Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Chemical Introduction of the Green Fluorescence: Imaging of Cysteine Cathepsins by an Irreversibly Locked GFP Fluorophore

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Inhibition assays for cathepsins L, S, K, and B

Cathepsin L inhibition assay. Human isolated cathepsin L (Enzo Life Sciences) was assayed spectrophotometrically (Cary 50 Bio, Varian) at 405 nm and at 37 ºC. Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, and 0.01% Brij 35. An enzyme stock solution of 135 µg/mL in 20 mM malonate buffer pH 5.5, 400 mM NaCl, and 1 mM EDTA was diluted 1:100 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. The inhibitor stock solution was prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-pNA was prepared with DMSO. The final concentration of DMSO was 2%, and the final concentration of the substrate was 100 µM (= 5.88 K_m). Assays were performed with a final concentration of 54 ng/mL of cathepsin L. Into a cuvette containing 940 µL assay buffer, inhibitor solution and DMSO in a total volume of 10 µL, and 10 µL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 40 µL of the cathepsin L solution.

Cathepsin S inhibition assay. Human recombinant cathepsin S (Calbiochem) was assayed spectrophotometrically (Cary 50 Bio, Varian) at 405 nm and at 37 ºC. Assay buffer was 50 mM sodium phosphate buffer pH 6.5, 50 mM NaCl, 2 mM EDTA, and 0.01% Triton X-100. An enzyme stock solution of 375 µg/mL in 35 mM potassium phosphate, 35 mM sodium acetate pH 6.5, 2 mM DTT, 2 mM EDTA, and 50% ethylene glycol was diluted 1:100 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. The inhibitor stock solution was prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-pNA was used in a final concentration of 100 µM (= 0.85 K_m). Assay was performed with a final concentration of 75 ng/mL of cathepsin S. Into a cuvette containing 960 µL assay buffer, inhibitor solution and DMSO in a total volume of 10 µL, and 10 µL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 20 µL of the cathepsin S solution.

Cathepsin K inhibition assay. Human recombinant cathepsin K (Enzo Life Sciences) was assayed fluorimetrically on a Monaco Safas spektrofluorometer flx. The wavelength for excitation was 360 nm and for emission 440 nm. An enzyme stock solution of 23 µg/mL in 50 mM sodium acetate pH 5.5, 50 mM NaCl, 0.5 mM EDTA, 5 mM DTT was diluted 1:100 with assay buffer (100 mM sodium citrate pH 5.0, 100 mM NaCl, 1 mM EDTA, 0.01% CHAPS) containing 5 mM DTT and incubated for 30 min at 37 ºC. The inhibitor stock solution was prepared in DMSO.

A 10 mM stock solution of the fluorogenic substrate Z-Leu-Arg-AMC was prepared with DMSO. The final concentration of DMSO was 2%, and the final concentration of the substrate was 40 µM (= 13.3 K_m). Assay was performed with a final concentration of 2 ng/mL of cathepsin K. Into a cuvette containing 970 µL assay buffer, inhibitor solution and DMSO in a total volume of 16 µL, and 4 µL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 10 µL of the cathepsin K solution.

Cathepsin B inhibition assay. Human isolated cathepsin B (Calbiochem) was assayed spectrophotometrically (Cary 50 Bio, Varian) at 405 nm and at 37 ºC. Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, 0.01% Brij 35. An enzyme stock solution of 1.81 mg/mL in 20 mM sodium acetate buffer pH 5.0, 1 mM EDTA was diluted 1:500 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. The inhibitor stock solution was prepared in DMSO. A 100 mM stock solution of the chromogenic substrate Z-Arg-Arg-pNA was prepared with DMSO. The final concentration of DMSO was 2% and the final concentration of the substrate was 500 µM (= 0.45 K_m). Assay was performed with a final concentration of 72 ng/mL of cathepsin B. Into a cuvette containing 960 µL assay buffer, inhibitor solution and DMSO in a total volume of 15 µL, and 5 µL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 20 µL of the cathepsin B solution.

Spectral properties of 12

Fig. Spectral properties of **12** (10 μM, 1% DMSO). (•) CH_2Cl_2; (•) MeOH; (•) H_2O.

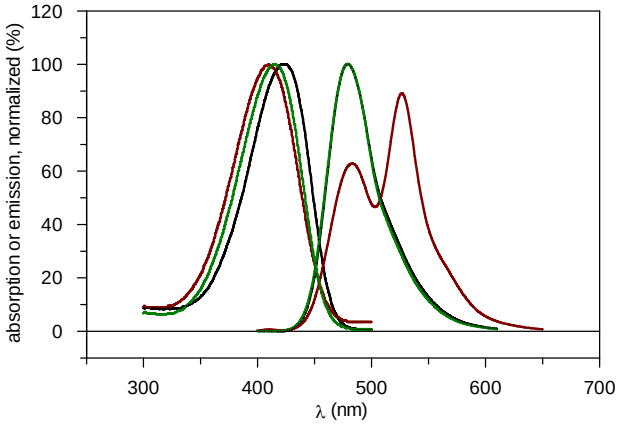
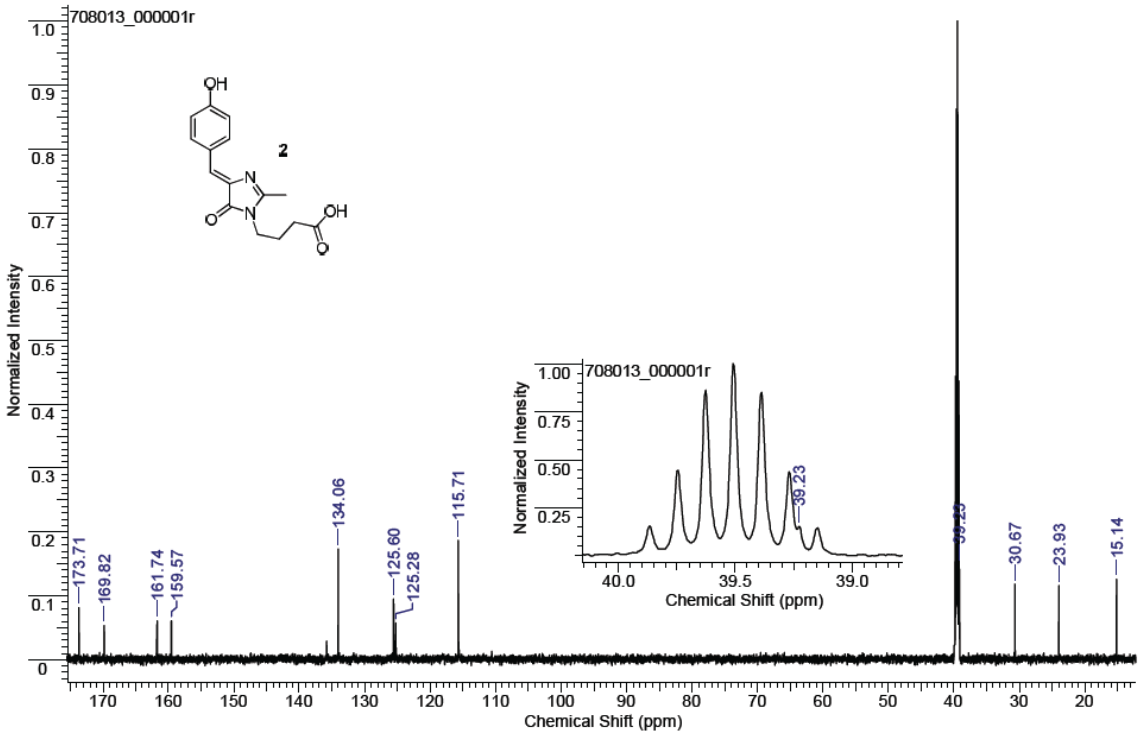


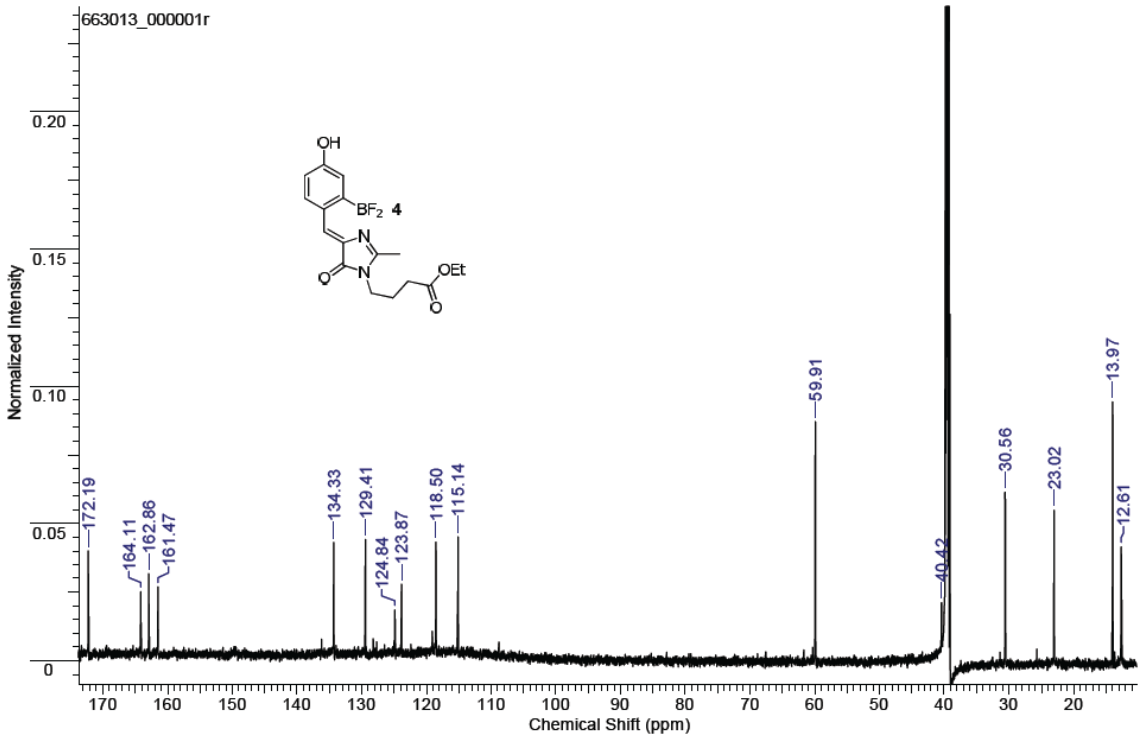
Table Absorption and emission maxima of **12**.

cmpd	$\lambda_{ex}(\lambda_{em})$ (nm)		
	CH_2Cl_2	MeOH	H_2O
12	423(480)	415(480)	410(486/528)

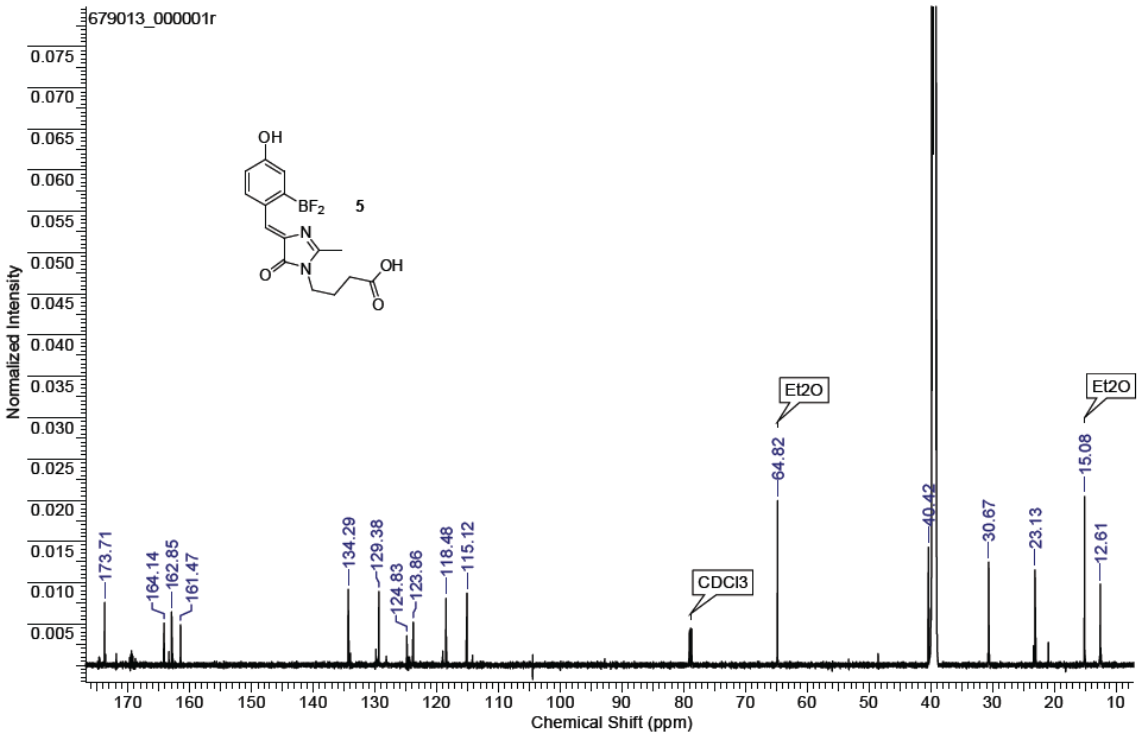
^{13}C NMR spectrum of compound **2**



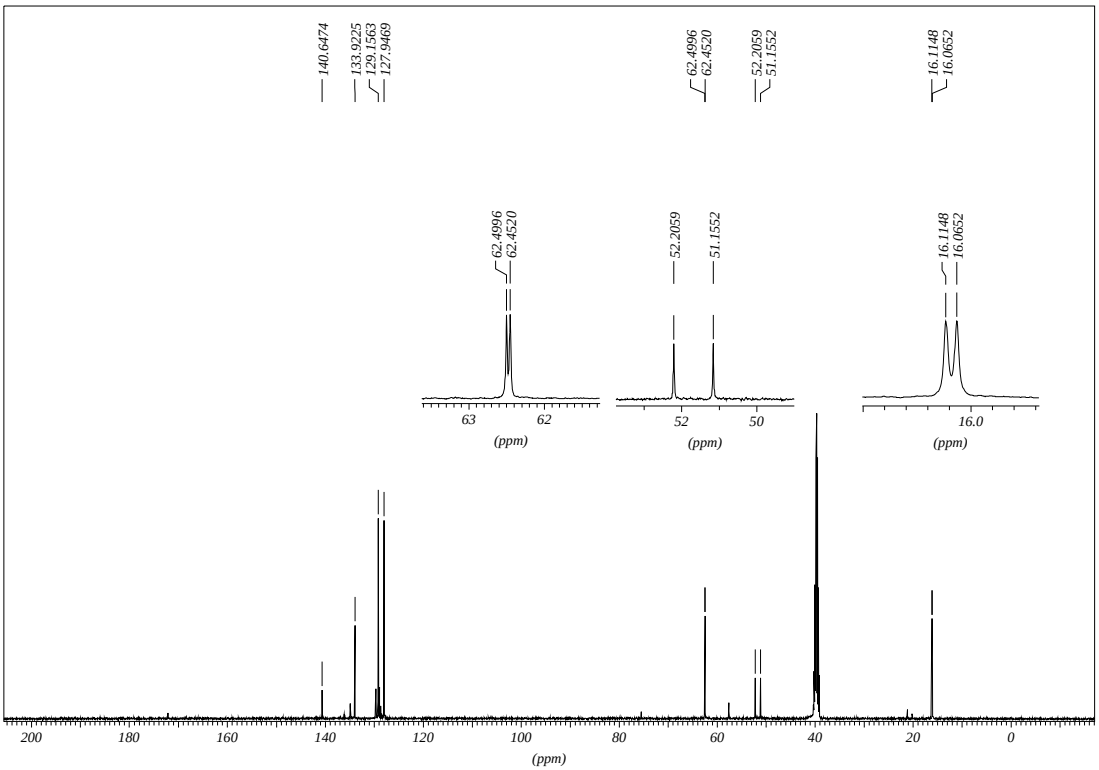
^{13}C NMR spectrum of **4**



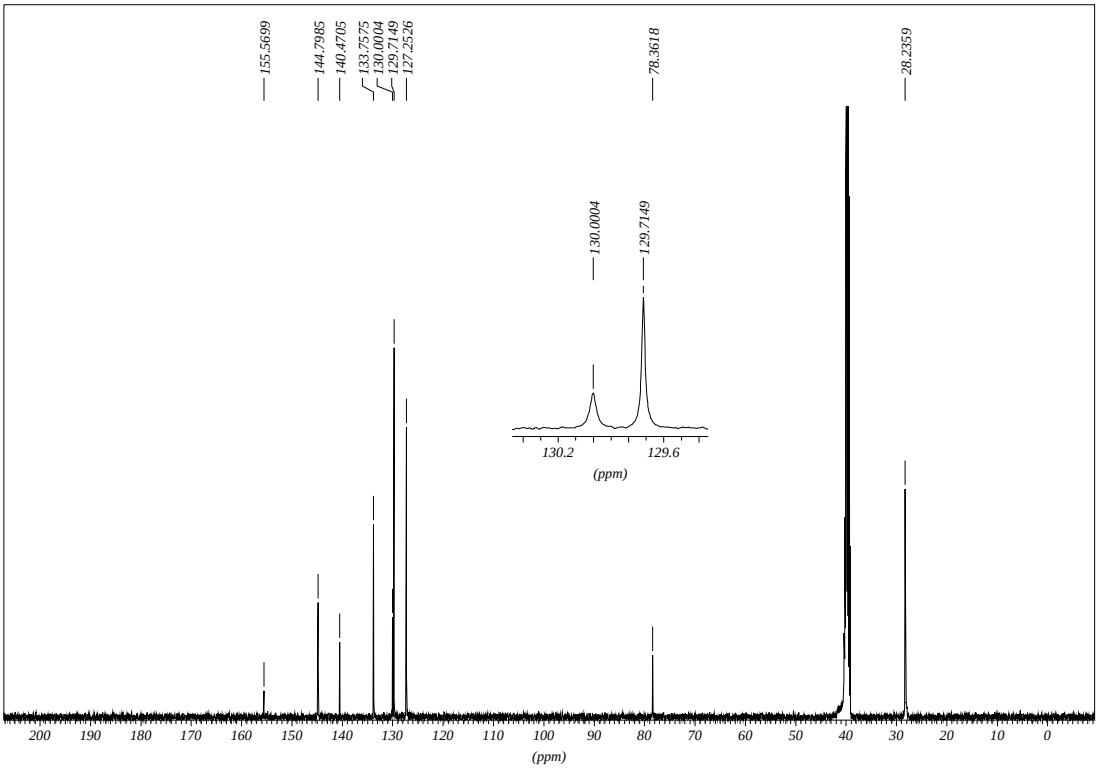
^{13}C NMR spectrum of **5**



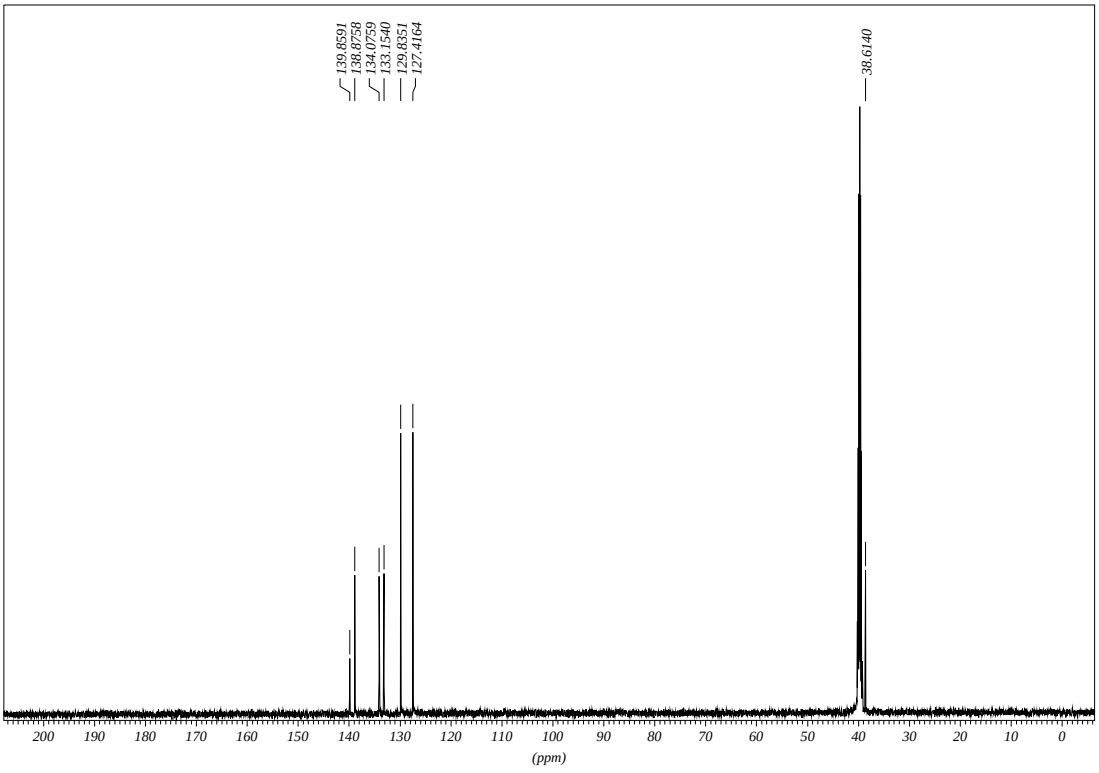
^{13}C NMR spectrum of **7**



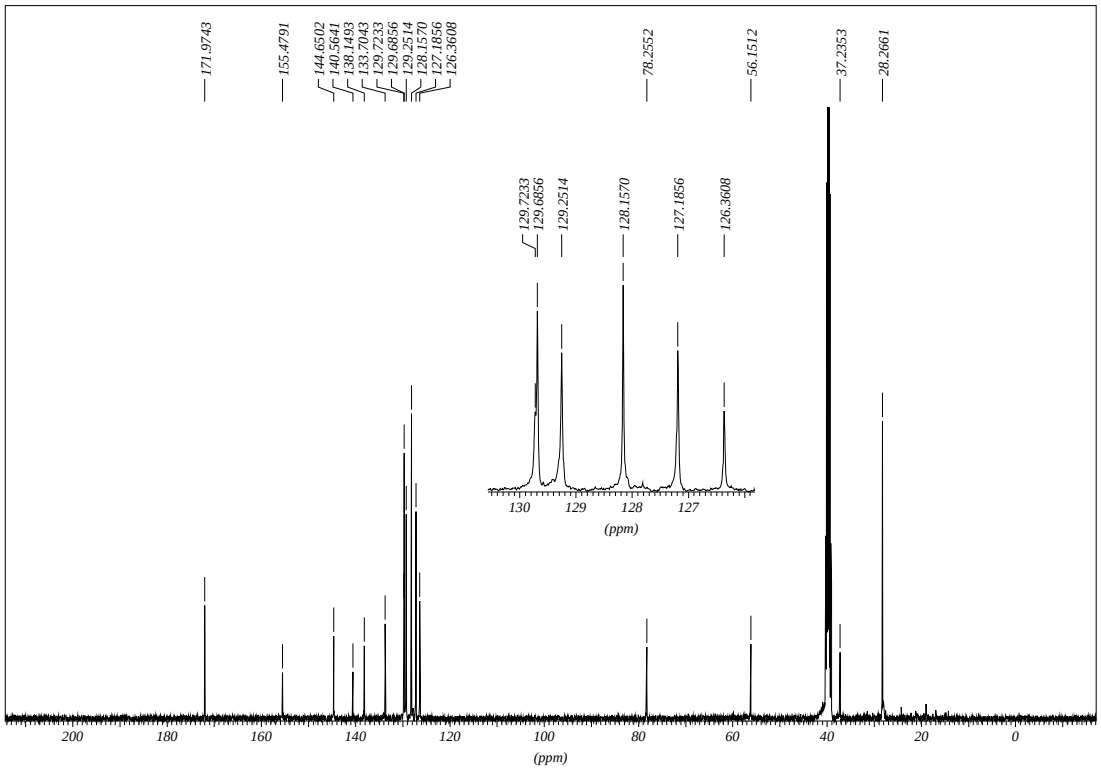
^{13}C NMR spectrum of **8**



^{13}C NMR spectrum of **9**



^{13}C NMR spectrum of **11**



^{13}C NMR spectrum of **12**