Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis of natural Urolithin M6, a Galloflavin mimetic as potential inhibitor of Lactate dehydrogenase A

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Electronic Supporting Information

Contents

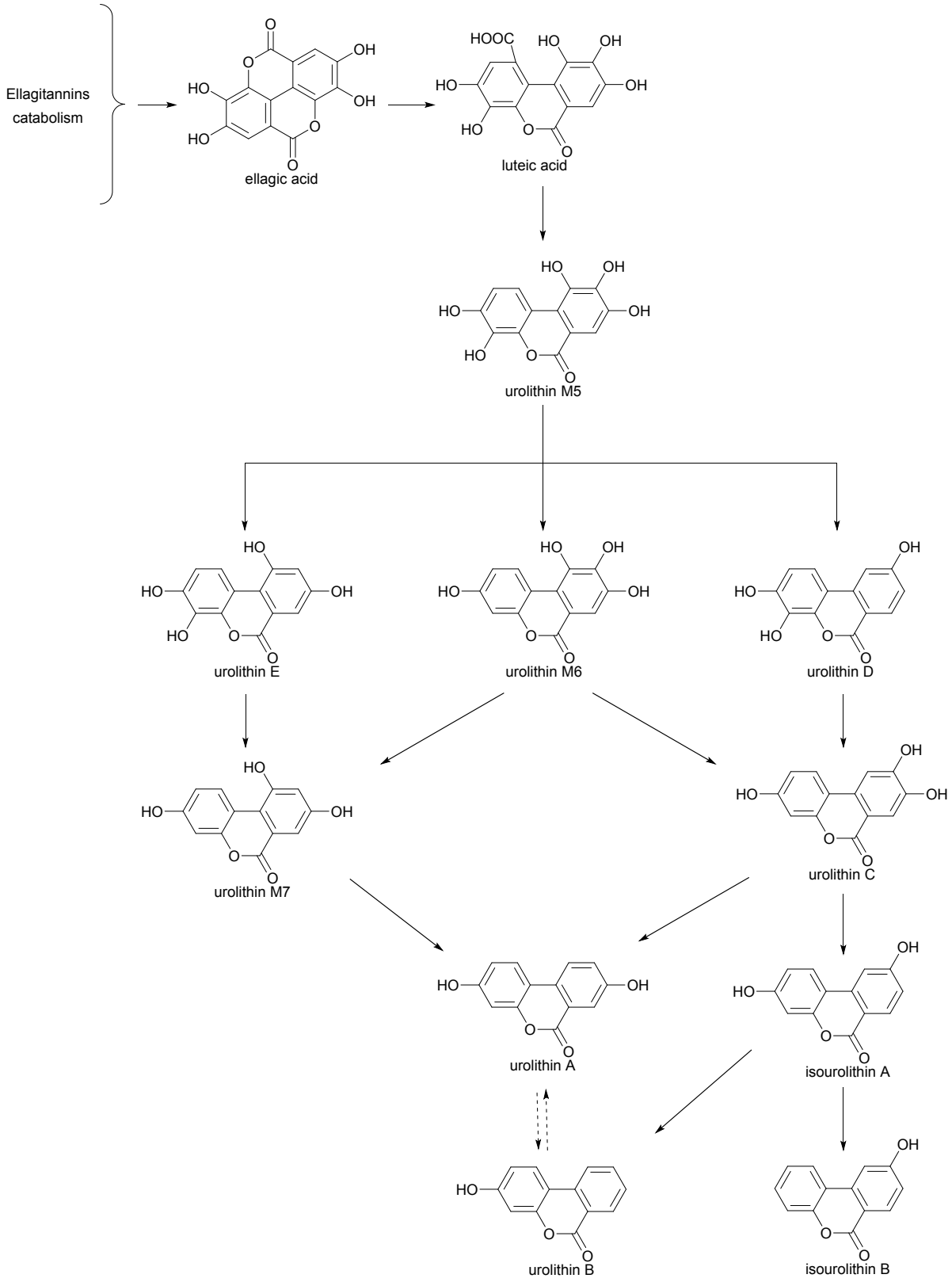


Fig.1 Gut microbiota metabolism of ellagitannins and ellagic acid (adapted from R. Garcia-Villalba, *et al., J Agric Food Chem*, 2013, **61**, 8797).

Detailed biological protocols

Enzymatic assays on purified human LDH-A

Purified LDH-A (from human liver) was obtained from Lee Biosolutions (St Louis, Missouri, USA). 20 mM stock solutions of compoundwas prepared in DMSO. It was added in scalar amounts (0 – 200 μM final concentrations) to a reaction mix containing 100 mM phosphate buffer pH 7.5, 0.015 U LDH / ml, 1 mM pyruvate and 150 μM NADH. For all the determinations (including those without the compounds), DMSO in the reaction mix was always kept to 0.6%. The enzymatic activity was measured by monitoring NADH oxidation for a period of 3 min. To avoid interference of the compounds with the UV reading of NADH oxidation, we adopted the procedure reported by Moran[7], which measured LDH activity by recording the decrease of NADH fluorescence. The assay was performed in 96-well white body plates, using a Fluoroskan Ascent FL reader (Labsystems). The concentration of compounds causing 50% inhibition of LDH activity (IC_{50}) was calculated from the second order polynomial regression of the experimental data, using the Prism 5 GraphPad software.

Cell culture conditions

Raji cells were grown as a suspension culture in RPMI 1640 containing 10% FBS, 100 U/ml penicillin/streptomycin, 4 mM glutamine and were maintained at a concentration of 1-2 x 10^5 viable cells/ml. All media and supplements were from Sigma. In all experiments, inhibitors were added to the culture media in the presence of 0.6% DMSO. The same amount of DMSO was always added to the control, untreated cultures.

Effects of the inhibitors on lactate production

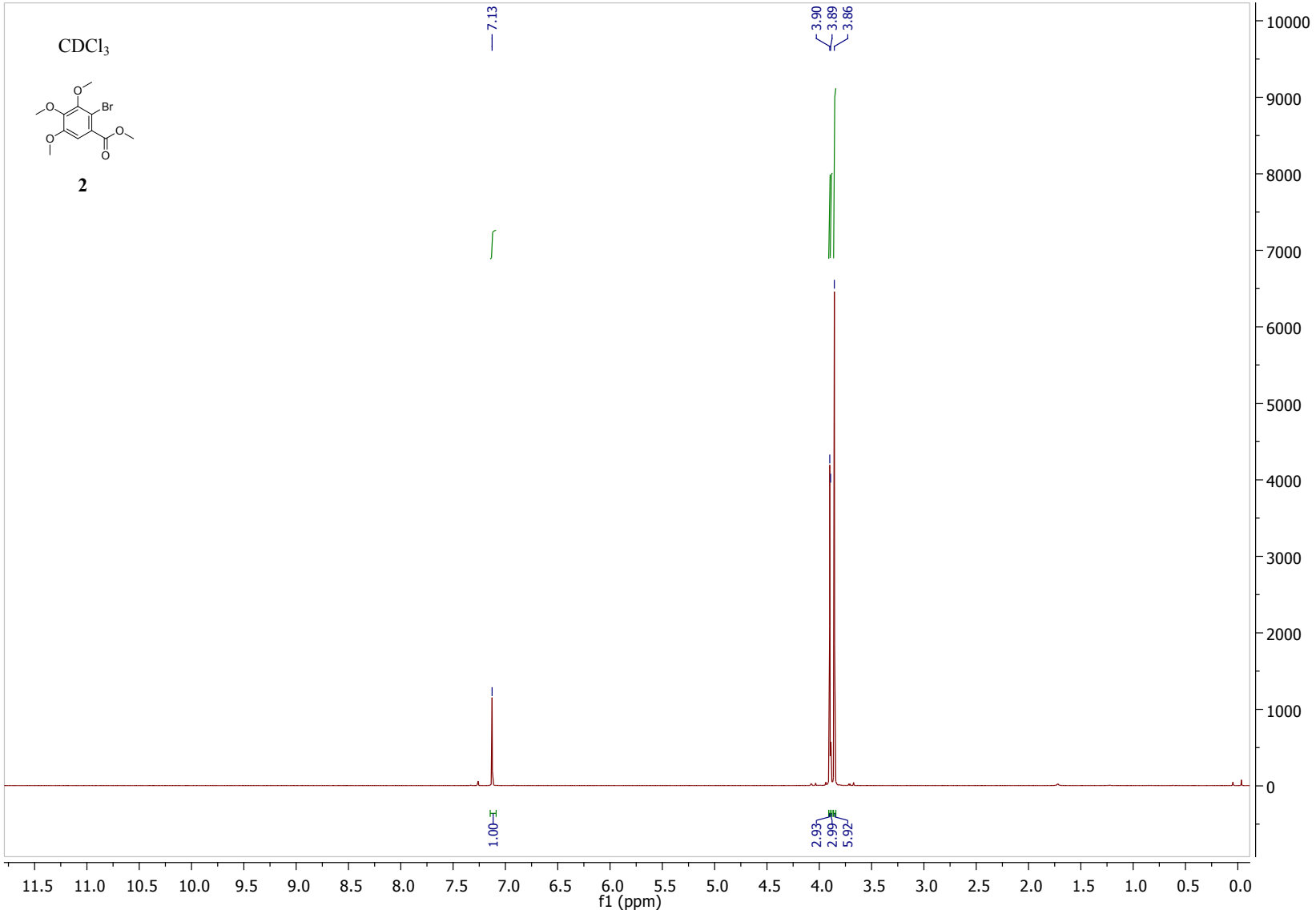
5 x 10^5 cells in 1 mL of culture medium were seeded in each well of a 6-well Nunclon plate. Scalar amounts of inhibitor (0-200 μM, tested in duplicate) were then added to the cultures. Lactate was measured in 3 untreated wells at the start of experiment (baseline value) and 3 h after incubation at 37°C. In each well, we simultaneously measured released in medium and intracellular lactate: at the end of incubation 100 μl of 100% trichloroacetic acid (TCA) solution was added; the cell lysate was collected and the well was washed with 1 mL 10% TCA. After centrifugation, lactate in the supernatant was measured according to the method of Barker and Summerson.[8] The amount of metabolite formed during the 3 h incubation with or without the inhibitor was calculated by subtracting the baseline value. The dose of compound causing 50% inhibition of lactate production (IC_{50}) was calculated from the second order polynomial regression of experimental data, using the Prism 5 GraphPad software.

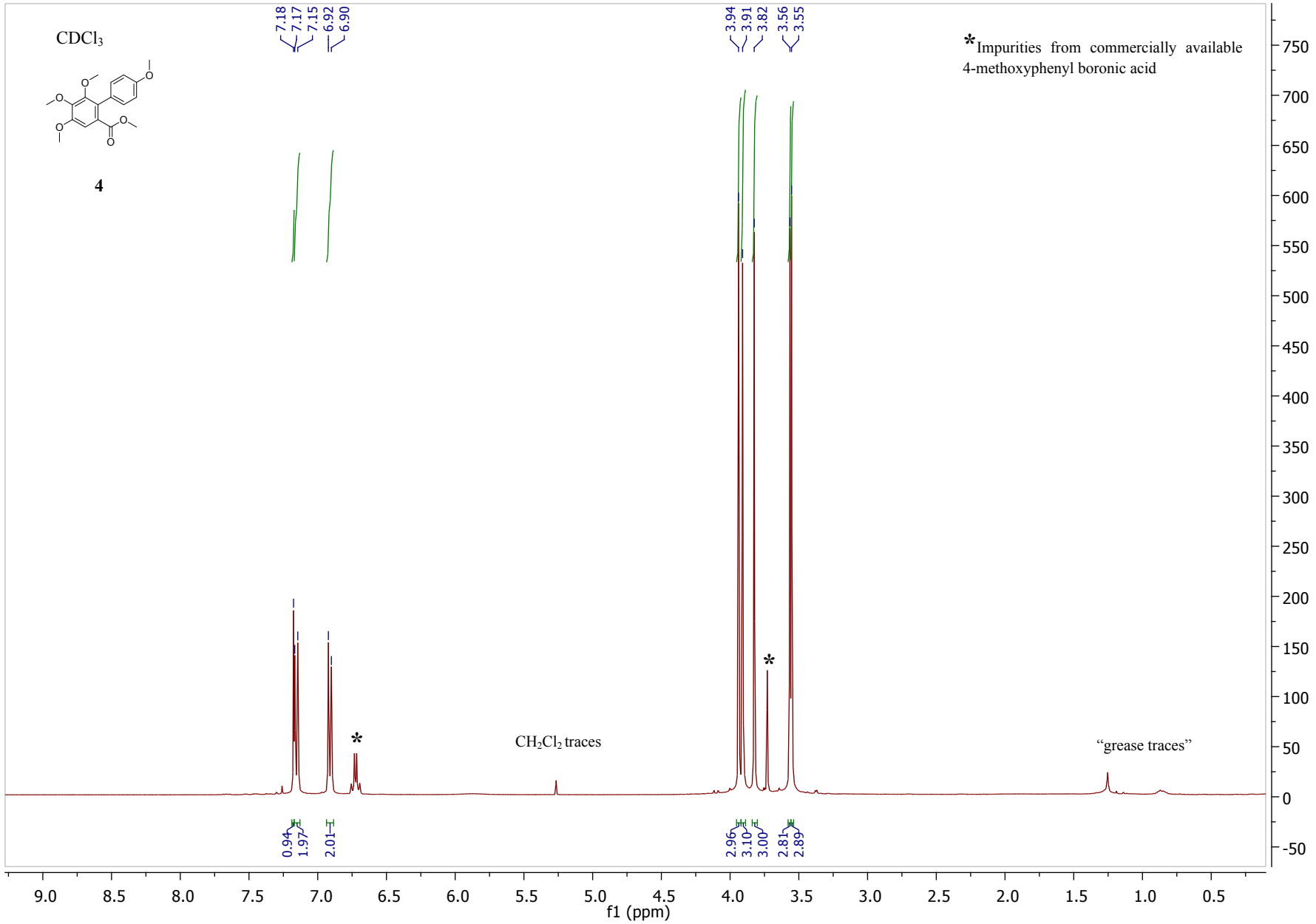
Effects of the inhibitors on cell growth or viability

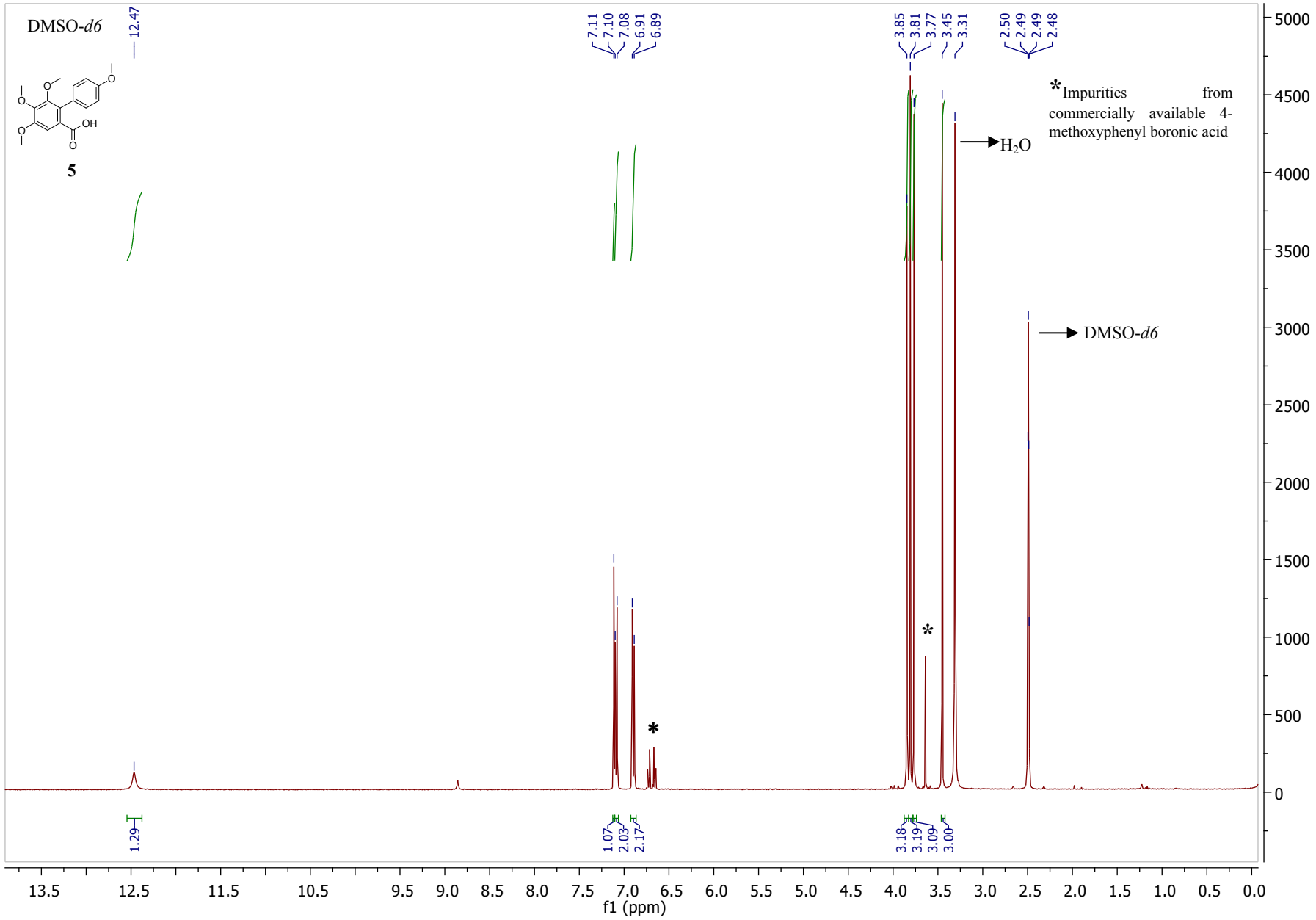
To study the effect of the inhibitor on cell proliferation, 1 x 10^5 cells were seeded in 24-multiwell plates and treated for 24 h at 37°C with scalar doses of inhibitors (0-200 μM, tested in duplicate). After incubation, cells were counted under a light microscope using a Neubauer chamber and their viability was determined by Trypan blue exclusion. The cell growth was calculated from the difference between the number of viable cells counted at 24 h and that at the beginning of experiment. Data were plotted as cell growth vs dose of compound. The dose causing 50% inhibition of cell growth (cell growth IC_{50}) was calculated by applying the second order polynomial regression to the experimental data.

^{1}H and ^{13}C NMR spectra (graphs) of compounds

^{1}H NMR (400 MHz)




CDCl3
4
7.18
7.17
7.15
6.92
6.90
3.94
3.91
3.82
3.56
3.55
*Impurities from commercially available 4-methoxyphenyl boronic acid
*
*
CH2Cl2 traces
"grease traces"
0.94
1.97
2.01
2.96
3.10
3.00
2.81
2.89
f1 (ppm)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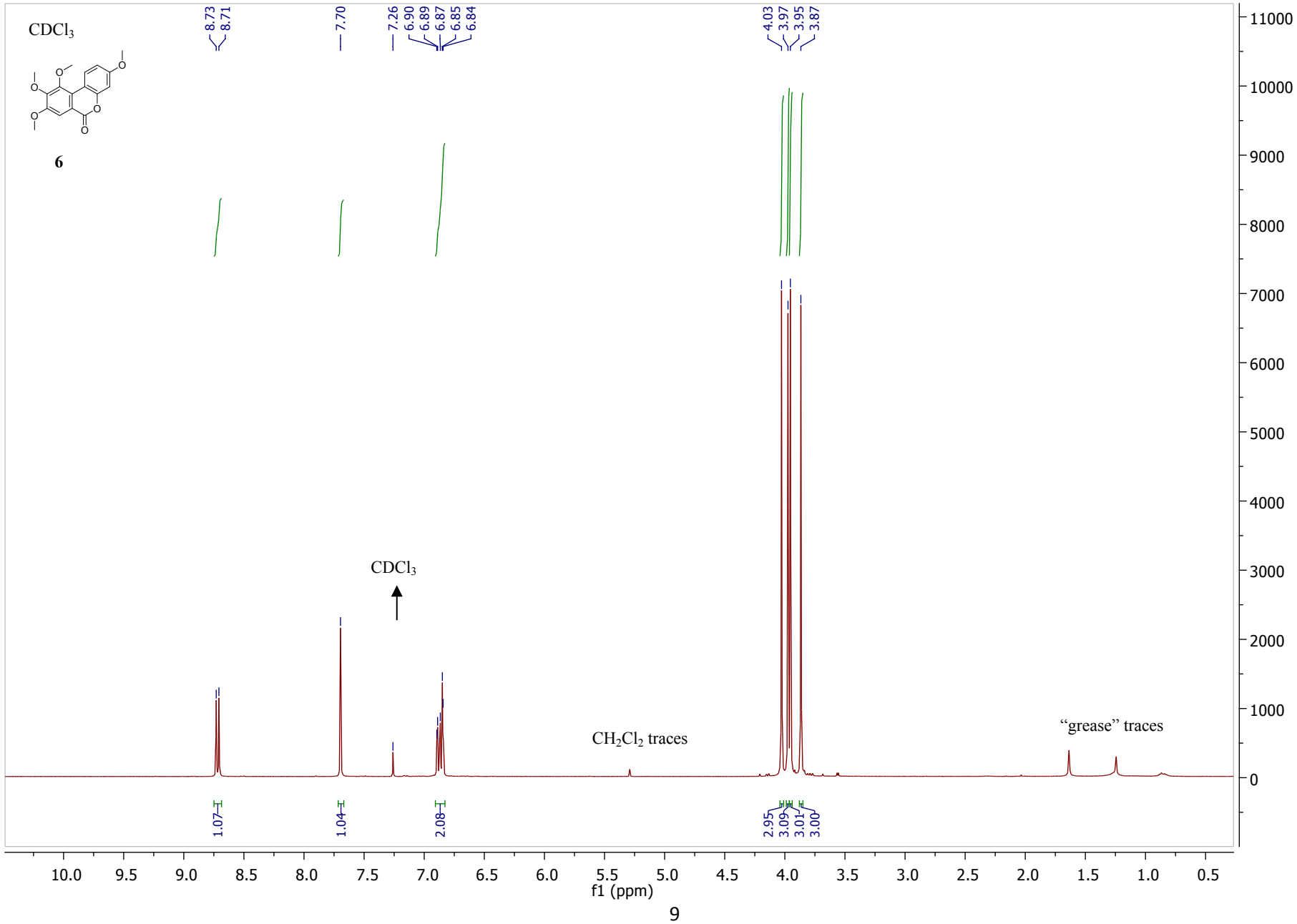
7.11
7.10
7.08
6.91
6.89
3.85
3.81
3.77
3.45
3.31
2.50
2.49
2.49
2.48
5
*Impurities from commercially available 4-methoxyphenyl boronic acid
H2O
DMSO-d6
*
*
1.29
1.07
2.03
2.17
3.18
3.19
3.09
3.00
f1 (ppm)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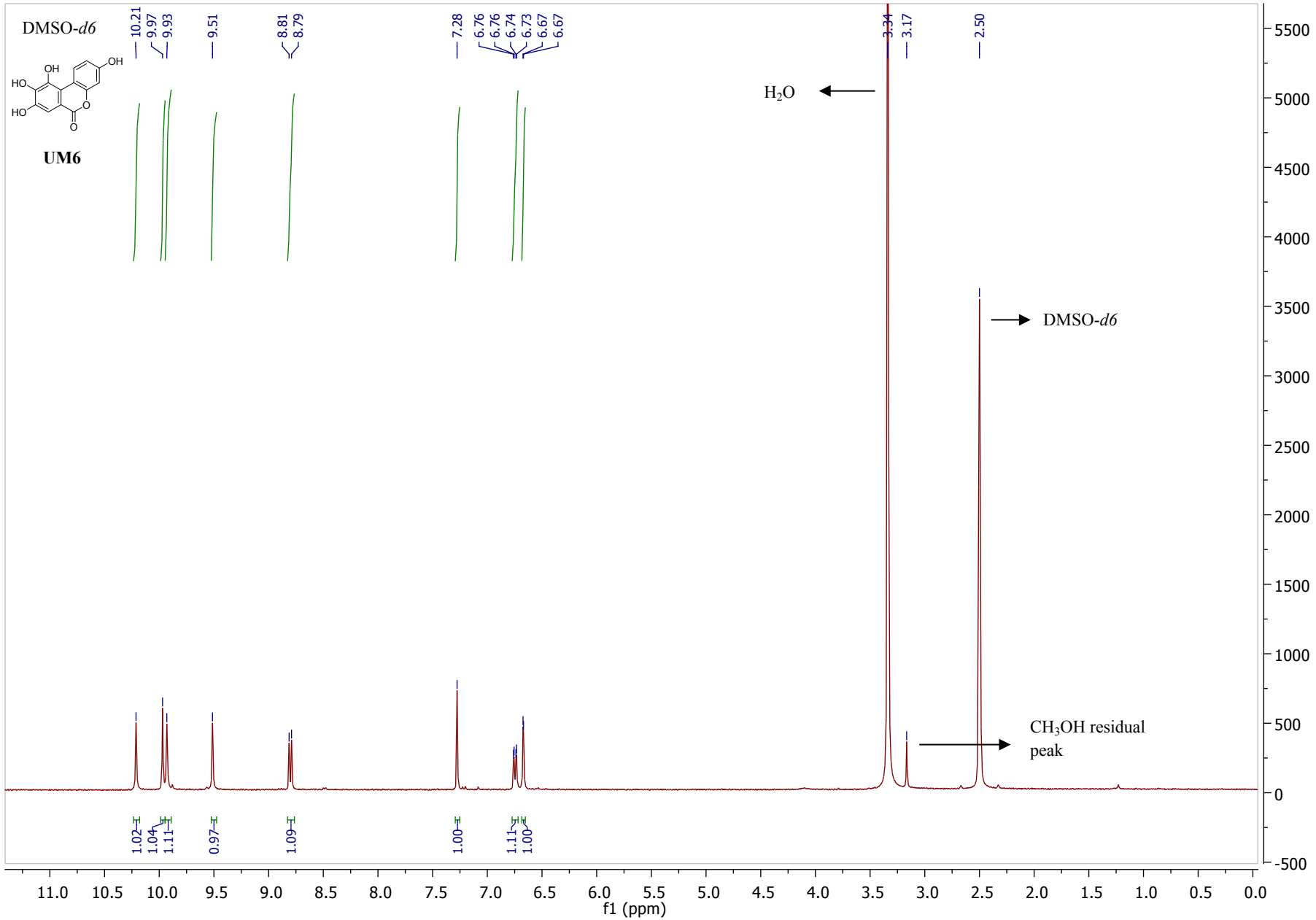
6
8.73
8.71
7.70
7.26
6.90
6.89
6.87
6.85
6.84
4.03
3.97
3.95
3.87
CDCl3
CH2Cl2 traces
"grease" traces
1.07
1.04
2.08
2.95
3.09
3.01
3.00
f1 (ppm)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UM6
10.21
9.97
9.93
9.51
8.81
8.79
7.28
6.76
6.76
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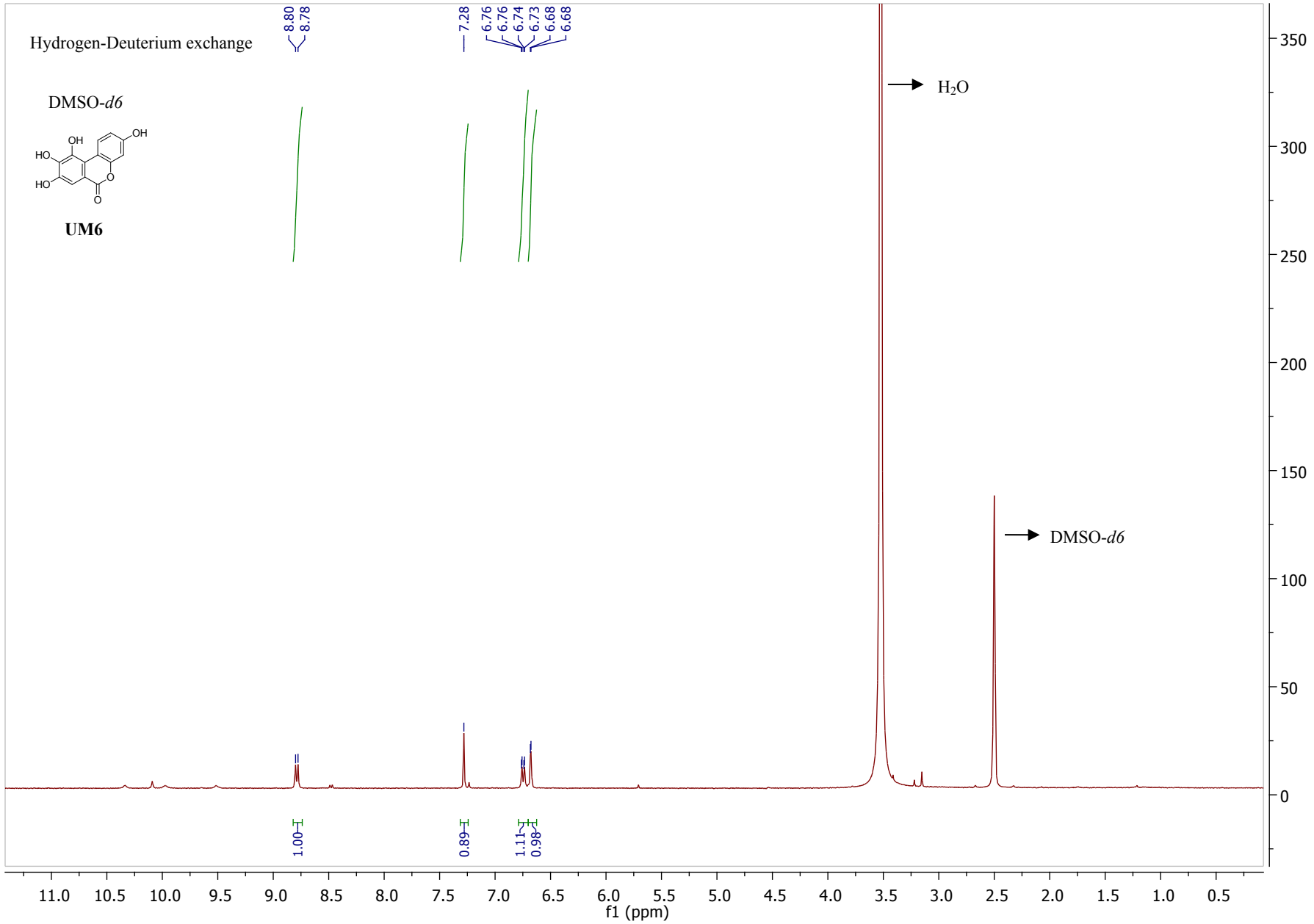
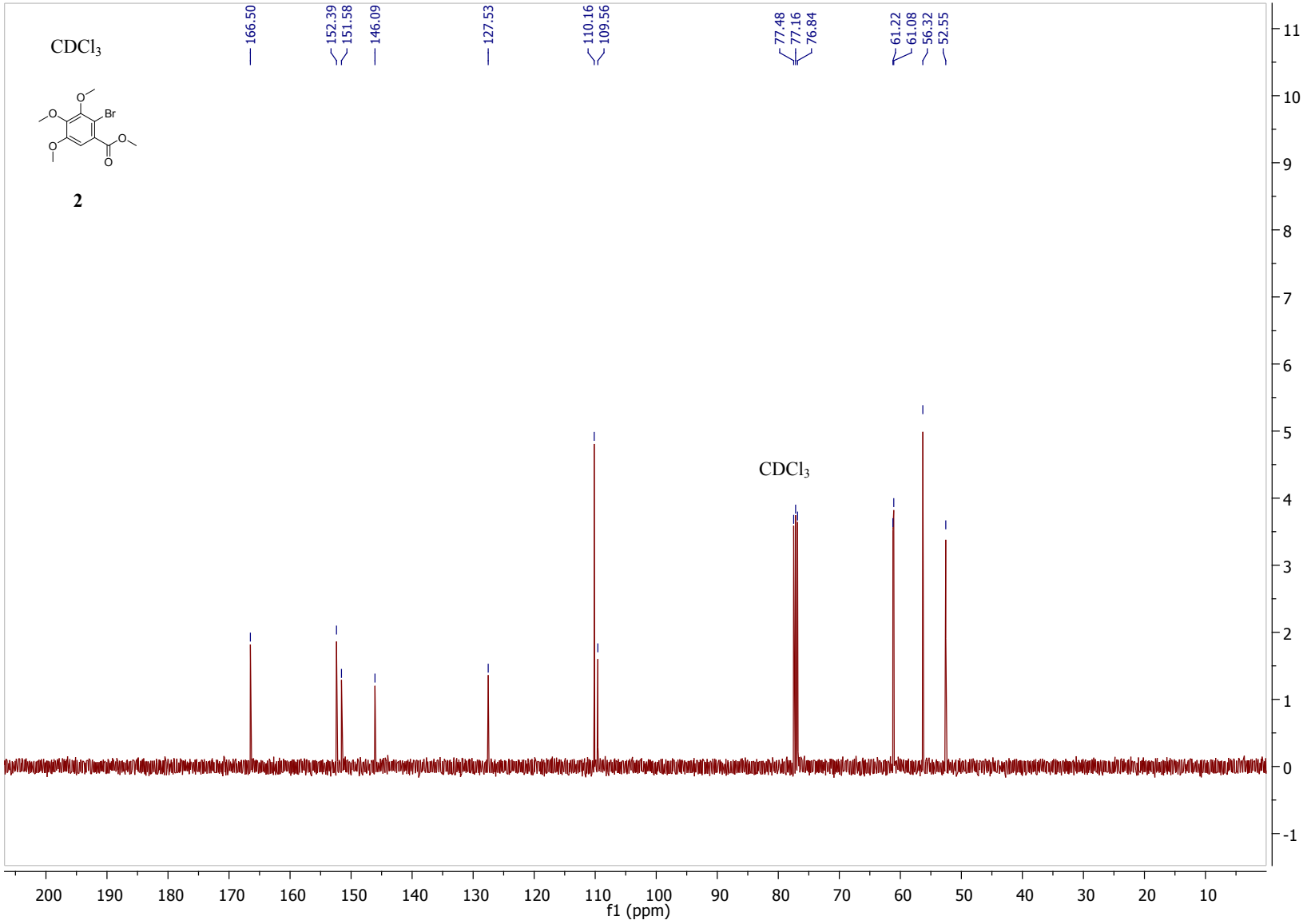
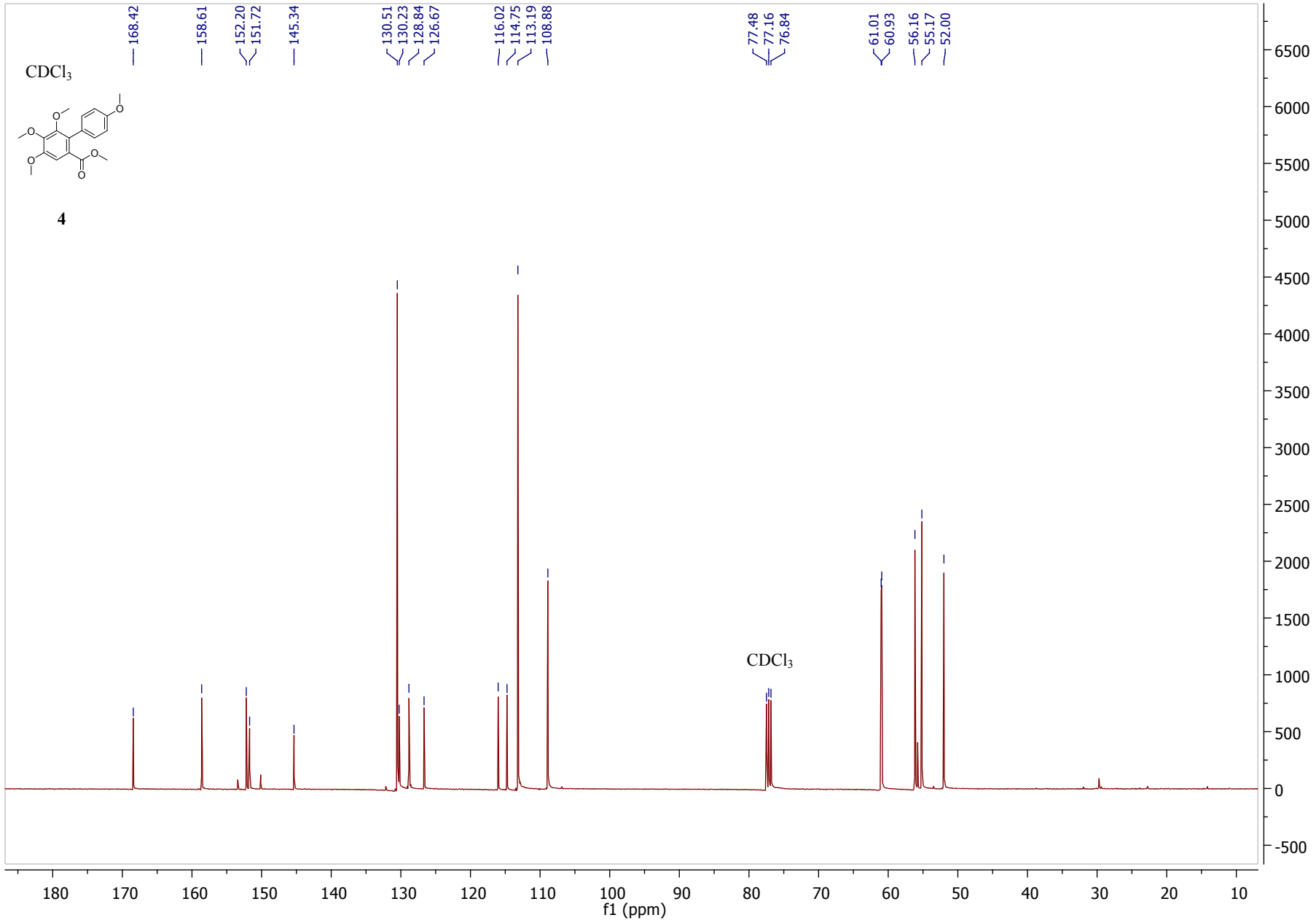
6.73
6.67
6.67
3.34
3.17
2.50
H2O
DMSO-d6
CH3OH residual peak
1.02
1.04
1.11
0.97
1.09
1.00
1.11
1.00
f1 (ppm)


Hydrogen-Deuterium exchange
DMSO-d6
OH
HO
HO
OH
O
O
UM6
8.80
8.78
7.28
6.76
6.76
6.74
6.73
6.68
6.68
H2O
DMSO-d6
1.00
0.89
1.11
0.98
350
300
250
200
150
100
50
0
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
f1 (ppm)

^{13}C NMR (100 MHz)

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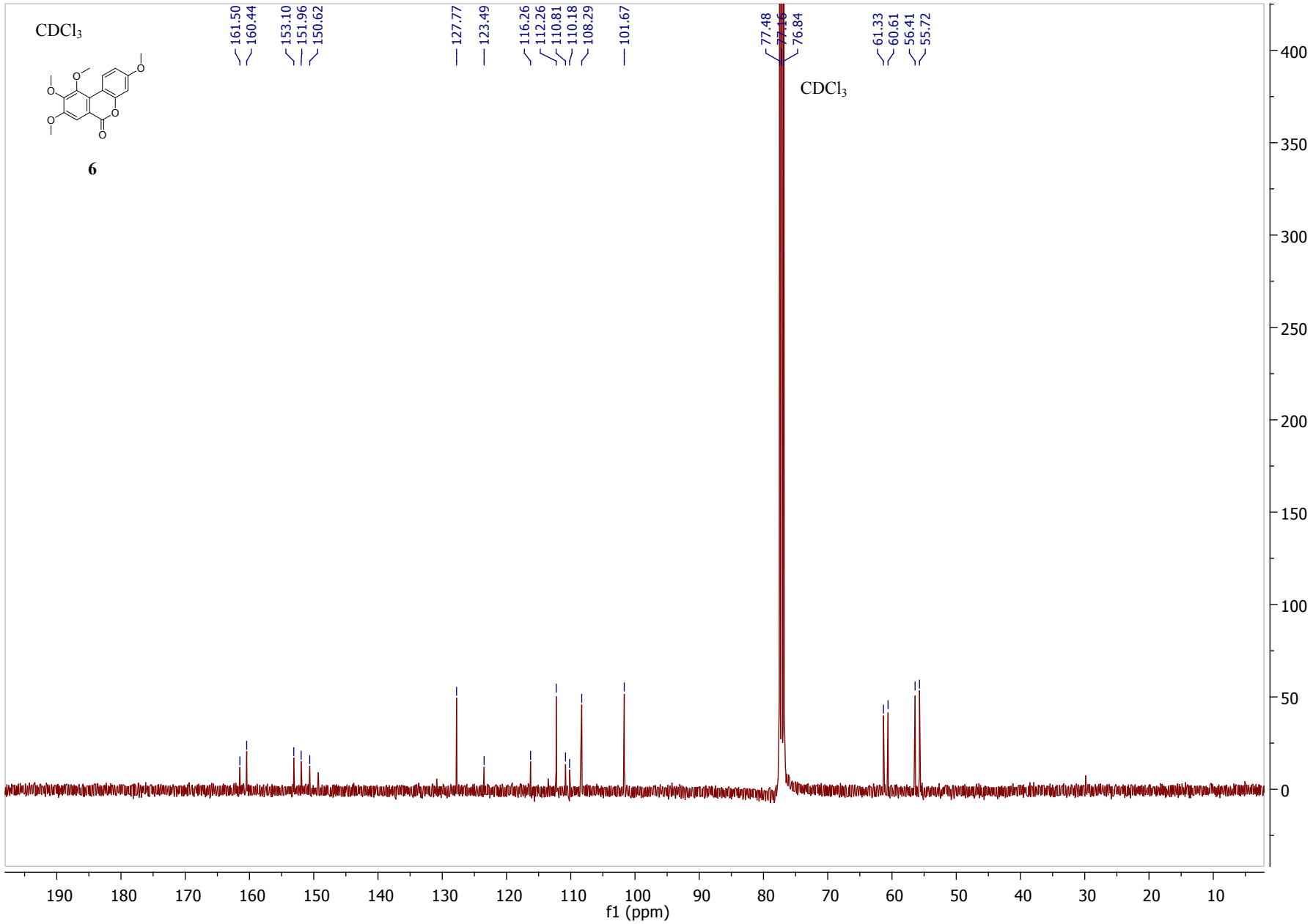
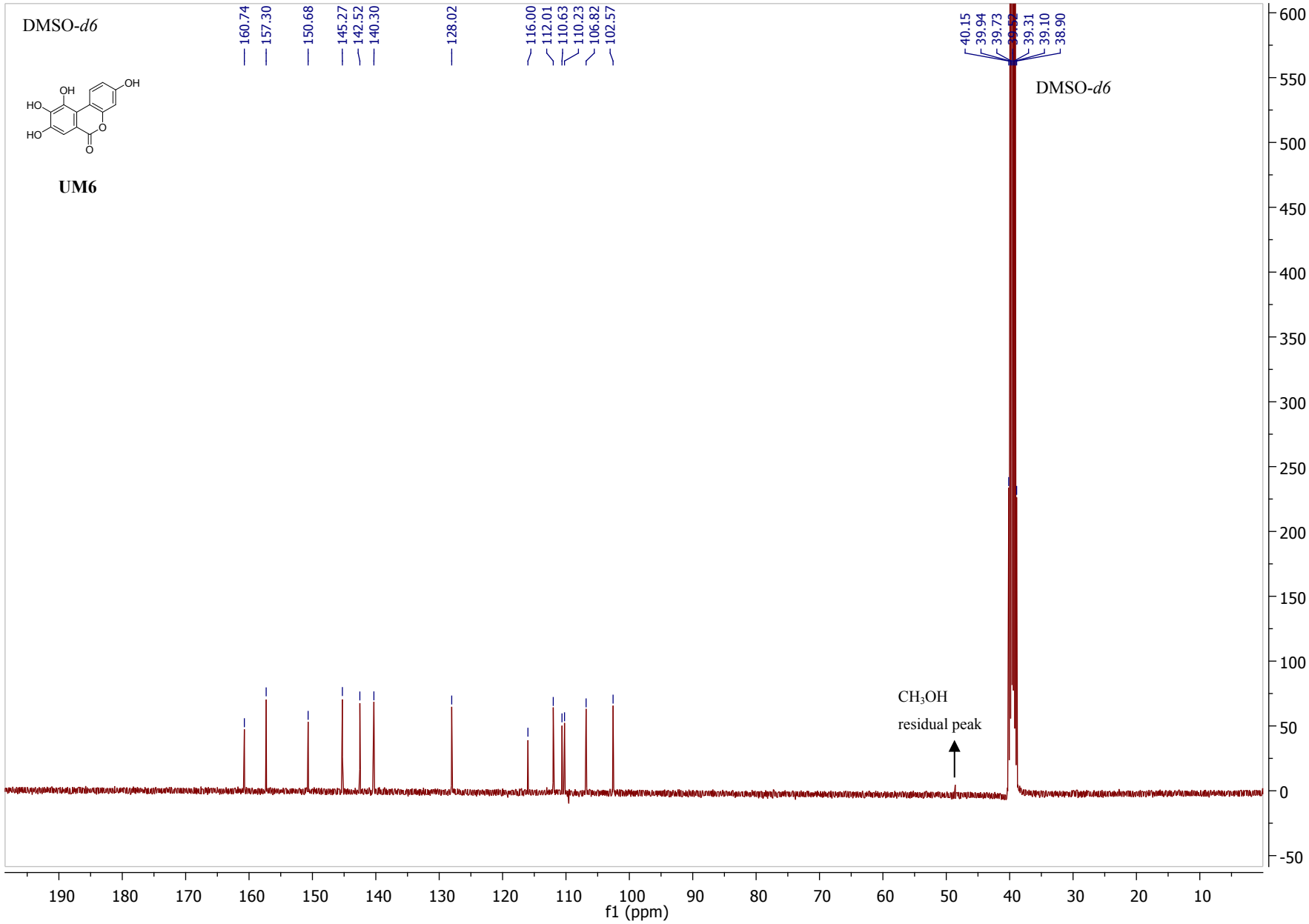
158.61
152.20
151.72
145.34
130.51
130.23
128.84
126.67
116.02
114.75
113.19
108.88
77.48
77.16
76.84
61.01
60.93
56.16
55.17
52.00
CDCl3
CDCl3
4
f1 (ppm)


CDCl3
161.50
160.44
153.10
151.96
150.62
127.77
123.49
116.26
112.26
110.81
110.18
108.29
101.67
77.48
77.16
76.84
CDCl3
61.33
60.61
56.41
55.72
400
350
300
250
200
150
100
50
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

6


DMSO-d6
160.74
157.30
150.68
145.27
142.52
140.30
128.02
116.00
112.01
110.63
110.23
106.82
102.57
40.15
39.94
39.73
39.52
39.31
39.10
38.90
DMSO-d6
OH
HO
HO
OH
O
O
UM6
CH3OH
residual peak
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)