Peptide-targeted dendrimeric prodrugs of 5-aminolevulinic acid: A novel approach towards enhanced accumulation of protoporphyrin IX for photodynamic therapy

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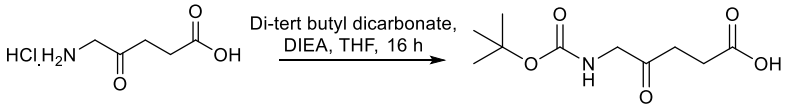
General information

Chemical reagents were purchased from Sigma, Aldrich, Fluka, Acros and Novabiochem. Anhydrous DCM was obtained by distillation over calcium hydride. All other solvents were purchased from Fisher Scientific. Analytical TLC was performed using silica gel 60 F_{254} pre-coated on aluminium sheets (0.25 mm thickness) and reverse-phase analytical TLC was performed with RP-18 F_{254s} pre-coated on aluminium sheets (0.27 mm thickness). Column chromatography was performed on silica gel 60 (35-70 micron) from Fisher Scientific. IR spectra were recorded on a Perkin-Elmer 782 infra-red spectrometer and values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Advance DPX 500MHz FT and Varian Mercury VX 400MHz spectrometers. *J* values are given in Hz. Mass spectrometry was performed using a microTOF instrument from Bruker Daltonics (Bremen, Germany). Analyses were performed at 35 ± 0.1 °C on a Gemini 5 µm C18 110A column, (150 x 4.6 mm - Phenomenex, UK), equipped with a SecurityGuard C18 (ODS) 4 x 3.0 mm ID guard column (Phenomenex, UK), at a flow rate of 1 mL/min. Semi-preparative RP-HPLC was performed on a Dionex HPLC system equipped with a Phenomenex Gemini 5 µm C-18 (250 x 10 mm) column with a flow rate of 2.5 mL min^{-1}. Mobile phase A was 0.1% TFA in water and mobile phase B was 0.1% TFA in MeCN. Gradient 1 was *T* = 0 min, *B* = 5%; *T* = 10 min, *B* = 95%; *T* = 15 min, *B* = 95%; *T* = 15.1 min, B = 5%; *T* = 18.1 min, *B* = 5%. Gradient 2 was *T* = 0 min, *B* = 5%; *T* = 25 min, *B* = 95%; *T* = 35 min, *B* = 95%; *T* = 35.1min, *B* = 5%.

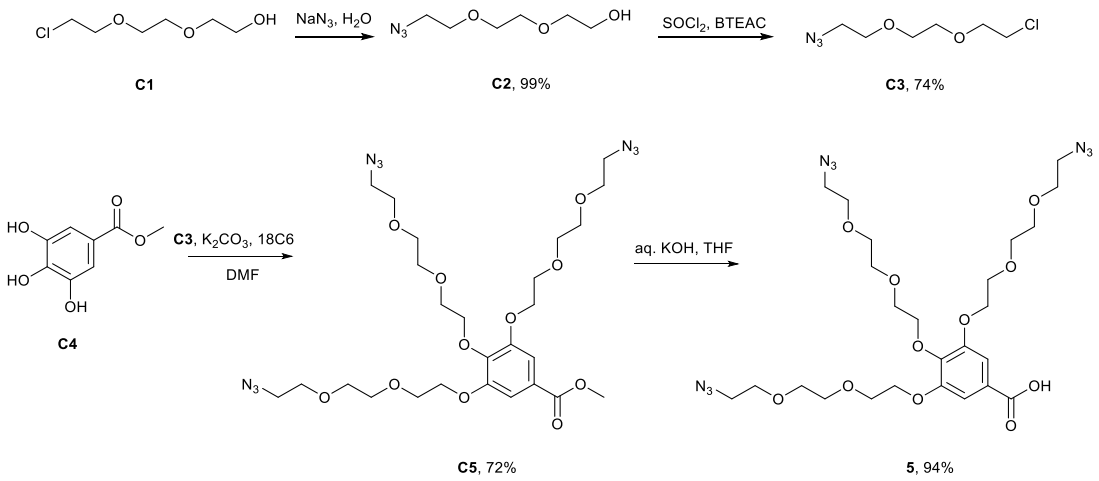
Synthesis of Boc-ALA, 3



ALA.HCl **3**, 78%

A stirred solution of ALA.HCl (1.68 g, 10.0 mmol) in dry THF (120 mL) was treated with di-*tert* butyl dicarbonate (4.66 g, 21.4 mmol). The mixture was cooled in an ice bath under N_2 and was then treated with a solution of DIEA (1.7 mL, 10.0 mmol) in dry THF (30 mL) added dropwise over 3 h. The mixture was then stirred overnight at RT, then the solvent was evaporated and the residue obtained was taken up in DCM (40 mL) and washed with citric acid (2 x 40 mL). The organic phase was dried over $MgSO_4$, filtered, and the solvent evaporated to give the crude product which was purified by column chromatography on silica gel eluting with 10% MeOH in DCM + 0.05% AcOH. Removal of solvent gave **3** as a yellow oil (1.70 g, 78%); R_f = 0.59 (10% MeOH in DCM); IR (film) 3373 (NH), 2976 (CH), 1713 (CO), 1685 (CO); ^{1}H NMR (400MHz, CD_3OD) δ 1.46 (s, 9H), 2.59 (t, *J* = 6.6, 2H), 2.74 (t, *J* = 6.4, 3H), 3.93 (s, 2H), 4.88 (br, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 28.50 , 28.68 , 35.03 , 50.79, 80.58, 158.40, 176.17, 207.49.

Synthesis of core azido unit, 5[1]



Scheme 1. Synthesis of the core unit 5 with terminal azido group.

2-[2-(2-azidoethoxy) ethoxy] ethanol, C2

A solution of 2-[2-(2-chloroethoxy) ethoxy] ethanol (1.53 g, 9.07 mmol) in H_2O (5 mL) was treated with sodium azide (1.18 g, 18.1 mmol) and the reaction mixture was heated at 75 °C while stirring for 48 h. The reaction mixture was then cooled down to RT and H_2O was evaporated under reduced pressure. The resulting residue was suspended in diethyl ether (10 mL) and filtered through a sintered funnel. The solvent was evaporated to obtain **C2** as a colourless liquid (1.58 g, 99%) which was used without further purification. IR (film) 3435 (OH), 2871 (CH), 2101 (N_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.2-3.4 (m, 2H), 3.50-3.80 (m, 10H).

1-azido-2-[2-(2-chloroethoxy) ethoxy] ethane, C3

A mixture of **C2** (1.30 g, 7.37 mmol) and benzyltriethylammonium chloride (BTEAC) (5.00 mg, 0.02 mmol) was heated at 65 °C in a three-necked round bottom flask under Ar. The reaction mixture was treated with $SOCl_2$ (4.30 mL, 59.2 mmol) added dropwise via an addition funnel, and the reaction was stirred for 3 h. After cooling at RT, excess $SOCl_2$ was removed under reduced pressure and the resulting crude product was suspended in phosphate buffer (50 mM, pH 7.0, 20 mL) and extracted with a mixture of EtOAc/petroleum ether (40 mL, 1:1). The organic layer was washed with phosphate buffer (20 mL) and dried over $MgSO_4$. The organic extract was filtered, and solvent was evaporated to give **C3** as a pale yellow liquid (1.05 g, 74%) which was used without further purification. IR (film) 2892 (CH), 2109 (N_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.33-3.40 (m, 2H), 3.58-3.77 (m, 10H).

Methyl 3,4,5-tris(2-(2-(2-azidoethoxy) ethoxy) ethoxy) benzoate, C5

A solution of **C3** (2.80 g, 14.5 mmol) in DMF (40 mL) was treated with methyl gallate **C4** (0.84 g, 4.59 mmol), dry K_2CO_3 (6.48 g, 46.8 mmol) and 18-crown-6 (0.10 g, 0.40 mmol) and the resulting suspension was heated at 80 °C under N_2 for 36 h. After cooling down to RT, the solvent was evaporated and the residue was extracted with EtOAc and H_2O (80 mL, 1:1). The aqueous phase was extracted with EtOAc (40 mL) and the combined organic phases were washed with brine (2 x 40 mL) and dried over $MgSO_4$. The organic extract was filtered, and solvent was evaporated to obtain the crude product (2.85 g). Purification by column chromatography on silica gel eluting with EtOAc gave **C5** as a dark yellow oil (2.16 g, 72%); R_f = 0.42 (EtOAc); IR (film) 2902 (CH), 2112 (N_3), 1718 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.37-3.42 (m, 6H), 3.59-3.86 (m, 27H), 4.16-4.22 (m, 6H), 7.26 (s, 2H).

3,4,5-*Tris*(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzoic acid, 5

A solution of **C4** (0.55 g, 0.84 mmol) in THF (6 mL) was treated with KOH (0.06 g, 1.05 mmol) in H_2O (1 mL) and the reaction was stirred at 50 °C for 24 h. The reaction mixture was then neutralized with 1M HCl (0.5 mL) and extracted with EtOAc/H_2O (1:1, 40 mL) and combined organics were dried over $MgSO_4$. The organic extract was filtered and evaporated under reduced pressure to give **5** as yellowish oil (0.5 g, 94%) which was used in next step without further purification. R_f = 0.3 (EtOAc: petroleum ether, 2:8); IR (film) 3025-2776 (OH broad), 2908 (CH), 2111 (N_3), 1705 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.36-3.40 (m, 6H), 3.64-3.90 (m, 24H), 4.20-4.27 (m, 6H), 7.36 (s, 2H).

HPLC for click reaction in the formation of ALA dendrimer 7

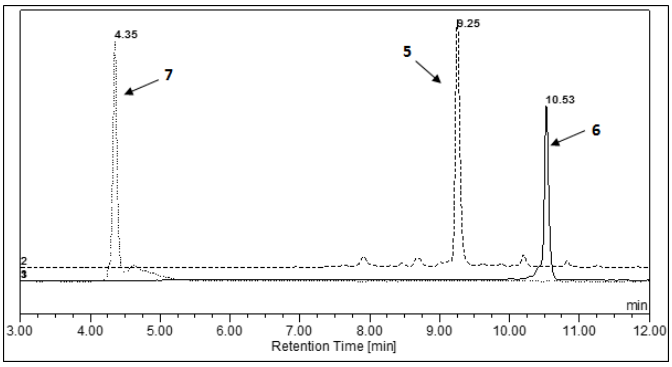


Figure S1. Overlay of HPLC chromatograms for crude reaction mixture for Boc-protected ALA conjugate **6** with azido starting material **5** and the final purified deprotected non-targeted ALA dendrimer **7**.

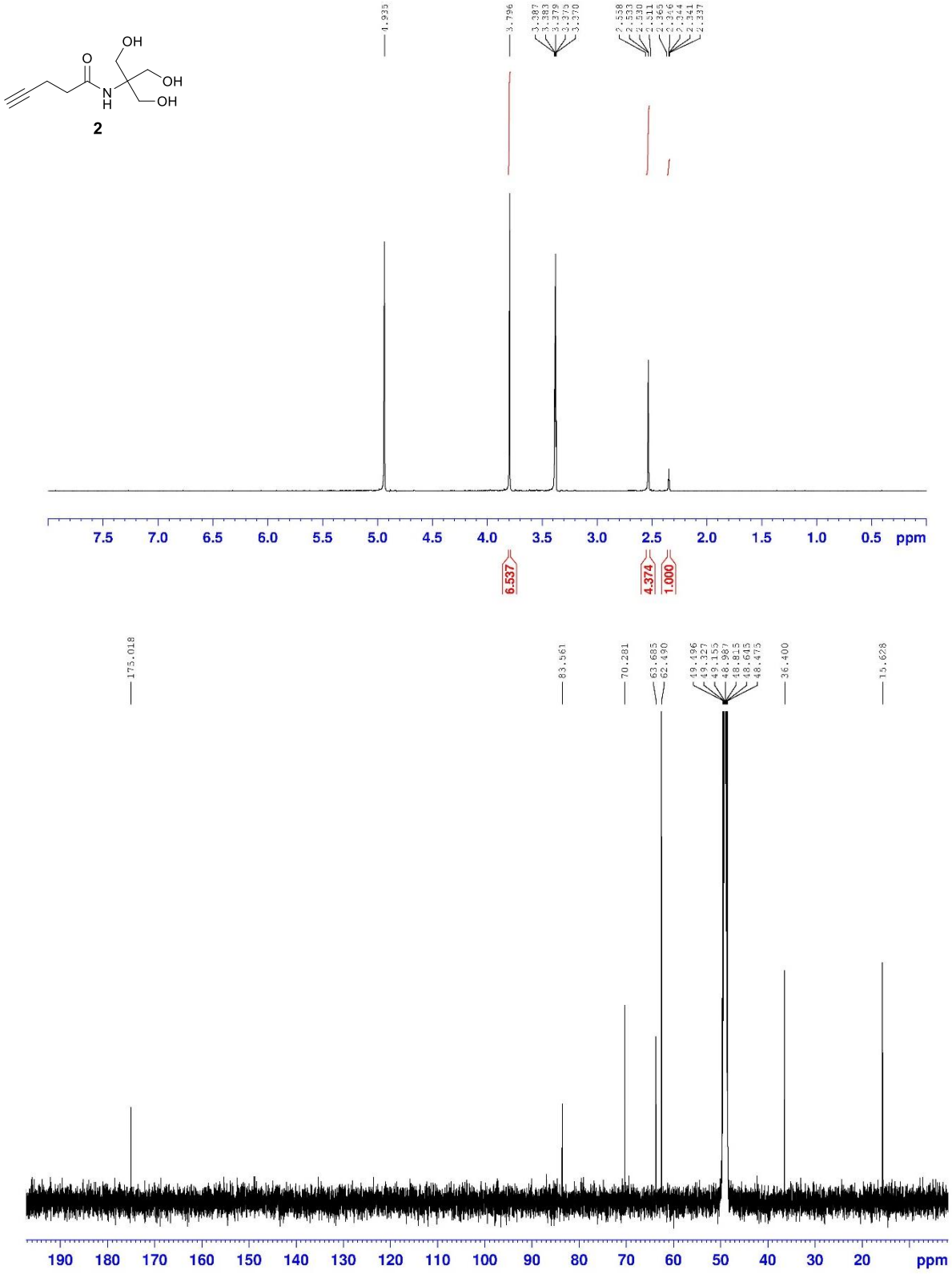
ALA-induced PpIX generation from 7 and 13

Table S1. ALA-induced PpIX generation from **7** (non-targeted) and **13** (EGFR-targeted) in MDA-MB-231 cells.

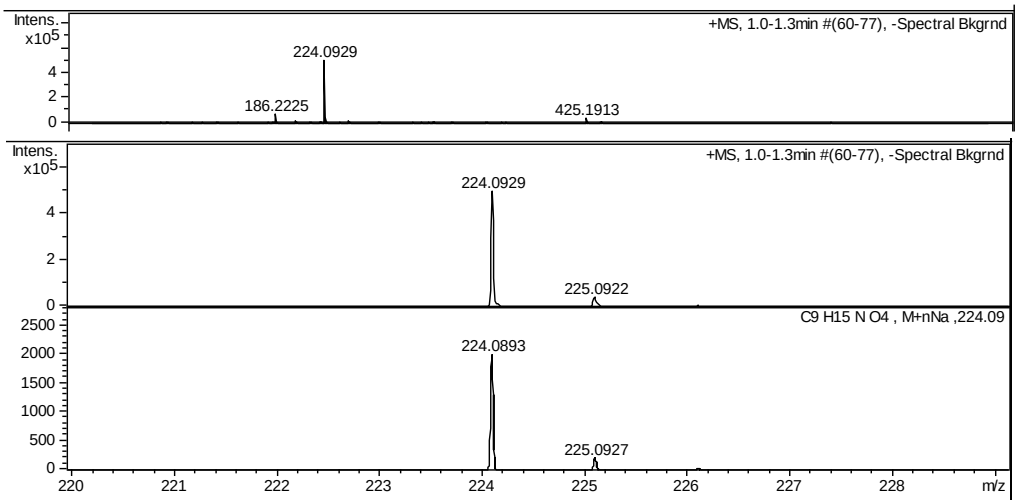
Compound	Fluorescence (a.u.)[a]		
	4 h	8 h	24 h
7	88.00 ± 6.25	144.33 ± 5.03	282.33 ± 8.08
13	201.33 ± 6.51	374.33 ± 8.33	500.33 ± 7.57

[a]MDA-MB-231 cells were treated with **7** or **13** (0.11 mM) and were incubated at 37 °C in the dark. Fluorescence readings were taken at 4, 8 and 24 h intervals. Results are expressed as mean fluorescence ± SD (n = 3).

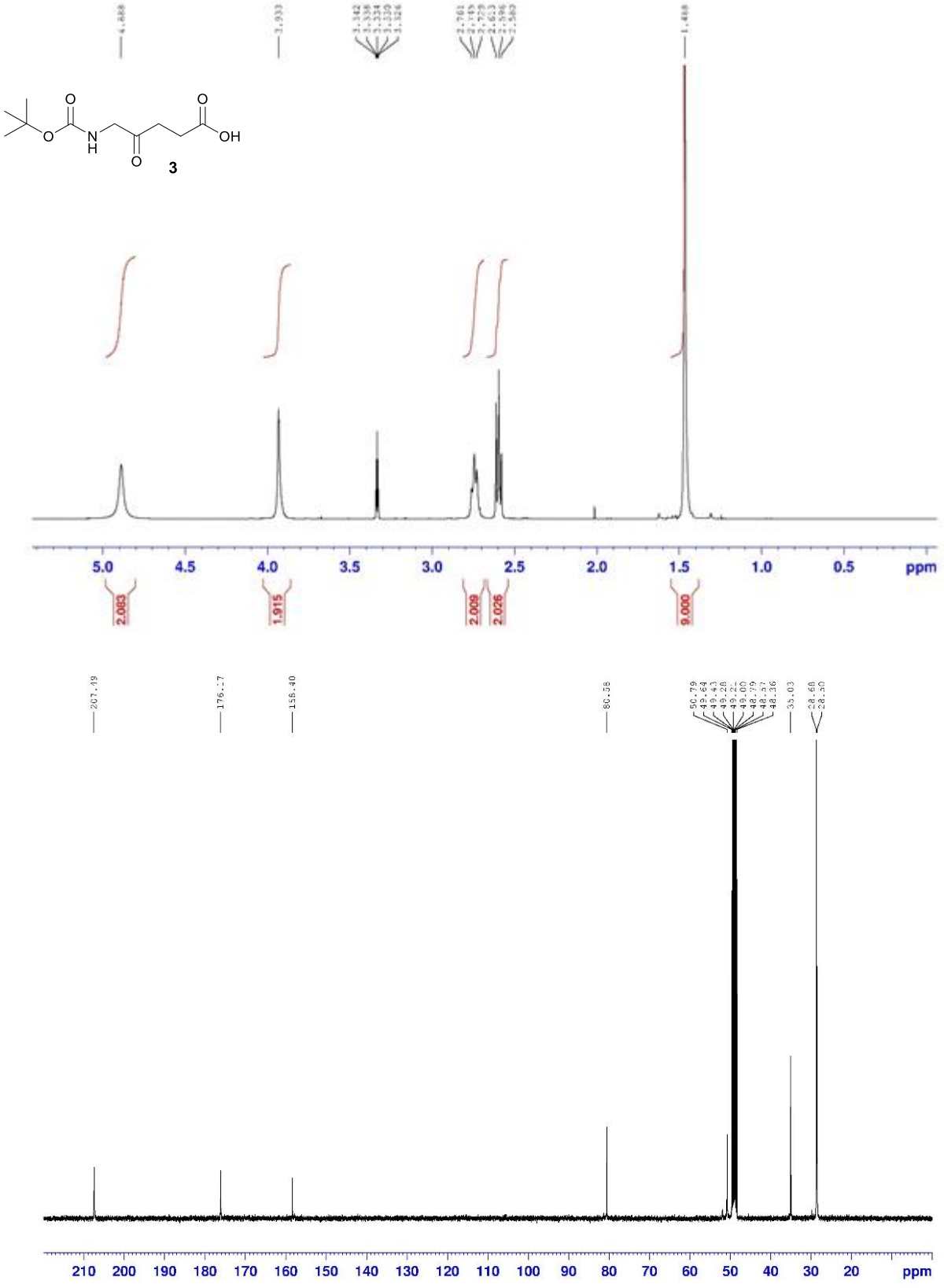
^{1}H NMR and ^{13}C NMR spectra of 2



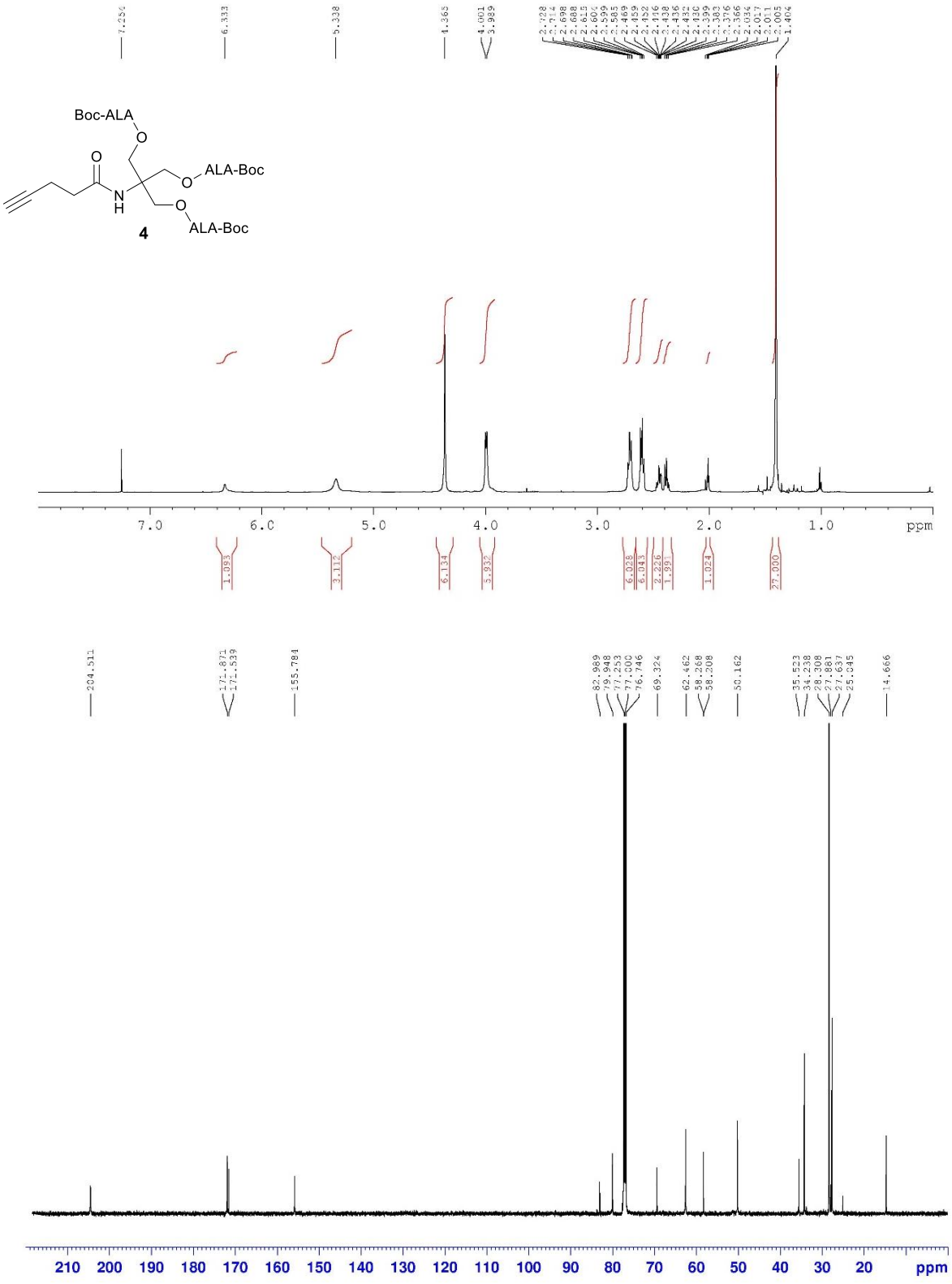
ESI-Mass spectra of 2



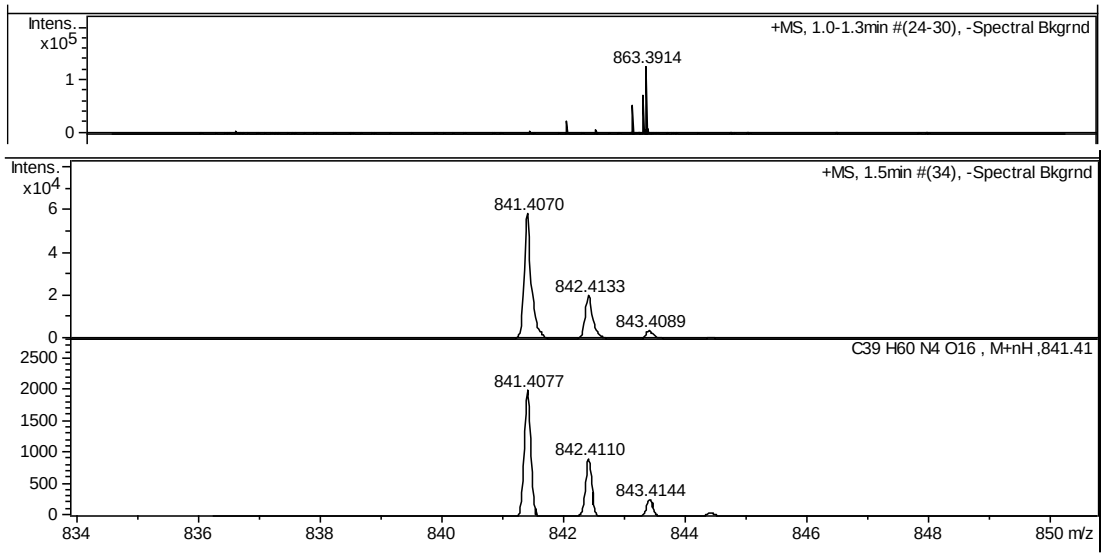
^{1}H NMR and ^{13}C NMR spectra of 3



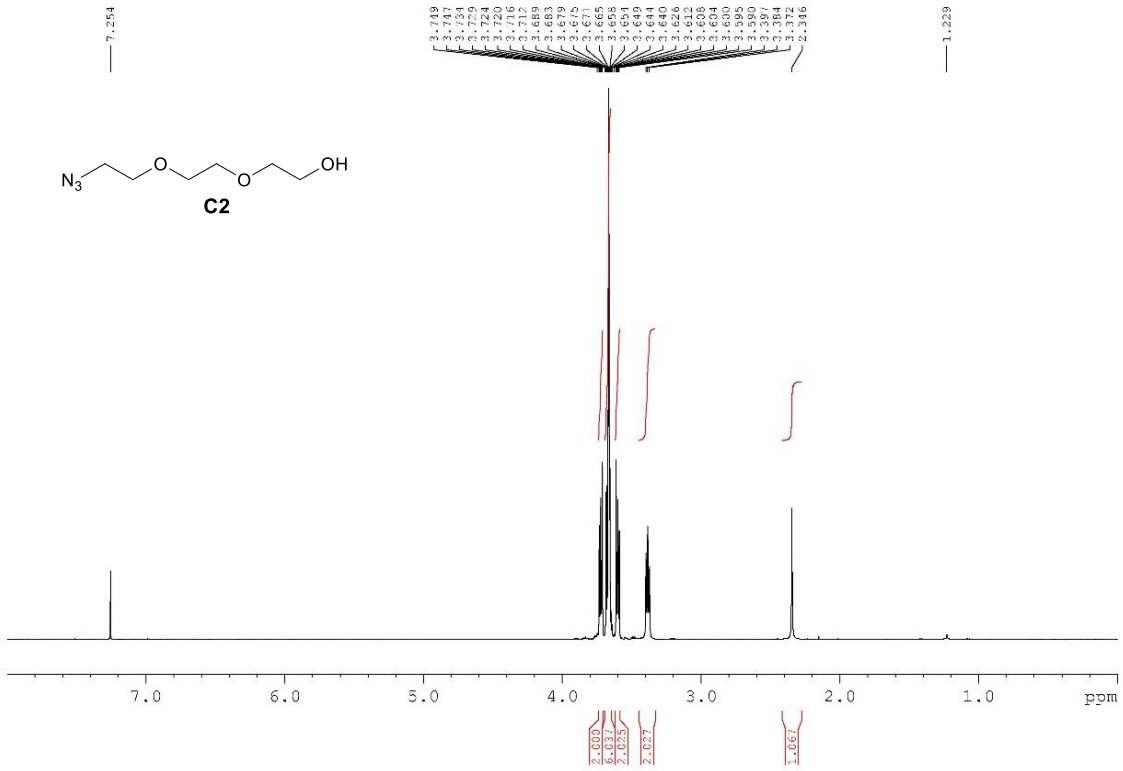
^{1}H NMR and ^{13}C NMR spectra of 4



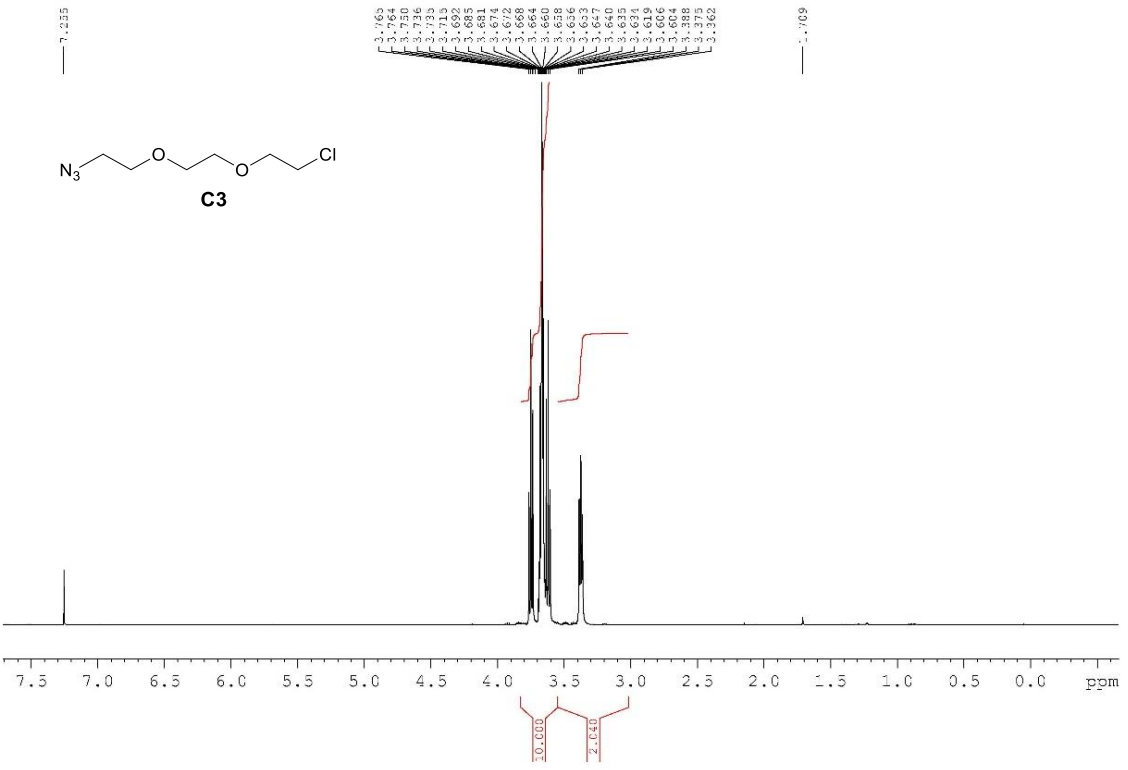
ESI-Mass spectra of 4



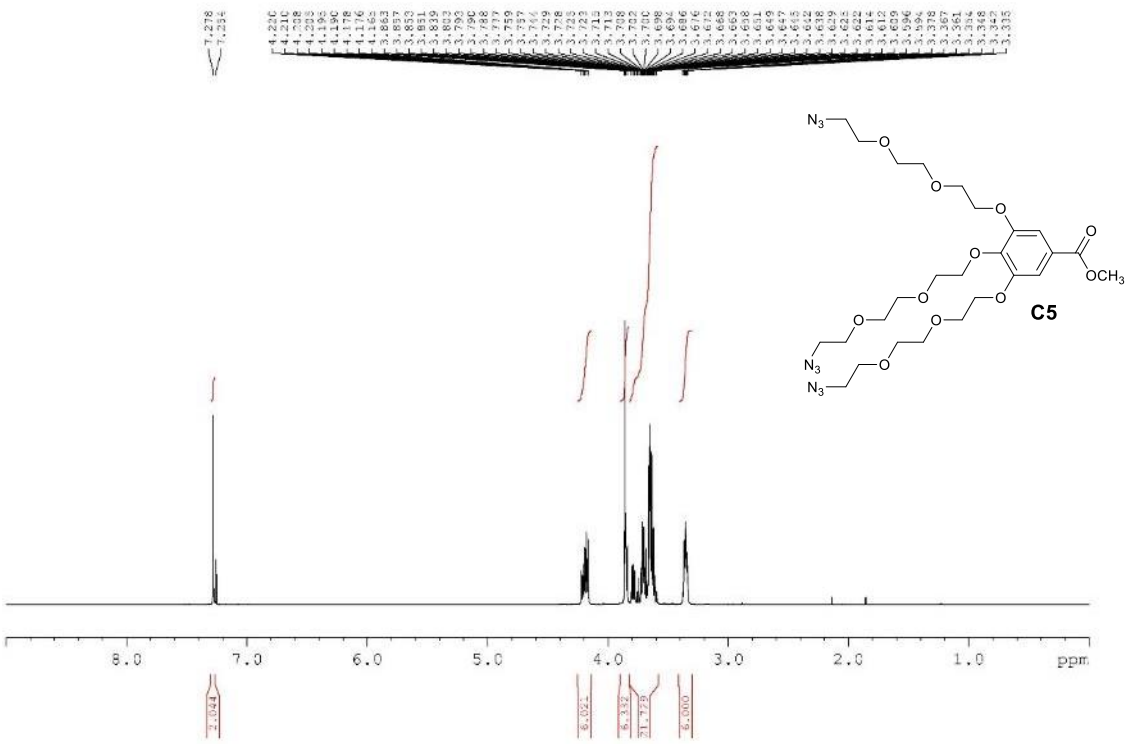
^{1}H NMR spectra of C2



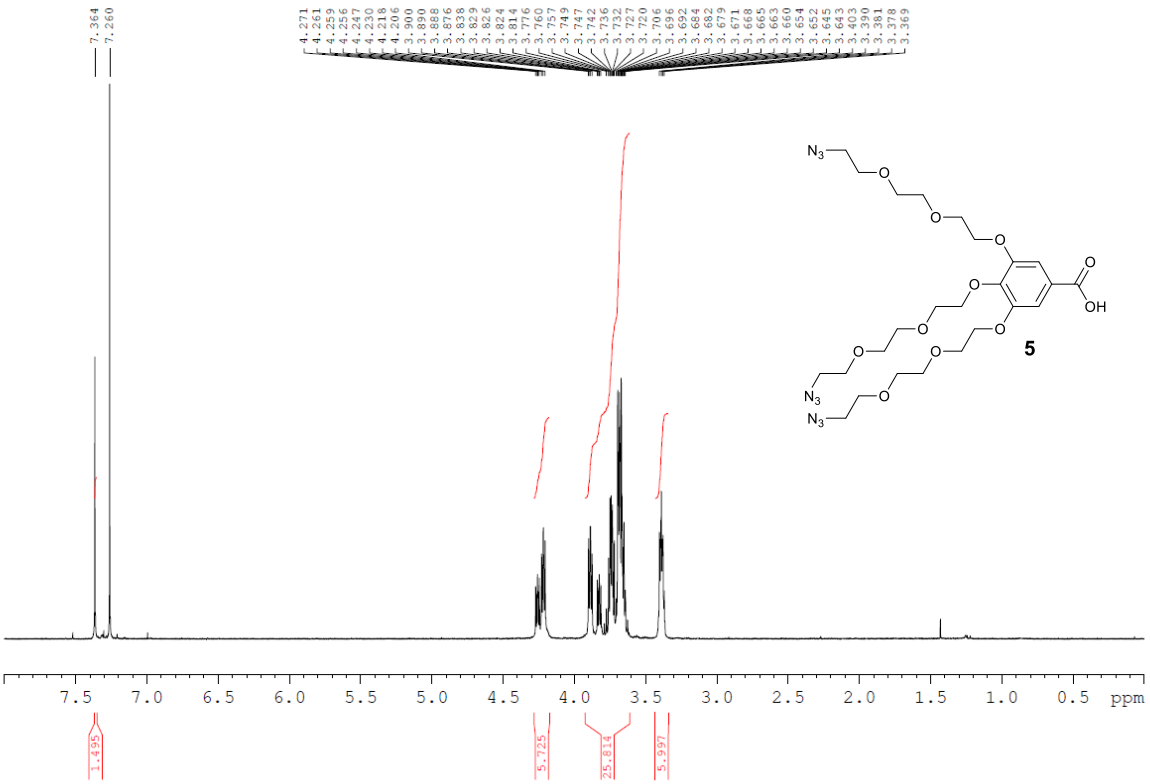
^{1}H NMR spectra of C3



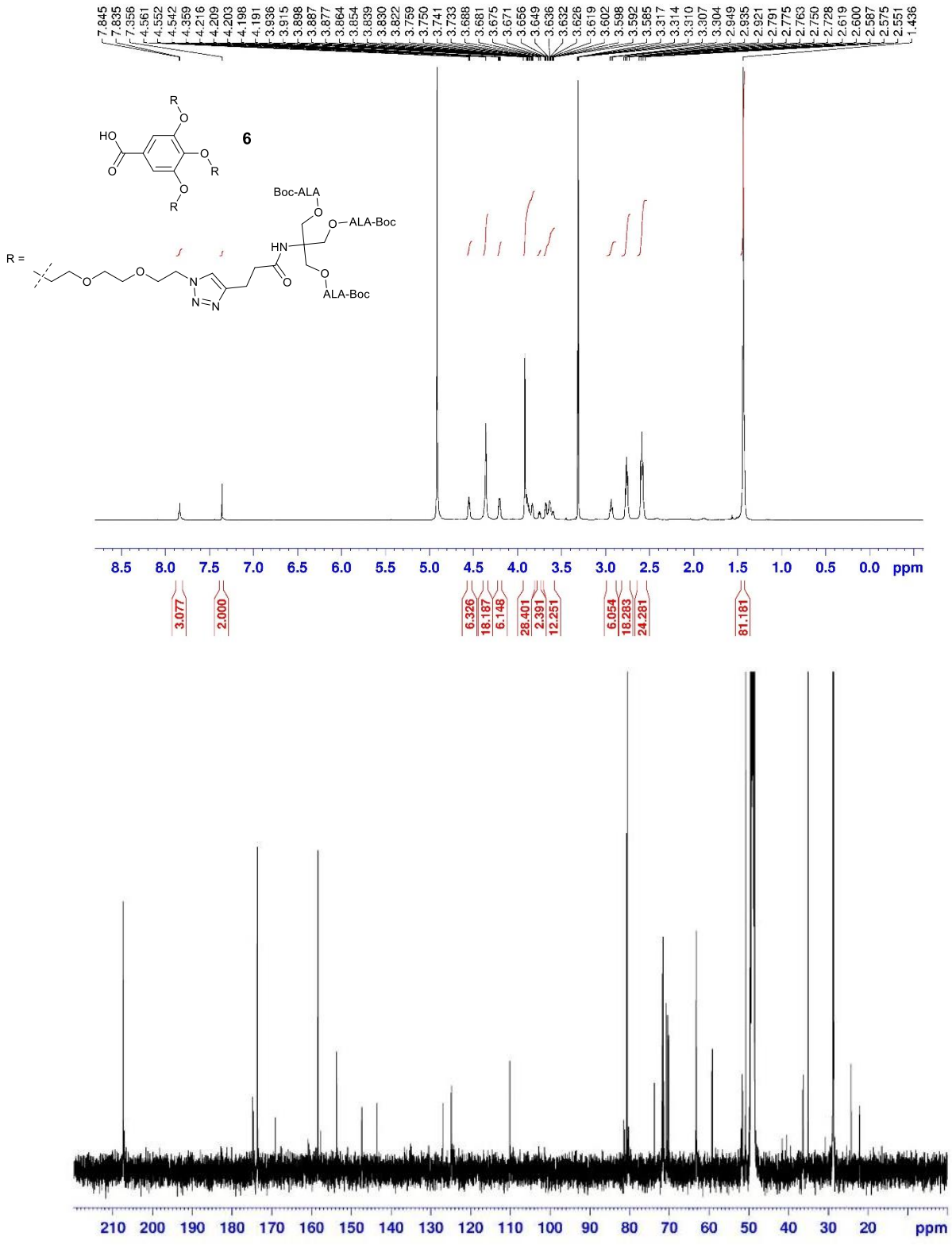
^{1}H NMR spectrum of C5



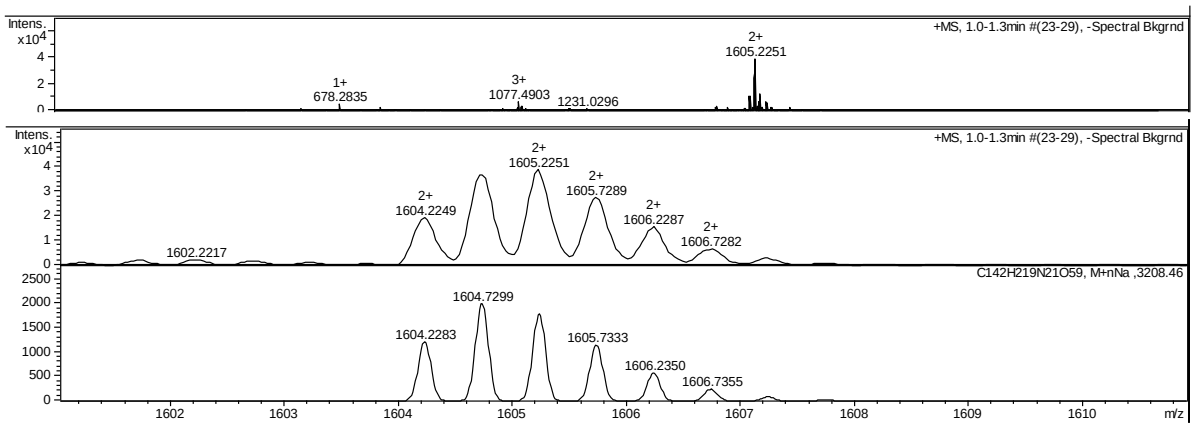
^{1}H NMR spectrum of 5

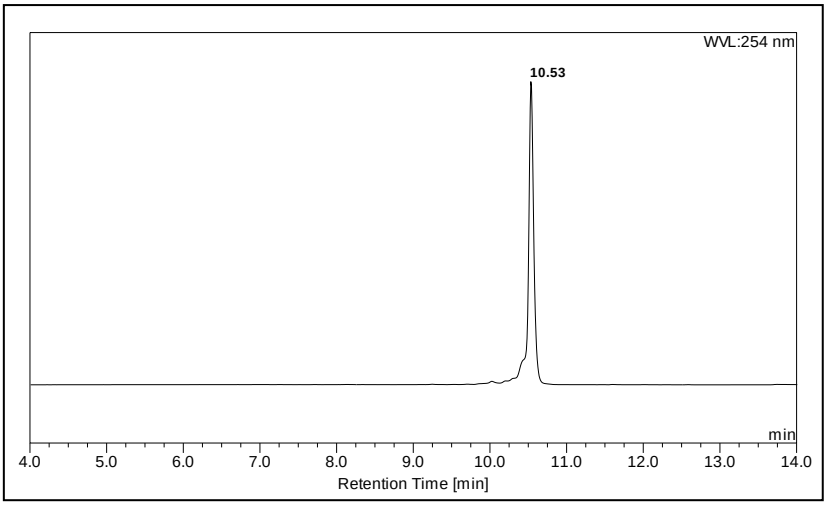


^{1}H NMR and ^{13}C NMR spectra of 6

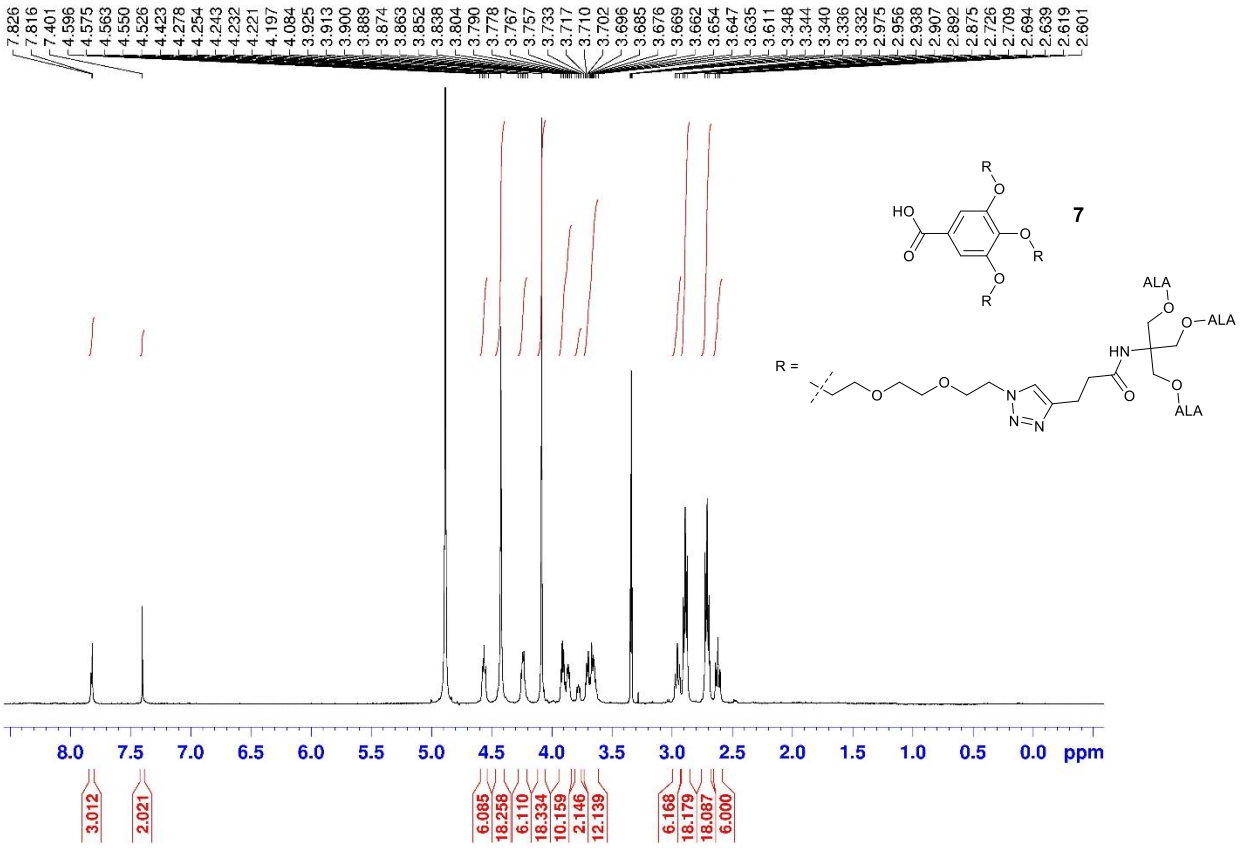


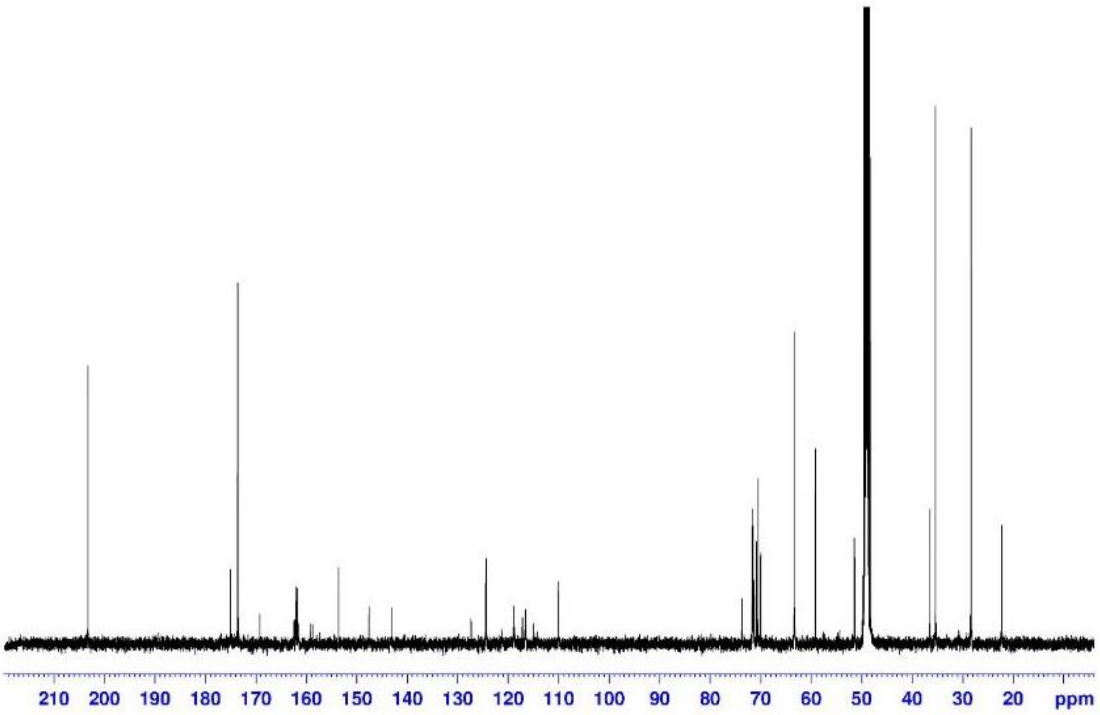
ESI-MS and HPLC chromatogram of 6





^{1}H NMR and ^{13}C NMR spectra of 7





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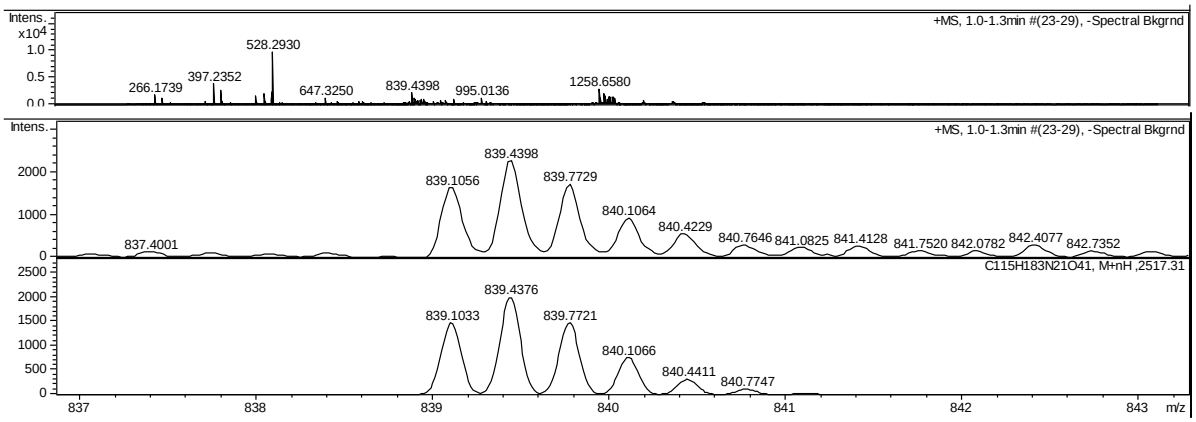
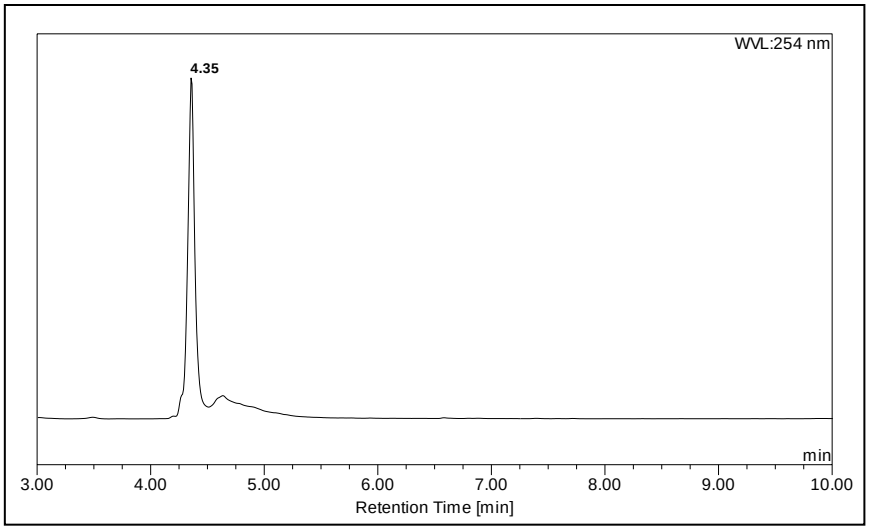
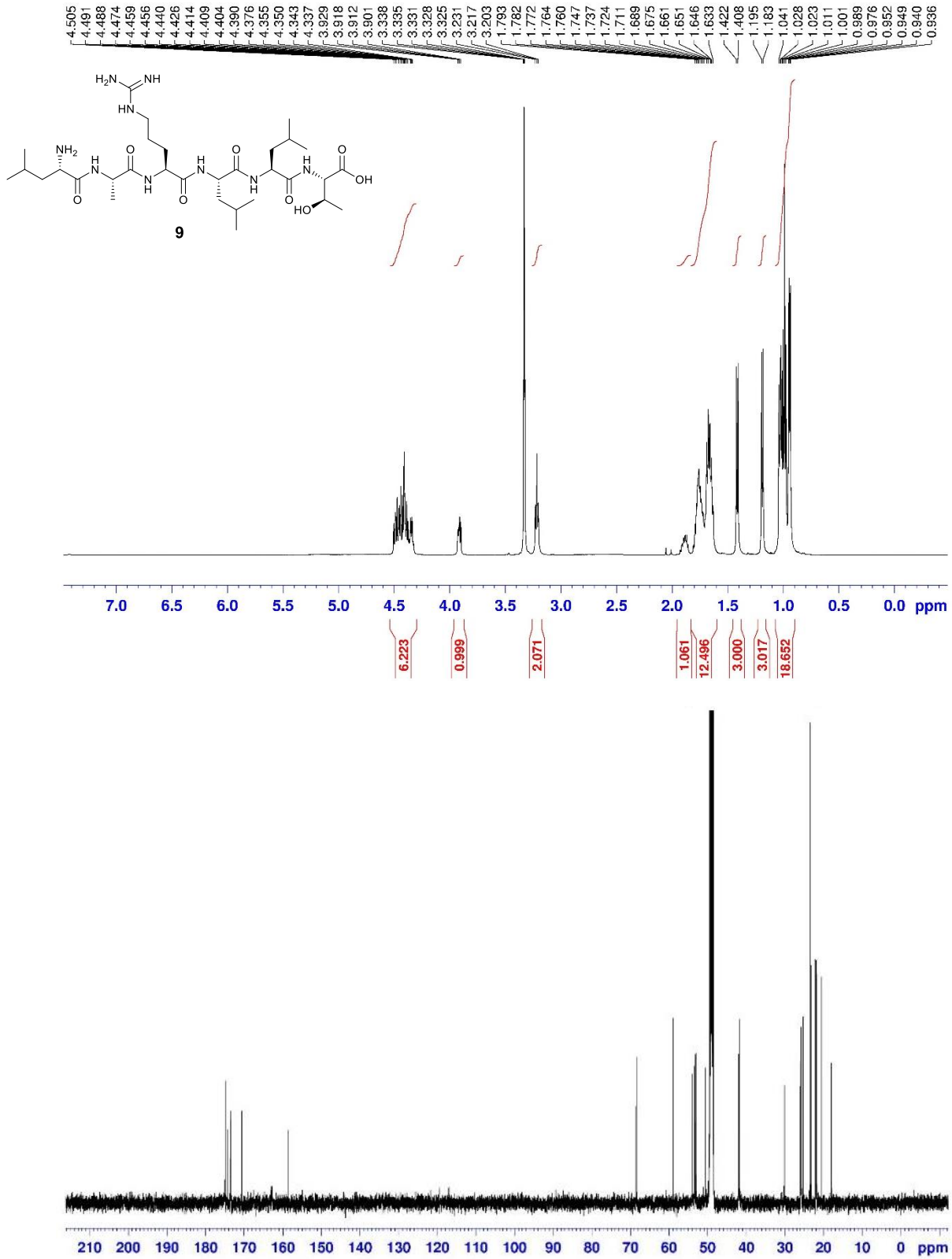
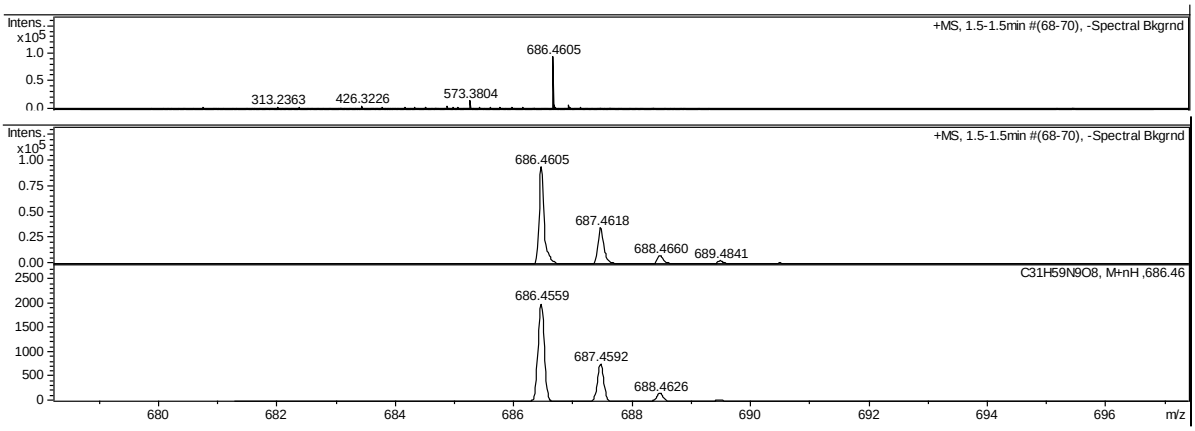


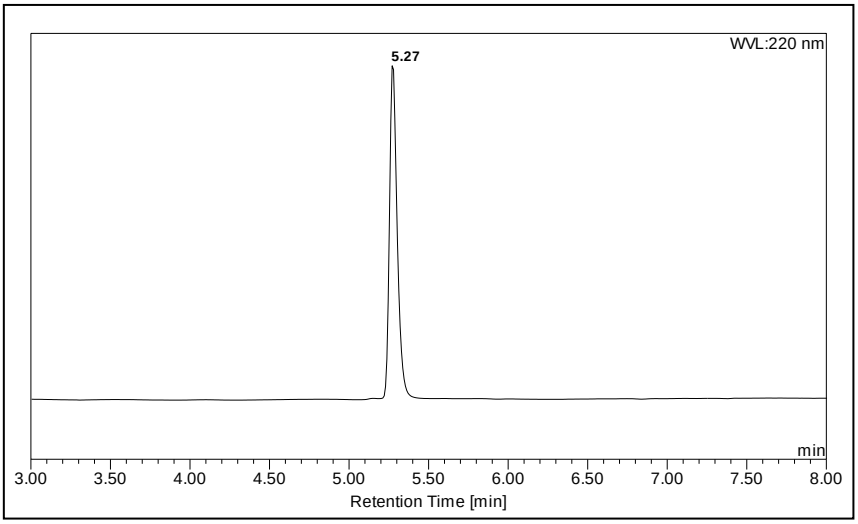


^{1}H NMR and ^{13}C NMR spectra of 9

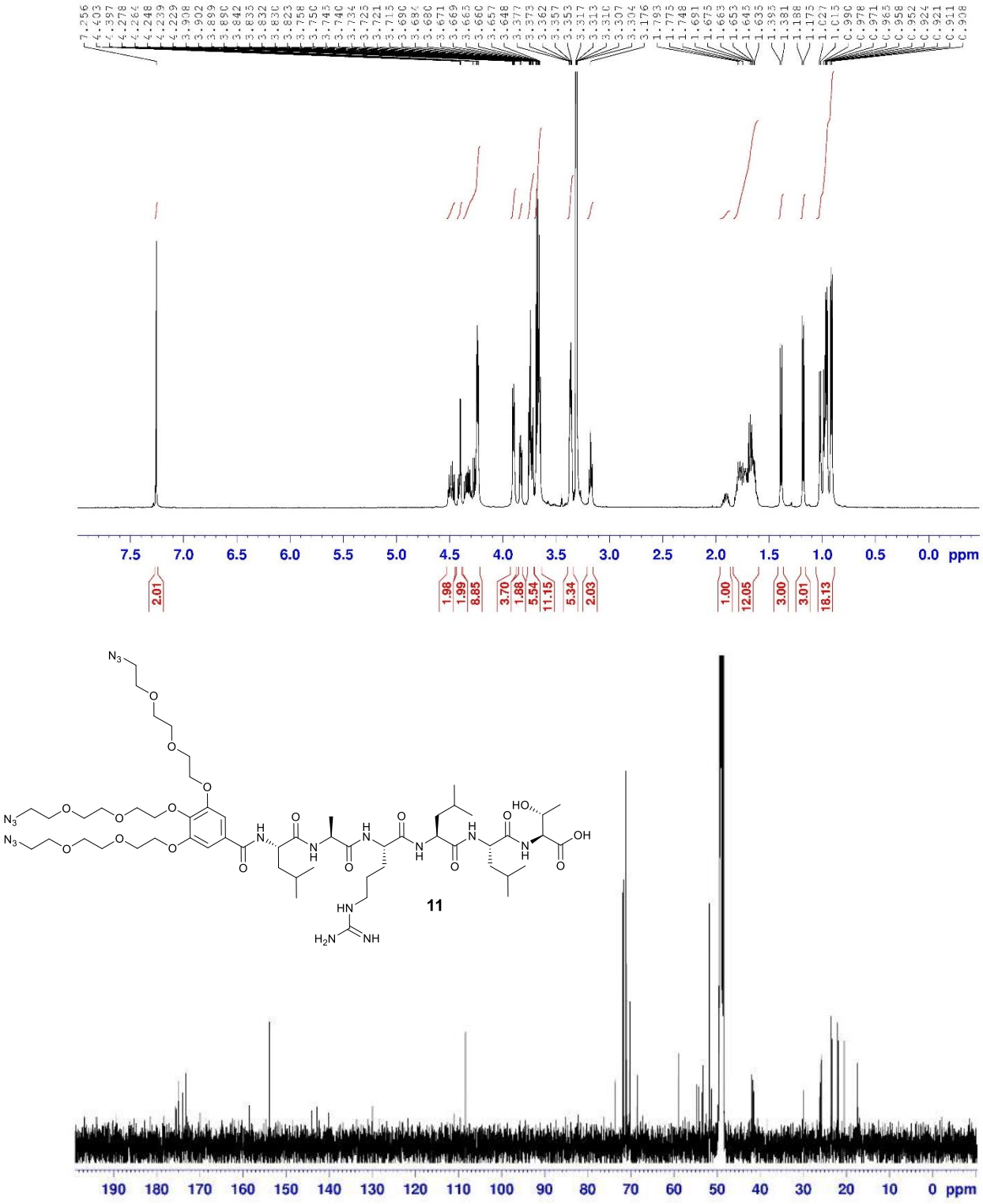


ESI-MS and HPLC chromatogram of 9





^{1}H NMR and ^{13}C NMR spectra of 11



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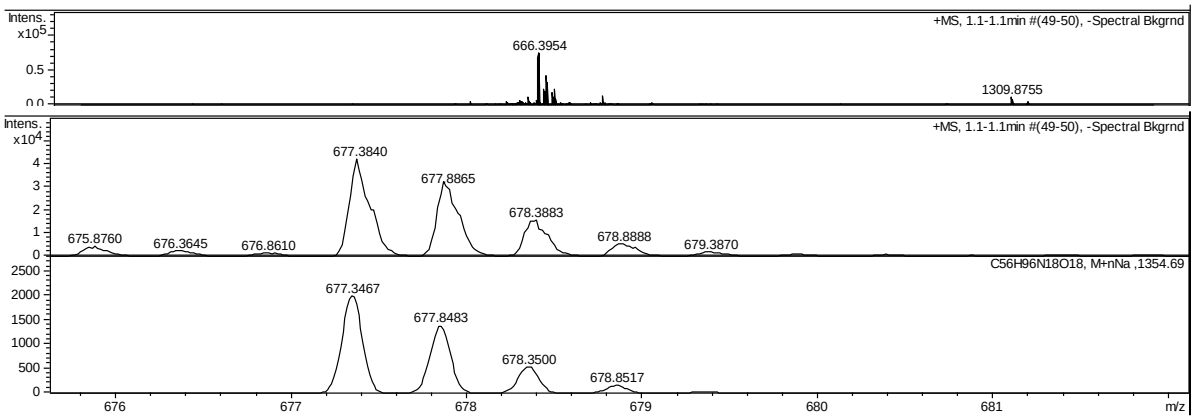

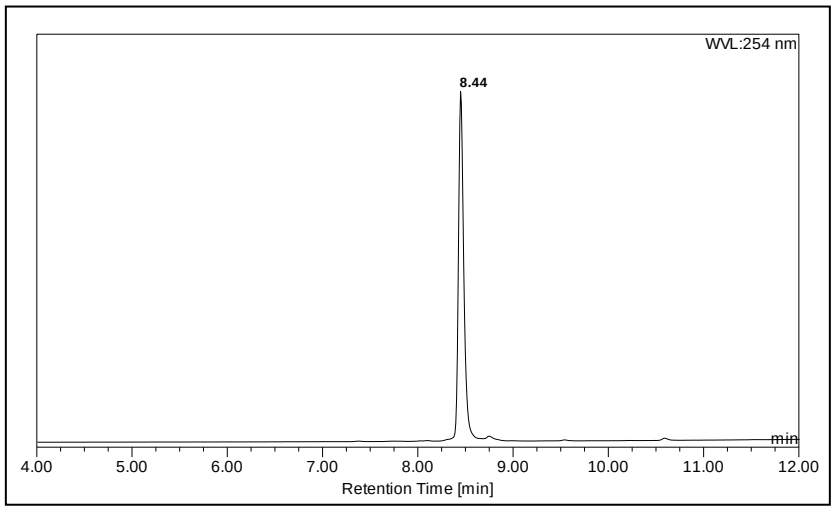

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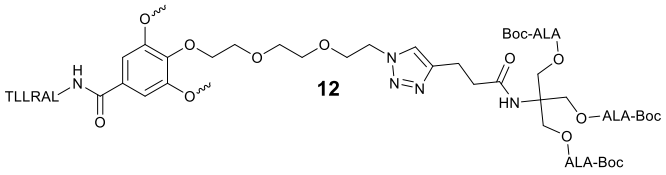
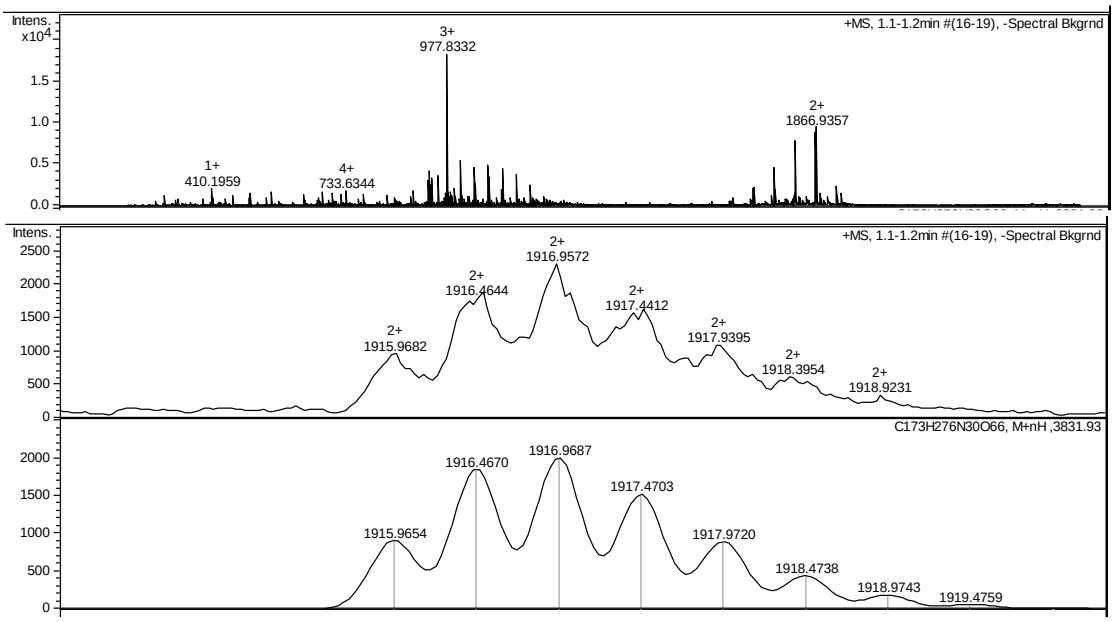
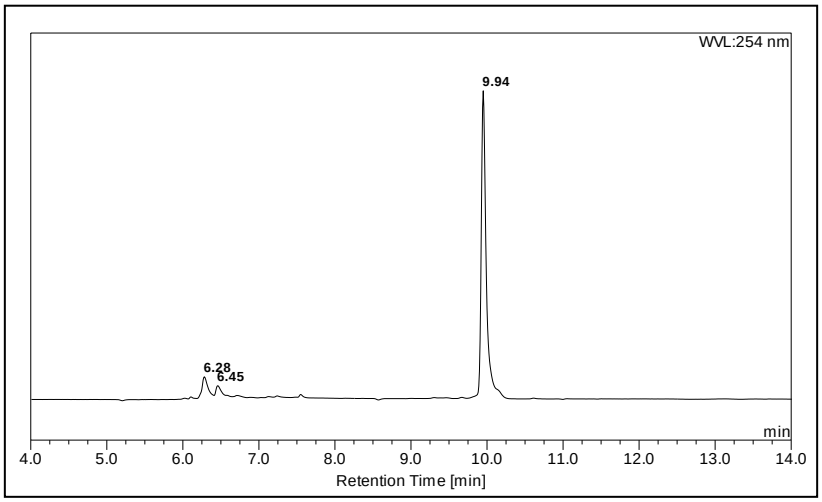
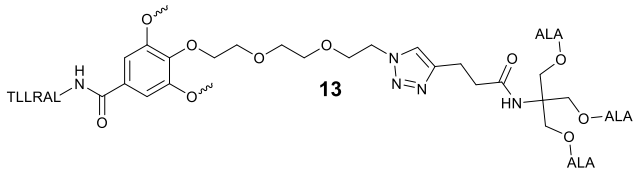
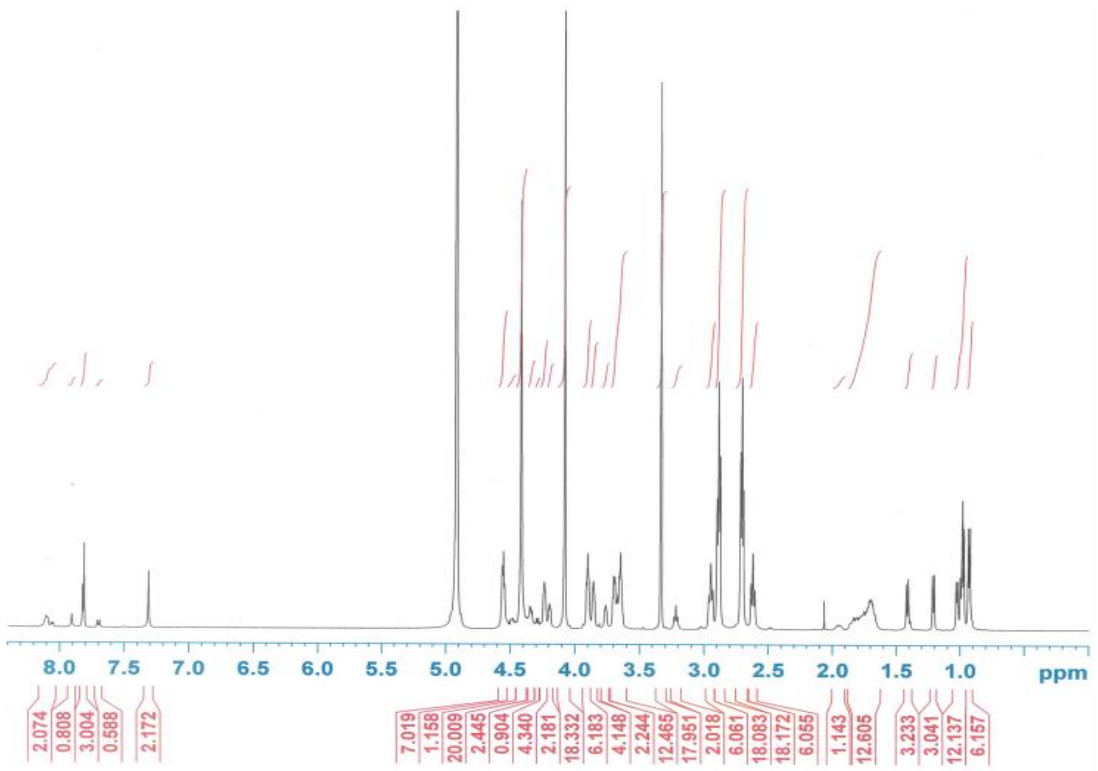


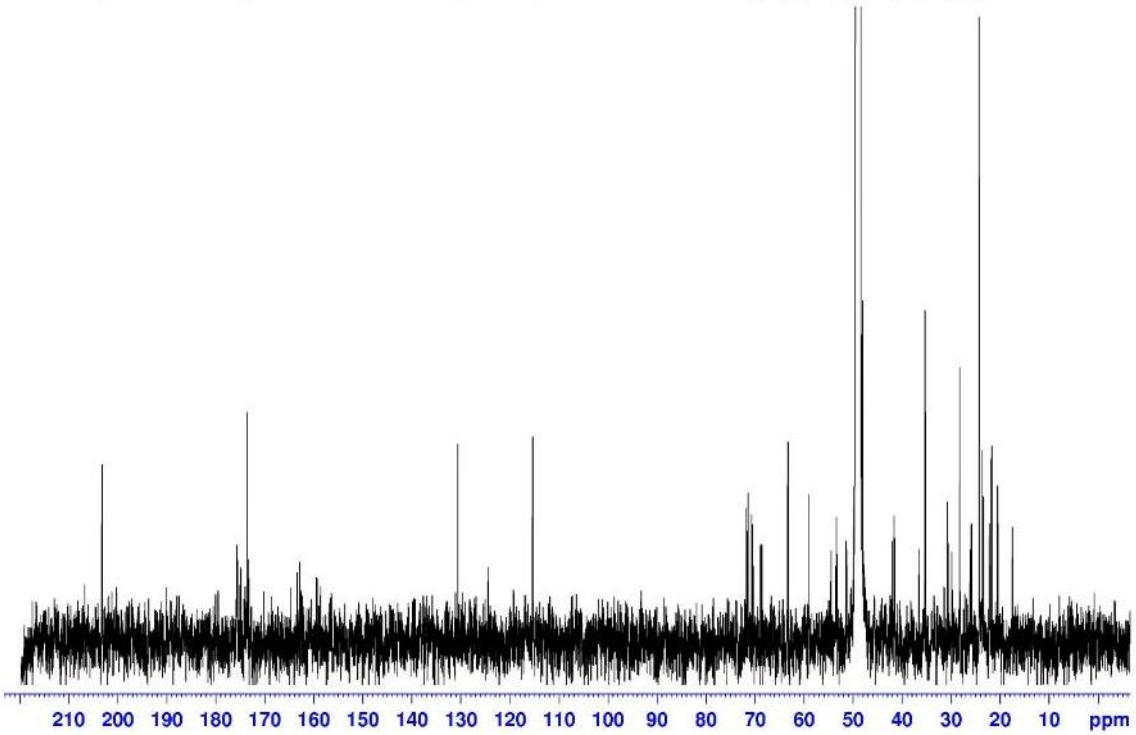




^{1}H NMR and ^{13}C NMR spectra of 13







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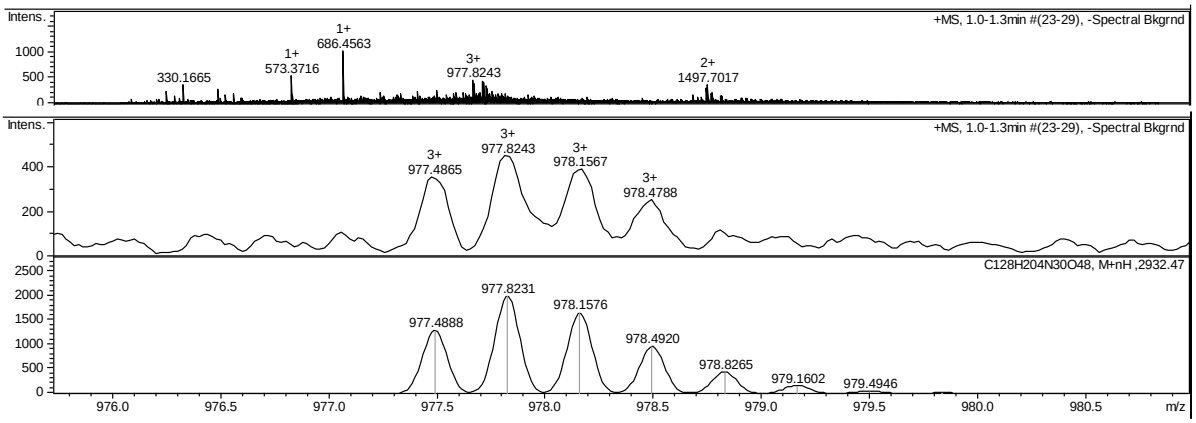

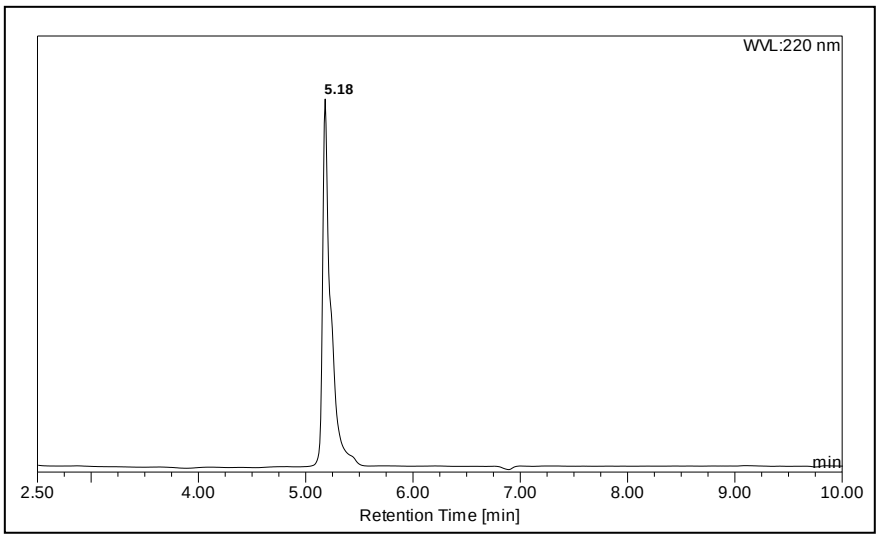

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[1] S. P. Amaral, M. Fernandez-Villamarin, J. Correa, R. Riguera and E. Fernandez-Megia, *Org. Lett.*, 2011, **13**, 4522-4525.