Supplementary material

Synthesis and *in silico* docking of new pyrazolo[*4,3-e*]pyrido[*1,2-a*]pyrimidine- based cytotoxic agents

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1. General

NMR spectra were recorded using the Agilent spectrometers DD2 500 MHz and VNMRS 400 MHz (δ given in ppm, J in Hz; typical experiments: APT, H-H-COSY, HMBC, HSQC, NOESY), MS spectra were taken on an Advion Expression CMS instrument. TLC was performed on silica gel (Macherey-Nagel, detection with cerium molybdate reagent); melting points are uncorrected (Leica hot stage microscope, or BUCHI melting point M-565), and elemental analyses were performed on a Foss-Heraeus Vario EL (CHNS) unit. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000 or on a Perkin-Elmer Spectrum Two (UATR Two Unit). The solvents were dried according to usual procedures.

2. Cytotoxicity assay (SRB assay)

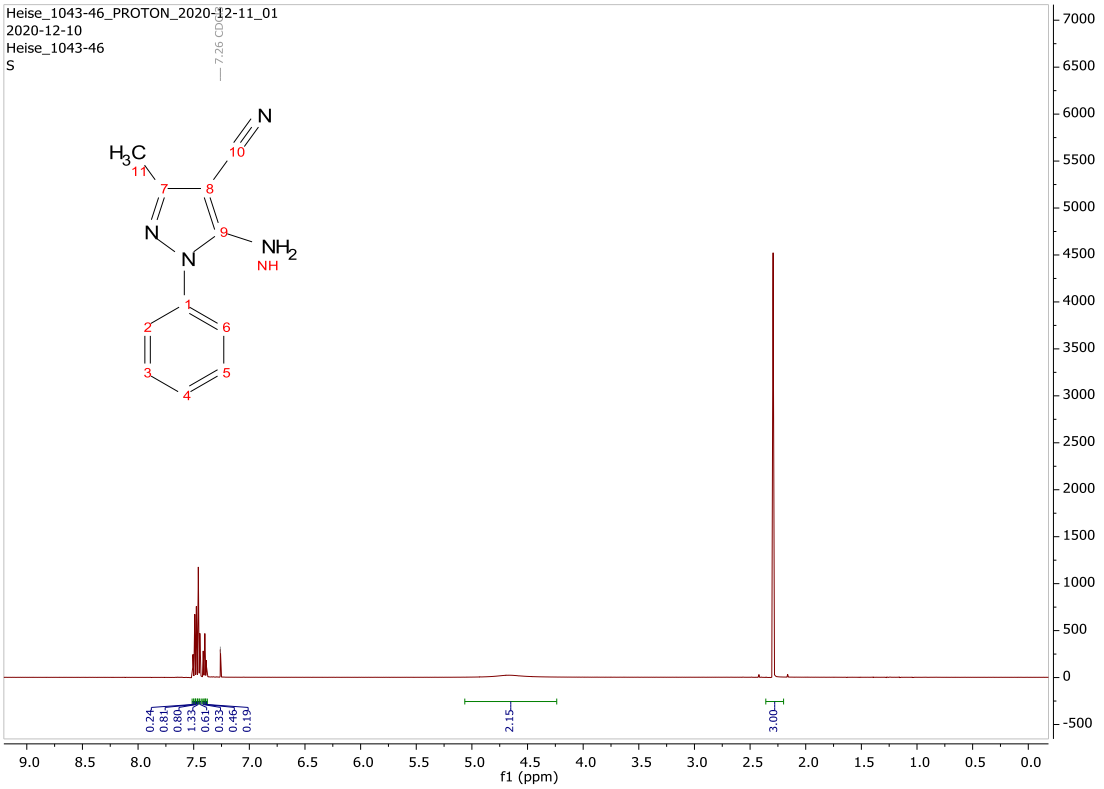
The cell lines were obtained from Department of oncology (Martin-Luther-University Halle Wittenberg). Cultures were maintained as monolayers in RPMI 1640 medium with L-glutamine (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 10% heat inactivated fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and penicillin/streptomycin (1%, Capricorn Scientific GmbH, Ebsdorfergrund, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (Kiton-Red S, ABCR) micro culture colorimetric assay using confluent cells in 96-well plates with the seeding of the cells on day 0 applying appropriate cell densities to prevent confluence of the

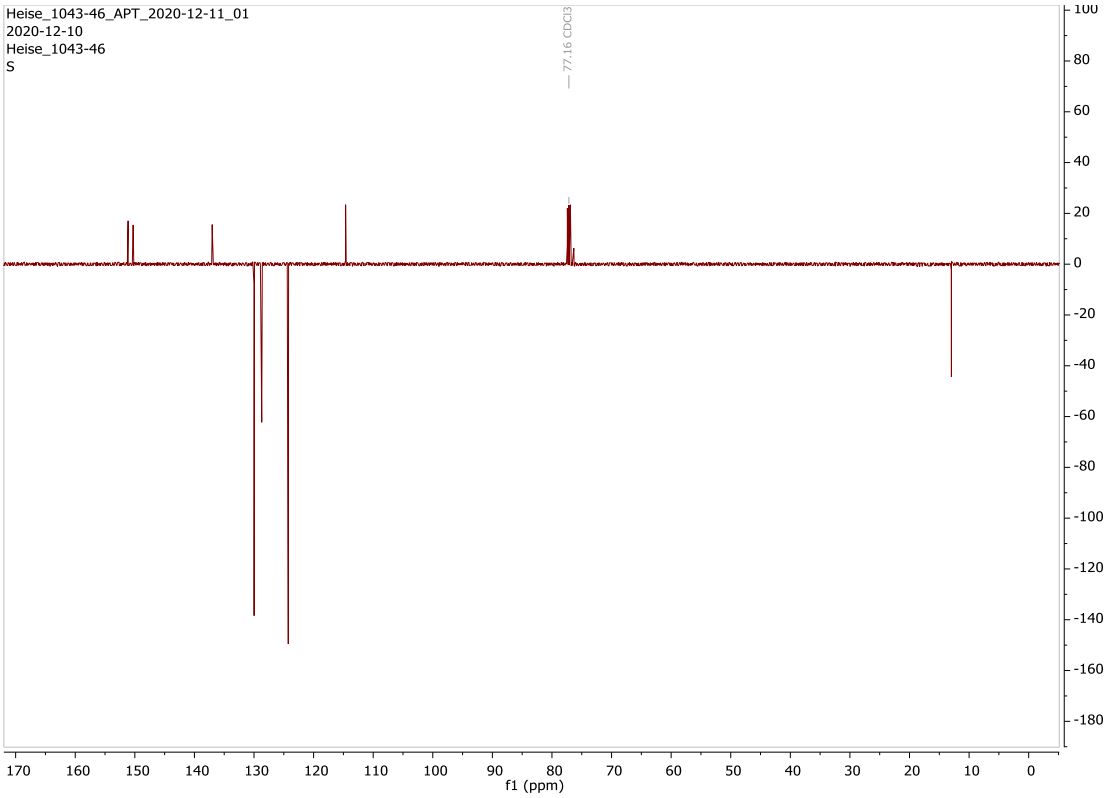
cells during the period of the experiment. On day 1, the cells were treated with six di↵erent concentrations (1, 3, 7, 12, 20 and 30 μM); thereby, the final concentration of DMSO was always < 0.5%, generally regarded as non-toxic to the cells. On day 4, the supernatant medium was discarded; the cells were fixed with 10 % trichloroacetic acid. After another day at 4 °C, the cells were washed in a strip washer and dyed with the SRB solution (100 μL, 0.4% in 1% acetic acid) for about 20 min to be followed by washing of the plates (four times, 1% acetic acid) and air-drying overnight. Furthermore, tris base solution (200 μL, 10 mM) was added to each well and absorbance was measured at λ = 570 nm employing a reader (96 wells, Tecan Spectra, Crailsheim, Germany). The EC_{50} values were averaged from three independent experiments performed each in triplicate calculated from semi logarithmic dose response curves applying a non-linear four-parameter Hills-slope equation (GraphPad Prism5; variables top and bottom were set to 100 and 0, respectively).

3. **Spectra of 3:**

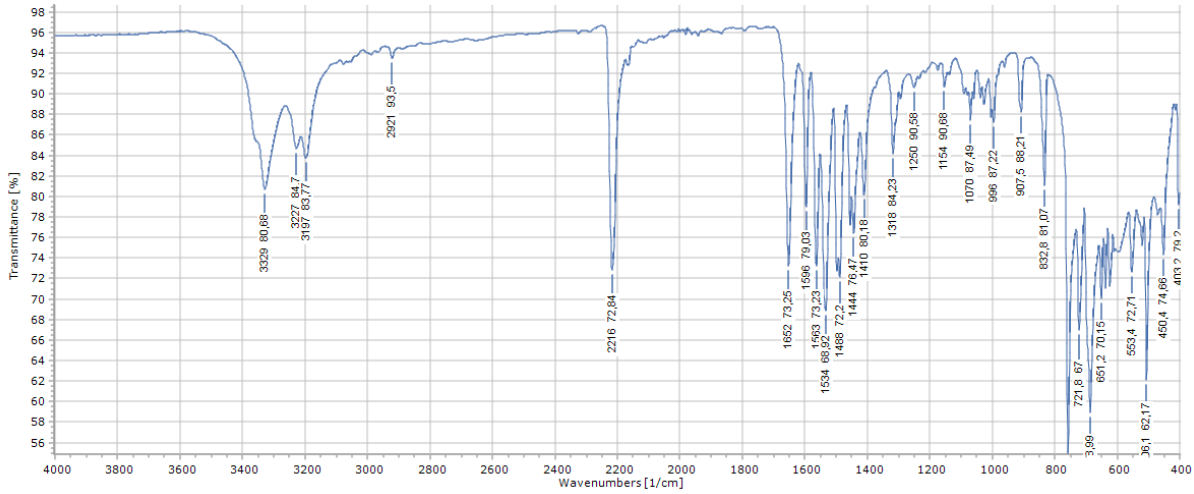
^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):

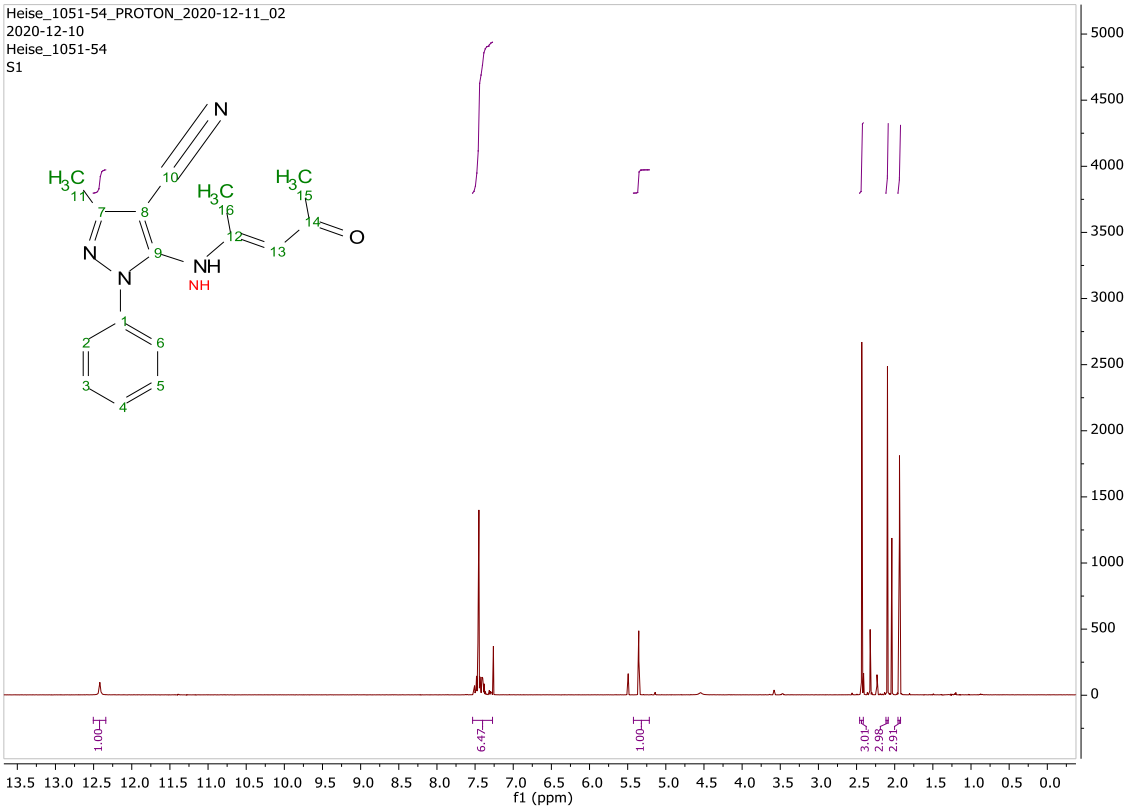


IR spectrum (ATR):

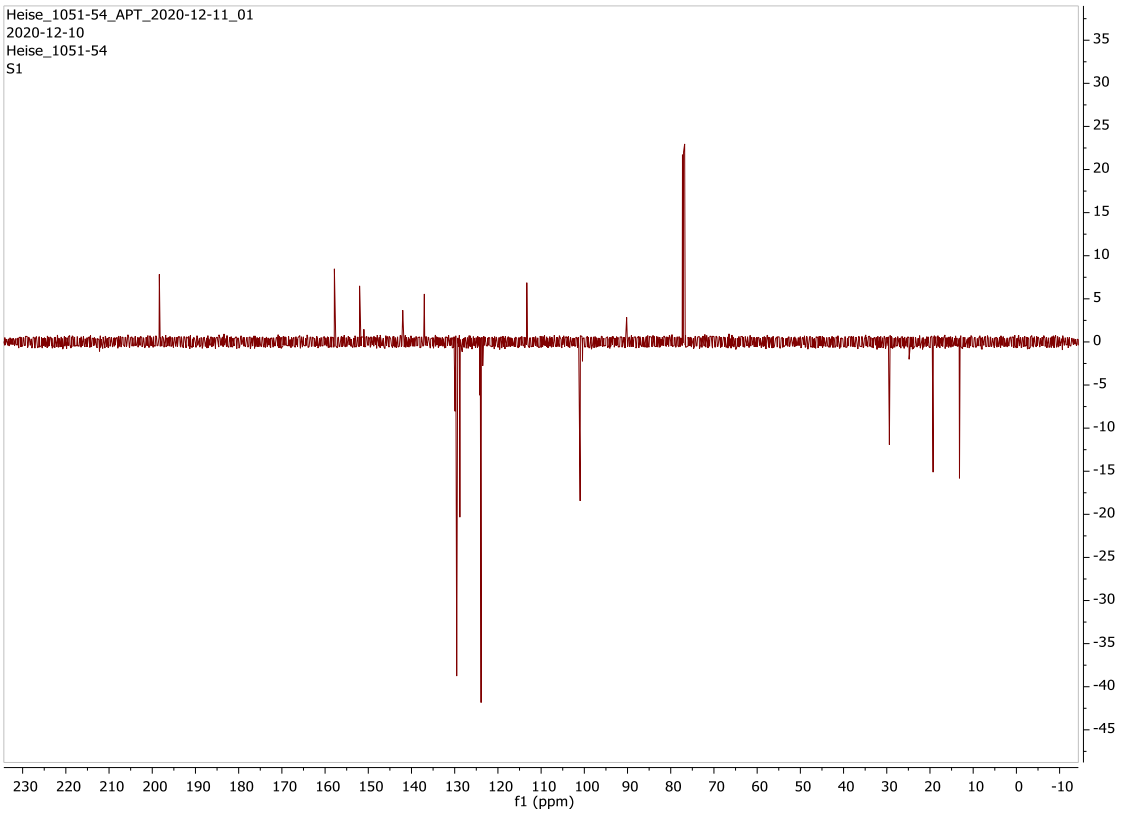


Spectra of 5:

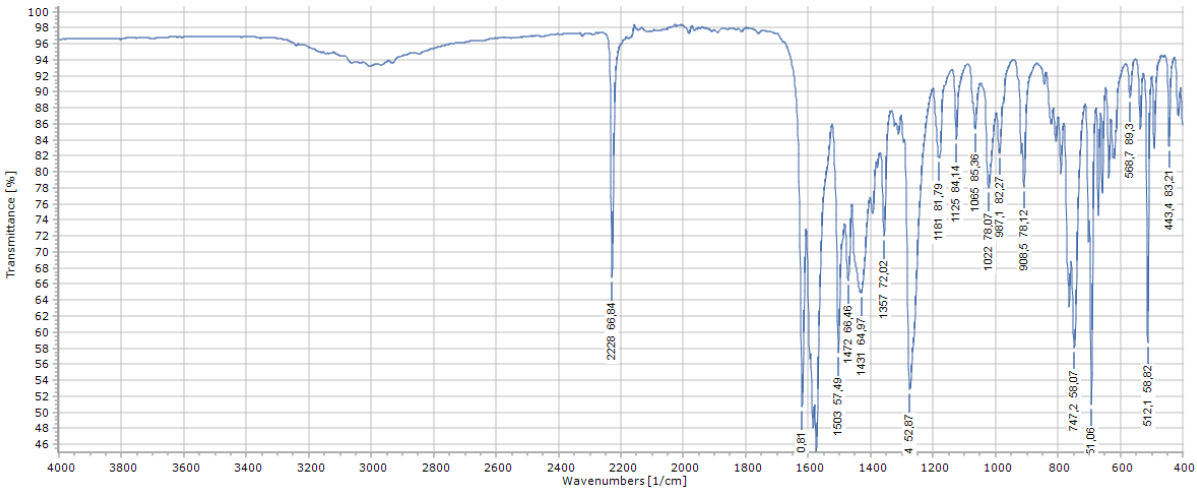
^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):

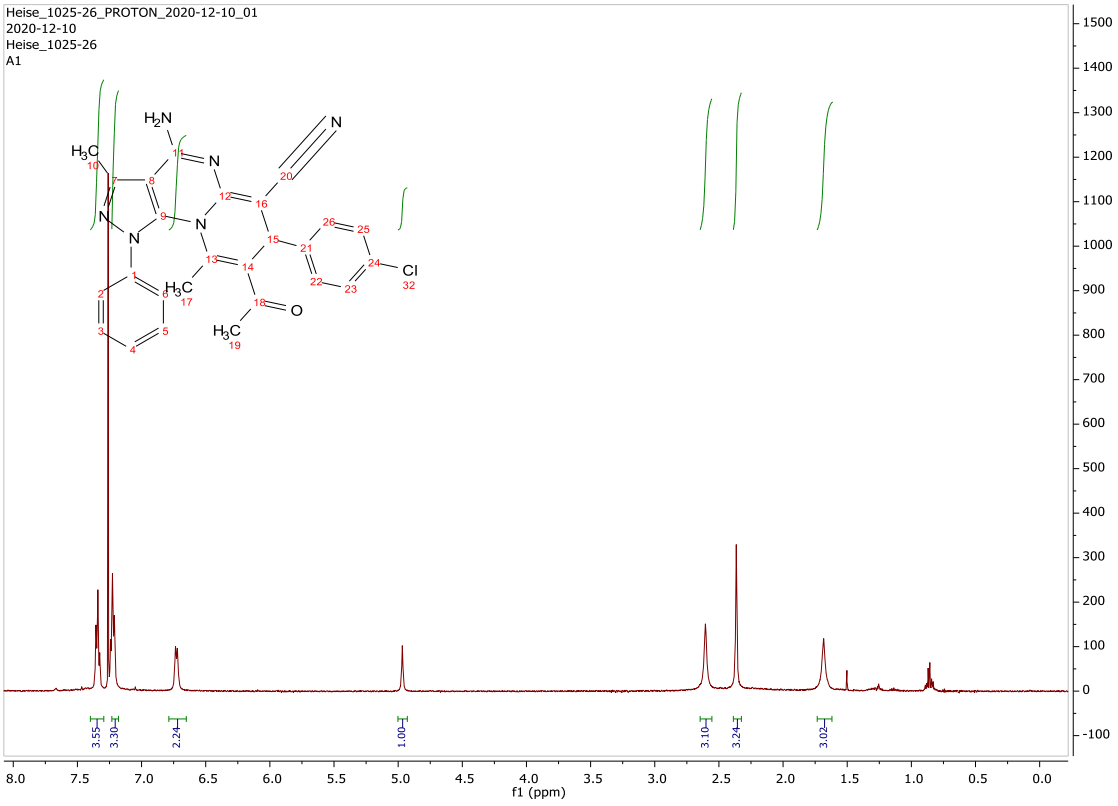


IR spectrum (ATR):

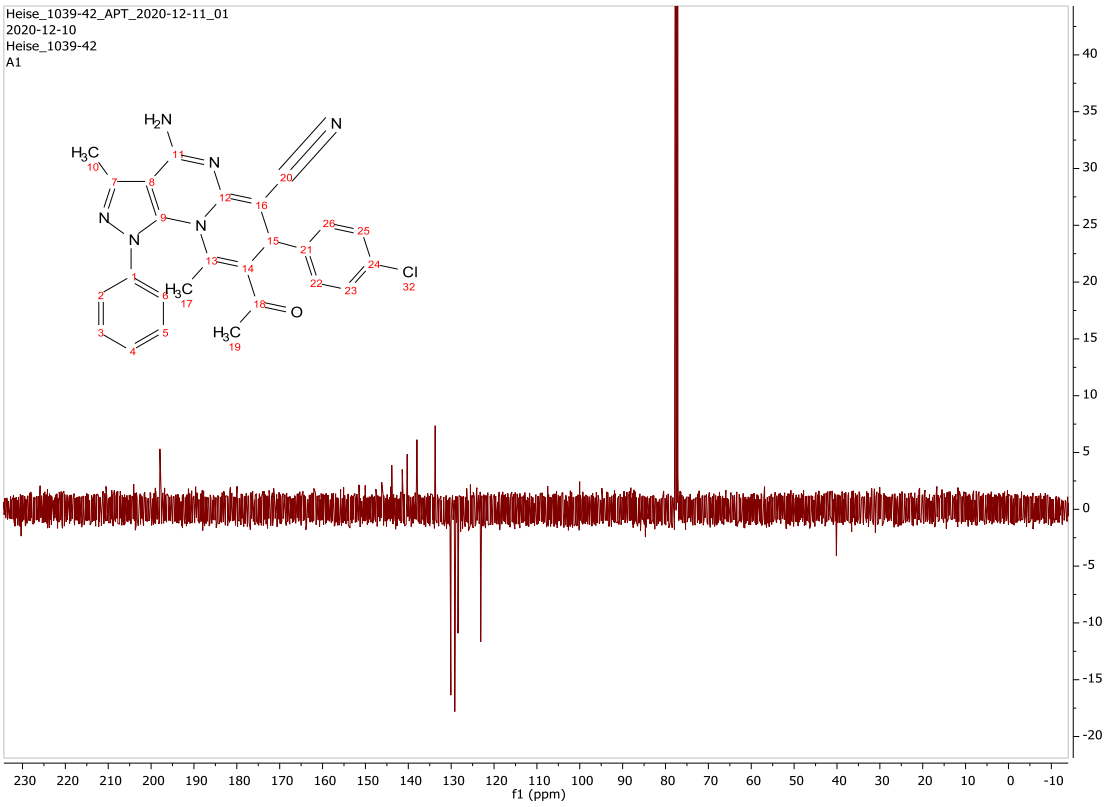


Spectra of 7a:

^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):



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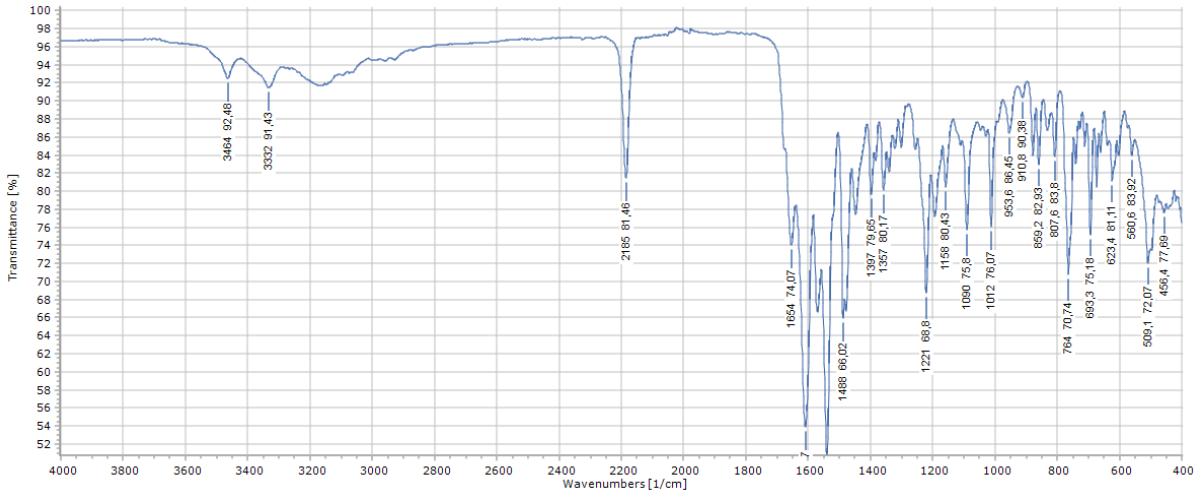

Spectra of 7b:

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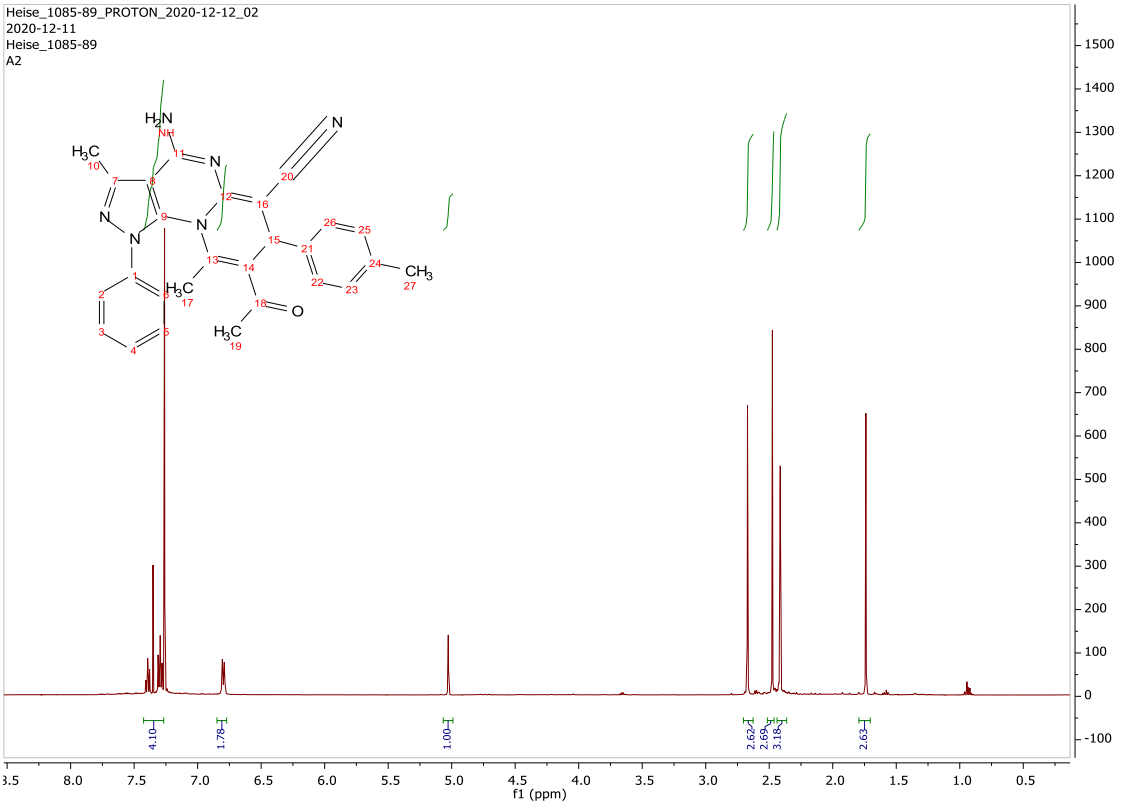
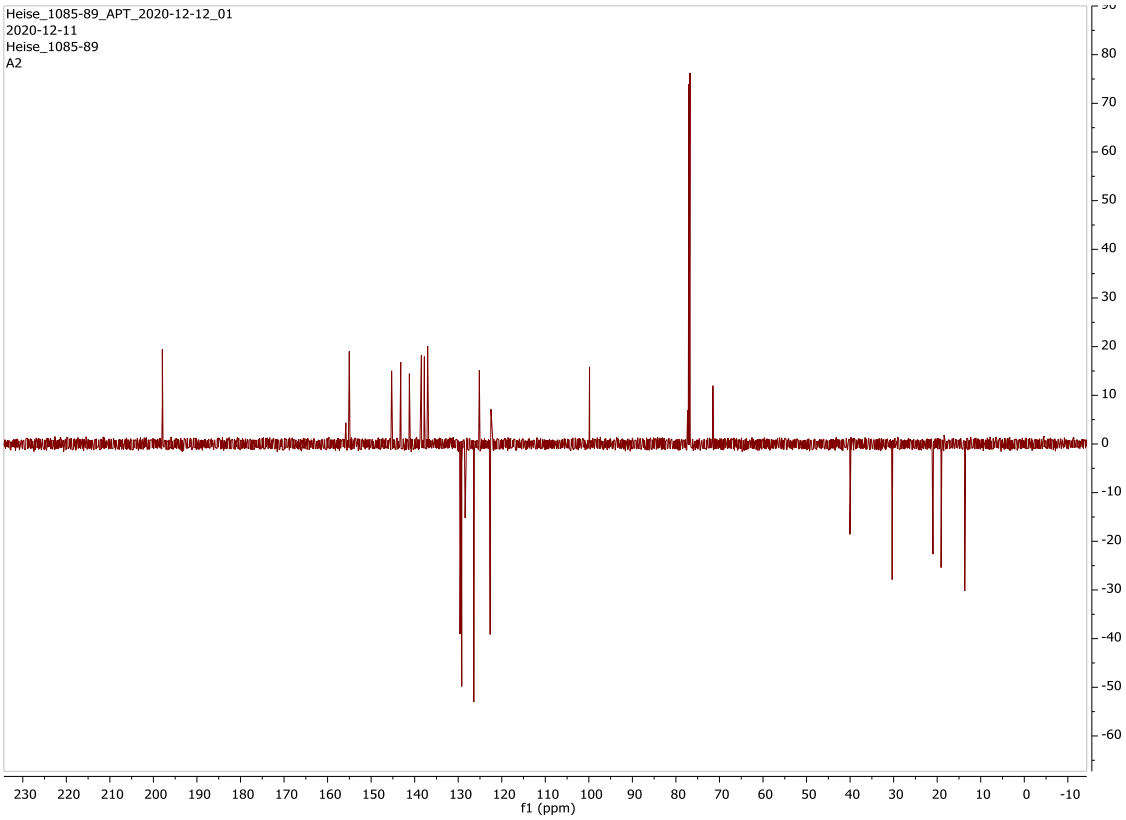
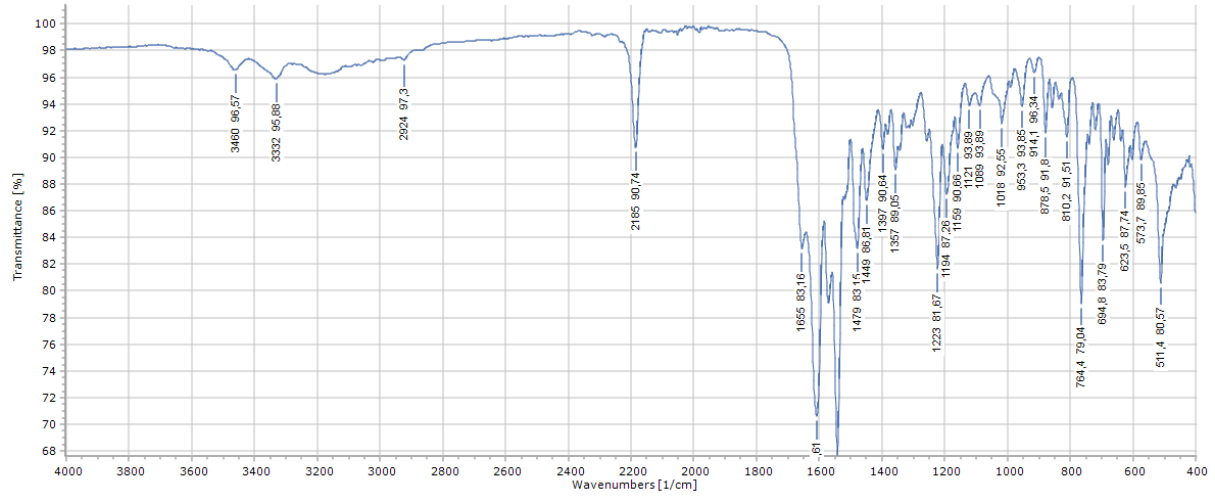


^{13}C NMR (126 MHz, $CDCl_3$):

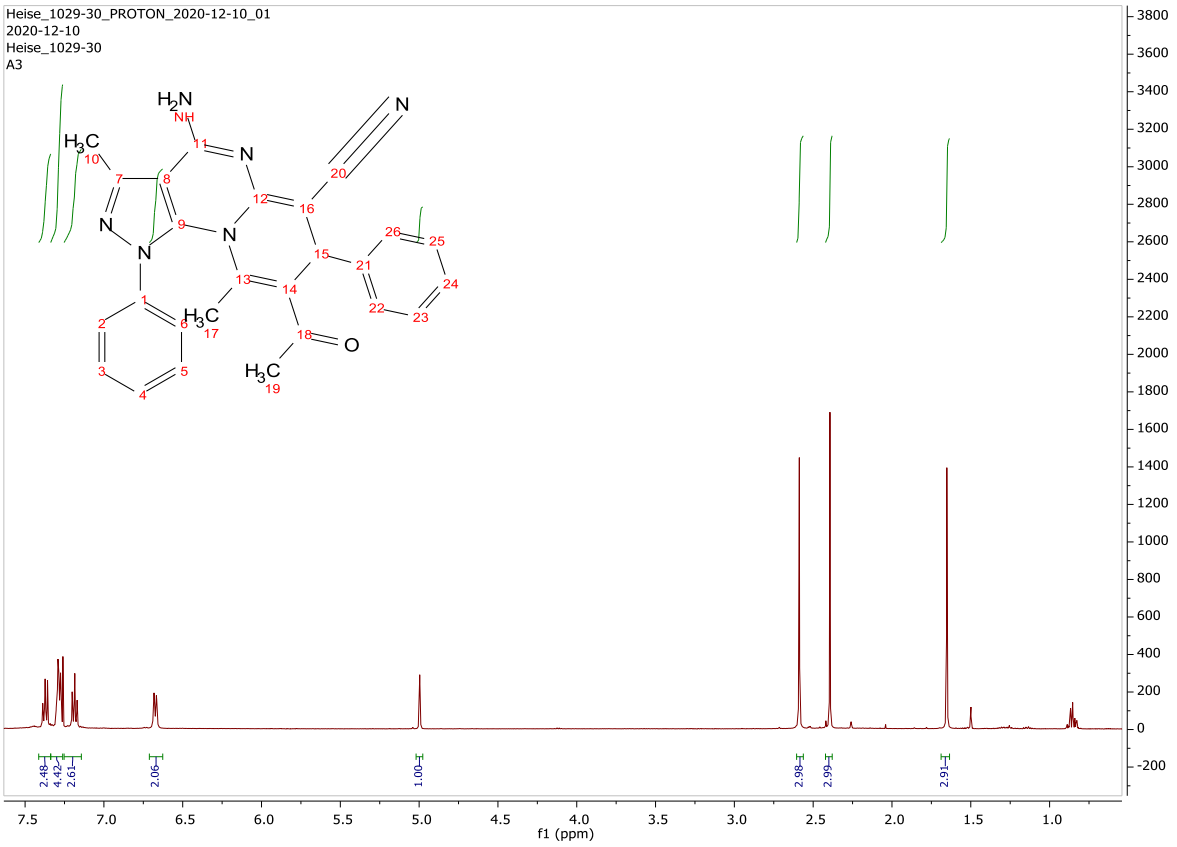


IR spectrum (ATR):

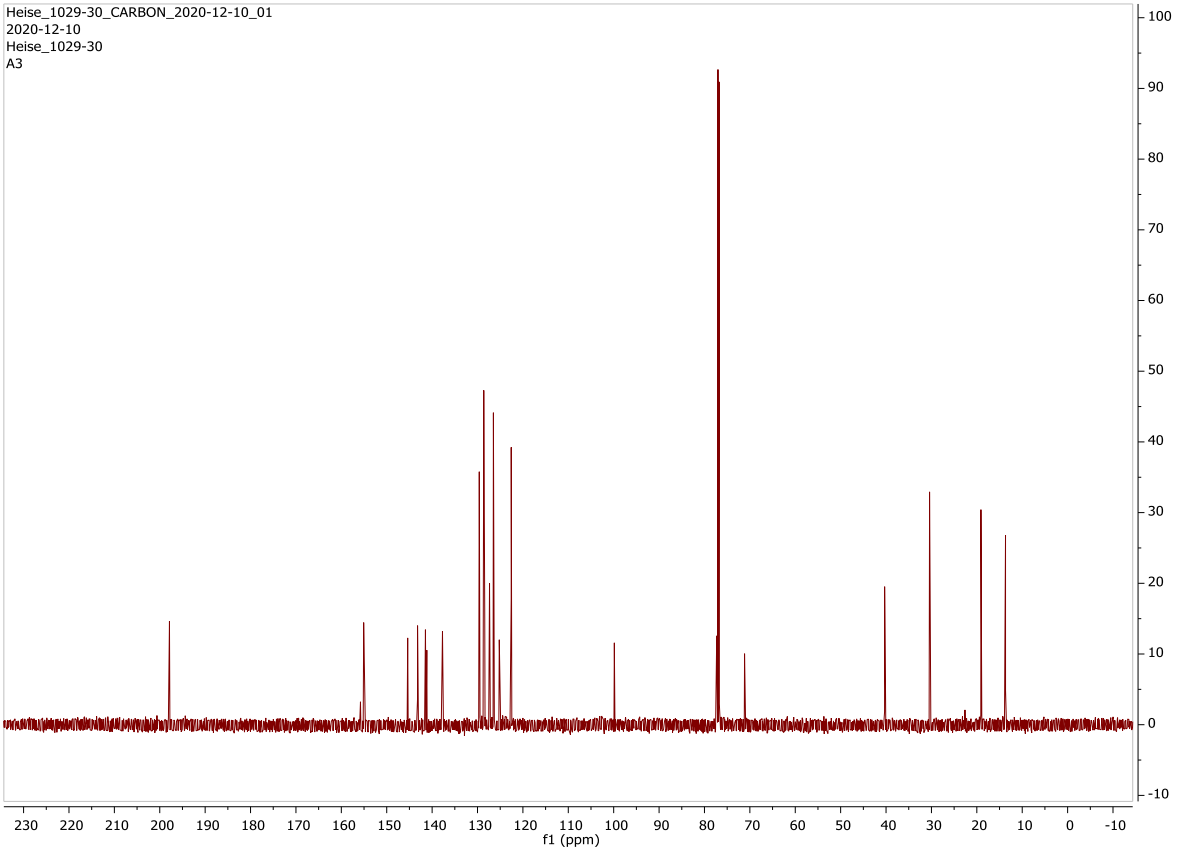


Spectra of 7c:

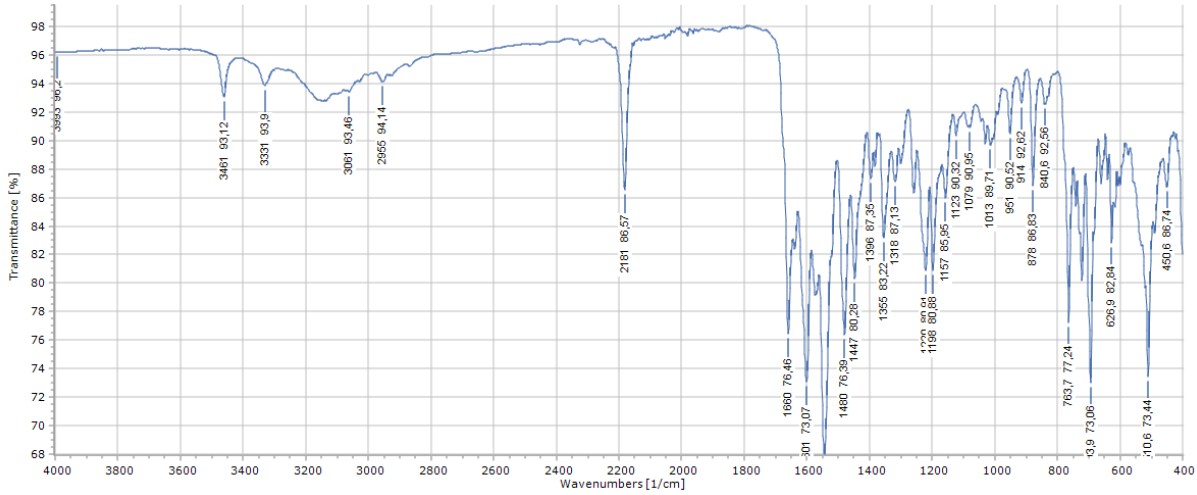
^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):



IR spectrum (ATR):



Spectra of 7d:

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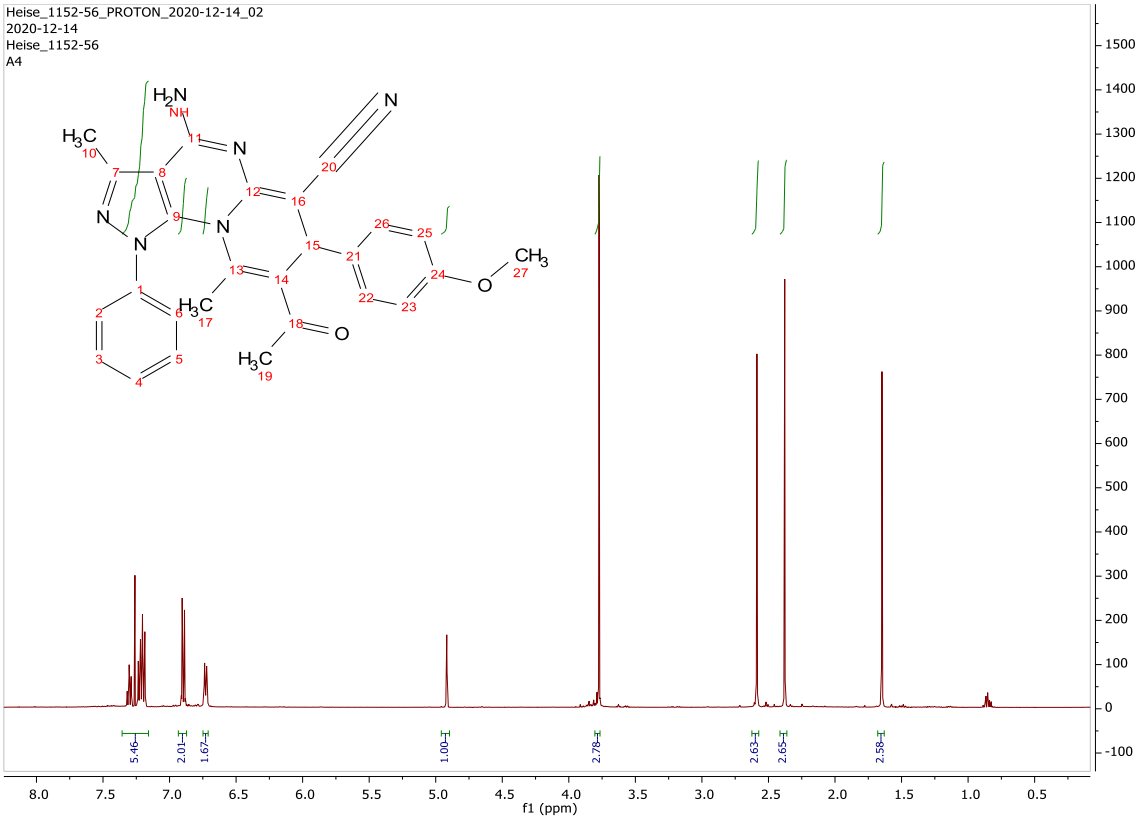
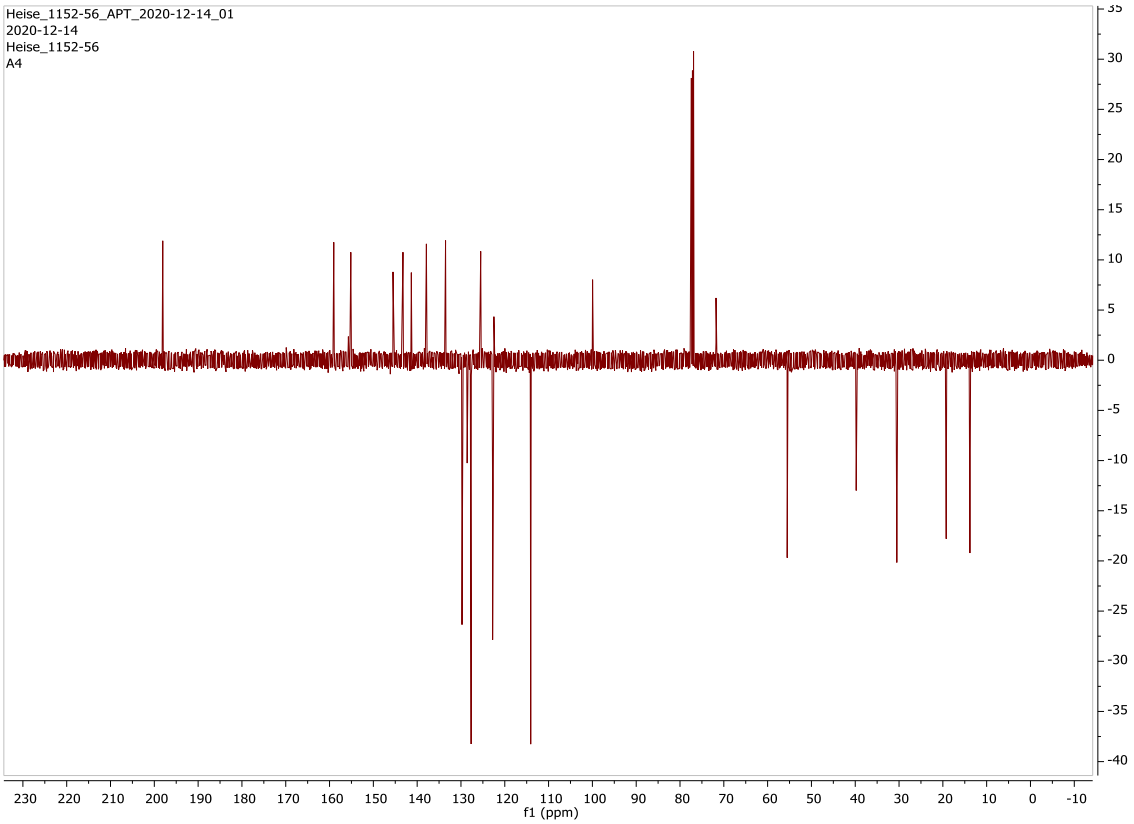
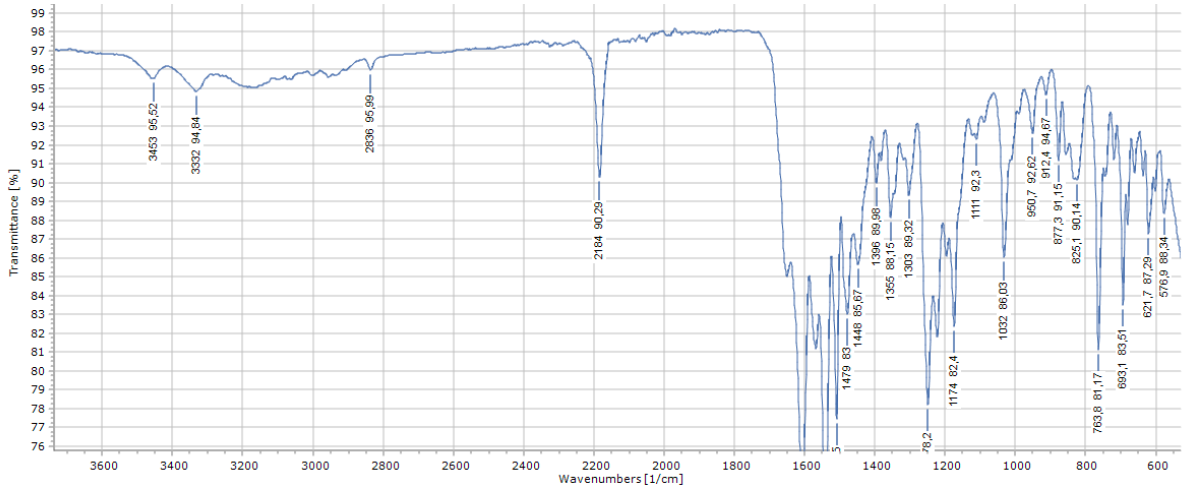


^{13}C NMR (126 MHz, $CDCl_3$):

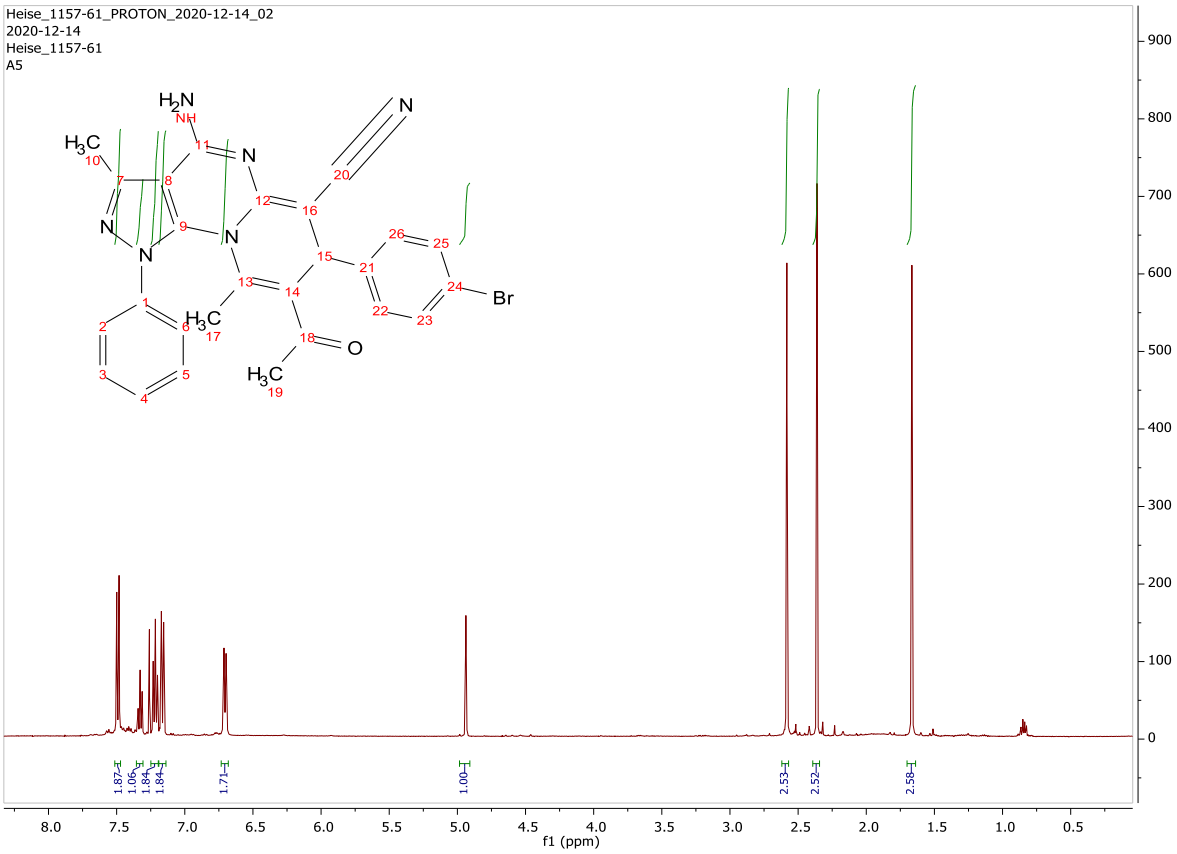


IR spectrum (ATR):

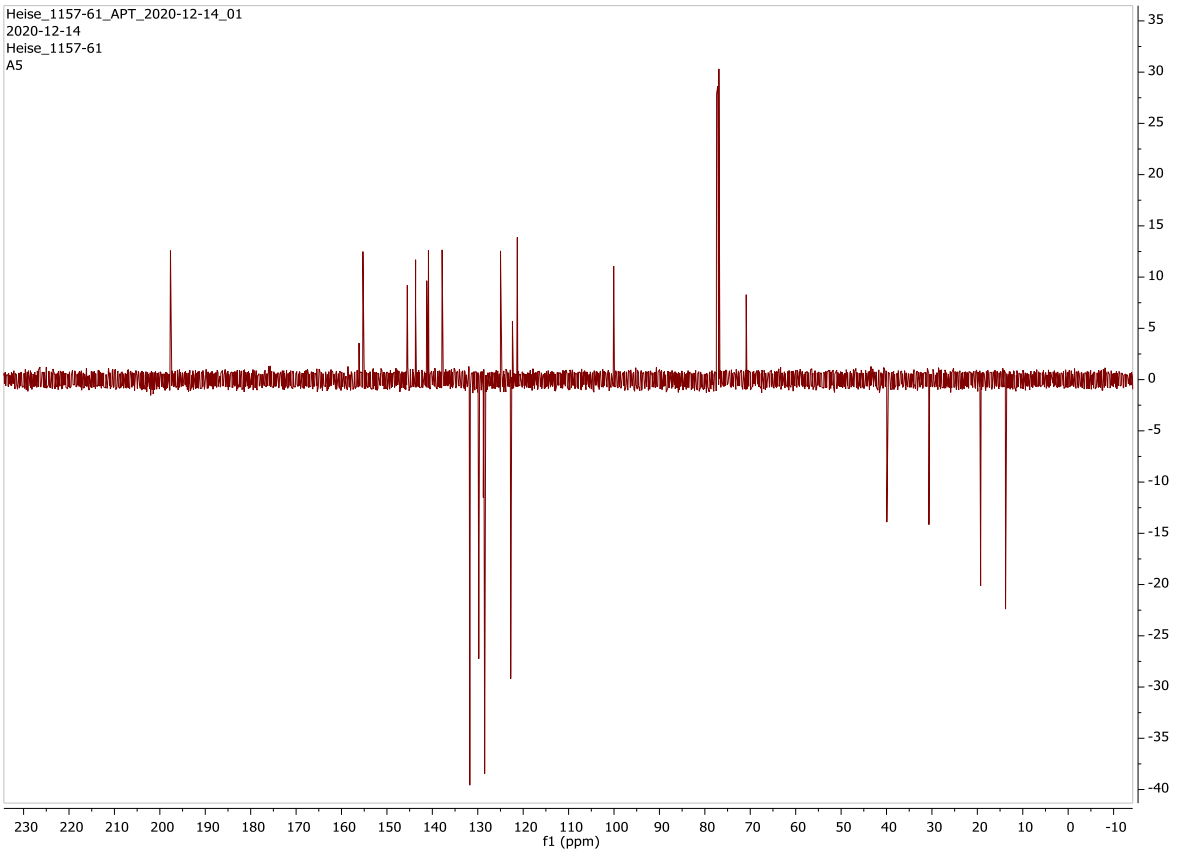


Spectra of 7e:

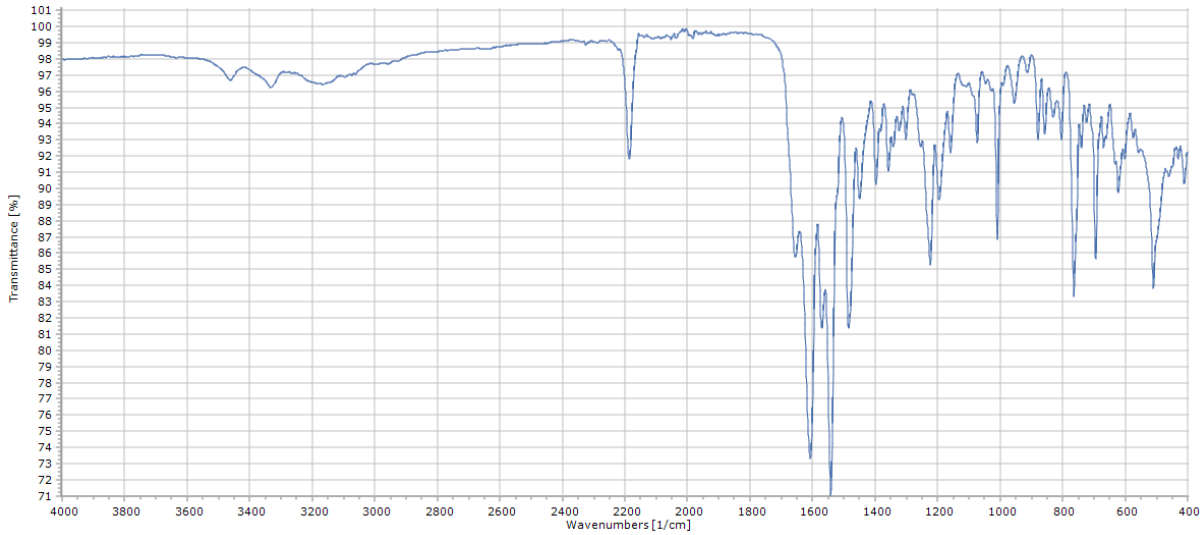
^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):



IR spectrum (ATR):



Spectra of 7f:

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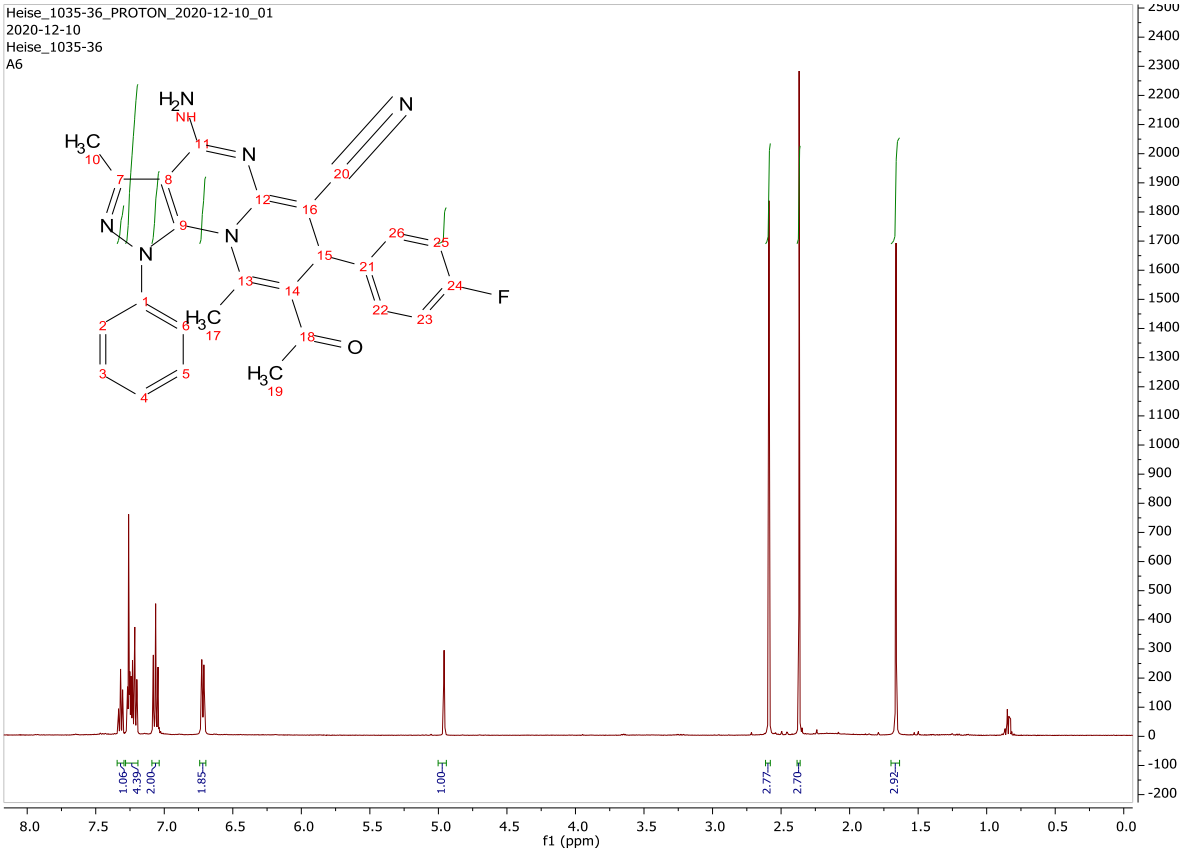
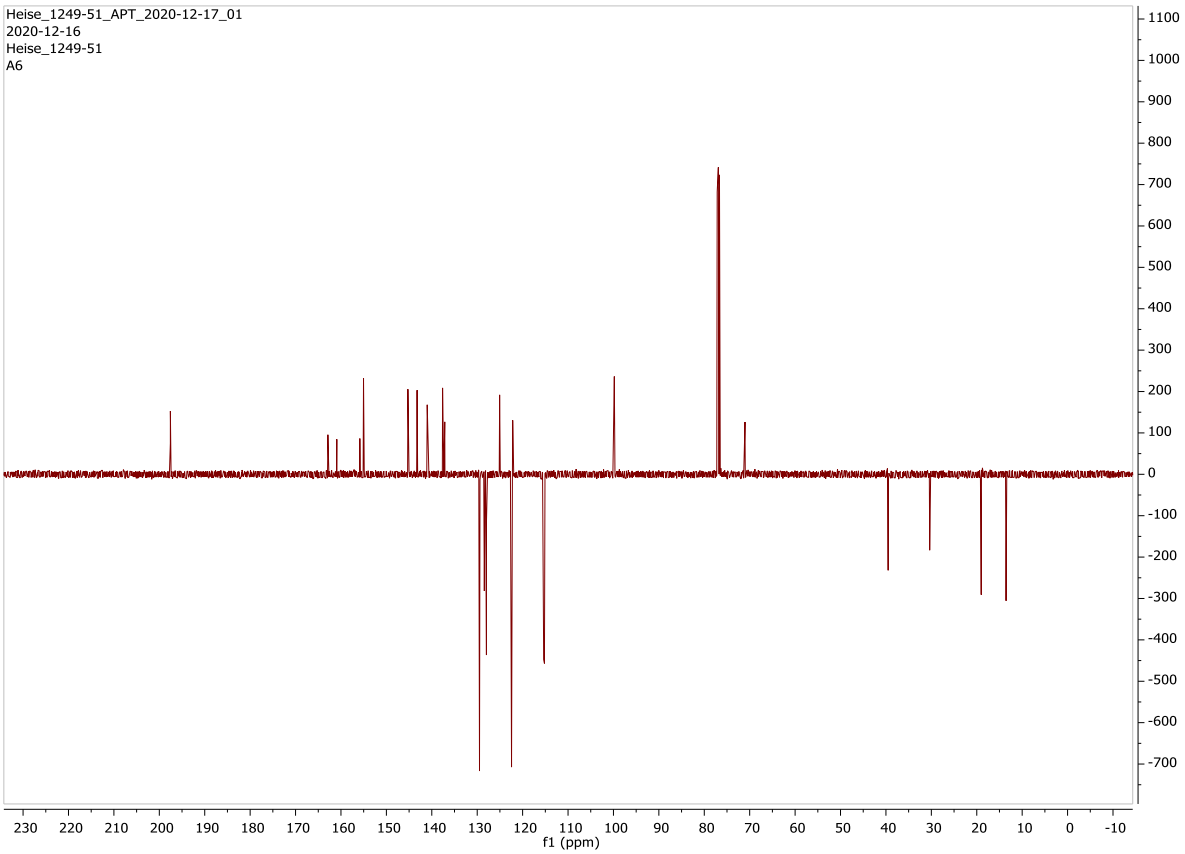
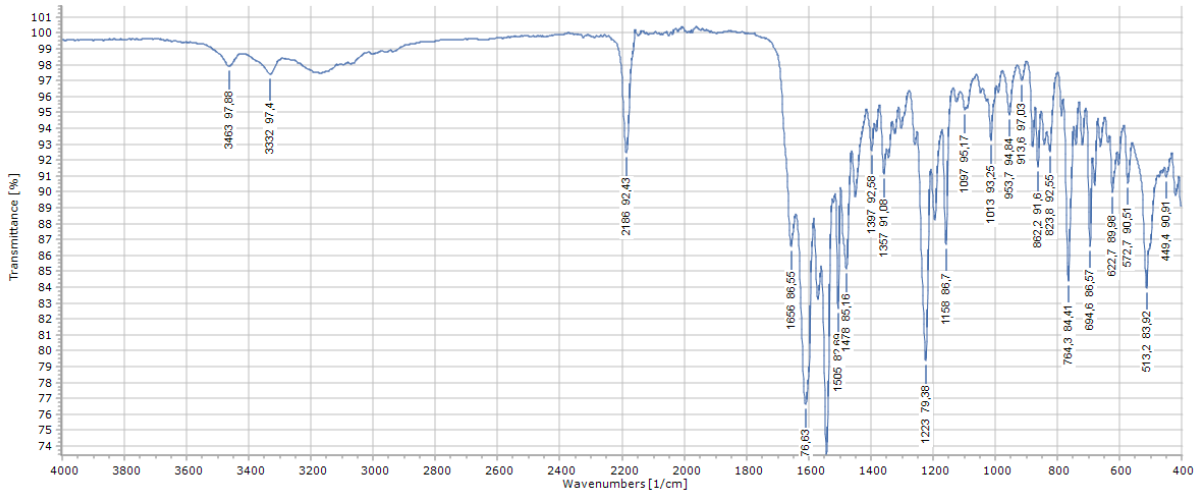


^{13}C NMR (126 MHz, $CDCl_3$):



IR spectrum (ATR):



Spectra of 7g:

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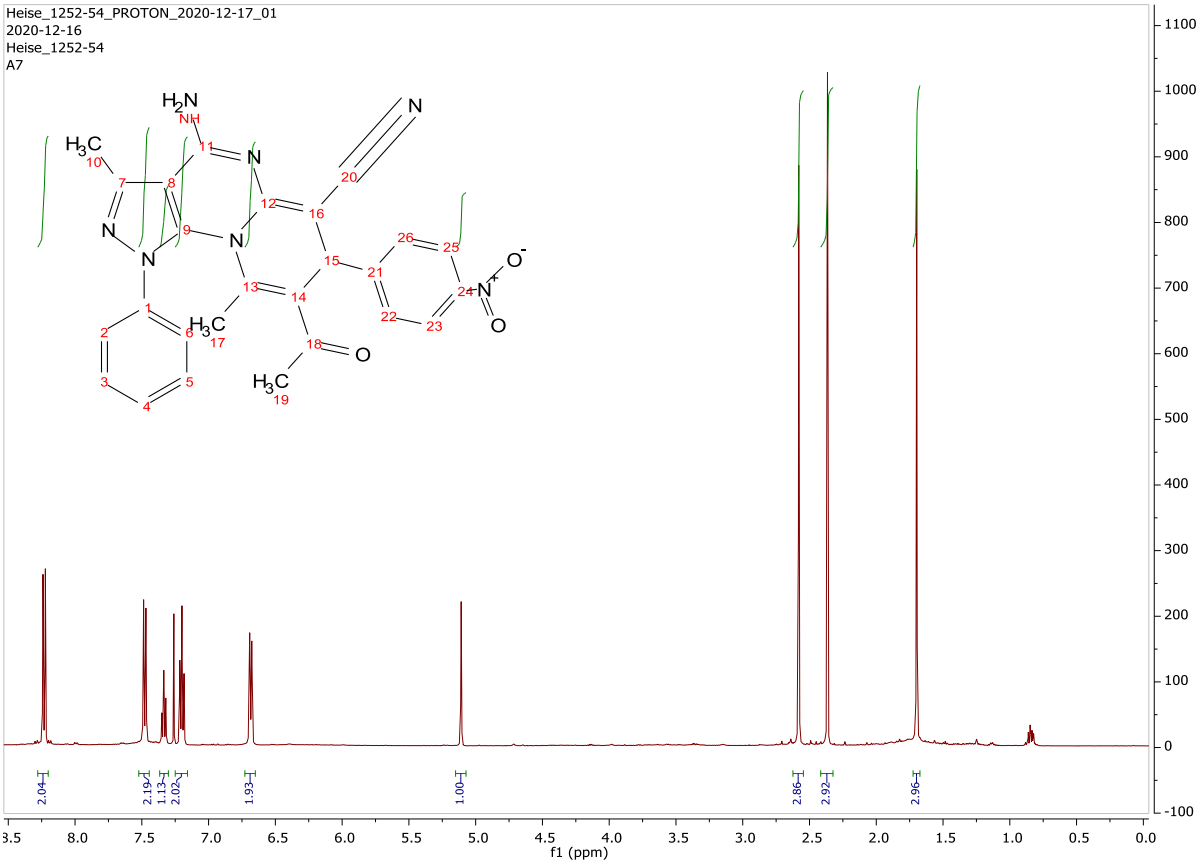
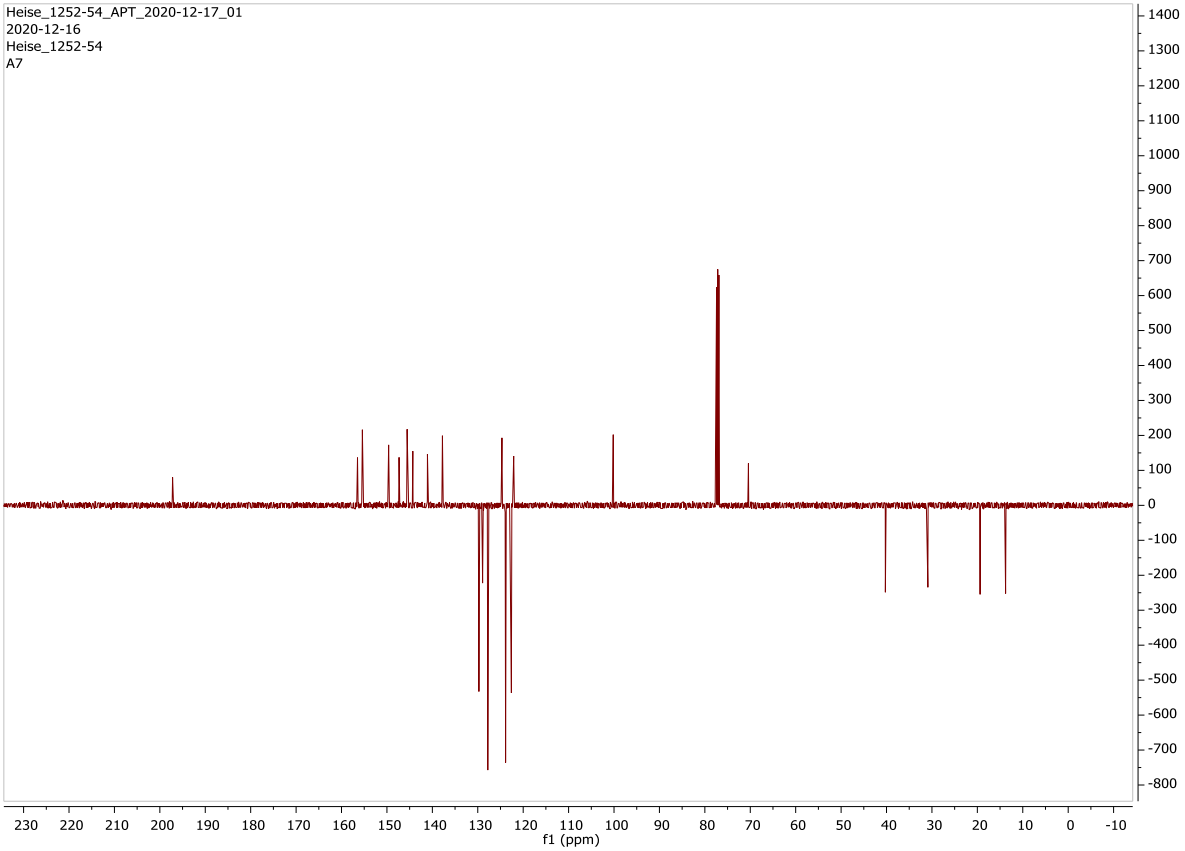
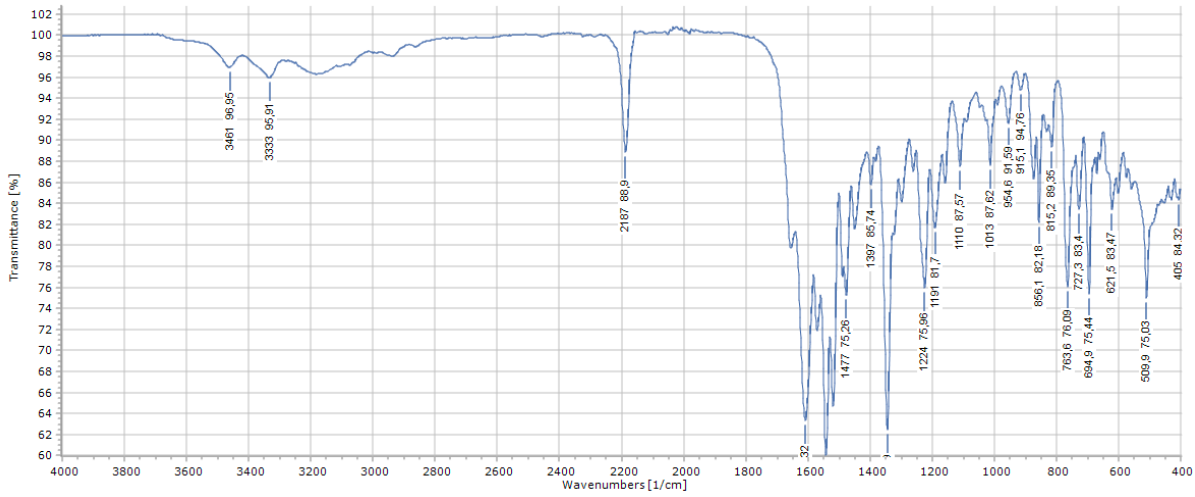


^{13}C NMR (126 MHz, $CDCl_3$):

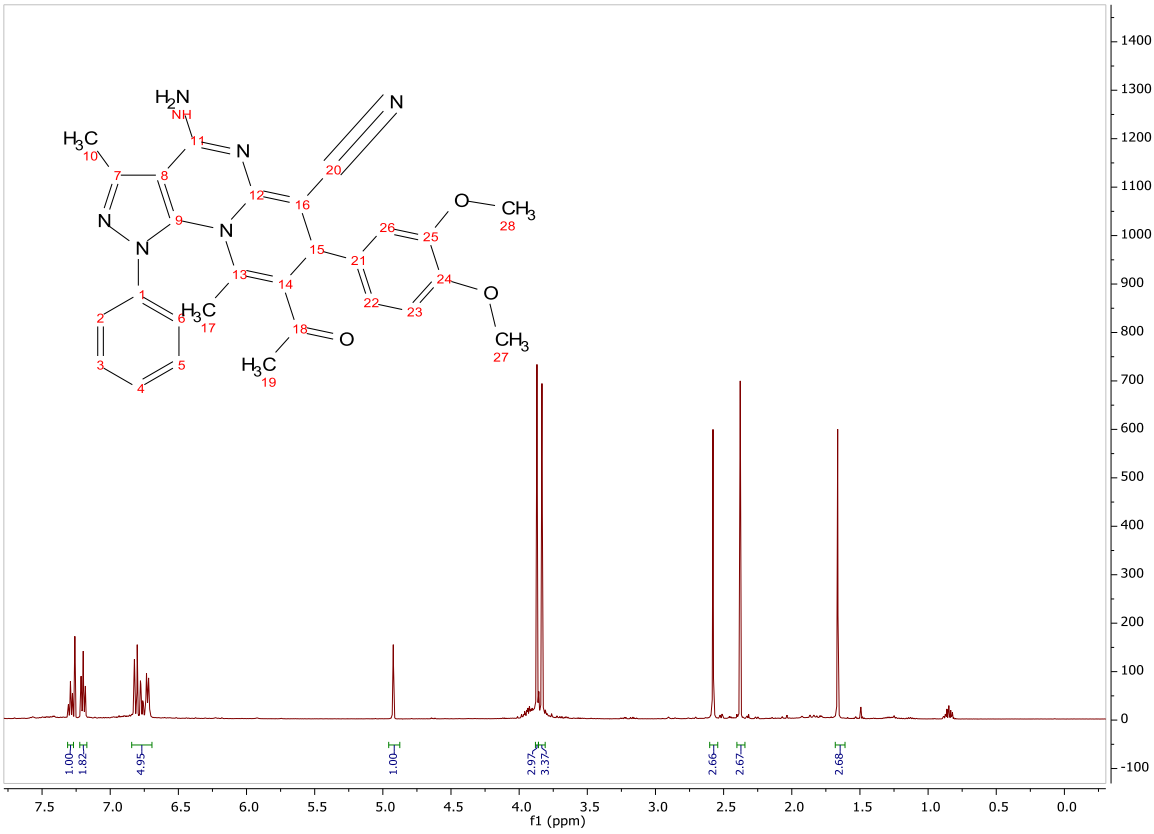


IR spectrum (ATR):

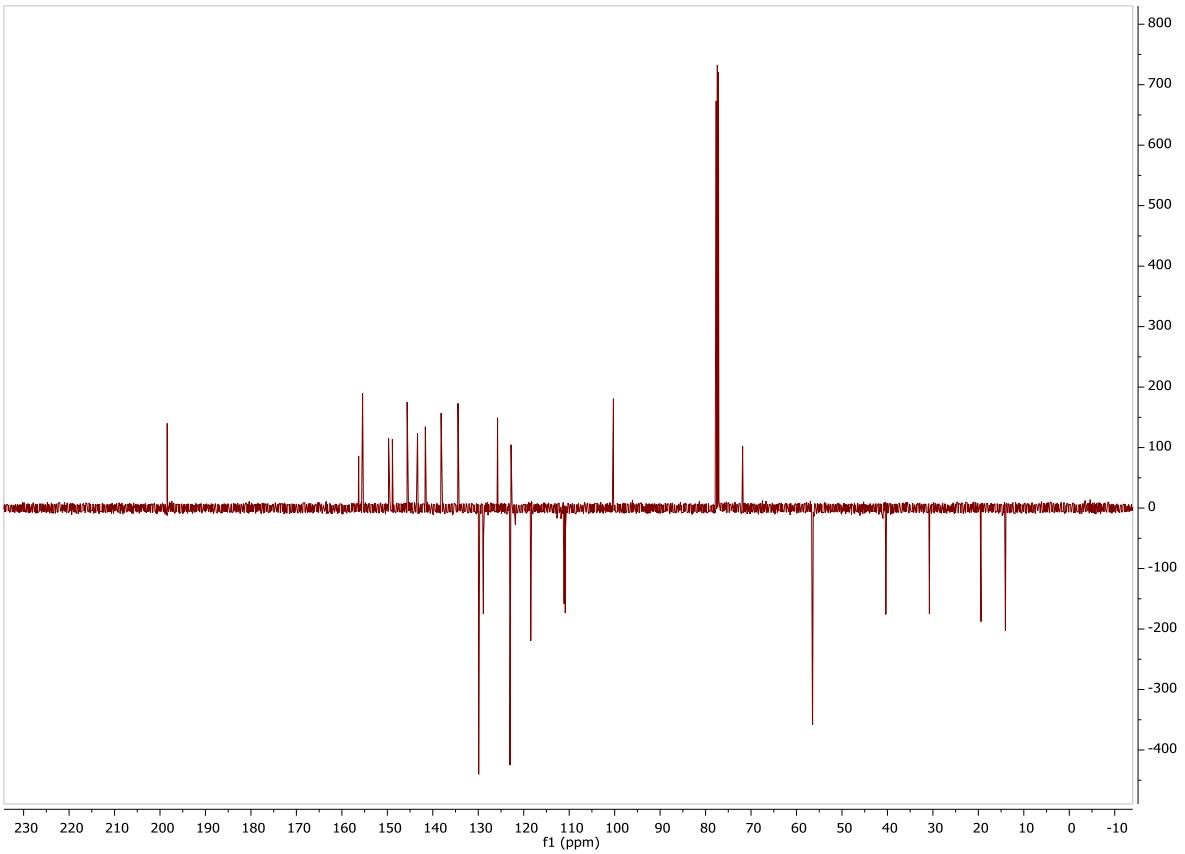


Spectra of 7h:

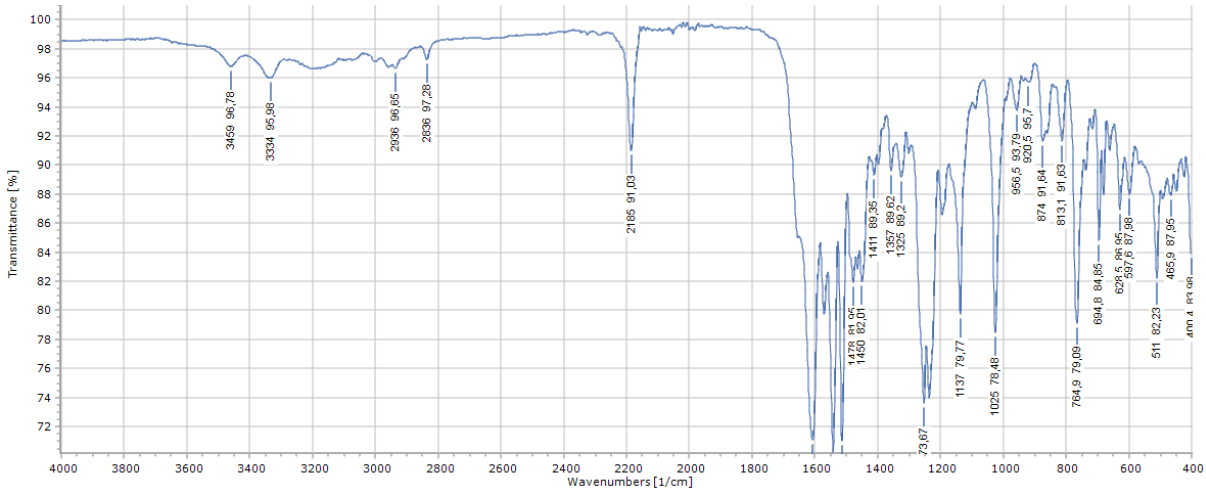
^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):

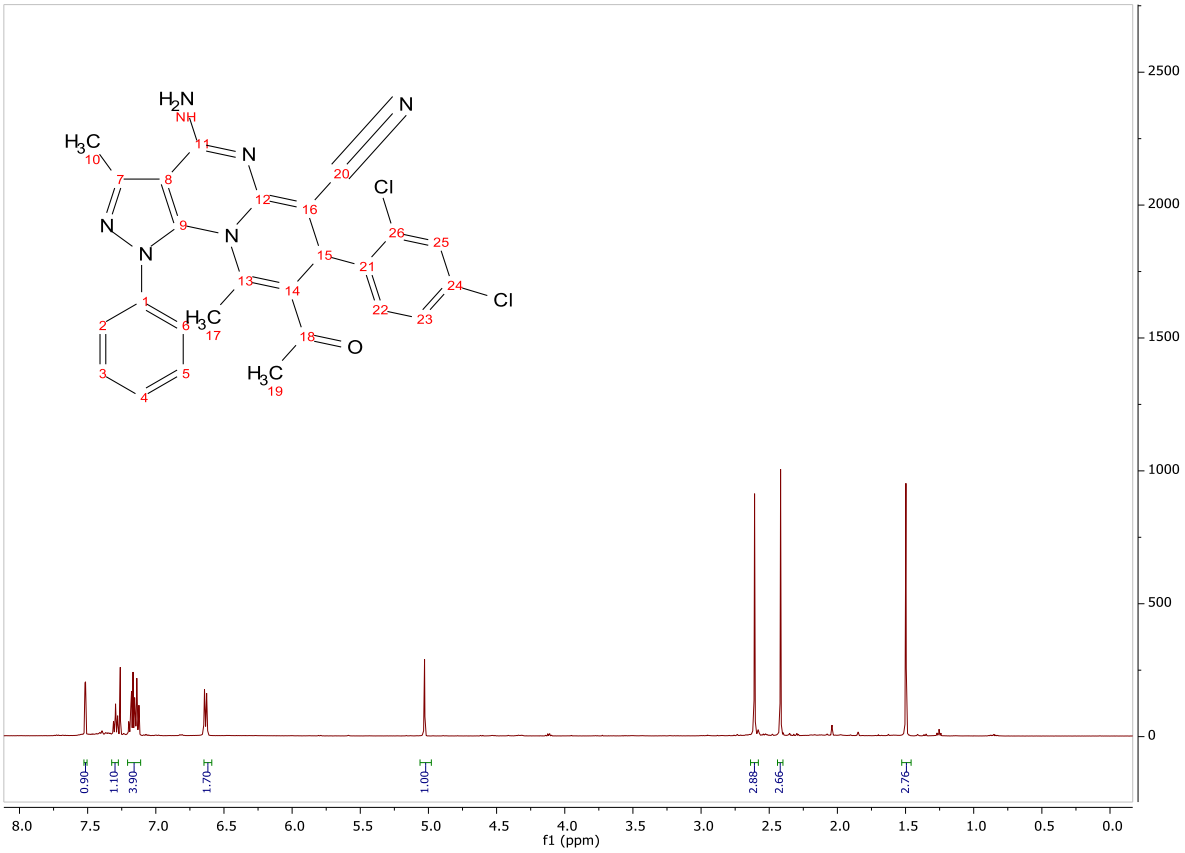


IR spectrum (ATR):



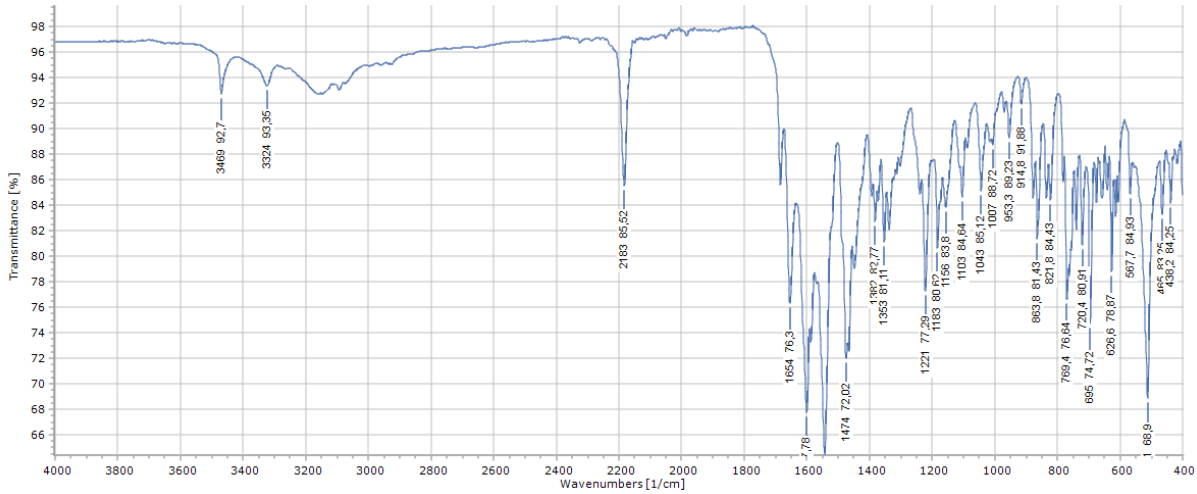
Spectra of 7i:

^{1}H NMR (500 MHz, $CDCl_3$):



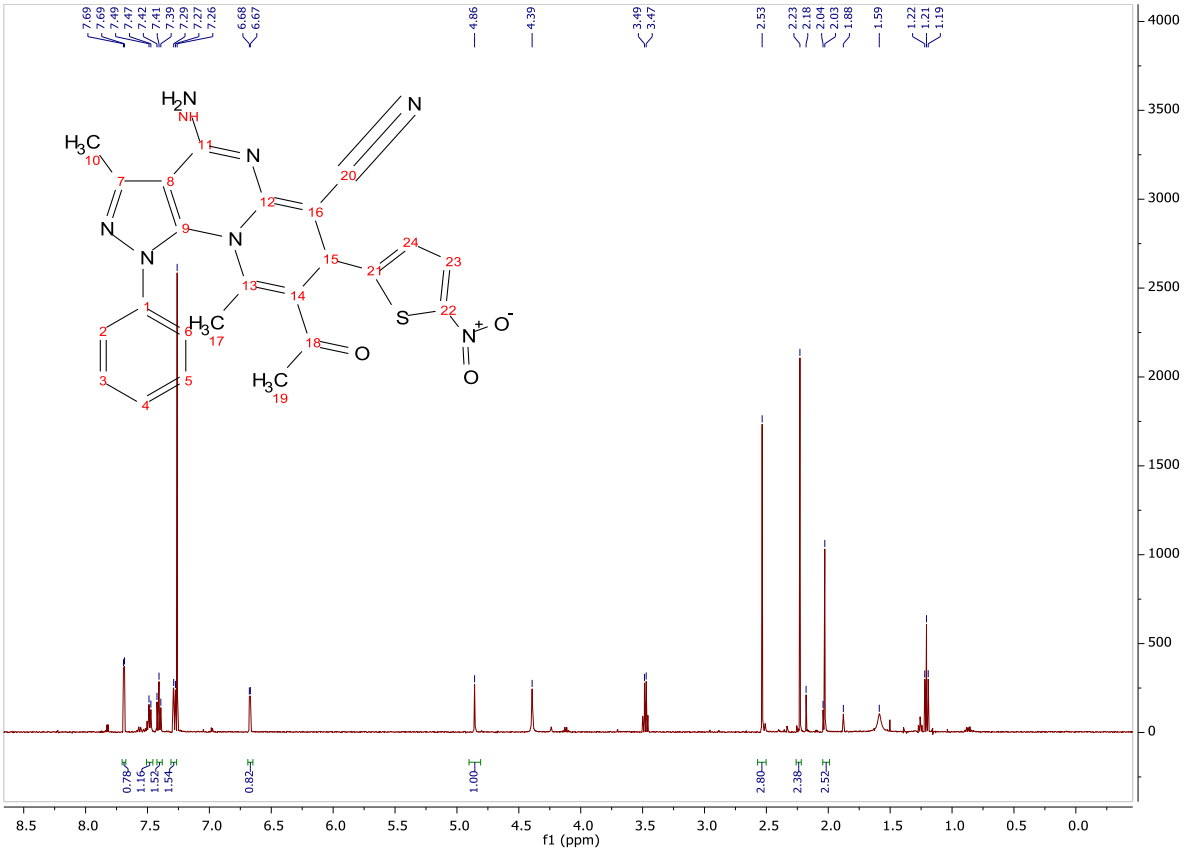
^{13}C NMR (126 MHz, $CDCl_3$):

IR spectrum (ATR):

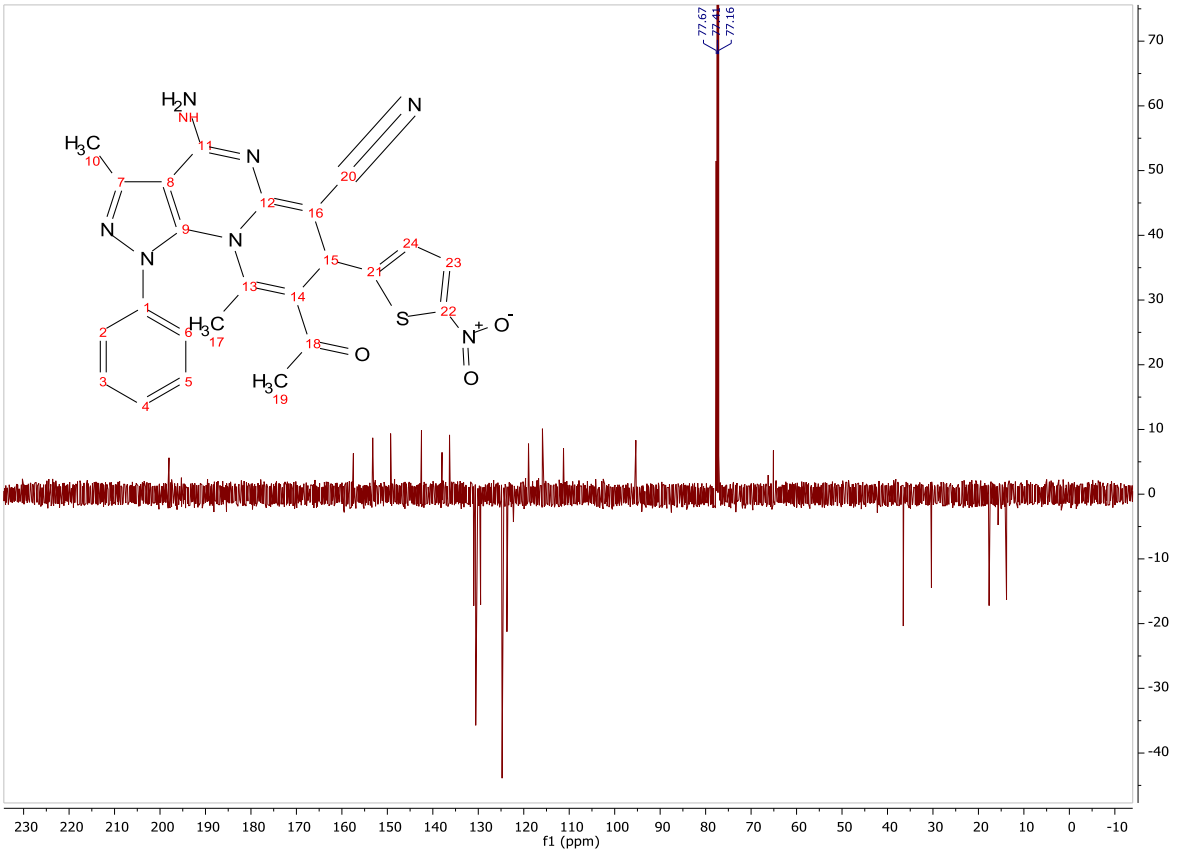


Spectra of 7j:

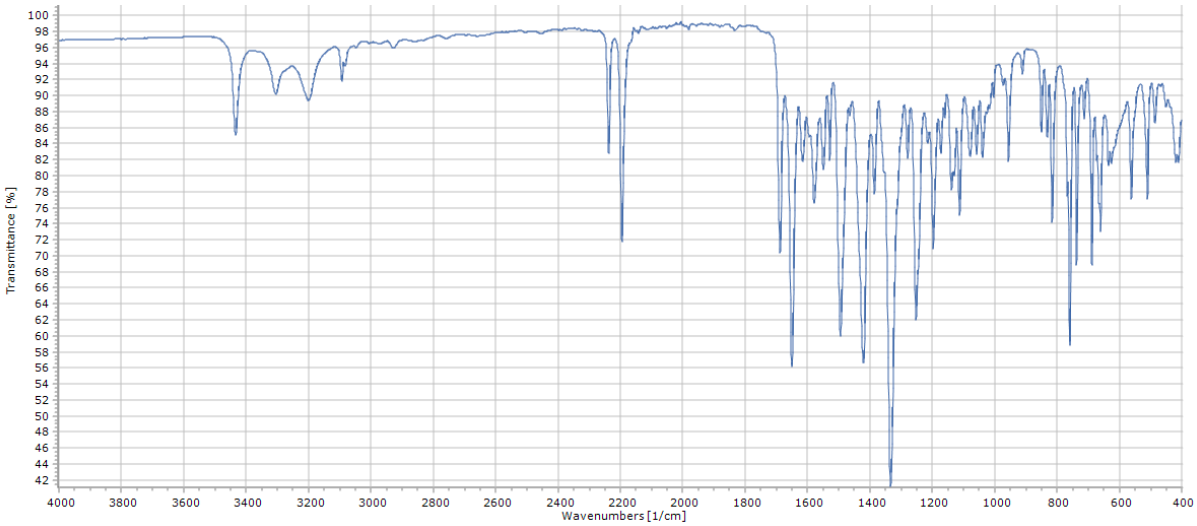
^{1}H NMR (500 MHz, $CDCl_3$):



^{13}C NMR (126 MHz, $CDCl_3$):



IR spectrum (ATR):



Spectra of 7k:

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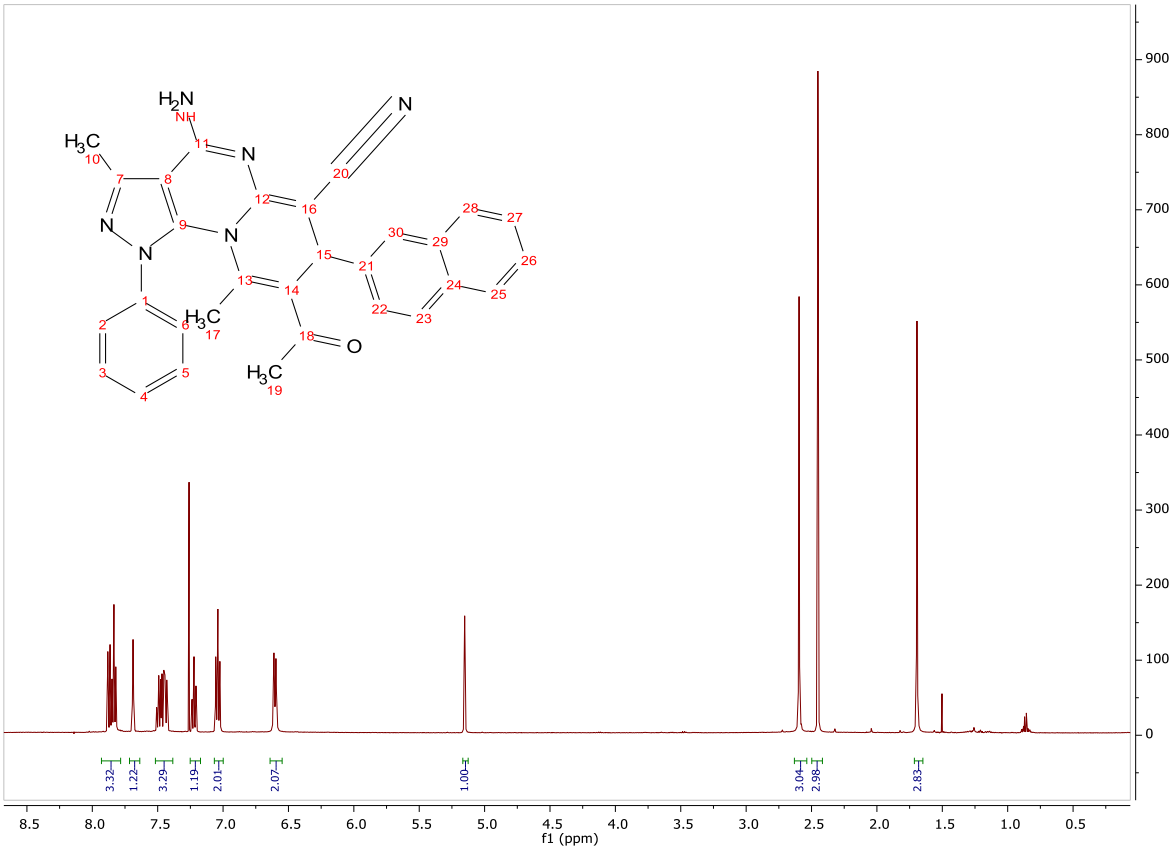
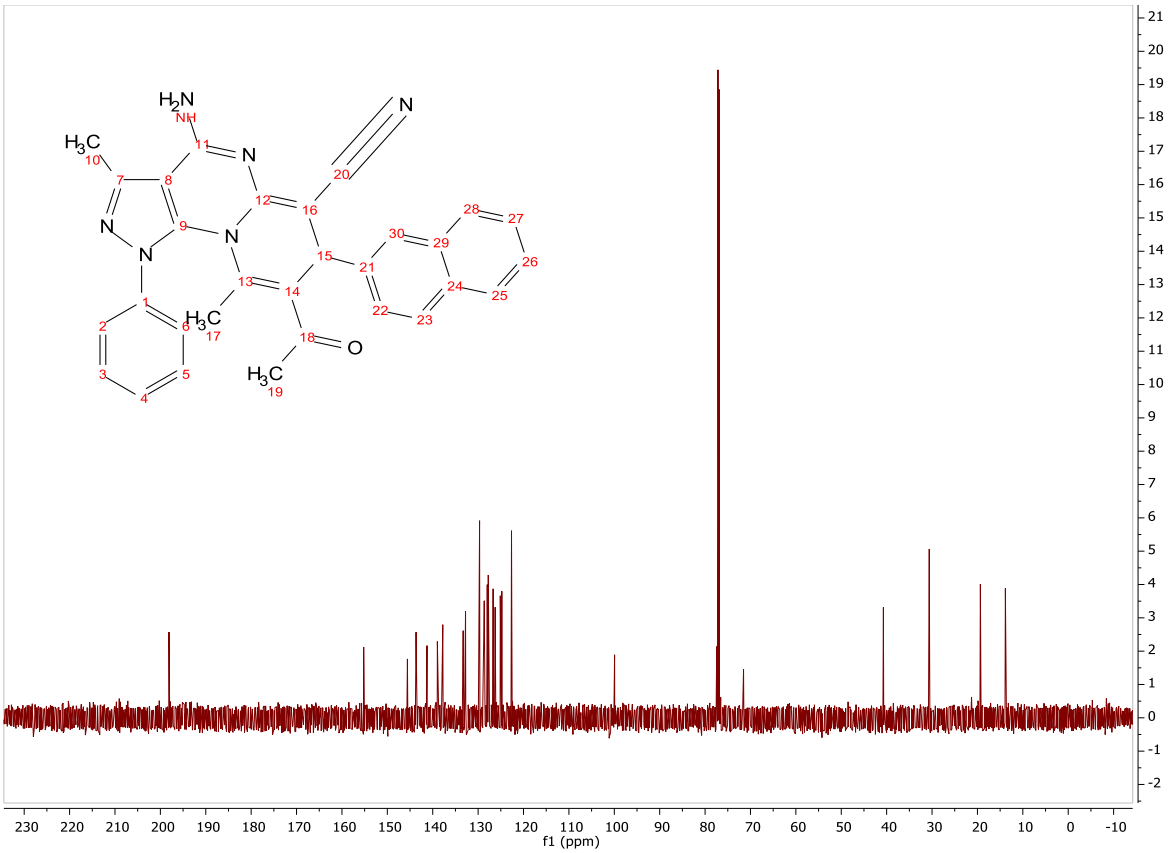
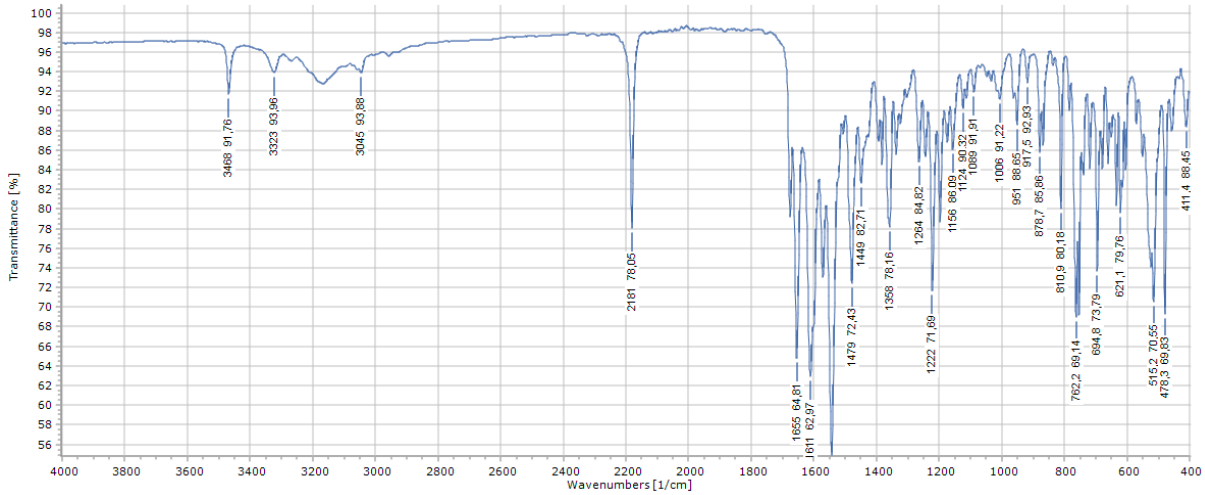


^{13}C NMR (126 MHz, $CDCl_3$):



IR spectrum (ATR):



Spectra of 7l:

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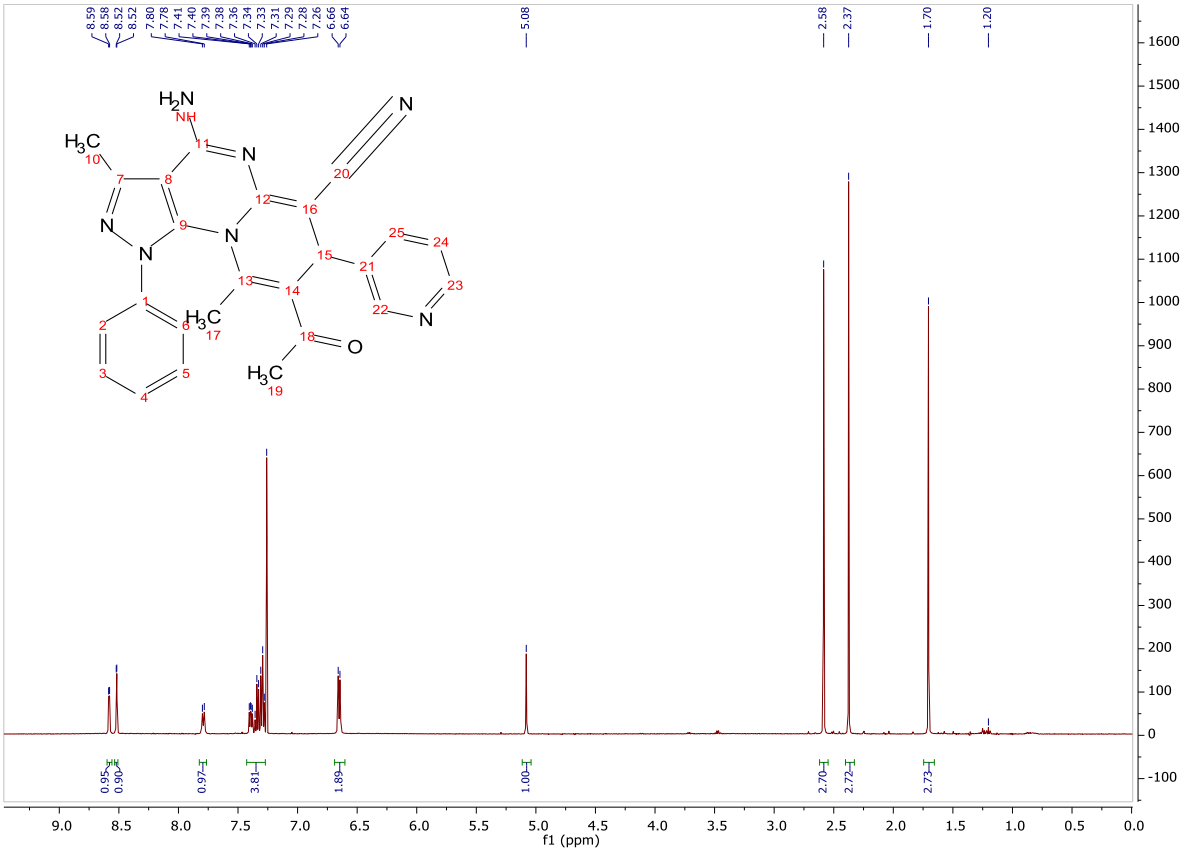
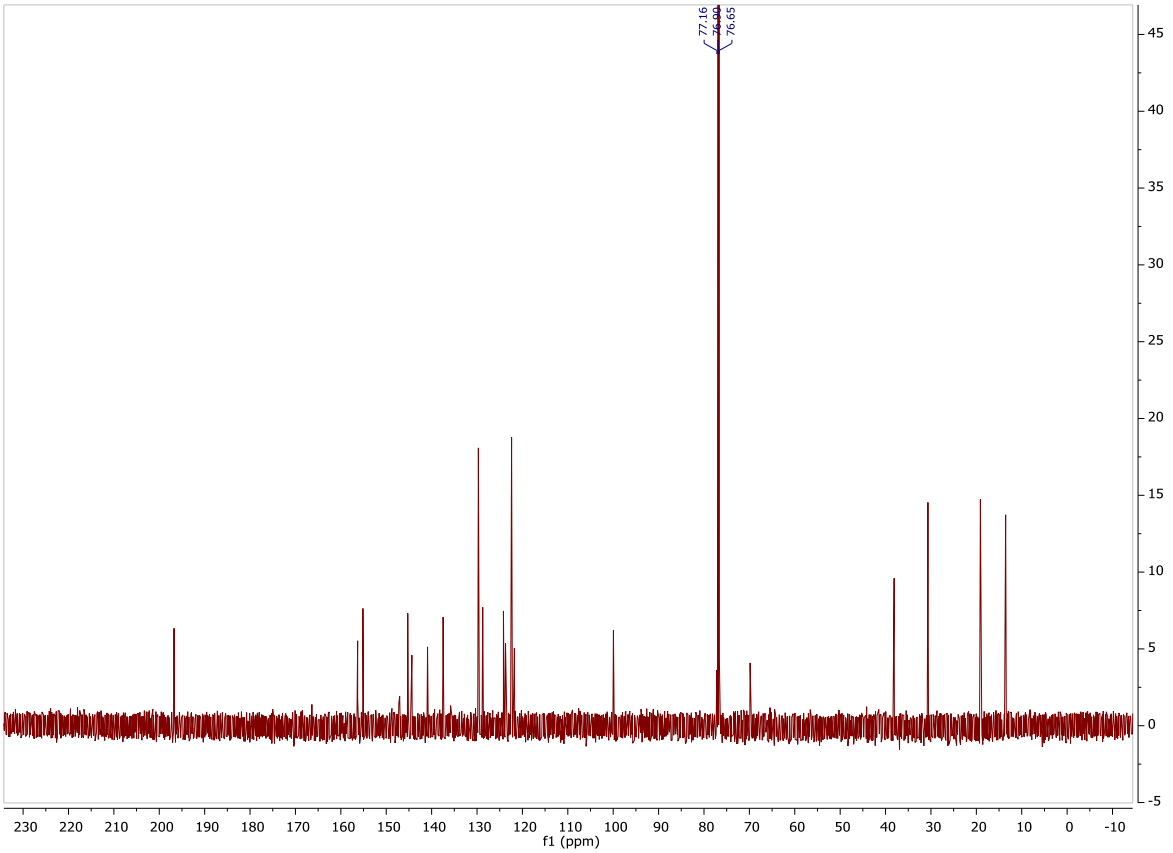


^{13}C NMR (126 MHz, $CDCl_3$):



IR spectrum (ATR):