Supporting Information

Chemical Constituents and Antioxidant Activity from the Stems of *Alyxia reinwardtii*

***Rec. Nat. Prod.* 6:3 (2012) 288-291**

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1. Antioxidant activity assays

1.1. DPPH radical scavenging activity: After isolation and purification, activities of pure compounds were quantified in this assay. Various concentrations of samples dissolved in methanolic (0.5 mL) were added to DPPH radical methanolic solution (0.2 mM, 1.0 mL). After 30 minutes incubation at room temperature in the dark, the absorbance was measured at 517 nm with a 96 well microplate. All tests were run in triplicate and calculated sample standard deviation. The scavenging activity was evaluated from the decrease value of 517 nm absorption, which was calculated by the following equation. The activity was shown as IC_{50} values that denote the concentration of sample required scavenging 50% DPPH free radicals.

$$\% \text{ Scavenging activity} = [1 - A_{sample}/A_{blank*}] \times 100$$

1. 2.Assay for scavenging activity of $O_2^{\bullet-}$ by xanthine oxidase: Superoxide anion radical was generated from xanthine-xanthine oxidase method with a slight modification. The reaction mixture consisted of 0.1 M phosphate buffer (pH 8.0) containing 0.4 mM xanthine, 0.24 mM nitroblue tetrazolium, and 0.049 units of xanthine oxidase in a final volume 1.0 mL. Samples at various concentrations in DMSO were added to the mixture (0.15 mL). After being incubated at 37° C for 20 minutes, the reaction was terminated by addition of 0.05 ml of 69 mM sodium dodecyl sulfate. The absorbance of formazen produced was determined at 560 nm, and scavenging activity on $O_2^{\bullet-}$ of each sample was estimated by the same equation as described before. The IC_{50} values were calculated from regression line.

$$\% \text{ Scavenging activity} = [1 - A_{Sample}/A_{blank*}] \times 100$$

1.3.Assay for inhibitory activity against xanthine oxidase: For studying of xanthine oxidase inhibitory activity, the rise in the absorbance at 290 nm due to uric acid production was measured in the absence of nitroblue tetrazolium. Allopurinol, which is a drug for gout treatment, was used as a standard for this assay. The inhibitory activity was shown as percent inhibition, which was estimated from the following equation. The IC_{50} values were determined from regression line.

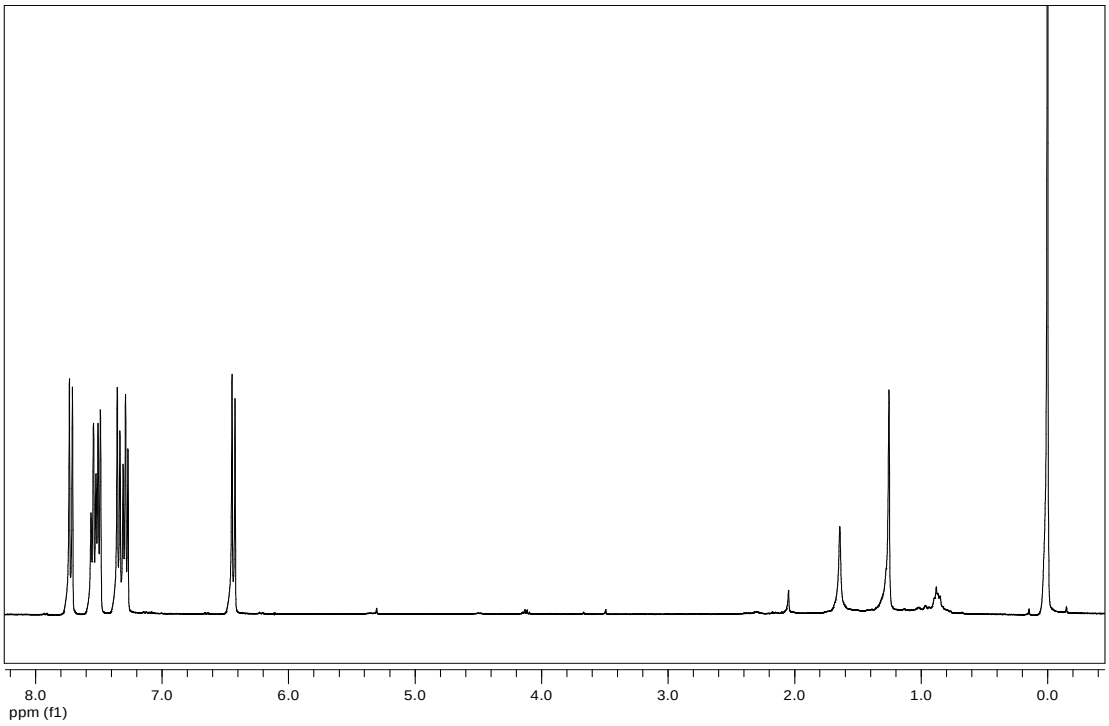
$$\% \text{ Inhibition} = [1 - A_{Sample}/A_{blank*}] \times 100$$

1.4.Ferric thiocyanate assay: This assay was slightly modified. The linoleic acid emulsion was prepared by vortex mixing 3.0 mL of linoleic acid with 3.0 mL of sodium dodecyl sulfate (SDS) as emulsifier and 200 mL of 30 % (v/v) ethanol. Each sample at various concentrations in ethanolic solution (0.5 mL) was mixed with 5 mL of emulsion and the final volume of the mixture was adjusted to 12.5 mL. The reaction mixture was incubated in a conical flask at 40°C in the dark. Aliquots of 0.05 mL were taken at eight hours during incubation and tested for lipid peroxidation products. The assay was carried out by adding 2.5 mL of 75 % ethanol, 0.1 mL of ammonium thiocyanate solution (30 % w/v), and 0.1 mL of ferrous chloride (0.1 % w/v) to 0.05 mL of sample. After the mixture was left for 3 minutes, the absorbance of the reaction mixture was measured at 500 nm. The activity was revealed as percent inhibition that was examined from the following equation. The IC_{50} values that denote the concentration of sample required scavenging 50% peroxyl radicals were calculated from regression line.

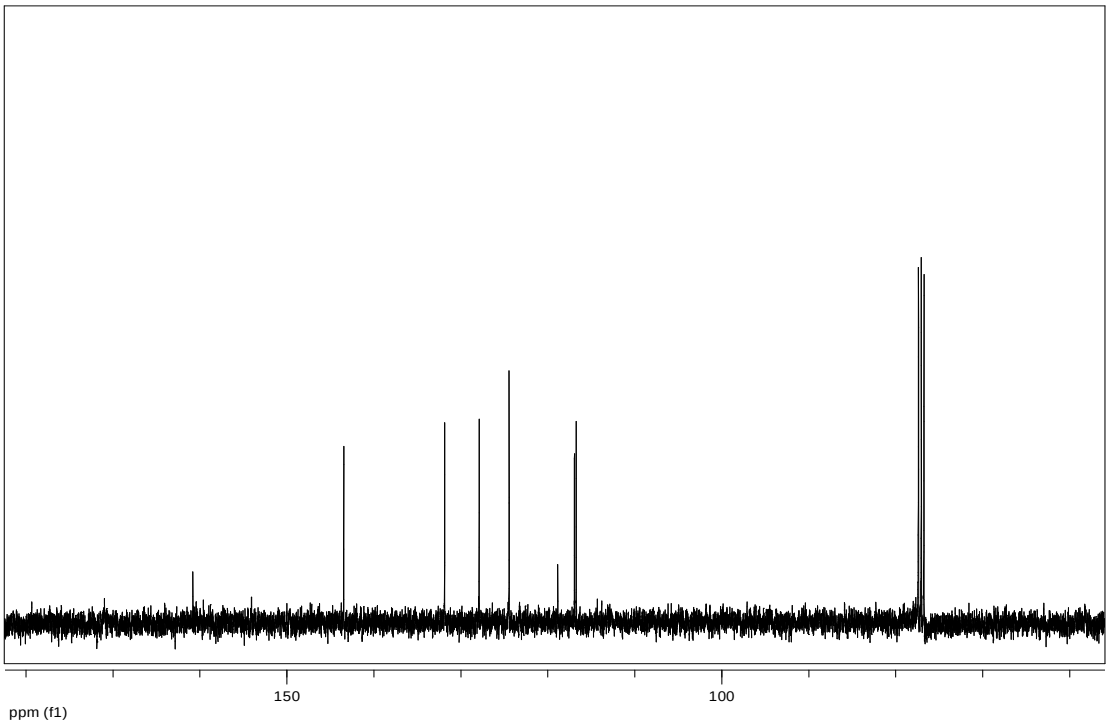
$$\% \text{ Inhibition} = [1 - A_{Sample}/A_{blank*}] \times 100$$

A_{blank*} = Absorbance of reaction mixtures were prepared without test compounds.

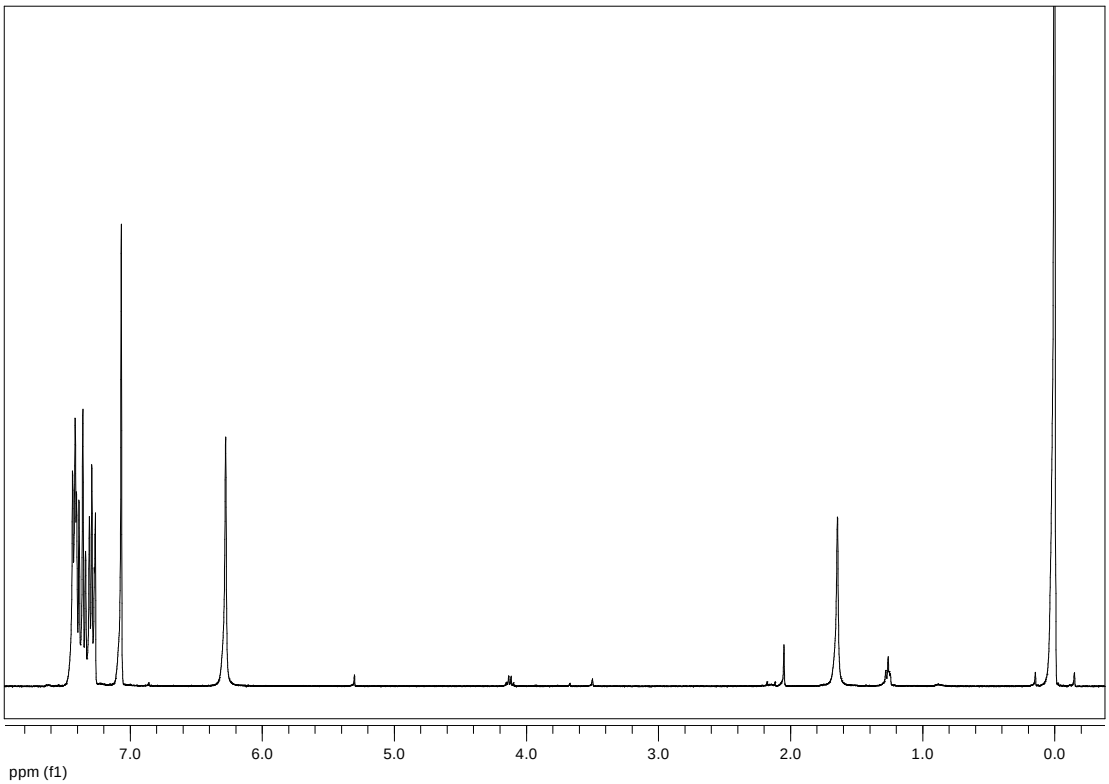
2. NMR data.



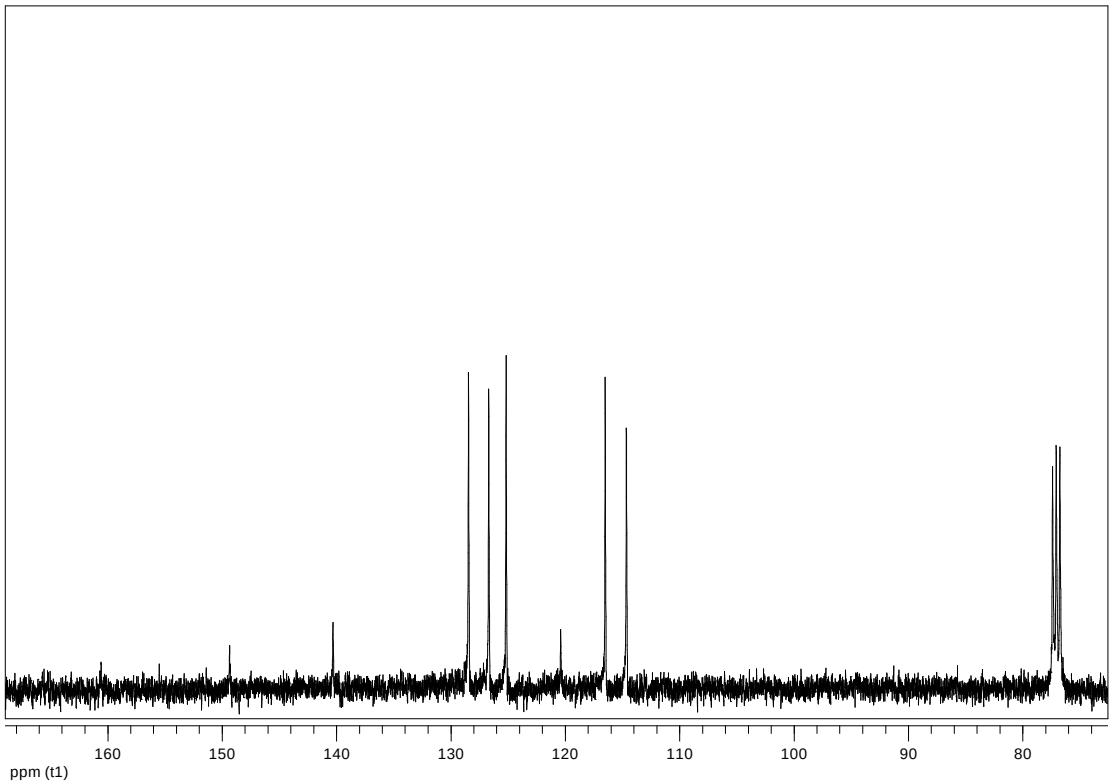
S. 1. The ^{1}H NMR spectrum ($CDCl_3$) of compound **1**



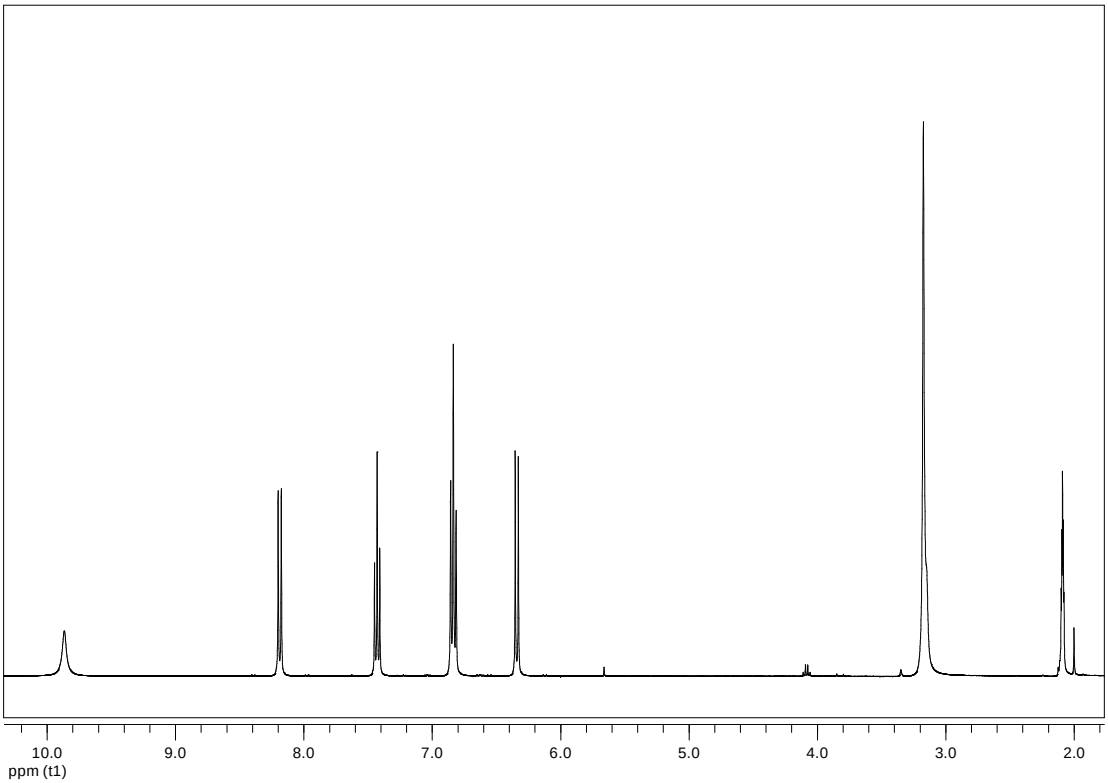
S. 2. The ^{13}C NMR spectrum ($CDCl_3$) of compound **1**



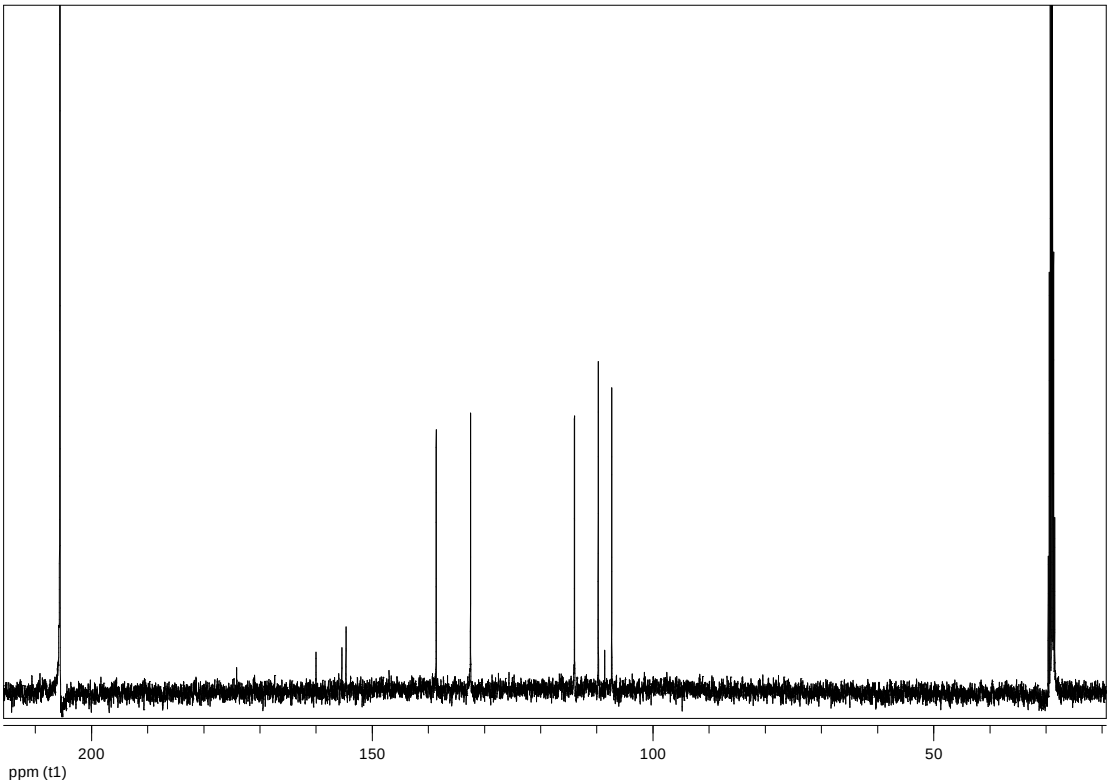
S.3. The ^{1}H NMR spectrum ($CDCl_3$) of compound **2**



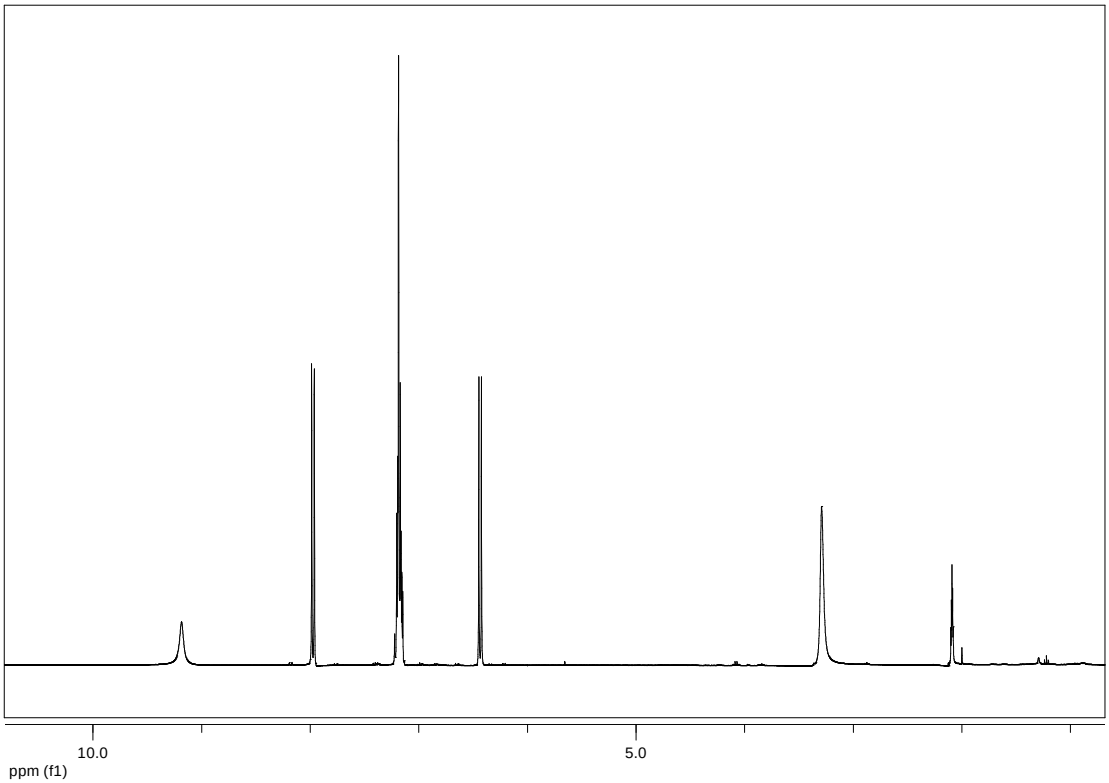
S. 4 The ^{13}C NMR spectrum ($CDCl_3$) of compound **2**



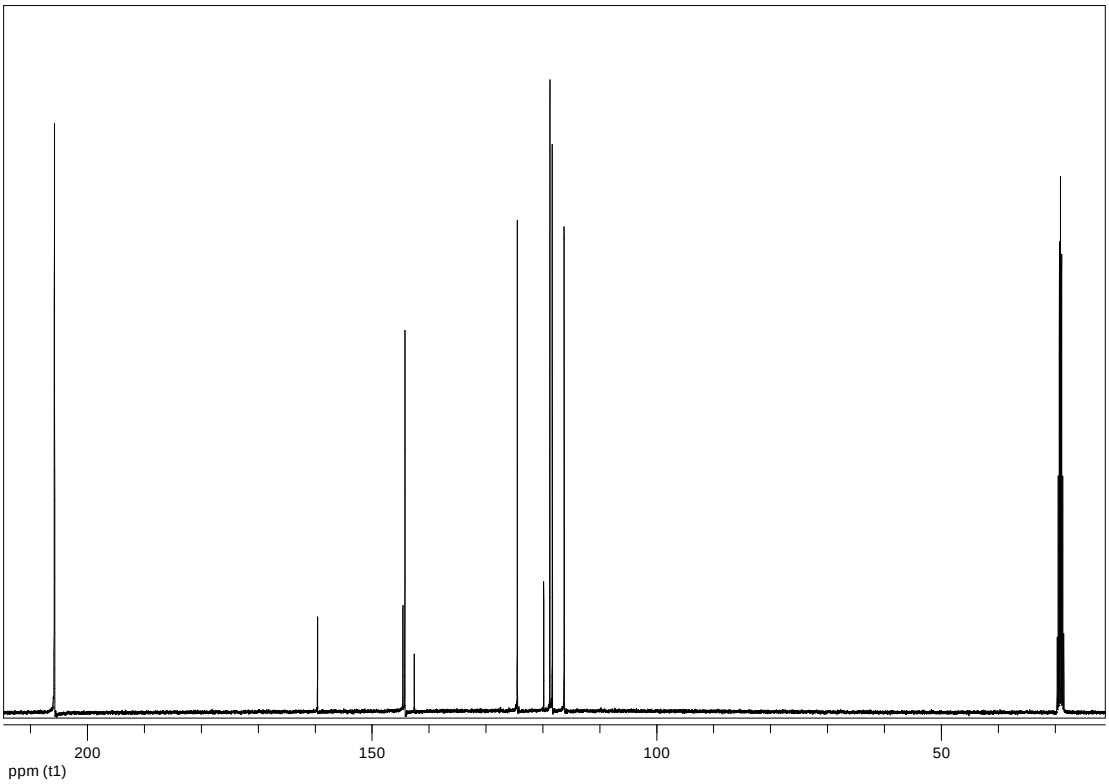
S. 5. The ^{1}H NMR spectrum (CD_3COCD_3) of compound **3**



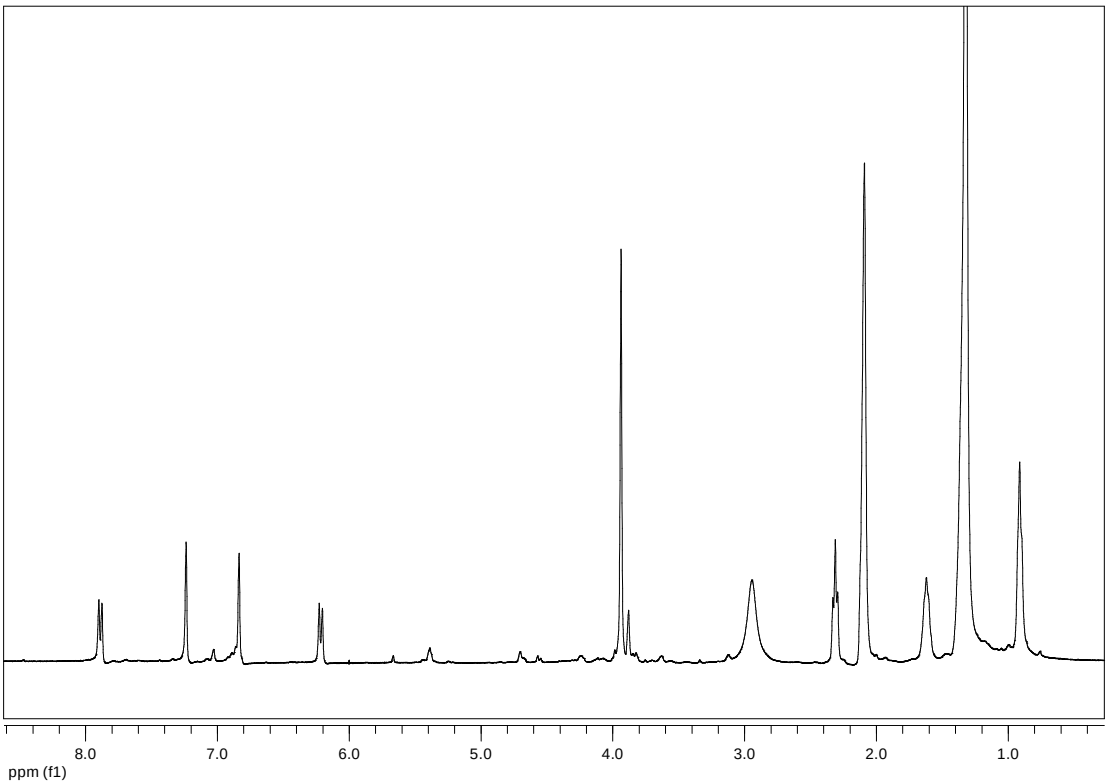
S. 6 The ^{13}C NMR spectrum (CD_3COCD_3) of compound **3**



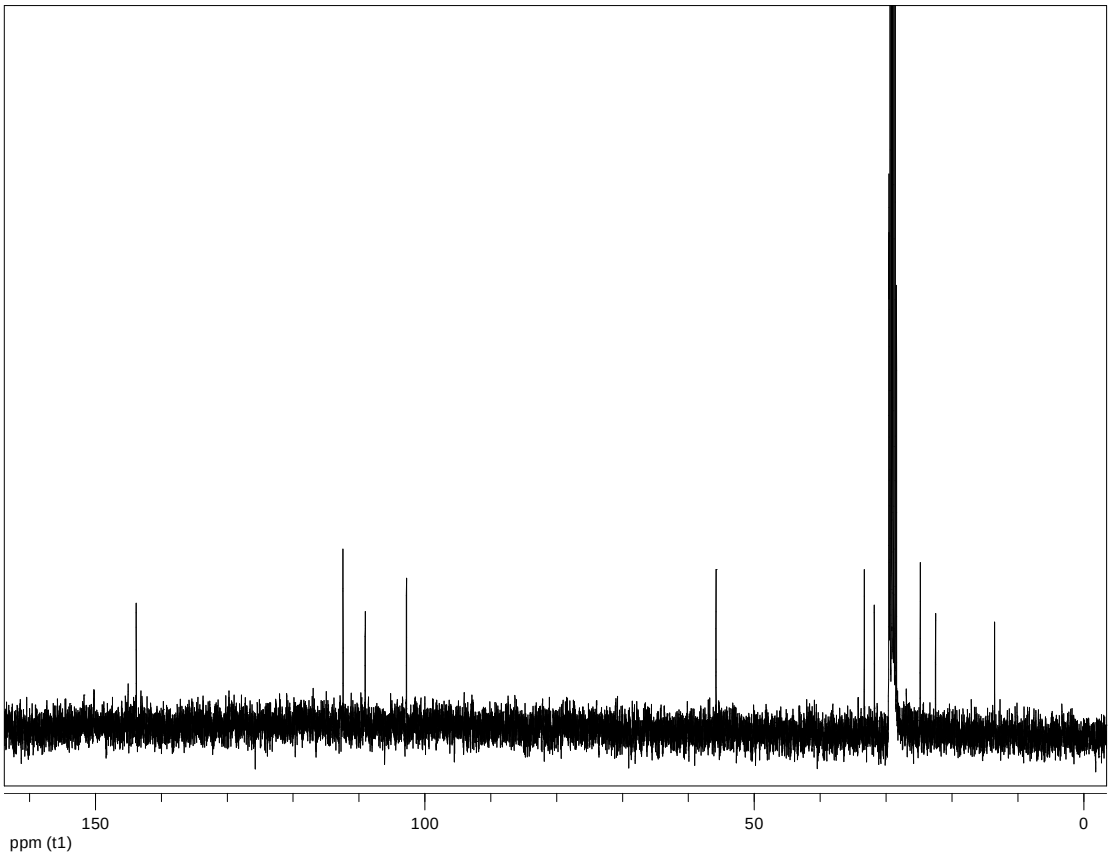
S. 7 The ^{1}H NMR spectrum (CD_3COCD_3) of compound **4**



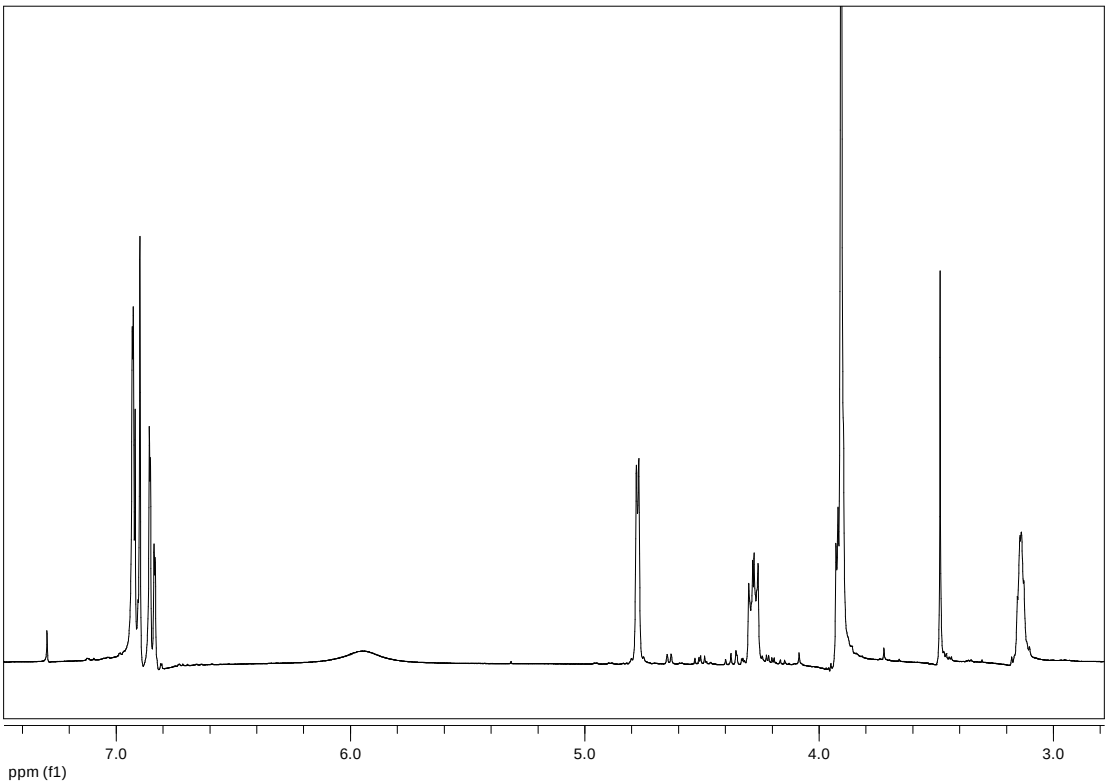
S. 8 The ^{13}C NMR spectrum (CD_3COCD_3) of compound **4**



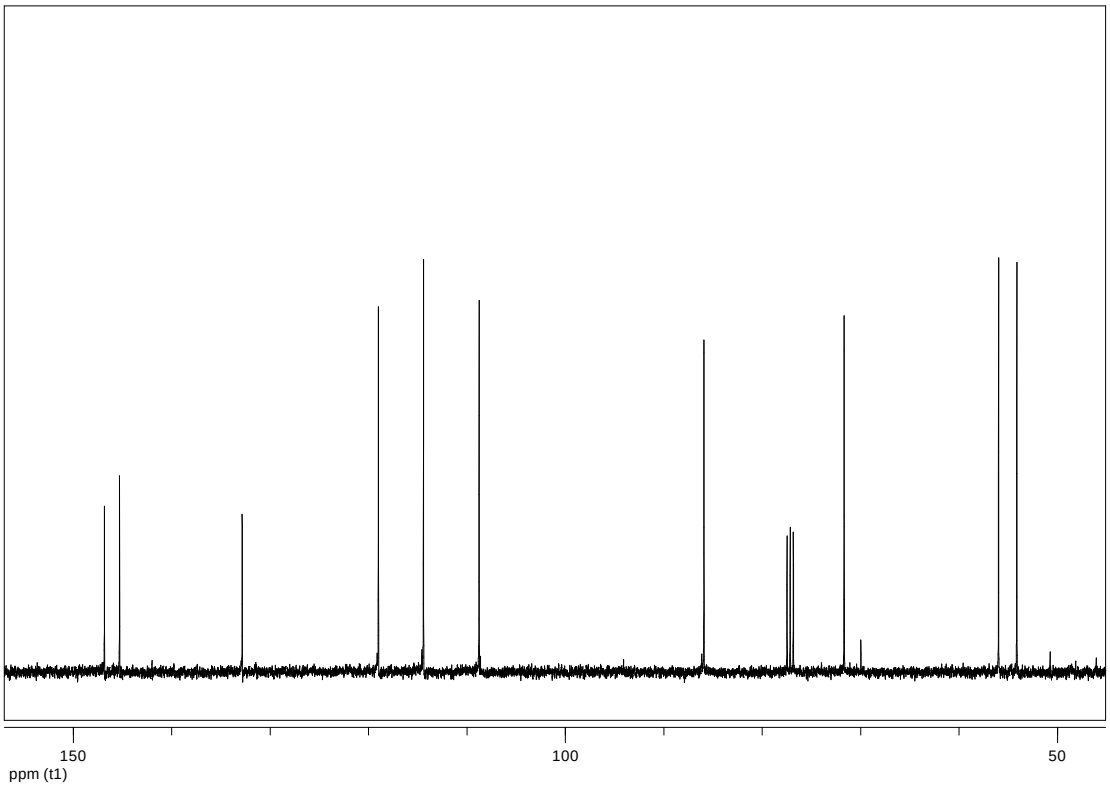
S. 9 The ^{1}H NMR spectrum (CD_3COCD_3) of compound **5**



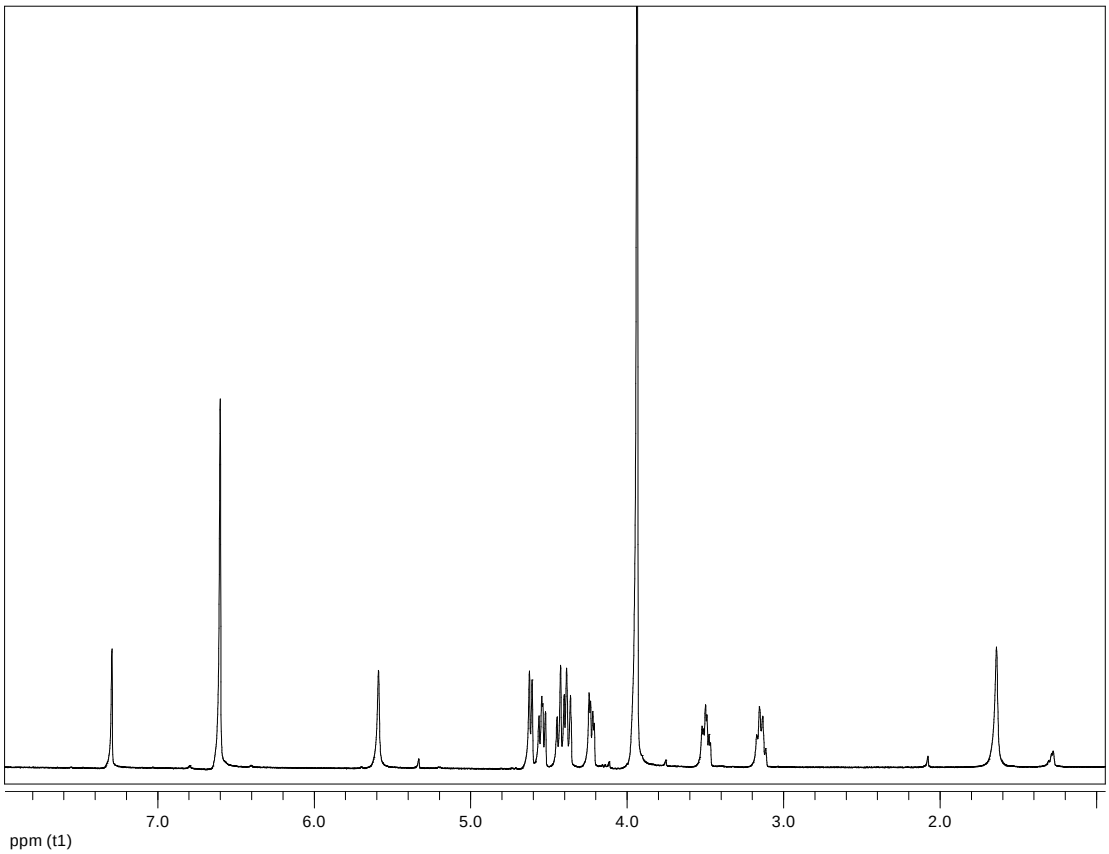
S. 10. The ^{13}C NMR spectrum (CD_3COCD_3) of compound **5**



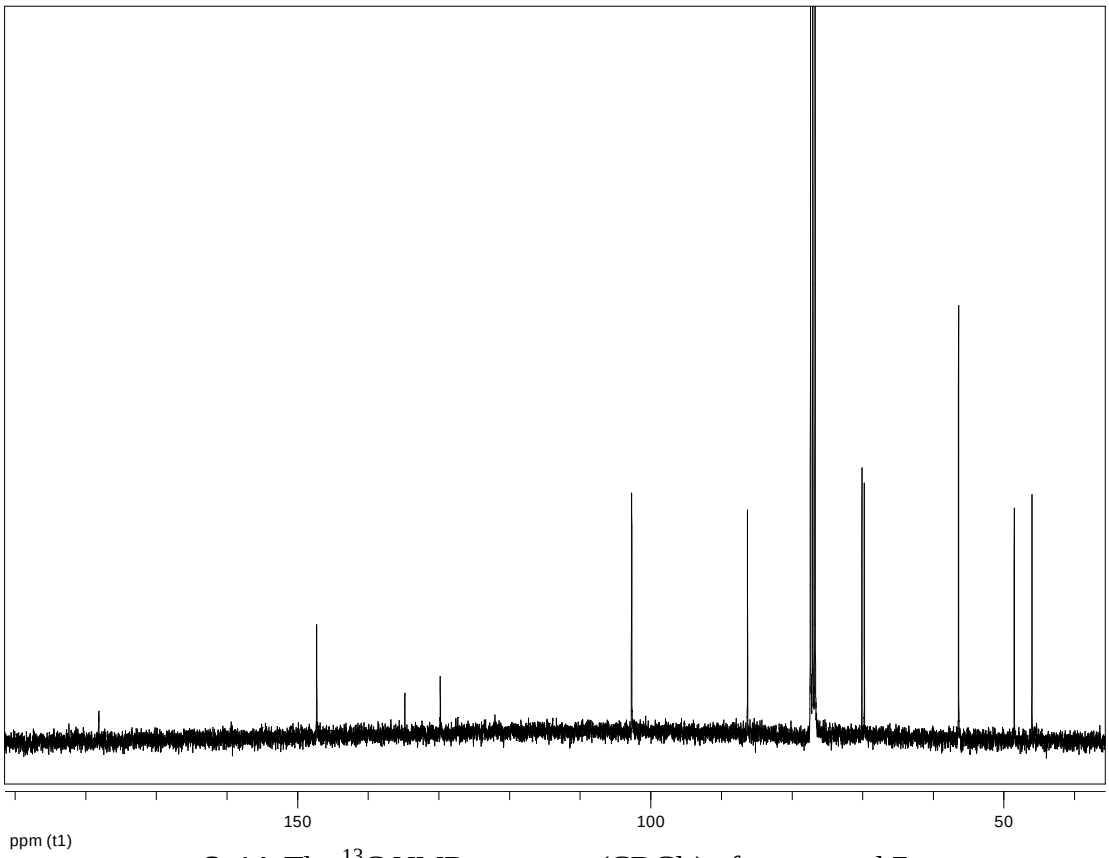
S. 11. The ^{1}H NMR spectrum ($CDCl_3$) of compound **6**



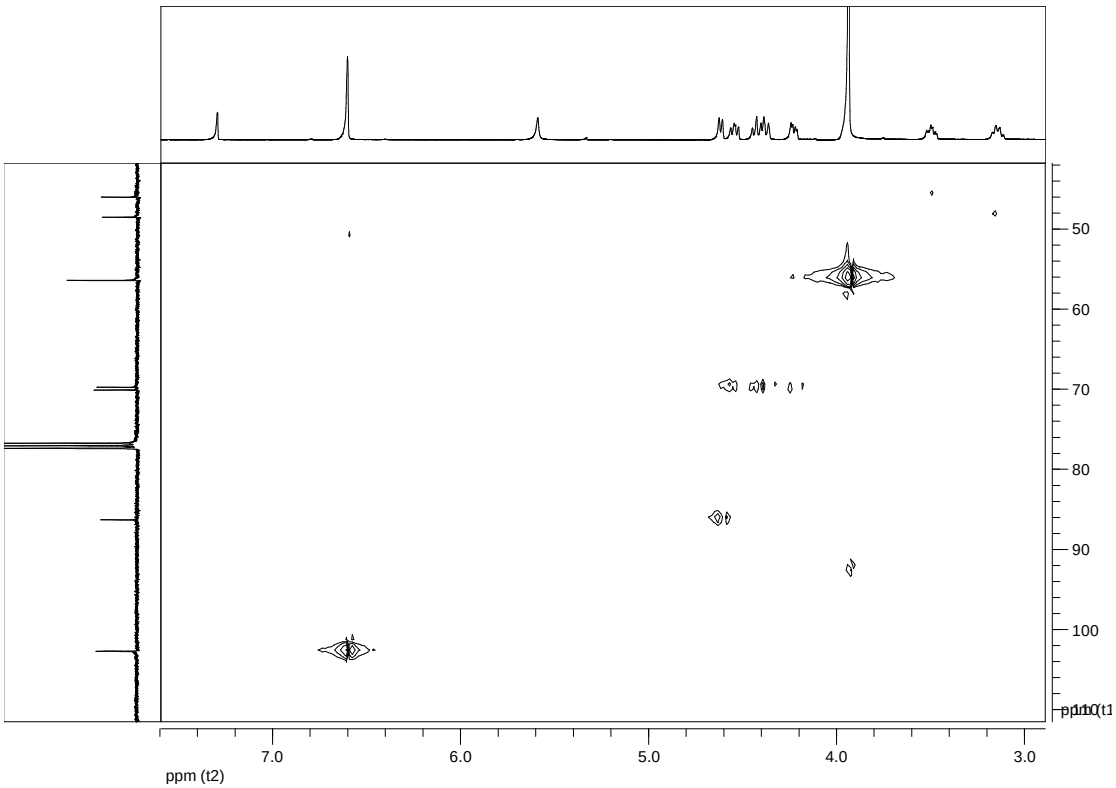
S. 12. The ^{13}C NMR spectrum ($CDCl_3$) of compound **6**



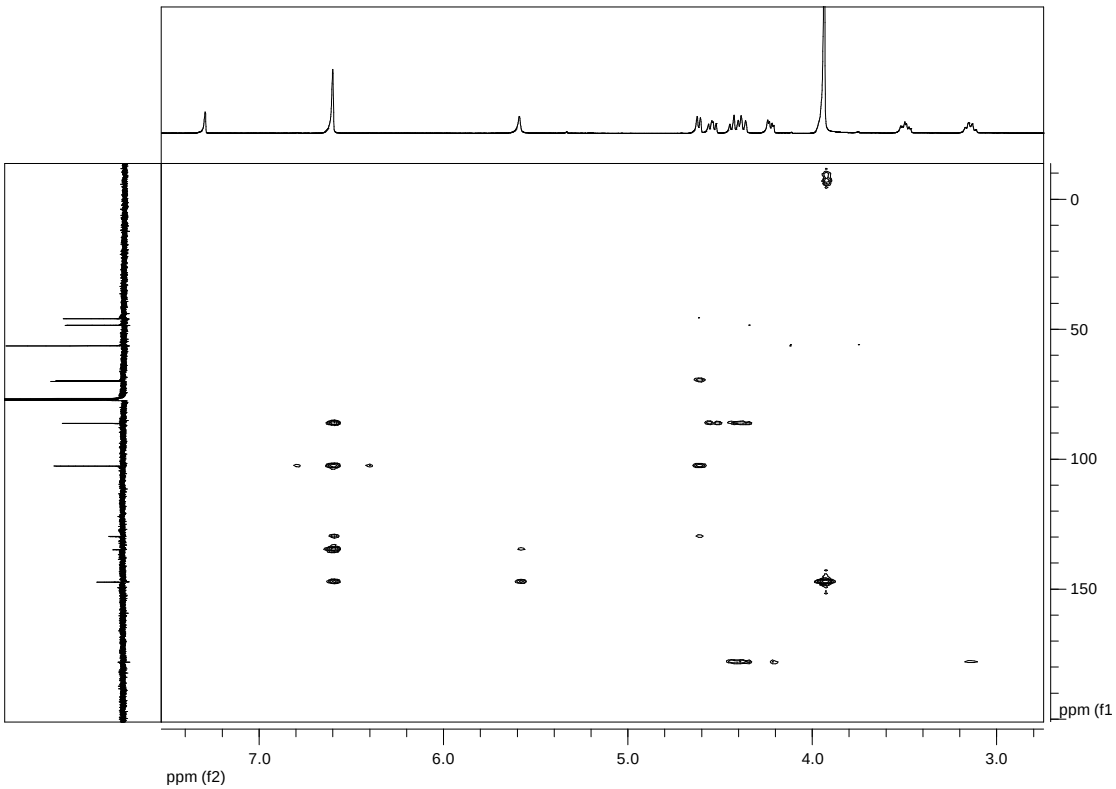
S. 13. The ^{1}H NMR spectrum ($CDCl_3$) of compound **7**



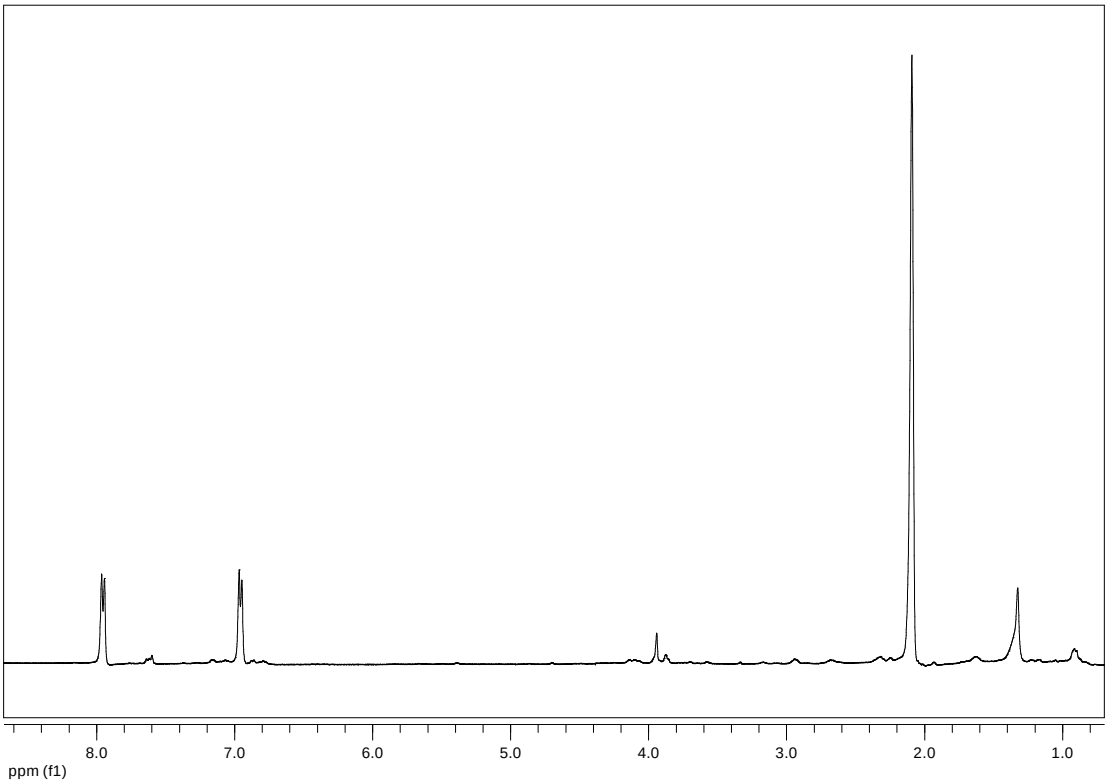
S. 14. The ^{13}C NMR spectrum ($CDCl_3$) of compound **7**



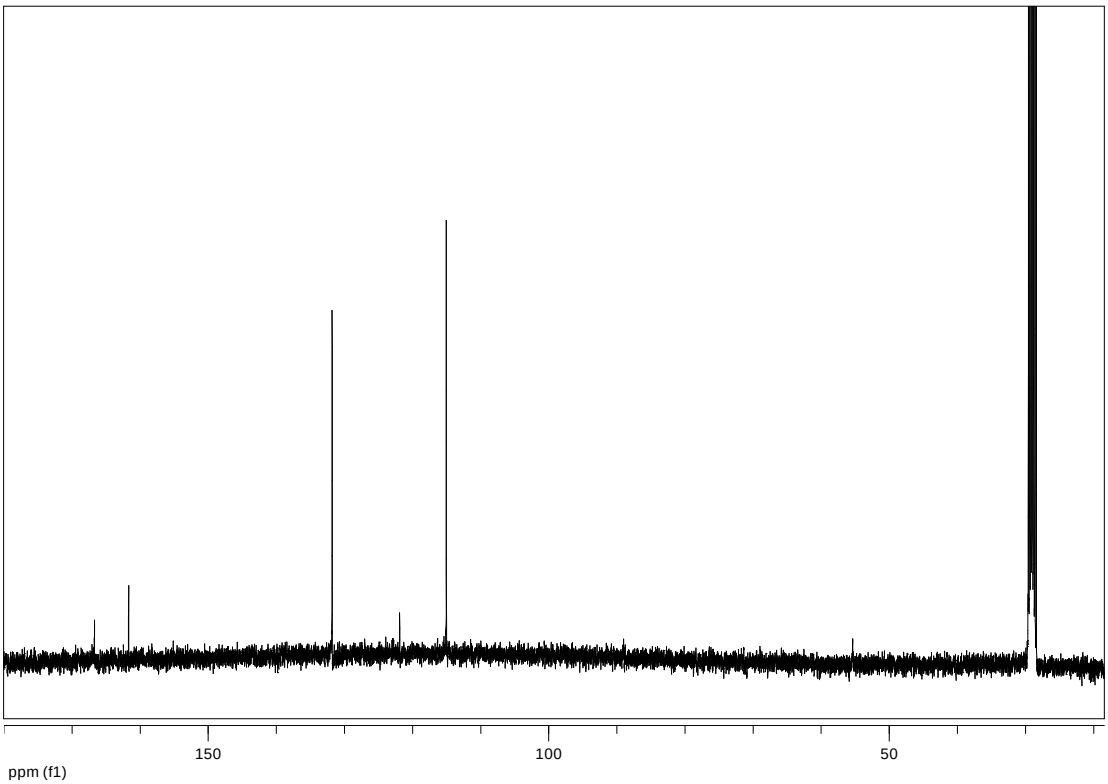
S. 15. The HMQC spectrum ($CDCl_3$) of compound **7**



S. 16. The HMBC spectrum ($CDCl_3$) of compound **7**



S.17. The ^{1}H NMR spectrum (CD_3COCD_3) of compound **8**



S. 18. The ^{13}C NMR spectrum (CD_3COCD_3) of compound **8**