Supporting Information

A Novel CUG^{exp}·MBNL1 Inhibitor with Therapeutic Potential for Myotonic Dystrophy Type 1

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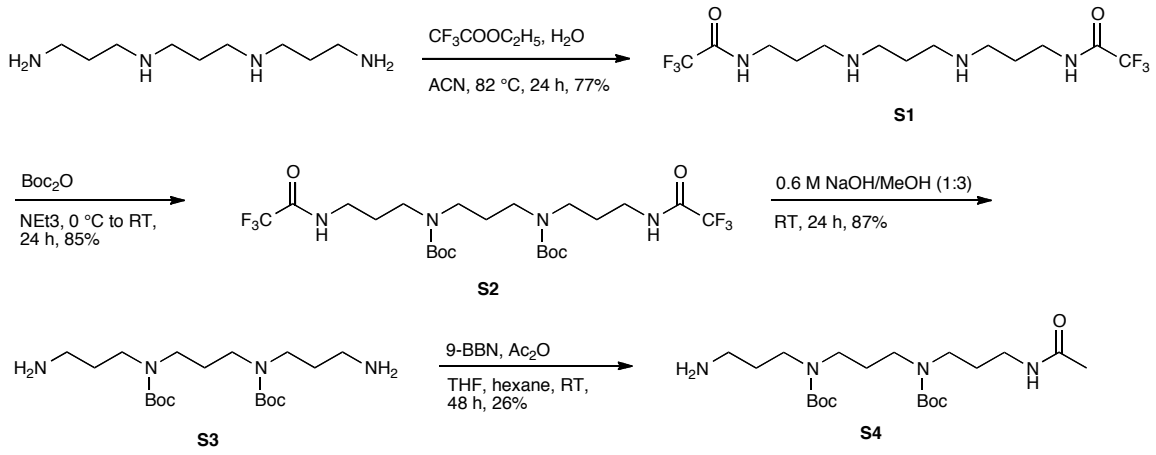
Instrumentation and chemicals

All reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were obtained from an anhydrous solvent dispensing system. For all reactions employing anhydrous solvents, glassware was oven-dried, cooled under vacuum, and then purged and conducted under dry nitrogen. Purified compounds were further dried under high vacuum (0.01–0.05 Torr) or lyophilized using a Labconco lyophilizer. Yields refer to purified and spectroscopically pure compounds. NMR spectra were recorded at 23 °C on either Varian Unity 500 or Varian Unity Inova 500NB, operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C acquisitions, respectively. NMR spectra were processed using MestReNova software. Chemical shifts are reported in ppm and referenced to the corresponding residual nuclei in the following deuterated solvents: $CDCl_3$ (7.26 ppm ^{1}H, 77.16 ppm ^{13}C); DMSO (2.50 ppm ^{1}H, 39.52 ppm ^{13}C); D_2O (4.79 ppm ^{1}H); CD_3OD (3.31 ppm ^{1}H, 50.41 ppm ^{13}C). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), sext (sextet), dd (doublet of doublets), ddd (doublet of doublet of doublets), td (triplet of doublets), dt (doublet of triplets), m (multiplet), b (broad). Integration is provided and coupling constants, *J*, are reported in Hertz (Hz). ESI mass spectra were recorded using the Quattro or ZMD mass spectrometer. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All compounds described herein gave NMR and mass spectral data in accord with their structures. Ligand **1** gave a HRMS within 1 ppm of calculated values.

Synthesis and characterization



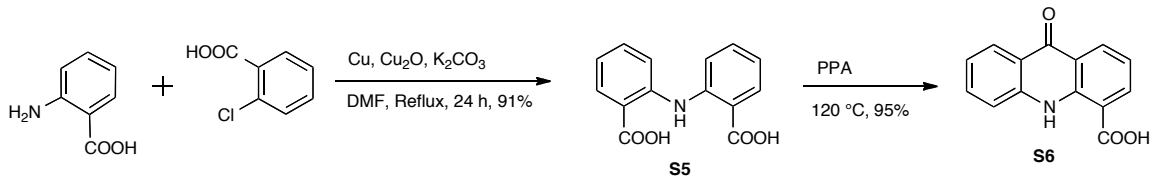
Supplementary Figure 1. Synthesis of *tert*-butyl (3-((3-acetamidopropyl)(*tert*-butoxycarbonyl)amino) propyl)(3-aminopropyl)carbamate (**S4**).

***N,N′*-((Propane-1,3-diylbis(azanediyl))bis(propane-3,1-diyl))bis(2,2,2-trifluoroacetamide) (S1).** Title compound was synthesized from *N,N′*-bis(3-aminopropyl)propane-1,3-diamine as described previously,[1] in 77% yield, with minor changes. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.61 (s, 2H), 8.75 (bs, 2H), 3.29 – 3.23 (m, 4H), 2.98 (t, J = 7.5 Hz, 4H), 2.92 (t, J = 7.5 Hz, 4H), 1.91 (p, J = 7.8 Hz, 2H), 1.82 (p, J = 7.0 Hz, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.40, 117.08, 44.63, 44.02, 36.61, 25.22, 22.56; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 381.2; found 381.2.

Di-*tert*-Butyl propane-1,3-diylbis((3-(2,2,2-trifluoroacetamido)propyl) carbamate) (S2). Title compound was synthesized as described previously,[1, 2] with minor changes. The product was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH, 98:2 to 95:5) in 85% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.42 (bs, 2H), 3.18 – 3.07 (m, 12H), 1.71 – 1.60 (m, 6H), 1.37 (s, 18H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 581.3; found 581.3.

Di-*tert*-Butyl propane-1,3-diylbis((3-aminopropyl)carbamate (S3). Title compound was synthesized as described previously,[1] with minor changes. The product was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH:NH_4OH, 80:19:1 to 67:30:2) to yield the pure product in 87% yield. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.28 (bs, 4H), 3.16 (bs, 4H), 2.69 (t, J = 6.4 Hz, 4H), 1.79 – 1.71 (m, 2H), 1.64 (p, J = 6.8 Hz, 4H), 1.45 (s, 18H); ^{13}C NMR (125 MHz, Chloroform-*d*) δ 155.29, 79.06, 44.44, 38.96, 32.34, 31.73, 28.17; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 389.3; found 389.3.

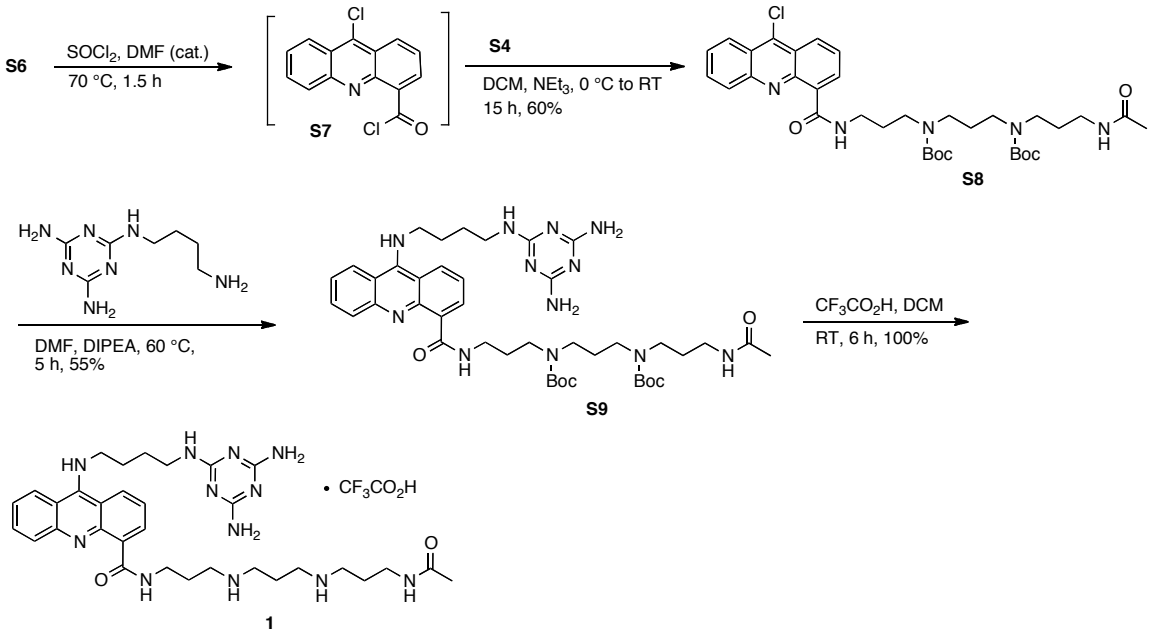
***tert*-Butyl (3-((3-acetamidopropyl)(*tert*-butoxycarbonyl)amino)propyl)(3-aminopropyl) carb-amate (S4).** Compound **S3** (1.1 g, 2.83 mmol, 1 eq.) was dissolved in THF (15 mL). A 0.5 M solution of 9-Borabicyclo(3.3.1)nonane (5.66 mL, 2.83 mmol, 1 eq.) was added and the reaction was stirred at room temperature for 3 hours.[3] Acetic anhydride (275 mg, 2.68 mmol, 0.95 eq.) was added and the reaction was allowed to proceed at room temperature overnight. The reaction was quenched with water (10 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over magnesium sulfate and filtered through celite. The solution was concentrated by rotary evaporation, purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH:NH4OH, 94:5:0.5 to 89:10:1) to afford **S4** (1.154 g, 26%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.32 – 3.10 (m, 10H), 2.71 (t, J = 6.7 Hz, 2H), 1.98 (s, 3H), 1.82 (s, 2H), 1.78 – 1.72 (m, 2H), 1.69 – 1.64 (m, 2H), 1.44 (s, 18H); m/z LRMS (ESI) calculated for $[M+H]^+$: 431.3; found 431.3.



Supplementary Figure 2. Synthesis of 9-oxo-9,10-dihydroacridine-4-carboxylic acid (**S6**).

2,2'-Azanediyldibenzoic acid (S5). Title compound was synthesized as described previously,[4] in 91% yield, with minor changes in the work-up procedure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.91 (dd, J = 7.9, 1.3 Hz, 2H), 7.50 – 7.40 (m, 4H), 6.95 (ddd, J = 8.0, 6.8, 1.5 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d6) δ 168.41, 143.58, 133.38, 131.81, 119.99, 117.56, 113.56; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 258.1; found 258.1.

9-Oxo-9,10-dihydroacridine-4-carboxylic acid (S6). Title compound was synthesized as described previously,[4] in 95% yield, with minor changes in the work-up procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (bs, 1H), 8.53 (dd, J = 8.0, 1.5 Hz, 1H), 8.45 (dd, J = 7.5, 1.6 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.83 – 7.73 (m, 2H), 7.41 – 7.29 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d6) δ 176.53, 169.14, 141.20, 139.92, 136.90, 134.11, 132.41, 125.89, 122.32, 121.63, 120.60, 120.24, 118.63, 115.01 *m/z* LRMS (ESI) calculated for $[M+H]^+$: 240.1; found 240.1.



Supplementary Figure 3. Synthesis of *N*-(3-((3-((3-acetamidopropyl)amino)propyl)amino)propyl)-9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide (**1**).

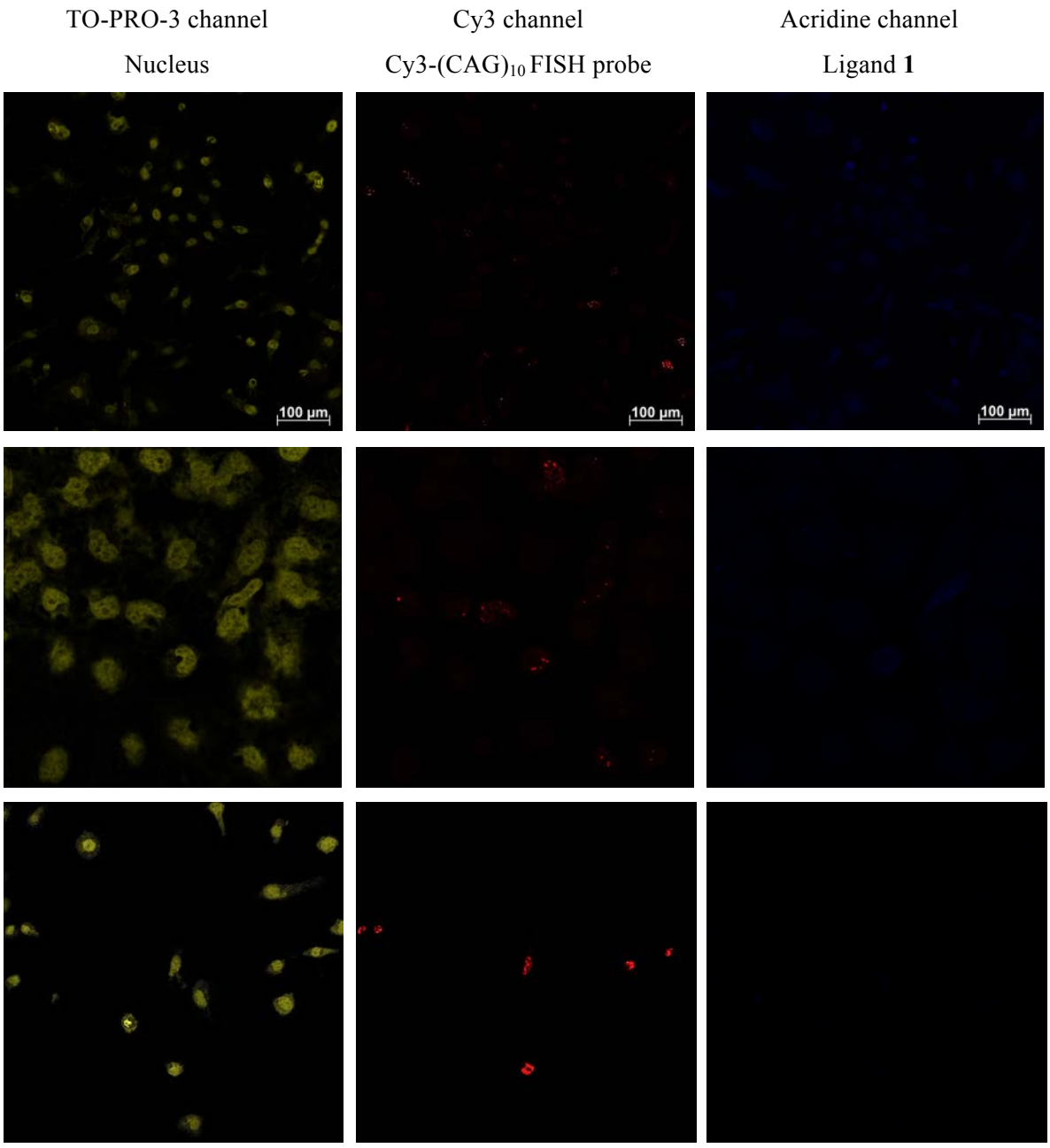
***tert*-Butyl (3-((3-acetamidopropyl)(*tert*-butoxycarbonyl)amino)propyl)(3-(9-chloroacridine-4-carboxamido)propyl)carbamate (S8).** A round-bottom flask, equipped with a stir bar, was charged with **S10** (600 mg, 2.5 mmol, 1 eq.) and freshly distilled thionyl chloride (3 mL, 41 mmol, 16.4 eq.). A catalytic amount of DMF was added and heated gently under reflux at 69 °C, stirring until homogeneous and then for 1 h. The excess thionyl chloride was distilled off and the last traces of it were removed azeotropically via co-evaporation with DCM (3 x 50 mL). It was left under vacuum (minimally) for 1 h to afford the crude intermediate as a yellow powder. The crude intermediate was dissolved in anhydrous DCM. Anhydrous triethylamine was added to the solution until the pH was 11 and it was cooled to 0 °C. Compound **S4** (1065 mg, 2.75 mmol, 1.1 eq.) was added and the solution was stirred at 0 °C for 2 hours and slowly warmed to room temperature overnight. The solvent was removed by rotary evaporation and the crude mixture was purified via flash chromatography (SiO2; CH_2Cl_2:MeOH, 98:2 to 95:5) to yield **S8** as a yellow solid (586 mg, 60%); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 670.3; found 670.3.

***tert*-Butyl (3-((3-acetamidopropyl)(*tert*-butoxycarbonyl)amino)propyl)(3-(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamido)propyl)carbamate (S9).** A round-bottom flask, equipped with a stir bar, was charged with **S8** (500 mg, 0.576 mmol, 1 eq.) and of *N*-(4-Aminobutyl)-[1,3,5]triazine-2,4,6-triamine (125 mg, 0.635 mmol, 1.1 eq.). DIPEA (163 mg, 1.26 mmol, 2.2 eq.) and anhydrous DMF (25 mL) were added. The solution was

heated at 70 °C for 5 hours. The solvent was removed by rotary evaporation and the product was purified via flash chromatography (Basic Alumina; DCM:Methanol:NH_4OH, from 95:4.9:0.1 to 90:9.5:0.5) to yield **S9** as a yellow solid (377 mg, 0.317 mmol, 55%); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 831.5; found 831.5.

***N*-(3-((3-((3-Acetamidopropyl)amino)propyl)amino)propyl)-9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide (1).** A round-bottom flask, equipped with a stir bar, was charged with **S9** (310mg, 0.261 mmol, 1eq.). TFA (30 mL) and anhydrous DCM (70 mL) were added and stirred at room temperature for 6 h. The solvents were removed to yield **1** as a yellow solid (437 mg, 0.261 mmol, 100%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 8.33 (d, J = 8.6 Hz, 1H), 8.16 (dd, J = 13.0, 8.0 Hz, 2H), 7.90 – 7.84 (m, 2H), 7.58 (d, J = 8.5 Hz, 1H), 7.46 (t, J = 7.9 Hz, 2H), 4.02 (t, J = 6.7 Hz, 2H), 3.56 (t, J = 7.0 Hz, 2H), 3.29 – 3.10 (m, 10H), 3.06 – 3.03 (m, 2H), 2.14 – 2.06 (m, 4H), 1.96 (s, 3H), 1.93 – 1.85 (m, 4H), 1.70 – 1.63 (m, 2H); ^{13}C NMR (125 MHz, Deuterium Oxide) δ 174.89, 168.78, 165.12, 163.37, 163.09, 162.80, 162.52, 159.99, 159.45, 158.02, 156.64, 136.13, 134.88, 120.06, 119.18, 118.71, 117.74, 115.41, 113.09, 48.84, 45.74, 45.38, 44.74, 40.38, 37.07, 36.16, 31.59, 25.78, 24.87, 22.89, 21.91; m/z HRMS (ESI) calculated for $[M+H]^+$: 631.3945; found 631.3944.

(a)



(b)

Total number of cells	64	32	18
Cells with foci	17 (27%)	8 (25%)	6 (33%)

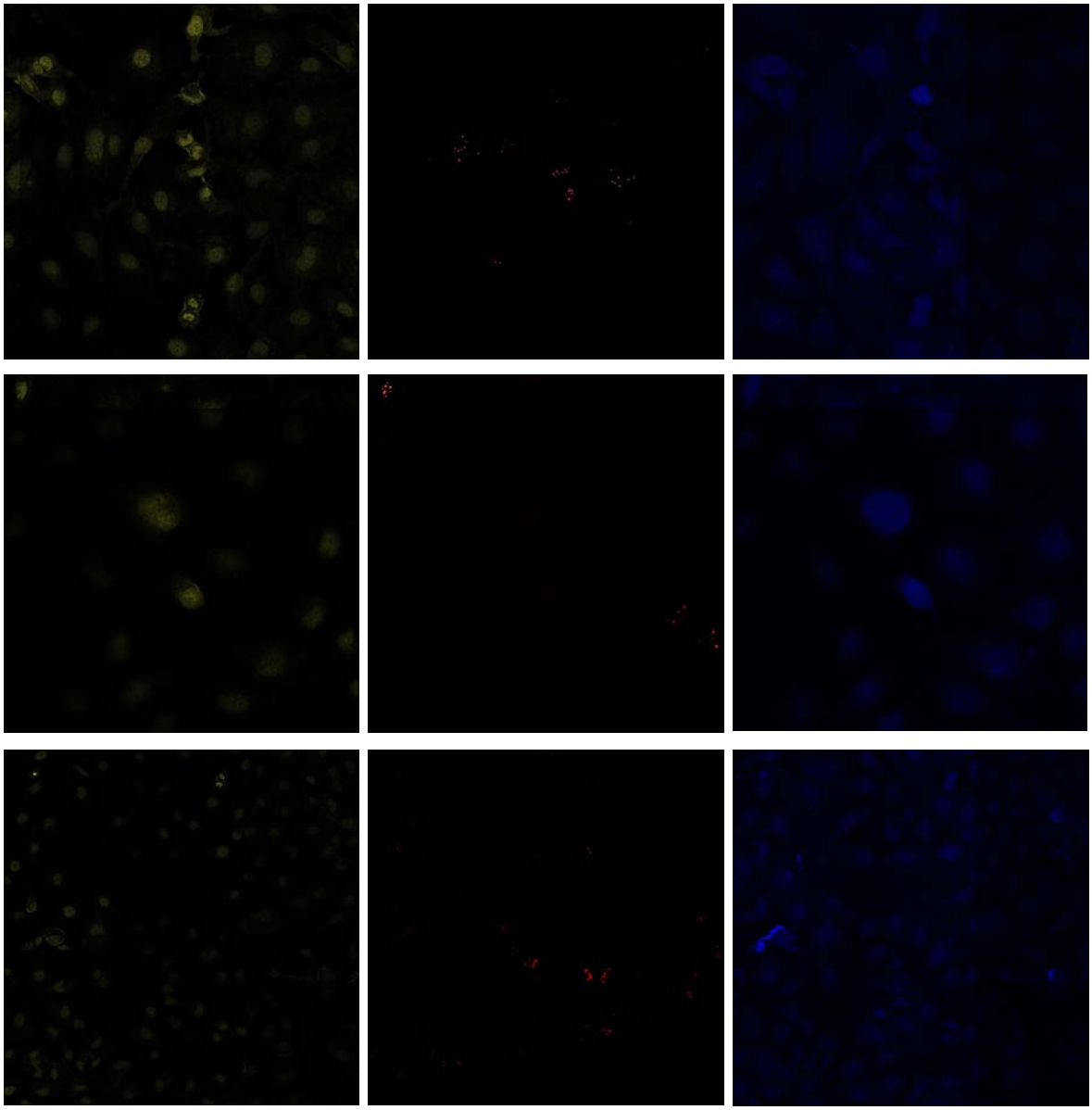
Supplementary Figure 4. CUG_{960} transfected HeLa cells (DM1 cell model) without ligand treatment. a) Three visible excitation channels (639, 555 and 408 nm) were used to detect To-Pro-3, Cy3 and acridine ring, respectively. These channels indicate the presence of nuclei, Cy3-$(CAG)_{10}$ FISH probe and **1**, respectively. b) 28% of DM1 model cells show CUG^{exp} nuclear foci in DM1 cell model.

(a)

TO-PRO-3 channel
Nucleus

Cy3 channel
Cy3-$(CAG)_{10}$ FISH probe

Acridine channel
Ligand **1**

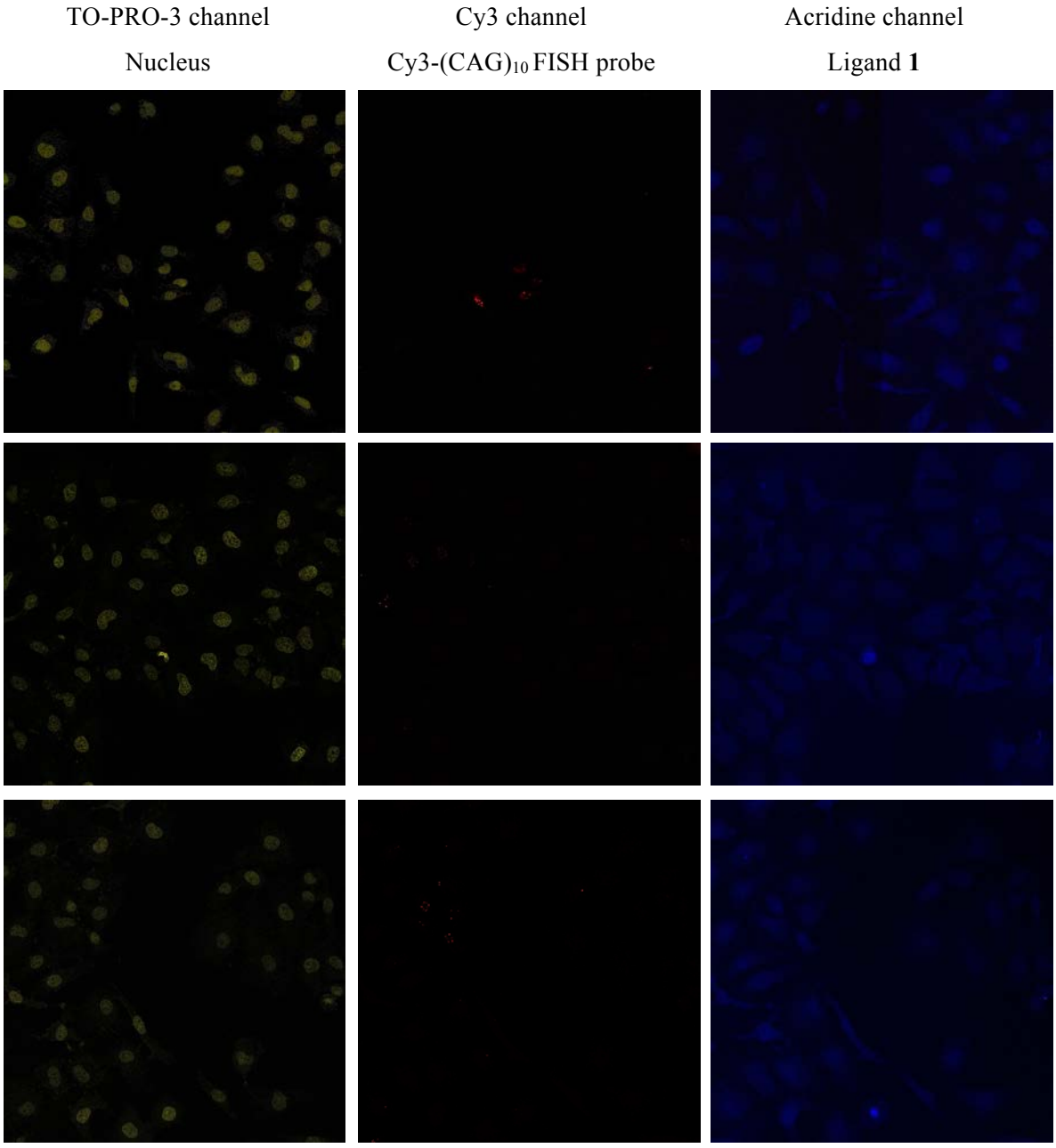


(b)

Total number of cells	49	18	114
Cells with foci	10 (20%)	3 (17%)	18 (16%)

Supplementary Figure 5. CUG_{960} transfected HeLa cells (DM1 cell model) treated with [**1**] = 20 µM. a) Three visible excitation channels (639, 555 and 408 nm) were used to detect To-Pro-3, Cy3 and acridine ring, respectively. These channels indicate the presence of nuclei, Cy3-$(CAG)_{10}$ FISH probe and **1**, respectively. b) 18% of DM1 model cells show CUG^{exp} nuclear foci in the presence of [**1**] = 20 µM.

(a)

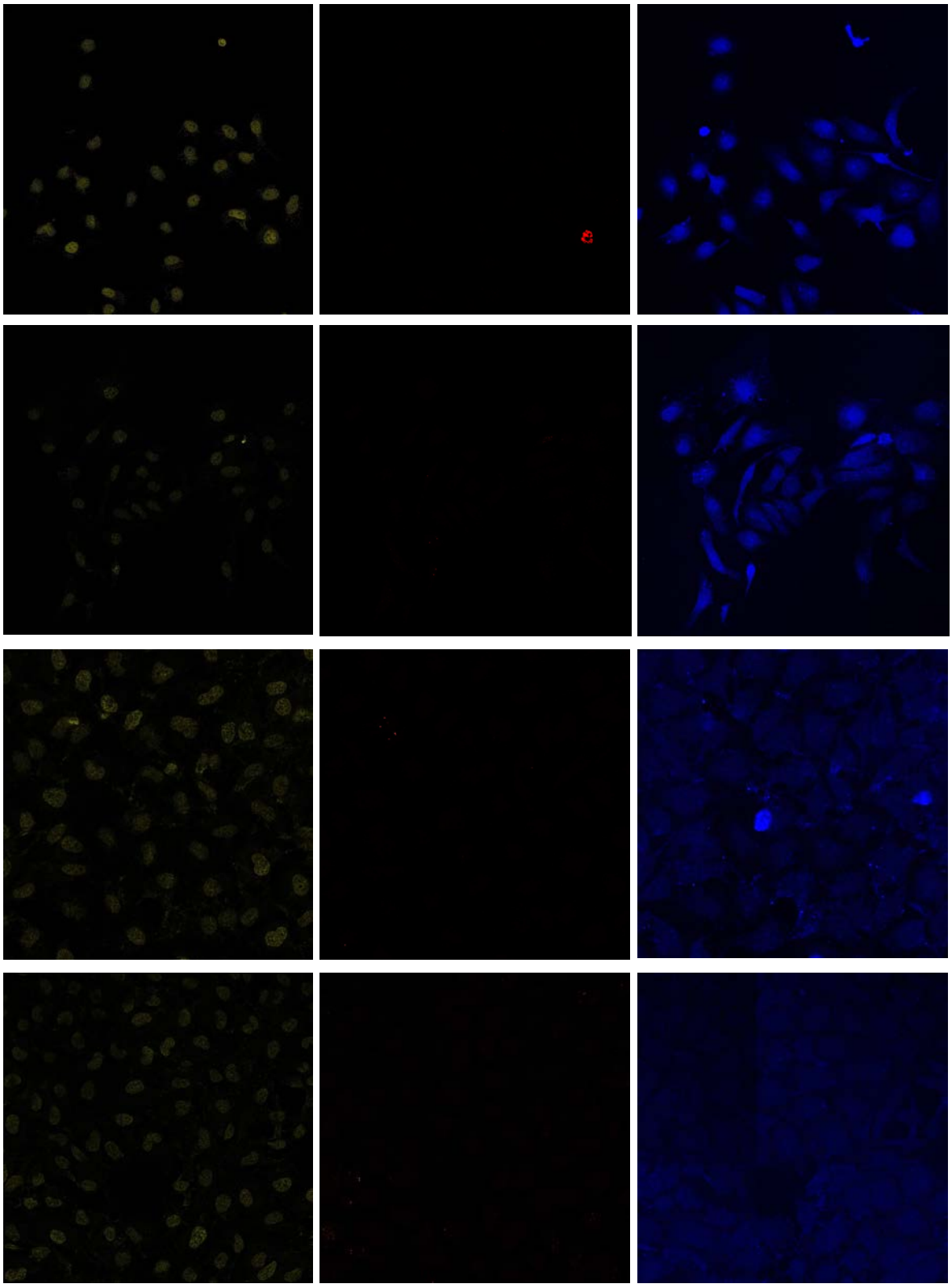


(b)

Total number of cells	50	58	43
Cells with foci	4 (8%)	6 (10%)	4 (9%)

Supplementary Figure 6. CUG_{960} transfected HeLa cells (DM1 cell model) treated with [**1**] = 50 µM. a) Three visible excitation channels (639, 555 and 408 nm) were used to detect To-Pro-3, Cy3 and acridine ring, respectively. These channels indicate the presence of nuclei, Cy3-$(CAG)_{10}$ FISH probe and **1**, respectively. b) 9% of DM1 model cells show CUG^{exp} nuclear foci in the presence of [**1**] = 50 µM.

(a)

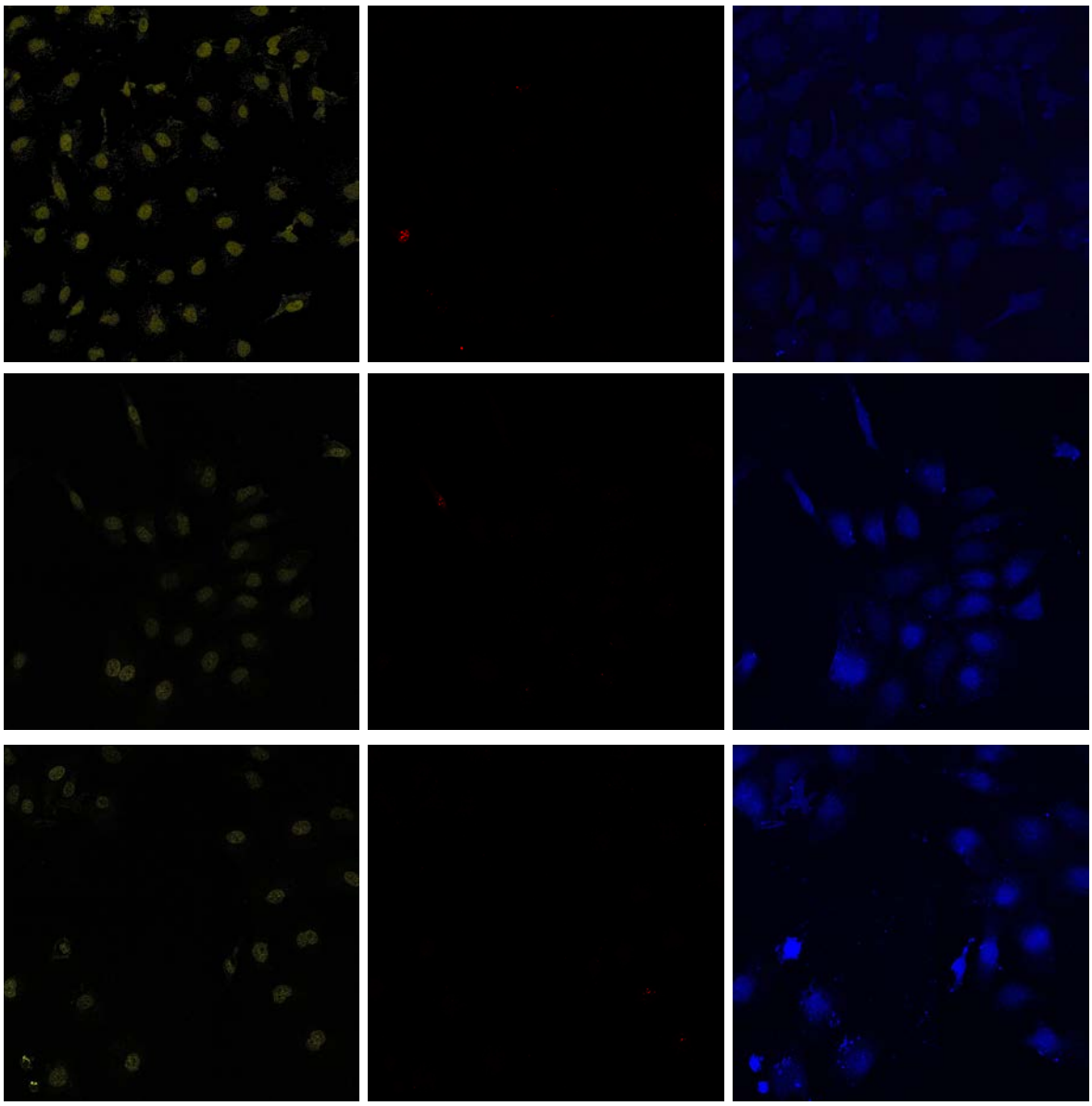

Cy3 channel
Acridine channel
Nucleus
Cy3-(CAG)10 FISH probe
Ligand 1

(b)

Total number of cells	30	32	63	94
Cells with foci	1 (3%)	2 (6%)	2 (3%)	5 (5%)

Supplementary Figure 7. CUG_{960} transfected HeLa cells (DM1 cell model) treated with [**1**] = 75 μM. a) Three visible excitation channels (639, 555 and 408 nm) were used to detect To-Pro-3, Cy3 and acridine ring, respectively. These channels indicate the presence of nuclei, Cy3-$(CAG)_{10}$ FISH probe and **1**, respectively. b) 4% of DM1 model cells show CUG^{exp} nuclear foci in the presence of [**1**] = 75 μM.

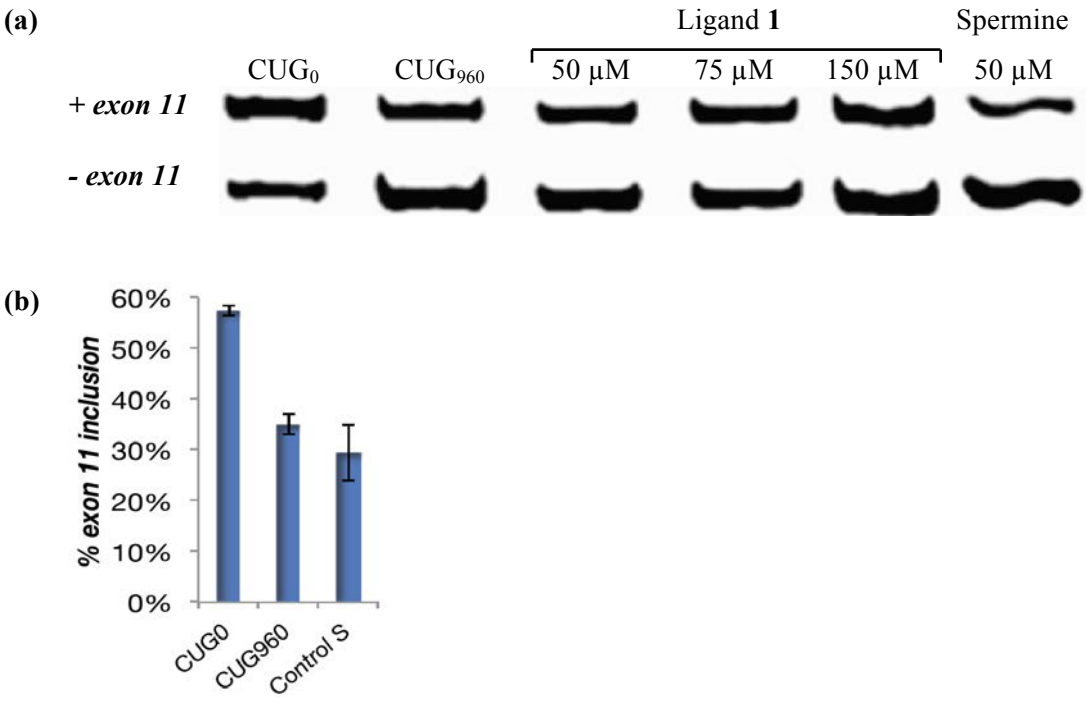
(a)



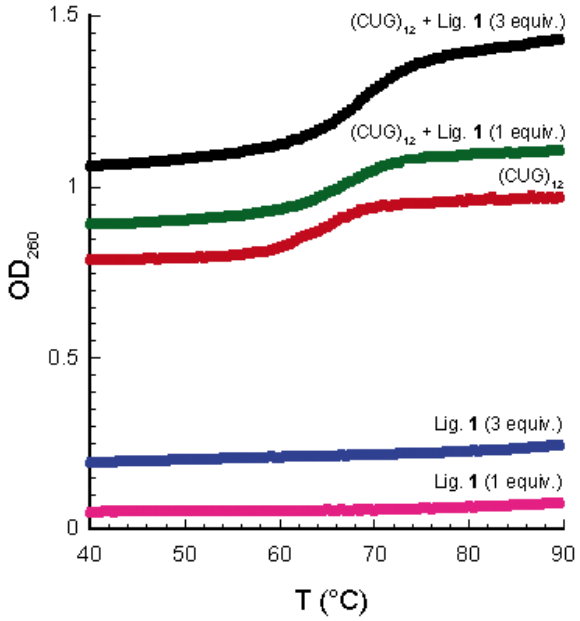
(b)

Total number of cells	50	27	26
Cells with foci	3 (6%)	1 (4%)	1 (4%)

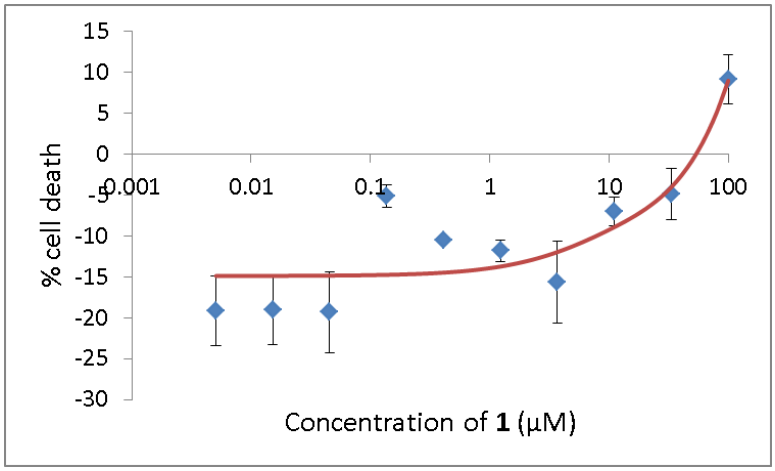
Supplementary Figure 8. CUG_{960} transfected HeLa cells (DM1 cell model) treated with [**1**] = 100 μM. a) Three visible excitation channels (639, 555 and 408 nm) were used to detect To-Pro-3, Cy3 and acridine ring, respectively. These channels indicate the presence of nuclei, Cy3-$(CAG)_{10}$ FISH probe and **1**, respectively. b) 5% of DM1 model cells show CUG^{exp} nuclear foci in the presence of [**1**] = 100 μM.



Supplementary Figure 9. Negative control ligand, spermine, at 50 µM does not improve the mis-splicing of IR in DM1 cell model. a) In this representative gel image of IR alternative splicing, two bands corresponding to IR isoforms A (+ exon 11) and B (- exon 11), respectively, are derived by reverse transcription-polymerase chain reaction (RT-PCR). DM1 cell model is treated with **1** at 50, 75 and 150 µM and spermine at 50 µM. **Note:** This image is derived from one gel but the lanes of interest have been cut and positioned adjacent to one another. b) A plot of the corresponding data shows no change in mis-splicing with spermine at 50 µM. The error bars represent mean ± standard error of 4-6 independent measurements for CUG_0 and CUG_{960} cells and 3 measurements for spermine-treated cells.



Supplementary Figure 10. Melting curves of $(CUG)_{12}$ in the absence and presence of one and three equivalents of **1**; ΔT_m of 2.5 °C and 5.5 °C were observed, respectively. **Note:** The assay was performed as described in the method section, however the flat part of the curve (10°C to 40°C) has been cut out and only The part of curve from 40°C to 90°C is shown here.

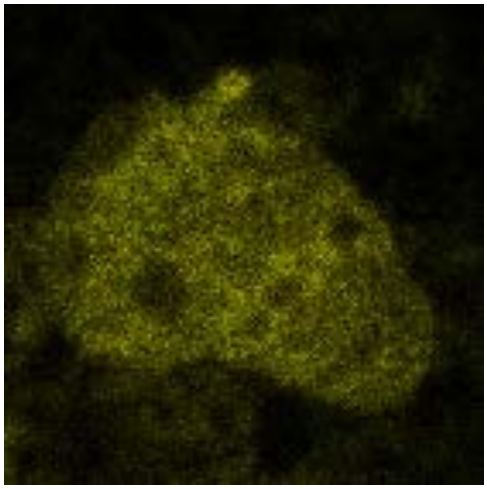


Supplementary Figure 11. Ligand **1** shows less than 10% cytotoxicity in concentrations up to 100 μM by the sulforhodamine B (SRB) assay. It was performed according to a previous method.[5] 98 μL of DMEM supplemented 10% FBS per well was placed in a 96-well plate. 2 μL of **1** with different concentrations from 0.5 μM to 10 mM were added, five repeats for each concentration. 100 μL of HeLa cell suspension of 10,000 cells per well were added in the 96-well plate. The cells were incubated at 37 °C for 24 h. 100 μL of cold 10% (wt/v) trichloroacetic acid were added in each well, followed by incubation at 4 °C for 1 h. The cells were washed twice with water and then air-dried. The cells were stained with 100 μL of 0.0057 % (wt/v) sulforhodamine B in 1% acetic acid at room temperature for 30 min. The plate was rinsed twice with 1% (v/v) acetic acid to remove unbound stain. The bound protein stain was solubilized in 200 μL of 10 mM Tris base, pH 10.5, and left for 30 min. The optical intensity was measured at 510 nm using a microplate reader. The cell growth inhibition was calculated using the following formula:

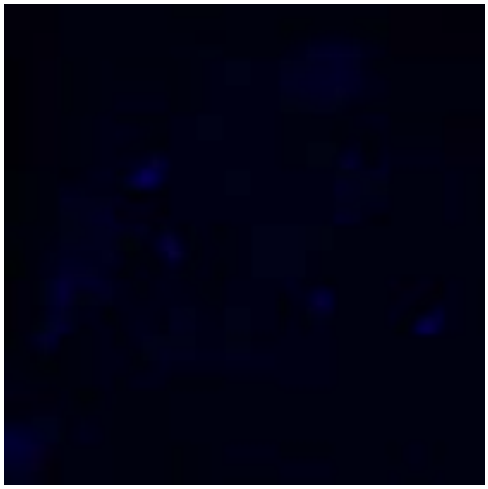
$$\%\ \text{Cell growth inhibition} = 100\% - \frac{\text{Mean}_{\text{OD sample}} - \text{Mean}_{\text{OD dead control}}}{\text{Mean}_{\text{OD live control}} - \text{Mean}_{\text{OD dead control}}} \times 100\%$$

The data was plotted using Excel. The curves were fitted using Table Curve (Systat).

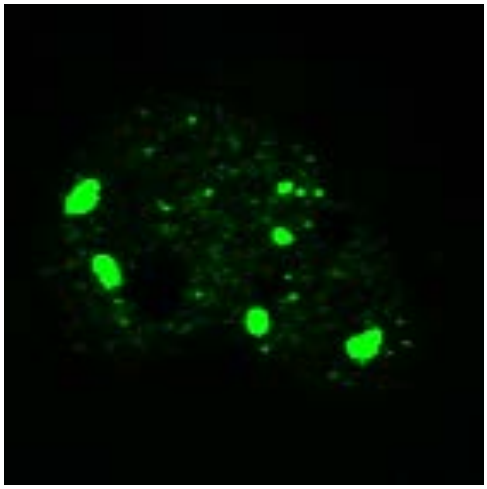
TO-PRO-3 channel/ Nucleus



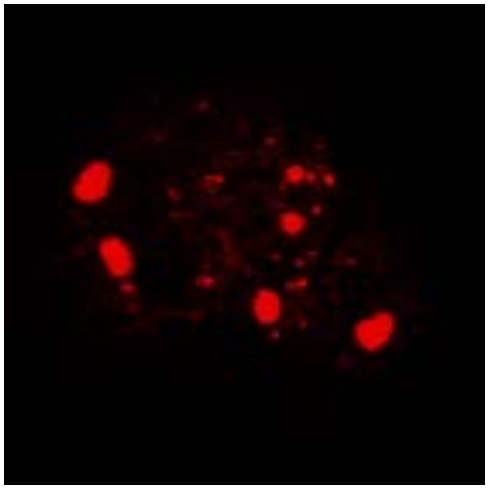
Acridine channel/ Ligand **1**



GFP channel/ GFP-MBNL1



Cy3 channel/ CUG^{exp}



Supplementary Figure 12. A magnified CUG_{960} transfected HeLa cell (DM1 cell model) showing ribonuclear foci.

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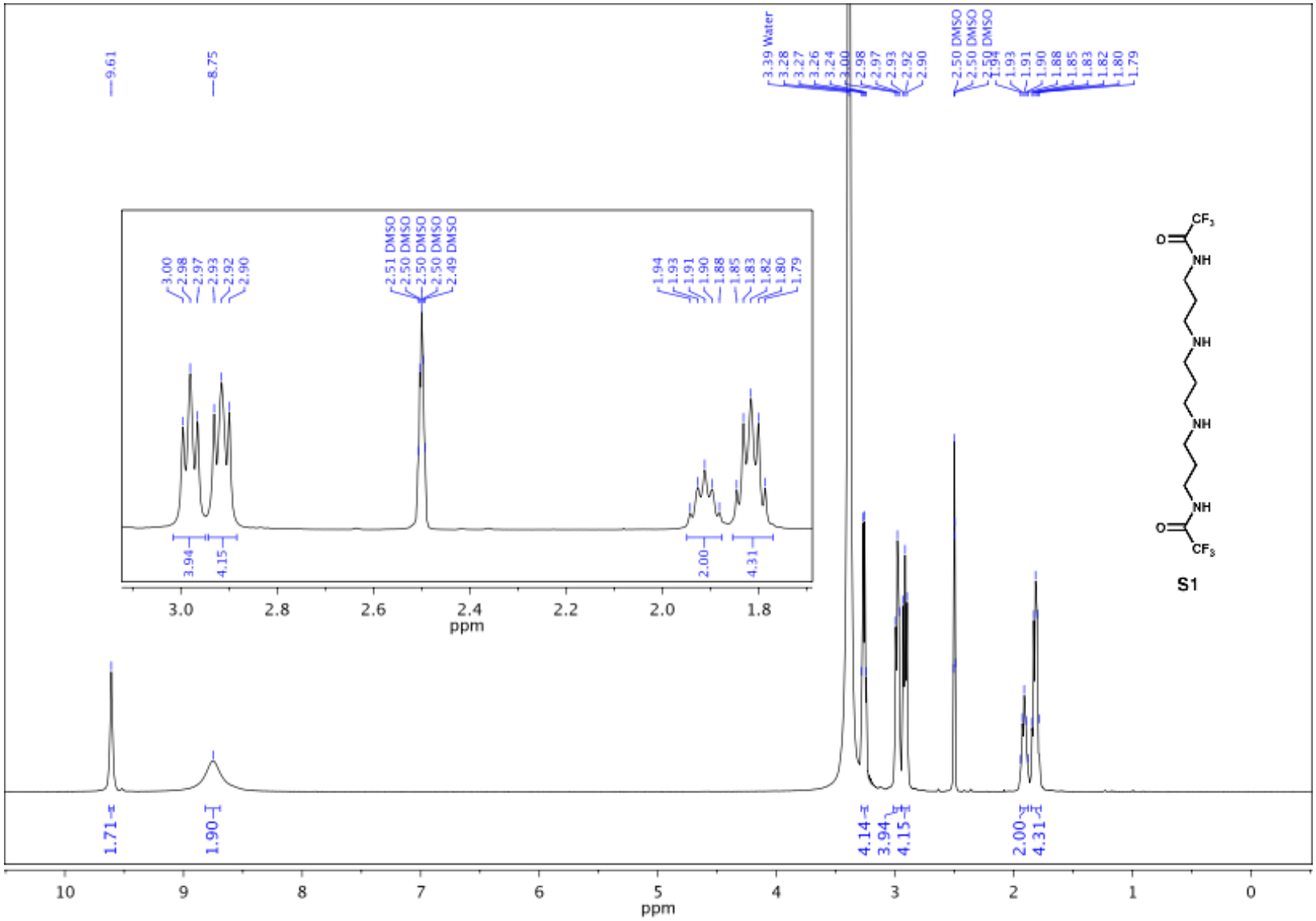
9.61
8.75
3.39 Water
3.28
3.27
3.26
3.24
3.00
2.98
2.97
2.93
2.92
2.90
2.50 DMSO
2.50 DMSO
2.50 DMSO
1.94
1.93
1.91
1.90
1.88
1.85
1.83
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2.4
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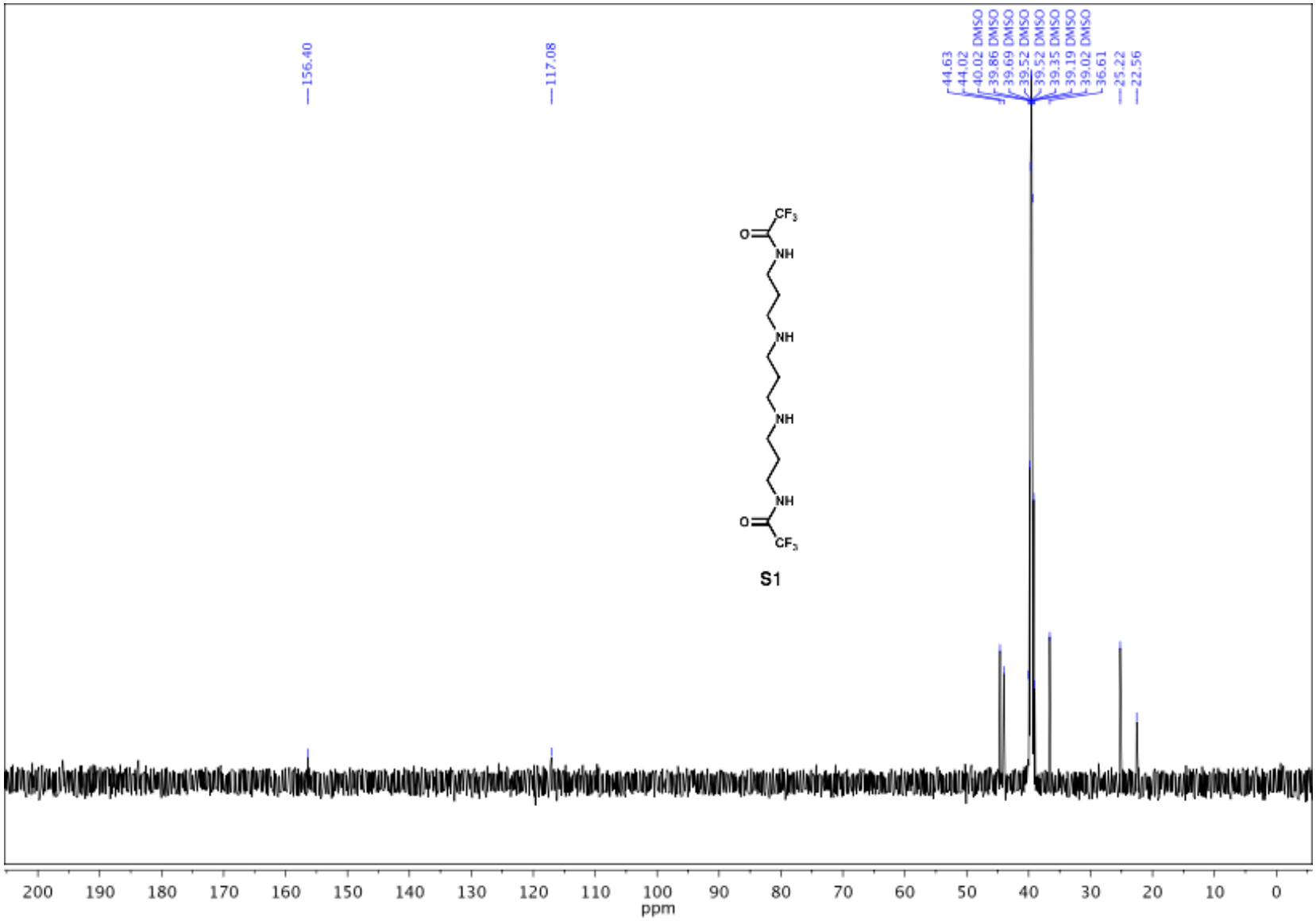
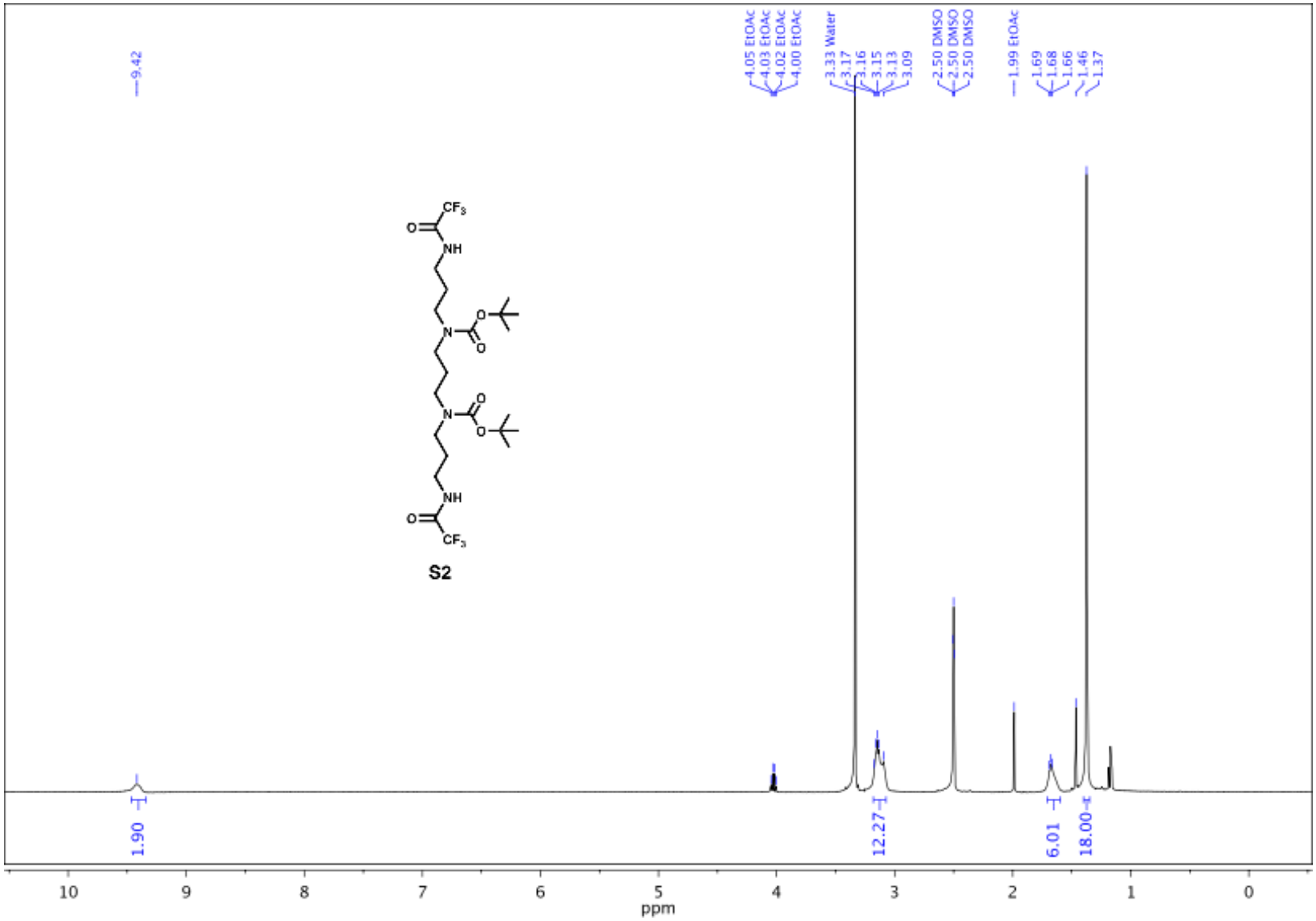
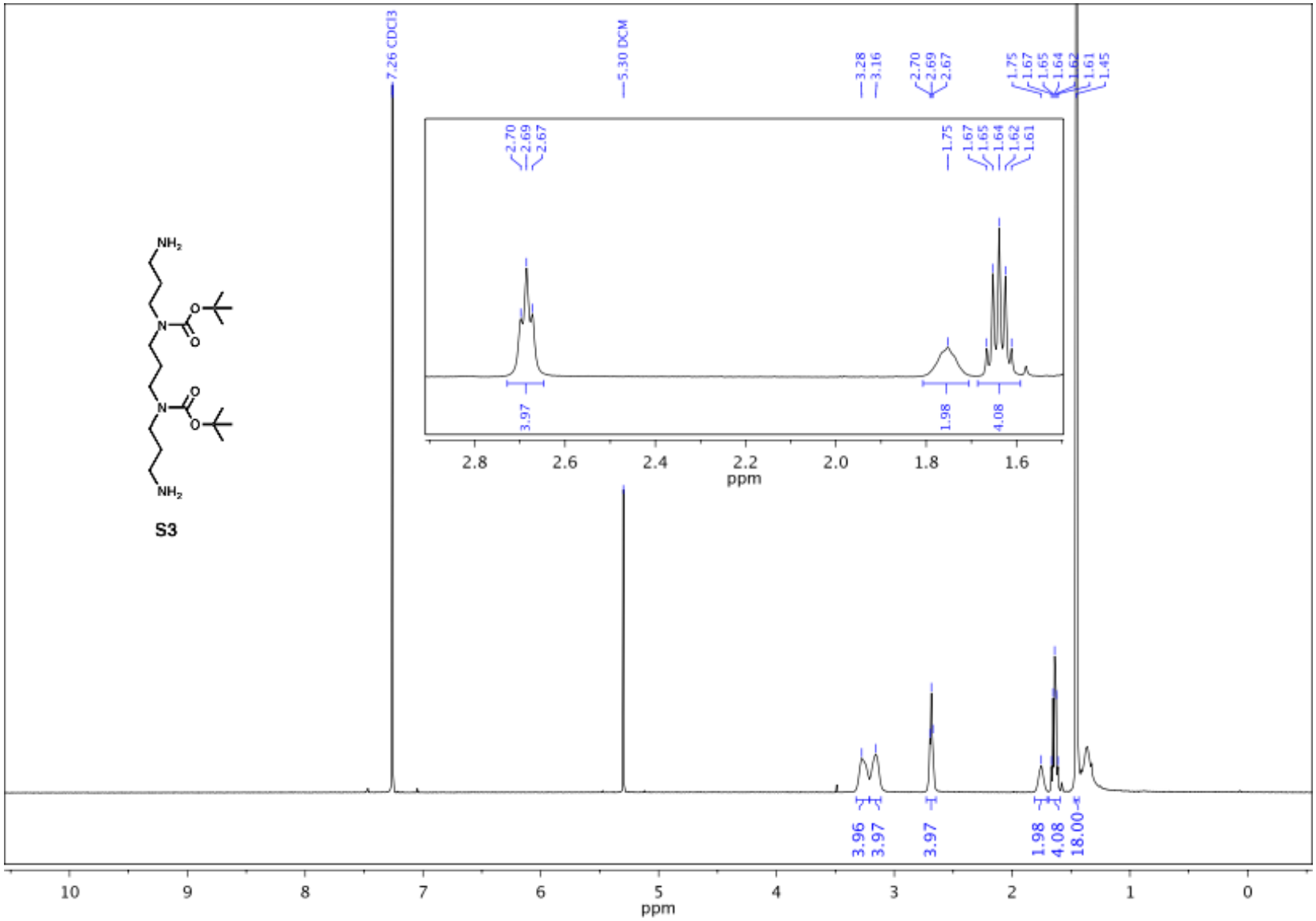
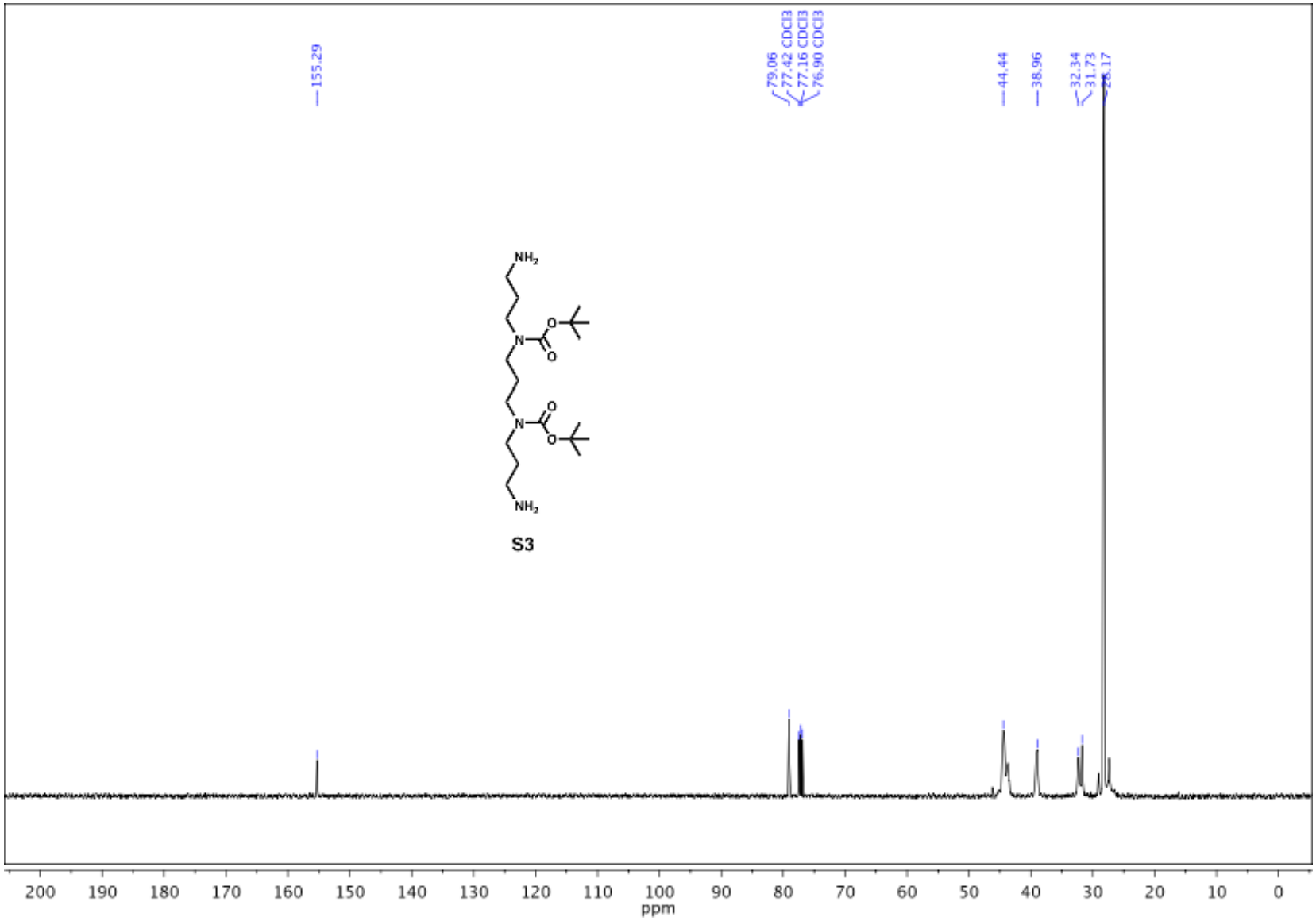
2
1
0
ppm

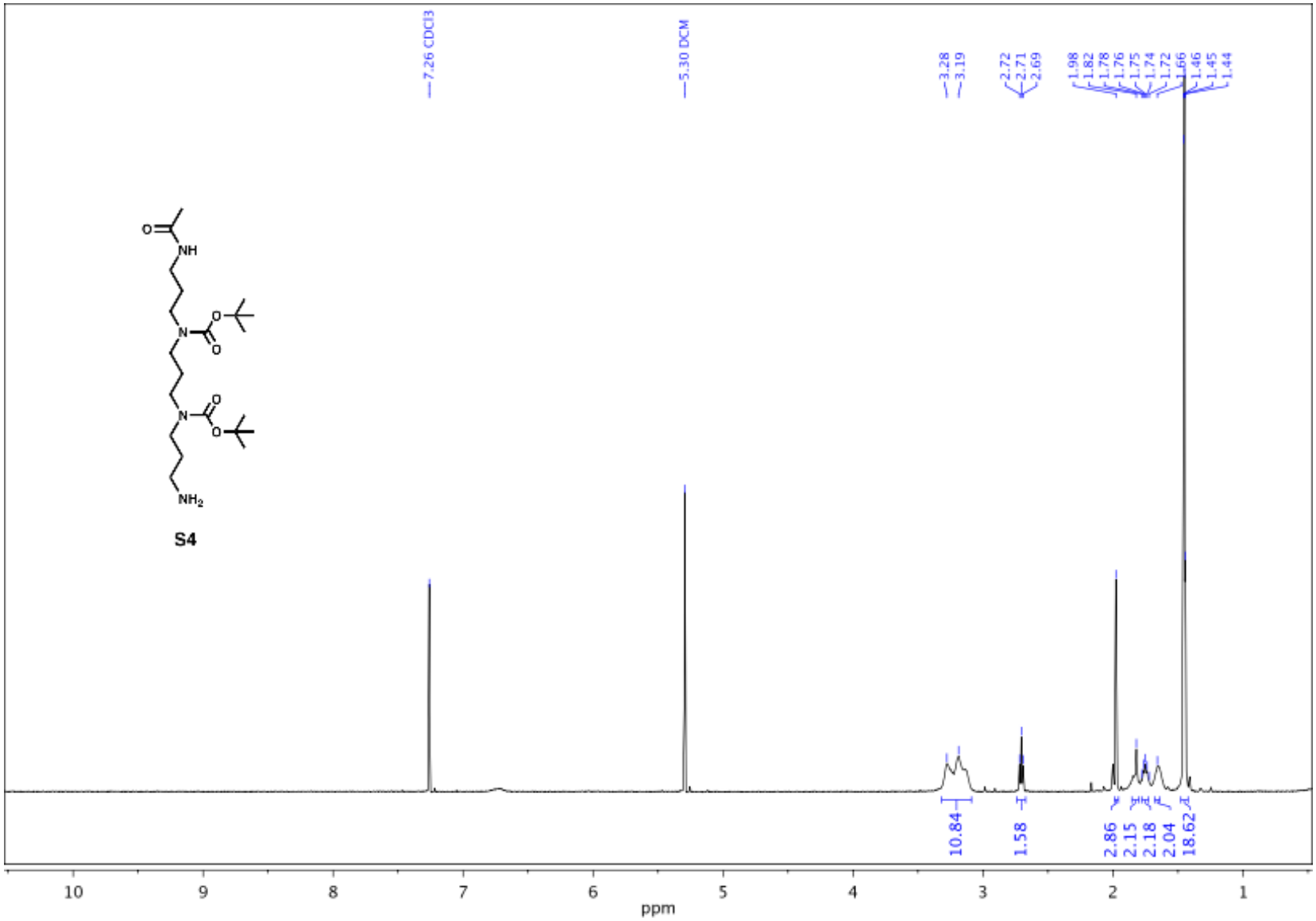

156.40
117.08
44.63
44.02
40.02 DMSO
39.86 DMSO
39.69 DMSO
39.52 DMSO
39.52 DMSO
39.35 DMSO
39.19 DMSO
39.02 DMSO
36.61
25.22
22.56
CF3
O
NH
NH
NH
NH
O
CF3
S1
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

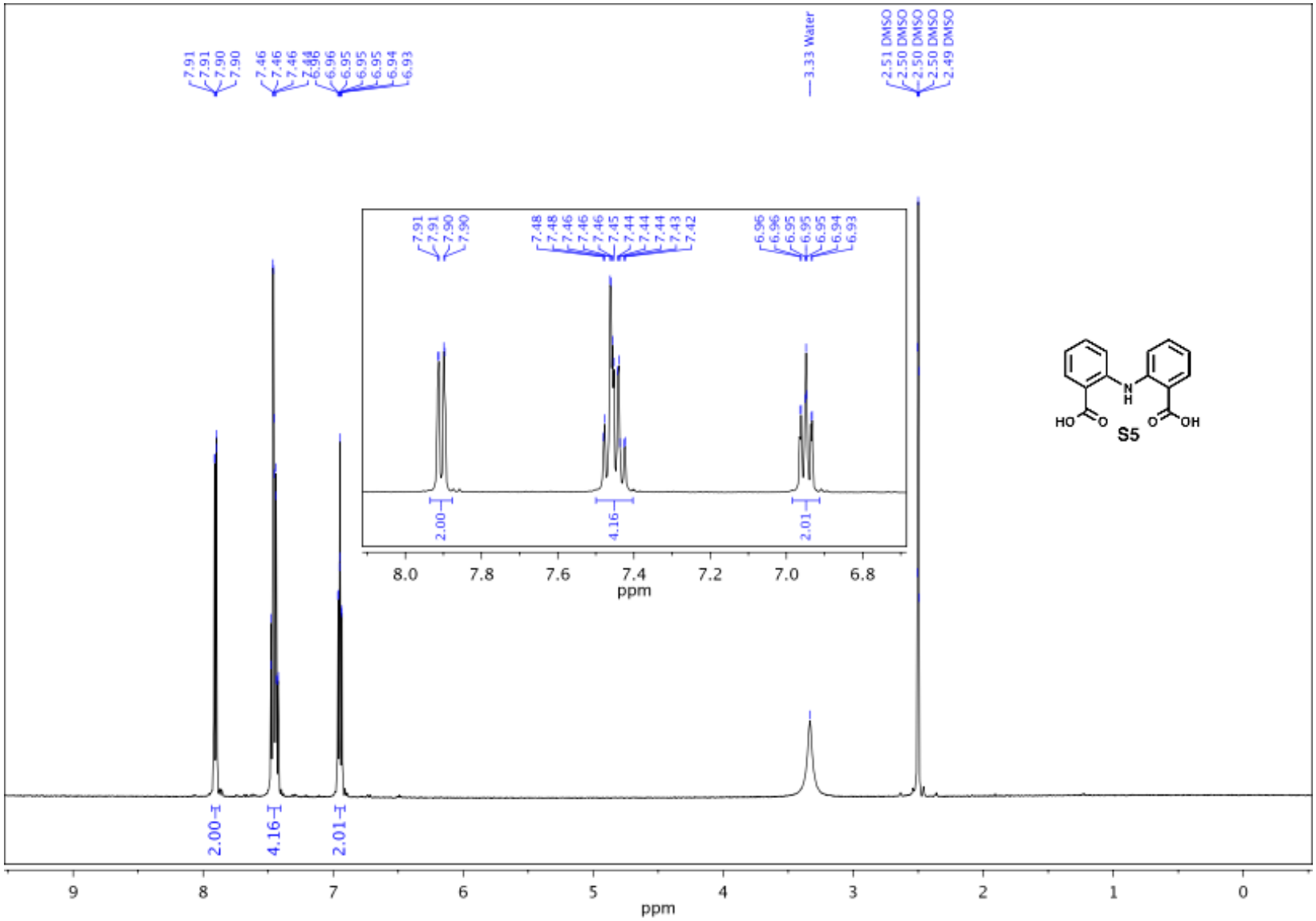

9.42
4.05 EtOAc
4.03 EtOAc
4.02 EtOAc
4.00 EtOAc
3.33 Water
3.17
3.16
3.15
3.13
3.09
2.50 DMSO
2.50 DMSO
2.50 DMSO
1.99 EtOAc
1.69
1.68
1.66
1.46
1.37
1.90
12.27
6.01
18.00
10
9
8
7
6
5
4
3
2
1
0
ppm

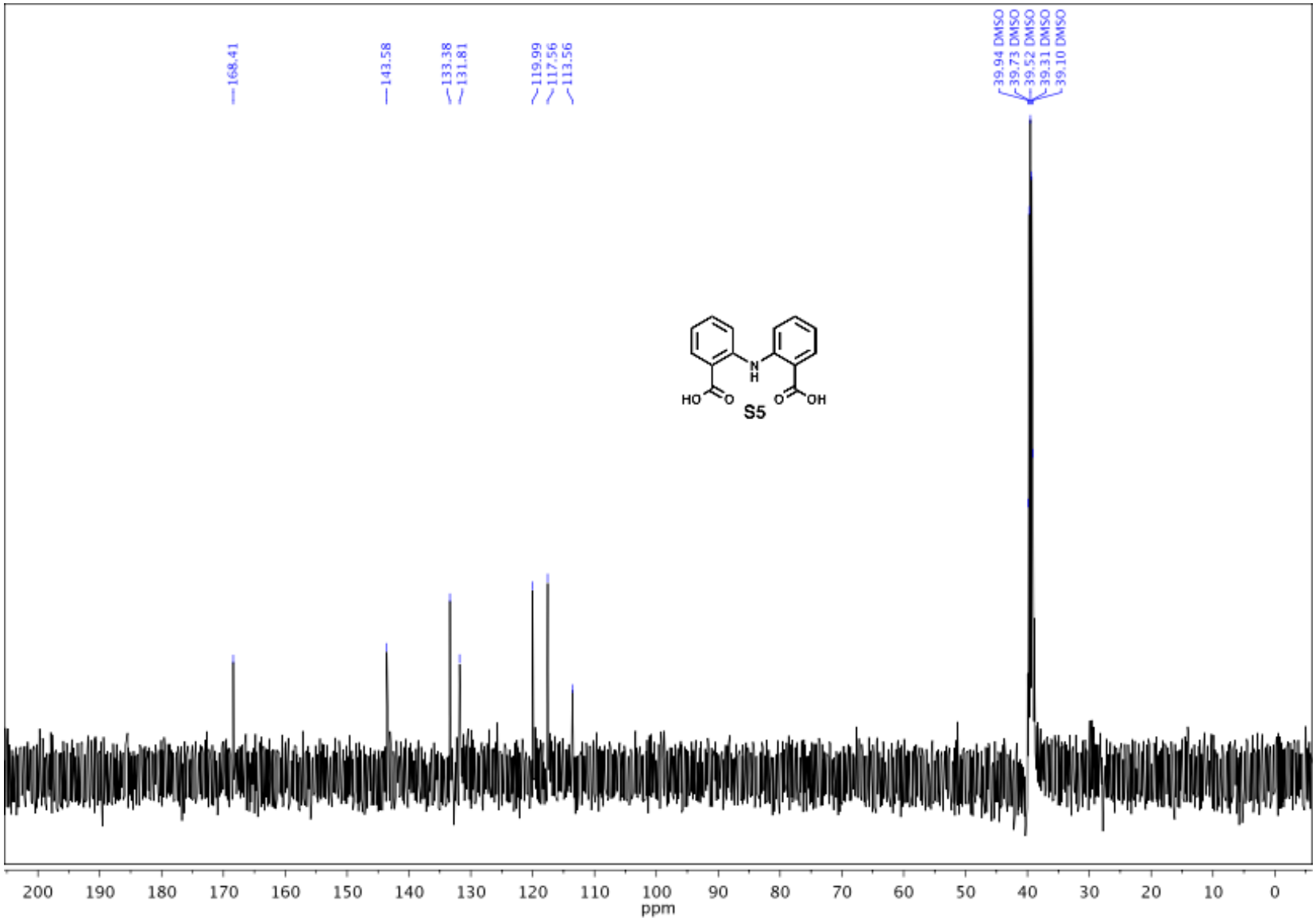
S2

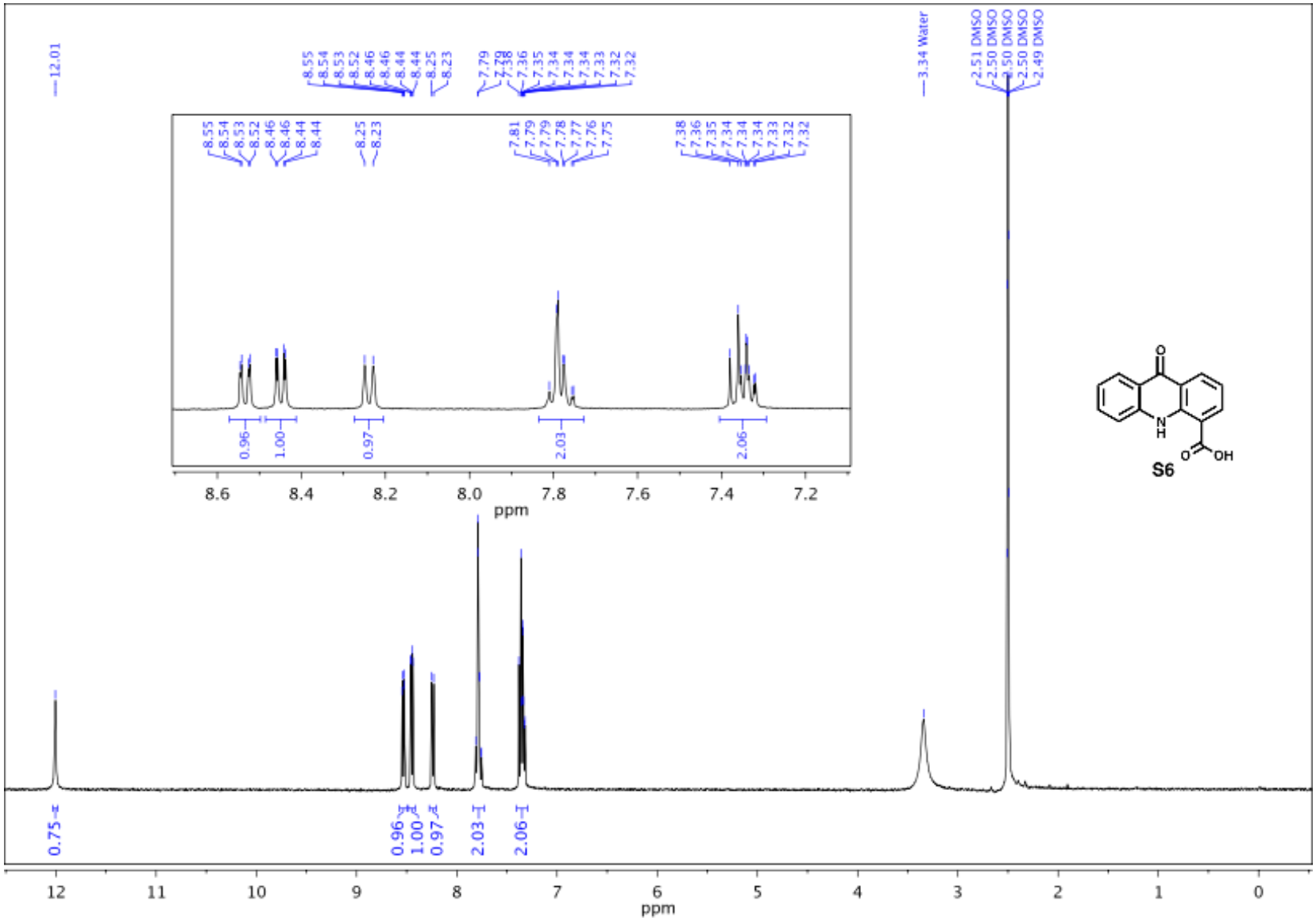

7.26 CDCl3
5.30 DCM
3.28
3.16
2.70
2.69
2.67
1.75
1.67
1.65
1.64
1.62
1.61
1.45
3.96
3.97
3.97
1.98
4.08
18.00
ppm
NH2
S3


155.29
79.06
77.42 CDCl3
77.16 CDCl3
76.90 CDCl3
44.44
38.96
32.34
31.73
28.17
NH2
O
N
O
O
O
N
O
O
NH2
S3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


7.26 CDCl3
5.30 DCM
3.28
3.19
2.72
2.71
2.69
1.98
1.82
1.78
1.76
1.75
1.74
1.72
1.66
1.46
1.45
1.44
10.84
1.58
2.86
2.15
2.18
2.04
18.62
ppm
S4


7.91
7.91
7.90
7.90
7.46
7.46
7.46
6.96
6.95
6.95
6.94
6.93
3.33 Water
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.00
4.16
2.01
ppm
S5


168.41
143.58
133.38
131.81
119.99
117.56
113.56
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
HO
O
N
H
O
OH
S5
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


12.01
8.55
8.54
8.53
8.52
8.46
8.46
8.44
8.44
8.25
8.23
7.79
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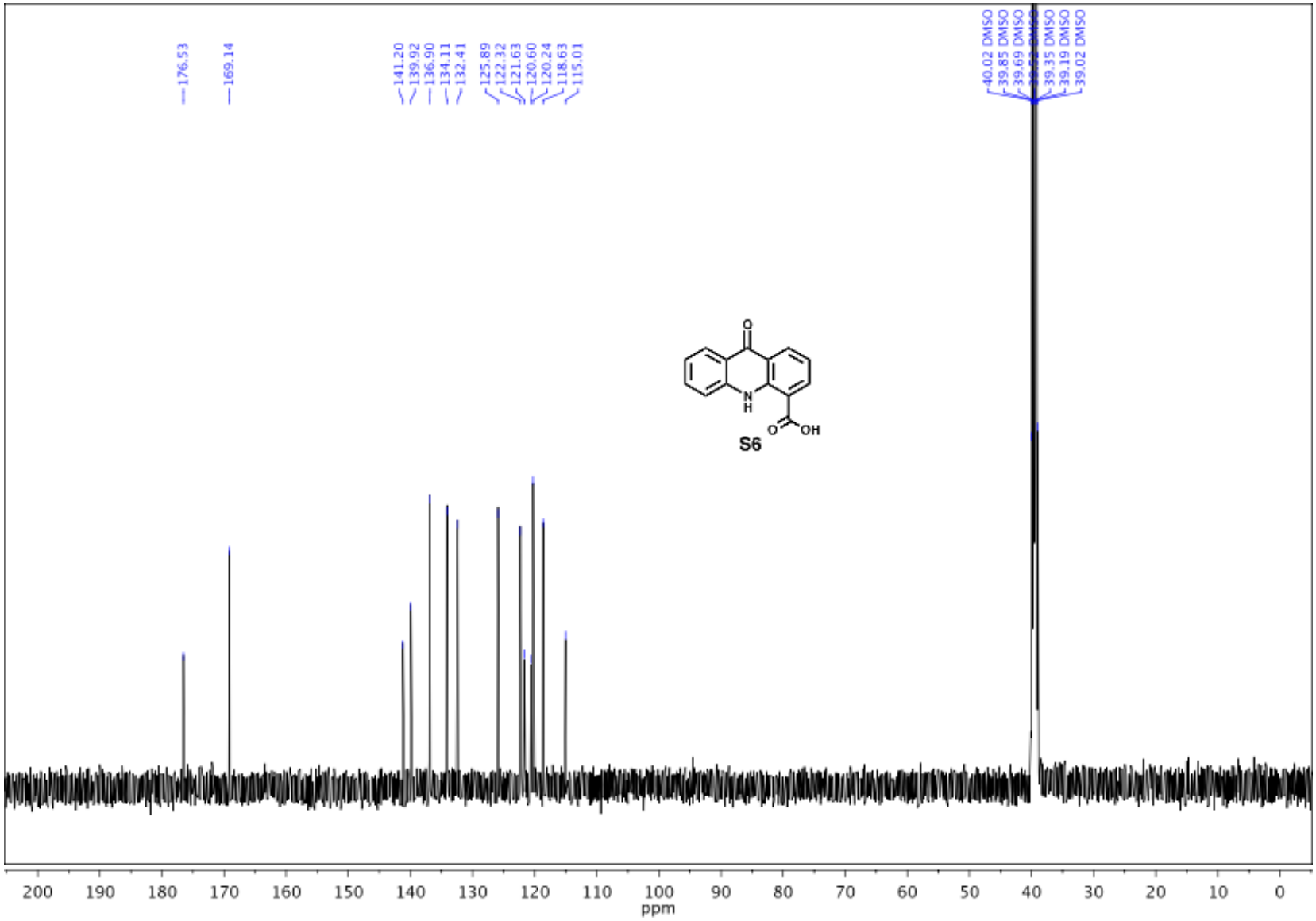
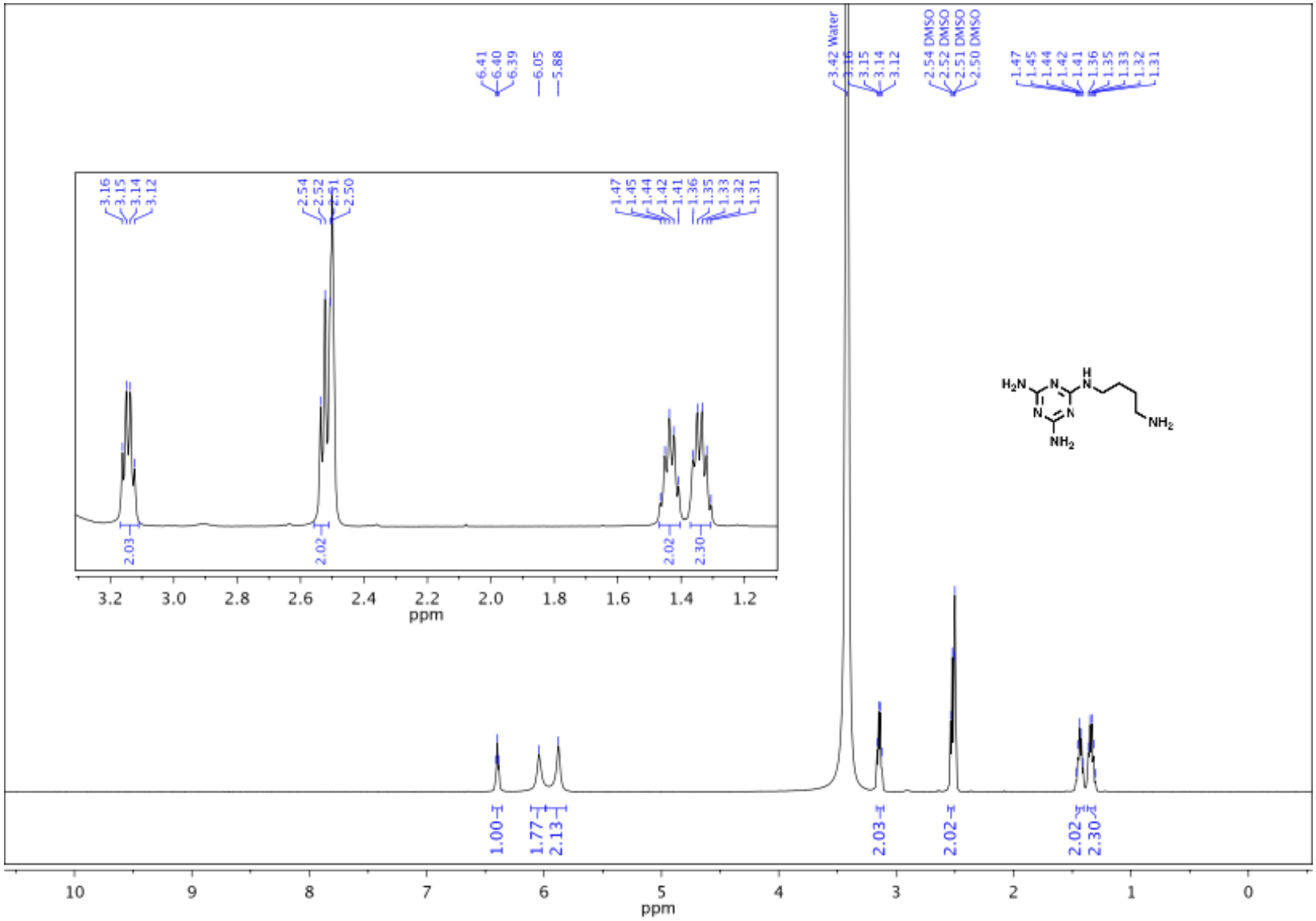
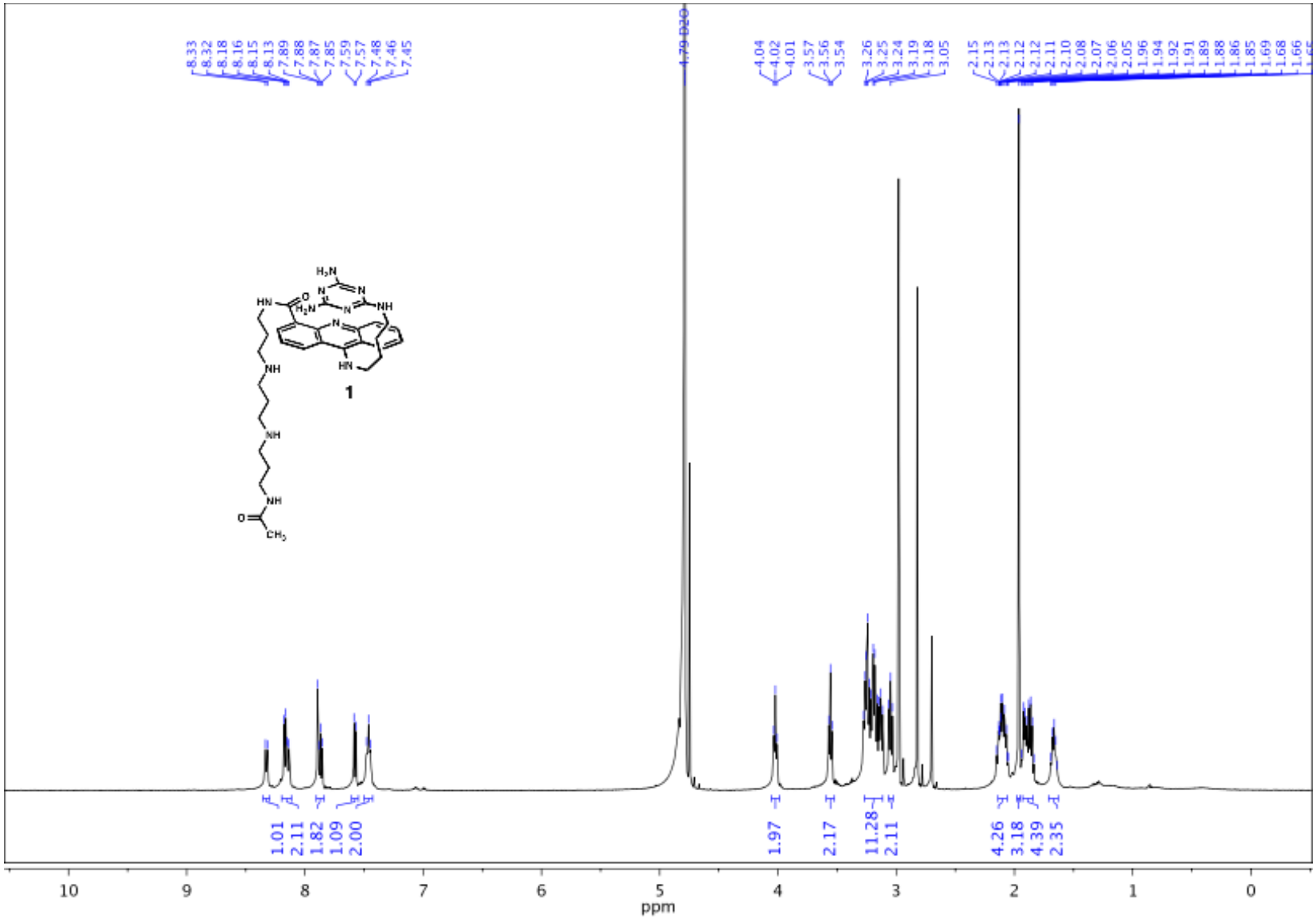
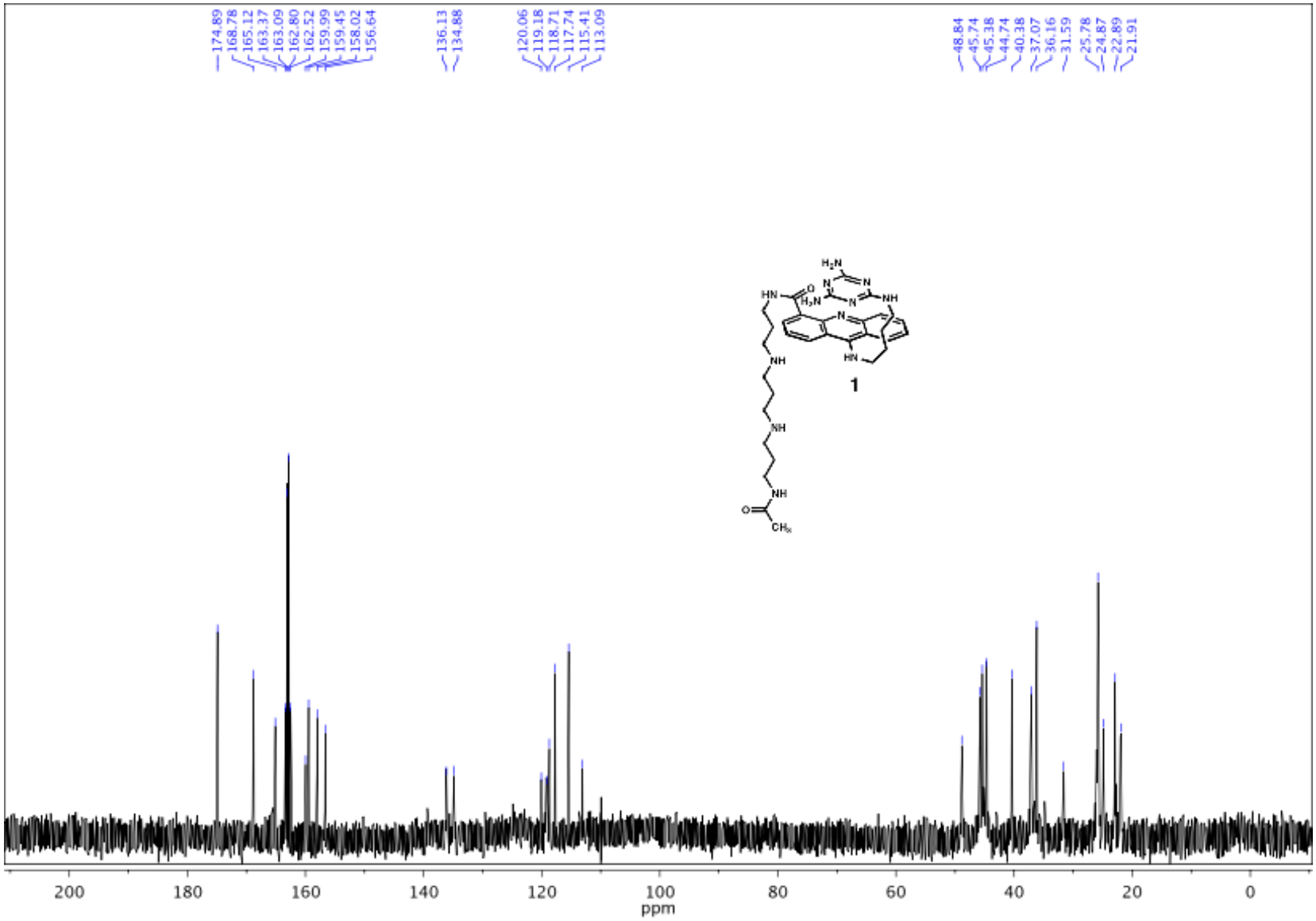
7.36
7.35
7.34
7.34
7.34
7.33
7.32
7.32
3.34 Water
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
0.96
1.00
0.97
2.03
2.06
ppm
S6
0.75
ppm


176.53
169.14
141.20
139.92
136.90
134.11
132.41
125.89
122.32
121.63
120.60
120.24
118.63
115.01
40.02 DMSO
39.85 DMSO
39.69 DMSO
39.52 DMSO
39.35 DMSO
39.19 DMSO
39.02 DMSO
O
N
H
O
OH
S6
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


6.41
6.40
6.39
6.05
5.88
3.42 Water
3.16
3.15
3.14
3.12
2.54 DMSO
2.52 DMSO
2.51 DMSO
2.50 DMSO
1.47
1.45
1.44
1.42
1.41
1.36
1.35
1.33
1.32
1.31
1.00
1.77
2.13
2.03
2.02
2.02
2.30
ppm


8.33
8.32
8.18
8.16
8.15
8.13
7.89
7.88
7.87
7.85
7.59
7.57
7.48
7.46
7.45
4.04
4.02
4.01
3.57
3.56
3.54
3.26
3.25
3.24
3.19
3.18
3.05
2.15
2.13
2.12
2.11
2.10
2.08
2.07
2.06
2.05
1.96
1.94
1.92
1.91
1.89
1.88
1.86
1.85
1.69
1.68
1.66
1.01
2.11
1.82
1.09
2.00
1.97
2.17
11.28
2.11
4.26
3.18
4.39
2.35
10
9
8
7
6
5
4
3
2
1
0
ppm
1


174.89
168.78
165.12
163.37
163.09
162.80
162.52
159.99
159.45
158.02
156.64
136.13
134.88
120.06
119.18
118.71
117.74
115.41
113.09
48.84
45.74
45.38
44.74
40.38
37.07
36.16
31.59
25.78
24.87
22.89
21.91
1
200
180
160
140
120
100
80
60
40
20
0
ppm