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

Supporting information

CARBOXYLATE ISOSTERES FOR CASPASE INHIBITORS: THE ACYLSULFONAMIDE CASE REVISITED

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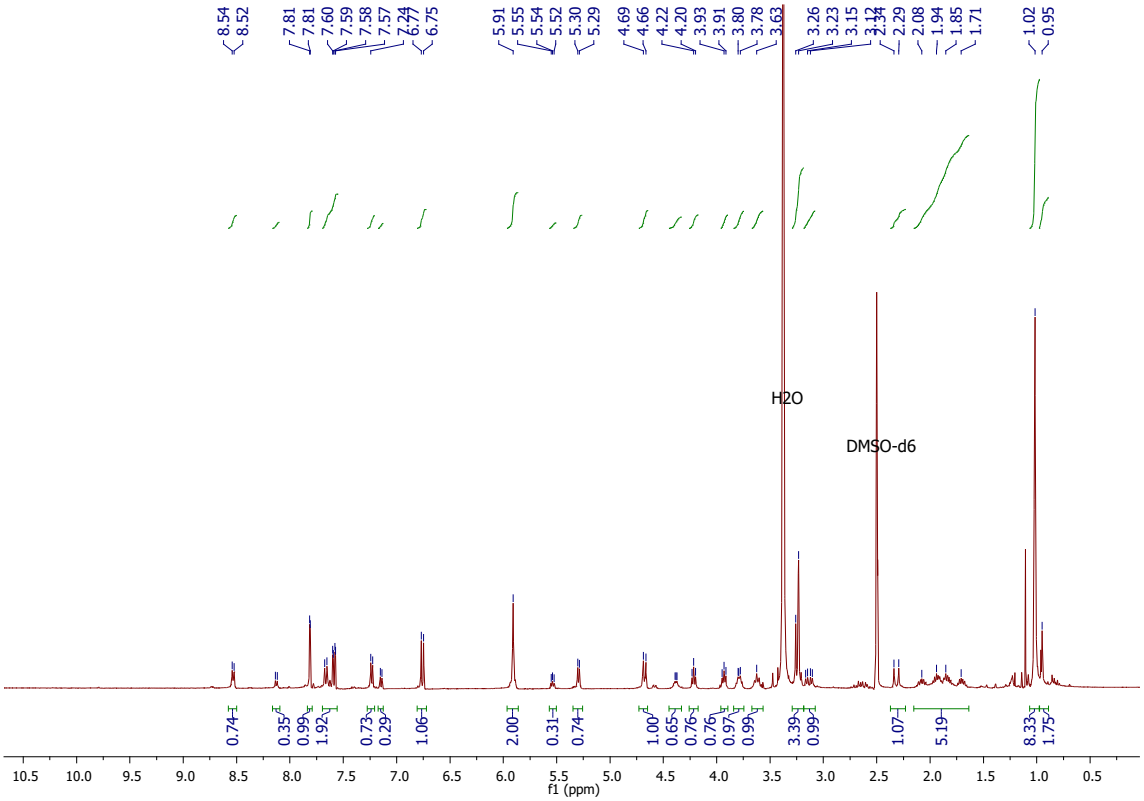
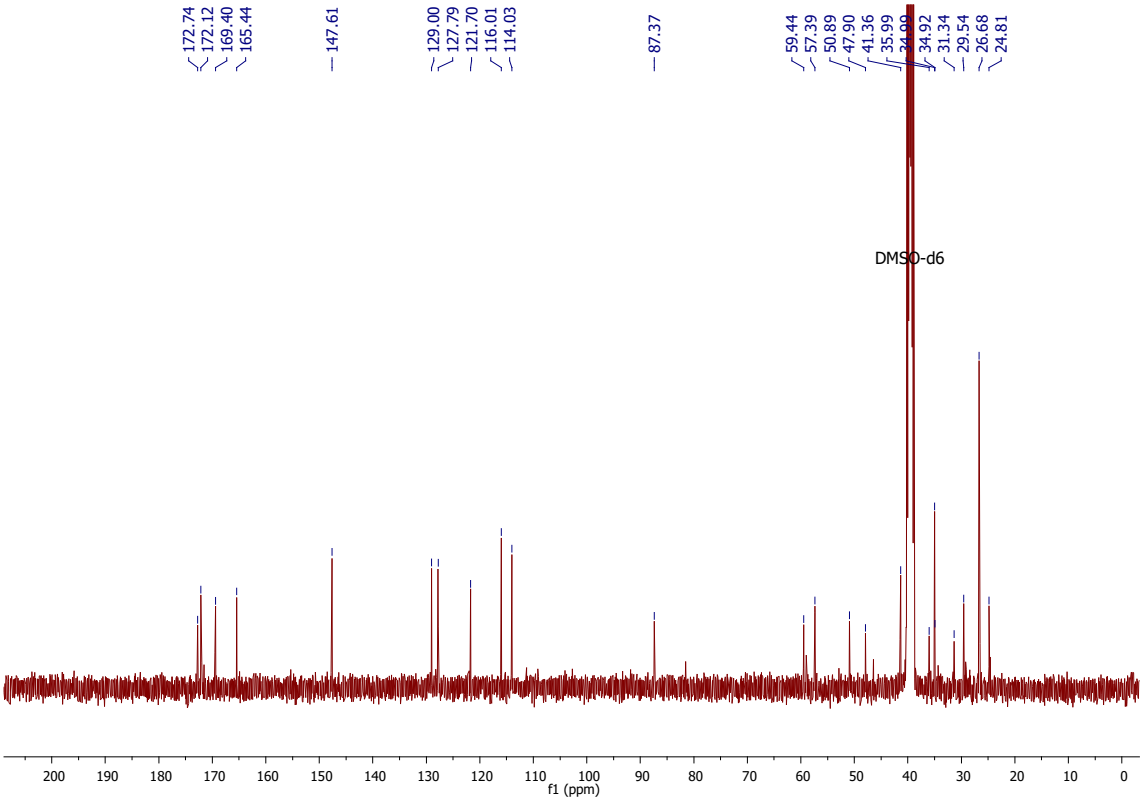
[d] *Department of Internal Medicine, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium*

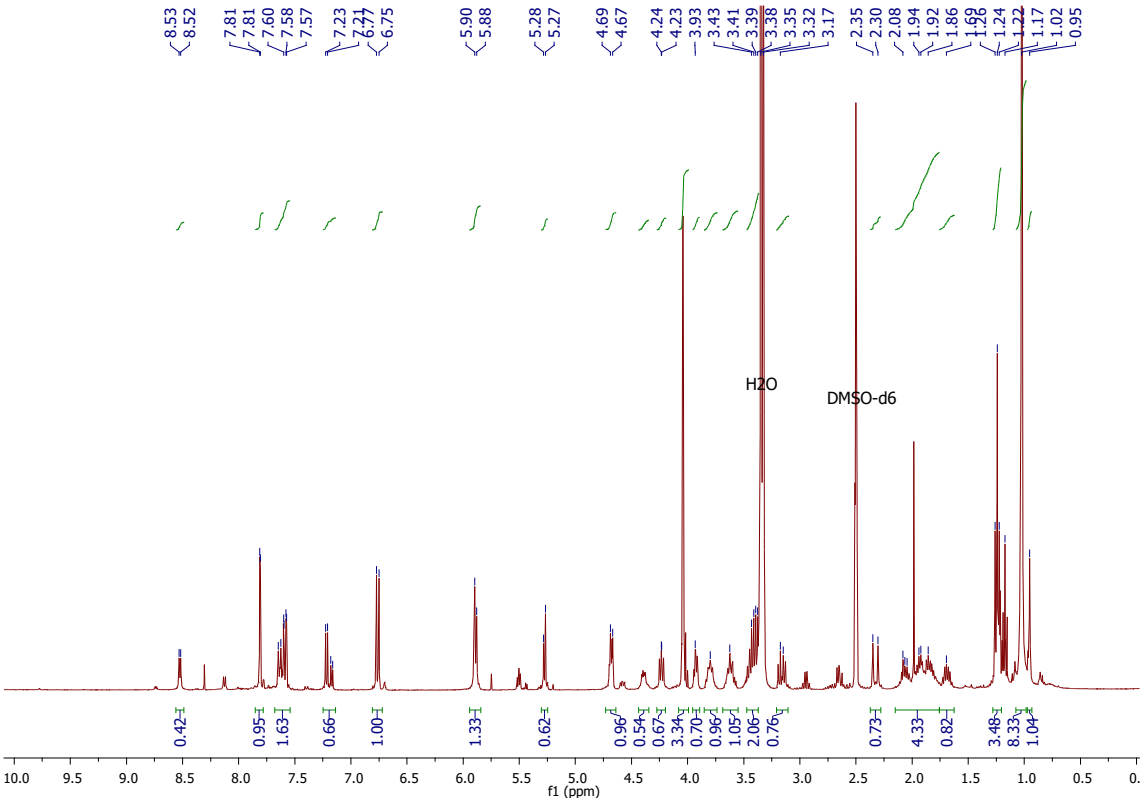
NMR spectroscopy

Compound **3**





Compound **4**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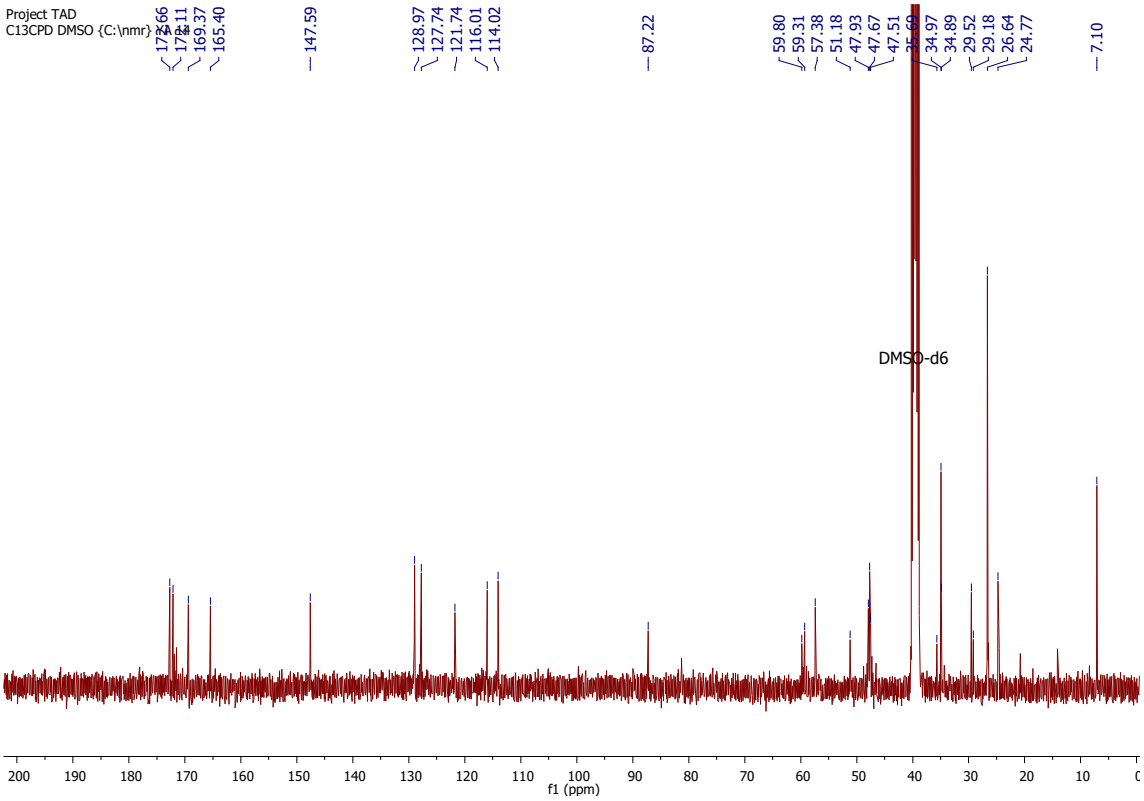
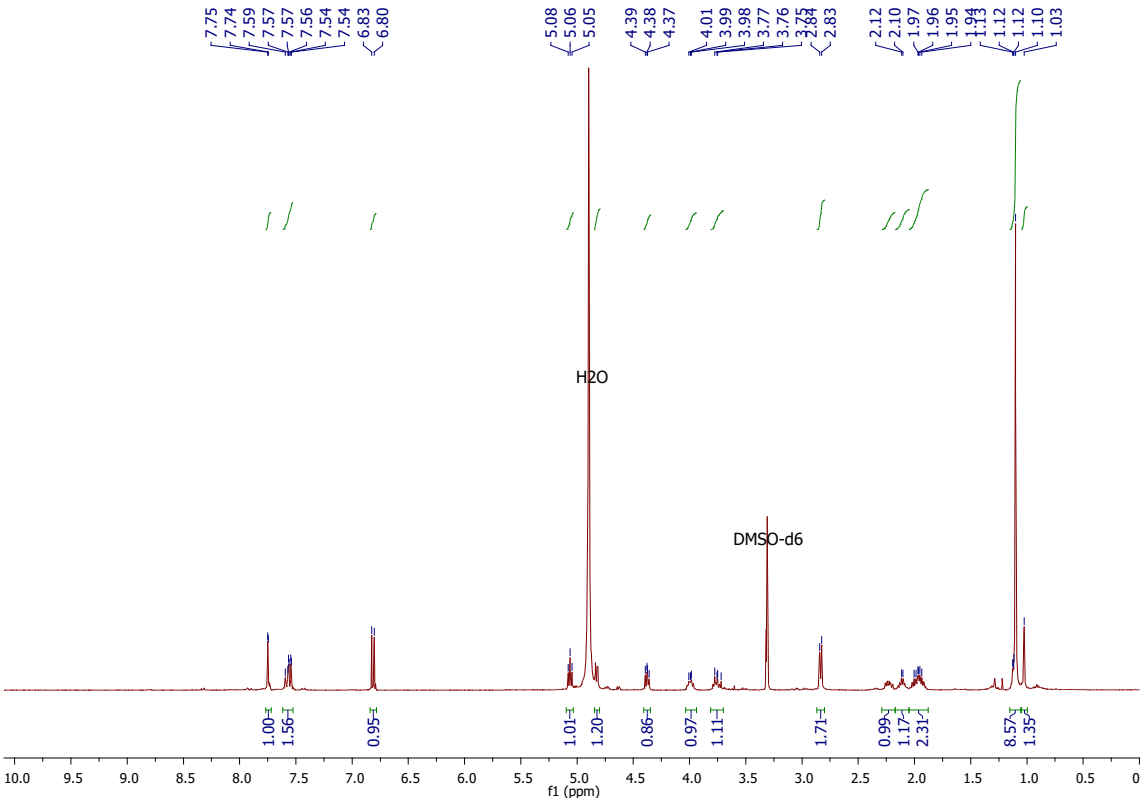
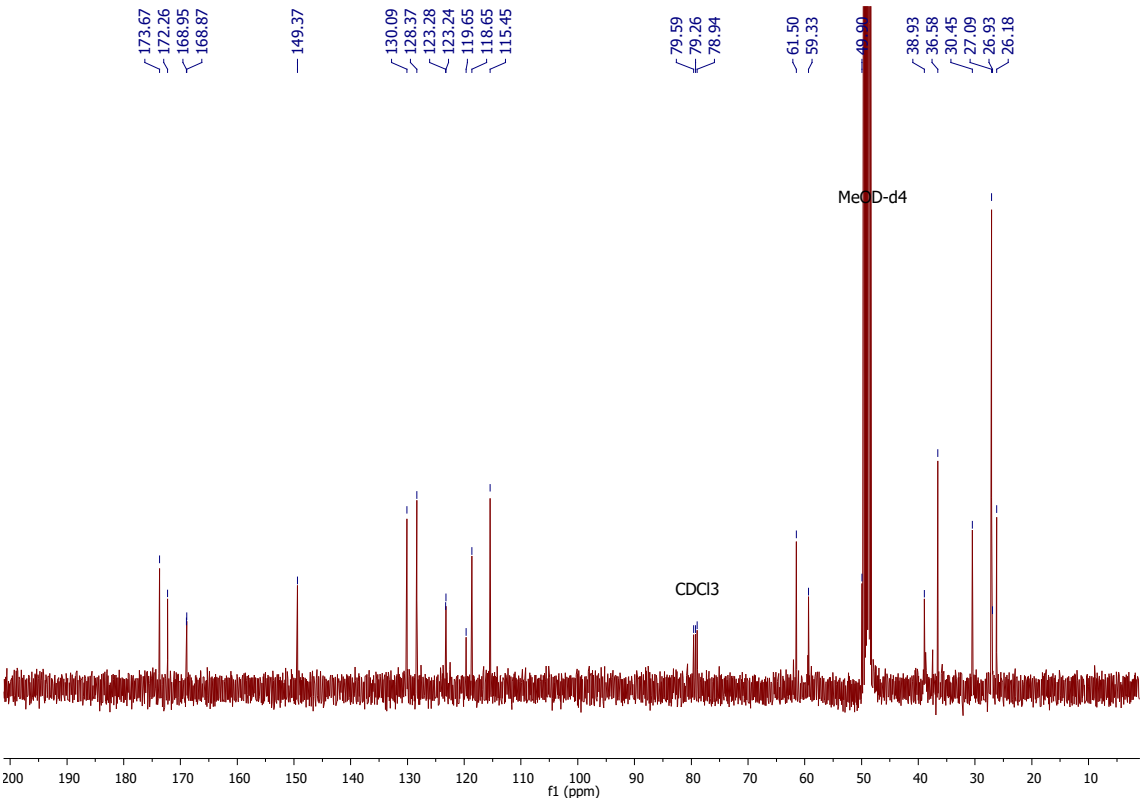
8.52
7.81
7.81
7.60
7.58
7.57
7.23
7.21
7.17
6.75
5.90
5.88
5.28
5.27
4.69
4.67
4.24
4.23
3.93
3.43
3.41
3.39
3.38
3.35
3.32
3.17
2.35
2.30
2.08
1.94
1.92
1.86
1.69
1.26
1.24
1.22
1.17
1.02
0.95
H2O
DMSO-d6
0.42
0.95
1.63
0.66
1.00
1.33
0.62
0.96
0.54
0.67
3.34
0.70
0.96
1.05
2.06
0.76
0.73
4.33
0.82
3.48
8.33
1.04
f1 (ppm)

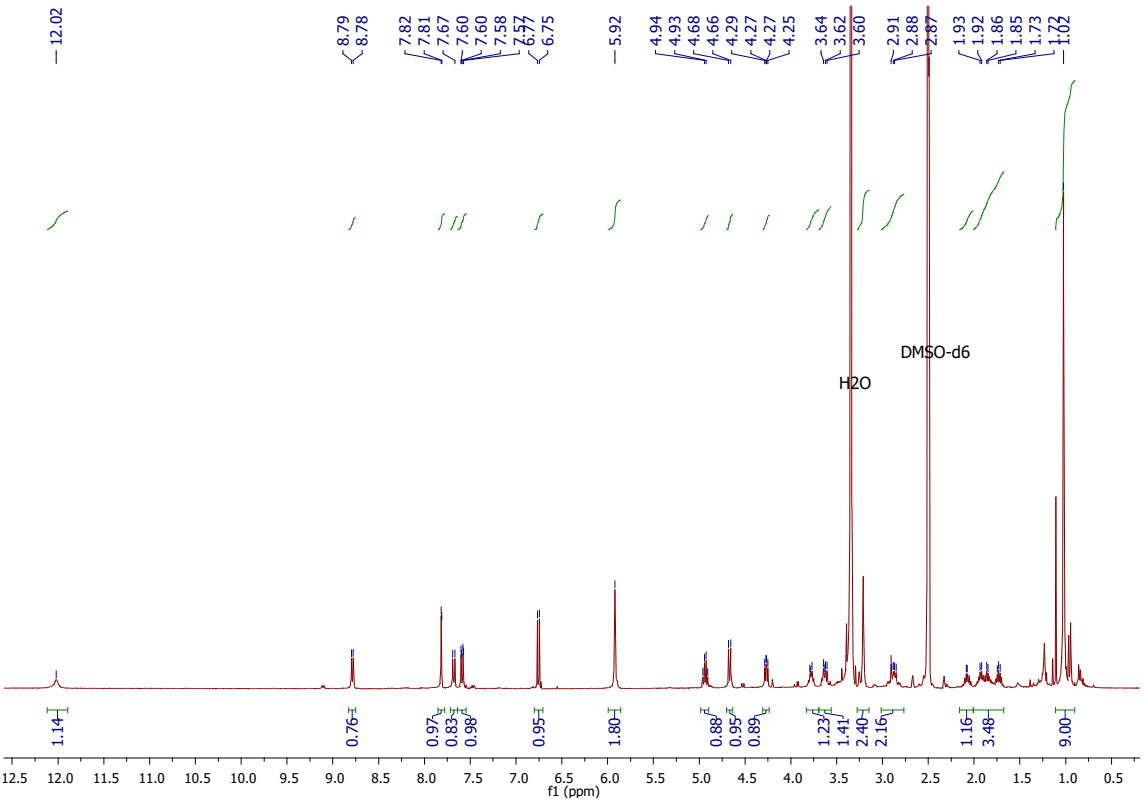

Project TAD
C13CPD DMSO {C:\nmr} TAD
173.66
173.11
169.37
165.40
147.59
128.97
127.74
121.74
116.01
114.02
87.22
59.80
59.31
57.38
51.18
47.93
47.67
47.51
35.69
34.97
34.89
29.52
29.18
26.64
24.77
7.10
DMSO-d6
f1 (ppm)

Compound **5**


7.75
7.74
7.59
7.57
7.57
7.56
7.54
7.54
6.83
6.80
5.08
5.06
5.05
4.39
4.38
4.37
4.01
3.99
3.98
3.77
3.76
3.75
3.74
2.84
2.83
2.12
2.10
1.97
1.96
1.95
1.94
1.13
1.12
1.12
1.10
1.03
H2O
DMSO-d6
1.00
1.56
0.95
1.01
1.20
0.86
0.97
1.11
1.71
0.99
1.17
2.31
8.57
1.35
f1 (ppm)


173.67
172.26
168.95
168.87
149.37
130.09
128.37
123.28
123.24
119.65
118.65
115.45
79.59
79.26
78.94
61.50
59.33
49.00
38.93
36.58
30.45
27.09
26.93
26.18
MeOD-d4
CDCl3
f1 (ppm)

Compound **6**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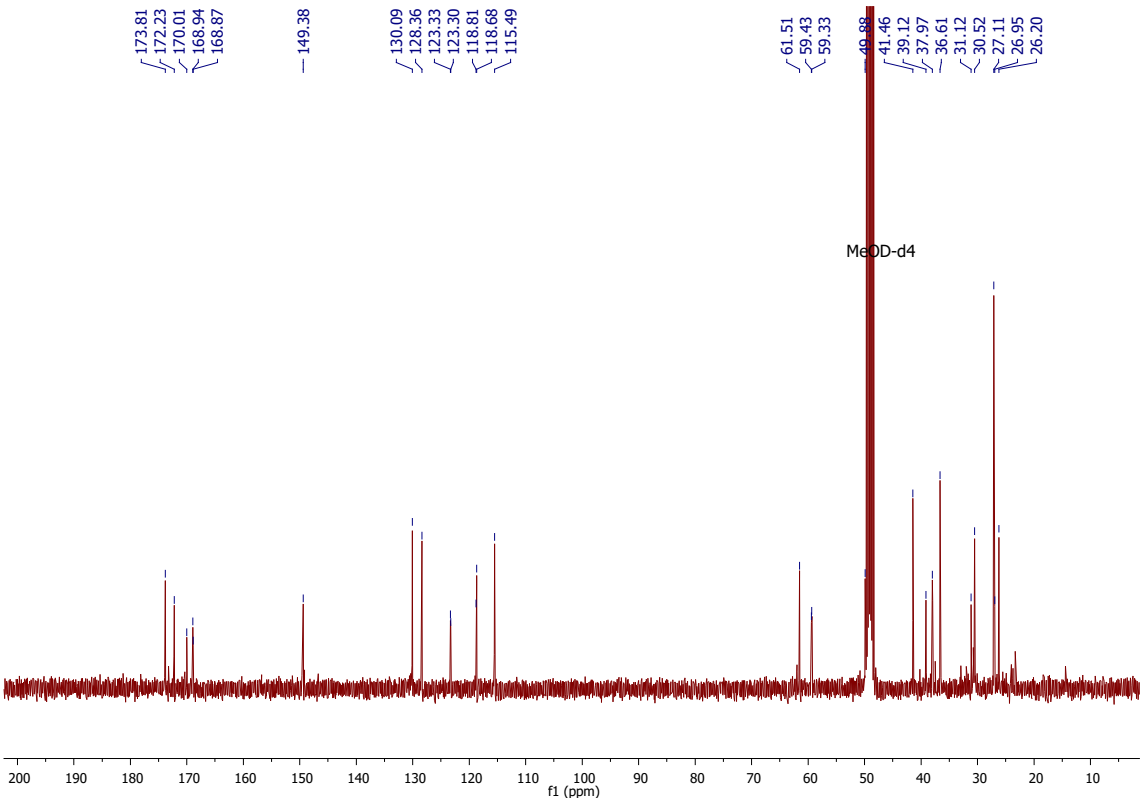
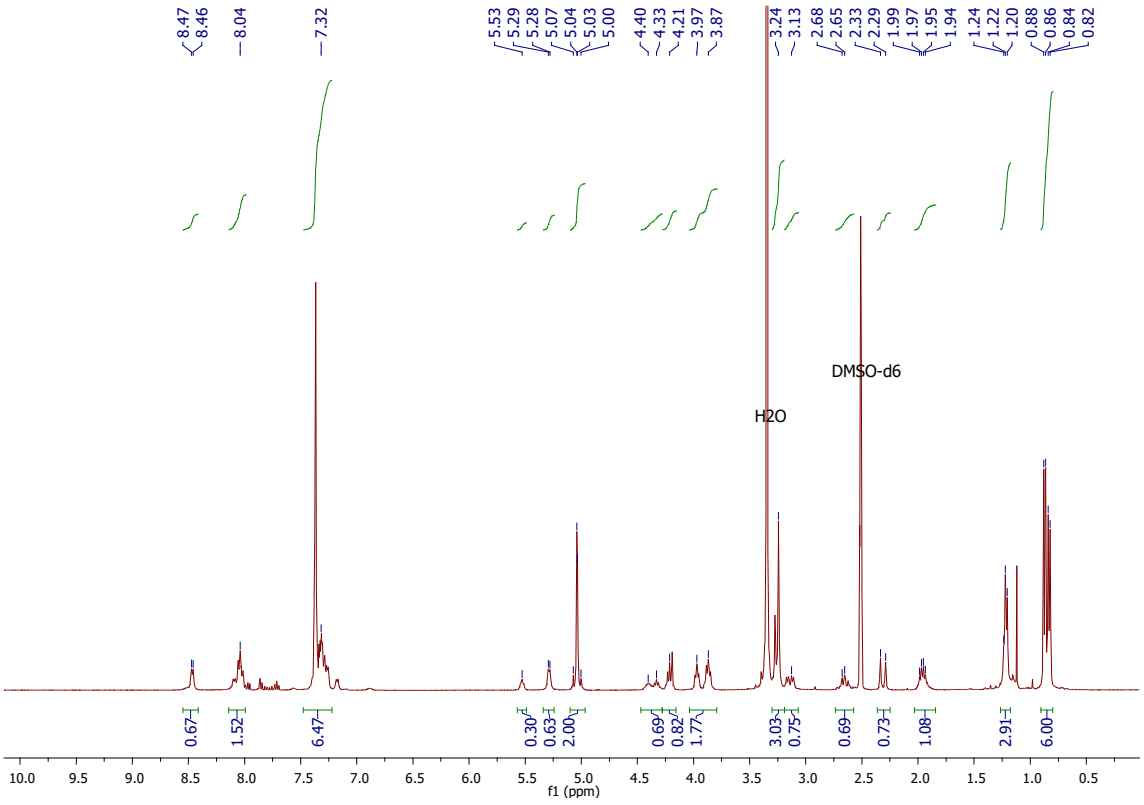
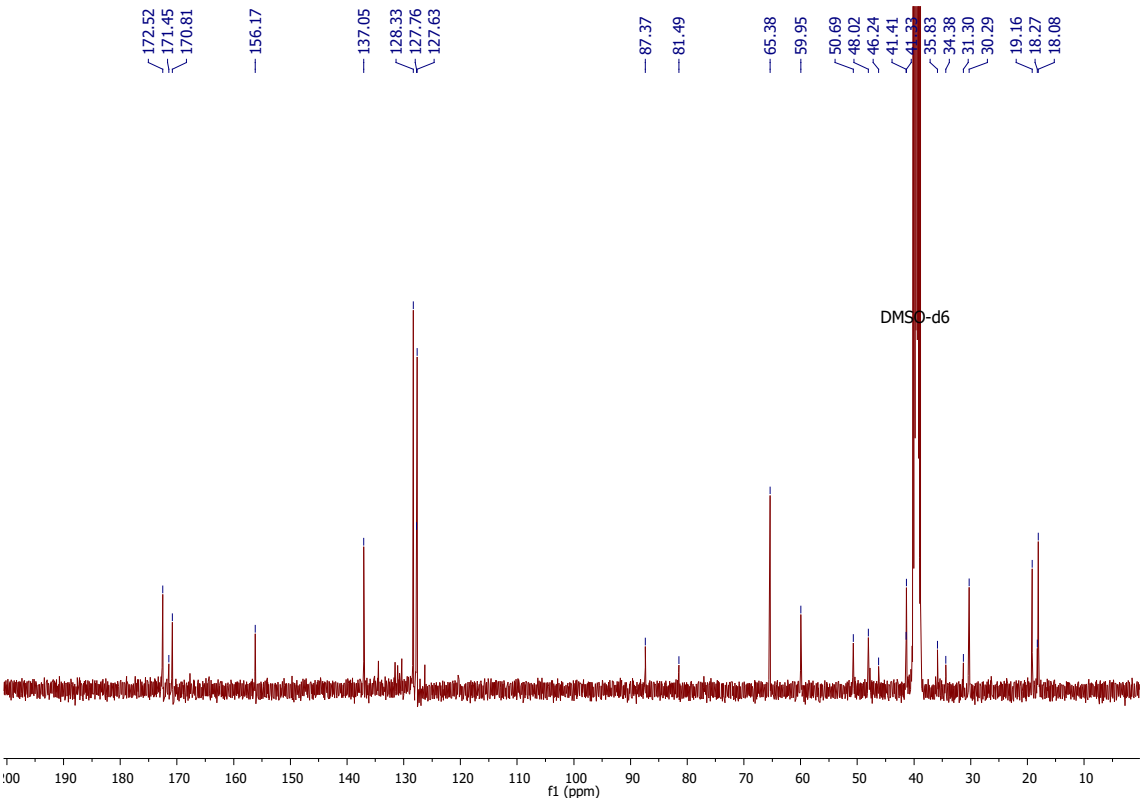
8.78
7.82
7.81
7.67
7.60
7.60
7.58
7.57
6.77
6.75
5.92
4.94
4.93
4.68
4.66
4.29
4.27
4.27
4.25
3.64
3.62
3.60
2.91
2.88
2.87
1.93
1.92
1.86
1.85
1.73
1.72
1.02
DMSO-d6
H2O
1.14
0.76
0.97
0.83
0.98
0.95
1.80
0.88
0.95
0.89
1.23
1.41
2.40
2.16
1.16
3.48
9.00
f1 (ppm)

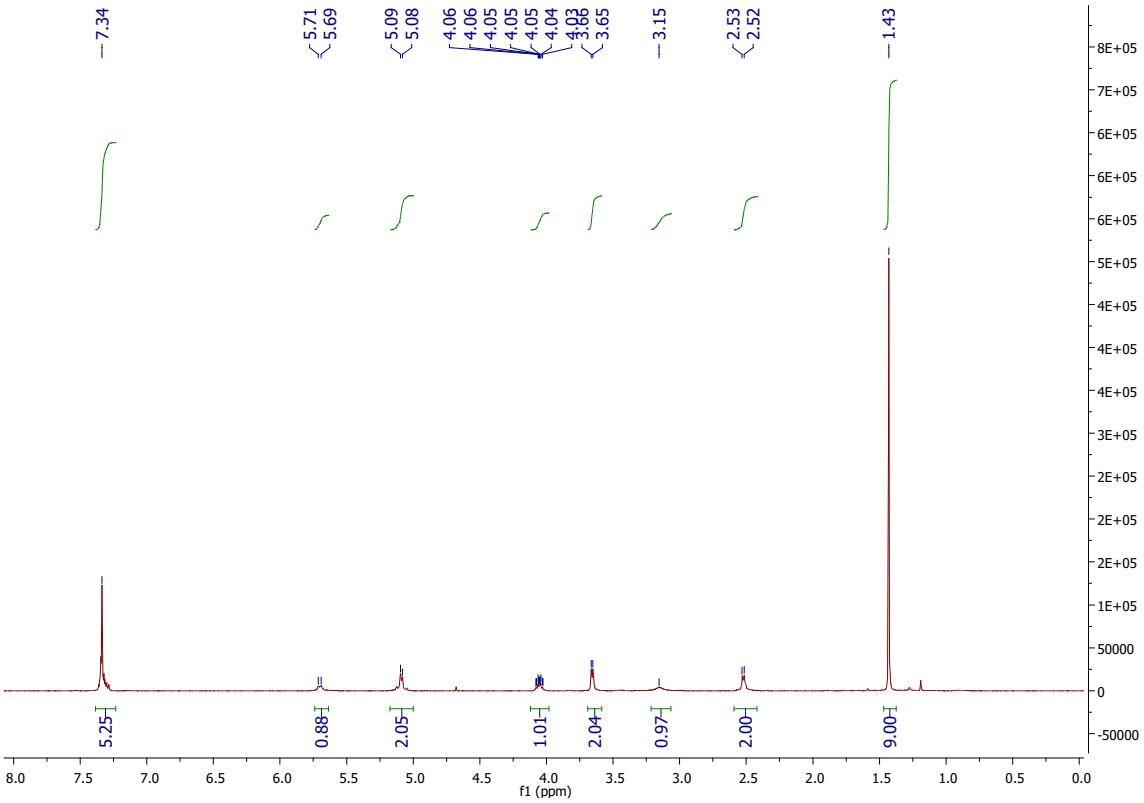

173.81
172.23
170.01
168.94
168.87
149.38
130.09
128.36
123.33
123.30
118.81
118.68
115.49
61.51
59.43
59.33
49.88
41.46
39.12
37.97
36.61
31.12
30.52
27.11
26.95
26.20
MeOD-d4
f1 (ppm)

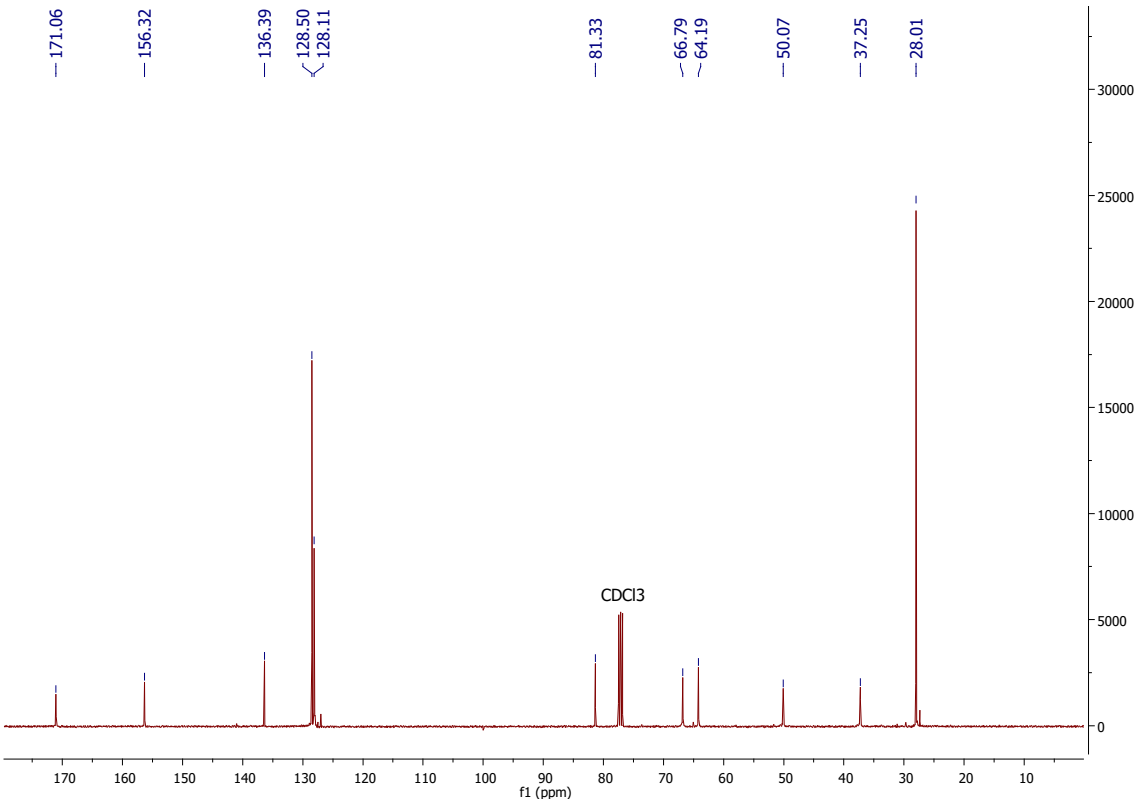
Compound **8**


8.47
8.46
8.04
7.32
5.53
5.29
5.28
5.07
5.04
5.03
5.00
4.40
4.33
4.21
3.97
3.87
3.24
3.13
2.68
2.65
2.33
2.29
1.99
1.97
1.95
1.94
1.24
1.22
1.20
0.88
0.86
0.84
0.82
DMSO-d6
H2O
0.67
1.52
6.47
0.30
0.63
2.00
0.69
0.82
1.77
3.03
0.75
0.69
0.73
1.08
2.91
6.00
f1 (ppm)

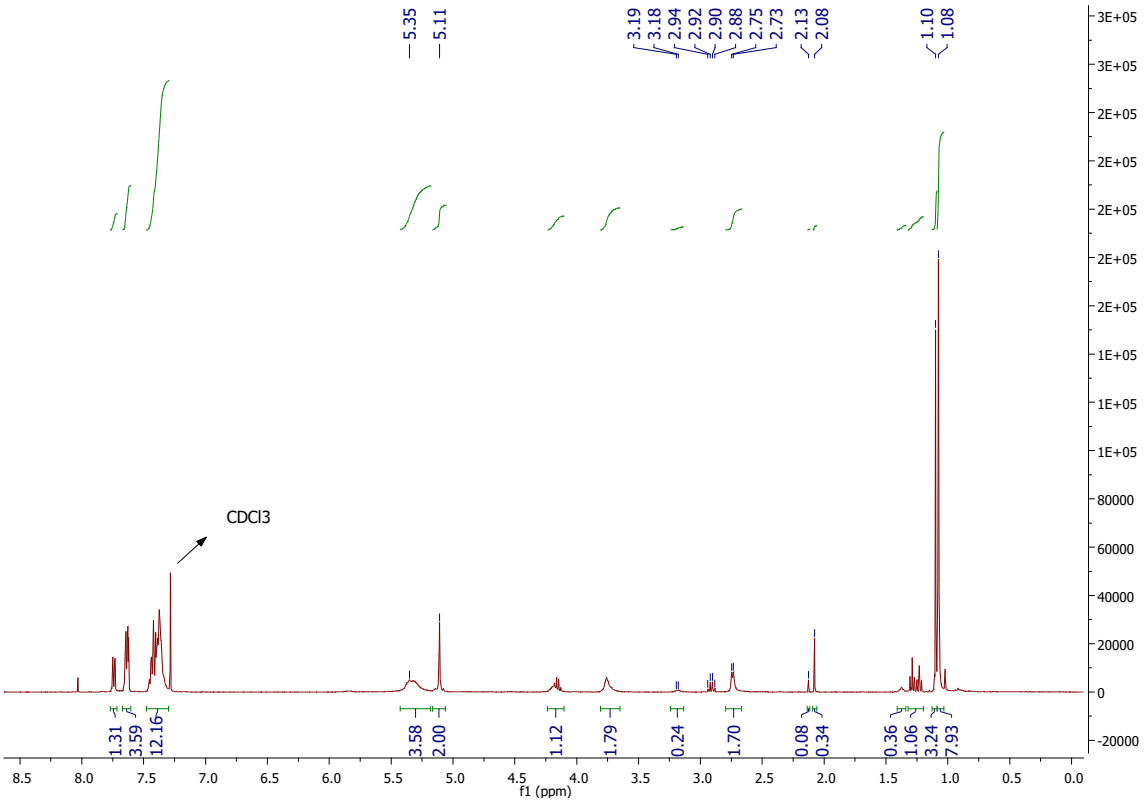

172.52
171.45
170.81
156.17
137.05
128.33
127.76
127.63
87.37
81.49
65.38
59.95
50.69
48.02
46.24
41.41
41.33
35.83
34.38
31.30
30.29
19.16
18.27
18.08
DMSO-d6
f1 (ppm)

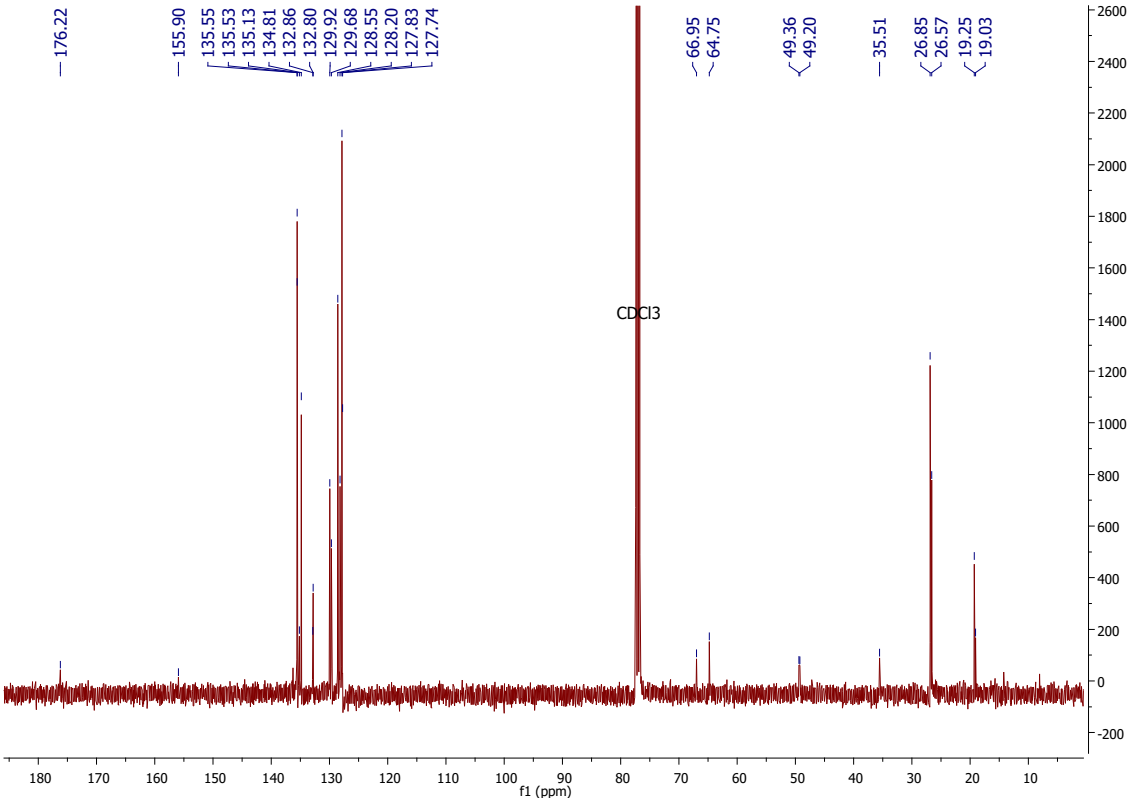
Compound **10**


7.34
5.71
5.69
5.09
5.08
4.06
4.06
4.05
4.05
4.05
4.04
3.68
3.65
3.15
2.53
2.52
1.43
5.25
0.88
2.05
1.01
2.04
0.97
2.00
9.00
f1 (ppm)

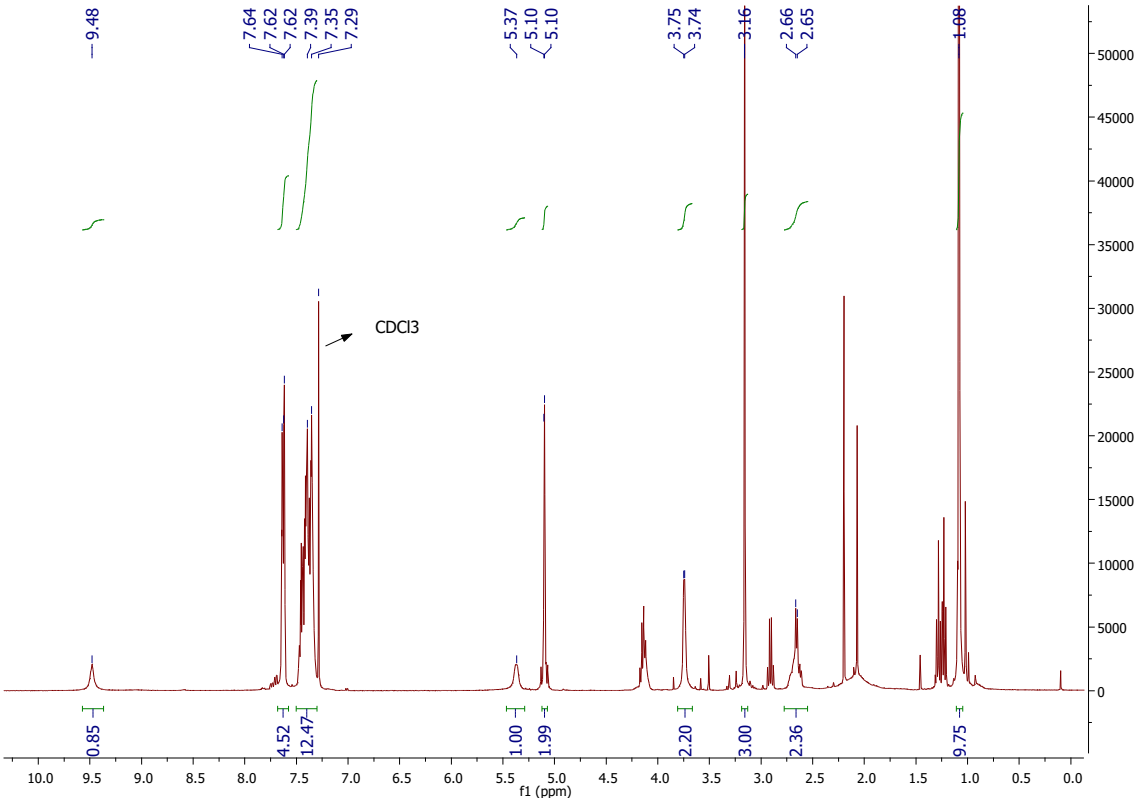

171.06
156.32
136.39
128.50
128.11
81.33
66.79
64.19
50.07
37.25
28.01
CDCl3
f1 (ppm)

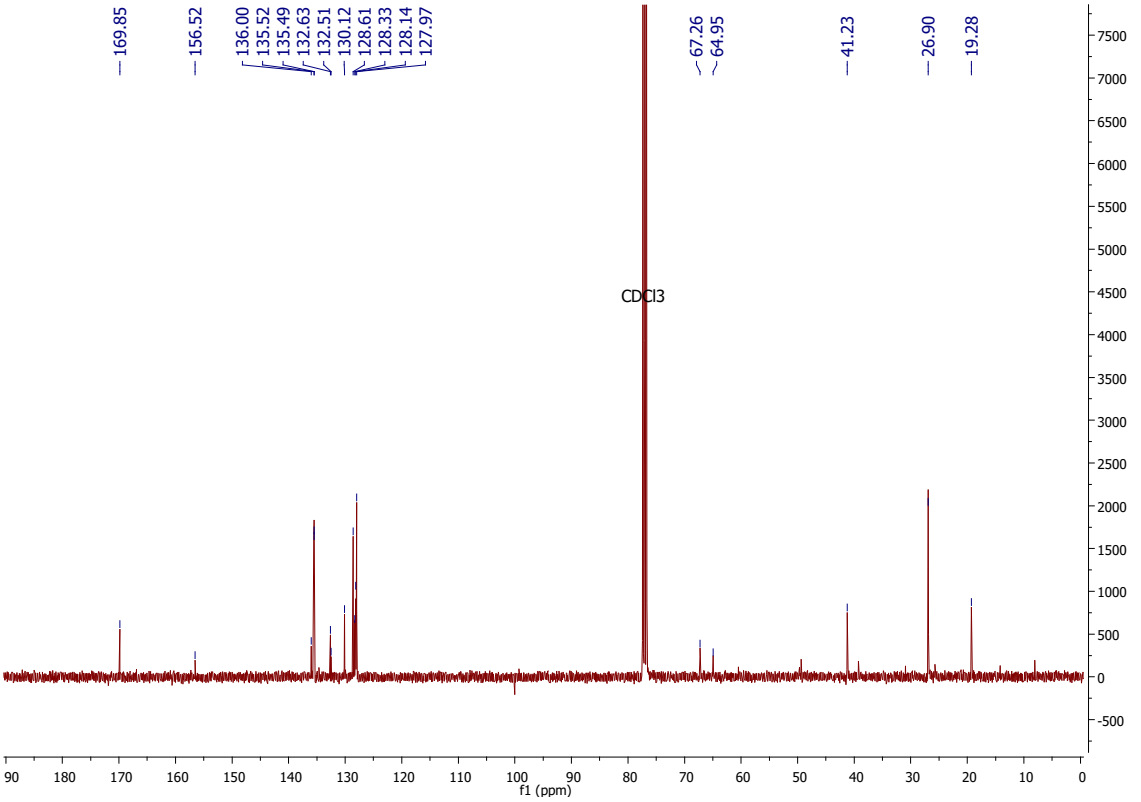
Compound **11**


5.35
5.11
3.19
3.18
2.94
2.92
2.90
2.88
2.75
2.73
2.13
2.08
1.10
1.08
CDCl3
1.31
3.59
12.16
3.58
2.00
1.12
1.79
0.24
1.70
0.08
0.34
0.36
1.06
3.24
7.93
f1 (ppm)

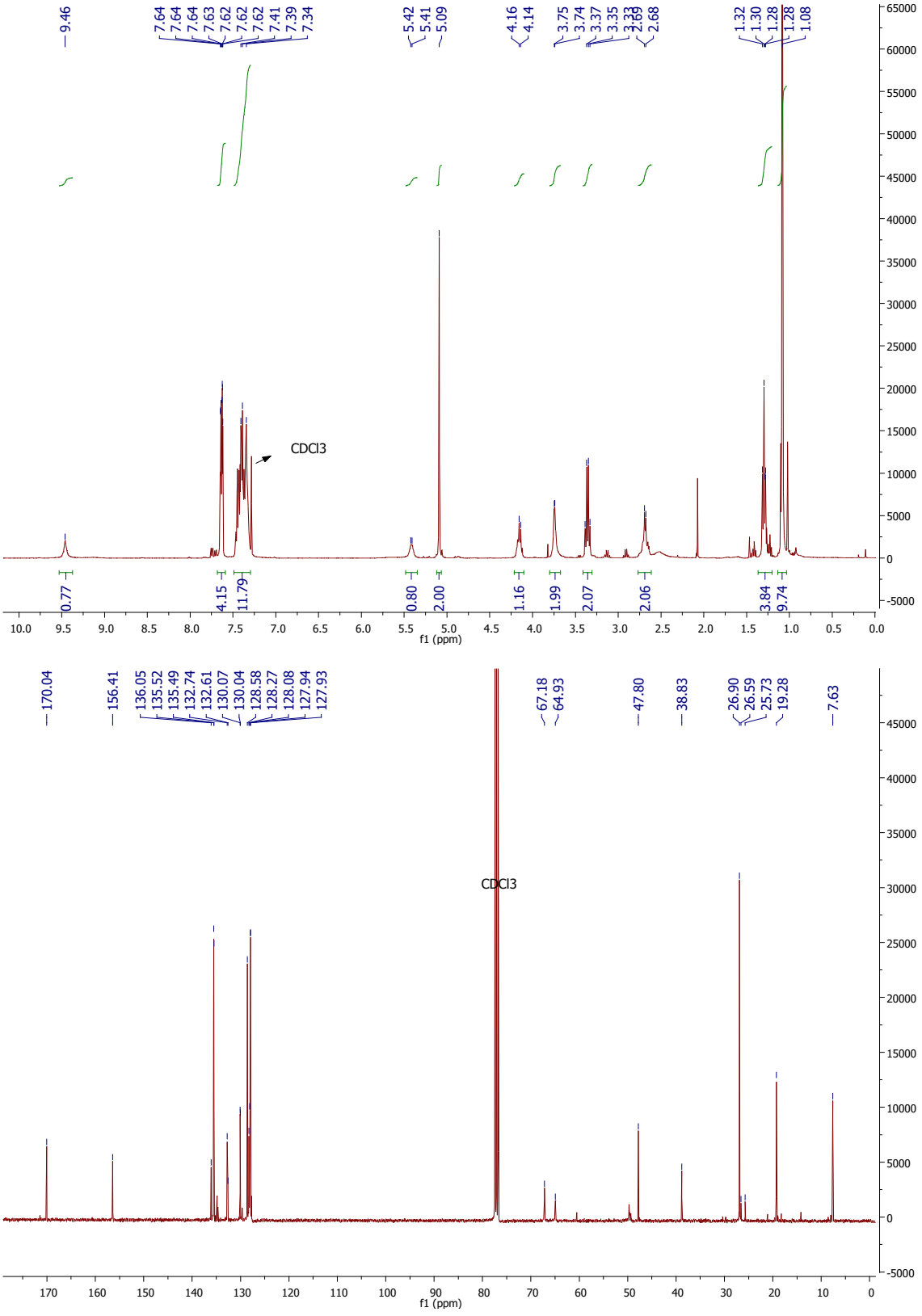

176.22
155.90
135.55
135.53
135.13
134.81
132.86
132.80
129.92
129.68
128.55
128.20
127.83
127.74
66.95
64.75
49.36
49.20
35.51
26.85
26.57
19.25
19.03
CDCl3
f1 (ppm)

Compound **12a**

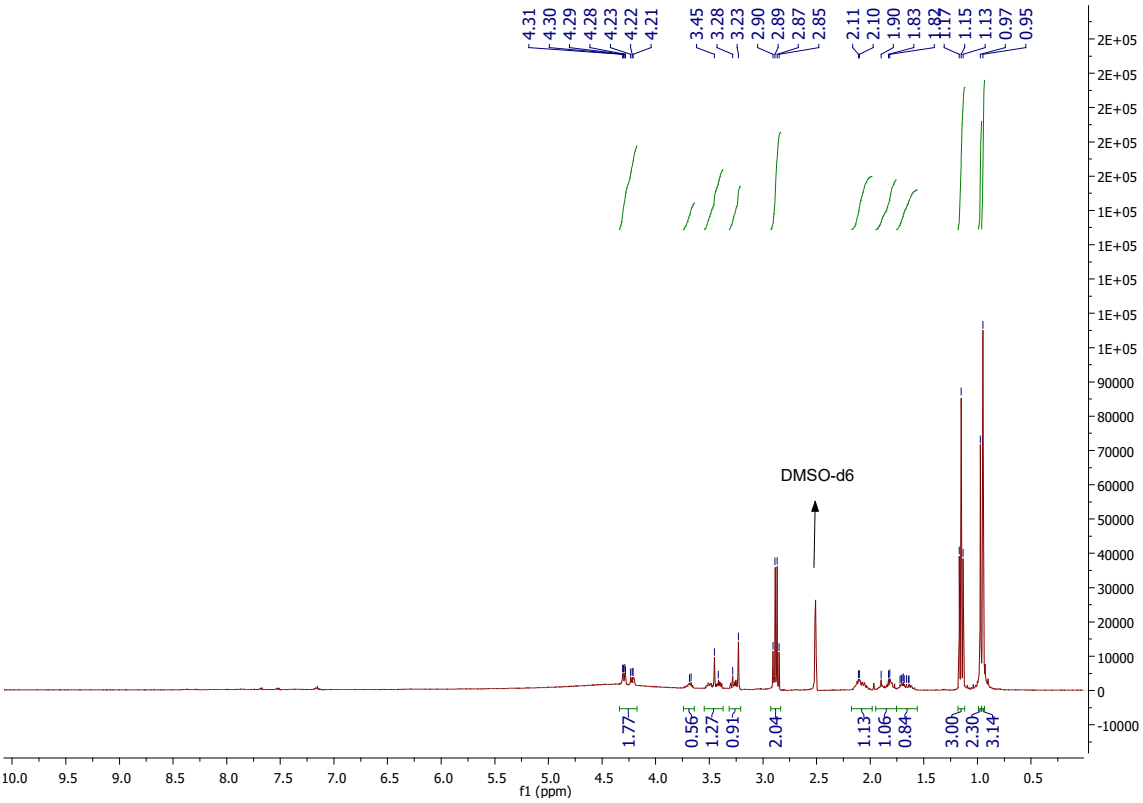


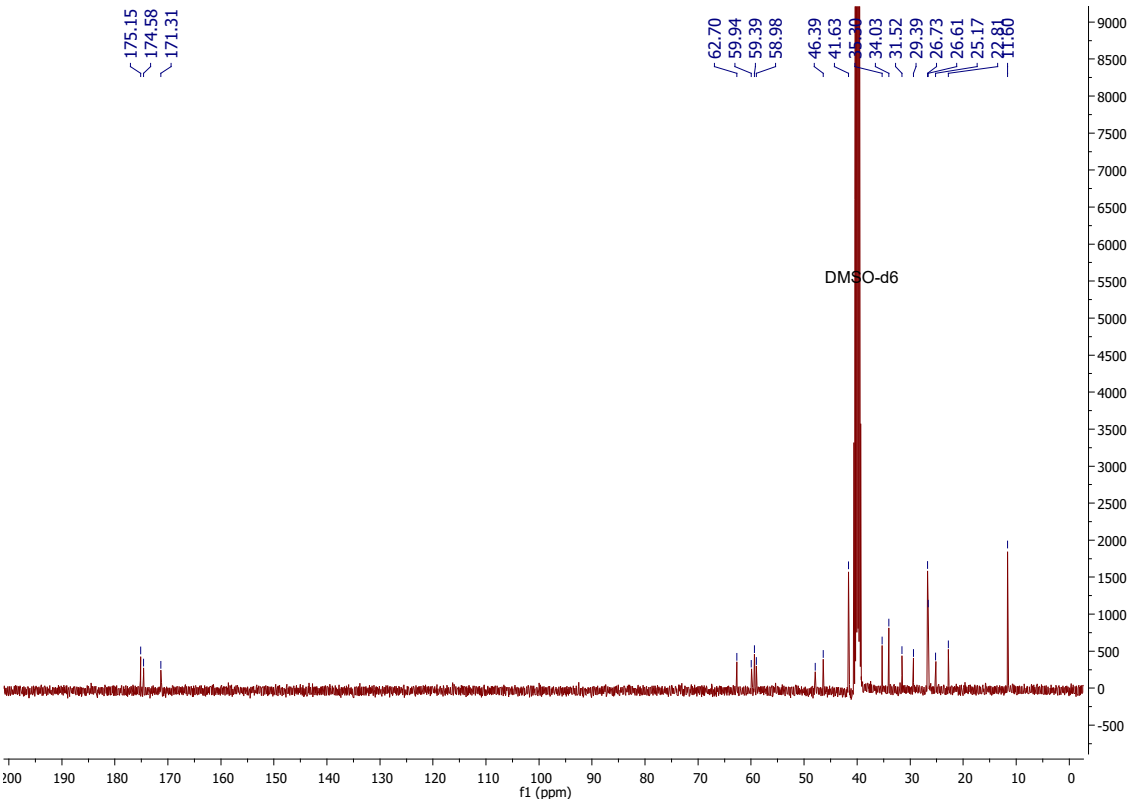


Compound **12b**

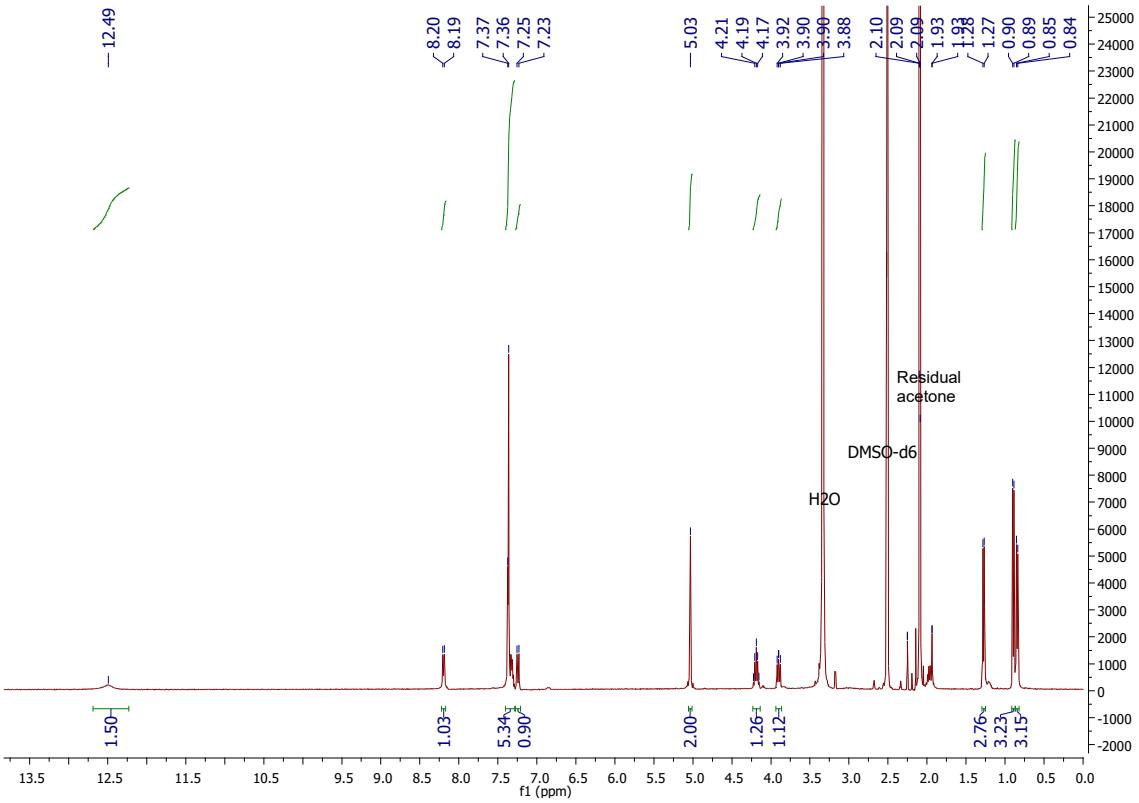


Compound **16**

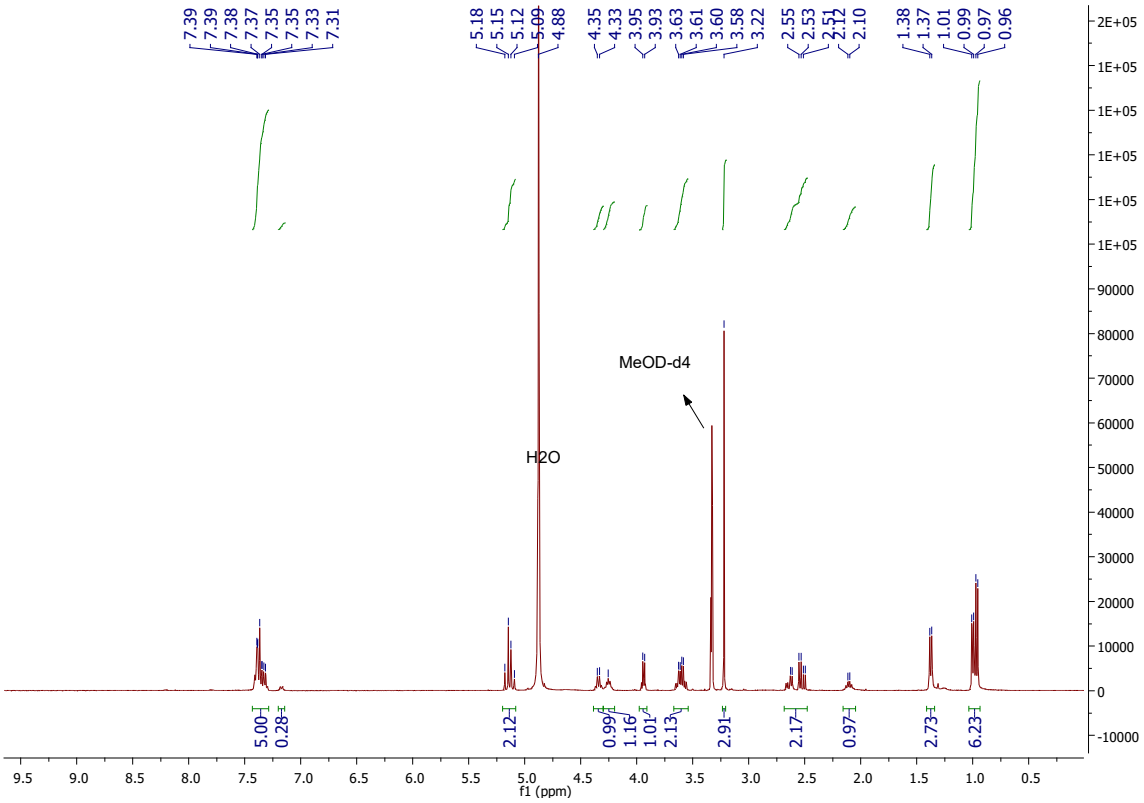

4.31
4.30
4.29
4.28
4.23
4.22
4.21
3.45
3.28
3.23
2.90
2.89
2.87
2.85
2.11
2.10
1.90
1.83
1.15
1.13
0.97
0.95
DMSO-d6
1.77
0.56
1.27
0.91
2.04
1.13
1.06
0.84
3.00
2.30
3.14
f1 (ppm)

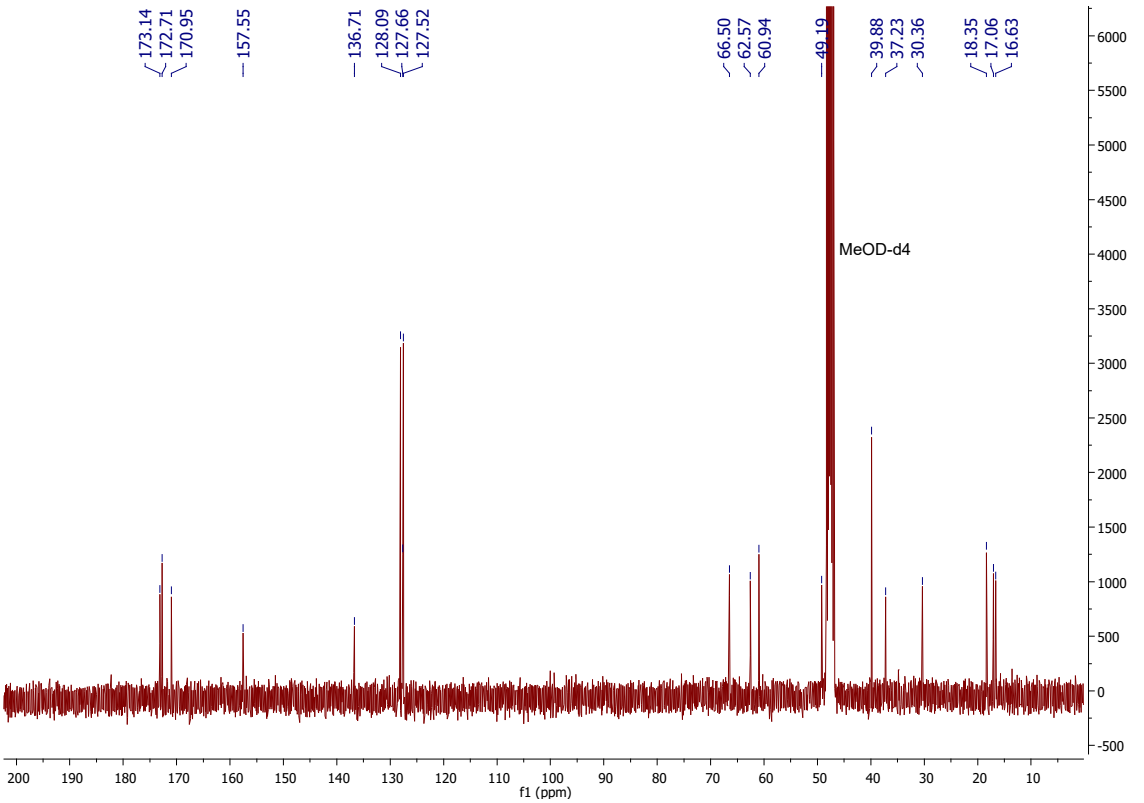

175.15
174.58
171.31
62.70
59.94
59.39
58.98
46.39
41.63
35.30
34.03
31.52
29.39
26.73
26.61
25.17
22.81
11.60
DMSO-d6
f1 (ppm)

Compound **22**

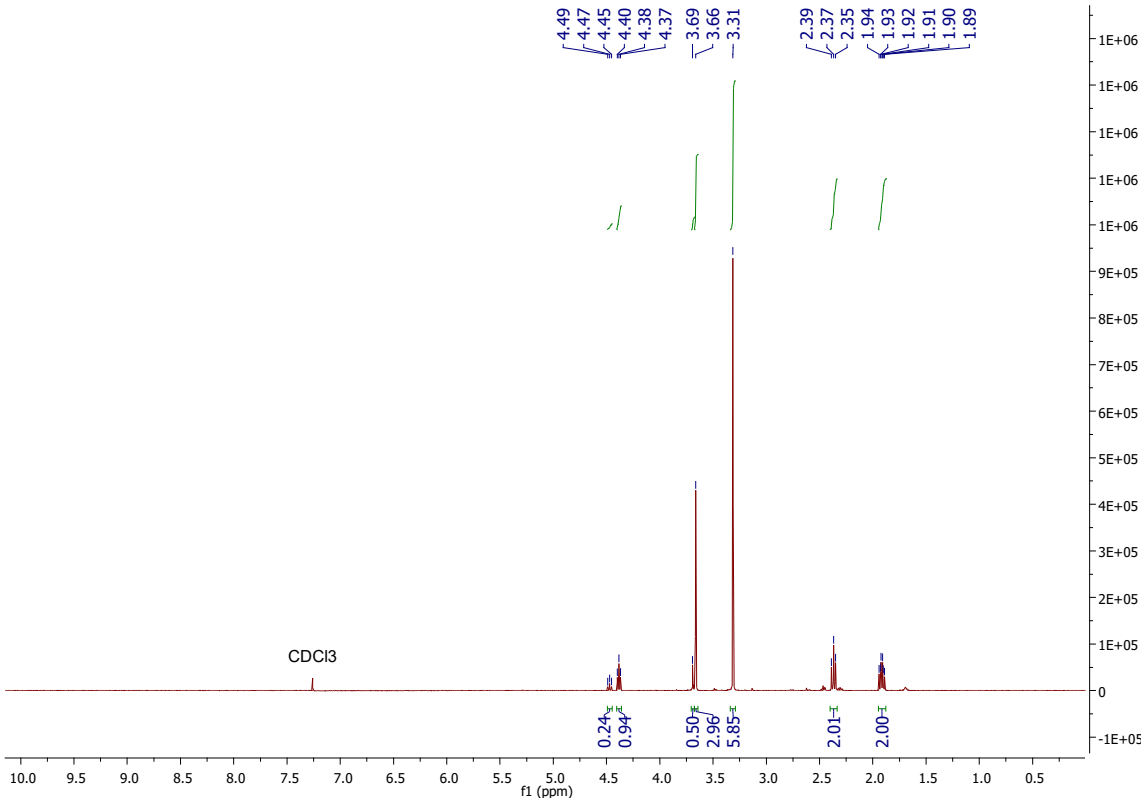

12.49
8.20
8.19
7.37
7.36
7.25
7.23
5.03
4.21
4.19
4.17
3.92
3.90
3.90
3.88
2.10
2.09
2.09
1.93
1.28
1.27
0.90
0.89
0.85
0.84
Residual acetone
DMSO-d6
H2O
1.50
1.03
5.34
0.90
2.00
1.26
1.12
2.76
3.23
3.15
f1 (ppm)

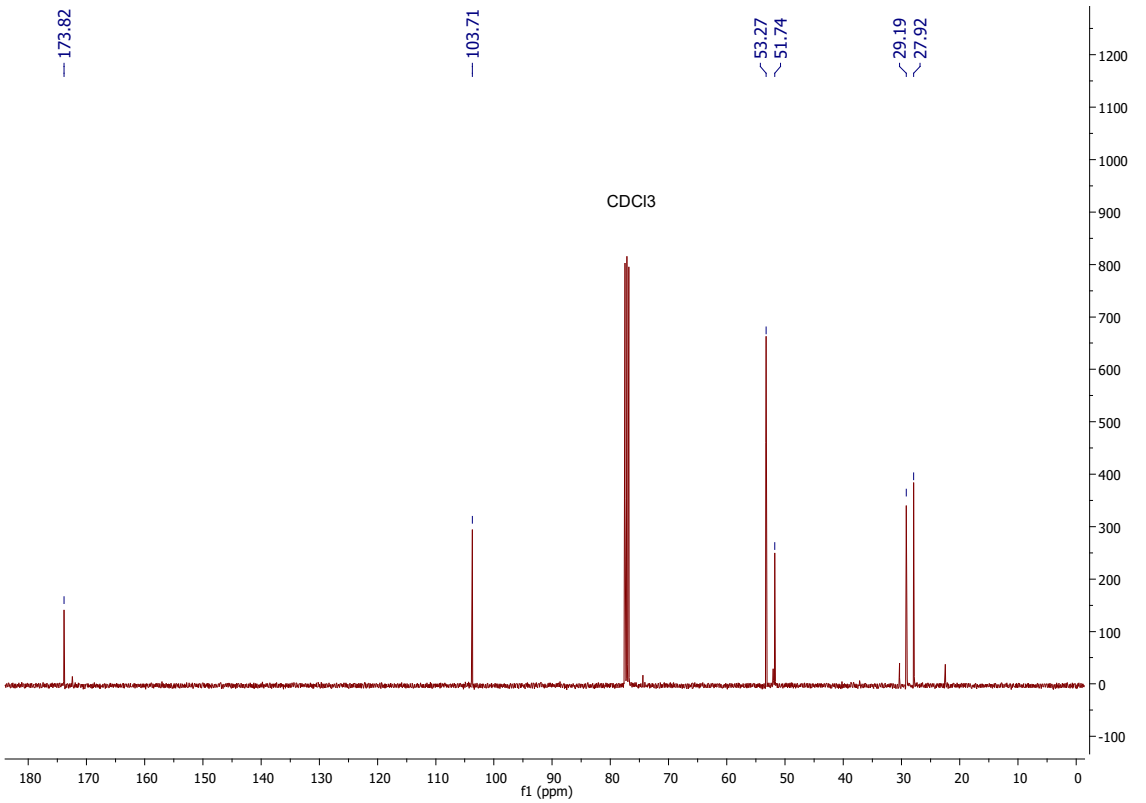
Compound **23**


7.39
7.39
7.38
7.37
7.35
7.35
7.33
7.31
5.18
5.15
5.12
5.09
4.88
4.35
4.33
3.95
3.93
3.63
3.61
3.60
3.58
3.22
2.55
2.53
2.51
2.12
2.10
1.38
1.37
1.01
0.99
0.97
0.96
MeOD-d4
H2O
5.00
0.28
2.12
0.99
1.16
1.01
2.13
2.91
2.17
0.97
2.73
6.23
f1 (ppm)

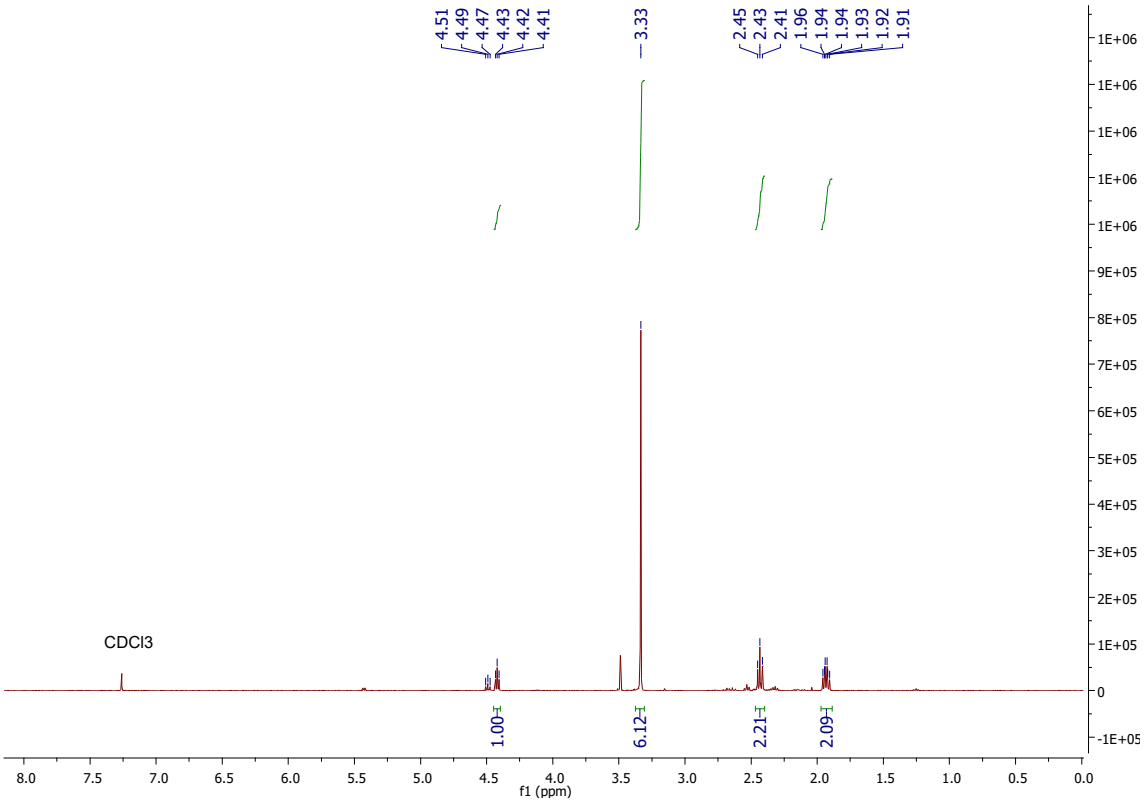

173.14
172.71
170.95
157.55
136.71
128.09
127.66
127.52
66.50
62.57
60.94
49.19
39.88
37.23
30.36
18.35
17.06
16.63
MeOD-d4
f1 (ppm)

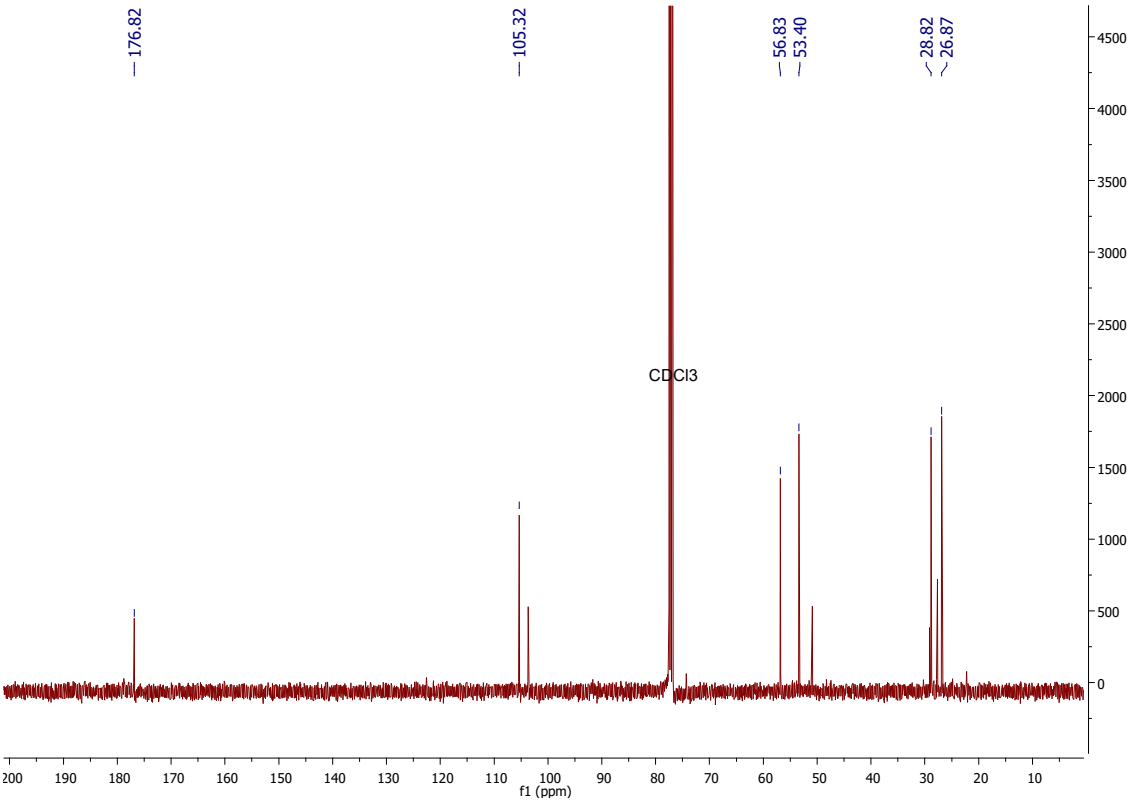
Comound **26**


4.49
4.47
4.45
4.40
4.38
4.37
3.69
3.66
3.31
2.39
2.37
2.35
1.94
1.93
1.92
1.91
1.90
1.89
CDCl3
0.24
0.94
0.50
2.96
5.85
2.01
2.00
f1 (ppm)

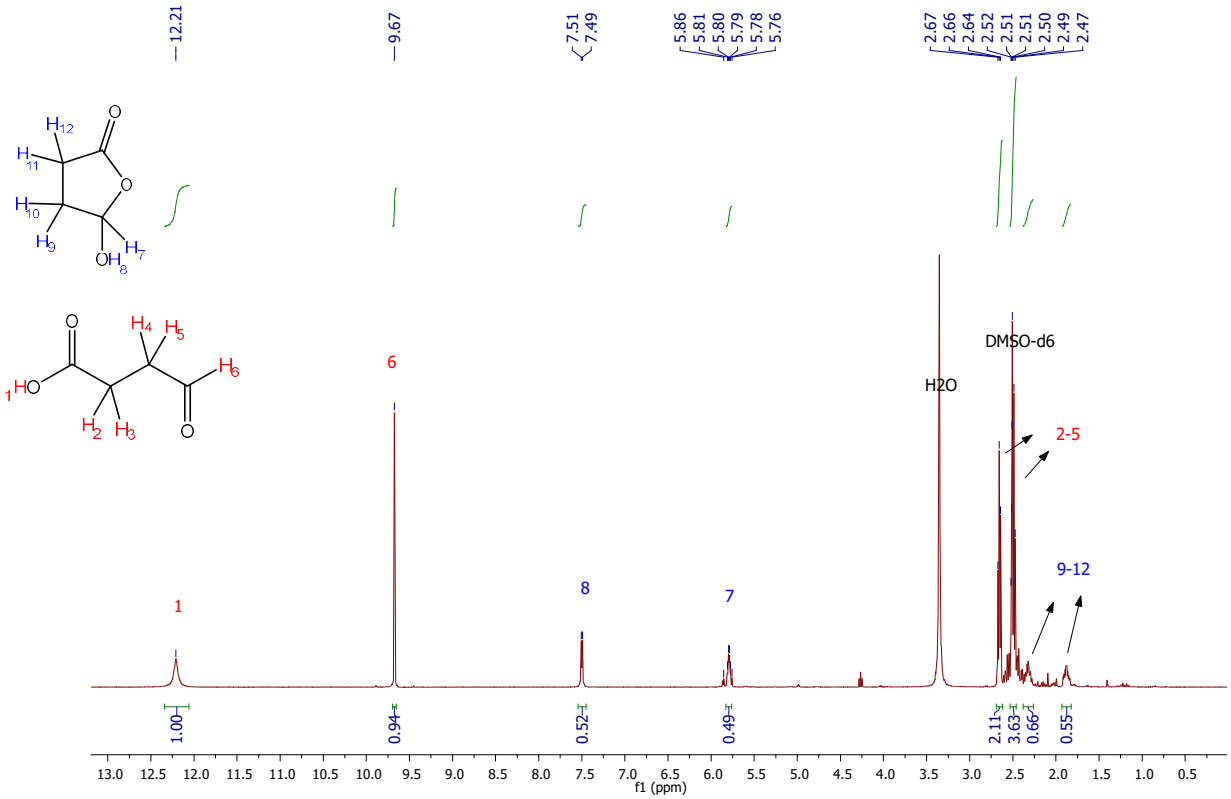

173.82
103.71
53.27
51.74
29.19
27.92
CDCl3
f1 (ppm)

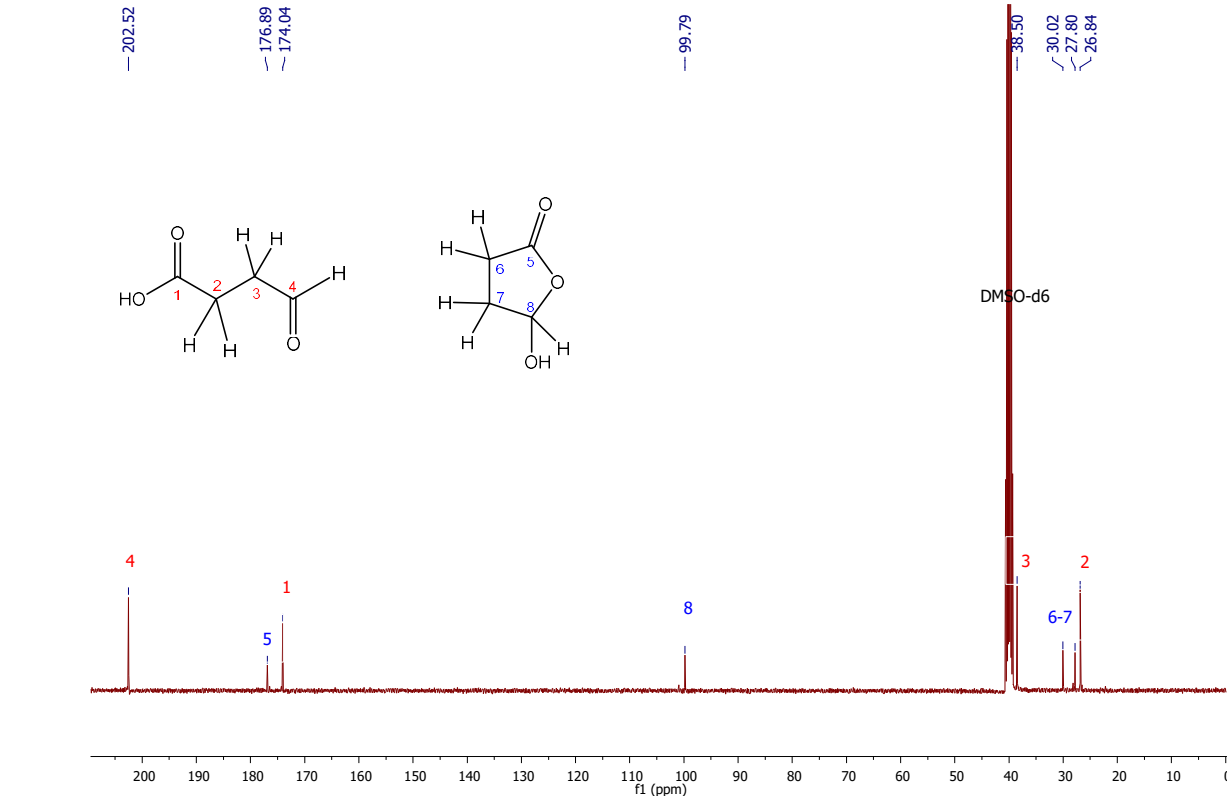
Compound **27**


4.51
4.49
4.47
4.43
4.42
4.41
3.33
2.45
2.43
2.41
1.96
1.94
1.94
1.93
1.92
1.91
CDCl3
1.00
6.12
2.21
2.09
f1 (ppm)

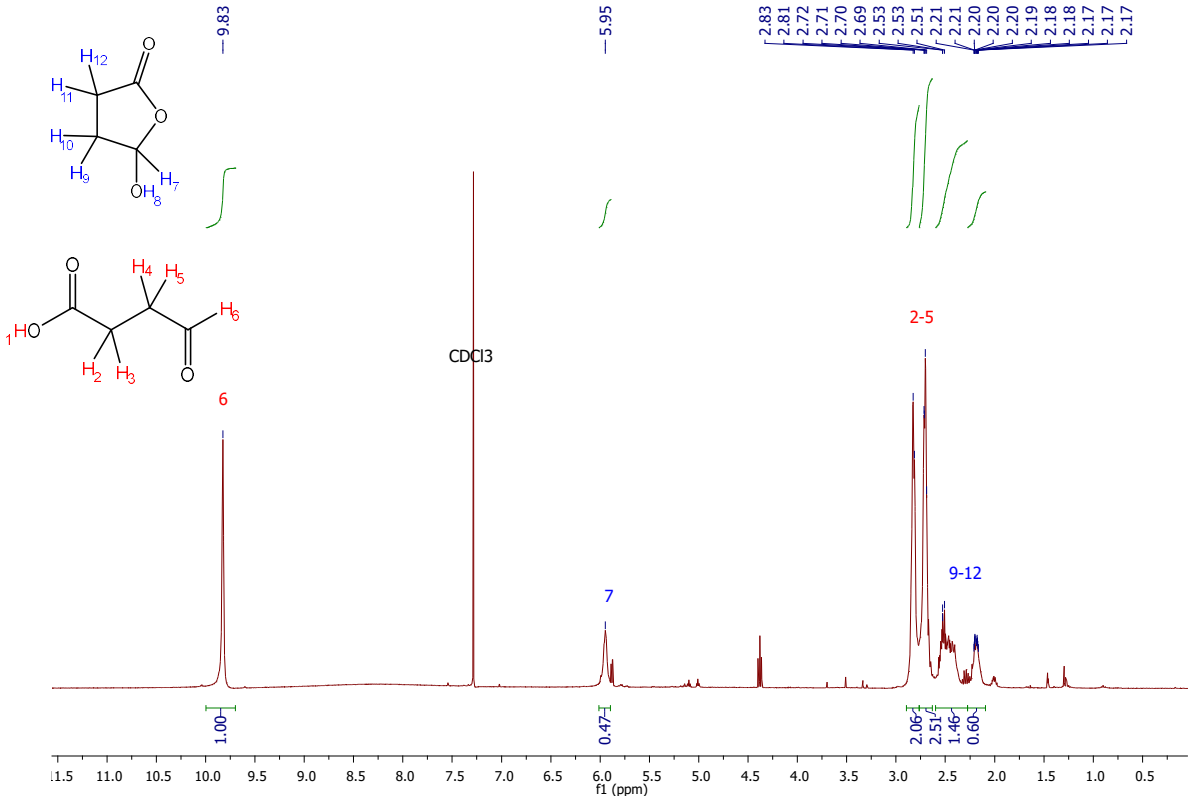

176.82
105.32
56.83
53.40
28.82
26.87
CDCl3
f1 (ppm)

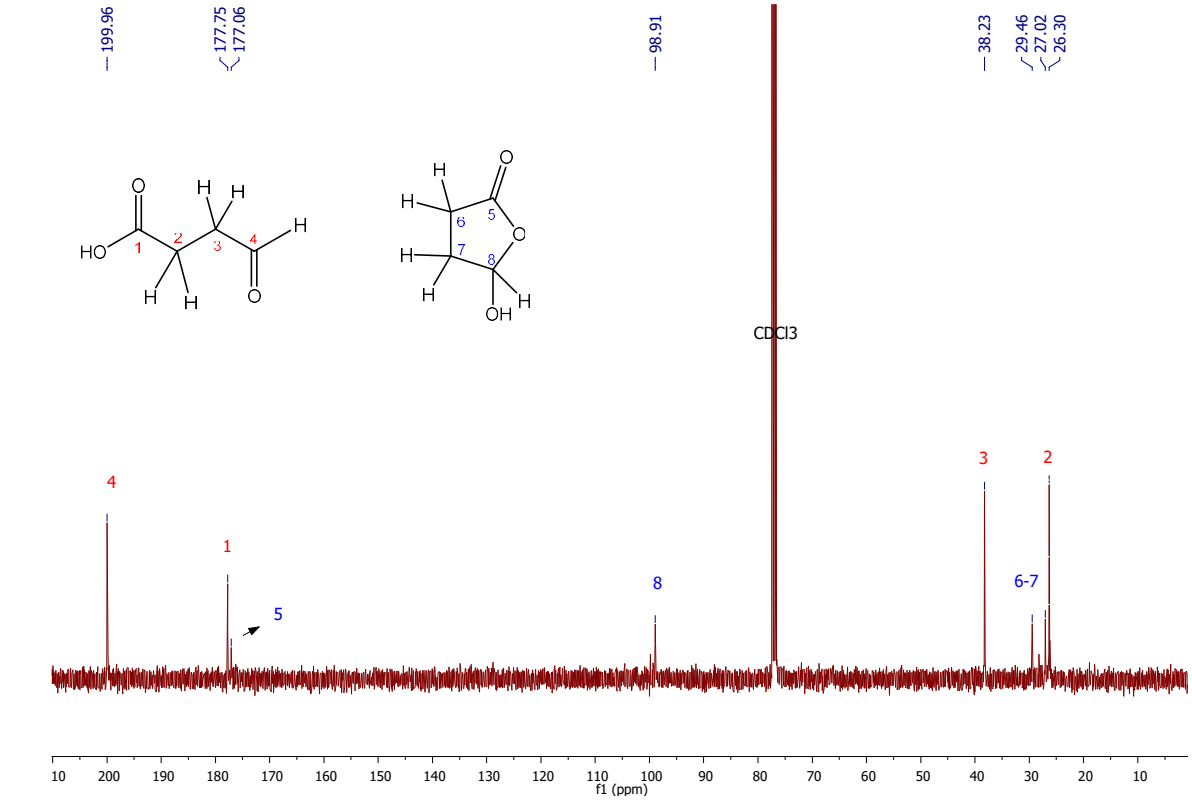
Compound **24**, **29a** or **29b** (in DMSO-d_6)



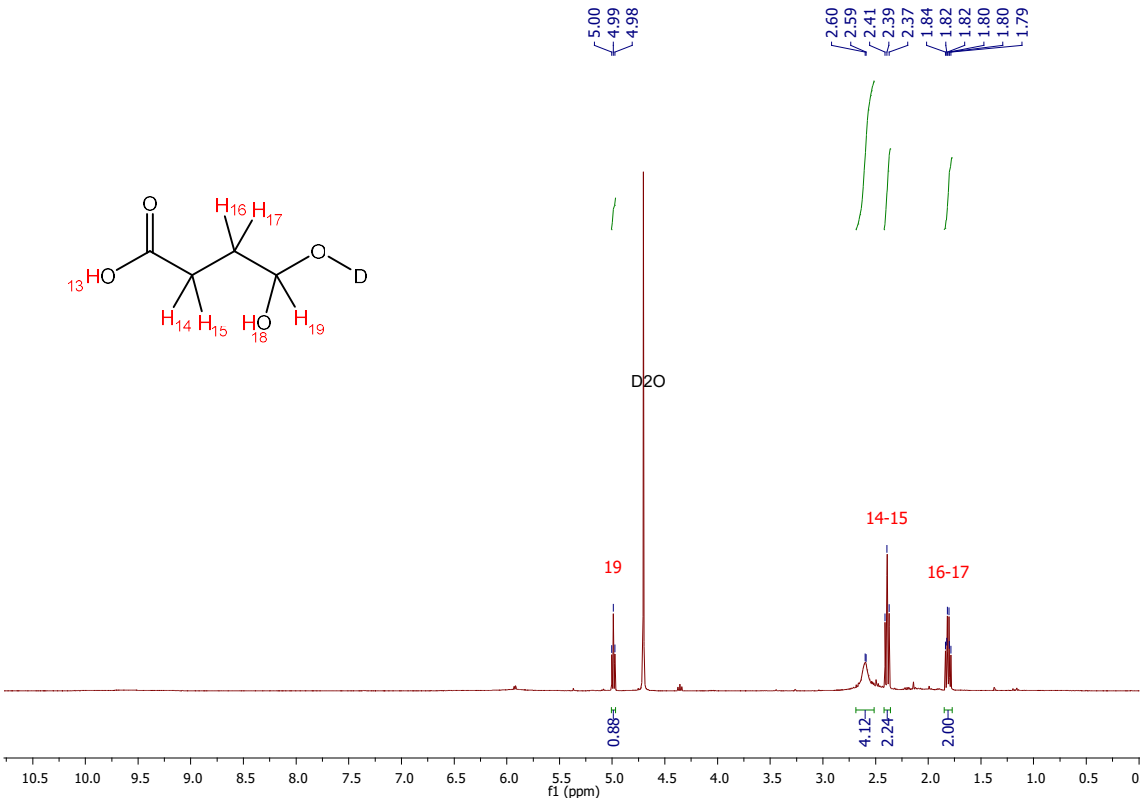


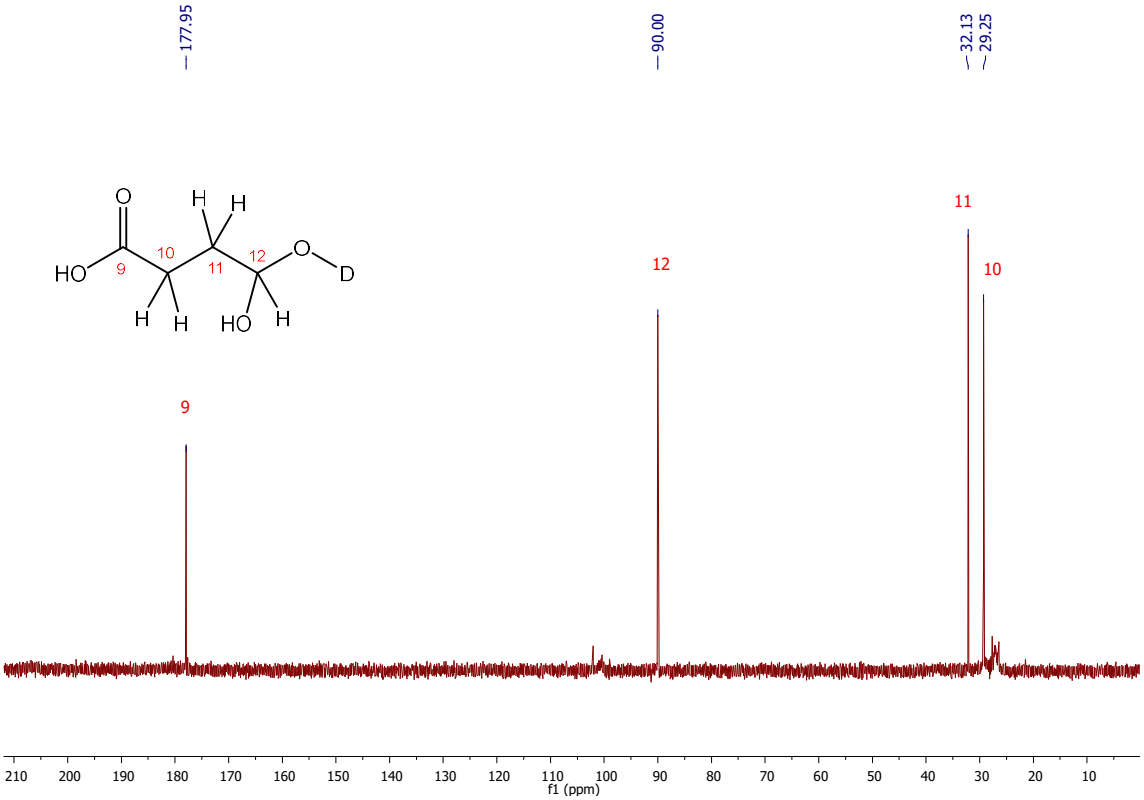
Compound **24, 29a** or **29b** (in $CDCl_3$)



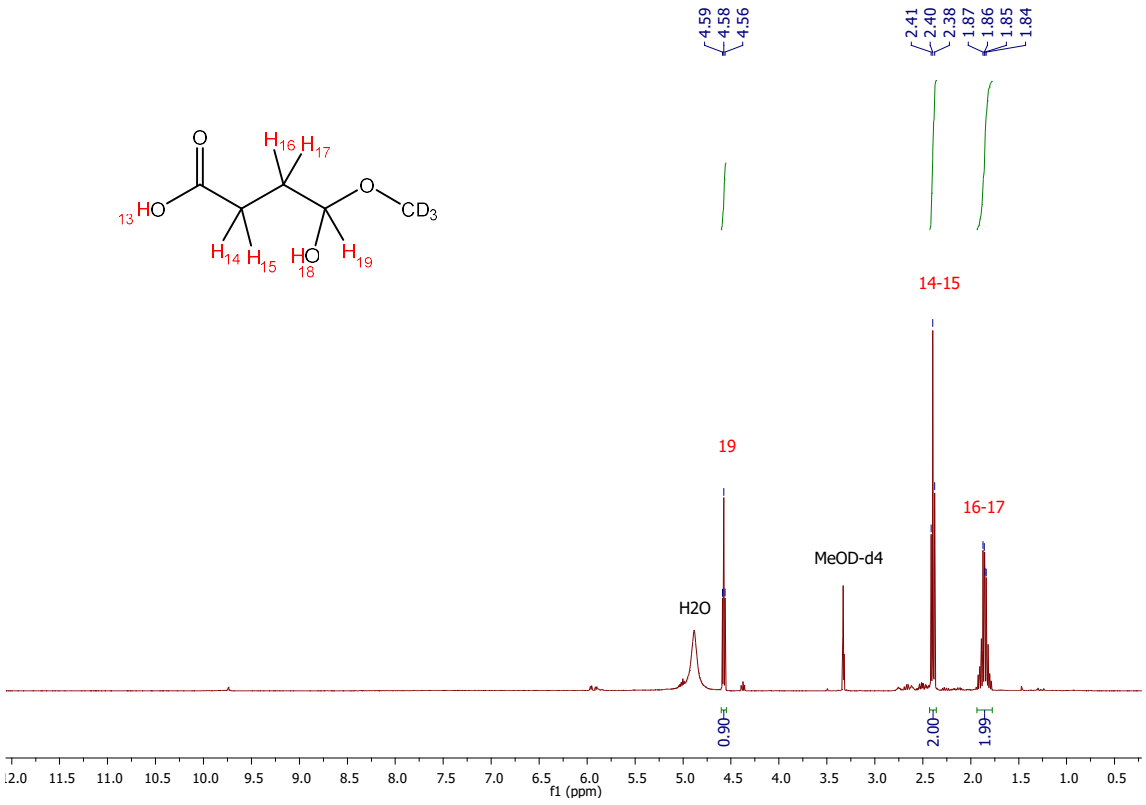


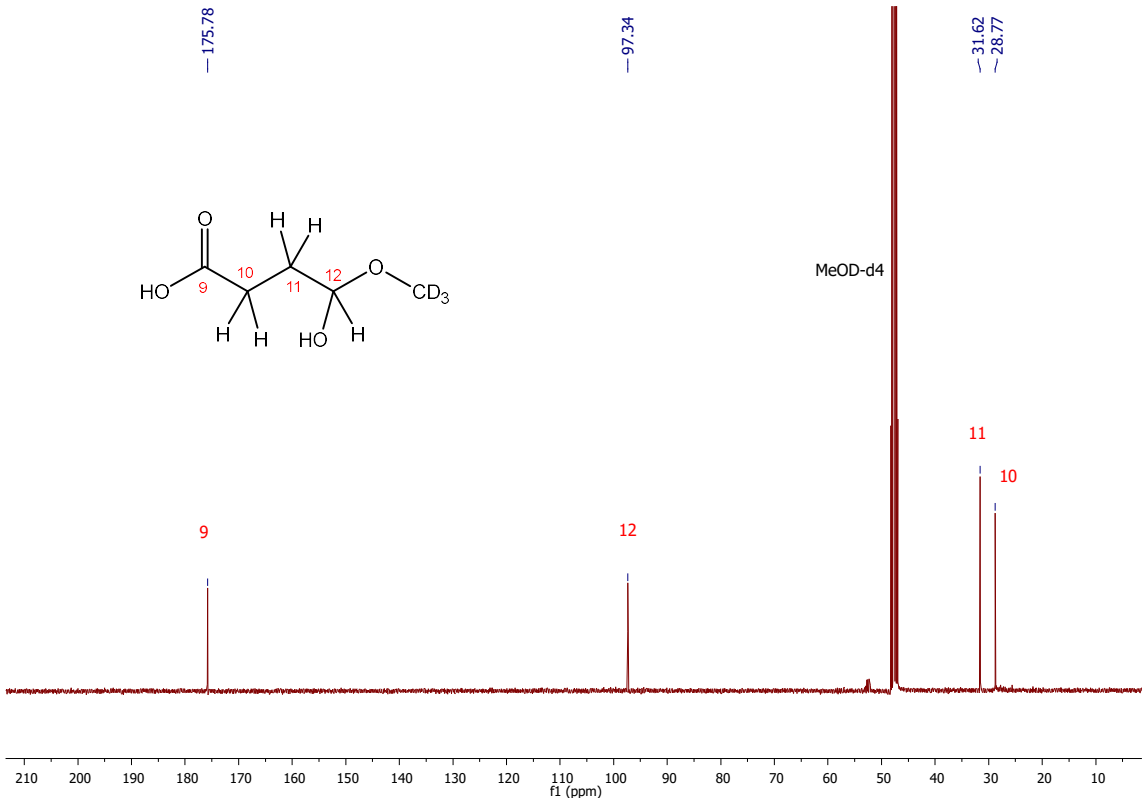
Compound **24**, **29a** or **29b** (in D_2O)



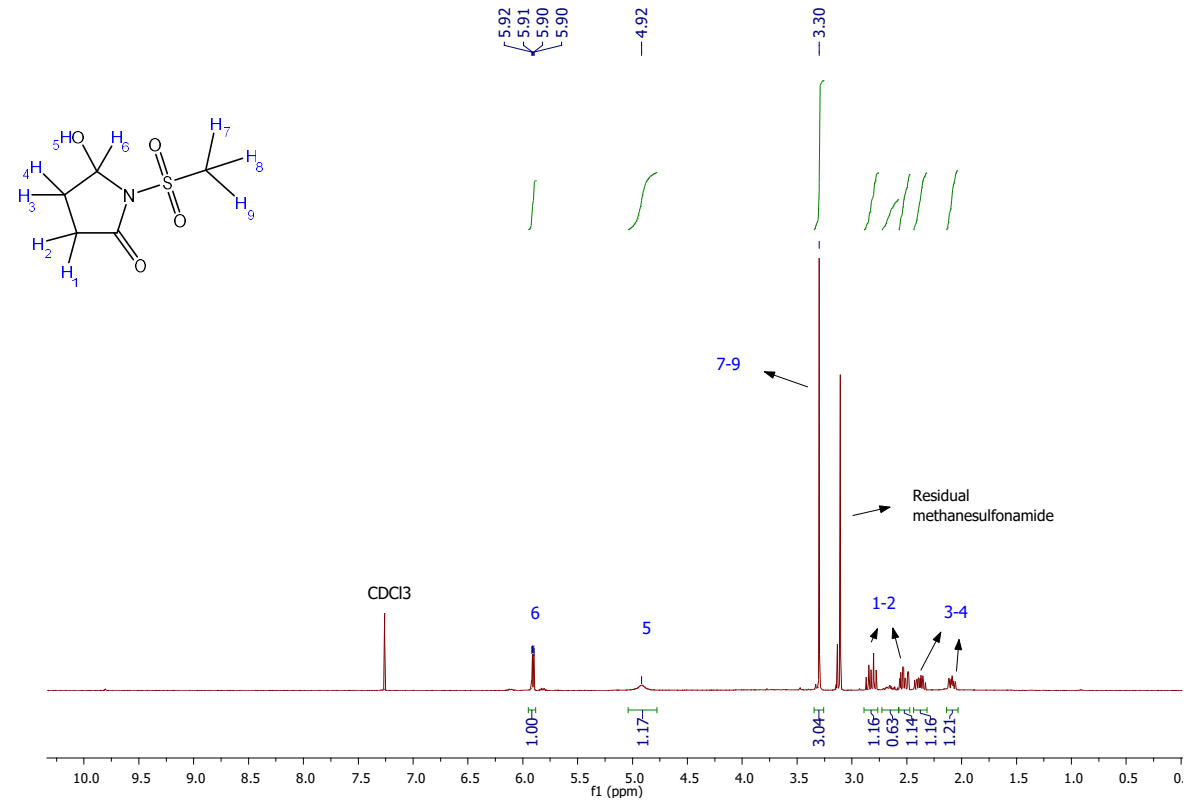


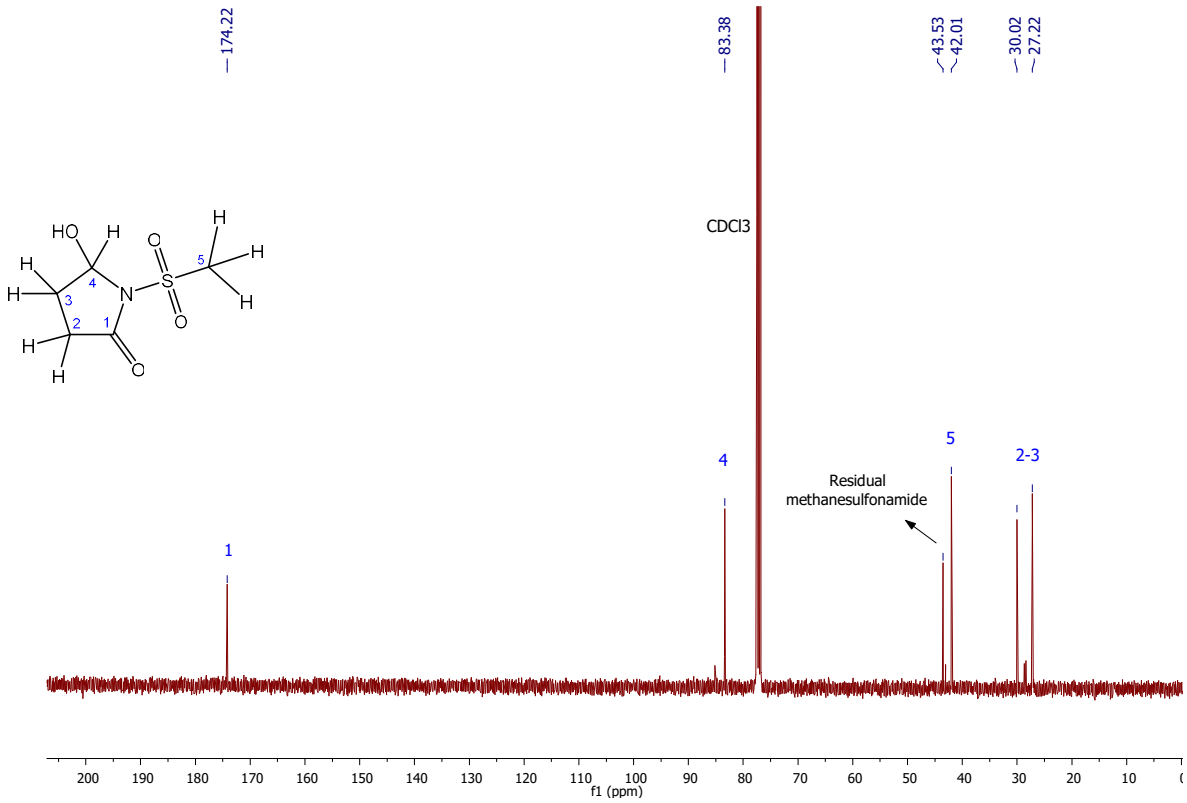
Compound **24**, **29a** or **29b** (in MeOD-d_4)



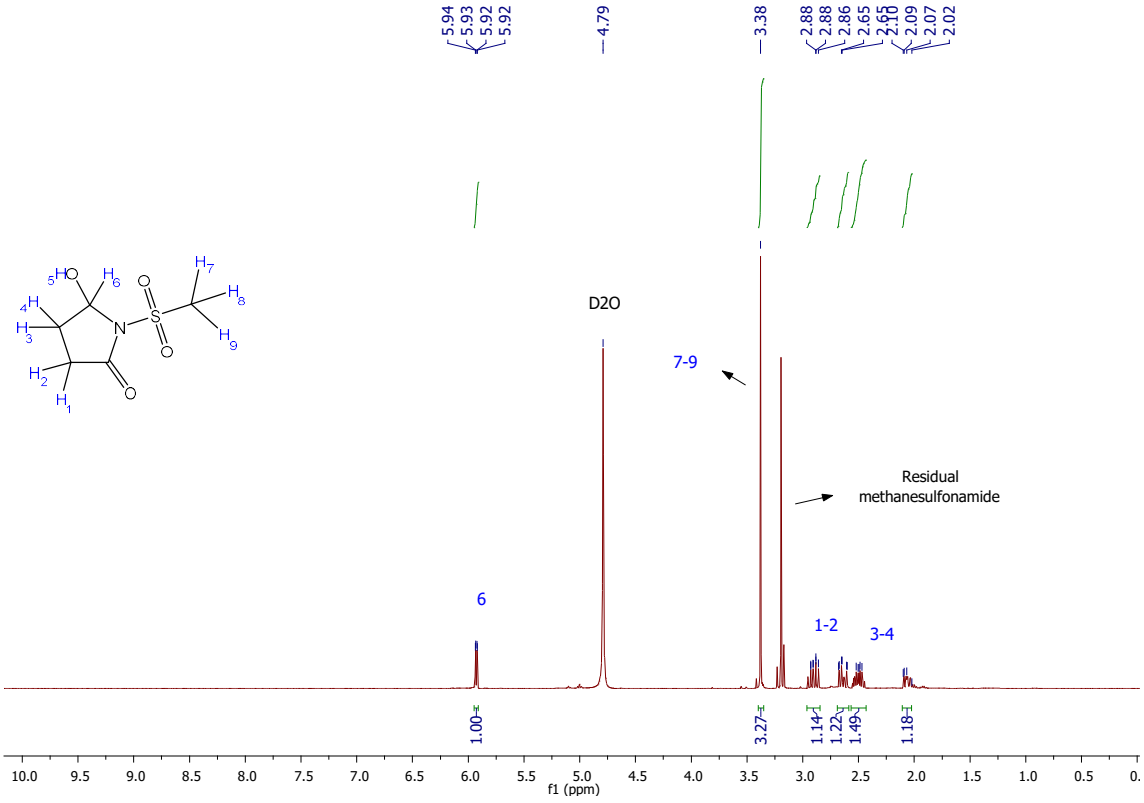


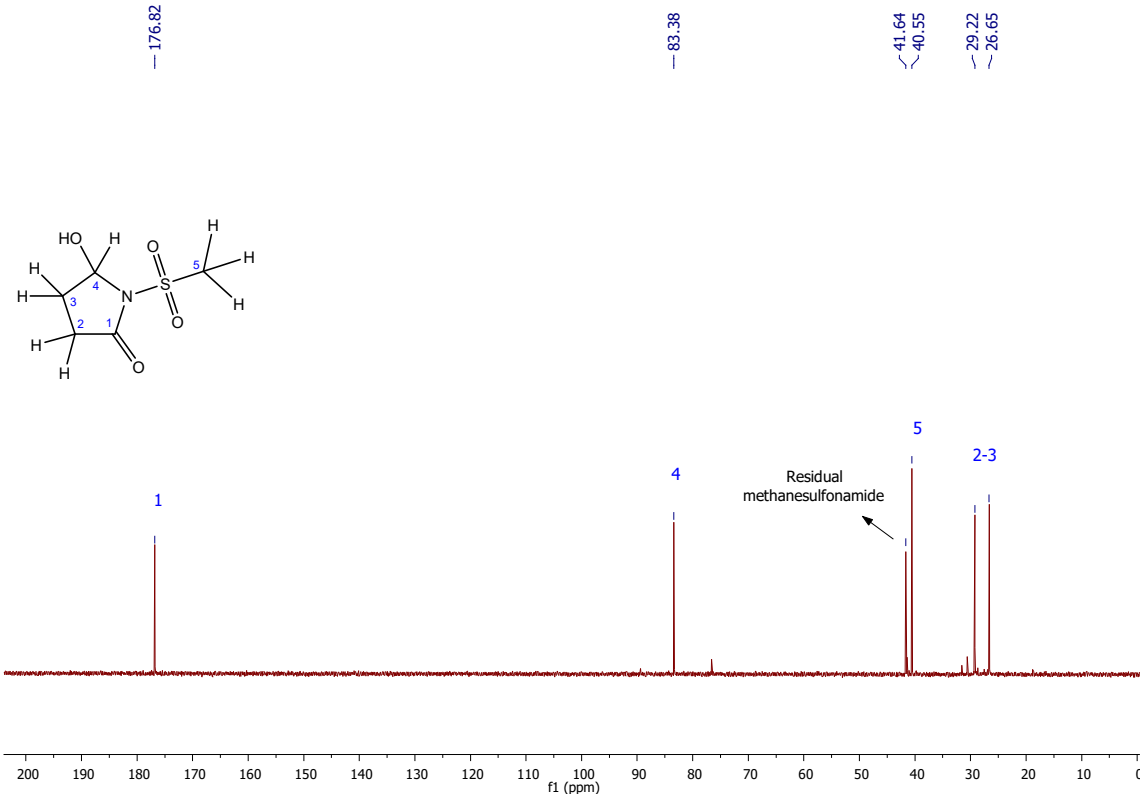
Compound **28**, **30a** or **30b** (in $CDCl_3$)



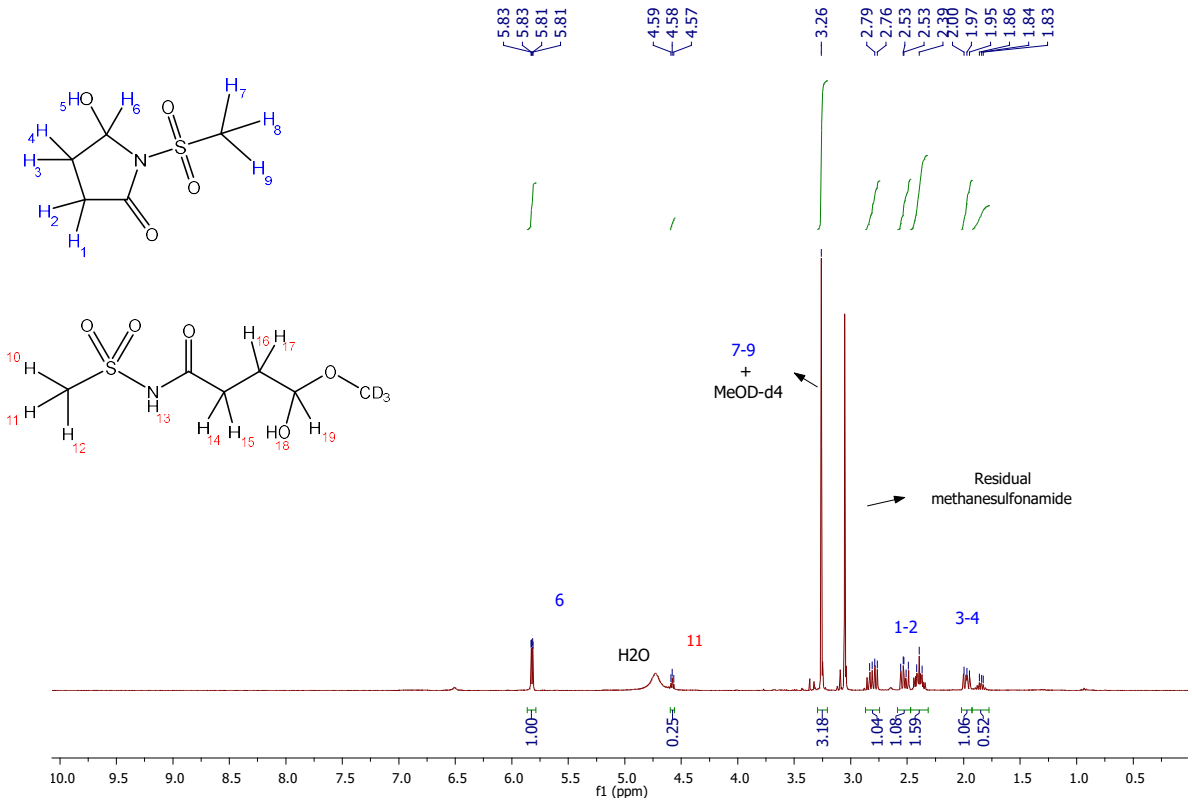


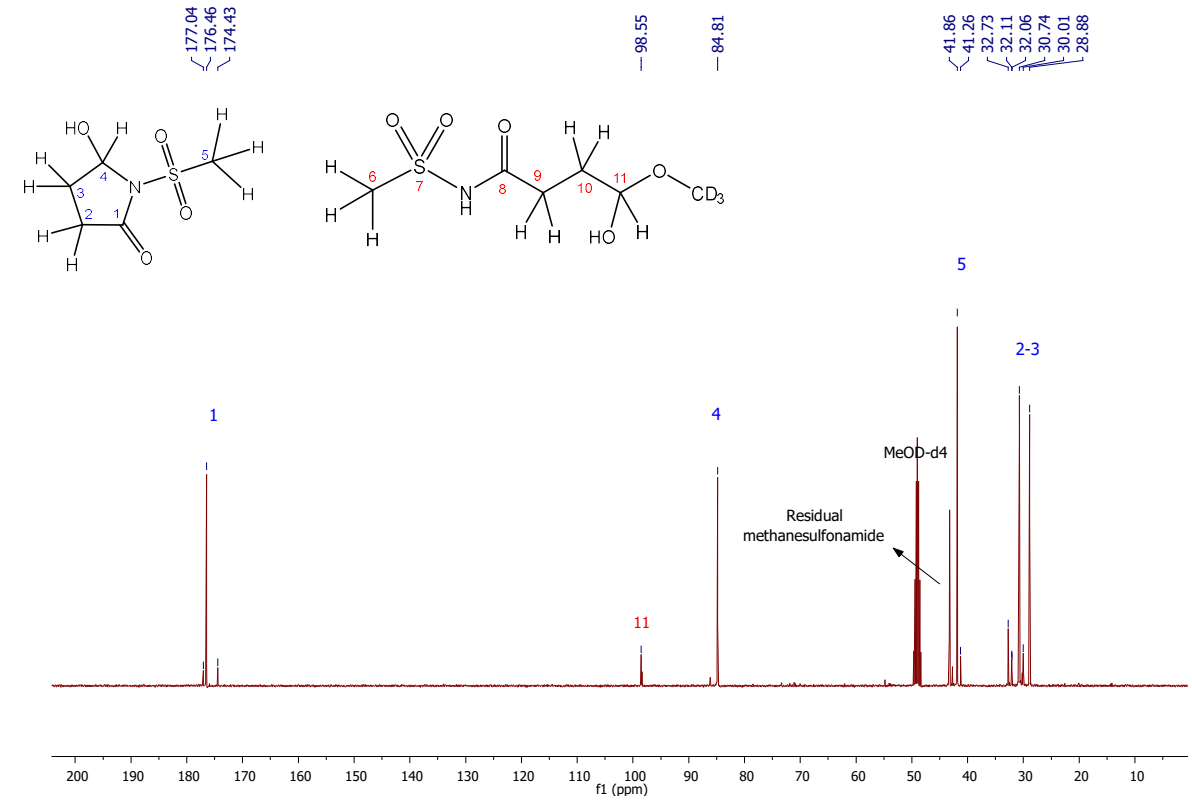
Compound **28**, **30a** or **30b** (in D_2O)





Compound **28**, **30a** or **30b** (in MeOD-d_4)

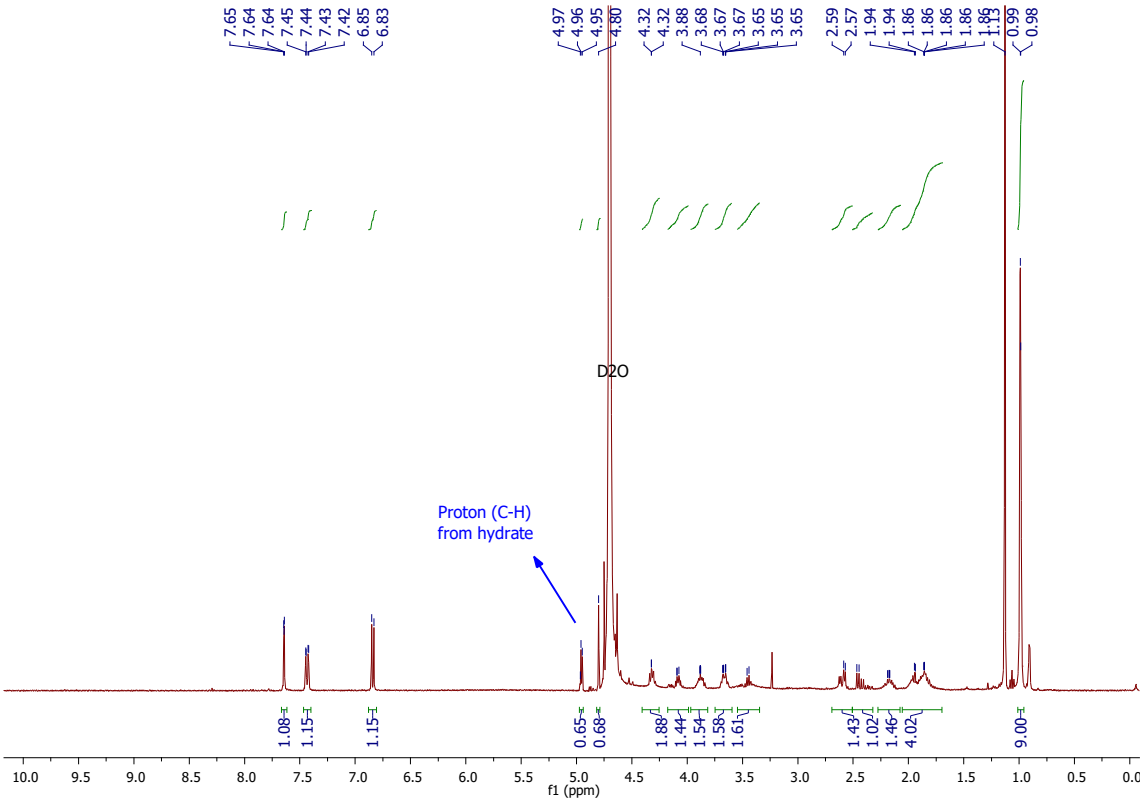




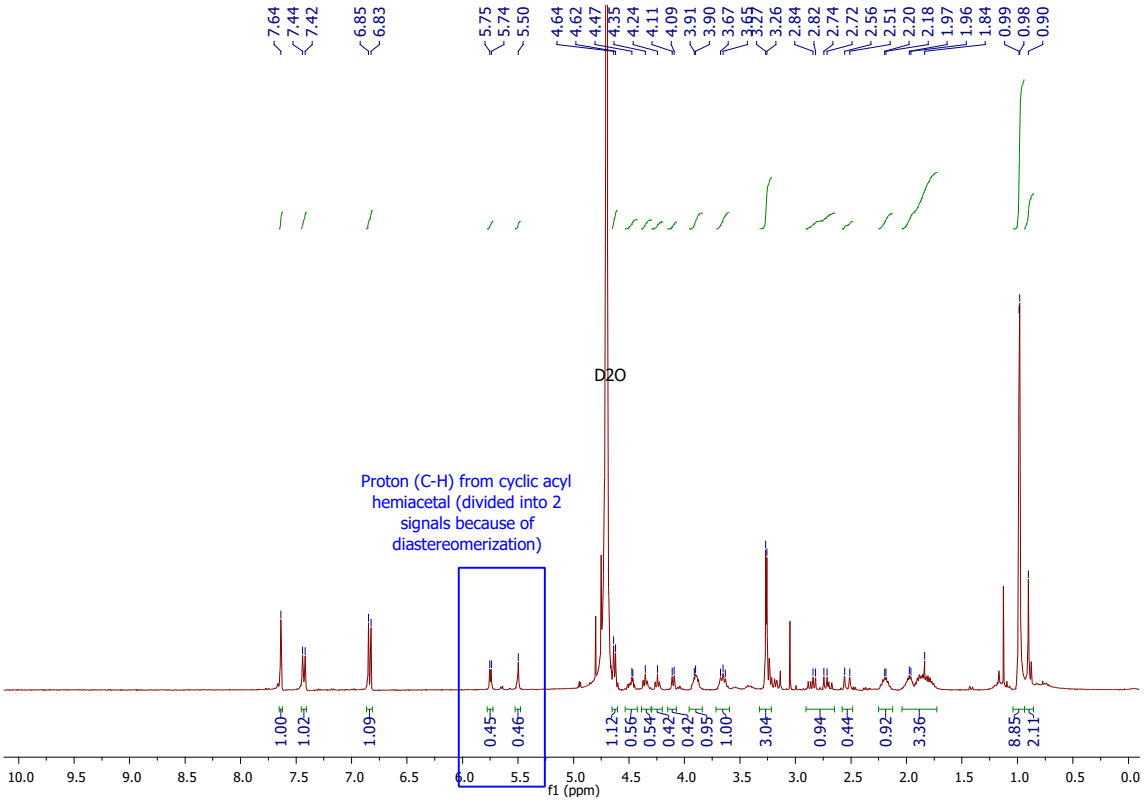
^{1}H NMR experiments in D_2O

1-2 mg of final compounds **2**, **3**, **7** and **8** were dissolved in D_2O, according to their solubility in this solvent. Centrifugation was done to eliminate any undissolved material, after which the liquid was transferred into an NMR tube. ^{1}H NMR was performed and the aldehydic proton (or this specific proton in any other formation) was highlighted in the spectrum for each compound. The chemical shift of this proton is comparable to the chemical shifts presented in **Table 3** (in case of carboxylic acid inhibitors) and **Table 4** (in case of acyl sulfonamide inhibitors) of this publication.

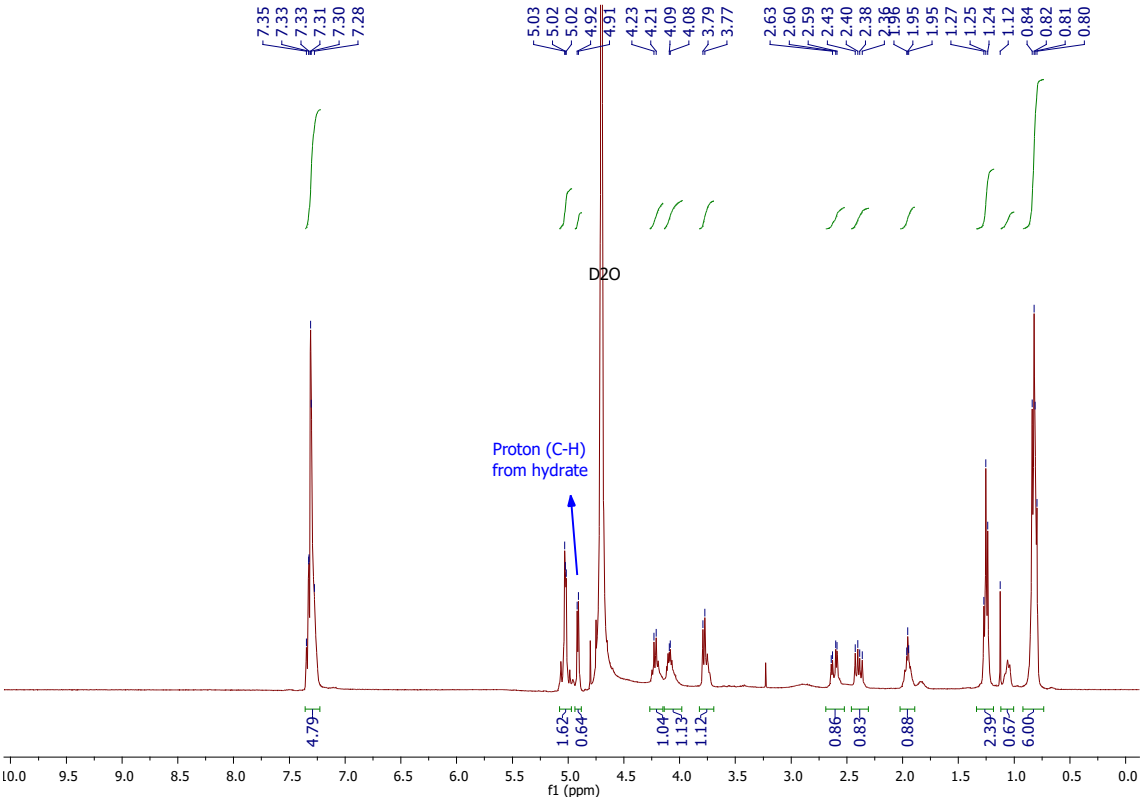
Compound **2**:



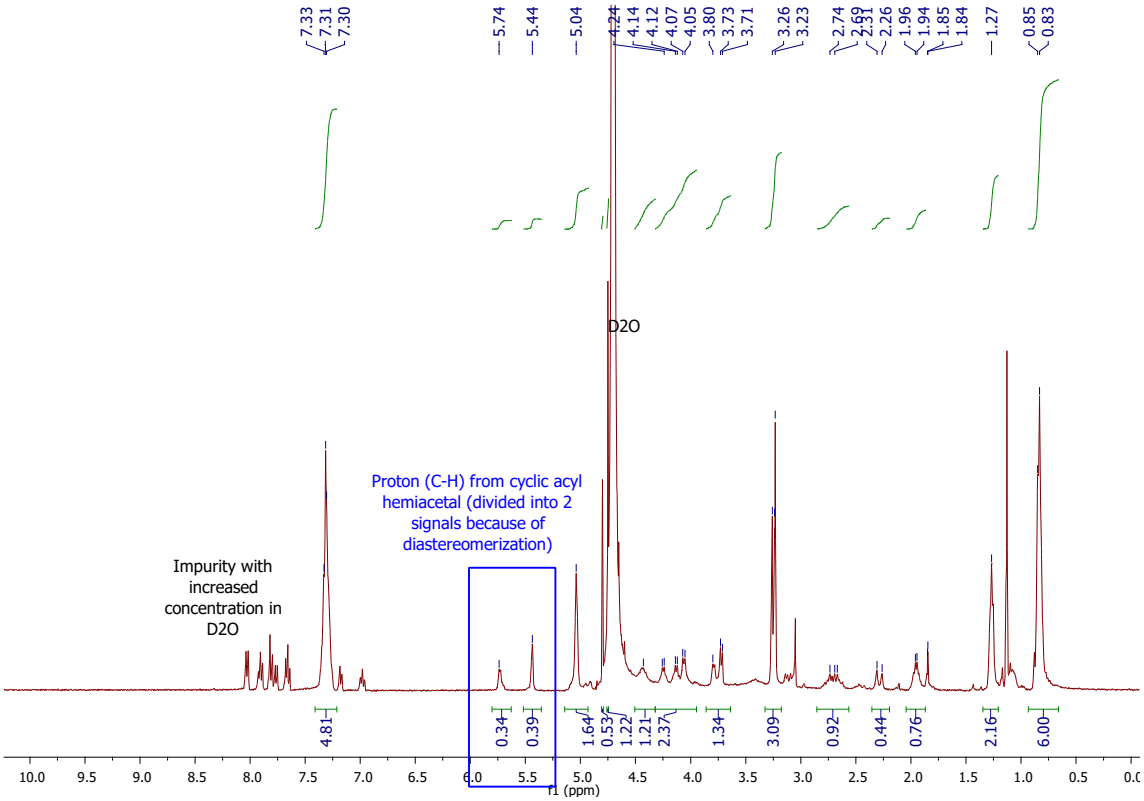
Compound **3**:



Compound **7**:



Compound **8**:


7.33
7.31
7.30
5.74
5.44
5.04
4.24
4.14
4.12
4.07
4.05
3.80
3.73
3.71
3.26
3.23
2.74
2.69
2.31
2.26
1.96
1.94
1.85
1.84
1.27
0.85
0.83
D2O
Proton (C-H) from cyclic acyl hemiacetal (divided into 2 signals because of diastereomerization)
Impurity with increased concentration in D2O
4.81
0.34
0.39
1.64
0.53
1.22
1.21
2.37
1.34
3.09
0.92
0.44
0.76
2.16
6.00
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)

Stability: Hydrolysis of acyl sulfonamide-aldehyde, into carboxylic acid-aldehyde

10 mM stock solutions in DMSO of compounds **3** and **4** were prepared. The stock solutions were diluted to a 50 µM solution with 10 mM PBS buffer (pH 7.4). The mixture was gently shaken at 37 °C. At different time points (0 min, 30 min, 1 h, 2 h, 3 h and 26 h) 100 µL was withdrawn which was analyzed with UPLC-MS. The percentage of parent acyl sulfonamide (**3**, **4**) remaining at each time point relative to the 0 min sample was then calculated from UPLC-MS peak area ratios. An additional measurement was done after 9 days, which was considered the time point for 100 % conversion. The percentage of carboxylate-based inhibitor (**2**) at each time point was calculated relative to the peak area at the 9 days time point. The samples were prepared in duplicate. UPLC-MS measurements were carried out on a Waters Acquity UPLC system coupled to a Waters TUV detector, ESI source and a Waters Acquity TQ Mass detector.