

Supporting Information

Novel Xanomeline-Containing Bitopic Ligands of Muscarinic Acetylcholine Receptors: Design, Synthesis and FRET Investigation

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² Institute for Molecular Cell Biology, Center for Molecular Biomedicine, University Hospital Jena, Friedrich Schiller University Jena, Hans Knoell Str. 2, 07745 Jena, Germany

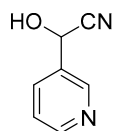
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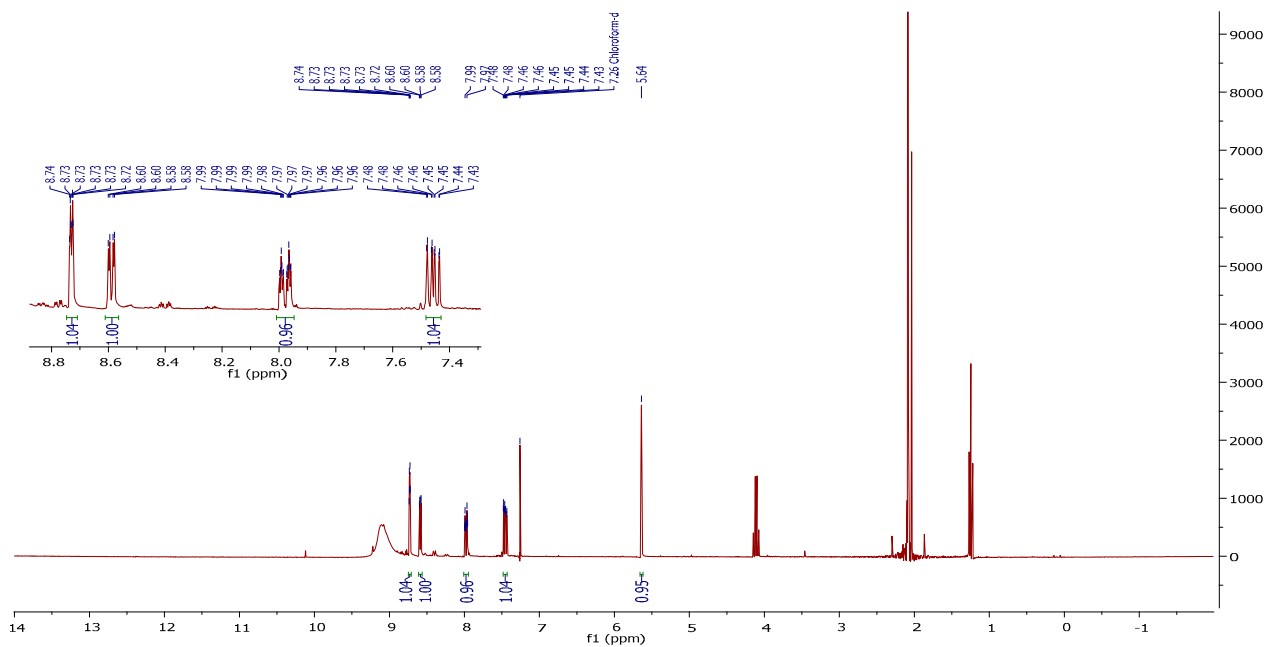
Table of contents

Compound 15	¹ H NMR, ¹³ C NMR	S3
Compound 16	¹ H NMR, ¹³ C NMR	S4
Compound 17	¹ H NMR, ¹³ C NMR	S5
Compound 18	¹ H NMR, ¹³ C NMR	S6
Compound 19	¹ H NMR, ¹³ C NMR	S7
Compound 10	¹ H NMR, ¹³ C NMR	S8
Compound 22-C3	¹ H NMR, ¹³ C NMR	S9
Compound 22-C5	¹ H NMR, ¹³ C NMR	S10
Compound 22-C7	¹ H NMR, ¹³ C NMR	S11
Compound 22-C9	¹ H NMR, ¹³ C NMR	S12
Compound 23-C3	¹ H NMR, ¹³ C NMR	S13
Compound 23-C5	¹ H NMR, ¹³ C NMR	S14
Compound 23-C7	¹ H NMR, ¹³ C NMR	S15
Compound 23-C9	¹ H NMR, ¹³ C NMR	S16
Compound 13-C3	¹ H NMR, ¹³ C NMR, HRMS	S17-S18
Compound 13-C5	¹ H NMR, ¹³ C NMR, HRMS	S19-S20
Compound 13-C7	¹ H NMR, ¹³ C NMR, HRMS	S21-S22
Compound 13-C9	¹ H NMR, ¹³ C NMR, HRMS	S23-S24
Compound 12-C3	¹ H NMR, ¹³ C NMR	S25
Compound 12-C3 Oxalate	¹ H NMR, ¹³ C NMR, Elemental analysis, HRMS.....	S26-S28
Compound 12-C5	¹ H NMR, ¹³ C NMR, HRMS	S29-S30
Compound 12-C7	¹ H NMR, ¹³ C NMR, HRMS	S31-S32
Compound 12-C9	¹ H NMR, ¹³ C NMR, HRMS	S33-S34

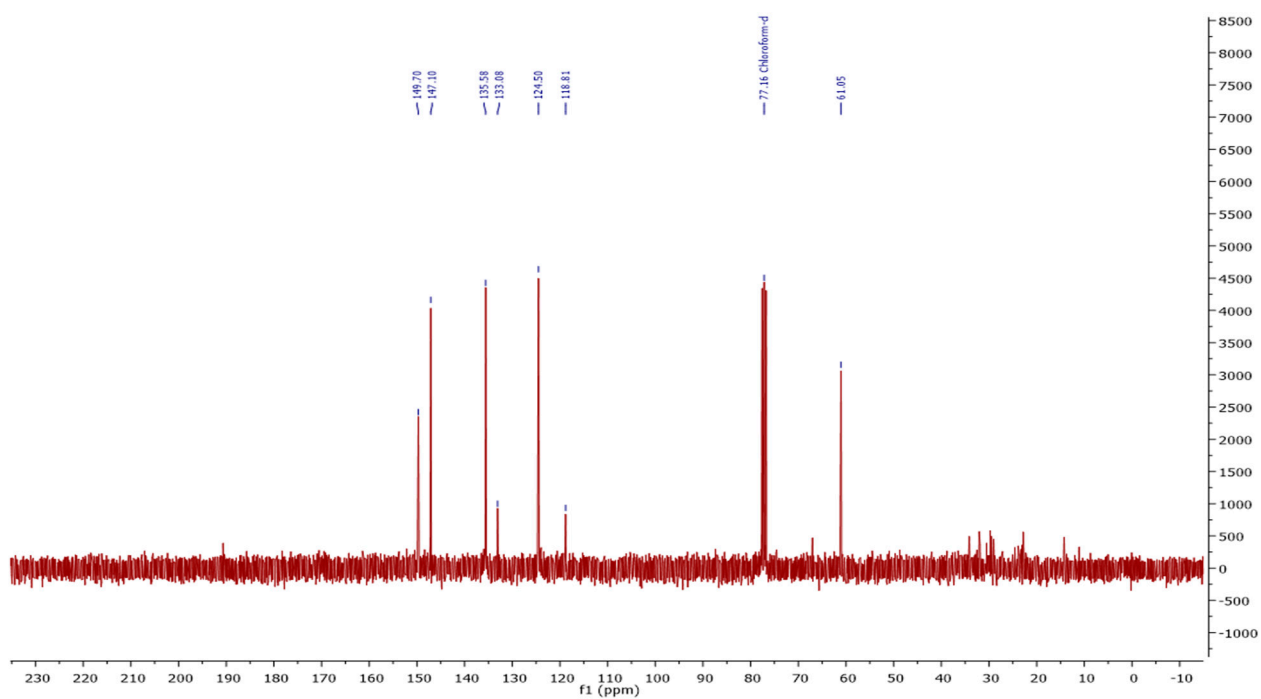
Compound 15



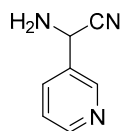
^1H NMR spectrum



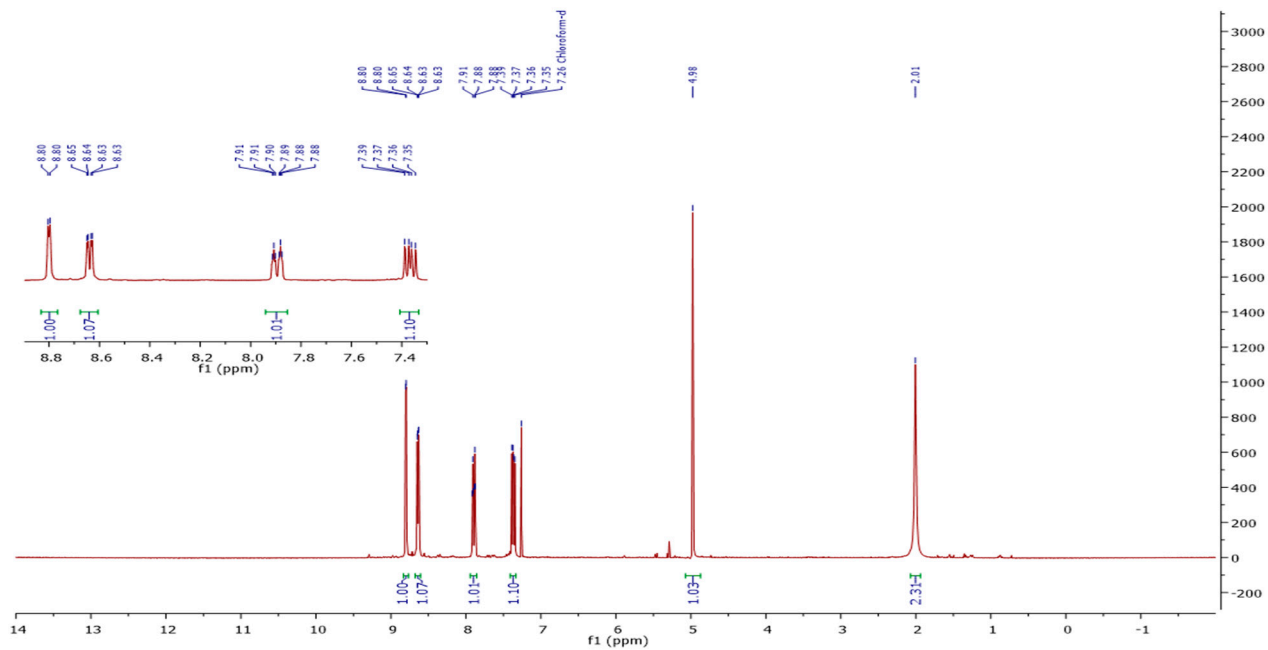
^{13}C NMR spectrum



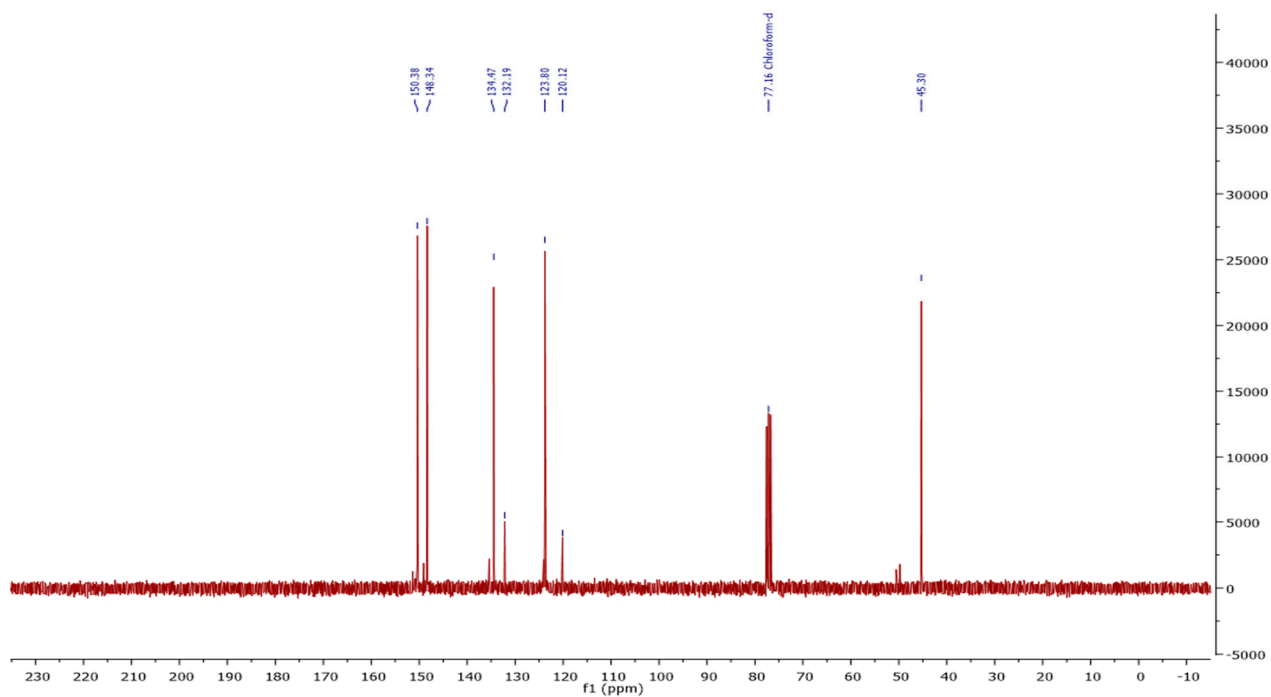
Compound 16



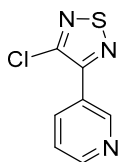
^1H NMR spectrum



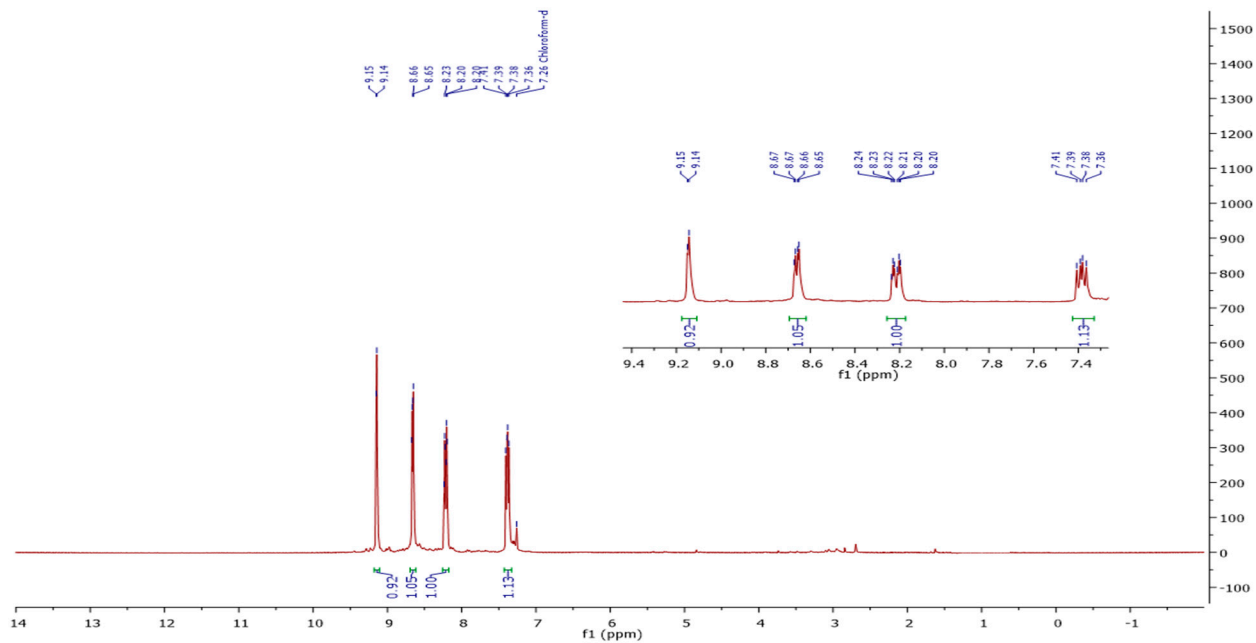
^{13}C NMR spectrum



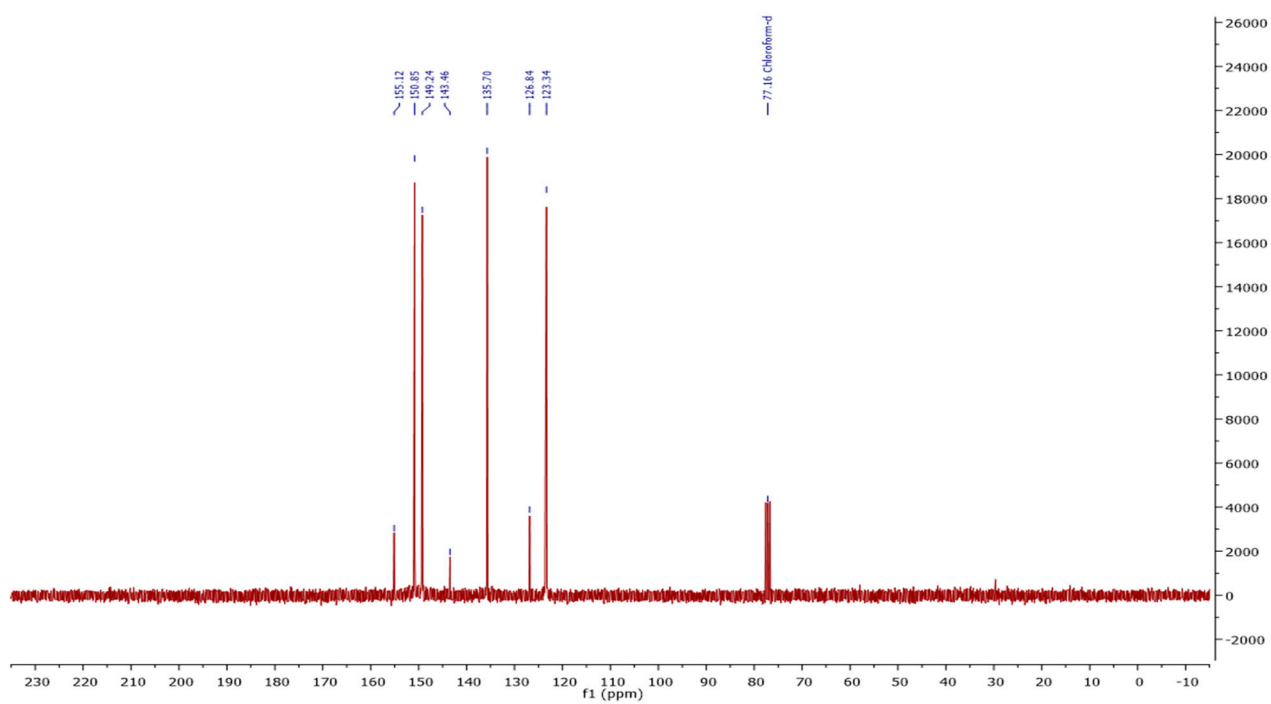
Compound 17



^1H NMR spectrum



^{13}C NMR spectrum

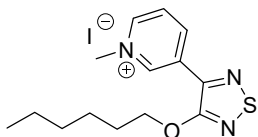


CCCCCOc1nc2ccccc2n1

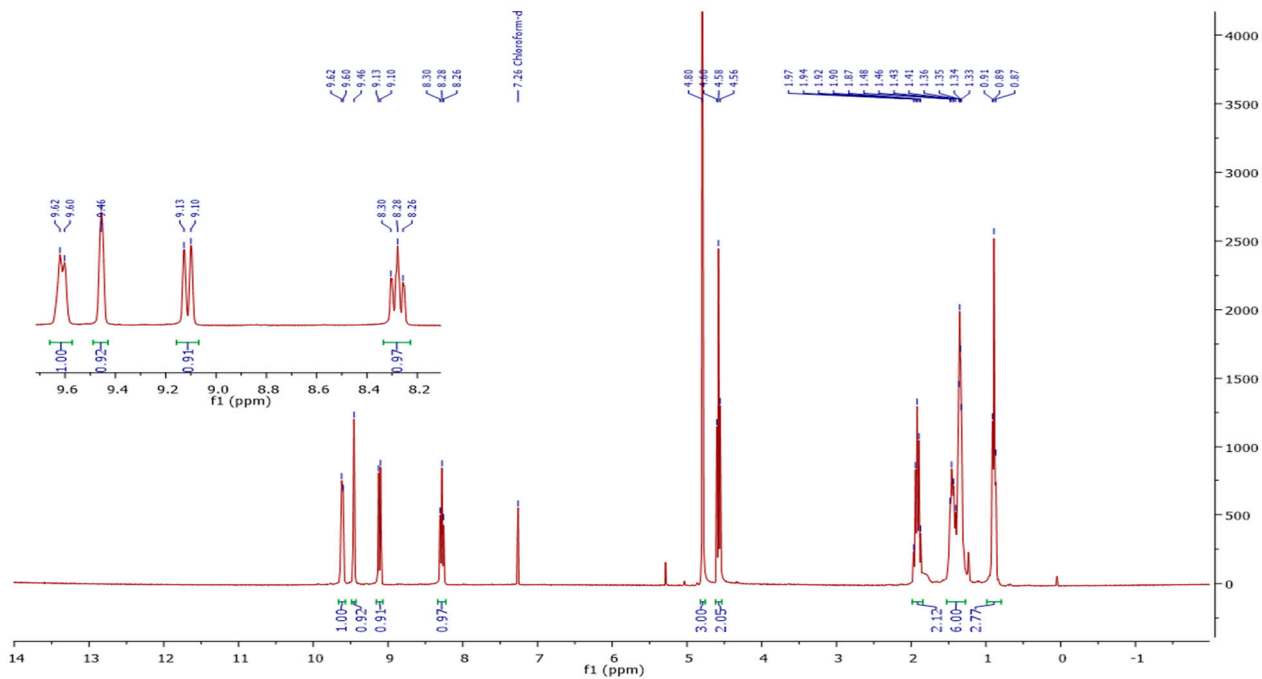
¹H NMR spectrum of compound 10 in CDCl₃. The spectrum shows peaks in the aromatic region (7.4-9.4 ppm) and aliphatic region (1.0-2.0 ppm). Integration values are provided for several peaks: 0.69, 0.77, 0.77, 0.86, 1.83, 1.88, 6.53, and 3.00. A solvent peak for CHCl₃ is visible at 7.26 ppm.

13C NMR spectrum of 77.16 Chloroform-d. The x-axis is chemical shift f1 (ppm) from 230 to -10. The y-axis is intensity from 0 to 30000. Peaks are labeled with their chemical shifts: 162.87, 150.13, 148.87, 144.57, 134.68, 127.69, 123.38, 77.16 Chloroform-d, 71.47, 31.46, 30.00, 25.71, 22.56, and 14.02.

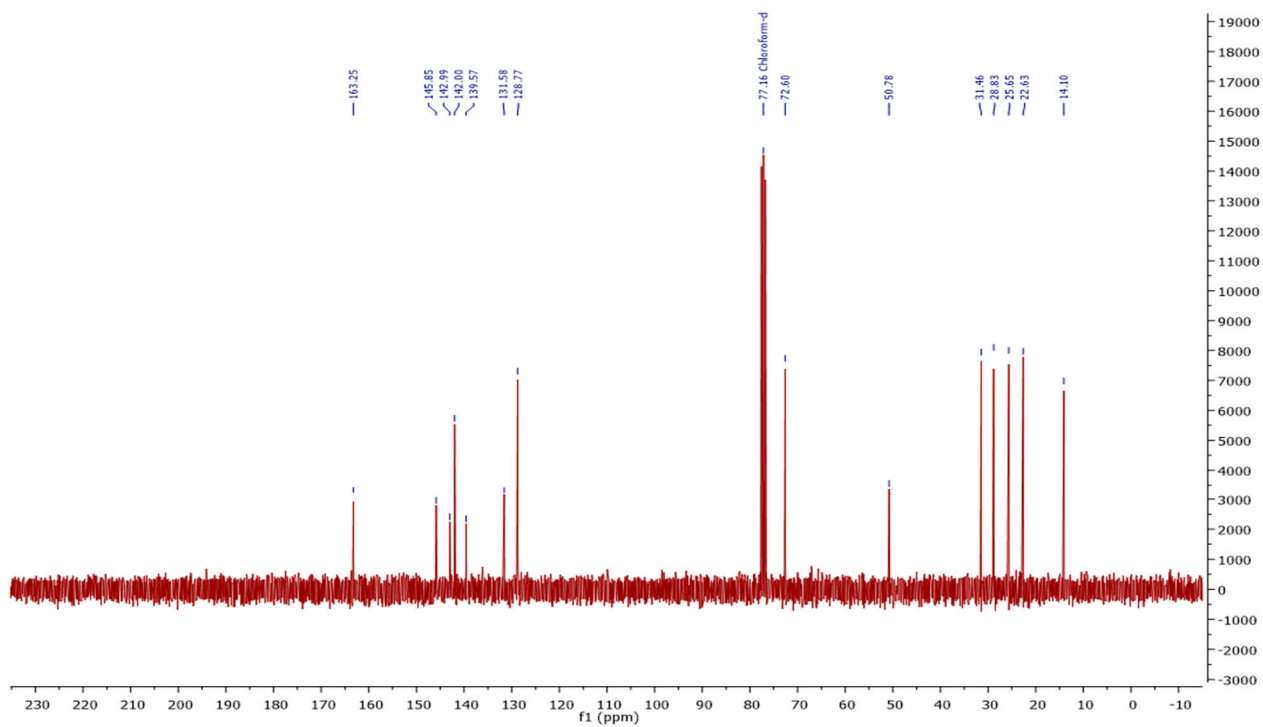
Compound 19



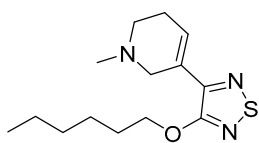
^1H NMR spectrum



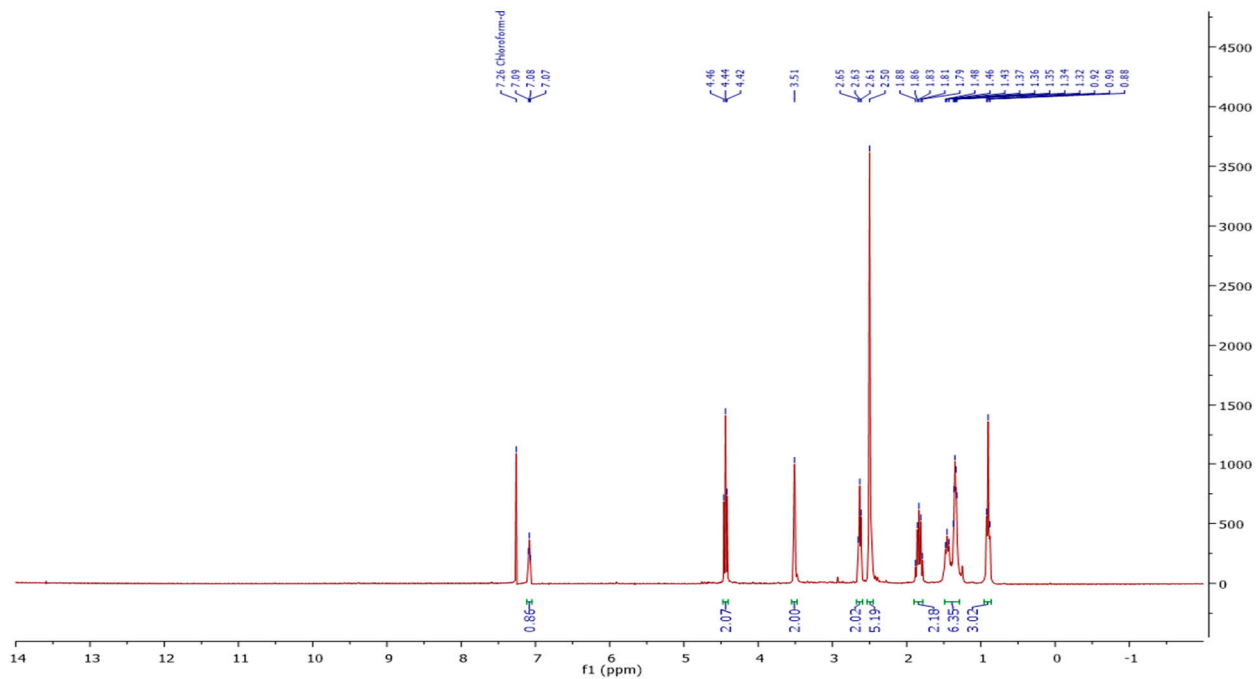
^{13}C NMR spectrum



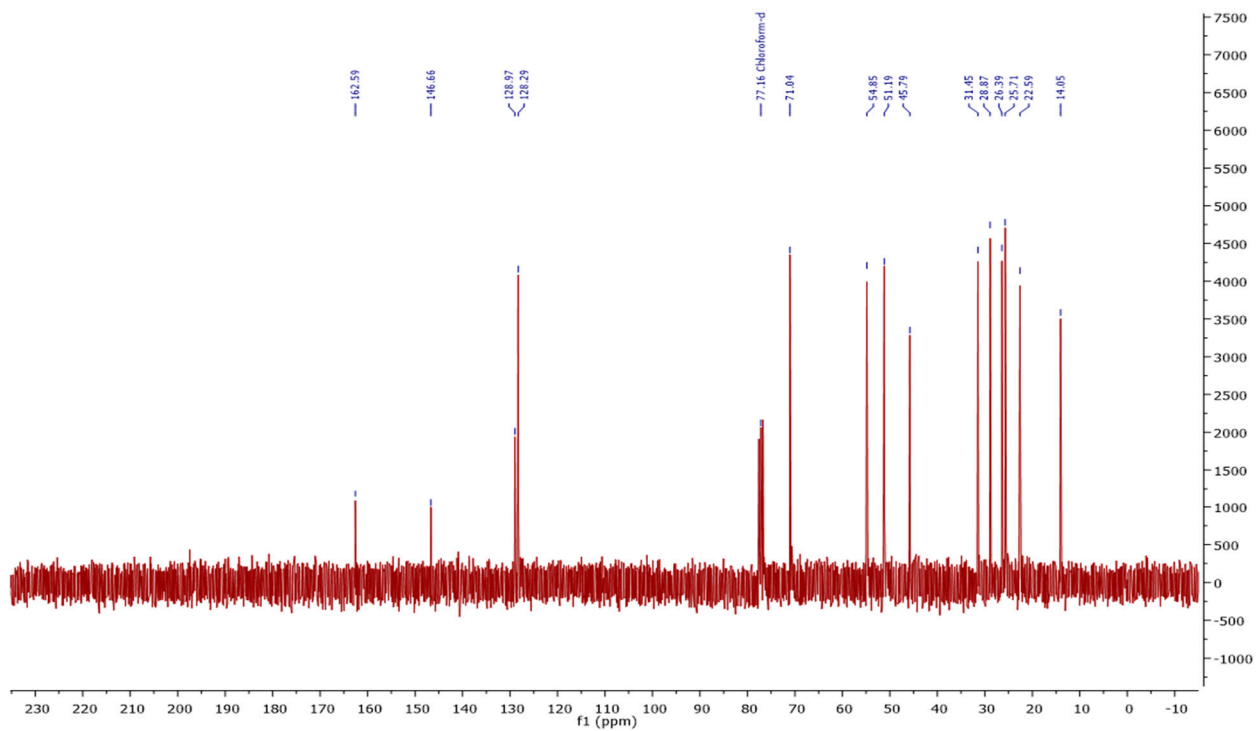
Compound 10



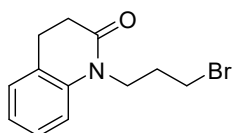
^1H NMR spectrum



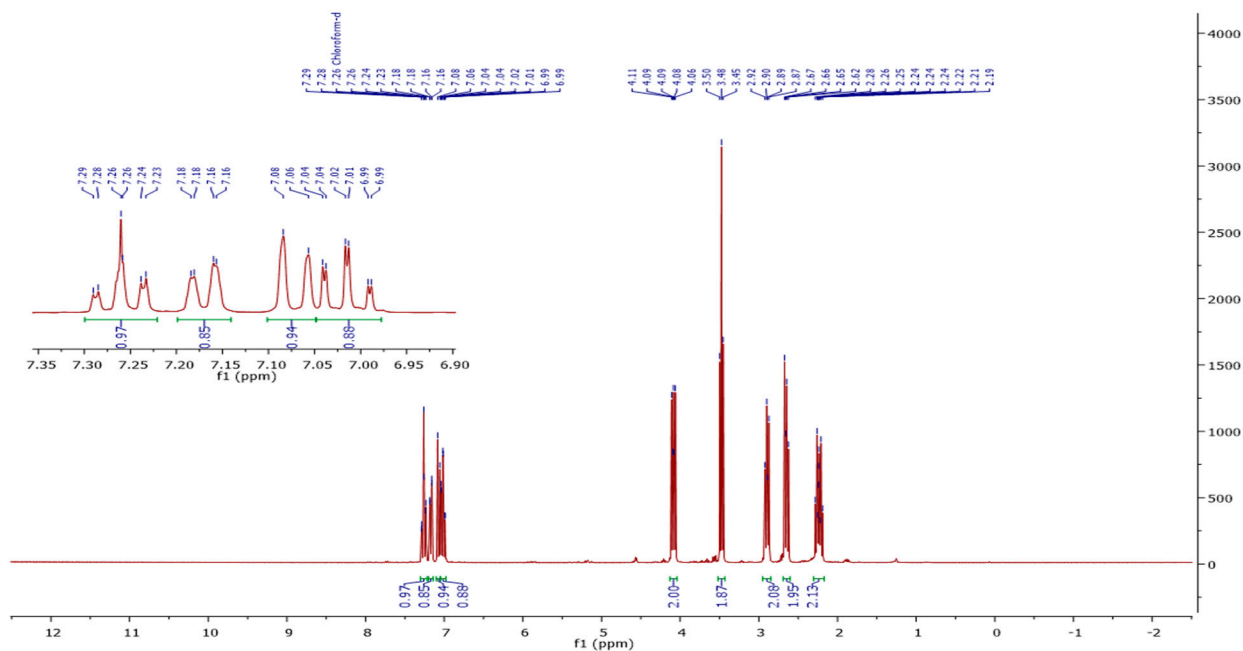
^{13}C NMR spectrum



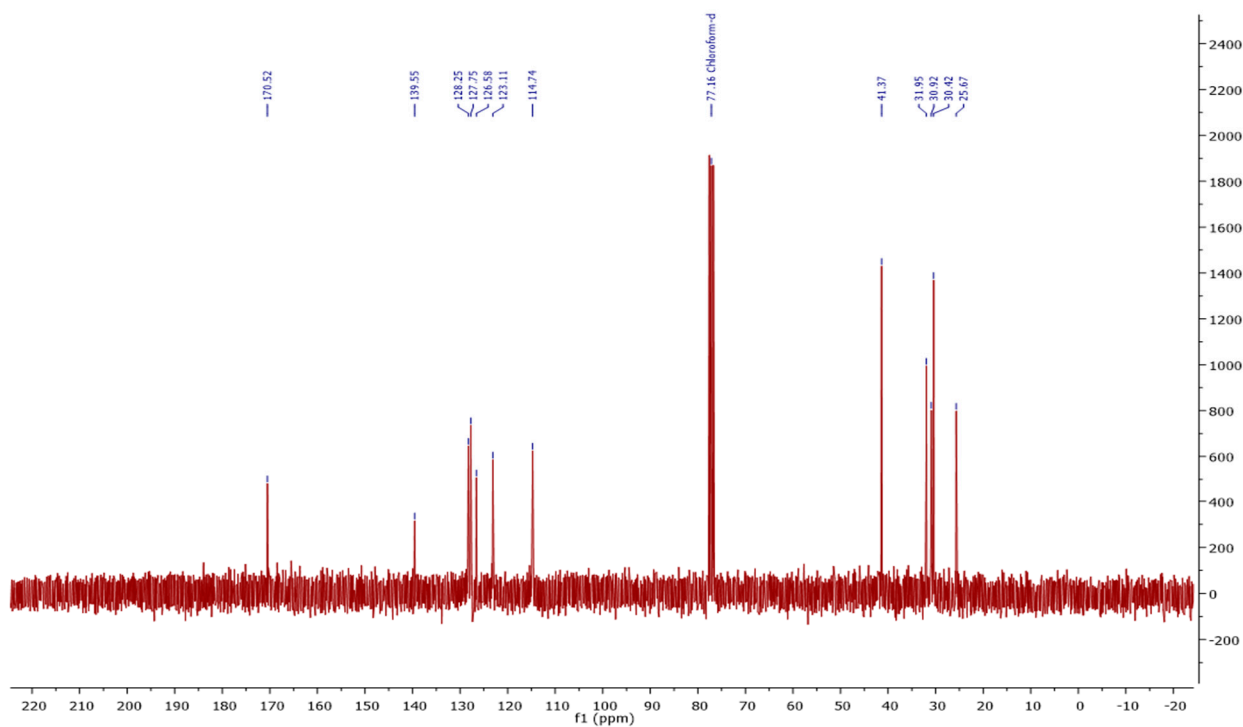
22-C3



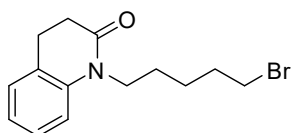
^1H NMR spectrum



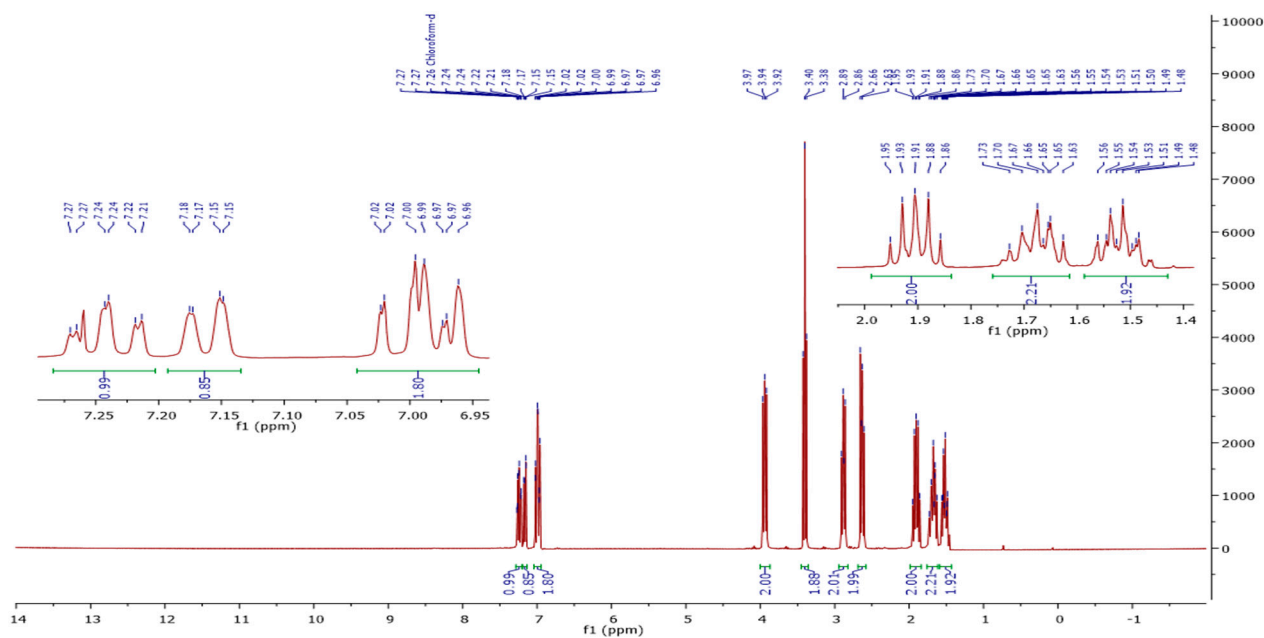
^{13}C NMR spectrum



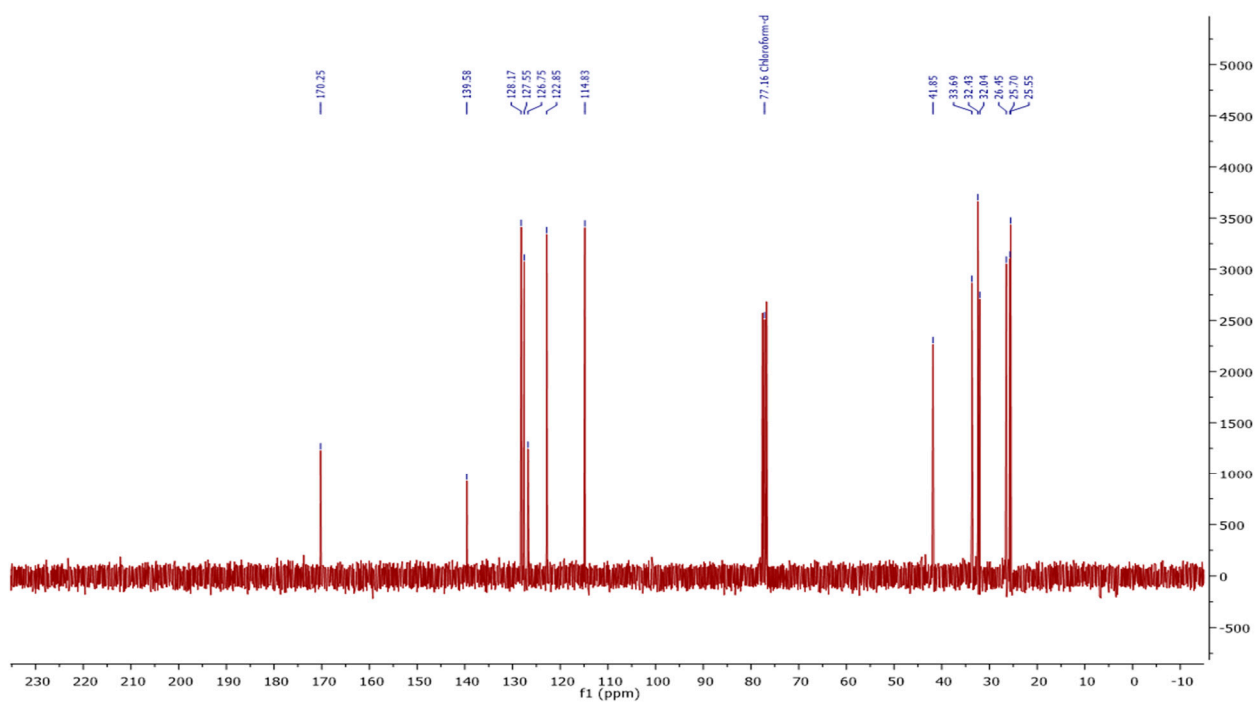
22-C5



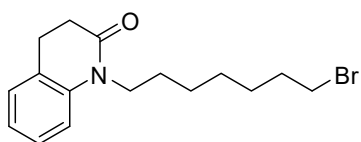
^1H NMR spectrum



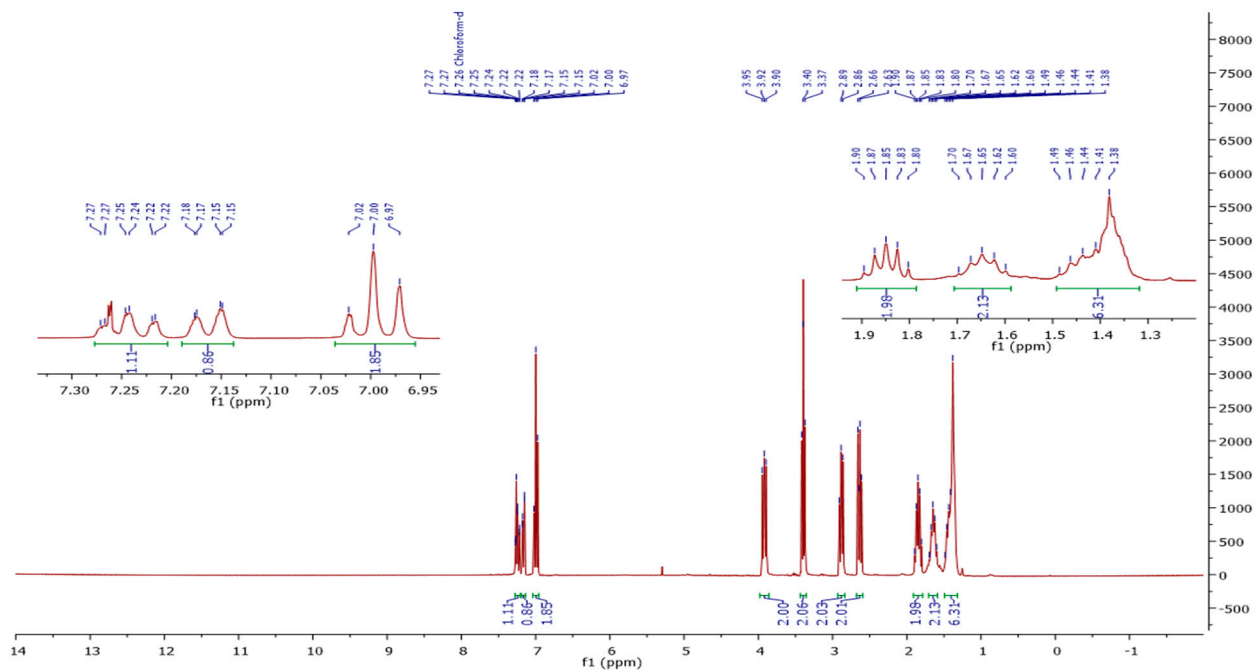
^{13}C NMR spectrum



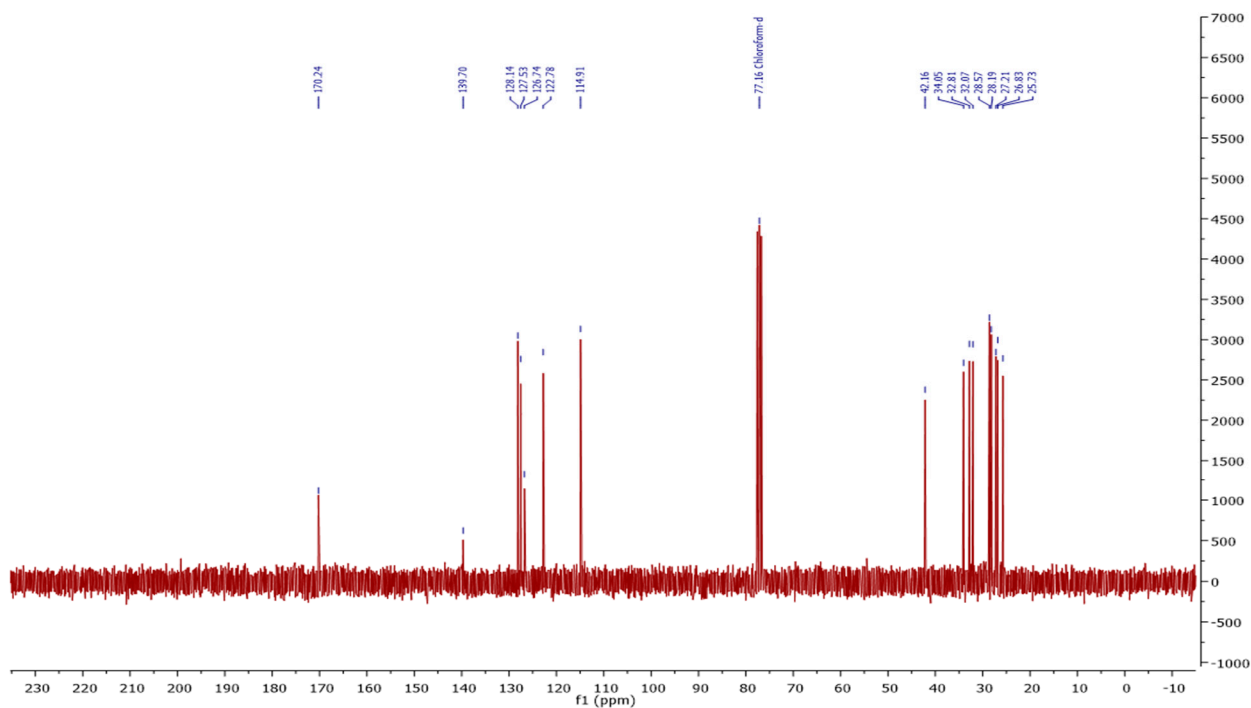
22-C7



^1H NMR spectrum



^{13}C NMR spectrum



BrCCCCCCCCN1C(=O)CCc2ccccc12

Figure 1 displays three ^1H NMR spectra of compound **1**. The top spectrum shows the full range from 14 to -1 ppm. The middle spectrum is an expansion of the aromatic region (7.35–6.95 ppm), and the bottom spectrum is an expansion of the aliphatic region (2.1–1.1 ppm). Integration values are provided for each peak group.

Chemical Shifts (ppm):

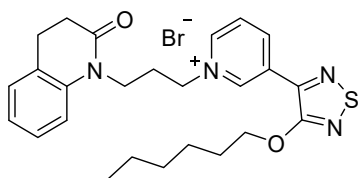
- Aromatic region (7.35–6.95 ppm):** 7.27, 7.24, 7.21, 7.17, 7.15, 7.02, 6.97.
- Aliphatic region (2.1–1.1 ppm):** 1.89, 1.87, 1.84, 1.82, 1.80, 1.66, 1.64, 1.62, 1.61, 1.59, 1.41, 1.39, 1.32, 1.31, 1.30, 1.26.

Integration values:

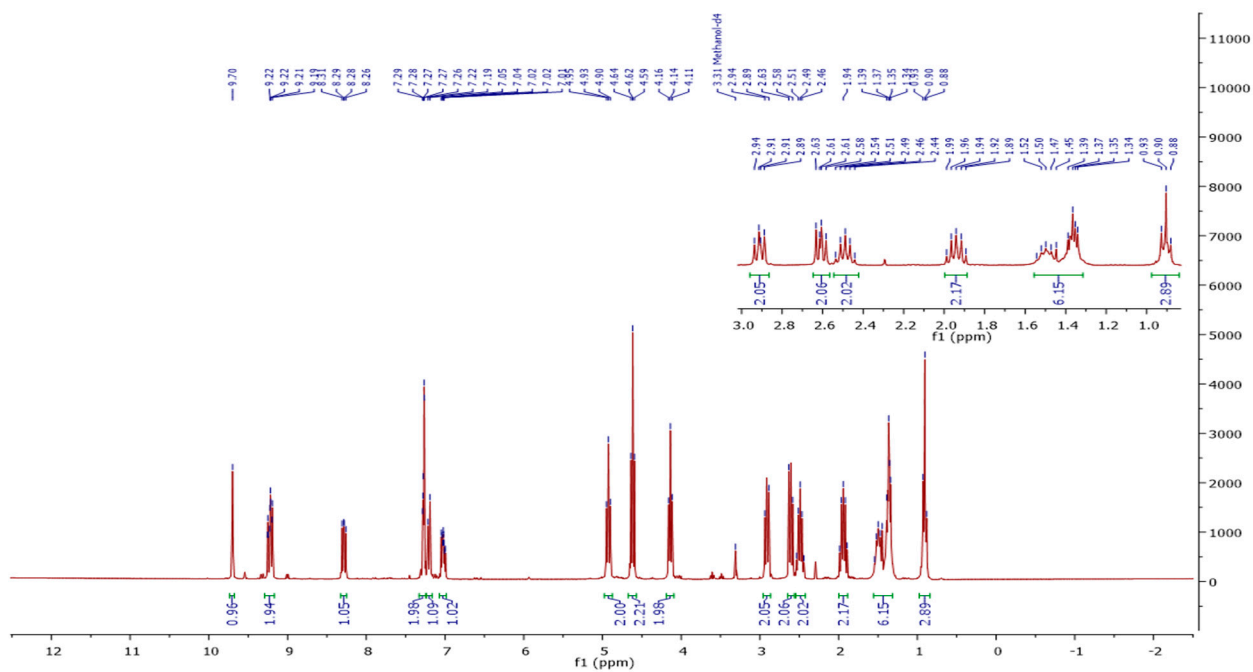
- Aromatic region (7.35–6.95 ppm):** 1.18, 0.87, 1.86.
- Aliphatic region (2.1–1.1 ppm):** 1.89, 2.36, 10.20.

13C NMR spectrum of 1,3-bis(4-chlorophenyl)propan-2-ol in CDCl₃. The spectrum shows peaks at 170.22, 139.78, 128.12, 127.52, 126.77, 122.75, 114.95, 77.16 (CDCl₃), 42.26, 34.13, 32.84, 32.10, 29.46, 29.24, 28.80, 28.27, 27.32, 26.99, and 25.76 ppm. The x-axis is labeled 'f1 (ppm)' and ranges from 230 to -10. The y-axis ranges from -500 to 6000.

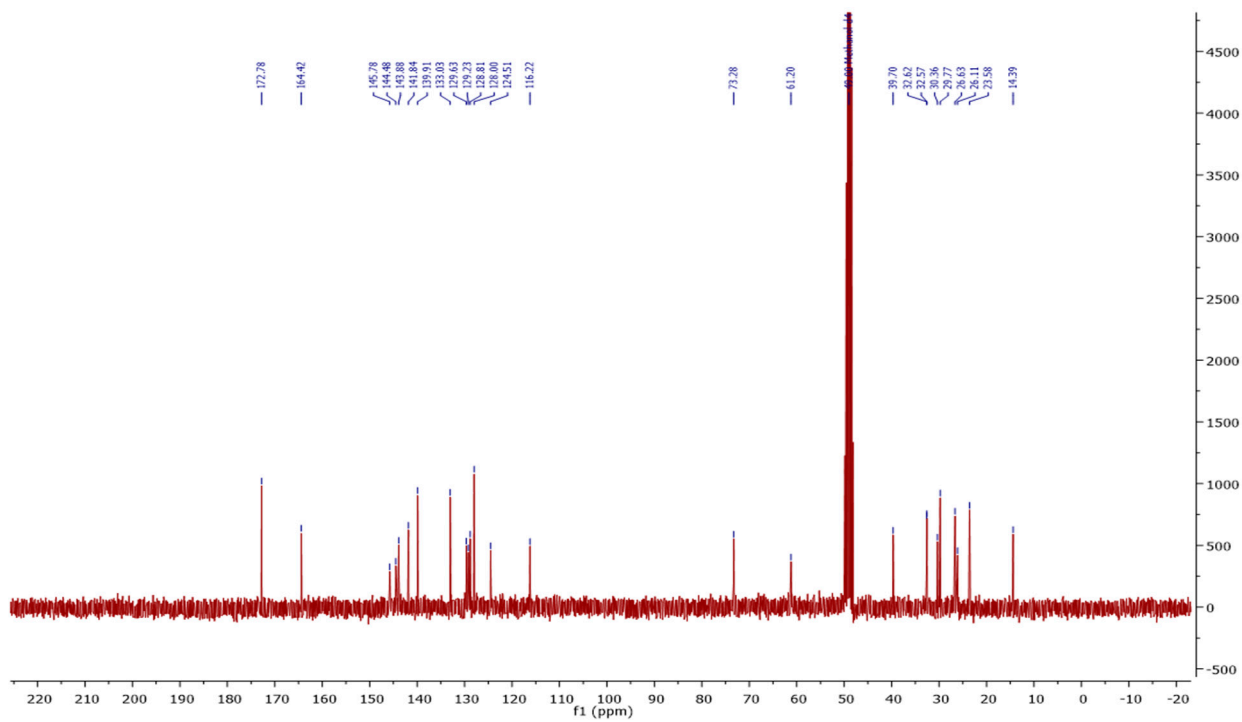
23-C3



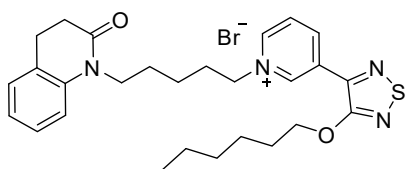
^1H NMR spectrum



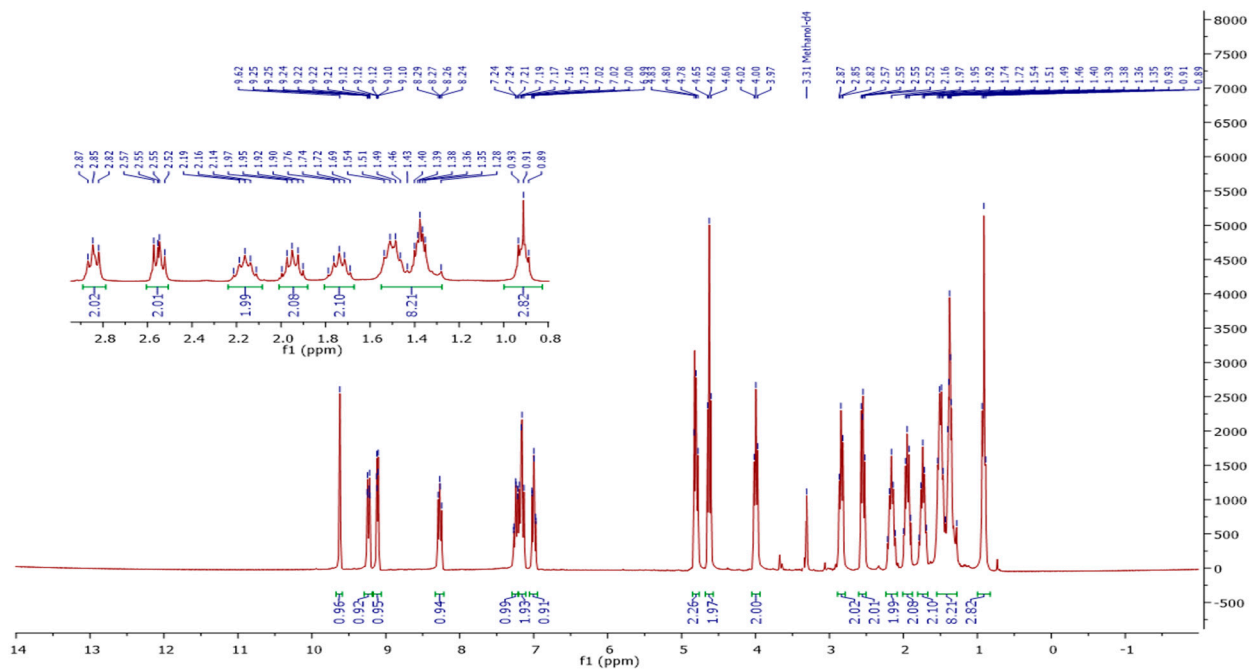
^{13}C NMR spectrum



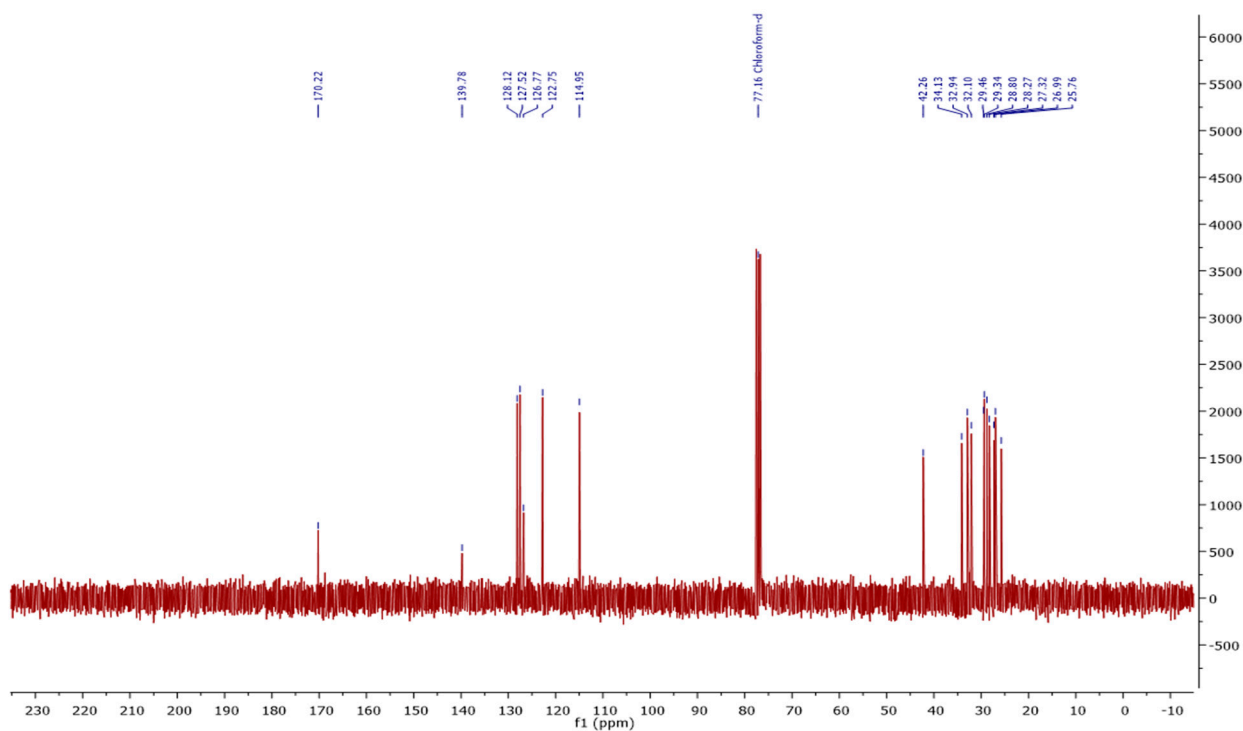
23-C5



^1H NMR spectrum



^{13}C NMR spectrum



CCCCCCCCN1C(=O)Cc2ccccc21.[Br-].CCCCCOc1ncnc1-c1ccc[n+]1

¹H NMR (400 MHz, CDCl₃)

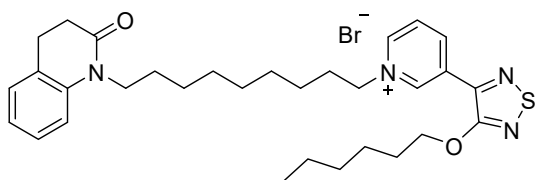
Chemical Shift (ppm)	Integration
3.94	2.10
3.89	2.10
3.85	2.10
3.82	2.10
3.78	2.10
3.75	2.10
3.72	2.10
3.68	2.10
3.65	2.10
3.62	2.10
3.58	2.10
3.55	2.10
3.52	2.10
3.48	2.10
3.45	2.10
3.42	2.10
3.38	2.10
3.35	2.10
3.32	2.10
3.28	2.10
3.25	2.10
3.22	2.10
3.18	2.10
3.15	2.10
3.12	2.10
3.08	2.10
3.05	2.10
3.02	2.10
2.98	2.10
2.95	2.10
2.92	2.10
2.88	2.10
2.85	2.10
2.82	2.10
2.78	2.10
2.75	2.10
2.72	2.10
2.68	2.10
2.65	2.10
2.62	2.10
2.58	2.10
2.55	2.10
2.52	2.10
2.48	2.10
2.45	2.10
2.42	2.10
2.38	2.10
2.35	2.10
2.32	2.10
2.28	2.10
2.25	2.10
2.22	2.10
2.18	2.10
2.15	2.10
2.12	2.10
2.08	2.10
2.05	2.10
2.02	2.10
1.98	2.10
1.95	2.10
1.92	2.10
1.88	2.10
1.85	2.10
1.82	2.10
1.78	2.10
1.75	2.10
1.72	2.10
1.68	2.10
1.65	2.10
1.62	2.10
1.58	2.10
1.55	2.10
1.52	2.10
1.48	2.10
1.45	2.10
1.42	2.10
1.38	2.10
1.35	2.10
1.32	2.10
1.28	2.10
1.25	2.10
1.22	2.10
1.18	2.10
1.15	2.10
1.12	2.10
1.08	2.10
1.05	2.10
1.02	2.10
0.98	2.10
0.95	2.10
0.92	2.10
0.88	2.10
0.85	2.10
0.82	2.10
0.78	2.10
0.75	2.10
0.72	2.10
0.68	2.10
0.65	2.10
0.62	2.10
0.58	2.10
0.55	2.10
0.52	2.10
0.48	2.10
0.45	2.10
0.42	2.10
0.38	2.10
0.35	2.10
0.32	2.10
0.28	2.10
0.25	2.10
0.22	2.10
0.18	2.10
0.15	2.10
0.12	2.10
0.08	2.10
0.05	2.10
0.02	2.10
0.98	2.10
0.95	2.10
0.92	2.10
0.88	2.10
0.85	2.10
0.82	2.10
0.78	2.10
0.75	2.10
0.72	2.10
0.68	2.10
0.65	2.10
0.62	2.10
0.58	2.10
0.55	2.10
0.52	2.10
0.48	2.10
0.45	2.10
0.42	2.10
0.38	2.10
0.35	2.10
0.32	2.10
0.28	2.10
0.25	2.10
0.22	2.10
0.18	2.10
0.15	2.10
0.12	2.10
0.08	2.10
0.05	2.10
0.02	2.10

¹³C NMR (100 MHz, CDCl₃)

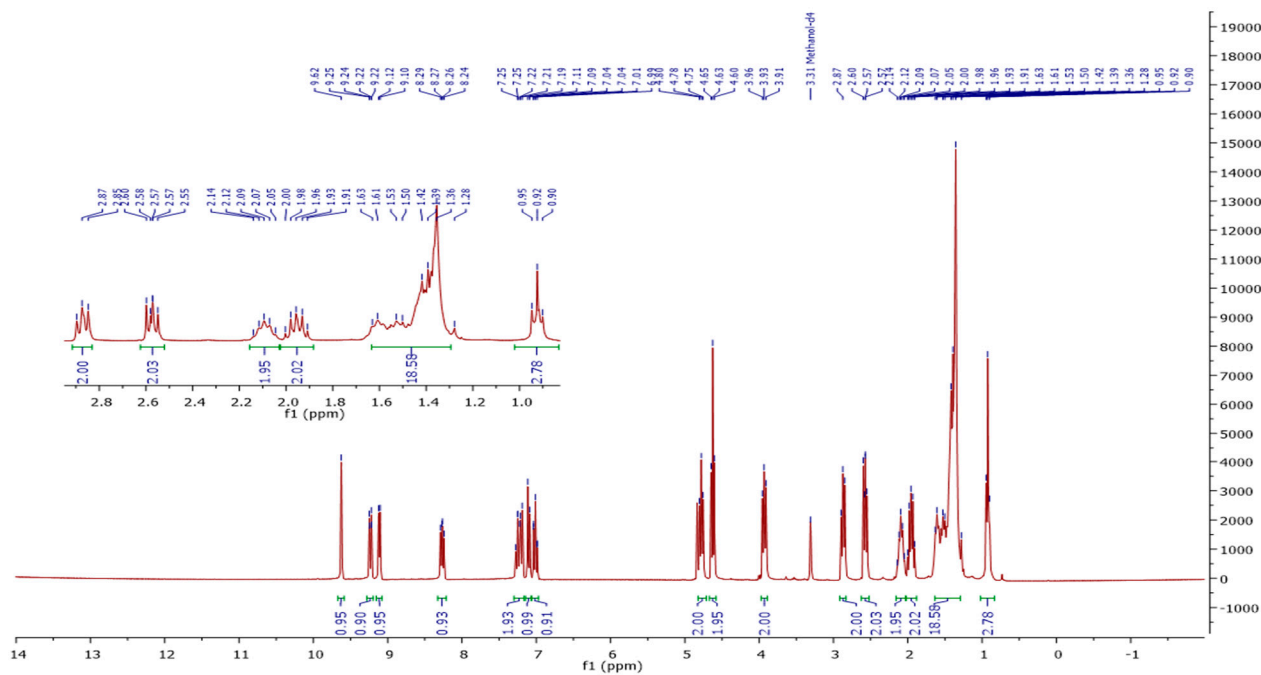
Chemical Shift (ppm)
21.11
21.09
21.07
21.05
21.03
21.01
20.99
20.97
20.95
20.93
20.91
20.89
20.87
20.85
20.83
20.81
20.79
20.77
20.75
20.73
20.71
20.69
20.67
20.65
20.63
20.61
20.59
20.57
20.55

172.09
164.30
146.64
144.11
143.88
143.88
140.04
137.85
129.67
128.99
128.58
128.58
124.12
116.26
73.21
63.28
49.00 Methanol-d4
42.14
32.68
32.52
29.72
27.54
26.14
24.08
23.54

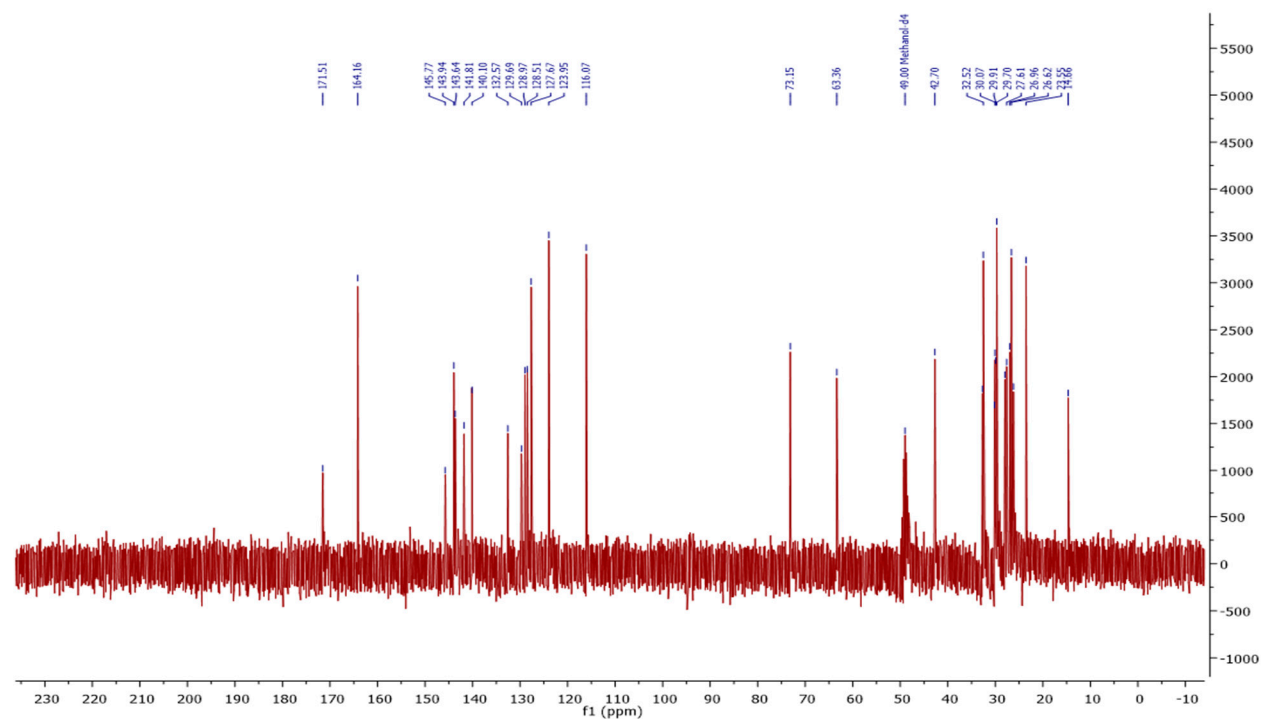
23-C9



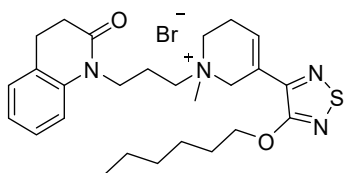
¹H NMR spectrum



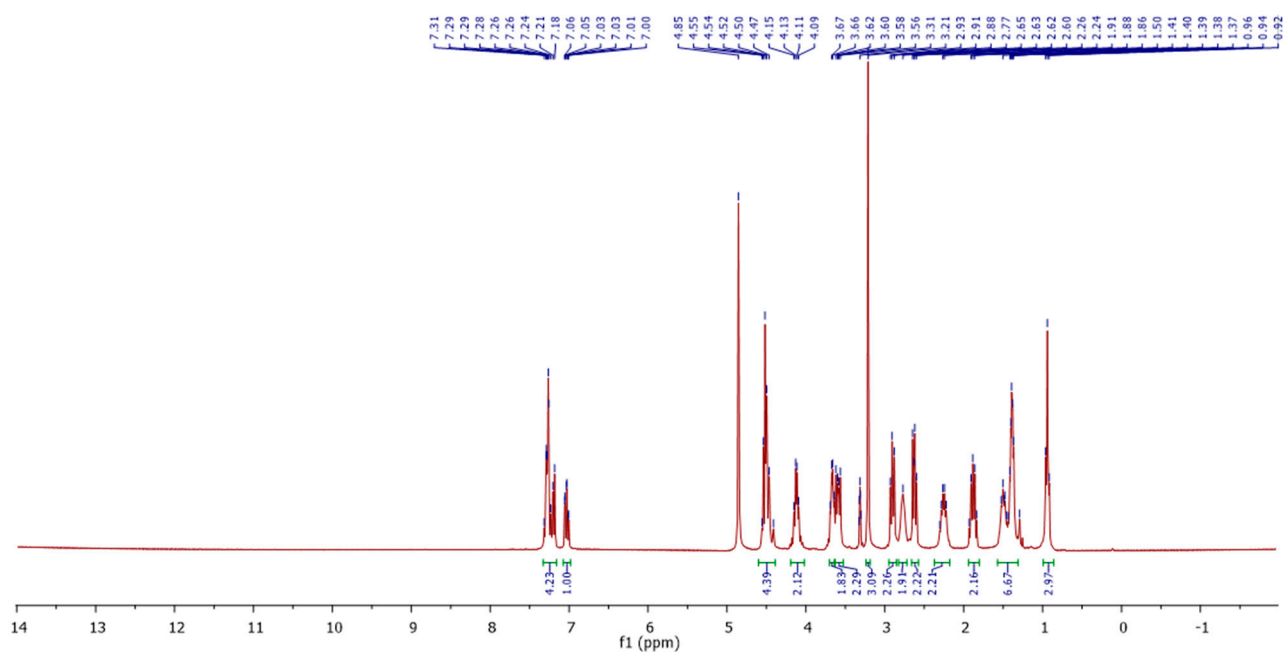
¹³C NMR spectrum



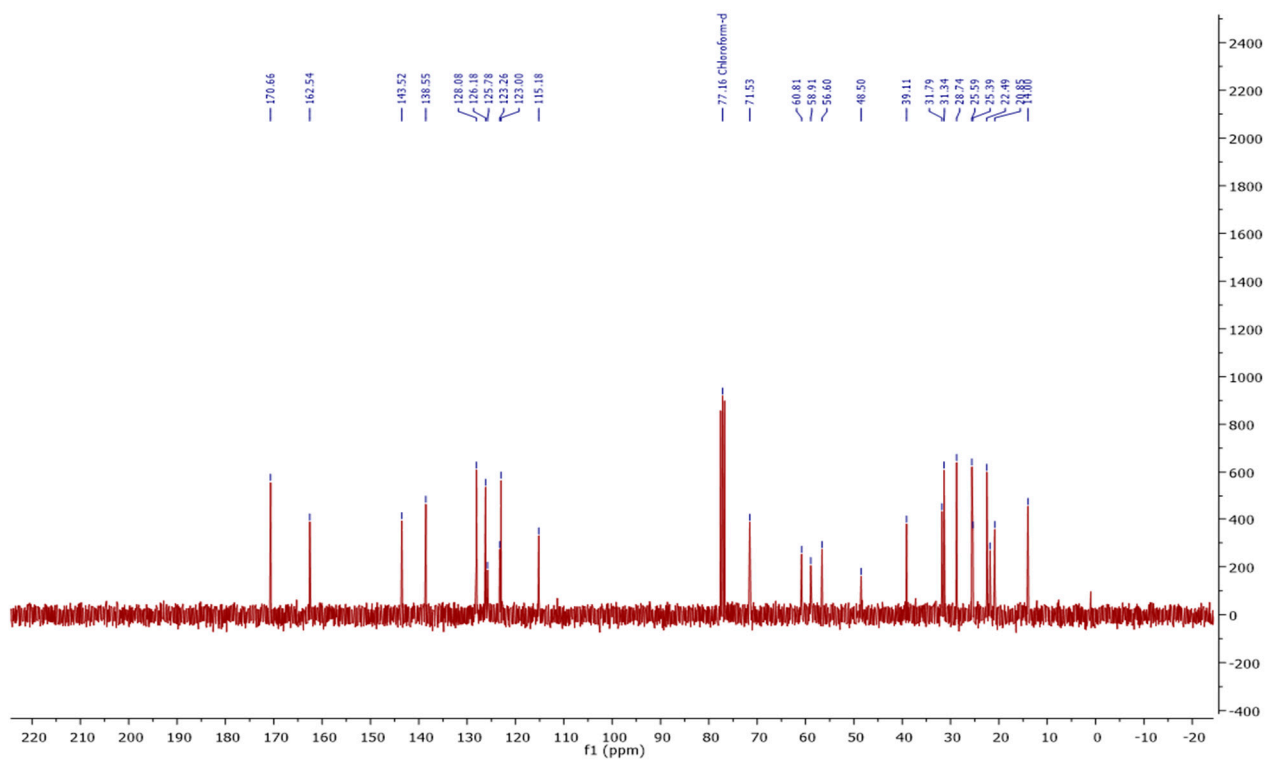
13-C3



^1H NMR spectrum

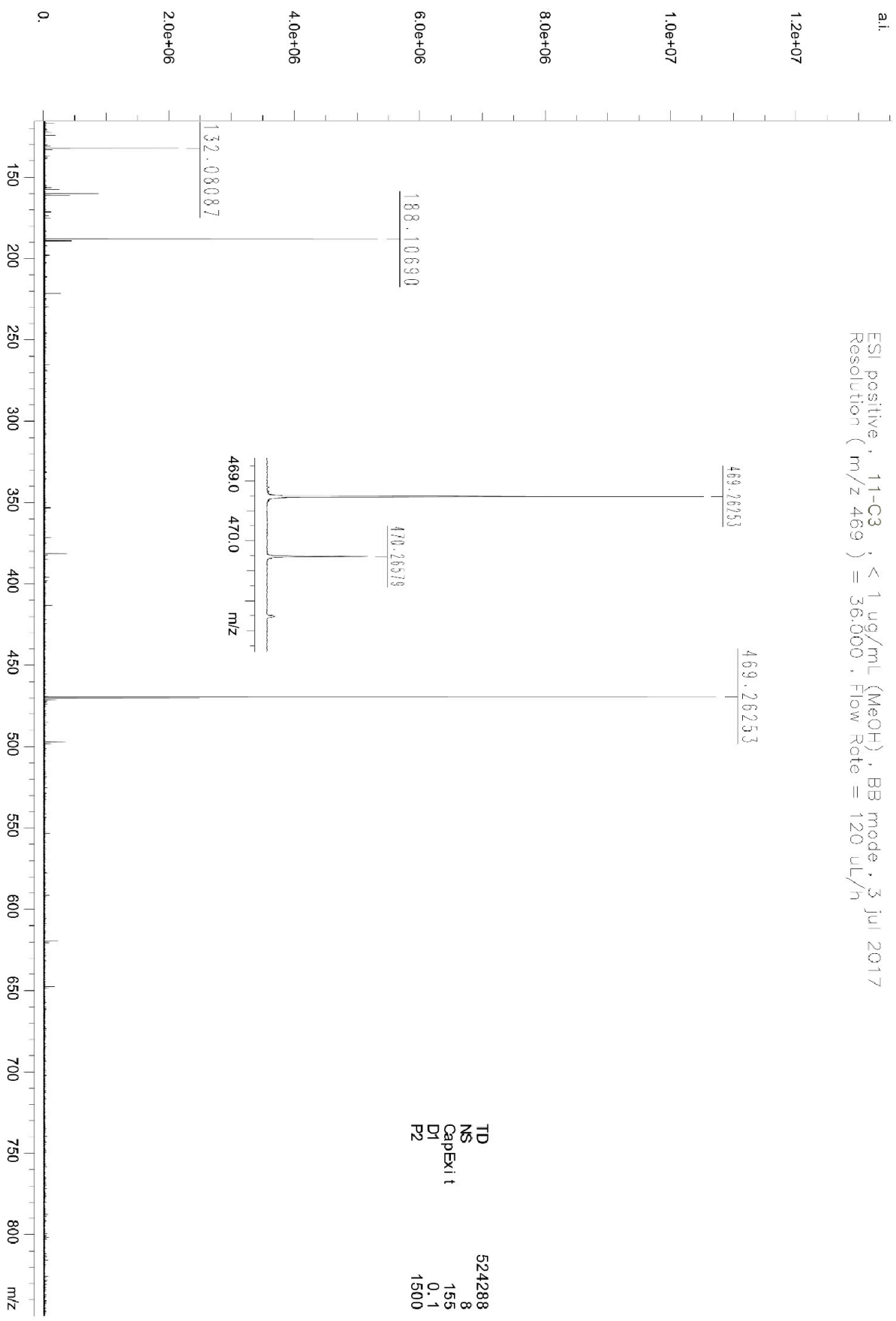


^{13}C NMR spectrum



a.i.

ESI positive, 11-C3, < 1 ug/mL (MeOH), BB mode, 3 jul 2017
Resolution (m/z 469) = 36.000, Flow Rate = 120 uL/h



CCCCCCCCN1CCCCC1C2=NC=NC=S2OCCCC3C=CC=C(C=C3)C(=O)N4CCCCC4

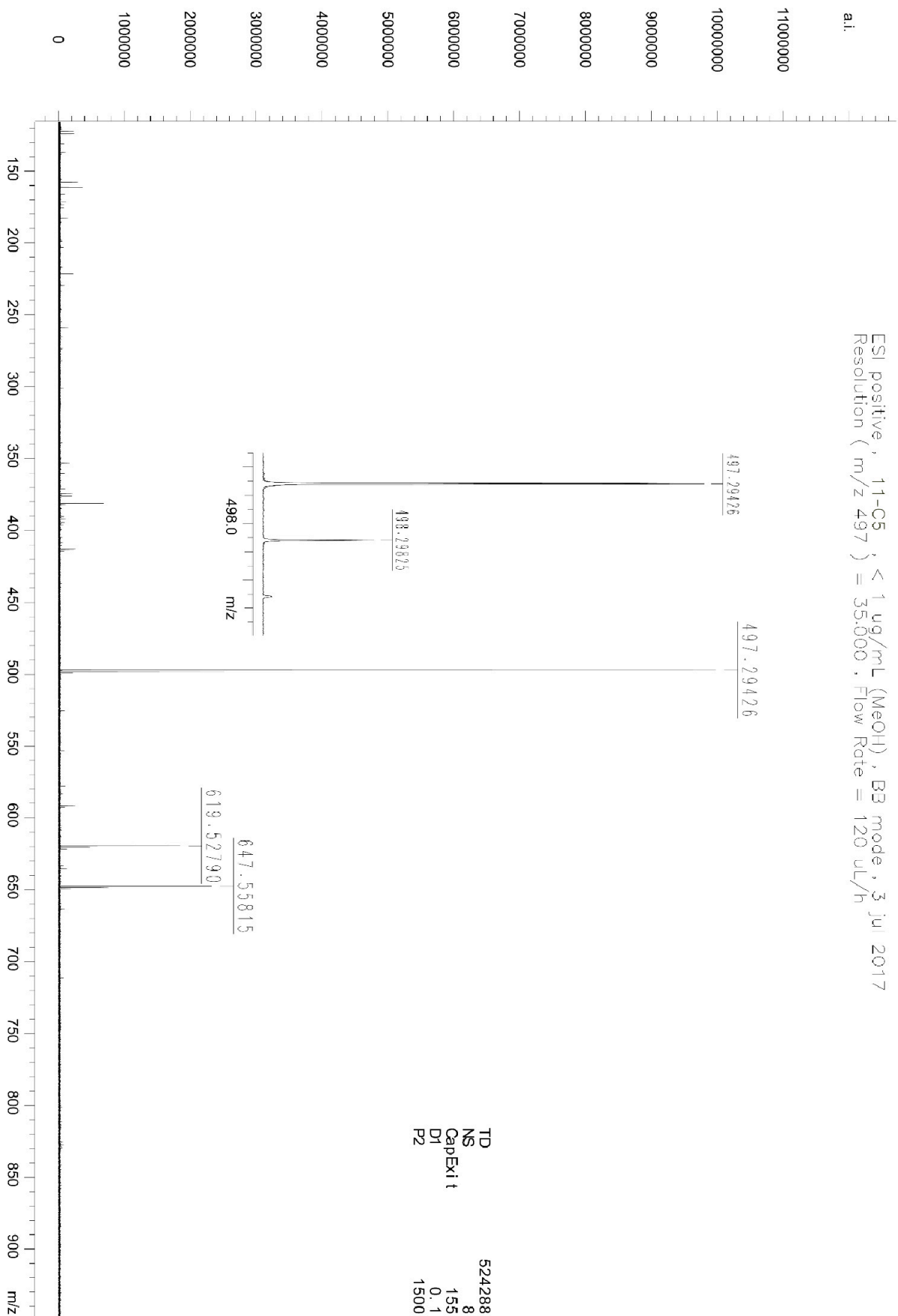
¹H NMR spectrum (CDCl₃) of compound 2b. The x-axis represents the chemical shift f1 (ppm) from 12 to -2. The y-axis represents the intensity from 0 to 10000. The spectrum shows several peaks with corresponding integrations and chemical shift values.

Chemical Shift (ppm)	Integration
7.26	2.00
7.17	1.04
7.16	2.06
7.14	
7.12	
7.11	
7.05	
7.05	
7.03	
7.02	
6.91	
6.88	
6.86	
6.85	
4.49	2.15
4.36	2.06
4.34	
4.32	
4.06	
4.04	
3.85	1.12
3.83	3.04
3.82	2.06
3.67	3.02
3.65	
3.64	
3.34	
2.76	4.22
2.74	2.17
2.50	
2.47	
1.74	6.17
1.36	
1.34	8.10
1.24	
1.22	
1.21	
0.79	3.00
0.77	

170.14
162.40
143.53
138.91
127.88
127.43
126.21
125.76
122.95
122.73
119.69
77.16 Chloroform-d
71.33
63.07
58.60
56.26
49.92
48.13
41.02
31.65
31.14
28.53
25.39
25.28
22.29
21.65

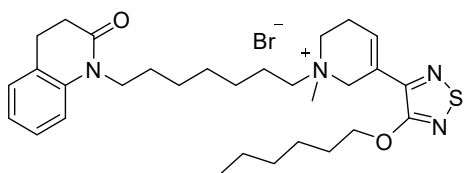
a.i.

ESI positive, 11-C5, < 1 ug/mL (MeOH), B3 mode, 3 Jul 2017
Resolution (m/z 497) = 35,000, Flow Rate = 120 uL/h

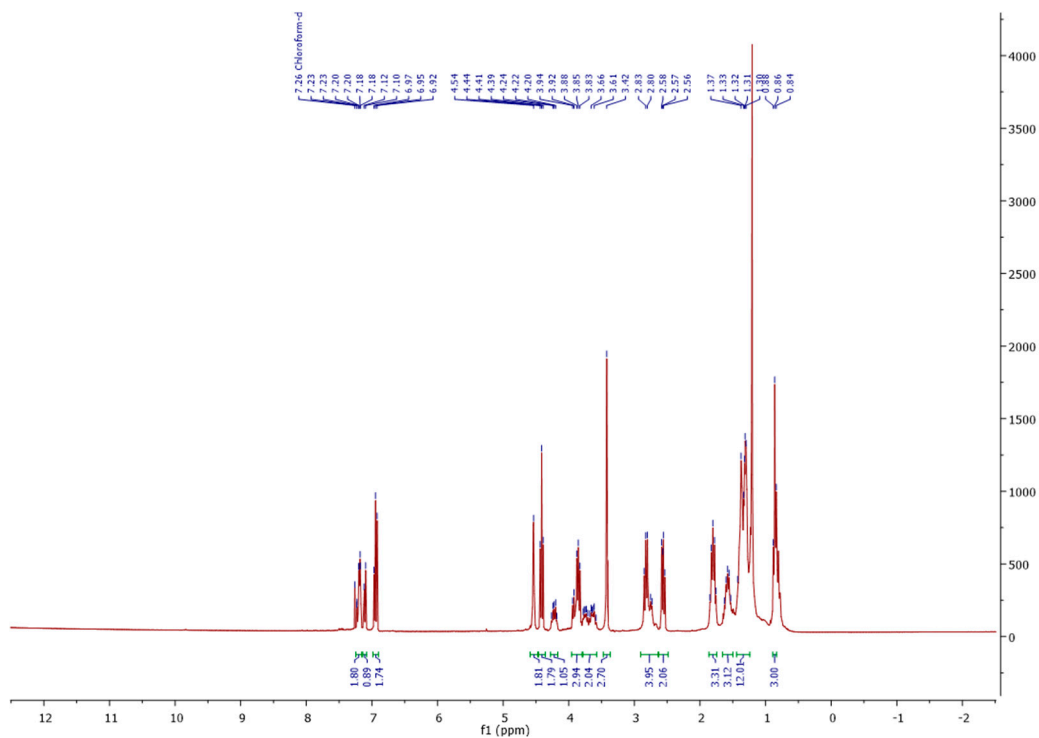


TD 524288
NS 8
CapExit 155
D1 0.1
P2 1500

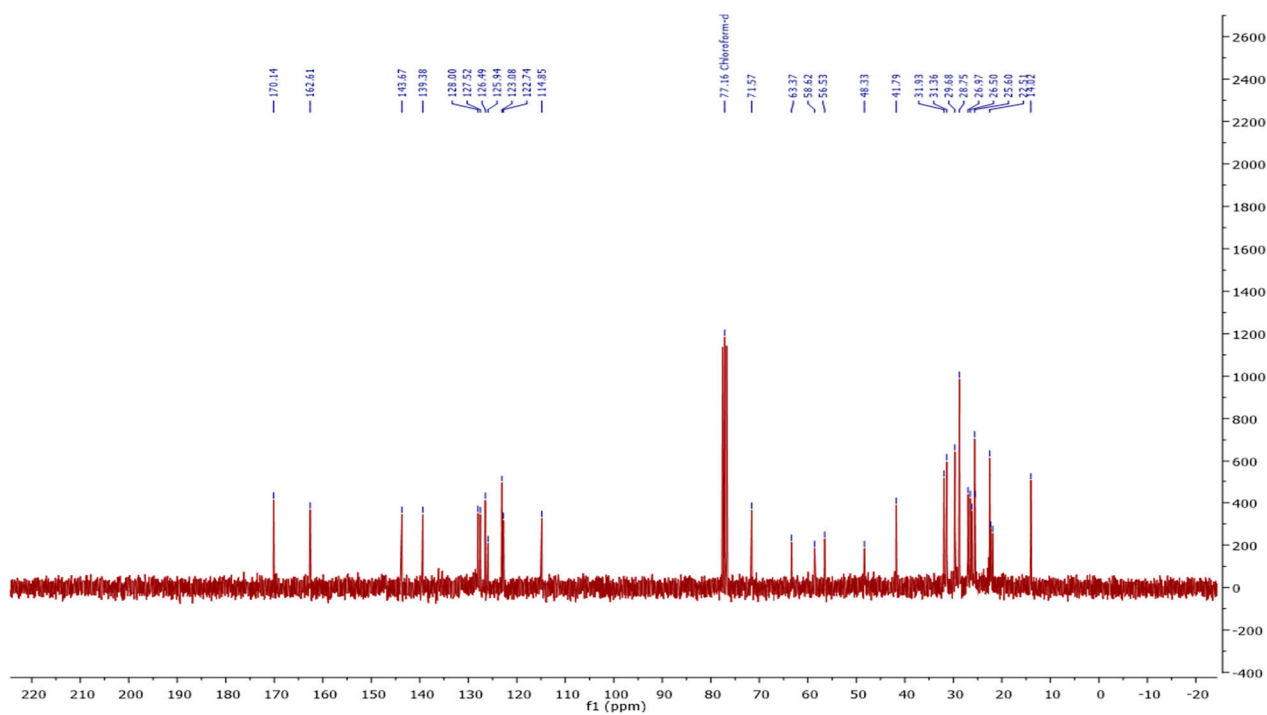
13-C7



^1H NMR spectrum

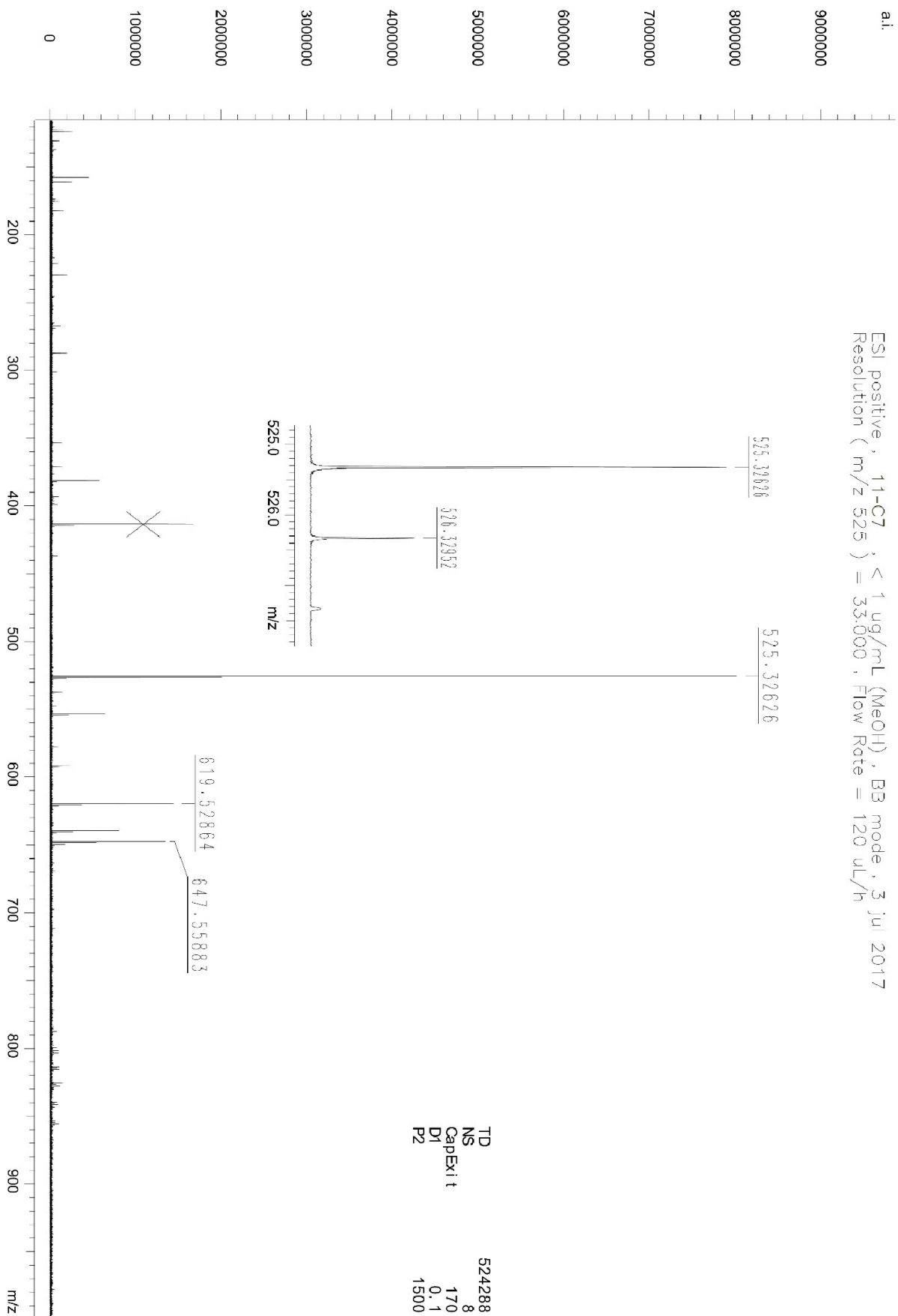


^{13}C NMR spectrum

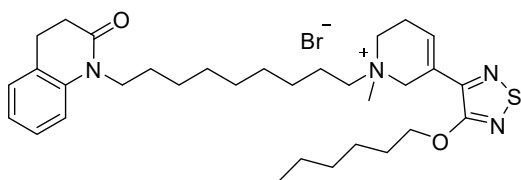


a.i.

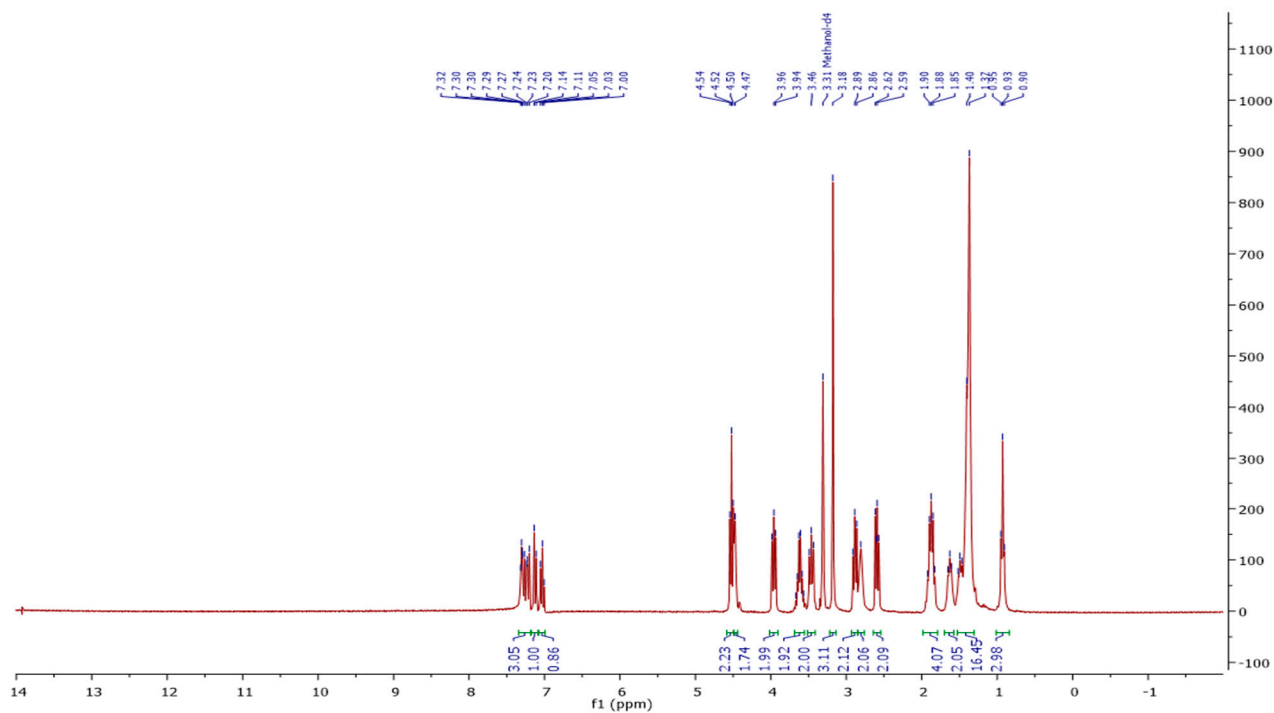
ESI positive, 11-C7; < 1 ug/mL (MeOH), B3 mode, 3 Jul 2017
Resolution (m/z 525) = 33.000, Flow Rate = 120 uL/h



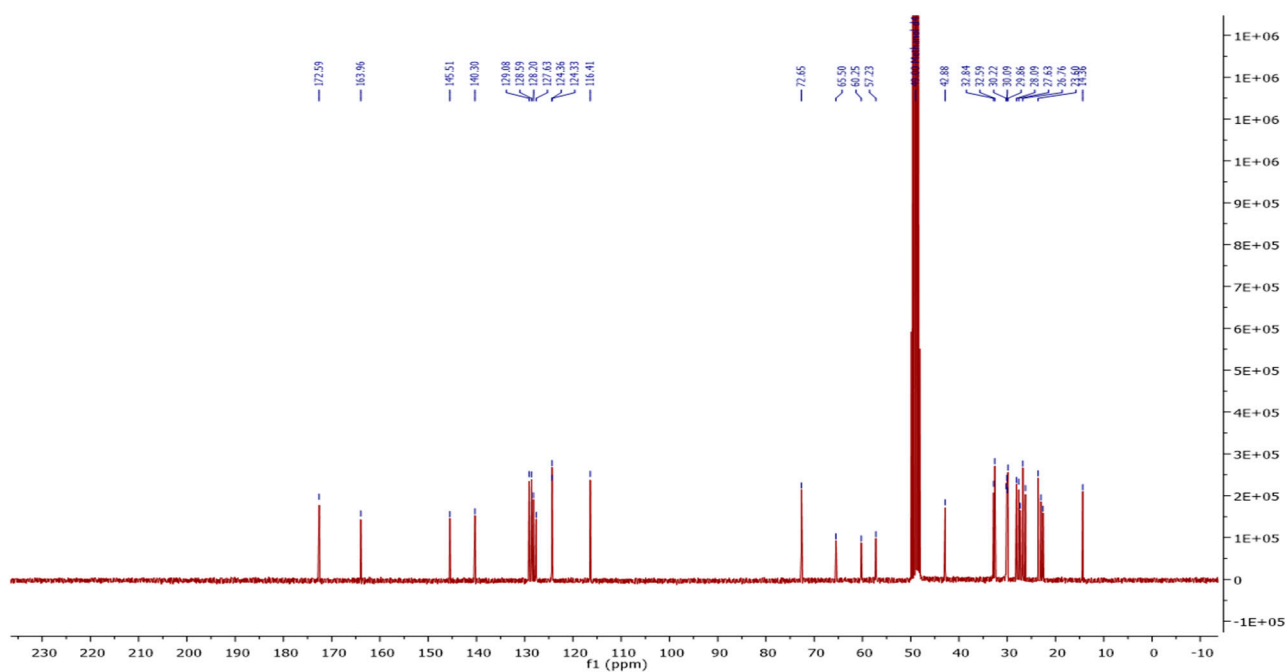
13-C9



^1H NMR spectrum

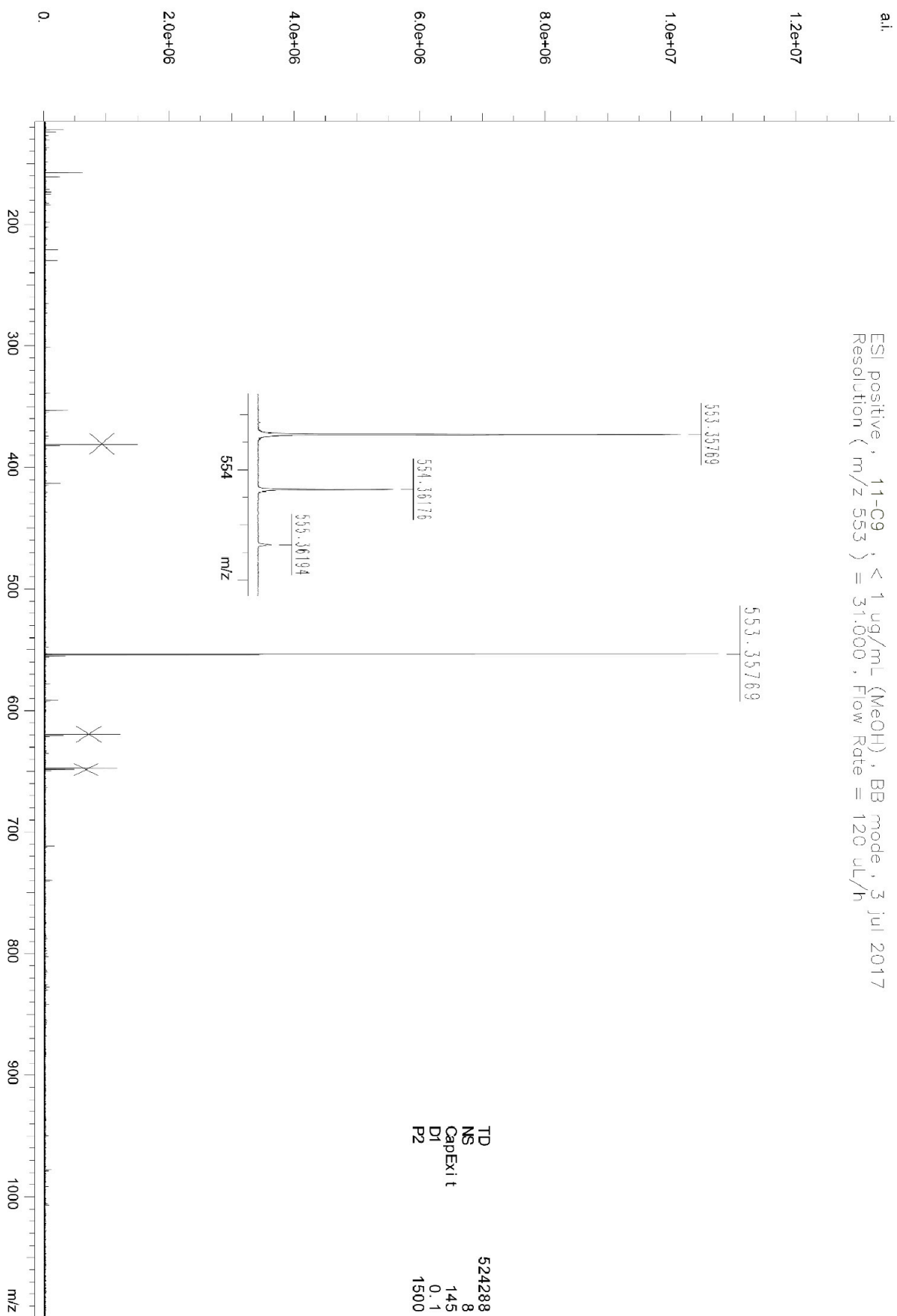


^{13}C NMR spectrum



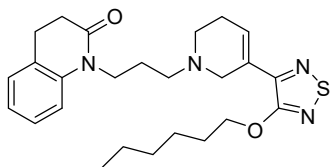
a).

ESI positive, 11-C9, < 1 µg/mL (MeOH), BB mode, 3 Jul 2017
Resolution (m/z 553) = 31,000, Flow Rate = 120 µL/h

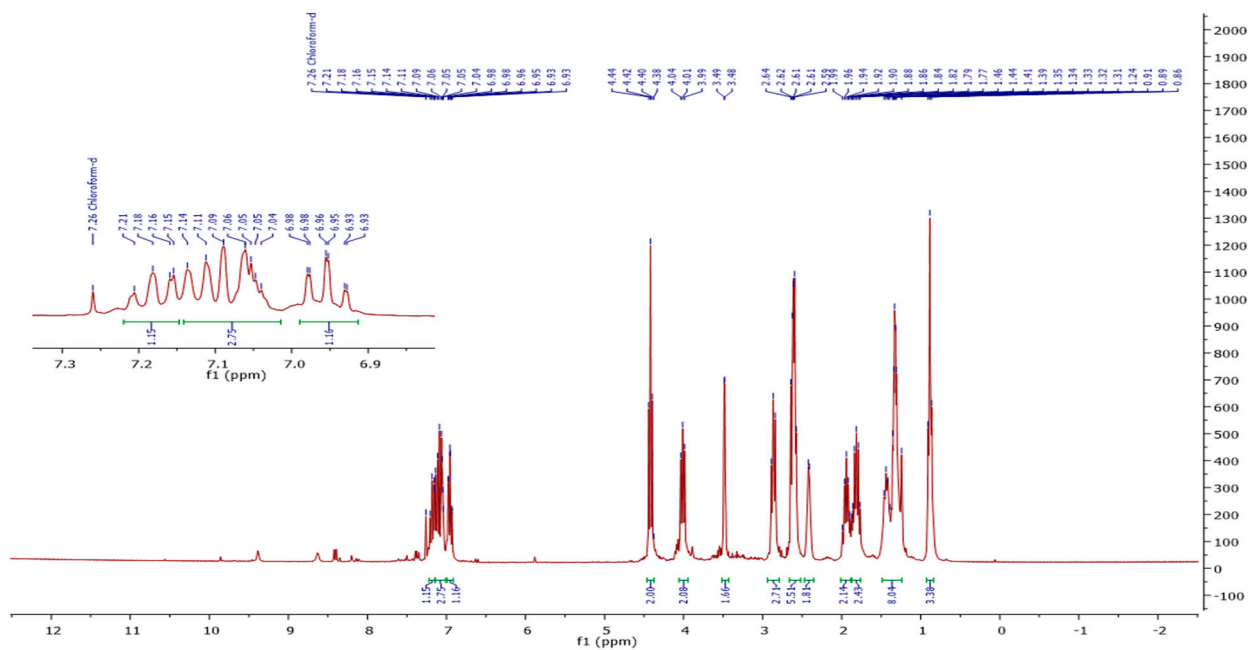


TD 524288
NS 8
CapExit 145
DI 0.1
P2 1500

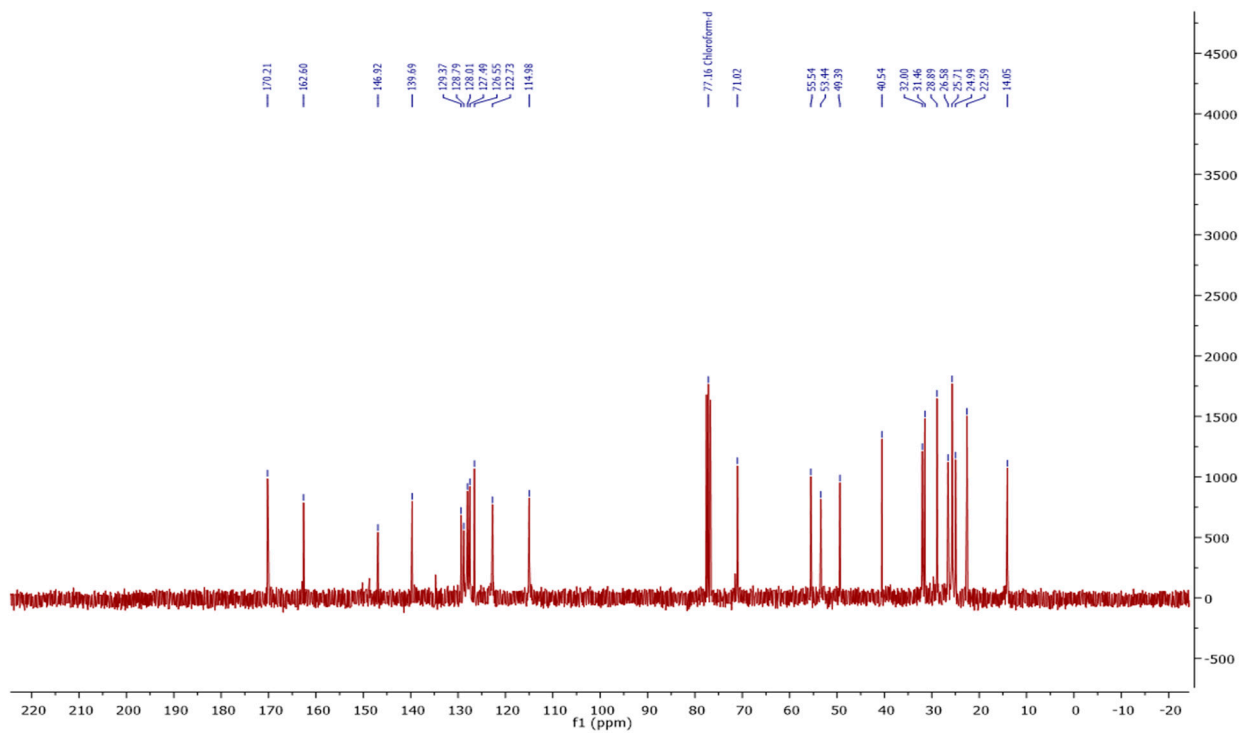
12-C3



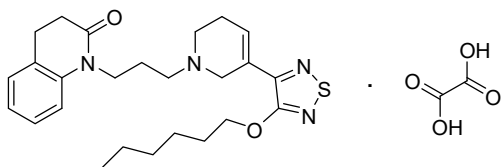
^1H NMR spectrum



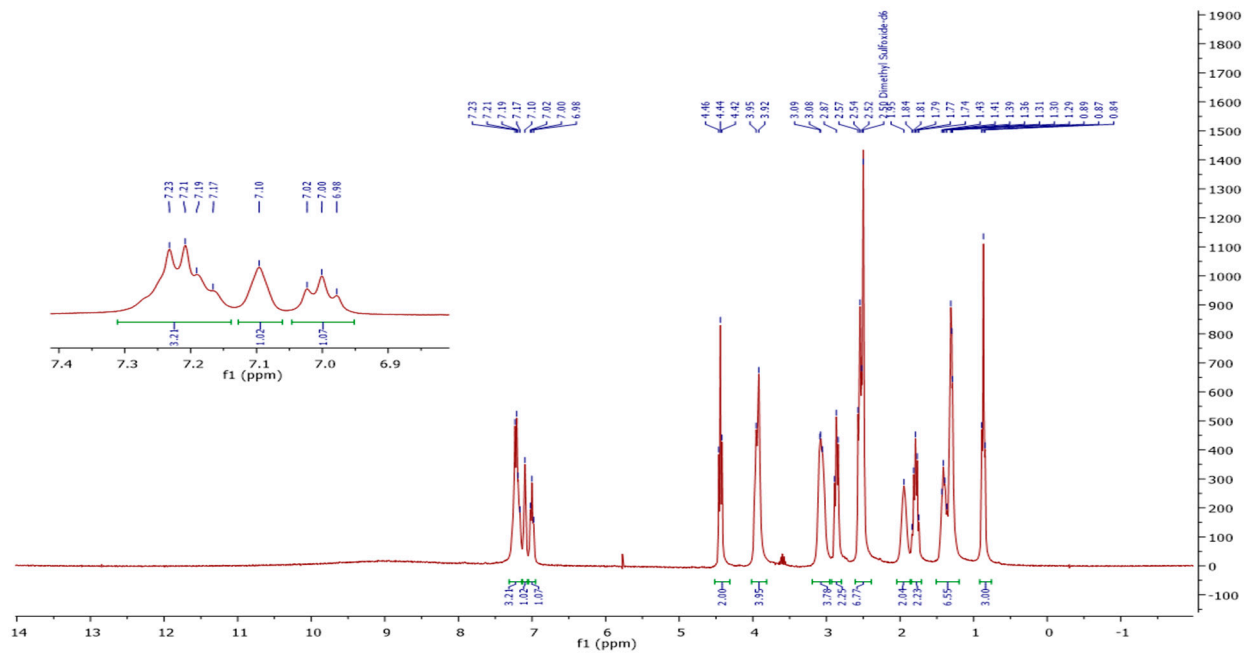
^{13}C NMR spectrum



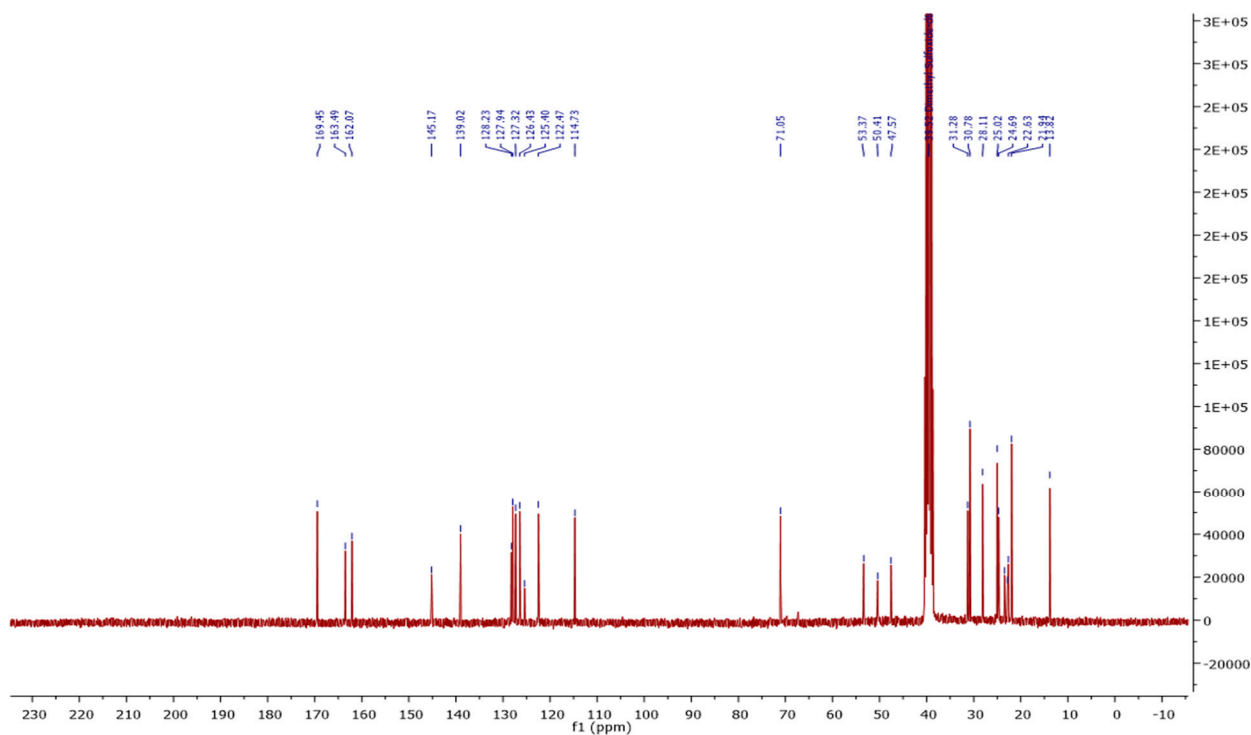
12-C3 Oxalate

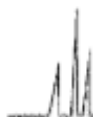


^1H NMR spectrum



^{13}C NMR spectrum



**Certificato di Analisi**
Certificate of Analysis

N°Campione/ Sample N°: 2016001294/LAB

Data apertura/ Registration date: 19/02/16

Richiedente/ Requested by:

Committente/ Company:

Dallanocce

UNIVERSITA' DI MILANO-Dip.Scienze
Farm."Pietro Pratesi"

Campione/ Sample:

Lotto/ Batch:

Q3X

Analisi - Analysis**Risultati****Specifiche**

Metodo Analitico - Analytical method

Results

Specifications

CHN

SOP - MET 019

Carbonio

58.23%

Idrogeno

6.57%

Azoto

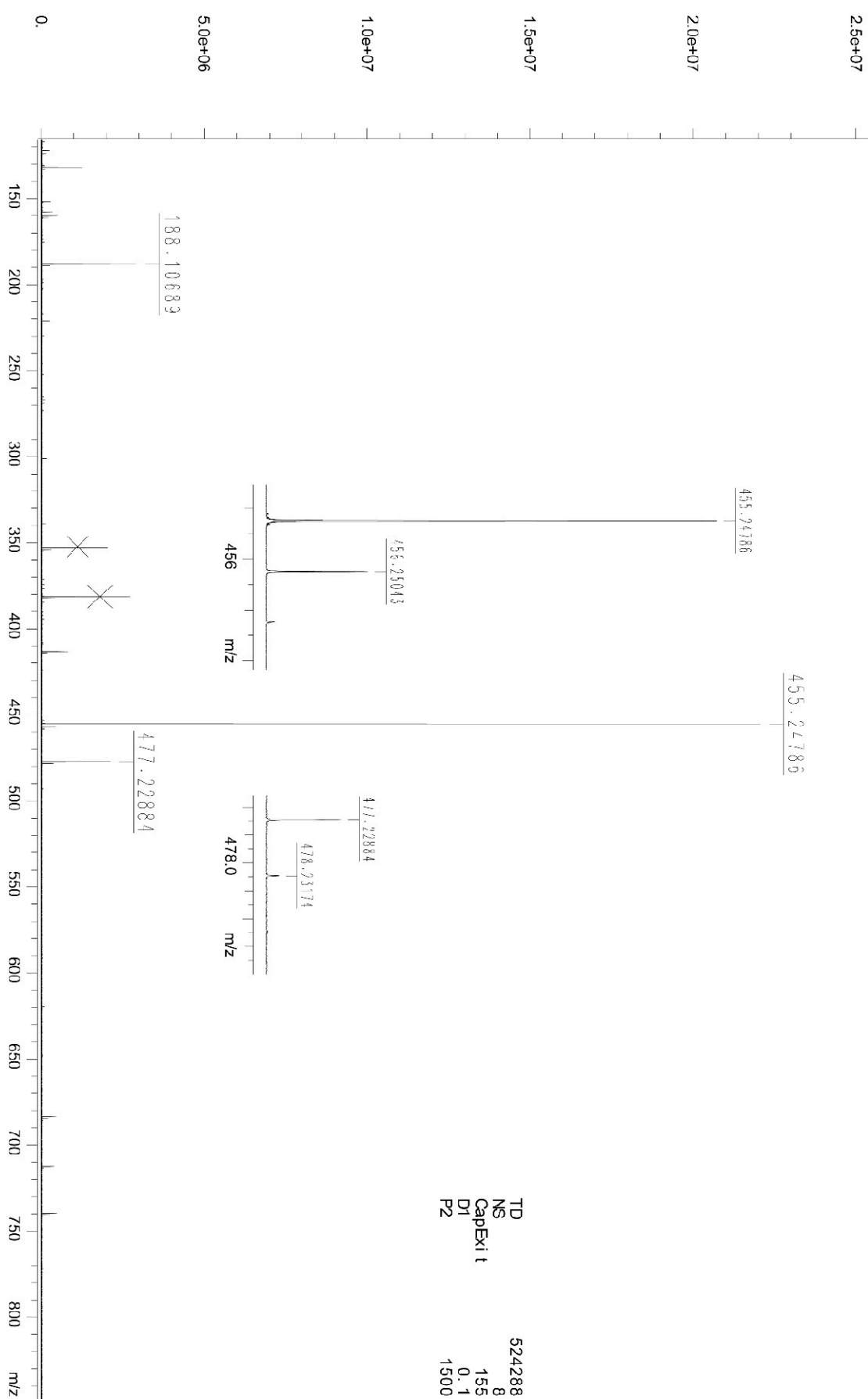
9.88%

Note Chiusura/ Notes:

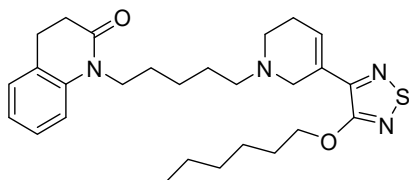
Calculated Elementary analysis for stoichiometry for 1:1

Anal. calcd for C₂₇H₃₆N₄O₆S: C, 59.54; H, 6.66; N, 10.29; found: C, 58,23; H, 6,57; N, 9.88.

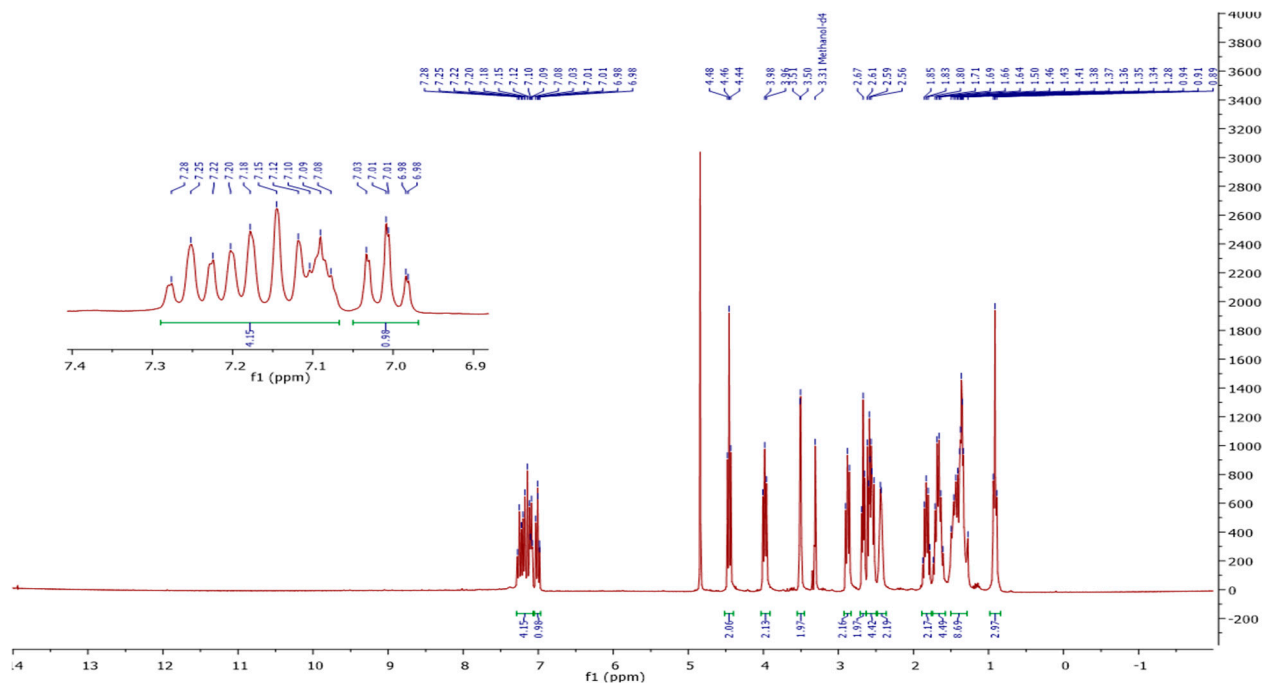
a.i. ESI positive, 10-C3, ~ 2 ug/mL (MeOH), B3 mode, 3 jul 2017
 Resolution (m/z 455) – 40.000, Flow Rate – 120 μ L/h



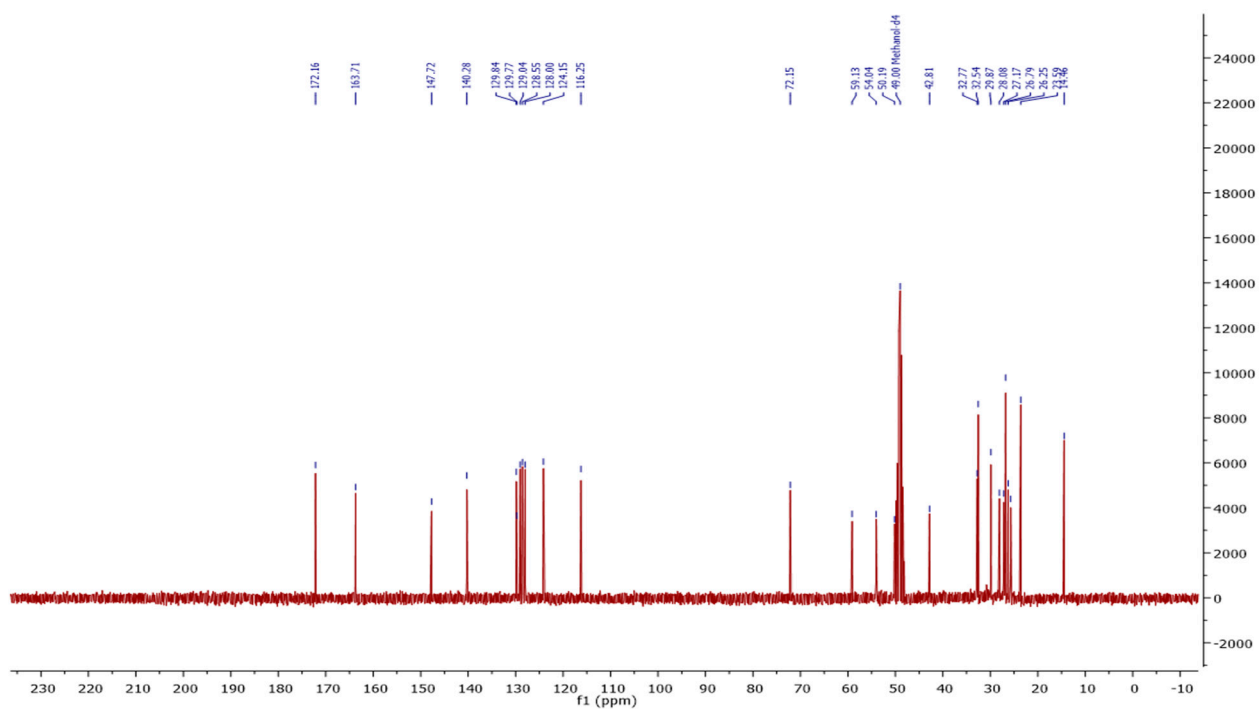
12-C5



^1H NMR spectrum

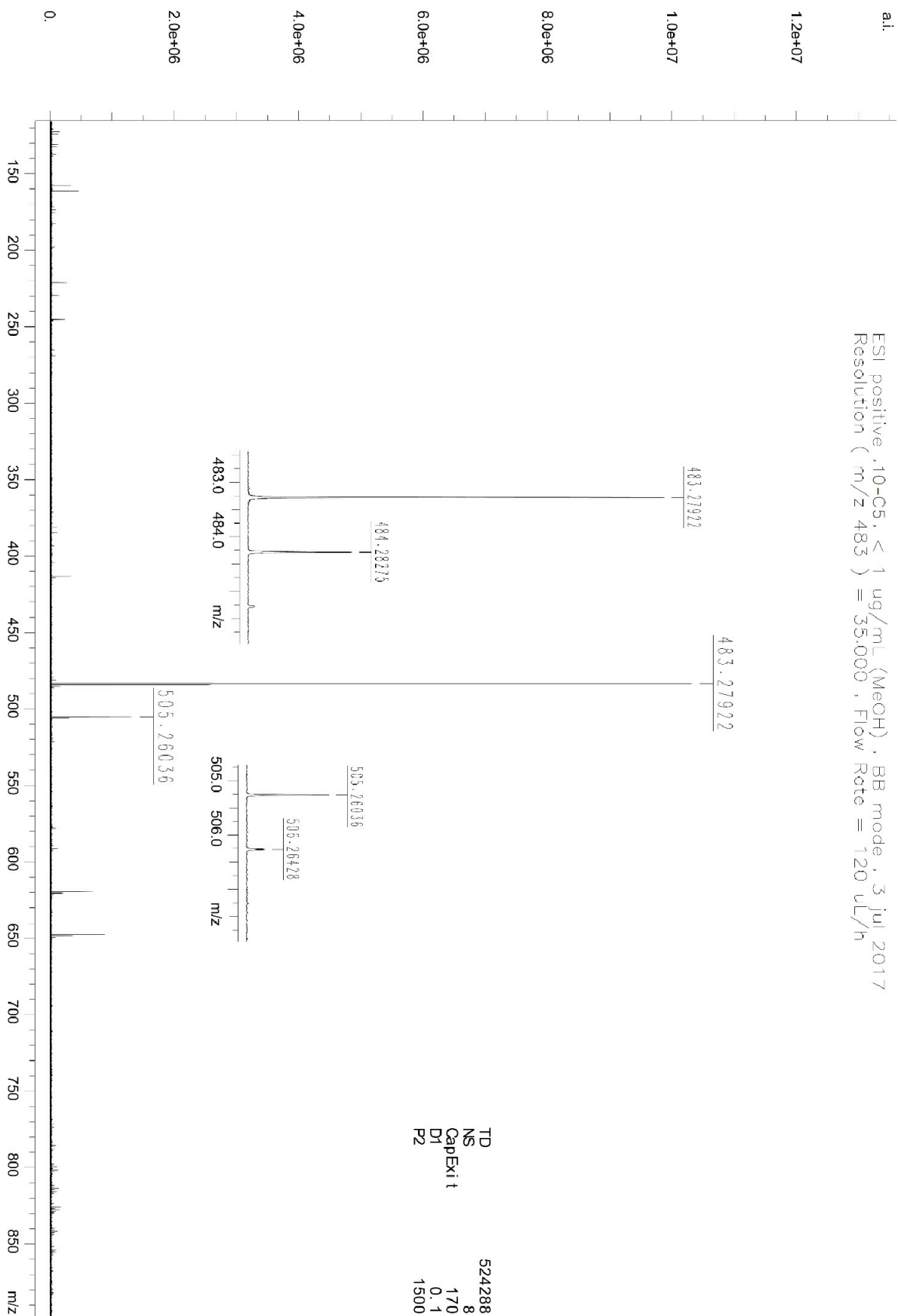


^{13}C NMR spectrum

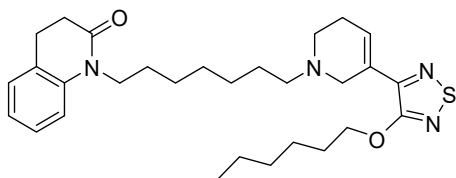


a.i.

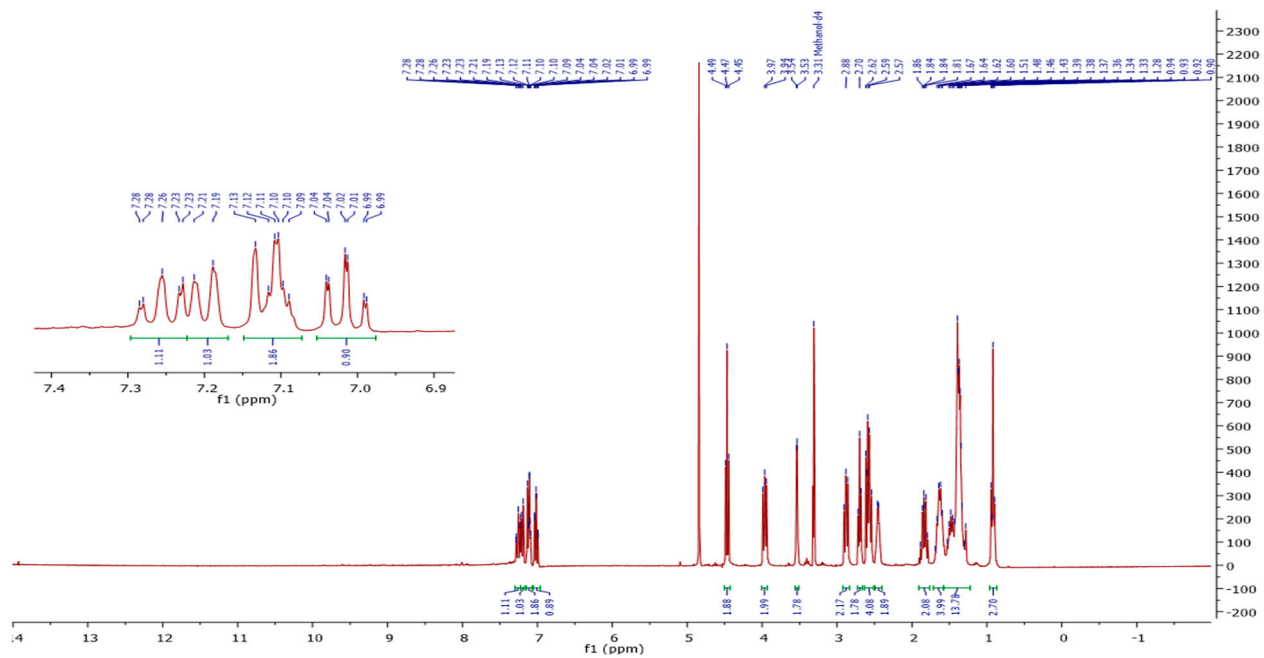
ESI positive, 10-C5, < 1 ug/mL (MeOH), BB mode, 3 jul 2017
Resolution (m/z 483) = 35,000 , Flow Rate = 120 uL/h



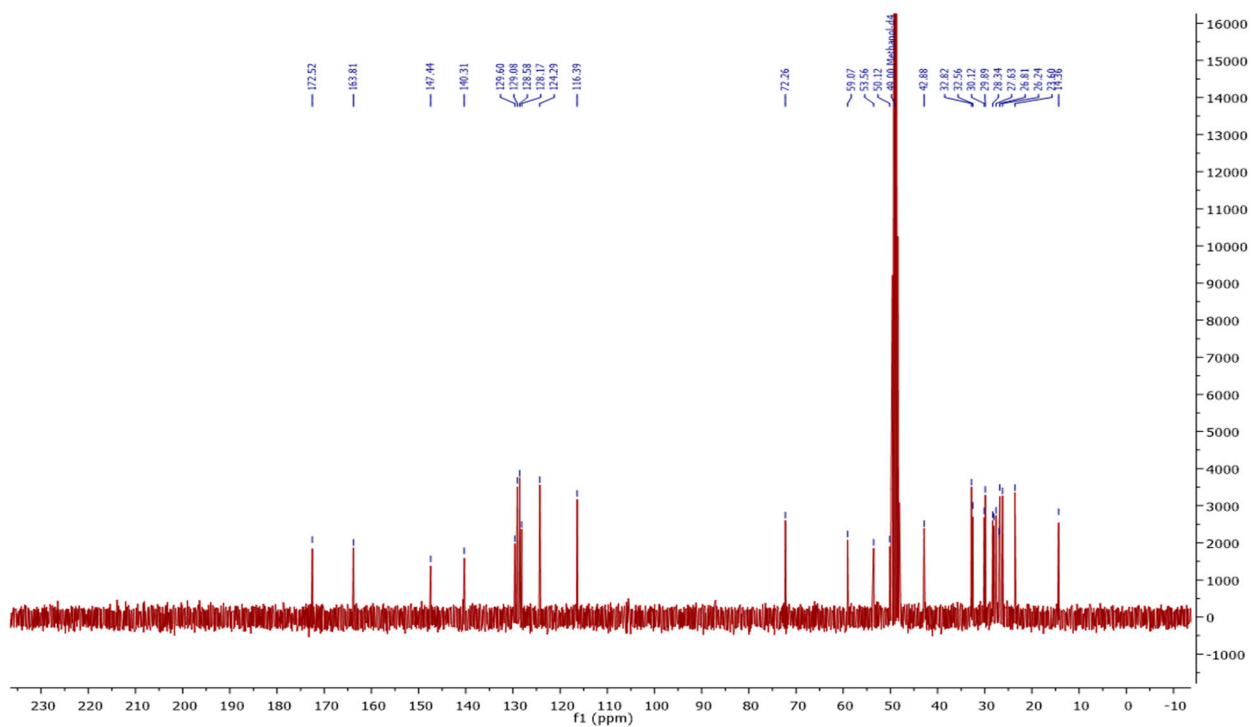
12-C7



^1H NMR spectrum

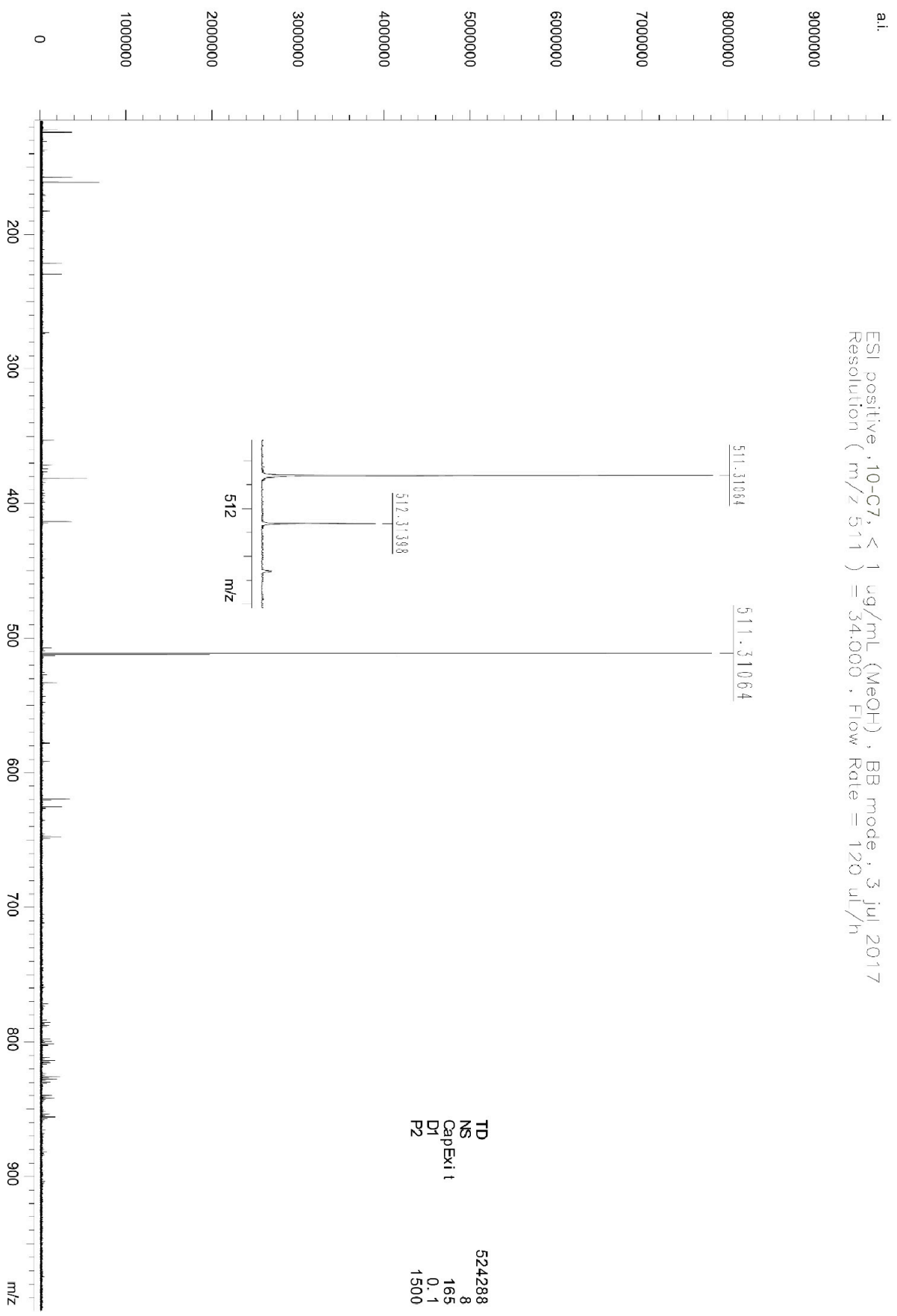


^{13}C NMR spectrum



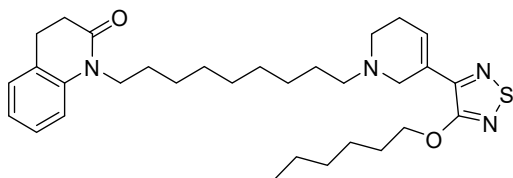
a.i.

ESI positive, 10-C7, < 1 µg/mL (MeOH), BB mode, 3 jul 2017
Resolution (m/z 511) = 34.000, Flow Rate = 120 µL/h

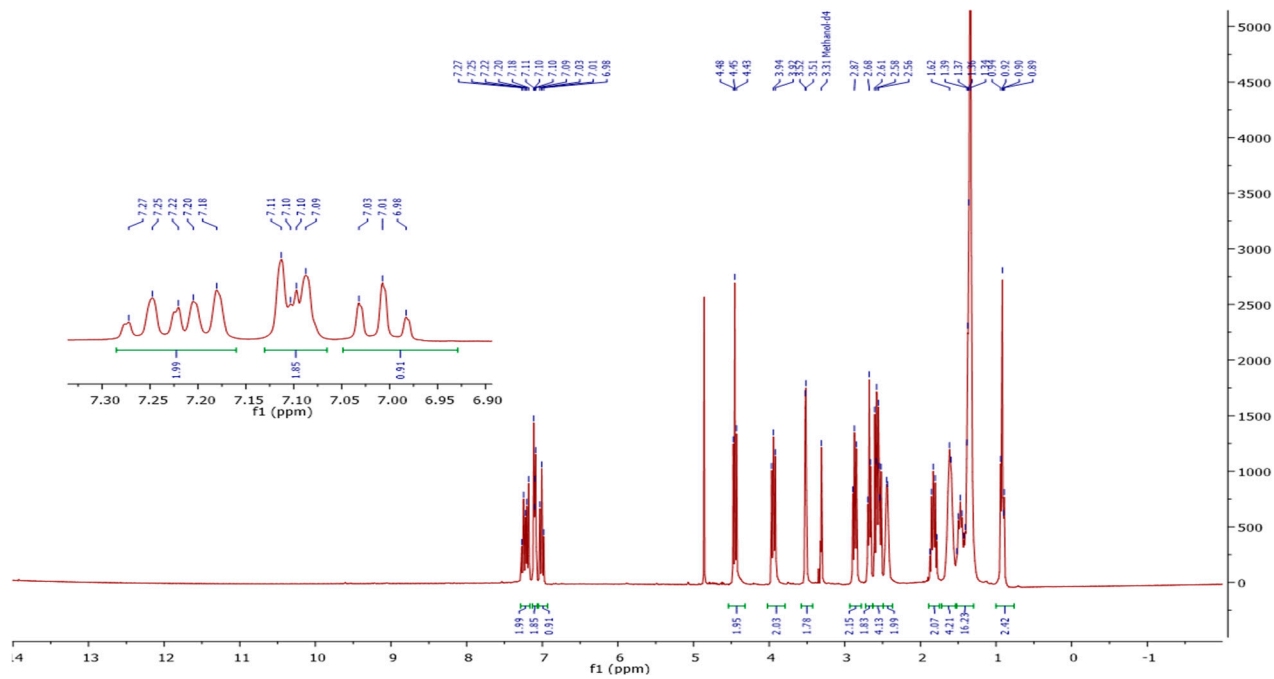


TD 524288
NS 8
CapExi t 165
D1 0.1
P2 1500

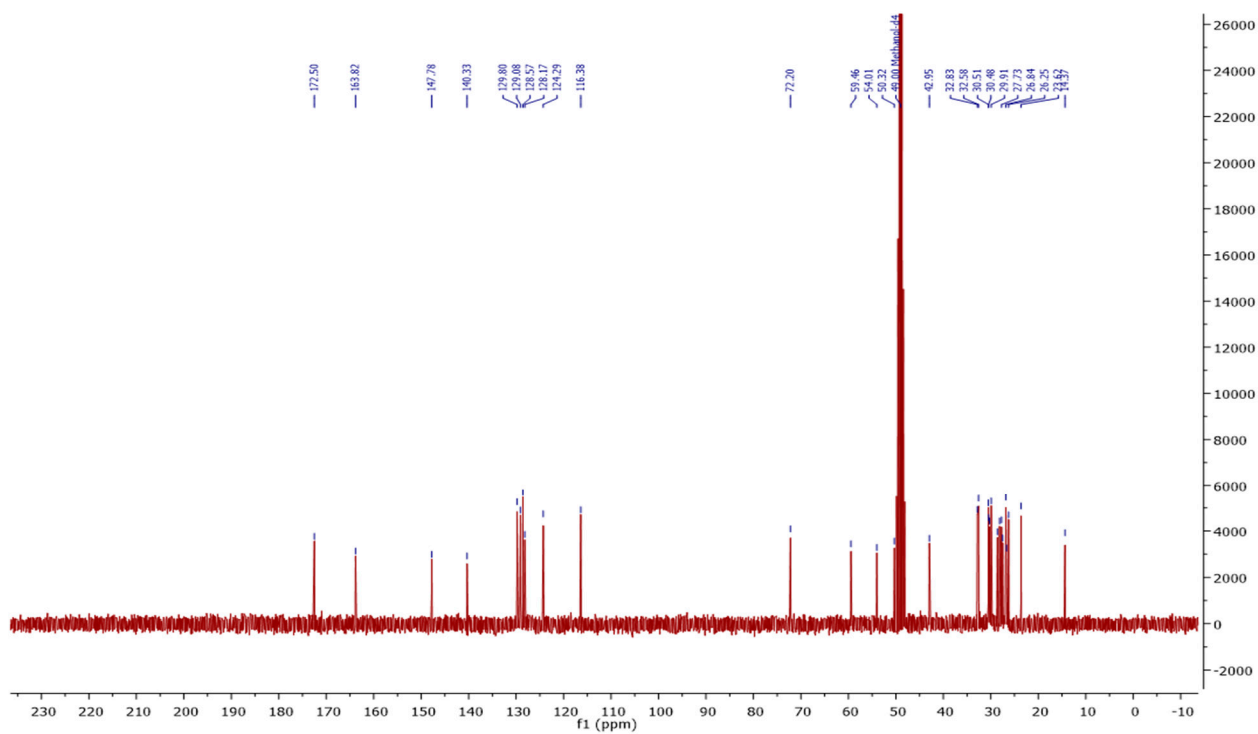
12-C9



^1H NMR spectrum



^{13}C NMR spectrum



a.i.

ESI positive, 10-C9, < 1 µg/mL (MeOH), BB mode, 3 jul 2017
Resolution (m/z 539) = 32,000 , Flow Rate = 120 µL/h

