $^{13}\text{C-NMR}$  spectrum of compound **5** in  $\text{CDCl}_3$

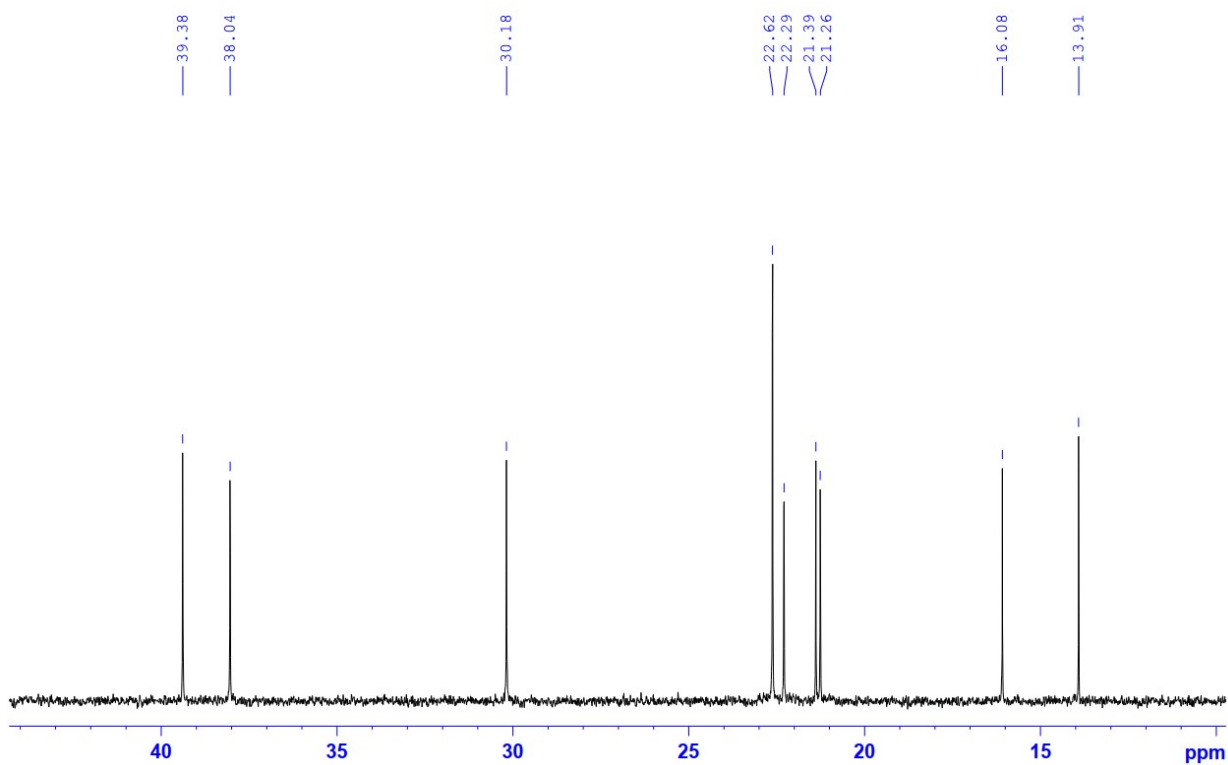


Figure S50. Expanded  $^{13}\text{C}$ -NMR spectrum of compound **5** in  $\text{CDCl}_3$

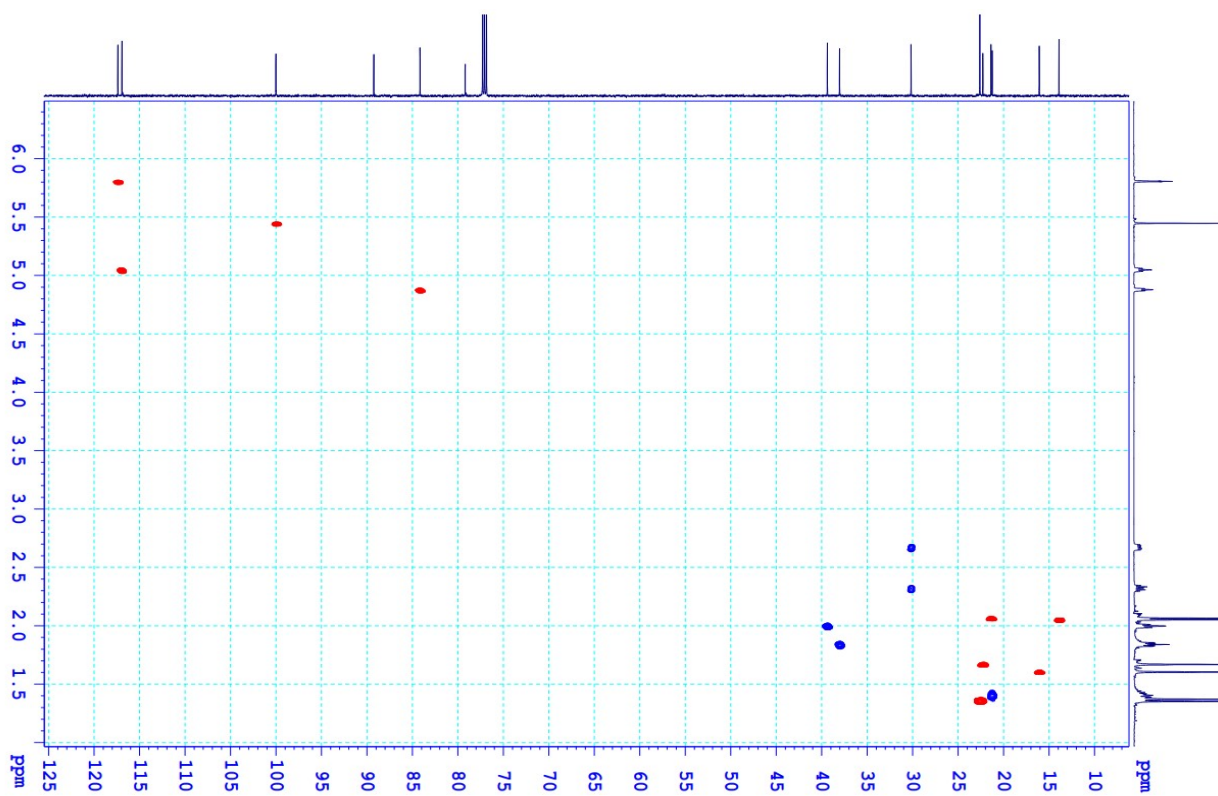


Figure S51. HSQC spectrum of compound **5**

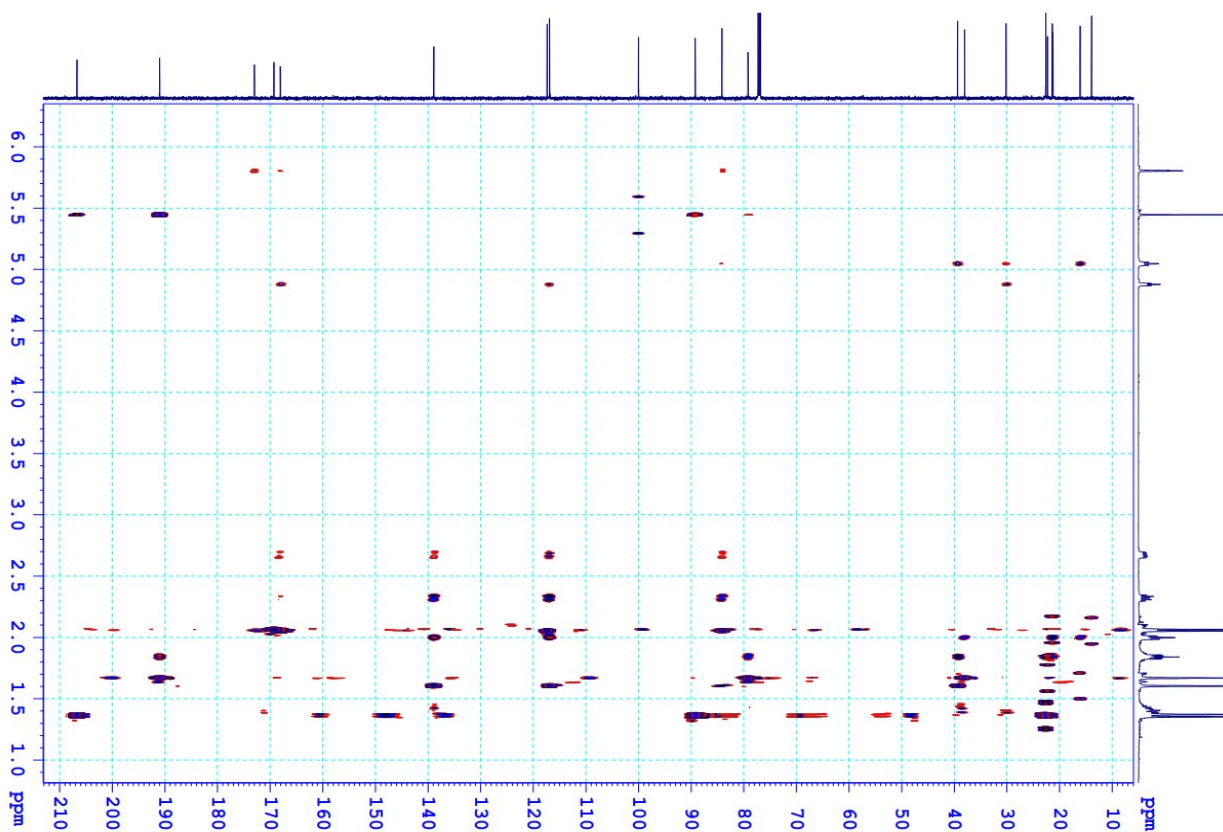


Figure S52. HMBC spectrum of compound 5

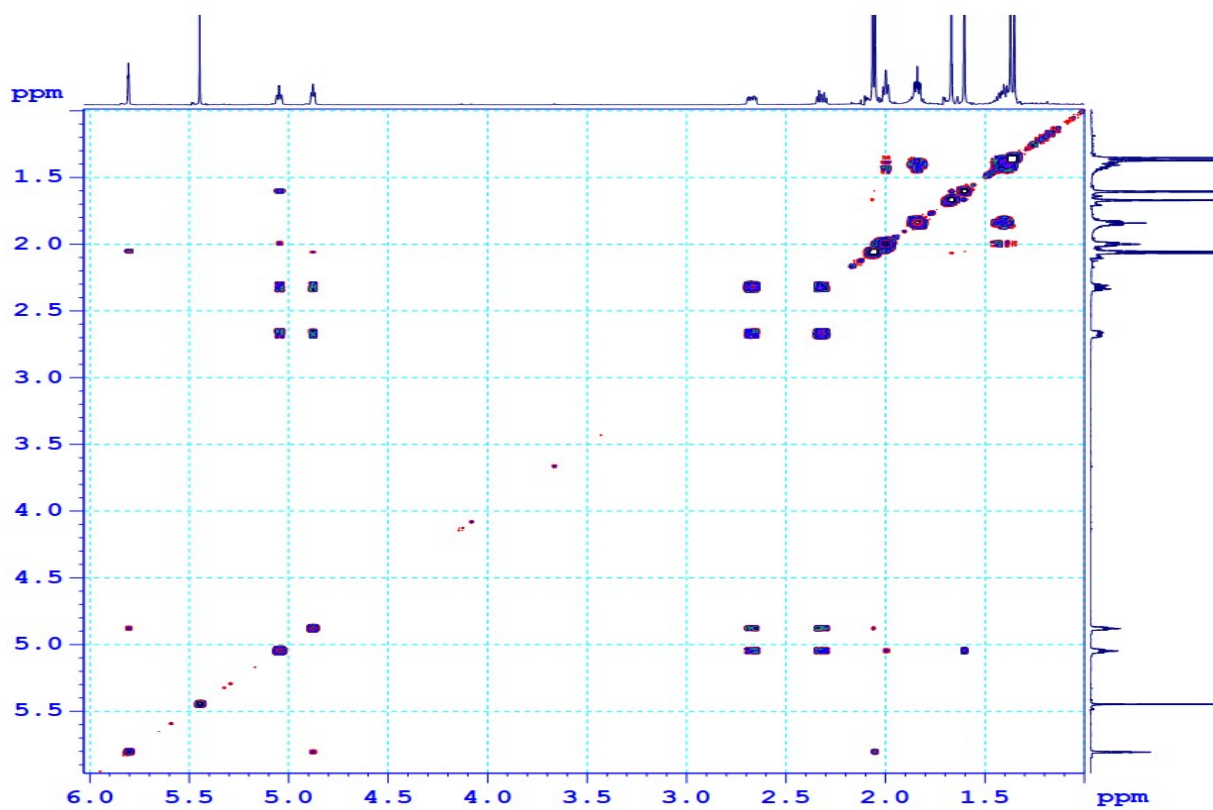


Figure S53. COSY spectrum of compound 5



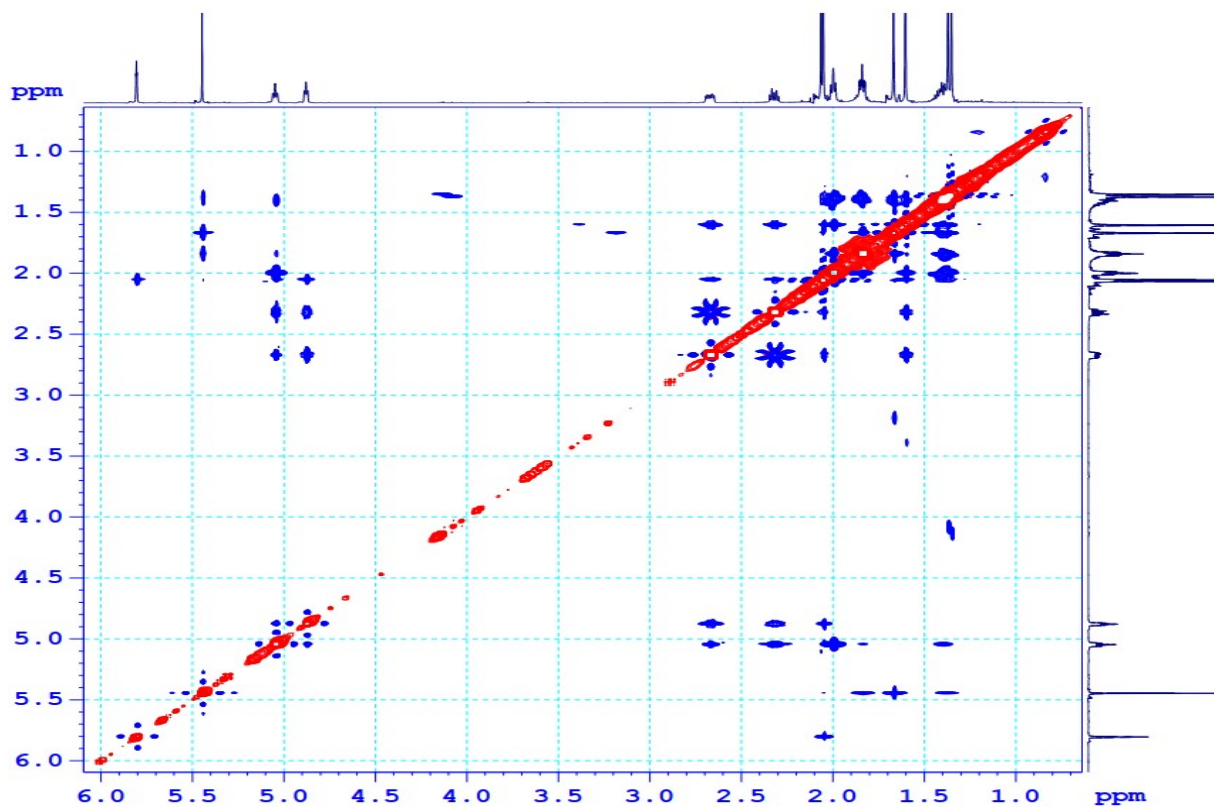


Figure S54. NOESY spectrum of compound 5

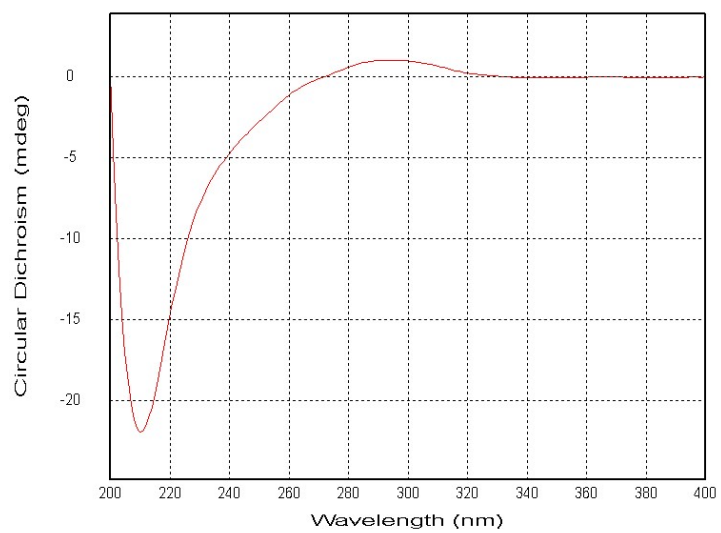


Figure S55. ECD spectrum of compound 5

## Nitric oxide assay

Nitric oxide assay was performed as previously described.<sup>1, 2</sup> In brief, RAW 264.7 cells were cultured in DMEM containing L-glutamine (2 mM), HEPES (10 mM), sodium pyruvate (1 mM), and fetal bovine serum (10%). The cells ( $2 \times 10^5$  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 (0.4-50  $\mu$ M) or vehicle and followed by LPS (1 $\mu$ g/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  $\mu$ L) 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.

## References

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