Supporting Information

N-acylated ciprofloxacin derivatives: synthesis and *in vitro* biological evaluation as antibacterial and anticancer agents

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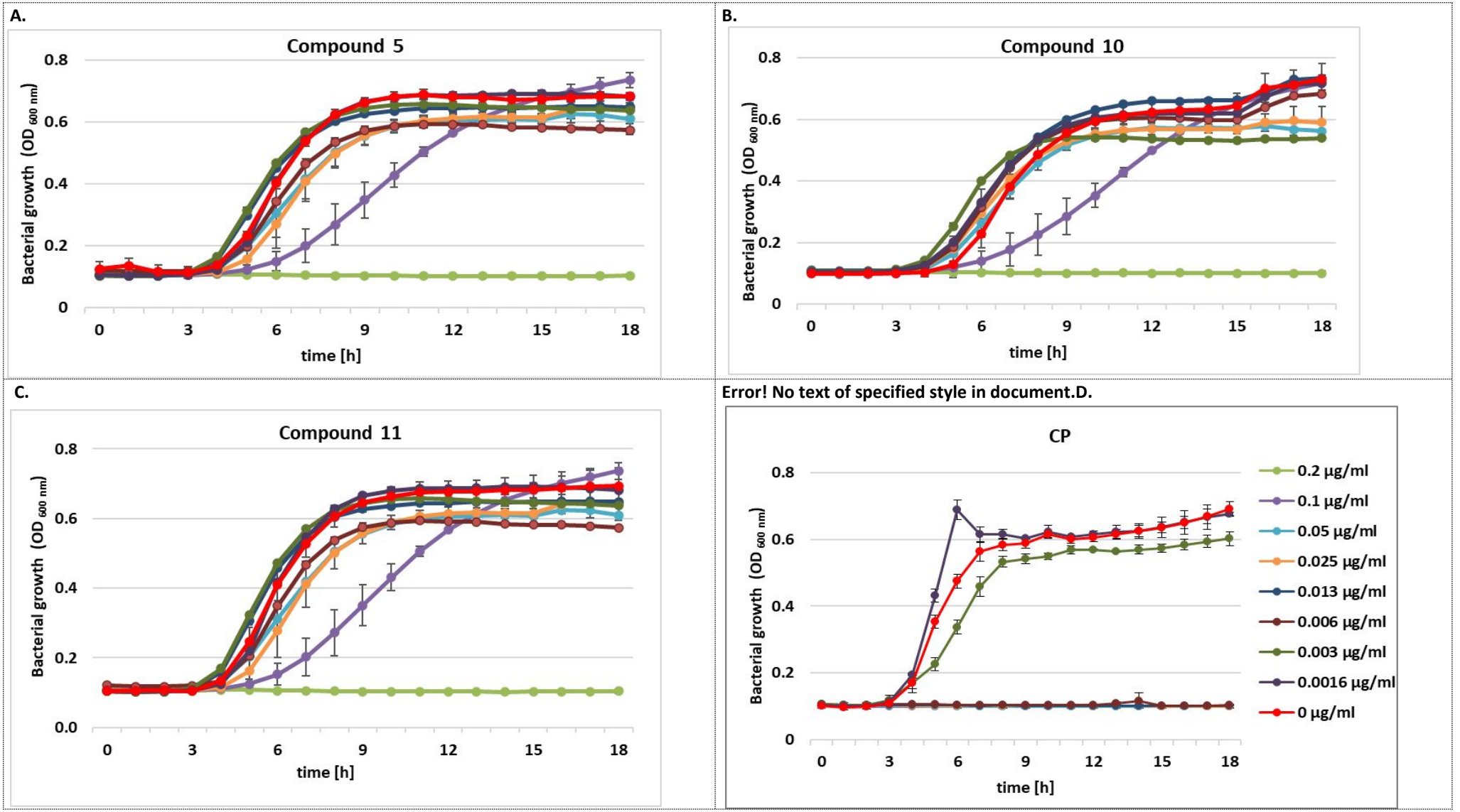


Figure S1. Growth curve analysis of *S. aureus* ATCC 6538 at absorbance of 600 nm (OD600) with or without different concentrations of **A.** compound **5**, **B.** compound **10**, **C.** compound **11**, **D.** CP for 18h. The growth curve data were plotted as average values with standard deviations of n = 3.

Table S1. MIC [ug/ml] values of compounds **5**, **10**, **11** and ciprofloxacin, used in biofilm eradication assay.

Bacteria strain	Concentration [µg/ml]															
	4 MIC				2 MIC				MIC				1/2 MIC			
	Compound															
	5	**10**	**11**	**CP**	**5**	**10**	**11**	**CP**	**5**	**10**	**11**	**CP**	**5**	**10**	**11**	**CP**
***E. coli* 25988**	0.1	0.1	0.1	0.1	0.05	0.05	0.05	0.03	0.025	0.025	0.025	0.015	0.013	0.0125	0.0125	0.0075
***S. aureus* 6538**	0.8	0.8	0.4	0.5	0.4	0.4	0.2	0.25	0.200	0.200	0.100	0.125	0.1	0.1	0.05	0.0625
***P. aeruginosa* 15442**	0.8	0.4	0.4	0.2	0.4	0.2	0.2	0.12	0.200	0.100	0.100	0.060	0.1	0.05	0.05	0.03

Table S2. DNA gyrase (PDB ID: 5BTC [1]) and DNA topoisomerase IV (PDB ID: 3RAD [2]) binding data based on docking results for compounds **1-13** and **CP**.

Compound	DNA gyrase		DNA topoisomerase IV	
	CS	**BE (kcal/mol)**	**CS**	**BE (kcal/mol)**
1	433	-7.86	660	-6.7200
2	360	-8.65	597	-7.3400
3	454	-7.64	714	-6.7100
4	409	-8.46	701	-6.8700
5	343	-9.12	662	-7.9100
6	340	-9.93	754	-8.2300
7	350	-10.79	647	-9.0800
8	408	-7.99	645	-7.4900
9	399	-8.48	672	-7.6300
10	418	-9.49	833	-8.5300
11	381	-10.29	830	-9.2100
12	392	-10.74	854	-10.0900
13	171	-12.46	219	-12.2000
CP	753	-7.26	698	-6.1700

CS = number of members of the largest cluster calculated for 1000 docking runs using RMSD cutoff tolerance = 3 Å.
BE = binding free energy values estimated using AutoDock4 energy function for the representative ligand structure of the largest cluster.

Table S3. Trypan blue assay. The effect of compounds **3**, **15** and **21** on live cell number and viability in PC3 and HaCaT cells. Cells were incubated for 72 h with tested compounds used in their IC_{50} concentrations, then cells were harvested, stained with trypan blue, and analyzed using cell counter. Data are expressed as the mean ± SD. „-" control without compound, [a]Human metastatic prostate cancer (PC3), [b]Human immortal keratinocyte cell line from adult human skin (HaCaT).

		Compound	Cell number x 10^6	Cell number (% of control)	Viability (%)
Cancer cell line	**PC3[a]**	**-**	2.4 ± 0.90	100	98 ± 1.01
		3	0.04 ± 0.01	1.97	52 ± 2.40
		15	0.3 ± 0.08	12.04	82 ± 2.64
		21	0.2 ± 0.03	6.22	78 ± 1.50
Normal cell line	**HaCaT[b]**	**-**	1.5 ± 0.45	100	97 ± 2.01
		3	0.2 ± 0.01	12.15	75 ± 2.23
		15	0.8 ± 0.10	48.38	90 ± 3.04
		21	0.3 ± 0.01	19.35	94 ± 4.01

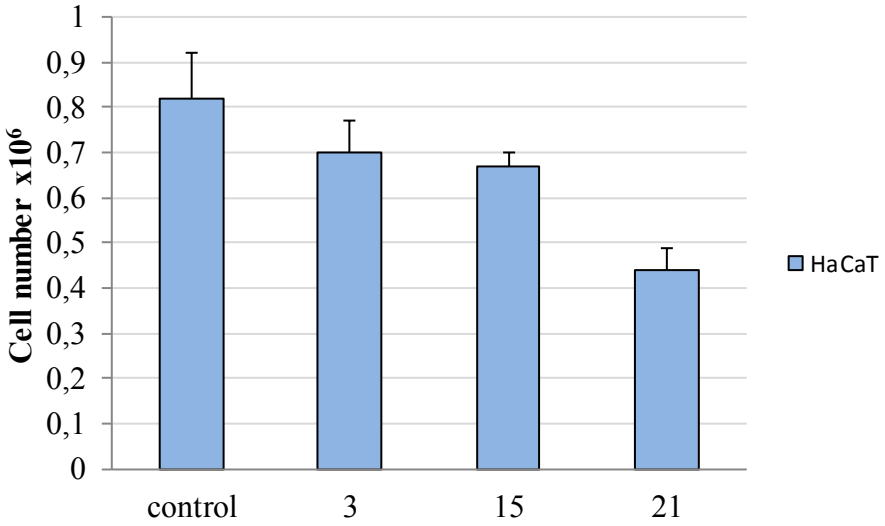


Figure S2. Trypan blue assay. The effect of compounds **3**, **15** and **21** on live cell number and viability in HaCaT cells. Compounds were used in their IC_{50} for PC3 cells, 2.02 µM, 15.7 µM and 4.8 µM, respectively.

Table S4. The effect of compounds **3**, **15** and **21** on early and late apoptosis or necrosis in PC3 and HaCaT cells detected with Annexin V-FITC/PI by flow cytometry. Cells which were Annexin V:FITC positive and PI negative were identified as early apoptosis, and Annexin V:FITC and PI positive as late apoptosis or necrosis. Data are expressed as the mean ± SD from 3 independent experiments.

PC3 cells				
Compound	Live cells	Early apoptosis	Late apoptosis	Dead cells
control	92.03 ± 1.04	7.61 ± 1.05	0.21 ± 0.06	0.15 ± 0.04
3	20.27 ± 0.45	1.51 ± 0.04	12.12 ± 2.18	66.10 ± 6.31
15	61.21 ± 2.06	1.15 ± 0.10	16.49 ± 2.65	21.15 ± 3.29
21	2.59 ± 0.20	28.22 ± 0.98	16.28 ± 2.80	52.91 ± 3.99

HaCaT cells				
Compound	Live cells	Early apoptosis	Late apoptosis	Dead cells
control	95.78 ± 0.77	2.30 ± 0.65	0.97 ± 0.22	0.95 ± 0.17
3	42.70 ± 2.81	10.07 ± 1.22	37.24 ± 2.27	9.99 ± 1.17
15	87.32 ± 2.20	11.63 ± 2.93	0.77 ± 0.05	0.3 ± 0.01
21	54.53 ± 3.34	10.52 ± 1.07	20.71 ± 4.34	14.24 ± 3.78

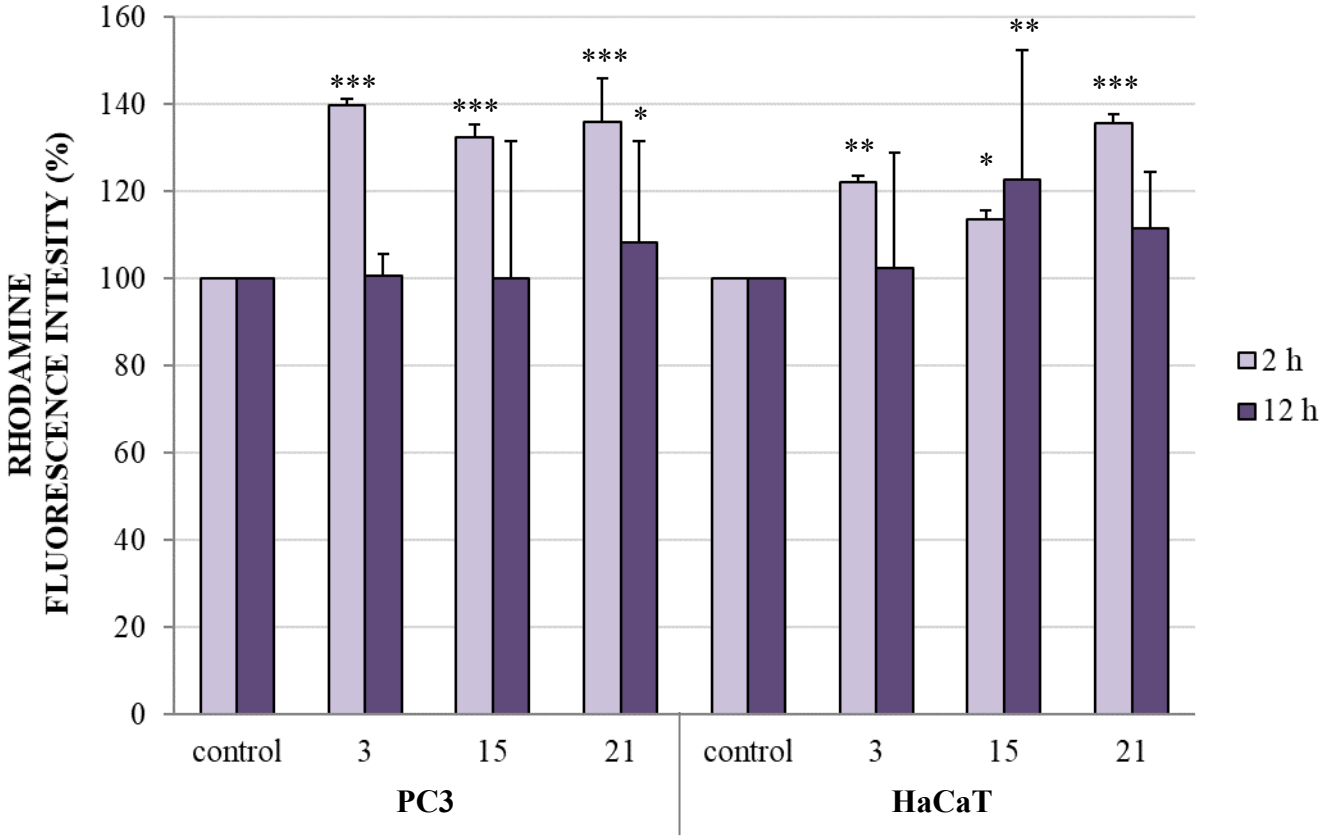
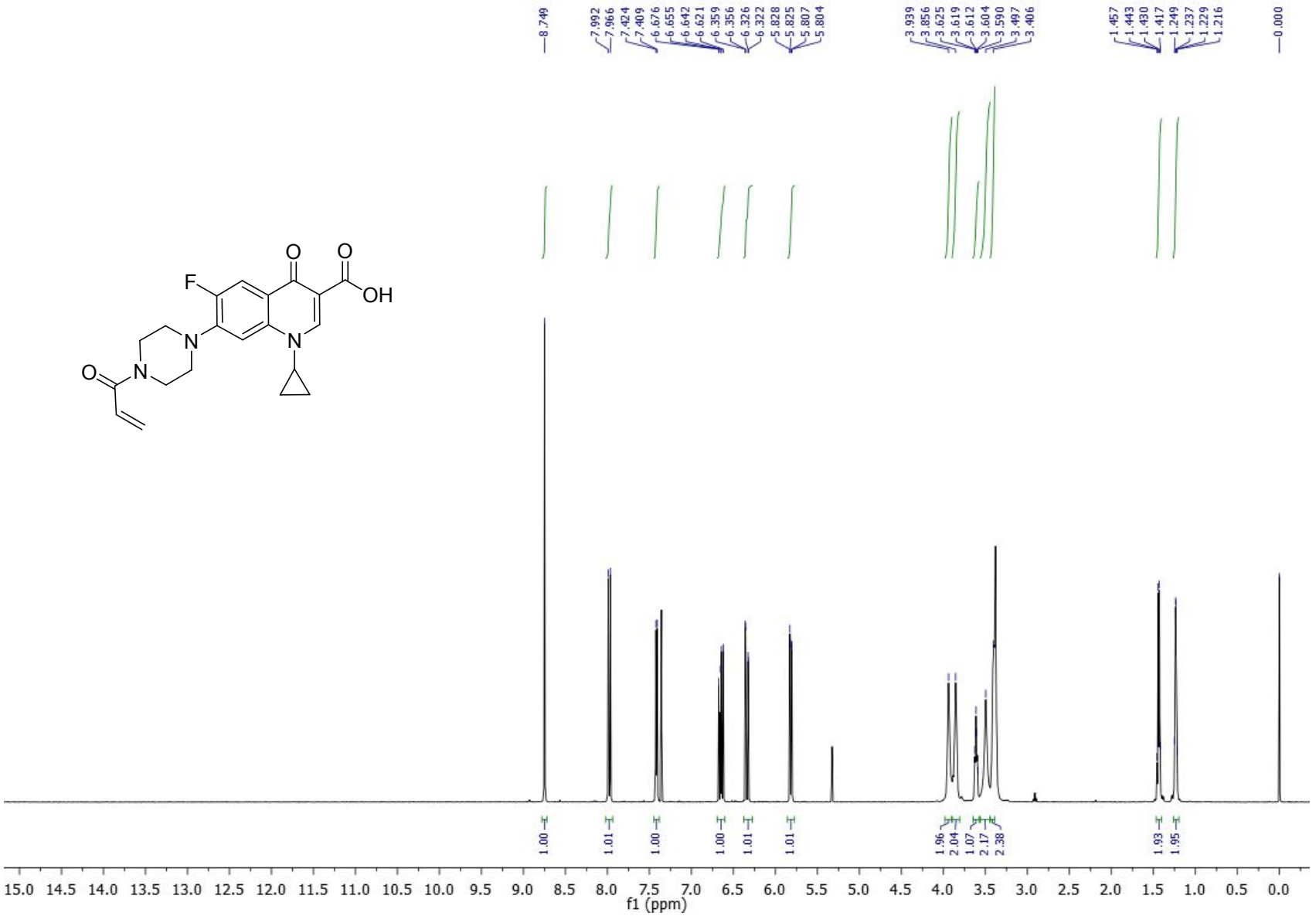


Figure S3. The effect of CP conjugates **3**, **15**, **21** on ROS production in PC3 and HaCaT cells. Cells were incubated with tested compounds at their IC_{50} concentration for 2 and 12 h. Fluorescence intensity (FI) of the probe was measured by rhodamine (5 μM). The results are expressed as mean ± SD of three experiments, each of them performed in triplicate. $^{***}p \leq 0.0001$, ** $p \leq 0.001$, *$p \leq 0.01$, as compared to the control.

^{1}H and ^{13}C NMR spectra of synthesized Ciprofloxacin derivatives **1-21.**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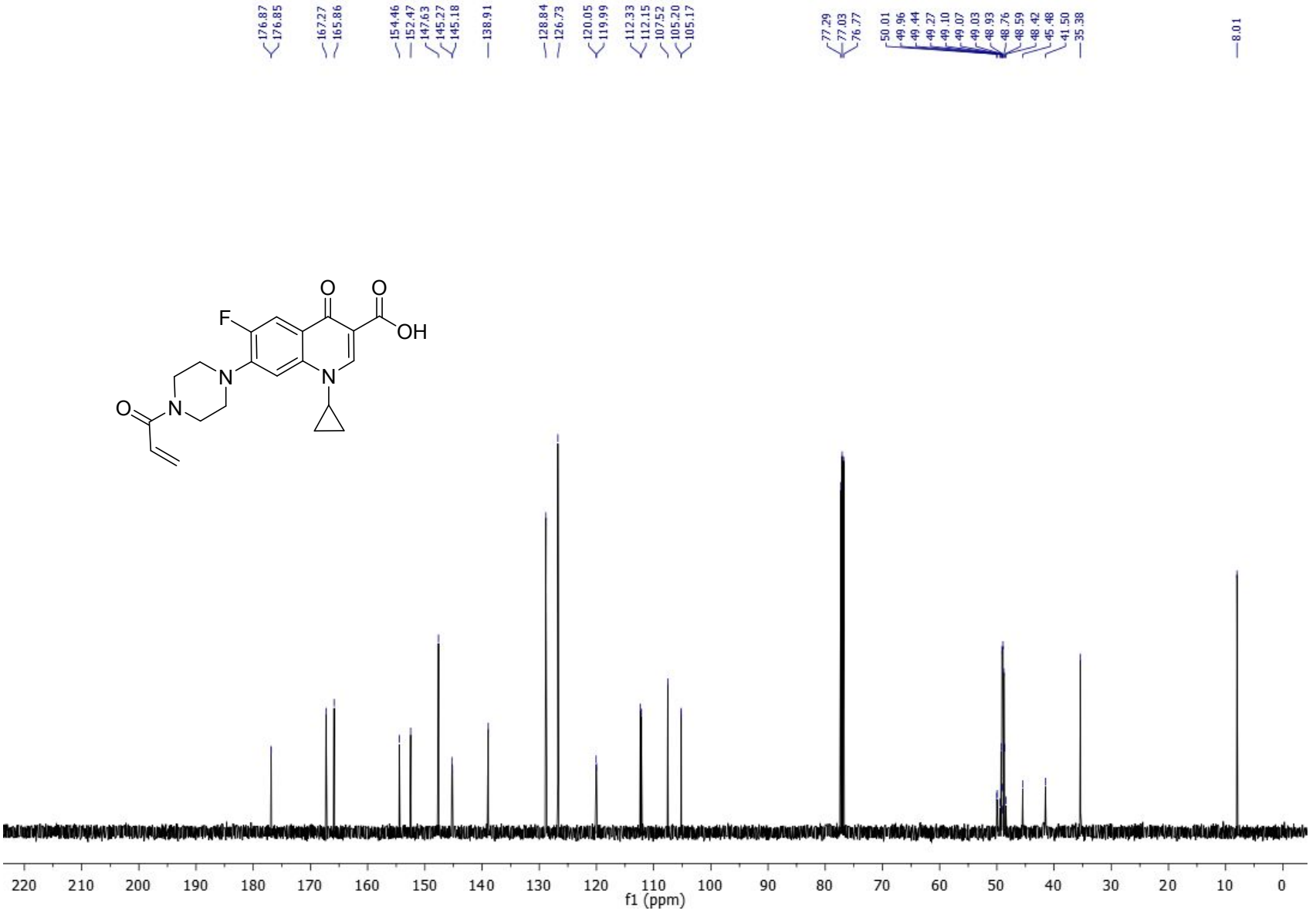
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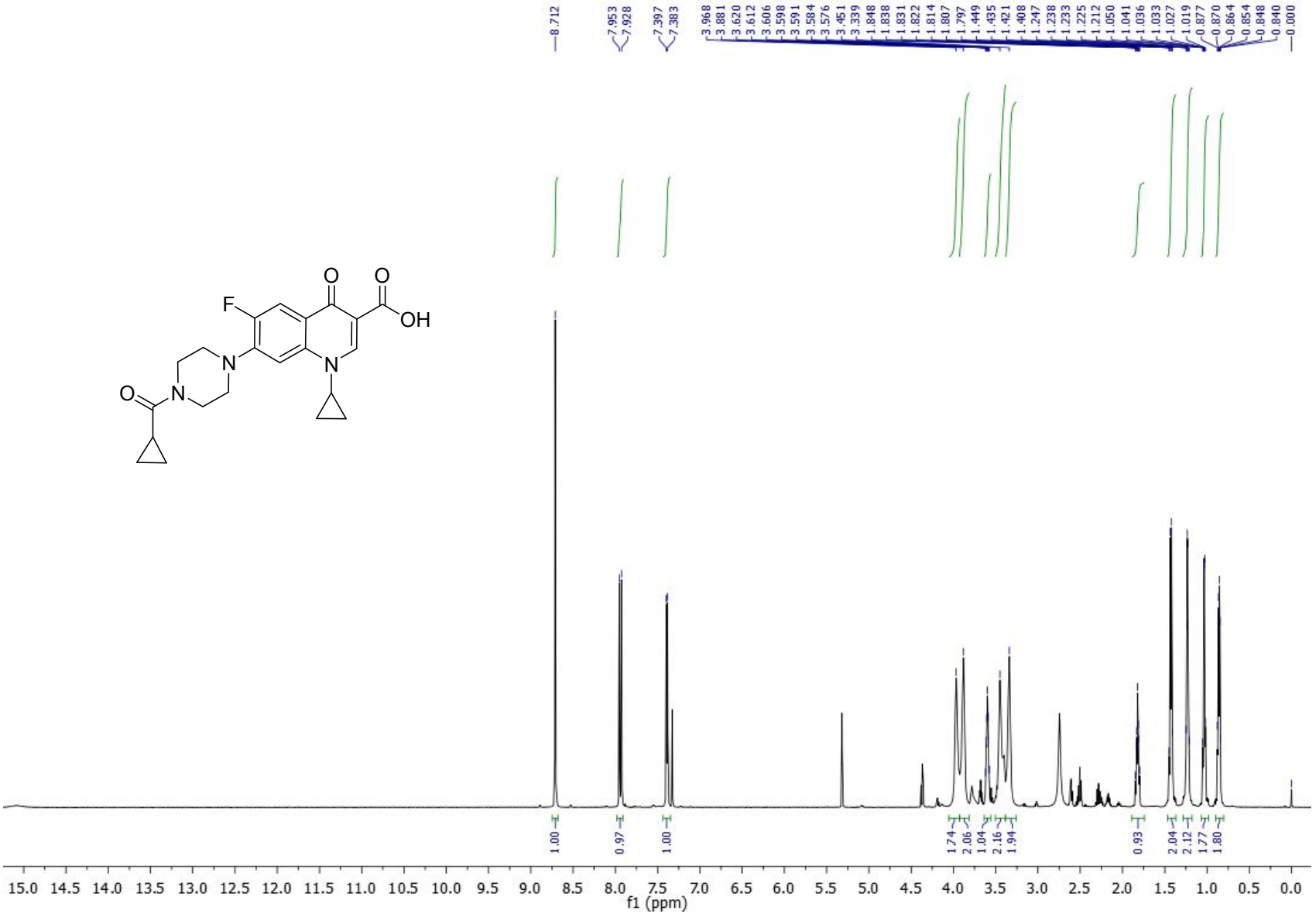
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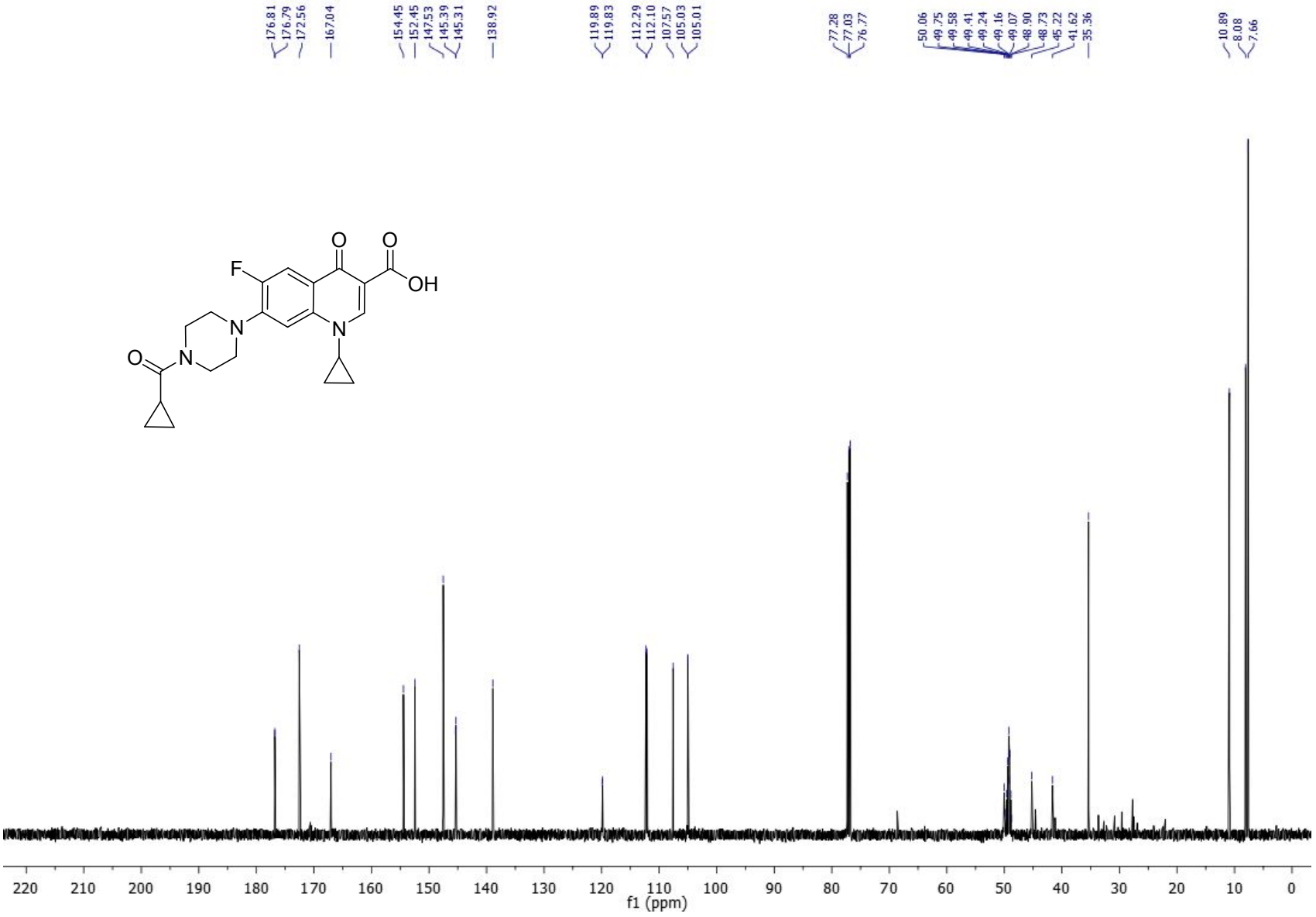
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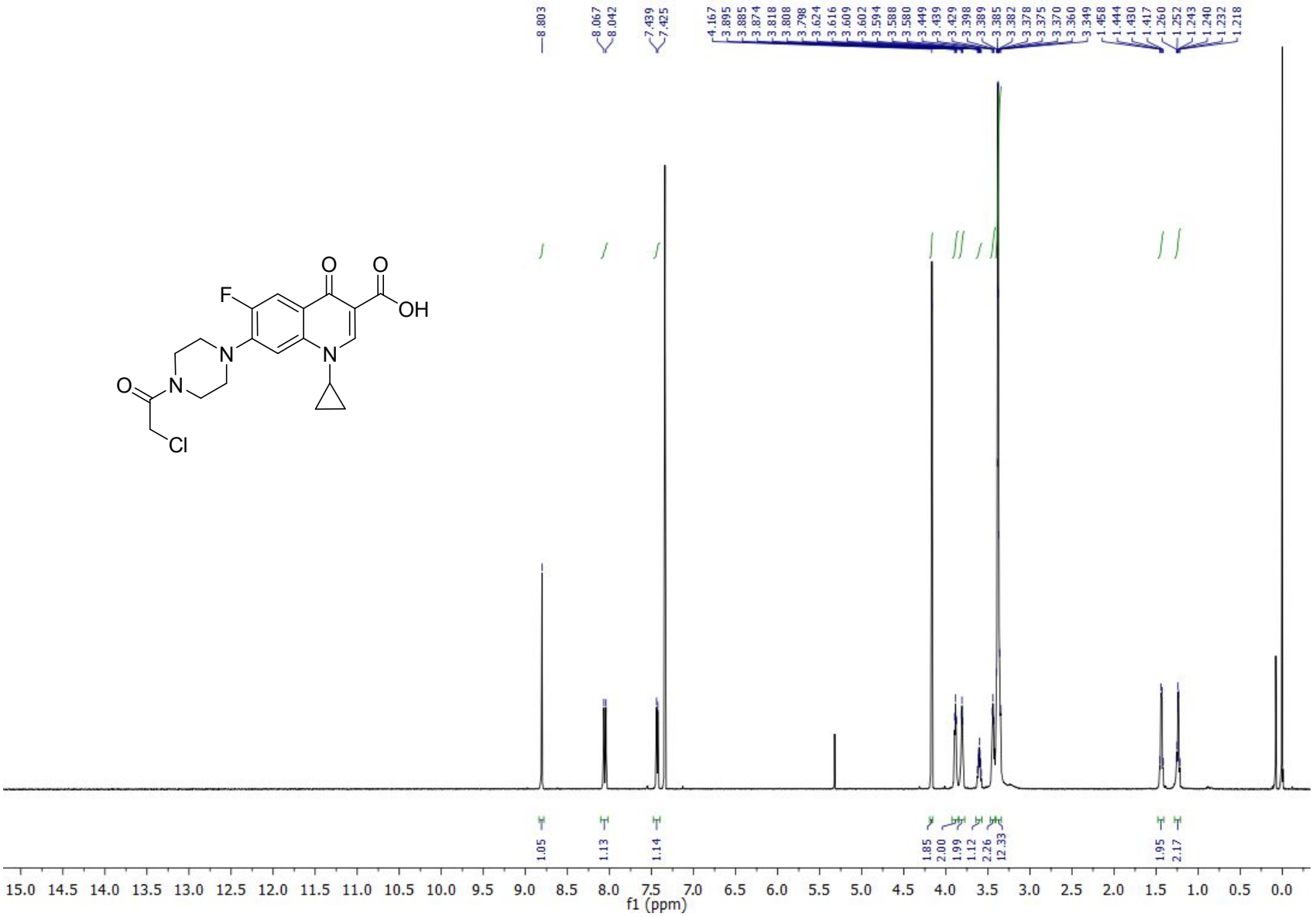
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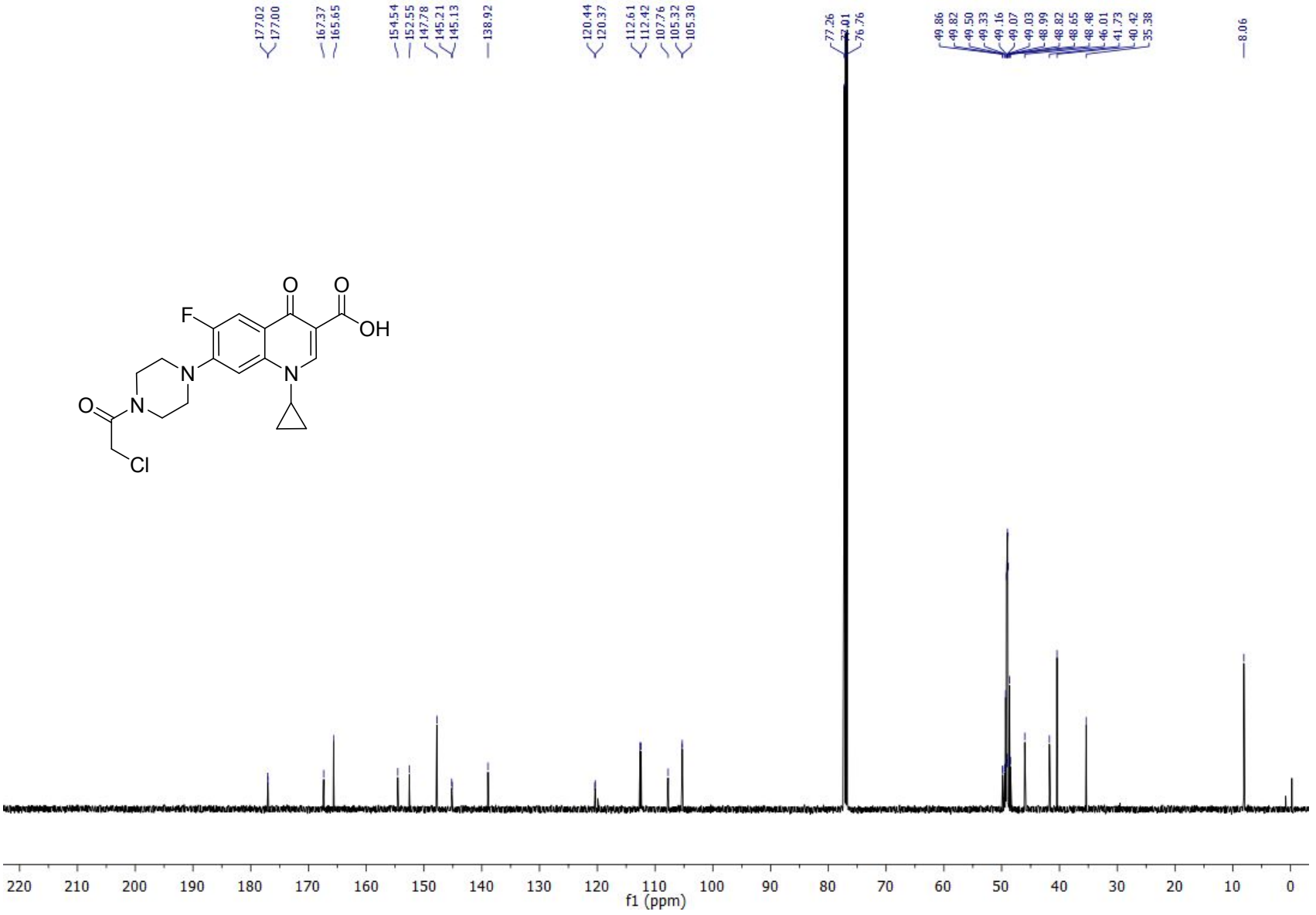
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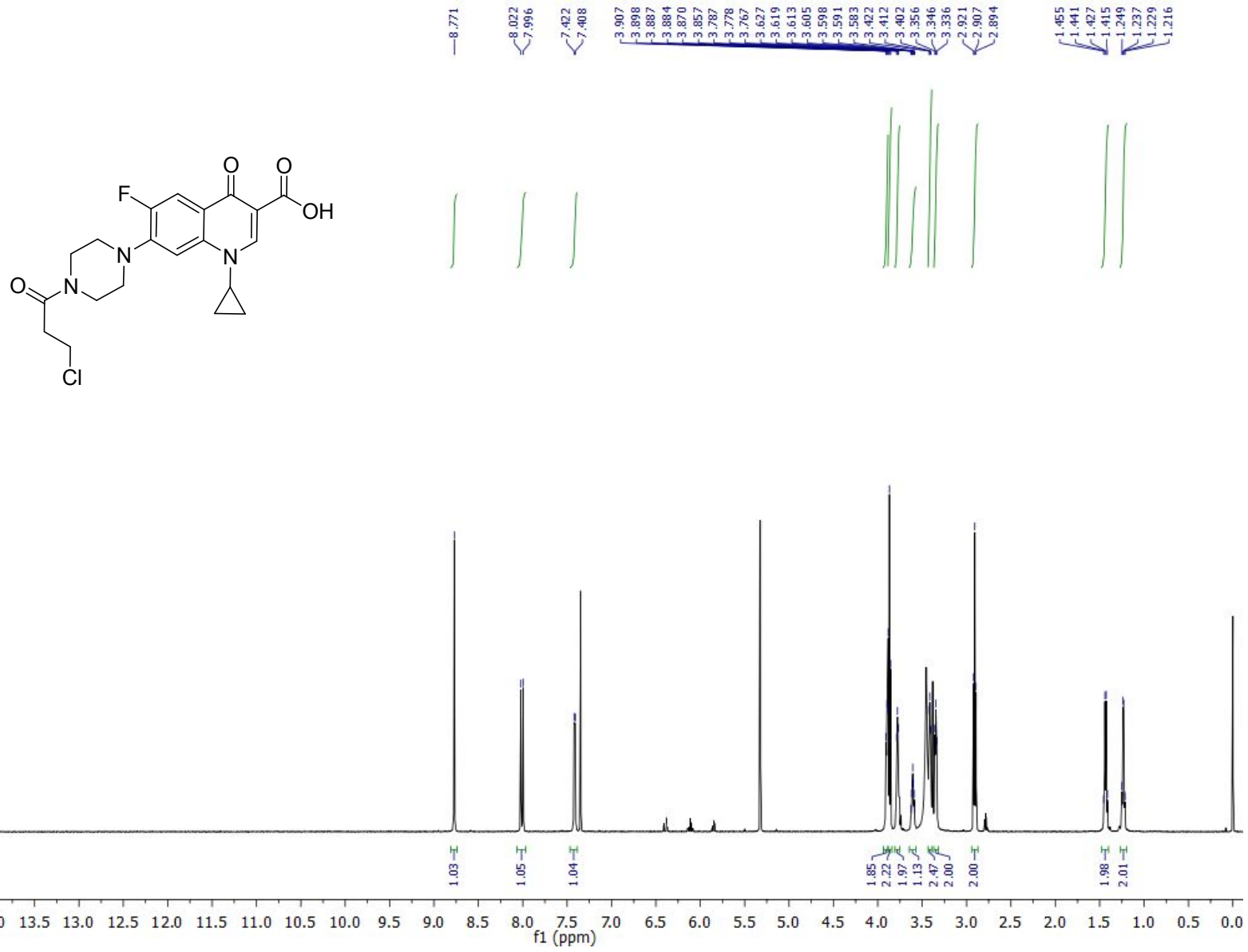
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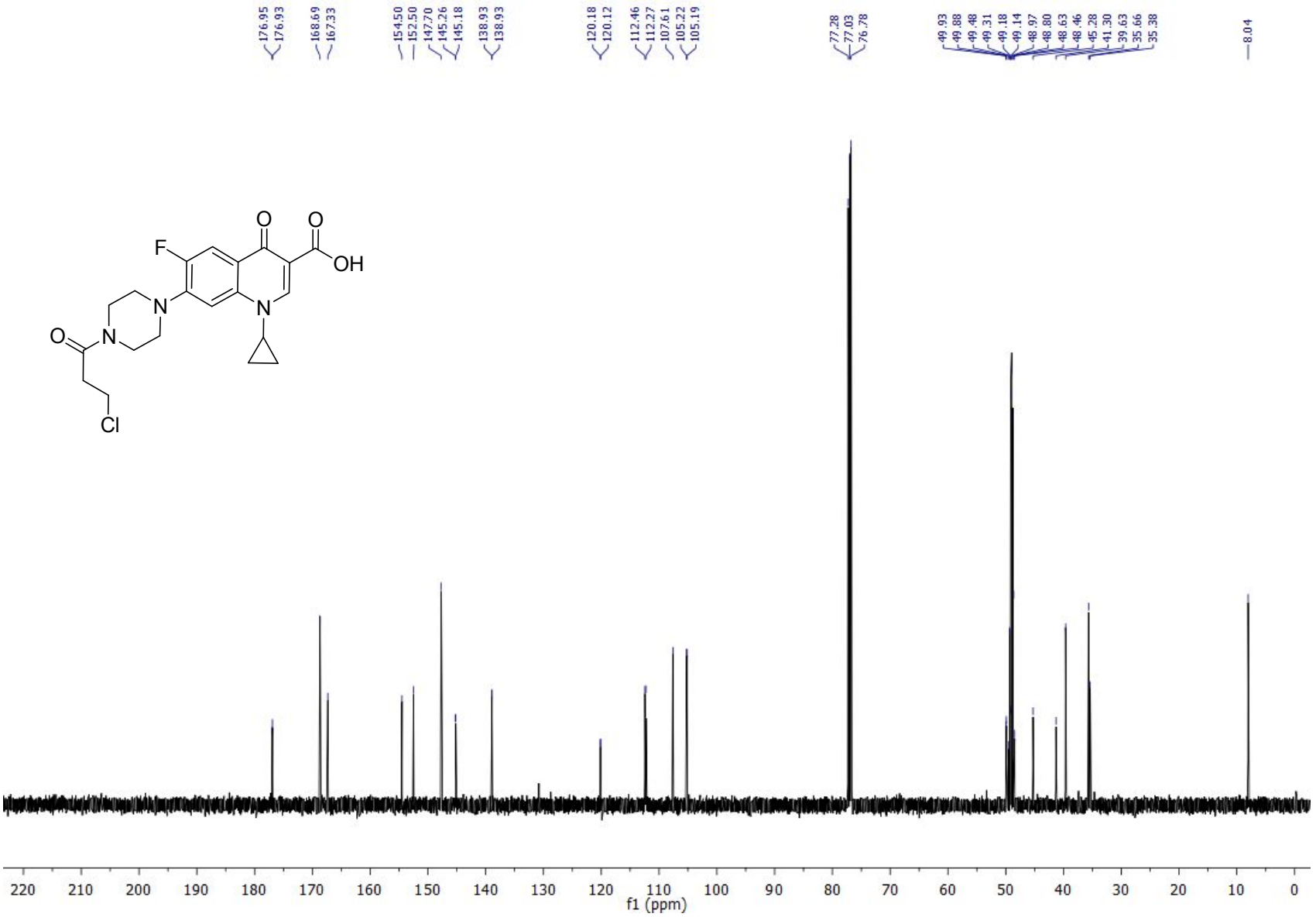
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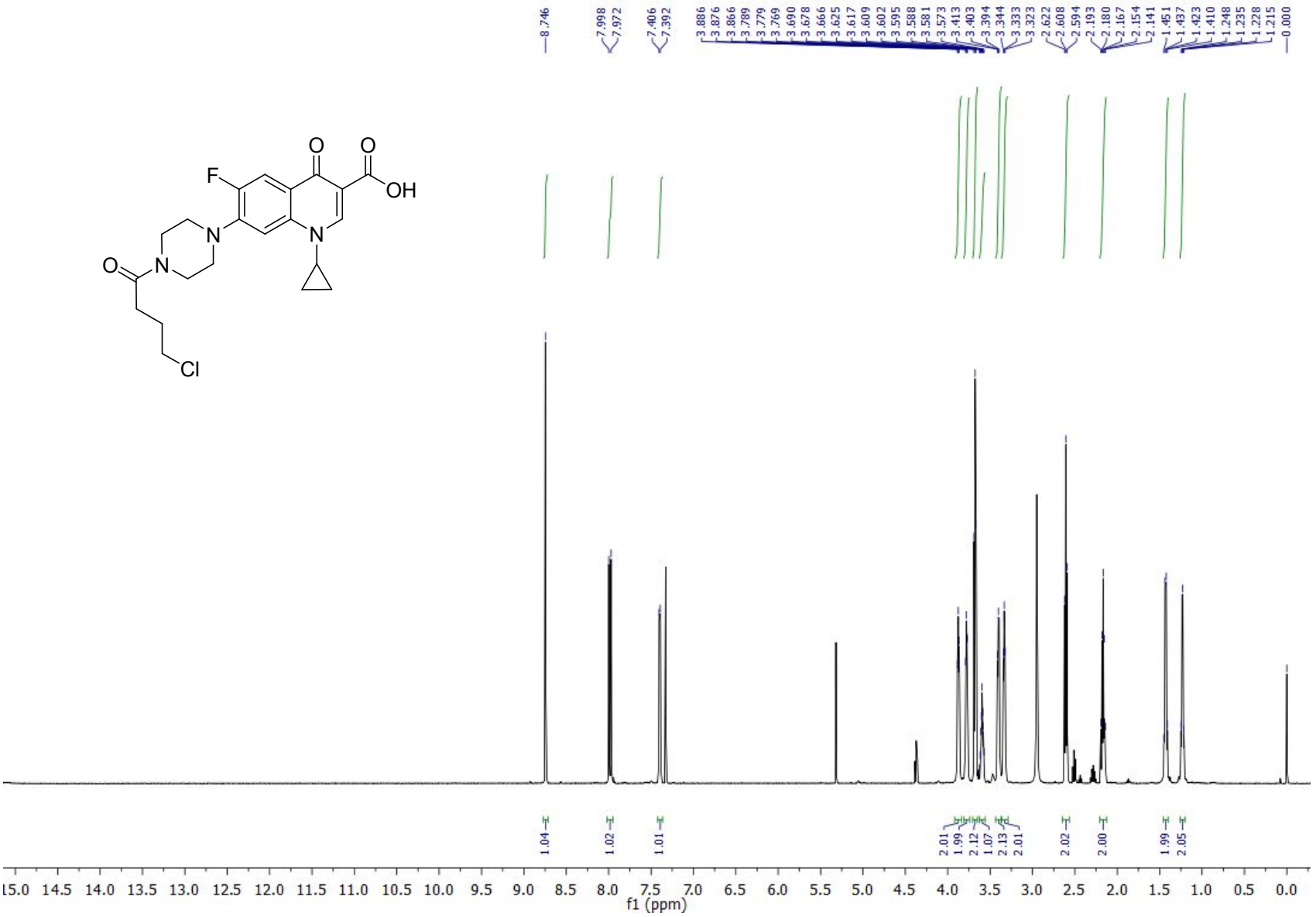
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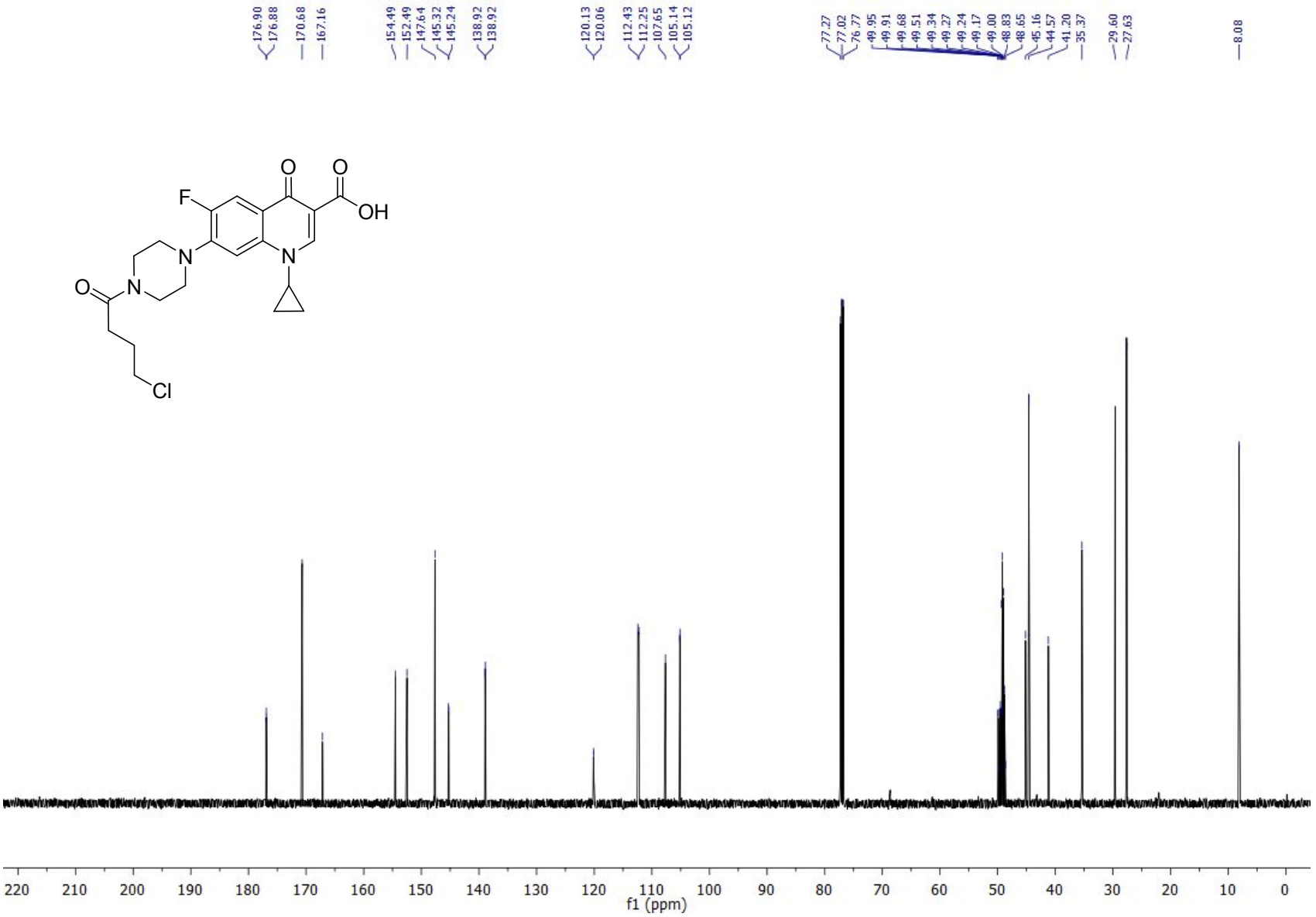
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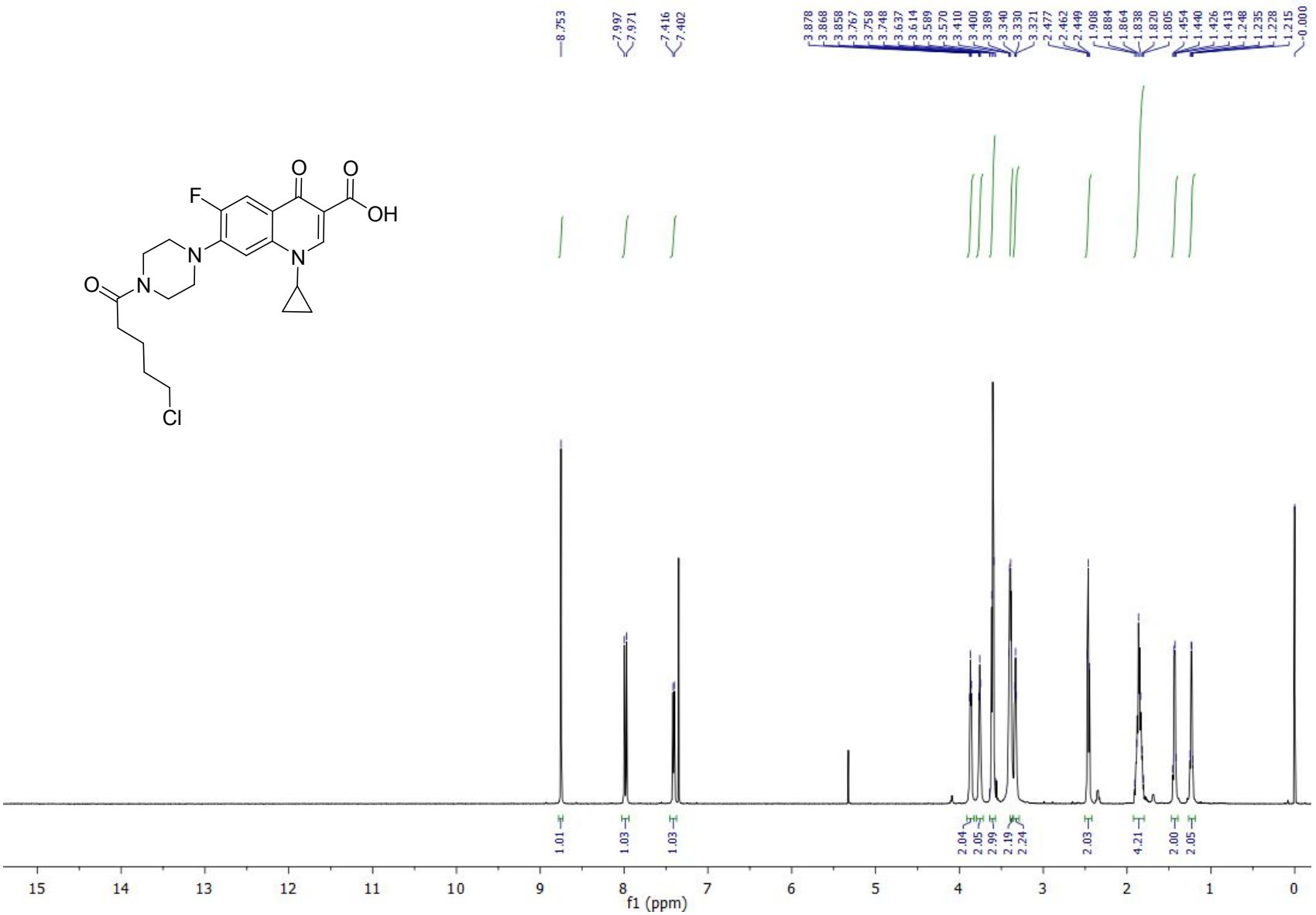
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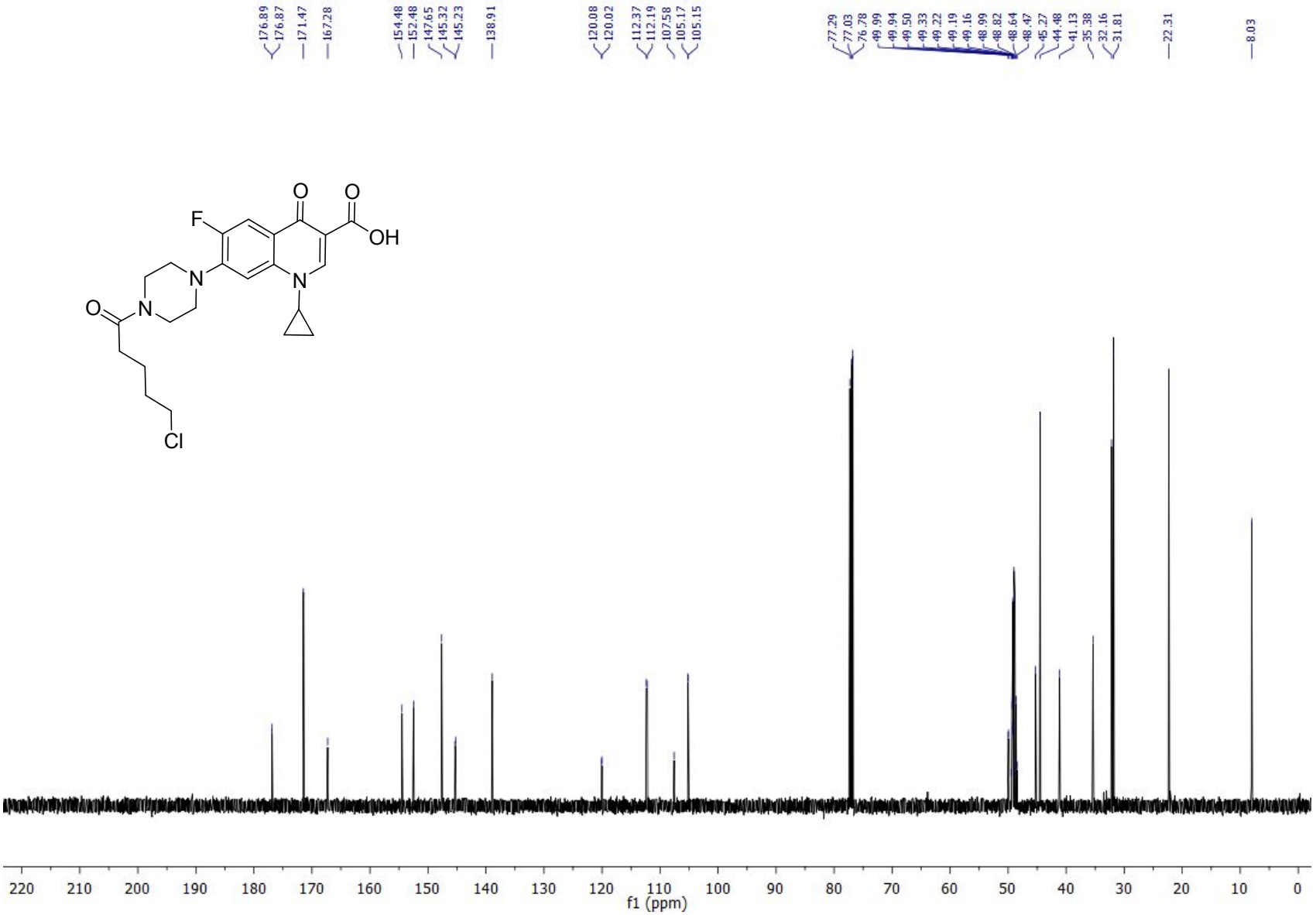
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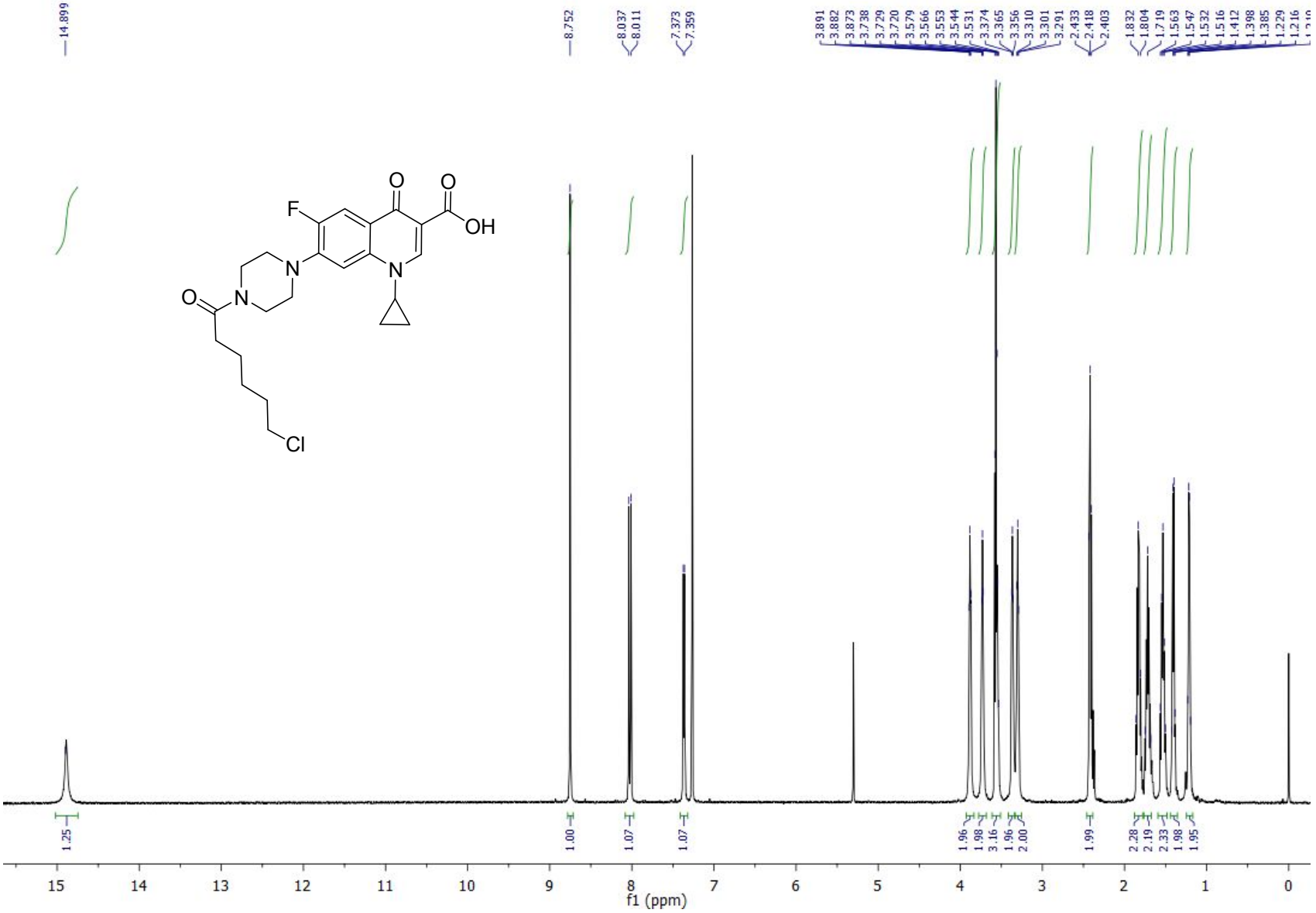
f1 (ppm)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3.531
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3.365
3.356
3.310
3.301
3.291
2.433
2.418
2.403
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1.385
1.229
1.216
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O
O
OH
N
N
O
N
Cl
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1.07
1.07
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1.98
3.16
1.96
2.00
1.99
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2.19
2.33
1.98
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4
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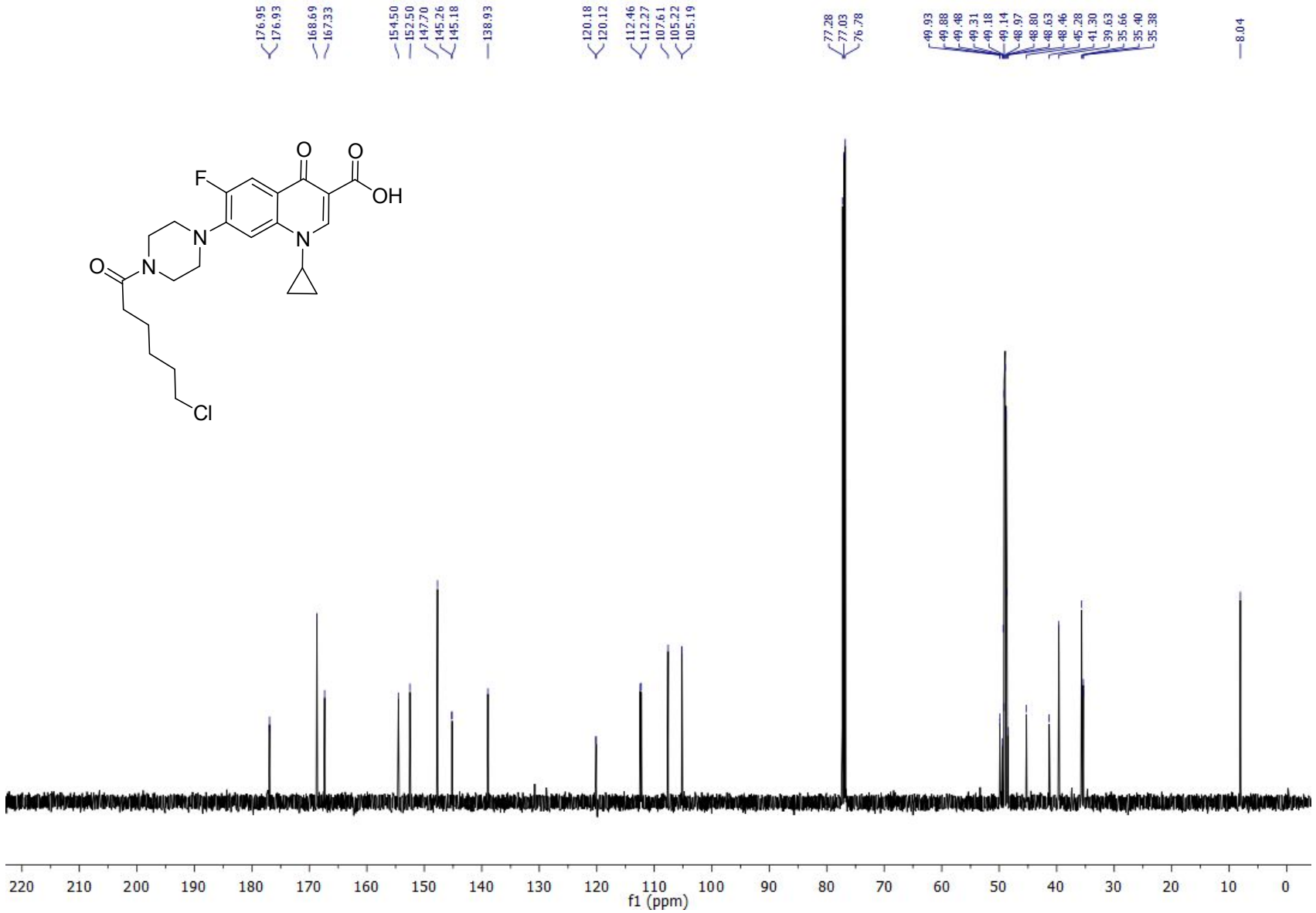
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48.46
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41.30
39.63
35.66
35.40
35.38
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F
O
O
OH
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N
O
N
Cl
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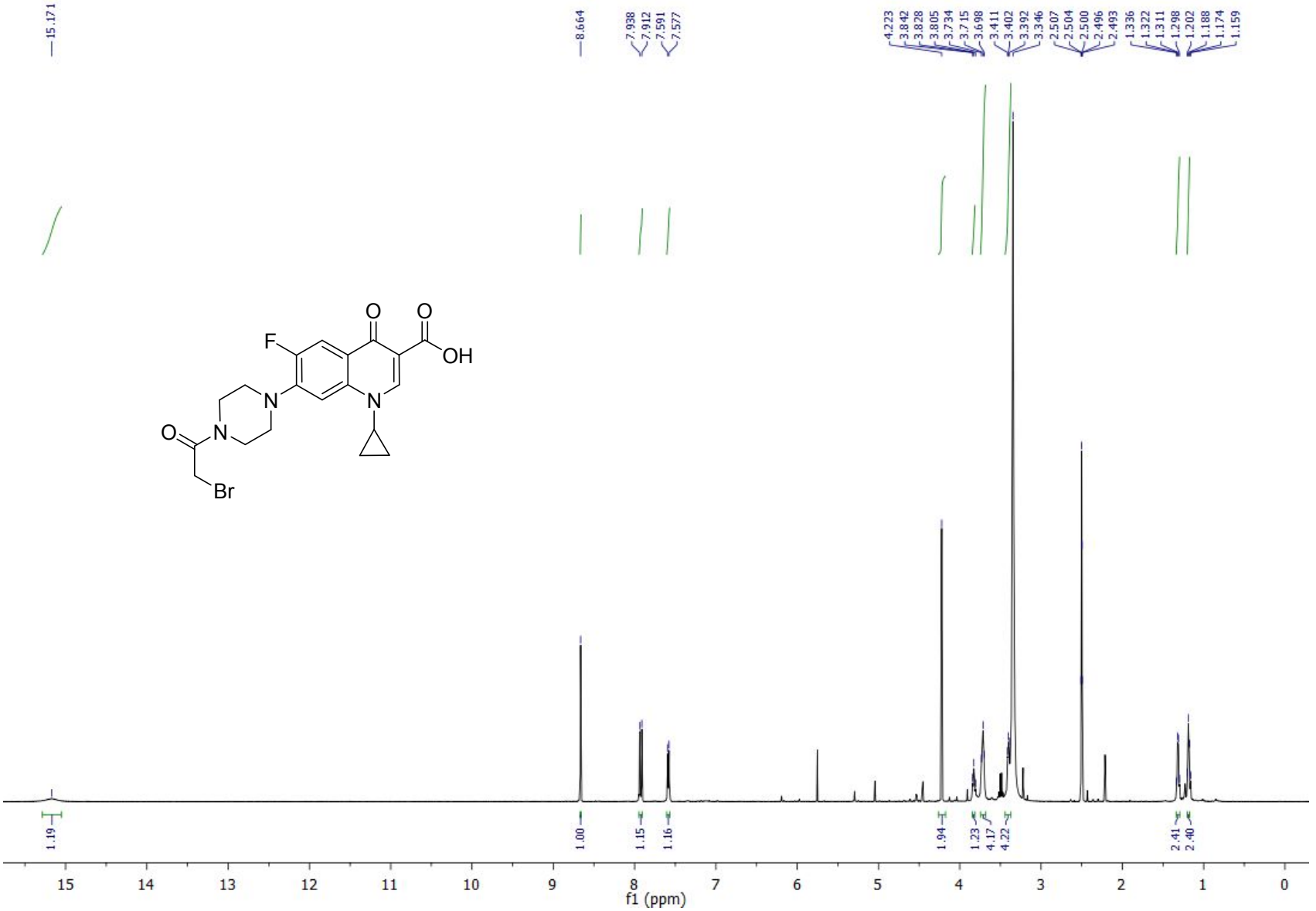
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3.411
3.402
3.392
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2.504
2.500
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2.493
1.336
1.322
1.311
1.298
1.202
1.188
1.174
1.159
F
O
O
OH
N
N
O
N
Br
1.19
1.00
1.15
1.16
1.94
1.23
4.17
4.22
2.41
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9
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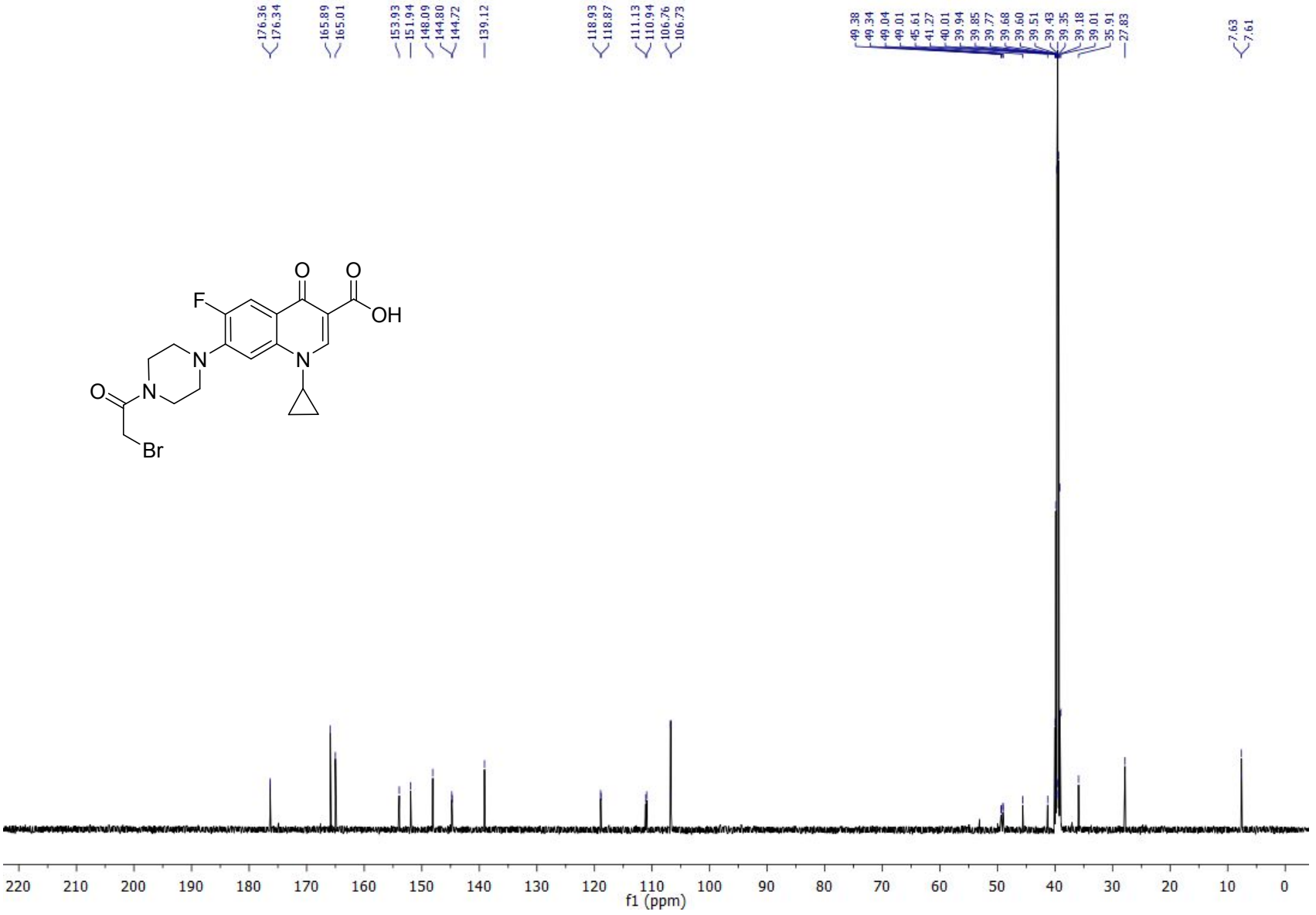
f1 (ppm)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40.01
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39.85
39.77
39.68
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39.43
39.35
39.18
39.01
35.91
27.83
7.63
7.61
F
O
O
OH
N
N
N
O
Br
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210
200
190
180
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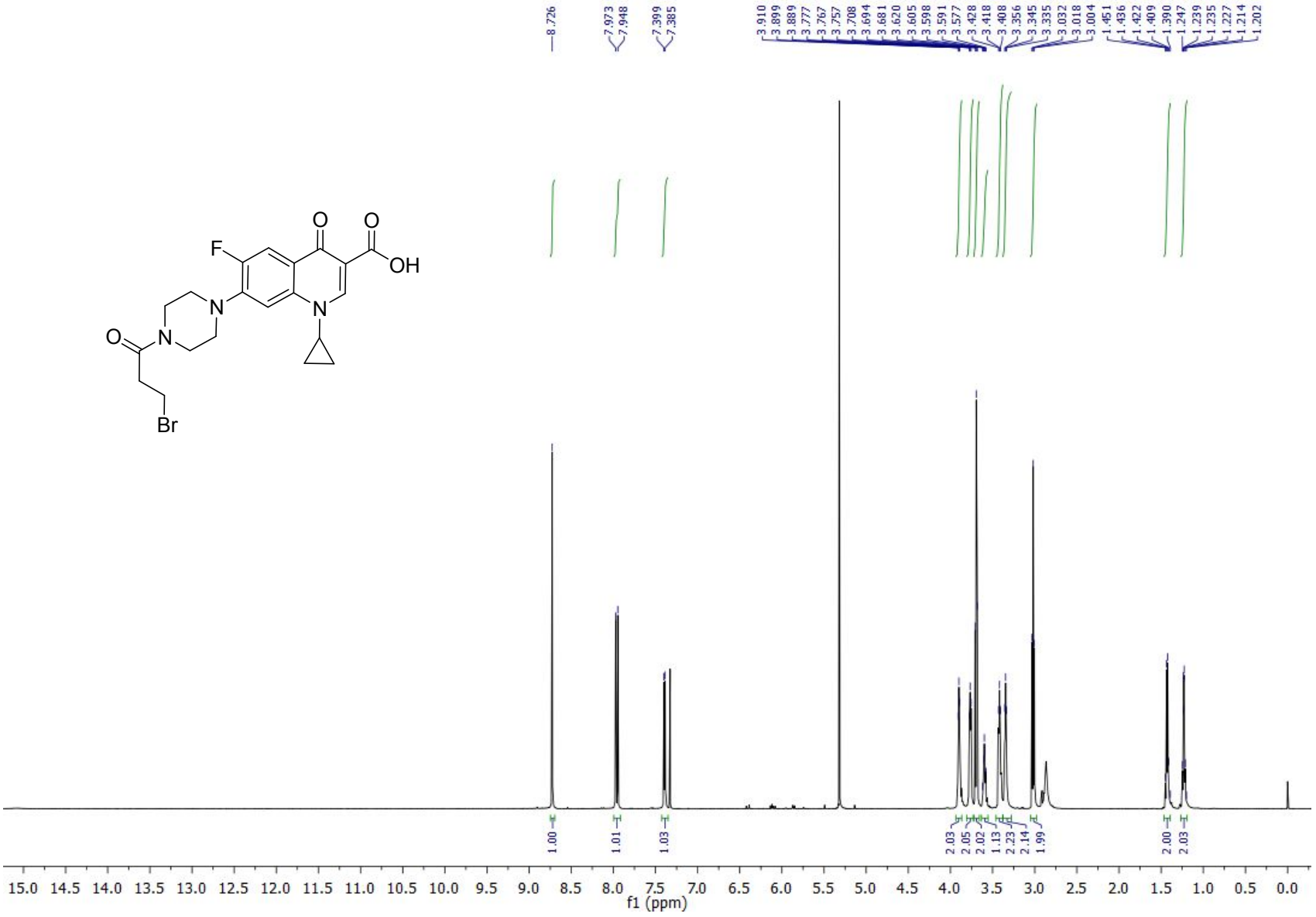
f1 (ppm)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3.605
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3.591
3.577
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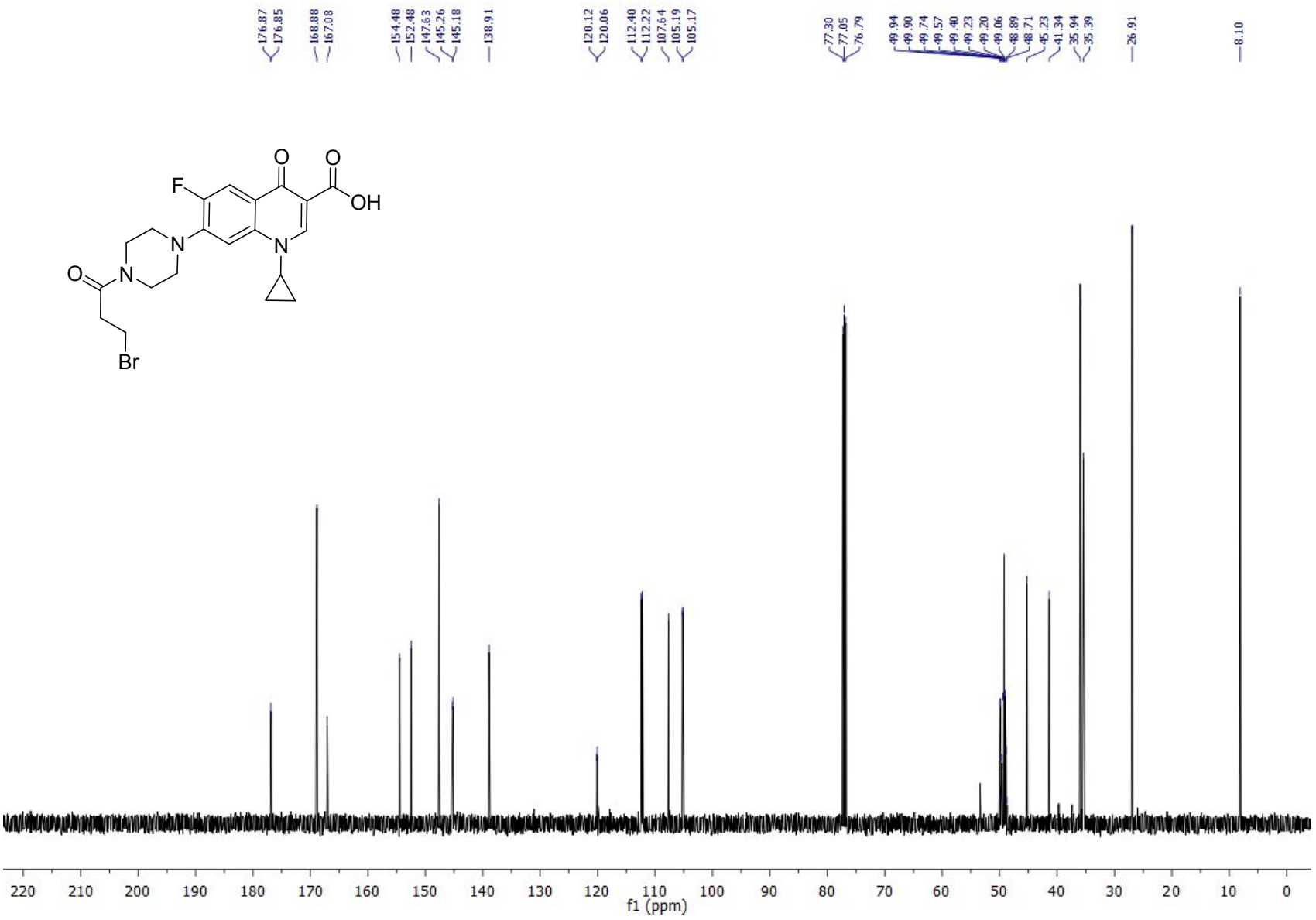
3.418
3.408
3.356
3.345
3.335
3.032
3.018
3.004
1.451
1.436
1.422
1.409
1.390
1.247
1.239
1.235
1.227
1.214
1.202
F
O
O
OH
N
N
O
N
Br
1.00
1.01
1.03
2.03
2.05
2.02
1.13
2.23
2.14
1.99
2.00
2.03
15.0 14.5 14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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120.12
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107.64
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105.17
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77.05
76.79
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49.57
49.40
49.23
49.20
49.06
48.89
48.71
45.23
41.34
35.94
35.39
26.91
8.10
F
O
O
OH
N
N
O
N
Br
220
210
200
190
180
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130
120
110
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90
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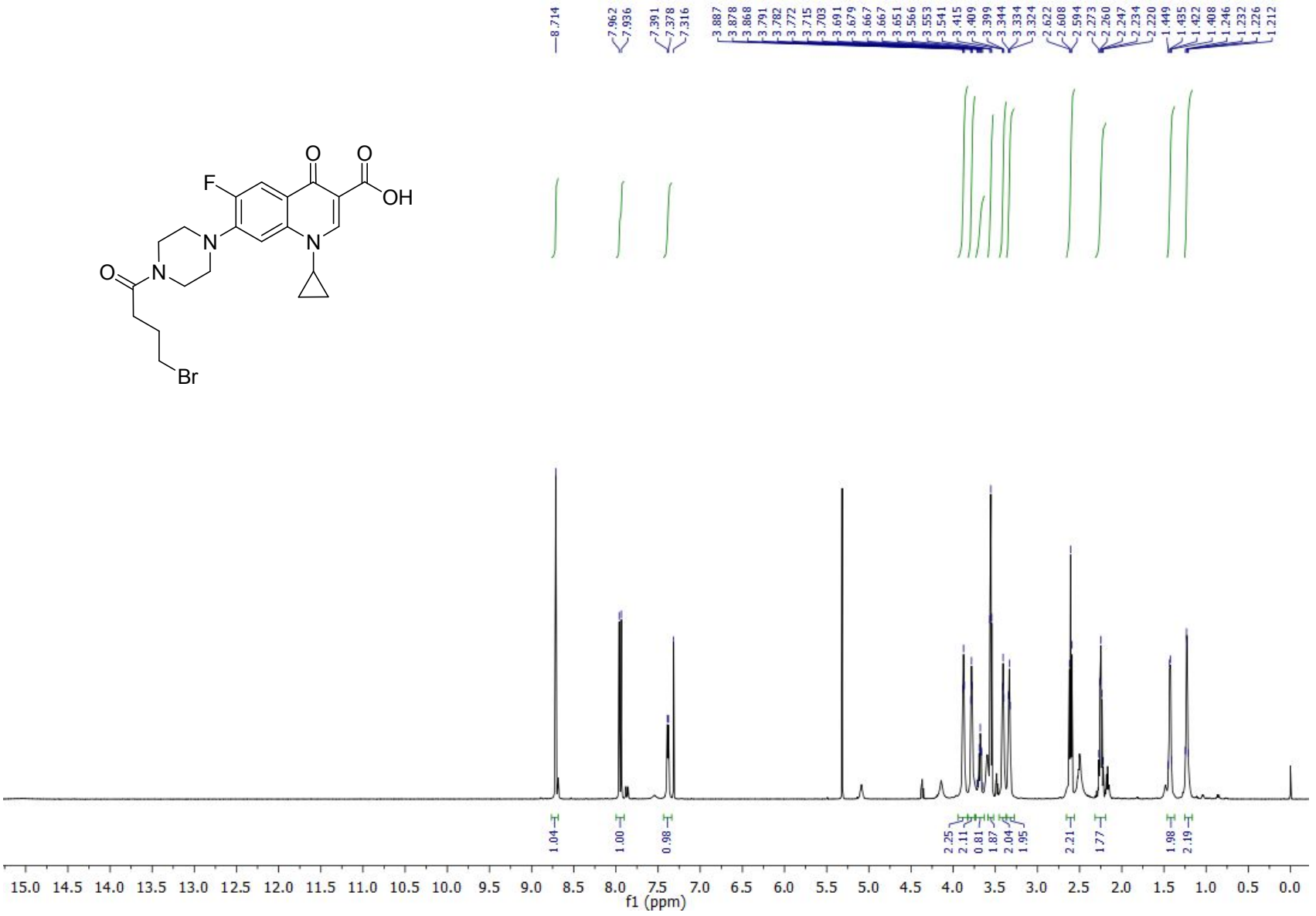
50
40
30
20
10
0
f1 (ppm)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3.679
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3.667
3.651
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3.553
3.541
3.415
3.409
3.399
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3.334
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2.247
2.234
2.220
1.449
1.435
1.422
1.408
1.246
1.232
1.226
1.212
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O
O
OH
N
N
O
N
Br
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1.00
0.98
2.25
2.11
0.81
1.87
2.04
1.95
2.21
1.77
1.98
2.19
15.0 14.5 14.0 13.5 13.0 12.5 12.0 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
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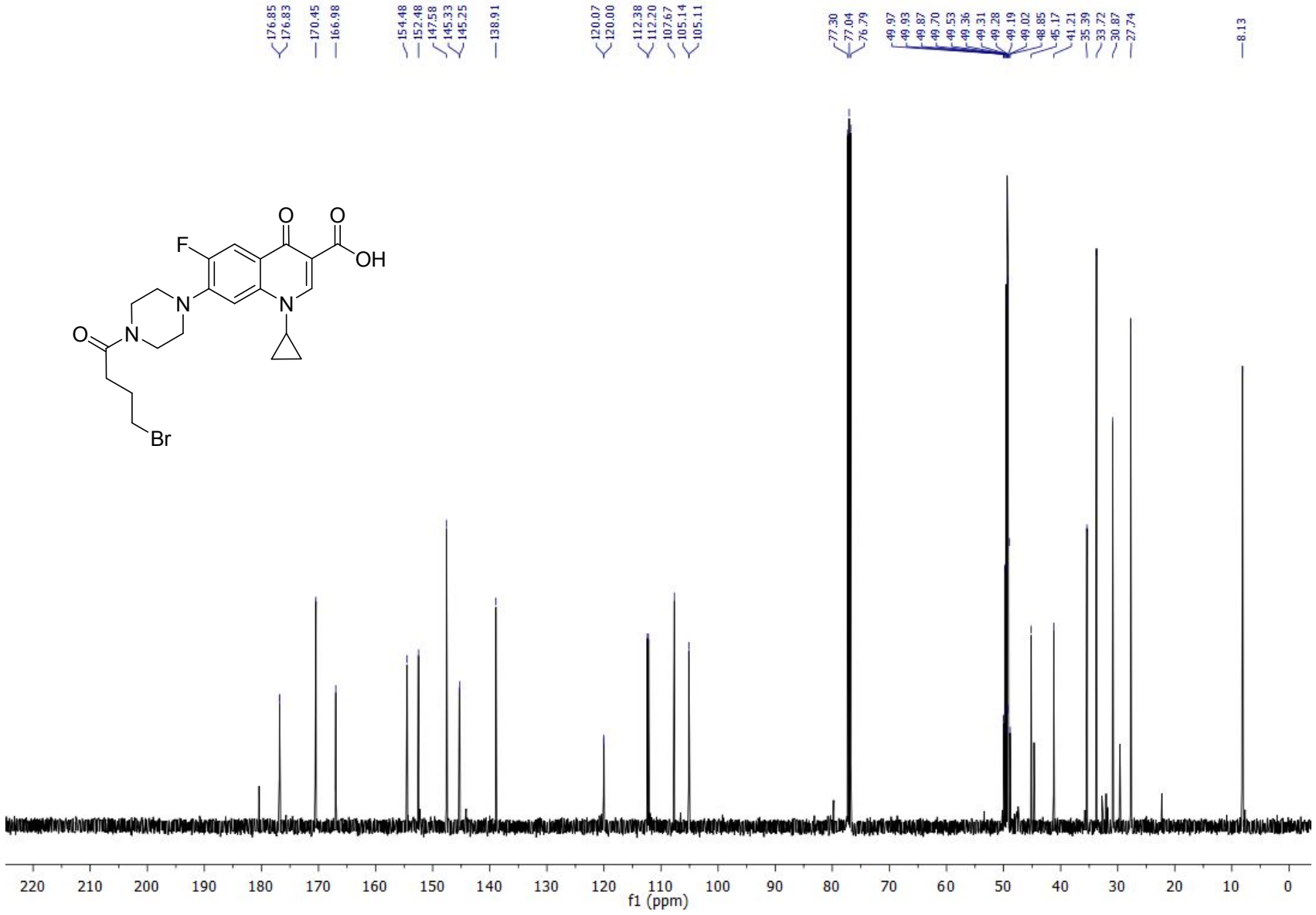
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49.36
49.31
49.28
49.19
49.02
48.85
45.17
41.21
35.39
33.72
30.87
27.74
8.13
F
O
O
OH
N
N
O
N
Br
220
210
200
190
180
170
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130
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110
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90
80
70
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50
40
30
20
10
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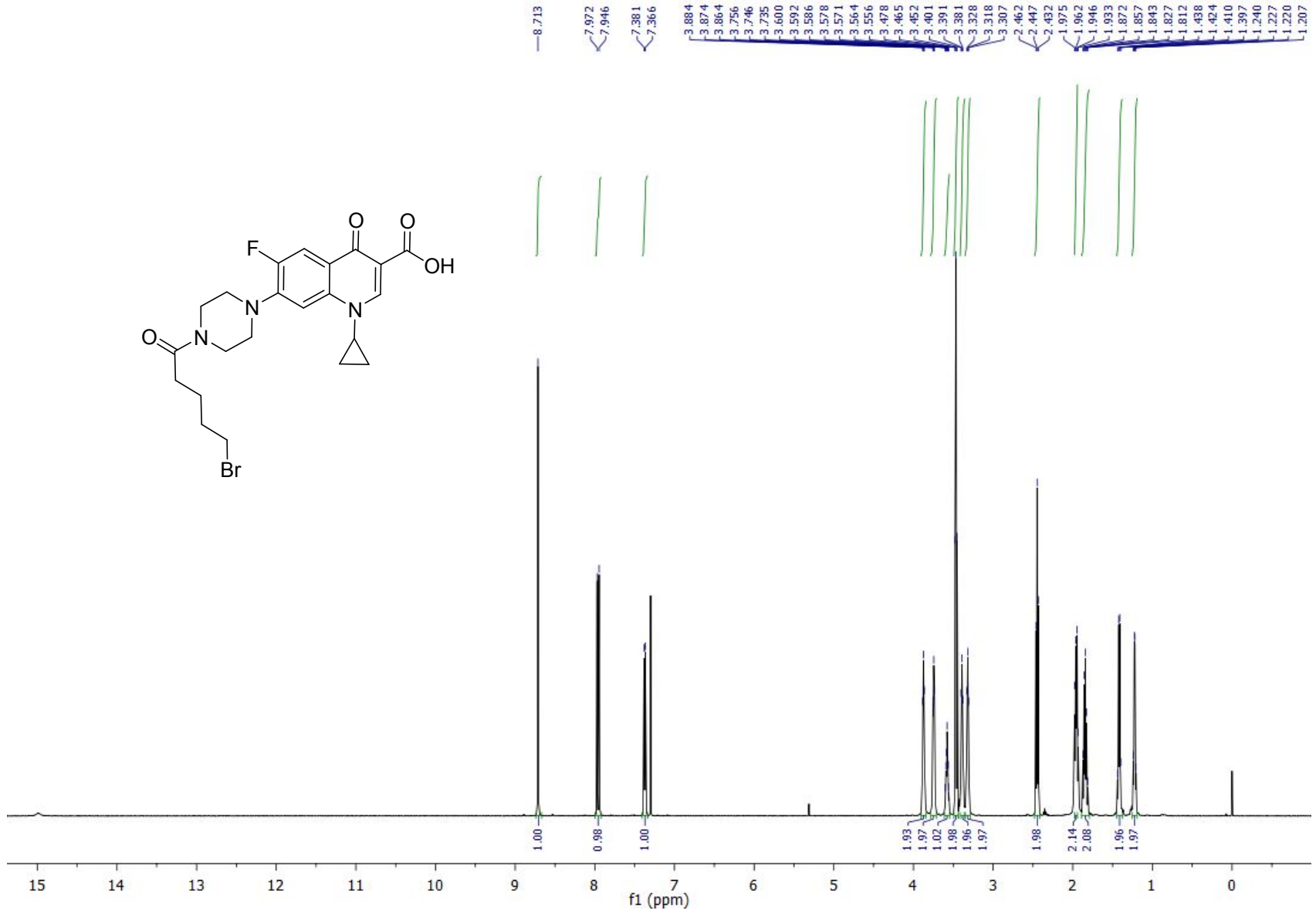
f1 (ppm)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3.600
3.592
3.586
3.578
3.571
3.564
3.556
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3.452
3.401
3.391
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3.318
3.307
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2.447
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1.962
1.946
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1.438
1.424
1.410
1.397
1.240
1.227
1.220
1.207
1.00
0.98
1.00
1.93
1.97
1.02
1.98
1.96
1.97
1.98
2.14
2.08
1.96
1.97
15
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13
12
11
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9
8
7
6
5
4
3
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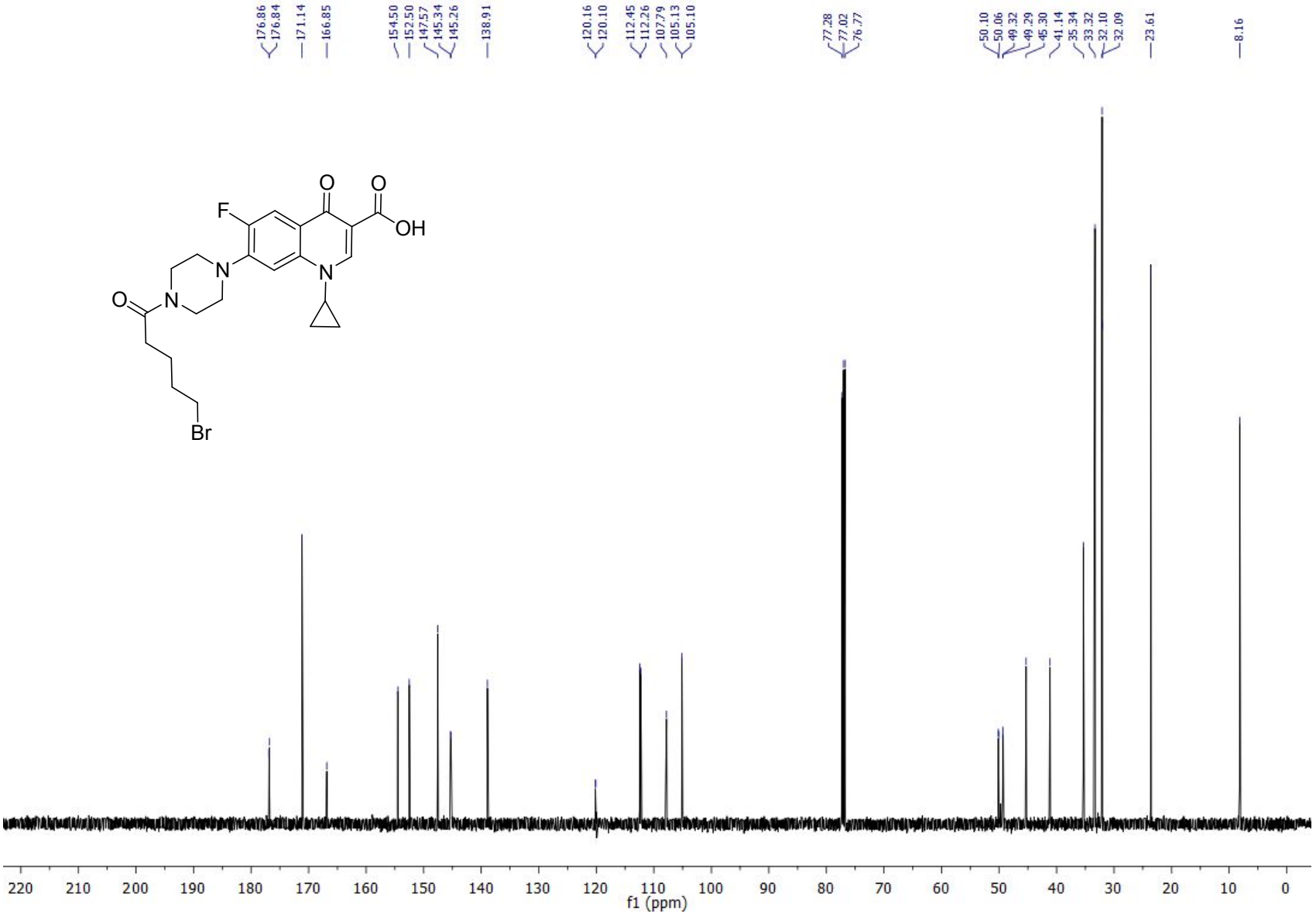
1
0
f1 (ppm)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145.26
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120.10
112.45
112.26
107.79
105.13
105.10
77.28
77.02
76.77
50.10
50.06
49.32
49.29
45.30
41.14
35.34
33.32
32.10
32.09
23.61
8.16
F
O
O
OH
N
N
O
N
Br
220
210
200
190
180
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150
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130
120
110
100
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50
40
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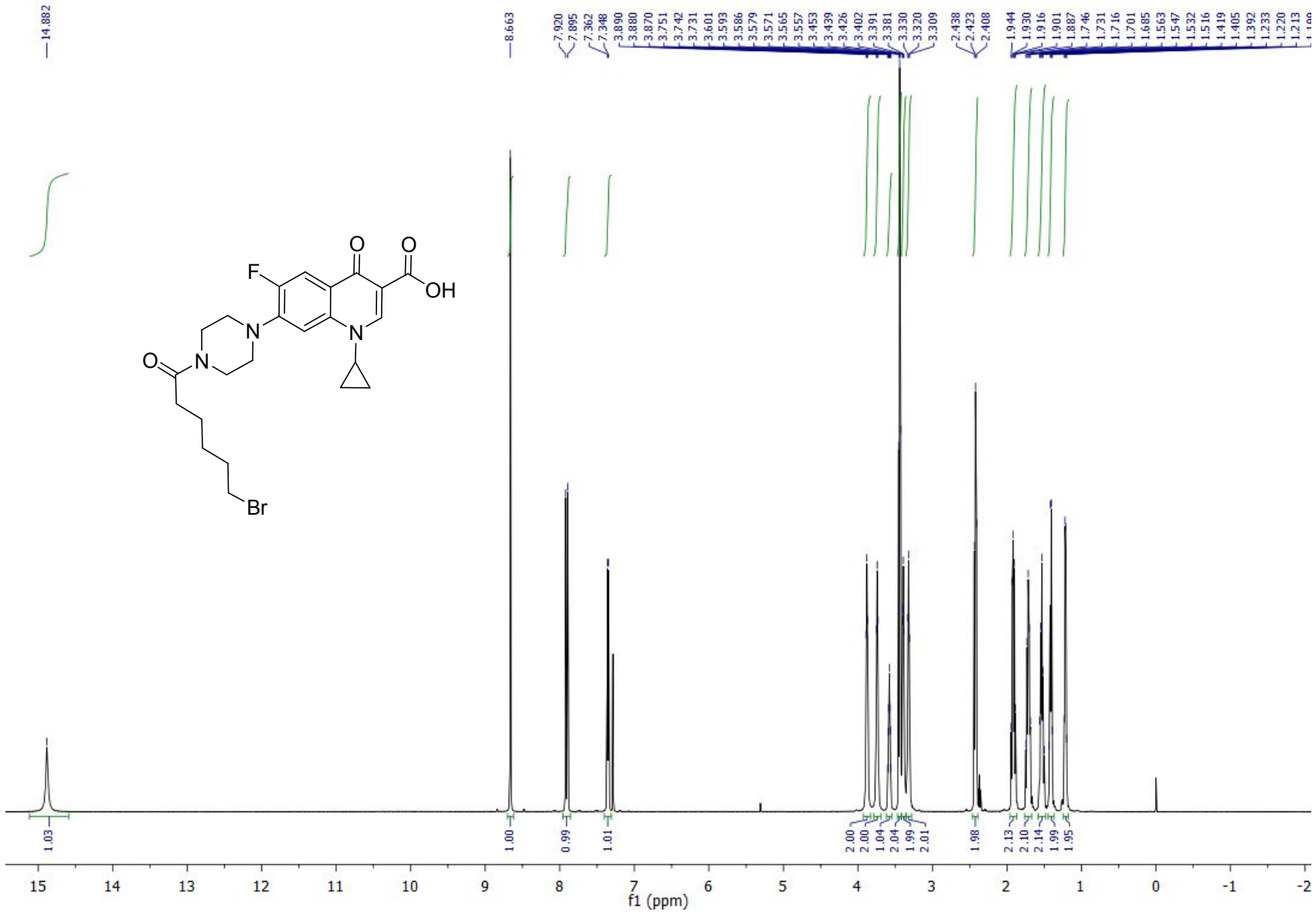
0
f1 (ppm)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3.320
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2.423
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1.563
1.547
1.532
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1.405
1.392
1.233
1.220
1.213
F
O
O
OH
N
N
O
N
Br
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1.00
0.99
1.01
2.00
2.00
1.04
2.04
1.99
2.01
1.98
2.13
2.10
2.14
1.99
1.95
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10
9
8
7
6
5
4
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2
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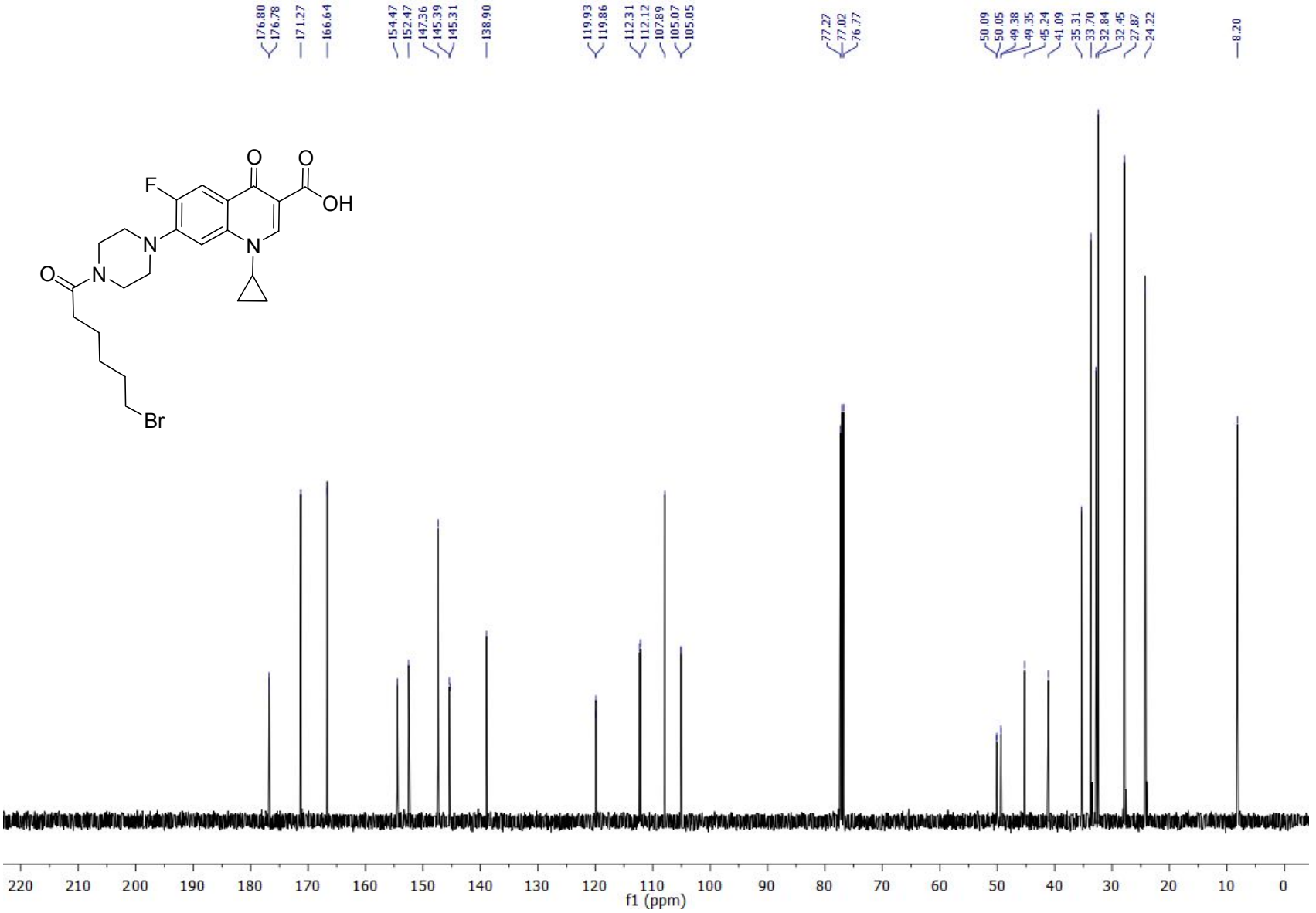
-1
-2
f1 (ppm)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145.31
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112.12
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105.05
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50.09
50.05
49.38
49.35
45.24
41.09
35.31
33.70
32.84
32.45
27.87
24.22
8.20
F
O
O
OH
N
N
O
N
Br
220
210
200
190
180
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130
120
110
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80
70
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50
40
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10
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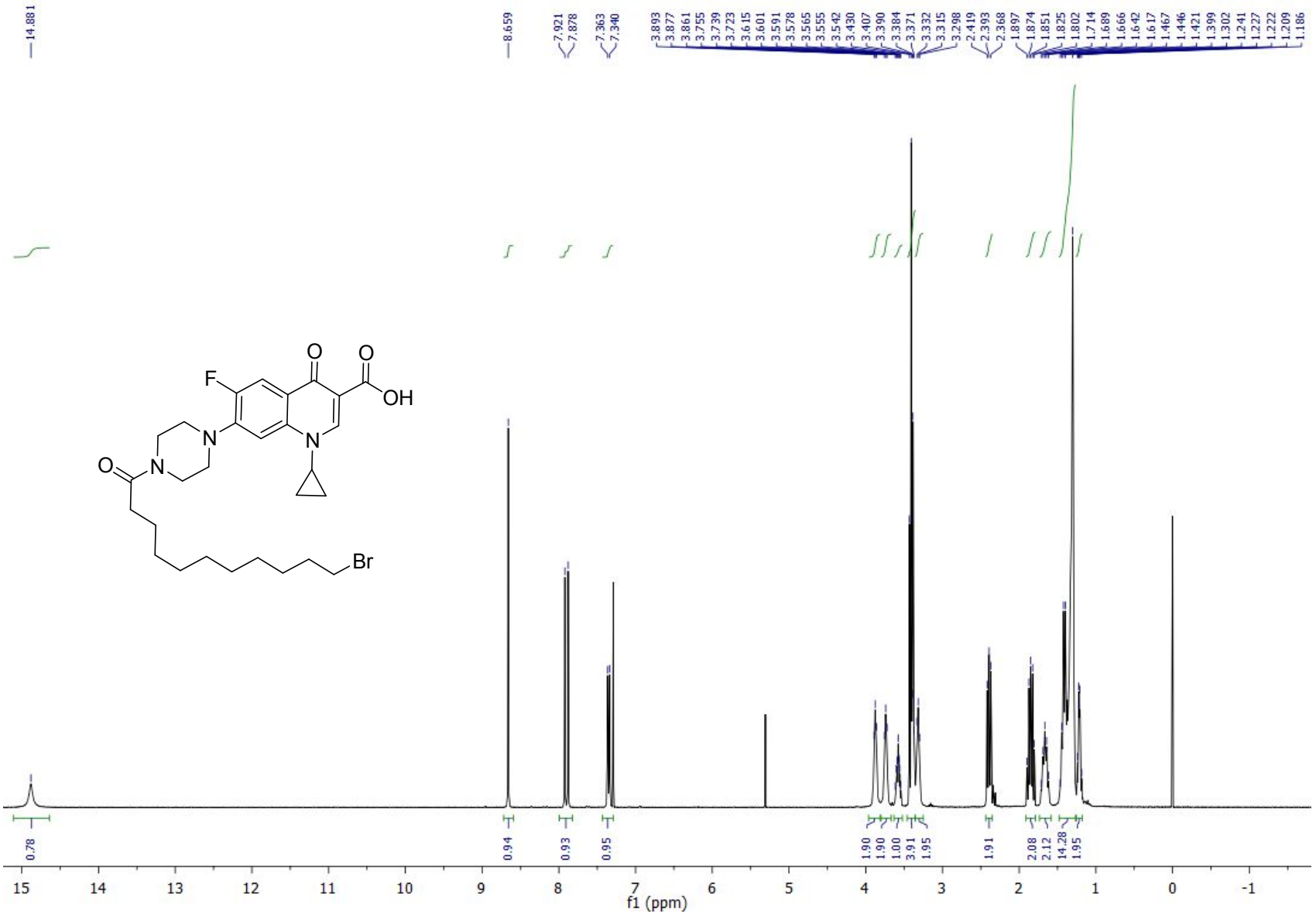
f1 (ppm)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3.601
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3.578
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3.430
3.407
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3.332
3.315
3.298
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2.393
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1.241
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1.209
1.186
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OH
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N
O
N
Br
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0.94
0.93
0.95
1.90
1.90
1.00
3.91
1.95
1.91
2.08
2.12
14.28
1.95
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14
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12
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8
7
6
5
4
3
2
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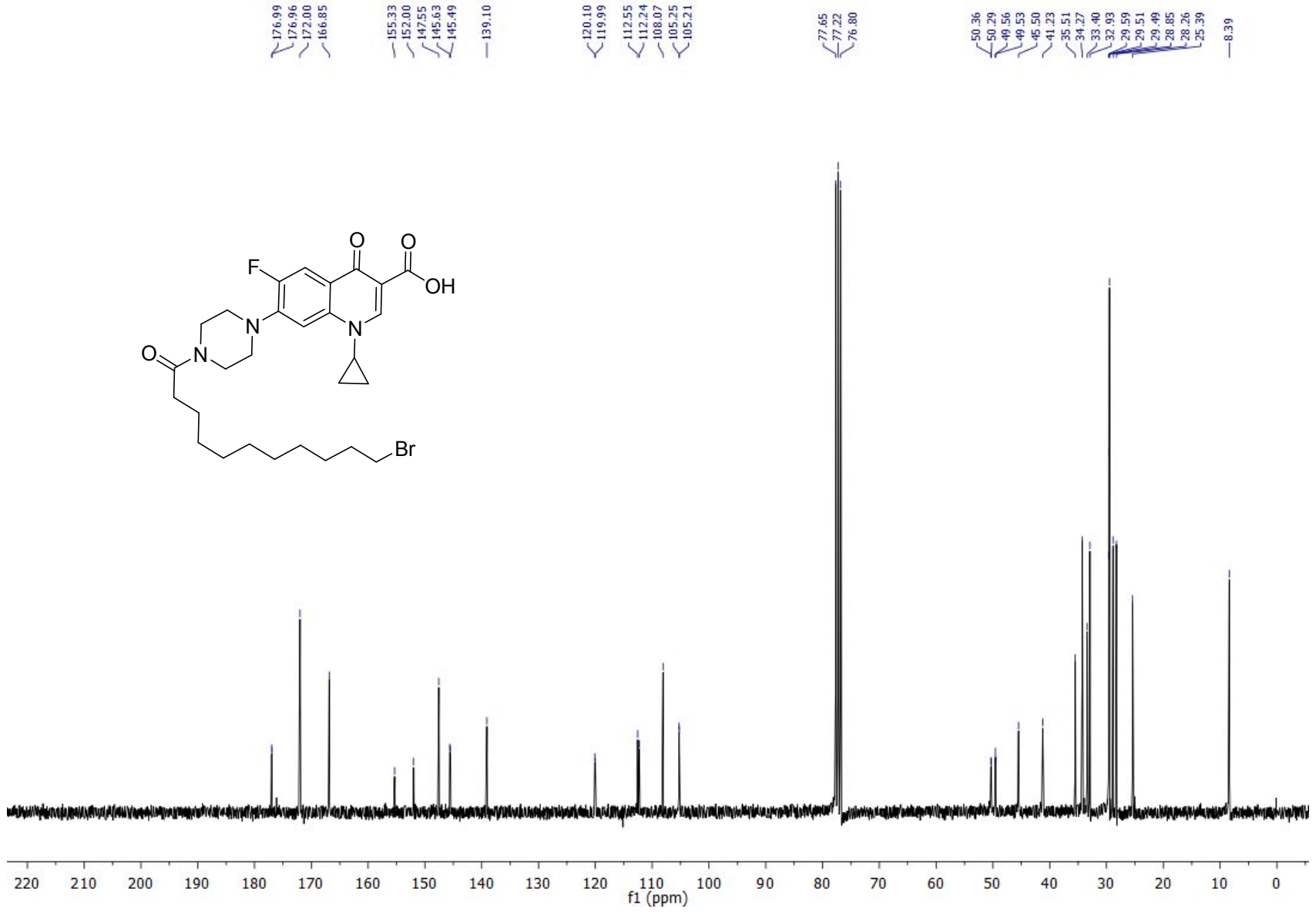
0
-1
f1 (ppm)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112.24
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76.80
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50.29
49.56
49.53
45.50
41.23
35.51
34.27
33.40
32.93
29.59
29.51
29.49
28.85
28.26
25.39
8.39
F
O
O
OH
N
N
O
N
Br
220
210
200
190
180
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130
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50
40
30
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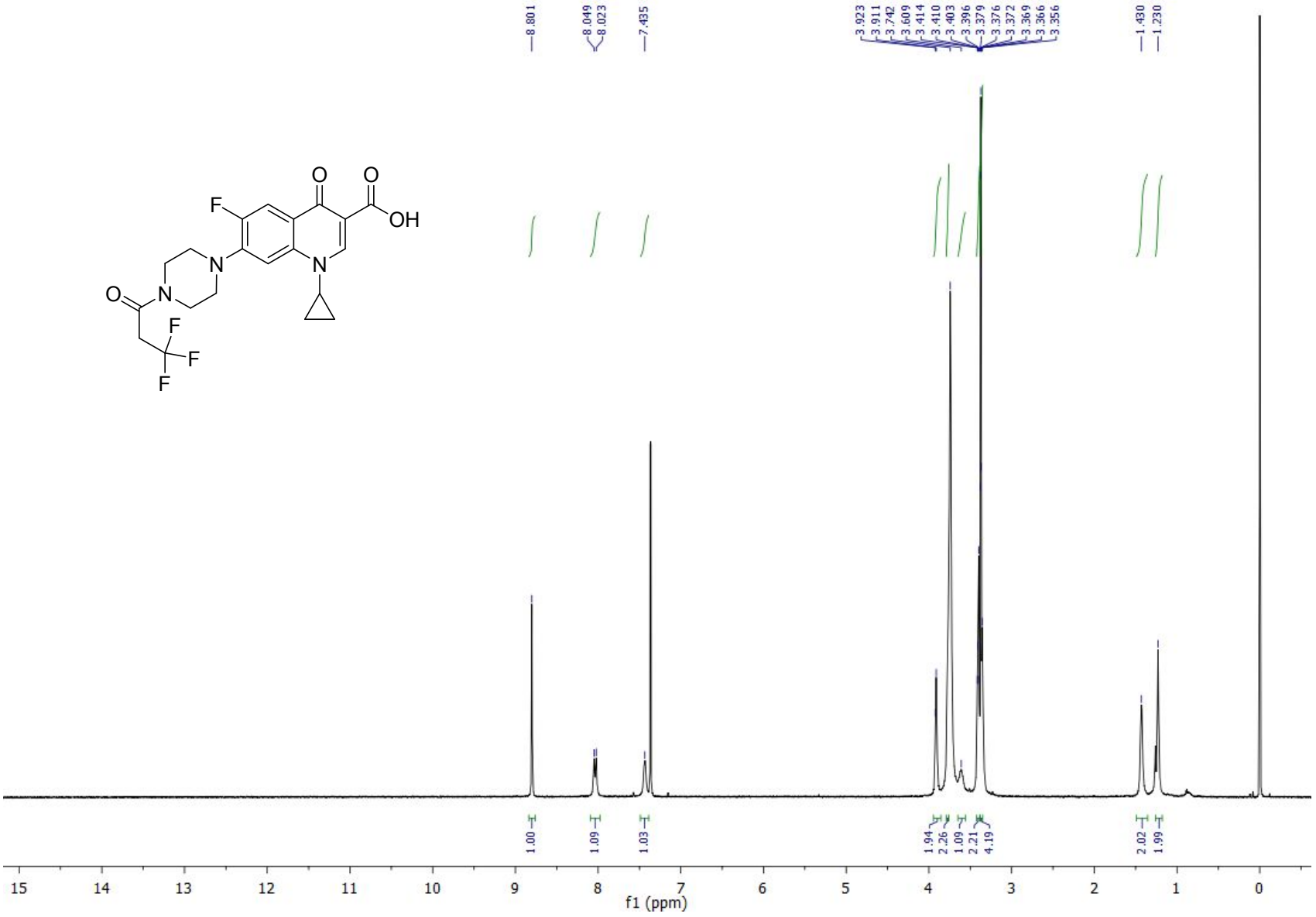
f1 (ppm)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3.379
3.376
3.372
3.369
3.366
3.356
1.430
1.230
1.00
1.09
1.03
1.94
2.26
1.09
2.21
4.19
2.02
1.99
15
14
13
12
11
10
9
8
7
6
5
4
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2
1
0
f1 (ppm)
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O
F
OH
N
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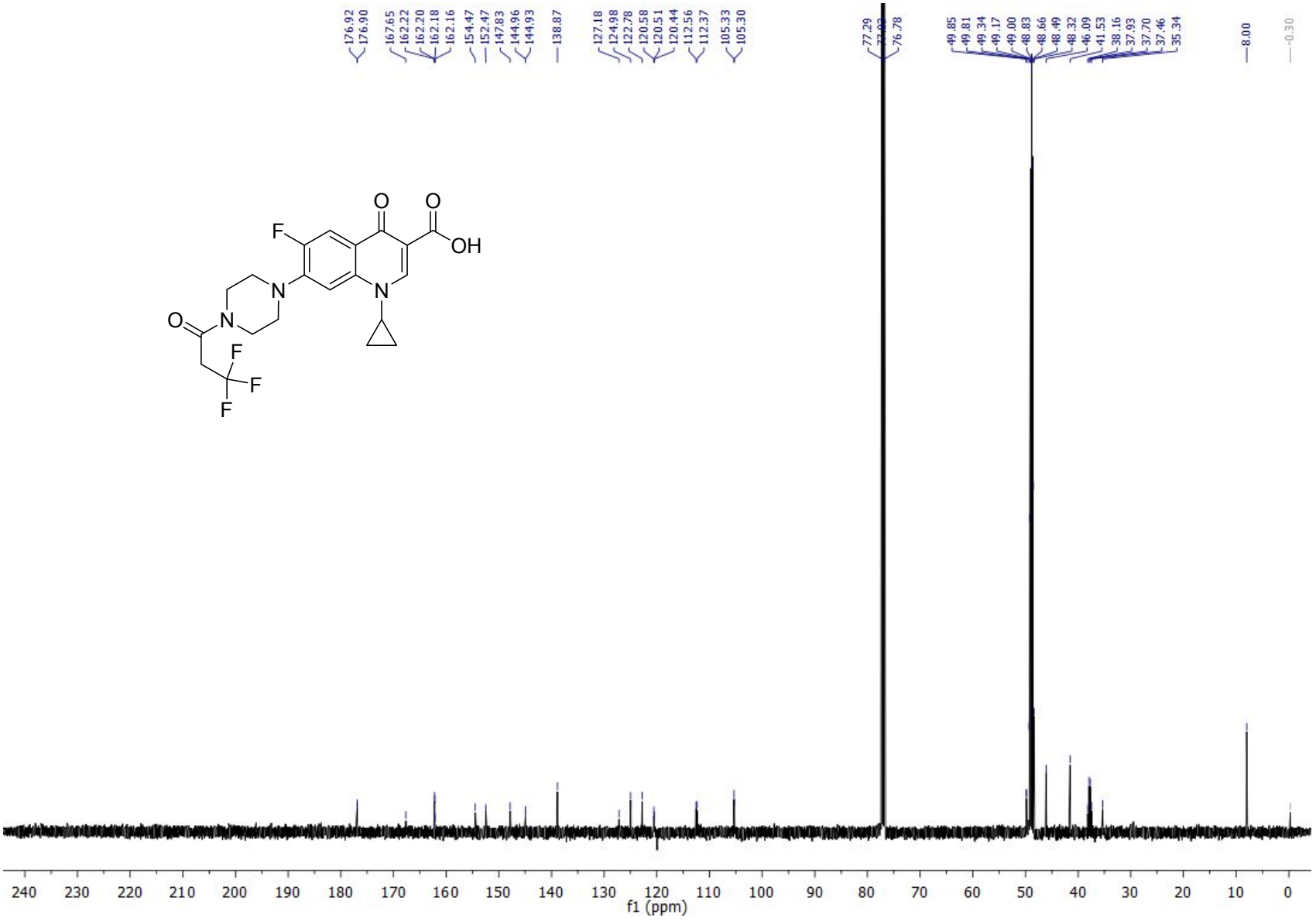
N
F
F
F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124.98
122.78
120.58
120.51
120.44
112.56
112.37
105.33
105.30
77.29
76.78
49.85
49.81
49.34
49.17
49.00
48.83
48.66
48.49
48.32
46.09
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O
O
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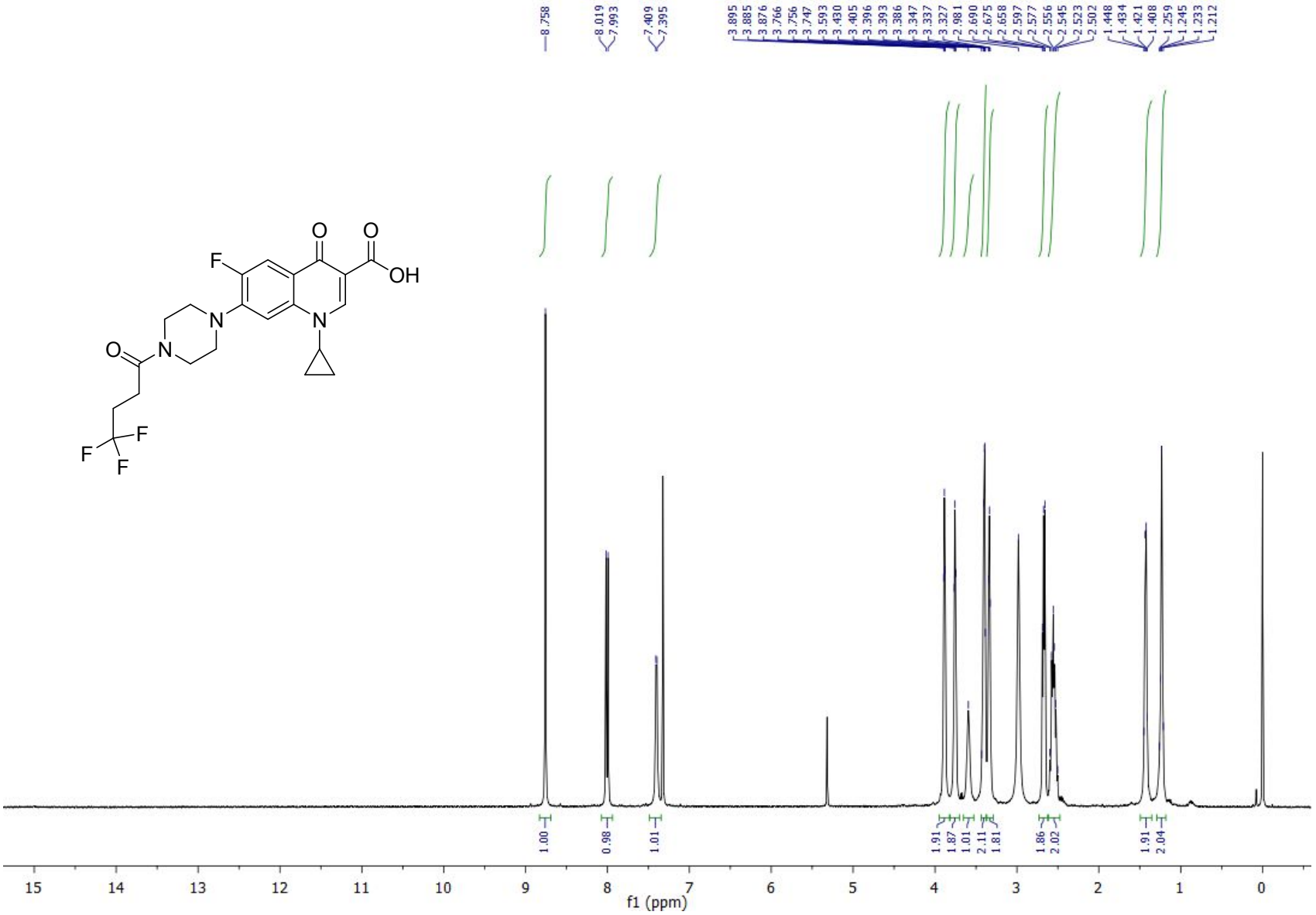
f1 (ppm)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3.393
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3.337
3.327
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2.597
2.577
2.556
2.545
2.523
2.502
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1.434
1.421
1.408
1.259
1.245
1.233
1.212
F
O
O
OH
N
N
O
N
F
F
F
1.00
0.98
1.01
1.91
1.87
1.01
2.11
1.81
1.86
2.02
1.91
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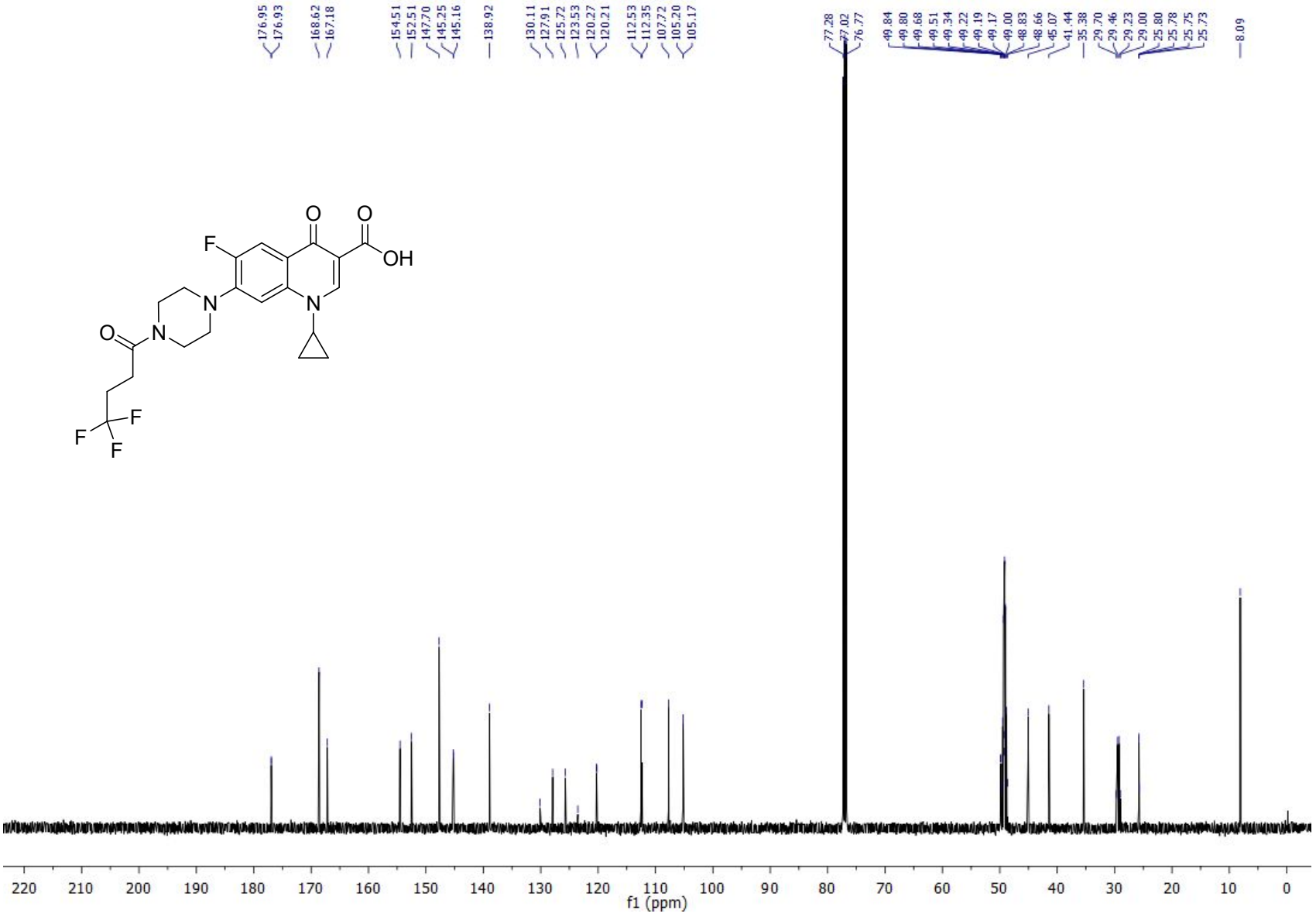
5
4
3
2
1
0
f1 (ppm)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49.22
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41.44
35.38
29.70
29.46
29.23
29.00
25.80
25.78
25.75
25.73
8.09
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O
F
OH
N
N
O
N
F
F
F
220
210
200
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180
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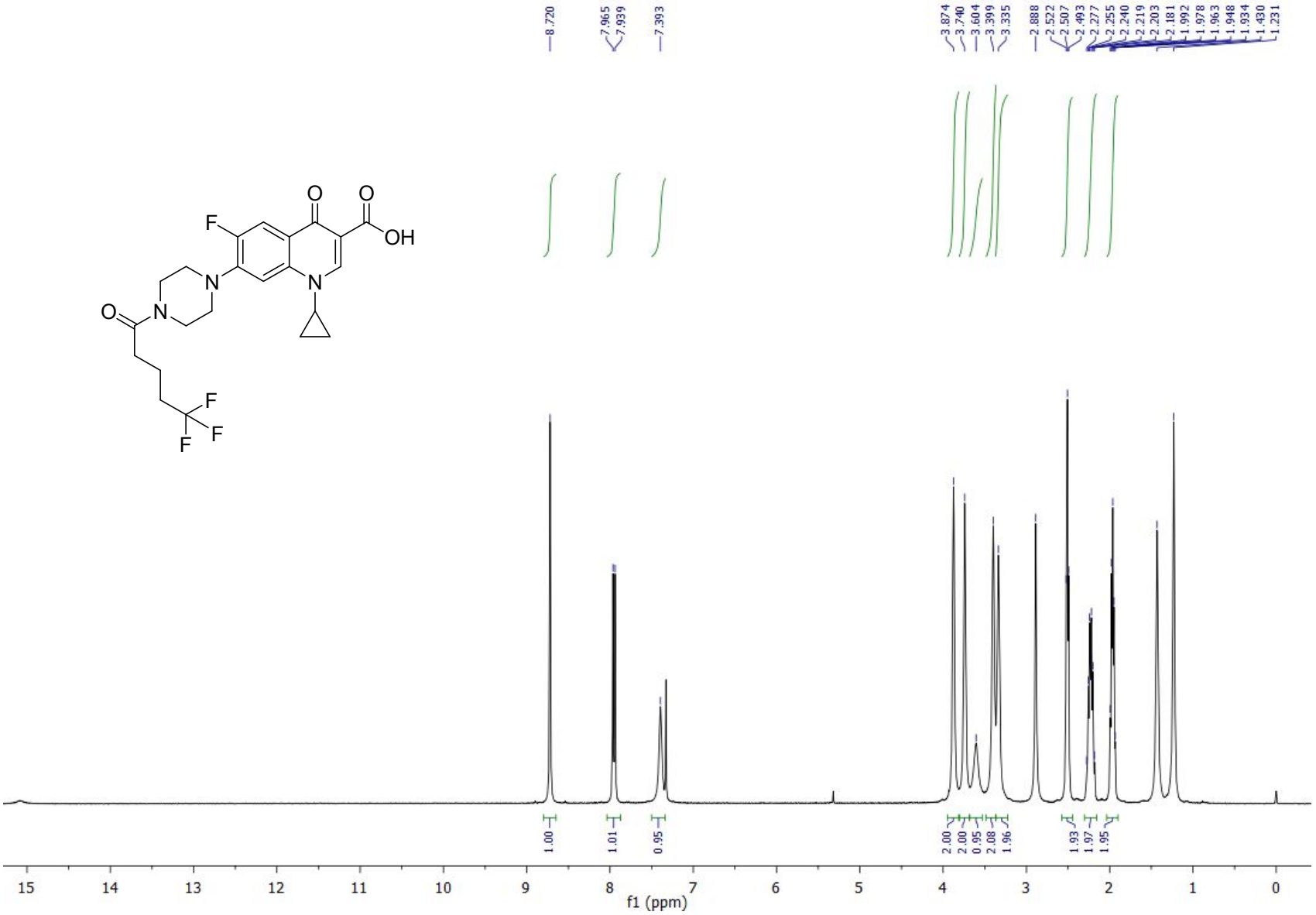
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2.277
2.255
2.240
2.219
2.203
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1.963
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1.934
1.430
1.231
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O
O
OH
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N
O
N
F
F
F
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1.01
0.95
2.00
2.00
0.95
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1.97
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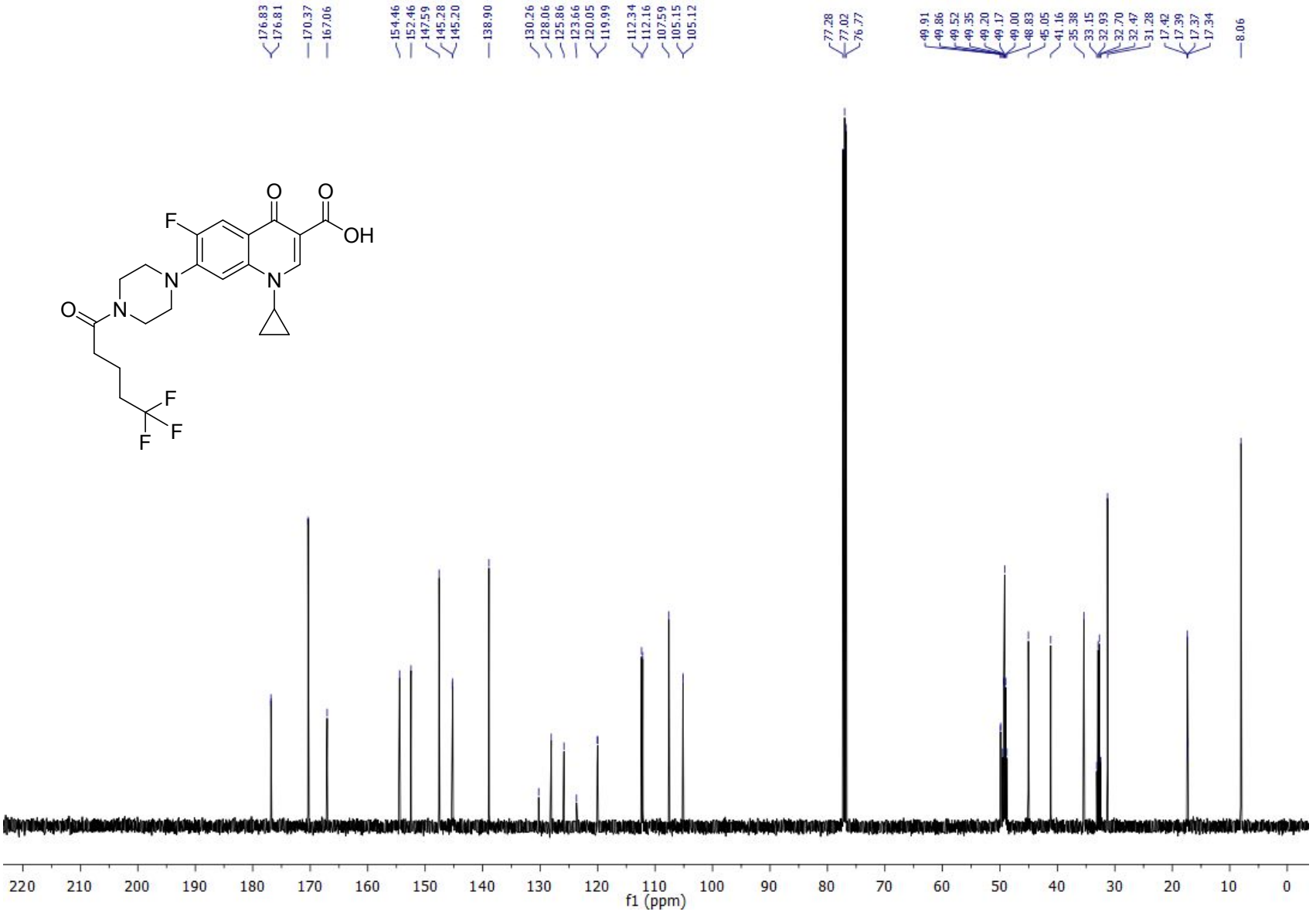
f1 (ppm)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119.99
112.34
112.16
107.59
105.15
105.12
77.28
77.02
76.77
49.91
49.86
49.52
49.35
49.20
49.17
49.00
48.83
45.05
41.16
35.38
33.15
32.93
32.70
32.47
31.28
17.42
17.39
17.37
17.34
8.06
O
O
F
OH
N
N
O
N
F
F
F
220
210
200
190
180
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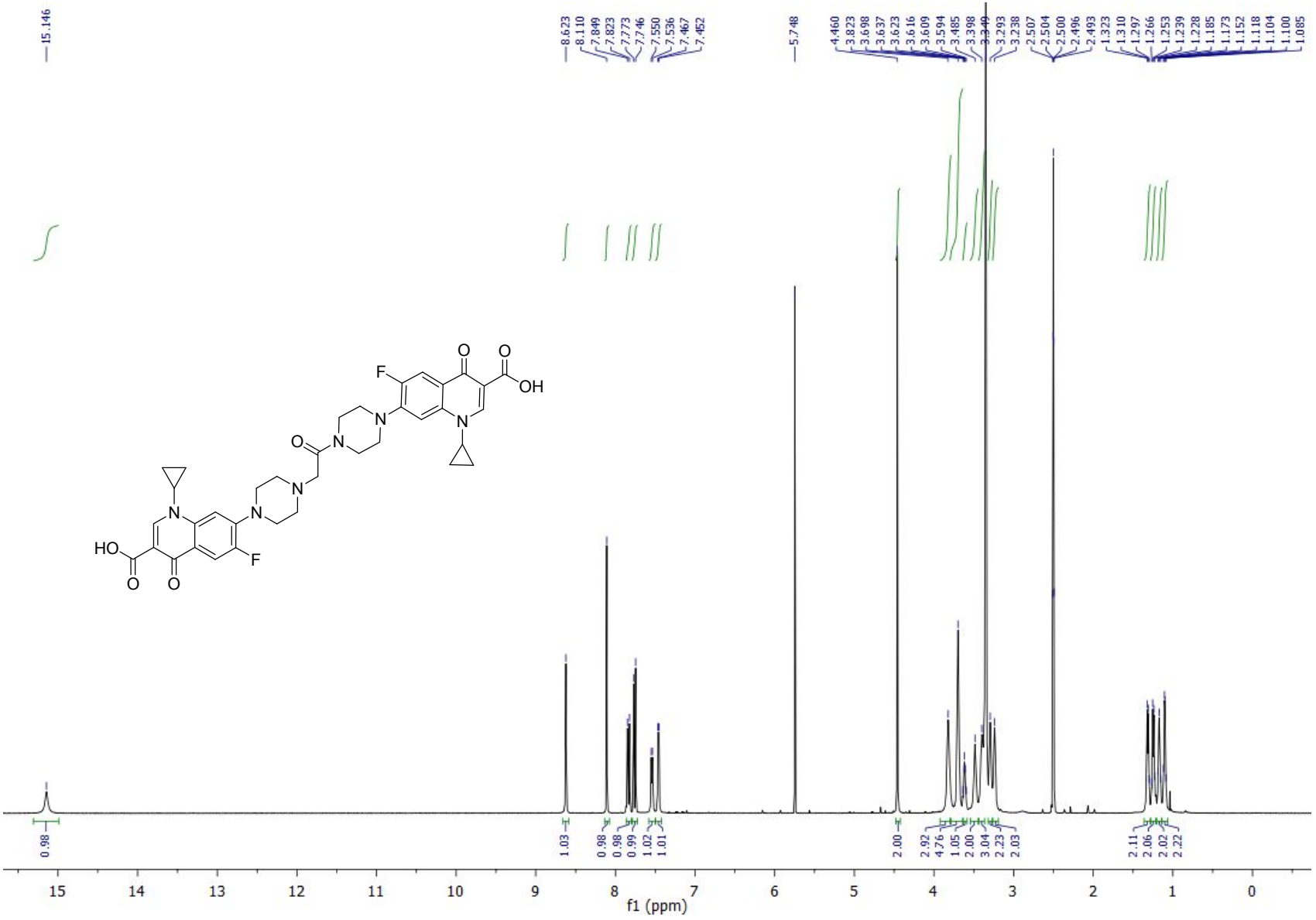
f1 (ppm)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4.460
3.823
3.698
3.637
3.623
3.616
3.609
3.594
3.485
3.398
3.349
3.293
3.238
2.507
2.504
2.500
2.496
2.493
1.323
1.310
1.297
1.266
1.253
1.239
1.228
1.185
1.173
1.152
1.118
1.104
1.100
1.085
0.98
1.03
0.98
0.98
0.99
1.02
1.01
2.00
2.92
4.76
1.05
2.00
3.04
2.23
2.03
2.11
2.06
2.02
2.22
15
14
13
12
11
10
9
8
7
6
5
4
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0
f1 (ppm)
HO
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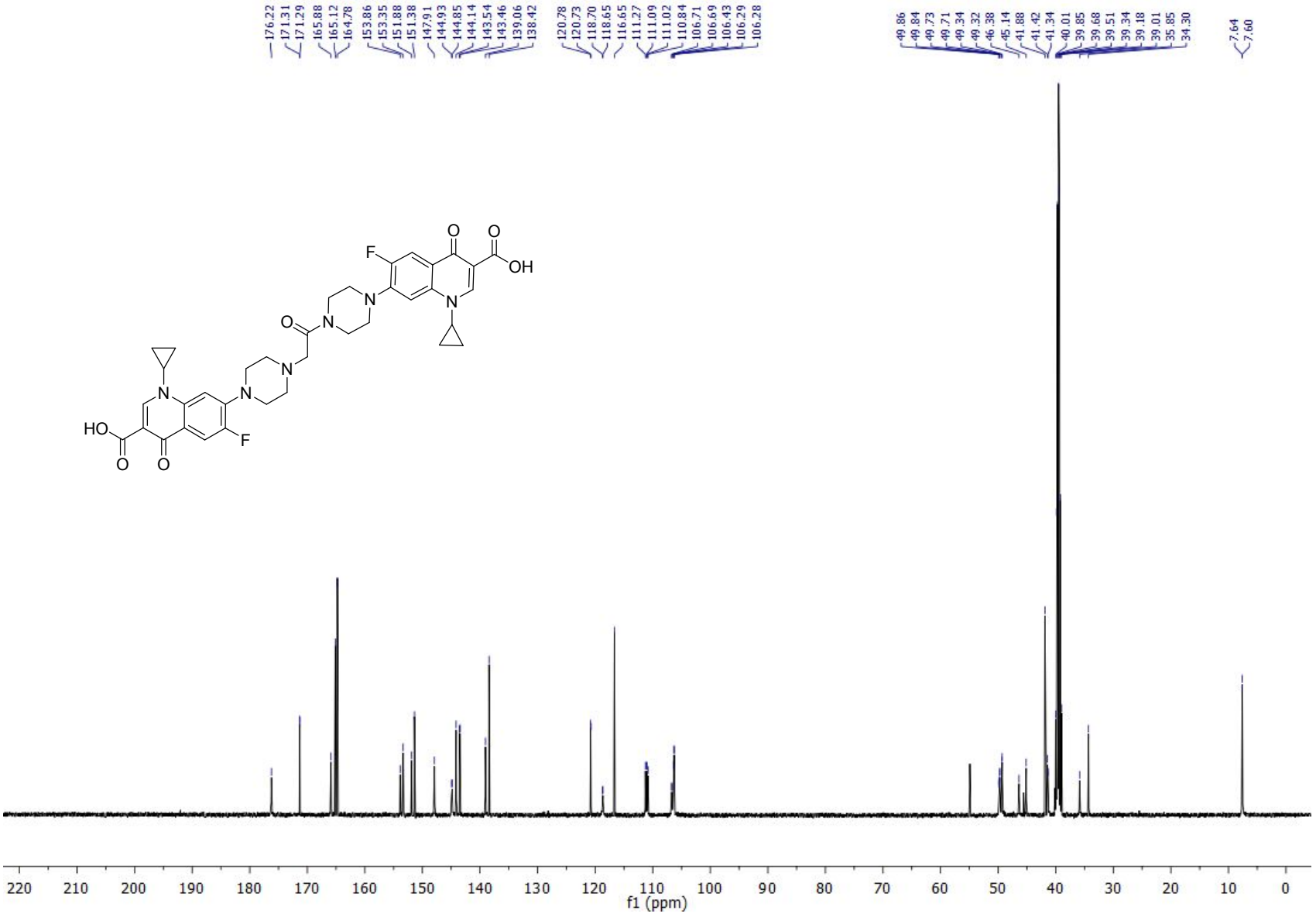
O
F
N
N
O
N
N
F
O
O
OH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144.14
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143.46
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118.65
116.65
111.27
111.09
111.02
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49.71
49.34
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46.38
45.14
41.88
41.42
41.34
40.01
39.85
39.68
39.51
39.34
39.18
39.01
35.85
34.30
7.64
7.60
F
O
O
OH
N
N
O
N
N
N
HO
O
O
F
220
210
200
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180
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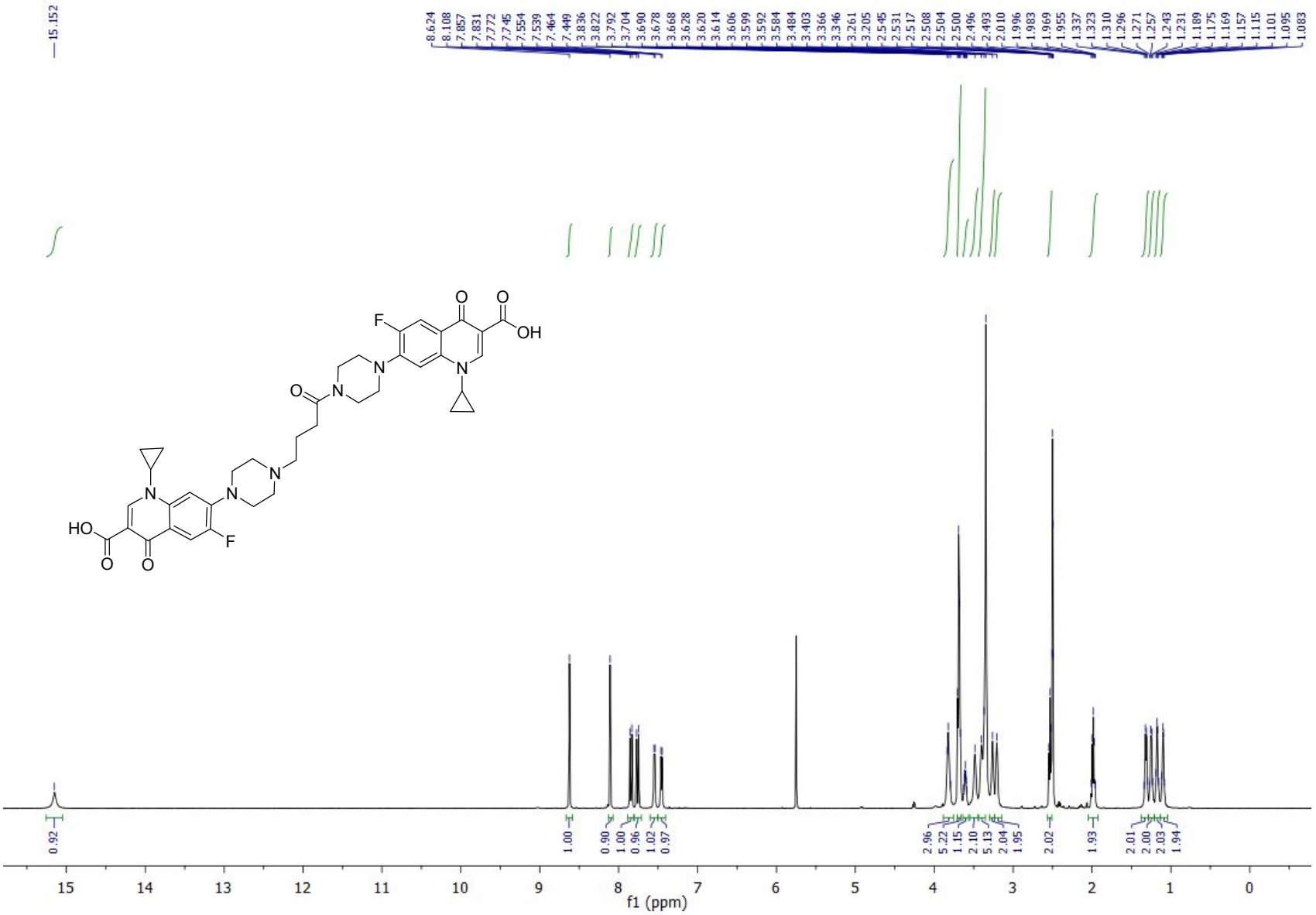
f1 (ppm)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3.792
3.704
3.690
3.678
3.668
3.628
3.620
3.614
3.606
3.599
3.592
3.584
3.484
3.403
3.366
3.346
3.261
3.205
2.545
2.531
2.517
2.508
2.504
2.500
2.496
2.493
2.010
1.996
1.983
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1.271
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OH
N
N
O
N
N
N
N
HO
O
O
F
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0.90
1.00
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1.02
0.97
2.96
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1.15
2.10
5.13
2.04
1.95
2.02
1.93
2.01
2.00
2.03
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9
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6
5
4
3
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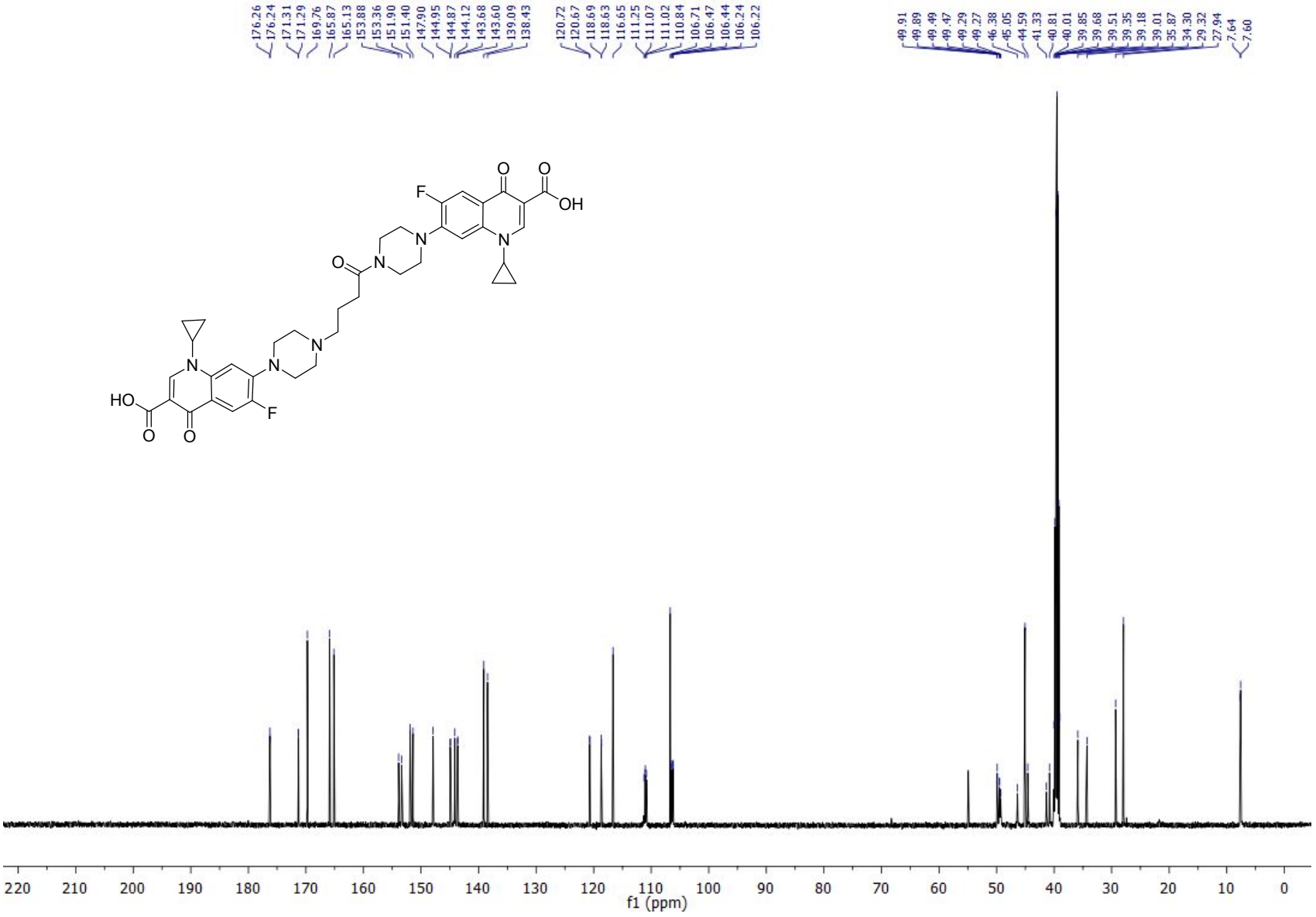
f1 (ppm)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144.87
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138.43
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118.63
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111.25
111.07
111.02
110.84
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49.47
49.29
49.27
46.38
45.05
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41.33
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39.85
39.68
39.51
39.35
39.18
39.01
35.87
34.30
29.32
27.94
7.64
7.60
F
O
O
OH
N
N
O
N
N
N
HO
O
O
F
220
210
200
190
180
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120
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40
30
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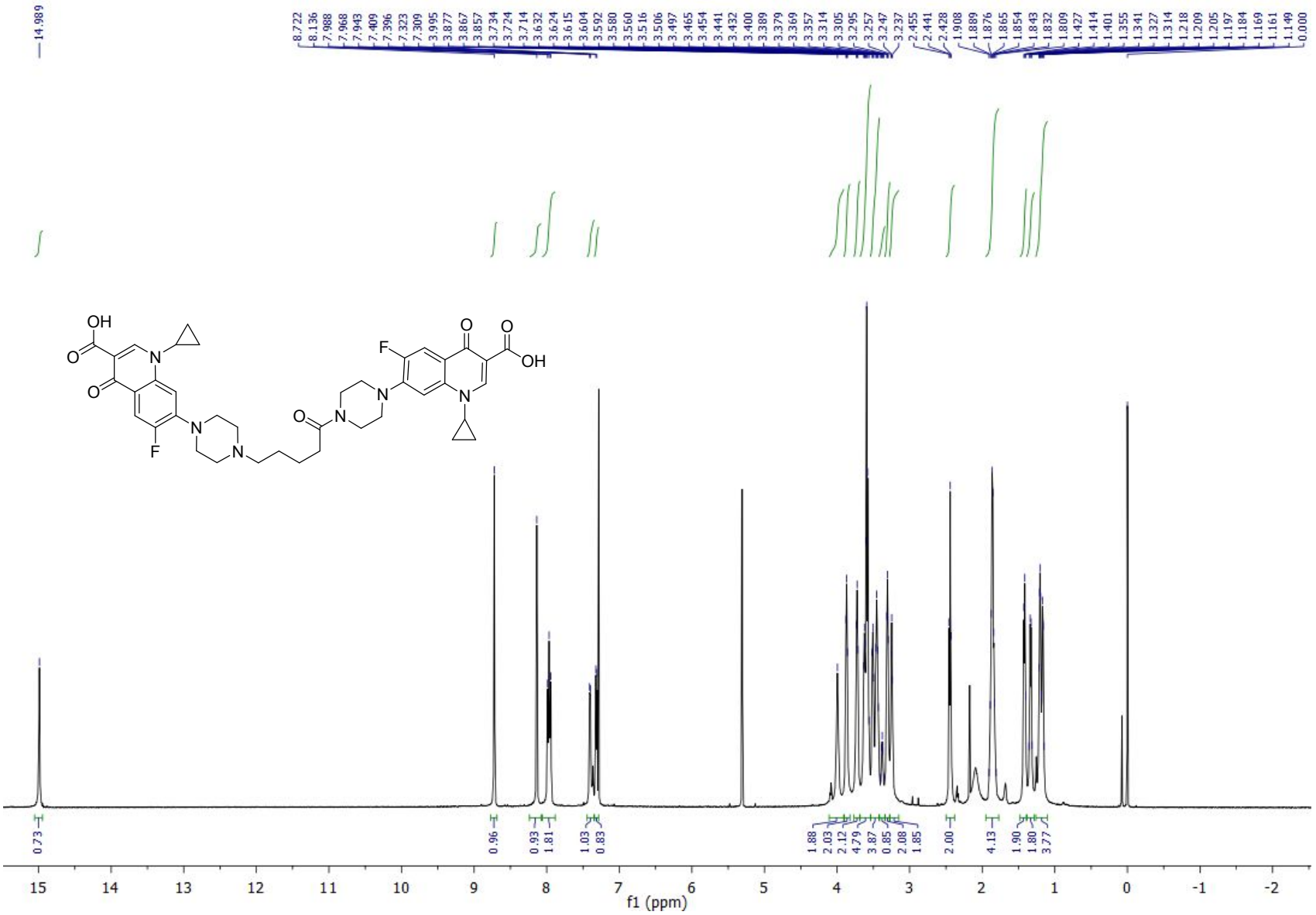
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3.724
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3.624
3.615
3.604
3.592
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3.560
3.516
3.506
3.497
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1.314
1.218
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1.169
1.161
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N
F
N
N
O
N
N
F
O
O
OH
N
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1.03
0.83
1.88
2.03
2.12
4.79
3.87
0.85
2.08
1.85
2.00
4.13
1.90
1.80
3.77
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12
11
10
9
8
7
6
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4
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2
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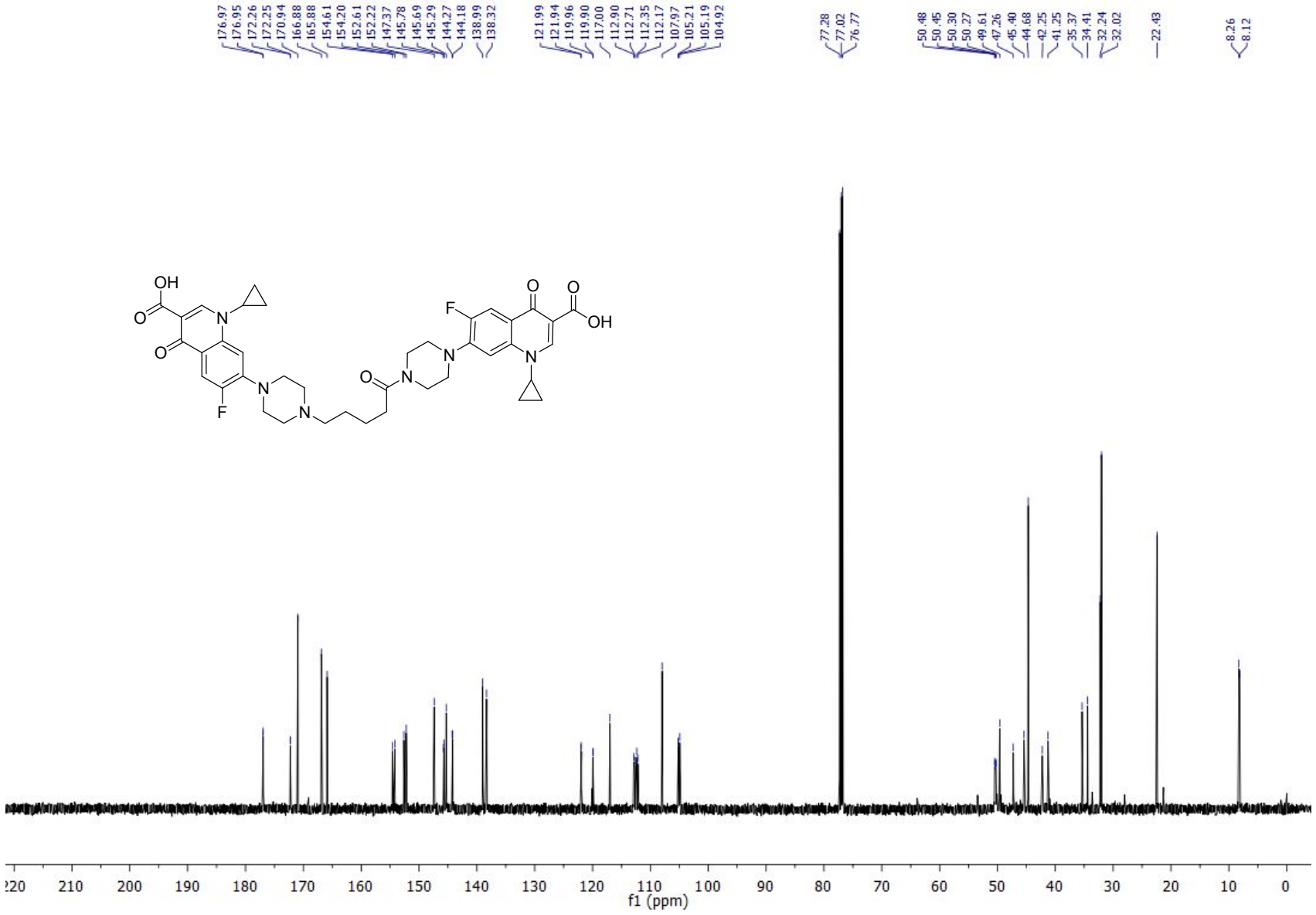
-2
f1 (ppm)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144.27
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121.94
119.96
119.90
117.00
112.90
112.71
112.35
112.17
107.97
105.21
105.19
104.92
77.28
77.02
76.77
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50.45
50.30
50.27
49.61
47.26
45.40
44.68
42.25
41.25
35.37
34.41
32.24
32.02
22.43
8.26
8.12
OH
O
O
N
N
F
N
N
O
N
F
O
O
OH
N
220
210
200
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180
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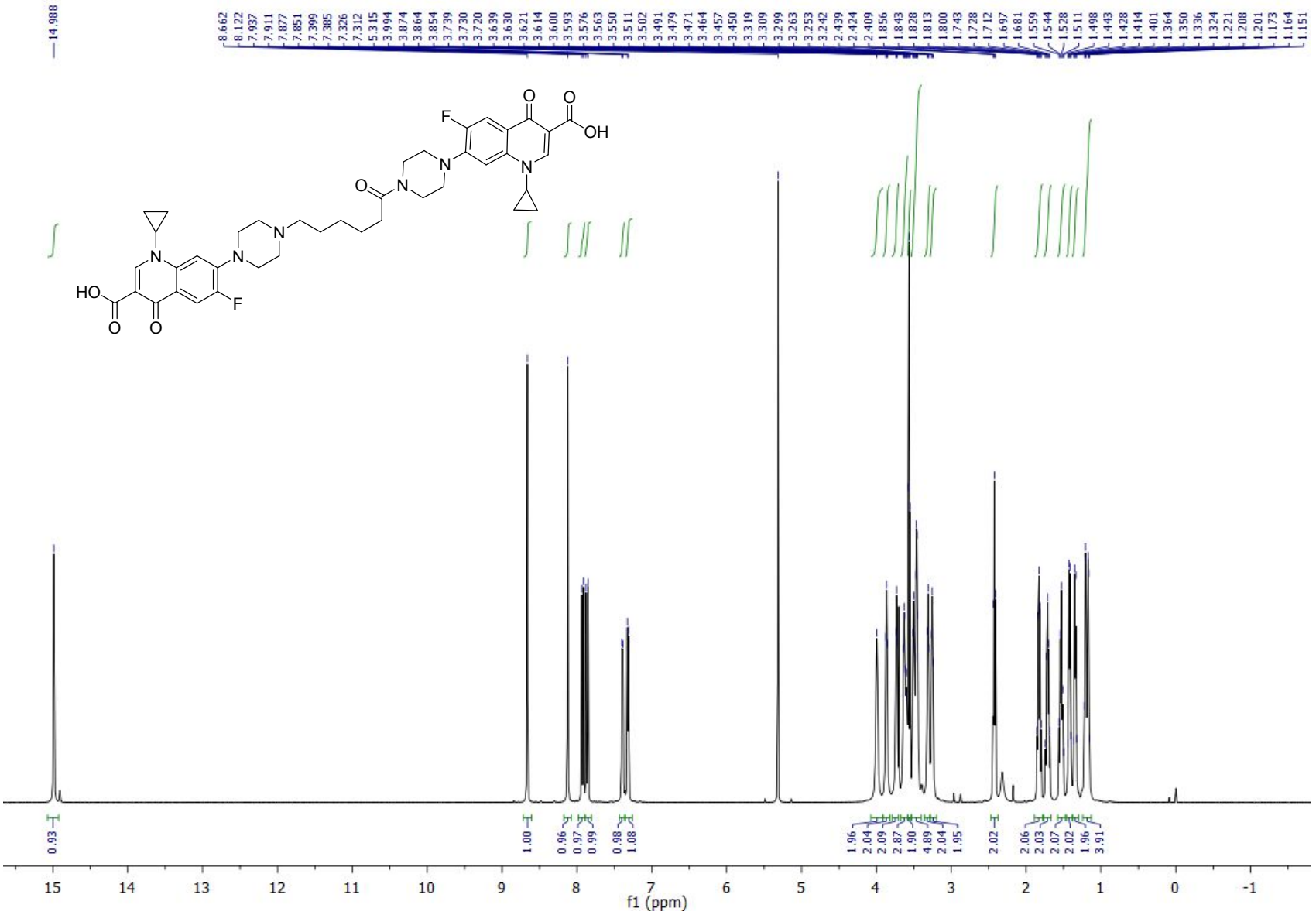
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3.854
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3.720
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3.630
3.621
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3.600
3.593
3.576
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3.511
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1.208
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1.151
F
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O
OH
N
N
O
N
N
HO
O
O
F
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1.00
0.96
0.97
0.99
0.98
1.08
1.96
2.04
2.09
2.87
1.90
4.89
2.04
1.95
2.02
2.06
2.03
2.07
2.02
1.96
3.91
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8
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6
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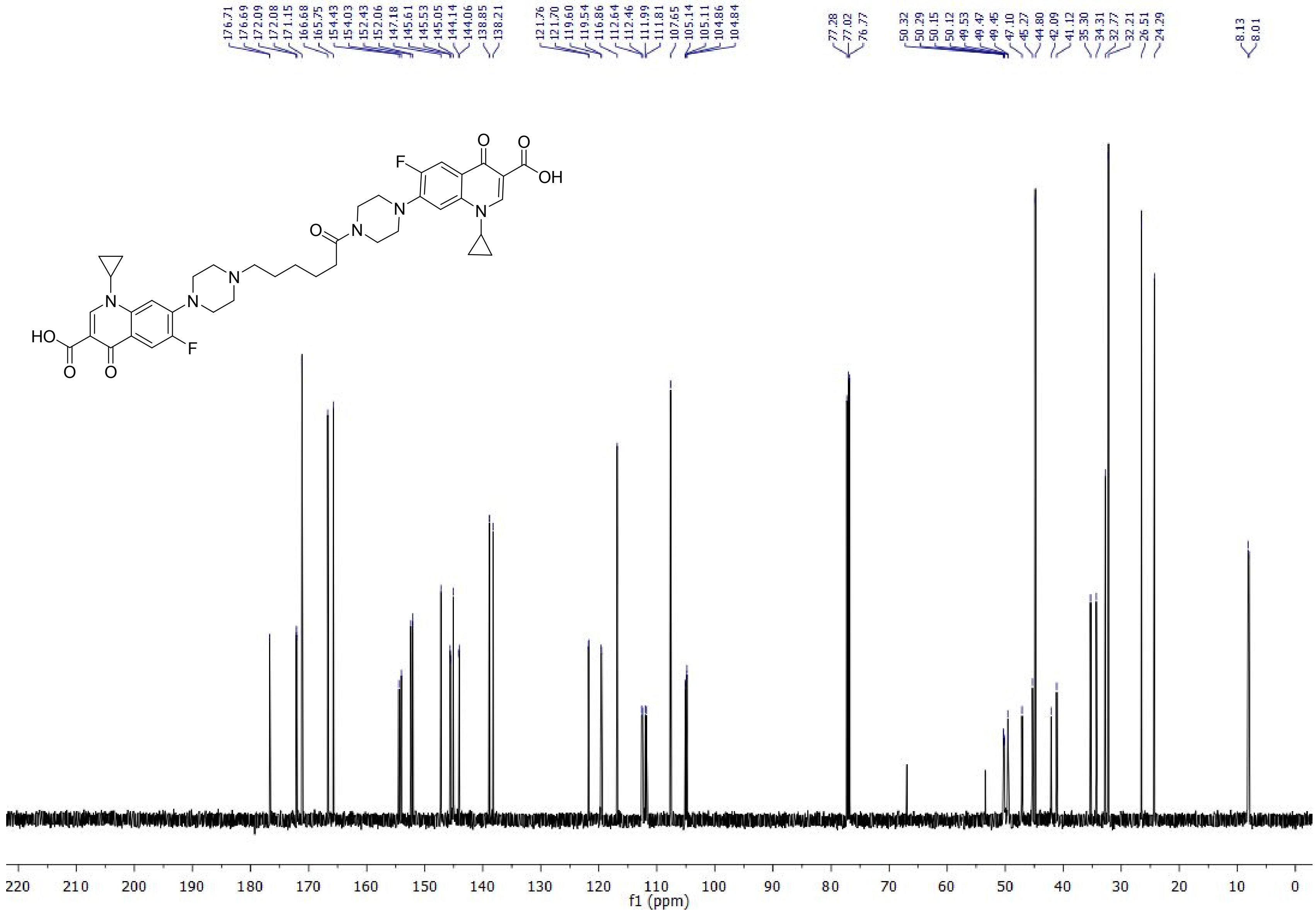
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104.86
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77.28
77.02
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49.47
49.45
47.10
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41.12
35.30
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32.77
32.21
26.51
24.29
8.13
8.01
F
O
O
OH
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N
O
N
N
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HO
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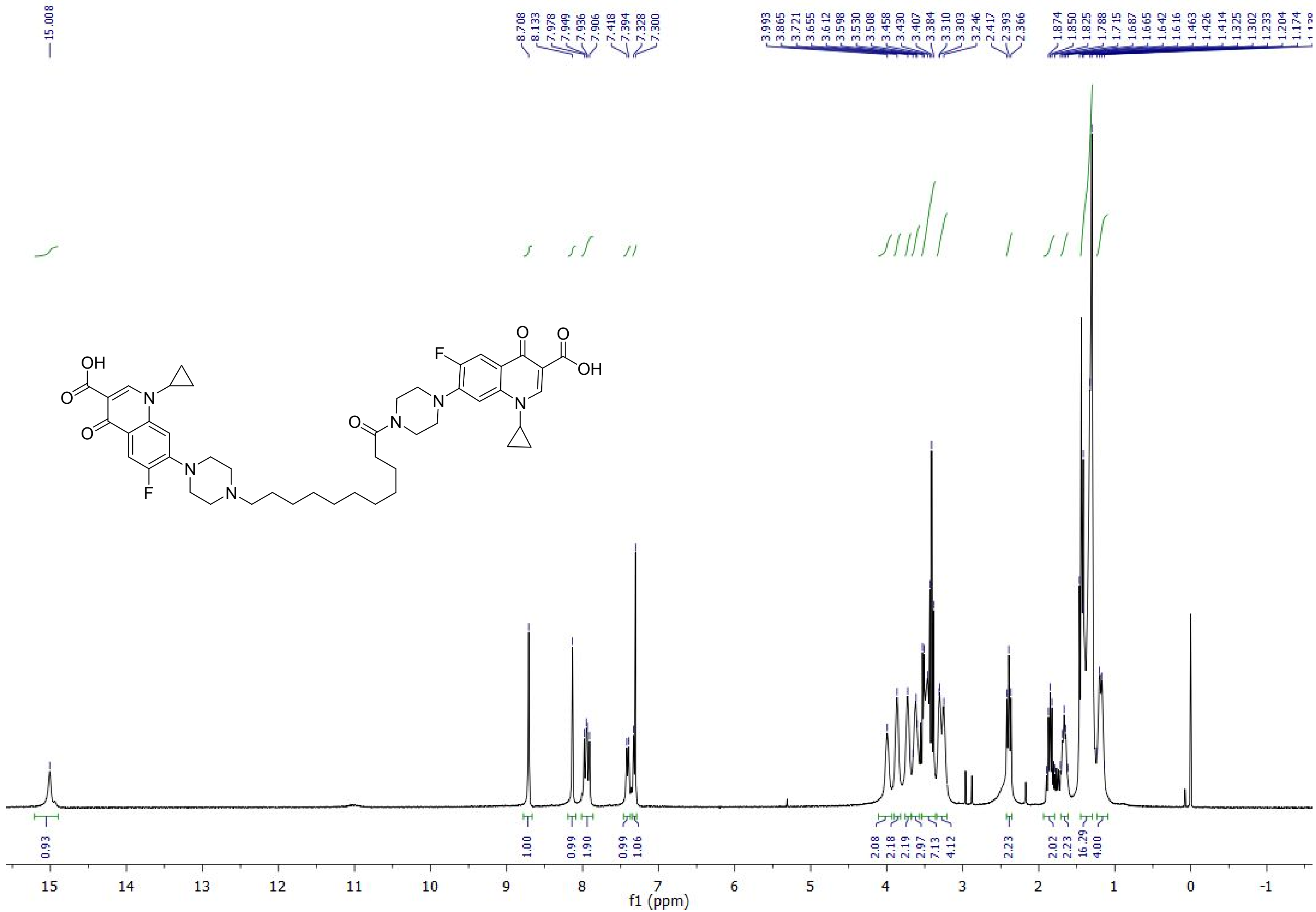
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3.458
3.430
3.407
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3.303
3.246
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1.850
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1.788
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1.642
1.616
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1.233
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1.90
0.99
1.06
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2.02
2.23
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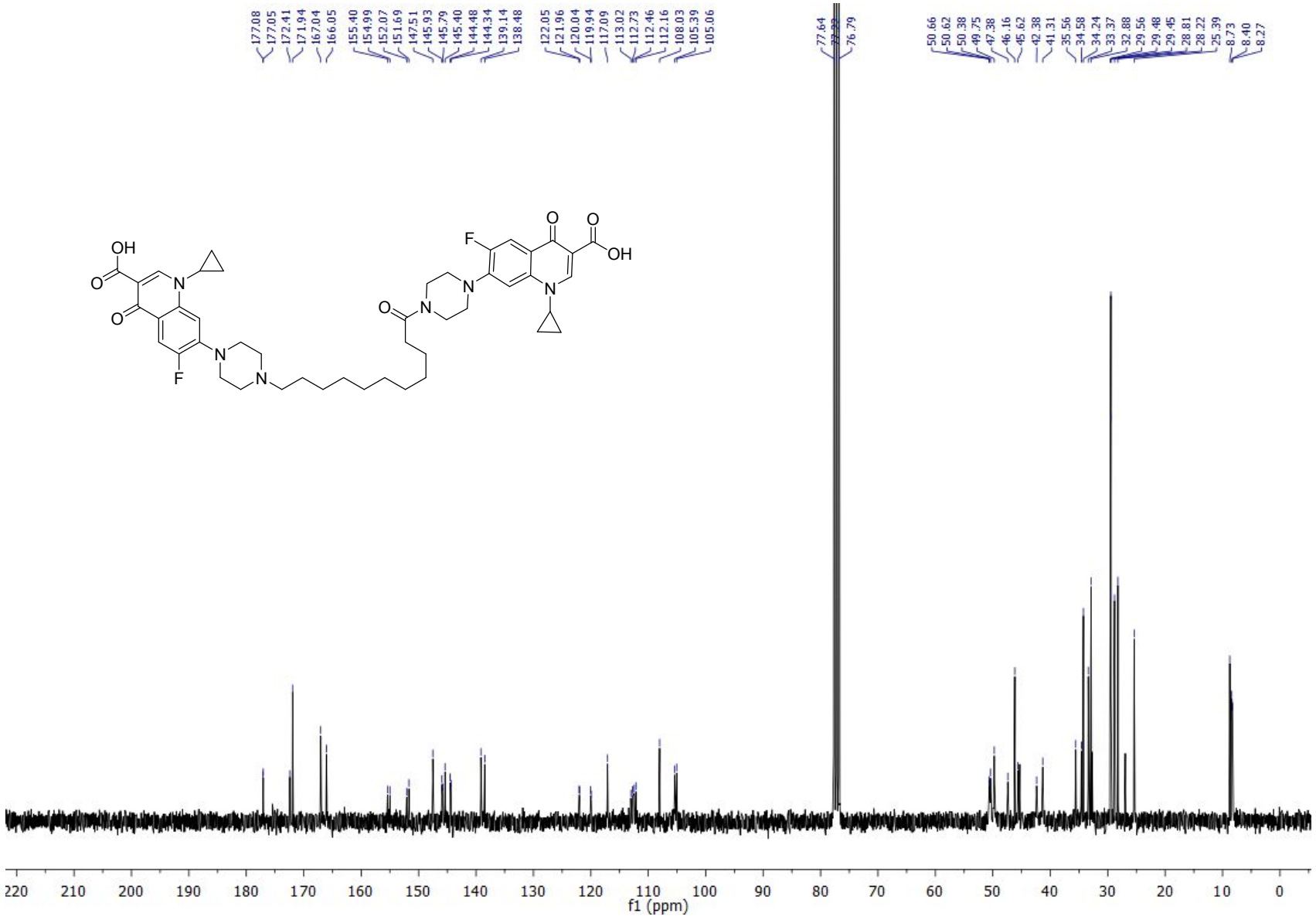
4.00
f1 (ppm)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117.09
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45.62
42.38
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29.56
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F
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O
N
N
F
O
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OH
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f1 (ppm)