

Phytochemical investigation and biofilm-inhibitory activity of Bachtari savory (*Satureja bachtiarica* Bunge) aerial parts

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Total Flavonoid Content

To quantify the total flavonoid content, the allumine chloride colorimetric assay was carried out and rutin was used as a reference. Following that, a 10-milliliter volumetric flask was filled with 1 mL of a sample with a concentration of 1 mg/mL and 4 mL of water. Following this, 0.3 mL of 5% NaNO₂ and 0.3 mL of 10% AlCl₃ were both added to the flask for 5 minutes. After 6 minutes, 2 mL of 1 M NaOH and the final 10 mL of water were added to the solution. After thoroughly combining the solutions, a UV-visible spectrophotometer was used to detect the absorbance at 510 nm in comparison to a blank control. The amount of flavonoids in the extract was expressed in rutin equivalents (RE), according to equation: $(A_e \times m_0 \times 10) / (A_0 \times m_e)$.

In the equation, A_e represents the average of the absorbance of the extract in triplicate, A_0 represents the average of the absorbance of the standard rutin solution in triplicate, m_e represents the weight of the plant extract that was analyzed in g, and m_0 represents the weight of the rutin in the solution in g. Flavonoid amount was expressed in mg/g plant extract in RE.

DPPH• Radical Scavenging Activity

For DPPH, the percentage of DPPH• radical scavenging activity (%) was plotted against the extract concentration (μg/mL) to determine the IC₅₀. In brief, a stock solution (1 mg/mL) of the extract was used in the range of 50–200 μg/mL, and an aliquot (5.0 μL) of the methanol solution containing different amounts of the extract was added to 195 μL of daily prepared DPPH• solution. Absorbance at 517 nm was measured on a UV-visible spectrophotometer (Multiskans skyhigh, Thermo Fisher Scientific, Milan, Italy) 10 min after starting the reaction.

All the experiments were performed in triplicate. Vitamin C provided as a positive control.

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SBL4-14 #170-188 RT: 1.58-1.62 AV: 7 NL: 5.46E7
T: FTMS - p ESI Full ms [190.00-1000.00]

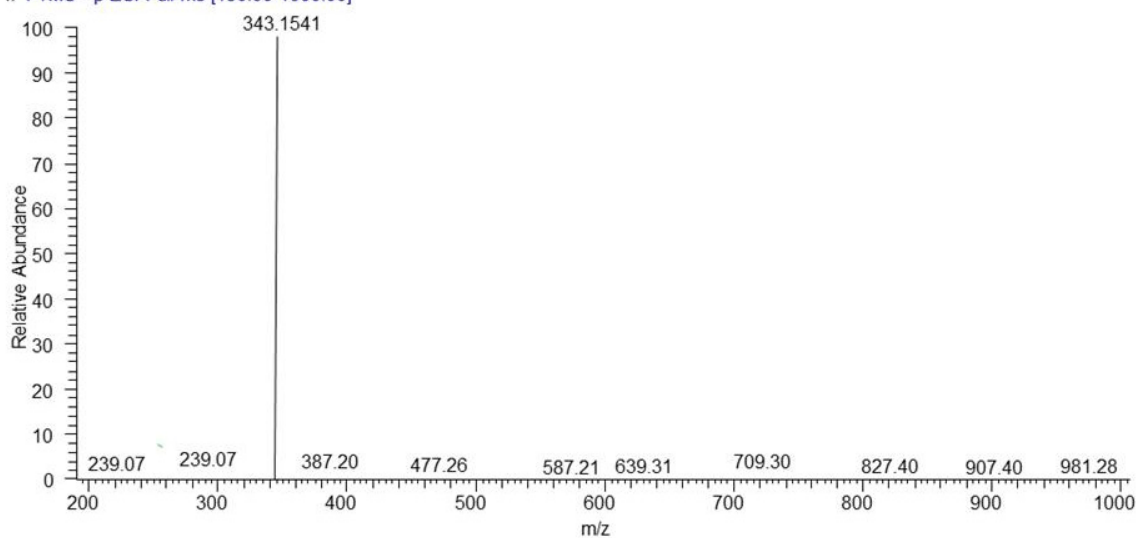


Figure S1. ESI/LTQOrbitrap spectrum of compound **37**, in negative ion mode.

SBL4-14 #106 RT: 0.93 AV: 1 SM: 7G NL: 1.42E7
F: FTMS - p ESI Full ms2 343.10@cid30.00 [90.00-1000.00]

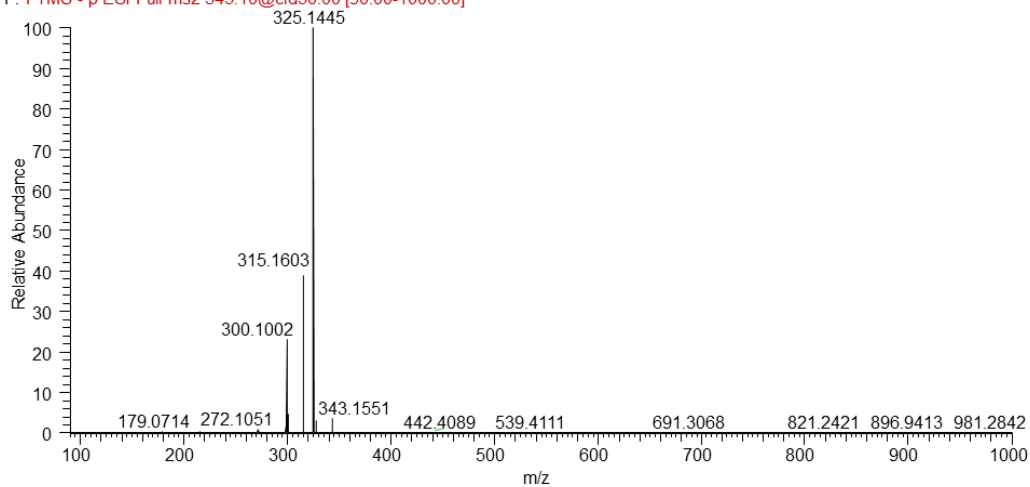


Figure S2. MS/MS spectrum of compound **37**

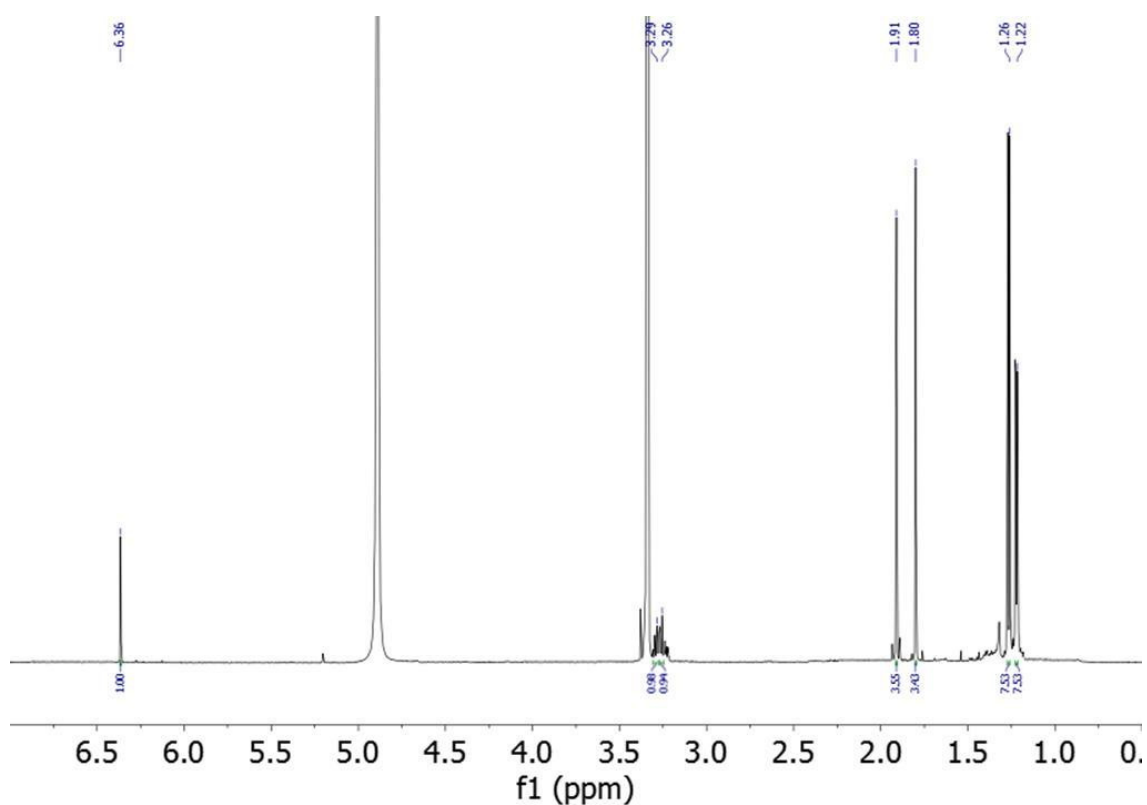


Figure S3. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **37**.

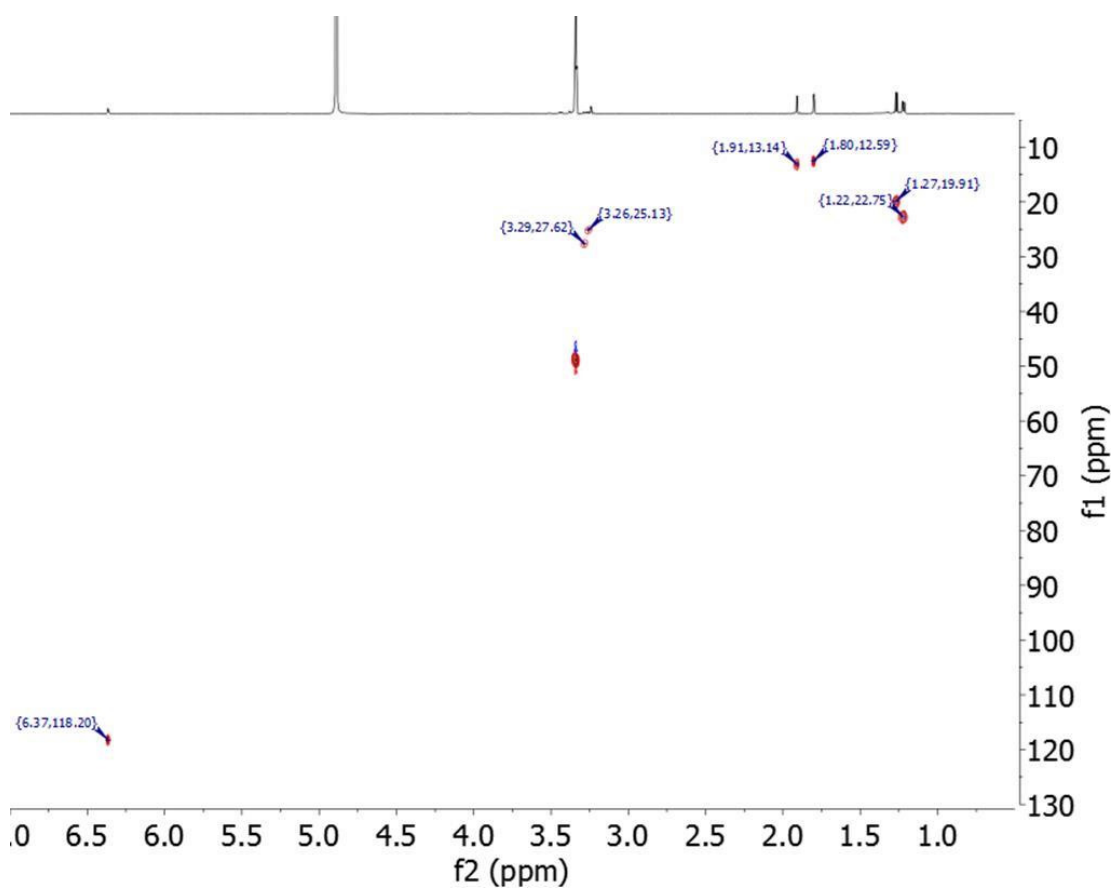


Figure S4. HSQC Spectrum (CD₃OD) of compound **37**.

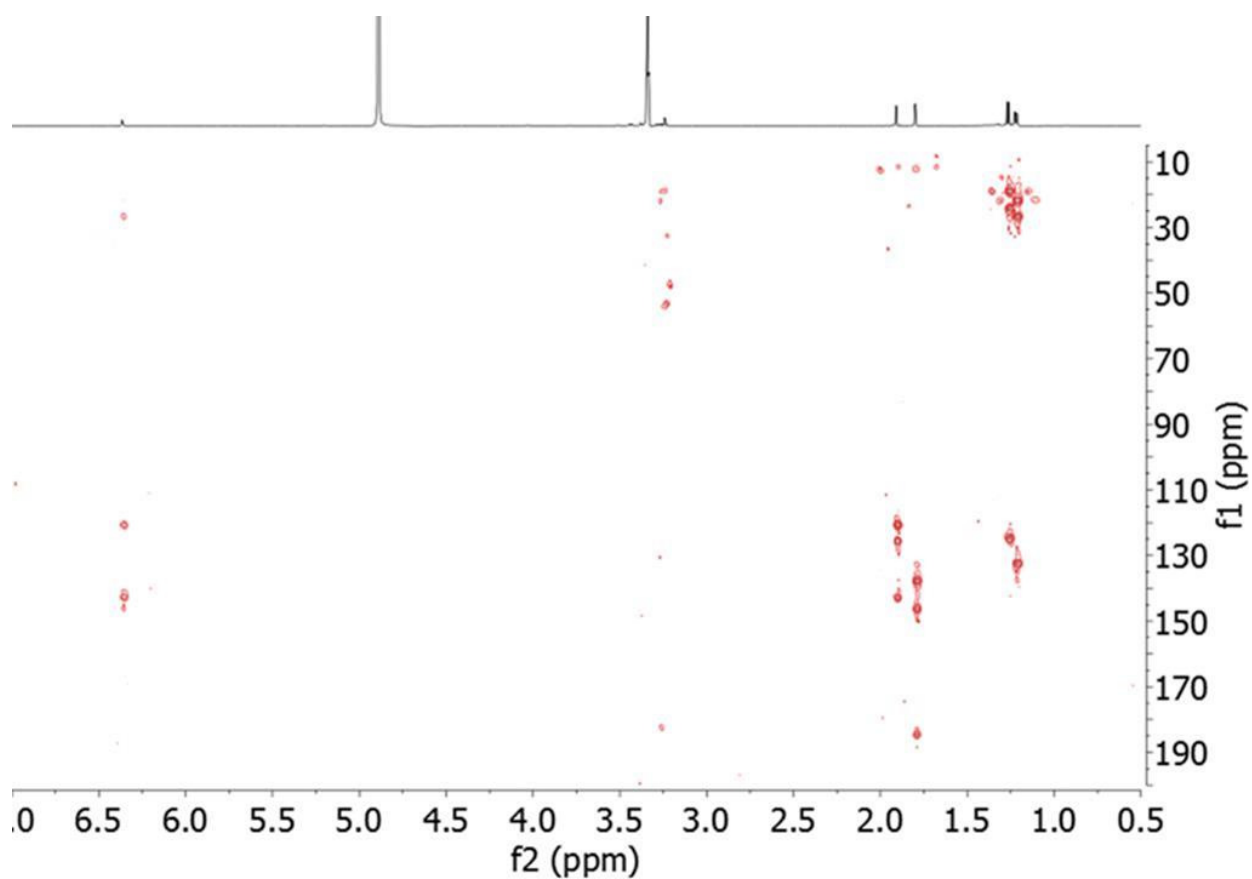


Figure S5. HMBC Spectrum (CD₃OD) of compound **37**.

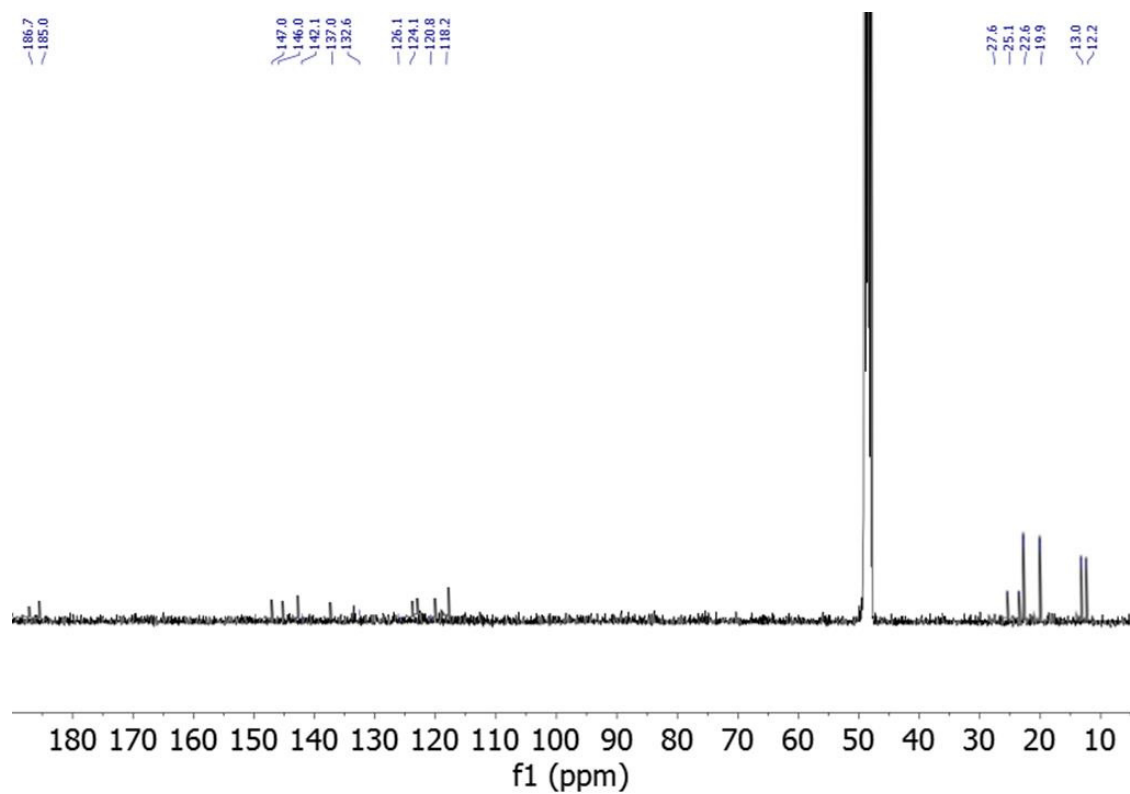


Figure S6. ¹³C NMR Spectrum (150 MHz, CD₃OD) of compound **37**.

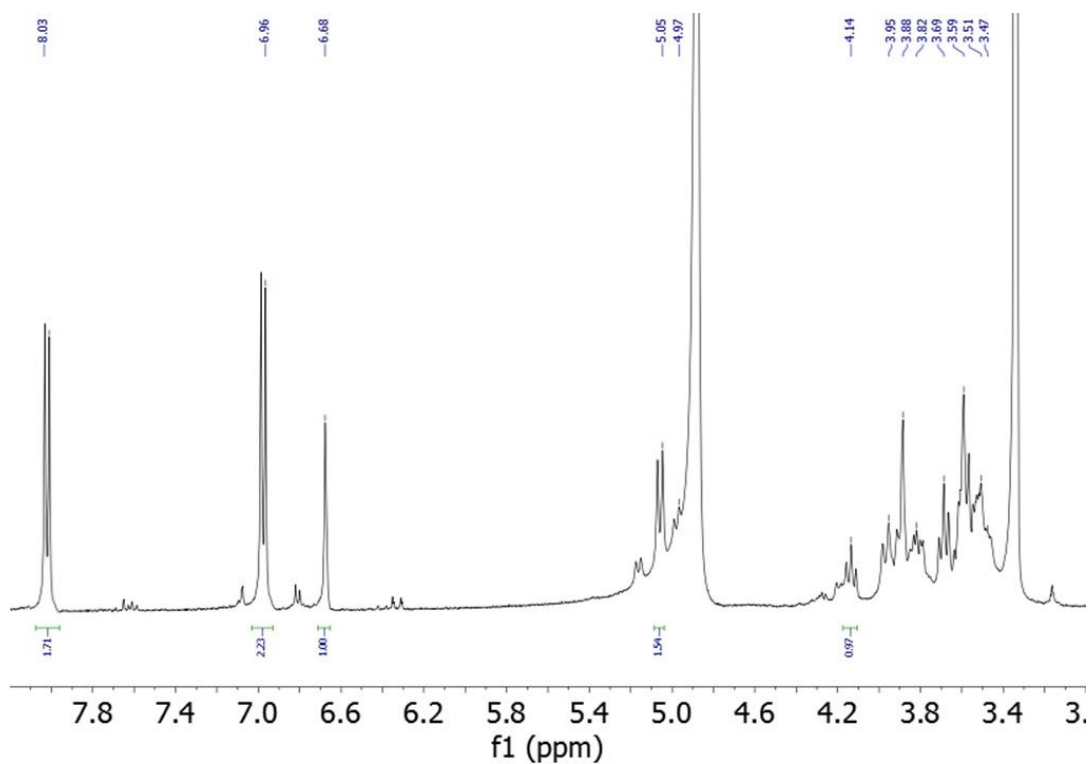


Figure S7. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **1**.

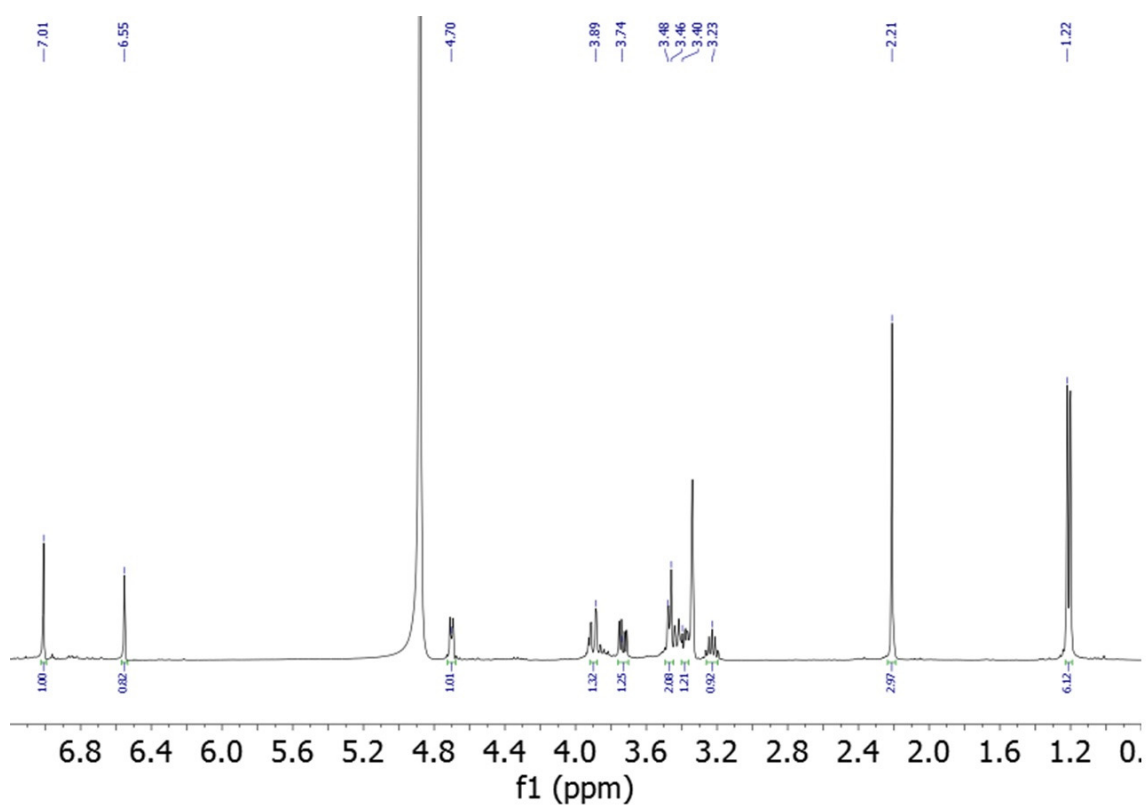


Figure S8. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **2**.

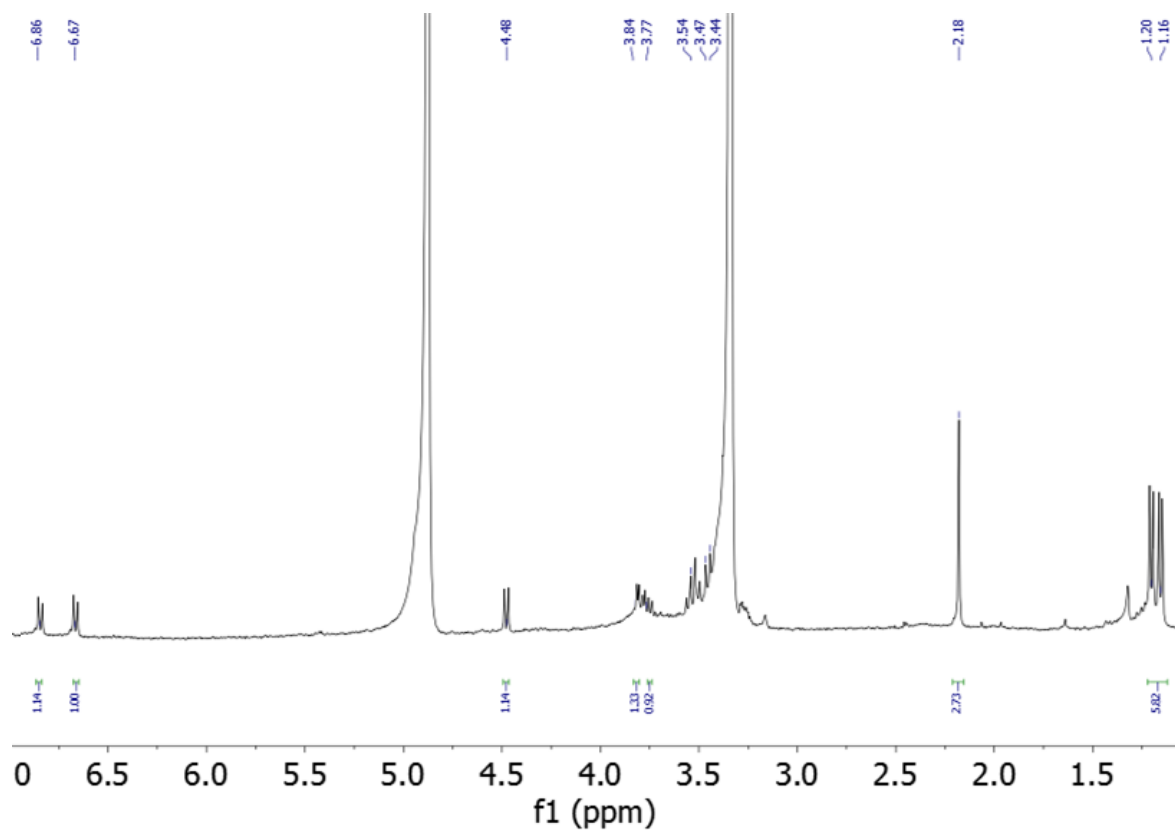


Figure S9. ^1H NMR Spectrum (600 MHz, CD_3OD) of compound **3**.

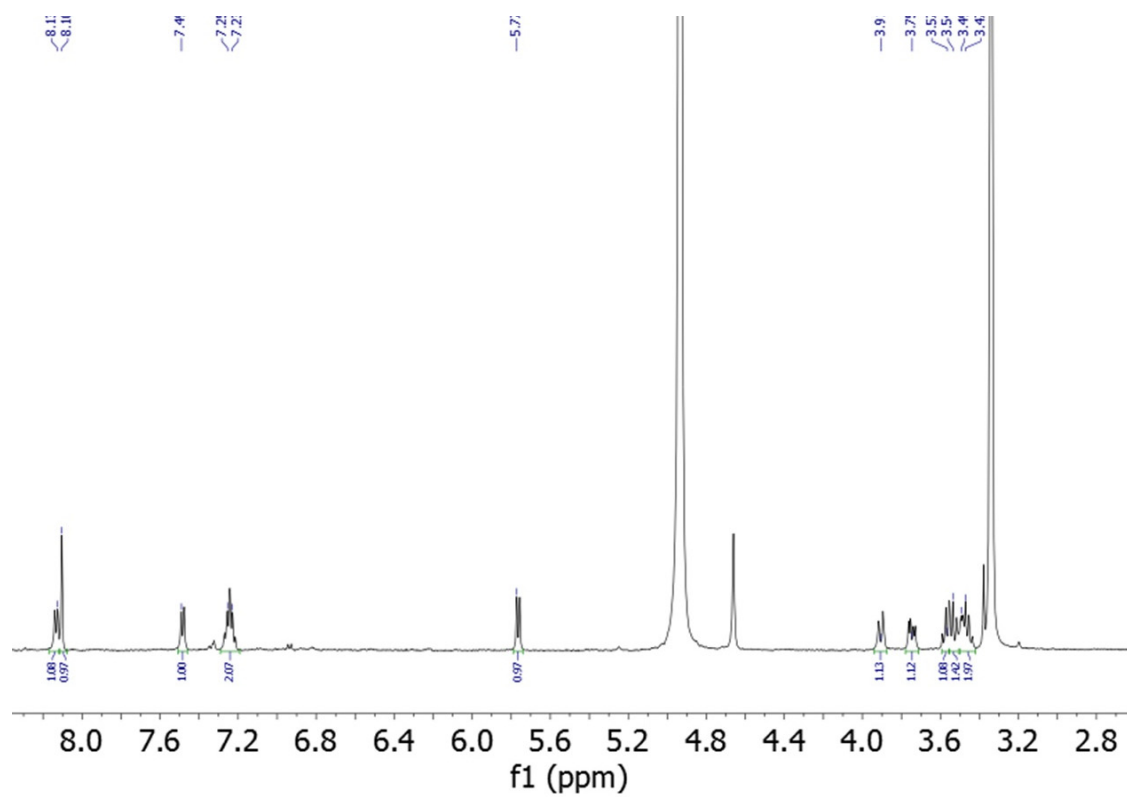


Figure S10. ^1H NMR Spectrum (600 MHz, CD_3OD) of compound **4**.

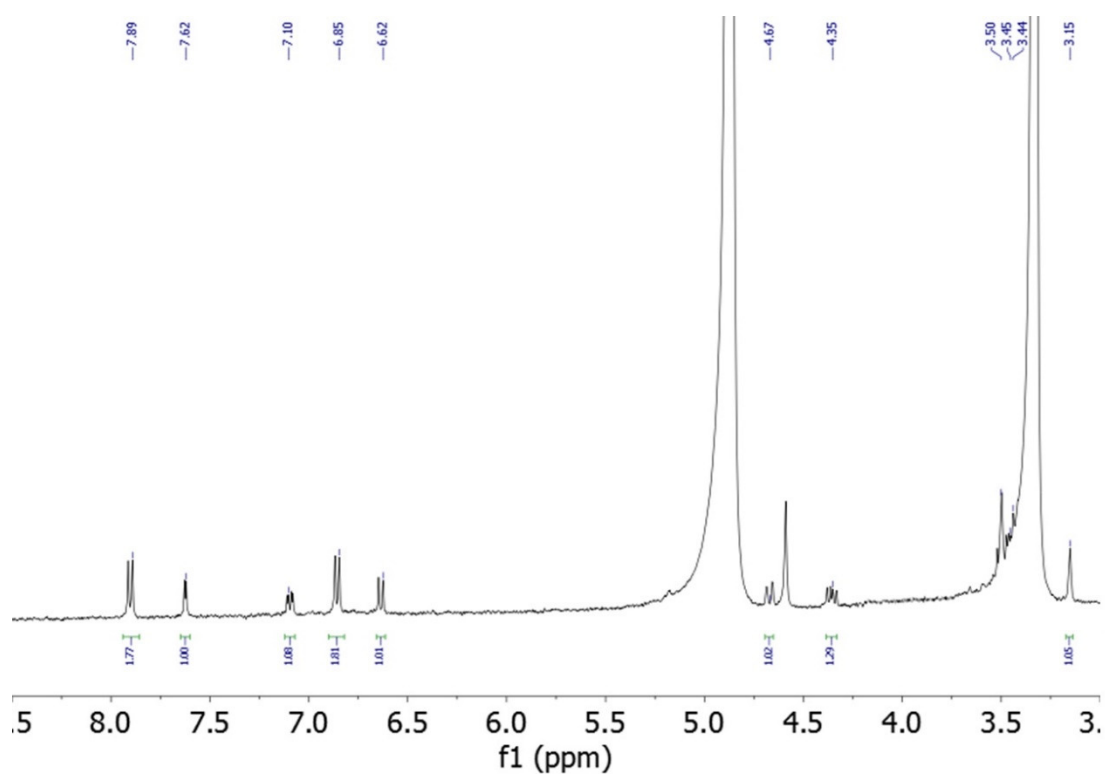


Figure S11. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **5**.

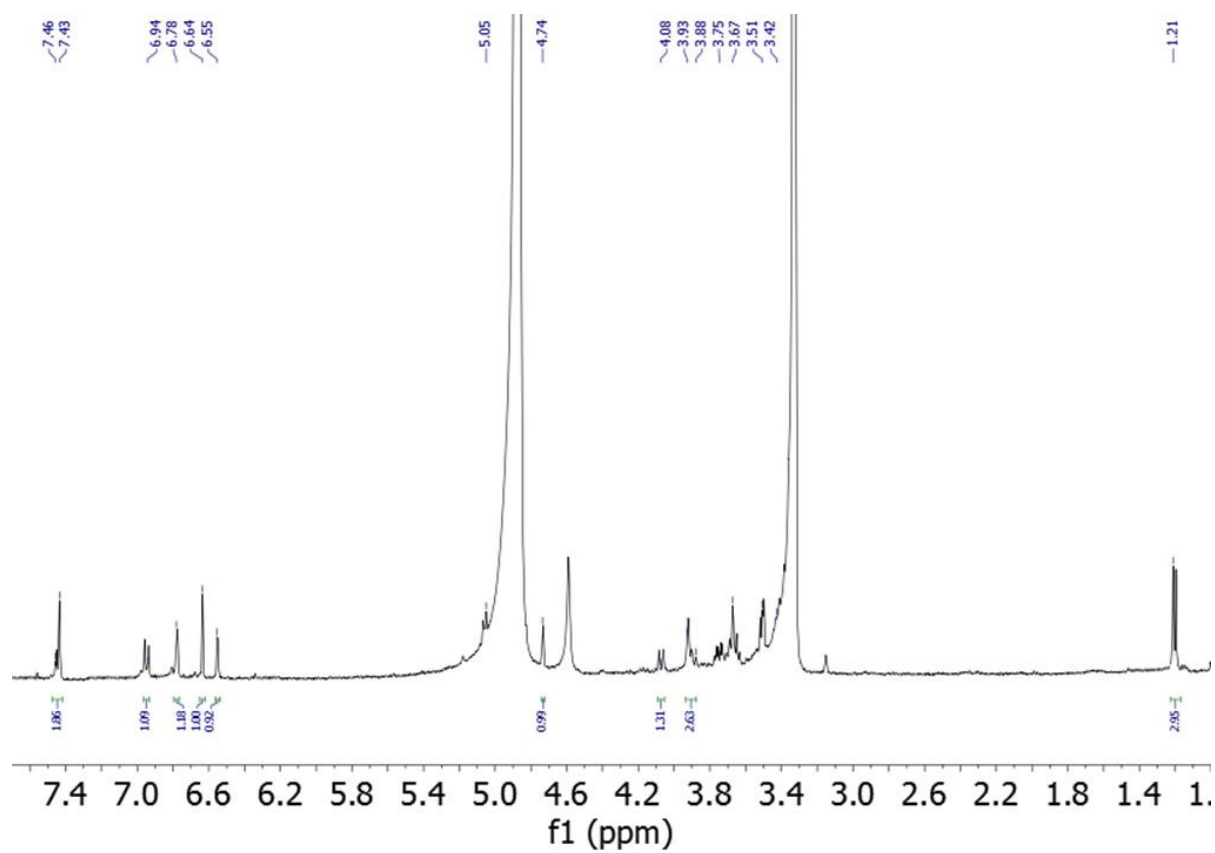


Figure S12. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **6**.

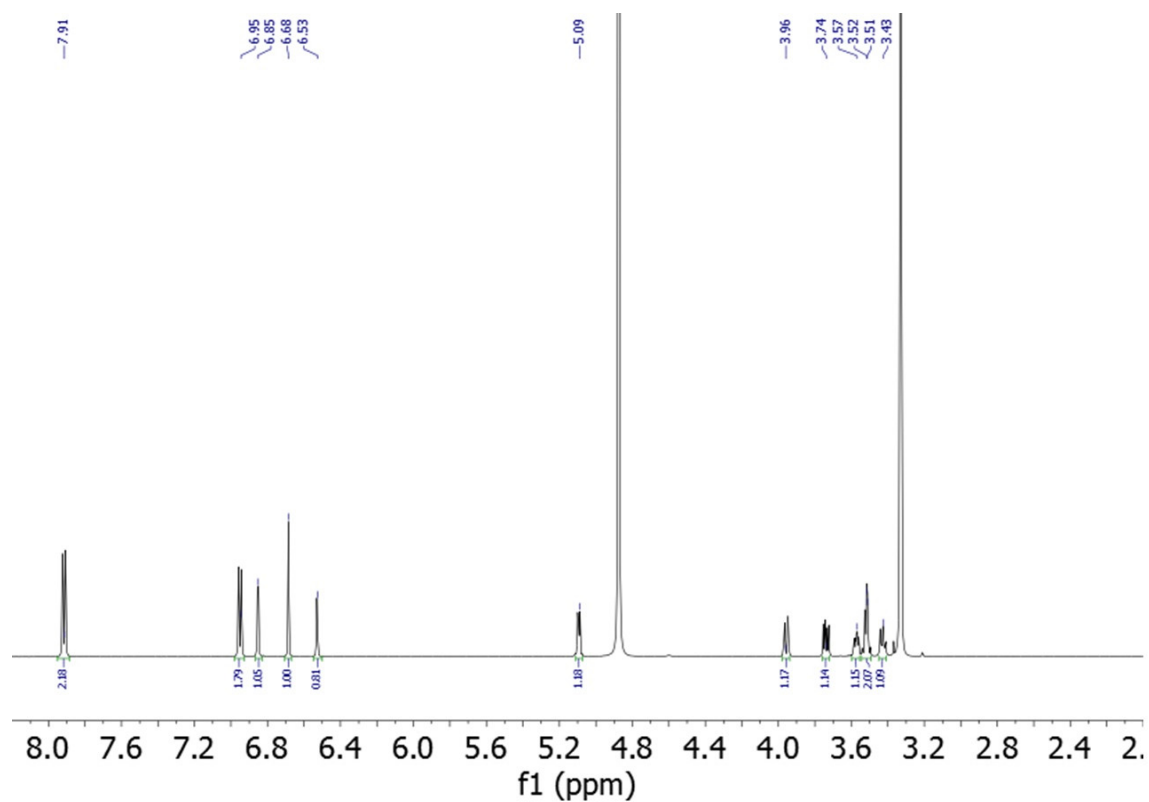


Figure S13. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound 7.

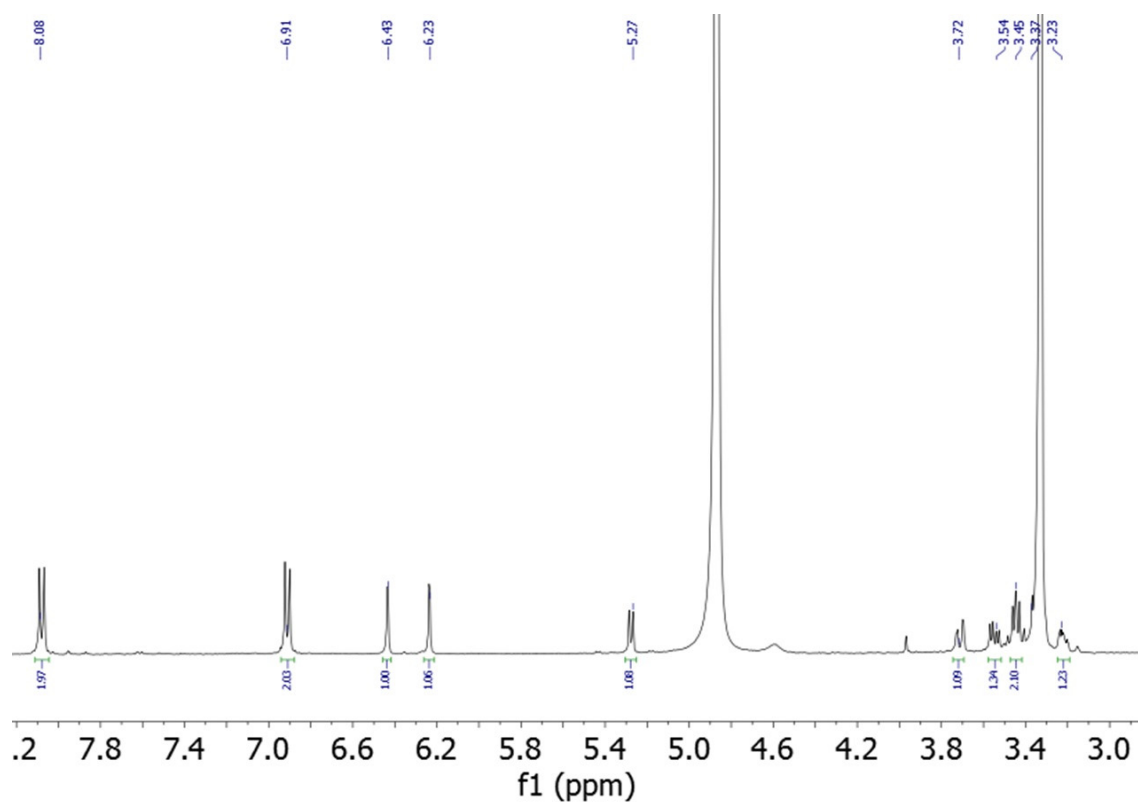


Figure S14. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound 8.

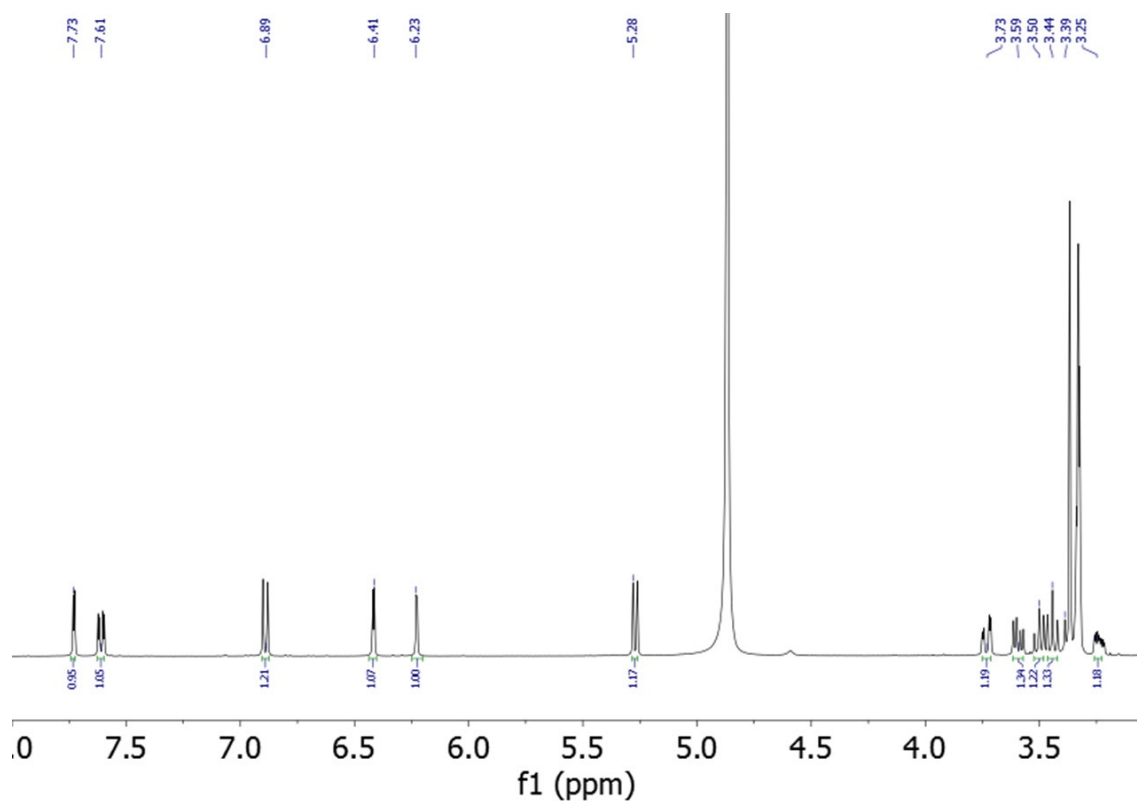


Figure S15. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **9**.

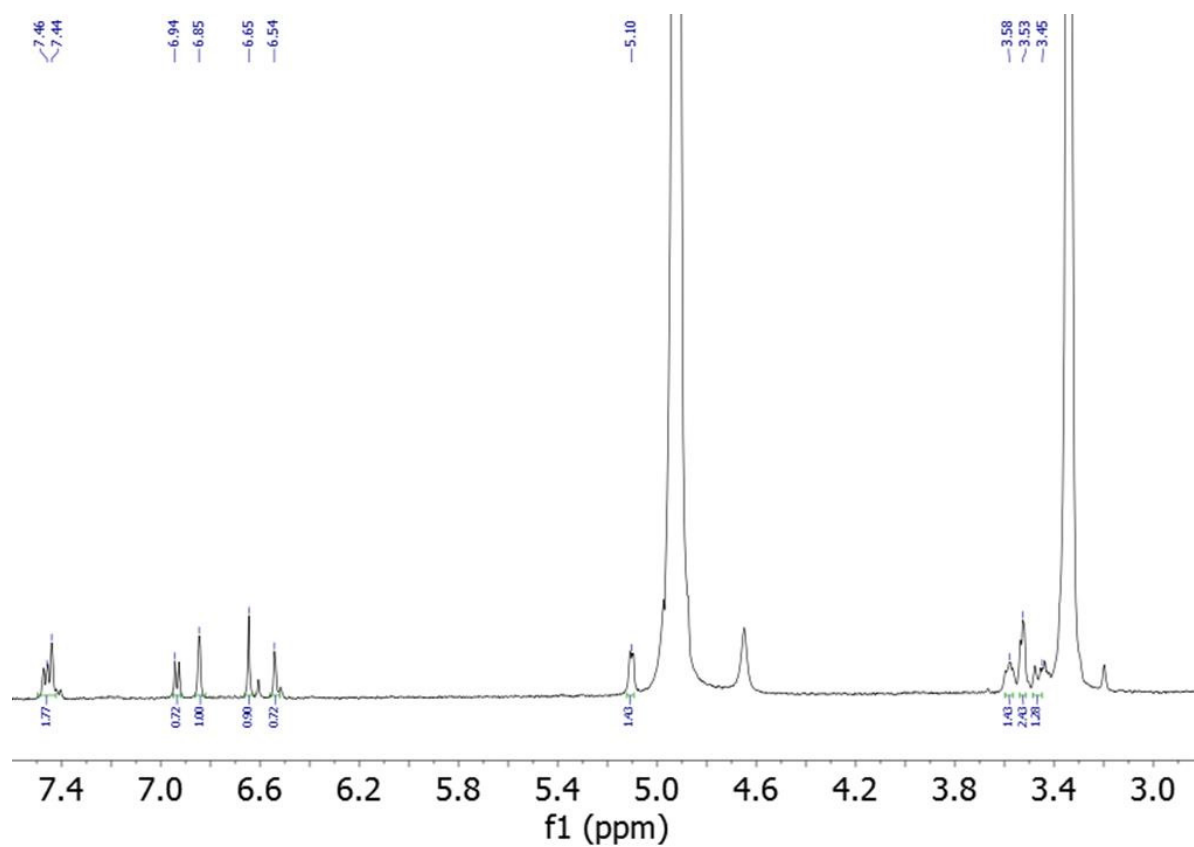


Figure S16. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **10**.

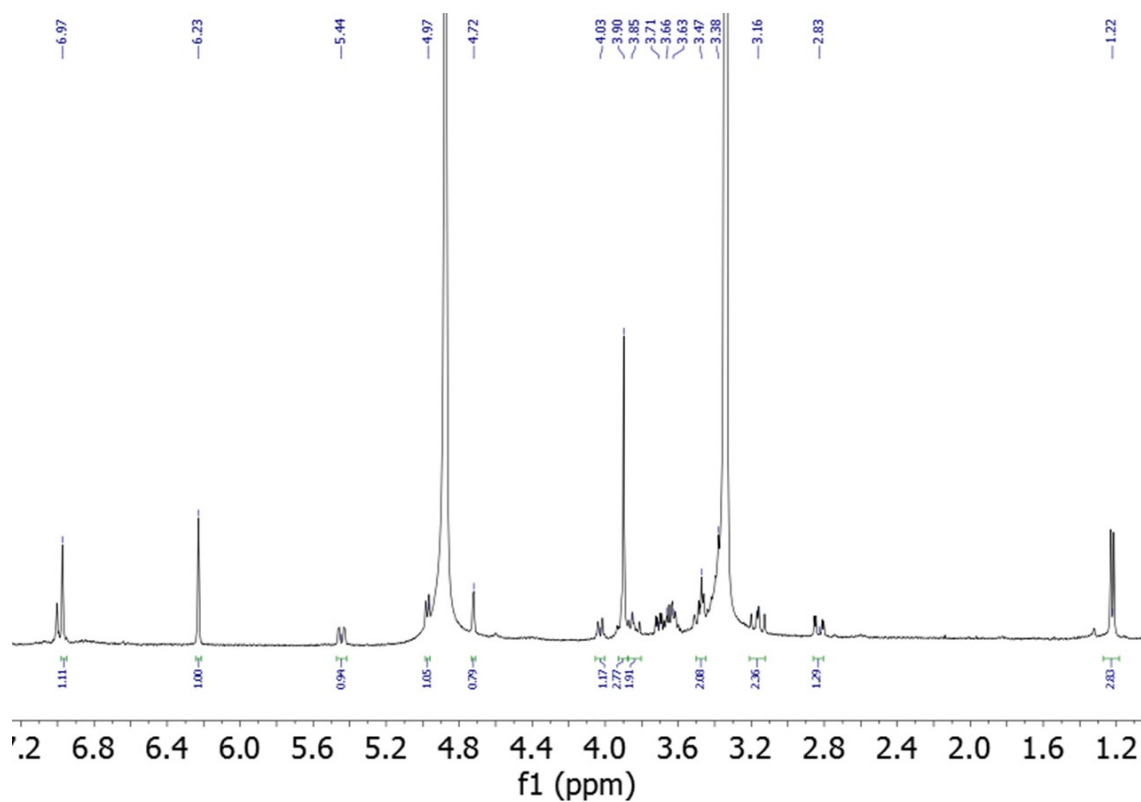


Figure S17. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound 12.

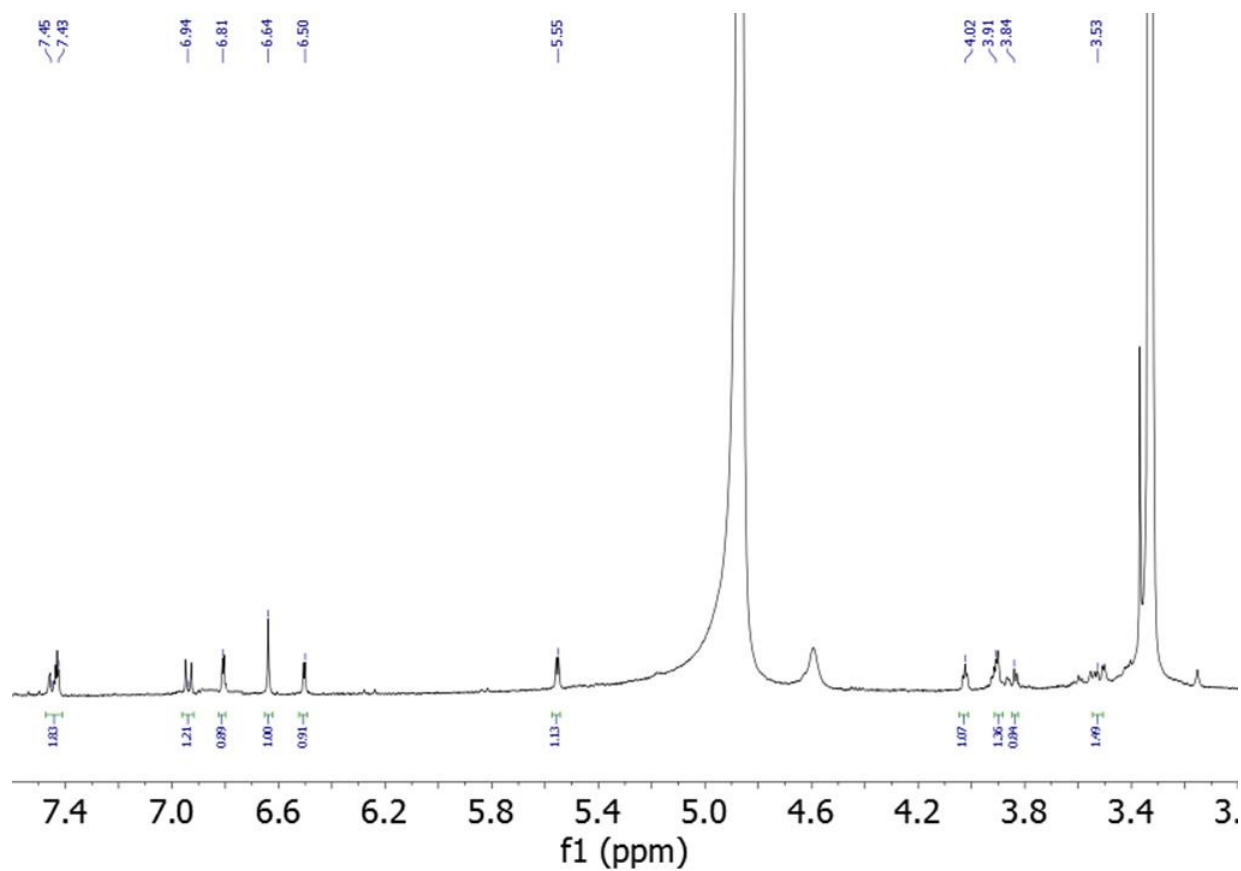


Figure S18. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound 13.

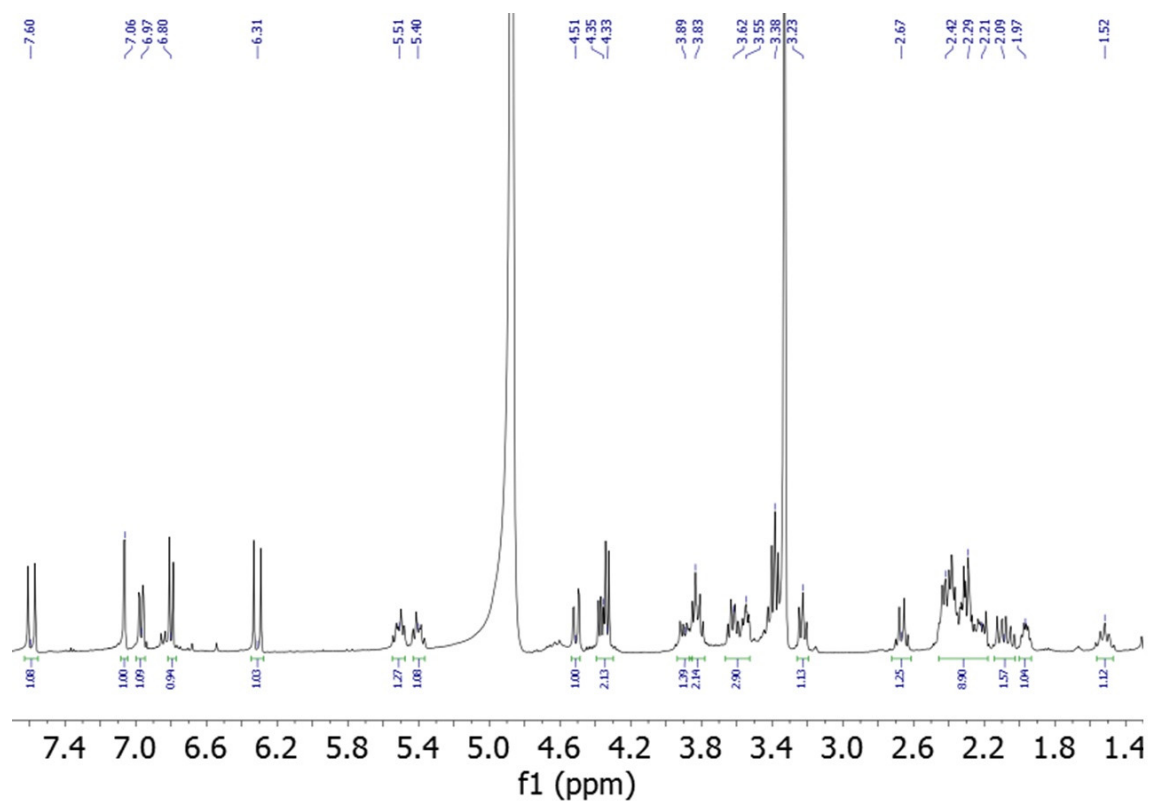


Figure S19. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **15**.

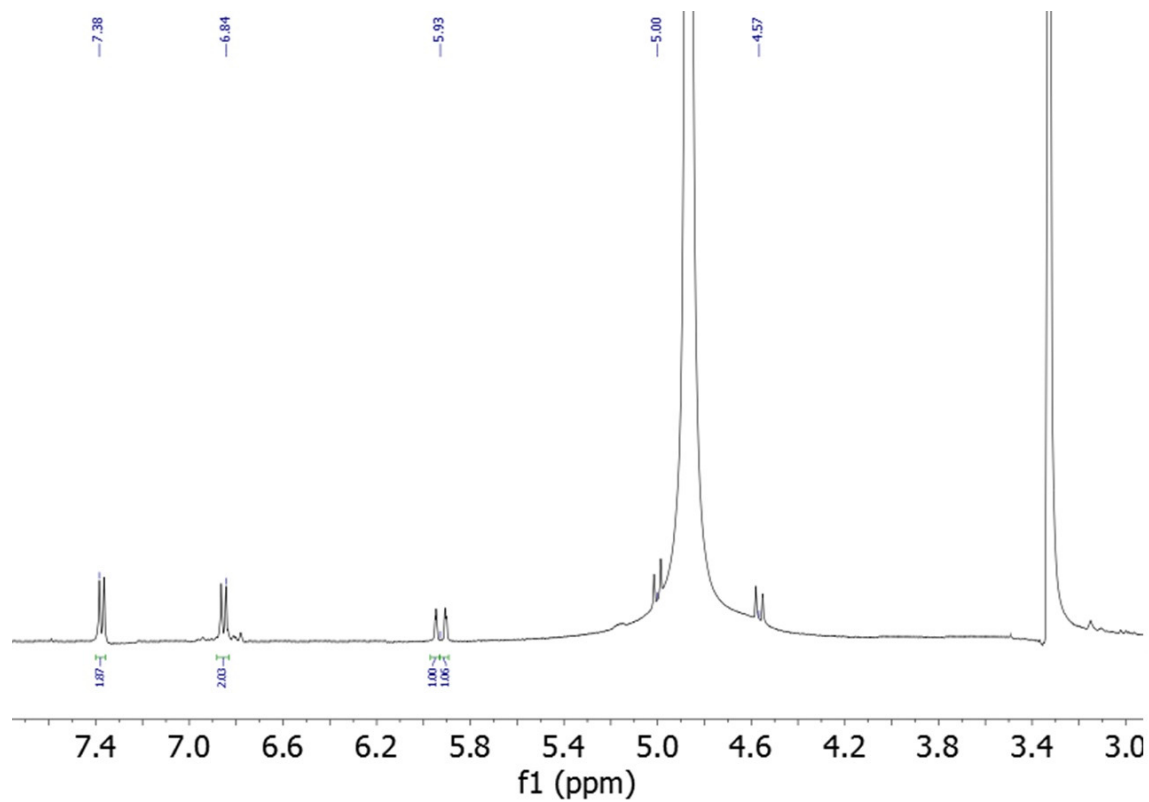


Figure S20. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **19**.

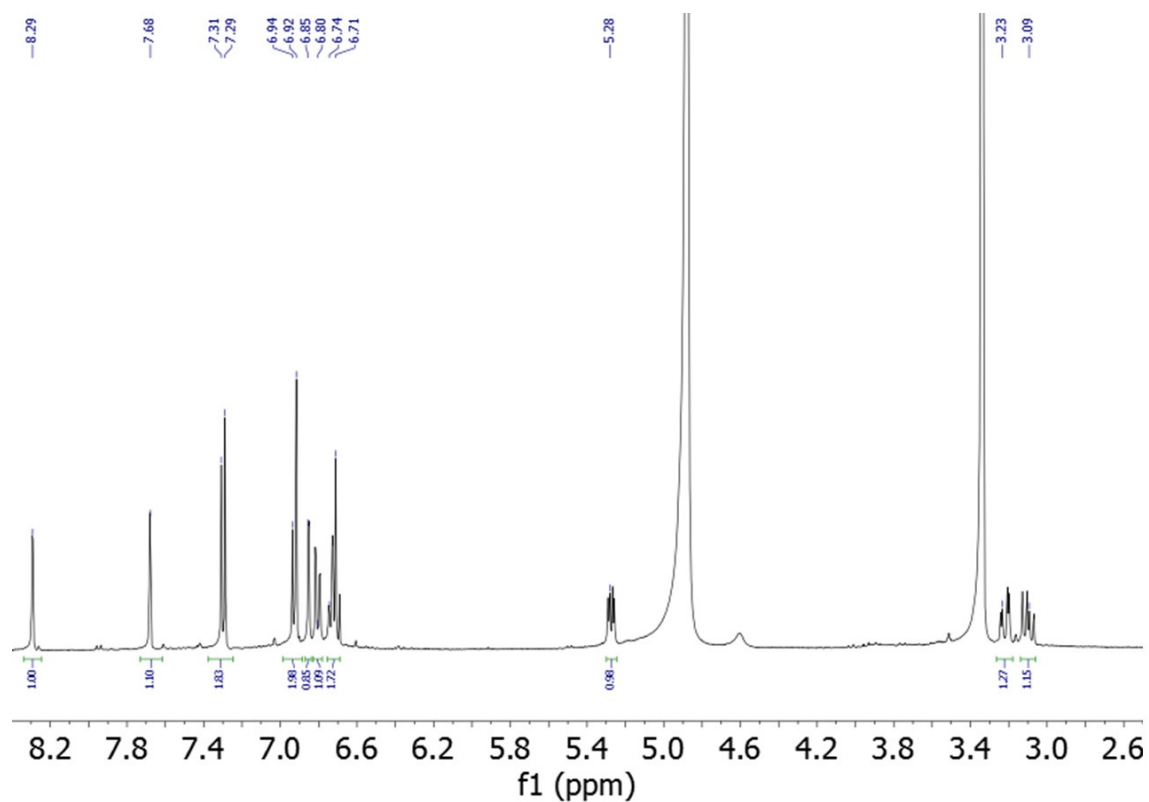


Figure S21. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **21**.

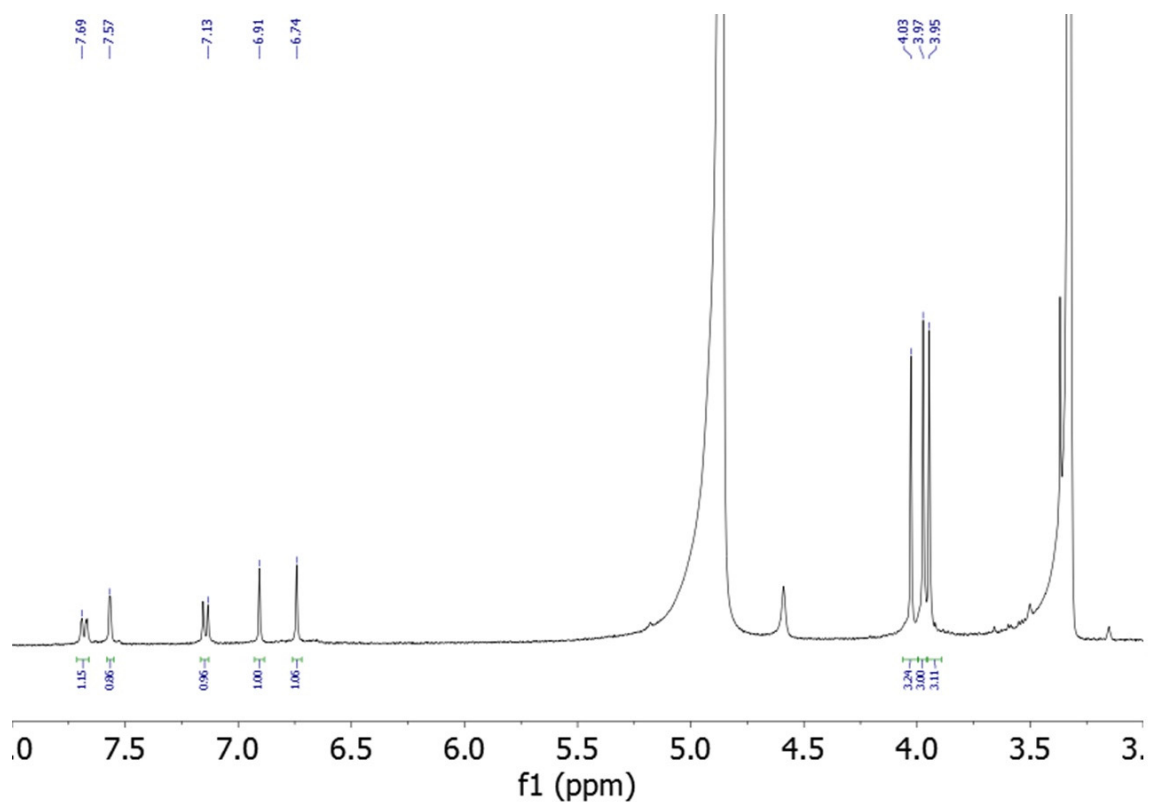


Figure S22. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **22**.

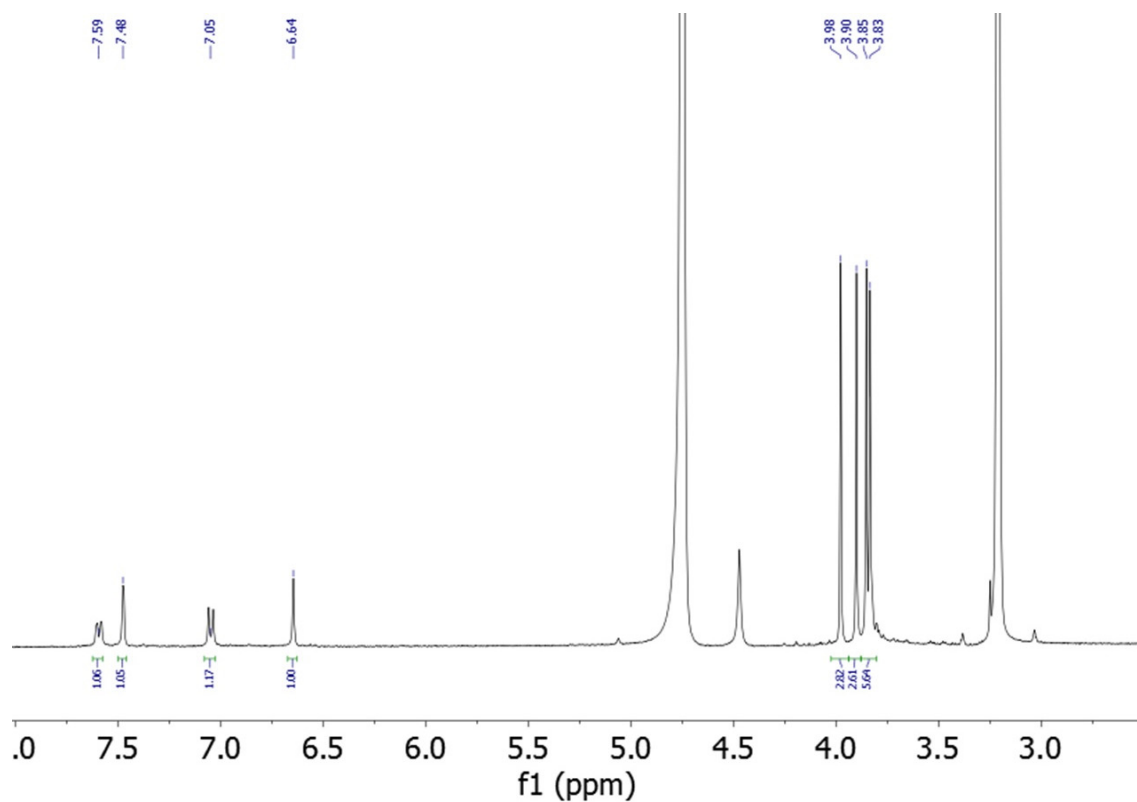


Figure S23. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **23**.

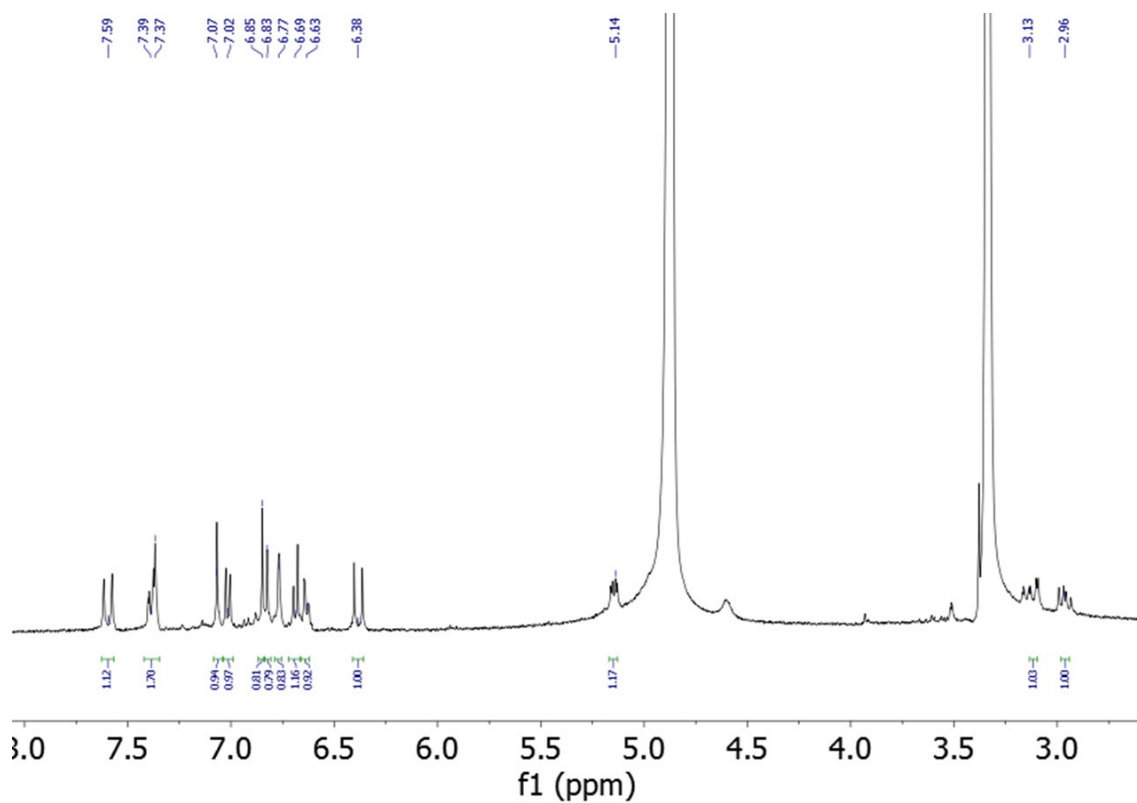


Figure S24. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **24**.

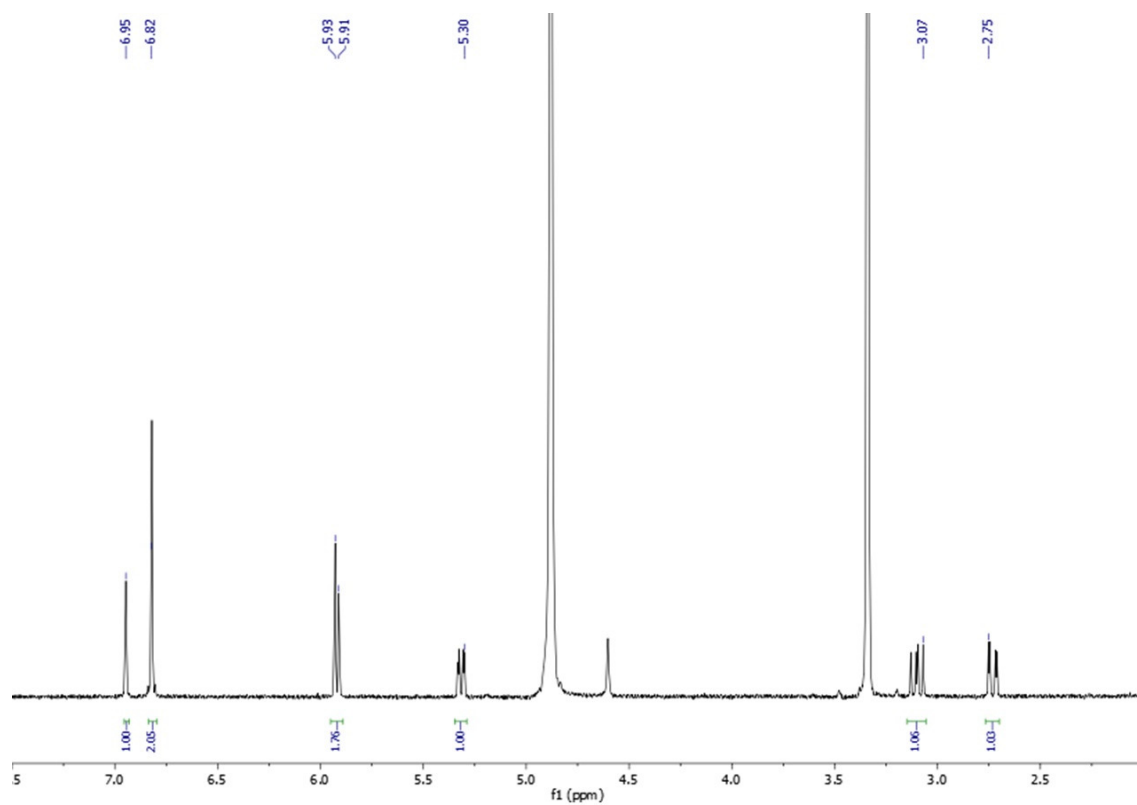


Figure S25. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **26**.

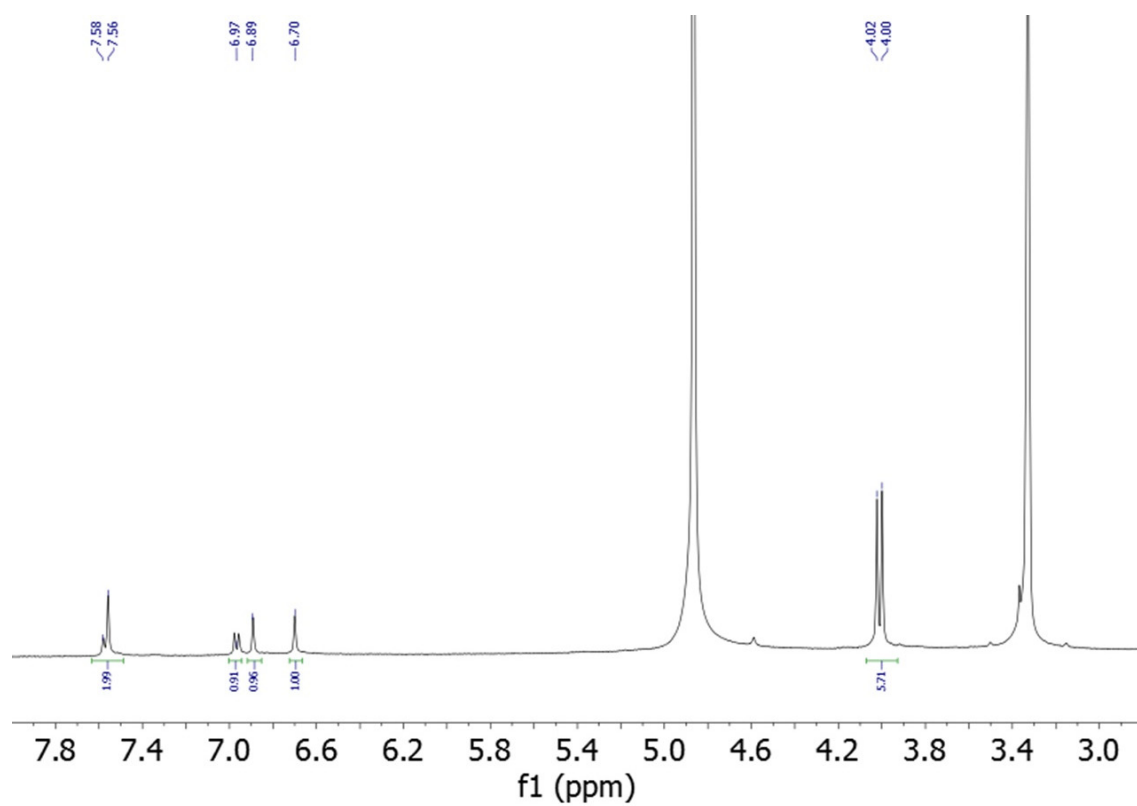


Figure S26. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **27**.

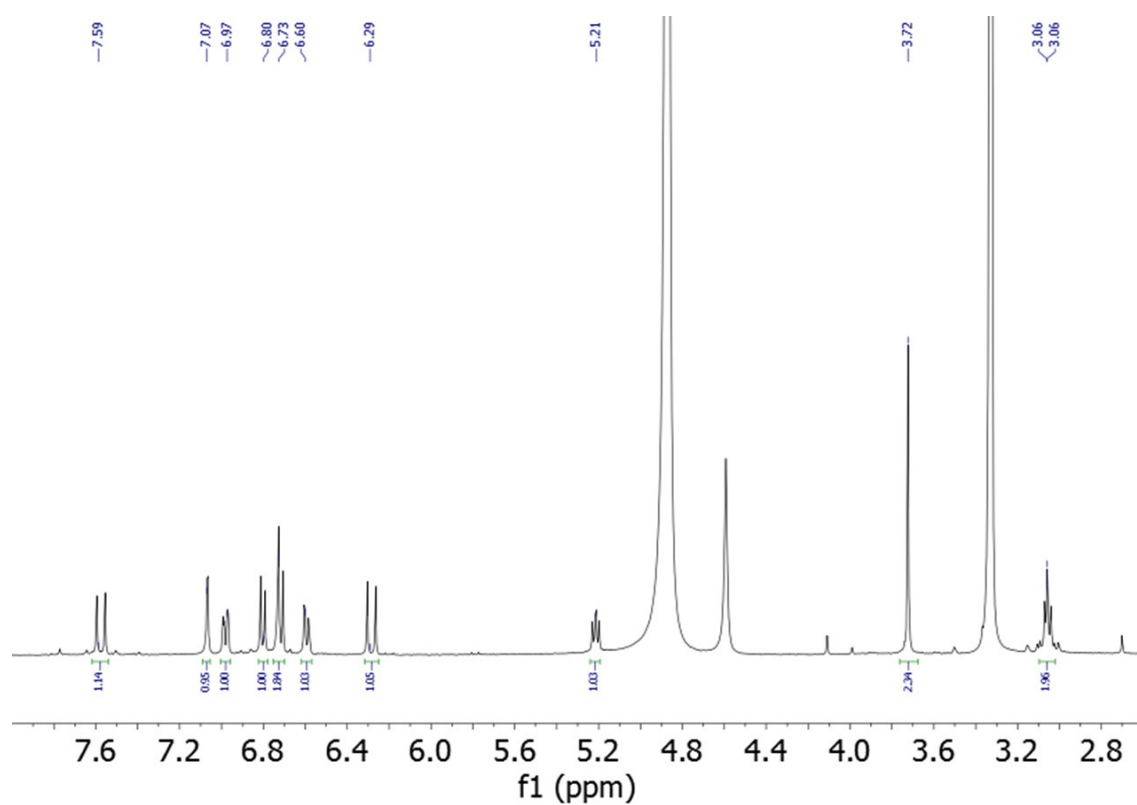


Figure S27. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **28**.

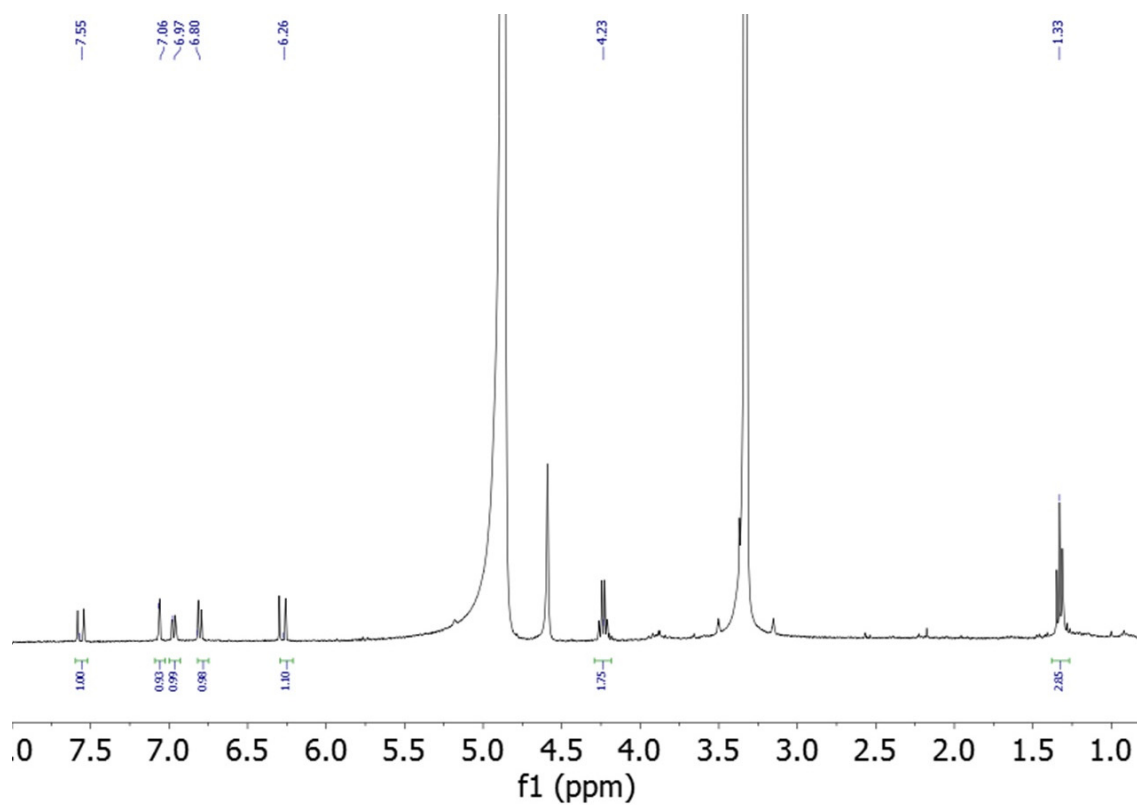


Figure S28. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **29**.

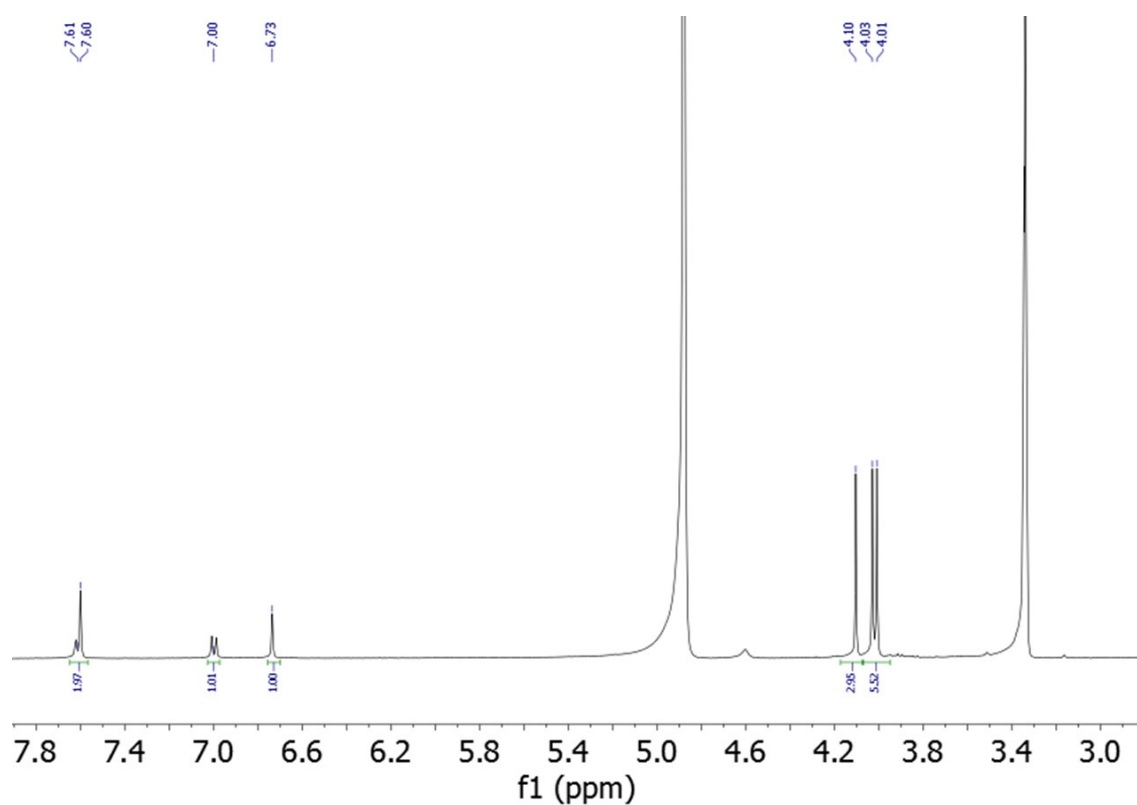


Figure S29. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **30**.

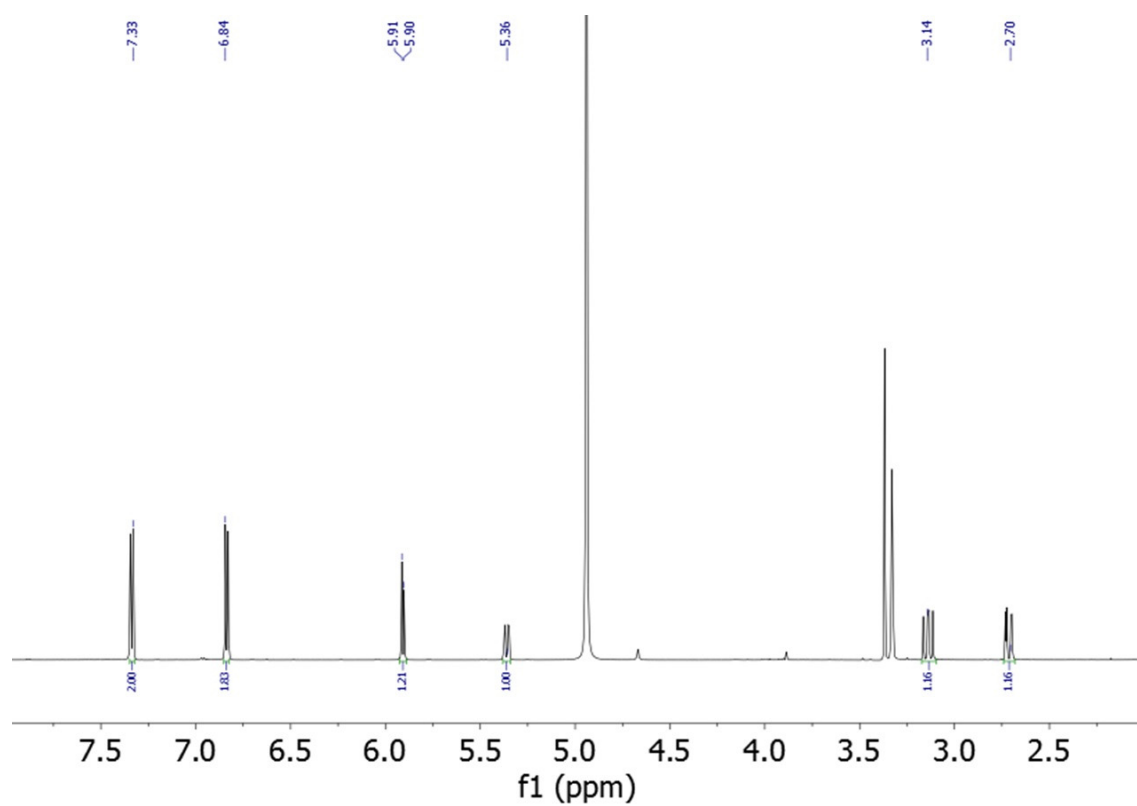


Figure S30. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **32**.

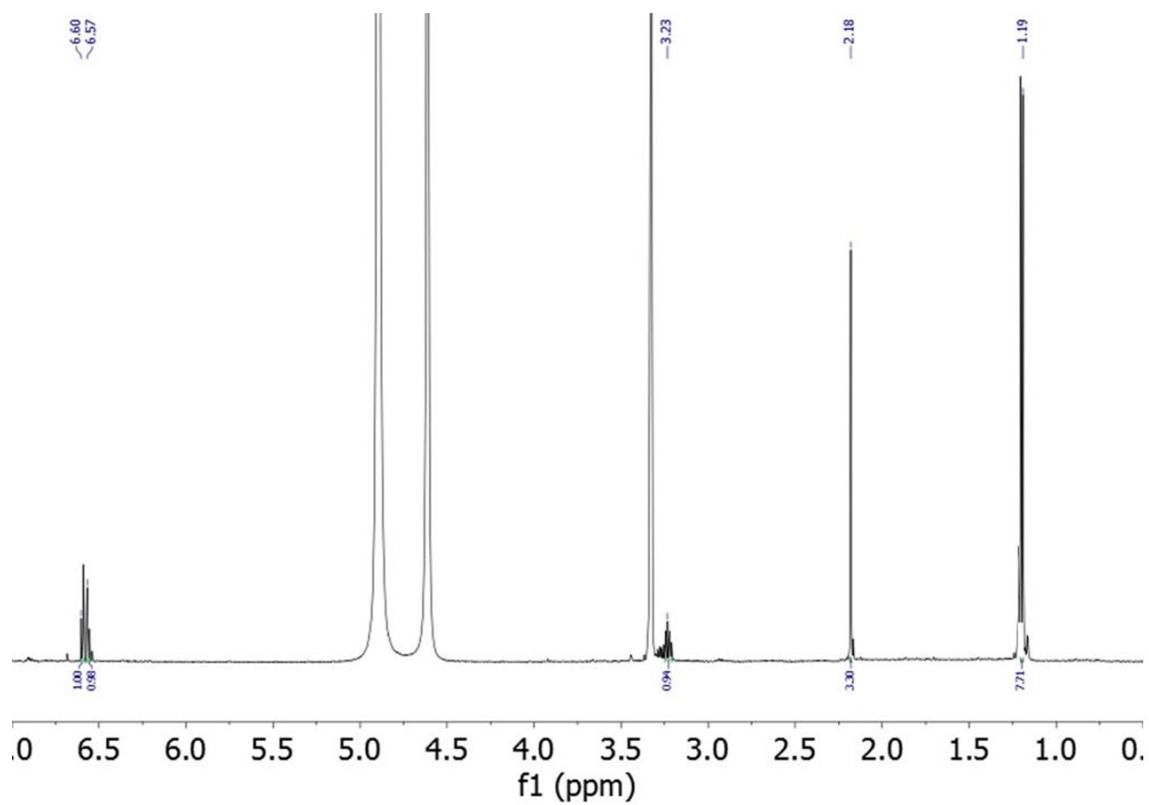


Figure S31. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **33**.

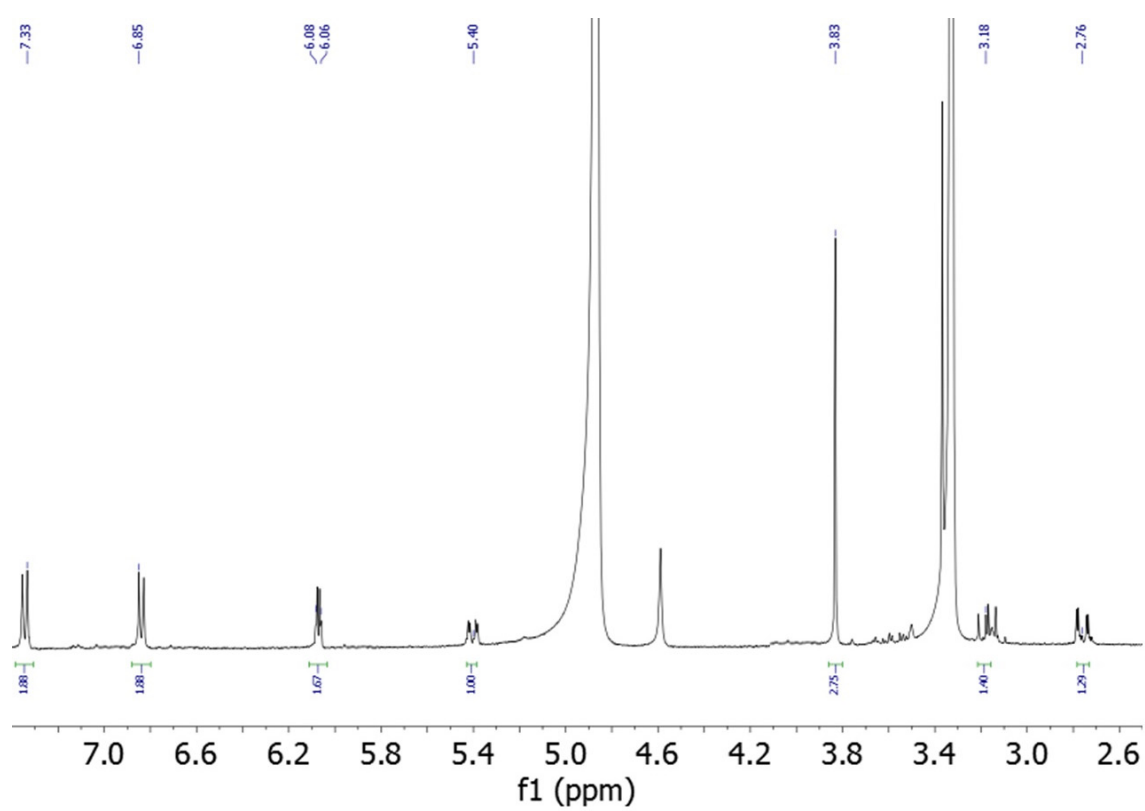


Figure S32. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **34**.

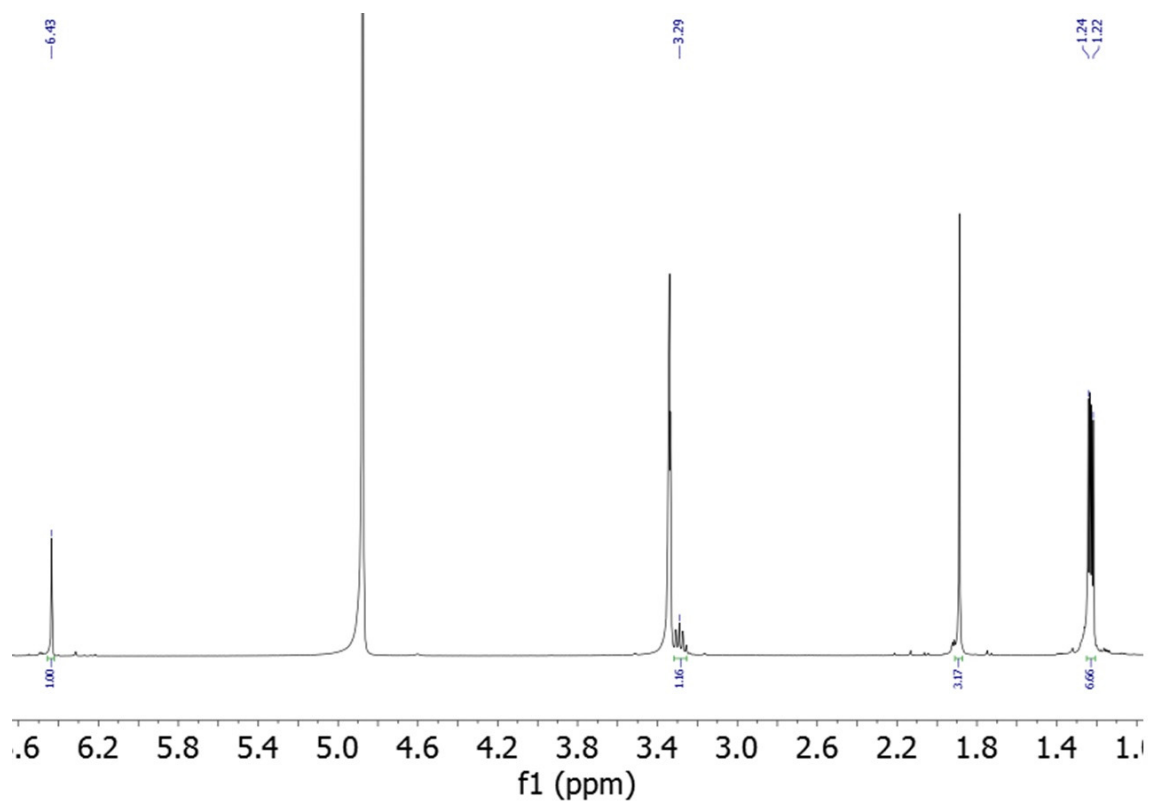


Figure S33. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **35**.

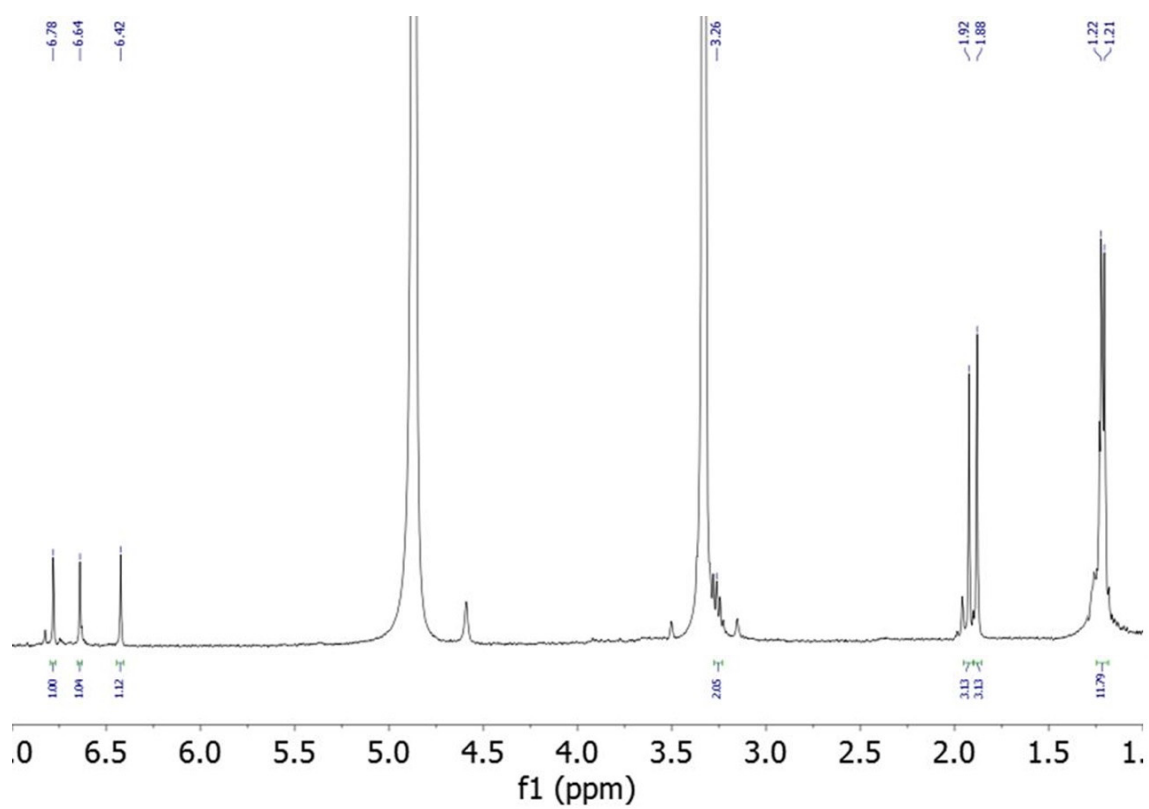


Figure S34. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **36**.

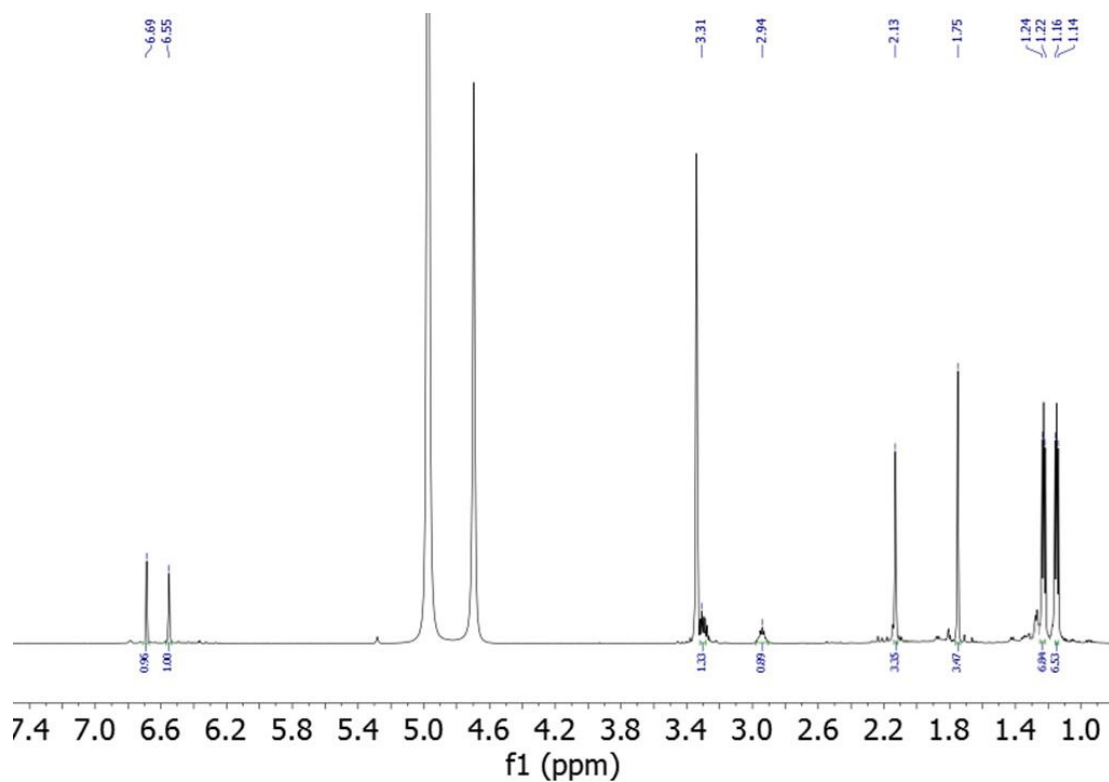


Figure S35. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **38**.

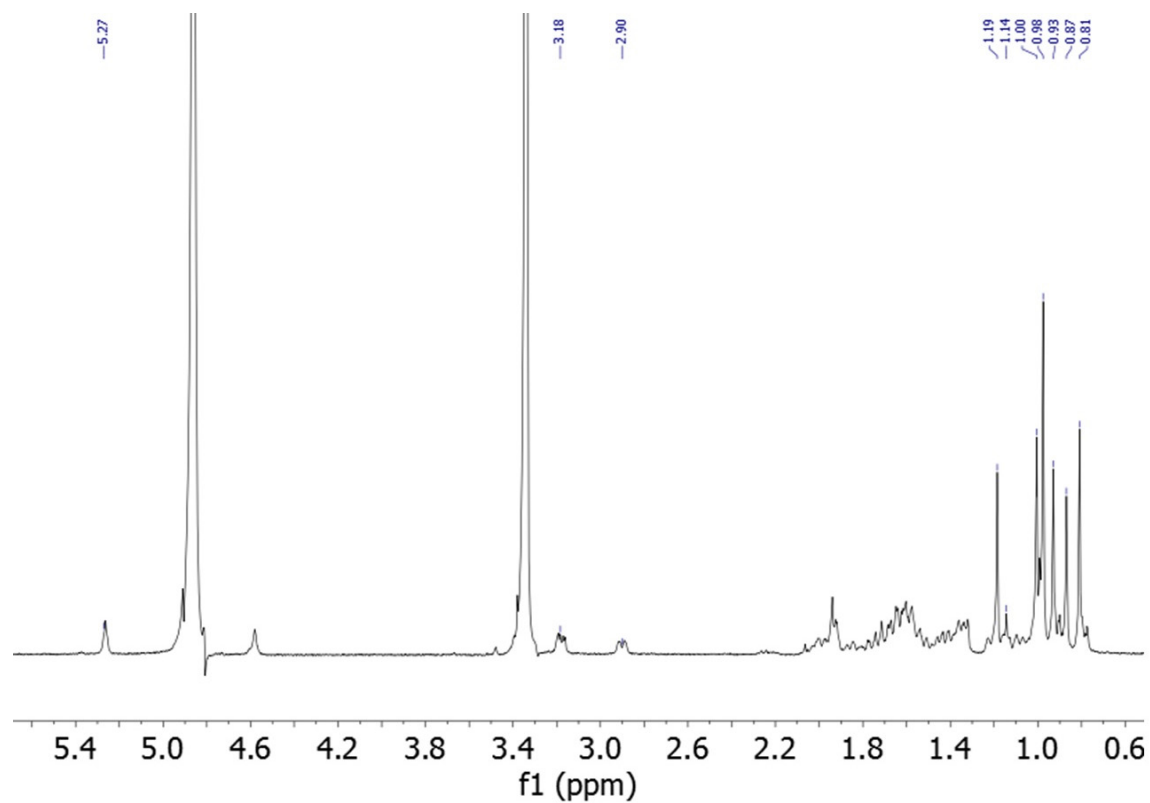


Figure S36. ¹H NMR Spectrum (600 MHz, CD₃OD) of compound **39**.