Supporting Information

Nanoparticle-Mediated Delivery of Micheliolide Analogs to Eliminate Leukemic Stem Cells in the Bone Marrow

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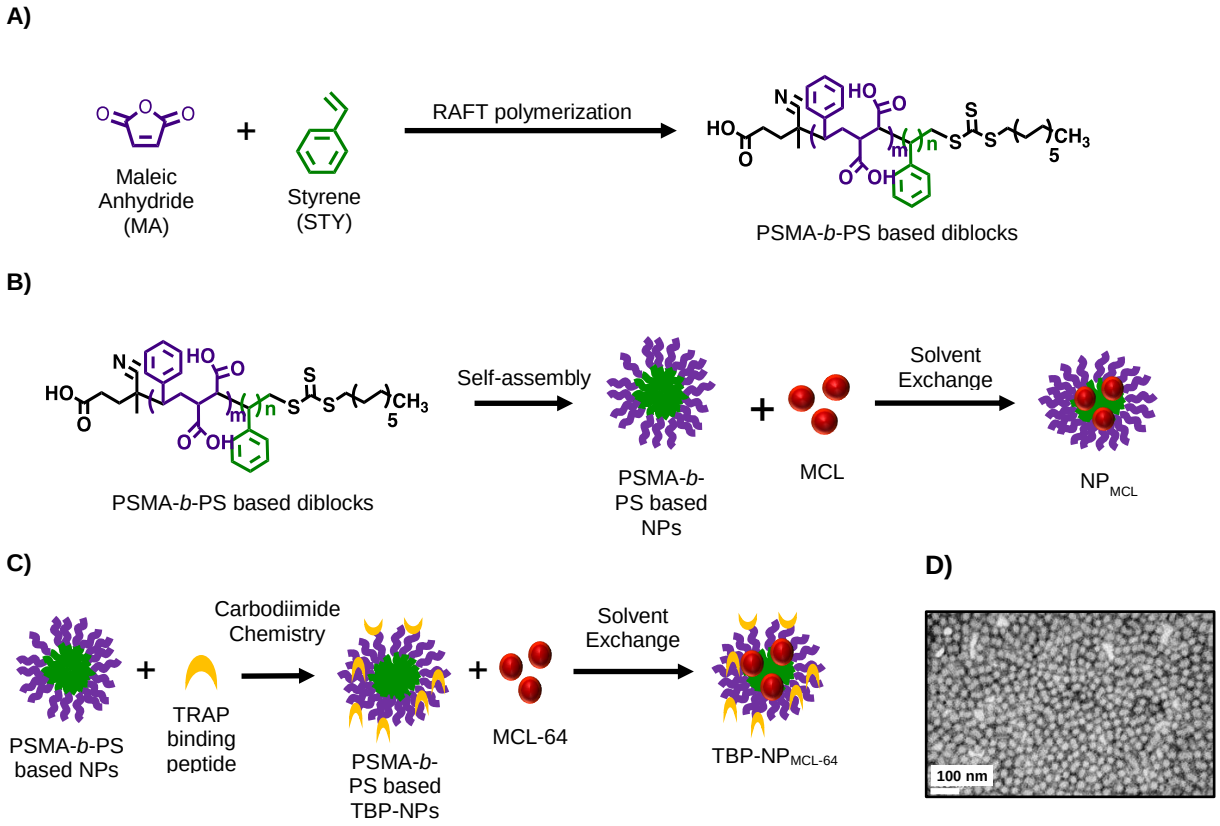
Schematic S1. RAFT polymerization schematic showing PSMA-*b*-PS nanoparticle self-assembly and subsequent loading with MCL analogs via solvent exchange [a]Amphiphilic PSMA-*b*-PS diblock copolymers were synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerizations using cyano-4-dodecysulfanyltrithiocarbonyl sulfanyl pentanoic acid (DCT) chain transfer agent. C) Schematic showing the development of TBP-NP$_{MCL-64}$ using in efficacy studies. D) Representative transmission electron microscopy (TEM) image of NPs used for studies.



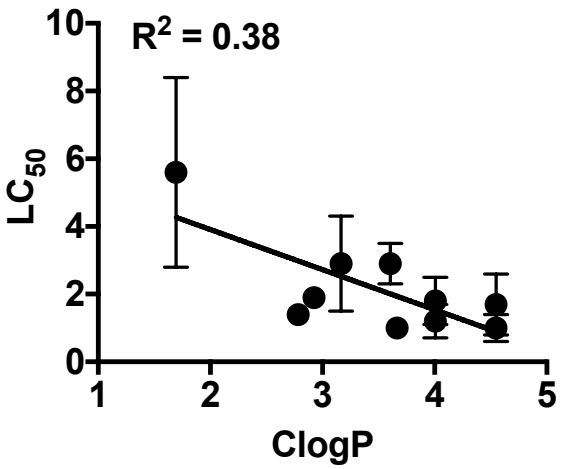


Figure S1: Relationship between LC_{50} and CLogP. Line of best fit drawn using non-linear regression function on Prism V7.

Solvent conditions alter loading capacity

Since solvent choice has been previously shown to impact PSMA-*b*-PS loading [27] , a subset of studies tested loading of MCL, MCL-13, and MCL-38 with acetone. The trends for the compounds were similar to loading with chloroform except that MCL and MCL-38 displayed reduced loading capacity of ~ 2-fold and ~ 5-fold while MCL-13 loading was unaffected. Since changing the solvent did not improve loading capacity of MCL, MCL-38 or MCL-13, acetone was omitted from loading experiments moving forward.

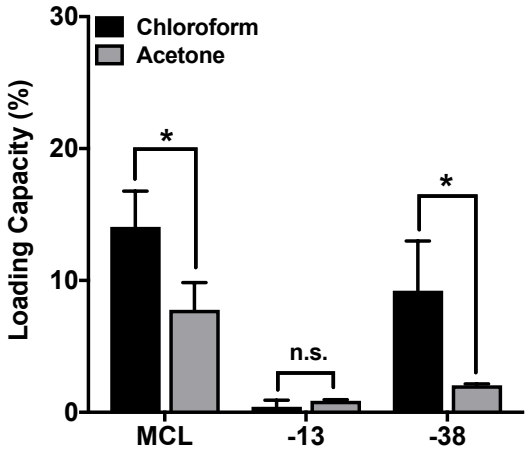


Figure S2: Solvent dependent loading conditions. B) Loading capacity for MCL and selected MCL analogs using acetone compared to chloroform.

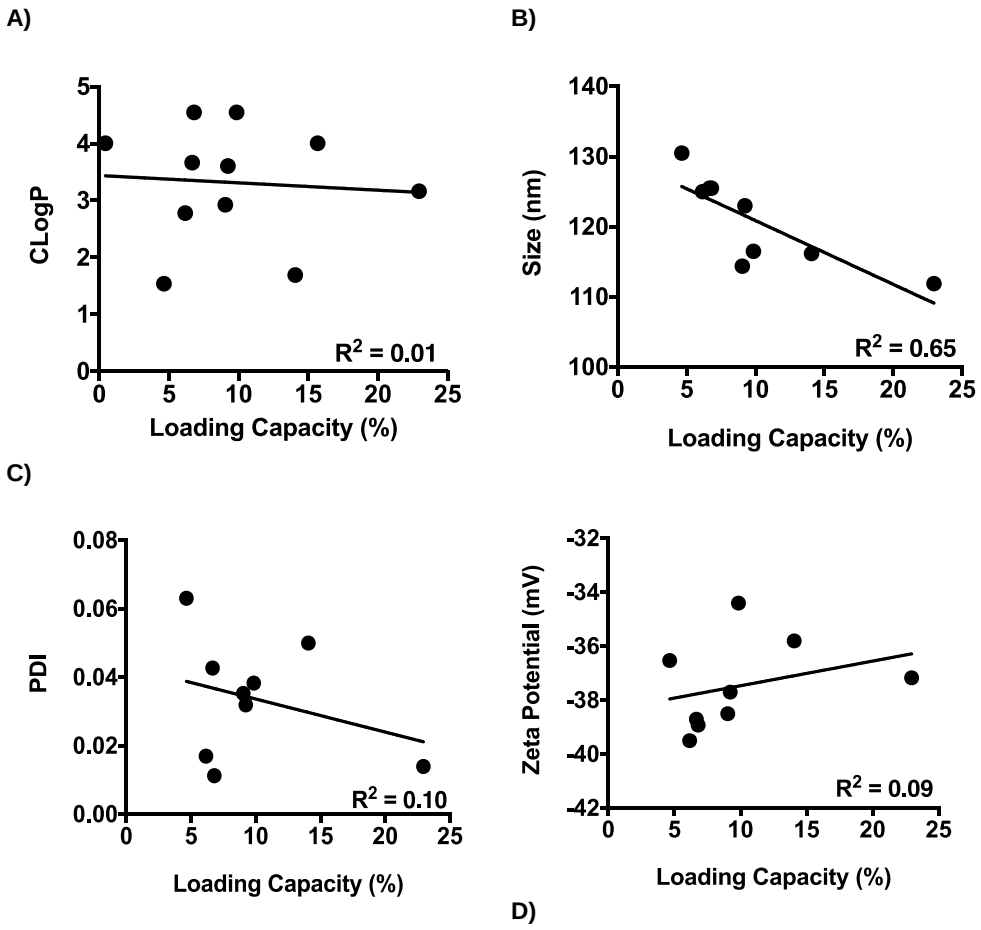


Figure S3: Relationship between loading capacity and drug and NP physiochemical properties. A) CLogP of compounds loaded, **B)** NP size after loading, **C)** polydispersity index of loaded NPs, **D)** Surface charge of loaded NPs. Line of best fit drawn using non-linear regression function.

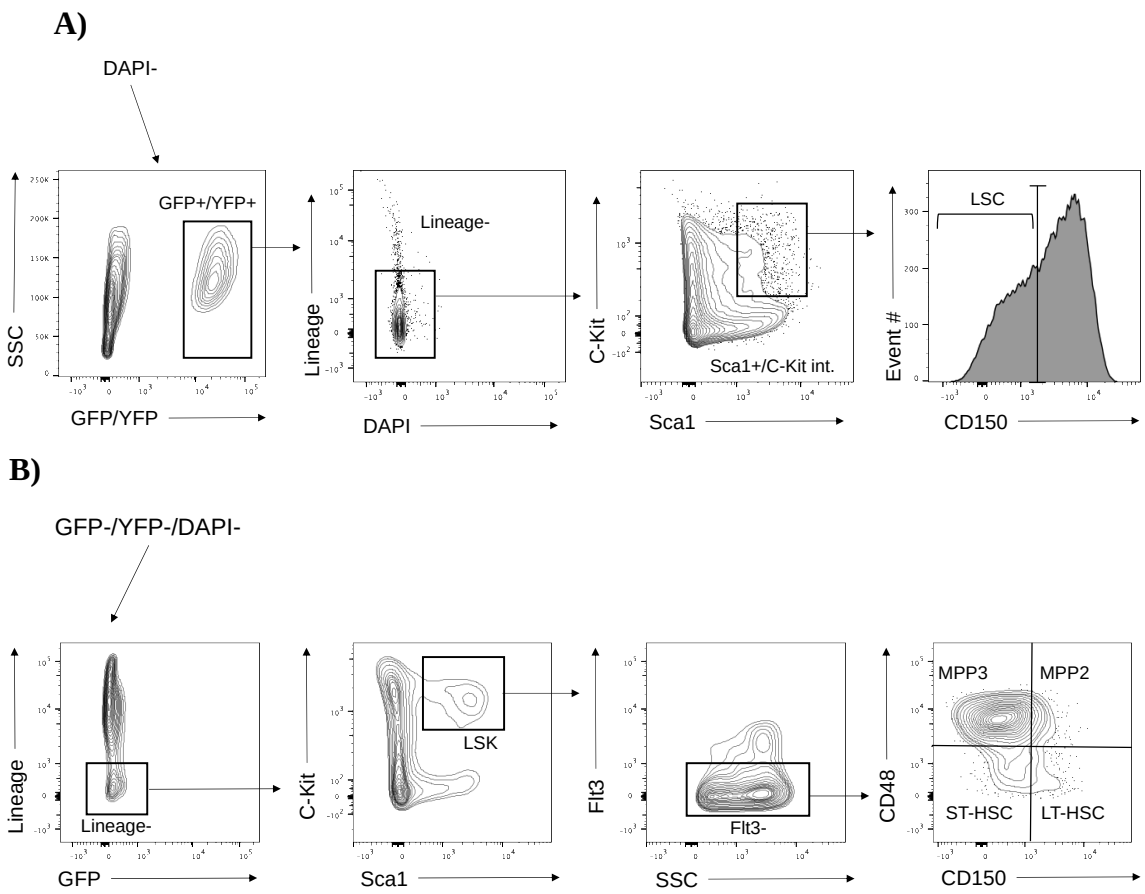


Figure S4: Gating strategy used for identifying A) LSCs, B) LSKs, and LT-HSCs.

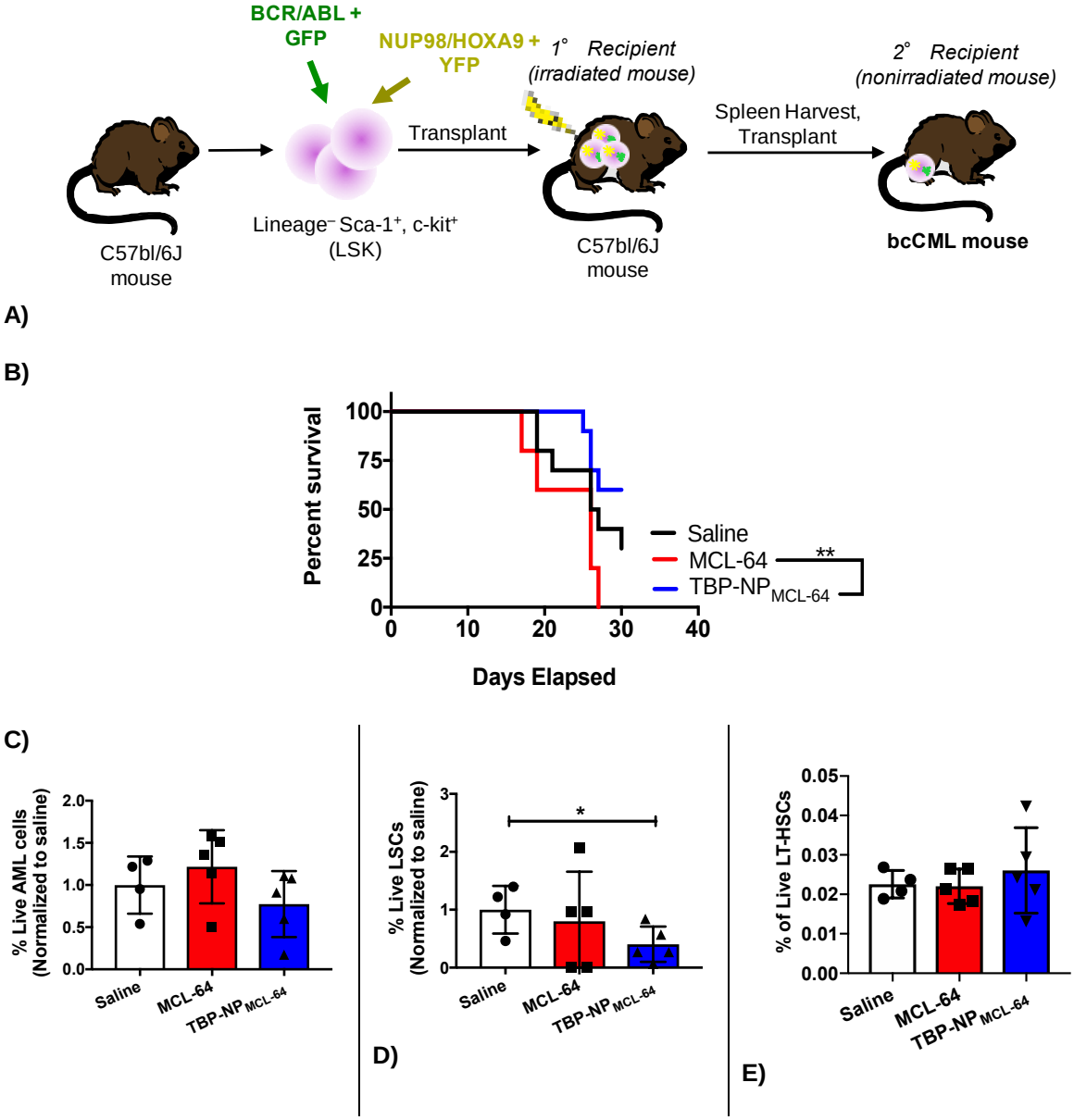


Figure S5: A) Schematic showing the initiation of the leukemia model used in these studies. **B)** bcCML mouse survival after treating with saline, free MCL-64 and NP loaded MCL-64 using 45 mg/kg free MCL and in TBP-NP. **C**) GFP+ bcCML cells in the marrow after treatment with 45 mg/kg MCL-64 as free drug and in NP. **D)** LSCs and E) LT-HSCs. Data represents mean ± std (n = 4- 5). * $p < 0.05$ represents statistical differences comparisons using unpaired *t*-tests.

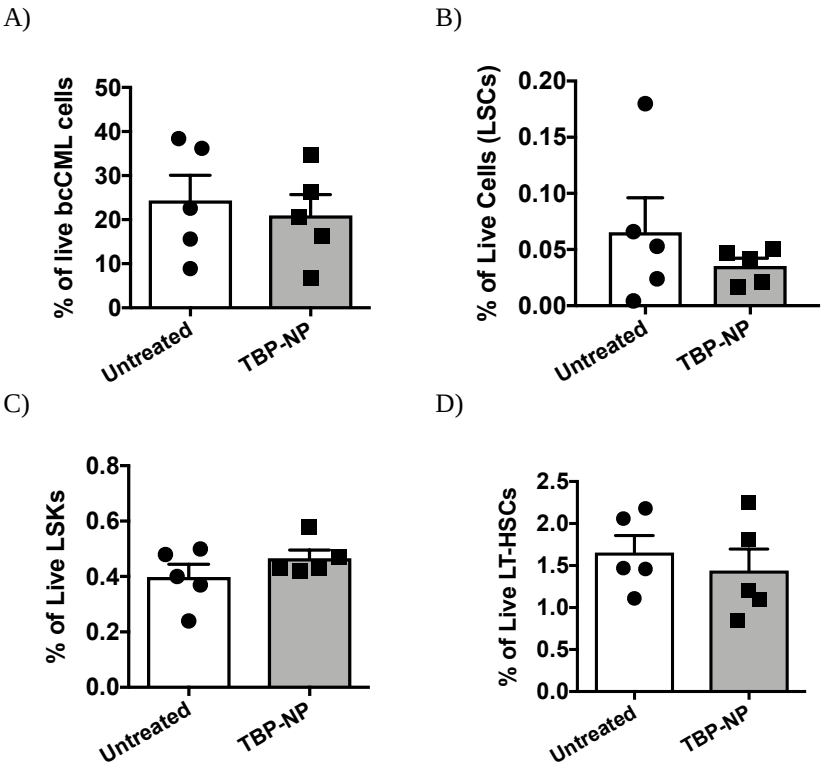


Figure S6: bcCML in the marrow after treatment with empty TBP-NPs. A) GFP+ bcCML cells, B) LSCs, C) LSKs, and D) LT-HSCs. Data represents mean ± SEM (n = 5). No statistical differences noted after statistical analysis via unpaired *t*-tests.

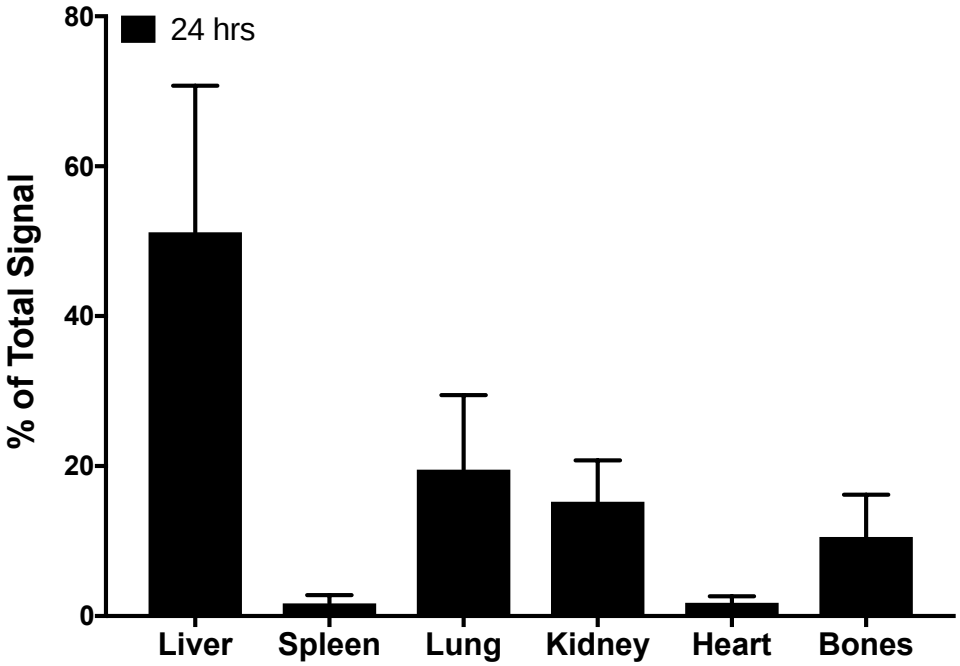
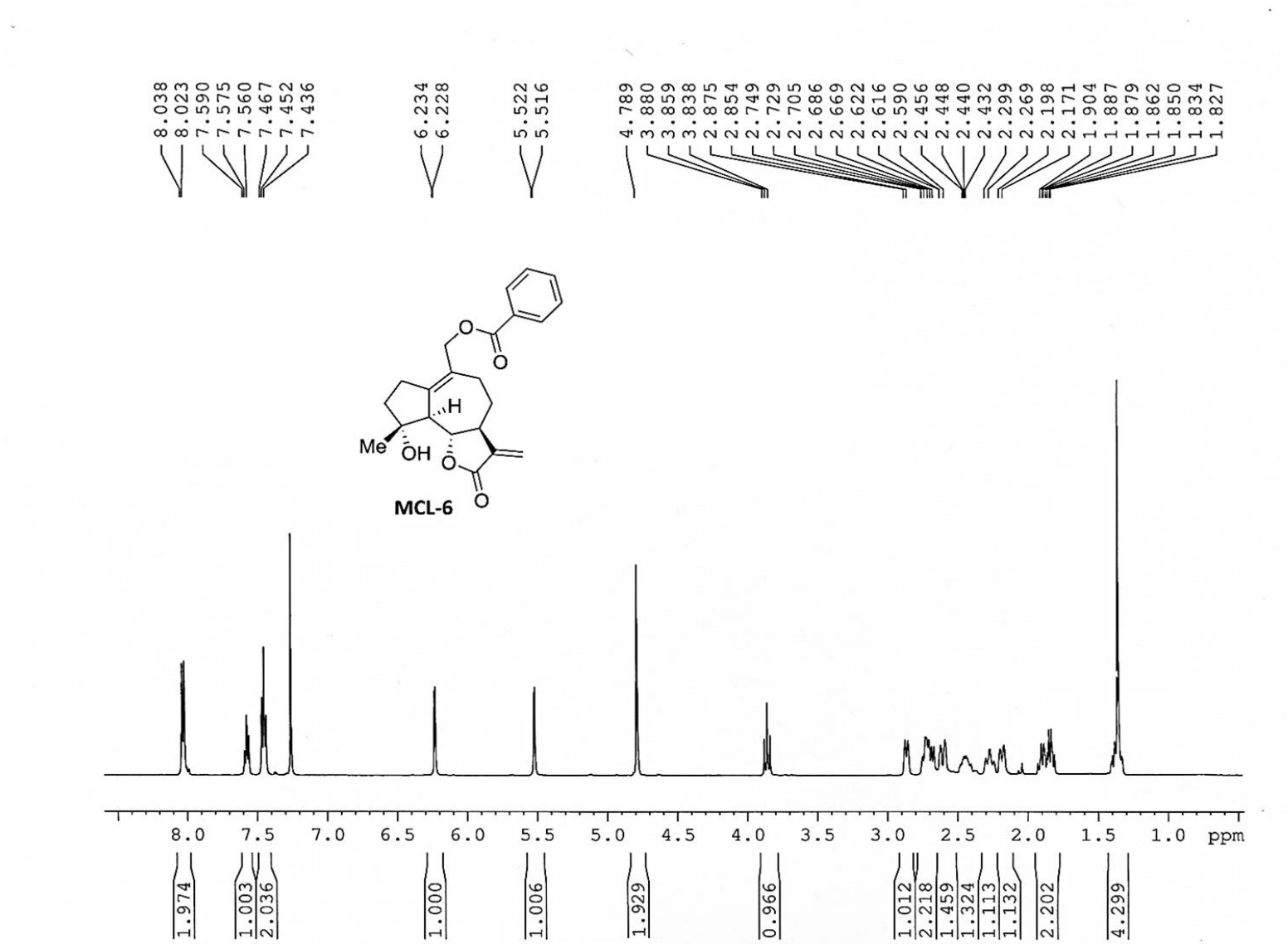
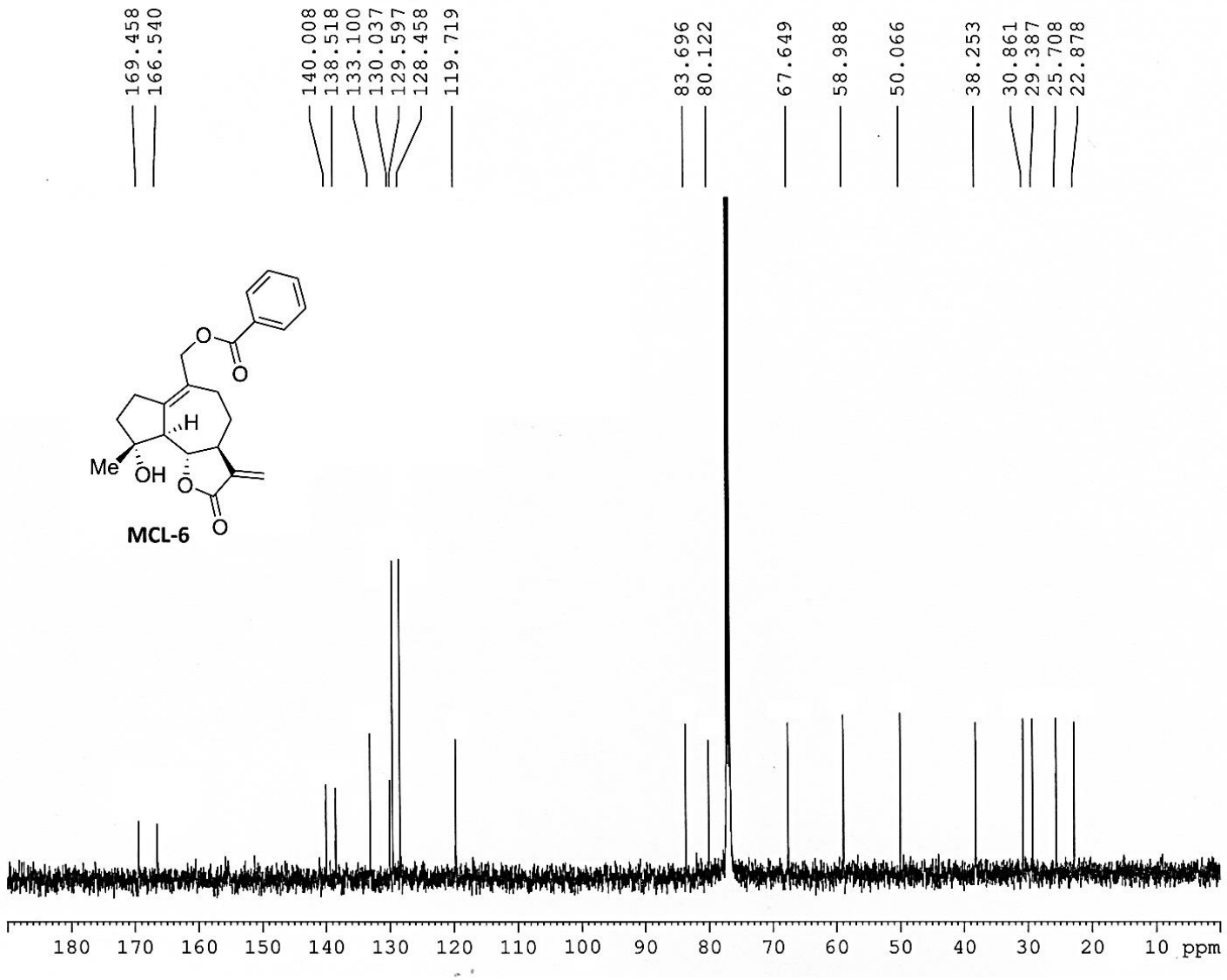


Figure S7: TBP-NPs accumulate in bone of the bcCML model. bcCML mice were injected at D7 with fluorescent TBP-NPs, and *in vivo* live imaging (IVIS) was performed 24 hours later after mice were sacrificed and tissues were collected. Data represents n = 5.

Figure S8: NMR spectra of MCL analogs synthesized.

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133.100
130.037
129.597
128.458
119.719
83.696
80.122
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58.988
50.066
38.253
30.861
29.387
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Me
OH
H
O
O
O
O
MCL-6
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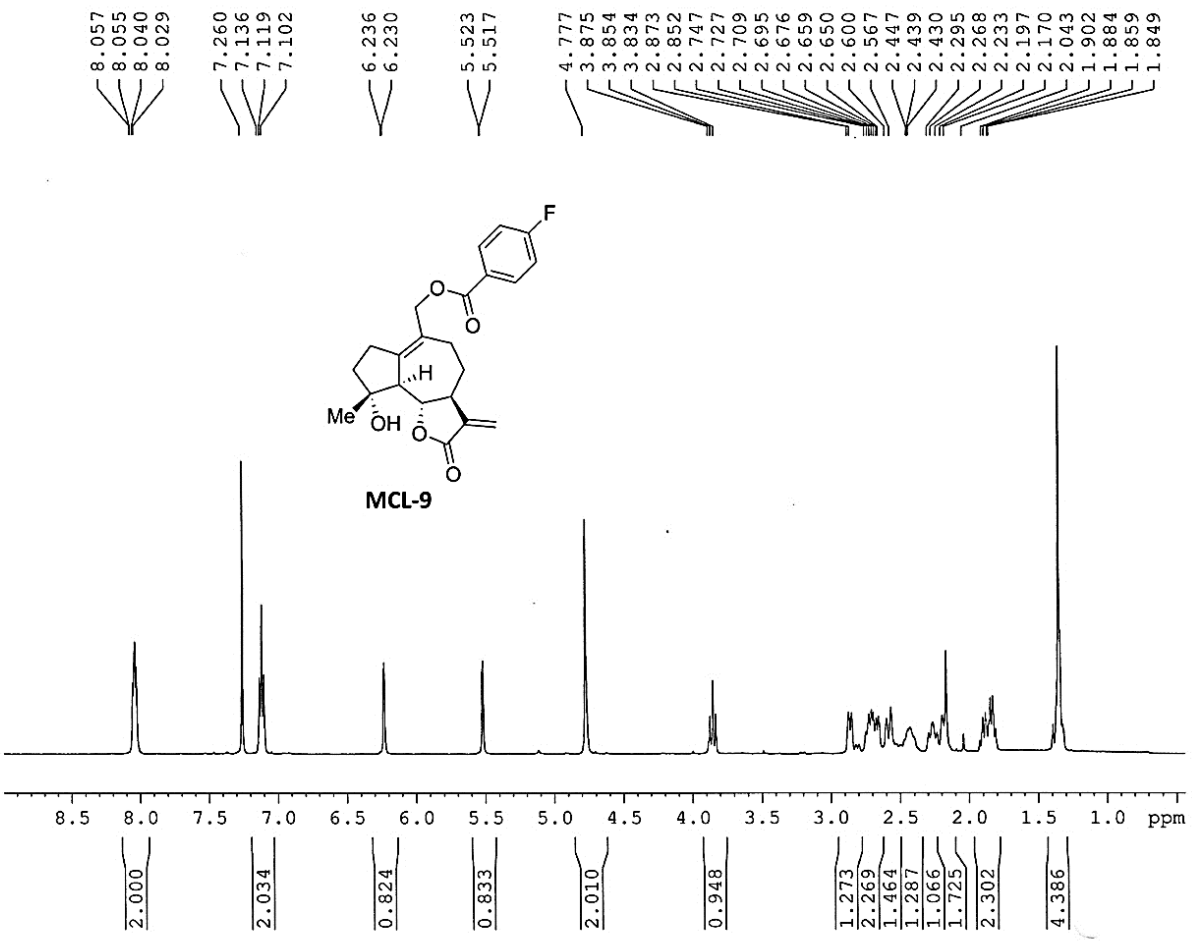
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6.230
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3.875
3.854
3.834
3.873
2.852
2.747
2.727
2.709
2.695
2.676
2.659
2.650
2.600
2.567
2.447
2.439
2.430
2.295
2.268
2.233
2.197
2.170
2.043
1.902
1.884
1.859
1.849
F
O
O
H
Me
OH
O
O
MCL-9
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6.5
6.0
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4.0
3.5
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1.0
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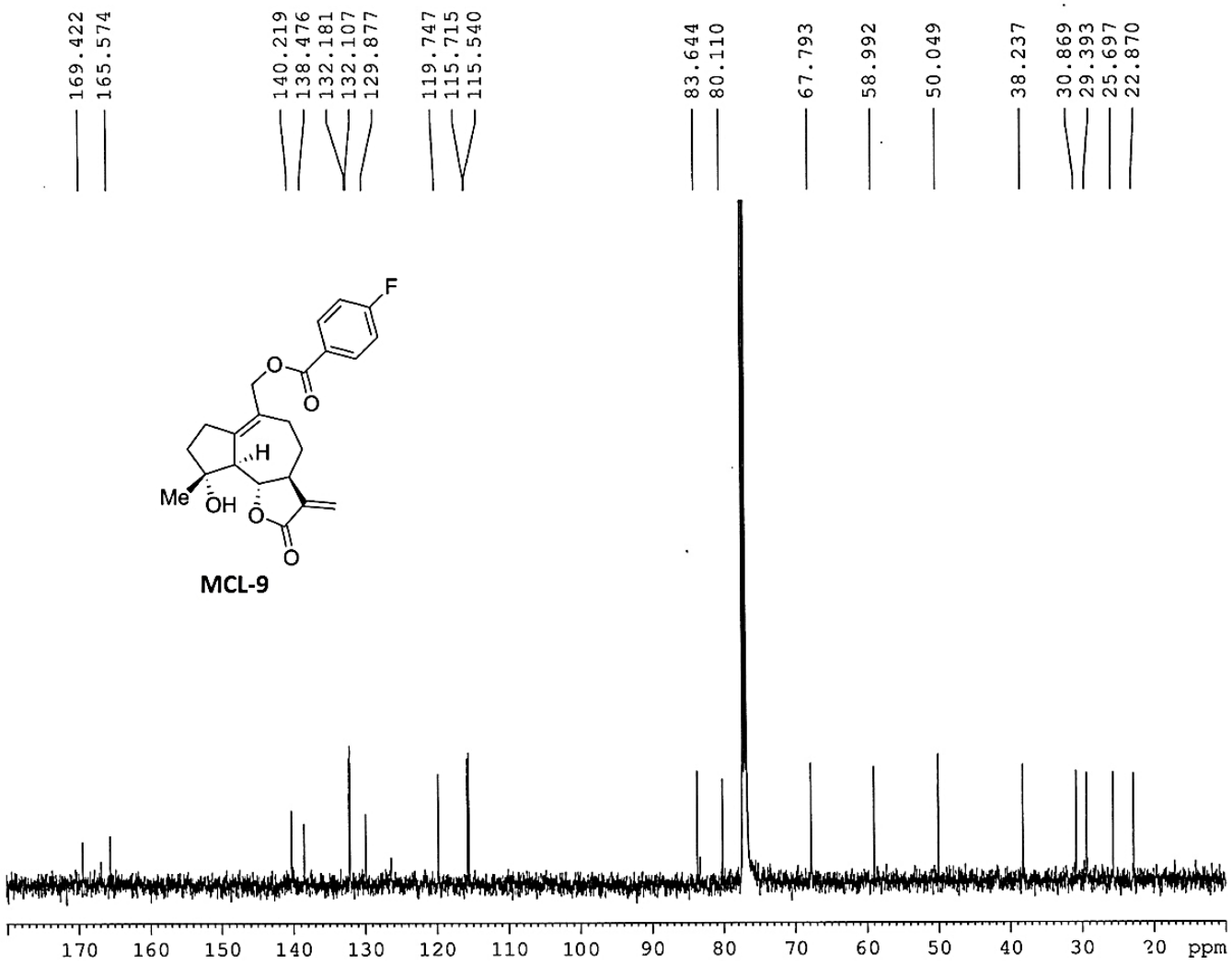
1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29.393
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22.870
F
O
O
H
Me
OH
O
O
MCL-9
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150
140
130
120
110
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70
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30
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ppm

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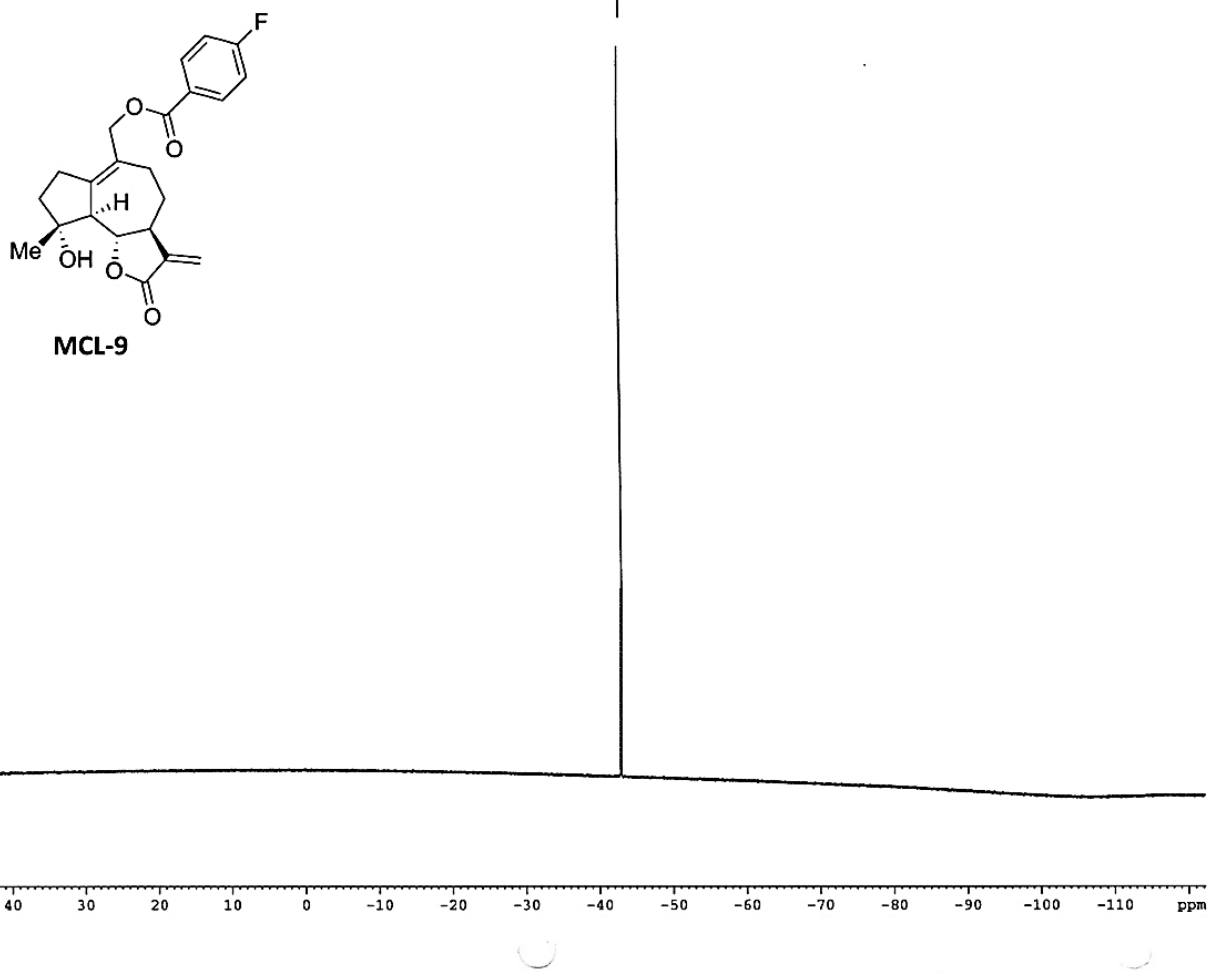
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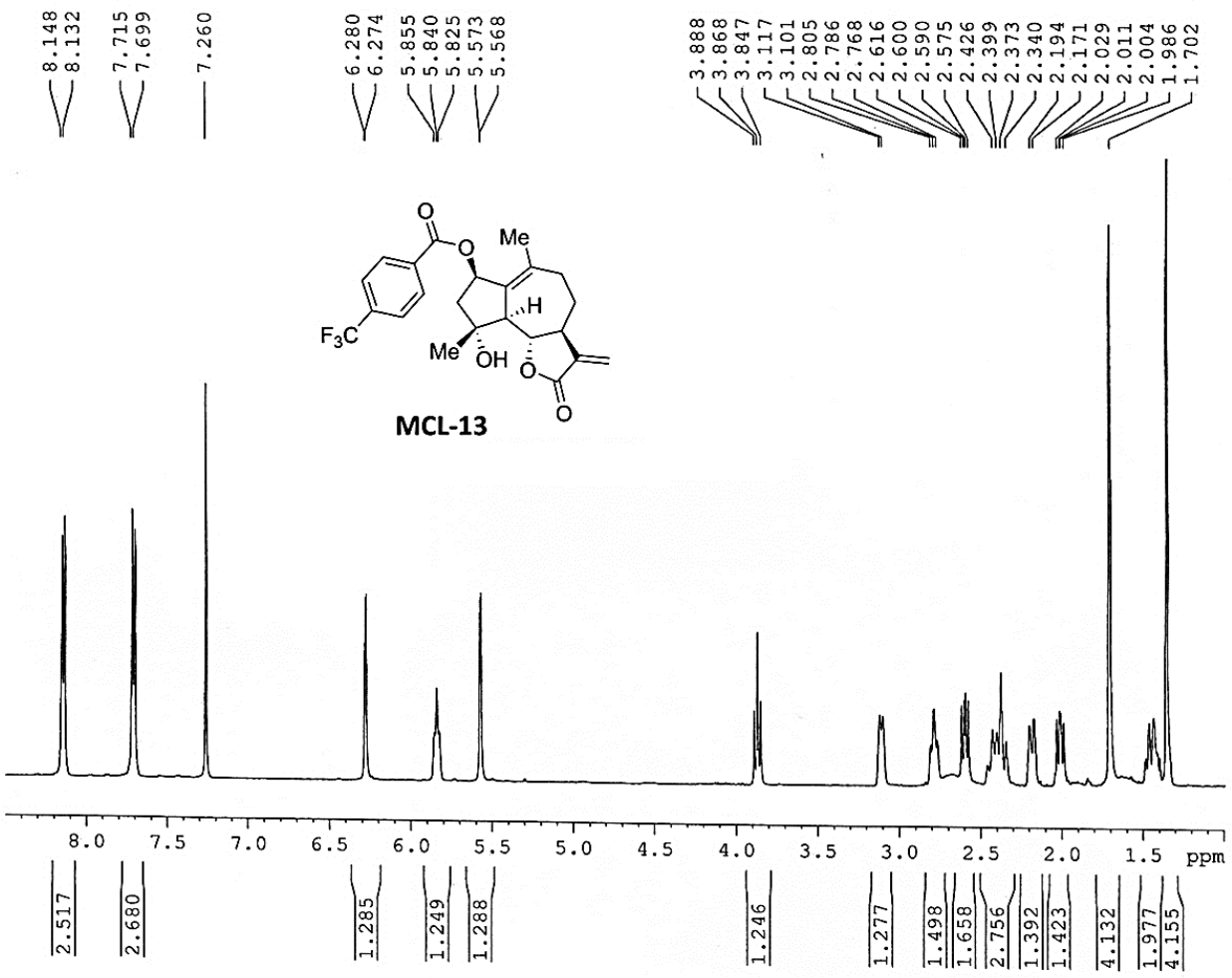
ppm

3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3.847
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F3C
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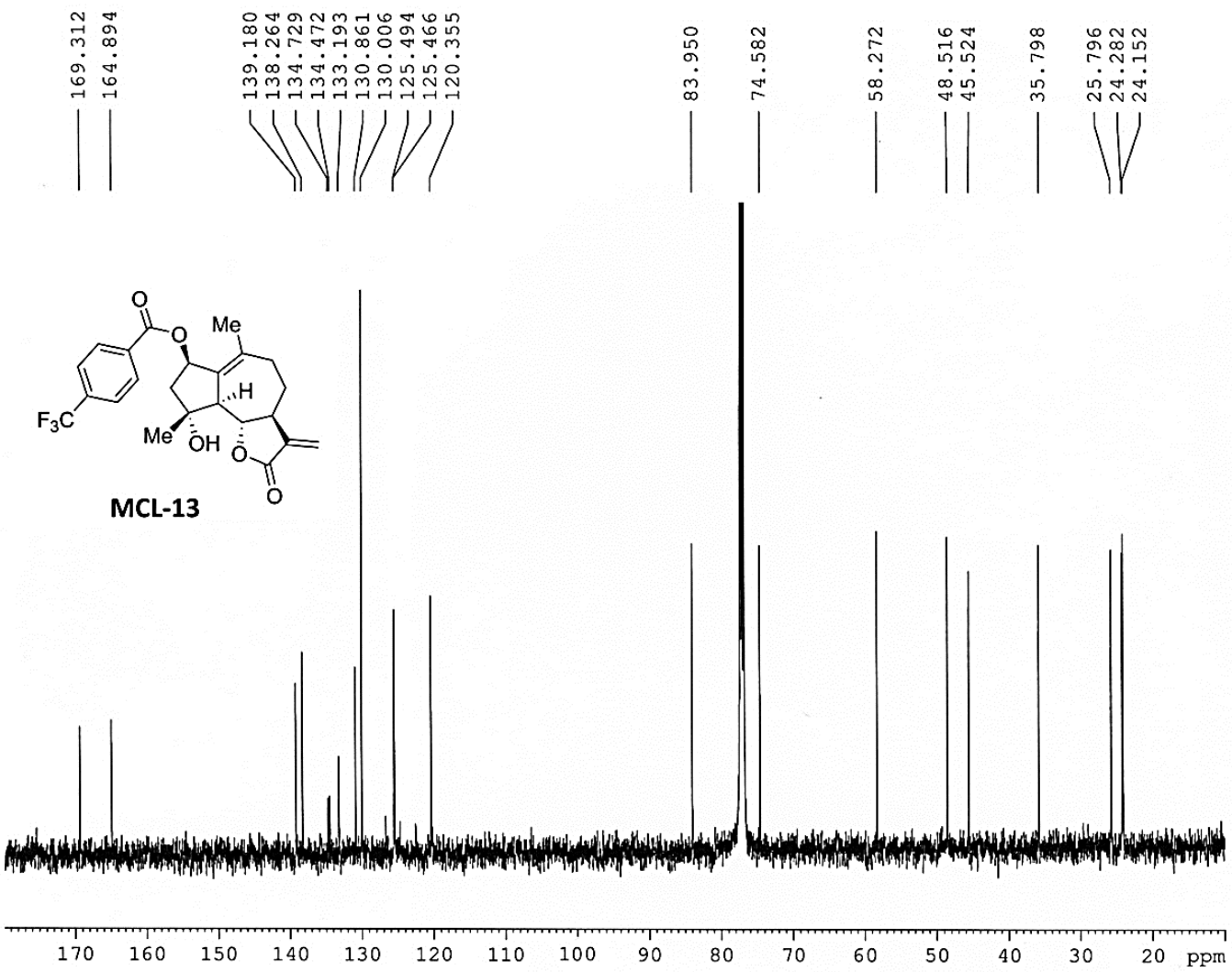
4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48.516
45.524
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24.282
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MCL-13
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50
40
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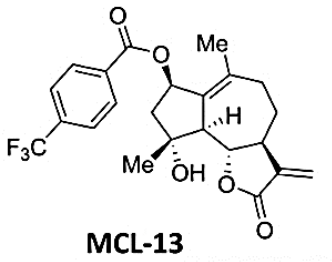
20
ppm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MCL-13

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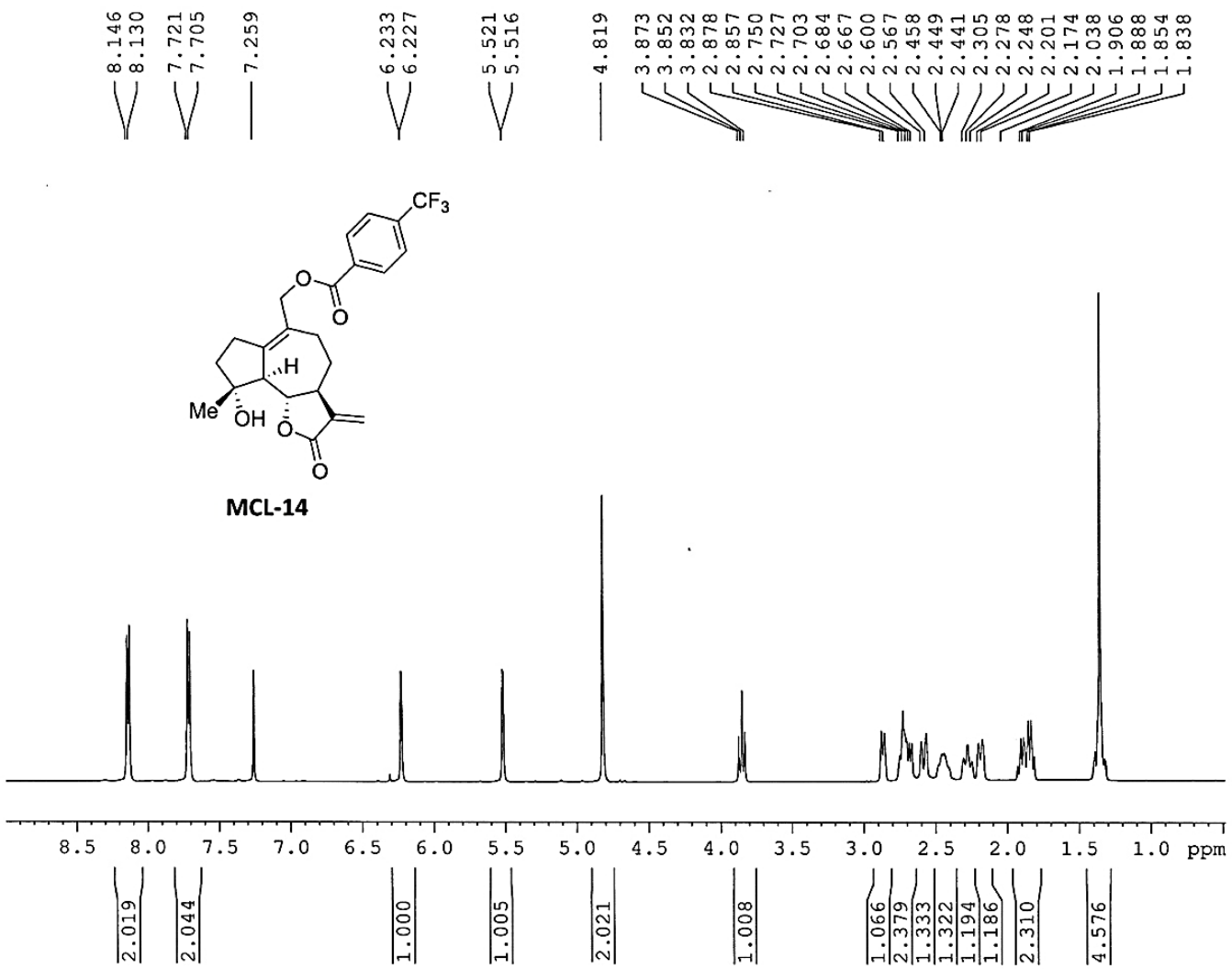
ppm

6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2.878
2.857
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2.684
2.667
2.600
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2.441
2.305
2.278
2.248
2.201
2.174
2.038
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1.888
1.854
1.838
CF3
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O
H
Me
OH
O
O
MCL-14
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1.5
1.0
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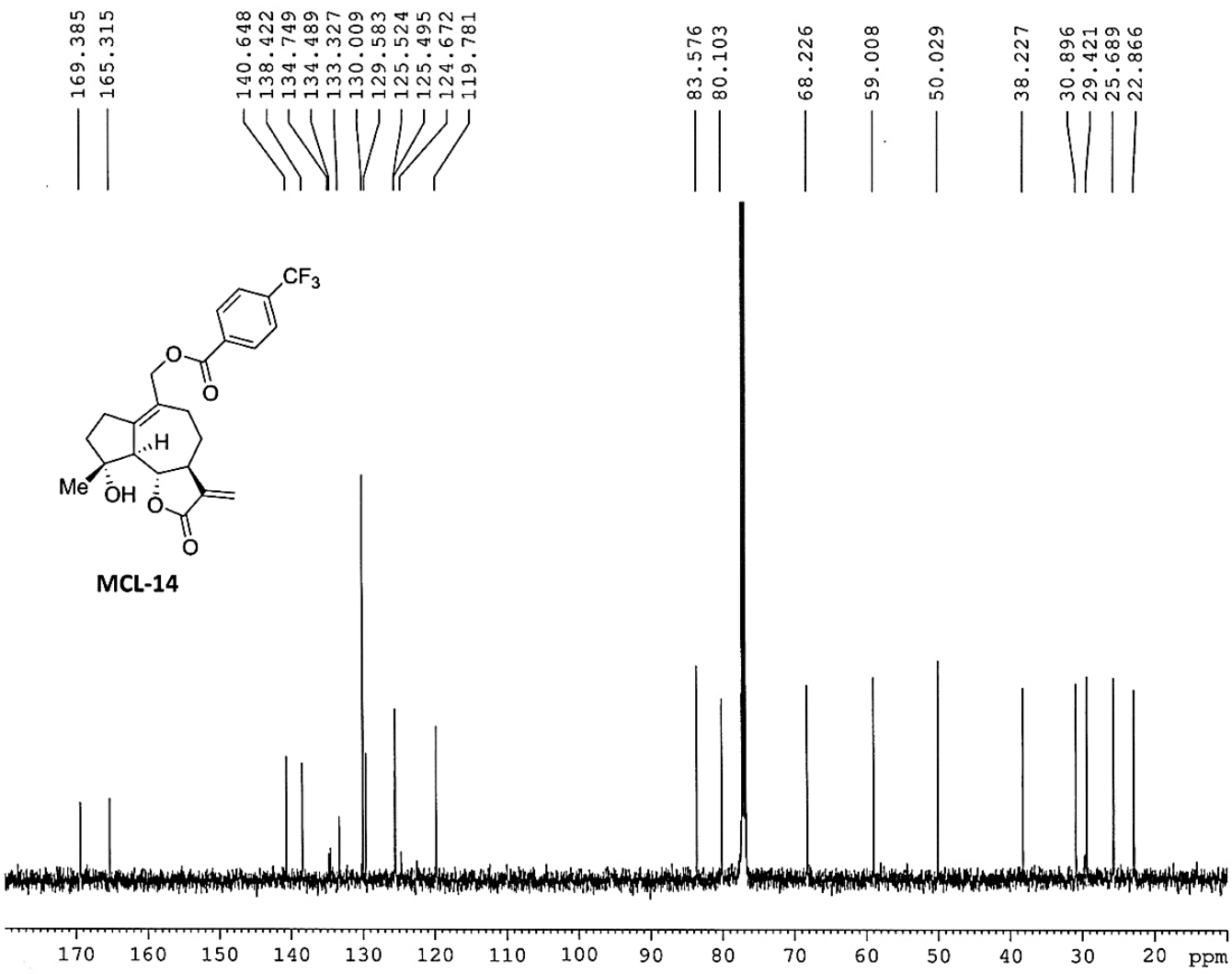
7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80.103
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38.227
30.896
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25.689
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H
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OH
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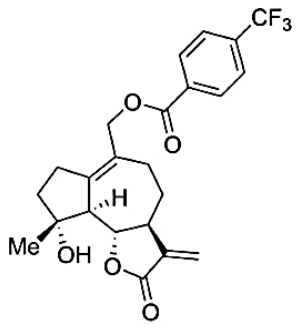
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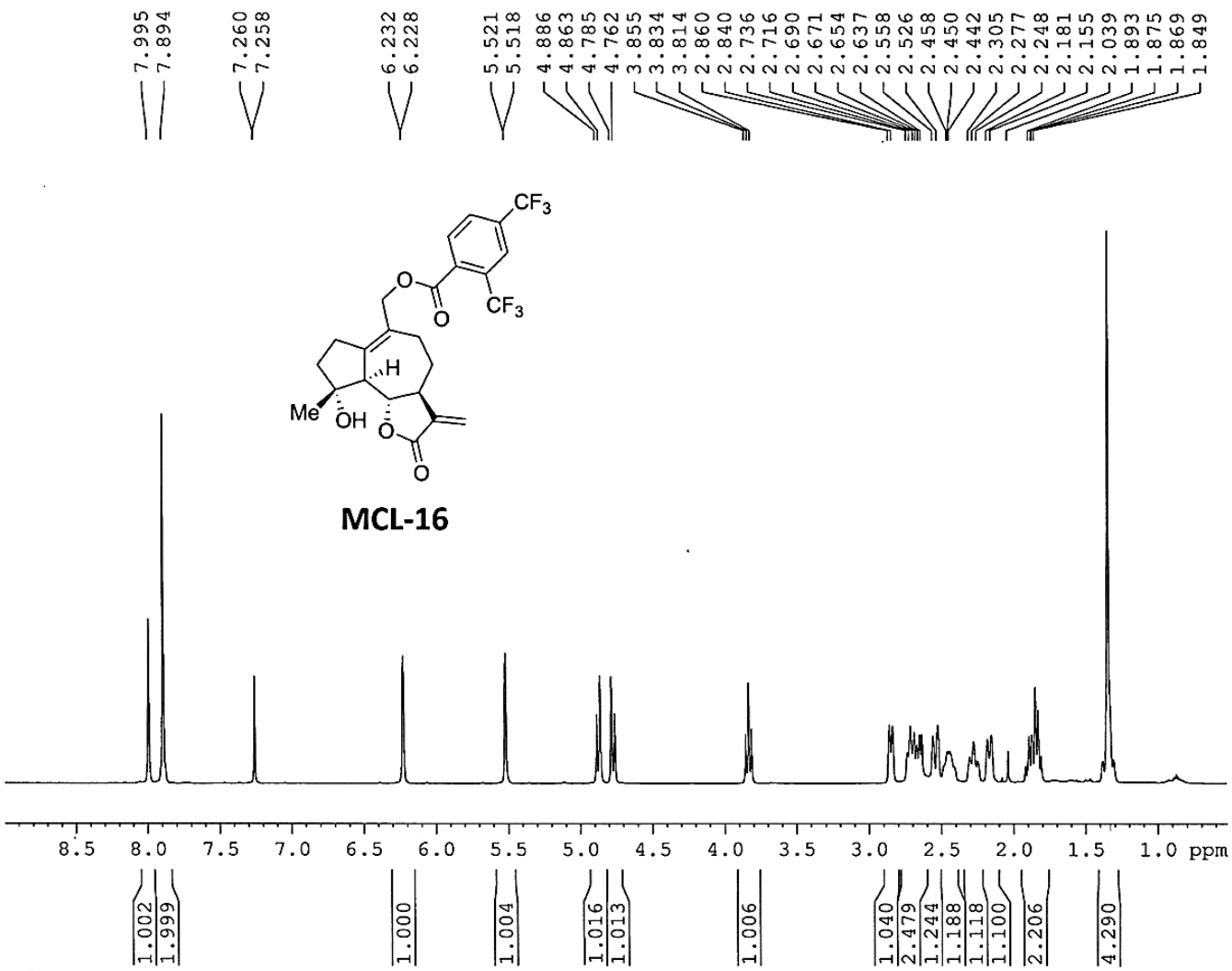
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2.736
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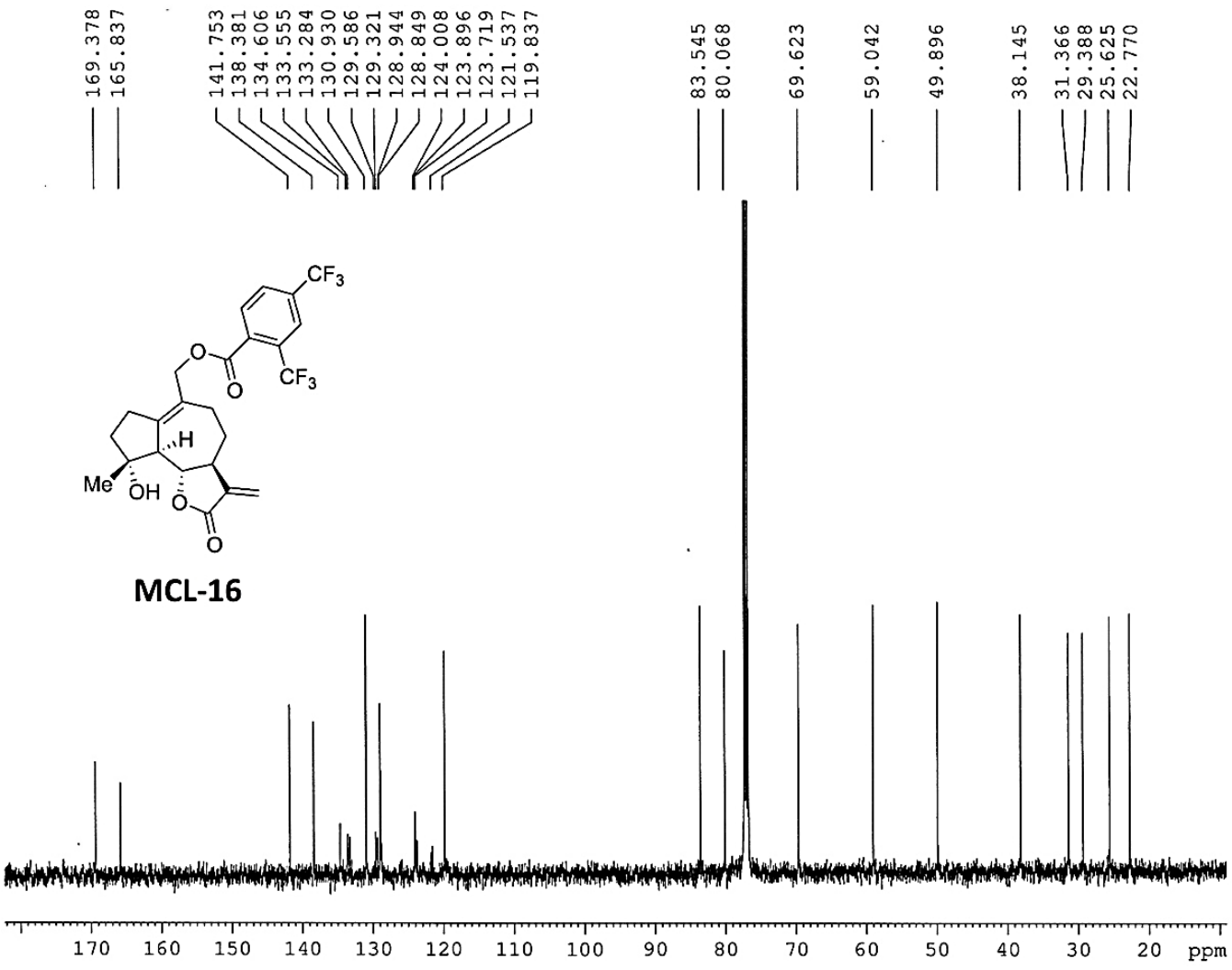
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123.896
123.719
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38.145
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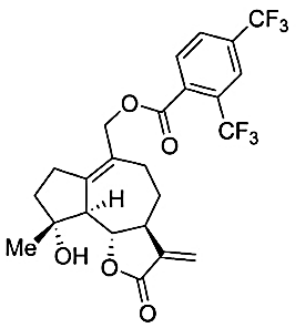
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MCL-16

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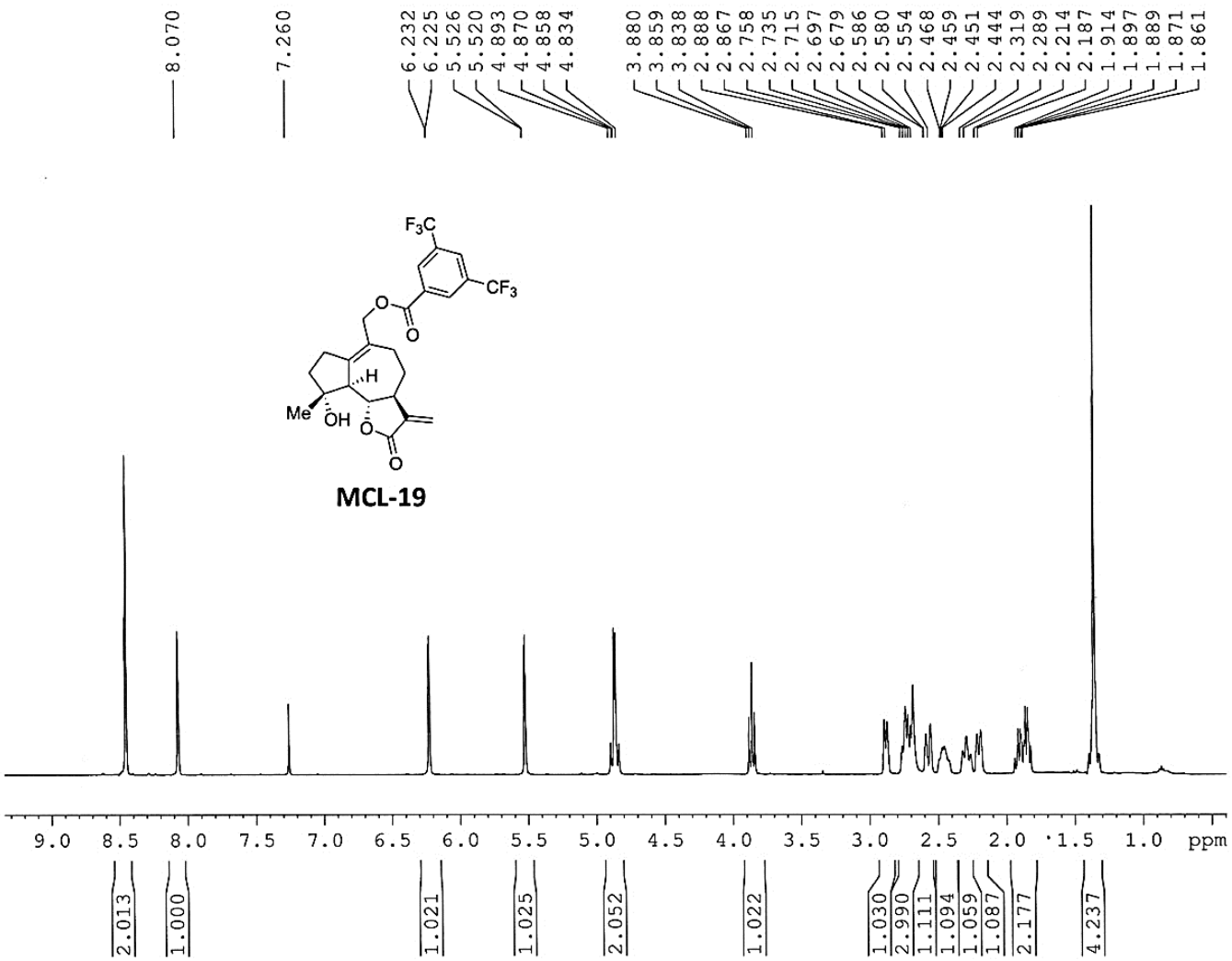
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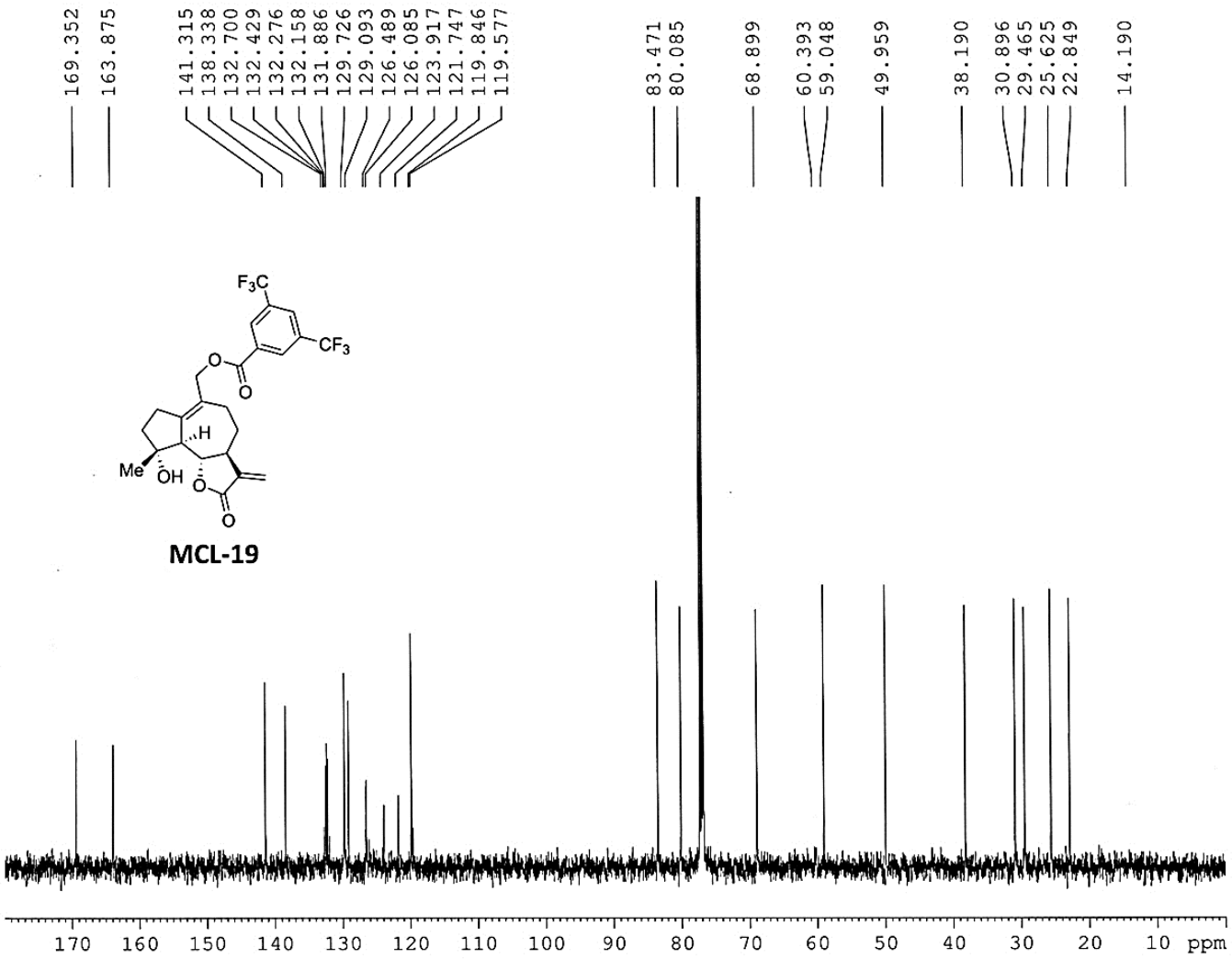
13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22.849
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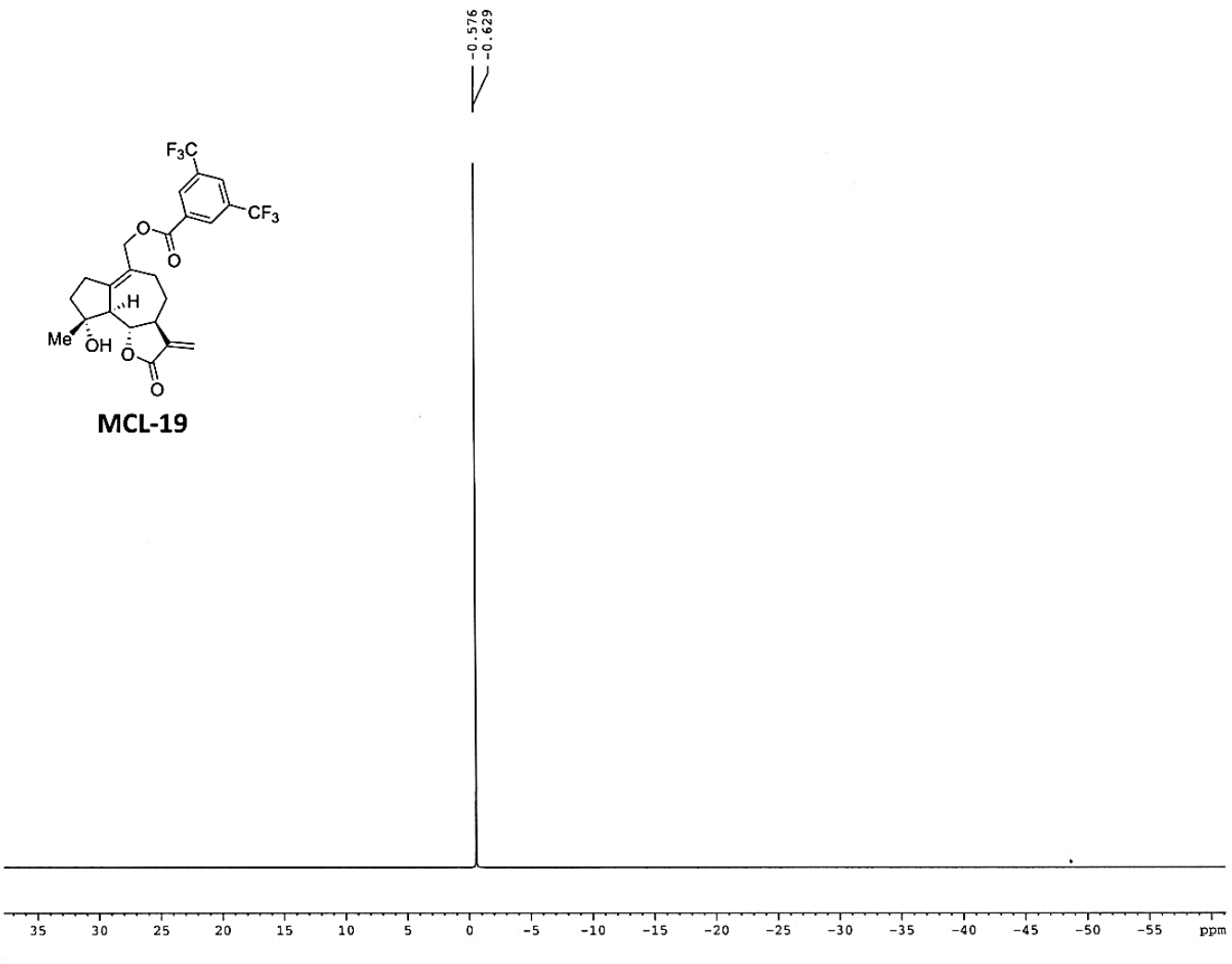
14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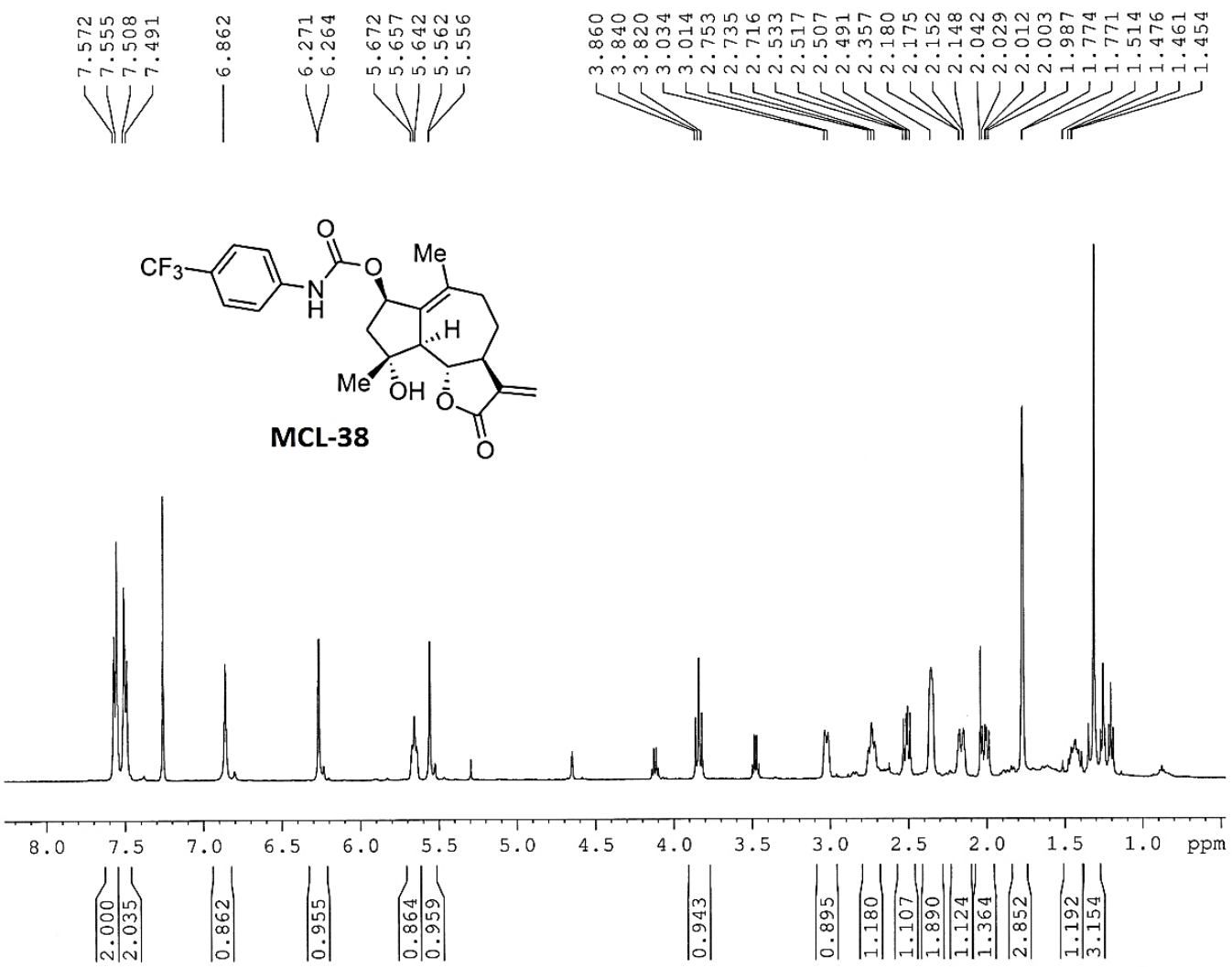
ppm

15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3.820
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2.012
2.003
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1.774
1.771
1.514
1.476
1.461
1.454
CF3
N
H
O
O
Me
H
Me
OH
O
O
MCL-38
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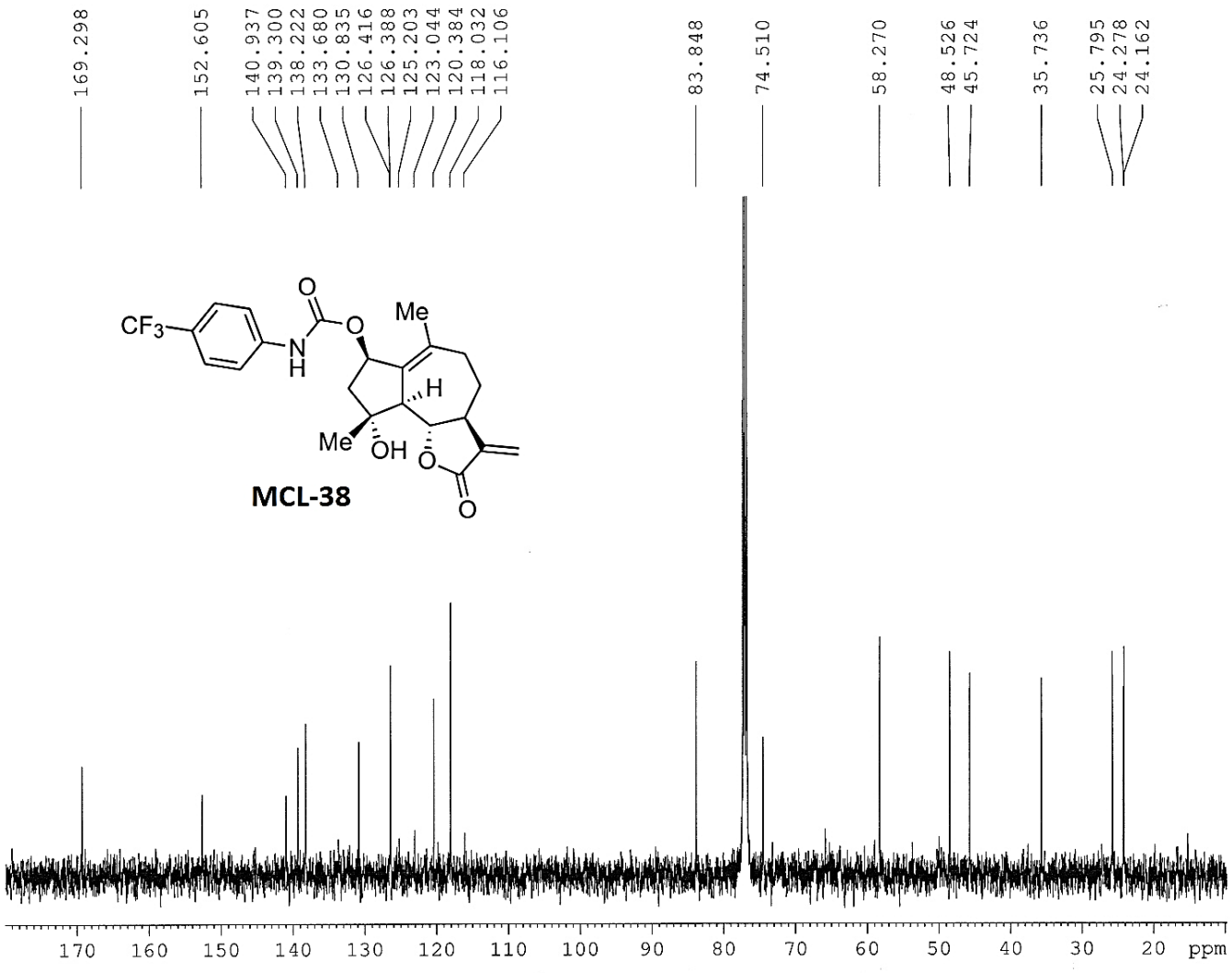
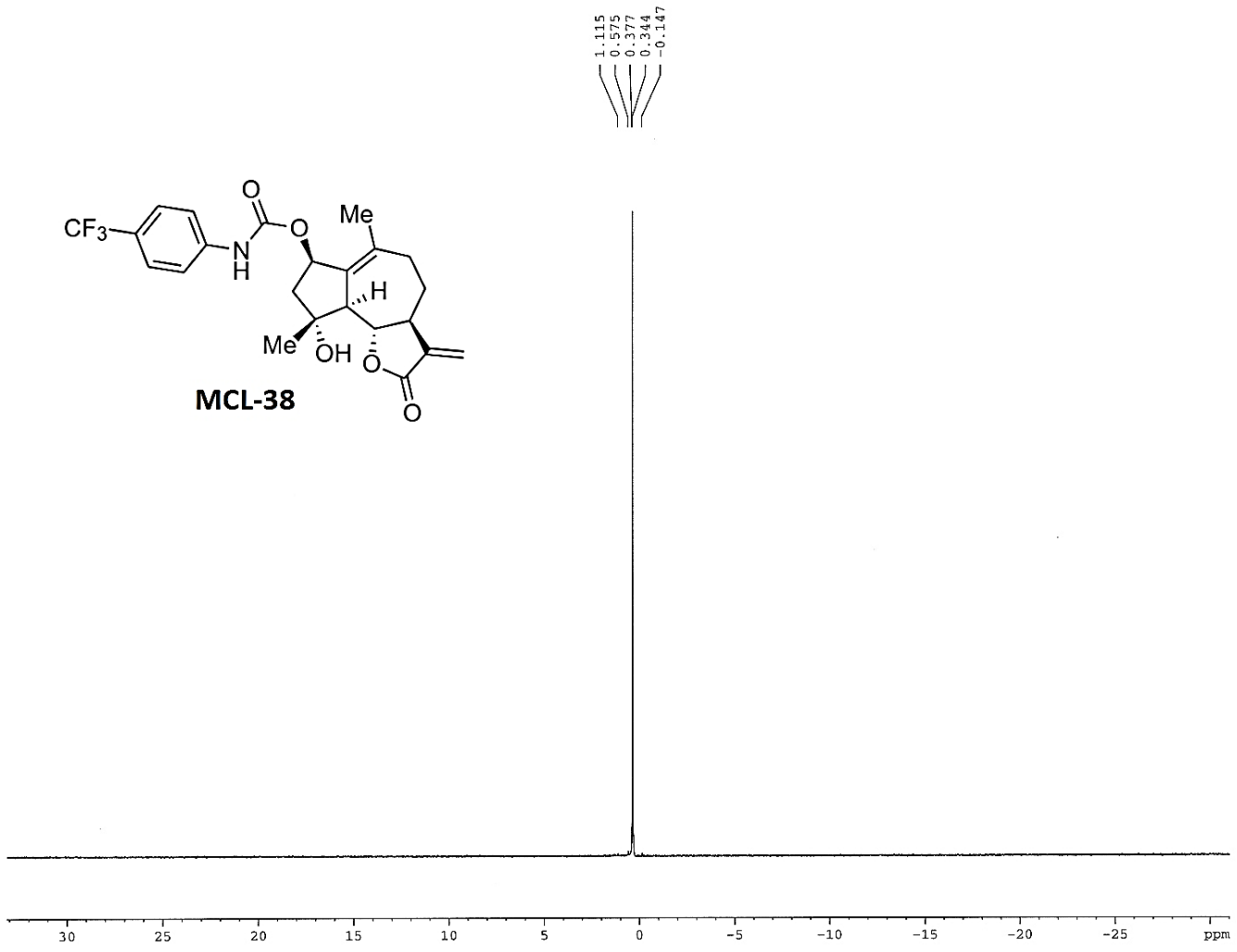
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
2.000
2.035
0.862
0.955
0.864
0.959
0.943
0.895
1.180
1.107
1.890
1.124
1.364
2.852
1.192
3.154

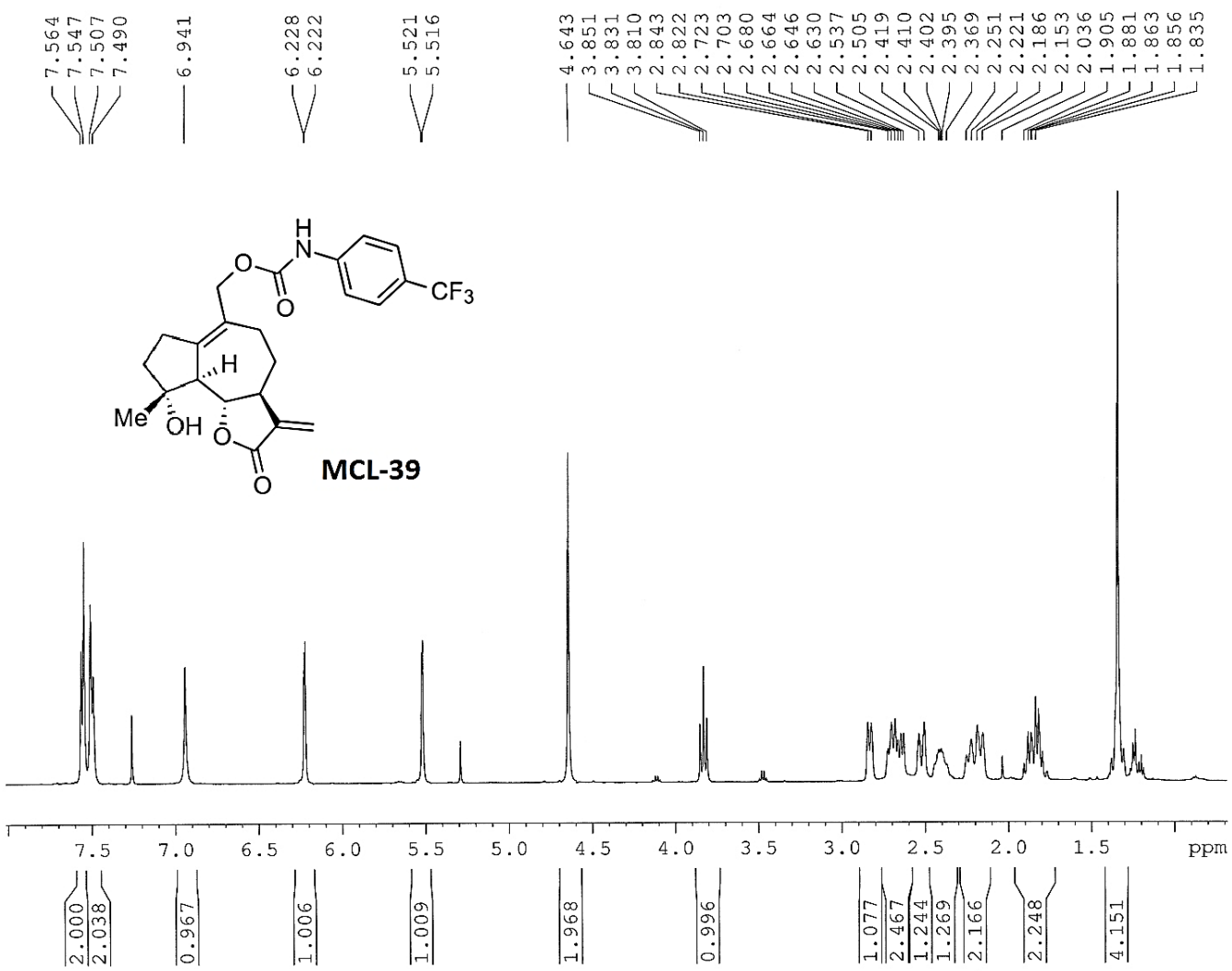
16


169.298
152.605
140.937
139.300
138.222
133.680
130.835
126.416
126.388
125.203
123.044
120.384
118.032
116.106
83.848
74.510
58.270
48.526
45.724
35.736
25.795
24.278
24.162
CF3
N
H
O
O
Me
H
Me
OH
O
O
MCL-38
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

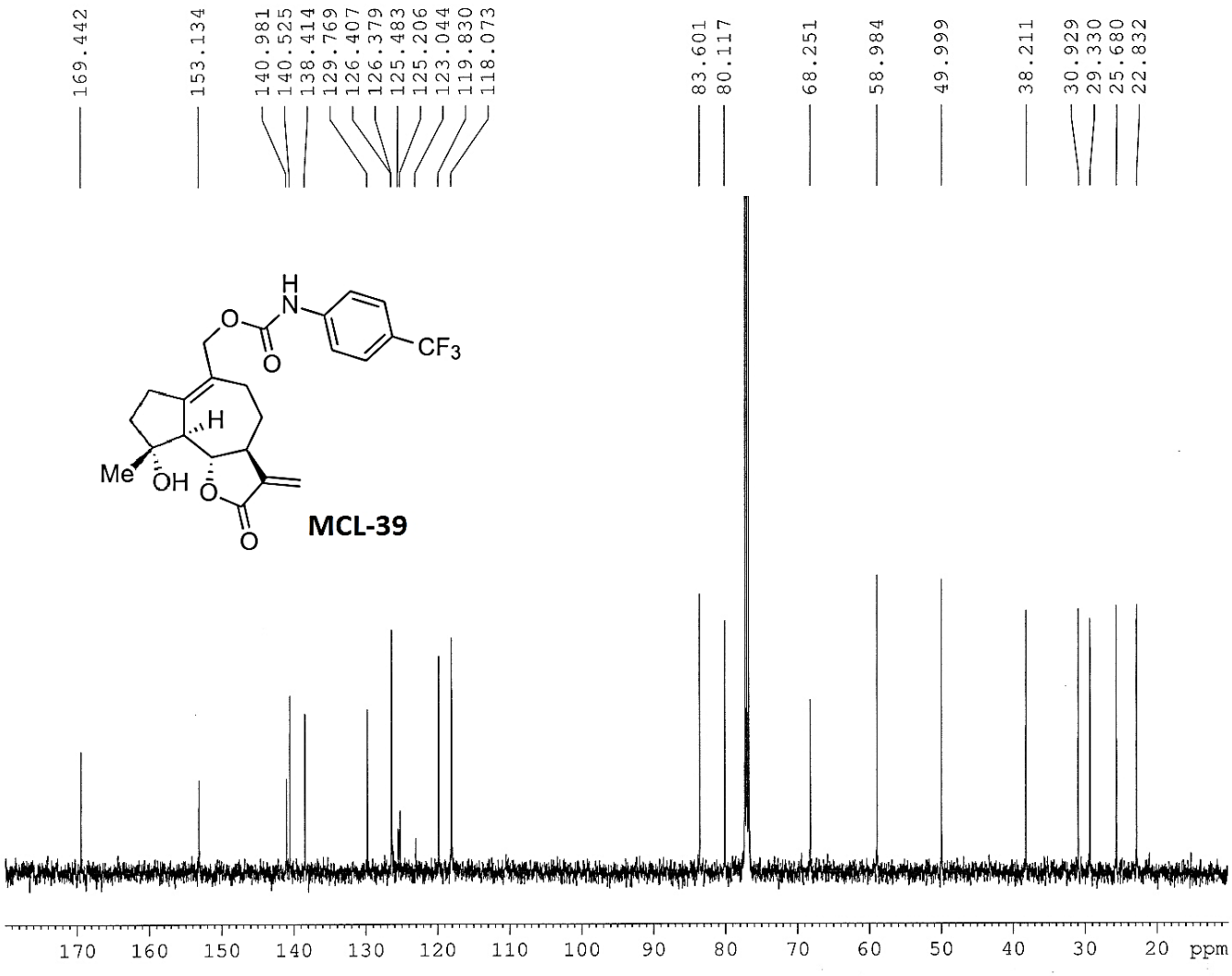
17


1.115
0.575
0.377
0.344
-0.147
CF3
N
H
O
O
Me
H
Me
OH
O
O
MCL-38
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
ppm

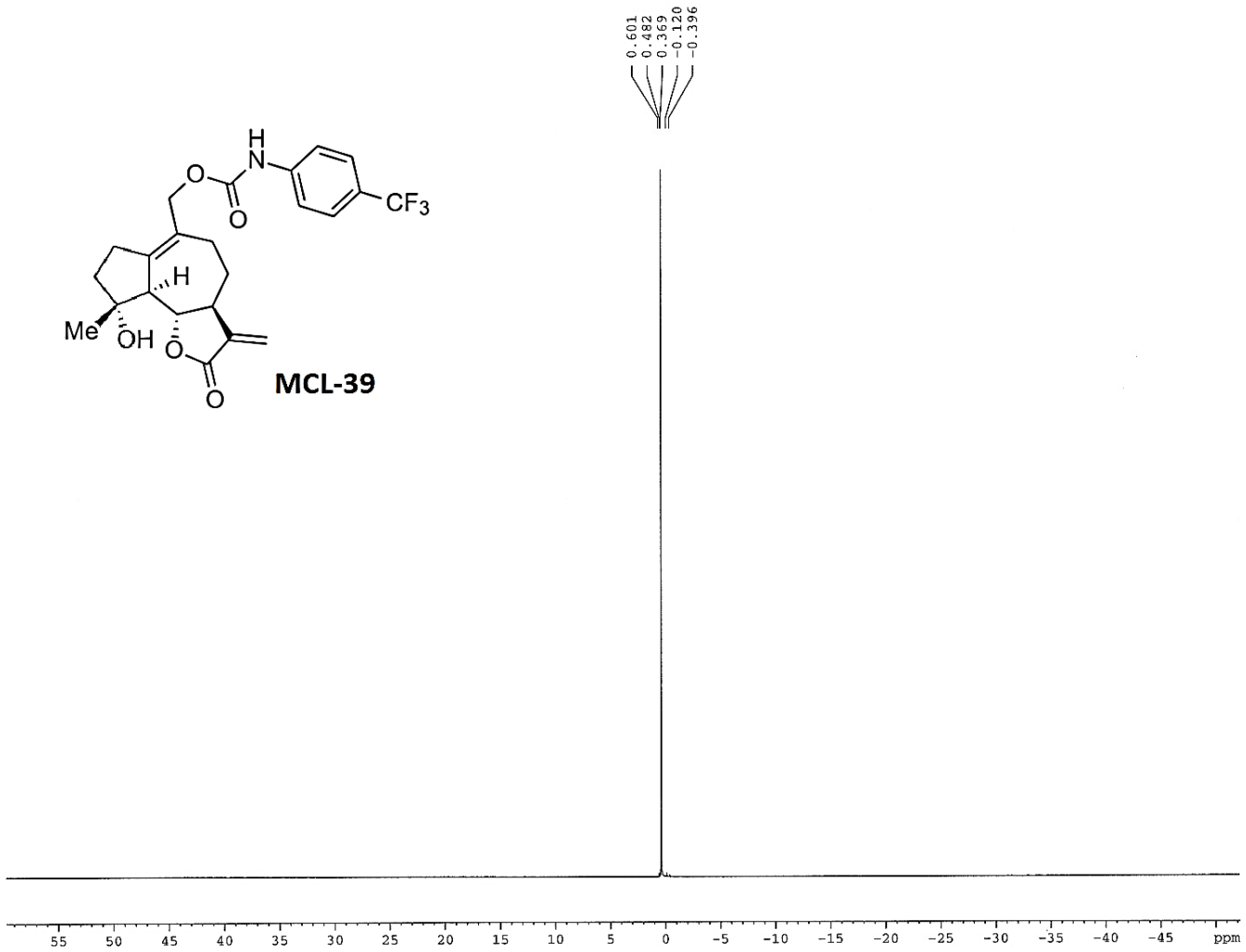
18


7.564
7.547
7.507
7.490
6.941
6.228
6.222
5.521
5.516
4.643
3.851
3.831
3.810
2.843
2.822
2.723
2.703
2.680
2.664
2.646
2.630
2.537
2.505
2.419
2.410
2.402
2.395
2.369
2.251
2.221
2.186
2.153
2.036
1.905
1.881
1.863
1.856
1.835
H
N
O
O
CF3
H
Me
OH
O
O
MCL-39
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
ppm
2.000
2.038
0.967
1.006
1.009
1.968
0.996
1.077
2.467
1.244
1.269
2.166
2.248
4.151

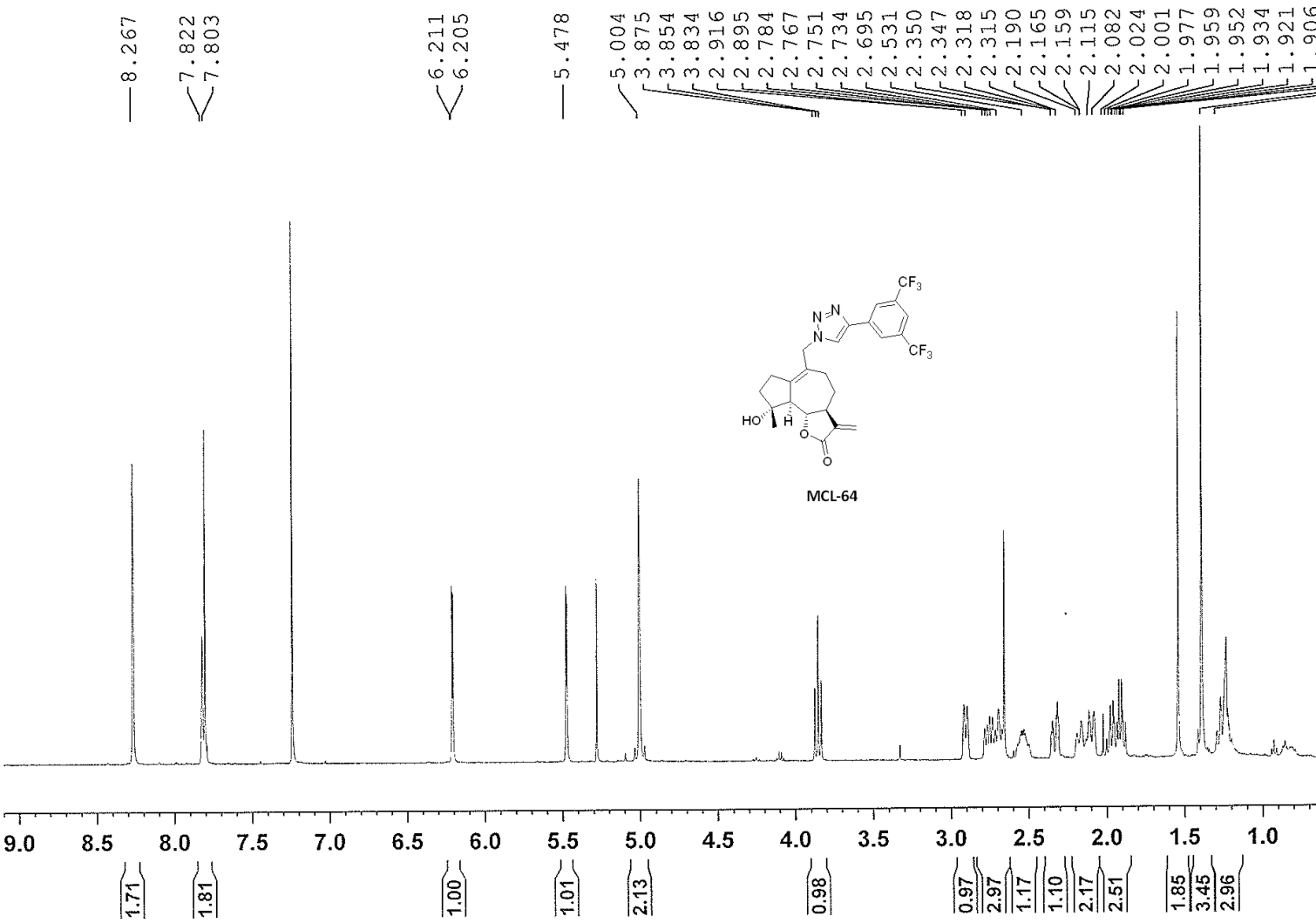
19


169.442
153.134
140.981
140.525
138.414
129.769
126.407
126.379
125.483
125.206
123.044
119.830
118.073
83.601
80.117
68.251
58.984
49.999
38.211
30.929
29.330
25.680
22.832
MCL-39
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

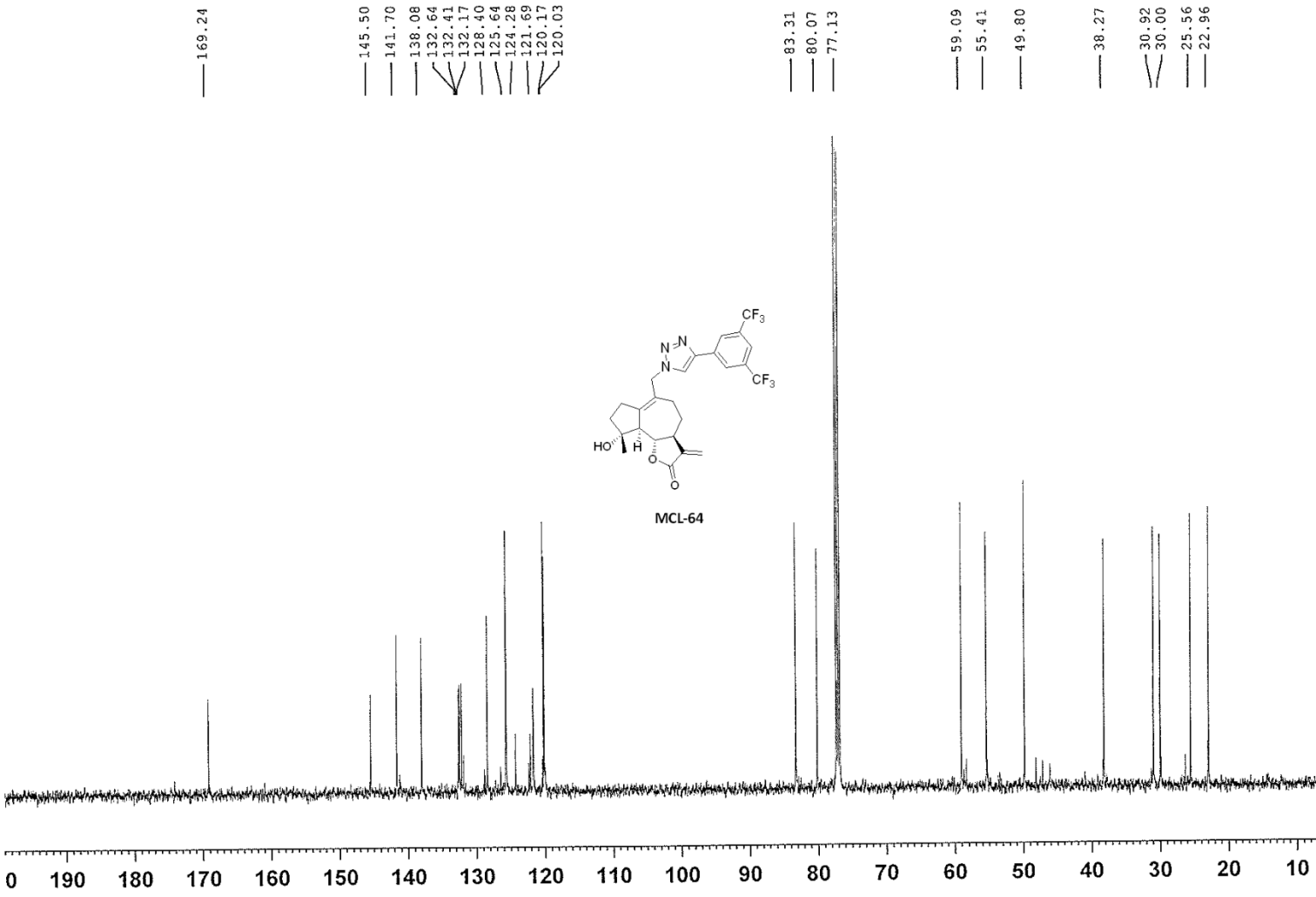
20


0.601
0.482
0.369
-0.120
-0.396
H
N
O
O
CF3
H
Me
OH
O
O
MCL-39
55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45 ppm

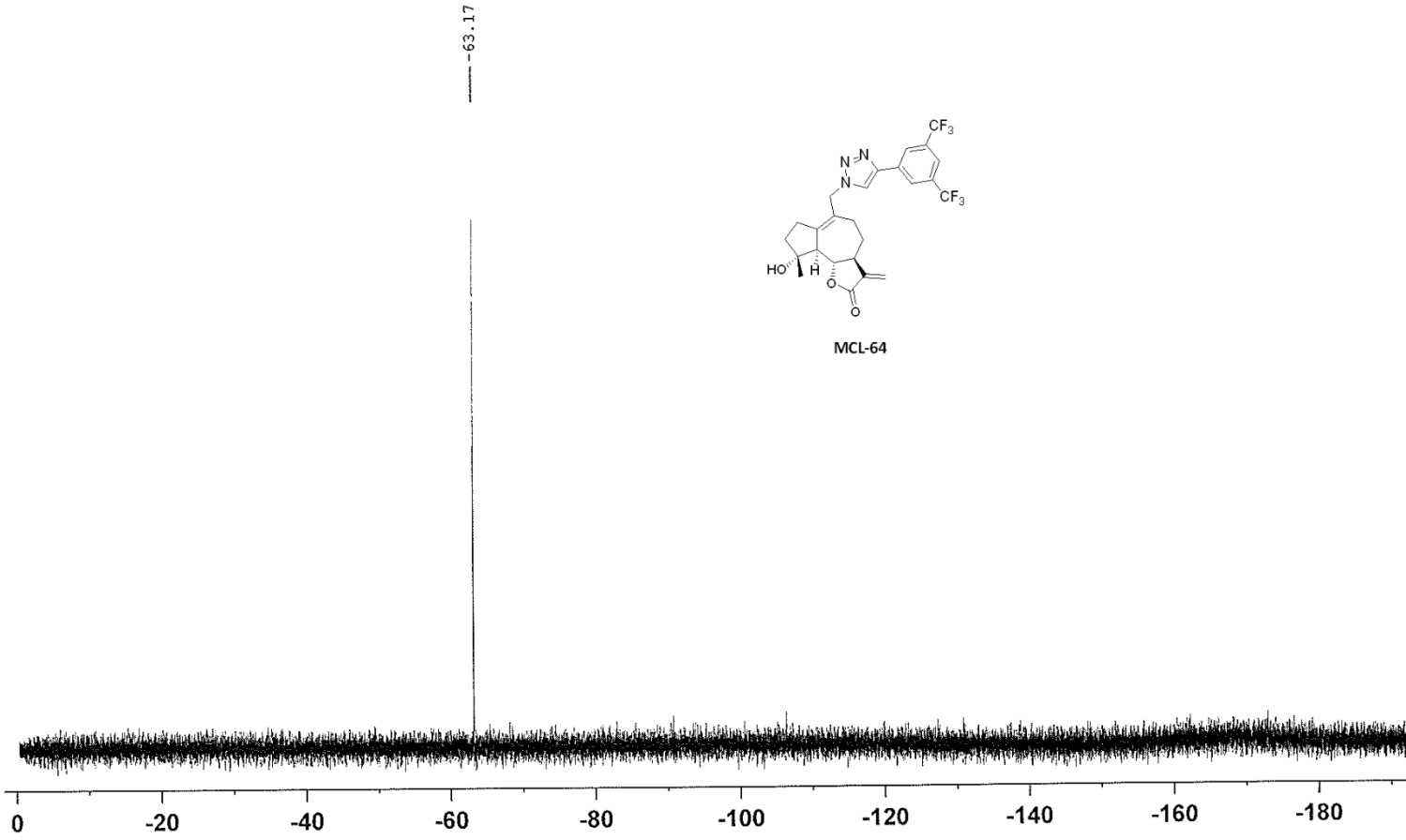
21


8.267
7.822
7.803
6.211
6.205
5.478
5.004
3.875
3.854
3.834
2.916
2.895
2.784
2.767
2.751
2.734
2.695
2.531
2.350
2.347
2.318
2.315
2.190
2.165
2.159
2.115
2.082
2.024
2.001
1.977
1.959
1.952
1.934
1.921
CF3
CF3
HO
H
O
O
MCL-64
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
1.71
1.81
1.00
1.01
2.13
0.98
0.97
2.97
1.17
1.10
2.17
2.51
1.85
3.45
2.96

22


169.24
145.50
141.70
138.08
132.64
132.41
132.17
128.40
125.64
124.28
121.69
120.17
120.03
83.31
80.07
77.13
59.09
55.41
49.80
38.27
30.92
30.00
25.56
22.96
CF3
CF3
HO
H
O
O
MCL-64
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

23


-63.17
CF3
CF3
N
N
N
HO
H
O
O
MCL-64
0
-20
-40
-60
-80
-100
-120
-140
-160
-180