Supporting Information

Discovery of Potent and Selective BRD4 Inhibitors Capable of Blocking TLR3-Induced Acute Airway Inflammation

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Allan R. Brasier[b,c,d] and Jia Zhou[a,c,d,*]

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Figure S1. Molecular docking of **35** into BRD2 BD1. Docking result of **35** with BRD2 BD1 (PDB code: 4A9M).

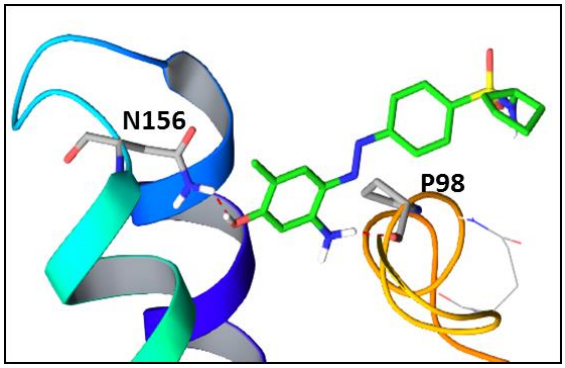
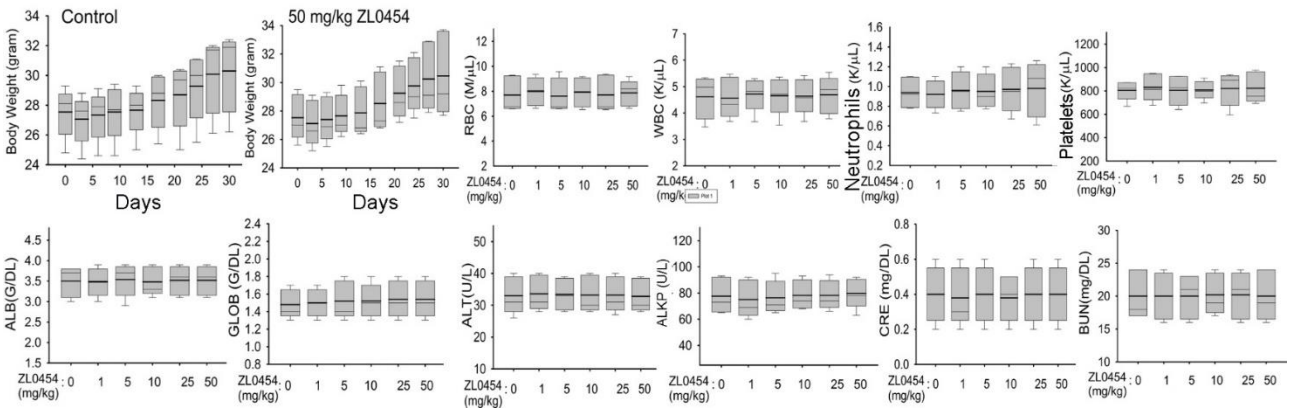


Figure S2. Assessment of chronic treatment of compound **35** on mouse physiology.



Groups of n = 5 mice were exposed to **35** from 0-50 mg/kg daily (IP). **A**. Effect on body weight. 30 d later, animals were sacrificed and hematological measurements were made (**B**) for red blood cells (RBCs), white blood cells (WBCs), neutrophils, and platelets. **C.** Liver function tests for albumin (ALB), globulin (GLOB), alanine aminotransferase (ALT), and alkaline phosphatase (ALKP). **D**. renal function. Data are shown as 10-90% confidence. No significant difference between groups (ANOVA).

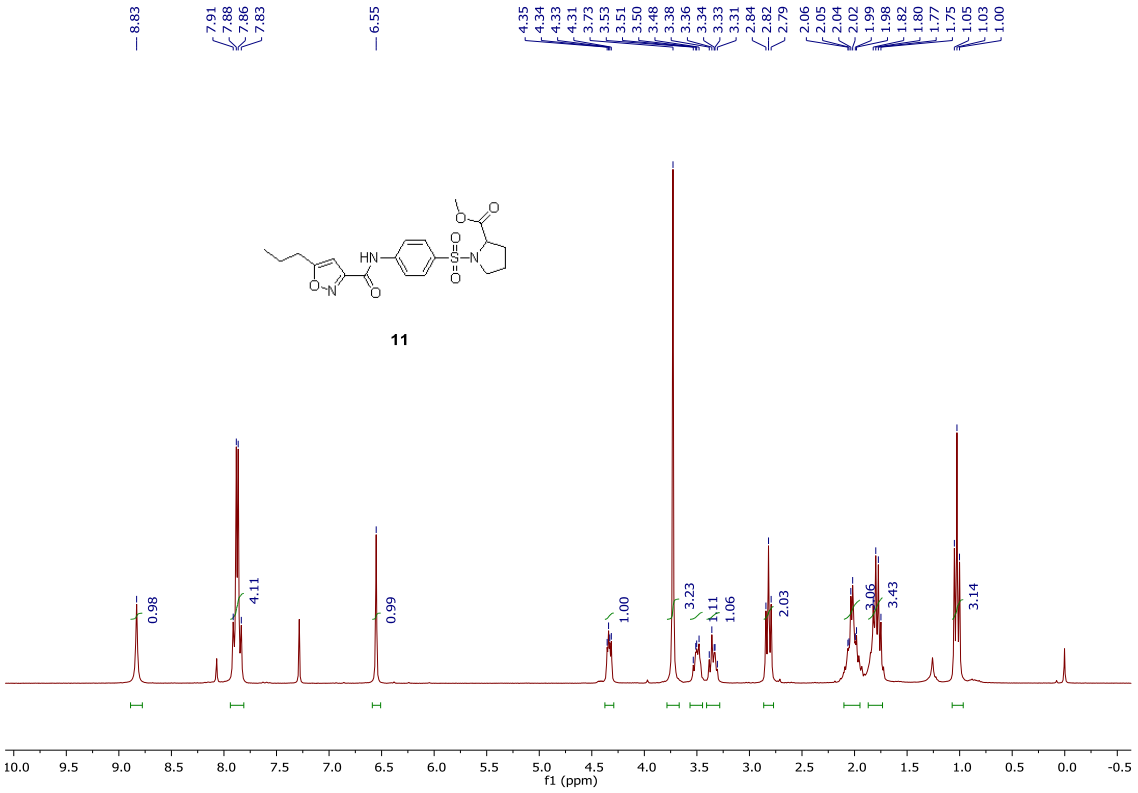
Table S1. Off-target screening of compound **35** through Eurofins Cerep panel assays[a]

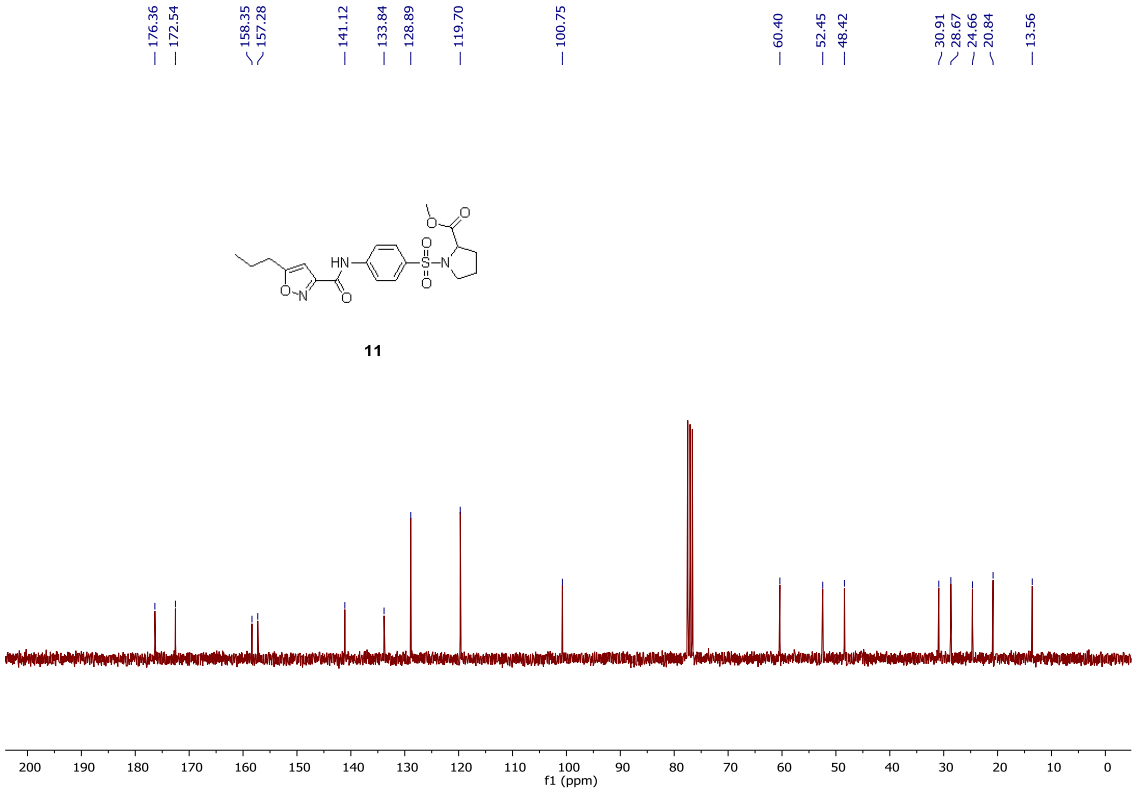
Receptors/Enzymes	%
A_{2A} (*h*) (agonist radioligand)	38.1
a_{1A} (*h*) (agonist radioligand)	17.2
a_{2A} (*h*) (agonist radioligand)	3.0
β_1 (*h*) (agonist radioligand)	7.4
B_2 (*h*) (agonist radioligand)	-2.4
BZD (central) (agonist radioligand)	8.3
CB_1 (*h*) (agonist radioligand)	24.7
CB_2 (*h*) (agonist radioligand)	59.3
CCK_1 (CCK_A) (*h*) (agonist radioligand)	17.4
D_1 (*h*) (antagonist radioligand)	21.9
D_{2s} (*h*) (agonist radioligand)	36.9
ETA (h) (agonist radioligand)	-4.3
NMDA (antagonist radioligand)	17.0
H_1 (*h*) (antagonist radioligand)	7.1
H_2 (*h*) (antagonist radioligand)	-10.1
MAO-A (antagonist radioligand)	49.8
M_1 (*h*) (antagonist radioligand)	-1.5
M_2 (*h*) (antagonist radioligand)	21.6
M_3 (*h*) (antagonist radioligand)	4.8
N neuronal $\alpha4\beta2$ (*h*) (agonist radioligand)	-2.9
δ (DOP) (h) (agonist radioligand)	-2.7
κ (KOP) (agonist radioligand)	8.9

μ (MOP) (*h*) (agonist radioligand)	17.4
5-HT_{1A} (*h*) (agonist radioligand)	-0.1
5-HT_{1B} (*h*) (antagonist radioligand)	-9.6
5-HT_3 (*h*) (antagonist radioligand)	-12.9
GR (*h*) (agonist radioligand)	15.0
V_{1a} (*h*) (agonist radioligand)	32.4
Ca^{2+} channel (L, dihydropyridine site) (antagonist radioligand)	42.0
Potassium Channel hERG (human)- [3H] Dofetilide	23.6
K_V channel (antagonist radioligand)	8.5
Na^+ channel (site 2) (antagonist radioligand)	27.7
5-HT transporter (*h*) (antagonist radioligand)	15.0
COX1(*h*)	41.6
COX1(*h*)	28.2
PDE3A (*h*)	46.0
PDE4D2 (*h*)	39.4
Lck kinase (*h*)	-20.6
acetylcholinesterase (*h*)	11.7
IKKα (*h*)	-14.2
IKKε (*h*) (IKBKE)	-26.2

[a]The panel assay screening was conducted by Eurofins Pharma Discovery Services (https://www.eurofinsdiscoveryservices.com/). Compound **35** was tested at the concentration of 10 μM. Compound binding was calculated as a % inhibition of the binding of a radioactively labeled ligand specific for each target. Compound enzyme inhibition effect was calculated as a % inhibition of control enzyme activity. Results are reported as mean of two independent measurements.

Copies of ^{1}H and ^{13}C NMR spectra

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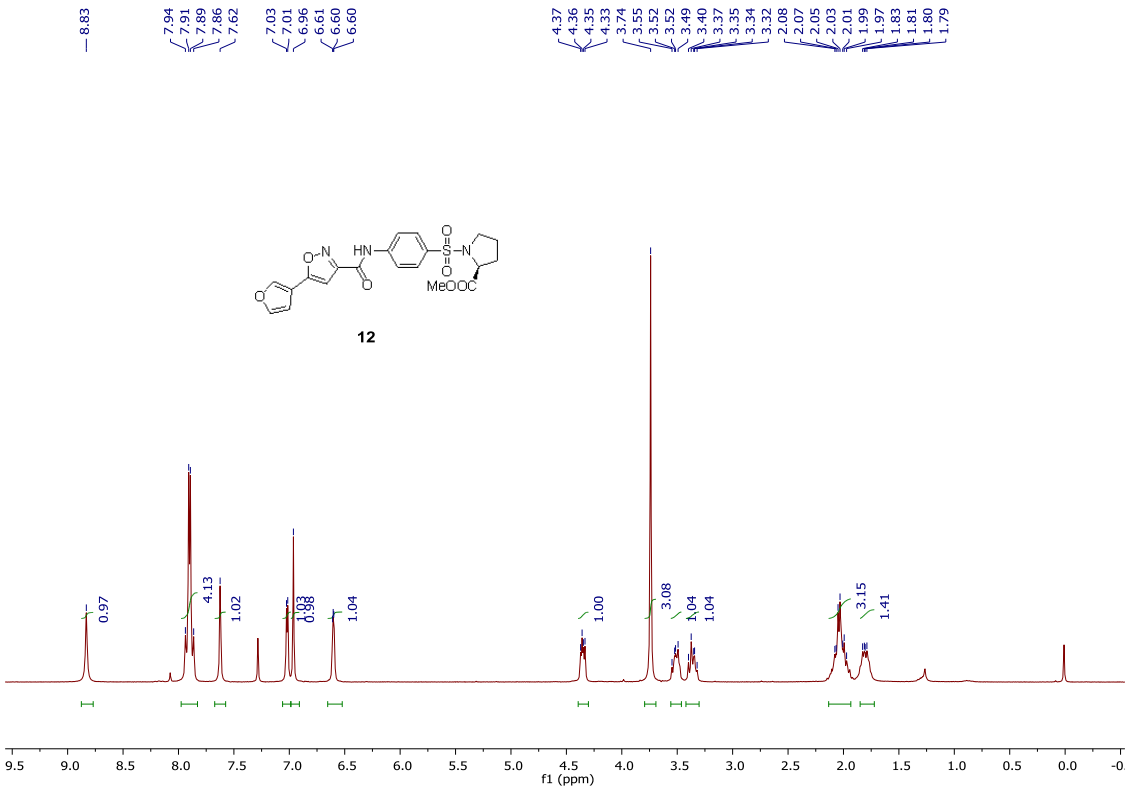
11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O
O
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O
O
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N
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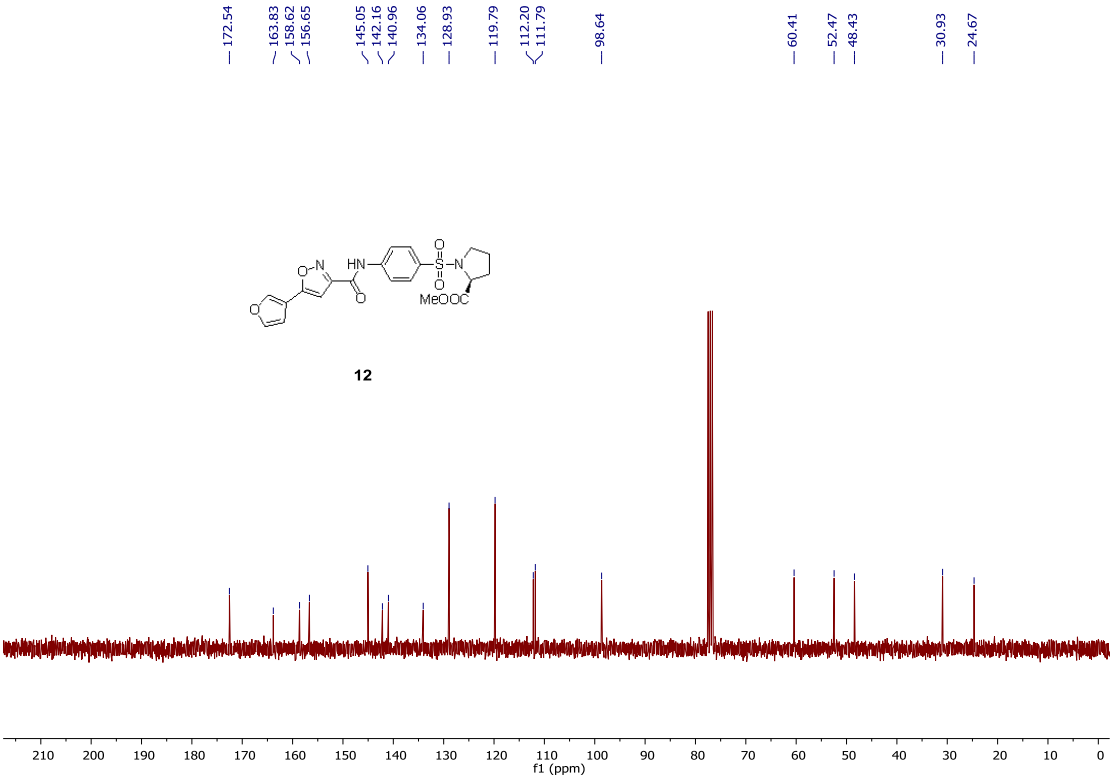
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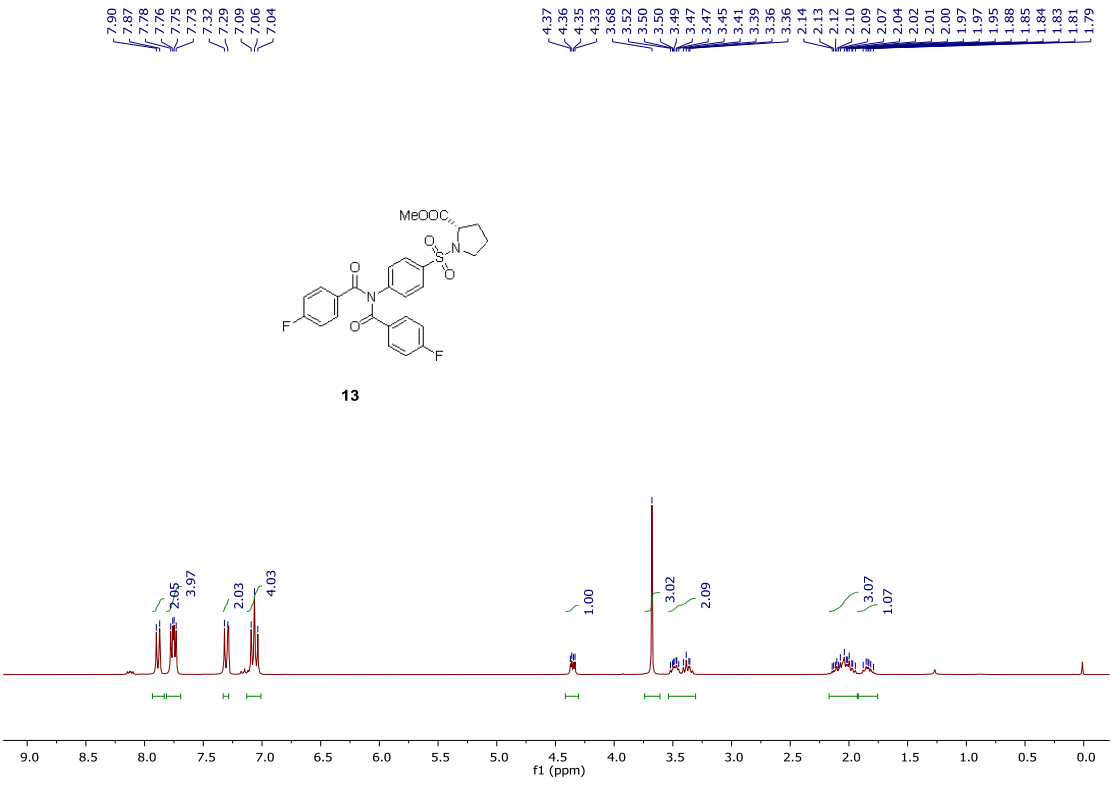
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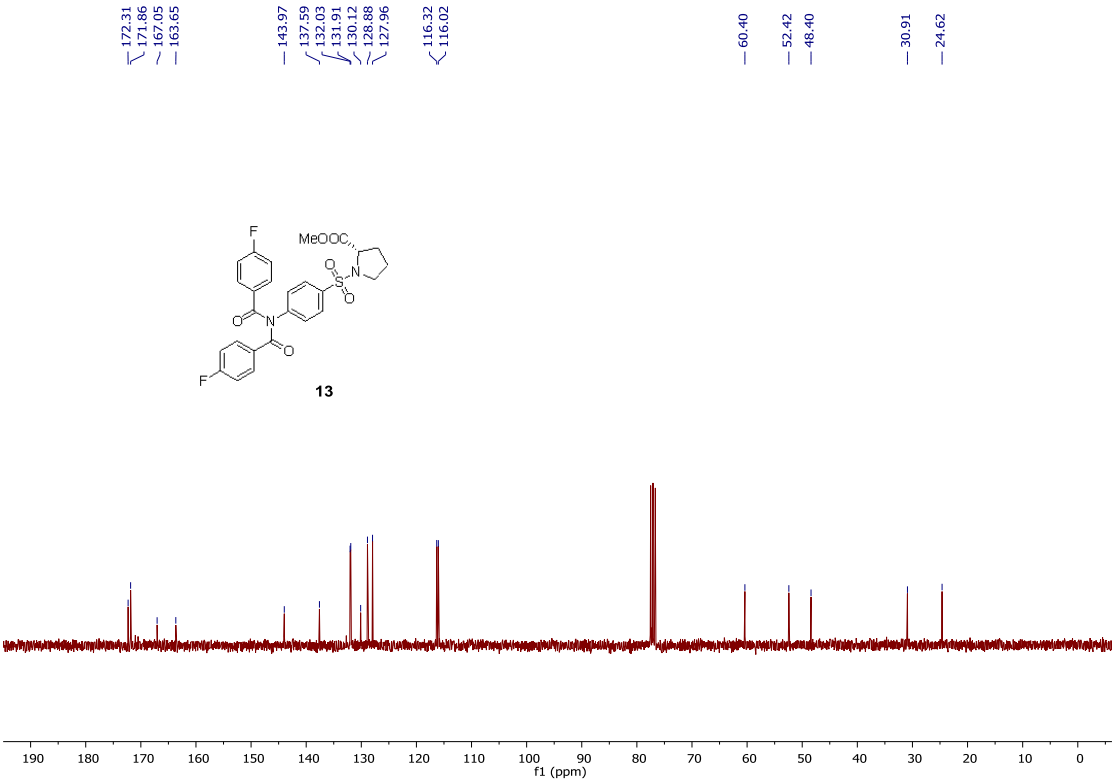
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F
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O
N
S
O
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N
O
F
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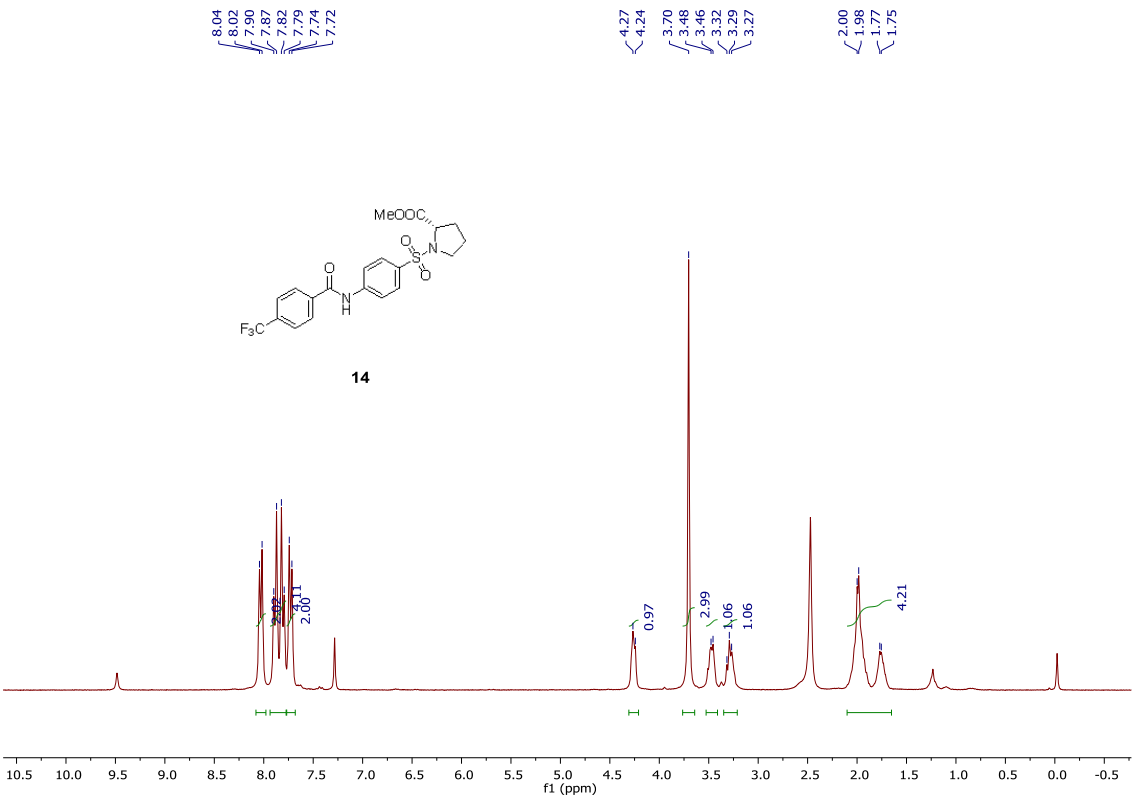
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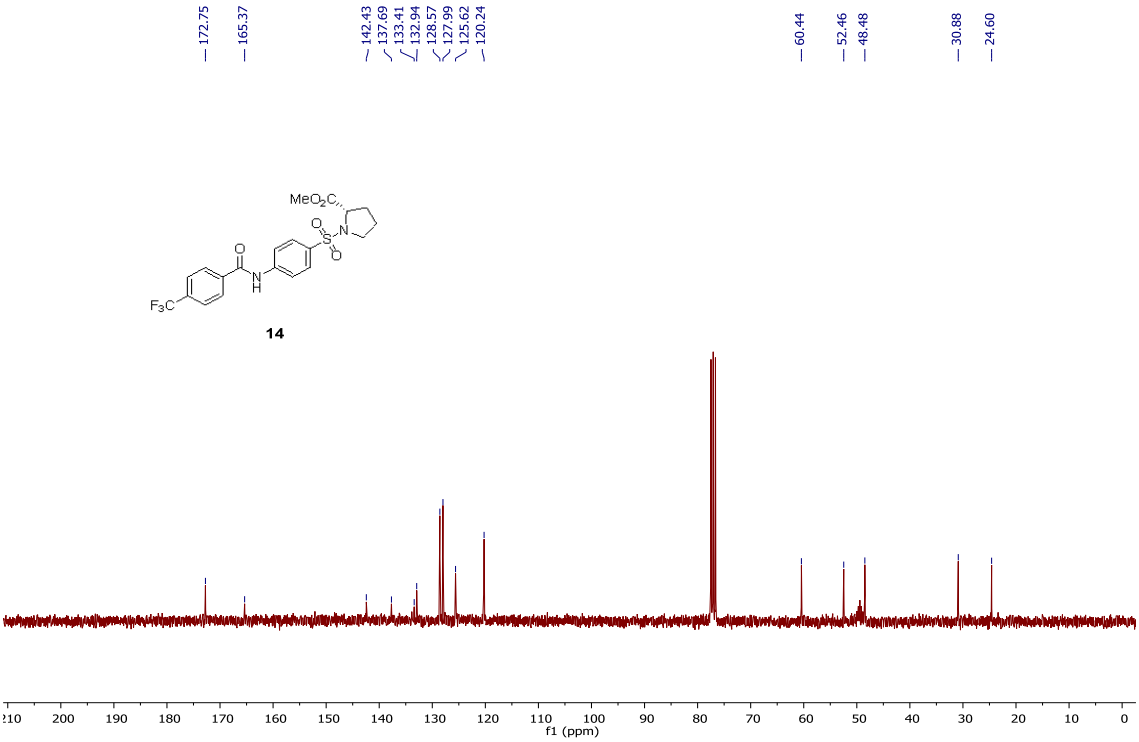
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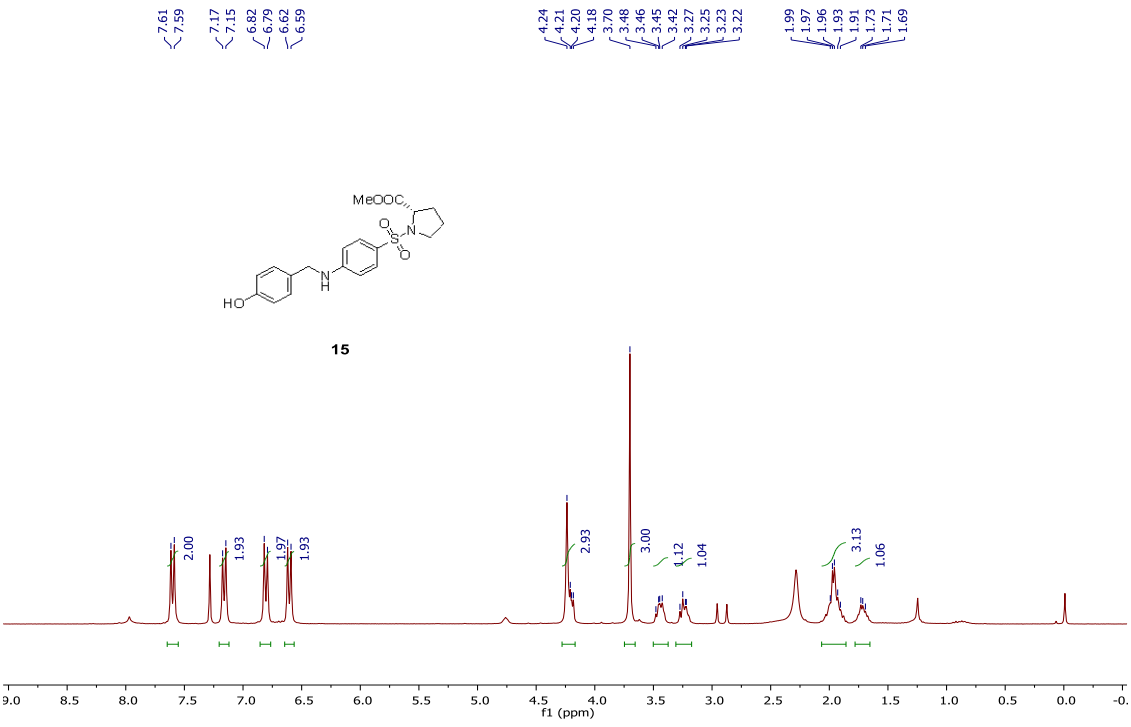
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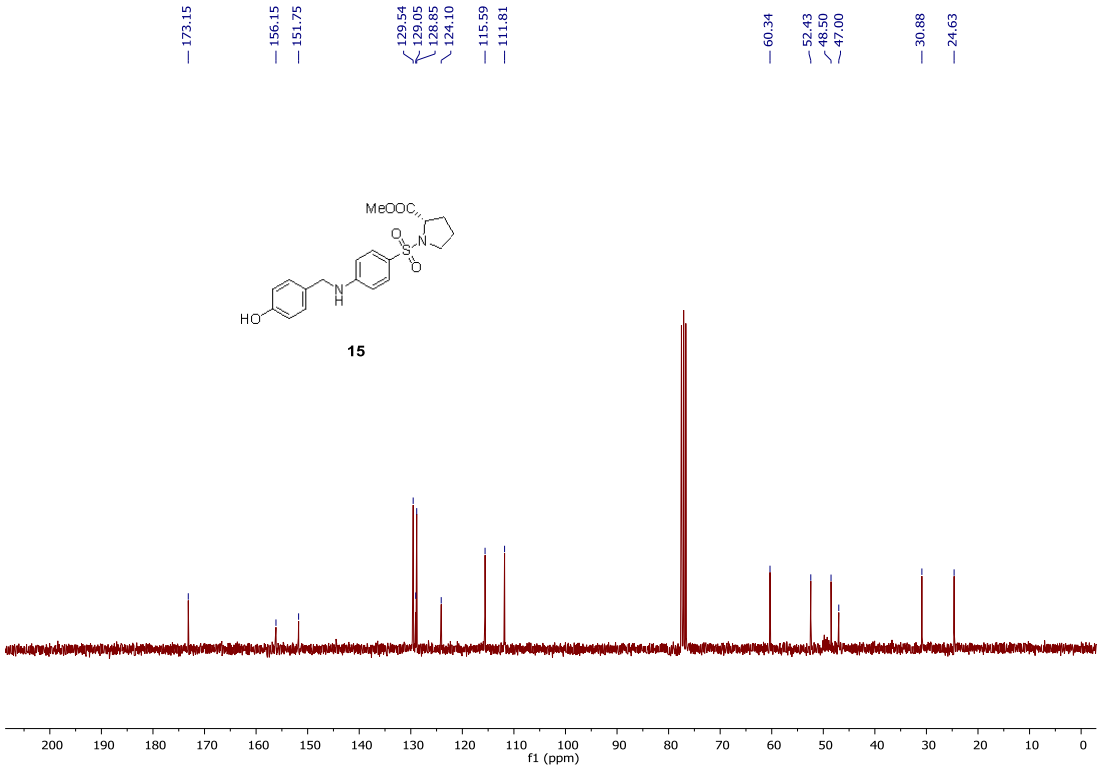
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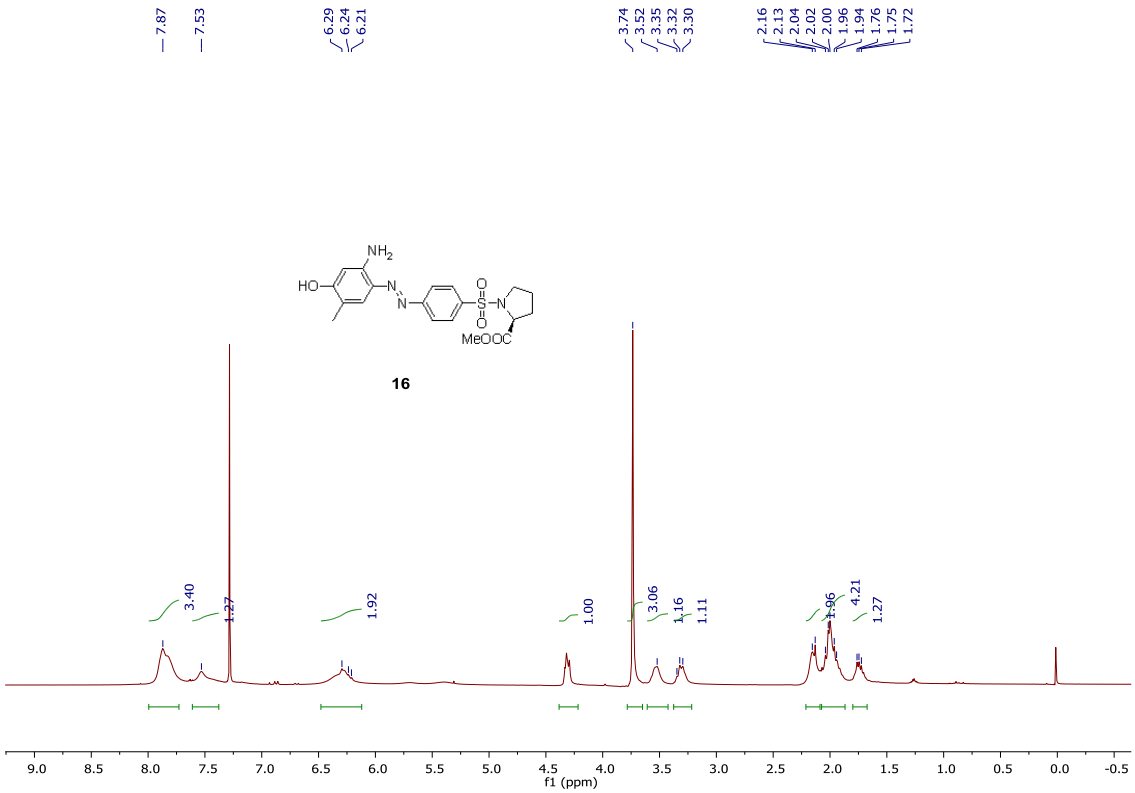
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MeOOC
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4.21
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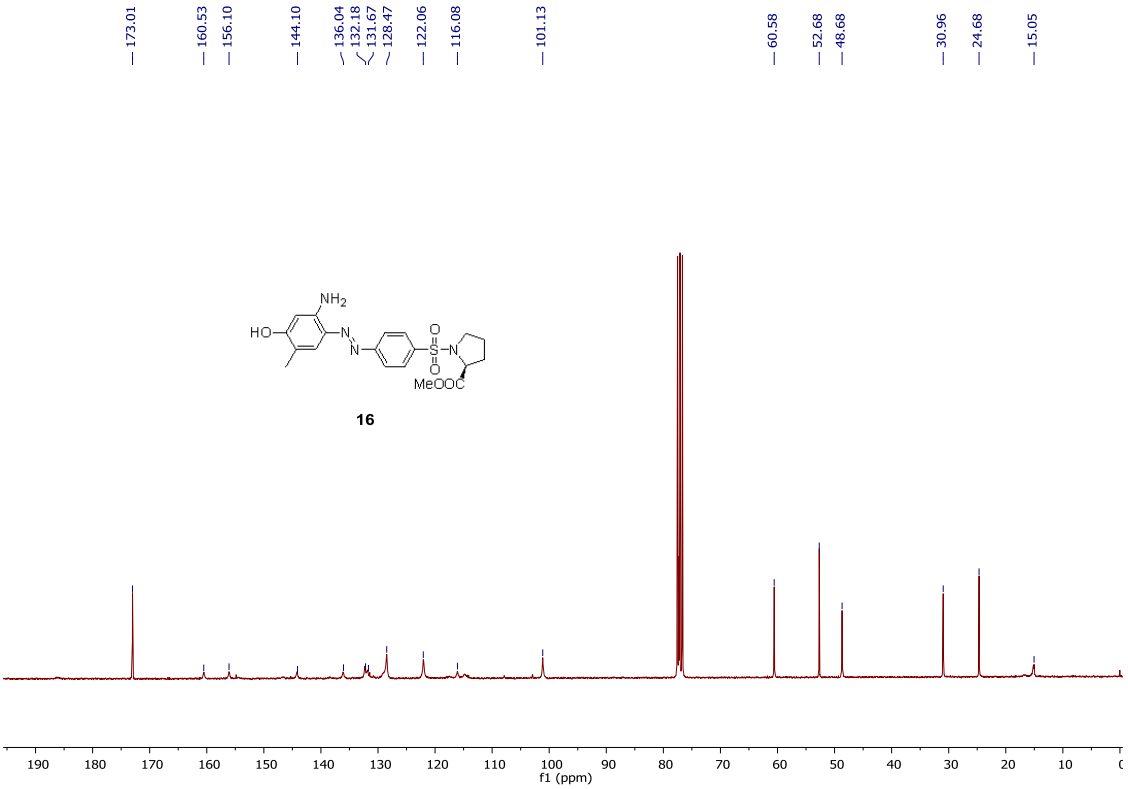
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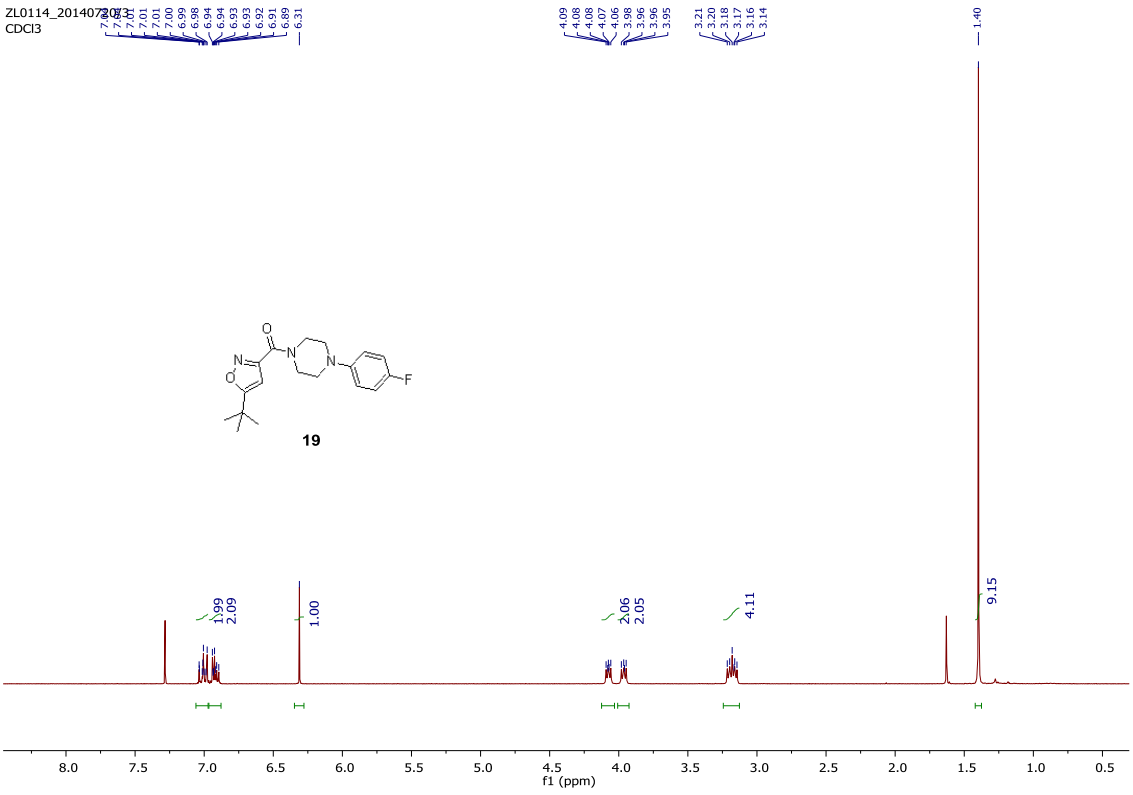
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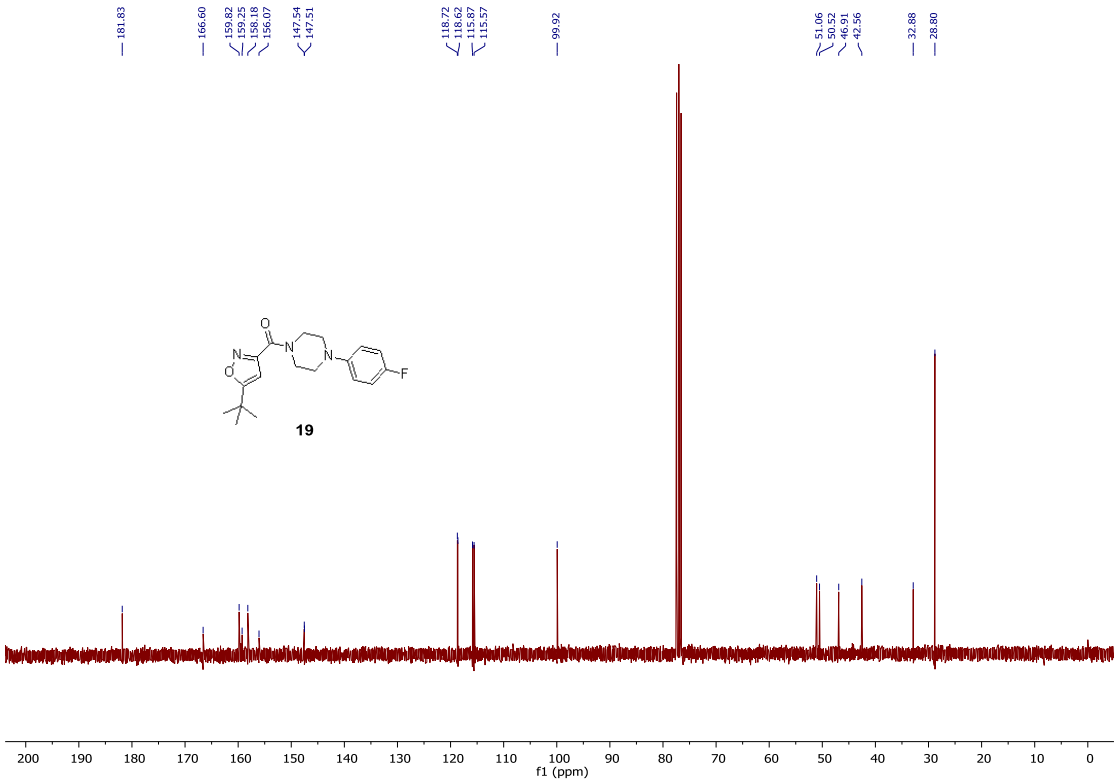
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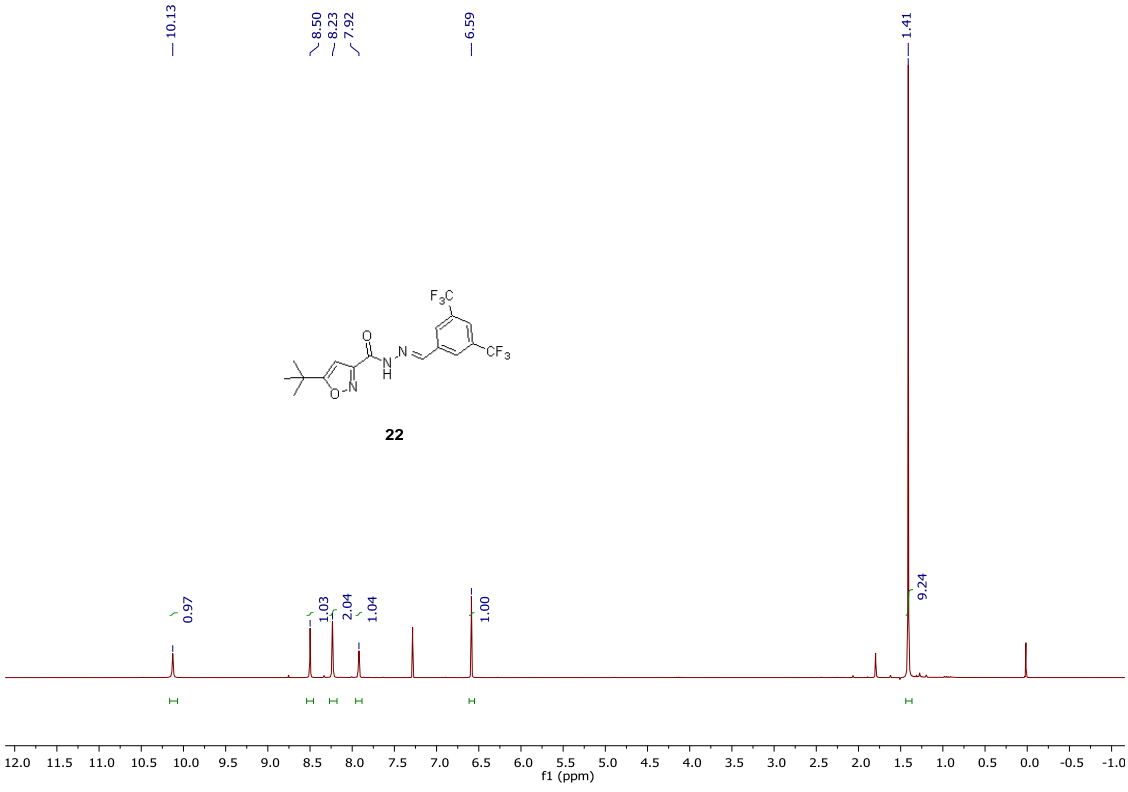
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1.04
1.00
9.24
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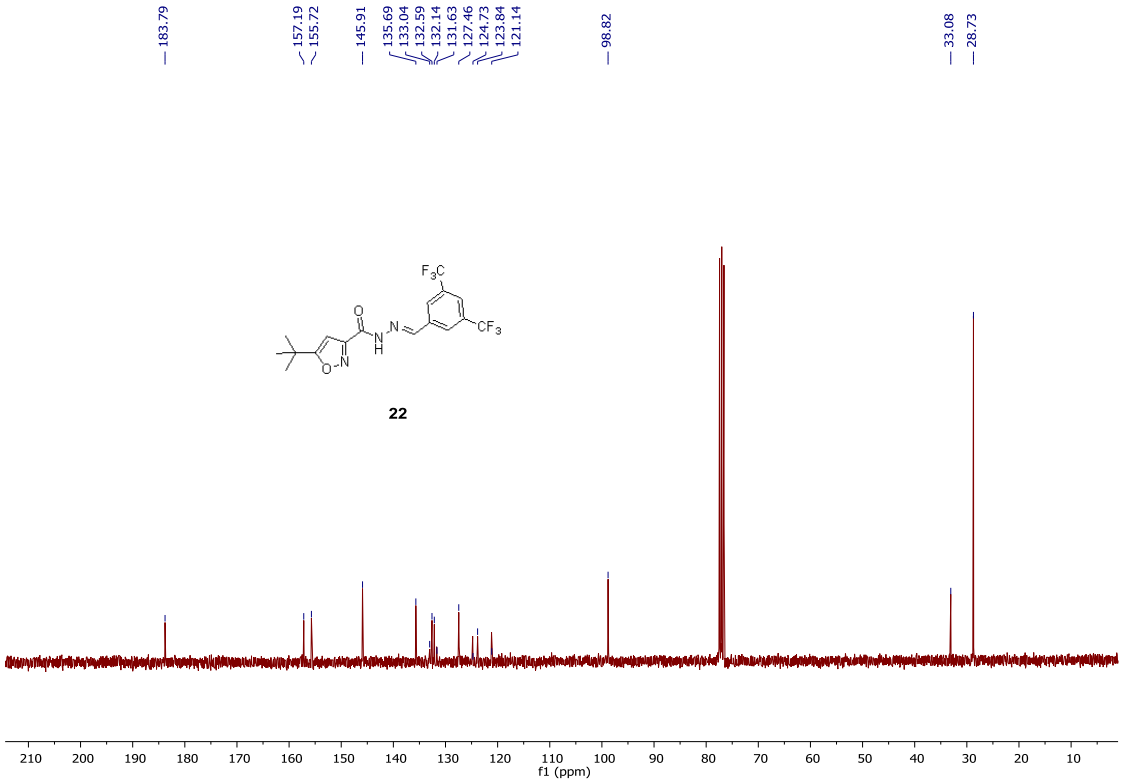
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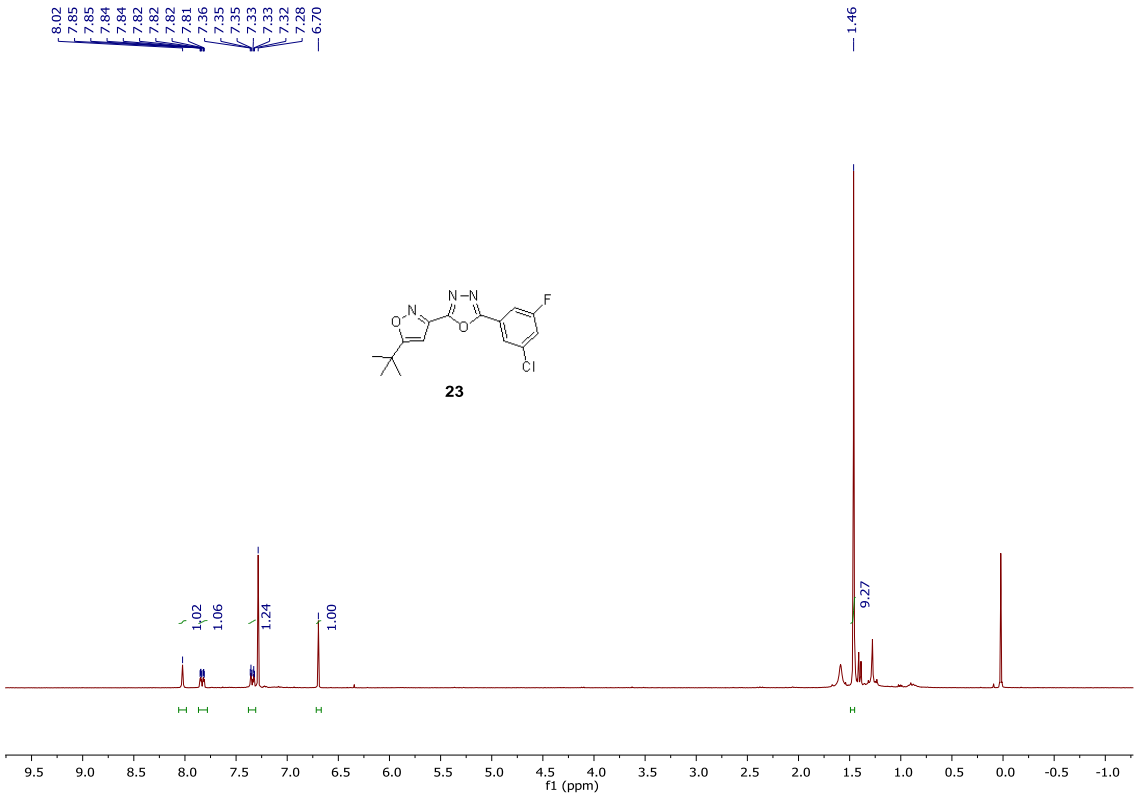
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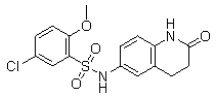

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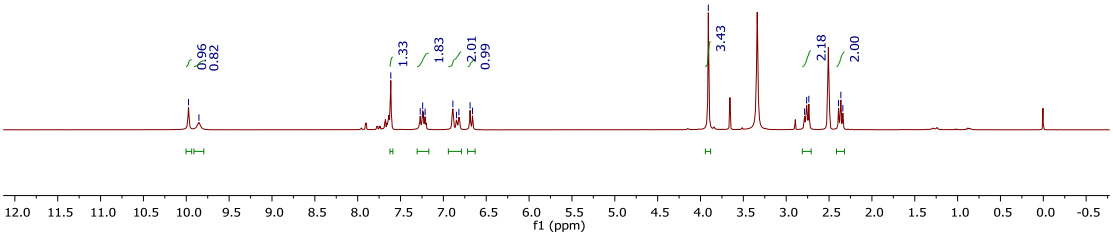

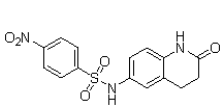

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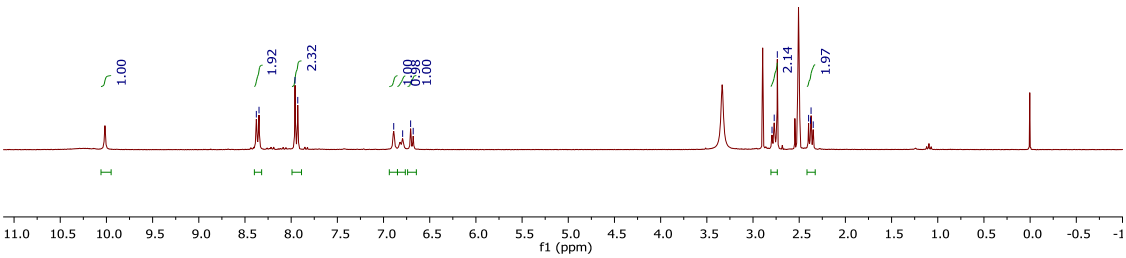

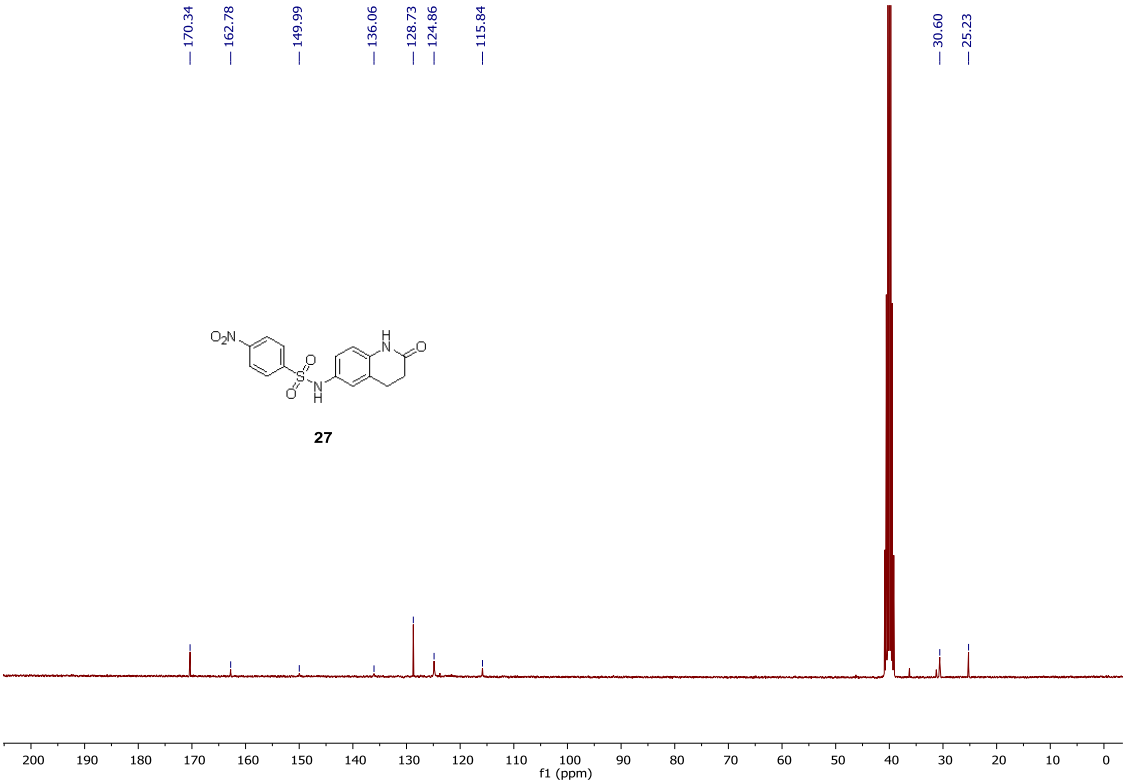
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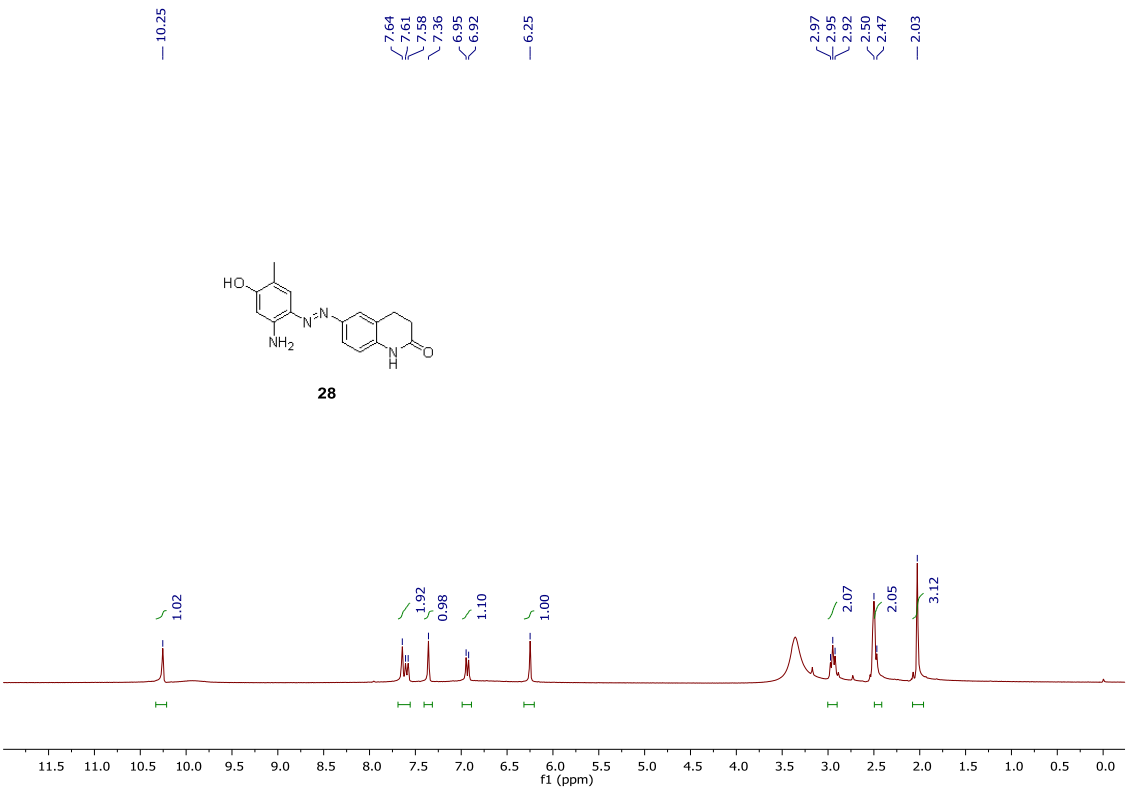
f1 (ppm)

27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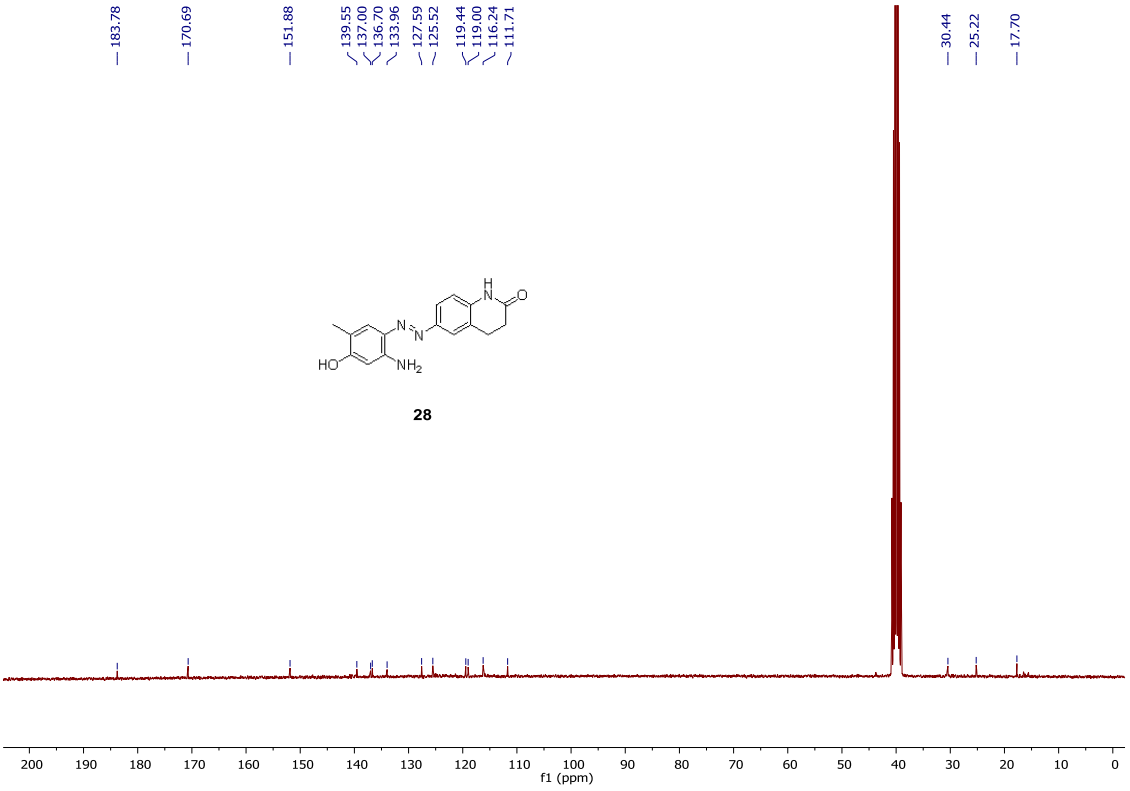
f1 (ppm)

28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119.00
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30.44
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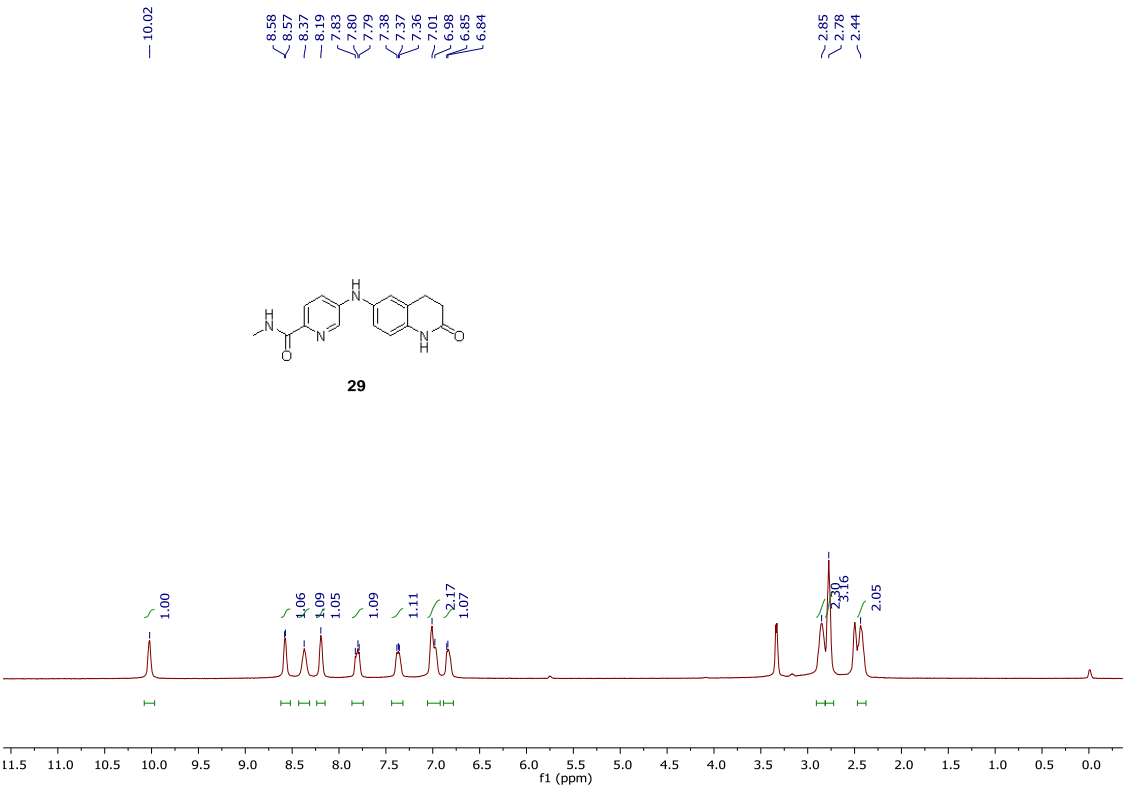
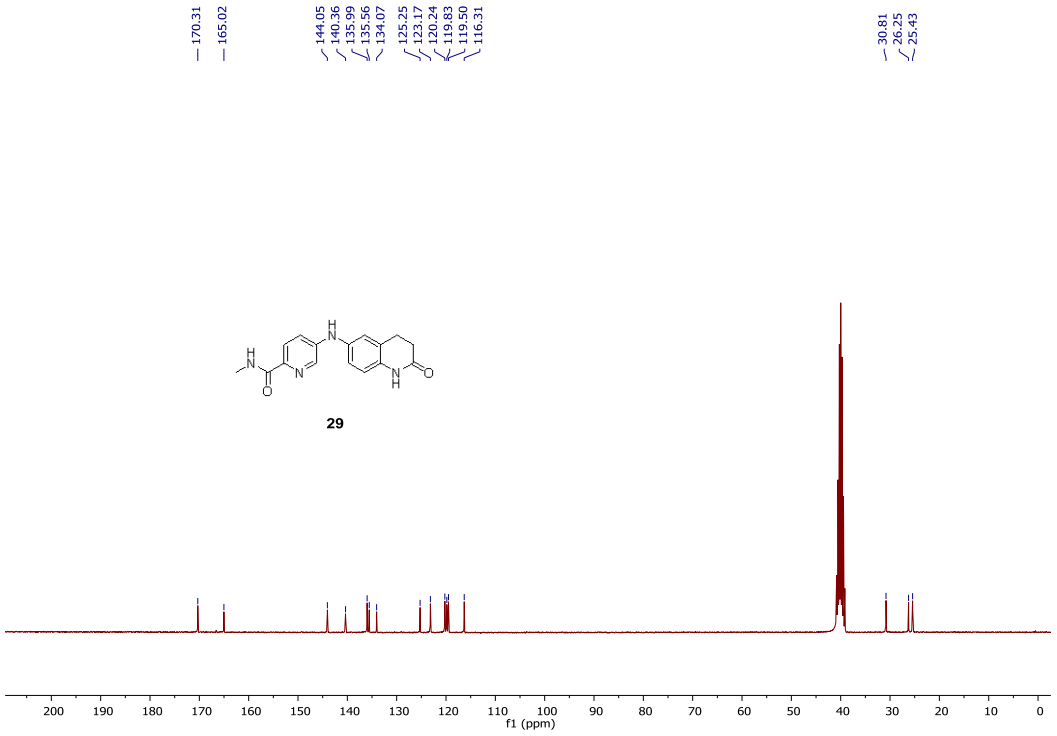
HO
NH2
f1 (ppm)

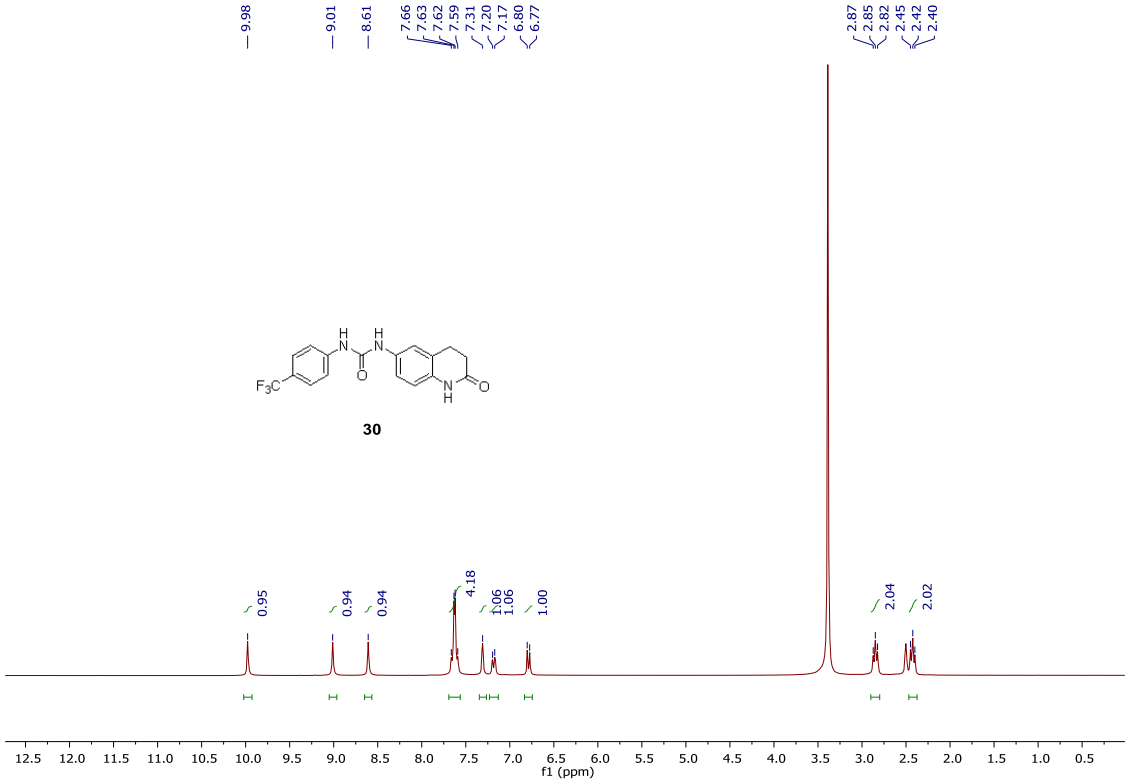
28


10.02
8.58
8.57
8.37
8.19
7.83
7.80
7.79
7.38
7.37
7.36
7.01
6.98
6.85
6.84
2.85
2.78
2.44
1.00
1.06
1.09
1.05
1.09
1.11
2.17
1.07
2.30
3.16
2.05
f1 (ppm)

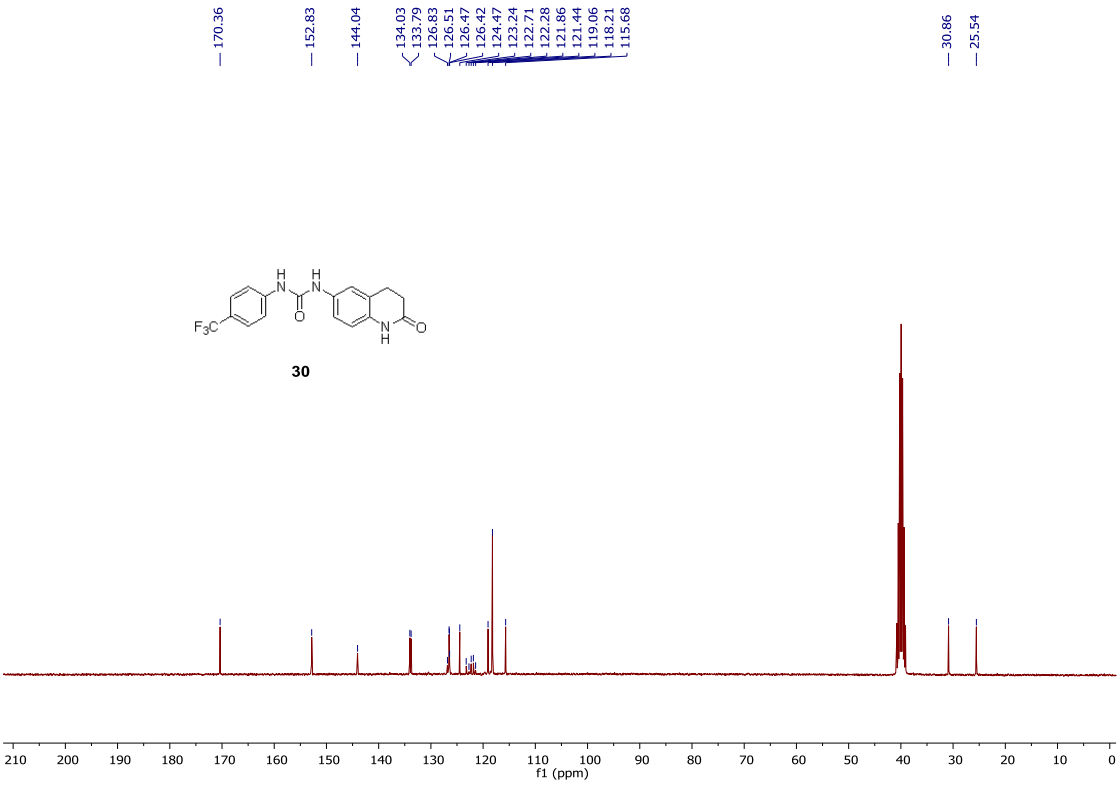
29


170.31
165.02
144.05
140.36
135.99
135.56
134.07
125.25
123.17
120.24
119.83
119.50
116.31
30.81
26.25
25.43
f1 (ppm)

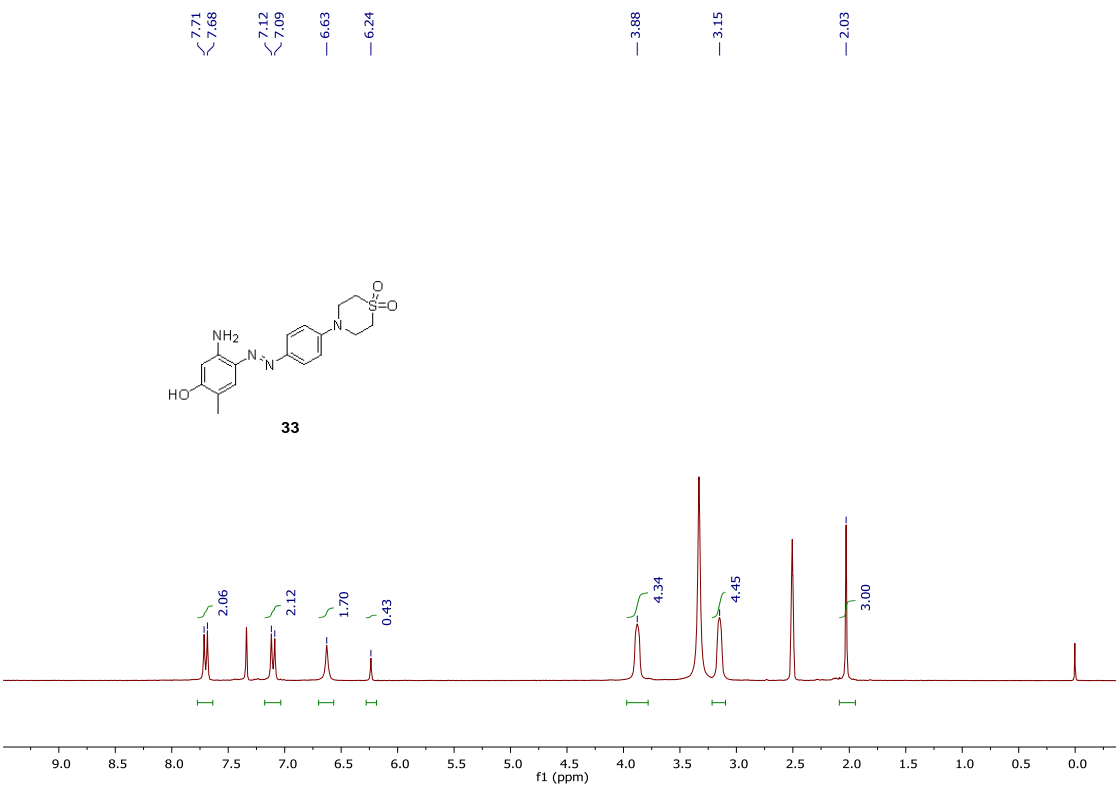
29


9.98
9.01
8.61
7.66
7.63
7.62
7.59
7.31
7.20
7.17
6.80
6.77
2.87
2.85
2.82
2.45
2.42
2.40
0.95
0.94
0.94
4.18
1.06
1.06
1.00
2.04
2.02
f1 (ppm)

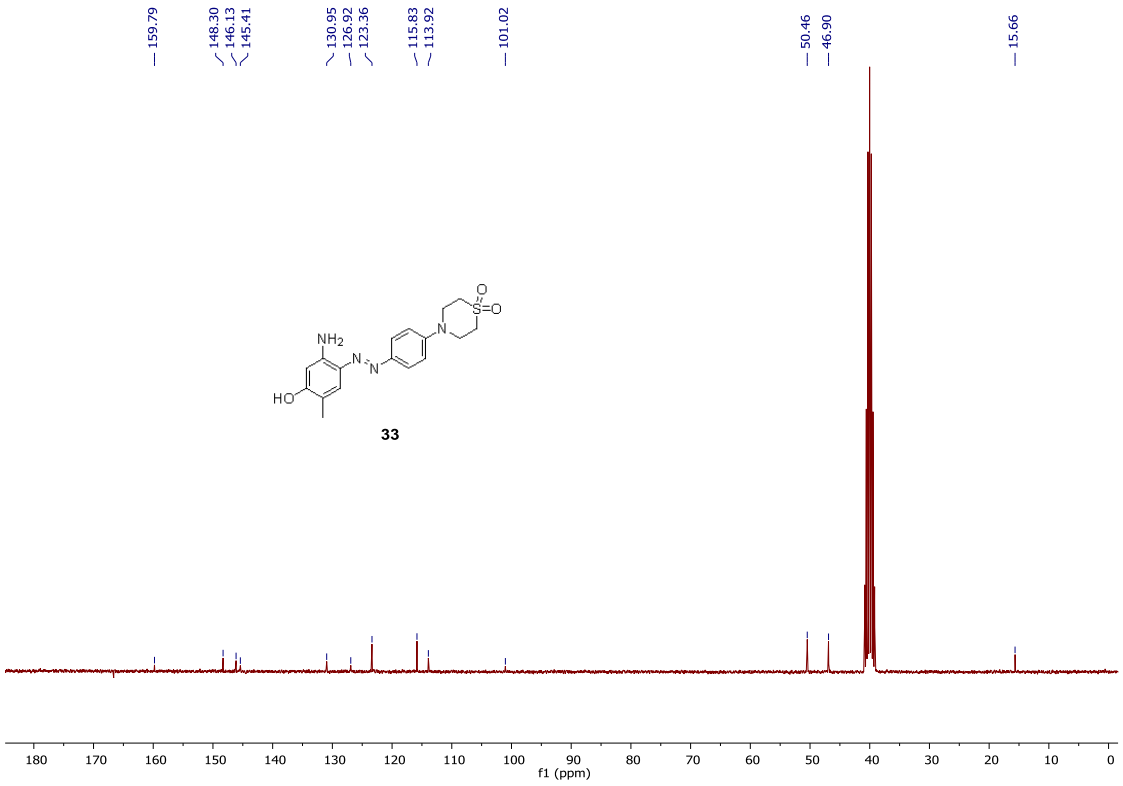
30


170.36
152.83
144.04
134.03
133.79
126.83
126.51
126.47
126.42
124.47
123.24
122.71
122.28
121.86
121.44
119.06
118.21
115.68
30.86
25.54
F3C
H
N
O
N
H
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

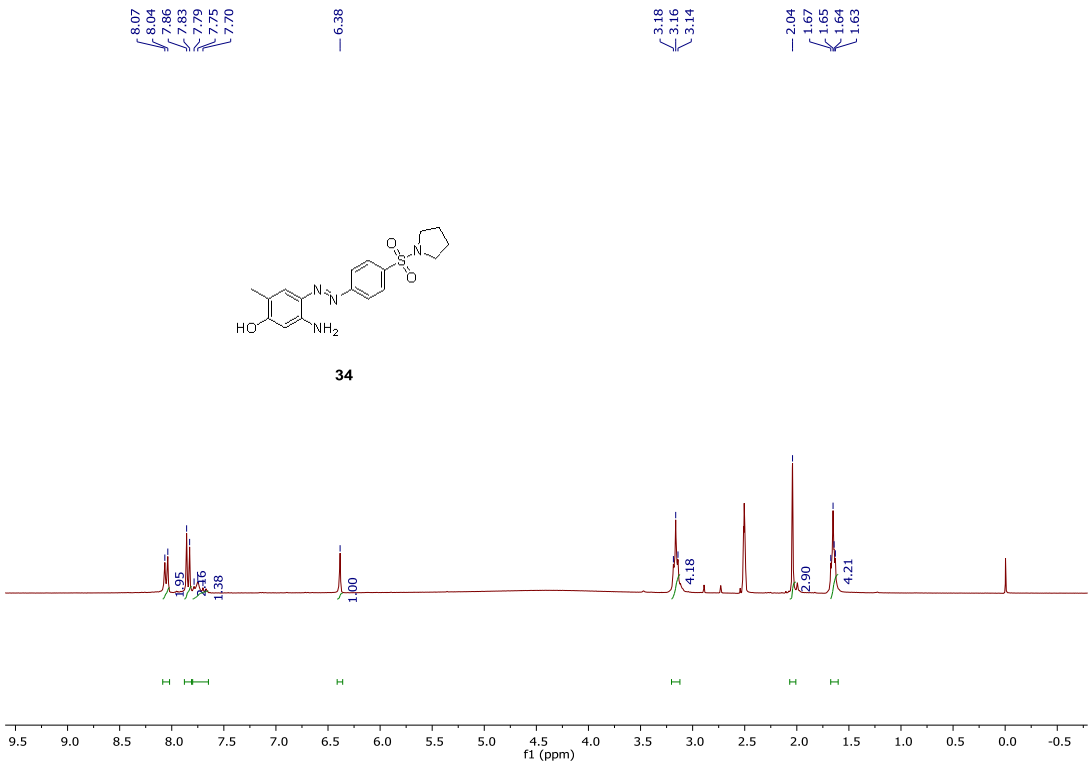
30


7.71
7.68
7.12
7.09
6.63
6.24
3.88
3.15
2.03
NH2
N
N
S
O
O
N
HO
2.06
2.12
1.70
0.43
4.34
4.45
3.00
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
f1 (ppm)

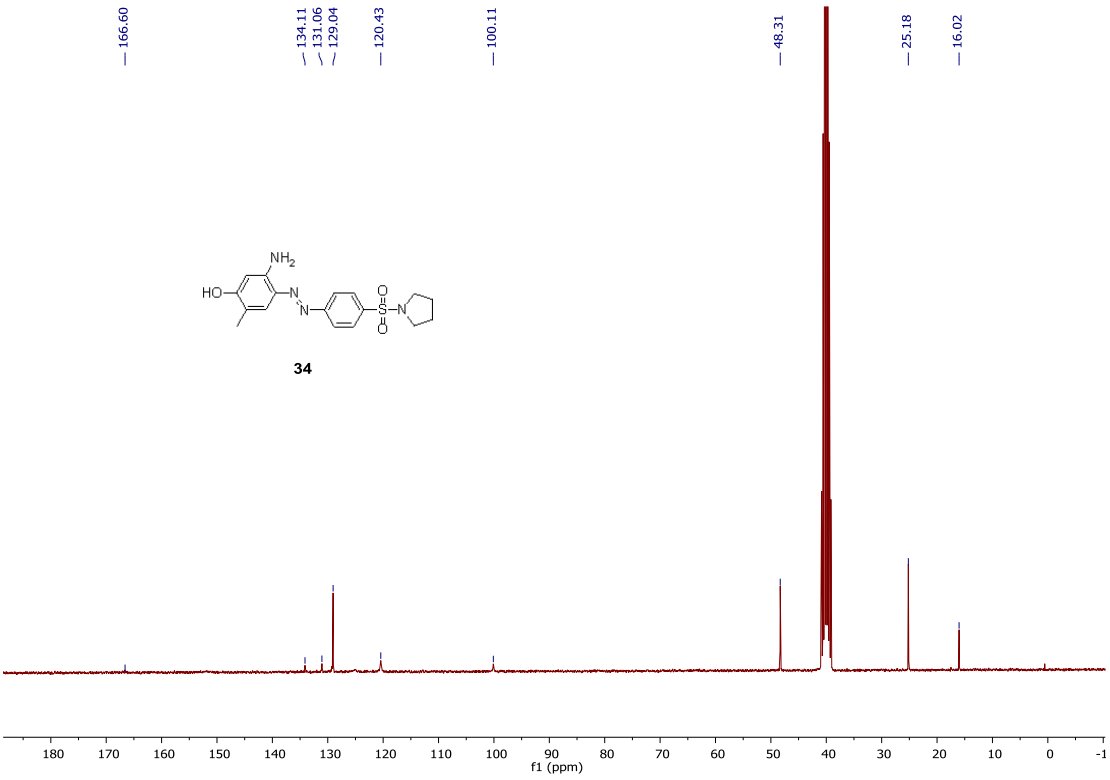
33

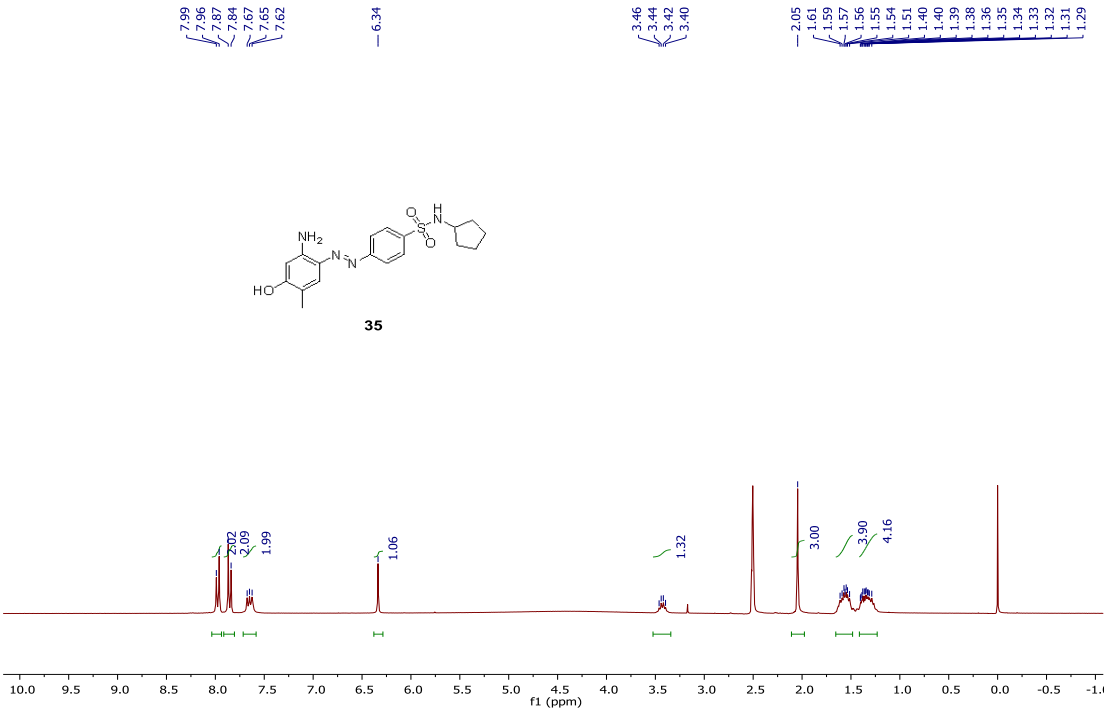

159.79
148.30
146.13
145.41
130.95
126.92
123.36
115.83
113.92
101.02
50.46
46.90
15.66
NH2
HO
N
N
O
S
O
N
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

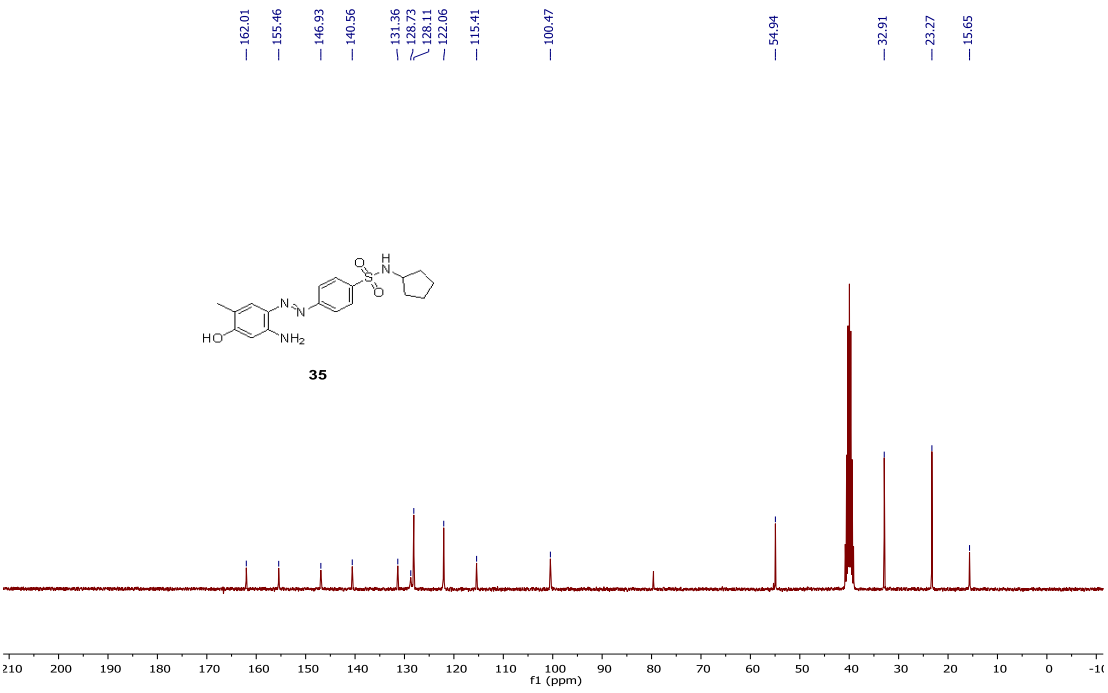
33


8.07
8.04
7.86
7.83
7.79
7.75
7.70
6.38
3.18
3.16
3.14
2.04
1.67
1.65
1.64
1.63
HO
NH2
N
N
O
S
O
N
0.95
0.96
1.38
1.00
4.18
2.90
4.21
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 0.0 -0.5
f1 (ppm)

34


166.60
134.11
131.06
129.04
120.43
100.11
48.31
25.18
16.02
NH2
HO
N
N
O
S
O
N
34
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)


7.99
7.96
7.87
7.84
7.67
7.65
7.62
6.34
3.46
3.44
3.42
3.40
2.05
1.61
1.59
1.57
1.56
1.55
1.54
1.51
1.40
1.40
1.39
1.38
1.36
1.35
1.34
1.33
1.32
1.31
1.29
NH2
HO
N
N
O
S
O
H
N
35
2.02
2.09
1.99
1.06
1.32
3.00
3.90
4.16
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 0.0 -0.5 -1.
f1 (ppm)


162.01
155.46
146.93
140.56
131.36
128.73
128.11
122.06
115.41
100.47
54.94
32.91
23.27
15.65
HO
NH2
N
N
S
O
O
H
N
35
f1 (ppm)