Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis and kinetic studies of cyclisation-based self-immolative spacers

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The Supporting Information reports on:

pKa measurements

All the reported self-immolative spacers are based on phenol cores. We determined the proton exchange constant for all of them by analysing the evolution of their UV-Vis absorption spectra on pH in CH_3CN/0.1 M Britton-Robinson buffer 1:1 (v:v) (figure 1 to 4).

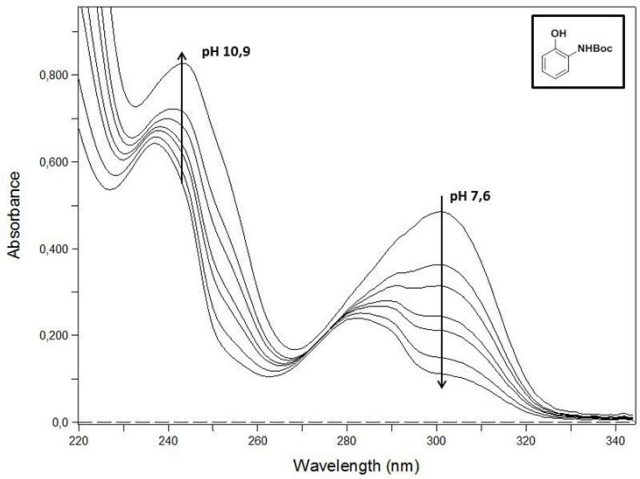


Figure 1: pH dependence of the absorption spectrum of 2-hydroxyphenolcarbamate 2-HPC^H (10 µM; pH from7.6 to 10.9).

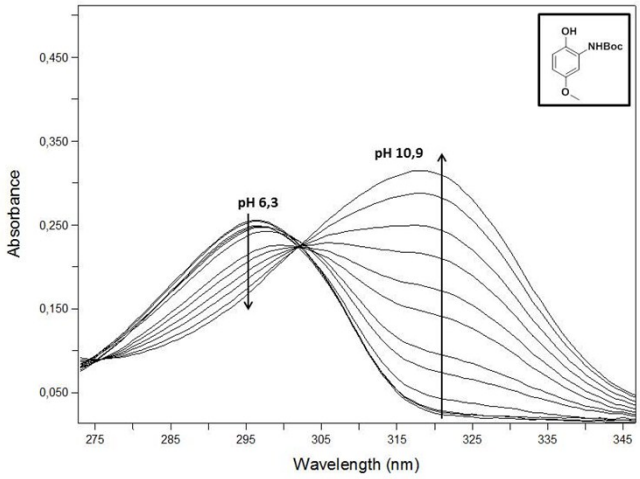


Figure 2: pH dependence of the absorption spectrum of 2-hydroxy-4-methoxy-phenolcarbamate 2-HPC^{OCH3} (10 µM; pH from 6.3 to 10.9)

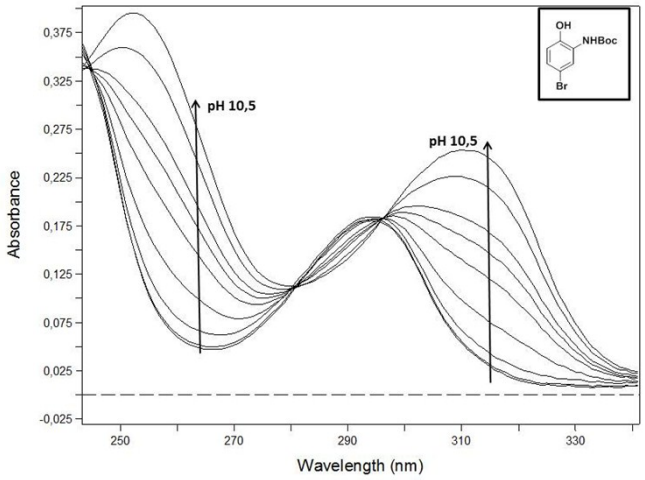


Figure 3: pH dependence of the absorption spectrum of 2-hydroxy-4-bromo-phenolcarbamate 2-HPC^{Br} (10 µM; pH from 6.4 to 10.5)

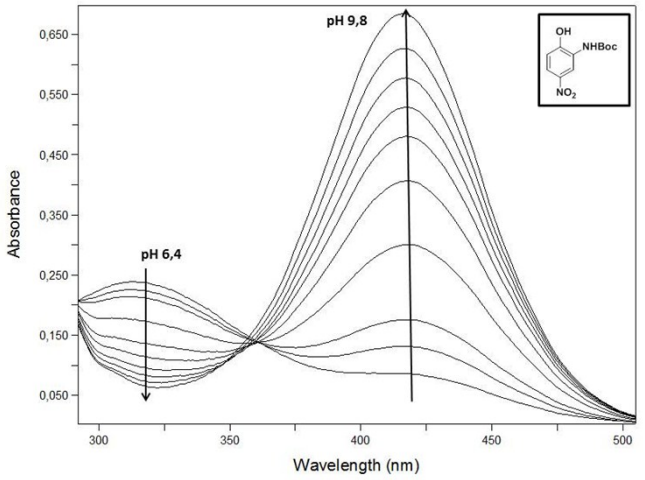
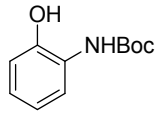
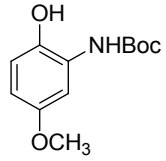
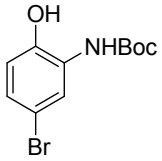
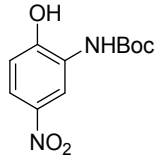
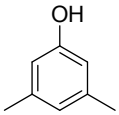


Figure 4: pH dependence of the absorption spectrum of 2-hydroxy-4-nitro-phenolcarbamate 2-HPC^{NO2} (10 µM; pH from 6.4 to 9.8)

The pH dependence of the absorption spectra of the phenols has been analysed with the SPECFIT/32™ Global Analysis System (Version 3.0 for 32-bit Windows systems) in order to extract the pKa. The extracted values are given in table 1.

Table 1: pKa of the phenol cores contained in cyclising self-immolative spacers.

Phenol	pKa
	10.7 ± 0.1
	10.8 ± 0.1
	10.0 ± 0.1
	7.6 ± 0.1
	10.2 (supplier data)

Irradiation experiments

The irradiations relying on one-photon excitation were performed in 54 µL homogeneously illuminated quartz fluorescence cuvettes (Hellma) without stirring at 293 K in CH_3CN/0.1 M Britton-Robinson pH 8 or pH 5 buffer 1/1 (v/v). Excitations were performed using a light-emitting diode (PE-2, CoolLED, Andover, United Kingdom; emission at 365±25 nm) and the resulting signals were observed at 660 nm.

Kinetic measurements

The self-immolation process is initiated by photocleavage of the 4,5-dimethoxy-2-nitrobenzyl moiety which involves a multi-step radical mechanism, but which can be considered as a single step for our kinetic measurement considering that the whole process occurs in the millisecond timescale (*Chem. Eur. J.,* **2006***, 12*, 6865-6879). Moreover, the 4,5-dimethoxy-2-nitrosobenzaldehyde formed in this reaction is not known to absorb at 365 nm or 660 nm (*J. Am. Chem. Soc.*, **1988,** *110*, 7170-7177), thus does not interfere with our measurements. DDAO emits a strong fluorescence in its free-phenol form, leading us to conclude that the contribution of the overall fluorescence could be due to the free fluorophore; however we cannot exclude a contribution of the intermediary uncaged compound. As in on our previous work (*Chem. Eur. J.*, **2013**, *19*, 11717-11724; supporting information, equation 13), the temporal evolution of the overall fluorescence signal could be fitted with the following equation:

$$I_F^{DDAO}(t) = I_F^{DDAO}(\infty)\left(q_i^{DDAO}\frac{C_i(t)}{C_i(0)} + \frac{C_{DDAO}(t)}{C_R(0)}\right) \quad (1)$$

With:

- $I_F^{DDAO}(t)$: Intensity of fluorescence (at 660 nm) at t time.
- $I_F^{DDAO}(\infty)$: Maximal intensity of fluorescence (at 660 nm) at t = ∞.
- q_i^{DDAO} : Brightness of the intermediary phenol formed after photocleavage.
- $C_i(t)$: Concentration of the intermediary phenol at t time
- $C_i(0)$: Initial concentration of the intermediary phenol
- $C_{DDAO}(t)$: Concentration of DDAO at t time
- $C_R(0)$: Initial concentration of the caged compound (2-HPC or TML derivatives)

Experimental curves

Figures 5-10 display the temporal evolution of the fluorescence emission from solutions initially containing the caged precursors and continuously submitted to illumination. They all exhibited the expected signal growth associated to quantitative DDAO liberation. Moreover the biexponential fit with equation (1) was found satisfactory, which enabled us to extract the k_2 values.

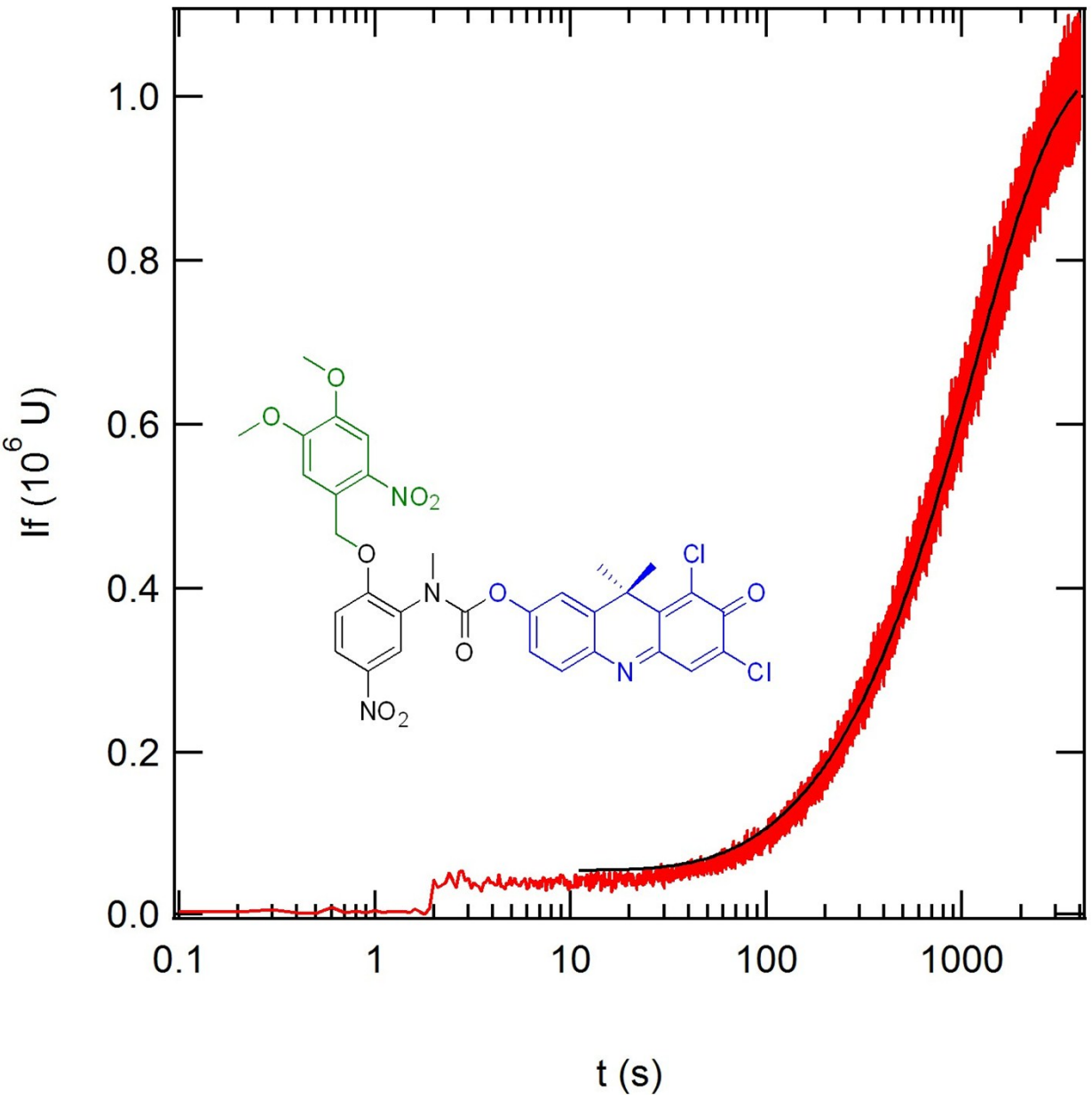


Figure 5: Temporal evolution of the fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of compound 2-HPC^{N02} *at* 1.4 10^{-9} Eins^{-1} *intensity of light and at 293K. Experimental data in red, fits with equation (1) in black. From the signal calibration with the fluorophore, we extracted 10.0 ±0.5 µM for the averaged final DDAO concentration, which demonstrated the DDAO liberation to be quantitative. From the fits, we retrieved k_2 = 5.2 ± 0.5 10^{-2} min^{-1}; Solvent: CH_3CN/0.1 M Britton-Robinson pH 5 buffer 1/1 (v/v).*

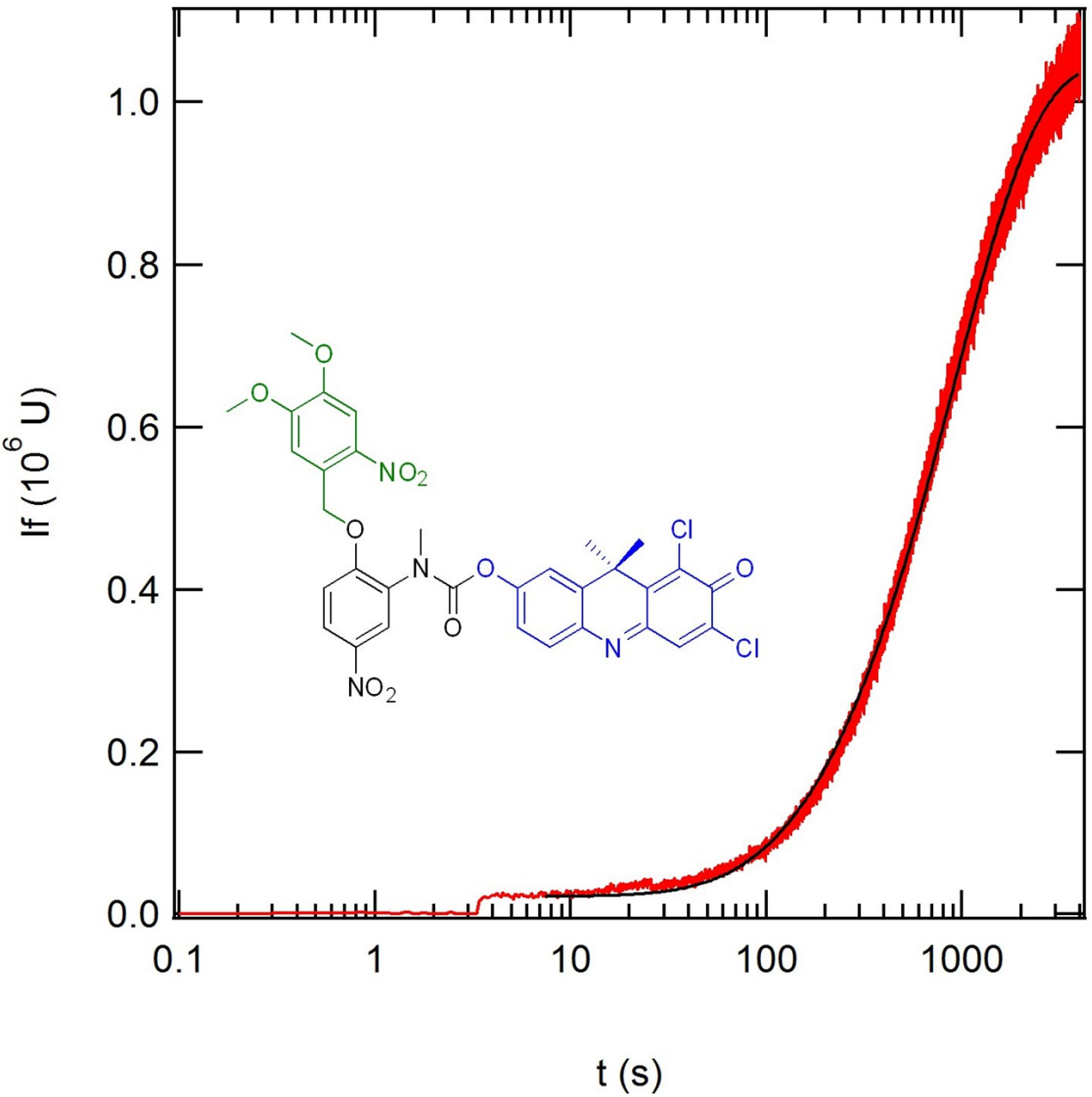


Figure 6: Temporal evolution of the fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of compound 2-HPC^{N02} *at* 1.4 10^{-9} Eins^{-1} *intensity of light and at 293K. Experimental data in red, fits with equation (1) in black. From the signal calibration with the fluorophore, we extracted 10.0 ±0.5 µM for the averaged final DDAO concentration, which demonstrated the DDAO liberation to be quantitative. From the fits, we retrieved* k_2 = 6.6 ± 0.5 10^{-2} min^{-1}*; Solvent:* CH_3CN*/0.1 M Britton-Robinson pH 8 buffer 1/1 (v/v).*

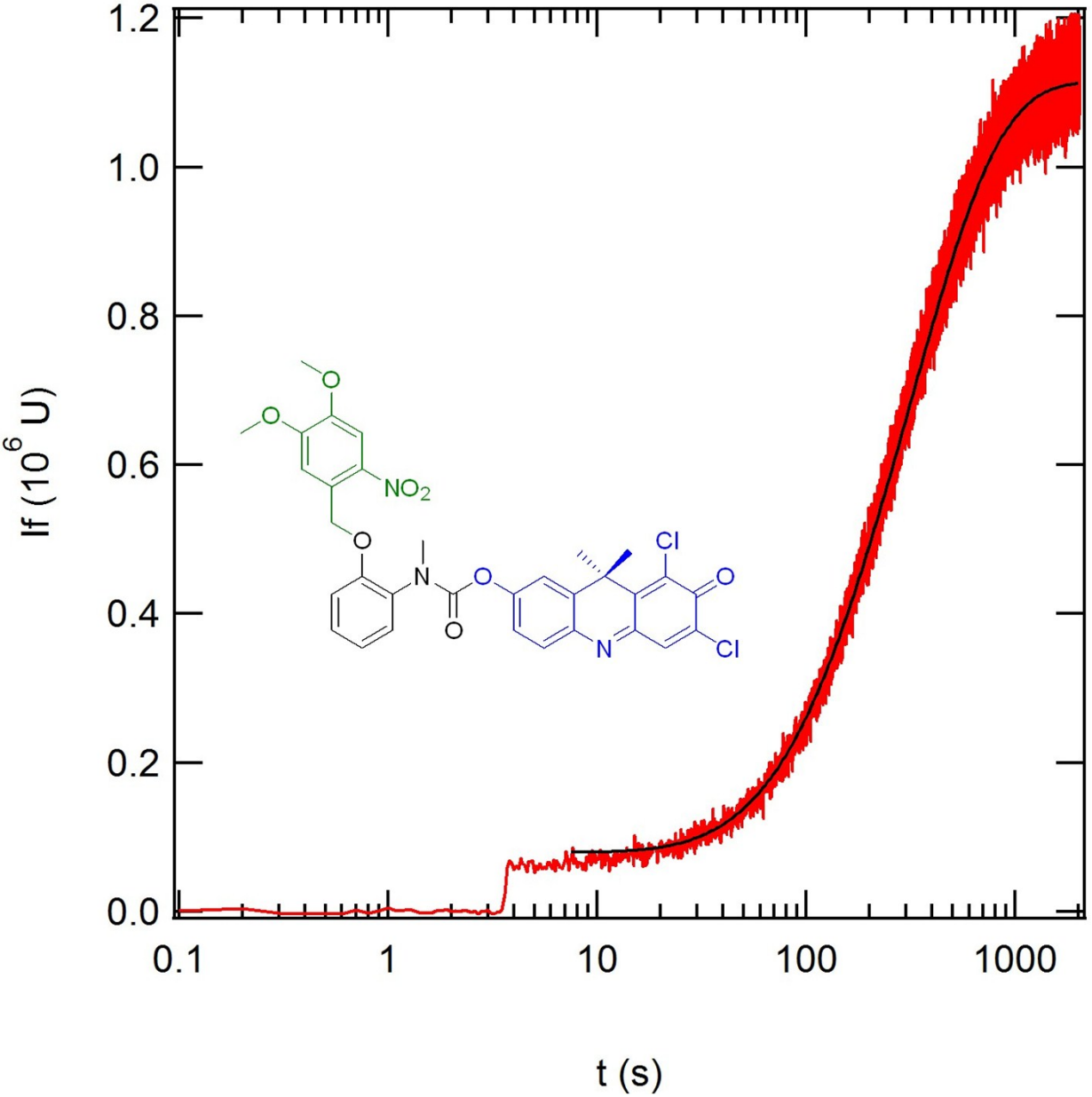


Figure 7: Temporal evolution of the fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of compound 2-HPC^{H} *at* 1.4 10^{-9} $Eins^{-1}$ *intensity of light and at 293K. Experimental data in red, fits with equation (1) in black. From the signal calibration with the fluorophore, we extracted 10.0 ±0.5 µM for the averaged final DDAO concentration, which demonstrated the DDAO liberation to be quantitative. From the fits, we retrieved* k_2 = 1.9 ± 0.2 10^{-1} min^{-1}*; Solvent:* CH_3CN*/0.1 M Britton-Robinson pH 8 buffer 1/1 (v/v).*

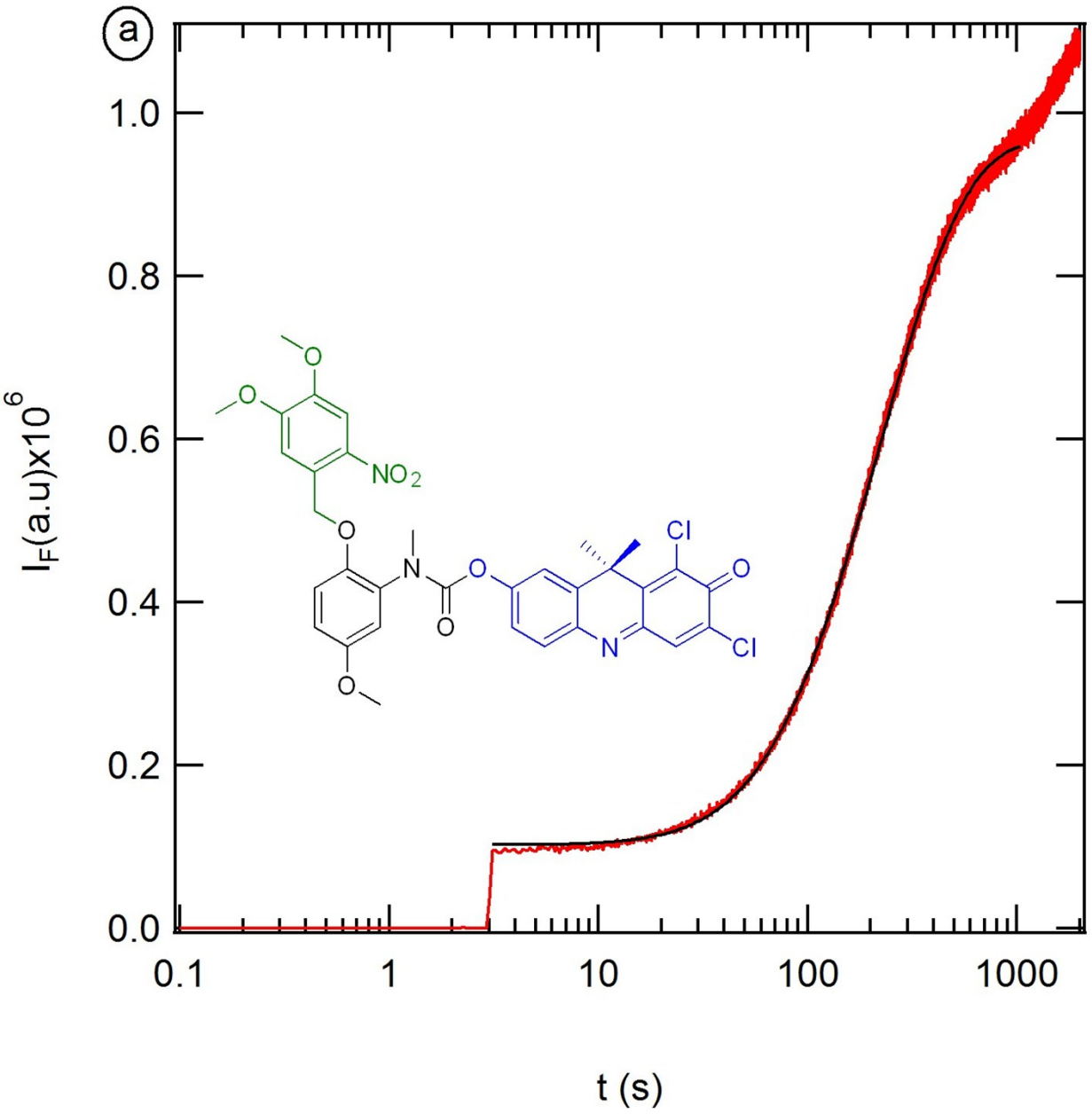


*Figure 8: Temporal evolution of the fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of compound 2-*HPC^{0CH3} *at* 1.4 10^{-9} Eins^{-1} *intensity of light and at 293K. Experimental data in red, fits with equation (1) in black. From the signal calibration with the fluorophore, we extracted 10.0 ±0.5 µM for the averaged final DDAO concentration, which demonstrated the DDAO liberation to be quantitative. From the fits, we retrieved k_2 = 2.9 ± 0.3 10^{-1} min^{-1}; Solvent: CH_3CN/0.1 M Britton-Robinson pH 8 buffer 1/1 (v/v).*

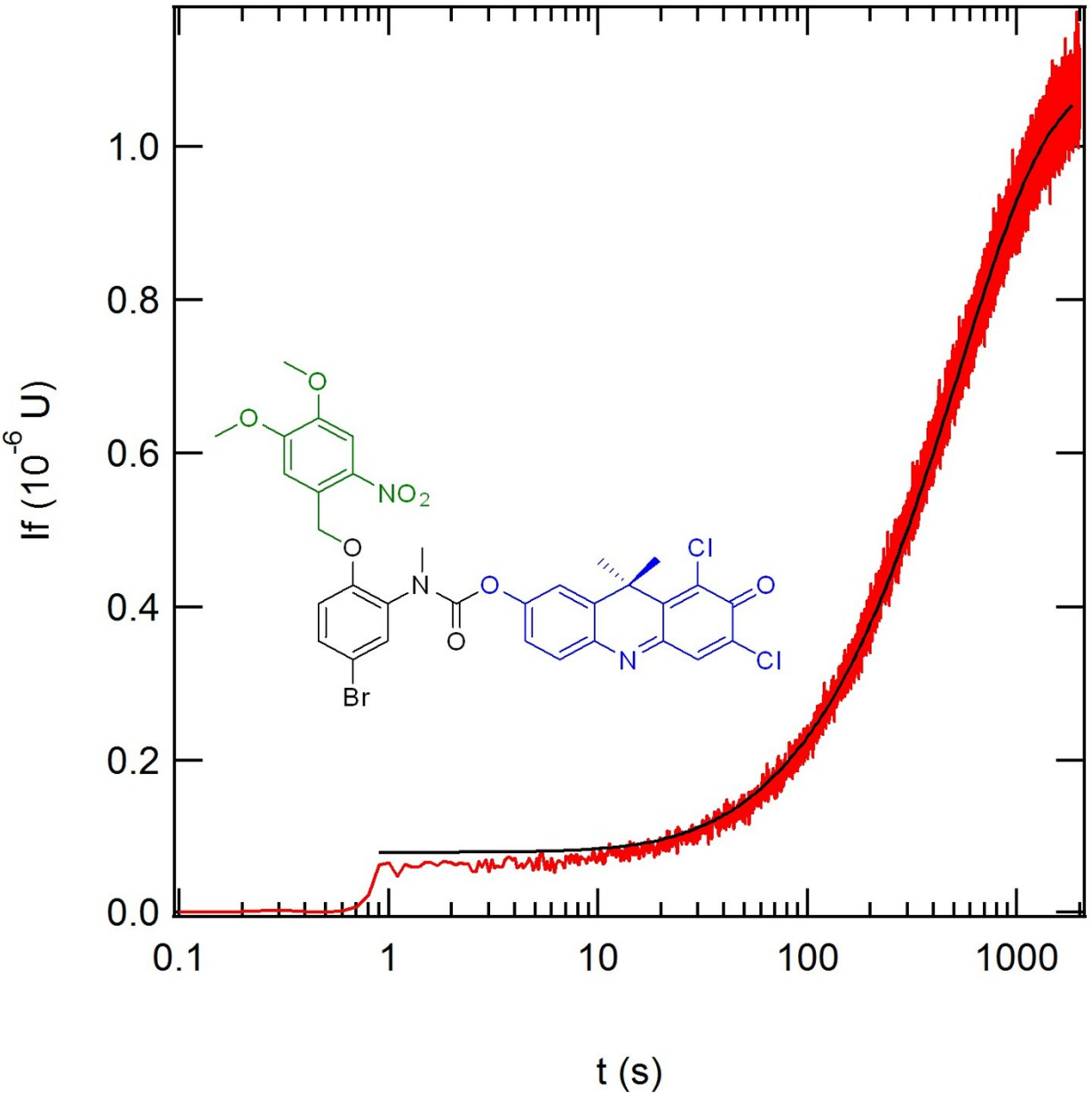


Figure 9: Temporal evolution of the fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of compound 2-HPC^{Br} *at* 1.4 10^{-9} Eins^{-1} *intensity of light and at 293K. Experimental data in red, fits with equation (1) in black. From the signal calibration with the fluorophore, we extracted 10.0 ±0.5 µM for the averaged final DDAO concentration, which demonstrated the DDAO liberation to be quantitative. From the fits, we retrieved k_2 = 1.1 ± 0.3 10^{-1} min^{-1}; Solvent: CH_3CN/0.1 M Britton-Robinson pH 8 buffer 1/1 (v/v).*

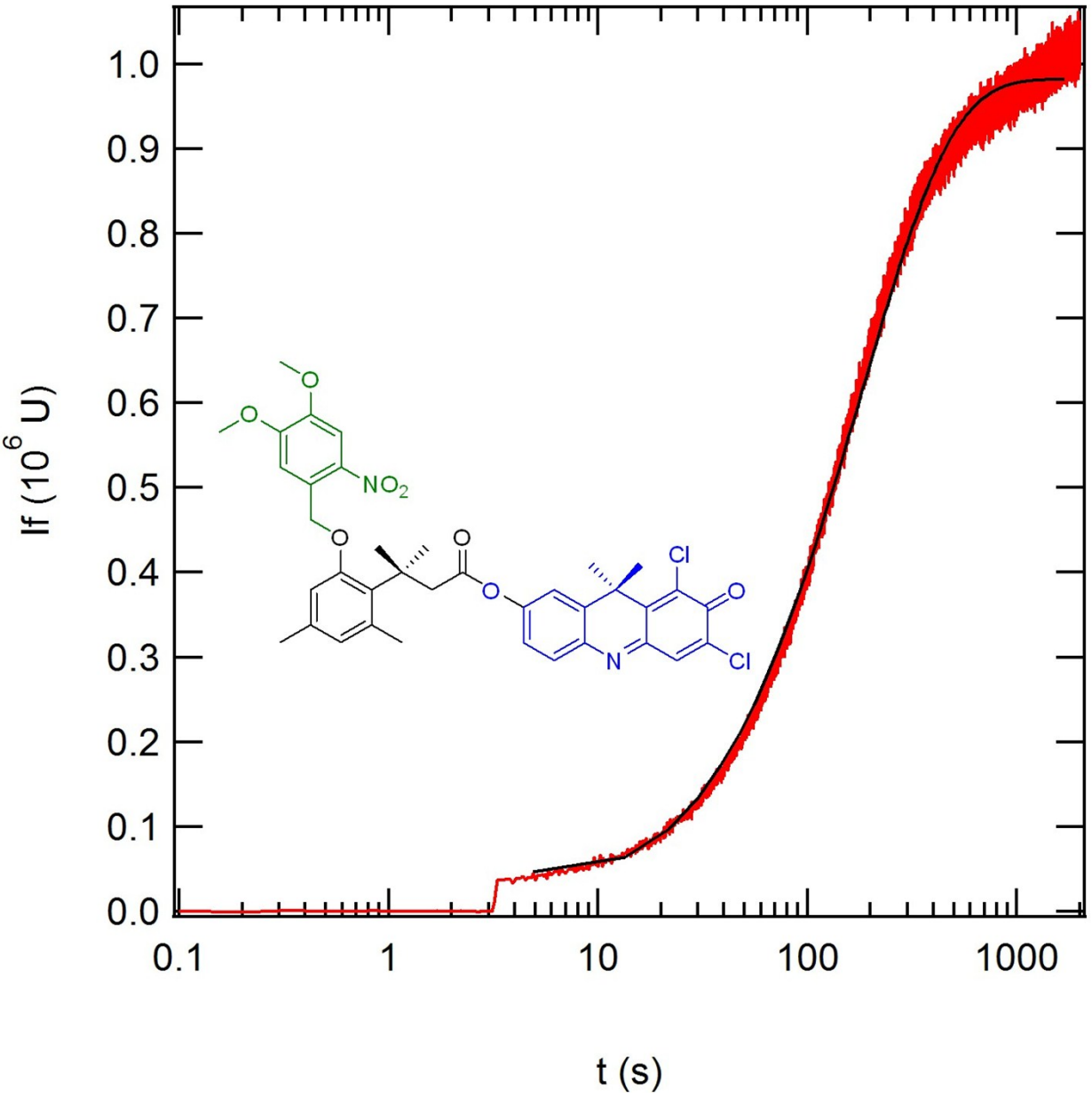


Figure 10: Temporal evolution of the fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of compound TML *at* 1.4 10^{-9} $Eins^{-1}$ *intensity of light and at 293K. Experimental data in red, fits with equation (1) in black. From the signal calibration with the fluorophore, we extracted 10.0 ±0.5 µM for the averaged final DDAO concentration, which demonstrated the DDAO liberation to be quantitative. From the fits, we retrieved k_2 = 3.3 ± 0.3 10^{-1} min^{-1}; Solvent: CH_3CN/0.1 M Britton-Robinson pH 8 buffer 1/1 (v/v).*

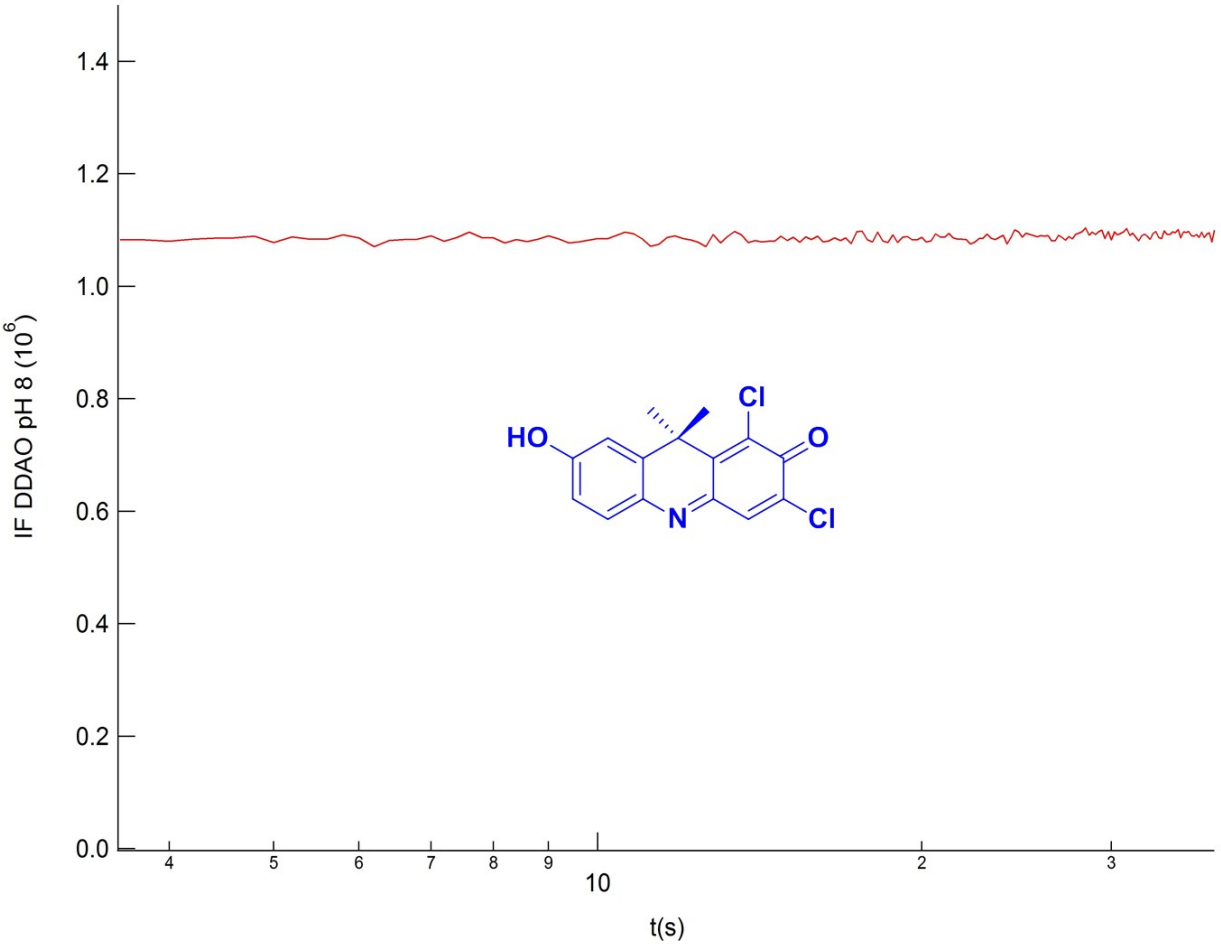


Figure 11: Fluorescence emission (λ_{em}=660 nm) by illuminating at λ_{exc}=365 ± 25 nm a 10.0 ± 0.3 µM solution of the free DDAO at 1.4 10^{-9} $Eins^{-1}$ *intensity of light and at 293K. Solvent: CH_3CN/0.1 M Britton-Robinson pH 8 buffer 1/1 (v/v) corresponding to the maximum fluorescence observed after decaging*

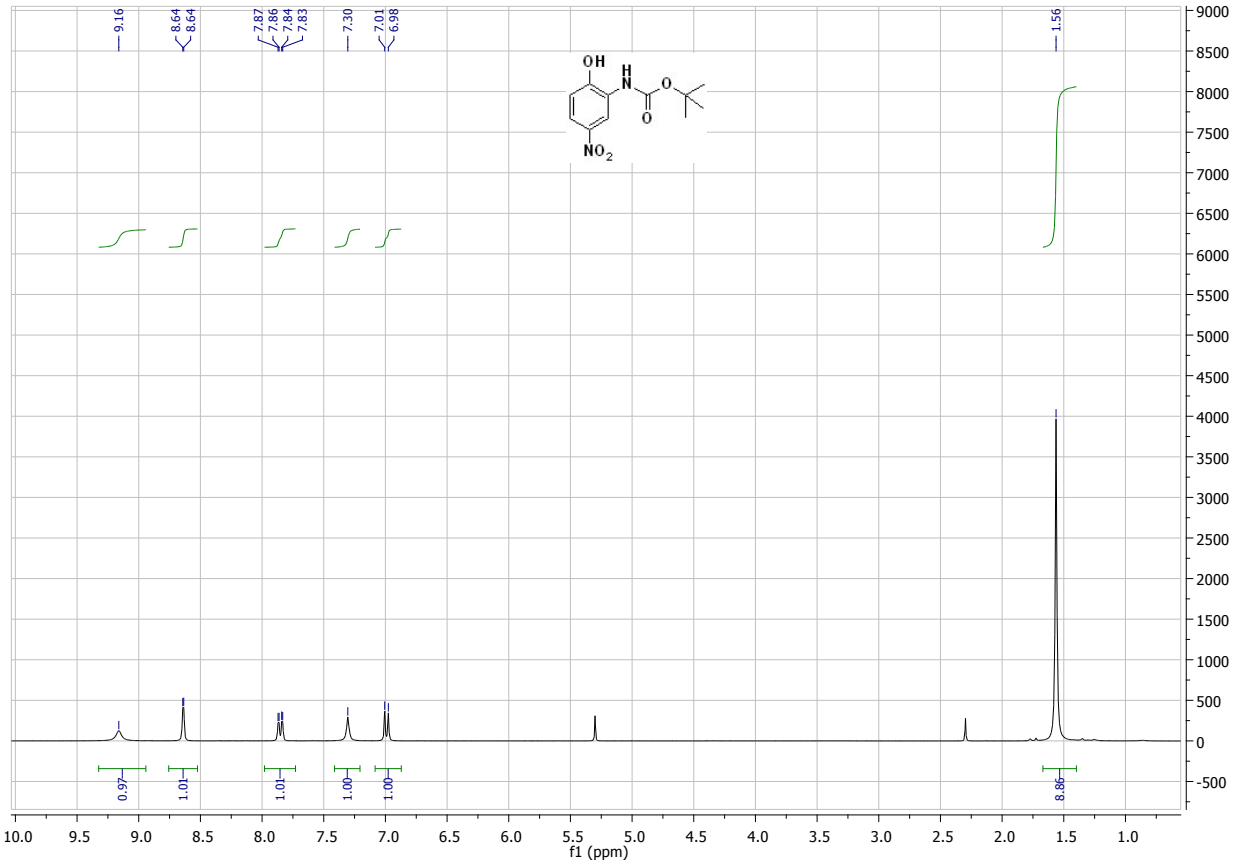


*Figure 12: ^{1}H NMR spectrum of compound **1***

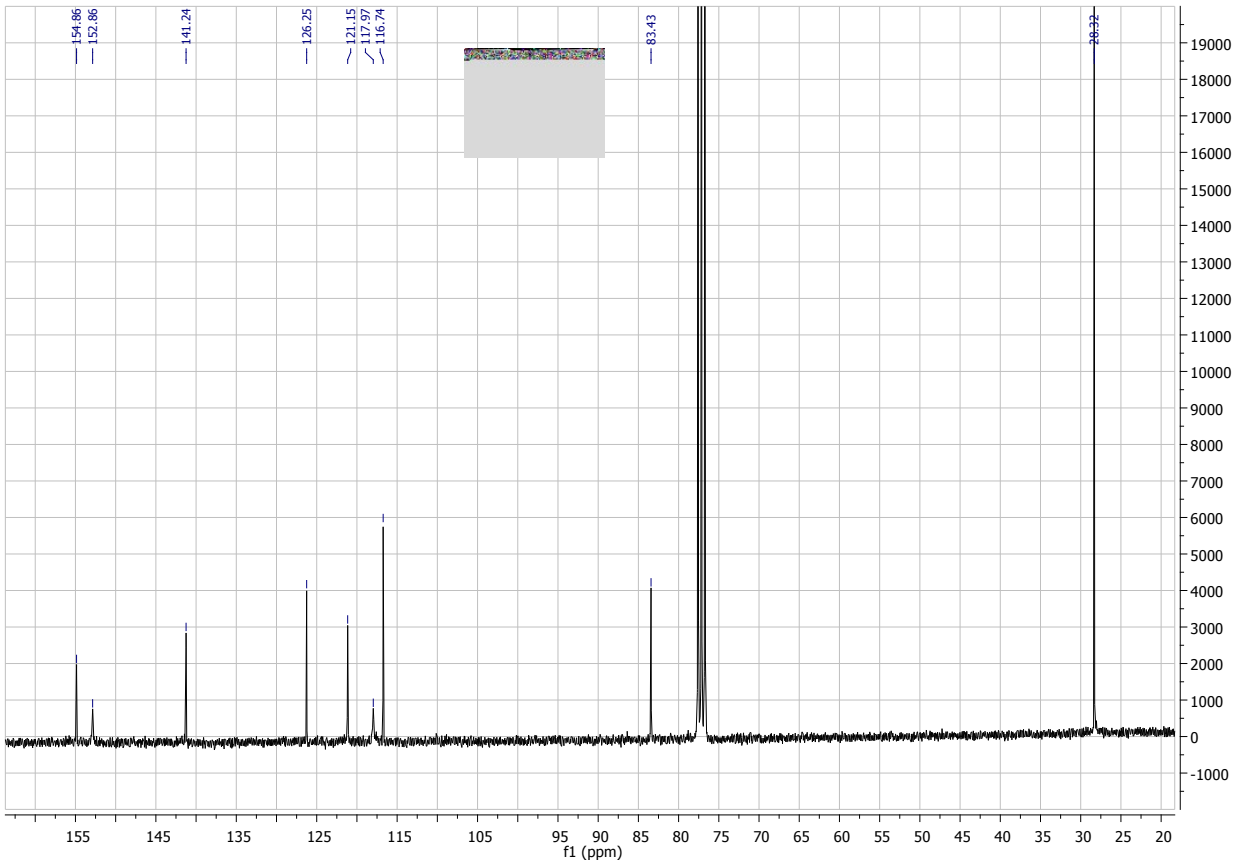
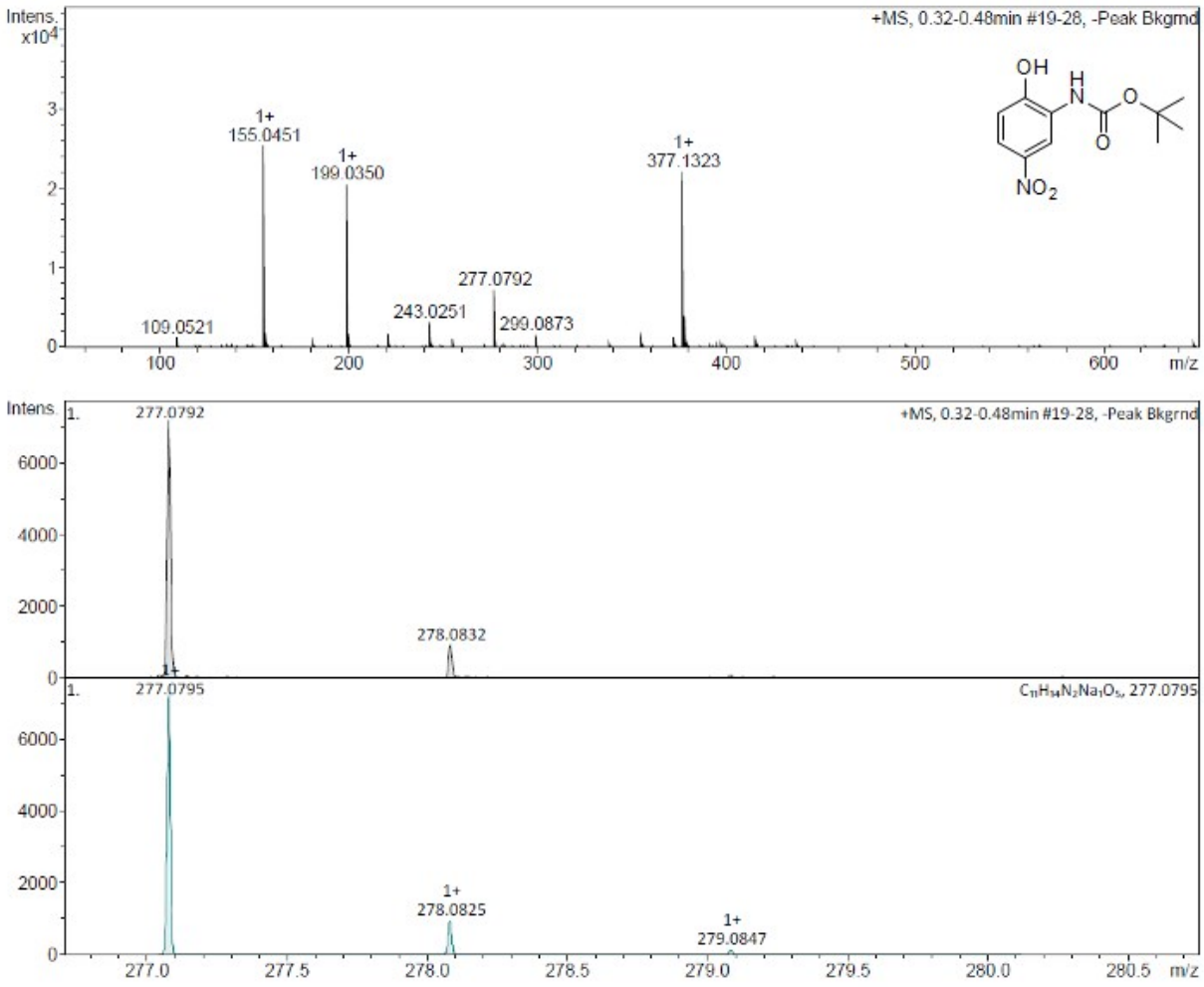


*Figure 13: ^{13}C NMR spectrum of compound **1***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
155.045130	1+	1	C6H7N2O3	155.045119	0.1	2.1	4.5	even
199.034987	1+	1	C7H7N2O5	199.034948	-0.2	1.1	5.5	even
255.097297	1+	1	C11H15N2O5	255.097548	1.0	757.5	5.5	even
277.079224	1+	1	C11H14N2NaO5	277.079492	1.0	10.4	5.5	even
355.149868	1+	1	C16H23N2O7	355.149978	-0.3	16.4	6.5	even
377.132251	1+	1	C16H22N2NaO7	377.131922	0.9	5.6	6.5	even

*Figure 14: HRMS spectrum of compound **1***

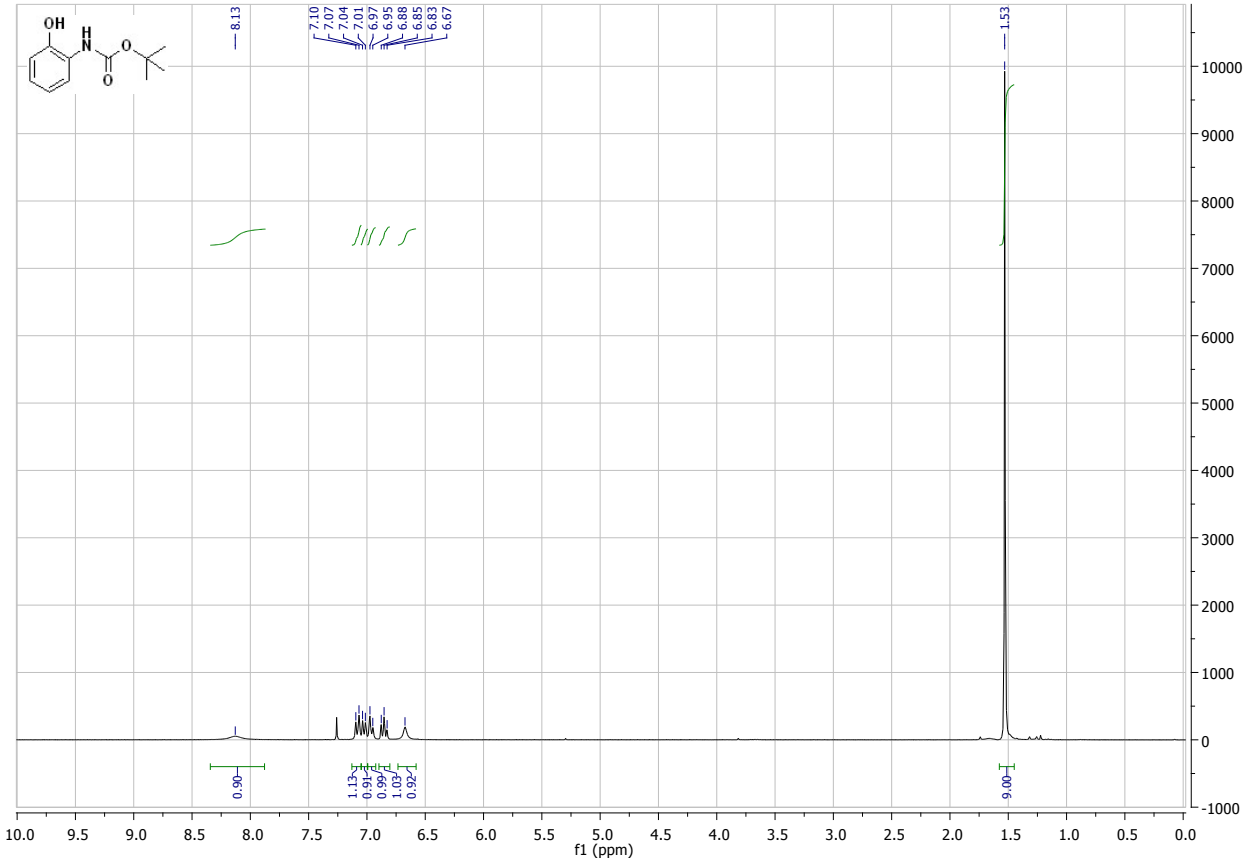


*Figure 15: ^{1}H NMR spectrum of compound **2***

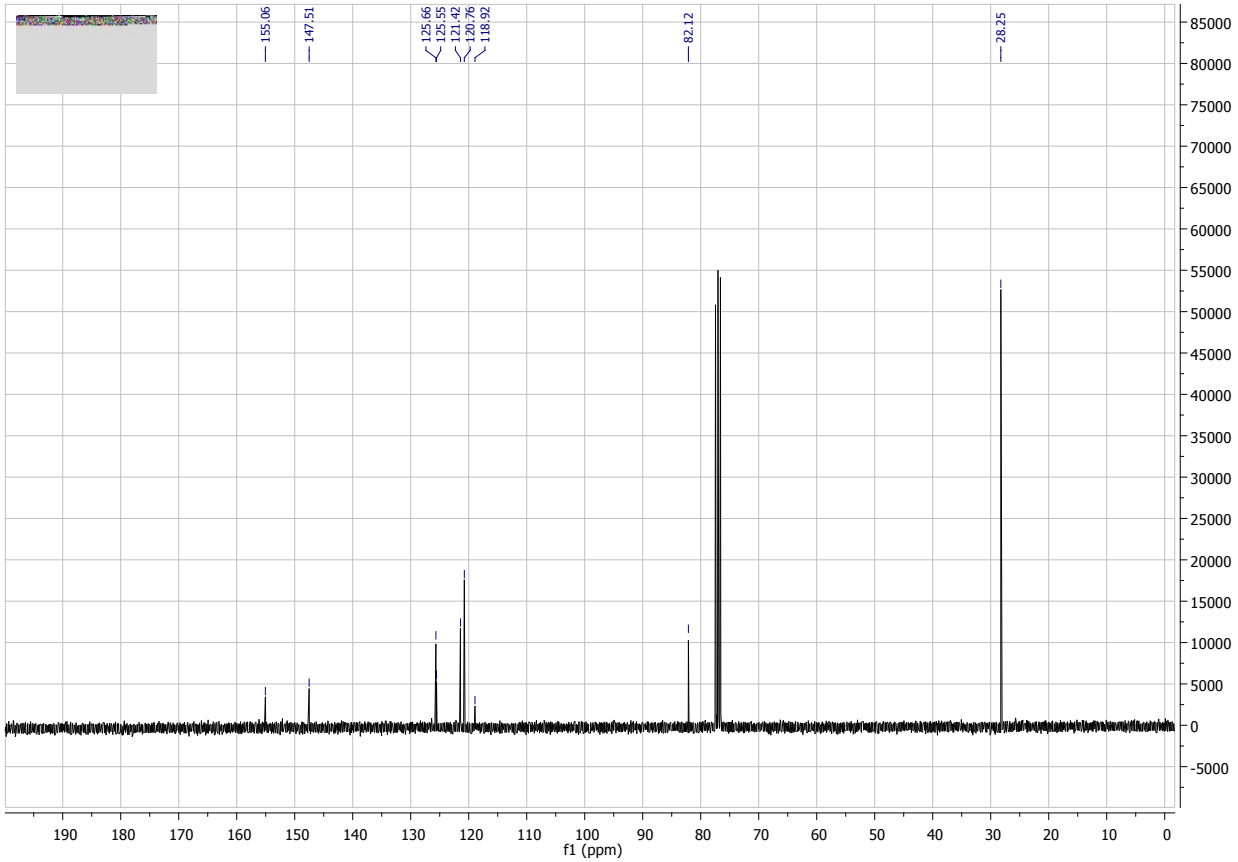
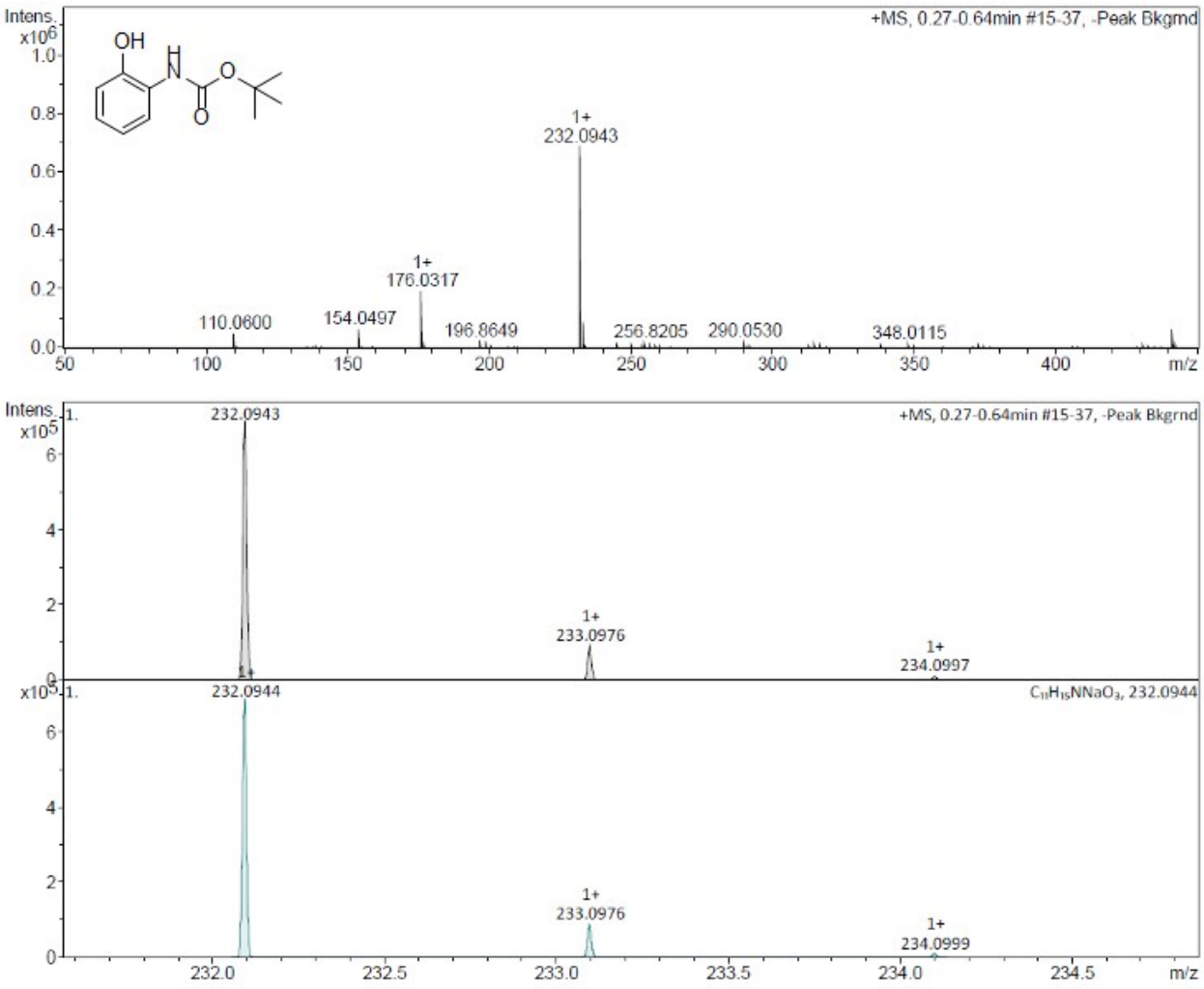


*Figure 16: ^{13}C NMR spectrum of compound **2***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
110.059969	1+	1	C6H8NO	110.060040	-0.6	13.2	3.5	even
154.049684	1+	1	C7H8NO3	154.049870	1.2	5.3	4.5	even
176.031698	1+	1	C7H7NNaO3	176.031814	-0.7	1.1	4.5	even
232.094275	1+	1	C11H15NNaO3	232.094414	0.6	3.4	4.5	even

*Figure 17: HRMS spectrum of compound **2***

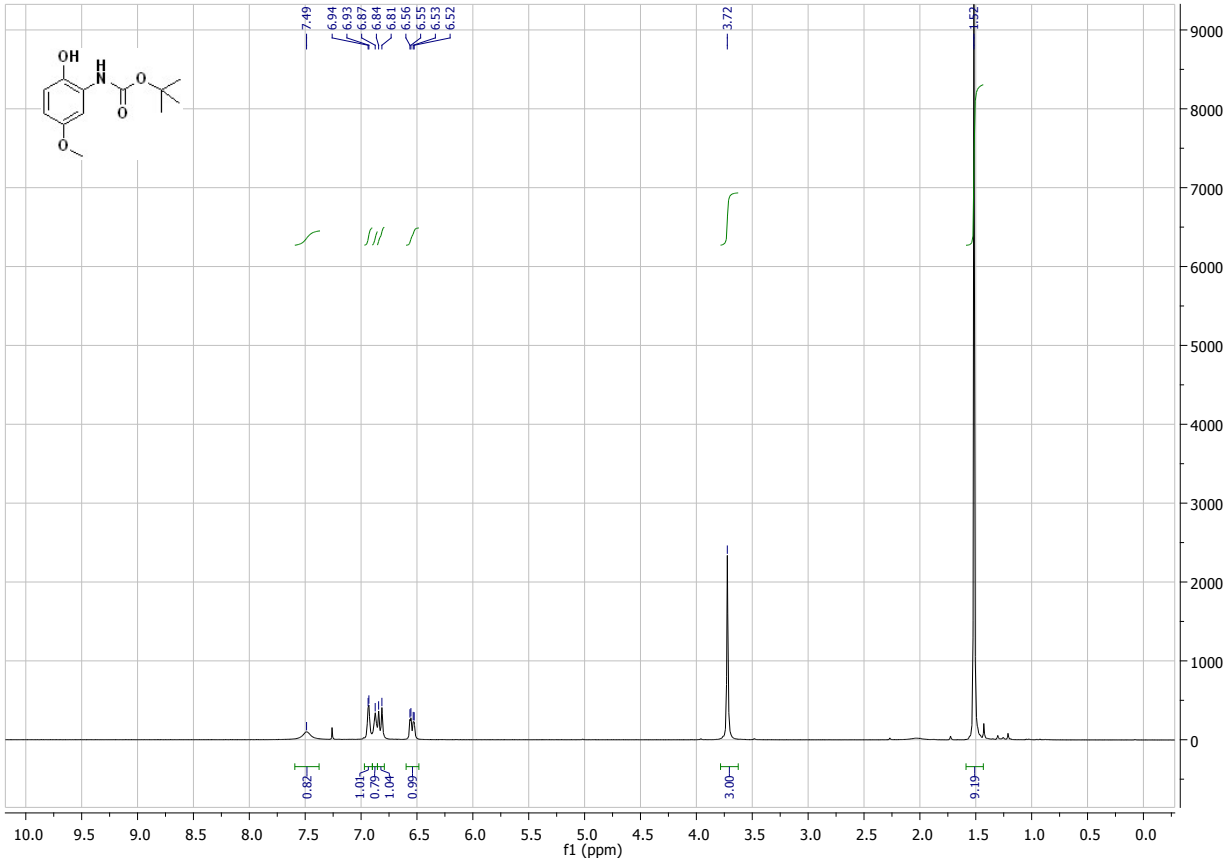


*Figure 18: ^{1}H NMR spectrum of compound **3***

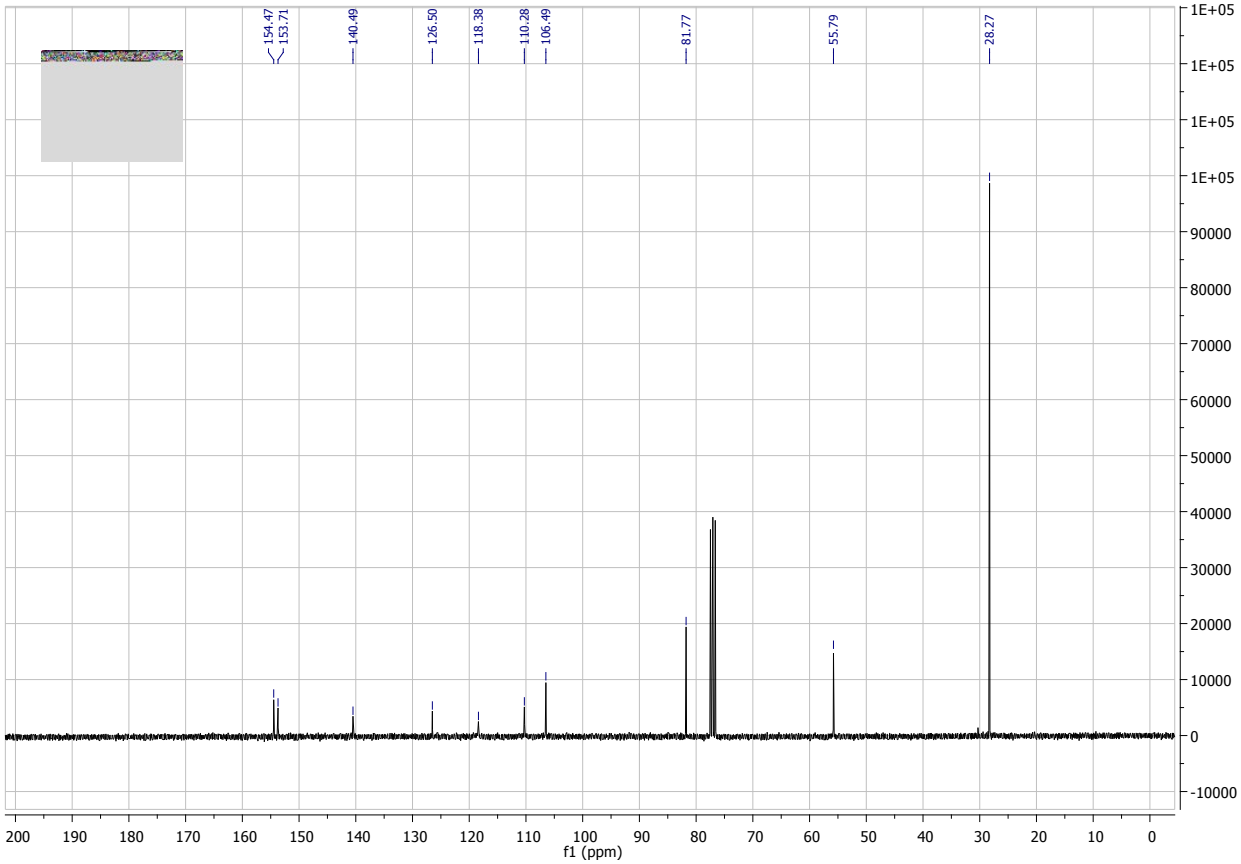
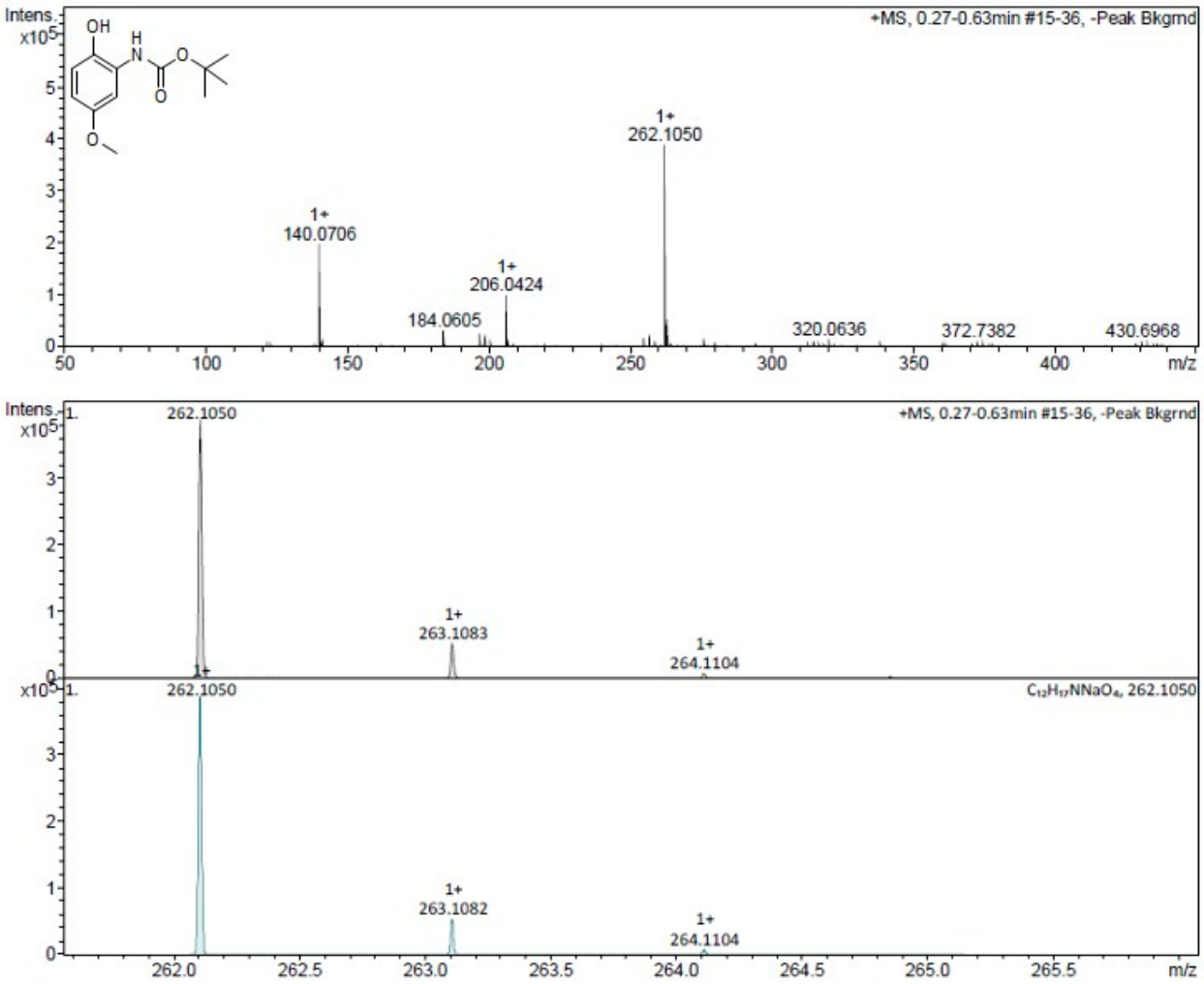


*Figure 19: ^{13}C NMR spectrum of compound **3***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
140.070596	1+	1	C7H10NO2	140.070605	-0.1	0.9	3.5	even
184.060484	1+	1	C8H10NO4	184.060434	-0.3	1.3	4.5	even
206.042441	1+	1	C8H9NNaO4	206.042378	0.3	1.2	4.5	even
262.104967	1+	1	C12H17NNaO4	262.104979	0.0	1.7	4.5	even

*Figure 20: HRMS spectrum of compound **3***

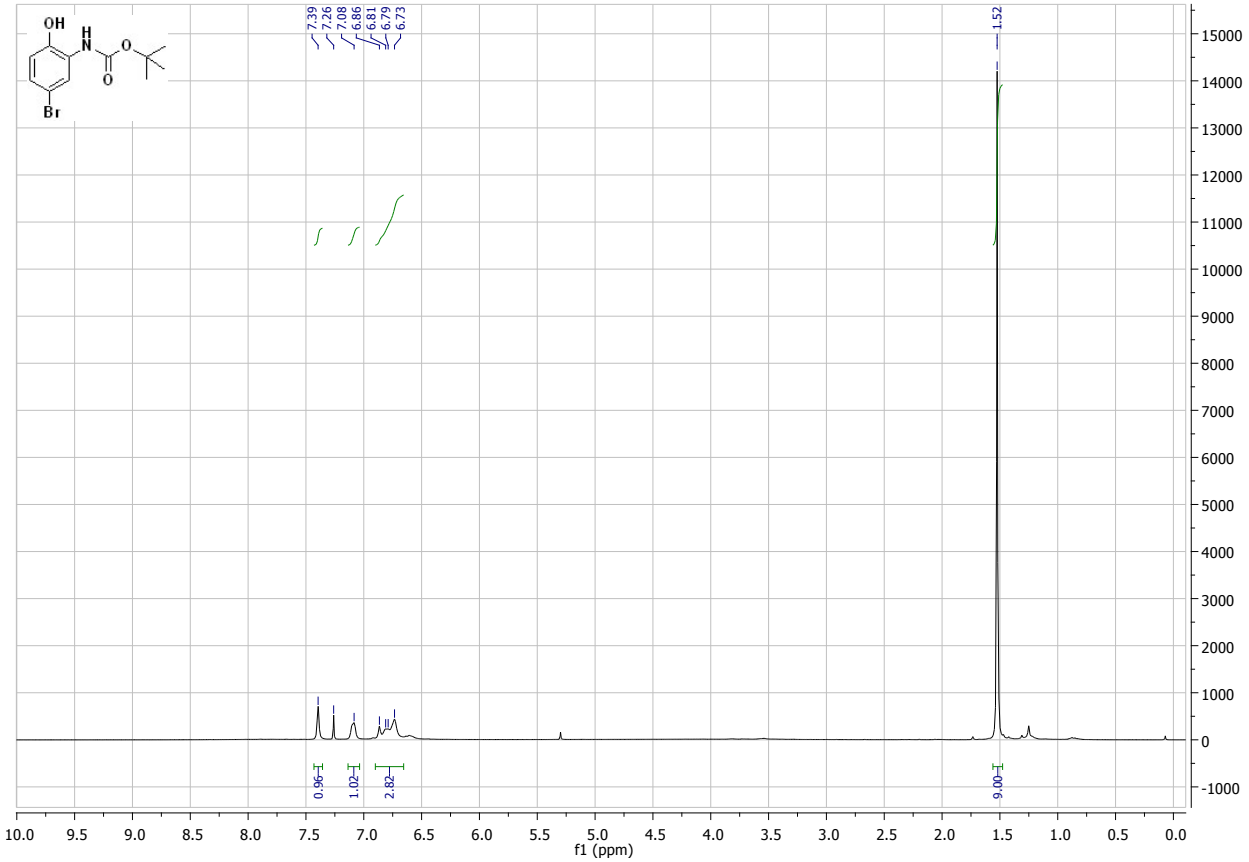


*Figure 21: ^{1}H spectrum of compound **4***

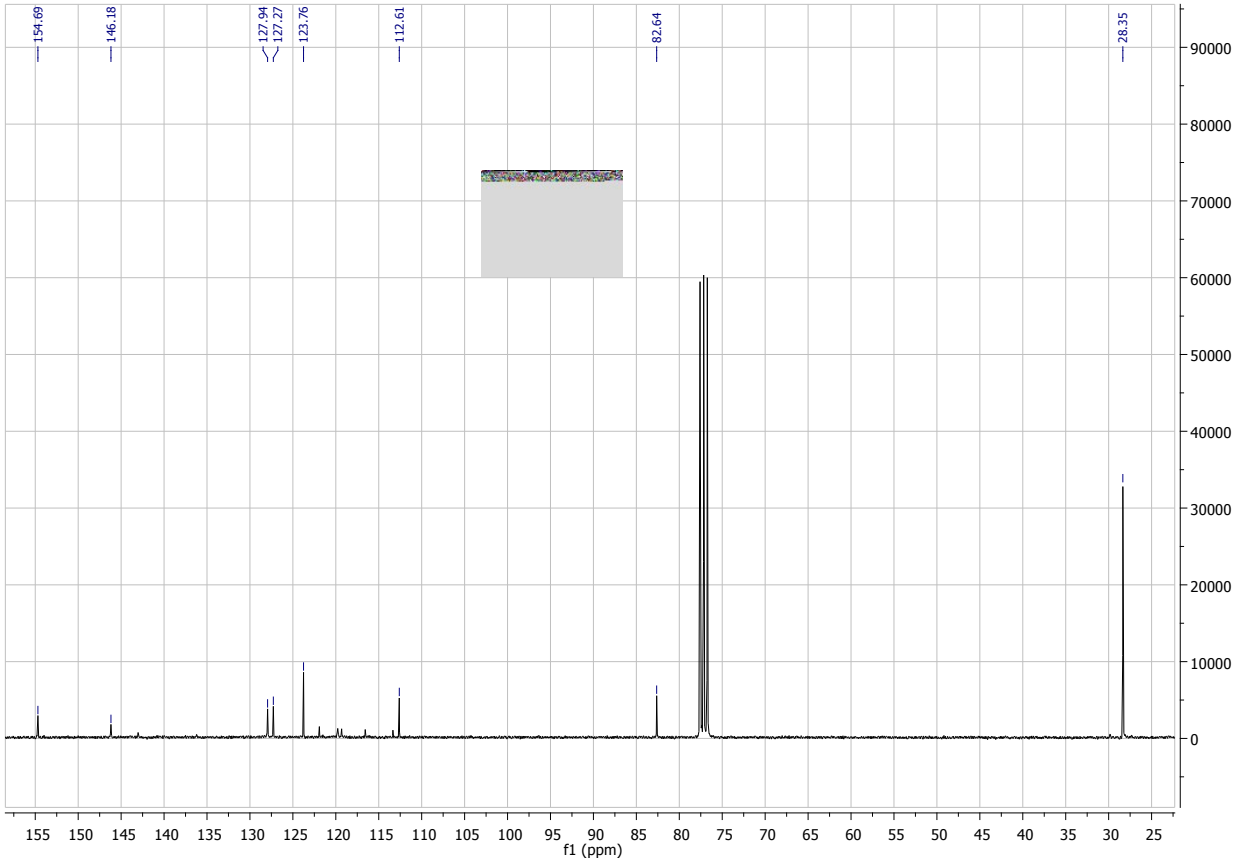
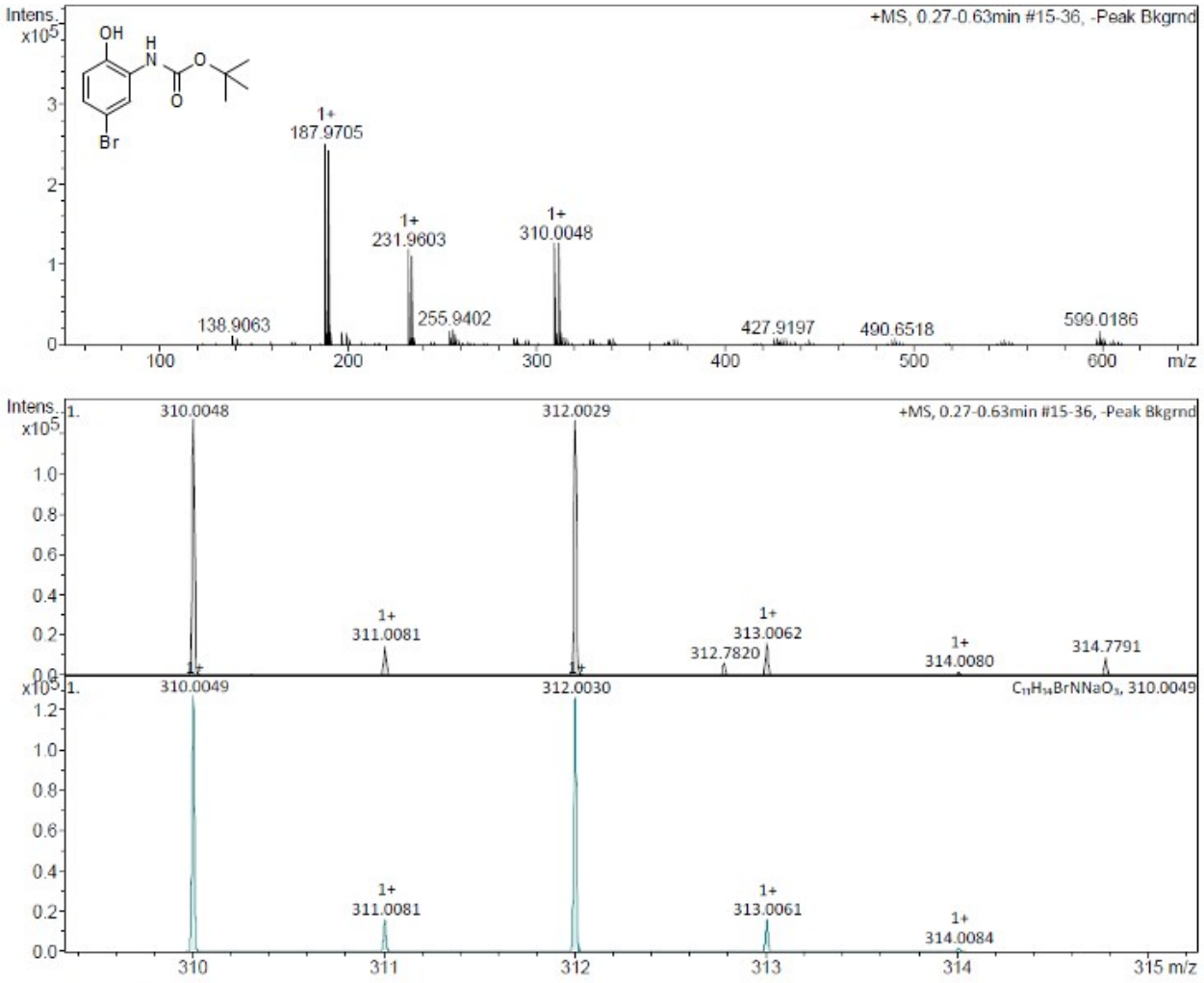


*Figure 22: ^{13}C NMR spectrum of compound **4***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
187.970464	1+	1	C6H7BrNO	187.970552	-0.5	4.8	3.5	even
231.960283	1+	1	C7H7BrNO3	231.960382	0.4	19.9	4.5	even
253.942247	1+	1	C7H6BrNNaO3	253.942326	-0.3	29.4	4.5	even
288.022884	1+	1	C11H15BrNO3	288.022982	-0.3	21.4	4.5	even
310.004756	1+	1	C11H14BrNNaO3	310.004926	0.5	6.2	4.5	even

*Figure 23: HRMS spectrum of compound **4***

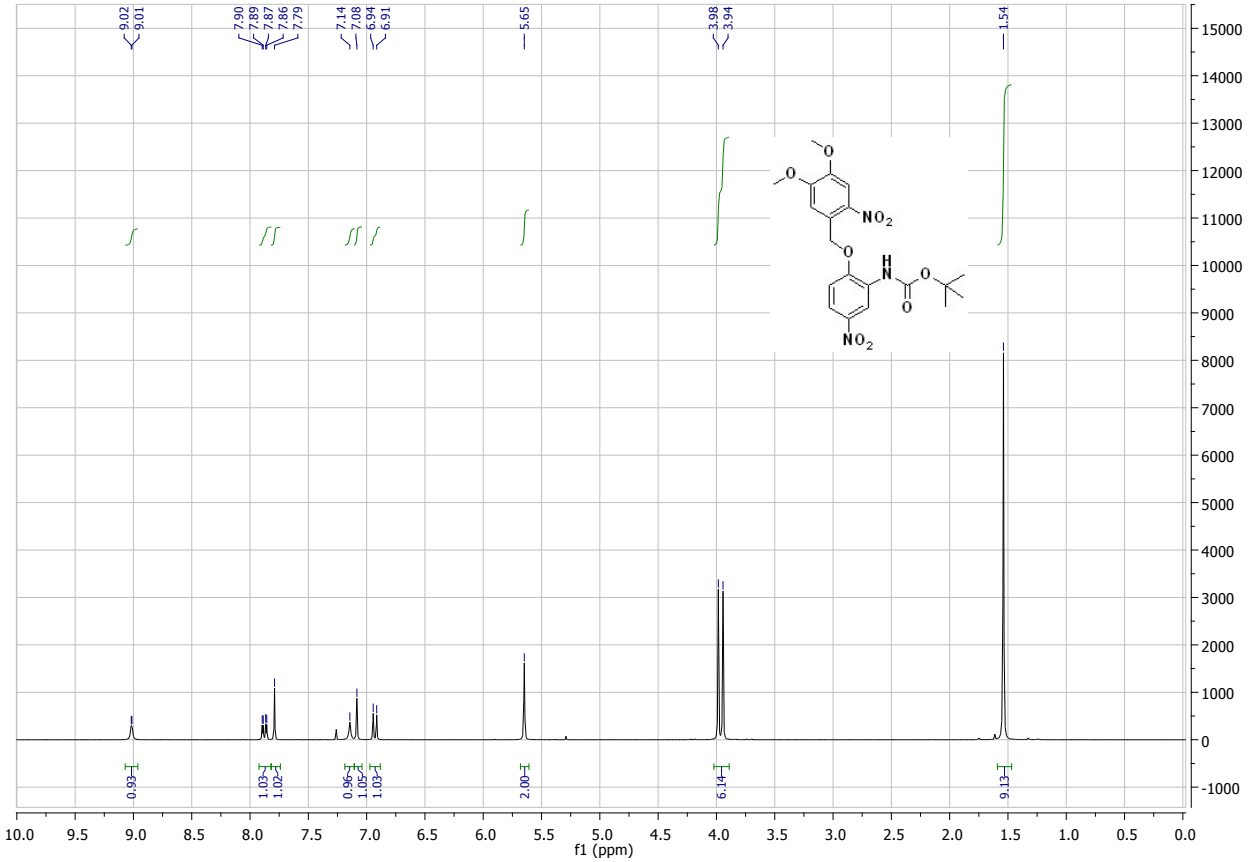


*Figure 24: ^{1}H NMR spectrum of compound **5***

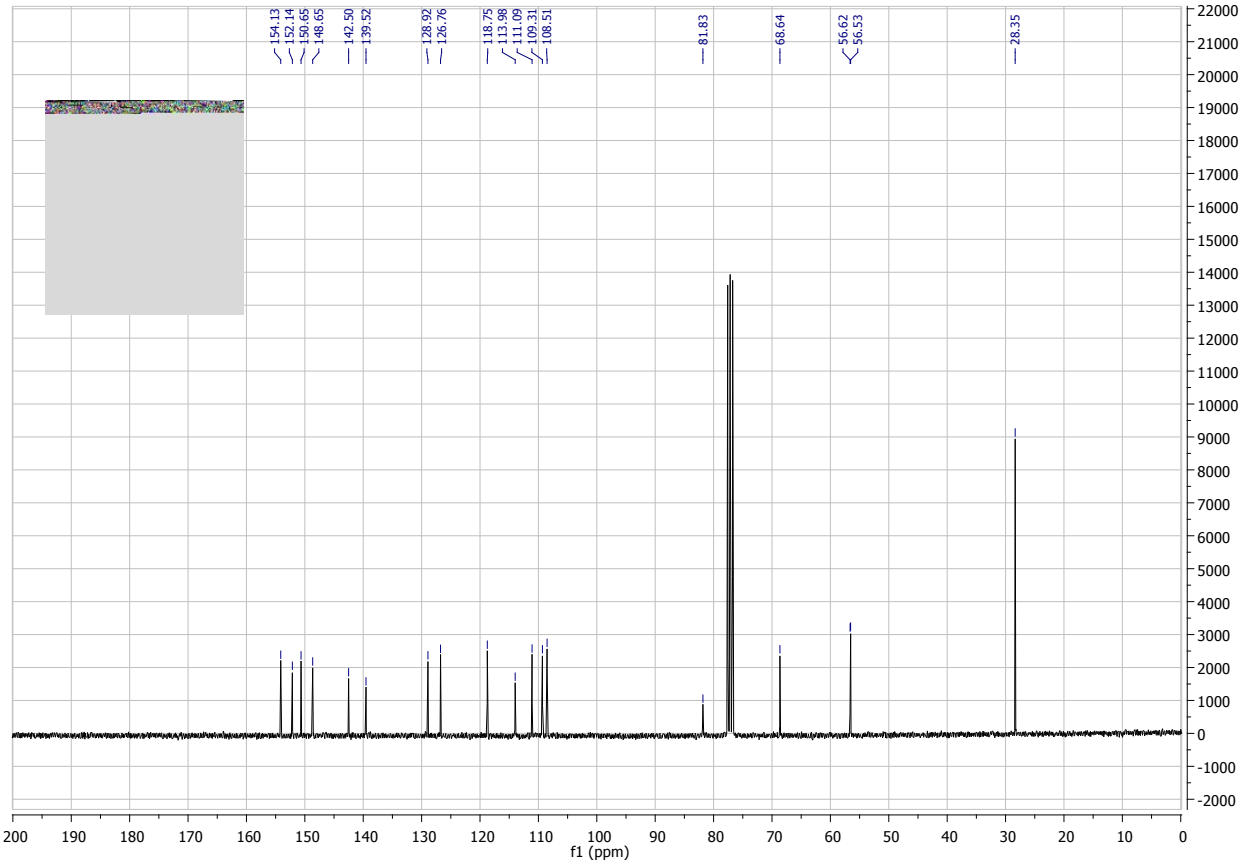
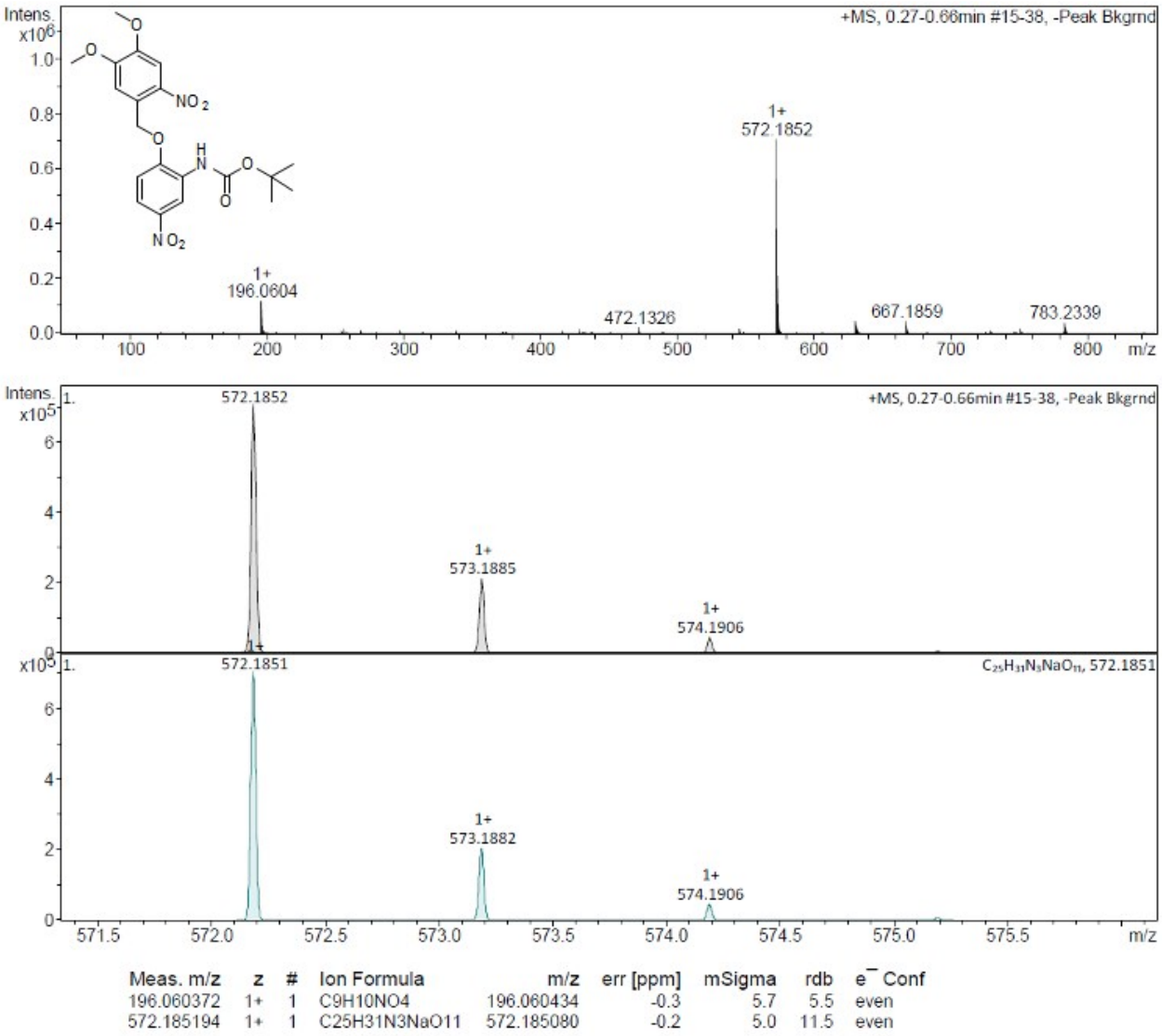


*Figure 25: ^{13}C NMR spectrum of compound **5***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060372	1+	1	C9H10NO4	196.060434	-0.3	5.7	5.5	even
572.185194	1+	1	C25H31N3NaO11	572.185080	-0.2	5.0	11.5	even

*Figure 26: HRMS spectrum of compound **5***

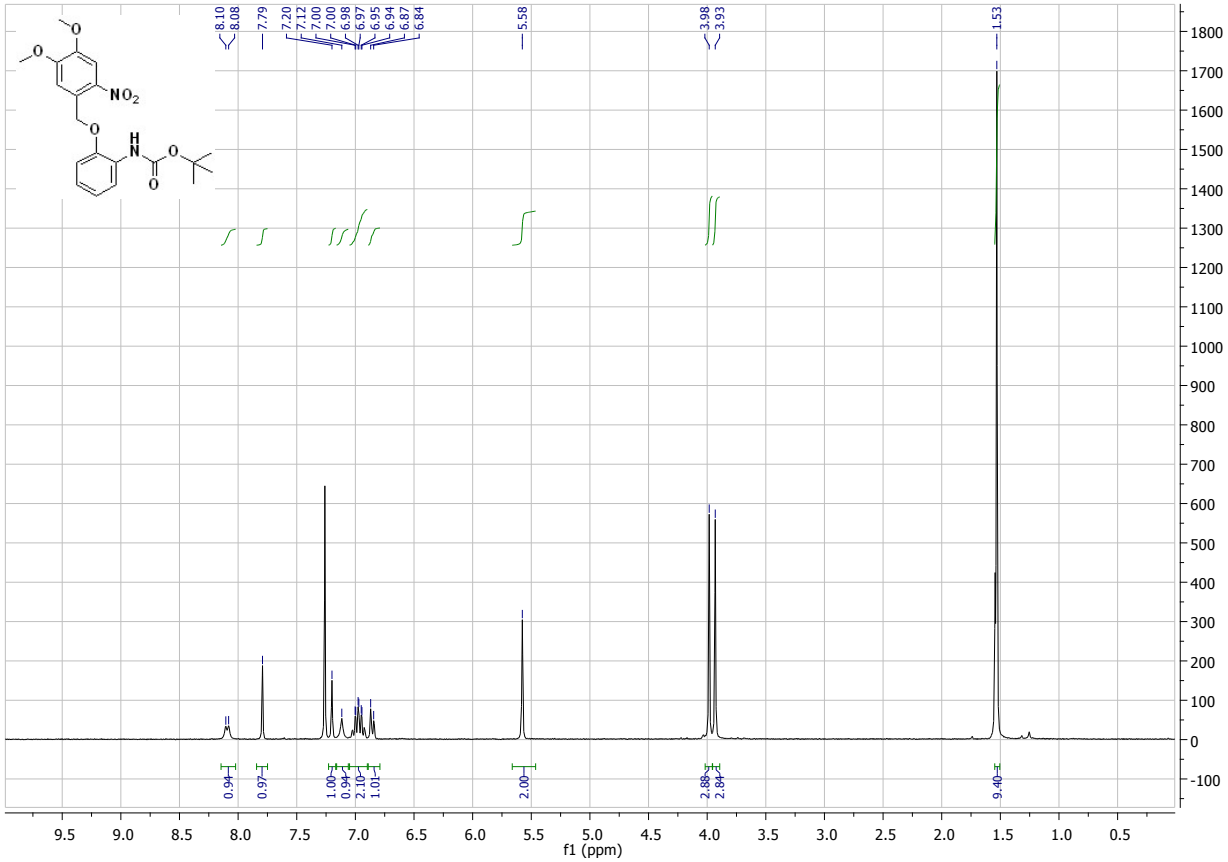


*Figure 27: ^{1}H NMR spectrum of compound **6***

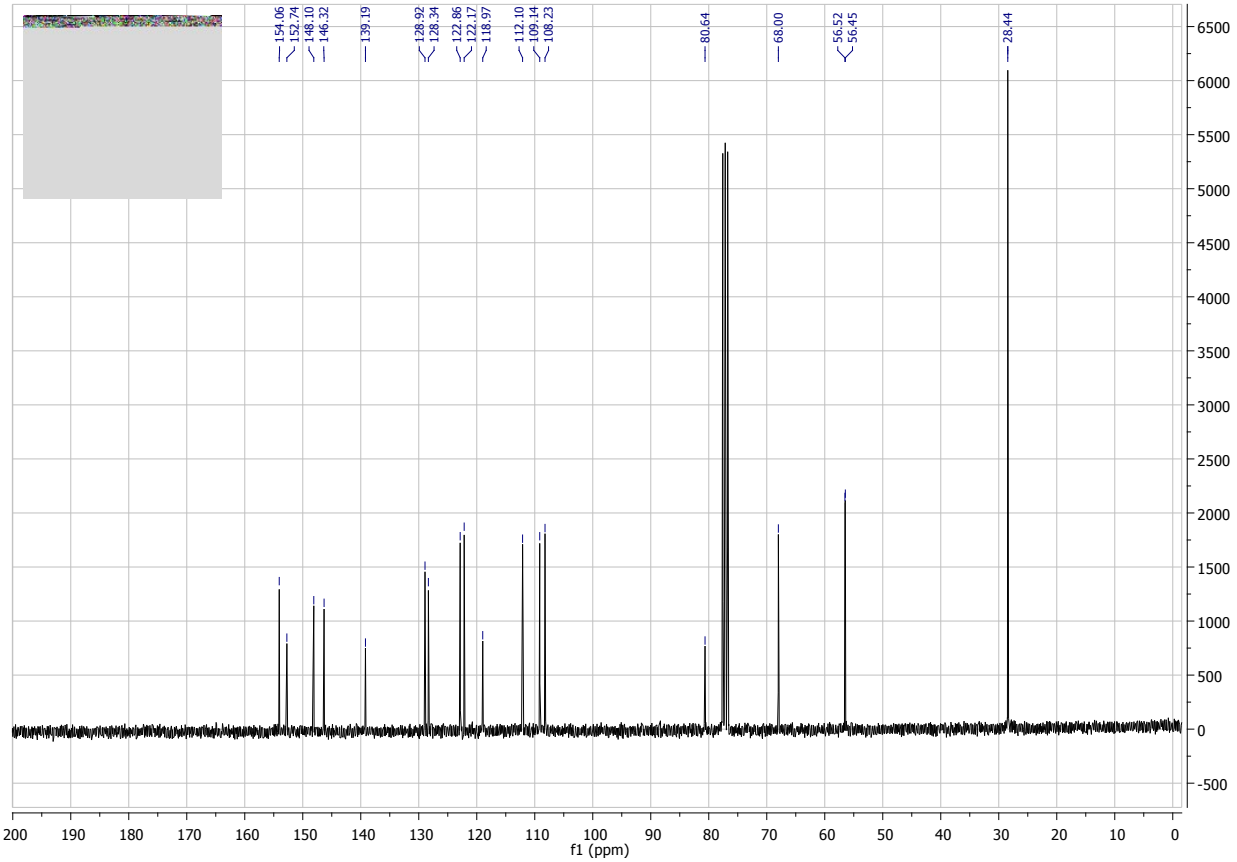
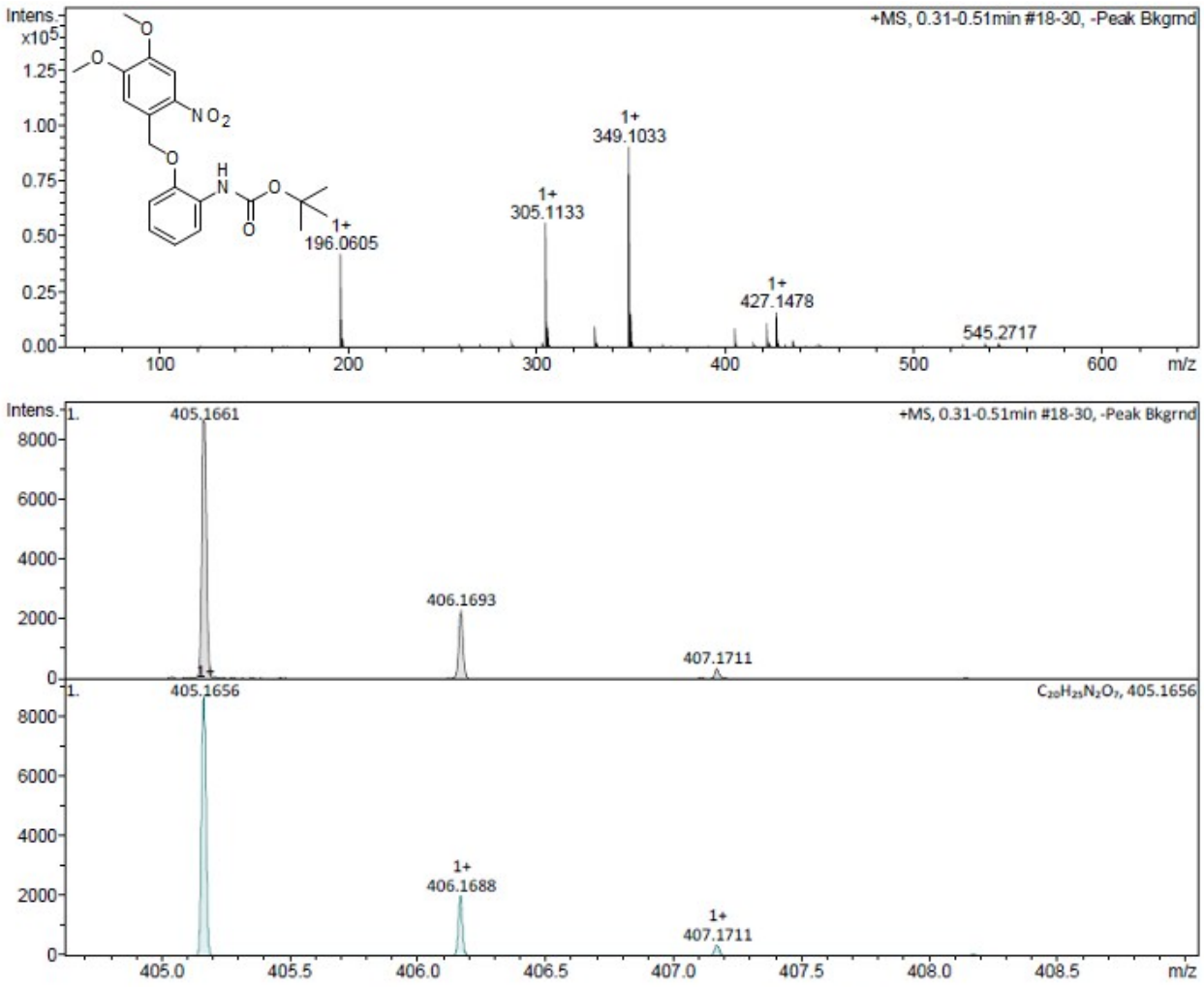


*Figure 28: ^{13}C NMR spectrum of compound **6***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060468	1+	1	C9H10NO4	196.060434	-0.2	1.4	5.5	even
305.113300	1+	1	C15H17N2O5	305.113198	0.3	9.9	8.5	even
331.092668	1+	1	C16H15N2O6	331.092463	-0.6	2.9	10.5	even
349.103286	1+	1	C16H17N2O7	349.103027	0.7	10.4	9.5	even
405.166137	1+	1	C20H25N2O7	405.165628	1.3	17.9	9.5	even
422.192037	1+	1	C20H28N3O7	422.192177	0.3	10.5	8.5	even
427.147797	1+	1	C20H24N2NaO7	427.147572	0.5	10.0	9.5	even

*Figure 29: HRMS spectrum of compound **6***

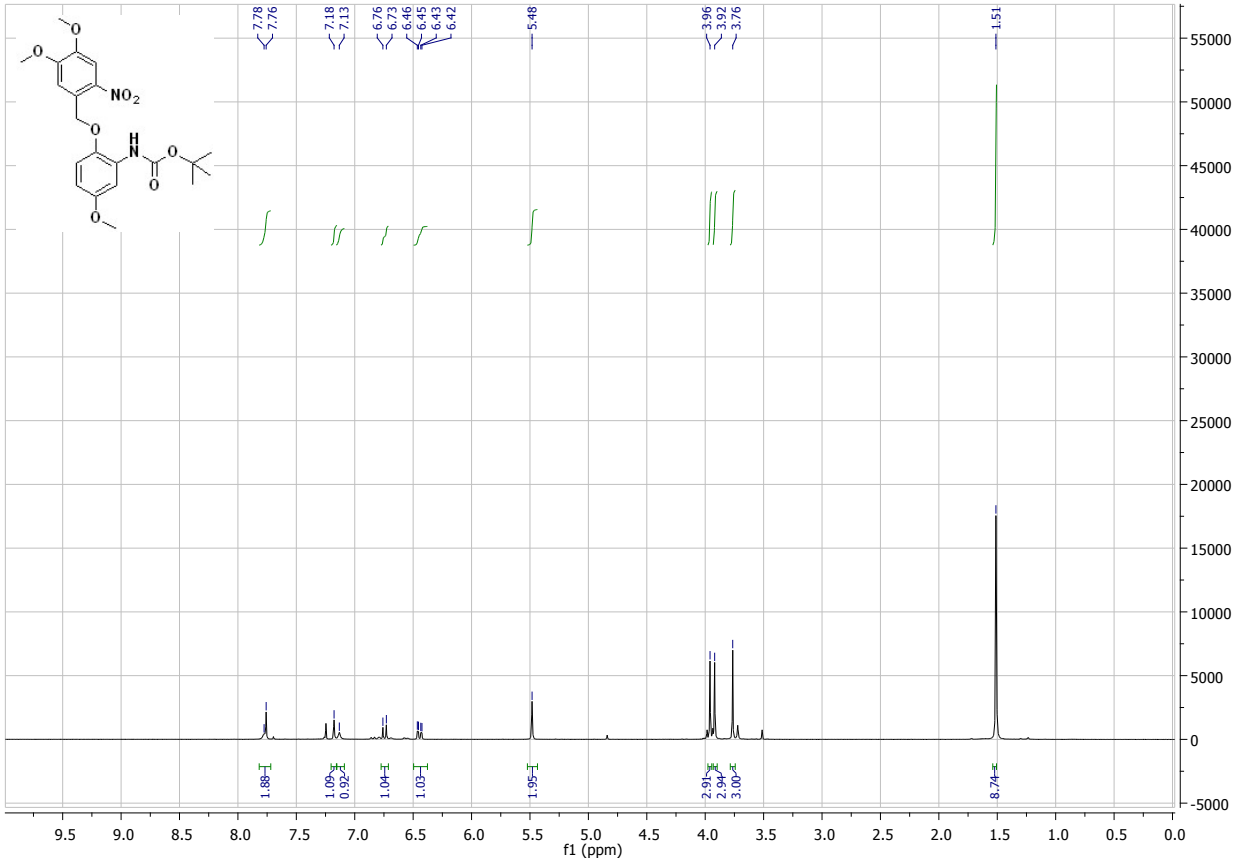


*Figure 30: ^{1}H NMR spectrum of compound **7***

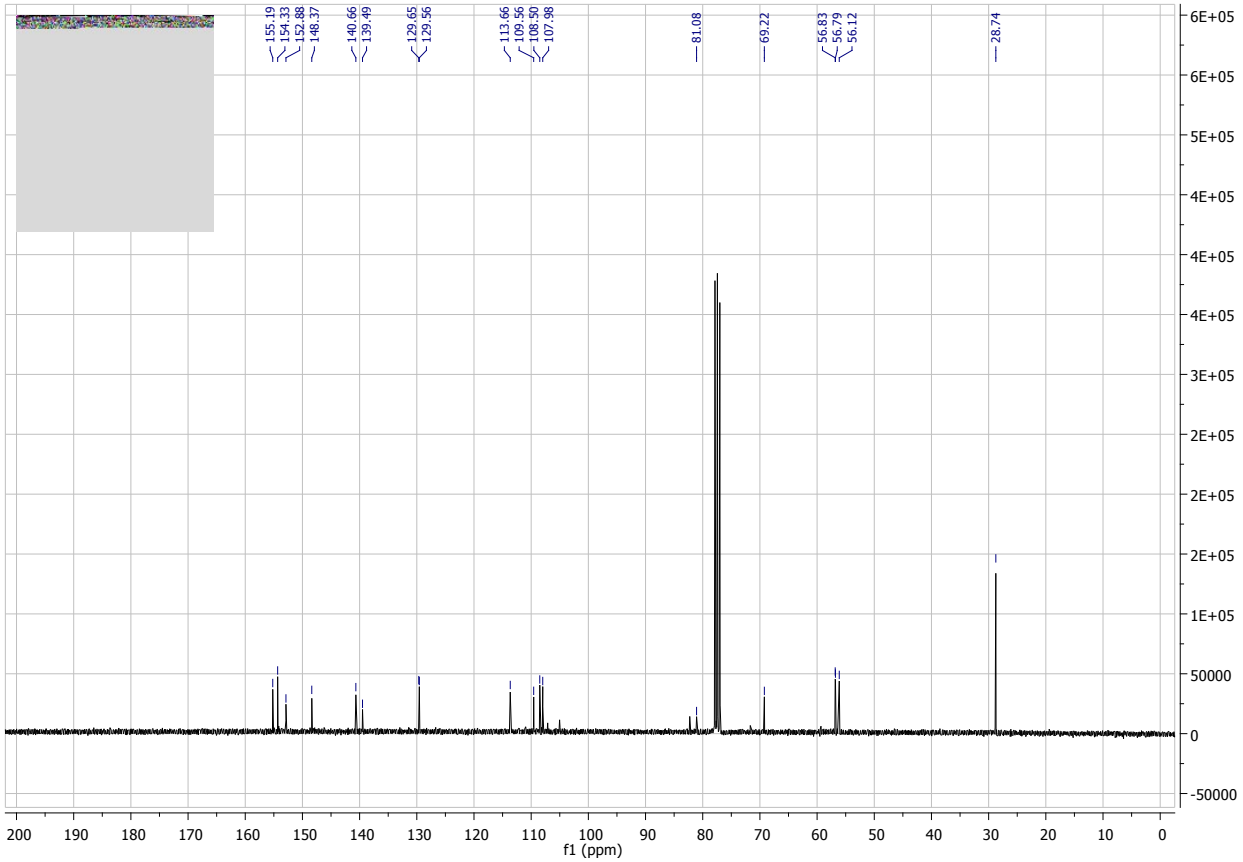
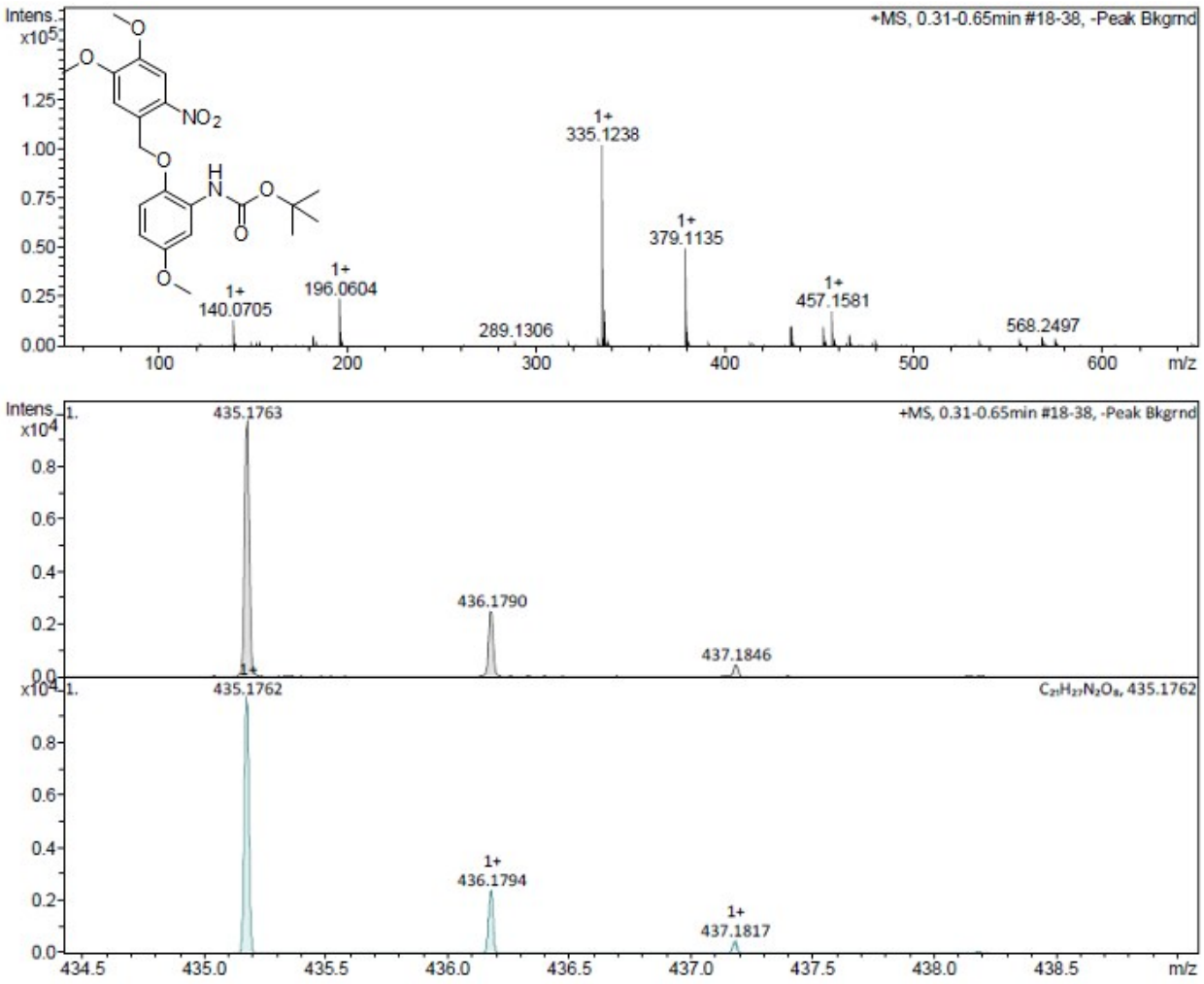


*Figure 31: ^{13}C NMR spectrum of compound **7***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
140.070472	1+	1	C7H10NO2	140.070605	1.0	4.5	3.5	even
182.081121	1+	1	C9H12NO3	182.081170	0.3	14.2	4.5	even
196.060379	1+	1	C9H10NO4	196.060434	0.3	9.6	5.5	even
335.123794	1+	1	C16H19N2O6	335.123763	-0.1	3.5	8.5	even
379.113549	1+	1	C17H19N2O8	379.113592	-0.1	3.6	9.5	even
435.176313	1+	1	C21H27N2O8	435.176192	-0.3	7.0	9.5	even
452.203095	1+	1	C21H30N3O8	452.202741	0.8	5.2	8.5	even
457.158135	1+	1	C21H26N2NaO8	457.158137	-0.0	6.9	9.5	even

*Figure 32: HRMS spectrum of compound **7***

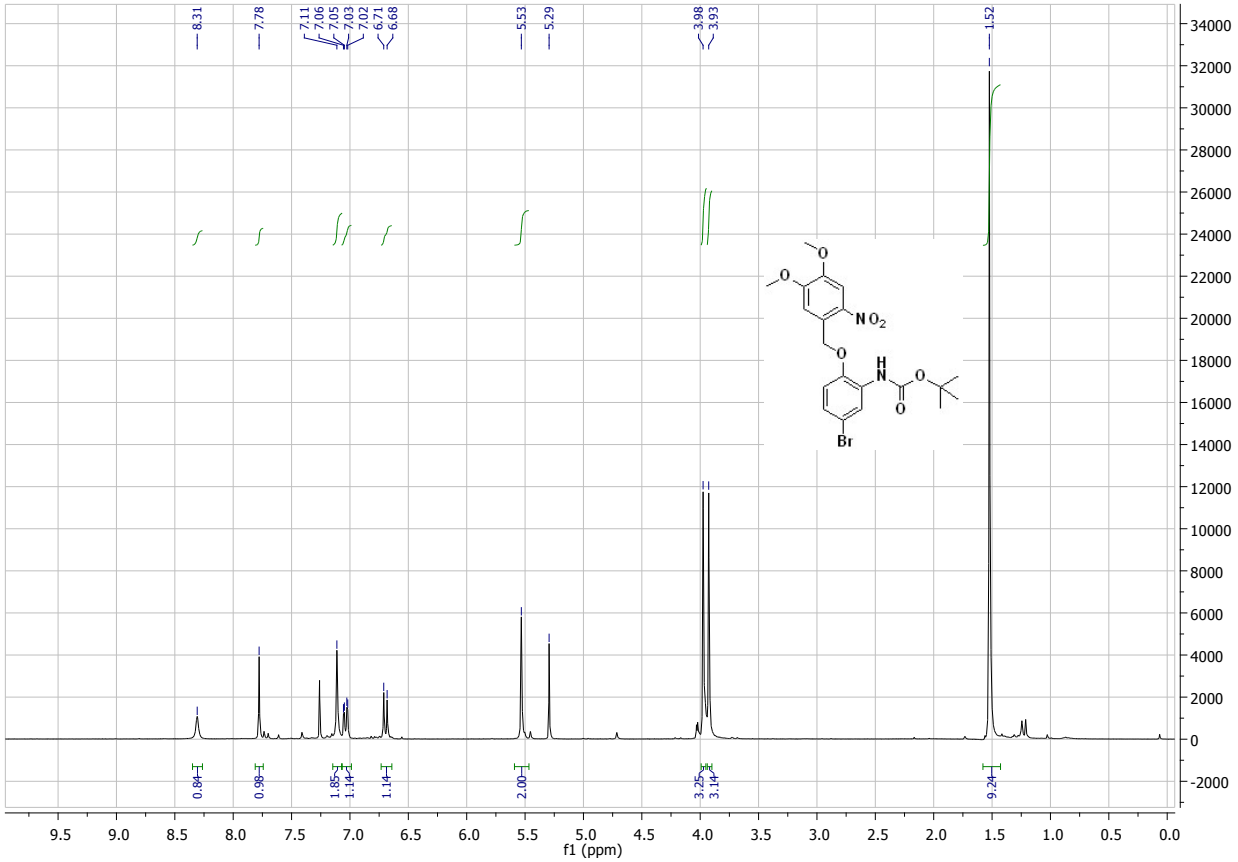


*Figure 33: ^{1}H NMR spectrum of compound **8***

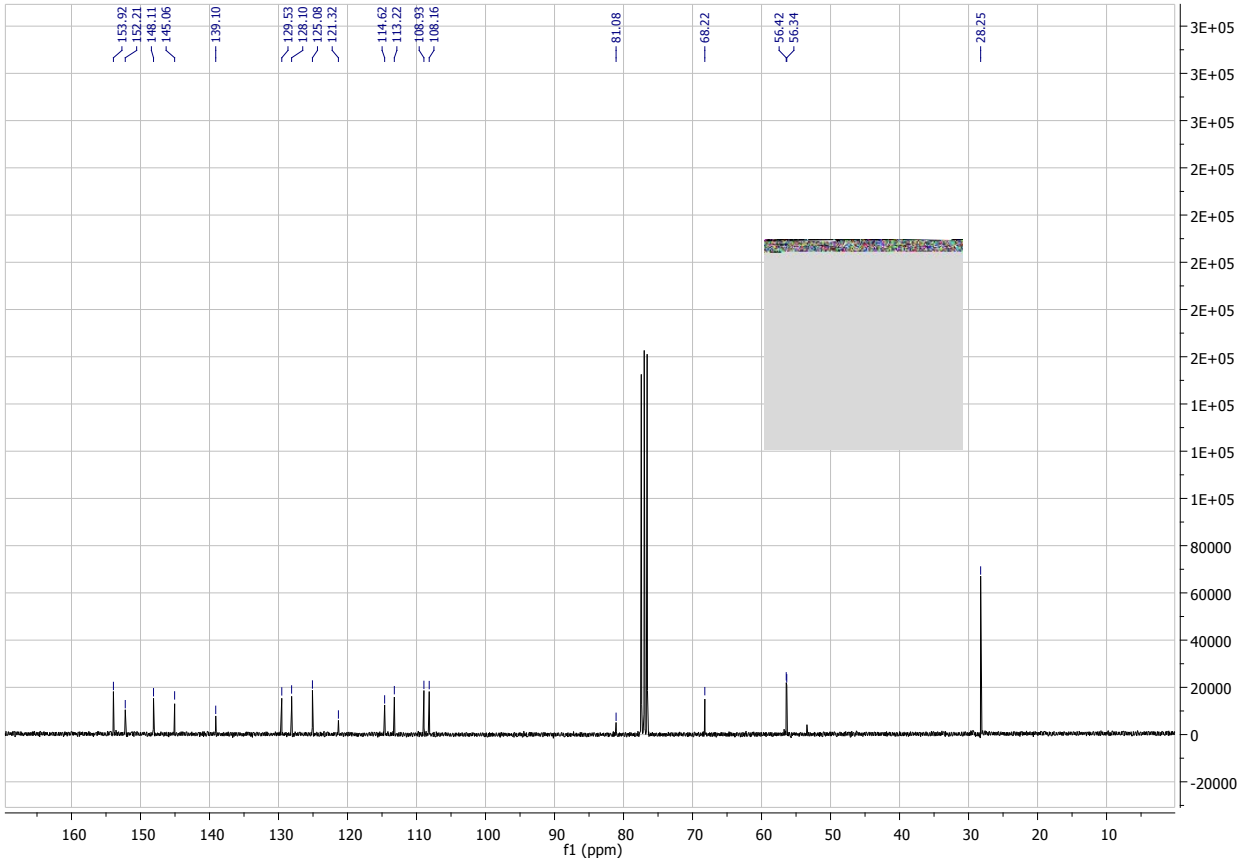
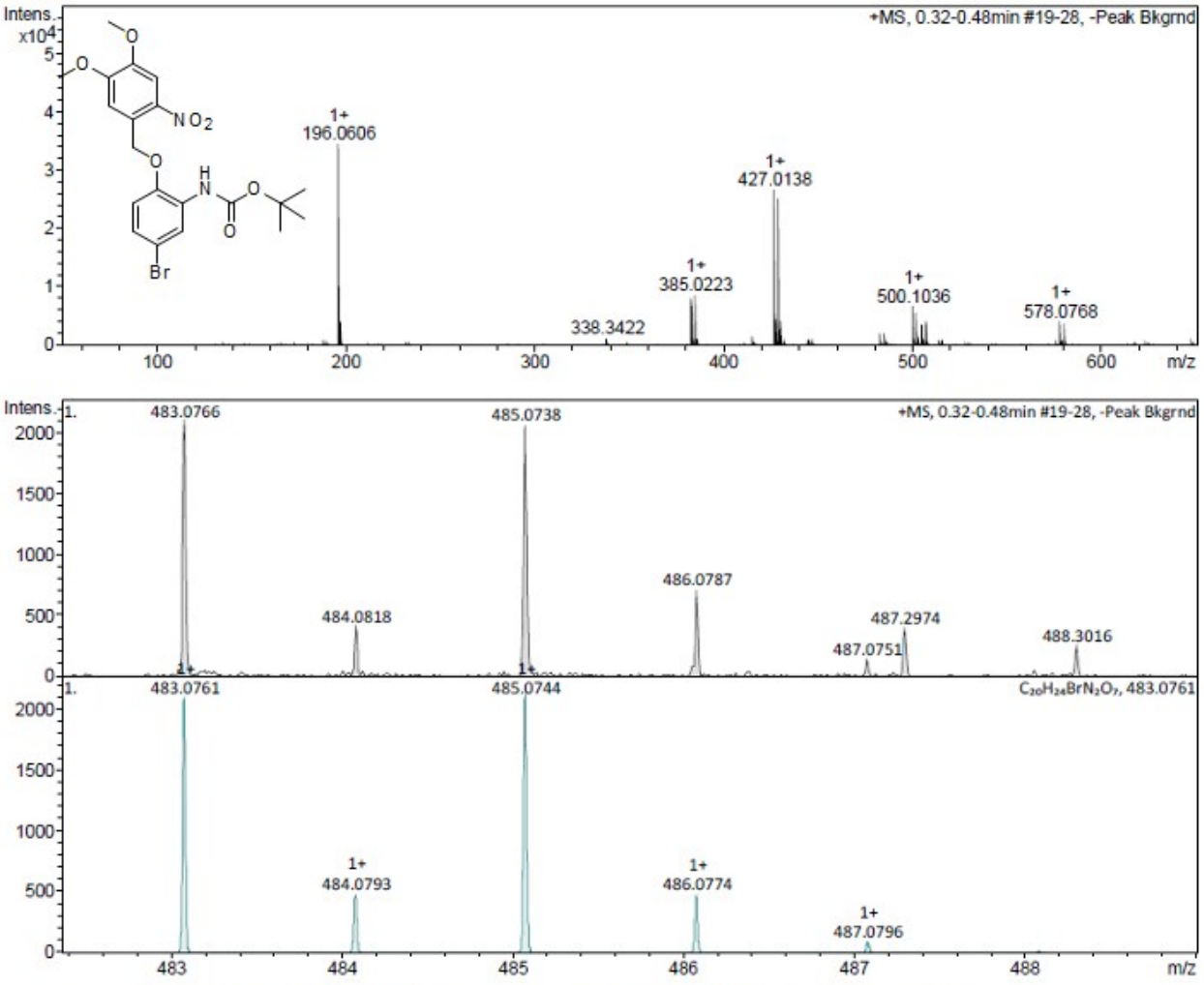


*Figure 34: ^{13}C NMR spectrum of compound **8***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
196.060596	1+	1	C9H10NO4	196.060434	-0.8	10.5	5.5	even
383.023526	1+	1	C15H16BrN2O5	383.023710	-0.5	41.4	8.5	even
427.013848	1+	1	C16H16BrN2O7	427.013539	0.7	26.9	9.5	even
483.076561	1+	1	C21H20BrN6O3	483.077477	1.9	45.3	14.5	even
	1+	2	C20H24BrN2O7	483.076140	0.9	48.9	9.5	even
500.103622	1+	1	C20H27BrN3O7	500.102689	1.9	61.1	8.5	even
	1+	2	C21H23BrN7O3	500.104026	0.8	63.1	13.5	even
505.058593	1+	1	C20H23BrN2NaO7	505.058084	-1.0	61.8	9.5	even

*Figure 35: HRMS spectrum of compound **8***

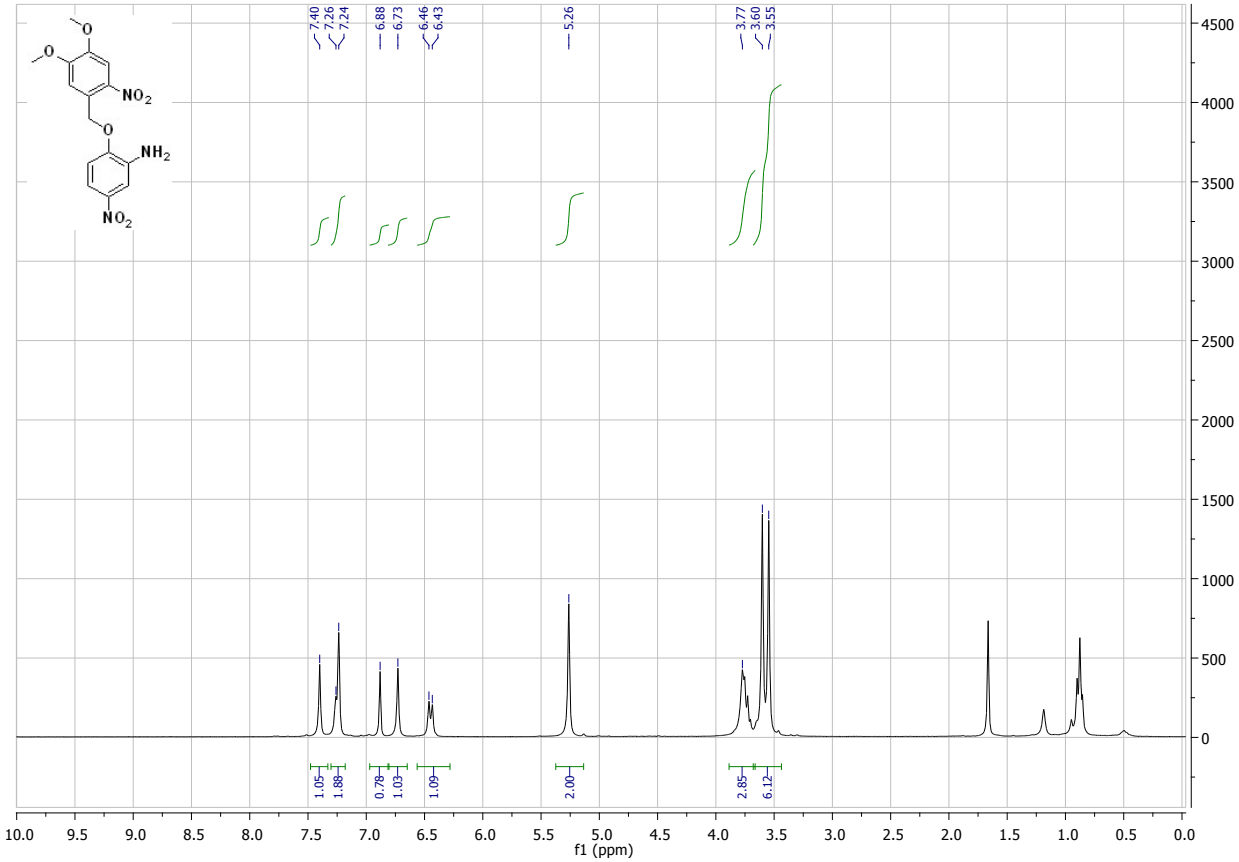


*Figure 36: [1]H NMR of compound **9***

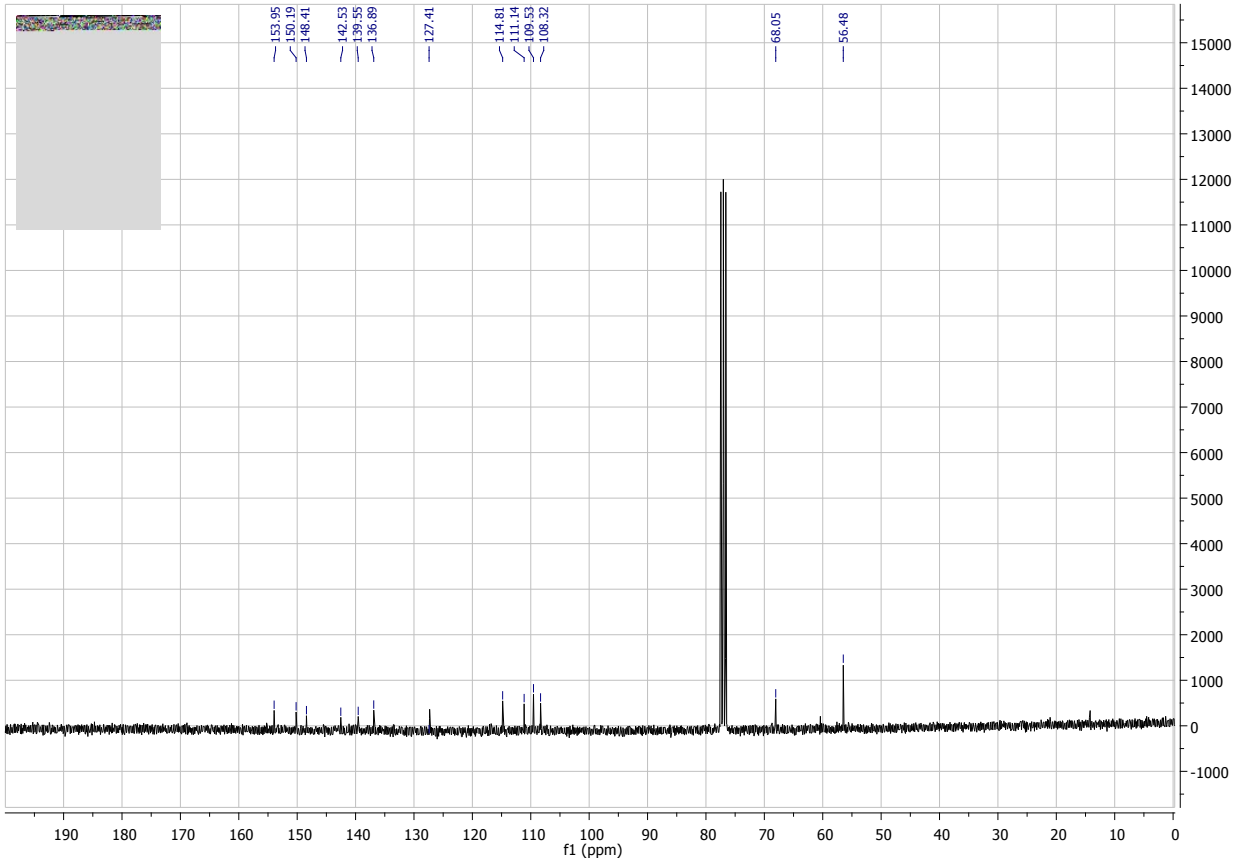
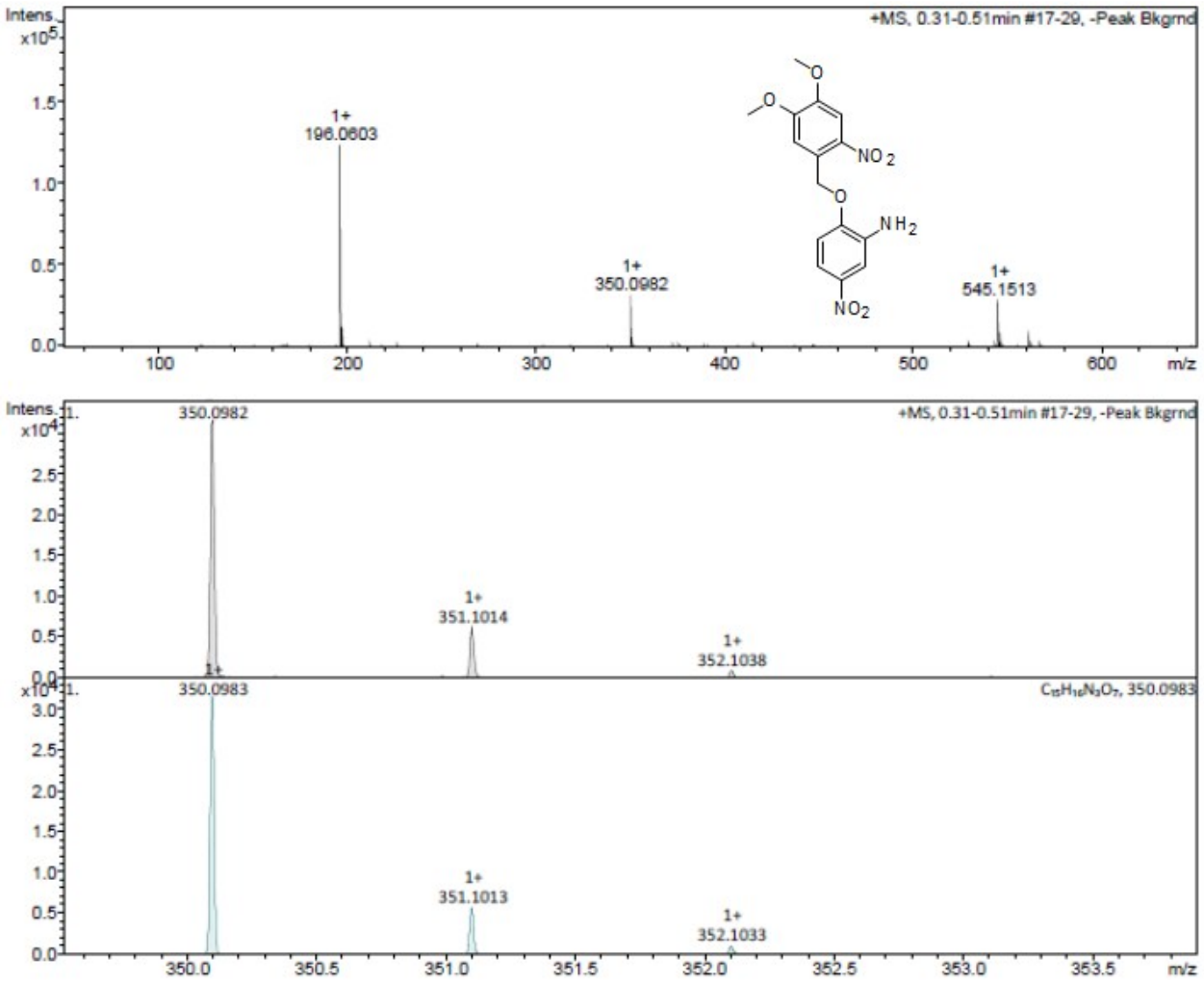


*Figure 37: ^{13}C NMR spectrum of compound **9***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060290	1+	1	C9H10NO4	196.060434	0.7	3.4	5.5	even
350.098225	1+	1	C15H16N3O7	350.098276	-0.1	9.5	9.5	even
545.151316	1+	1	C24H25N4O11	545.151434	0.2	13.3	14.5	even
	1+	2	C37H17N6	545.150921	0.7	60.2	32.5	even
567.133051	1+	1	C24H24N4NaO11	567.133378	0.6	22.4	14.5	even
	1+	2	C23H28NaO15	567.132041	1.8	33.8	9.5	even
	1+	3	C37H16N6Na	567.132865	0.3	51.2	32.5	even

*Figure 38: HRMS spectrum of compound **9***

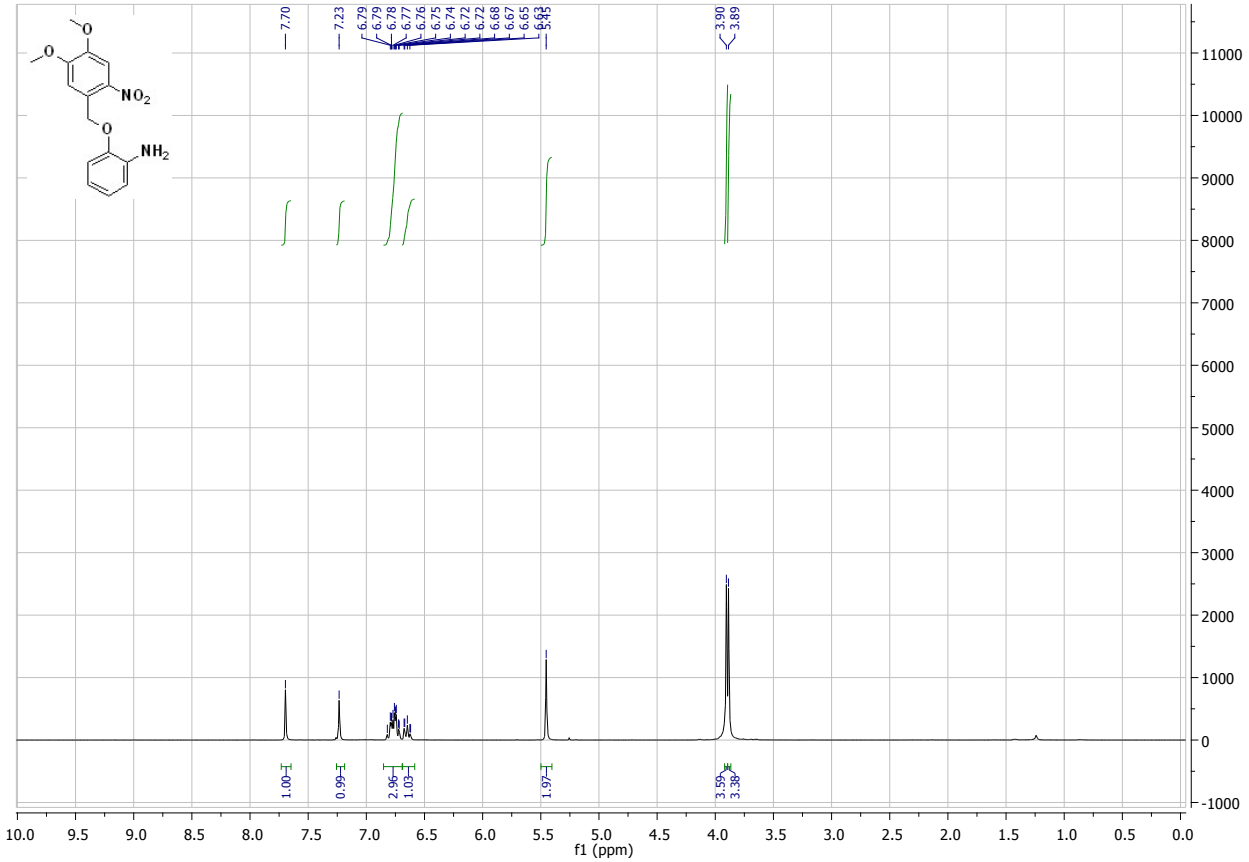


*Figure 39: ^{1}H NMR spectrum of compound **10***

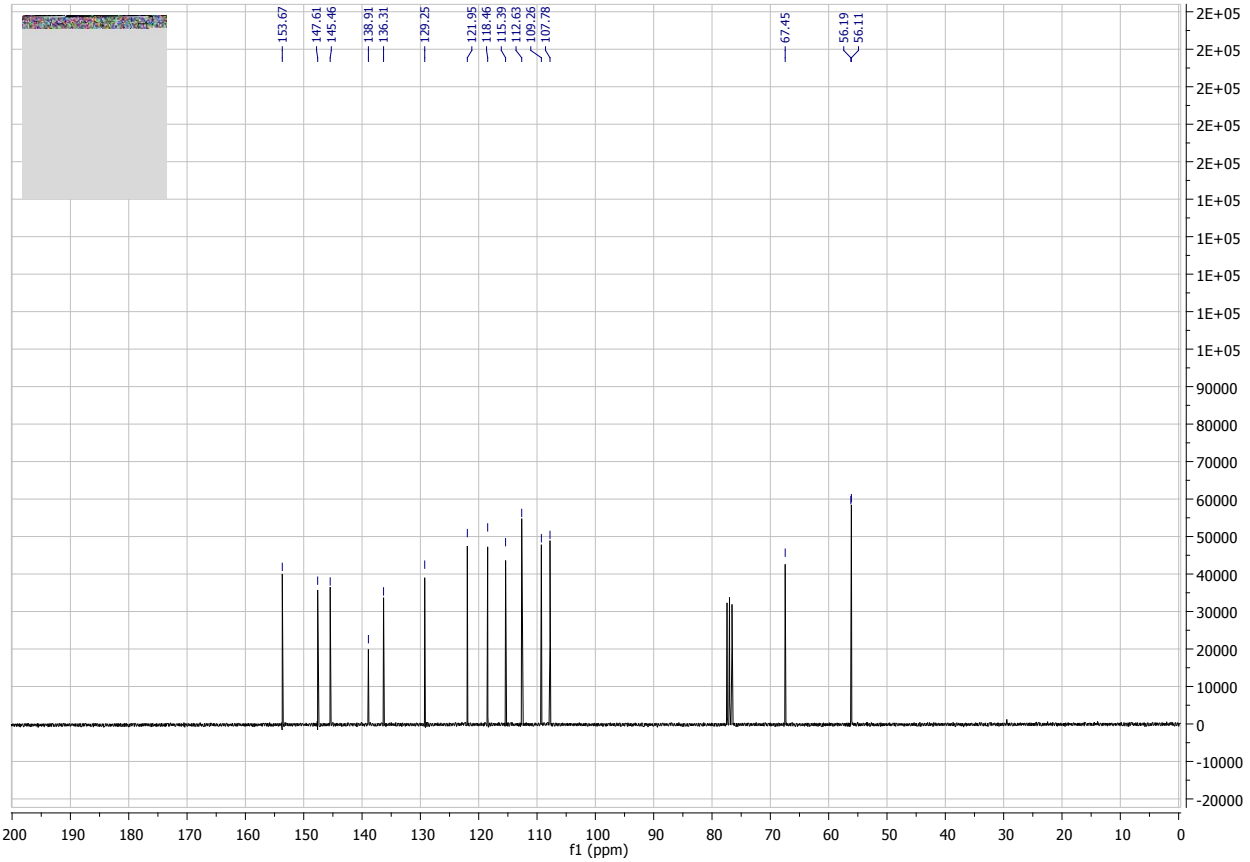
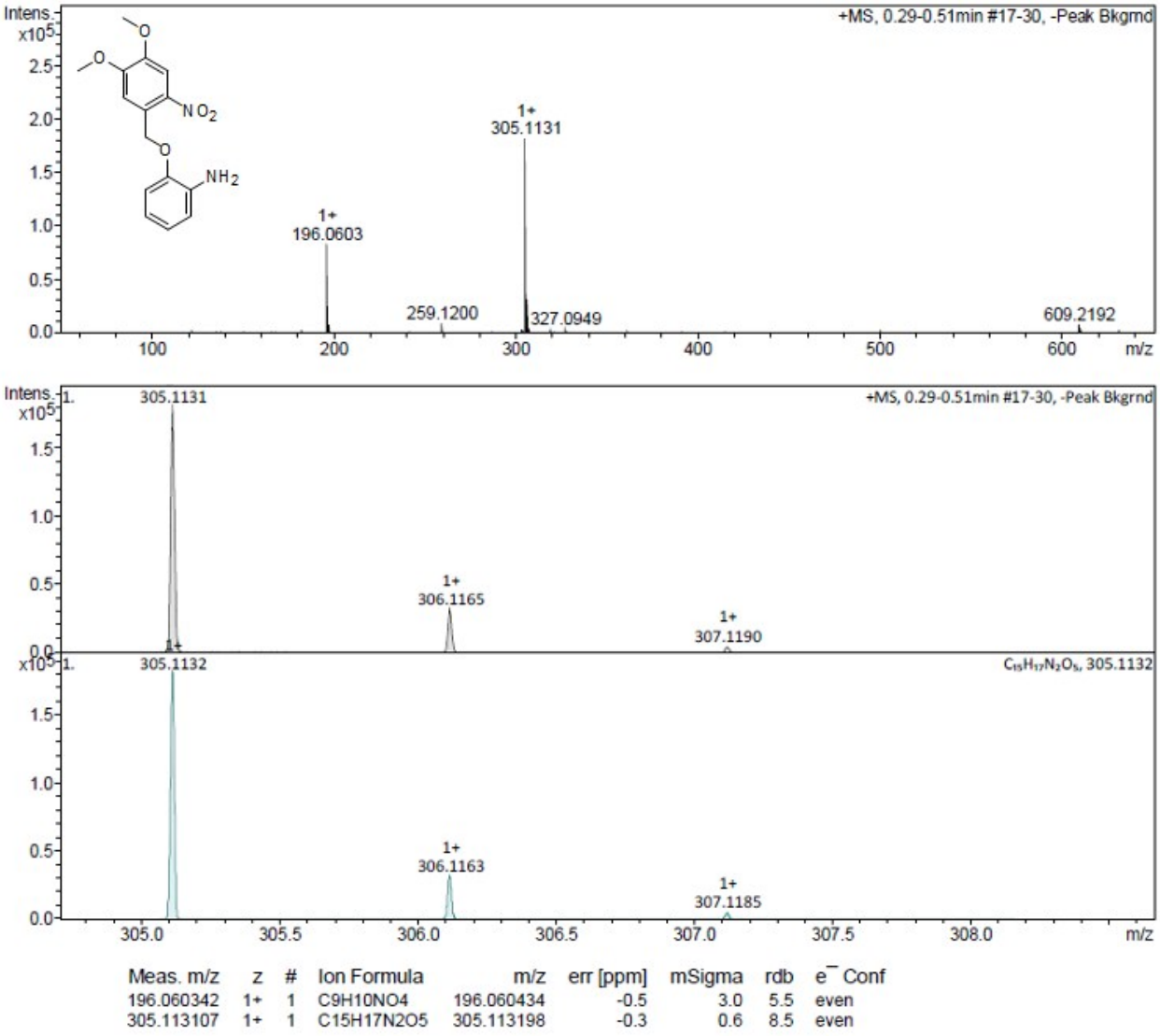


*Figure 40: ^{13}C NMR spectrum of compound **10***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060342	1+	1	C9H10NO4	196.060434	-0.5	3.0	5.5	even
305.113107	1+	1	C15H17N2O5	305.113198	-0.3	0.6	8.5	even

*Figure 41: HRMS spectrum of compound **10***

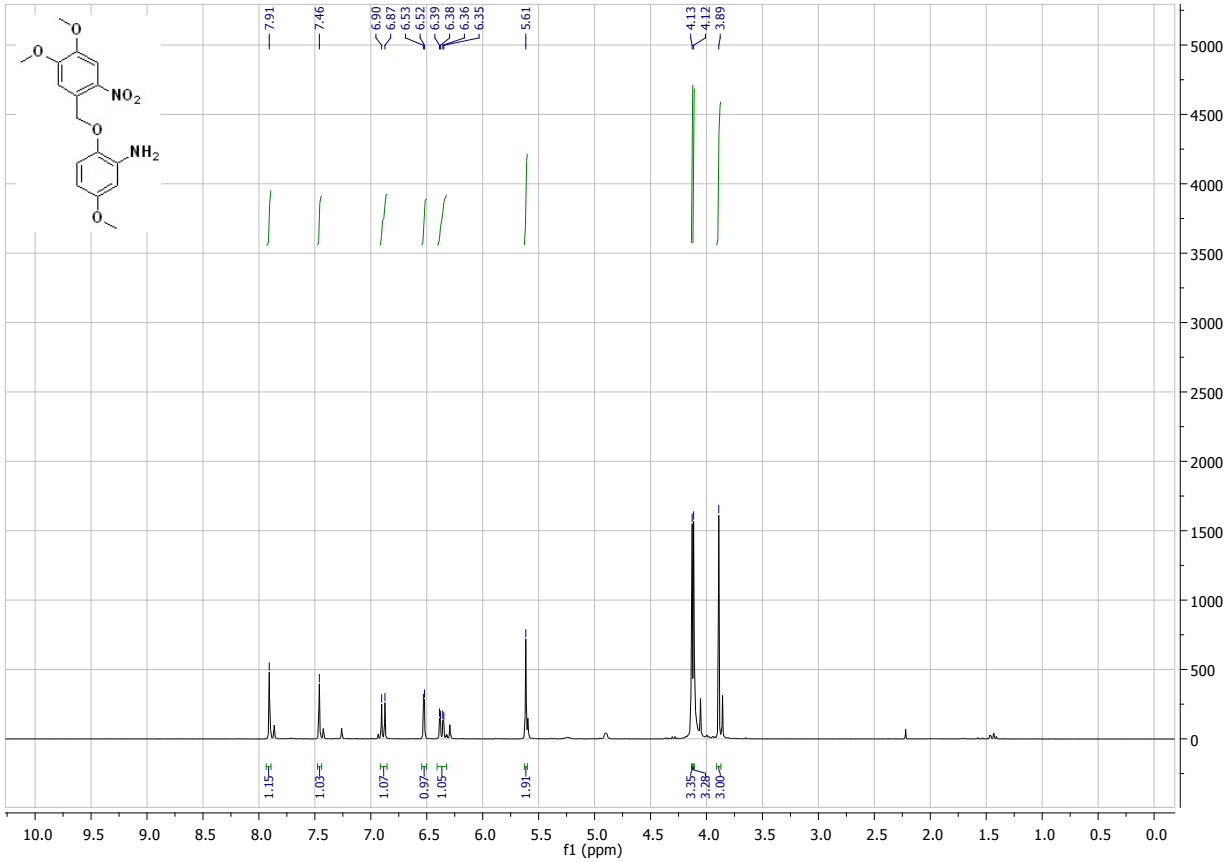


*Figure 42: ^{1}H NMR spectrum of compound **11***

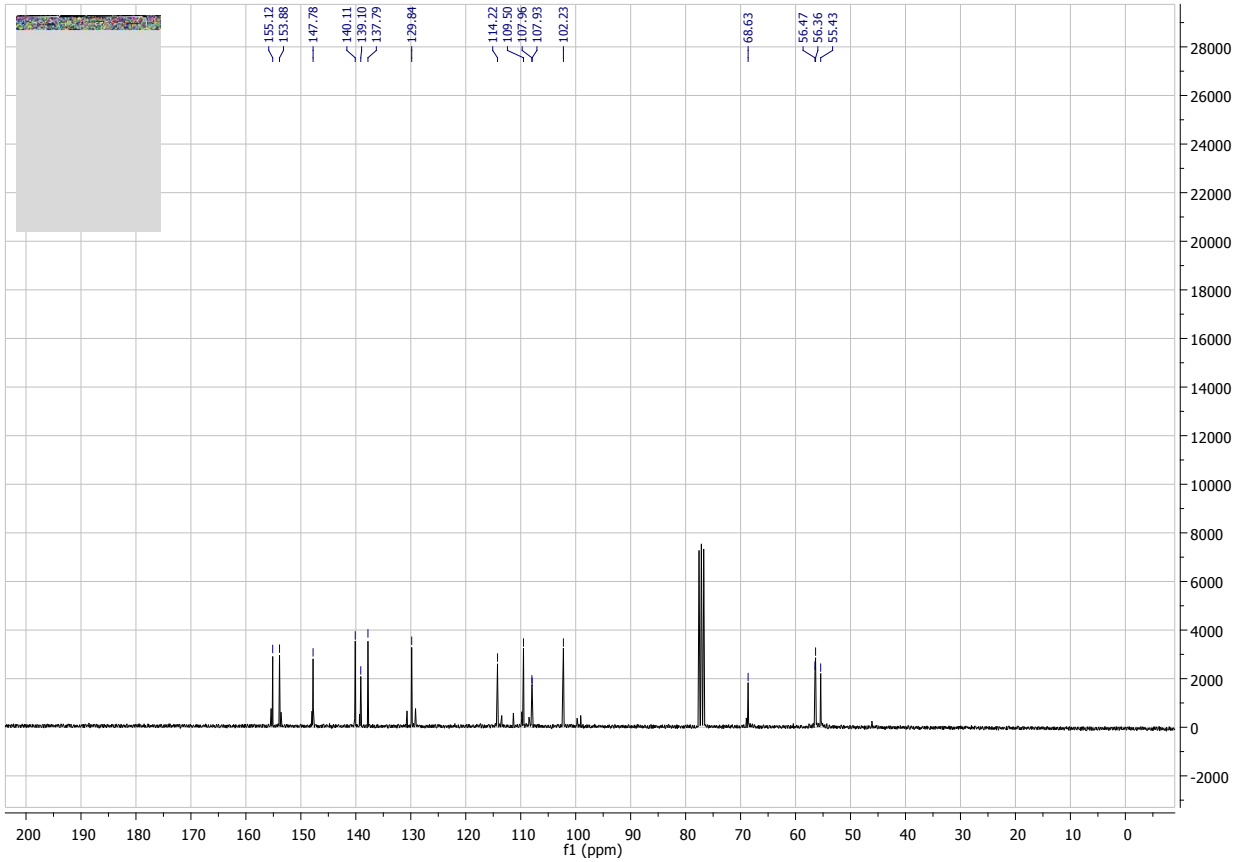
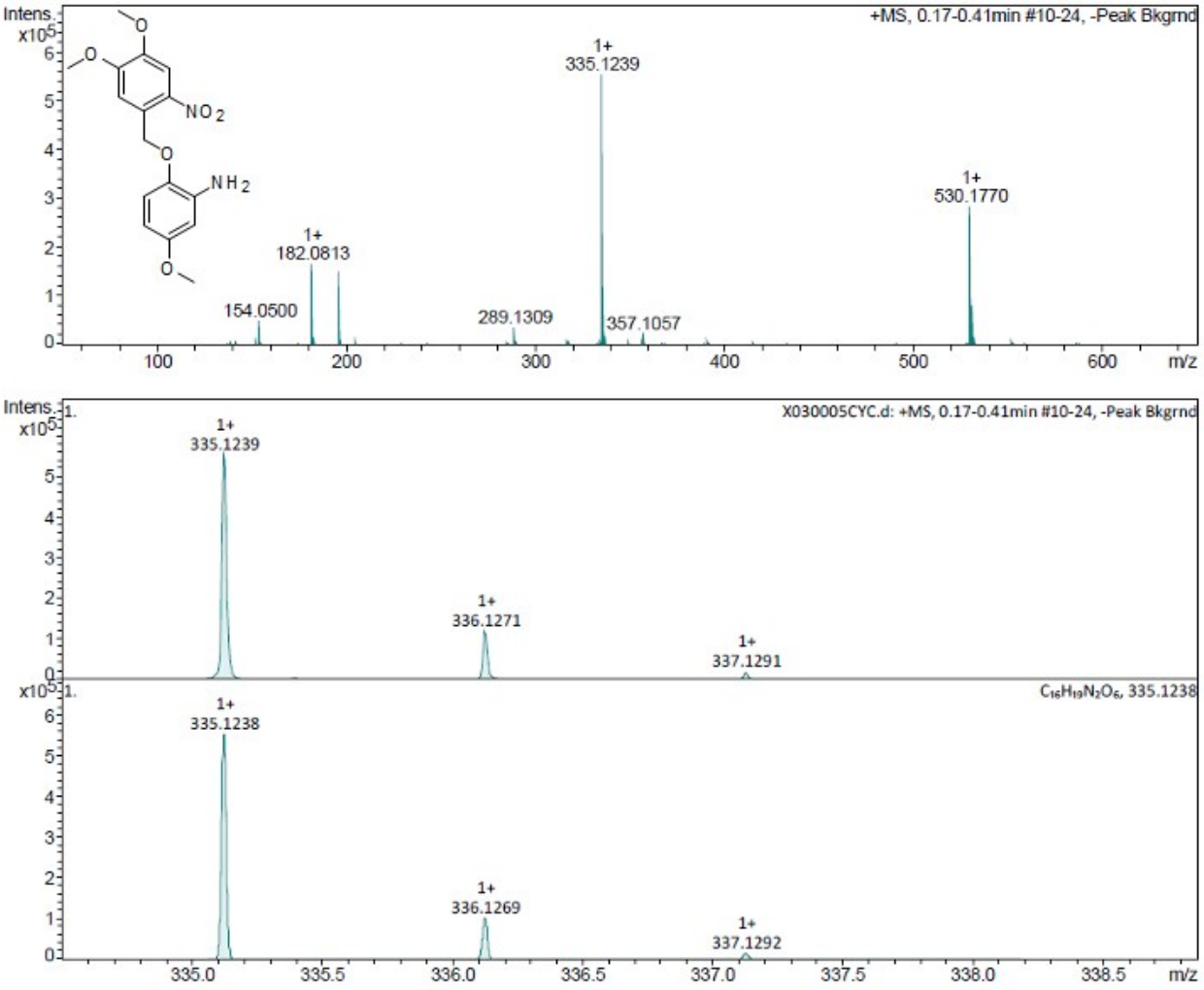


*Figure 43: ^{13}C NMR spectrum of compound **11***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
154.050029	1+	1	C7H8NO3	154.049870	-1.0	5.6	5.0	even
182.081253	1+	1	C9H12NO3	182.081170	-0.5	1.5	5.0	even
196.060590	1+	1	C9H10NO4	196.060434	-0.8	2.4	6.0	even
335.123878	1+	1	C16H19N2O6	335.123763	-0.3	17.2	9.0	even
357.105660	1+	1	C16H18N2NaO6	357.105707	0.1	10.0	9.0	even
530.176979	1+	1	C25H28N3O10	530.176921	-0.1	0.9	14.0	even

*Figure 44: HRMS spectrum of compound **11***

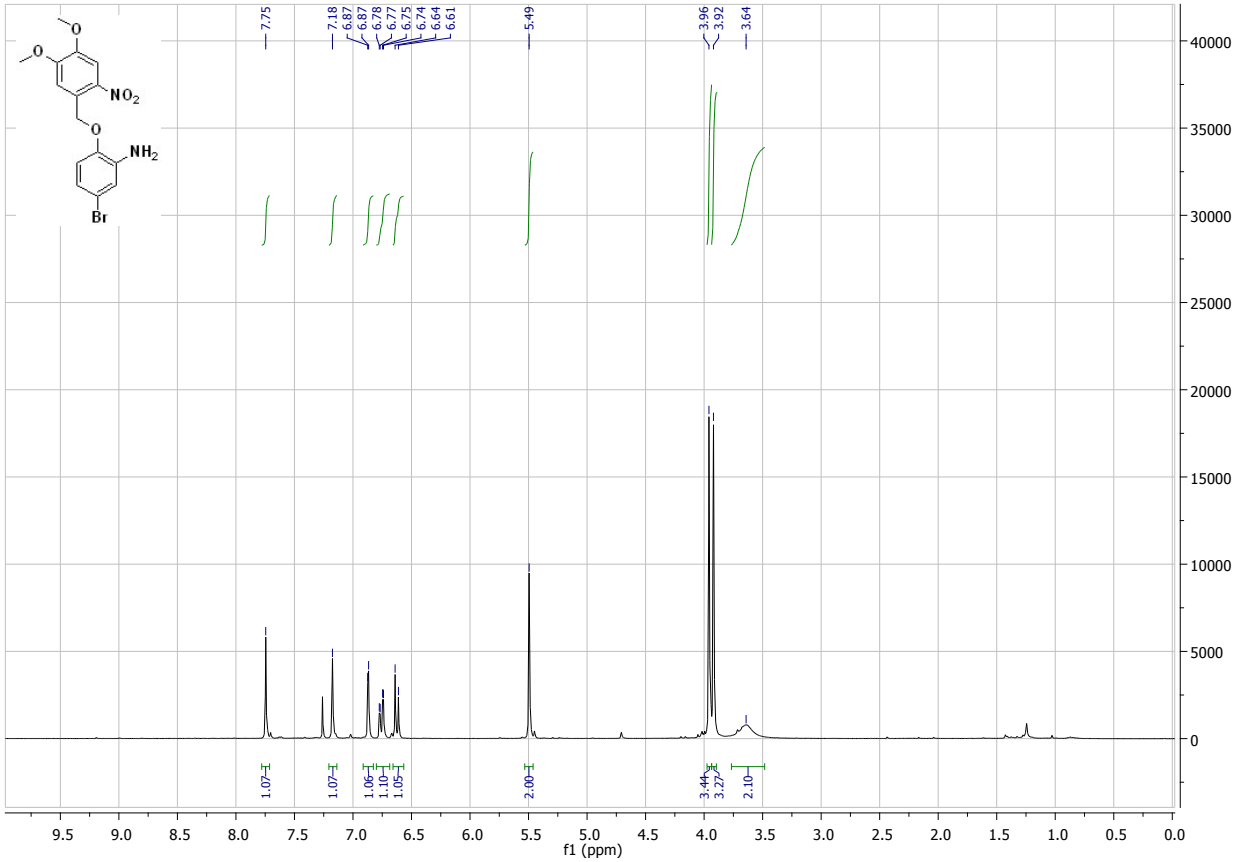


*Figure 45: ^{1}H NMR spectrum of compound **12***

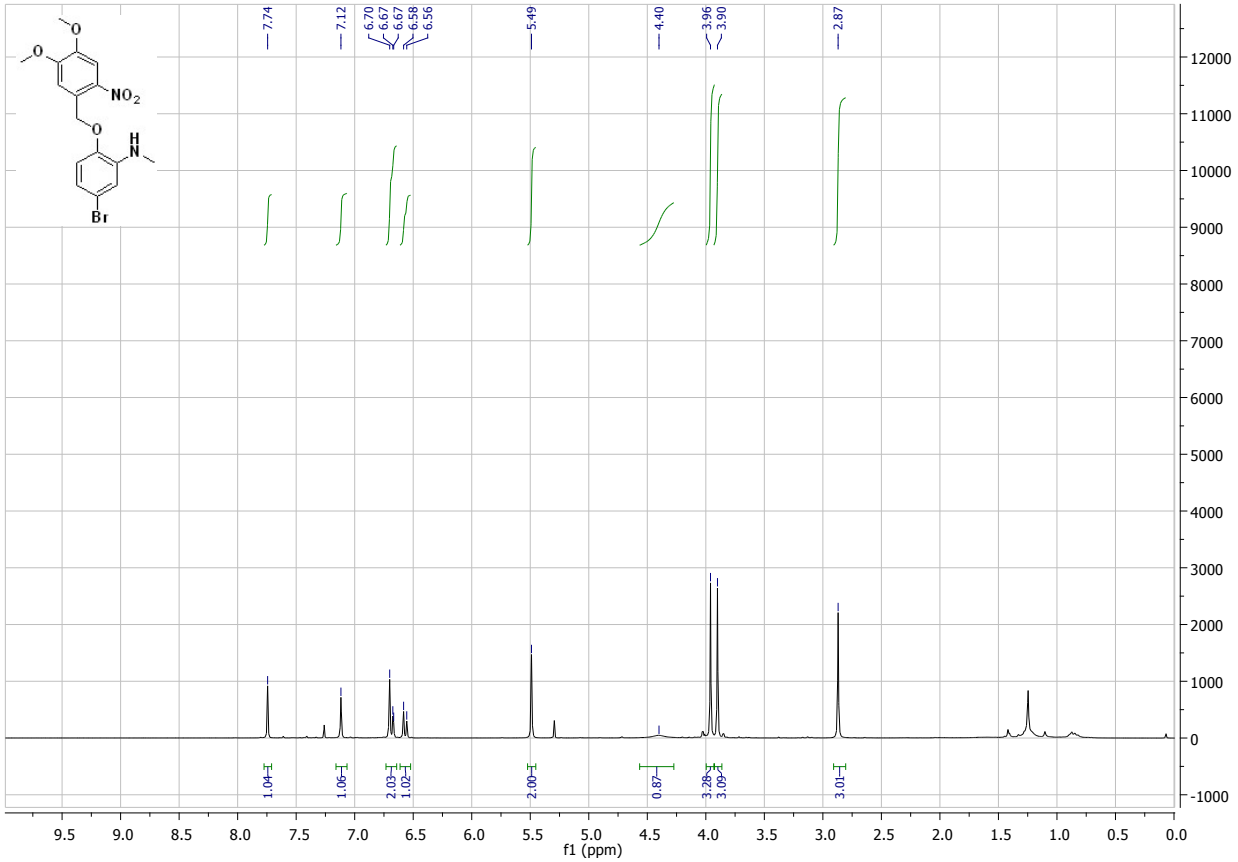
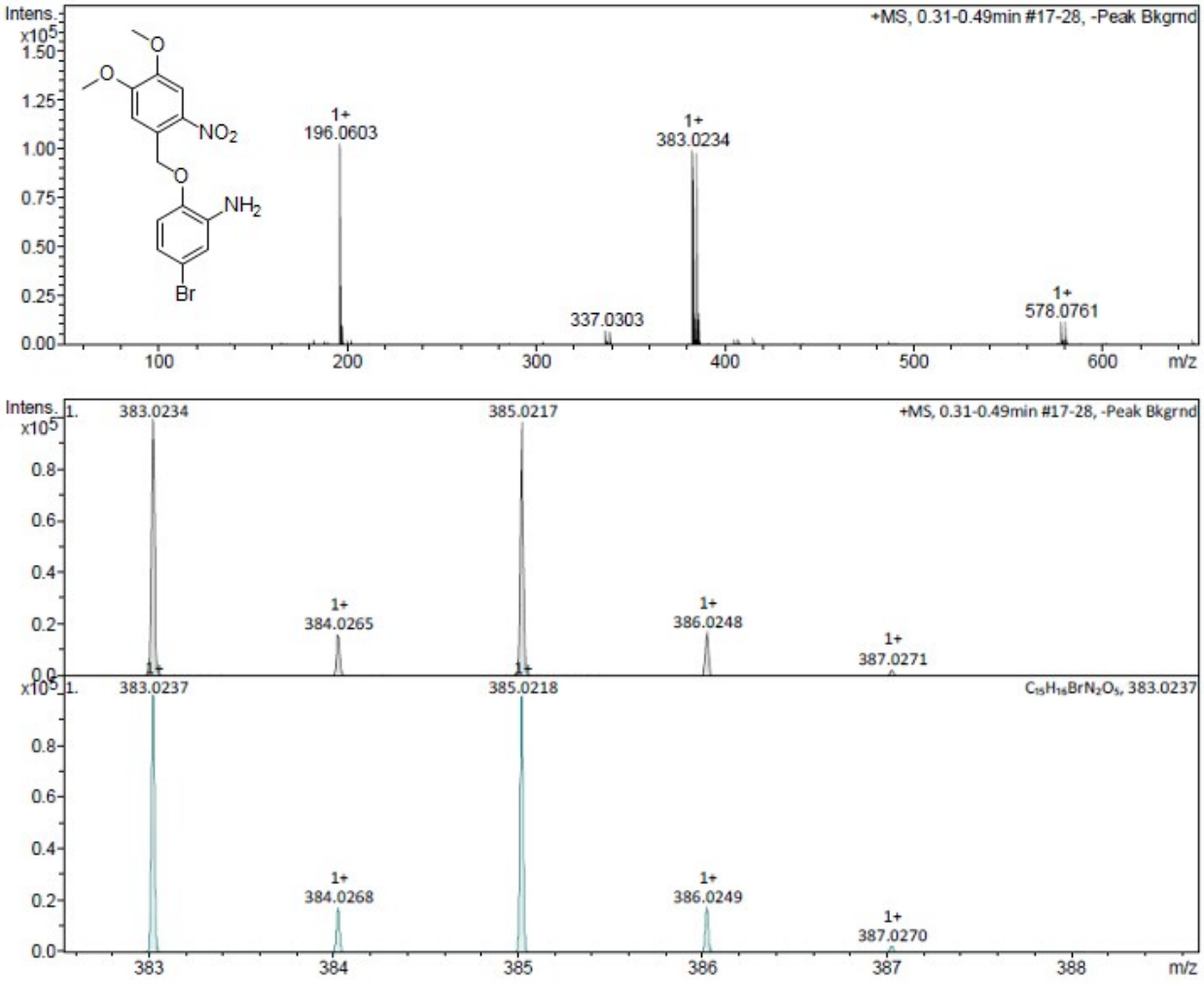


*Figure 46: ^{13}C NMR spectrum of compound **12***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060259	1+	1	C9H10NO4	196.060434	-0.9	2.9	5.5	even
337.030322	1+	1	C13H14BrN4O2	337.029464	-2.5	44.4	8.5	even
383.023369	1+	1	C15H16BrN2O5	383.023710	0.9	8.9	8.5	even
578.076143	1+	1	C21H17BrN13O3	578.075520	-1.1	17.3	19.5	even
	1+	2	C24H25BrN3O9	578.076868	-1.3	19.4	13.5	even
	1+	3	C36H21BrNO2	578.075017	1.9	79.7	26.5	even

*Figure 47: HRMS spectrum of compound **12***

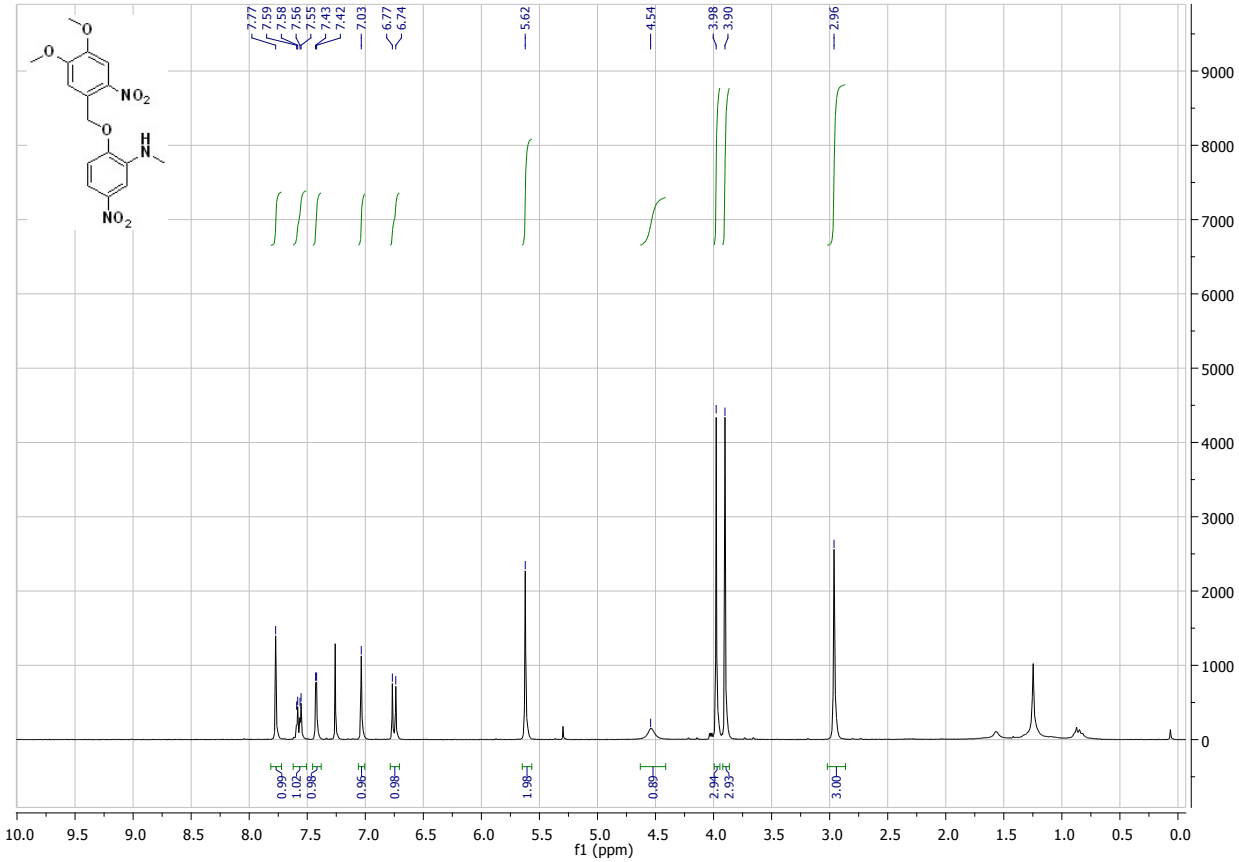


*Figure 48: ^{1}H NMR spectrum of compound **13***

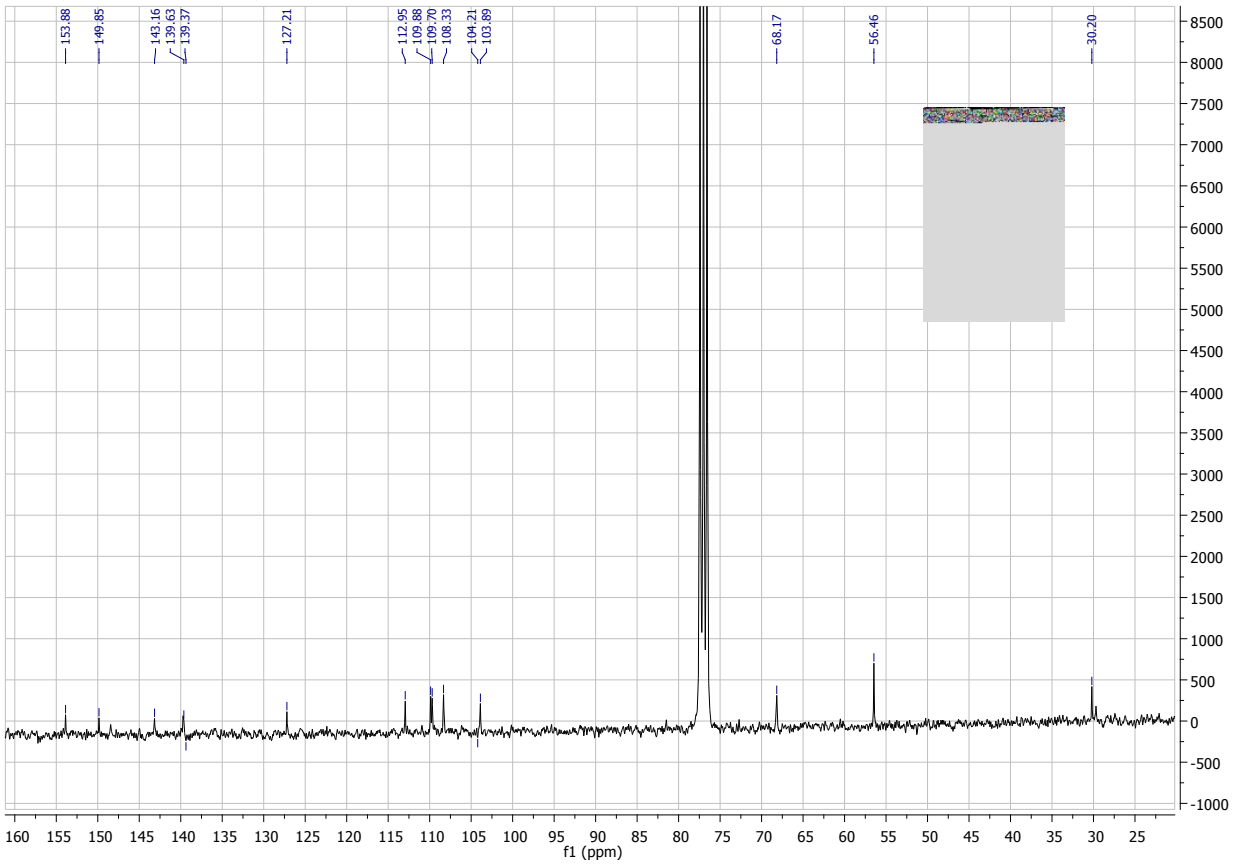
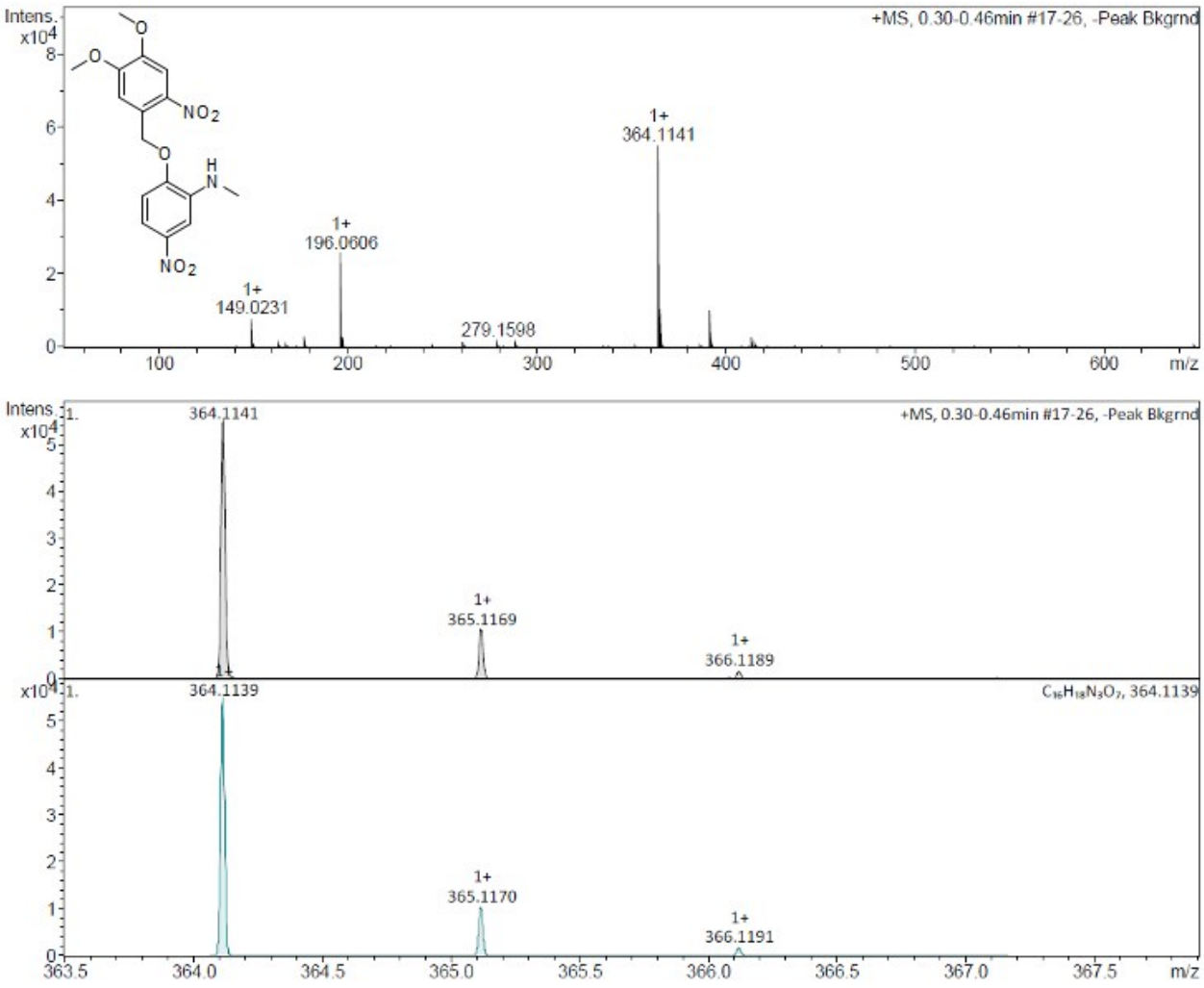


*Figure 49: ^{13}C NMR spectrum of compound **13***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
149.023104	1+	1	C8H5O3	149.023320	-1.5	14.4	6.5	even
177.054501	1+	1	C10H9O3	177.054621	0.7	8.1	6.5	even
196.060604	1+	1	C9H10NO4	196.060434	-0.9	0.8	5.5	even
364.114066	1+	1	C16H18N3O7	364.113926	-0.4	0.4	9.5	even
391.284912	1+	1	C24H39O4	391.284286	-1.6	16.0	5.5	even

*Figure 50: HRMS spectrum of compound **13***

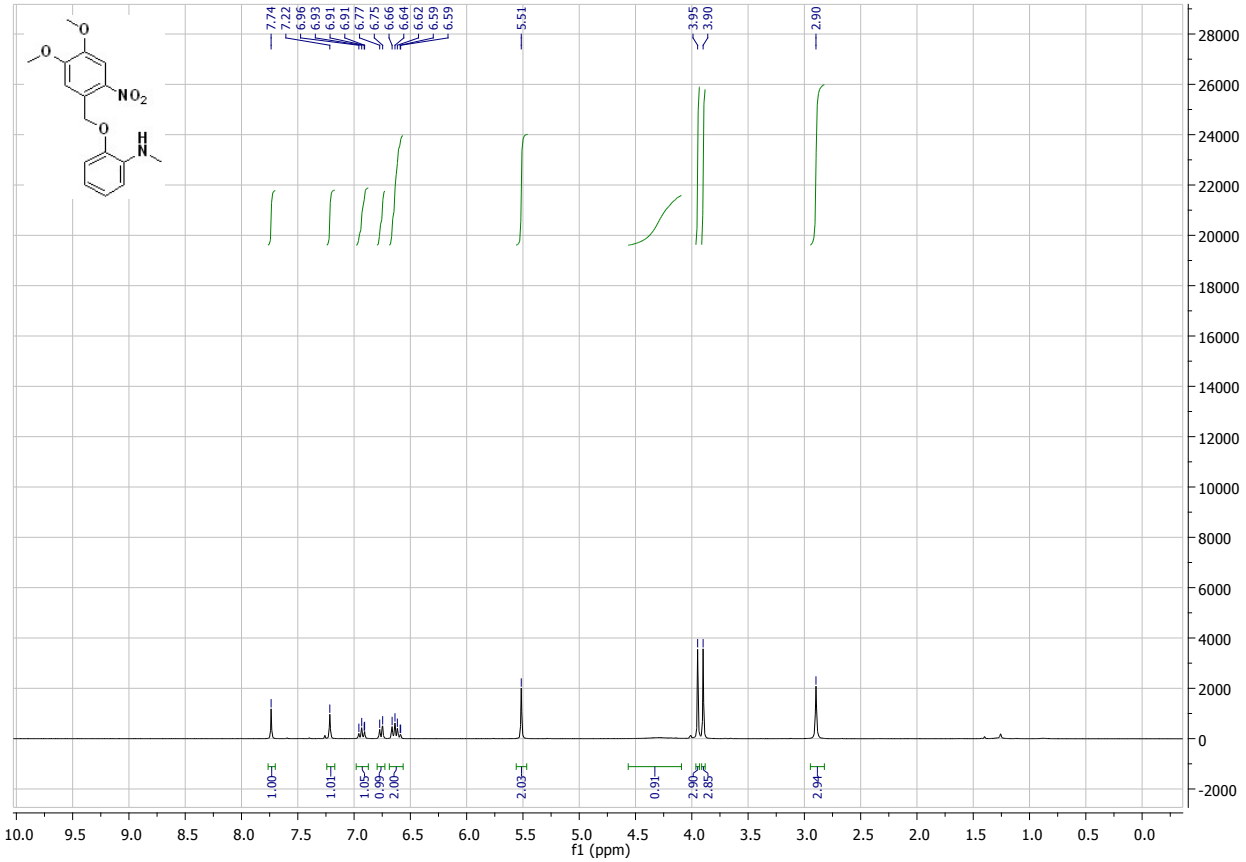


*Figure 51: ^{1}H NMR spectrum of compound **14***

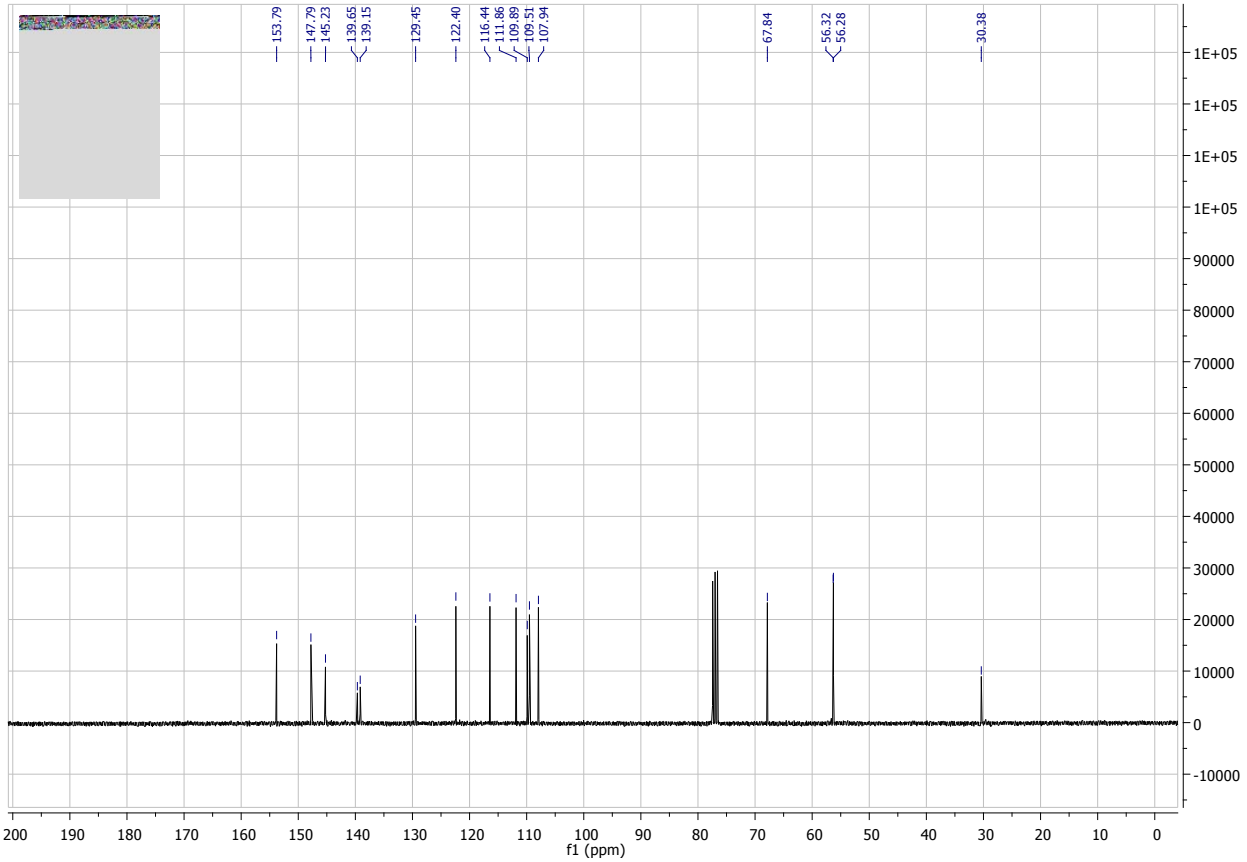
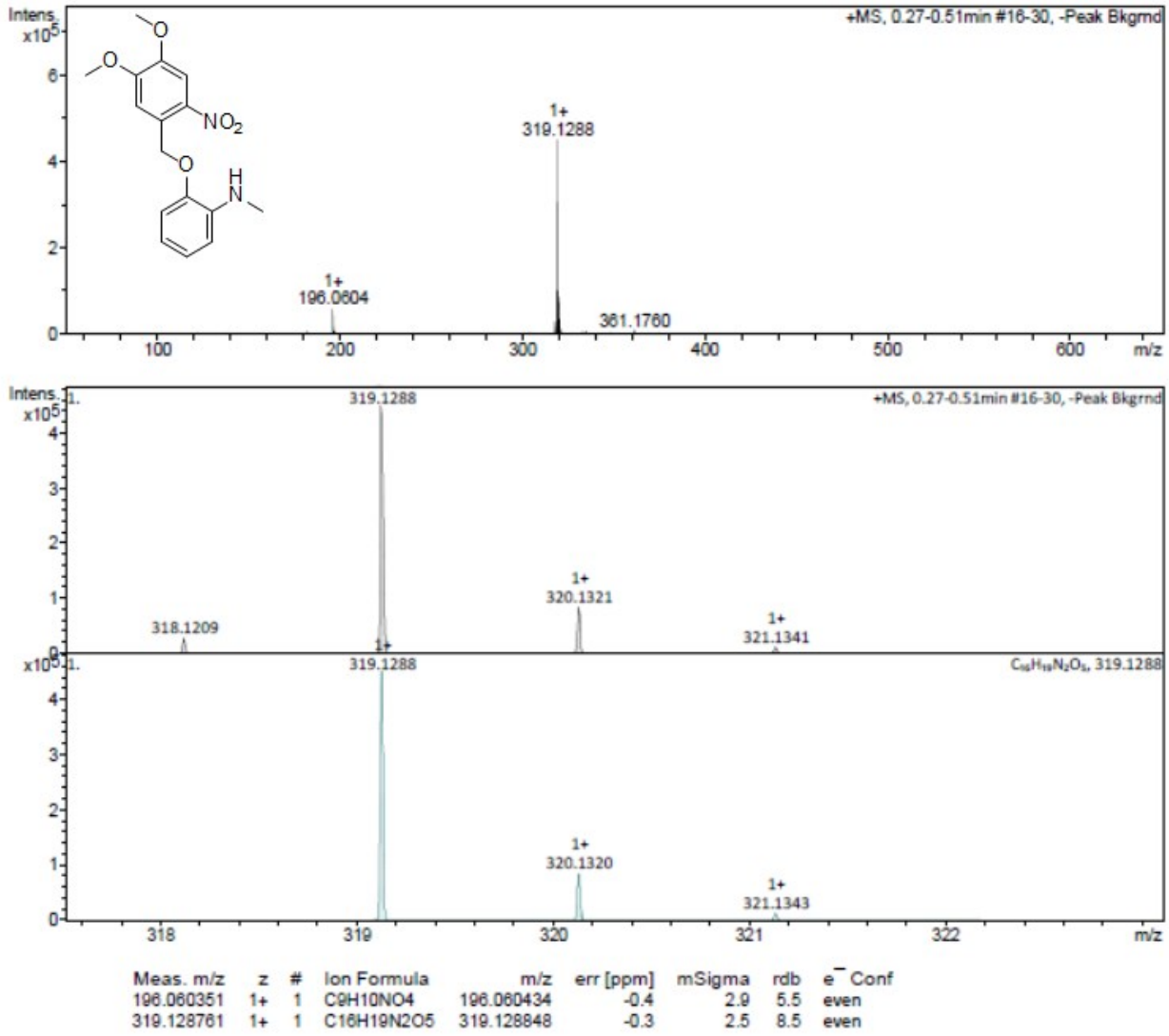


*Figure 52: ^{13}C NMR of compound **14***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060351	1+	1	C9H10NO4	196.060434	-0.4	2.9	5.5	even
319.128761	1+	1	C16H19N2O5	319.128848	-0.3	2.5	8.5	even

*Figure 53: HRMS spectrum of compound **14***

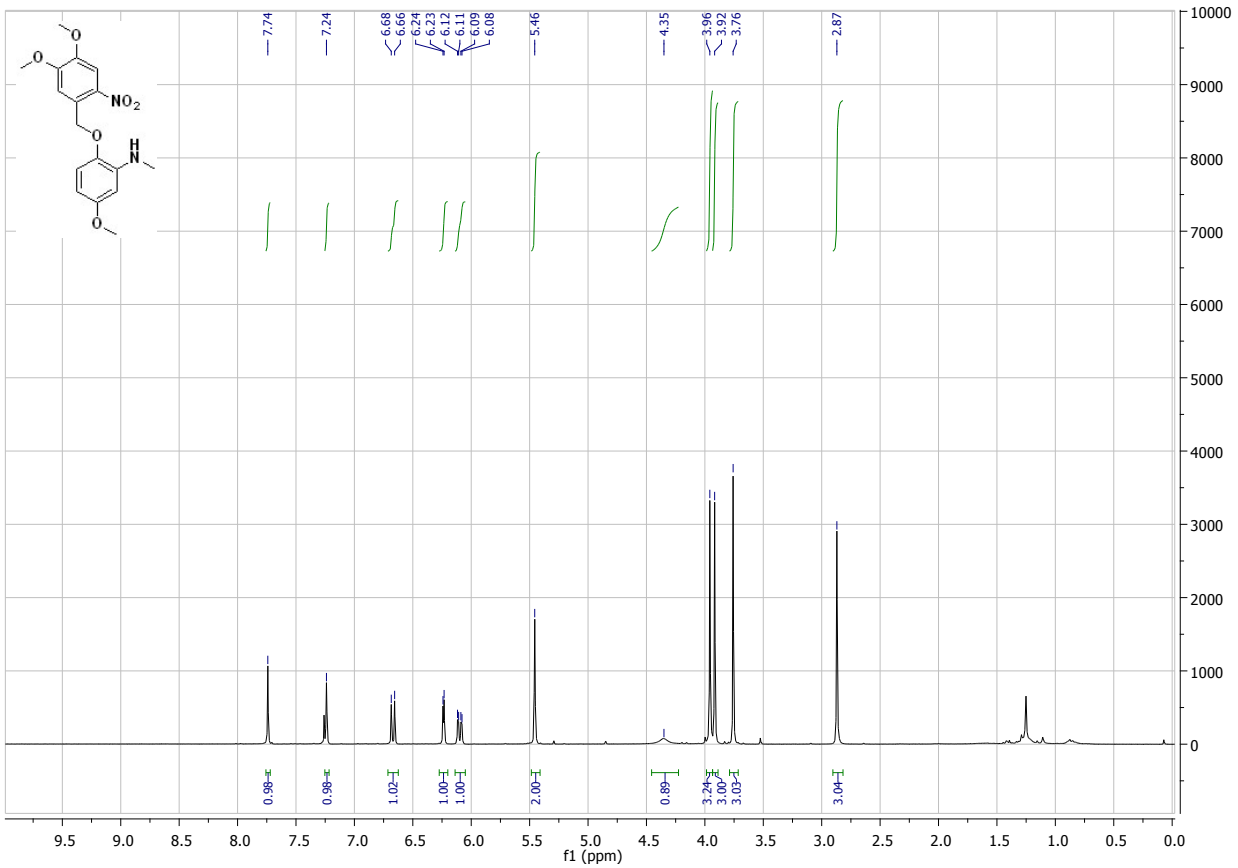


*Figure 54: ^{1}H NMR spectrum of compound **15***

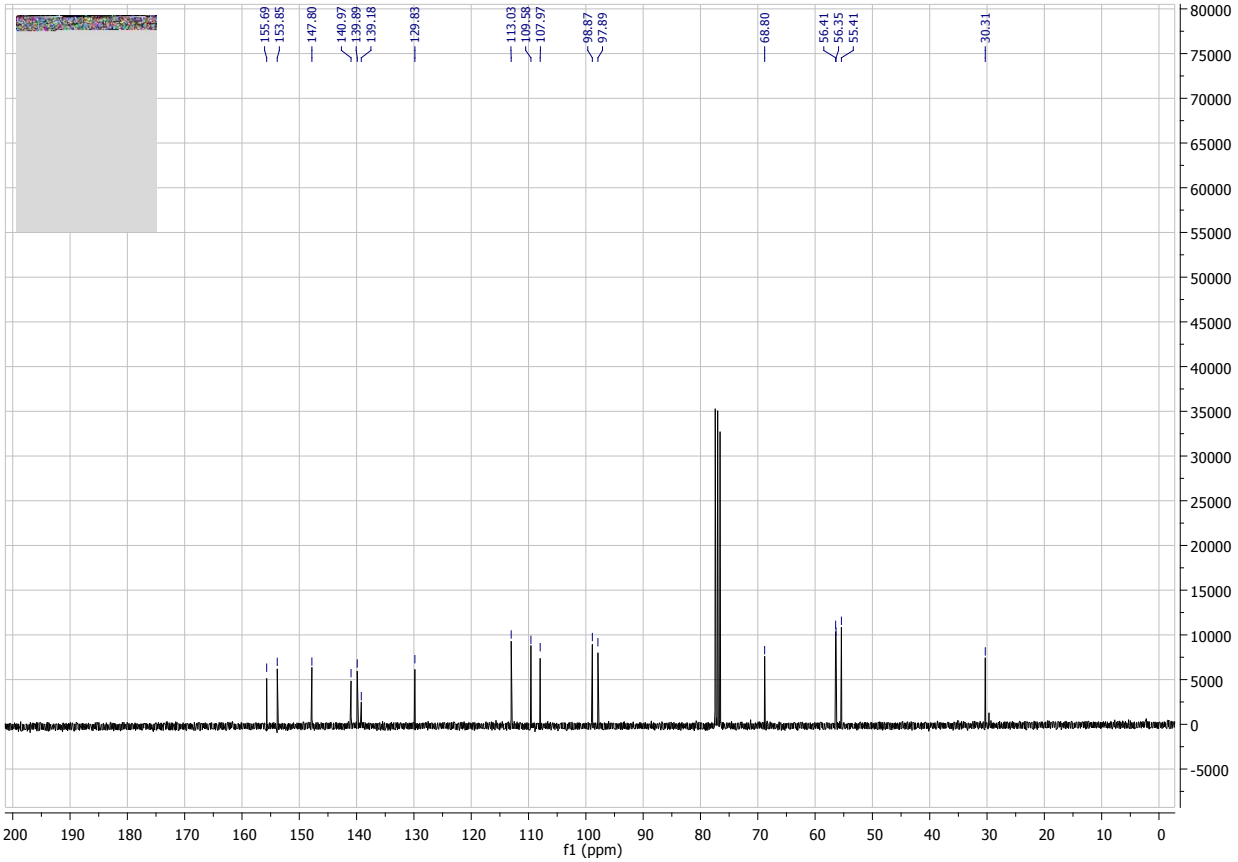
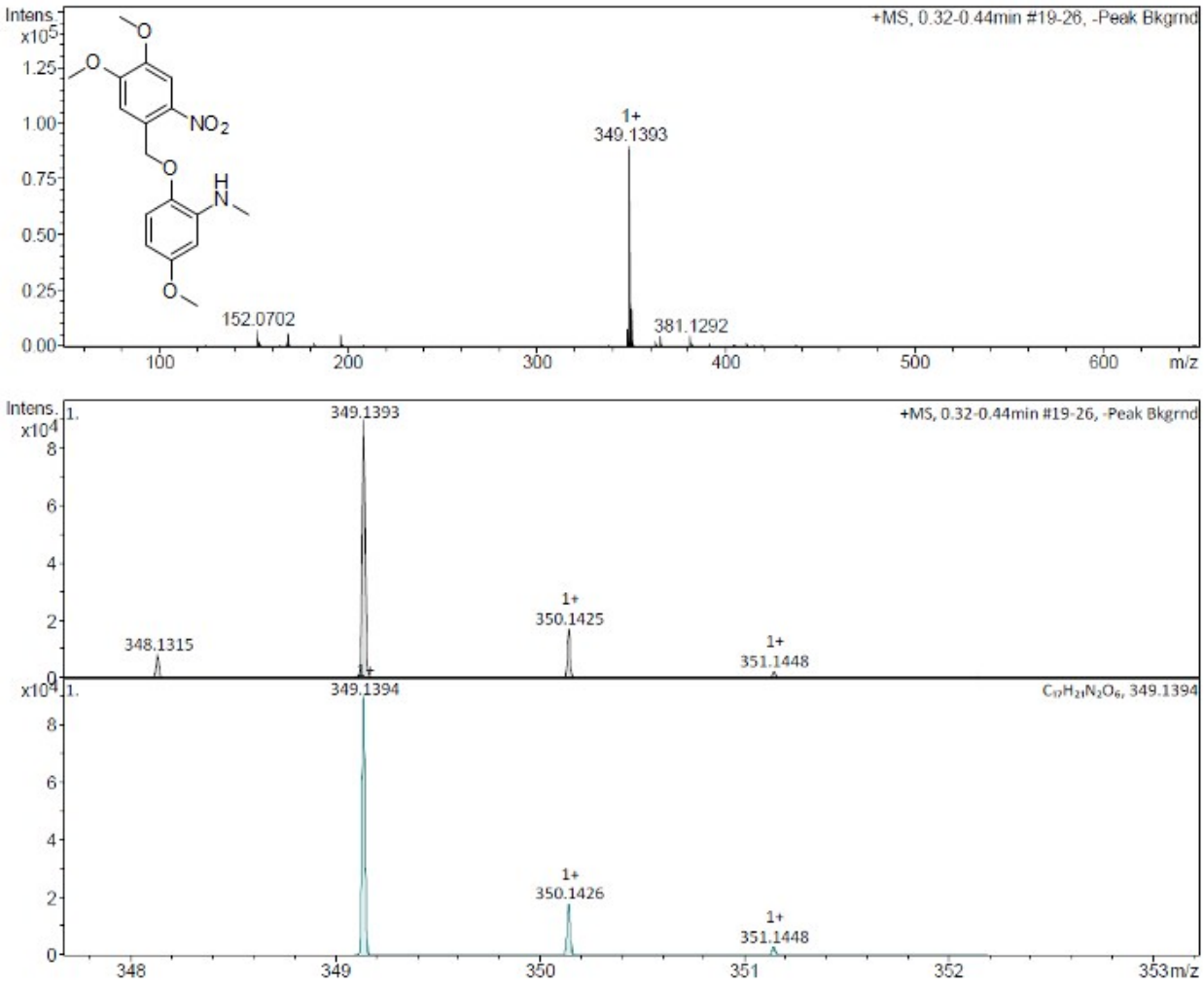


*Figure 55: ^{13}C NMR spectrum of compound **15***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
152.070198	1+	1	C8H10NO2	152.070605	-2.7	84.1	4.5	even
168.065340	1+	1	C8H10NO3	168.065520	1.1	29.8	4.5	even
196.060440	1+	1	C9H10NO4	196.060434	-0.0	21.4	5.5	even
349.139290	1+	1	C17H21N2O6	349.139413	-0.4	4.2	8.5	even
365.134710	1+	1	C17H21N2O7	365.134327	-1.0	6.1	8.5	even
	1+	2	C18H17N6O3	365.135665	-2.6	7.5	13.5	even
381.129158	1+	1	C17H21N2O8	381.129242	-0.2	9.7	8.5	even

*Figure 56: HMRS spectrum of compound **15***

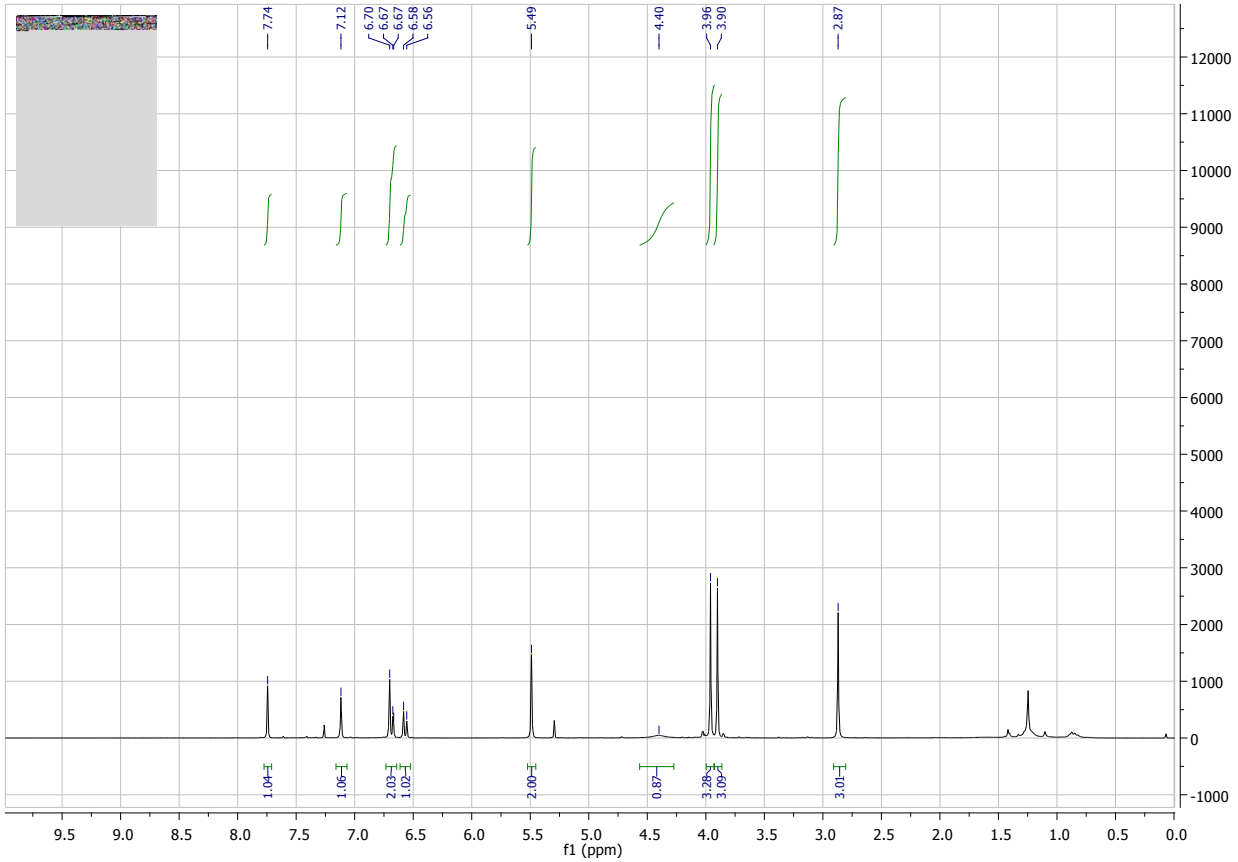


*Figure 57: ^{1}H NMR spectrum of compound **16***

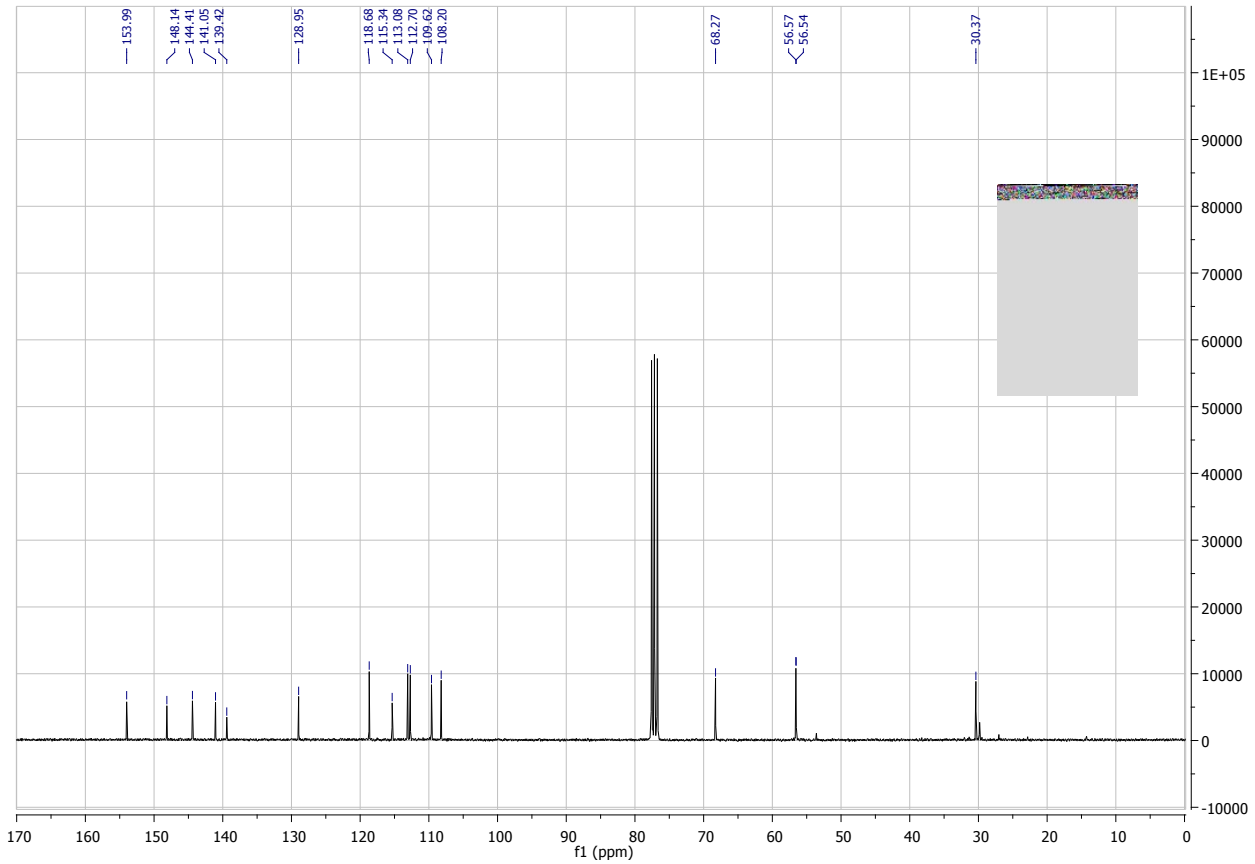
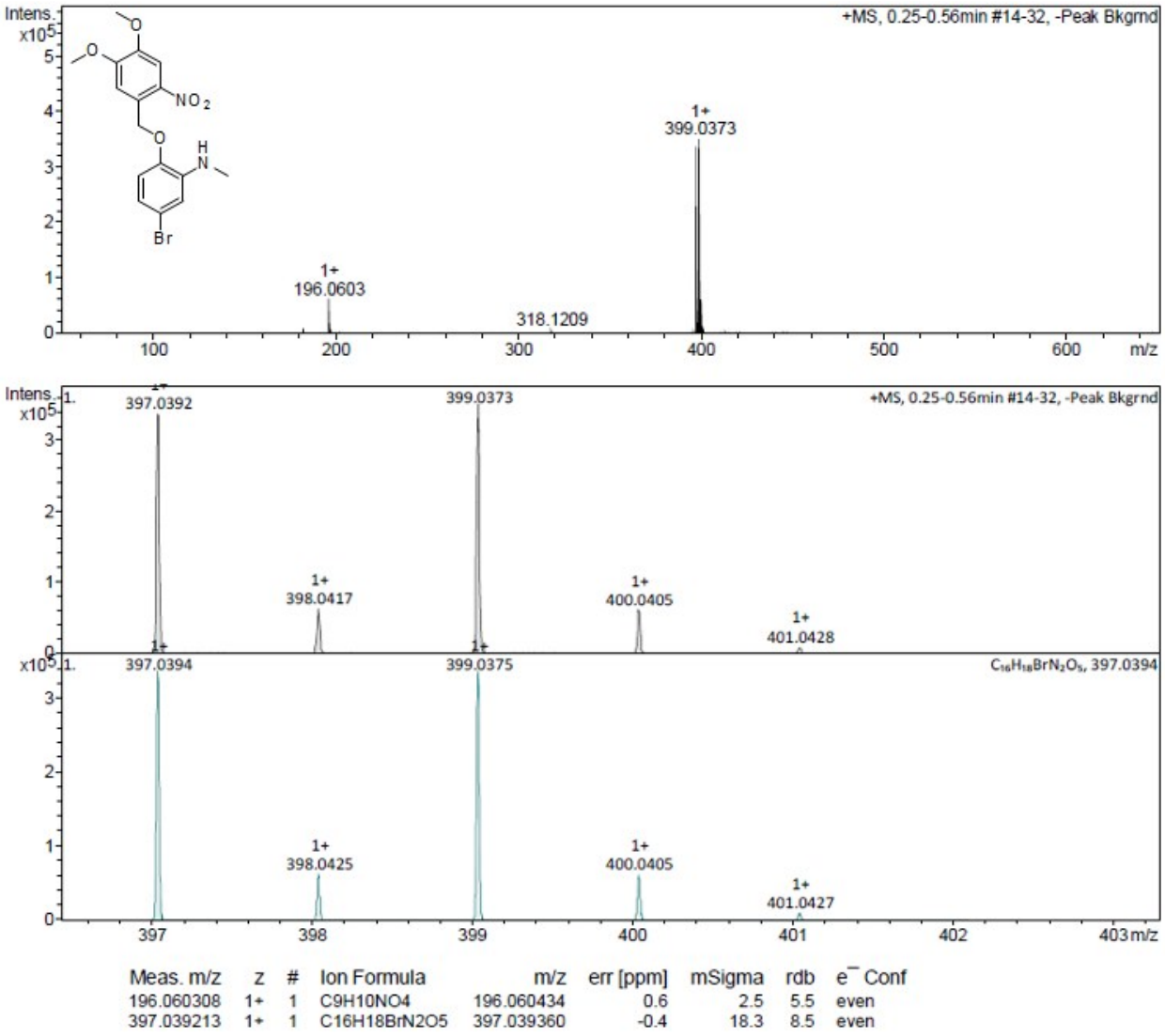


*Figure 58: ^{13}C NMR spectrum of compound **16***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
196.060308	1+	1	C9H10NO4	196.060434	0.6	2.5	5.5	even
397.039213	1+	1	C16H18BrN2O5	397.039360	-0.4	18.3	8.5	even

*Figure 59: HRMS spectrum of compound **16***

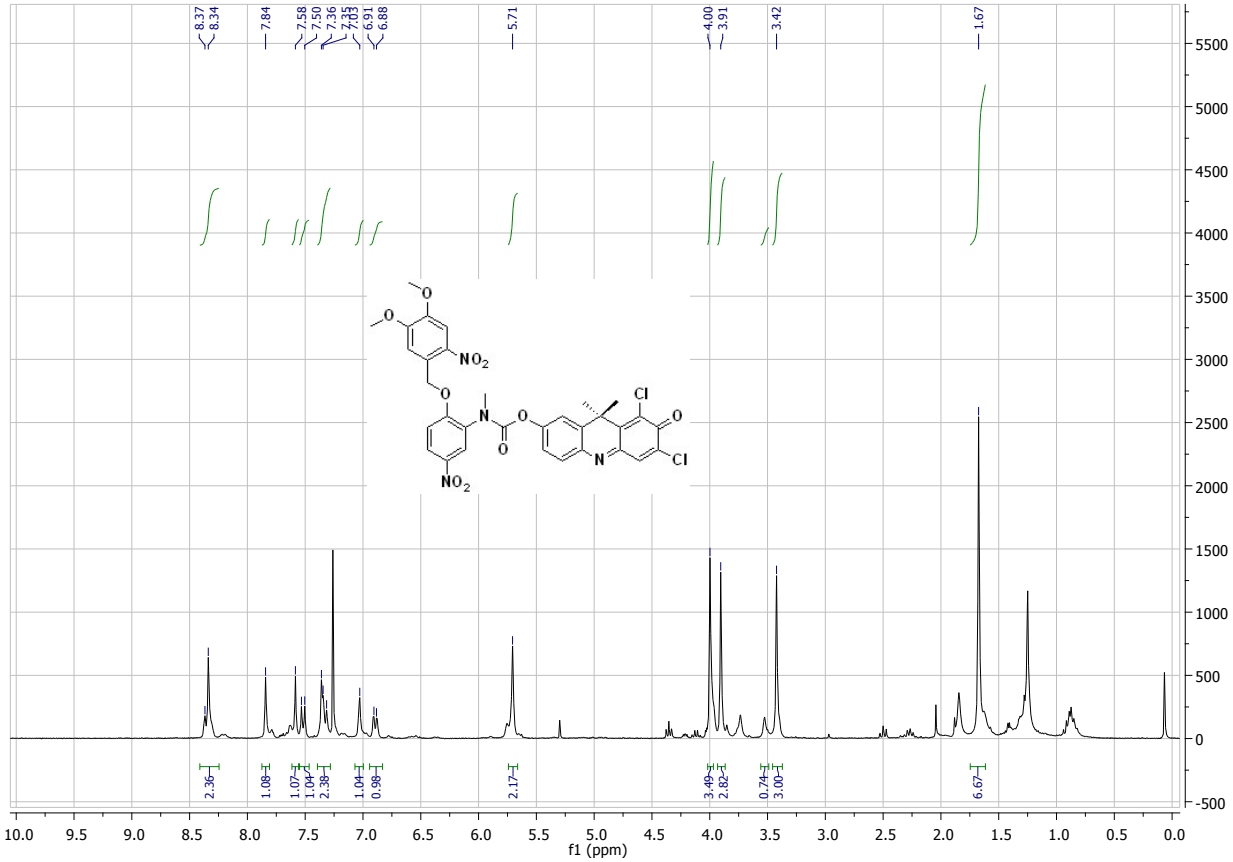


*Figure 60: ^{1}H NMR spectrum of compound **2-HPC^{NO2}***

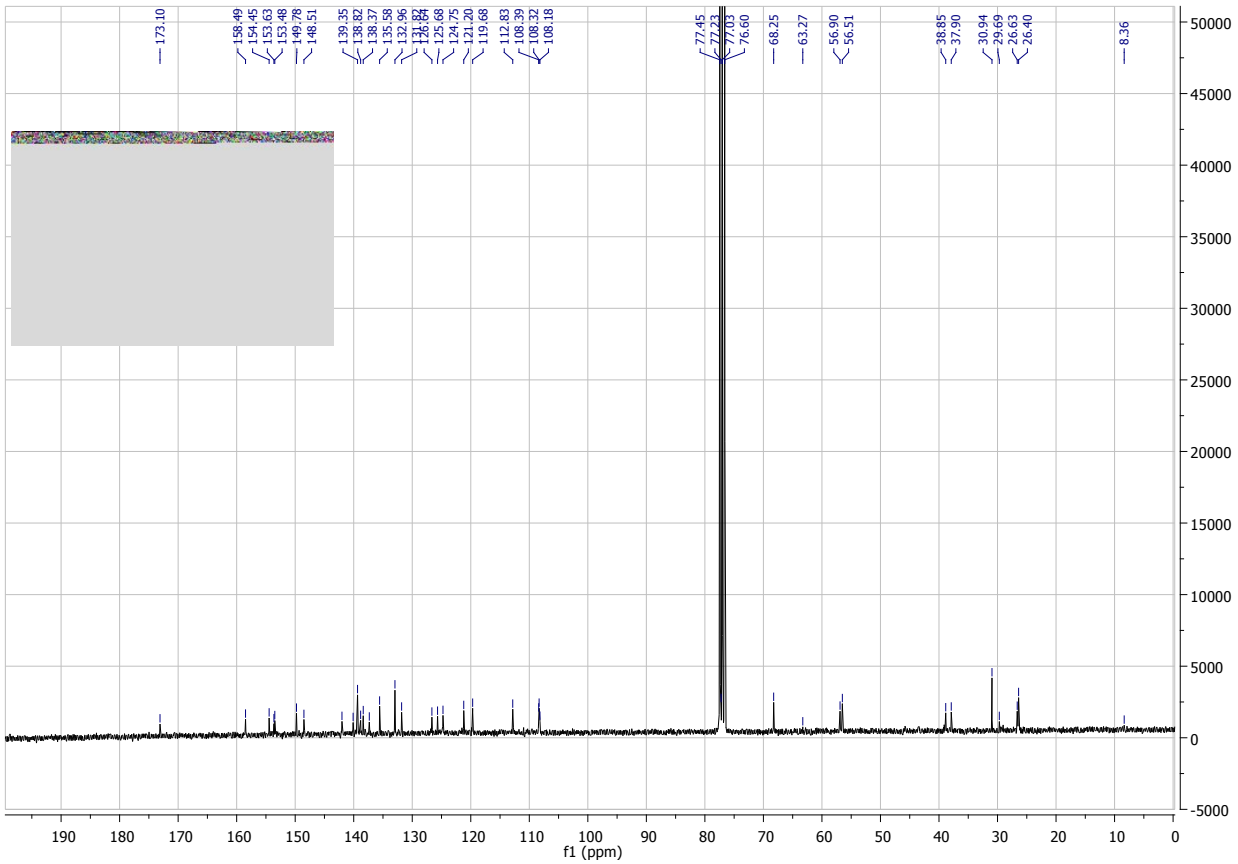
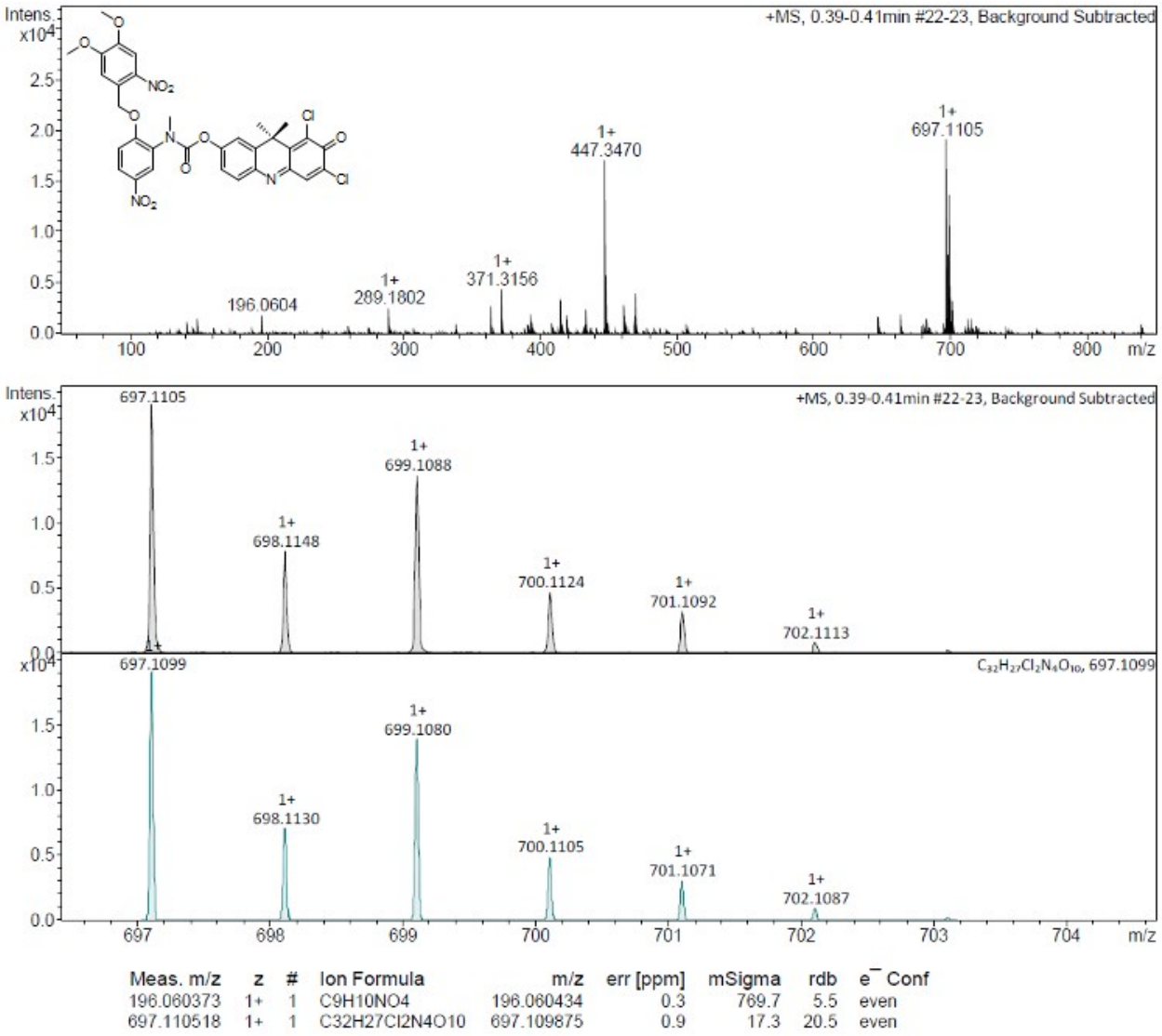


*Figure 61: ^{13}C NMR spectrum of compound **2-HPC^{NO2}***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
196.060373	1+	1	C9H10NO4	196.060434	0.3	769.7	5.5	even
697.110518	1+	1	C32H27Cl2N4O10	697.109875	0.9	17.3	20.5	even

*Figure 62: HRMS spectrum of compound **2-HPC^{NO2}***

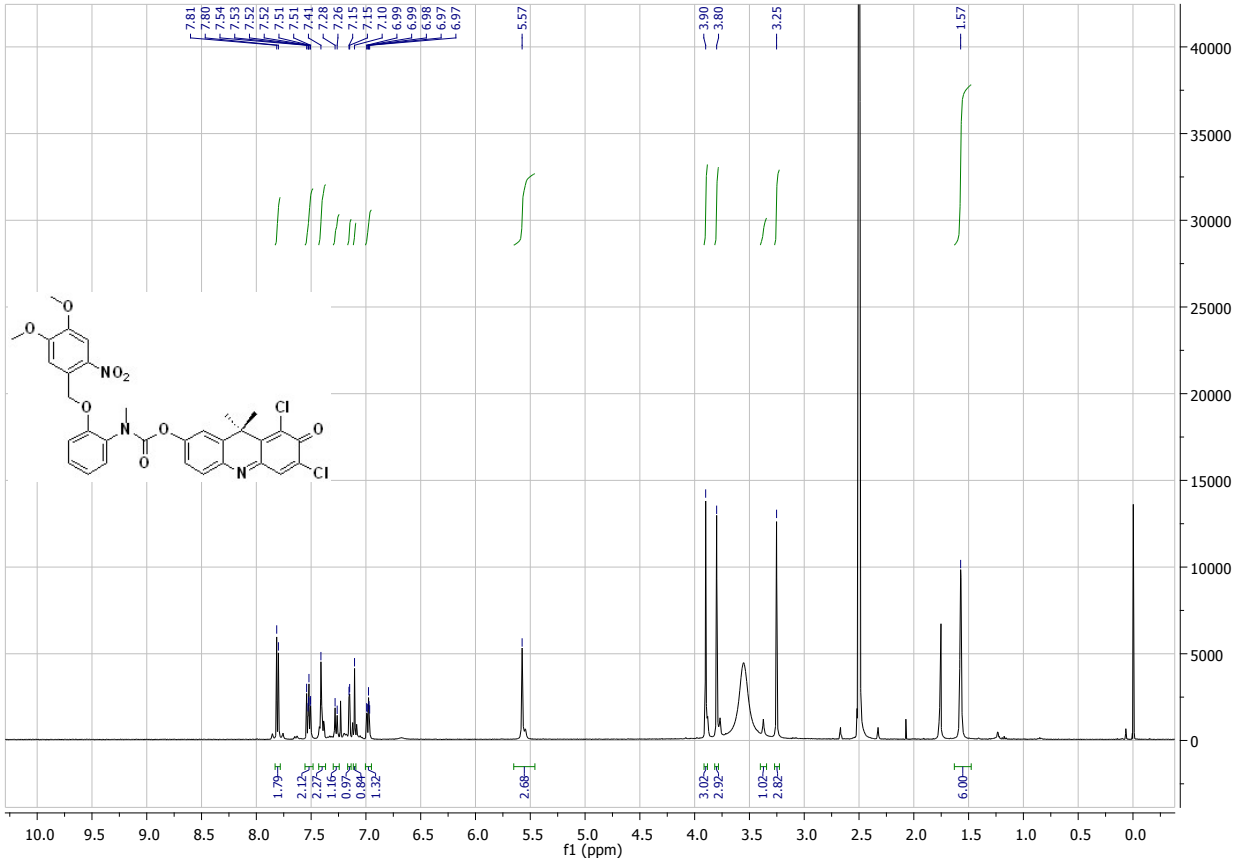


*Figure 63: ^{1}H NMR spectrum of compound **2-HPC^{H}***

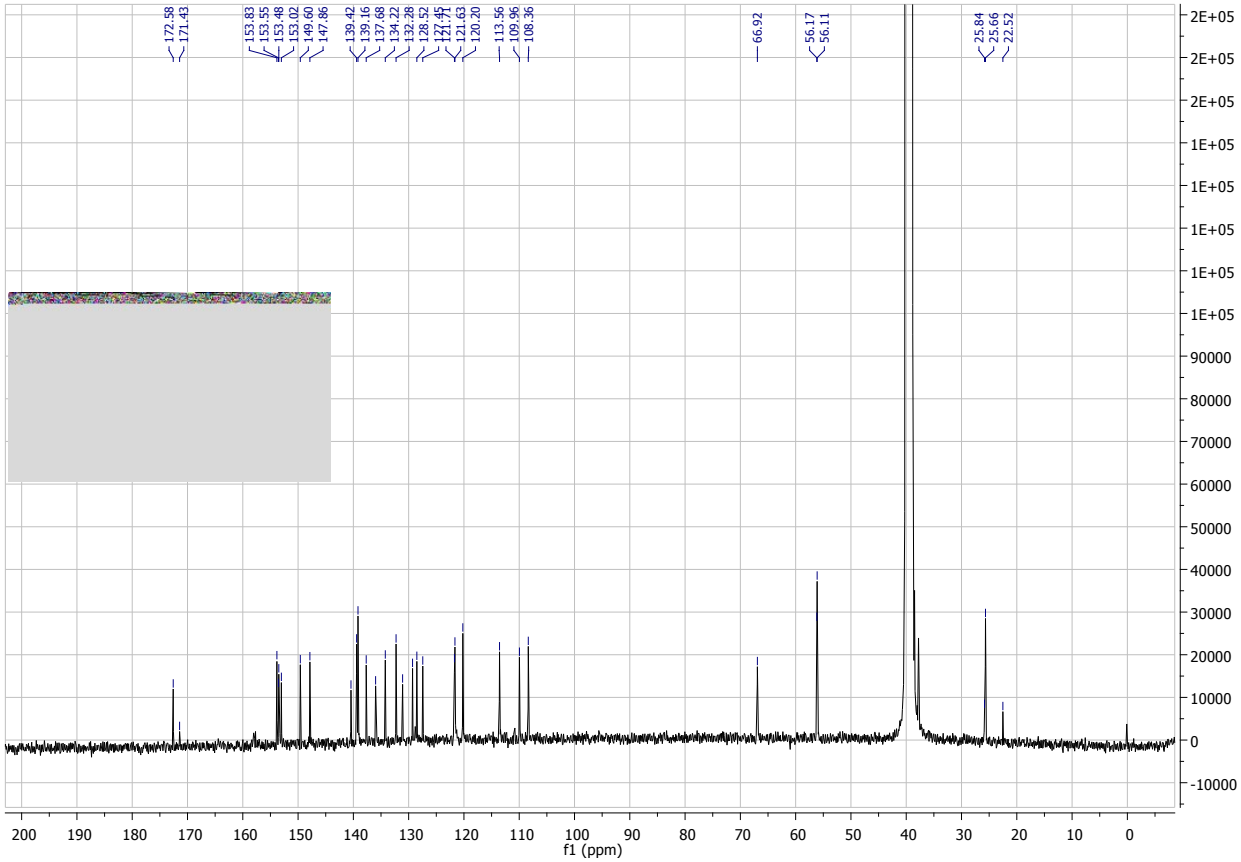
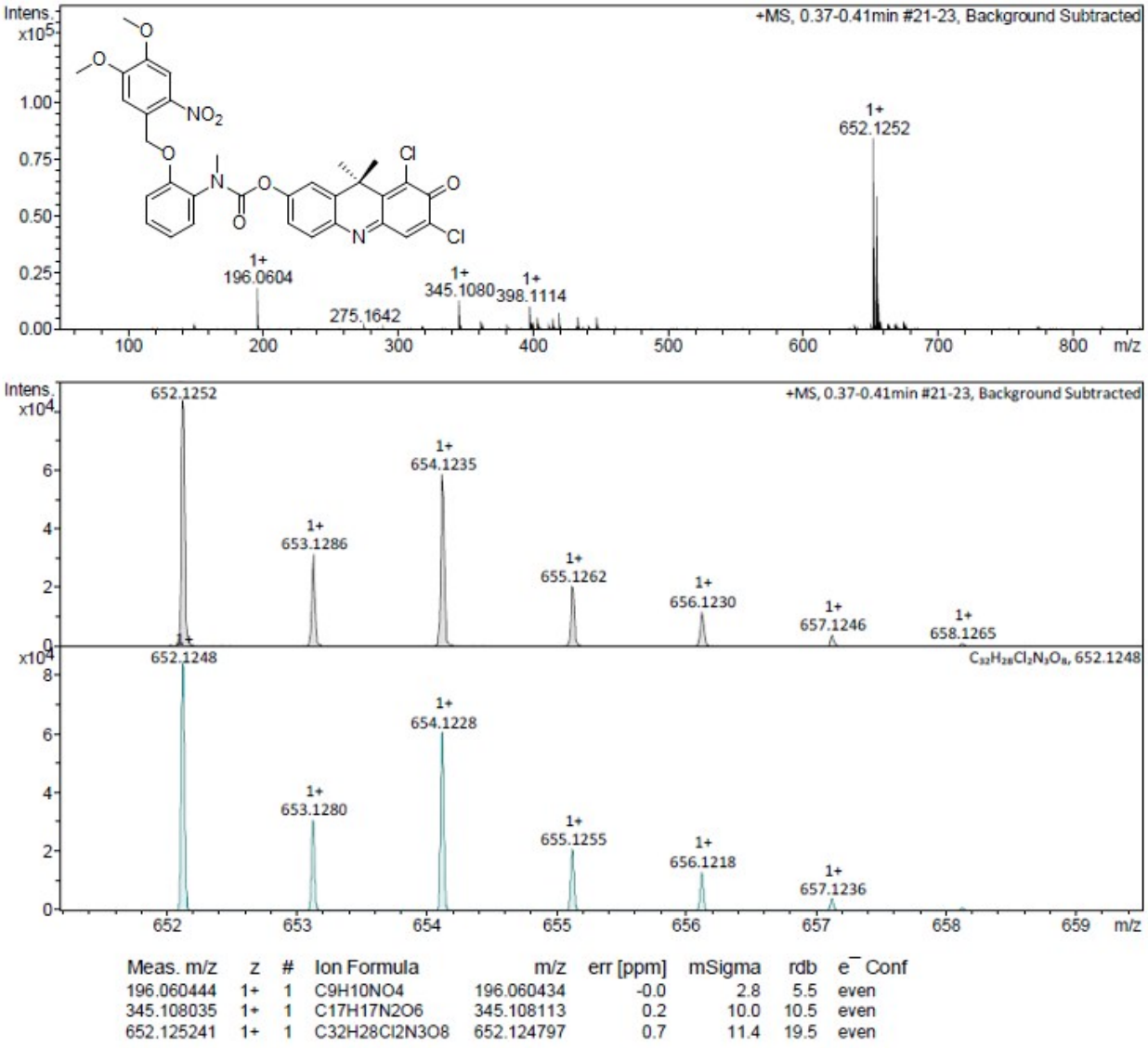


*Figure 64: ^{13}C NMR spectrum of compound **2-HPC^{H}***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
196.060444	1+	1	C9H10NO4	196.060434	-0.0	2.8	5.5	even
345.108035	1+	1	C17H17N2O6	345.108113	0.2	10.0	10.5	even
652.125241	1+	1	C32H28Cl2N3O8	652.124797	0.7	11.4	19.5	even

*Figure 65: HRMS spectrum of compound **2-HPC**^{H}*

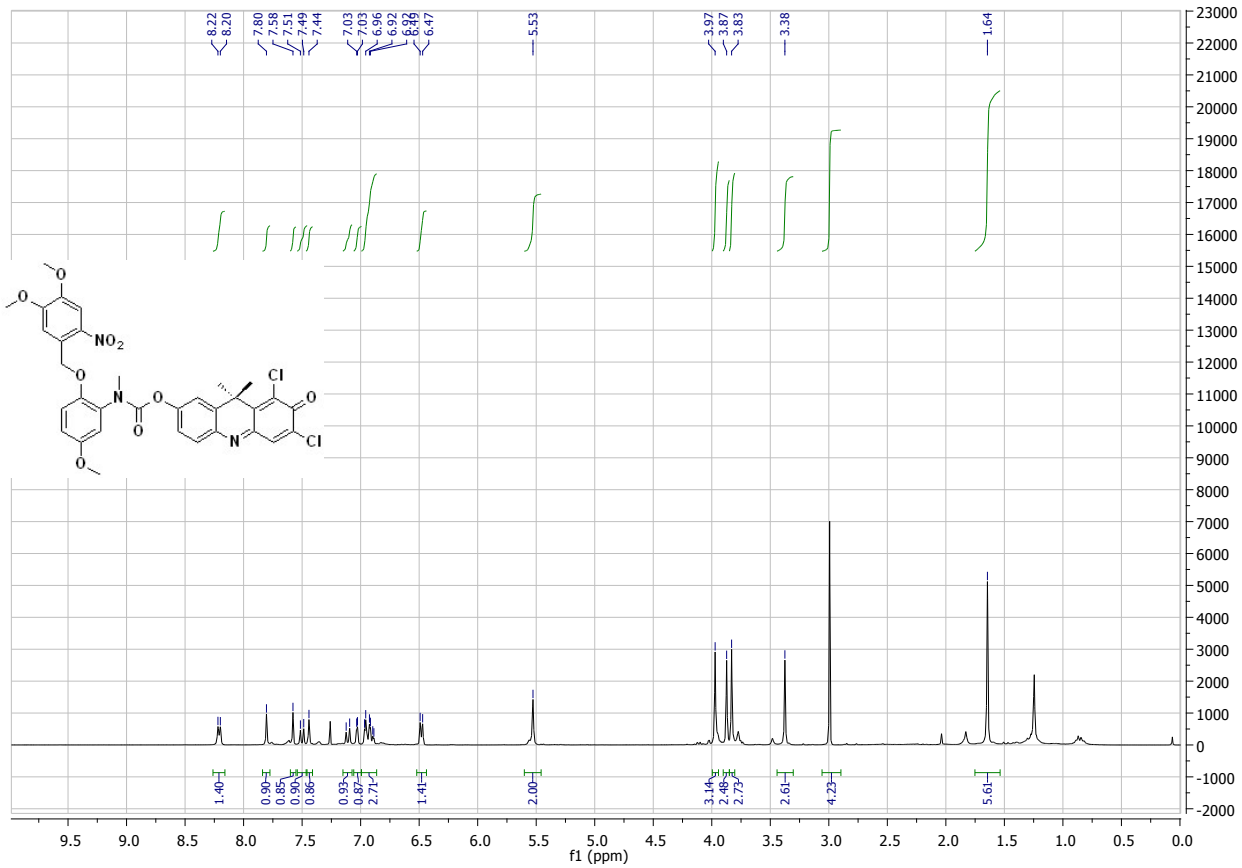


Figure 66: [1]H NMR spectrum of compound ***2-HPC^{OCH3}***

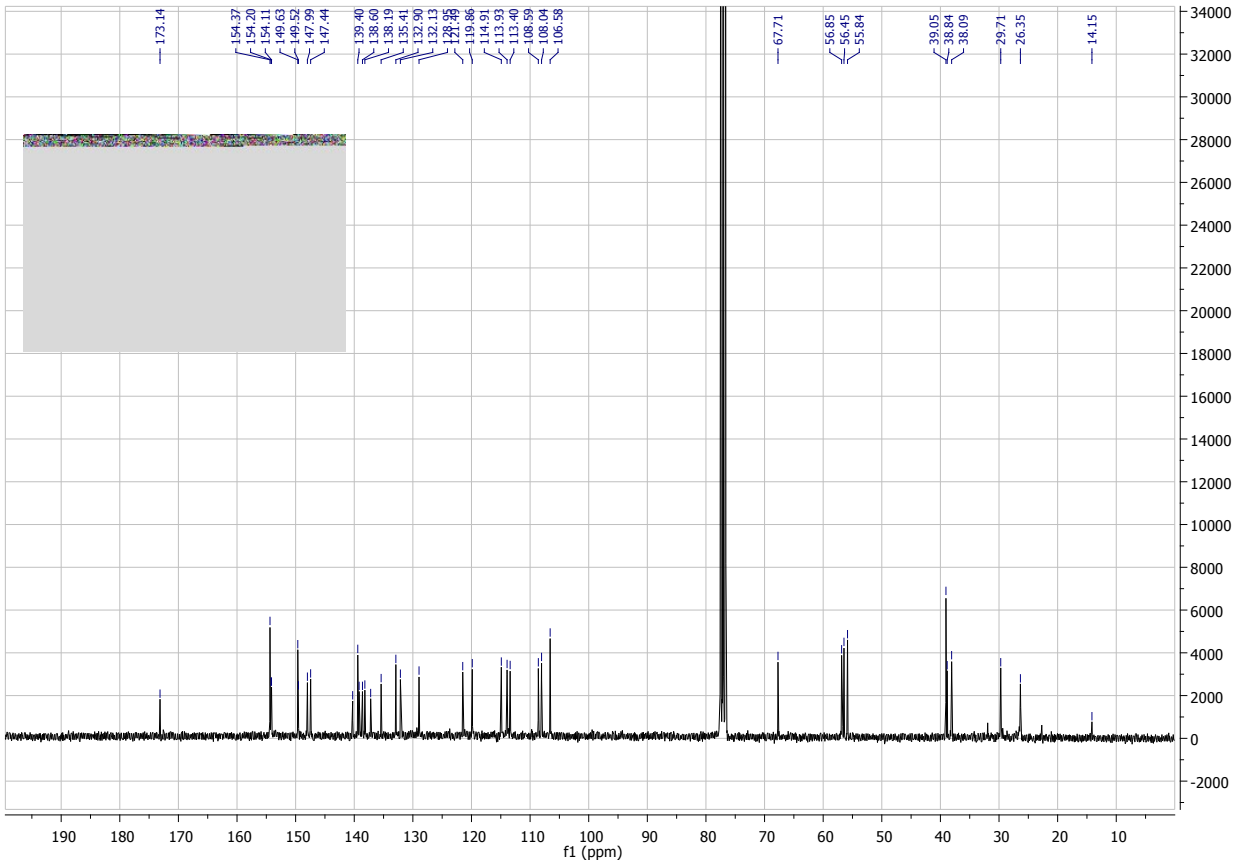
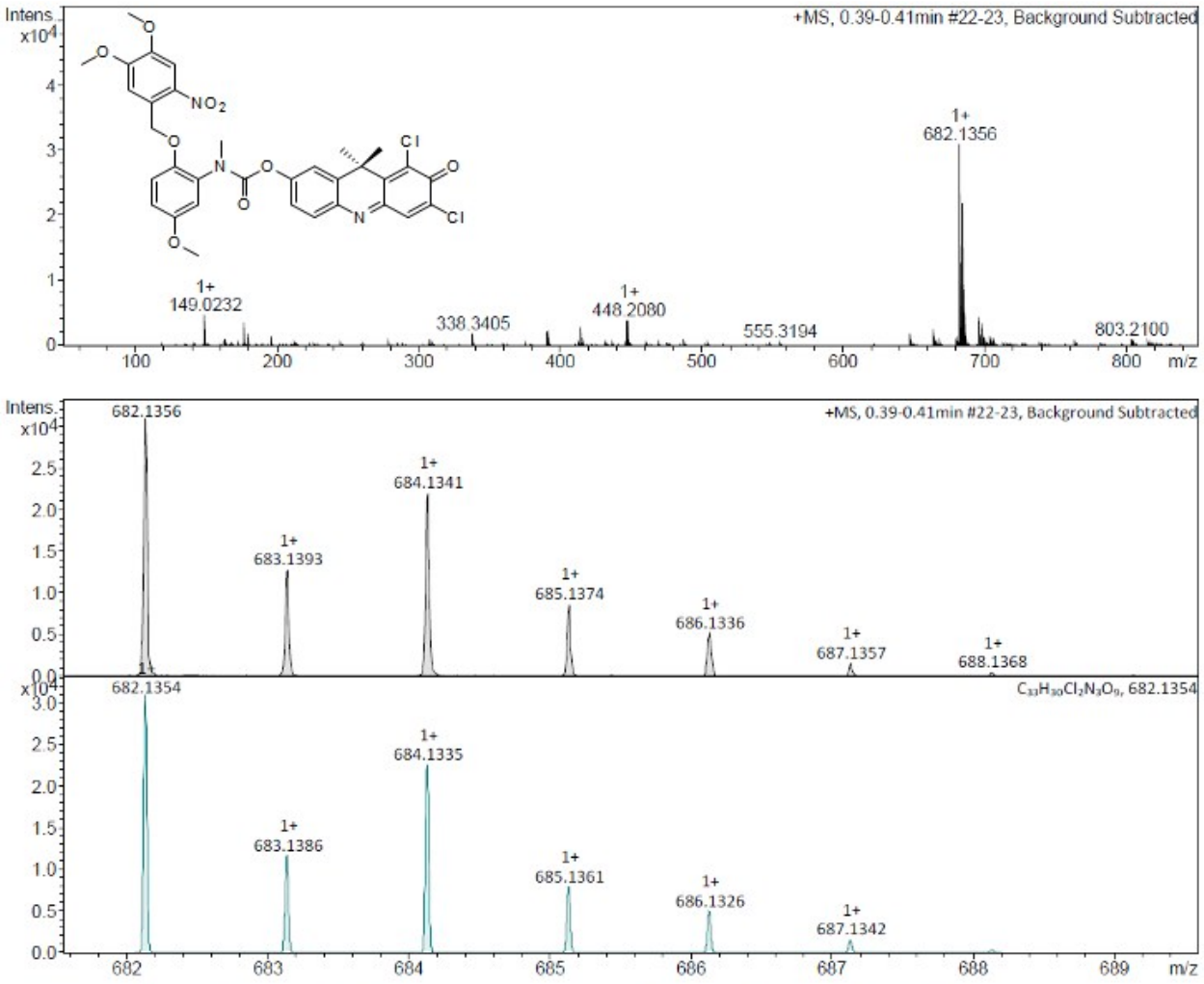


*Figure 67: ^{13}C NMR spectrum of compound **2-HPC^{OCH3}***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
682.135602	1+	1	C34H26Cl2N7O5	682.136699	1.6	9.1	24.5	even
	1+	2	C33H30Cl2N3O9	682.135361	0.4	17.5	19.5	even
696.115432	1+	1	C34H24Cl2N7O6	696.115963	0.8	43.7	25.5	even
	1+	2	C33H28Cl2N3O10	696.114626	1.2	47.5	20.5	even
	1+	3	C31H16Cl2N17	696.114615	1.2	48.6	31.5	even

*Figure 68: HRMS spectrum of compound **2-HPC^{OCH3}***

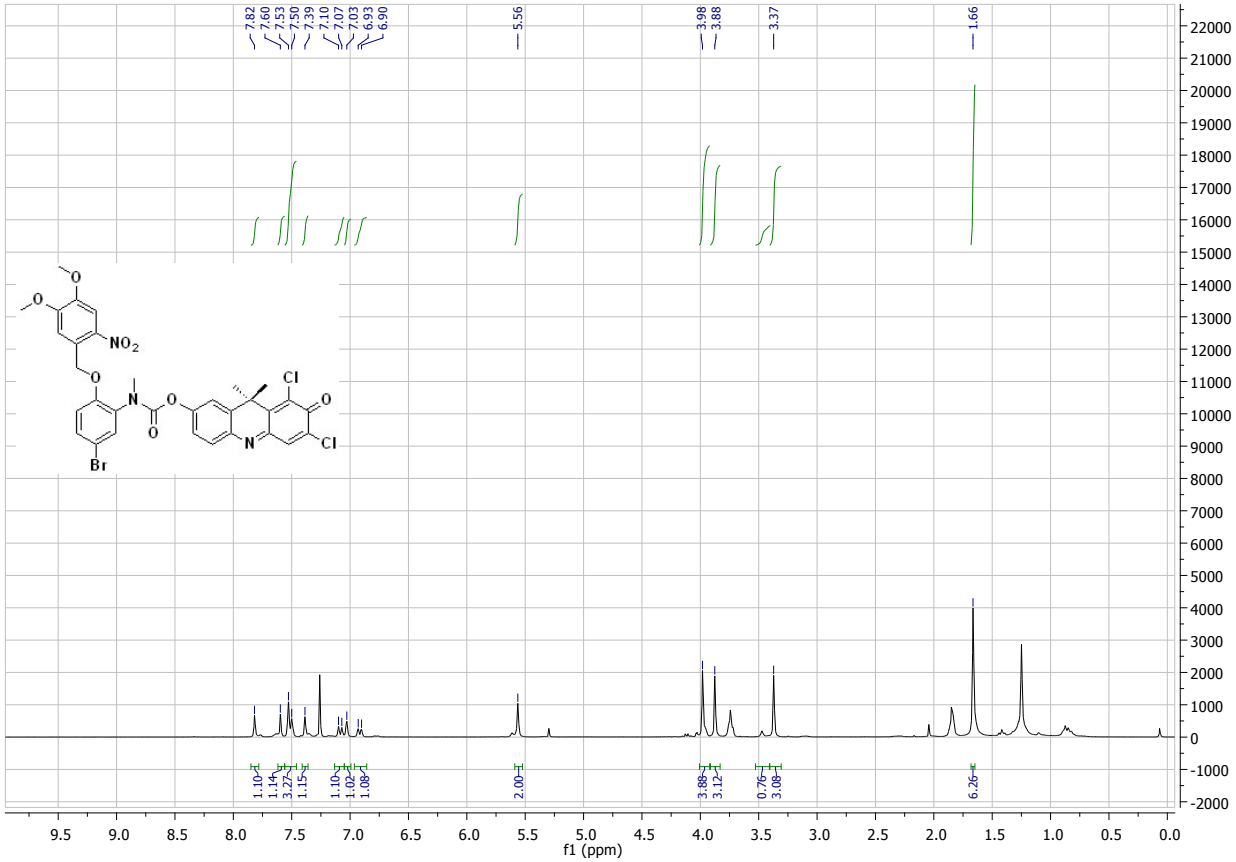


*Figure 69: ^{1}H NMR spectrum of compound **2-HPC^{Br}***

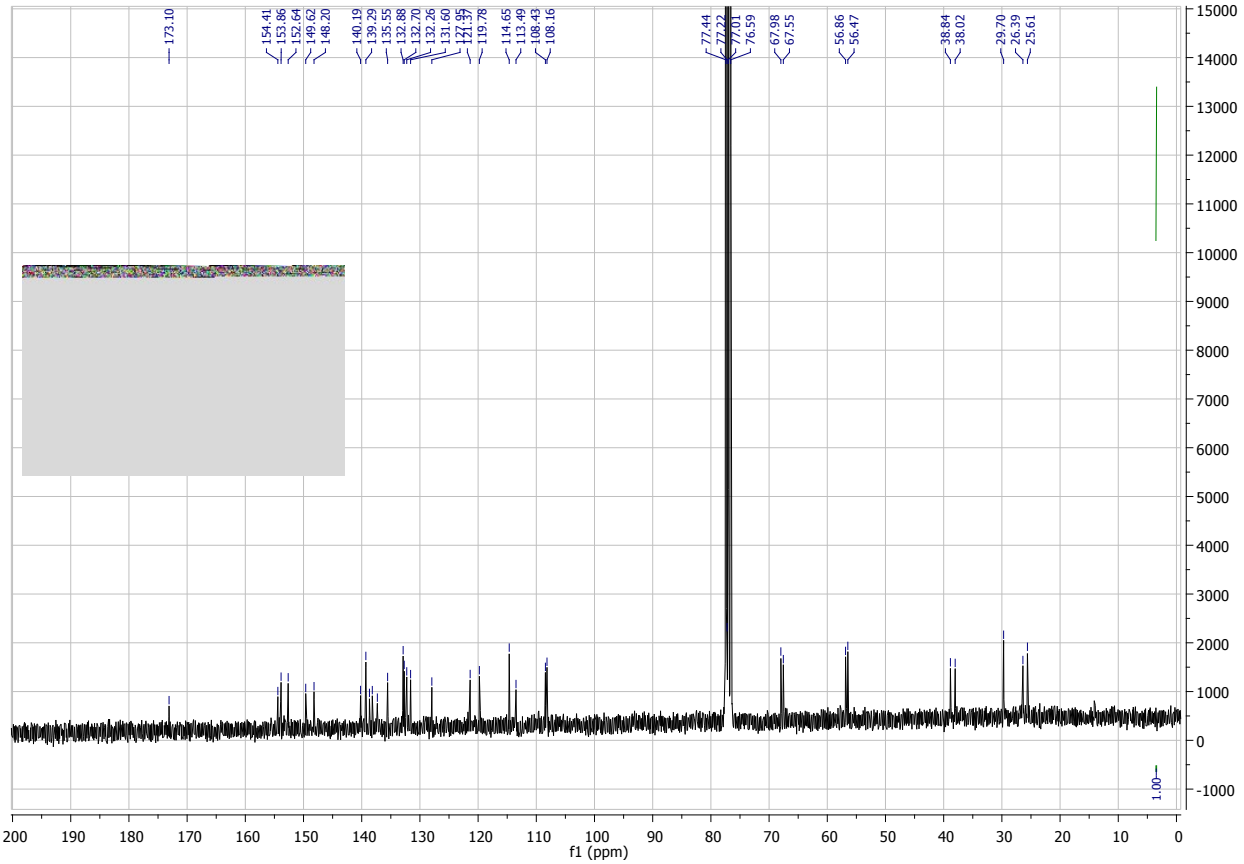
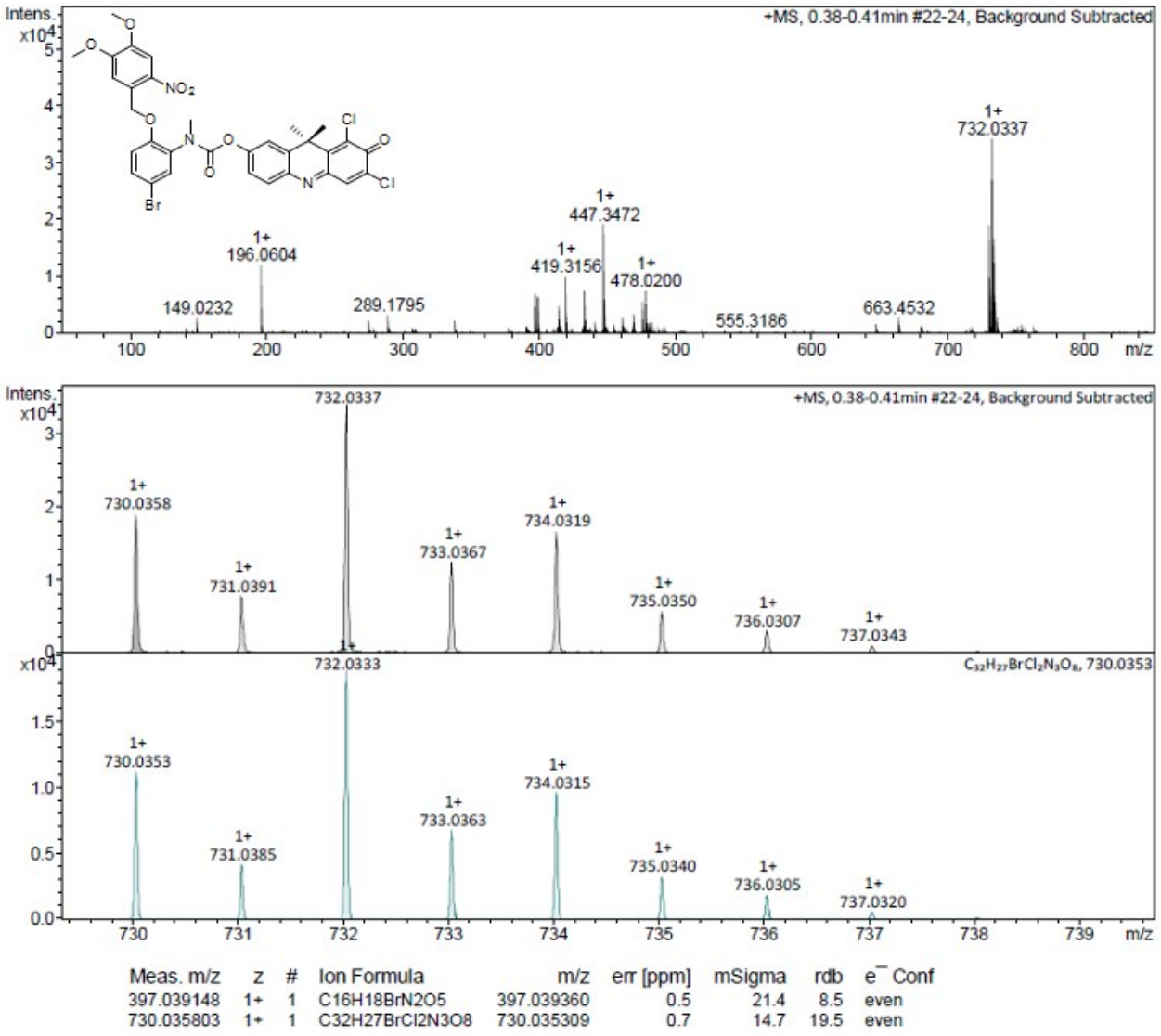


*Figure 70: ^{13}C NMR spectrum of compound **2-HPC^{Br}***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
397.039148	1+	1	C16H18BrN2O5	397.039360	0.5	21.4	8.5	even
730.035803	1+	1	C32H27BrCl2N3O8	730.035309	0.7	14.7	19.5	even

*Figure 71: HRMS spectrum of compound **2-HPC^{Br}***

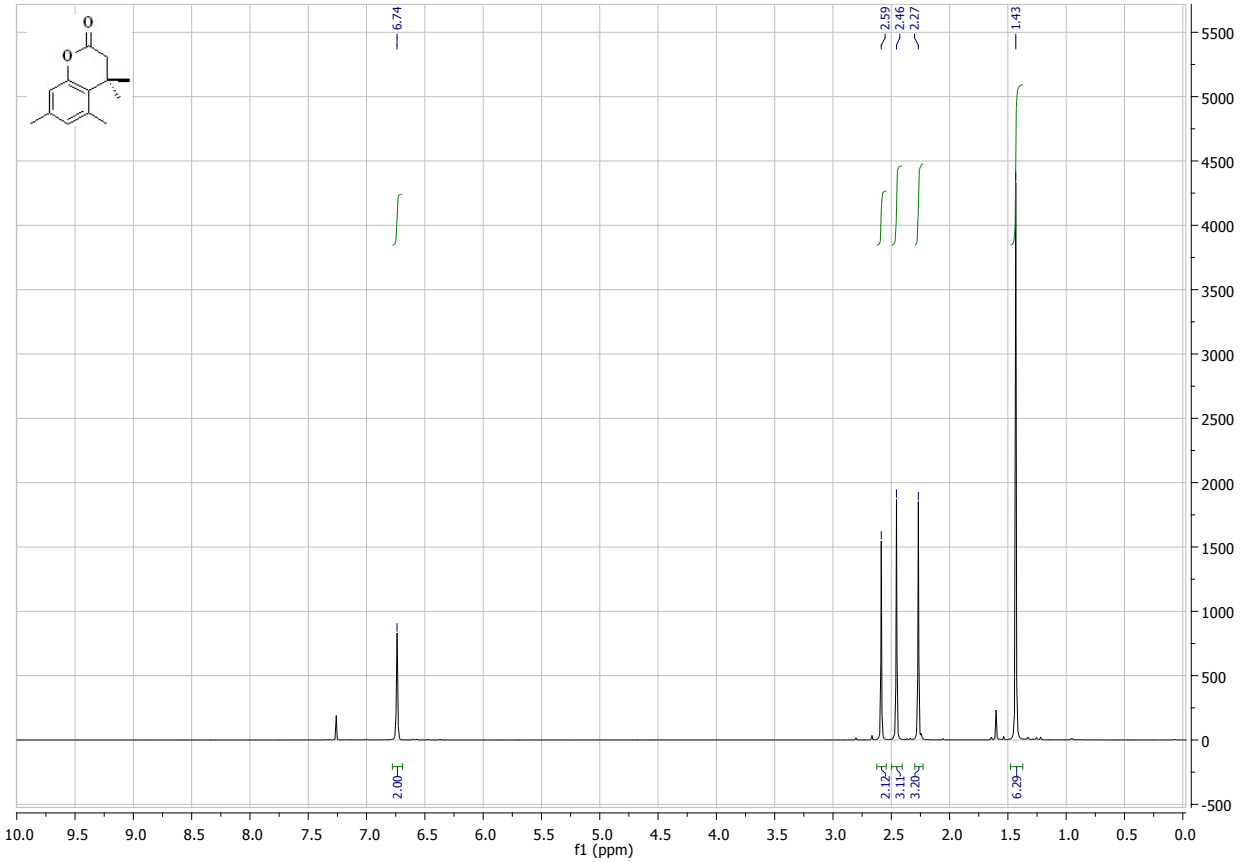


*Figure 72: ^{1}H NMR spectrum of compound **17***

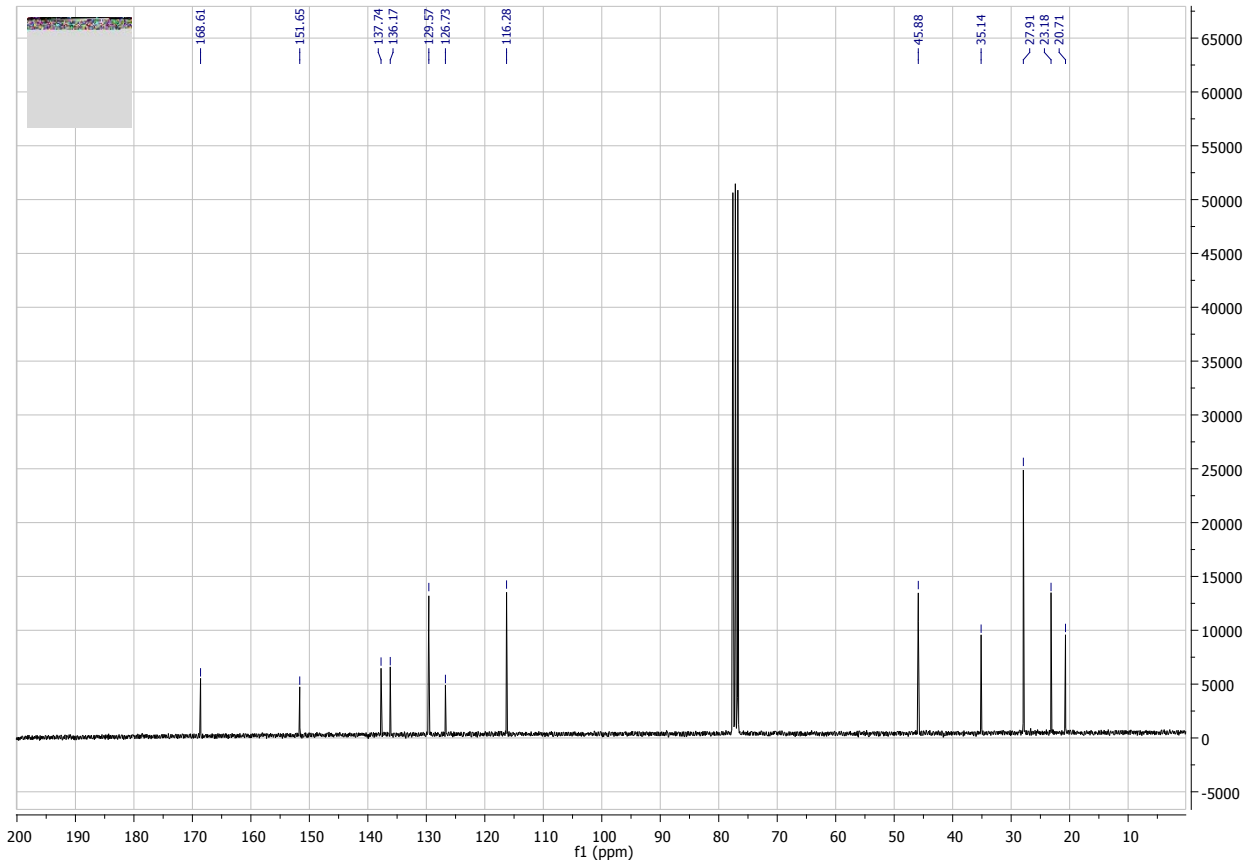
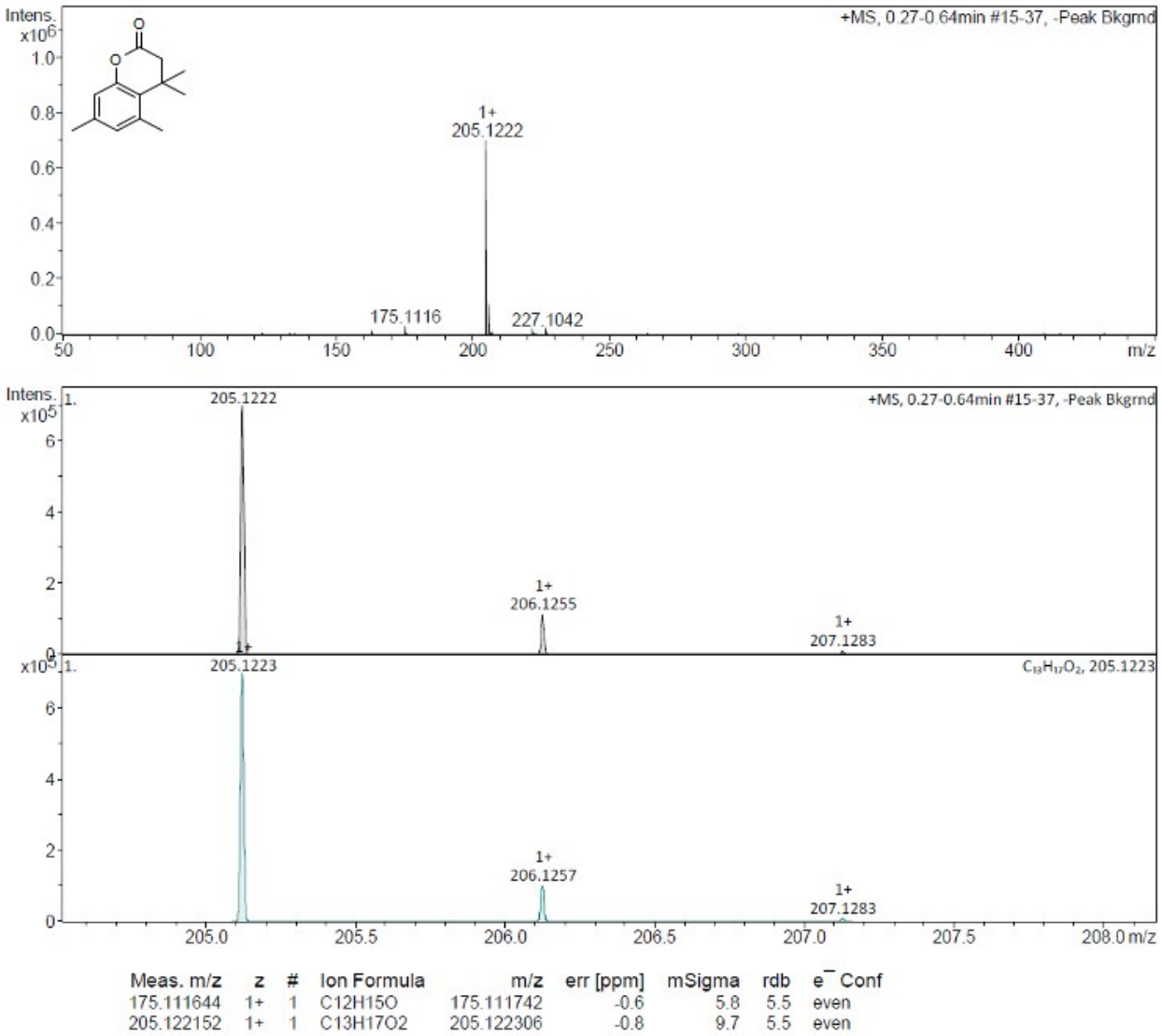


*Figure 73: ^{13}C NMR spectrum of compound **17***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
175.111644	1+	1	C12H15O	175.111742	-0.6	5.8	5.5	even
205.122152	1+	1	C13H17O2	205.122306	-0.8	9.7	5.5	even

*Figure 74: HRMS spectrum of compound **17***

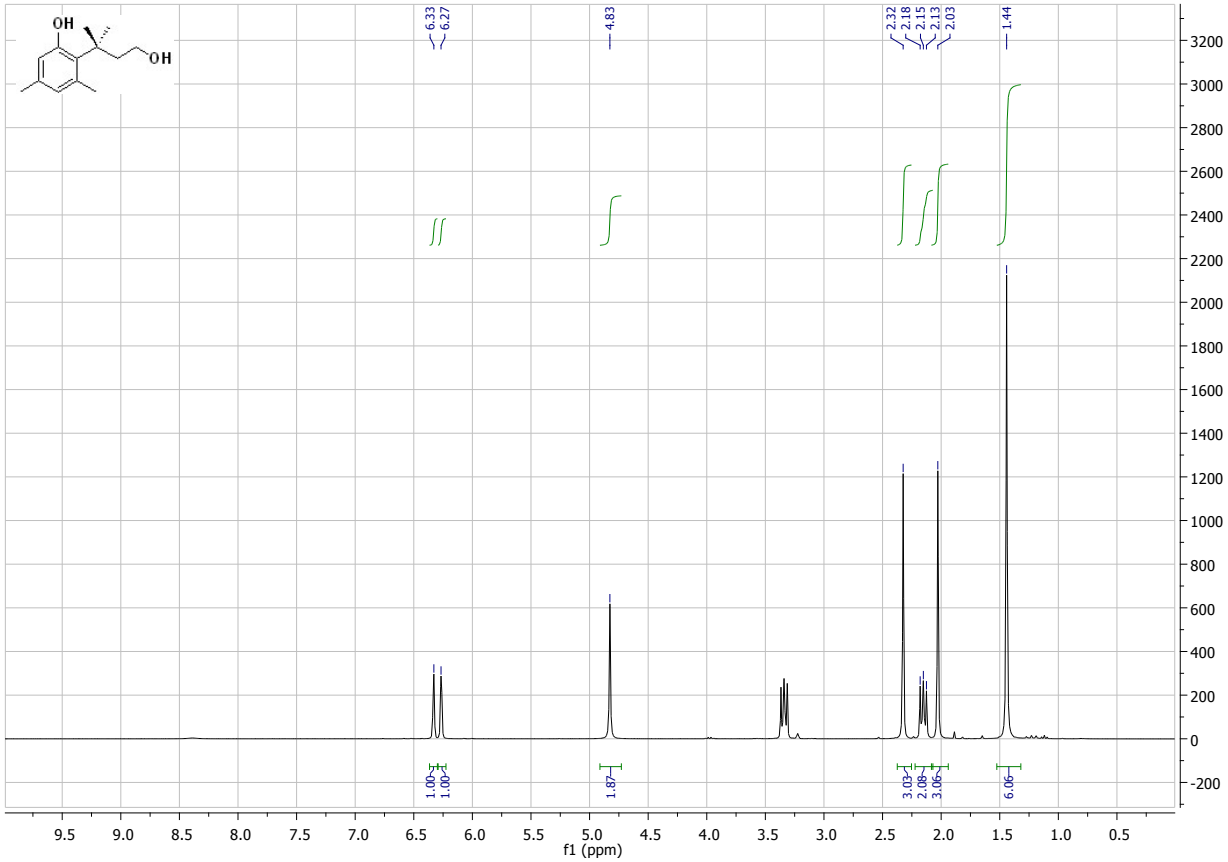


*Figure 75: ^{1}H NMR spectrum of compound **18***

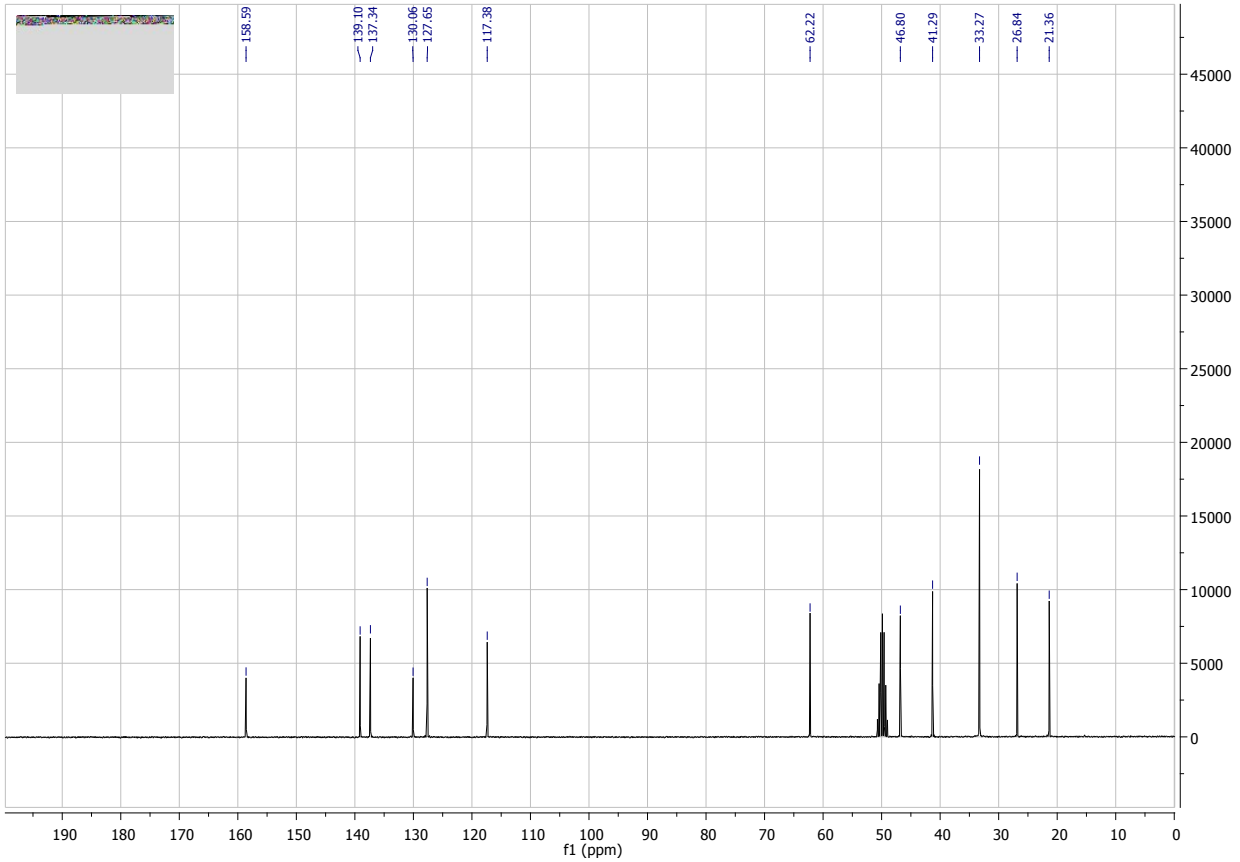
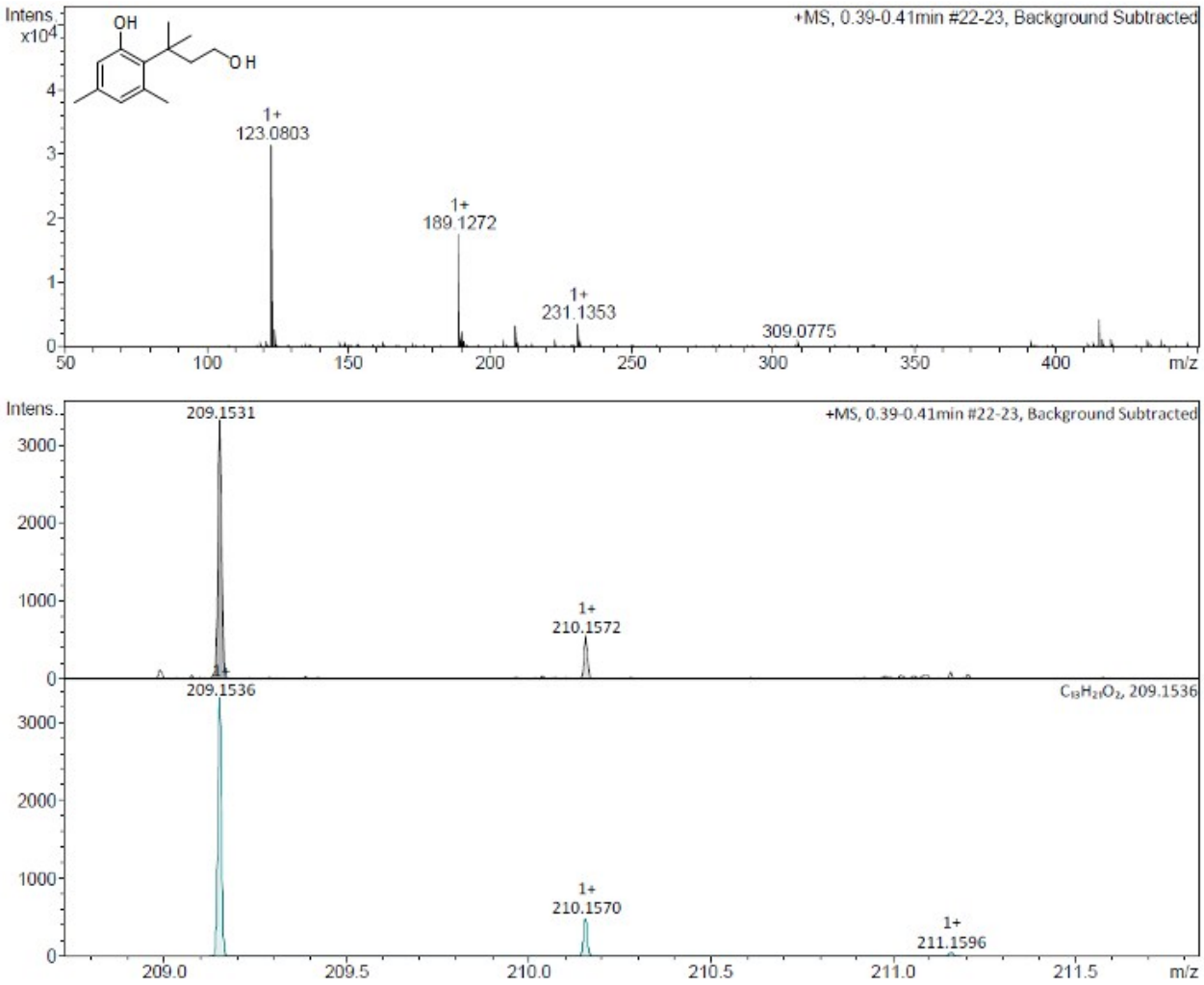


*Figure 76: ^{13}C NMR spectrum of compound **18***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
123.080274	1+	1	C8H11O	123.080441	-1.4	0.8	3.5	even
189.127219	1+	1	C13H17O	189.127392	-0.9	6.9	5.5	even
209.153099	1+	1	C13H21O2	209.153606	2.4	10.6	3.5	even
231.135303	1+	1	C13H20NaO2	231.135551	-1.1	22.7	3.5	even

*Figure 77: HRMS spectrum of compound **18***

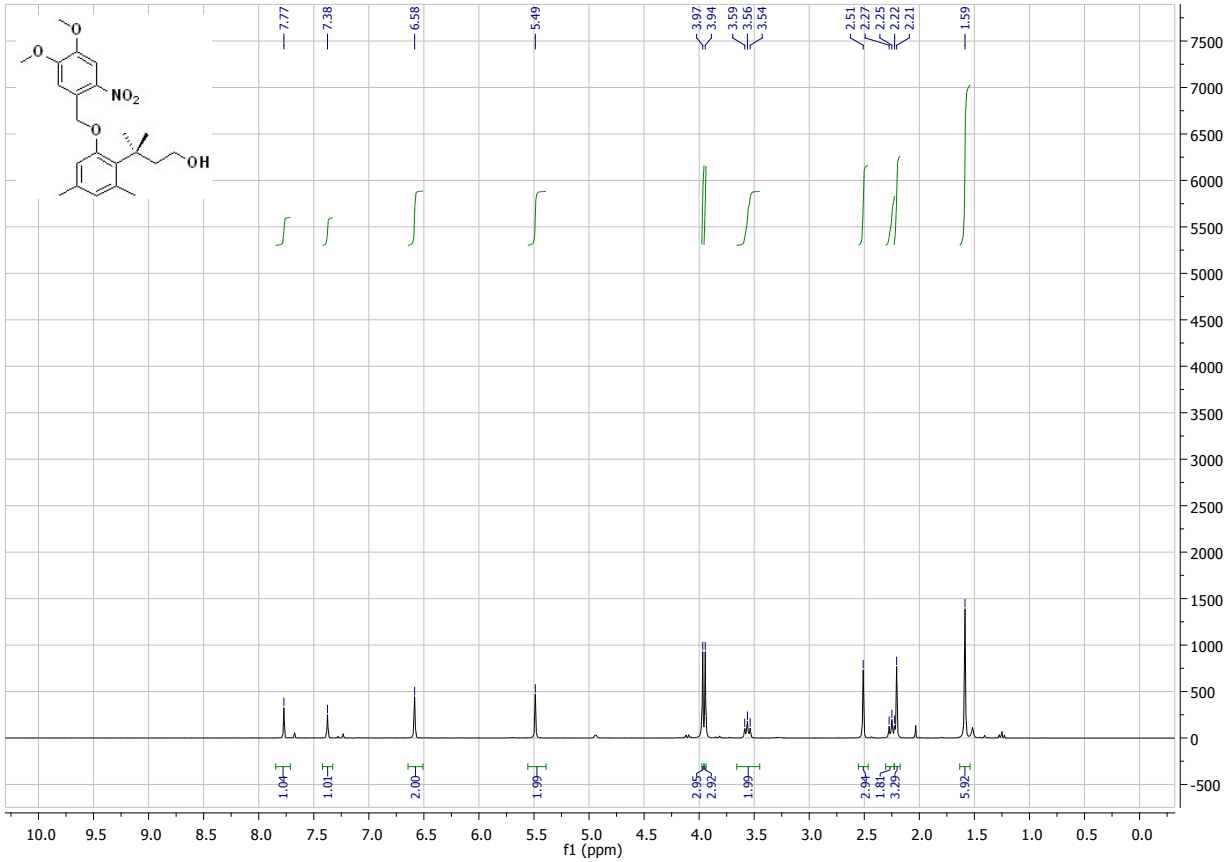


*Figure 78: ^{1}H NMR spectrum of compound **19***

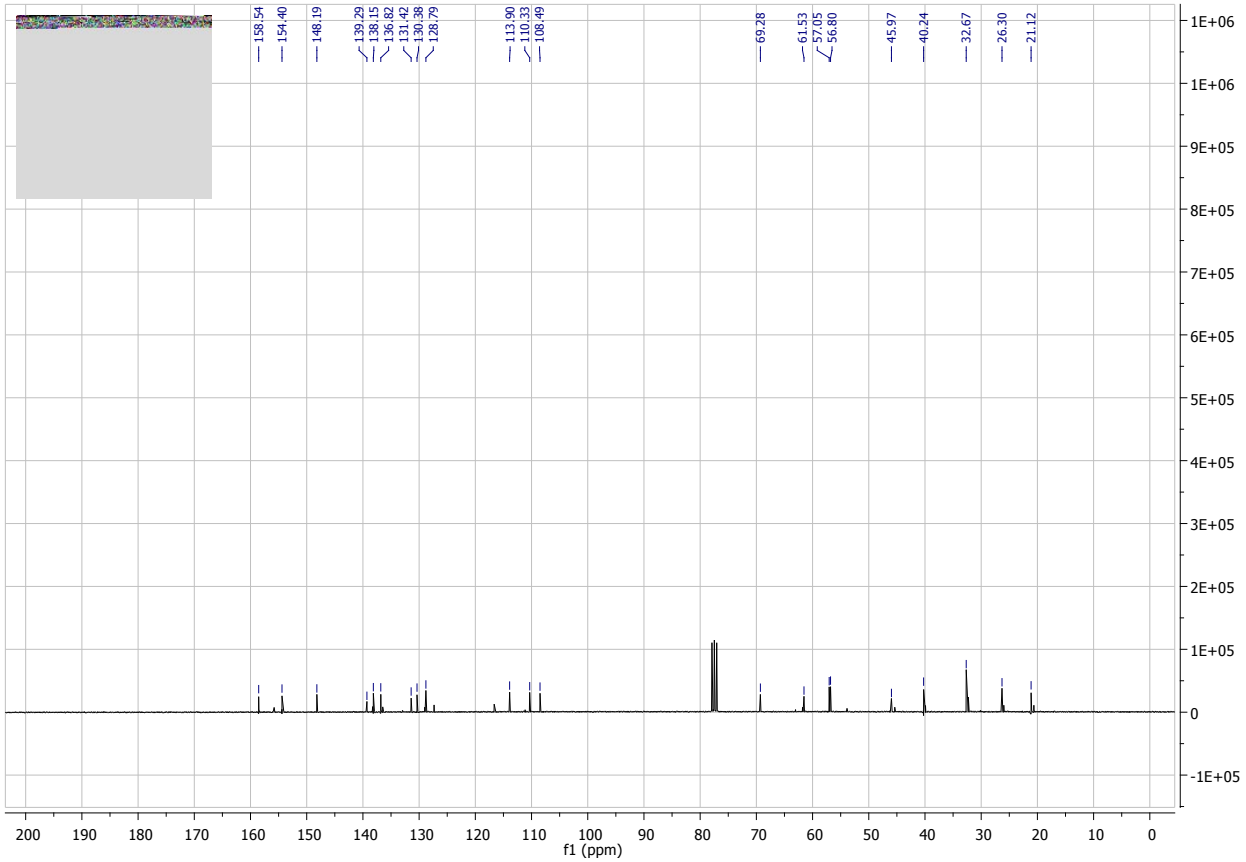
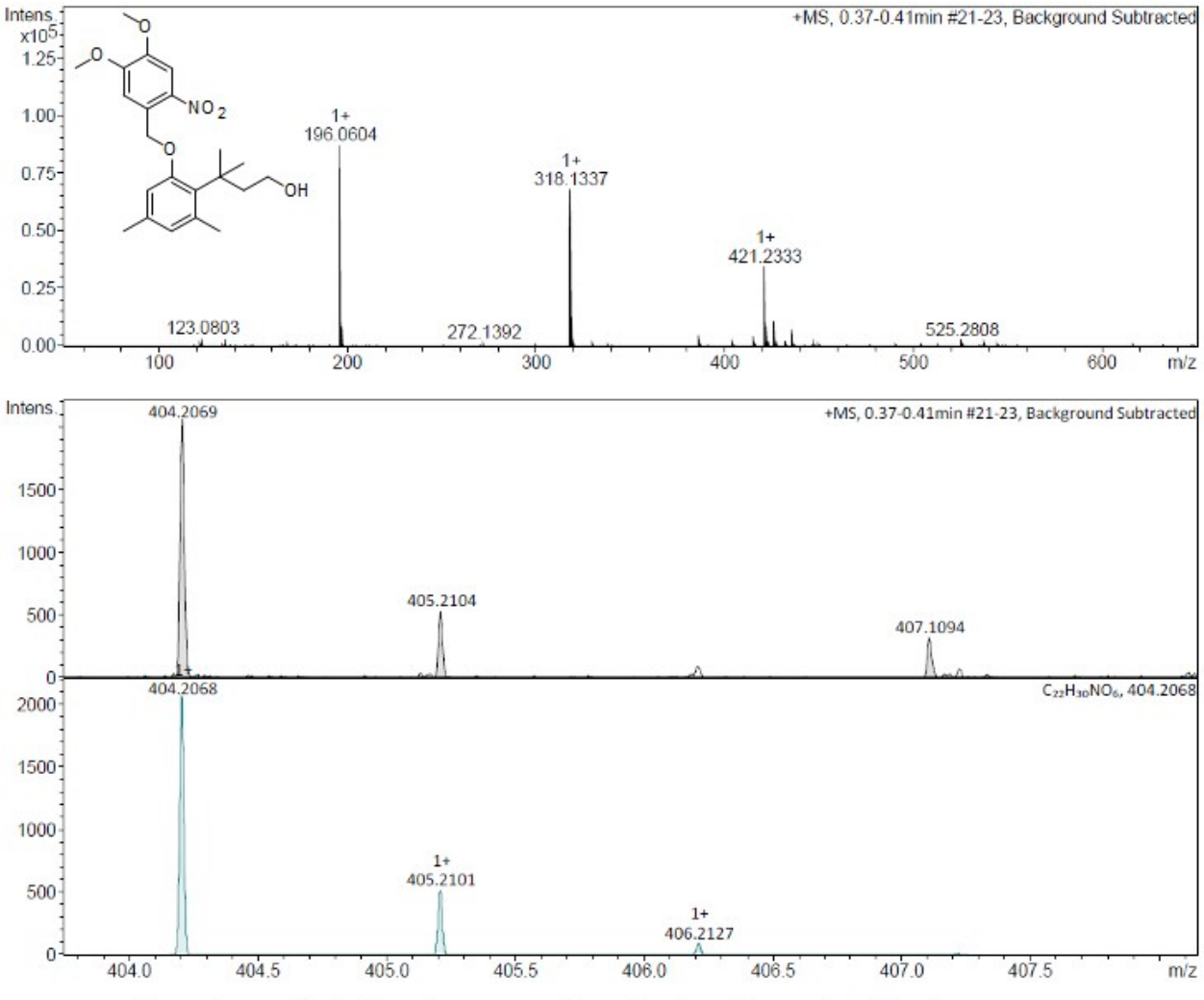


*Figure 79: ^{13}C NMR spectrum of compound **19***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060358	1+	1	C9H10NO4	196.060434	-0.4	2.0	5.5	even
318.133675	1+	1	C17H20NO5	318.133599	0.2	0.3	8.5	even
404.206862	1+	1	C22H30NO6	404.206764	-0.2	21.5	8.5	even
421.233332	1+	1	C22H33N2O6	421.233313	0.0	3.4	7.5	even
426.188692	1+	1	C22H29NNaO6	426.188708	0.0	14.3	8.5	even

*Figure 80: HRMS spectrum of compound **19***

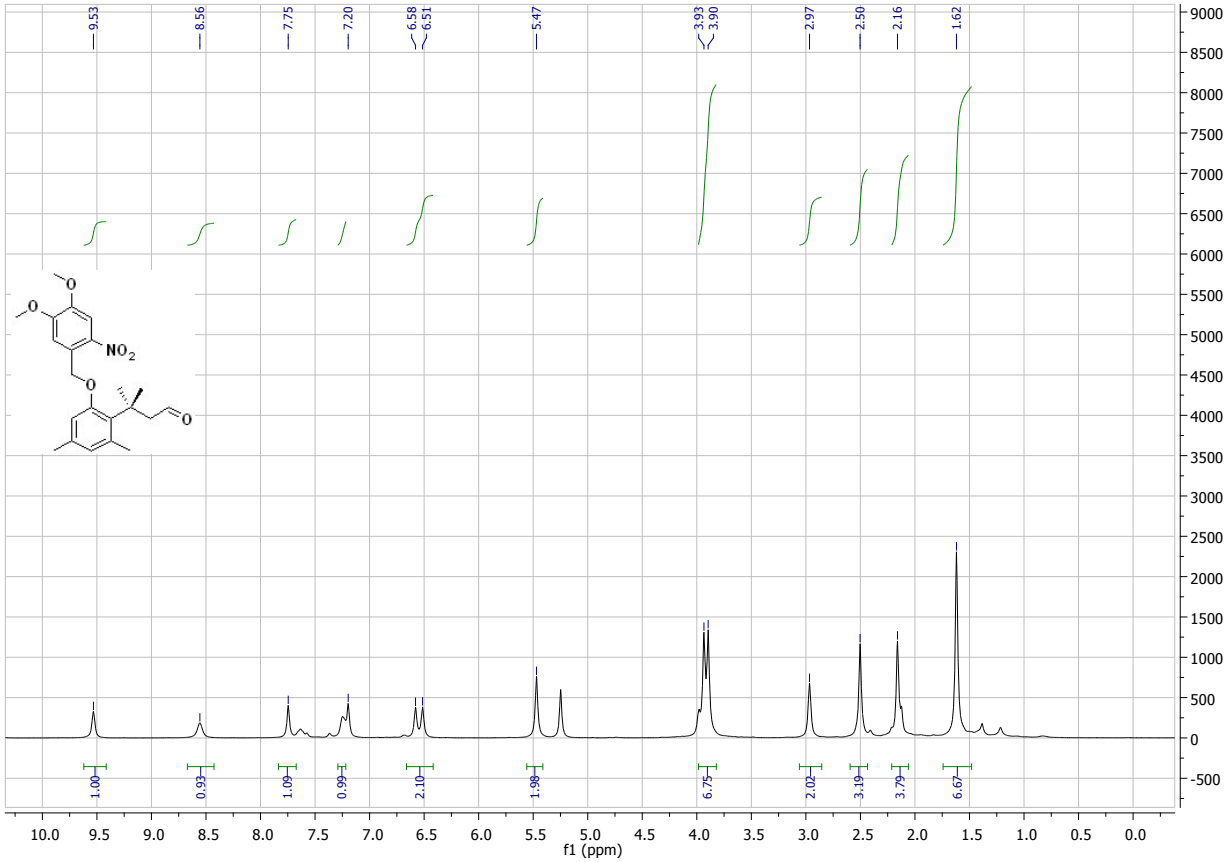


*Figure 81: ^{1}H NMR spectrum of compound **20***

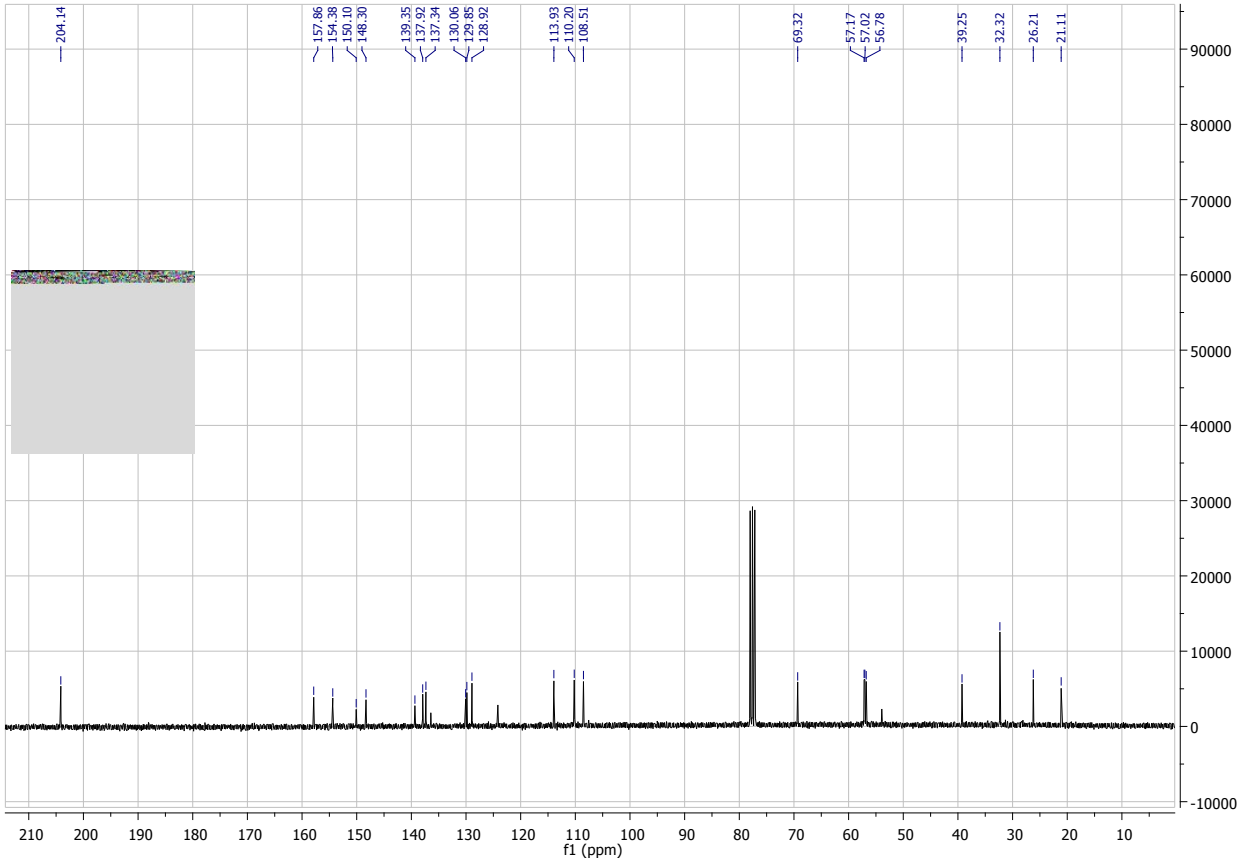
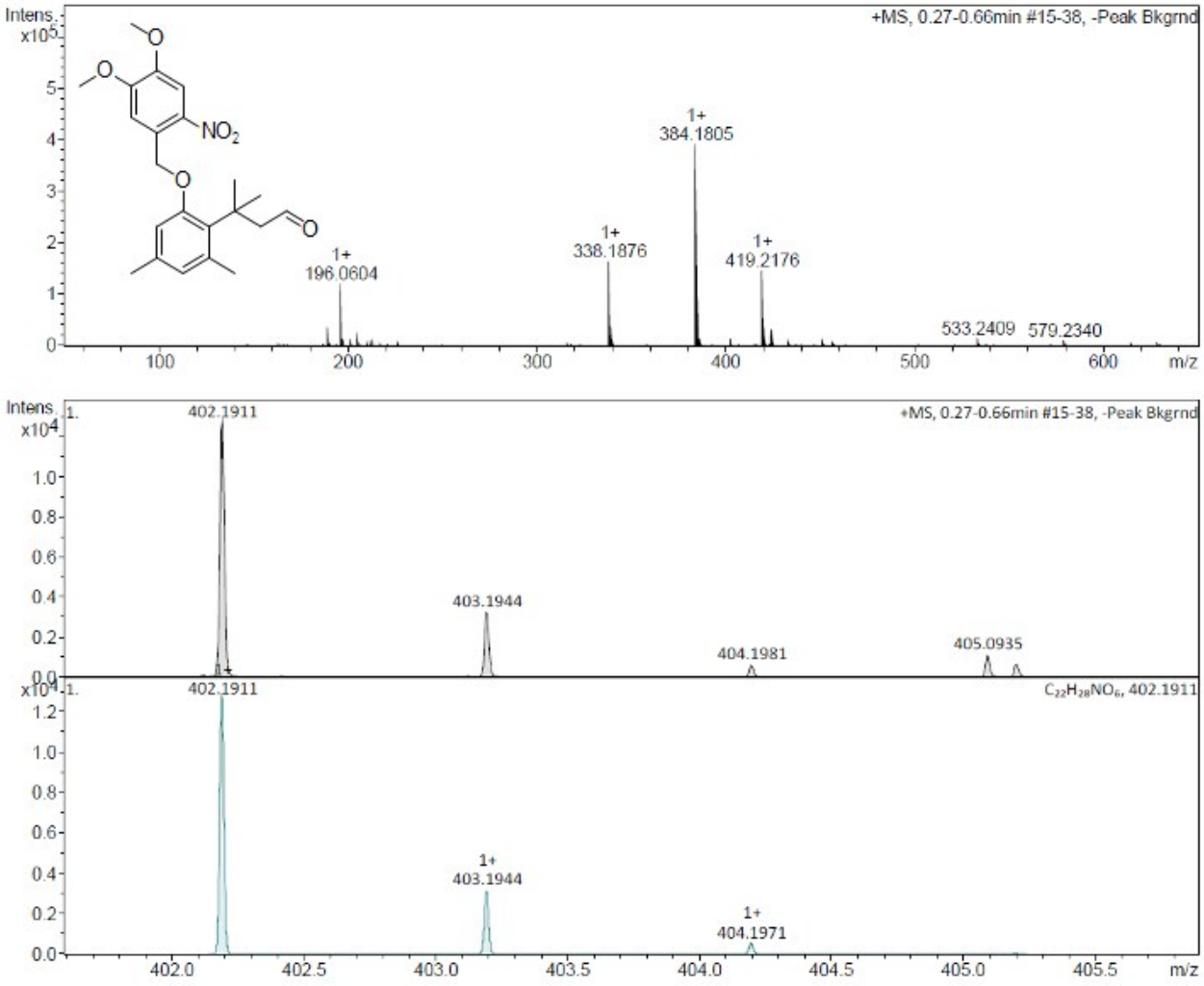


*Figure 82: ^{13}C NMR spectrum of compound **20***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
189.127064	1+	1	C13H17O	189.127392	1.7	4.5	5.5	even
196.060354	1+	1	C9H10NO4	196.060434	-0.4	1.9	5.5	even
338.187591	1+	1	C22H26O3	338.187646	-0.2	5.1	10.0	odd
384.180513	1+	1	C22H26NO5	384.180549	0.1	1.1	10.5	even
402.191105	1+	1	C22H28NO6	402.191114	0.0	24.7	9.5	even
419.217647	1+	1	C22H31N2O6	419.217663	-0.0	3.6	8.5	even
424.173201	1+	1	C22H27NNaO6	424.173058	0.3	5.8	9.5	even

*Figure 83: HRMS spectrum of compound **20***

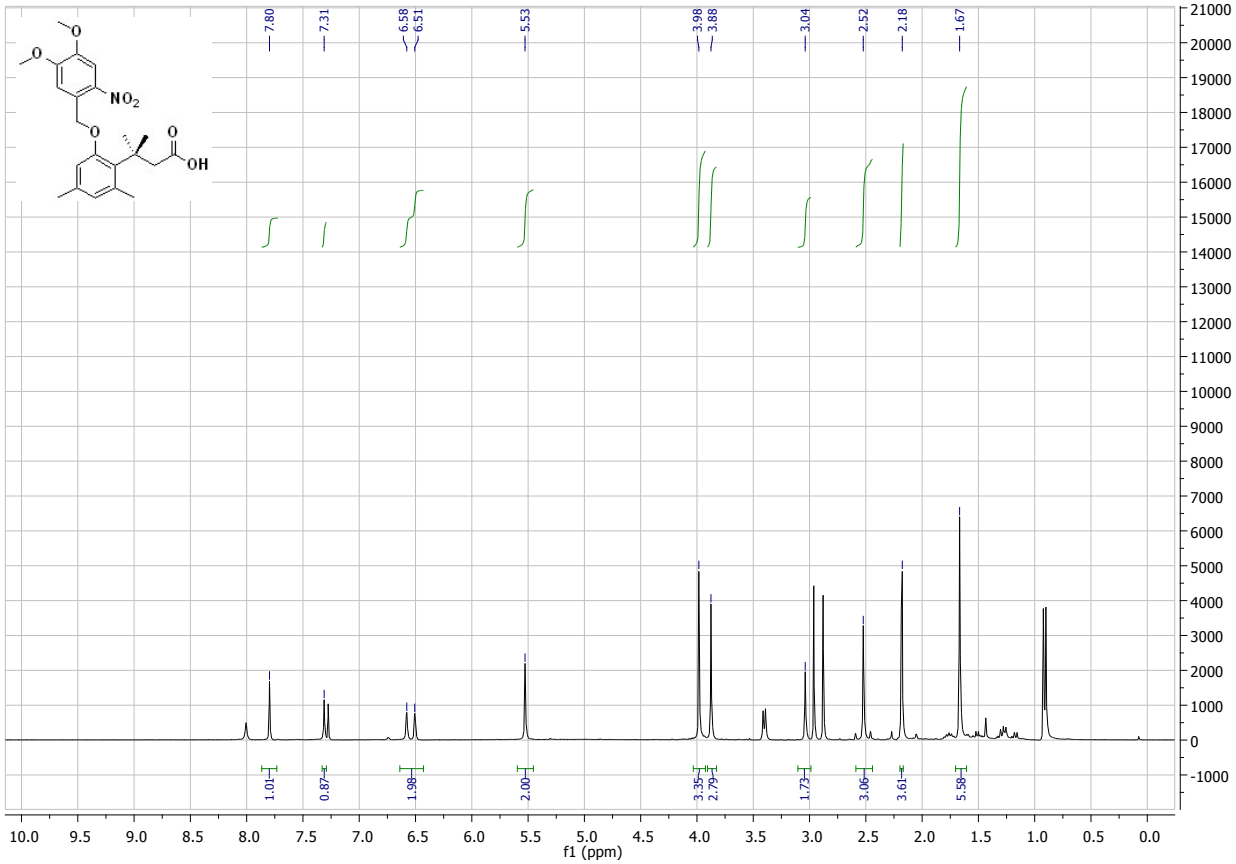


*Figure 84: ^{1}H NMR of compound **21***

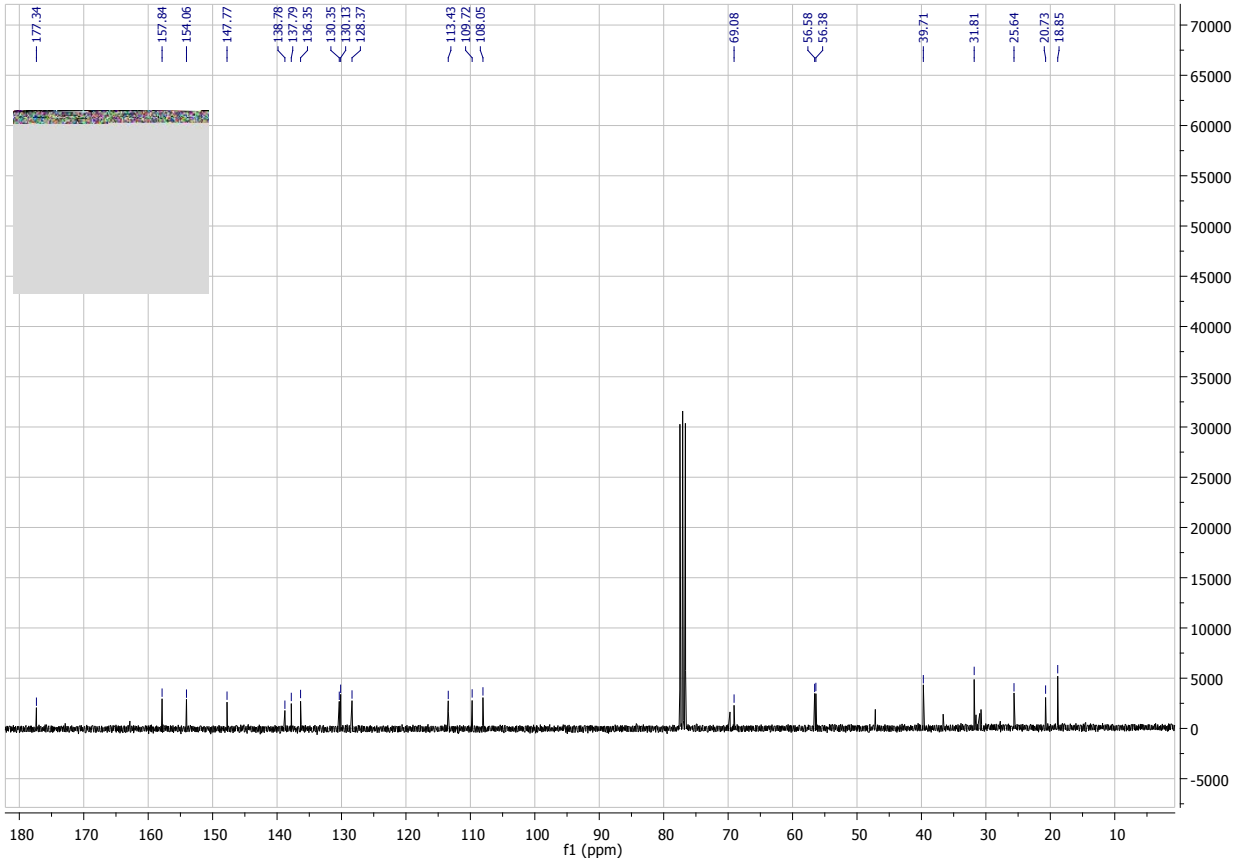
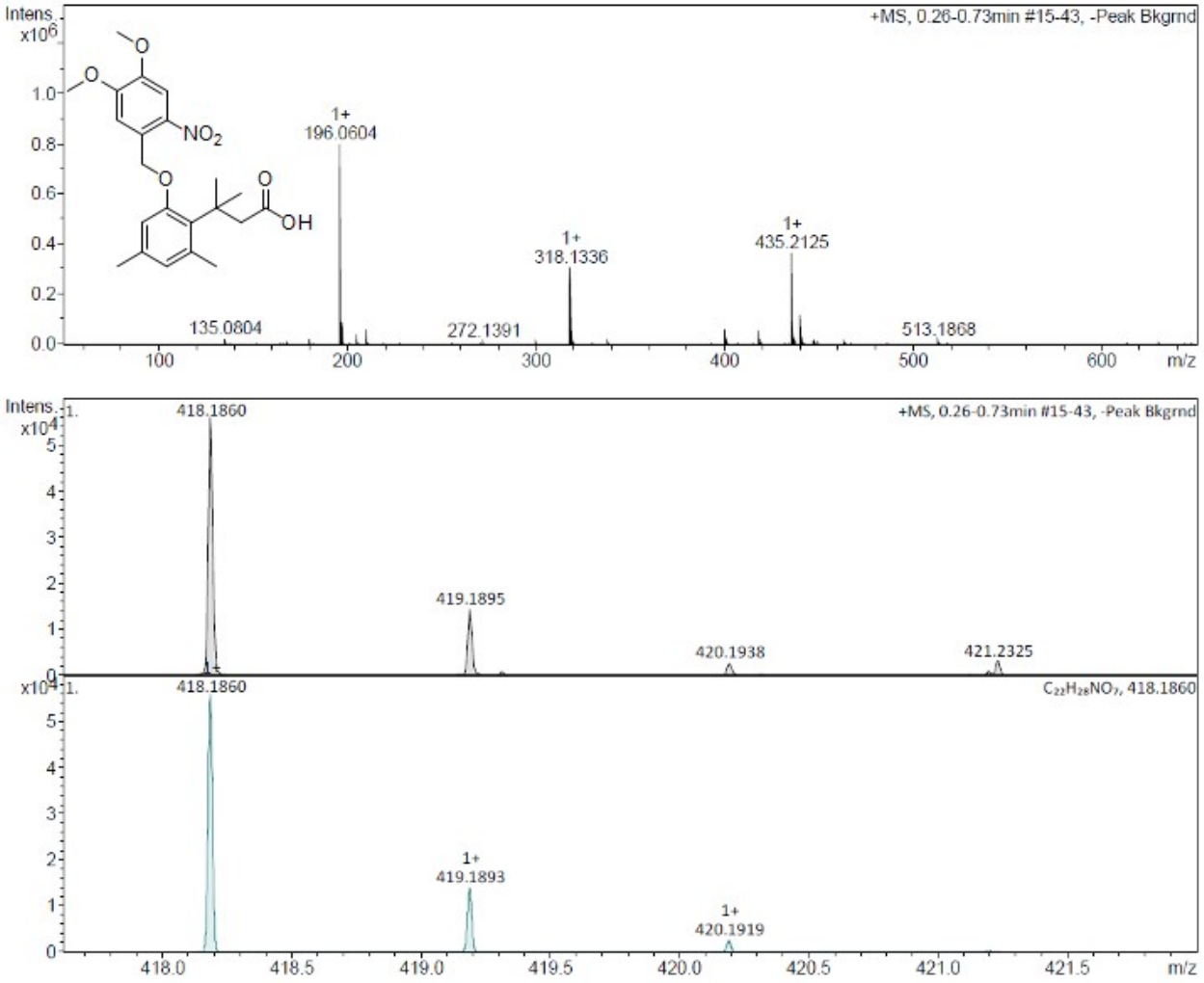


*Figure 85: ^{13}C NMR spectrum of compound **21***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
196.060447	1+	1	C9H10NO4	196.060434	-0.1	2.1	5.5	even
318.133557	1+	1	C17H20NO5	318.133599	0.1	2.1	8.5	even
400.175408	1+	1	C22H26NO6	400.175464	-0.1	9.3	10.5	even
418.186015	1+	1	C22H28NO7	418.186029	-0.0	7.4	9.5	even
435.212453	1+	1	C22H31N2O7	435.212578	0.3	1.0	8.5	even
440.167926	1+	1	C22H27NNaO7	440.167973	0.1	4.0	9.5	even

*Figure 86: HRMS spectrum of compound **21***

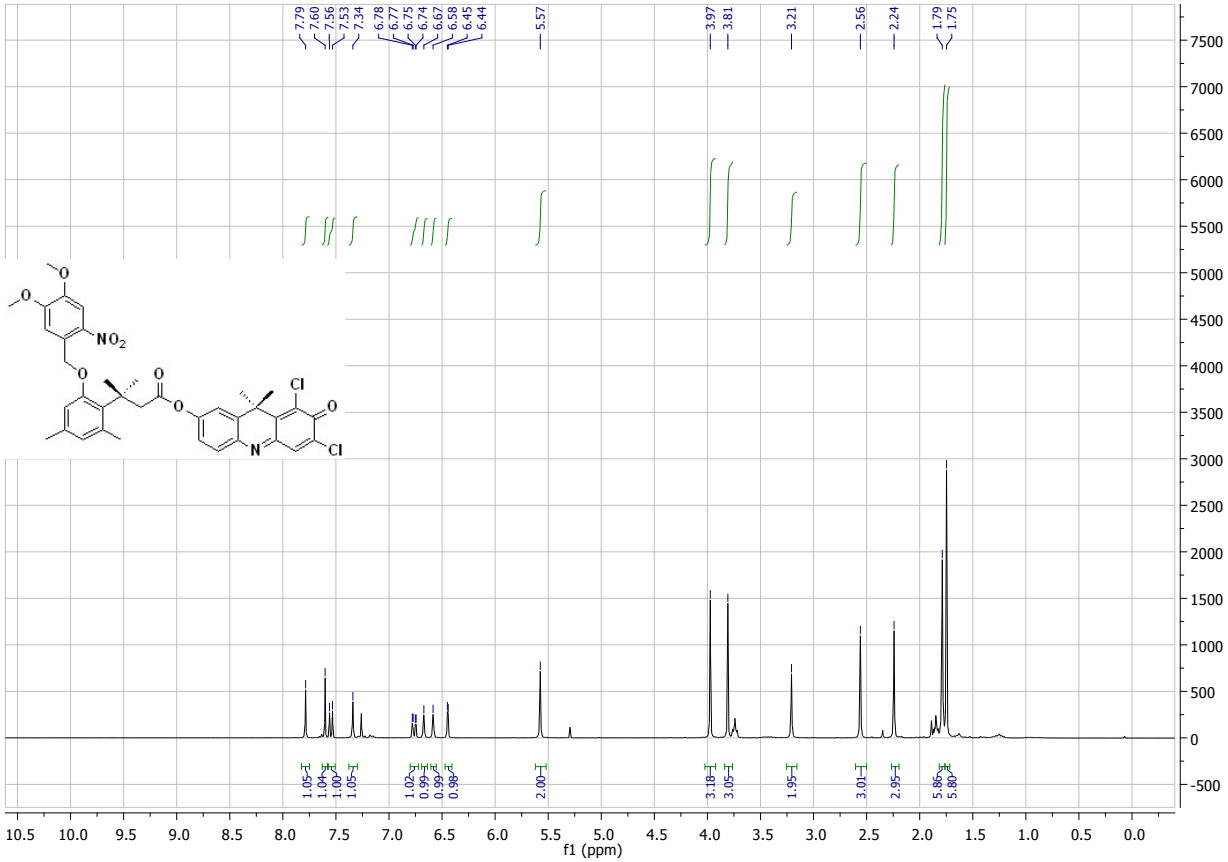


*Figure 87: ^{1}H NMR spectrum of compound **TML***

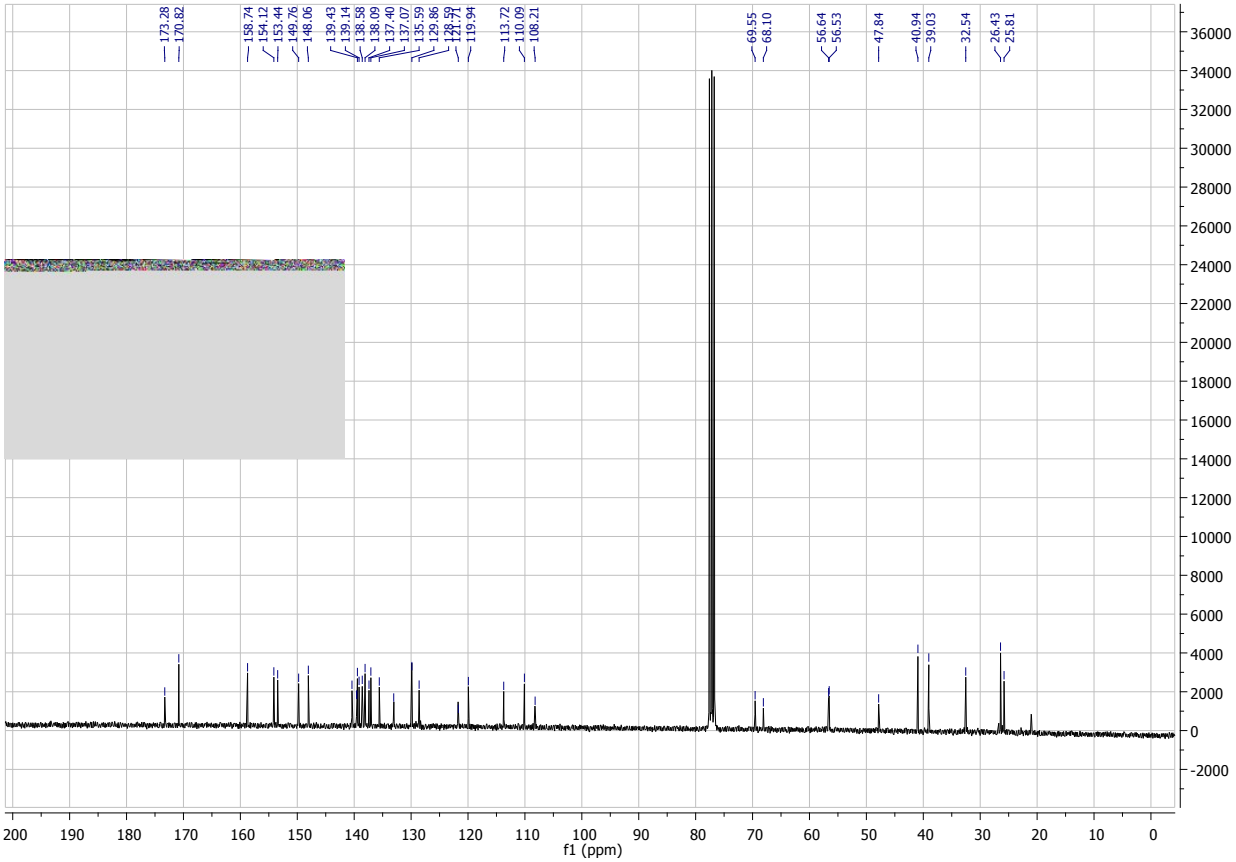
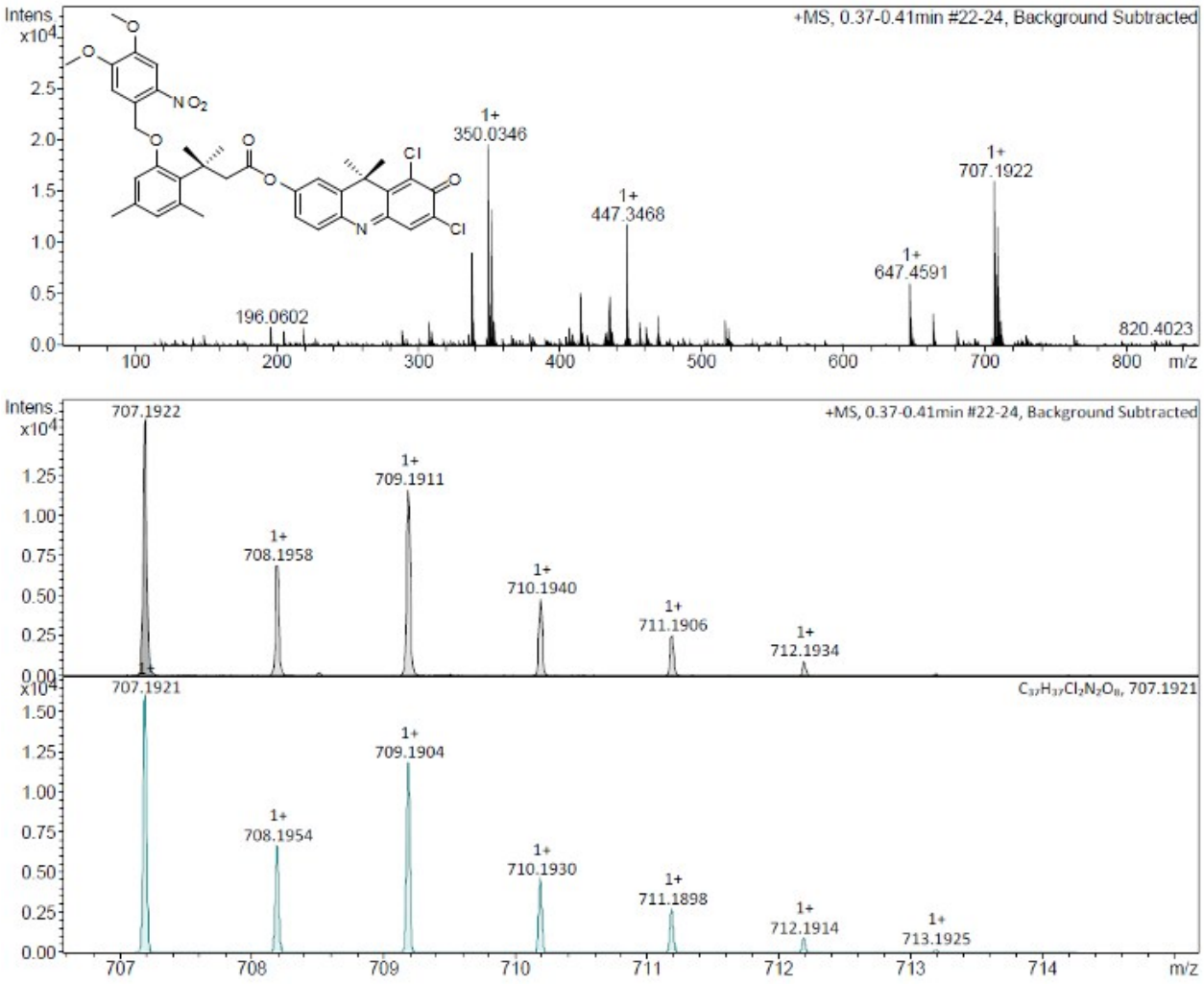


*Figure 88: ^{13}C NMR spectrum of compound **TML***



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
350.034571	1+	1	C17H14Cl2NO3	350.034525	0.1	11.6	10.5	even
707.192168	1+	1	C38H33Cl2N6O4	707.193485	-1.9	10.3	24.5	even
	1+	2	C34H29Cl2N12O2	707.190800	-1.9	11.4	25.5	even
	1+	3	C37H37Cl2N2O8	707.192148	0.0	12.8	19.5	even

*Figure 89: HRMS spectrum of compound **TML***

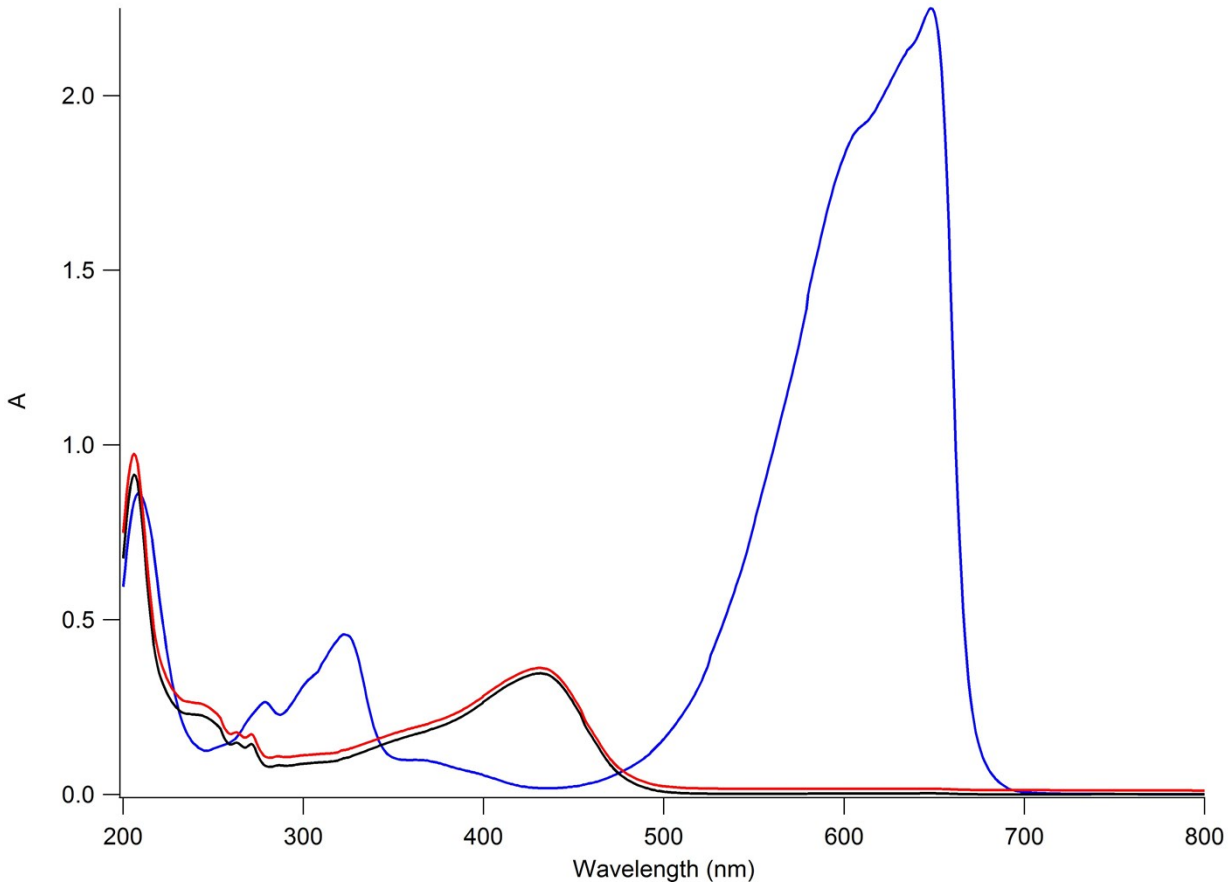


*Figure 90: UV absorbance of free DDAO (blue) and **TML** at 0 (black) and 1000s (red) in acetonitrile/buffer: no absorption at 640 nm corresponding to free DDAO appeared, which proves the stability of the **TML** ester during this time*

The purity of the compounds was evaluated by HPLC (Waters ACQUITY UPLC BEH C18 1.7µm VanGuard® Pre-Column 3/Pk 2.1 x 5mm Column). Conditions of HPLC analysis introduce 2 peaks when purified carbamate and ester linked DDAO compounds are analysed. Thus, the purity was calculated by the sum of these 2 peaks.

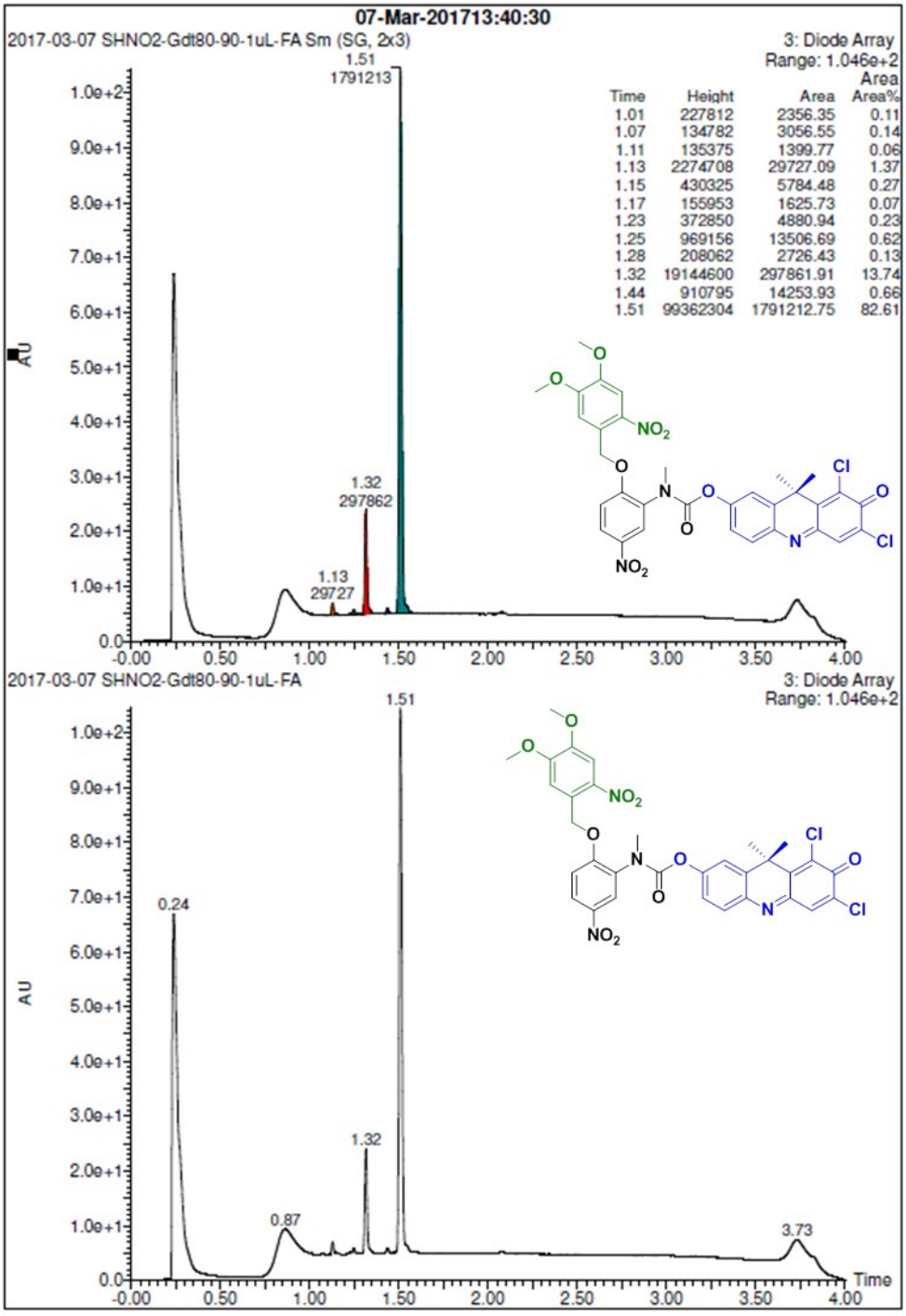


*Figure 91: HPLC spectrum of **2-HPC^{NO2}**, showing retention time of 1.51 min in 80 to 90% gradient of CH_3CN/water/0.1% formic acid. The purity of the desired compound is 96.4%.*

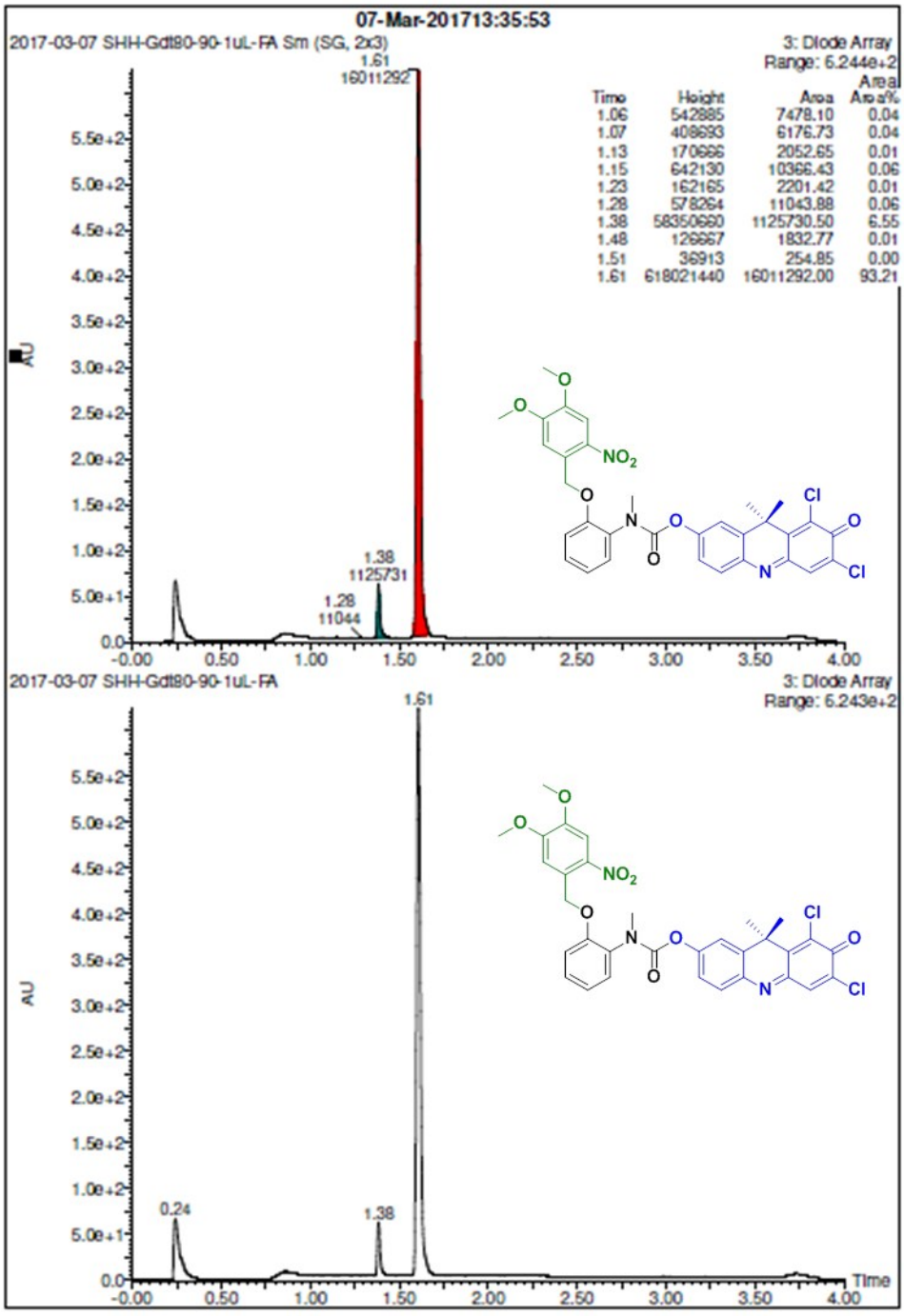


*Figure 92: HPLC spectrum of **2-HPC^{H}**, showing retention time of 1.61 min in 80 to 90% gradient of CH_3CN/water/0.1% formic acid. The purity of the desired compound is 99.8%.*

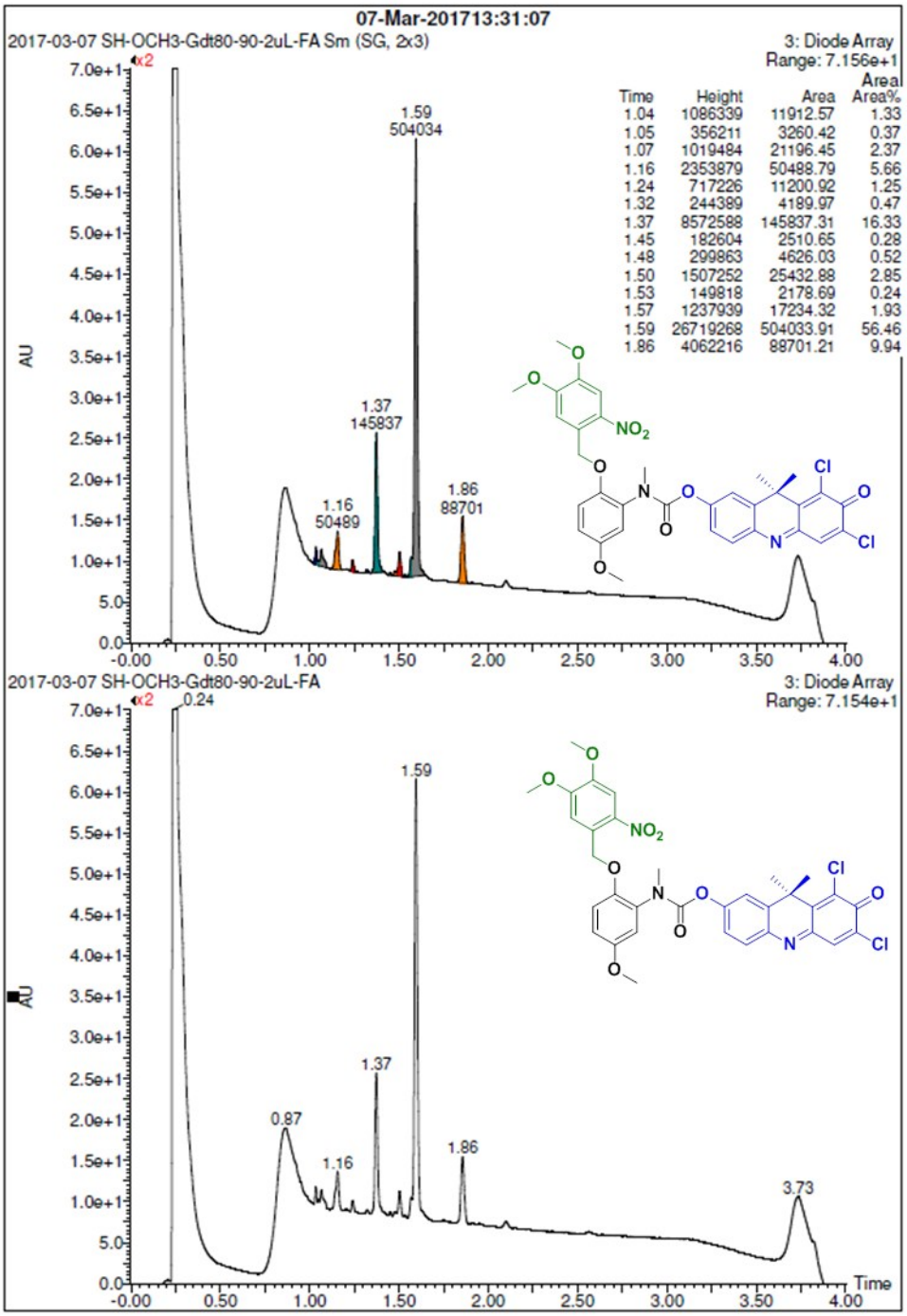


*Figure 93: HPLC spectrum of **2-HPC^{OCH3}**, showing retention time of 1.59 min in 80 to 90% gradient of CH_3CN/water/0.1% formic acid. The purity of the desired compound is 72.8%.*

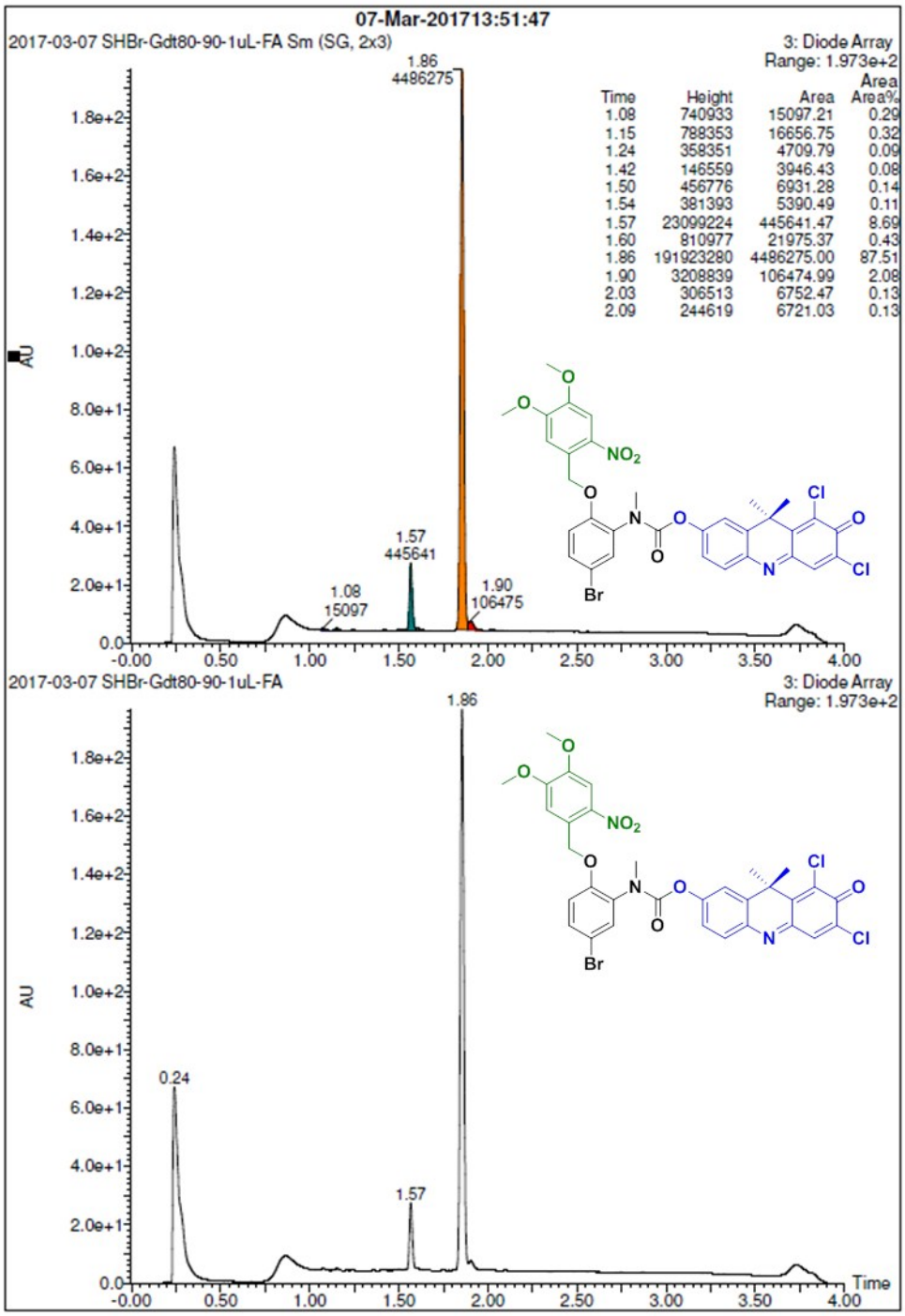


*Figure 94: HPLC spectrum of **2-HPC^{Br}**, showing retention time of 1.96 min in 80 to 90% gradient of CH_3CN/water/0.1% formic acid. The purity of the desired compound is 96.2%.*

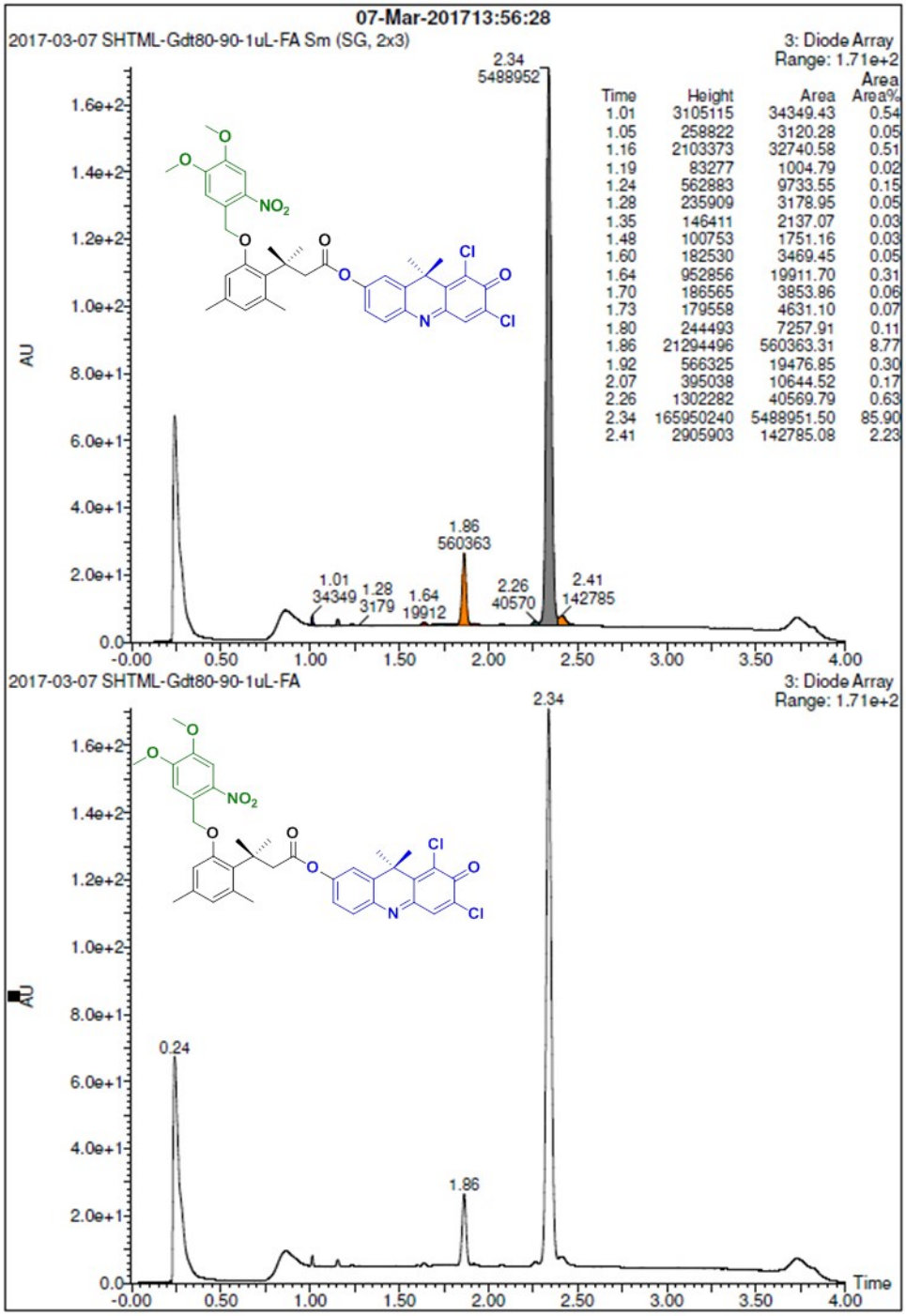


*Figure 95: HPLC spectrum of **TML**, showing retention time of 2.34 min in 80 to 90% gradient of CH_3CN/water/0.1% formic acid. The purity of the desired compound is 94.7%.*