Supplemental Information:

A novel chemical inducer of *Streptococcus* quorum sensing acts by inhibiting the pheromone-degrading endopeptidase PepO

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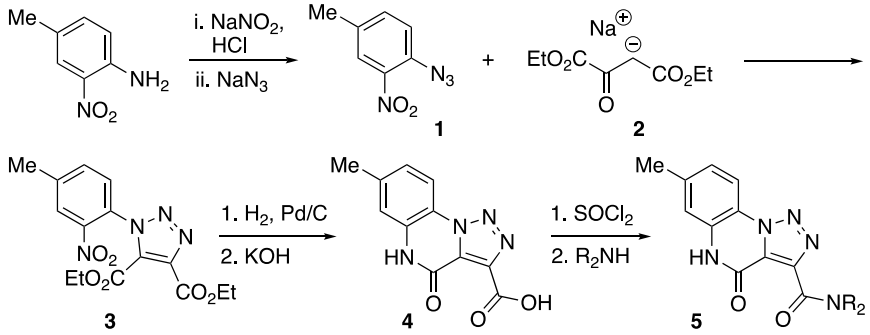
Supporting Information

Contents:

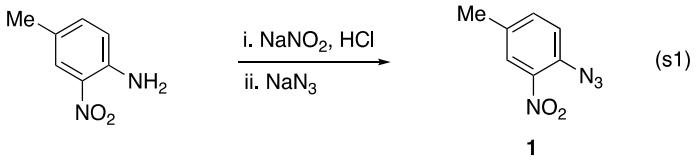
General. ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature using 500 MHz or 300 MHz spectrometers. The data are reported as follows: chemical shift in ppm from internal tetramethylsilane on the δ scale, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz) and integration. High-resolution mass spectra were obtained by peak matching. Melting points are reported uncorrected. Infrared spectroscopy was obtained using a diamond attenuated total reflectance (ATR) accessory. Analytical thin layer chromatography was performed on 0.25 mm extra hard silica gel plates with UV254 fluorescent indicator. Liquid chromatography was performed using forced flow (flash chromatography) of the indicated solvent system on 60Å (40 – 60 μm) mesh silica gel (SiO_2). Medium pressure liquid chromatography (MPLC) was performed to force flow the indicated solvent system down columns that had been packed with 60Å (40 – 60 μm) mesh silica gel (SiO_2). All reactions were carried out under an atmosphere of nitrogen in glassware, which had been oven-dried. Unless otherwise noted, all reagents were commercially obtained and, where appropriate, purified prior to use. Acetonitrile, Methanol, Toluene, THF, Et_2O, and CH_2Cl_2 were dried by filtration through alumina according to the procedure of Grubbs.[1] Metal salts were stored in a nitrogen atmosphere dry box.

I. Preparation of P516-0475 Analogs.

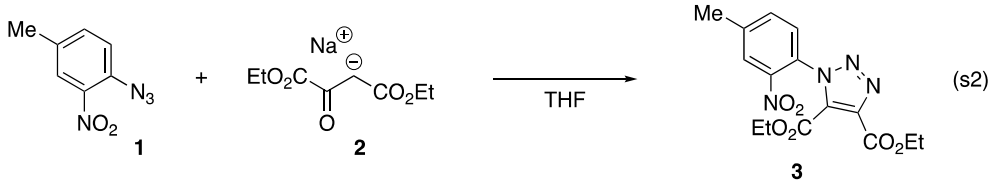
The P516-0475 analogs were prepared by the synthesis in Scheme s1.



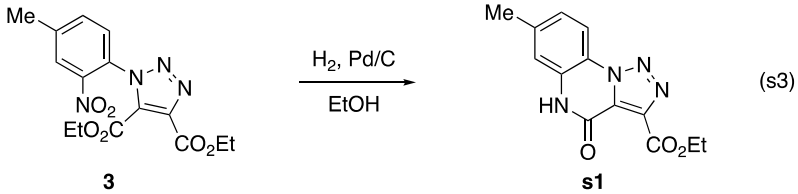
Scheme s1. Synthesis of P516-0475 analogs



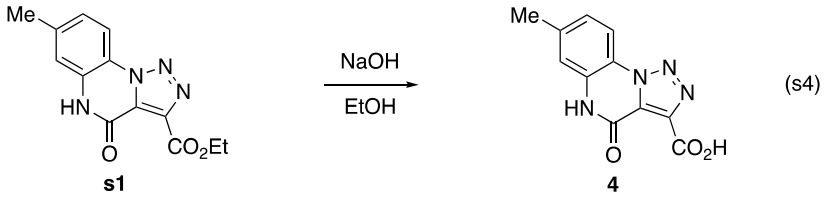
4-Azido-2-nitrotoluene 1.[2] To a cooled (0 °C) solution of 6.09 g of 2-nitro-4-toluidine (40.2 mmol) in 160 mL of water was added 90 mL of a 37% w/w aq soln of HCl followed by the dropwise addition of 20 mL of an aq soln of sodium nitrite (2.99 g, 42.0 mmol). After 10 min, 2.60 g of sodium azide (40.0 mmol) was added in portions. After 1 h, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature. After 1 h, the resulting solution was extracted with 3 × 40 mL of dichloromethane. The combined organic phases were washed with H_2O and brine. The resulting organic phase was dried over Na_2SO_4, filtered, and the filtrate was concentrated *in vacuo* to form the aryl azide, which matched the characterization data reported by Vijayakumar and co-workers.[2]



1-(4-Methyl-2-nitrophenyl)-4,5-diethylester 1*H*-1,2,3-triazole-4,5-dicarboxylic acid 3.[3] To a warmed (35 °C) suspension of diethyl oxalacetate sodium salt **2** (4.73 g, 22.5 mmol) in 60 mL of THF was added dropwise a solution of 4-azido-2-nitrotoluene **1** (2.67 g, 15.0 mmol) in 50 mL of THF. After addition the reaction mixture was heated to 50 °C. After 7 h, the reaction mixture was cooled to room temperature and then concentrated *in vacuo*. The residue was diluted with 25 mL of H_2O, and the resulting mixture was extracted with 3 × 20 mL of dichloromethane. The combined organic phases were dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. Purification by MPLC (1:2 EtOAc:hexanes) afforded the product (5.22 g, quant).[3]



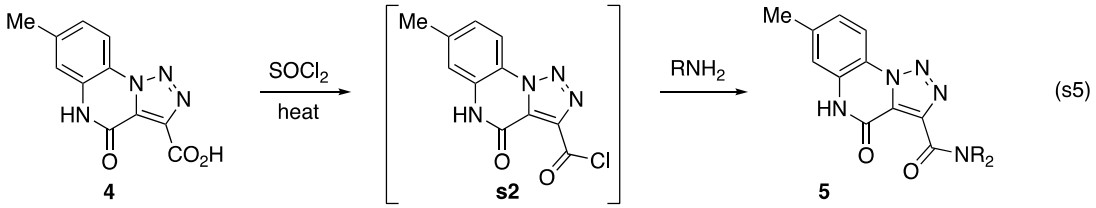
4,5-Dihydro-7-methyl-4-oxo-ethyl ester [1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxylic acid s1.[3] To a solution of triazole **3** (5.00 g, 14.3 mmol) in 300 mL of EtOH was added 0.430 g of Pd/C (10 wt % of Pd) and a balloon of H_2 was attached. When adsorption of the hydrogen volume was completed (approximately 15 h as monitored using thin layer chromatography), the reaction mixture was filtered. The precipitate was washed with 3 × 20 mL of hot dichloromethane. The dichloromethane washings were concentrated *in vacuo* to afford the product (4.98 g, quant).[3]



s1 4 (s4)

4,5-Dihydro-7-methyl-4-[1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxylic acid 4.[4] To a solution of ethyl ester **s1** (4.80 g, 13.8 mmol) in 20 mL of EtOH was added 20 mL of a 10 wt % aq soln of NaOH. The resulting mixture was heated to reflux. After 4 h, the reaction mixture was cooled to room temperature and then concentrated *in vacuo*. The resulting residue was diluted with H_2O and acidified to a pH 2 to precipitate the acid, which were collected by filtration and washed with water to produce the product (3.75 g, quant).[4]

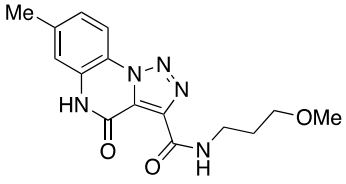
1. General procedure for amide formation



4 s2 5 (s5)

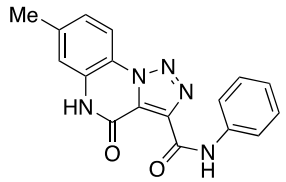
A solution of acid **4** (0.2 mmol) in 2 mL of $SOCl_2$ was heated to reflux. After 1 h, the reaction mixture was cooled to room temperature and then concentrated *in vacuo* to produce the acid chloride **s2** as a thick viscous oil, which was dissolved in 5 mL of toluene. To the resulting solution was added dropwise a solution of the amine (0.3 mmol) and 1.0 mL of triethylamine (7.0 mmol) in 2 mL of toluene. The resulting mixture was heated to reflux. After 20 h, the reaction mixture was cooled to room temperature. The resulting precipitation was isolated by filtration and was washed with toluene and water. The resulting solid was dissolved in hot EtOH and water and filtered to produce the amide product.

2. Characterization data for P516-0475 analogs.



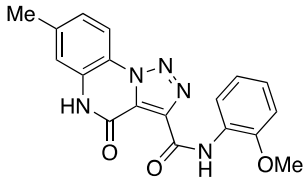
P516-0475

4,5-Dihydro-*N*-(3-methoxypropyl)-7-methyl-4-oxo-[1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxamide P516-0475. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, 3-methoxypropylamine (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a yellow amorphous solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.57 (br s, 1H) 10.08 (s, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.23 (s, 2H), 3.41 (t, J = 6.7 Hz, 2H, 3.32 (s, 2H), 3.24 (s, 3H), 2.39, (s, 3H), 1.77 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4, 155.8, 141.3, 140.3, 129.2, 125.9, 125.1, 119.5, 117.2, 116.2, 69.8, 58.4, 36.3, 29.5, 21.5.



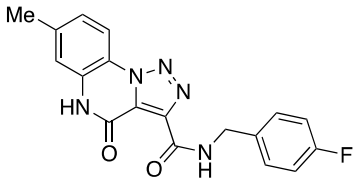
DSW0420C

4,5-Dihydro-7-methyl-4-oxo-*N*-phenyl-[1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxamide DSW0420C. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, aniline (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.80 (br s, 1H), 12.34 (s, 1H), 8.31 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 8.0 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.15 (t, J = 7.5 Hz, 1H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.5, 156.3, 141.4, 140.6, 138.9, 129.7, 129.1, 126.2, 125.3, 124.6, 119.8, 119.6, 117.4, 116.4, 21.5.



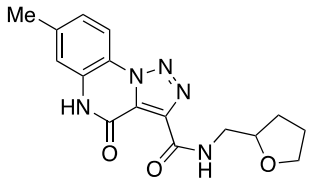
DSW0420D

4,5-Dihydro-7-methyl-4-oxo-*N*-(2-methoxyphenyl)-[1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxamide DSW0420D. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, 2-methoxyaniline (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77 (br s, 1H), 12.04 (s, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.28 (m, 2H), 7.12 (m, 2H), 6.98 (t, J = 6.5 Hz, 1H) 3.88 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.6, 156.0, 149.9, 141.1, 140.4, 134.1, 129.3, 128.0, 125.9, 121.7, 120.9, 119.6, 117.2, 116.4, 114.8, 111.9, 56.3, 21.5.



DSW0421D

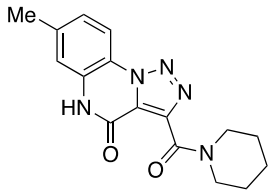
4,5-Dihydro-7-methyl-4-oxo-*N*-(4-fluorophenyl)methyl-[1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxamide DSW0421D. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, 4-fluorobenzylamine (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.58 (br s, 1H), 10.50 (s, 1H), 8.27 (m, 1H), 7.42 (t, J = 8.0 Hz, 2H), 7.27 (t, J = 8.0 Hz, 2H), 7.16 (s, 2H), 4.58 (s, 2H), 2.42 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.8 (d, J_{CF} = 240.9 Hz), 158.5, 155.8, 140.7 (d, J_{CF} = 89.9 Hz), 135.4, 129.9, 129.8, 129.1, 126.0, 125.3, 119.5, 117.2, 116.3, 115.6 (d, J_{CF} = 21.1 Hz), 42.2, 21.5.



DSW0421C

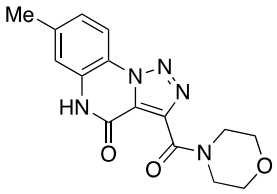
4,5-Dihydro-7-methyl-4-oxo-*N*-[(tetrahydro-2-furanyl)methyl]-[1,2,3]-triazole[1,5-*a*]quinoxaline-3-carboxamide DSW0421C. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, (tetrahydro-2-furanyl)methylamine (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a white solid: ^{1}H

NMR (500 MHz, DMSO-d_6) δ 12.57 (br s, 1H), 10.19 (br s, 1H), 8.24 (m, 1H), 7.25 (br s, 2H), 3.98 (br s, 1H), 3.80 (br s, 1H), 3.63 (br s, 1H), 3.46 (br s, 2H), 2.48 (s, 3H), 1.81 – 1.94 (m, 3H), 1.59 (br s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.5, 155.9, 141.1, 140.4, 129.1, 125.9, 125.1, 119.5, 117.2, 116.3, 77.4, 67.9, 43.2, 28.9, 25.8, 21.5.



DSW0421E

3-(1-Piperidinylcarbonyl)-[1,2,3]-triazole[1,5-*a*]quinoxalin-4(5*H*)-one DSW0421E. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, piperidine (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.06 (br s, 1H), 8.16 (br s, 1H), 7.19 (br s, 2H), 3.64 (br s, 2H), 3.32 (br s, 3H), 3.21 (br s, 2H), 2.38 (br s, 3H), 1.58 (br s, 4H), 1.41 (2H); δ 12.58 (br s, 1H), 10.50 (s, 1H), 8.27 (m, 1H), 7.42 (t, J = 8.0 Hz, 2H), 7.27 (t, J = 8.0 Hz, 2H), 7.16 (s, 2H), 4.58 (s, 2H), 2.32 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.9, 153.6, 141.6, 140.0, 129.8, 125.1, 124.3, 119.4, 117.1, 116.1, 47.8, 42.6, 26.3, 25.7, 24.4, 21.5.



DSW0421F

3-(4-Morpholinylcarbonyl)-[1,2,3]-triazole[1,5-*a*]quinoxalin-4(5*H*)-one DSW0421F. The general procedure was followed using 0.2 mmol of acid **4**, 2 mL of $SOCl_2$, morpholine (0.3 mmol), 1.0 mL of triethylamine in 7 mL of toluene to afford the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (br s, 1H), 8.18 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 6.5 Hz, 2H), 3.68 (br s, 4H), 3.50 (br s, 2H), 3.30 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.3, 153.7, 140.7, 140.0, 129.8, 125.2, 124.7, 119.3, 117.0, 116.1, 66.6, 66.4, 47.2, 42.4, 21.5.

References.

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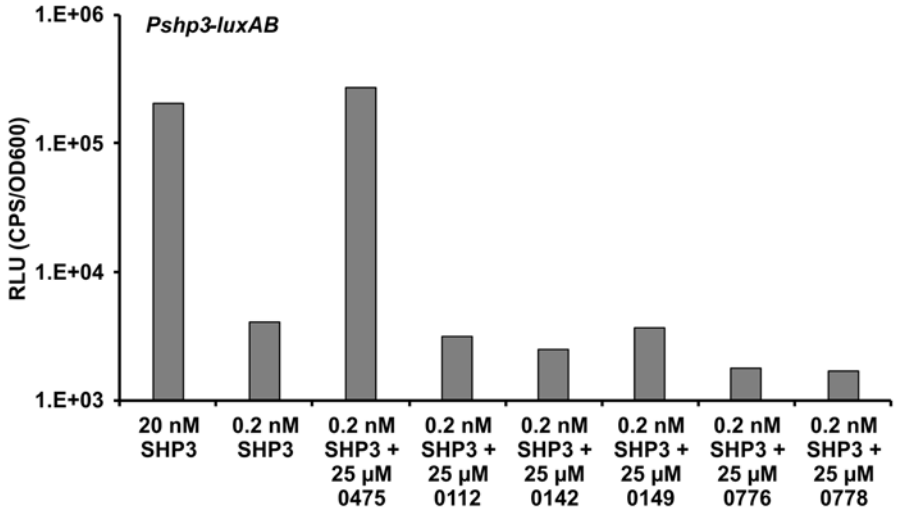
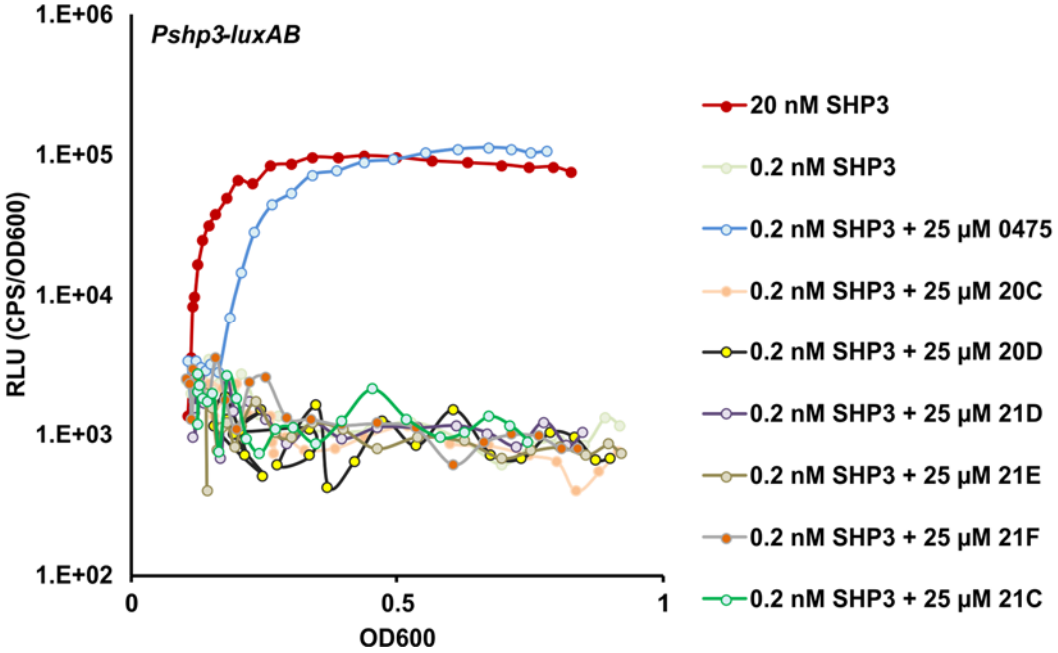
4 Bertelli, L., Biagi, G., Giorgi, I., Manera, C., Livi, O., Scartoni, V., Betti, L., Giannaccini, G., Trincavelli, L. & Barili, P. L. 1,2,3-Triazolo[1,5-a]quinoxalines: synthesis and binding to benzodiazepine and adenosine receptors. *Eur. J. Med. Chem.* **33**, 113-122, doi:http://dx.doi.org/10.1016/S0223-5234(98)80036-6 (1998).

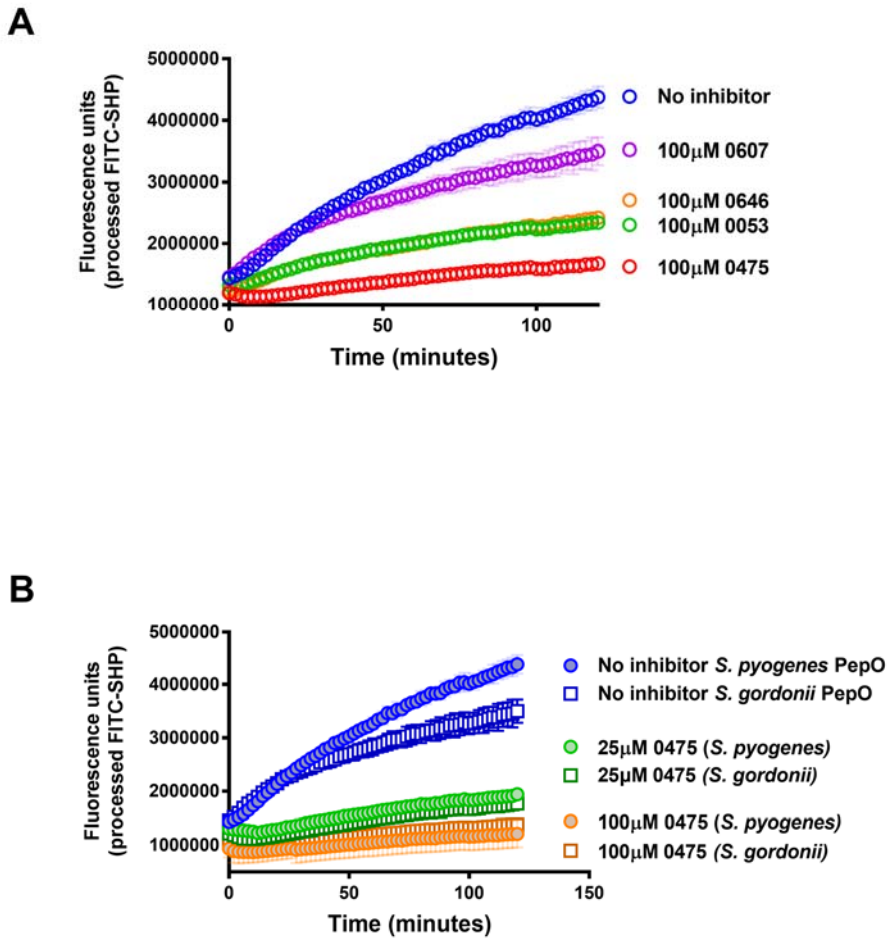
Supplemental Figure 1. Analogs of 0475 not displaying activity. Cultures of JCC157, which contains *Pshp3-luxAB,* were treated with: DMSO, 20 nM SHP3, or 25 µM of **(A)** library compounds 0475, 0112, 0142, 0149, 0776, or 0778 **(B)** designed synthetic analogs DSW-20C, 20D, 21D, 21E, 21F, and 21C. Plotted as maximum relative light units (RLU) from a single time point. Experiment was conducted in triplicate.

A



B





Supplemental Figure 2. Activity of 0475 analogs, and inhibition of PepO ortholog. (A) 0475 analogs were tested for inhibition of *S. pyogenes* rPepO. 10 nM rPepO was preincubated with each analog before 100 μM FAM-SHP2-QXL peptide was added to the reactions and fluorescence emission was measured over time. Reactions were conducted in triplicate with standard error indicated as bars. **(B)** 0475 was compared for its activity on rPepO from *Streptococcus pyogenes* (circles) and *Streptococcus gordonii* (squares). 10 nm rPepO was preincubated with each concentration of 0475 (0, 25μM, 100μM) before 100 μM FAM-SHP2-QXL peptide was added to the reactions and fluorescence emission was measured over time. Reactions were conducted in triplicate with standard error indicated as bars.

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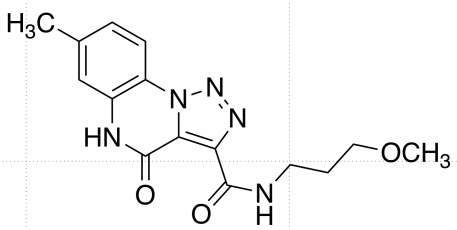
H1 standard parameters, BBO probe.



independently synthesized P516-0475

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8.2350
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7.2298
3.4214
3.4081
3.3926
3.3180
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2.4795
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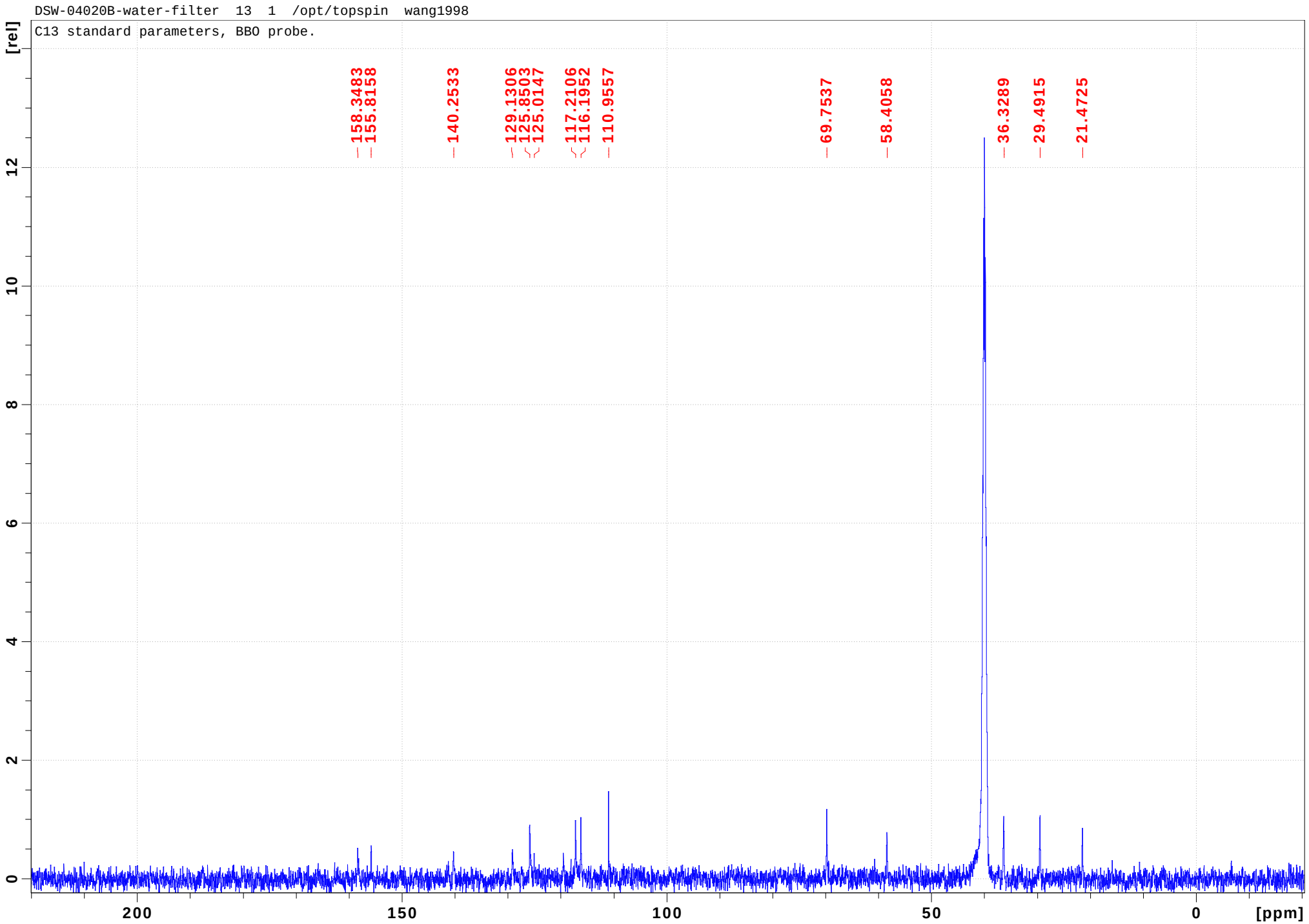
[ppm]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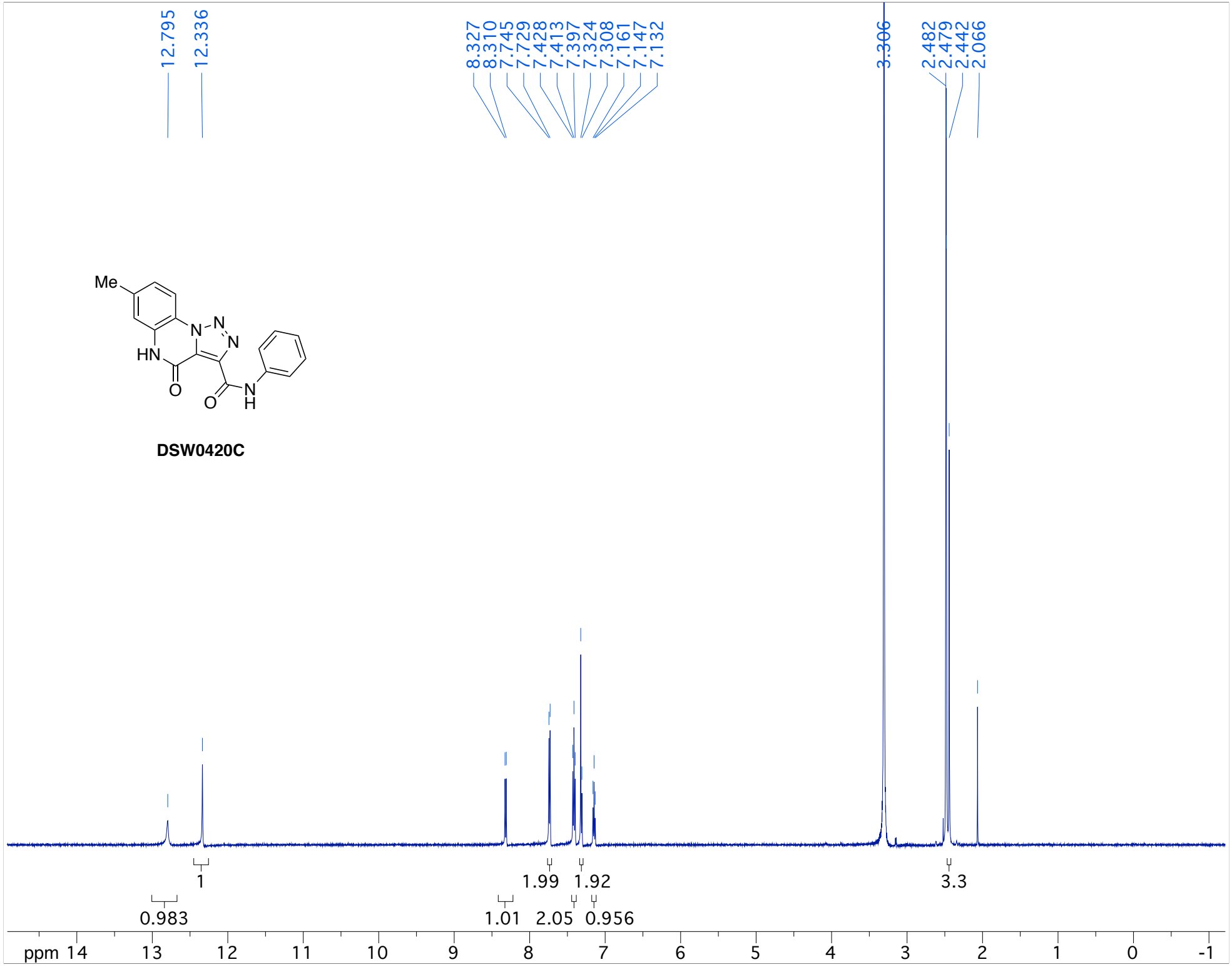
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N
N
HN
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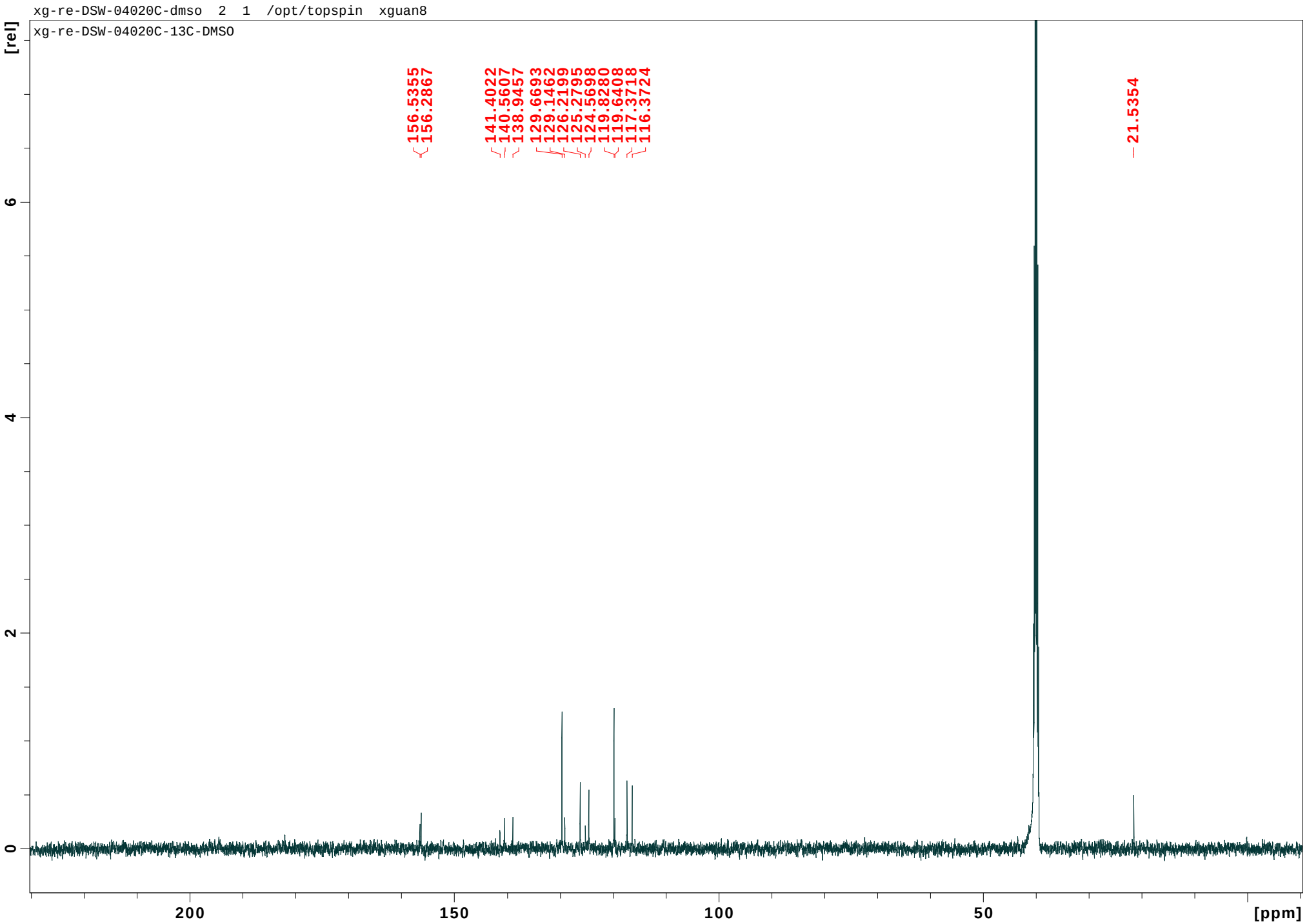
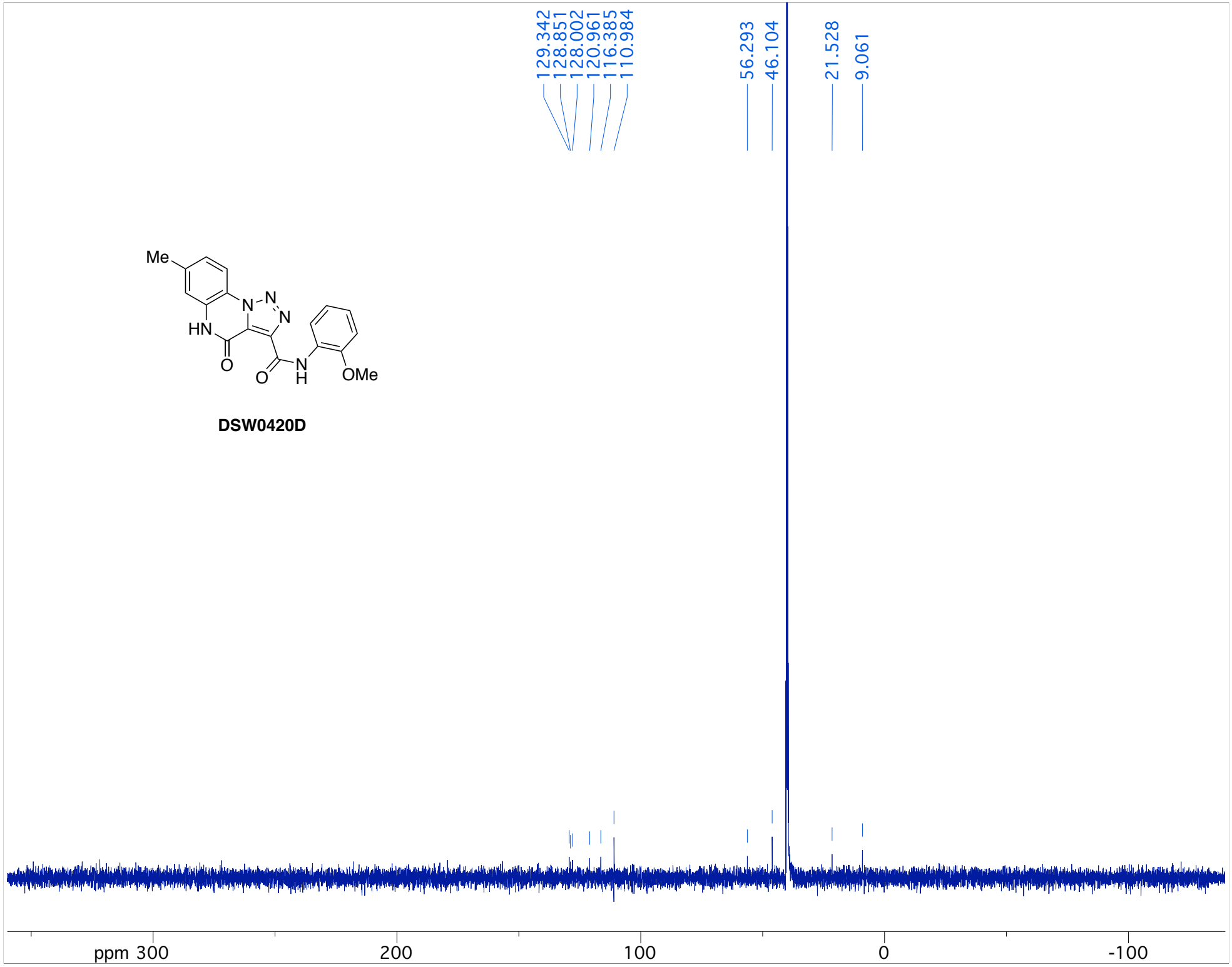
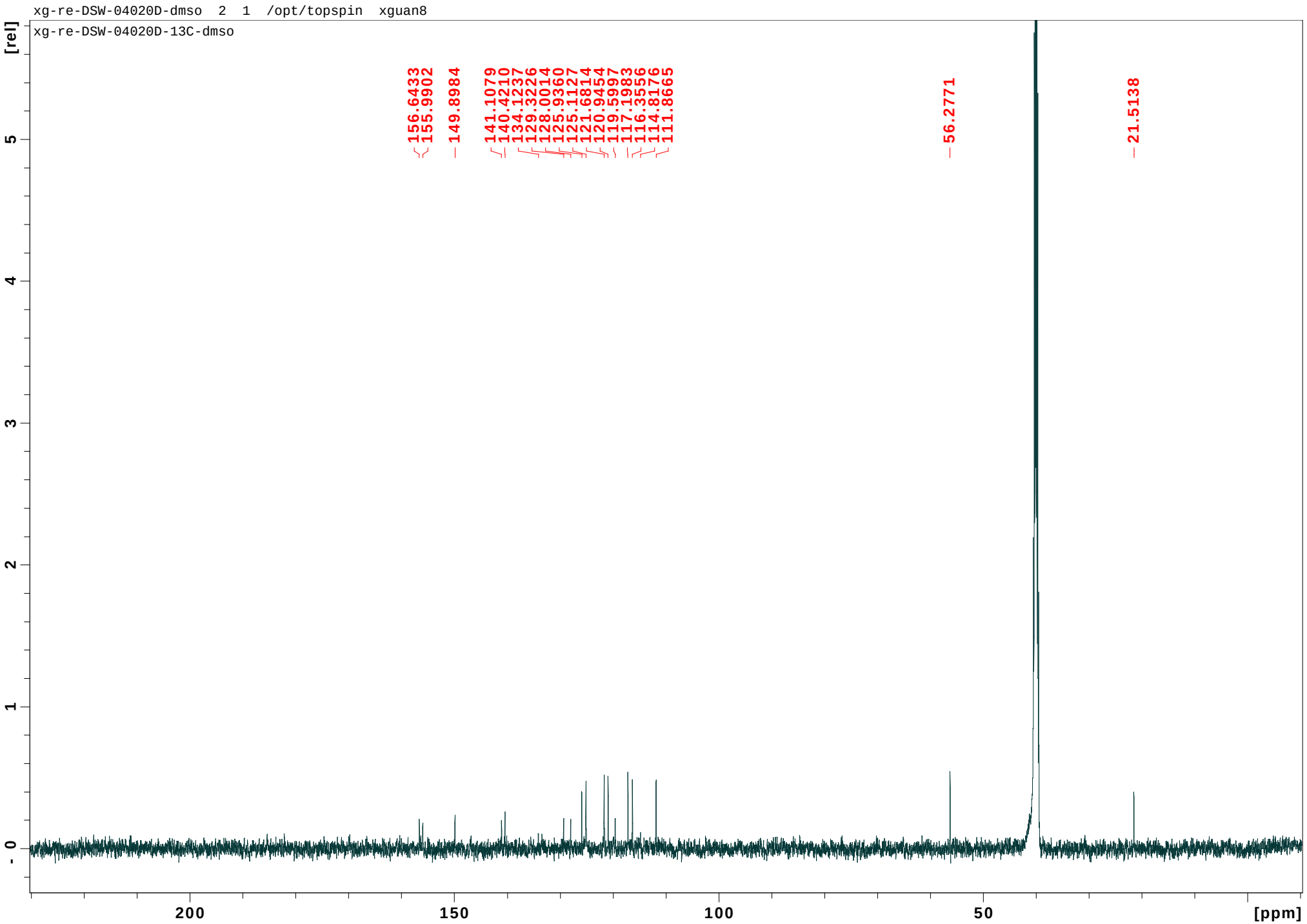
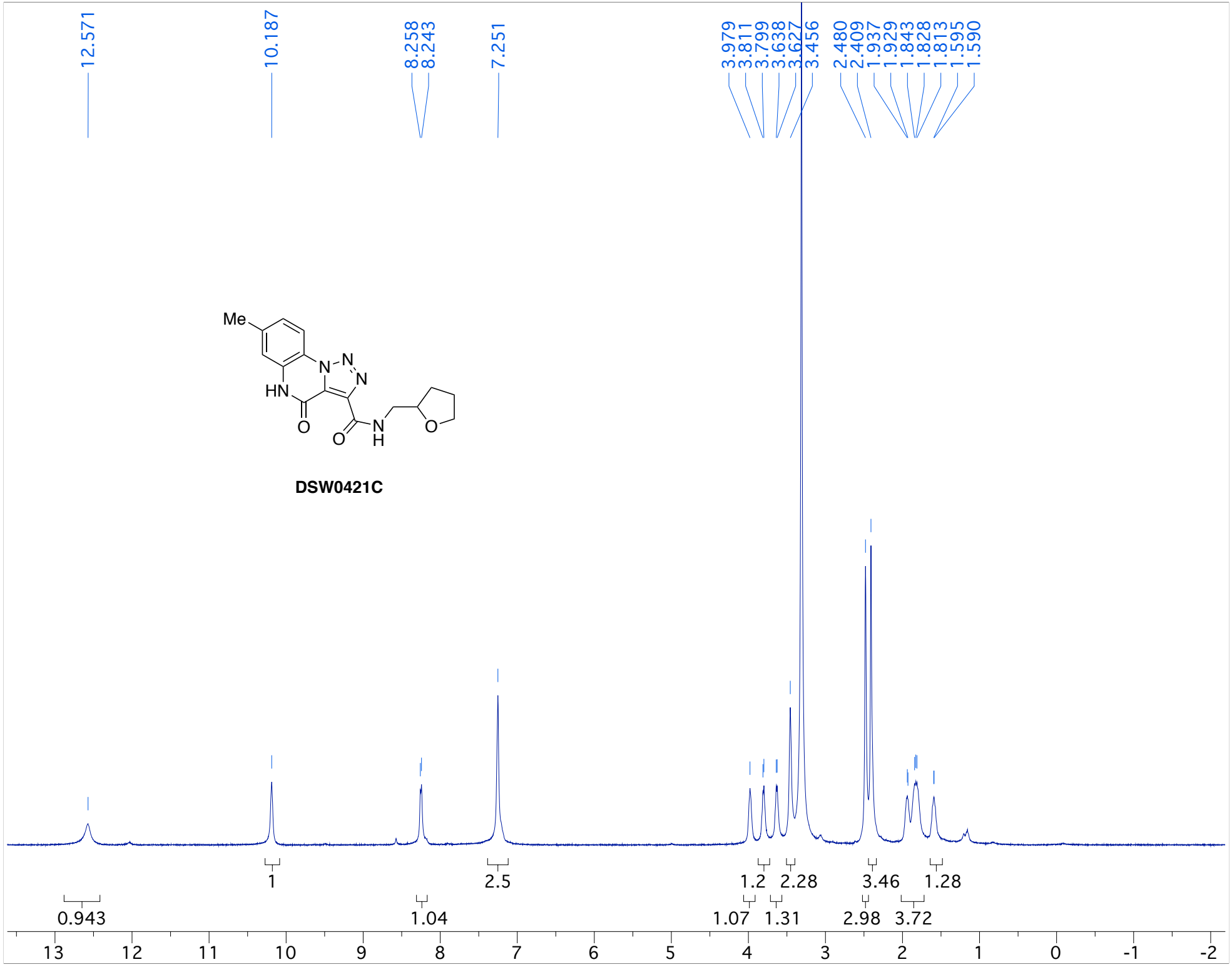
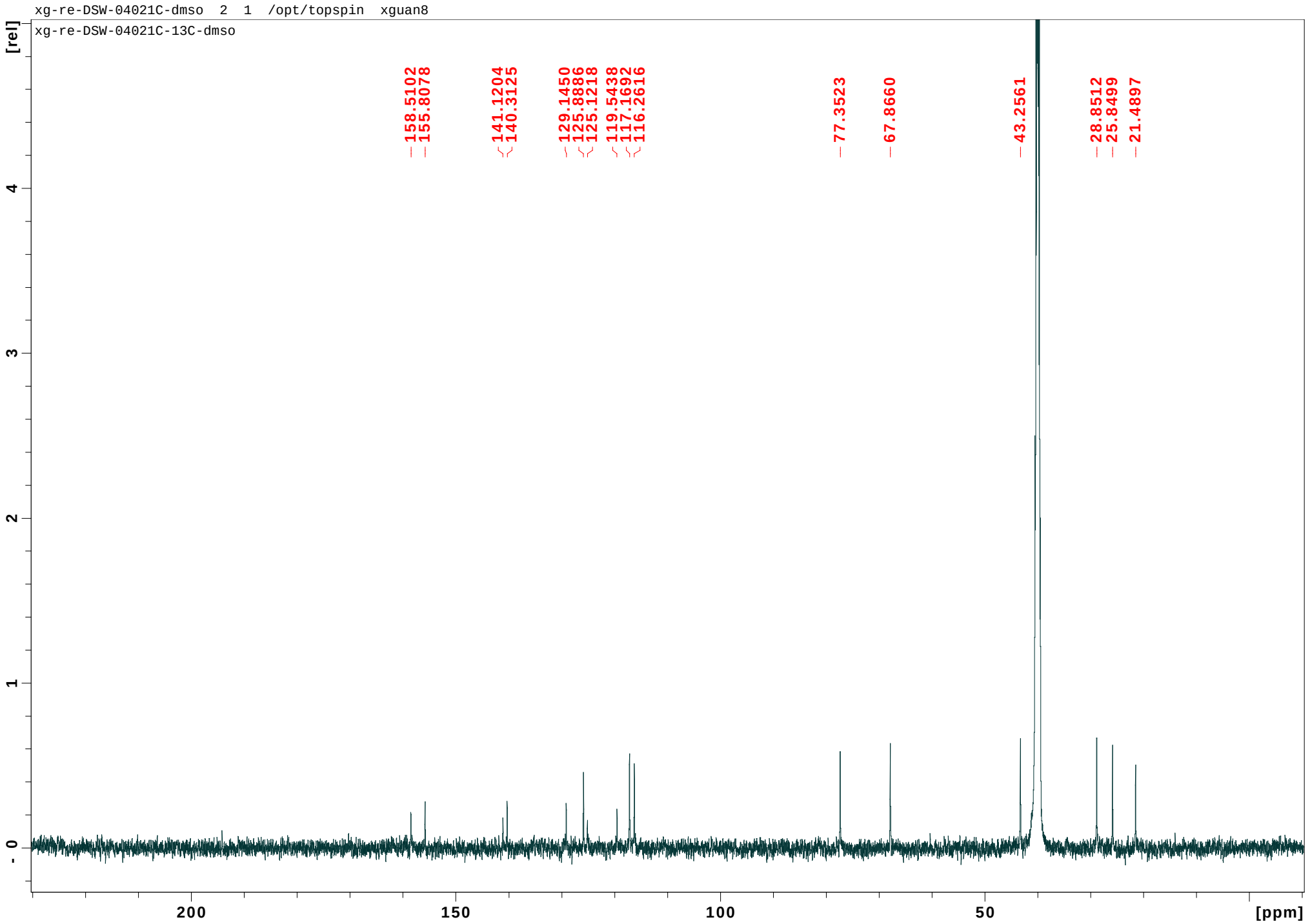
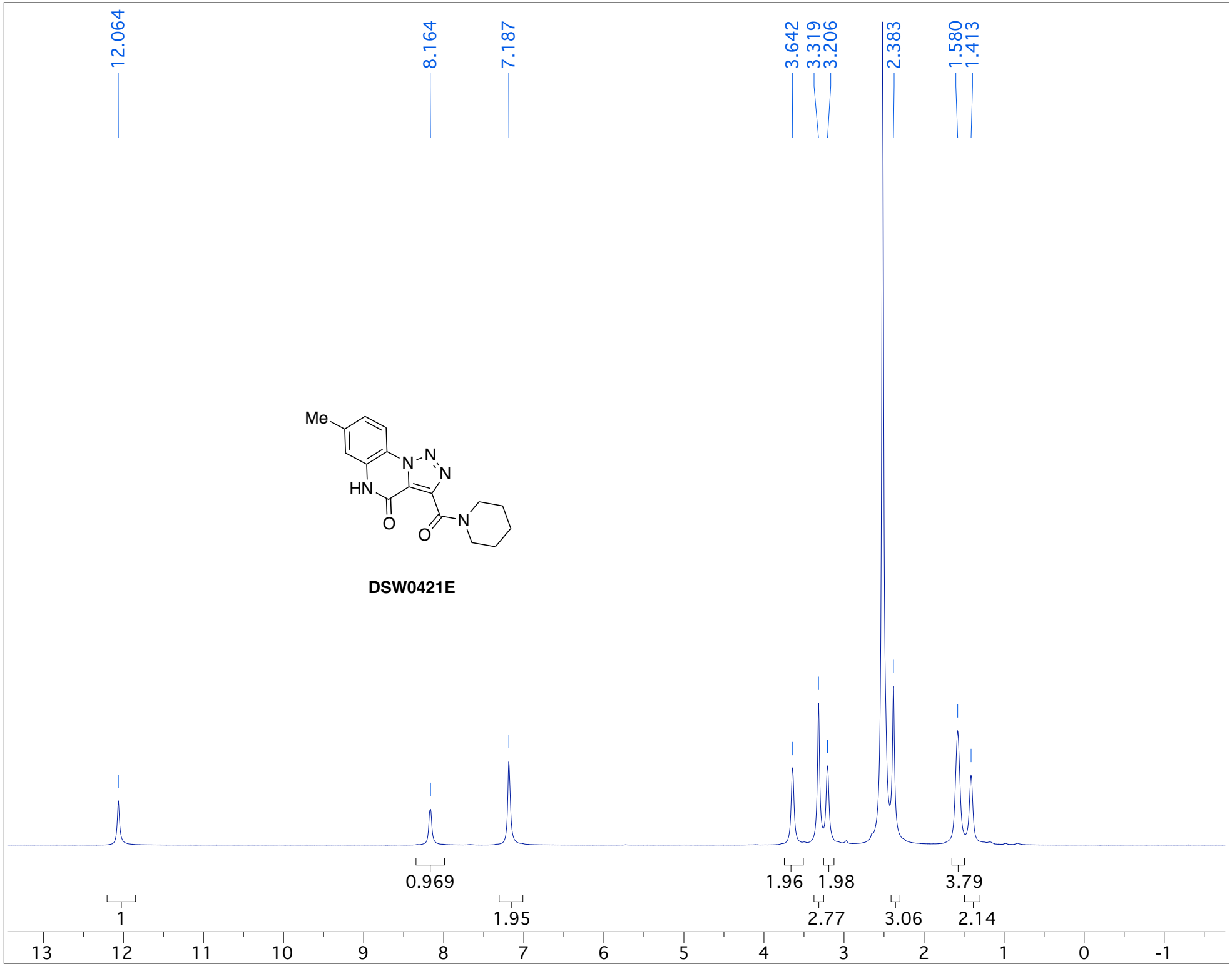

xg-re-DSW-04020C-dmso 2 1 /opt/topspin xguan8
xg-re-DSW-04020C-13C-DMSO
[rel]
156.5355
156.2867
141.4022
140.5607
138.9457
129.6693
129.1462
126.2199
125.2795
124.5698
119.8280
119.6408
117.3718
116.3724
21.5354
0
2
4
6
200
150
100
50
[ppm]

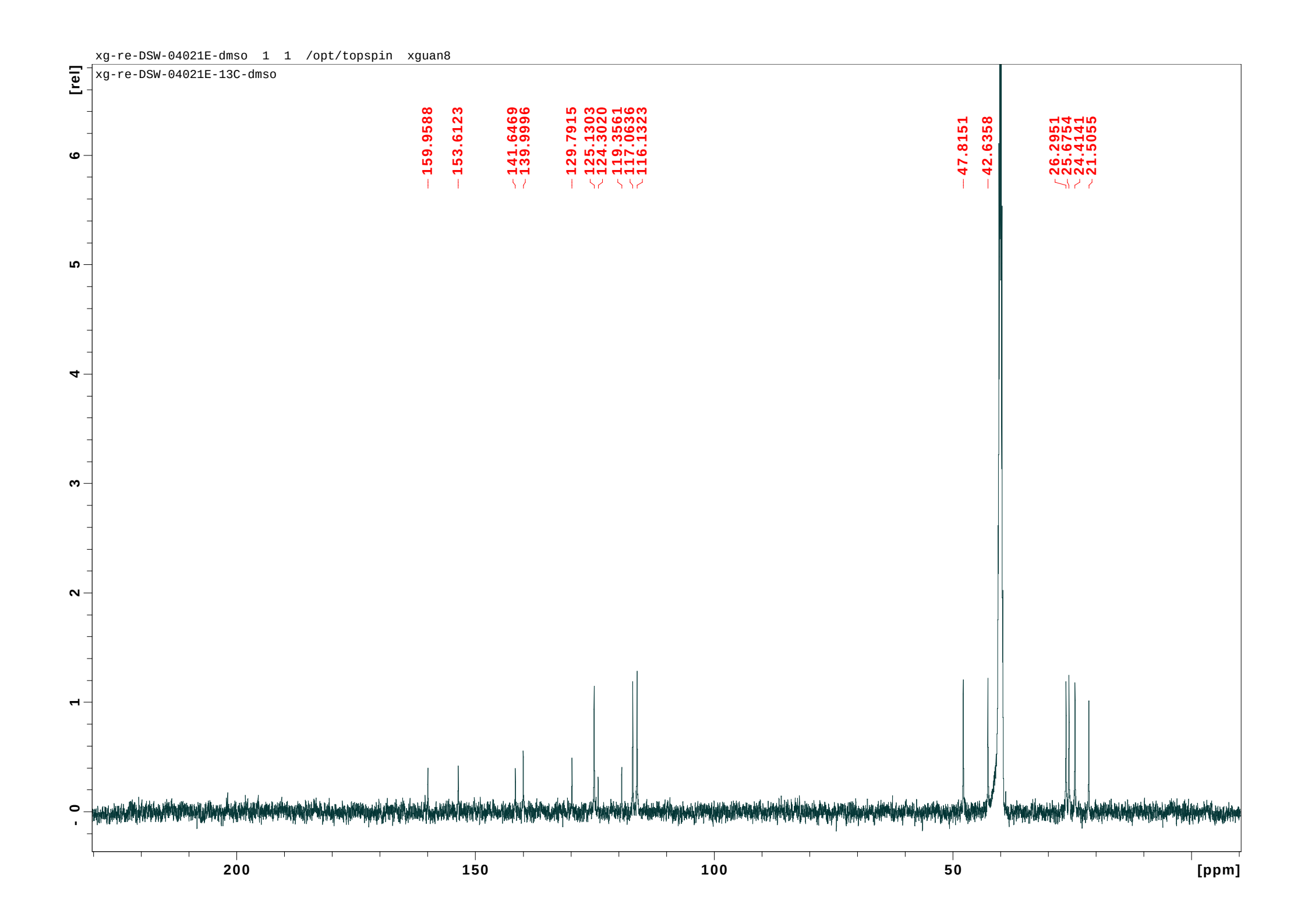

129.342
128.851
128.002
120.961
116.385
110.984
56.293
46.104
21.528
9.061
Me
HN
N
N
N
O
O
N
H
OMe
DSW0420D
ppm 300
200
100
0
-100

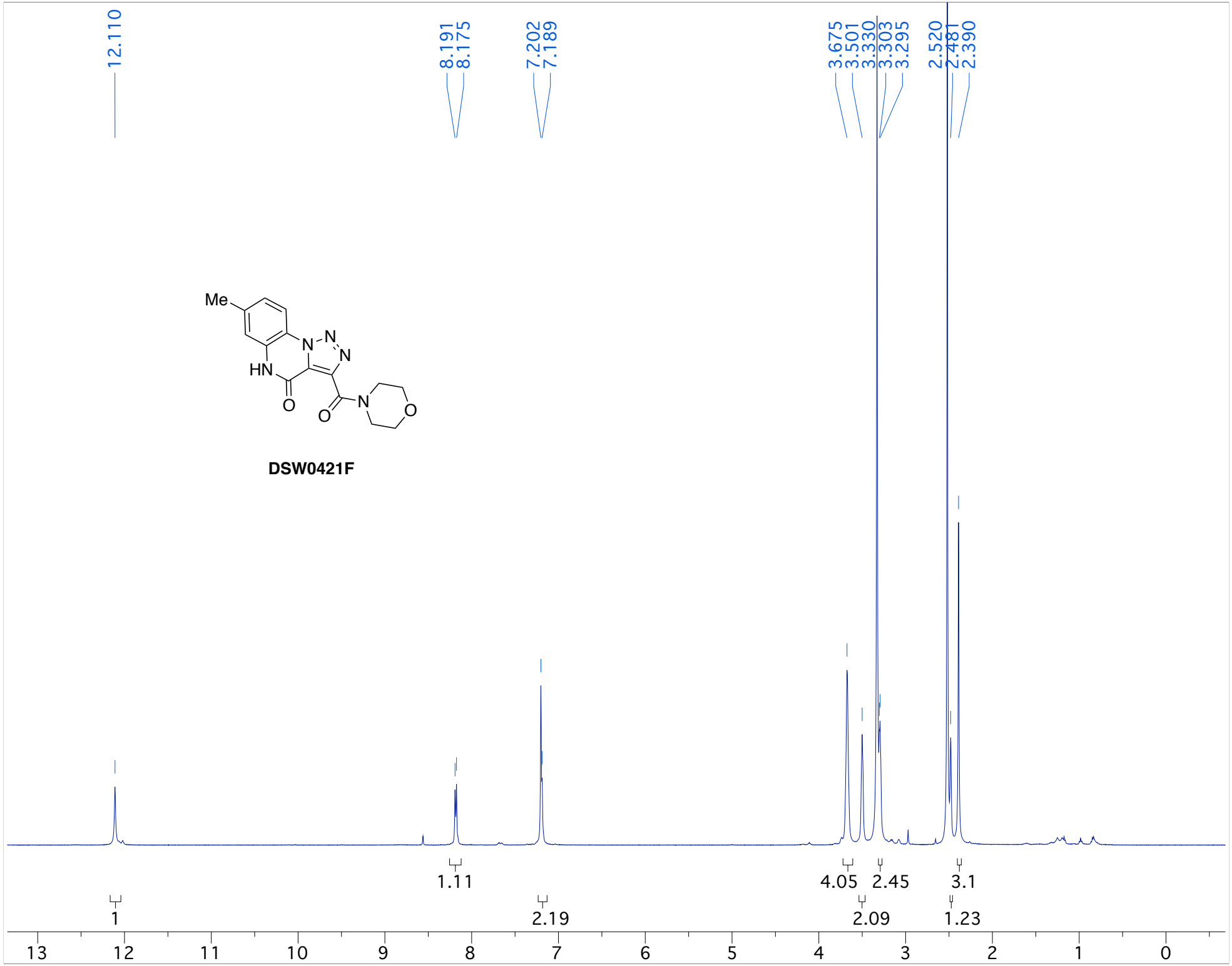

xg-re-DSW-04020D-dmso 2 1 /opt/topspin xguan8
xg-re-DSW-04020D-13C-dmso
[rel]
156.6433
155.9902
149.8984
141.1079
140.4210
134.1237
129.3226
128.0014
125.9360
125.1127
121.6814
120.9454
119.5997
117.1983
116.3556
114.8176
111.8665
56.2771
21.5138
- 0
1
2
3
4
5
200
150
100
50
[ppm]

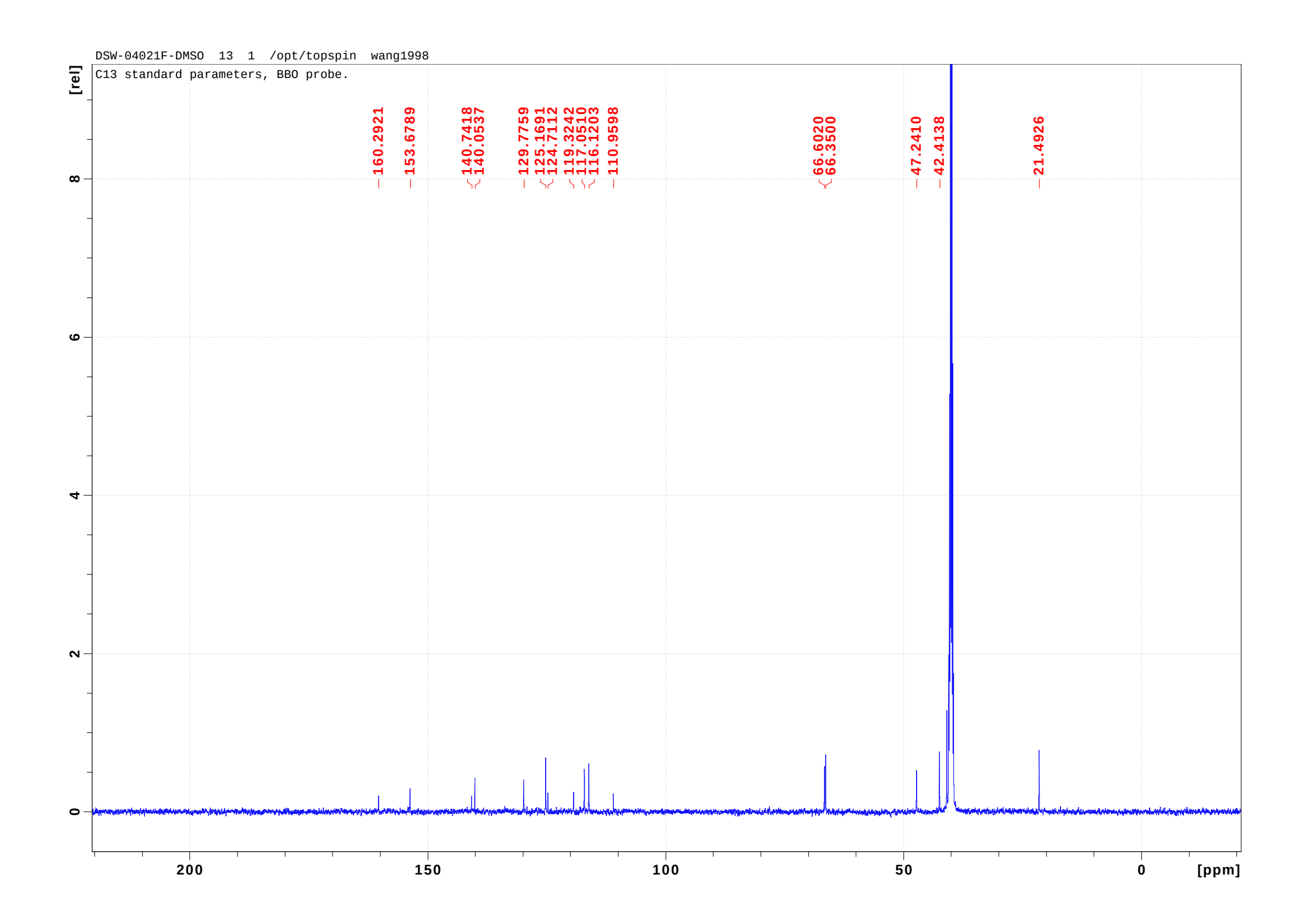

12.571
10.187
8.258
8.243
7.251
3.979
3.811
3.799
3.638
3.627
3.456
2.480
2.409
1.937
1.929
1.843
1.828
1.813
1.595
1.590
Me
HN
N
N
N
O
O
N
H
O
DSW0421C
1
2.5
1.2
2.28
3.46
1.28
0.943
1.04
1.07
1.31
2.98
3.72
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2


xg-re-DSW-04021C-dmso 2 1 /opt/topspin xguan8
xg-re-DSW-04021C-13C-dmso
[rel]
- 0
1
2
3
4
158.5102
155.8078
141.1204
140.3125
129.1450
125.8886
125.1218
119.5438
117.1692
116.2616
77.3523
67.8660
43.2561
28.8512
25.8499
21.4897
200
150
100
50
[ppm]


12.064
8.164
7.187
3.642
3.319
3.206
2.383
1.580
1.413
Me
N
N
N
HN
O
O
N
DSW0421E
0.969
1.96
1.98
3.79
1
1.95
2.77
3.06
2.14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1


xg-re-DSW-04021E-dmso 1 1 /opt/topspin xguan8
xg-re-DSW-04021E-13C-dmso
159.9588
153.6123
141.6469
139.9996
129.7915
125.1303
124.3020
119.3561
117.0636
116.1323
47.8151
42.6358
26.2951
25.6754
24.4141
21.5055
[rel]
6
5
4
3
2
1
- 0
200
150
100
50
[ppm]


12.110
8.191
8.175
7.202
7.189
3.675
3.501
3.330
3.303
3.295
2.520
2.481
2.390
Me
HN
O
N
N
N
O
N
O
DSW0421F
1.11
4.05
2.45
3.1
1
2.19
2.09
1.23
13
12
11
10
9
8
7
6
5
4
3
2
1
0


DSW-04021F-DMSO 13 1 /opt/topspin wang1998
C13 standard parameters, BBO probe.
[rel]
160.2921
153.6789
140.7418
140.0537
129.7759
125.1691
124.7112
119.3242
117.0510
116.1203
110.9598
66.6020
66.3500
47.2410
42.4138
21.4926
0
2
4
6
8
200
150
100
50
0
[ppm]