Supporting Information

A new cyclopamine glucuronide prodrug with improved kinetics of drug release.

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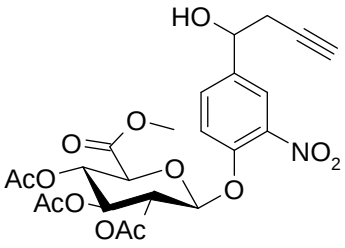
General experimental section

All reactions were performed under an atmosphere of N_2. Unless otherwise stated, solvents used were of HPLC quality. Chemicals were of analytical grade from commercial sources and were used without further purification. The reaction progress was monitored on precoated silica gel TLC plates MACHEREY-NAGEL ALUGRAM® SIL G/UV_{254} (0.2 mm silica gel 60). Spots were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of 3 g of phosphomolibdic acid in 100 mL of ethanol followed by heating with a hot gun. Flash column chromatography was performed using MACHEREY-NAGEL silica gel 60 (15-40 µm) as the stationary phase. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 Avance III instrument, equipped with an ultra shielded magnet and a BBFO 5 mm broadband probe. Chemical shifts (δ) are reported in parts per million (ppm) from high to low field and referenced to residual solvent. Coupling constant (*J*) are reported in hertz (Hz). Standard abbreviations indicating multiplicity are used as follows: br = broad, s = singlet, d = doublet, t = triplet, m = multiplet. Melting points were measured on a Büchi Melting Point B-545 instrument and are uncorrected. High resolution ESI mass spectrometry was carried out by the CRMPO (Centre Régional de Mesures Physiques de l'Ouest), at the University of Rennes 1. Analytical RP-HPLC was carried out on a Dionex Ultimate 3000 system equipped with a UV/Visible variable wavelength detector and with a reverse-phase column chromatography Acclaim$^{(R)}$ (C18, 250x4.6 mm, 5 µm, 120 Å) at 30°C and 1 mL.min^{-1}. Gradient eluent was composed of A (0.2% TFA in water) and B (CH_3CN). LCMS analysis were performed with a Waters instrument composed with a Waters 2695 Separator module, a Waters 2489 UV/Visible variable wavelength detector and a Waters 3100 Mass detector. The reverse-phase column chromatography used was an Acclaim$^{(R)}$ (C18, 250x4.6 mm, 5 µm, 120 Å) at 30°C and 1 mL.min^{-1}. Gradient eluent was composed of A (0.2% TFA in water) and B (CH_3CN). Preparative reverse-phase HPLC for **2b** was performed with a VWR LaPrep system. Solvent flow 4 mL.min^{-1} was applied to a semi-preparative column ACE® C18-AR (100x10 cm, 5 µm). Gradient eluent was composed of A (0.2% TFA in water) and B (CH_3CN). Method: linear gradient beginning with A/B = 80/20 v/v, reaching A/B = 0/100 v/v within 30 min.. All chromatograms were recorded at 254 nm.



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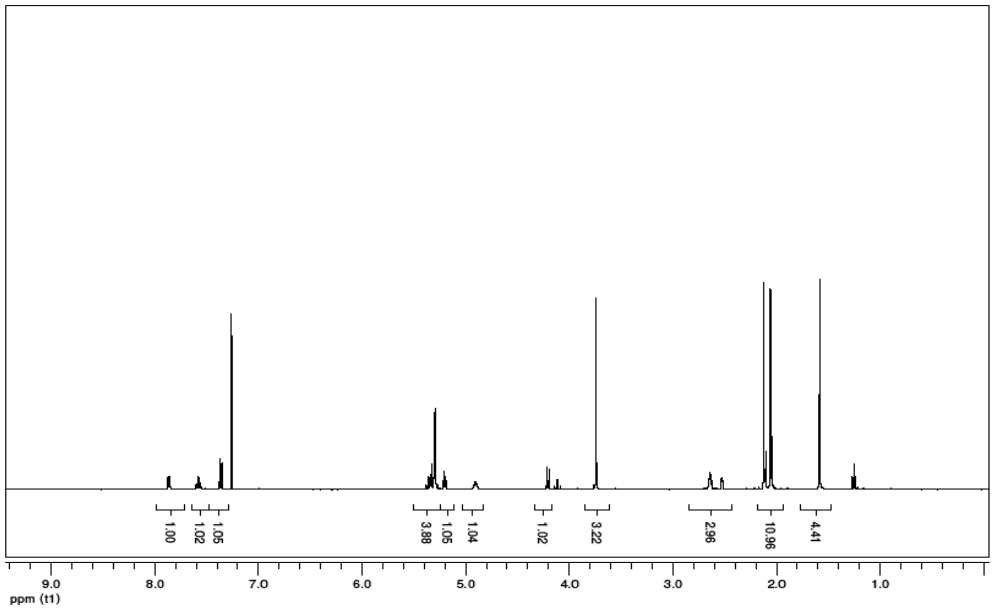
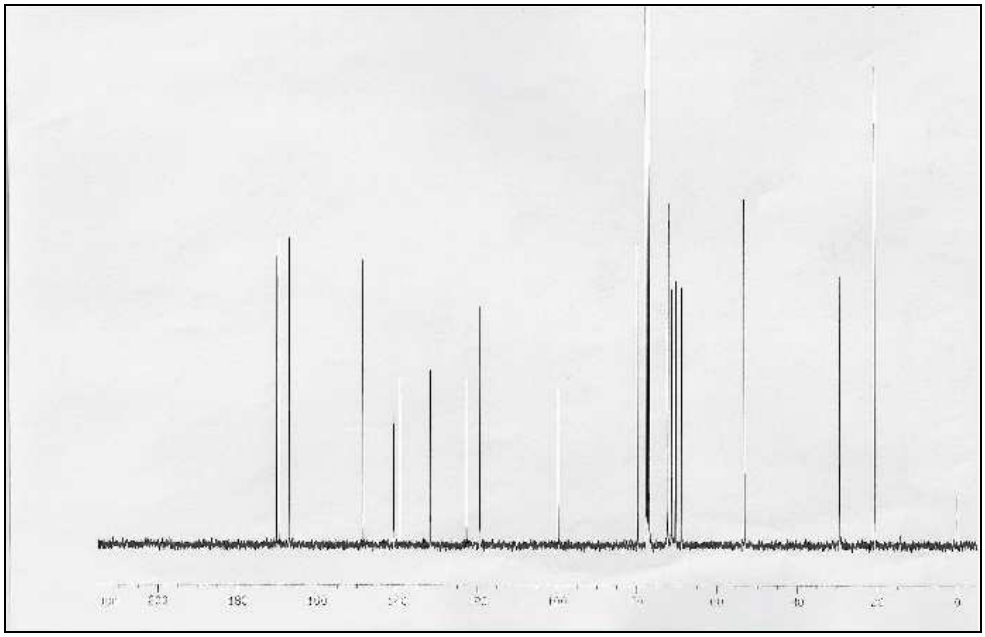
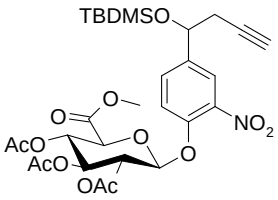


^{1}H NMR spectrum of **8**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **8**, 100 MHz, $CDCl_3$



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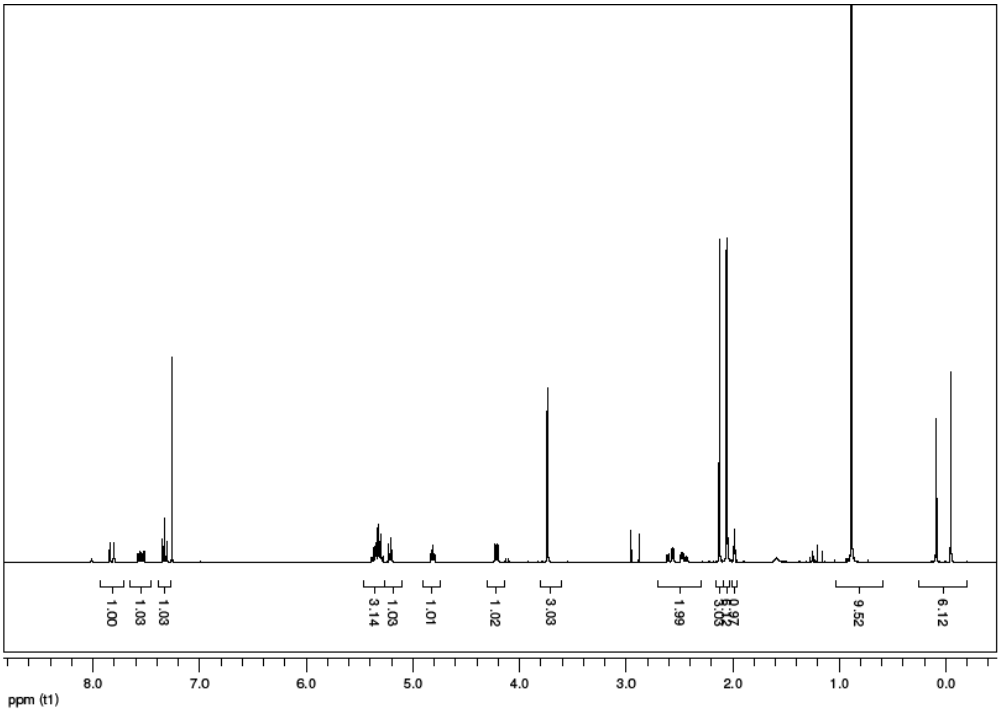
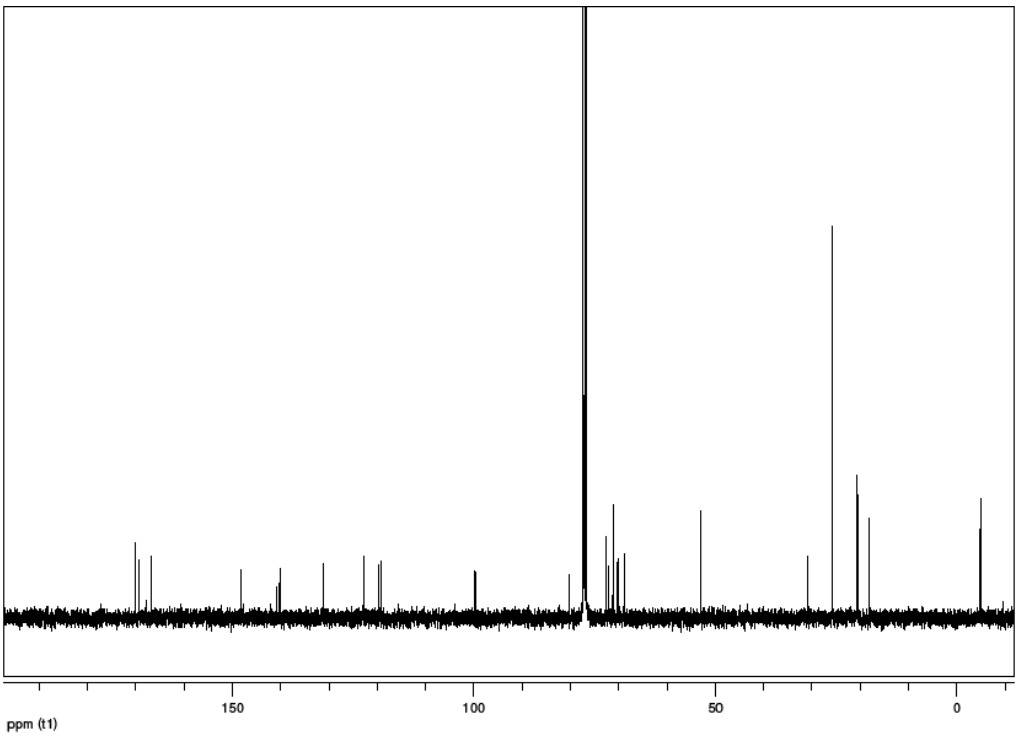
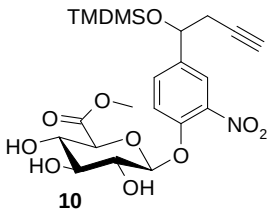


^{1}H NMR spectrum of **9**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **9**, 100 MHz, $CDCl_3$



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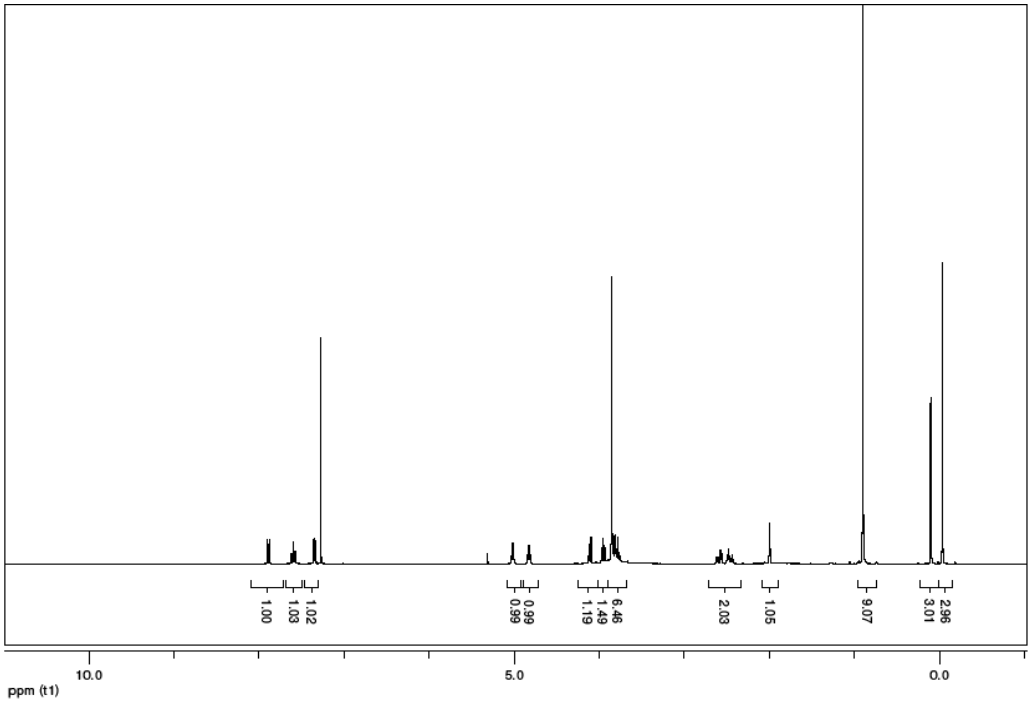
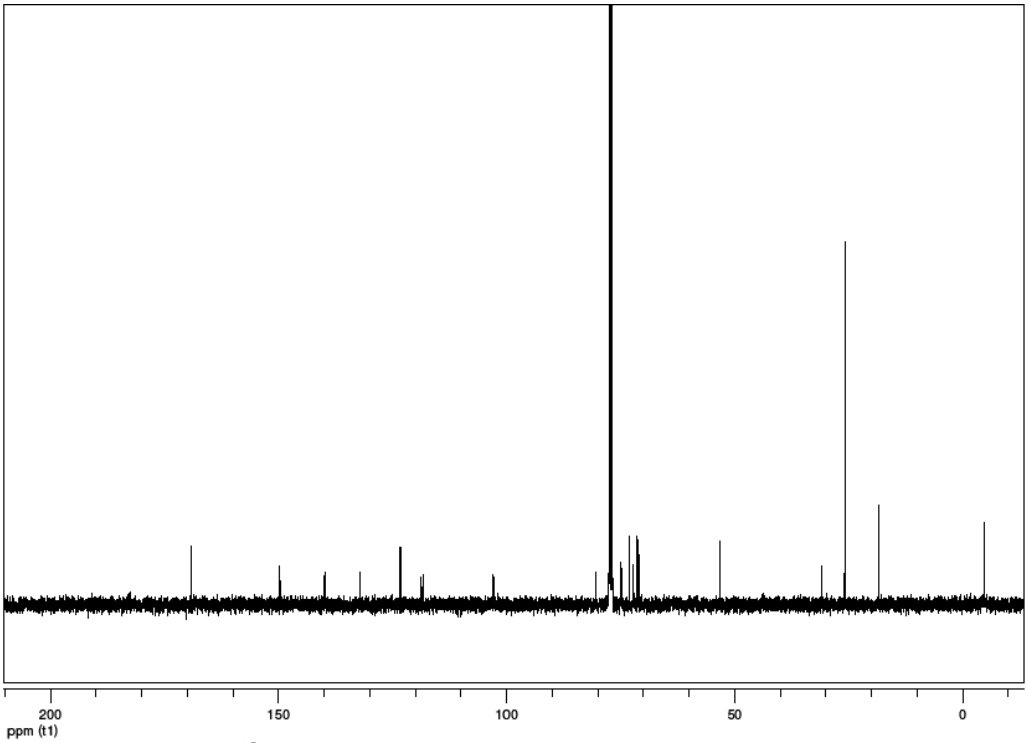
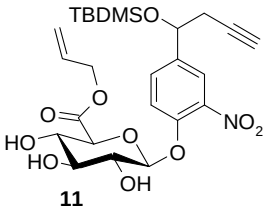


^{1}H NMR spectrum of **10**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **10**, 100 MHz, $CDCl_3$



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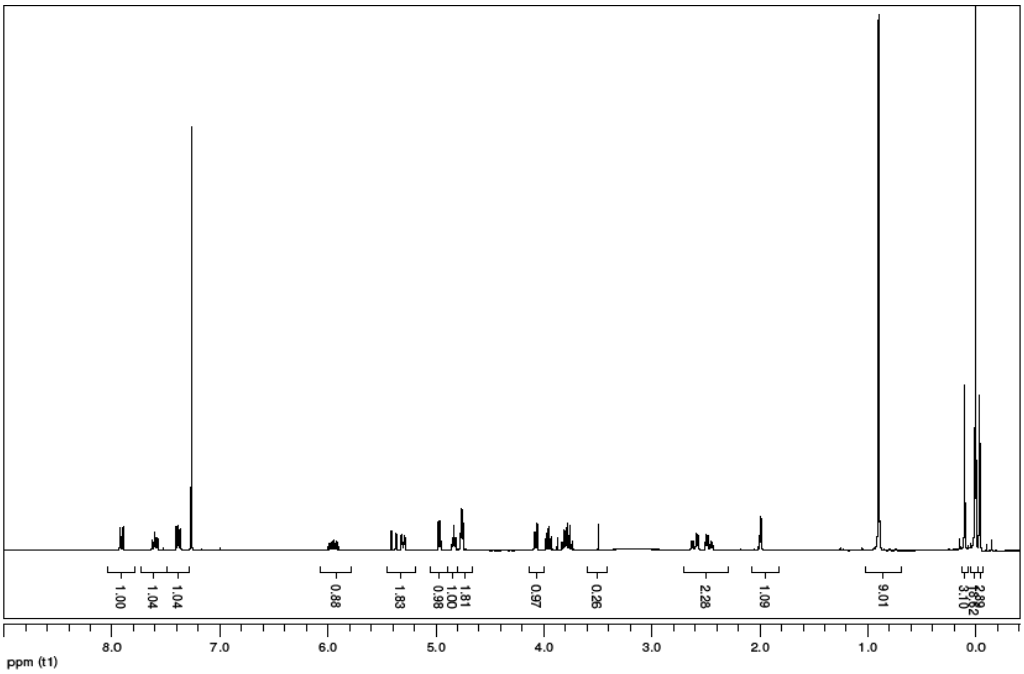
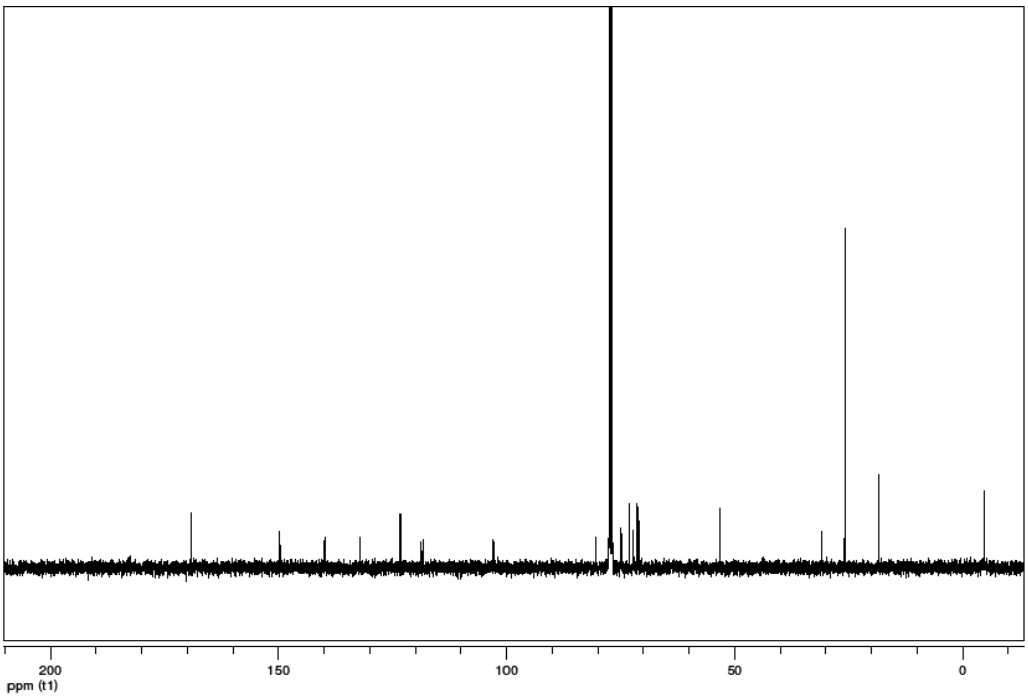
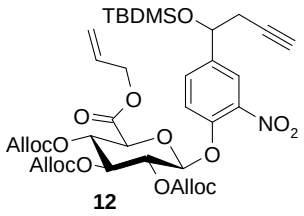


^{1}H NMR spectrum of **11**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **11**, 100 MHz, $CDCl_3$



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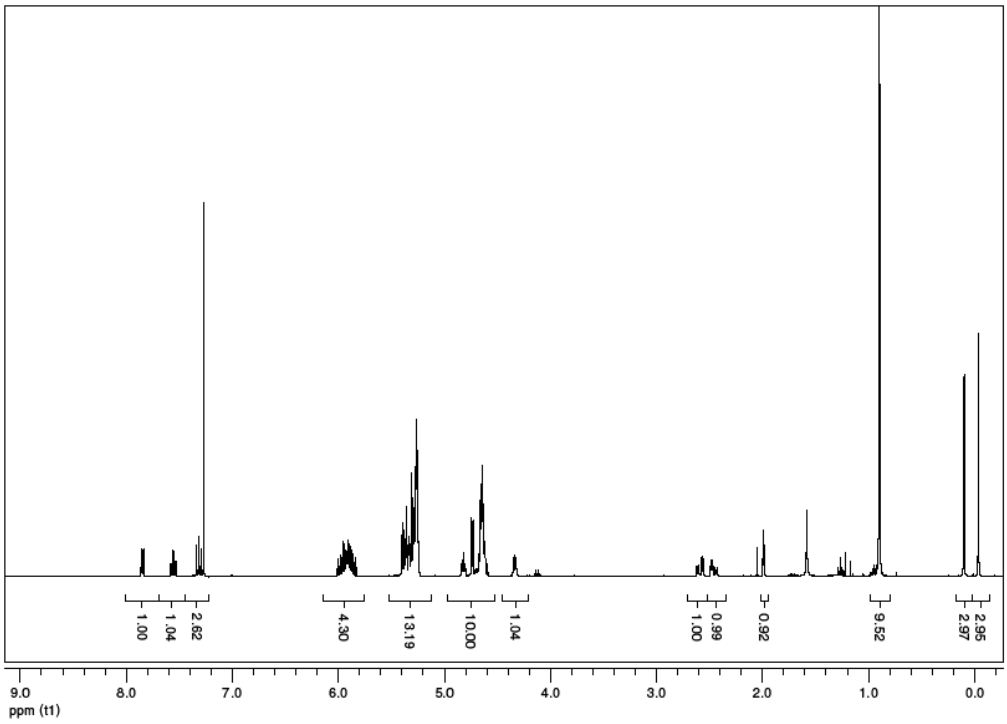
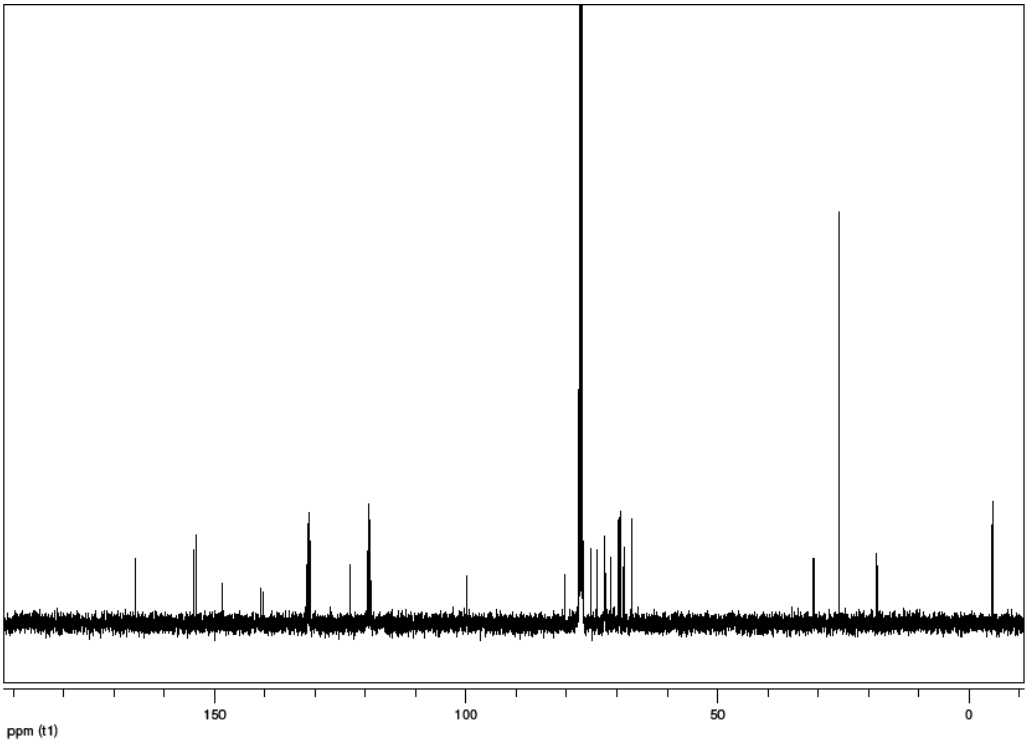
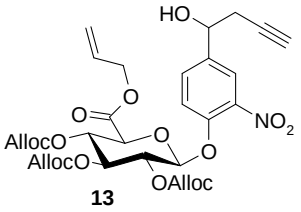


^{1}H NMR spectrum of **12**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **12**, 100 MHz, $CDCl_3$



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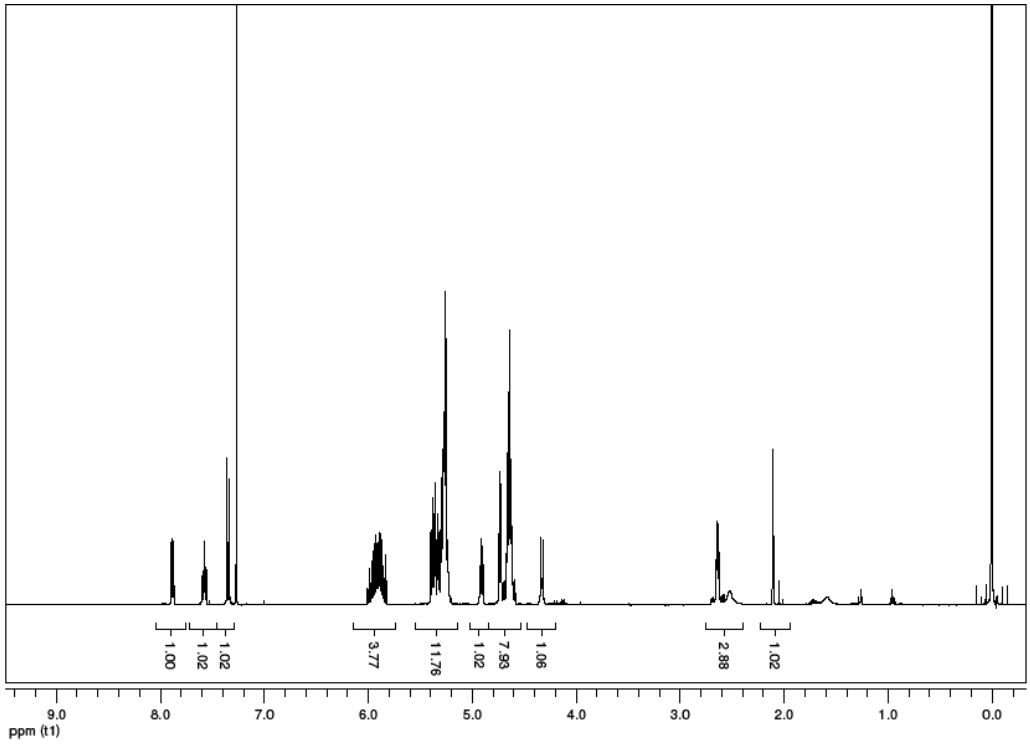
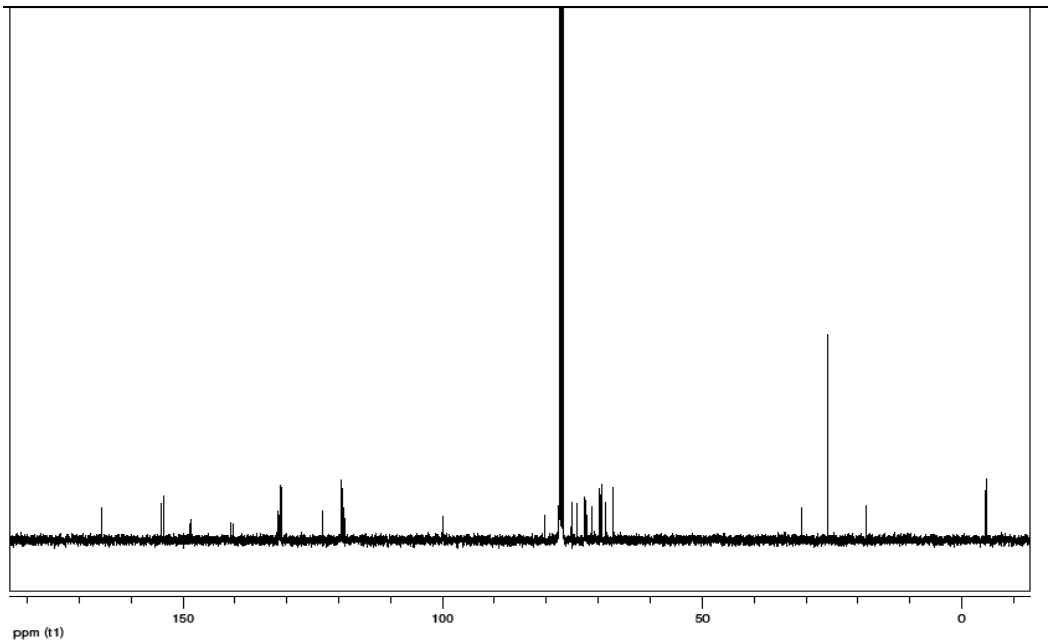
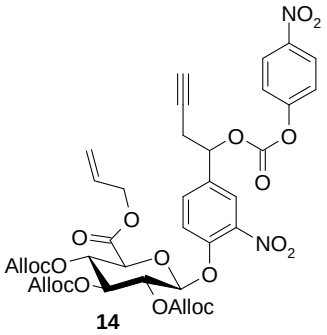


^{1}H NMR spectrum of **13**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **13**, 100 MHz, $CDCl_3$



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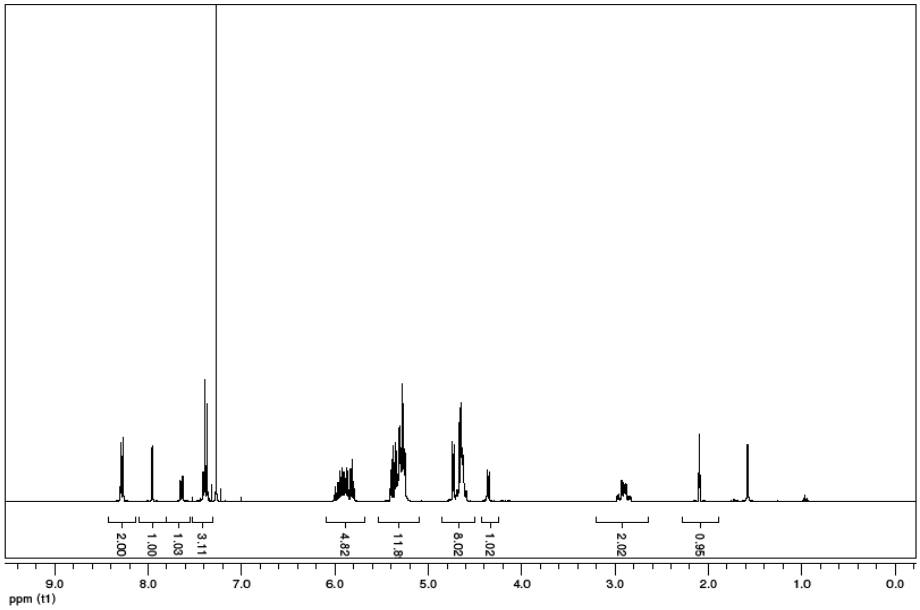
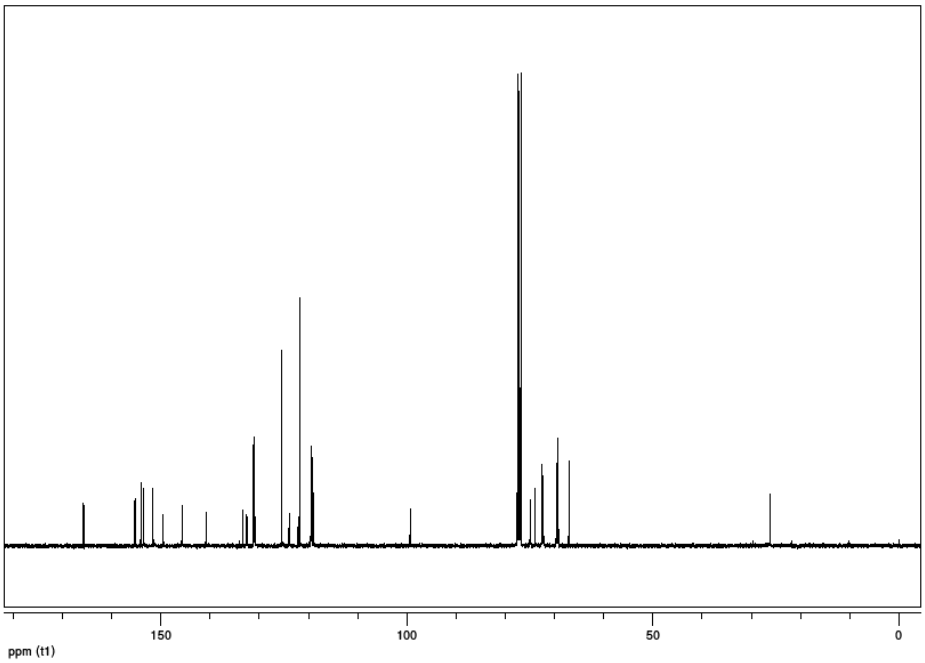
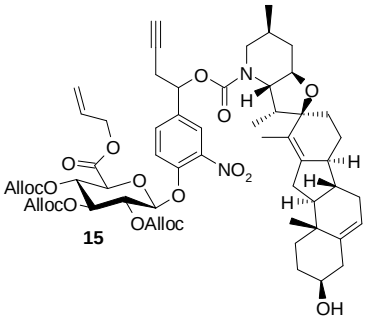


^{1}H NMR spectrum of **14**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **14**, 100 MHz, $CDCl_3$



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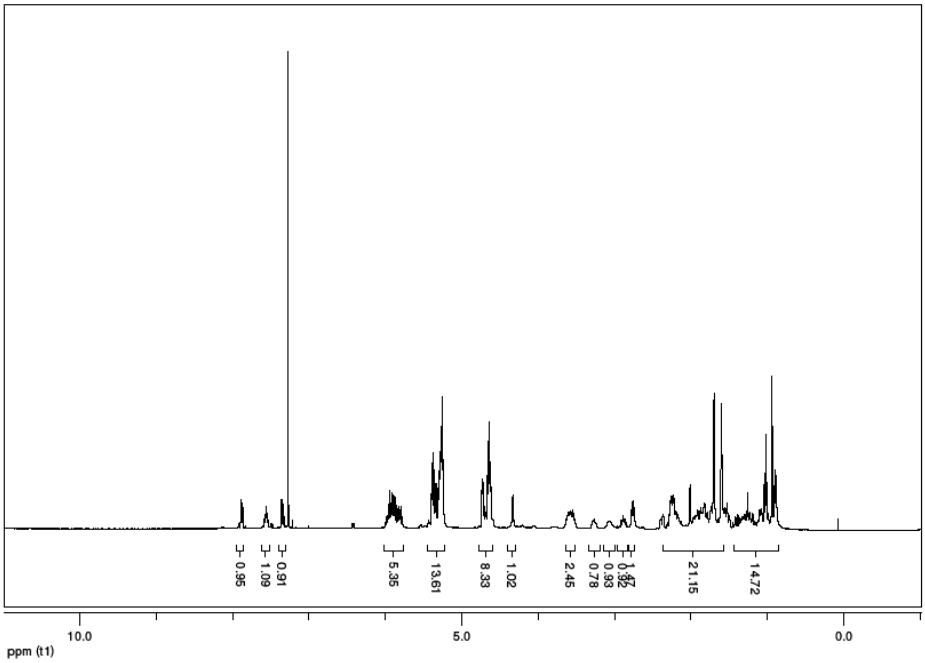
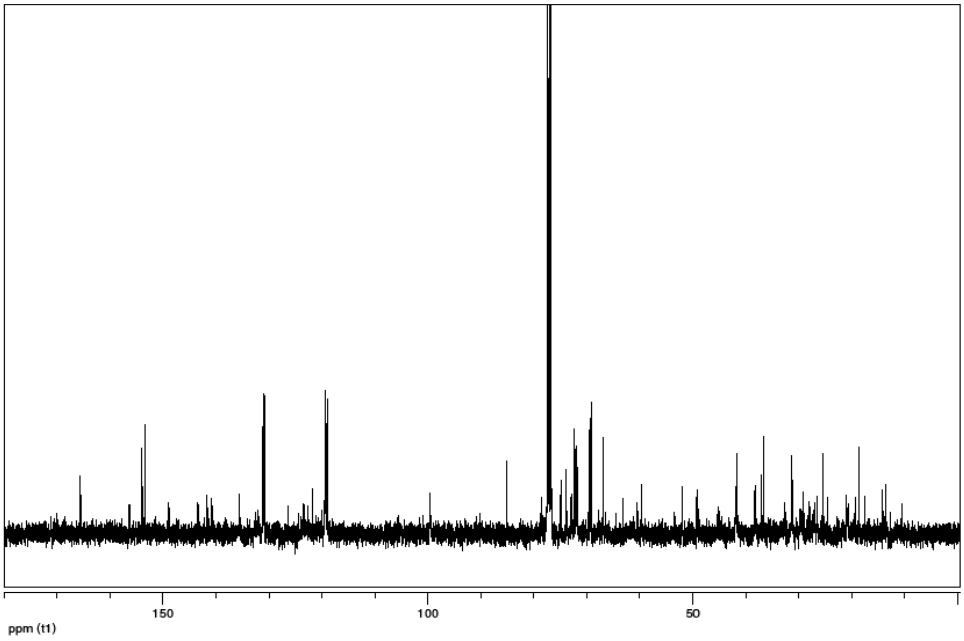
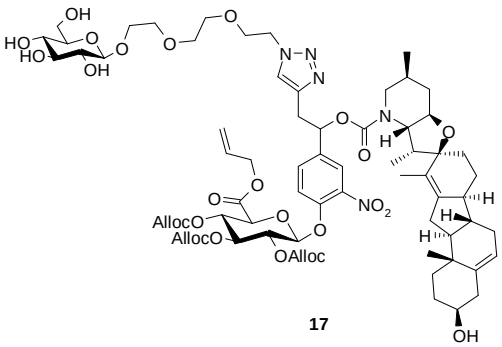


^{1}H NMR spectrum of **15**, 400 MHz, $CDCl_3$



^{13}C NMR spectrum of **15**, 100 MHz, $CDCl_3$



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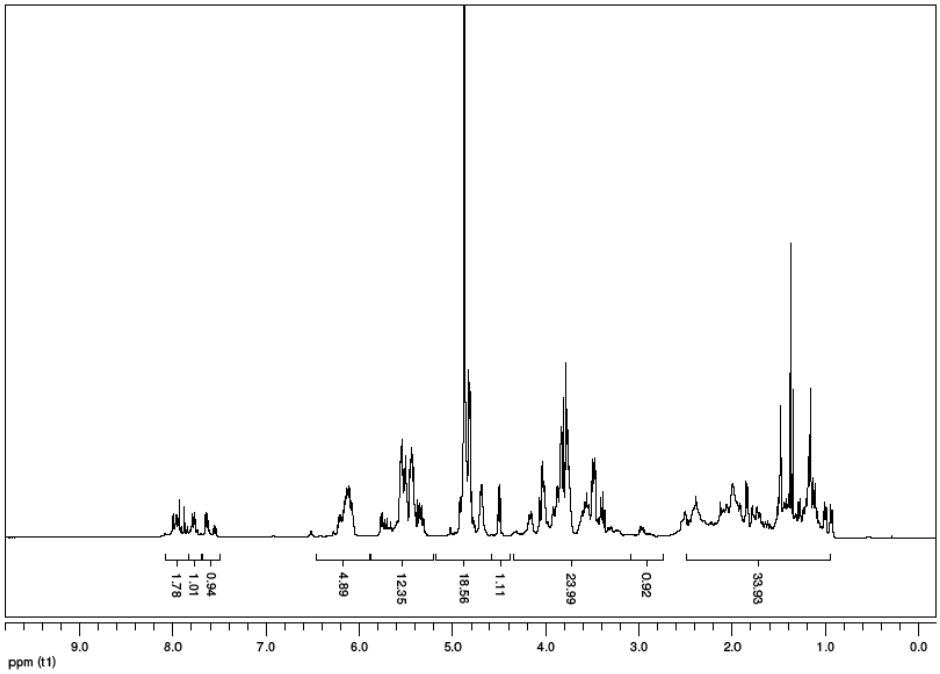
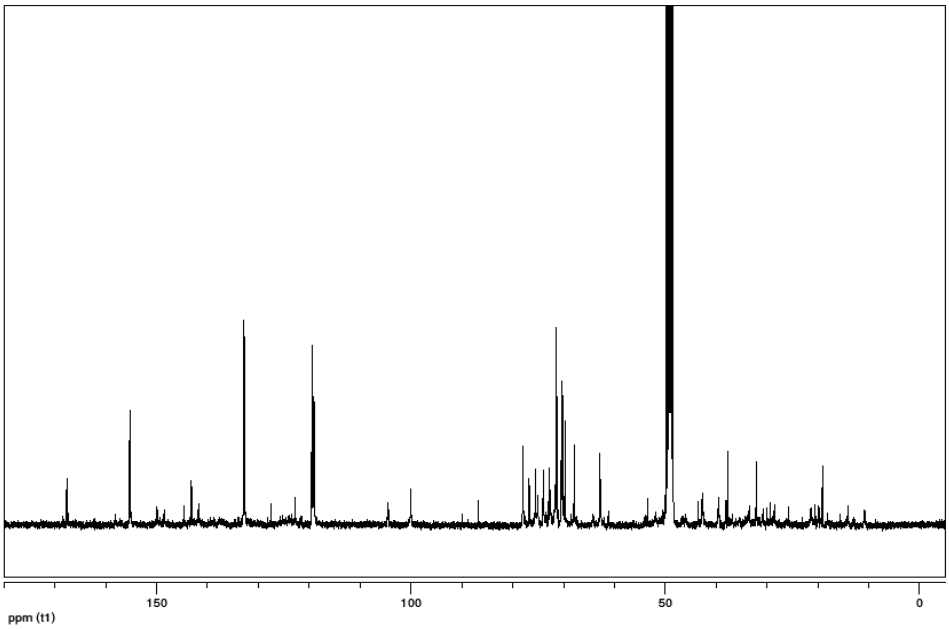
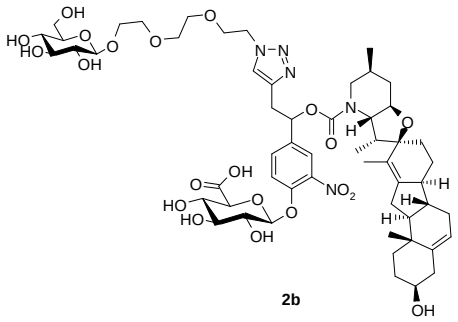


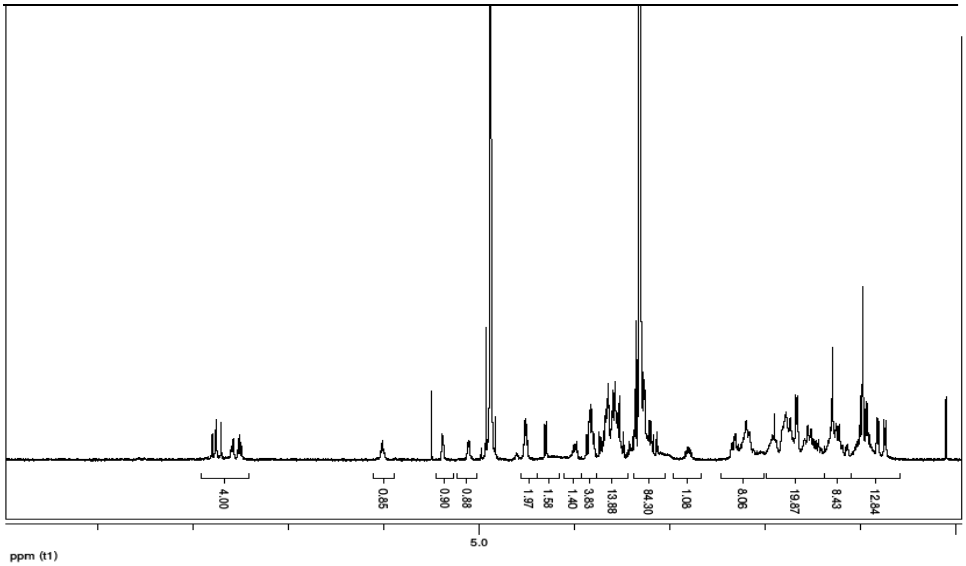
^{1}H NMR spectrum of **17**, 400 MHz, CD_3OD, 313 K



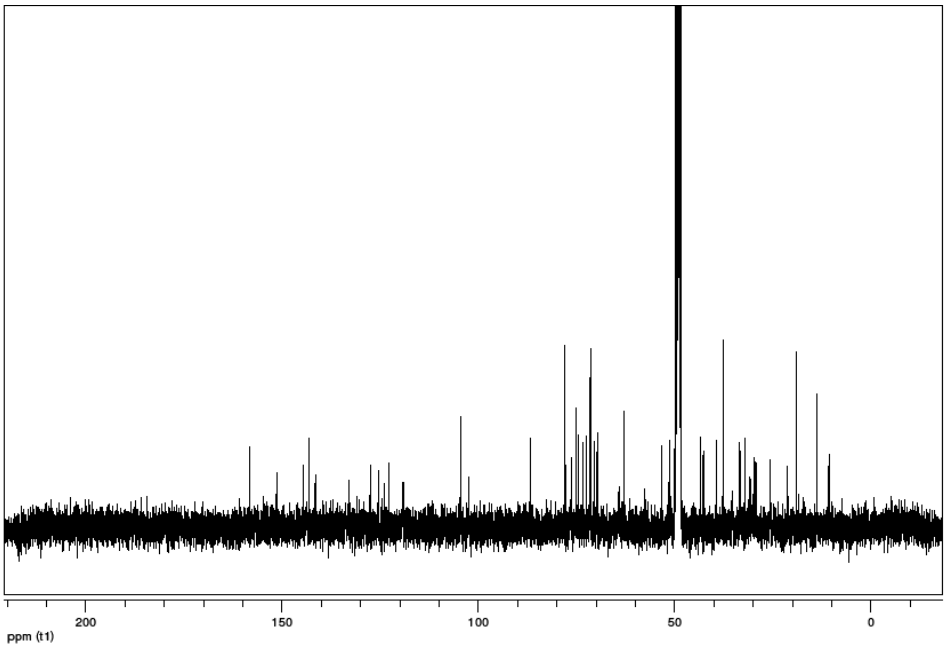
^{13}C NMR spectrum of **17**, 100 MHz, CD_3OD



2b



^{1}H NMR spectrum of **2b**, 400 MHz, CD_3OD



^{13}C NMR spectrum of **2b**, 100 MHz, CD_3OD

LRESI-SM spectra