Supporting Information

Fluorinated Amino-Derivatives of the Sesquiterpene Lactone, Parthenolide, as ^{19}F NMR Probes in Deuterium-Free Environments

James R. Woods,[†] Huaping Mo,[†,‡] Andrew A. Bieberich,[†] Tanja Alavanja,[§] and David A. Colby[*,†,§]

[†]*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana 47907,* [‡]*Interdepartmental NMR Facility, Purdue University, West Lafayette, Indiana 47907, and* [§]*Department of Chemistry, Purdue University, West Lafayette, Indiana 47907*

Table of Contents

I. Methods for GI_{50}, TGI, and LC_{50} Determination

Three day cancer cell growth inhibition (CCGI) assay

The Molecular Discovery and Evaluation (MDE) Shared Resource, within the Purdue University Center for Cancer Research (PUCCR) has developed a standardized cell growth inhibition assay in an effort to produce statistically reliable dose response characterizations of novel compounds. The assay design and data analysis workflow determine three standard descriptors of compound effect upon tissue culture: 50% growth inhibition (GI_{50}), total growth inhibition (TGI) and 50% cell death relative to the Day 0 population (LC_{50}). Final determination of these values includes error propagation across replicate plate wells for all controls and treatments. GI_{50}, TGI and LC_{50} values are calculated as estimated parameter values during nonlinear regression of a sigmoid inhibitory response model against replicate data for a ten-step, two-fold dilution series of each compound. By using this design, the values for GI_{50}, TGI and LC_{50} can be tested for difference between compounds using an Extra Sum of Squares F test within the Graphpad Prism environment. This is essential for demonstrating whether synthetic analogues achieve significantly different activity relative to parent compounds.

CCGI 96-well plate design and assembly for Day 0 and Day 3

One complete unit of the plate design screens two compounds in parallel. On Day 0, two 96 well plates are seeded with HL-60 cells in RPMI 1640 + 10% FBS using 5,000 cells per well. The "Day 0" plate contains alternating sections of medium-only control wells (plate columns 1–3 and 7–9) and wells containing medium + cells (plate columns 4–6 and 10–12). Medium volume is 100 µl per well. The "Day 3" plate has one column of medium-only control wells (plate column

1) followed by eleven columns of cells in medium. Medium volume is 80 µl per well in the “Day 3” plate; this allows for a subsequent application of 20 µl medium containing either vehicle control (plate column 2) or a compound dilution series (plate columns 3–12). Therefore, final culture volume for the “Day 3” plate is 100 µl per well, matching the “Day 0” plate. Dilution series for two different experimental compounds are applied in parallel; Compound 1 is located in wells E–H of plate columns 3–12, and Compound 2 is located in wells A–D of plate columns 3–12. Plates are incubated at 37 °C under 5% CO_2 atmosphere. All components are distributed to plate wells using a Biomek 3000 Laboratory Automation Workstation (Beckman Coulter) located inside a SterilGARD III Advance Class II Biological Safety Cabinet.

Immediately after assembly of the “Day 0” and “Day 3” plates, the “Day 0” plate is treated with an MTT assay as follows: 20 µl medium containing 0.25% MTT (m/v) is applied to every well across the plate. MTT is applied in a controlled light environment. The plate is then incubated for four hours, after which each well receives 100 µl Isopropanol containing 0.1 N HCl and 10% Triton X-100. Formazan crystals are allowed to solubilize in the dark at room temperature overnight, and absorbance at 570 nm is subsequently read for every well on Day 1 using a SpectraMax Plus 384 plate reader (Molecular Devices).

Dilution series for experimental compounds are formulated as follows: First, a 10 mM solution of compound is made in DMSO and filter sterilized. (All DMSO and cell culture medium subsequently used for dilution and creation of the master compound plate must be filter sterilized.) A ten-step, two-fold dilution series (including the 10 mM stock as Step 1) is then created from this stock by serial two-fold dilution in DMSO. The original 10 mM stock and each of the nine subsequent dilutions are then added to cell culture medium at a concentration of 2.5% (v/v). This means that each concentration in the series is reduced by 1:40. When a compound dilution series is added to the “Day 3” plate, the addition of 20 µl of each dilution step to 80 µl of medium results in a further reduction in concentration by 1:5. Each well has a final working DMSO concentration of 0.5%. The dilution series for the compound has final working concentrations beginning at 50 µM and decreasing by a factor of two for each subsequent step. Serial dilutions and assembly of compound master plates are performed with the Biomek 3000.

On Day 3 the same MTT assay, as described above, is applied to all wells of the “Day 3” plate. Ab 570 nm for the “Day 3” plate is read on Day 4.

Cell growth normalization with error propagation

For each set of four treatment replicate wells on the “Day 3” plate, the Day 3 cell population is expressed as a percent change relative to the mean per-well Day 0 cell population. The algorithm is described below.

1. For the “Day 0” plate data, mean and standard deviation are calculated separately for wells with medium-only control and medium + cells. A background-subtracted Day 0 cell population is then determined by subtracting the mean medium-only Ab 570 value from the mean value for medium + cells.

2. For any group of four replicate treatment wells on the “Day 3” plate (including the vehicle control cells in plate column 2), a background-subtracted Day 3 cell population is calculated by subtracting the mean Ab 570 value for plate column 1 (8 wells of medium only control) from the mean value for the four replicate treatment wells.

3. The background-subtracted mean value for a replicate group is then divided by the mean background-subtracted Day 0 cell population value and converted to a percentage.

4. Basic rules for arithmetic propagation of standard deviation are followed to calculate the correct standard deviation for mean Day 3 cell density of each replicate group:

$$\sigma_{treatment} = \left(\sqrt{\left(\frac{\sqrt{\sigma_4^2 + \sigma_3^2}}{\bar{x}_4 - \bar{x}_3} \right)^2 + \left(\frac{\sqrt{\sigma_2^2 + \sigma_1^2}}{\bar{x}_2 - \bar{x}_1} \right)^2} \right) \left(\frac{\bar{x}_4 - \bar{x}_3}{\bar{x}_2 - \bar{x}_1} \right) 100$$

where:

$\bar{x}_1$ is the mean Ab 570 nm for Day 0 medium-only control wells.
$\bar{x}_2$ is the mean Ab 570 nm for Day 0 wells with medium + cells.
$\bar{x}_3$ is the mean Ab 570 nm for Day 3 medium-only control wells.
$\bar{x}_4$ is the mean Ab 570 nm for a treatment replicate group.
σ_1 is the standard deviation for Day 0 medium-only control wells.
σ_2 is the standard deviation for Day 0 wells with medium + cells.
σ_3 is the standard deviation for Day 3 medium-only control wells.
σ_4 is the standard deviation for a treatment replicate group.

Estimating GI_{50}, TGI and LC_{50} as parameters of a sigmoid inhibitor model through nonlinear regression

After normalized Day 3 cell densities have been calculated for each compound dilution series, dose response characteristics are determined by using nonlinear regression tools within GraphPad Prism (Graphpad Software). Three user defined equations have been created within the Analysis section of Graphpad Prism for estimating GI_{50}, TGI and LC_{50}, respectively, as parameters of a sigmoid inhibitory dose response model. Each was created by editing the pre-existing model called “log(agonist) vs. response – FindECanything”. When entering data into GraphPad Prism, all compound concentrations must be converted to $\log_{10}$ values of molarity.

log(inhibitor) vs. response – Find GI50

To create this model, edit “log(agonist) vs. response – FindECanything” so that its equations are as follows:

F=100*(((((Top-100)/2)+100)-Bottom)/(Top-Bottom))
logEC50=logECF - (1/HillSlope)*log(F/(100-F))

Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))

Set the Rules for Initial Values as:

Parameter Name	Initial Value	Rule
Top	1.0	*YMAX
Bottom	1.0	*YMIN
logECF	1.0	*(Value of X at YMID)
HillSlope	1.0	*SIGN(YATXMAX – YATXMIN)

Set Default Constraints as:

Parameter Name	Constraint Type	Value
Top	No constraint	
Bottom	No constraint	
logECF	No constraint	
HillSlope	Must be less than	0.0

After running this model on a data set, GI_{50} is reported in the GraphPad Prism Results sheet as the best-fit value of ECF. The value is reported as molarity, i.e. 5.210e-007 = 521 nanomolar. If GI_{50} was not physically achieved during the assay, the model will fail to converge.

log(inhibitor) vs. response – Find TGI

To create this model, edit "log(agonist) vs. response – FindECanything" so that its equations are as follows:

F=100*(100-Bottom)/(Top-Bottom)
logEC50=logECF-(1/HillSlope)*log(F/(100-F))
Y=Bottom+(Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))

Rules for Initial Values and Default Constraints are set as shown for "log(inhibitor) vs. response – Find GI50". After running this model on a data set, TGI is reported in the GraphPad Prism Results sheet as the best-fit value of ECF. If TGI was not physically achieved during the assay, the model will fail to converge.

log(inhibitor) vs. response – Find LC50

To create this model, edit "log(agonist) vs. response – FindECanything" so that its equations are as follows:

F=100*((50-Bottom)/(Top-Bottom))
logEC50=logECF-(1/HillSlope)*log(F/(100-F))
Y=Bottom+(Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))

Rules for Initial Values and Default Constraints are set as shown for "log(inhibitor) vs. response – Find GI50". After running this model on a data set, LC50 is reported in the GraphPad Prism Results sheet as the best-fit value of ECF. If LC_{50} was not physically achieved during the assay, the model will fail to converge.

To create the proper plot environment within GraphPad Prism for running each model, choose "XY" as the table and graph type. For Y the value options, choose "Enter and plot error values already calculated elsewhere", and in the pull-down menu labeled "Enter" choose the option "Mean, SD, N".

Tests of difference for GI_{50}, TGI and LC_{50} between pairs of compounds

It is possible to run a test in order to determine whether any of the three dose response parameters differs significantly between two compounds. To do so, create a GraphPad Prism file as described above with data for two compounds entered into the same Data Table. For analysis, choose to run the model described above that matches the parameter to be tested. After the desired model is highlighted in the menu under the "Fit" tab of the window called "Parameters: Nonlinear Regression", click the "Compare" tab. Then choose the following options:

What question are you asking?
Do the best fit values of selected parameters differ between data sets?

Comparison method
Extra sum-of-squares F test. Select the simpler model unless P value less than: 0.05.

Choose one or more parameters
logECF

A section labeled Comparison of Fits reports the test results within the Results sheet that is generated when the model is run. "logECF" is actually logGI50/TGI/LC50, depending upon which model has been run.

II. X-ray Crystallographic Data

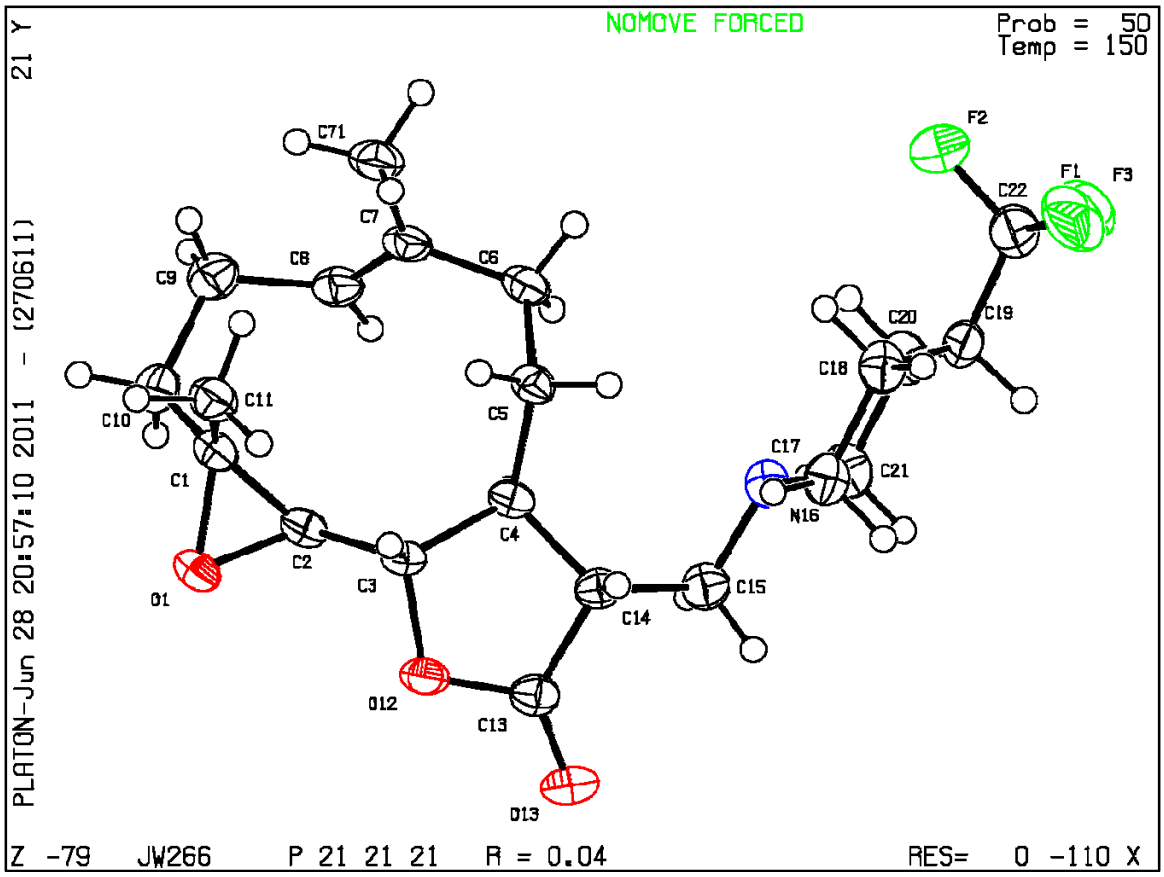
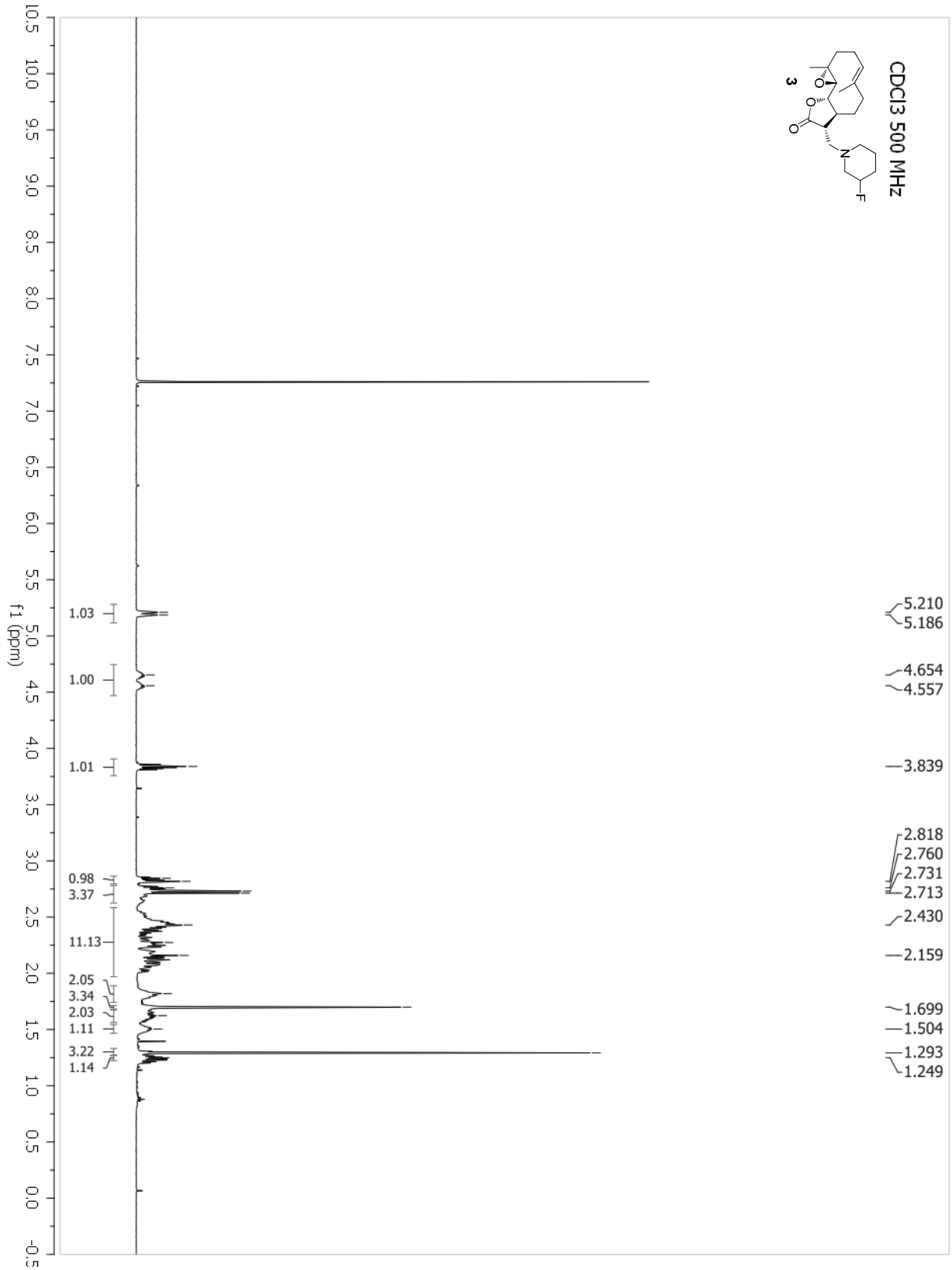
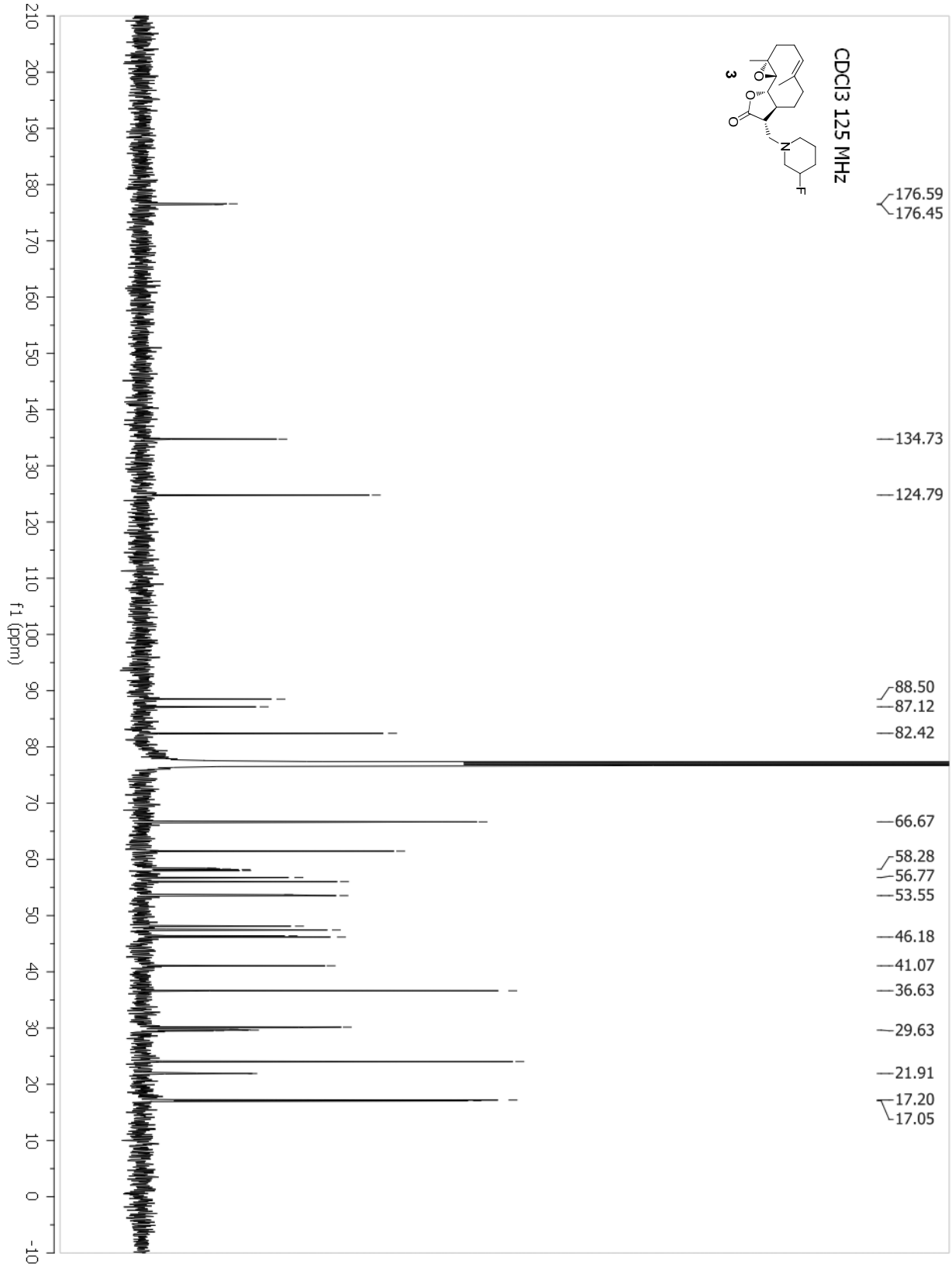
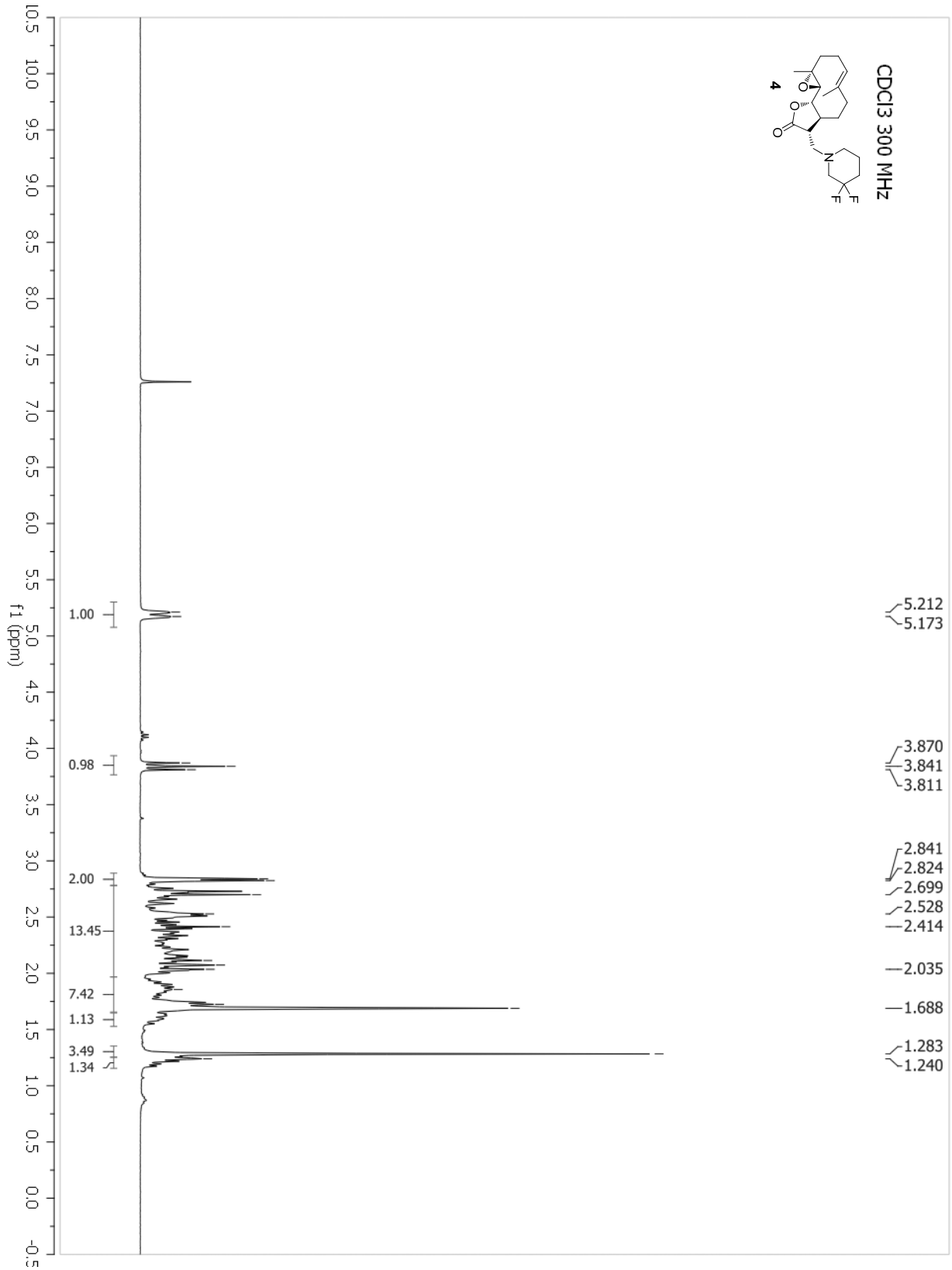
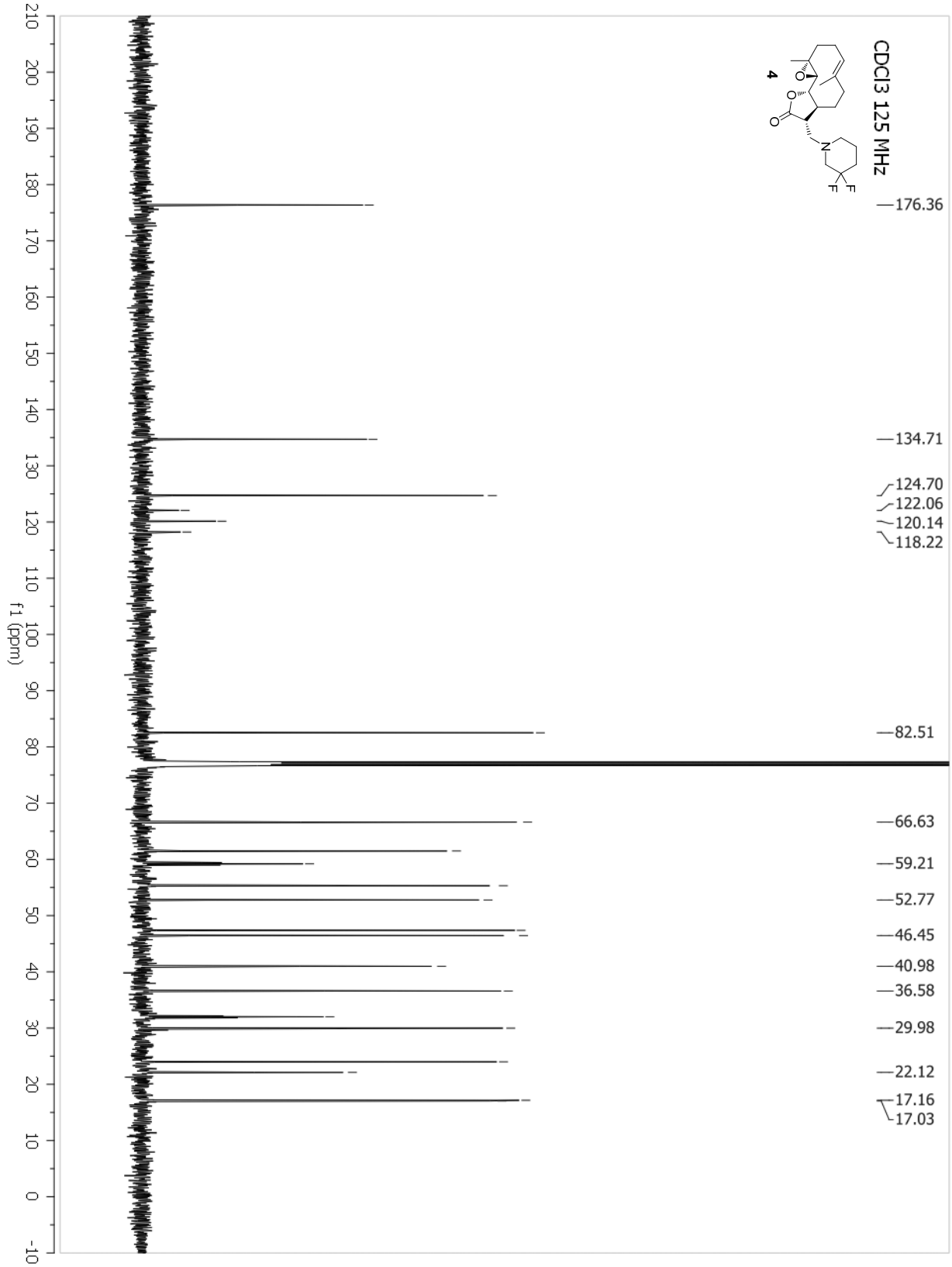


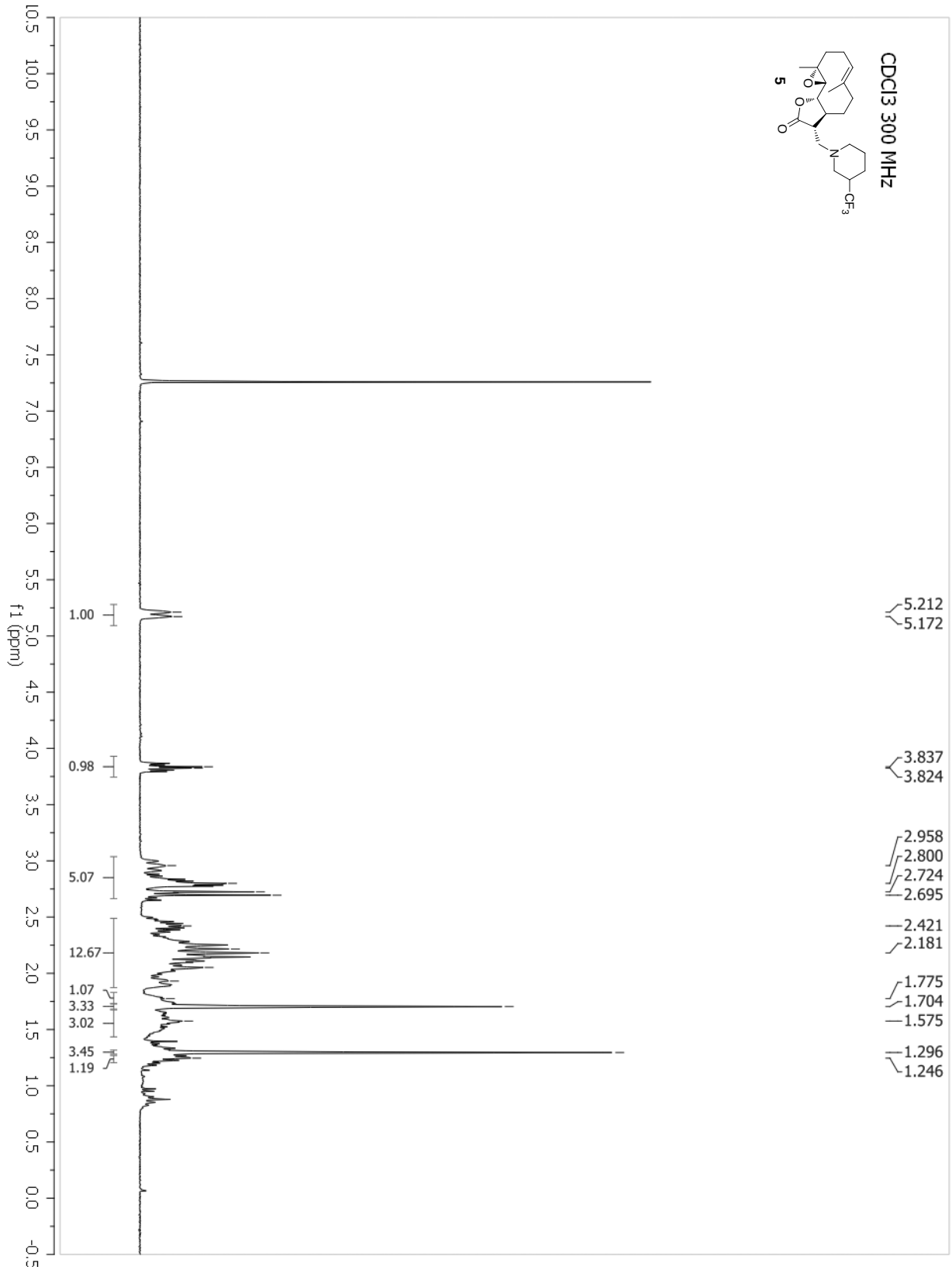
Figure S1. ORTEP Diagram of **8**

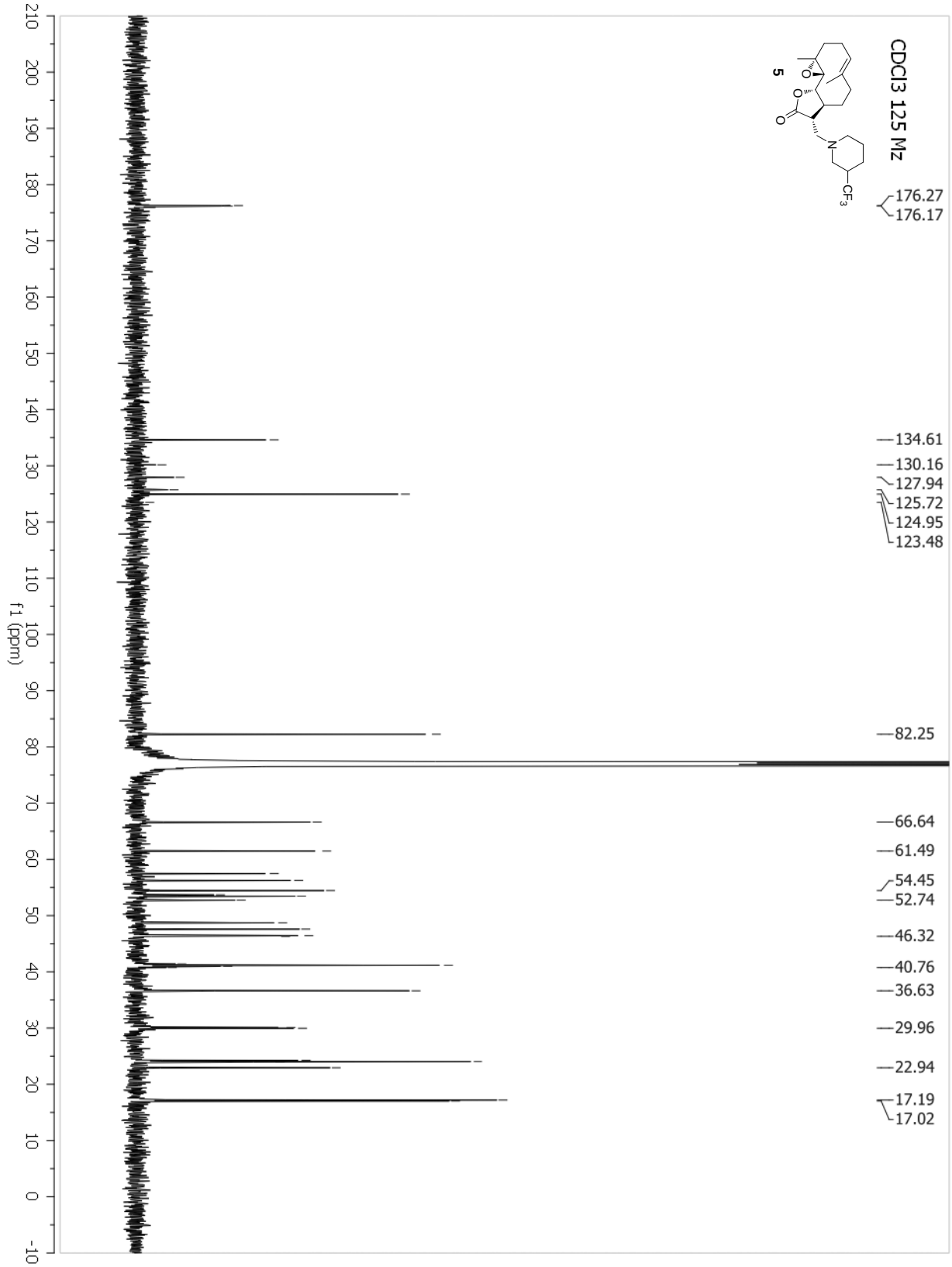

CDCl3 500 MHz
3
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1.03
1.00
1.01
0.98
3.37
11.13
2.05
3.34
2.03
1.11
3.22
1.14
5.210
5.186
4.654
4.557
3.839
2.818
2.760
2.731
2.713
2.430
2.159
1.699
1.504
1.293
1.249

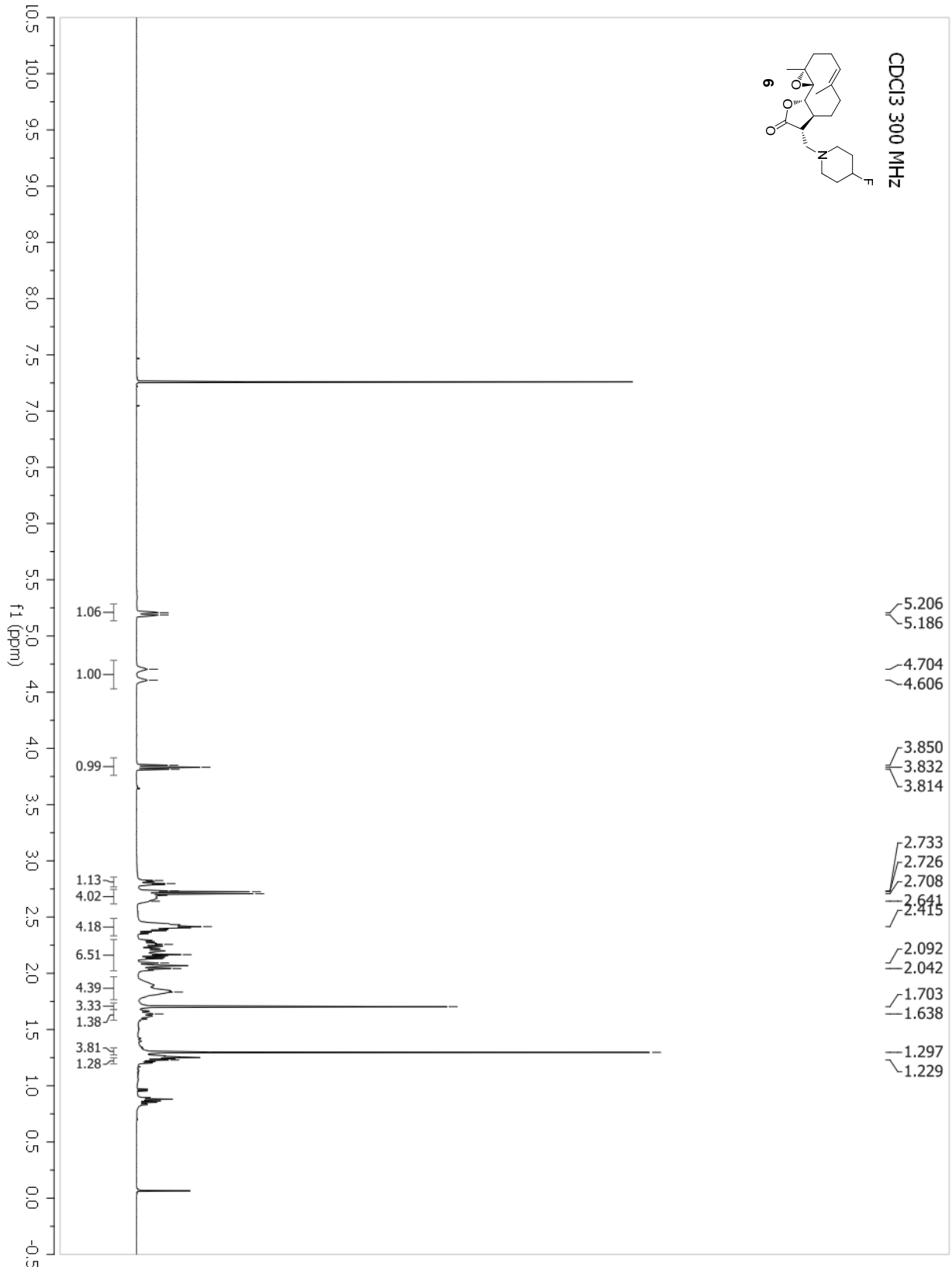

CDCl3 125 MHz
3
176.59
176.45
134.73
124.79
88.50
87.12
82.42
66.67
58.28
56.77
53.55
46.18
41.07
36.63
29.63
21.91
17.20
17.05
f1 (ppm)

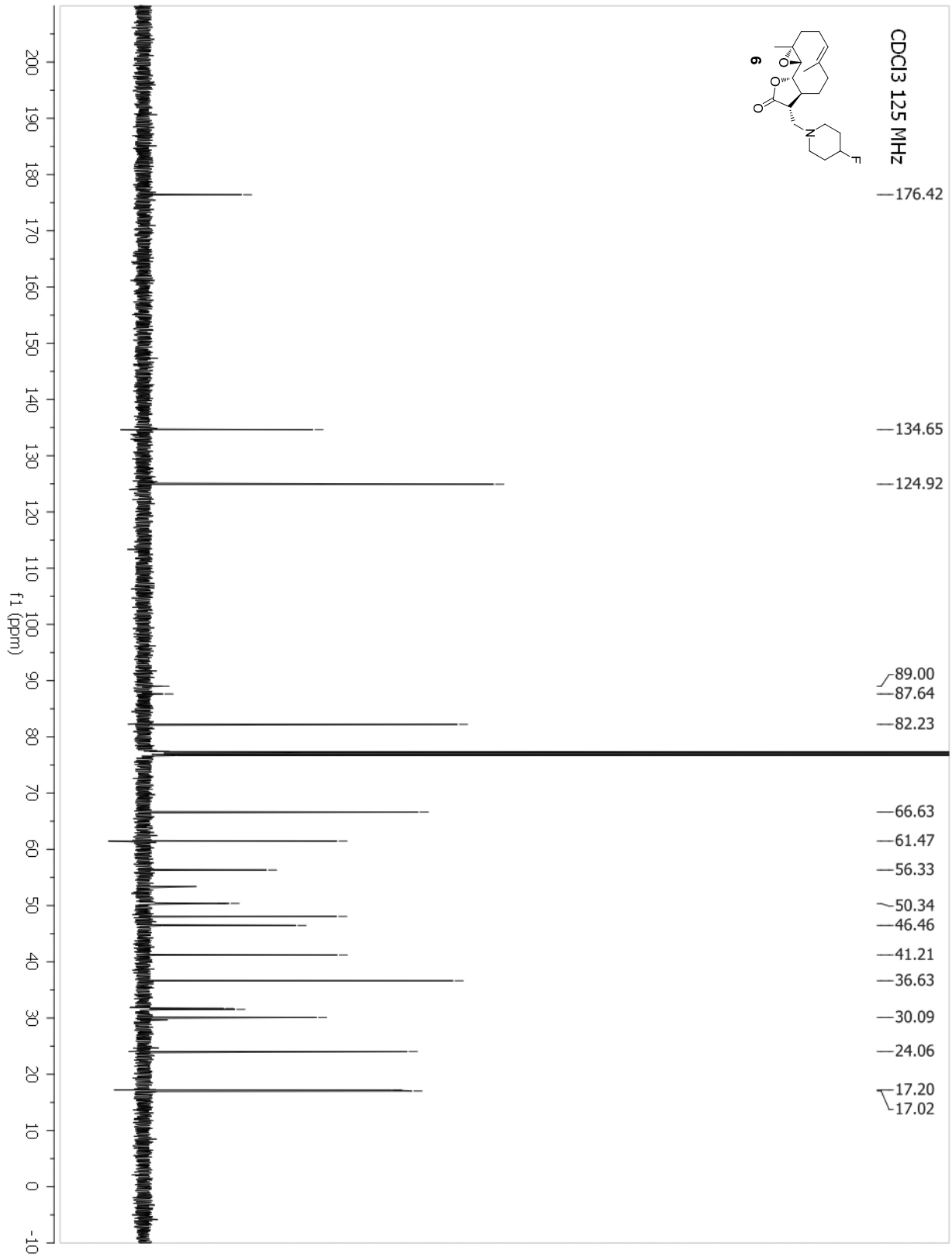

CDCl3 300 MHz
4
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1.00
0.98
2.00
13.45
7.42
1.13
3.49
1.34
5.212
5.173
3.870
3.841
3.811
2.841
2.824
2.699
2.528
2.414
2.035
1.688
1.283
1.240

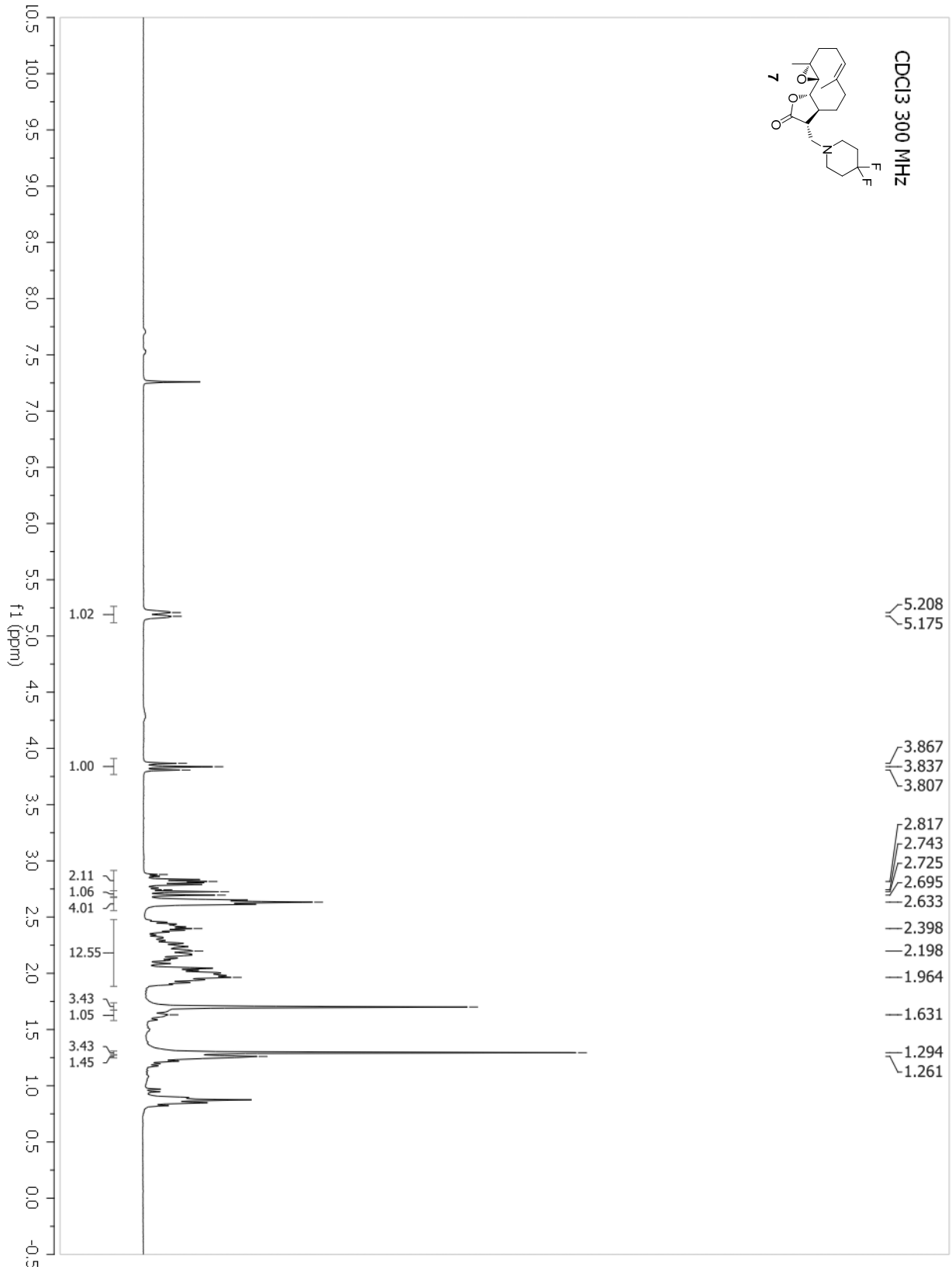

CDCl3 125 MHz
4
176.36
134.71
124.70
122.06
120.14
118.22
82.51
66.63
59.21
52.77
46.45
40.98
36.58
29.98
22.12
17.16
17.03
f1 (ppm)

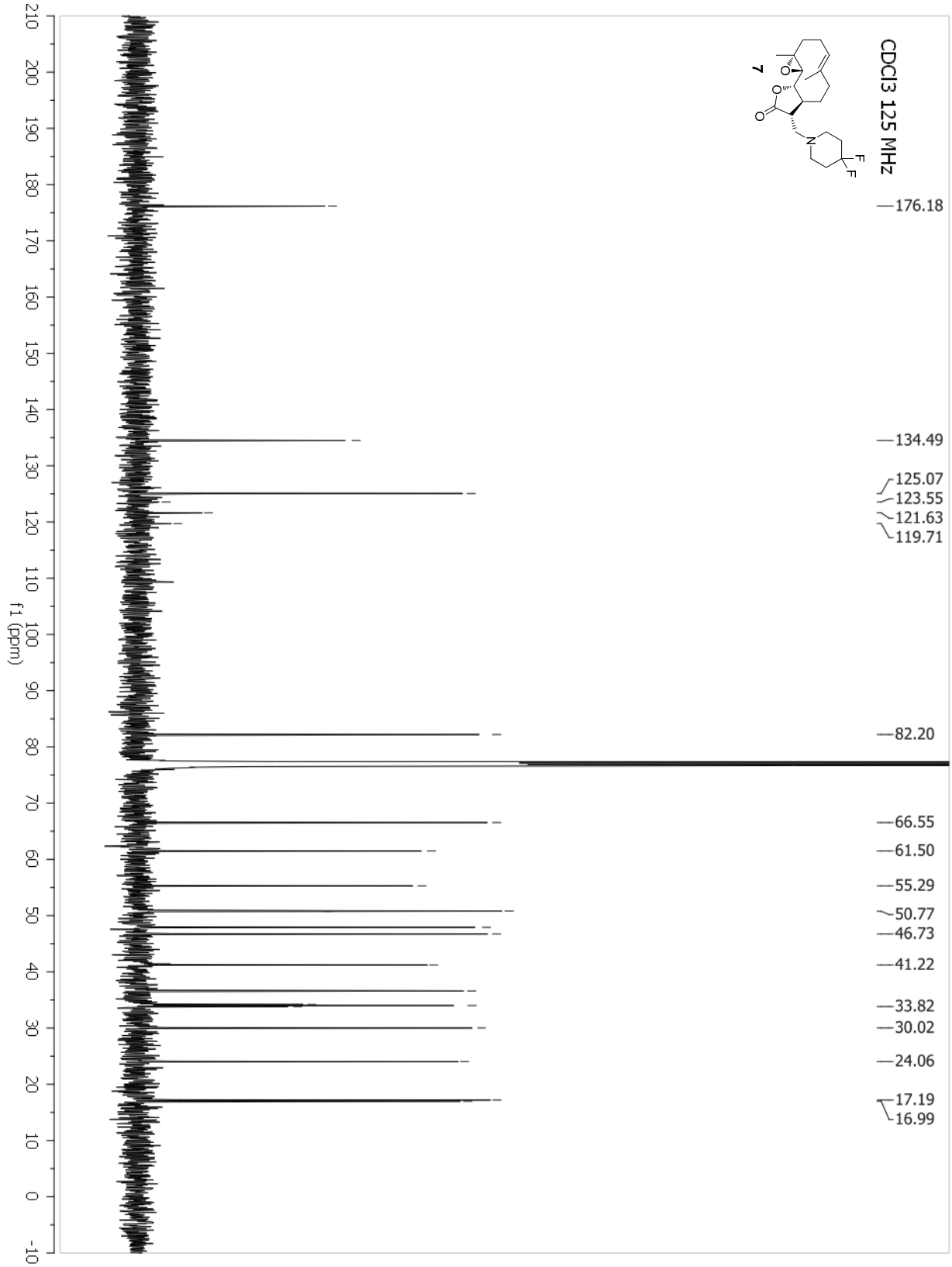

CDCl3 300 MHz
5
f1 (ppm)
1.00
0.98
5.07
12.67
1.07
3.33
3.02
3.45
1.19
5.212
5.172
3.837
3.824
2.958
2.800
2.724
2.695
2.421
2.181
1.775
1.704
1.575
1.296
1.246

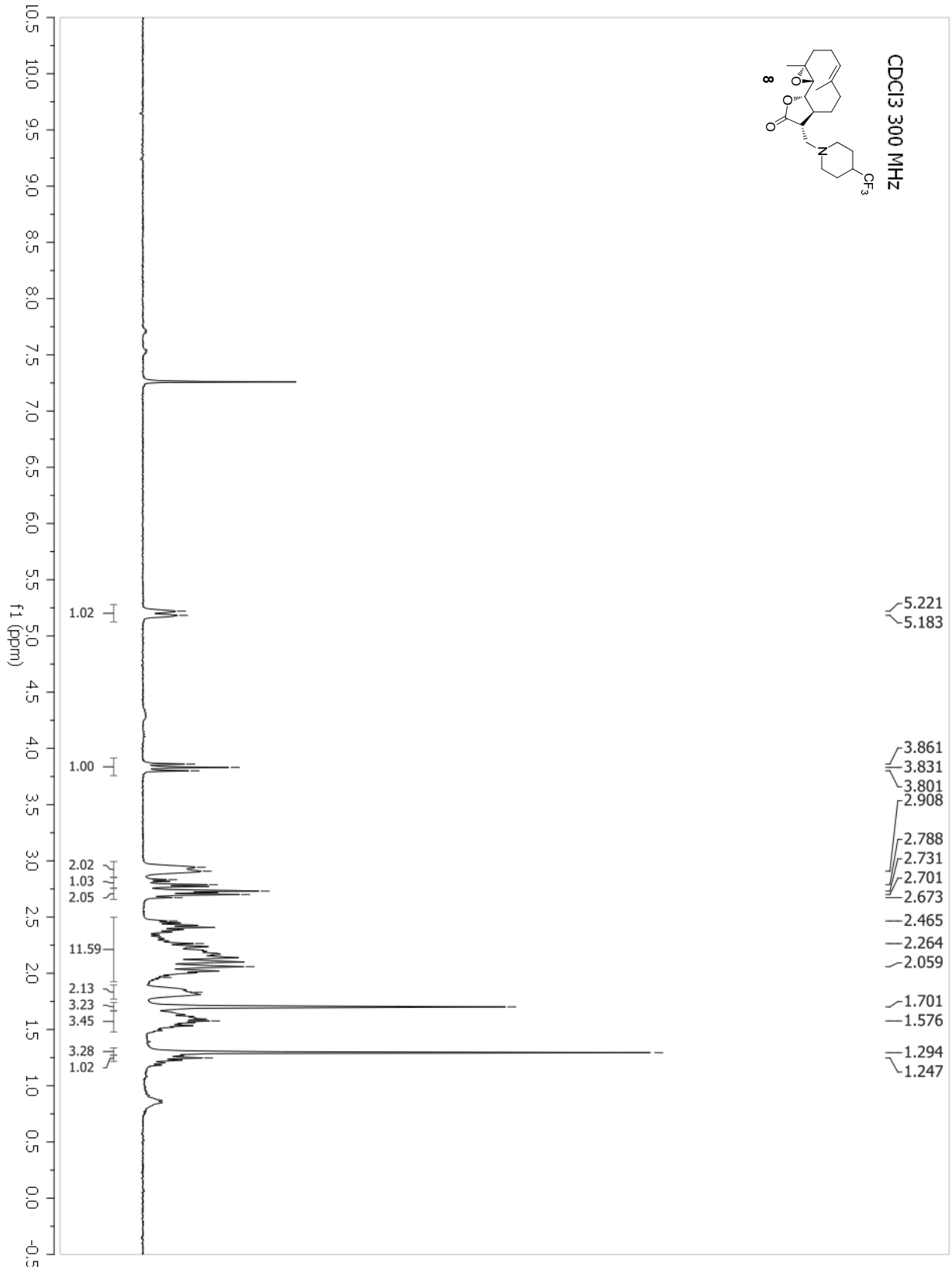

CDCl3 125 Mz
5
176.27
176.17
134.61
130.16
127.94
125.72
124.95
123.48
82.25
66.64
61.49
54.45
52.74
46.32
40.76
36.63
29.96
22.94
17.19
17.02
f1 (ppm)

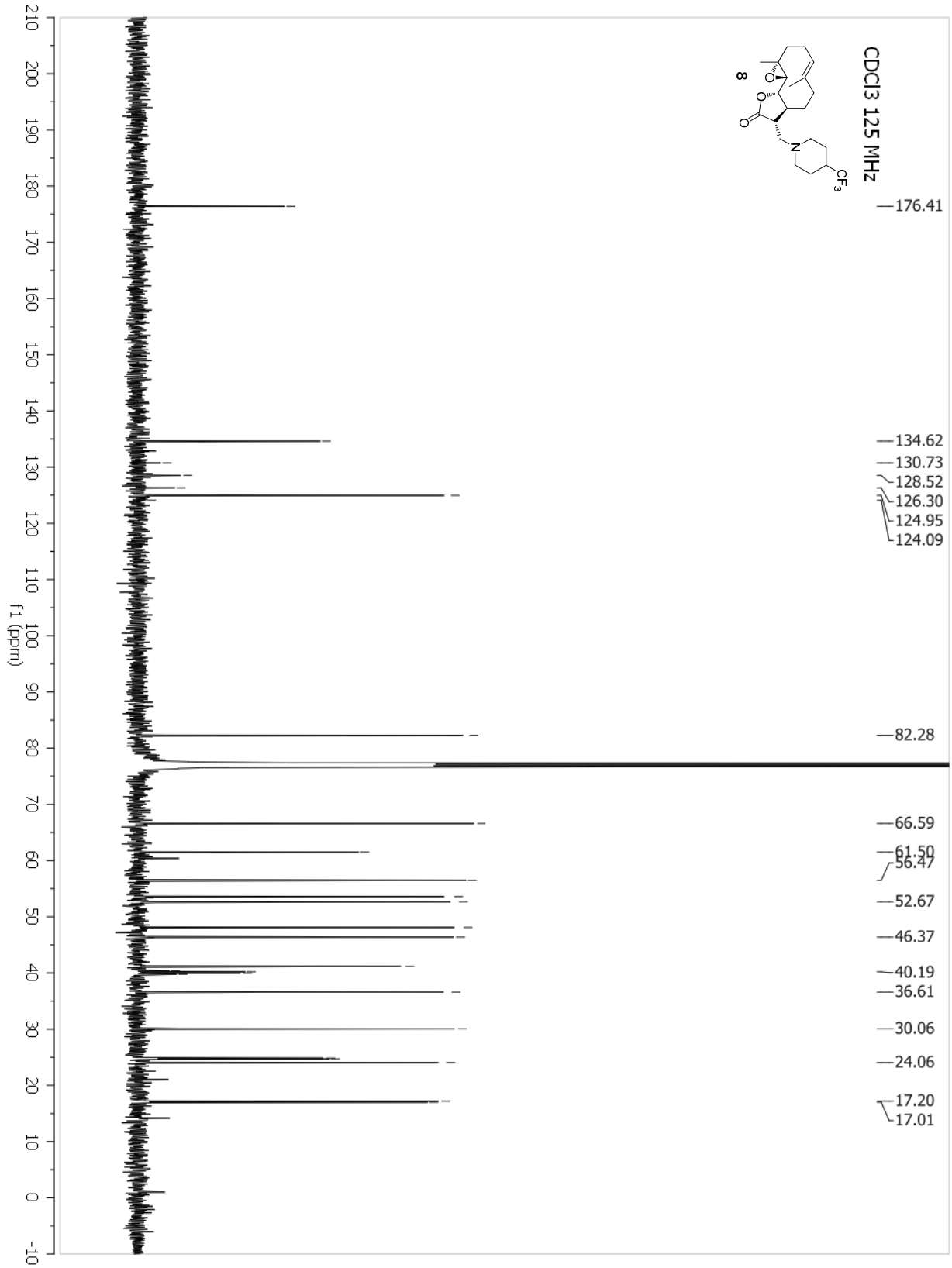

CDCl3 300 MHz
6
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1.06
1.00
0.99
1.13
4.02
4.18
6.51
4.39
3.33
1.38
3.81
1.28
5.206
5.186
4.704
4.606
3.850
3.832
3.814
2.733
2.726
2.708
2.641
2.415
2.092
2.042
1.703
1.638
1.297
1.229

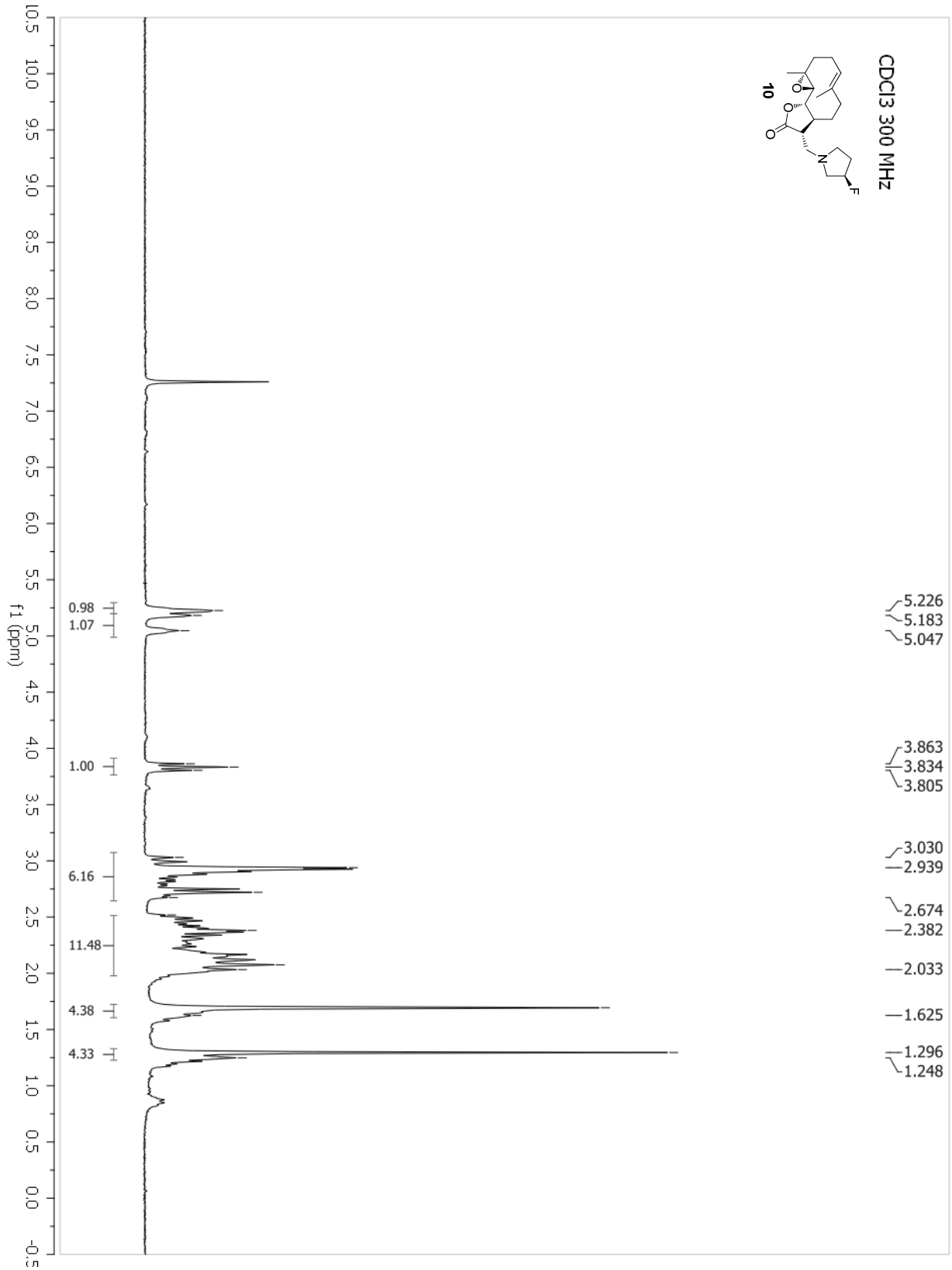

CDCl3 125 MHz
6
176.42
134.65
124.92
89.00
87.64
82.23
66.63
61.47
56.33
50.34
46.46
41.21
36.63
30.09
24.06
17.20
17.02
f1 (ppm)

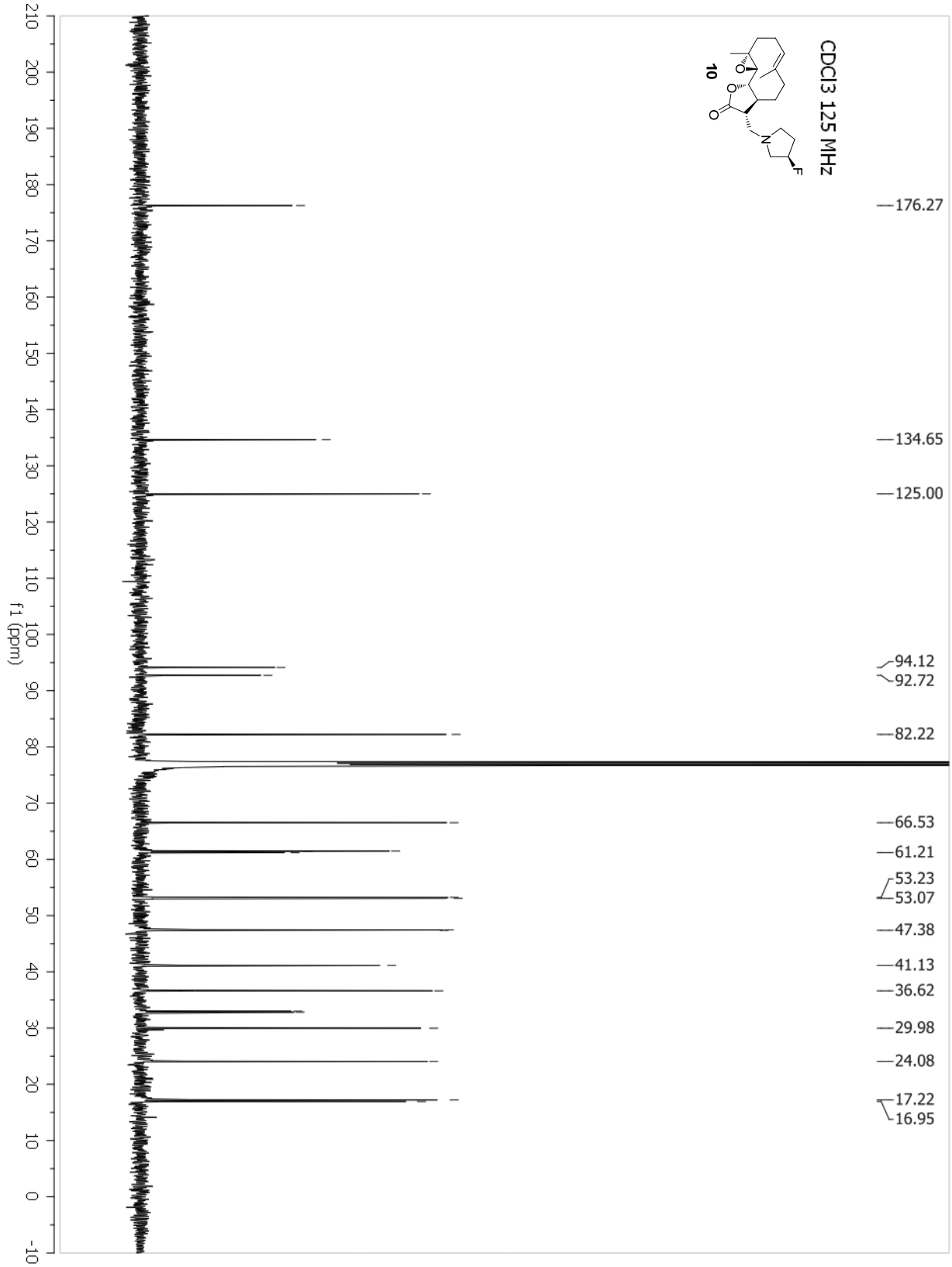

CDCl3 300 MHz
7
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1.02
1.00
2.11
1.06
4.01
12.55
3.43
1.05
3.43
1.45
5.208
5.175
3.867
3.837
3.807
2.817
2.743
2.725
2.695
2.633
2.398
2.198
1.964
1.631
1.294
1.261

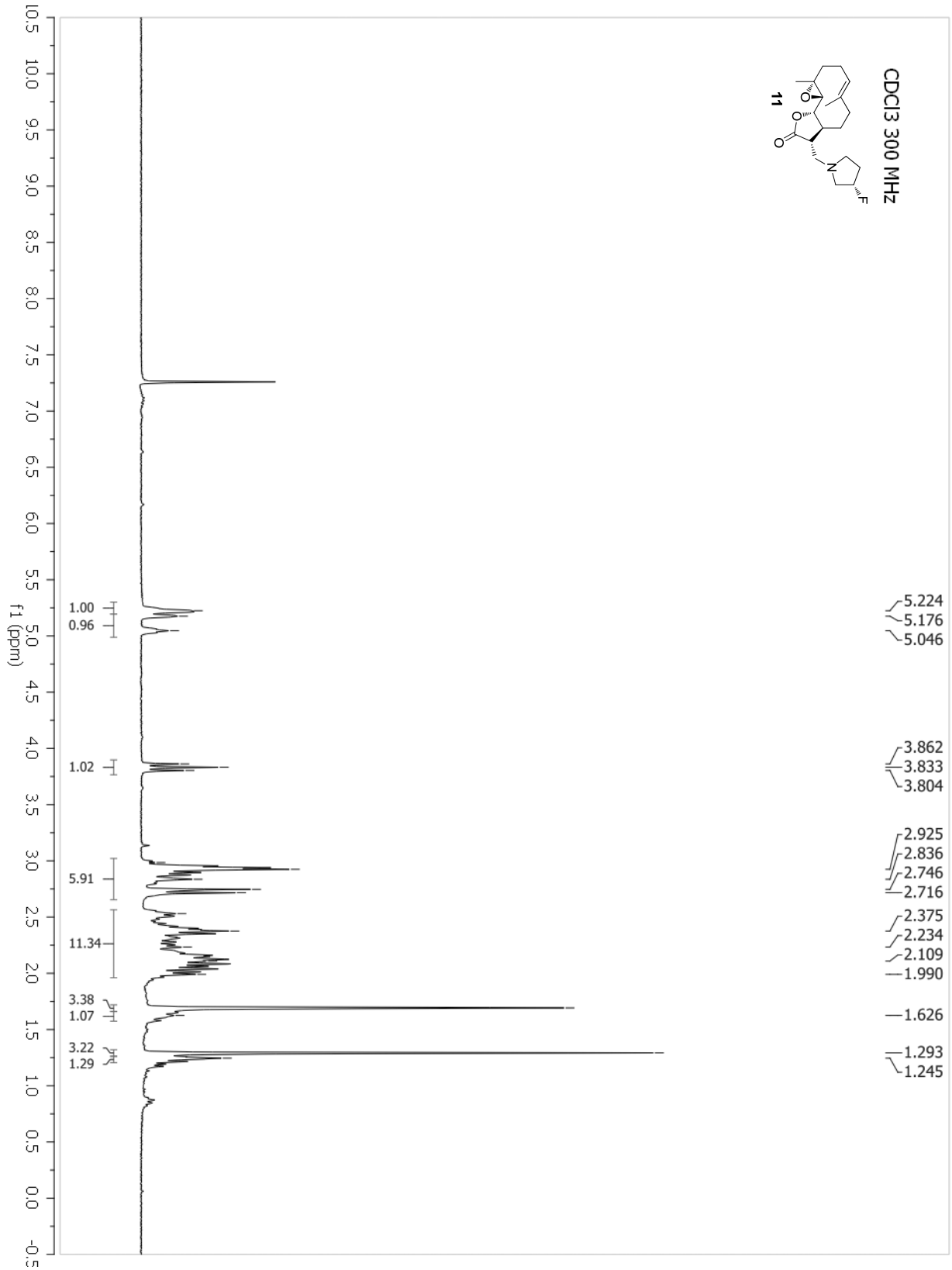

CDCl3 125 MHz
7
176.18
134.49
125.07
123.55
121.63
119.71
82.20
66.55
61.50
55.29
50.77
46.73
41.22
33.82
30.02
24.06
17.19
16.99
f1 (ppm)

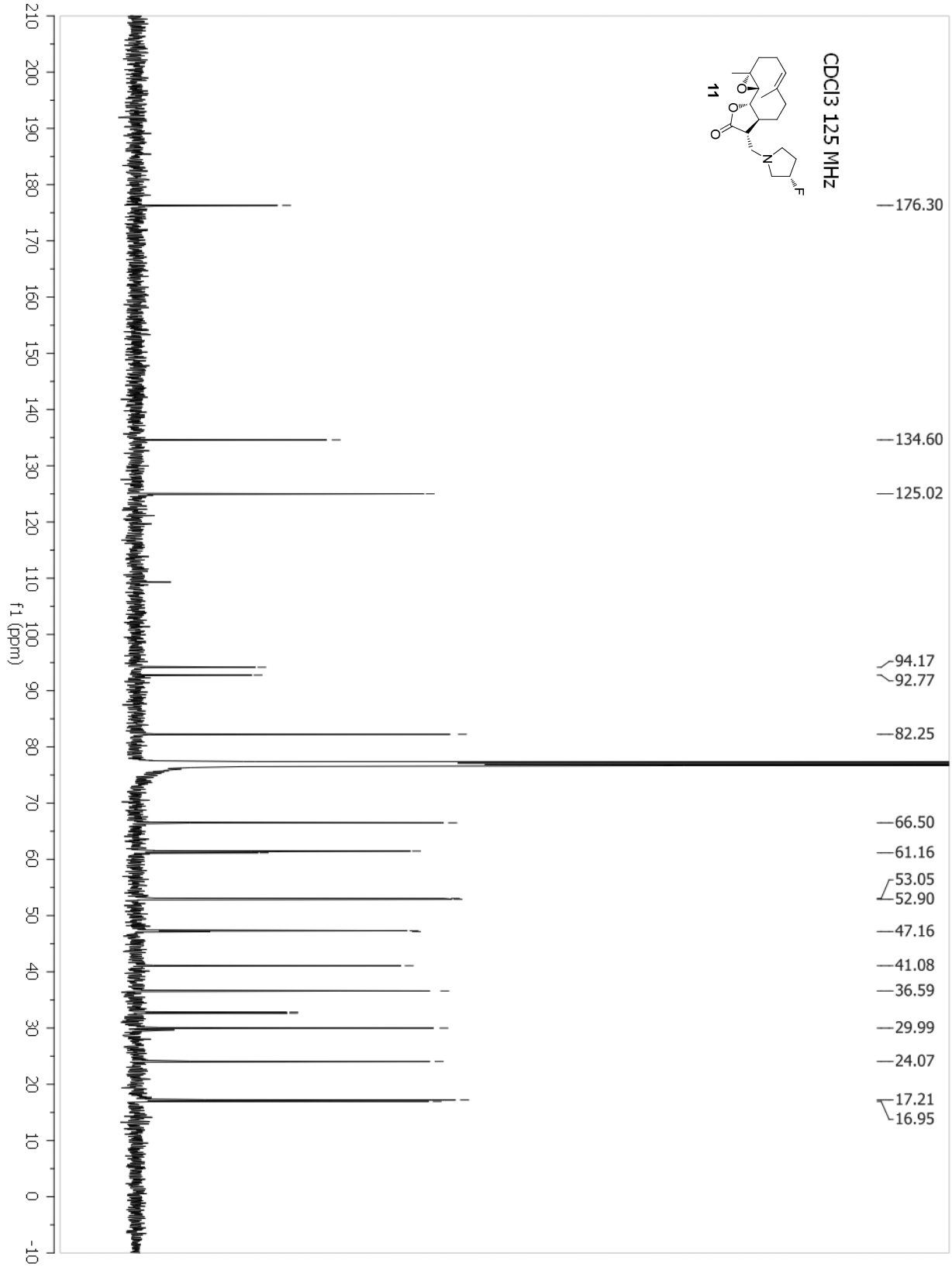

CDCl3 300 MHz
8
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1.02
1.00
2.02
1.03
2.05
11.59
2.13
3.23
3.45
3.28
1.02
5.221
5.183
3.861
3.831
3.801
2.908
2.788
2.731
2.701
2.673
2.465
2.264
2.059
1.701
1.576
1.294
1.247

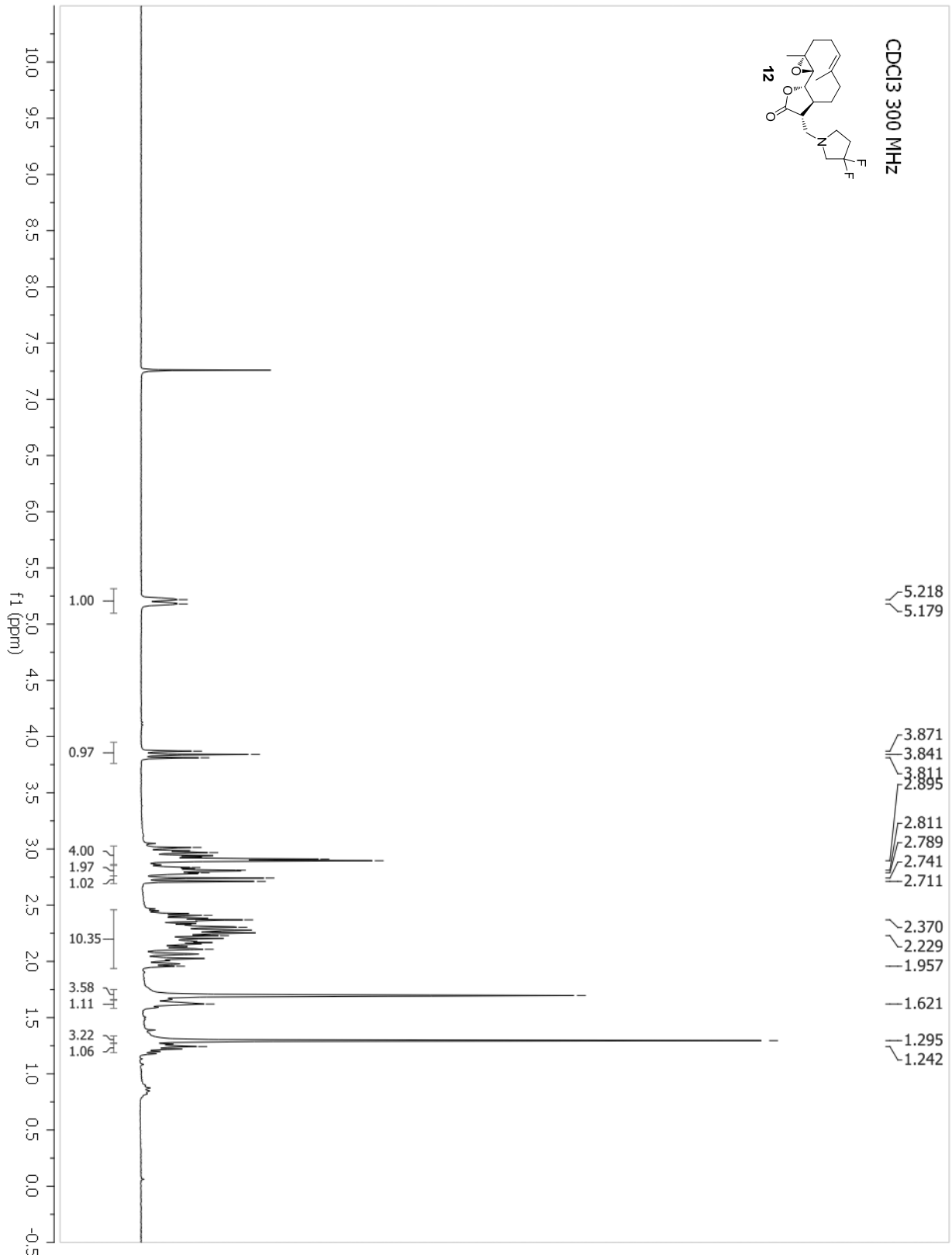

CDCl3 125 MHz
8
176.41
134.62
130.73
128.52
126.30
124.95
124.09
82.28
66.59
61.50
56.47
52.67
46.37
40.19
36.61
30.06
24.06
17.20
17.01
f1 (ppm)

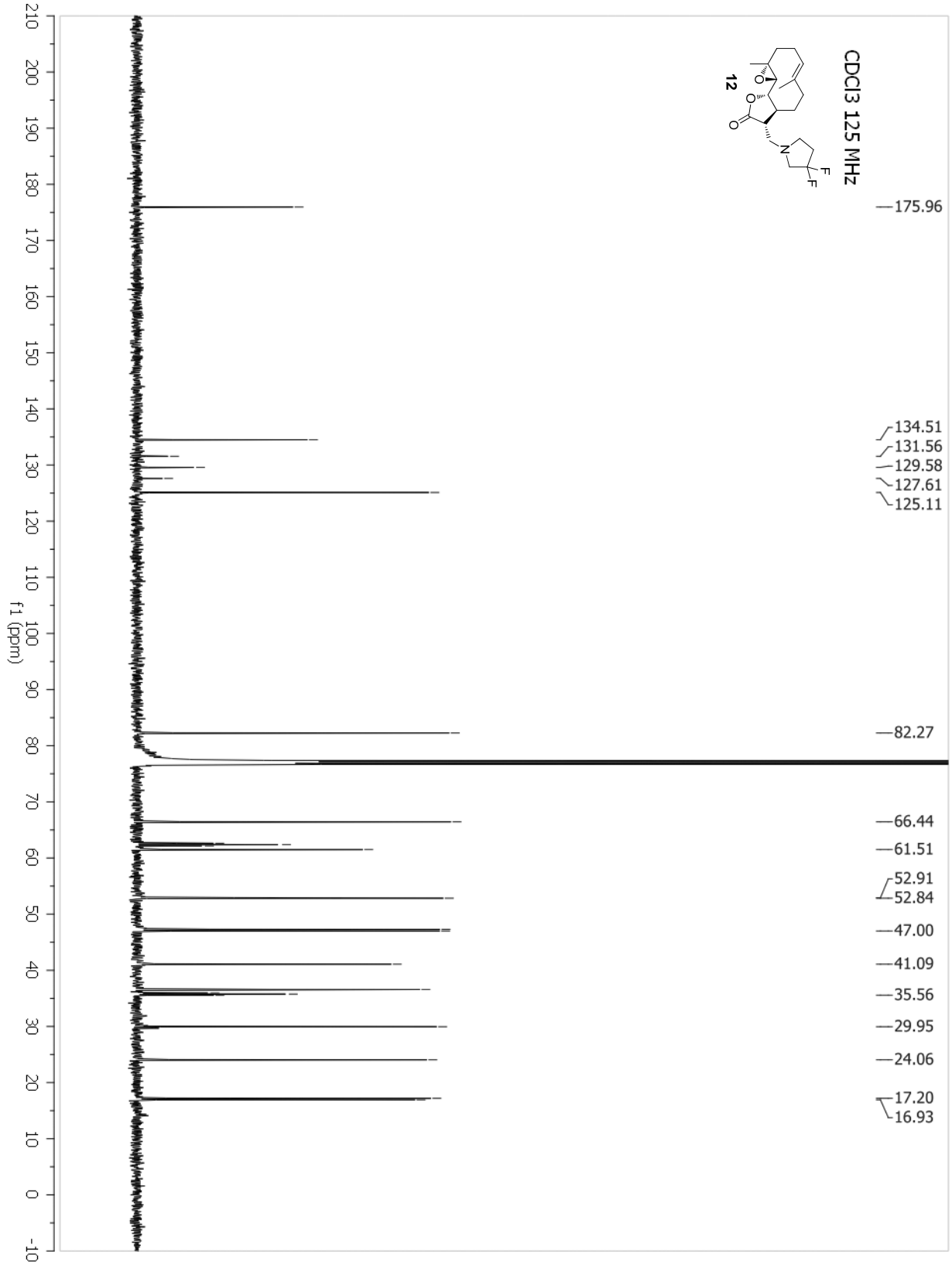

CDCl3 300 MHz
10
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.98
1.07
1.00
6.16
11.48
4.38
4.33
5.226
5.183
5.047
3.863
3.834
3.805
3.030
2.939
2.674
2.382
2.033
1.625
1.296
1.248


CDCl3 125 MHz
10
176.27
134.65
125.00
94.12
92.72
82.22
66.53
61.21
53.23
53.07
47.38
41.13
36.62
29.98
24.08
17.22
16.95
f1 (ppm)


CDCl3 300 MHz
11
f1 (ppm)
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
-0.5
1.00
0.96
1.02
5.91
11.34
3.38
1.07
3.22
1.29
5.224
5.176
5.046
3.862
3.833
3.804
2.925
2.836
2.746
2.716
2.375
2.234
2.109
1.990
1.626
1.293
1.245


CDCl3 125 MHz
11
176.30
134.60
125.02
94.17
92.77
82.25
66.50
61.16
53.05
52.90
47.16
41.08
36.59
29.99
24.07
17.21
16.95
f1 (ppm)


CDCl3 300 MHz
12
f1 (ppm)
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
1.00
0.97
4.00
1.97
1.02
10.35
3.58
1.11
3.22
1.06
5.218
5.179
3.871
3.841
3.811
2.895
2.811
2.789
2.741
2.711
2.370
2.229
1.957
1.621
1.295
1.242


CDCl3 125 MHz
12
175.96
134.51
131.56
129.58
127.61
125.11
82.27
66.44
61.51
52.91
52.84
47.00
41.09
35.56
29.95
24.06
17.20
16.93
f1 (ppm)