Electronic Supplementary Material (ESI) for Chemical Science.
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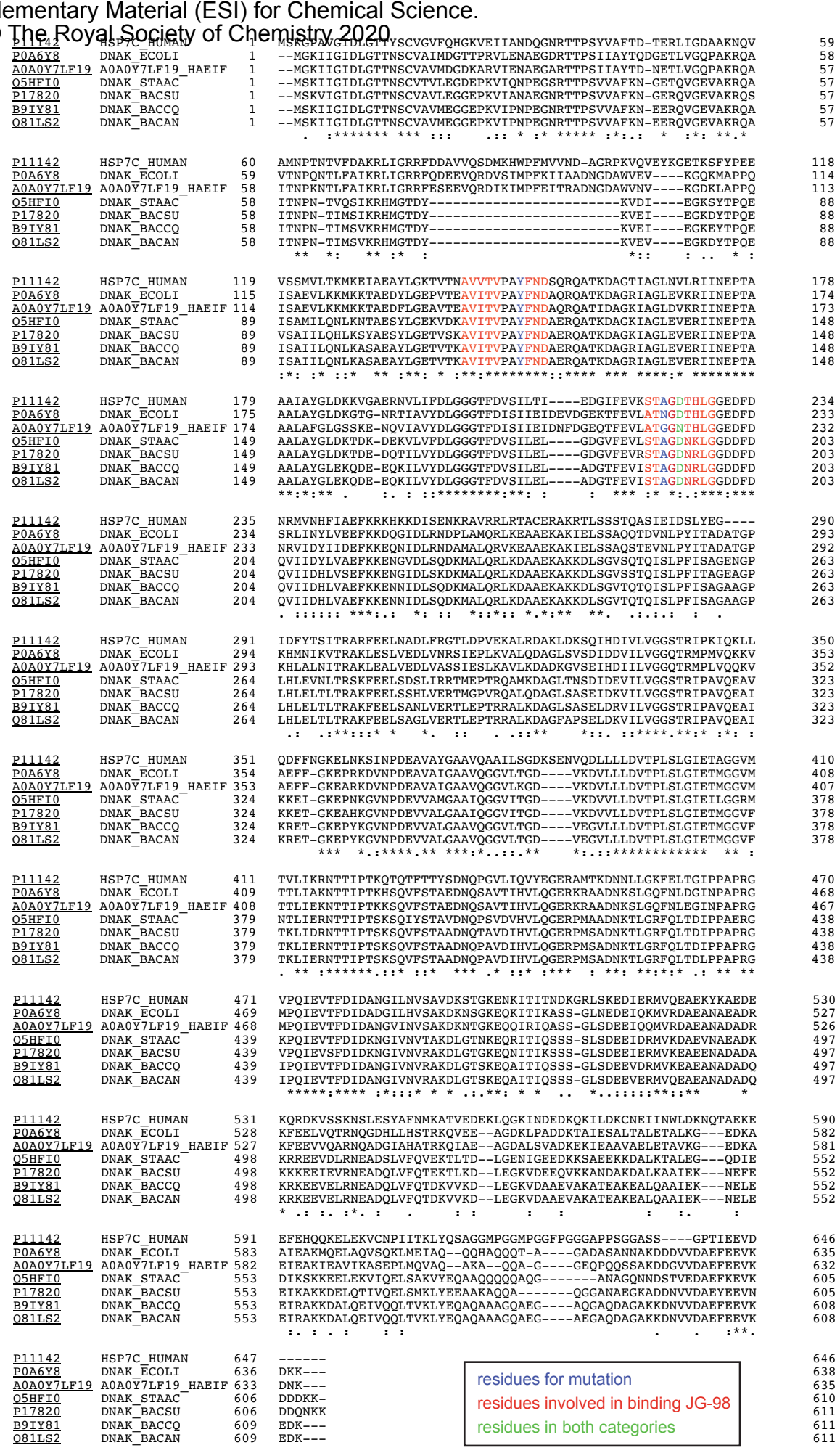
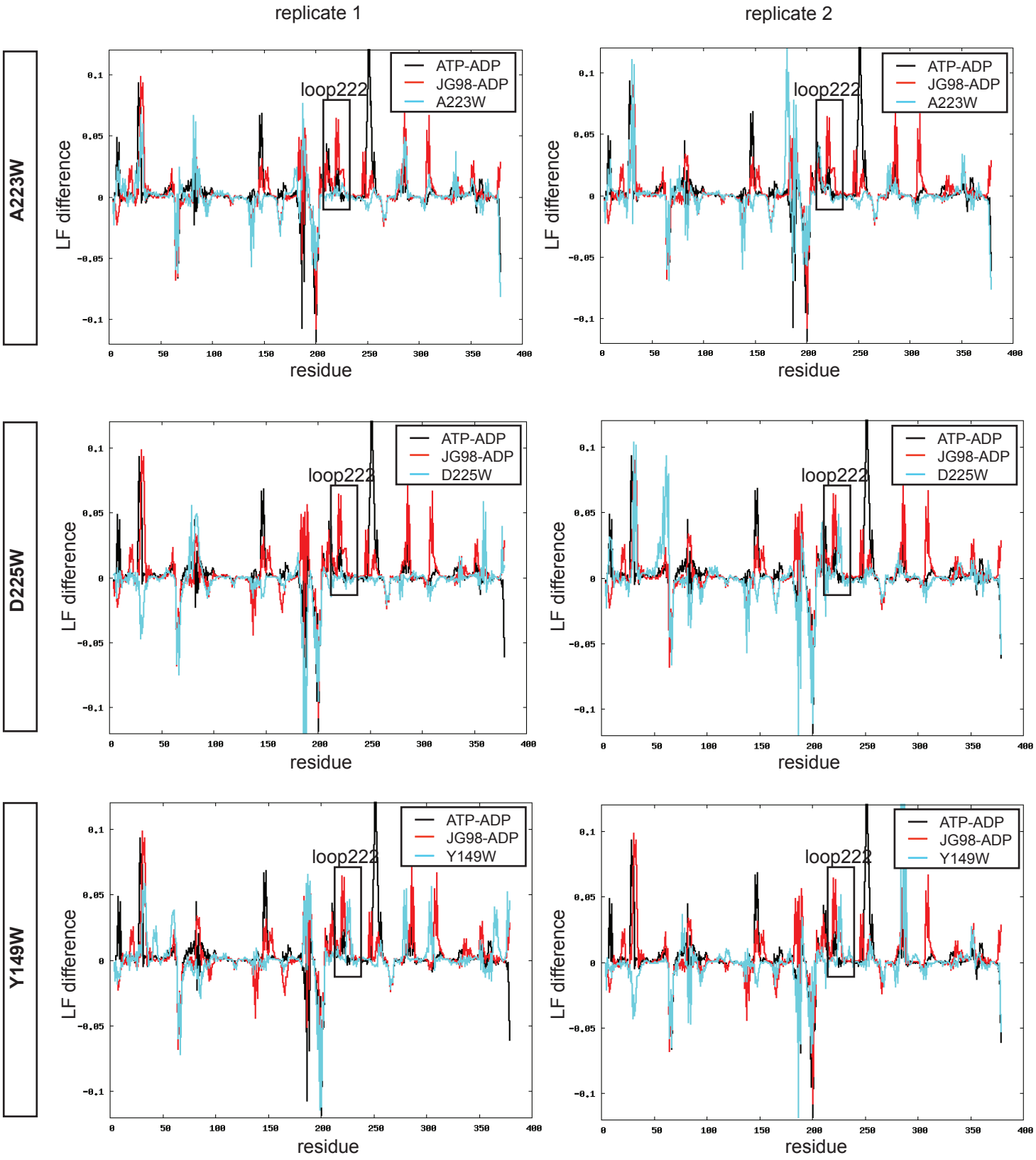
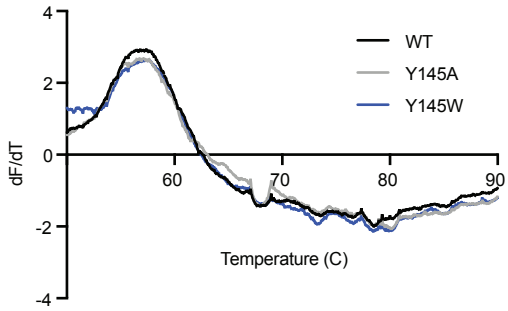


Supplemental Figure 1. Alignment of Hsp70 family members from humans (Hsc70, HSPA8) and multiple prokaryotes (DnaKs). The conserved residues that contact JG-98 are highlighted. Also, the location of Y149/Y145 and other mutated sites are shown.



Supplemental Figure 2. Y148W, but not A223W or D225W, partially mirrors the motions in the NBD that accompany the ADP-bound or compound-bound states. Two replicates are shown and the position of loop 222 (see Fig 1) is highlighted.

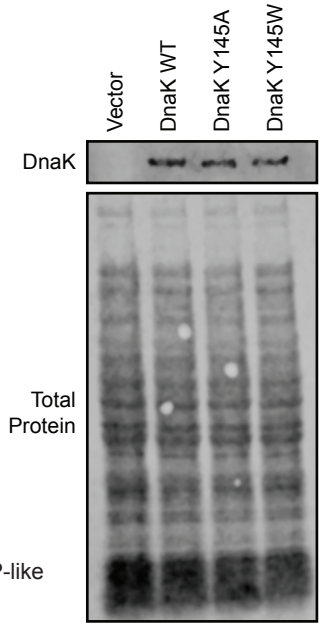
(A) DnaK mutants are properly folded, based on DSF



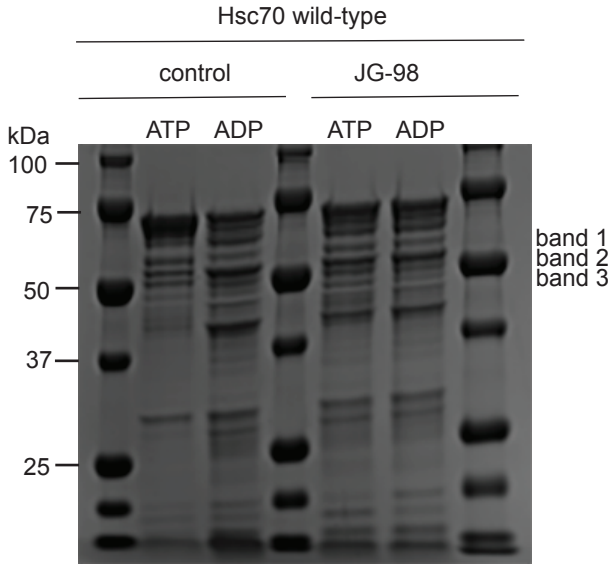
(B) ATPase activity of DnaK Y145W is relatively resistant to JG-48

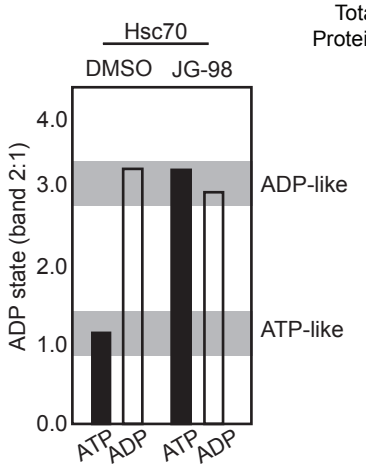
DnaK	JG-48 inhibition of $Vmax_J$ (%)
WT	35%
Y145W	8%

(D) DnaK mutants are expressed at similar levels



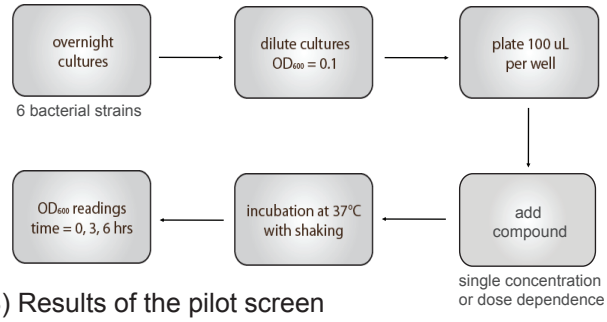
(C) JG-98 stabilizes the ADP-like conformer of Hsc70



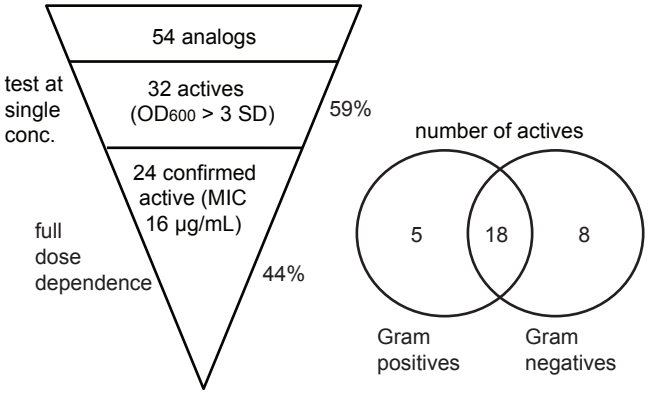


Supplemental Figure 3. Controls for partial proteolysis and bacterial complementation experiments. (A) Differential scanning fluorimetry results on DnaK and its mutants show that the proteins are properly folded (Tm values ~ 58 °C). Results are the average of experiments performed in triplicate. (B) ATPase activity of WT DnaK and Y145W DnaK was measured in the presence of DnaJ and JG-48. The Trp mutant was relatively resistant to the inhibitor, as expected. (C) Partial proteolysis experiments showing that JG-98 (10 μM) is able to stabilize the ADP-like state, even if ATP (1 mM) is added. The quantification is the result of duplicate experiments and is the ratio of band intensity from band 2:1. Band intensities were determined using Image J. (D) Arabinose- mediated induction of DnaK and its mutants in the Δ*dnaK E. coli* strain, showing similar expression levels.

(A) Workplan for MIC measurements



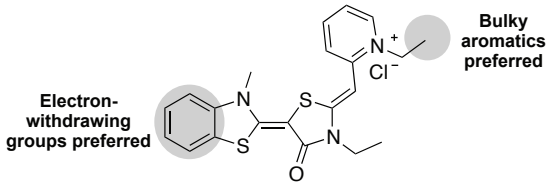
(B) Results of the pilot screen



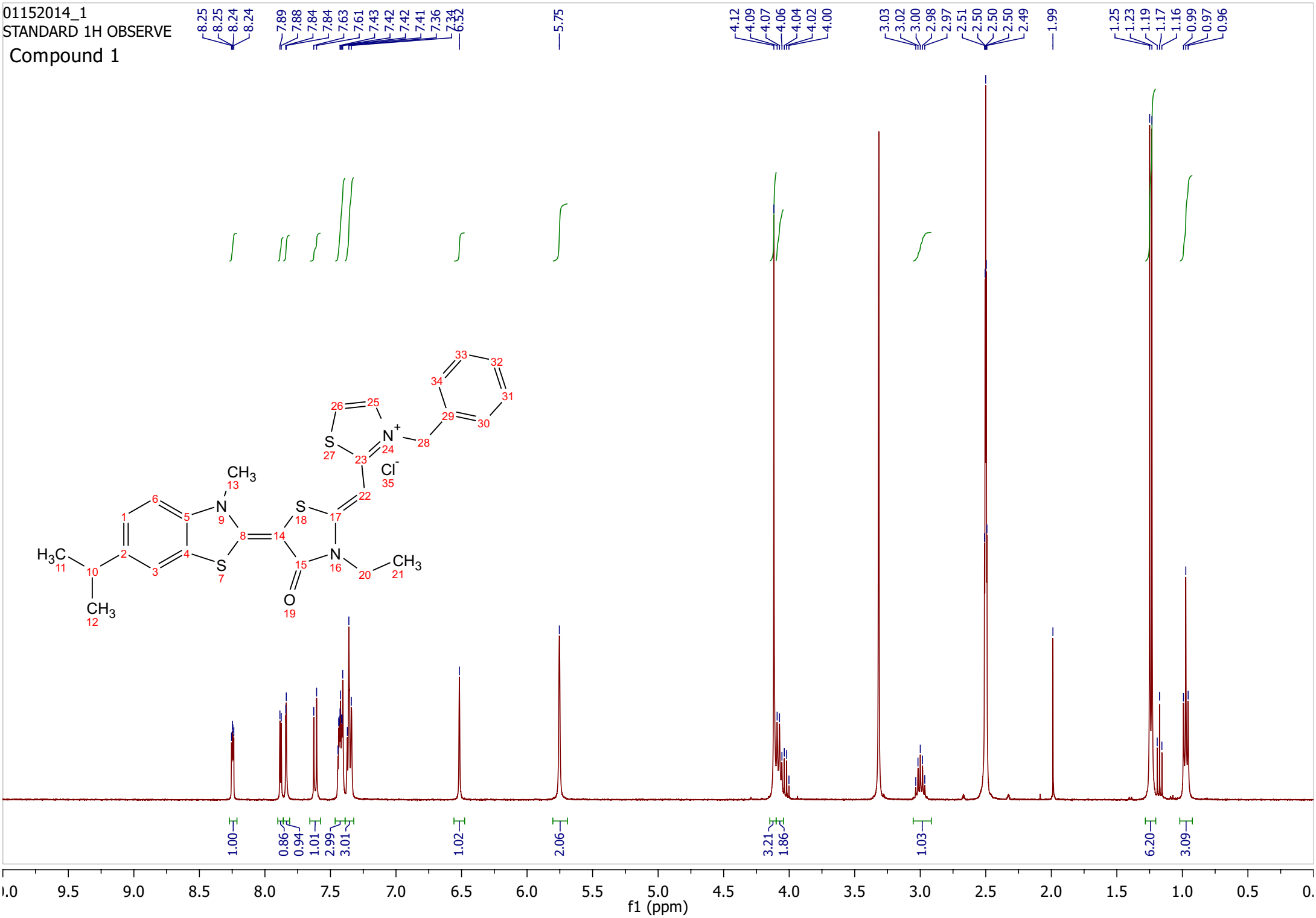
(C) Confirmed actives (MIC values < 16 µg/mL)

analog	*B. subtilis*	*B. anthracis*	*B. cereus*	*S. aureus*	*E. coli*	*H. influenza*
MKT077	---	---	>16	>16	---	16
YM1	---	>16	---	>16	---	16
YM9	---	---	---	---	---	>16
YM21	---	---	---	---	---	>16
JG12	>16	>16	---	>16	---	16
JG18	---	>16	---	---	---	>16
JG31	---	>16	---	---	---	16
JG33	>16	16	16	16	---	8
JG35	---	>16	---	16	---	16
JG37	---	---	---	---	---	>16
JG38	---	>16	>16	---	---	16
JG41	---	16	>16	>16	---	8
JG43	16	8	8	8	---	8
JG58	---	---	---	---	---	---
JG60	---	>16	>16	>16	---	>16
JG61	---	---	---	---	---	16
JG66	---	>16	---	>16	---	---
JG70	8	8	8	8	---	16
JG71	>16	---	---	4	>16	---
JG73	16	8	16	16	>16	8
JG74	---	8	8	8	>16	4
JG78	---	4	8	4	---	8
JG81	>16	>16	>16	>16	---	16
JG83	8	4	16	8	---	4
JG86	---	16	---	>16	---	8
JG91	>16	16	>16	16	---	4
JG96	>16	8	8	8	---	16
JG97	16	4	8	8	---	16
JG98	2	---	---	---	---	---
JG111	---	16	---	>16	---	---
JG121	>16	>16	---	---	---	16
JG123	---	---	---	---	---	>16
tetracycline	2	0.25	0.5	1	4	1
DMSO	>16	>16	>16	>16	>16	>16

(D) Structure-activity relationships from the pilot screen

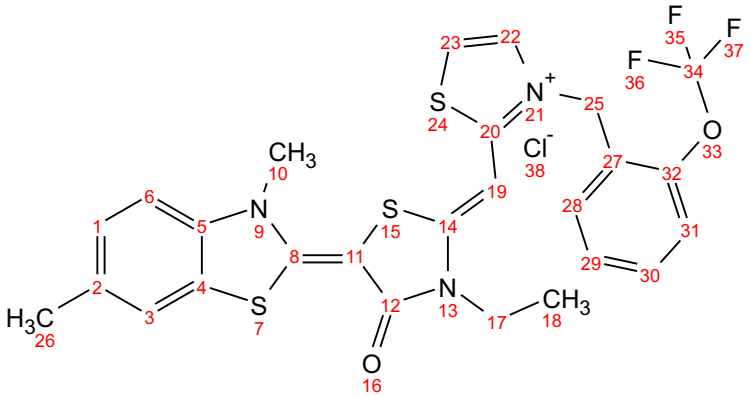


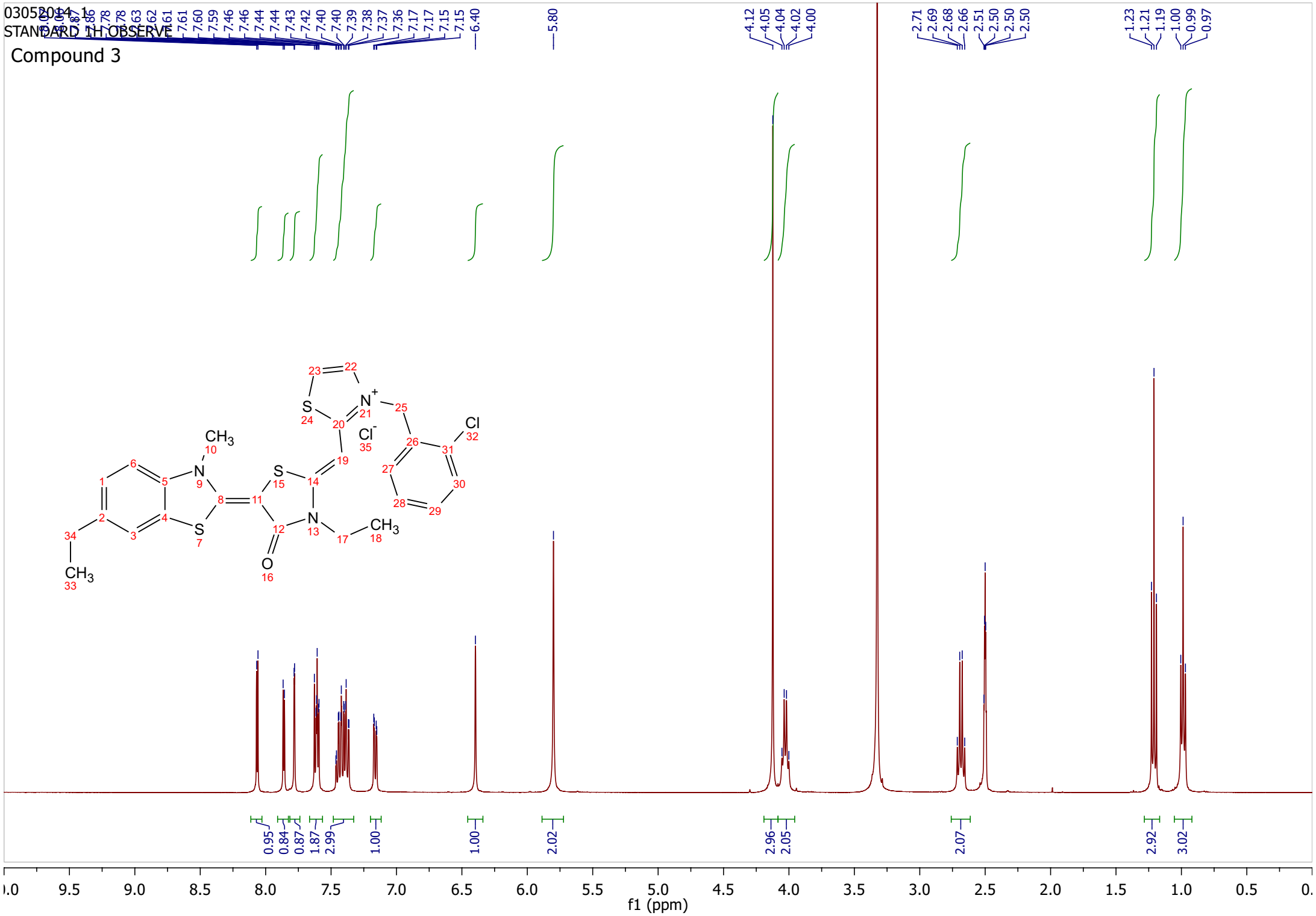
Supplemental Figure 4. Pilot screen of JG-98 analogs against a panel of bacteria. (A) Schematic of the method for determination of anti-bacterial activity. In the pilot screen, 54 compounds were tested at a single concentration (16 µg/mL). If the compound reduced growth by 3SD of the negative control (1% DMSO) for any of the 6 bacterial strains, then it was selected for MIC determination (12 concentrations). Confirmed actives were defined as those with MIC values of 16 µg/mL or better. Compounds that were not active in the initial screen were not retested for dose dependence (---). All pilot screen experiments performed at 37 °C. Compounds are described in Li *et al.* 2013 *ACS Med. Chem. Lett.* 4:1042.


01152014_1
STANDARD 1H OBSERVE
Compound 1
8.25
8.25
8.24
8.24
7.89
7.88
7.84
7.84
7.63
7.61
7.43
7.42
7.42
7.41
7.36
7.34
6.52
5.75
4.12
4.09
4.07
4.06
4.04
4.02
4.00
3.03
3.02
3.00
2.98
2.97
2.51
2.50
2.50
2.50
2.49
1.99
1.25
1.23
1.19
1.17
1.16
0.99
0.97
0.96
1.00
0.86
0.94
1.01
2.99
3.01
1.02
2.06
3.21
1.86
1.03
6.20
3.09
f1 (ppm)

02252014_2
STANDARD 1H OBSERVE

Compound 2

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8.86
7.78
7.78
7.63
7.62
7.61
7.61
7.60
7.59
7.46
7.46
7.44
7.44
7.43
7.42
7.40
7.40
7.39
7.38
7.37
7.36
7.17
7.17
7.15
7.15
6.40
5.80
4.12
4.05
4.04
4.02
4.00
2.71
2.69
2.68
2.66
2.51
2.50
2.50
2.50
1.23
1.21
1.19
1.00
0.99
0.97
0.95
0.84
0.87
1.87
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1.00
1.00
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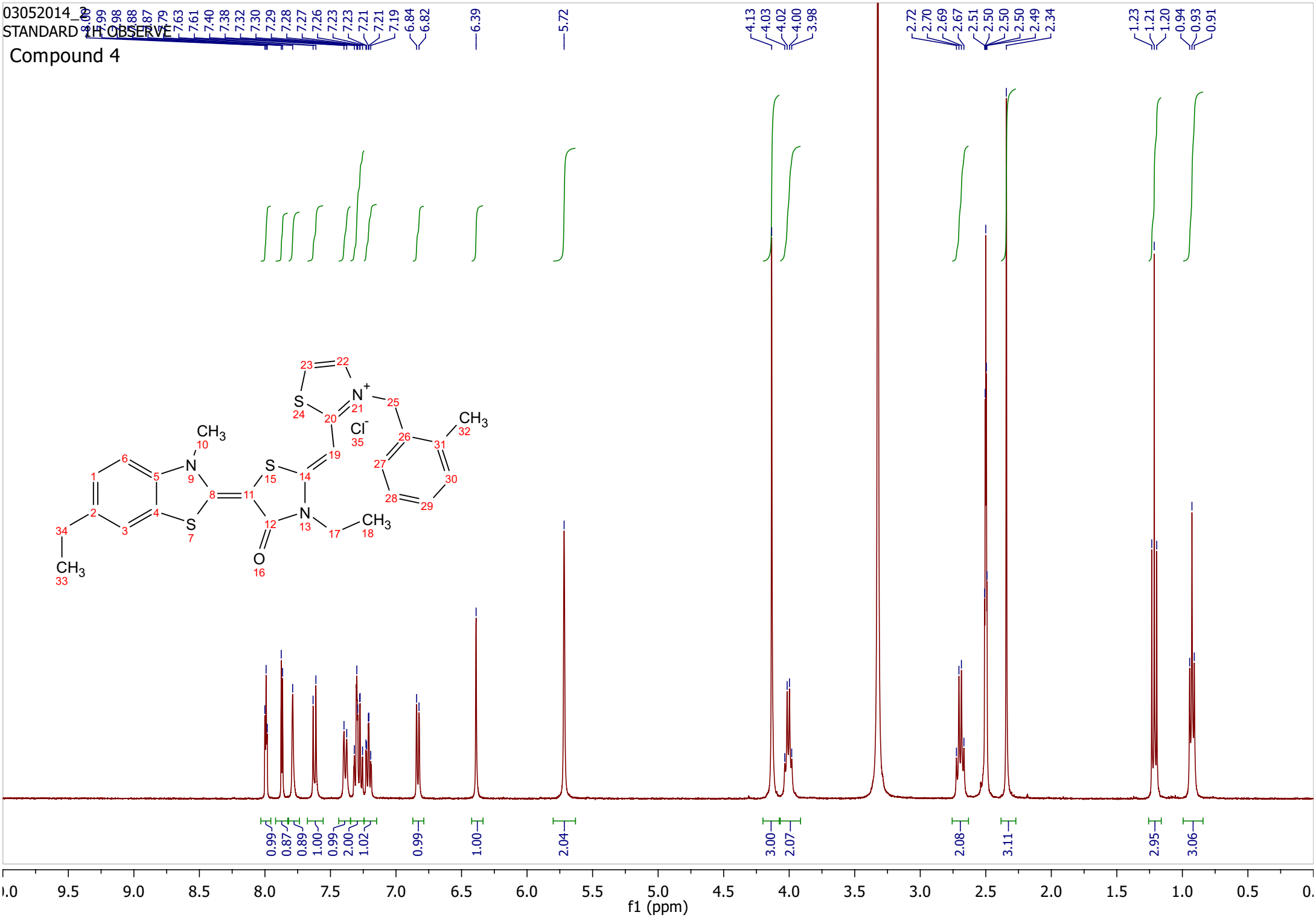
2.96
2.05
2.07
2.92
3.02
f1 (ppm)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7.99
7.98
7.88
7.87
7.79
7.63
7.61
7.40
7.38
7.32
7.30
7.29
7.28
7.27
7.26
7.23
7.23
7.21
7.21
7.19
6.84
6.82
6.39
5.72
4.13
4.03
4.02
4.00
3.98
2.72
2.70
2.69
2.67
2.51
2.50
2.50
2.50
2.49
2.34
1.23
1.21
1.20
0.94
0.93
0.91
0.99
0.87
0.89
1.00
0.99
2.00
1.02
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1.00
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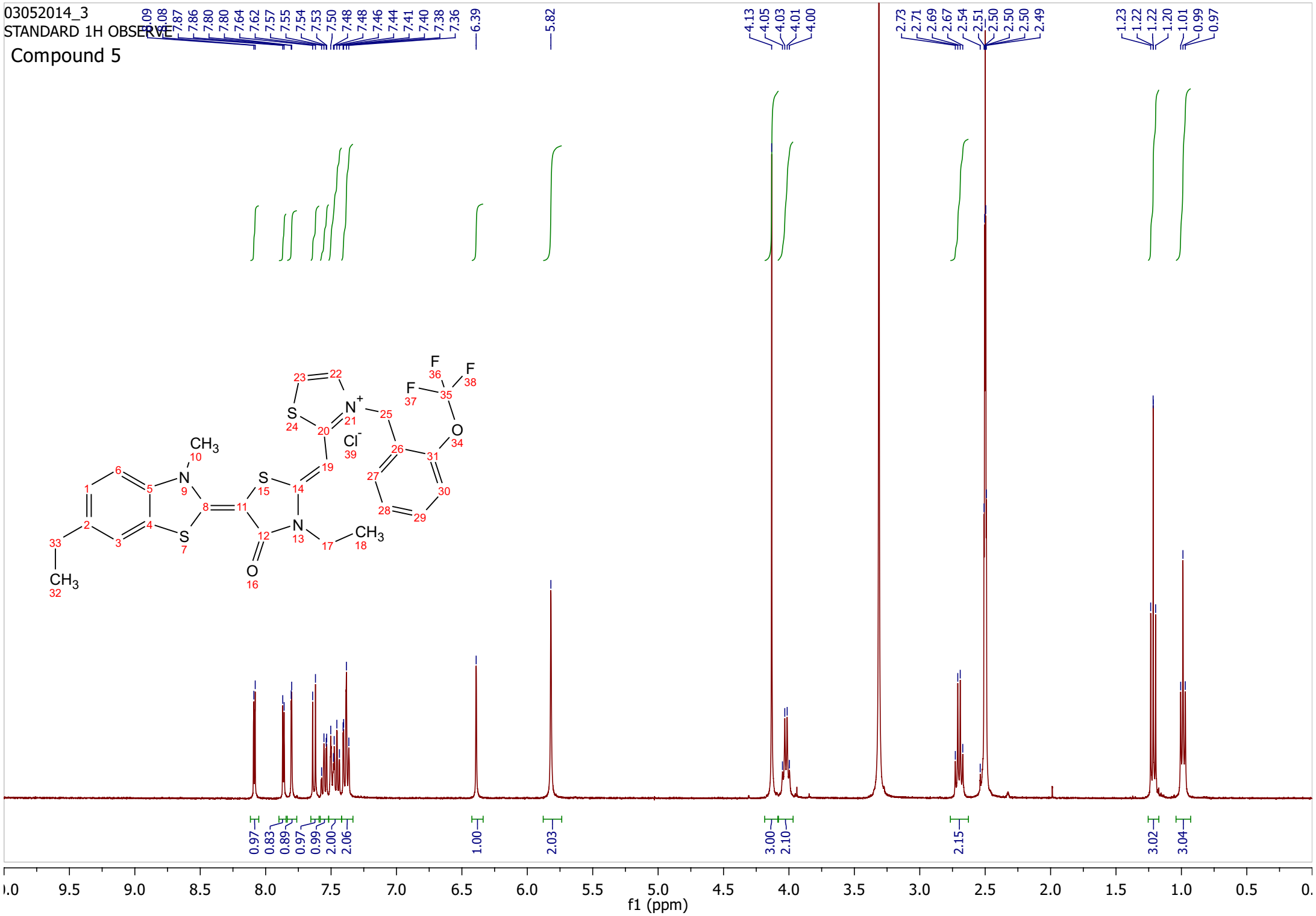
2.95
3.06
f1 (ppm)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9.08
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7.80
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7.62
7.57
7.55
7.54
7.53
7.50
7.48
7.48
7.46
7.44
7.41
7.40
7.38
7.36
6.39
5.82
4.13
4.05
4.03
4.01
4.00
2.73
2.71
2.69
2.67
2.54
2.51
2.50
2.50
2.50
2.49
1.23
1.22
1.22
1.20
1.01
0.99
0.97
0.97
0.83
0.89
0.97
0.99
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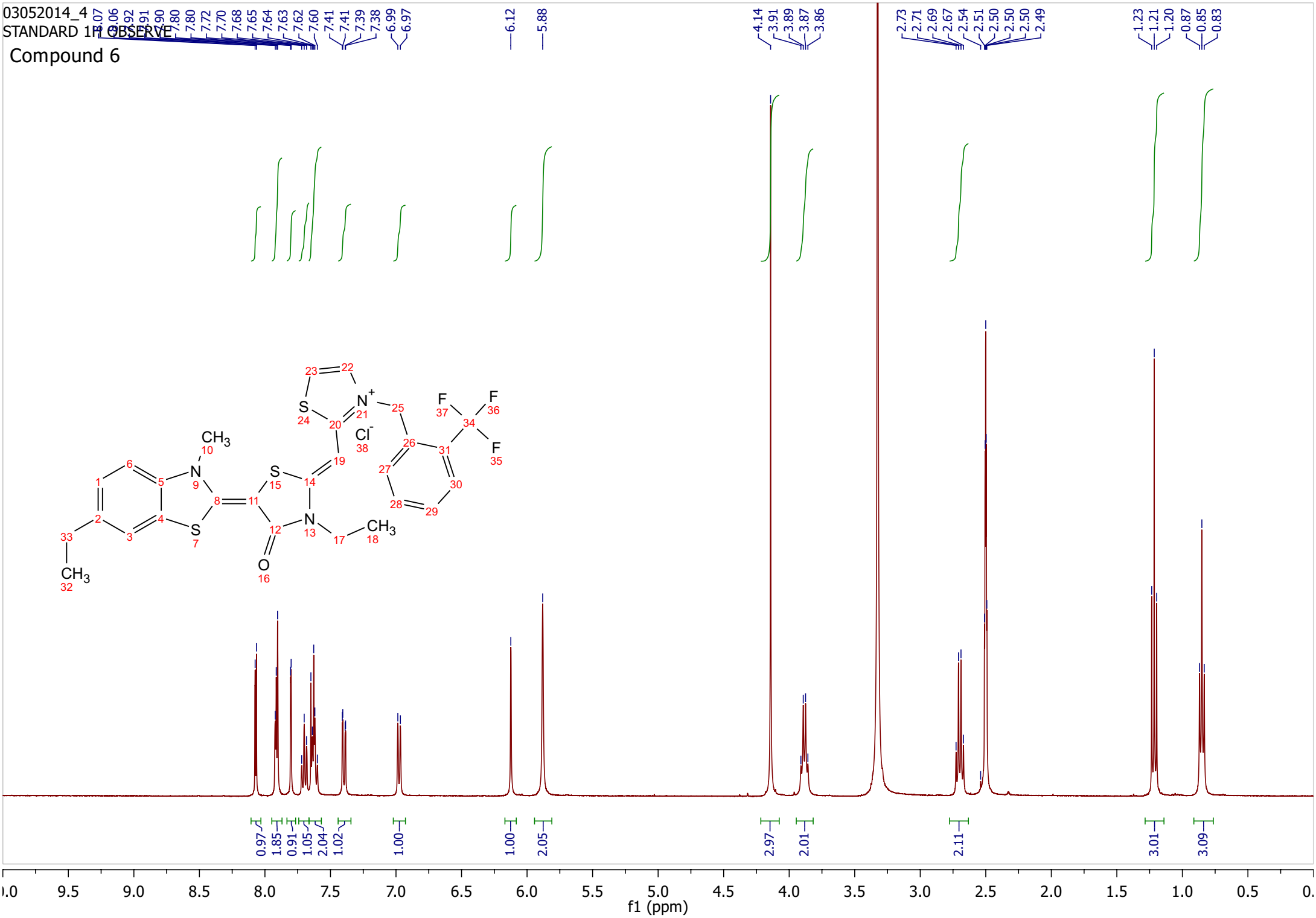
2.06
1.00
2.03
3.00
2.10
2.15
3.02
3.04
f1 (ppm)


03052014_4
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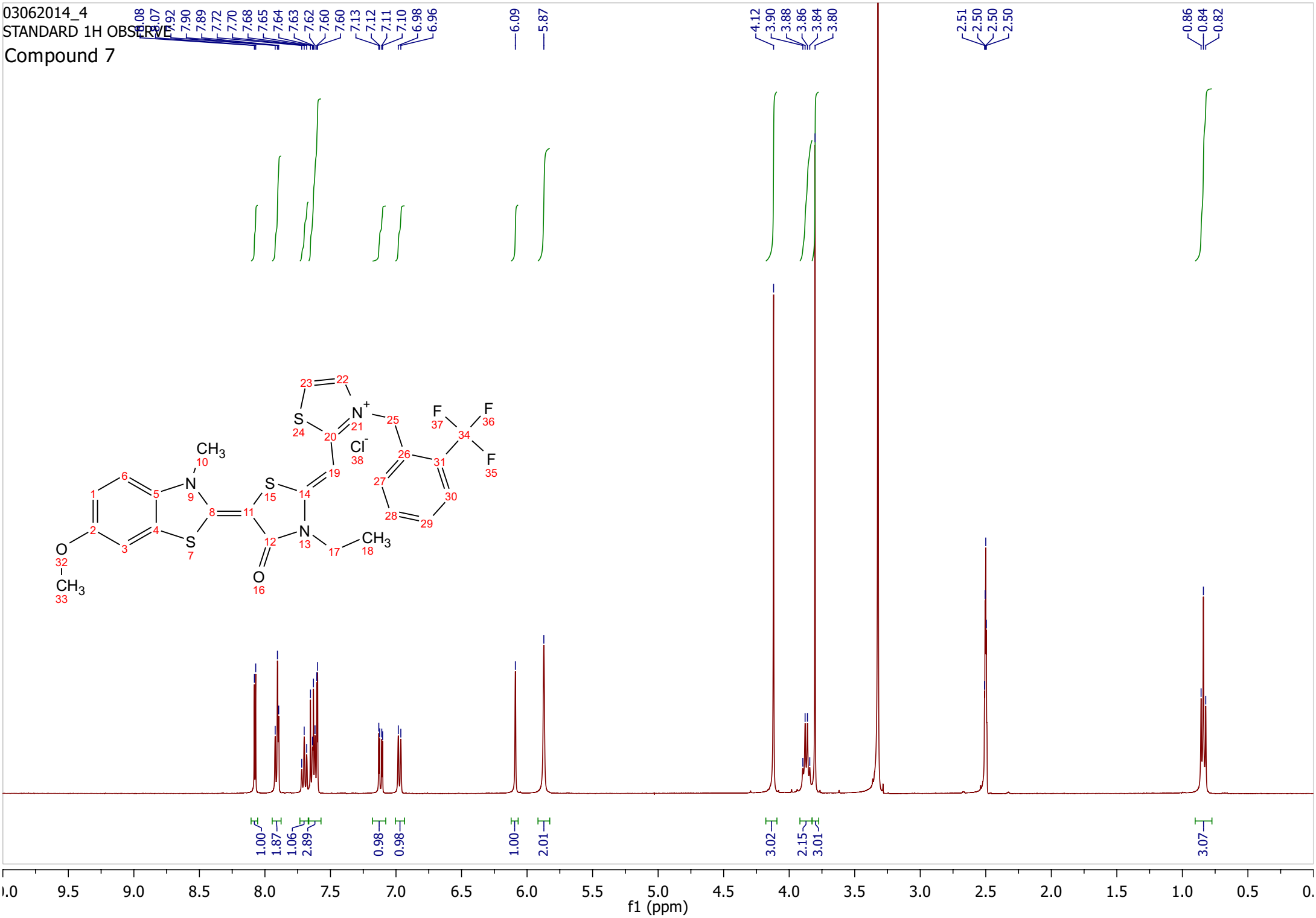
8.06
7.92
7.91
7.90
7.80
7.80
7.72
7.70
7.68
7.65
7.64
7.63
7.62
7.60
7.41
7.41
7.39
7.38
6.99
6.97
6.12
5.88
4.14
3.91
3.89
3.87
3.86
2.73
2.71
2.69
2.67
2.54
2.51
2.50
2.50
2.50
2.49
1.23
1.21
1.20
0.87
0.85
0.83
0.97
1.85
0.91
1.05
2.04
1.02
1.00
1.00
2.05
2.97
2.01
2.11
3.01
3.09
f1 (ppm)


03062014_4
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Compound 7
8.08
8.07
7.92
7.90
7.89
7.72
7.70
7.68
7.65
7.64
7.63
7.62
7.60
7.60
7.13
7.12
7.11
7.10
6.98
6.96
6.09
5.87
4.12
3.90
3.88
3.86
3.84
3.80
2.51
2.50
2.50
2.50
0.86
0.84
0.82
1.00
1.87
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1.00
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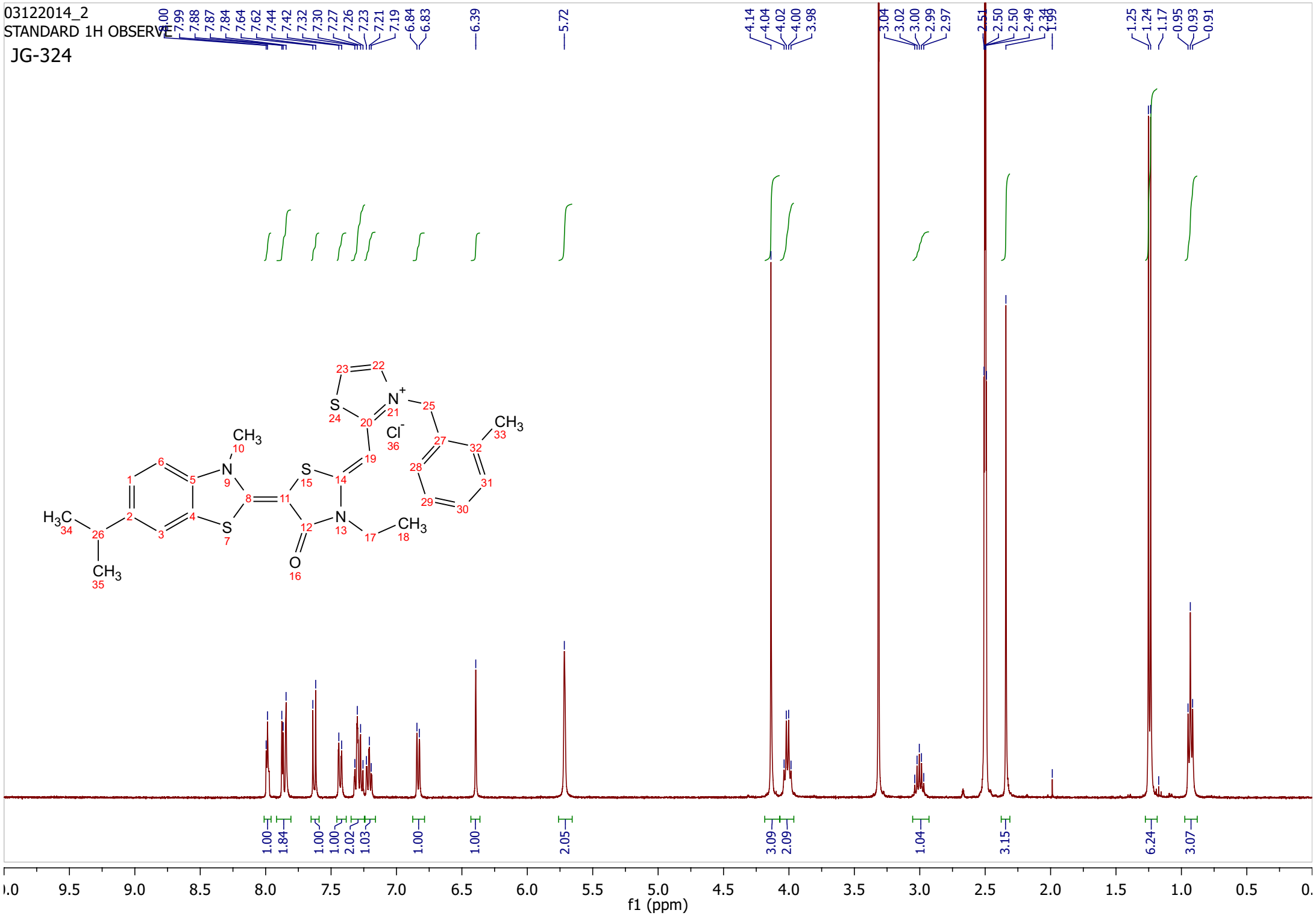
3.07
f1 (ppm)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JG-324
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7.99
7.88
7.87
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7.64
7.62
7.44
7.42
7.32
7.30
7.27
7.26
7.23
7.21
7.19
6.84
6.83
6.39
5.72
4.14
4.04
4.02
4.00
3.98
3.04
3.02
3.00
2.99
2.97
2.51
2.50
2.50
2.49
2.34
1.99
1.25
1.24
1.17
0.95
0.93
0.91
1.00
1.84
1.00
1.00
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2.09
1.04
3.15
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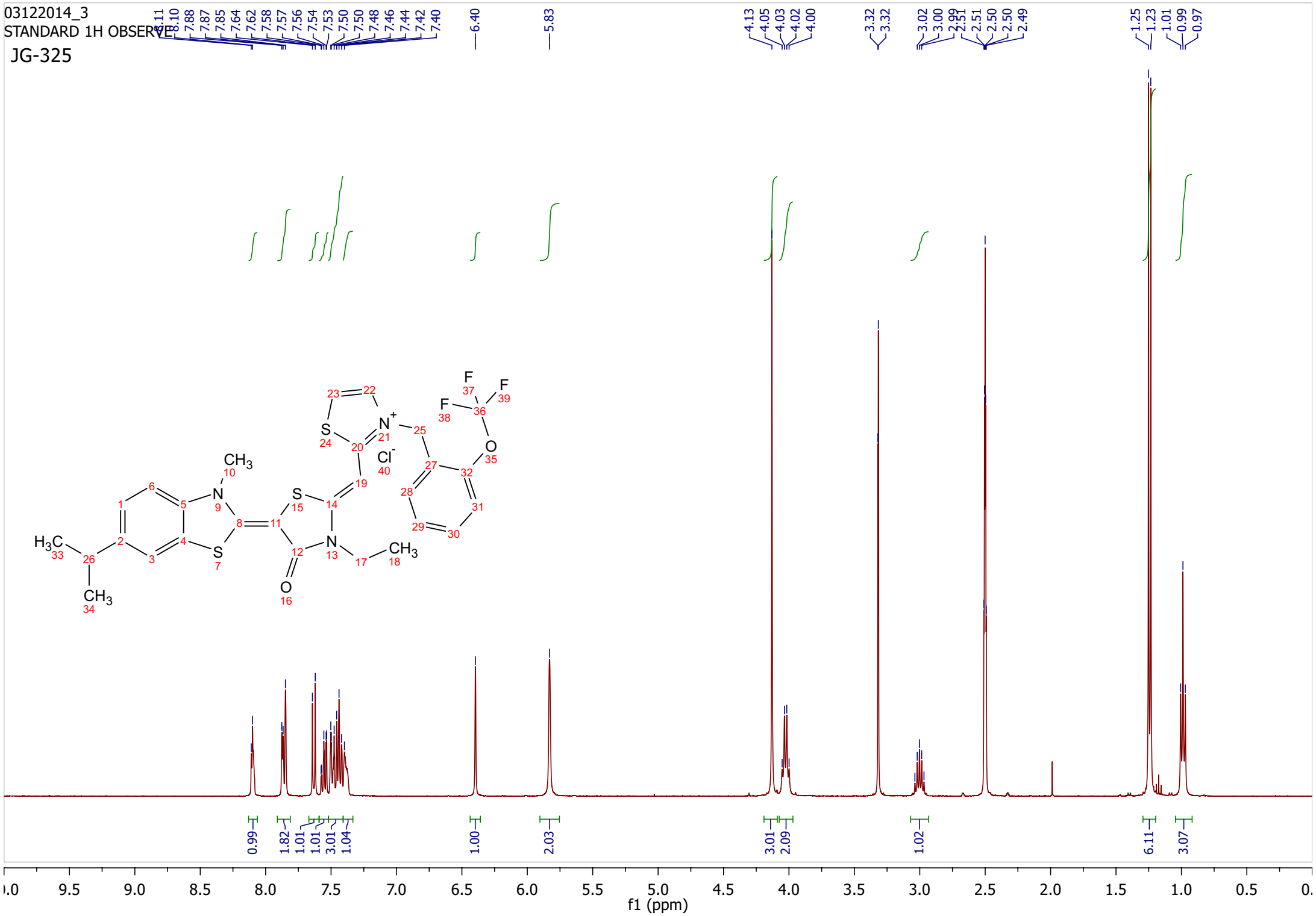
3.07
f1 (ppm)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8.10
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7.57
7.56
7.54
7.53
7.50
7.50
7.48
7.46
7.44
7.42
7.40
6.40
5.83
4.13
4.05
4.03
4.02
4.00
3.32
3.32
3.02
3.00
2.99
2.51
2.51
2.50
2.50
2.49
1.25
1.23
1.01
0.99
0.97
0.99
1.82
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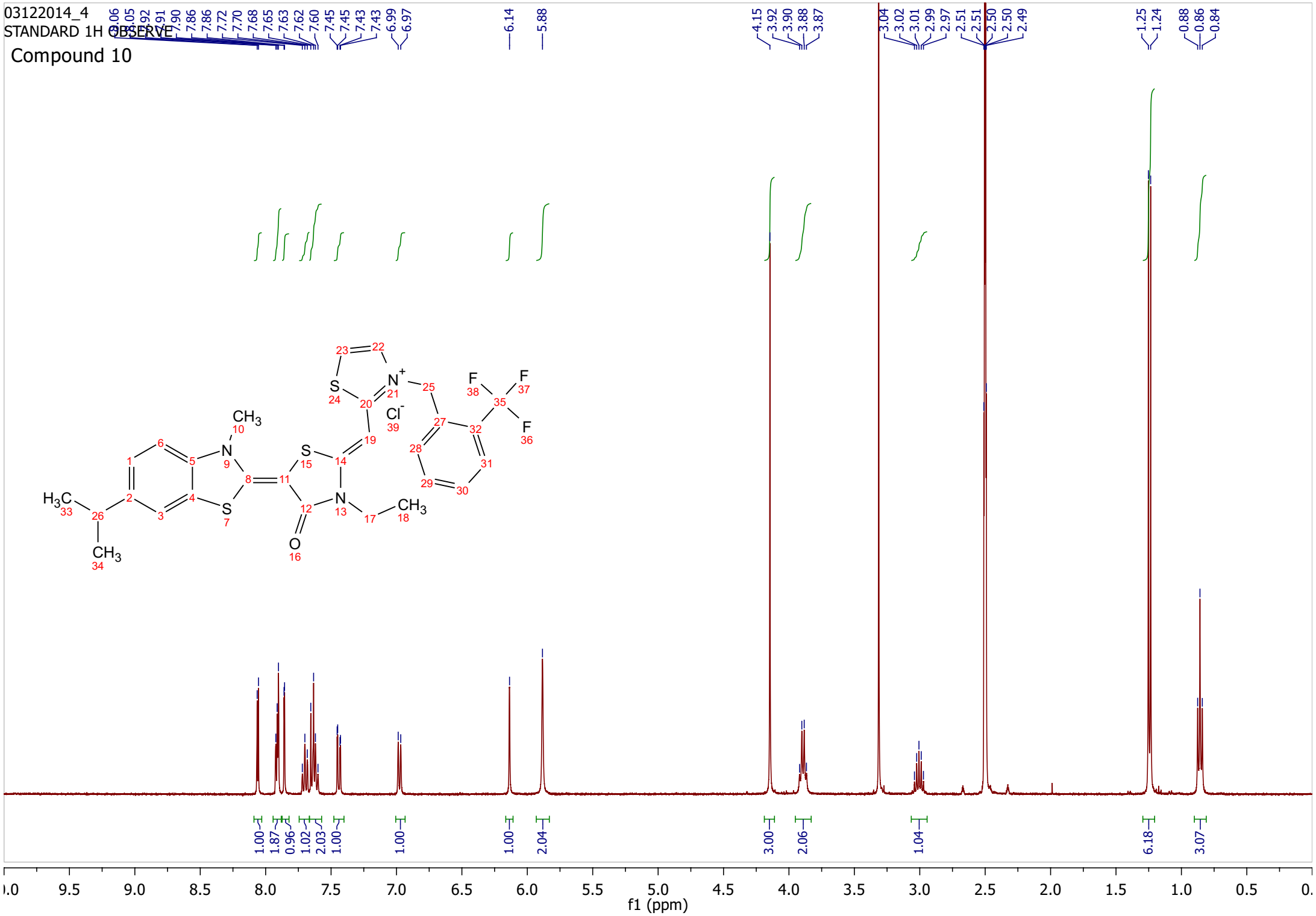
3.07
f1 (ppm)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7.70
7.68
7.65
7.63
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7.45
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7.43
6.99
6.97
6.14
5.88
4.15
3.92
3.90
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3.04
3.02
3.01
2.99
2.97
2.51
2.51
2.50
2.50
2.49
1.25
1.24
0.88
0.86
0.84
1.00
1.87
0.96
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1.04
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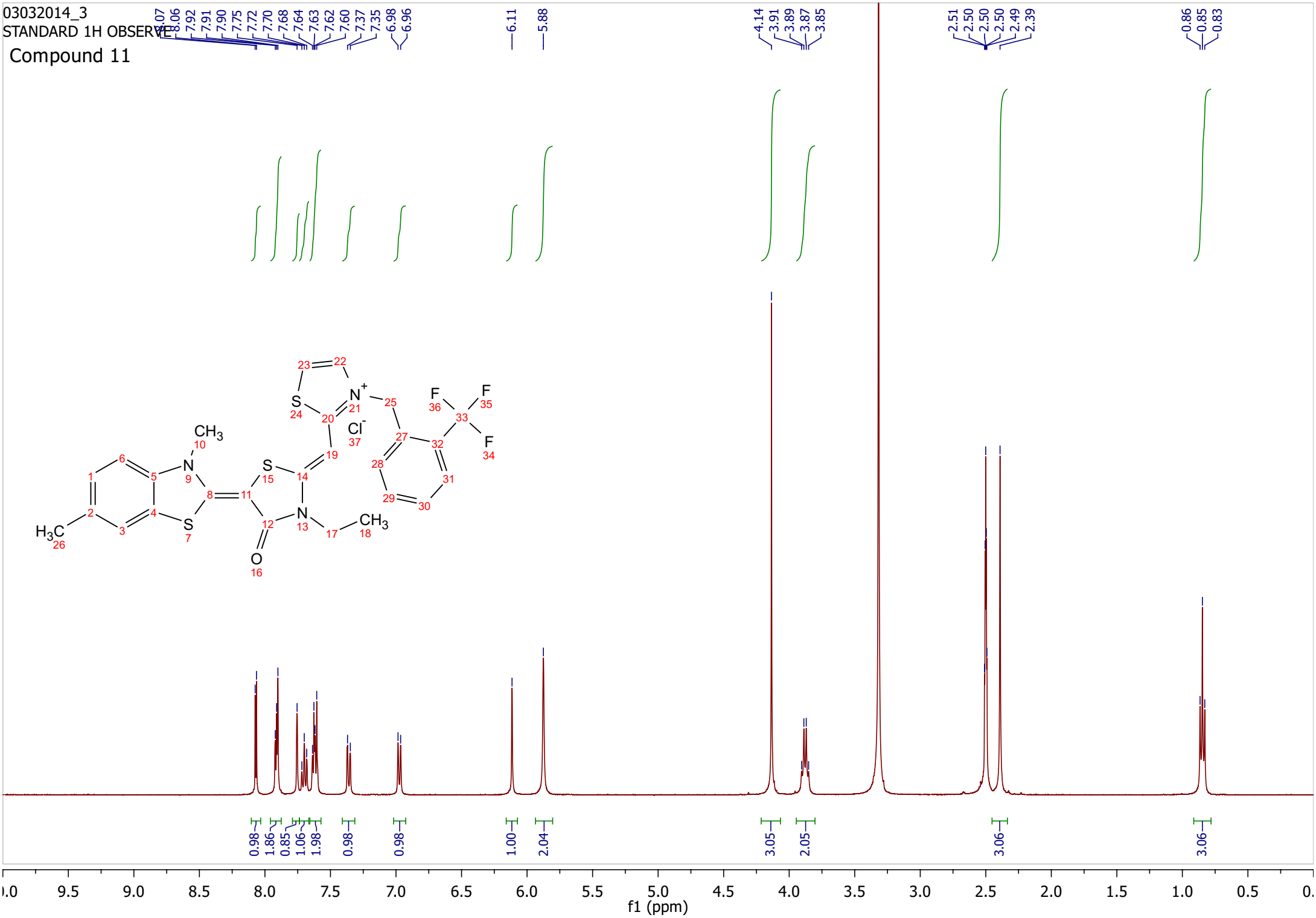
3.07
f1 (ppm)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7.64
7.63
7.62
7.60
7.37
7.35
6.98
6.96
6.11
5.88
4.14
3.91
3.89
3.87
3.85
2.51
2.50
2.50
2.50
2.49
2.39
0.86
0.85
0.83
0.98
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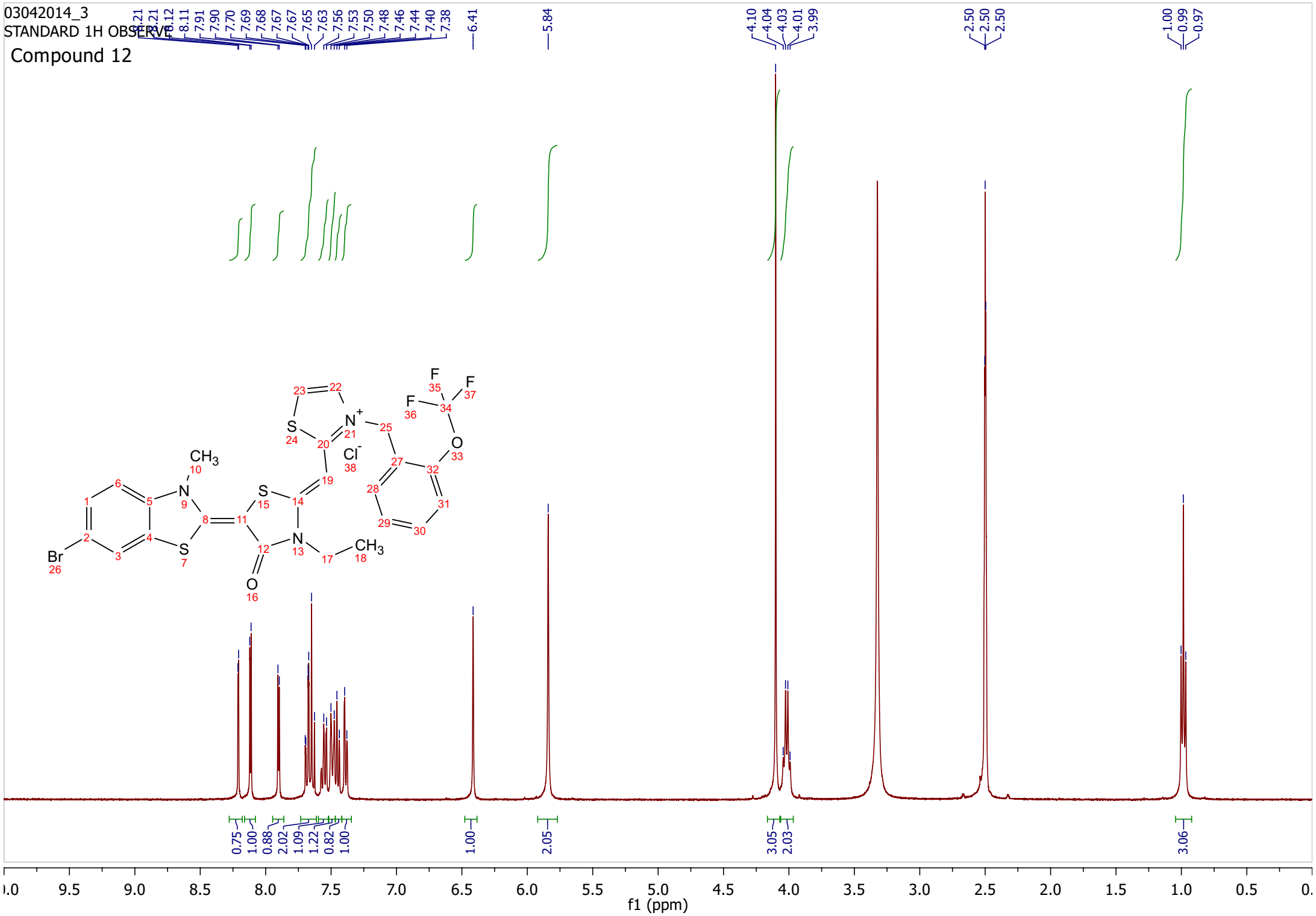
3.06
f1 (ppm)


03042014_3
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Compound 12
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8.11
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7.90
7.70
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7.68
7.67
7.67
7.65
7.63
7.56
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7.50
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7.46
7.44
7.40
7.38
6.41
5.84
4.10
4.04
4.03
4.01
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2.50
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0.97
0.75
1.00
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1.00
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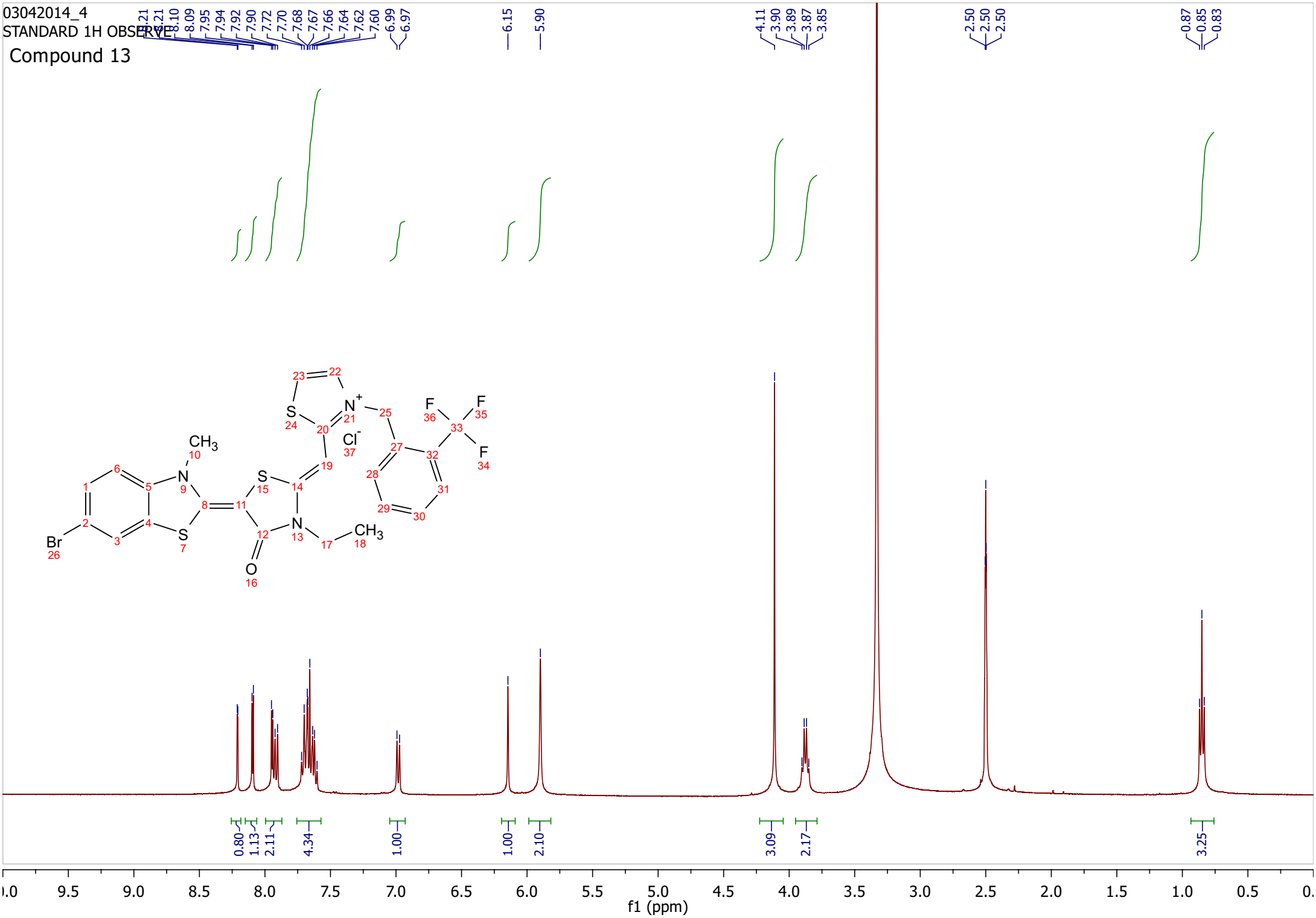
3.05
2.03
3.06
f1 (ppm)


03042014_4
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Compound 13
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7.67
7.66
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6.97
6.15
5.90
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2.50
0.87
0.85
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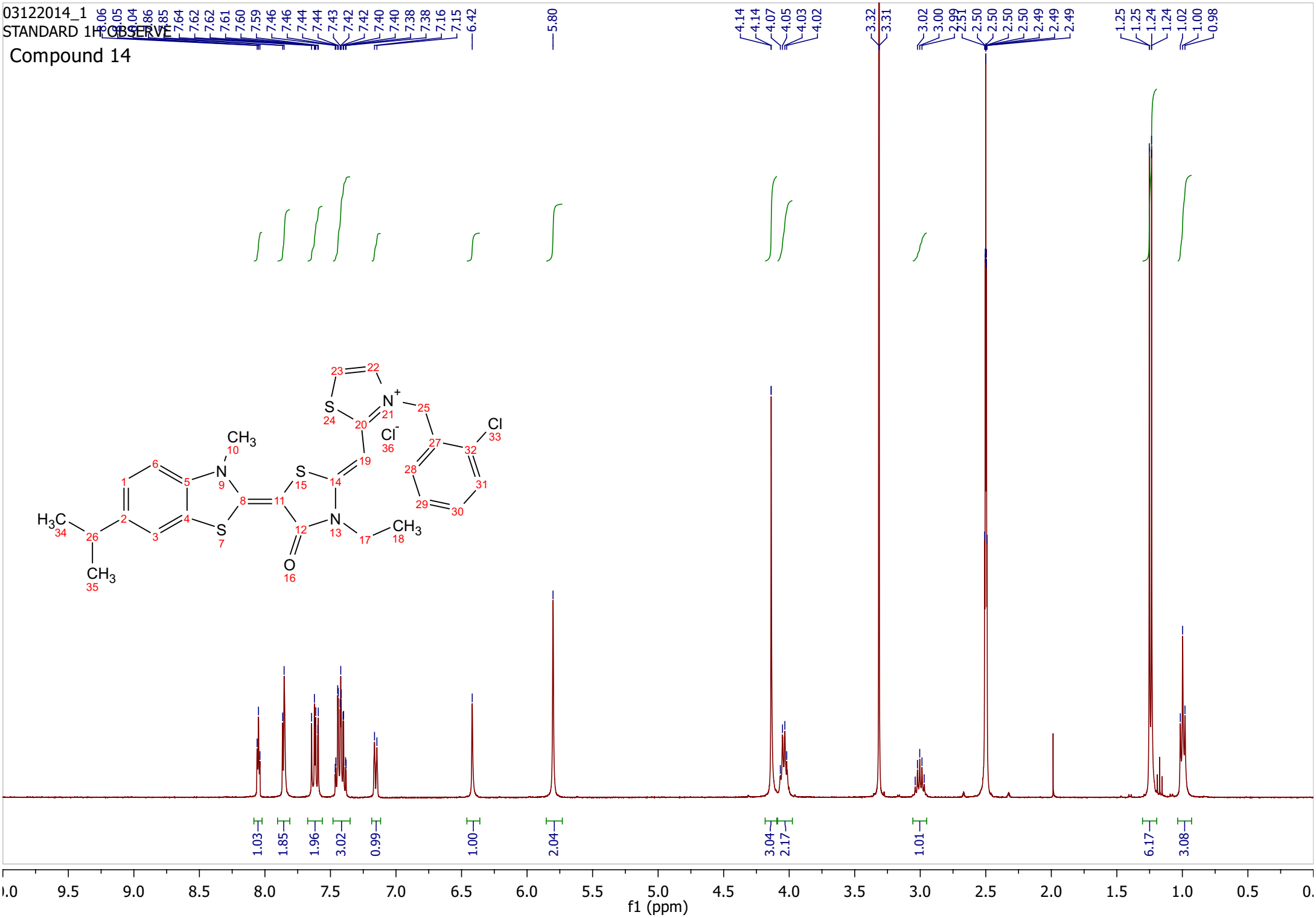
f1 (ppm)


03122014_1
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Compound 14
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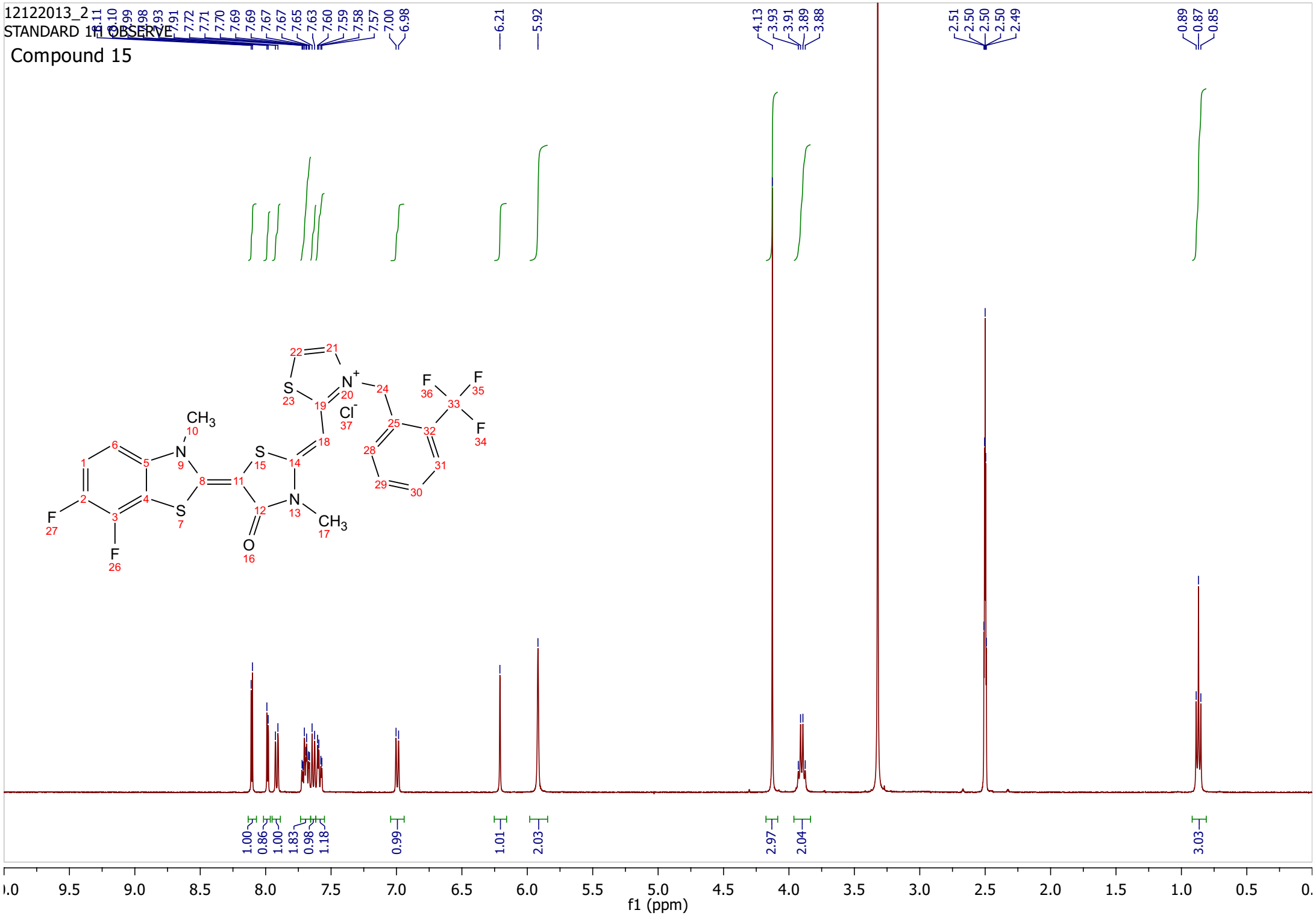
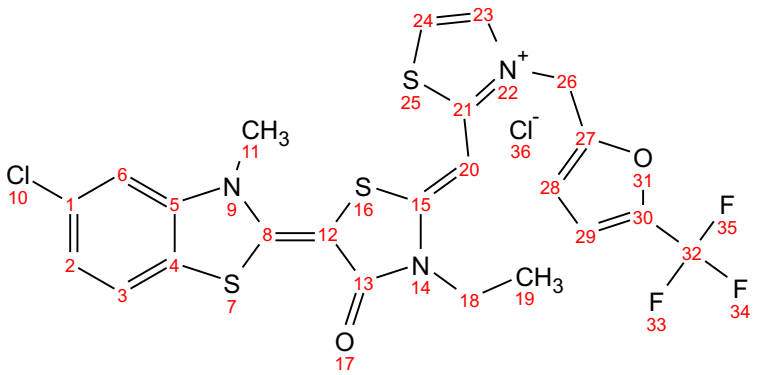
5.80
4.14 4.14 4.07 4.05 4.03 4.02
3.32 3.31
3.02 3.00 2.99 2.51 2.50 2.50 2.50 2.50 2.49 2.49 2.49
1.25 1.25 1.24 1.24 1.02 1.00 0.98
1.03 1.85 1.96 3.02 0.99 1.00 2.04 3.04 2.17 1.01 6.17 3.08
f1 (ppm)

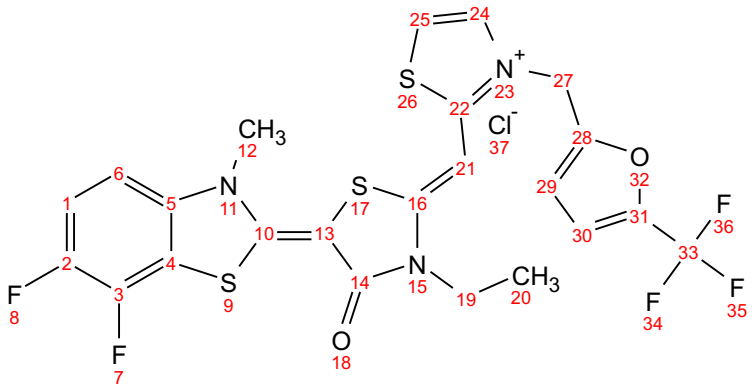

12122013_2
STANDARD 1H OBSERVE
Compound 15
8.11
8.10
7.99
7.98
7.93
7.91
7.72
7.71
7.70
7.69
7.69
7.67
7.67
7.65
7.63
7.60
7.59
7.58
7.57
7.00
6.98
6.21
5.92
4.13
3.93
3.91
3.89
3.88
2.51
2.50
2.50
2.50
2.49
0.89
0.87
0.85
1.00
0.86
1.00
1.83
0.98
1.18
0.99
1.01
2.03
2.97
2.04
3.03
f1 (ppm)

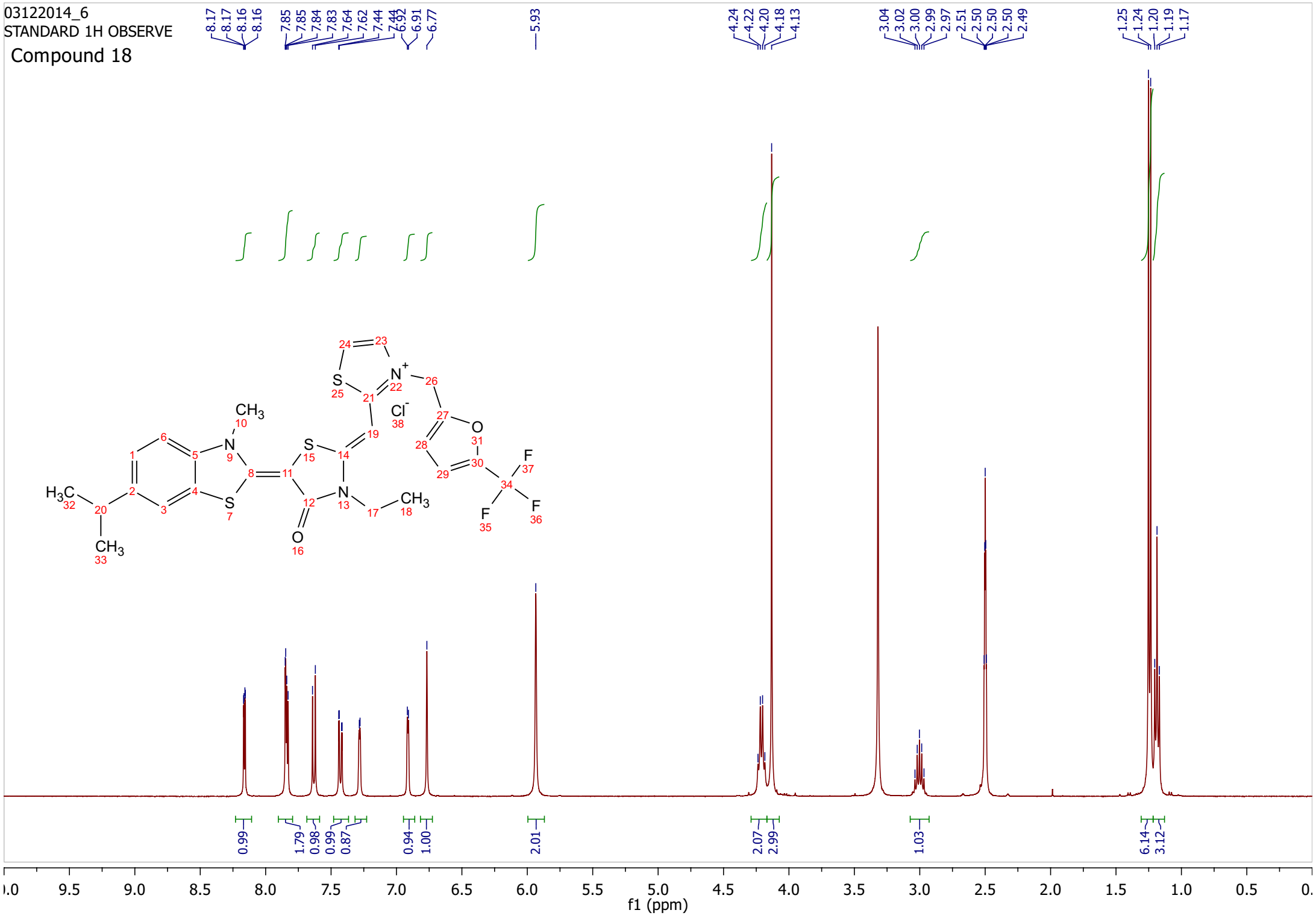
11062013_1
STANDARD 1H OBSERVE

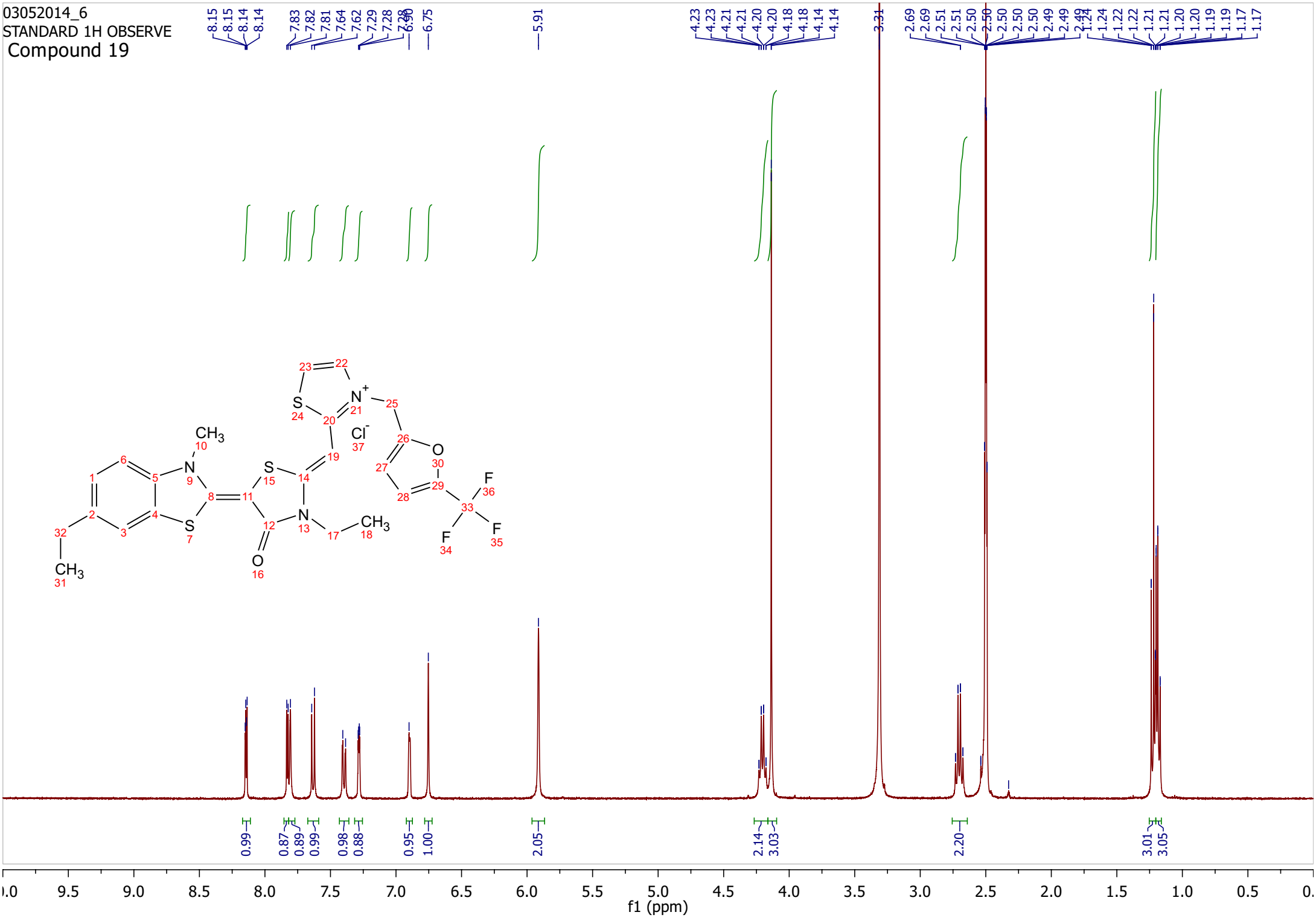
Compound 16

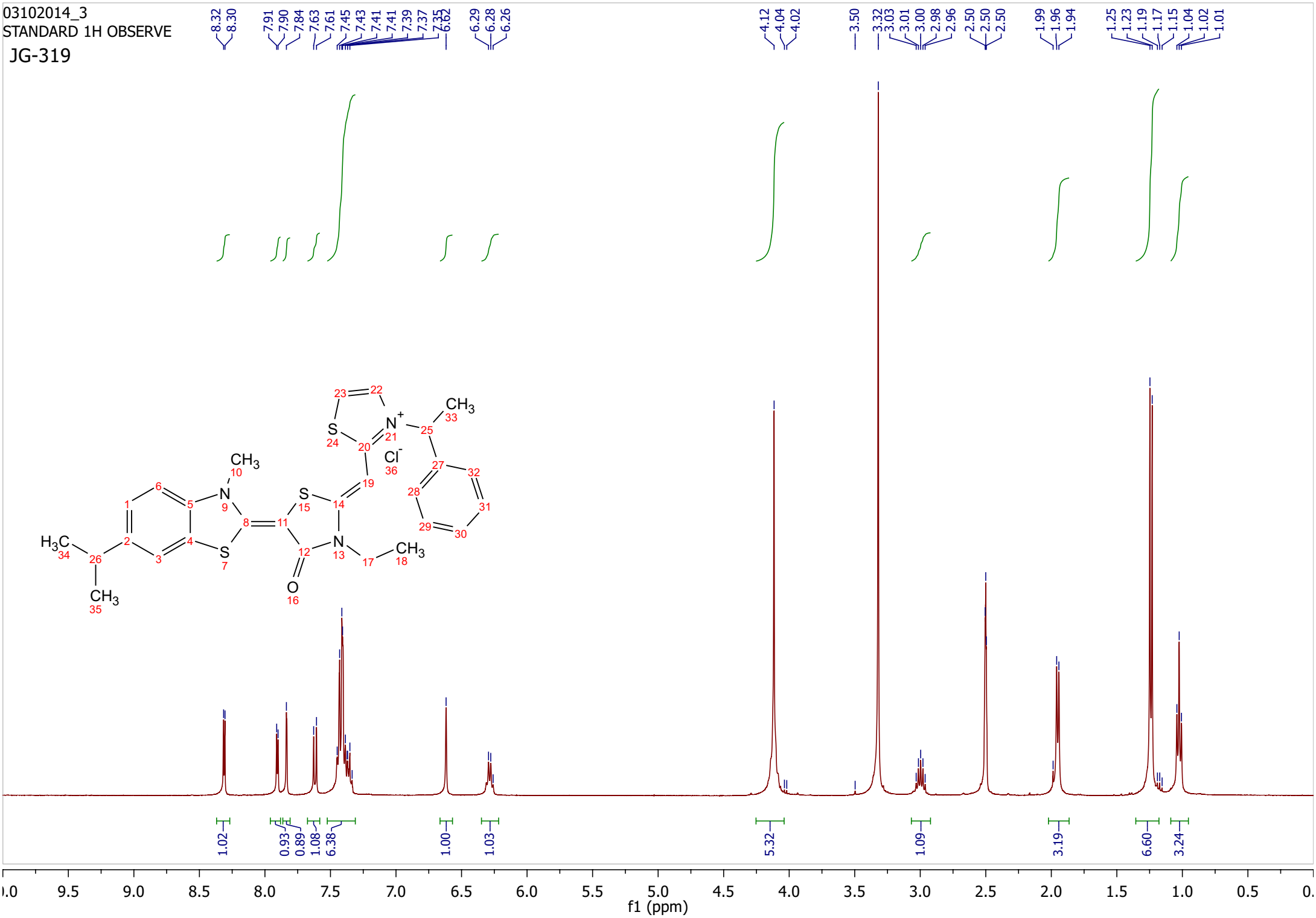


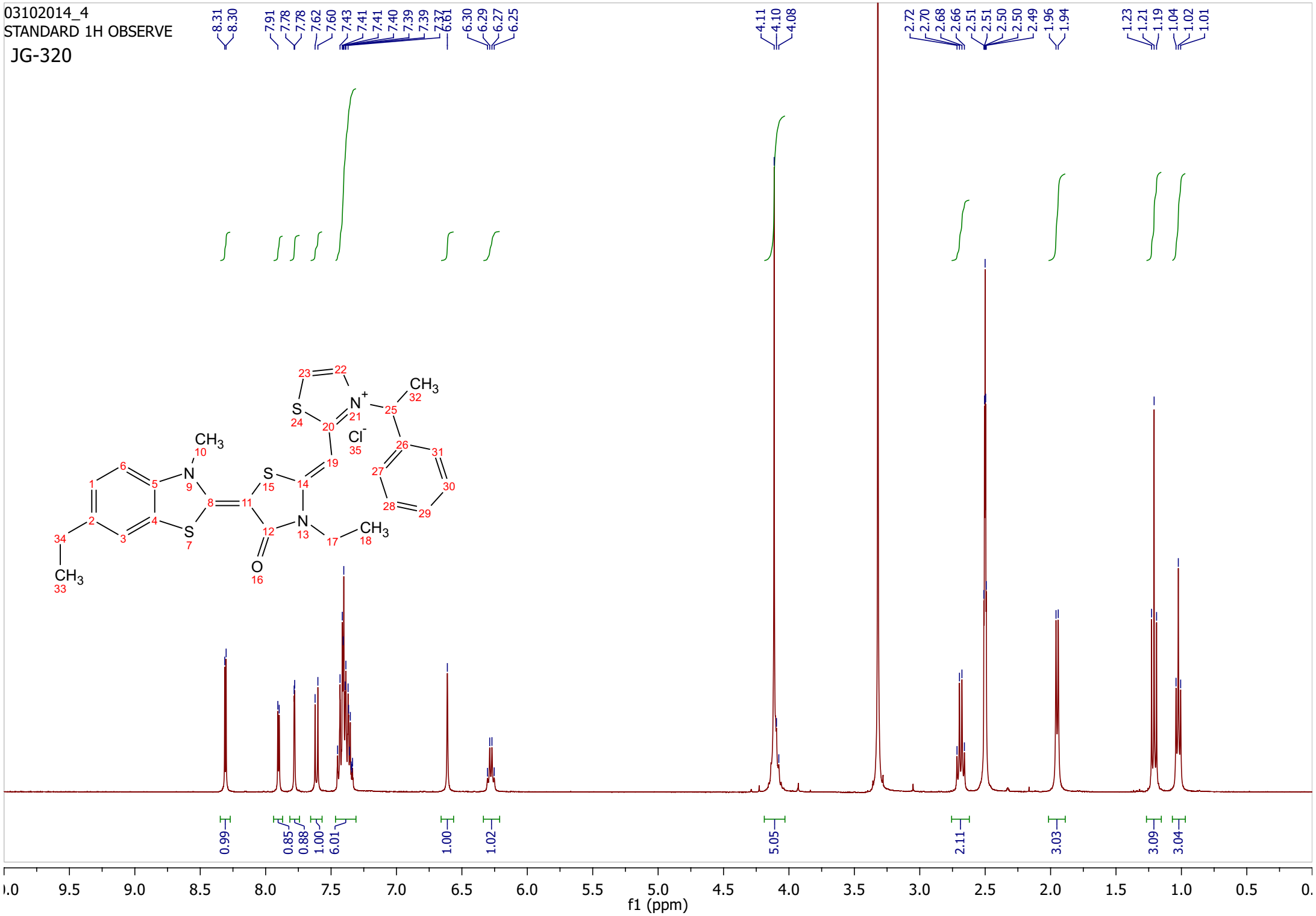
12132013_1
STANDARD 1H OBSERVE
Compound 17

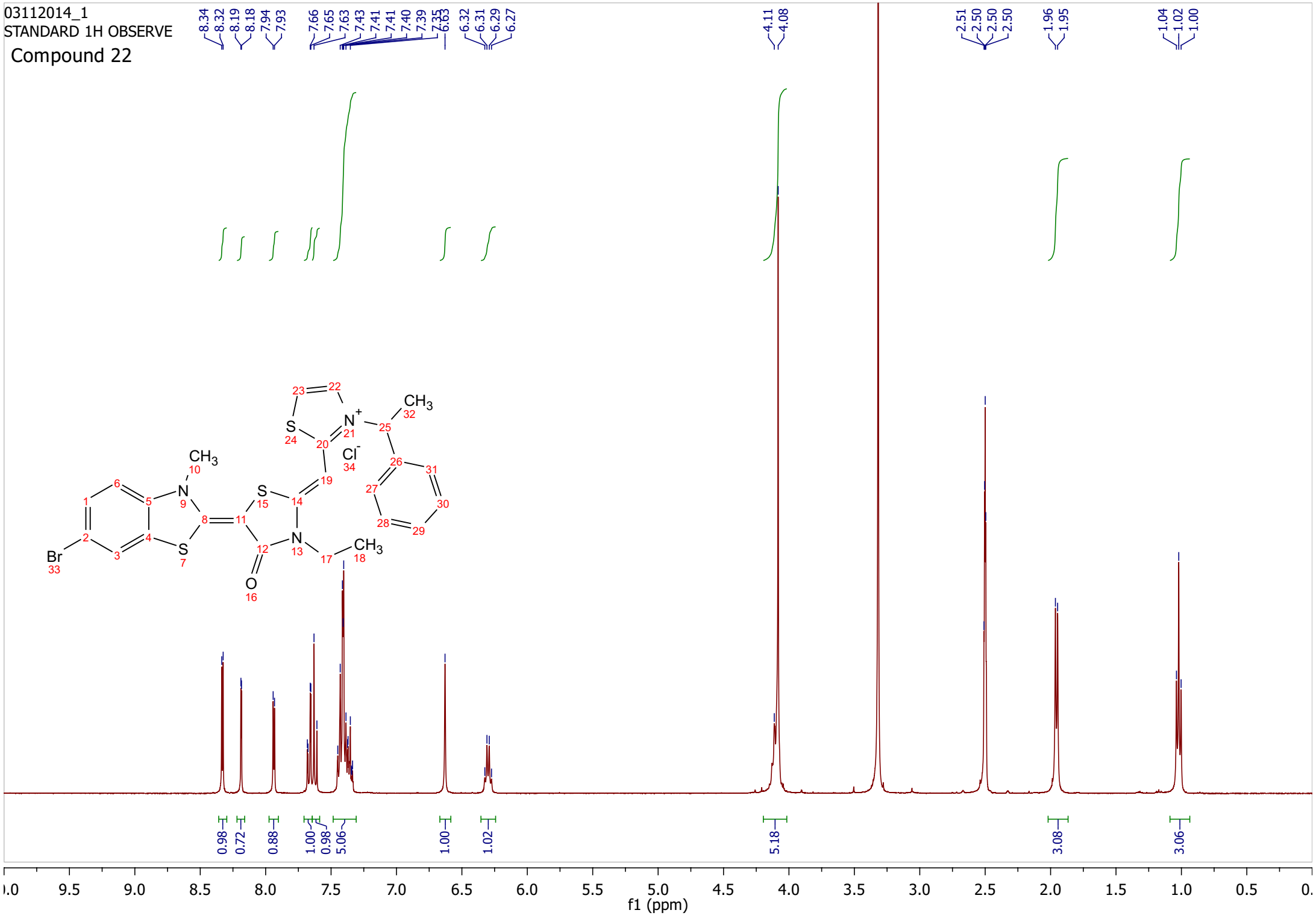


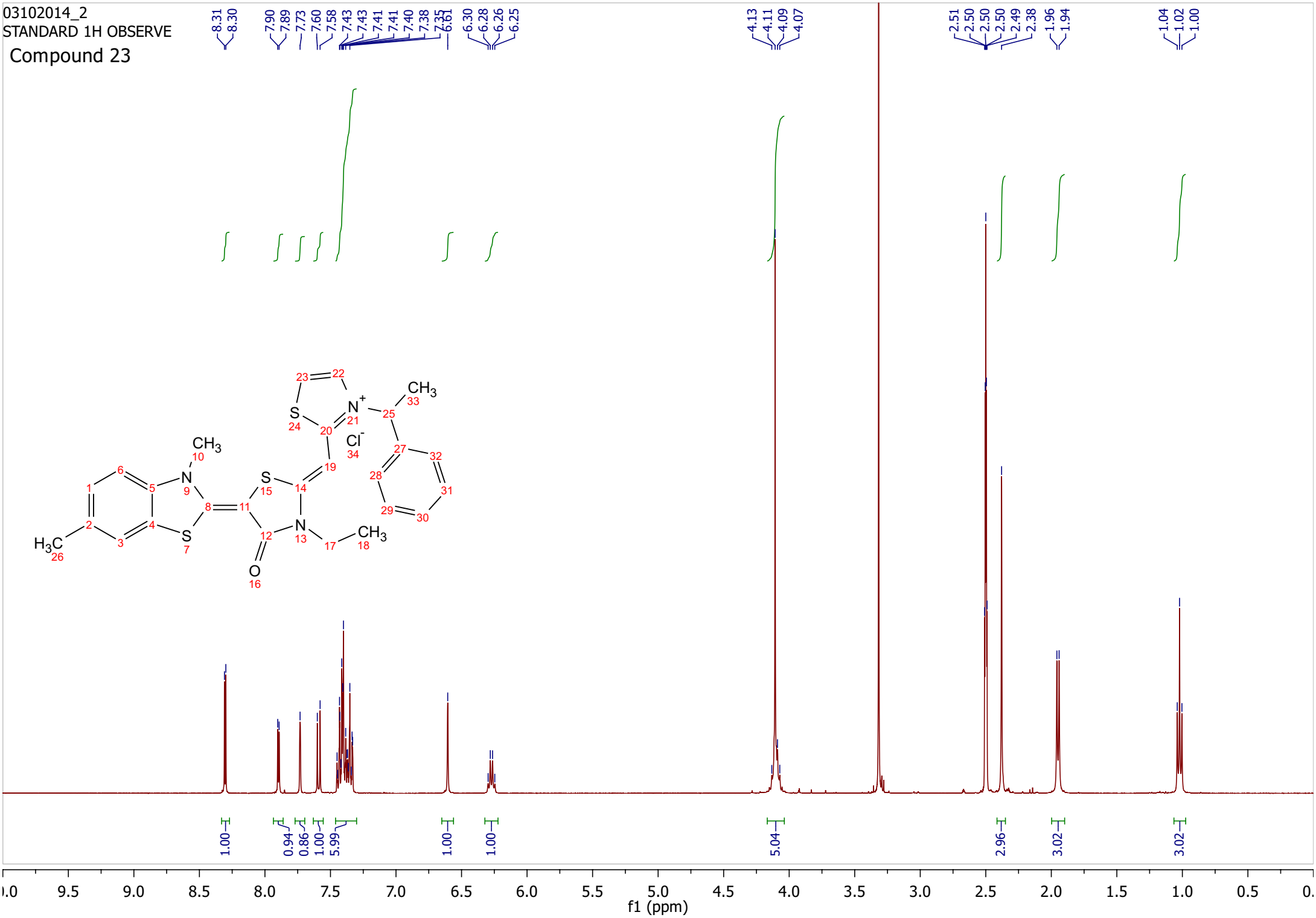

03122014_6
STANDARD 1H OBSERVE
Compound 18
8.17
8.17
8.16
8.16
7.85
7.85
7.84
7.83
7.64
7.62
7.44
7.44
6.92
6.91
6.77
5.93
4.24
4.22
4.20
4.18
4.13
3.04
3.02
3.00
2.99
2.97
2.51
2.50
2.50
2.50
2.49
1.25
1.24
1.20
1.19
1.17
0.99
1.79
0.98
0.99
0.87
0.94
1.00
2.01
2.07
2.99
1.03
6.14
3.12
f1 (ppm)

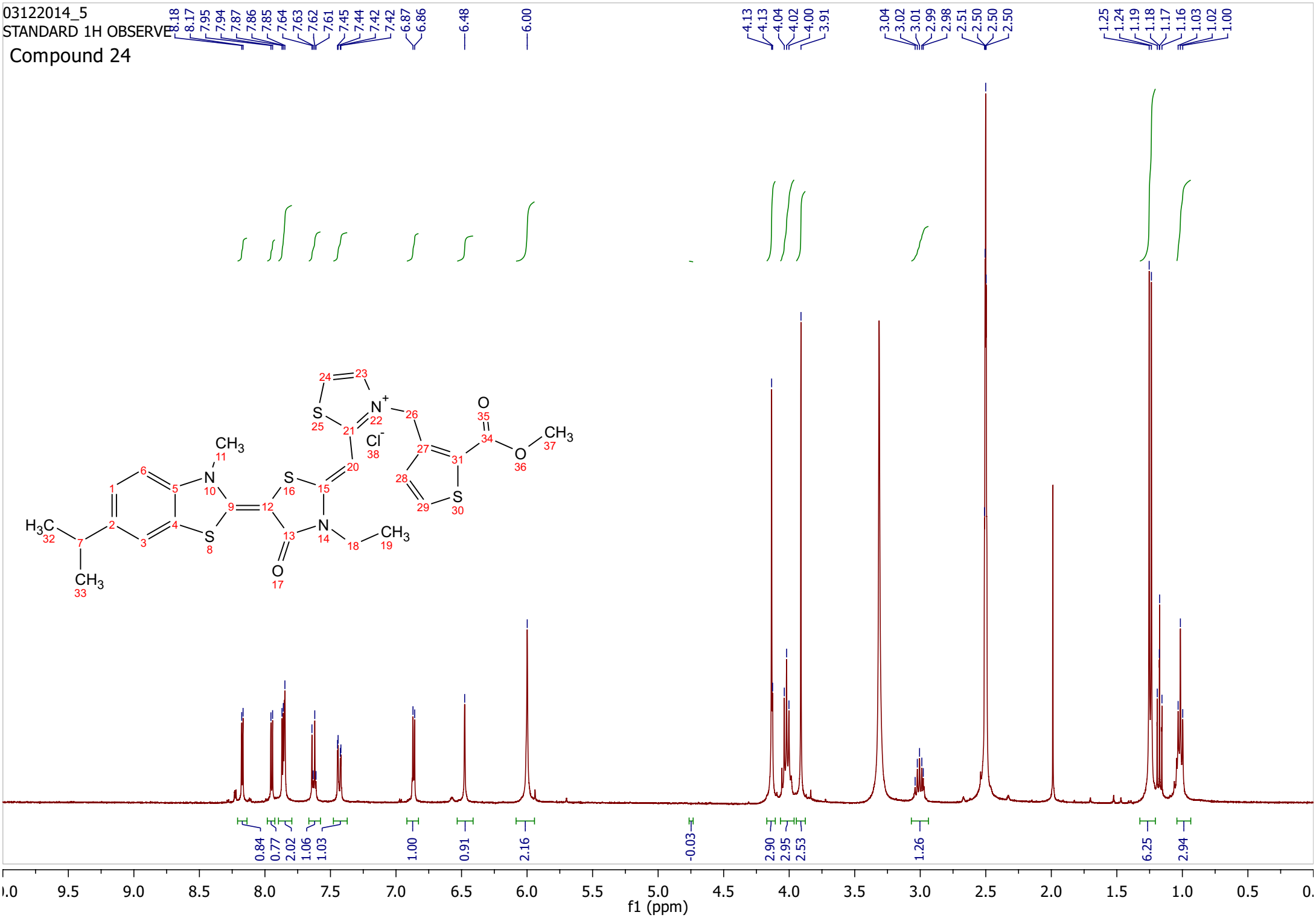

03052014_6
STANDARD 1H OBSERVE
Compound 19
8.15 8.15 8.14 8.14
7.83 7.82 7.81 7.64 7.62 7.29 7.28 6.90 6.75
5.91
4.23 4.23 4.21 4.21 4.20 4.20 4.18 4.18 4.14 4.14
3.31
2.69 2.69 2.51 2.51 2.50 2.50 2.50 2.50 2.50 2.49 2.49 1.24 1.24 1.22 1.22 1.21 1.21 1.20 1.20 1.19 1.19 1.17 1.17
0.99 0.87 0.89 0.99 0.98 0.88 0.95 1.00 2.05 2.14 3.03 2.20 3.01 3.05
f1 (ppm)

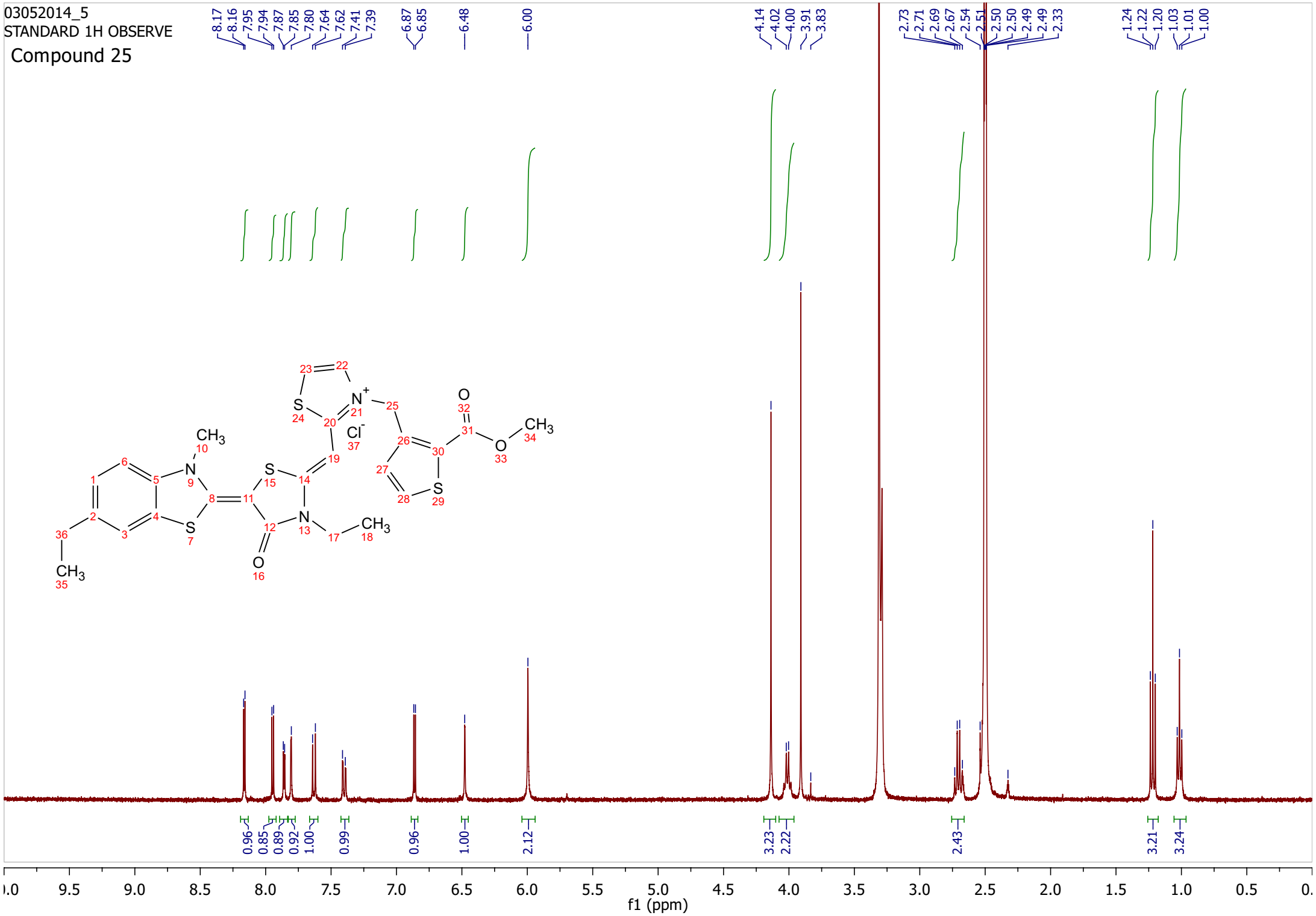

03102014_3
STANDARD 1H OBSERVE
JG-319
8.32
8.30
7.91
7.90
7.84
7.63
7.61
7.45
7.43
7.41
7.41
7.39
7.37
7.35
6.62
6.29
6.28
6.26
4.12
4.04
4.02
3.50
3.32
3.03
3.01
3.00
2.98
2.96
2.50
2.50
2.50
1.99
1.96
1.94
1.25
1.23
1.19
1.17
1.15
1.04
1.02
1.01
1.02
0.93
0.89
1.08
6.38
1.00
1.03
5.32
1.09
3.19
6.60
3.24
f1 (ppm)


03102014_4
STANDARD 1H OBSERVE
JG-320
8.31
8.30
7.91
7.78
7.78
7.62
7.60
7.43
7.41
7.41
7.40
7.39
7.39
7.37
6.61
6.30
6.29
6.27
6.25
4.11
4.10
4.08
2.72
2.70
2.68
2.66
2.51
2.51
2.50
2.50
2.49
1.96
1.94
1.23
1.21
1.19
1.04
1.02
1.01
0.99
0.85
0.88
1.00
6.01
1.00
1.02
5.05
2.11
3.03
3.09
3.04
f1 (ppm)

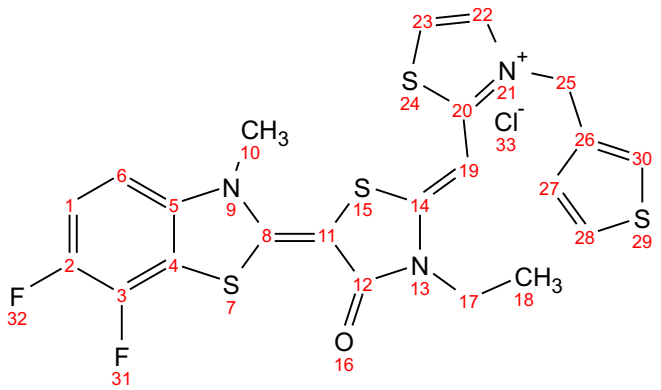

03112014_1
STANDARD 1H OBSERVE
Compound 22
8.34
8.32
8.19
8.18
7.94
7.93
7.66
7.65
7.63
7.43
7.41
7.41
7.40
7.39
7.35
6.83
6.32
6.31
6.29
6.27
4.11
4.08
2.51
2.50
2.50
2.50
1.96
1.95
1.04
1.02
1.00
0.98
0.72
0.88
1.00
0.98
5.06
1.00
1.02
5.18
3.08
3.06
f1 (ppm)


03102014_2
STANDARD 1H OBSERVE
Compound 23
8.31
8.30
7.90
7.89
7.73
7.60
7.58
7.43
7.43
7.41
7.41
7.40
7.38
7.35
6.61
6.30
6.28
6.26
6.25
4.13
4.11
4.09
4.07
2.51
2.50
2.50
2.50
2.49
2.38
1.96
1.94
1.04
1.02
1.00
1.00
0.94
0.86
1.00
5.99
1.00
1.00
5.04
2.96
3.02
3.02
f1 (ppm)

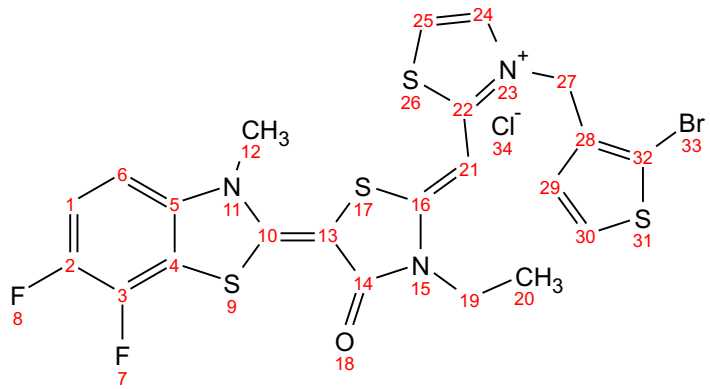

03122014_5
STANDARD 1H OBSERVE
Compound 24
8.18
8.17
7.95
7.94
7.87
7.86
7.85
7.64
7.63
7.62
7.61
7.45
7.44
7.42
7.42
6.87
6.86
6.48
6.00
4.13
4.13
4.04
4.02
4.00
3.91
3.04
3.02
3.01
2.99
2.98
2.51
2.50
2.50
2.50
1.25
1.24
1.19
1.18
1.17
1.16
1.03
1.02
1.00
0.84
0.77
2.02
1.06
1.03
1.00
0.91
2.16
-0.03
2.90
2.95
2.53
1.26
6.25
2.94
f1 (ppm)


03052014_5
STANDARD 1H OBSERVE
Compound 25
8.17
8.16
7.95
7.94
7.87
7.85
7.80
7.64
7.62
7.41
7.39
6.87
6.85
6.48
6.00
4.14
4.02
4.00
3.91
3.83
2.73
2.71
2.69
2.67
2.54
2.51
2.50
2.50
2.49
2.49
2.33
1.24
1.22
1.20
1.03
1.01
1.00
0.96
0.85
0.89
0.92
1.00
0.99
0.96
1.00
2.12
3.23
2.22
2.43
3.21
3.24
f1 (ppm)

12122013_1
STANDARD 1H OBSERVE
Compound 26

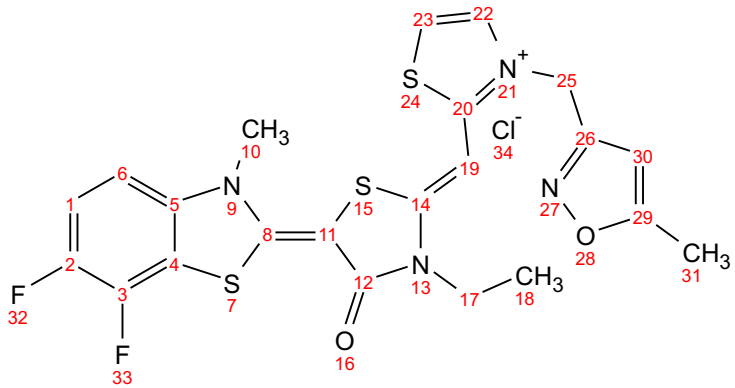


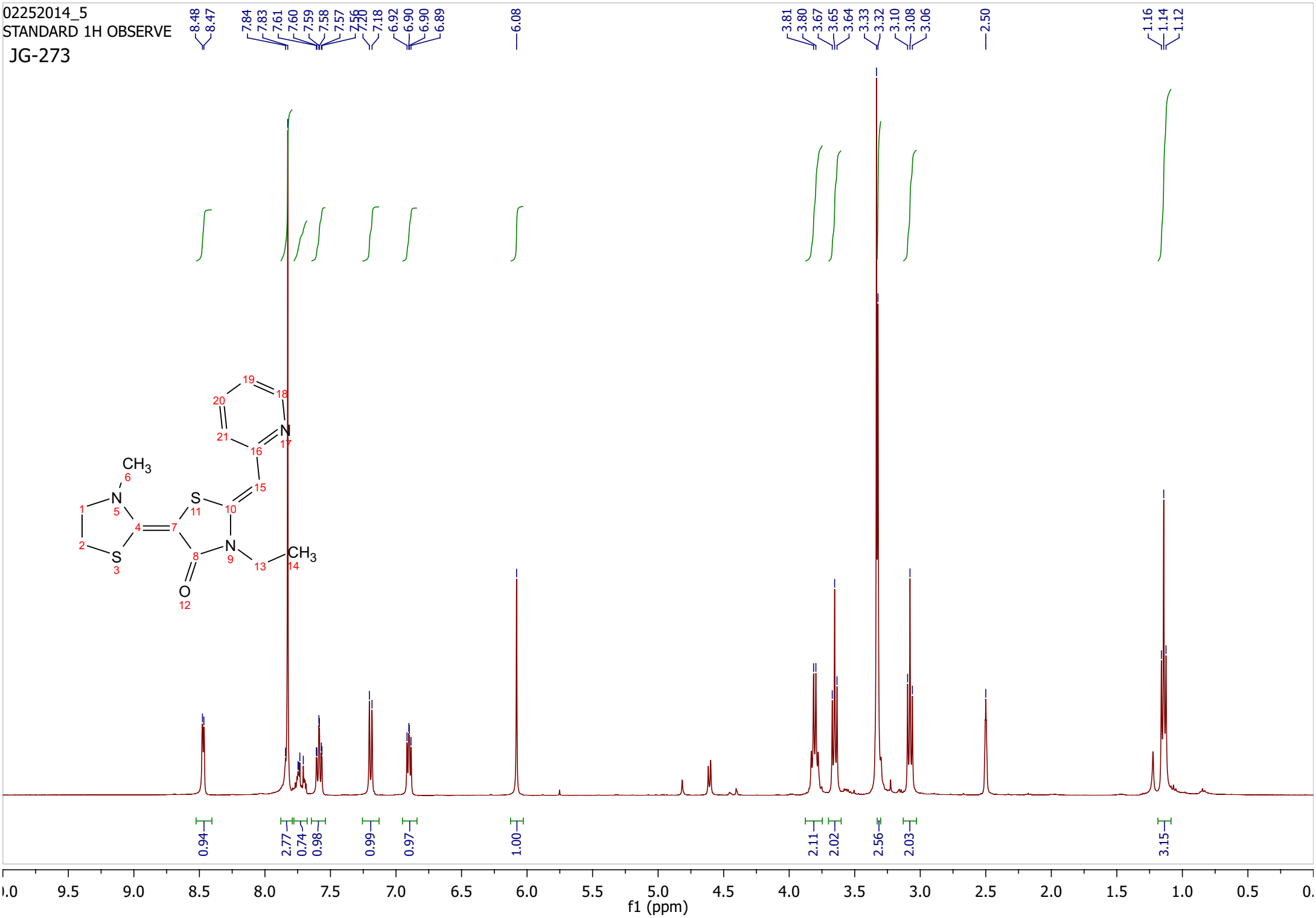
12132013_2
STANDARD 1H OBSERVE
Compound 27



f1 (ppm)

12062013_6
STANDARD 1H OBSERVE
Compound 28




02252014_5
STANDARD 1H OBSERVE
JG-273
8.48
8.47
7.84
7.83
7.61
7.60
7.59
7.58
7.57
7.56
7.20
7.18
6.92
6.90
6.90
6.89
6.08
3.81
3.80
3.67
3.65
3.64
3.33
3.32
3.10
3.08
3.06
2.50
1.16
1.14
1.12
0.94
2.77
0.74
0.98
0.99
0.97
1.00
2.11
2.02
2.56
2.03
3.15
f1 (ppm)