Supporting Information

SYNTHESIS AND CYTOTOXIC EVALUATION OF STEROIDAL ENDOPEROXIDE DERIVATIVES WITH HYDRAZIDE SIDE CHAIN AS ANTICANCER AGRNTS

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Effect of 6k on the cell morphological changes

In this work, the changes of the morphological characters in HepG2 cells were studies by Hoechst33342/PI staining, which to prove that the inhibitory activity of compound **6k** was related to the inducing of cell apoptosis. As shown in Figure S1, staining the cells with PI incorporation (red) and Hoechst 33342 nuclei staining (blue) showed the typical features of apoptosis such as chromatin condensation, and formation of apoptotic bodies.

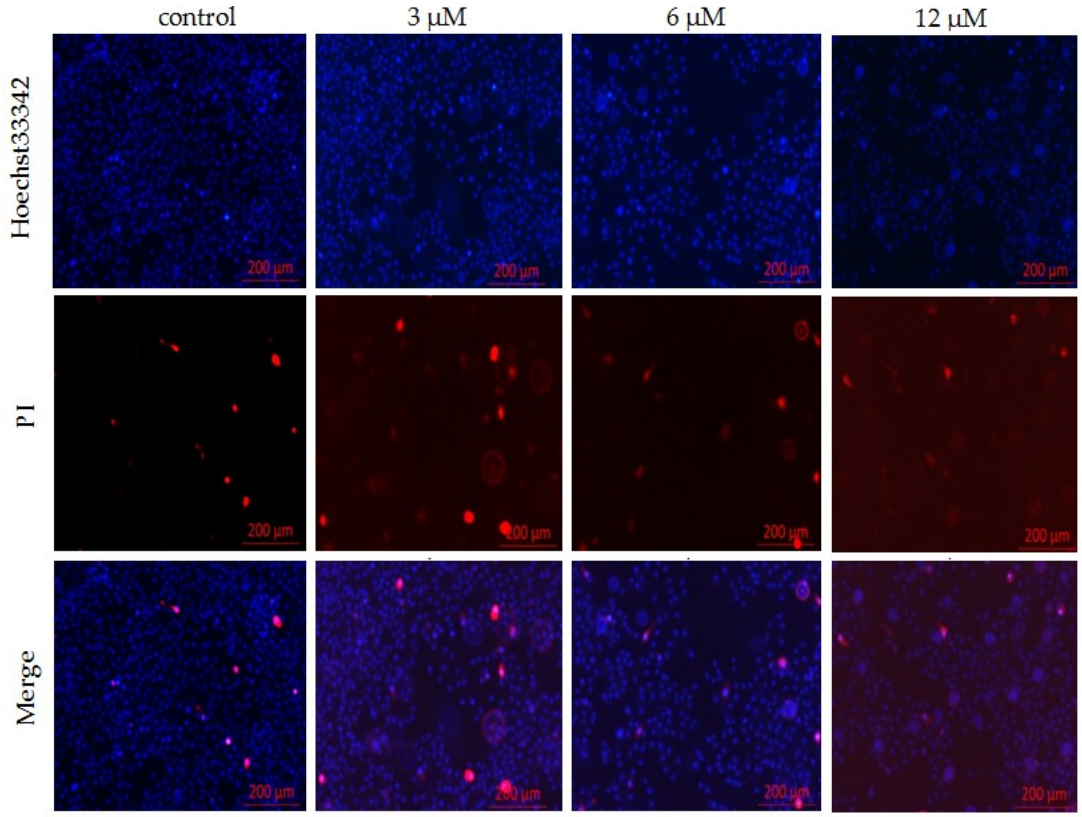
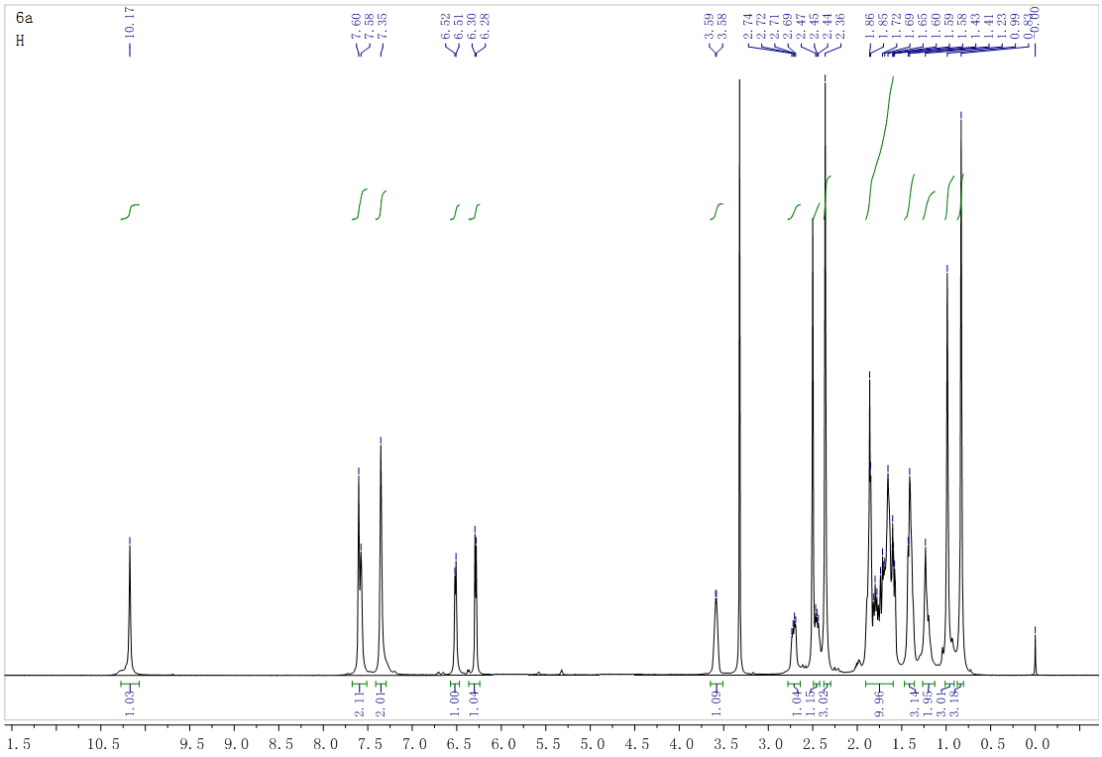
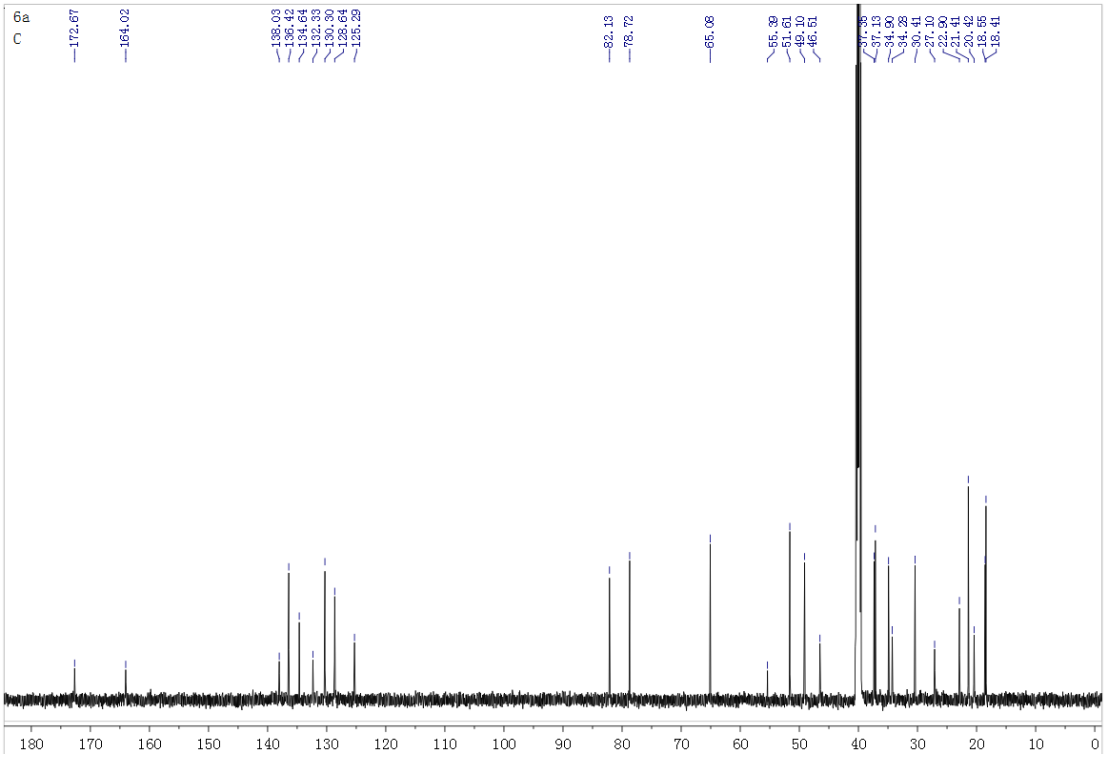


Figure S1. Compound **6k** induced morphological changes of HepG2 cells. HepG2 cells were treated with compound **6k** and then stained with Hoechst 33342/PI to reflect changes in cell morphology. Images were taken on a fluorescence microscope and depict PI incorporation (red) and Hoechst 33342 nuclei staining (blue). (Scale bar: 200 μm).

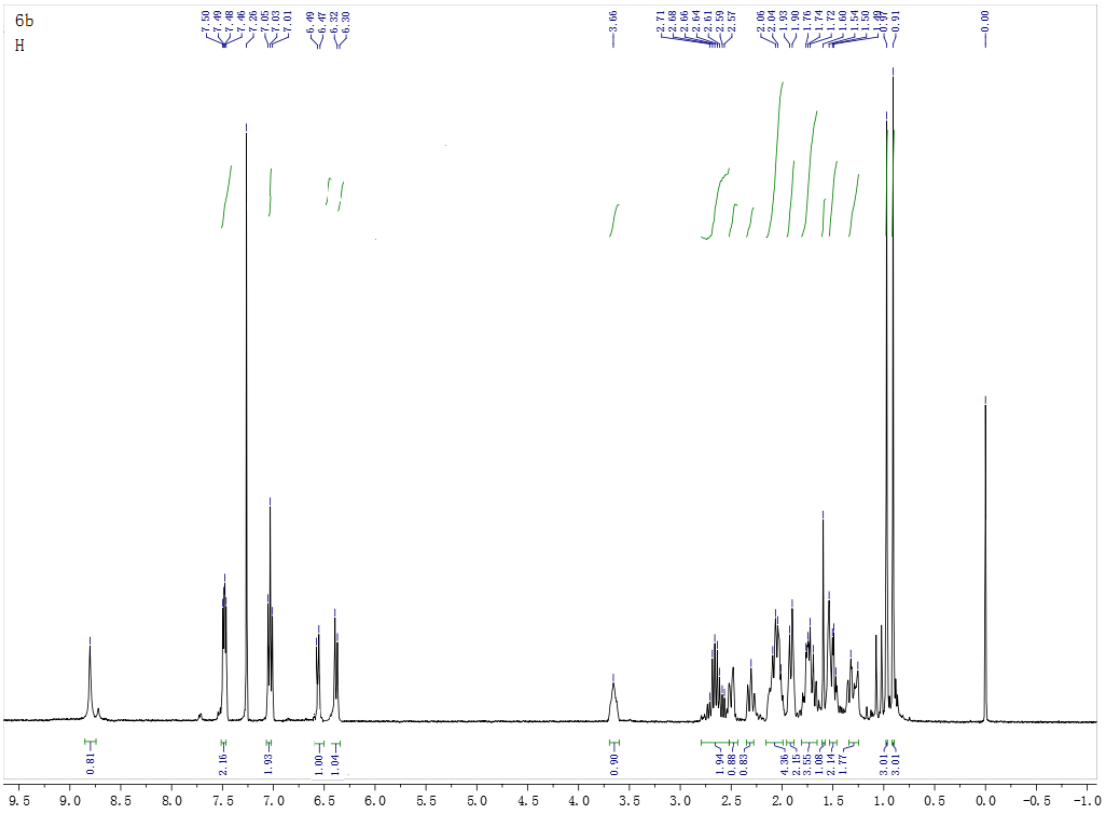
The morphological changes of cells were tested with Hoechst 33342/PI double stain kit (Solarbio, Basingstoke, England). HepG2 cells were seeded in 6-well plates (2×10^5 cells/well). After stabilization for 24 h, the cells were treated with compound **6k** (0, 3, 6 and 12 μM) for 48 h. Then the cells were fixed with 4% formaldehyde for 1 h at 4°C, and the cells were treated with 5 μL Hoechst33342 and 5 μL PI at 37°C in the dark for 20 min. Then the morphological changes of HepG2 cells were observed using a fluorescence microscope.

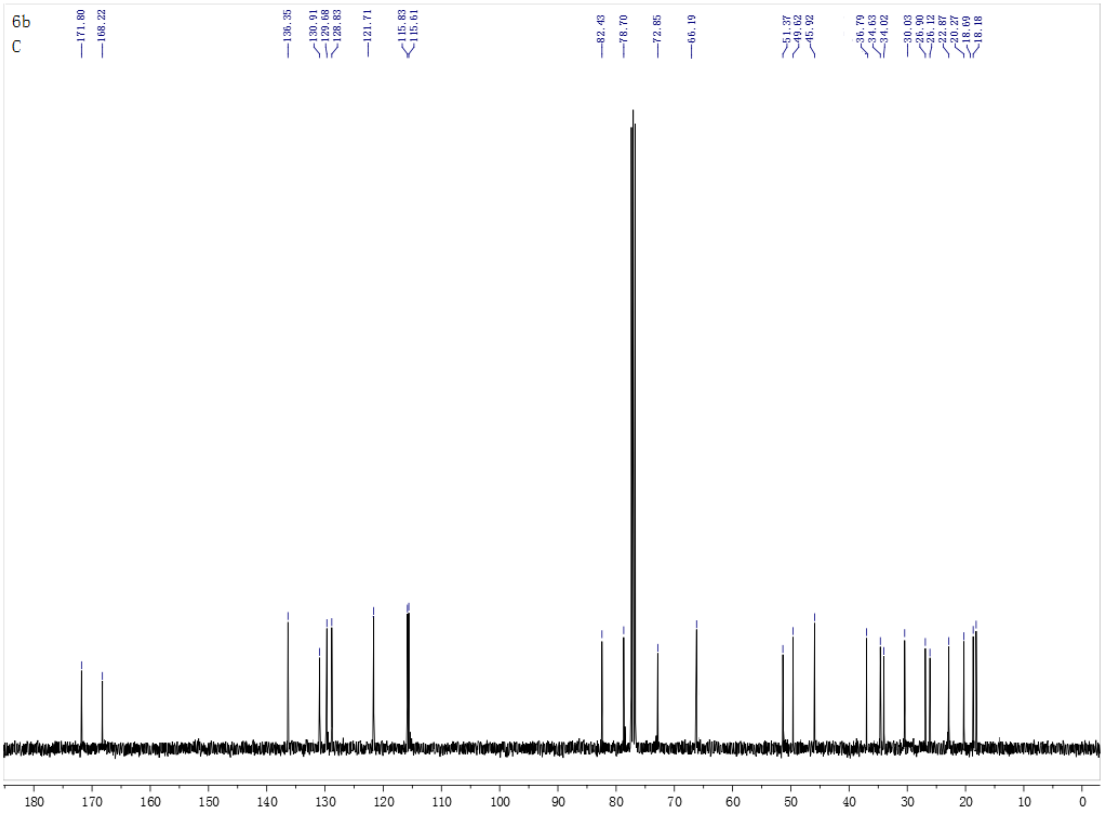
Compound **6a**


6a
H
10.17
7.60
7.58
7.35
6.52
6.51
6.30
6.28
3.59
3.58
2.74
2.72
2.71
2.69
2.47
2.45
2.44
2.36
1.86
1.85
1.72
1.69
1.65
1.60
1.59
1.58
1.43
1.41
1.23
0.99
0.82
0.80
1.03
2.11
2.01
1.00
1.04
1.09
1.04
1.15
3.02
9.96
3.14
1.95
3.05
3.18

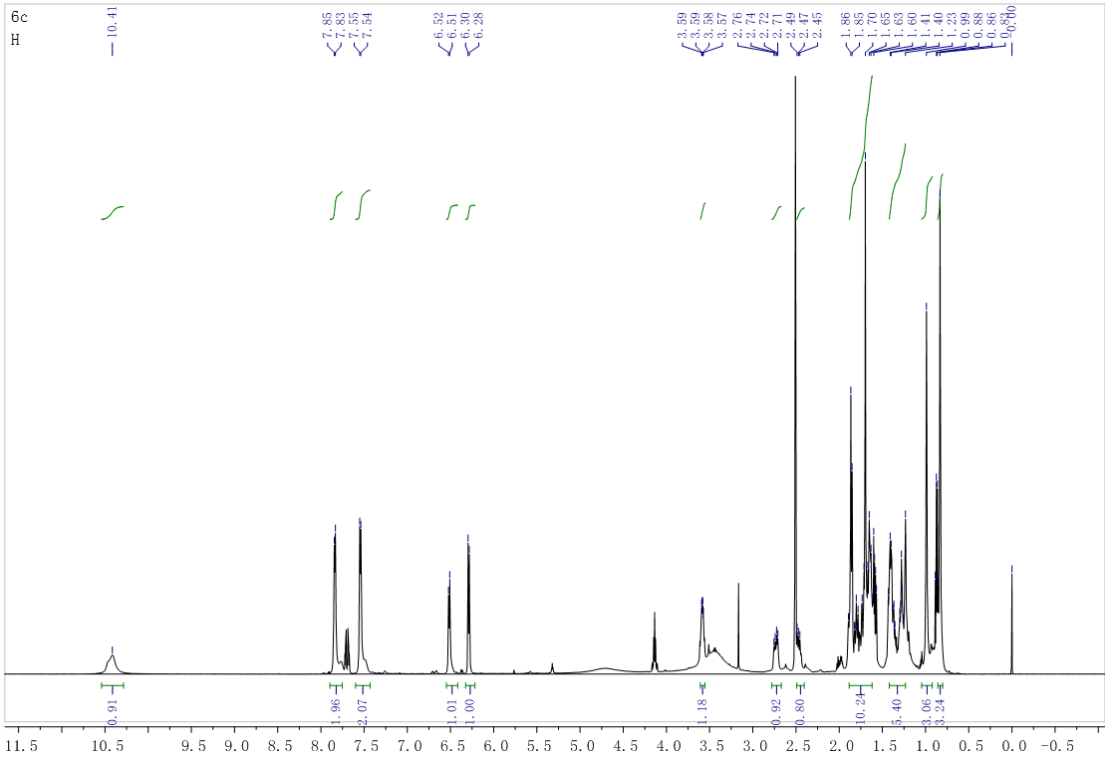

6a
C
172.67
164.02
138.03
136.42
134.64
132.33
130.30
128.64
125.29
82.13
78.72
65.08
55.39
51.61
49.10
46.51
37.36
37.13
34.90
34.28
30.41
27.10
22.90
21.41
20.42
18.55
18.41

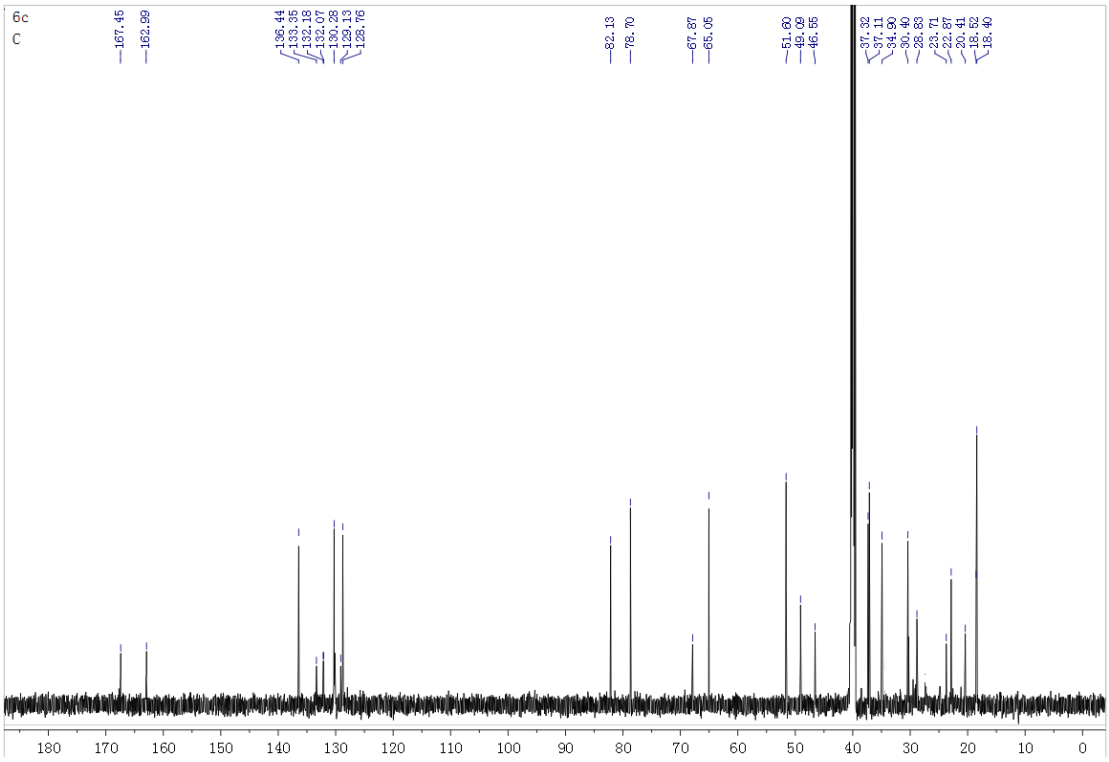
Compound **6b**



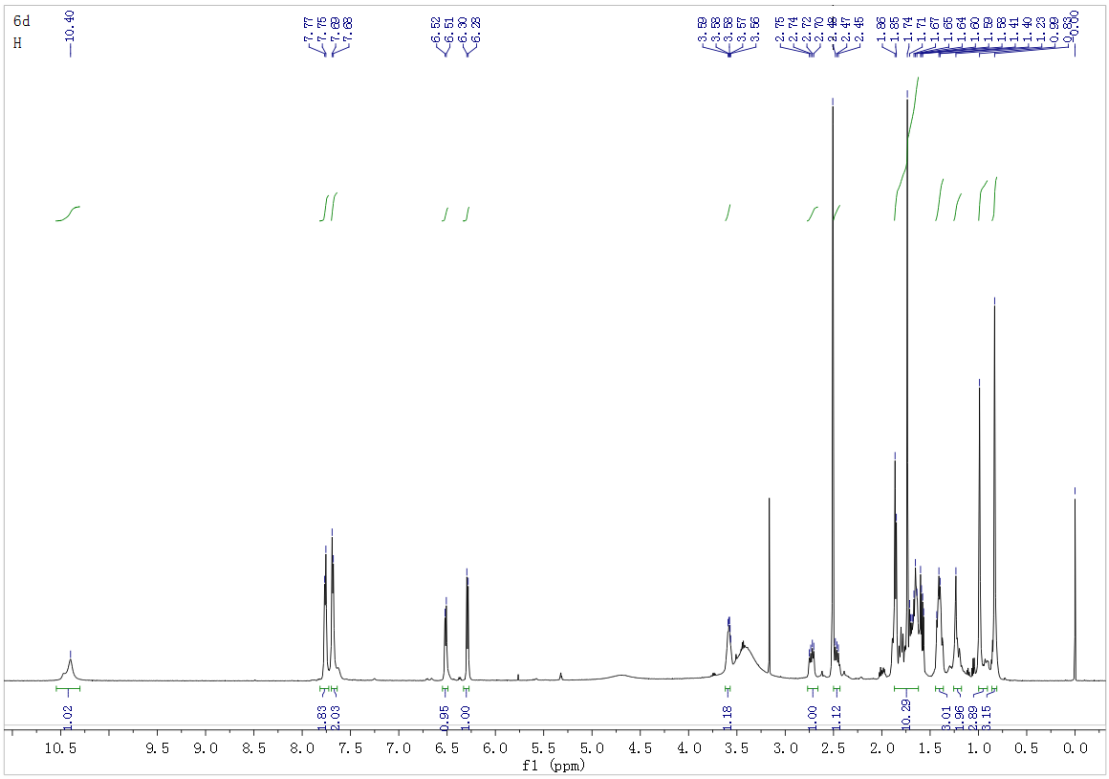


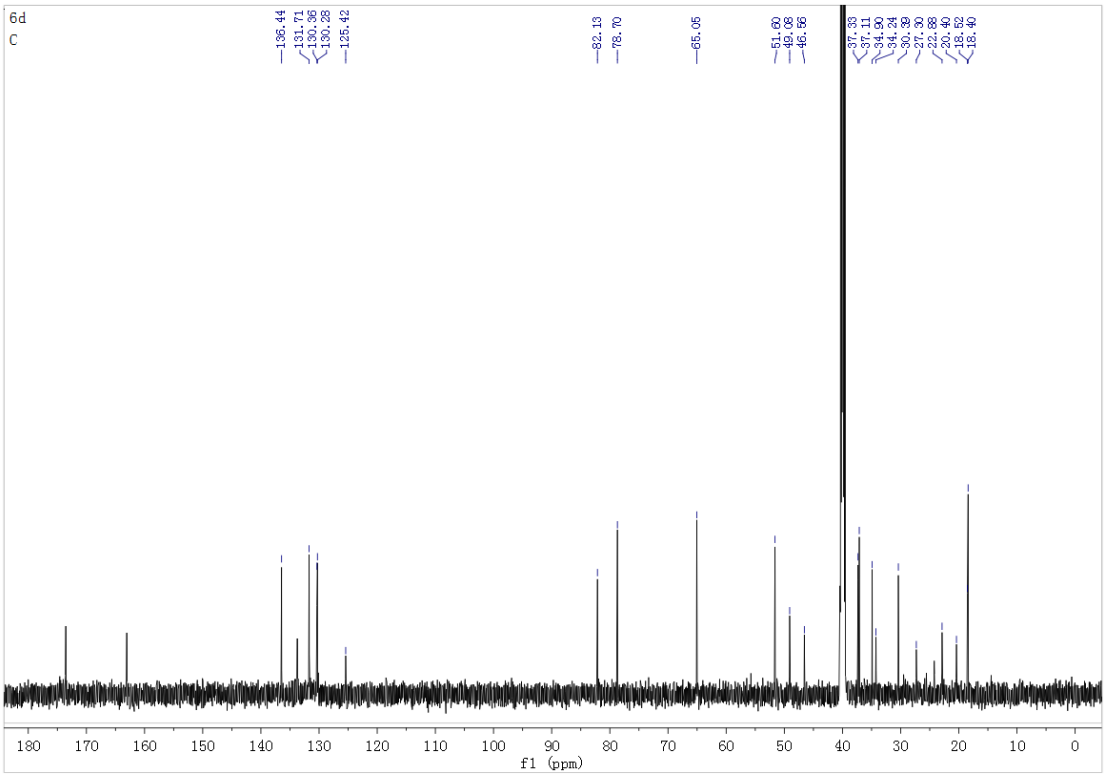
Compound **6c**


6c
H
10.41
7.85
7.83
7.55
7.54
6.52
6.51
6.30
6.28
3.59
3.59
3.58
3.57
2.76
2.74
2.72
2.71
2.49
2.47
2.45
1.86
1.85
1.70
1.65
1.63
1.60
1.41
1.40
1.23
0.89
0.88
0.86
0.83
0.00
0.91
1.96
2.07
1.01
1.00
1.18
0.92
0.80
10.24
5.40
3.06
3.24

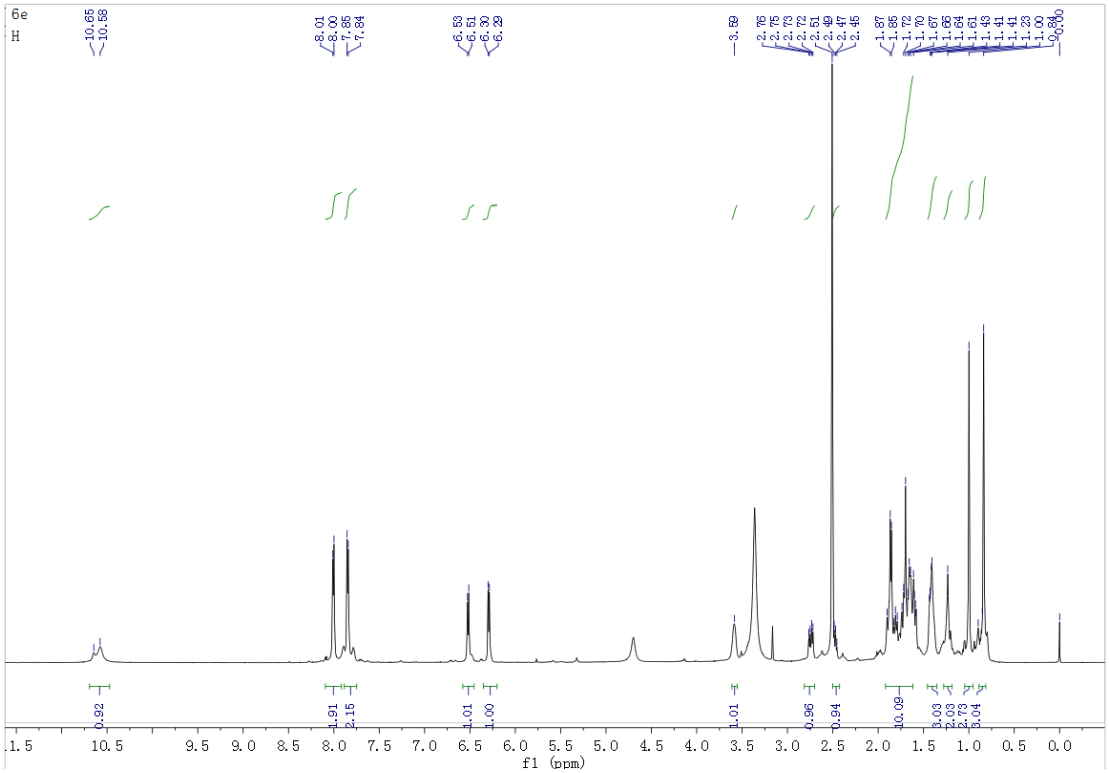

6c
C
167.45
162.99
136.44
133.35
132.18
132.07
130.28
129.13
128.76
82.13
78.70
67.87
65.05
51.60
49.09
46.55
37.32
37.11
34.90
30.40
28.83
23.71
22.87
20.41
18.52
18.40

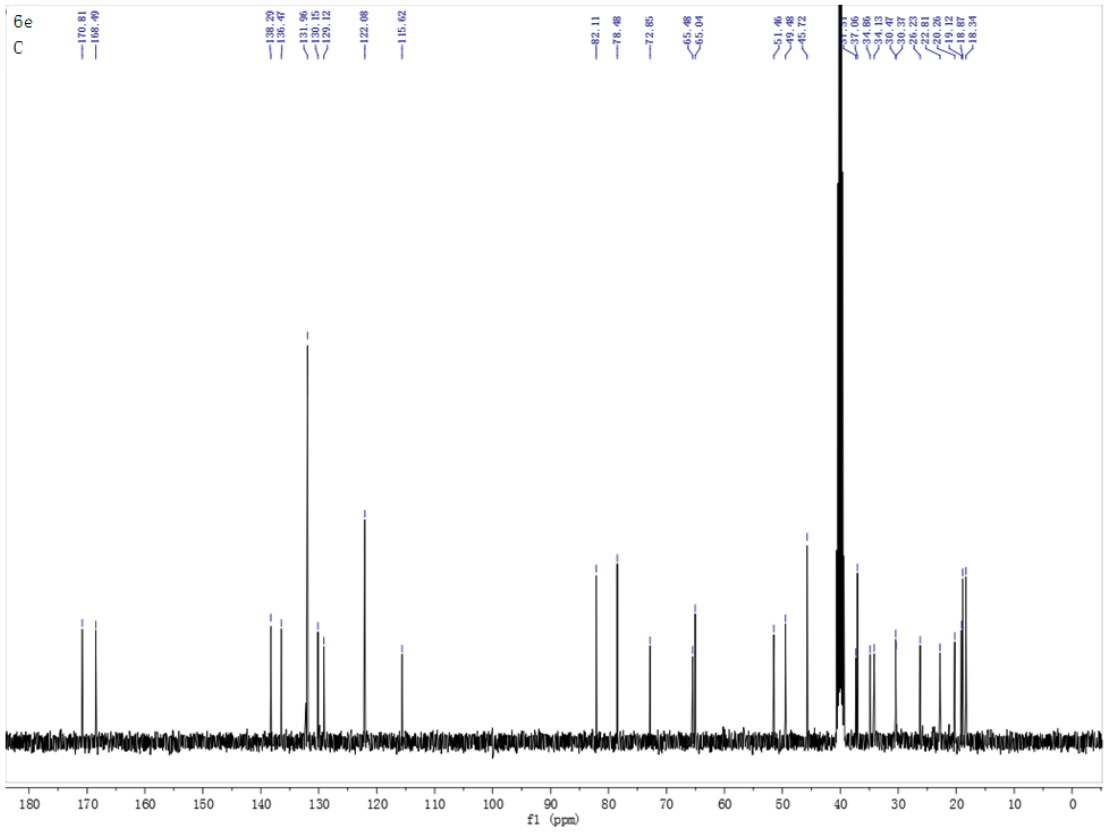
Compound **6d**



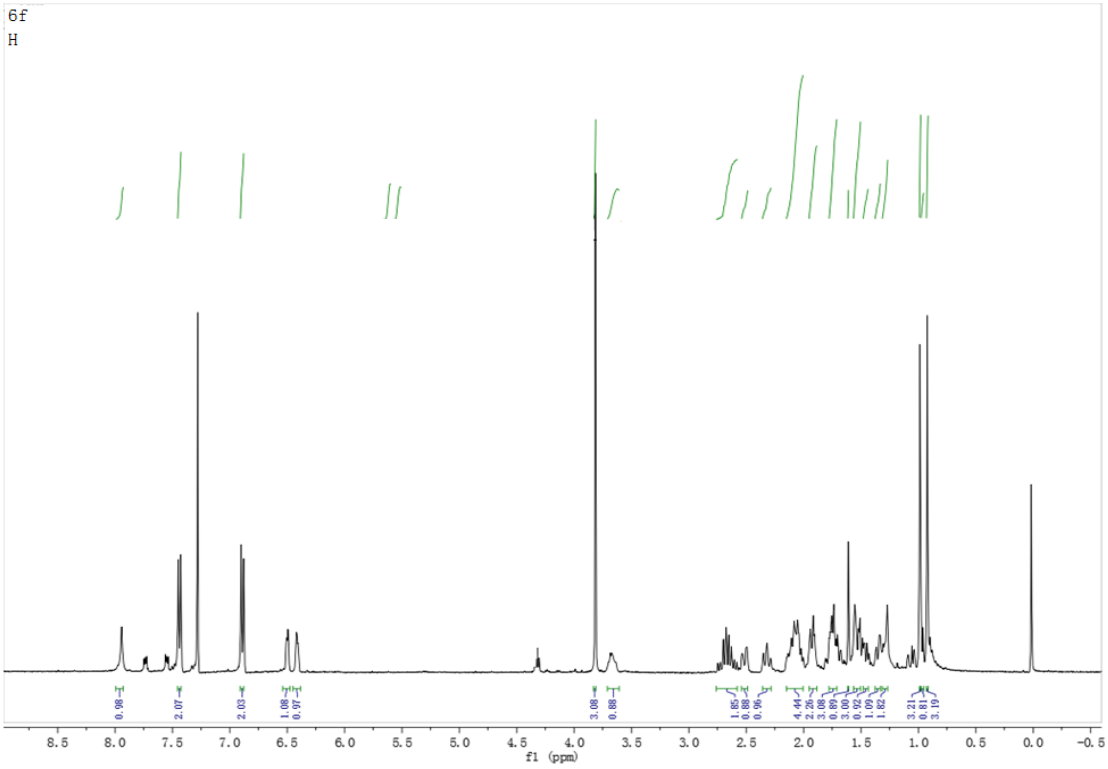


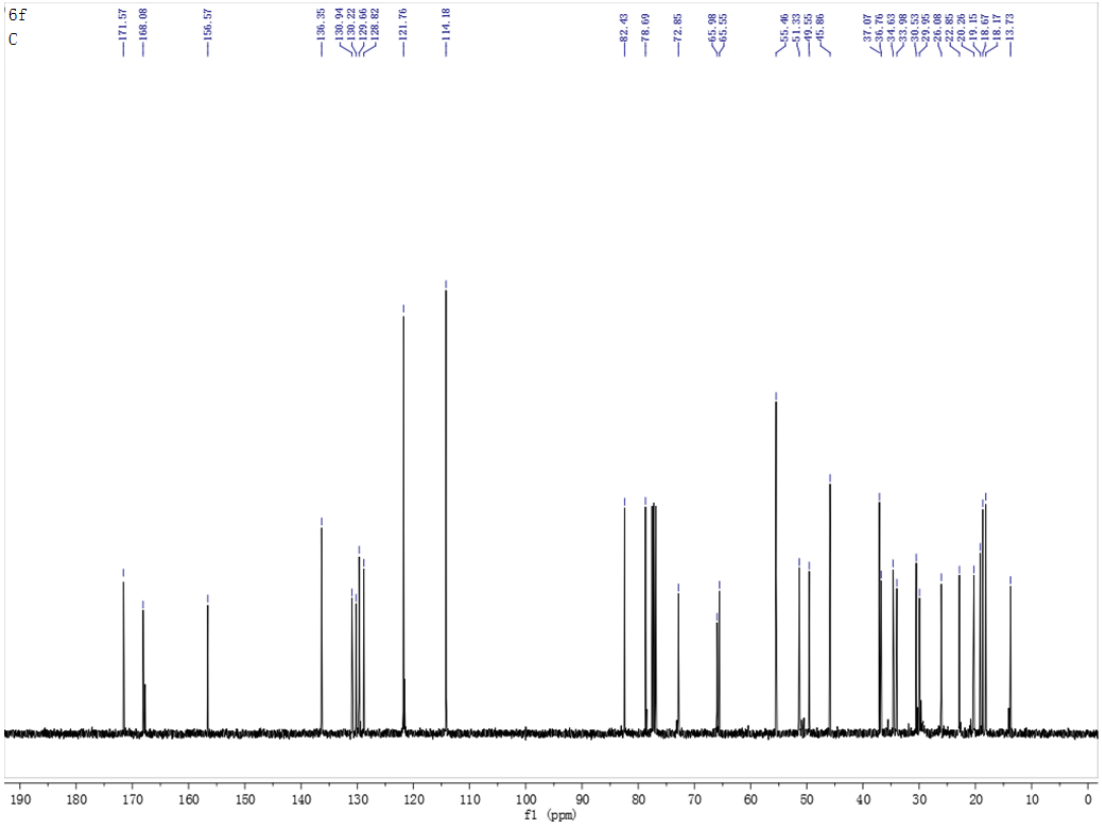
Compound **6e**



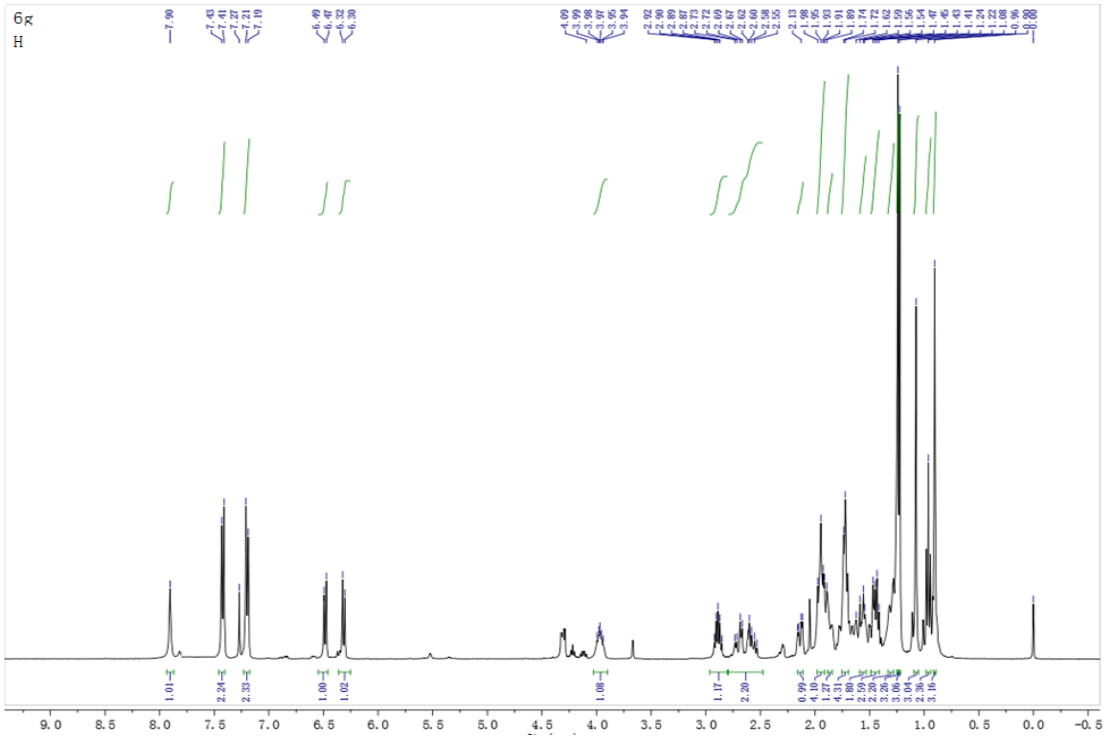


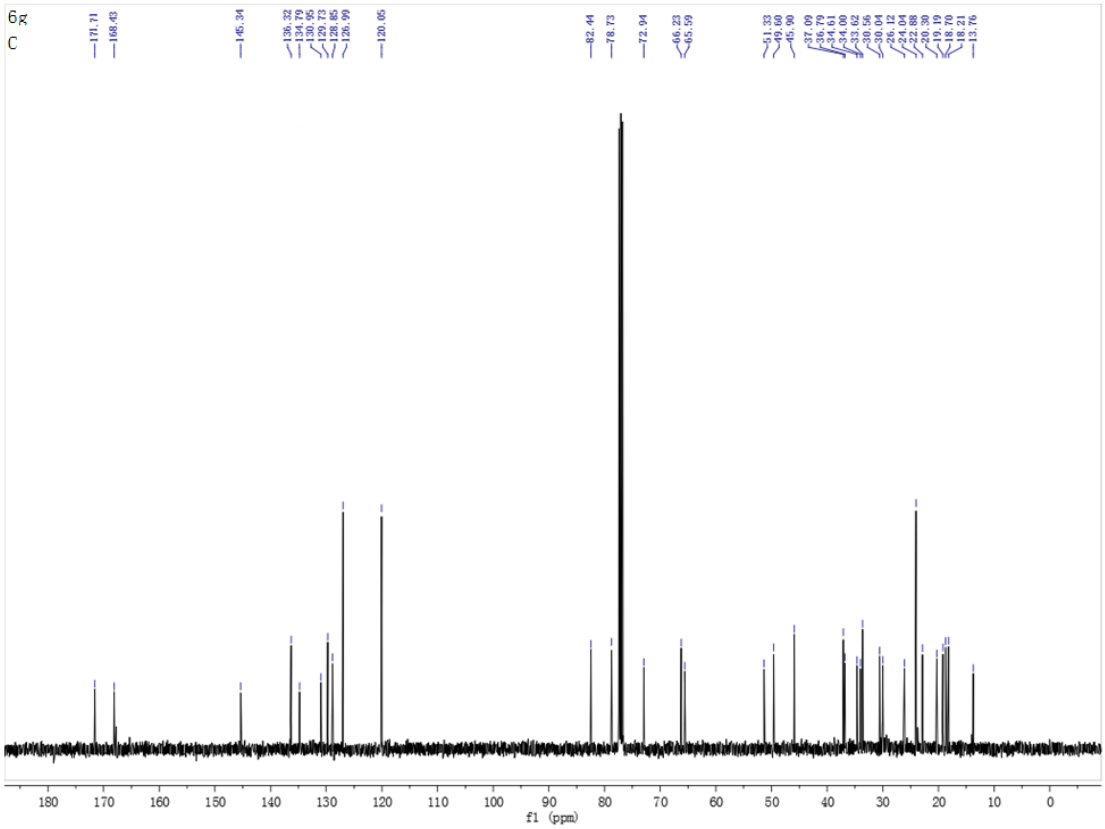
Compound **6f**


6f
H
f1 (ppm)

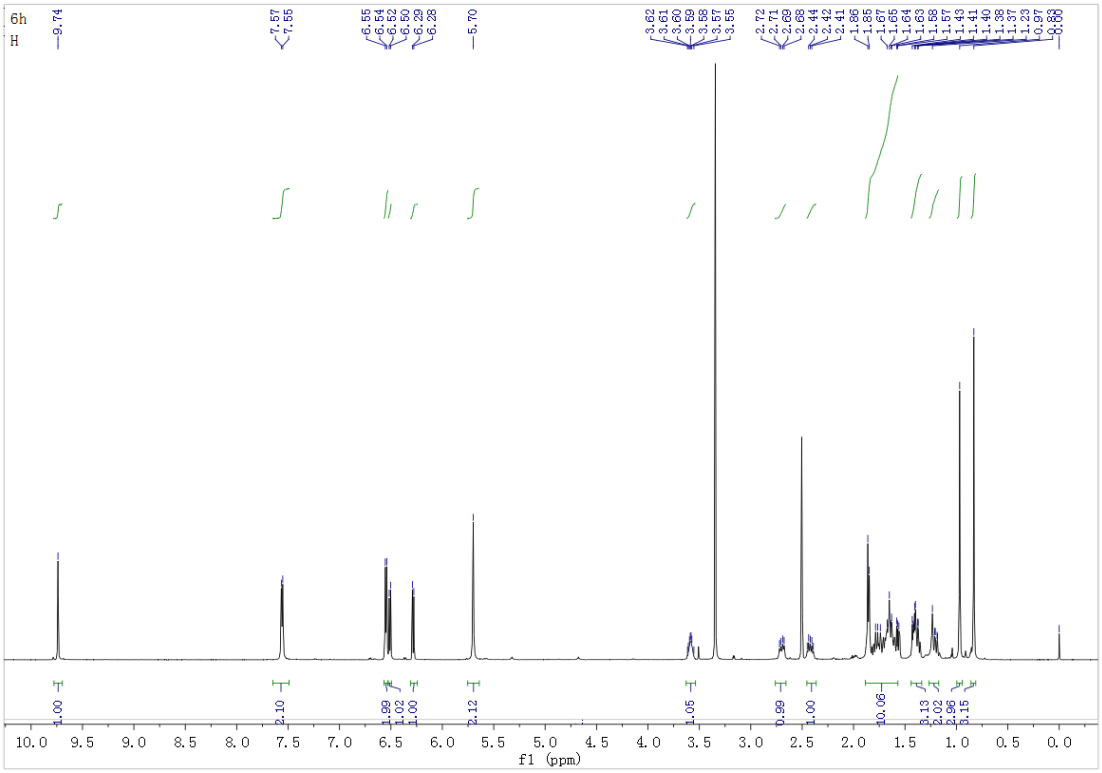

6f
C
171.57
168.08
156.57
136.35
130.94
130.22
129.66
128.82
121.76
114.18
82.43
78.69
72.85
65.98
65.55
55.46
51.33
49.55
45.86
37.07
36.76
34.63
33.98
30.53
29.95
26.08
22.85
20.35
19.15
18.67
18.17
13.73
f1 (ppm)

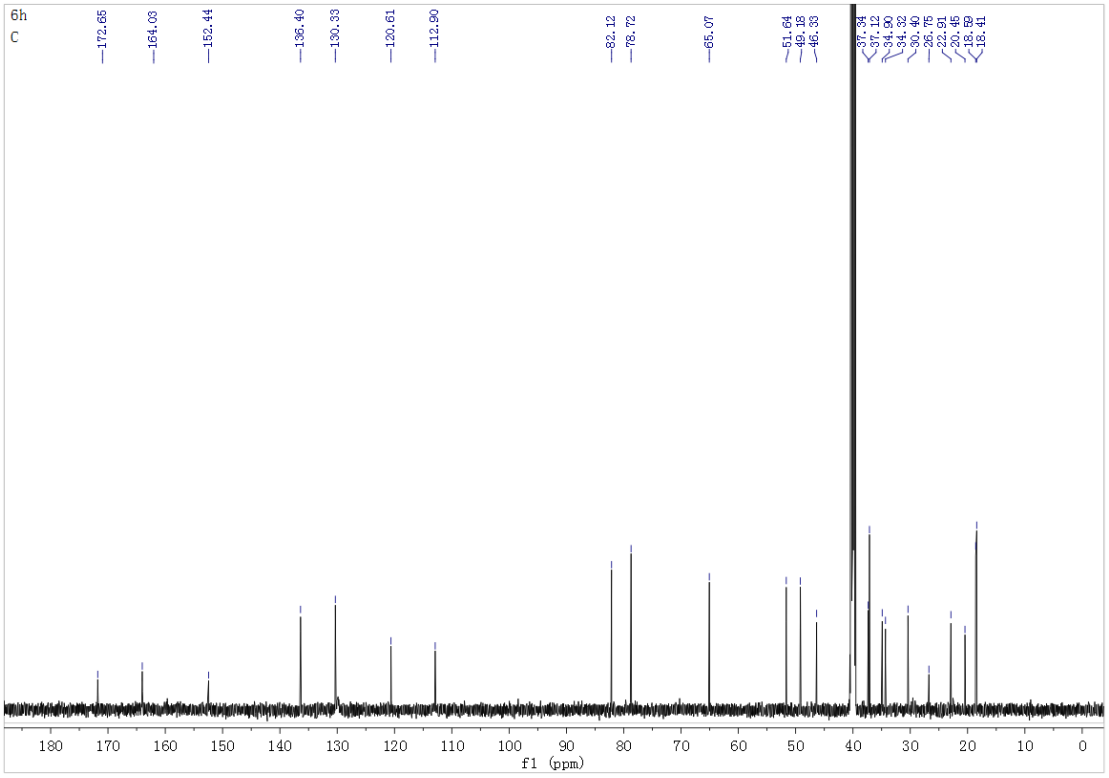
Compound **6g**



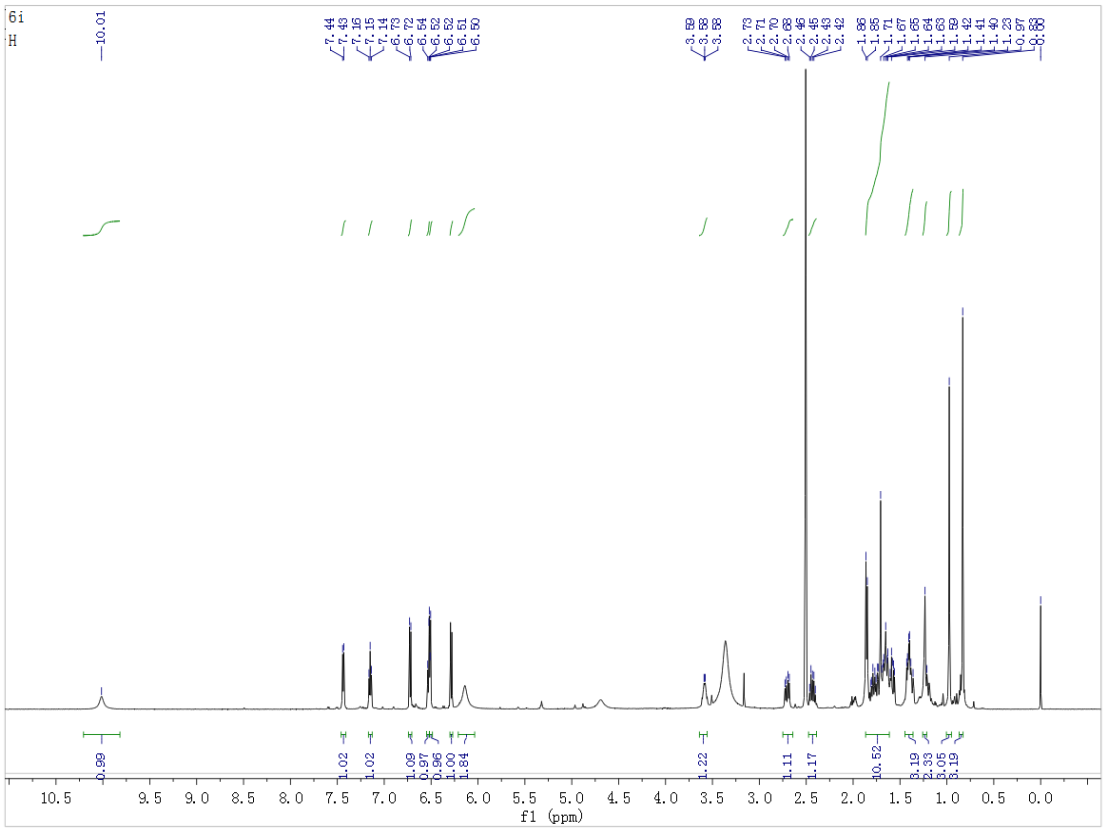


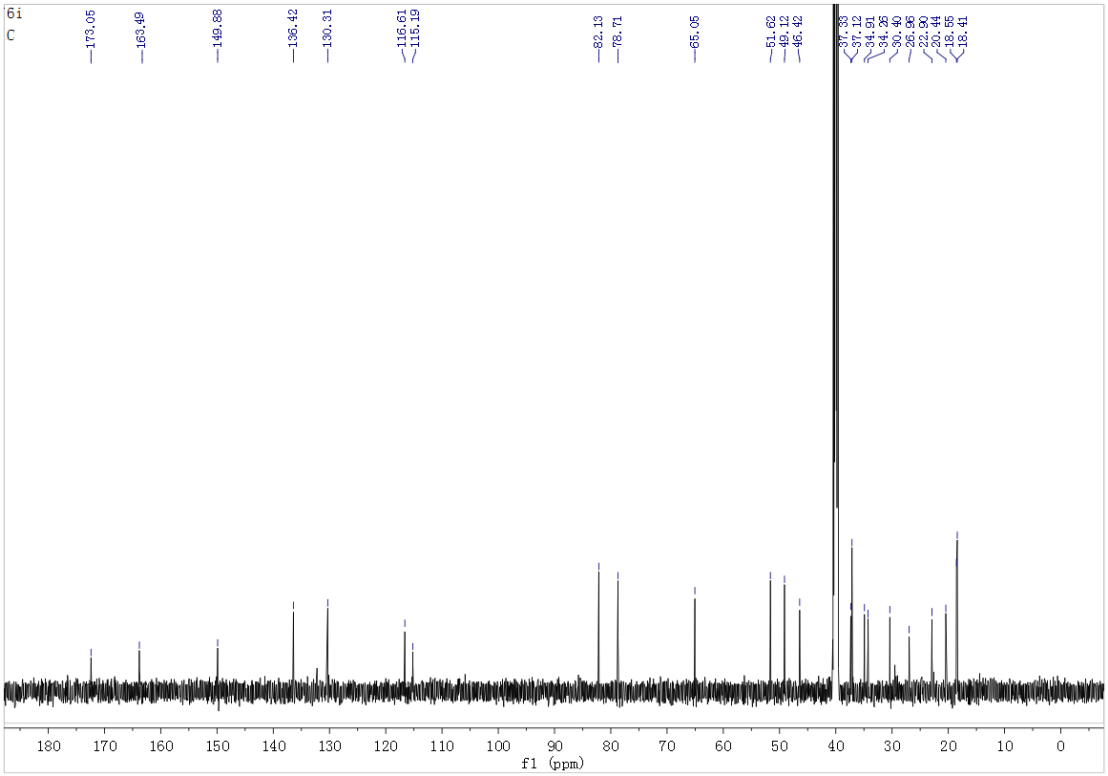
Compound **6h**


6h
H
9.74
7.57
7.55
6.55
6.54
6.52
6.50
6.29
6.28
5.70
3.62
3.61
3.60
3.59
3.58
3.57
3.56
2.72
2.71
2.69
2.68
2.44
2.42
2.41
1.86
1.85
1.67
1.65
1.64
1.63
1.58
1.57
1.43
1.41
1.40
1.38
1.37
1.23
0.97
0.86
1.00
2.10
1.99
1.02
1.00
2.12
1.05
0.99
1.00
10.06
3.13
2.02
2.96
3.15
f1 (ppm)

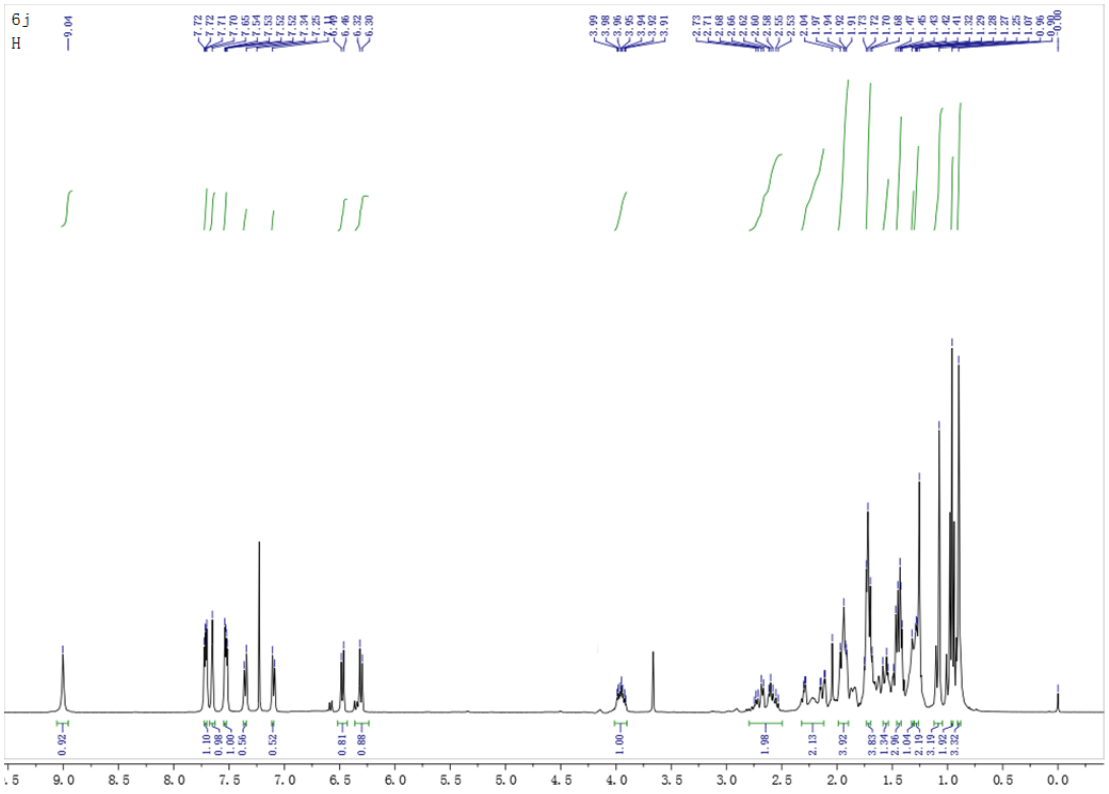

6h
C
172.65
164.03
152.44
136.40
130.33
120.61
112.90
82.12
78.72
65.07
51.64
49.18
46.33
37.34
37.12
34.90
34.32
30.40
26.75
22.91
20.45
18.59
18.41
f1 (ppm)

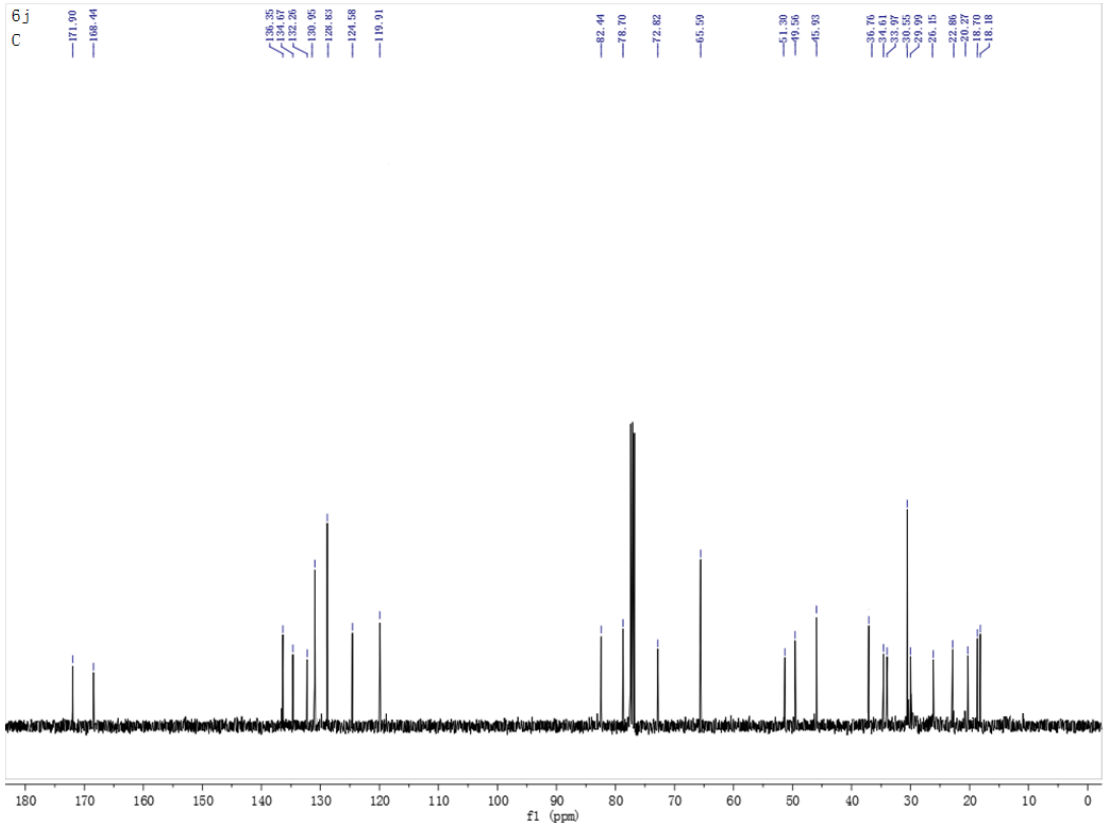
Compound **6i**


6i
H
10.01
7.44
7.43
7.16
7.15
7.14
6.73
6.72
6.64
6.62
6.62
6.61
6.60
3.89
3.88
3.88
2.73
2.71
2.70
2.68
2.46
2.45
2.43
2.42
1.86
1.85
1.71
1.67
1.65
1.64
1.63
1.59
1.42
1.41
1.40
1.23
0.87
0.86
0.99
1.02
1.02
1.09
0.97
0.96
1.00
1.84
1.22
1.11
1.17
10.52
3.19
2.33
3.05
3.19
f1 (ppm)

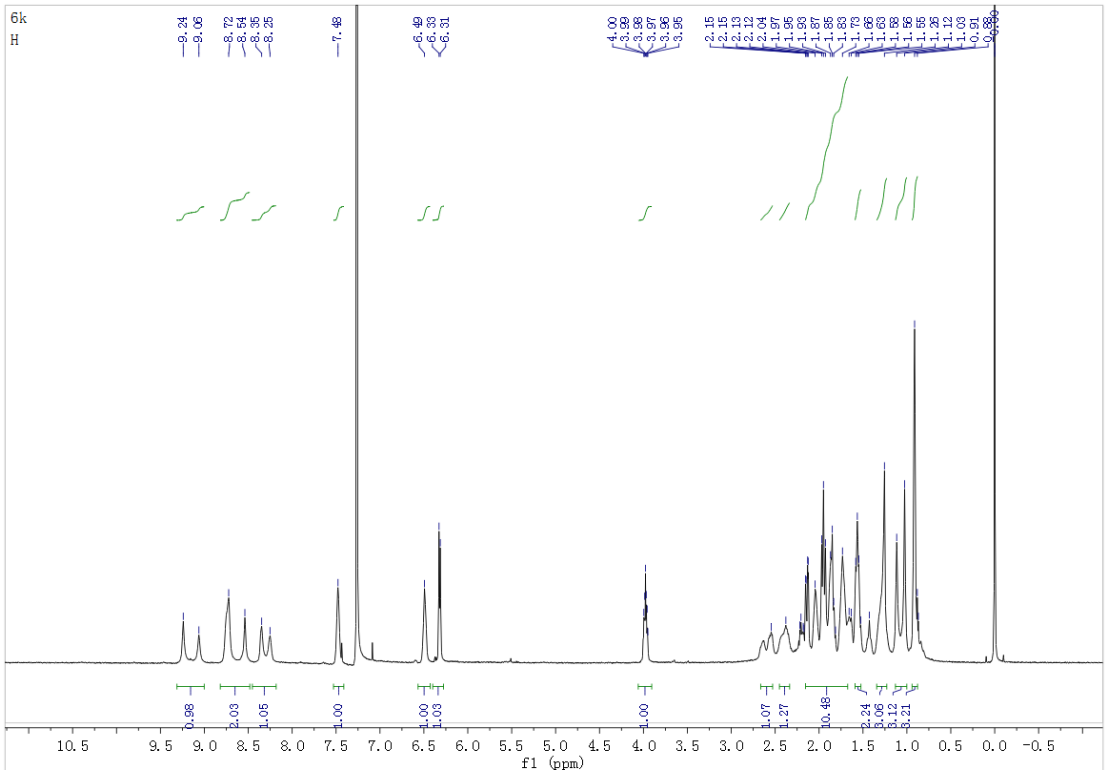

6i
C
173.05
163.49
149.88
136.42
130.31
116.61
115.19
82.13
78.71
65.05
51.62
49.12
46.42
37.38
37.12
34.91
34.25
30.40
26.96
22.90
20.44
18.55
18.41
f1 (ppm)

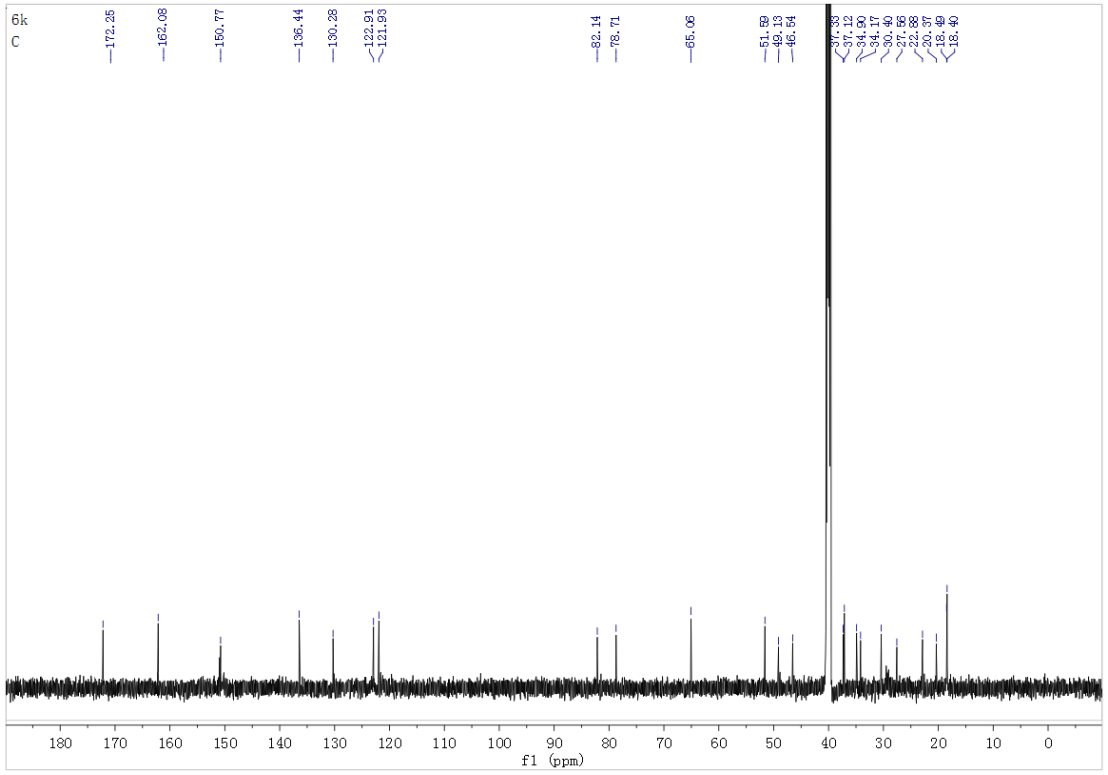
Compound **6j**


6j
H
9.04
7.72
7.71
7.70
7.65
7.54
7.53
7.52
7.34
7.25
6.46
6.32
6.30
3.99
3.98
3.96
3.95
3.94
3.92
3.91
2.73
2.71
2.68
2.66
2.62
2.60
2.58
2.55
2.53
2.04
1.97
1.94
1.92
1.91
1.73
1.72
1.70
1.68
1.67
1.65
1.43
1.42
1.41
1.32
1.29
1.28
1.27
1.25
1.07
0.96
0.00
0.92
1.10
0.98
1.00
0.56
0.52
0.81
0.88
1.00
1.98
2.13
3.92
3.83
1.94
2.06
2.04
2.19
3.19
1.92
3.32
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


6j
C
171.90
168.44
136.35
134.67
132.26
130.95
126.83
124.58
119.91
82.44
78.70
72.82
65.59
51.30
49.56
45.93
36.76
34.64
33.97
30.55
29.99
26.15
22.86
20.27
18.70
18.18
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Compound **6k**





Compound **6l**