Synthesis, study of antileishmanial and antitrypanosomal activity of imidazo pyridine fused triazole analogues

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Experimental section

1. Materials and methods

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All chemical reagents and solvents are purchased from Aldrich, Alfa Aesar, Finar. The solvents and reagents were of LR grade. All the solvents were dried and distilled before use. Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 100-200 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 101 MHz respectively using a Bruker AV 400 spectrometer (Bruker CO., Switzerland) in $CDCl_3$ and DMSO-*d6* solution with tetramethylsilane as the internal standard and chemical shift values (δ) were given in ppm. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-*d6*. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Melting points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. Elemental analyses were performed by ElementarAnalysensysteme GmbH vario MICRO cube CHN Analyzer. Mass spectra (ESI-MS) were recorded on Schimadzu LCMS 8040 MS/ESI mass spectrometer.

2. General Procedure and analytical Data

Representative procedure for the synthesis of compounds 2a and 2b

General procedure for preparation of **2a** and **2b** from **1a** and **1b**:

A solution of 2-amino-4-picoline (**1a**) (10.0 g, 91.5 mmol) and ethyl-2-chloroacetoacetate (7.93 g, 45.8 mmol) were dissolved in 92 mL of 1,2-dimethoxyethane (DME) and heated for 36 h at reflux. The precipitated 2-amino-4-picoline hydrochloride salt was collected through filtration and washed with hexane. The filtrate liquor was concentrated in vacuo and residue was dissolved in CH_2Cl_2 and washed with 5% acetic acid solution (2×) and brine. The organic layer was dried over anhydrous sodium sulfate (Na_2SO_4) and then concentrated under reduced pressure. The Crude material was purified by silica gel column chromatography with 20% ethyl acetate: CH_2Cl_2 solvent system to yield 7.6 g (76%) of ethyl 2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylate (**1b**) as a tan solid. mp 59-61°C; ^{1}H NMR (300 MHz, $CDCl_3$) 9.14 δ (d, J= 7.1 Hz, 1H), 7.34 (s, 1H), 6.78 (dd, J= 7.1, 1.7 Hz,

1H), 4.40 (q, J= 7.1, 7.1, 7.1 Hz, 2H), 2.66 (s, 3H), 2.42 (s, 3H), 1.42 (t, J= 7.1, 7.1 Hz, 3H). HRMS (EI), M+1 calcd. for $C_{12}H_{15}N_2O_2$, 219.1155; found 219.1128. The same procedure was followed to get **2b** from **1b**.

General procedure for preparation of **3a** and **3b** from **2a** and **2b**:

The ethyl 2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylate (**2a**) (6.4 g, 29.3 mmol) was dissolved in 64 mL of ethanol; 1M LiOH (60 mL, 60 mmol) was added and reaction was heated to reflux for 35 hours. The resulting solution was concentrated to dryness and then made acidic (pH~4-5) with the addition of 4 N HCl; the precipitated compound was collected by filtration and dried to give 4.1 grams (79%) of 2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylic acid (**3a**), an off-white solid. M.P. 181-183°C; ^{1}H NMR (300 MHz, CD_3OD) δ 9.52 (d, *J*= 7.1 Hz, 1H), 7.73 (d, *J*= 1.8, 0.9, 0.9 Hz, 1H), 7.48 (dd, *J*= 7.1, 1.3 Hz, 1H), 2.81 (s, 3H), 2.63 (s, 3H). HRMS (EI), M+1 calcd. for $C_{10}H_{11}N_2O_2$, 191.0815; found 191.0837. Retention time = 0.6-0.7 minutes (mobile phase: 60% water: acetonitrile). A similar procedure followed to get **3b** from **2b**.

General procedure for preparation of **4a** and **4b** from **3a** and **3b**:

EDC.HCl (1.20 Equiv) and *N*, *N*- diisopropylethylamine (2.5 equiv.) were added to a solution of N-Boc piperazine (1 equiv.), acid compound (**3a** or **3b**) (1 equiv.) and HOBt (1.2 equiv.) in DMF (8V) and stirred for 24 hours at room temperature under nitrogen. Ethyl acetate was added to the crude reaction mixture and washed with a 10% bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate (Na_2SO_4) and then concentrated under reduced pressure. The crude material was purified by column by using 40 % Ethyl acetate in hexane as eluents. The gummy solid of (tert-butyl 4-(2-methylimidazo[1,2-a]pyridine-3-carbonyl)piperazine-1-carboxylate) **4a.** ^{1}H NMR (300 MHz, CD_3OD) δ 9.50 (d, *J*= 7.1 Hz, 1H), 7.75 (d, *J*= 1.8, 0.9, 0.9 Hz, 1H), 7.46 (dd, *J*= 7.1, 1.3 Hz, 1H), 2.79 (s, 3H), 2.6 (s, 3H), 1.40 (s, 9H). HRMS (EI), M+1 calcd. $C_{18}H_{24}N_4O_3$, 345.190; found 345.097. The yield of **4a** and **4b**: 72% and 65% respectively.

General procedure for preparation of **5a** and **5b** from **4a** and **4b**:

The **Boc-compound (4a or 4b) (**1g) was dissolved in dichloromethane and treated with **4M** HCl-dioxane (2 mL) for 4h. The solvent was distilled under reduced pressure. The solid compound was collected through Buchner filtration to give ((2-methylimidazo[1,2-a]pyridin-3-yl)(piperazin-1-yl)methanone hydrochloride) **5a** ^{1}H NMR (300 MHz, CD_3OD) δ 9.50 (d, *J*= 7.1 Hz, 1H), 7.75 (d, *J*= 1.8, 0.9, 0.9 Hz, 1H), 7.46 (dd, *J*= 7.1, 1.3 Hz, 1H), 3.45 (s, 1H), 2.79 (s, 3H), 2.6 (s, 3H). HRMS (EI), M+1 calcd. $C_{13}H_{17}ClN_4O$, 281.11; found 281.25. Same procedure for the **5b** and obtained 64 to 72% yield.

General procedure for preparation of **6a** and **6b** from **5a** and **5b**

5a/**5b** was dissolved in DMF and then K_2CO_3 and propargyl bromide (80% in toluene) (1.5 eq) was added. The reaction mixture was heated to 100 °C for12h. Once the reaction complete, ethyl acetate was added to the reaction mixture and washed with water. The organic layer was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The crude material was purified by column by using 60 % Ethyl acetate in hexane as eluents to yield 70%. ^{1}H NMR (300 MHz, CD_3OD) δ 9.50 (d, *J*= 7.1 Hz, 1H), 7.75 (d, *J*= 1.8, 0.9, 0.9 Hz, 1H), 7.46 (dd, *J*= 7.1, 1.3 Hz, 1H), 3.15 (s, 1H), 3.46(s, 2H), 2.79 (s, 3H), 2.6 (s, 3H). HRMS (EI), M+1 calcd. $C_{16}H_{18}N_4O$, 283.15; found 283.35

General procedure for preparation of **7a-j** and **8a-p** from **6a** and **6b**:

A solution of the alkyne **(6a**/**6b)** (1.0 equiv.) in DMF: H_2O (8:2) was reacted with different substituted azides (1.5 equiv.) in the presence of sodium ascorbate (0.01 equiv.) and $CuSO_4{\cdot}5H_2O$ (0.02 equiv.). The reaction mixture was stirred at rt for 7-12 h. The reaction mixture was monitored by TLC. Once completion of the reaction, as indicated by TLC, the reaction was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure and the crude residue was purified by column chromatography by using 70-90% ethyl acetate in hexane as eluent to get title compounds **7a-j** and **8a-p**.

General procedure for preparation of **9a** and **9b** from **5a** and **5b**:

5a/**5b** was dissolved in DMF and then K_2CO_3 and 2-azidoethyl 4-methylbenzenesulfonate (1.5 eq) were added. The reaction mixture was heated to 100 °C for12h. Once the reaction is complete, as indicated by TLC, ethyl acetate was added to the reaction mixture and washed with water. The organic layer was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The crude material was purified by column chromatography, using 60 % Ethyl acetate in hexane as eluents to yield **9a** as a gummy solid ((4-(2-azidoethyl)piperazin-1-yl)(2-methylimidazo[1,2-a]pyridin-3-yl)methanone). ^{1}H NMR (300 MHz, CD_3OD) δ 9.50 (d, *J*= 7.1 Hz, 1H), 7.75 (d, *J*= 1.8, 0.9, 0.9 Hz, 1H), 7.46 (dd, *J*= 7.1, 1.3 Hz, 1H), 2.79 (s, 3H), 2.6 (s, 3H), 2.3 (t, 2H), 1.9 (t, 2H). HRMS (EI), M+1 calcd. $C_{15}H_{19}N_7O$, 314.17; found 314.27. Same procedure for the compound 9b.

General procedure for preparation of **10a-d** and **11a-e** from **9a** and **9b**:

A solution of azide **9a**/**9b** (1.0 equiv.) in DMF: H_2O (8:2) is reacted with various substituted acetylenes (1.5 equiv.) in the presence of sodium ascorbate (0.01 equiv.) and $CuSO_4{\cdot}5H_2O$ (0.02 equiv.) The reaction mixture is stirred at rt for 7 to 12h. Once completion of the reaction, as indicated by TLC, the reaction was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous sodium sulfate concentrated under reduced pressure and the crude residue was purified by column chromatography using 70-90% ethyl acetate in hexane as eluent to get title compounds **10a-d** and **11a-e**.

3. Biological Procedures:

Cytotoxicity assay

HeLa cell cytotoxicity studies were carried out as described previously. Briefly, the cells were cultured in DMEM supplemented with 10% fetal calf serum and 2 mM L-glutamine. Cells were plated at initial cell concentration of 2×10^4 cells / well and incubated with the compounds for ~65 h prior to addition of Alamar Blue solution for further 5 h [1].

In-vitro **antileishmanial activity**

Anti-leishmanial activity of the titled analogues was determined by evaluating their inhibition activity against promastigote forms of *Leishmania major* LV9 strain. Compounds were screened against promastigote forms of the Leishmania strains to determine their effective concentration (EC_{50}) values. Anti-leishmanial drug miltefosine was used as standard for comparison purpose. Anti-promastigote activity of these titled derivatives was determined by Alamar blue assay method. *L. major* promastigotes were cultured at 37°C in M199 medium supplemented with 10% heat-inactivated fetal calf serum [2]. Parasites were incubated with serial dilutions of compounds for 72 h, followed by Alamar blue based assay as previously described [3].

***In-vitro* antitrypanosomal activity**

The titled compounds were tested on bloodstream forms of *Trypanosoma brucei,* which was cultured in HMI-9 medium (pH 7.4) supplemented with 10% heat-inactivated Fetal Calf Serum (FCS, BioSera) and 14 µl/L of 13.4 M βmercaptoethanol (Sigma) [4]. In a flow cabinet through filtration medium was sterilized. The resultant *T. brucei* cultures were incubated at 37 ºC and 5% CO_2 and passaged in vented flasks three times a week. The assays were performed by alamar blue assay method [5] in 96-well plates with 1 × 10^5 cell/ well in the presence of 23 doubling dilutions of test compound, and one well for each dilution series receiving growth medium only, for 48 h at 37 °C/5% CO_2. The alamar blue solution was added for a further 24 h incubation before fluorescence was measured in a Biotex plate reader, λ_{ex} 540 nm, λ_{em} 590 nm. EC_{50} values were calculated by non-linear regression to a sigmoidal curve with variable slope using Grafit software.

***In-silico* prediction of ADME and Toxicity parameters**

The ADMET parameters of the titled compounds were *in silico* predicted using Qikprop module of Schrodinger. The diverse parameters predicted were molecular weight (M.Wt.), total solvent accessible surface area (SASA), number of hydrogen bond donor (HBD), number of hydrogen bond acceptor (HBA), octanol / water partition coefficient (log P), aqueous solubility (Log S), predicted apparent Caco-2 cell permeability in nm/sec (P Caco) and number of rotatable bonds (Rot) [6, 7]. SMILES format of the

compounds was generated by using OSIRIS DataWarrior. All the related toxicity parameters were also predicted by the same software [8].

4. Molecular docking study

Molecular docking study was carried out using Schrodinger software [9] (Version 2019-1, Schrodinger) installed on Intel Xenon W 3565 processor and Ubuntu enterprise version 14.04 as an operating system. The selected target protein structure was retrieved from the RCSB protein data bank (www.rcsb.org) [10]. Targeted ligands were drawn using ChemDraw 18.0 software.

Ligand preparation

The ligands used as an input for docking study was sketched using ChemDraw software and cleaned up the structures for bond alignment, ligands incorporated into the workstation, the energy was minimized using OPLS3e force field in Ligprep [11] (Version 2019-1, Schrodinger). This minimization helps to assign bond orders, the addition of hydrogens to the ligands, and conversion of 2D to 3D structure for further docking studies. The generated output file (best conformations of the ligands) was further used for docking studies.

Protein preparation

Protein was retrieved from the RCSB site (https://www.rcsb.org/structure/2JK6) [12]. Protein was prepared using a protein preparation wizard [13] (Version 2019-1, Schrodinger). Hydrogen atom was added to the proteins, and charges were assigned. Generated Het states using Epik at pH 7.0 ±2.0. Pre-processed the protein and refined, modified the protein by analysing the workspace, water molecules, and other heteroatoms were examined, non-significant atoms were excluded from the crystal structure of the protein. Finally, the protein was minimized by using OPLS3e force filed

Receptor grid generation

A receptor grid was generated around the protein by picking the inhibitory ligand (X-ray pose of the ligand in the protein). The centroid of the ligand was selected to create a grid box around it, and the Vander Waals radius of receptor atoms was scaled to 1.00 Å with a partial atomic charge of 0.25.

Docking validation

The most straightforward way of validating the accuracy of specified parameters for docking study is to re-dock the co-crystallized ligand back into the binding site of the protein and calculate the root mean square deviation (RMSD) value between the crystallographic orientation and the docked pose. RMSD calculation is a convenient method to use in order to follow how much a structure has diverged from its initial geometry. The lower the RMSD value between the docked pose to that of its crystallographic orientation is an indication of the suitability of the docking protocols. Therefore, prior to screening of all ligands, the co-crystal structures of PDB-2JK6 (FAD molecule), was chosen and re-docked back into the same active site. The RMSD value between the crystallographic orientation and the best-docked pose was generated. The RMSD value of the selected targets was found to be 0.20 Å respectively. The lower RMSD value indicates that the docking protocol could be reliable for the final docking studies of the test compounds against the selected target.

Docking and analysis

Molecular docking was performed using the above-prepared ligand and protein as input. The results of the docking study were analysed with the help of XP Visualiser (Version 2019-1, Schrodinger). Docking studies of the designed and synthesized molecules were performed by using the Glide module [14] in Schrodinger. All docking calculations were performed using Extra Precision (XP) mode. A scaling factor of 0.8 and a partial atomic charge of less than 0.15 was applied to the atoms of the protein. Glide docking score was used to determine the best-docked confirmation from the output. The interactions of these docked conformations were investigated further using XP visualizer.

5. X-ray crystallographic studies:

Single crystal X-ray crystallographic structure of compound 8f:

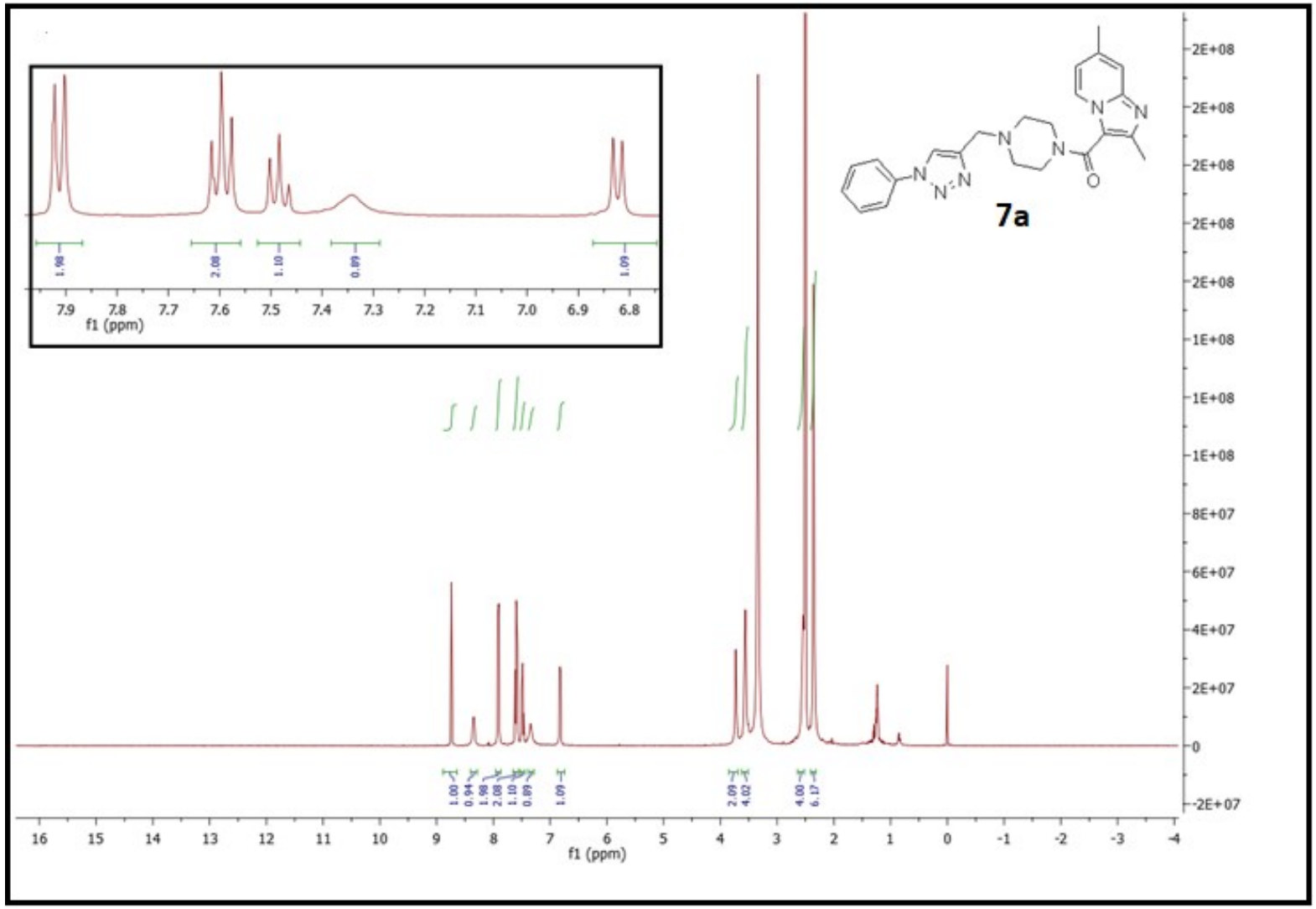
The suitable crystals of compound **8f** for single crystal X-ray diffraction (SCXRD) study were grown from the mixture of methanol and dichloromethane (1:3). The SCXRD measurements were performed on the Rigaku XtaLAB P200 diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The data was collected and reduced using CrysAlisPro (Rigaku Oxford Diffraction) software. The data collection was carried out at 100 K and the structures were solved using Olex2 with the ShelX structure solution program using Direct Methods and refined with the ShelXL refinement package using Least Squares minimization. The basic crystallographic data is shown in **Table 1.**

Table 1. **Single crystal data of compound 8f**

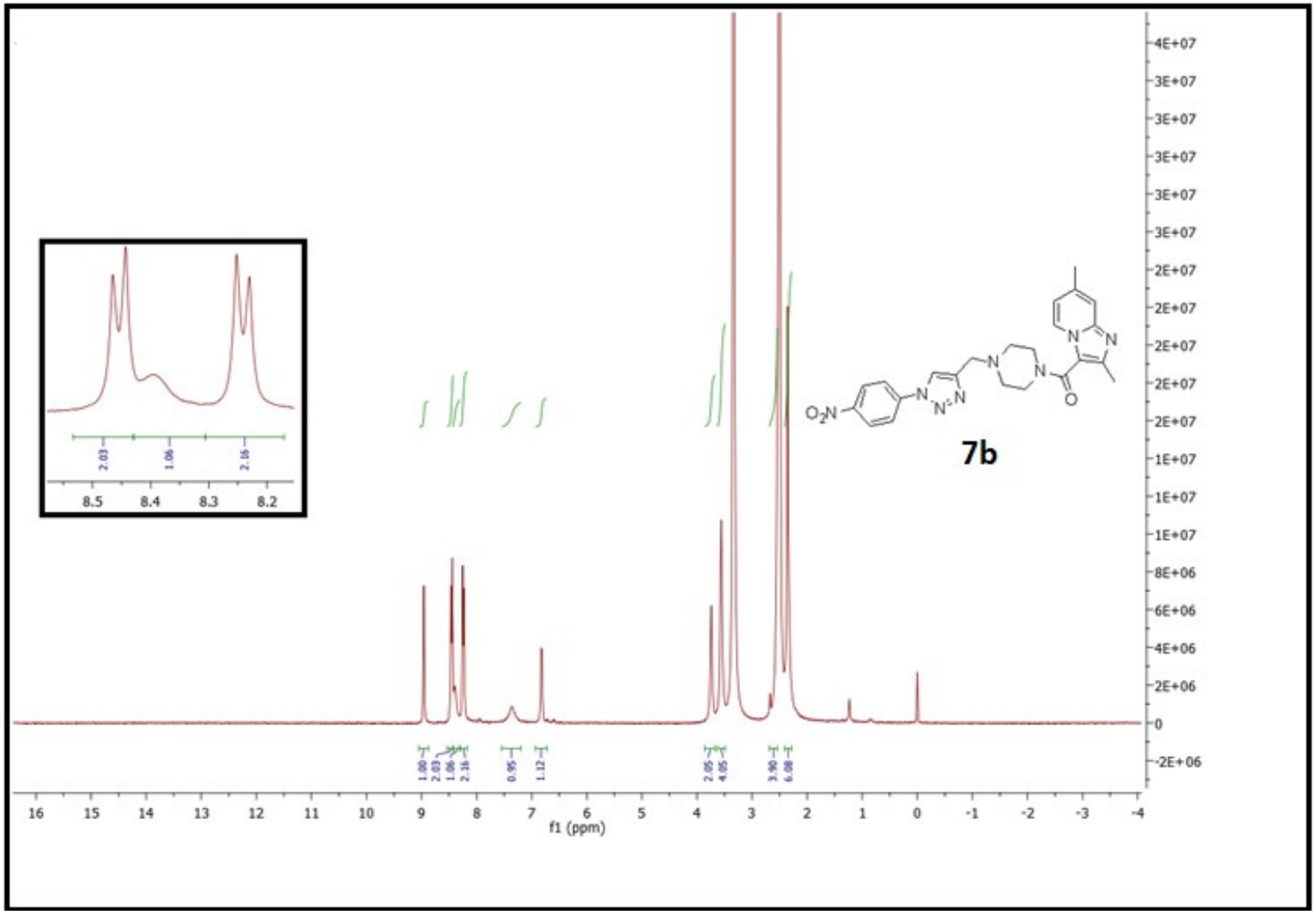
Empirical formula	$C_{23}H_{23}BrCl_3N_7O$
Formula weight	599.75
Temperature/K	100.0
Crystal system	orthorhombic
Space group	Pbca
a/Å	17.2953(7)
b/Å	13.8502(5)
c/Å	20.9305(7)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	5013.8(3)
Z	8
ρ_{calc}g/cm^3	1.5889
μ/mm^{-1}	1.992
F(000)	2434.0

Crystal size/mm^3	0.3 × 0.2 × 0.2
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	6.62 to 59.64
Index ranges	-23 ≤ h ≤ 22, -18 ≤ k ≤ 19, -28 ≤ l ≤ 22
Reflections collected	37545
Independent reflections	6649 [R_{int} = 0.0292, R_{sigma} = 0.0204]
Data/restraints/parameters	6649/2/344
Goodness-of-fit on F^2	1.039
Final R indexes [I>=2σ (I)]	R_1 = 0.0292, wR_2 = 0.0725
Final R indexes [all data]	R_1 = 0.0411, wR_2 = 0.0791
Largest diff. peak/hole / e Å^{-3}	1.19/-0.60

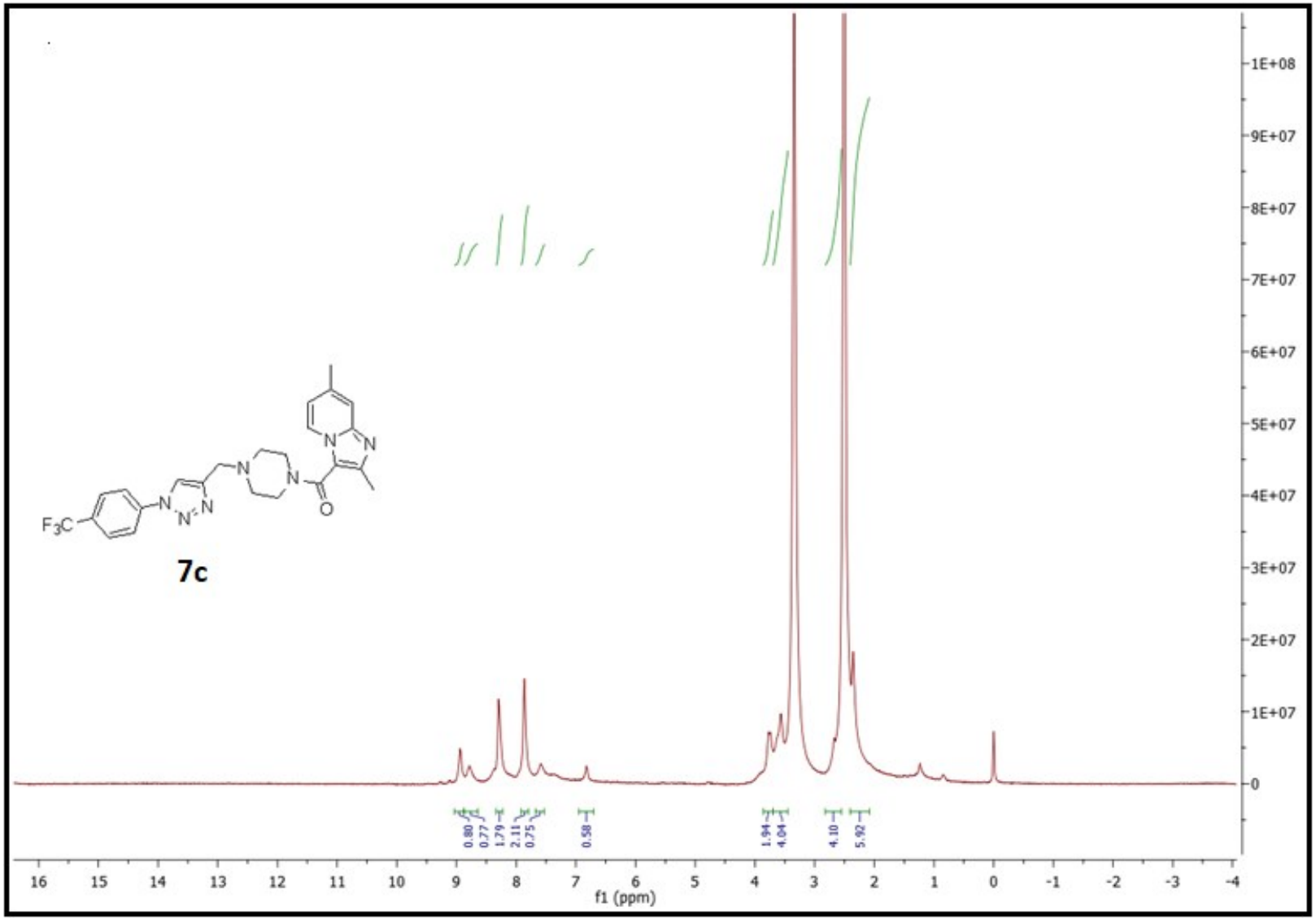
6. **^{1}H NMR spectras of intermediate compounds and final compounds:**



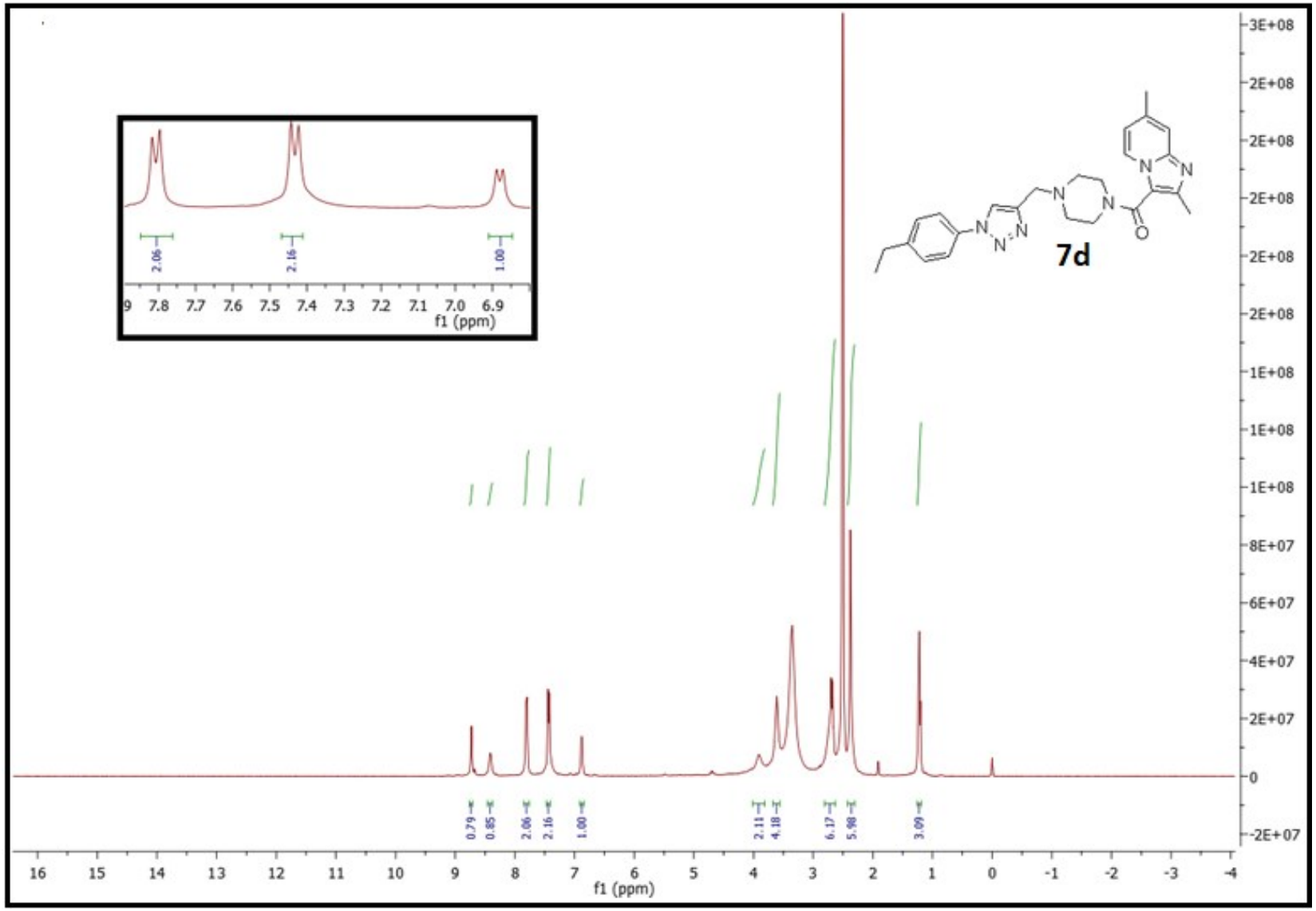
^{1}HNMR of 7a



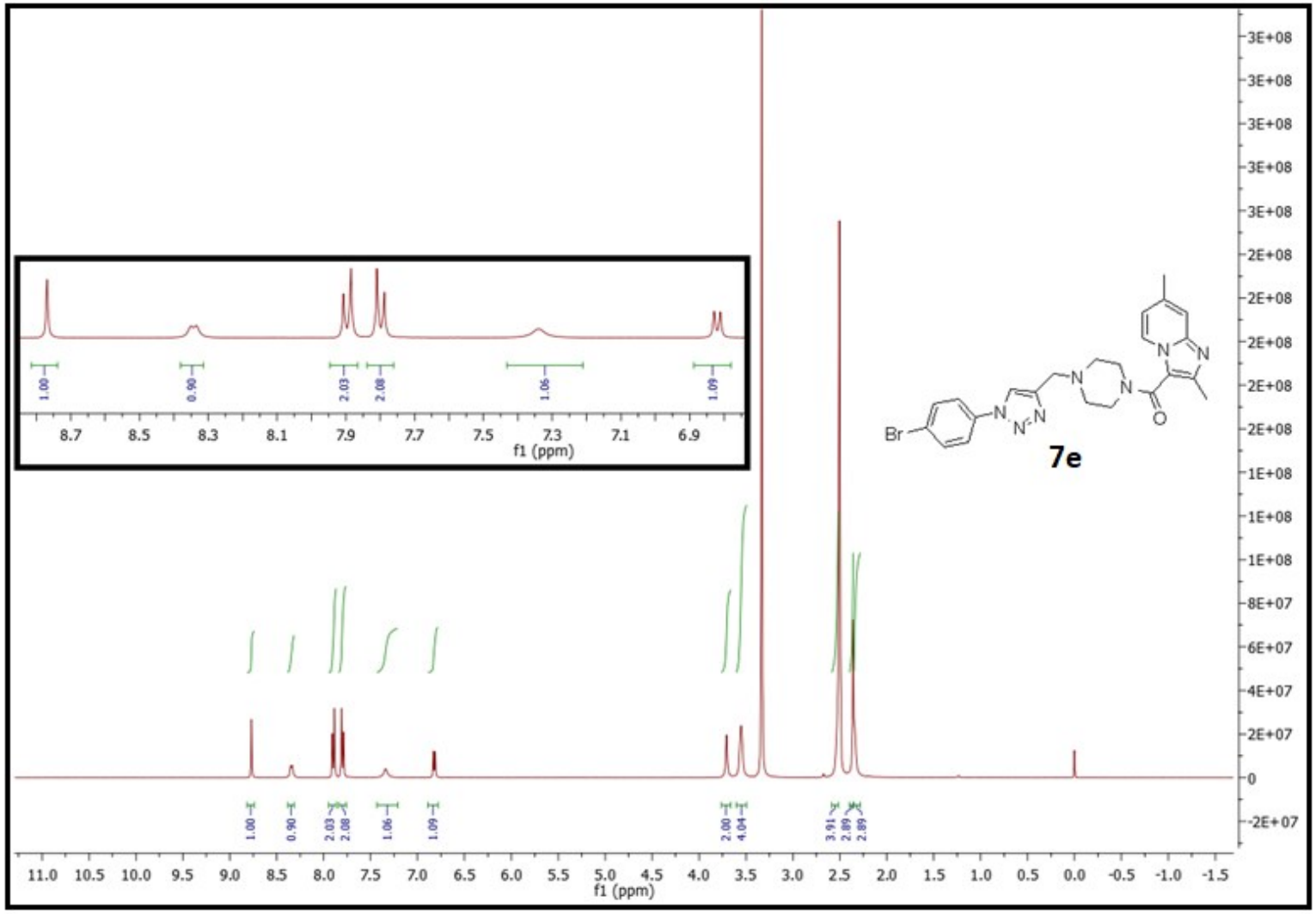
[1]HNMR of 7b


7c
F3C
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
0.80
0.77
1.79
2.11
0.75
0.58
1.94
4.04
4.10
5.92
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
f1 (ppm)

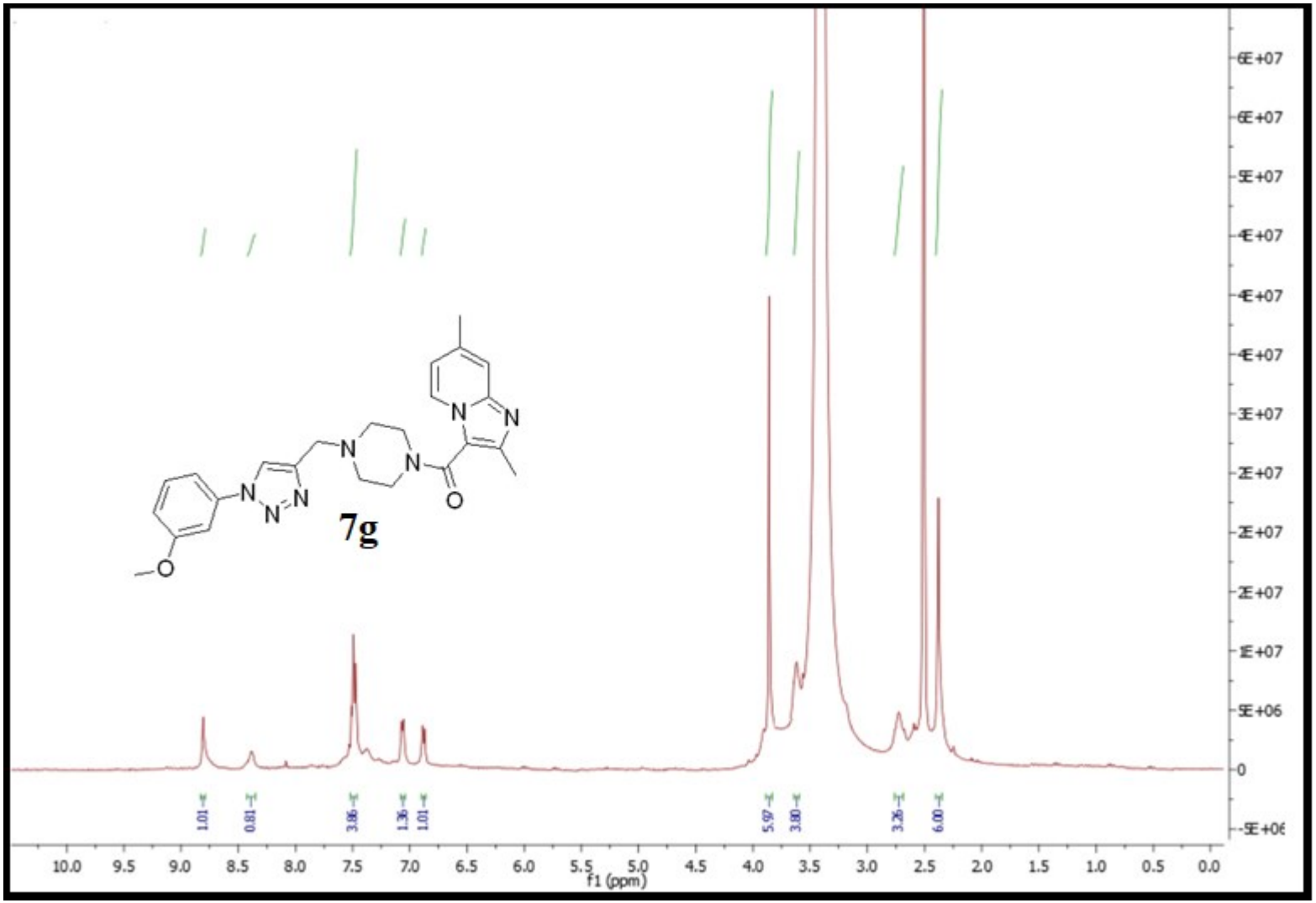
[1]HNMR of 7c



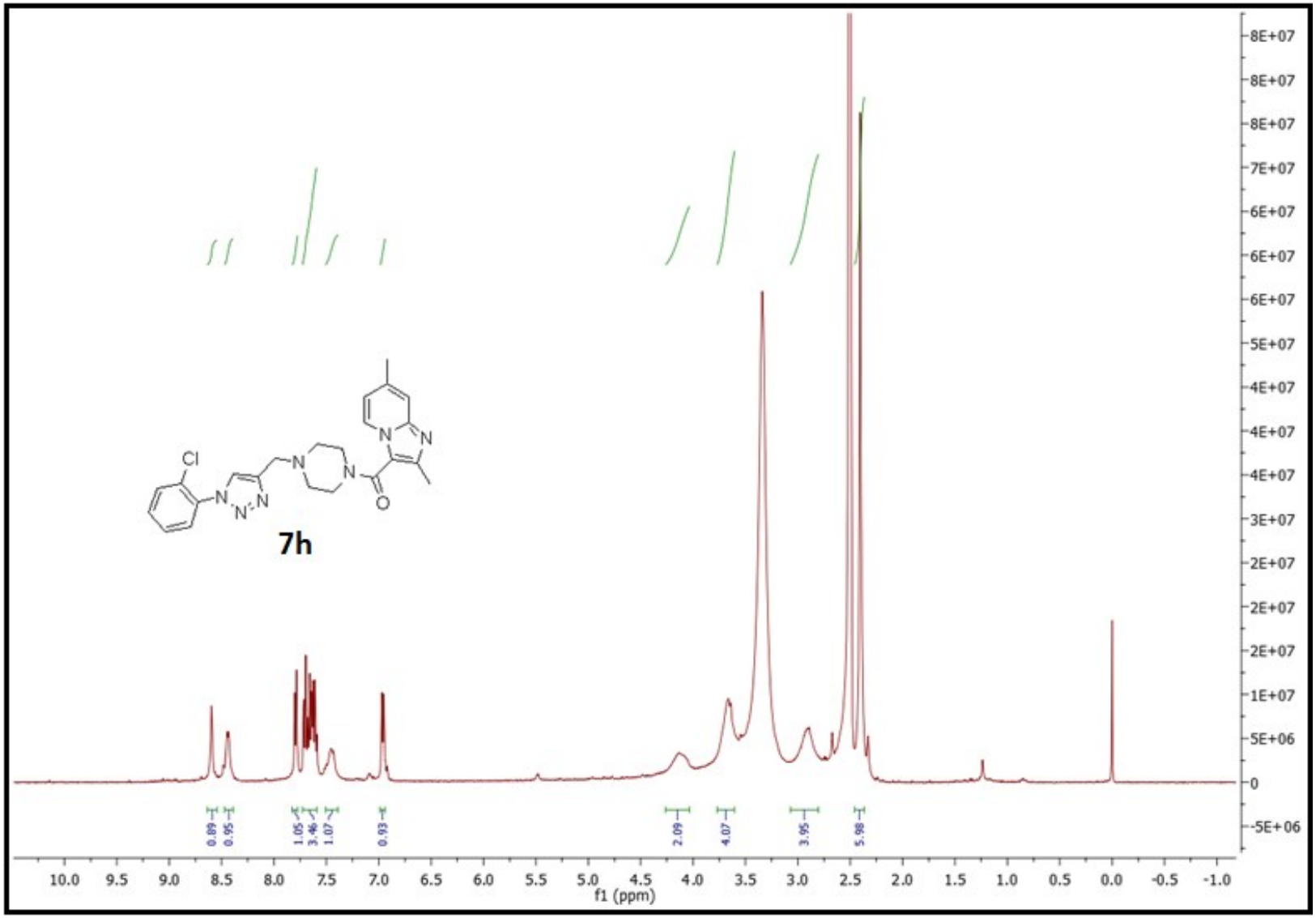
[1]HNMR of 7d



[1]HNMR of 7e



[1]HNMR of 7g


7h
Cl
N
N
N
N
N
N
O
8E+07
8E+07
8E+07
7E+07
6E+07
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6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
0.89
0.95
1.05
3.46
1.07
0.93
2.09
4.07
3.95
5.98
10.0
9.5
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8.5
8.0
7.5
7.0
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6.0
5.5
5.0
4.5
4.0
3.5
3.0
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1.5
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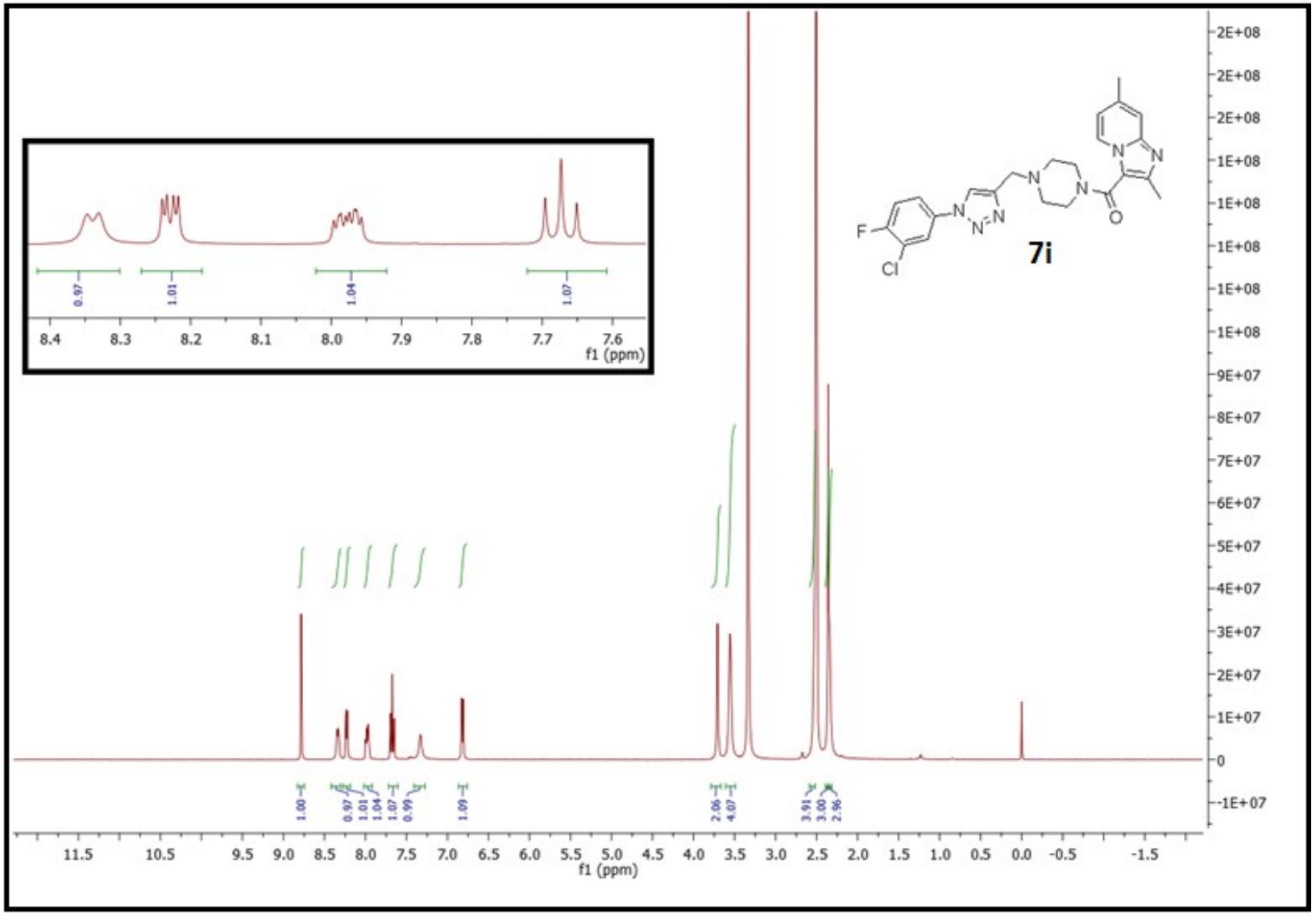
-1.0
f1 (ppm)

^{1}HNMR of 7h


7i
F
Cl
N
N
N
N
O
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1.01
1.04
1.07
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
f1 (ppm)
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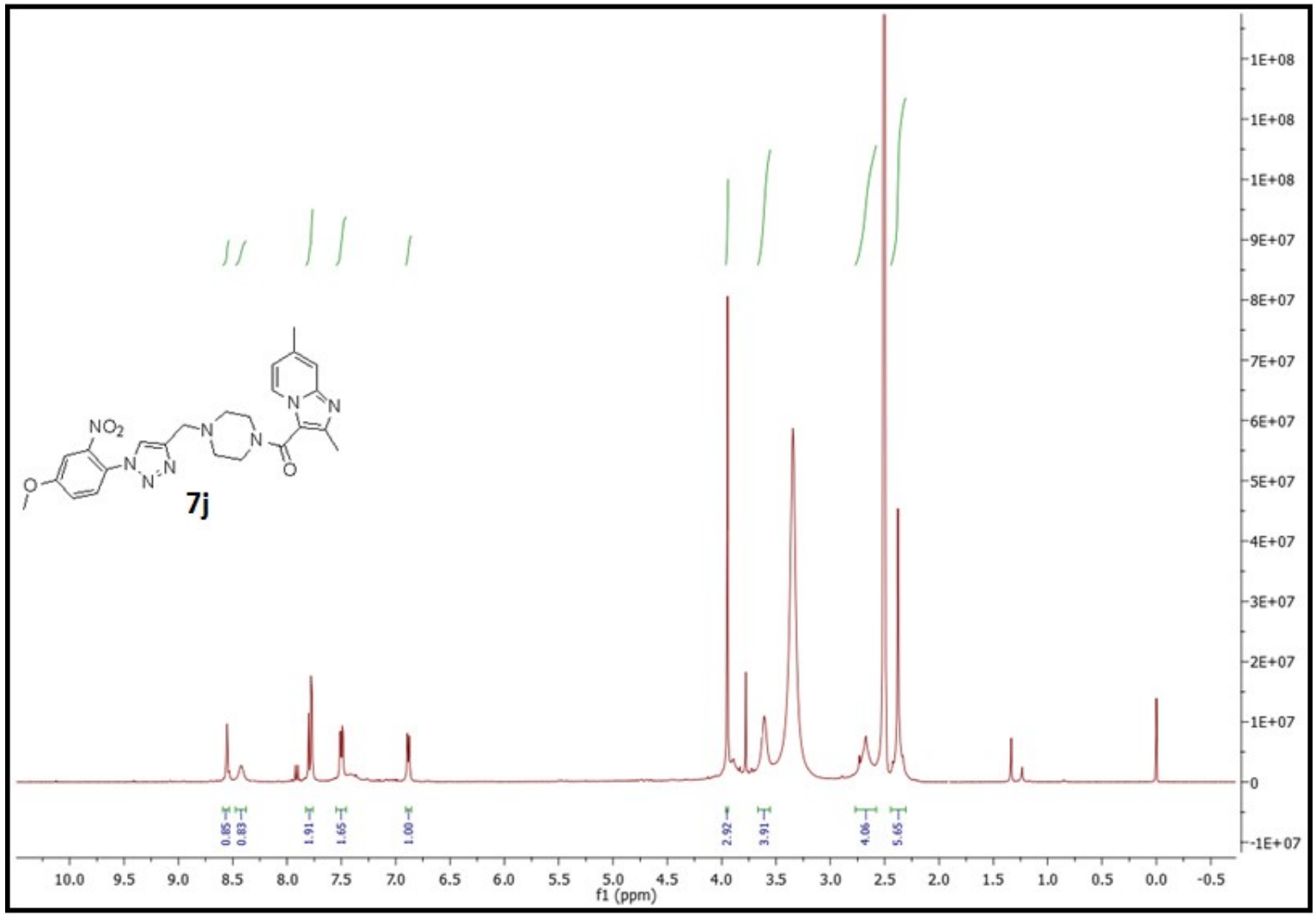
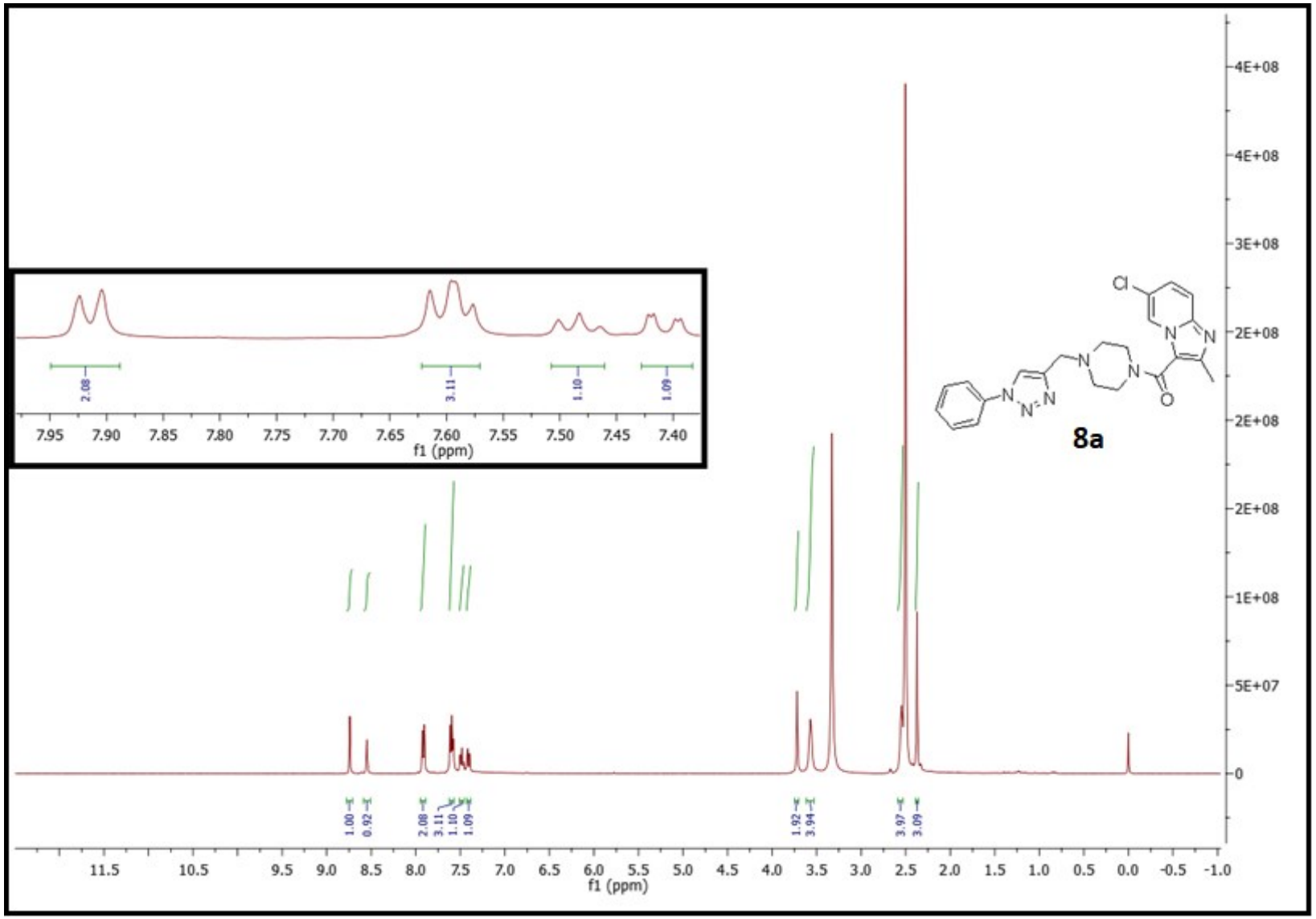
0.97
1.01
1.04
1.07
0.99
1.09
2.06
4.07
3.91
3.00
2.96
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.5
f1 (ppm)
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07

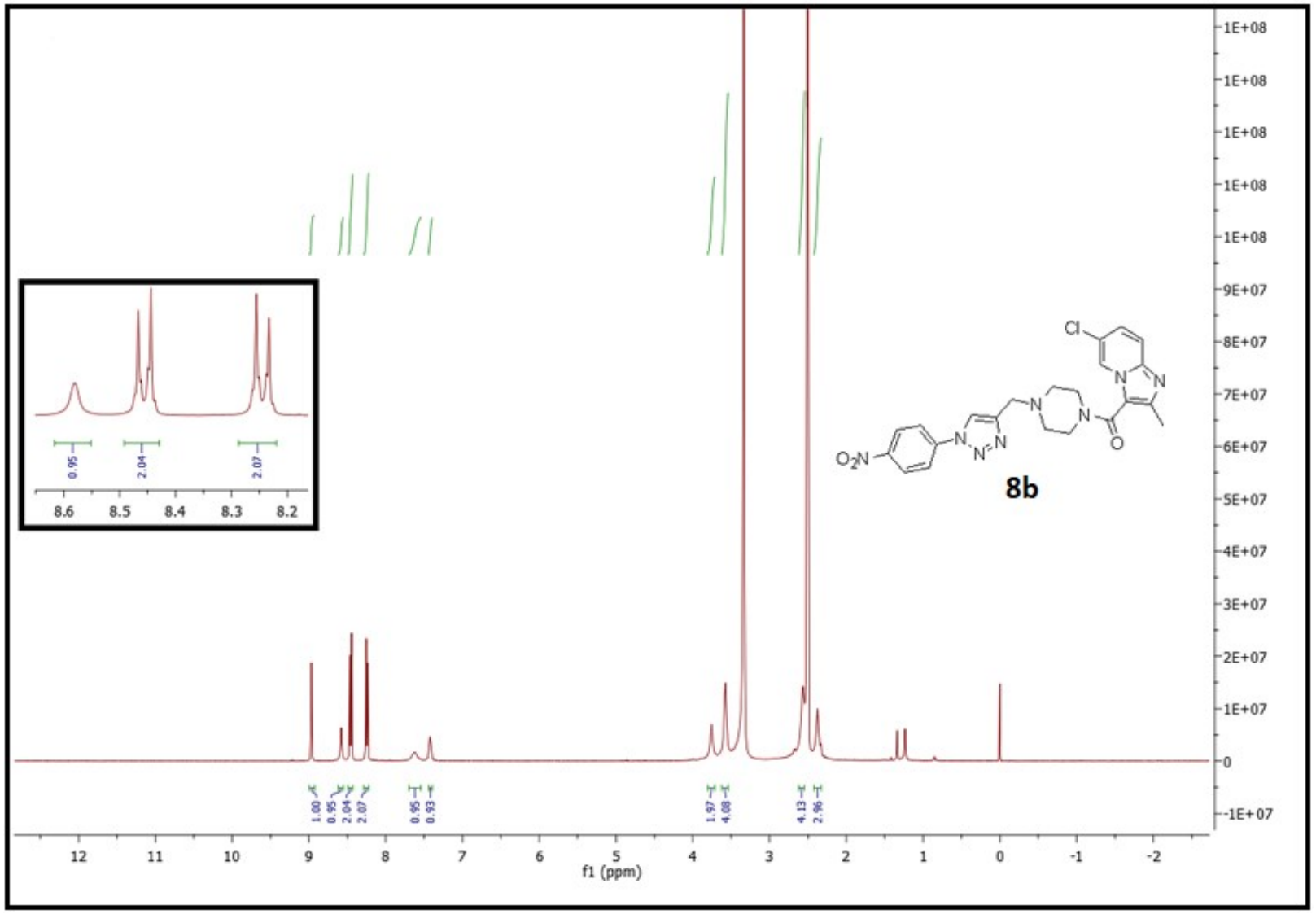
[1]HNMR of 7i


7j
NO2
O
N
N
N
N
N
O
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
0.85
0.83
1.91
1.65
1.00
2.92
3.91
4.06
5.65
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

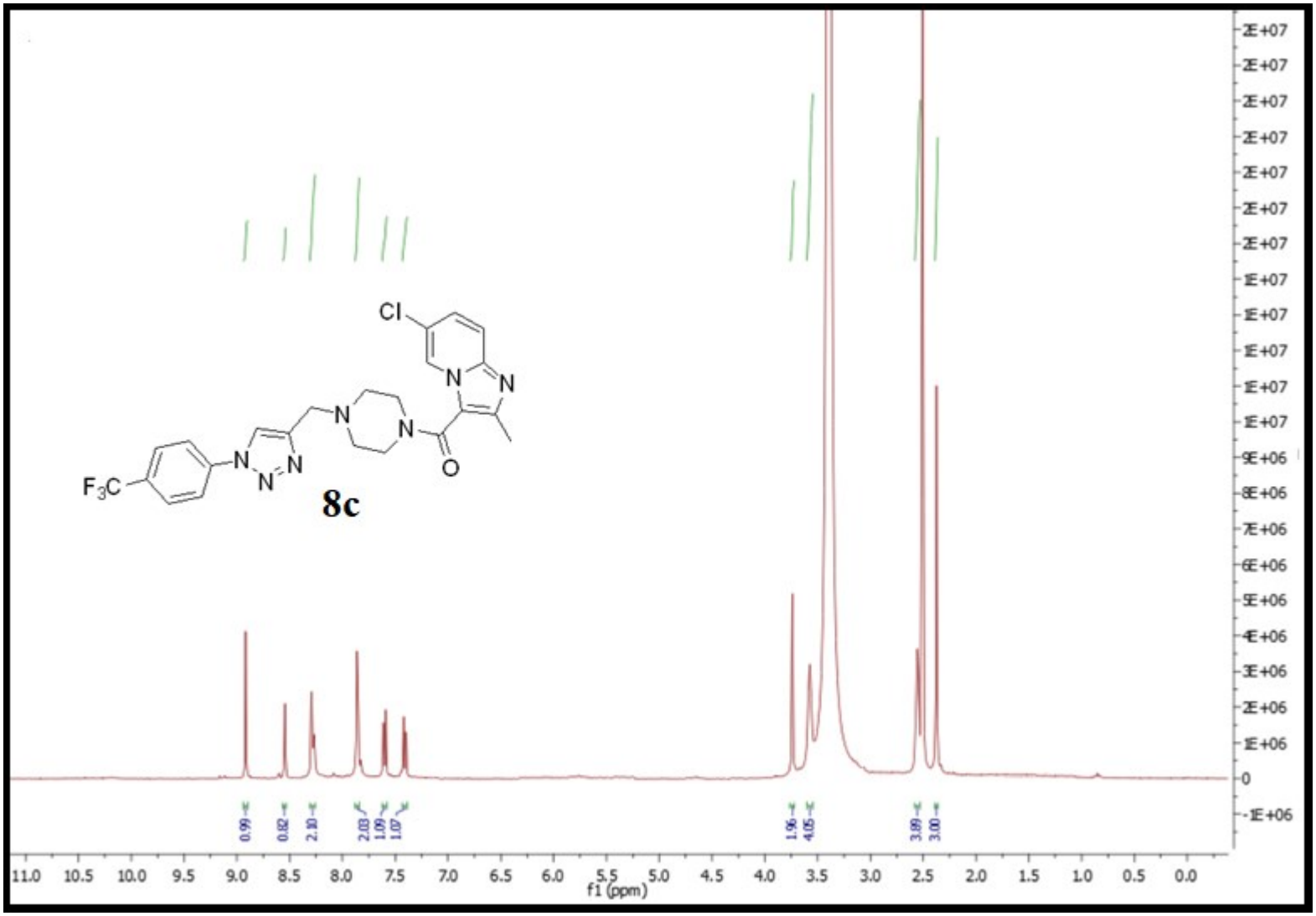
[1]HNMR of 7j



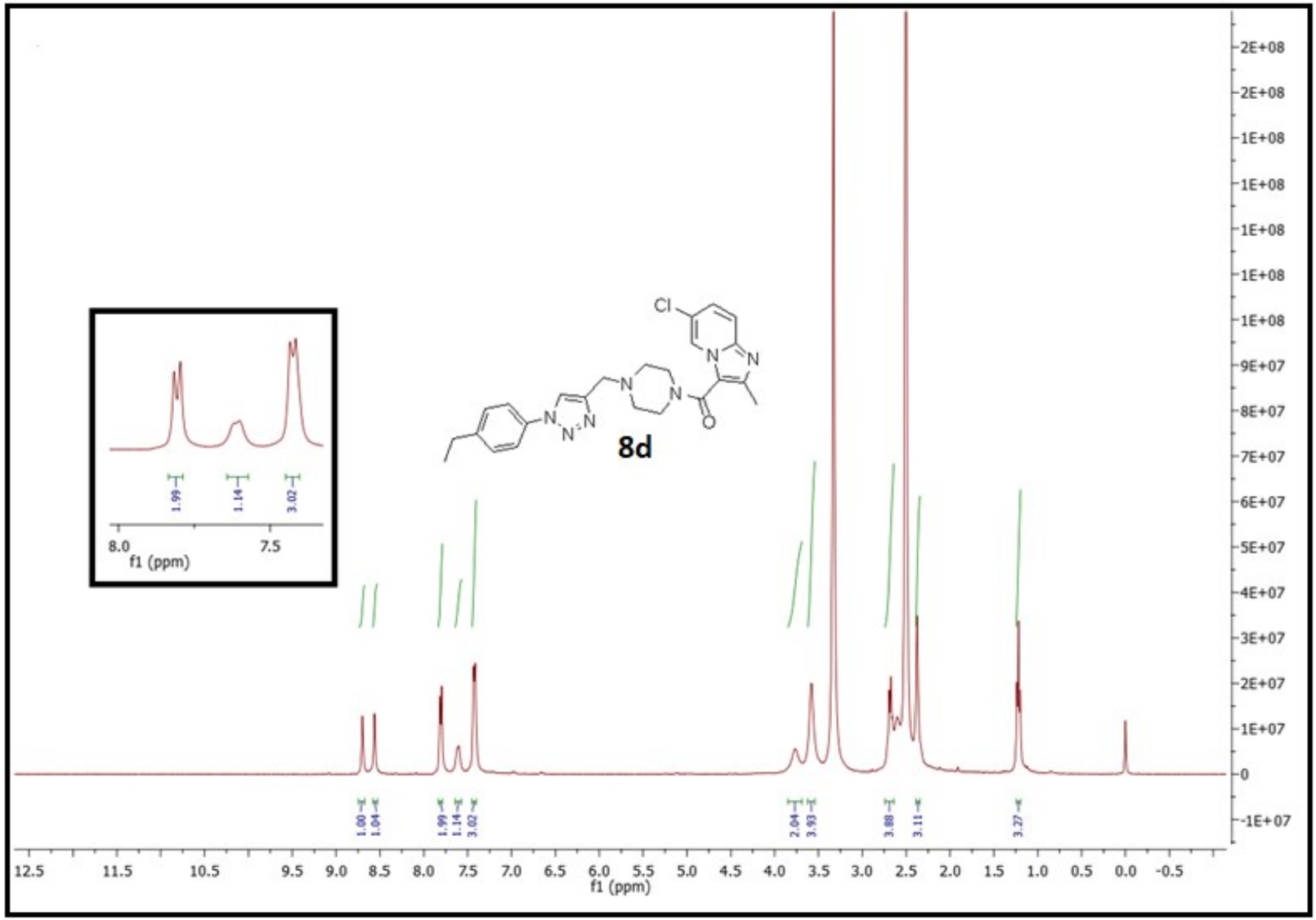
[1]HNMR of 8a



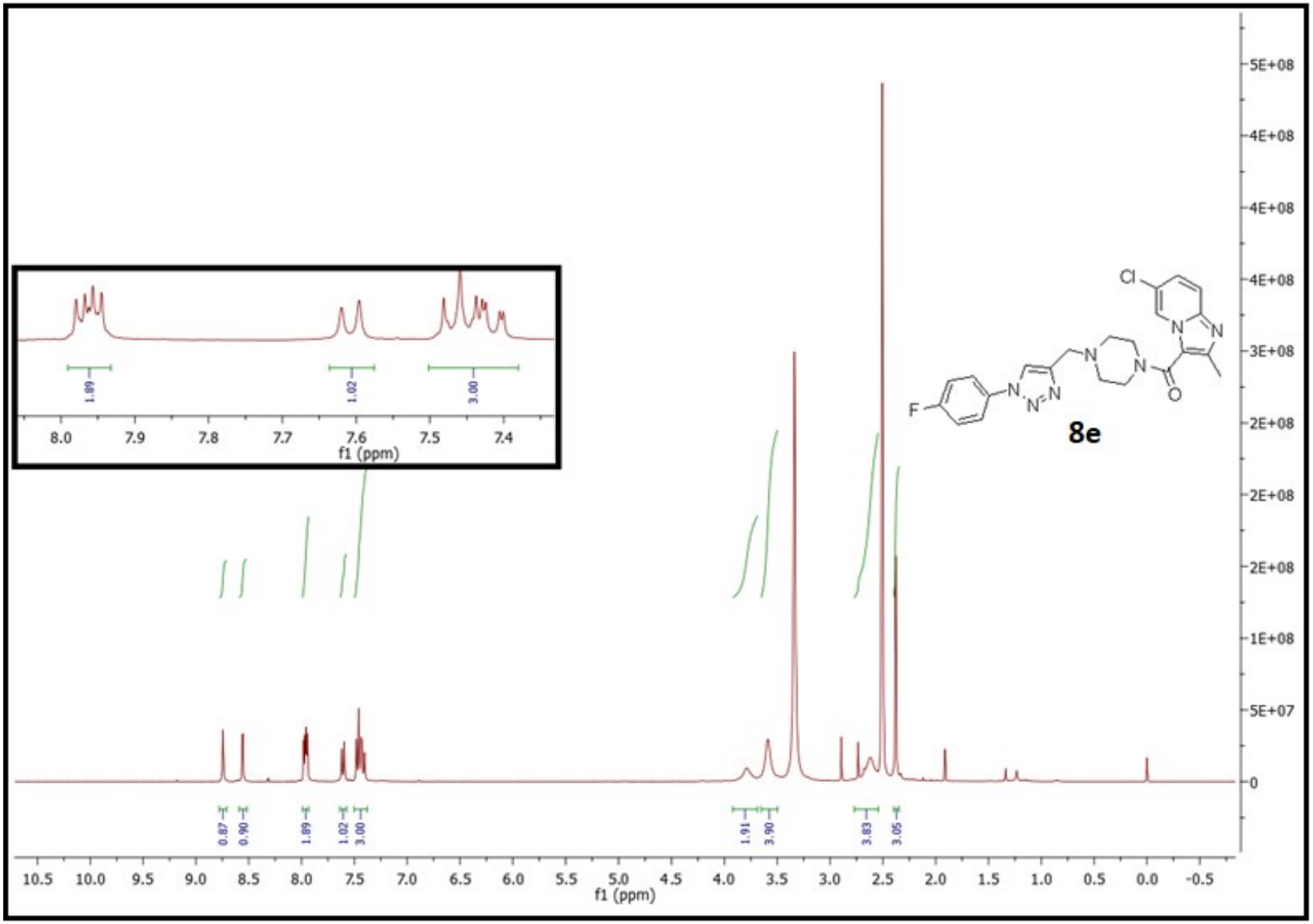
[1]HNMR of 8b


Cl
N
N
N
N
N
N
O
F3C
8c
0.99
0.82
2.10
2.03
1.09
1.07
1.96
4.05
3.89
3.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
2E+07
2E+07
2E+07
2E+07
2E+07
2E+07
2E+07
1E+07
1E+07
1E+07
1E+07
1E+07
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0
-1E+06

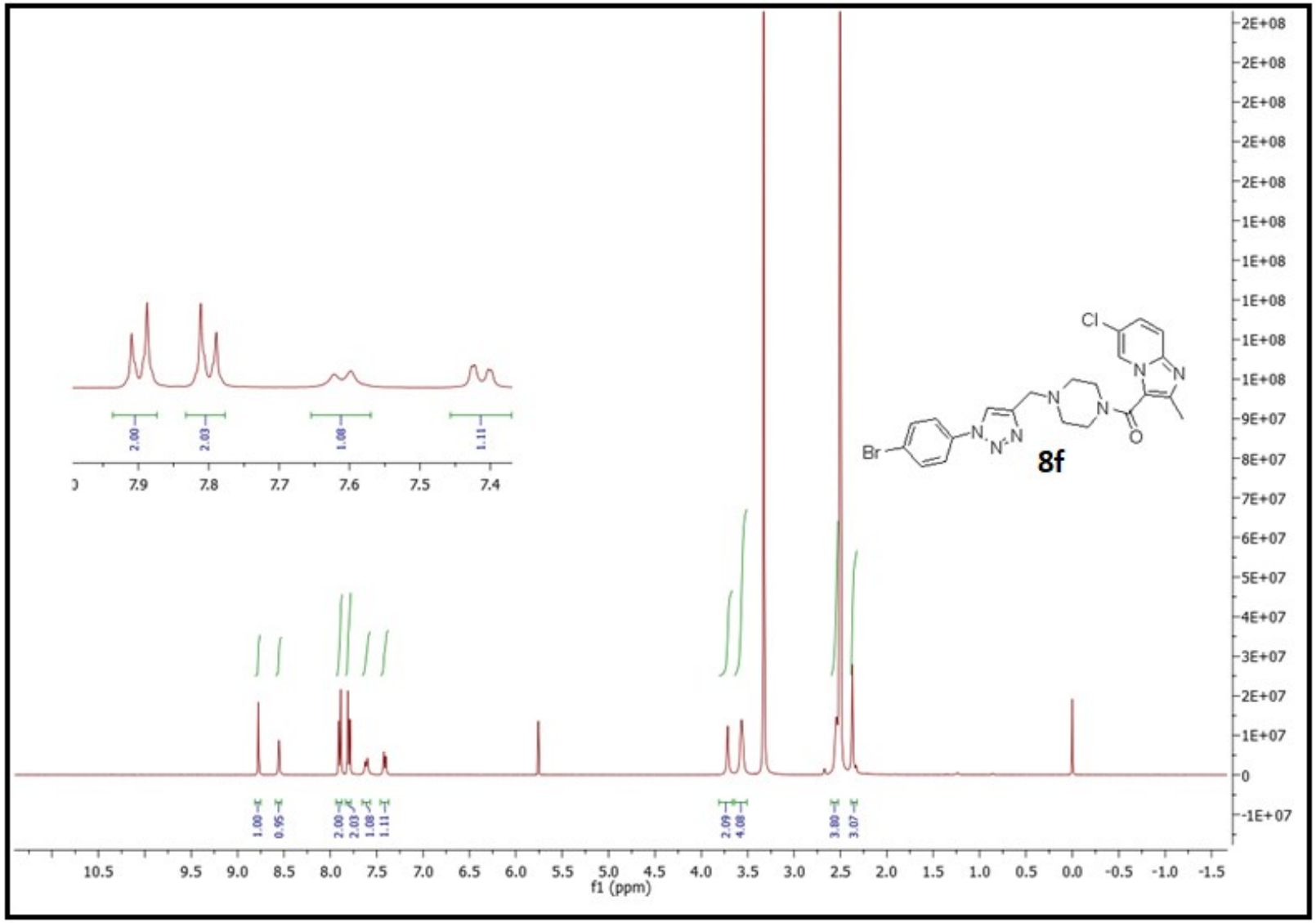
[1]HNMR of 8c


8d
Cl
N
N
N
N
N
N
O
1.99
1.14
3.02
8.0
7.5
f1 (ppm)
1.00
1.04
1.99
1.14
3.02
2.04
3.93
3.88
3.11
3.27
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)
2E+08
2E+08
1E+08
1E+08
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07

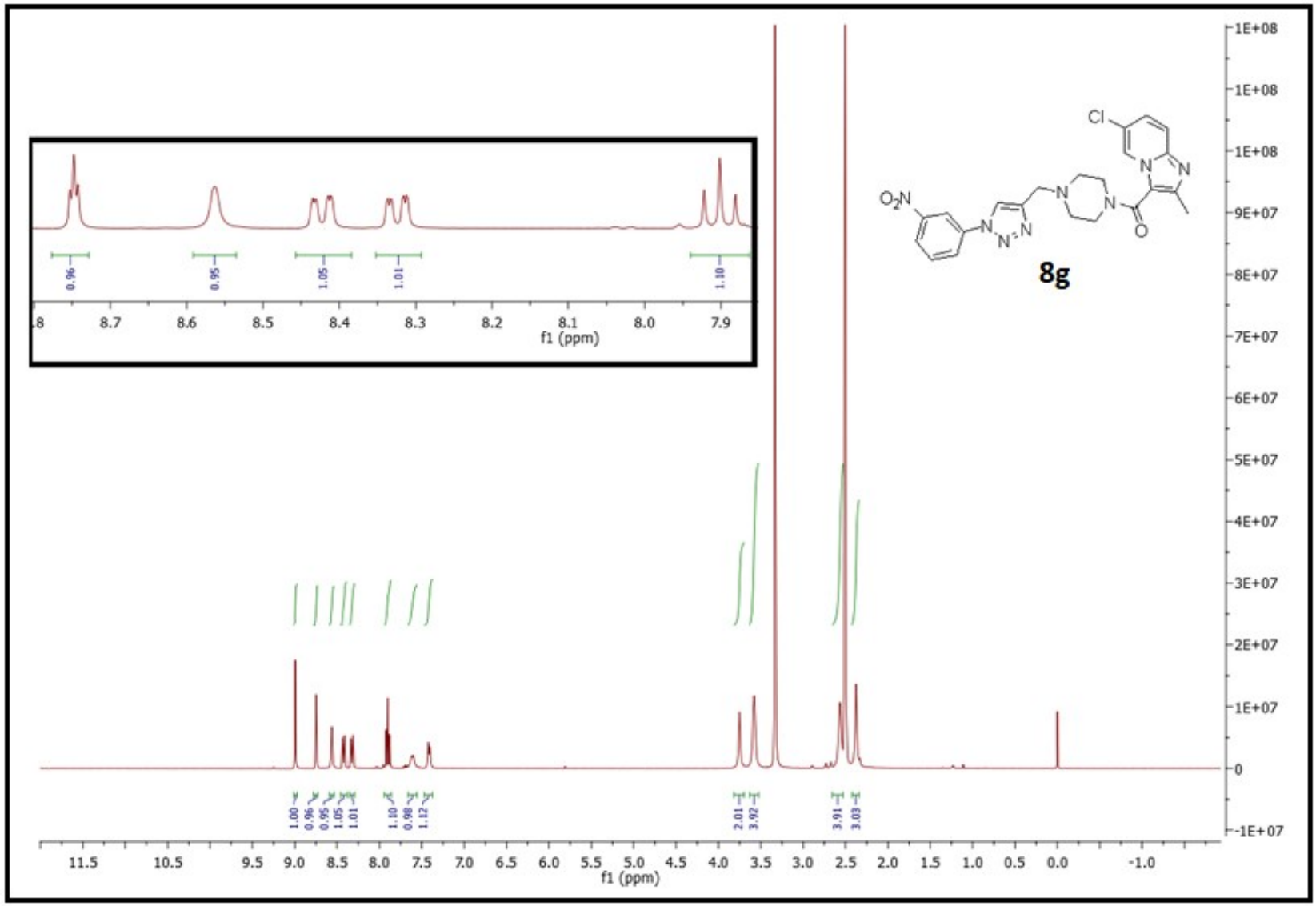
[1]HNMR of 8d



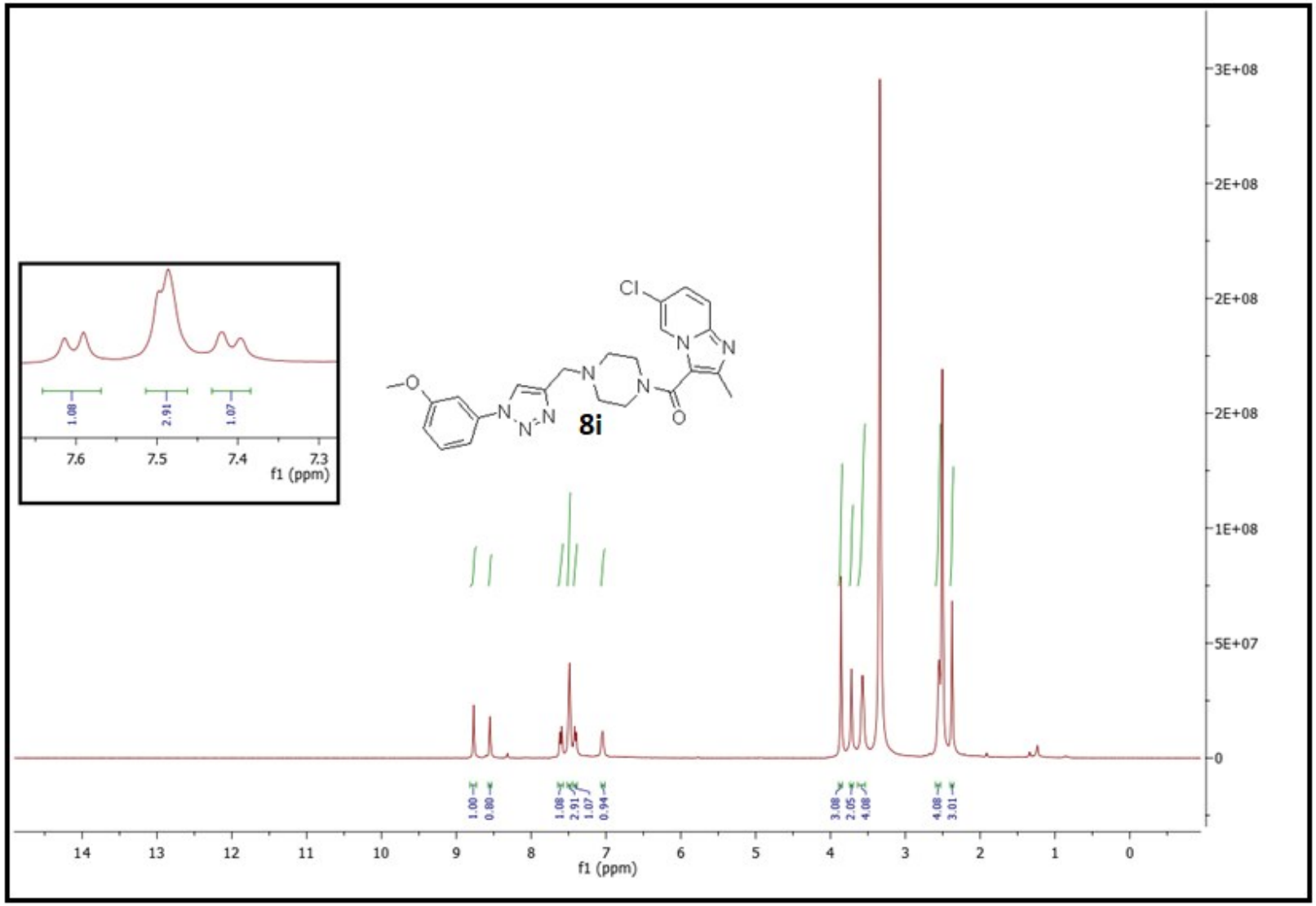
^{1}HNMR of 8e



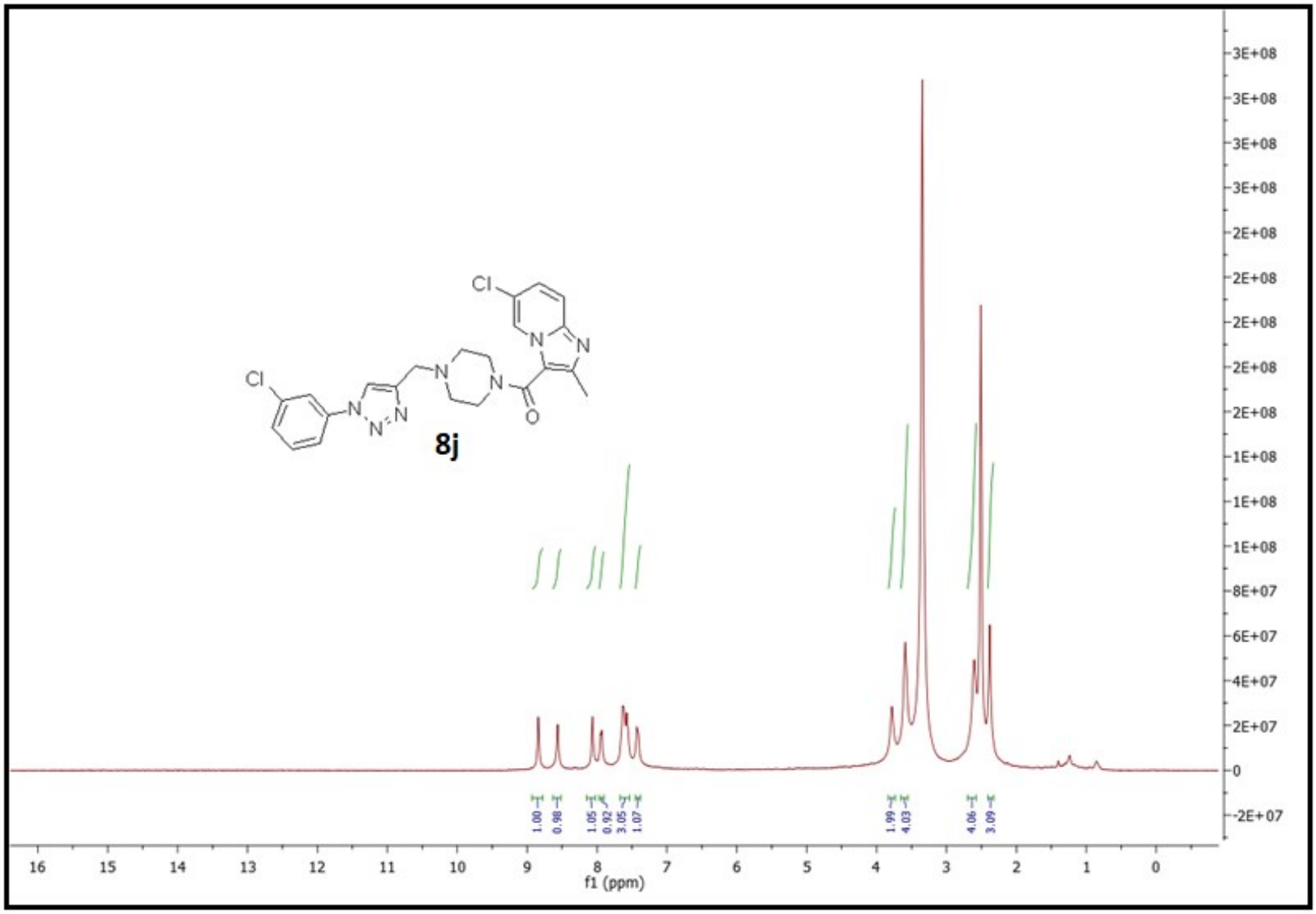
[1]HNMR of 8f



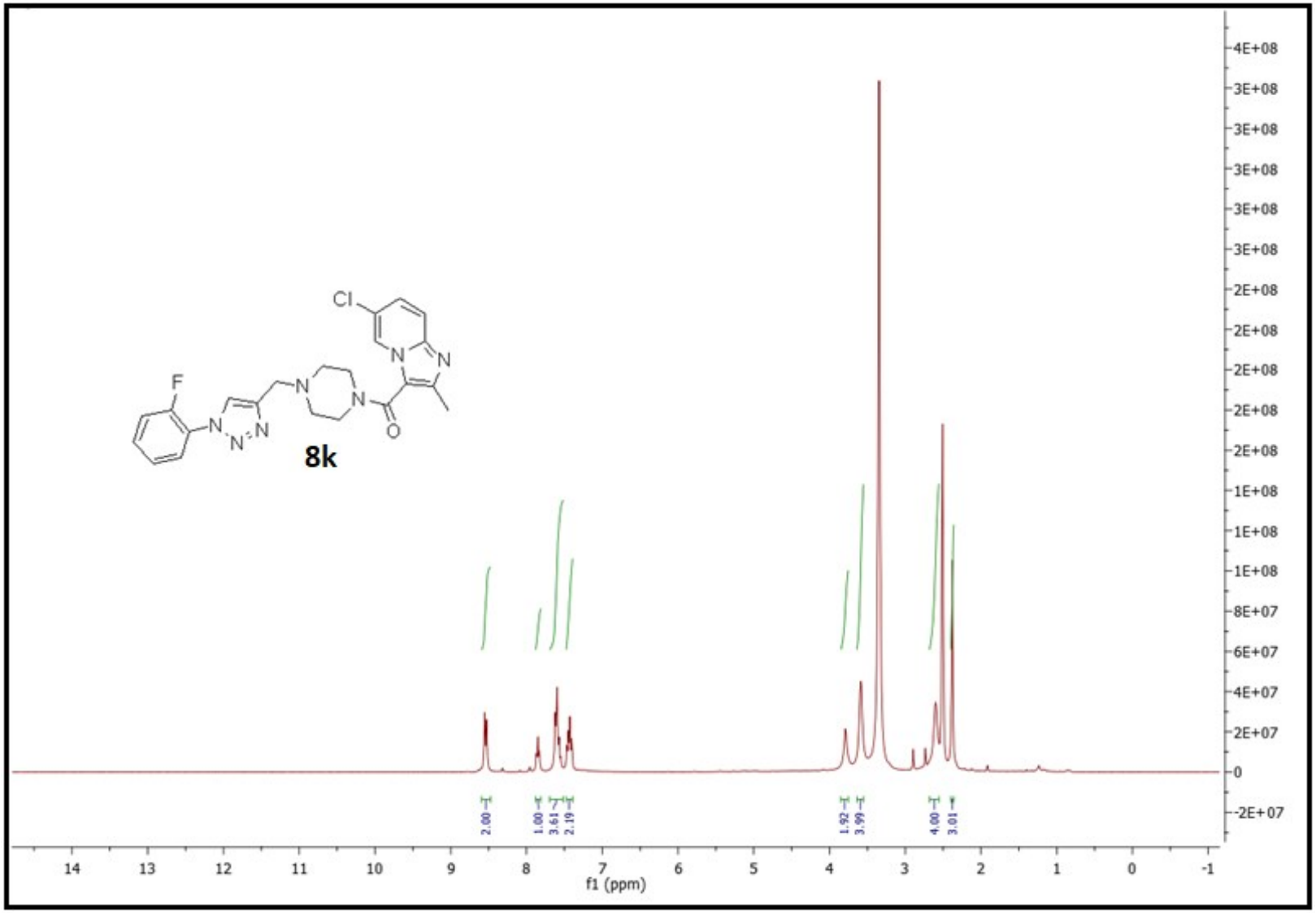
[1]HNMR of 8g


8i
Cl
O
N
N
N
N
N
O
1.00
2.91
1.07
7.6
7.5
7.4
7.3
f1 (ppm)
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
1.00
0.80
1.08
2.91
1.07
0.94
3.08
2.05
4.08
4.08
3.01
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

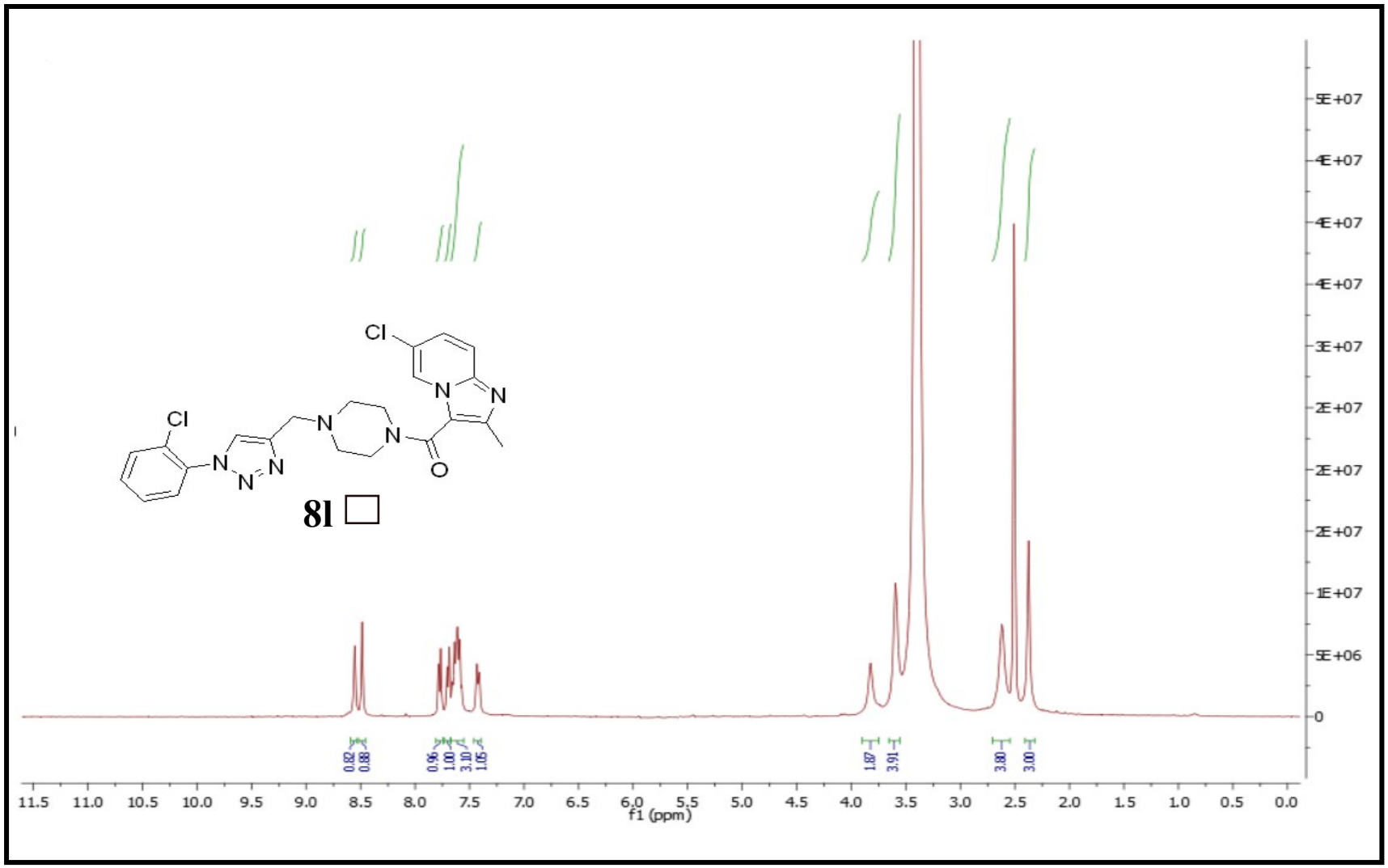
^{1}HNMR of 8i


Cl
Cl
N
N
N
N
N
N
O
8j
3E+08
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
1.00
0.98
1.05
0.92
3.05
1.07
1.99
4.03
4.06
3.09
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

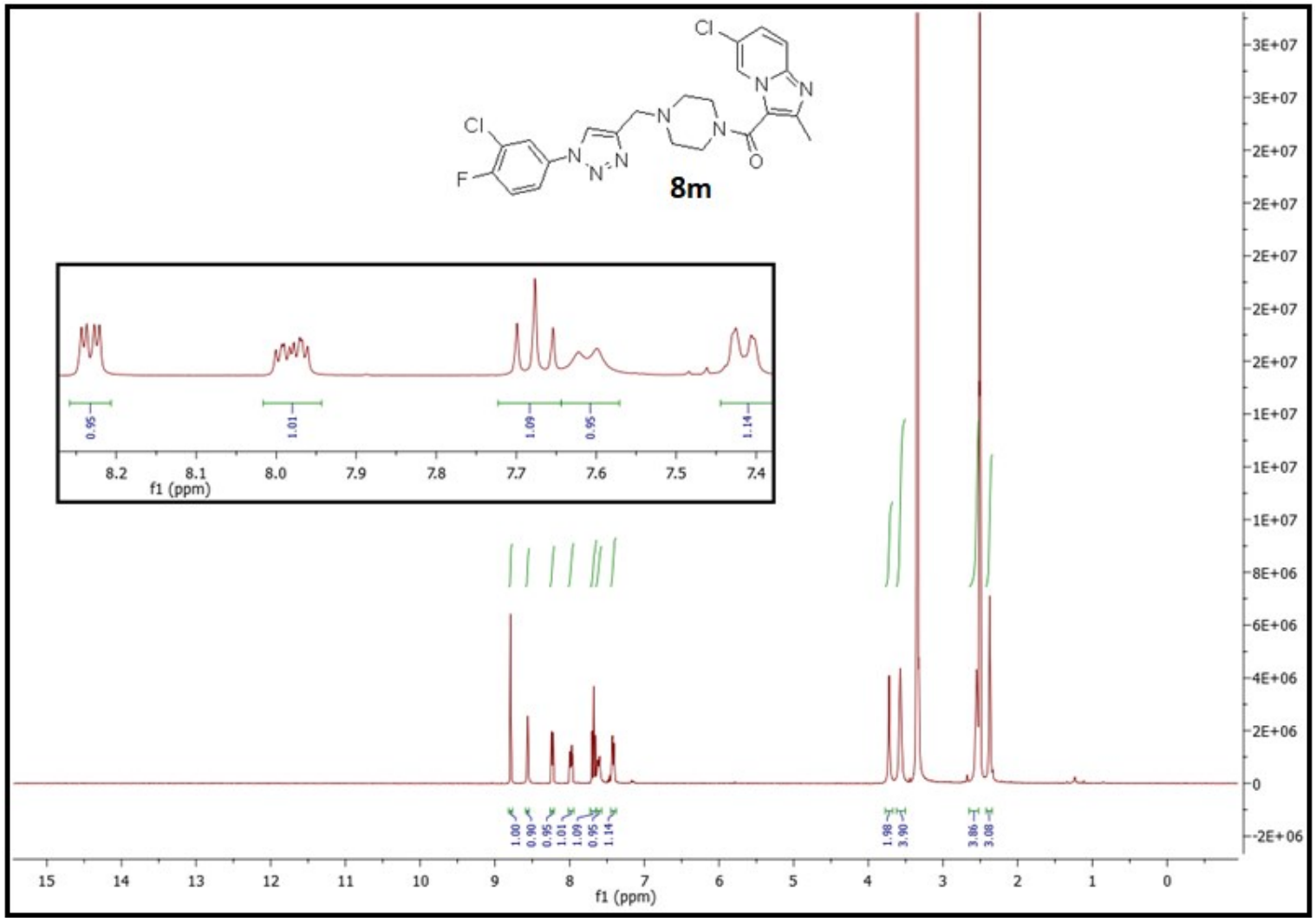
[1]HNMR of 8j


Cl
F
N
N
N
N
N
N
O
8k
4E+08
3E+08
3E+08
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
2.00
1.00
3.61
2.19
1.92
3.99
4.00
3.01
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

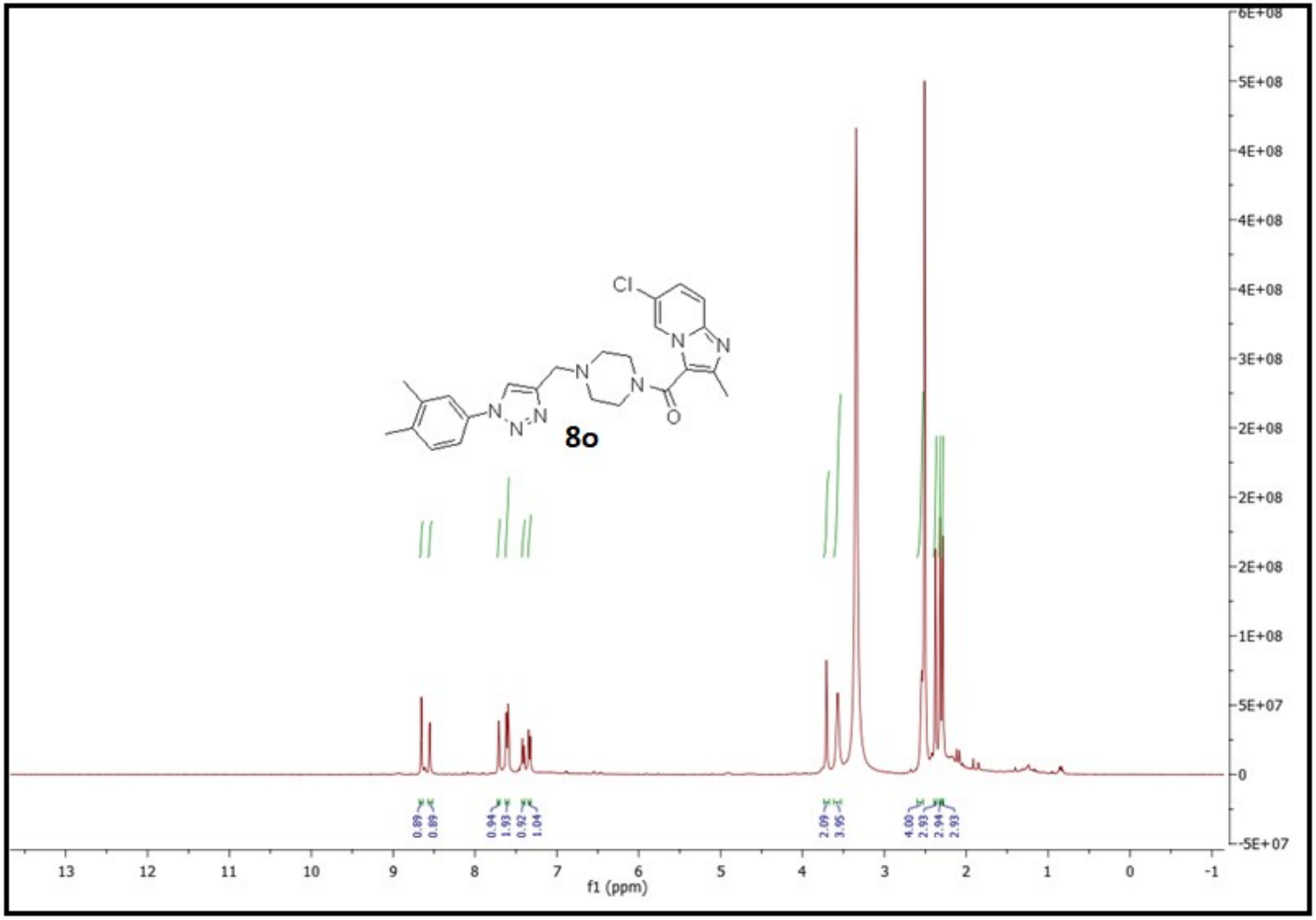
[1]HNMR of 8k


Cl
N
N
N
N
N
N
Cl
O
8l
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
0.82
0.88
0.96
1.00
3.10
1.05
1.87
3.91
3.80
3.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

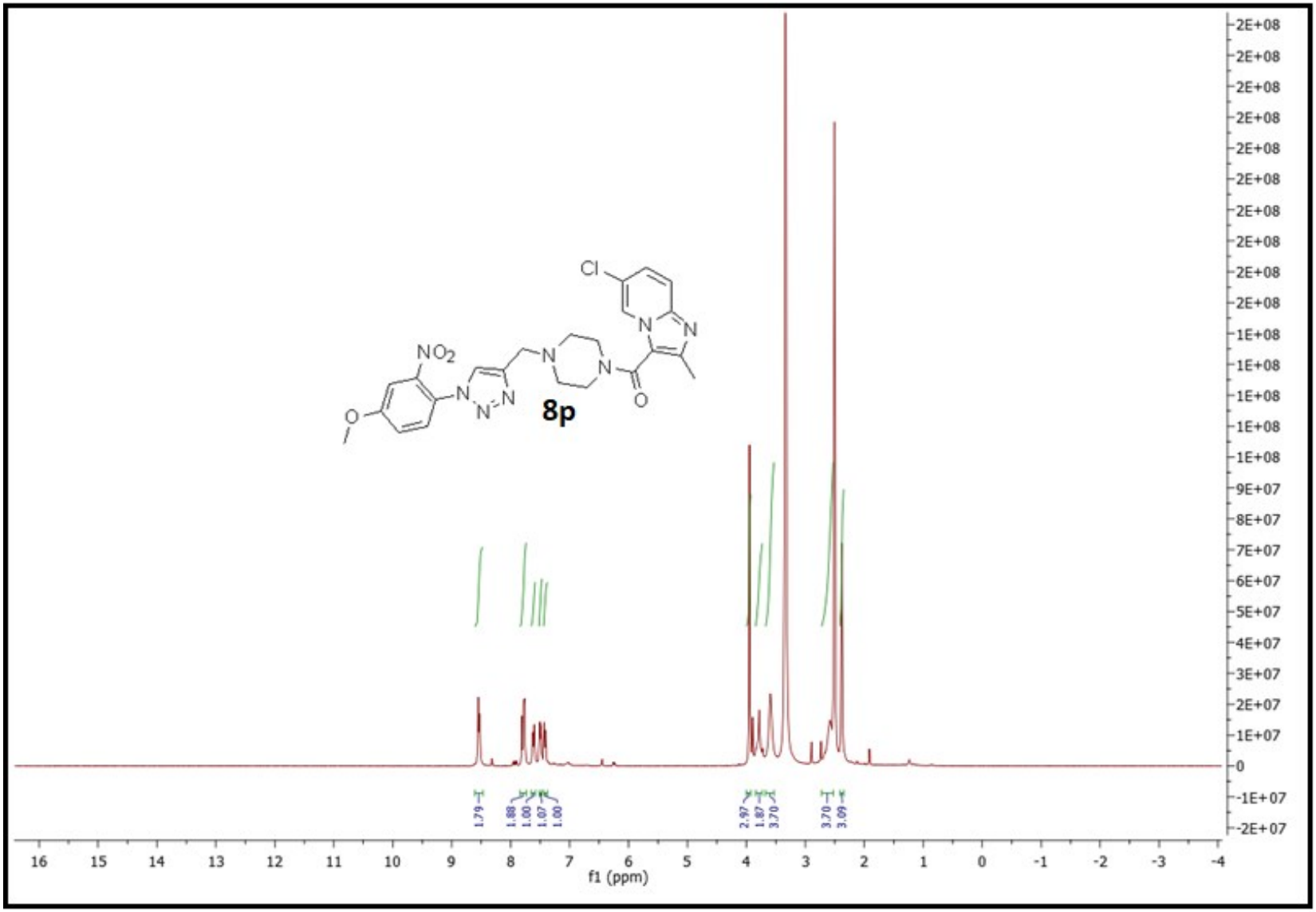
^{1}HNMR of 8l


Cl
Cl
F
N
N
N
N
N
N
O
8m
0.95
1.01
1.09
0.95
1.14
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
f1 (ppm)
1.00
0.90
0.95
1.01
1.09
0.95
1.14
1.98
3.90
3.86
3.08
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)
3E+07
3E+07
2E+07
2E+07
2E+07
2E+07
2E+07
1E+07
1E+07
1E+07
1E+07
8E+06
6E+06
4E+06
2E+06
0
-2E+06

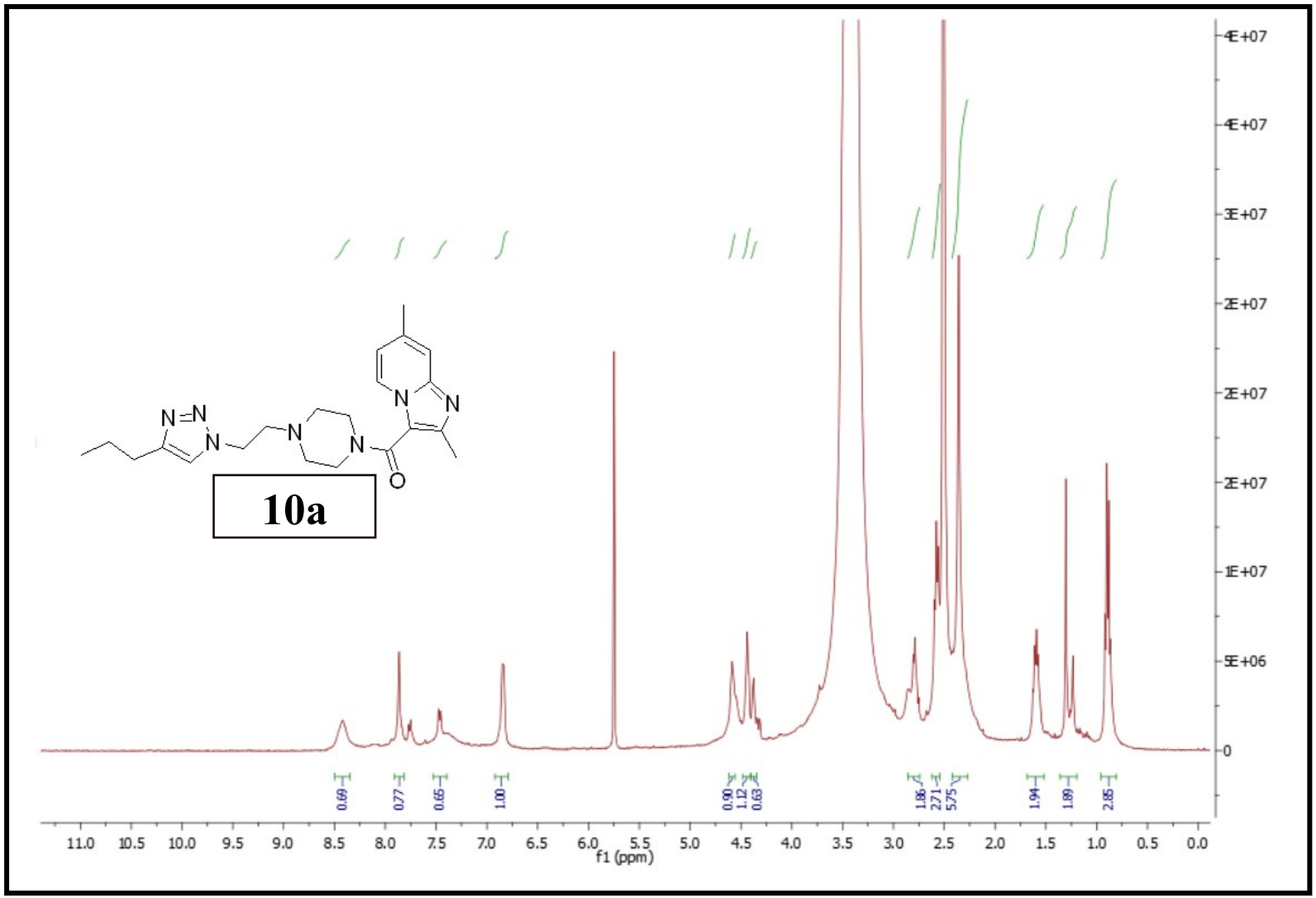
^{1}HNMR of 8m


Cl
N
N
N
N
N
N
O
8o
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
0.89
0.89
0.94
1.93
0.92
1.04
2.09
3.95
4.00
2.93
2.94
2.93
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

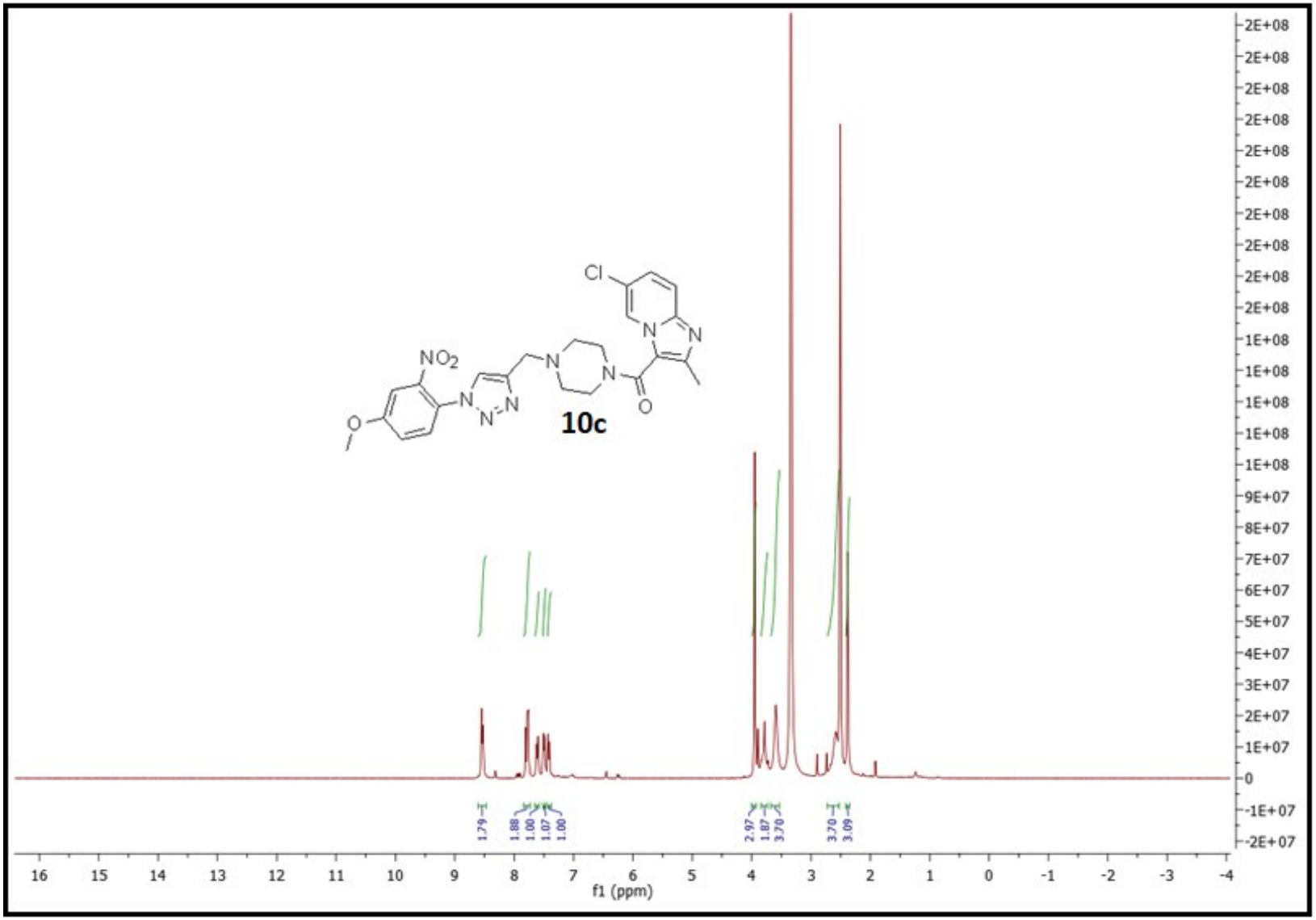
[1]HNMR of 8o


Cl
NO2
O
8p
1.79
1.88
1.00
1.07
1.00
2.97
1.87
3.70
3.70
3.09
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)
2E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
-2E+07

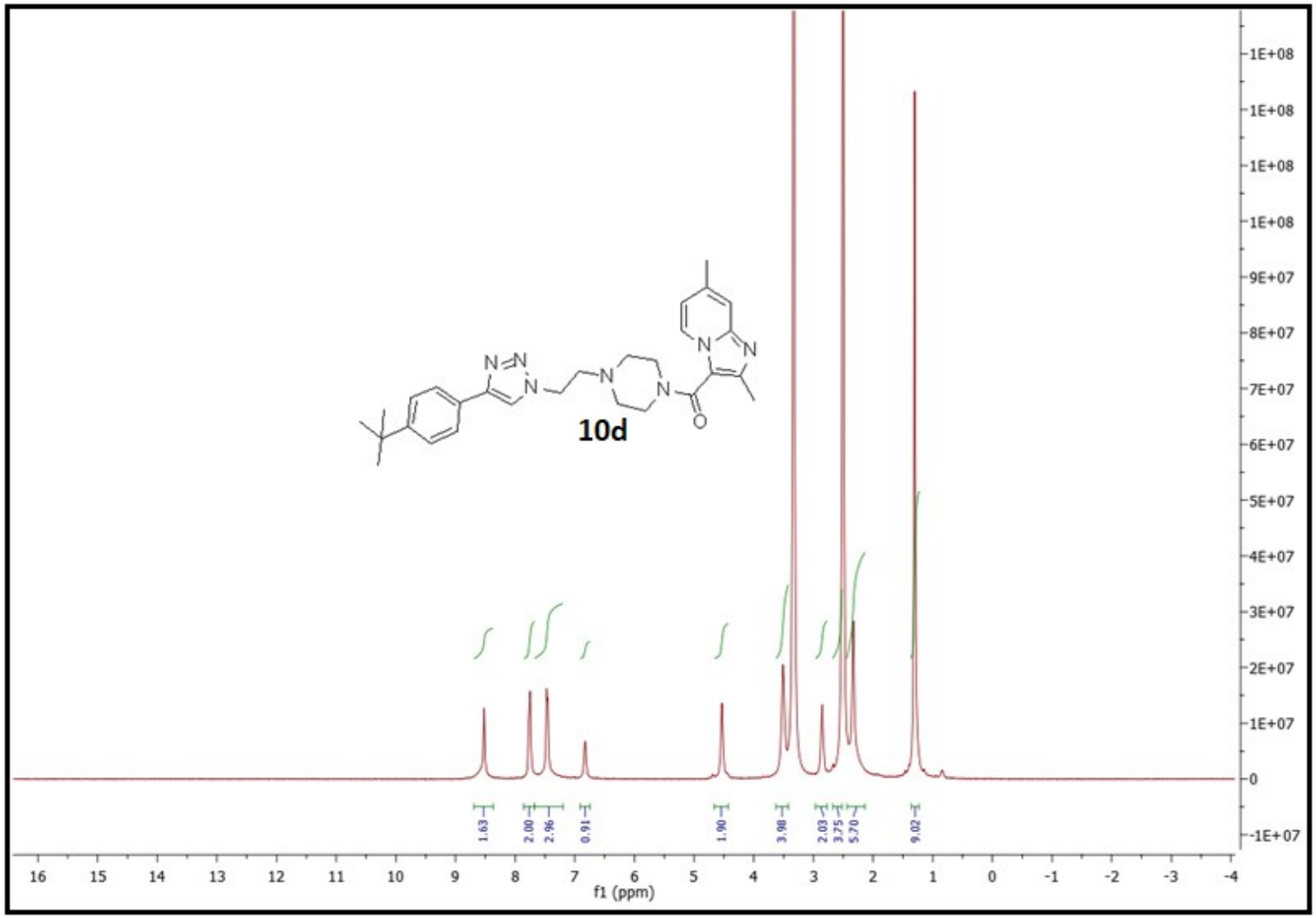
[1]HNMR of 8p


10a
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
0.69
0.77
0.65
1.00
0.90
1.12
0.63
1.86
2.71
5.75
1.94
1.89
2.85
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

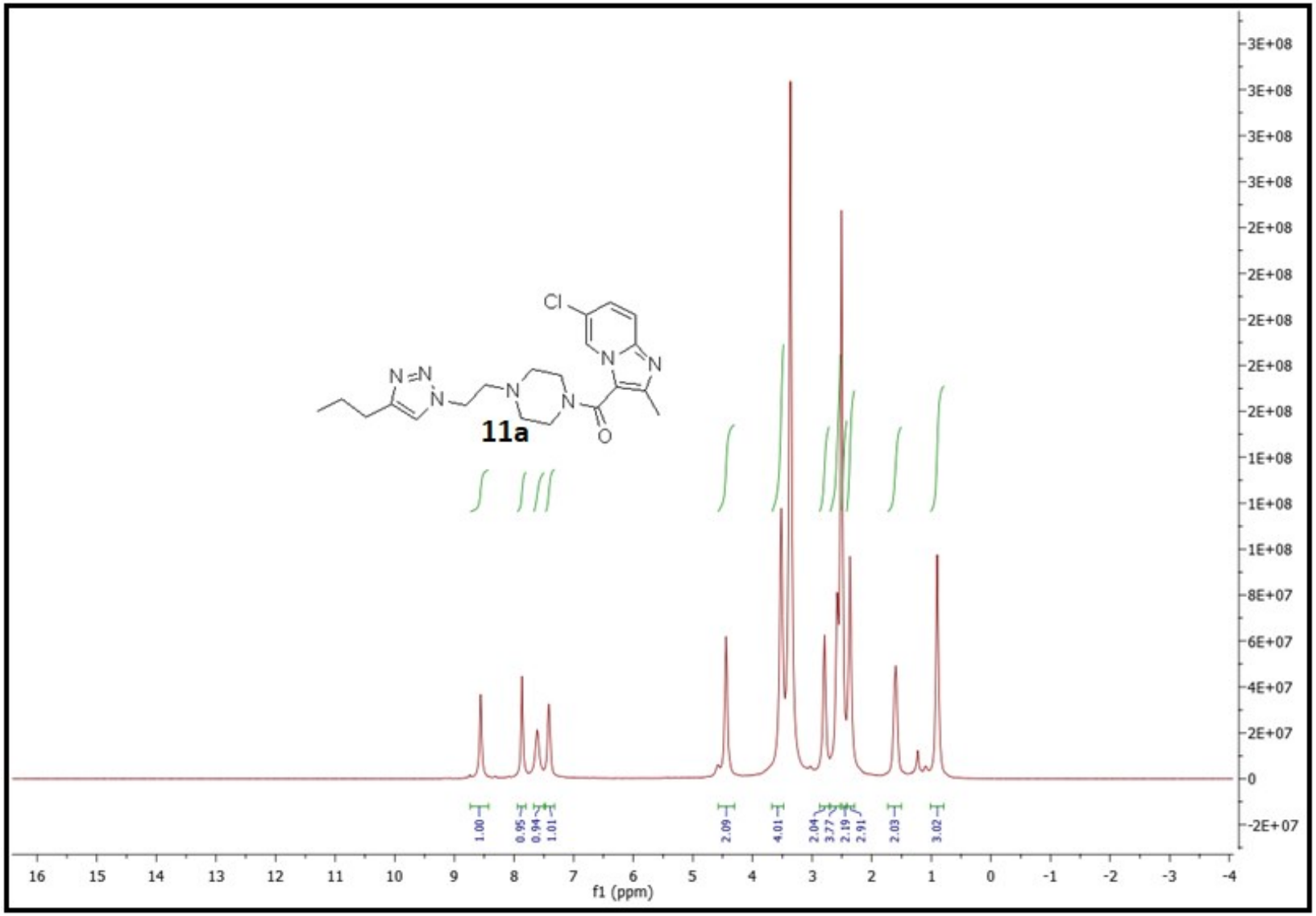
[1]HNMR of 10a



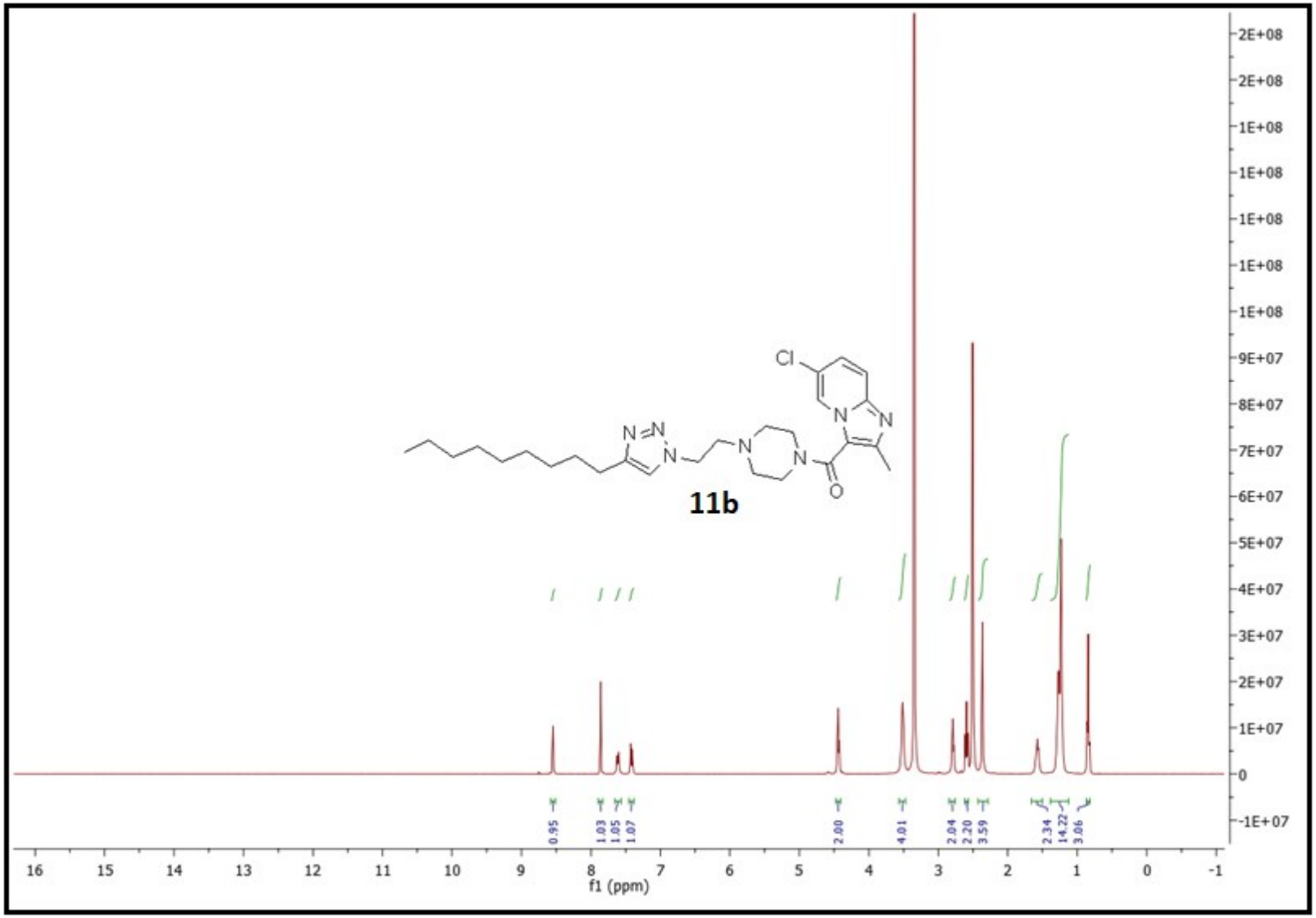
[1]HNMR of 10c


10d
1E+08
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
1.63
2.00
2.96
0.91
1.90
3.98
2.03
3.75
5.70
9.02
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

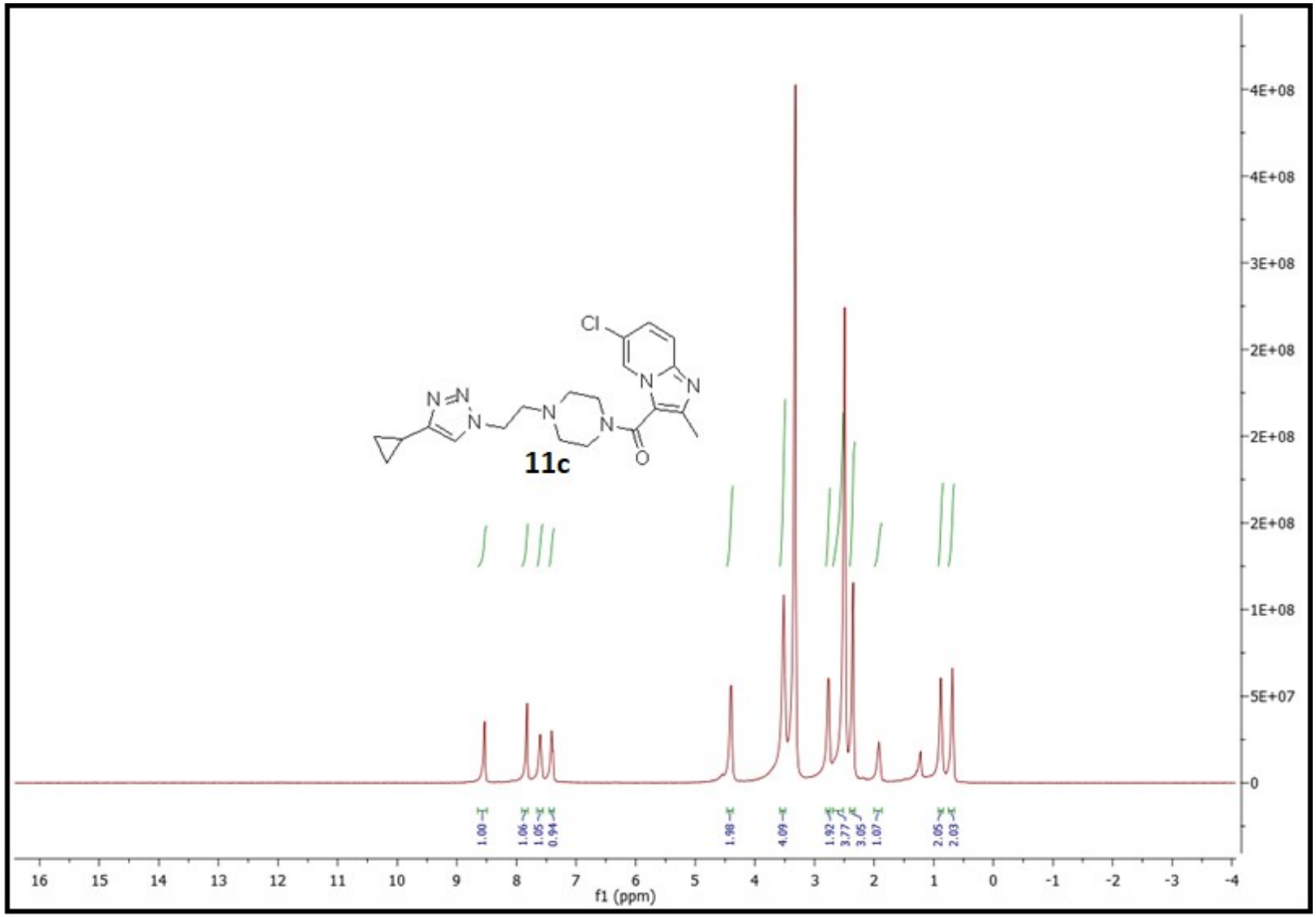
[1]HNMR of 10d


11a
Cl
N
N
N
N
N
O
1.00
0.95
0.94
1.01
2.09
4.01
2.04
3.77
2.19
2.91
2.03
3.02
f1 (ppm)

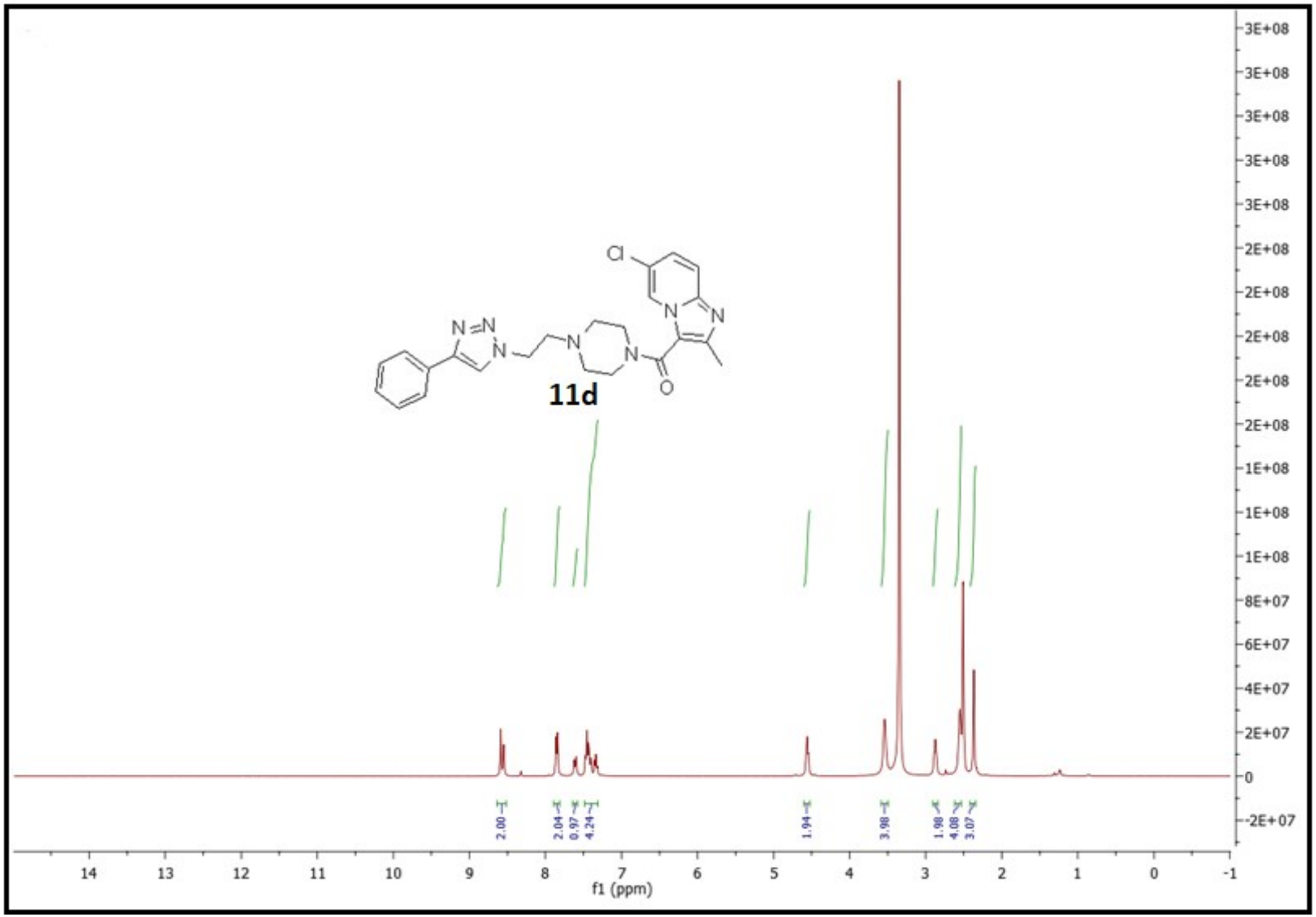
[1]HNMR of 11a


11b
Cl
N
N
N
N
N
N
O
0.95
1.03
1.05
1.07
2.00
4.01
2.04
2.20
3.59
2.34
14.22
3.06
f1 (ppm)

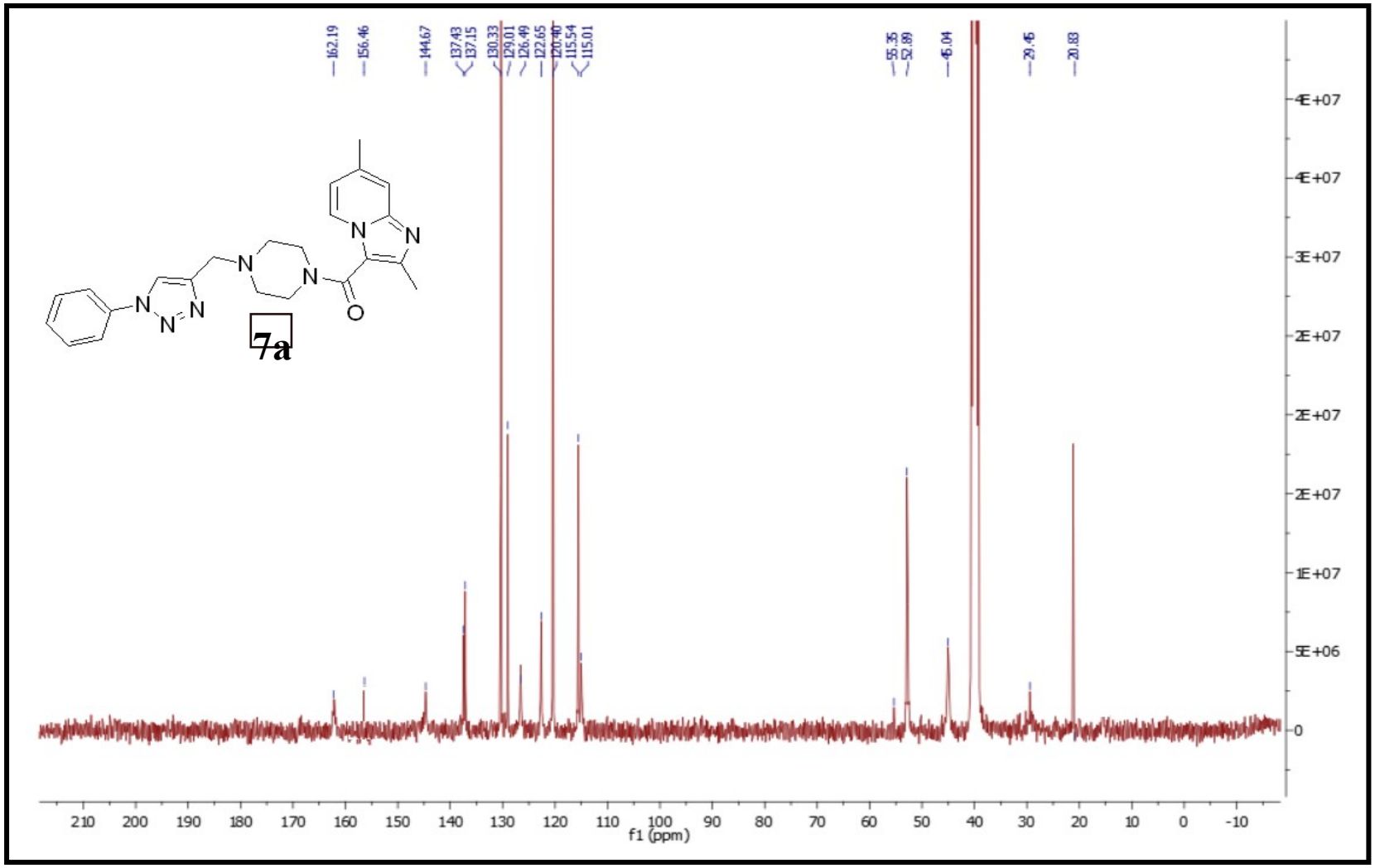
[1]HNMR of 11b


Cl
N
N
N
N
N
N
O
11c
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
1.00
1.06
1.05
0.94
1.98
4.09
1.92
3.77
3.05
1.07
2.05
2.03
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
f1 (ppm)

[1]HNMR of 11c

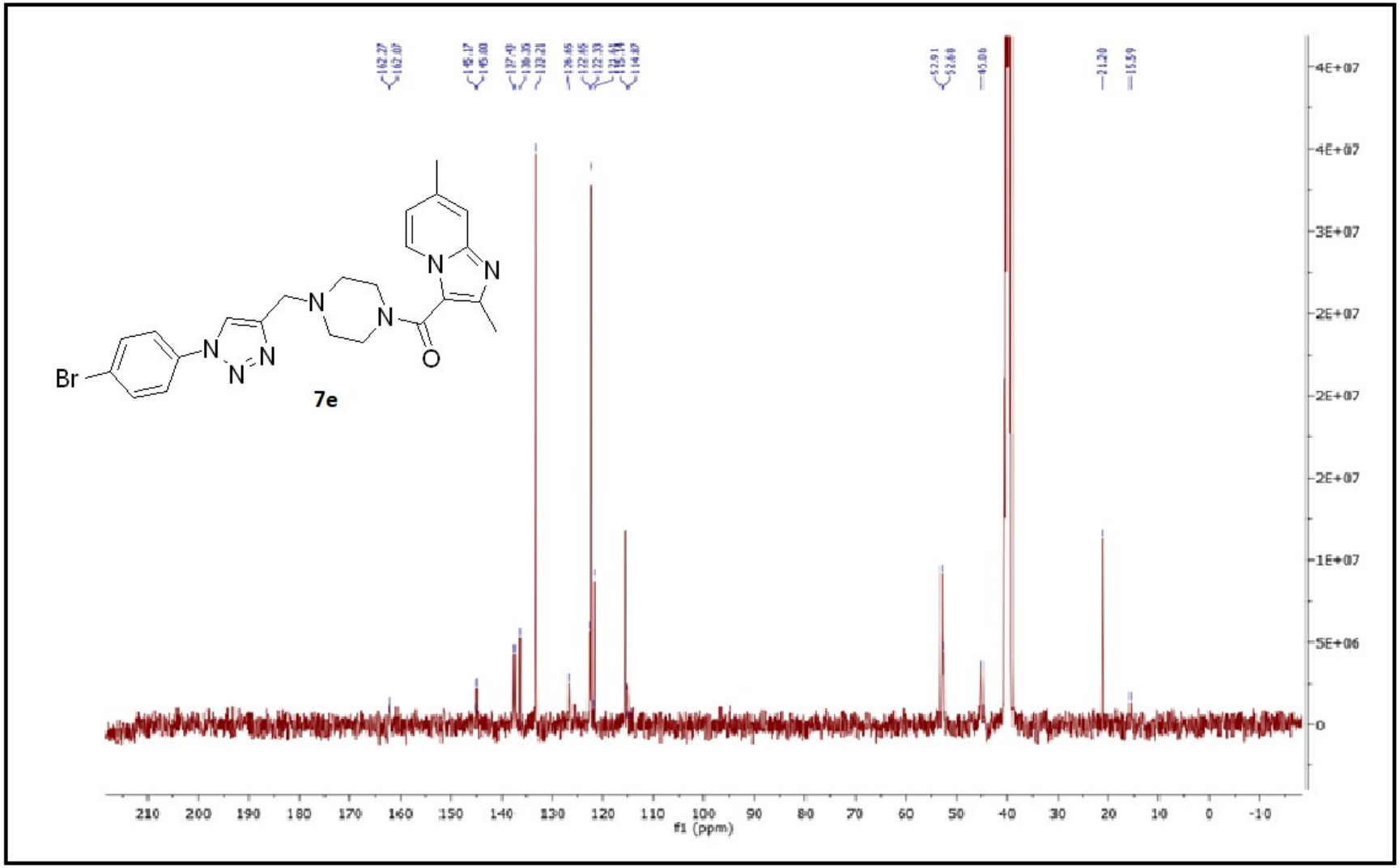


[1]HNMR of 11d

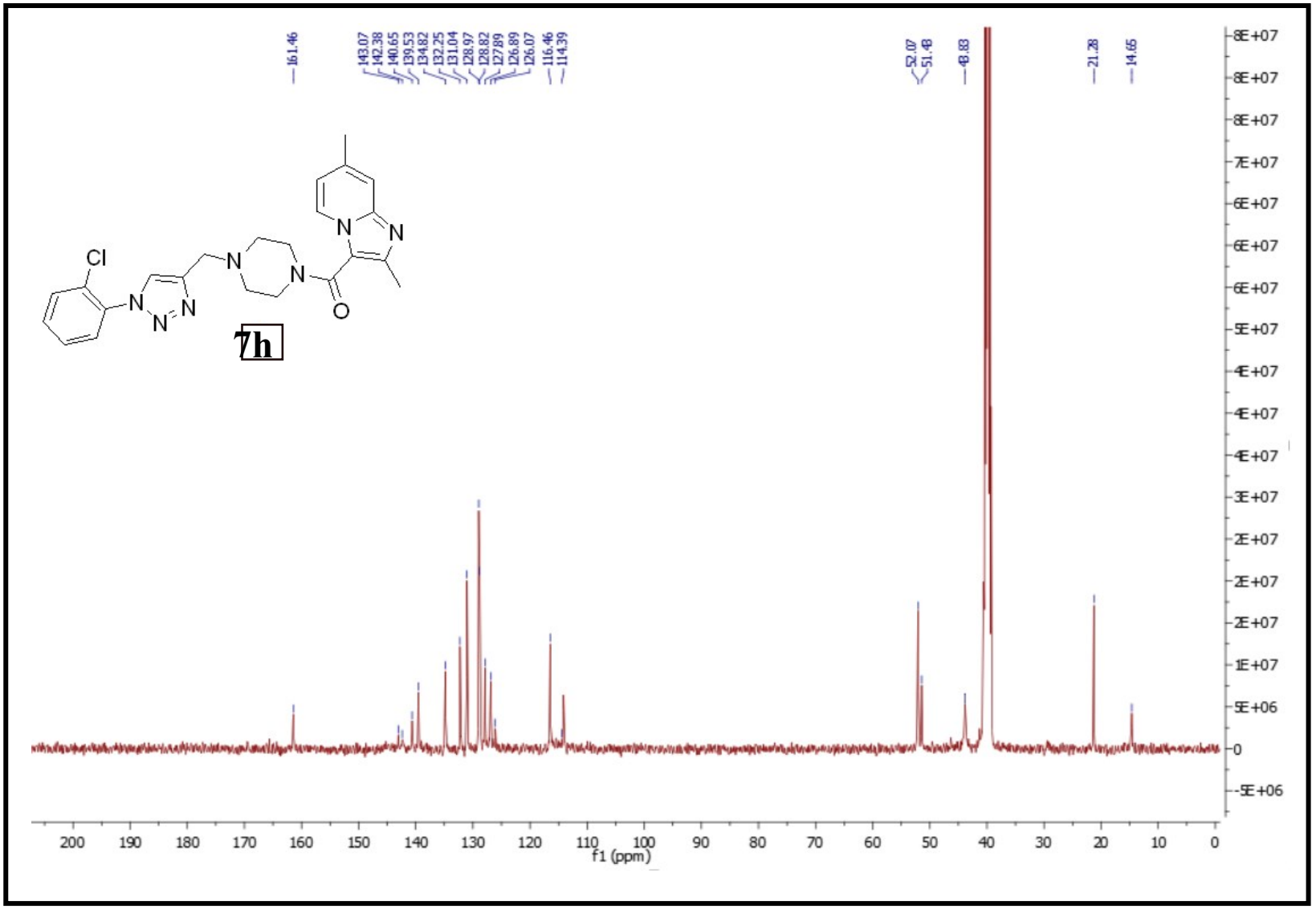


7. ^{13}CNMR spectras of intermediate compounds and final compounds:

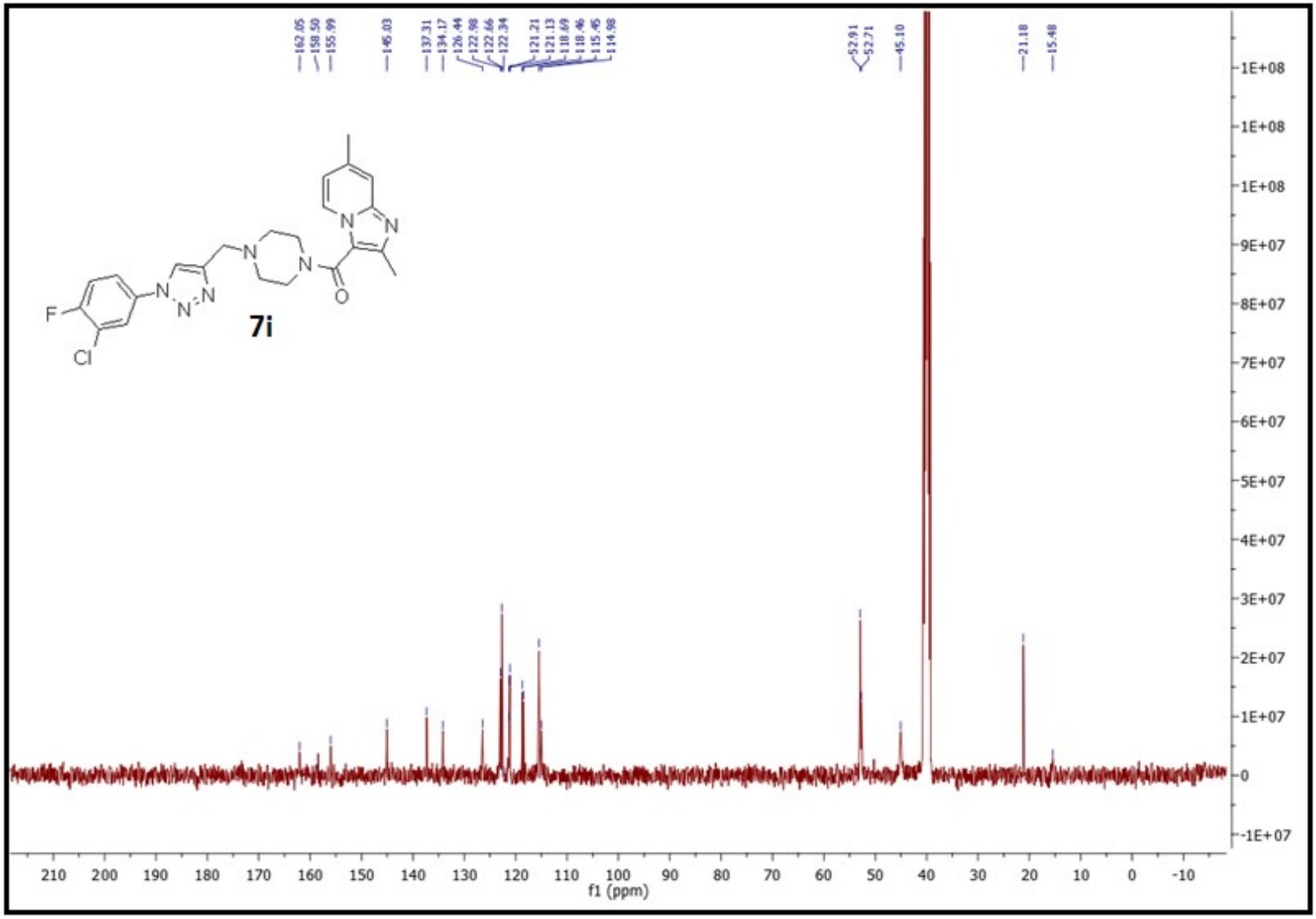
^{13}CNMR of 7a



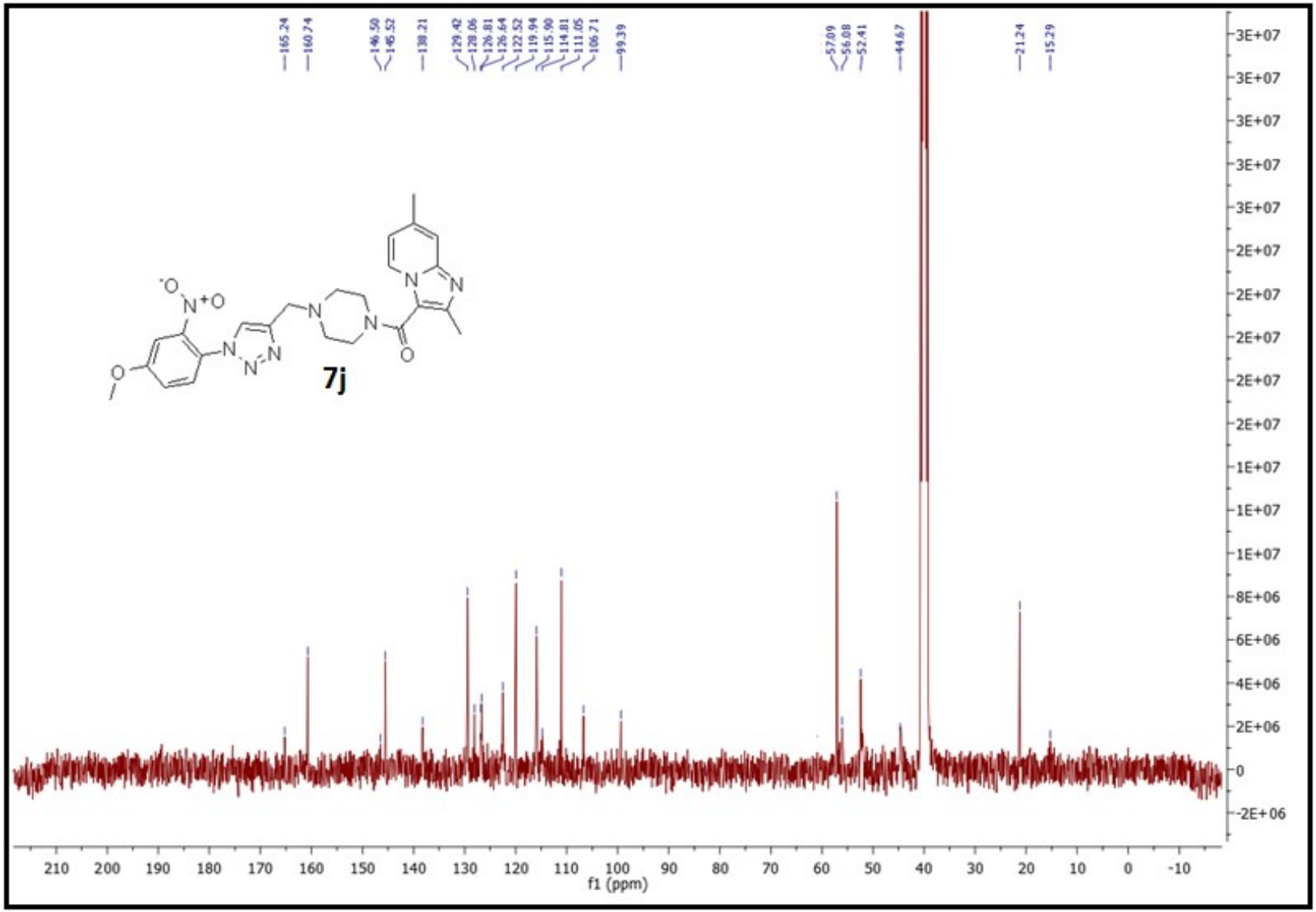
^{13}CNMR of 7e


161.46
143.07
142.38
140.65
139.53
134.82
132.25
131.04
128.97
128.82
127.89
126.89
126.07
116.46
114.39
52.07
51.48
48.83
21.28
14.65
Cl
N
N
N
N
N
N
N
O
7h
8E+07
8E+07
8E+07
7E+07
6E+07
6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

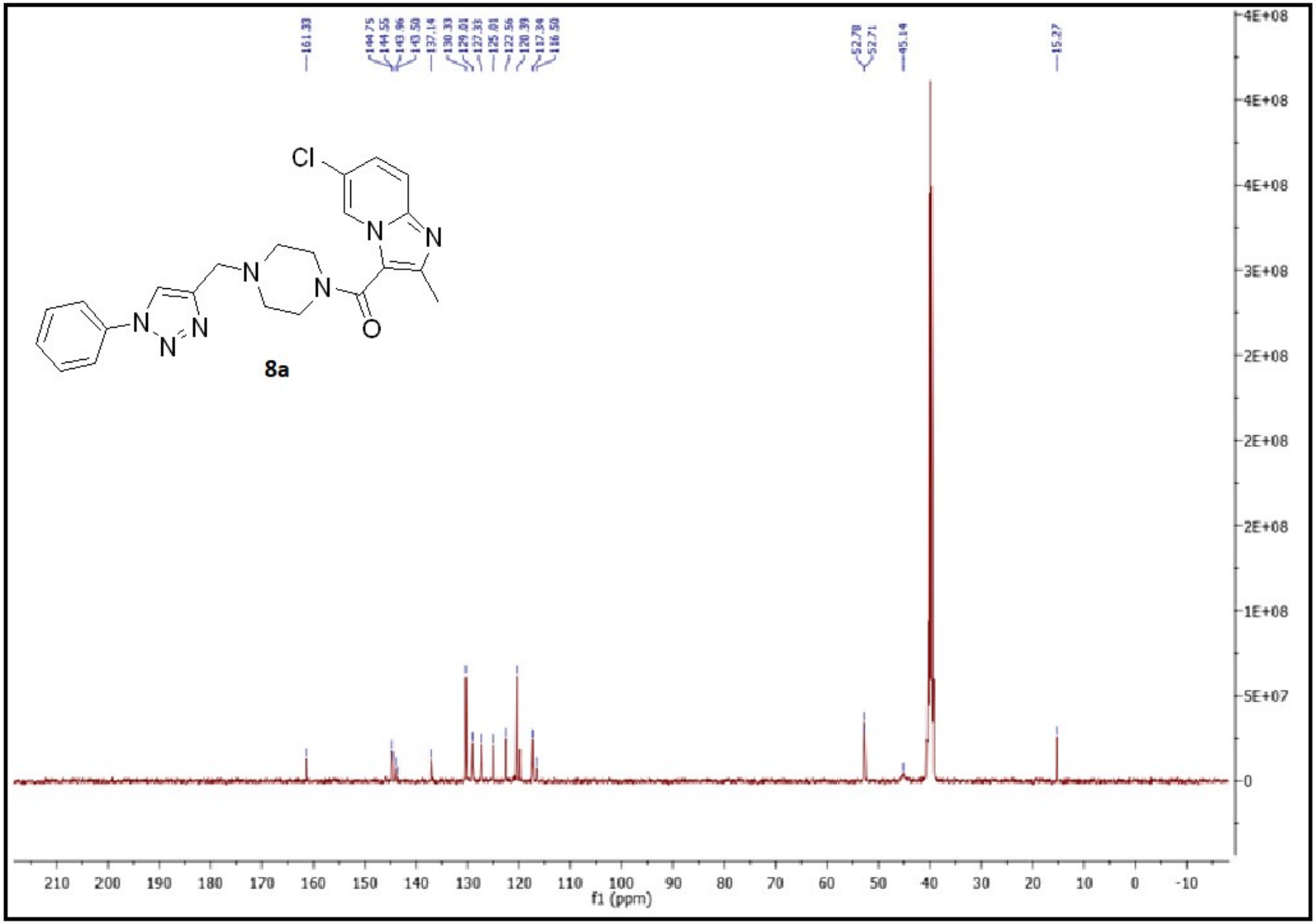
^{13}CNMR of 7h


162.05
158.50
155.99
145.03
137.31
134.17
126.44
122.98
122.66
122.34
121.21
121.13
118.69
118.46
115.45
114.98
52.91
52.71
45.10
21.18
15.48
7i
F
Cl
N
N
N
N
N
O
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

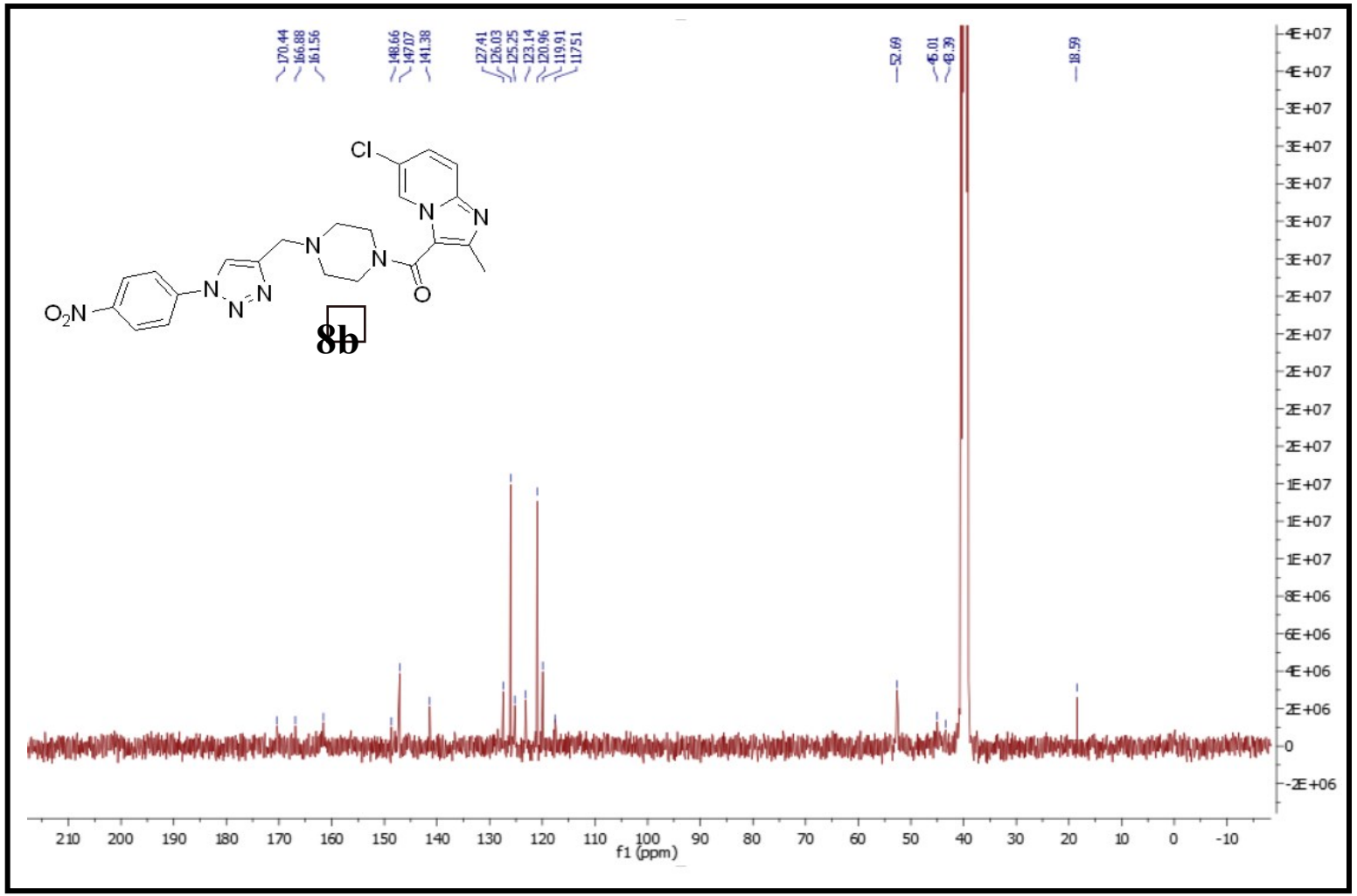
^{13}CNMR of 7i


165.24
160.74
146.50
145.52
138.21
129.42
128.06
126.81
126.64
122.52
119.94
115.90
114.81
111.05
106.71
99.39
57.09
56.08
52.41
44.67
21.24
15.29
7j
3E+07
2E+07
1E+07
8E+06
6E+06
4E+06
2E+06
0
-2E+06
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

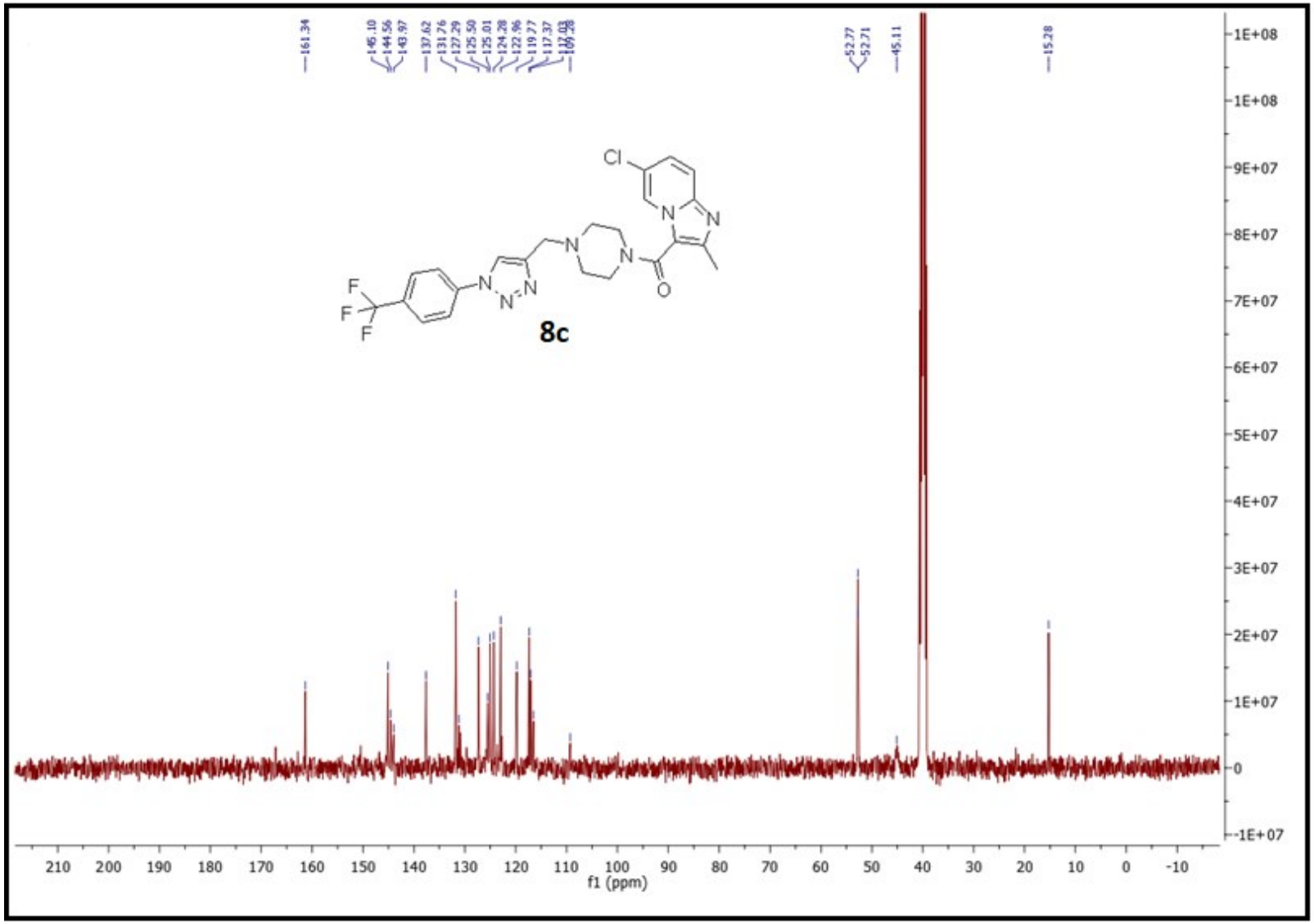
^{13}CNMR of 7j


161.33
144.75
144.55
143.96
143.50
137.14
130.33
129.01
127.35
125.01
122.56
120.39
117.84
116.50
52.78
52.71
45.14
15.27
Cl
N
N
N
N
N
N
O
8a
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

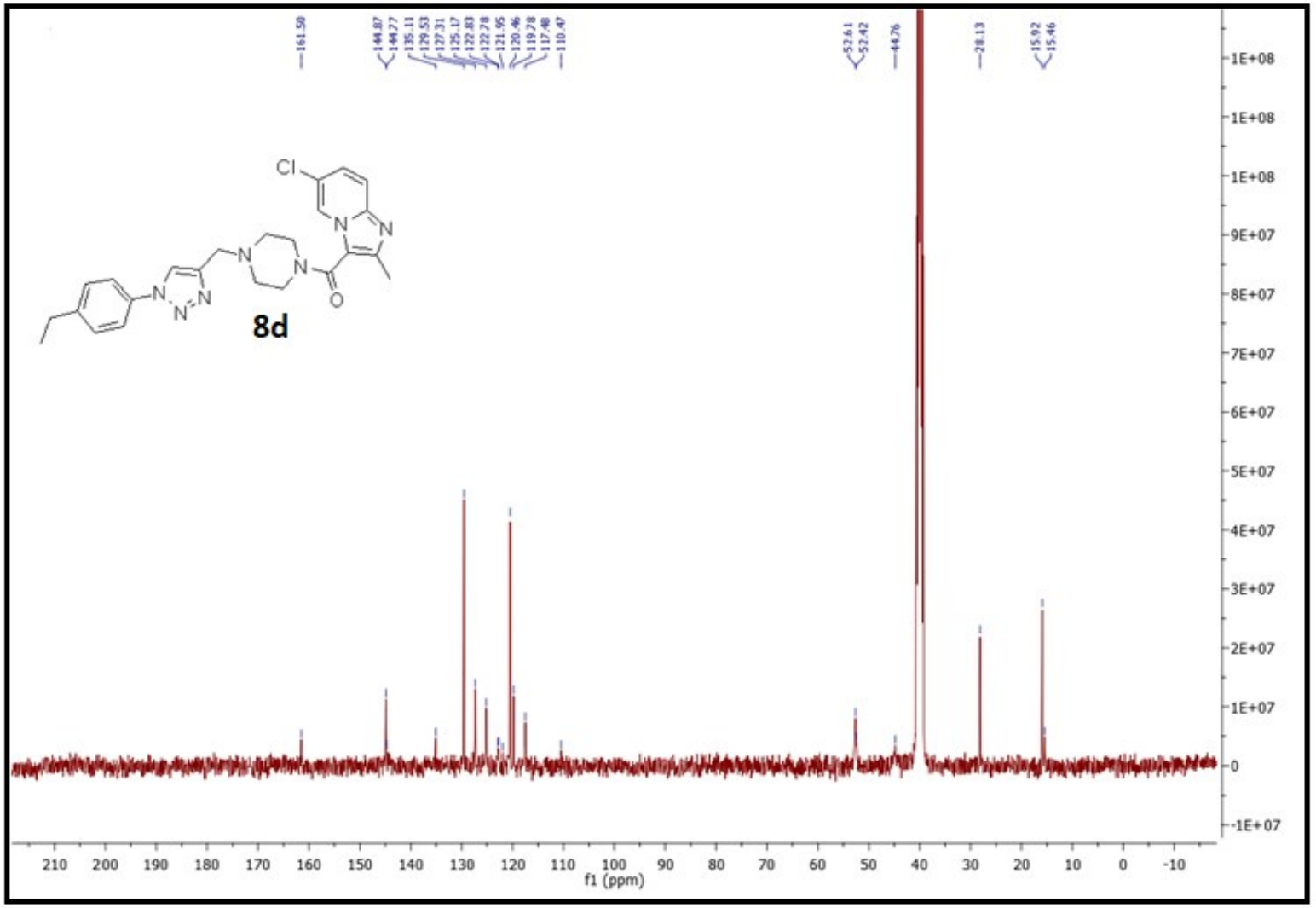
^{13}CNMR of 8a


170.44
166.88
161.56
148.66
147.07
141.38
127.41
126.03
125.25
123.14
120.96
119.91
117.51
52.69
46.01
43.39
18.59
Cl
N
N
N
N
N
N
O
O2N
8b
4E+07
4E+07
3E+07
3E+07
3E+07
3E+07
3E+07
2E+07
2E+07
2E+07
2E+07
2E+07
2E+07
1E+07
1E+07
1E+07
8E+06
6E+06
4E+06
2E+06
0
-2E+06
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

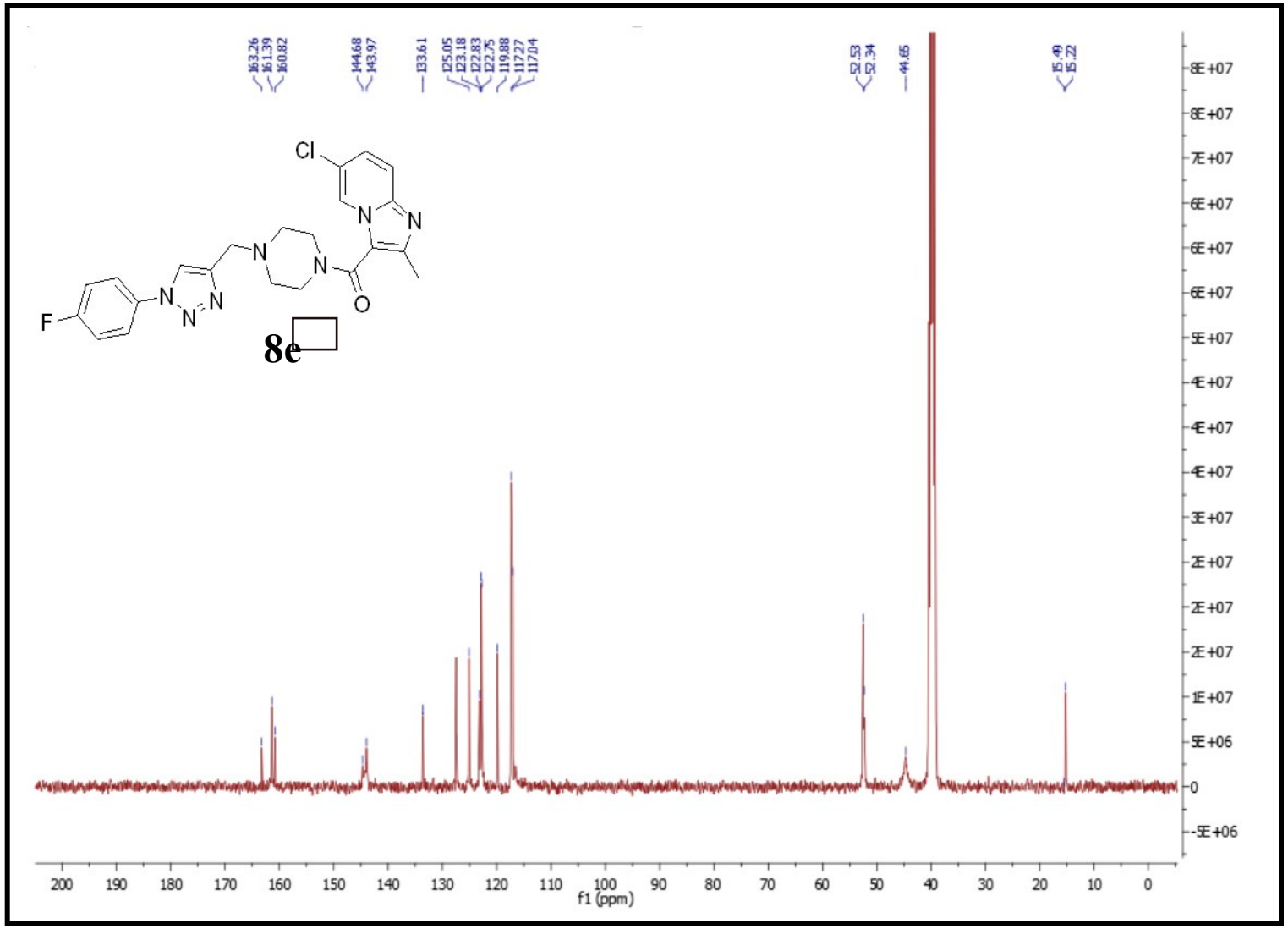
^{13}CNMR of 8b


161.34
145.10
144.56
143.97
137.62
131.76
127.29
125.50
125.01
124.28
122.96
119.77
117.37
52.77
52.71
45.11
15.28
Cl
N
N
N
N
N
O
F
F
F
8c
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

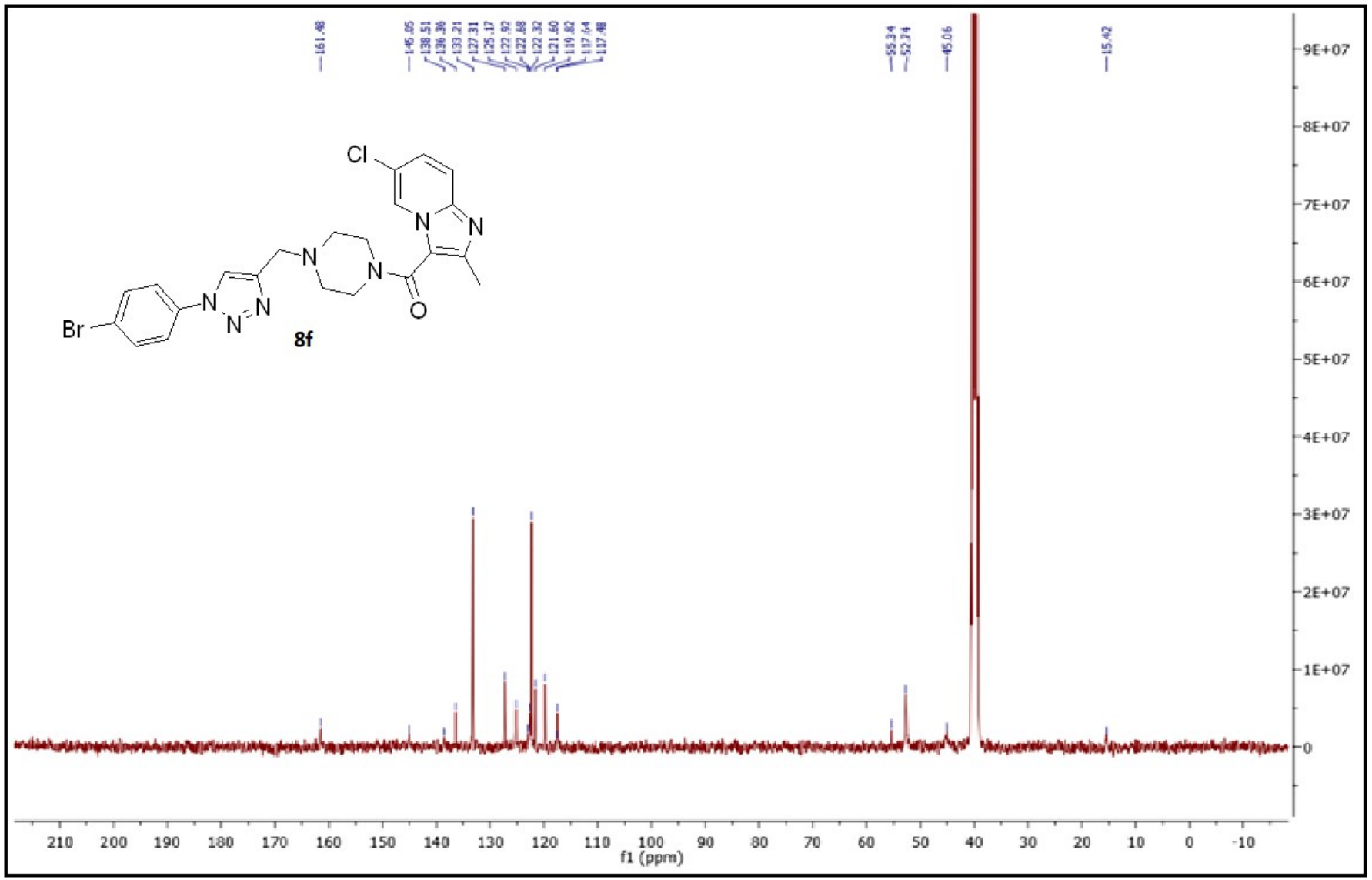
^{13}CNMR of 8c


161.50
144.87
144.77
135.11
129.53
127.31
125.17
122.83
122.78
121.95
120.46
119.78
117.48
110.47
52.61
52.42
44.76
28.13
15.92
15.46
Cl
N
N
N
N
N
N
O
8d
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

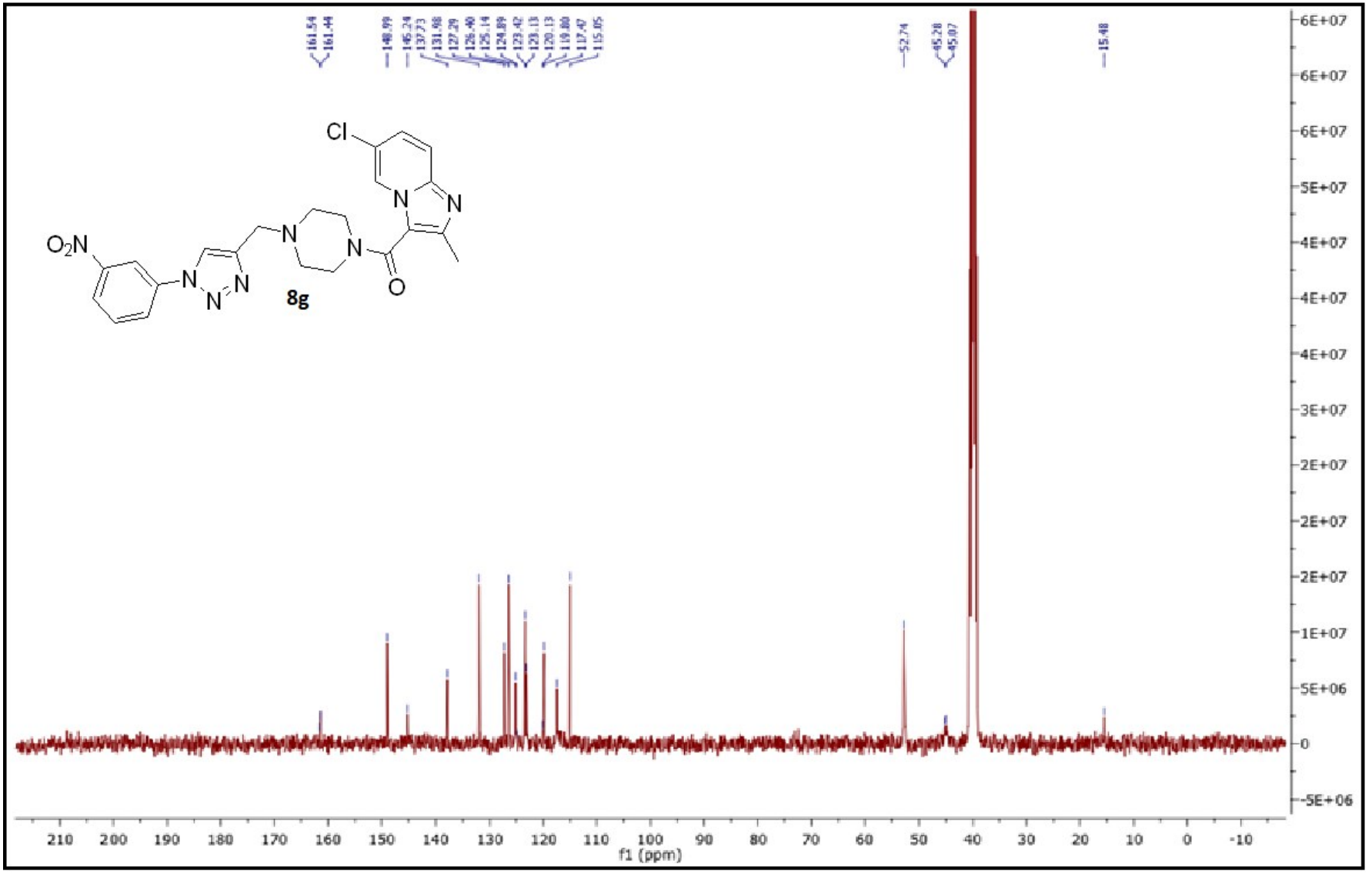
^{13}CNMR of 8d


163.26
161.39
160.82
144.68
143.97
133.61
125.05
123.18
122.83
122.75
119.88
117.27
117.04
52.53
52.34
44.65
15.49
15.22
Cl
N
N
N
N
N
N
O
F
8e
8E+07
8E+07
7E+07
6E+07
6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

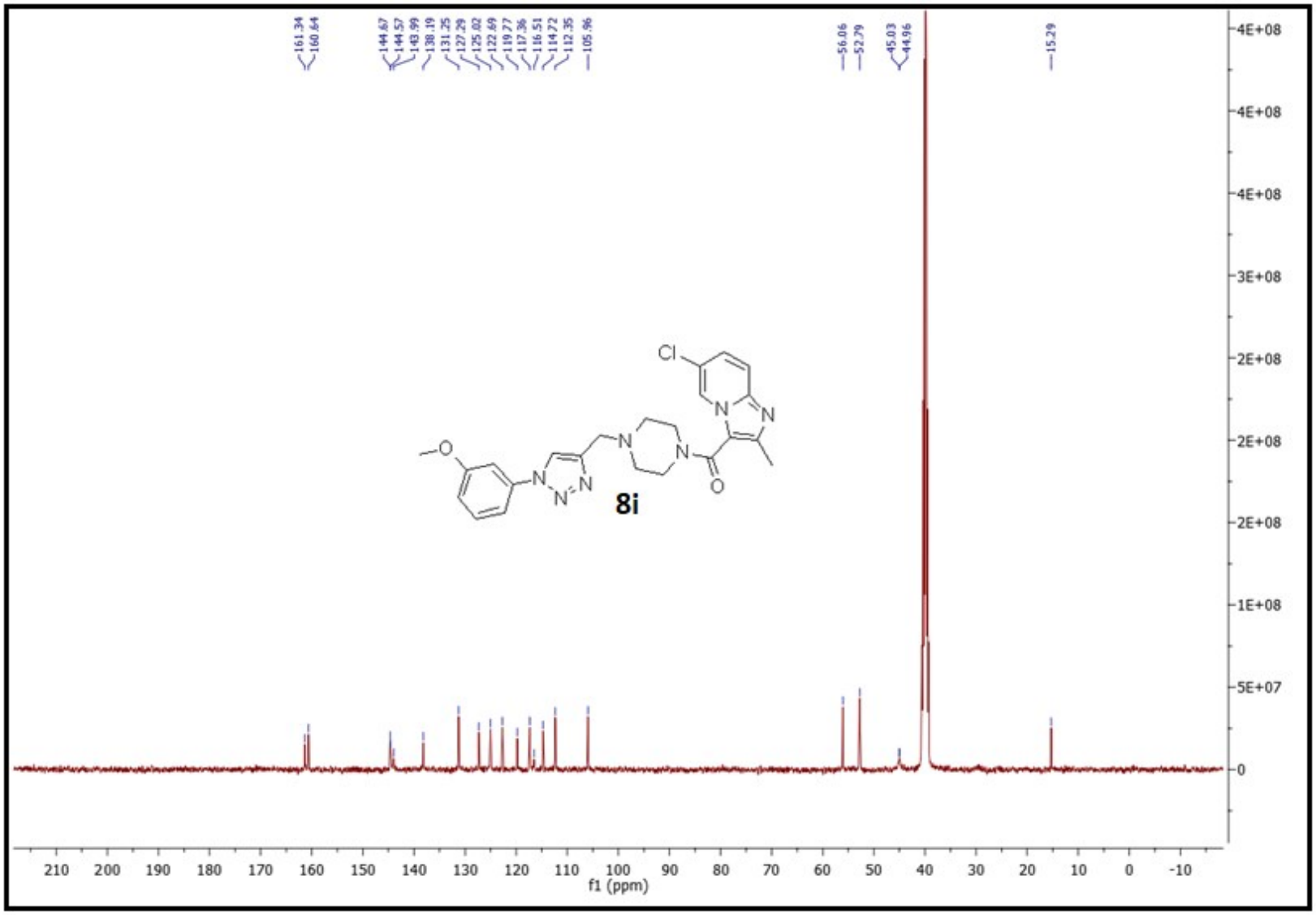
^{13}CNMR of 8e


161.48
145.65
138.51
136.36
133.21
127.31
125.17
122.92
122.68
122.32
121.60
119.82
117.64
117.48
55.34
52.74
45.06
15.42
Cl
N
N
N
N
N
N
N
O
Br
8f
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

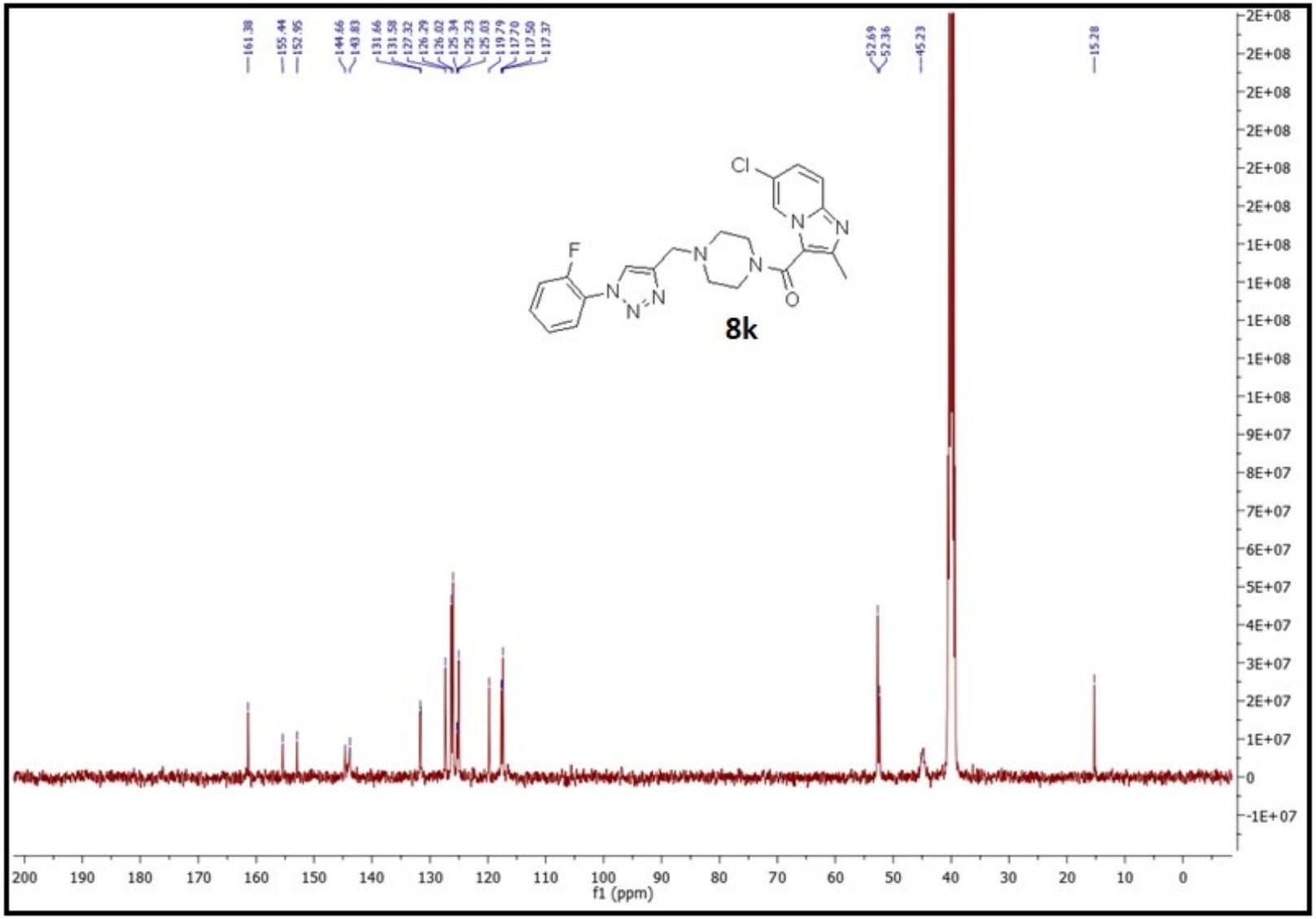
^{13}CNMR of 8f


161.54
161.44
148.99
145.24
137.73
131.98
127.29
126.40
125.14
124.89
123.42
120.13
119.88
117.47
115.05
52.74
45.28
45.07
15.48
Cl
N
N
N
N
N
N
O
O2N
8g
6E+07
6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

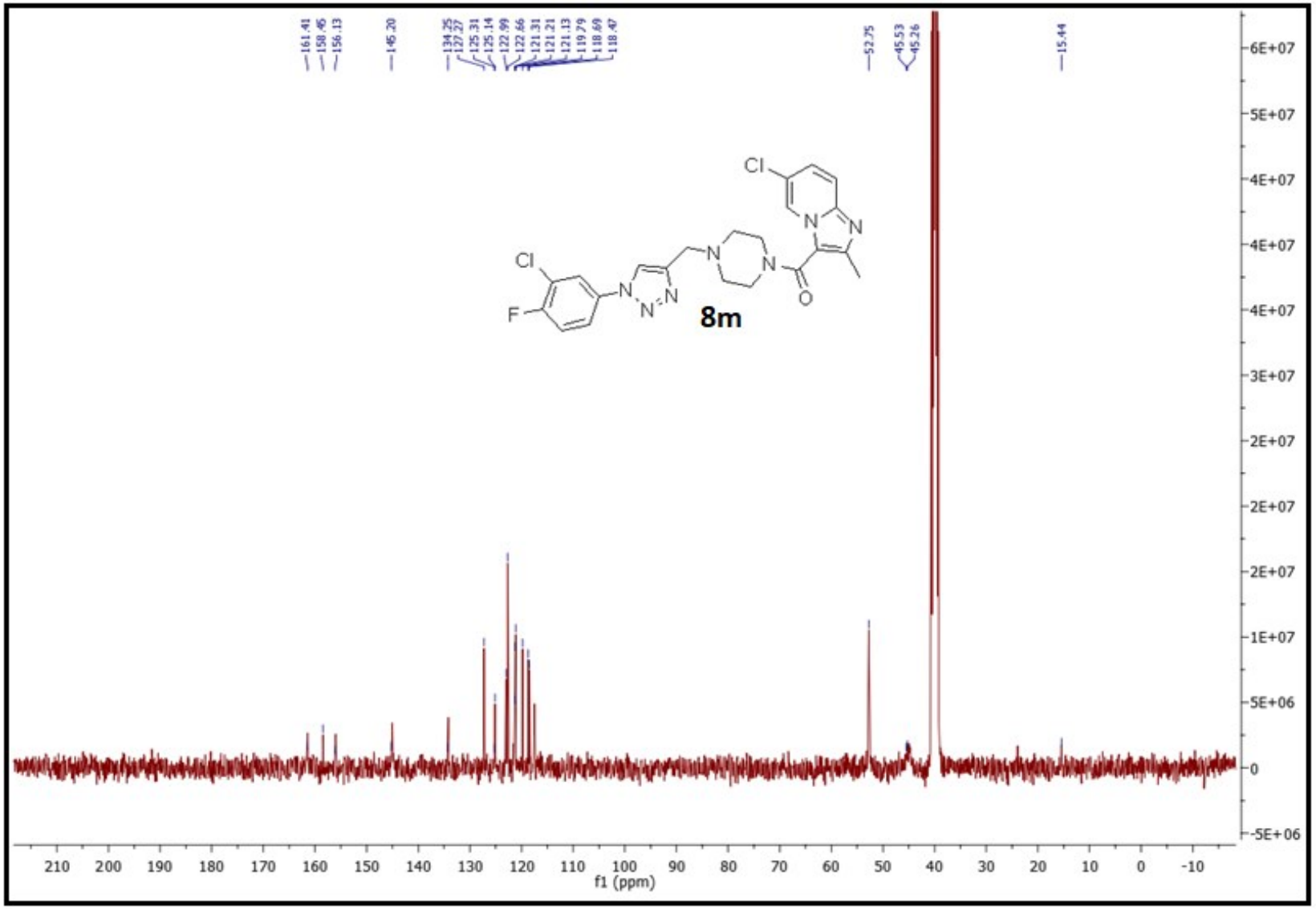
^{13}CNMR of 8g


161.34
160.64
144.67
144.57
143.99
138.19
131.25
127.29
125.02
122.69
119.77
117.36
116.51
114.72
112.35
105.96
56.06
52.79
45.03
44.96
15.29
Cl
O
N
N
N
N
N
O
8i
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

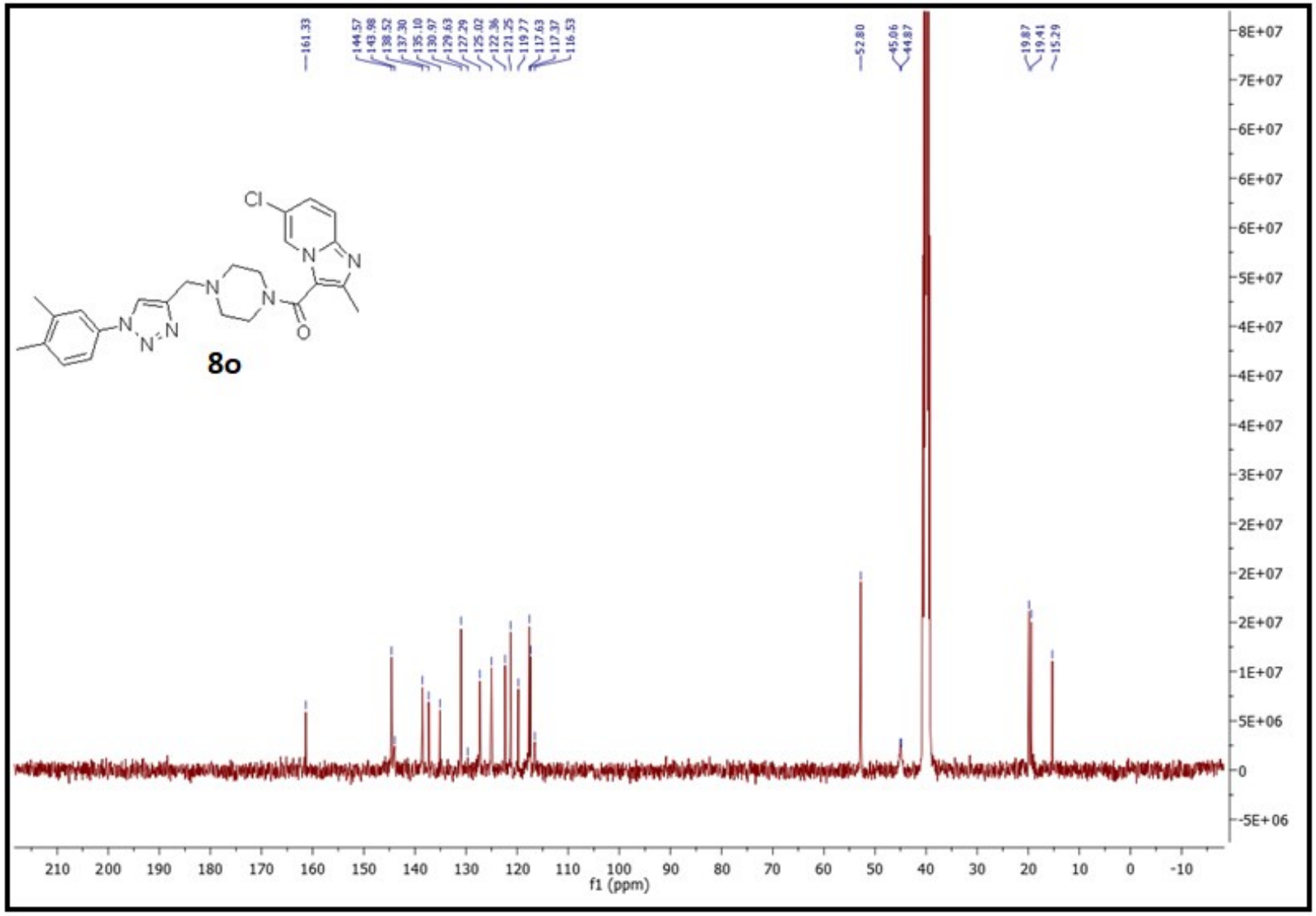
^{13}CNMR of 8i


161.38
155.44
152.95
144.66
143.83
131.66
131.58
127.32
126.29
126.02
125.34
125.23
125.03
119.79
117.70
117.50
117.37
52.69
52.36
45.23
15.28
Cl
F
N
N
N
N
N
O
8k
2E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

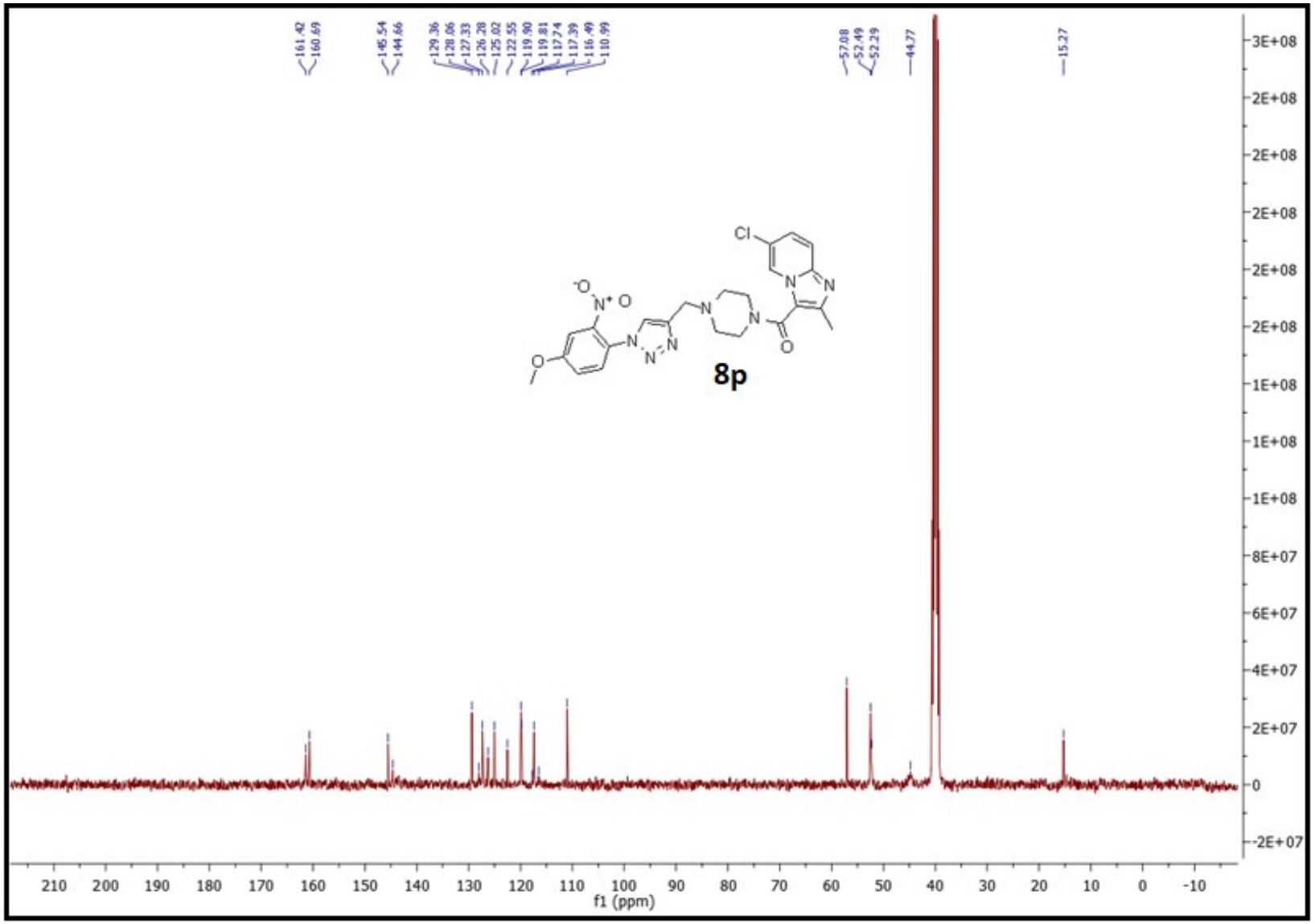
^{13}CNMR of 8k


161.41
158.45
156.13
145.20
134.25
127.27
125.31
125.14
122.99
122.66
121.31
121.21
121.13
119.79
118.69
118.47
52.75
45.53
45.26
15.44
Cl
Cl
F
N
N
N
N
N
O
8m
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

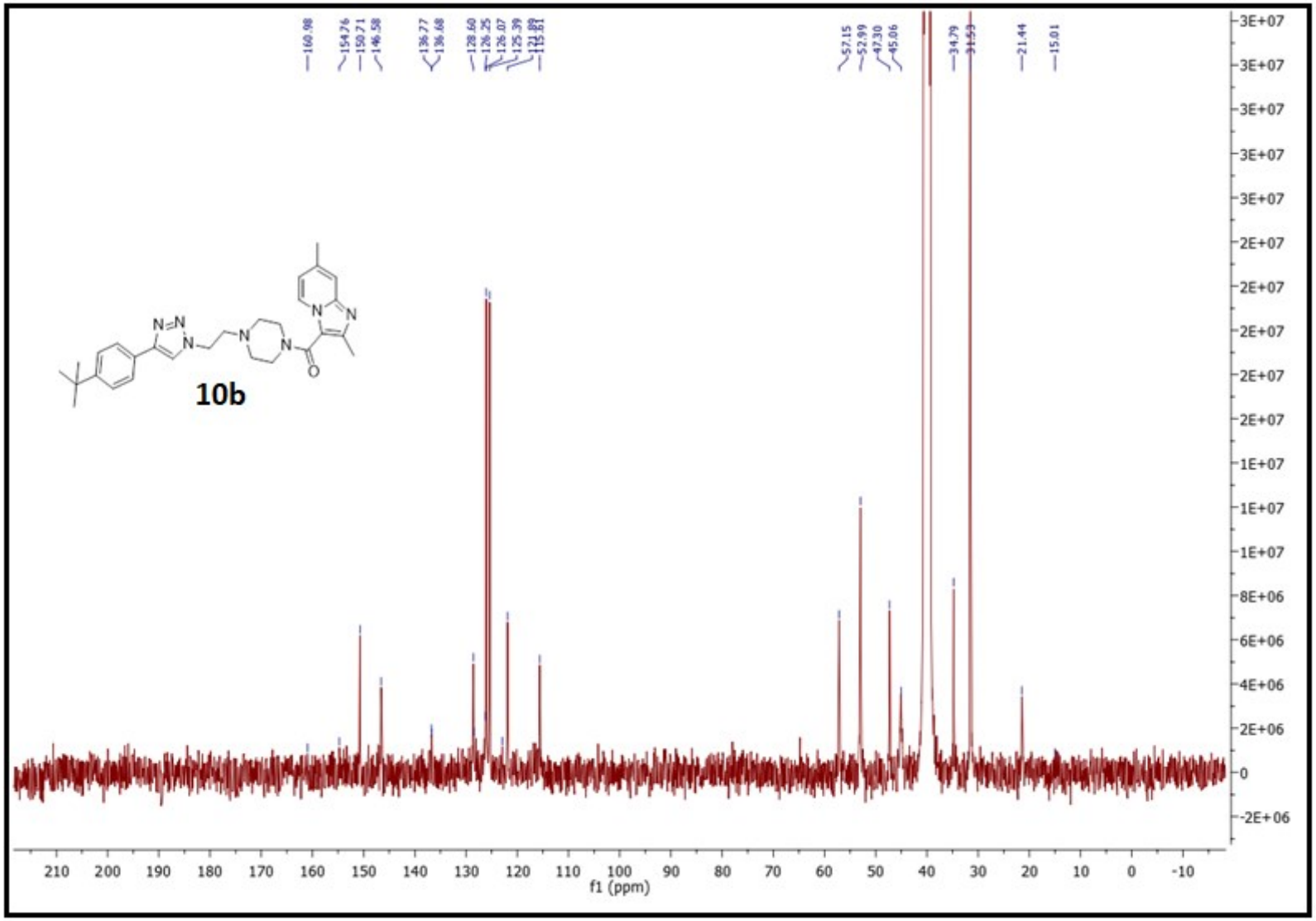
^{13}CNMR of 8m


161.33
144.57
143.98
138.52
137.30
135.10
130.97
129.63
127.29
125.02
122.36
121.25
119.77
117.63
117.37
116.53
52.80
45.06
44.87
19.87
19.41
15.29
Cl
N
N
N
N
N
N
O
8o
8E+07
7E+07
6E+07
6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

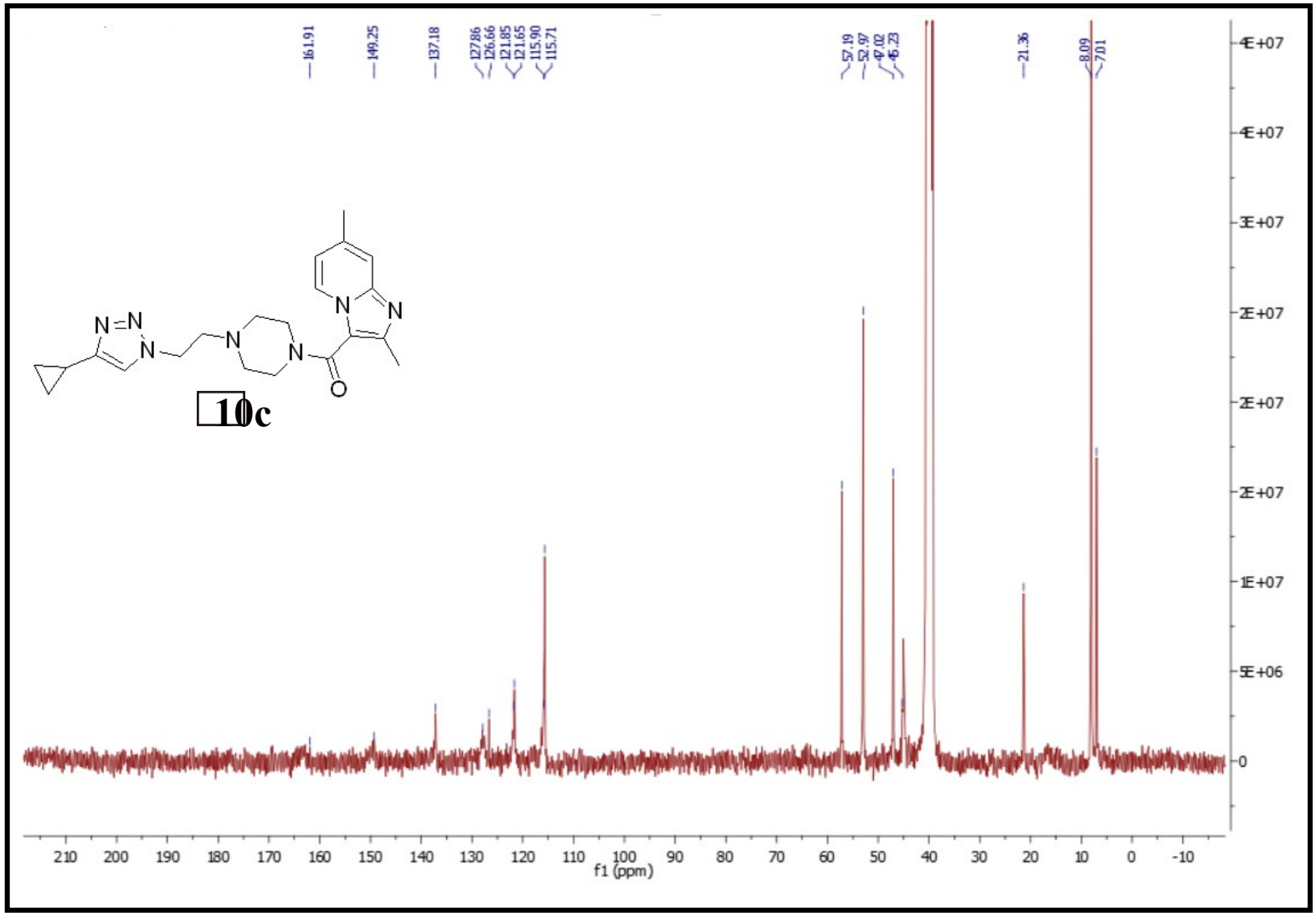
^{13}CNMR of 8o


161.42
160.69
145.54
144.66
129.36
128.06
127.33
126.28
125.02
122.55
119.90
119.81
117.74
117.39
116.49
110.99
57.08
52.49
52.29
44.77
15.27
8p
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

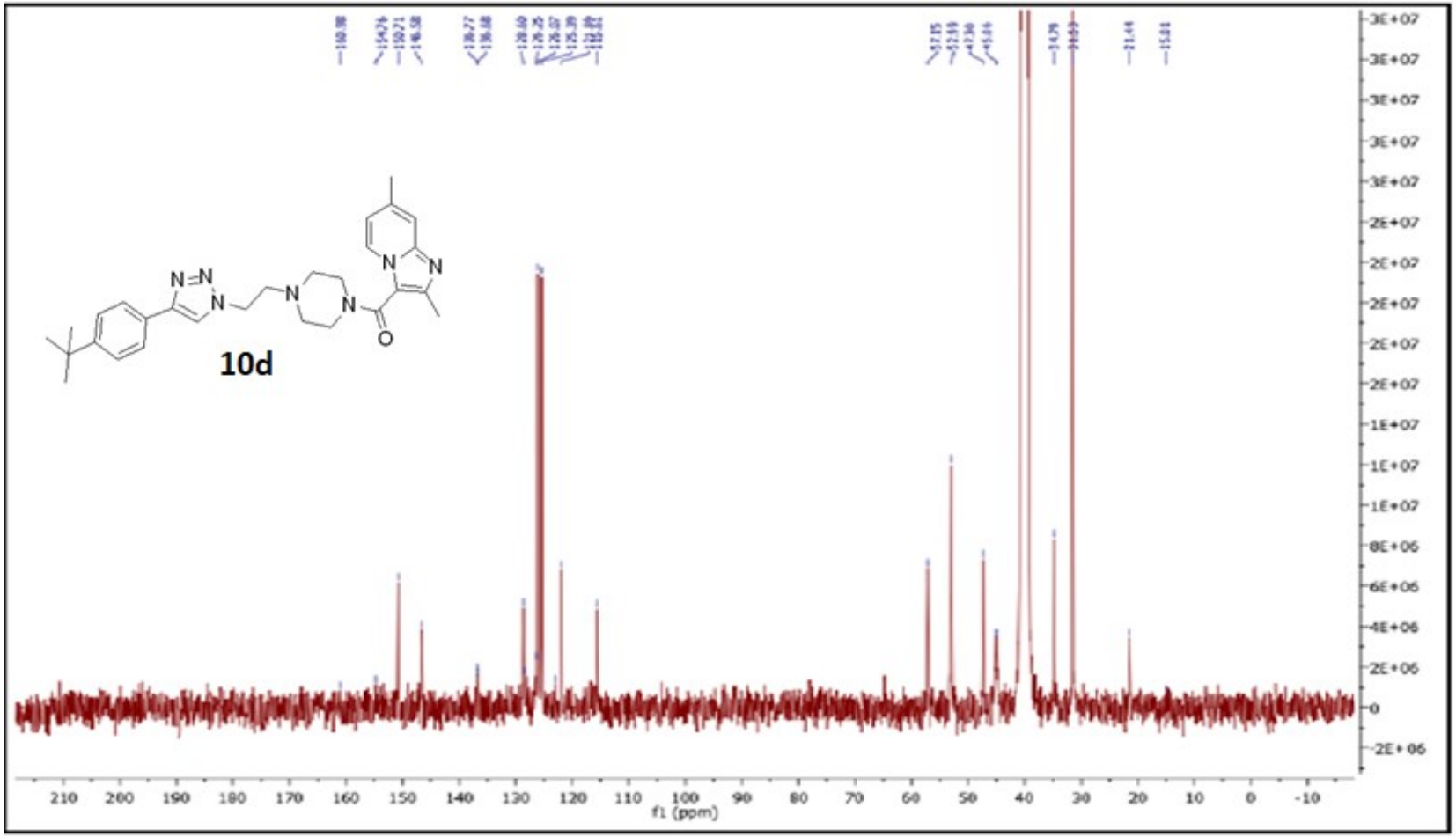
^{13}CNMR of 8p



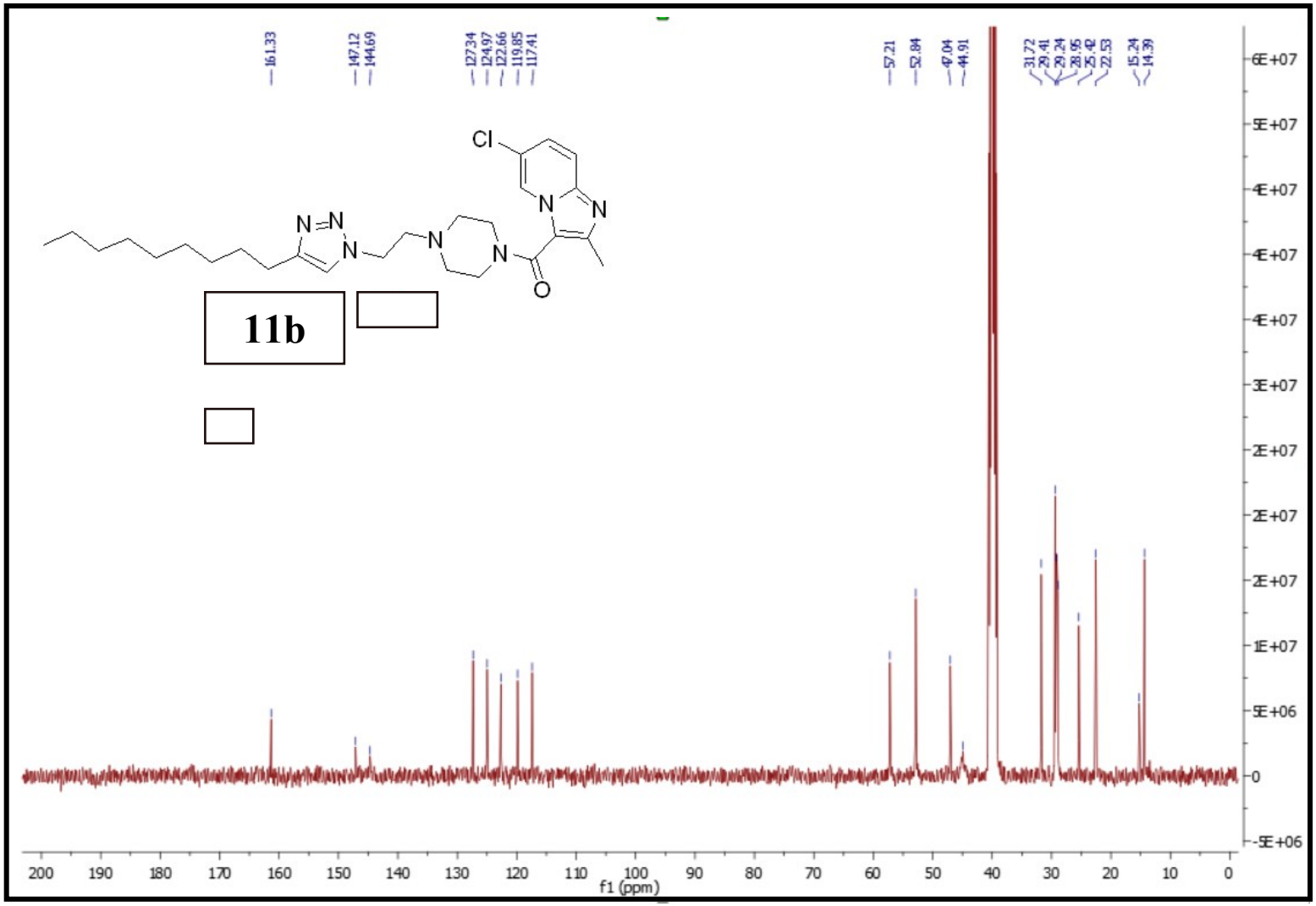
^{13}CNMR of 10b



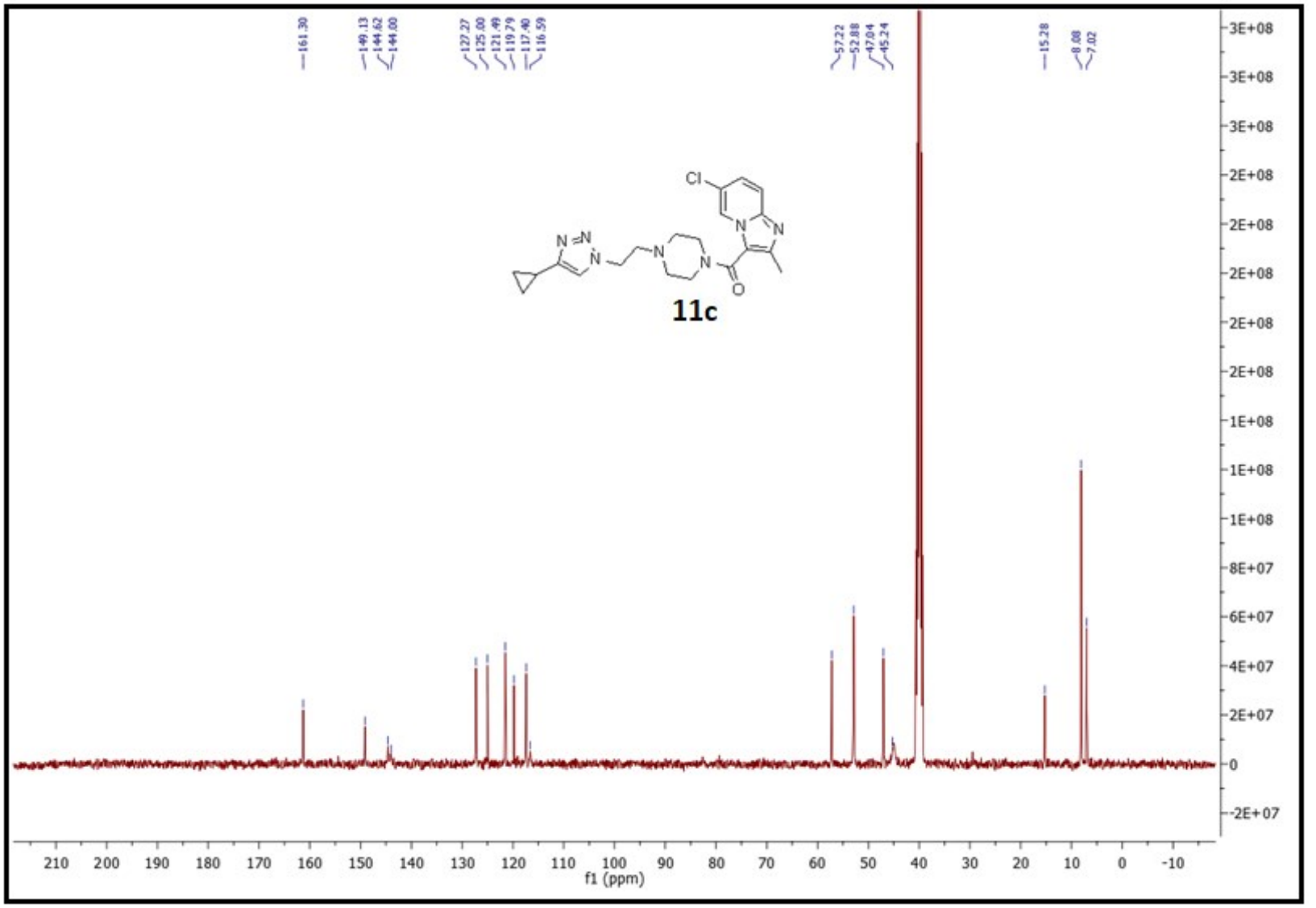
^{13}CNMR of 10c



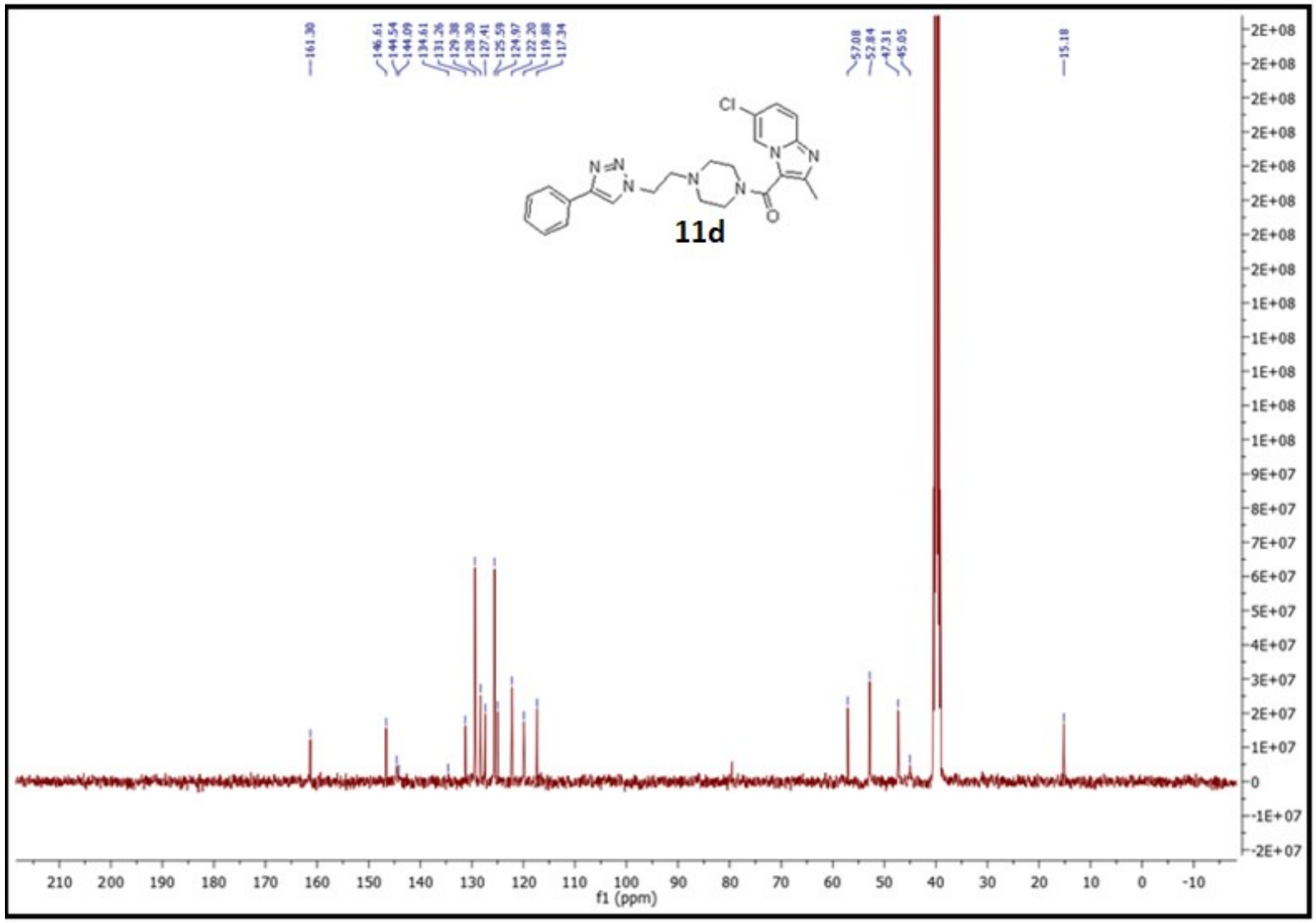
^{13}CNMR of 10d



^{13}CNMR of 11b

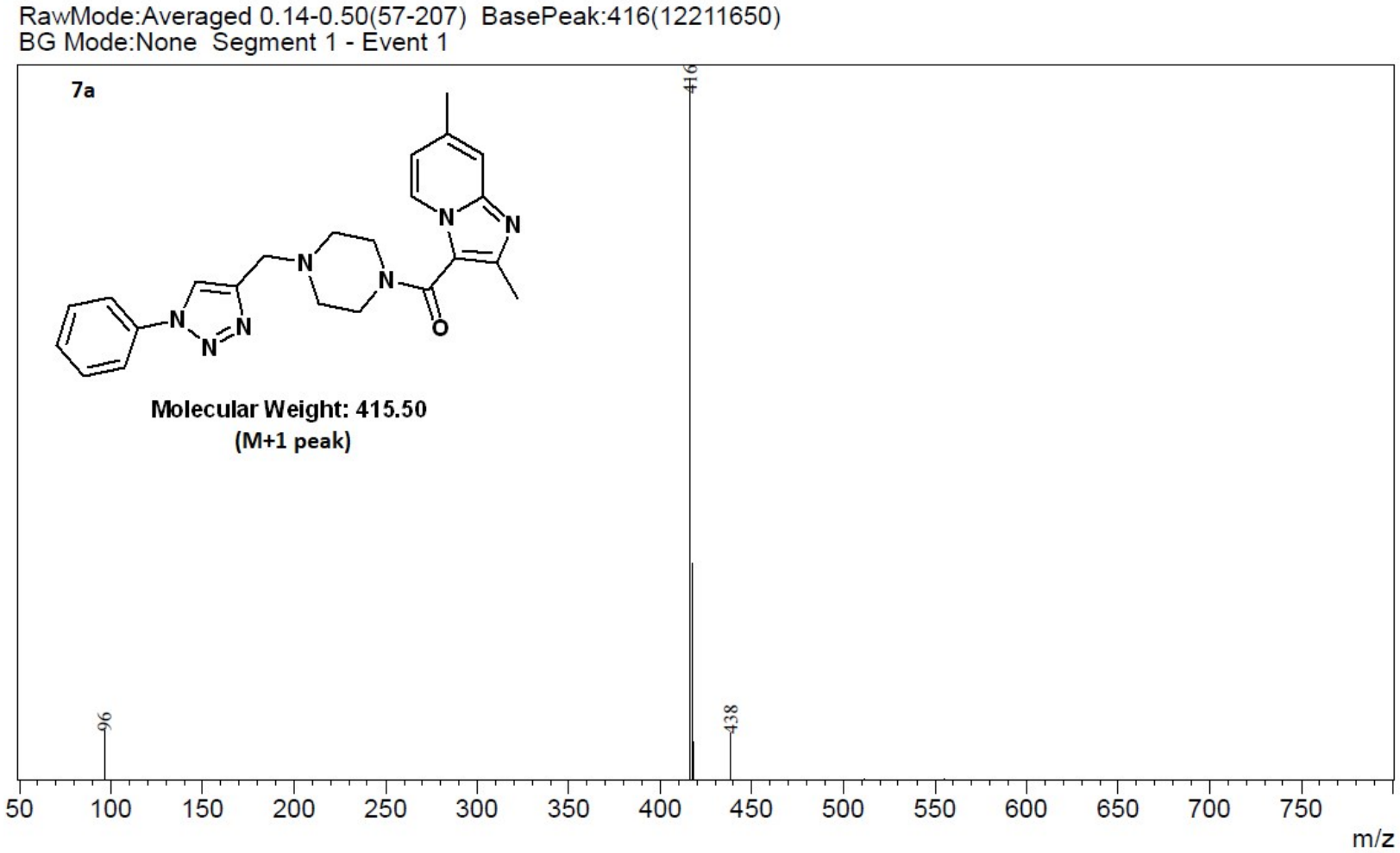


^{13}CNMR of 11c

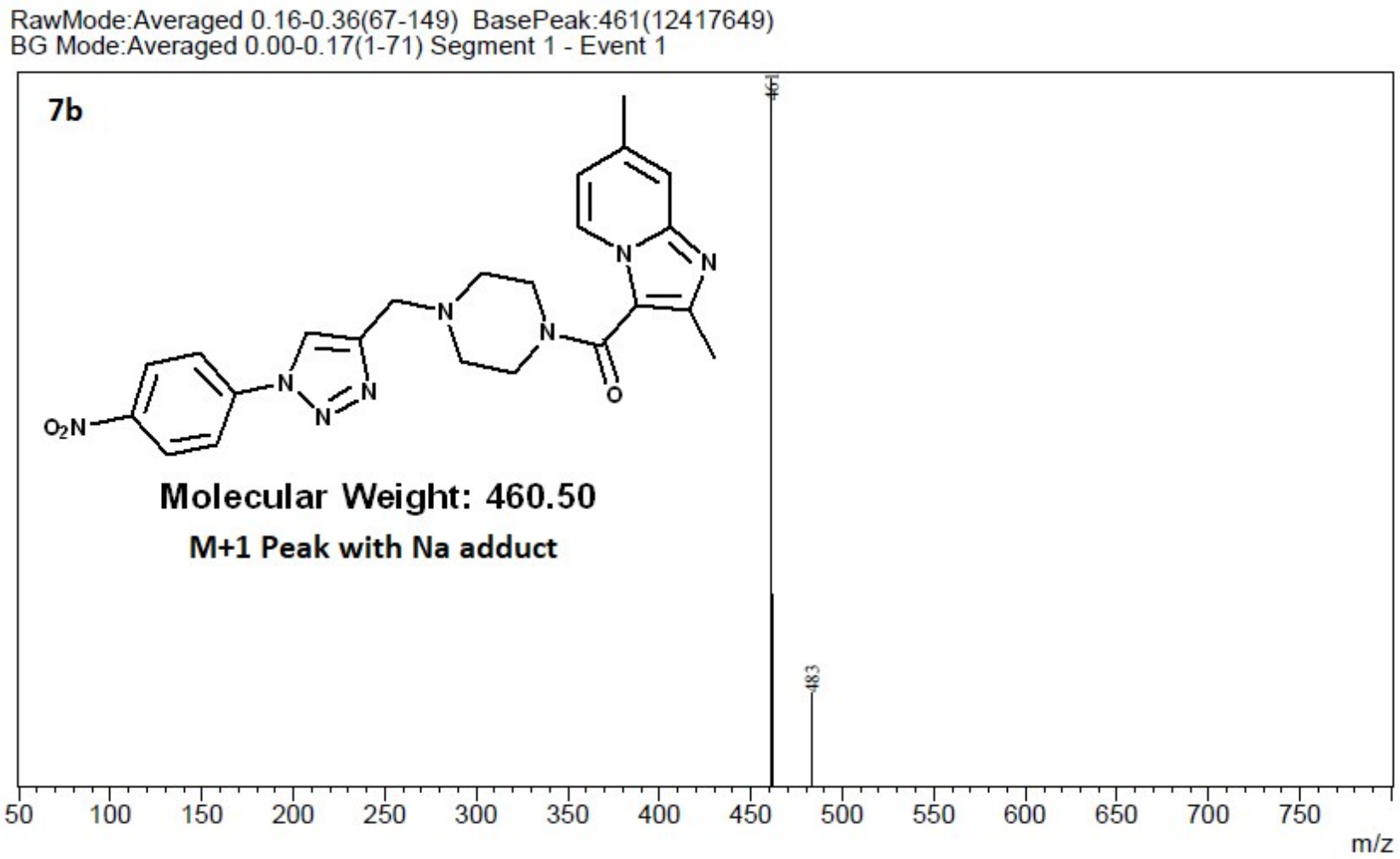


^{13}CNMR of 11d

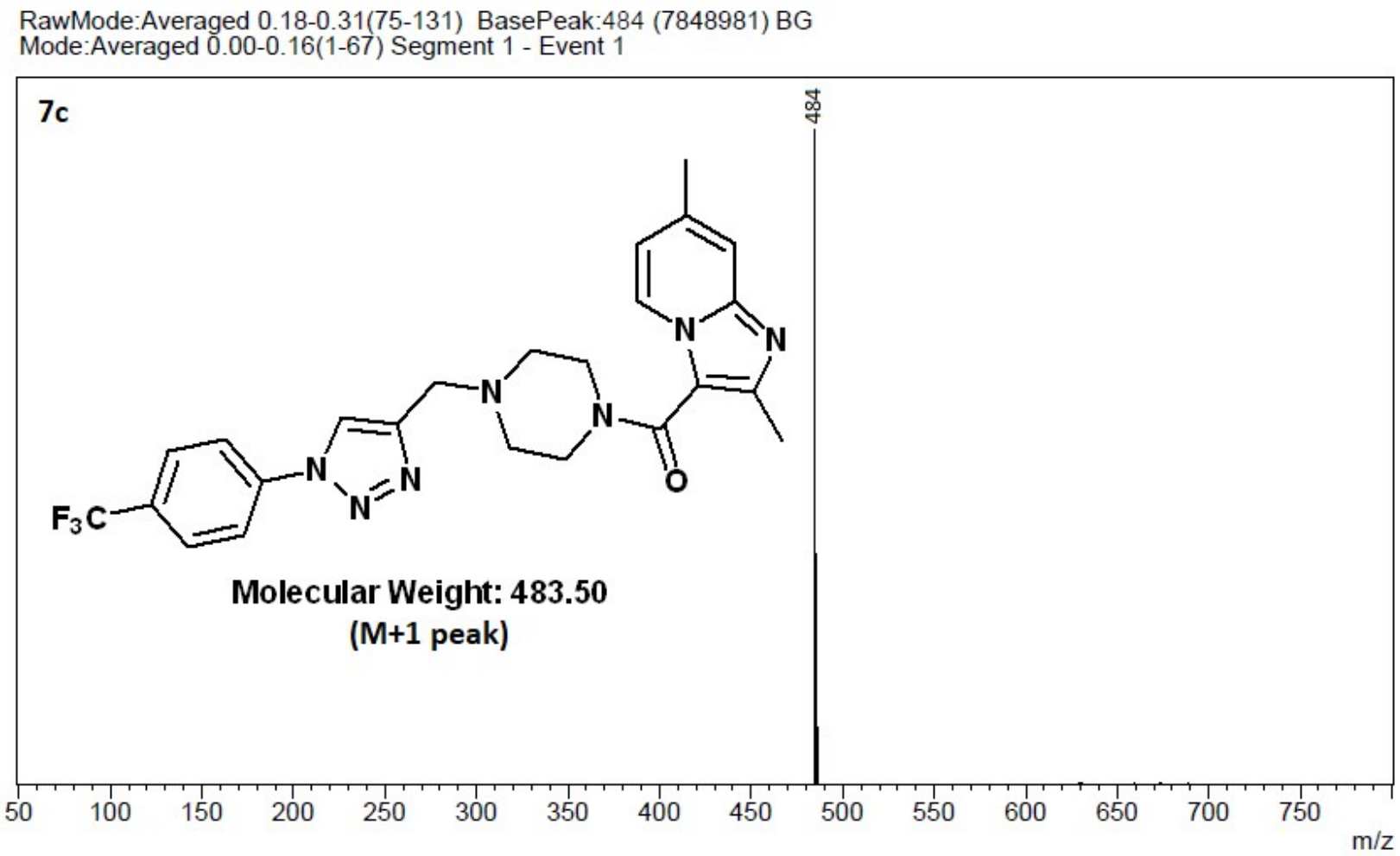
8. Mass spectras:



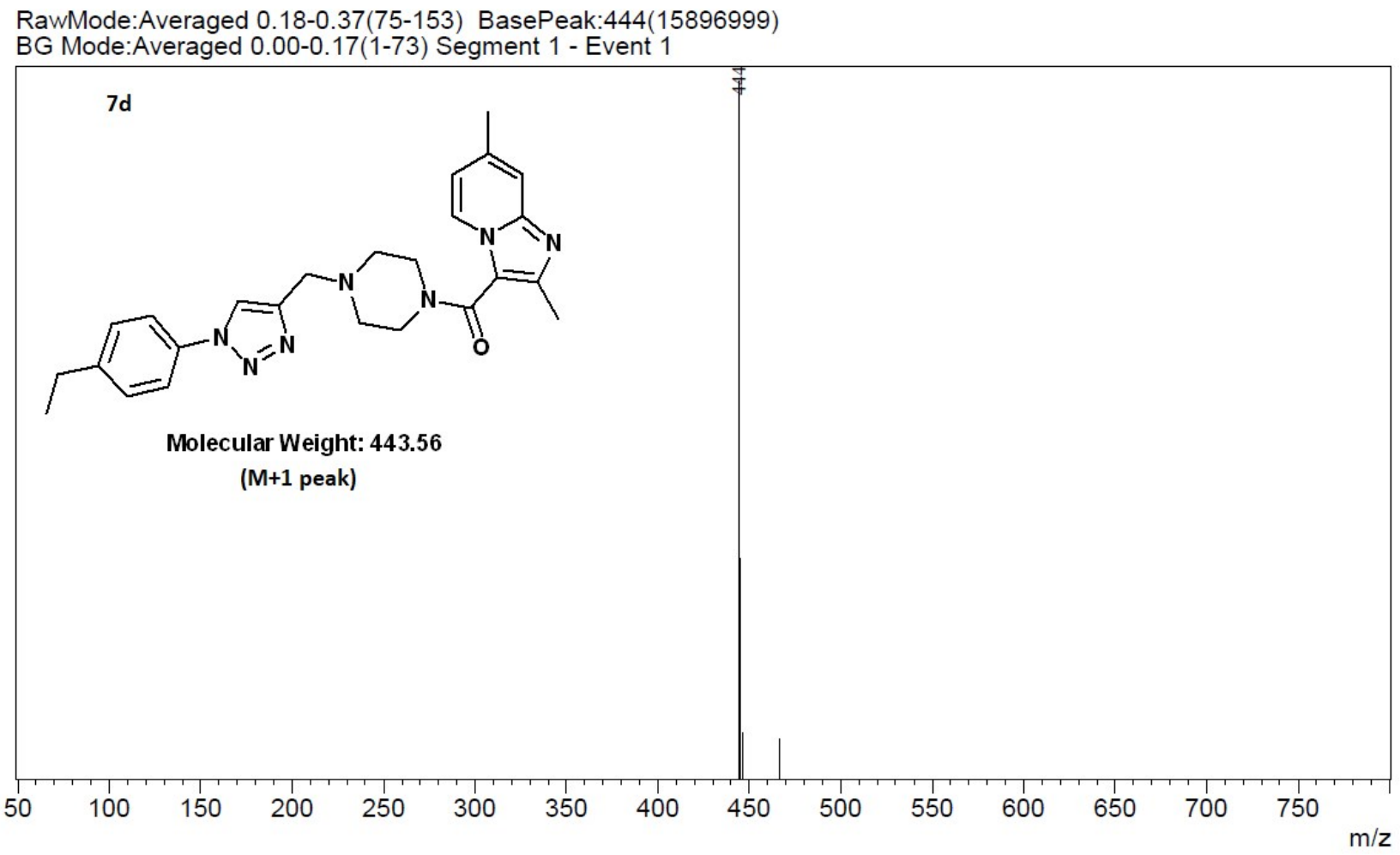
Mass spectra of compound 7a



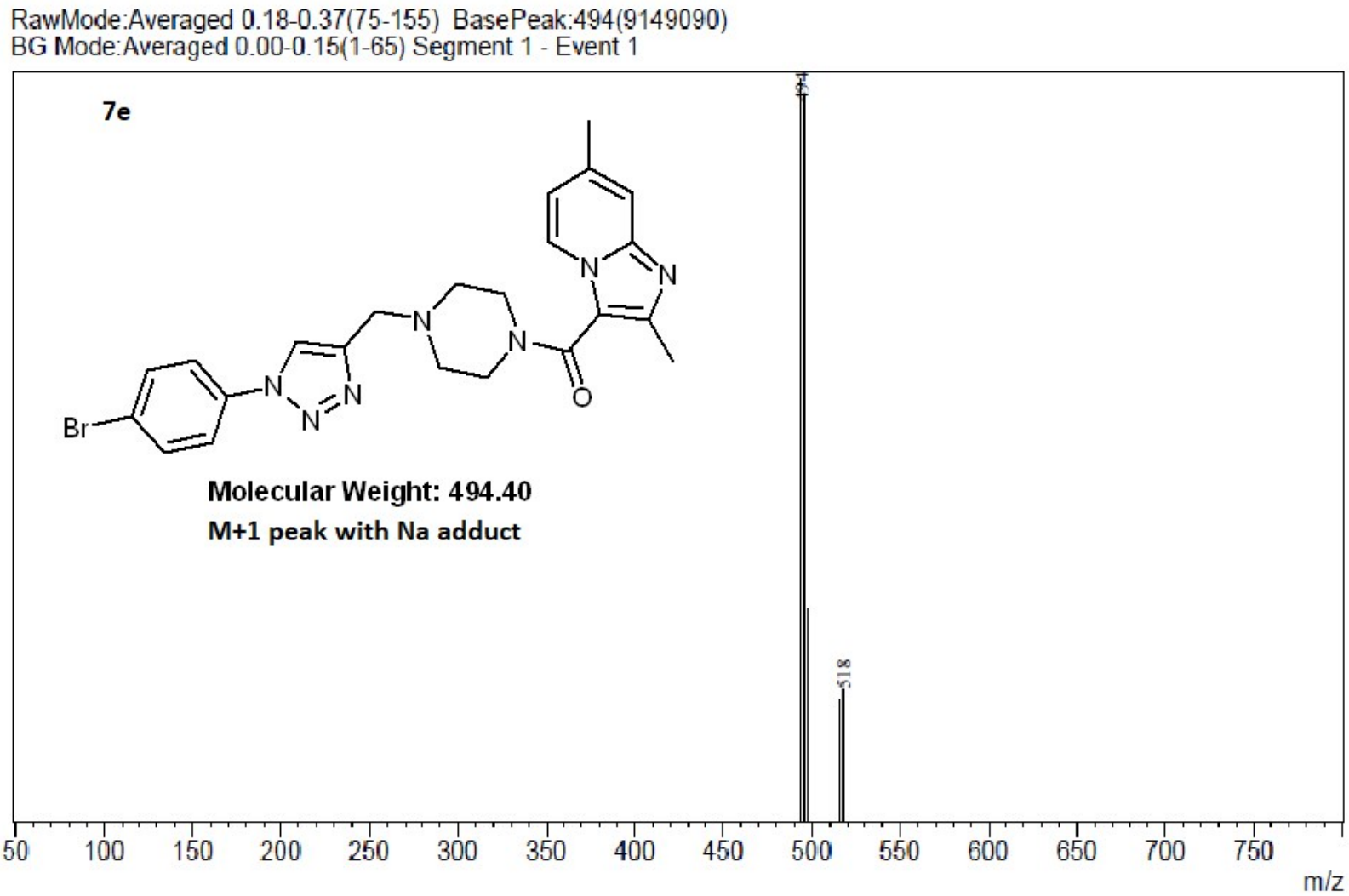
Mass spectra of compound 7b



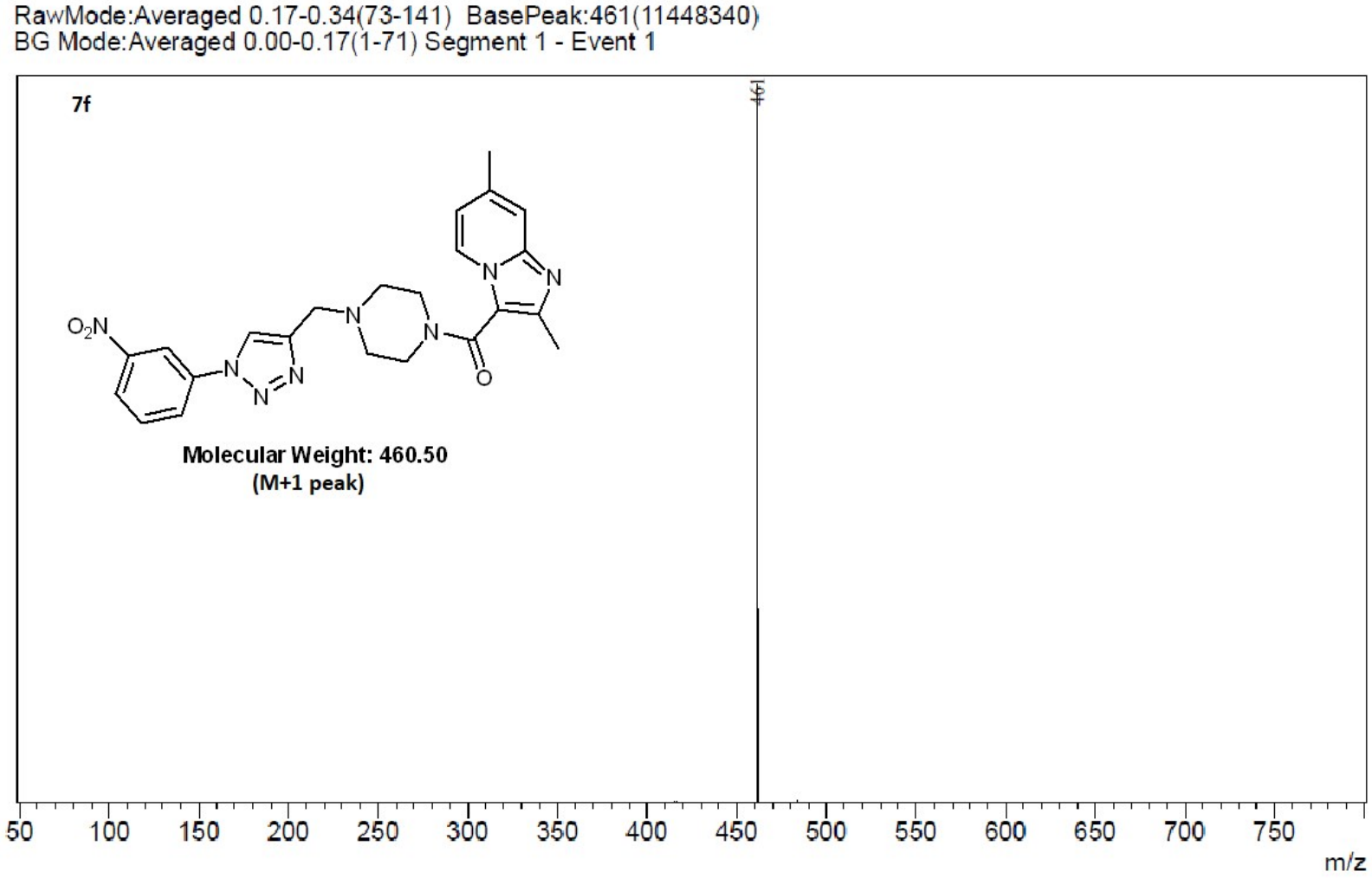
Mass spectra of compound 7c



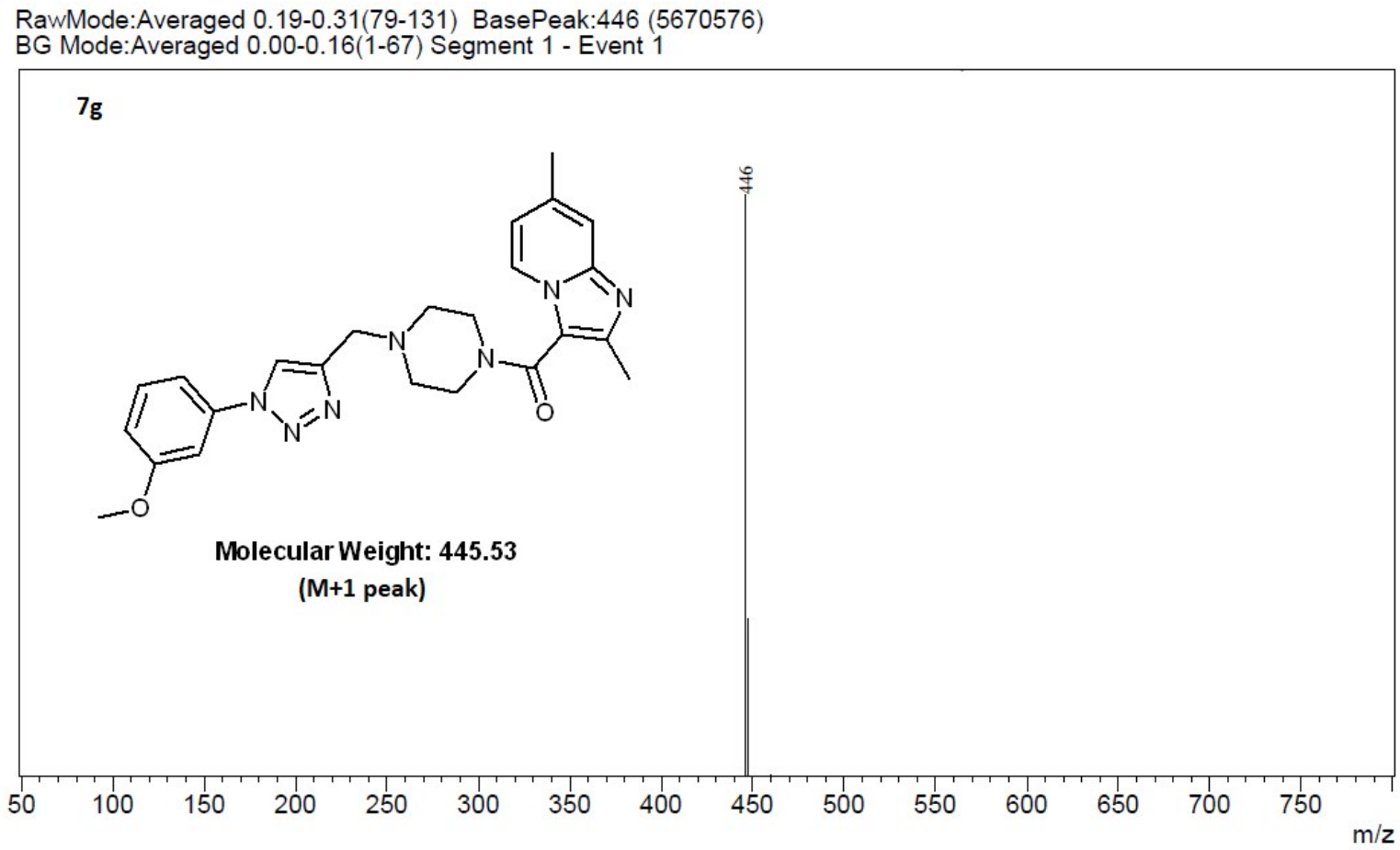
Mass spectra of compound 7d



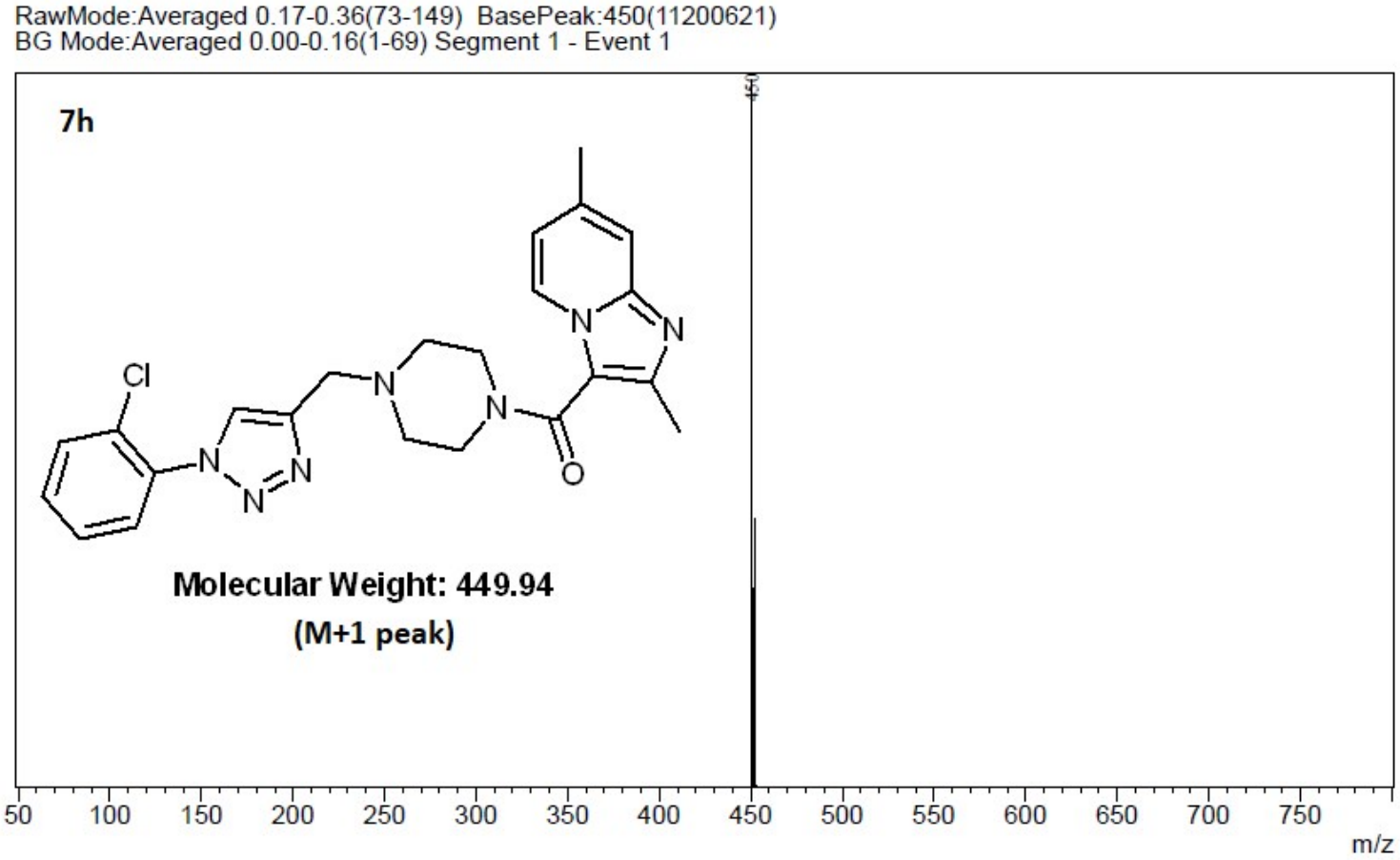
Mass spectra of compound 7e



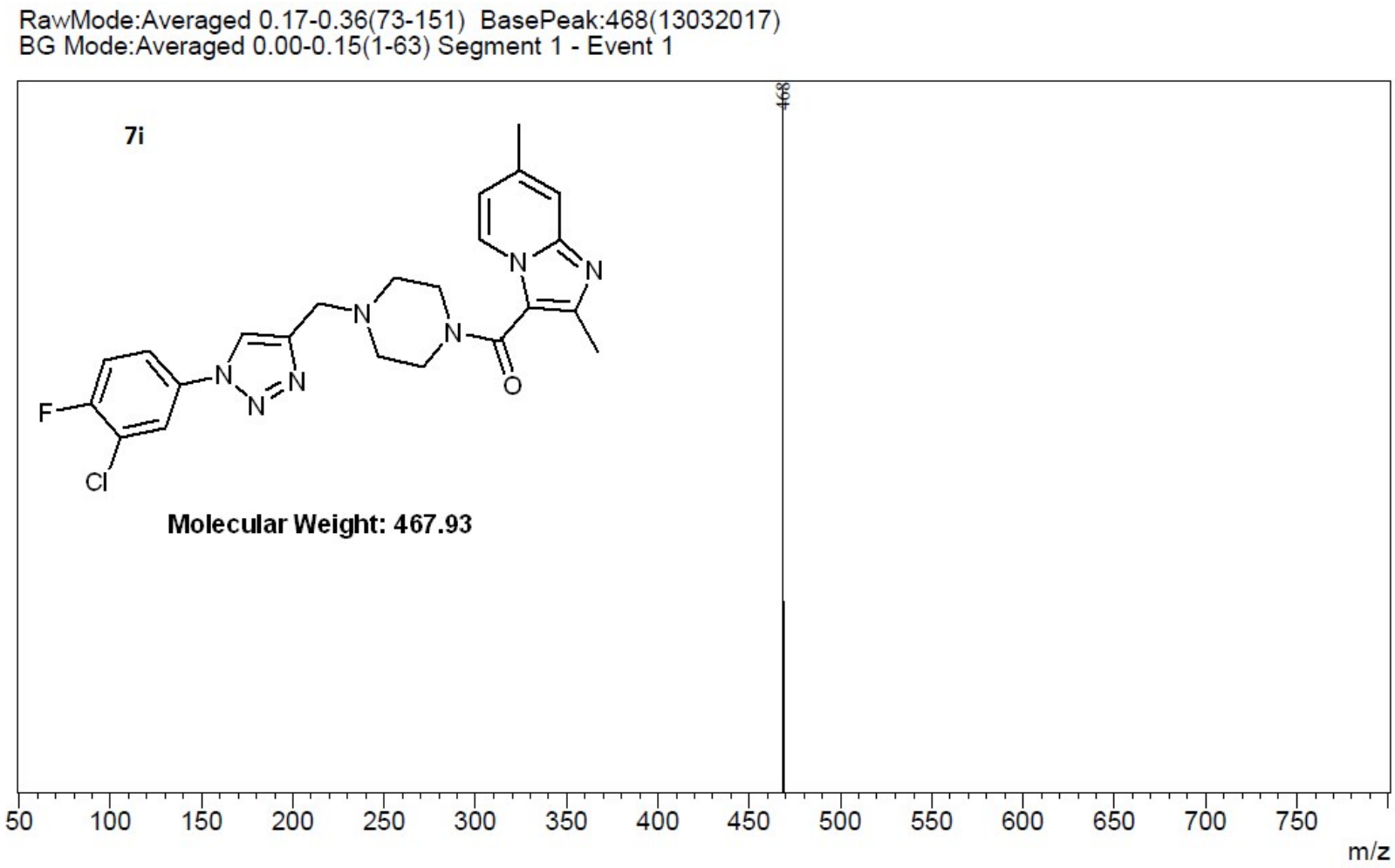
Mass spectra of compound 7f



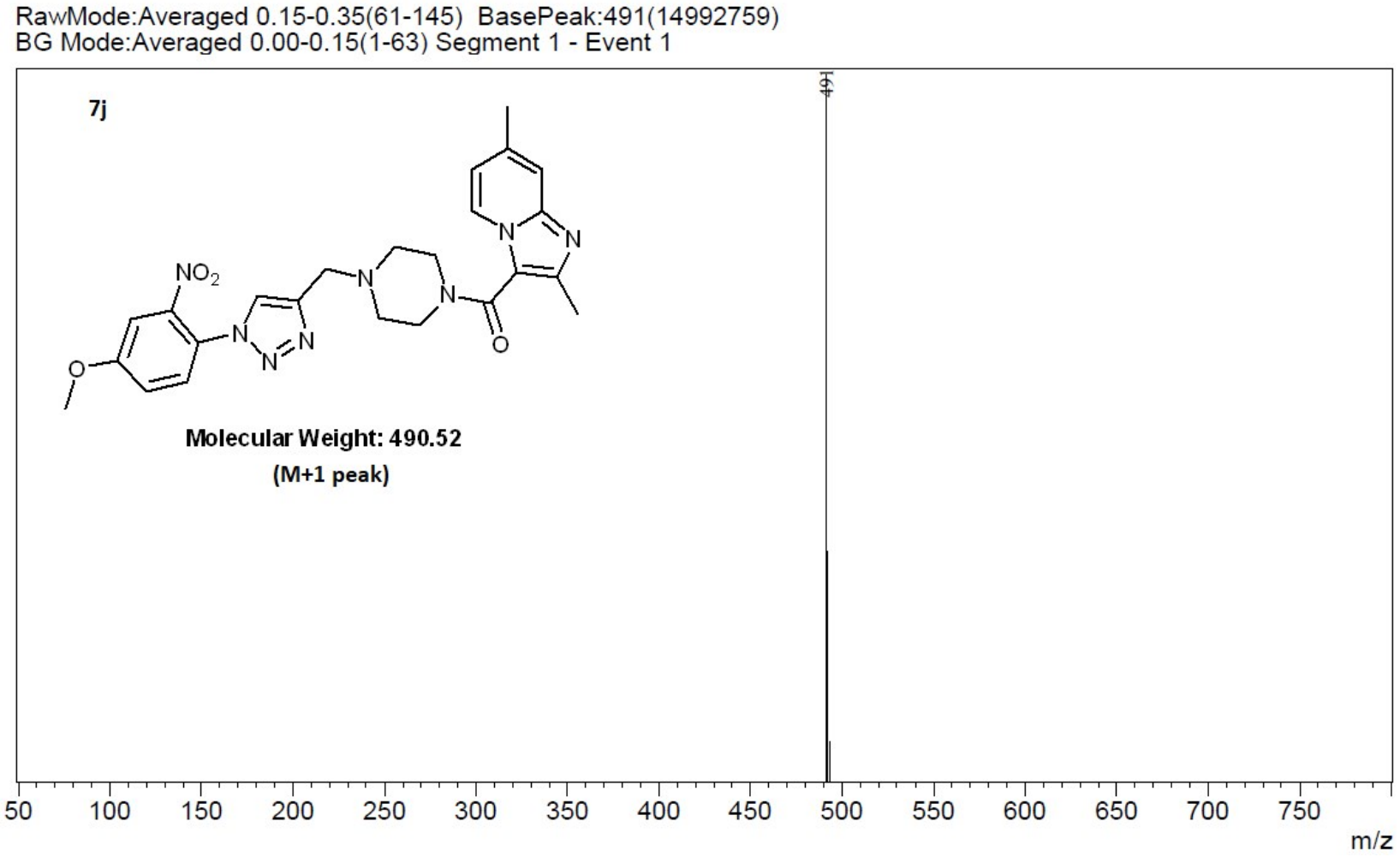
Mass spectra of compound 7g



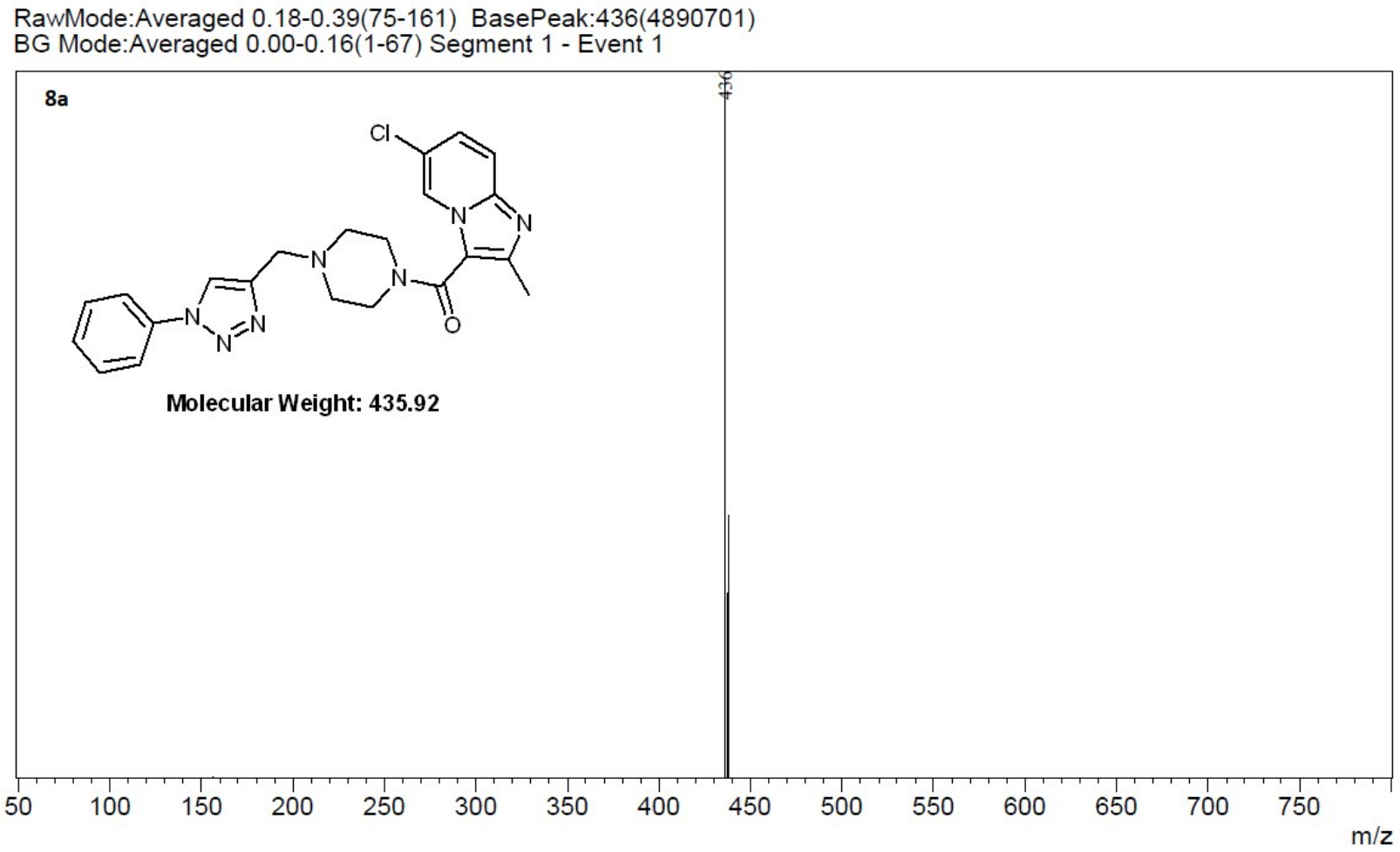
Mass spectra of compound 7h



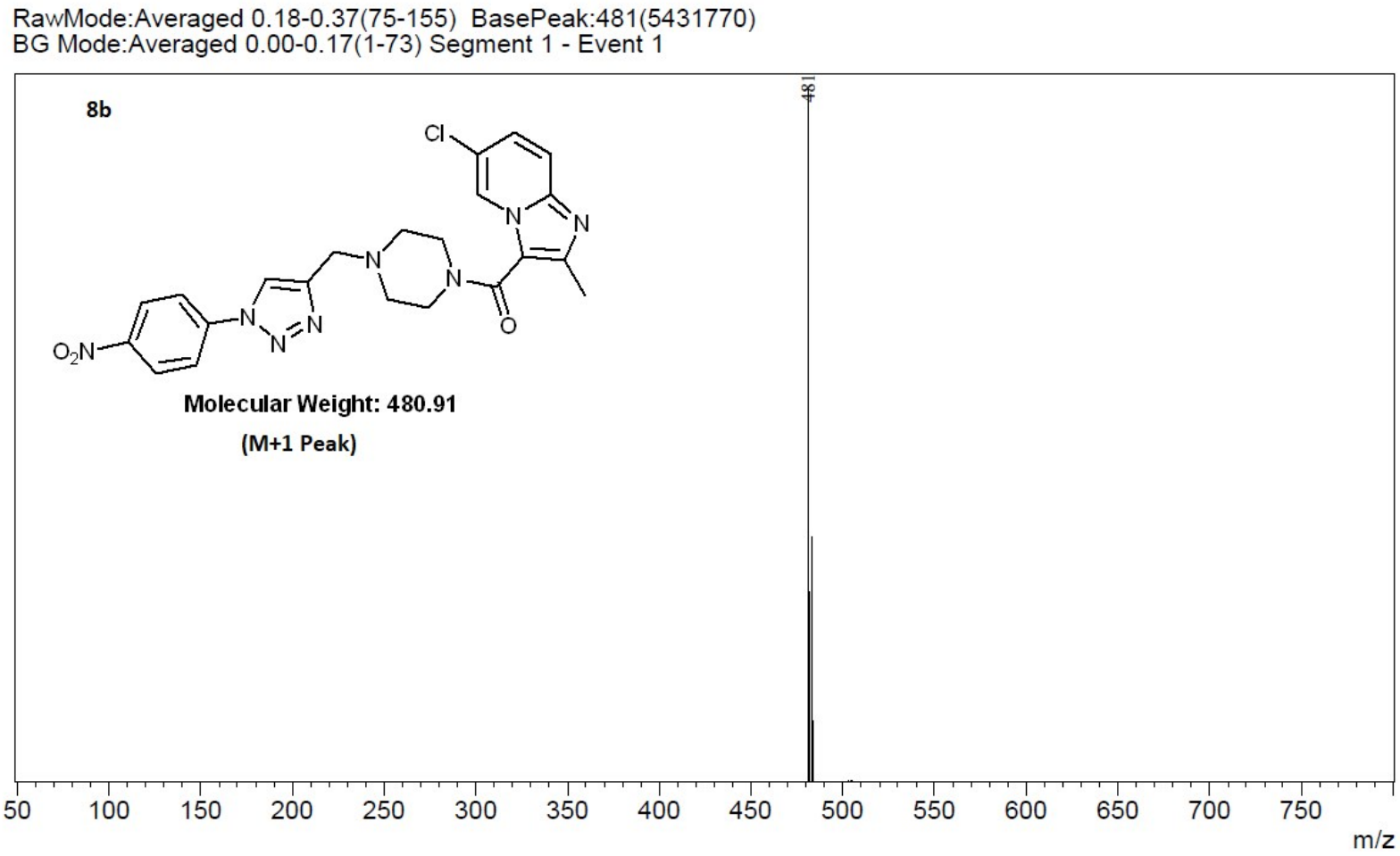
Mass spectra of compound 7i



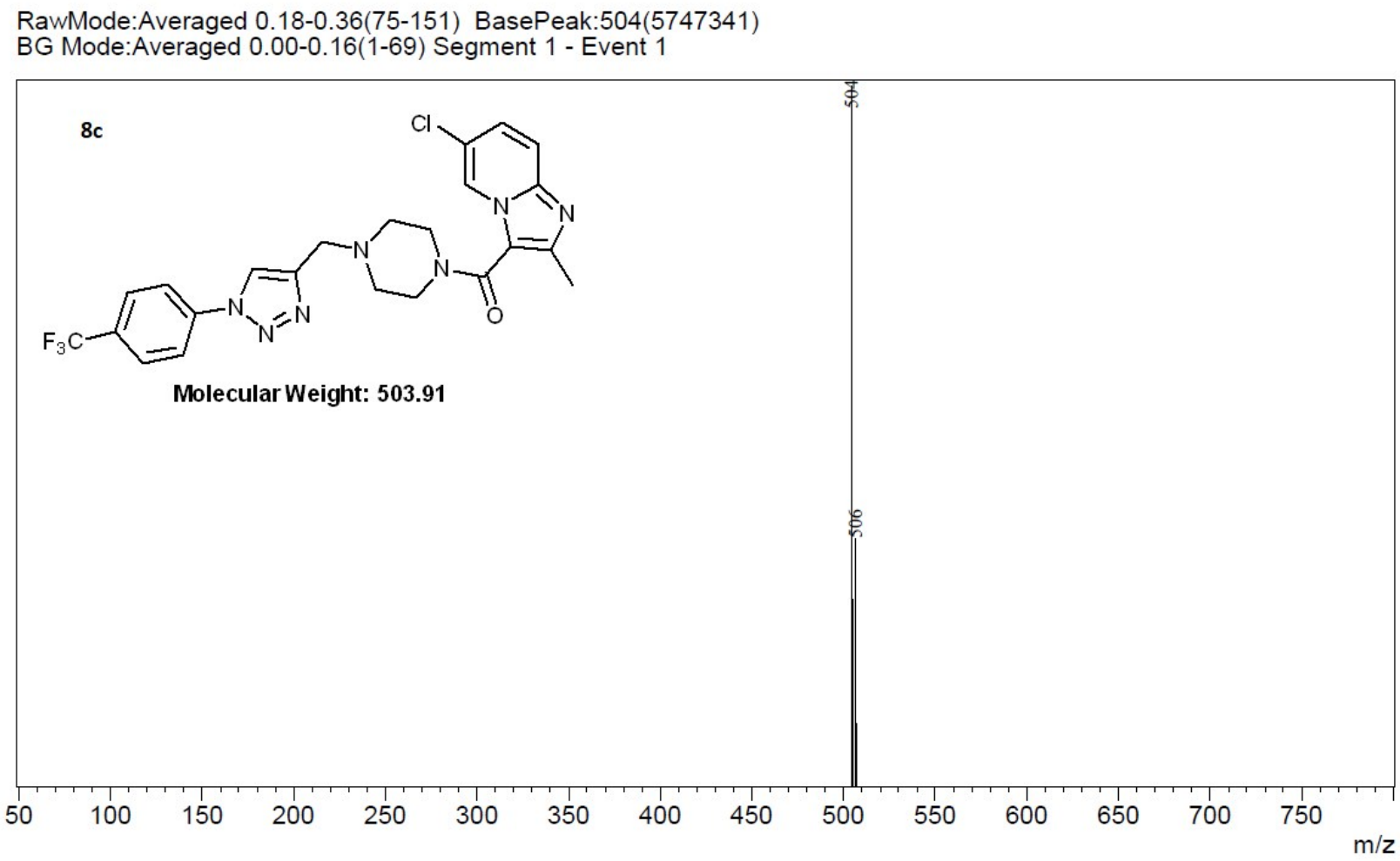
Mass spectra of compound 7j



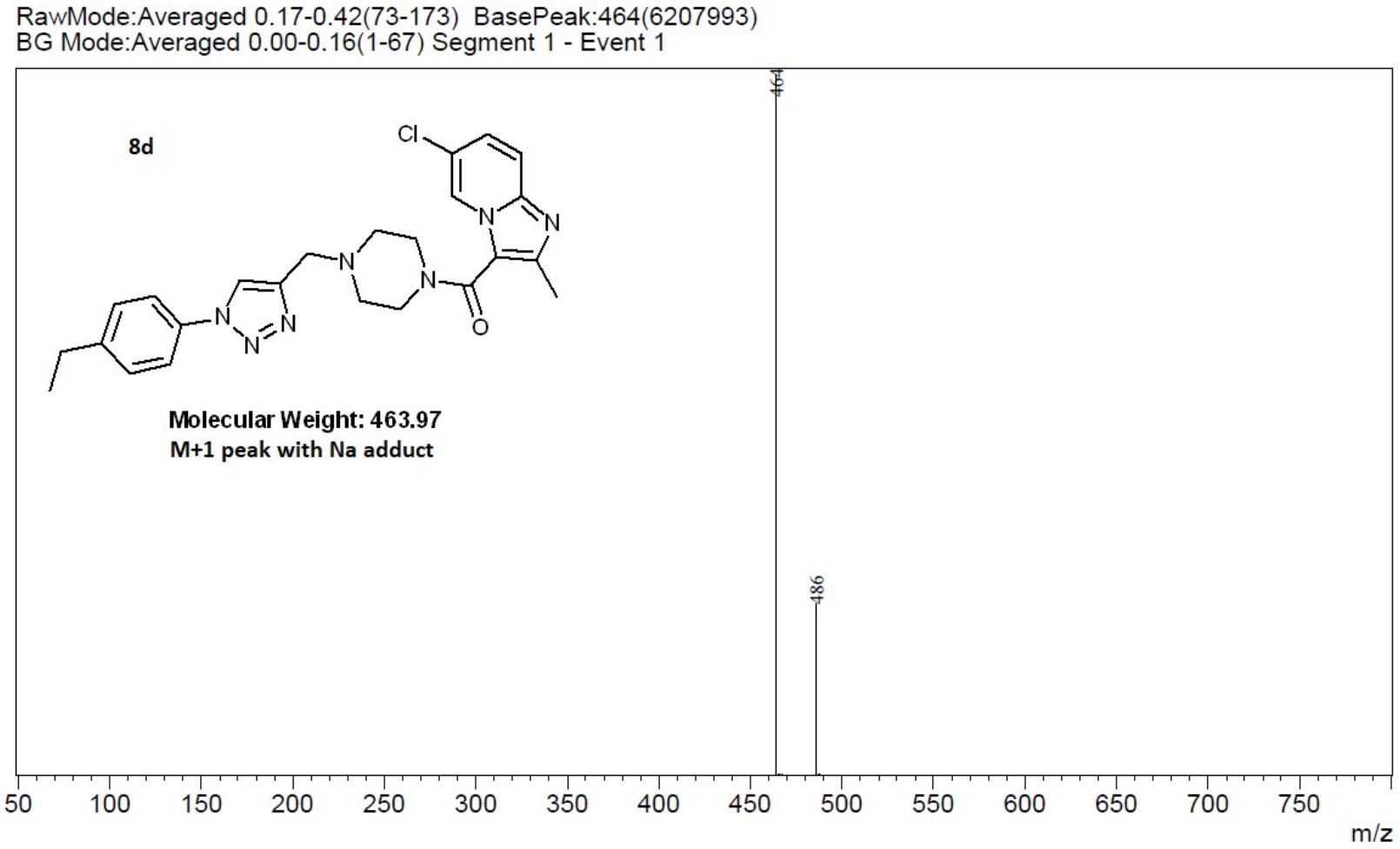
Mass spectra of compound 8a



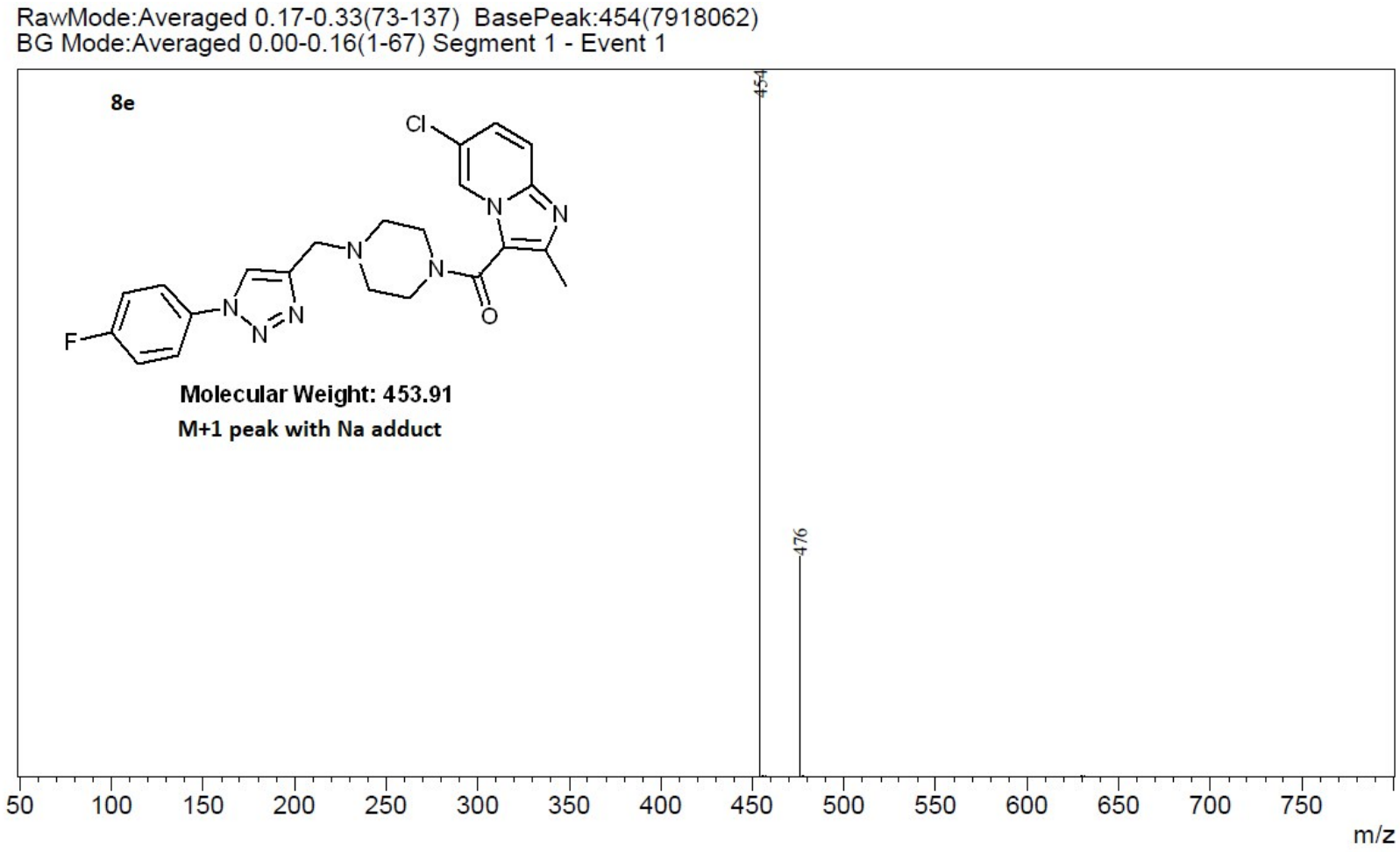
Mass spectra of compound 8b



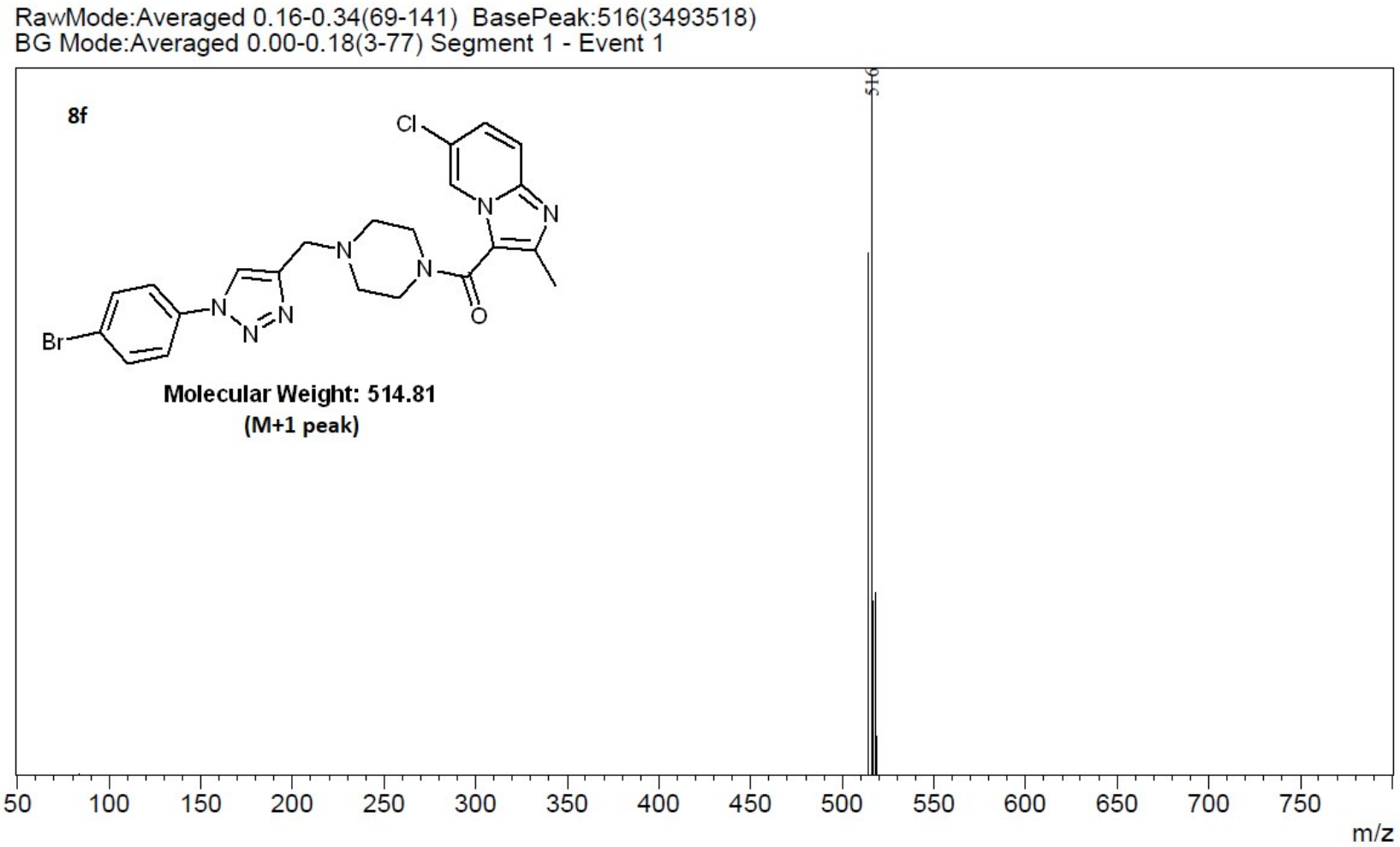
Mass spectra of compound 8c



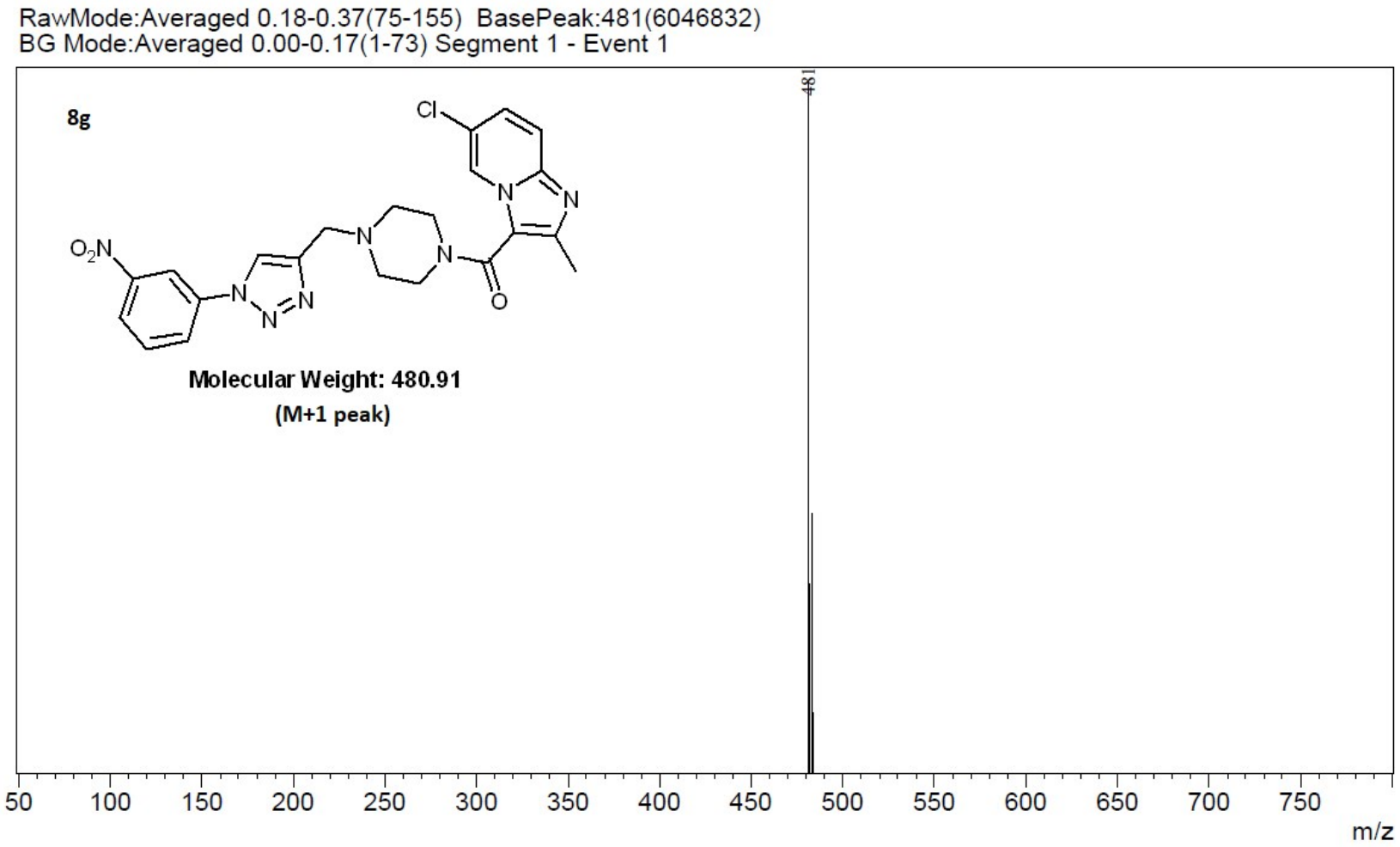
Mass spectra of compound 8d



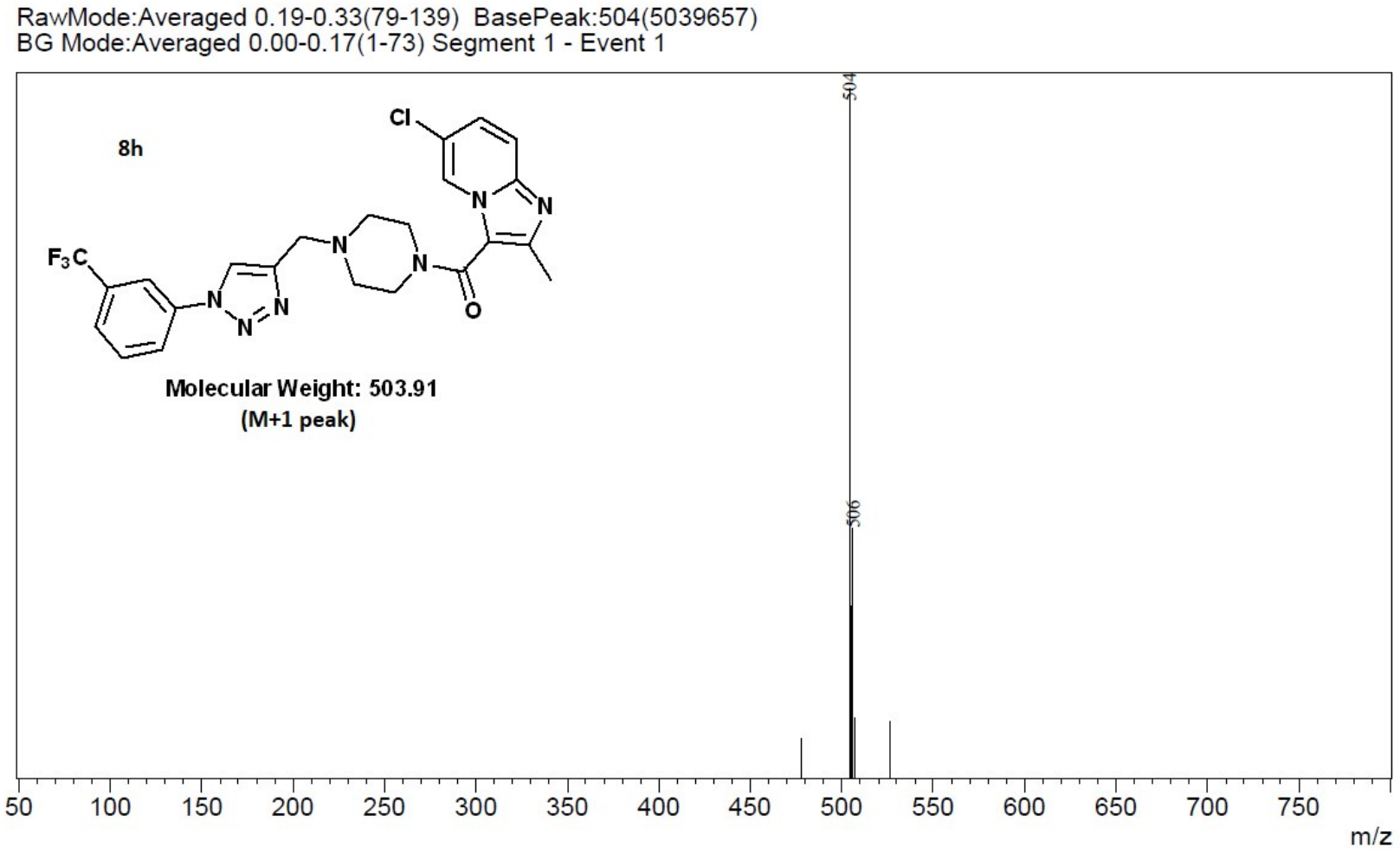
Mass spectra of compound 8e



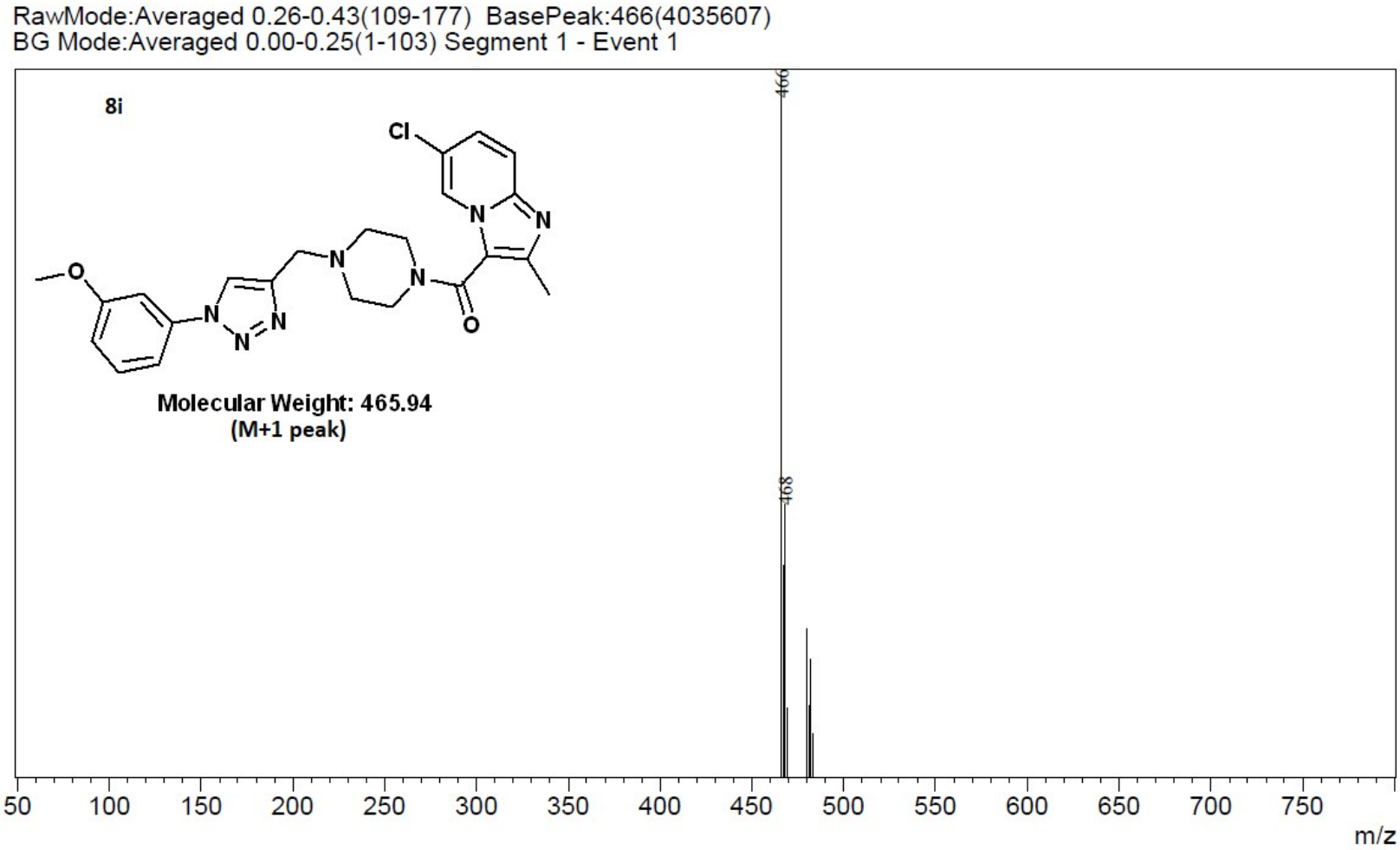
Mass spectra of compound 8f



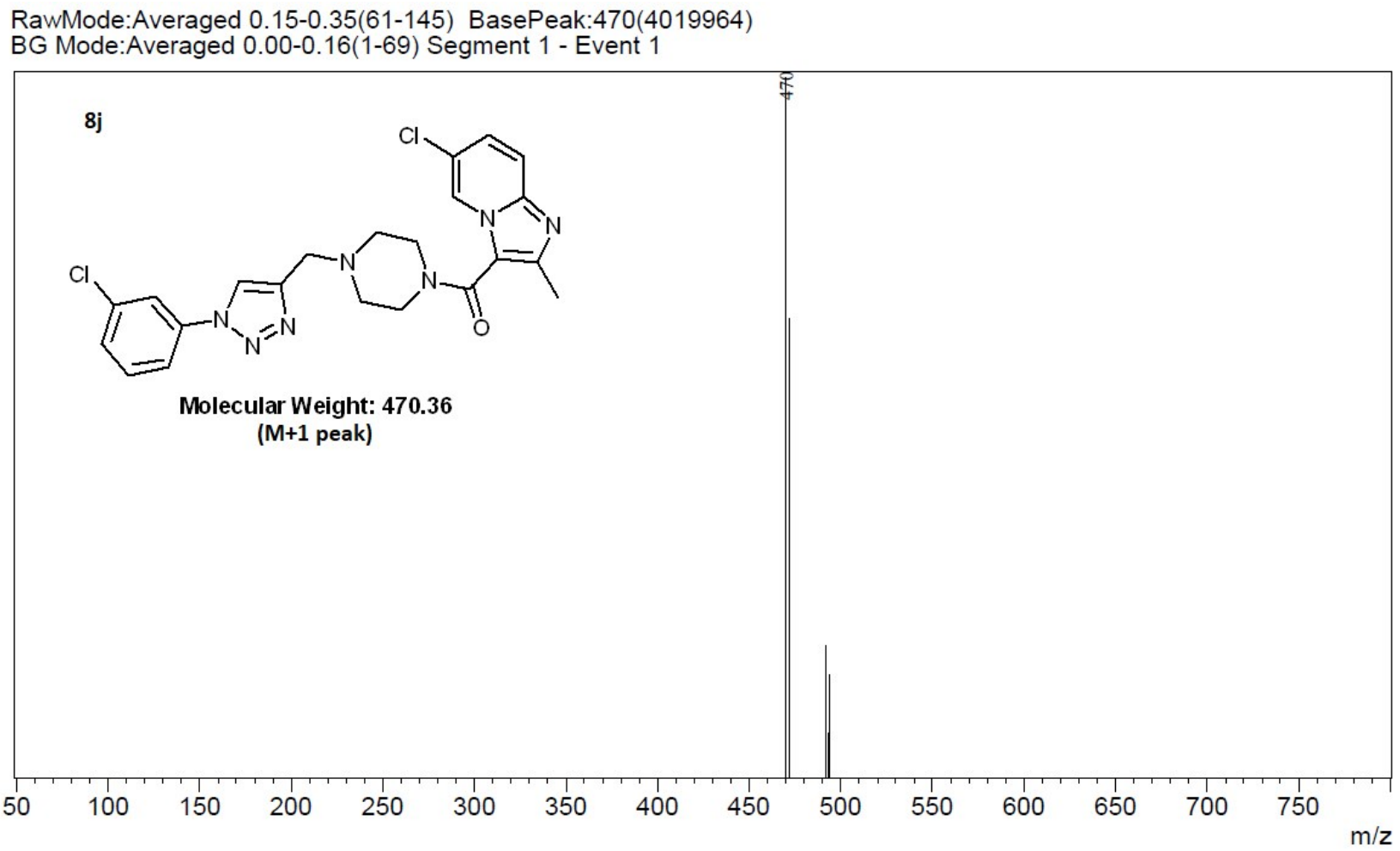
Mass spectra of compound 8g



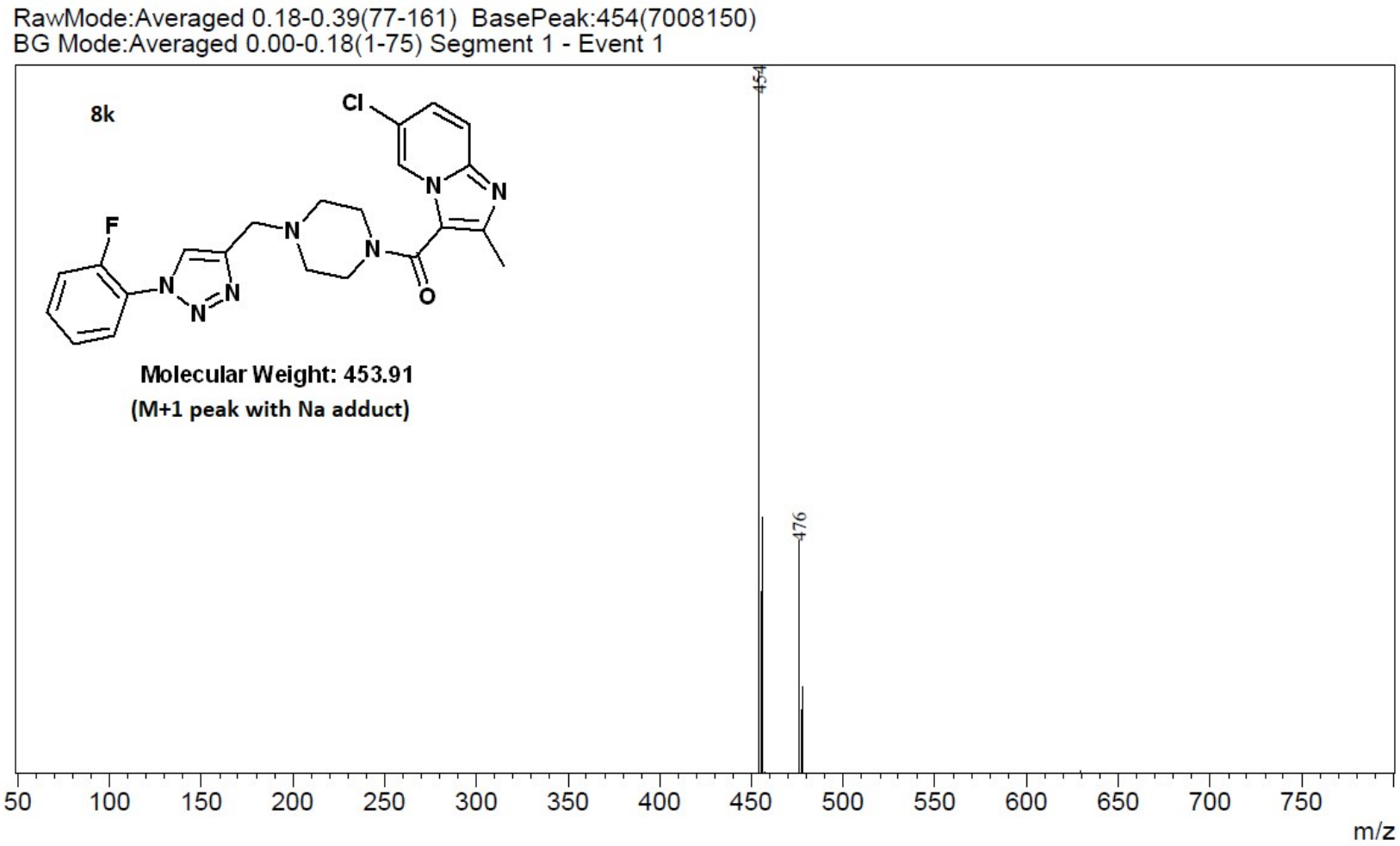
Mass spectra of compound 8h



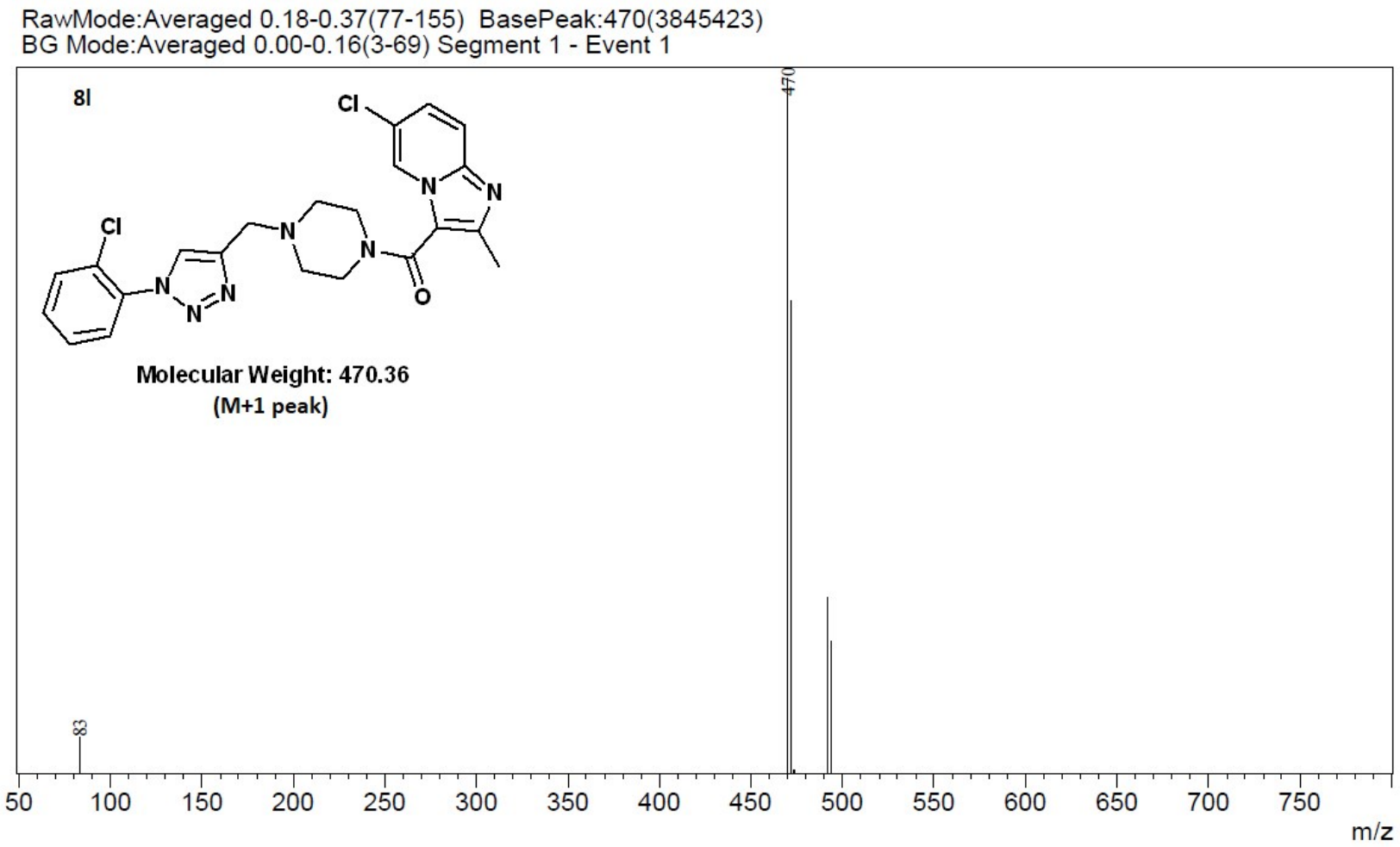
Mass spectra of compound 8i



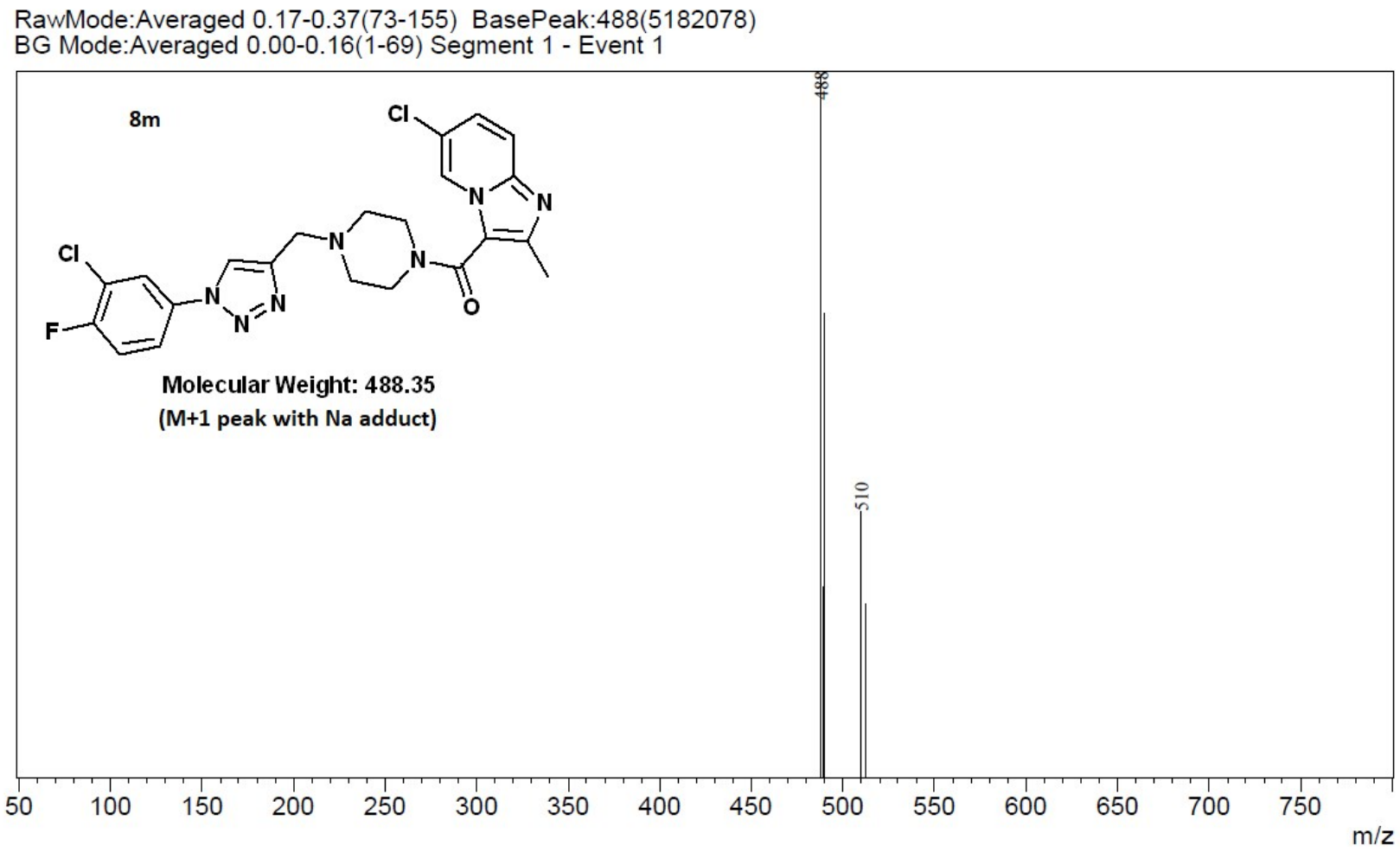
Mass spectra of compound 8j



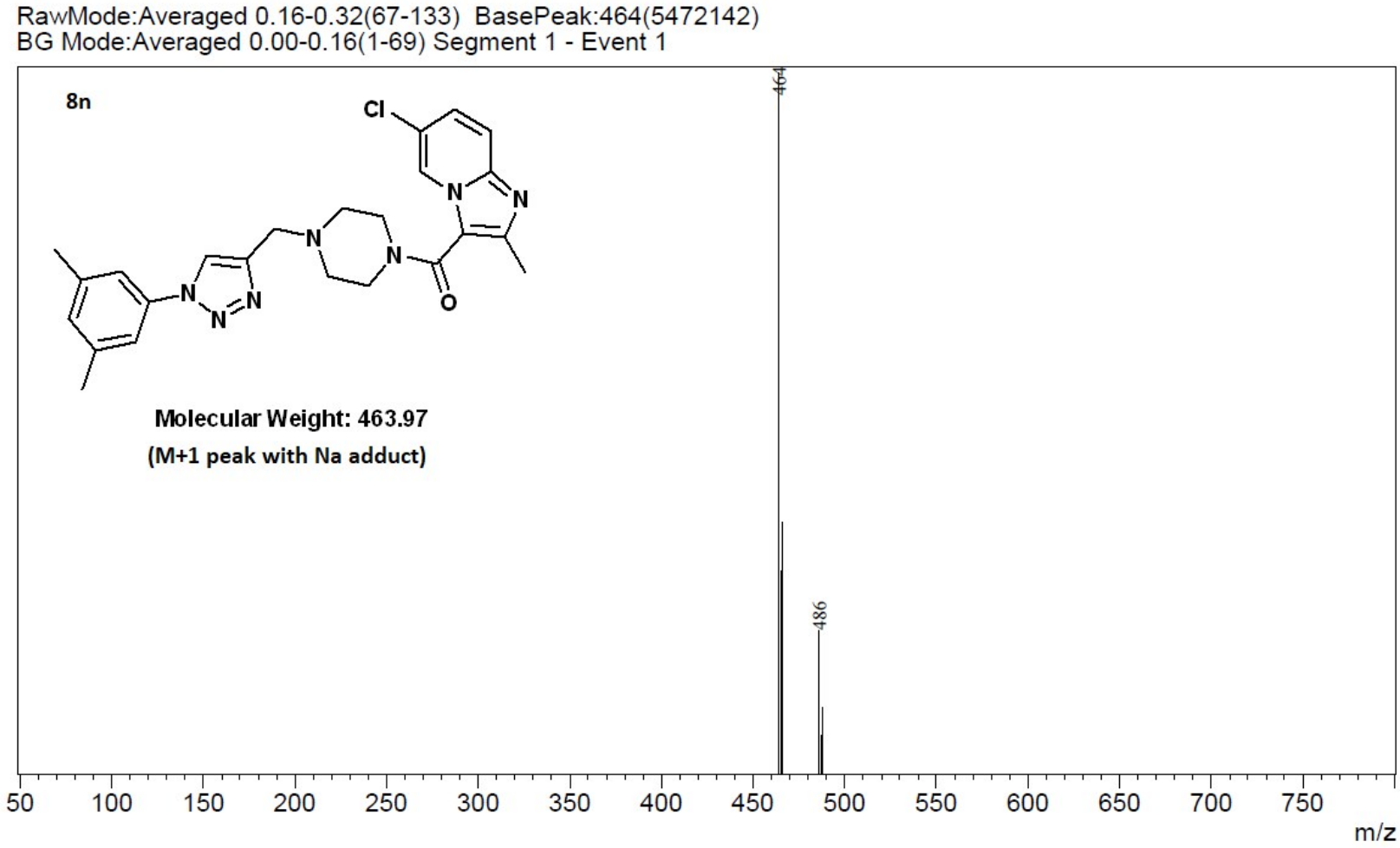
Mass spectra of compound 8k



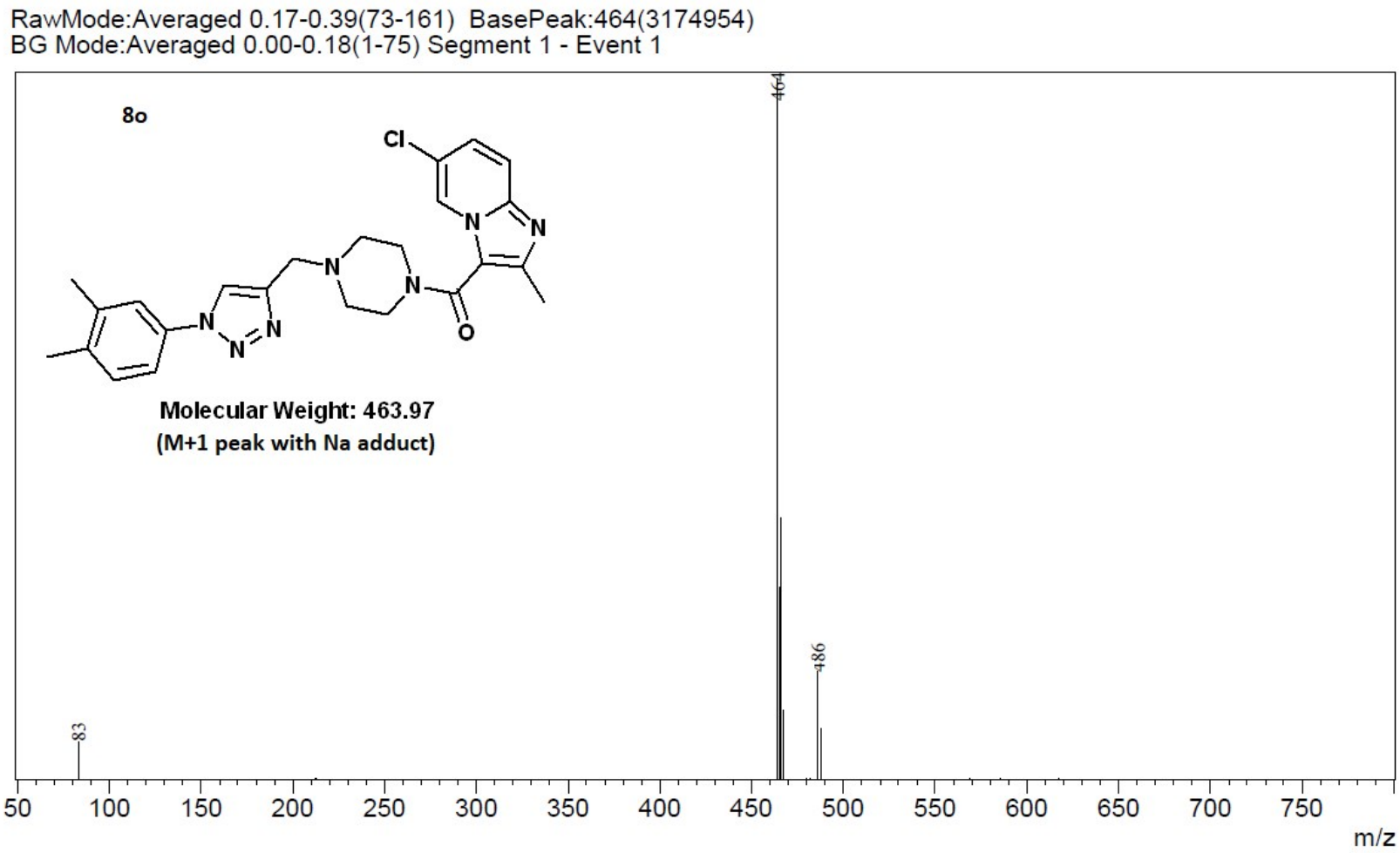
Mass spectra of compound 8l



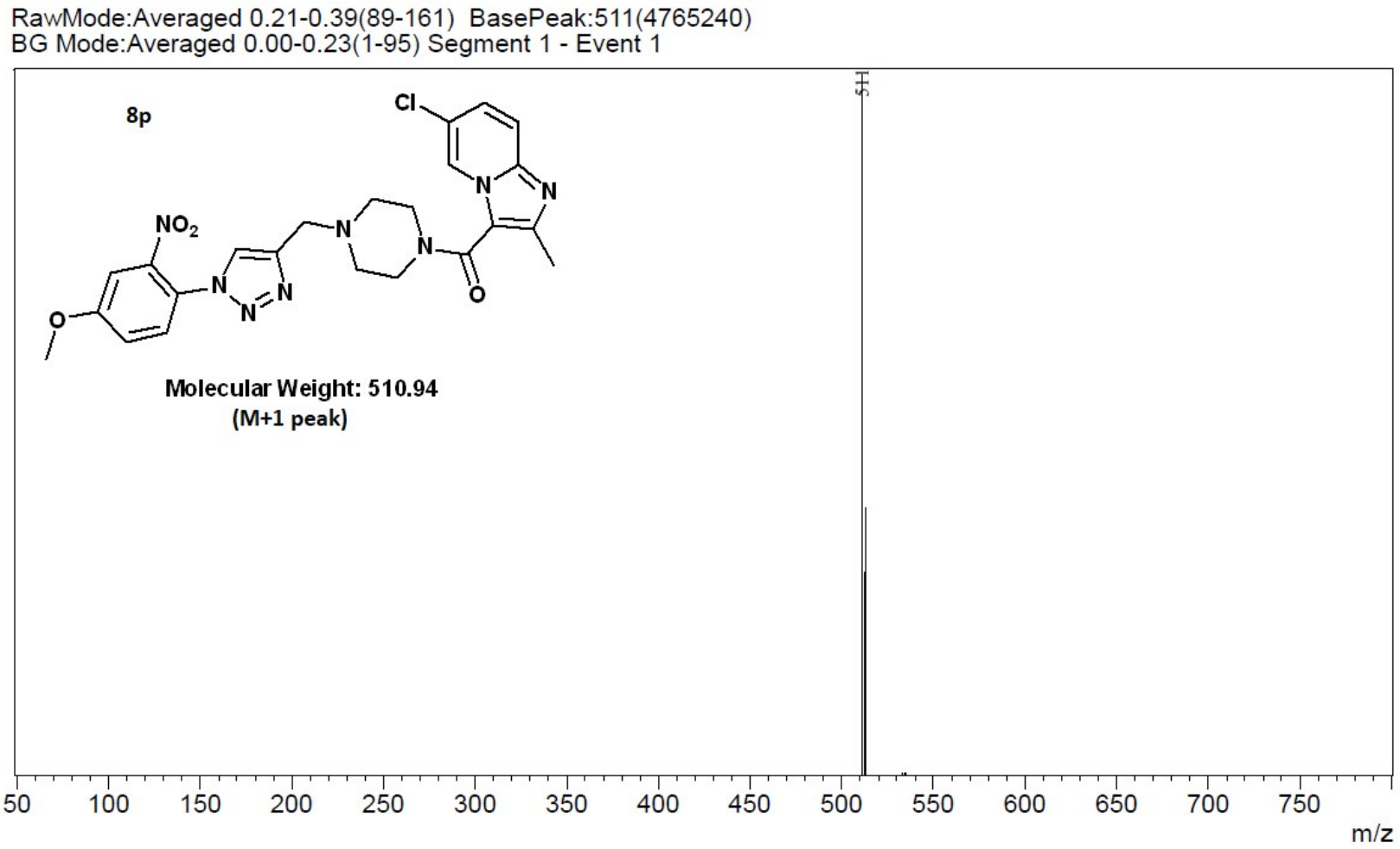
Mass spectra of compound 8m



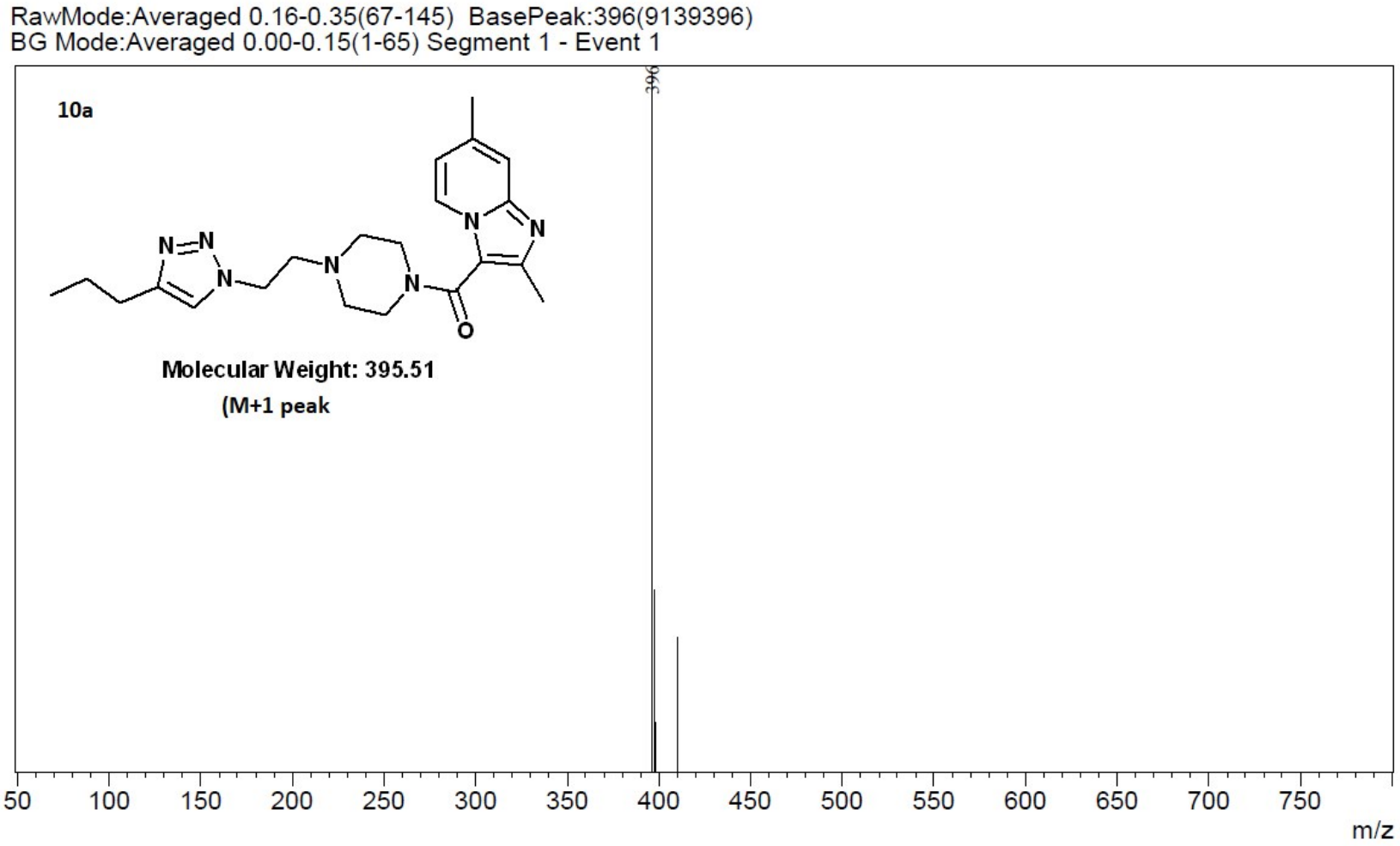
Mass spectra of compound 8n



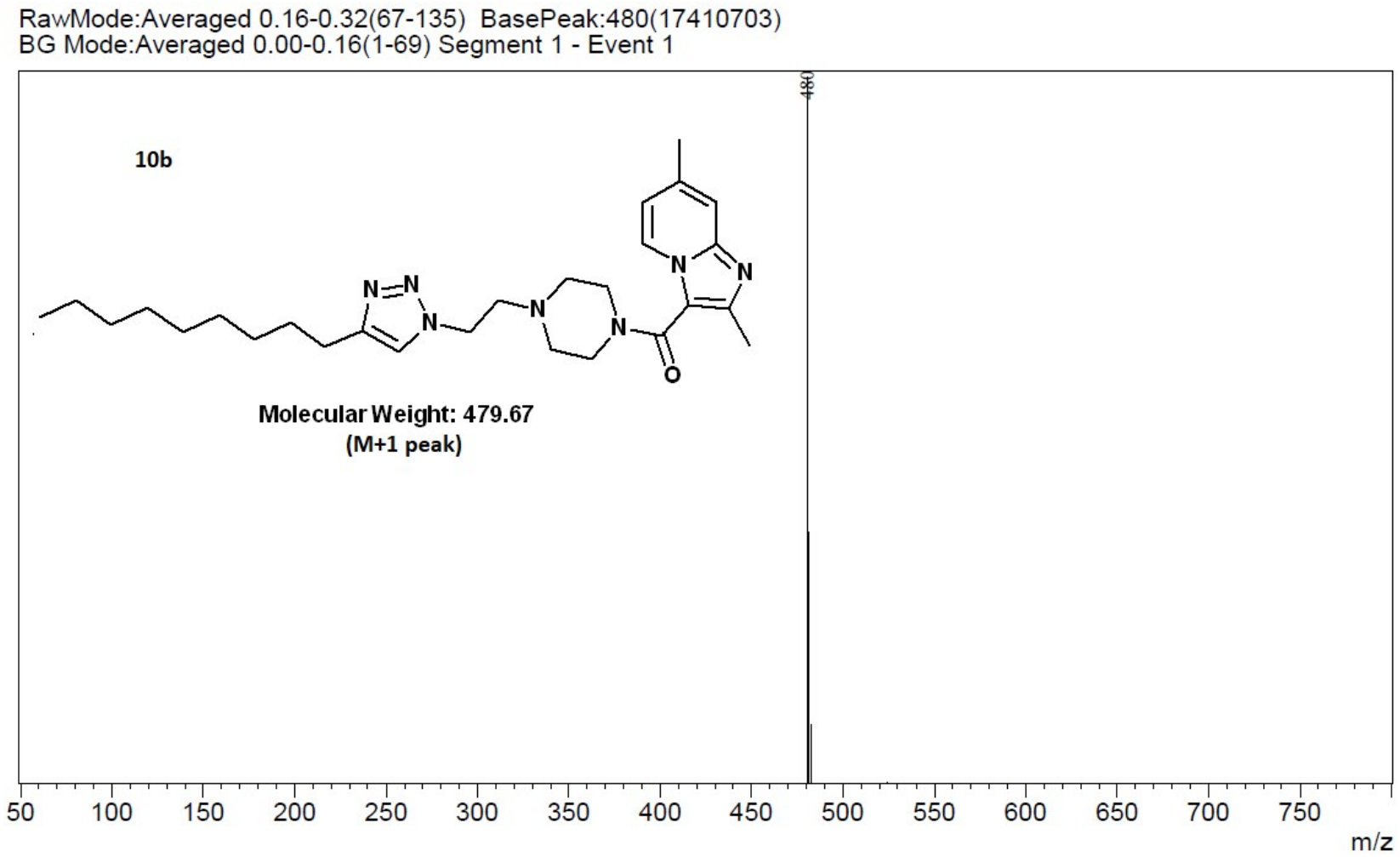
Mass spectra of compound 8o



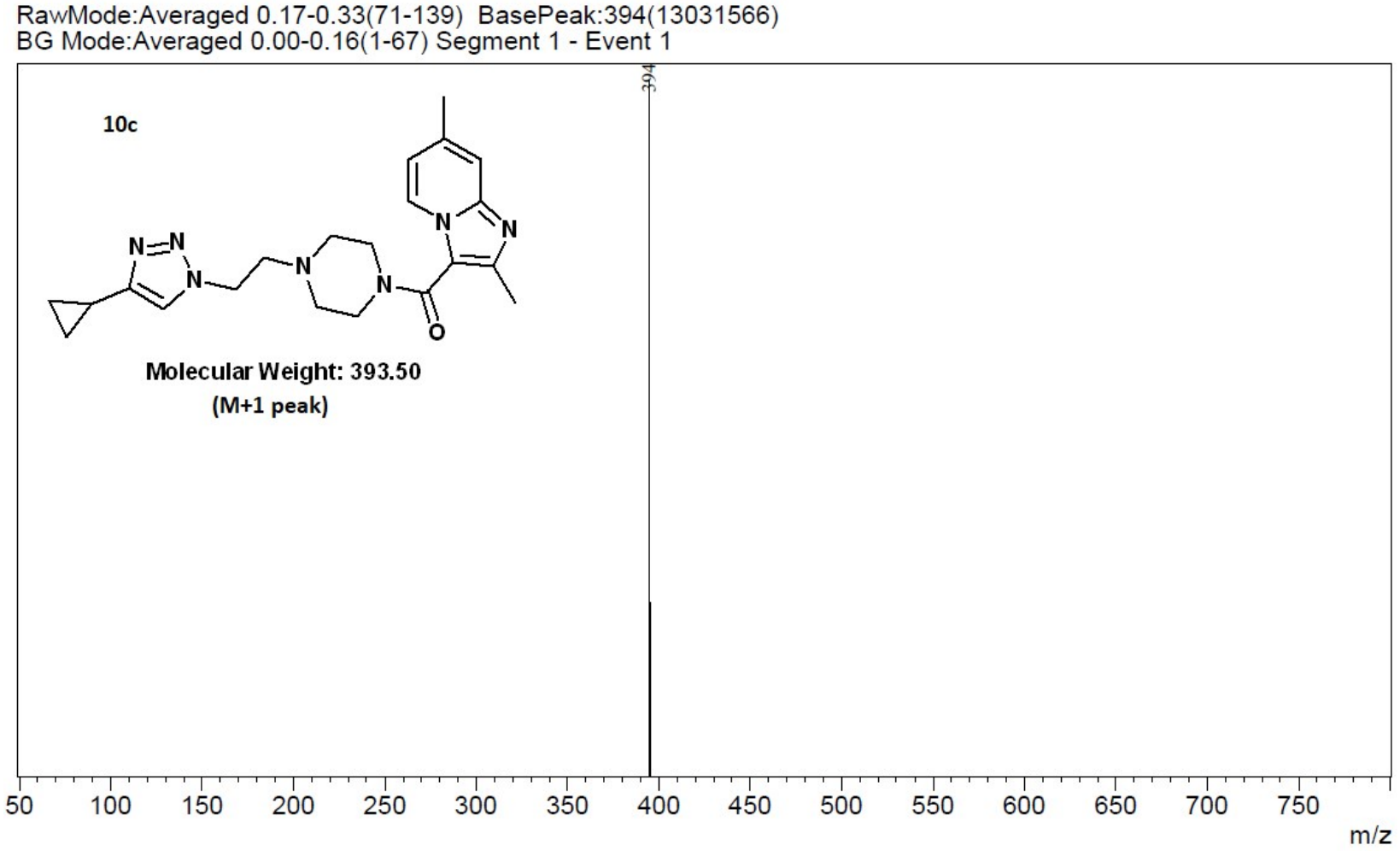
Mass spectra of compound 8p



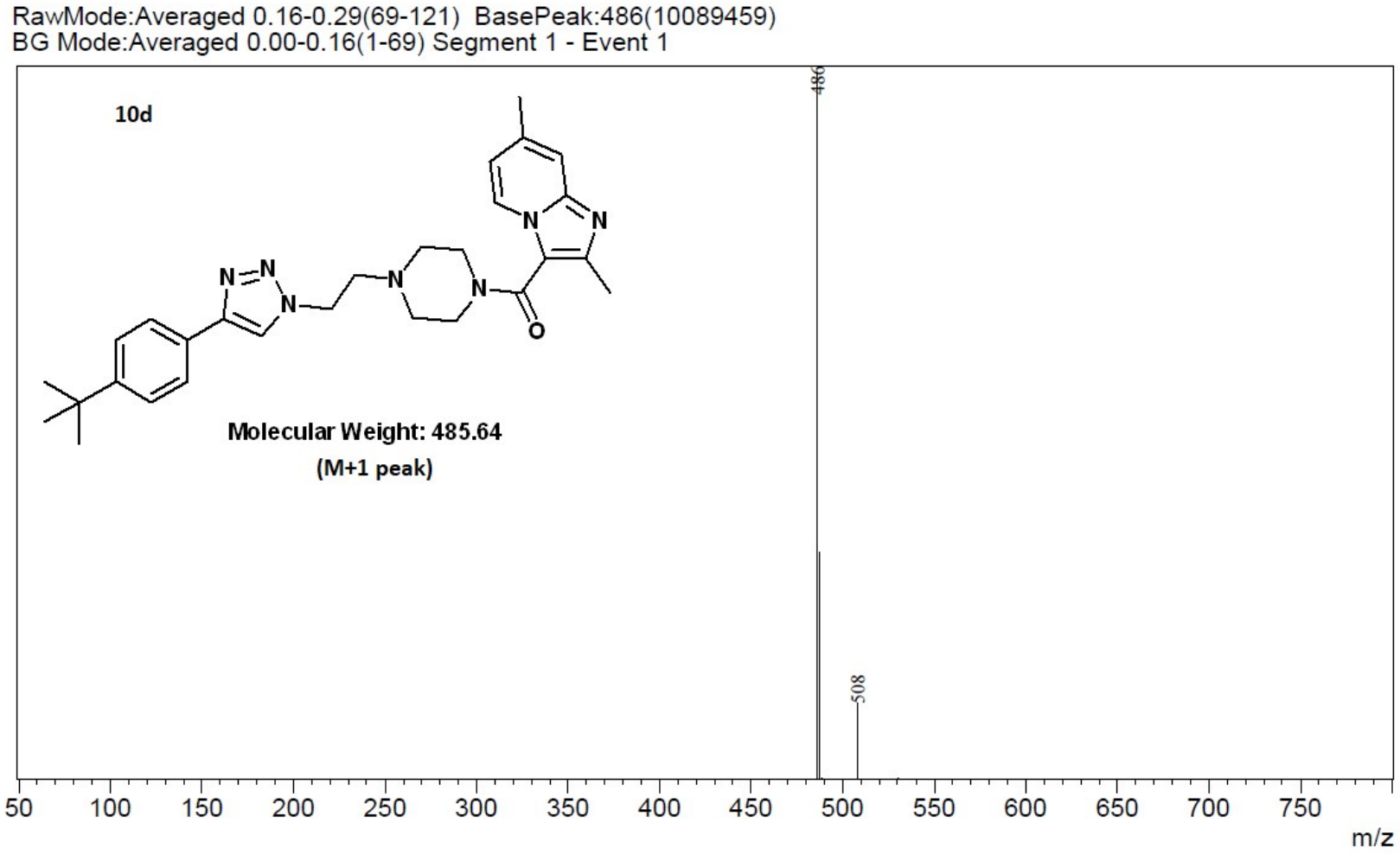
Mass spectra of compound 10a



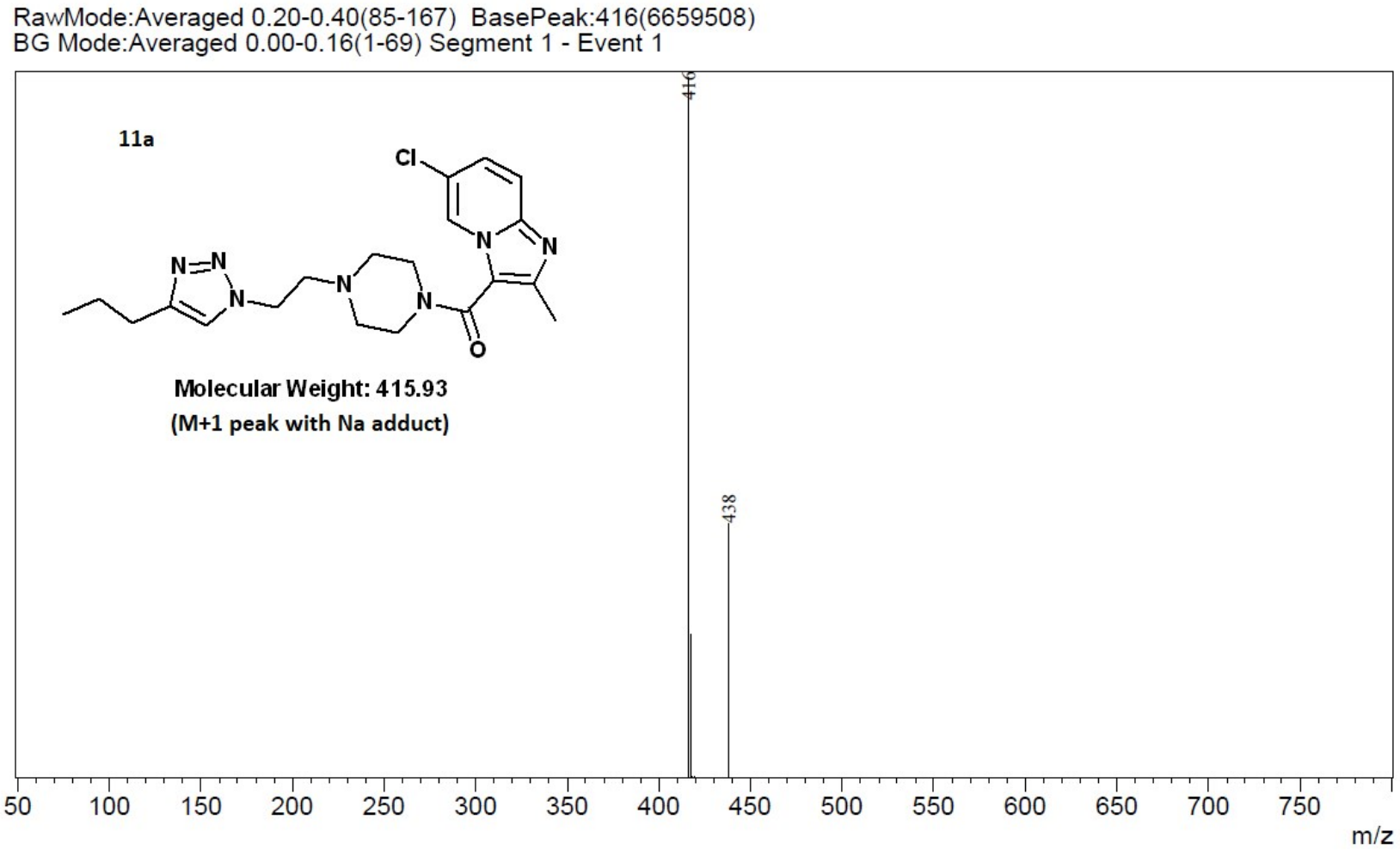
Mass spectra of compound 10b



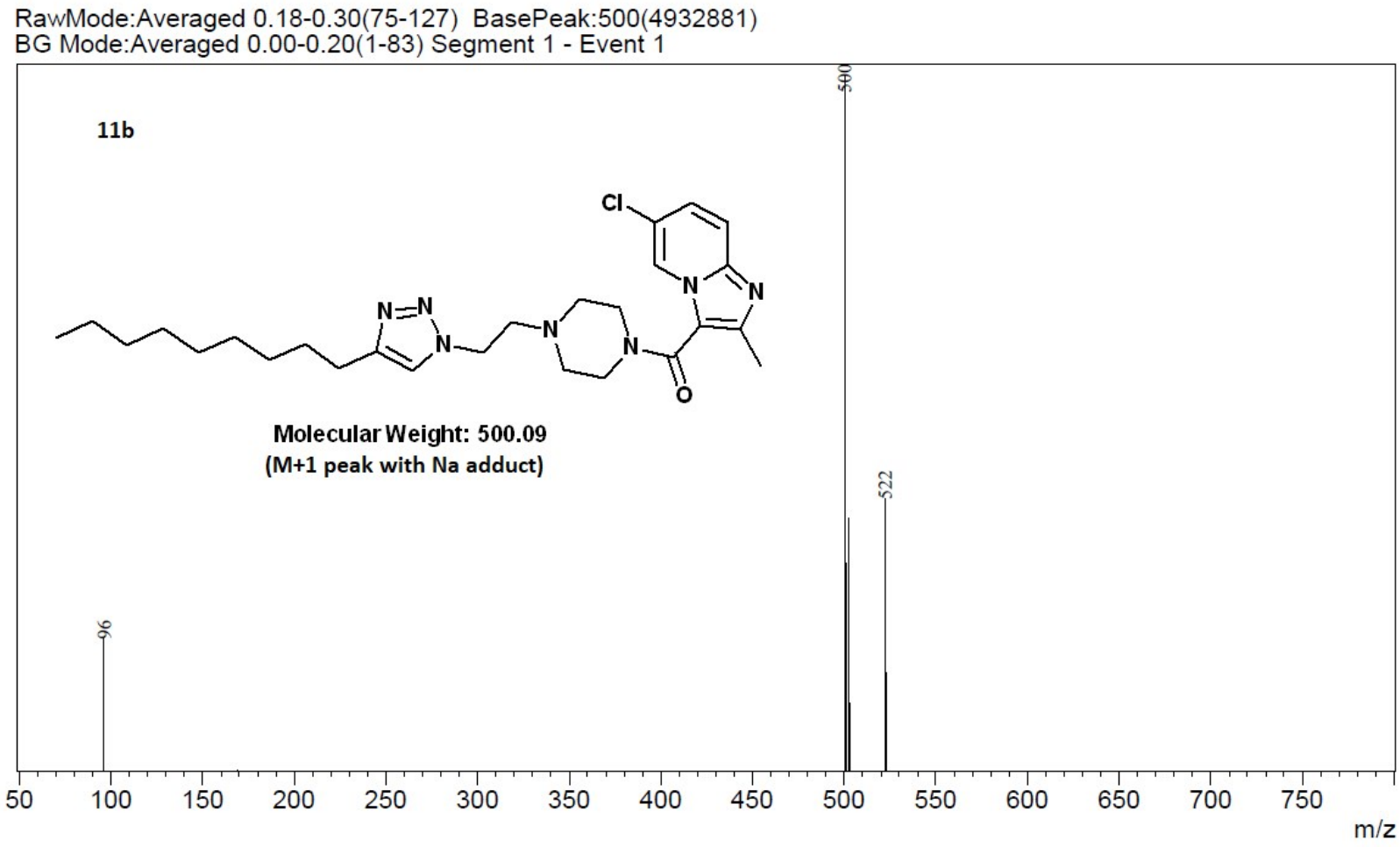
Mass spectra of compound 10c



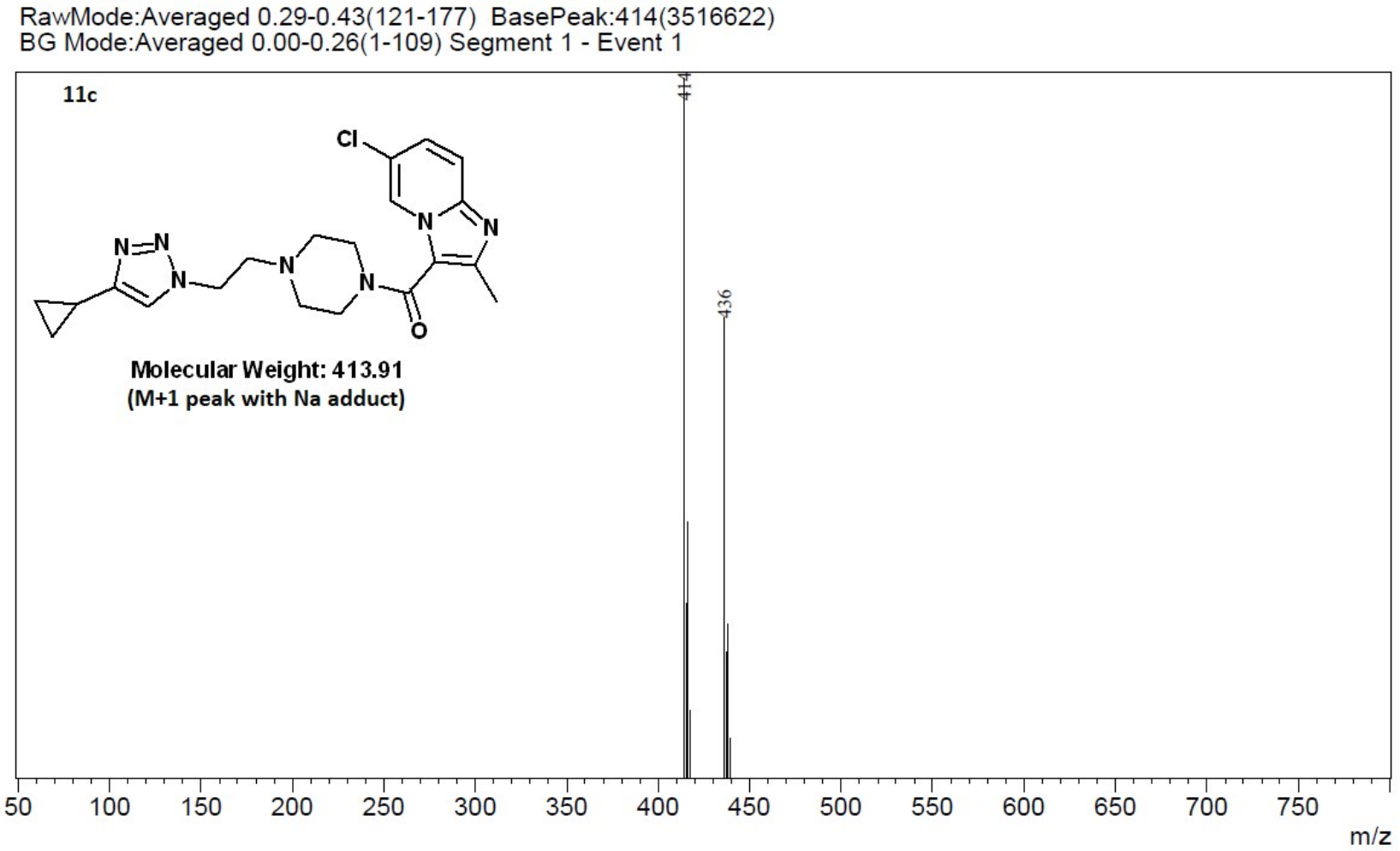
Mass spectra of compound 10d



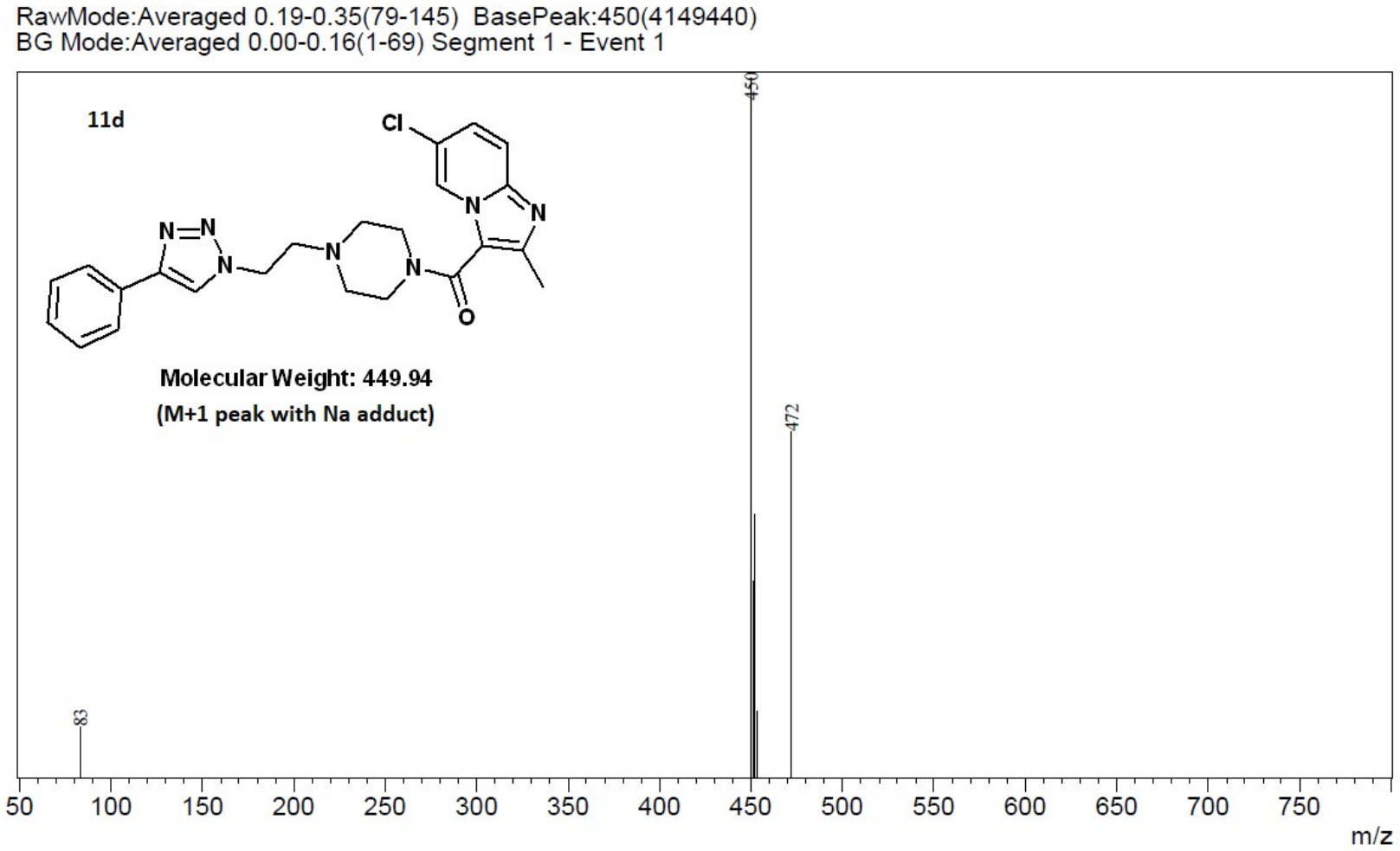
Mass spectra of compound 11a



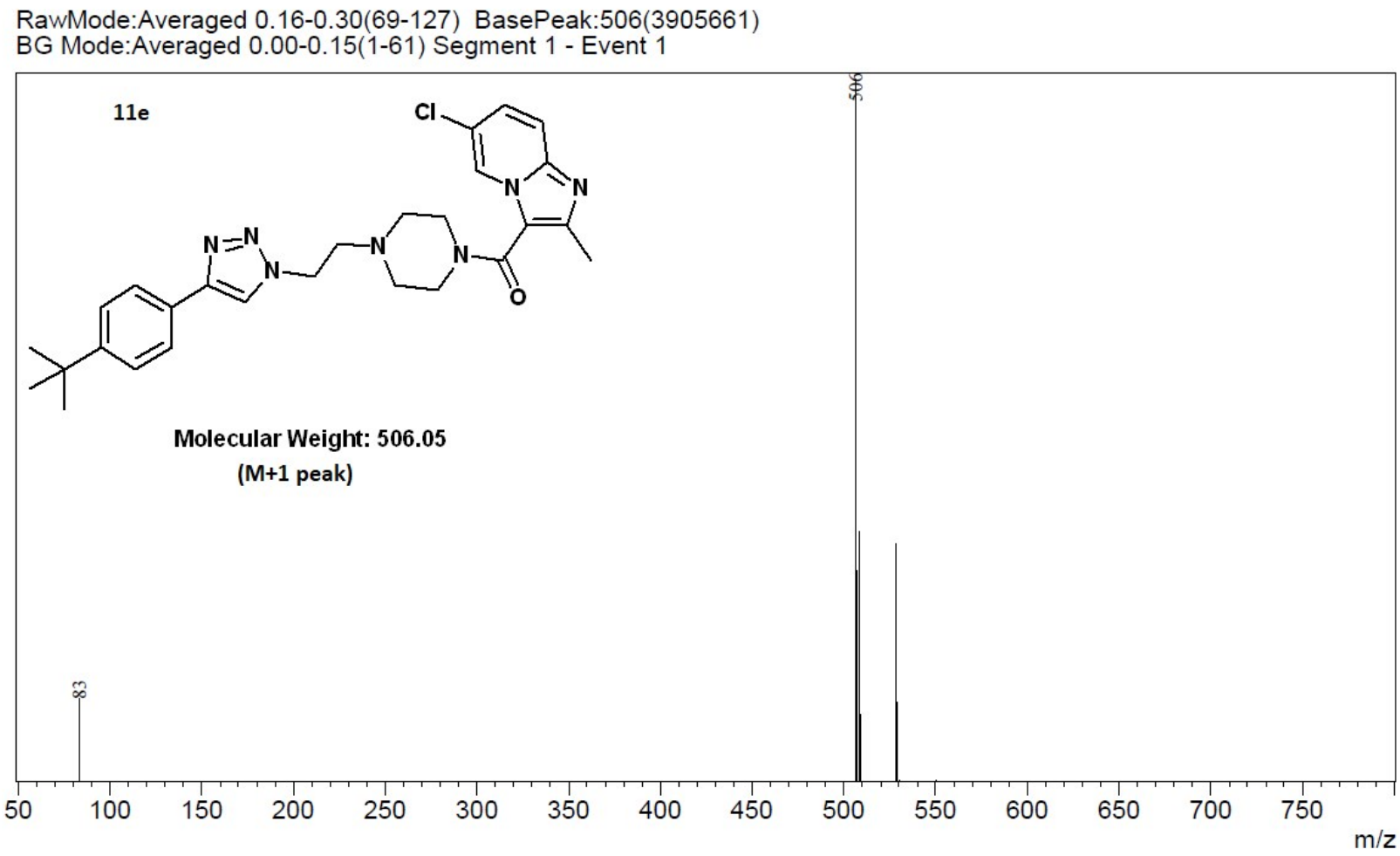
Mass spectra of compound 11b



Mass spectra of compound 11c



Mass spectra of compound 11d



Mass spectra of compound 11e

9. References

[1] P. Ashok, S. Chander, T. K. Smith, R. Prakash Singh, P. N. Jha and M. Sankaranarayanan, *Bioorg. Chem.,* 2019, **84**, 98–105.

[2] S. O. Oyola, K. J. Evans, T. K. Smith, B.A. Smith, J. D. Hilley, J. C. Mottram, P. M. Kaye and D. F. Smith, *PLoS One*, 2012, **7**, e51300.

[3] M. De Rycker, I. Hallyburton, J. Thomas, L. Campbell, S. Wyllie, D. Joshi, S. Cameron, I. H. Gilbert, P. G. Wyatt, J. A. Frearson, A. H. Fairlamb and D. W. Gray, *Antimicrob. Agents Chemother.*, 2013, **57,** 2913–2922.

[4] H. Hirumi and K. Hirumi, *The Journal of Parasitology*, 1989, **75**, 985-989.

[5] B. Rodenko, A. M. Van Der Burg, M. J. Wanner, M. Kaiser, R. Brun, M. Gould, H. P. De Koning and G. J. Koomen, *Antimicrob. Agents Chemother.*, 2007, **51**, 3796–3802.

[6] Schrödinger Release 2019-1: QikProp, Schrödinger, LLC, New York, NY, 2019.

[7] QikProp Descriptors and Properties PISA, 2015, 2–4.

[8] T. Sander, J. Freyss, M. Von Korff and C. Rufener, *J. Chem. Inf. Model.*, 2015, **55,** 460–473.

[9] Schrödinger Release 2019-1: Maestro, Schrödinger, LLC, New York, NY, 2019.

[10] S. K. Burley, H. M. Berman, C. Bhikadiya, C. Bi, L. Chen, L. Di Costanzo, C. Christie, K. Dalenberg, J. M. Duarte, S. Dutta, Z. Feng, S. Ghosh, D. S. Goodsell, R. K. Green, V. Guranović, D. Guzenko, B. P. Hudson, T. Kalro, Y. Liang, R. Lowe, H. Namkoong, E. Peisach, I. Periskova, A. Prlić, C. Randle, A. Rose, P. Rose, R. Sala, M. Sekharan, C. Shao, L. Tan, Y. P. Tao, Y. Valasatava, M. Voigt, J. Westbrook, J. Woo, H. Yang, J. Young, M. Zhuravleva and C. Zardecki, *Nucleic Acids Res.,* 2019, **47**, D464–D474.

[11] Schrödinger Release 2019-1: LigPrep, Schrödinger, LLC, New York, NY, 2019.

[12] P. Baiocco, G. Colotti, S. Franceschini and A. Ilari, *J. Med. Chem.*, 2009, **52**, 2603–2612.

[13] Schrödinger Release 2019-1: Schrödinger Suite 2019-1 Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2019.

[14] R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, D. E. Shaw, P. Francis and P. S. Shenkin, *J. Med. Chem.*, 2004, **47**, 1739–1749.