

Article

Synthesis and antioxidative properties of 1,2,3,4-tetrahydropyridine derivatives with different substituents in 4-position

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1. Folin-Ciocalteu assay

1.1 Preliminary procedures

- Prepare a solution of Na_2CO_3 2 % (w/v) by weighting 2 g of Na_2CO_3 and dissolve in 100 mL of distilled water.
- Dilute FCR with distilled water to obtain a solution 50 % (v/v).
- Prepare a solution of Gallic acid (standard) 6 mM by weighting 1 mg of gallic acid (PM = 170,12) and solubilize in 1 mL of distilled water.

The quantities of solution of gallic acid 6 mM used are:

- 10 μL (10 μg) + 90 μL di H_2O
- 30 μL (30 μg) + 70 μL di H_2O
- 50 μL (50 μg) + 50 μL di H_2O
- 70 μL (70 μg) + 30 μL di H_2O

The final volumes of the sample and standard must be equal to 100 μL .

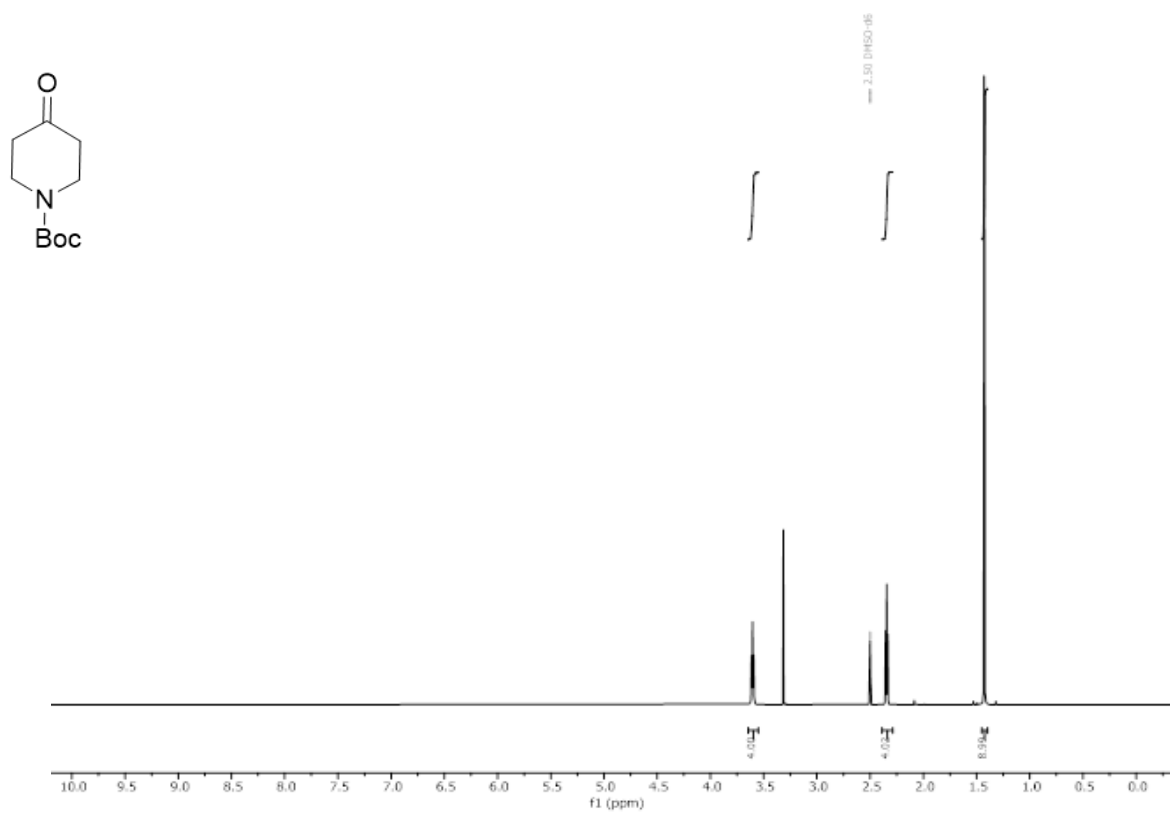
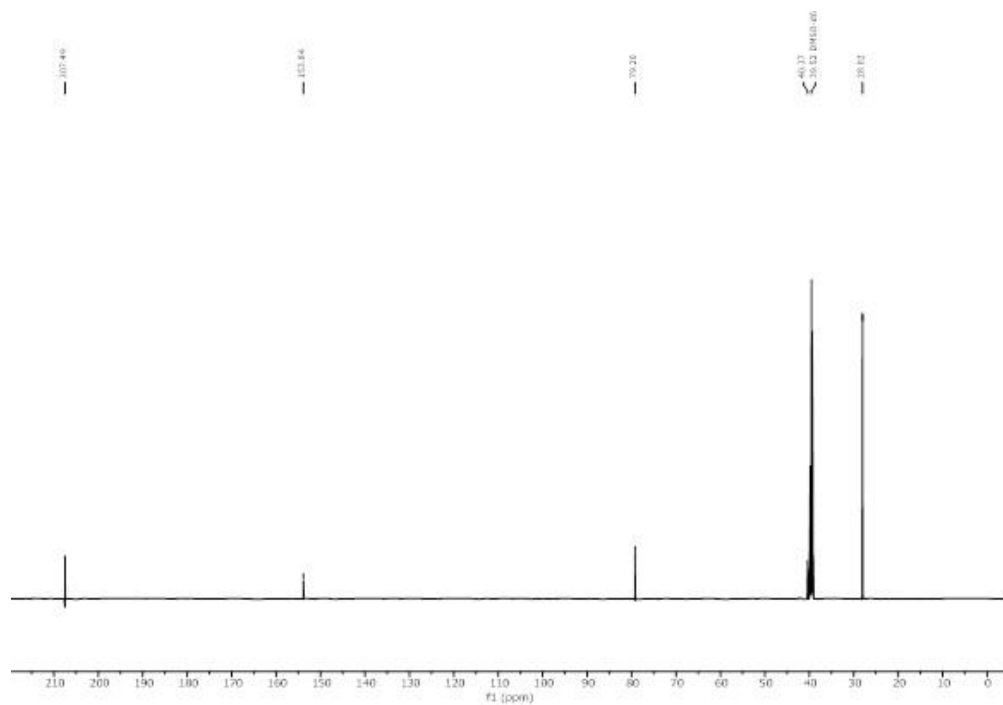
1.2 General procedure

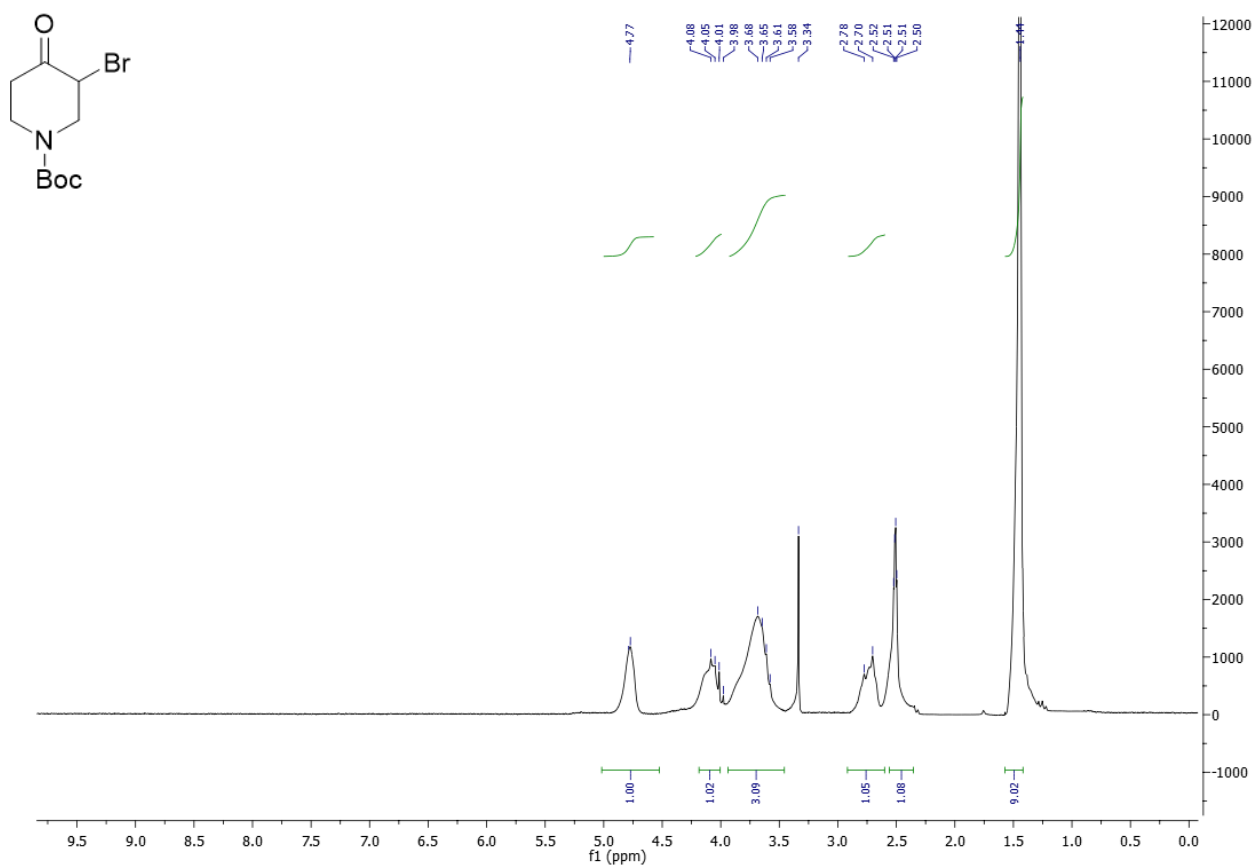
- In each tube add the exact volume of distilled water to reach 100 μL then add sample or standard.
- Prepare the standard with 100 μL .
- Add 3 mL of 2% Na_2CO_3 (w/v) solution.
- Add 100 μL of FCR solution 50 % (v/v).
- Stirring and incubation at RT in dark place for 60 min
- Measure the optical density with an UV-spectrophotometer at 765 nm.
- Create the curve for data analysis
- Express the results in mg GAE/g sample.

1.3 Determination of the TPC of compounds 21

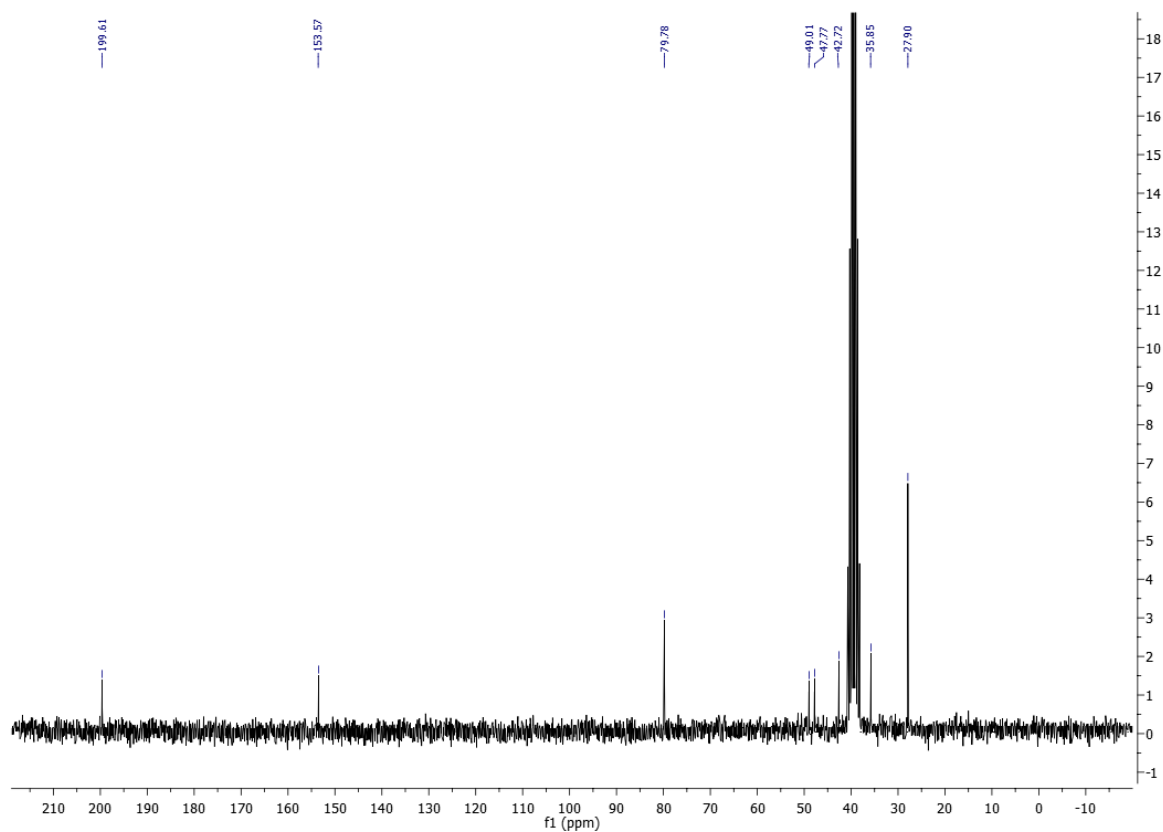
The samples were solubilized in DMSO to obtain a 100 mM solution. For each sample two aliquots of 1 μL and 100 μL were used and the volumes were adjusted to 100 μL with distilled water. To the samples 3 mL of 2% Na_2CO_3 (w/v) solution were added followed by addition of 100 μL of FCR. The samples were incubated for 60 min in a dark place at RT. After 60 min no color change was observed indicating the absence of antioxidant activity.

2. NMR spectra

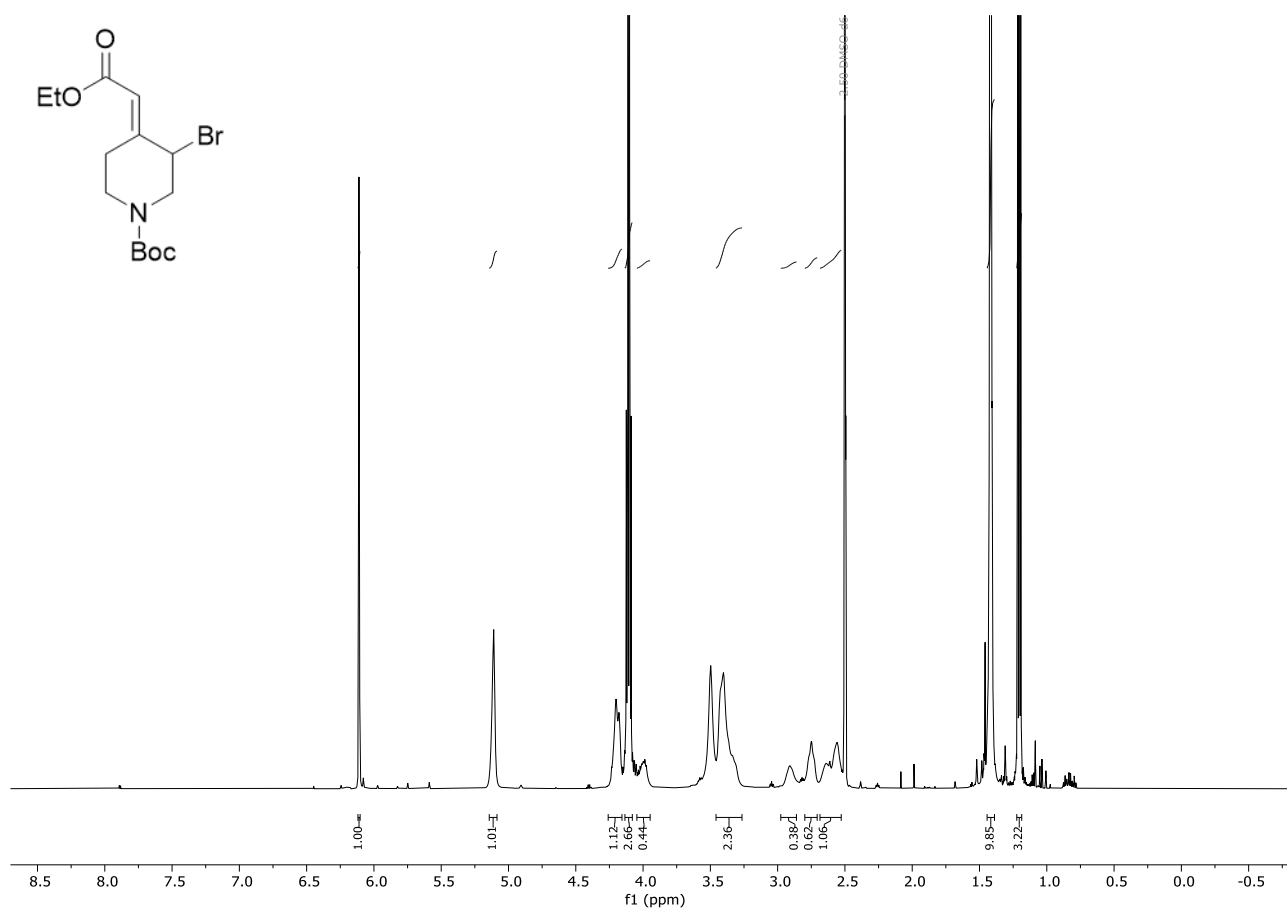
¹H NMR spectrum (DMSO-d₆) of **18**¹³C NMR spectrum (DMSO-d₆) of **18**



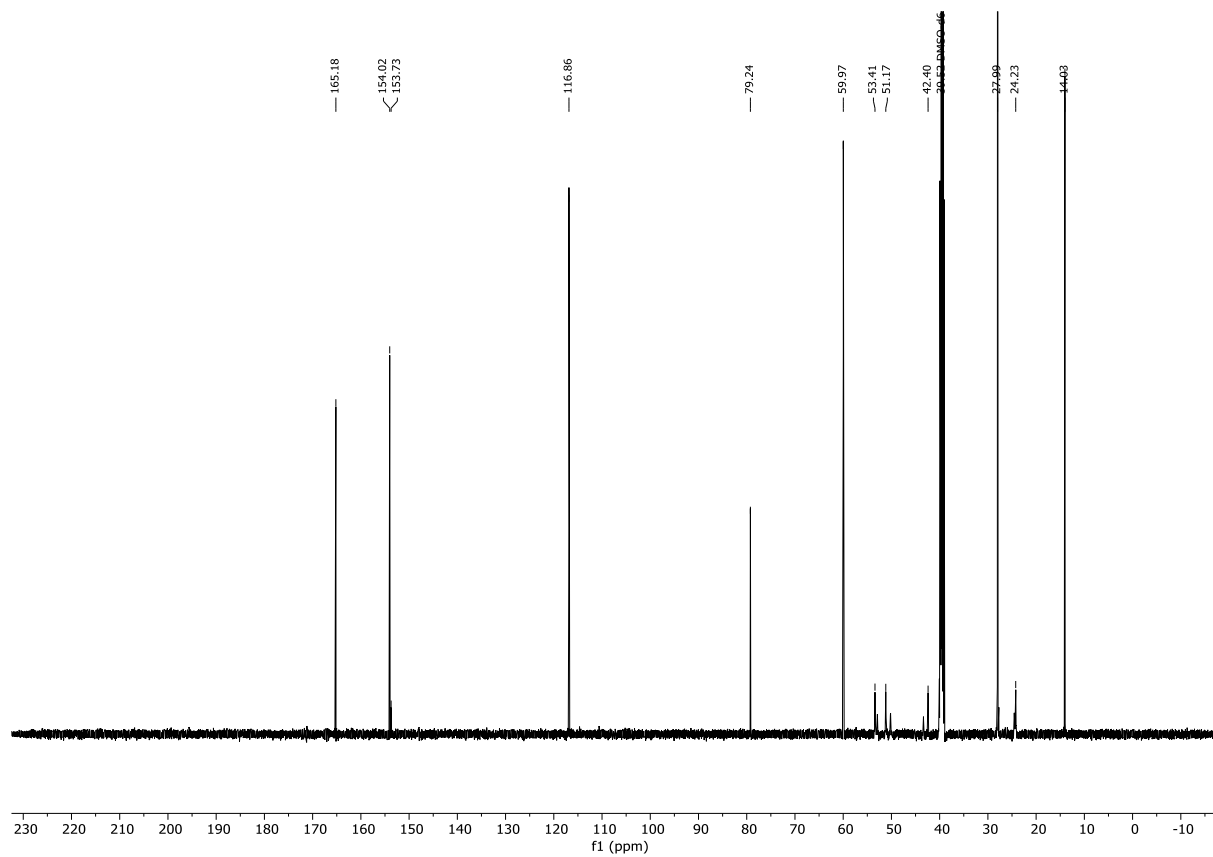
^1H NMR spectrum (DMSO- d_6) of 17.



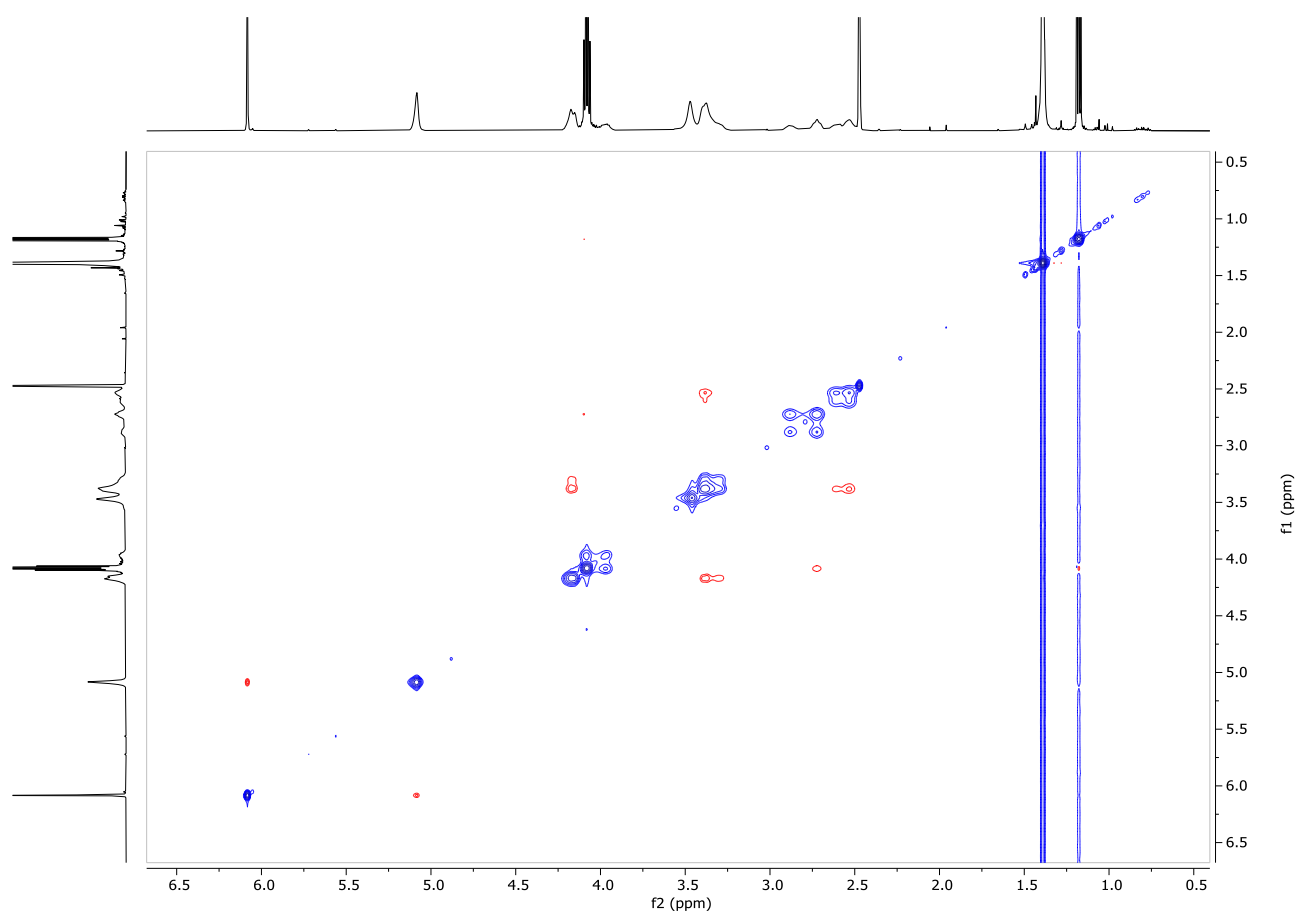
^{13}C NMR spectrum (DMSO- d_6) of 17

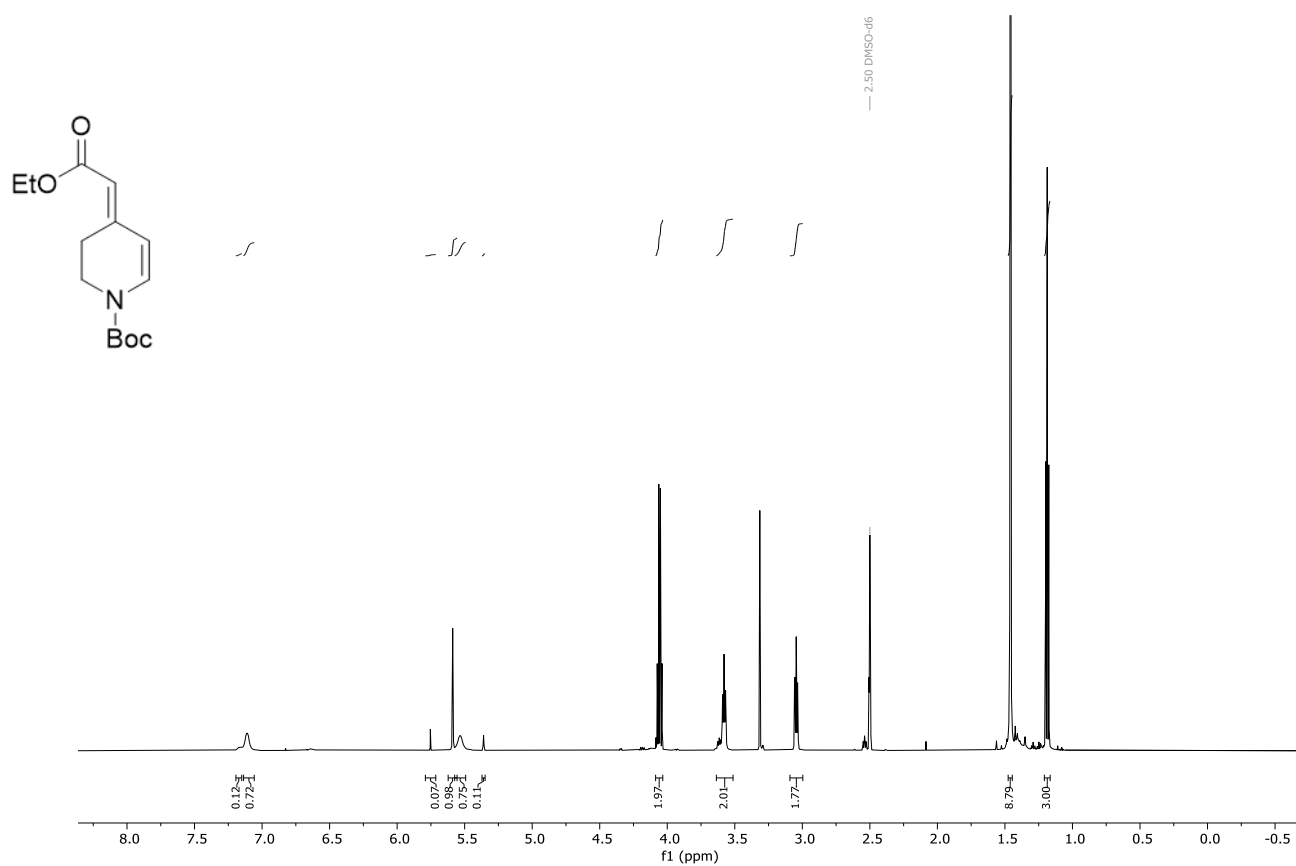


¹H NMR spectrum (DMSO-d₆) of **16**.

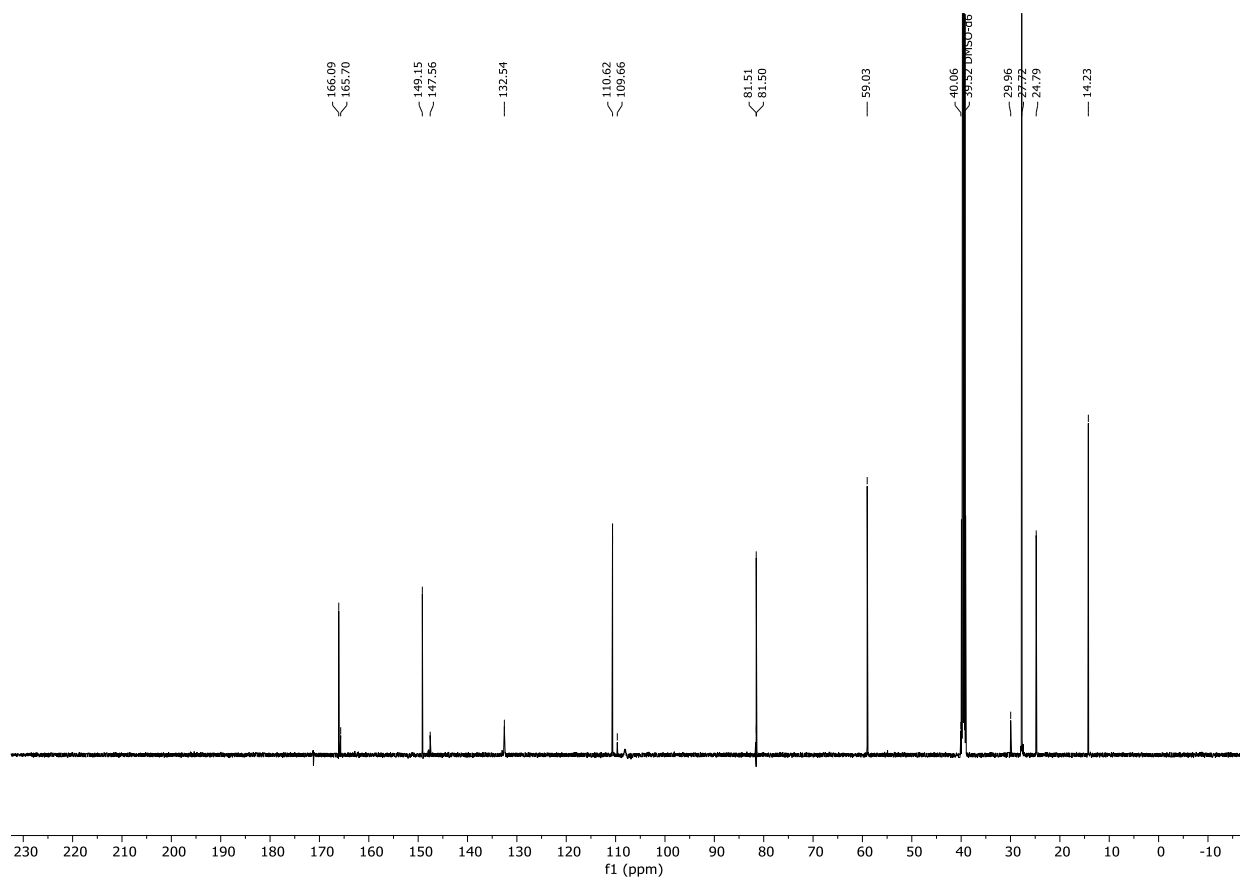


¹³C NMR spectrum (DMSO-d₆) of **16**.

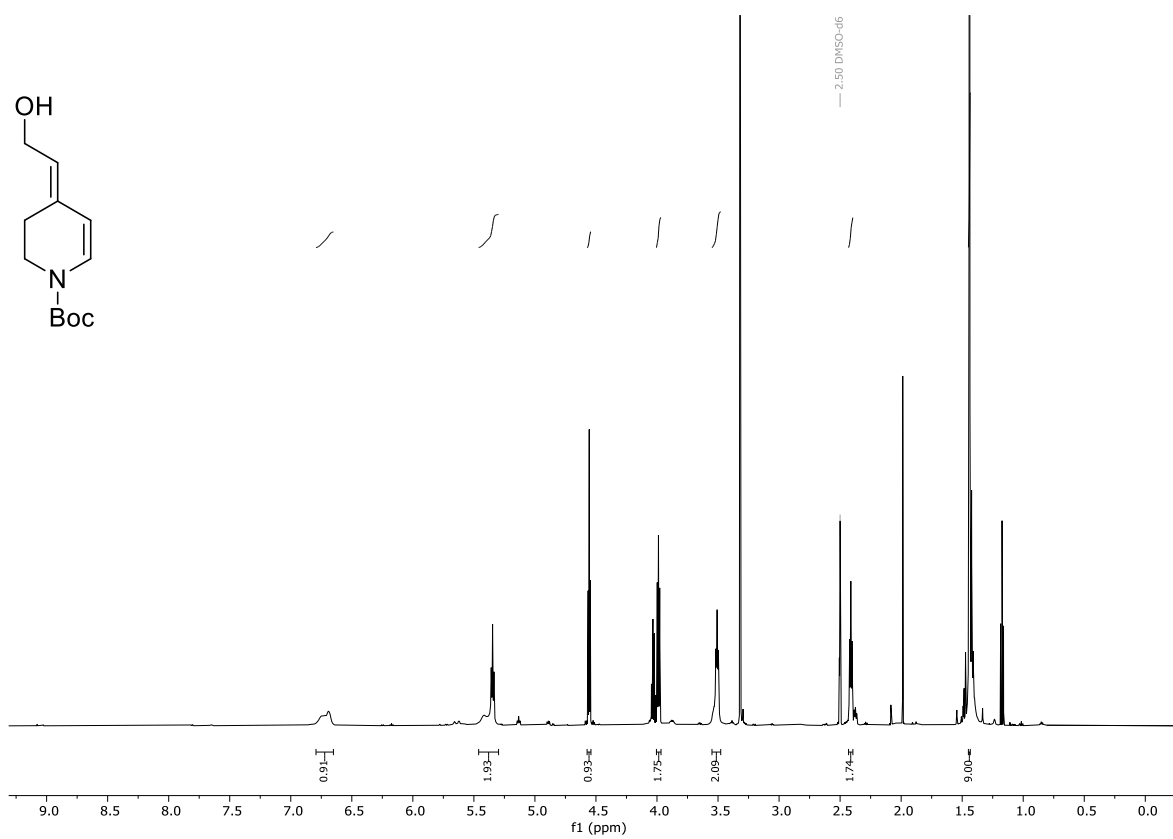
NOESY spectrum of **16**



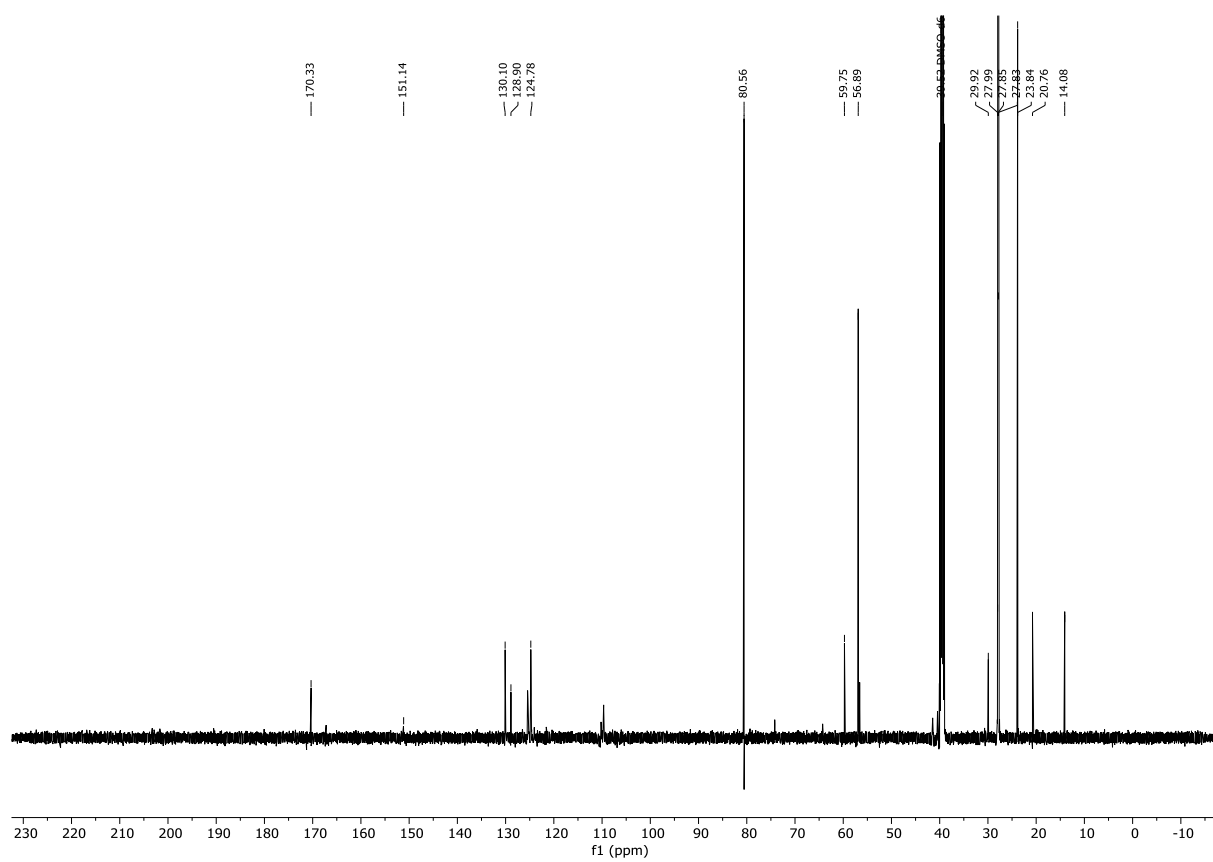
¹H NMR spectrum (DMSO-d₆) of **15**.



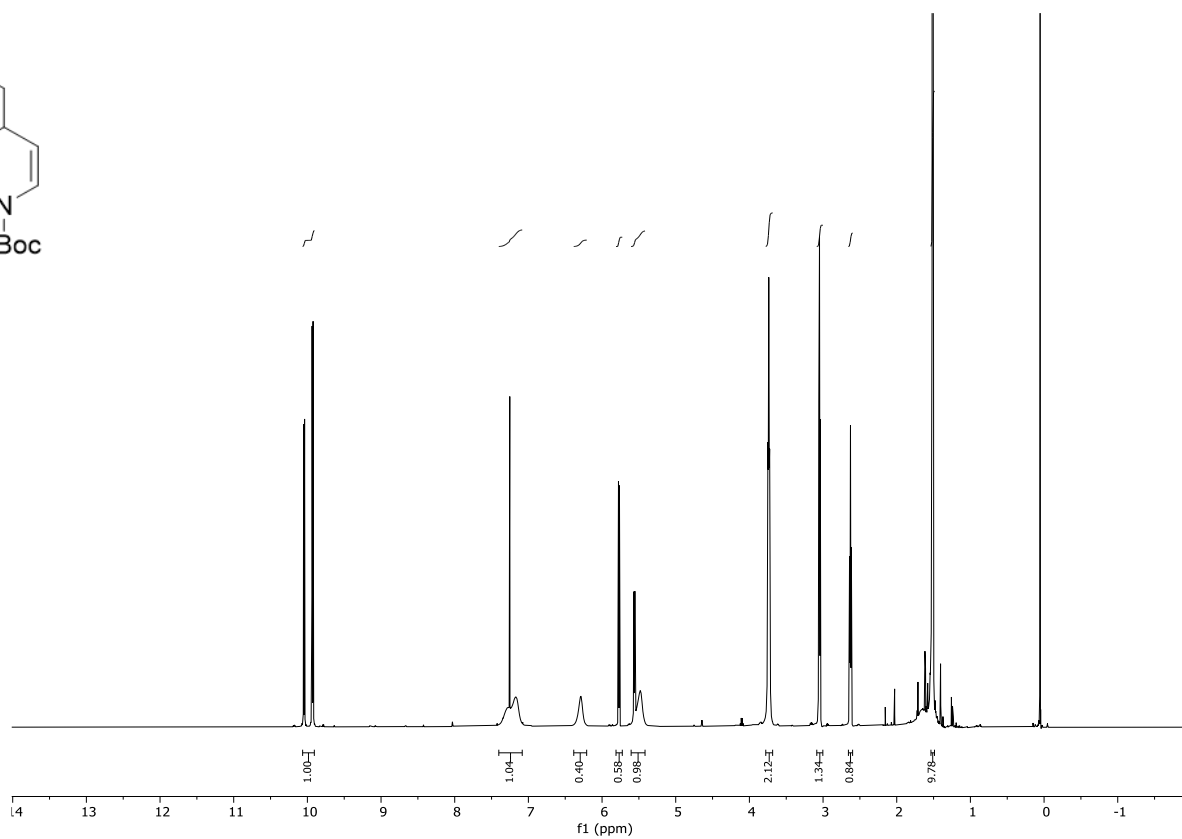
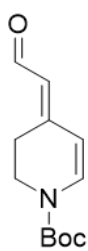
¹³C NMR spectrum (DMSO-d₆) of **15**.



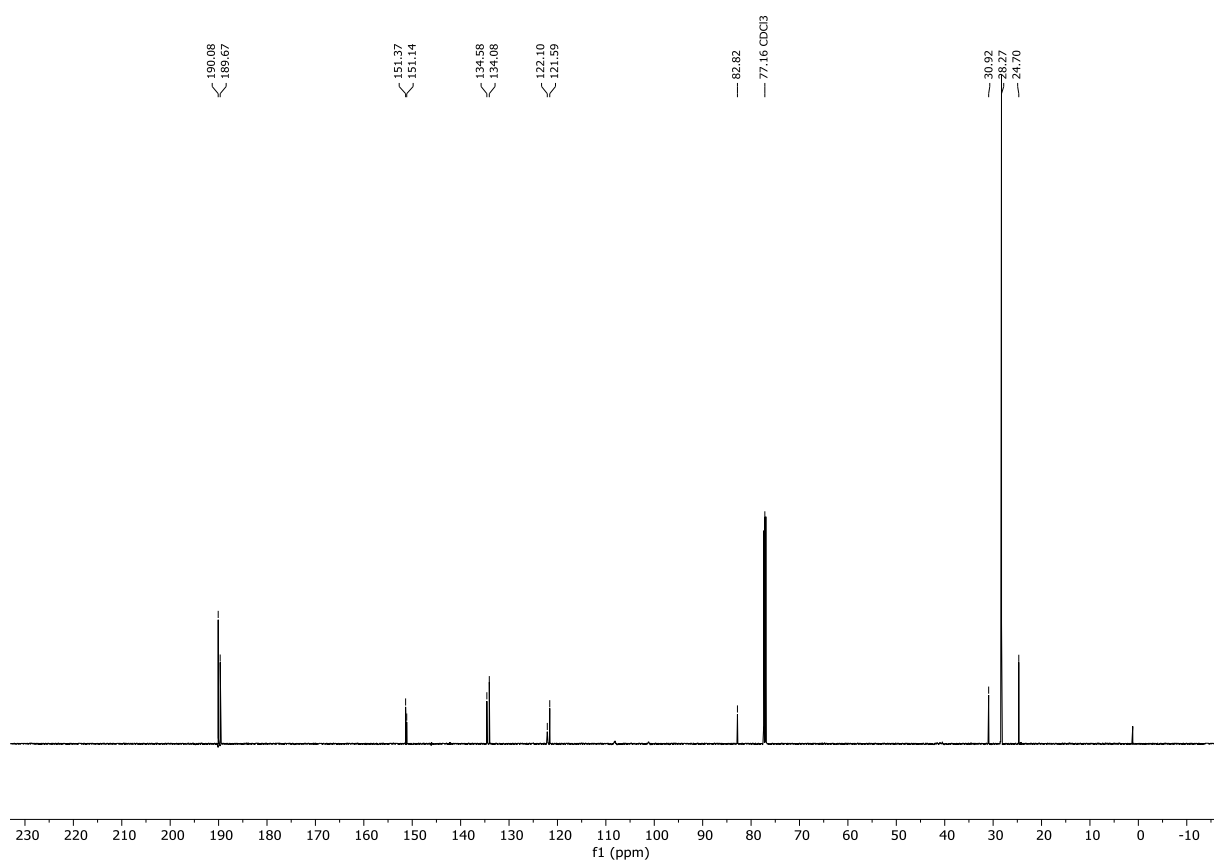
¹H NMR spectrum (DMSO-d₆) of **20**.



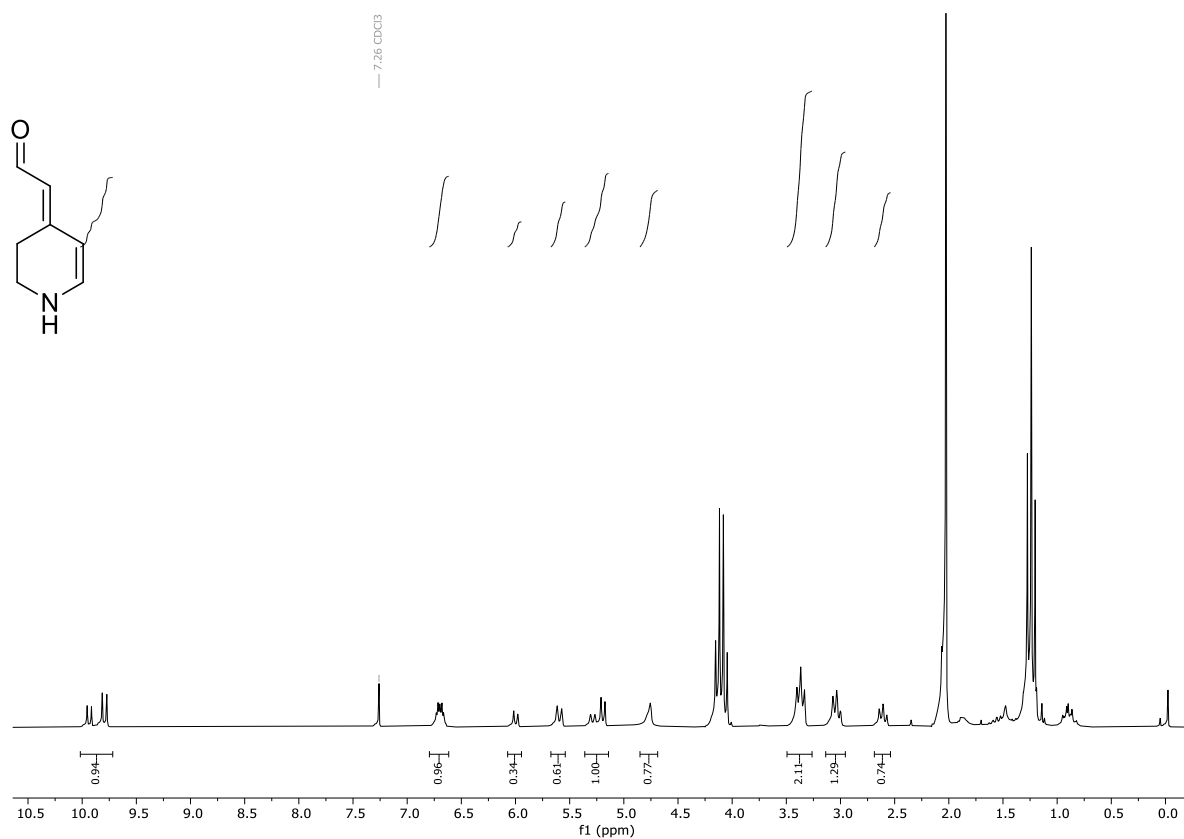
¹³C NMR spectrum (DMSO-d₆) of **20**.



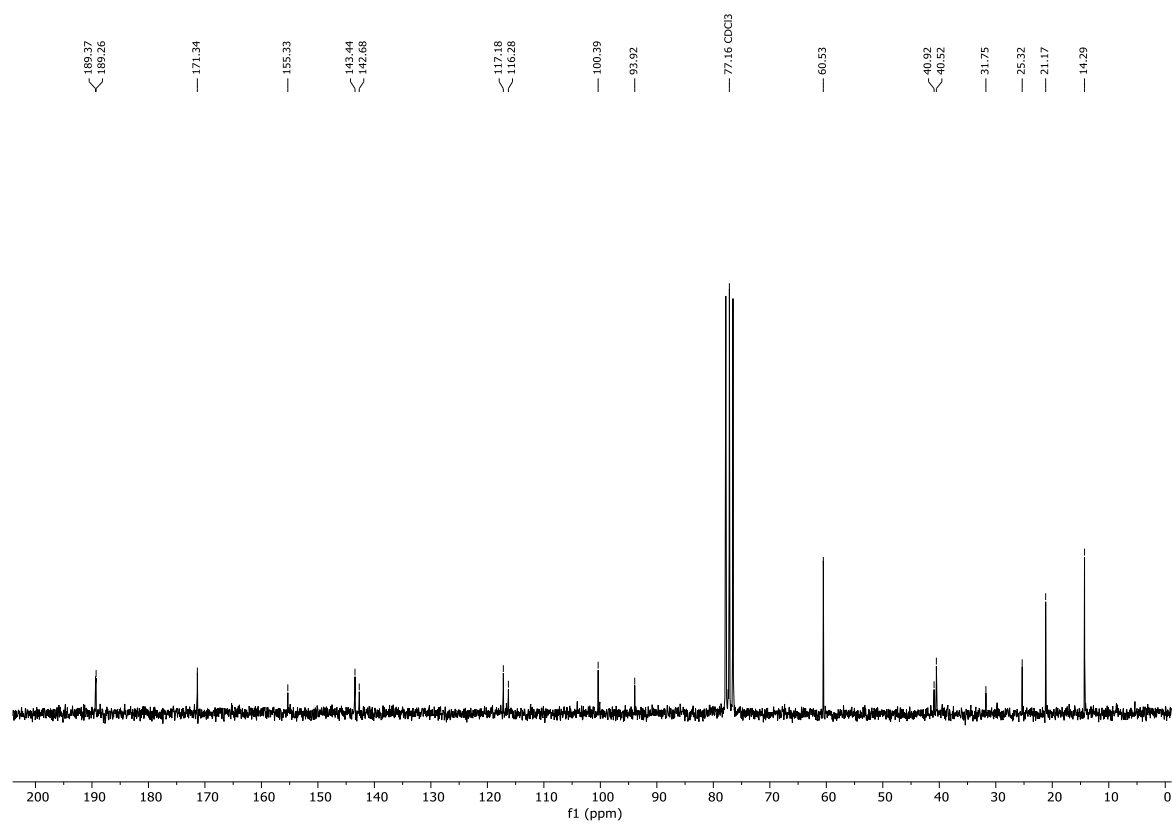
^1H NMR spectrum (CDCl_3) of **21**.



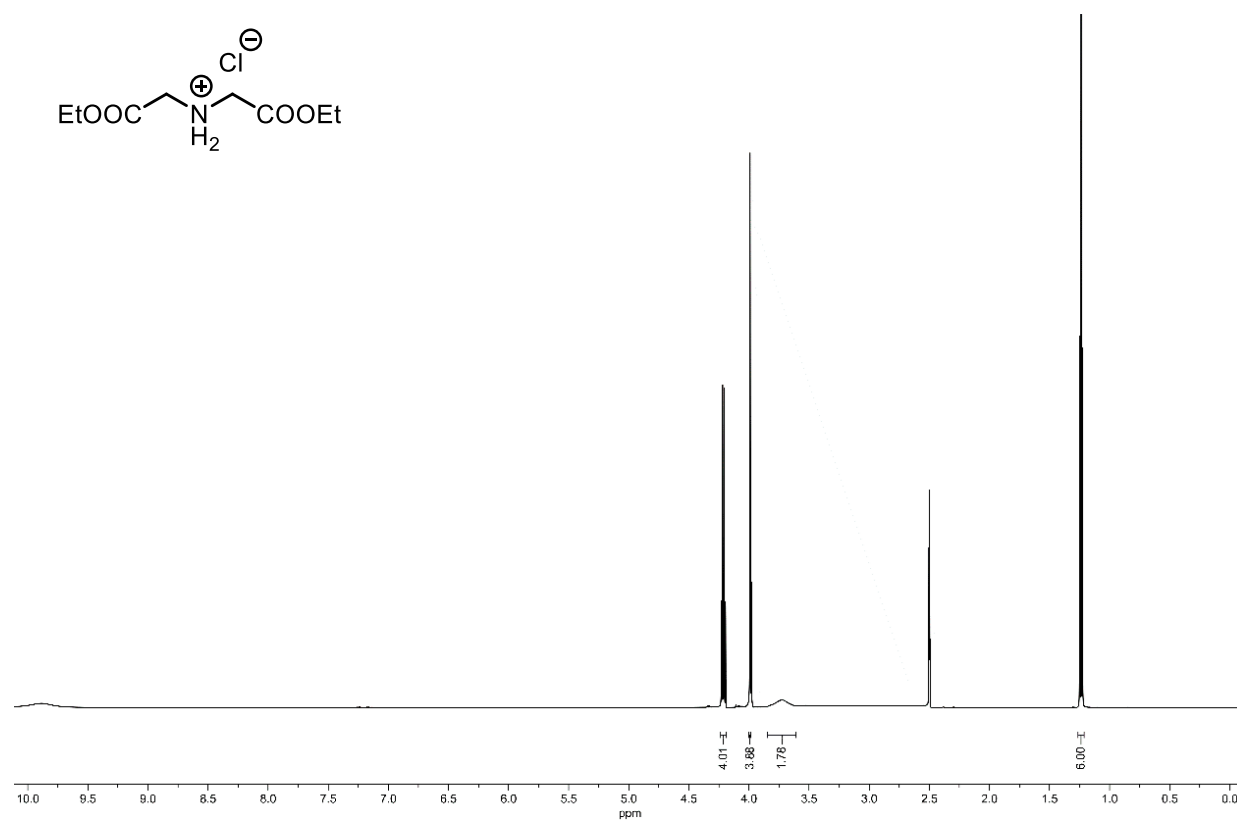
^{13}C NMR spectrum (CDCl_3) of **21**.



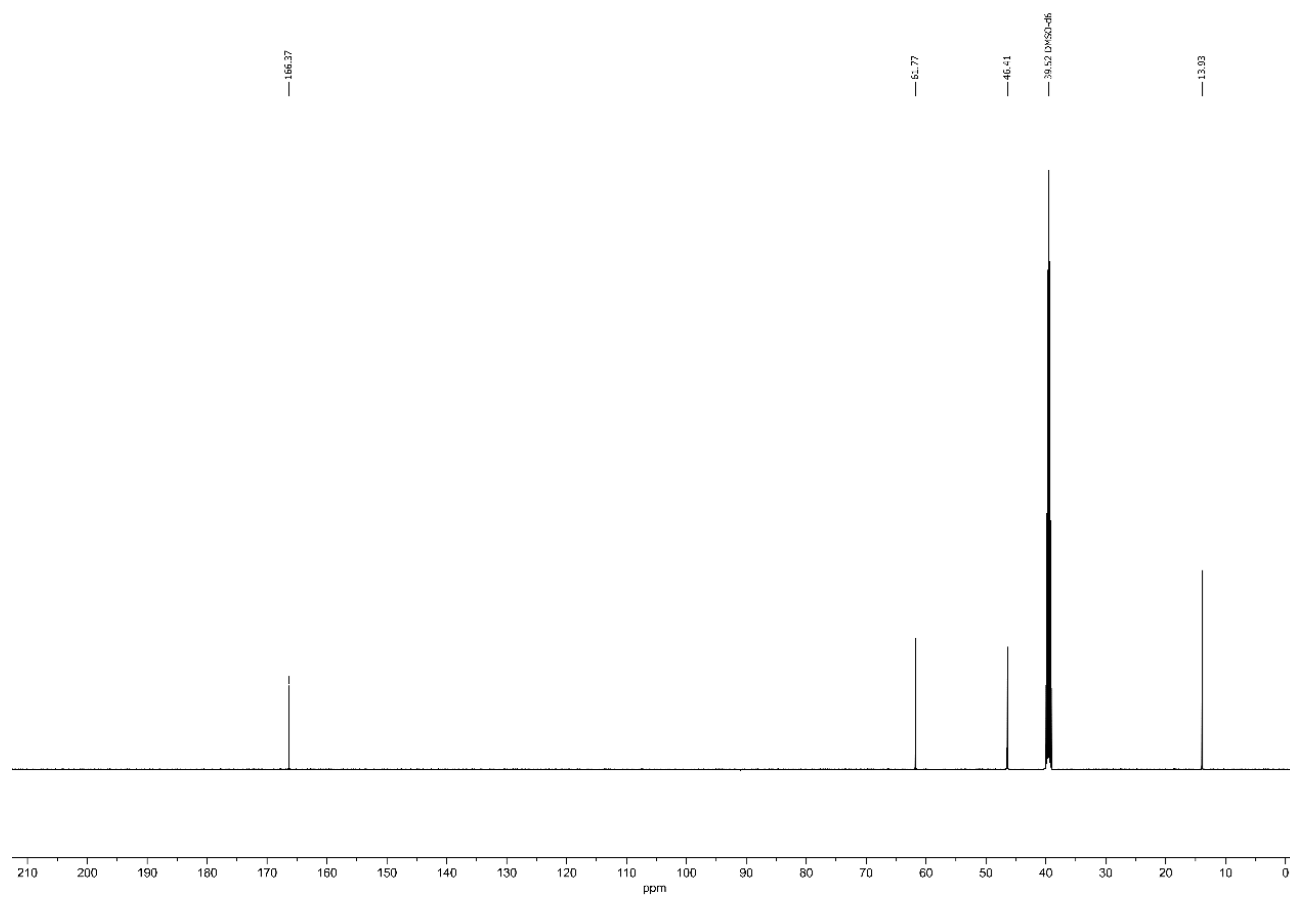
^1H NMR spectrum (CDCl_3) of **13**.



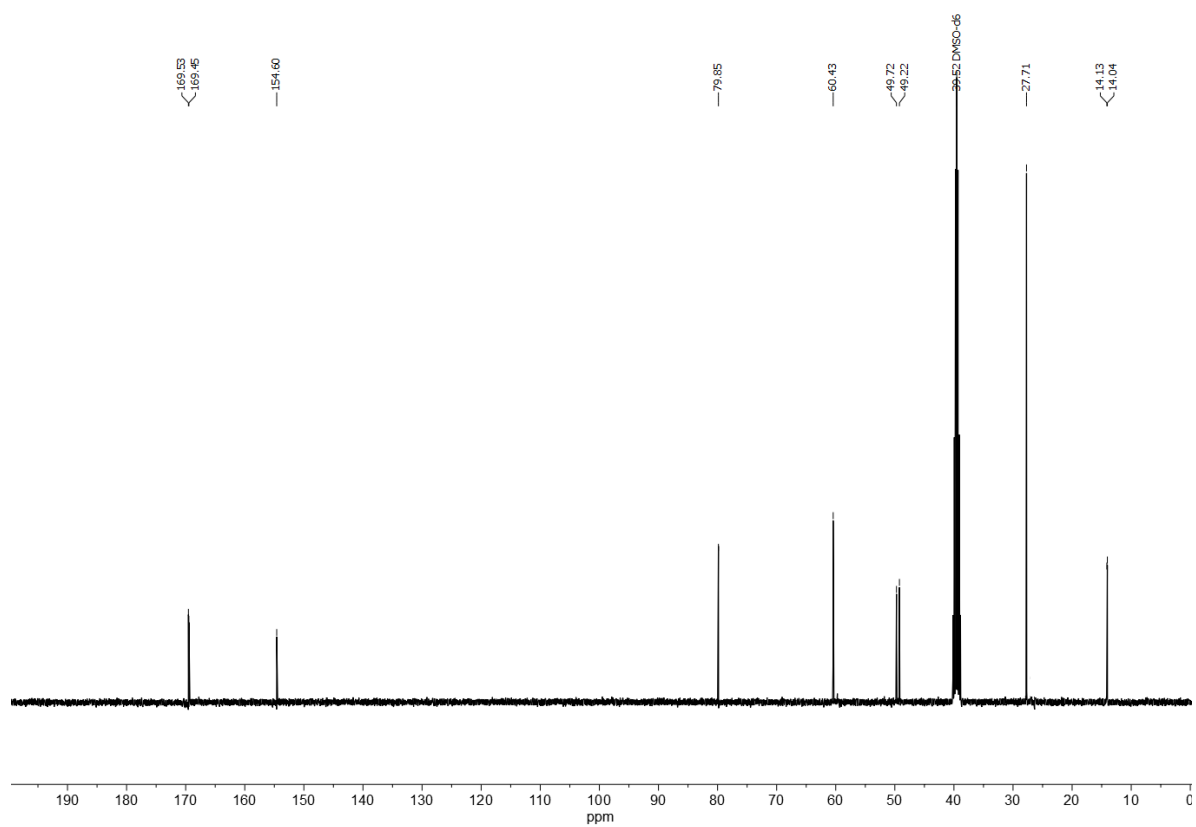
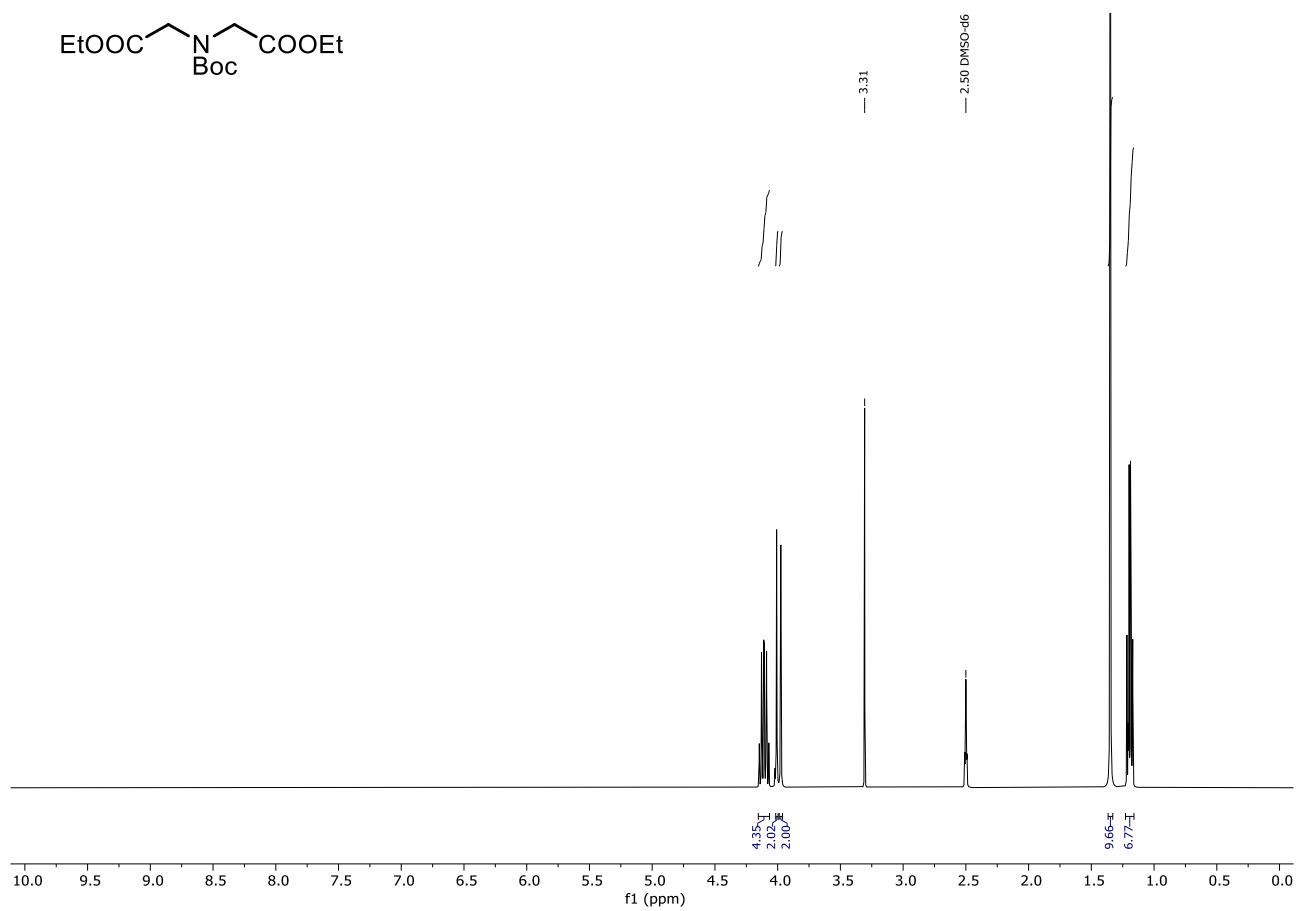
^{13}C NMR spectrum (CDCl_3) of **13**.

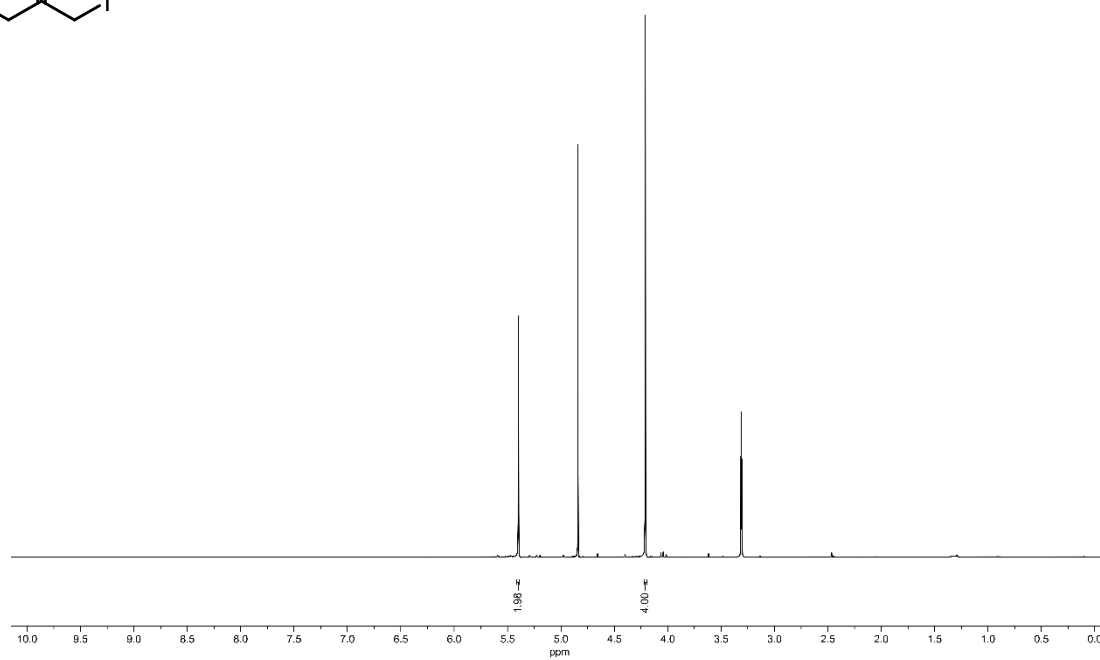
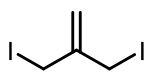


¹H NMR spectrum (DMSO-*d*₆) of **28**.

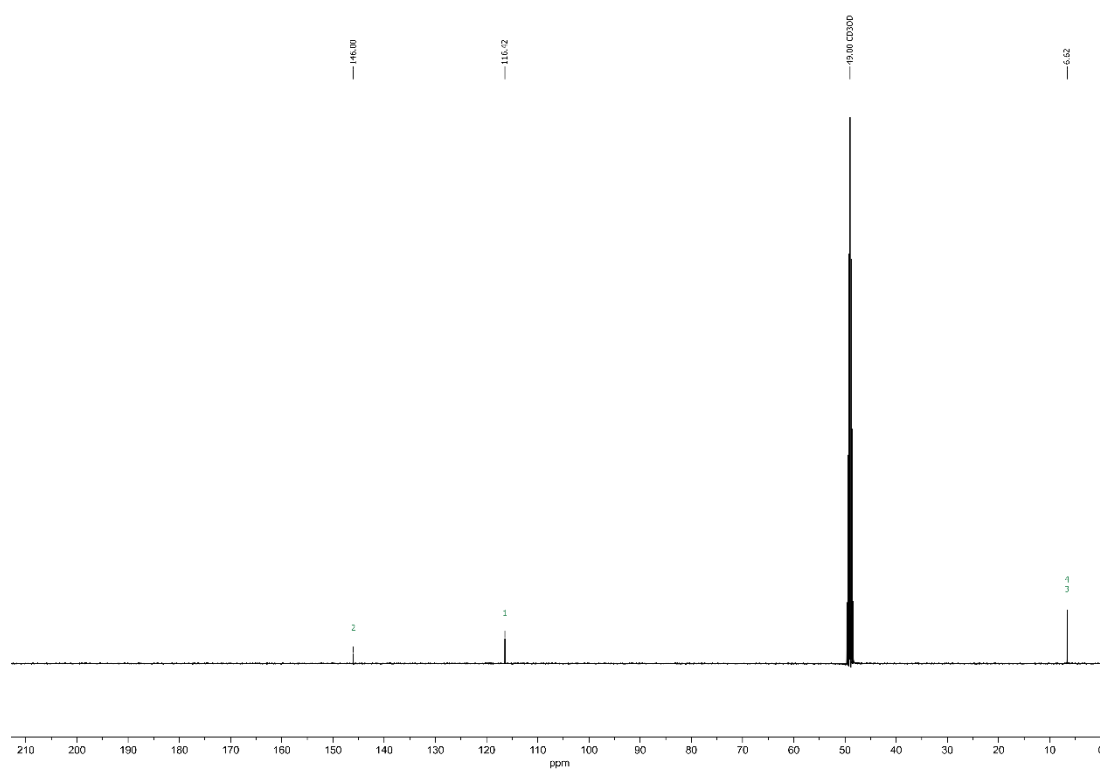


¹³C NMR spectrum (DMSO-*d*₆) of **28**.

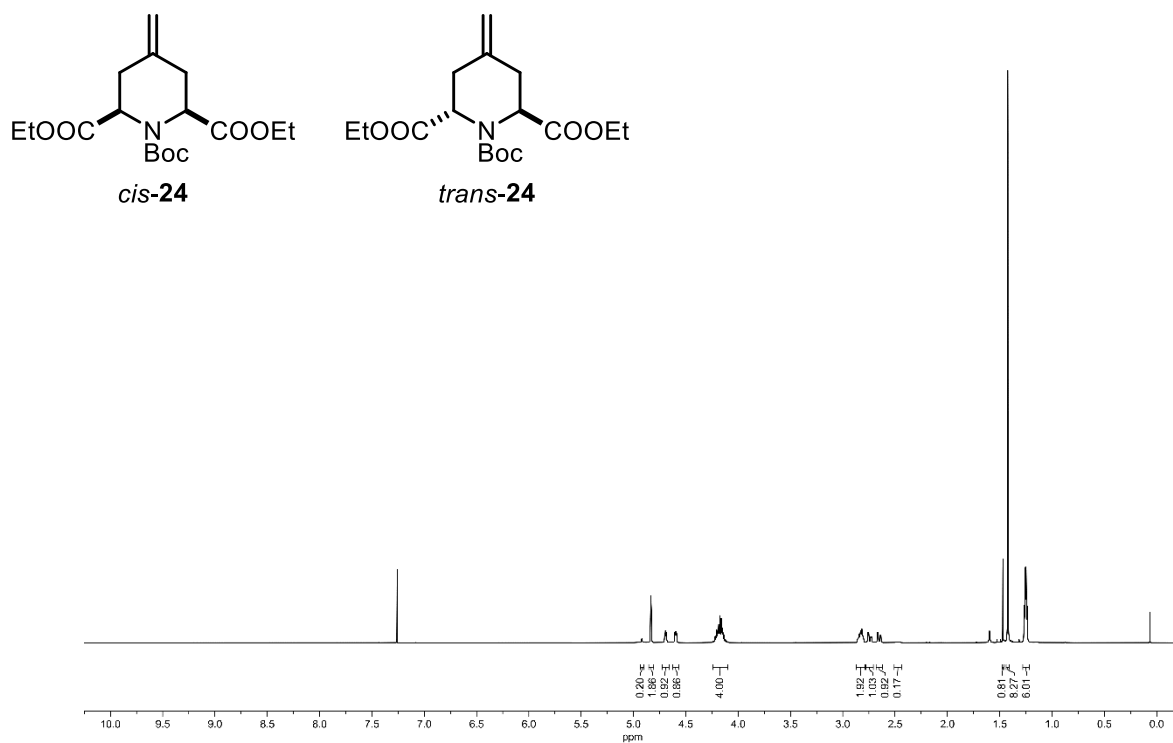




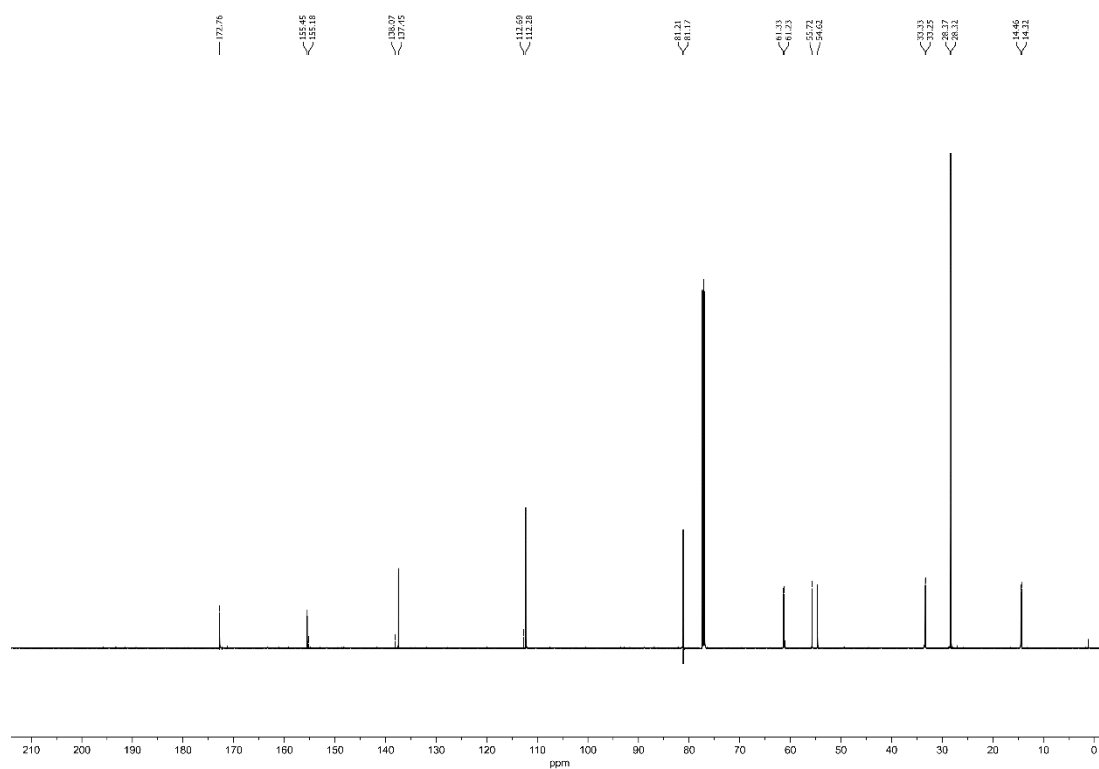
¹H NMR spectrum (CD₃OD) of **29**.



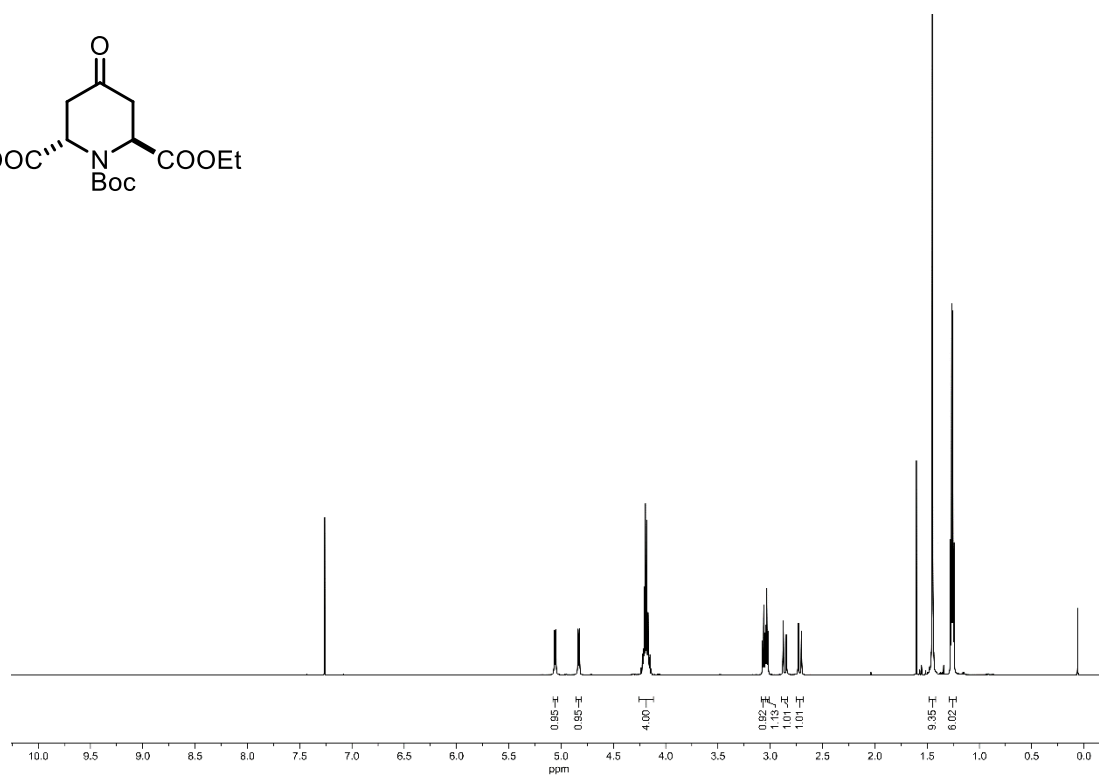
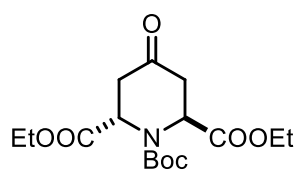
¹³C NMR spectrum (CD₃OD) of **29**.



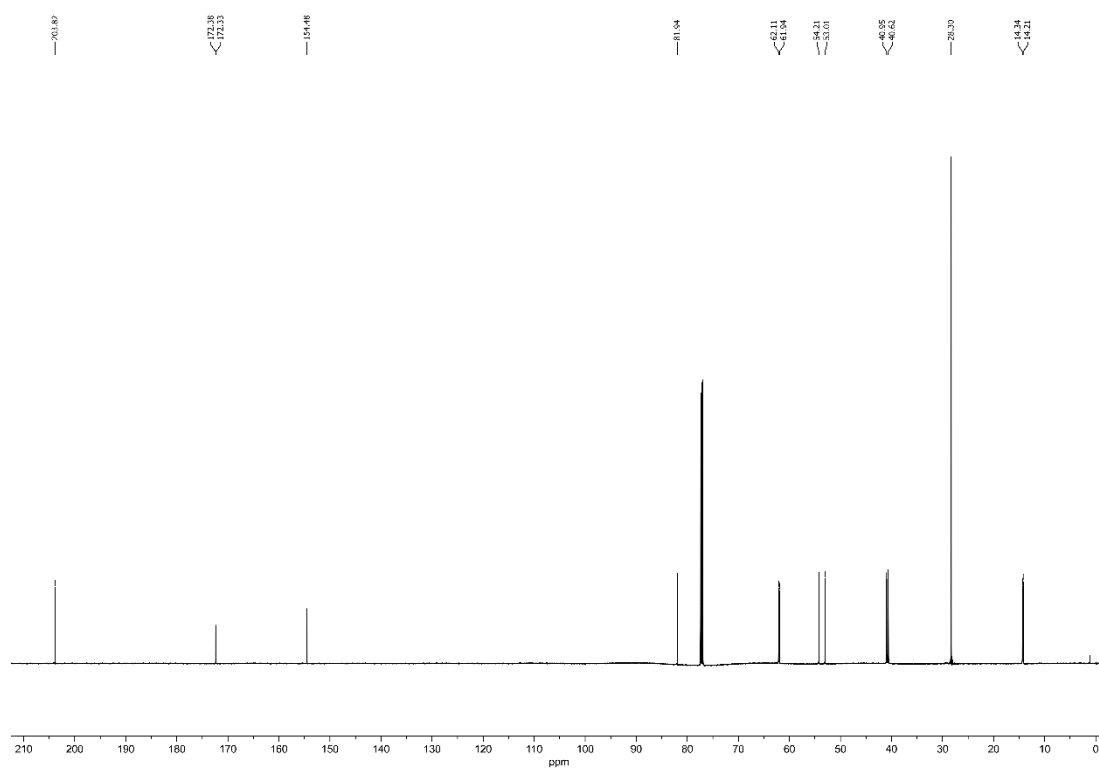
^1H NMR spectrum (CDCl_3) of **24**. The diastereomers were obtained in a 9:1 ratio.



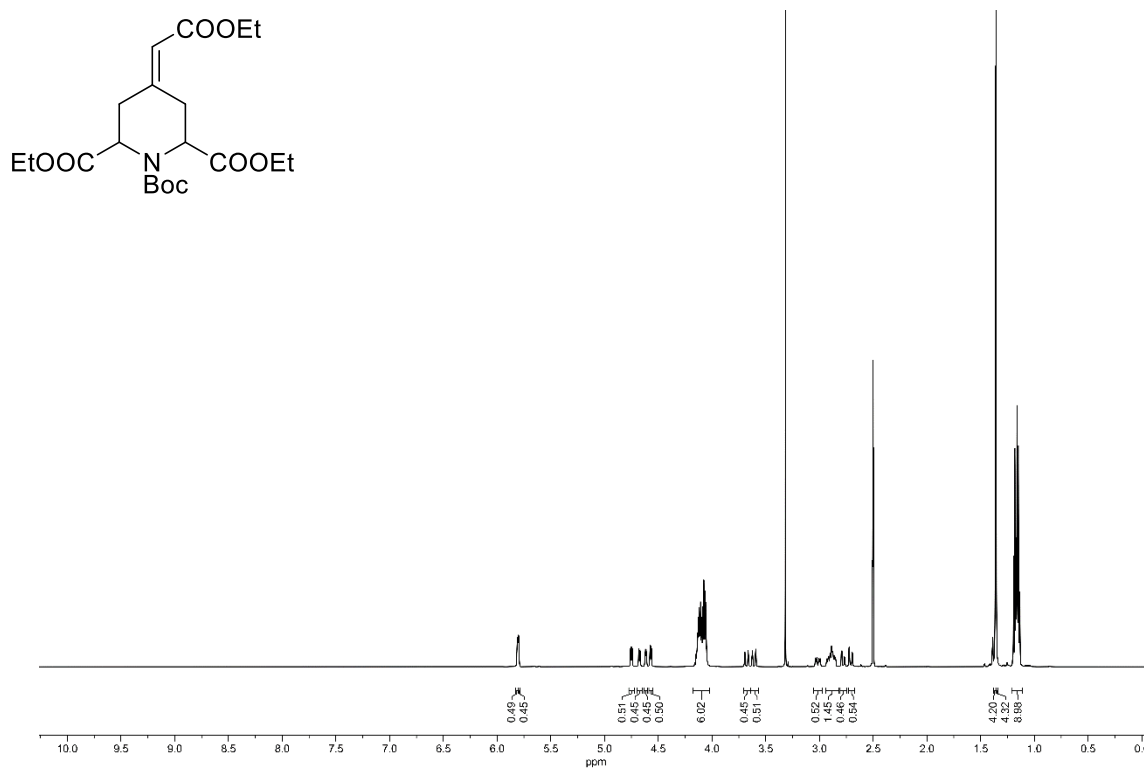
^{13}C NMR spectrum (CDCl_3) of **24**. The diastereomers were obtained in a 9:1 ratio.



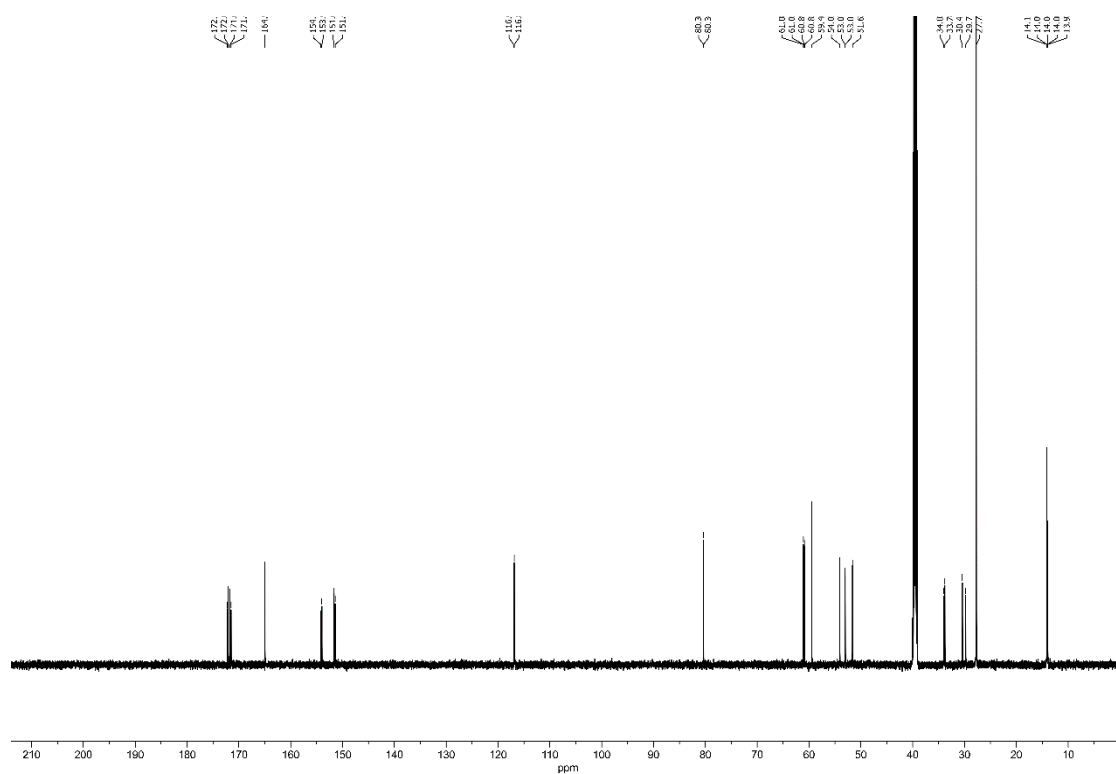
^1H NMR spectrum (CDCl_3) of **30**.



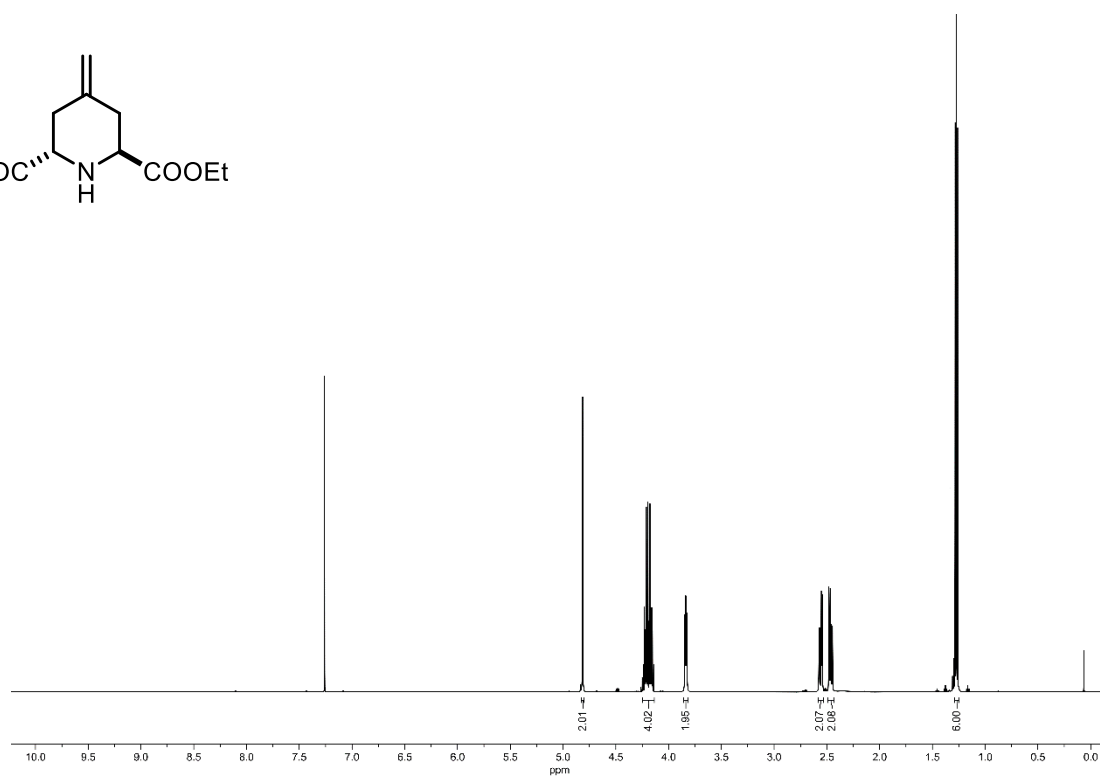
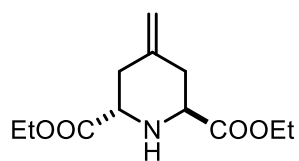
^{13}C NMR spectrum (CDCl_3) of **30**.



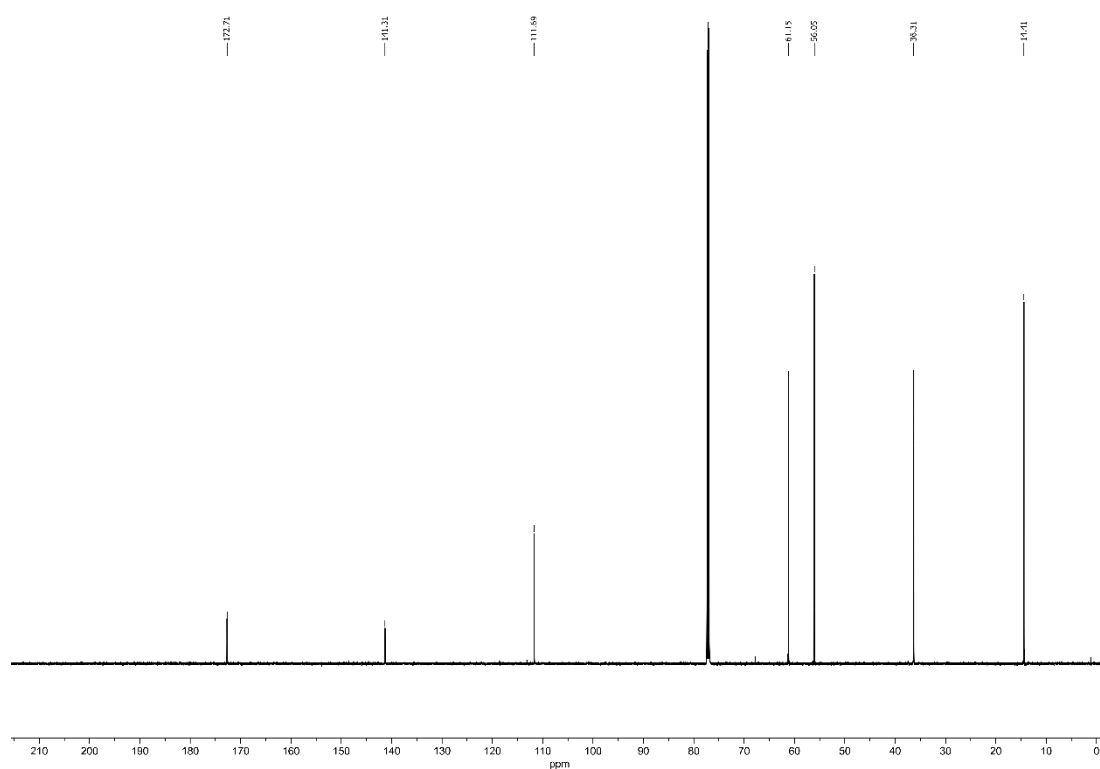
¹H NMR spectrum (DMSO-*d*₆) of **31**.



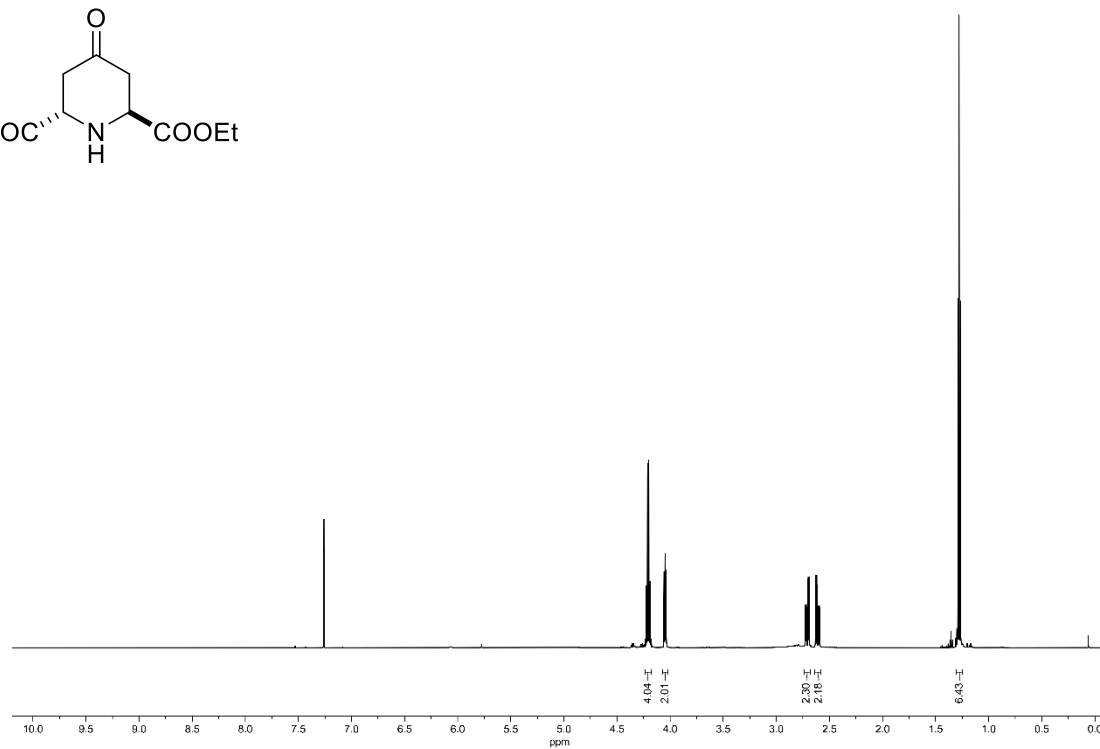
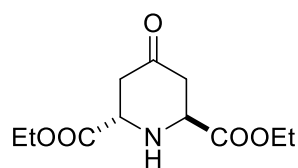
¹³C NMR spectrum (DMSO-*d*₆) of **31**.



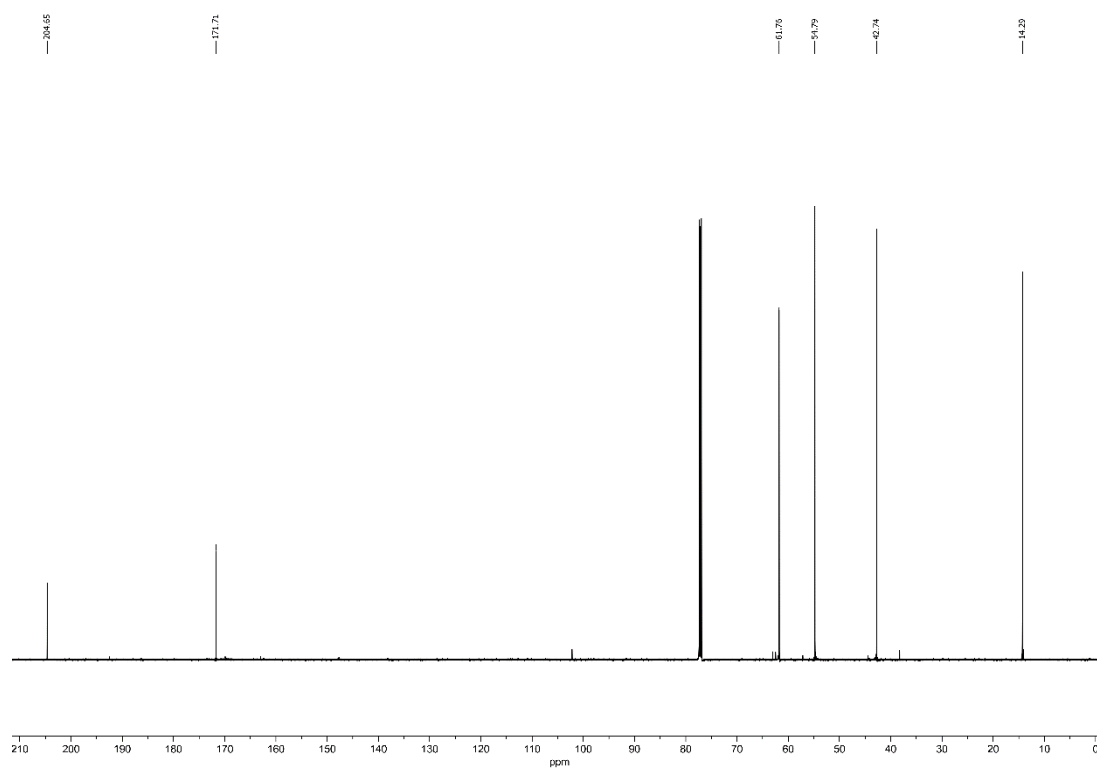
^1H NMR spectrum (CDCl_3) of **32**.



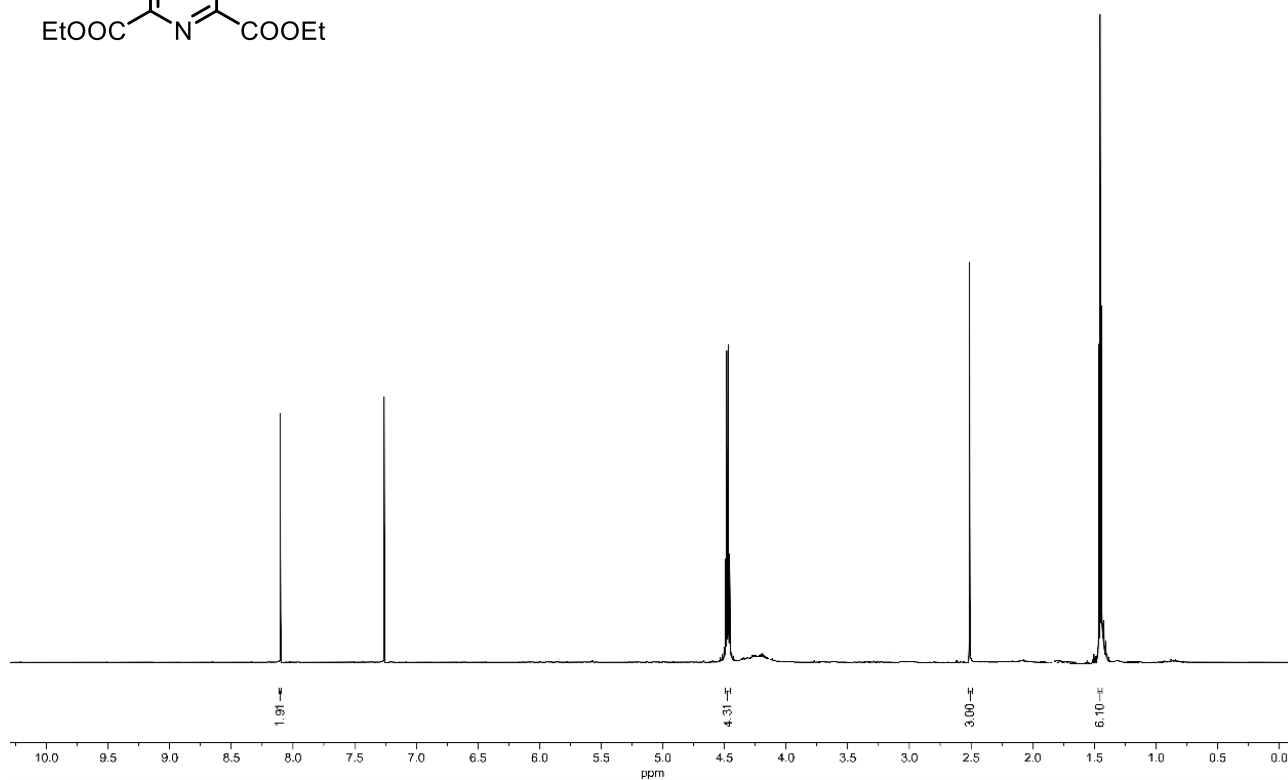
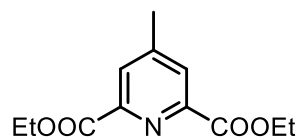
^{13}C NMR spectrum (CDCl_3) of **32**.



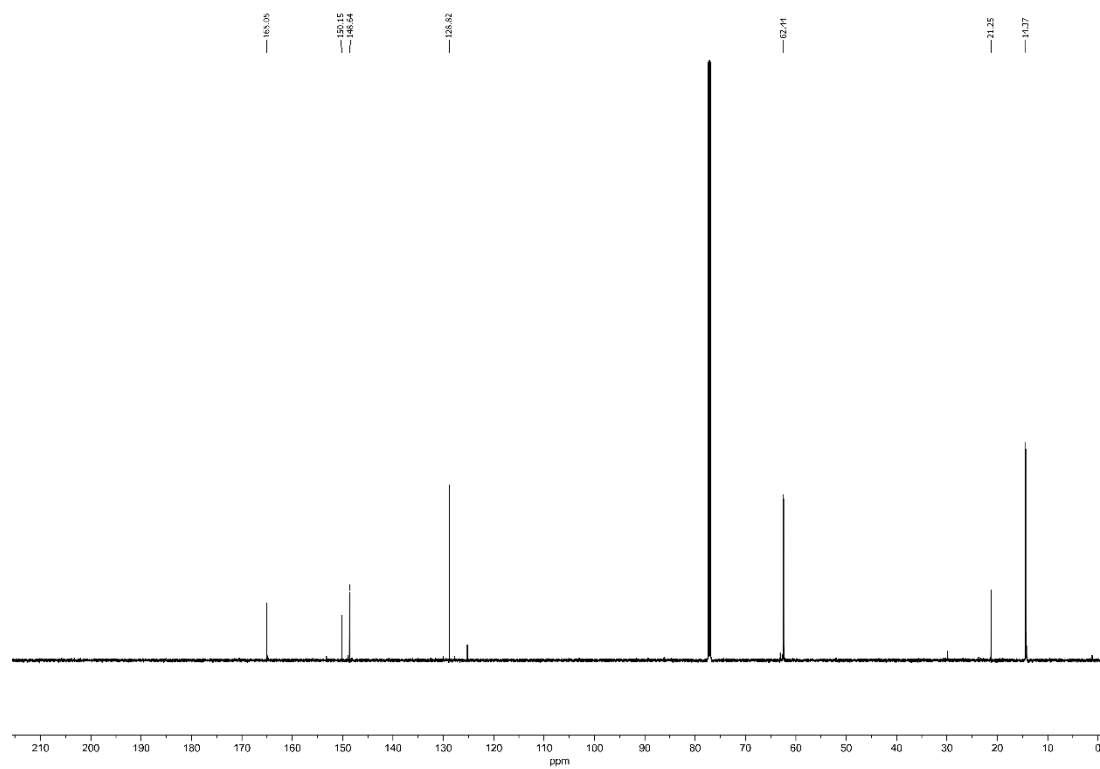
^1H NMR spectrum (CDCl_3) of **33**.



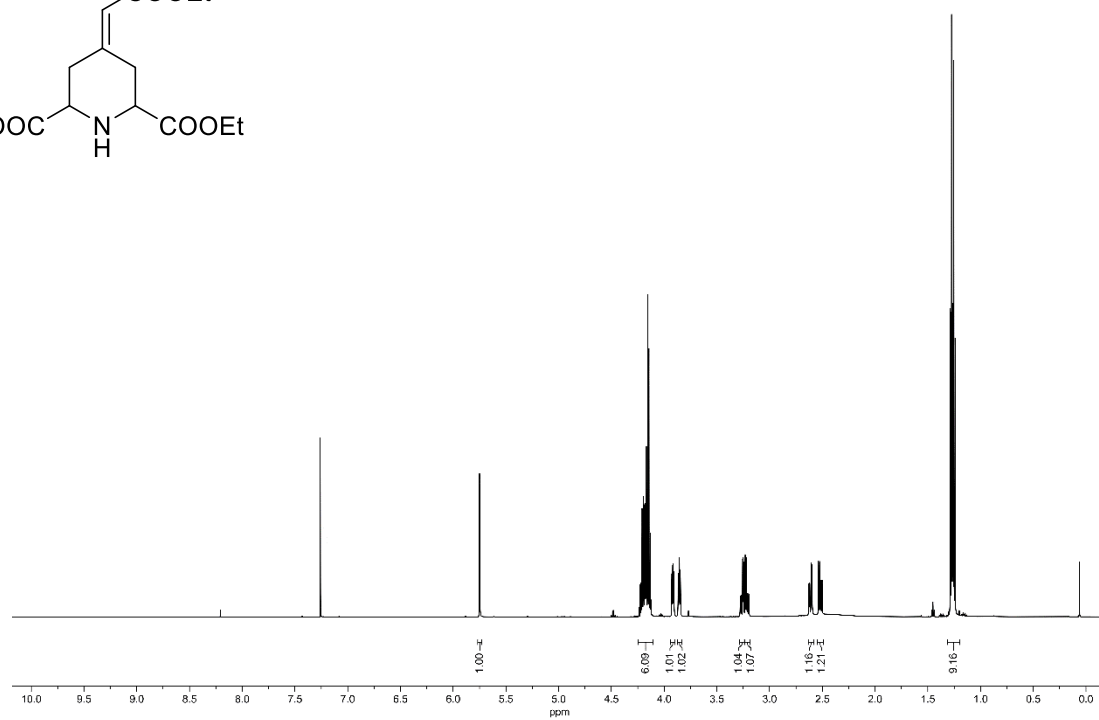
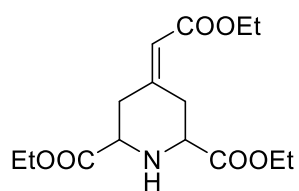
^{13}C NMR spectrum (CDCl_3) of **33**.



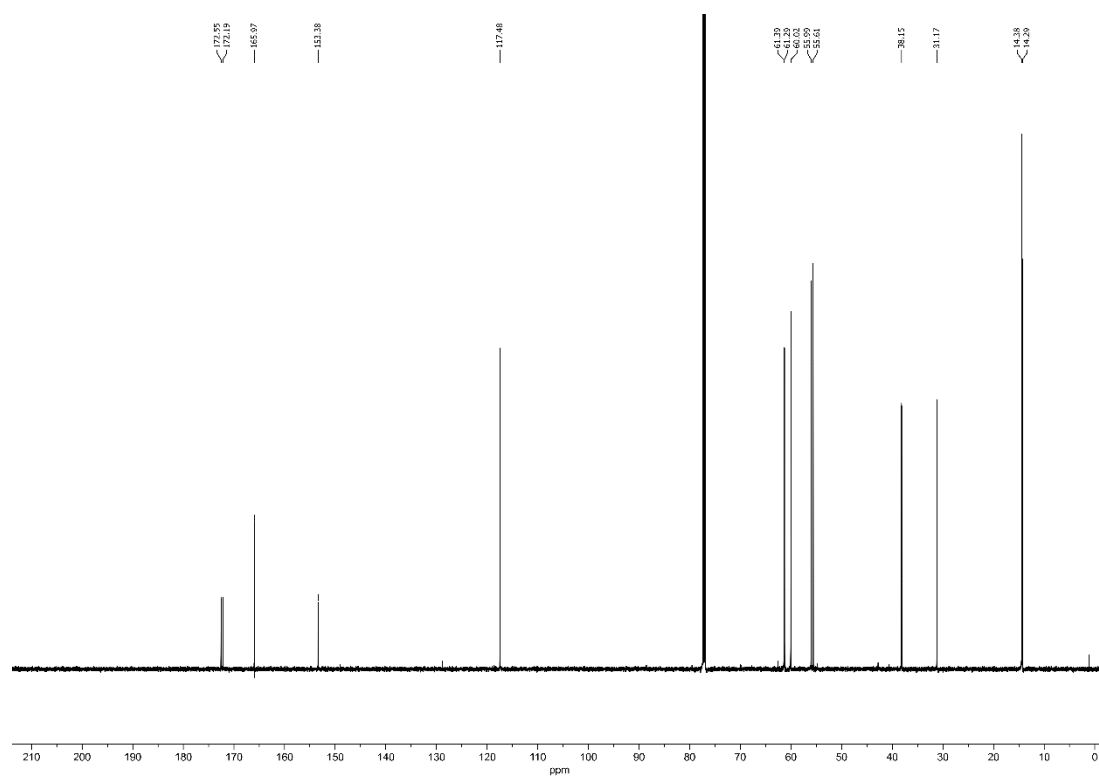
^1H NMR spectrum (CDCl_3) of **38**.



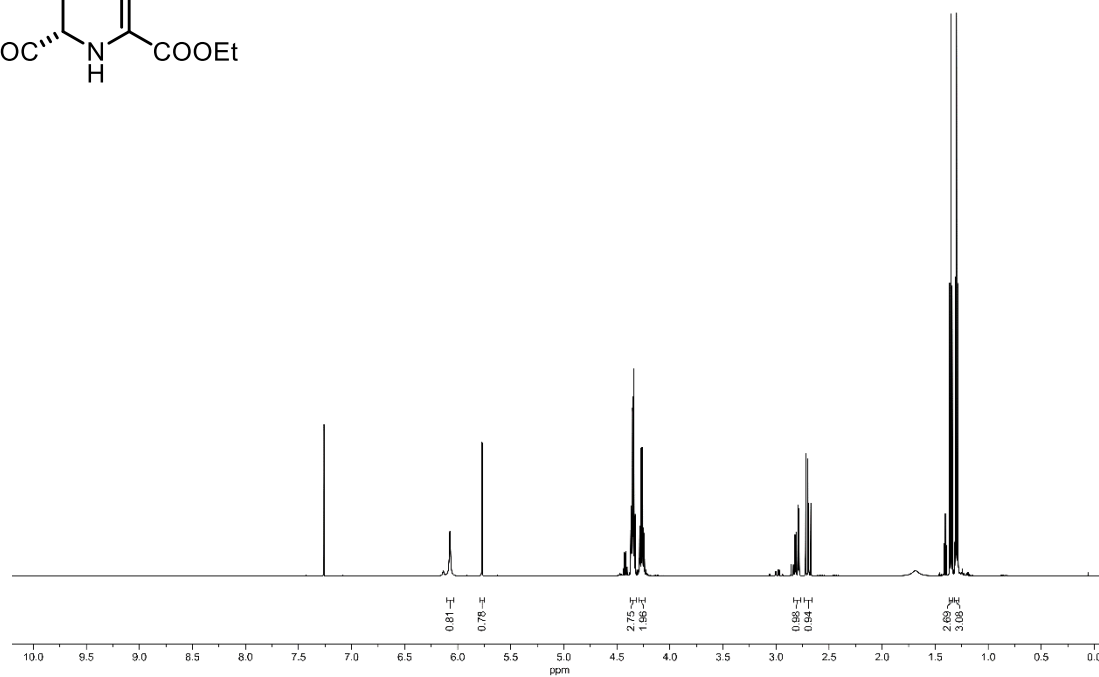
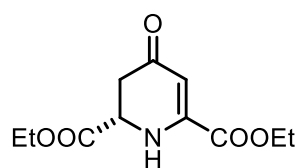
^{13}C NMR spectrum (CDCl_3) of **38**.



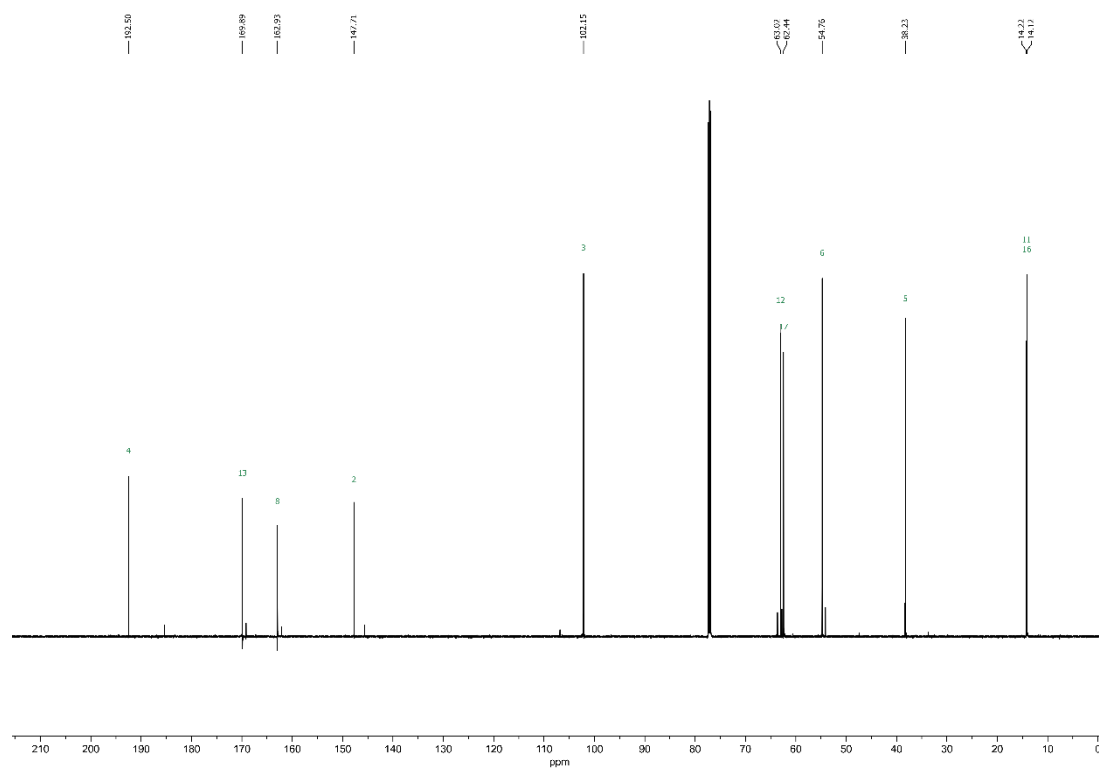
^1H NMR spectrum (CDCl_3) of **34**.



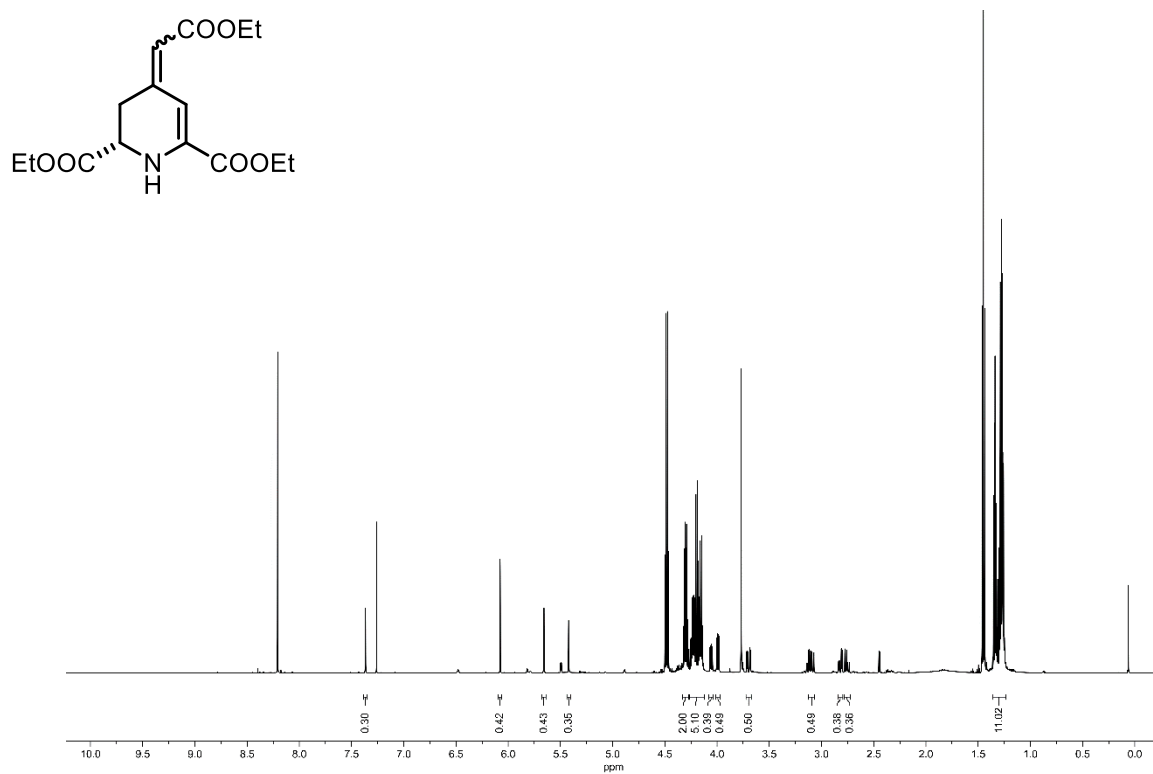
^{13}C NMR spectrum (CDCl_3) of **34**.



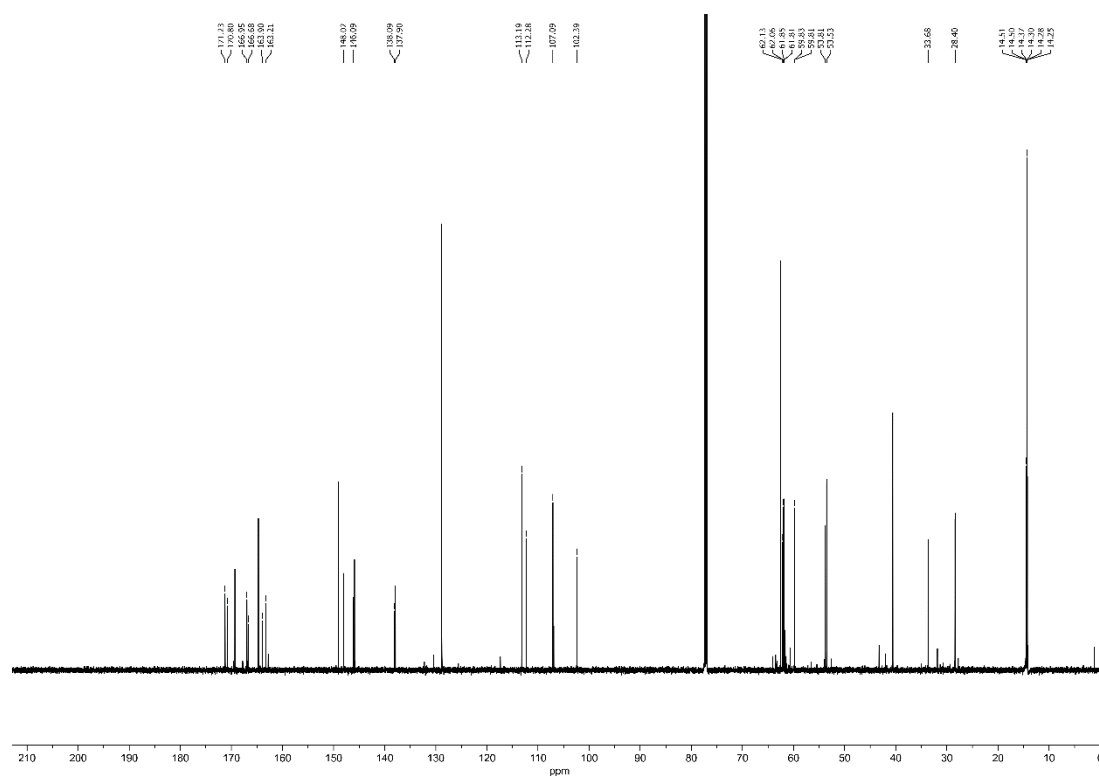
¹H NMR spectrum (CDCl₃) of **36**.



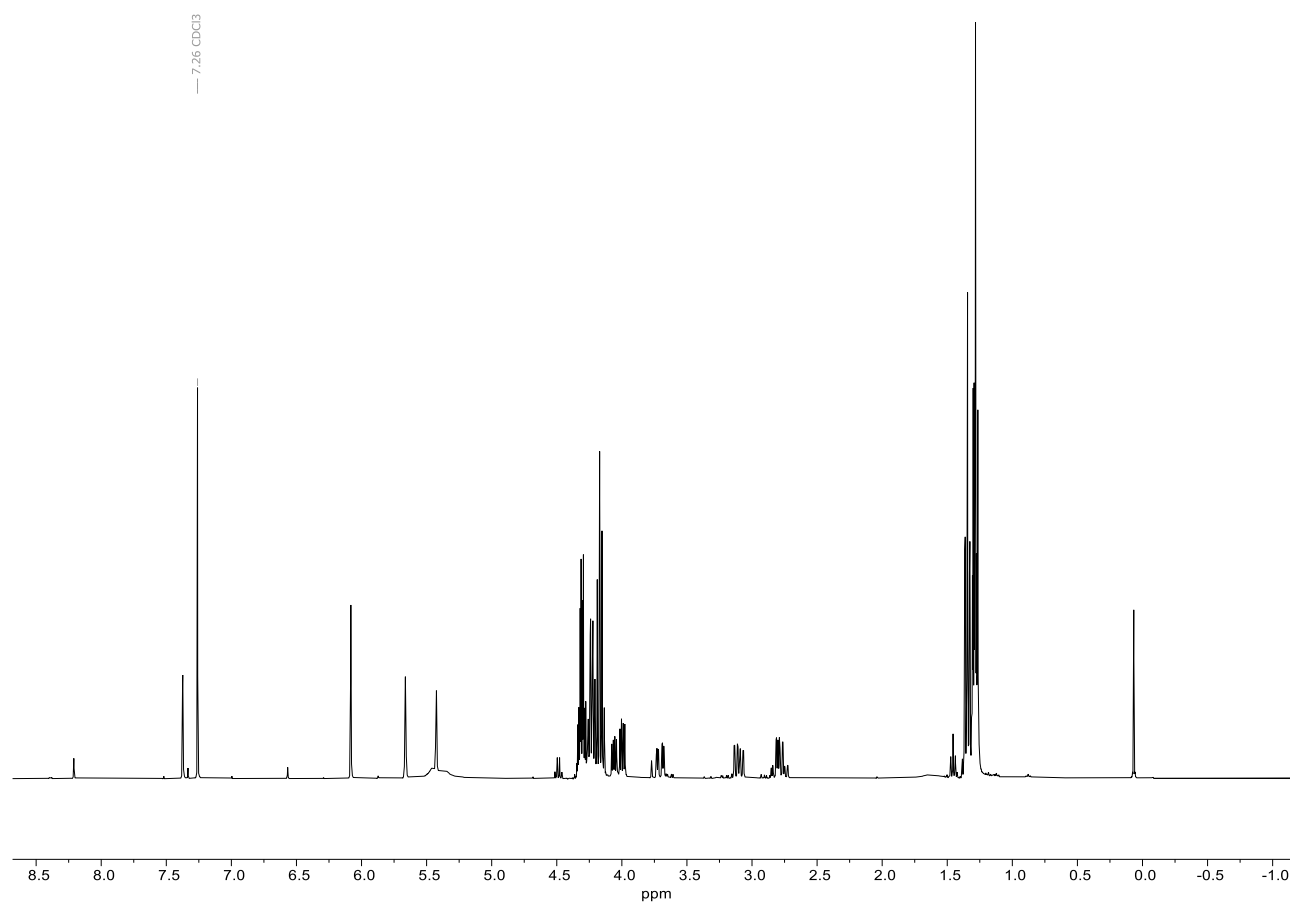
¹³C NMR spectrum (CDCl₃) of **36**.



¹H NMR spectrum (CDCl₃) of **37**. The spectrum contains a mixture of *E/Z* isomers and its oxidized form **40**.



¹³C NMR spectrum (CDCl₃) of **37**. The spectrum contains a mixture of *E/Z* isomers and its oxidized form **40**.



^1H NMR spectrum (CDCl_3) of **37**, 30 minutes after 2nd FC.