Supporting Information

Thieno[2,3-*d*]pyrimidine-based Positive Allosteric Modulators of Human MAS-Related G Protein-Coupled Receptor X1 (MRGPRX1)

Ilyas Berhane,[†,‡] Niyada Hin,[†] Ajit G. Thomas,[†] Qian Huang,[§] Chi Zhang,[§] Vijayabhaskar Veeravalli,[†,‡] Ying Wu,[†] Justin Ng,[†] Jesse Alt,[†] Camilo Rojas,[†] Hiroe Hihara,[¶] Mika Aoki,[¶] Kyoko Yoshizawa,[¶] Tomoki Nishioka,[¶] Shuichi Suzuki,[¶] Shao-Qiu He,[§] Qi Peng,[#] Yun Guan,[§] Xinzhong Dong,[#] Srinivasa N. Raja,[§] Barbara S. Slusher,[†,‡,#] Rana Rais,[,†,‡] and Takashi Tsukamoto[*,†,‡]*

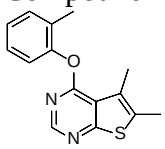
[†]Johns Hopkins Drug Discovery, [‡]Department of Neurology, [§]Department of Anesthesiology and Critical Care Medicine, and [#]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD 21205, USA

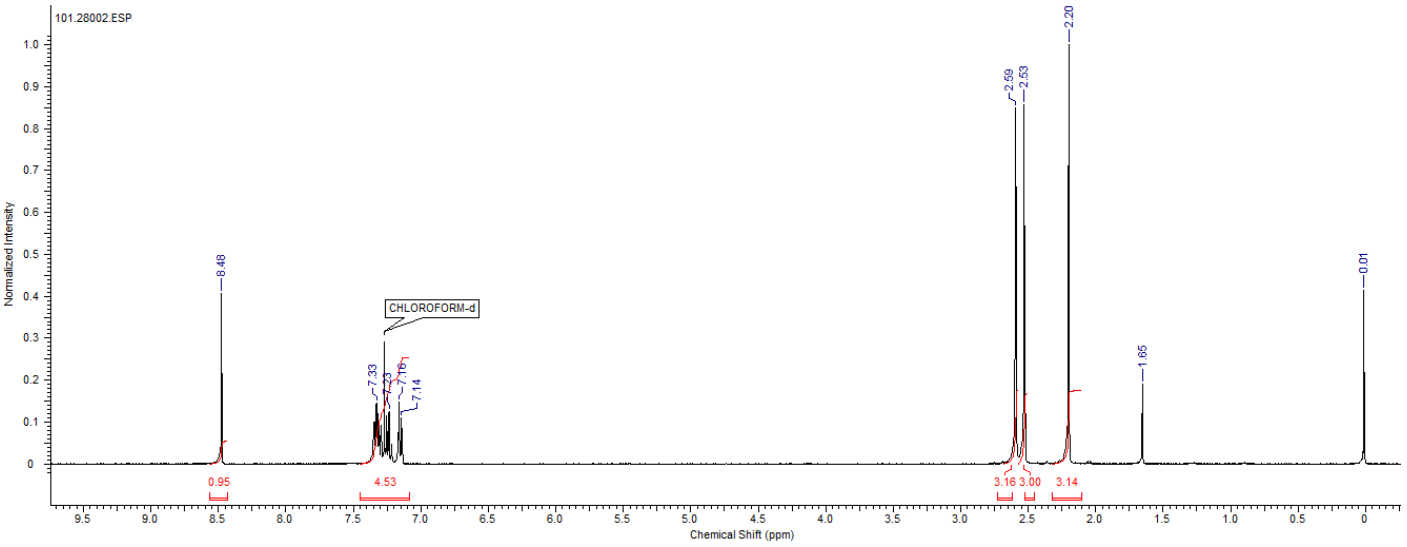
[¶]Tsukuba Research Laboratories, Eisai Co., Ltd., Tsukuba, Ibaraki 300-2635, Japan

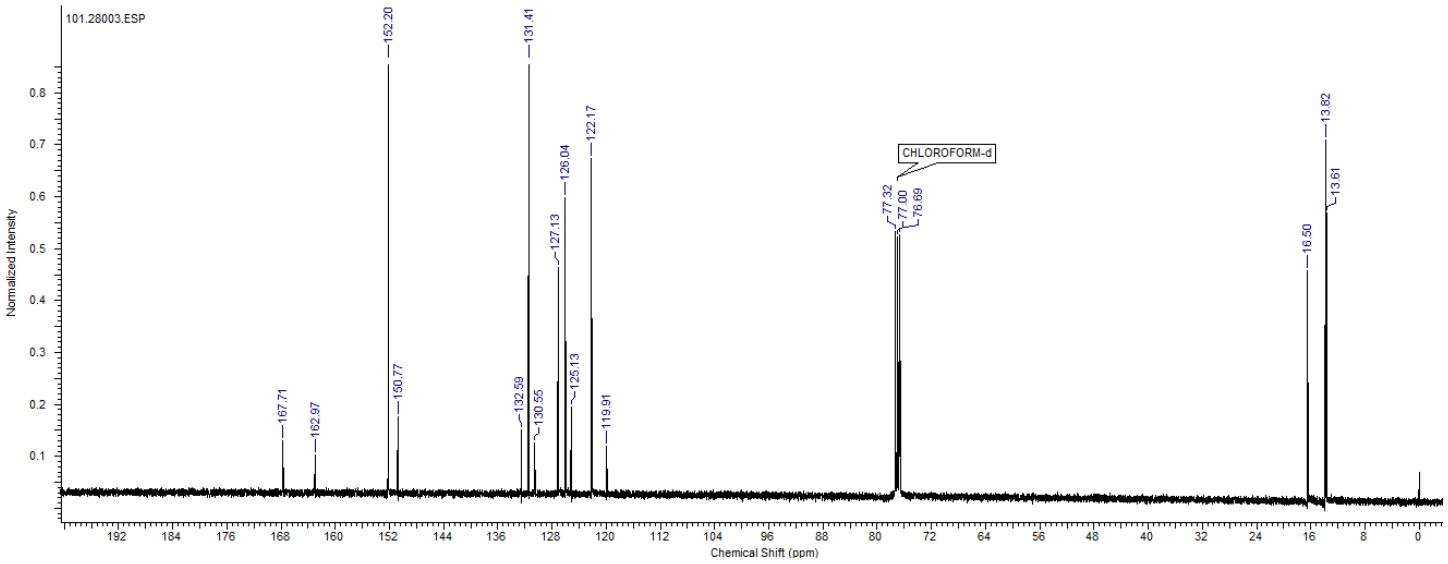
*Corresponding Authors:
Rana Rais; Email: rrais2@jhmi.edu
Takashi Tsukamoto; Email: ttsukamoto@jhmi.edu

Contents

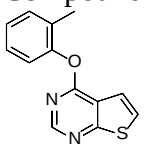
Compound **1a**

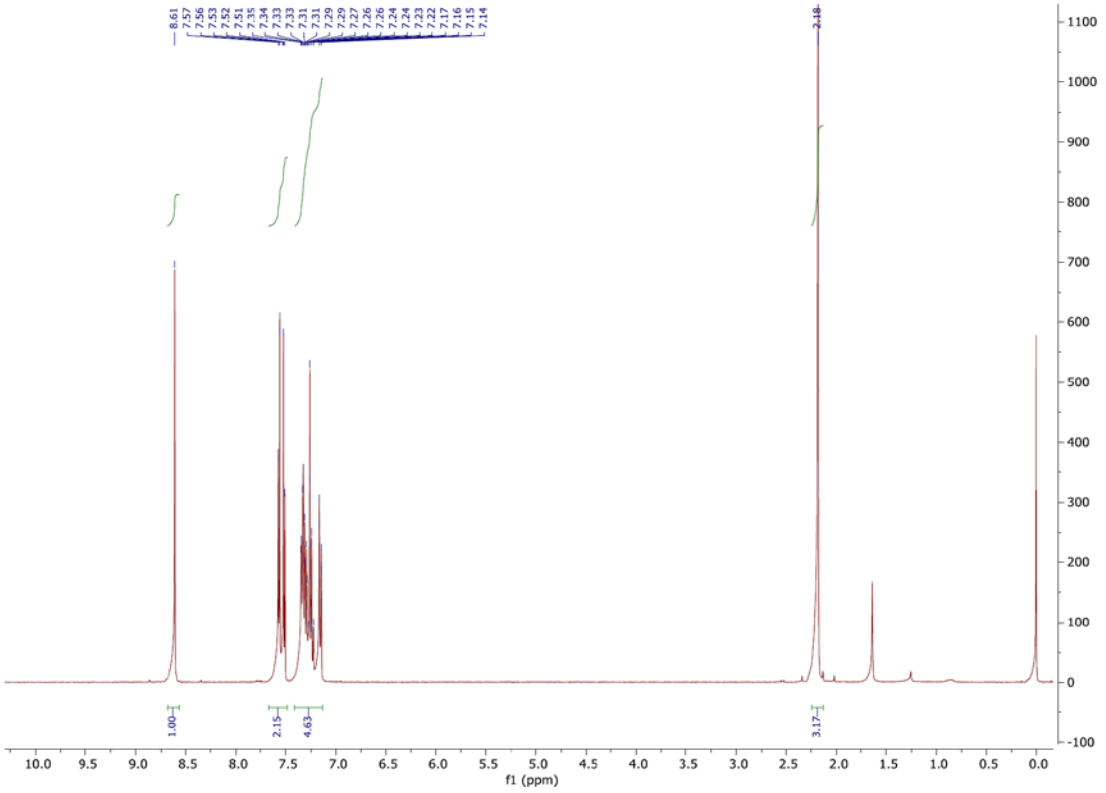

O
N
N
S

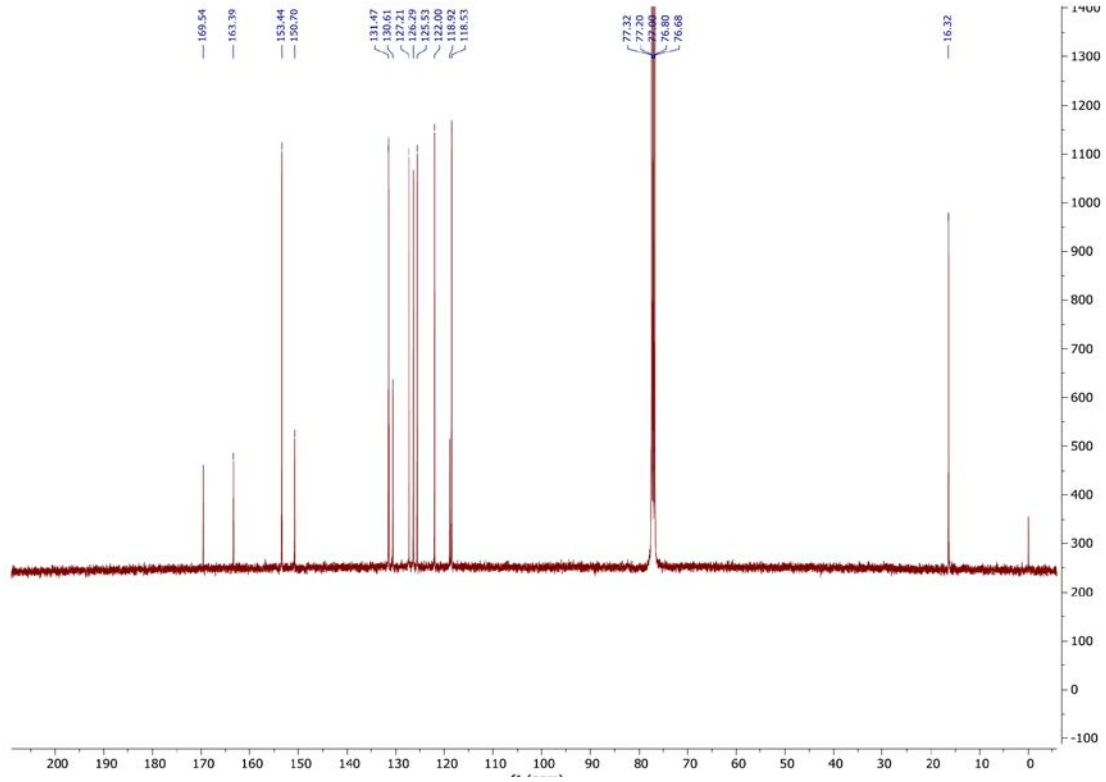

101.28002.ESP
Normalized Intensity
Chemical Shift (ppm)
8.48
CHLOROFORM-d
7.33
7.23
7.16
7.14
2.59
2.53
2.20
1.65
0.01
0.95
4.53
3.16
3.00
3.14


101.28003.ESP
Normalized Intensity
Chemical Shift (ppm)
152.20
131.41
126.04
122.17
127.13
125.13
119.91
167.71
162.97
160.77
132.59
130.55
CHLOROFORM-d
77.32
77.00
76.69
13.82
13.61
16.50

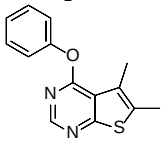
Compound **1b**

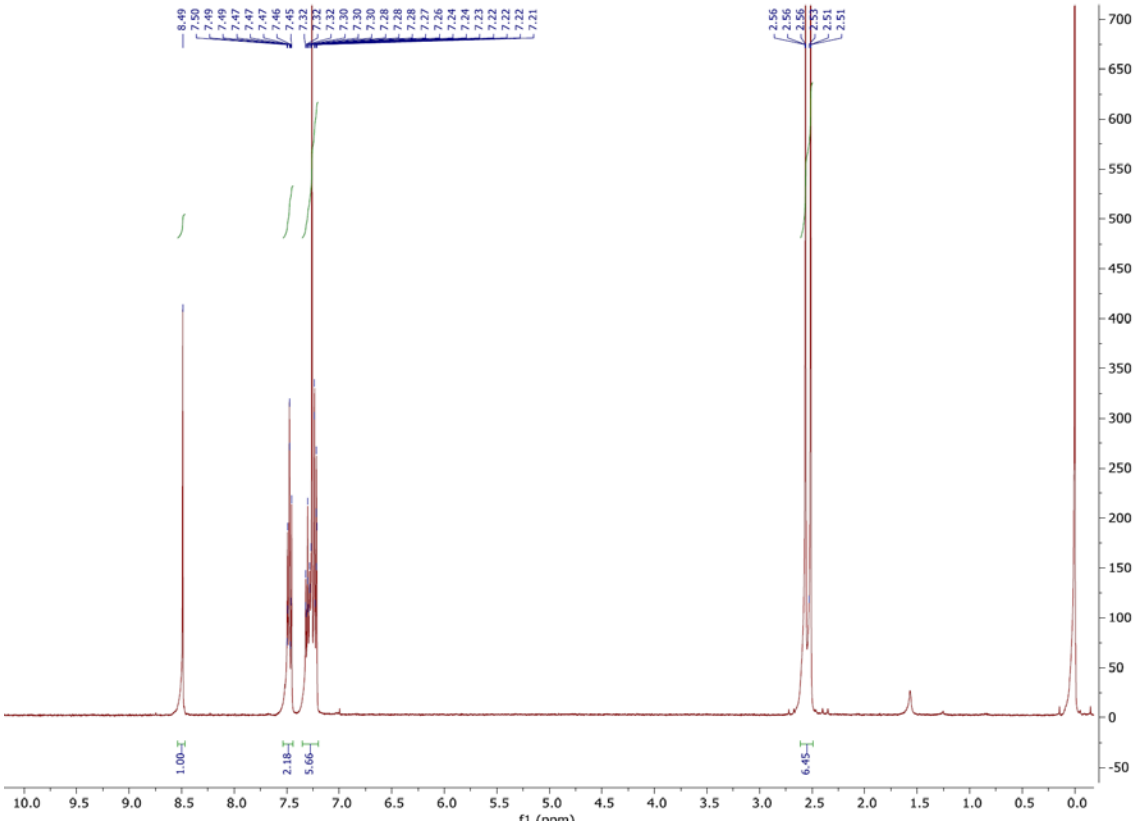

O
N
N
S

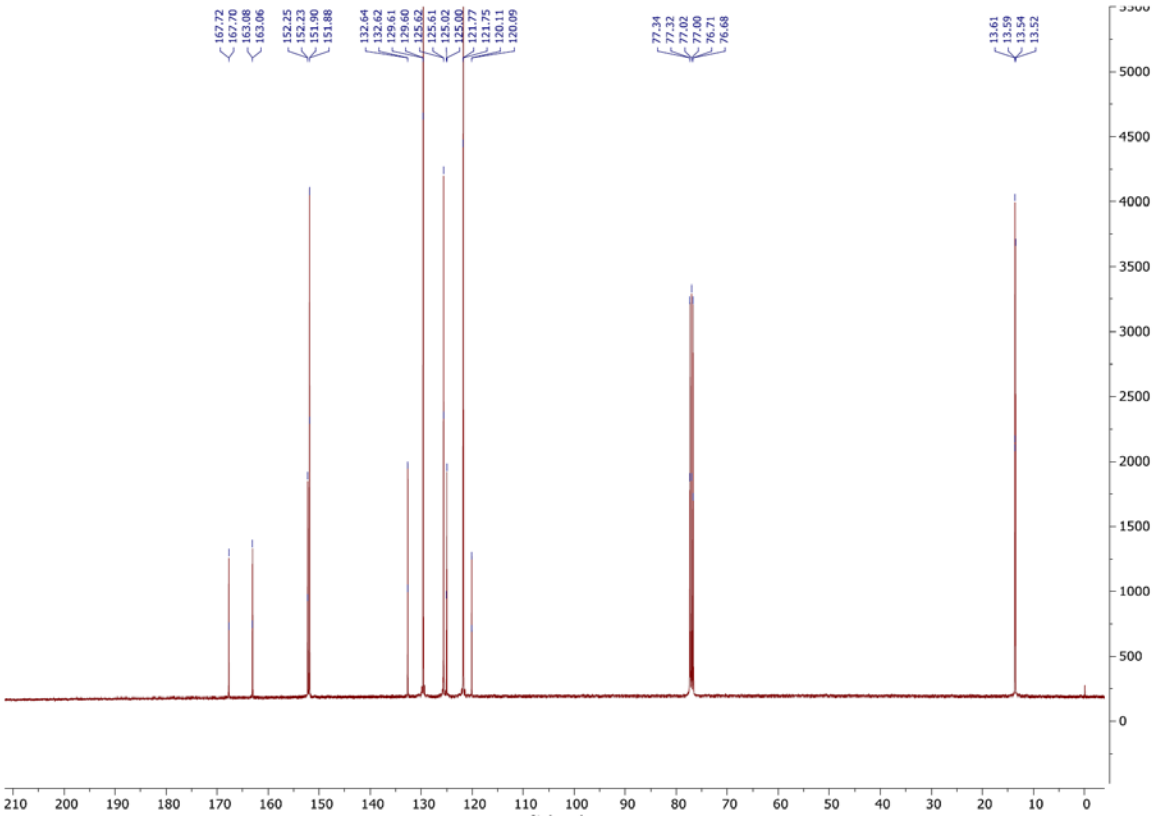

8.61
7.57
7.56
7.53
7.52
7.51
7.35
7.34
7.33
7.33
7.31
7.31
7.29
7.29
7.27
7.26
7.26
7.24
7.24
7.23
7.22
7.17
7.16
7.15
7.14
2.18
1.00
2.15
4.63
3.17
f1 (ppm)


169.54
163.39
153.44
150.70
131.47
130.61
127.21
126.29
125.53
122.00
118.82
118.53
77.32
77.20
77.00
76.80
76.68
16.32

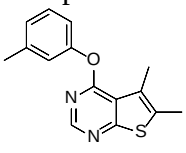
Compound **1c**

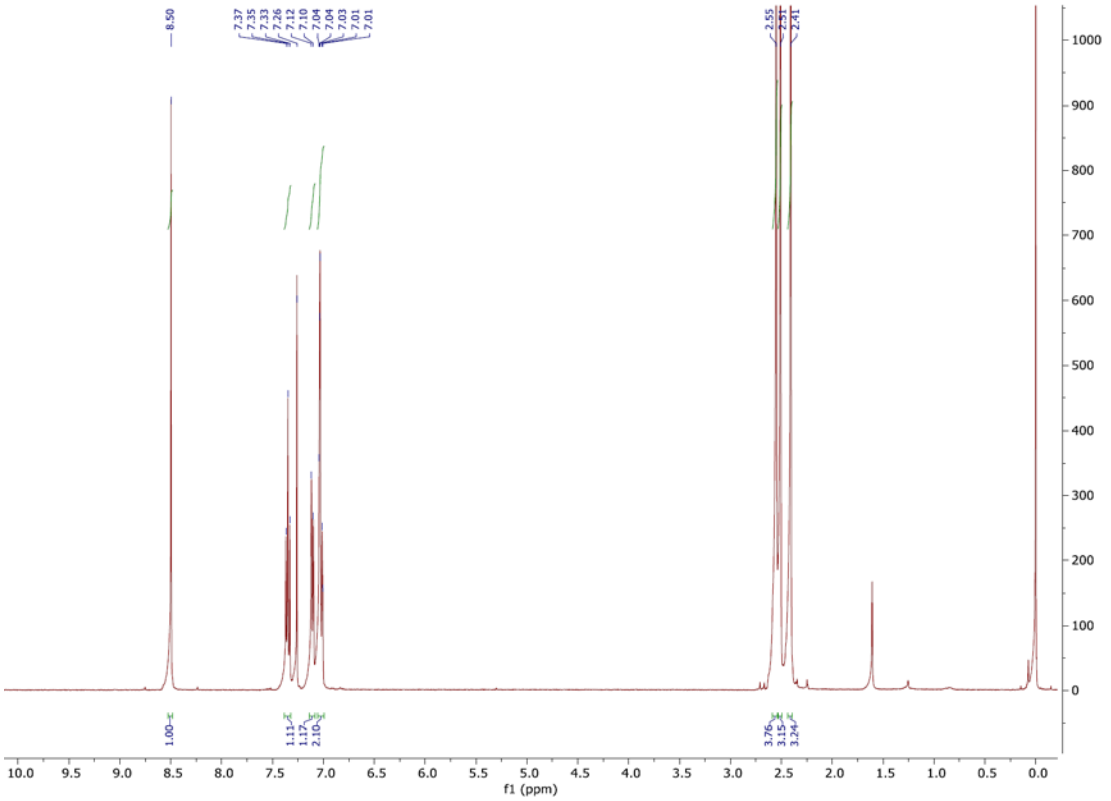


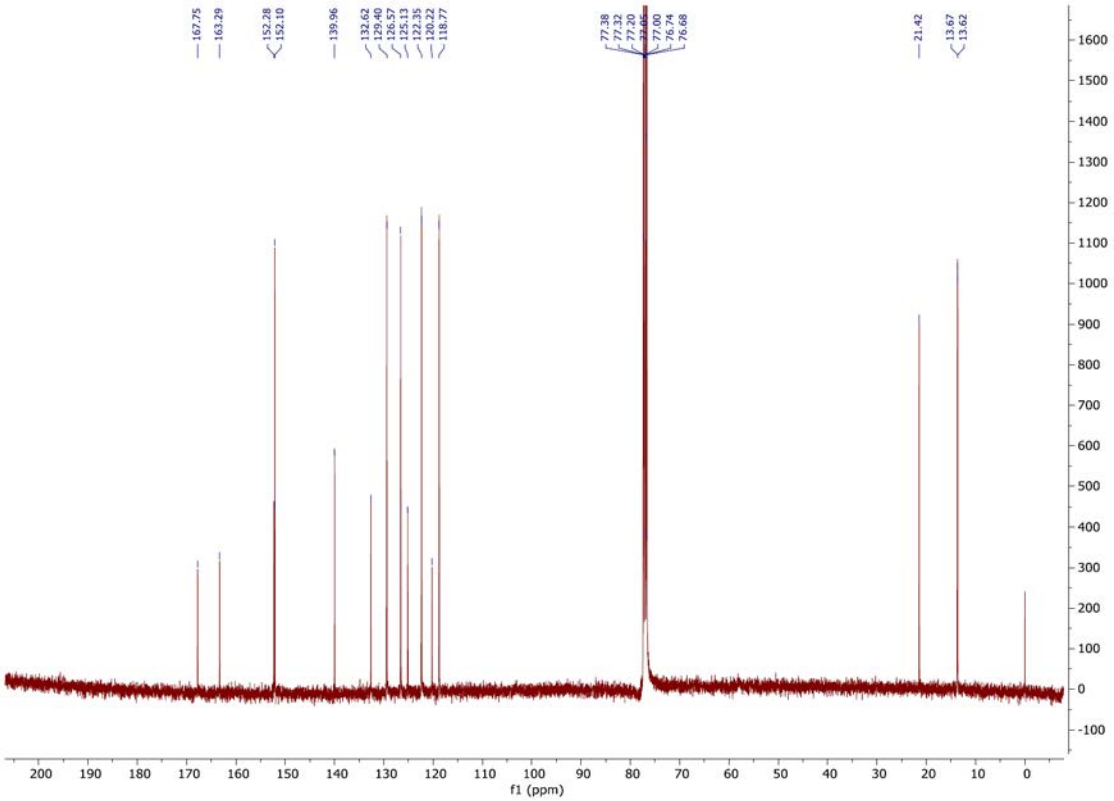

8.49
7.50
7.49
7.49
7.47
7.47
7.47
7.46
7.45
7.32
7.32
7.32
7.30
7.30
7.30
7.28
7.28
7.28
7.27
7.26
7.24
7.24
7.23
7.22
7.22
7.22
7.21
2.56
2.56
2.56
2.55
2.51
2.51
1.00
2.18
5.66
6.45
f1 (ppm)


167.72
167.70
163.08
163.06
152.25
152.23
151.90
151.88
132.64
132.62
129.61
129.60
125.62
125.61
125.02
125.00
121.77
121.75
120.11
120.09
77.34
77.32
77.02
77.00
76.71
76.68
13.61
13.59
13.54
13.52

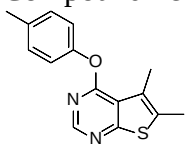
Compound **1d**

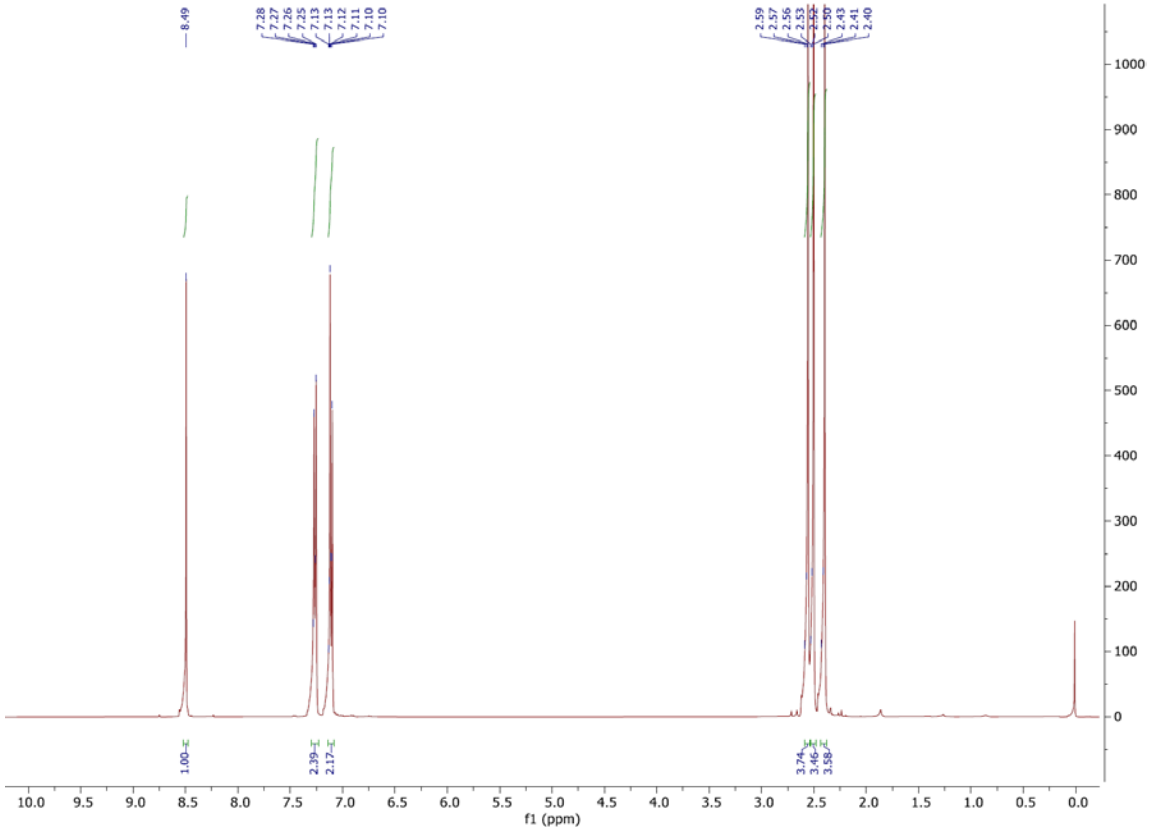

O
N
N
S

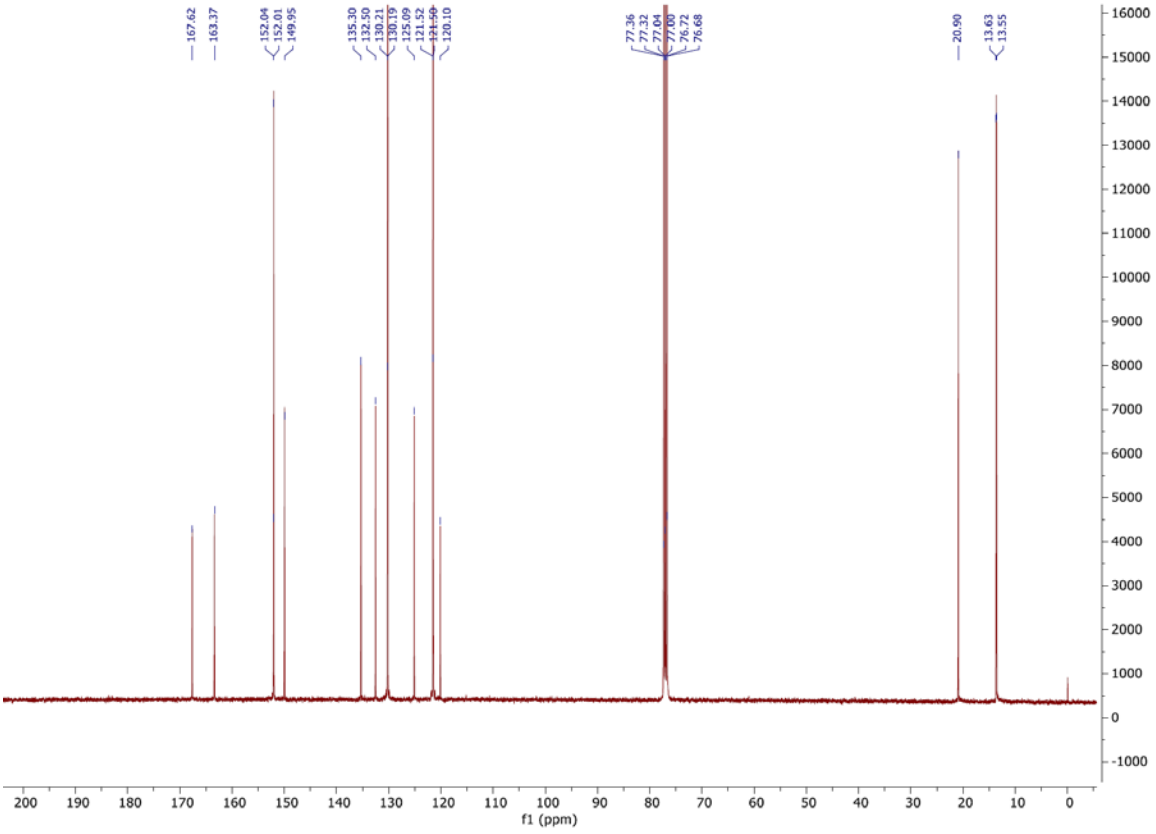

8.50
7.37
7.35
7.33
7.26
7.12
7.10
7.04
7.04
7.03
7.01
7.01
2.55
2.51
2.41
1.00
1.11
1.17
2.10
3.76
3.15
3.24
f1 (ppm)


167.75
163.29
152.28
152.10
139.96
132.62
129.40
126.57
125.13
122.35
120.22
118.77
77.38
77.32
77.20
77.00
76.74
76.68
21.42
13.67
13.62
f1 (ppm)

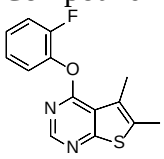
Compound **1e**

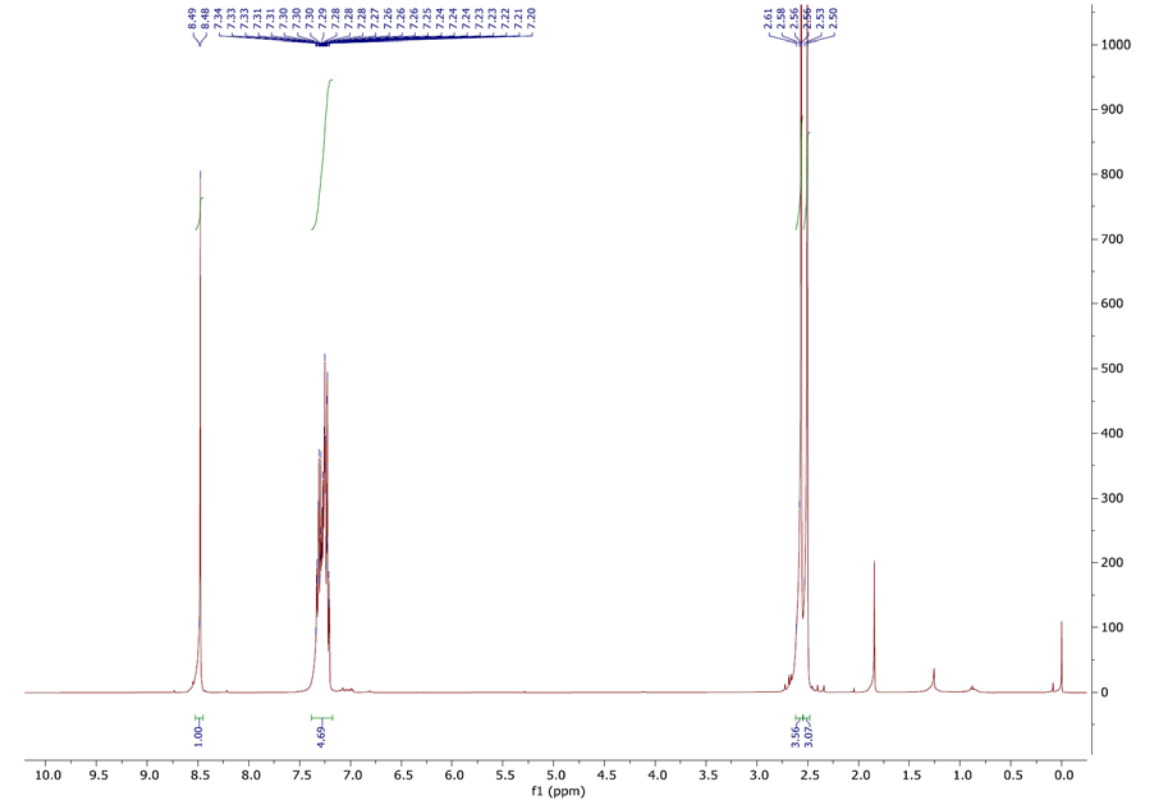

O
N
N
S

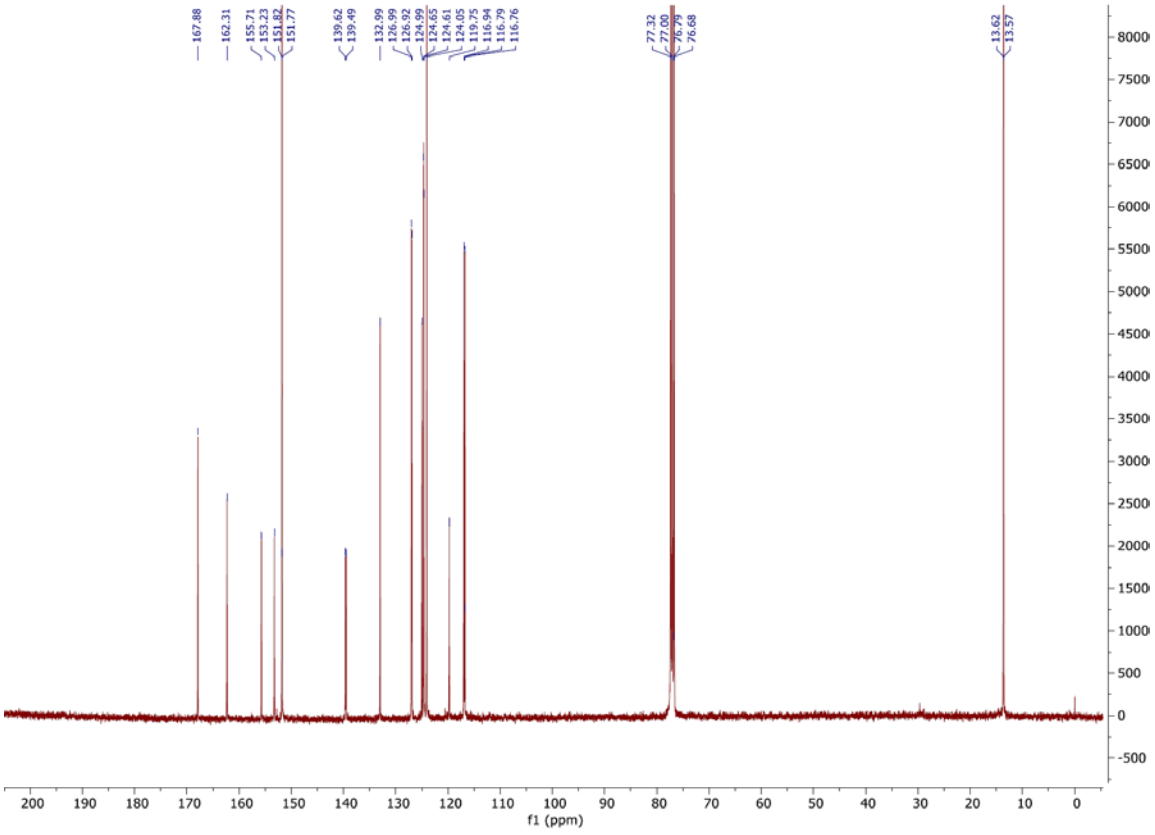

8.49
7.28
7.27
7.26
7.25
7.13
7.13
7.12
7.11
7.10
7.10
2.59
2.57
2.56
2.53
2.52
2.50
2.43
2.41
2.40
1.00
2.39
2.17
3.74
3.46
3.58
f1 (ppm)


167.62
163.37
152.04
152.01
149.95
135.30
132.50
130.21
130.19
125.09
121.52
121.50
120.10
77.36
77.32
77.04
77.00
76.72
76.68
20.90
13.63
13.55
f1 (ppm)

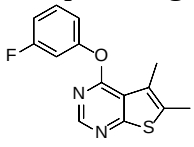
Compound **1f**

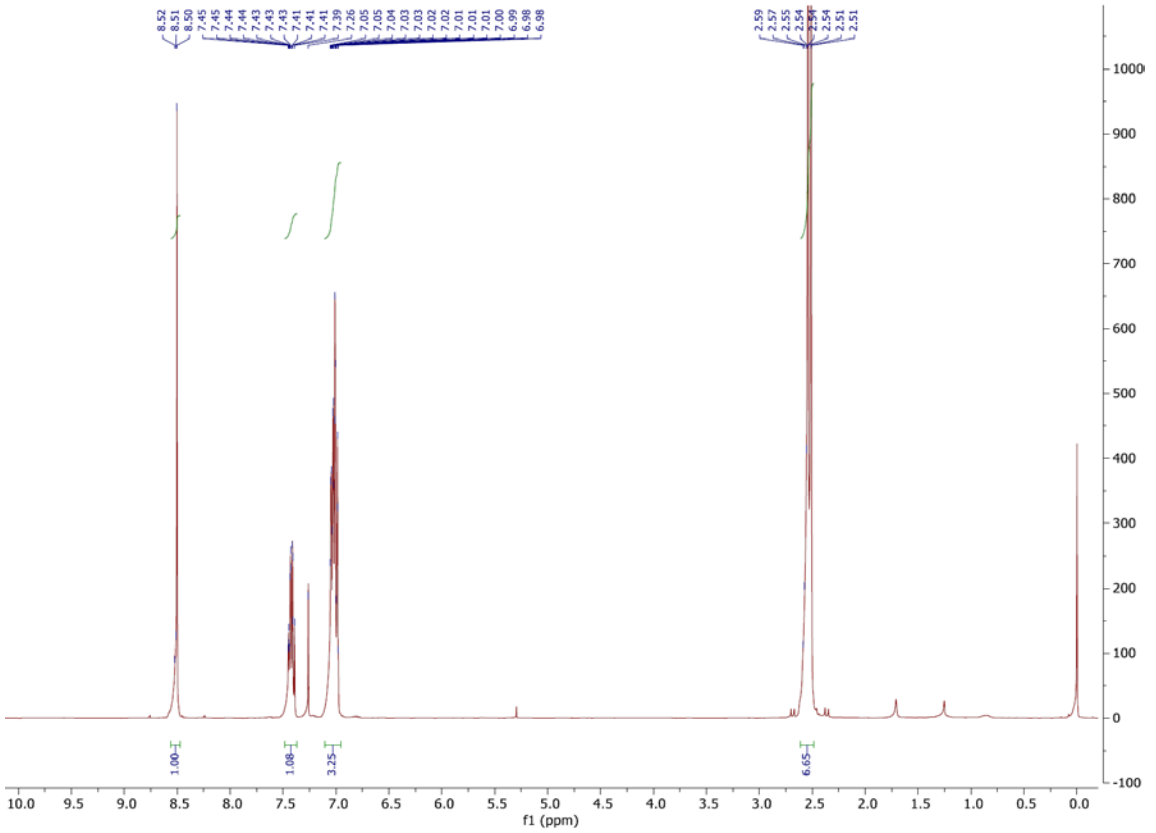

F
O
N
N
S

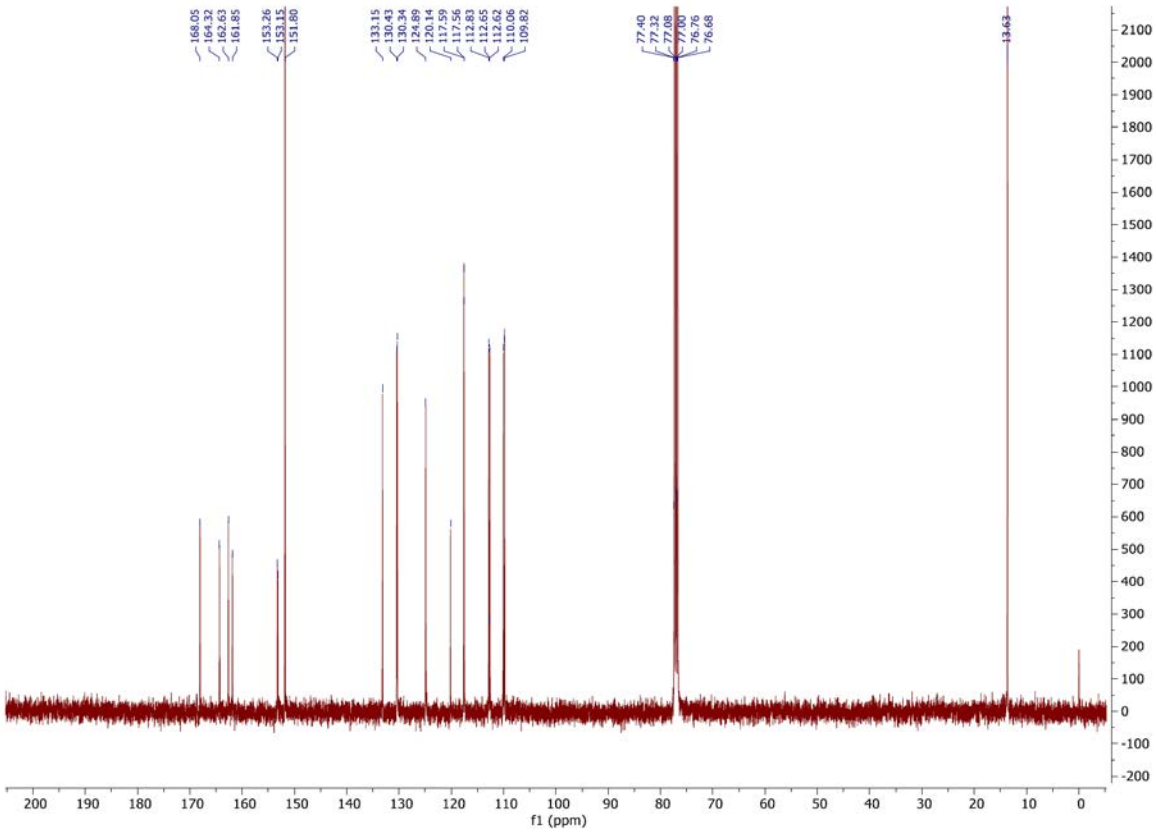

8.49
8.48
7.34
7.33
7.33
7.31
7.31
7.30
7.30
7.30
7.29
7.28
7.28
7.28
7.27
7.26
7.26
7.26
7.25
7.24
7.24
7.24
7.23
7.23
7.22
7.21
7.20
2.61
2.58
2.56
2.56
2.53
2.50
1.00
4.69
3.56
3.07
f1 (ppm)


167.88
162.31
155.71
153.23
151.82
151.77
139.62
139.49
132.99
126.99
126.92
124.99
124.65
124.61
124.05
119.75
116.94
116.79
116.76
77.32
77.00
76.79
76.68
13.62
13.57
f1 (ppm)

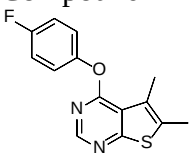
Compound **1g**

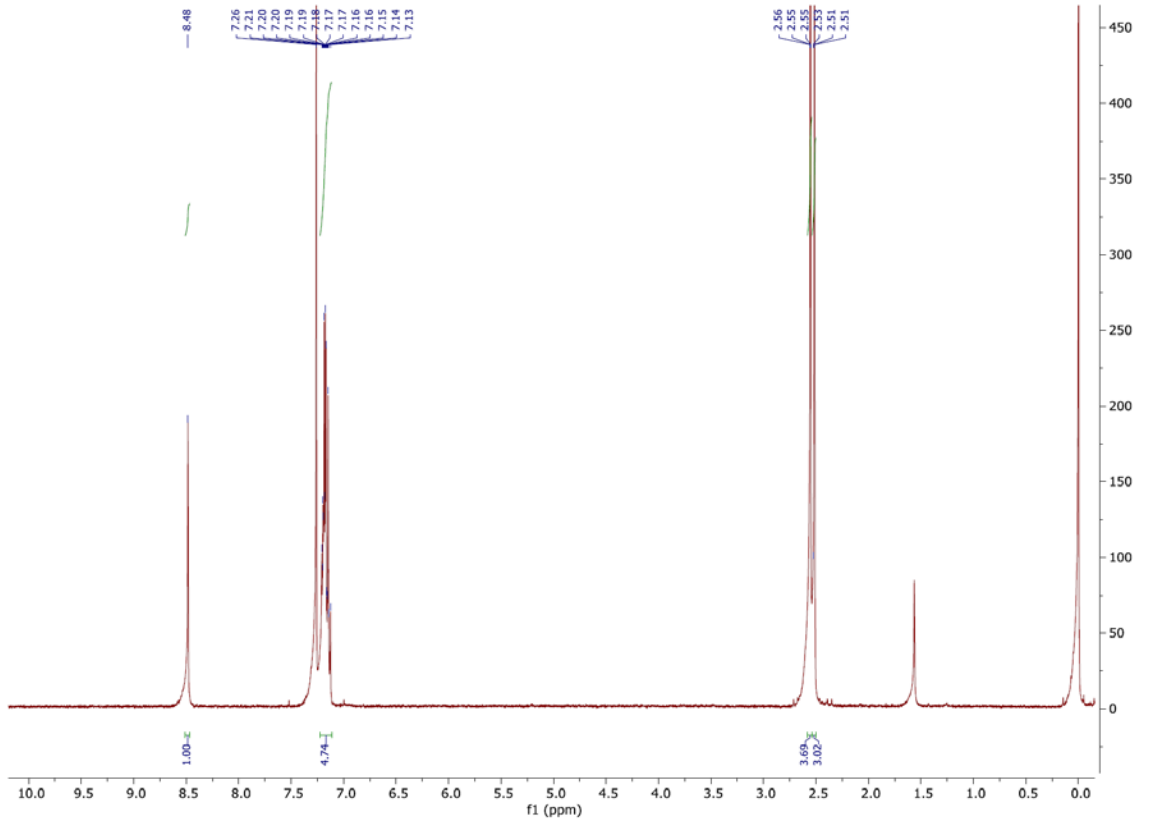

F
O
N
N
S

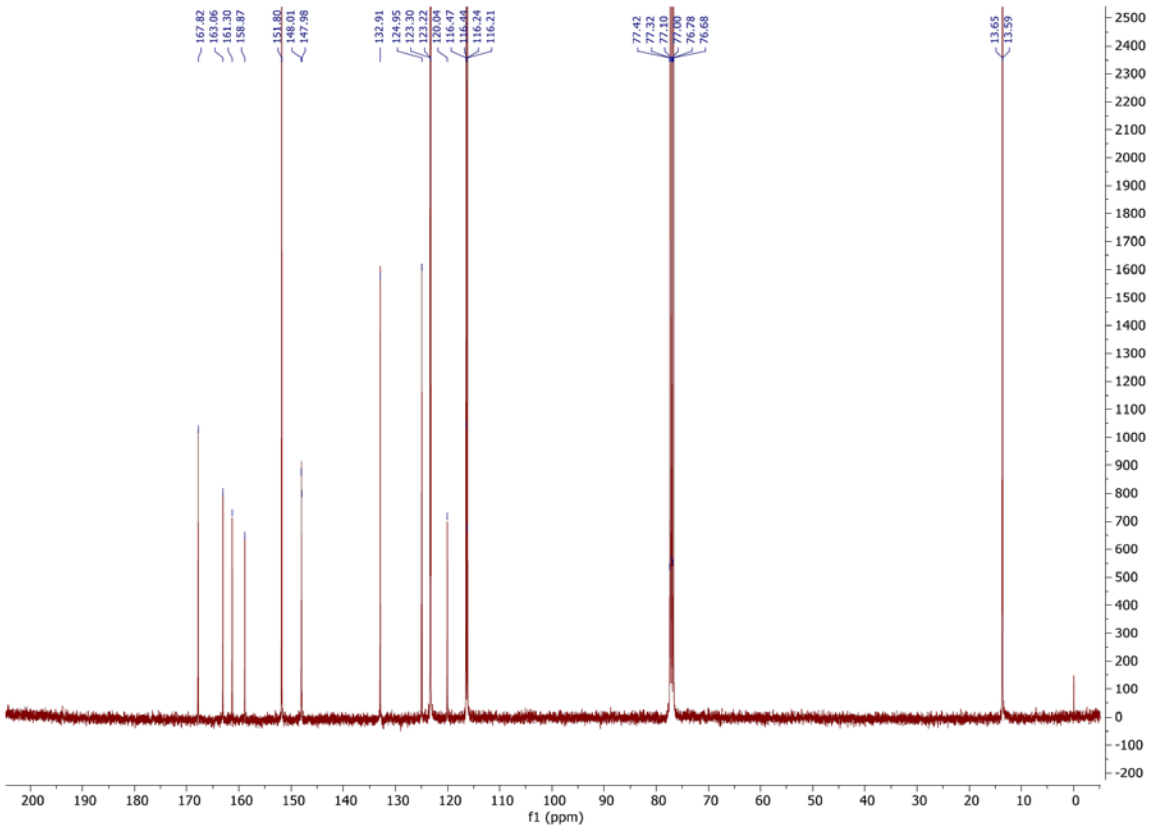

8.52
8.51
8.50
7.45
7.45
7.44
7.44
7.43
7.43
7.43
7.41
7.41
7.41
7.39
7.26
7.05
7.05
7.04
7.03
7.03
7.02
7.02
7.01
7.01
7.00
6.99
6.98
6.98
2.59
2.57
2.55
2.54
2.54
2.51
2.51
1.00
1.08
3.25
6.65
f1 (ppm)


168.05
164.32
162.63
161.65
153.26
153.15
151.80
133.15
130.43
130.34
124.89
120.14
117.59
117.56
112.83
112.65
112.62
110.06
109.82
77.40
77.32
77.08
77.00
76.76
76.68
13.63
f1 (ppm)

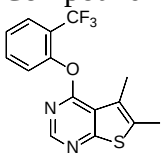
Compound **1h**

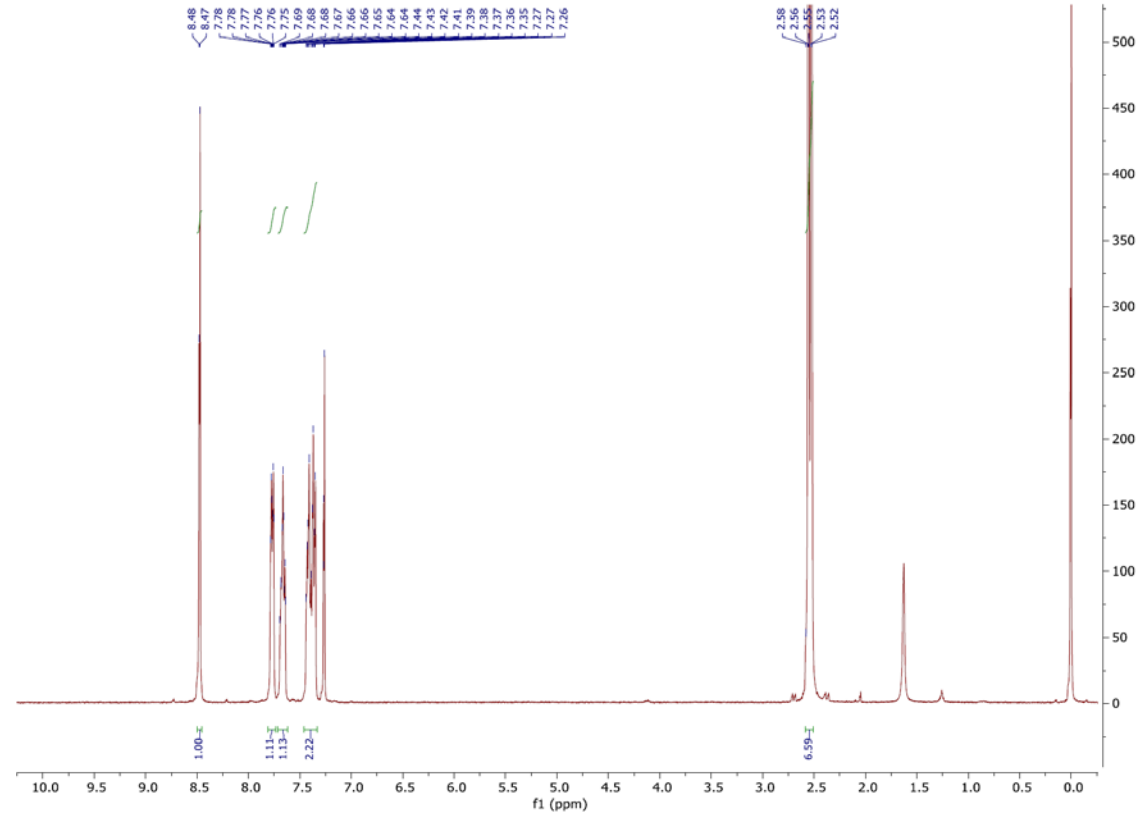

F
O
N
N
S

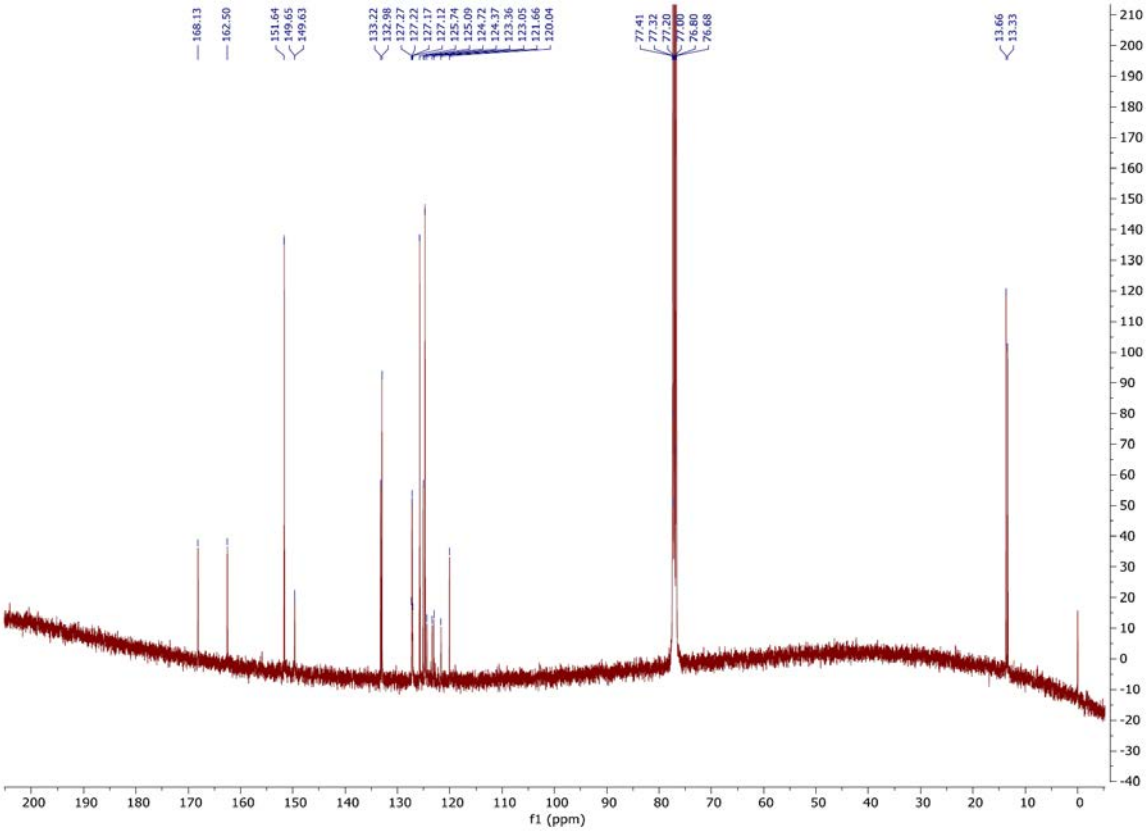

8.48
7.26
7.21
7.20
7.20
7.19
7.19
7.18
7.17
7.17
7.17
7.16
7.16
7.15
7.14
7.13
2.56
2.55
2.55
2.53
2.51
2.51
1.00
4.74
3.69
3.02
f1 (ppm)


167.82
163.06
161.30
158.87
151.80
148.01
147.98
132.91
124.95
123.30
123.22
120.04
116.47
116.44
116.24
116.21
77.42
77.32
77.10
77.00
76.78
76.68
13.65
13.59
f1 (ppm)

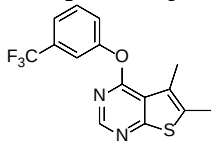
Compound **1i**

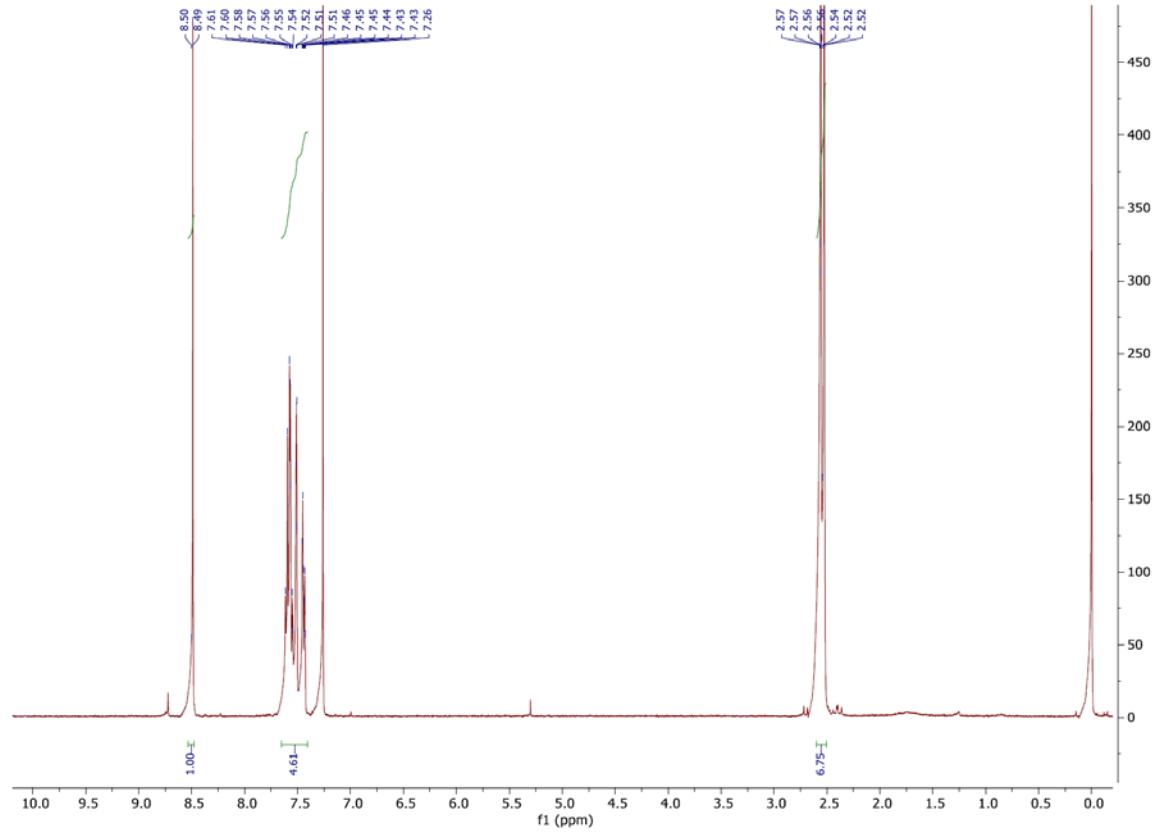

CF3
O
N
N
S

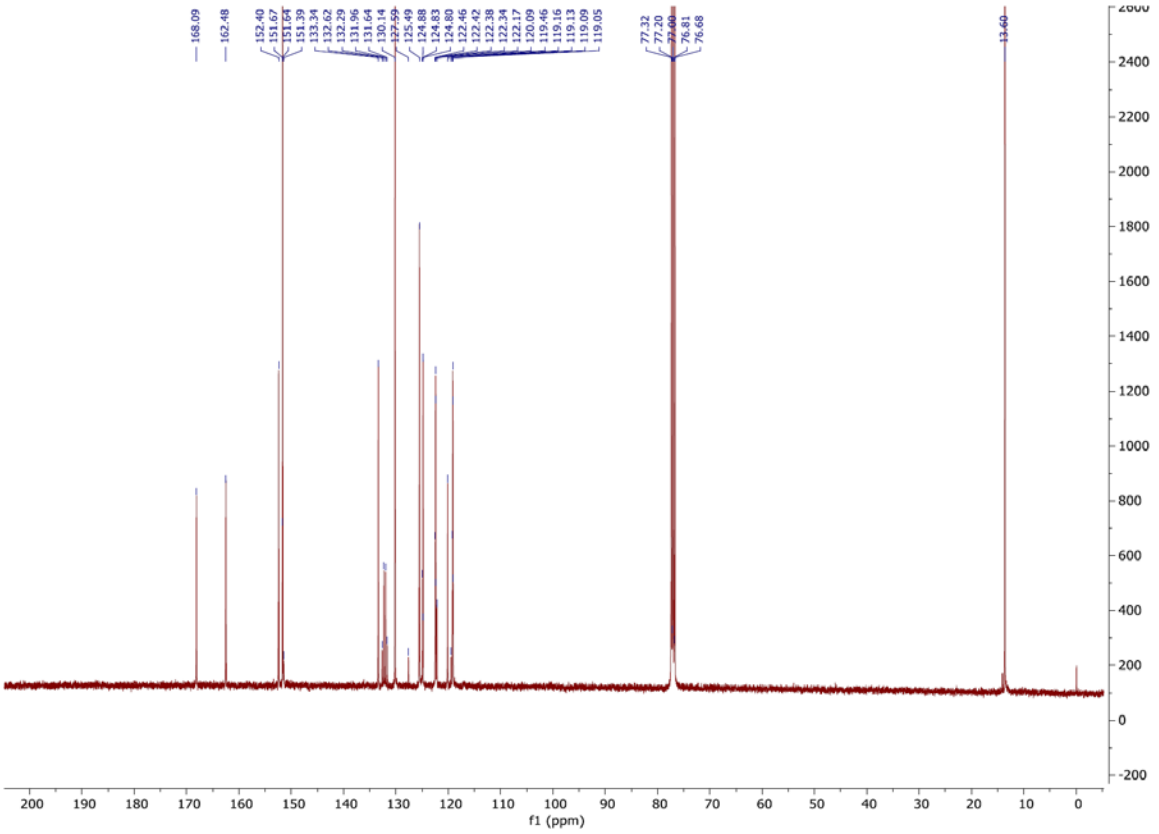

8.48
8.47
7.78
7.78
7.77
7.76
7.76
7.75
7.69
7.68
7.68
7.67
7.66
7.66
7.65
7.64
7.44
7.43
7.42
7.41
7.39
7.38
7.37
7.36
7.35
7.27
7.27
7.26
2.58
2.56
2.55
2.53
2.52
1.00
1.11
1.13
2.22
6.59
f1 (ppm)


168.13
162.50
151.64
149.65
149.63
133.22
132.98
127.27
127.22
127.17
127.12
125.74
125.09
124.72
124.37
123.36
123.05
121.66
120.04
77.41
77.32
77.20
77.00
76.80
76.68
13.66
13.33
f1 (ppm)

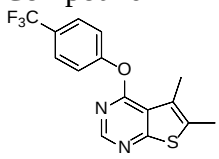
Compound **1j**

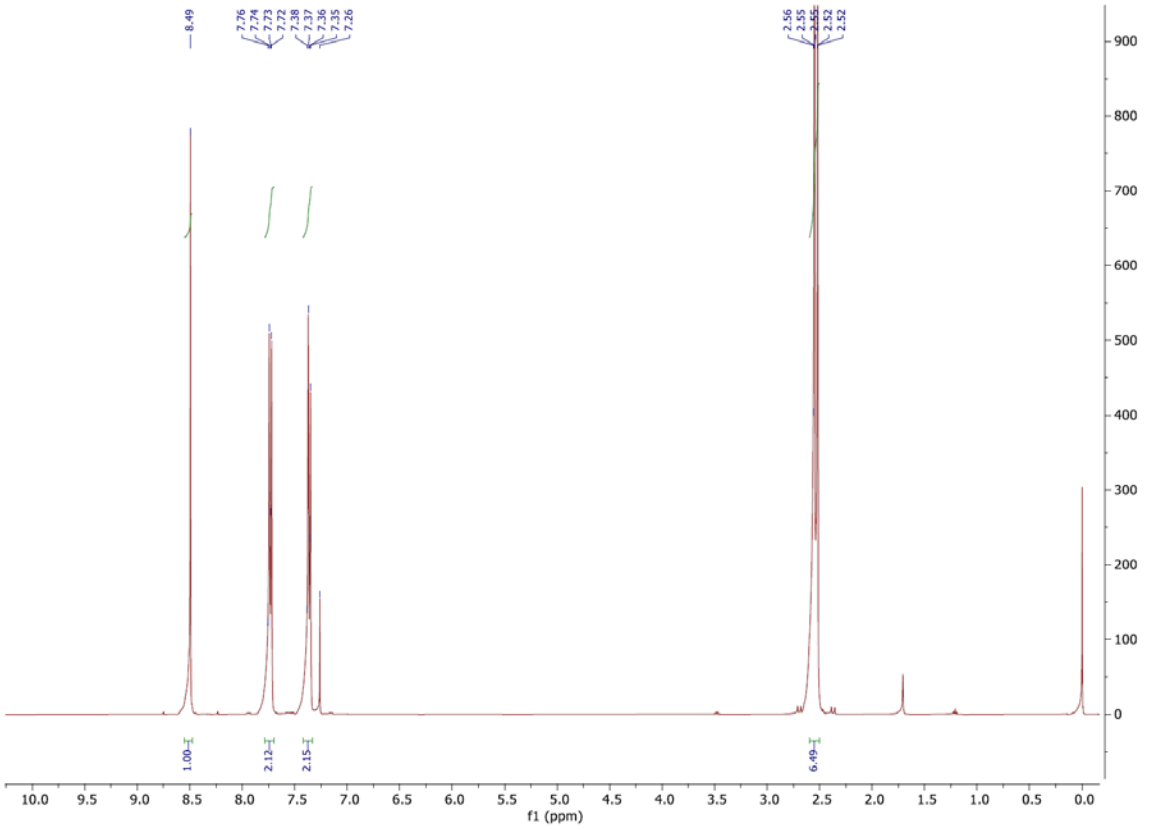

F3C
O
N
N
S

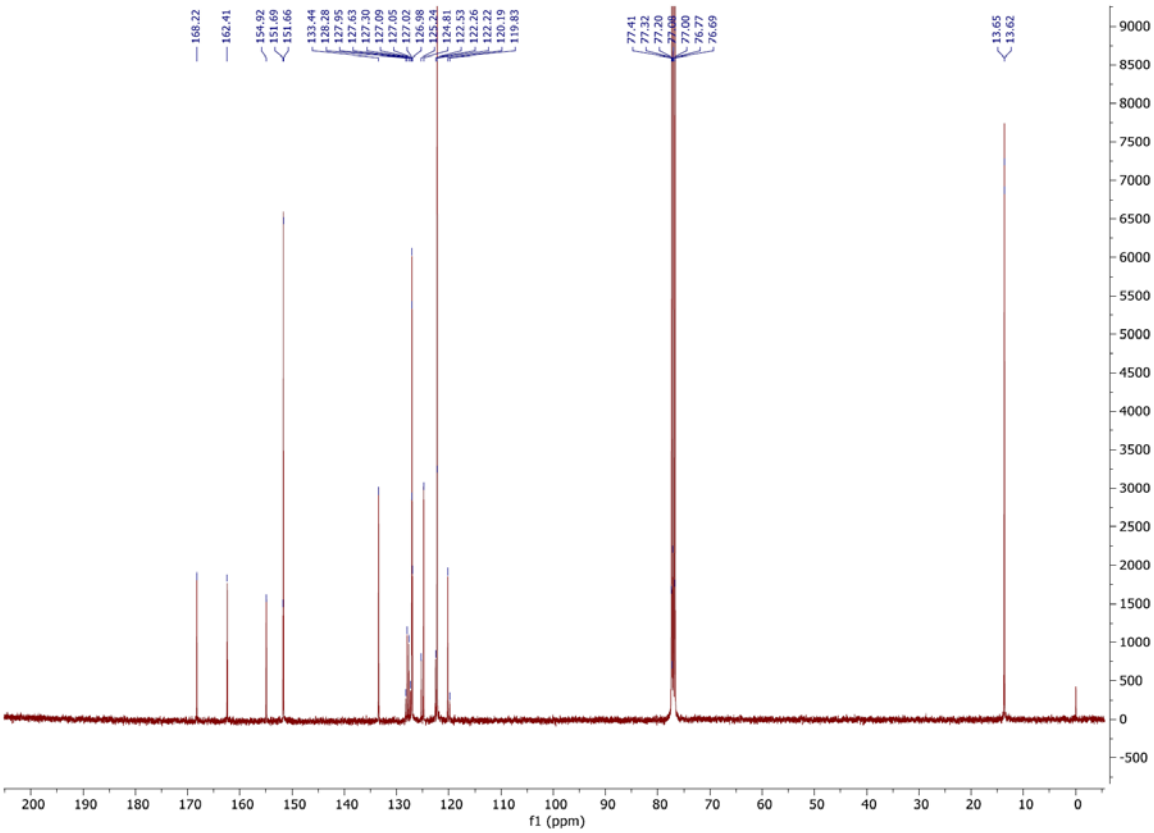

8.50
8.49
7.61
7.60
7.58
7.57
7.56
7.55
7.54
7.52
7.51
7.51
7.46
7.45
7.45
7.44
7.43
7.43
7.26
2.57
2.57
2.56
2.56
2.54
2.52
2.52
1.00
4.61
6.75
f1 (ppm)


168.09
162.48
152.40
151.67
151.64
151.39
133.34
132.62
132.29
131.96
131.64
130.14
77.32
77.20
76.81
76.68
13.60
f1 (ppm)

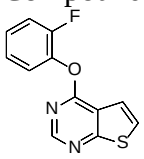
Compound **1k**

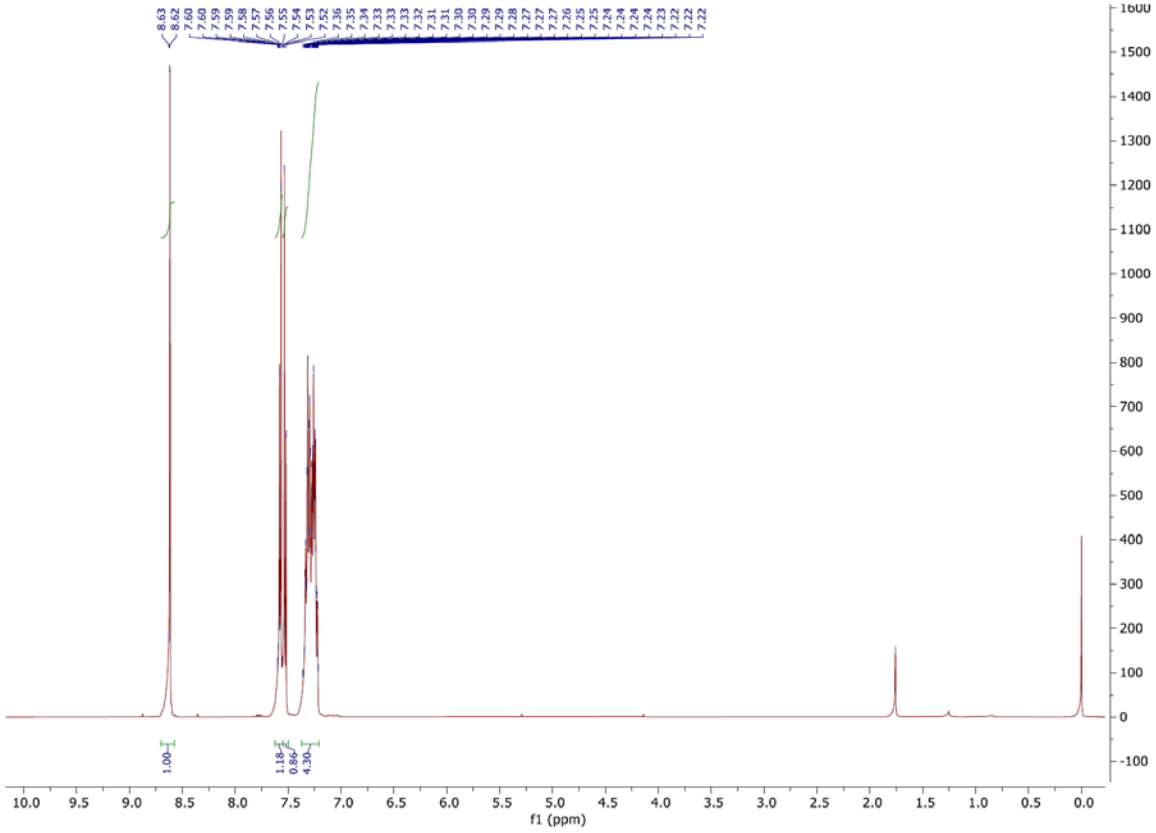

F3C
O
N
N
S

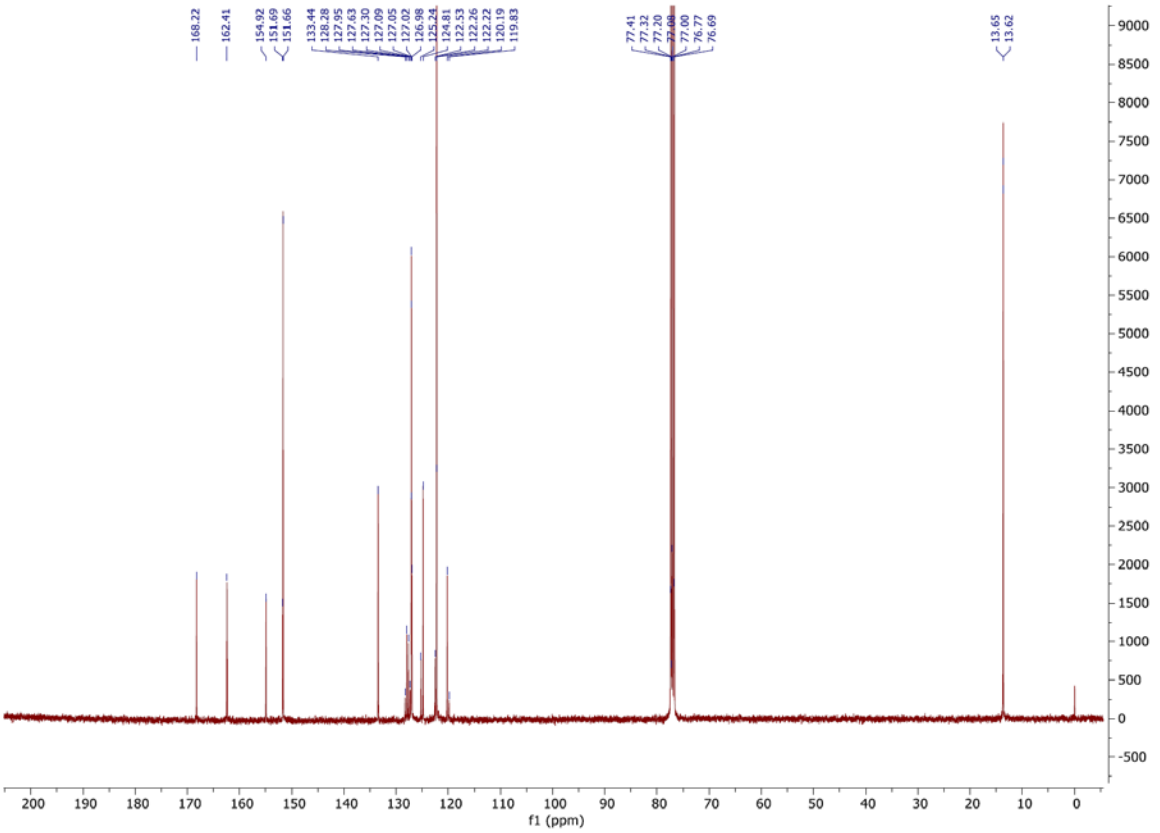

8.49
7.76
7.74
7.73
7.72
7.38
7.37
7.36
7.35
7.26
2.56
2.55
2.55
2.52
2.52
1.00
2.12
2.15
6.49
f1 (ppm)


168.22
162.41
154.92
151.69
151.66
133.44
128.28
127.95
127.63
127.30
127.09
127.05
127.02
126.98
125.24
124.81
122.53
122.26
122.22
120.19
119.83
77.41
77.32
77.20
77.00
76.77
76.69
13.65
13.62
f1 (ppm)

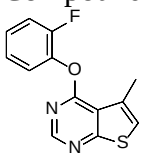
Compound **1l**

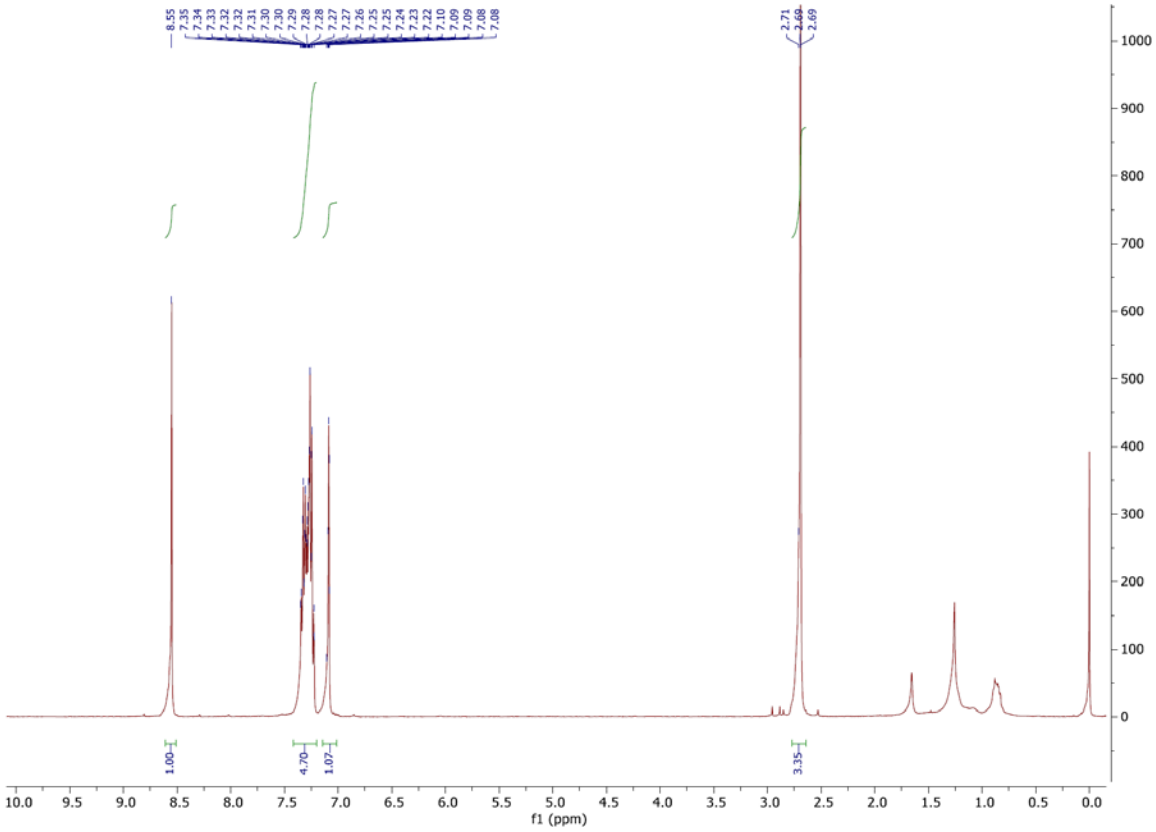

F
O
N
N
S

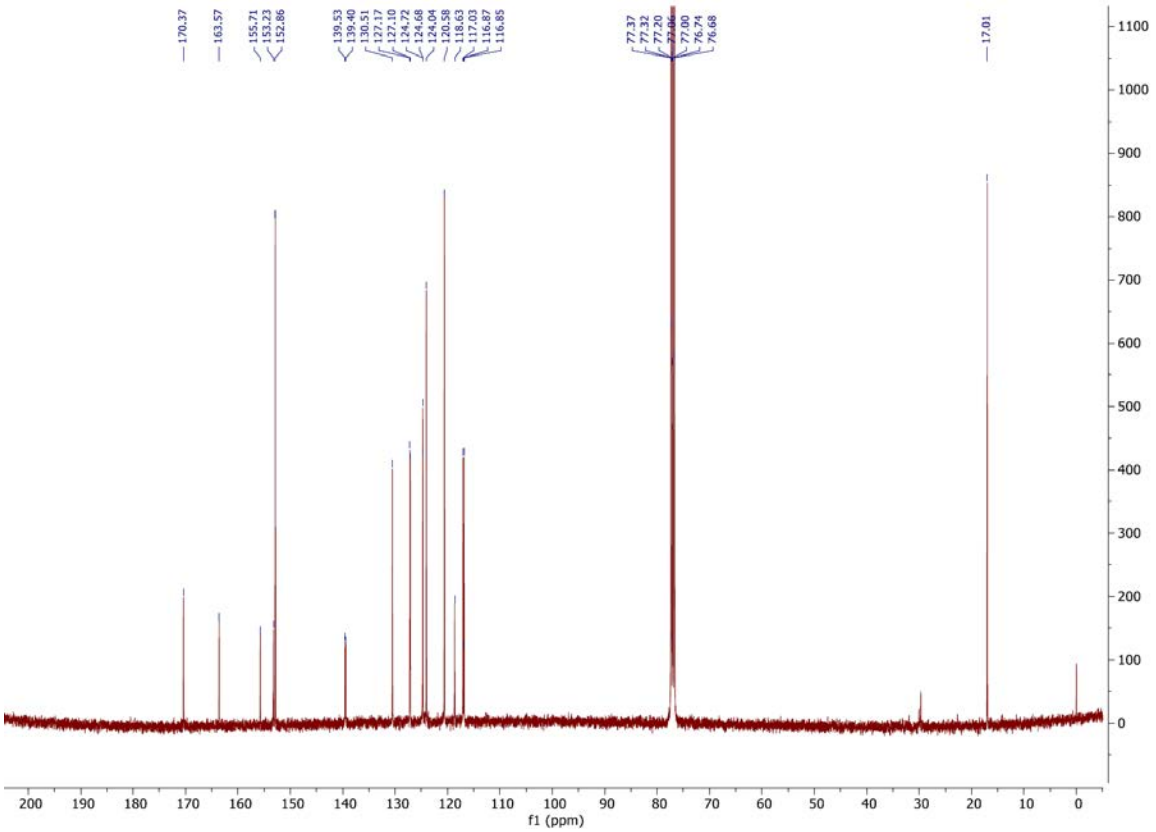

8.63
8.62
7.60
7.60
7.60
7.59
7.59
7.58
7.57
7.56
7.55
7.54
7.53
7.52
7.36
7.35
7.34
7.33
7.33
7.33
7.32
7.31
7.31
7.30
7.30
7.29
7.29
7.28
7.27
7.27
7.27
7.26
7.25
7.25
7.24
7.24
7.24
7.24
7.23
7.22
7.22
7.22
1.00
1.18
0.86
4.30
f1 (ppm)


168.22
162.41
154.92
151.69
151.66
133.44
129.38
129.05
127.63
127.30
127.09
127.05
127.02
126.98
125.24
124.81
122.53
122.26
122.22
120.19
119.83
77.41
77.32
77.20
77.00
76.77
76.69
13.65
13.62
f1 (ppm)

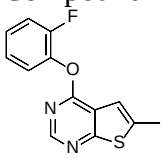
Compound **1m**

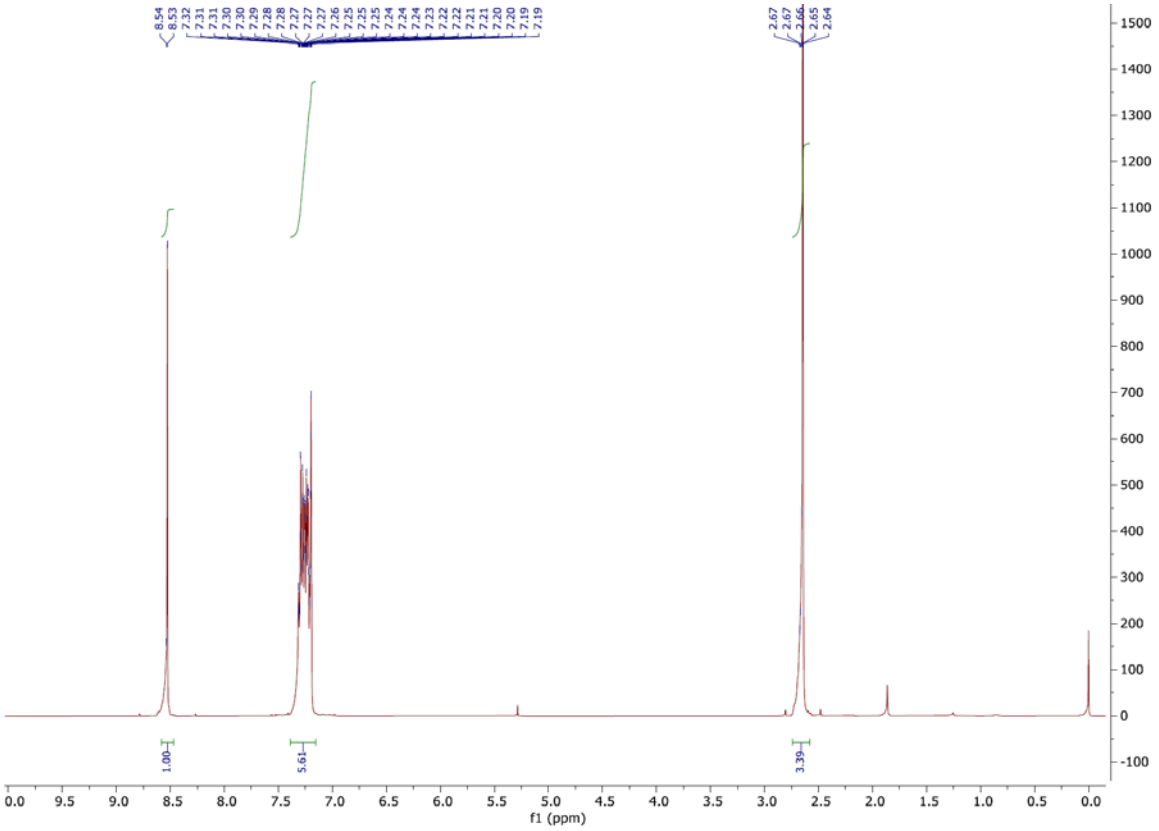

F
O
N
N
S

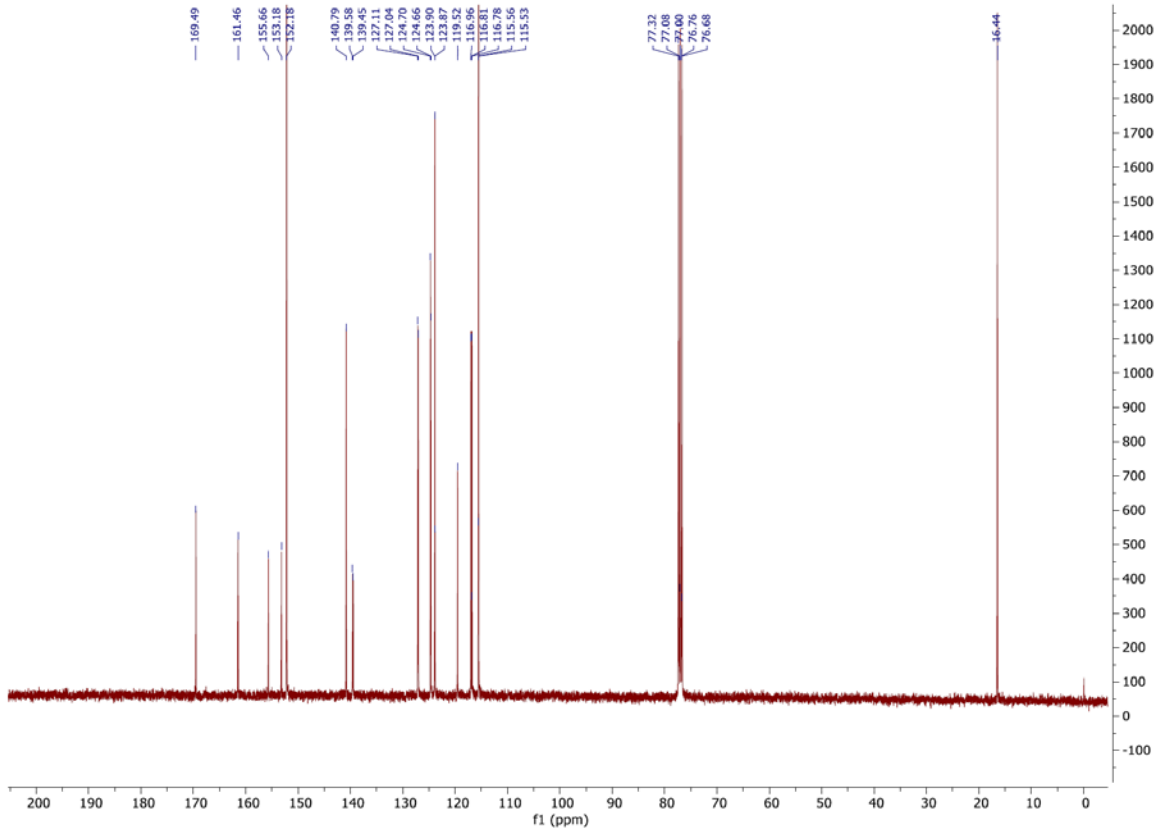

8.55
7.35
7.34
7.33
7.32
7.32
7.31
7.30
7.30
7.29
7.28
7.28
7.27
7.27
7.26
7.25
7.25
7.24
7.23
7.22
7.10
7.09
7.09
7.08
7.08
2.71
2.69
2.69
1.00
4.70
1.07
3.35
f1 (ppm)


170.37
163.57
155.71
153.23
152.86
139.53
139.40
130.51
127.17
127.10
124.72
124.68
124.04
120.58
118.63
117.03
116.87
116.85
77.37
77.32
77.20
77.00
76.74
76.68
17.01
f1 (ppm)

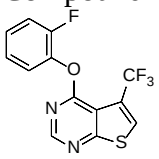
Compound **1n**

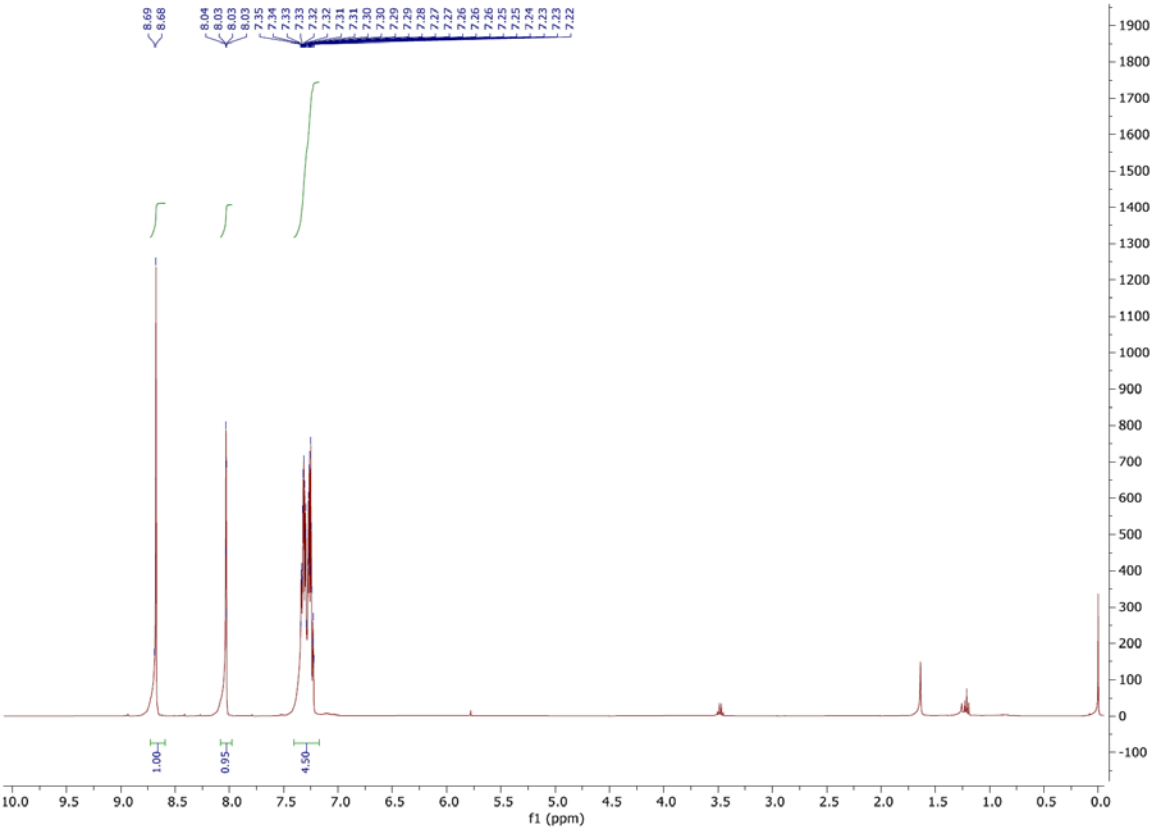

F
O
N
N
S

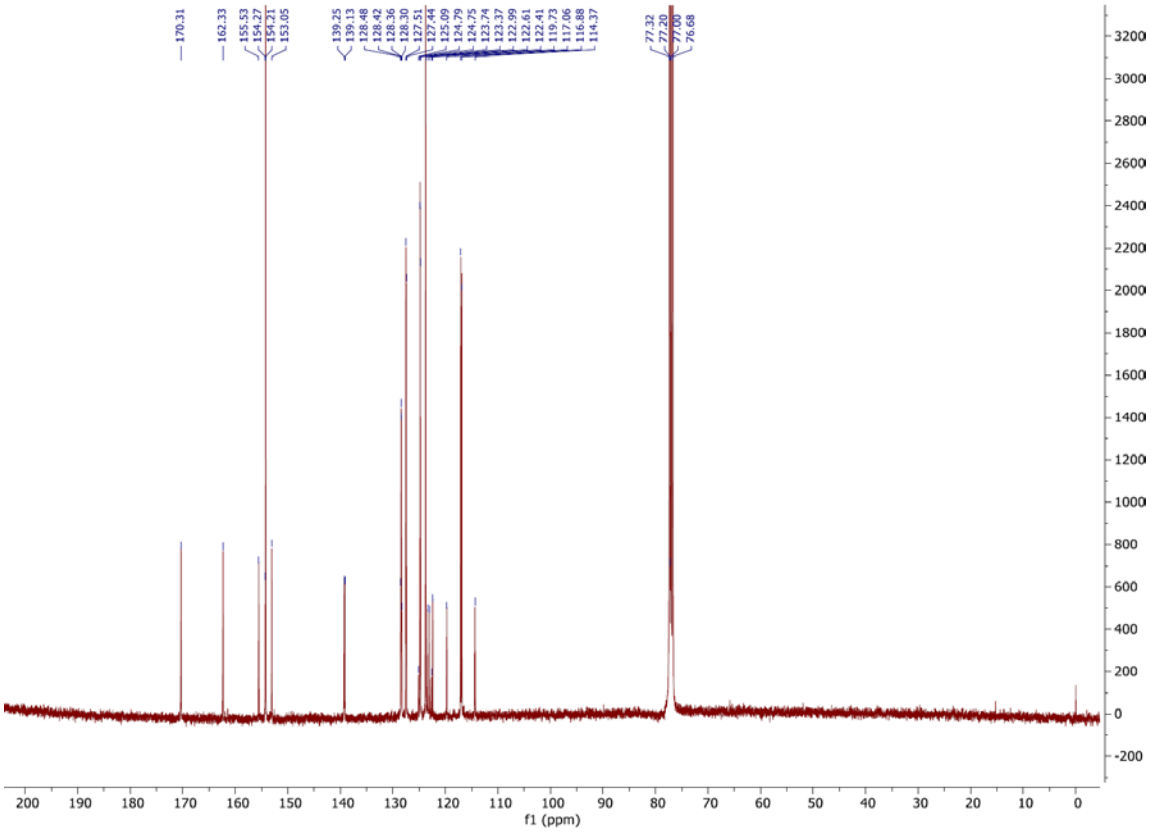

8.54
8.53
7.32
7.31
7.31
7.30
7.30
7.29
7.28
7.28
7.27
7.27
7.27
7.26
7.25
7.25
7.25
7.24
7.24
7.24
7.23
7.22
7.22
7.21
7.21
7.20
7.20
7.19
7.19
2.67
2.67
2.66
2.65
2.64
1.00
5.61
3.39
f1 (ppm)


169.49
161.46
155.66
153.18
152.18
140.79
139.58
139.45
127.11
127.04
124.70
124.66
123.90
123.87
119.52
116.96
116.81
116.78
115.56
115.53
77.32
77.08
77.00
76.76
76.68
16.44
f1 (ppm)

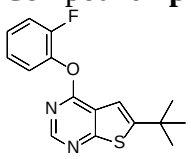
Compound **1o**

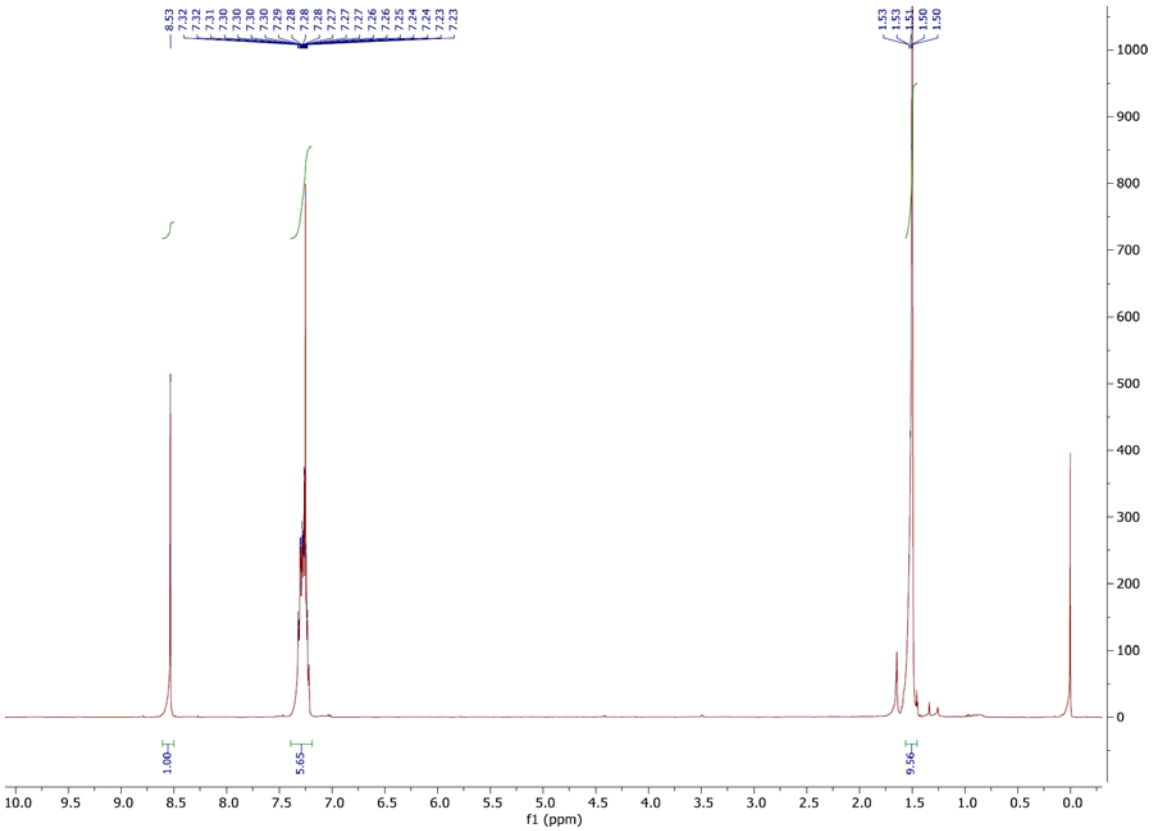

F
O
CF3
N
N
S

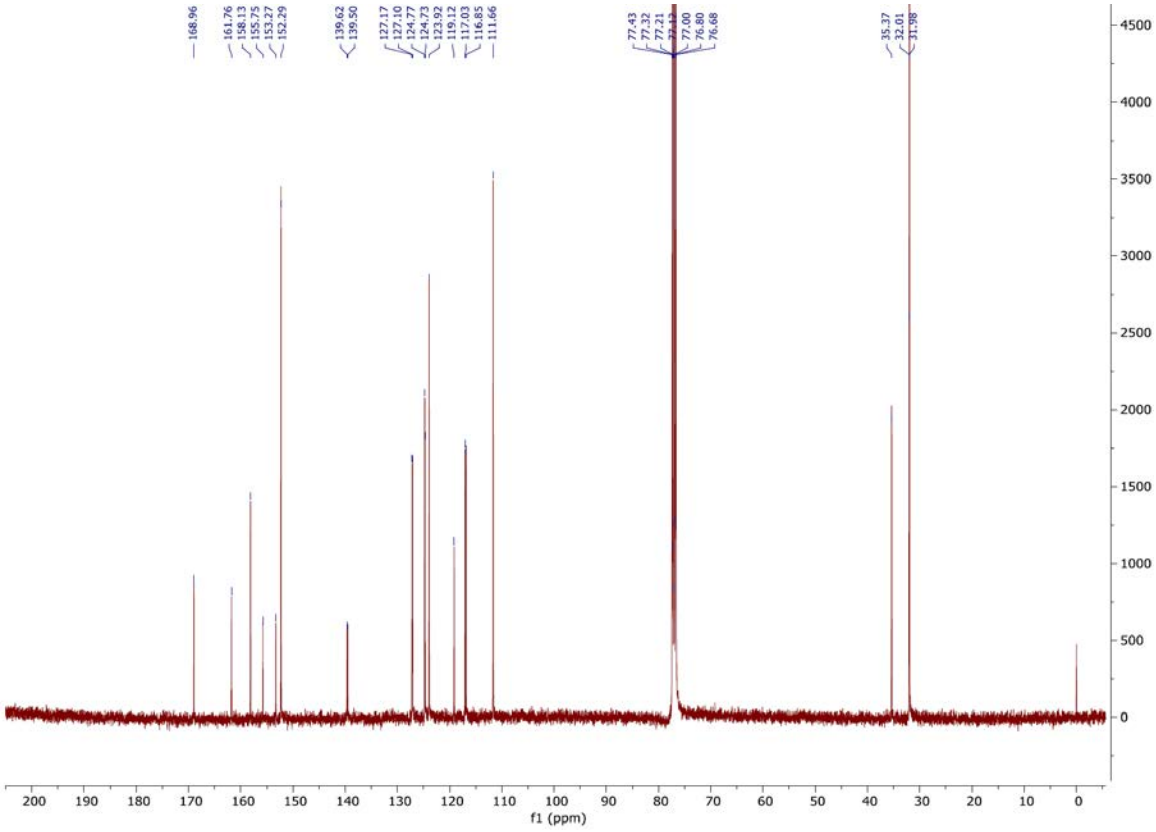

8.69
8.68
8.04
8.03
8.03
8.03
7.35
7.34
7.33
7.33
7.32
7.32
7.31
7.31
7.30
7.30
7.29
7.29
7.28
7.27
7.27
7.26
7.26
7.26
7.25
7.25
7.24
7.23
7.23
7.22
1.00
0.95
4.50
f1 (ppm)


170.31
162.33
155.53
154.27
154.21
153.05
139.25
139.13
128.48
128.42
128.36
128.30
127.51
127.44
125.09
124.79
124.75
123.74
123.37
122.99
122.61
122.41
119.73
117.06
116.88
114.37
77.32
77.20
77.00
76.68
f1 (ppm)

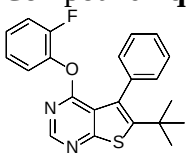
Compound **1p**

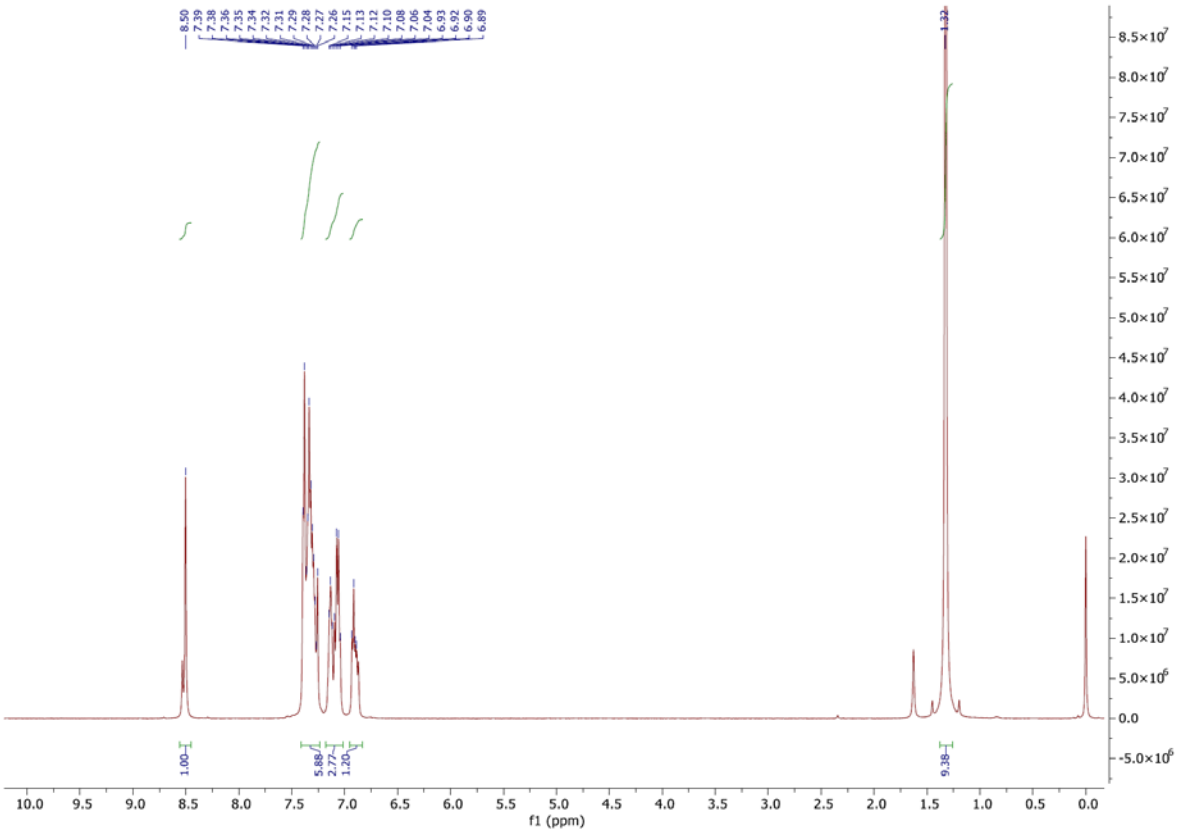

F
O
N
N
S

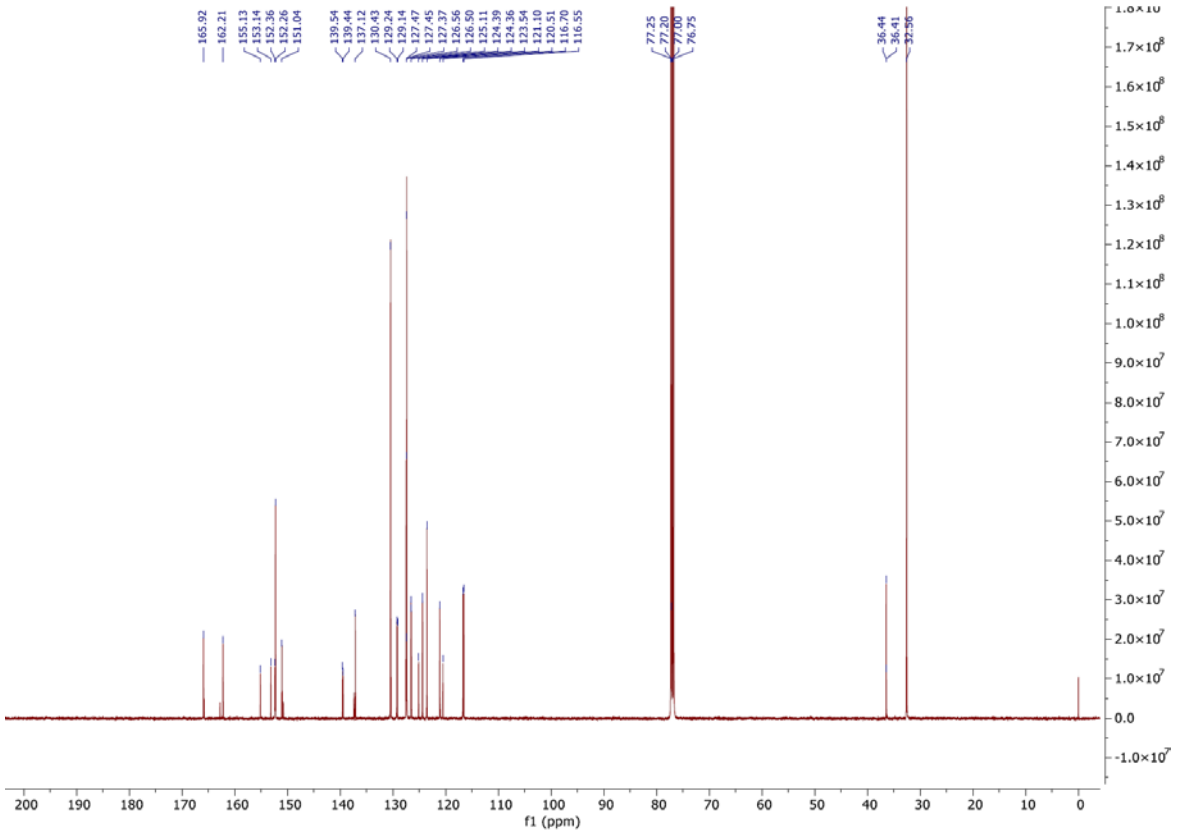

8.53
7.32
7.32
7.32
7.31
7.30
7.30
7.30
7.30
7.29
7.28
7.28
7.28
7.27
7.27
7.27
7.26
7.26
7.25
7.24
7.24
7.23
7.23
1.53
1.53
1.51
1.50
1.50
1.00
5.65
9.56
f1 (ppm)


168.96
161.76
158.13
155.75
153.27
152.29
139.62
139.50
127.17
127.10
124.77
124.73
123.92
119.12
117.03
116.85
111.66
77.43
77.32
77.21
77.00
76.80
76.68
35.37
32.01
31.98
f1 (ppm)

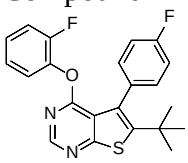
Compound **1q**

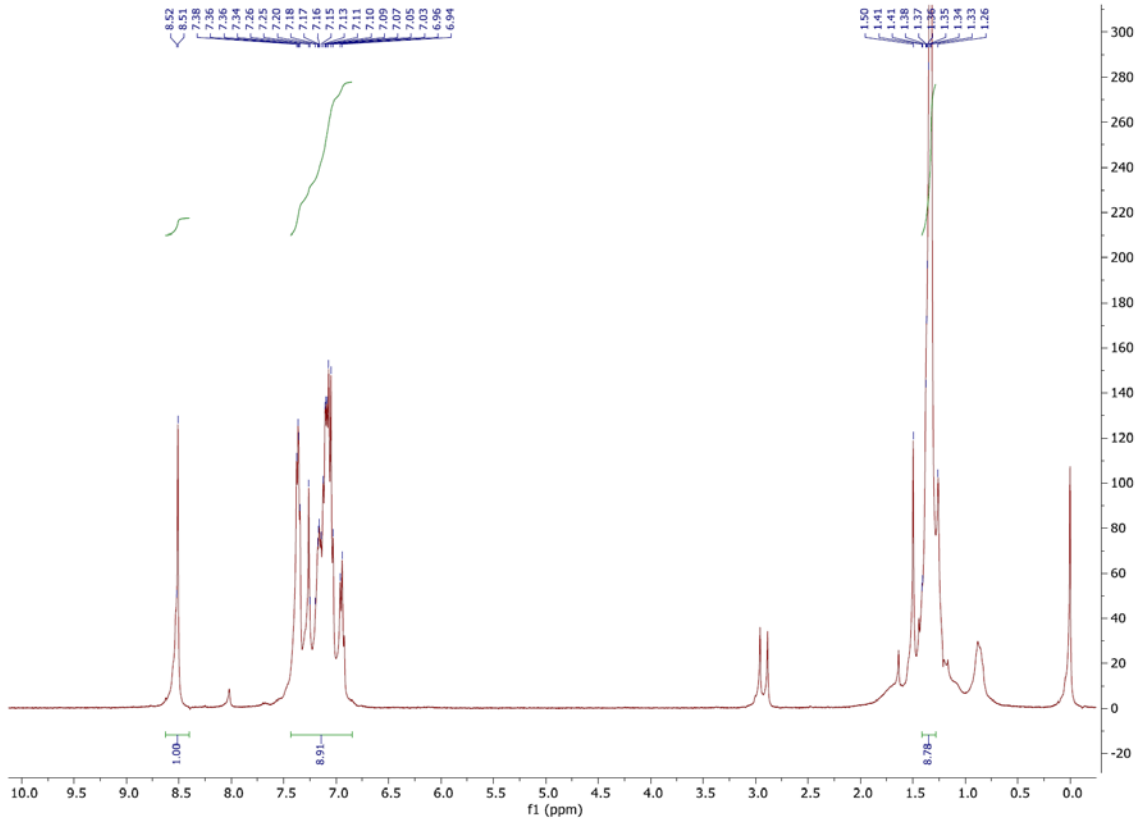

F
O
N
N
S

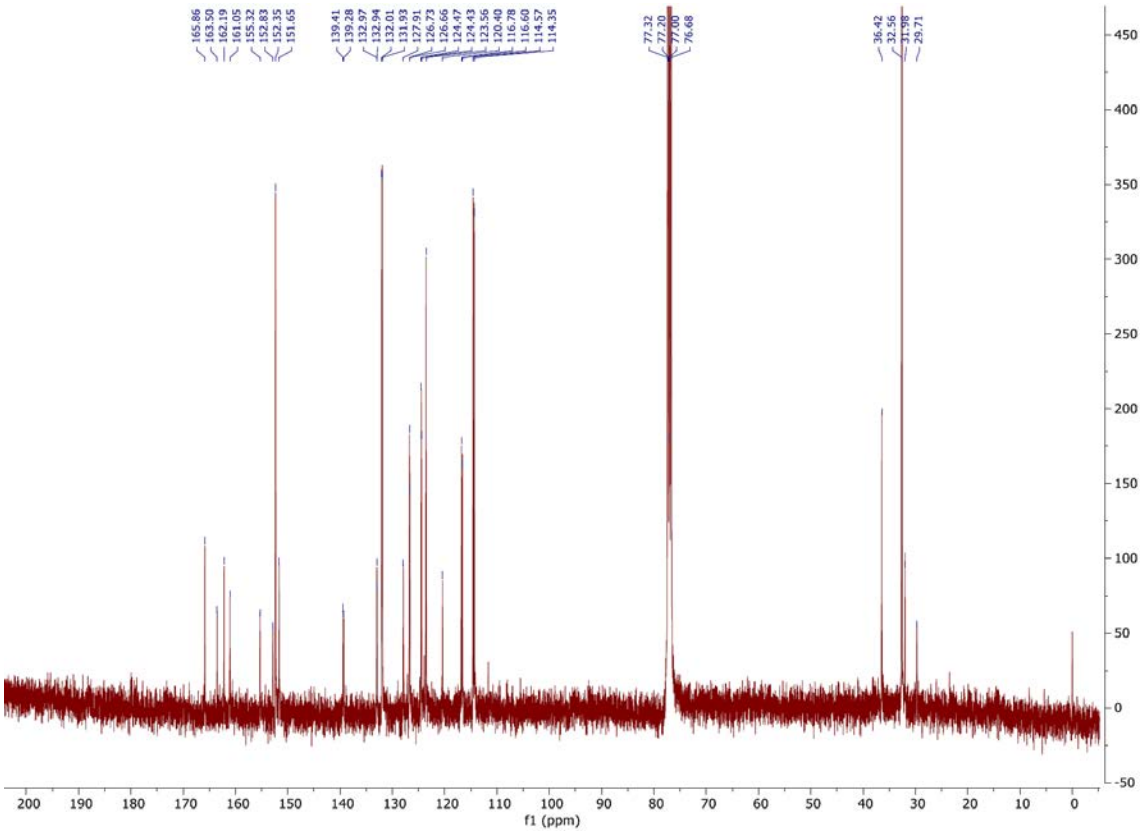

8.50
7.39
7.38
7.36
7.35
7.34
7.32
7.31
7.29
7.28
7.27
7.26
7.15
7.13
7.12
7.10
7.08
7.06
7.04
6.93
6.92
6.90
6.89
1.32
1.00
5.88
2.77
1.20
9.38
f1 (ppm)


165.92
162.21
155.13
153.14
152.36
152.26
151.04
139.54
139.44
137.12
130.43
129.24
127.44
127.47
127.37
126.56
126.50
125.11
124.39
124.36
123.54
121.10
120.51
116.70
116.55
77.25
77.20
77.00
76.75
36.44
36.41
32.56
f1 (ppm)

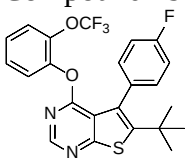
Compound **1r**

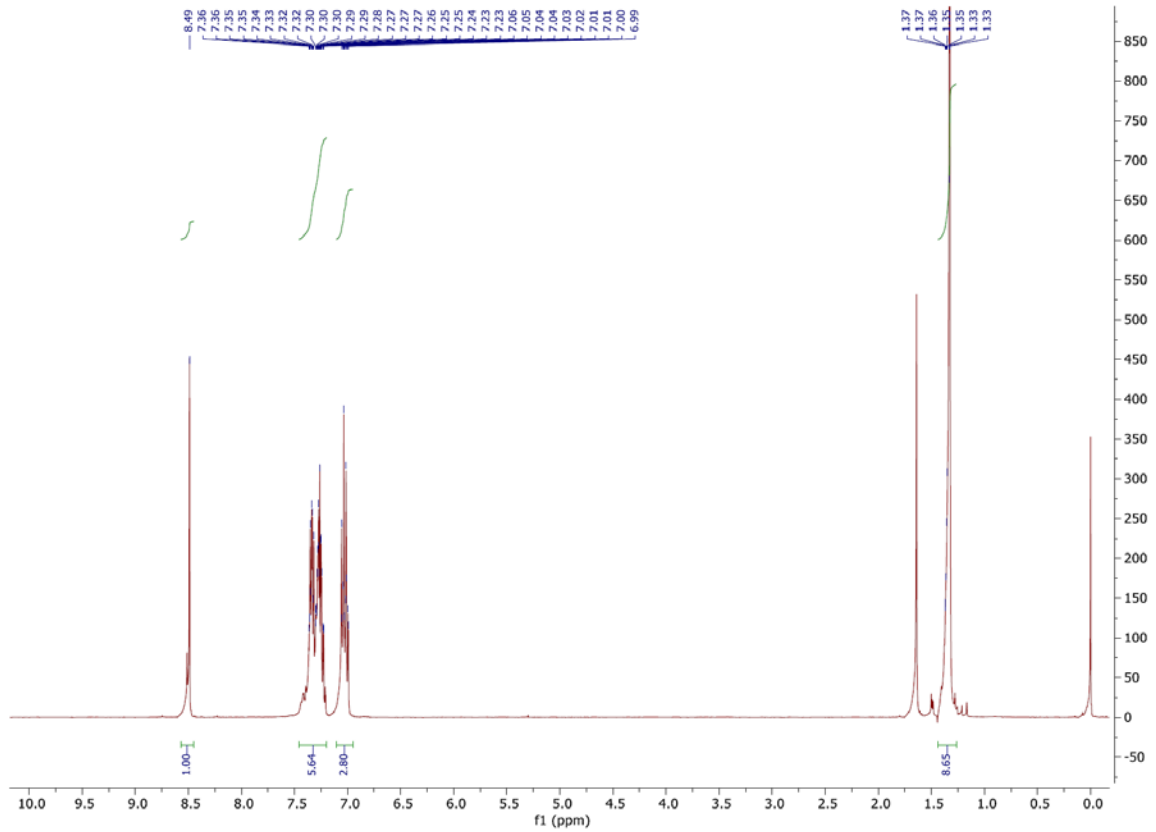

F
F
O
N
N
S

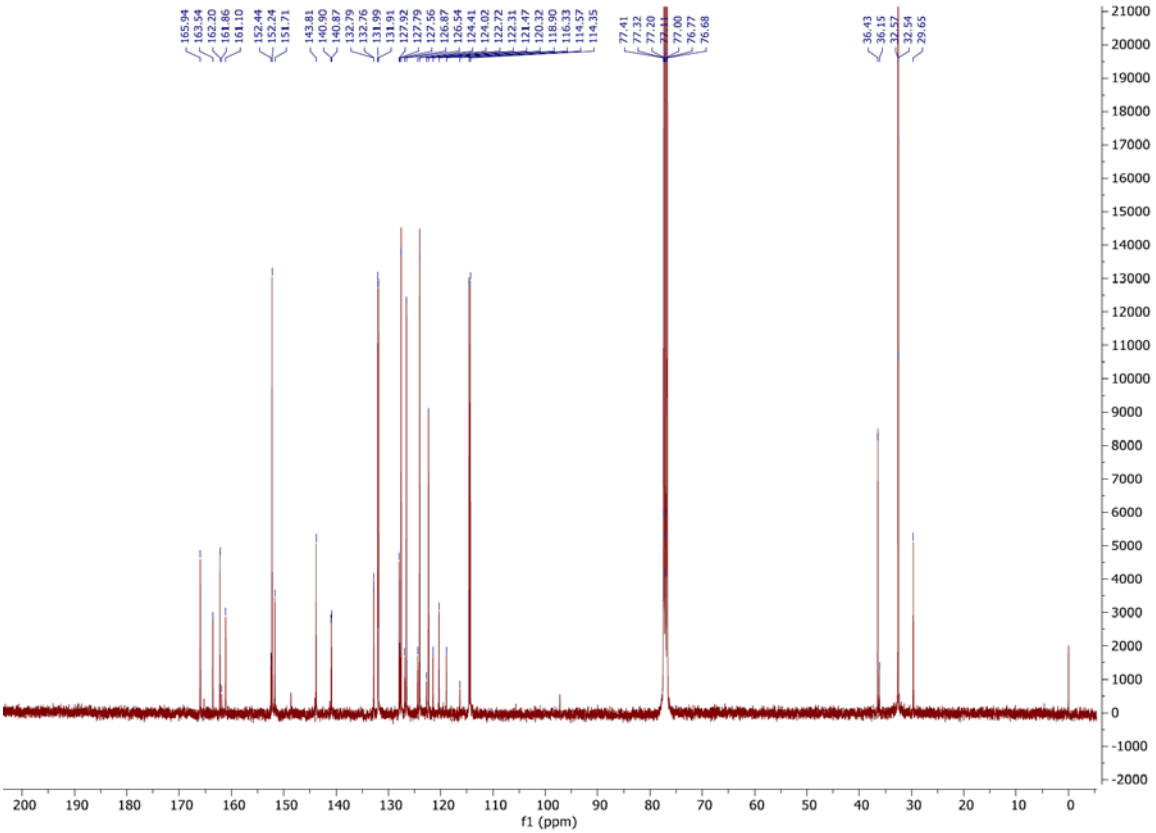

8.52
8.51
7.38
7.36
7.36
7.34
7.26
7.25
7.20
7.18
7.17
7.16
7.15
7.13
7.11
7.10
7.09
7.07
7.05
7.03
6.96
6.94
1.50
1.41
1.41
1.38
1.37
1.36
1.35
1.34
1.33
1.26
1.00
8.91
8.78
f1 (ppm)


165.86
163.50
162.19
161.05
155.32
152.83
152.35
151.65
139.41
139.28
132.97
132.94
132.01
131.93
127.91
126.73
126.66
124.47
124.43
123.56
120.40
116.78
116.60
114.57
114.35
77.32
77.20
77.00
76.68
36.42
32.56
31.98
29.71
f1 (ppm)

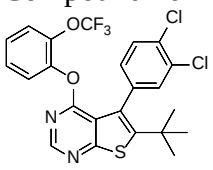
Compound **1s**

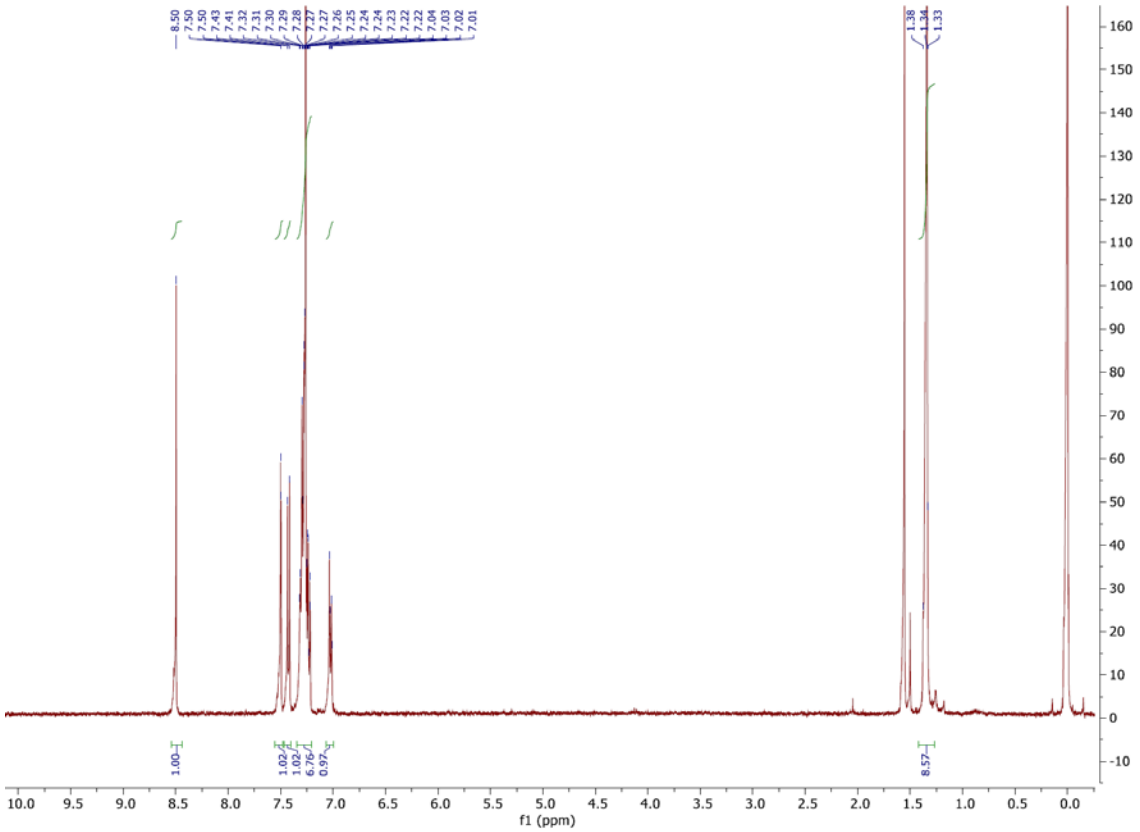

OCF3
F
O
N
N
S

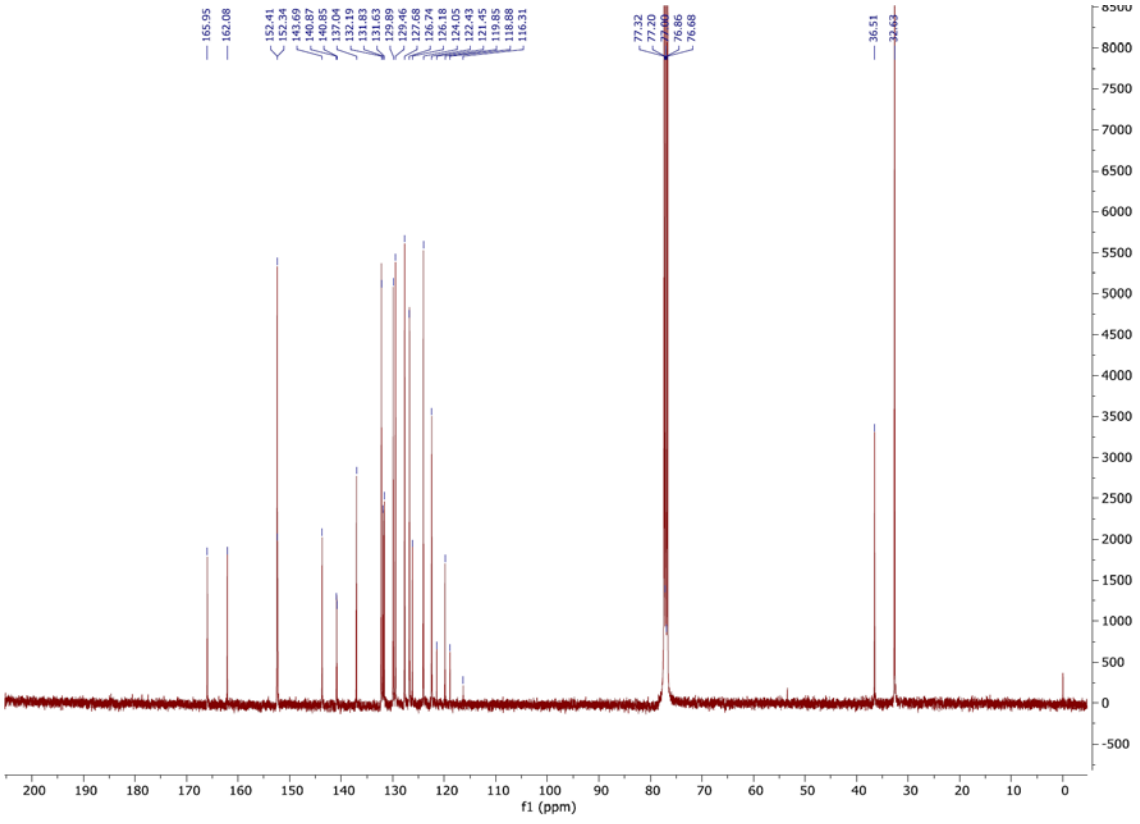

8.49
7.36
7.36
7.35
7.35
7.35
7.34
7.33
7.32
7.32
7.30
7.30
7.30
7.29
7.29
7.28
7.27
7.27
7.27
7.26
7.25
7.25
7.24
7.23
7.23
7.06
7.05
7.04
7.04
7.03
7.02
7.01
7.01
7.00
6.99
1.37
1.37
1.36
1.36
1.35
1.35
1.33
1.33
1.00
5.64
2.80
8.65
f1 (ppm)


165.94
163.54
162.20
161.86
161.10
152.44
152.24
151.71
143.81
140.90
140.87
132.79
132.76
131.99
131.91
127.92
127.79
127.56
126.87
126.54
124.41
124.02
122.72
122.31
121.47
120.32
118.90
116.33
114.57
114.35
77.41
77.32
77.20
77.00
76.77
76.68
36.43
36.15
32.52
32.54
29.65
f1 (ppm)

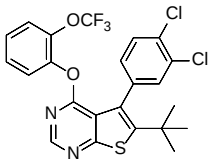
Compound **1t**


OCF3
Cl
Cl
O
N
N
S

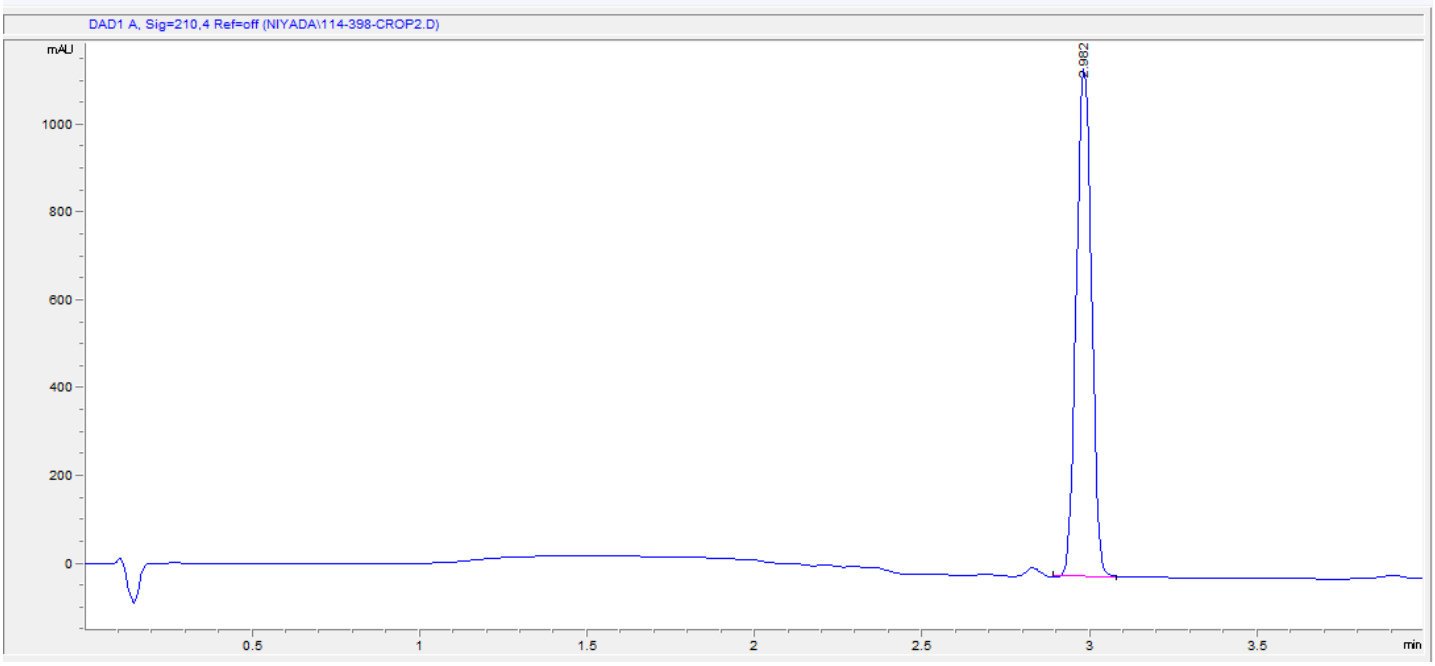

8.50
7.50
7.50
7.50
7.43
7.41
7.32
7.31
7.30
7.29
7.28
7.27
7.27
7.26
7.25
7.24
7.24
7.23
7.22
7.22
7.04
7.03
7.02
7.01
1.38
1.34
1.33
1.00
1.02
1.02
6.76
0.97
8.57
f1 (ppm)


165.95
162.08
152.41
152.34
143.69
140.87
140.85
137.04
132.19
131.83
131.63
129.89
129.46
127.68
126.74
126.18
124.05
122.43
121.45
119.85
118.88
116.31
77.32
77.20
76.86
76.68
36.51
32.63
f1 (ppm)

Compound **1t**

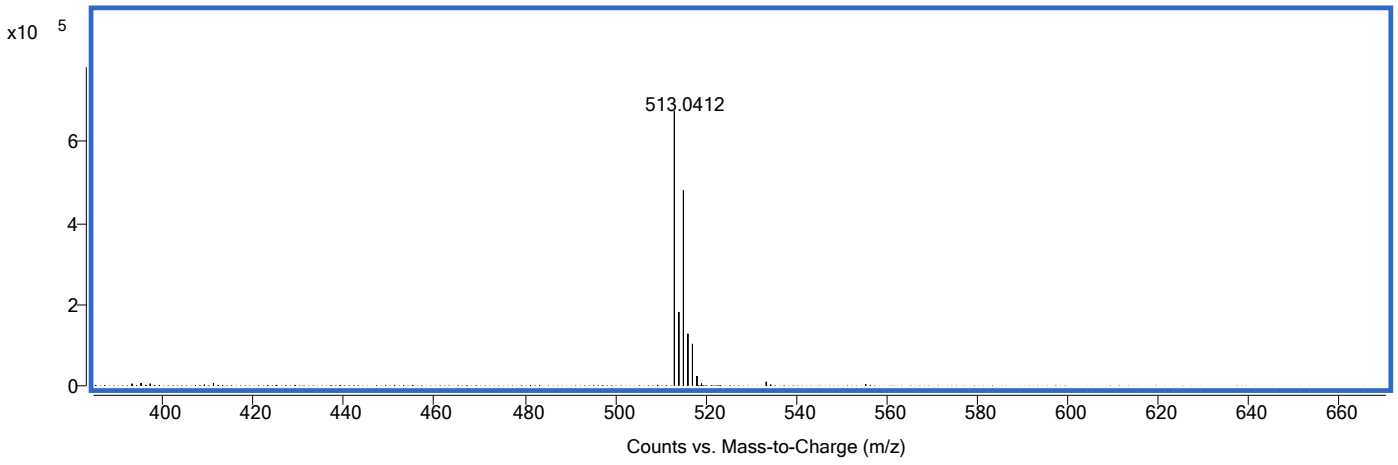


HPLC Trace



HRMS

HRMS was recorded on an Agilent 6520 QTOF coupled to an Agilent 1290 LC, $[M+H]^+$ for $C_{23}H_{18}Cl_2F_3N_2O_2S$: calcd 513.0418, found 513.0412.



Dose response curves of compound 1t in MRGPRX1 assay in the presence and absence of BAM8-22

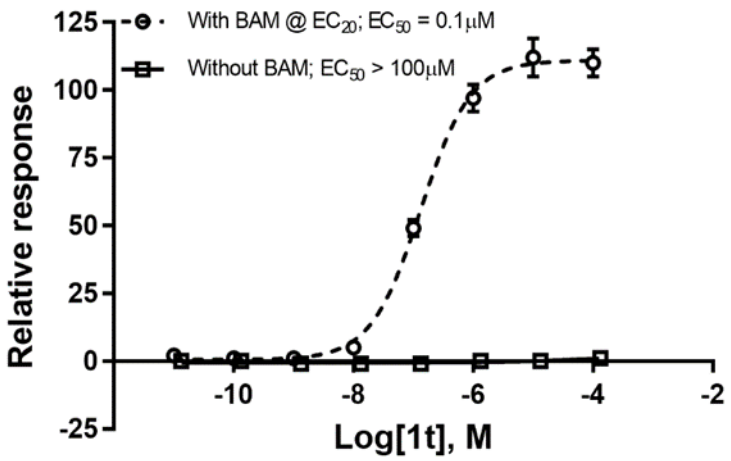


Figure S1. Dose response curves (with respect to compound **1t**) obtained from HEK293 cells expressing MRGPRX1 in the presence and absence of BAM8-22 at EC_{20} concentration.

Cell based Ca^{2+} imaging assay to determine the selectivity of compound 1t over MRPGRX2. Ca^{2+} imaging assay was employed to determine the positive allosteric effect of compound **1t** at MRGPRX2 for its agonist compound 48/80 using a HEK293 cell line that stably expresses MRGPRX2. Cells were incubated with Ca^{2+} sensitive dye Fluo4 solution at 37 °C for 30 min and at RT for 30 min after removing media. 5 µM of compound **1t** were added to the assay buffer with dye for 80 sec followed by adding 0.3 µg/ml compound 48/80 (EC_{20} of the agonist activating MRGPRX2) for 75 sec and recorded the change of fluorescence by Flexstation3 imaging plate reader (Figure S2). 10 µg/ml compound 48/80 was used to activate MRGPRX2 cells at maximum level. The assay was repeated 3 times.

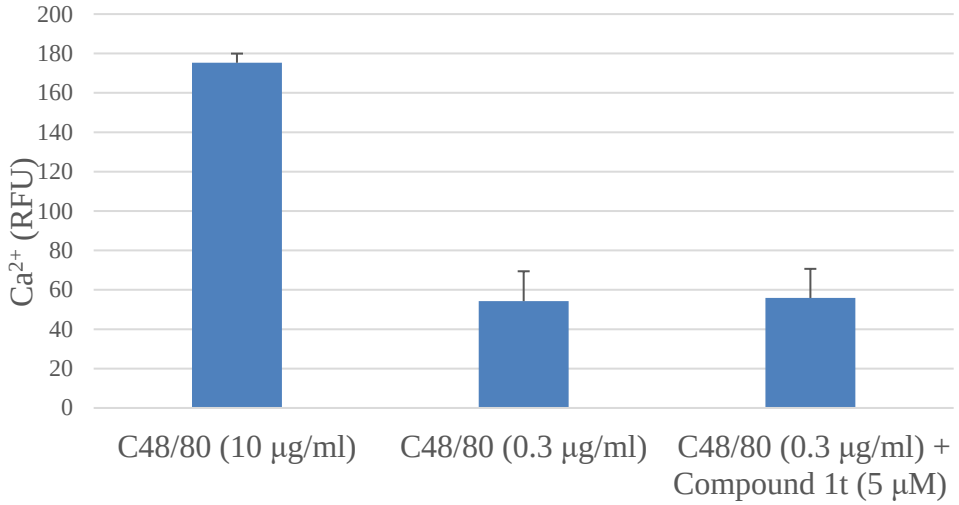


Figure S2. Effect of compound **1t** on the MRGPRX2 agonist activity of compound 48/80 (EC_{20} concentration).

Off-target activity of compound 1t. Compound **1t** showed >50% inhibition at 10 μM in five binding assays (out of 44 assays included in SafetyScreen44™ Panel) as listed in Table S1.

Table S1. Five binding assays in SafetyScreen44™ Panel in which compound **1t** exhibited >50% inhibition at 10 μM

Assay	% Inhibition of Control Specific Binding
CB1 (human) − agonist radioligand	63.6%
CCK_1 (CCK_A) (human) − agonist radioligand	62.2%
Potassium Channel hERG (human) − [^{3}H]Dofetilide	57.1%
Norepinephrine transporter (human) − antagonist radioligand	50.4%
Dopamine transporter (human) − antagonist radioligand	52.6%

Acute behavioral effects of compound 1t in naïve MRGPRX1 mice. To examine if compound **1t** elicits any adverse effect, spontaneous behaviors of awake, free-moving MRGPRX1 mice were video recorded during 0-1 hour post-drug treatment. Acute toxicity and time-dependent changes in other overt behavioral effects were monitored, including failure to groom, vocalization, scratching, flinching, shiver, aggression upon handling. Central side effects were monitored including sedation, hyperactivity, anorexia, and respiratory depression in drug group vs vehicle group. The number of scratching bouts (itch response) was counted and binned in five-minute intervals during this period. Compared to vehicle, compound **1t** at this oral dose (100 mg/kg) caused no overt adverse effects in naïve MRGPRX1 mice including itch side effects as evident from the lack of spontaneous scratch bouts (Table S2).

Table S2. Safety monitoring at 0-1 hour following administration of compound **1t** (100 mg/kg, n=4, po) and vehicle (n=4, po) in naïve MRGPRX1 mice

	Vehicle			**Compound 1t**		
Post-drug time	**0-10 min**	**25-35 min**	**50-60 min**	**0-10 min**	**25-35 min**	**50-60 min**
	Average ± SEM	Average ± SEM	Average ± SEM	average ± SEM	average ± SEM	average ± SEM
Vocalization times	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00
wipe/groom bouts	2.50 ± 3.00	4.25 ± 5.19	2.50 ± 1.91	2.75 ± 1.71	3.50 ± 1.00	2.00 ± 1.41
paw lick/flinch	0.00 ± 0.00	1.00 ± 1.41	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
scratch bouts	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
shiver times	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
sedation (Y/N)	N	N	N	N	N	N
hyperactivity (Y/N)	N	N	N	N	N	N

Open field test of compound 1t in naïve MRGPRX1 mice. Spontaneous exploration was measured as an indicator of locomotor activity with using the open field test. Mice were placed in an open field Plexiglas chamber (40 x 40 x 33 cm) and their movements were recorded with an automated video tracking system (Panlab SMART 3.0 software, Harvard Apparatus, Barcelona, Spain). Mice were individually placed in the center of the apparatus at the beginning of each test and allowed to explore freely for 10 minutes. Locomotor activity was then estimated from parameters including total distance travelled, time spent in center zone and number of center zone entries, which were determined by the software. The arena was wiped with 75% ethanol before and after each test to eliminate scents left by other mice. Neither compound **1t** (100 mg/kg, po) nor vehicle significantly impaired exploratory performance in MRGPRX1 mice, as compared to pre-drug (Figure S3A-E).

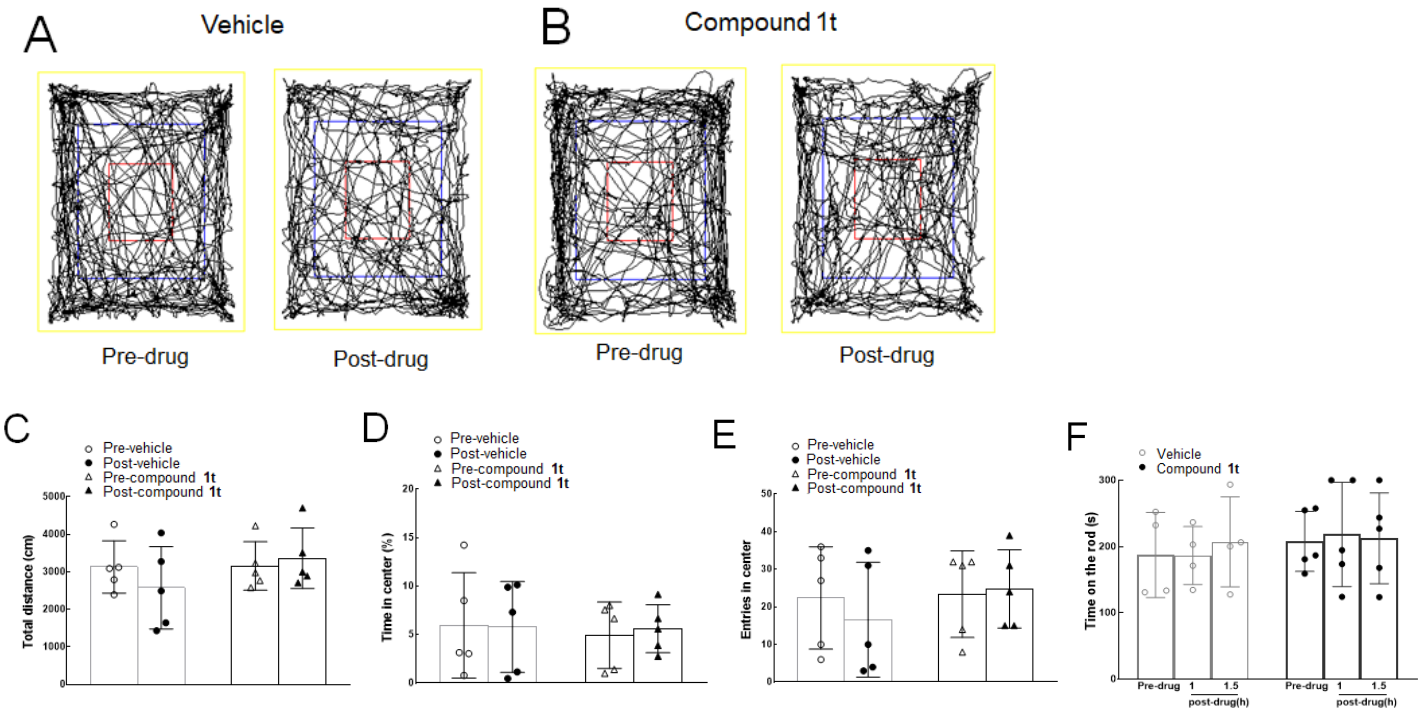


Figure S3. Oral administration of compound 1t did not impair exploratory activity of naïve MRGPRX1 mice. (A-B) Example traces show exploratory activity of naïve MRGPRX1 in the open field test (10-minute duration) before and 1h after oral gavage of vehicle (A, n = 5) and compound 1t (B, 100 mg/kg, po, n = 5). The area between the outer black box and the inner blue box is the border periphery, the area between the blue box and the red box is the internal periphery, and the red box constitutes the center. **(C)** The total distance traveled, **(D)** the time (%) spent in the center, and **(E)** the number of entries into the center before and after each drug treatment. **(F)** In the rotarod test, the time that naïve MRGPRX1 mice remained on the accelerating rod 1h and 1.5h after vehicle or compound 1t administration (100 mg/kg, po, n=6/group) was unchanged from baseline. Two-way mixed model ANOVA with Bonferroni post hoc test. Data are expressed as mean ± SD.

Rotarod test of compound 1t in naïve MRGPRX1 mice.

Motor coordination, ataxia and equilibrium were measured using a rotarod apparatus (Ugo Basile, Varese, Italy). Mice were placed in the testing environment, in their home cage, and allowed to acclimate for at least 1 hour to minimize effects of stress on behavior during testing. Mice underwent a 2d training period (2 trials per mouse, 4 rpm rotarod for 10 min per trial, 20 min interval between each trial) before the test. Mice were placed in separate lanes on a rod rotating at 4rpm such that the animals were able to walk forward and keep their balance. During testing, the speed of rotation was accelerated from 4 to 40 rpm over 300 s for each trial. Animals' performance on the rod was examined before, 1h and 1.5 h after drug administration. The time (in seconds) that each animal remained on the accelerating rod without falling was recorded. This procedure was repeated for three trials and the averages of the three trials were used for data analysis. The time that naïve MRGPRX1 mice remained on the accelerating rod 1h to 1.5h after compound **1t** (100 mg/kg, po) or vehicle administration was also compared to pre-drug level (Figure S3F).