Supporting Information

Isothiocyanate-Containing Hybrid Androgen Receptor (AR) Antagonist Downregulates AR and Induces Ferroptosis in GSH–Deficient Prostate Cancer Cells

Zhihui Qin,[a]∗ Siyu Ou, [a],# Liping Xu, [a],# Kathleen Sorensen,[a] Yingxue Zhang,[b] Zhe Yang,[b] Wen-Yang Hu,[c] Fei Chen[a] and Gail S. Prins[c]

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^{1}H, ^{13}C-NMR and HRMS spectra of the synthetic products

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Figure S1. The predicted interactions of 12a (A) and 13 (B) with AR. Both compounds form identical hydrogen bonds and π-π interaction with AR, compared to **12b**. Homology model was constructed by aligning the agonistic form of AR (2AMB) with glucocorticoid receptor antagonistic conformation (1NHZ). Docked **12a** and **13** are colored in blue and yellow, respectively. Drug-interacting AR residues are depicted as sticks. Dashed lines indicate hydrogen bonds.

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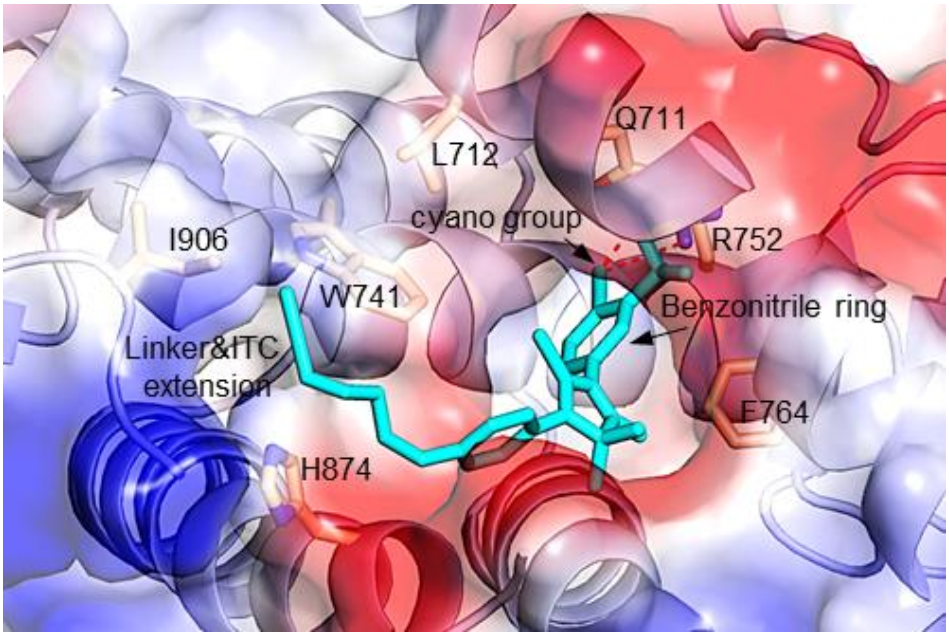

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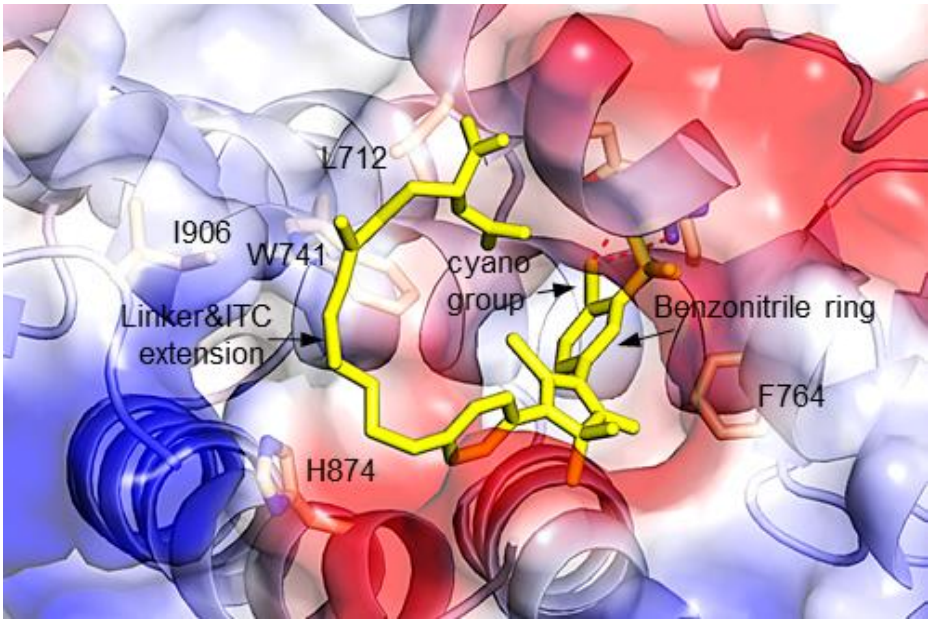

Figure S2. Differential selectivity of 13 and 12b in cell culture. LNCaP and RWPE-1 cells were treated with **12b** or **13** for 72 h, respectively. Cell viability was measured using MTT assay. ns, not significant; **, P<0.01; ****, P<0.0001.

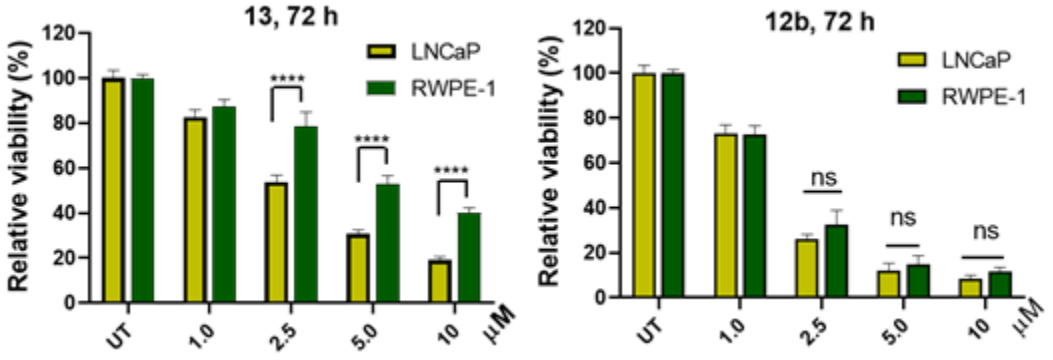

Table S1. The calculated interaction index of 13 and BSO in treating VCaP and 22Rv1 cells. The interaction index is defined as (% viable cells treated with drug combination)/[(% viable cells treated with drug 1)×(% viable cells treated with drug 2)]. Cells were pretreated with BSO for 16 h, followed by the co-treatment for 24 h. Viability was assessed using MTT assay.

Interaction Index (VCaP)

2-63 (μM)	BSO (μM)		
	2.5	5	10
1	0.83	0.81	0.70
2.5	0.62	0.46	0.42
5	0.43	0.34	0.33

Interaction Index (22Rv1)

2-63 (μM)	BSO (μM)		
	2.5	5.0	10
2.5	0.81	0.70	0.67
5.0	0.63	0.60	0.60
10	0.70	0.68	0.66

Figure S3. **13 and BSO combination induces ferroptosis in PCa cells. 13** plus BSO-caused viability loss is rescued by antioxidants, iron chelator and HO-1 inhibitor. VCaP (**A**) or 22Rv1 (**B**) cells were treated with **13** (VCaP, 2.5 μM; 22Rv1, 5 μM), DFO (10 μM), α-Tocopherol (α-Toc, 100 μM), ferrostatin-1 (Fer-1, 0.5 μM) or ZnPP (3 μM) individually or in combination for 24 h. BSO (10 μM) was added 16 h prior to other agents. Values stand for mean±SD (n=6-8). ****, P<0.0001. Cell viability was assessed using MTT assay.

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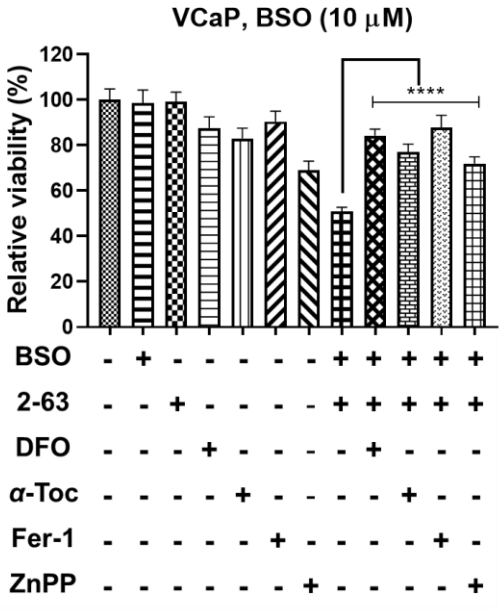

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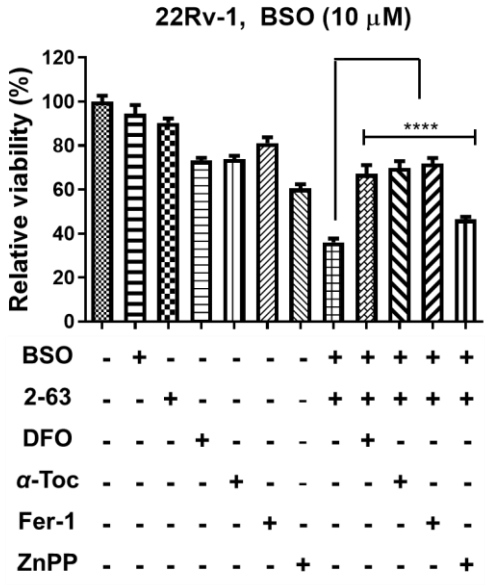

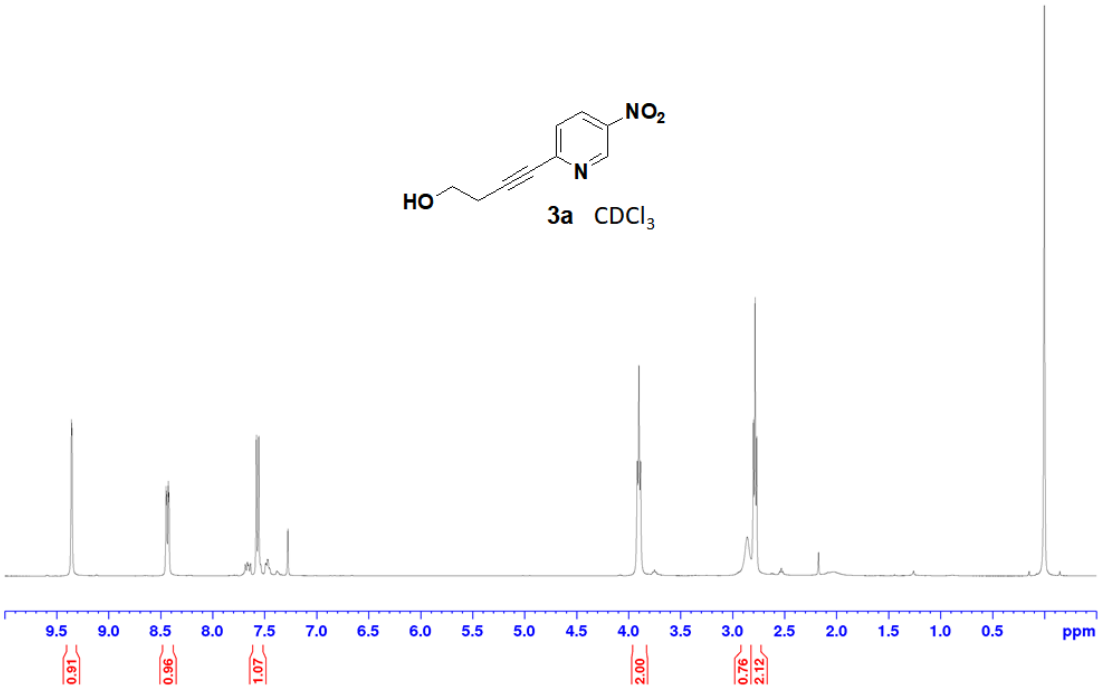
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0.96
1.07
2.00
0.76
2.12


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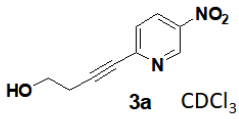
-0.00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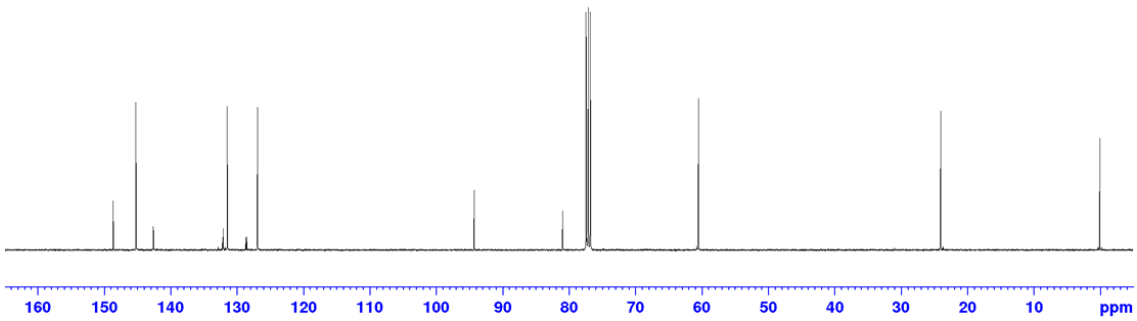
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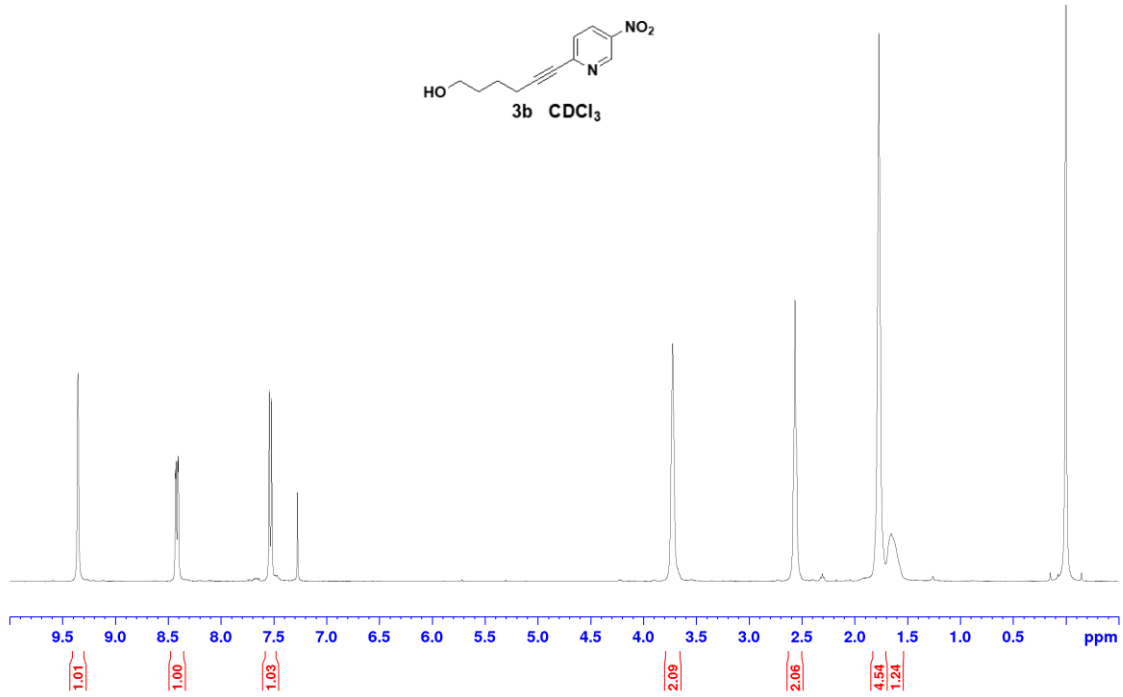
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4.0
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3.0
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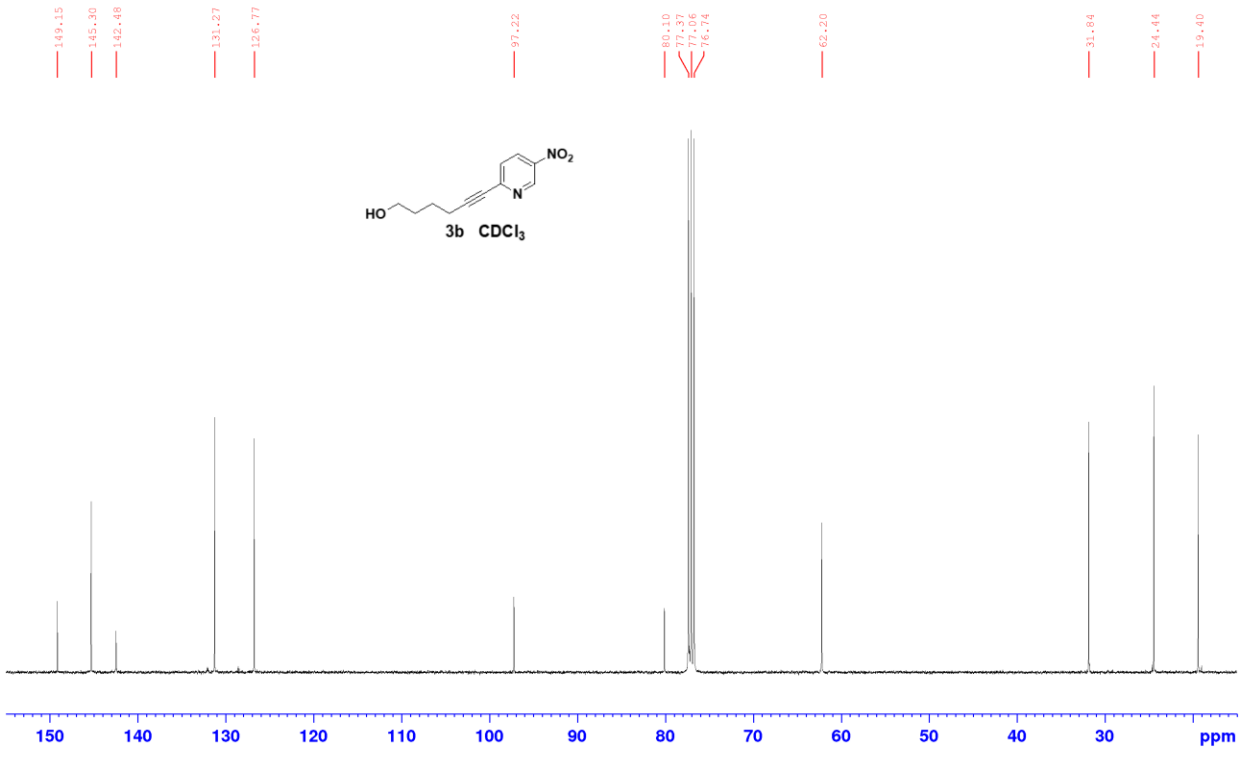
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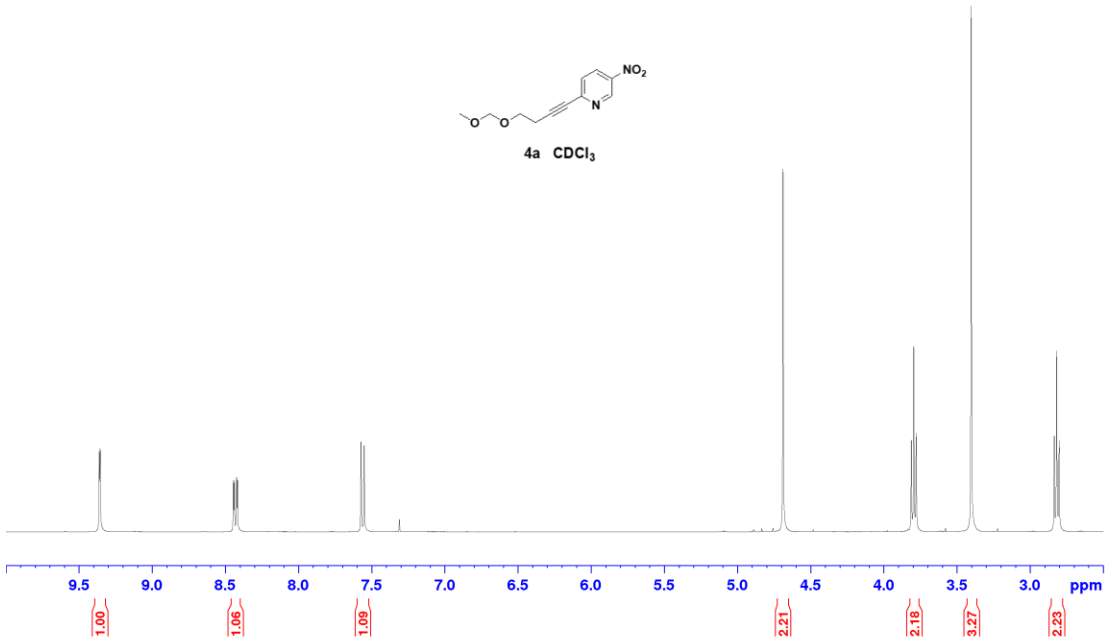
ppm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O
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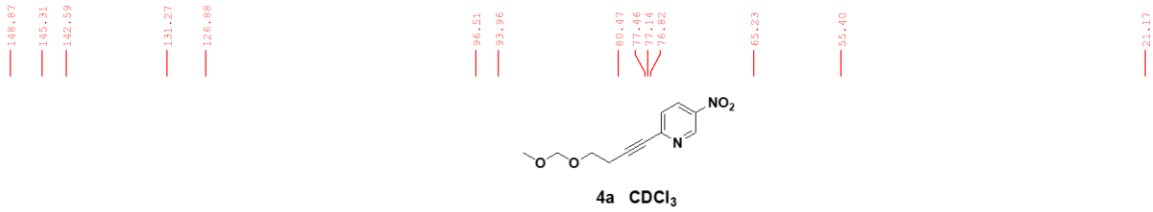
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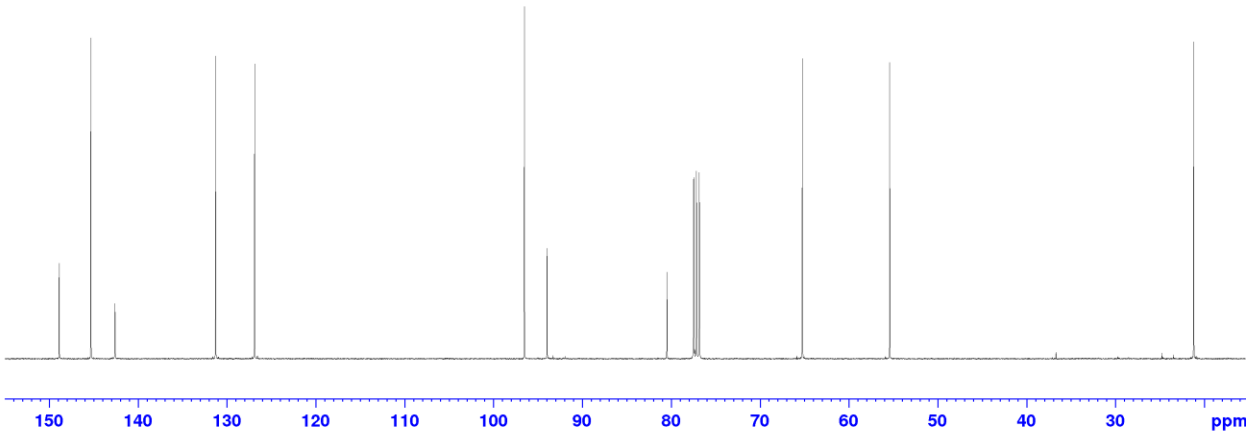
O
O

4a $CDCl_3$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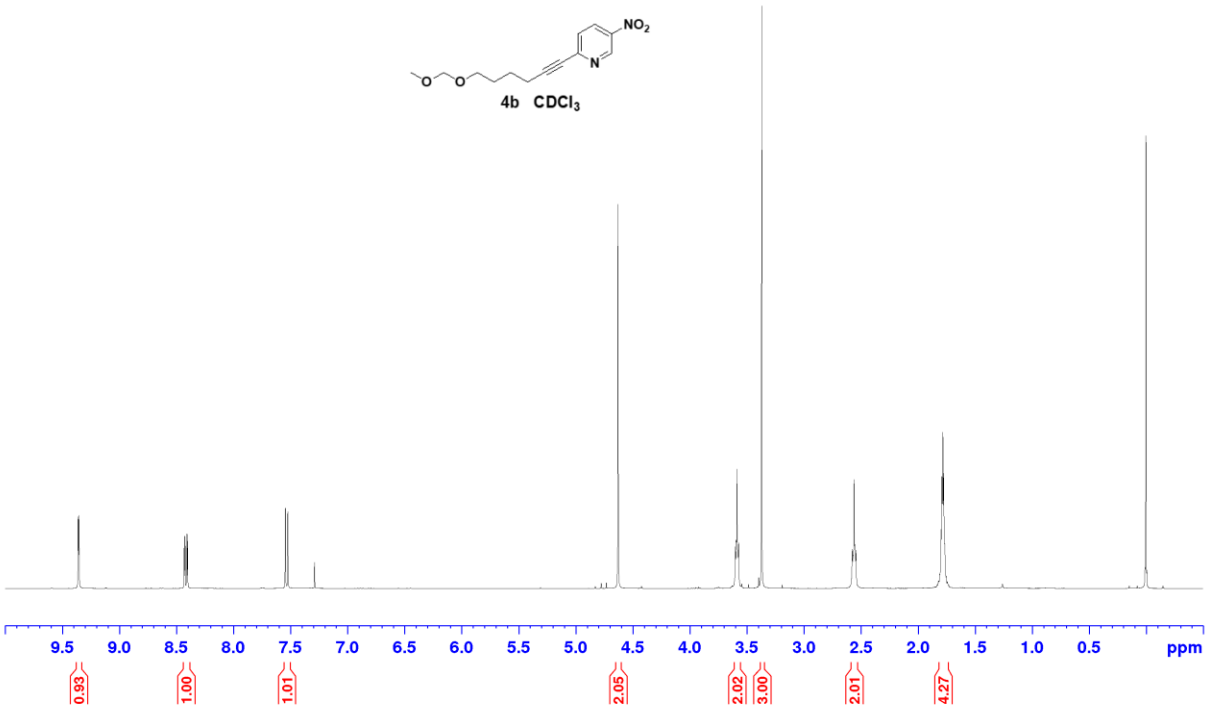
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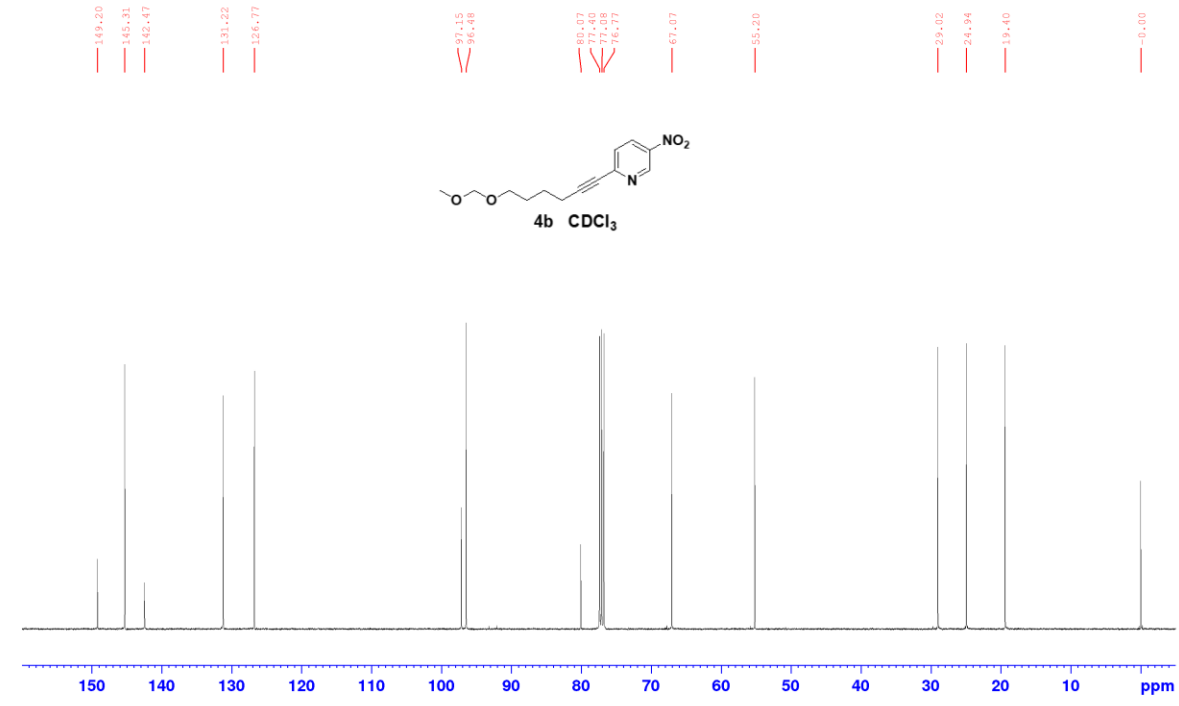
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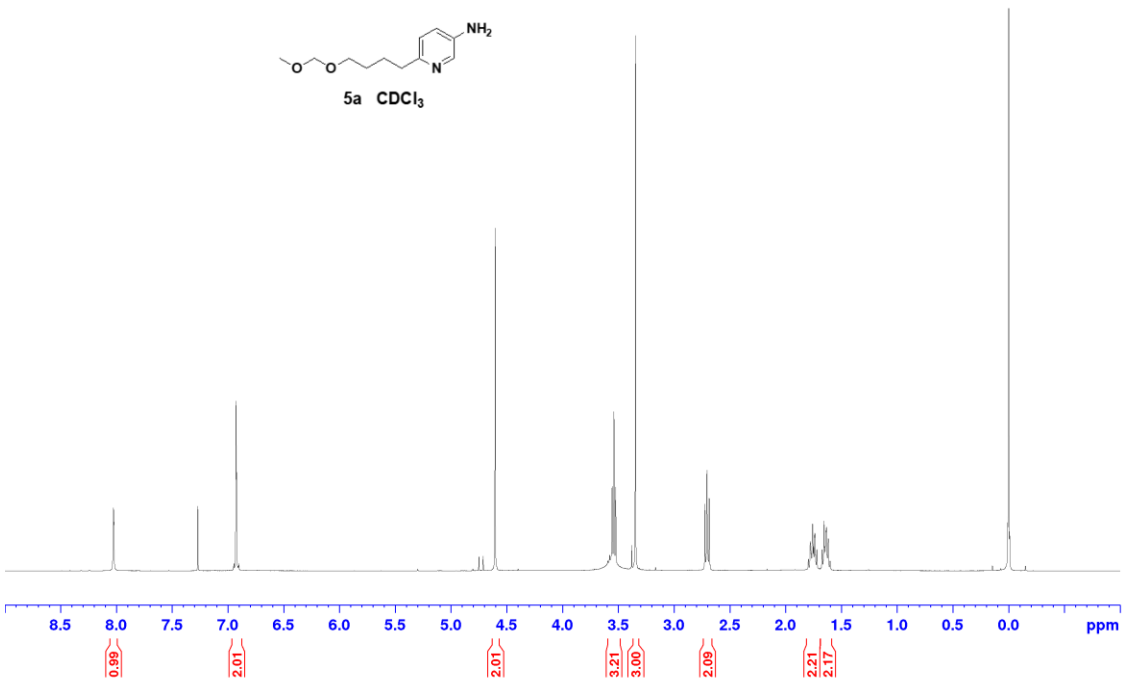
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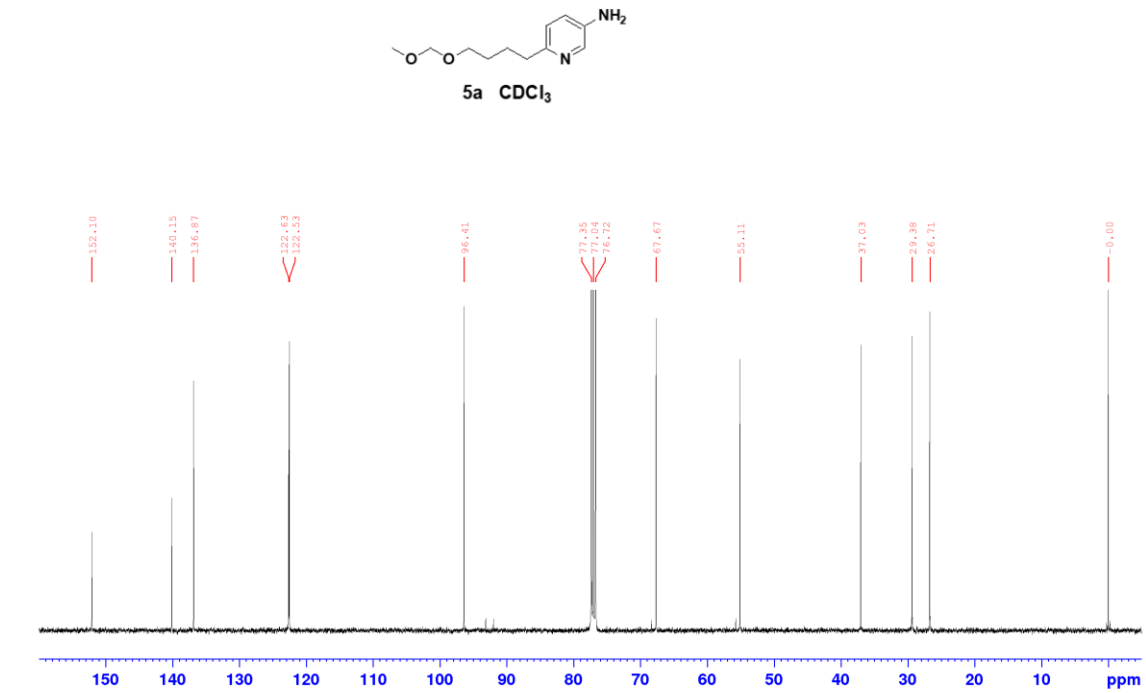
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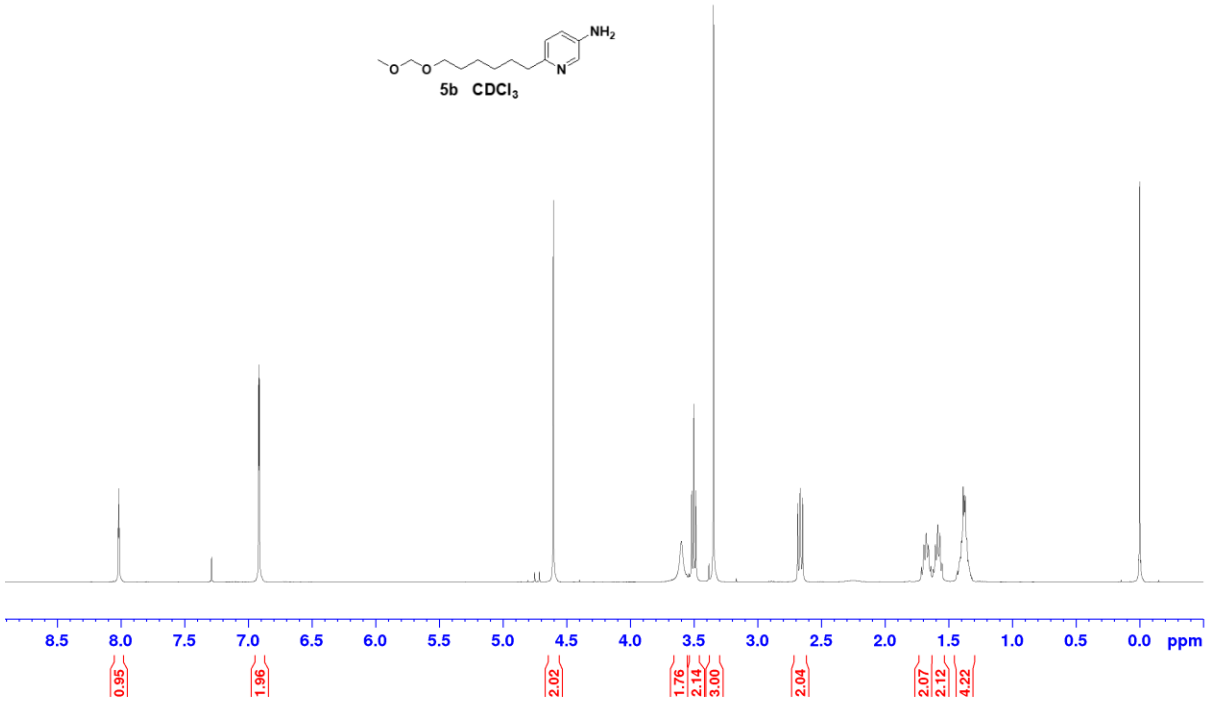
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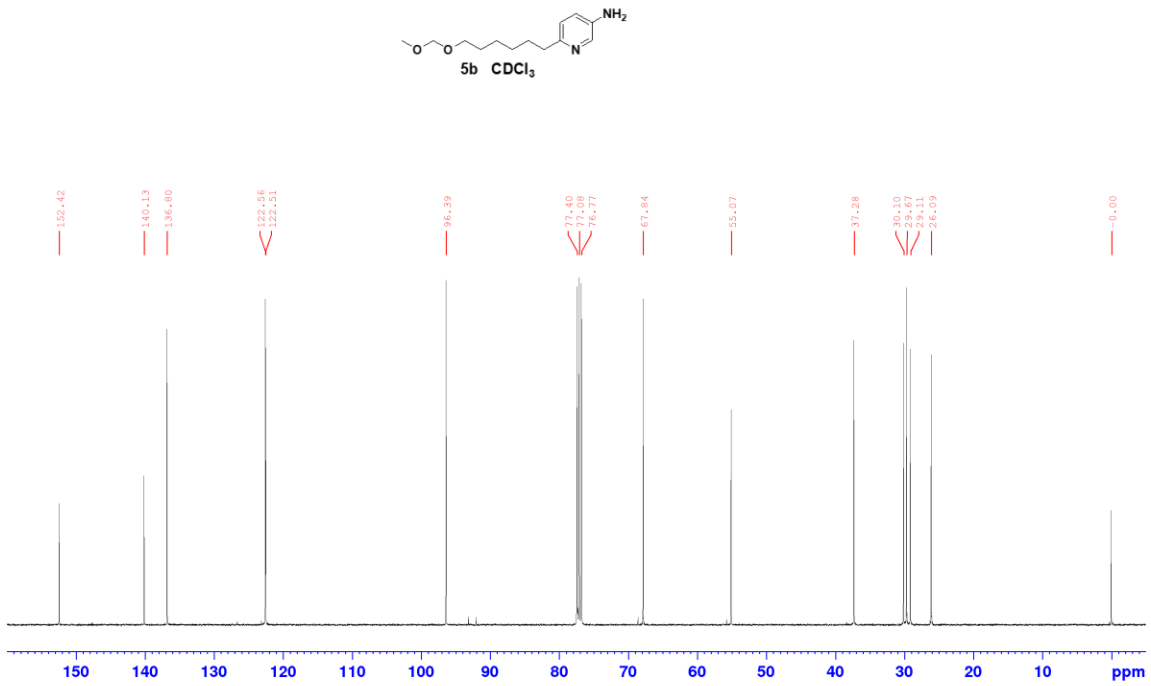
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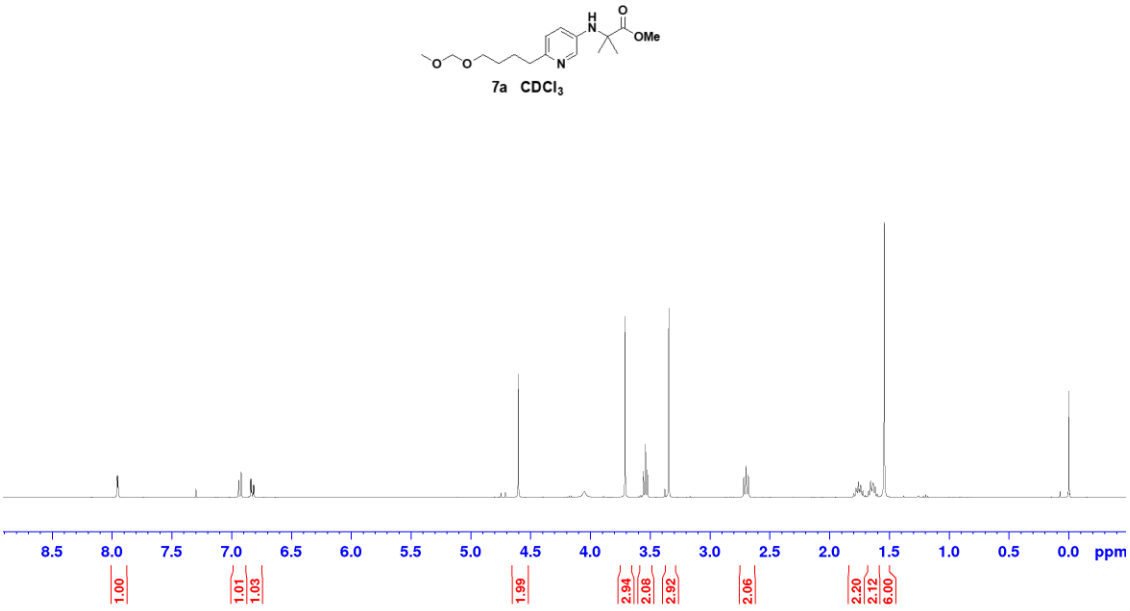
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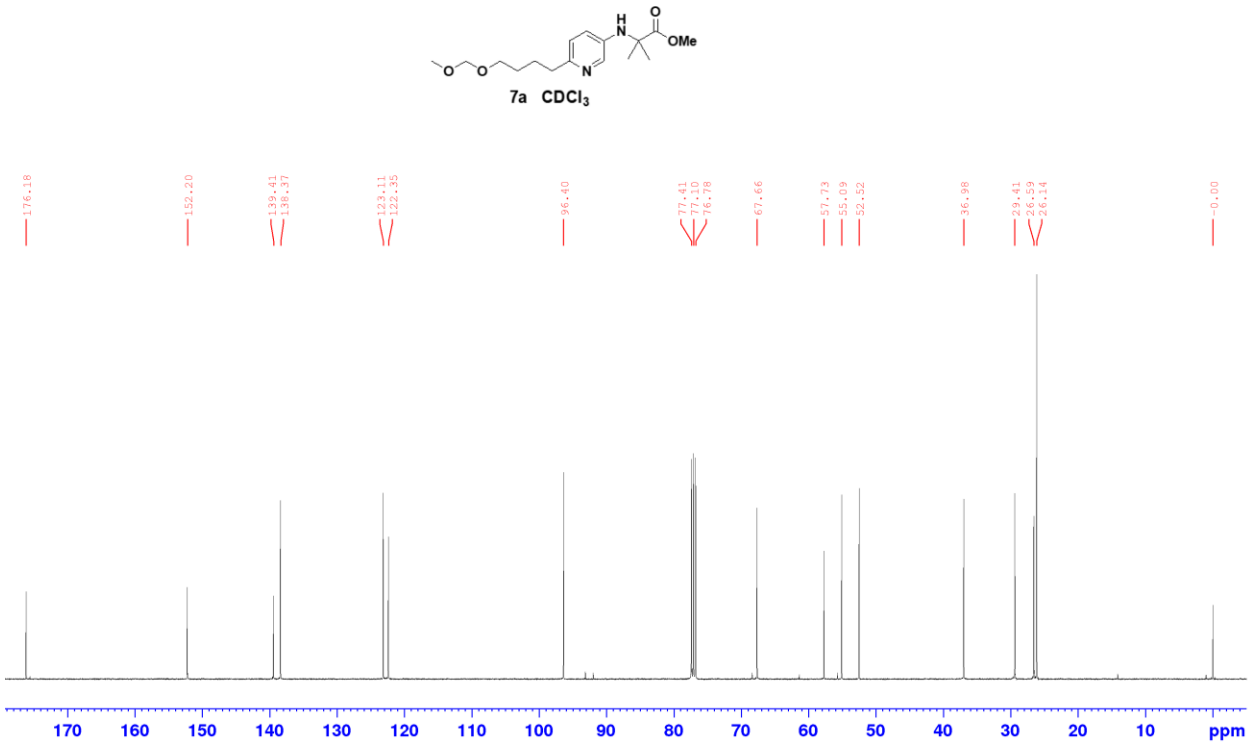
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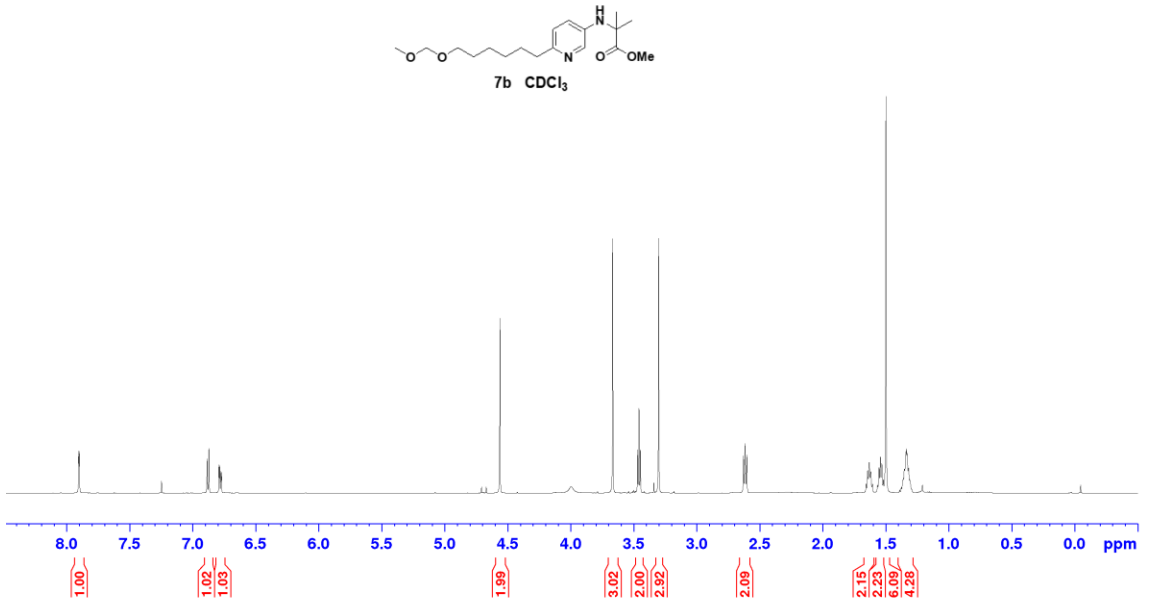
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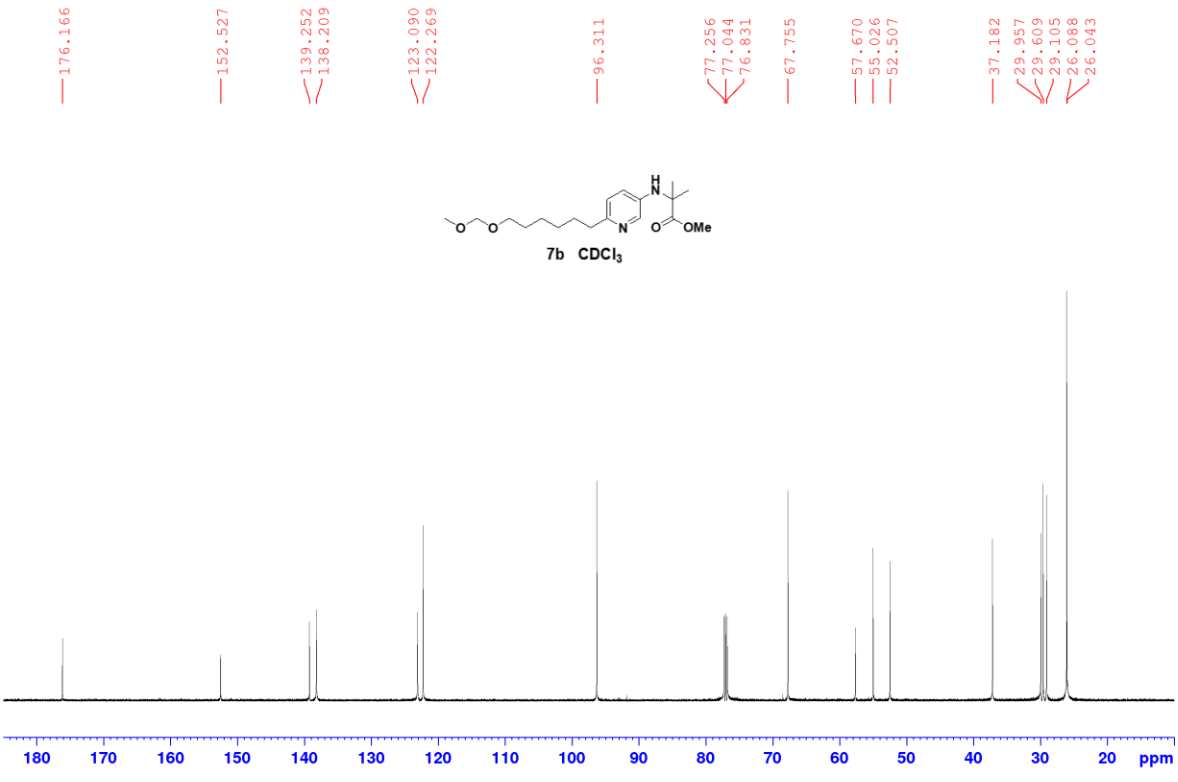
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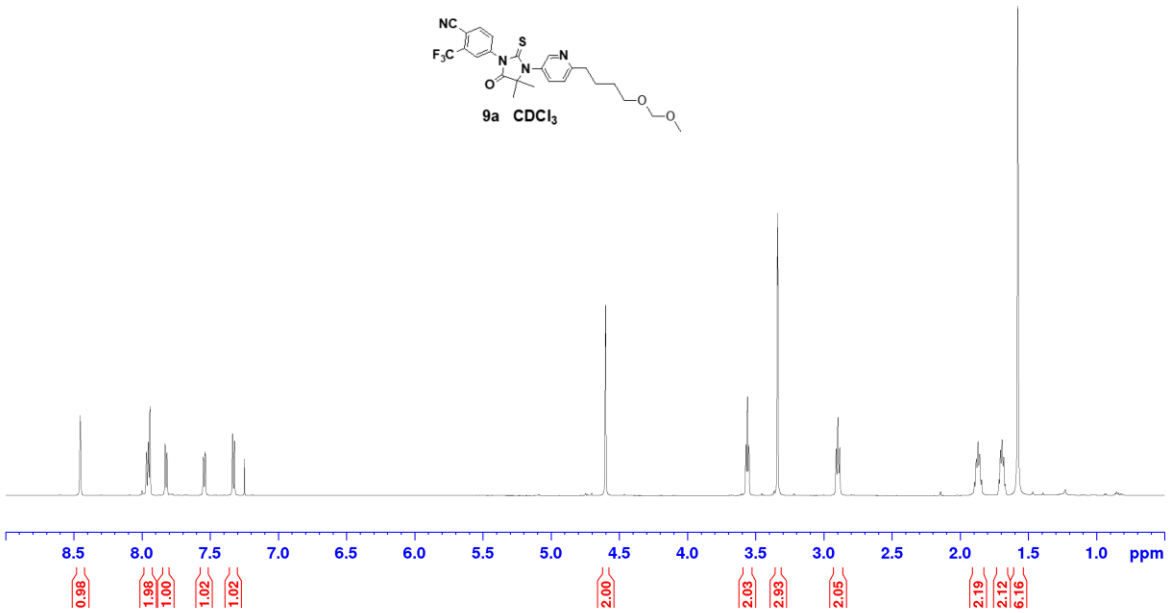
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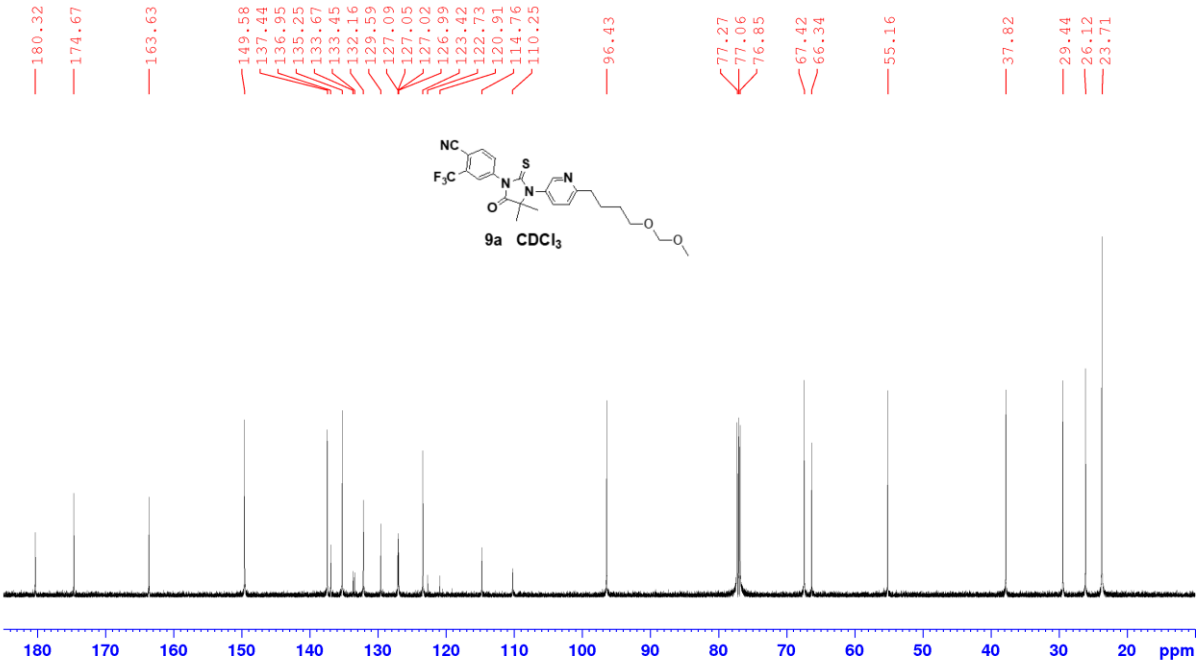
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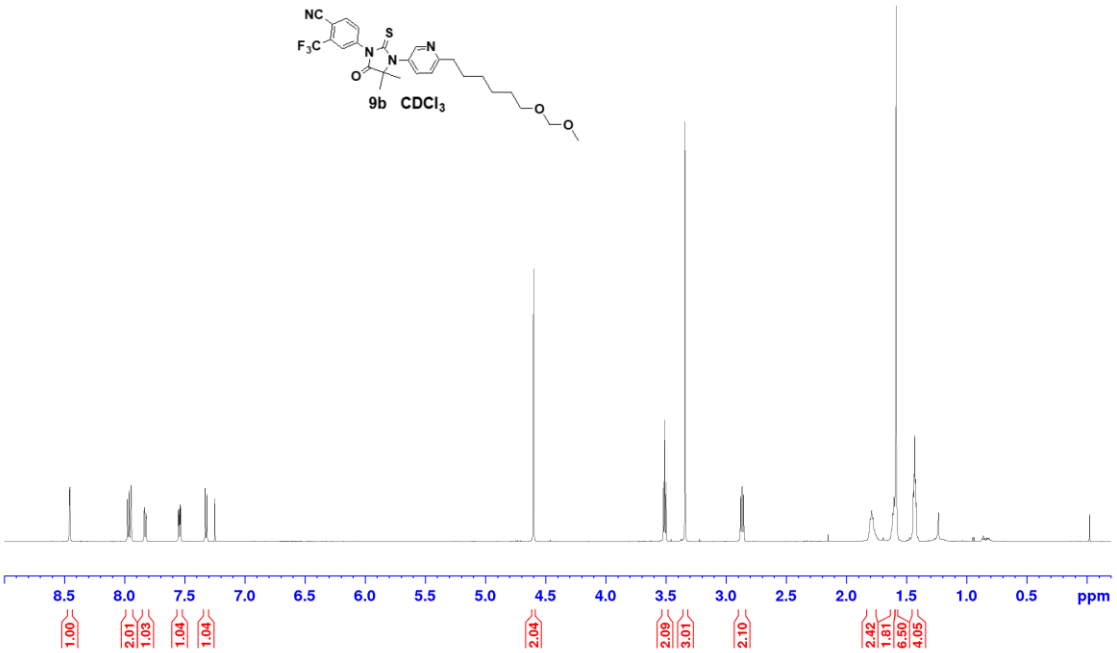
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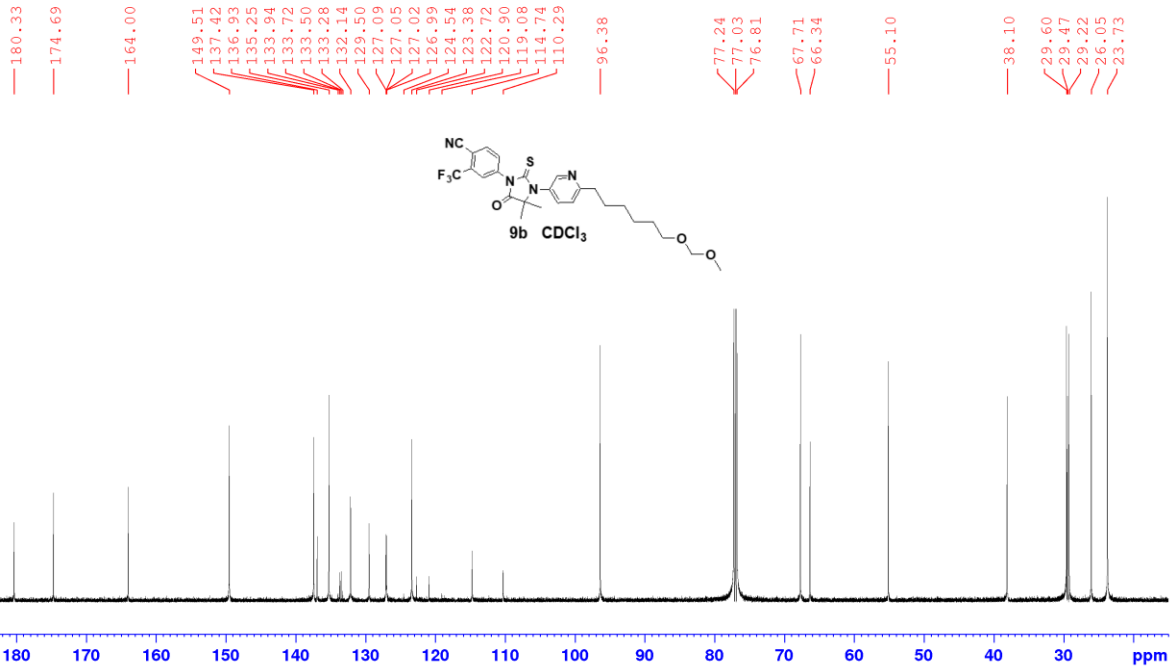
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77.24
77.03
76.81
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66.34
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F3C
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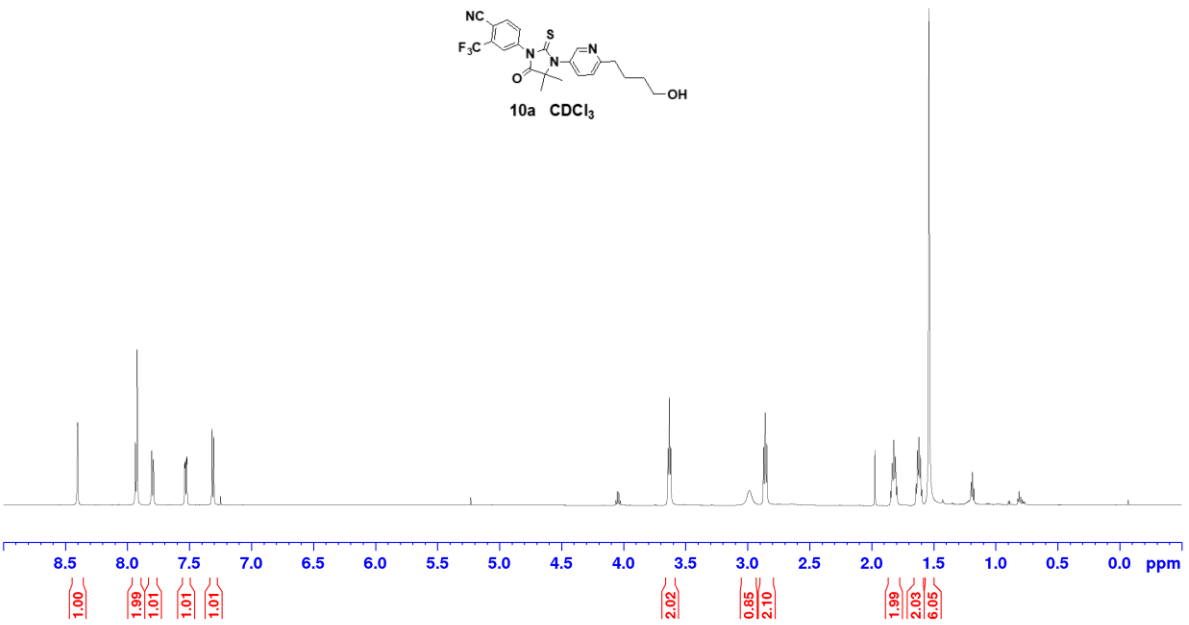
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5.0
4.5
4.0
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2.0
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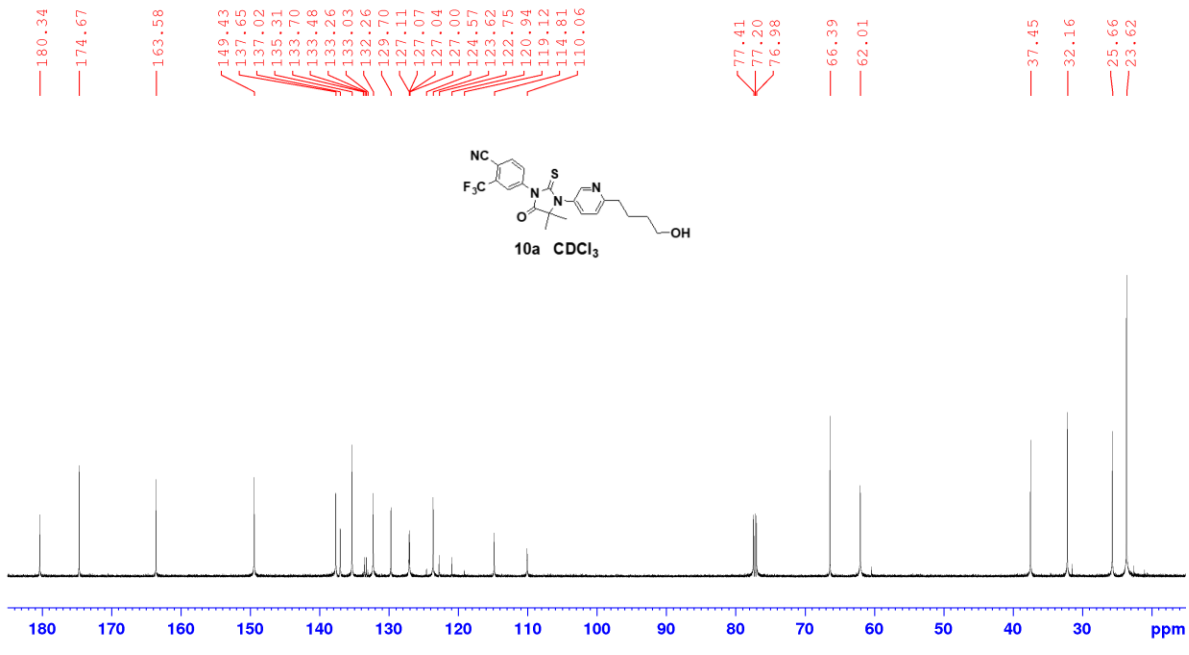
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F3C
S
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O
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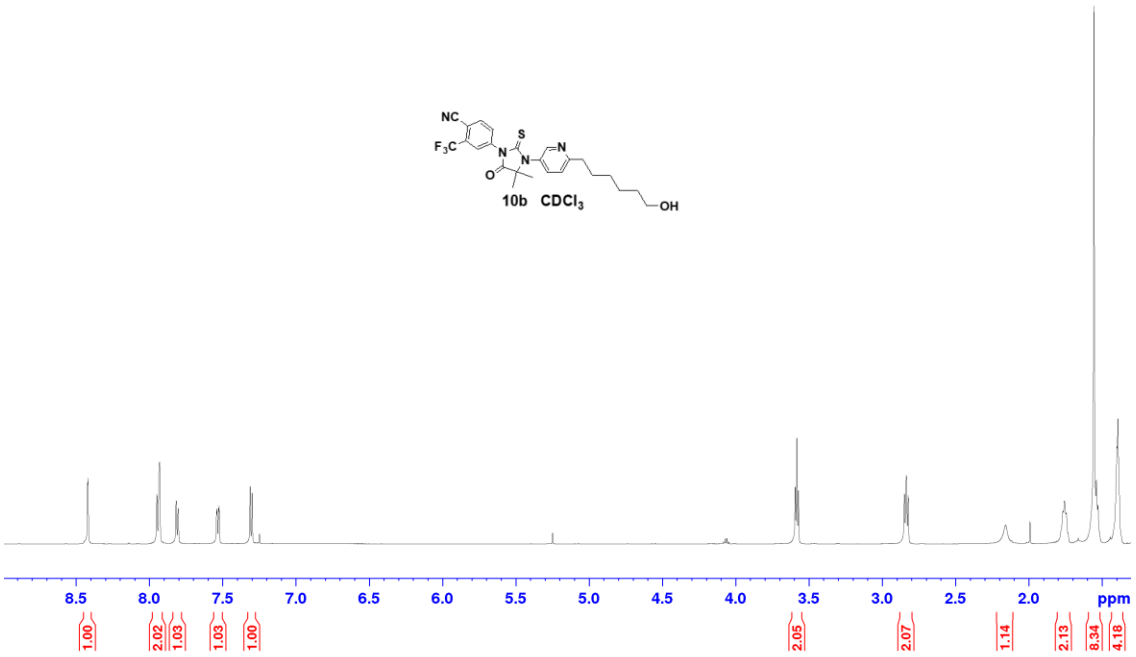
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5.0
4.5
4.0
3.5
3.0
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2.0
ppm
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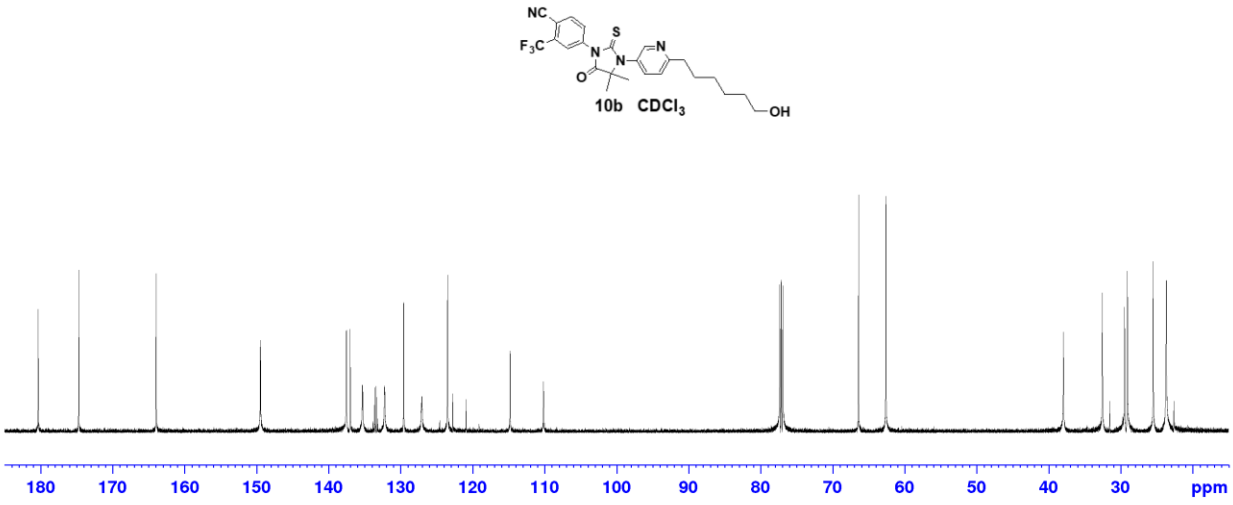
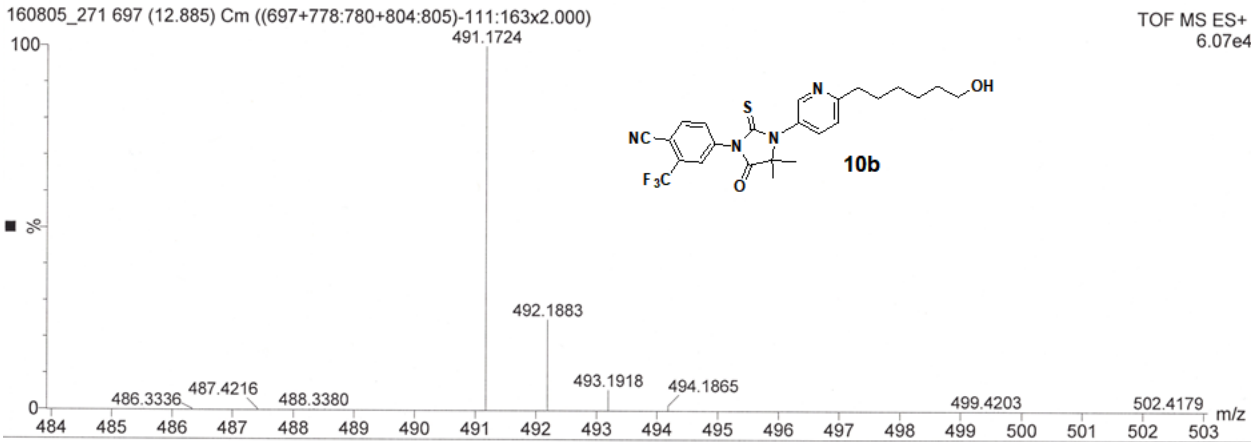
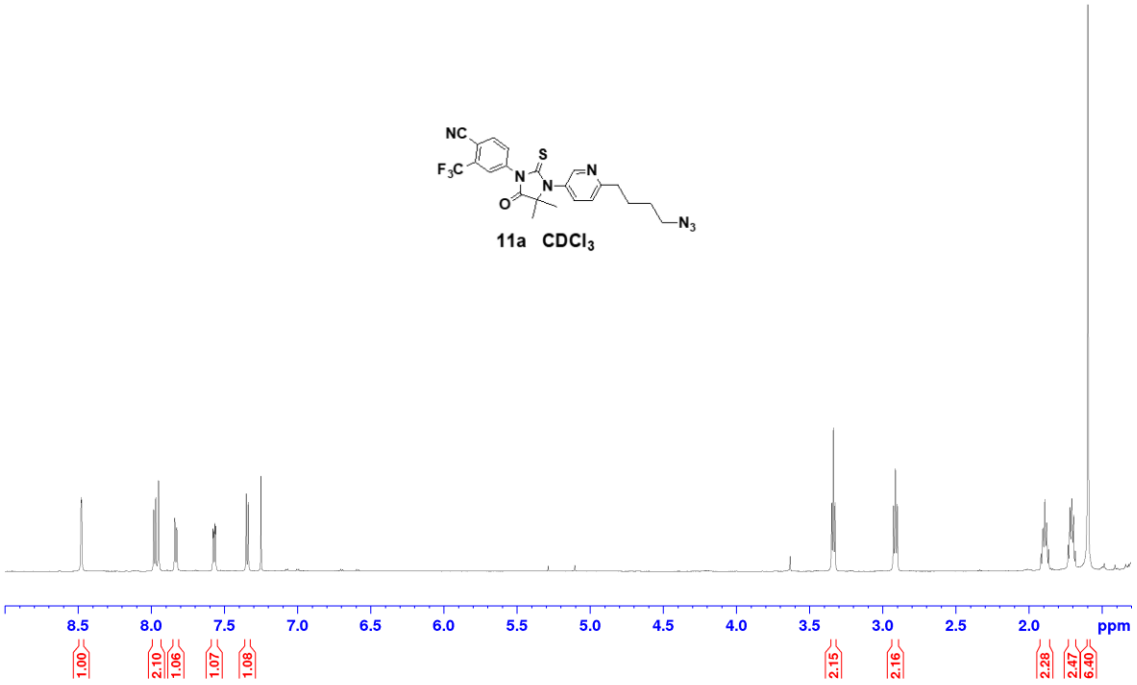
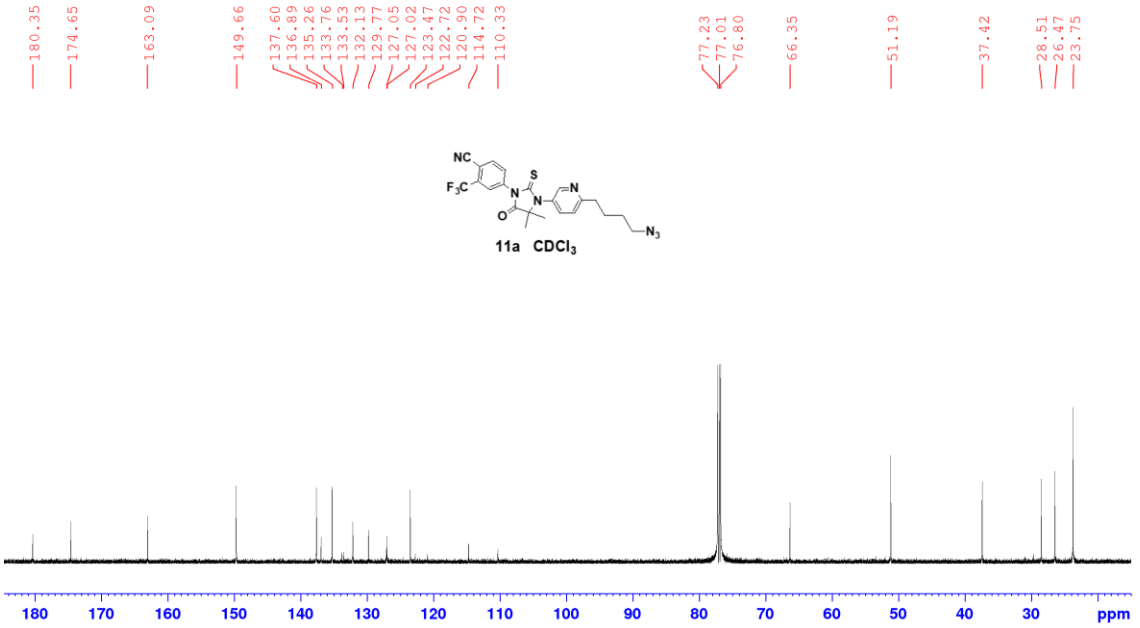
1.03
1.03
1.00
2.05
2.07
1.14
2.13
8.34
4.18

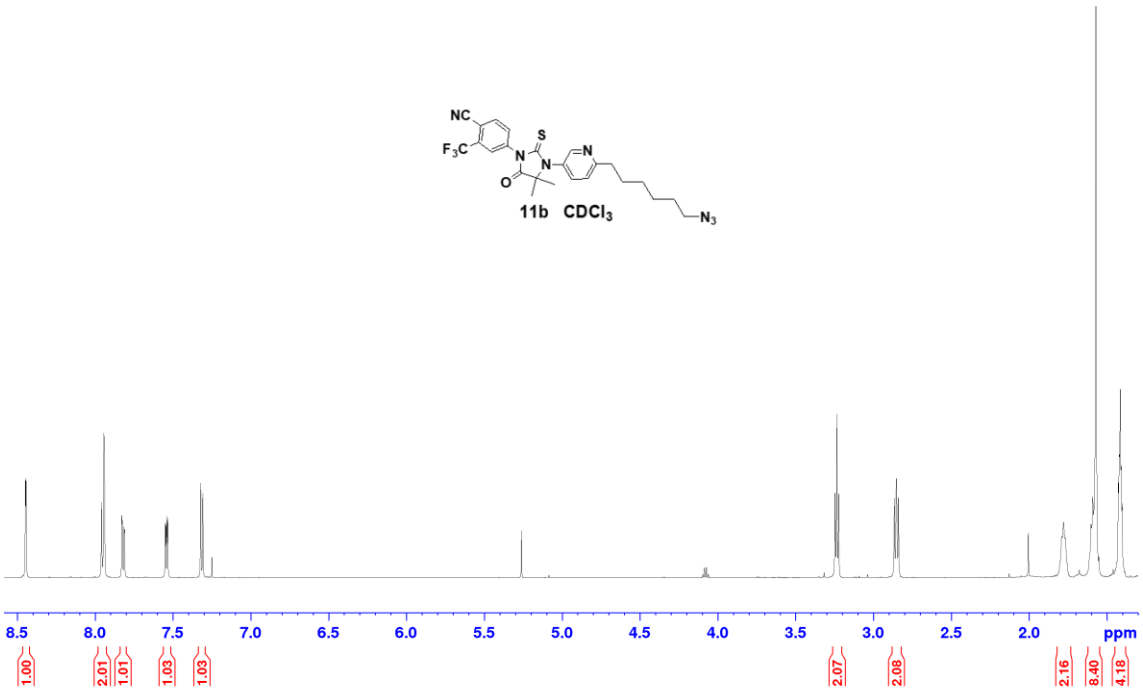

180.33
174.69
163.93
149.48
149.43
137.51
136.99
135.31
133.81
133.58
133.36
133.14
132.19
129.55
127.10
127.07
127.04
127.00
124.56
123.46
122.74
120.92
119.10
114.78
110.16
77.33
77.12
76.90
66.37
62.59
37.94
32.52
29.44
29.04
25.46
23.68
23.64

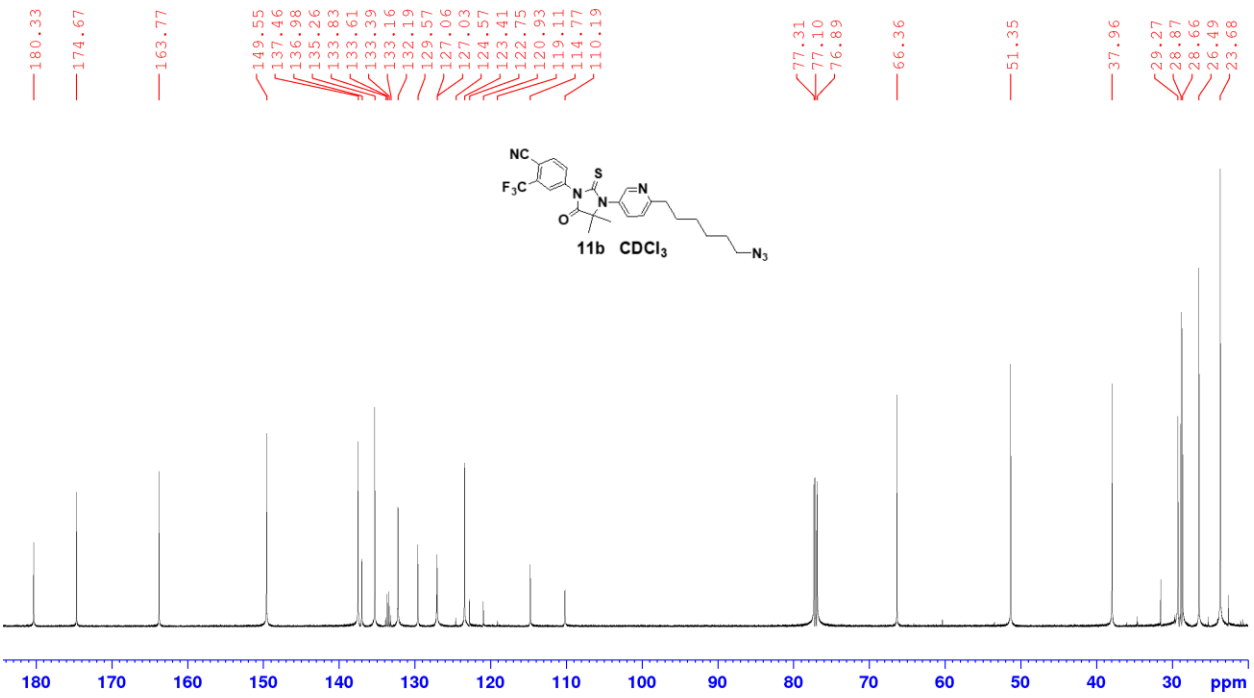

NC
F3C
S
N
N
O
OH
10b CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

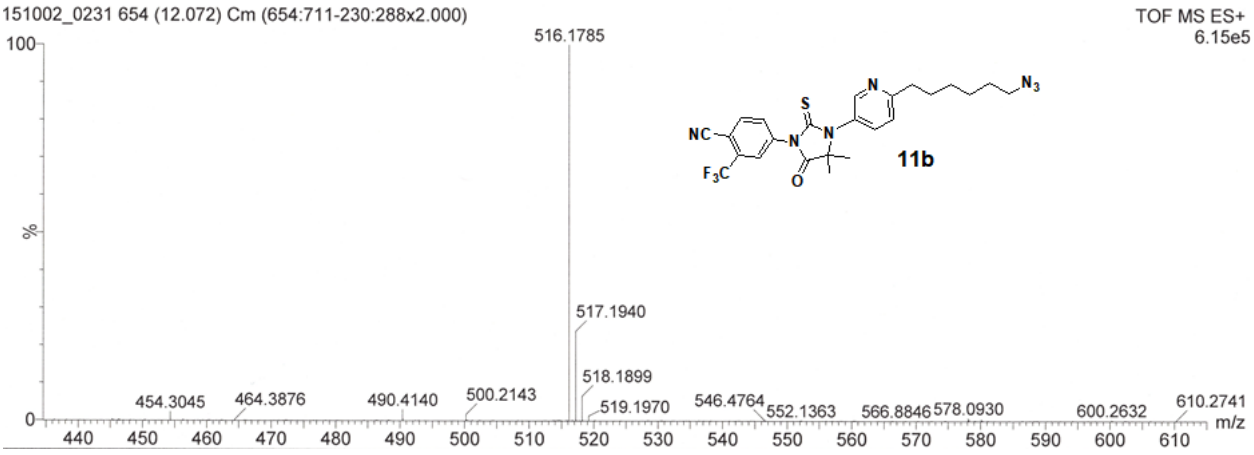

160805_271 697 (12.885) Cm ((697+778:780+804:805)-111:163x2.000)
TOF MS ES+
6.07e4
100
%
0
491.1724
492.1883
493.1918
494.1865
486.3336
487.4216
488.3380
499.4203
502.4179
m/z
484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503
NC
F3C
S
N
O
OH
10b

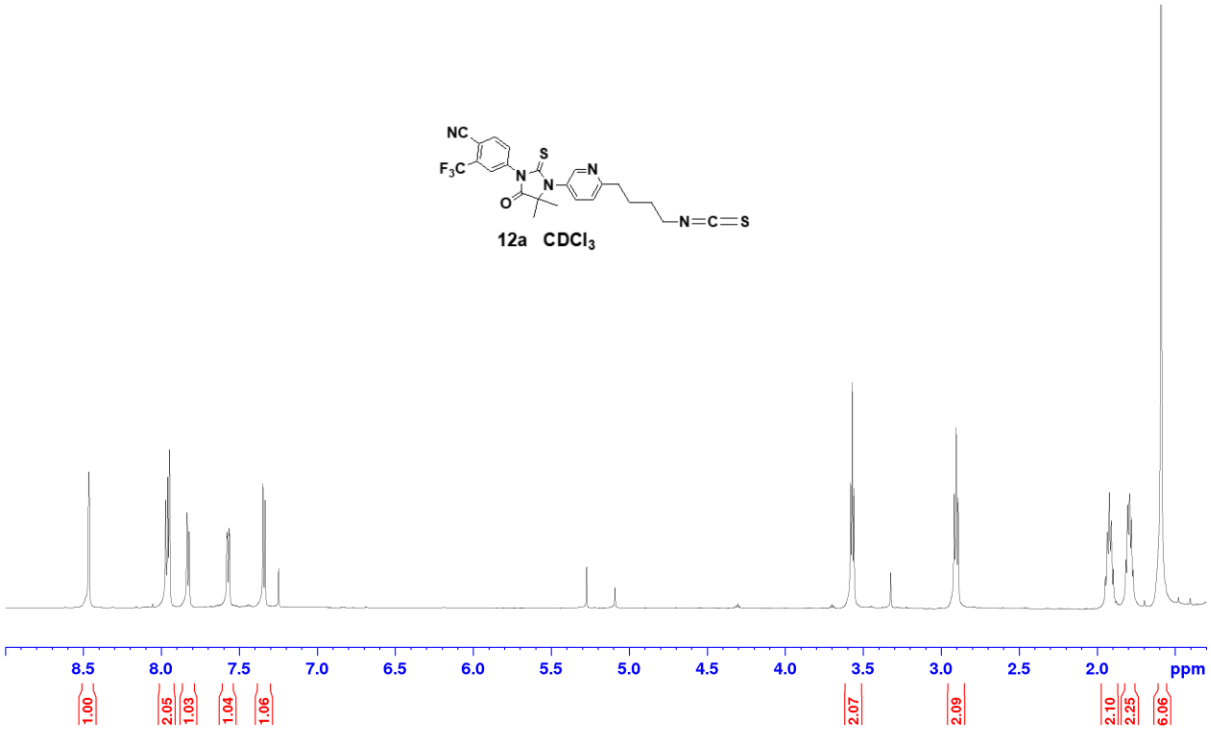

NC
F3C
S
N
O
N3
11a CDCl3
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
1.00
2.10
1.06
1.07
1.08
2.15
2.16
2.28
2.47
6.40

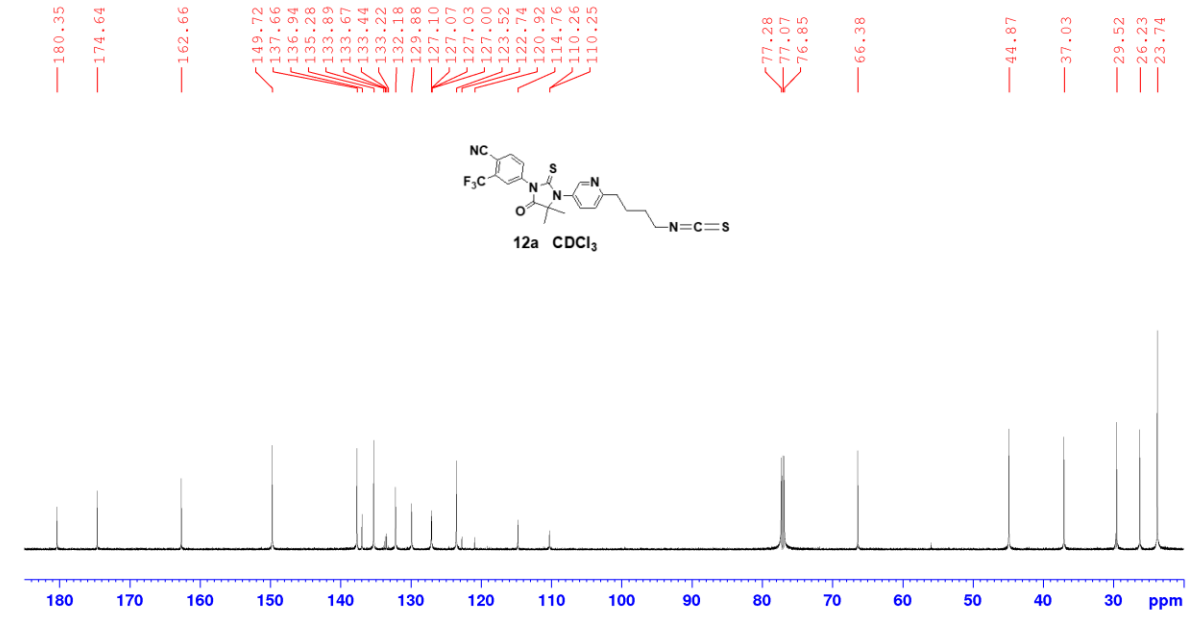

180.35
174.65
163.09
149.66
137.60
136.89
135.26
133.76
133.53
132.13
129.77
127.05
127.02
123.47
122.72
120.90
114.72
110.33
77.23
77.01
76.80
66.35
51.19
37.42
28.51
26.47
23.75
NC
F3C
S
N
O
N3
11a CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

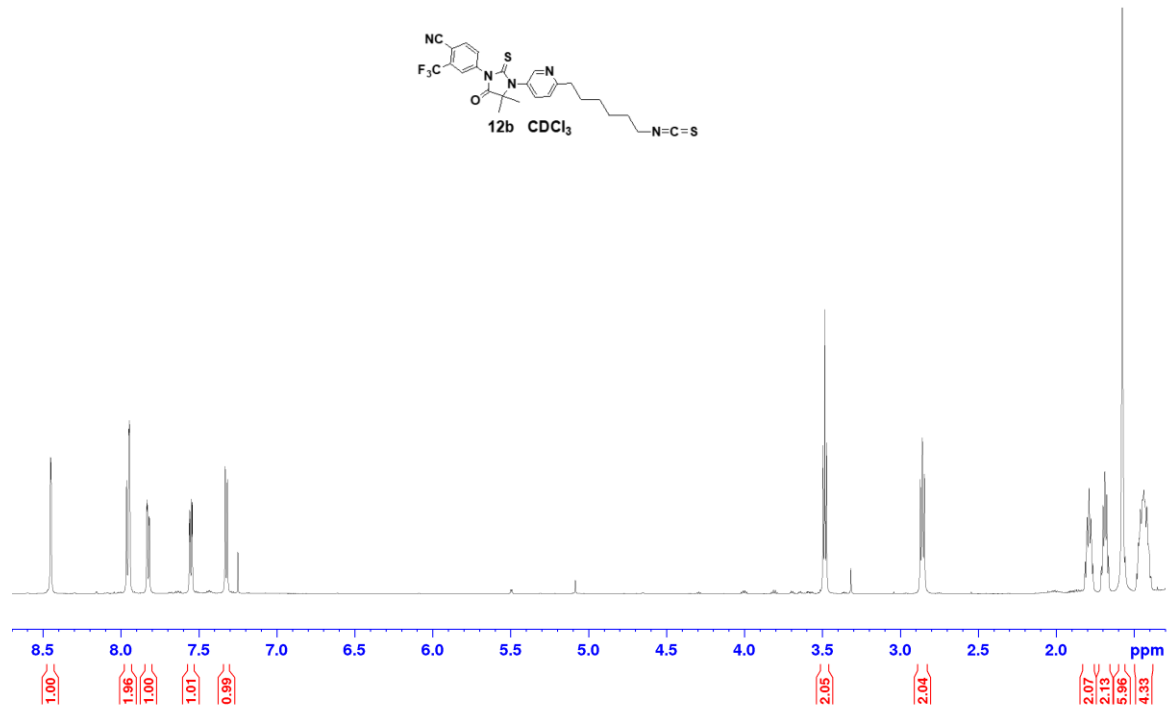

NC
F3C
S
N
O
N3
11b CDCl3
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
2.01
1.01
1.03
1.03
2.07
2.08
2.16
8.40
4.18

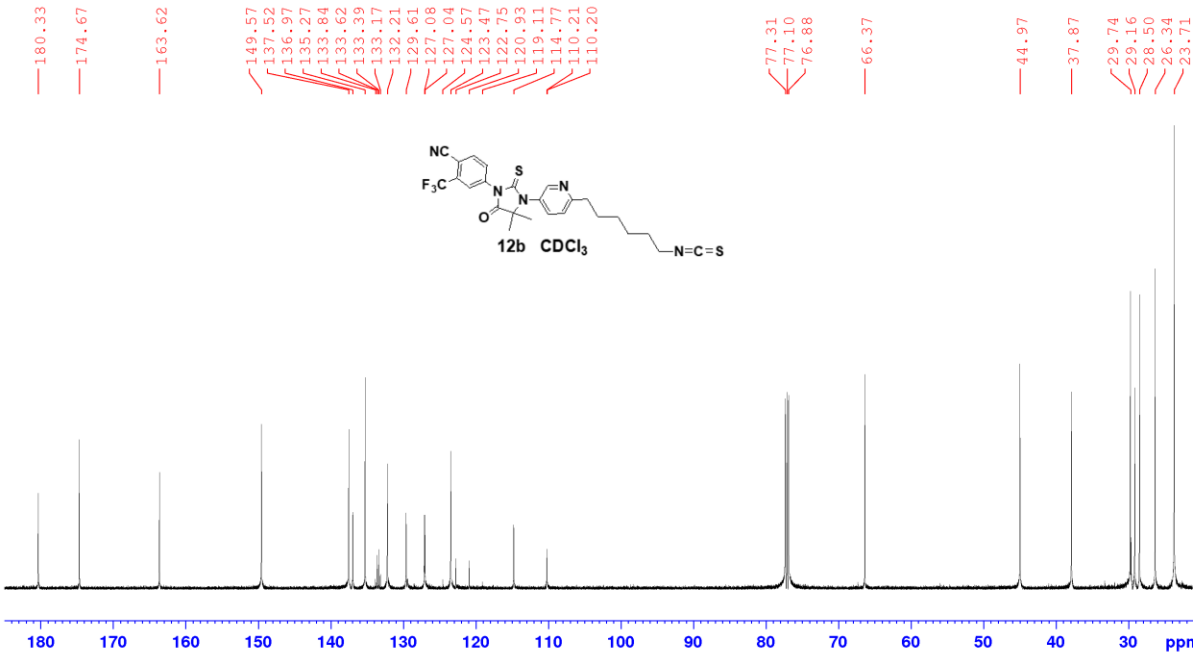

180.33
174.67
163.77
149.55
137.46
136.98
135.26
133.83
133.61
133.39
133.16
132.19
129.57
127.06
127.03
124.57
123.41
122.75
120.93
119.11
114.77
110.19
77.31
77.10
76.89
66.36
51.35
37.96
29.27
28.87
28.66
26.49
23.68
11b CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

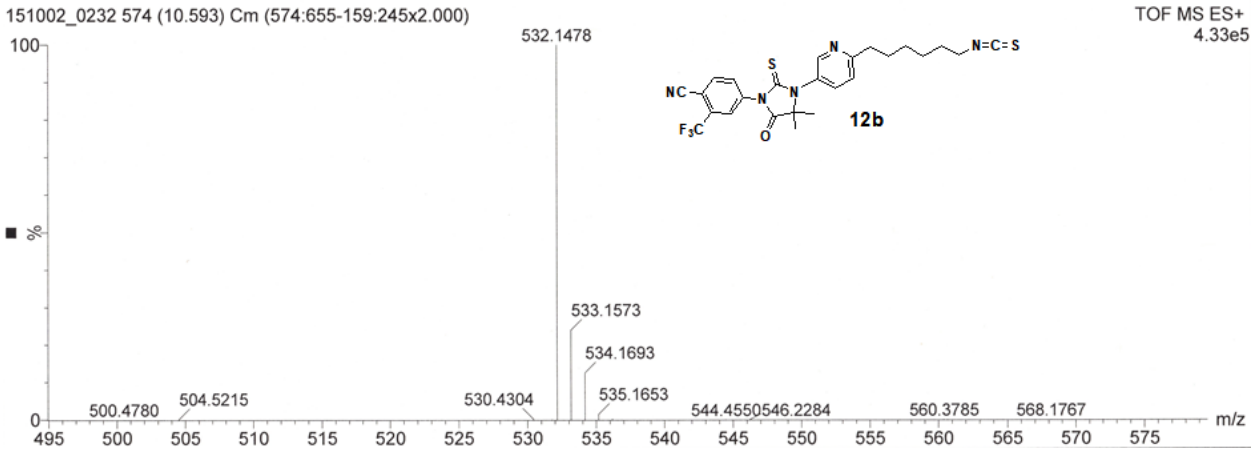

151002_0231 654 (12.072) Cm (654:711-230:288x2.000)
TOF MS ES+
6.15e5
100
%
0
516.1785
517.1940
518.1899
519.1970
454.3045
464.3876
490.4140
500.2143
546.4764
552.1363
566.8846
578.0930
600.2632
610.2741
m/z
11b

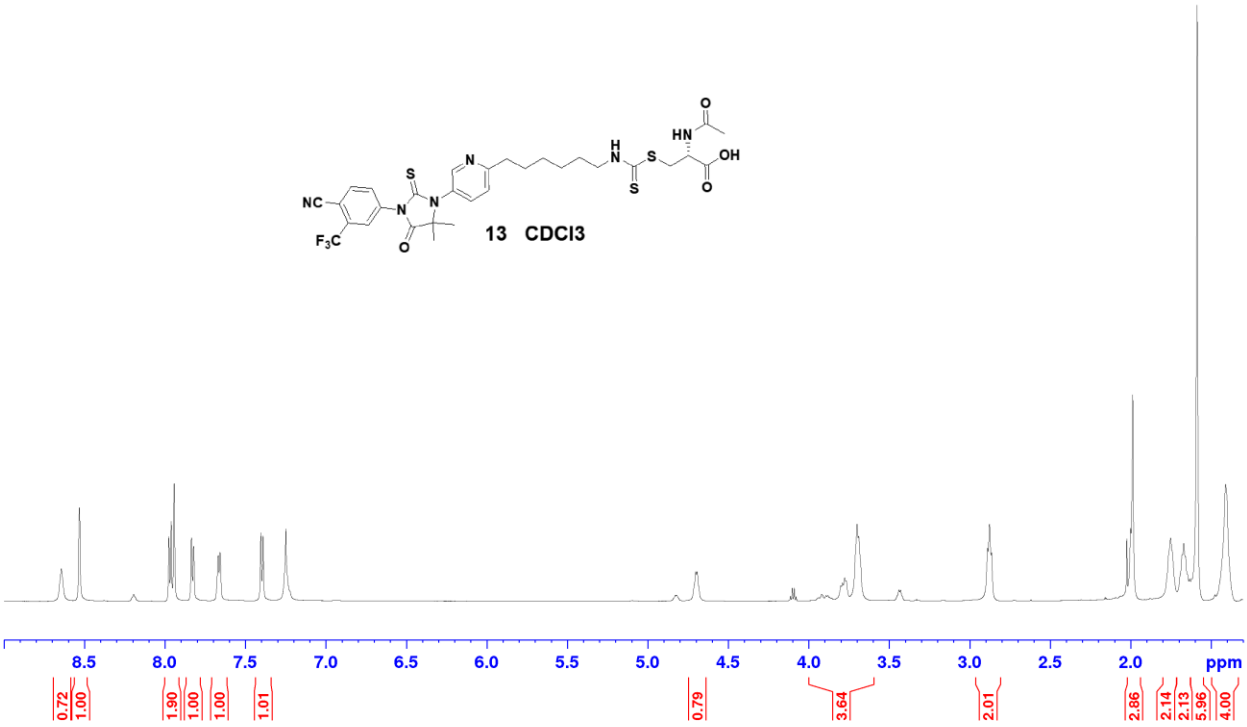

NC
F3C
S
N
N
O
N
N=C=S
12a CDCl3
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
2.05
1.03
1.04
1.06
2.07
2.09
2.10
2.25
6.06

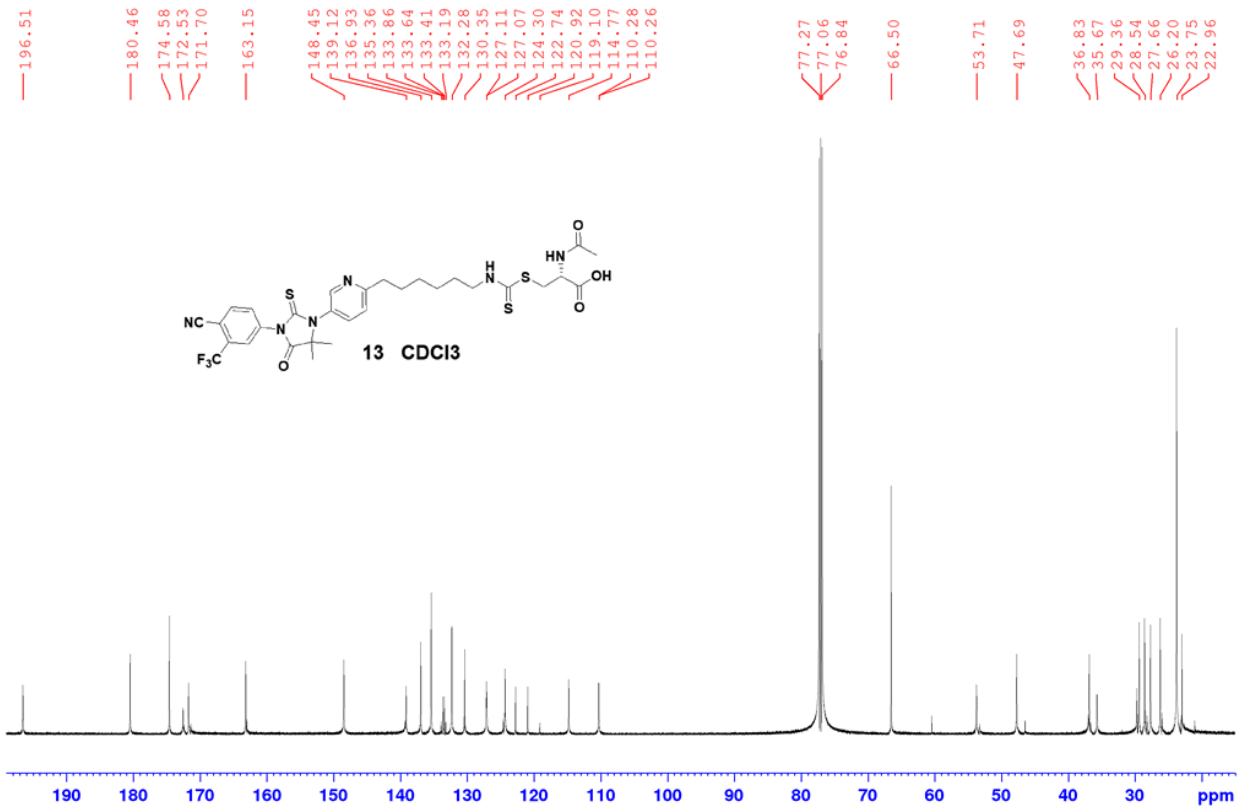

180.35
174.64
162.66
149.72
137.66
136.94
135.28
133.89
133.67
133.44
133.22
132.18
129.88
127.10
127.07
127.03
127.00
123.52
122.74
120.92
114.76
110.26
110.25
77.28
77.07
76.85
66.38
44.87
37.03
29.52
26.23
23.74
NC
F3C
S
N
N
O
N
N=C=S
12a CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

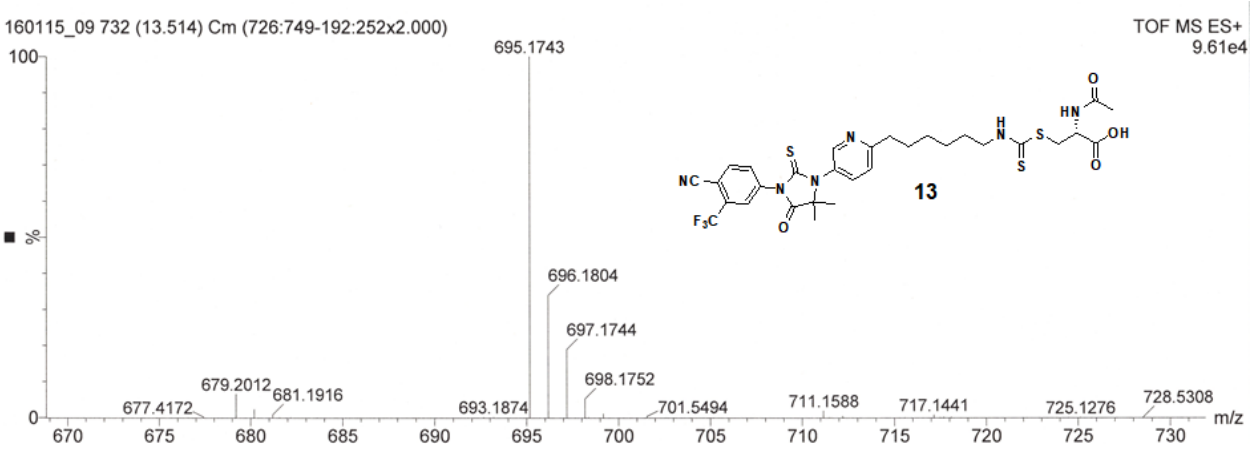

12b CDCl3
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
1.96
1.00
1.01
0.99
2.05
2.04
2.07
2.13
5.96
4.33


180.33
174.67
163.62
149.57
137.52
136.97
135.27
133.84
133.62
133.39
133.17
132.21
129.61
127.08
127.04
124.57
123.47
122.75
120.93
119.11
114.77
110.21
110.20
77.31
77.10
76.88
66.37
44.97
37.87
29.74
29.16
28.50
26.34
23.71
12b CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm


151002_0232 574 (10.593) Cm (574:655-159:245x2.000)
TOF MS ES+
4.33e5
100
%
0
532.1478
533.1573
534.1693
535.1653
500.4780
504.5215
530.4304
544.4550546.2284
560.3785
568.1767
m/z
495
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
12b


13 CDCl3
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
ppm
0.72
1.00
1.90
1.00
1.00
1.01
0.79
3.64
2.01
2.86
2.14
2.13
5.96
4.00


196.51
180.46
174.58
172.53
171.70
163.15
148.45
139.12
136.93
135.36
133.86
133.64
133.41
133.19
132.28
130.35
127.11
127.07
124.30
122.74
120.92
119.10
114.77
110.28
110.26
77.27
77.06
76.84
66.50
53.71
47.69
36.83
35.67
29.36
28.54
27.66
26.20
23.75
22.96
13 CDCl3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm


160115_09 732 (13.514) Cm (726:749-192:252x2.000)
TOF MS ES+
9.61e4
100
%
0
695.1743
696.1804
697.1744
698.1752
679.2012
681.1916
677.4172
693.1874
701.5494
711.1588
717.1441
725.1276
728.5308
13
670
675
680
685
690
695
700
705
710
715
720
725
730
m/z