Supplementary Information for:

New AMD3100 derivatives for CXCR4 targeted molecular imaging studies: synthesis, anti-HIV-1 evaluation and binding affinities.

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General Considerations

Ligand purifications and HPLC analysis:
Flash Chromatography was performed using the Automatic Reveleris® Flash Chromatography System (GRACE) equipped with a multiple channel detection (UV (254 nm and/or 215 nm) and ELSD (Evaporative Light Scattering Detection)). Reveleris® 12g Silica Flash Cartridges and Reveleris® C18 RP 12g Cartridges were used for normal and reversed phase liquid chromatography respectively.
RP-HPLC analyses were performed according to the following method: C_{18} column (Merck Chromolith® High Resolution, 50-4.6 mm) with [MeCN/0.1 % TFA] and [H_2O/0.1 % TFA] as eluents [100 % H_2O/0.1 % TFA (2 min), followed by linear gradient from 0 to 100 % (5 min) of [MeCN/0.1 % TFA], a return to initial condition by linear gradient from 100 to 0 % (0.5 min) and 100 % [H_2O/0.1 % TFA] (3 min)] at a flow rate of 1.0 $mL.min^{-1}$.
Semi-preparative RP-HPLC was performed according to the following method: C_8 column (Macherey-Nagel NUCLEODUR, 5 µm, 10 x 250 mm) with [MeCN/0.1 % TFA] and [H_2O/0.1 % TFA] as eluents: linear gradient from 0 to 60 % (45 min) of [MeCN/0.1 % TFA], linear gradient from 60 to 100 % (3 min) of [MeCN/0.1 % TFA], 100 % [MeCN/0.1 % TFA] (4 min), a return to initial condition by linear gradient from 100 to 0 % (2 min) and 100 % [H_2O/0.1 % TFA] (2 min) at a flow rate of 2.7 $mL.min^{-1}$. UV-vis detection with an Ultimate 3000 diode array detector at 214, 254, 280, 300 nm.

NMR, mass spectrometry measurements, photophysic measurements: The ^{1}H, ^{11}B, ^{13}C and ^{19}F NMR spectra were recorded at 298 K, 300 K or 330 K on BRUKER Avance 300, 500, 600 spectrometers using predeuterated solvents as internal standard. Matrix-Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry was carried out using a Bruker Daltonics Proflex III spectrometer and High Resolution Electrospray Mass Spectrometry (HRMS-ESI) was carried out using a LTQ-Orbitrap (Thermo). Absorption spectra were recorded on a JASCO V-630 Bio UV-Vis spectrophotometer. Emission and excitation spectra were recorded on a JASCO FP-8500 fluorescence spectrometer.

Elemental Analysis: The elemental analyses were performed with Fisons EA-1108 CHNS elemental analyzer instrument.

Quantum yield measurements : Measurement were performed in a solution of PBS (phosphate buffer saline) + 0.05 % NaN3. The sample concentration were chosen to obtain an absorbance included between 0.08 and 0.05. The fluorescence quantum yield (Φ_F) measurements were performed with a slit width of 5-5 nm for compound **10** and **12** and 5-2.5 nm for compound **11** for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectra of the sample relative to a known standard and the following equation was used to calculate Φ_F :

$$\Phi_F(sample) = \Phi_F(standard) \times \left(\frac{I\,(sample)}{I\,(standard)}\right) \times \left(\frac{A\,(standard)}{A(sample)}\right) \times \left(\frac{n\,(sample)^2}{n(standard)^2}\right)$$

where Φ_F is the reported quantum yield of the standard, I is the integrated emission spectrum, A is the absorbance at the excitation wavelenght and n is the refractive index of the solvents used. Rhodamine 6G (Φ_F = 0.78 in water, λ_{exc} = 488 nm) was used as the standard.

Characterization of compounds 1 to 15

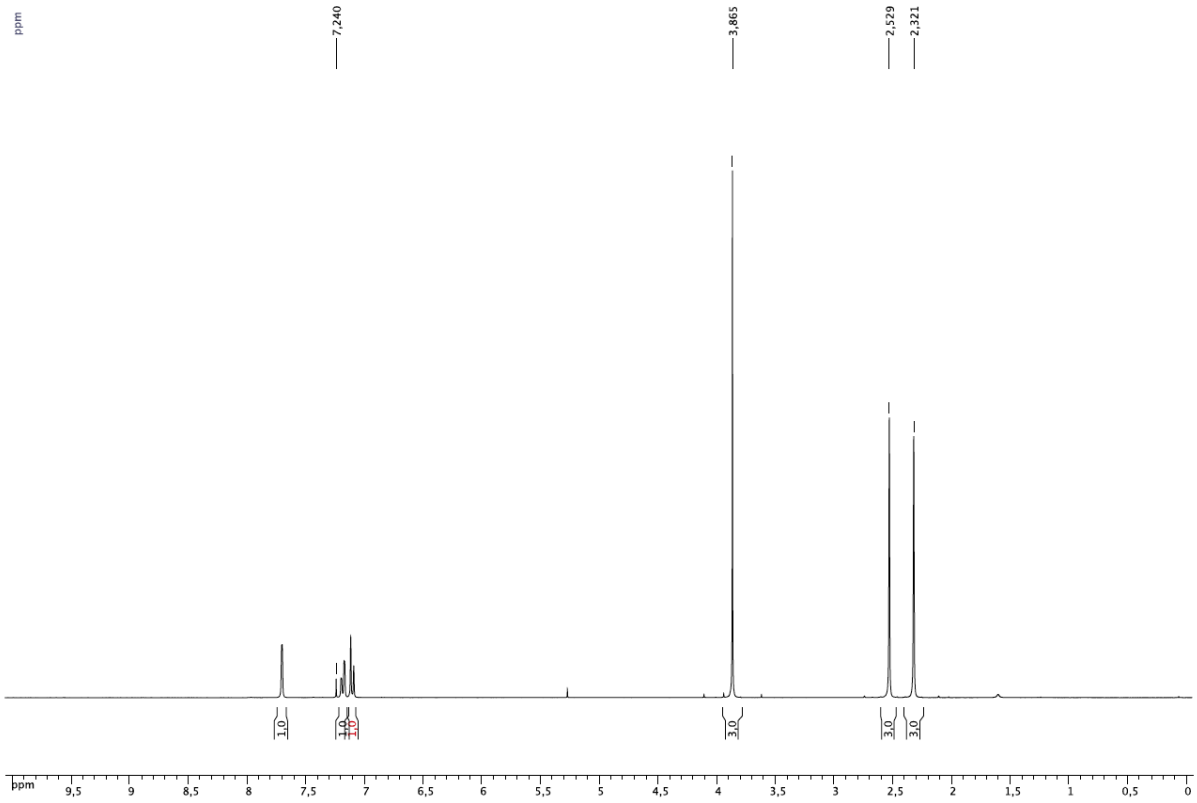


Figure 1 : ^{1}H NMR spectrum of compound **1** ($CDCl_3$, 300 MHz, 300 K)

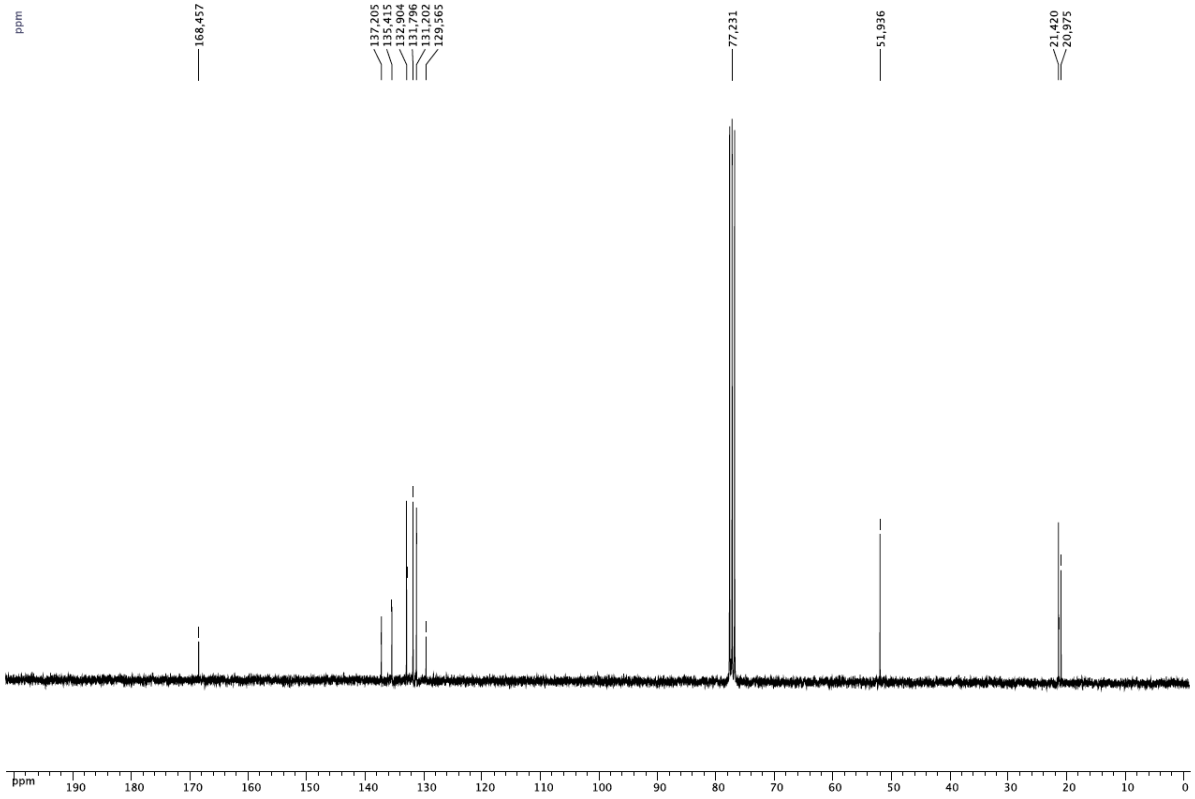


Figure 2 : $^{13}C\ \{^{1}H\}$ NMR spectrum of compound **1** ($CDCl_3$, 75 MHz, 300 K)

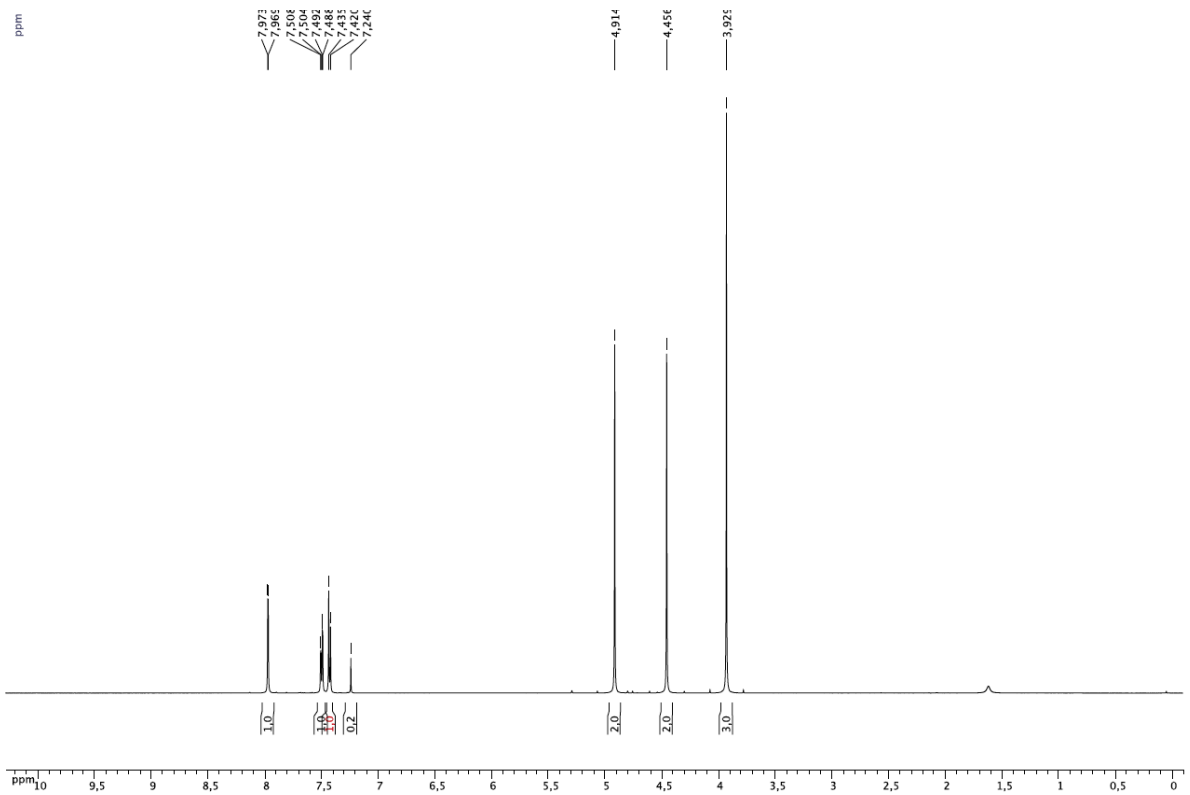


Figure 3 : ^{1}H NMR spectrum of compound **2** ($CDCl_3$, 500 MHz, 300 K)

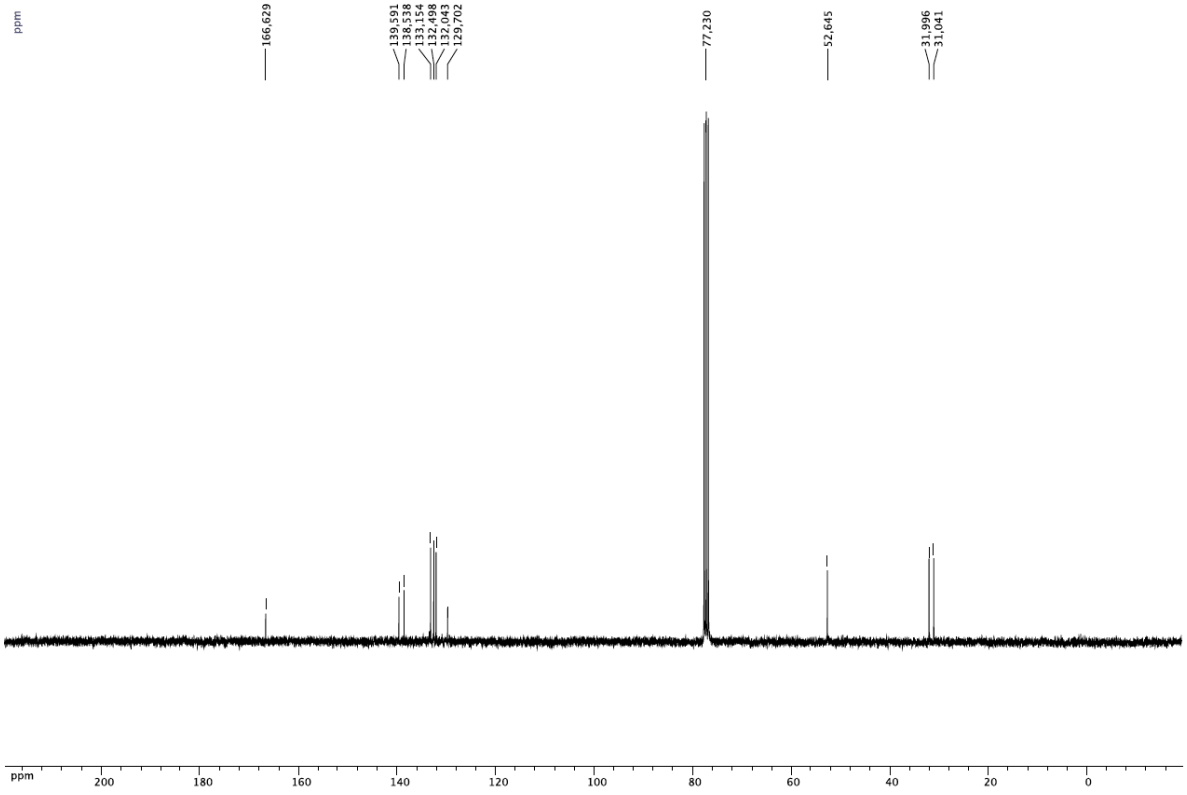


Figure 4 : $^{13}C\ \{^{1}H\}$ NMR spectrum of compound **2** ($CDCl_3$, 75 MHz, 300 K)

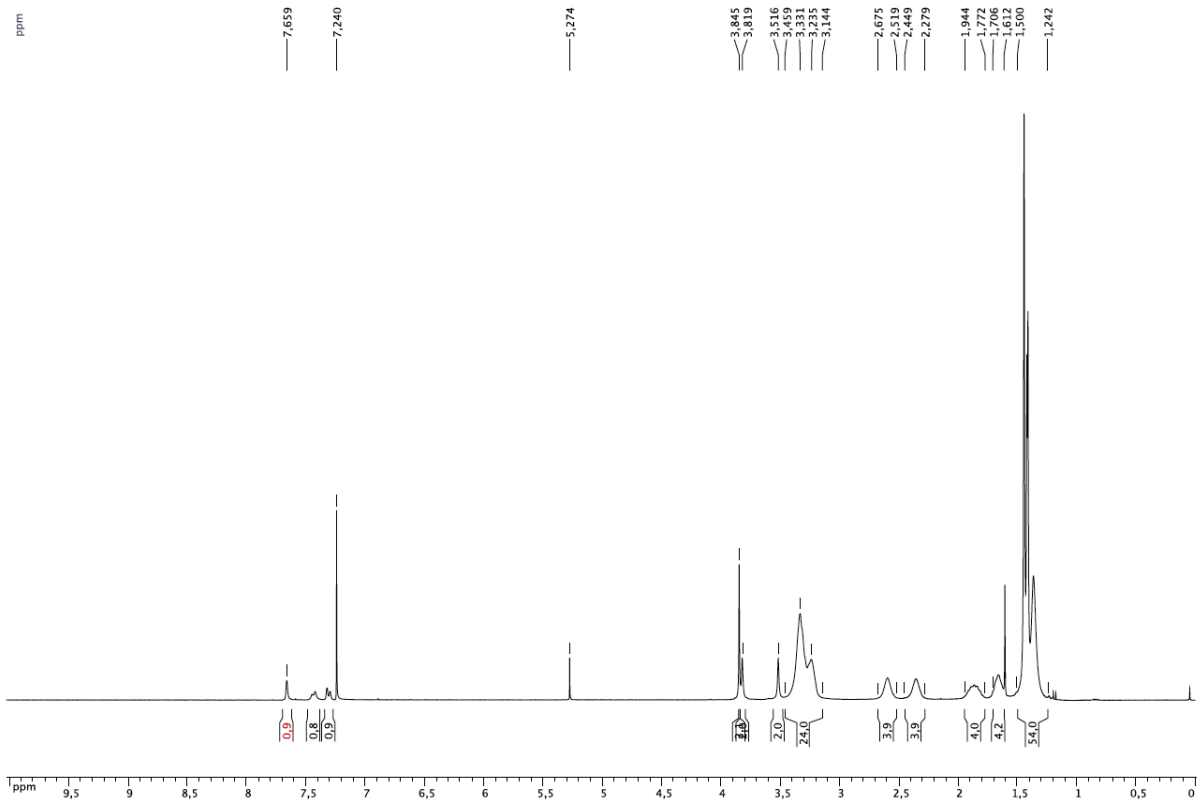


Figure 5 : ^{1}H NMR spectrum of compound **3** ($CDCl_3$, 300 MHz, 300 K)

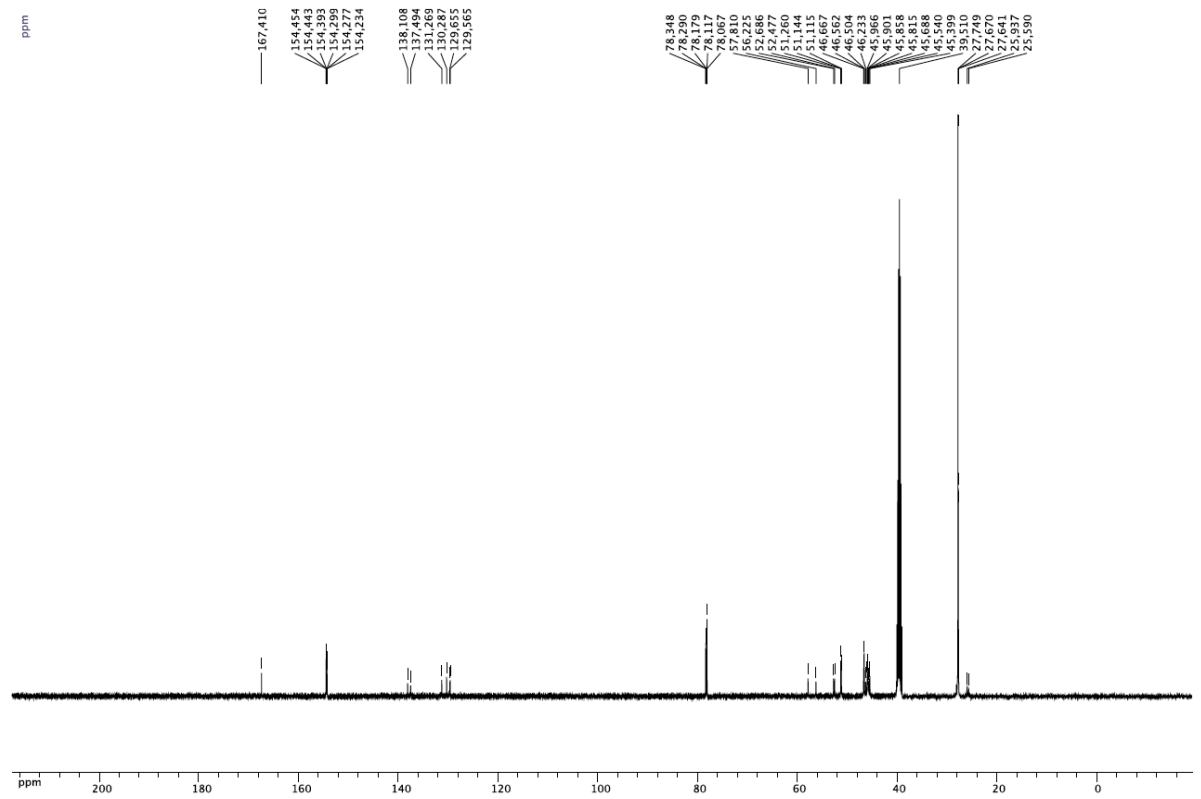
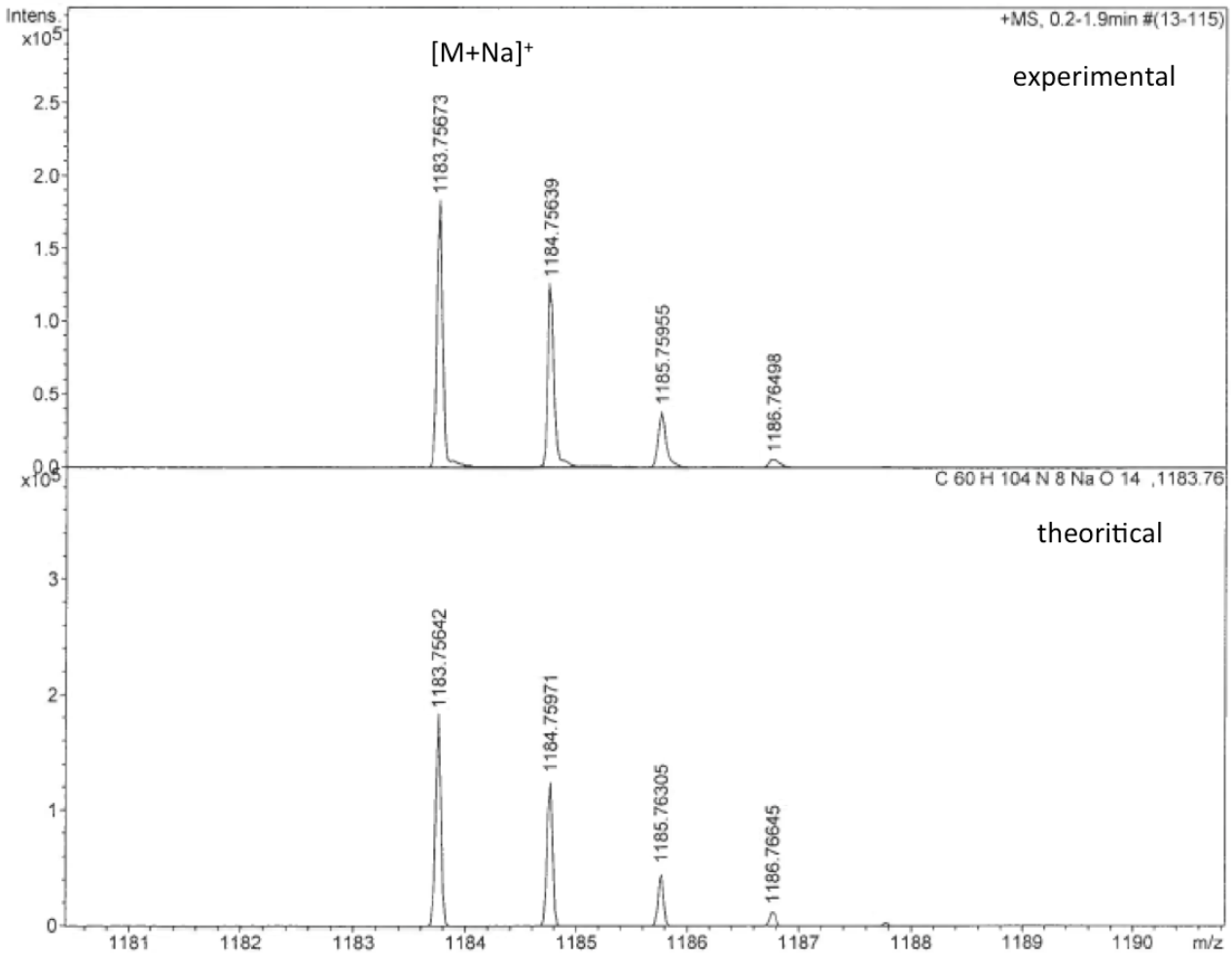
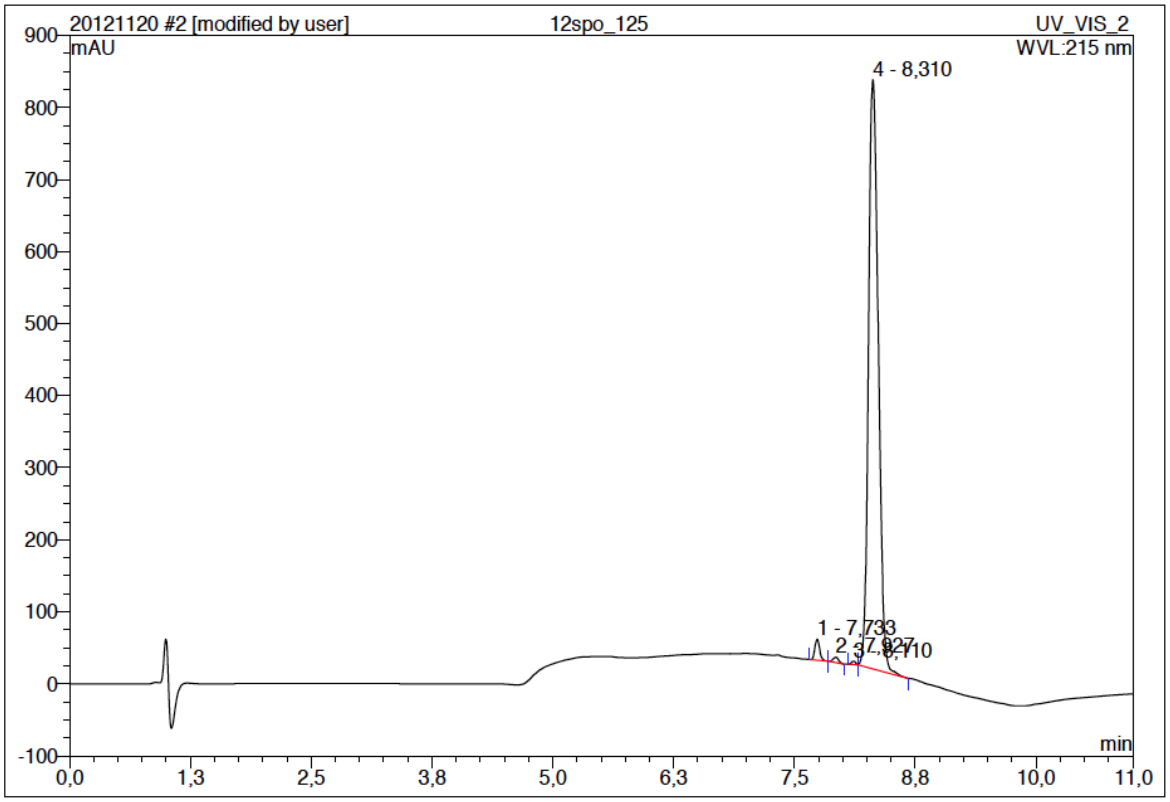


Figure 6 : ^{13}C {^{1}H} NMR spectrum of compound **3** (DMSO, 125 MHz, 343 K)



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1183.75673	1	C 60 H 104 N 8 Na O 14	100.00	1183.75642	-0.3	-0.3	30.7	12.5	even	ok

Figure 7 : HR-MS (ESI) analysis of compound **3**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7,73	n.a.	28,985	1,688	1,72	n.a.	BMB
2	7,93	n.a.	7,328	0,498	0,51	n.a.	BMB
3	8,11	n.a.	4,444	0,209	0,21	n.a.	BMb
4	8,31	n.a.	817,450	95,983	97,57	n.a.	bMB
Total:			858,207	98,378	100,00	0,000	

Figure 8 : HPLC analysis of compound **3**

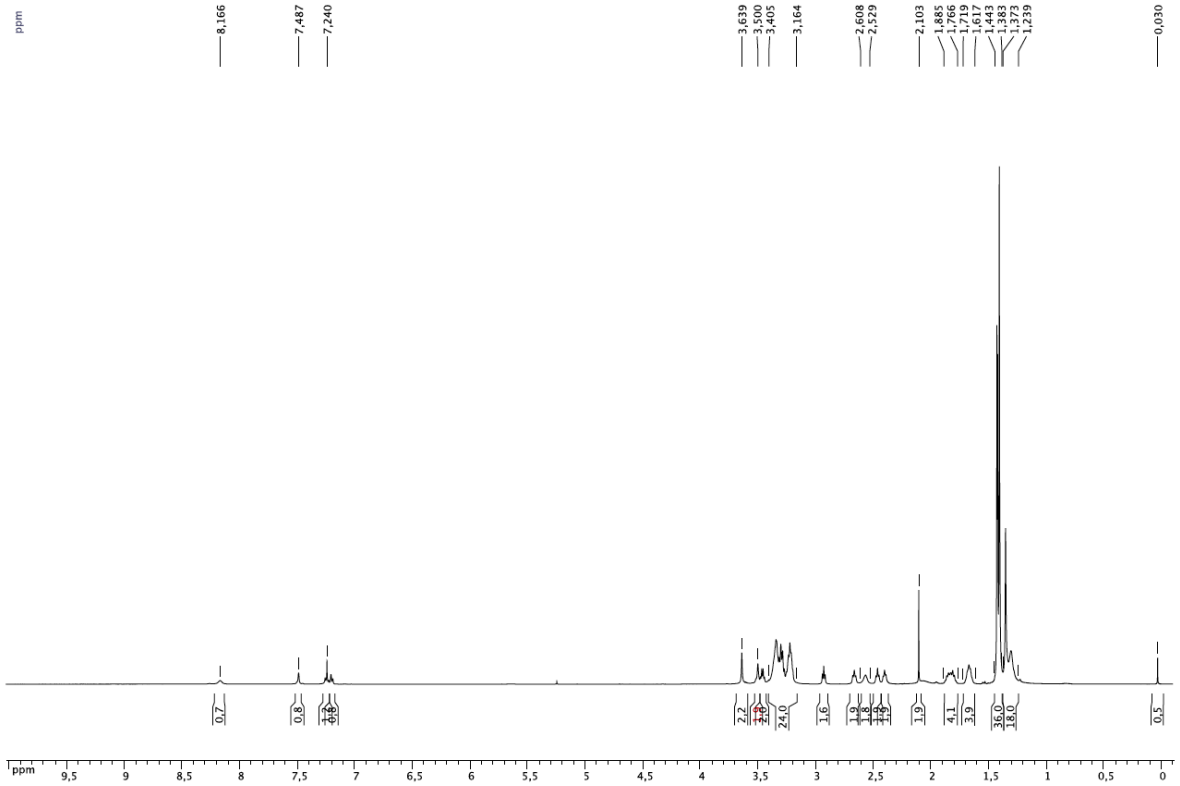


Figure 9 : ^{1}H NMR spectrum of compound **4** ($CDCl_3$, 500 MHz, 324 K)

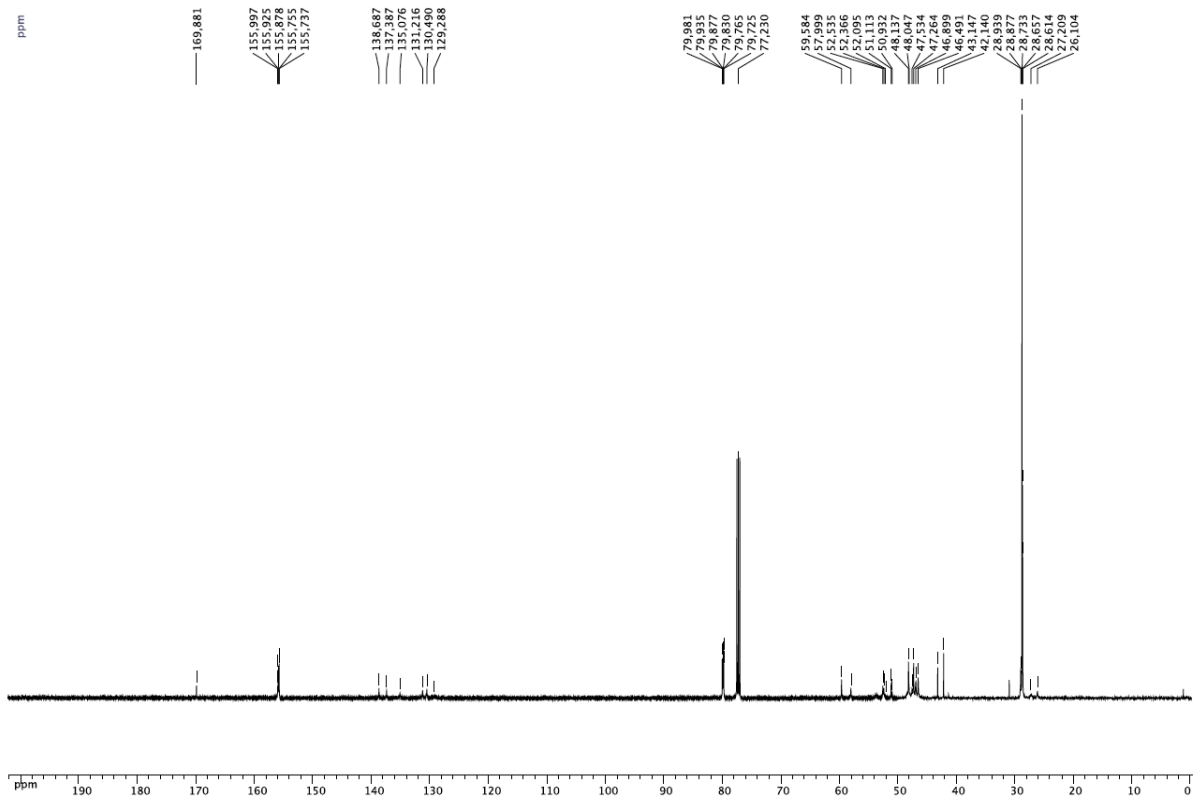


Figure 10 : ^{13}C {^{1}H} NMR spectrum of compound **4** ($CDCl_3$, 500 MHz, 324 K)

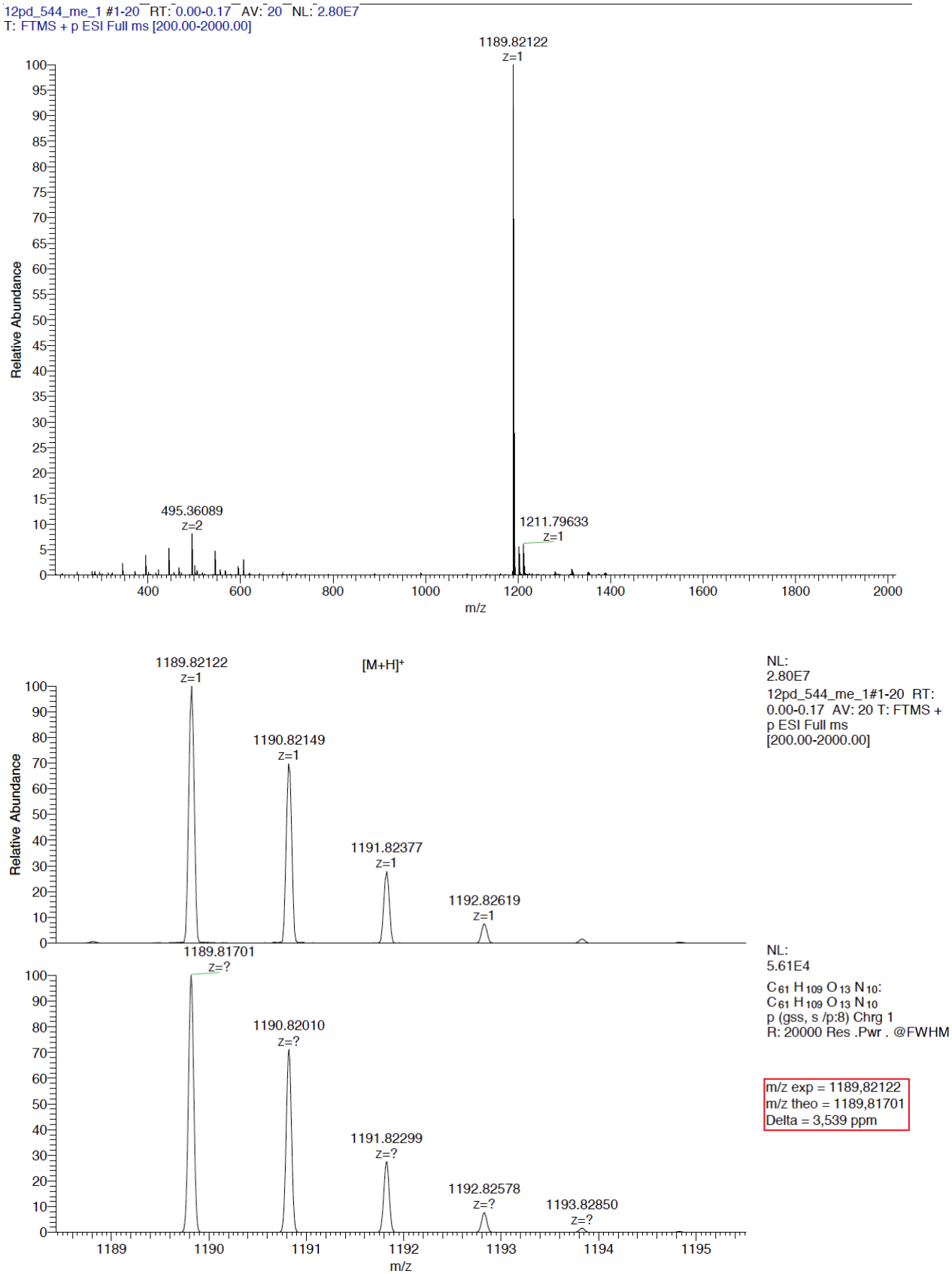
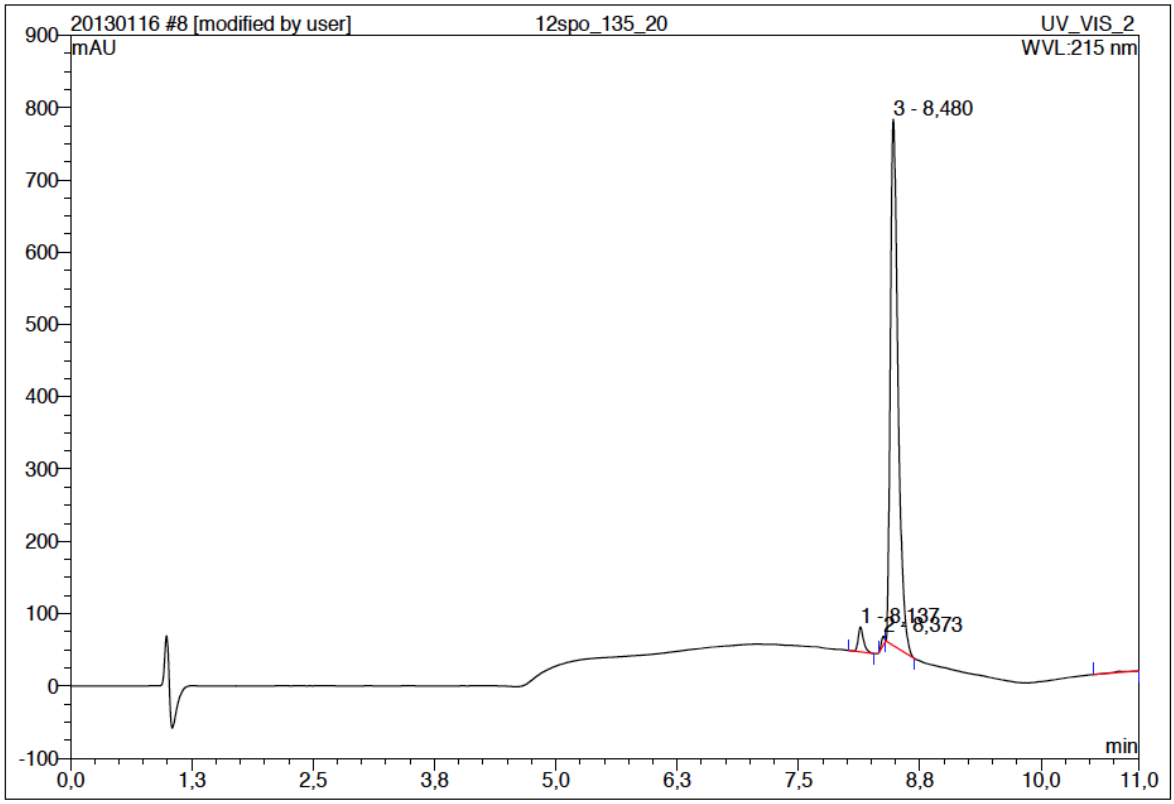


Figure 11 : HR-MS (ESI) analysis of compound **4**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8,14	n.a.	34,403	2,197	3,17	n.a.	BMB*
2	8,37	n.a.	11,687	0,442	0,64	n.a.	BMb
3	8,48	n.a.	728,289	66,538	95,86	n.a.	bMB
4	11,00	n.a.	0,000	0,232	0,33	n.a.	BMB
Total:			774,379	69,410	100,00	0,000	

Figure 12 : HPLC analysis of compound **4**

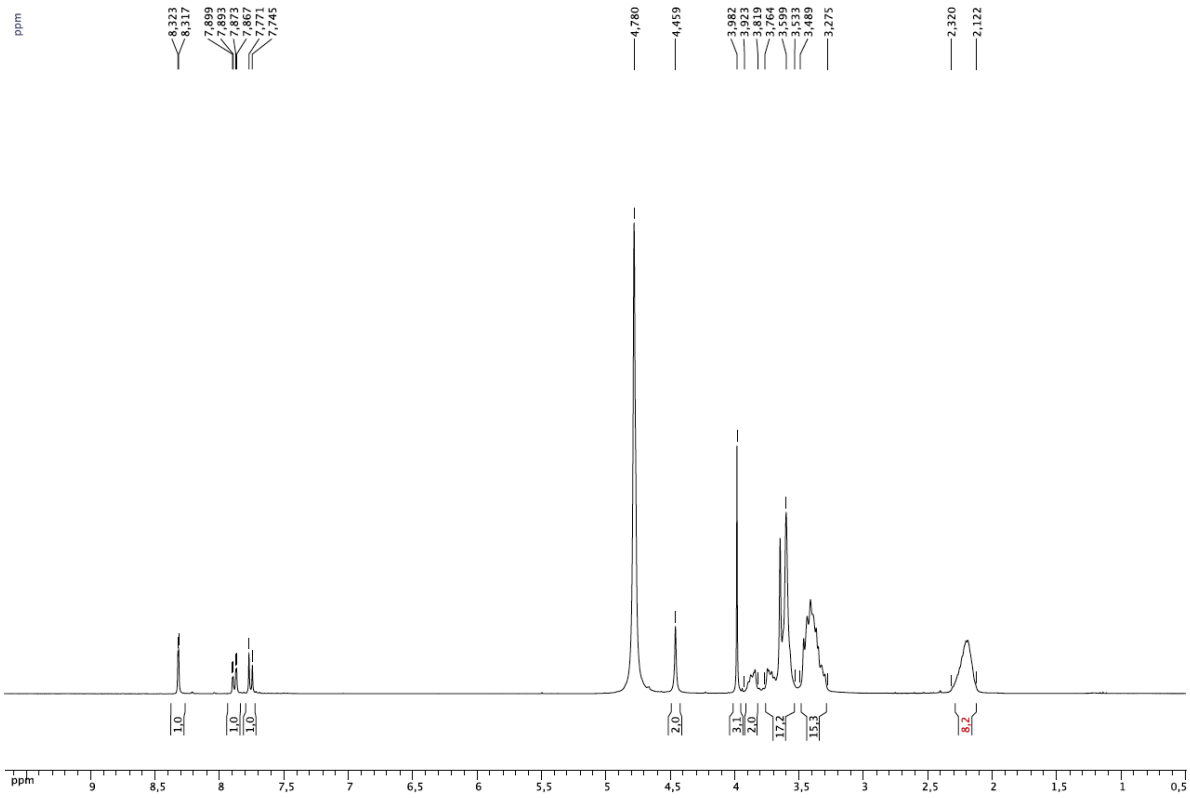


Figure 13 : ^{1}H NMR spectrum of compound **5** (D_2O, 300 MHz, 300 K)

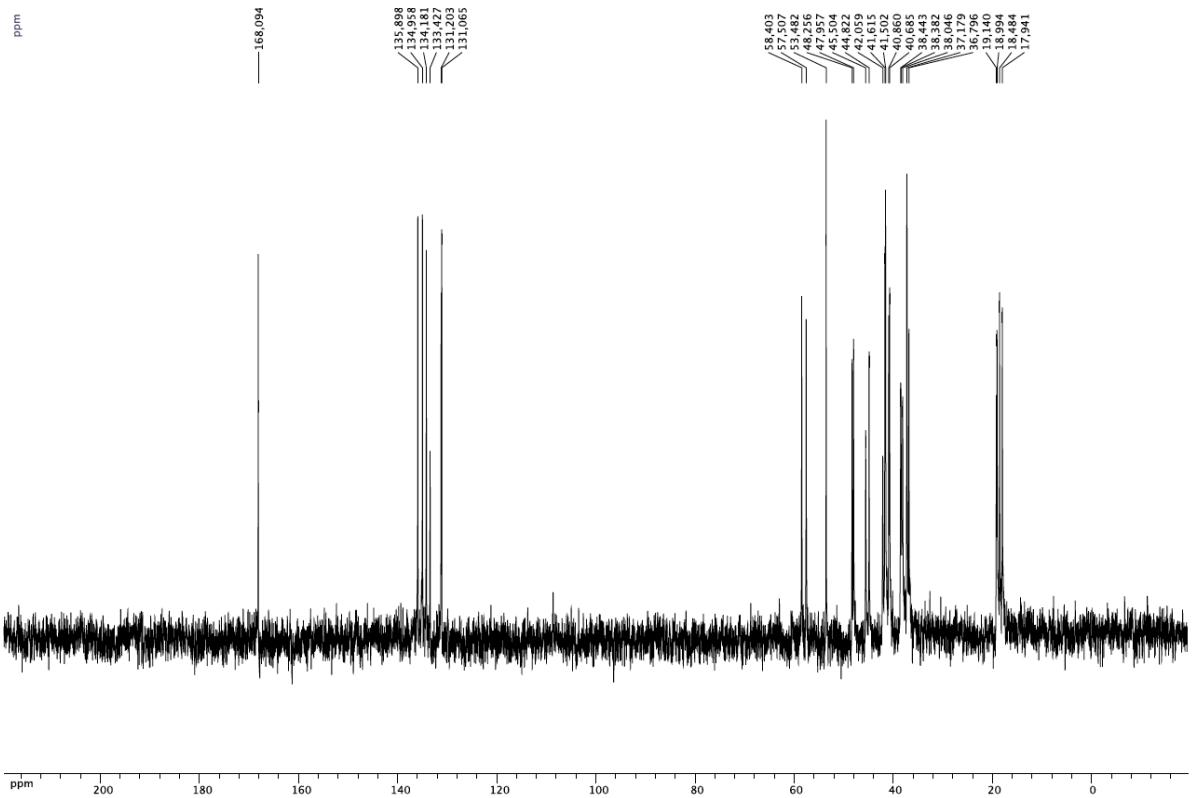


Figure 14 : ^{13}C {^{1}H} NMR spectrum of compound **5** (D_2O, 300 MHz, 300 K)

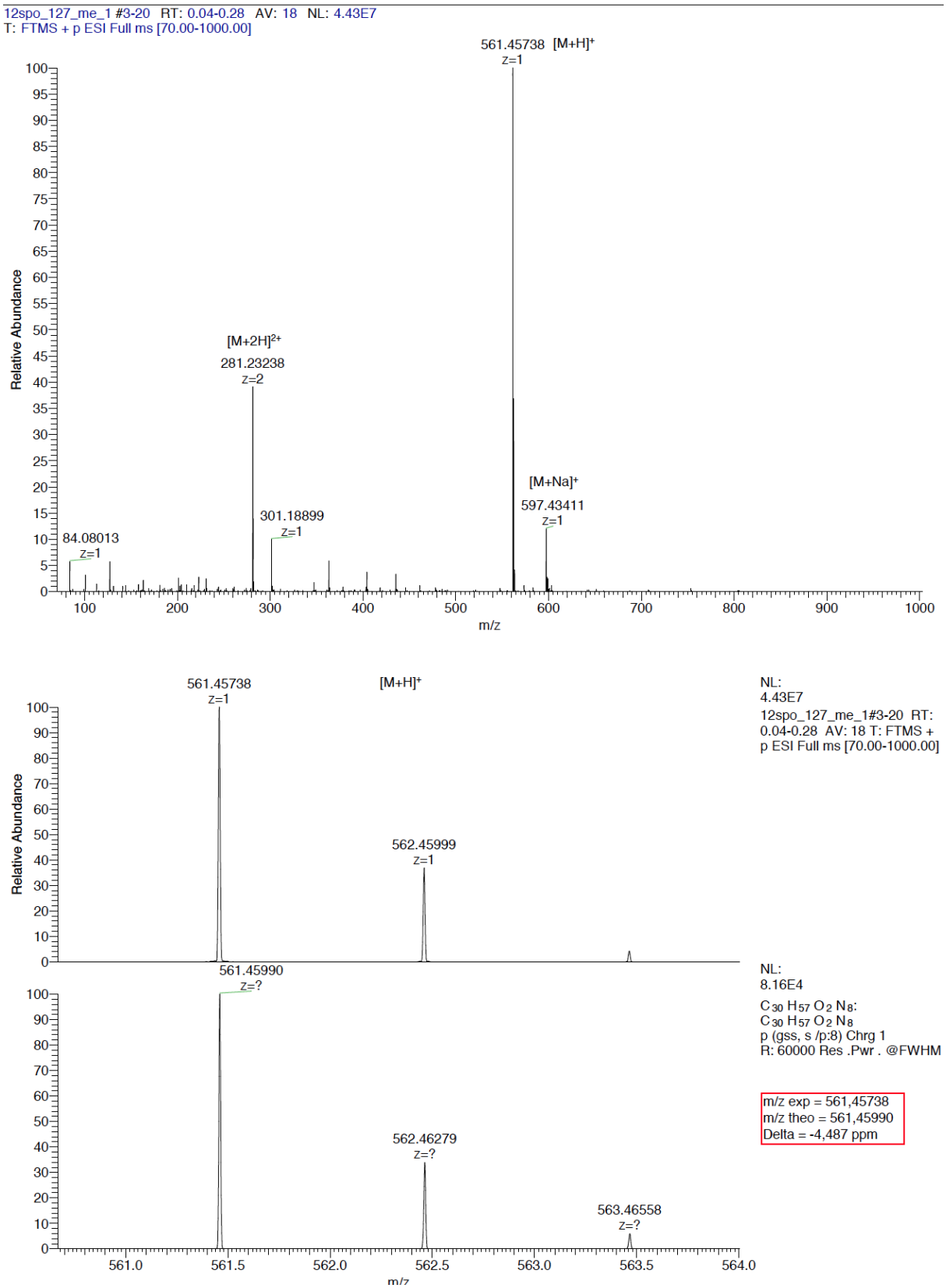
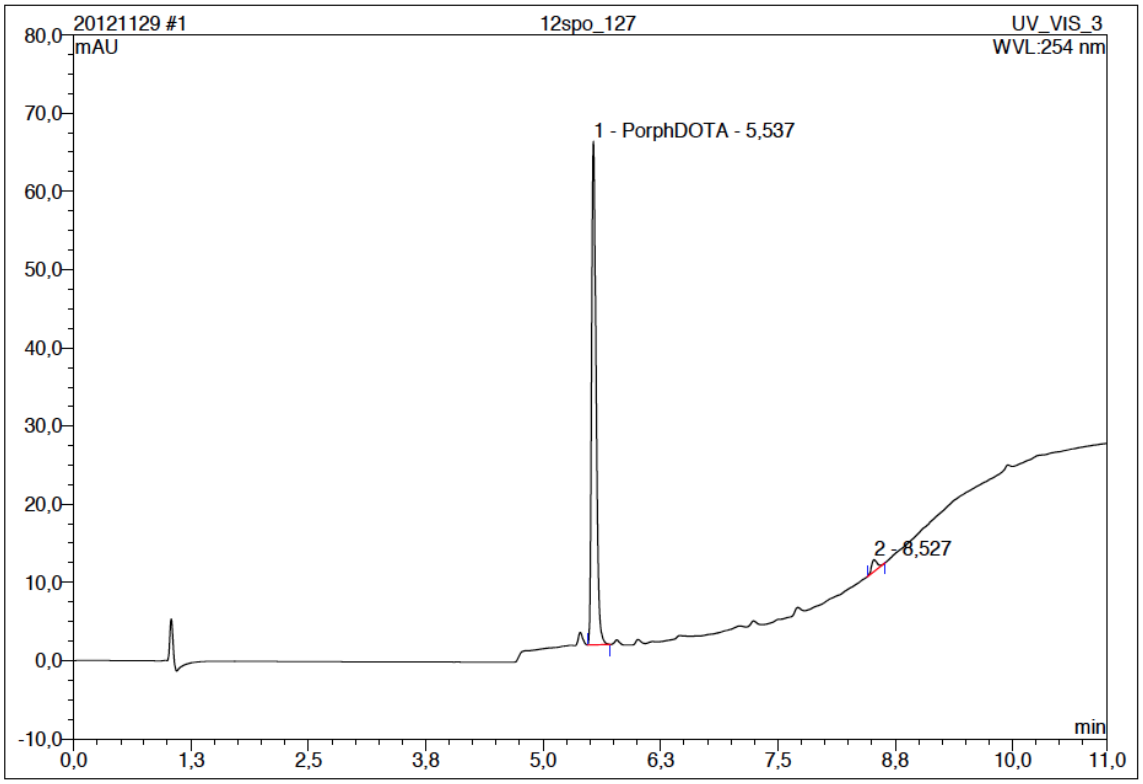


Figure 15 : HR-MS (ESI) analysis of compound **5**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,54	PorphDOTA	64,358	3,553	97,22	n.a.	BMB
2	8,53	n.a.	1,471	0,101	2,78	n.a.	BMB
Total:			65,829	3,654	100,00	0,000	

Figure 16 : HPLC analysis of compound **5**

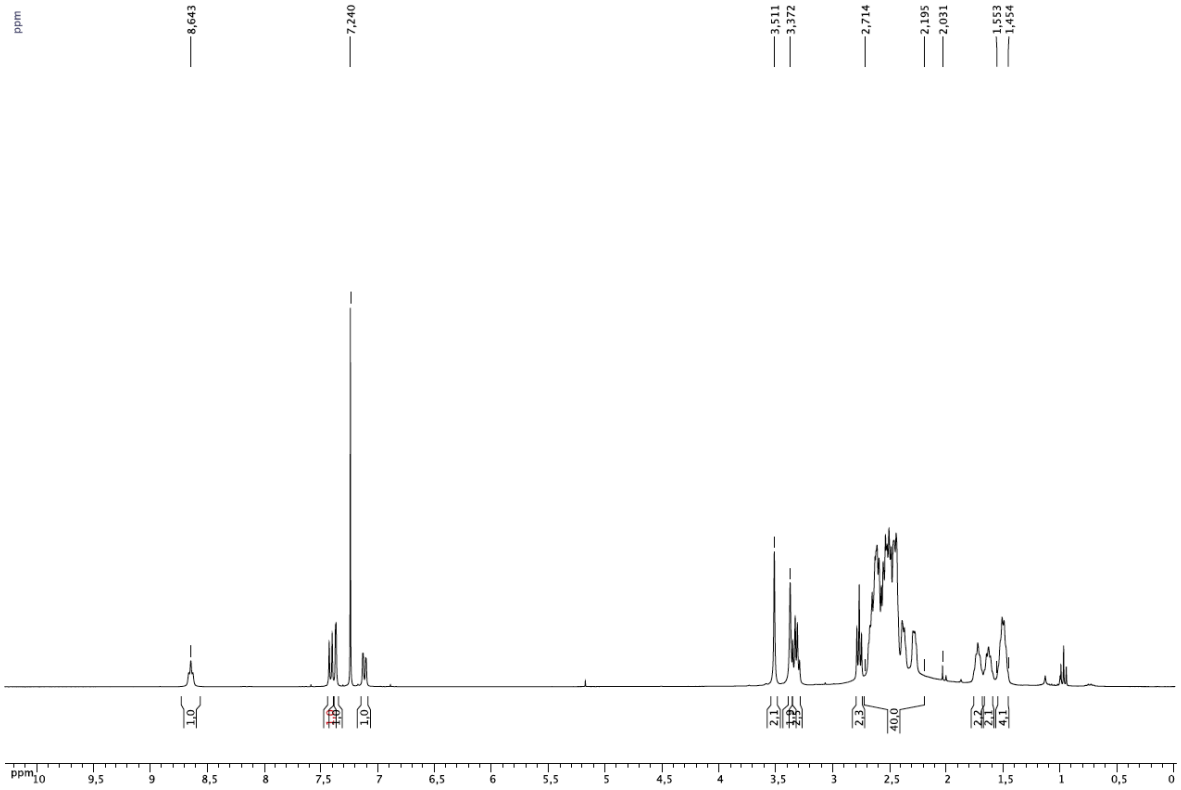


Figure 17 : ^{1}H NMR spectrum of compound **6** ($CDCl_3$, 300 MHz, 300 K)

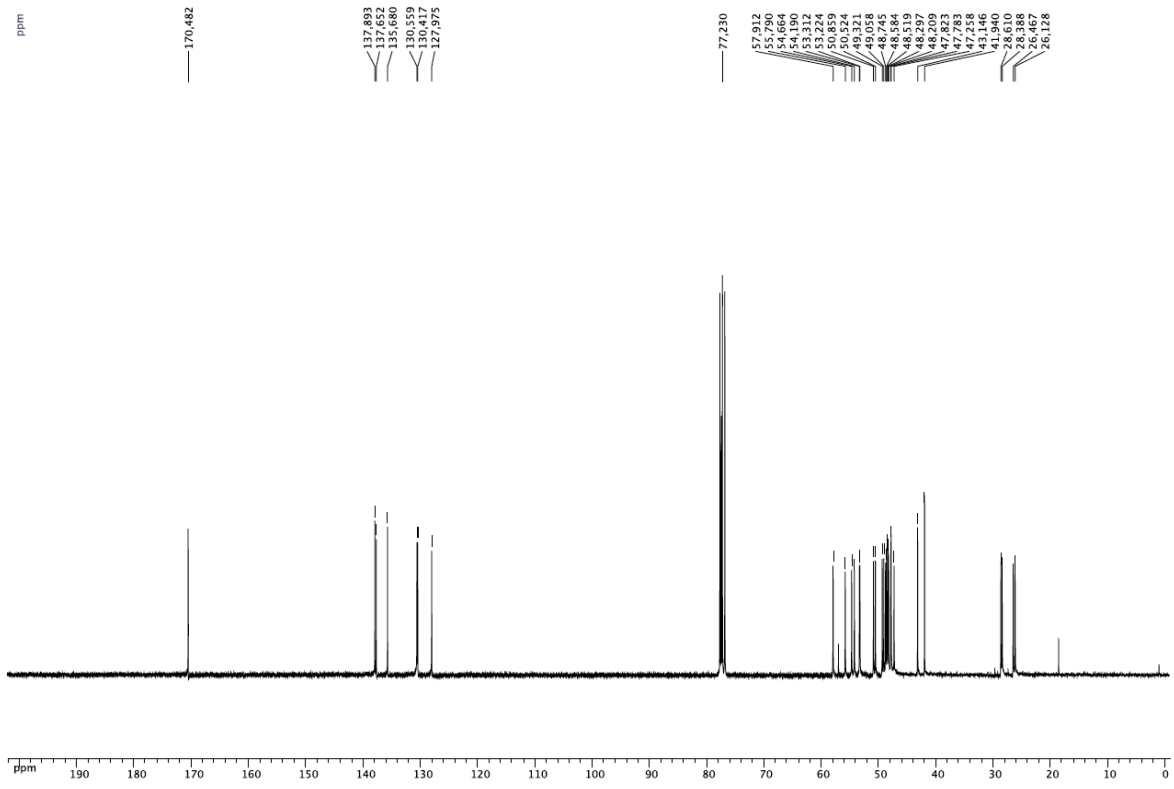
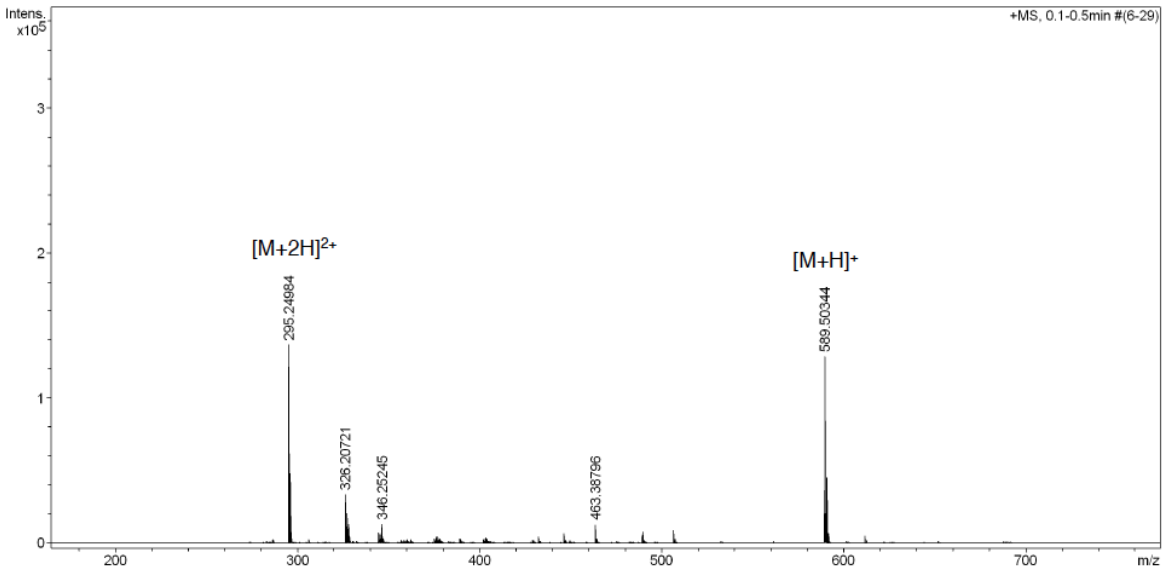
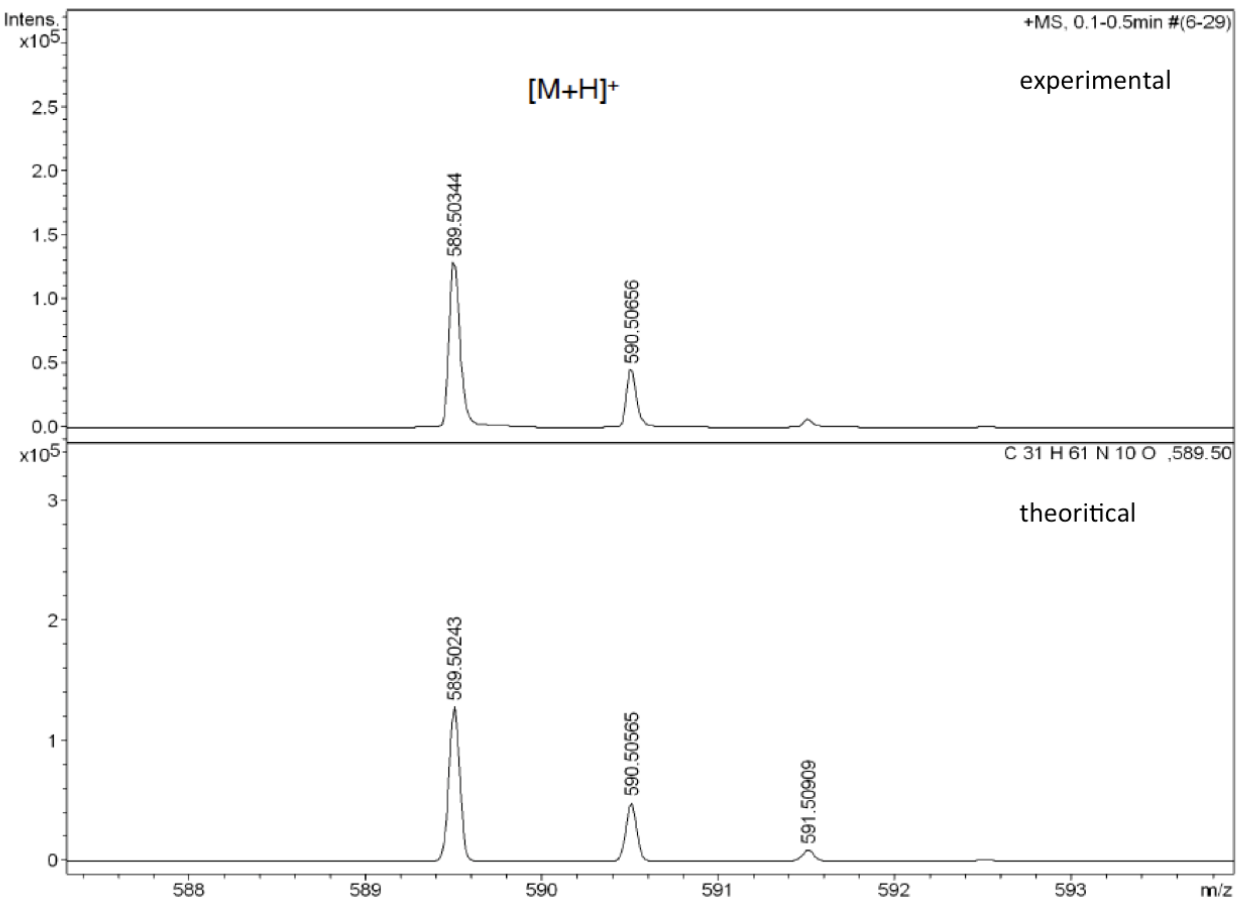


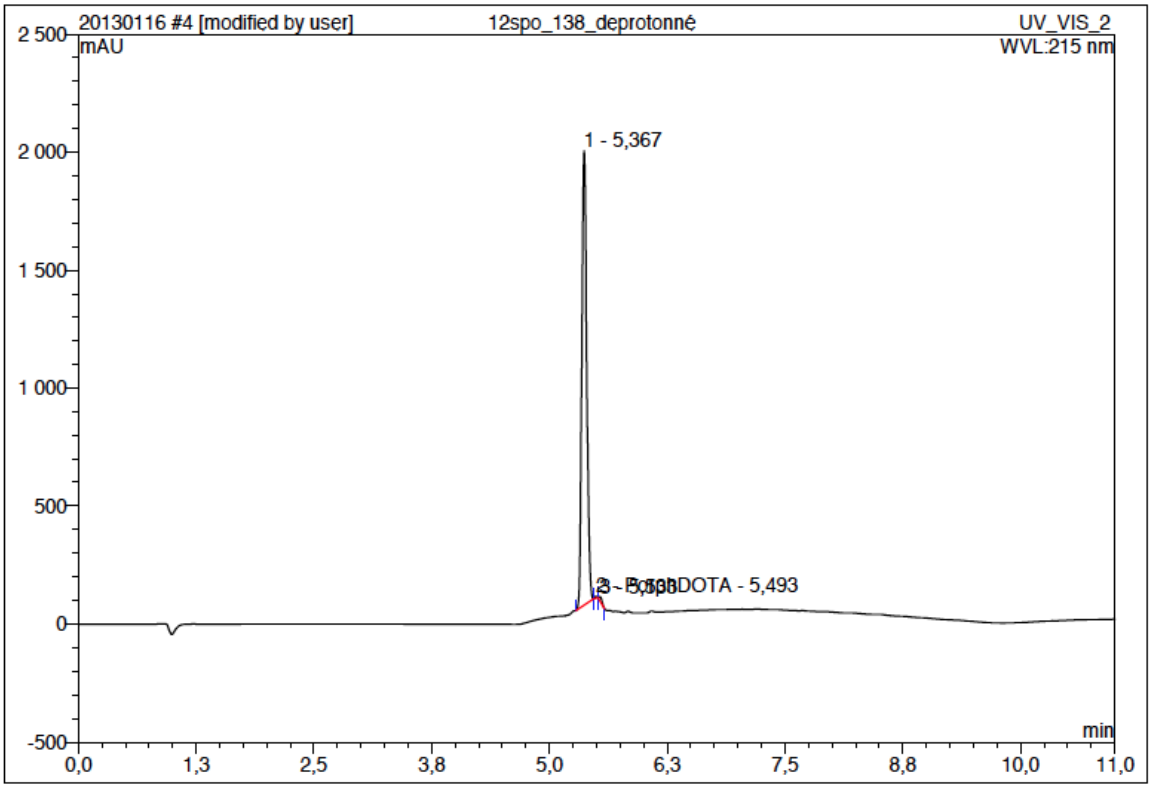
Figure 18 : $^{13}C\ \{^{1}H\}$ NMR spectrum of compound **6** ($CDCl_3$, 300 MHz, 300 K)





Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
589.50344	1	C 31 H 61 N 10 O	100.00	589.50243	-1.0	-1.7	38.0	6.5	even	ok

Figure 19 : HR-MS (ESI) analysis of compound **6**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,37	n.a.	1924,809	119,738	99,29	n.a.	BMb*
2	5,49	PorphDOTA	8,751	0,211	0,17	n.a.	bMb
3	5,53	n.a.	15,780	0,651	0,54	n.a.	bMB
Total:			1949,340	120,599	100,00	0,000	

Figure 20 : HPLC analysis of compound **6**

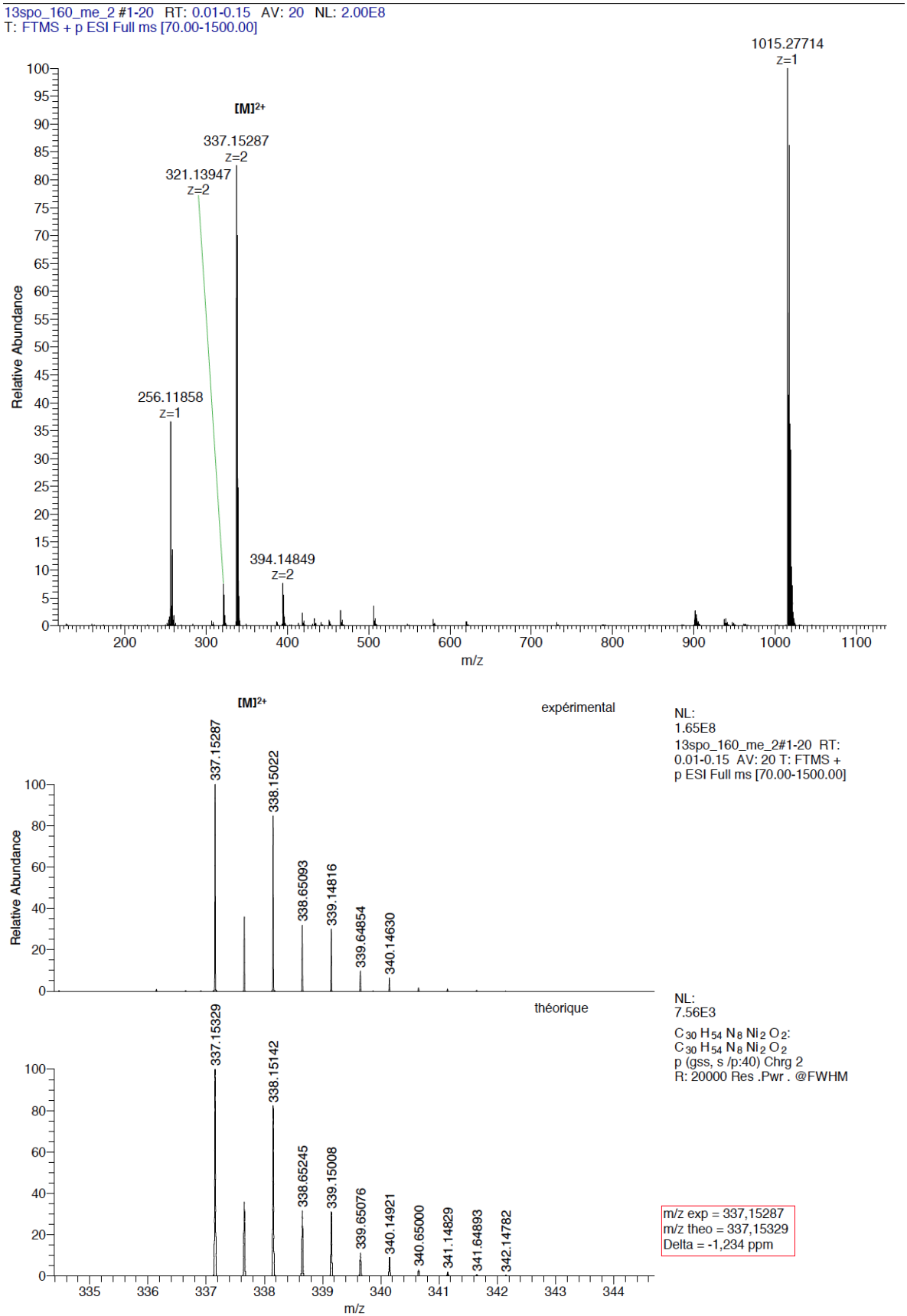
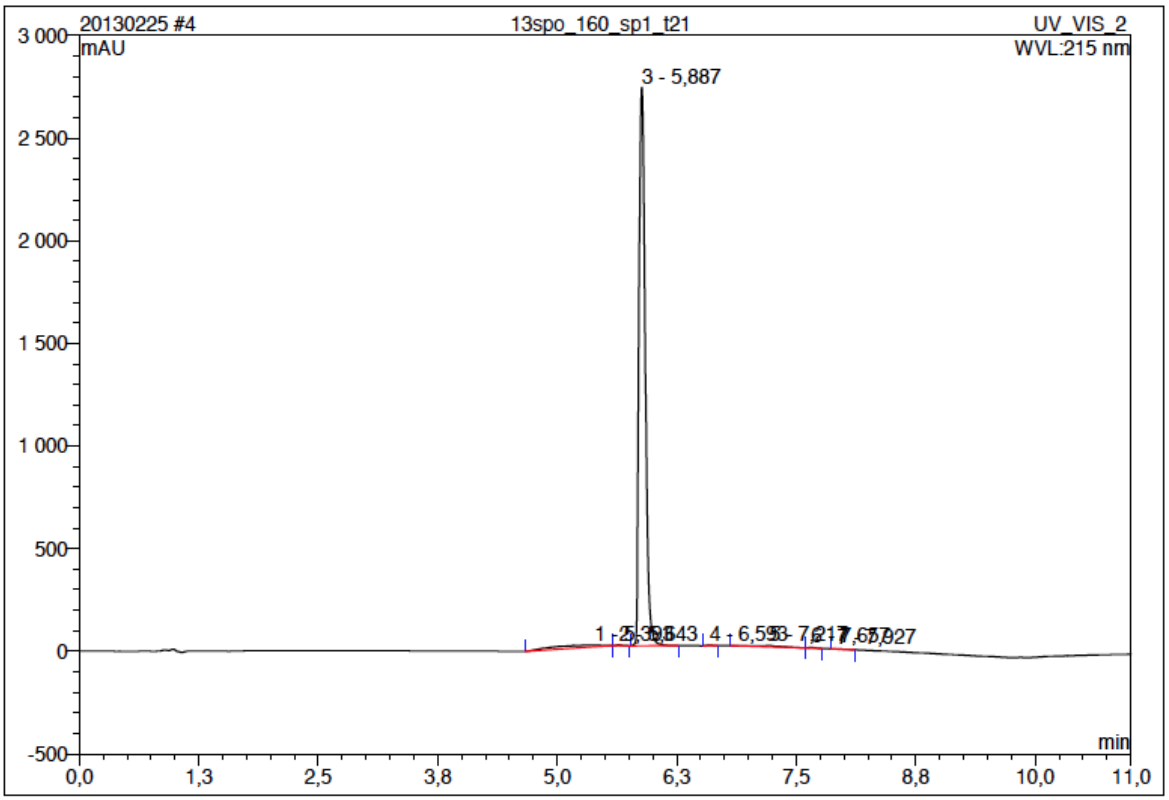


Figure 21 : HR-MS (ESI) analysis of compound **7**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,39	n.a.	7,765	7,487	3,48	n.a.	BMb
2	5,64	n.a.	3,352	0,208	0,10	n.a.	bMB
3	5,89	n.a.	2720,563	205,104	95,38	n.a.	BMB
4	6,59	n.a.	1,909	0,141	0,07	n.a.	BMB
5	7,22	n.a.	5,913	1,658	0,77	n.a.	BMb
6	7,66	n.a.	3,662	0,285	0,13	n.a.	bMB
7	7,93	n.a.	1,375	0,166	0,08	n.a.	BMB
Total:			2744,539	215,049	100,00	0,000	

Figure 22 : HPLC analysis of compound **7**

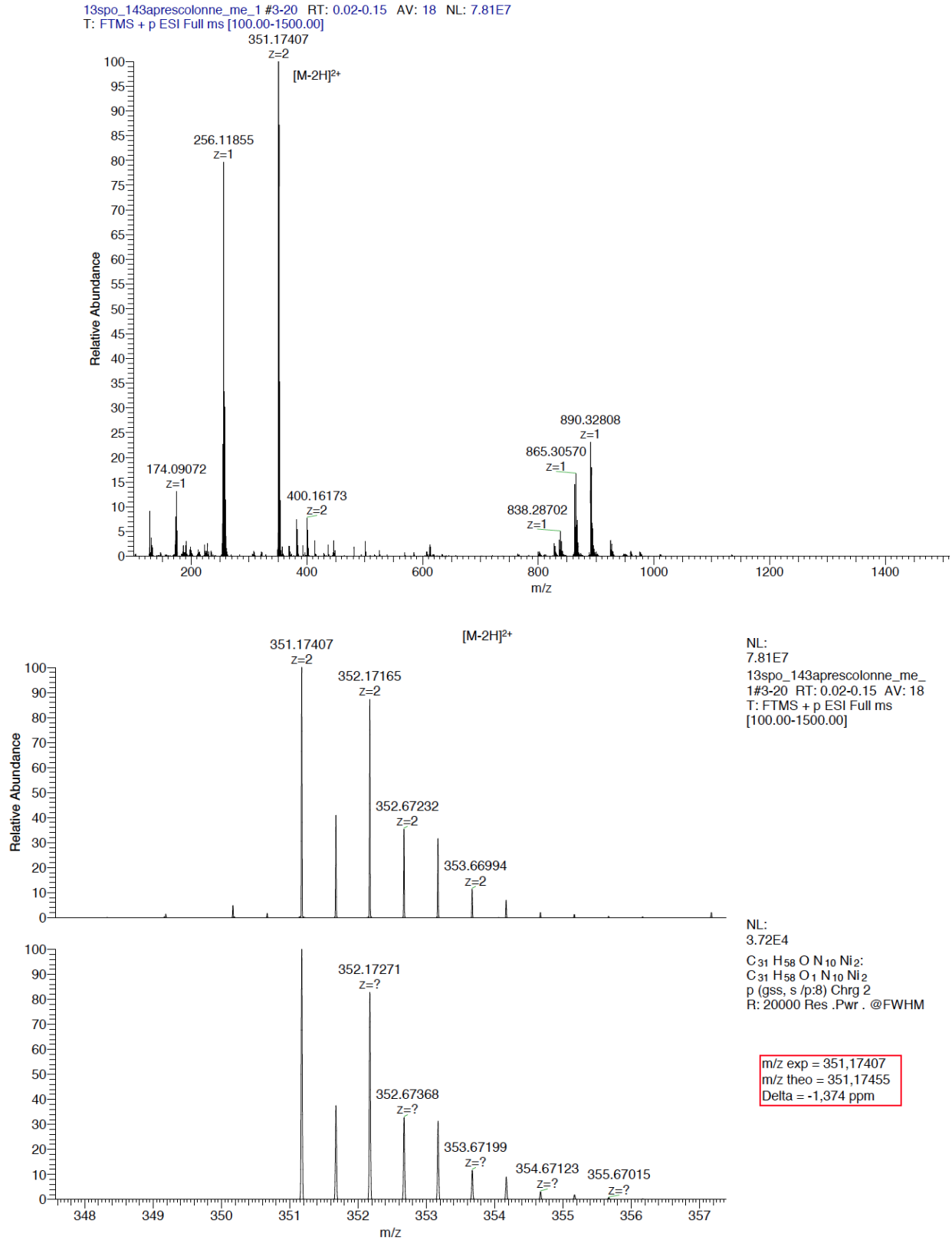
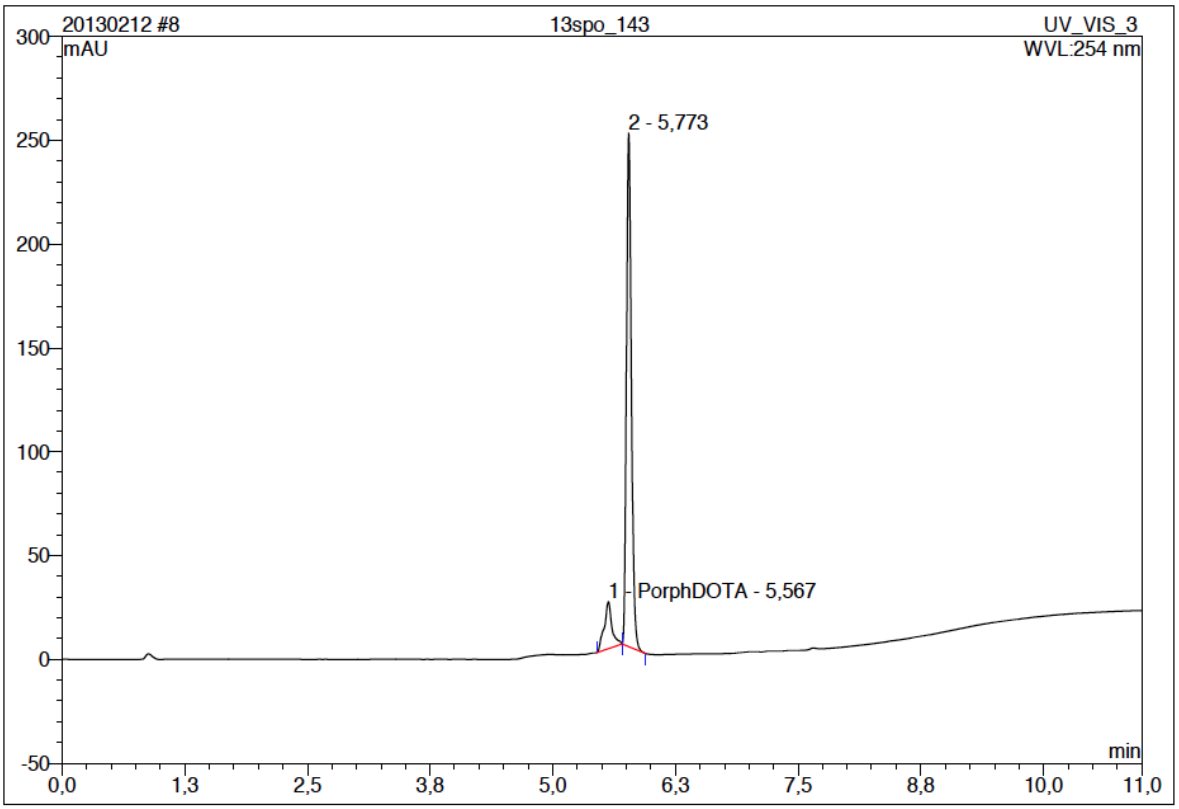


Figure 23 : HR-MS (ESI) analysis of compound **8**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,57	PorphDOTA	22,521	2,053	12,45	n.a.	BMb
2	5,77	n.a.	247,118	14,441	87,55	n.a.	bMB
Total:			269,640	16,494	100,00	0,000	

Figure 24 : HPLC analysis of compound **8**

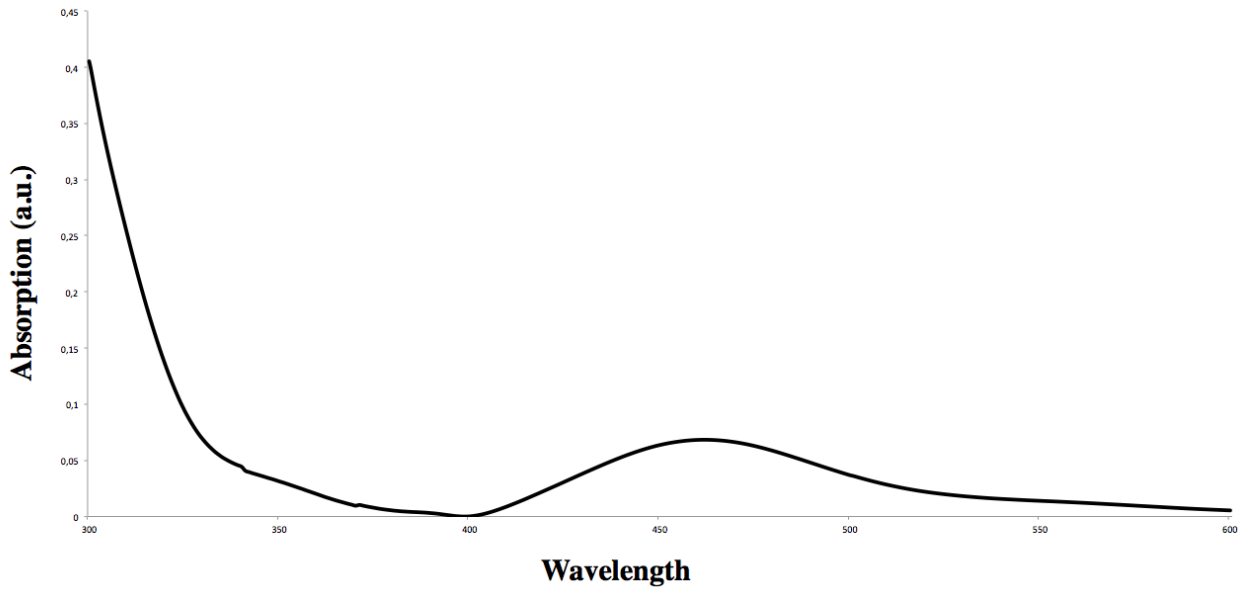


Figure 25 : Absorption spectra of compound **8** in PBS.

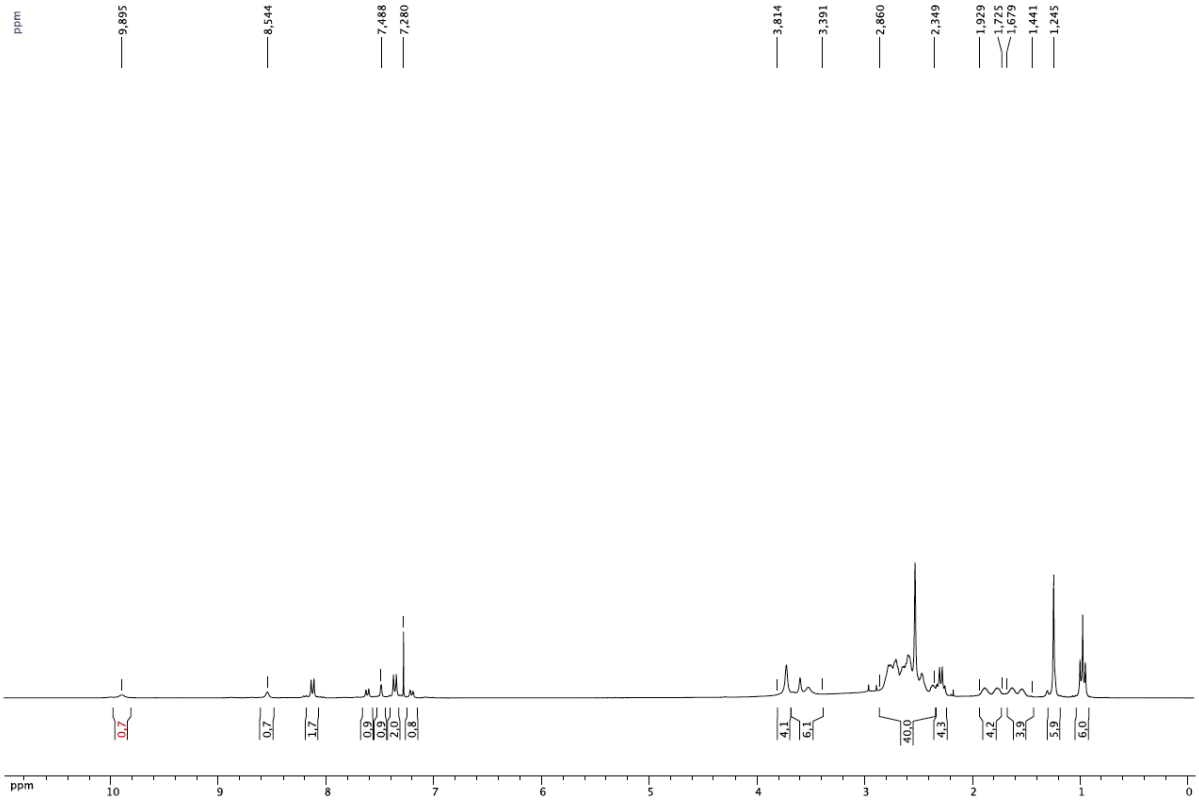


Figure 26 : ^{1}H NMR spectrum of compound **10** ($CDCl_3$, 300 MHz, 300 K)

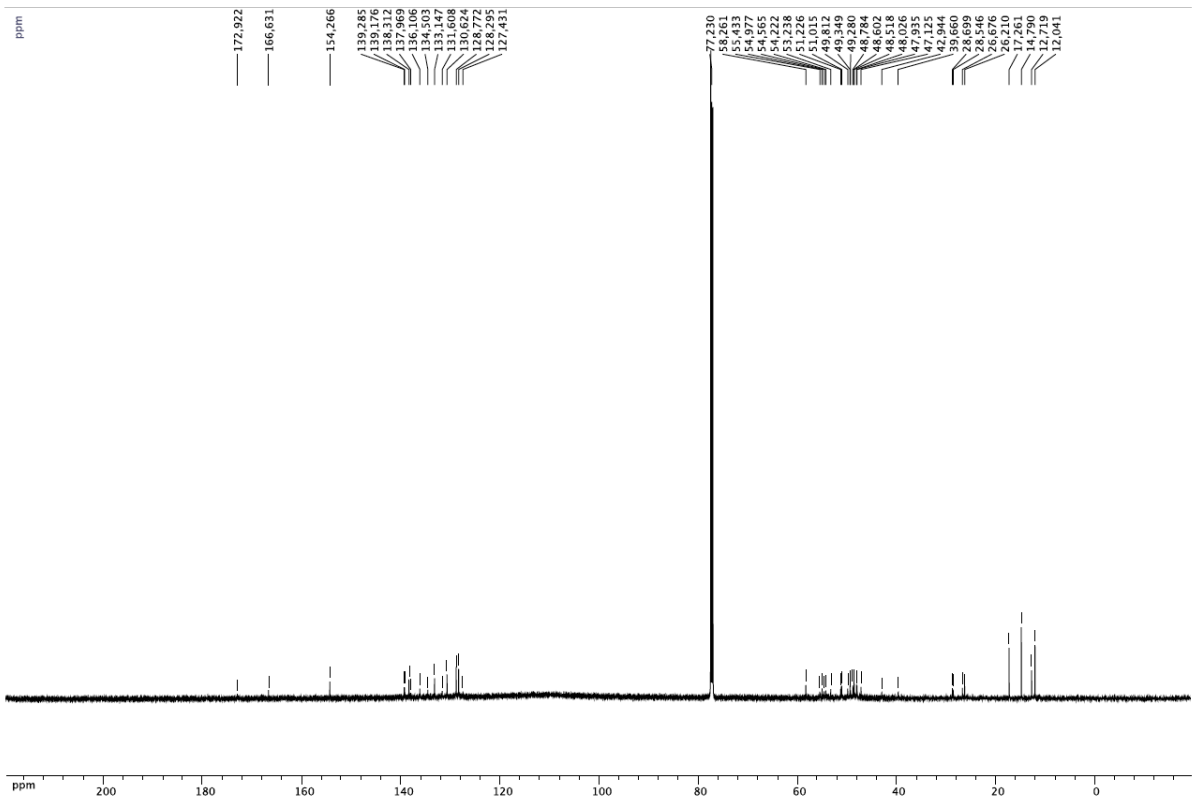


Figure 27 : ^{13}C {^{1}H} NMR spectrum of compound **10** ($CDCl_3$, 300 MHz, 300 K)

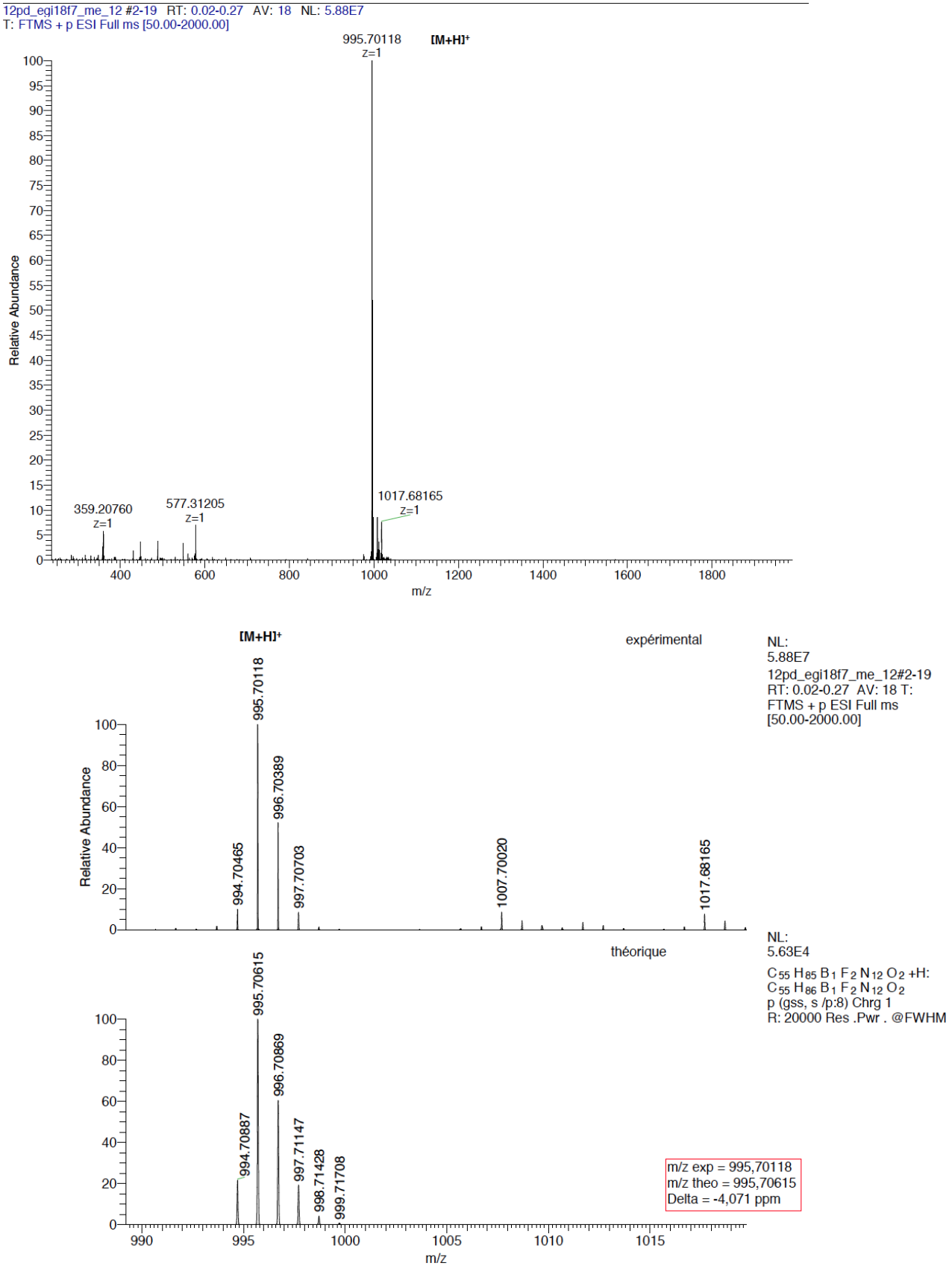


Figure 28 : HR-MS (ESI) analysis of compound **10**

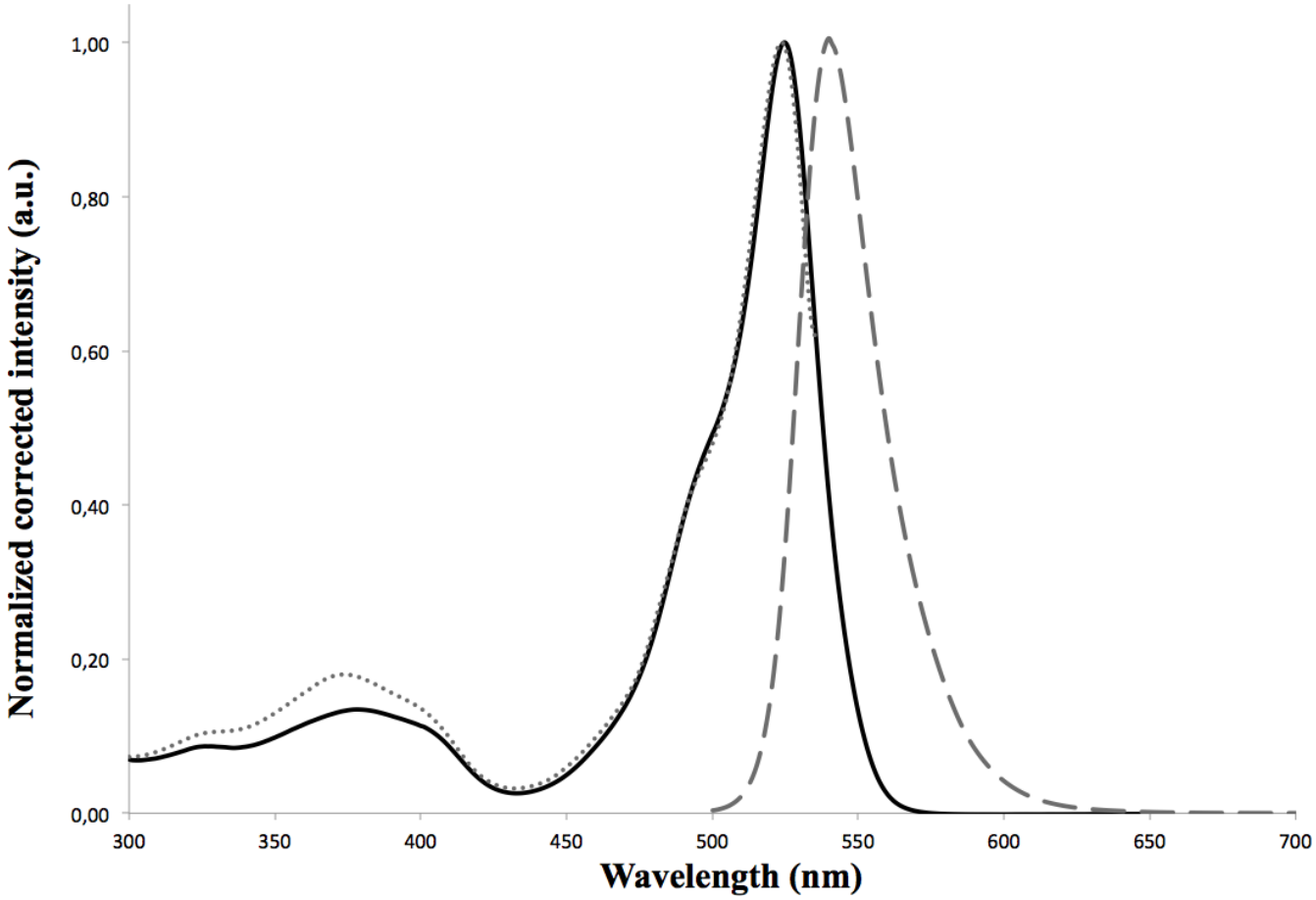
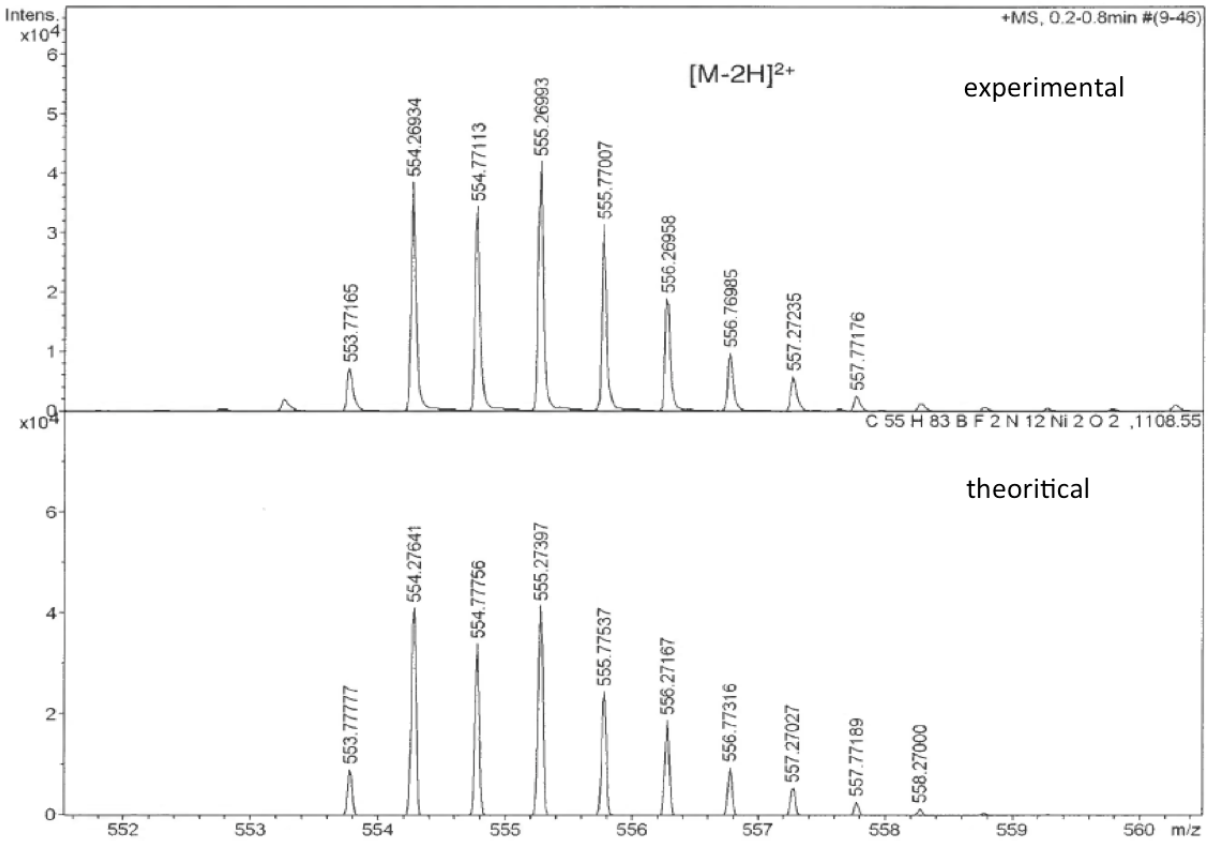


Figure 29 : Absorption (plain line), emission (large dashed line) and excitation (small dashed line) spectra of compound **10** in PBS.



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
554.26934	1	C 55 H 83 B F 2 N 12 Ni 2 O 2	100.00	554.27640	6.6	11.9	584.3	20.0	even	ok

Figure 30 : HR-MS (ESI) analysis of compound **11**

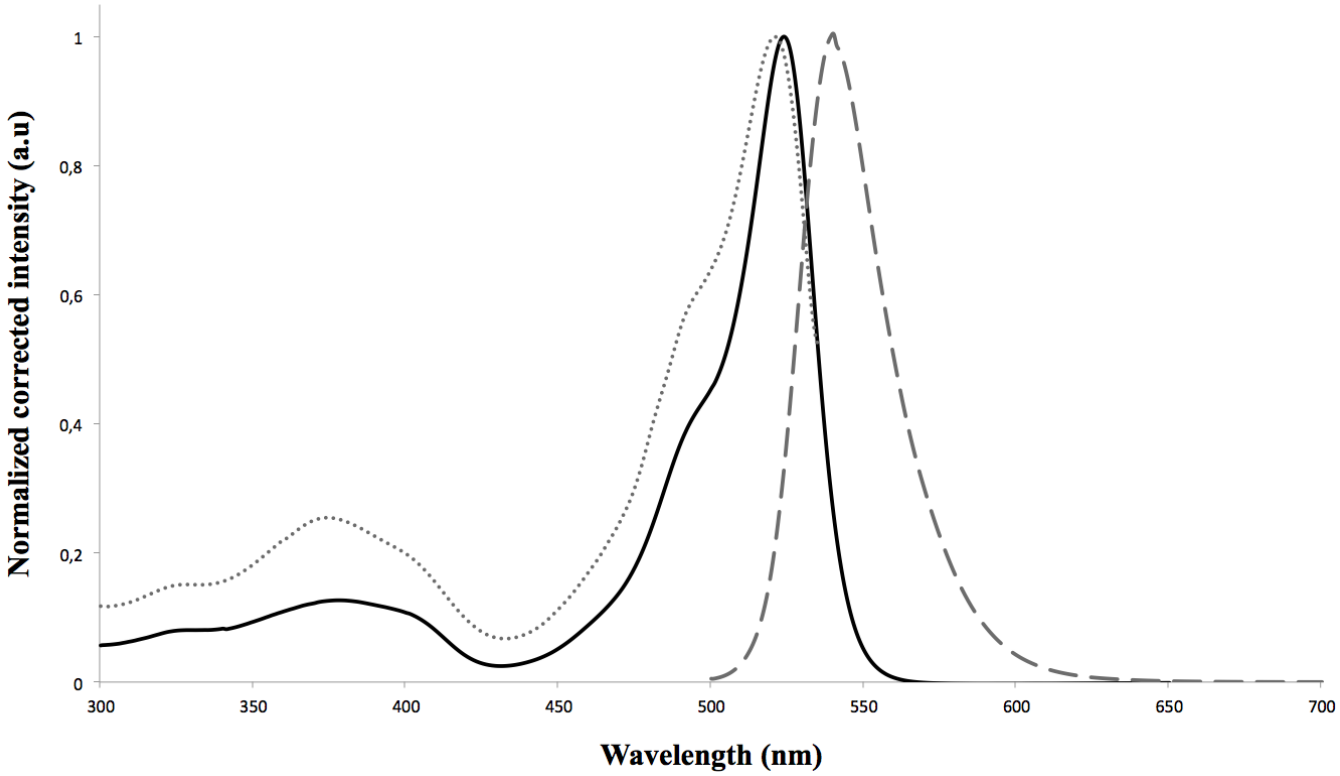
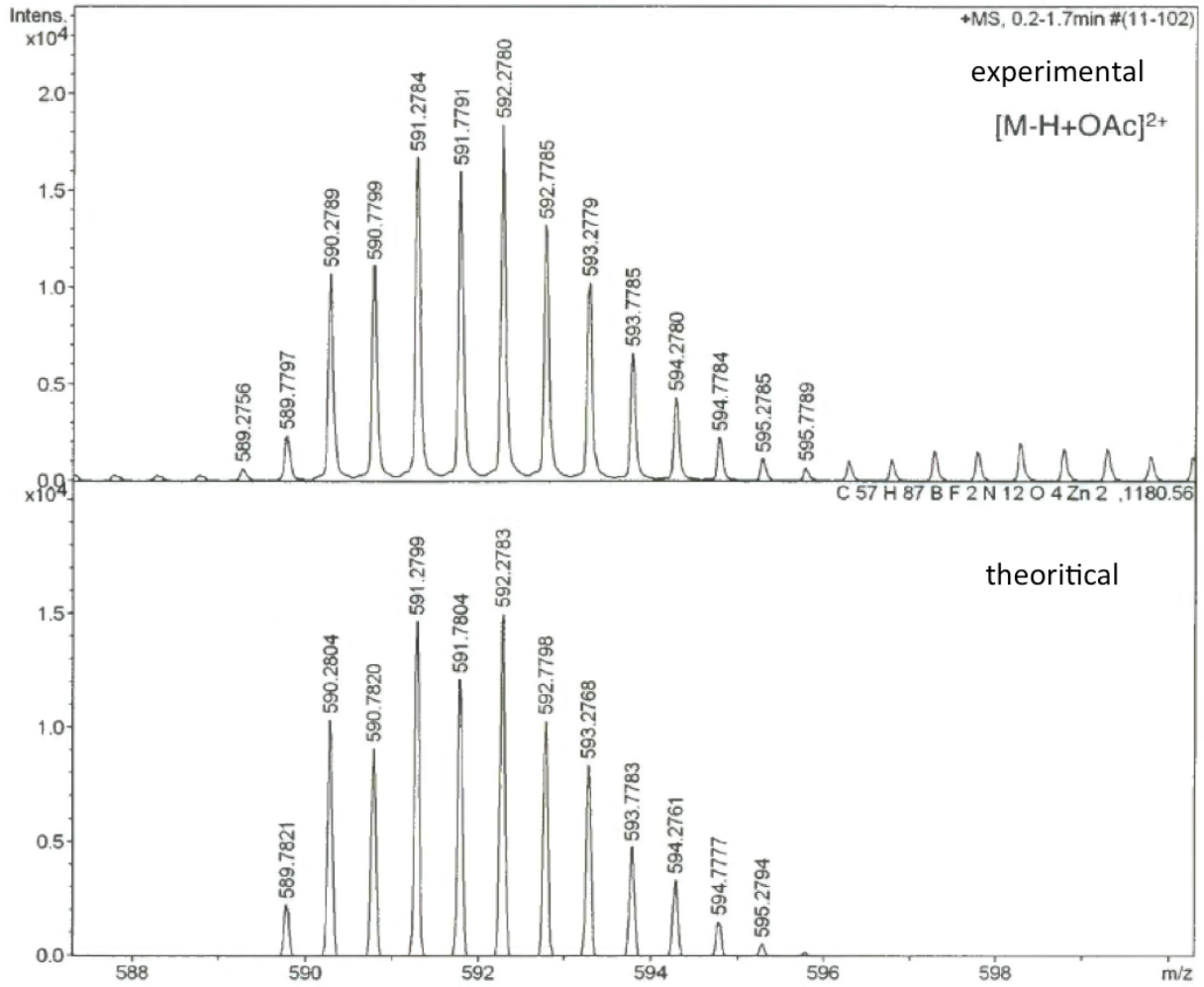


Figure 31 : Absorption (plain line), emission (large dashed line) and excitation (small dashed line) spectra of compound **11** in PBS.



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
590.2789	1	C 57 H 87 B F 2 N 12 O 4 Zn 2	100.00	590.2804	1.4	2.4	43.8	20.0	even	ok

Figure 32 : HR-MS (ESI) analysis of compound **12**

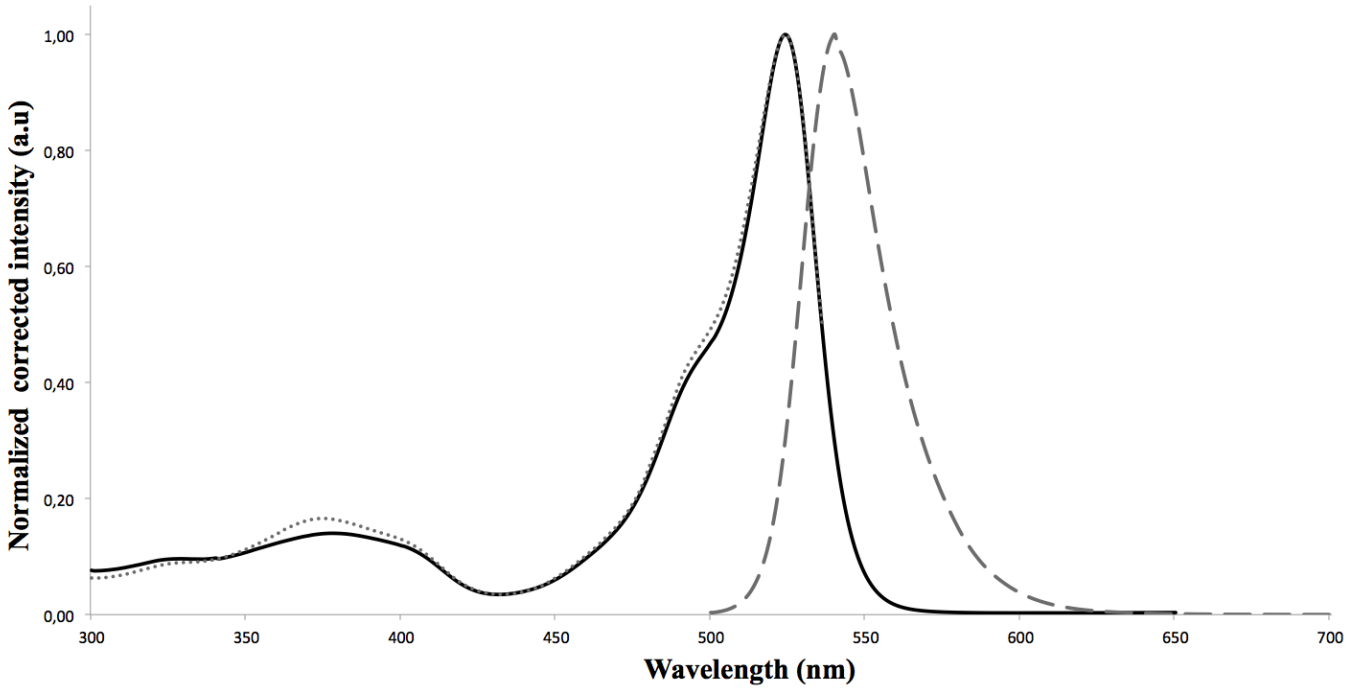


Figure 33 : Absorption (plain line), emission (large dashed line) and excitation (small dashed line) spectra of compound **12** in PBS.

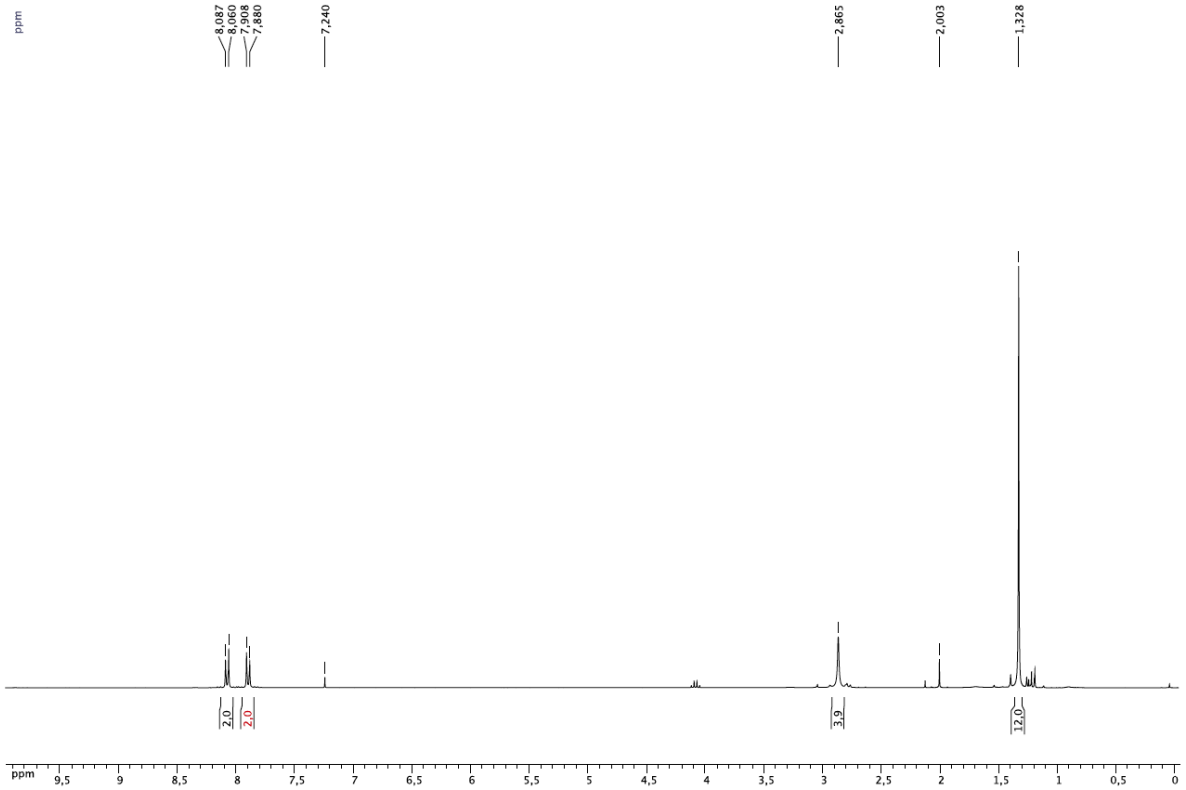


Figure 34 : ^{1}H NMR spectrum of compound **13** ($CDCl_3$, 300 MHz, 300 K)

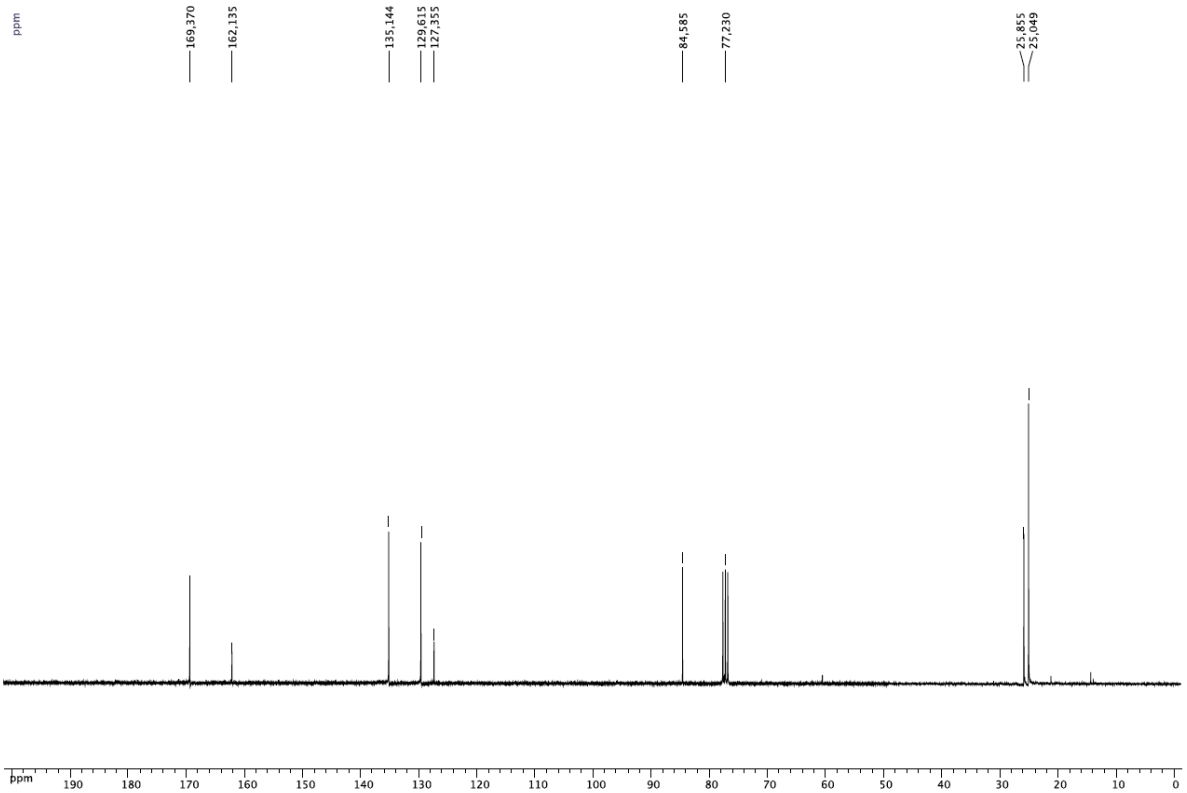


Figure 35 : ^{13}C {^{1}H} NMR spectrum of compound **13** ($CDCl_3$, 300 MHz, 300 K)

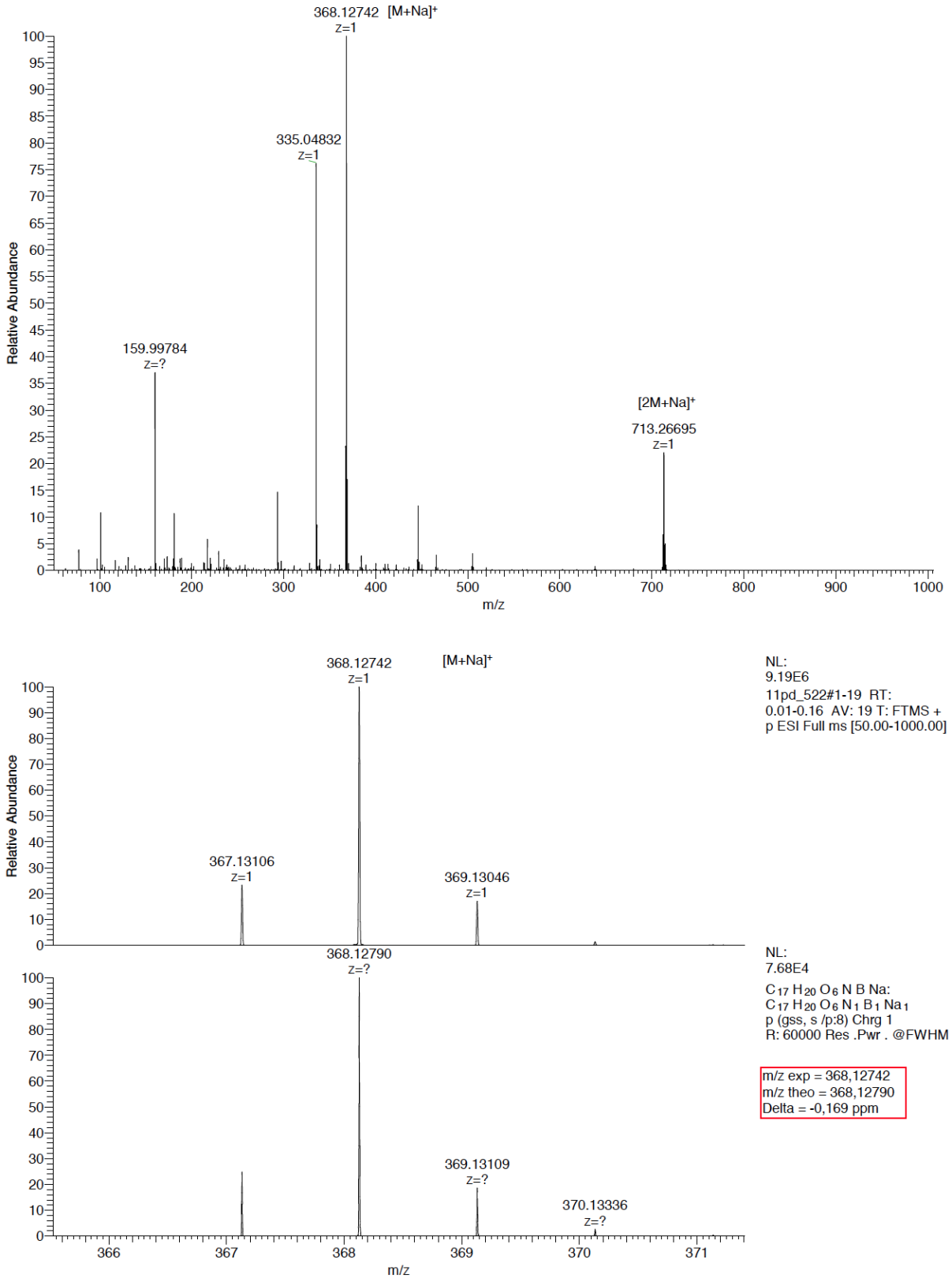


Figure 36 : HR-MS (ESI) analysis of compound **13**

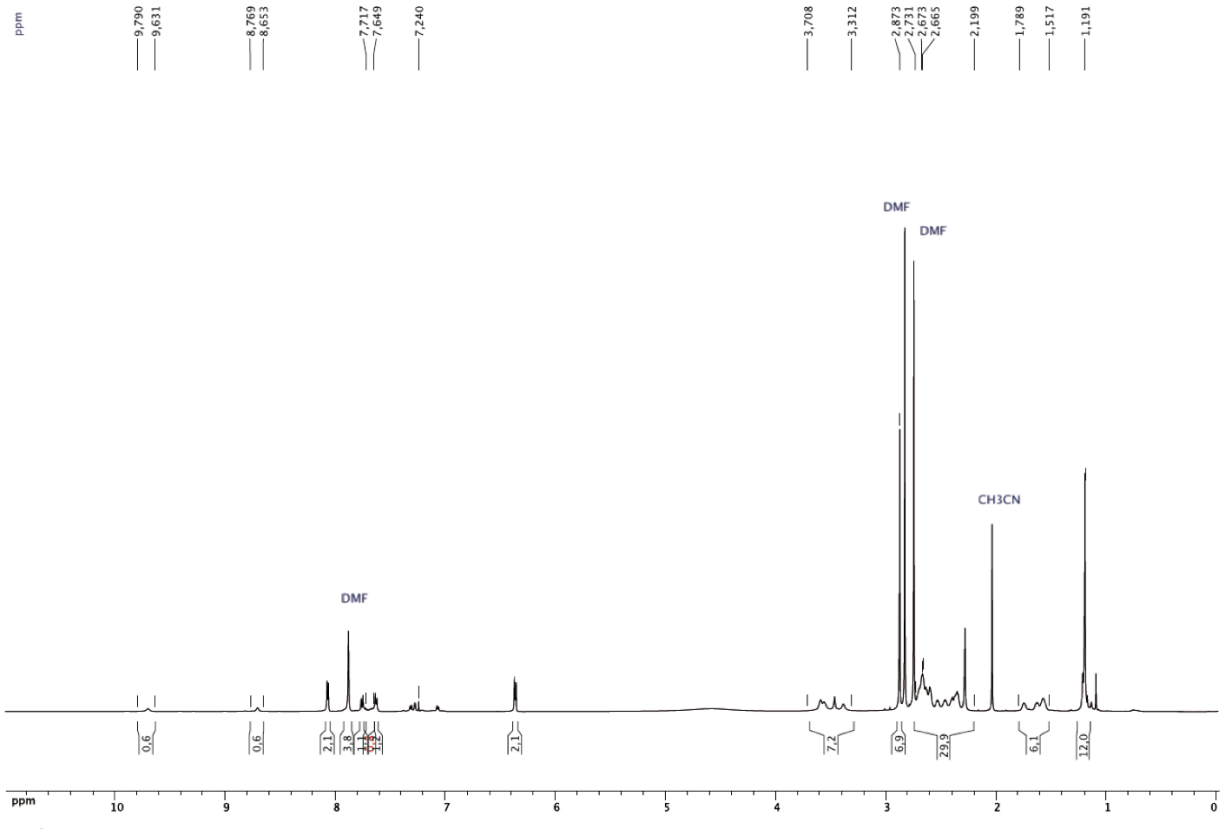


Figure 37 : ^{1}H NMR spectrum of compound **14** ($CDCl_3$, 500 MHz, 300 K)

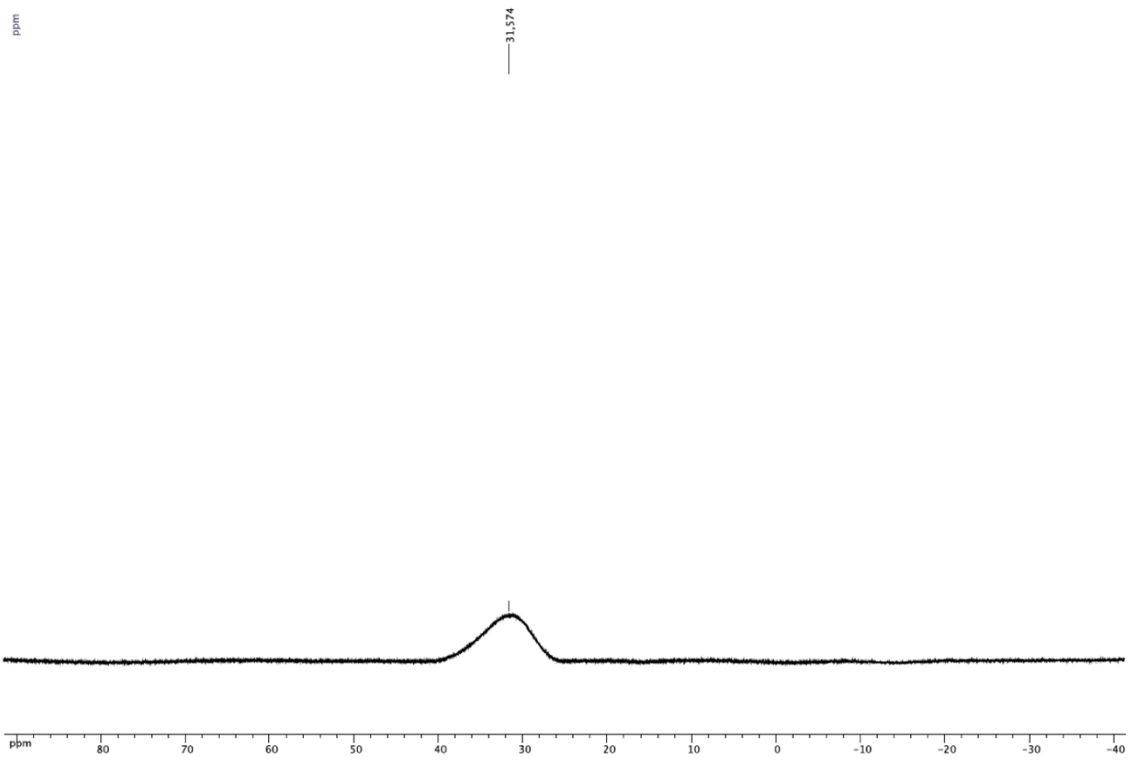


Figure 38 : ^{11}B NMR spectrum of compound **14** ($CDCl_3$, 160 MHz, 300 K)

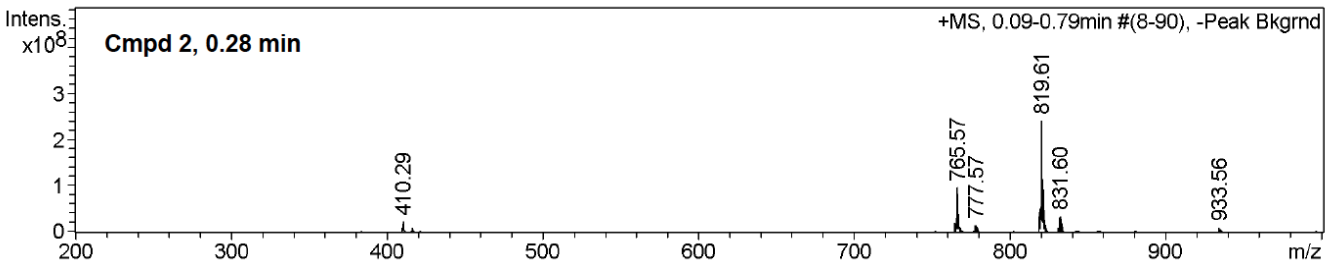


Figure 39 : ESI-MS analysis of compound **14**

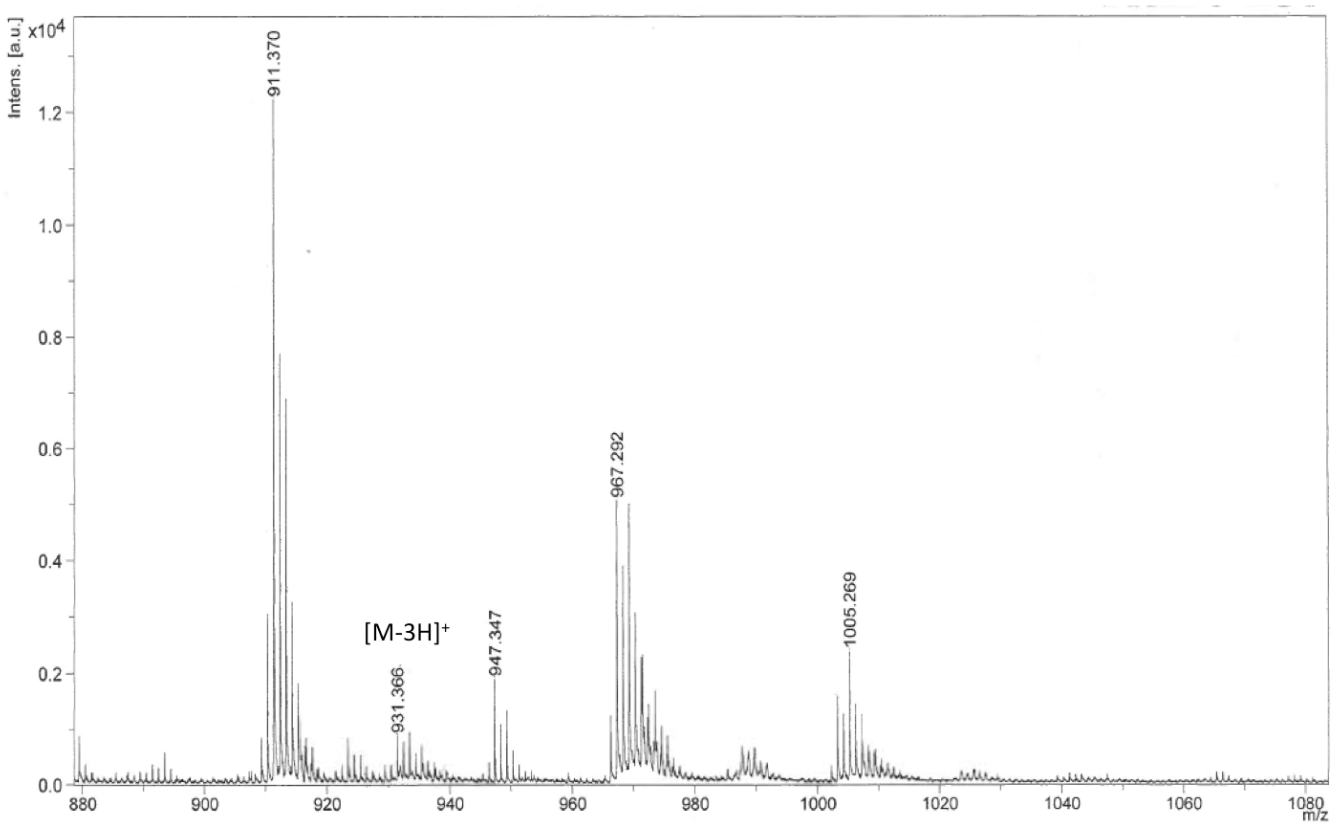


Figure 40 : MALDI-TOF analysis of compound **15**

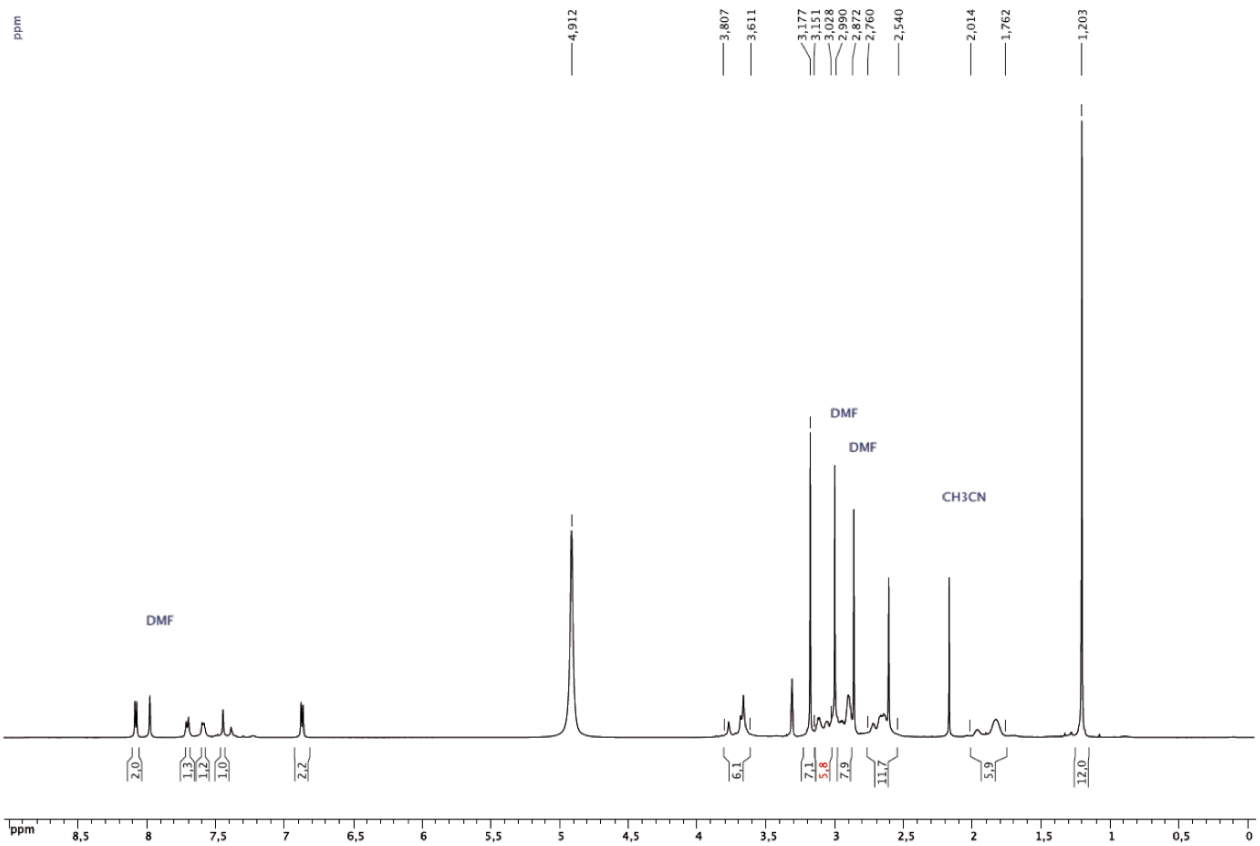


Figure 41 : ^{1}H NMR spectrum of compound **16** (CD_3OD, 160 MHz, 300 K)

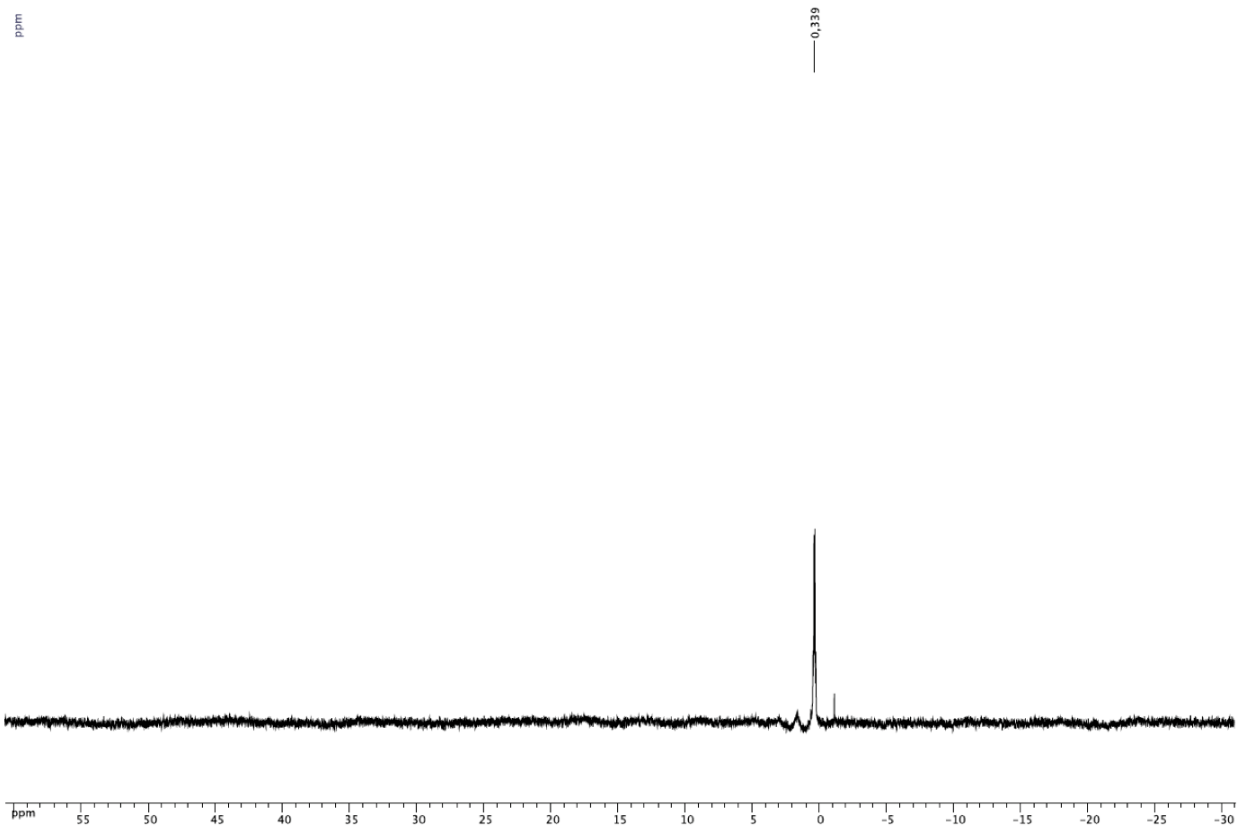


Figure 42 : ^{11}B NMR spectrum of compound **16** (CD_3OD, 160 MHz, 300 K)

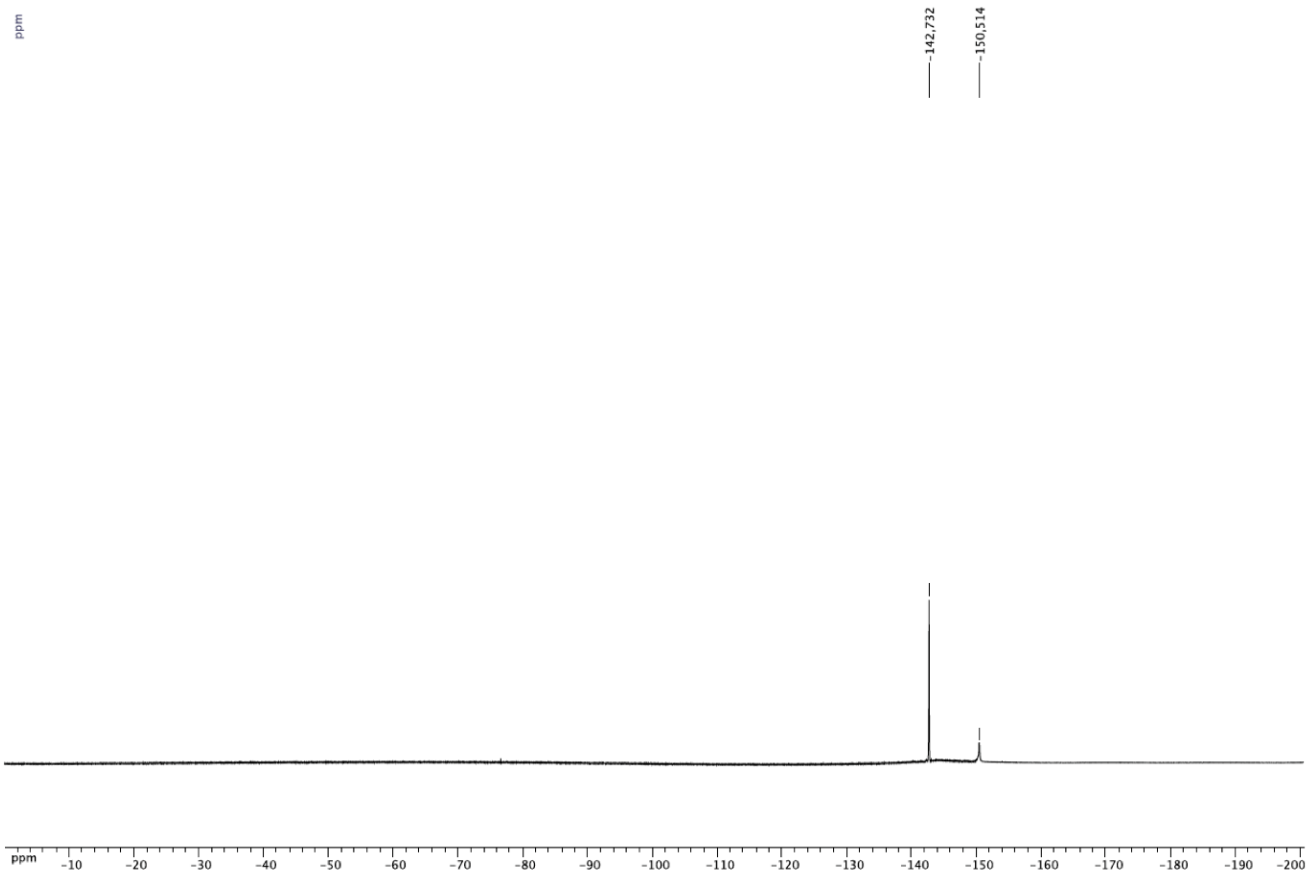


Figure 43 : ^{19}F NMR spectrum of compound **16** (CD_3OD, 202 MHz, 300 K)

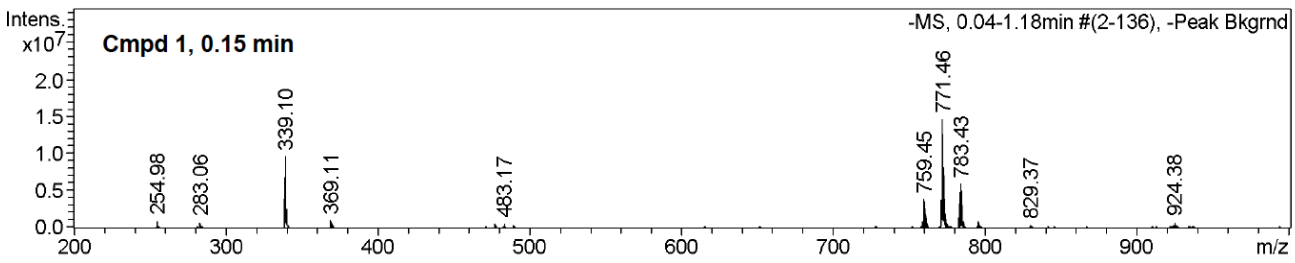


Figure 44 : ESI-MS analysis of compound **16**

Flow cytometry analysis of compound 5 to 8 and AMD3100.8 HCl

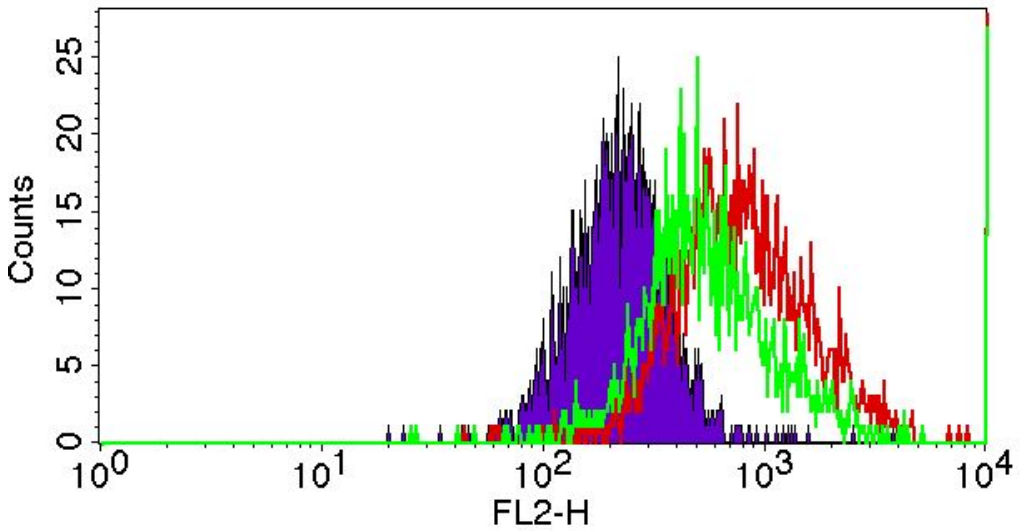


Figure 45 : Flow cytometric analysis of the binding of 12G-5 mAb in competition with compound **5** (20 µM) in Jurkat cells.

Negative control (purple), positive control (red), 12G-5 mAb in competition with compound **5** (light green) are shown.

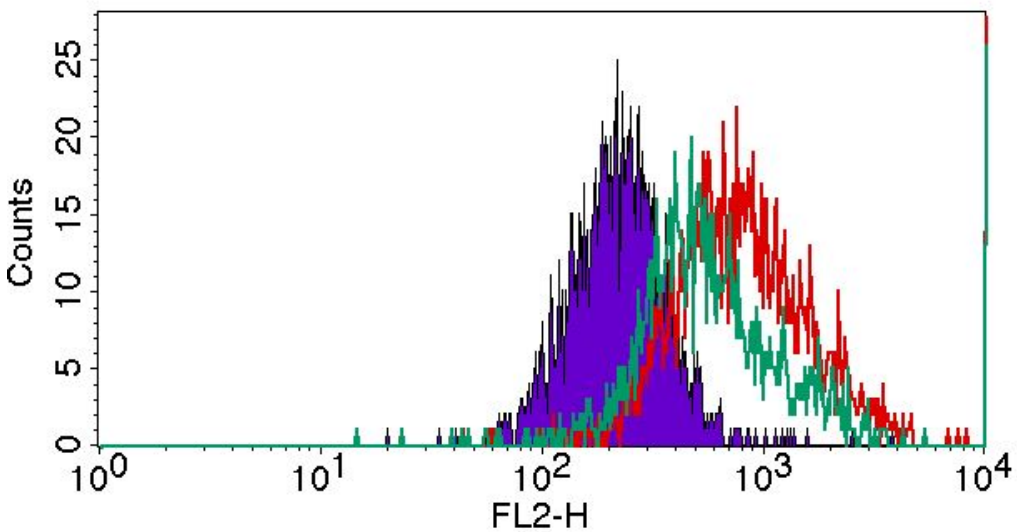


Figure 46 : Flow cytometric analysis of the binding of 12G-5 mAb in competition with compound **6** (20 µM) in Jurkat cells.

Negative control (purple), positive control (red), 12G-5 mAb in competition with compound **6** (dark green) are shown.

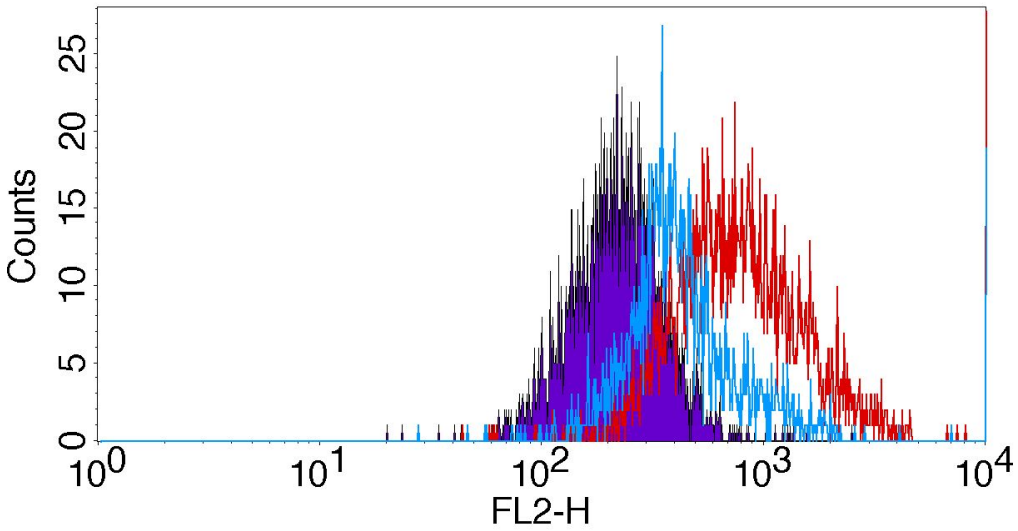


Figure 47 : Flow cytometric analysis of the binding of 12G-5 mAb in competition with compound **7** (20 µM) in Jurkat cells.

Negative control (purple), positive control (red), 12G-5 mAb in competition with compound **7** (light blue) are shown.

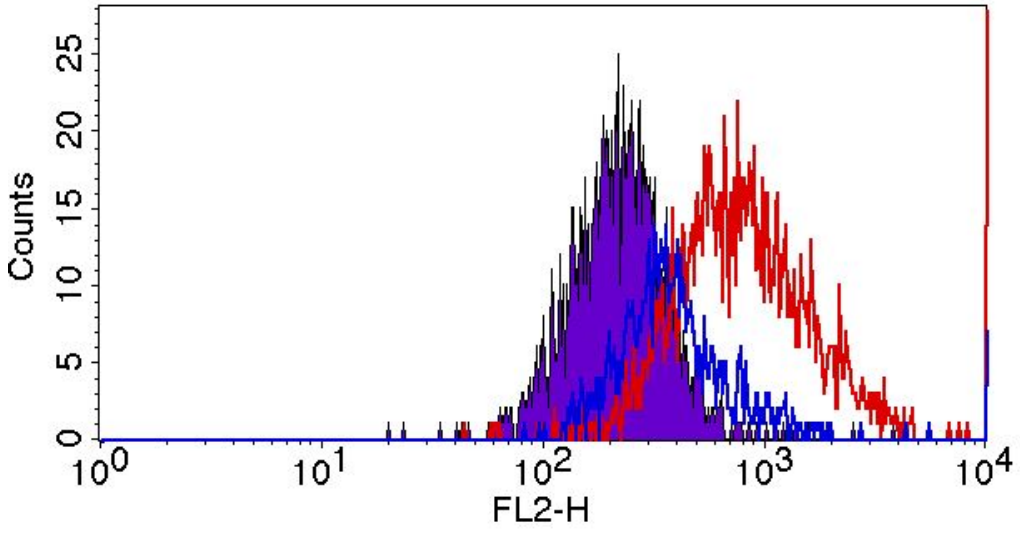


Figure 48 : Flow cytometric analysis of the binding of 12G-5 mAb in competition with compound **8** (20 µM) in Jurkat cells.

Negative control (purple), positive control (red), 12G-5 mAb in competition with compound **8** (dark blue) are shown.

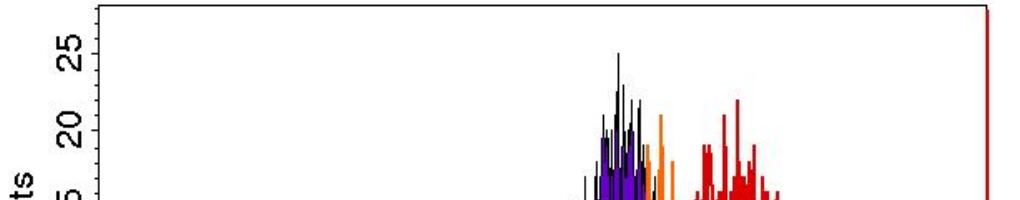


Figure 49 : Flow cytometric analysis of the binding of 12G-5 mAb in competition with **AMD3100.8 HCl** (20 μM) in Jurkat cells.

Negative control (purple), positive control (red), 12G-5 mAb in competition with **AMD3100.8 HCl** (orange) are shown.