Supplementary Information

Discovery of new 1,4,6-trisubstituted-1*H*-pyrazolo[3,4-*b*]pyridines with anti-tumor efficacy in mouse model of breast cancer

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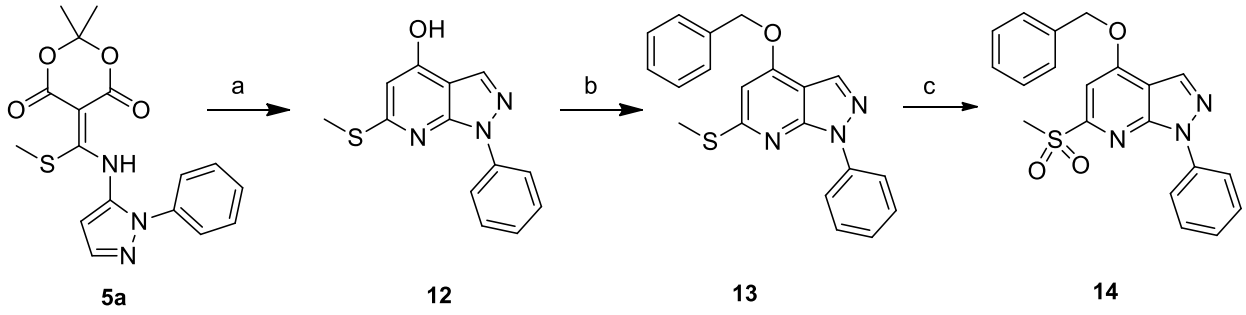
Attempt for an alternative synthetic pathway

During the initial investigation of the synthetic procedures, we have also performed the cyclization of **5a** prior the introduction of aniline, in order to have access to the more versatile 6-methylthiopyrazolopyridinol intermediate **12** (Scheme SI1). This compound was converted in the 4-benzyloxyderivative **13** however, our attempts to substitute the 6-methylthiogroup of **13**, or even the corresponding oxidized 6-methylsulfonylderivative **14**, were not successful.



Scheme S1. Reagents and conditions. a) Ph_2O, reflux, Ar, 85%; b) 1)K_2CO_3, DMF, 2) benzylbromide, 50^oC, 57%;, c) mCPBA, CH_2Cl_2, rt, 98%.

Experimental data of compounds 12-14

6-(Methylthio)-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridin-4-ol **(12)**

This derivative was prepared by a method analogous to that described for the synthesis of **7a**. Chromatographic purification (silica gel 40-60µm) was performed using a mixture of cyclohexane / EtOAc (9/1 to 6/4, v/v) as the eluent. Yield 85%. Pale white solid, mp. 154-156°C (EtOAc/*n*-pentane). ^{1}H-NMR (600 MHz, DMSO-d_6) δ (ppm) 2.59 (s, 3H, SCH_3), 6.51 (s, 1H, H-5), 7.31 (t, 1H, *J*= 7.0 Hz, phenyl H-4), 7.54 (t, 2H, *J*= 8.1 Hz, phenyl H-3, H-5), 8.28-8.29 (m, 3H, H-3, phenyl H-2, H-6), 11.78 (brs, 1H, D_2O exch., OH). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) 12.94, 100.38, 106.41, 120.08, 125.58, 129.06, 132.60, 139.37, 151.83, 158.73, 161.77.HR-MS (ESI) m/z calculated for $C_{13}H_{12}N_3OS$ $[M+H]^+$: 258.0696; found258.0696.

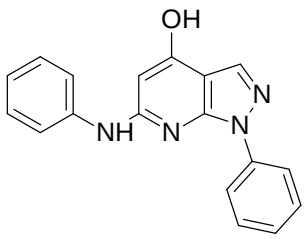
6-(Methylthio)-4-benzyloxy-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine **(13)**

This derivative was prepared by a method analogous to that described for the synthesis of **8a**. Chromatographic purification (silica gel 40-60µm) was performed using a mixture of cyclohexane / EtOAc (1/1, v/v) as the eluent. Yield 57%. White solid, mp. 138-140°C (CH_2Cl_2/Et_2O). ^{1}H-NMR (600 MHz, $CDCl_3$) δ (ppm) 2.67 (s, 3H, SCH_3), 5.22 (s, 2H, CH_2), 6.54 (s, 1H, H-5), 7.29 (t, 1H, *J*= 7.4 Hz, phenyl H-4), 7.39-7.53 (m, 7H, benzyloxy 5H, phenyl H-3, H-5), 8.12 (s, 1H, H-3), 8.33 (d, 2H, *J*= 7.7 Hz, phenyl H-2,H-6). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) 13.70, 70.69, 98.17, 106.97, 121.01, 125.85, 127.77, 128.70, 128.93, 128.99, 132.14, 135.43, 139.88, 152.09, 159.24, 162.88.HR-MS (ESI) m/z calculated for $C_{20}H_{18}N_3OS$ $[M+H]^+$: 348.1165; found 348.1164.

6-(Methylsulfonyl)-4-benzyloxy-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine **(14)**

To a solution of **13** (40 mg, 0.12 mmol) in dichloromethane (4 mL) was added 3-chloroperoxybenzoic acid (m-CPBA, 59 mg, 0.34 mmoL) and the mixture was stirred for 3h at room temperature. It was then neutralized with a Na_2CO_3 solution (150 mg in 40 mL H_2O) and extracted with dichloromethane. The organic layer was washed with brine, dried (Na_2SO_4) and the solvent was vacuum evaporated to provide pure **14** (42 mg, 98%) as a white solid. Mp. 144°C (Et_2O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 3.34 (s, 3H, SO_2CH_3), 5.41 (s, 2H, CH_2), 7.36 (t, 1H, *J*= 7.4 Hz, phenyl H-4), 7.40-7.43 (m, 1H, benzyloxy 1H), 7.46 (t, 2H, *J*= 6.8 Hz, phenyl H-3, H-5), 7.50-7.55 (m, 5H, benzyloxy 4H, H-5), 8.22 (d, 2H, *J*= 7.8 Hz, phenyl H-2, H-6), 8.34 (s, 1H, H-3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 39.97, 71.86, 96.50, 110.97, 121.39, 126.85, 128.10, 129.10, 129.15, 129.32, 132.33, 134.48, 139.09, 150.67, 159.32, 161.70.HR-MS (ESI) m/z calculated for $C_{20}H_{18}N_3O_3S$ $[M+H]^+$: 380.1063; found 380.1060.

Spectral data of the new derivatives



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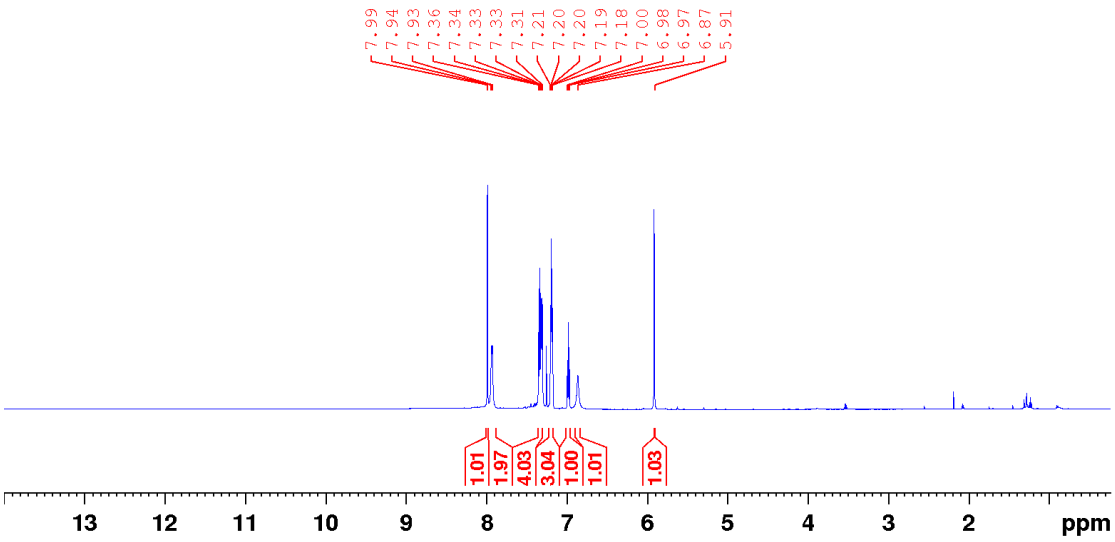

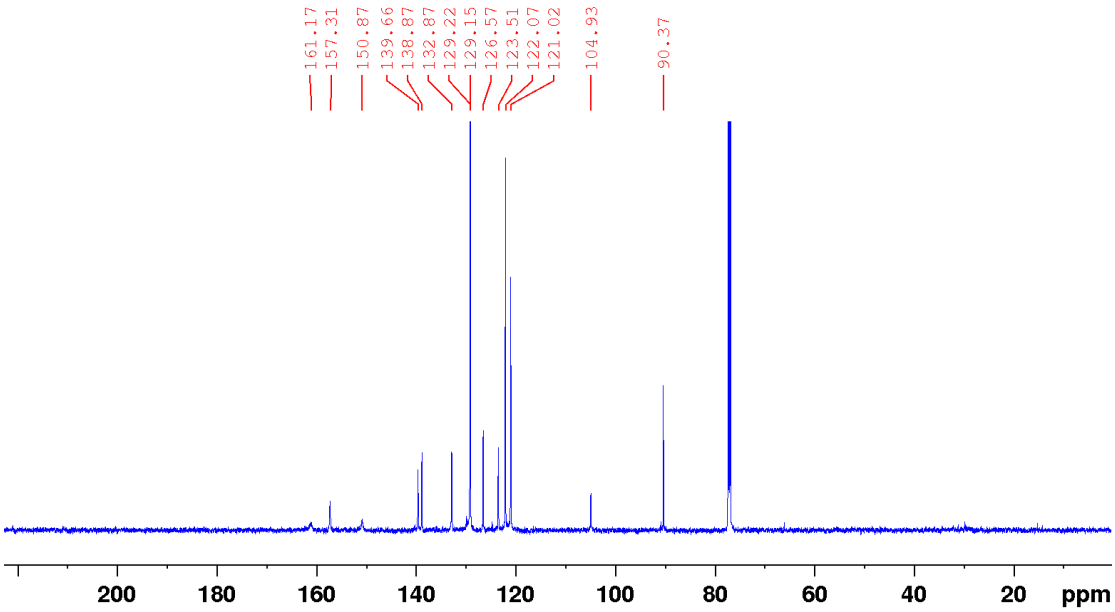

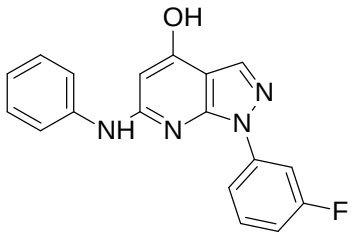
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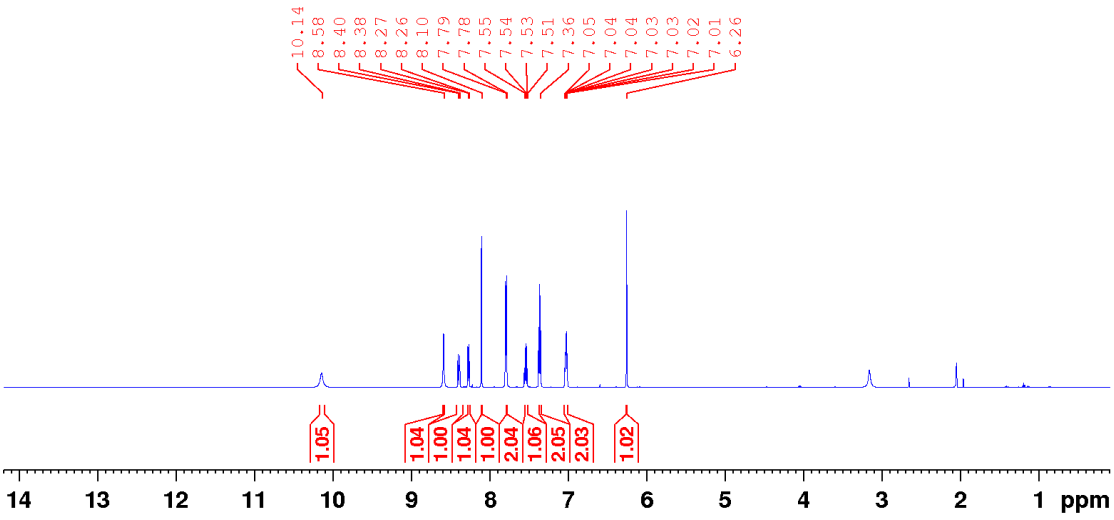
7b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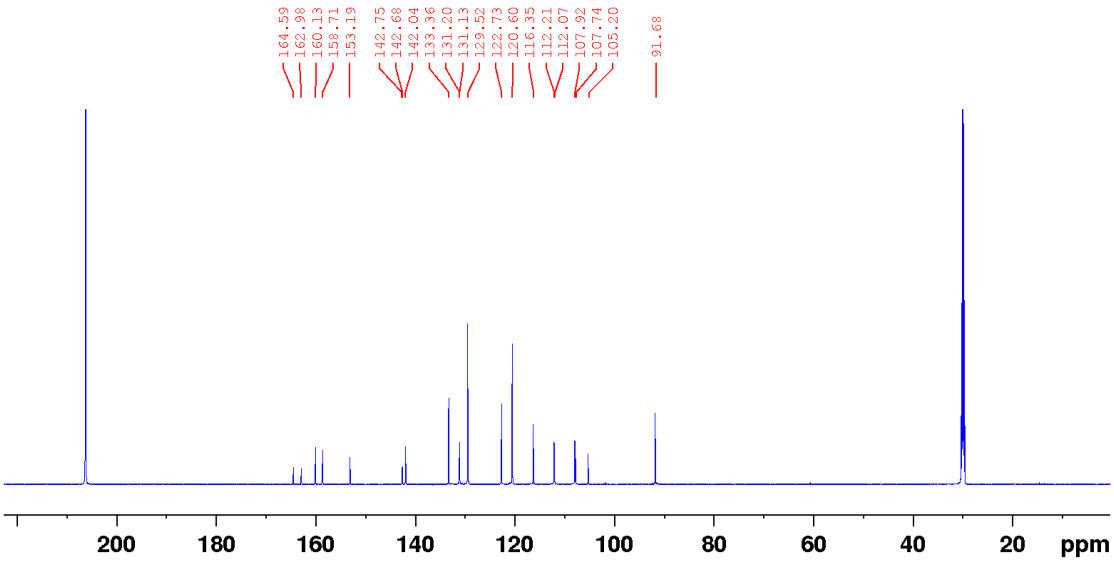
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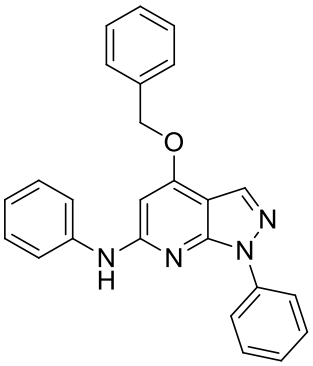
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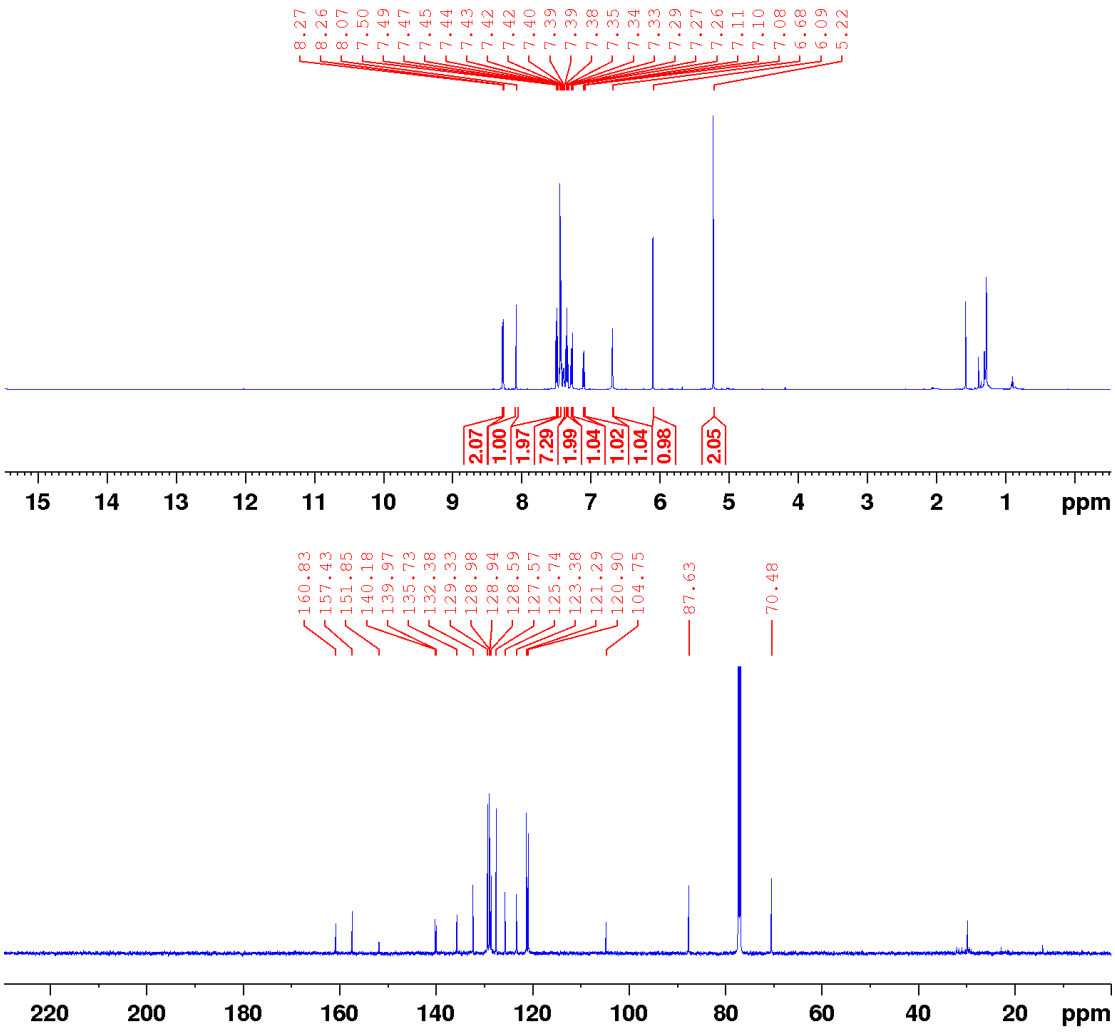
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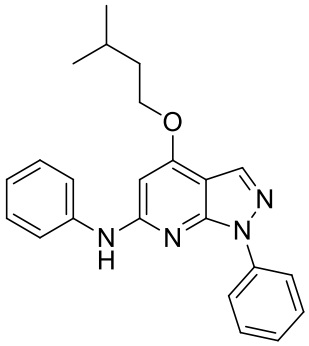
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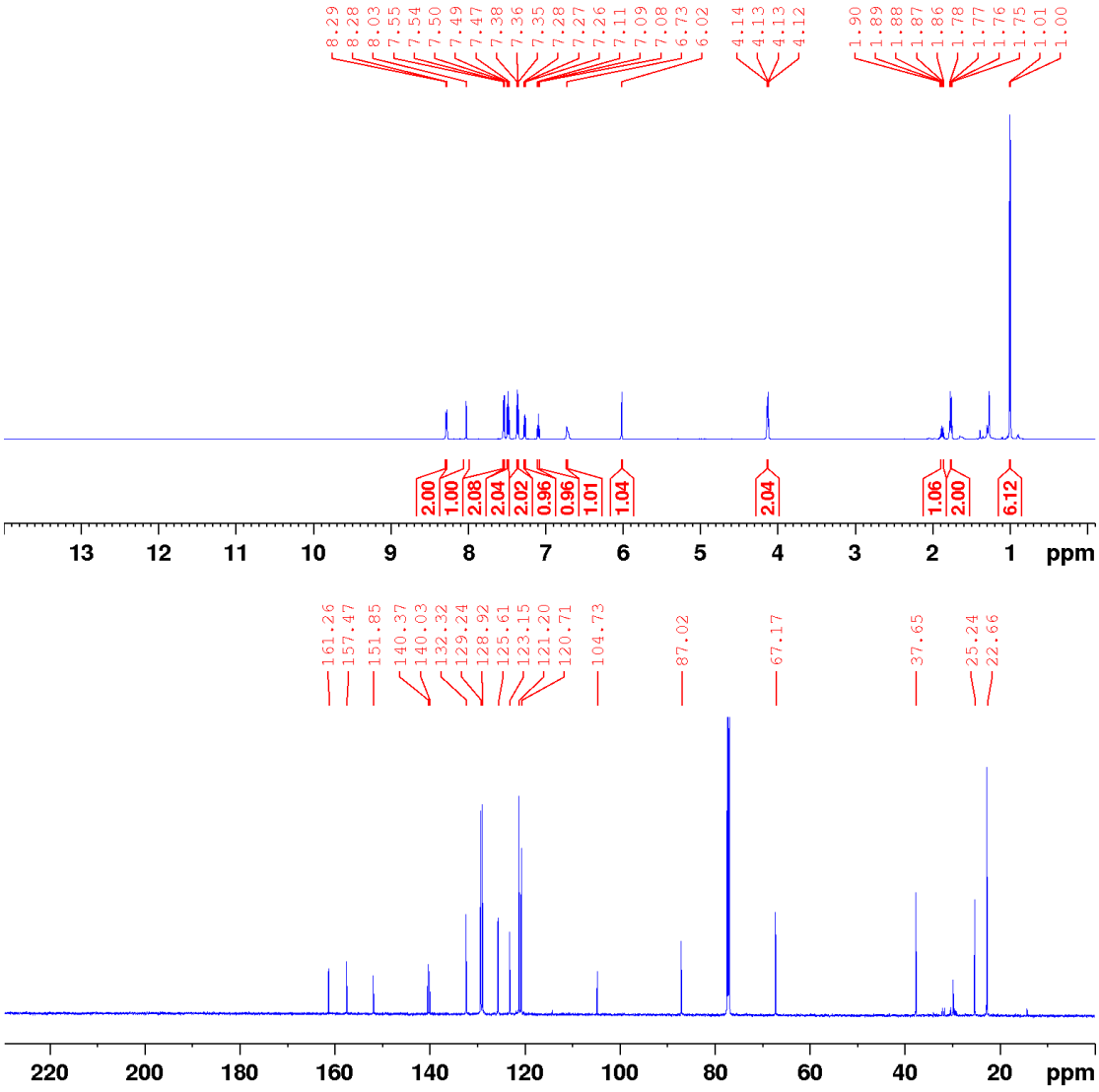
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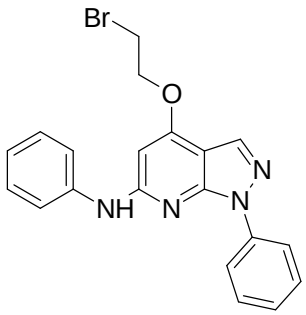
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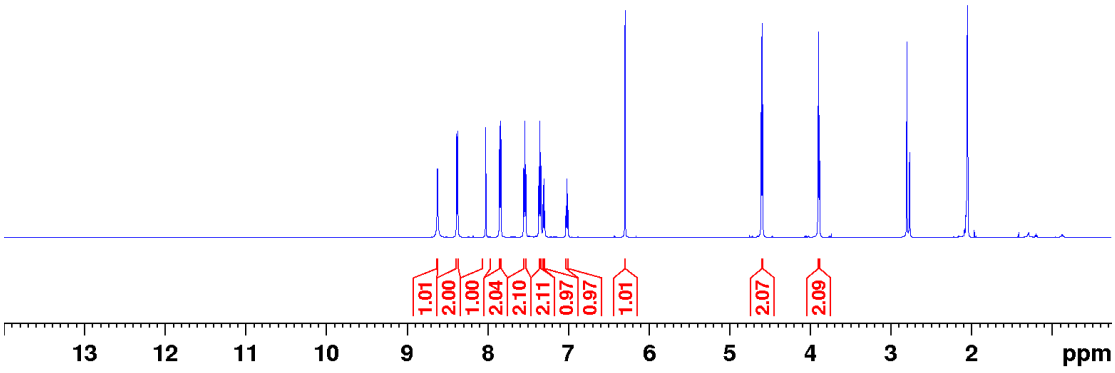
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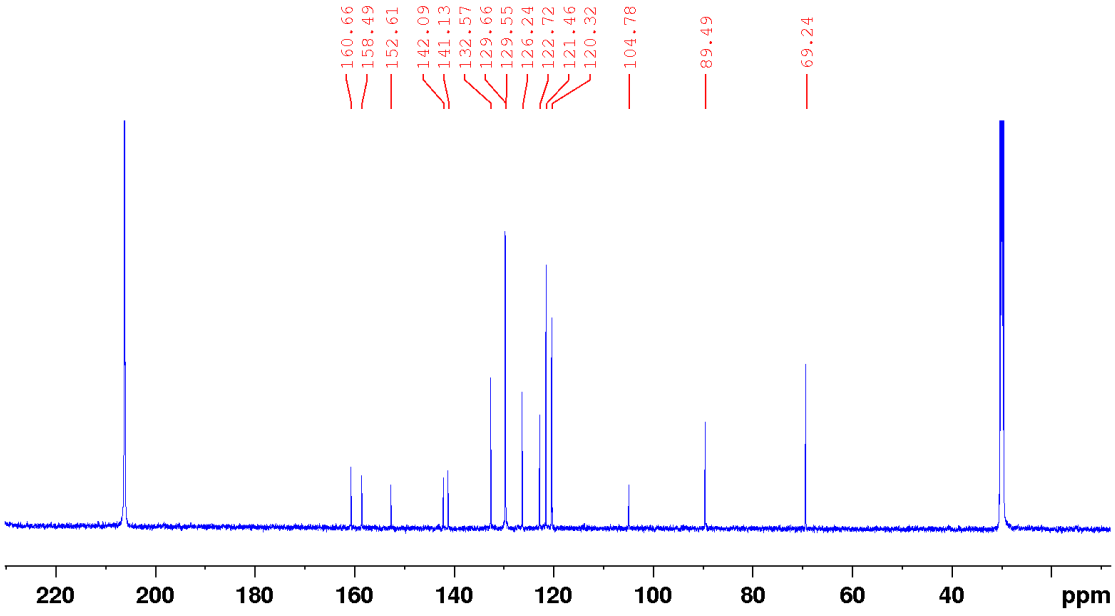
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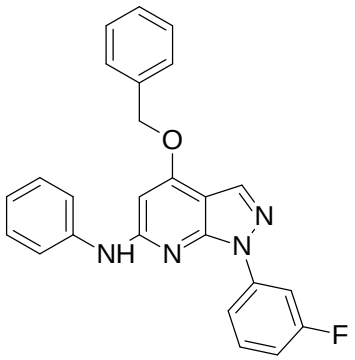
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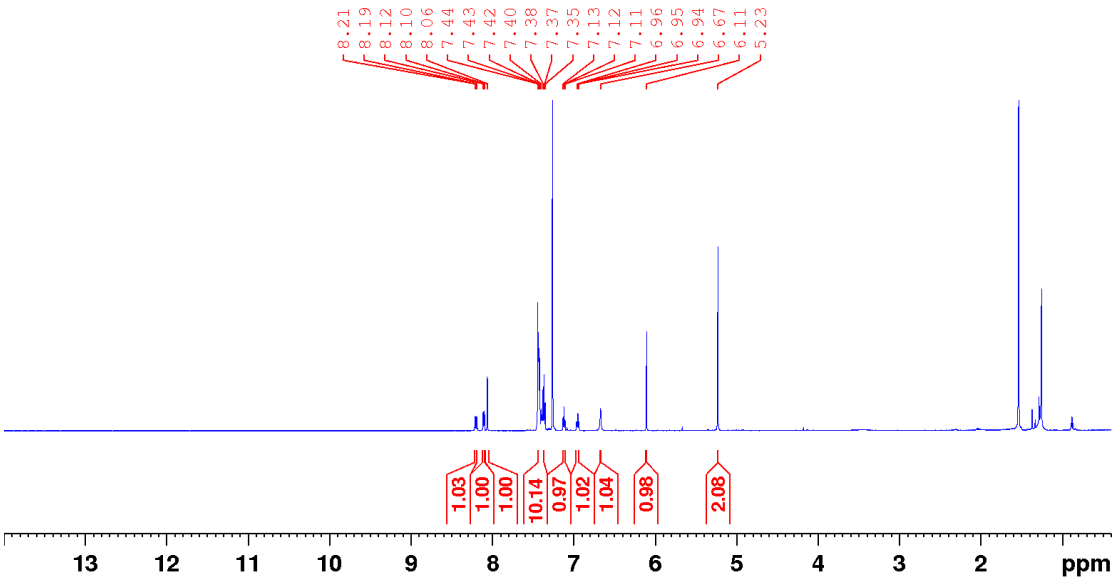
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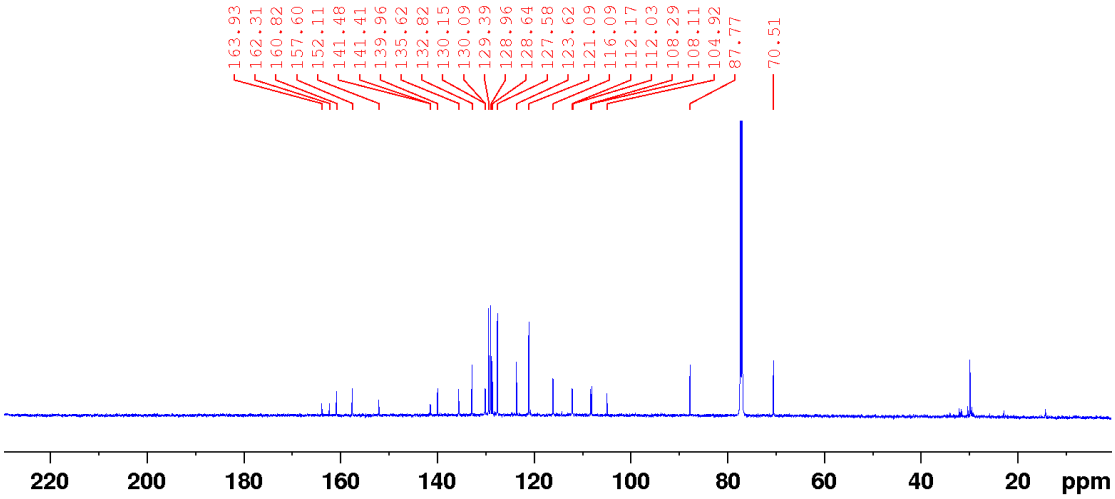
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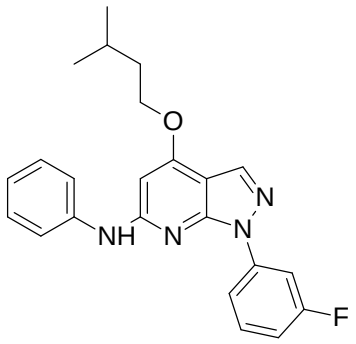
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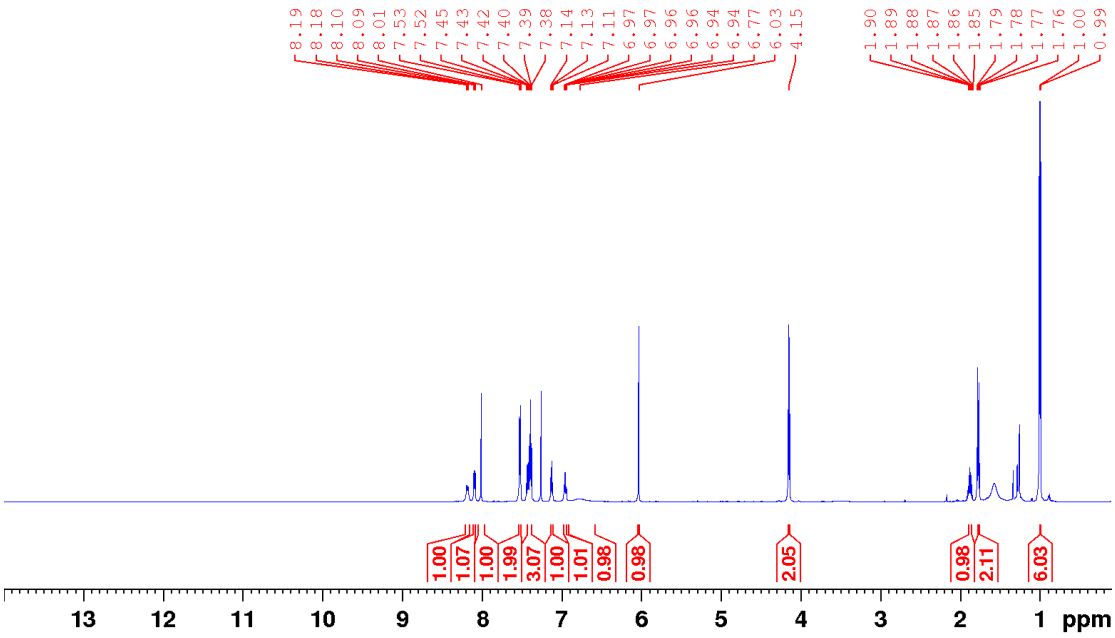
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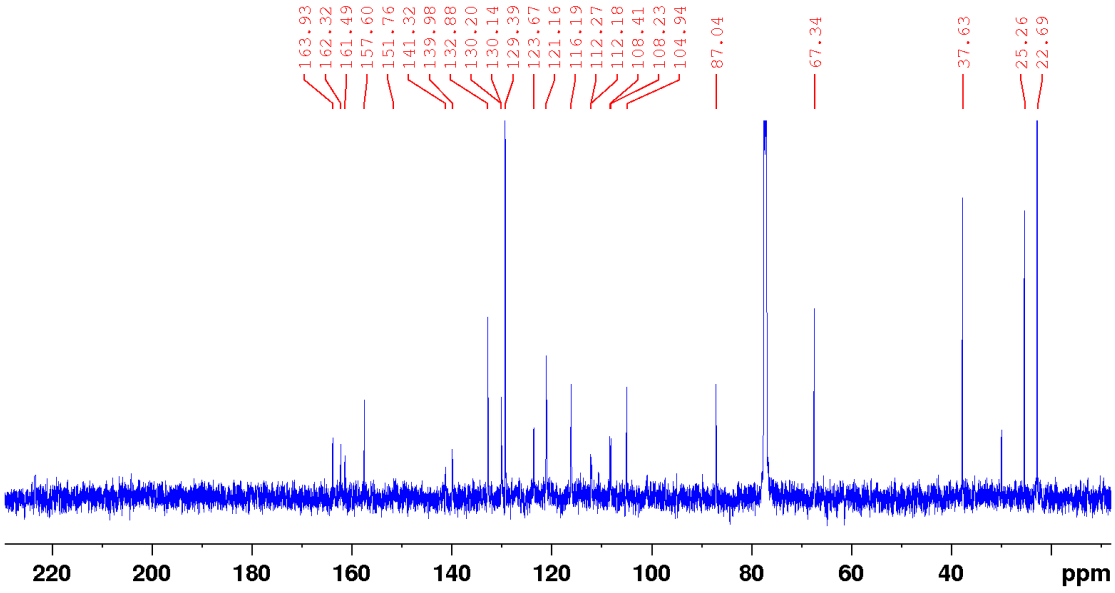
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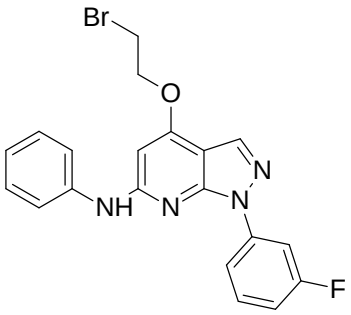
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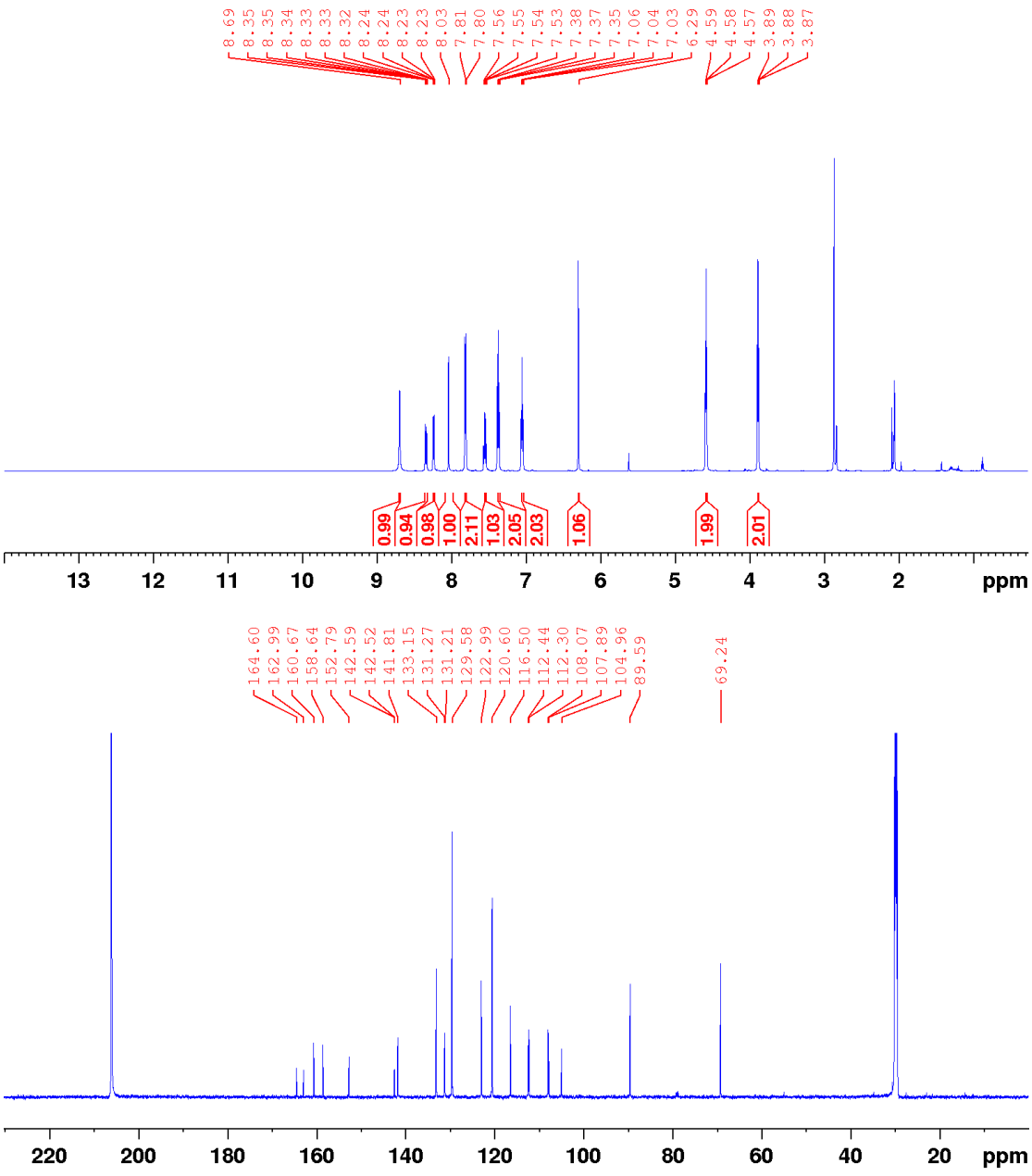
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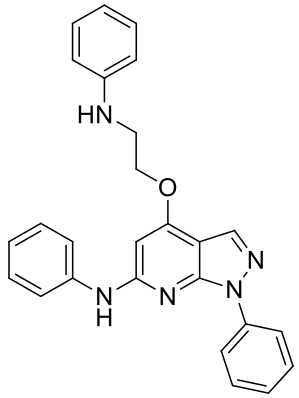
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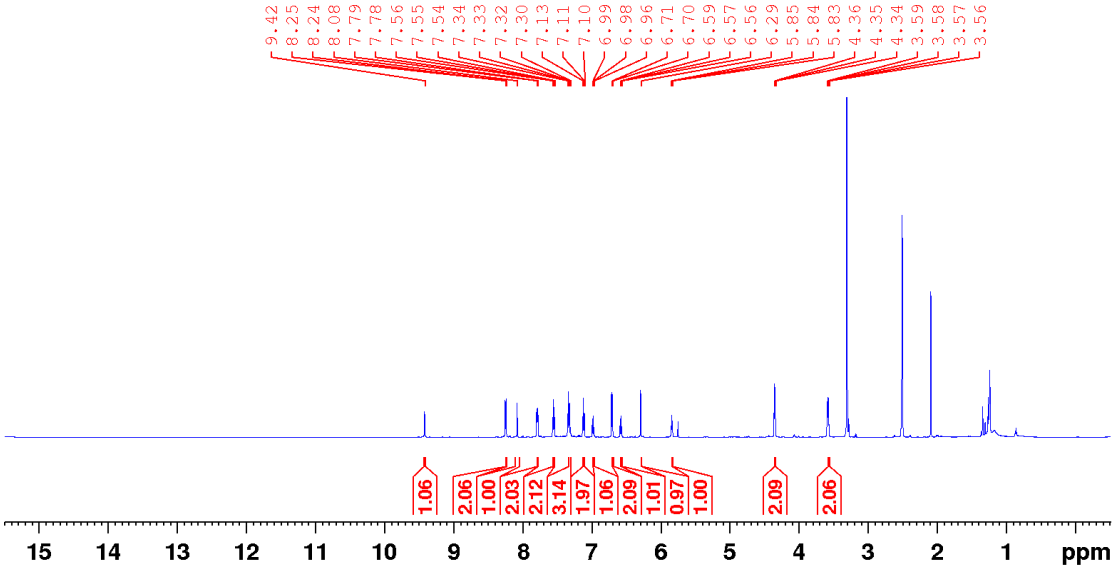
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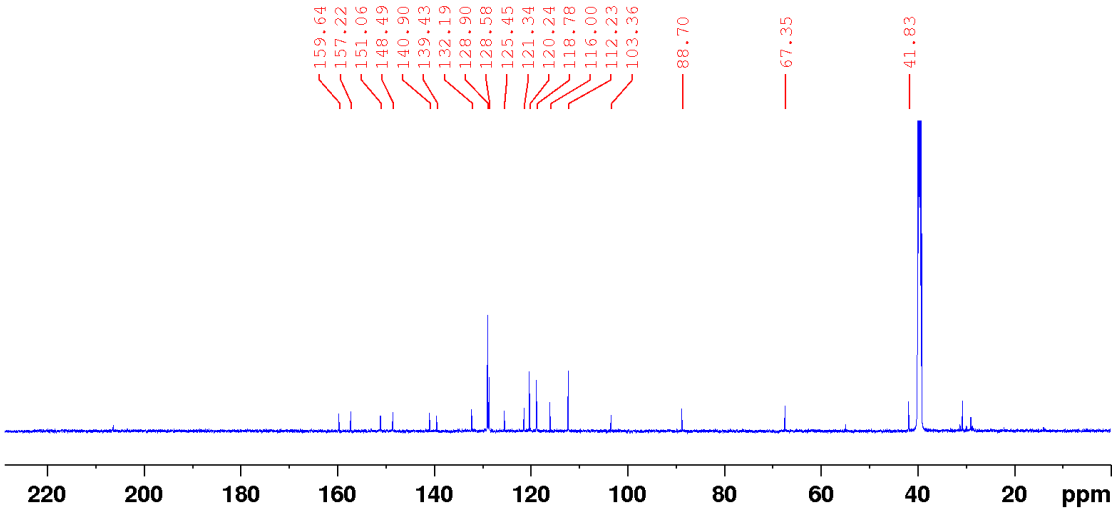
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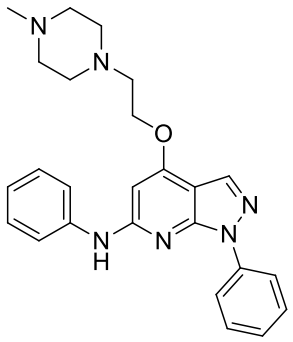
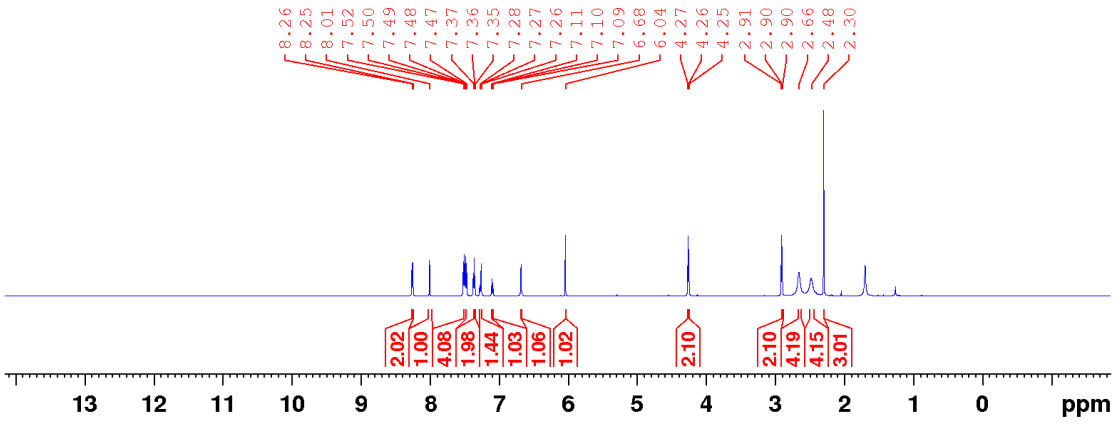


9b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7.35
7.28
7.27
7.26
7.11
7.10
7.09
6.68
6.04
4.27
4.26
4.25
2.91
2.90
2.90
2.66
2.48
2.30
2.02
1.00
4.08
1.98
1.44
1.03
1.06
1.02
2.10
2.10
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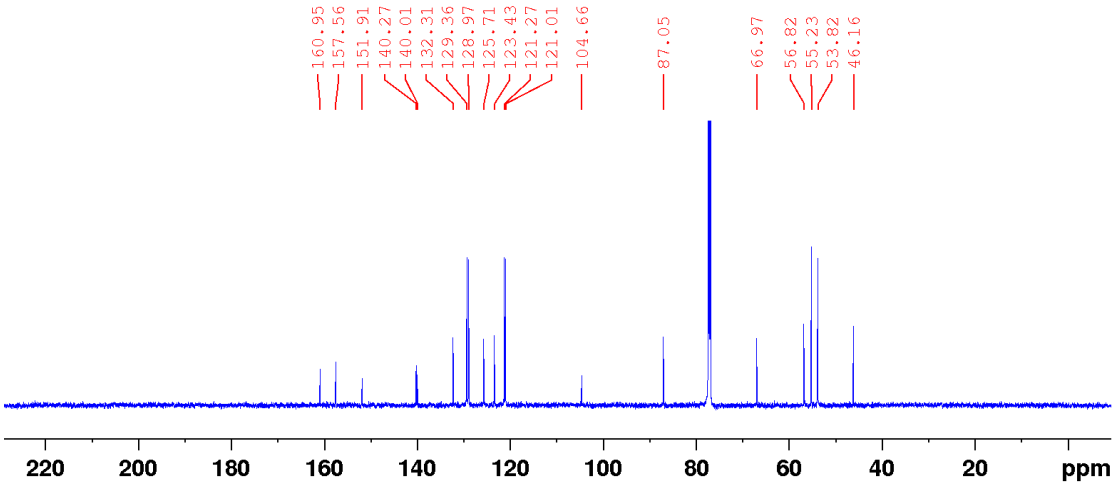
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157.56
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132.31
129.36
128.97
125.71
123.43
121.27
121.01
104.66
87.05
66.97
56.82
55.23
53.82
46.16
220
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180
160
140
120
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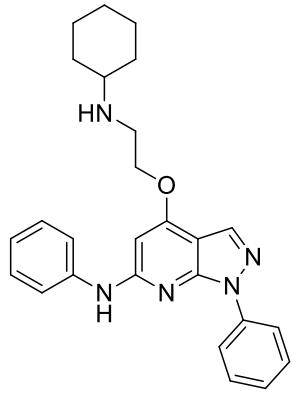
80
60
40
20
ppm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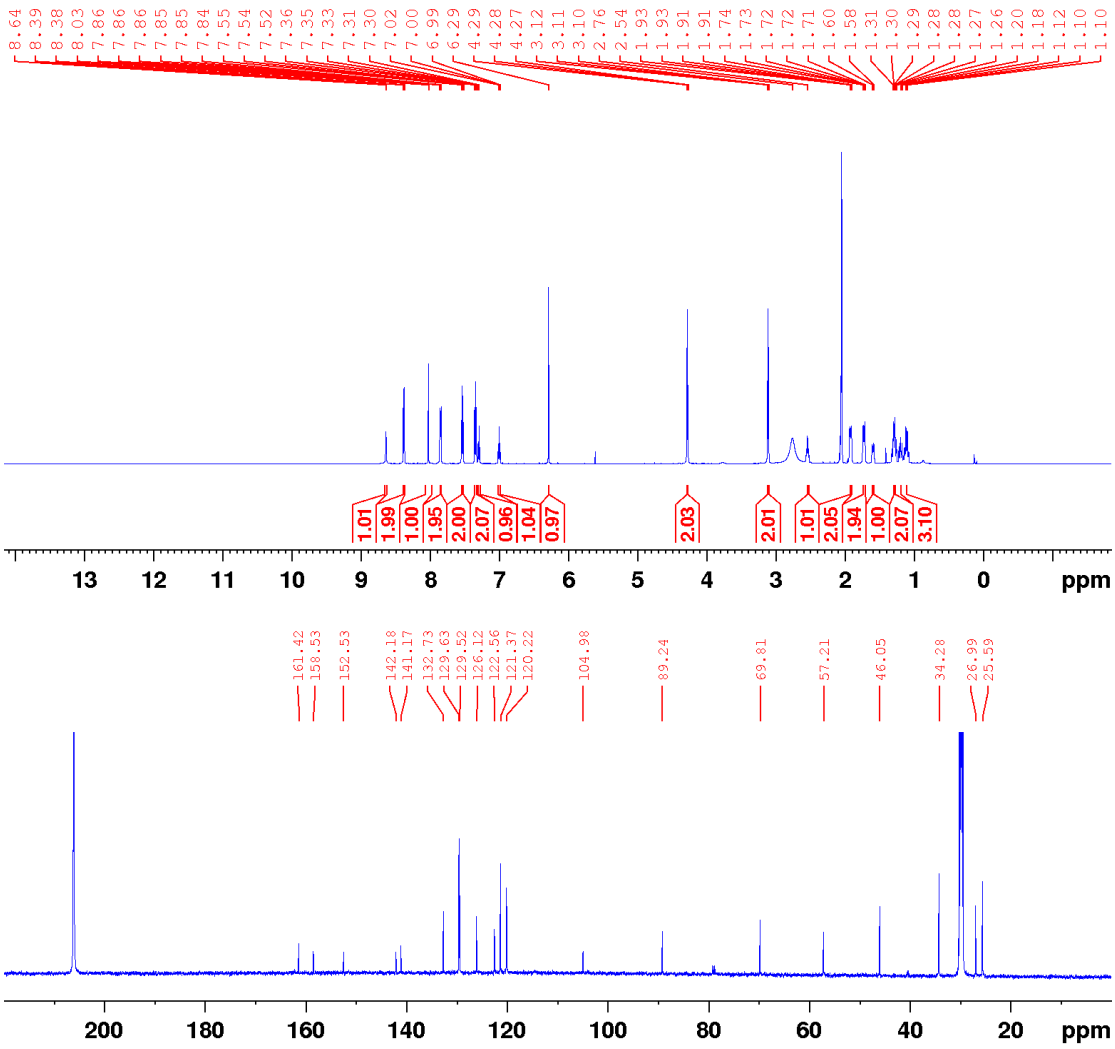
N
N
H
N

9c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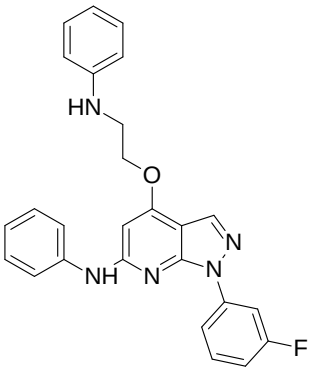
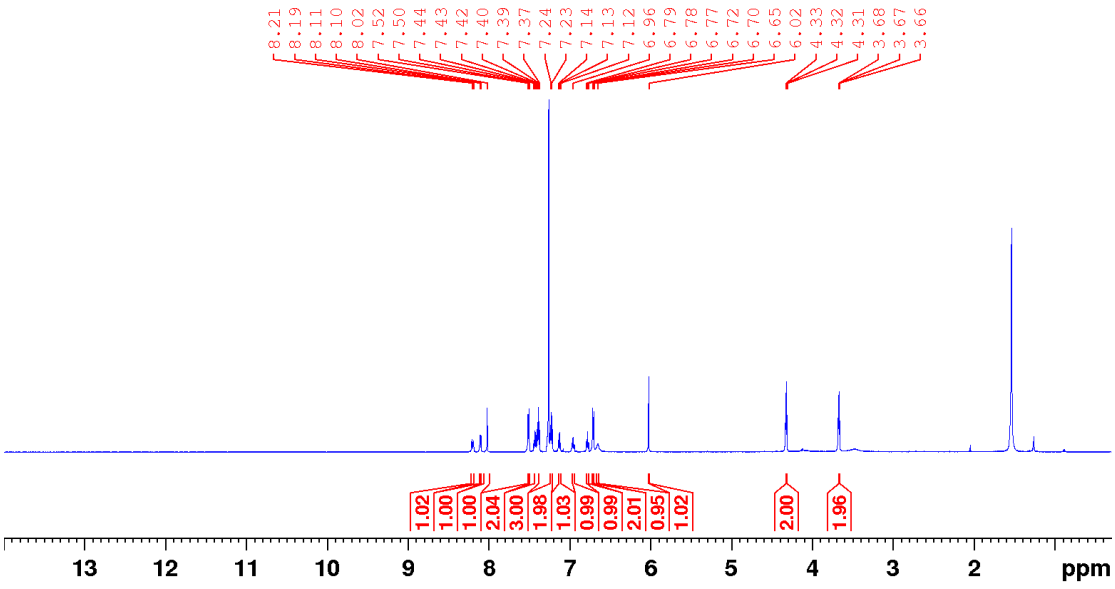
1.01 1.99 1.00 1.95 2.00 2.07 0.96 1.04 0.97 2.03 2.01 1.01 2.05 1.94 1.00 2.07 3.10
13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

161.42 158.53 152.53 142.18 141.17 132.73 129.63 129.52 126.12 122.56 121.37 120.22 104.98 89.24 69.81 57.21 46.05 34.28 26.99 25.59
200 180 160 140 120 100 80 60 40 20 ppm


HN
O
NH
N
N
N
F

9d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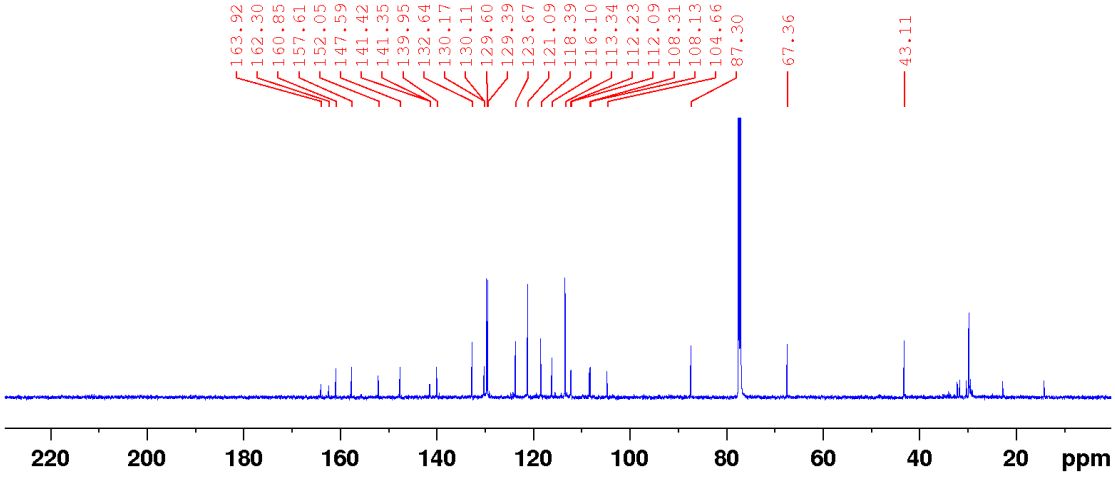
7.50
7.44
7.43
7.42
7.40
7.39
7.37
7.24
7.23
7.14
7.13
7.12
6.96
6.79
6.78
6.77
6.72
6.70
6.65
6.02
4.33
4.32
4.31
3.68
3.67
3.66
1.02
1.00
1.00
2.04
3.00
1.98
1.03
0.99
0.99
2.01
0.95
1.02
2.00
1.96
13
12
11
10
9
8
7
6
5
4
3
2
ppm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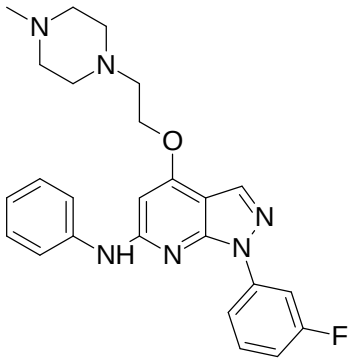
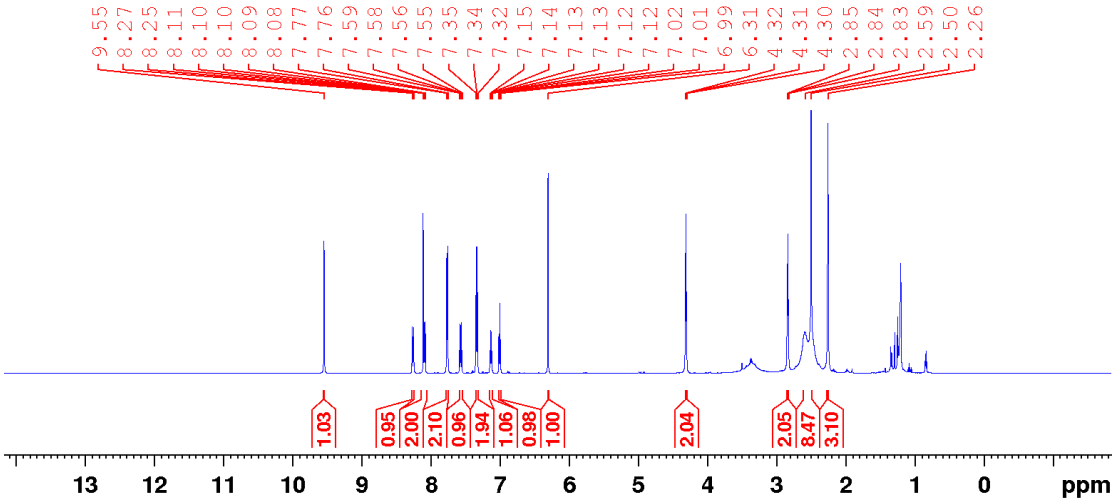
147.59
141.42
141.35
139.95
132.64
130.17
130.11
129.60
129.39
123.67
121.09
118.39
116.10
113.34
112.23
112.09
108.31
108.13
104.66
87.30
67.36
43.11
220
200
180
160
140
120
100
80
60
40
20
ppm


N
N
O
N
NH
N
N
F

9e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8.10
8.09
8.08
7.77
7.76
7.59
7.58
7.56
7.55
7.35
7.34
7.32
7.15
7.14
7.13
7.13
7.12
7.12
7.02
7.01
6.99
6.31
4.32
4.31
4.30
2.85
2.84
2.83
2.59
2.50
2.26
1.03
0.95
2.00
2.10
0.96
1.94
1.06
0.98
1.00
2.04
2.05
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3.10
13
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10
9
8
7
6
5
4
3
2
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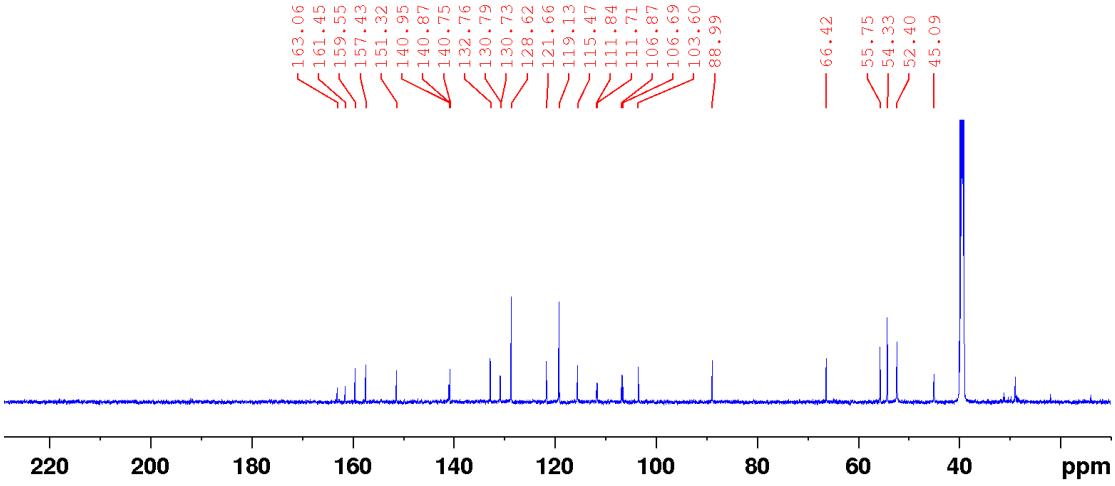
ppm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140.95
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140.75
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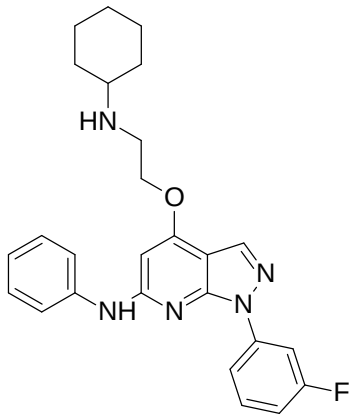
130.79
130.73
128.62
121.66
119.13
115.47
111.84
111.71
106.87
106.69
103.60
88.99
66.42
55.75
54.33
52.40
45.09
220
200
180
160
140
120
100
80
60
40
ppm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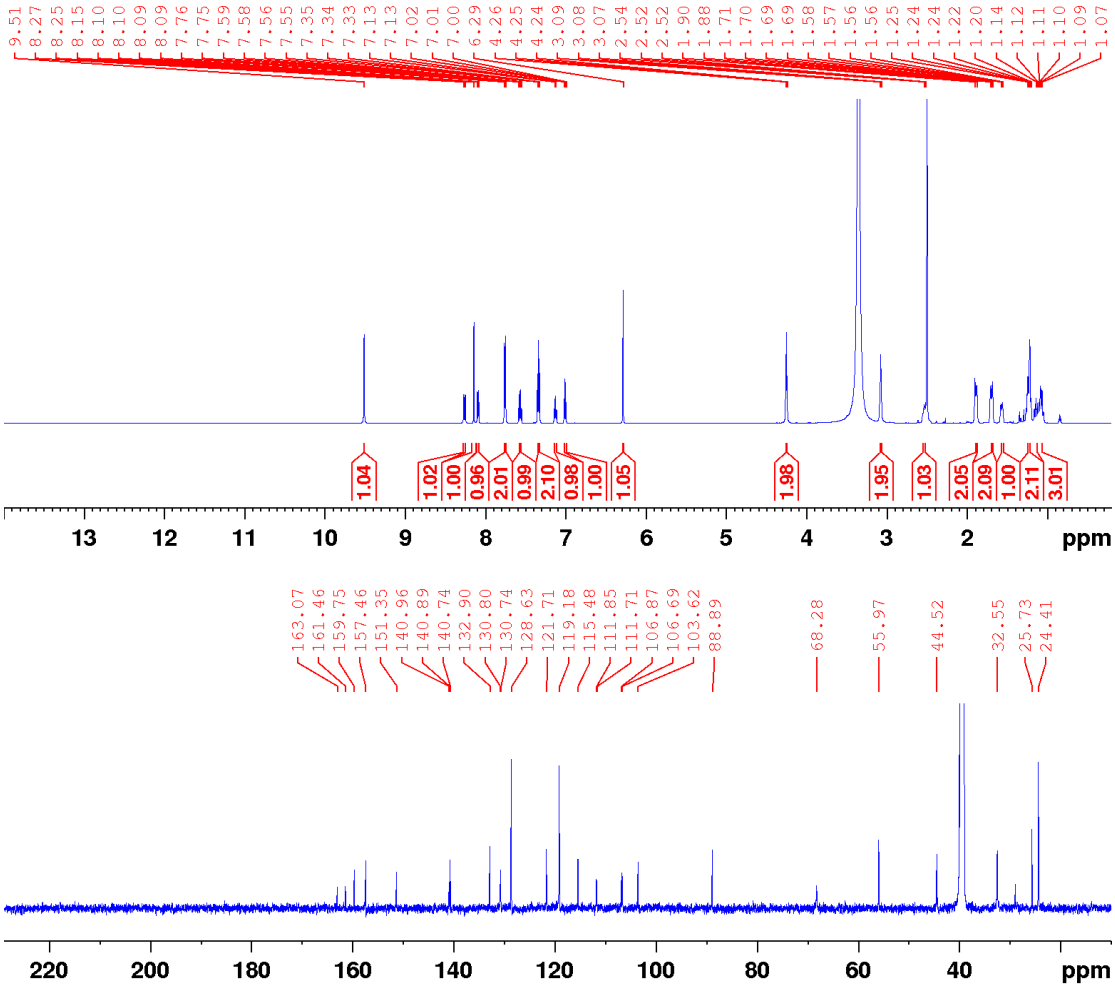
N
NH
N
F

9f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8.09
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7.58
7.56
7.55
7.35
7.34
7.33
7.13
7.13
7.02
7.01
7.00
6.29
4.26
4.25
4.24
3.09
3.08
3.07
2.54
2.52
2.52
1.90
1.88
1.71
1.70
1.69
1.69
1.58
1.57
1.56
1.56
1.25
1.24
1.24
1.22
1.20
1.14
1.12
1.11
1.10
1.09
1.07
1.04
1.02
1.00
0.96
2.01
0.99
2.10
0.98
1.00
1.05
1.98
1.95
1.03
2.05
2.09
1.00
2.11
3.01
13
12
11
10
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ppm
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159.75
157.46
151.35
140.96
140.89
140.74
132.90
130.80
130.74
128.63
121.71
119.18
115.48
111.85
111.71
106.87
106.69
103.62
88.89
68.28
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32.55
25.73
24.41
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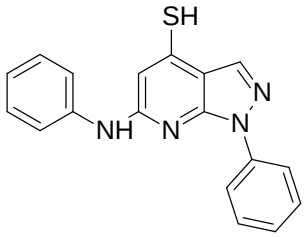
80
60
40
ppm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NH
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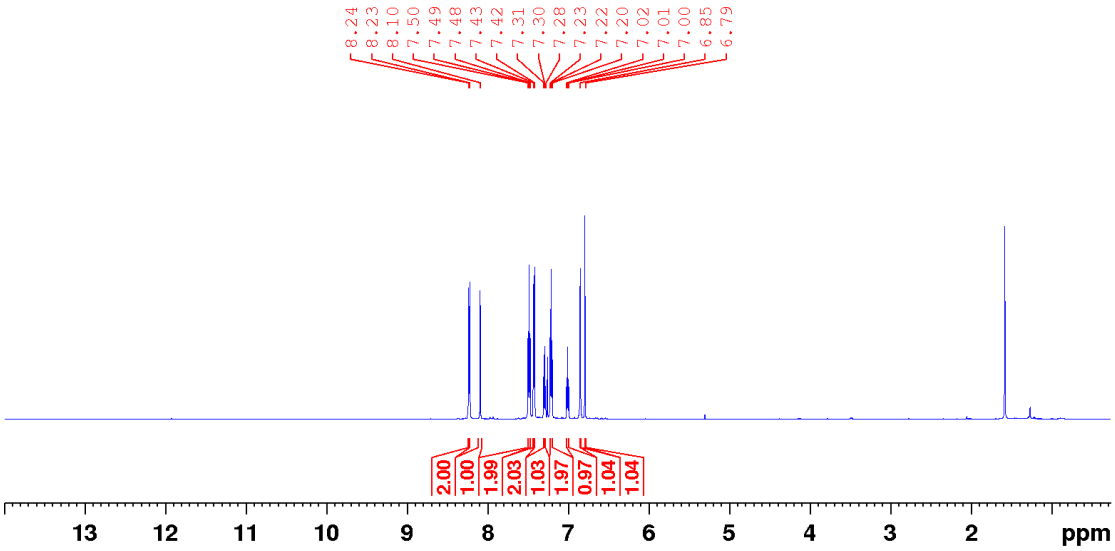
N

10a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7.48
7.43
7.42
7.31
7.30
7.28
7.23
7.22
7.20
7.02
7.01
7.00
6.85
6.79
2.00
1.00
1.99
2.03
1.03
1.97
0.97
1.04
1.04
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12
11
10
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8
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4
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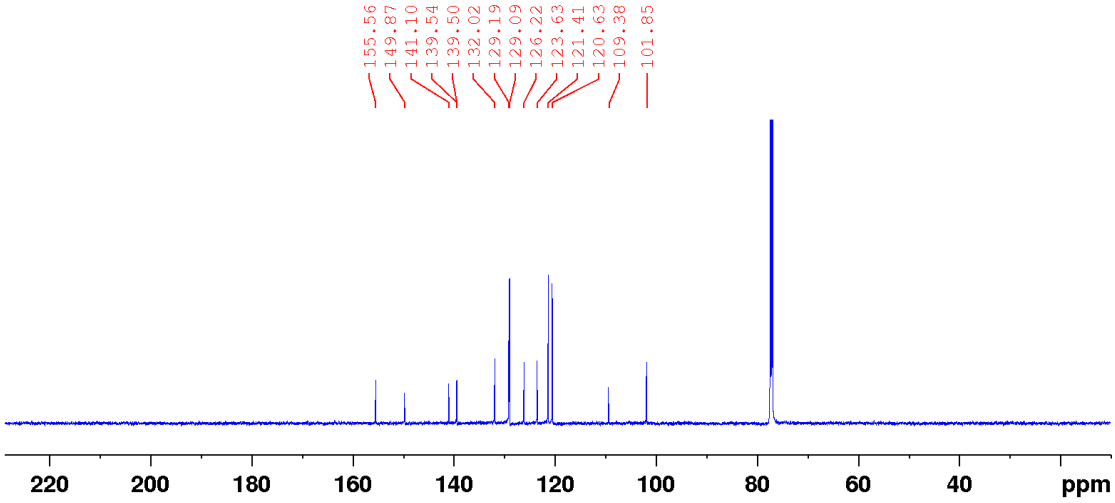
ppm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149.87
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139.54
139.50
132.02
129.19
129.09
126.22
123.63
121.41
120.63
109.38
101.85
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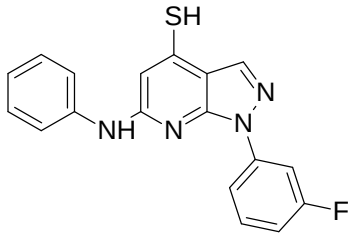
160
140
120
100
80
60
40
ppm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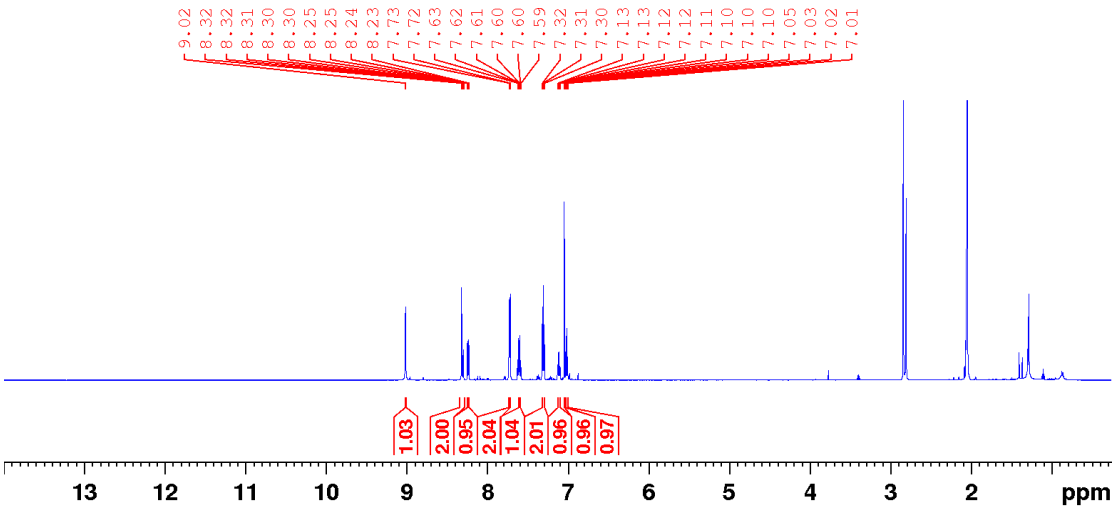
N
F

10b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7.60
7.60
7.59
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7.30
7.13
7.13
7.12
7.12
7.11
7.10
7.10
7.10
7.05
7.03
7.02
7.01
1.03
2.00
0.95
2.04
1.04
2.01
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0.96
0.97
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12
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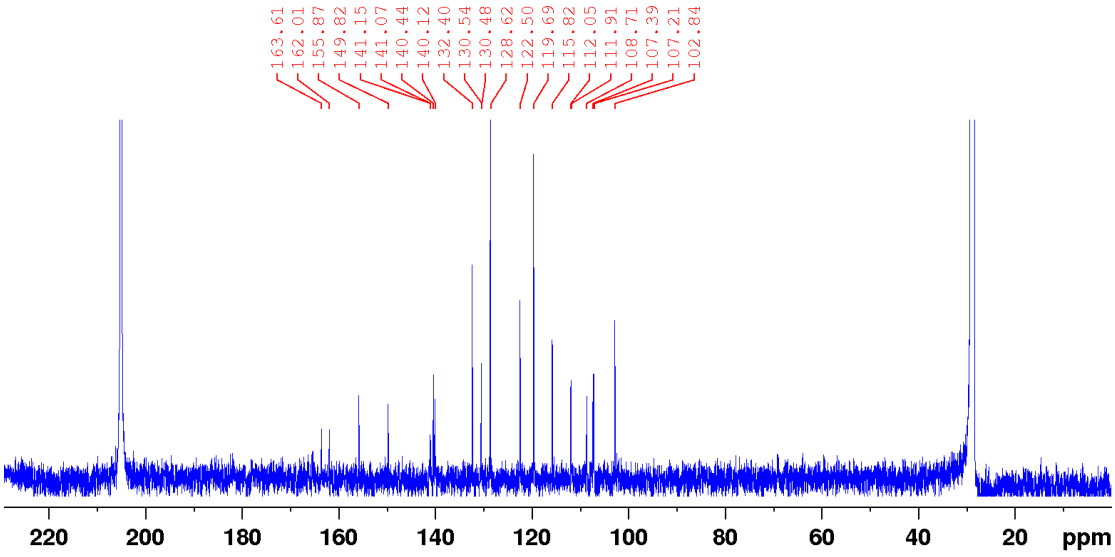
4
3
2
ppm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130.48
128.62
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112.05
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20
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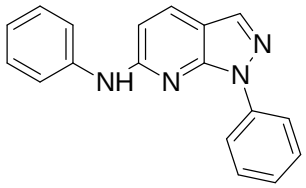
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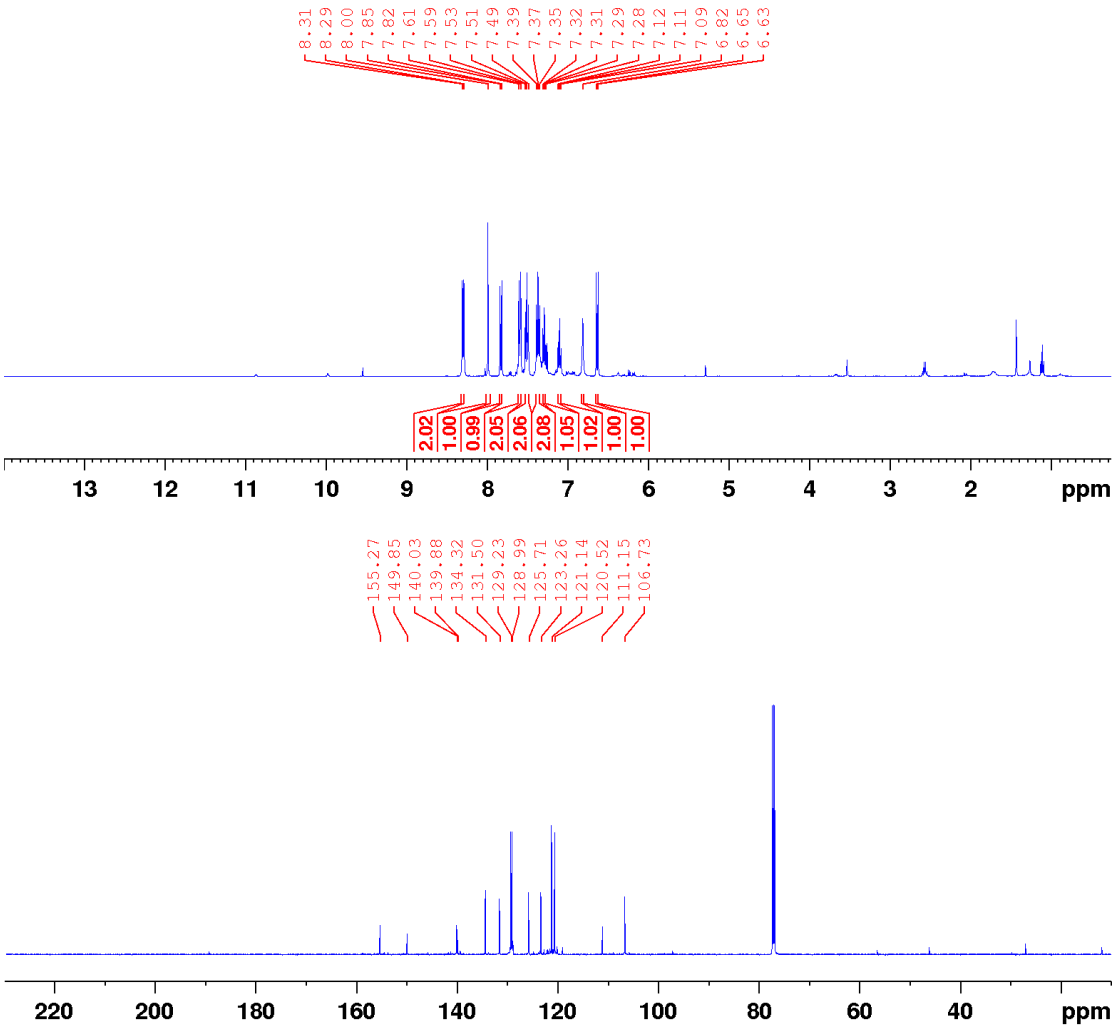
11a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7.39
7.37
7.35
7.32
7.31
7.29
7.28
7.12
7.11
7.09
6.82
6.65
6.63
2.02
1.00
0.99
2.05
2.06
2.08
1.05
1.02
1.00
1.00
13
12
11
10
9
8
7
6
5
4
3
2
ppm
155.27
149.85
140.03
139.88
134.32
131.50
129.23
128.99
125.71
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120.52
111.15
106.73
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140
120
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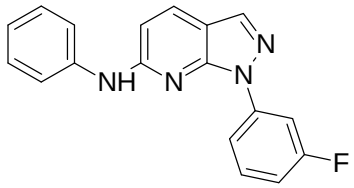
80
60
40
ppm


NH
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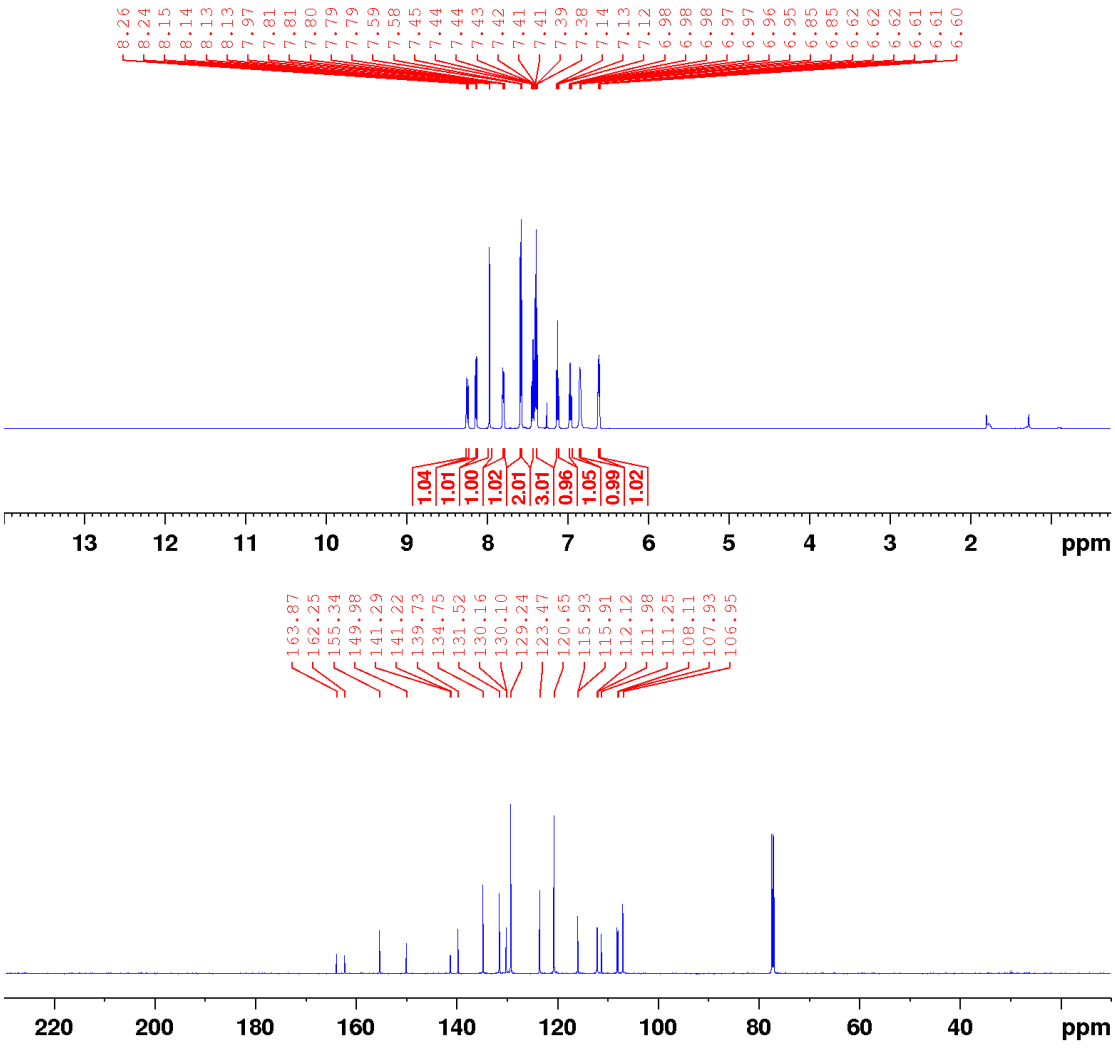
N
N
F

11b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7.58
7.45
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7.44
7.43
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7.41
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7.13
7.12
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6.98
6.98
6.97
6.97
6.96
6.95
6.85
6.85
6.62
6.62
6.62
6.61
6.61
6.60
1.04
1.01
1.00
1.02
2.01
3.01
0.96
1.05
0.99
1.02
13
12
11
10
9
8
7
6
5
4
3
2
ppm

163.87
162.25
155.34
149.98
141.29
141.22
139.73
134.75
131.52
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130.10
129.24
123.47
120.65
115.93
115.91
112.12
111.98
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106.95
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140
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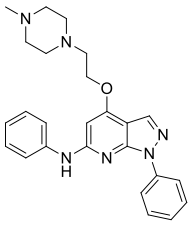
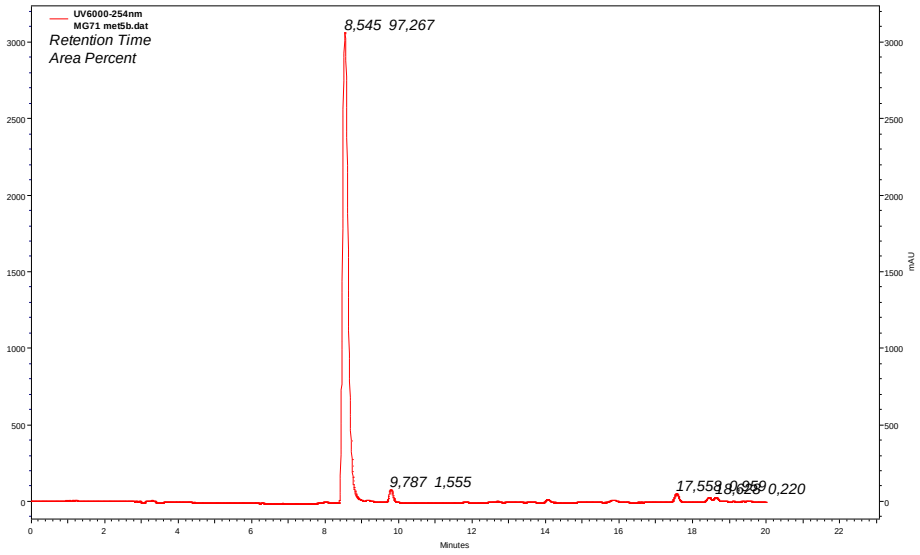
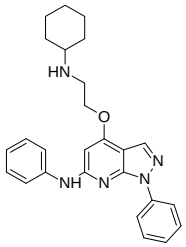
100
80
60
40
ppm

HPLC of the compounds tested in vivo



9b




HN
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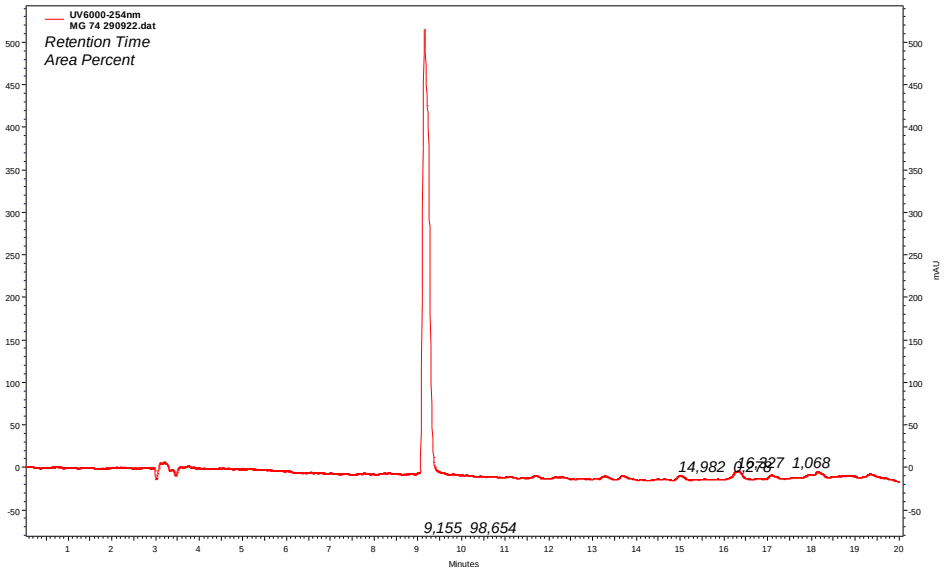
N
N

9c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Retention Time
Area Percent
mAU
Minutes
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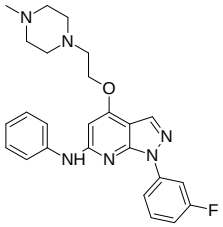
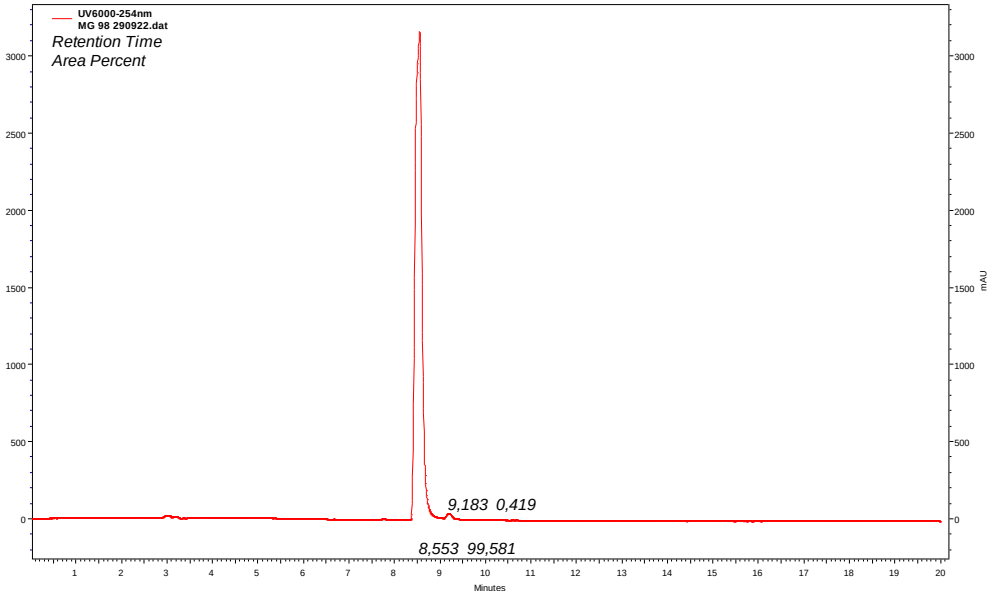
14,982 0,278
16,327 1,068


N
N
O
NH
N
N
N
F

9e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MG 98 290922.dat
Retention Time
Area Percent
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8,553 99,581
Minutes
mAU