

Fluorescent 2-(pyridin-2-yl)vinyl pyridine dyes and their thermo-controlled release

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Optimization of Heck cross-coupling reaction

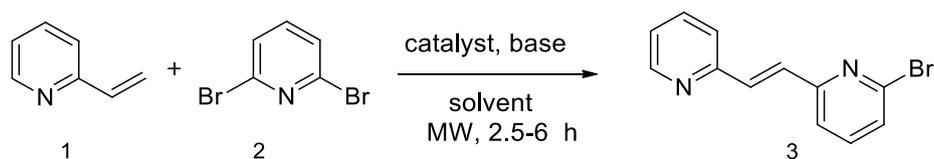


Table S1. Optimizing the Heck cross-coupling between **1** and **2**

Entry	PdCl ₂ [mol %]	Pd(OAc) ₂ [mol %]	K ₂ CO ₃ [mmol]	NaOAc [mmol]	Cs ₂ CO ₃ [mmol]	TBAB [mmol]	DMF/H ₂ O [v/v]	PPh ₃ [mol %]	MW [°C]	Yield [%]
1	1	-	2	-	-	5	3/0	2	80	18
2	1	-	2	-	-	1	3/0	2	80	16
3	1	-	2	-	-	1	3/0	2	120	-
4	1	-	2	-	-	1	8/0.8	2	120	-
5	5	-	2	-	-	1	8/0.8	-	120	23
6	2	-	2	-	-	1	2/1	-	120	22
7	2	-	2	-	-	1	2/1	-	140	27
8	2	-	-	1.5	-	1	0.5	-	120	20
9	-	2	-	1.5	-	1	0.5	-	120	38
10	-	2	-	-	-	1	-	-	120	-
11	-	2	-	1.5	-	1	1	-	120	40
12	-	A) 5, B) 10	-	1.5	-	1	0.5	-	120	A)~38, B) 40
13	-	2	-	1.5	-	1	0.5	6	120	40
14	-	2	-	1.5	-	1	0.5	-	120	38
15	-	2	-	-	1.5	1	0.5	-	120	33
16	-	-	-	-	1.5	1	1	6	120	50-60
17	-	2	-	-	1.5	0.1	1	6	120	60

^aGeneral Conditions: unless otherwise stated, reactions were performed using 2-vinylpyridine (1.2 mmol), 2,6-dibromopyridine (1.0 mmol), catalyst (1–5 mol%), base (1.5 mmol), TBAB (0.5–1 mmol) in 1.0 mL solvent, MW; ^bisolated yield. MW = microwave heating. TBAB = tetrabutylammonium bromide. DMF = *N,N*-dimethylformamide.

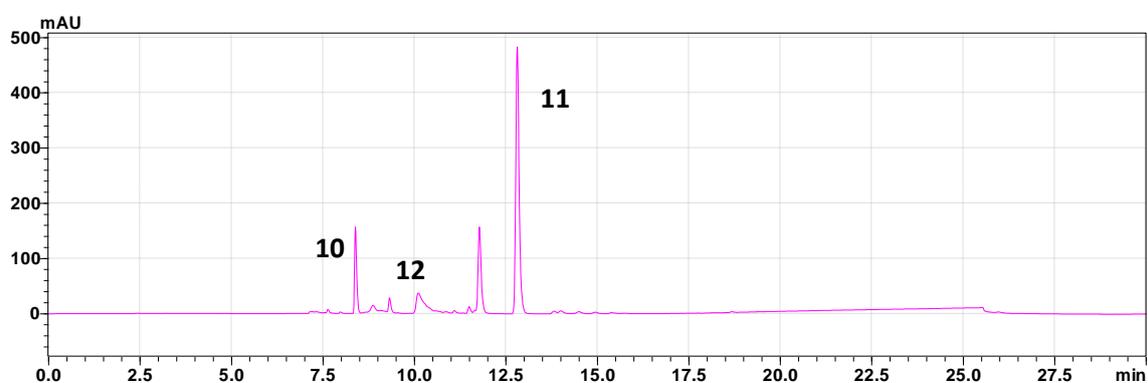
Table S2. Retention times of each compound mixture upon HPLC analysis.

	Retention time [min.]
Compound 11	13.5
Compound 12	9.6
Compound 10	8.5

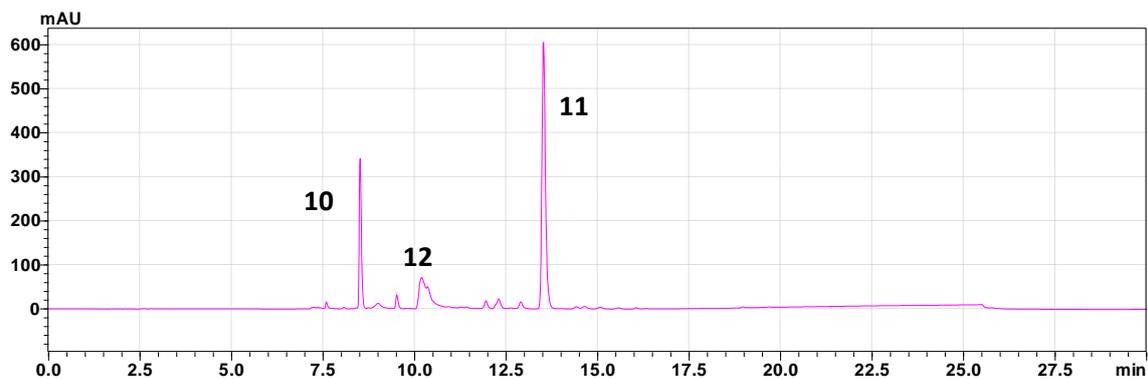
Figure S1. HPLC Chromatograms – thermal releasing of 11

1. Temperature dependence

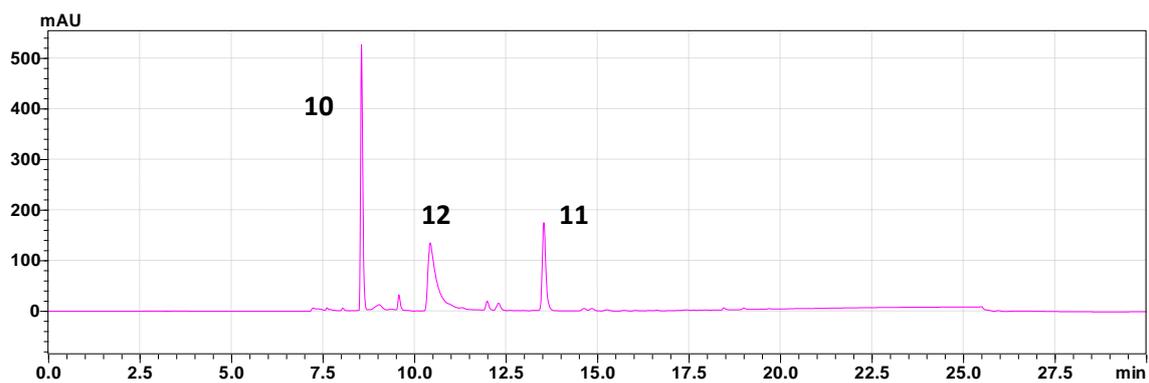
Probe 11 – heated for 2h in 30°C



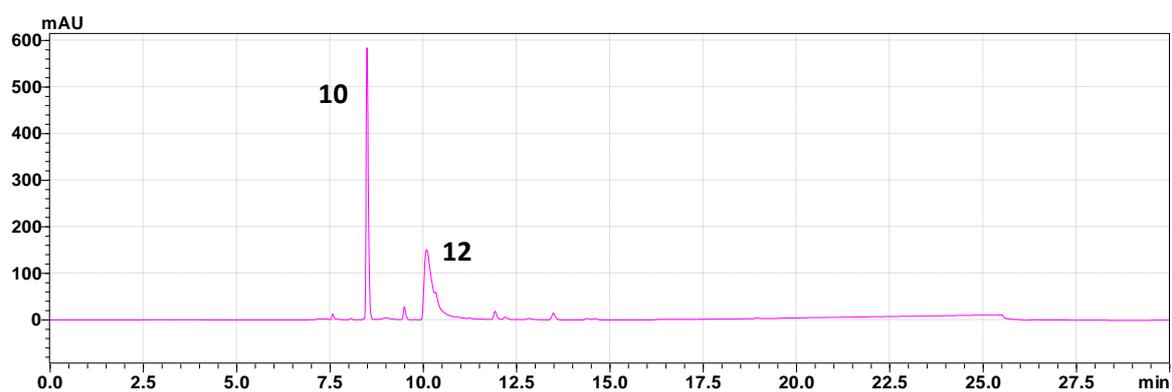
Probe 11 - heated for 2h in 40°C



Probe 11 - heated for 2h in 50°C

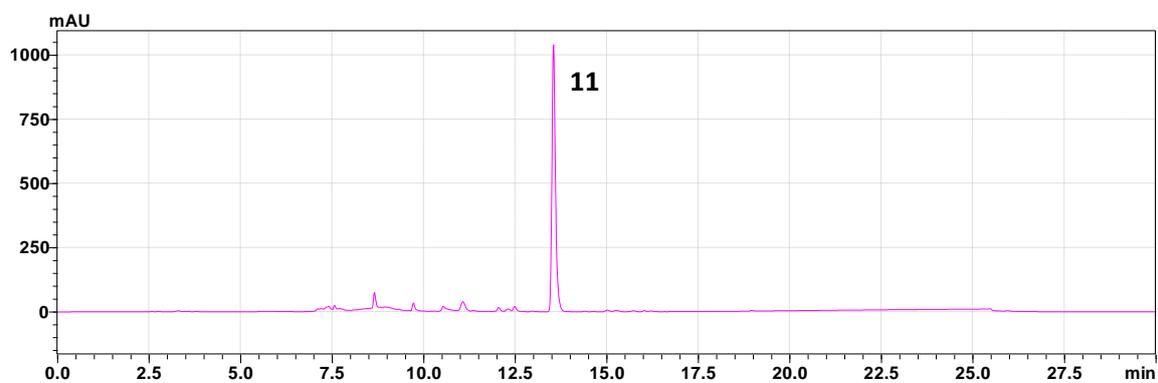


Probe 11 - heated for 2h in 60°C

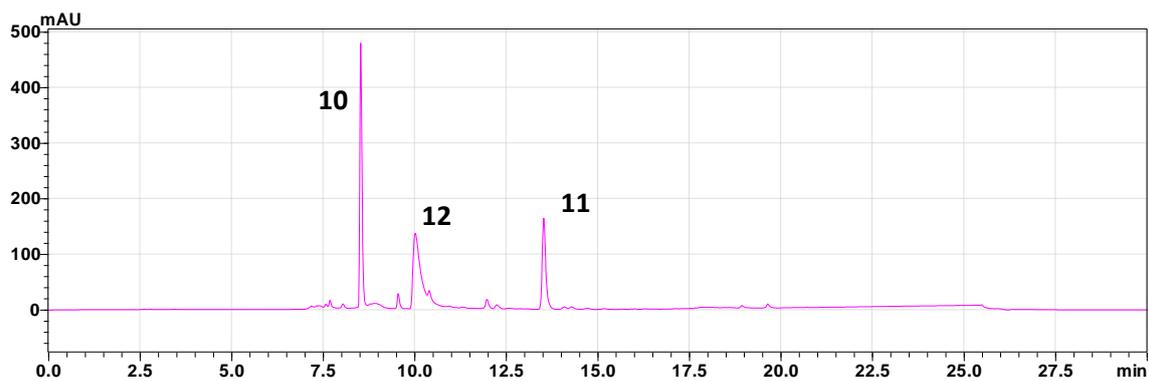


2. Time dependance

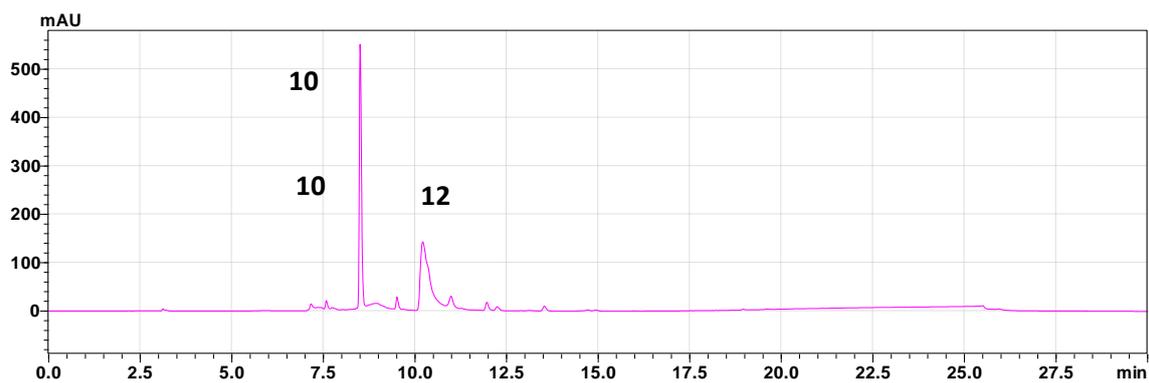
Probe 11 - room temperature



Probe 11 – heated 5 min. in 90°C



Probe 11 - heated 10 min. in 90°C



Probe 11 - heated 15 min. in 90°C

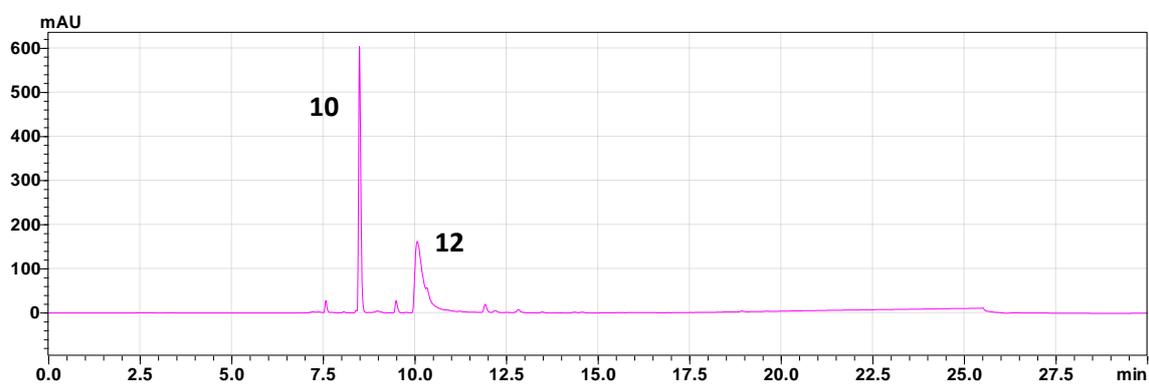
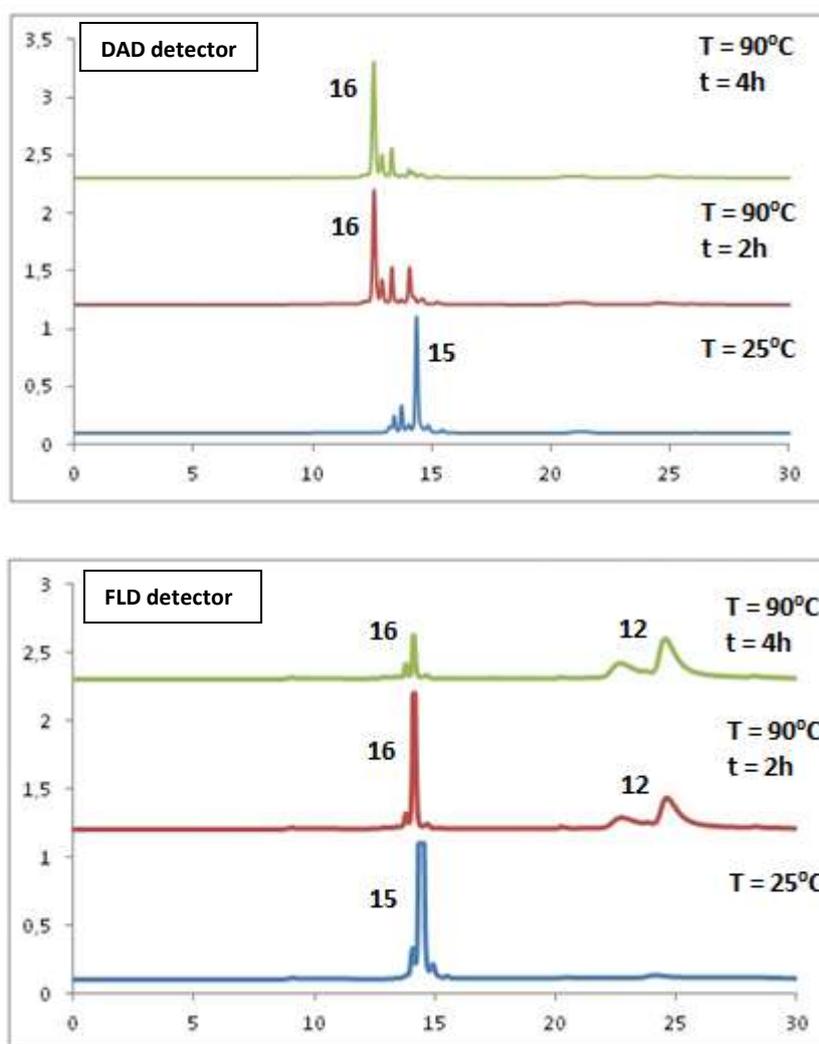


Table S3. Retention times of each compound in the reaction mixture during dye removal.

Compound	R _t [min.]
Labeled oligonucleotide (15)	14.49
5'-monophosphate oligonucleotide (16)	12.57
Cyclic form of dye (12)	22.64-24.52

Figure S2. HPLC Chromatograms depicting thermal releasing of **12** from oligonucleotide 5'-monophosphate. We observed total dye removal after 4 hours of heating the probe in 90°C, appearing as a new signal with different retention time and without any fluorescence emission on FLD detector (**16**).



X-ray crystallographic data

Data collections were performed with the wavelength of 0.8943 Å at 100 K on a P13 beamline of the EMBL facility localized in DESY, Hamburg, Germany. Corrections for Lorentz-polarization effect and for absorption were used. Unit cell parameters were determined by a least-square fit of 2567 reflections of highest intensity, chosen from the whole experiment. SIR92 [1] was used for structures solution. Refinement with the full-matrix procedure on F^2 was carried out in SHELXL97 [2]. The function $\sum w(|F_o|^2 - |F_c|^2)^2$, where $w^{-1} = [\sigma^2(F_o)^2 + A \cdot P^2 + B \cdot P]$ and $P = [\text{Max}(F_o^2, 0) + 2F_c^2]/3$, was minimized. All non-hydrogen atoms were refined anisotropically, while positions of hydrogen atoms were found on the difference electron density map. Crystallographic data (without structure factors) has been deposited with the Cambridge Crystallographic Data Centre, No. CCDC-1916777. Copies of this information may be obtained free of charge from: The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK. FAX: +44(1223)336-033, email: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk.

[1] A. Altomare, G. Cascarano, C. Giacovazzo & A. Gualardi, *J. Appl. Cryst.*, **26**, 343, 1993

[2] G. M. Sheldrick, *Acta Cryst.* **A64**, 112, 2008.

Single crystals suitable for X-ray crystallography were obtained by diffusion of **5b** in DCM and slow evaporation of the solvent. Afterward, the probe was kept in a refrigerator for crystallization. Thin crystals were obtained within a night.

Table S4. X-ray crystallographic data and refinement statistics

	5b
Empirical formula	C ₁₅ H ₁₇ N ₃ O
Formula weight	255.31
T [K]	100
λ [Å]	0.89429
Crystal system	Monoclinic
Space group	P2 ₁ /c
F(000)	544
μ [mm ⁻¹]	0.08
Z	4
a [Å]	12.880 (3)
b [Å]	16.510 (3)
c [Å]	6.395 (1)
β [°]	99.631 (30)
V [Å ³]	1340.72
ρ_{calc} [g cm ⁻³]	1.265
Collected data	2797
Unique data / R _{int}	2690
No. of parameters	240
Goof	1.166
R / wR ² (I > 4 σ)	0.0413 / 0.1053
R / wR ² (all data)	0.0459 / 0.1317
Largest diff. peak/holes [e Å ⁻³]	0.29 / -0.29

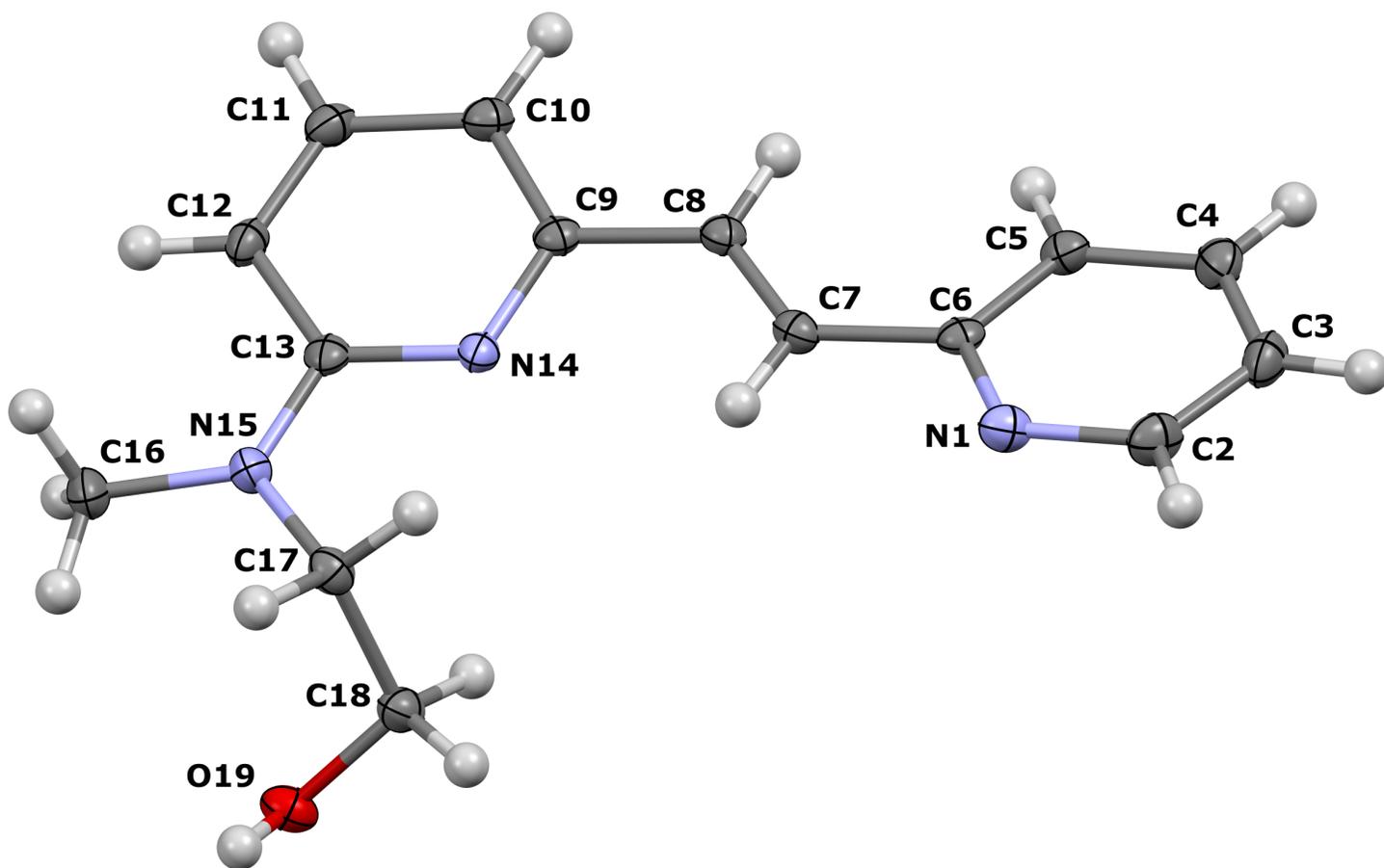


Figure S3. Thermal ellipsoid plot of the atom numbering-scheme of 5b. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

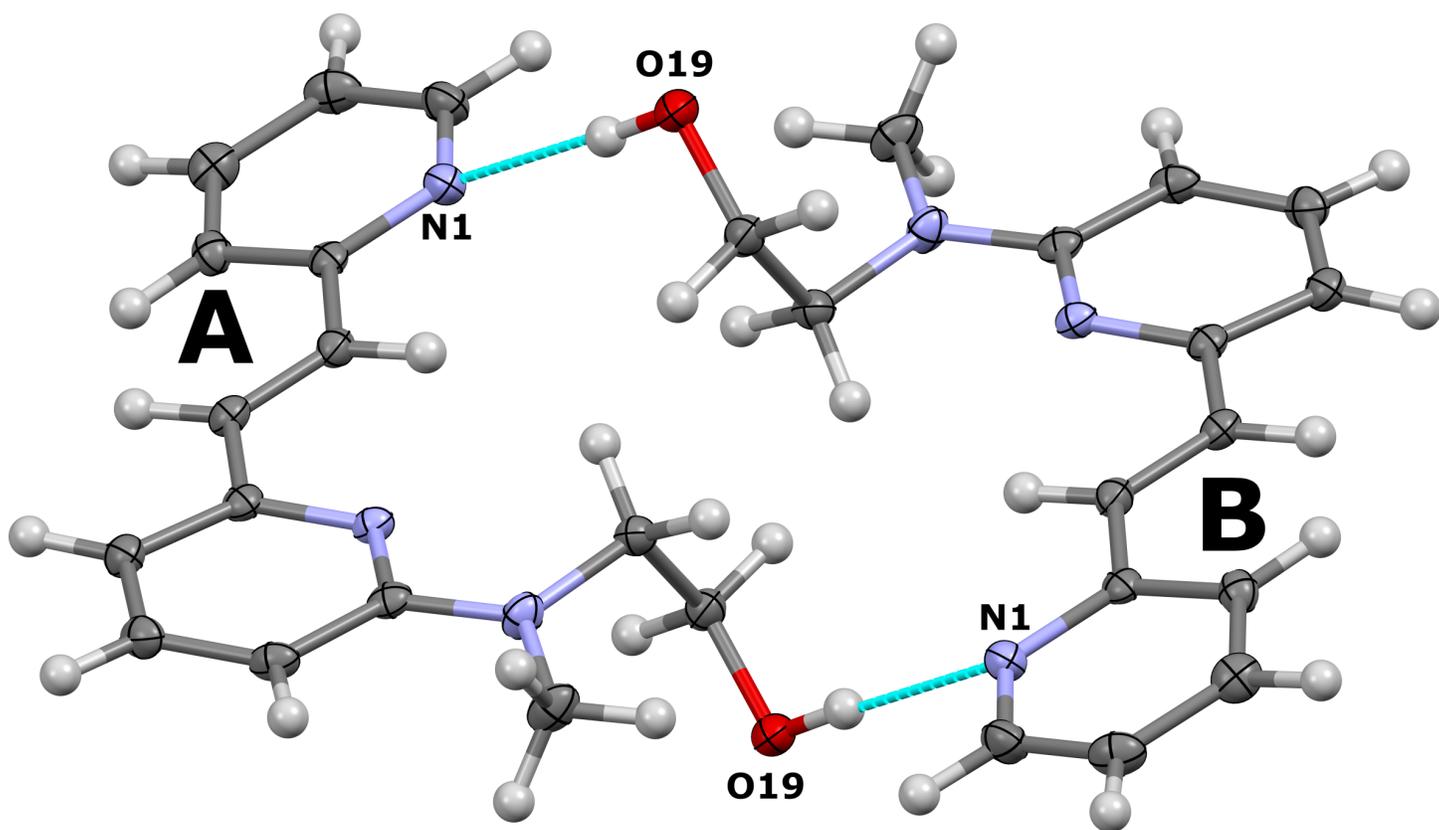
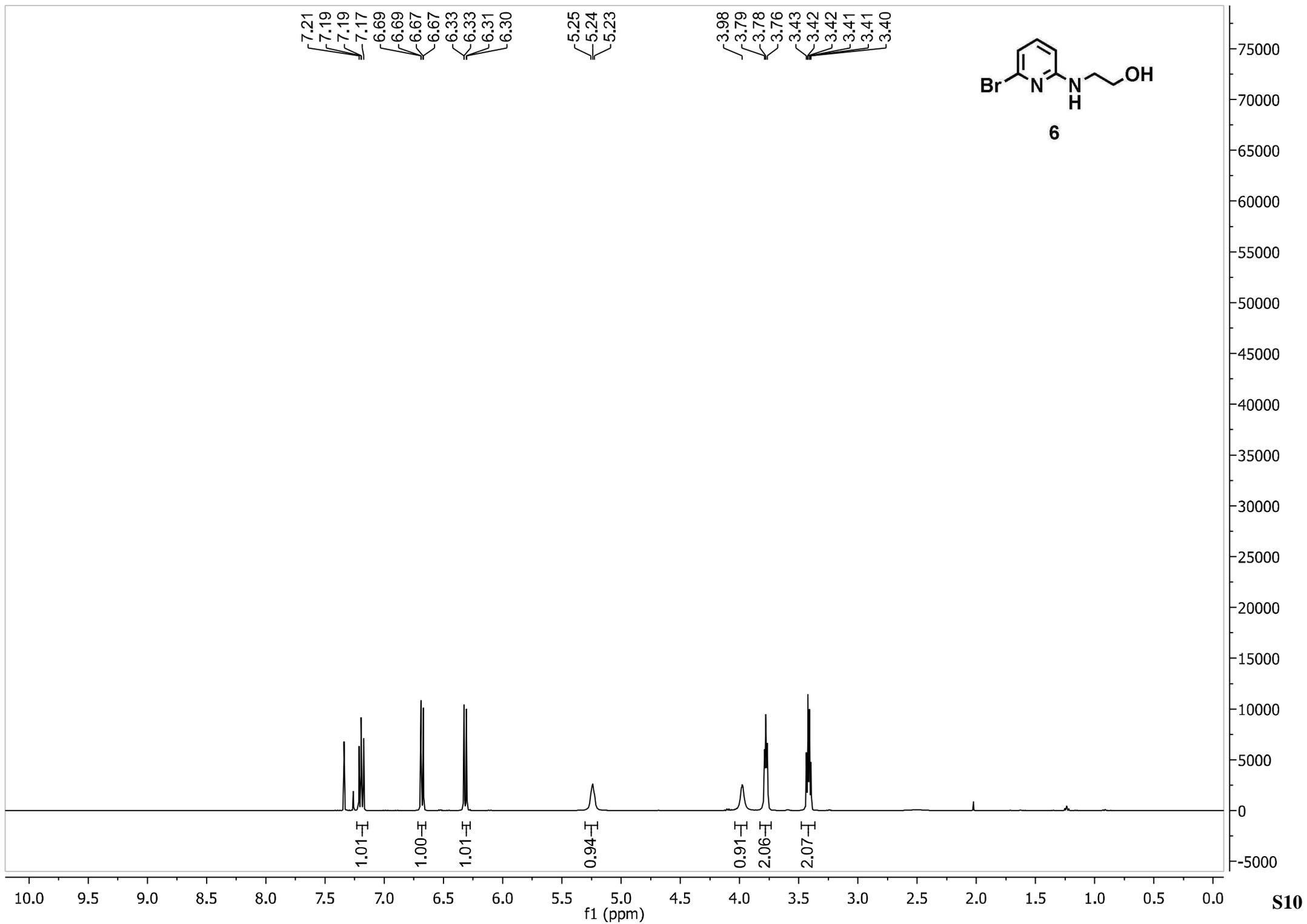
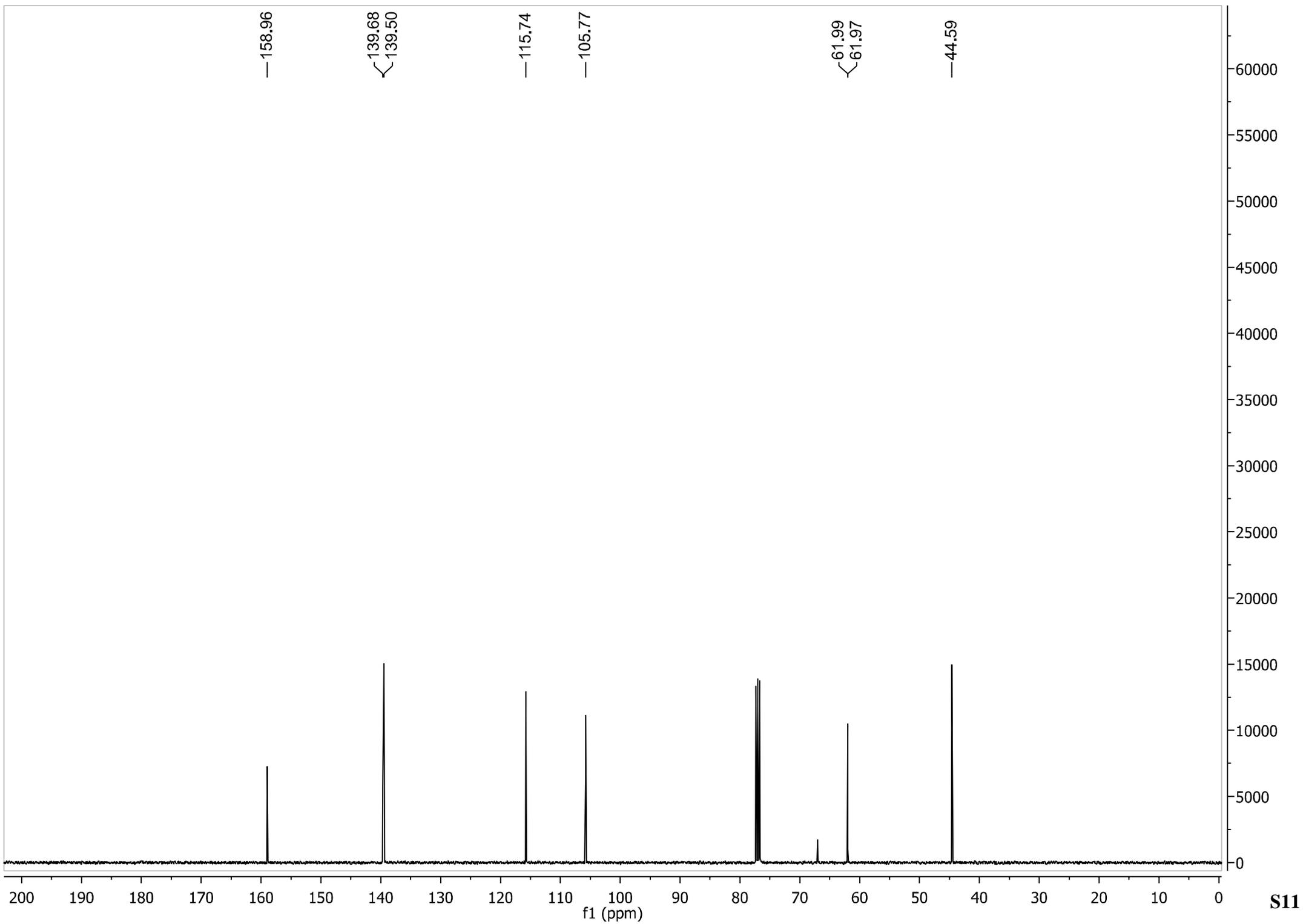


Figure S4. Thermal ellipsoid plot of the intermolecular hydrogen bonds (blue lines) between two symmetry-related molecules A and B. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.





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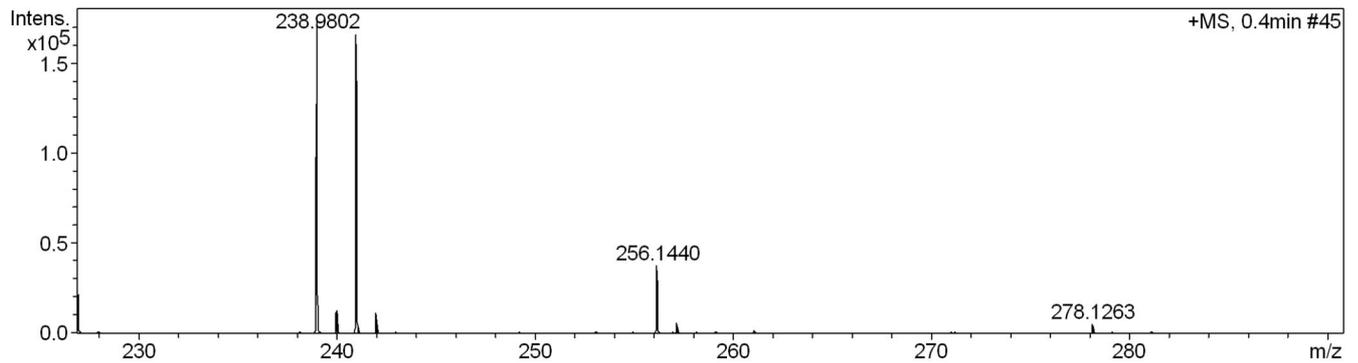
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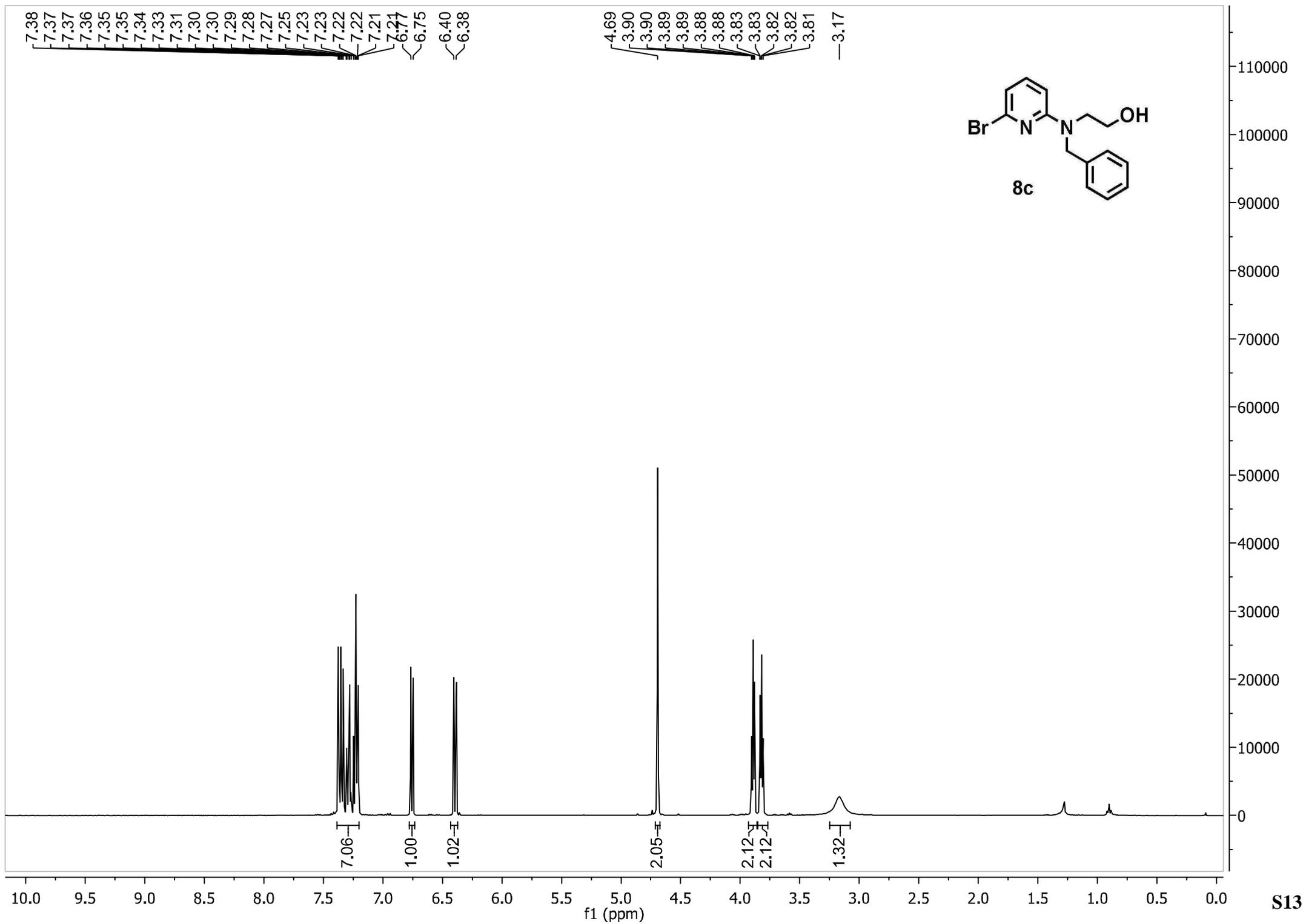
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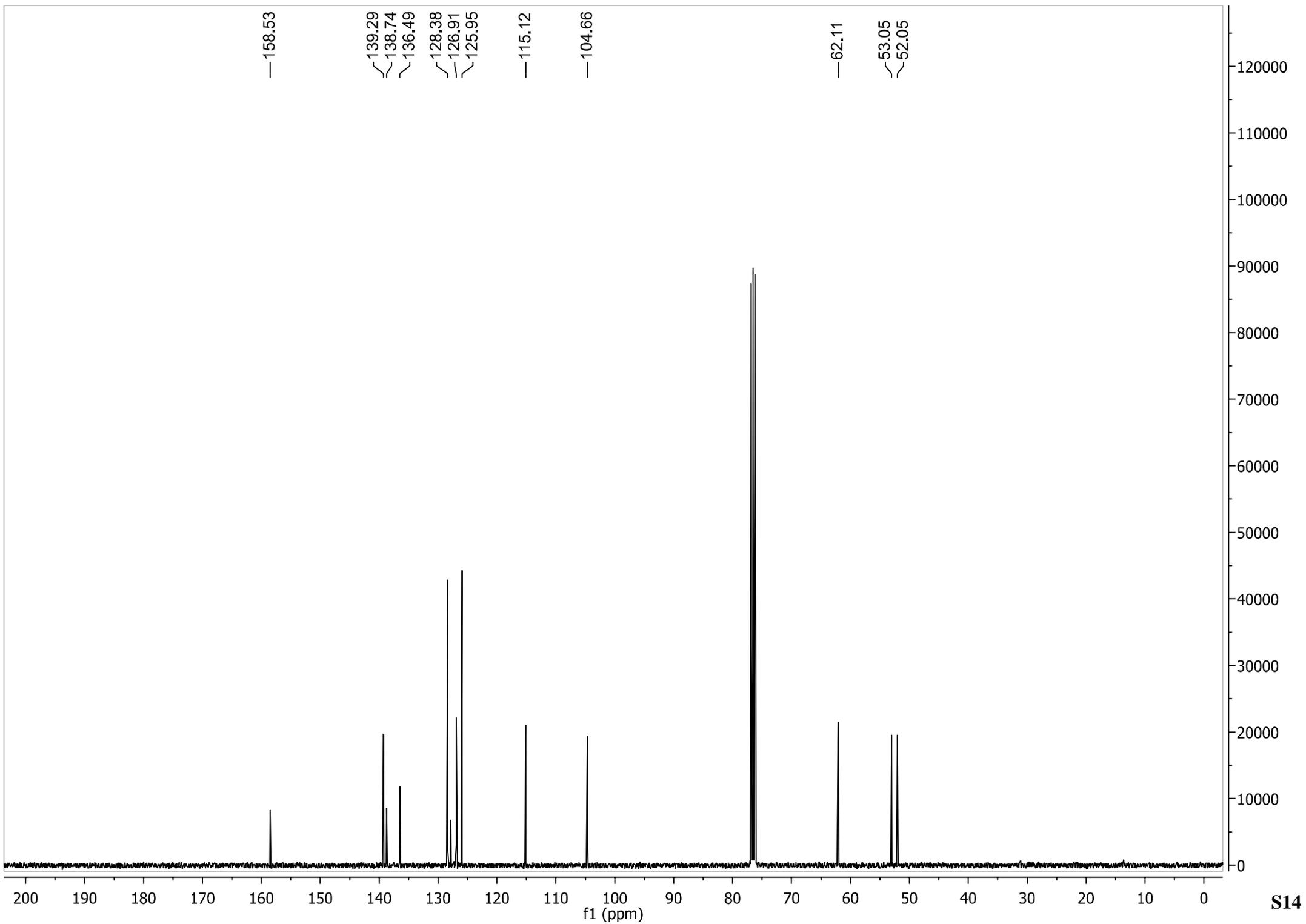
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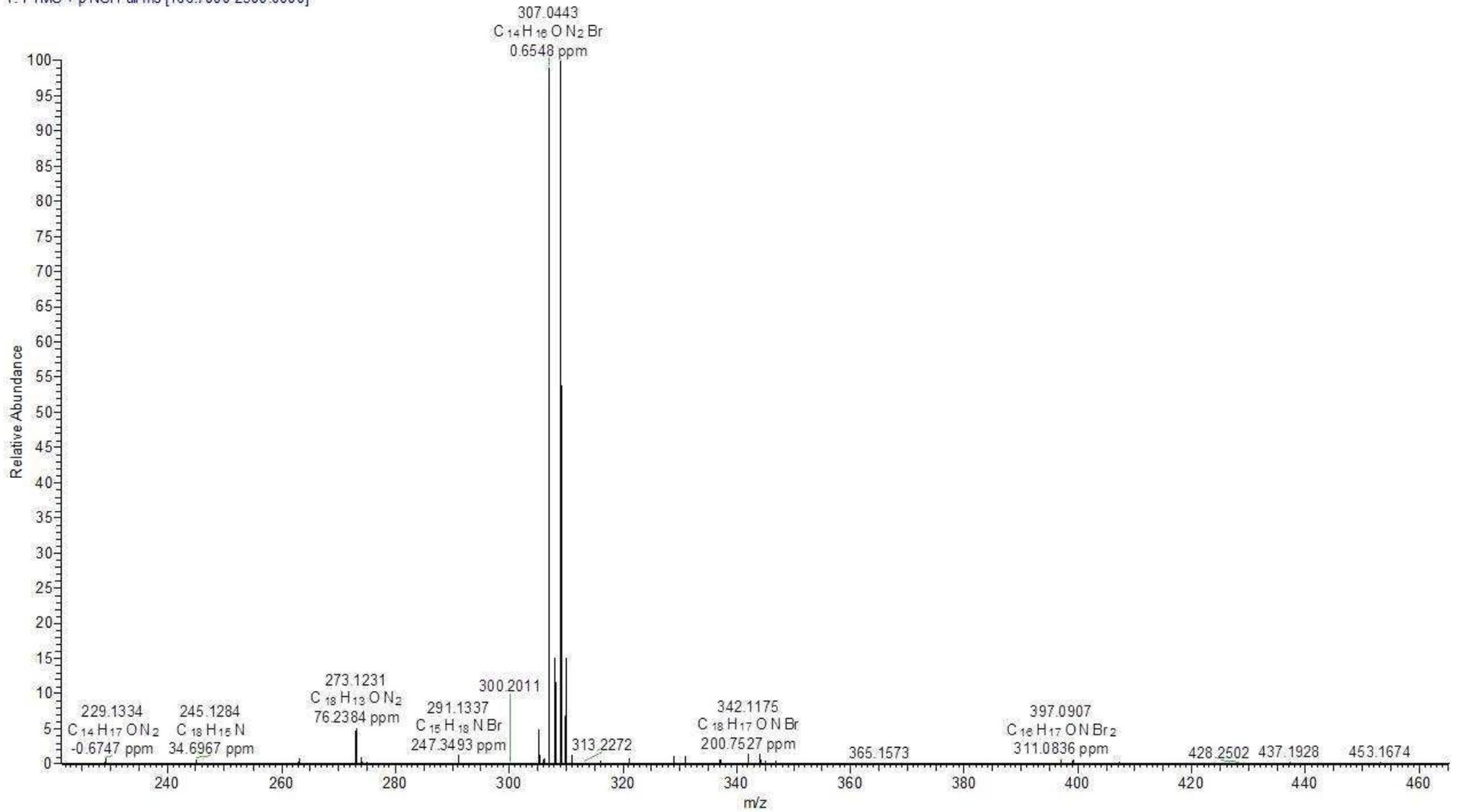


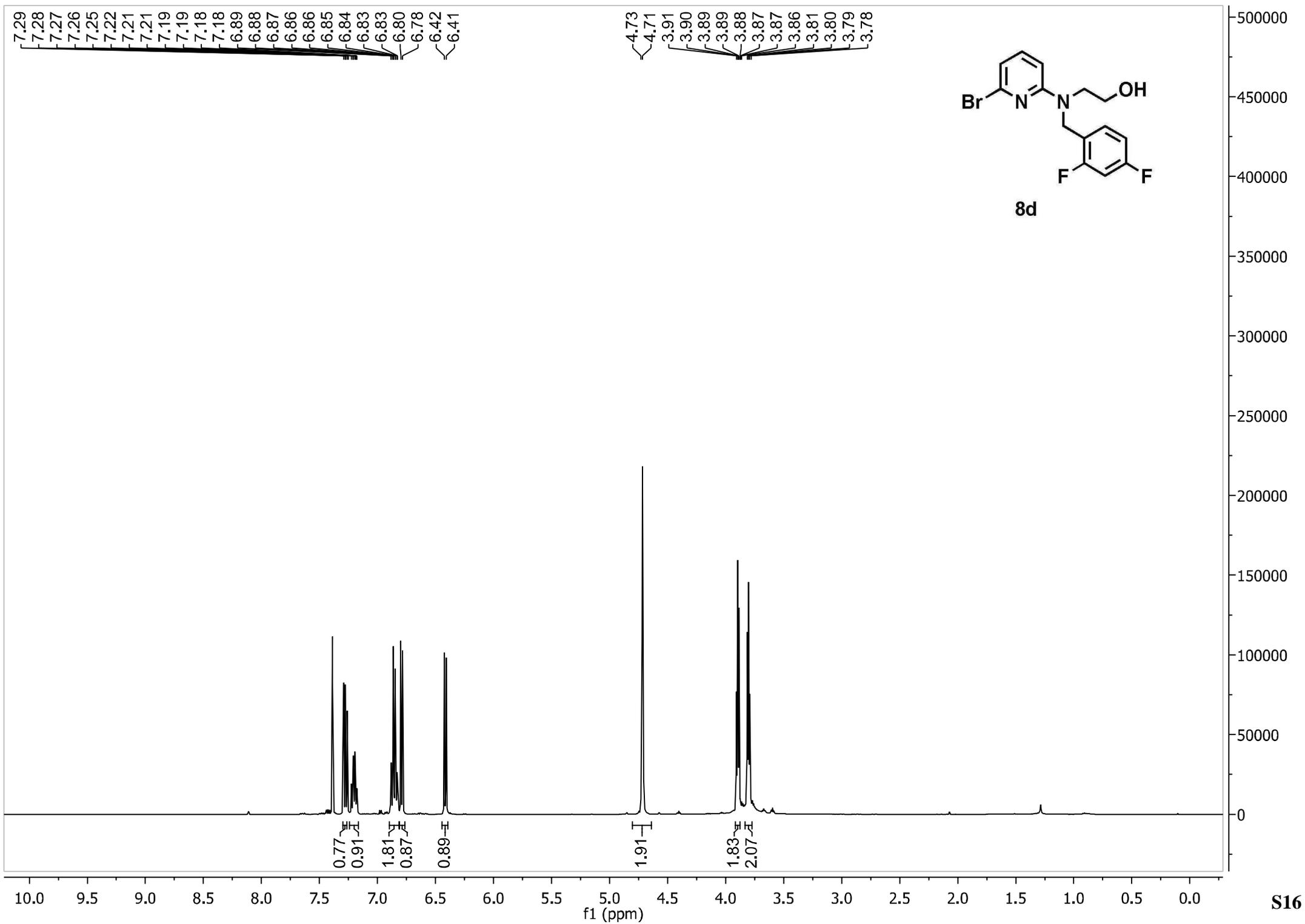
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238.9802 1	C 7 H 9 Br N 2 Na O	238.9790	-4.8	-4.0	3.5	ok	even	13.8	13.6	1.1	6.4	1.2	842.7

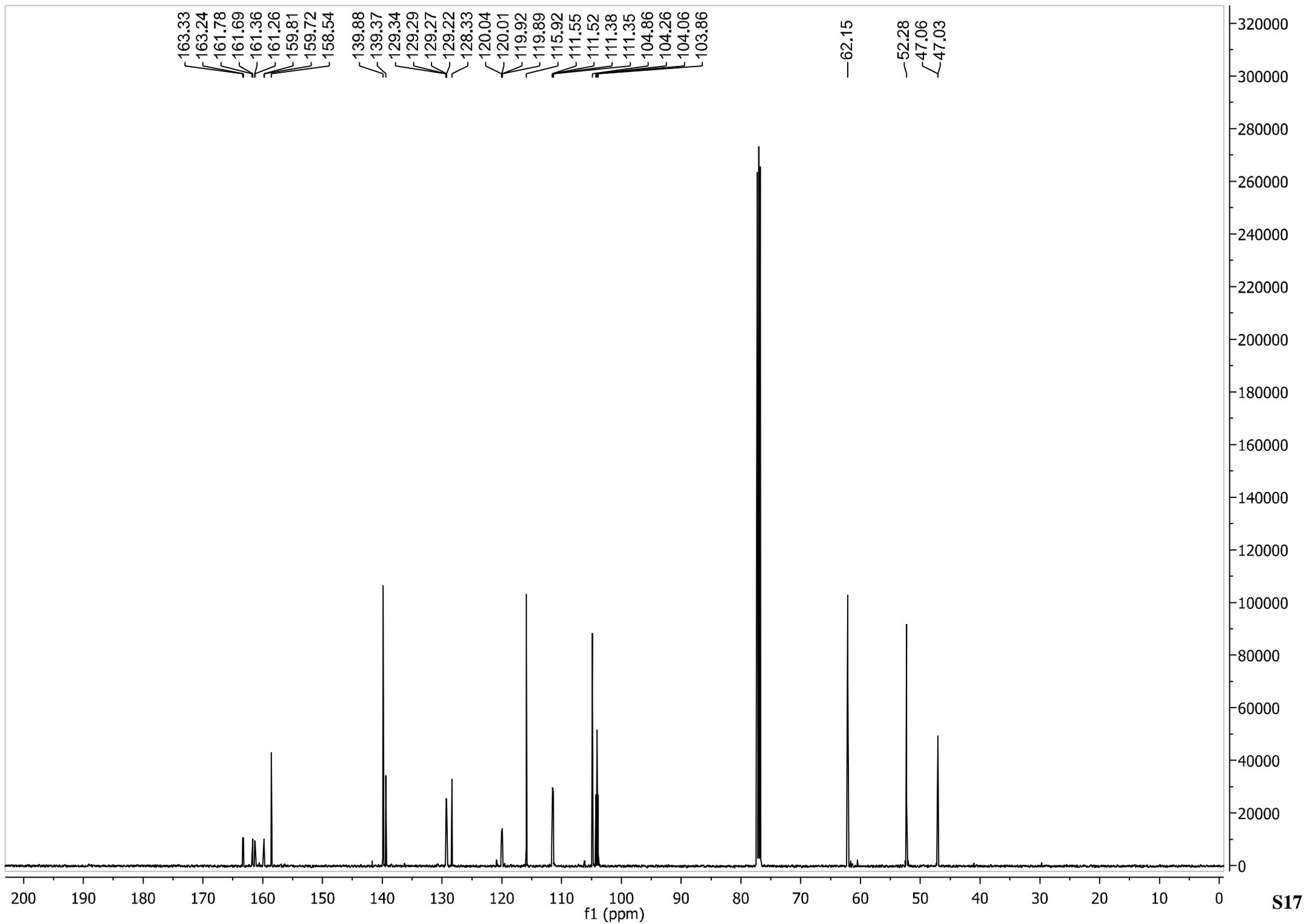




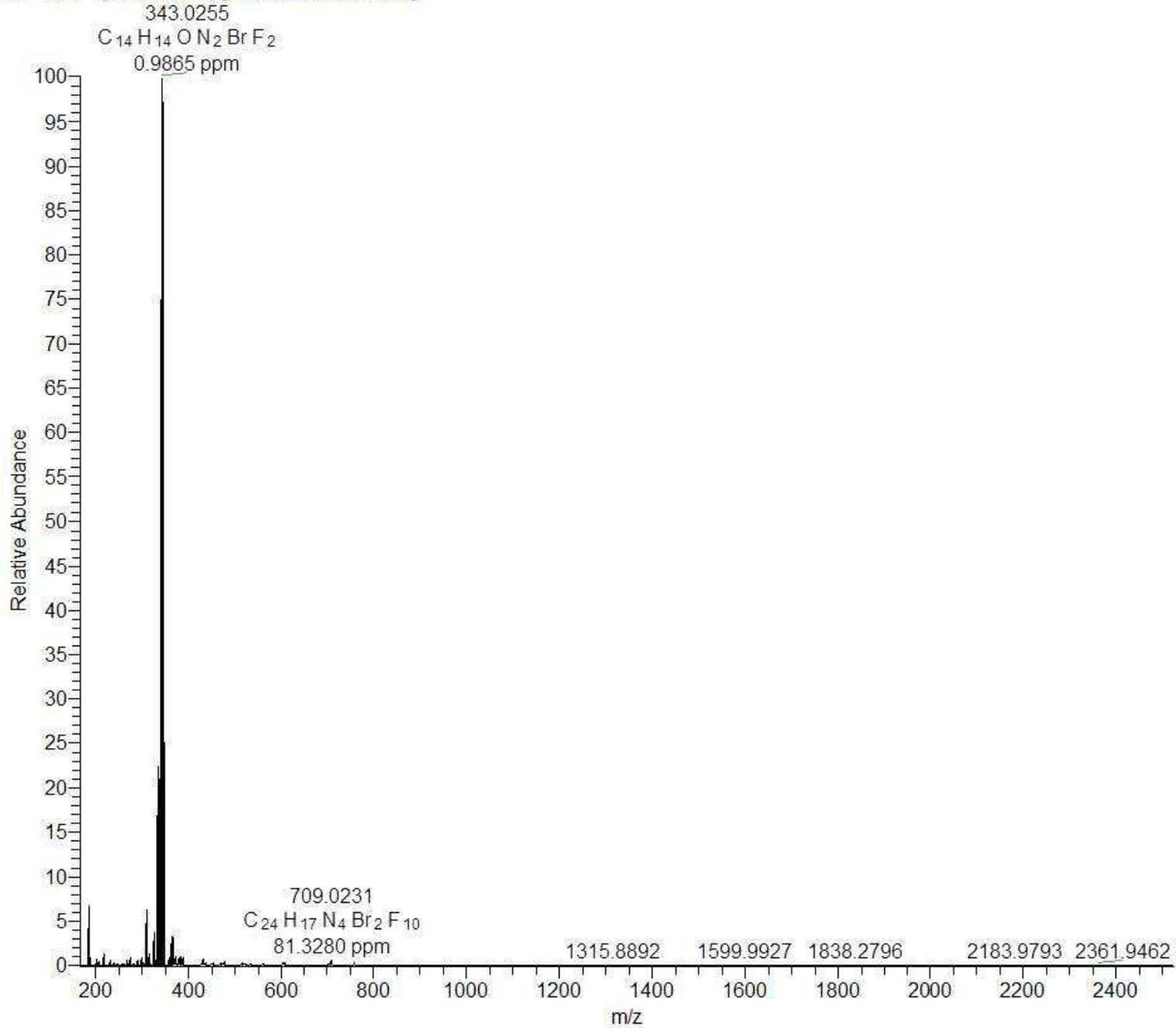
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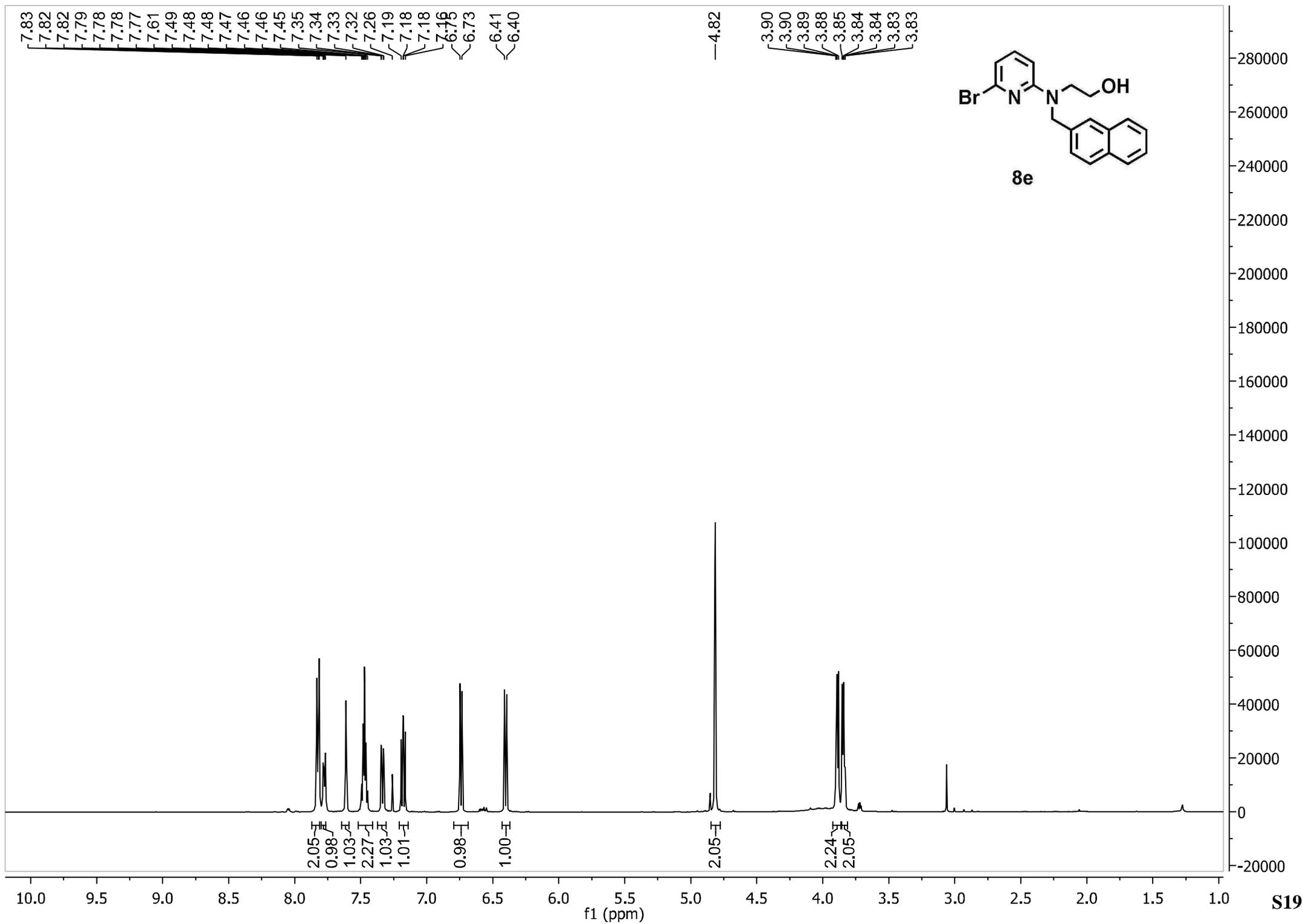


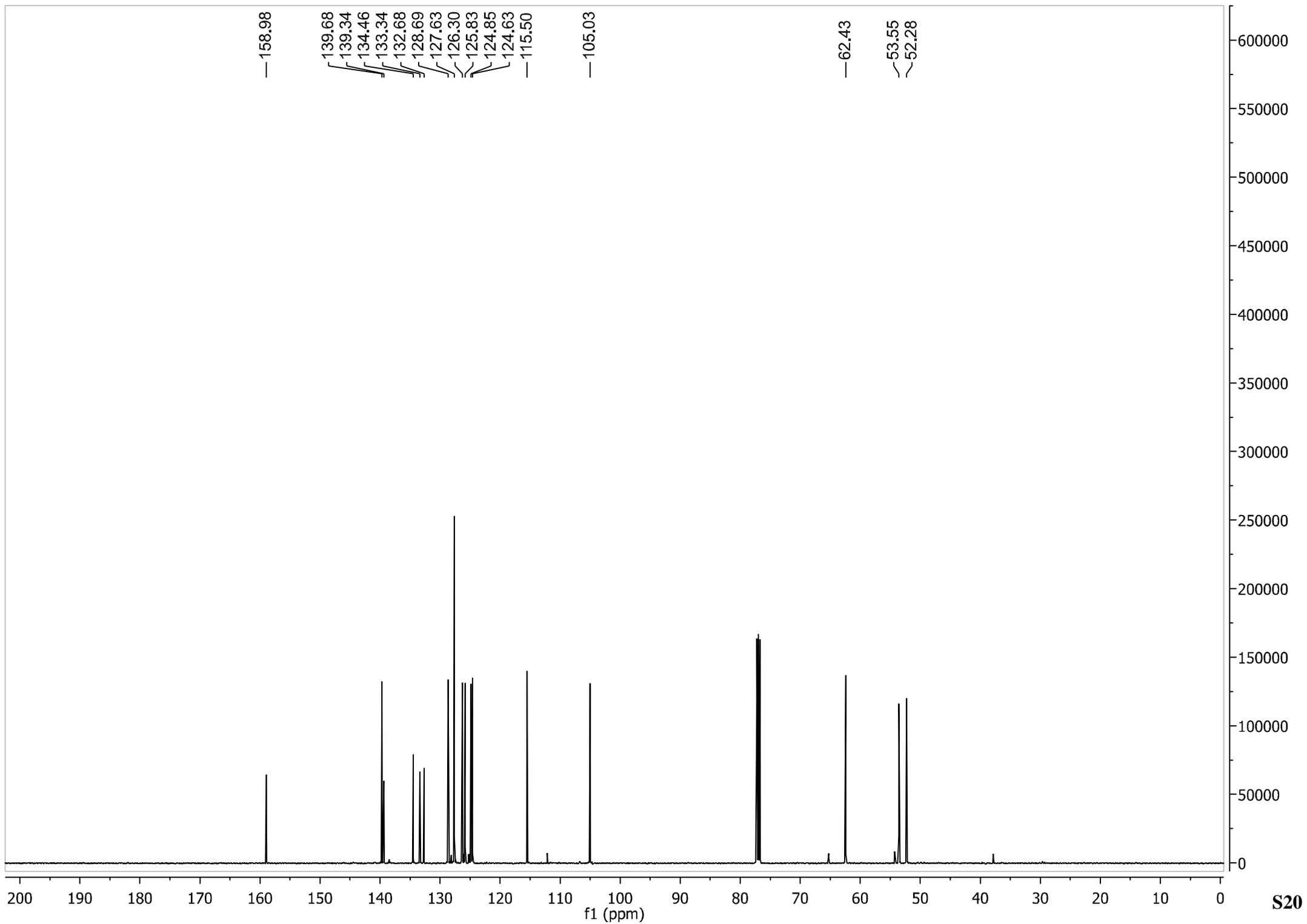




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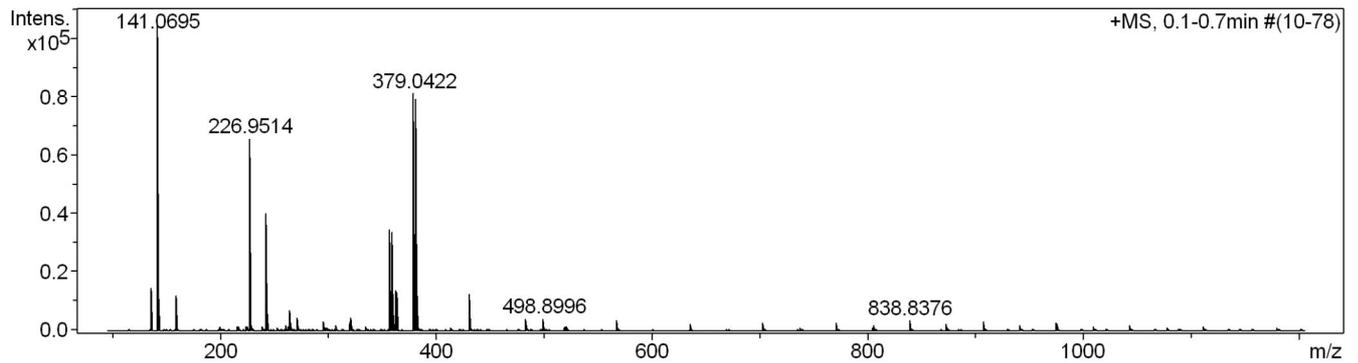
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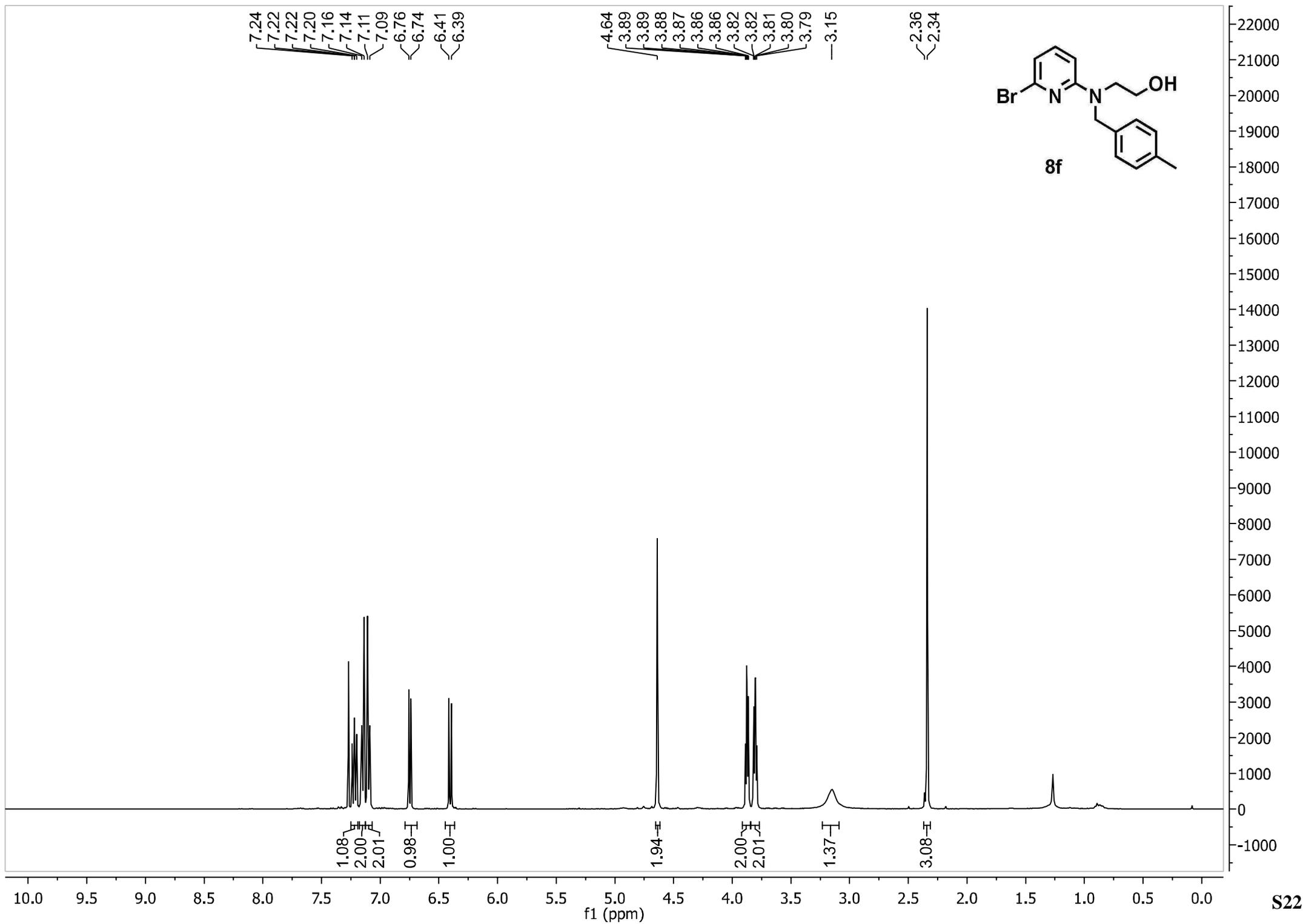
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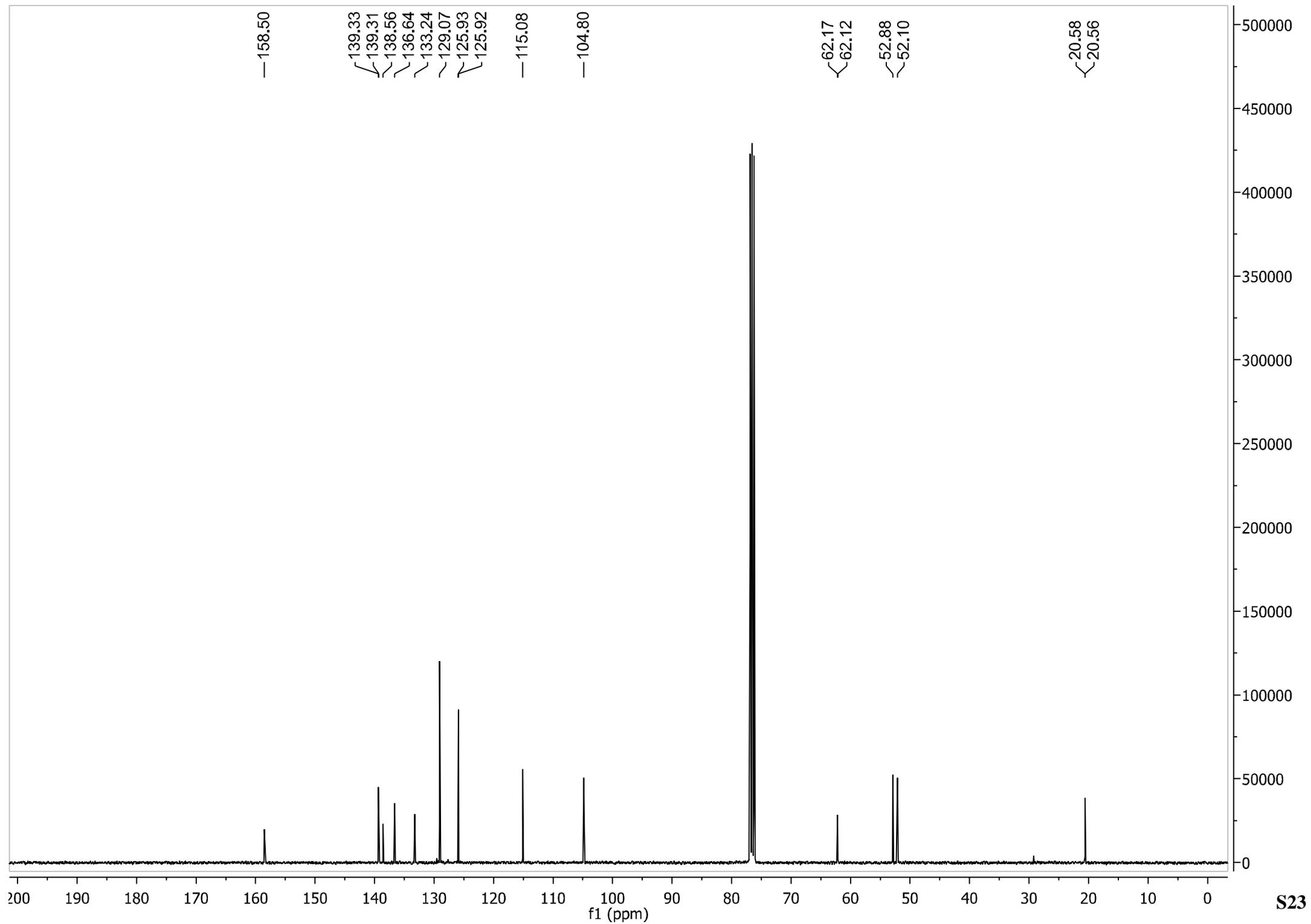
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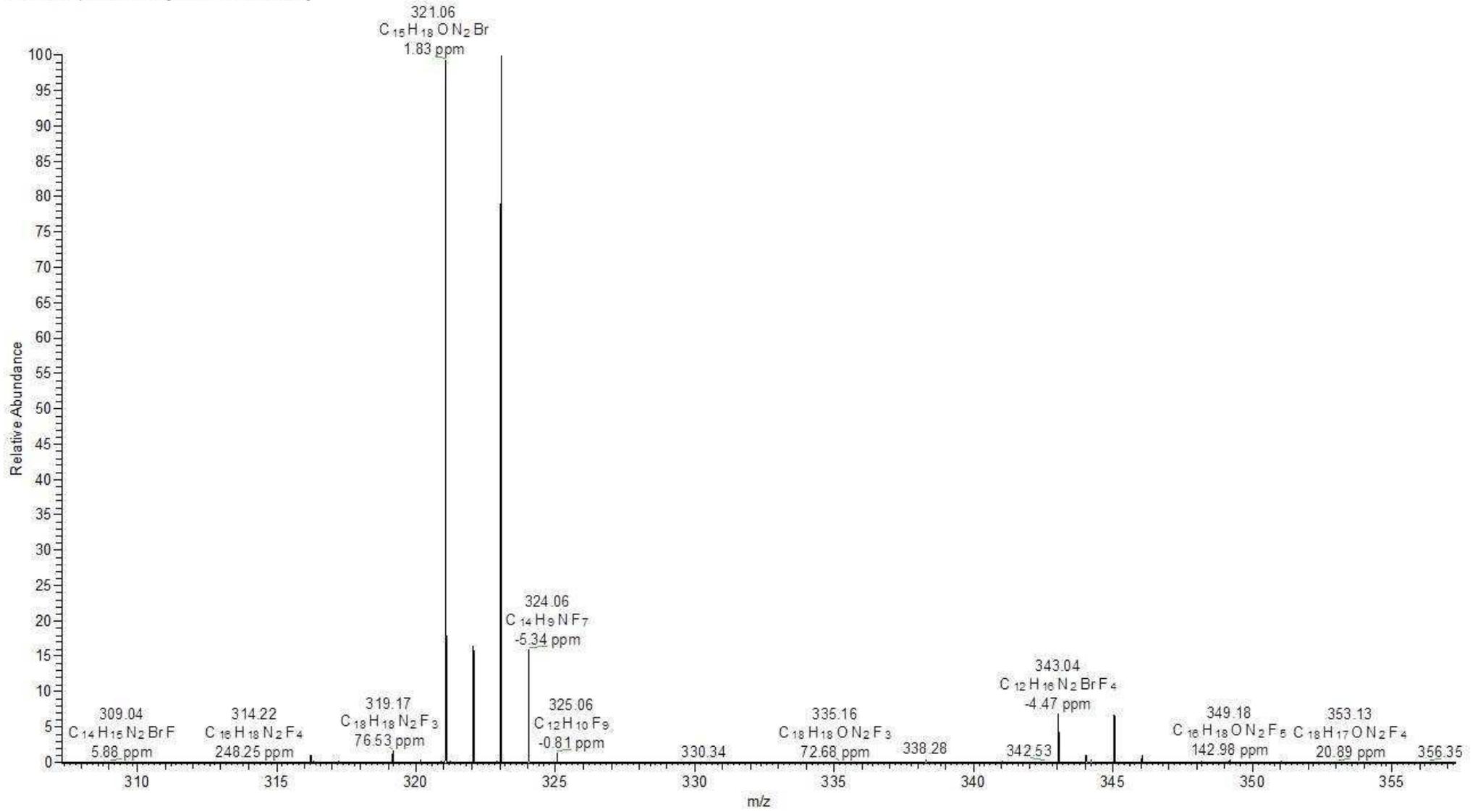


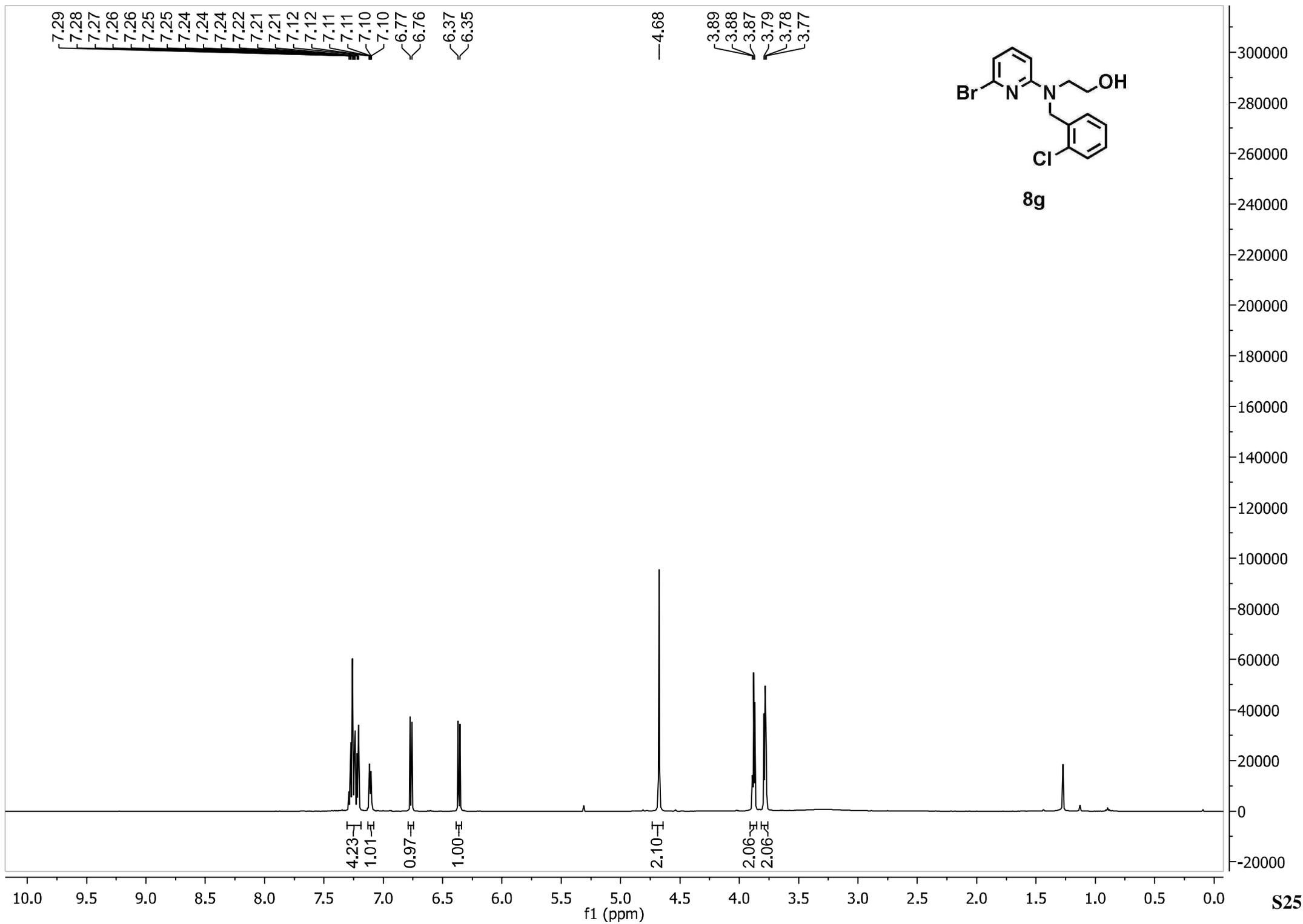
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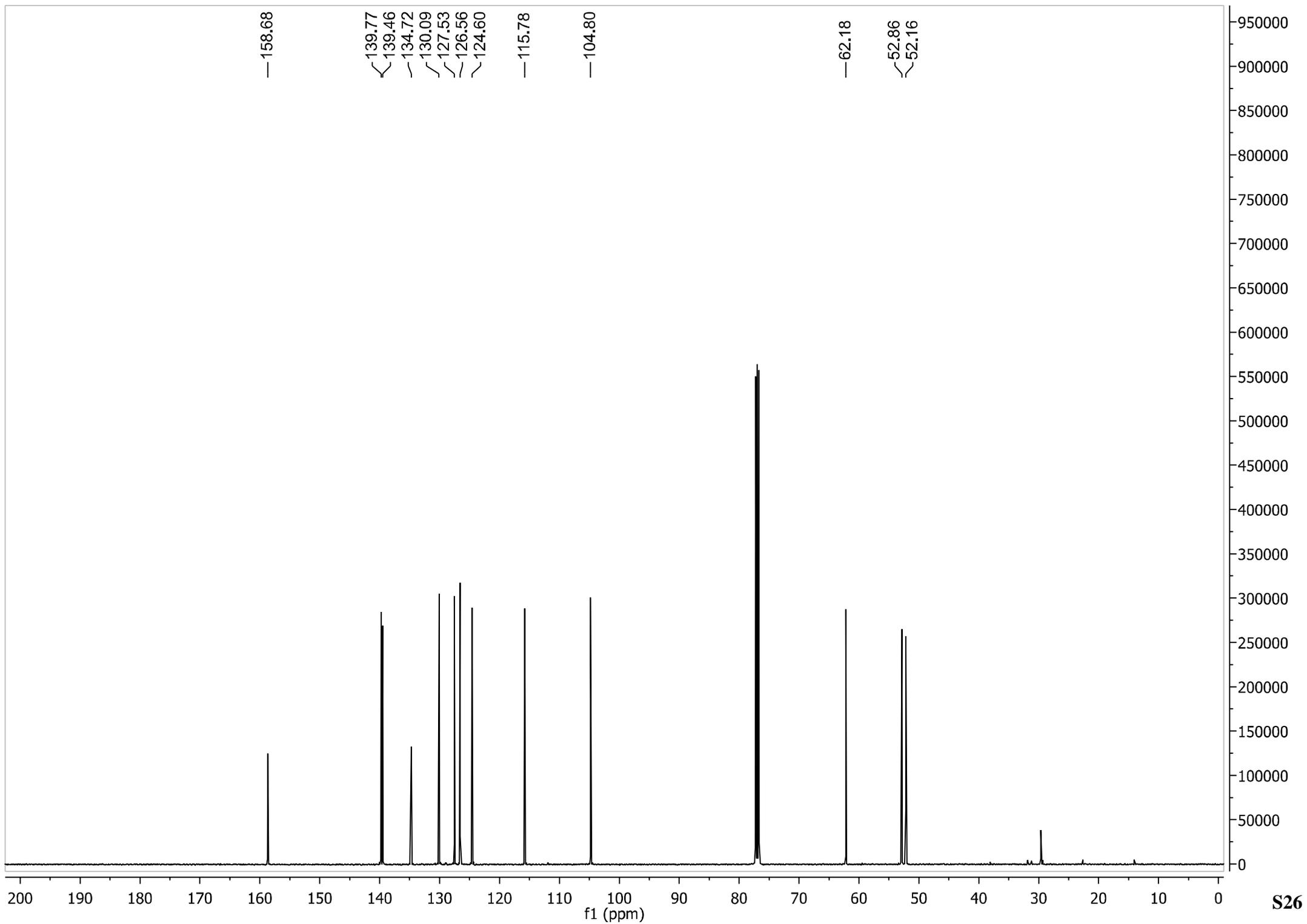


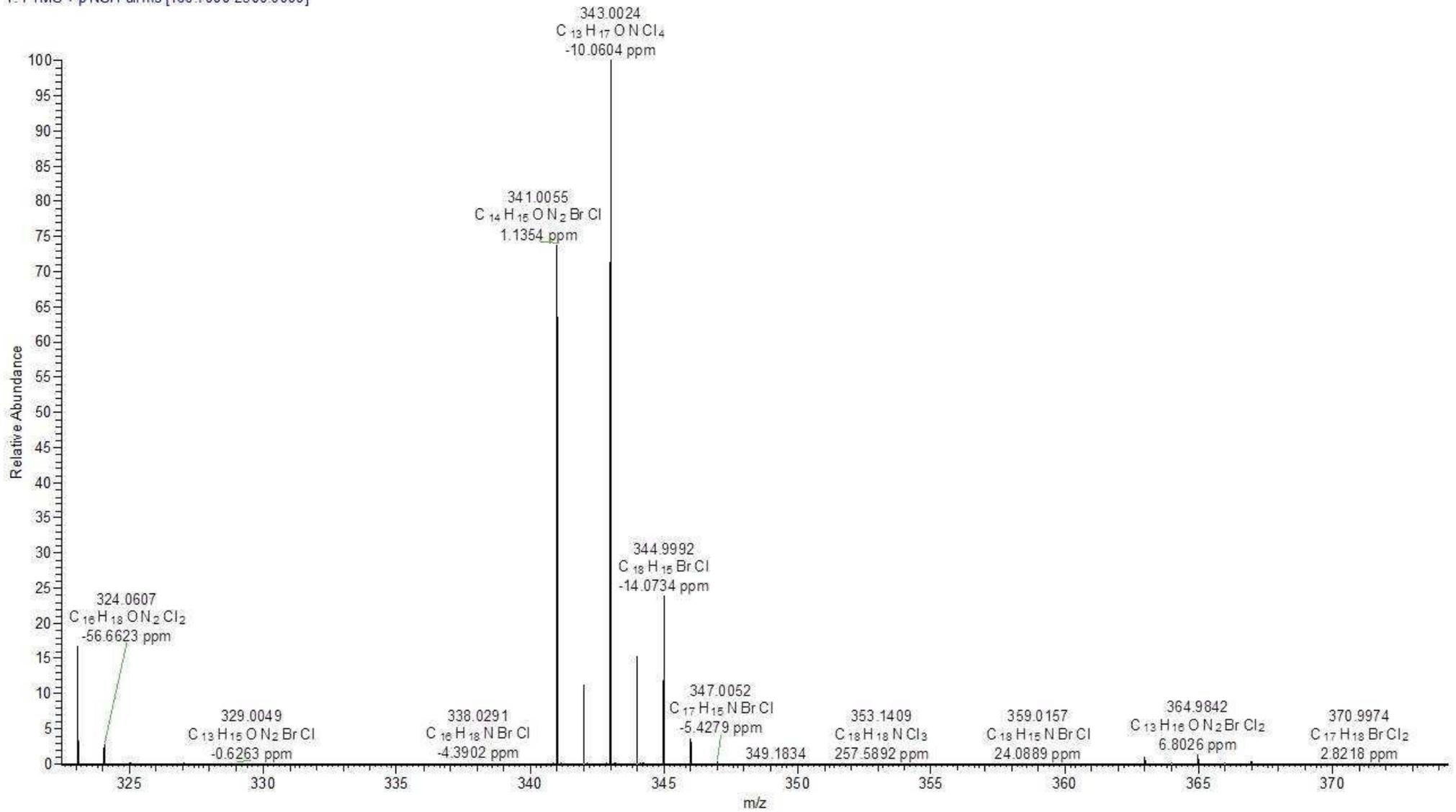


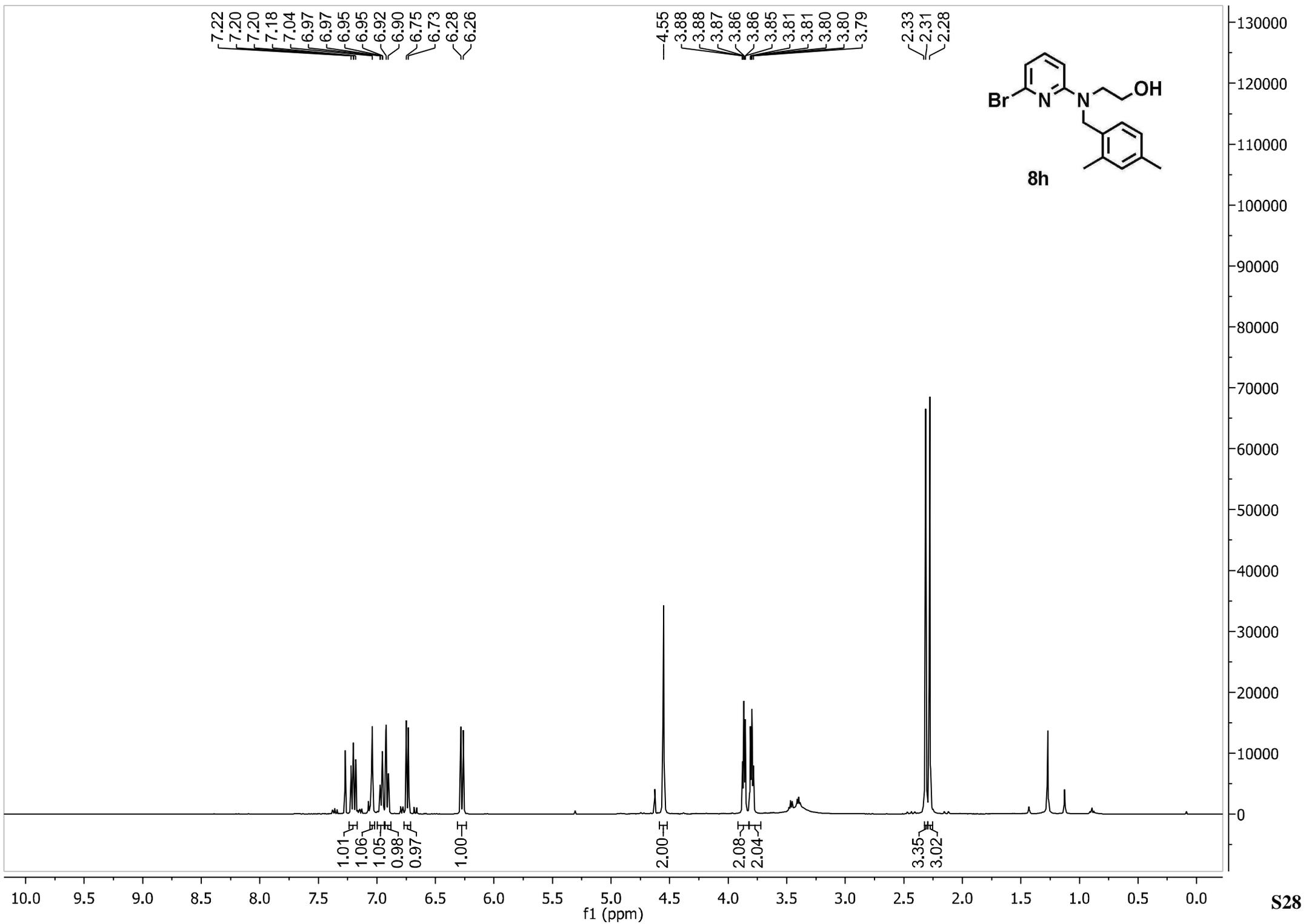
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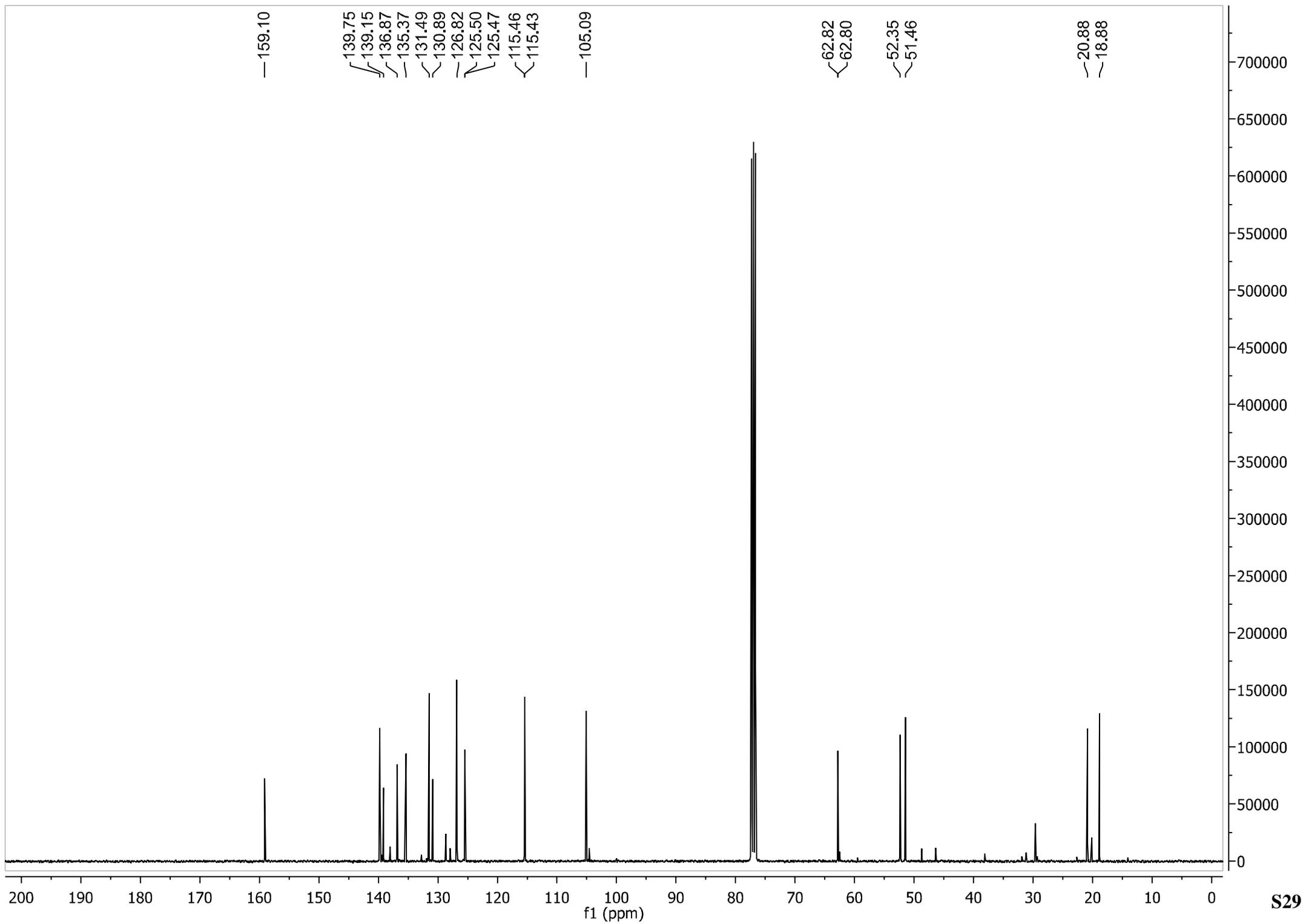




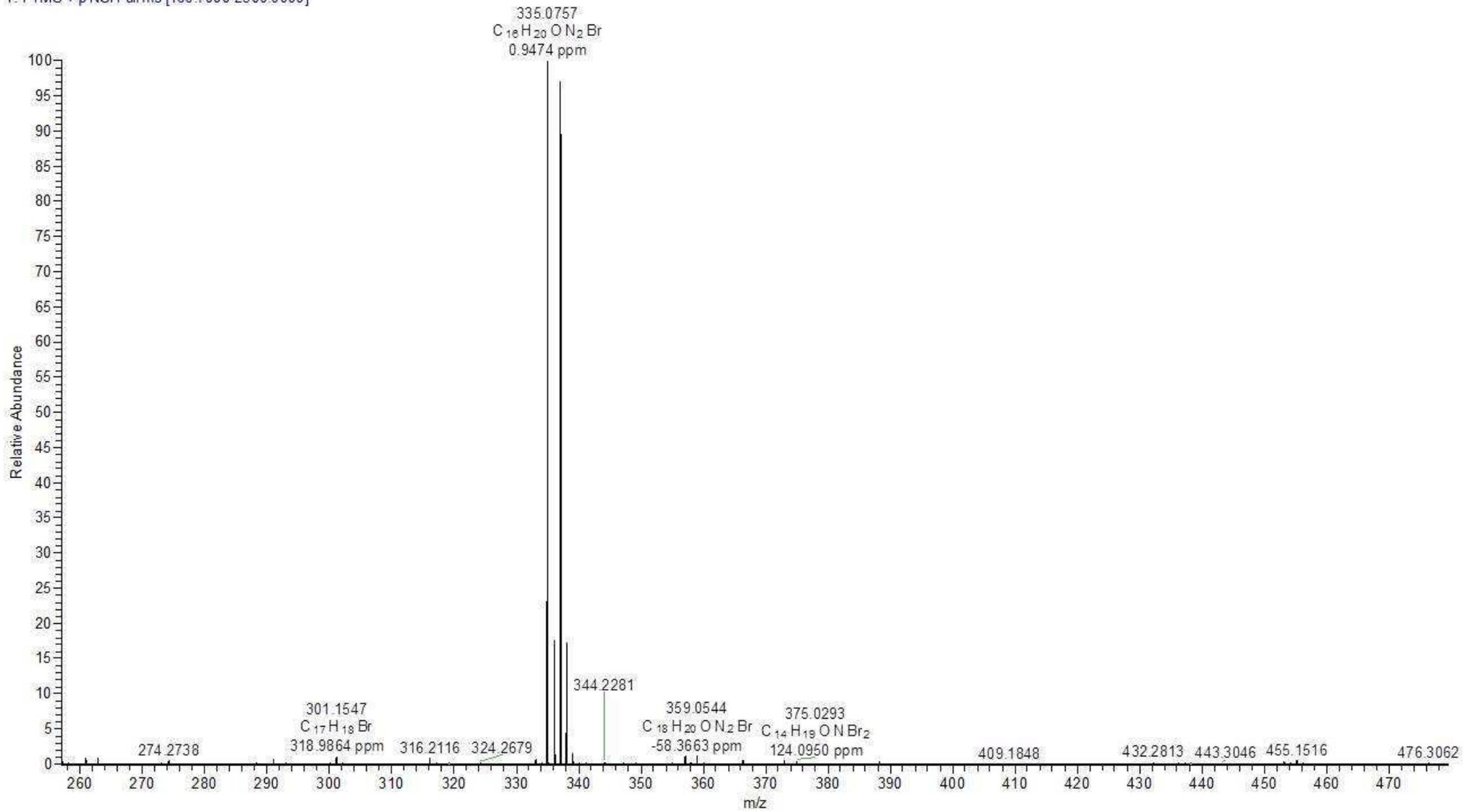


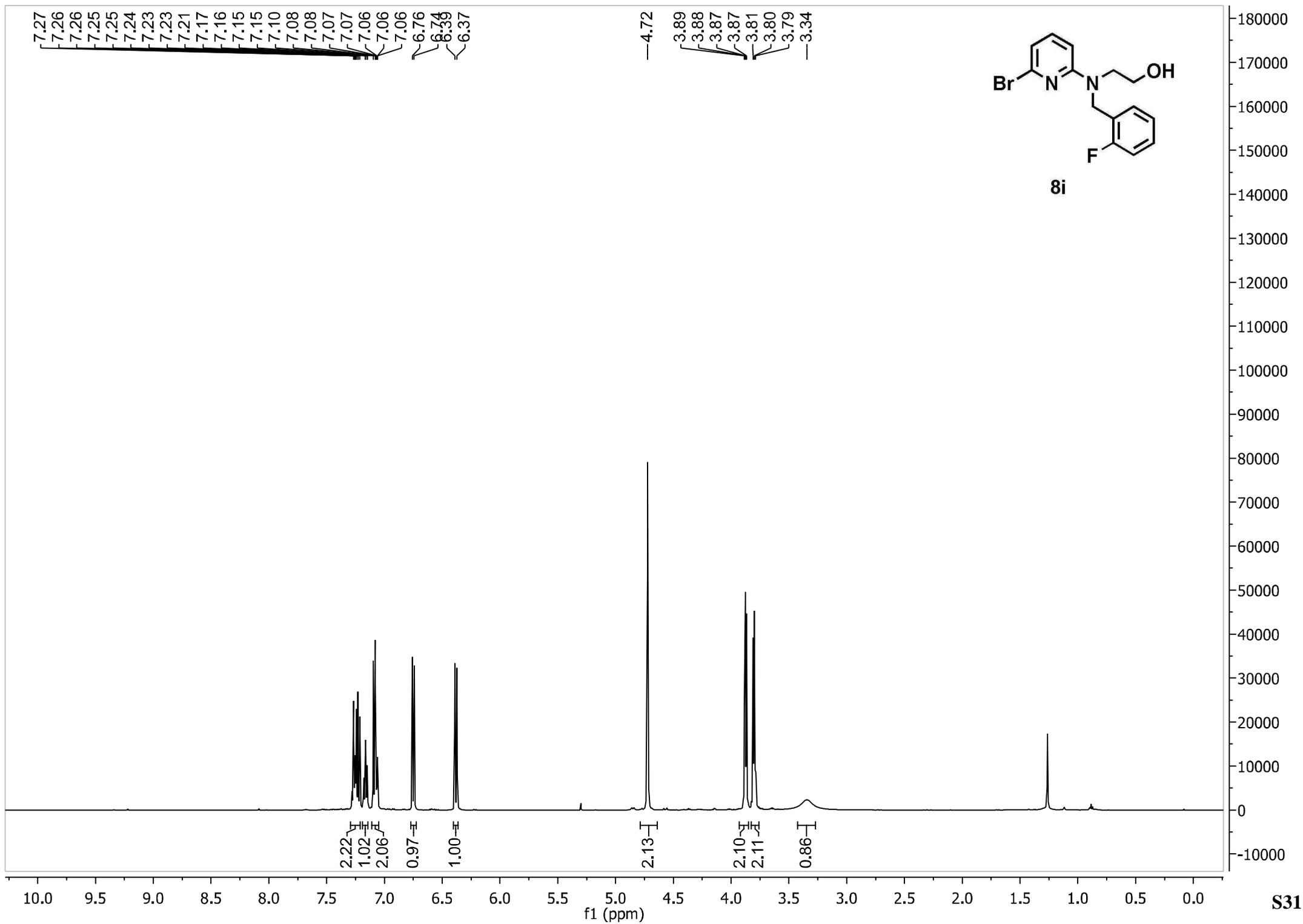


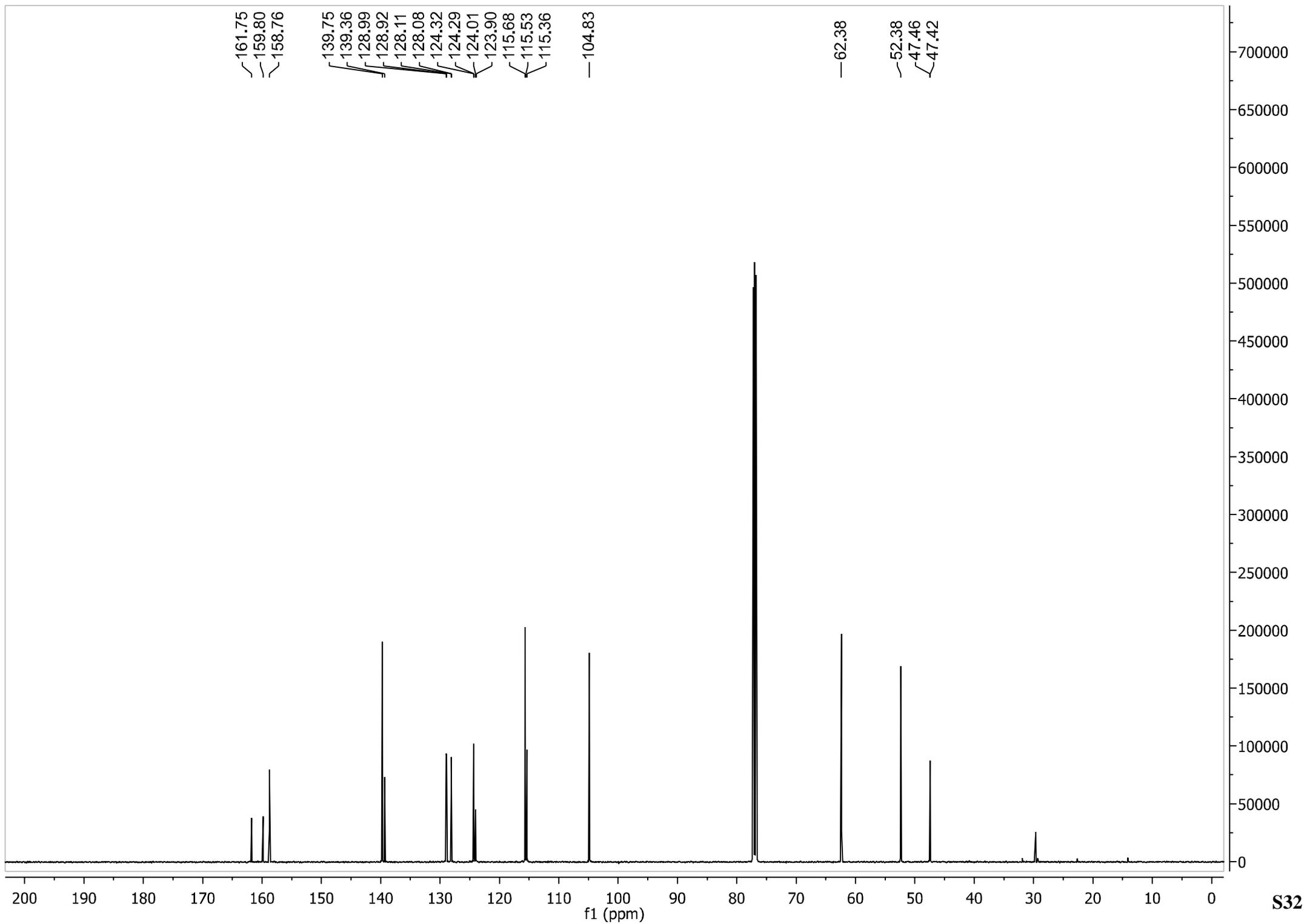




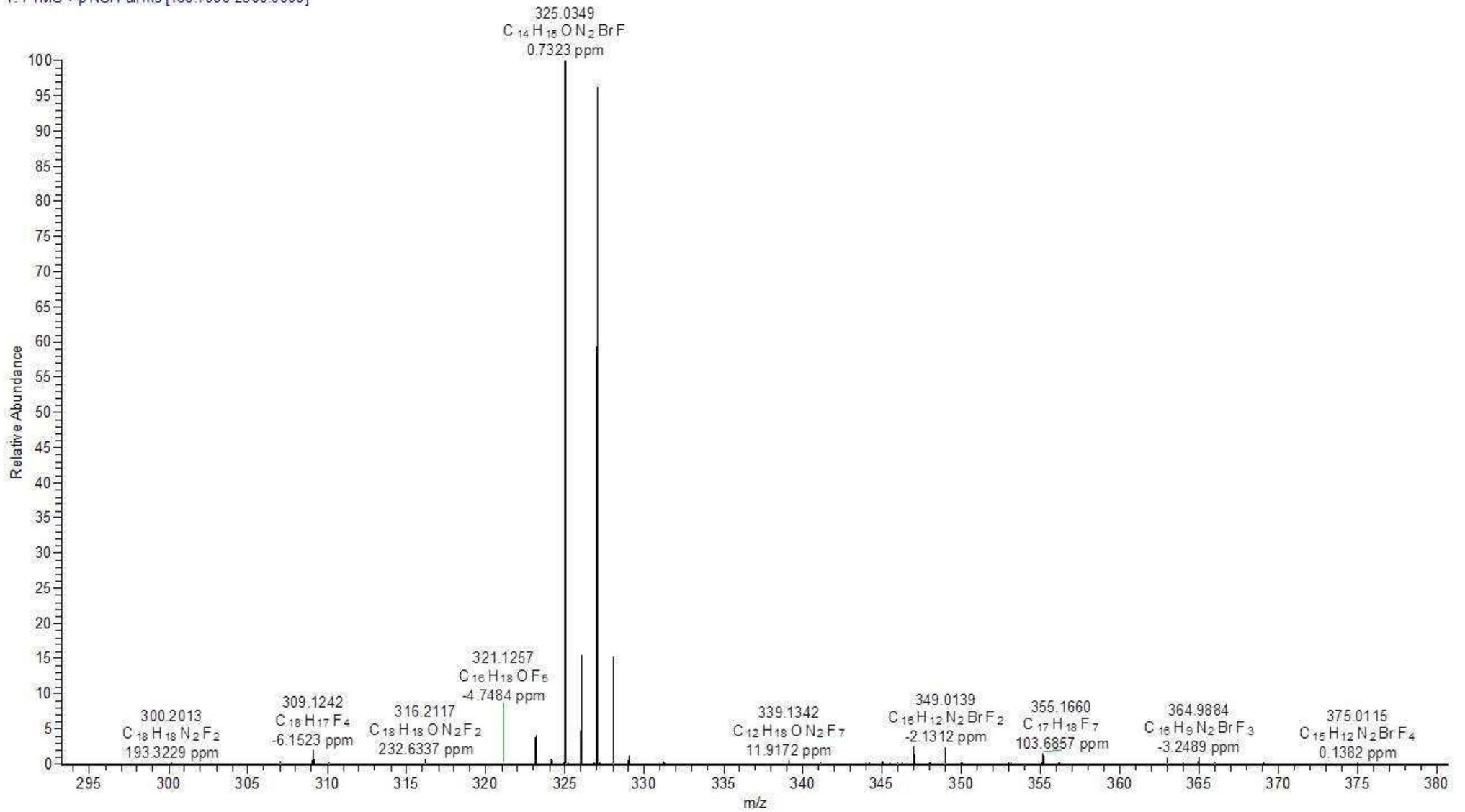
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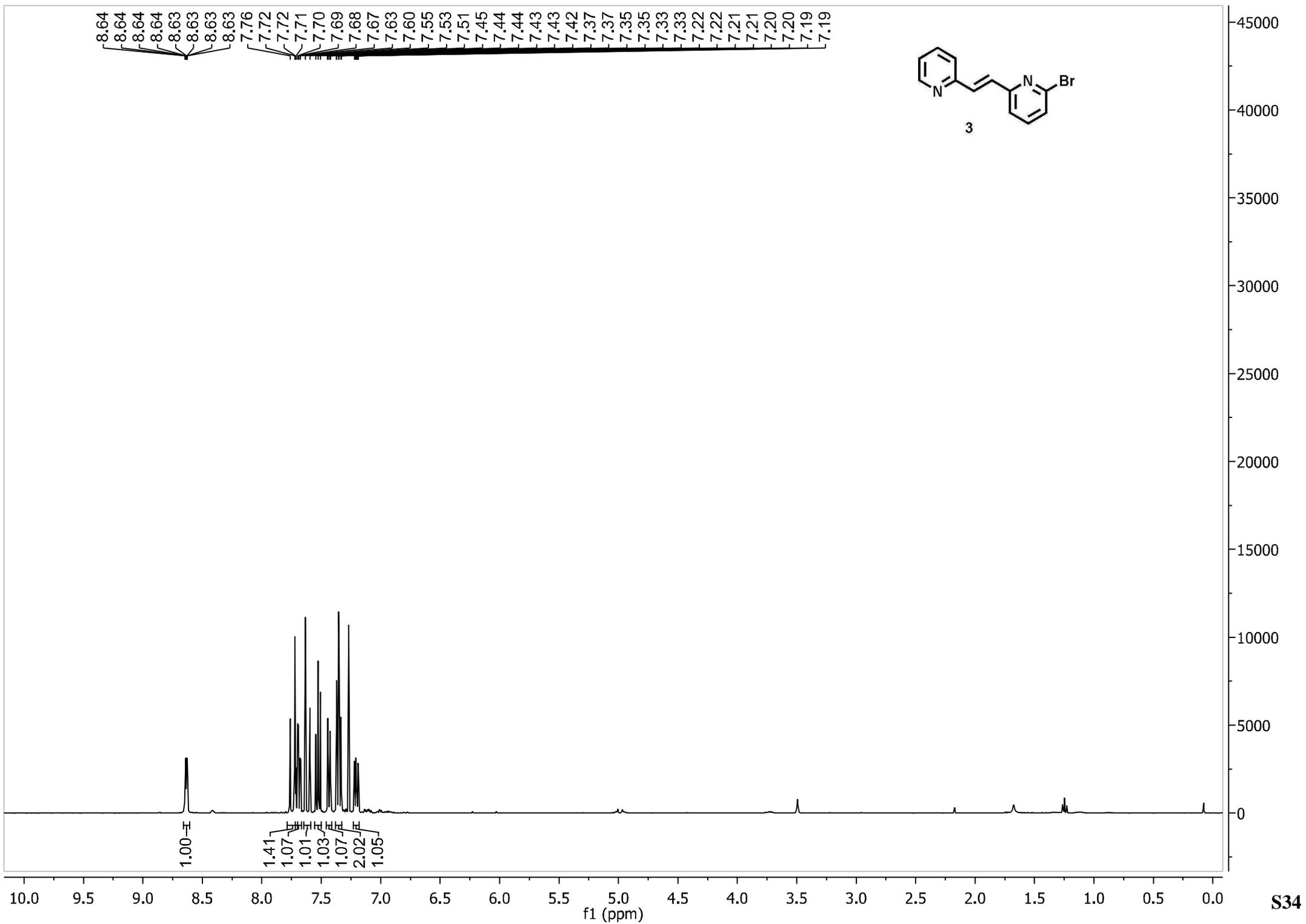


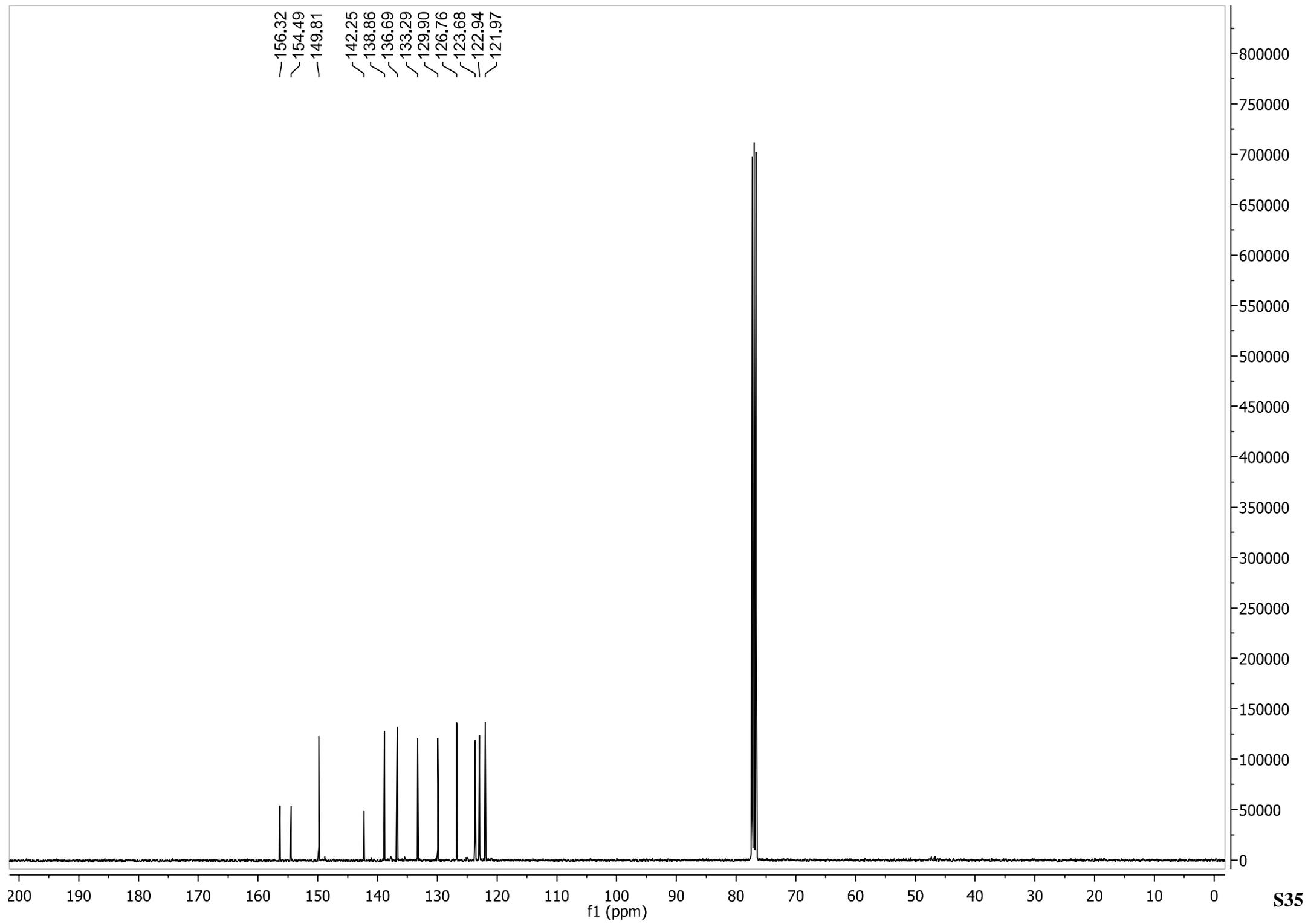




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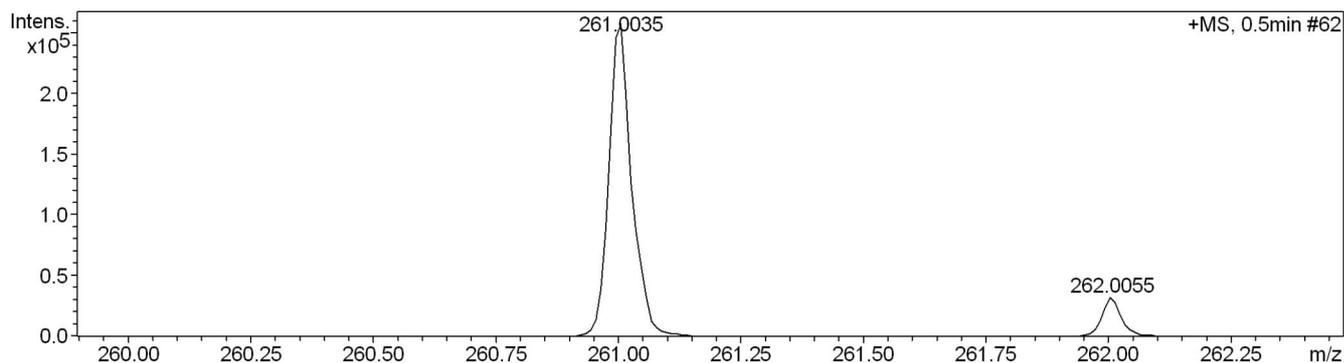
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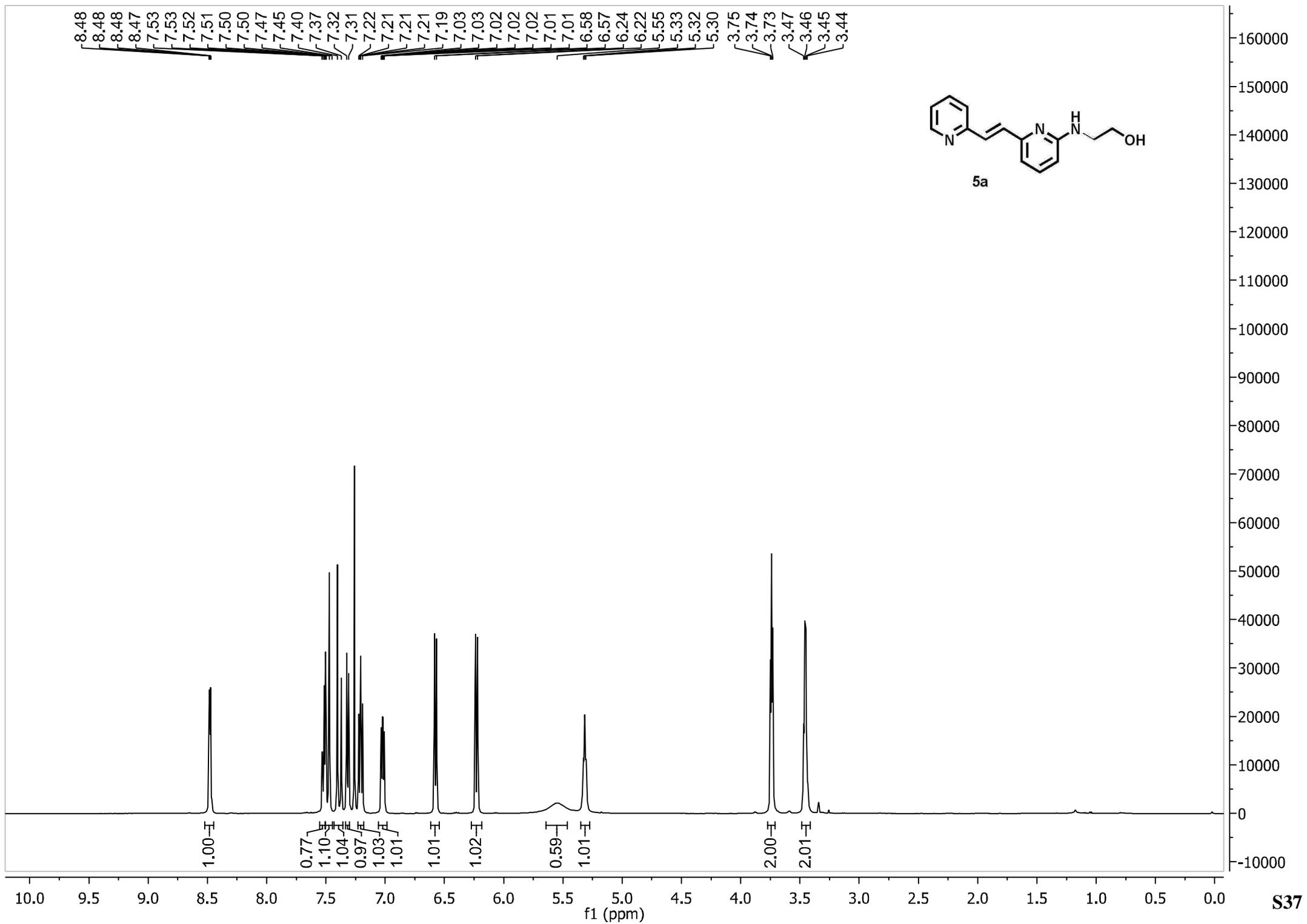
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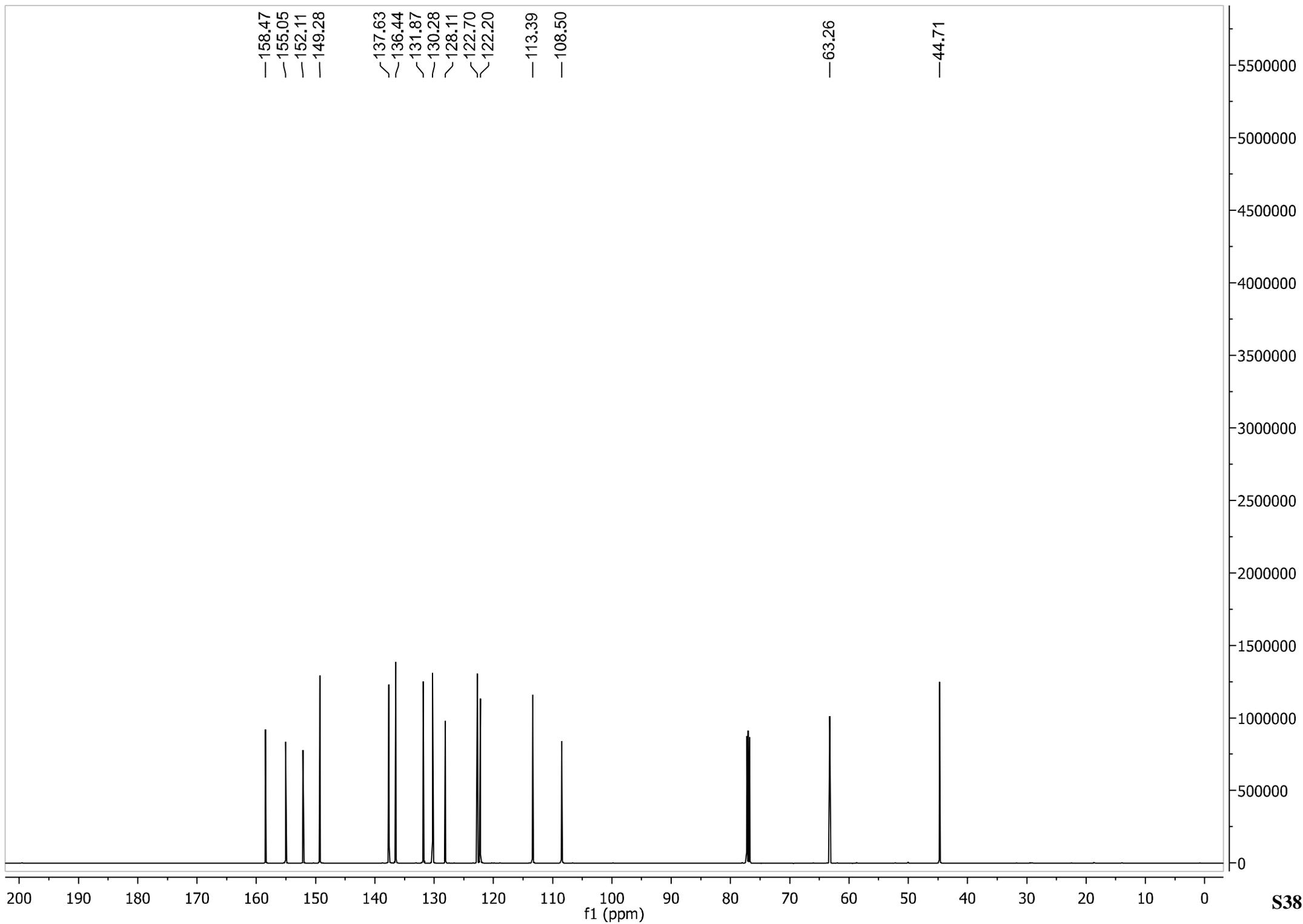
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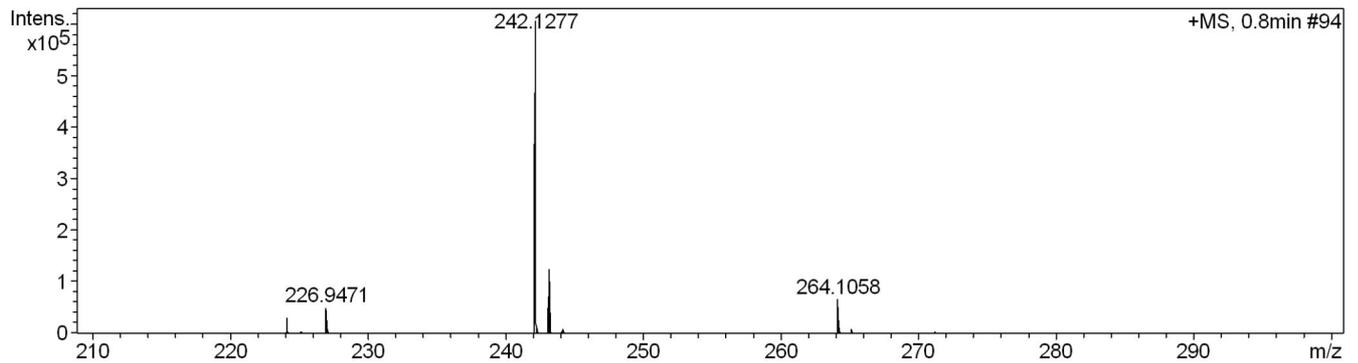
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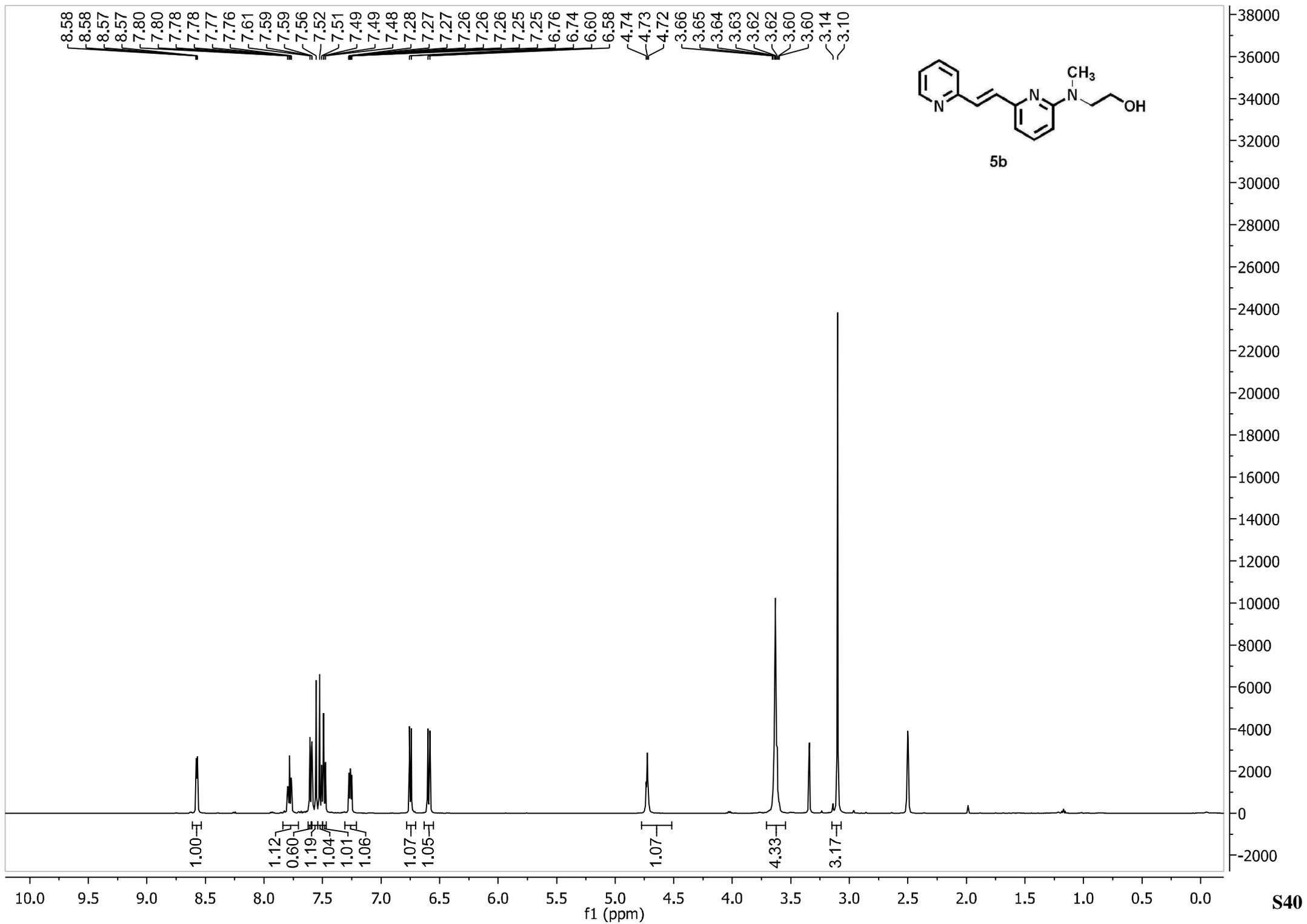
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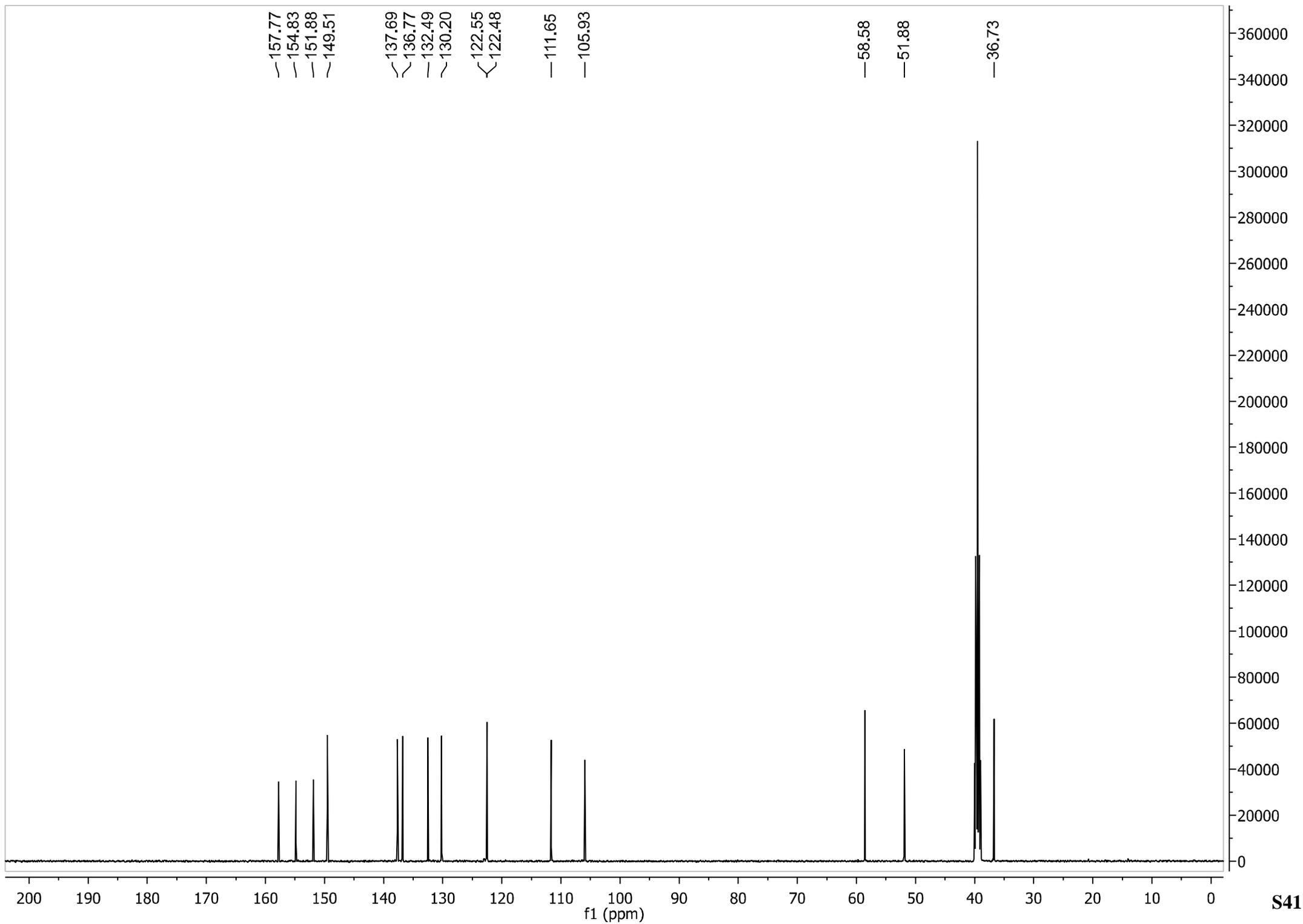
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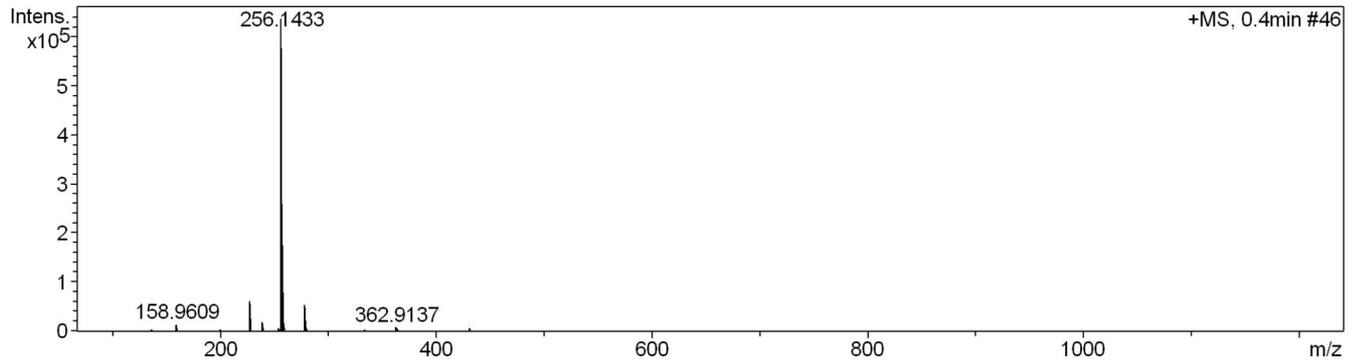
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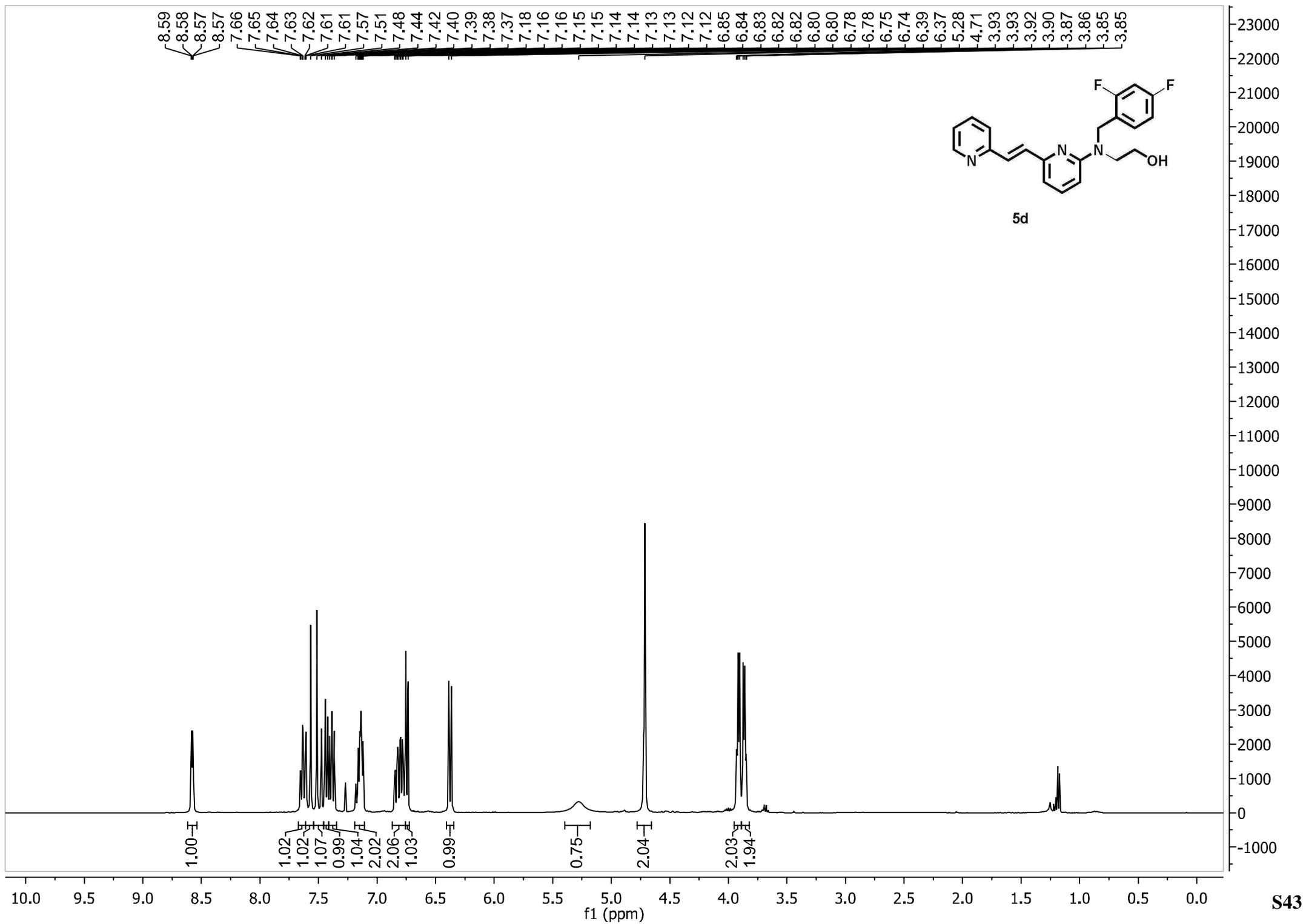
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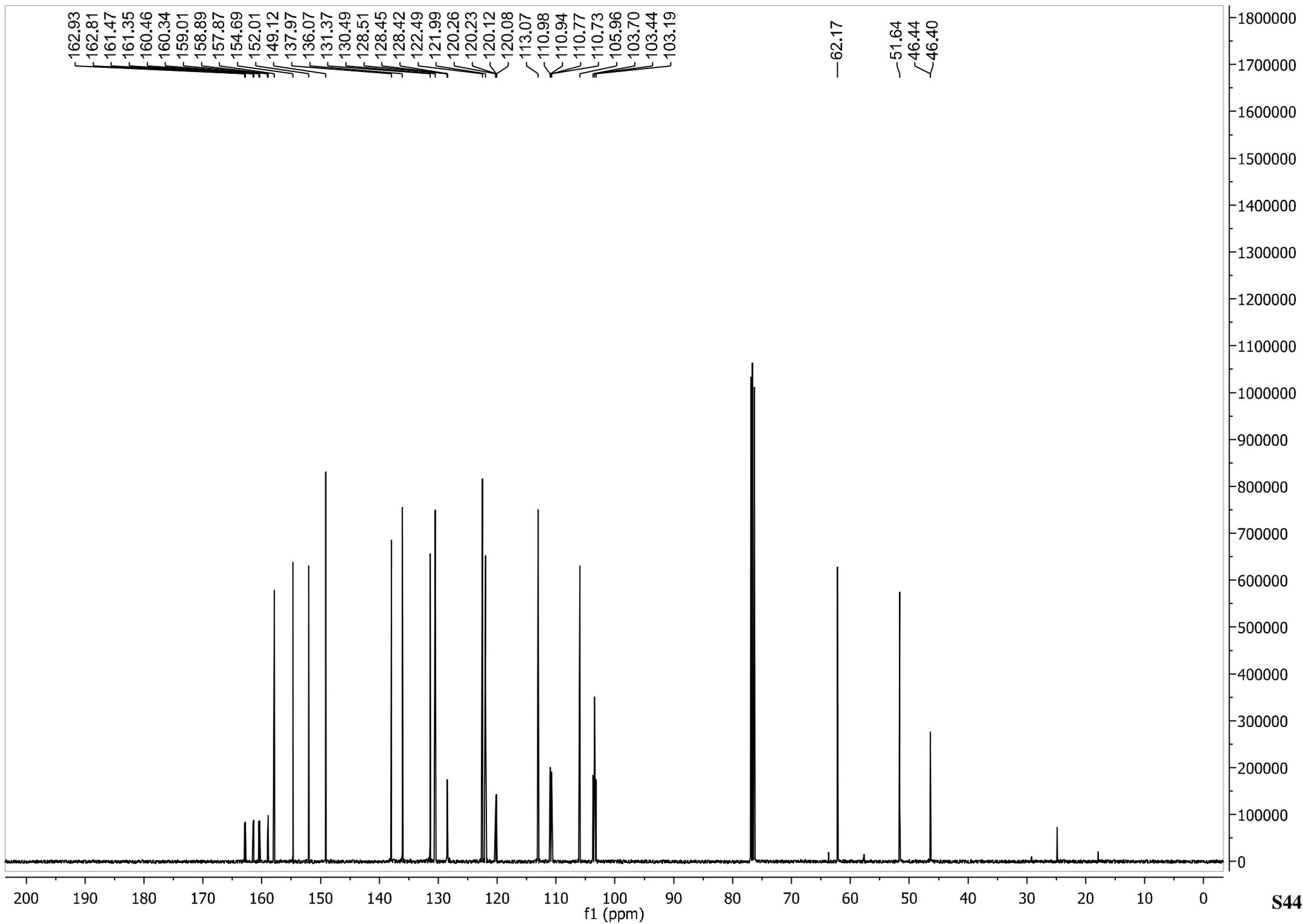
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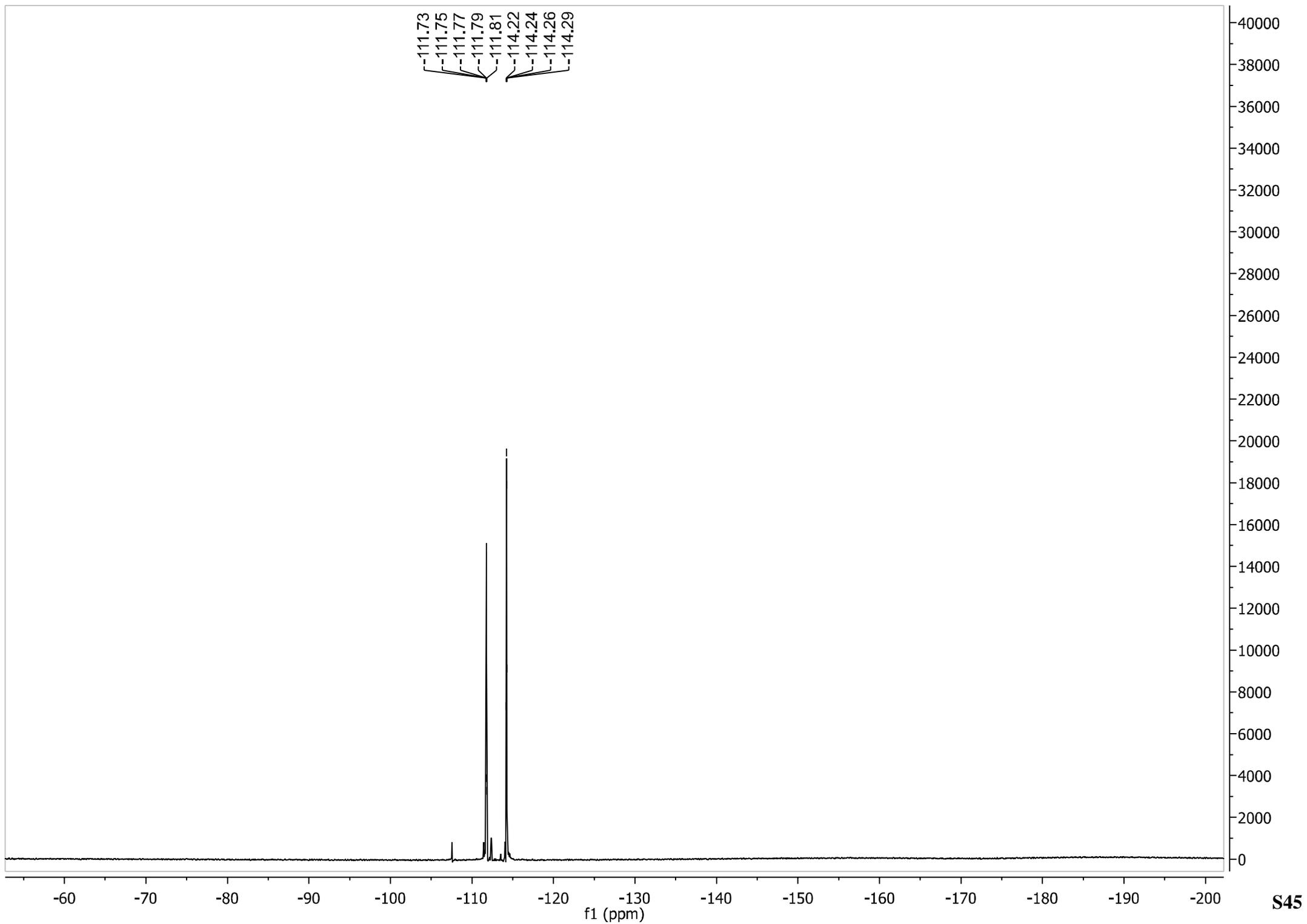
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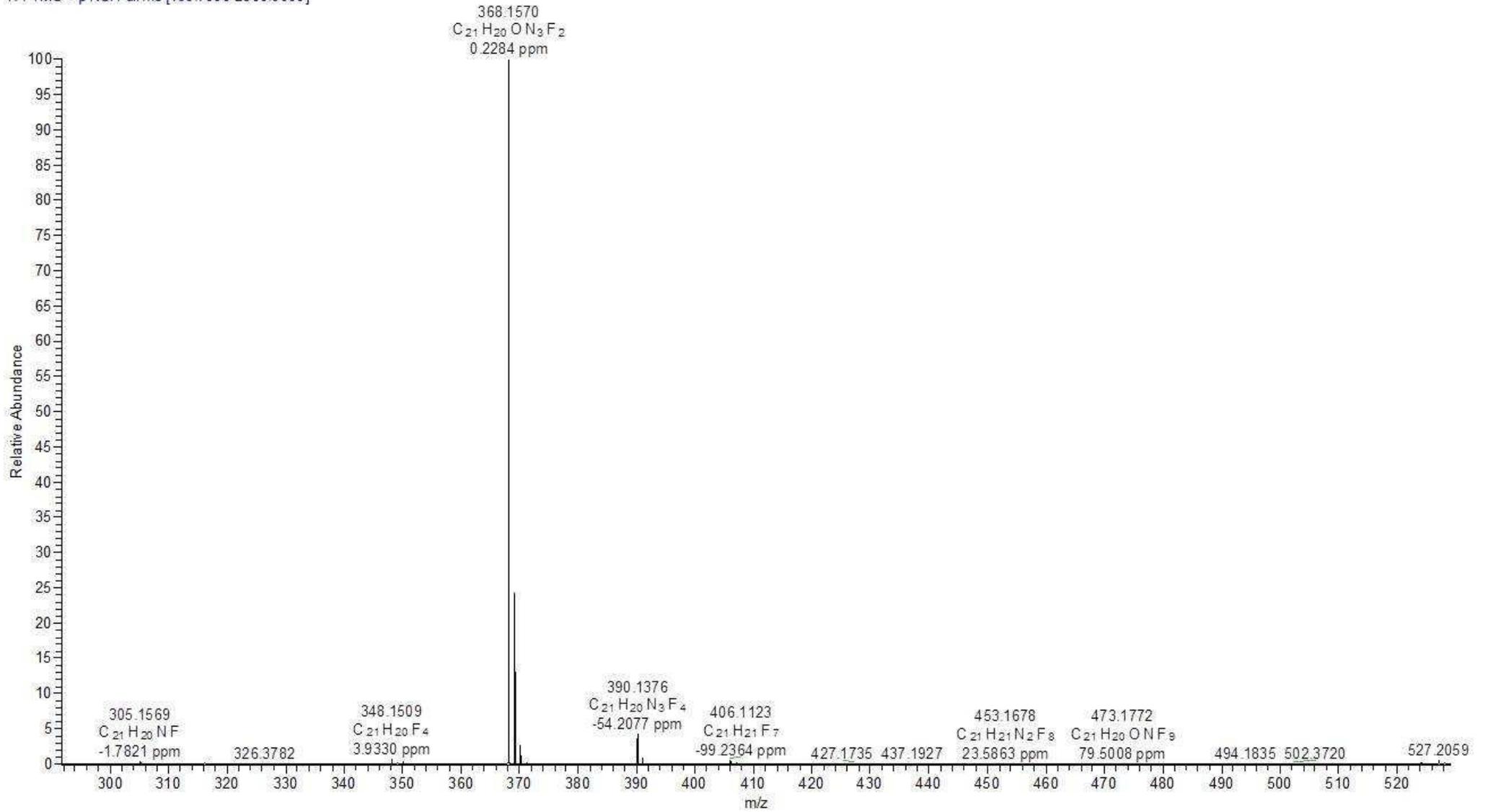
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256.1433	1	C ₁₅ H ₁₈ N ₃ O	256.1444	4.3	8.8	8.5	ok	even	75.9	123.9	3.1	49.3	4.8	842.7

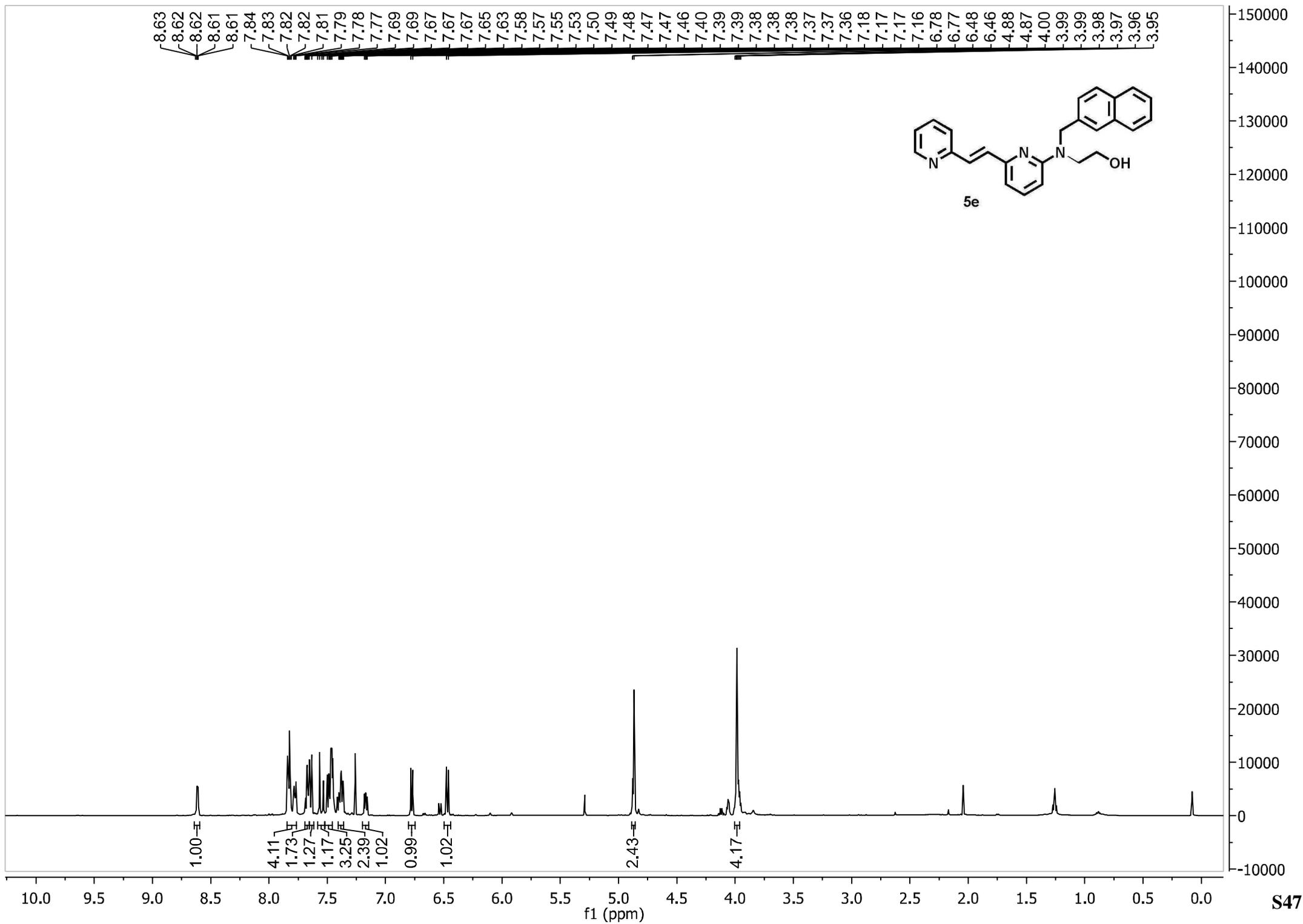


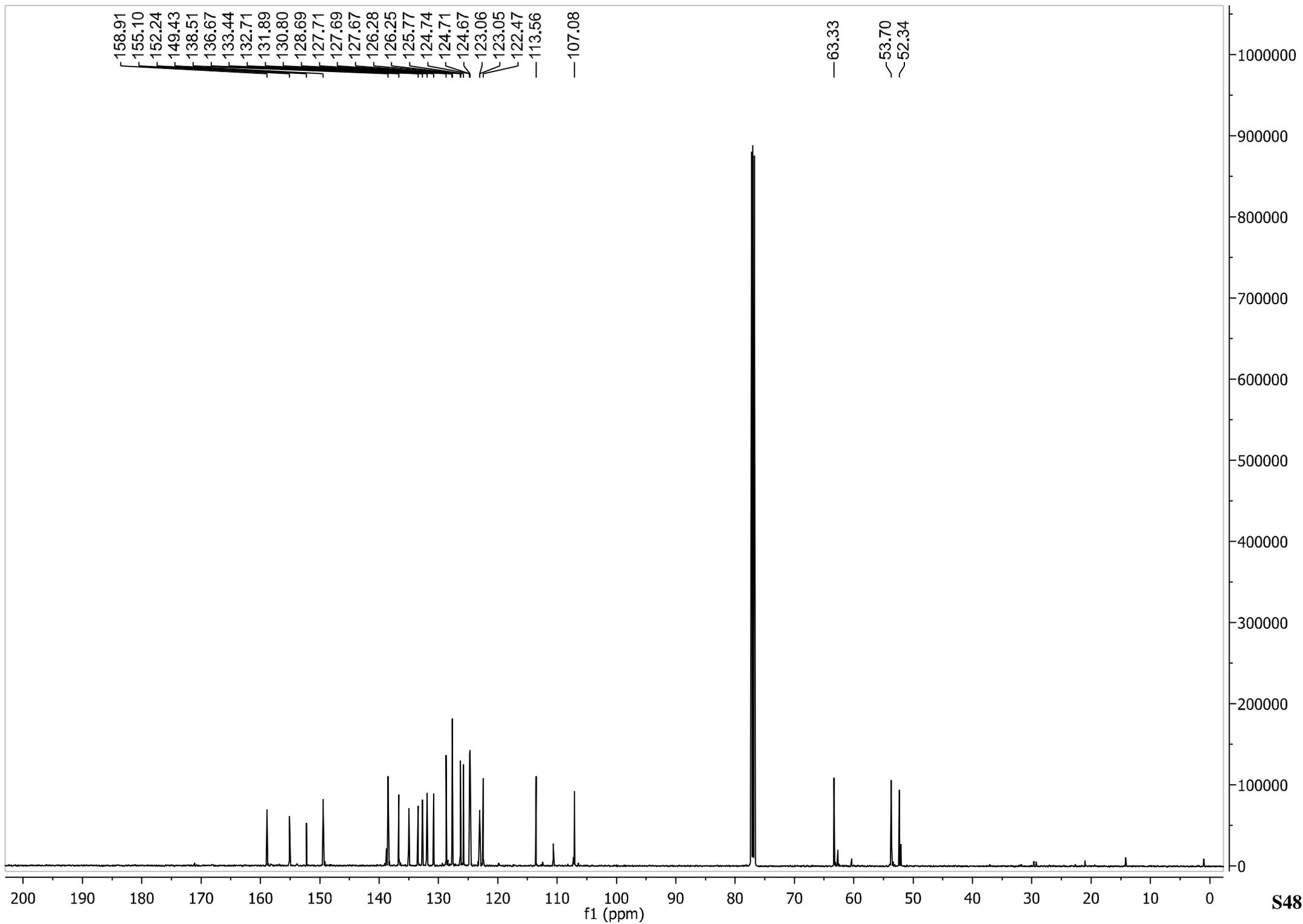




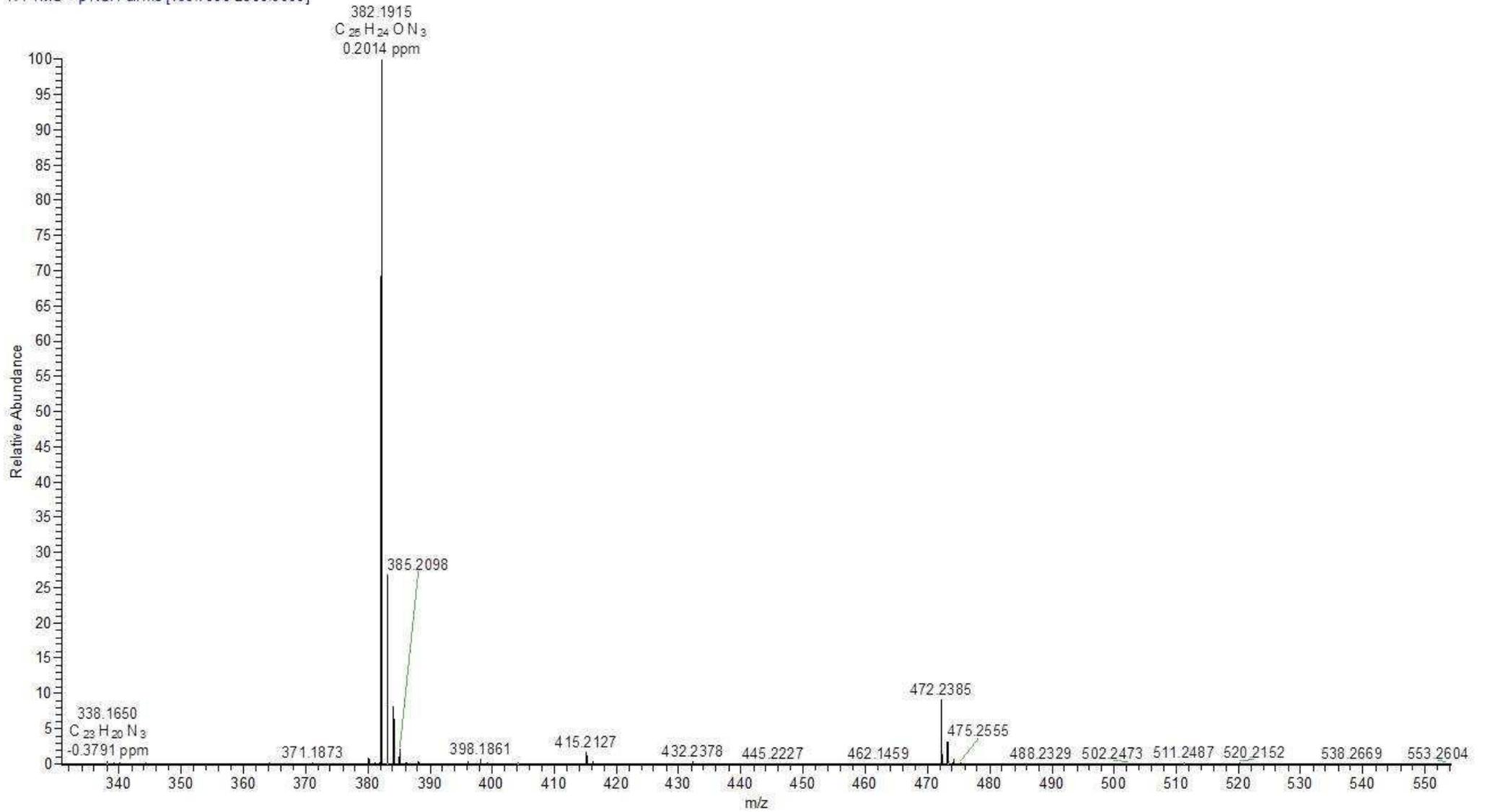
aw15 #4-294 RT: 0.01-0.68 AV: 291 NL: 9.43E9
T: FTMS + p NSI Full ms [166.7000-2500.0000]

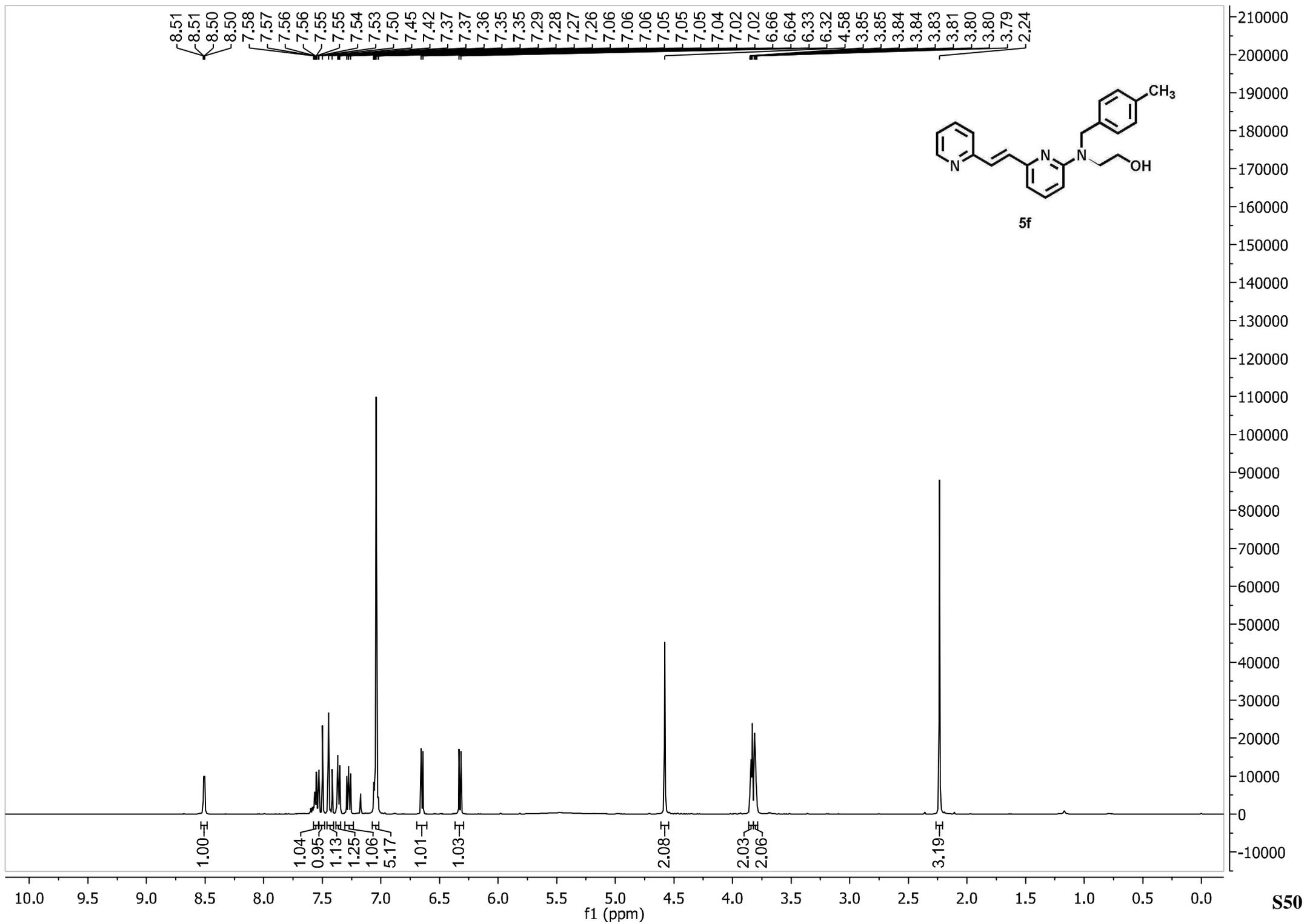


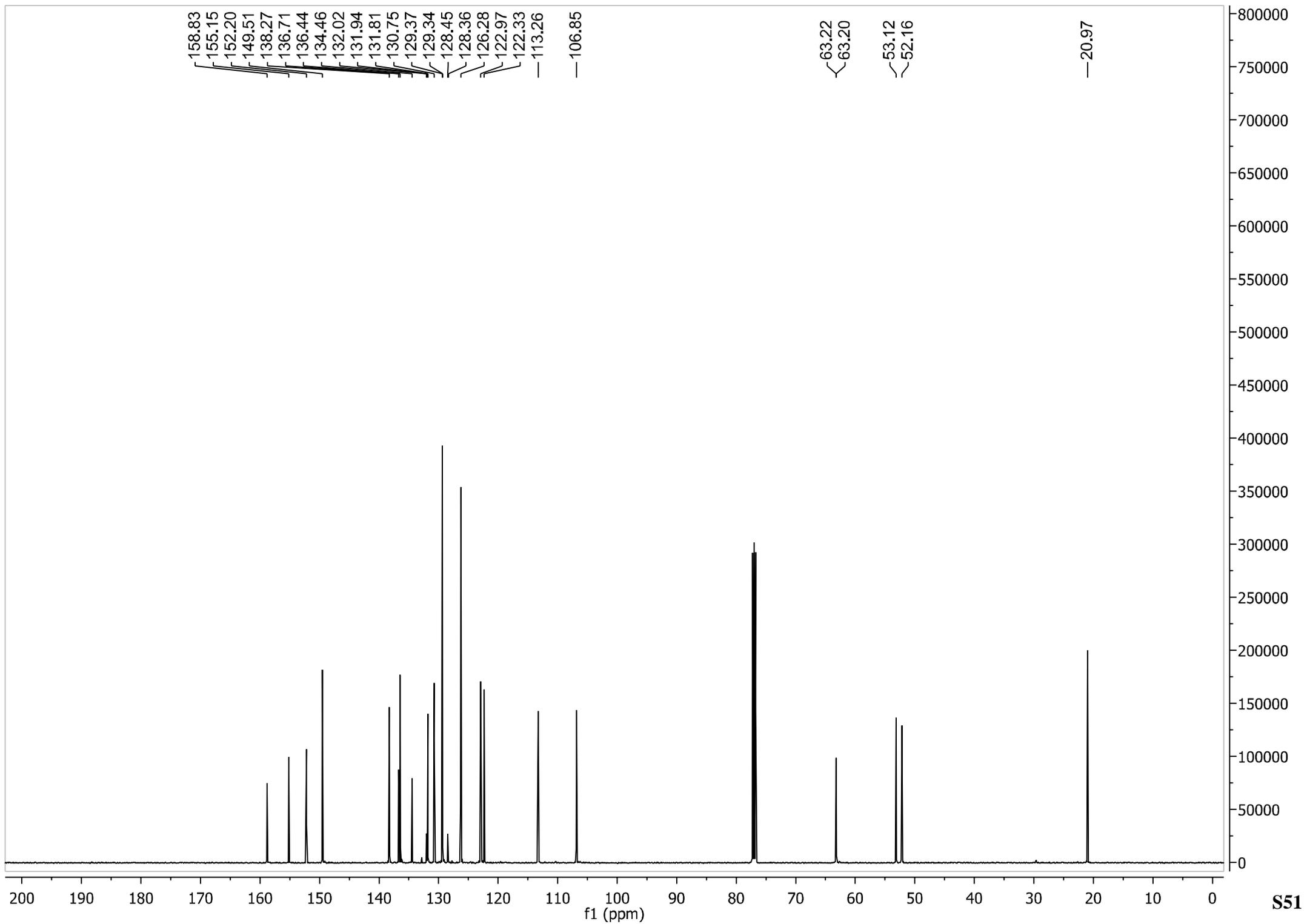




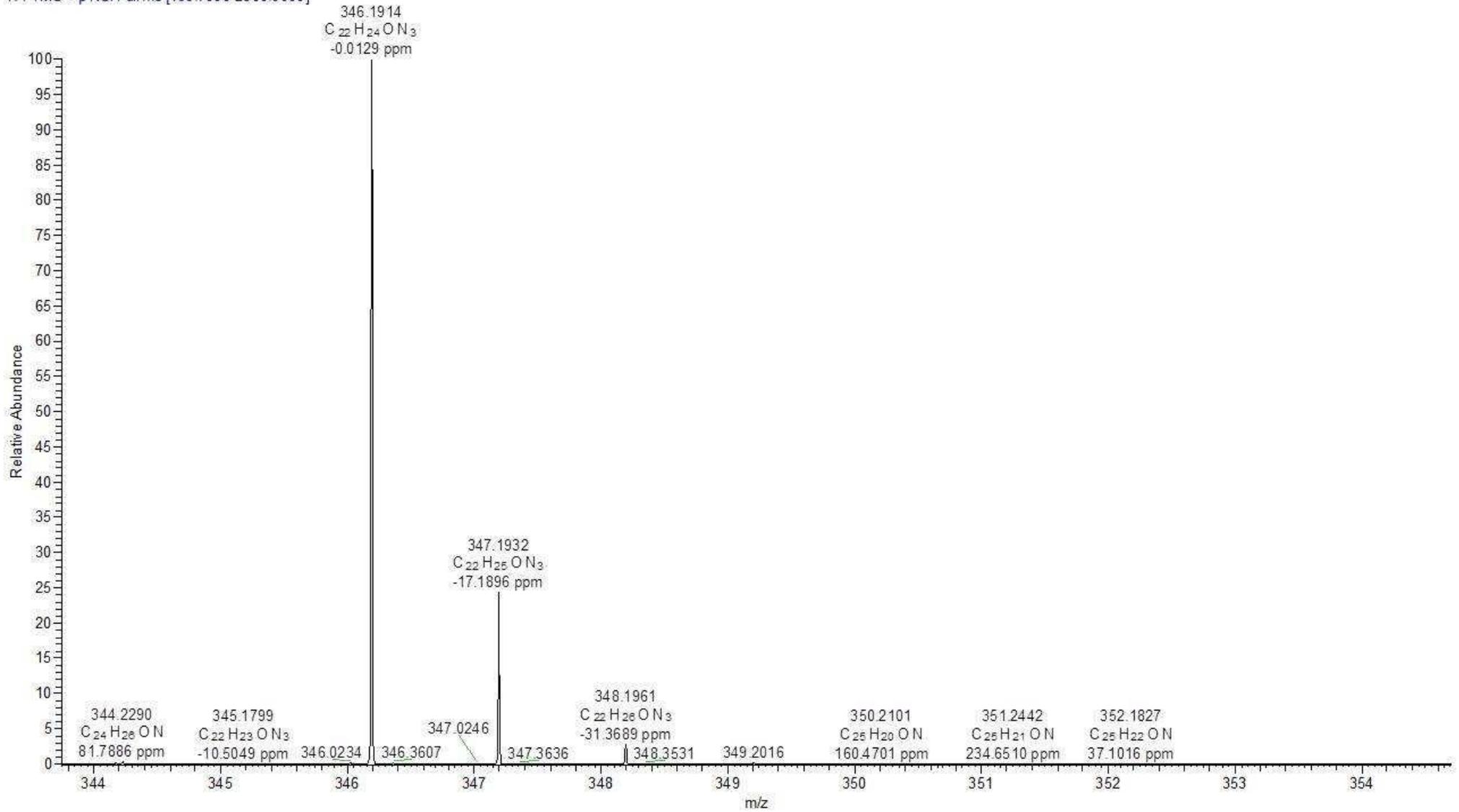
aw14 #8-337 RT: 0.02-0.78 AV: 330 NL: 3.70E9
T: FTMS + p NSI Full ms [166.7000-2500.0000]

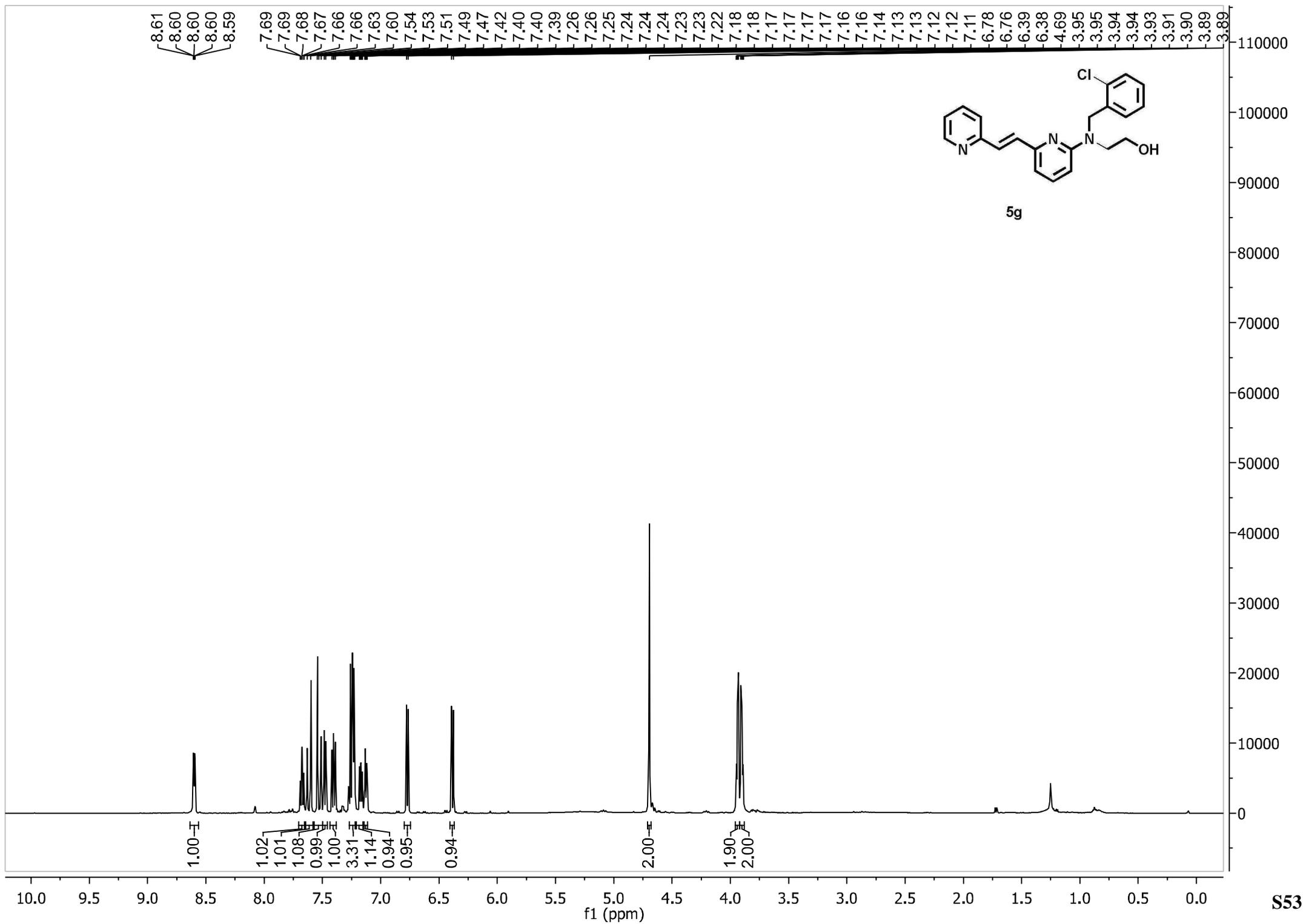


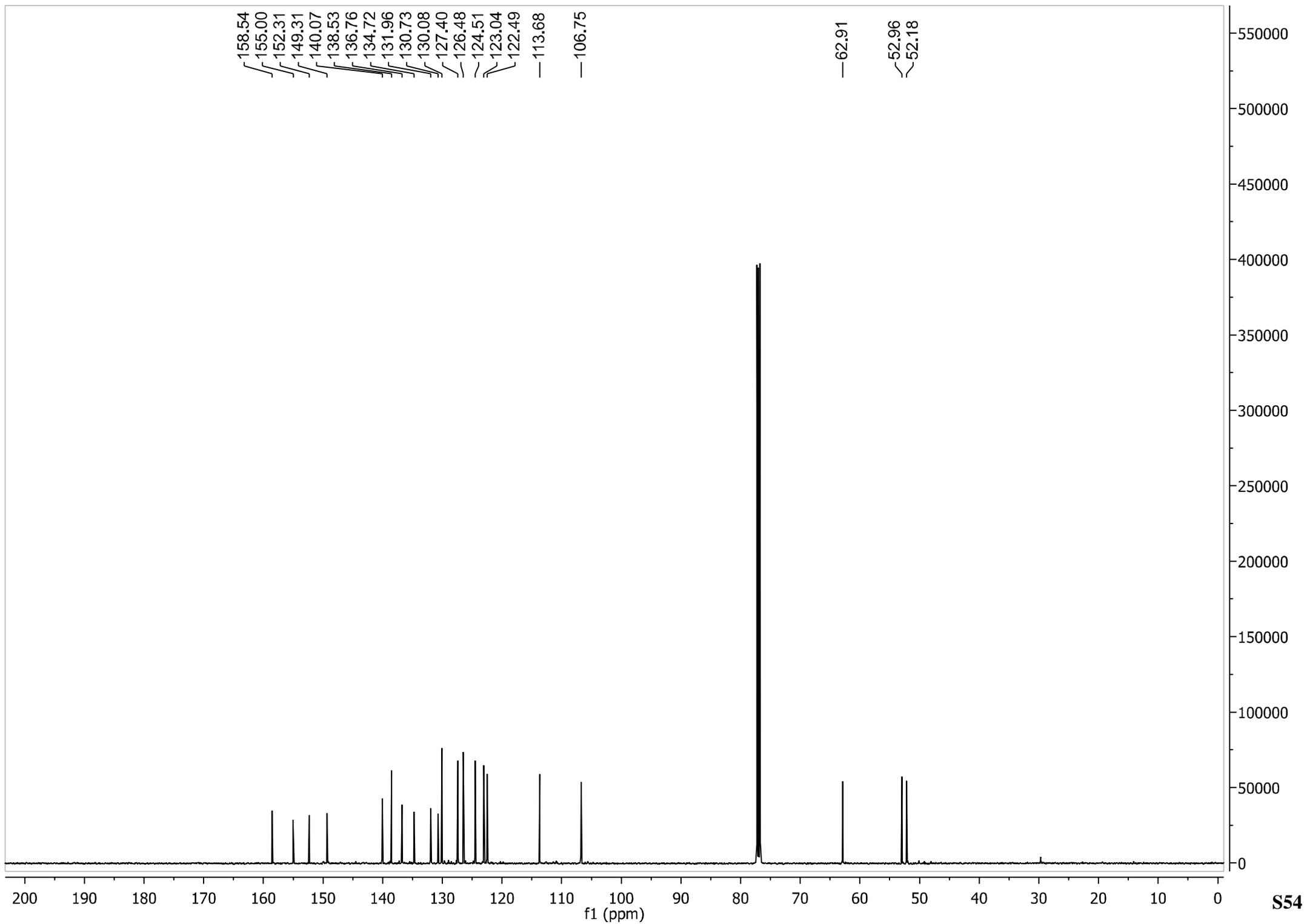




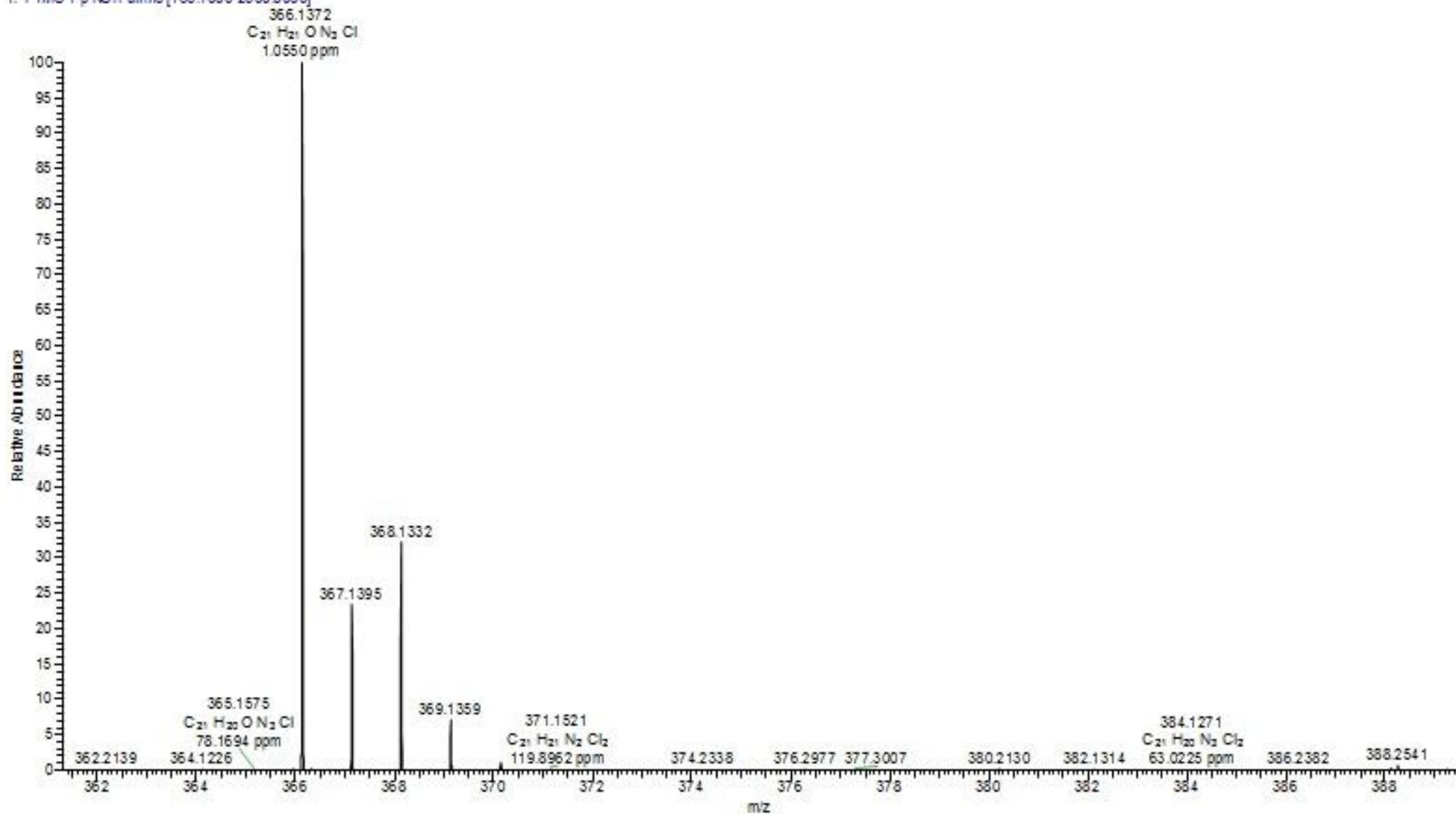
aw16 180516122613 #3-311 RT: 0.01-0.72 AV: 309 NL: 5.62E9
T: FTMS + p NSI Full ms [166.7000-2500.0000]

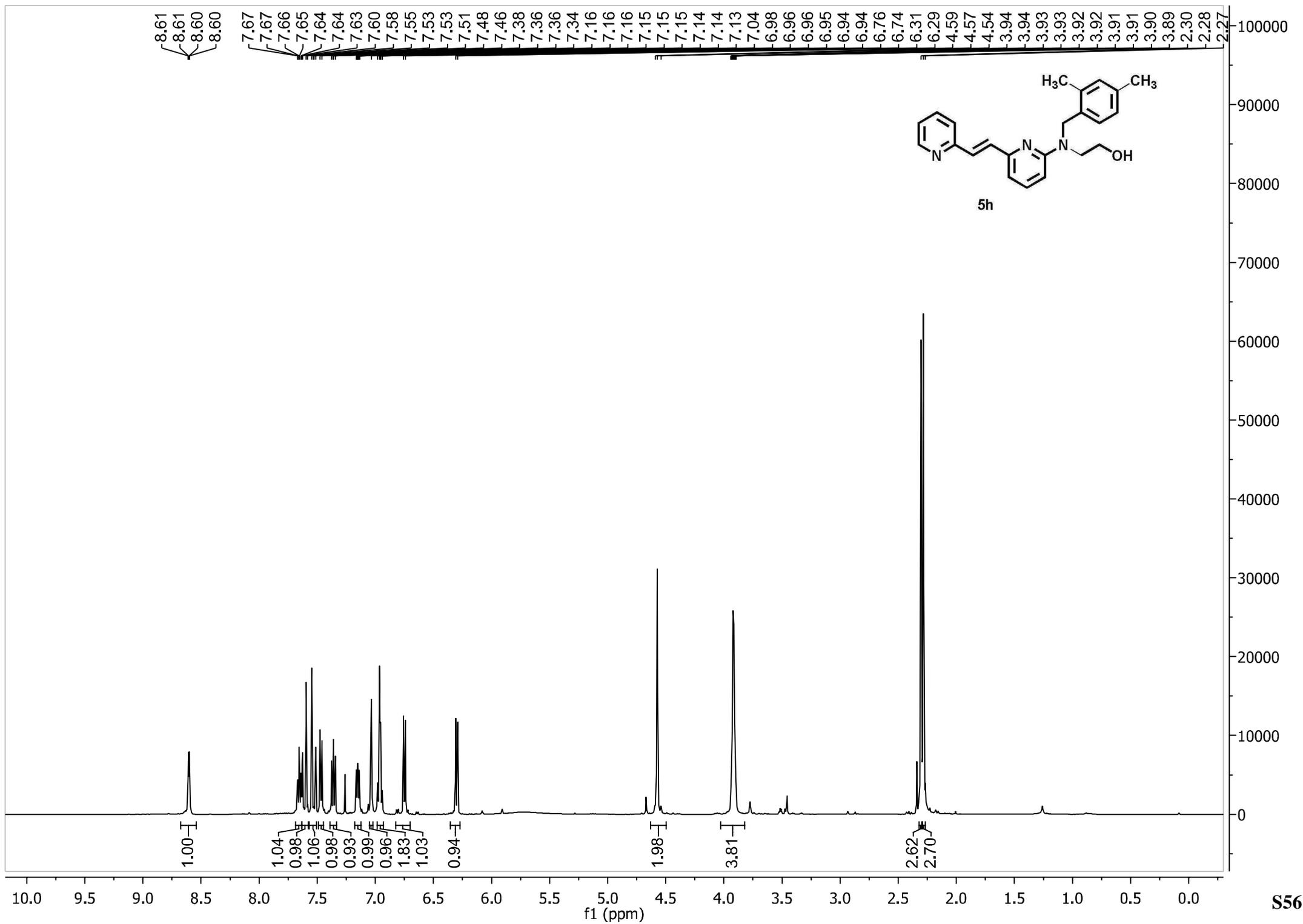


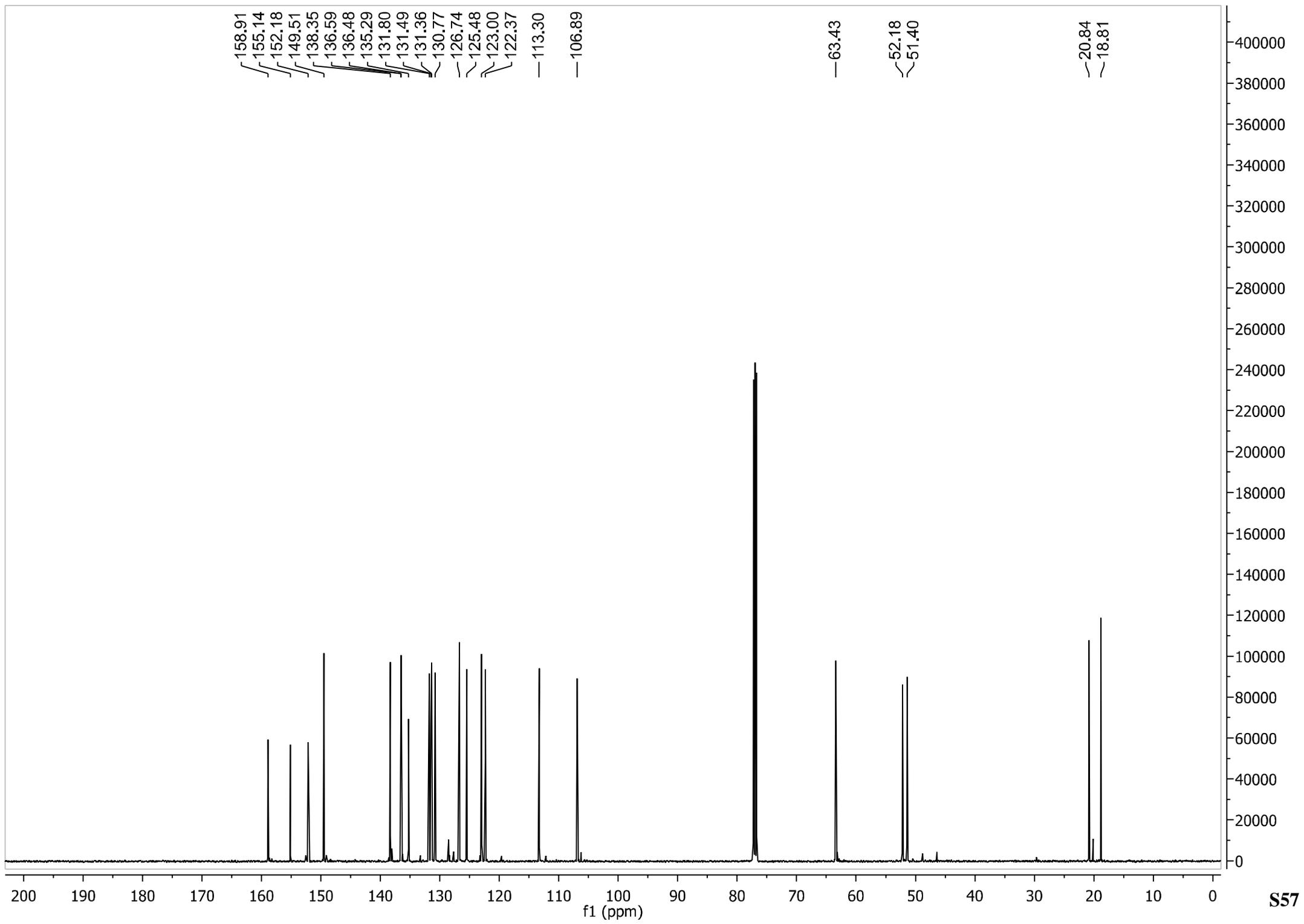




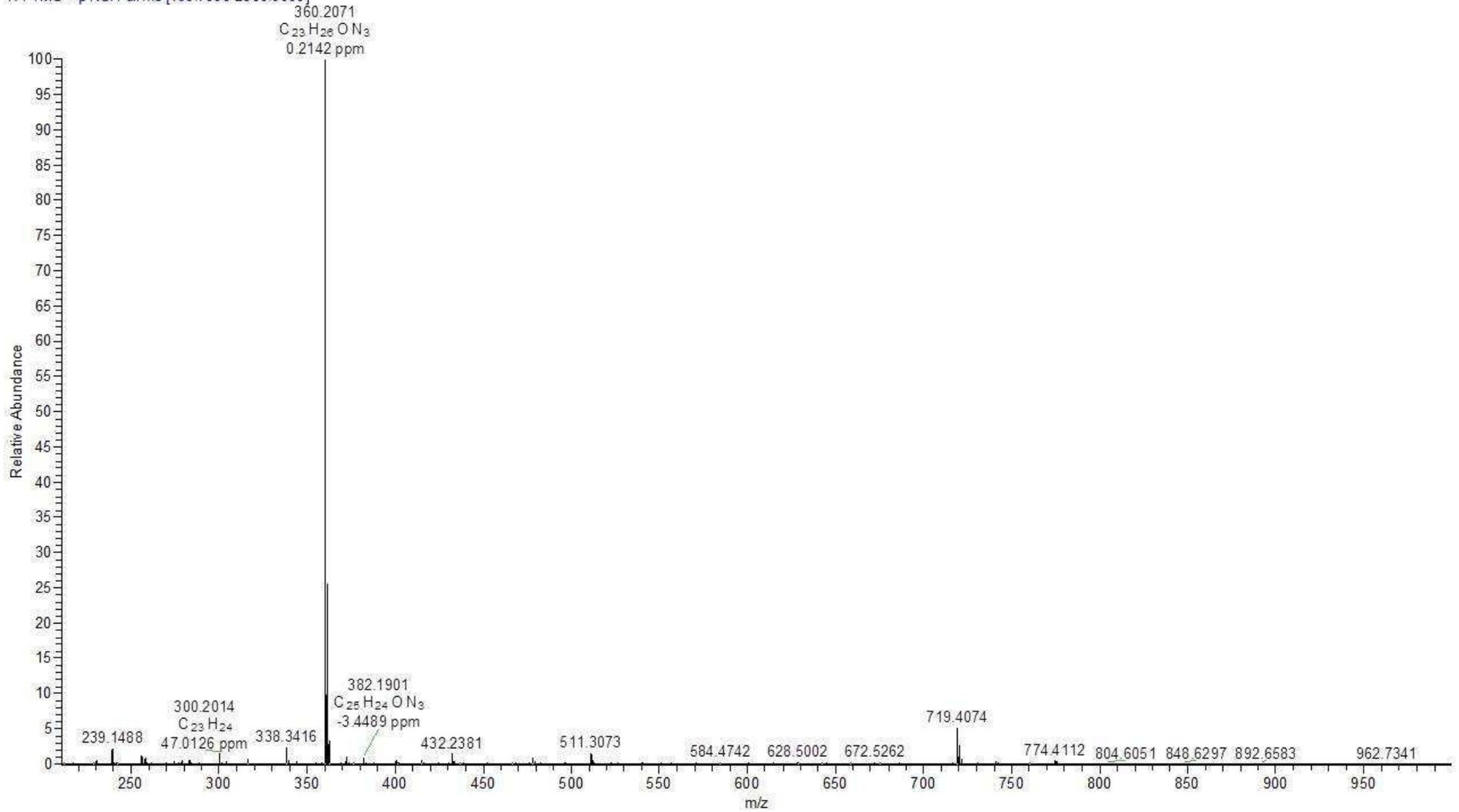
aw17 #4-361 RT: 0.01-0.84 AV: 358 NL: 2.99E9
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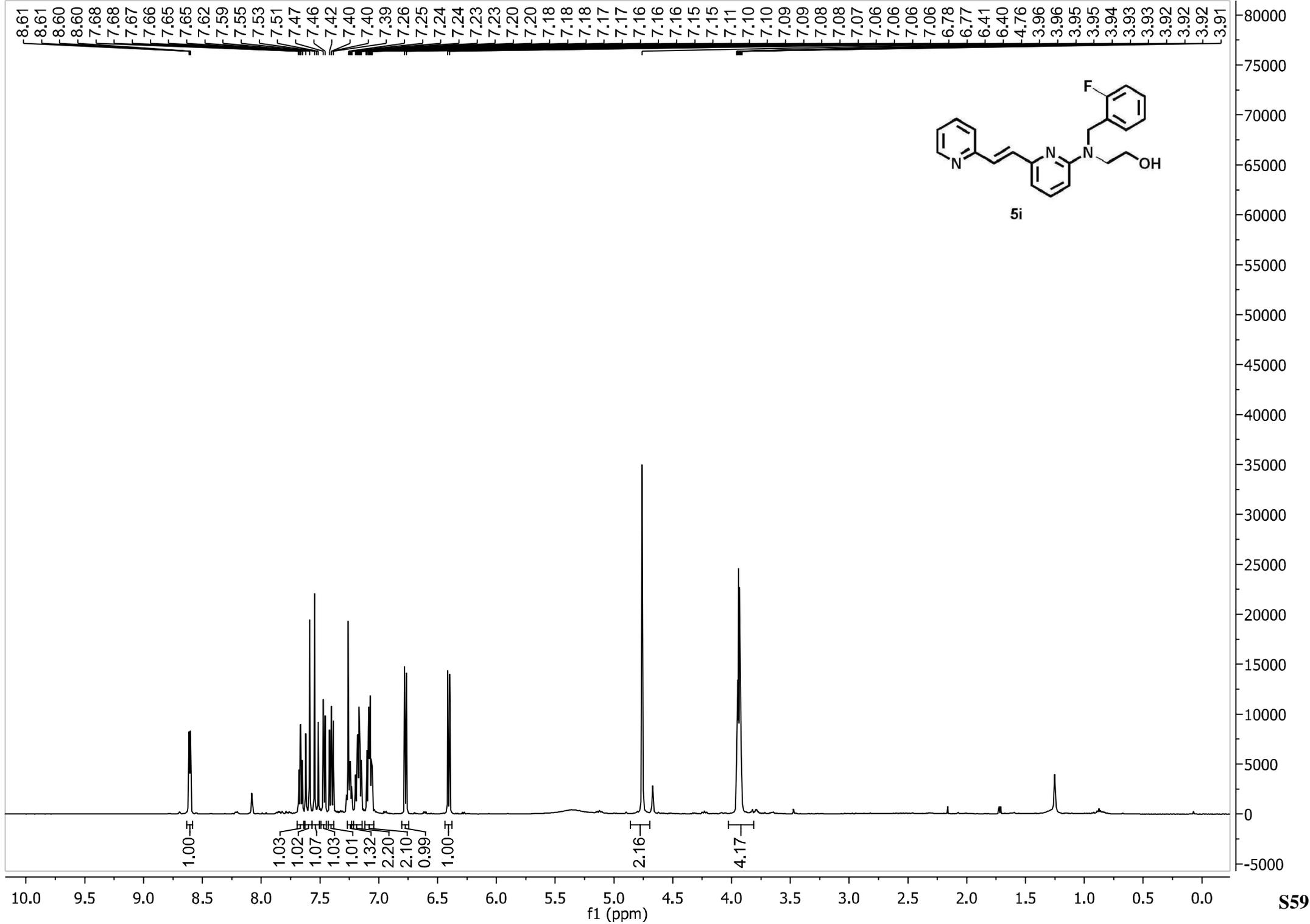


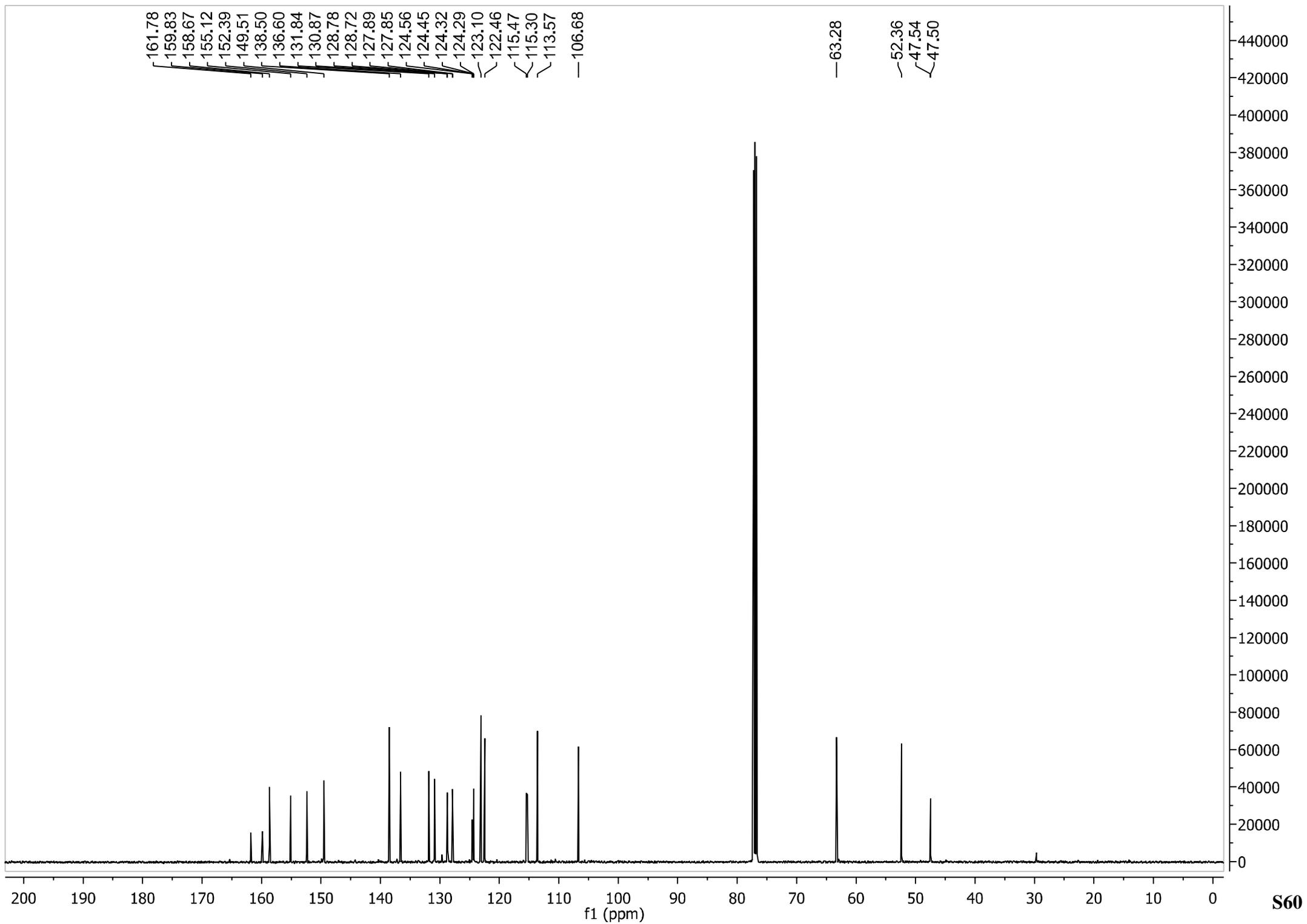


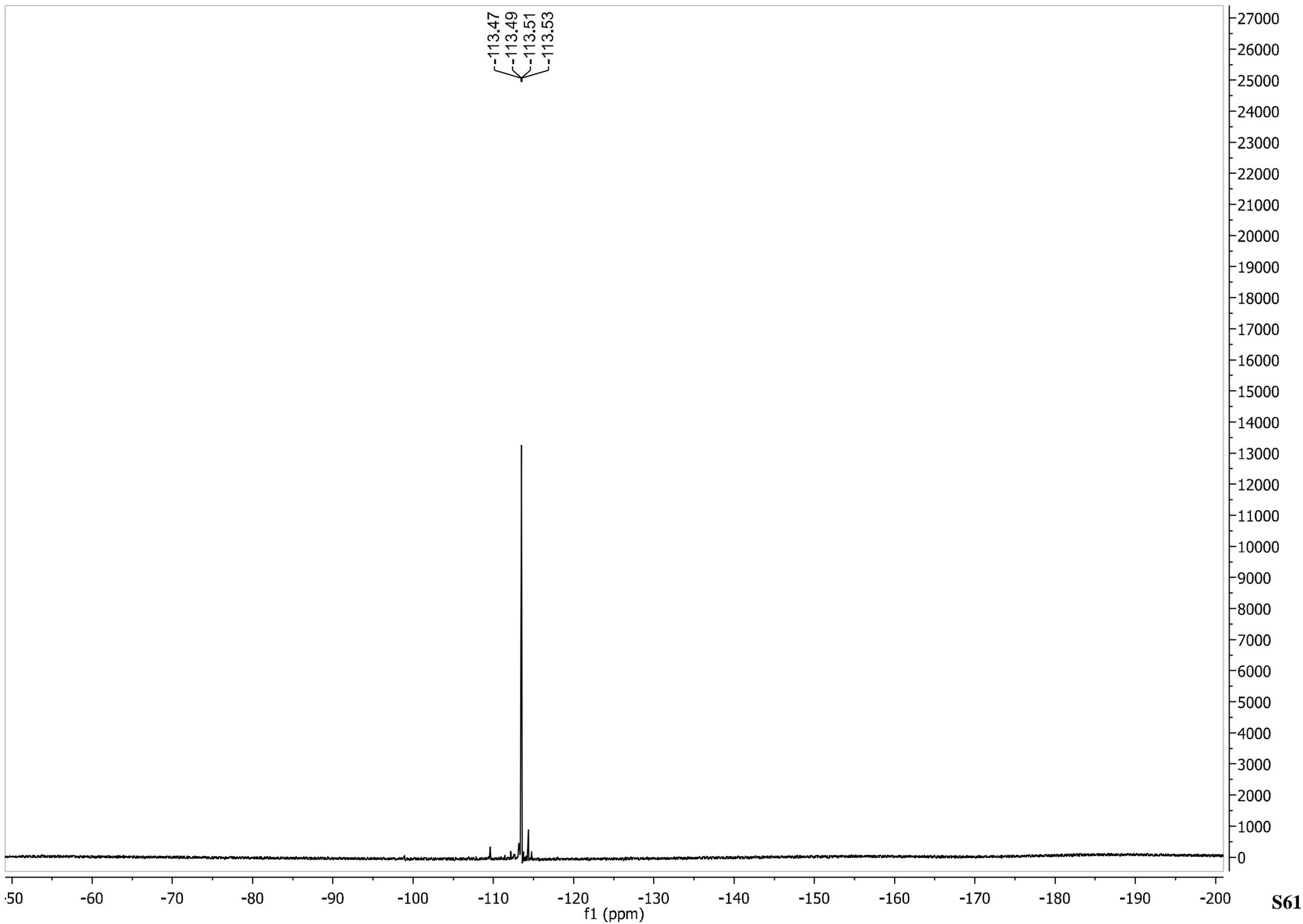


aw18 #6-325 RT: 0.01-0.75 AV: 320 NL: 4.03E9
T: FTMS + p NSI Full ms [166.7000-2500.0000]

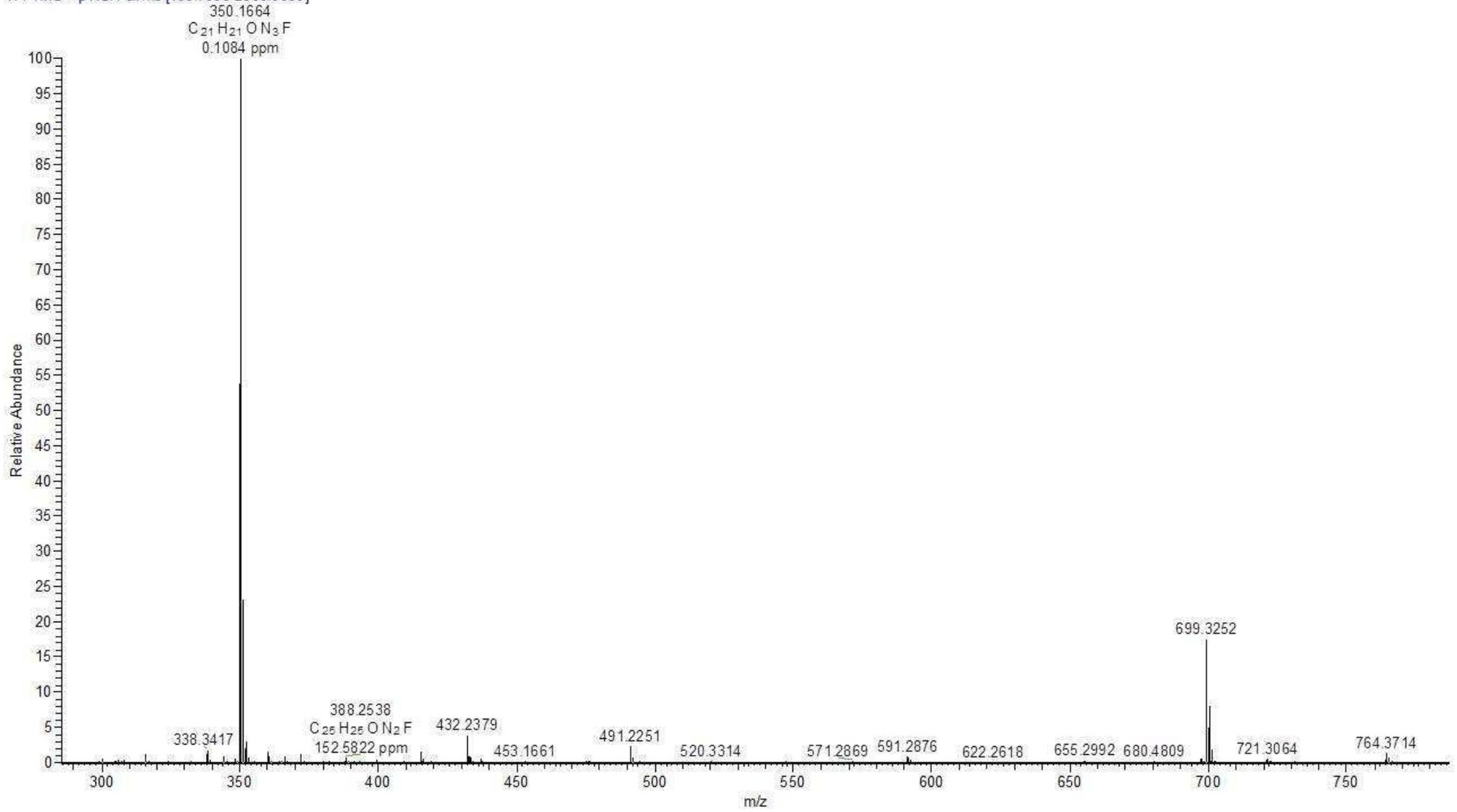


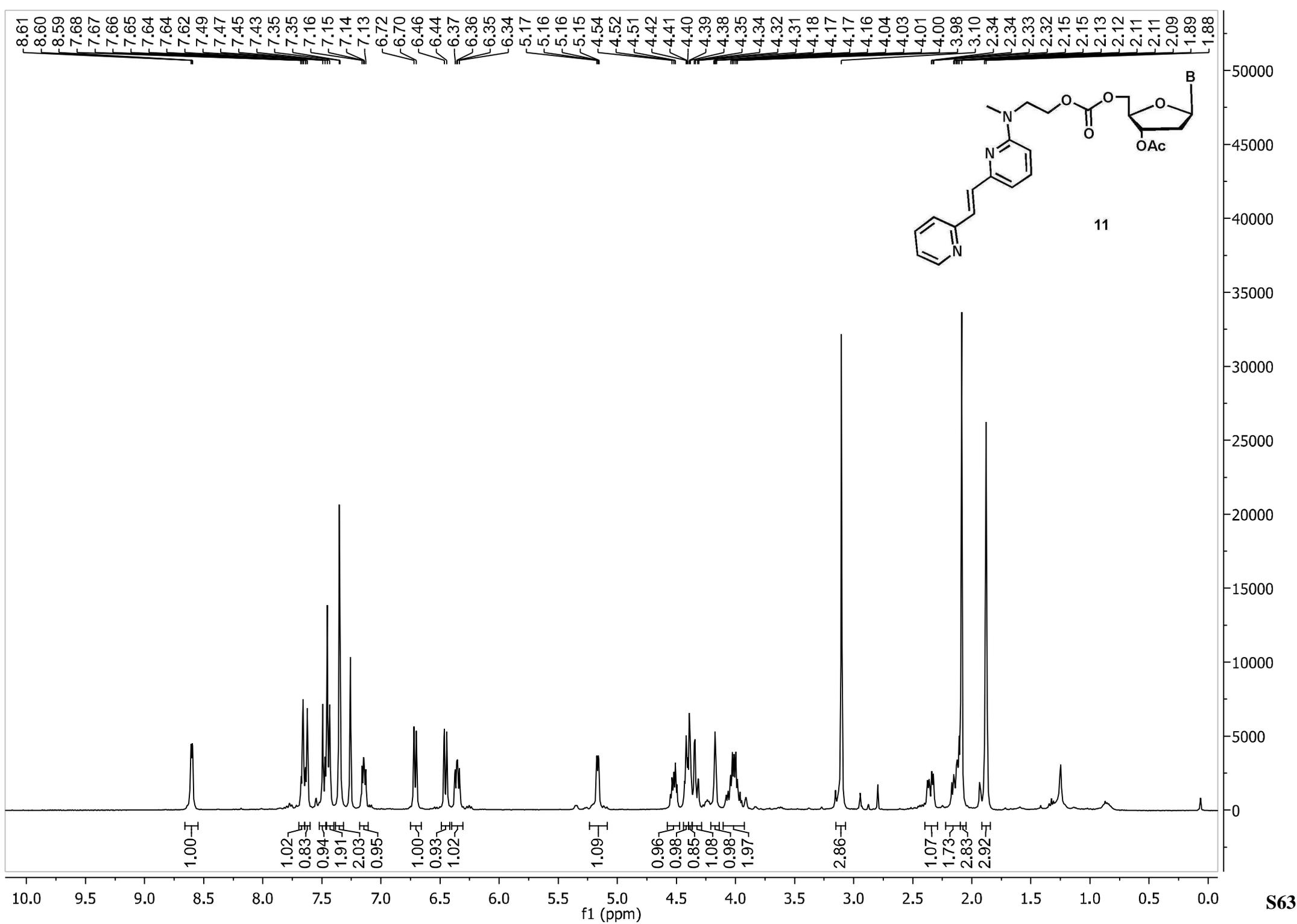


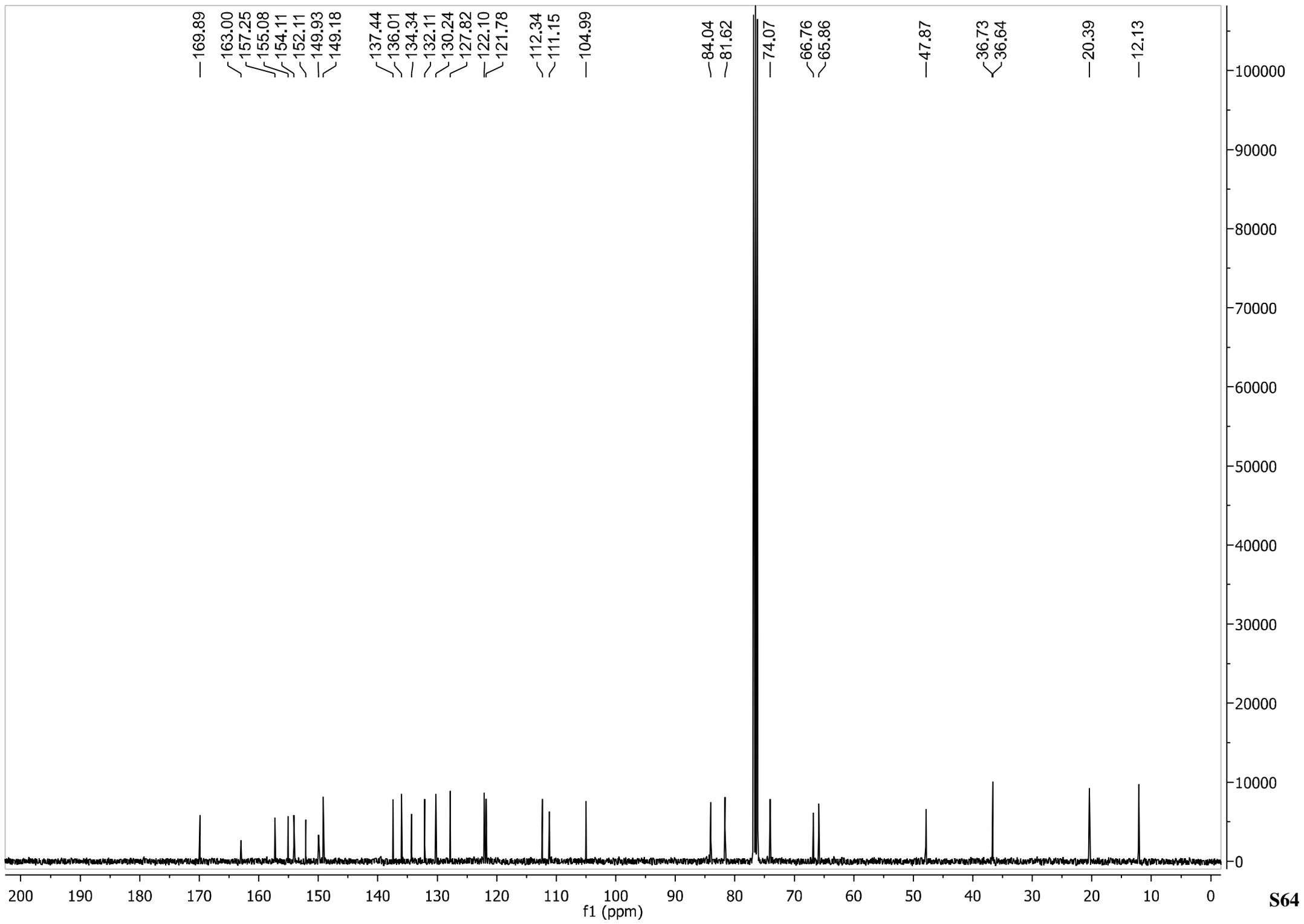




aw19 #6-266 RT: 0.01-0.62 AV: 261 NL: 2.52E9
T: FTMS + p NSI Full ms [166.7000-2500.0000]







Mass Spectrum SmartFormula Report

Analysis Info

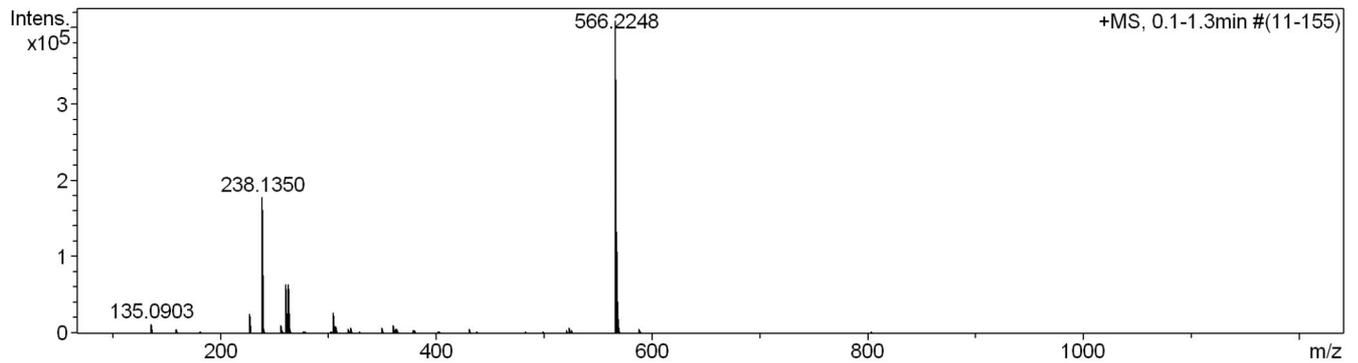
Analysis Name D:\Data\Lukasz\kuba zleconelwegan_5_1.d
 Method ewelina.m
 Sample Name DMSO
 Comment

Acquisition Date 3/10/2017 1:41:26 PM

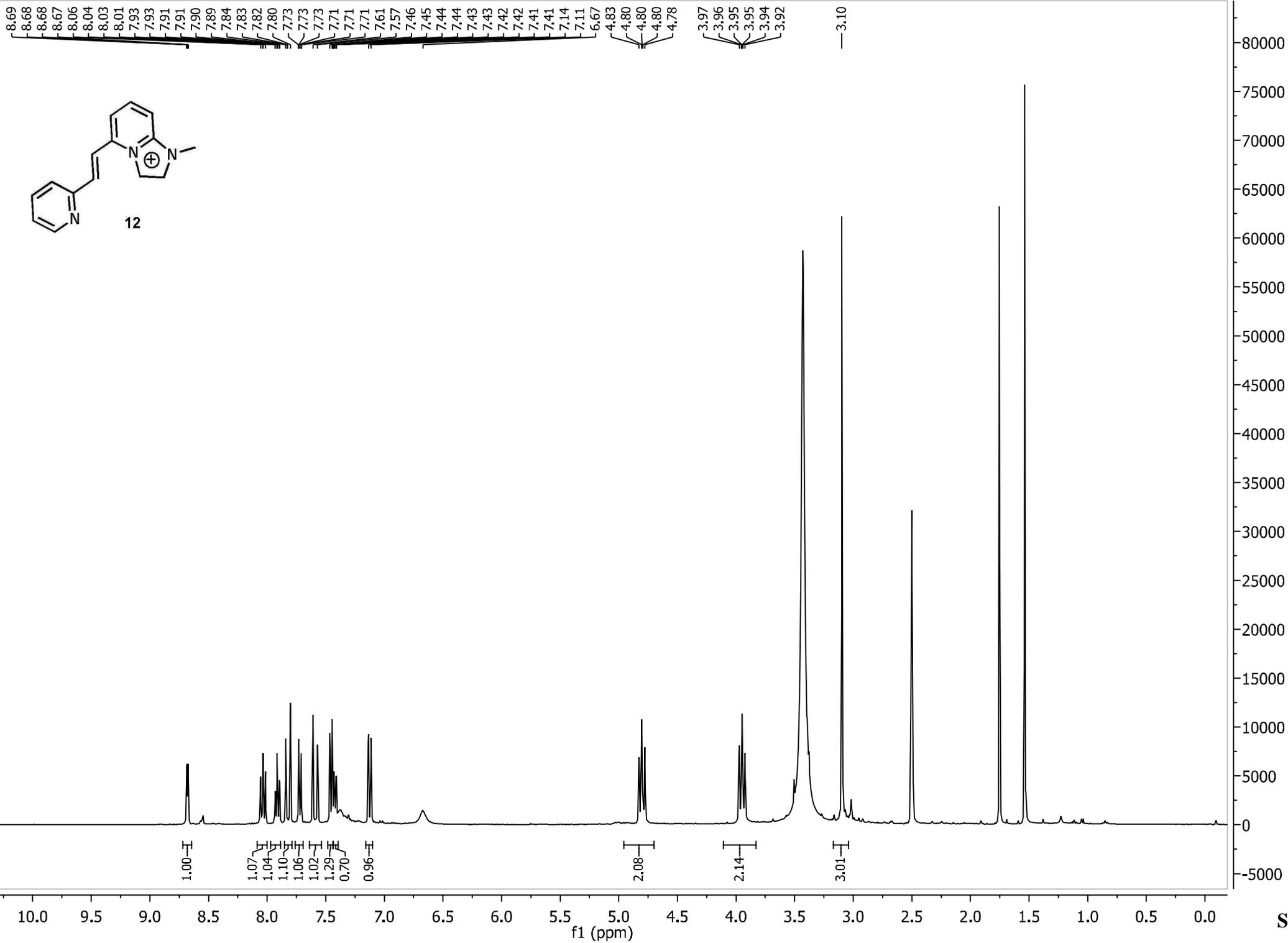
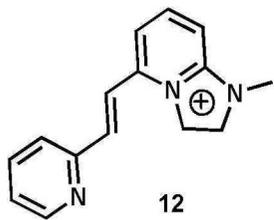
Operator Bruker Customer
 Instrument / Ser# micrOTOF-Q 128

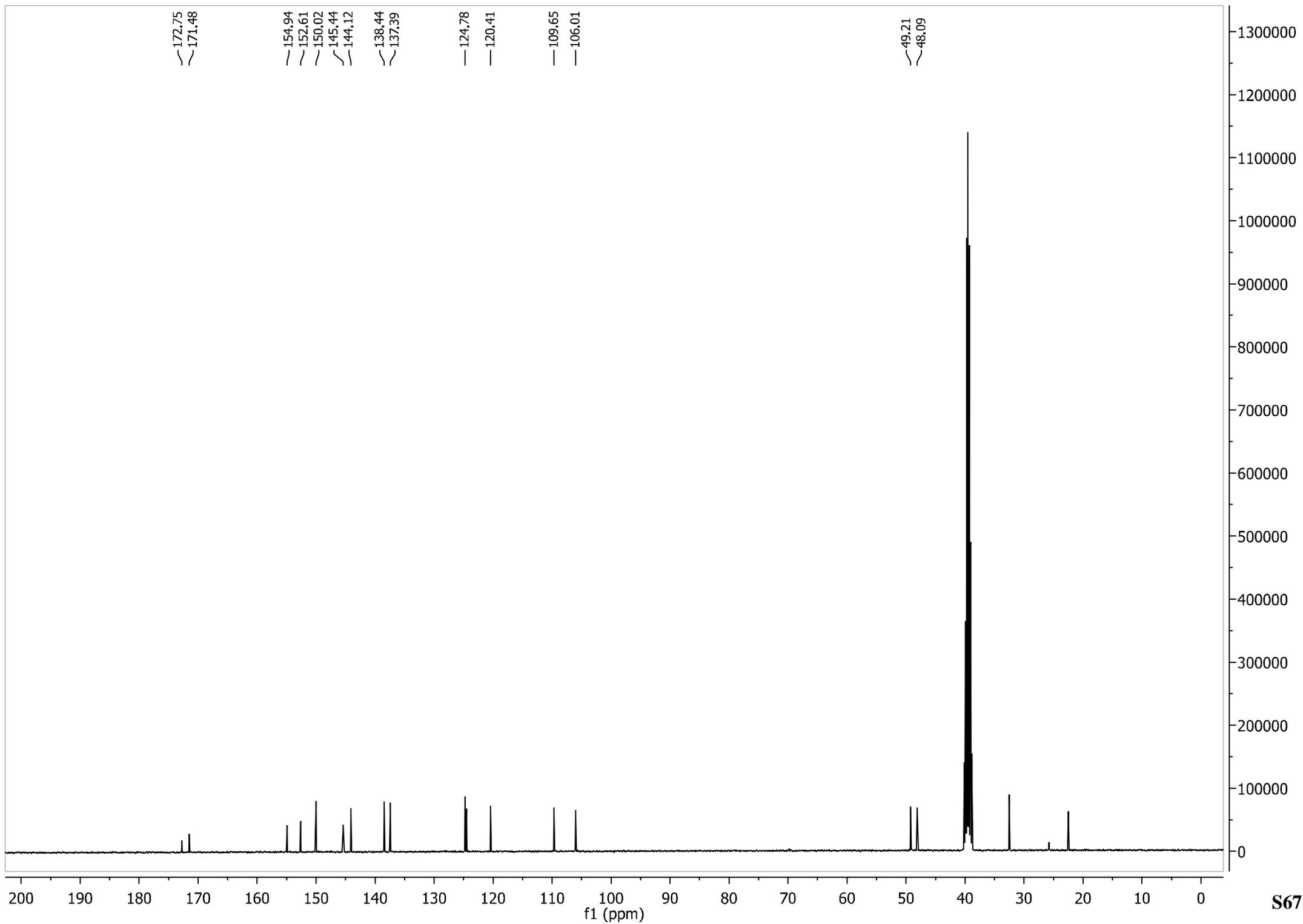
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1200 m/z	Set Collision Cell RF	200.0 Vpp	Set Divert Valve	Waste

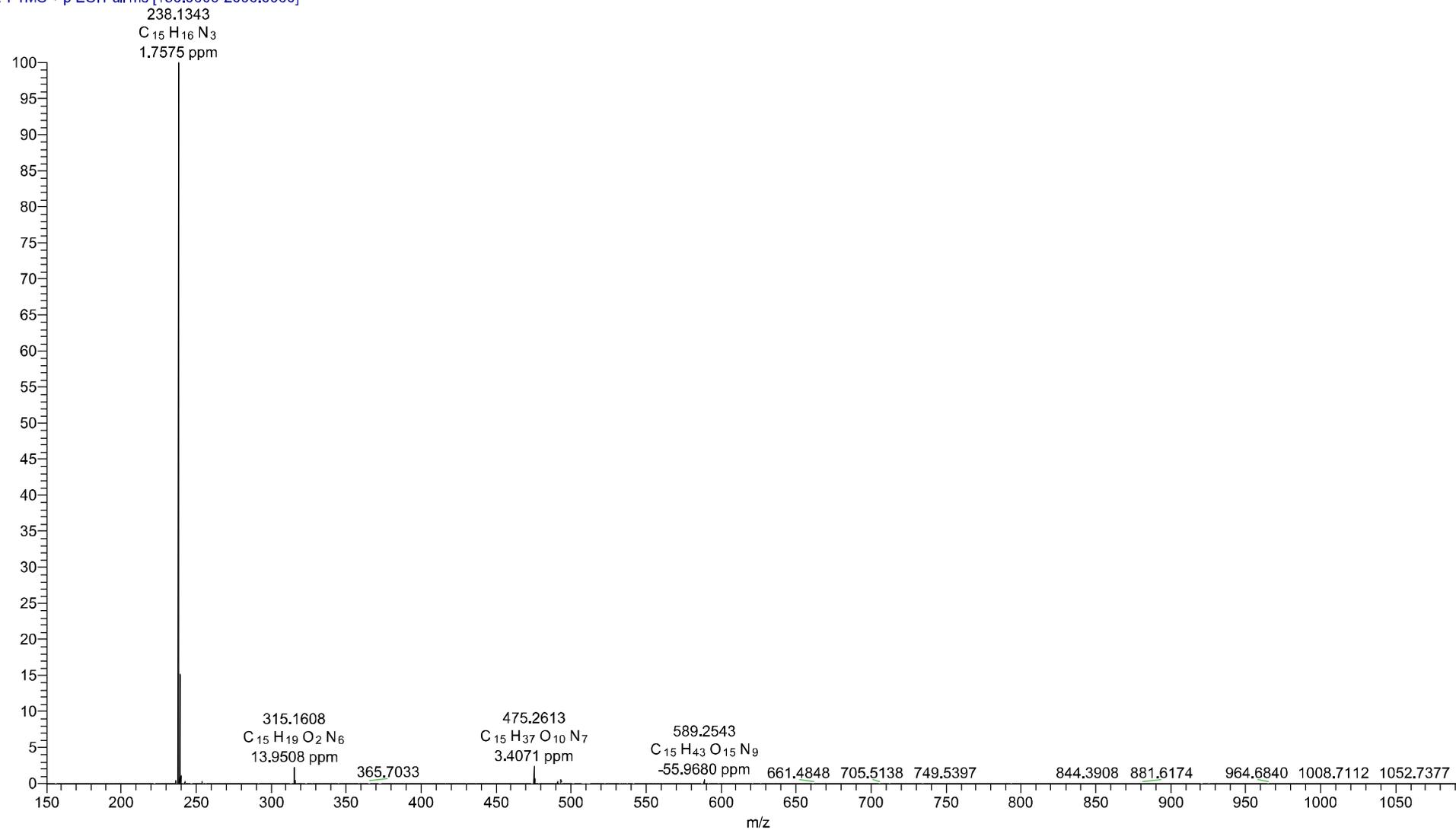


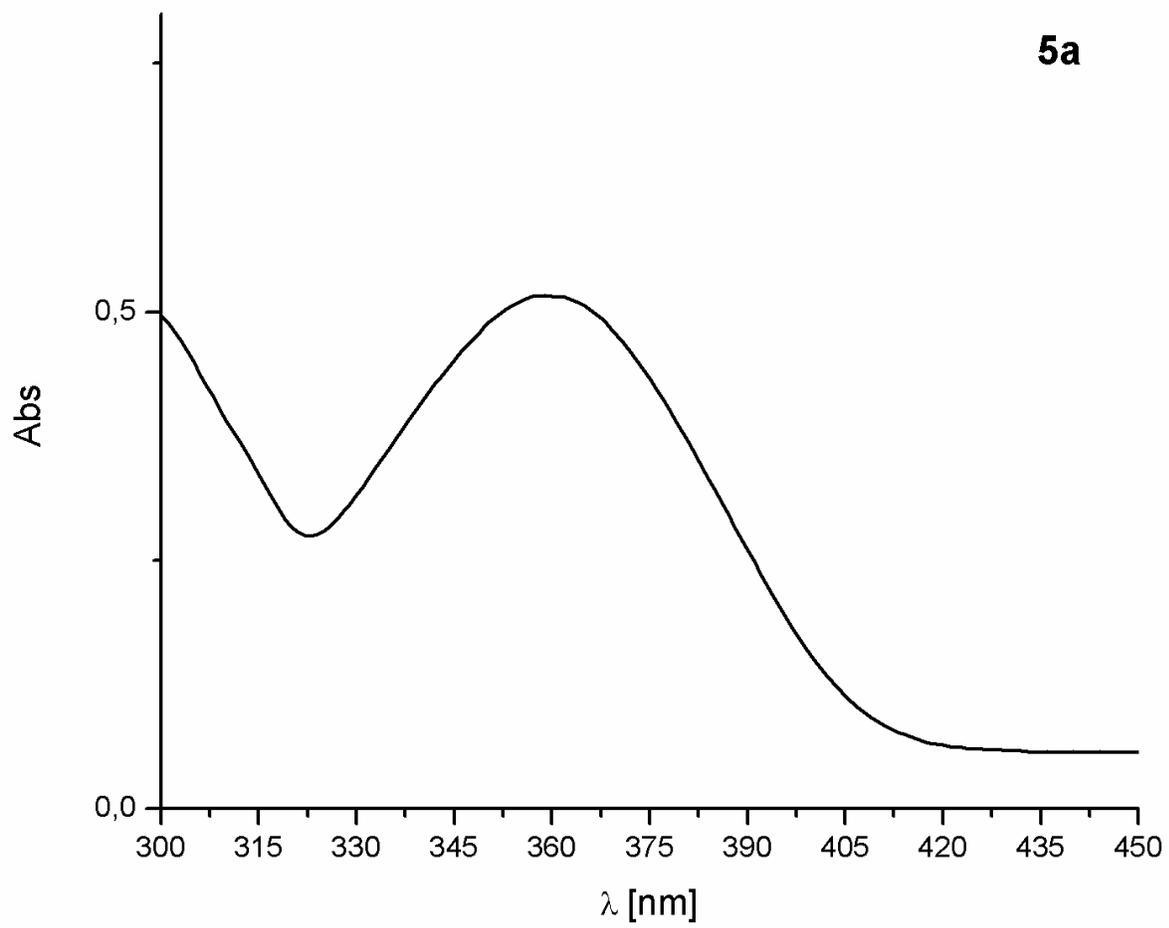
Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma	Std I	Std Me an m/z	Std I Var Norm	Std m/z Diff	Std Com b Dev
566.2248	1	C ₂₈ H ₃₂ N ₅ O ₈	566.2245	-0.5	0.2	15.5	ok	even	6.5	12.9	0.7	5.3	1.8	842.7

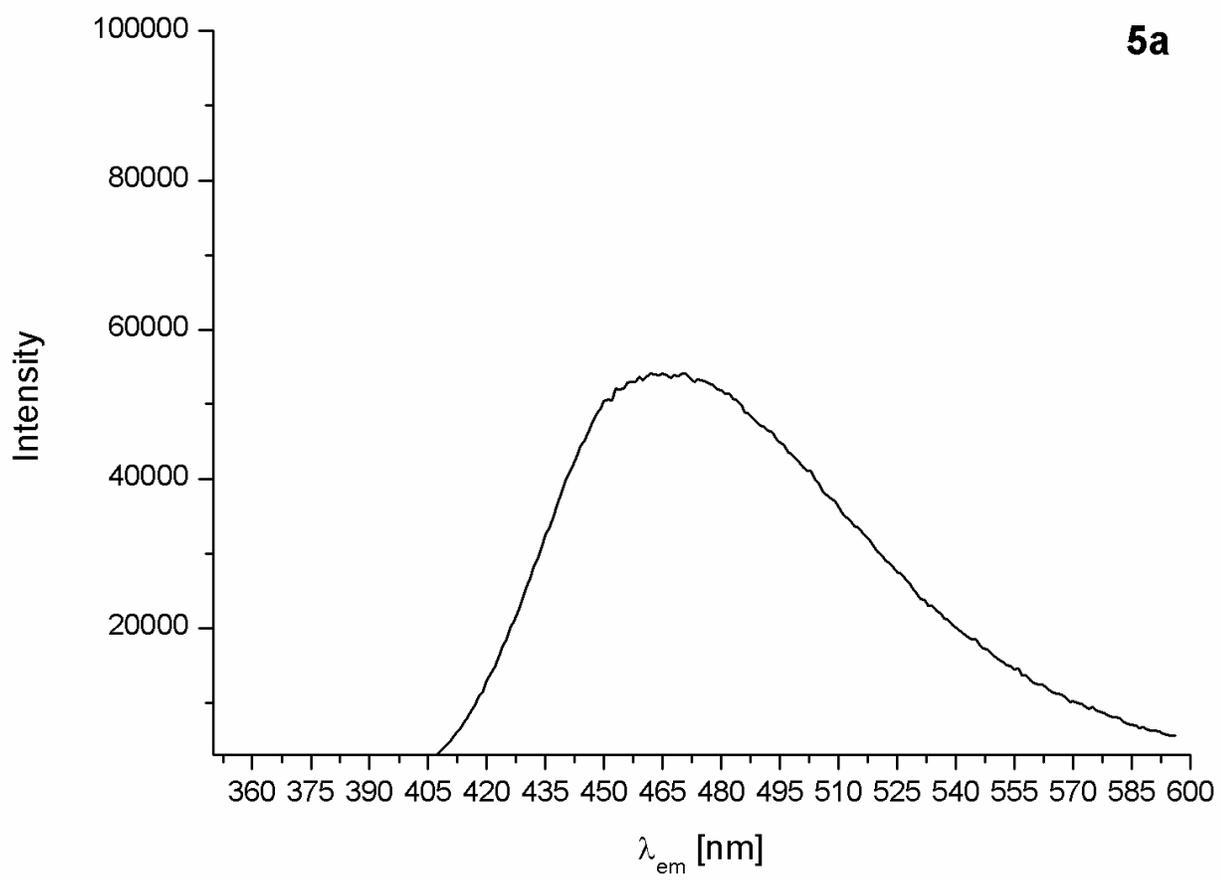


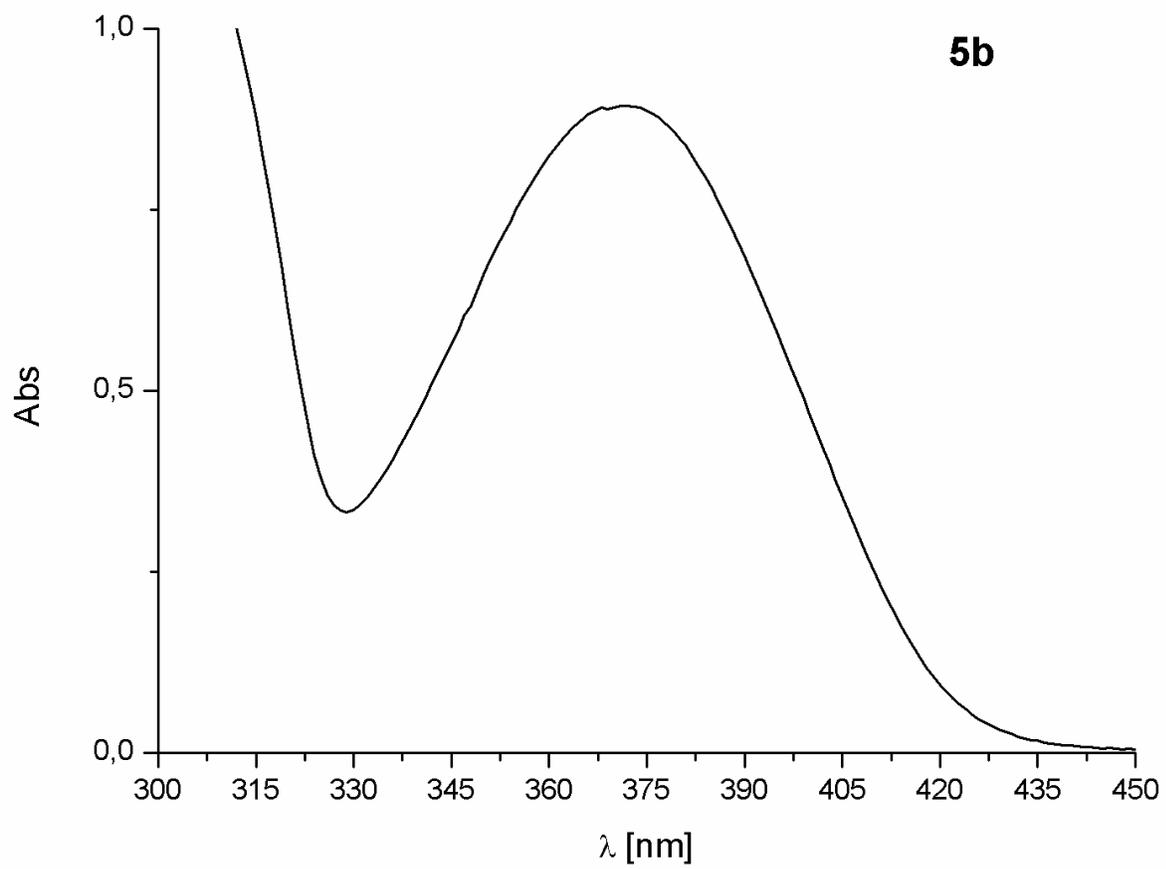


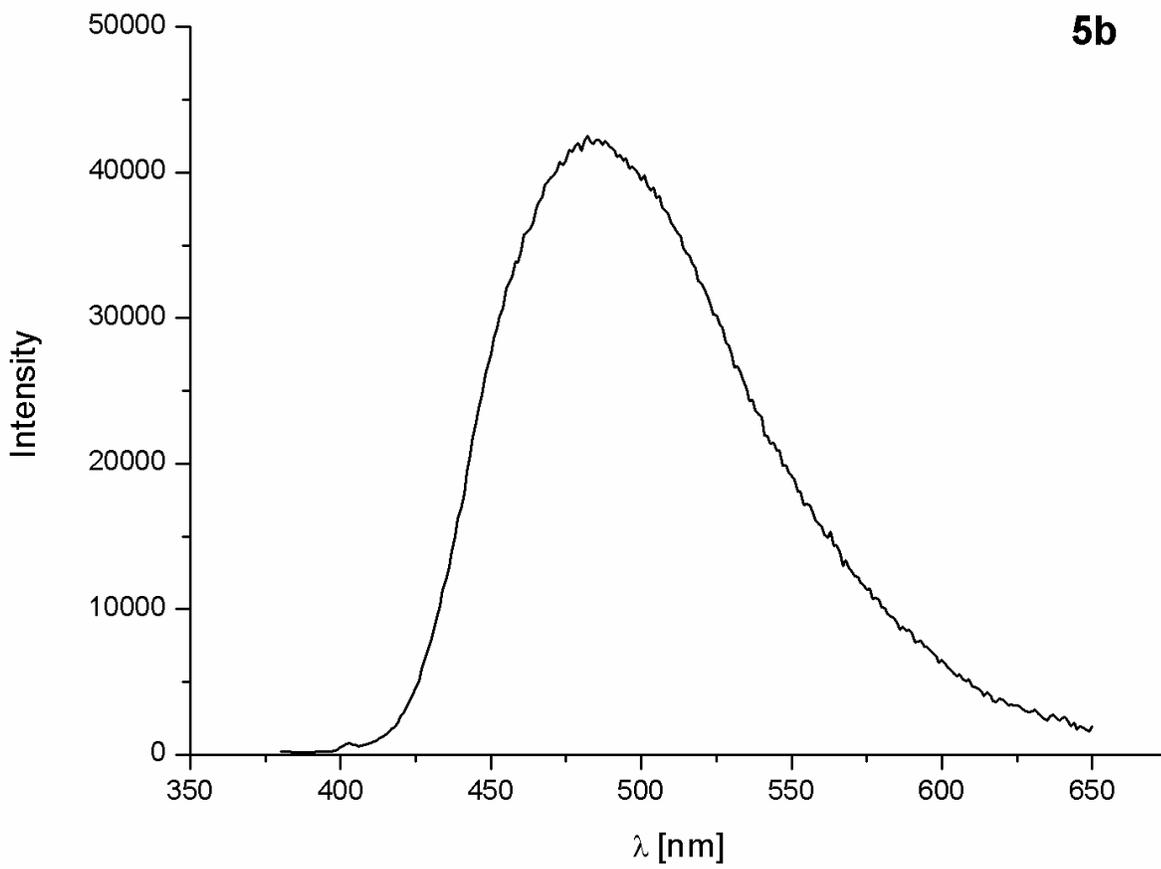
AW_cykliczny12 #129 RT: 0.57 AV: 1 NL: 1.13E10
T: FTMS + p ESI Full ms [150,0000-2000,0000]

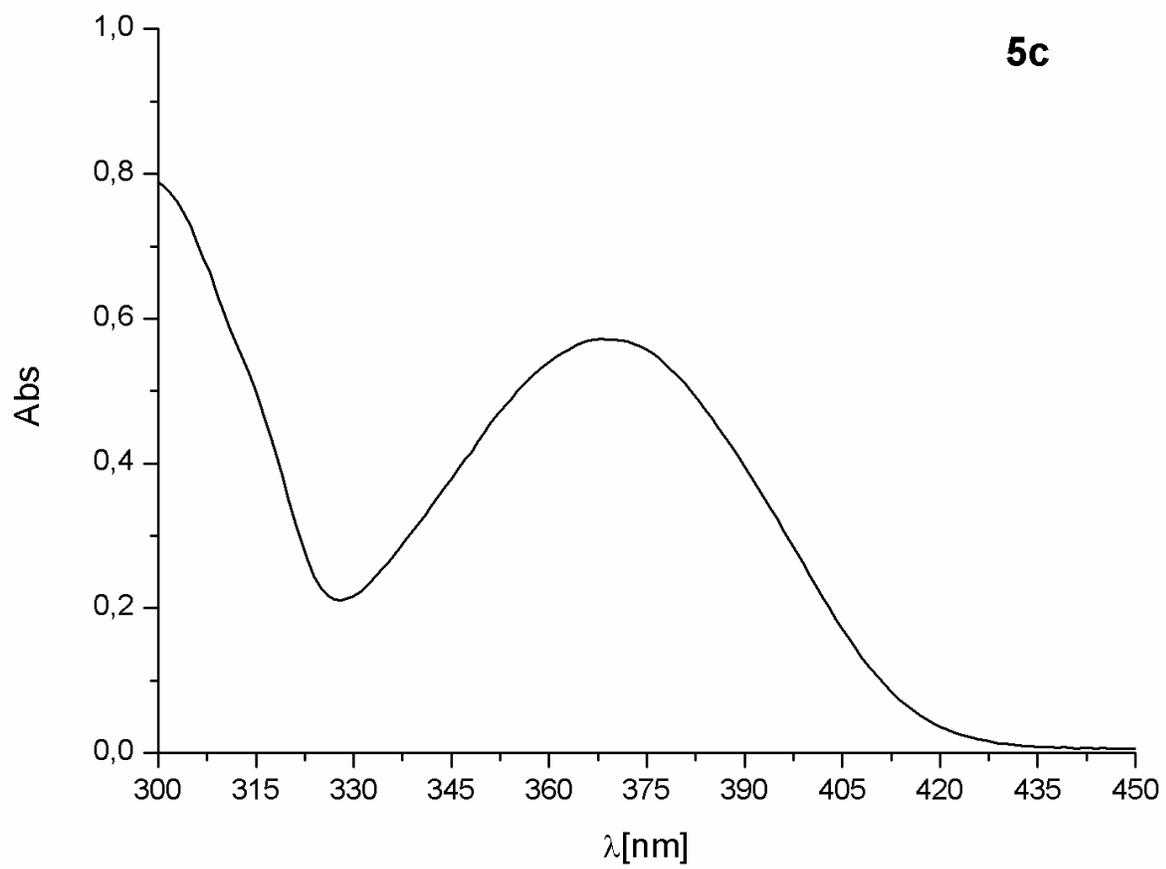


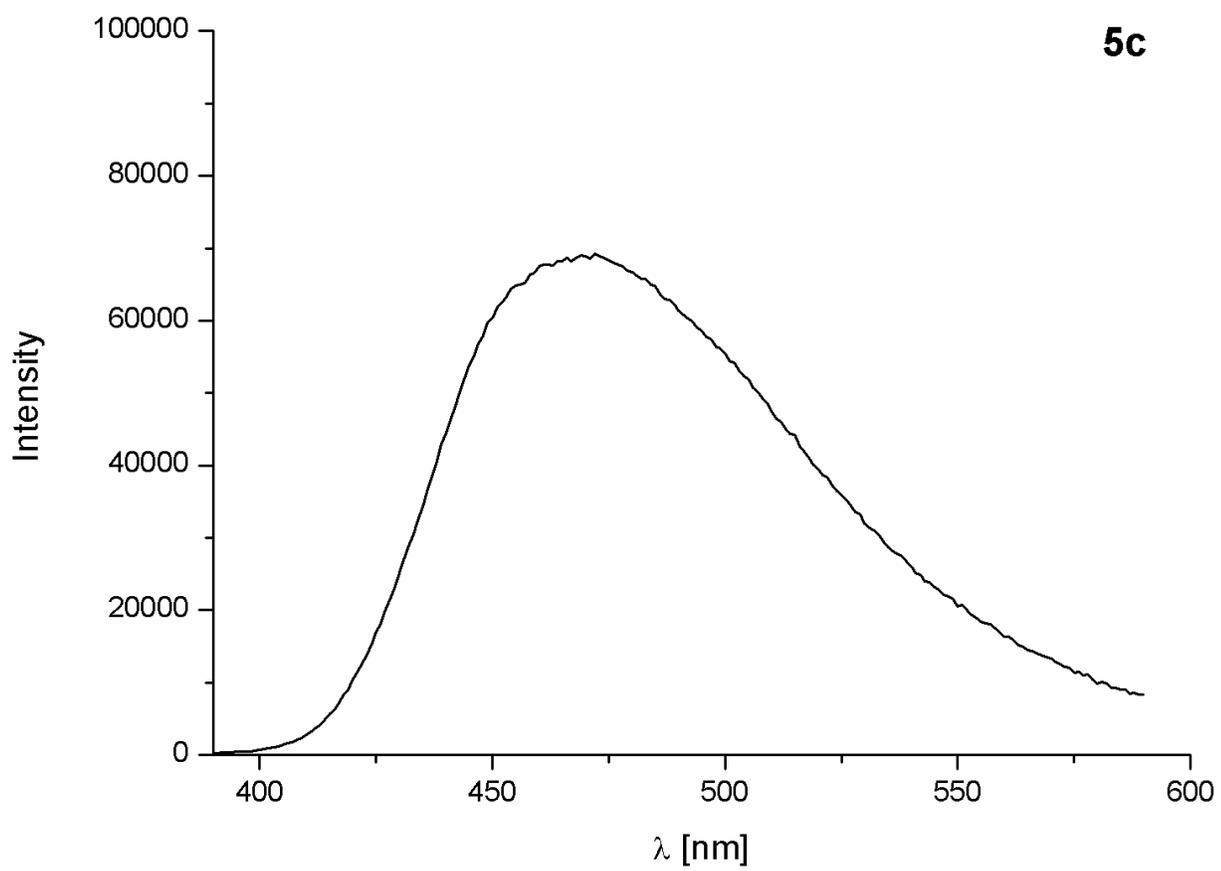


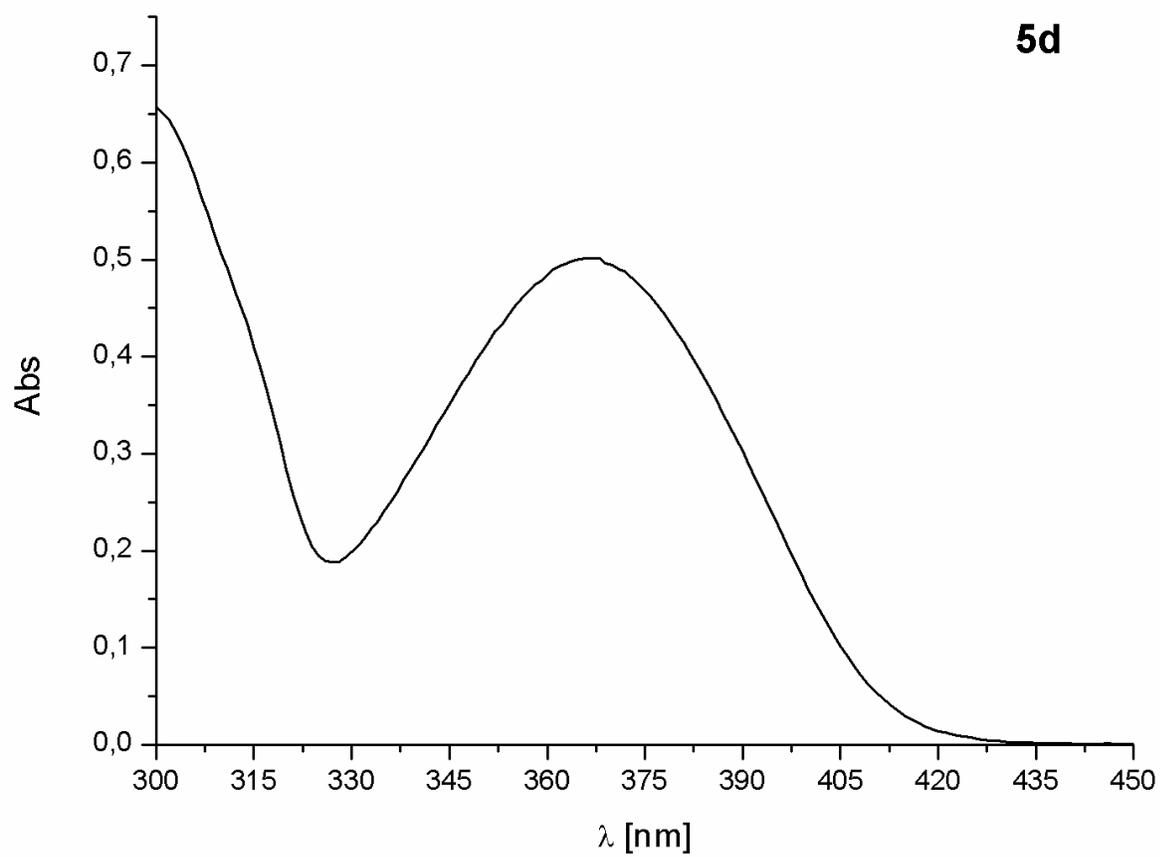


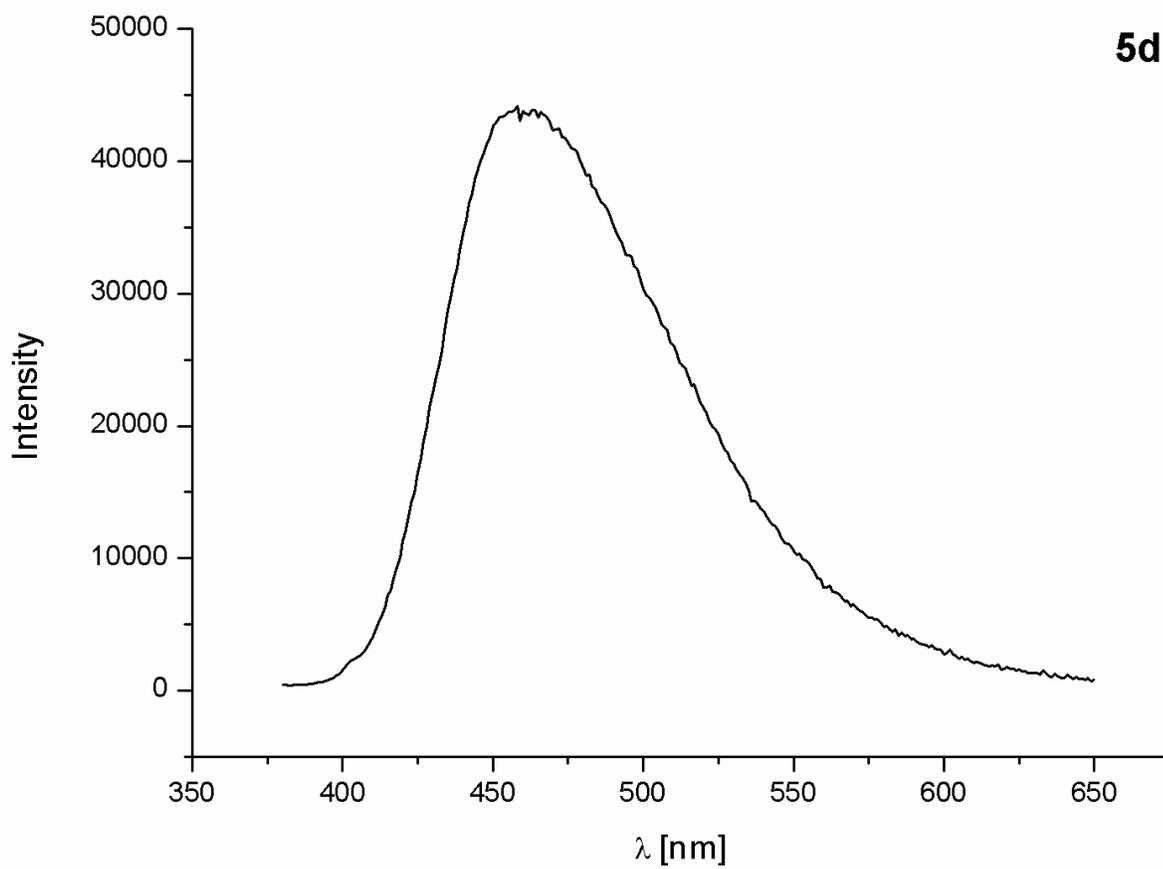


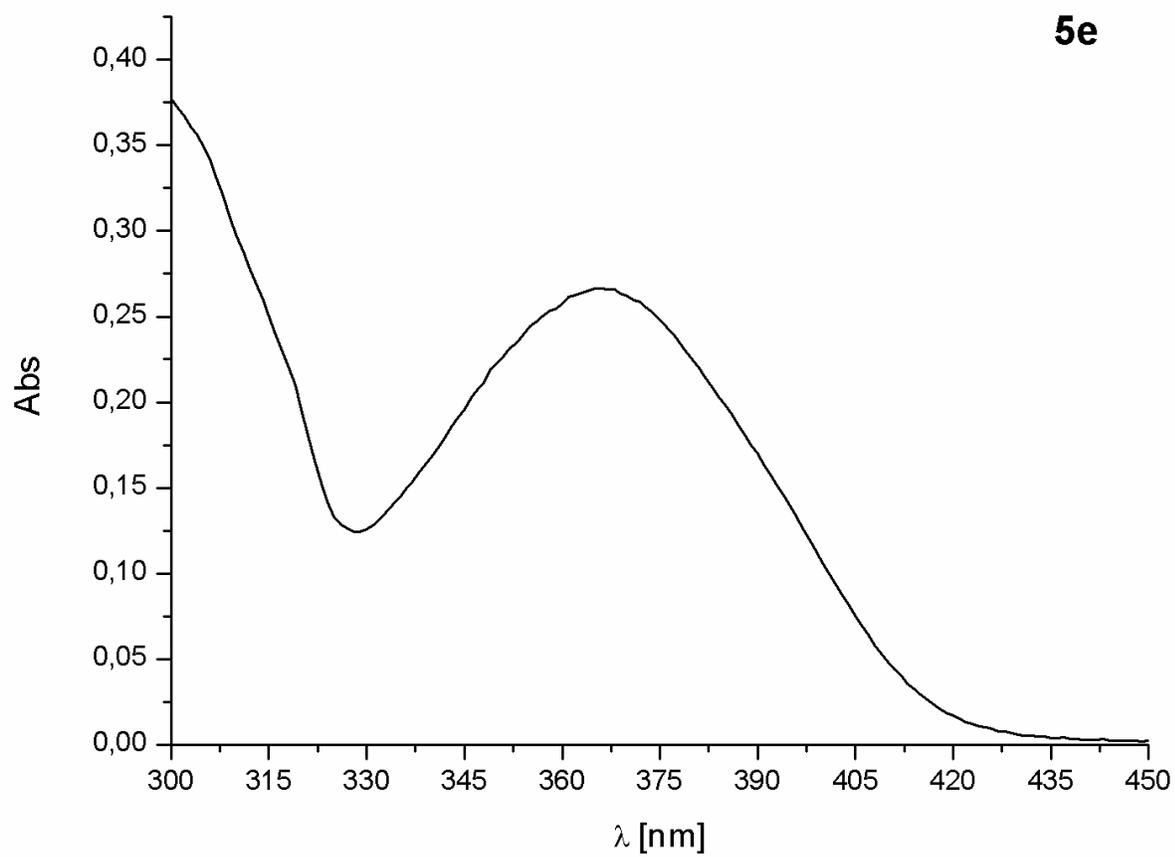


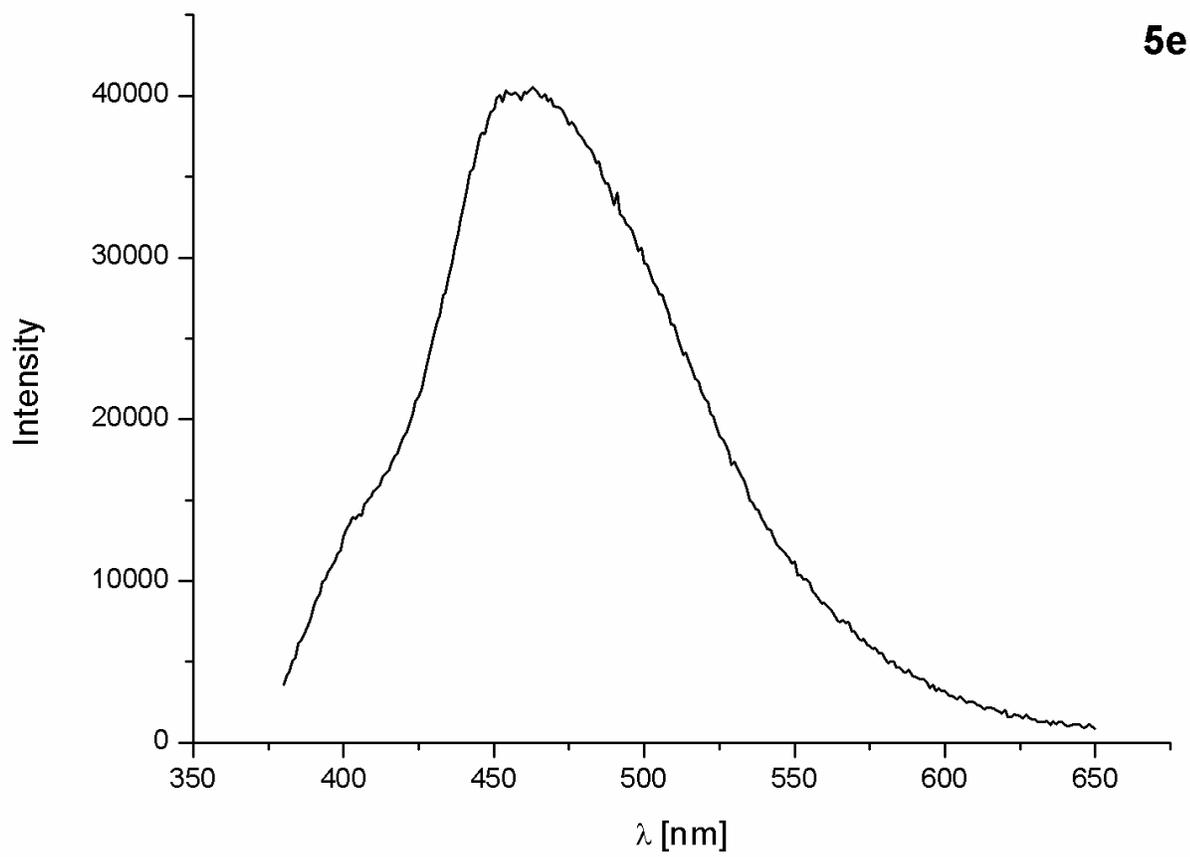




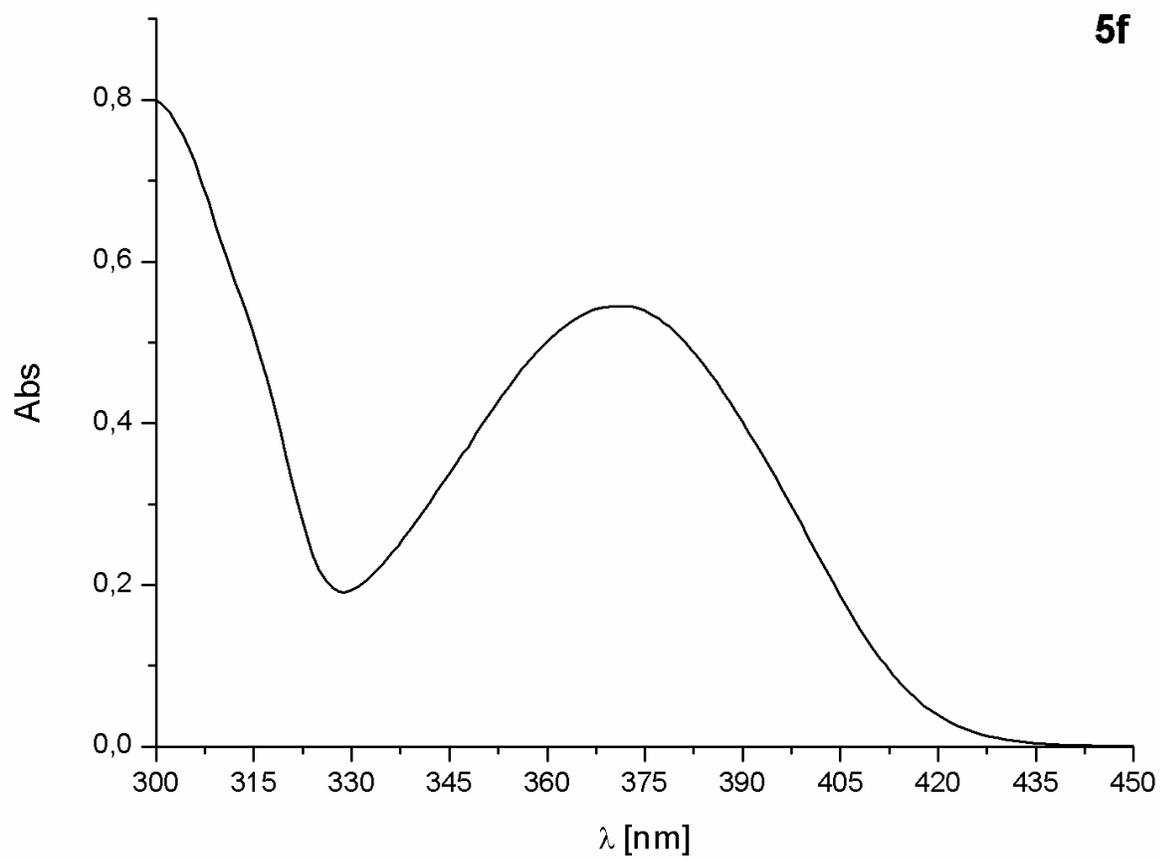


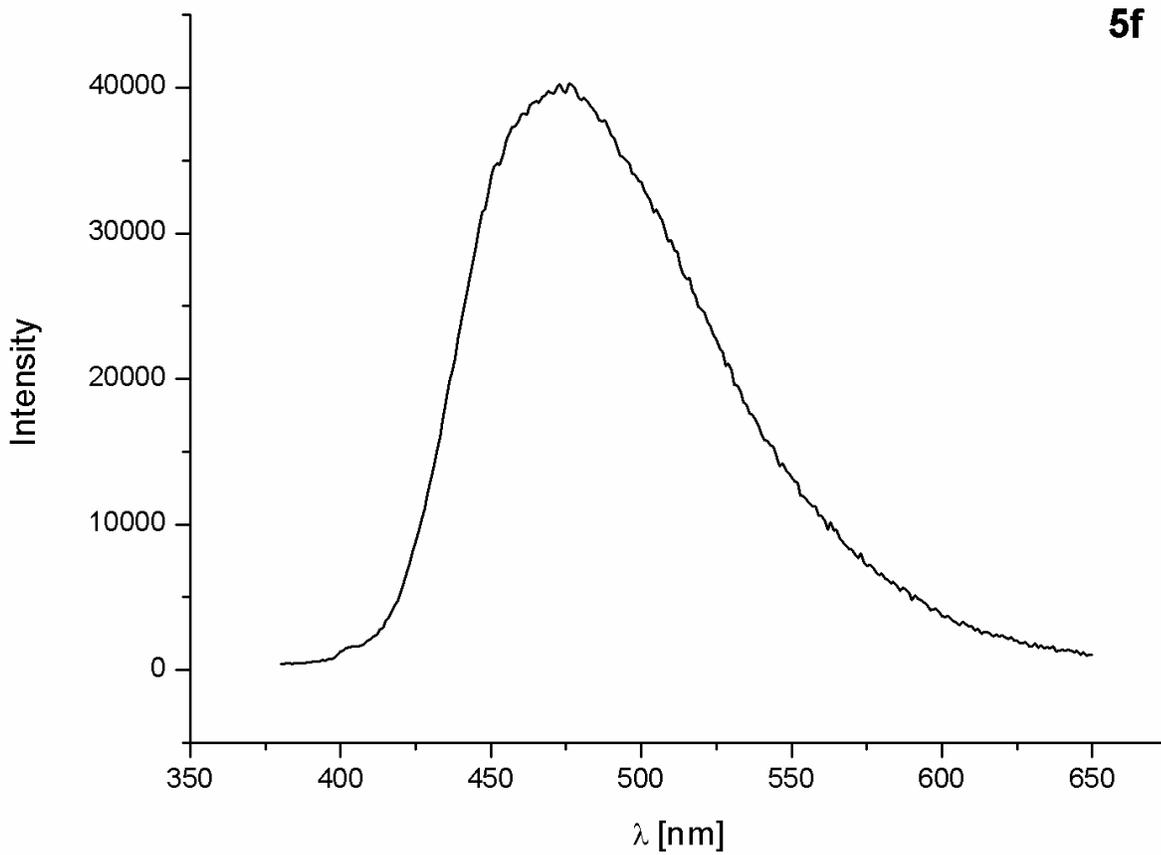


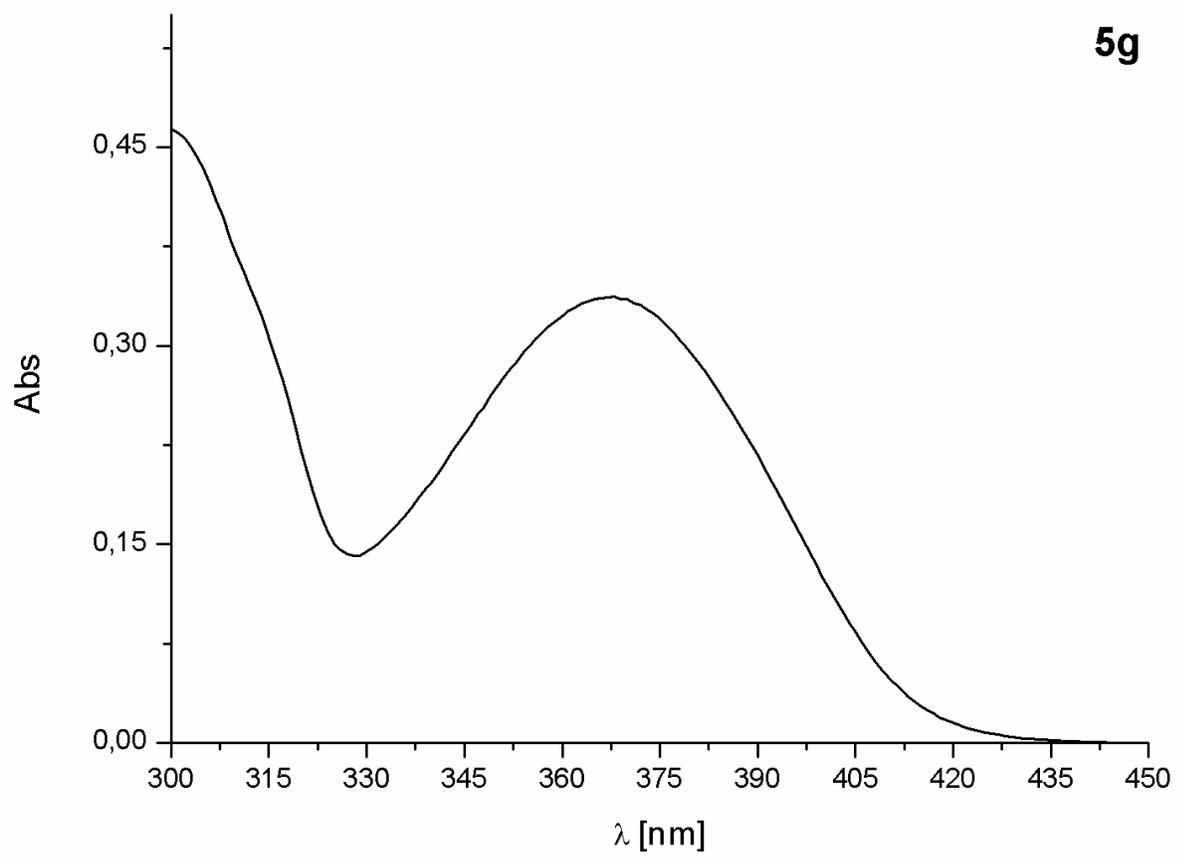


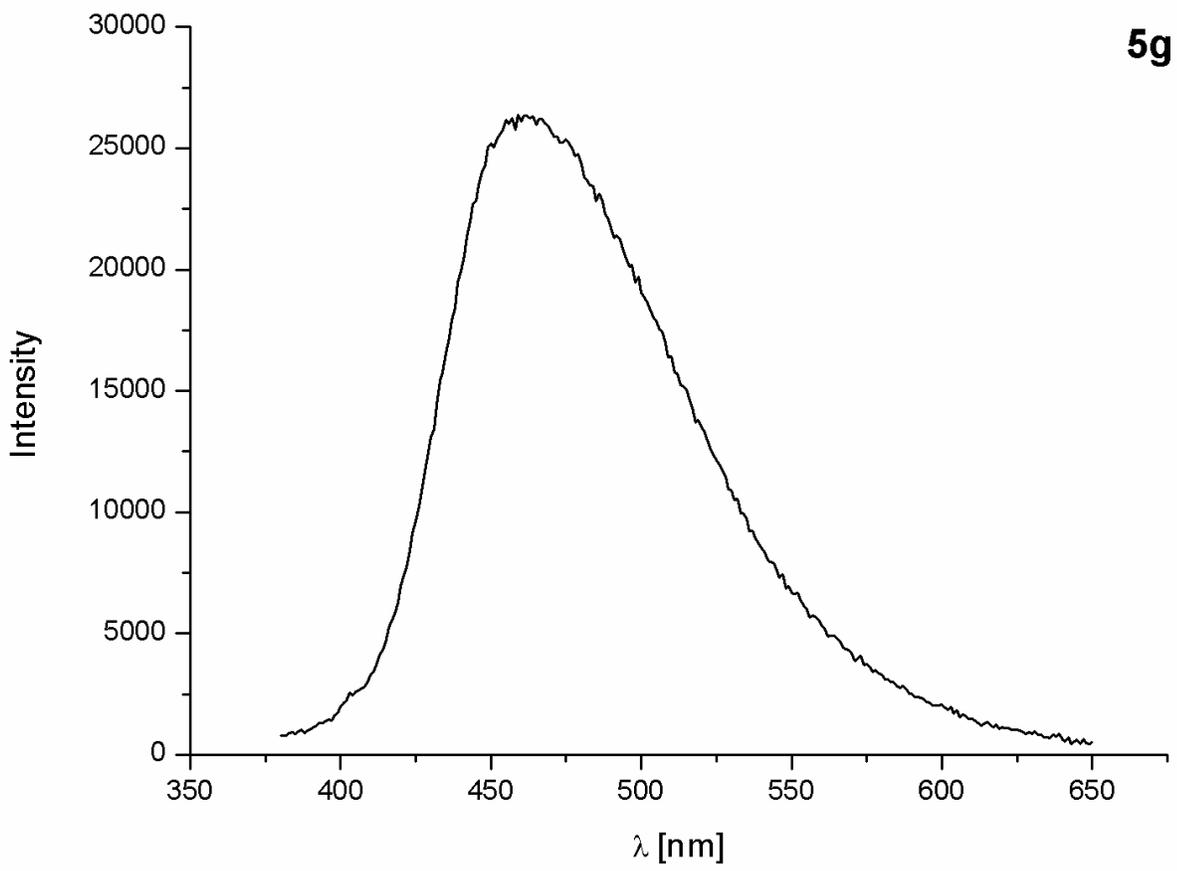


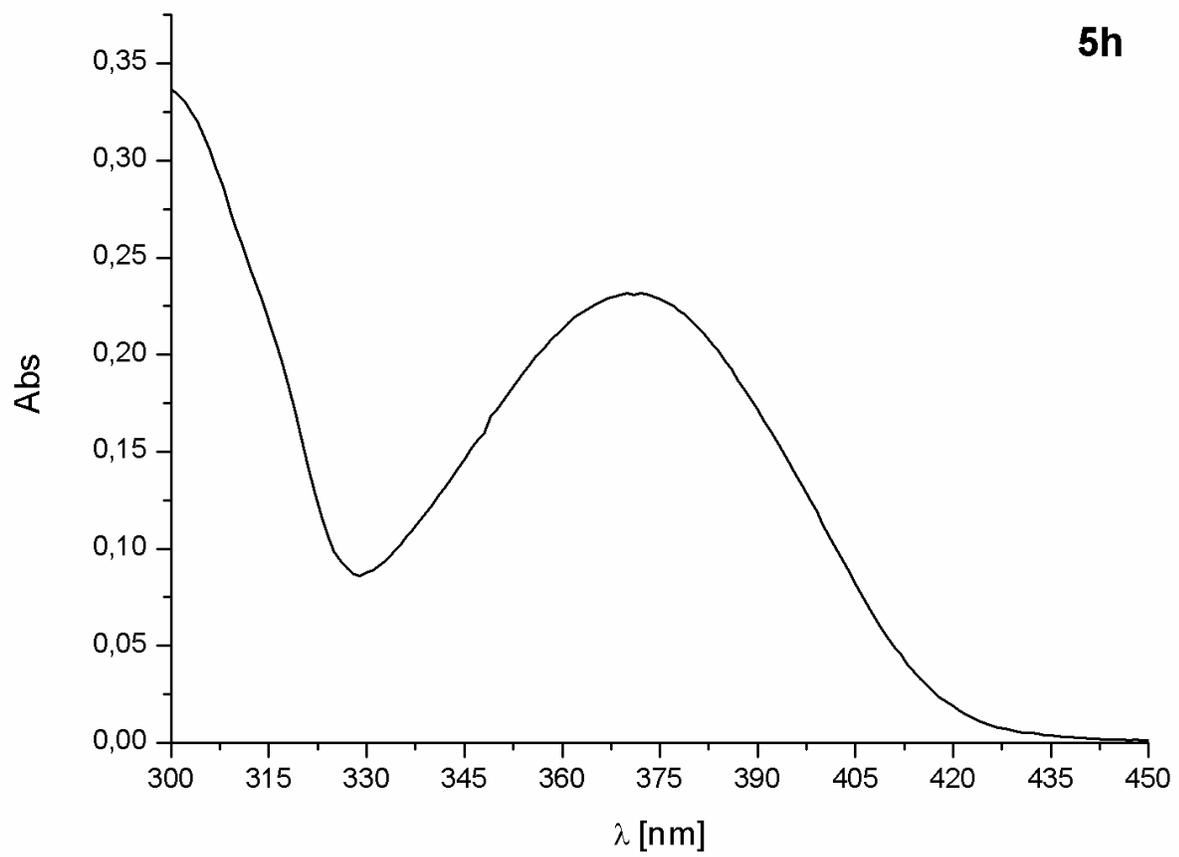
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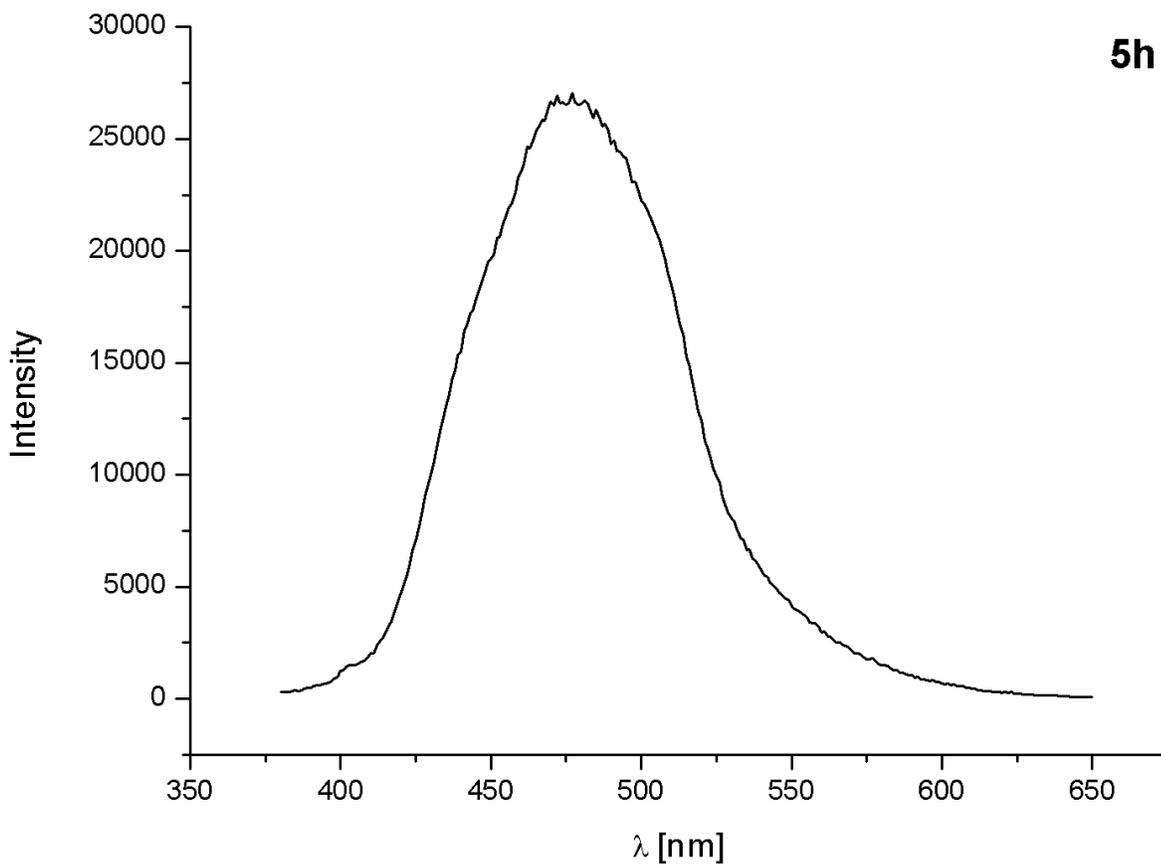


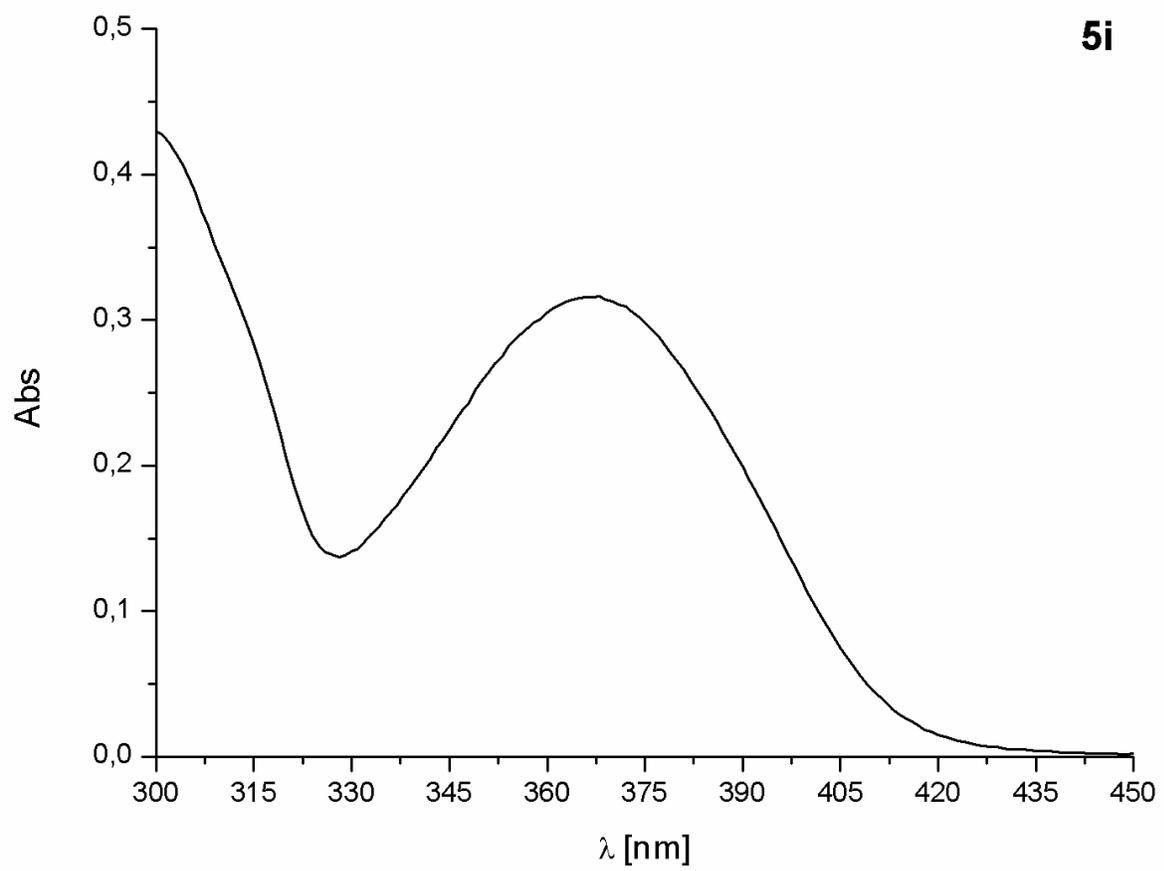


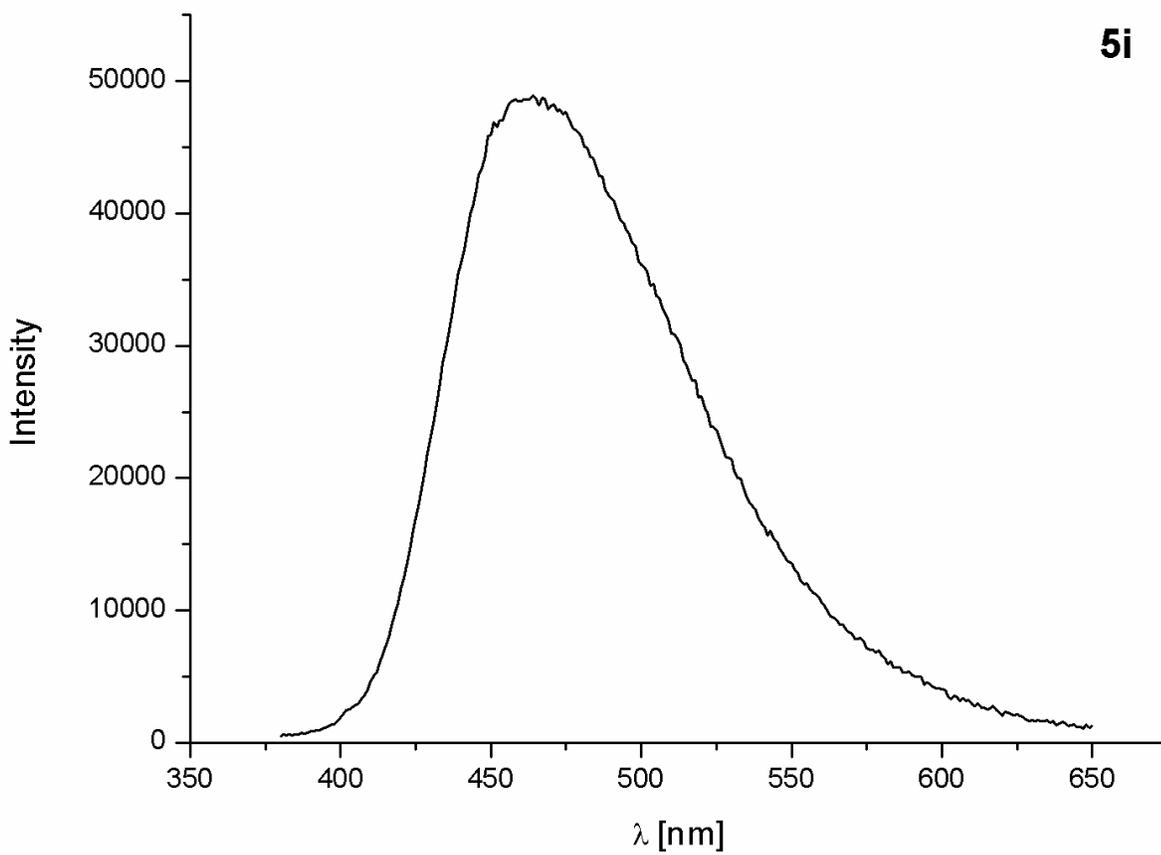


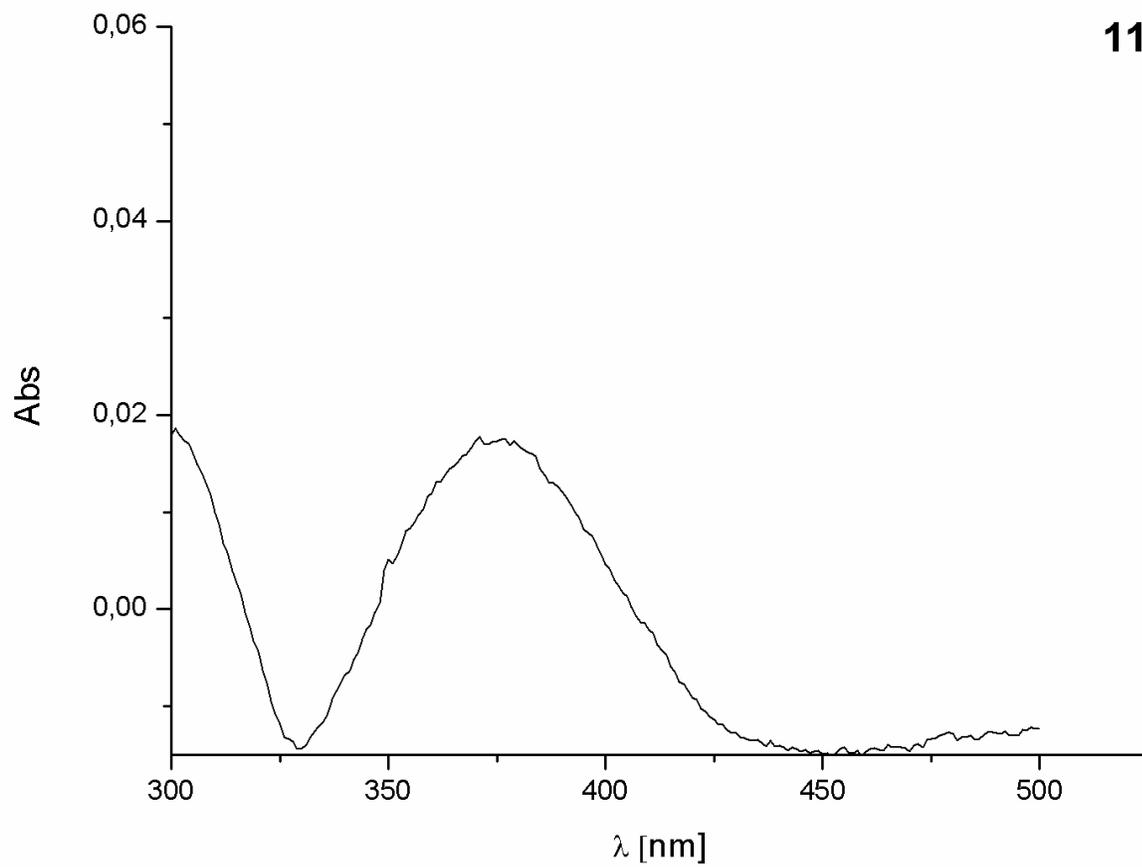


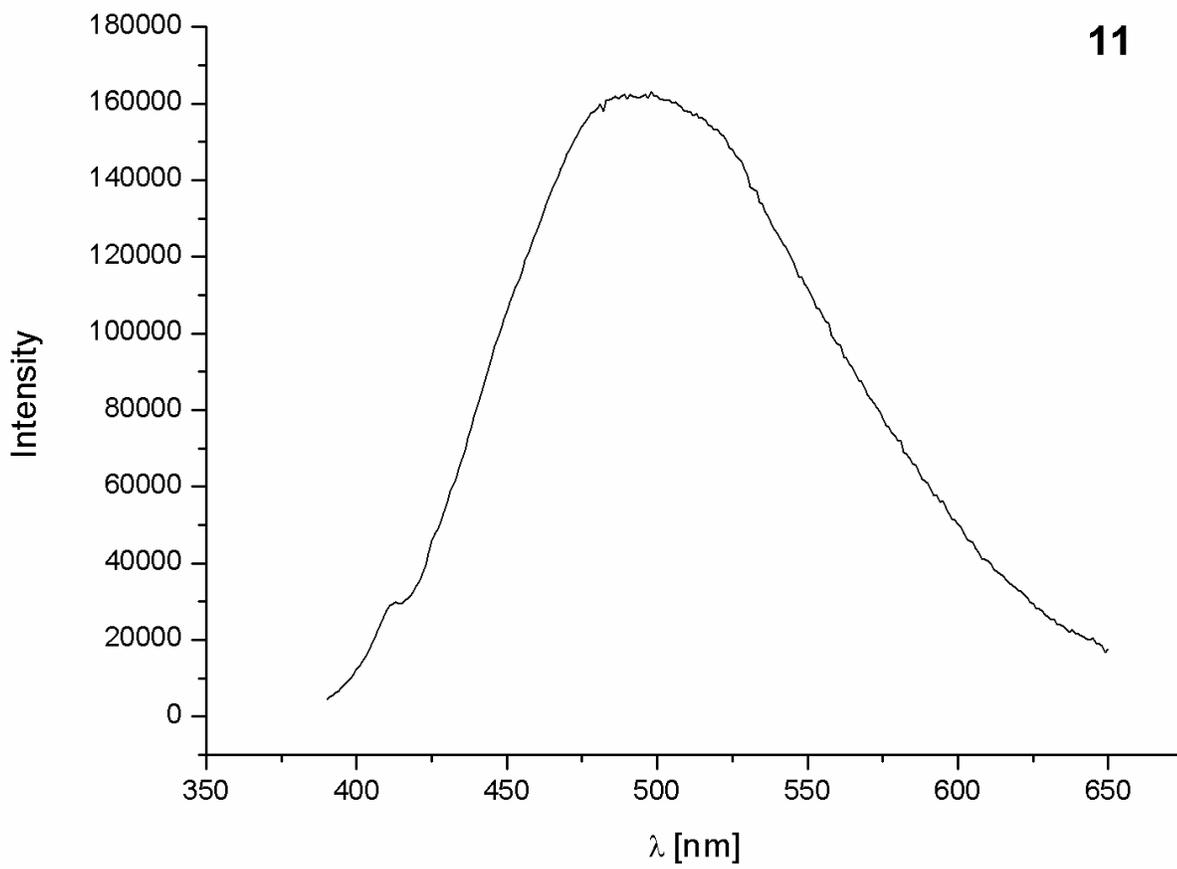












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