Structure Guided Lead Generation toward Nonchiral *M. tuberculosis* Thymidylate Kinase Inhibitors

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Supporting information

1: Determination of the IC_{50}-values and plot for compound 44.

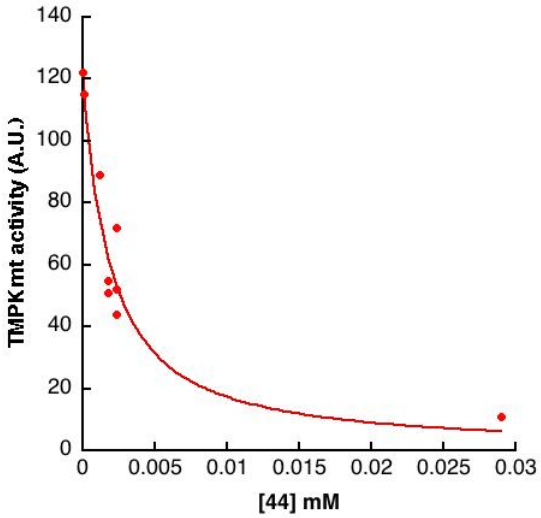
Table S1: Determination of the IC_{50}-values.

N°	ID	IC50 (µM)	Data-points	Number of concentra-tions tested	Maximum concentra-tion tested (mM)	Minimum concentra-tion tested (mM)	Coefficient of determina-tion
1	**LS2077**	10 ± 2	8	6	0.1	0.0077	0.965
2	**LS2048**	6.1 ± 0.5	5	3	0.1	0.0044	0.998
3	**LS3147**	11 ± 1	5	3	0.0705	0.00094	0.999
4	**LS2049**	29 ± 3	6	4	0.1	0.038	0.996
5	**LS2061**	NI at 0.8 mM	2	1	0.8	0.8	NA
6	**LS2062**	> 2 mM	3	2 (precipita-tion issue)	1	0.8	NA
7	**LS3115**	944 ± 105	6	3	0.47	0.188	0.993
8	**LS3145**	1910 ± 417	5	3	0.723	0.241	0.990
9	**LS3141**	1142 ± 39	7	4	0.651	0.086	0.999
10	**LS3116**	832 ± 100	9	4	0.765	0.48	0.968
11	**LS4069**	72 ± 3	6	4	0.216	0.0432	0.997
12	**LS4068**	> 2mM	6	4	0.726	0.0726	NA
13	**LS2067**	235 ± 8	7	4	0.355	0.1	0.998
14	**LS2068**	353 ± 52	8	4	0.3	0.1	0.983
15	**LS2161**	138 ± 18	5	2	0.2	0.1	0.988
16	**LS3005**	72 ± 7	5	3	0.1	0.04	0.990
17	**LS3001**	168 ± 35	5	2	0.1	0.04	0.990
18	**LS3008**	113 ± 20	4	2	0.164	0.1	0.986

19	**LS3009**	328 ± 21	4	3	0.3	0.1	0.997
20	**LS3012**	40 ± 7	10	7	0.1	0.0176	0.930
21	**LS3004**	7.1 ± 1.2	6	3	0.02	0.005	0.976
22	**LS3013**	35 ± 7	5	3	0.1	0.01	0.981
23	**LS3023**	141 ± 3	4	2	0.155	0.1	0.999
24	**LS3020**	142 ± 26	4	3	0.1	0.0213	0.995
25	**LS3015**	96 ± 16	5	2	0.1	0.04	0.972
26	**LS3016**	75 ± 3	5	3	0.1	0.04	0.996
27	**LS3014**	49 ± 1	4	2	0.055	0.028	1
28	**LS4037**	143 ± 16	5	3	0.237	0.071	0.992
29	**LS2160**	114 ± 8	5	3	0.2	0.1	0.995
30	**LS3003**	254 ± 47	4	2	0.2	0.15	0.995
31	**LS3011**	129 ± 1	4	2	0.2	0.1	1
32	**LS3010**	1115 ± 93	5	3	0.5	0.1	0.998
33	**LS3002**	38 ± 3	6	3	0.1	0.025	0.992
34	**LS2162**	20 ± 3	6	3	0.1	0.02	0.984
35	**LS3007**	9.0 ± 1.6	6	3	0.1	0.005	0.977
36	**LS3097**	17 ± 2	5	3	0.048	0.0038	0.980
37	**LS3067**	25 ± 3	7	4	0.0311	0.01295	0.968
38	**LS3095**	27 ± 3	5	3	0.025	0.005	0.991
39	**LS3061**	26 ± 8	7	4	0.0082	0.00295	0.965
40	**LS3062**	25 ± 3	5	3	0.0242	0.00726	0.988
41	**LS3006**	23 ± 3	4	2	0.1	0.02	0.993
42	**LS3078**	1.1 ± 0.1	6	5	0.00456	0.000684	0.983

43	**LS3110**	0.95 ± 0.08	5	3	0.00198	0.00079	0.992
44	**LS3133**	1.8 ± 0.4	9	6	0.029	0.000115	0.917

Figure S1: The plot of the IC_{50} determination of compound **44**.



2: Data collection and refinement statistics.

Table S2: Data collection and refinement statistics

	***Mt*TMPK/1**	***Mt*TMPK/43**
PDB code	5NQ5	5NR7
Synchrotron beamline	PX1, Soleil	P14, Petra III
Wavelength (Å)	0.97857	0.9763
Space group	$P3_221$	$P3_2$
Unit-cell parameters (Å, °)	a = 71.39, b = 71.39, c = 133.75, α = β = 90, γ = 120	a = 73.63, b = 73.63, c = 72.86, α = β = 90, γ = 120
Resolution range (Å)	50-2.85 (2.92-2.85)	50-2.35 (2.41-2.35)
Number of measurements	70891 (5262)	155550 (11434)
Unique reflections	9634 (681)	18395 (1359)
Completeness (%)	99.5 (99.6)	100 (100)
R_{meas} (I) [a](%)	6.3 (127.6)	15.3 (126.7)
CC(1/2) [b](%)	99.9 (72.5)	99.8 (37.5)
Mean I/σ(I)	17.0 (1.6)	11.1 (1.6)
R_{work} (%)	20.08	19.23
R_{free} [c](%)	23.93	22.55
Asymmetric unit content	Monomer	Dimer
Non-hydrogen protein atoms	1383	2668
Non-hydrogen ligand atoms	29	70
Solvent molecules	0	97

RSCC ligand [d](%)	97	**43** in chain A = 93 **43** in chain B = 88
Rmsd bonds [e](Å)	0.001	0.002
Rmsd angles [e](°)	0.302	0.474
Ramachandran favored [e](%)	97.3	97.8
Ramachandran allowed [e](%)	2.7	2.2
Ramachandran outliers [e](%)	0	0
Average B, all atoms (Å^2)	111	55
Wilson B-factor (Å^2)	103.9	44.7
Ligand average B-factor [f](Å^2)	95	**43** in chain A = 68 **43** in Chain B = 97

Values in parentheses are for the high resolution shell

[a] Redundancy-independent merging R factor or R_{meas}

[b] CC(1/2) values were calculated using the program XSCALE

[c] R_{free} is the cross-validation R factor calculated for the test set of 5 % of unique reflections

[d] Calculated using the Twilight validation tool

[e] Calculated using Molprobity

[f] Calculated as the median value of B factors of atoms in the group

3: Ligand efficiency calculation for compound 2, 15 - 41.

Table S3: Ligand efficiency calculation.

Code	IC50 (μM)	HA (heavy atoms)	IC50(mol)	LE (ligand efficiency)
2	6.1	29	0.0000061	0.25
15	138	22	0.000138	0.24
16	72	23	0.000072	0.25
17	168	23	0.000168	0.22
18	113	24	0.000113	0.23
19	328	25	0.000328	0.19
20	40	23	0.00004	0.26
21	7.1	24	0.0000071	0.29
22	35	24	0.000035	0.25
23	141	25	0.000141	0.21
24	142	27	0.000142	0.20
25	96	22	0.000096	0.25
26	75	22	0.000075	0.26
27	49	22	0.000049	0.27
28	143	25	0.000143	0.21
29	114	21	0.000114	0.26
30	254	21	0.000254	0.23
31	129	23	0.000129	0.23
32	1115	22	0.001115	0.18
33	38	26	0.000038	0.23
34	20	26	0.00002	0.25
35	9	26	0.000009	0.27
36	17	26	0.000017	0.25
37	25	26	0.000025	0.24
38	27	26	0.000027	0.24
39	26	26	0.000026	0.24
40	25	26	0.000025	0.24
41	23	25	0.000023	0.25

Note: The equation used for LE caculation is as follows,[1, 2]

$$LE = (1.37/HA) \times pIC_{50}$$

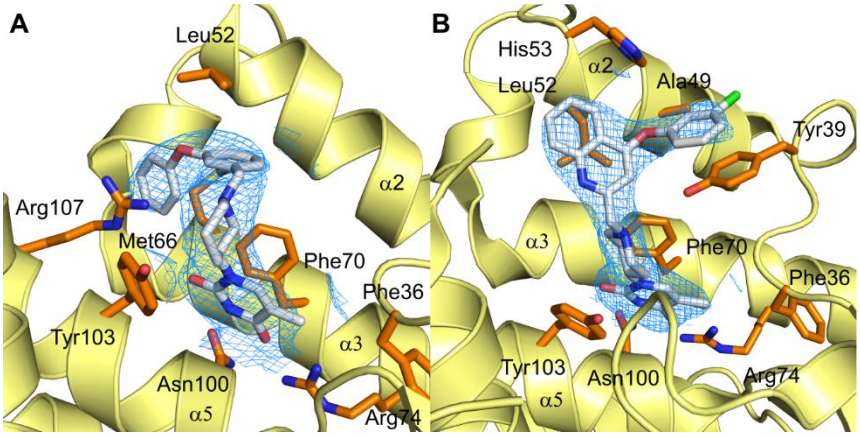


Figure S2. Composite electron density maps of bound inhibitors in *Mt*TMPK active site. (A) *Mt*TMPK/compound **1** cocrystal structure. The 2Fo-Fc Fourier difference electron density map for compound **1** (stick representation, carbon atoms in white) and calculated at the end of the refinement procedure is contoured to +1.4 sigma and represented with a blue mesh. (B) *Mt*TMPK/compound **43** cocrystal structure. The 2Fo-Fc Fourier difference electron density map for compound **43** (stick representation, carbon atoms in white) and calculated at the end of the refinement procedure is contoured to +1.0 sigma and represented with a blue mesh. Protein is depicted in pale yellow cartoon representation. Key interacting residues are represented in stick conformation and colored in orange for carbon atoms.

References

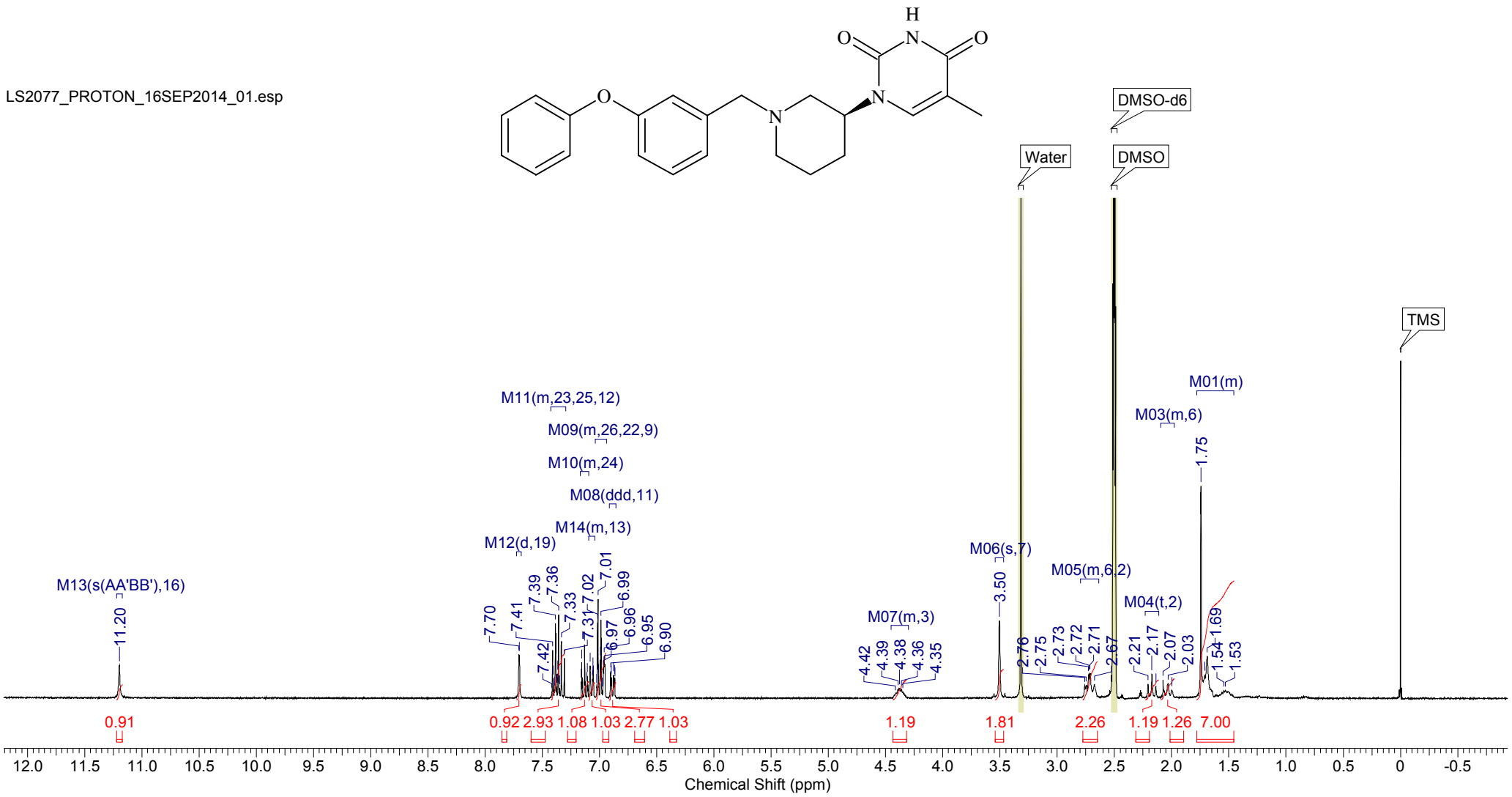
1. Hopkins, A. L.; Groom, C. R.; Alex, A. Ligand efficiency: a useful metric for lead selection. *Drug Discovery Today* **2004,** 9, 430-431.

2. Hopkins, A. L.; Keseru, G. M.; Leeson, P. D.; Rees, D. C.; Reynolds, C. H. The role of ligand efficiency metrics in drug discovery. *Nat Rev Drug Discov* **2014,** 13, 105-121.

^{1}H and ^{13}C NMR Spectra of compound 1 – 44

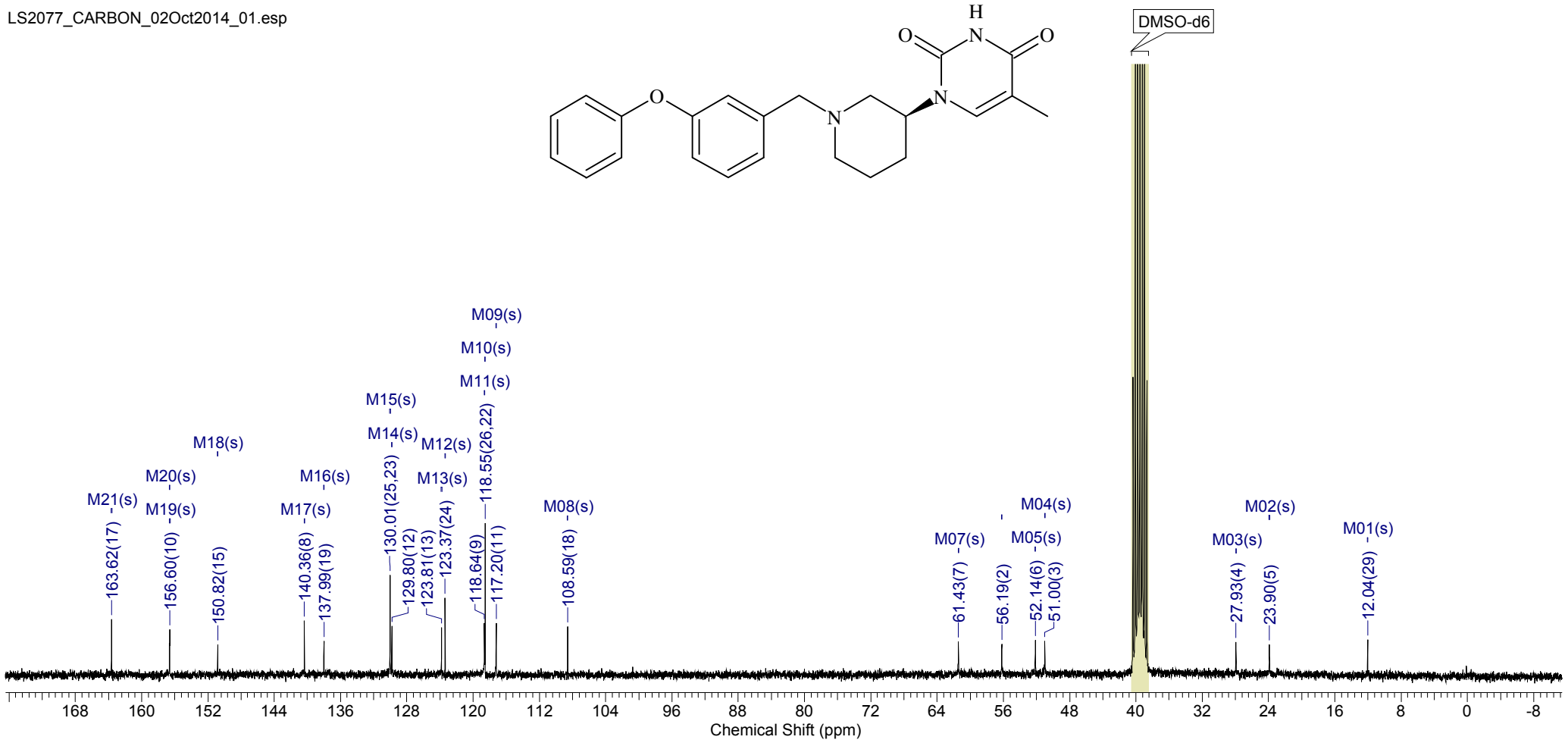
Compound 1

^{1}HNMR 300 MHz DMSO-d_6



Compound 1

^{13}CNMR 75 MHz DMSO-d_6



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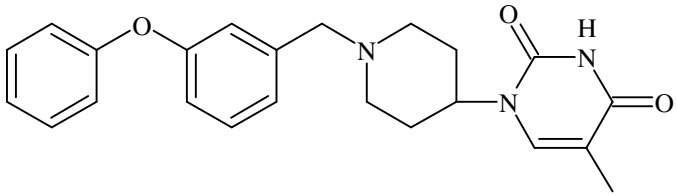

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Compound 2

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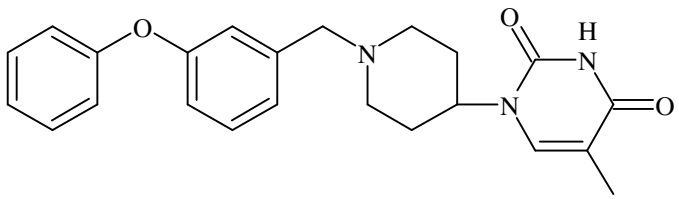

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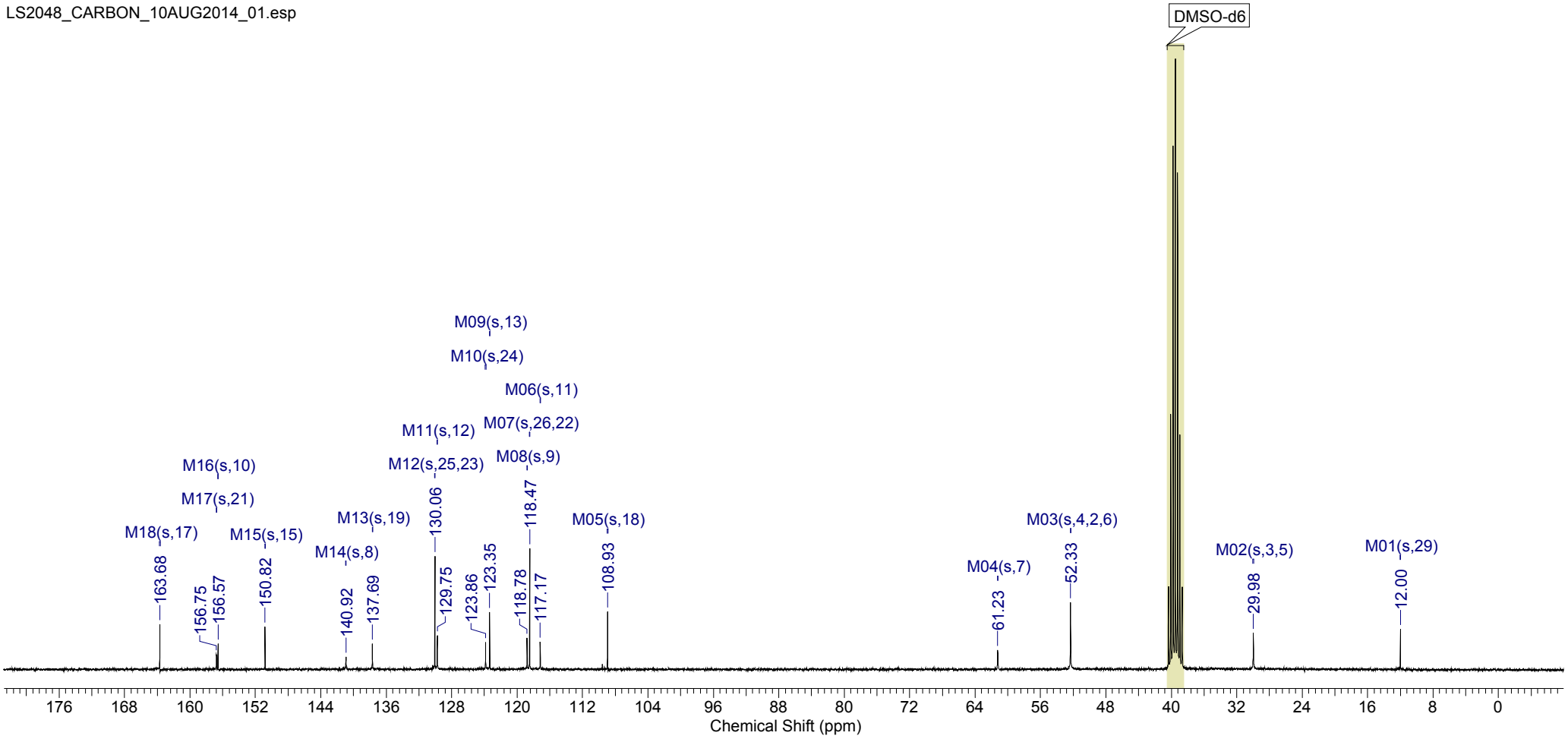

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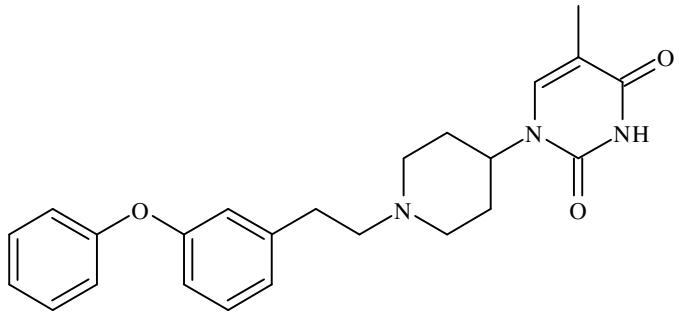

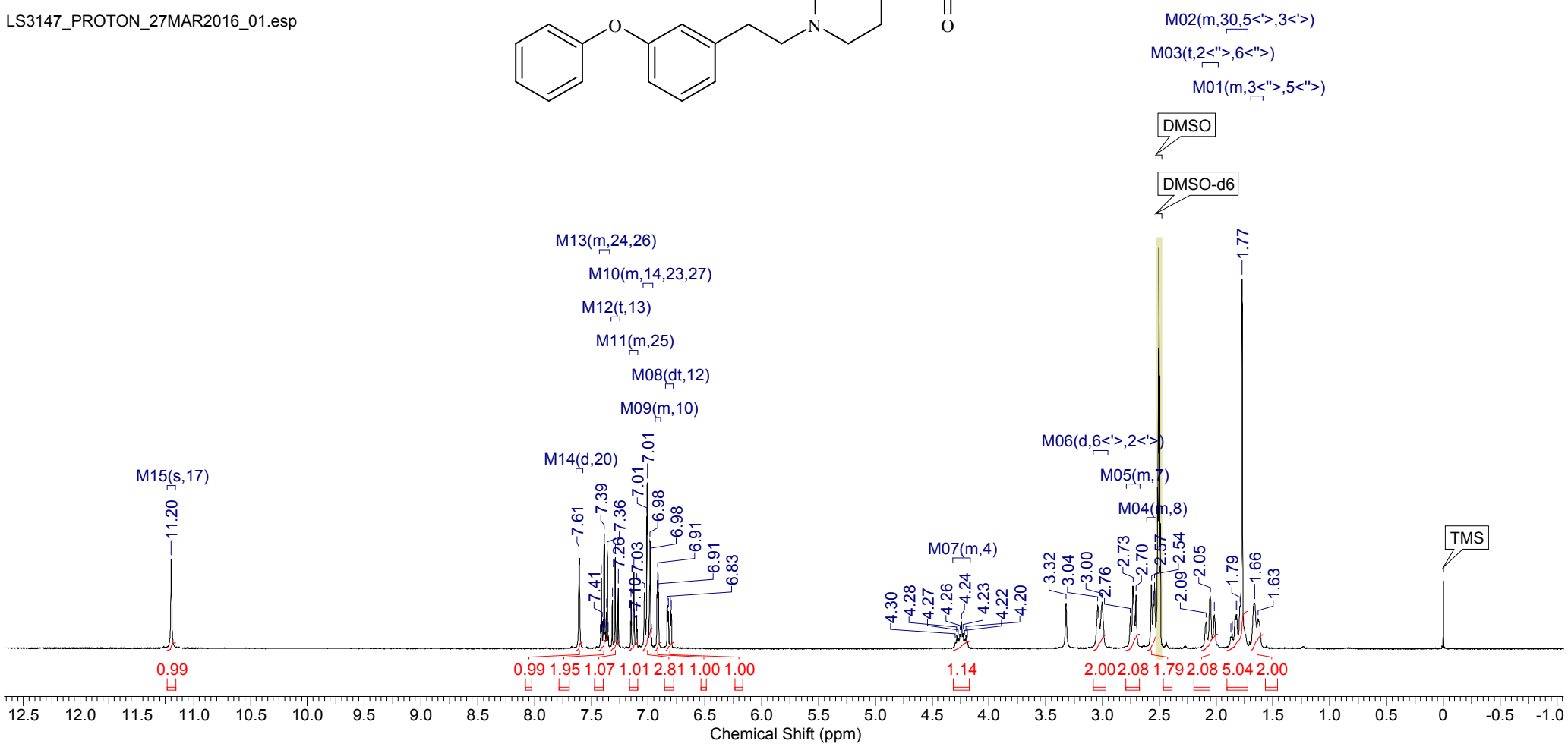

Compound 3

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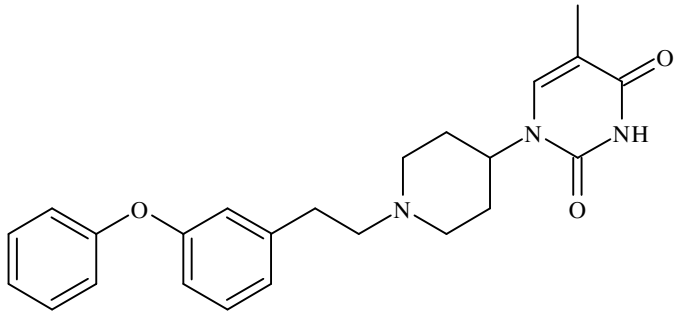

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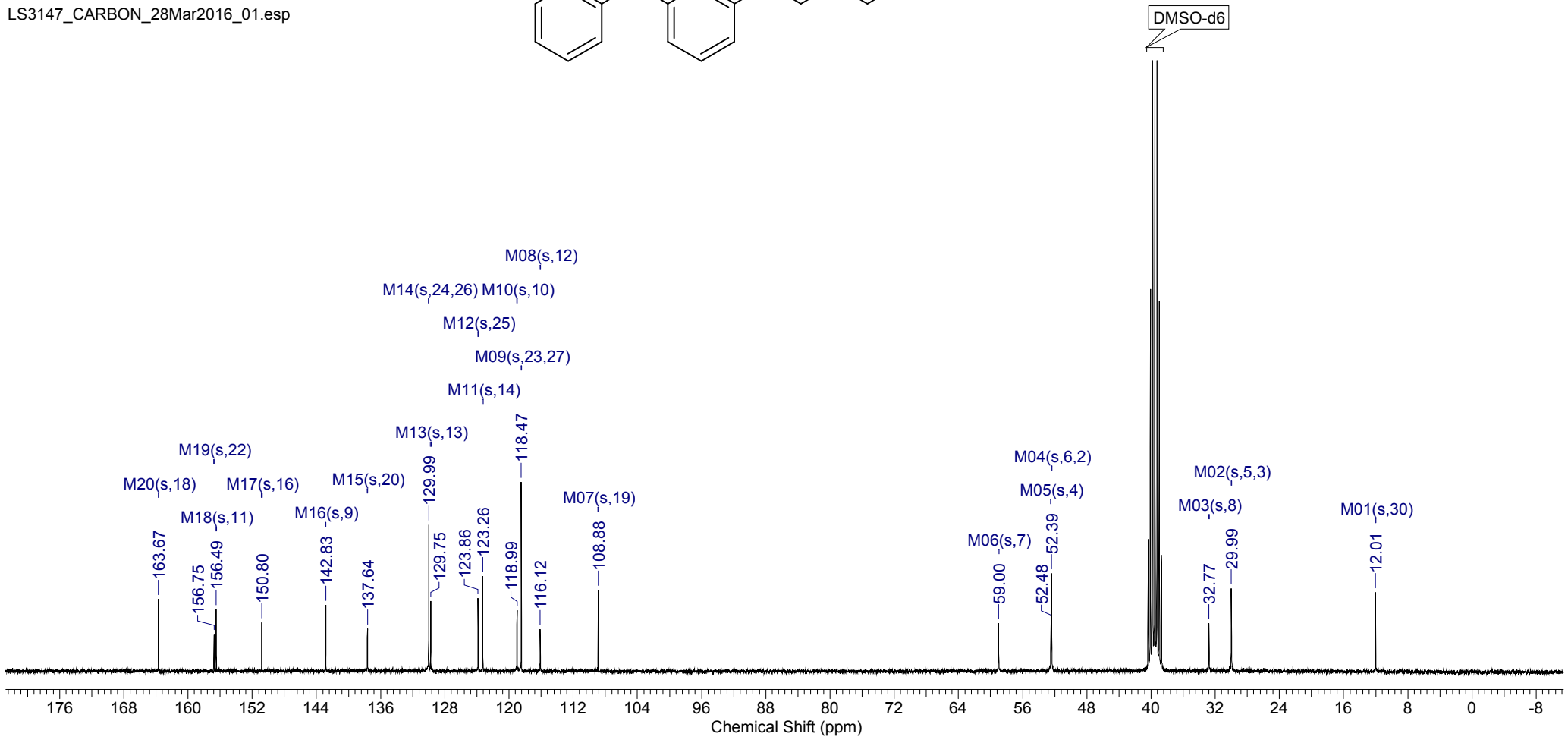

Compound 4

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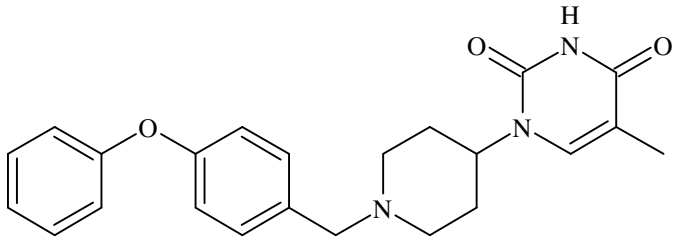

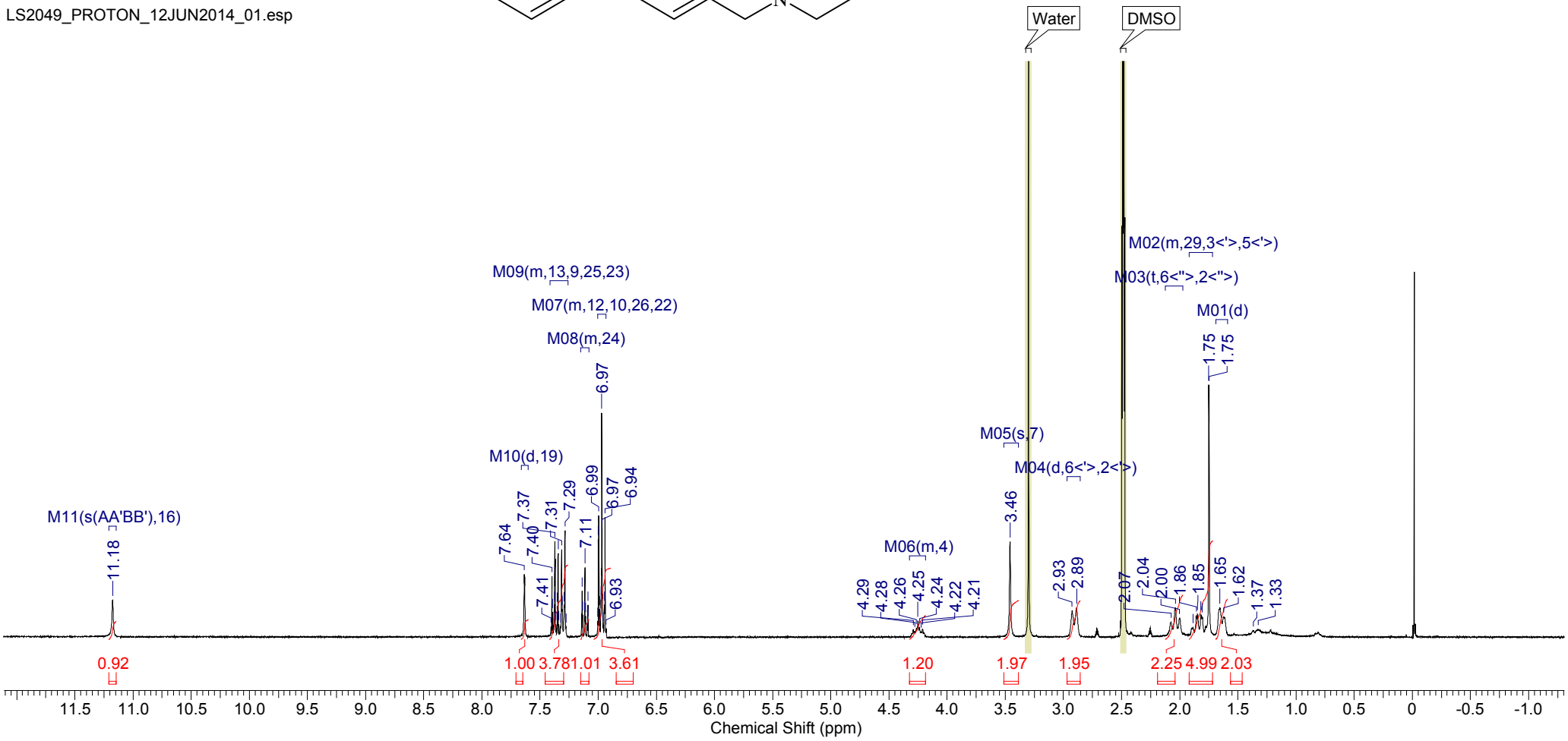

Compound 4

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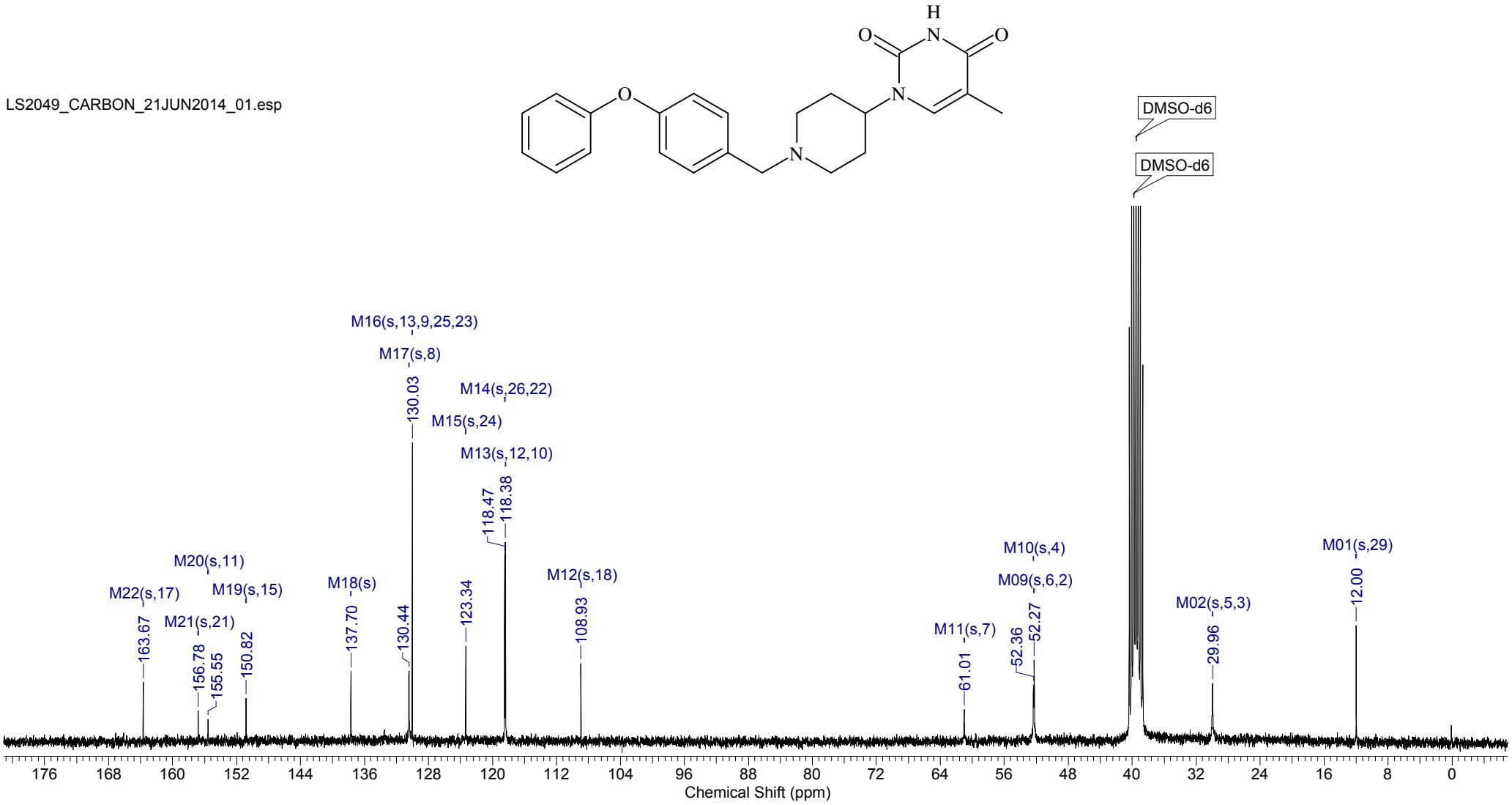

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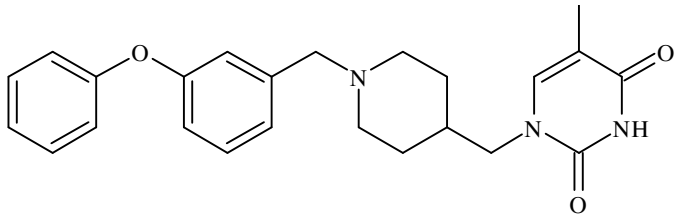

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Compound 5

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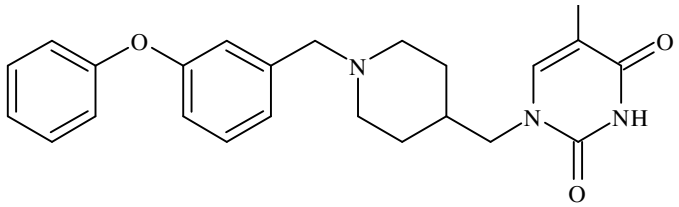

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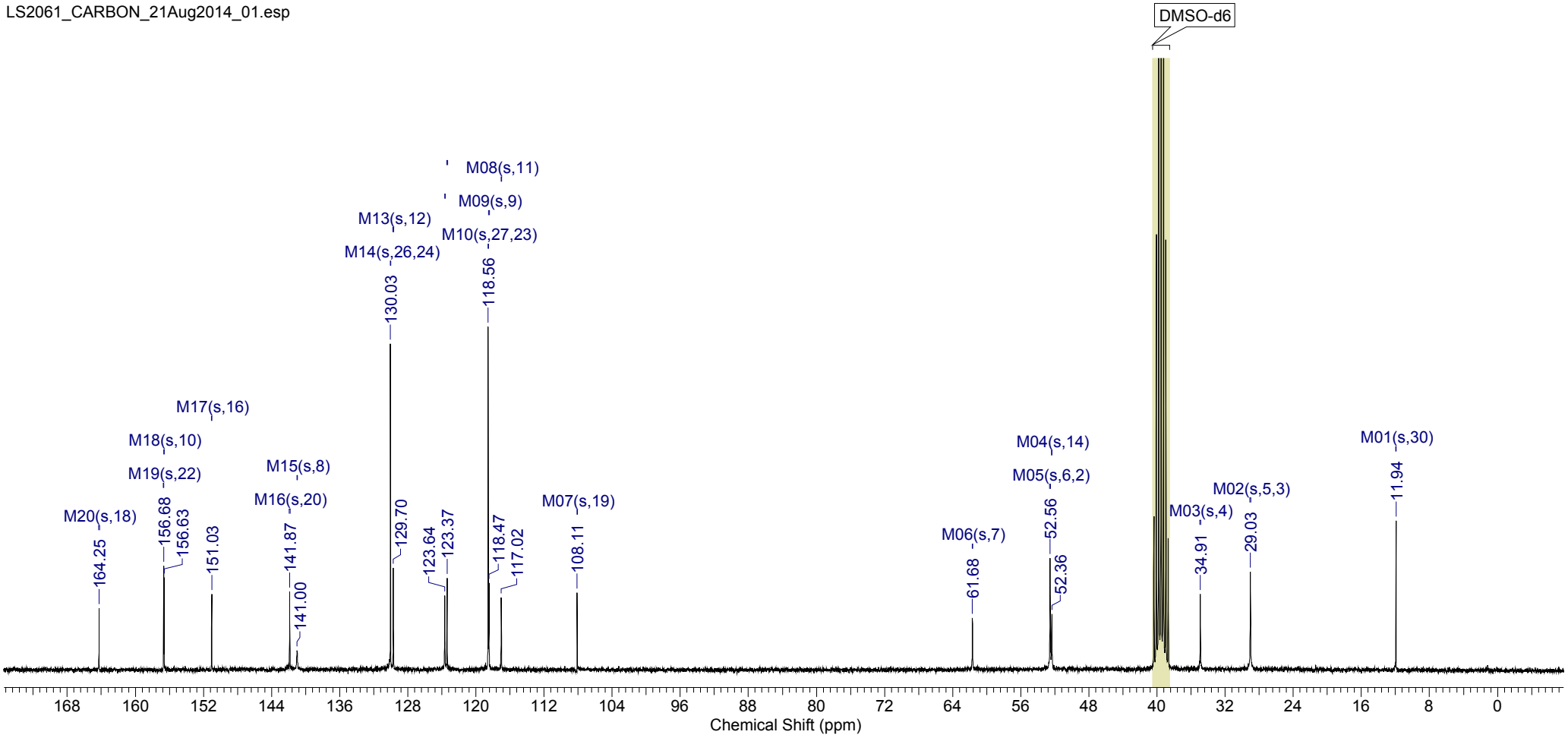

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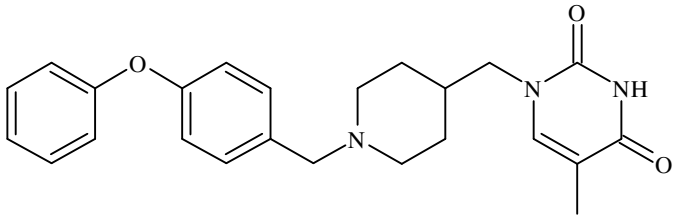

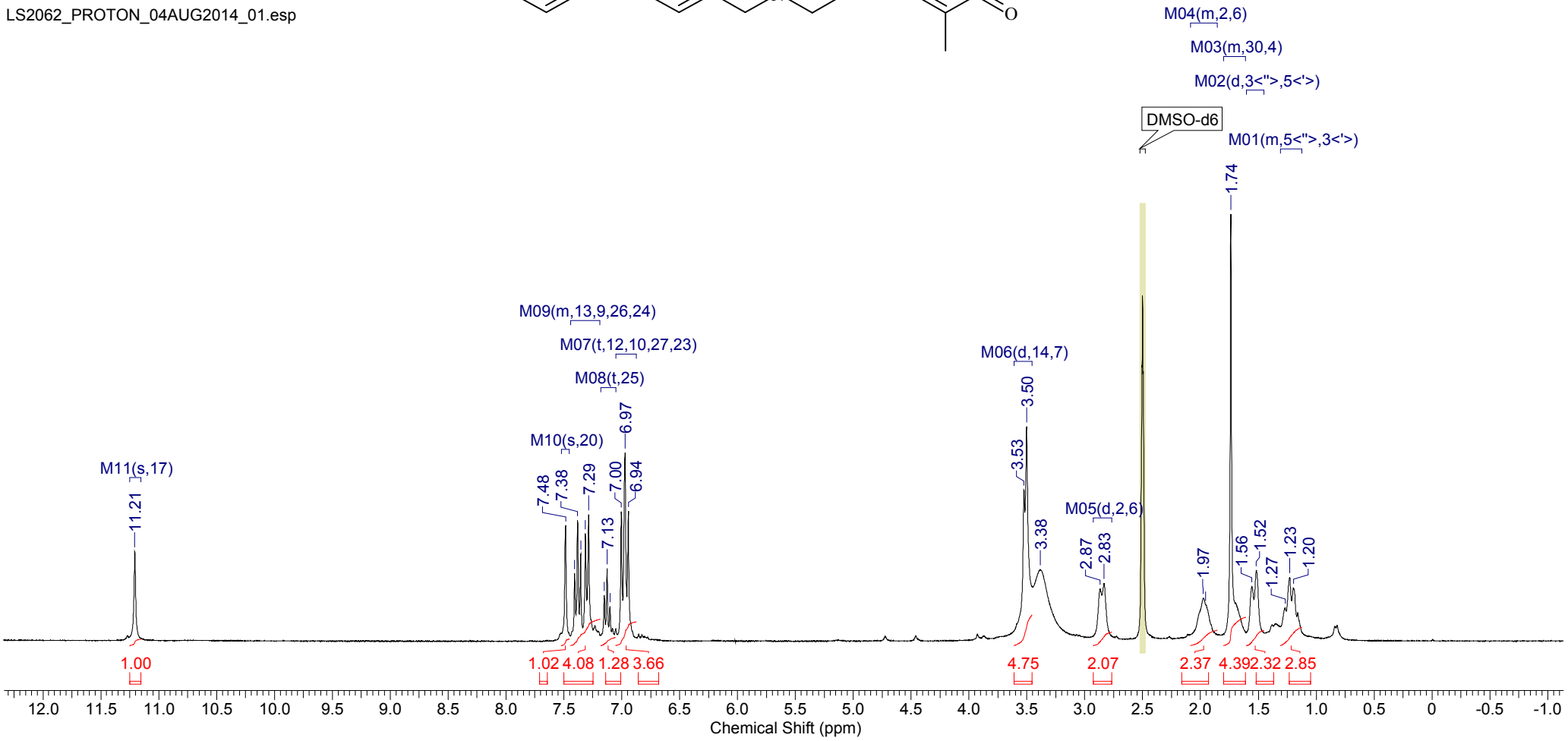

Compound 6

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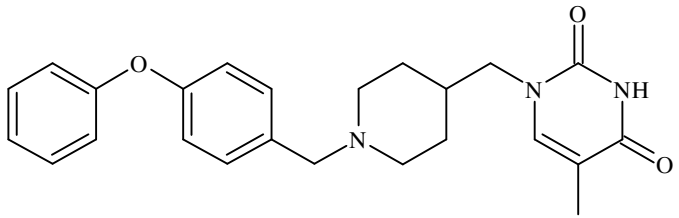

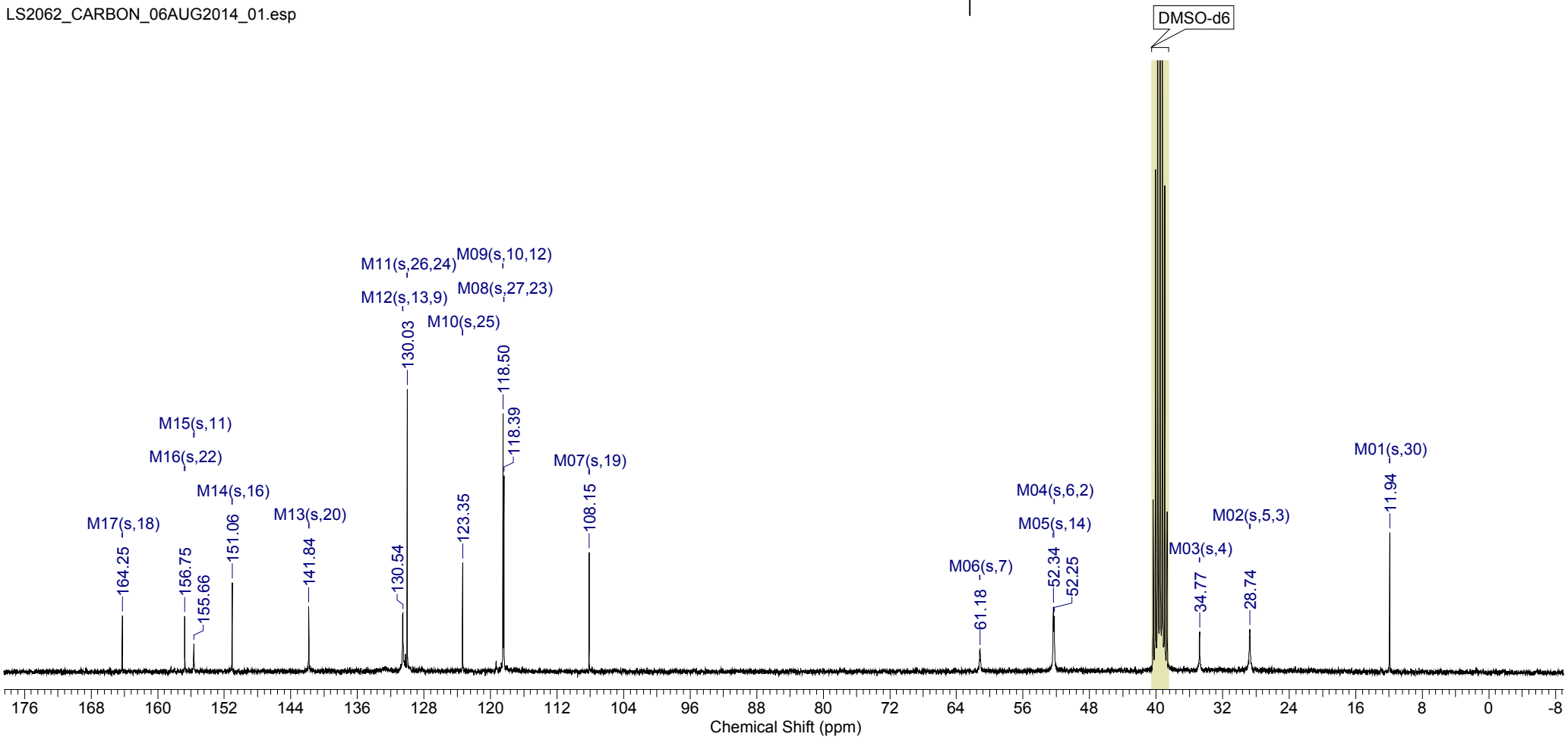

Compound 7

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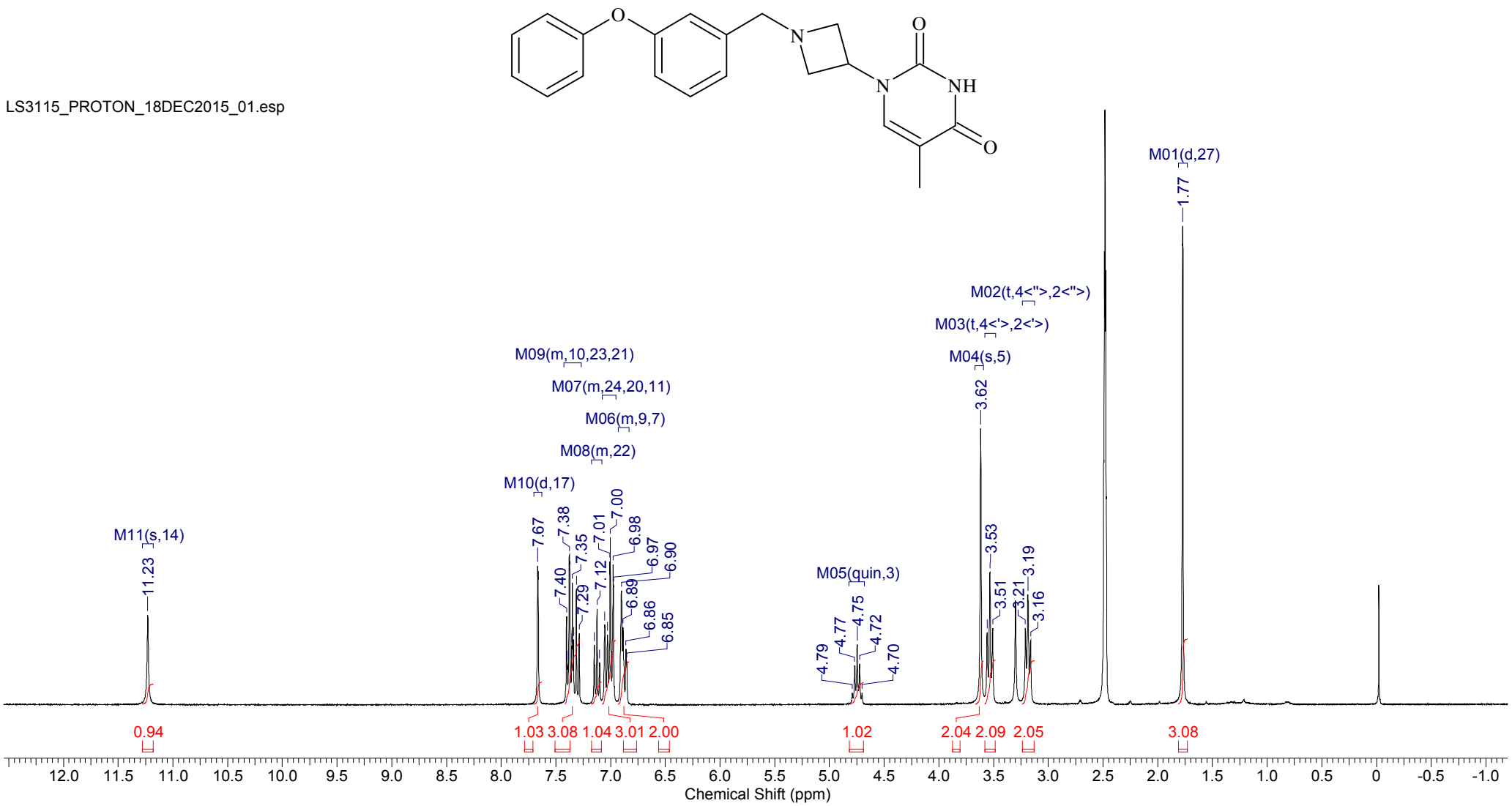

Compound 7

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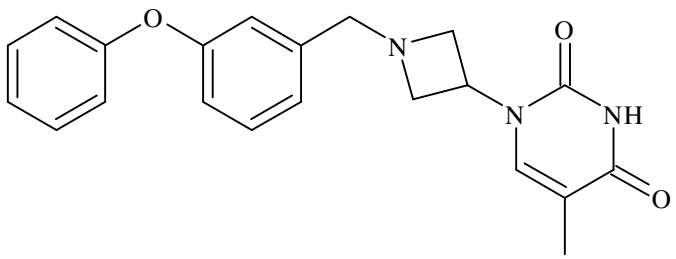

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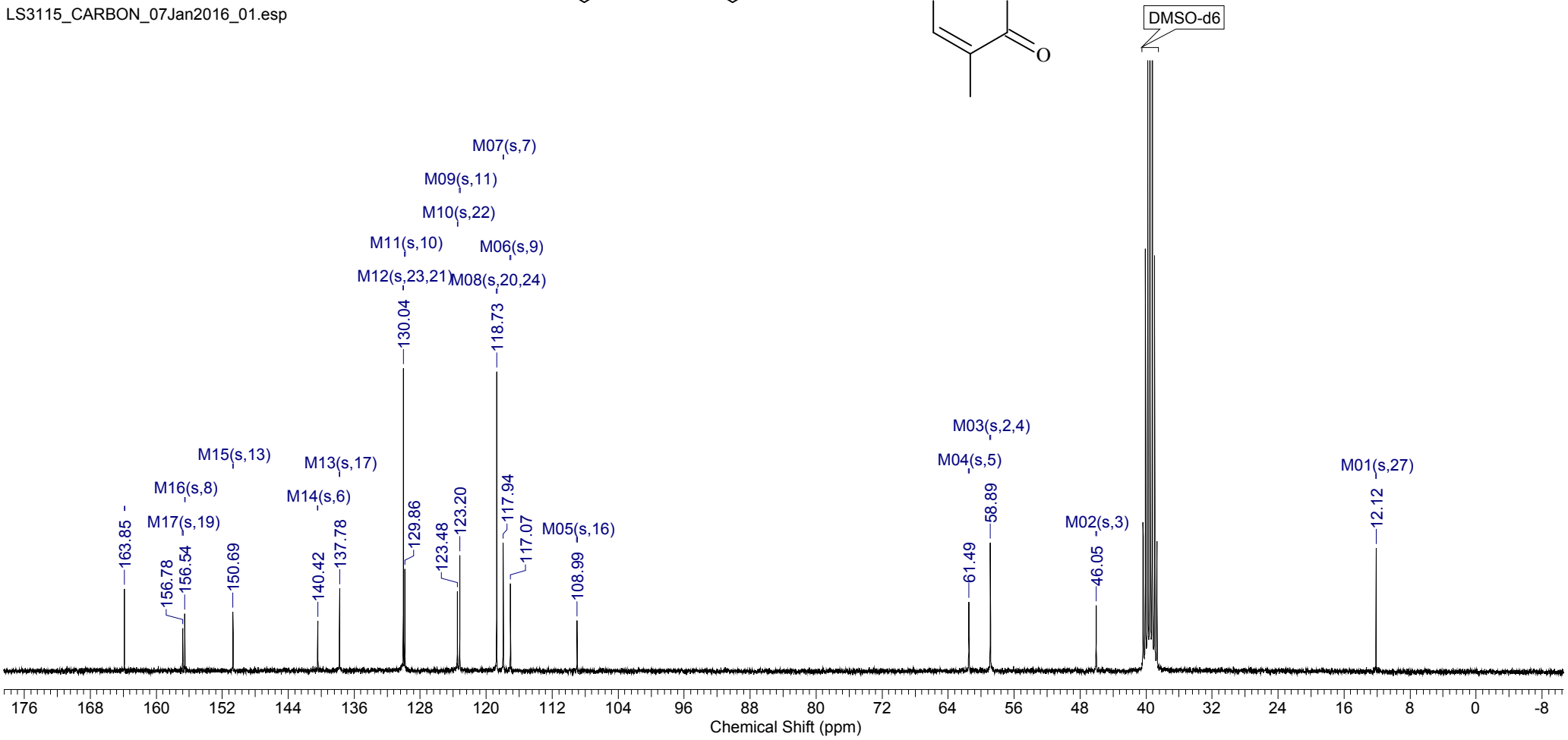

Compound 8

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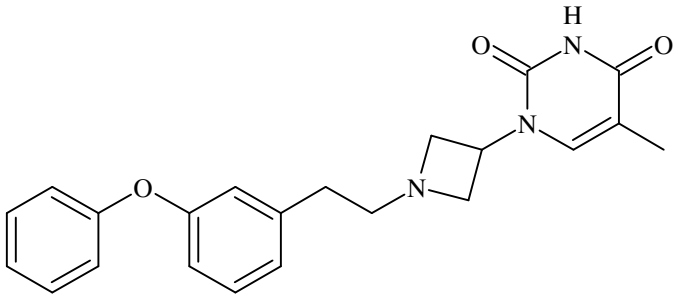

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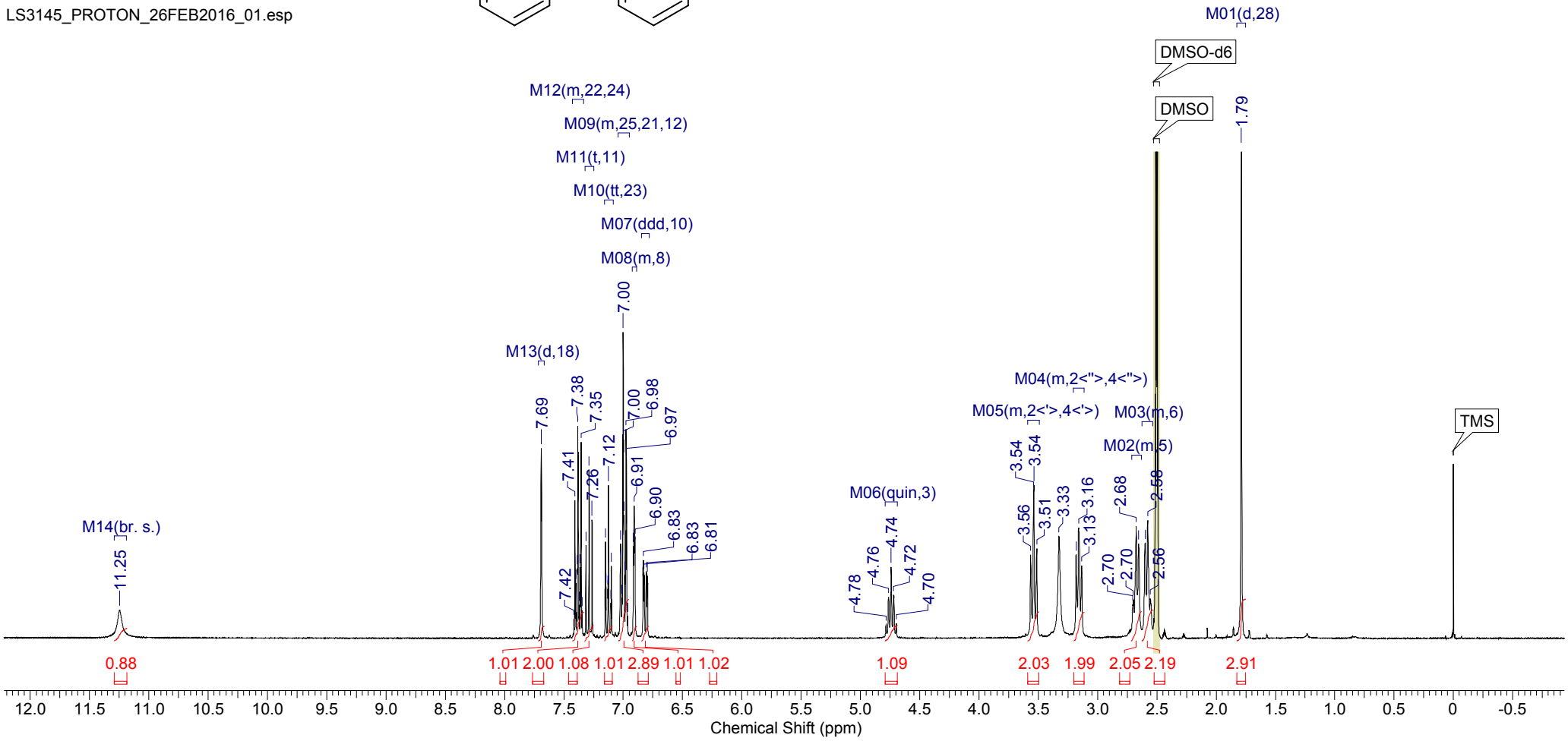

Compound 8

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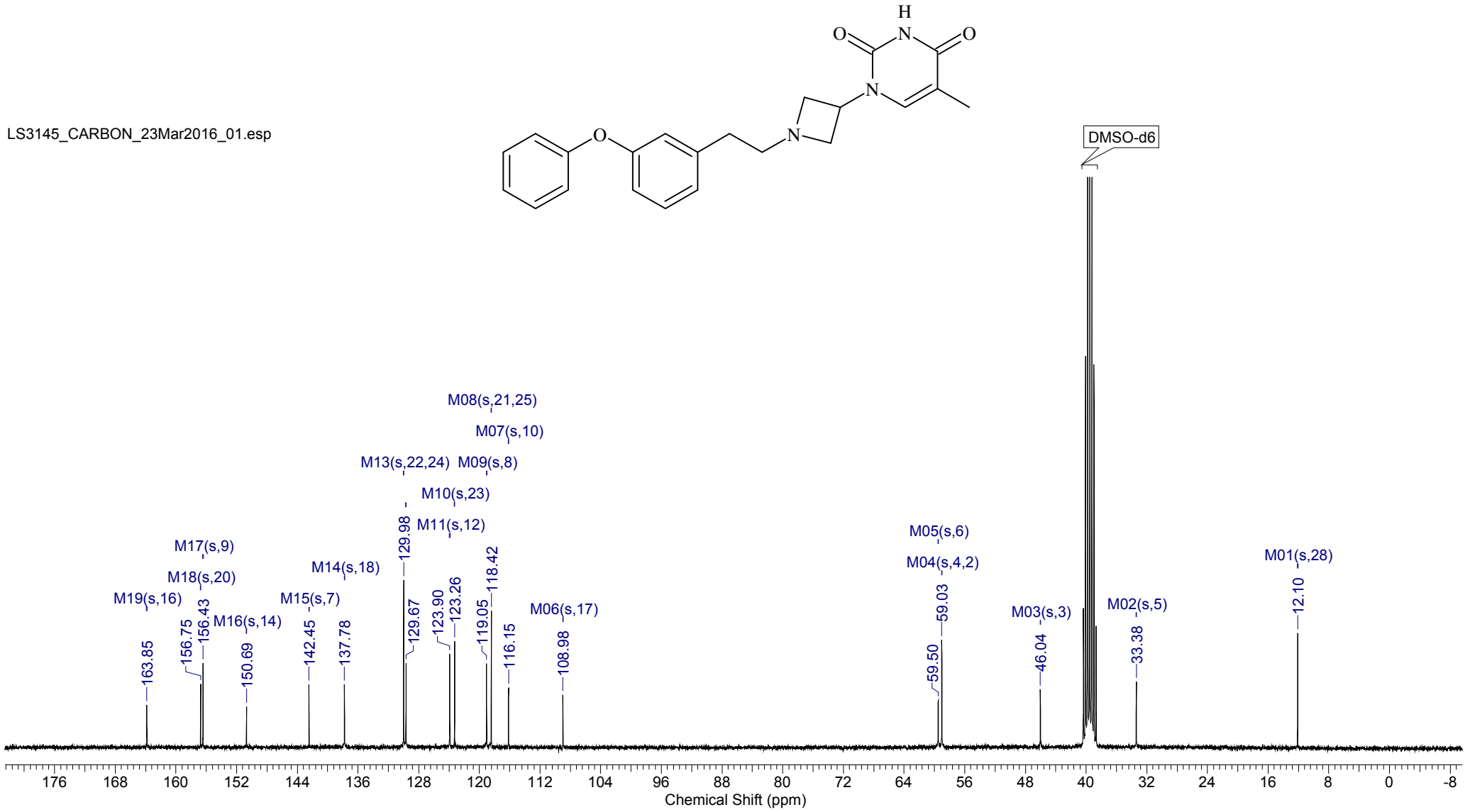

Compound 9

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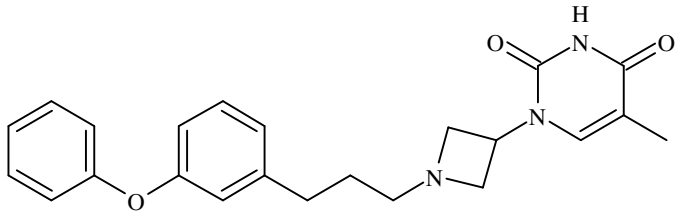

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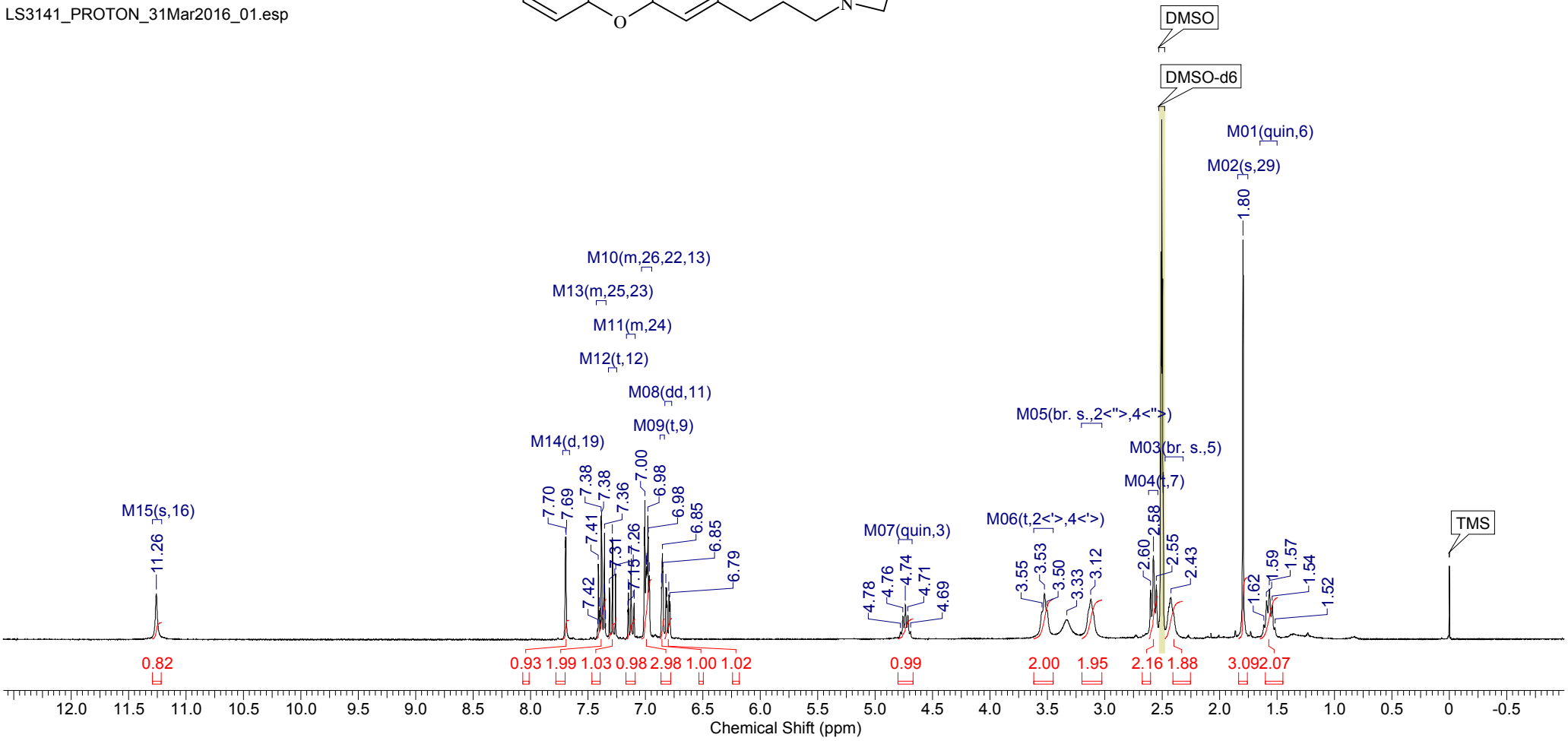
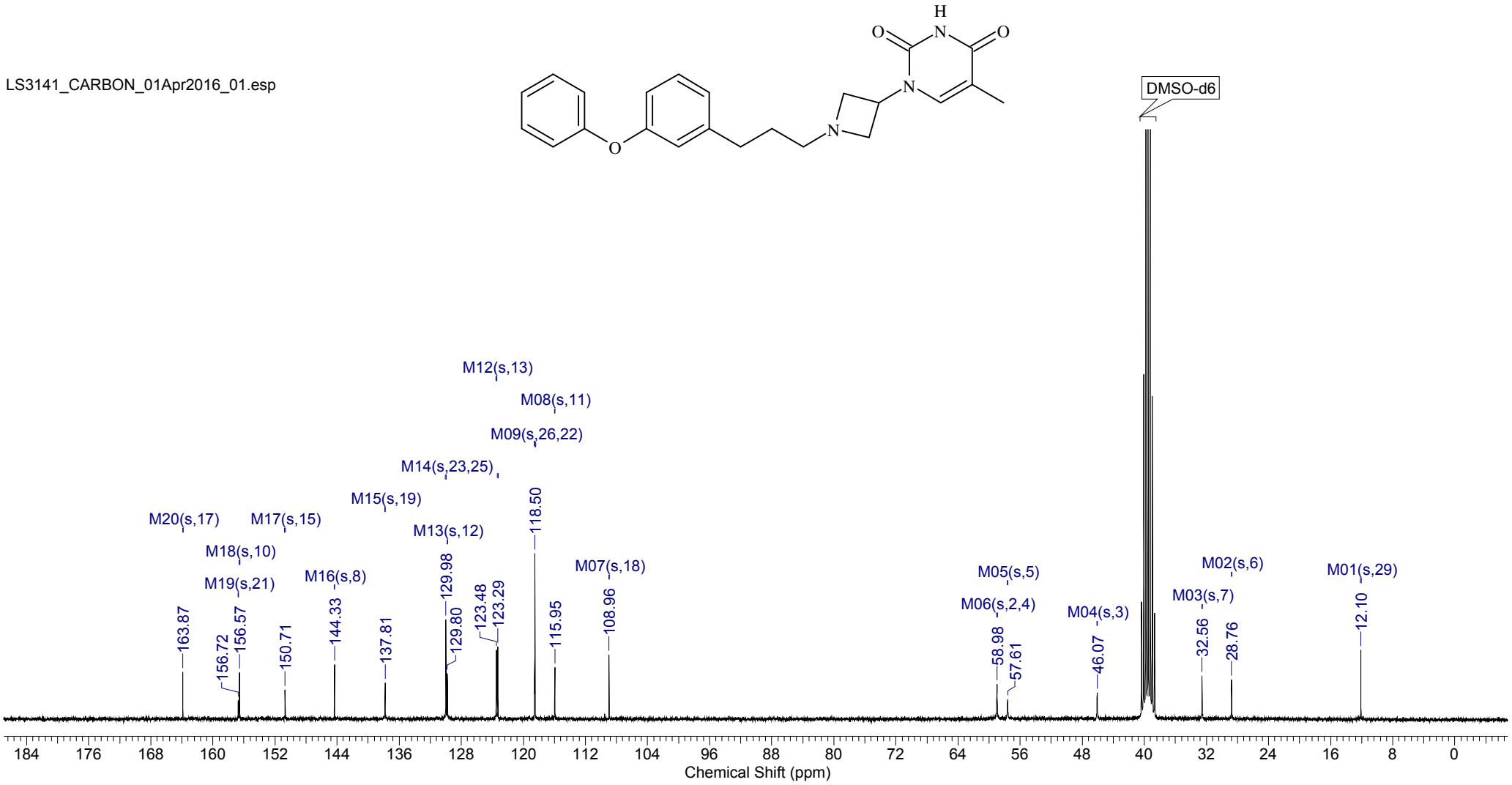


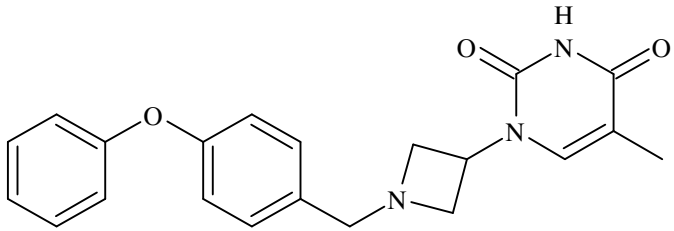
Compound 9

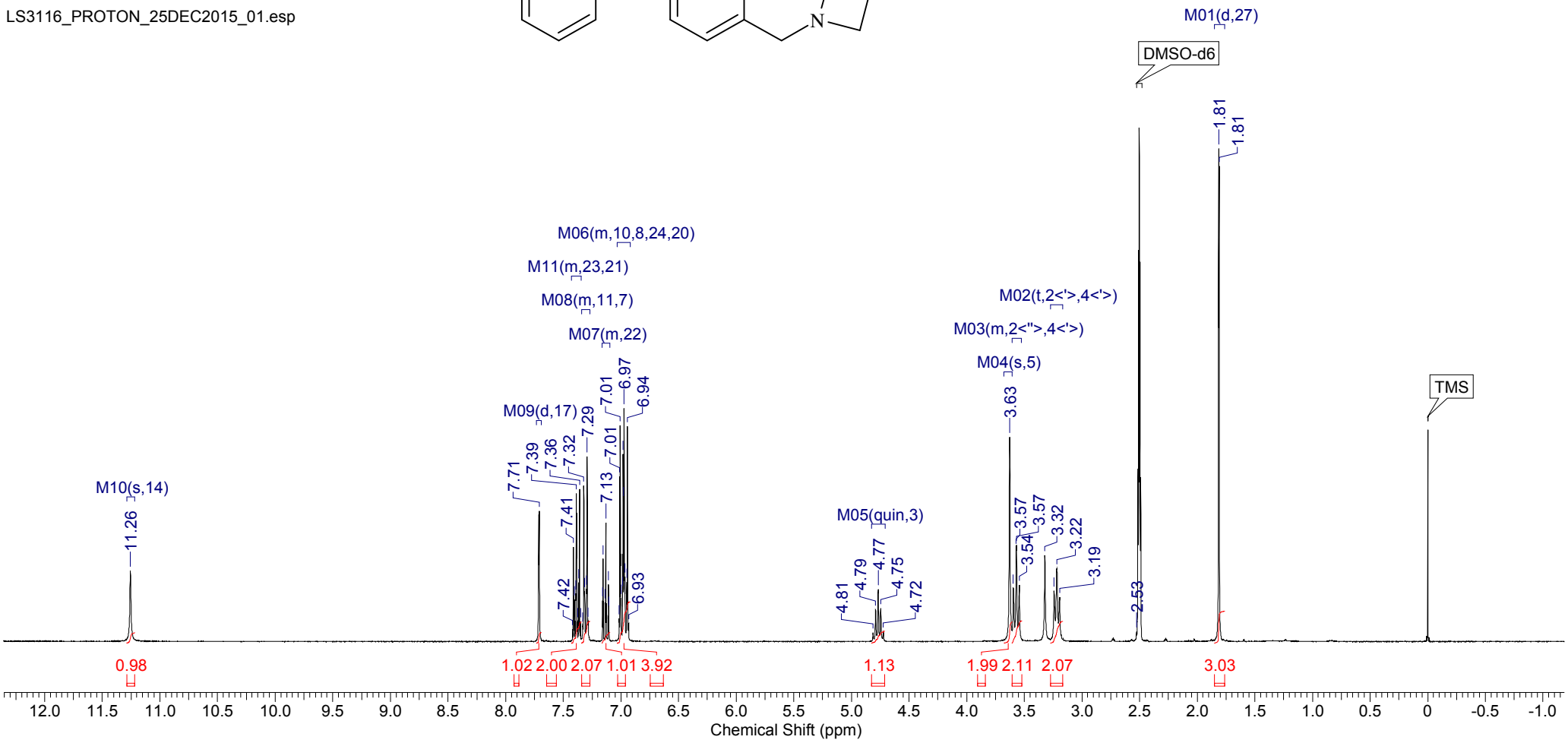
^{13}CNMR 75 MHz DMSO-d_6



Compound 10

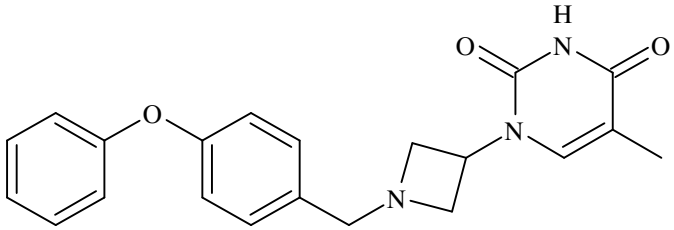
^{1}HNMR 300 MHz DMSO-d$_6$



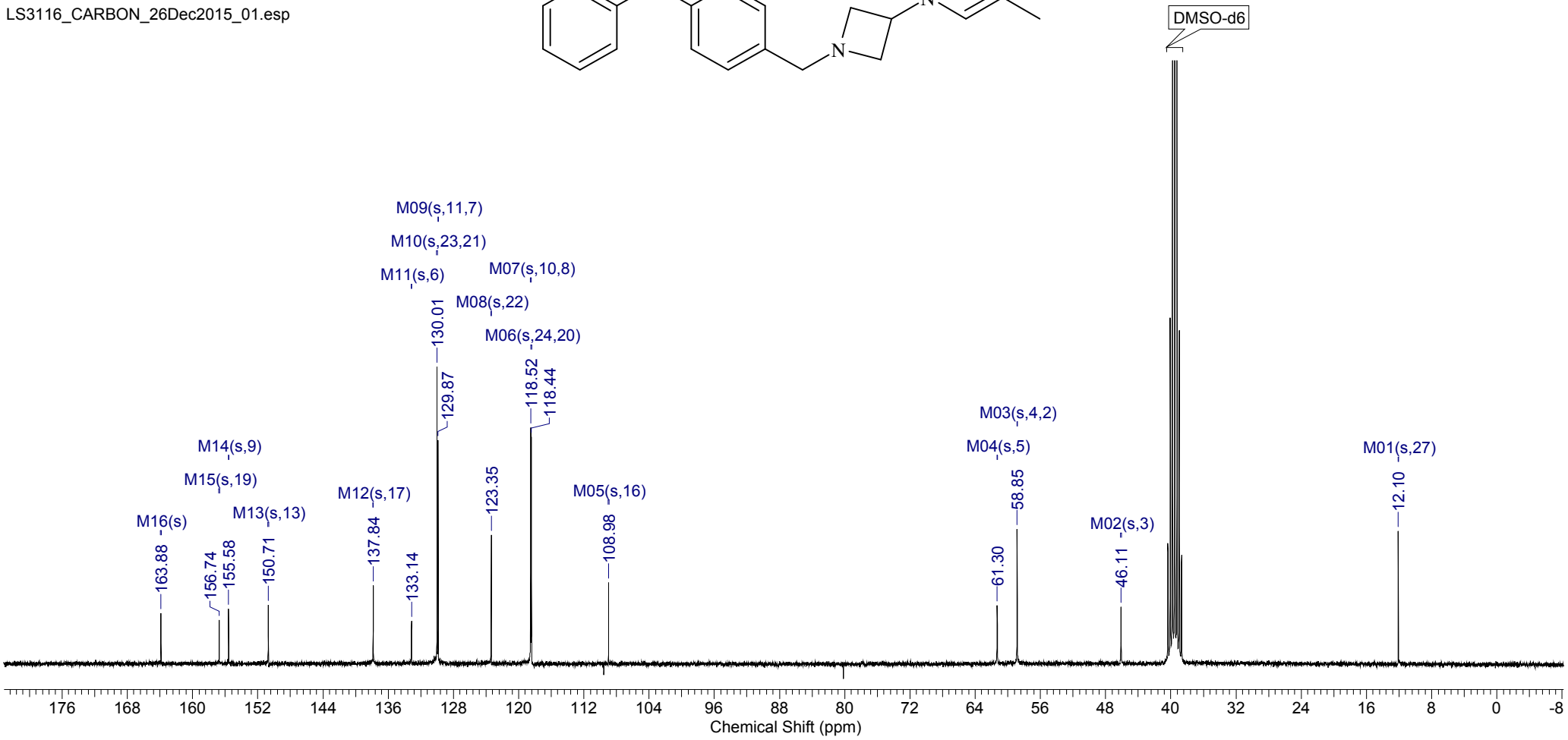


Compound 10

^{13}CNMR 75 MHz DMSO-d_6

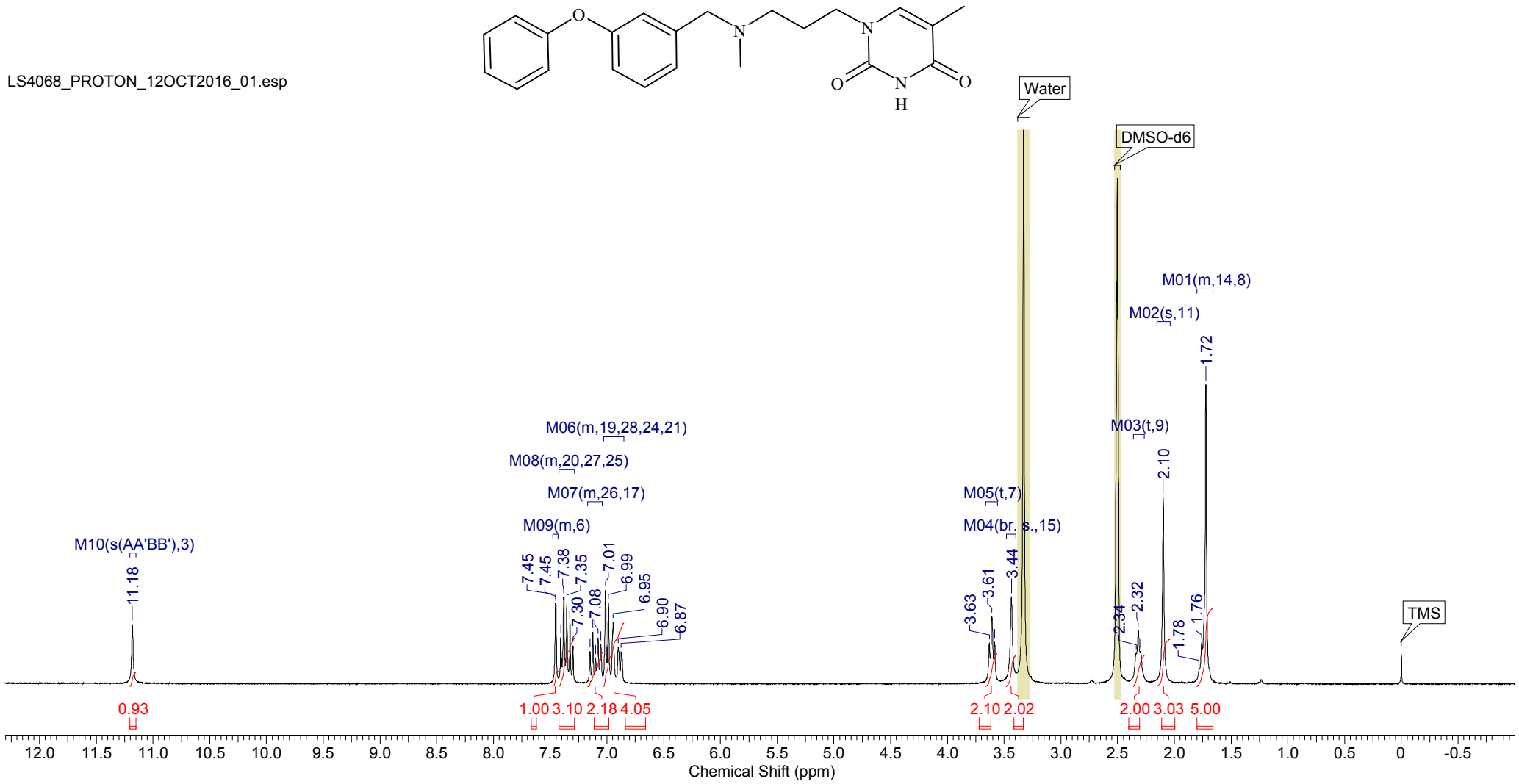


LS3116_CARBON_26Dec2015_01.esp



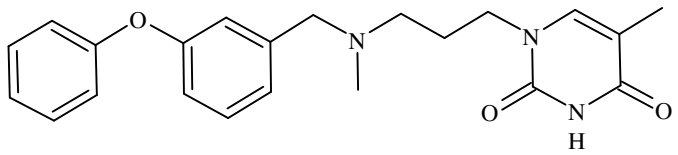
Compound 11

^{1}HNMR 300 MHz DMSO-d_6



Compound 11

^{13}CNMR 75 MHz DMSO-d_6



LS4068_CARBON_13Oct2016_01.esp

DMSO-d6

M11(s,26)
M12(s,17)
M13(s,20)
M08(s,19)
M14(s,27,25)
M09(s,21)
M17(s,18,23)
M19(br. s.,16)
M18(s,4)
M15(s,6)
M16(s,2)
M07(s,5)
M05(s,9)
M03(s,11)
M06(s,15)
M04(s,7)
M02(s,8)
M01(s,14)

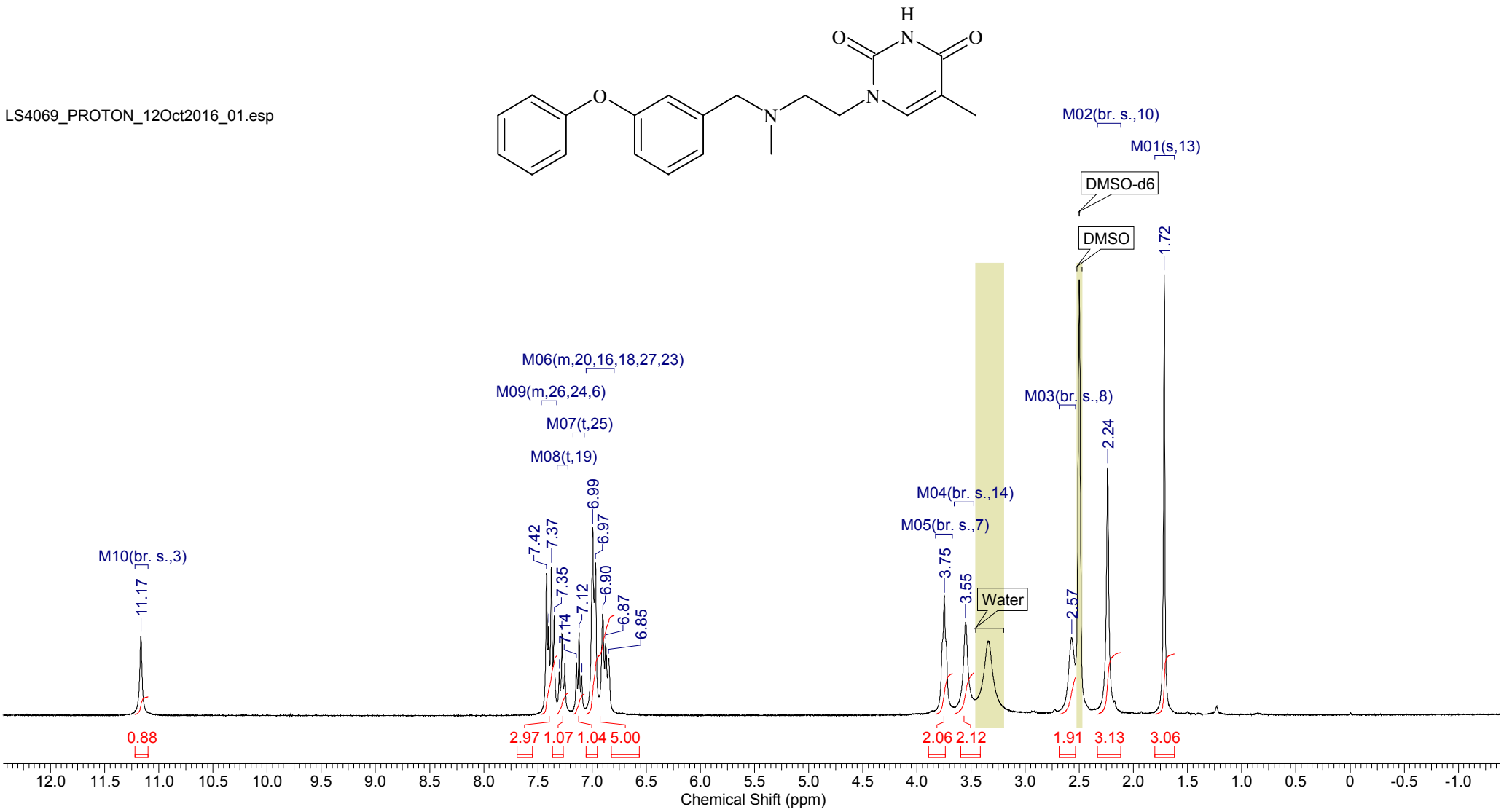
164.30
156.68
150.82
141.57
141.43
130.03
129.72
123.74
123.40
118.64
118.56
117.08
108.24
61.01
53.47
45.66
41.53
26.01
11.94

176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0 -8

Chemical Shift (ppm)

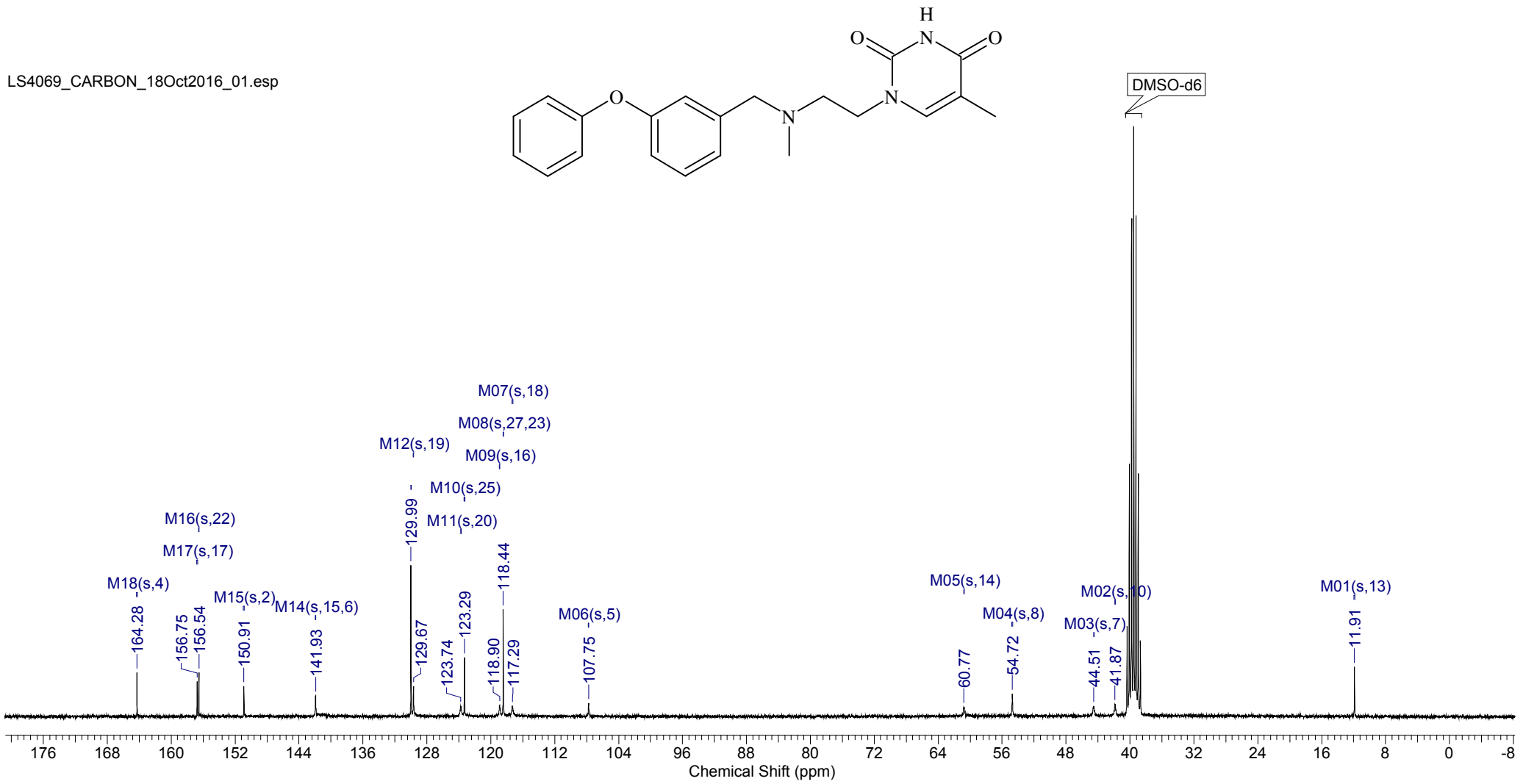
Compound 12

^{1}HNMR 300 MHz DMSO-d_6



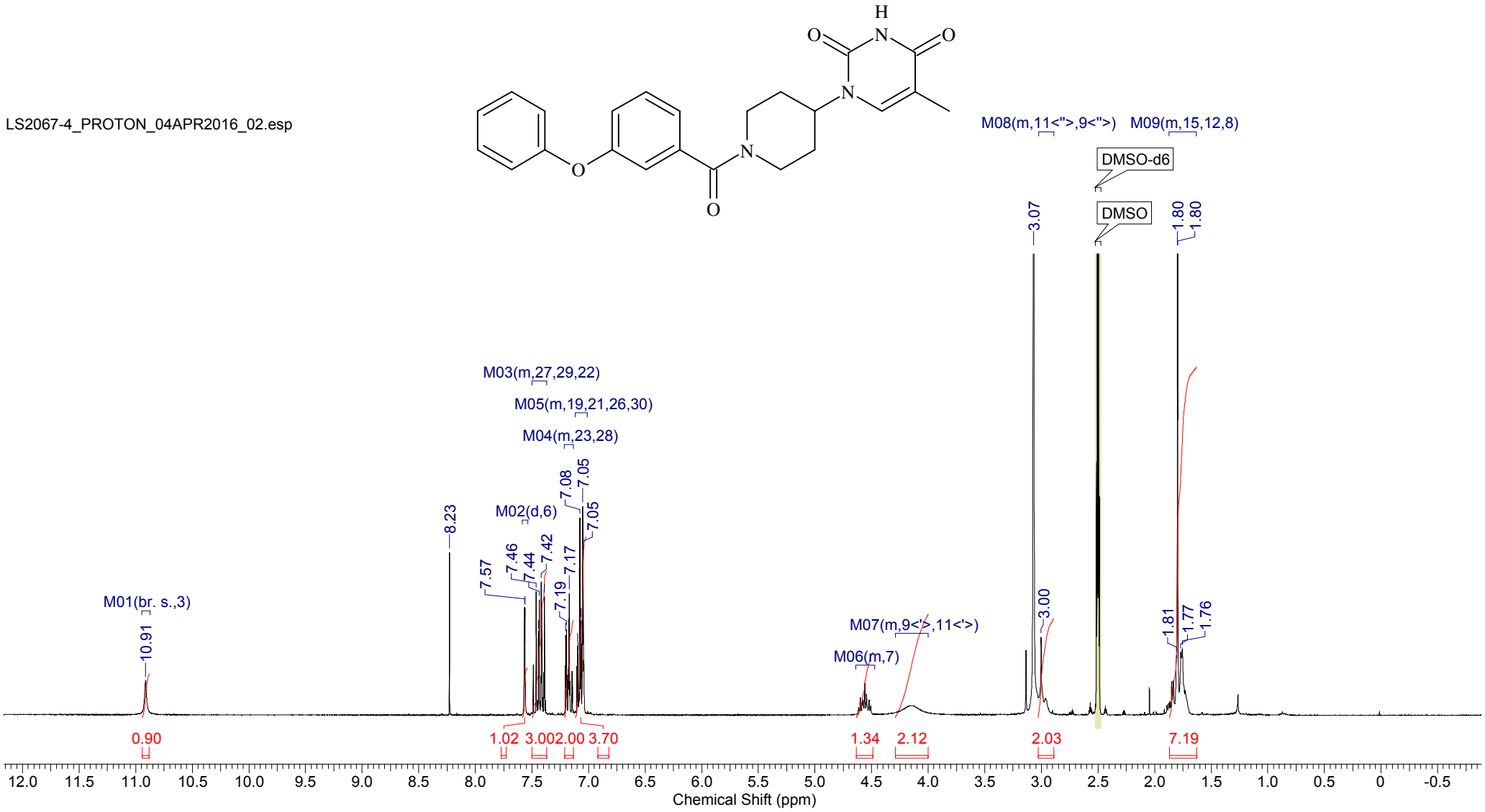
Compound 12

^{13}CNMR 75 MHz DMSO-d_6



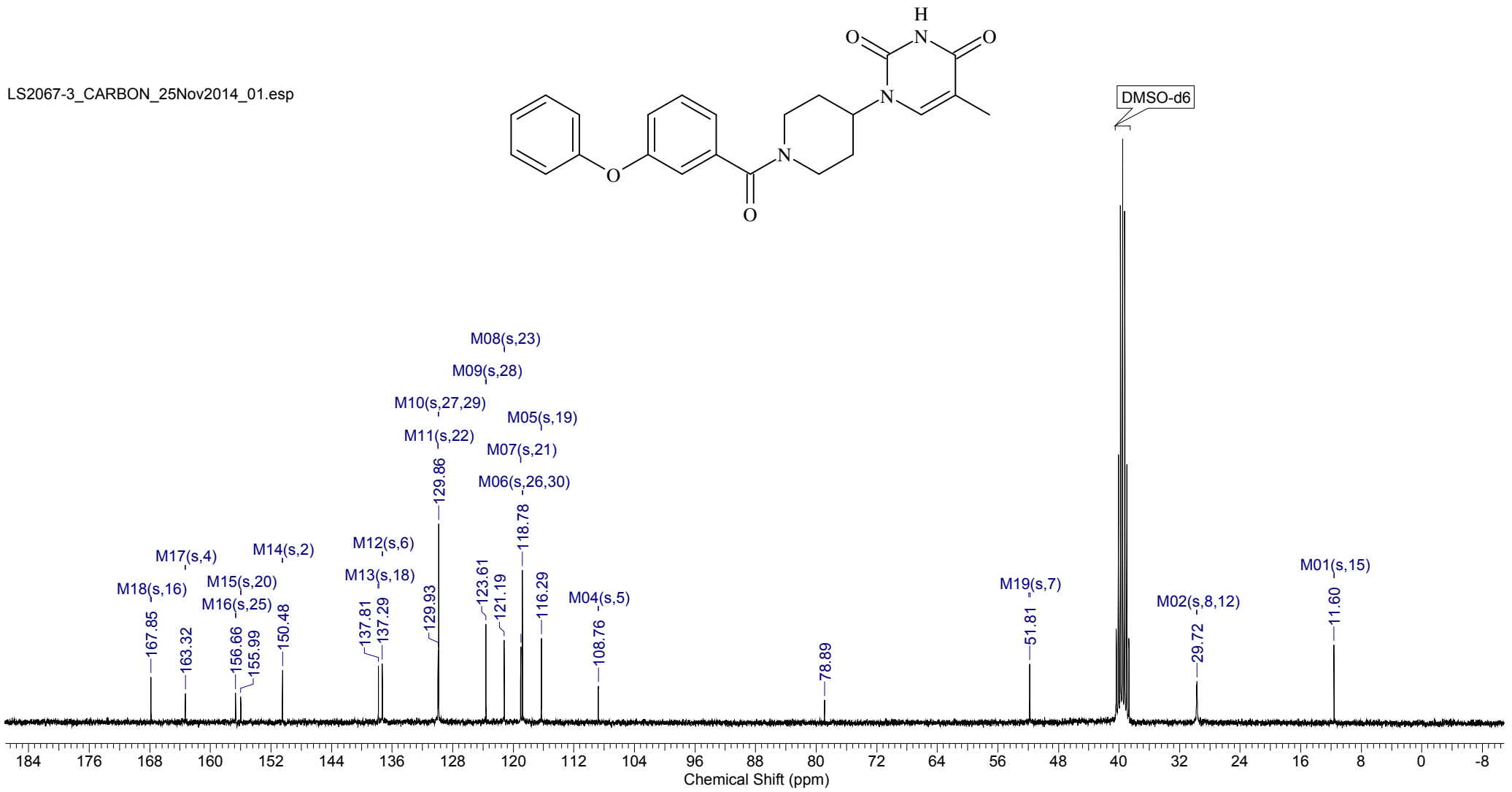
Compound 13

^{1}HNMR 300 MHz DMSO-d_6



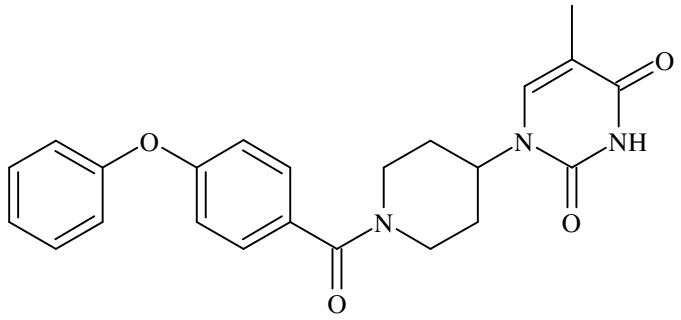
Compound 13

^{13}CNMR 75 MHz DMSO-d$_6$

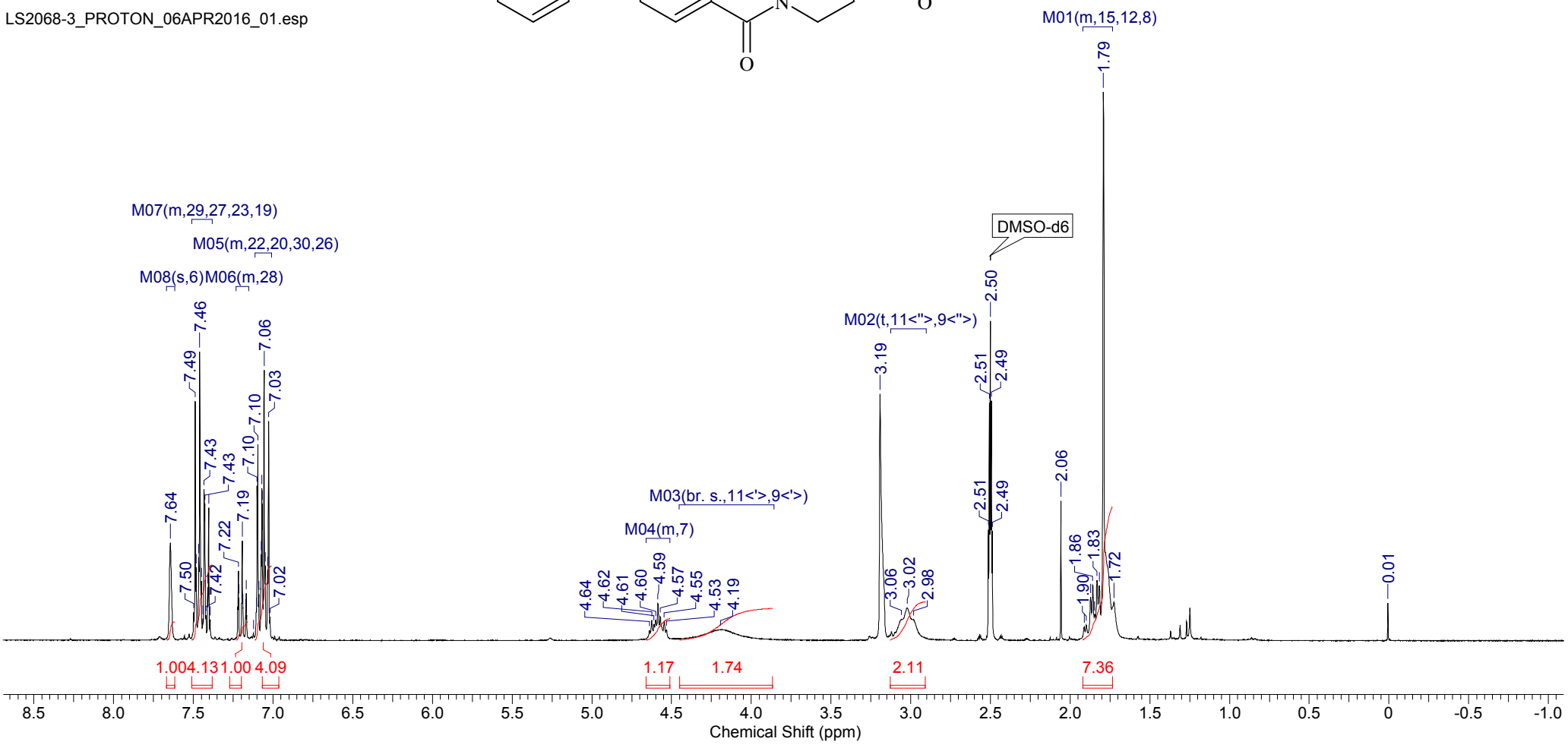


Compound 14

^{1}HNMR 300 MHz DMSO-d$_6$



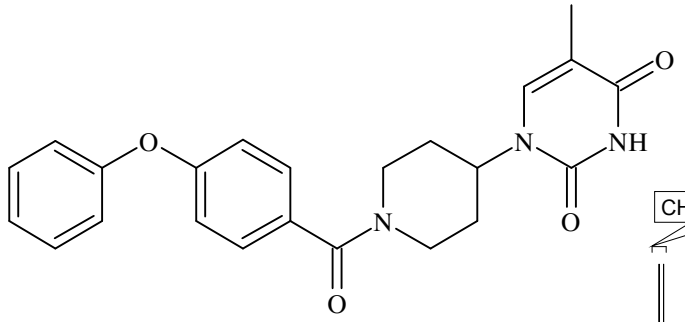
LS2068-3_PROTON_06APR2016_01.esp

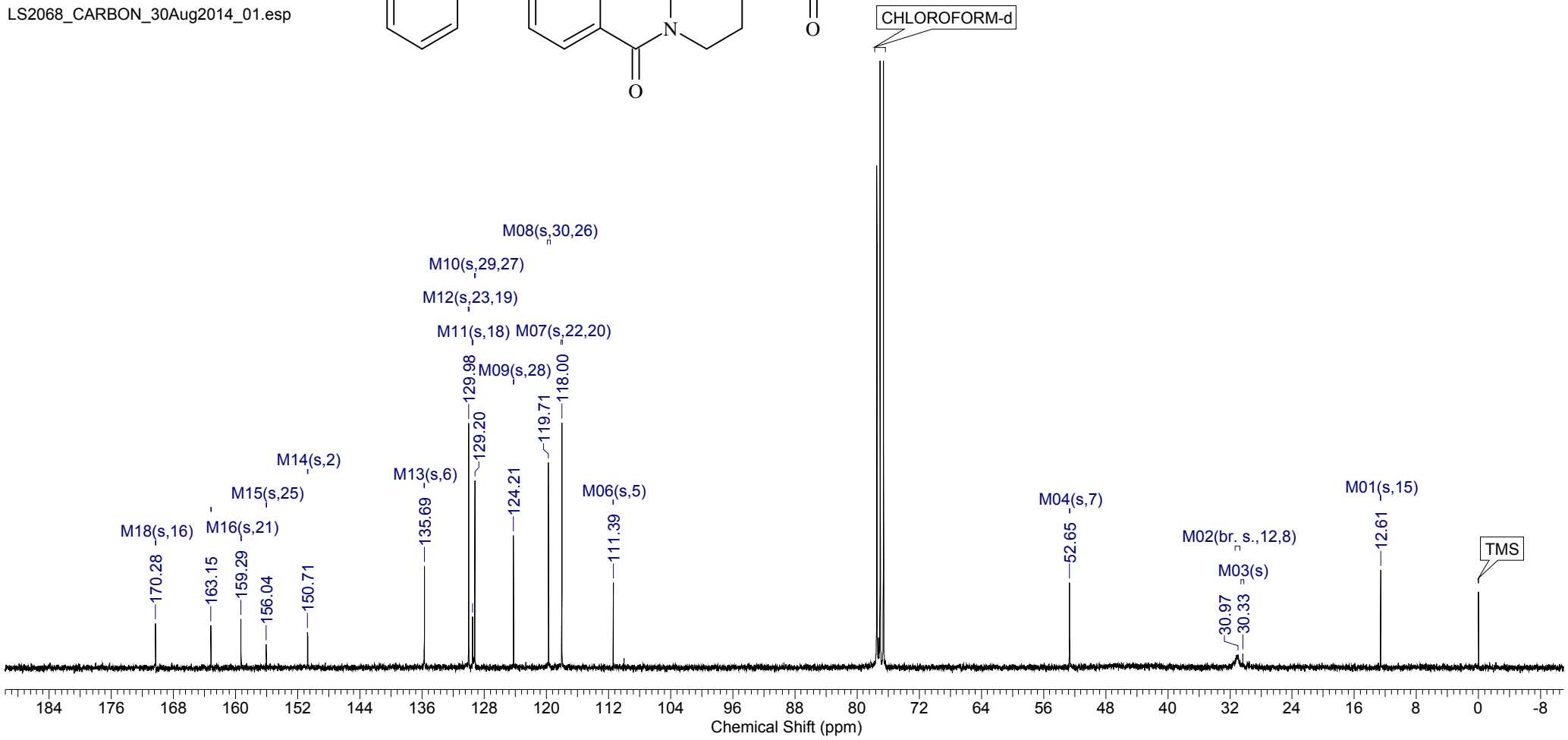


Compound 14

^{13}CNMR 75 MHz $CDCl_3$

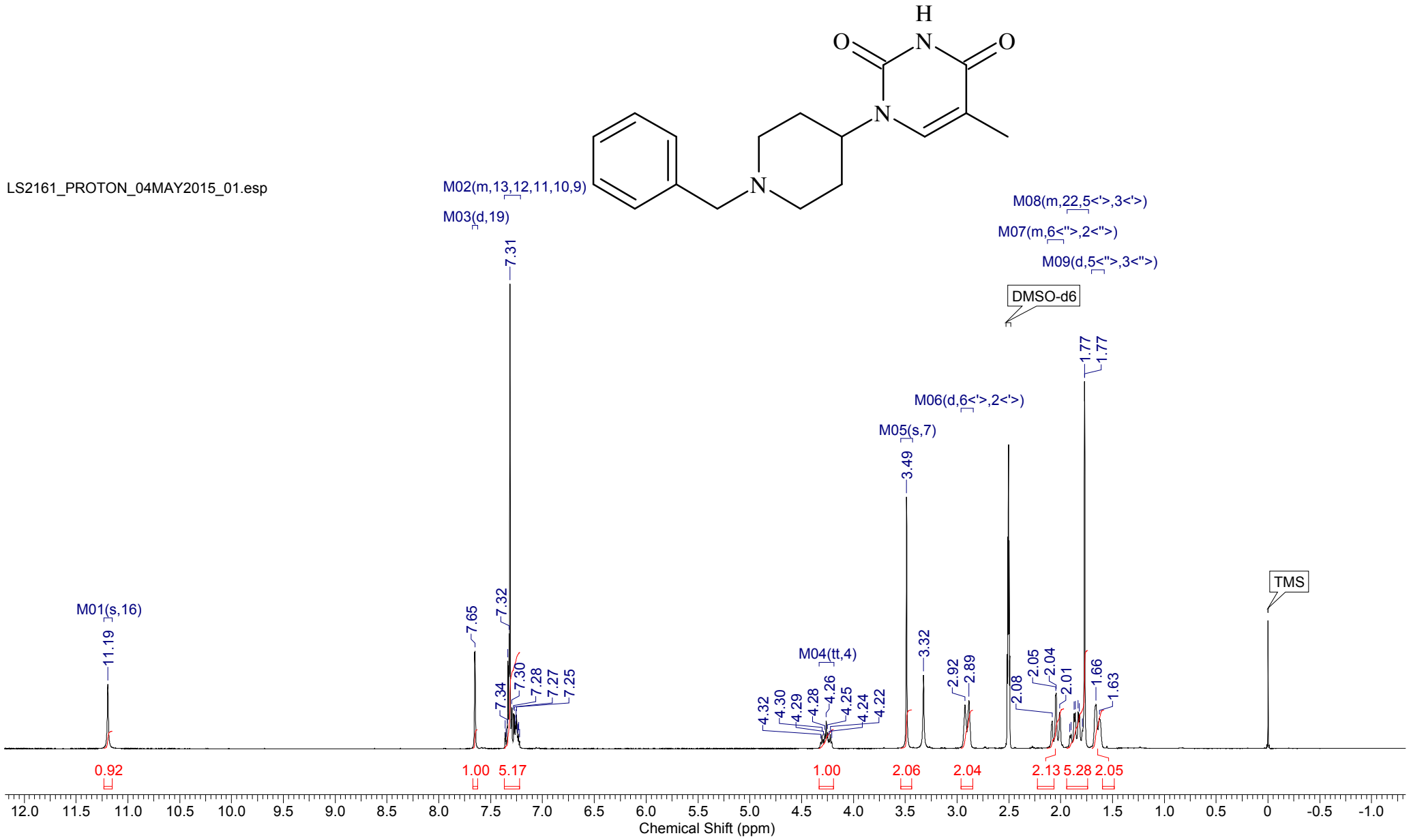
LS2068_CARBON_30Aug2014_01.esp





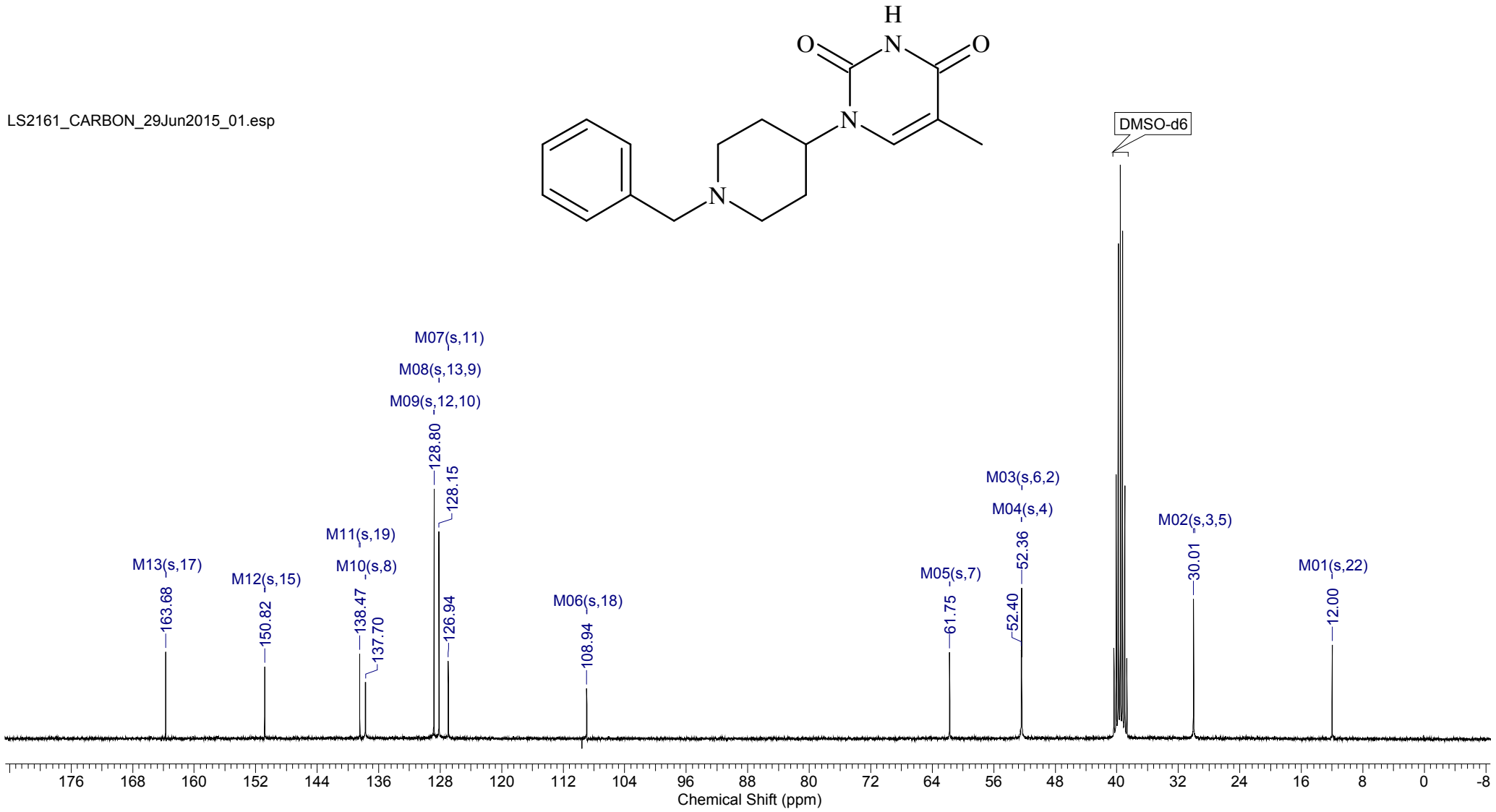
Compound 15

^{1}HNMR 300 MHz DMSO-d_6



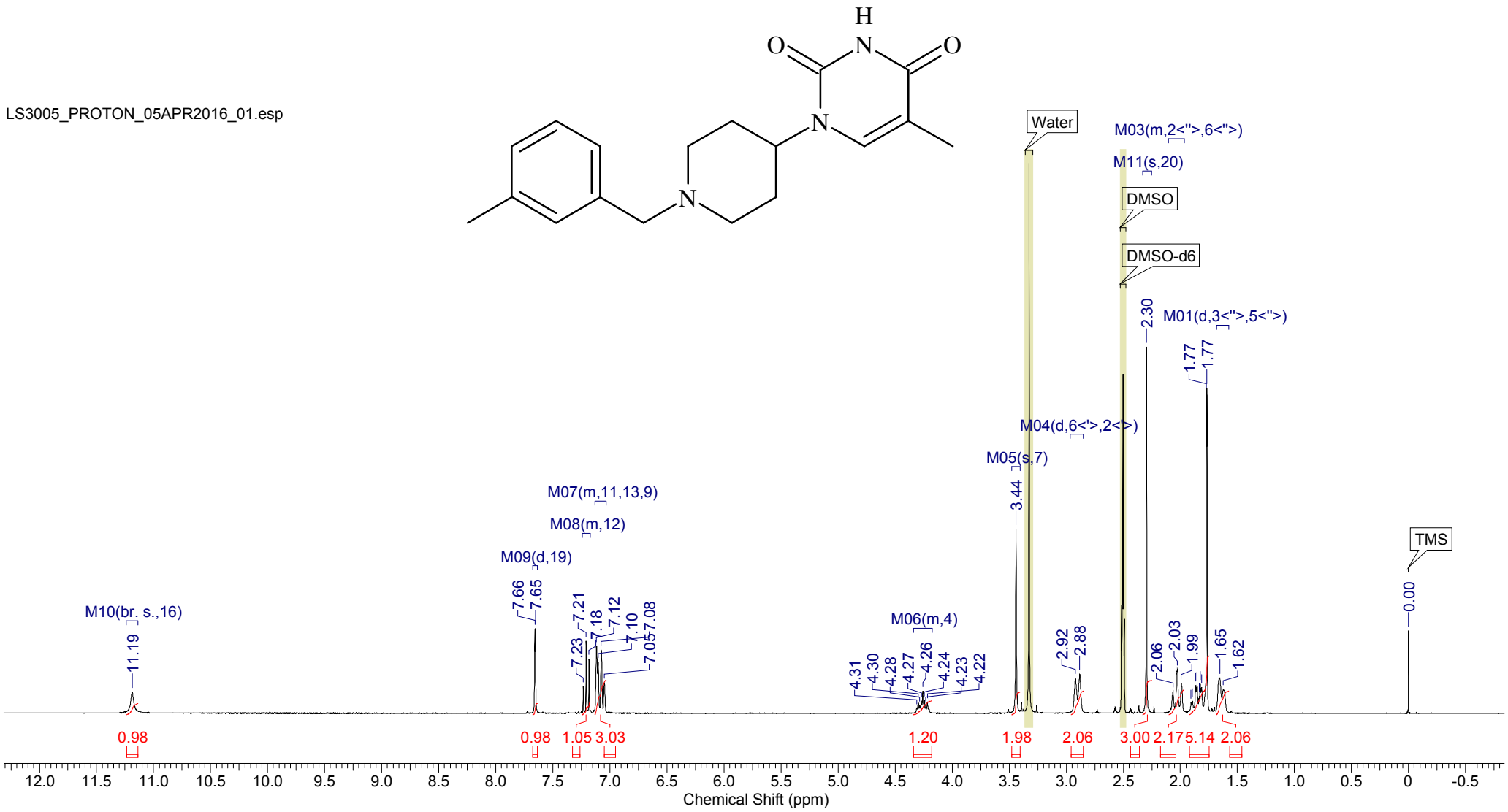
Compound 15

^{13}CNMR 75 MHz DMSO-d_6



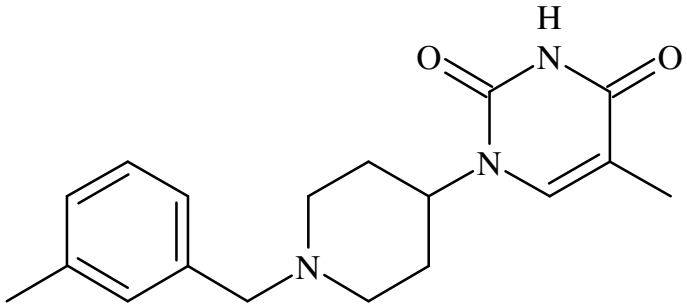
Compound 16

^{1}HNMR 300 MHz DMSO-d_6

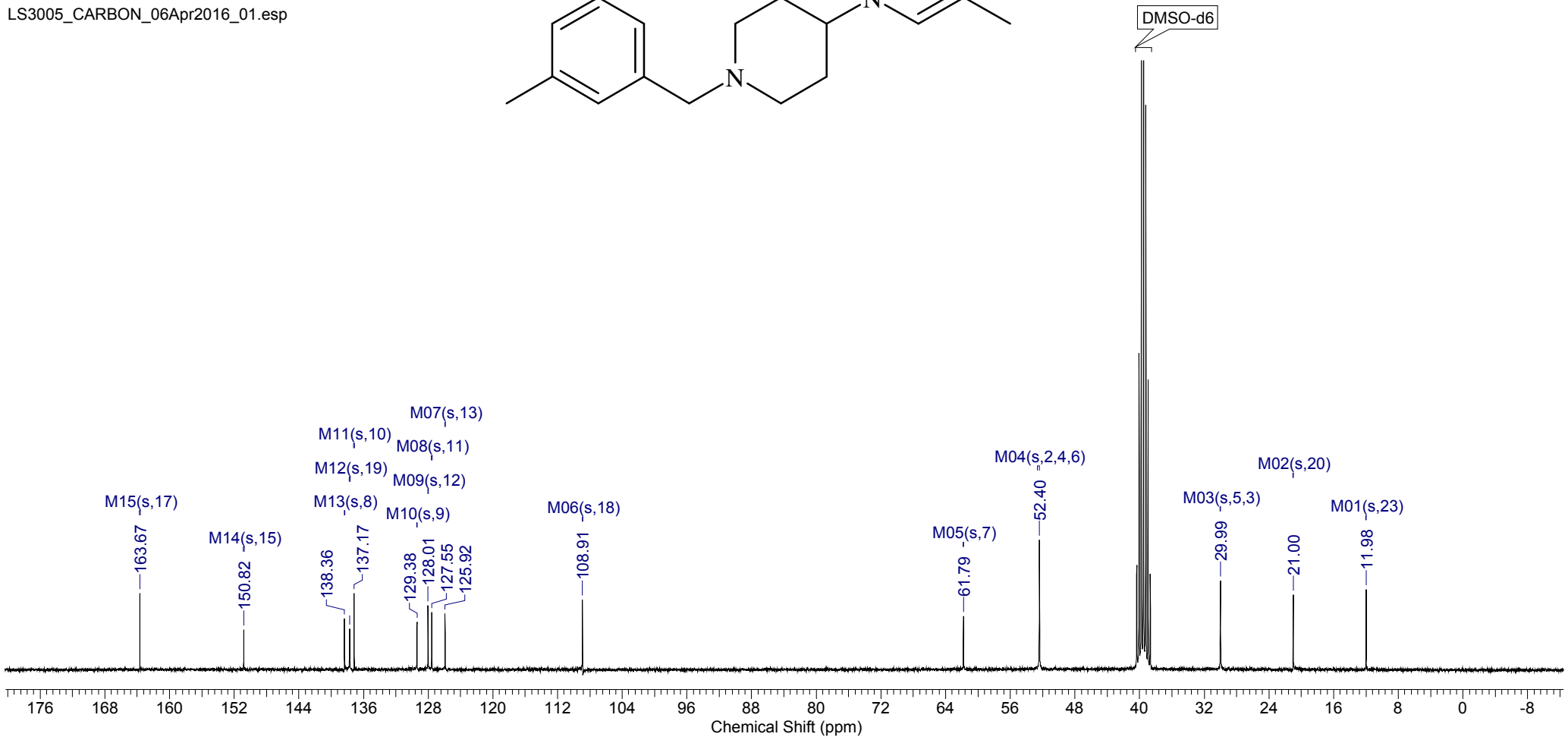


Compound 16

^{13}CNMR 75 MHz DMSO-d_6

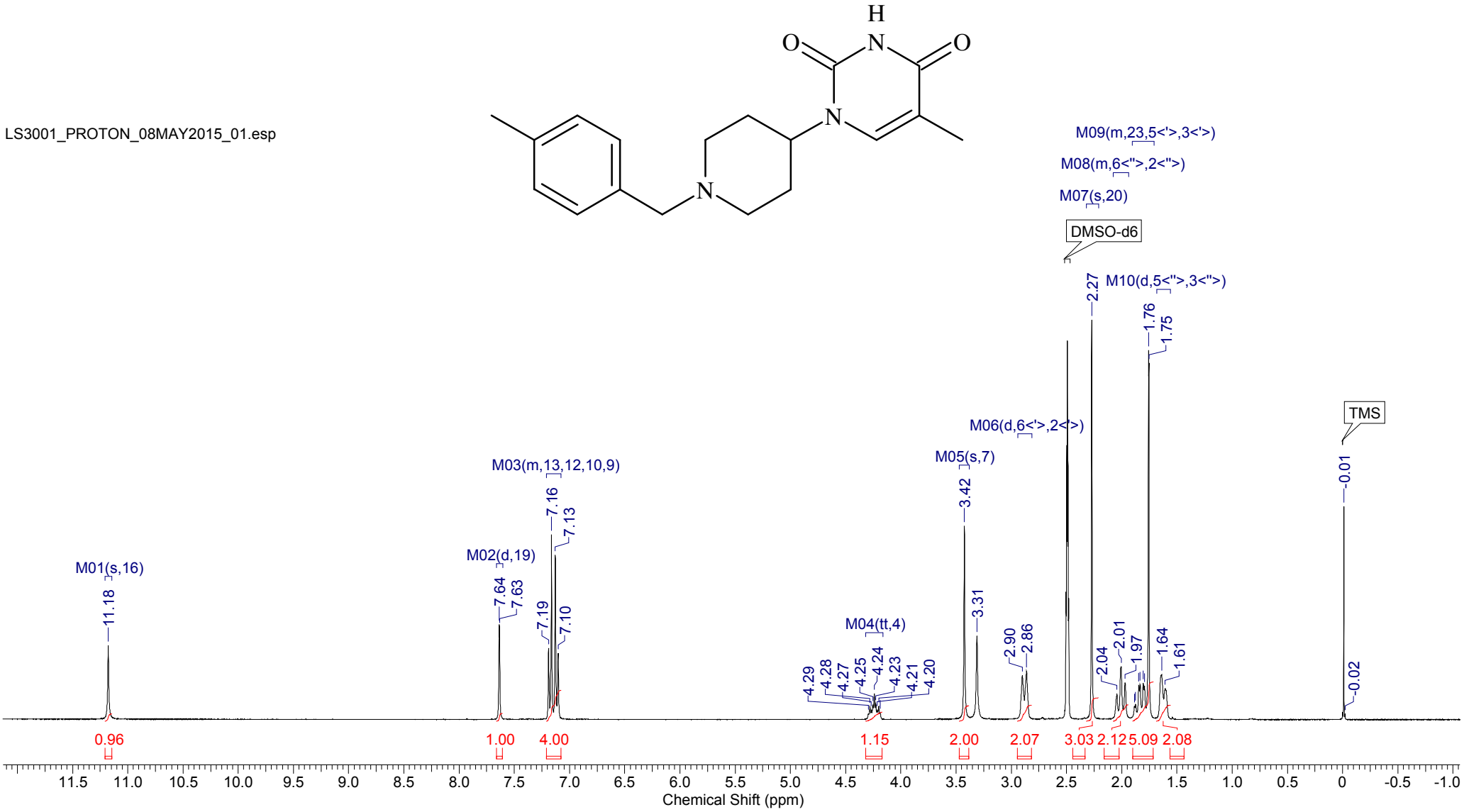


LS3005_CARBON_06Apr2016_01.esp



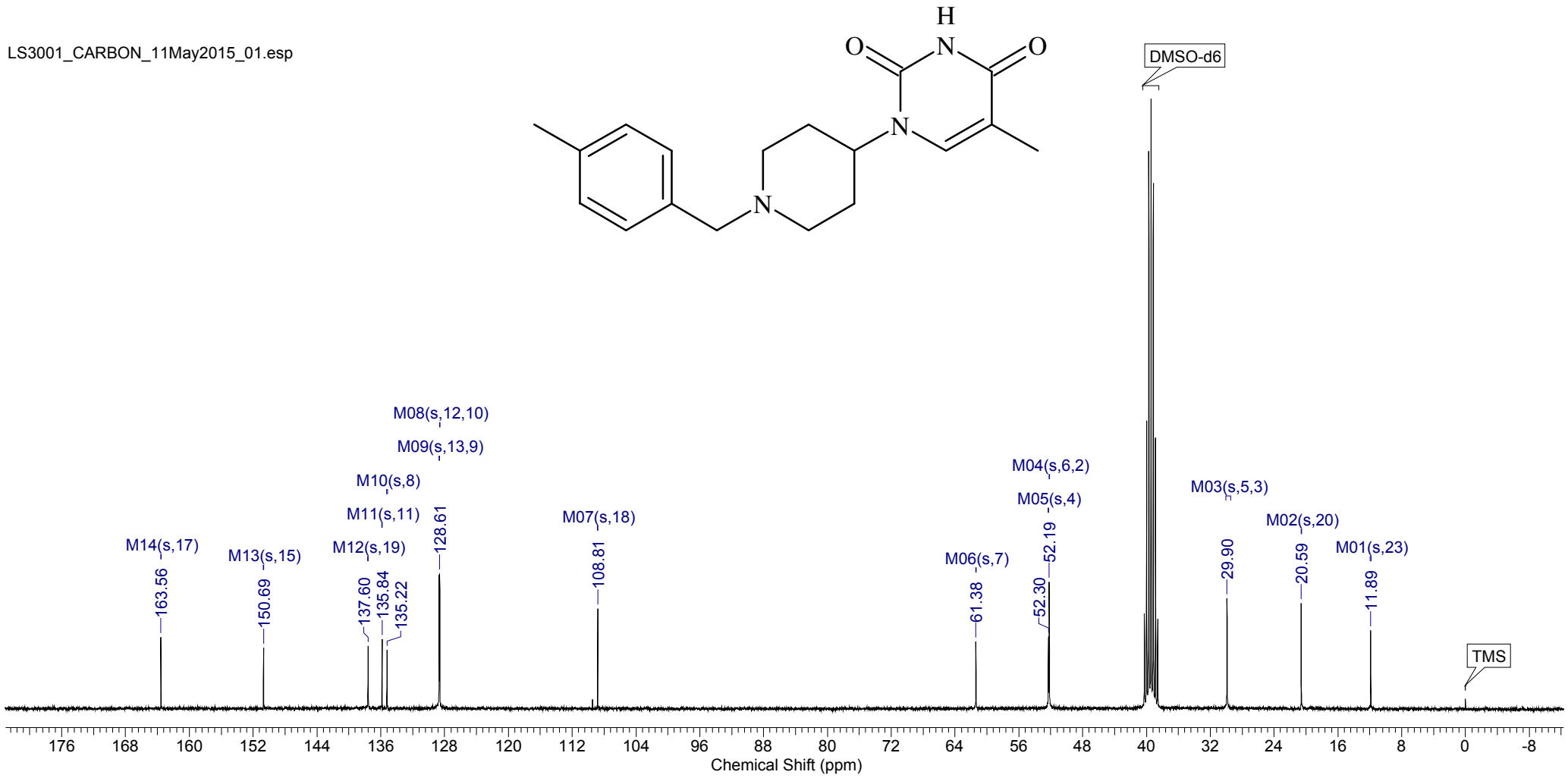
Compound 17

^{1}HNMR 300 MHz DMSO-d_6



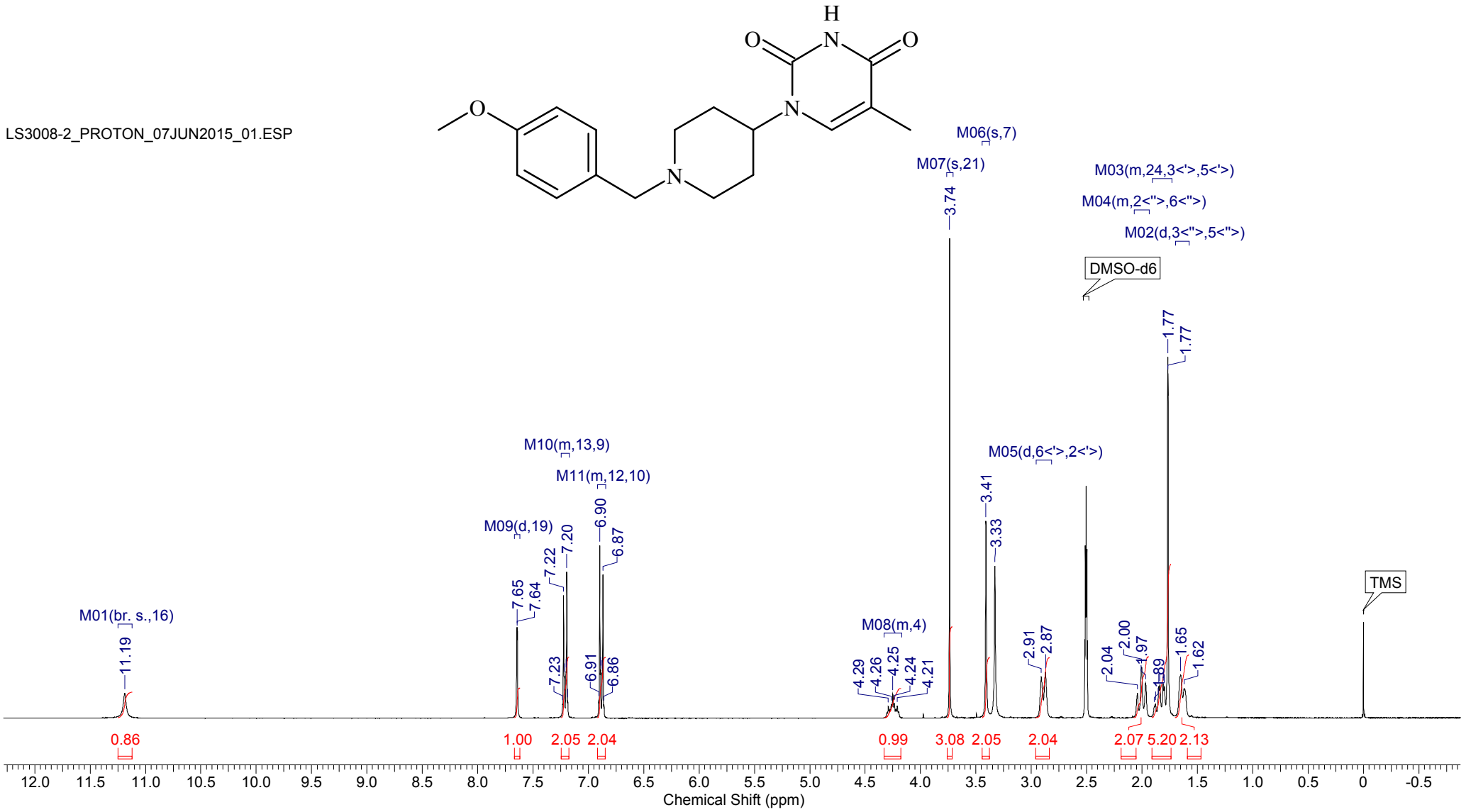
Compound 17

^{13}CNMR 75 MHz DMSO-d_6



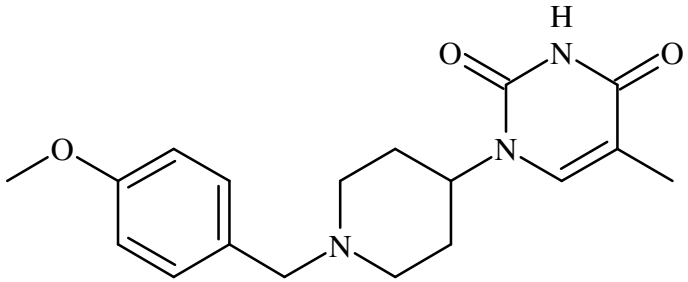
Compound 18

^{1}HNMR 300 MHz DMSO-d_6

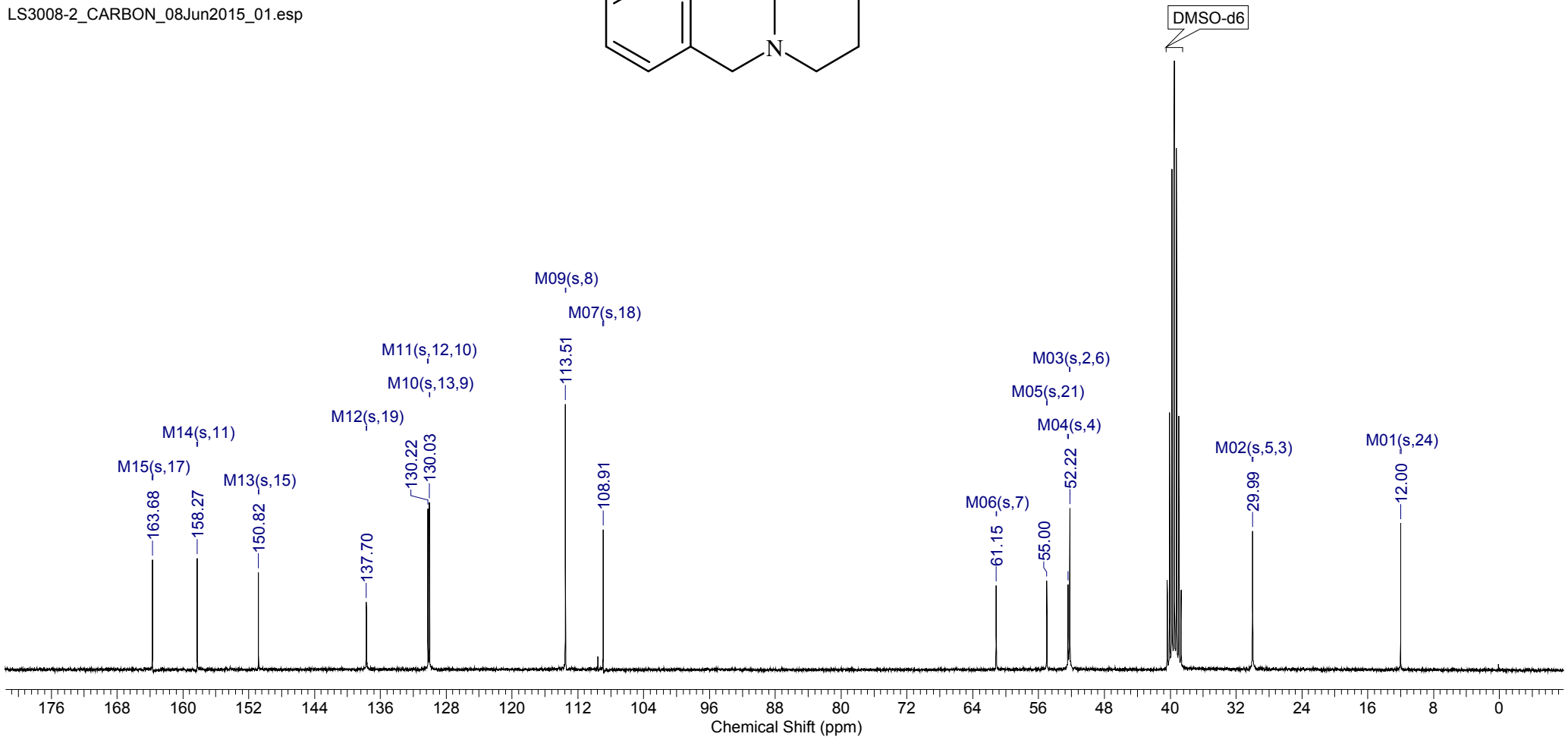


Compound 18

^{13}CNMR 75 MHz DMSO-d_6

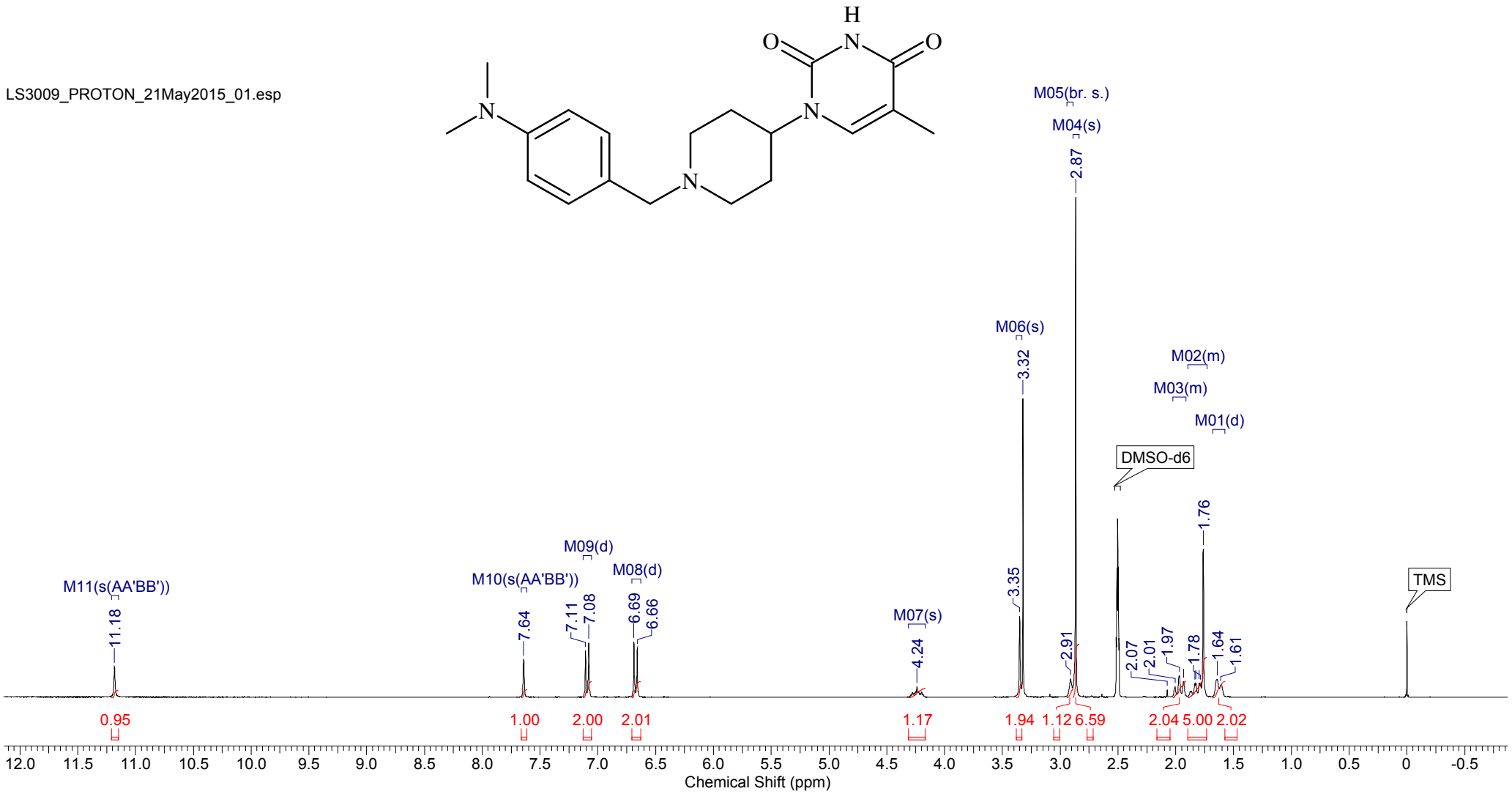


LS3008-2_CARBON_08Jun2015_01.esp



Compound 19

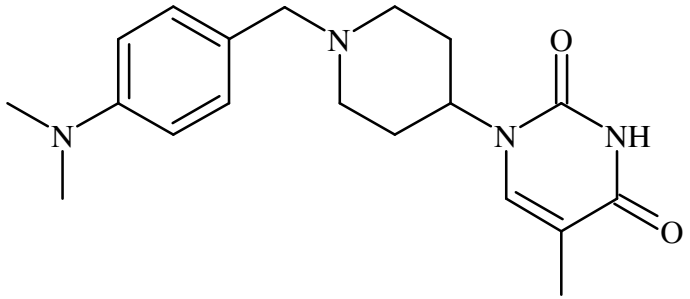
^{1}HNMR 300 MHz DMSO-d_6



Compound 19

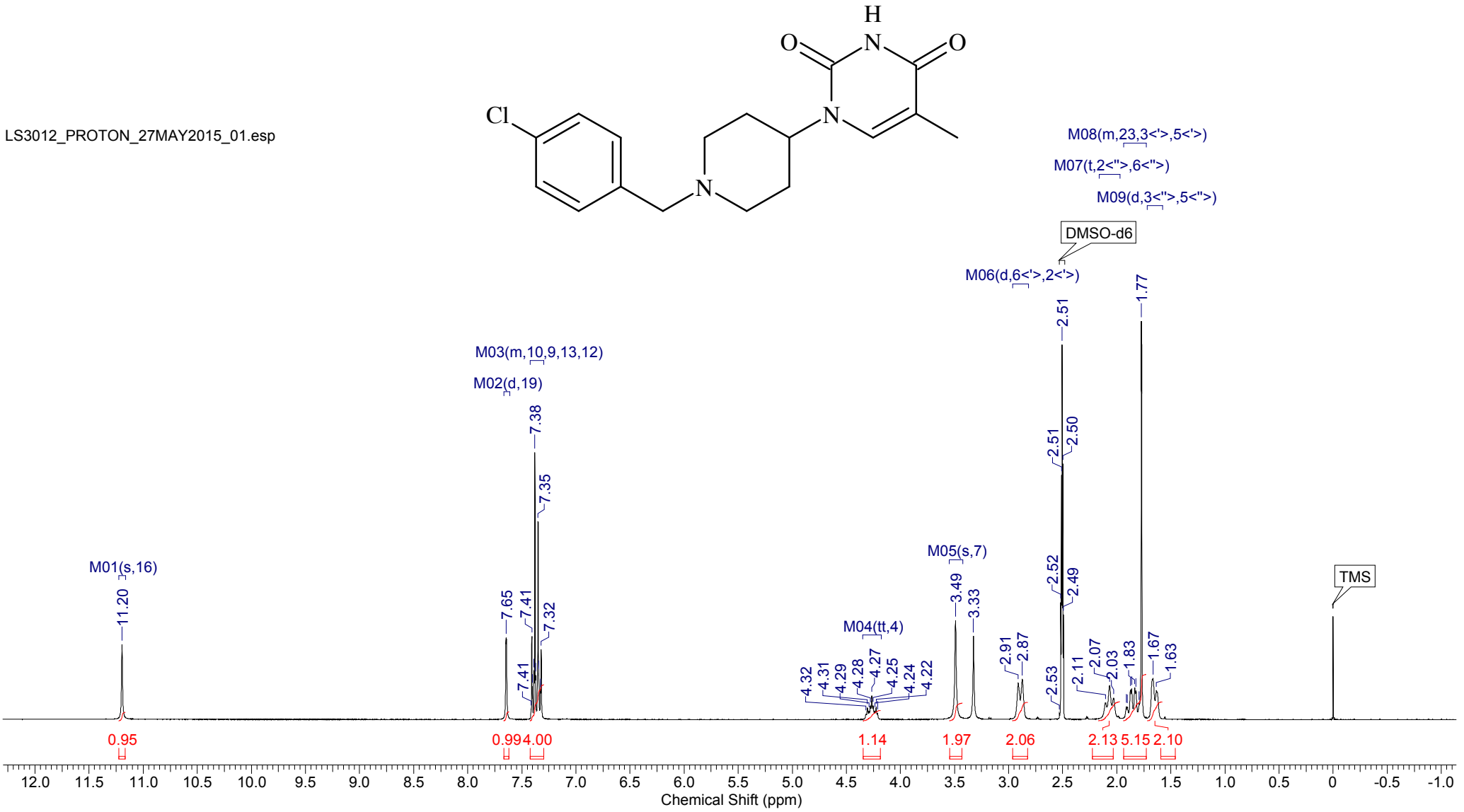
^{13}CNMR 75 MHz DMSO-d_6

LS3009_CARBON_22May2015_01.esp



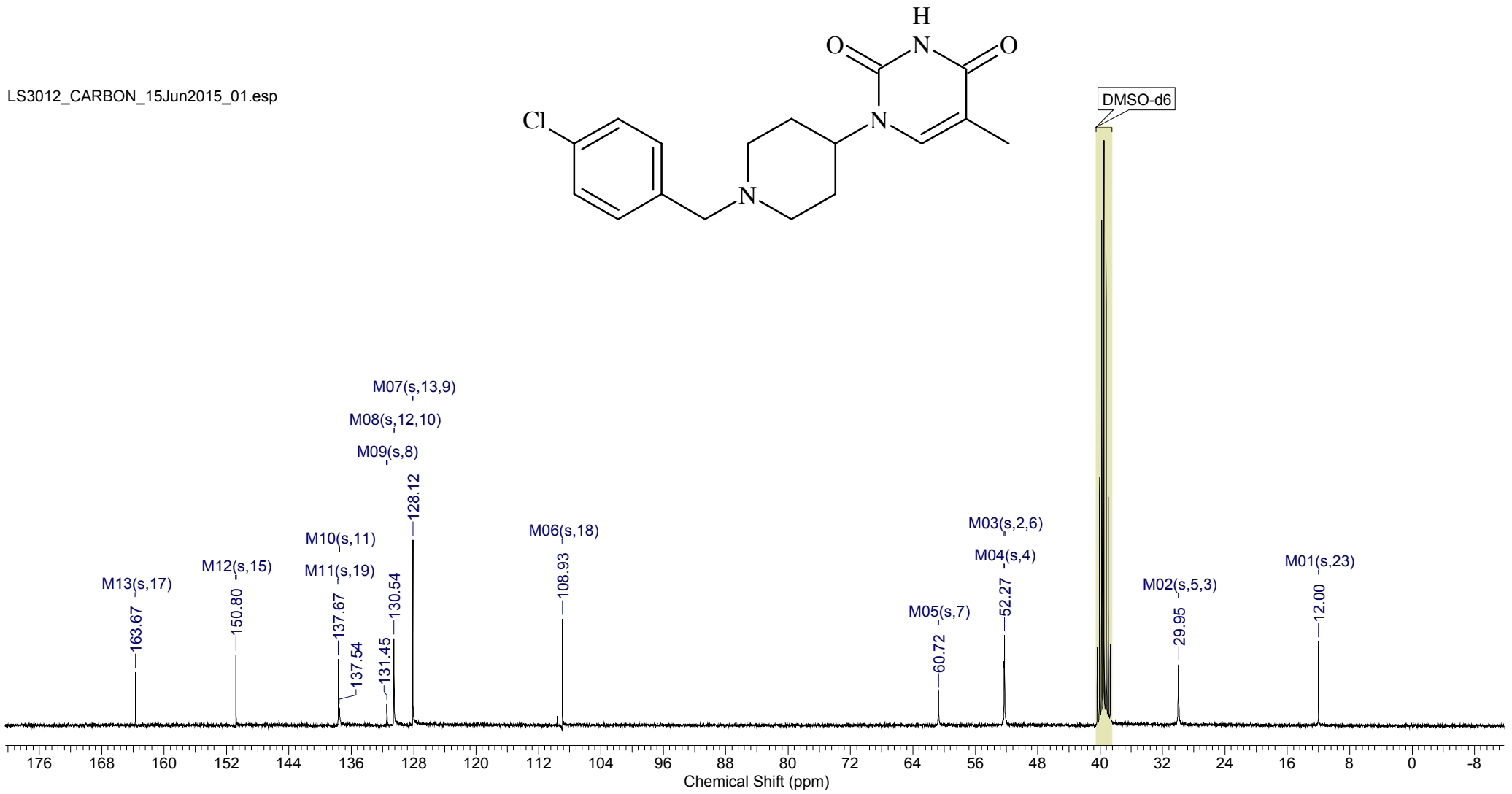
Compound 20

^{1}HNMR 300 MHz DMSO-d_6



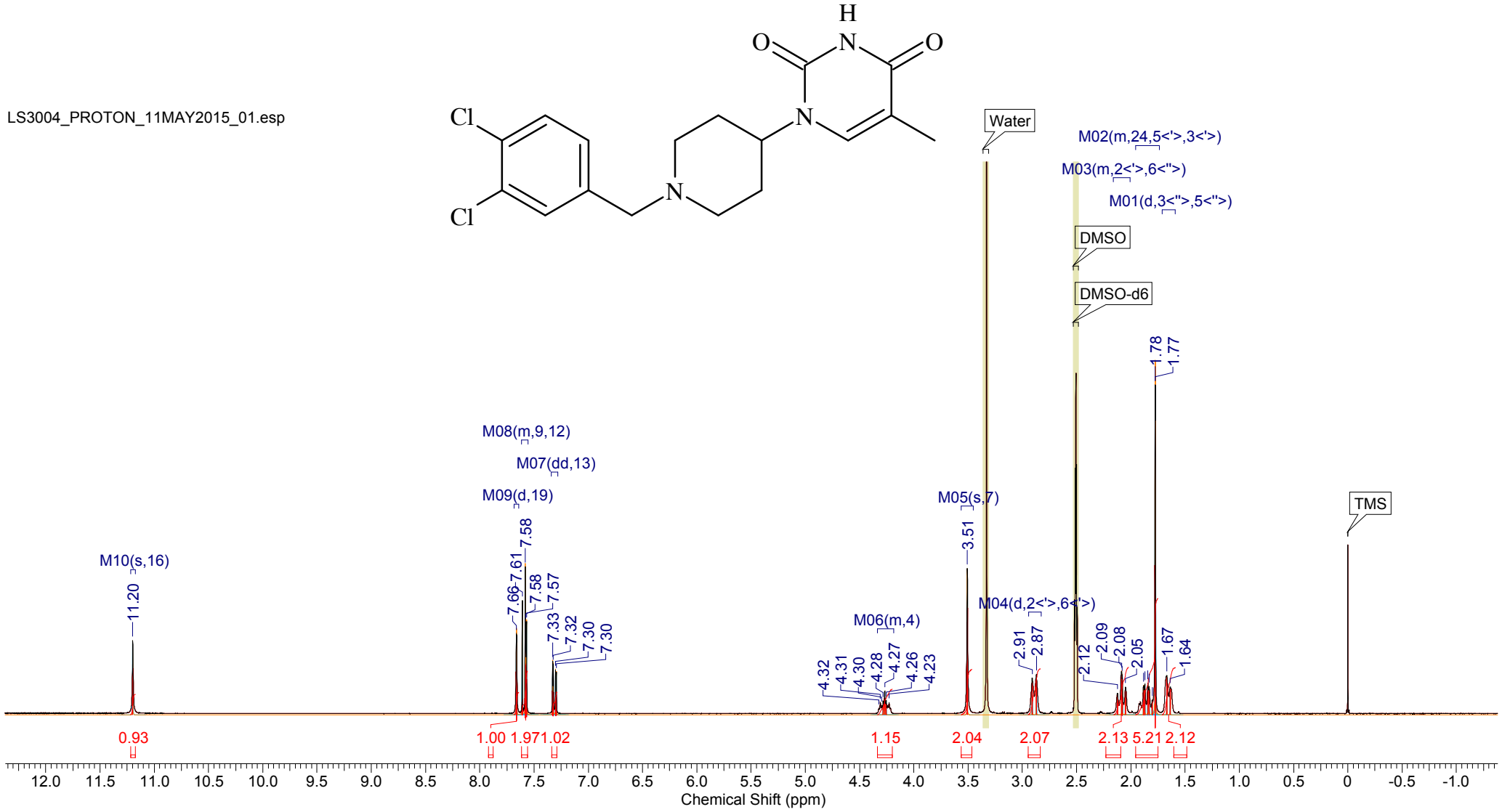
Compound 20

^{13}CNMR 75 MHz DMSO-d_6



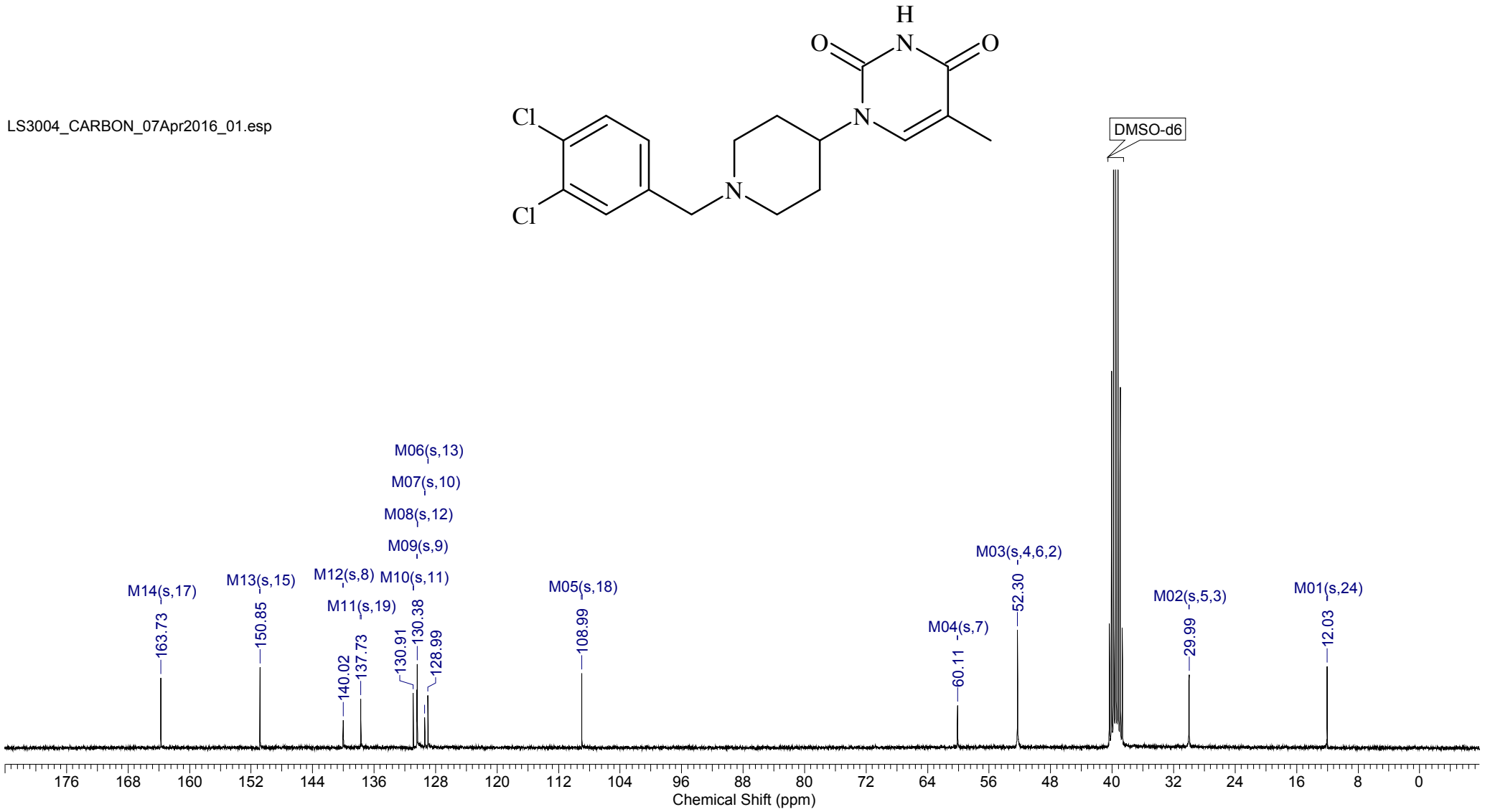
Compound 21

^{1}HNMR 300 MHz DMSO-d_6



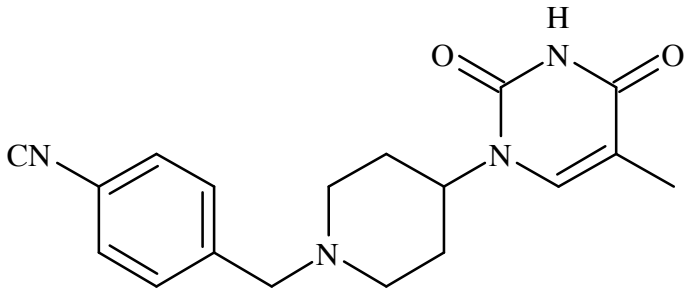
Compound 21

^{13}CNMR 75 MHz DMSO-d_6

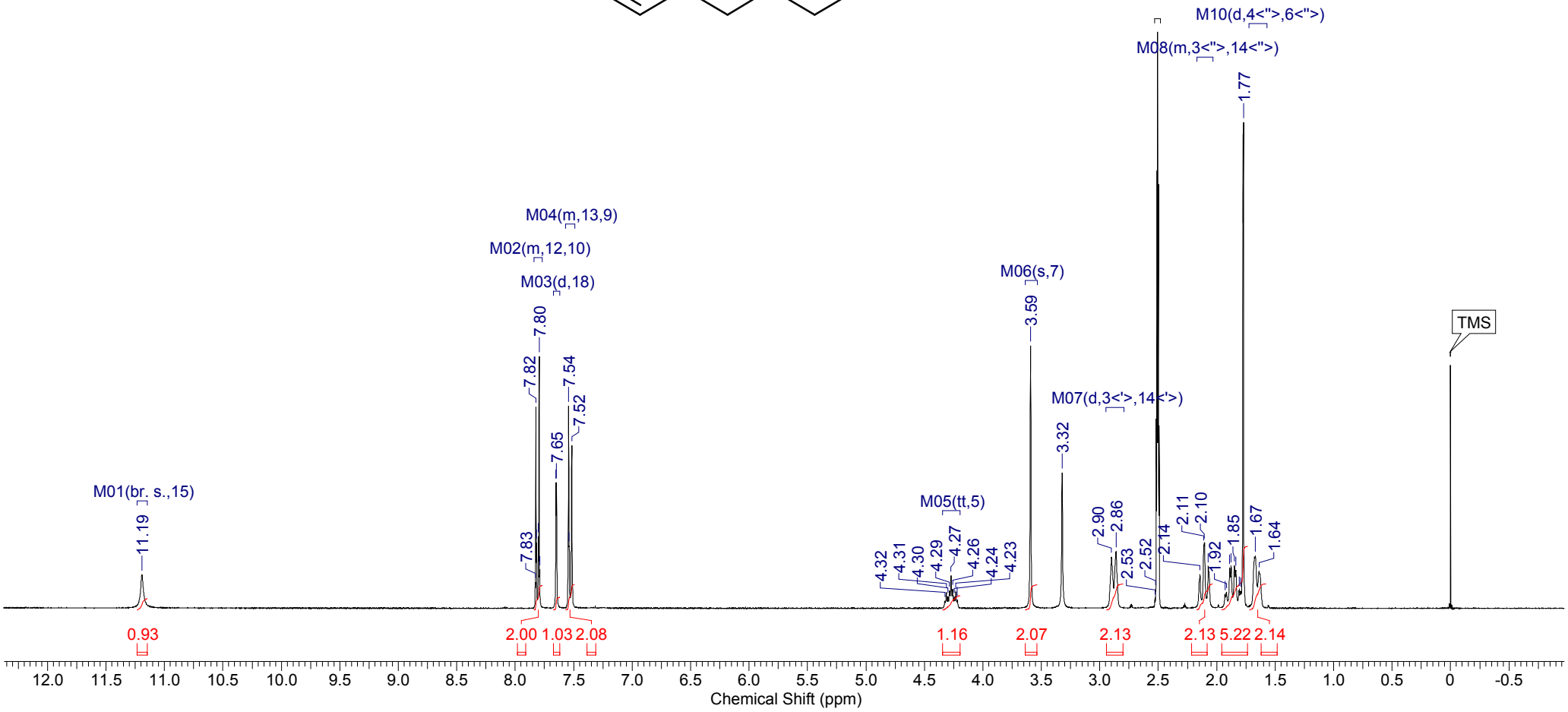


Compound 22

^{1}HNMR 300 MHz DMSO-d_6

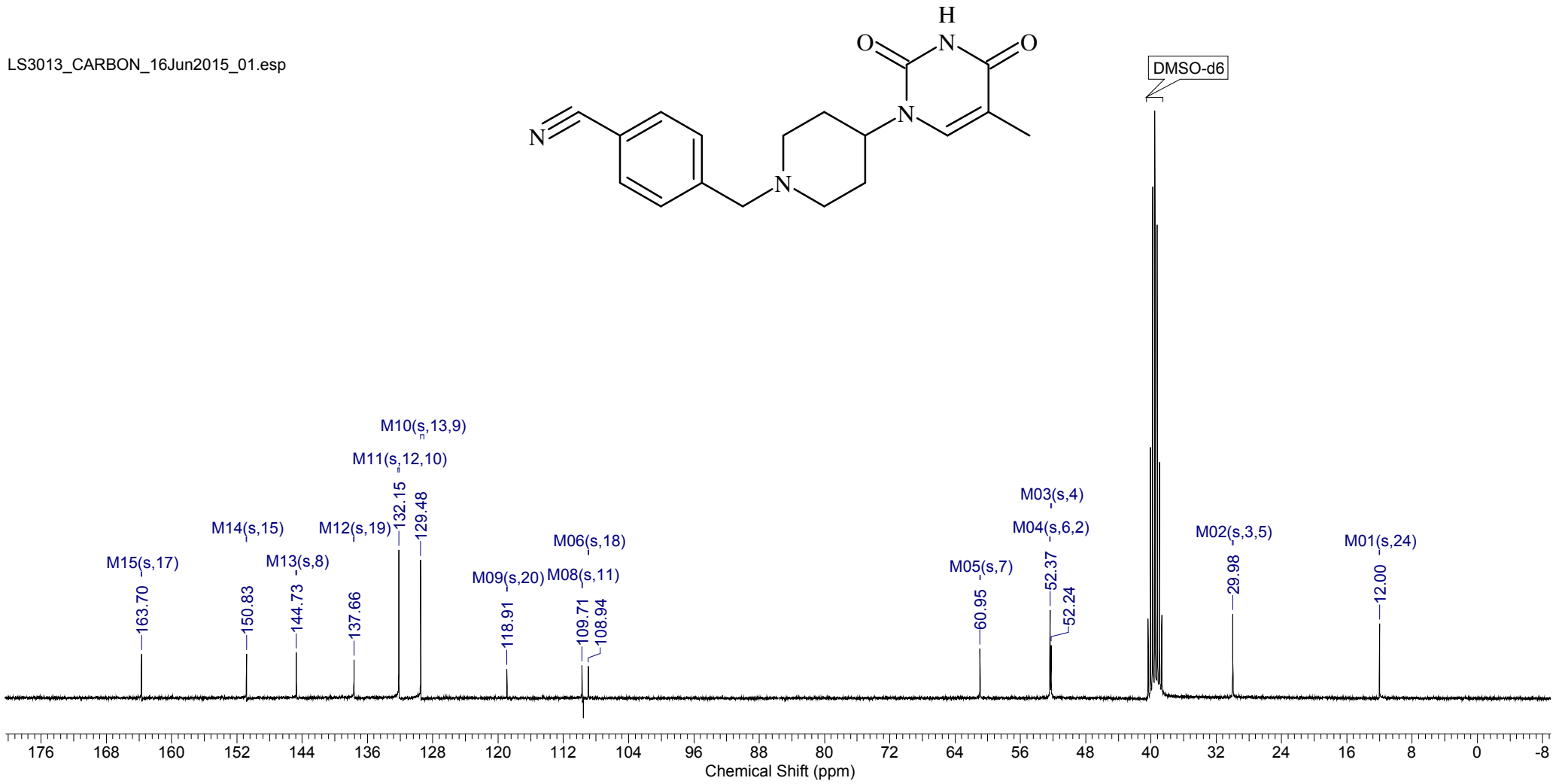


LS3013_PROTON_29MAY2015_01.esp



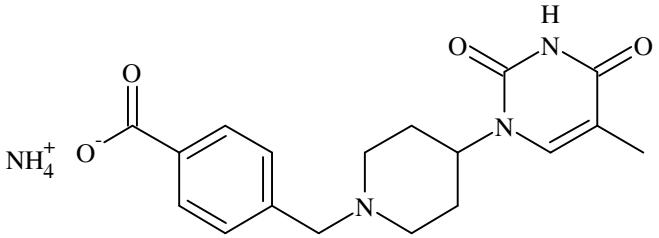
Compound 22

^{13}CNMR 75 MHz DMSO-d$_6$

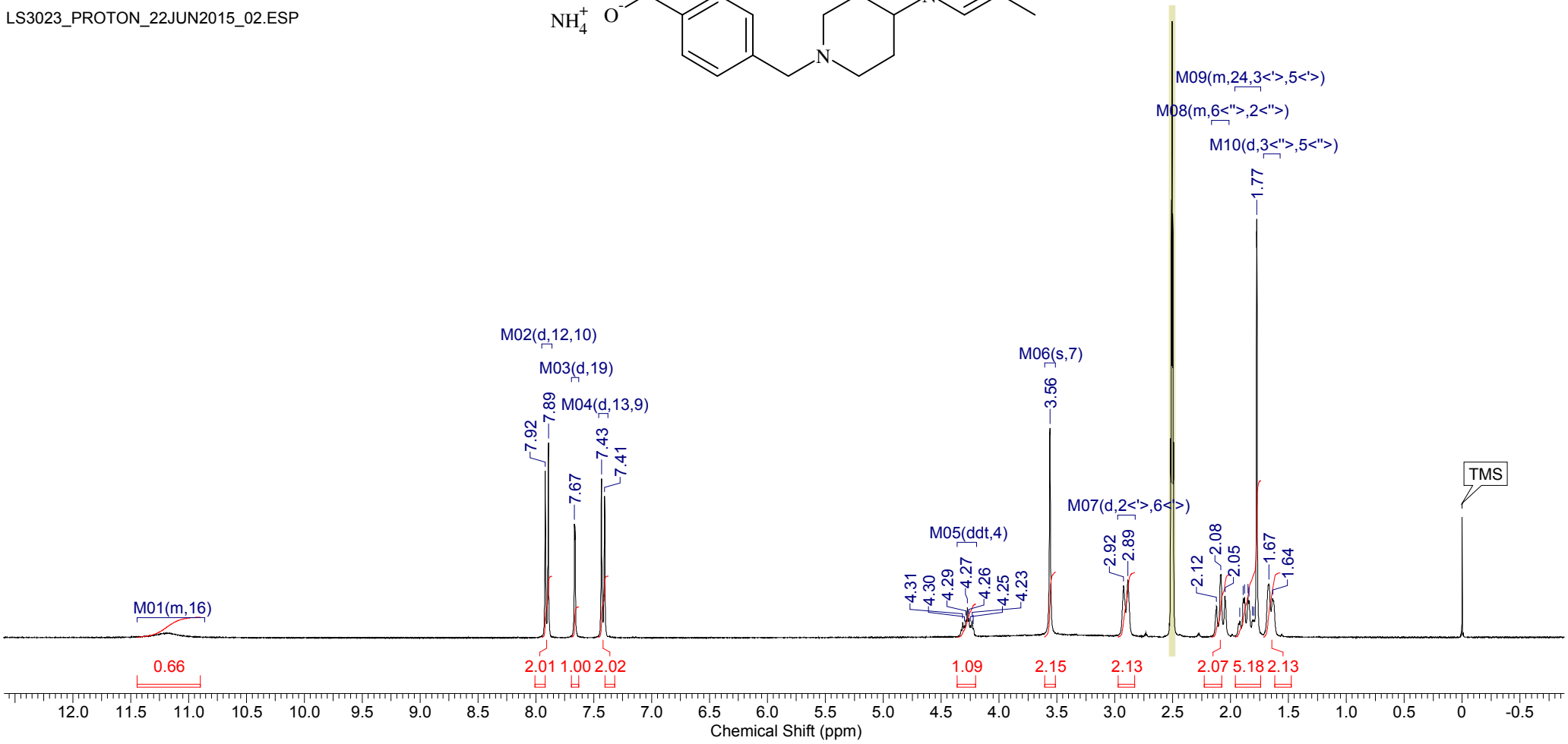


Compound 23

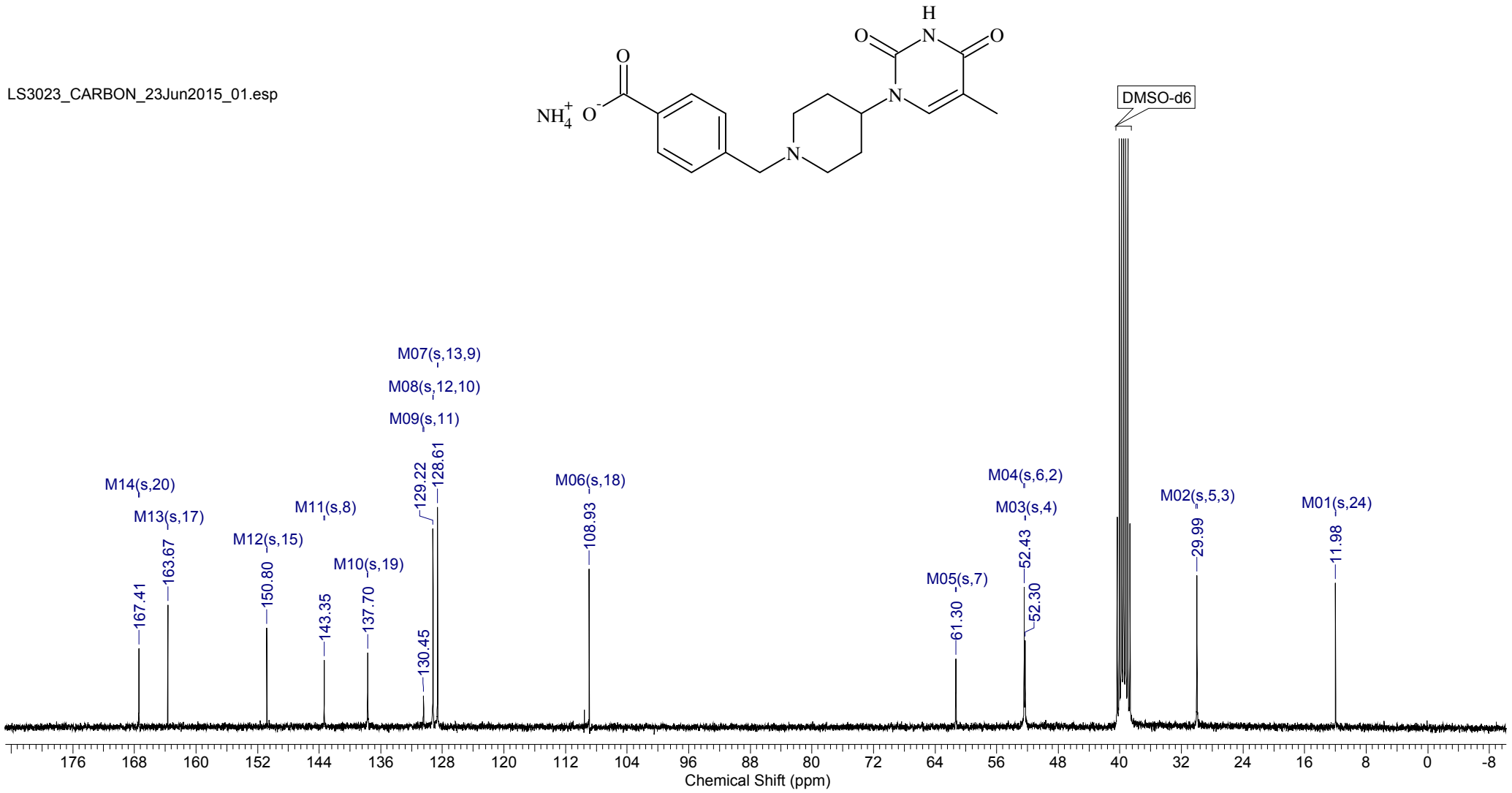
^{1}HNMR 300 MHz DMSO-d_6



LS3023_PROTON_22JUN2015_02.ESP

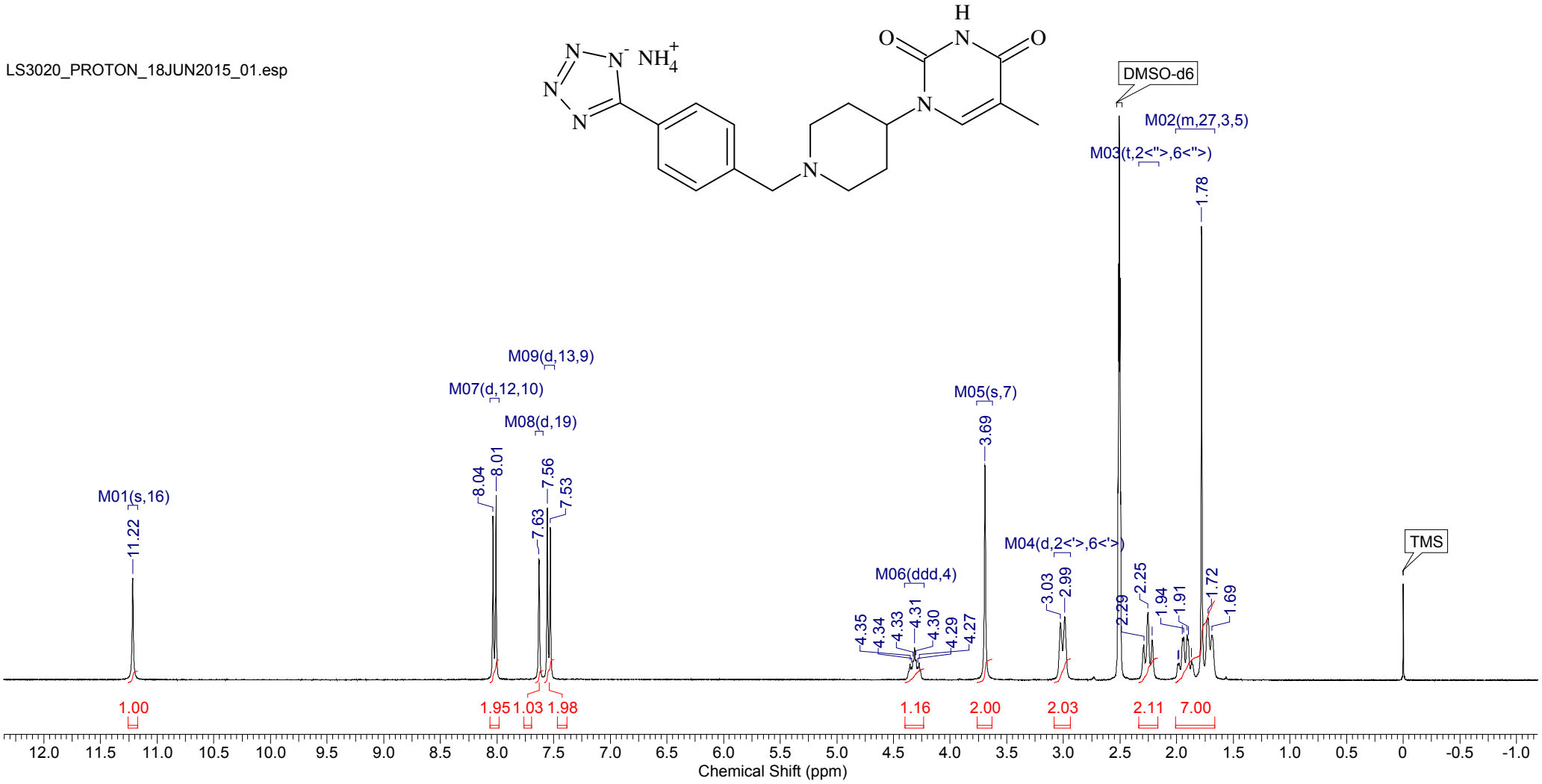


Compound 23


LS3023_CARBON_23Jun2015_01.esp
DMSO-d6
M14(s,20)
M13(s,17)
M12(s,15)
M11(s,8)
M10(s,19)
M07(s,13,9)
M08(s,12,10)
M09(s,11)
M06(s,18)
M05(s,7)
M04(s,6,2)
M03(s,4)
M02(s,5,3)
M01(s,24)
167.41
163.67
150.80
143.35
137.70
130.45
129.22
128.61
108.93
61.30
52.43
52.30
29.99
11.98
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
-8
Chemical Shift (ppm)

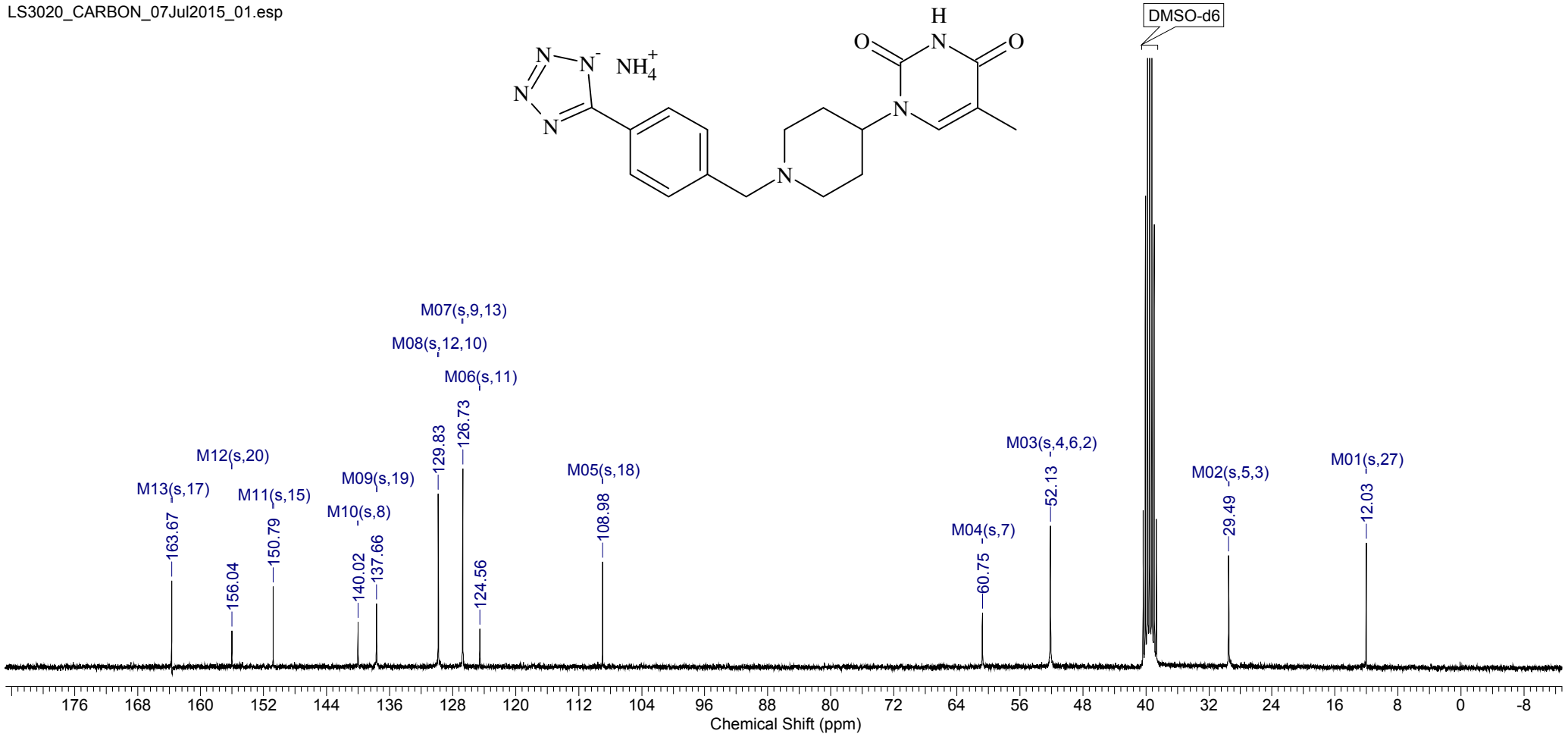
Compound 24

^{1}HNMR 300 MHz DMSO-d_6



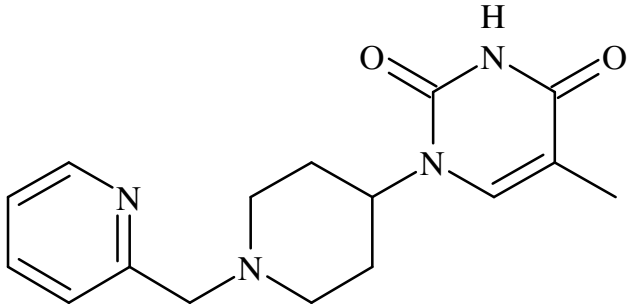
Compound 24

^{13}CNMR 75 MHz DMSO-d_6

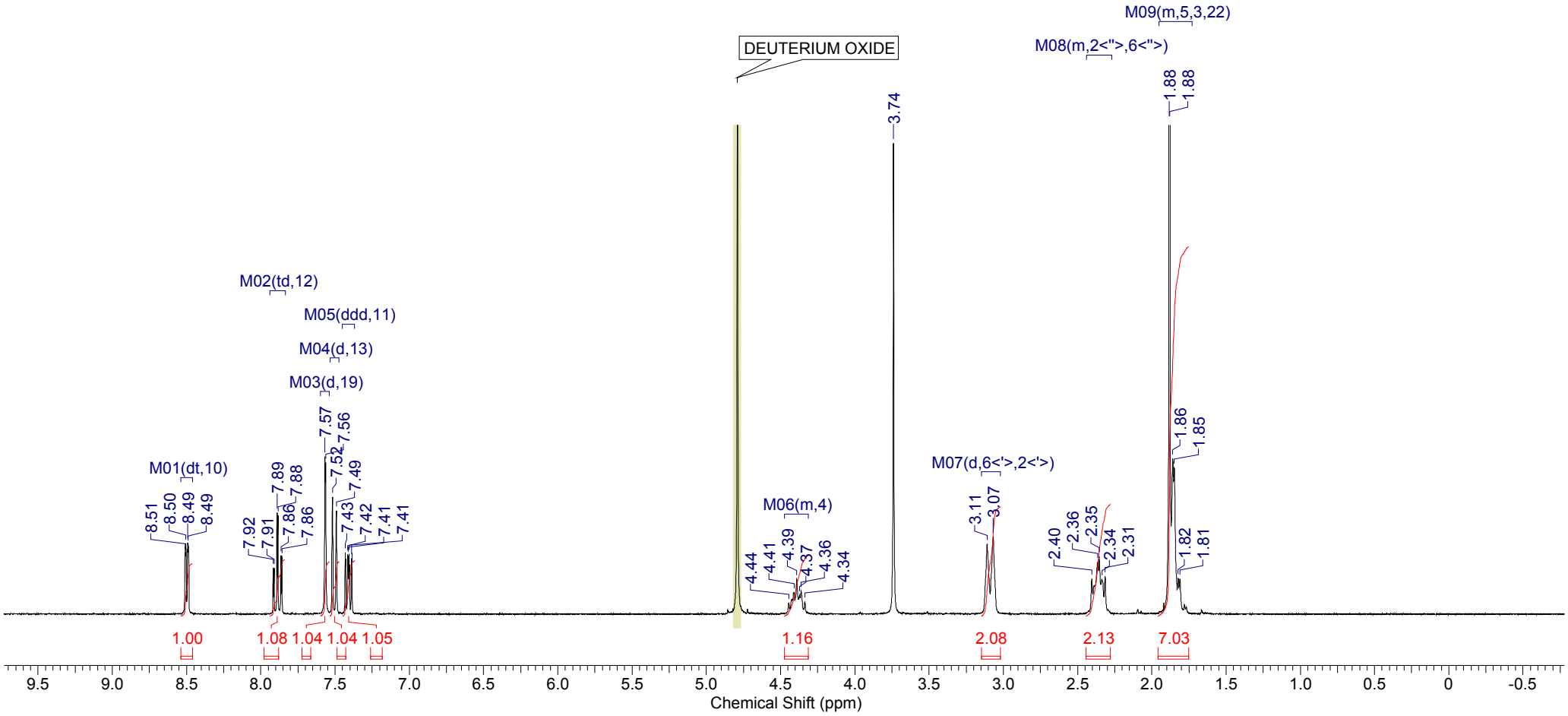


Compound 25

^{1}HNMR 300 MHz D_2O

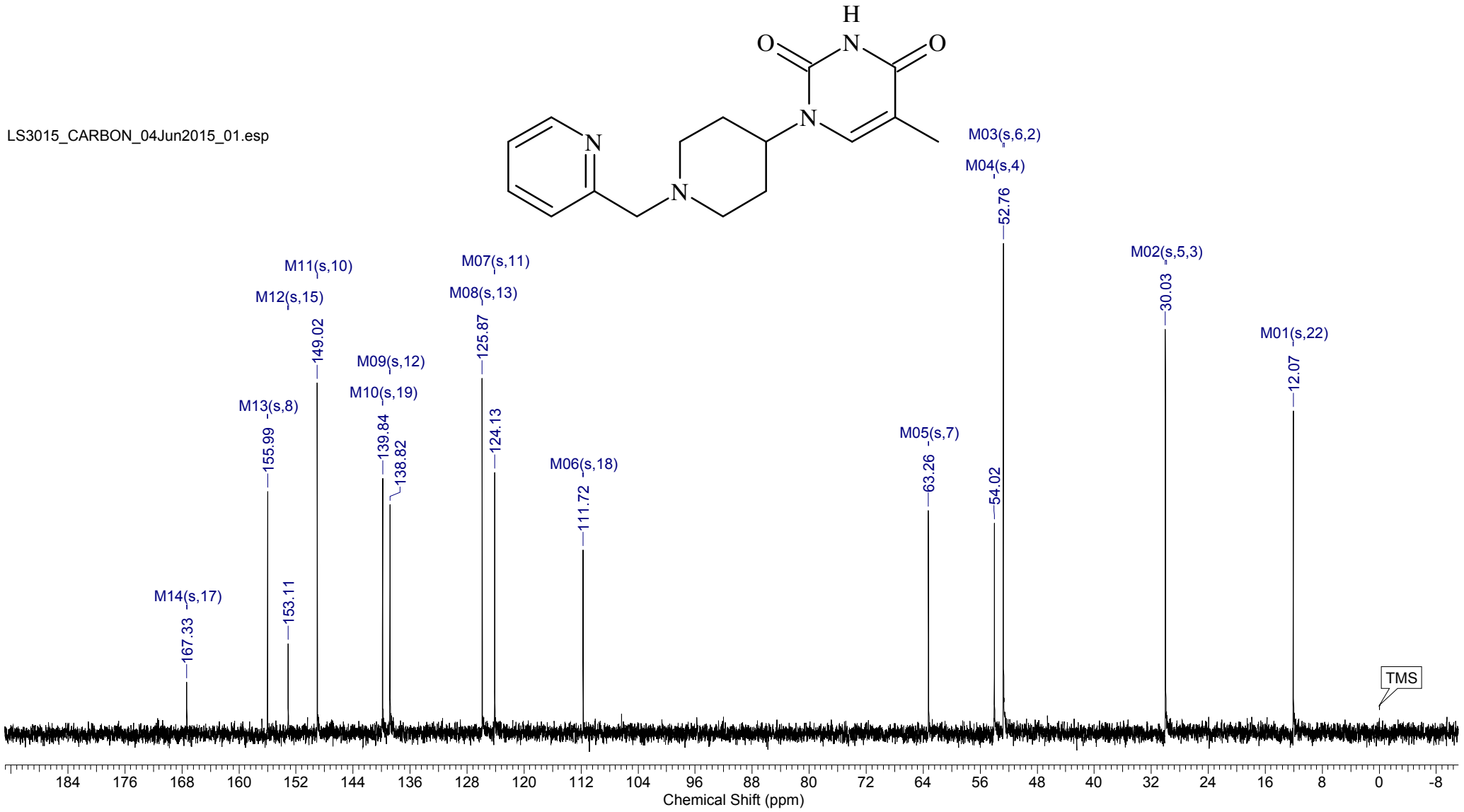


LS3015_PROTON_03JUN2015_01.esp



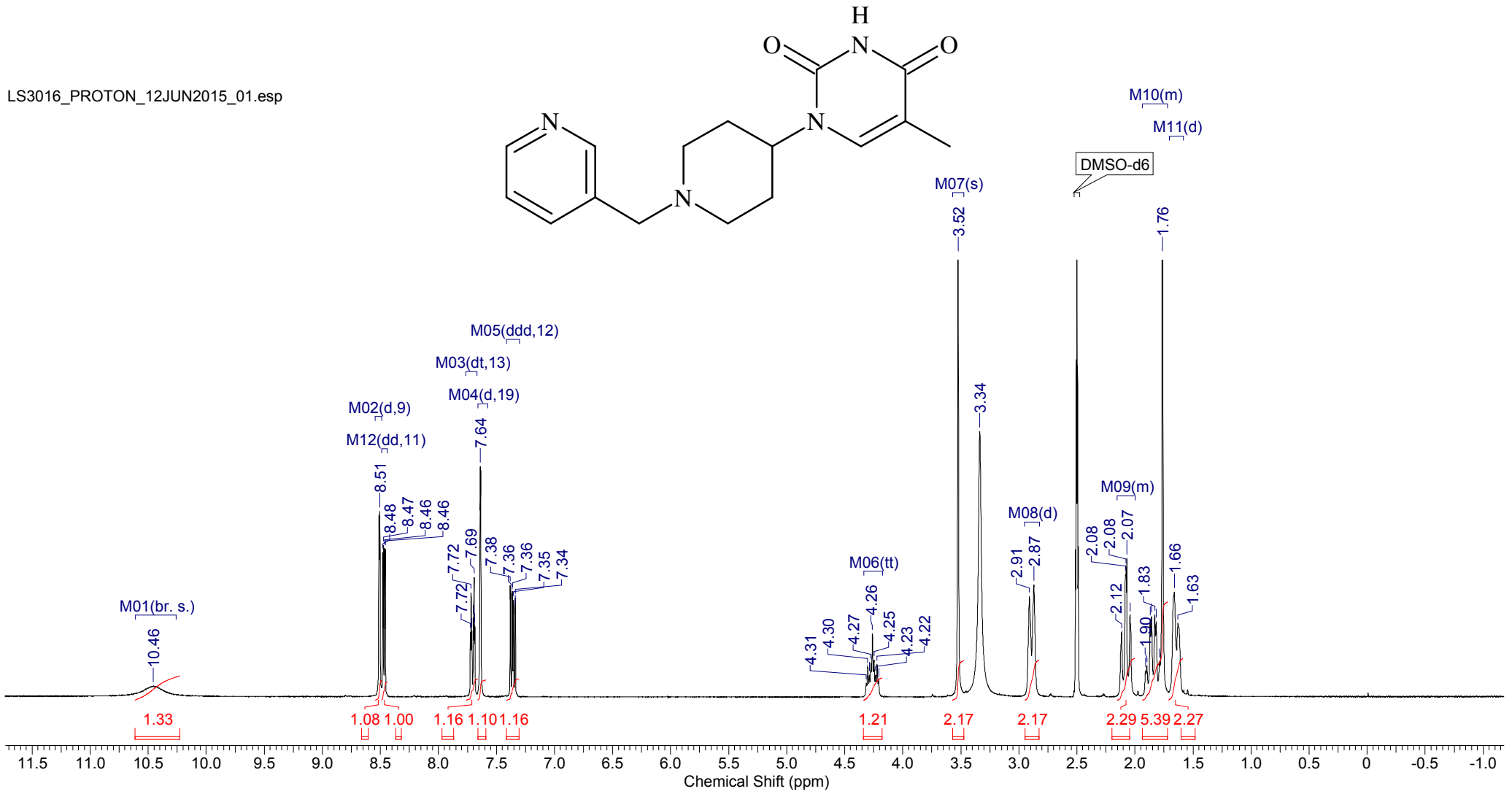
Compound 25

^{13}CNMR 75 MHz D_2O



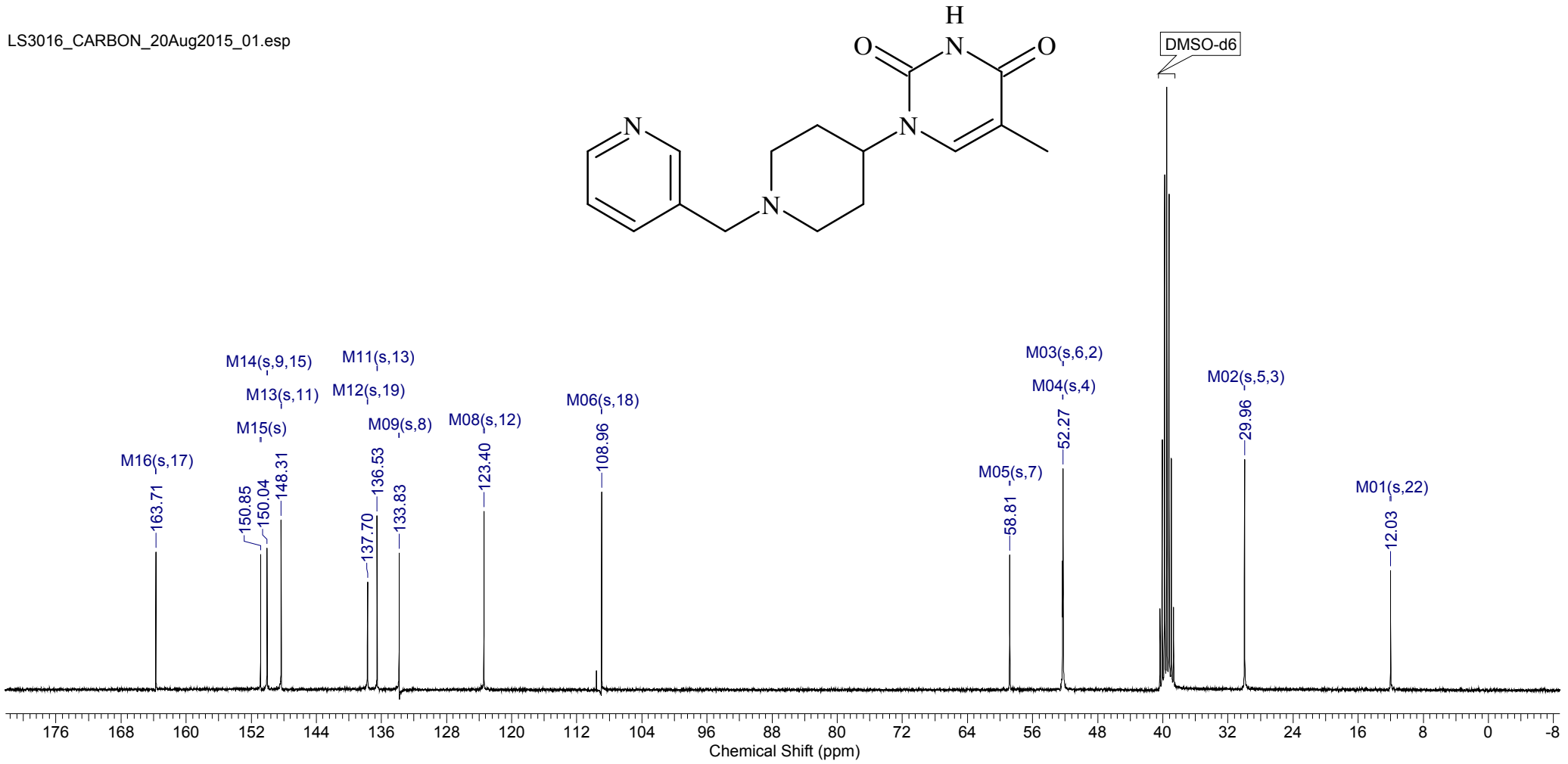
Compound 26

^{1}HNMR 300 MHz DMSO-d_6



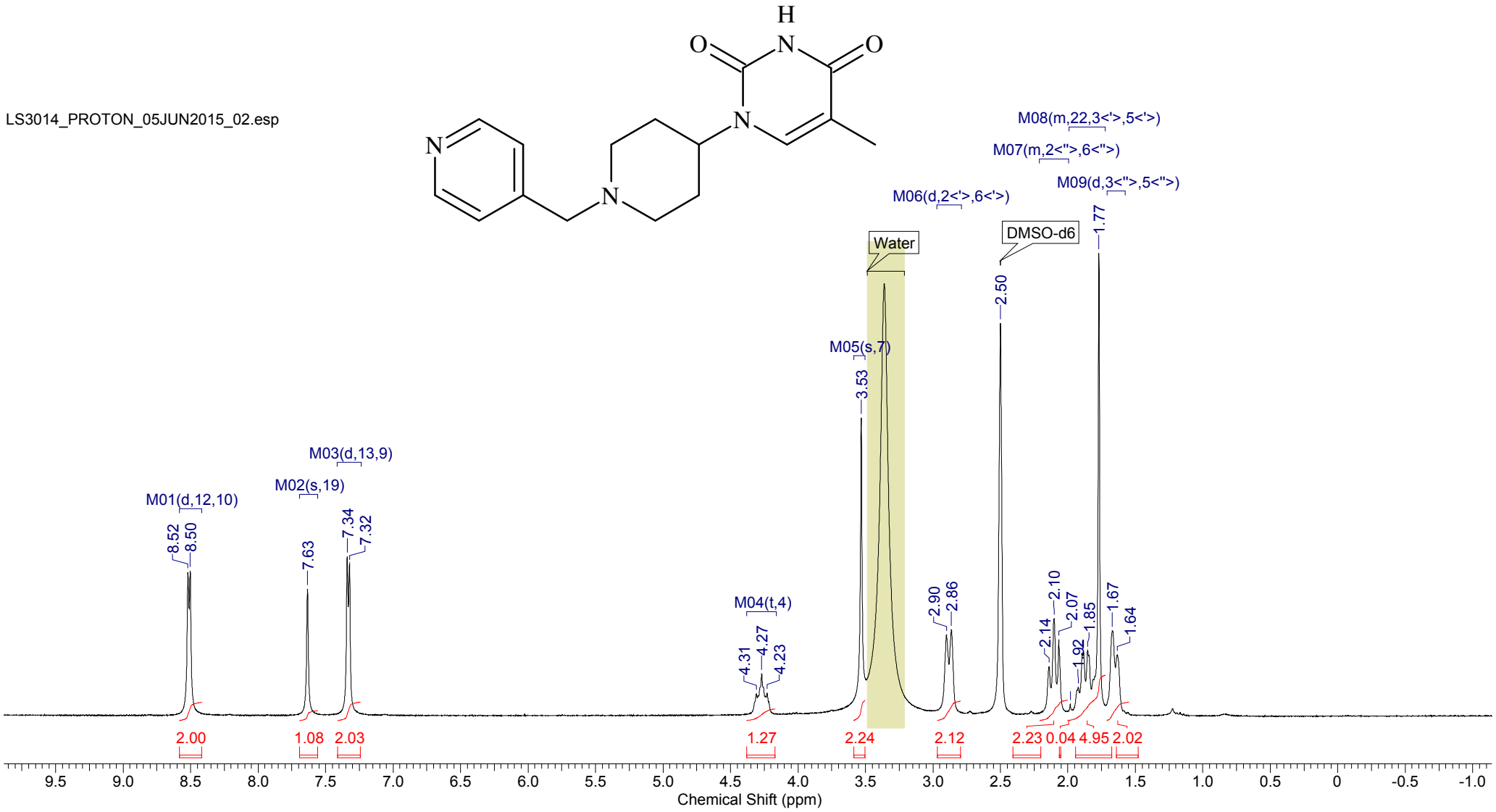
Compound 26

^{13}CNMR 75 MHz DMSO-d_6



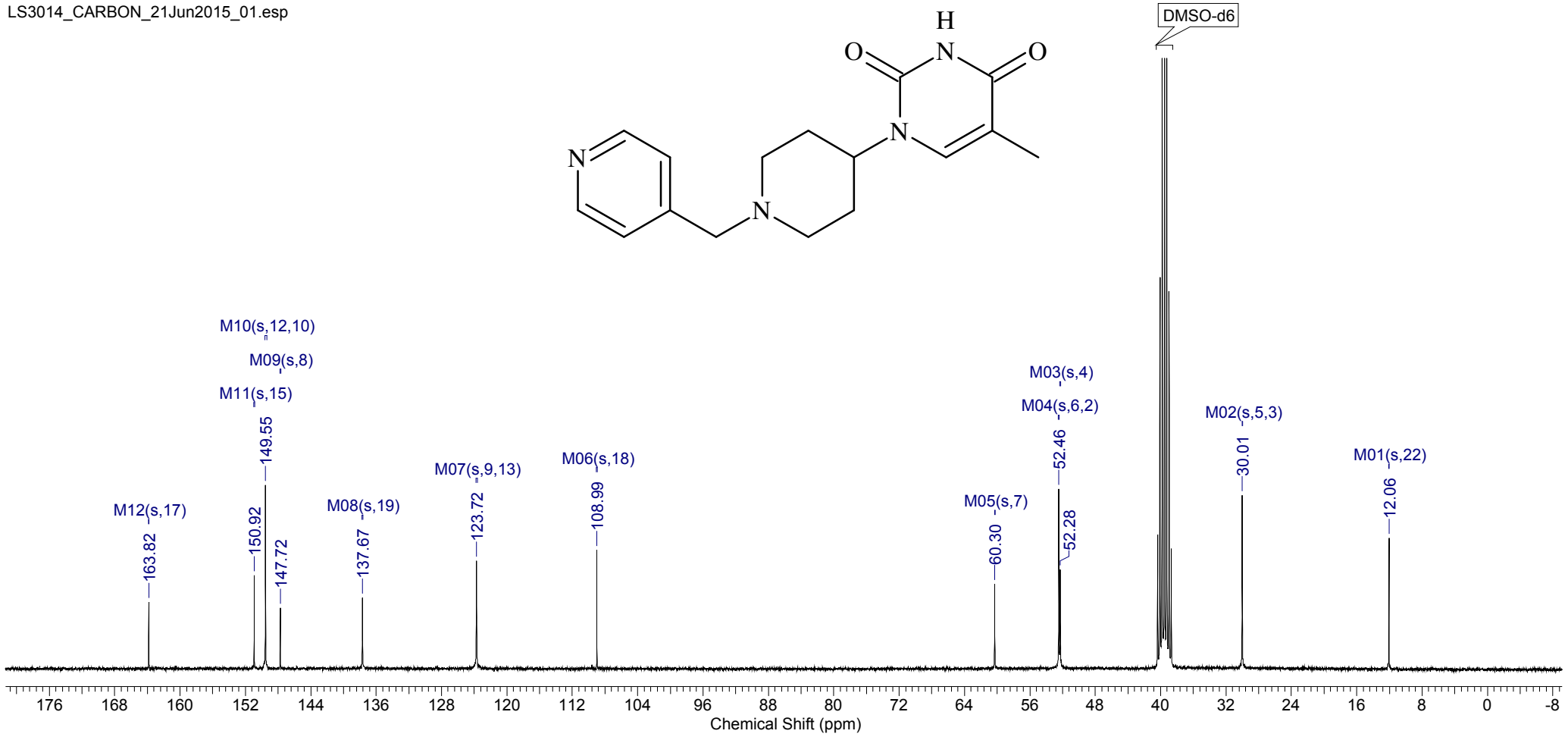
Compound 27

^{1}HNMR 300 MHz DMSO-d_6



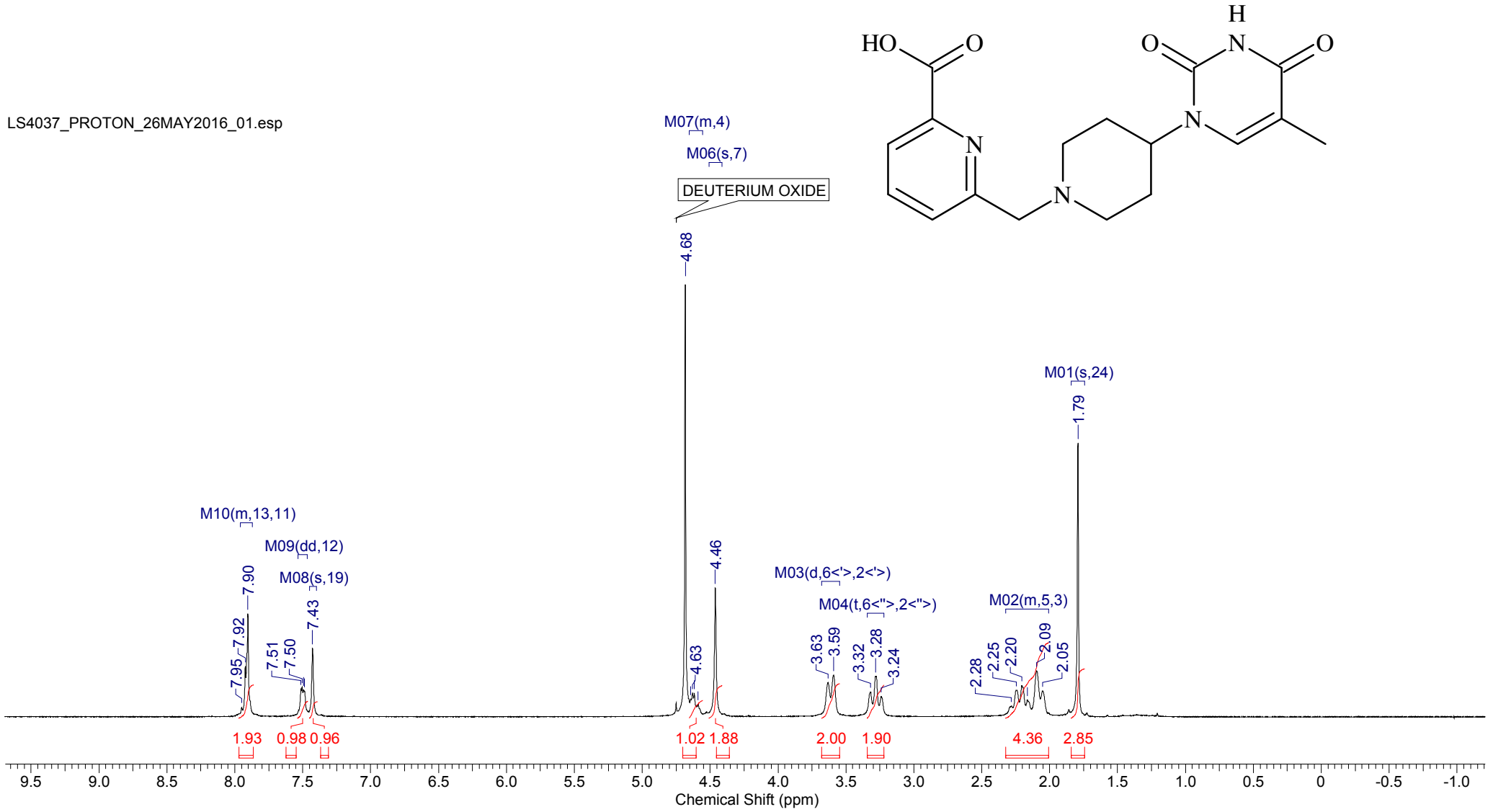
Compound 27

^{13}CNMR 75 MHz DMSO-d_6



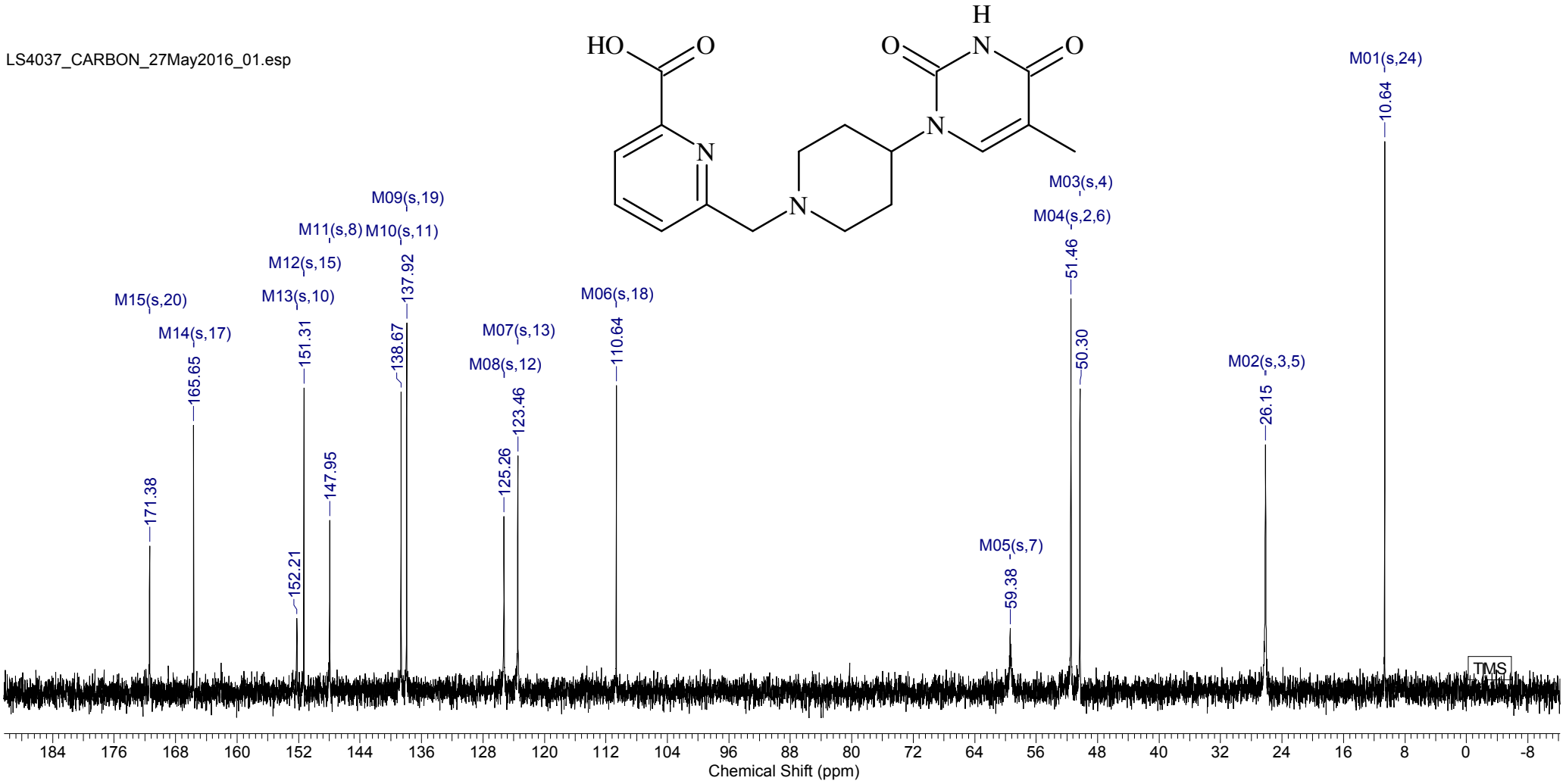
Compound 28

^{1}HNMR 300 MHz D_2O



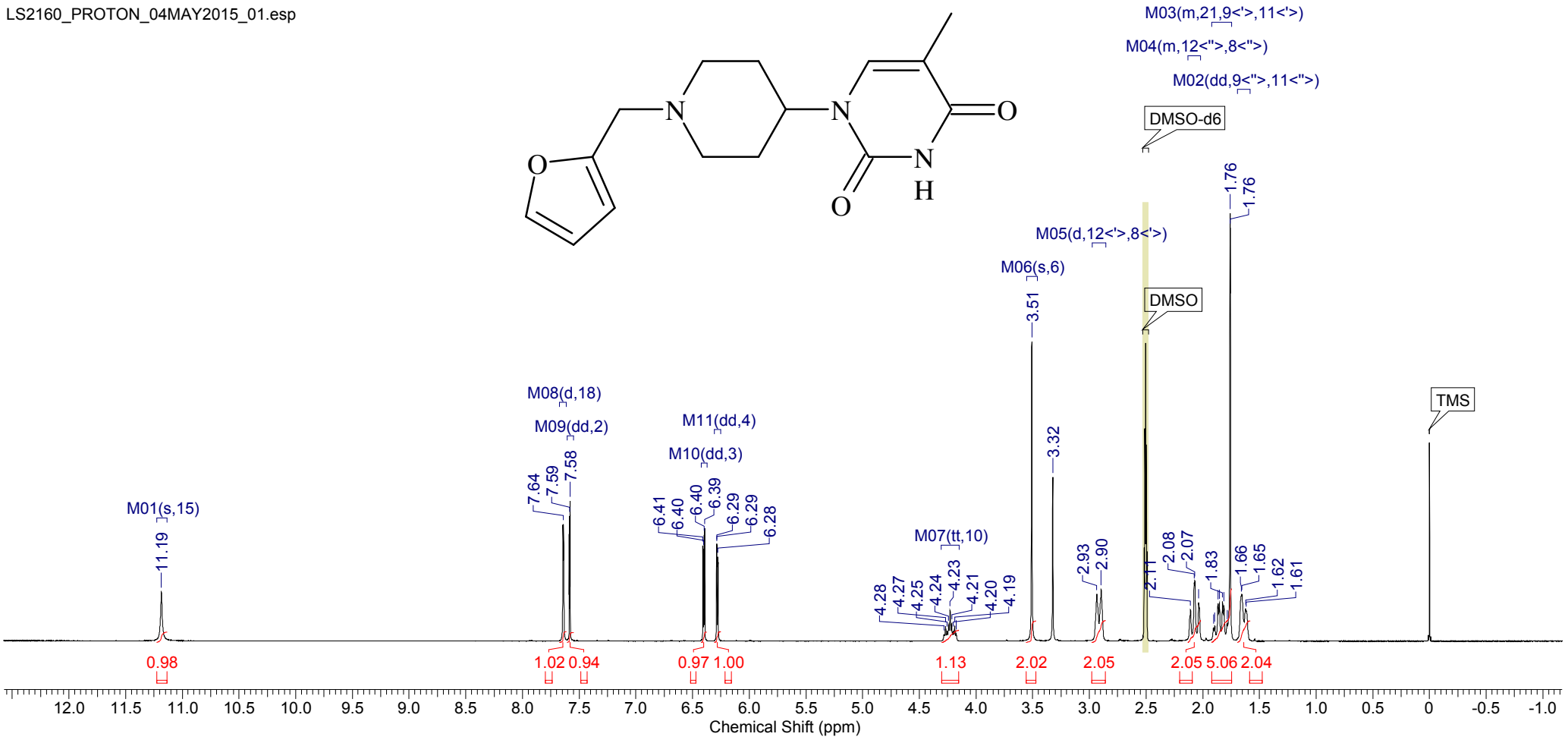
Compound 28

^{13}CNMR 75 MHz D_2O



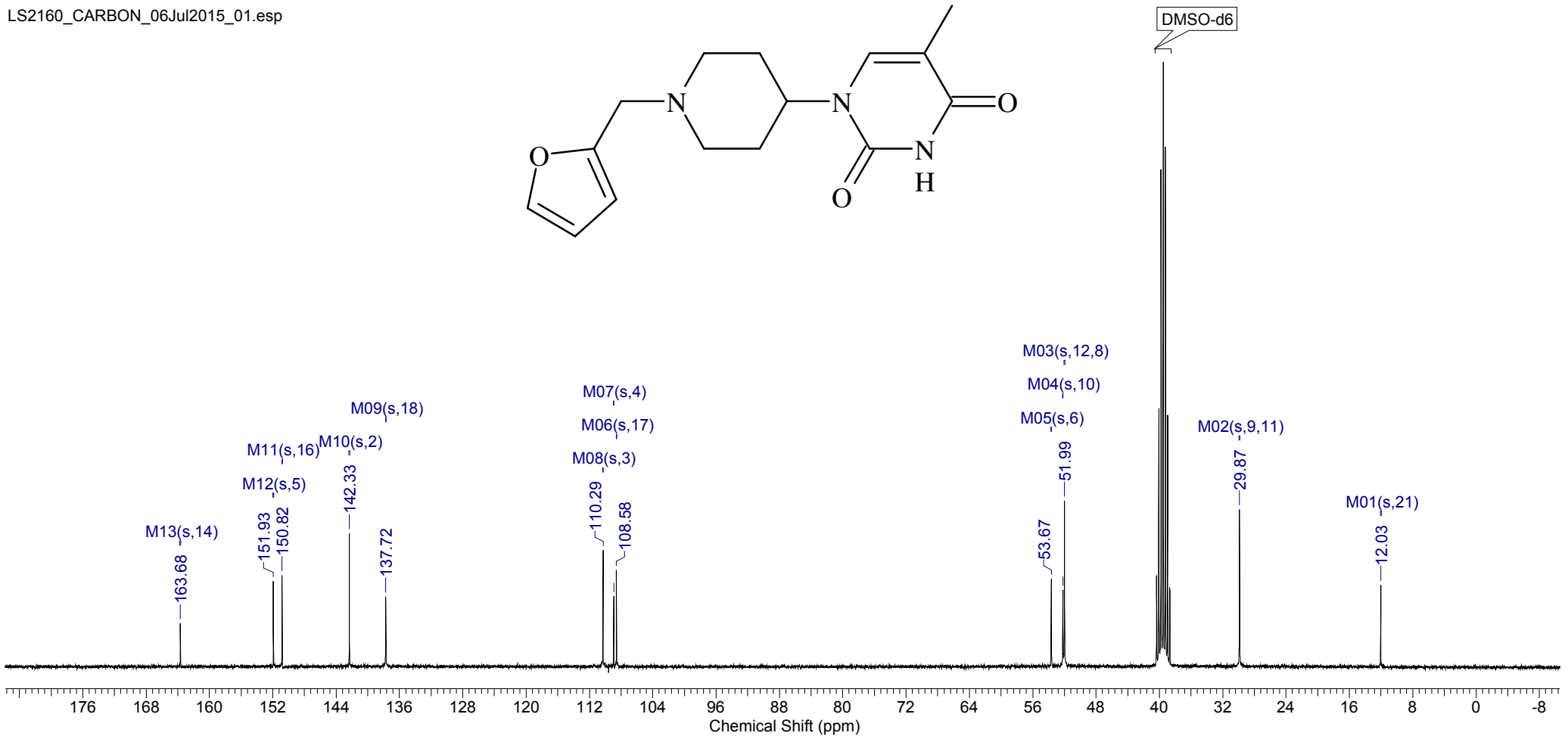
Compound 29

^{1}HNMR 300 MHz DMSO-d_6



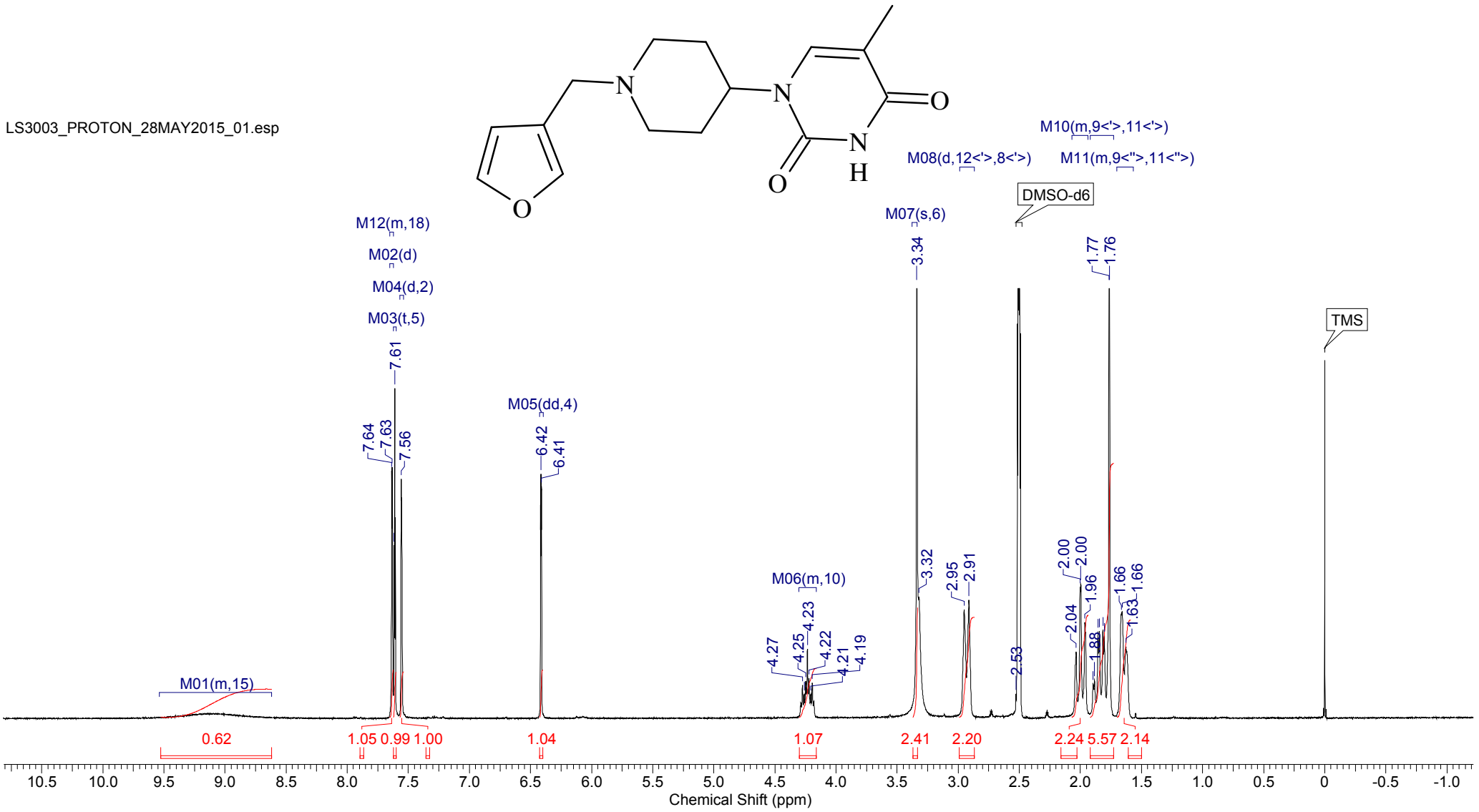
Compound 29

^{13}CNMR 75 MHz DMSO-d_6



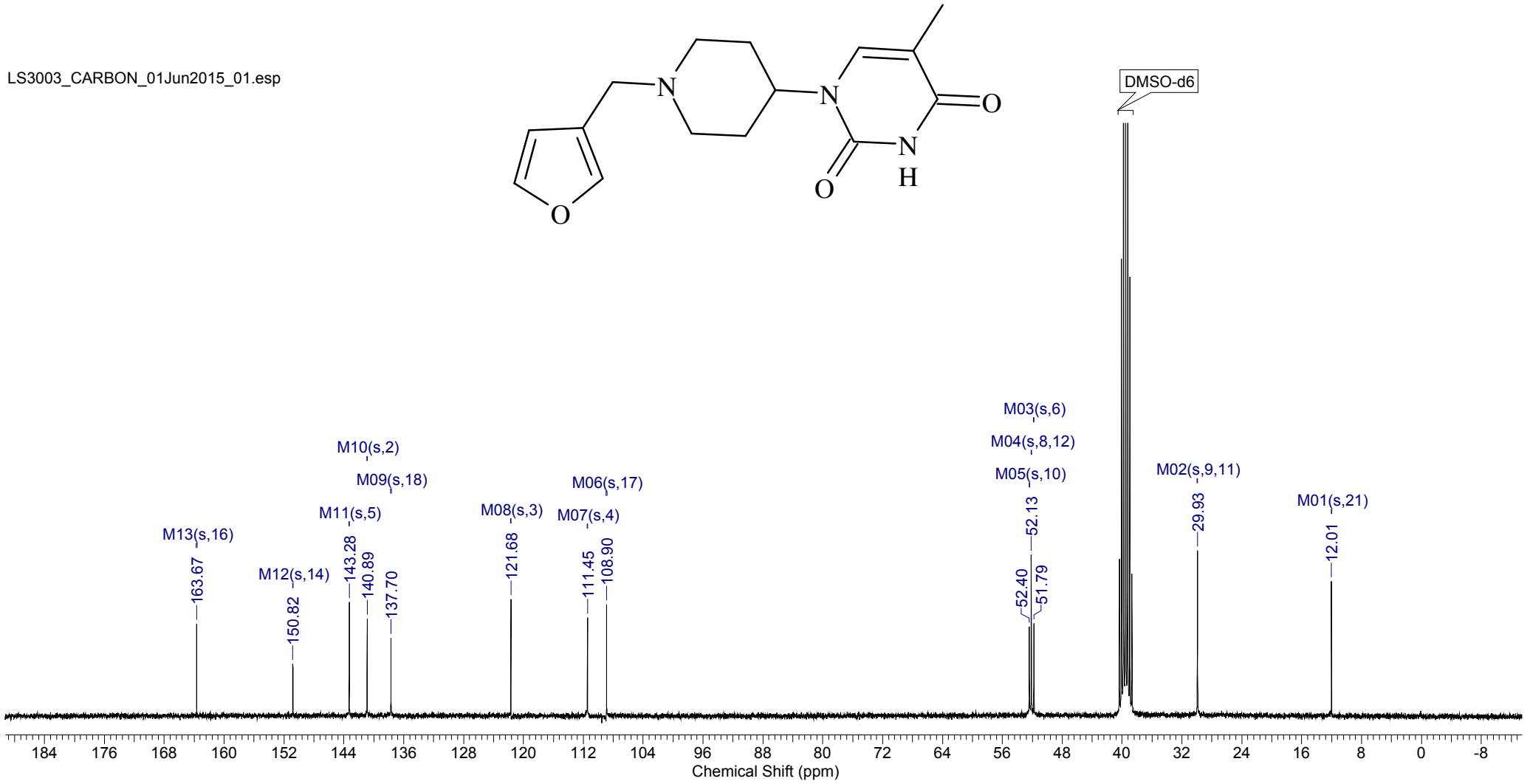
Compound 30

^{1}HNMR 300 MHz DMSO-d$_6$



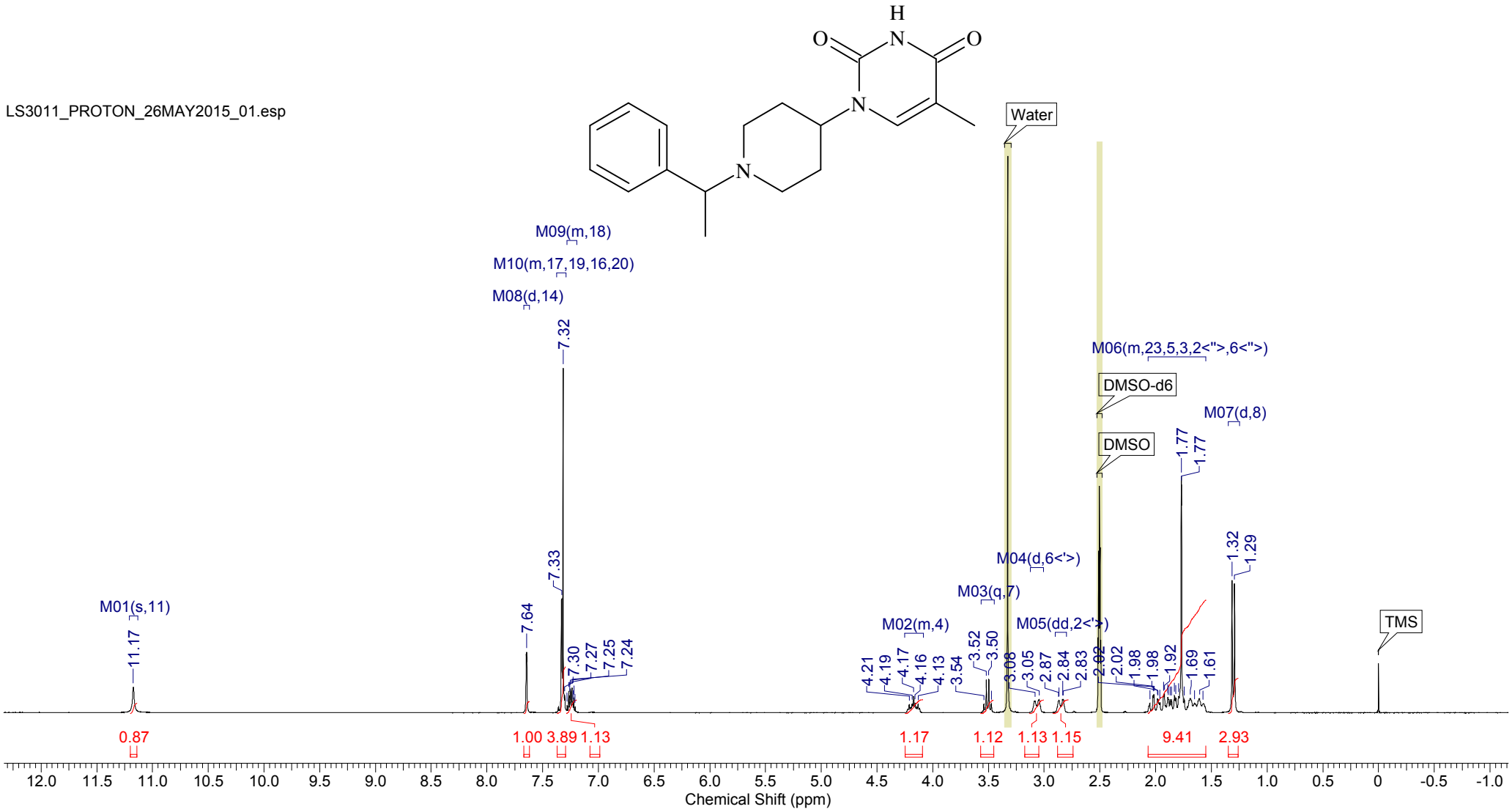
Compound 30

^{13}CNMR 75 MHz DMSO-d_6



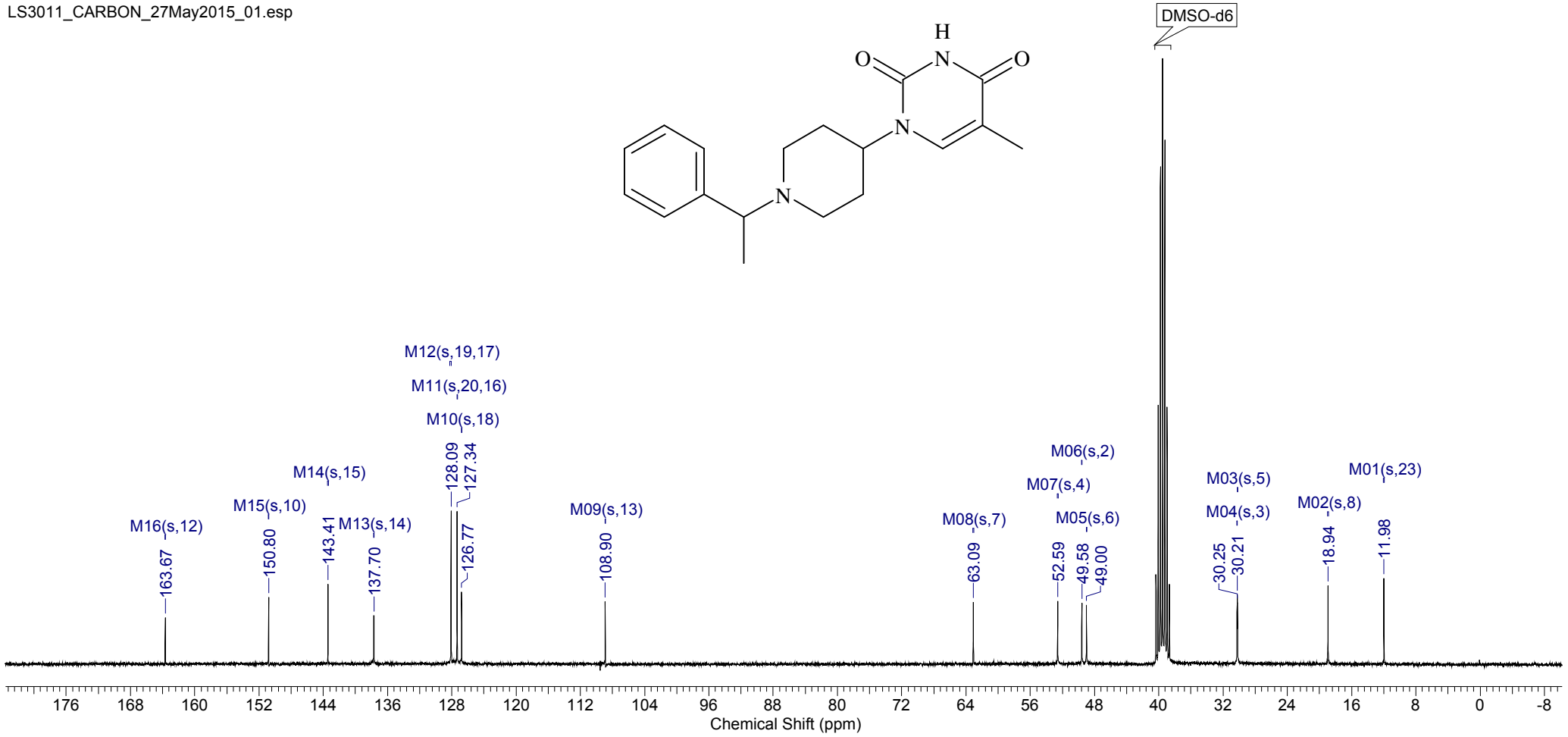
Compound 31

^{1}HNMR 300 MHz DMSO-d_6



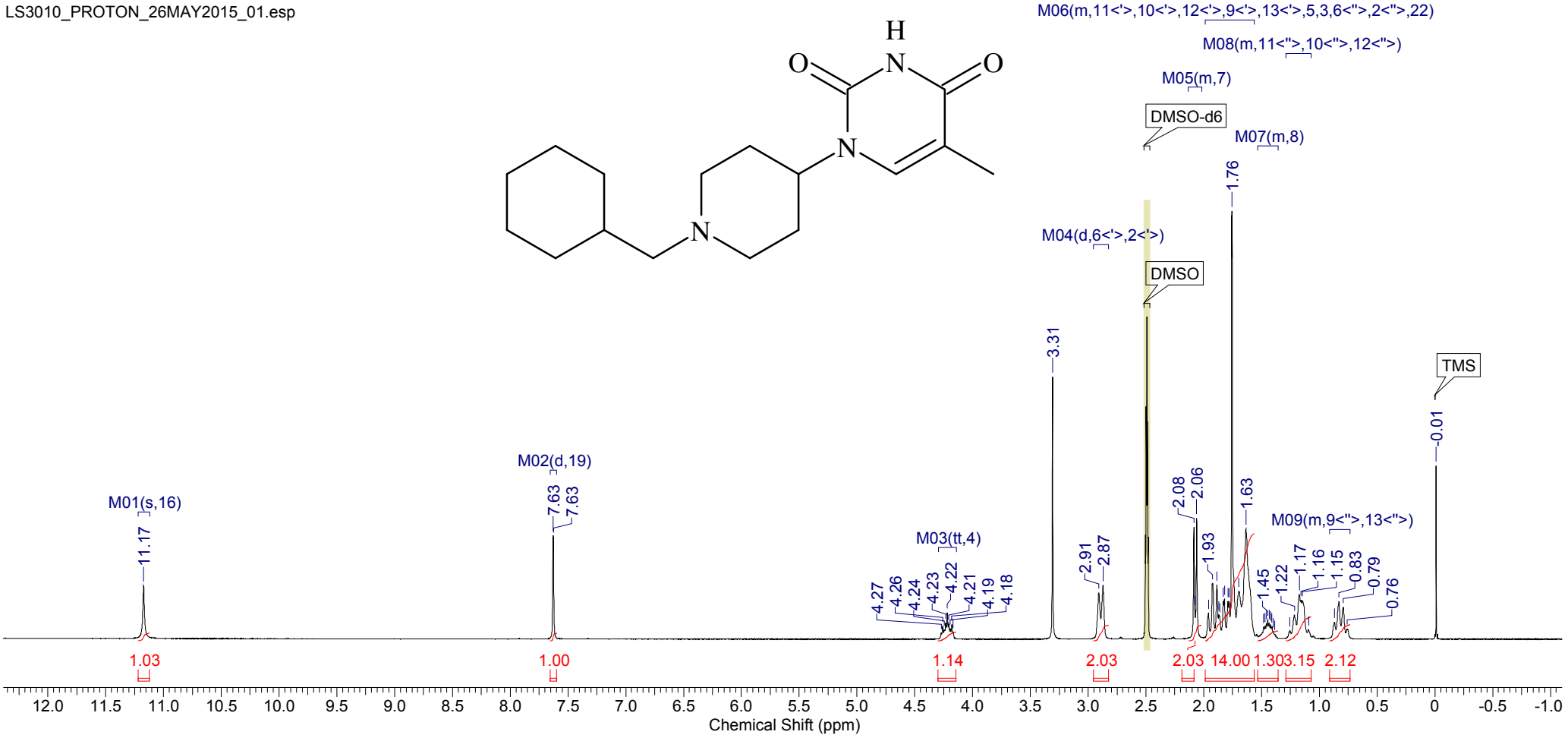
Compound 31

^{13}CNMR 75 MHz DMSO-d_6



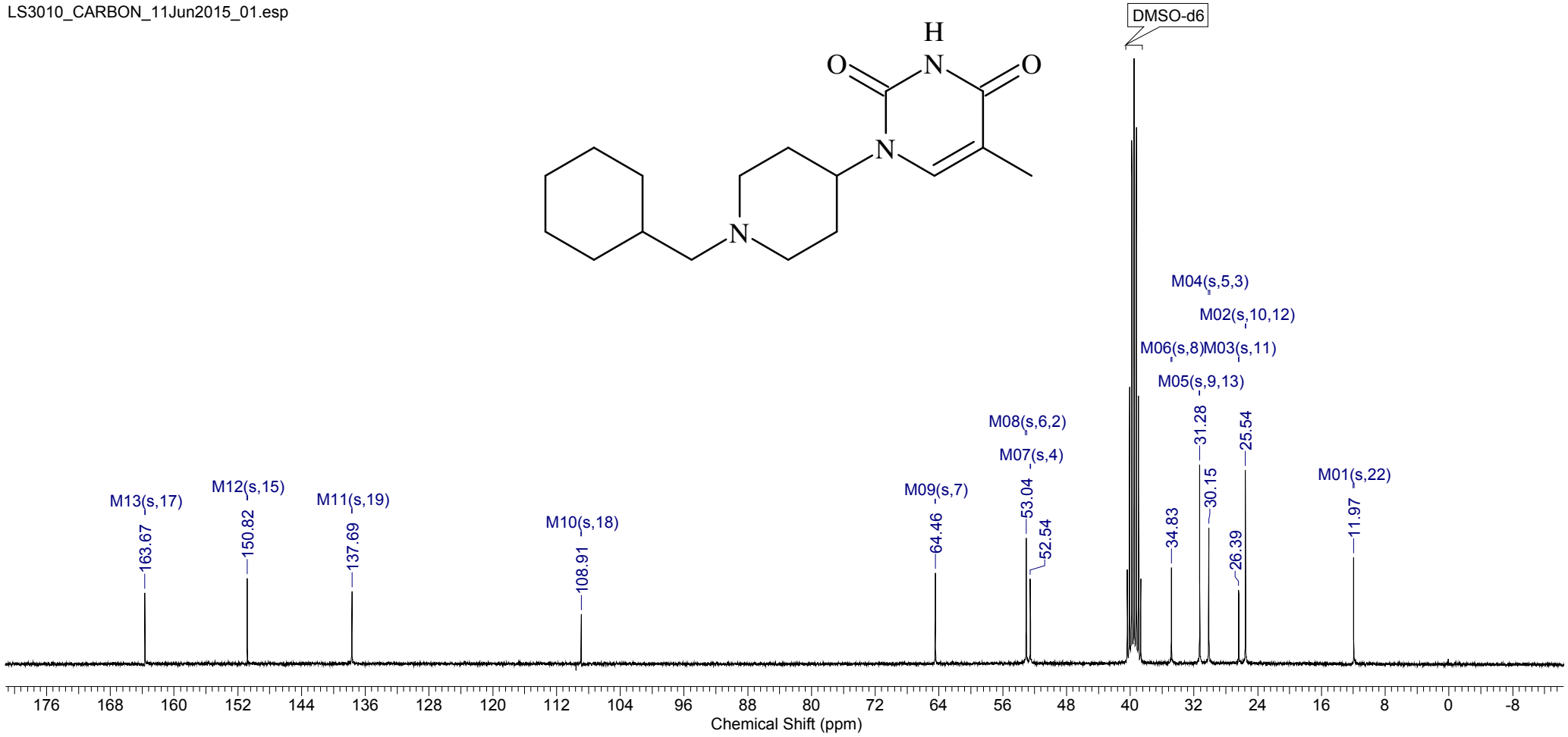
Compound 32

^{1}HNMR 300 MHz DMSO-d_6



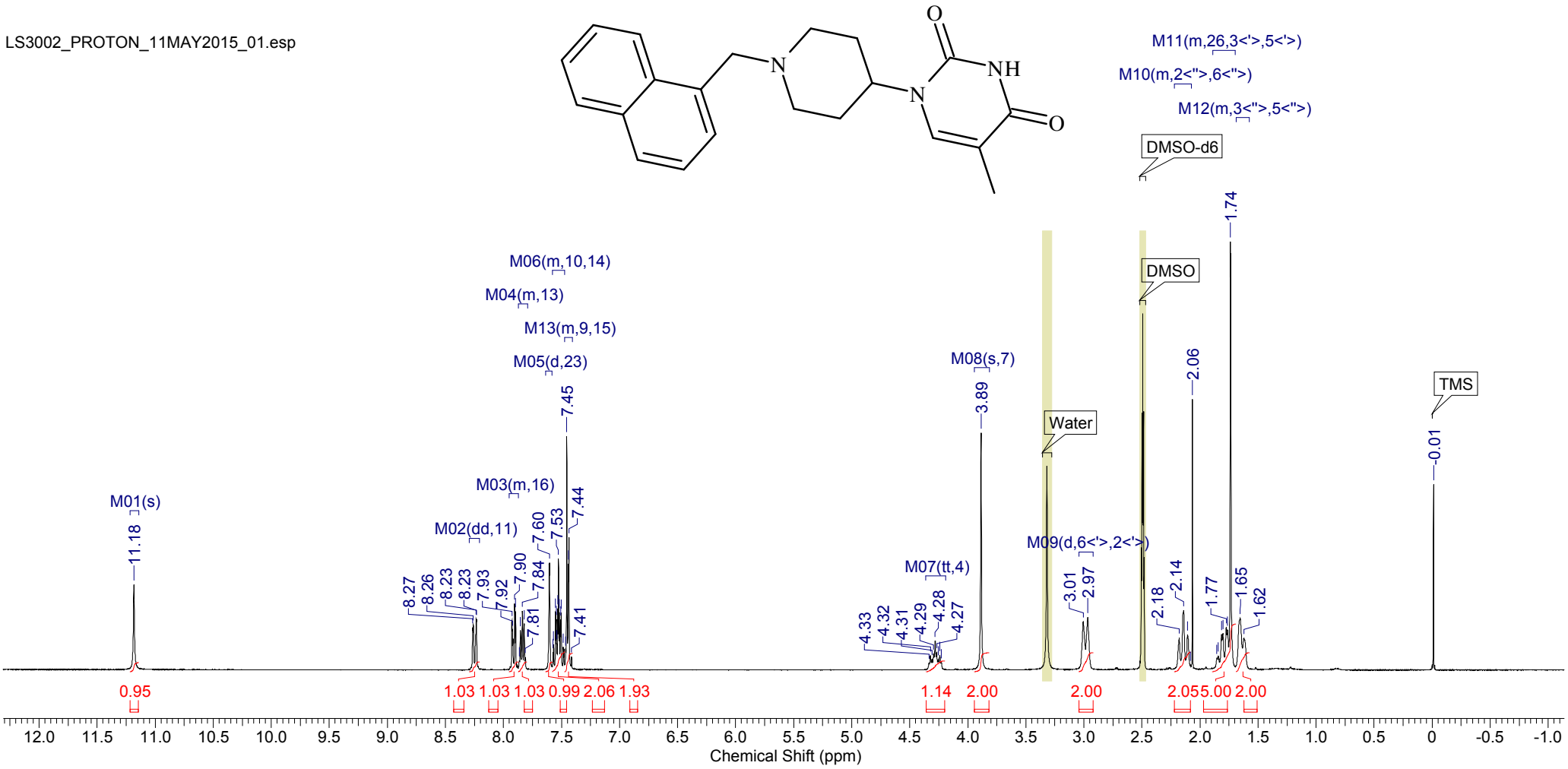
Compound 32

^{13}CNMR 75 MHz DMSO-d_6



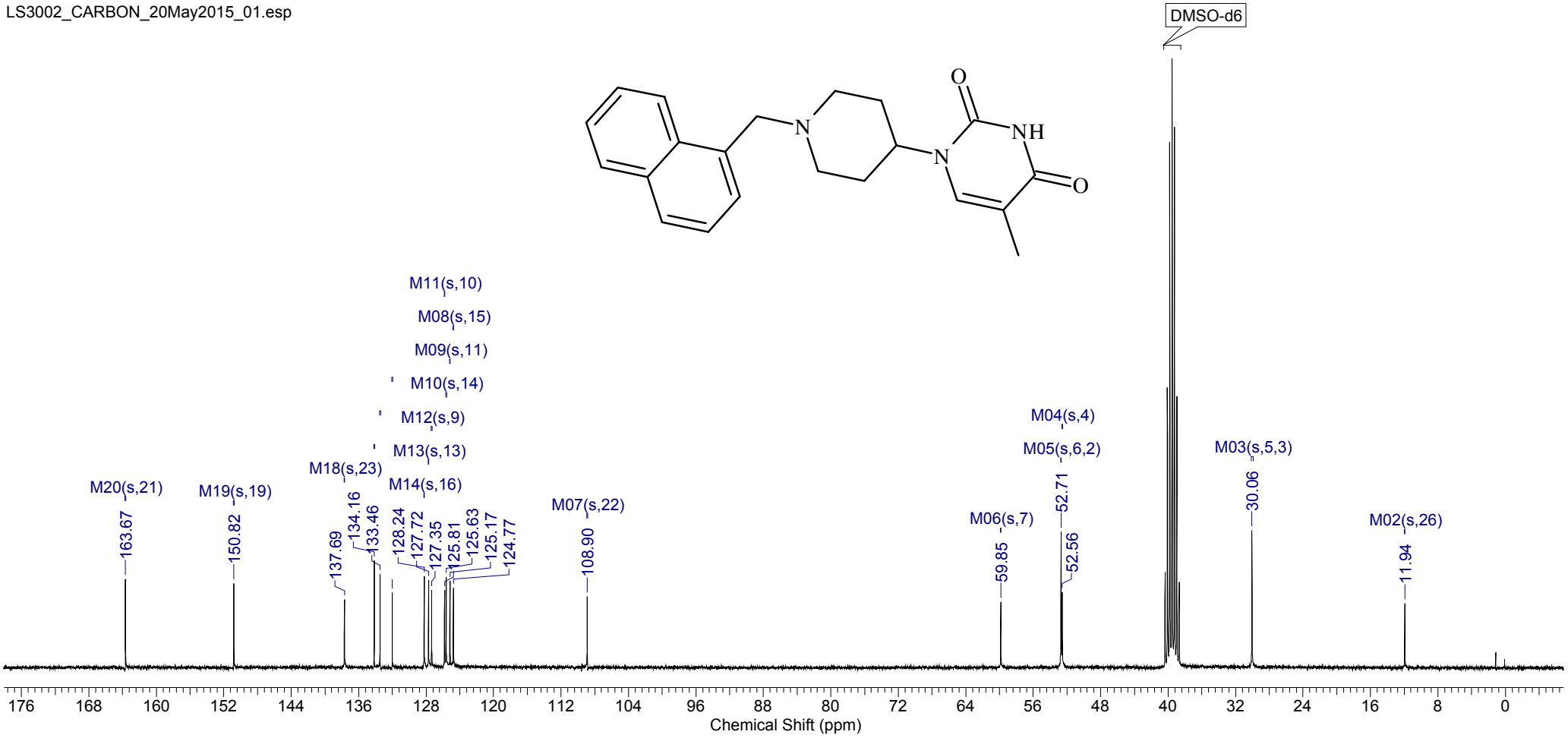
Compound 33

^{1}HNMR 300 MHz DMSO-d$_6$



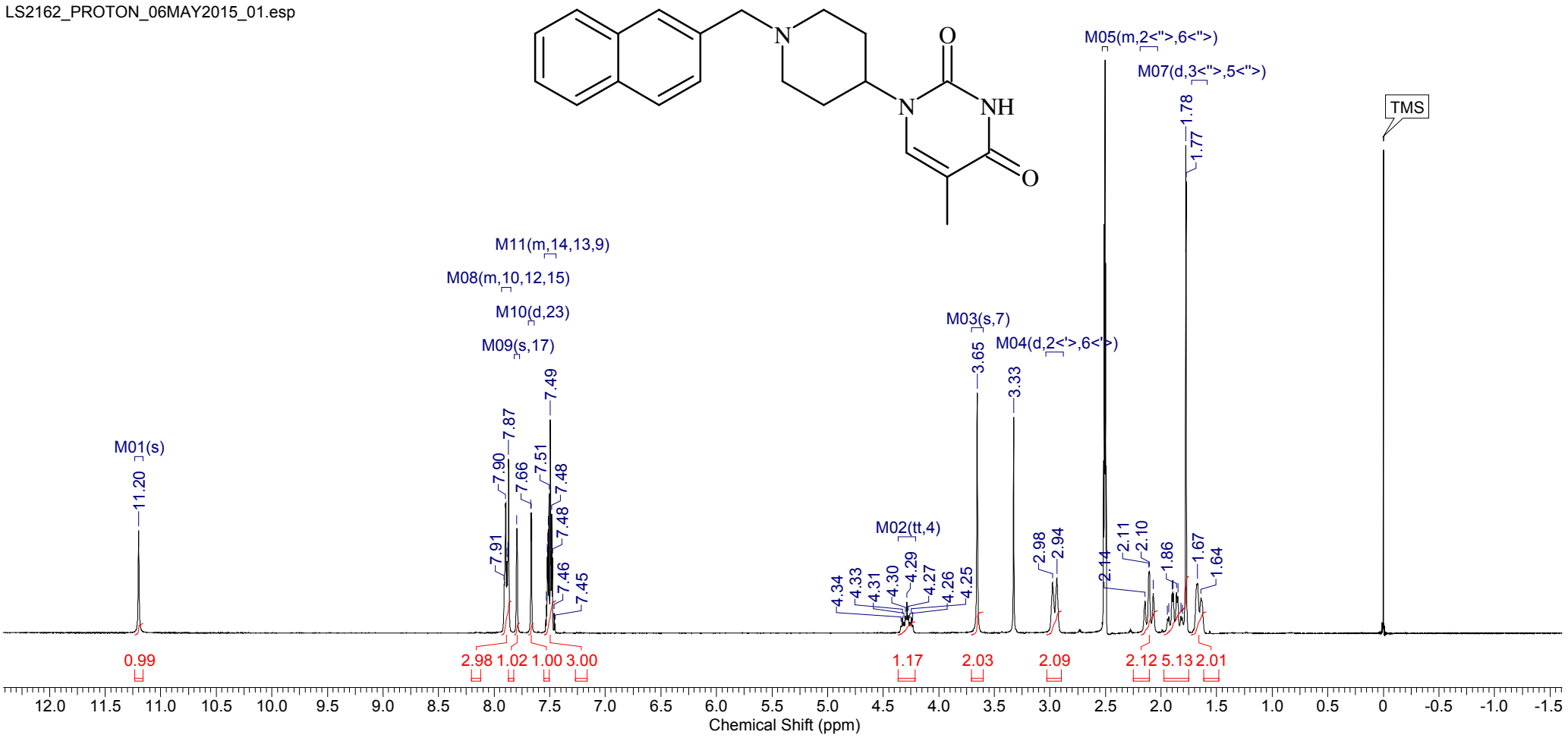
Compound 33

^{13}CNMR 75 MHz DMSO-d_6



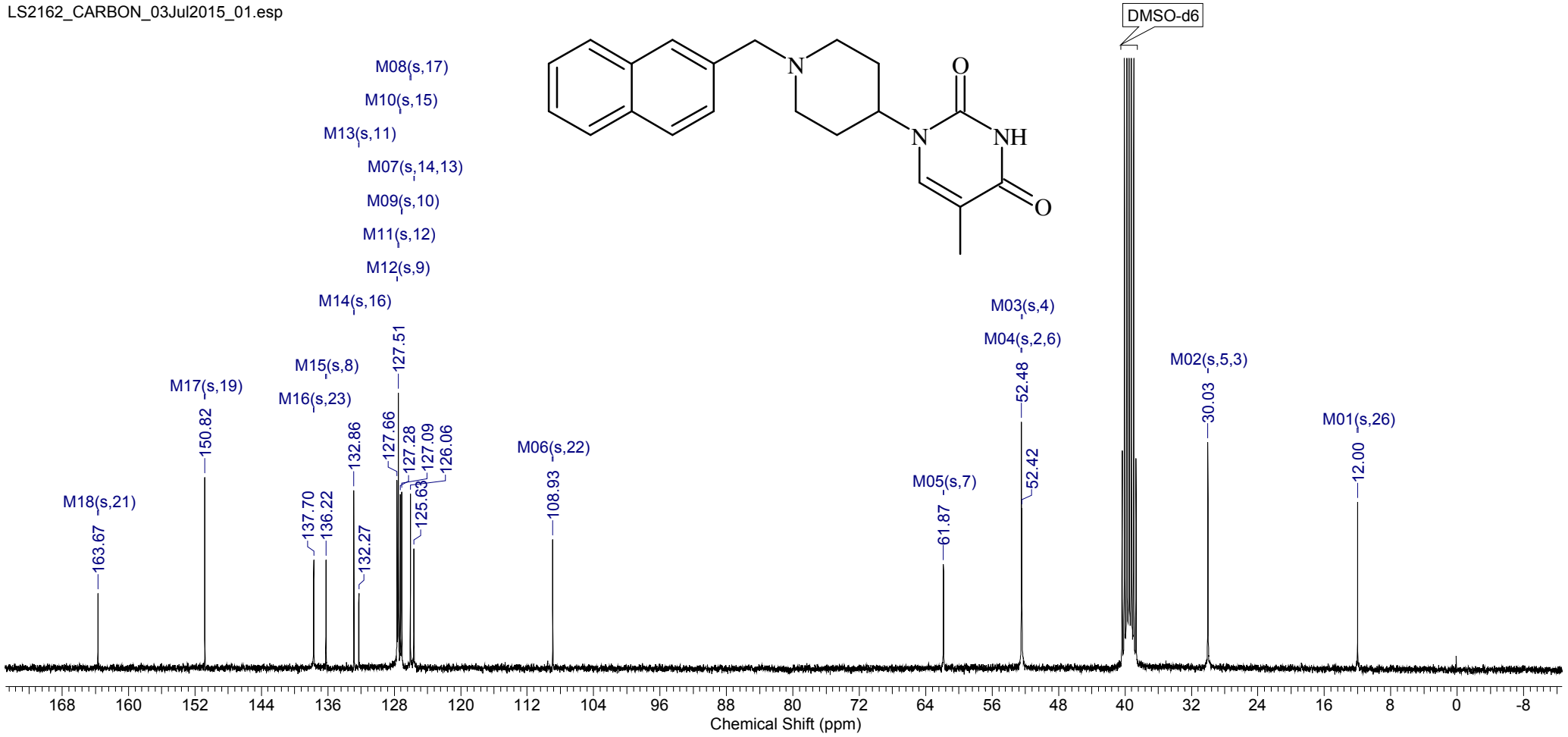
Compound 34

^{1}HNMR 300 MHz DMSO-d_6



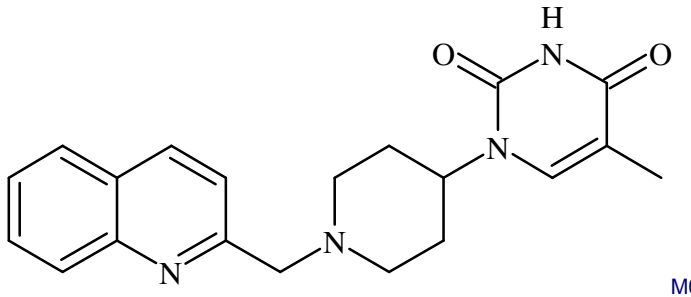
Compound 34

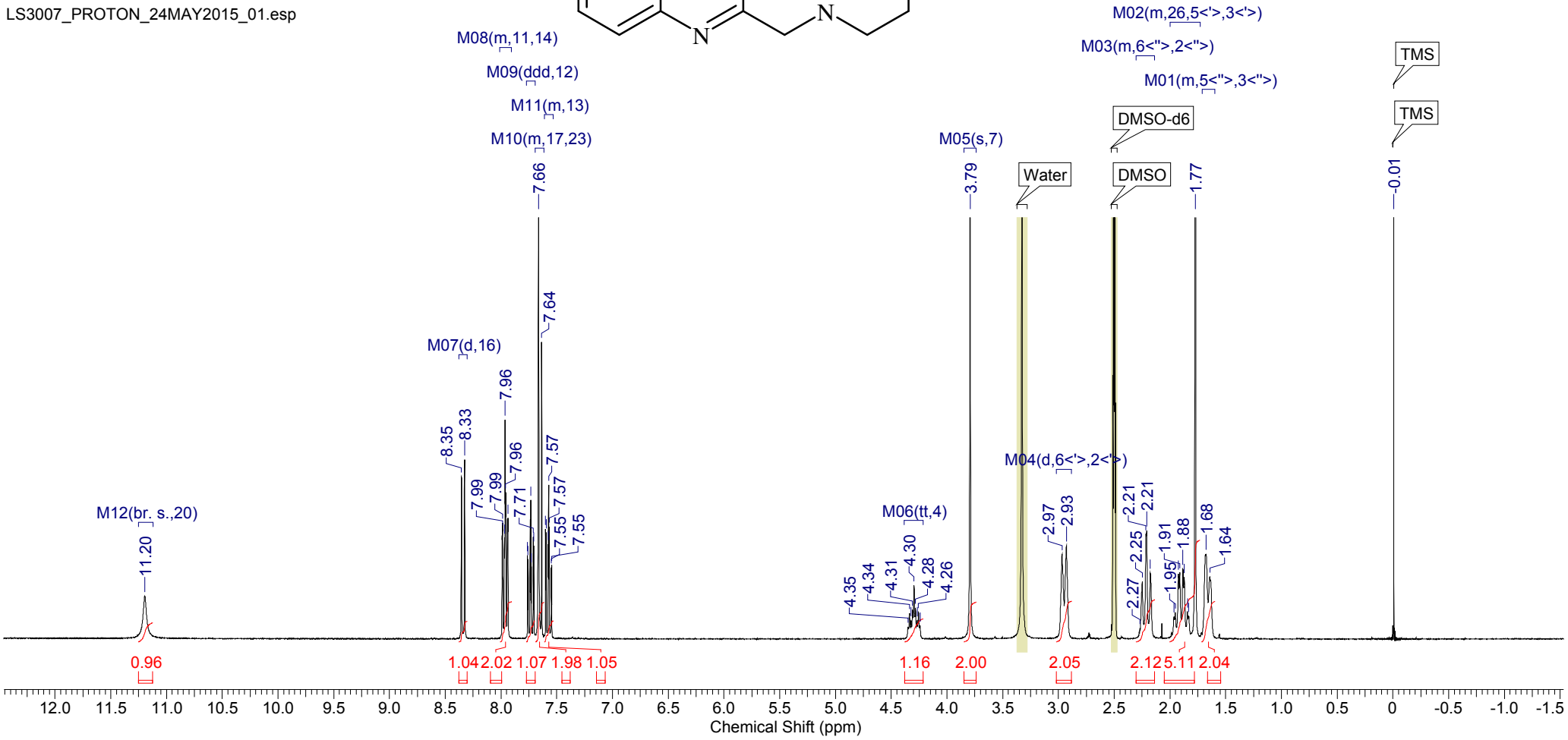
^{13}CNMR 75 MHz DMSO-d_6



Compound 35

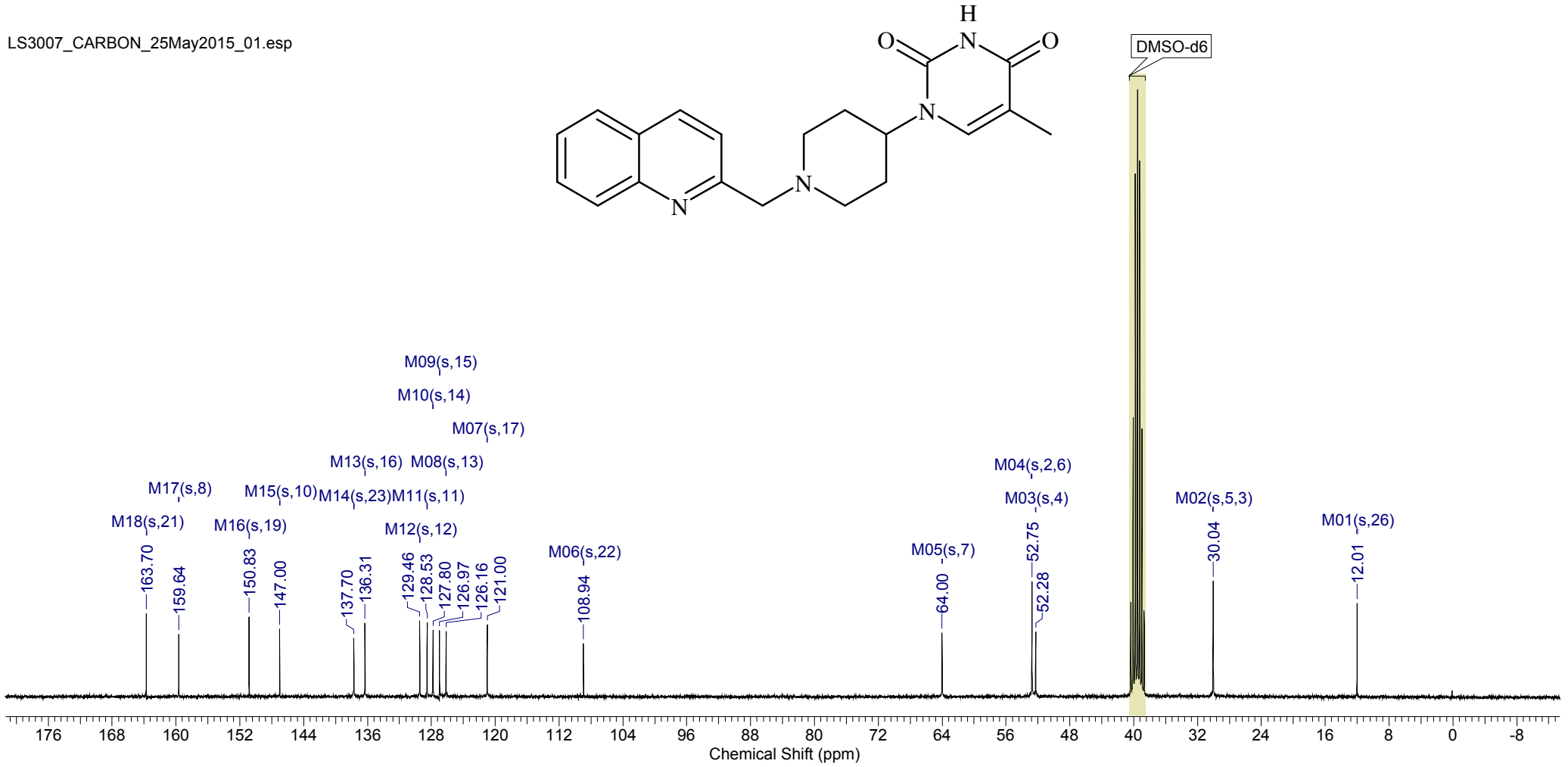
^{1}HNMR 300 MHz DMSO-d$_6$





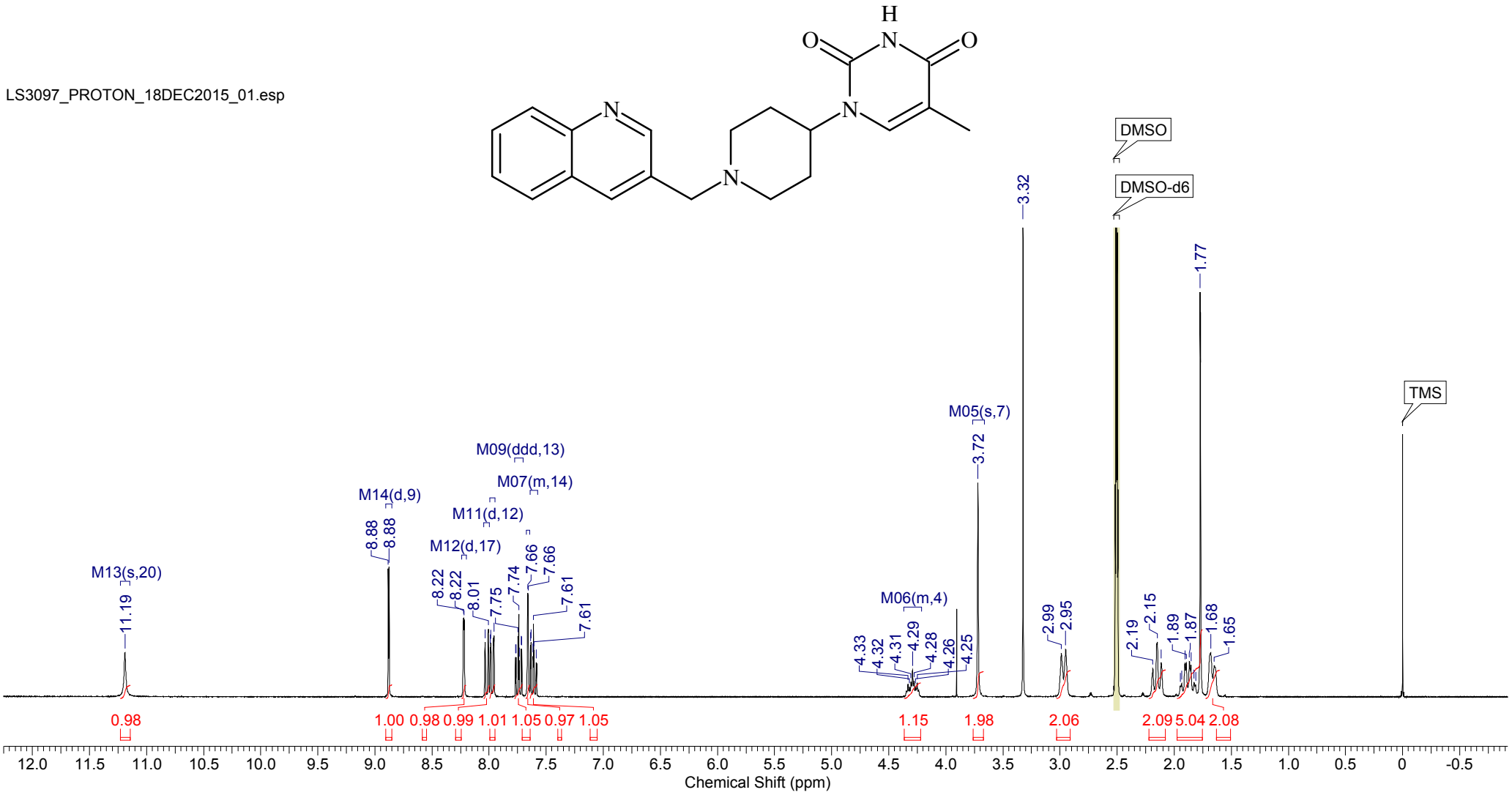
Compound 35

^{13}CNMR 75 MHz DMSO-d_6



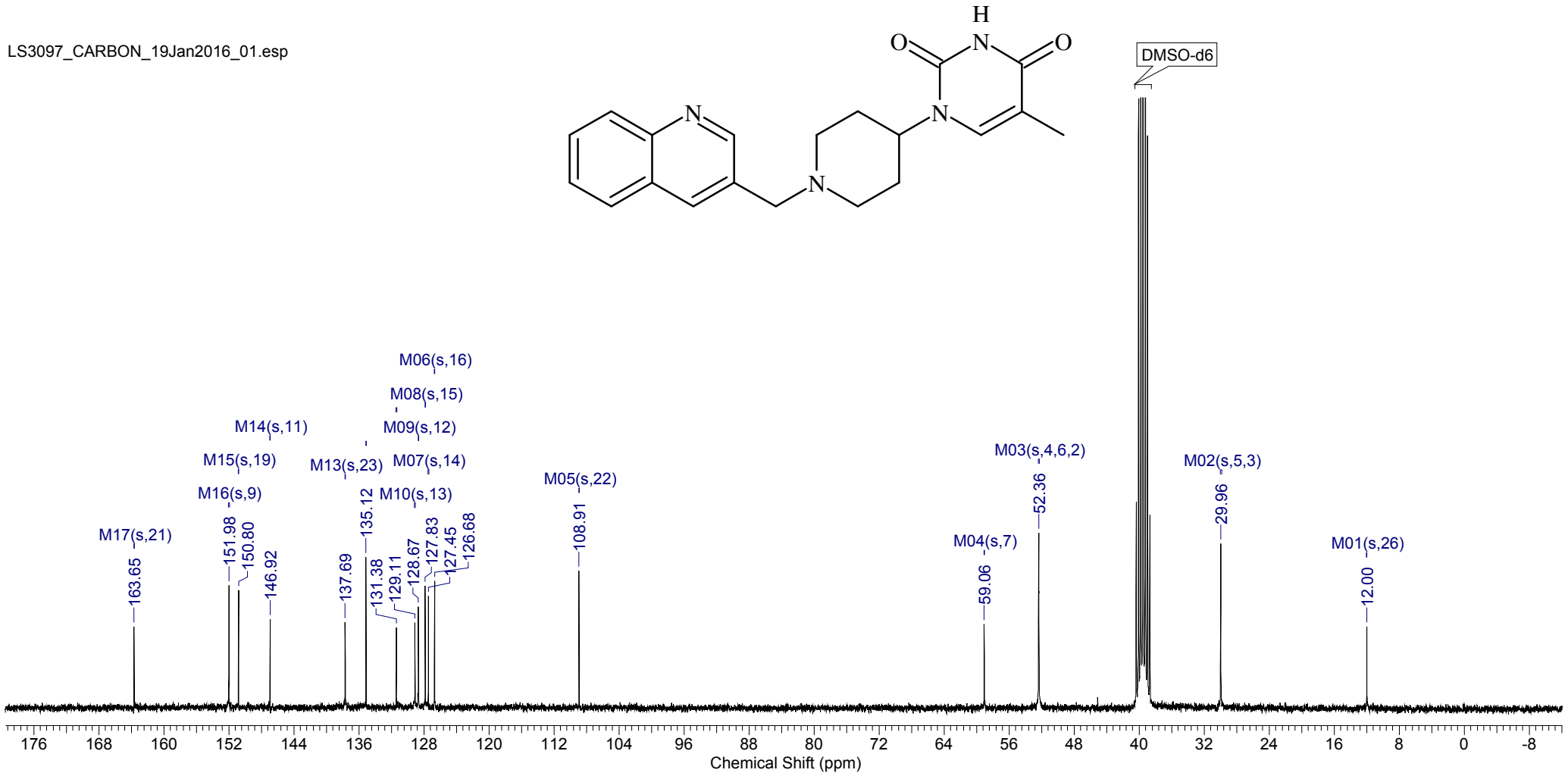
Compound 36

^{1}HNMR 300 MHz DMSO-d_6



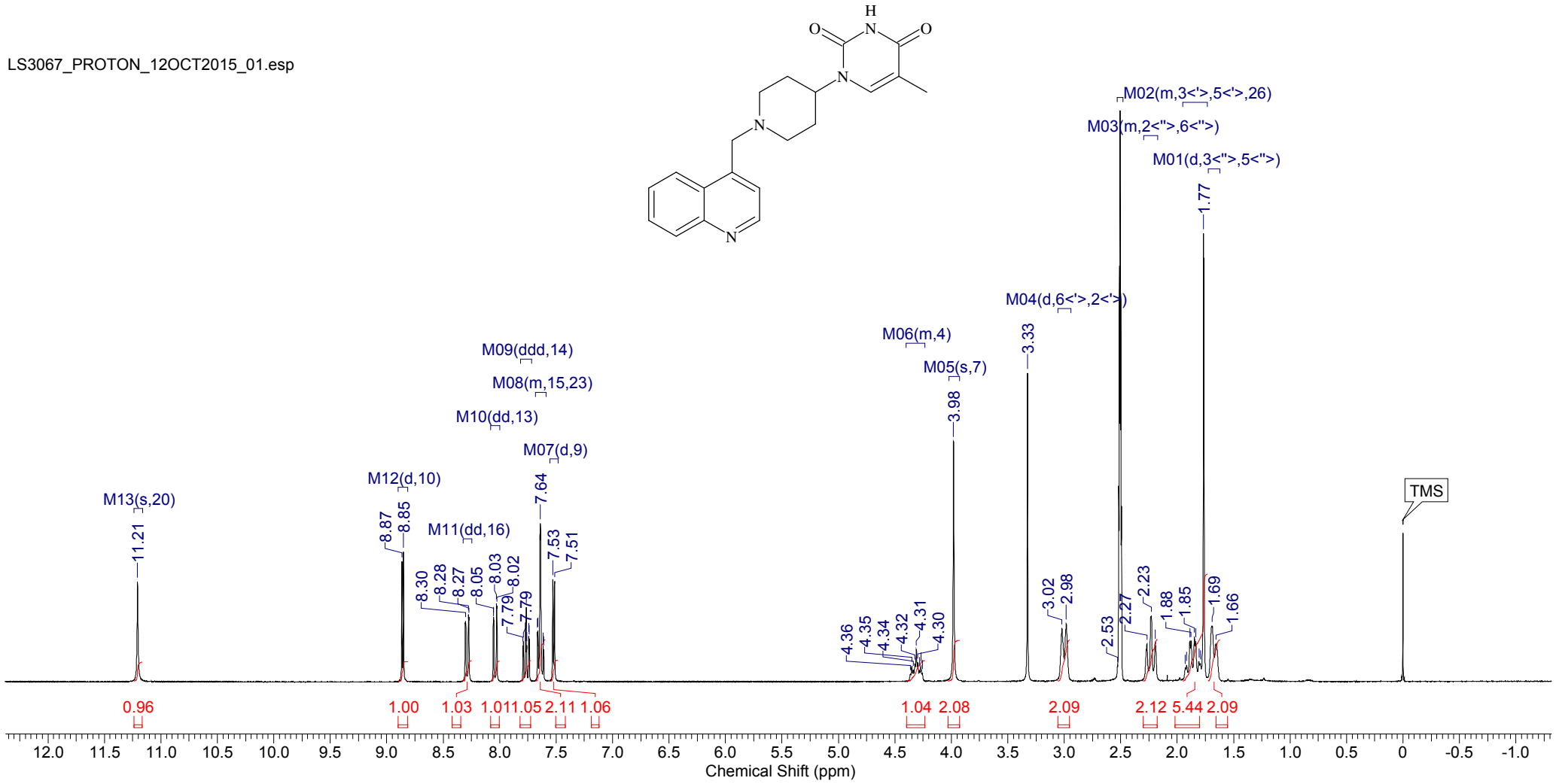
Compound 36

^{13}CNMR 75 MHz DMSO-d_6



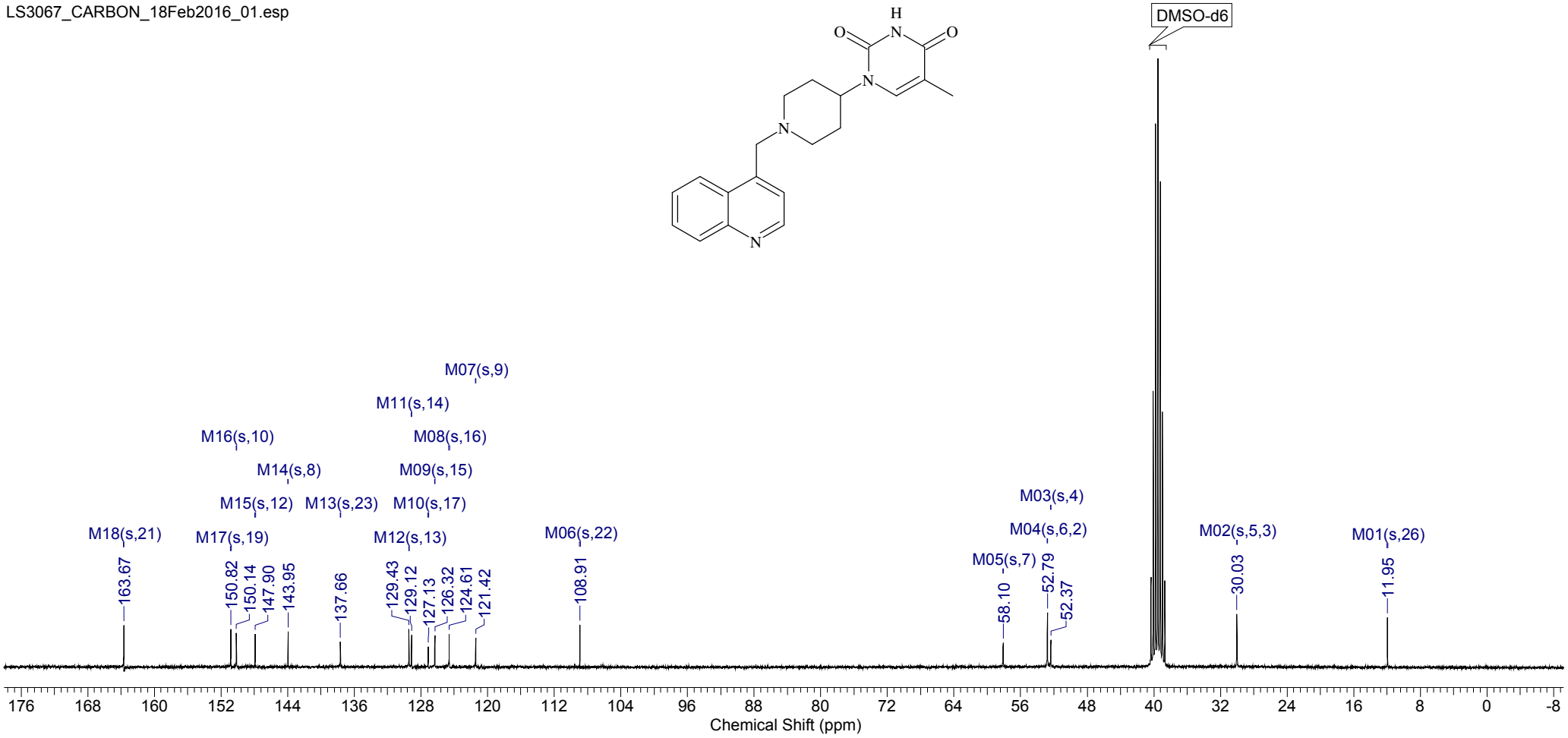
Compound 37

^{1}HNMR 300 MHz DMSO-d_6



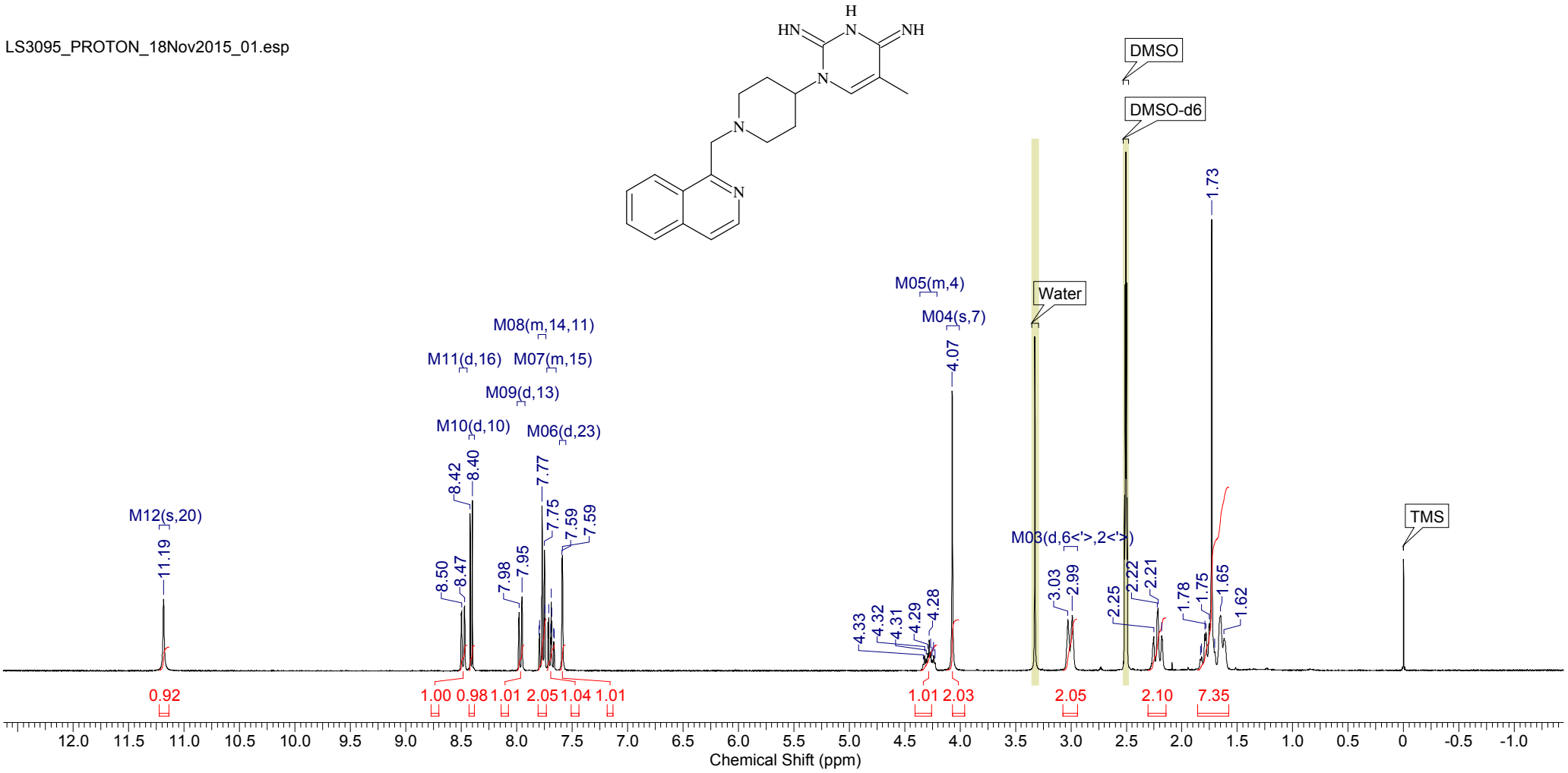
Compound 37

^{13}CNMR 75 MHz DMSO-d_6



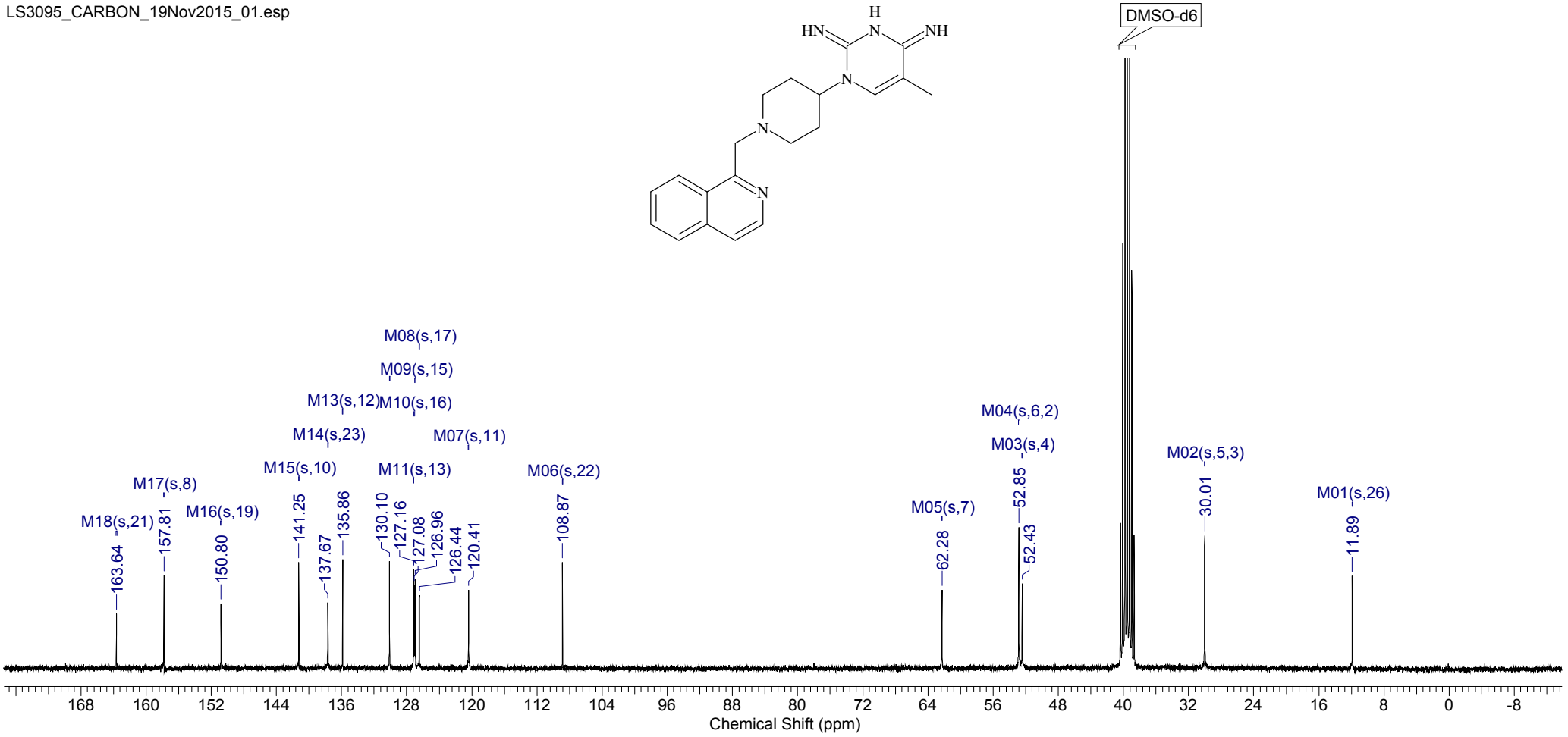
Compound 38

^{1}HNMR 300 MHz DMSO-d_6



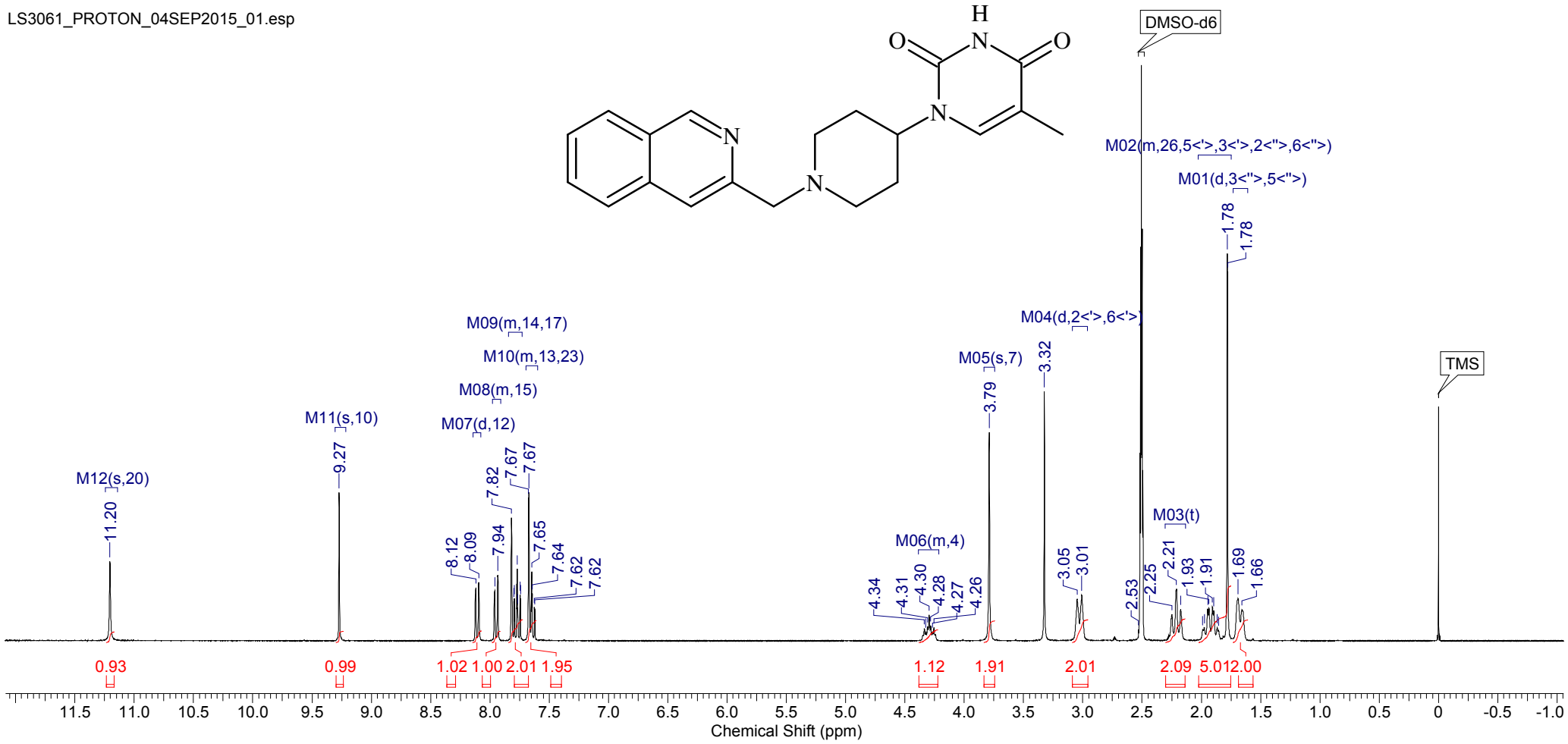
Compound 38

^{13}CNMR 75 MHz DMSO-d_6



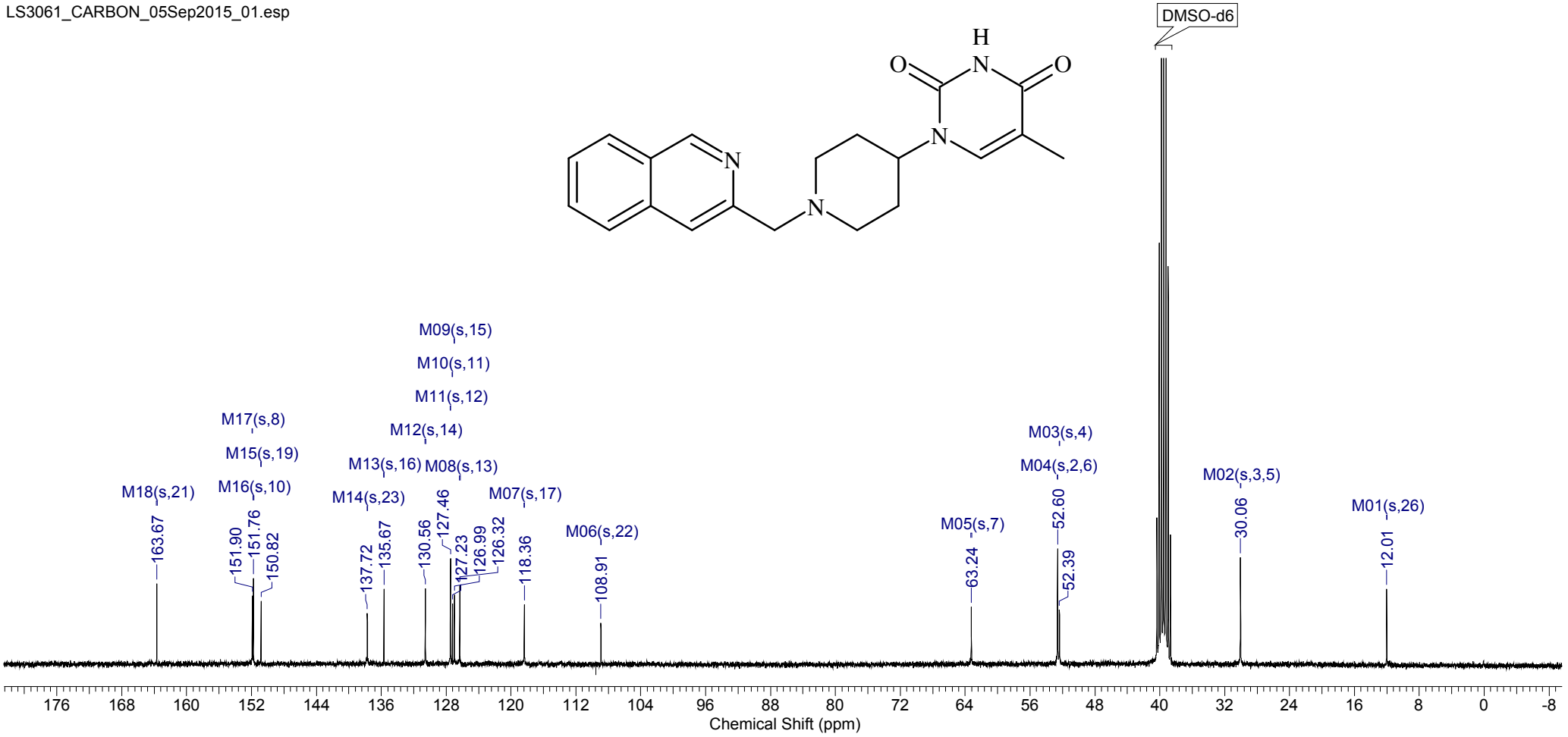
Compound 39

^{1}HNMR 300 MHz DMSO-d_6



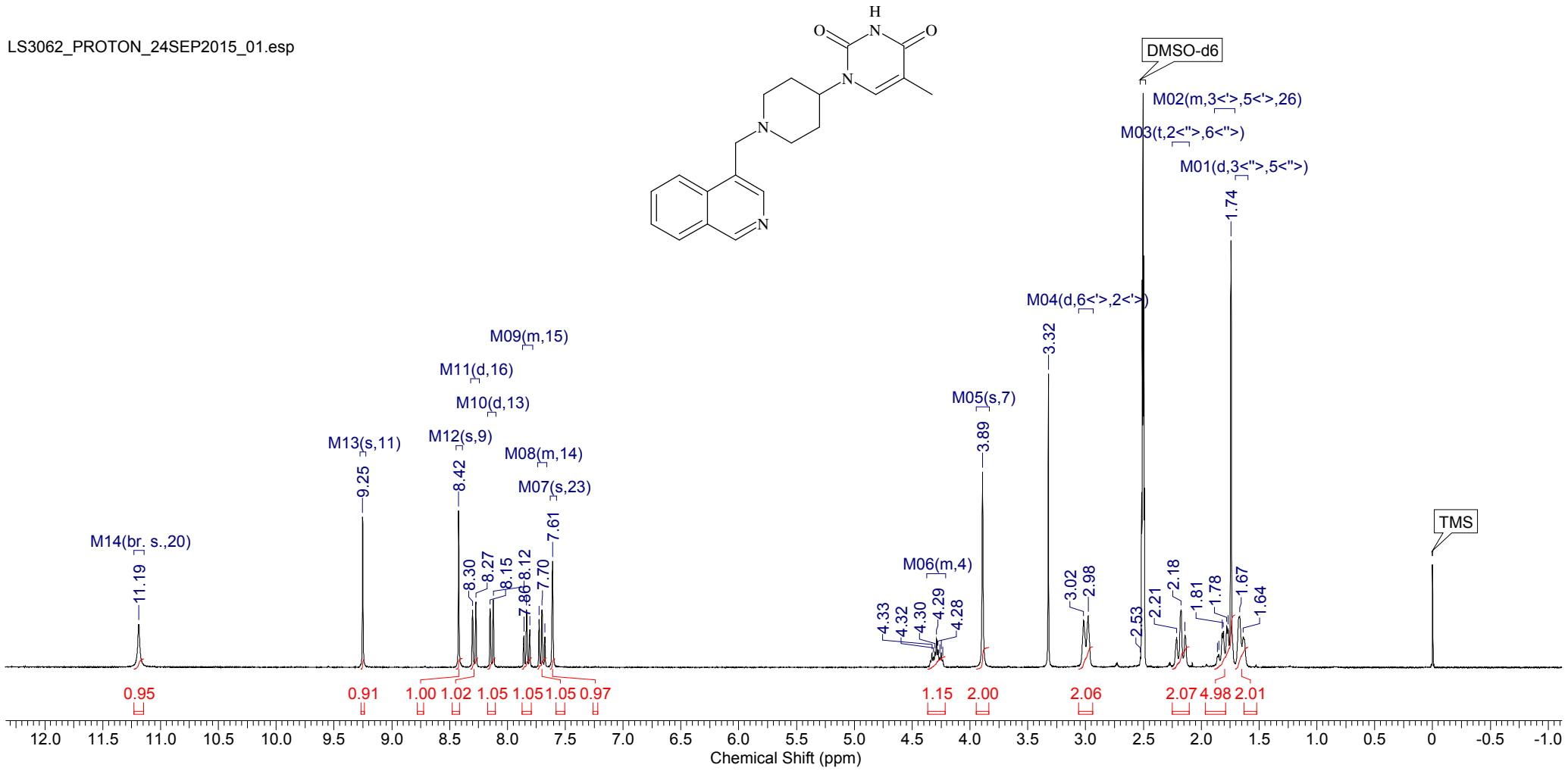
Compound 39

^{13}CNMR 75 MHz DMSO-d_6



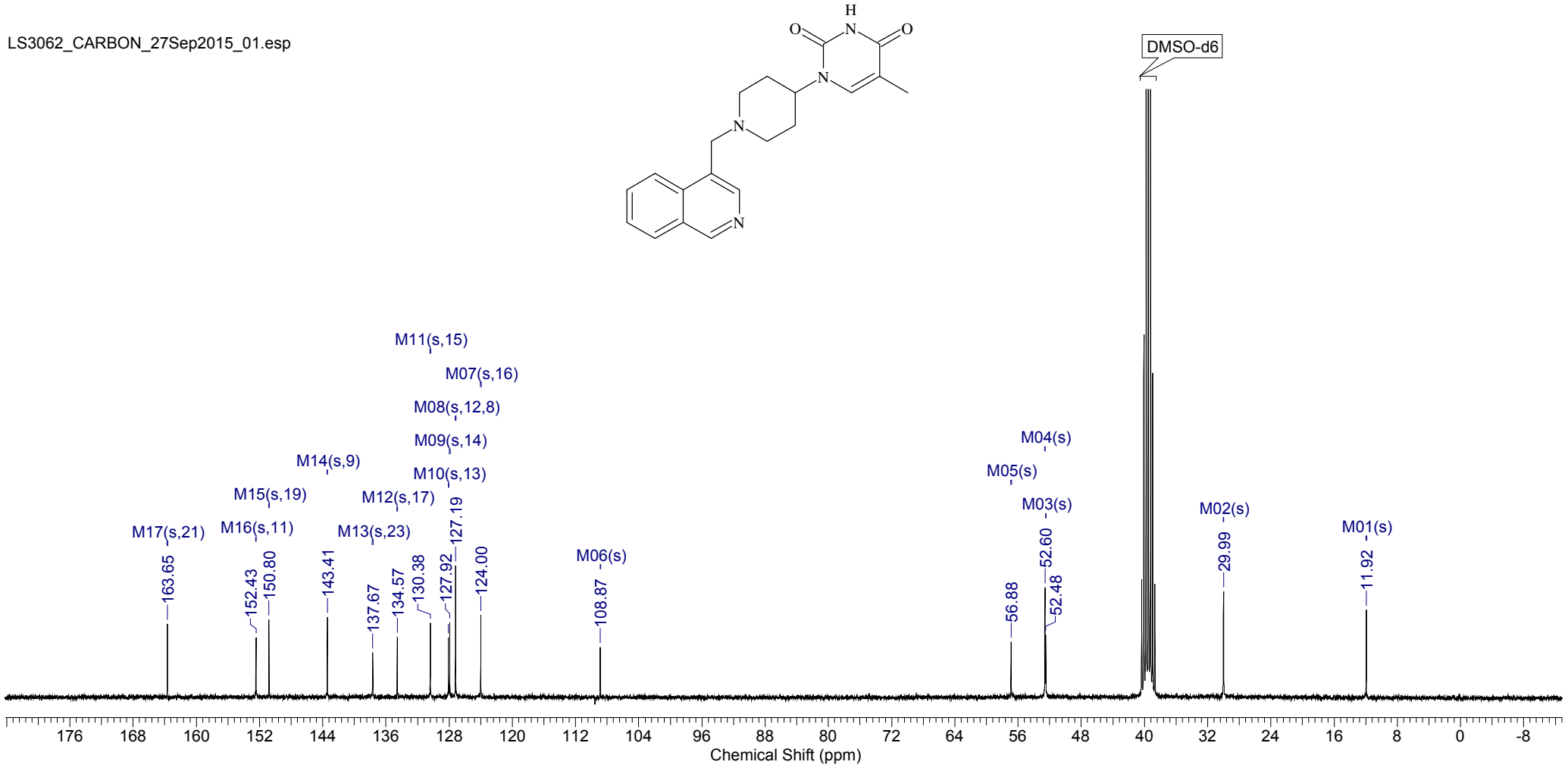
Compound 40

^{1}HNMR 300 MHz DMSO-d_6



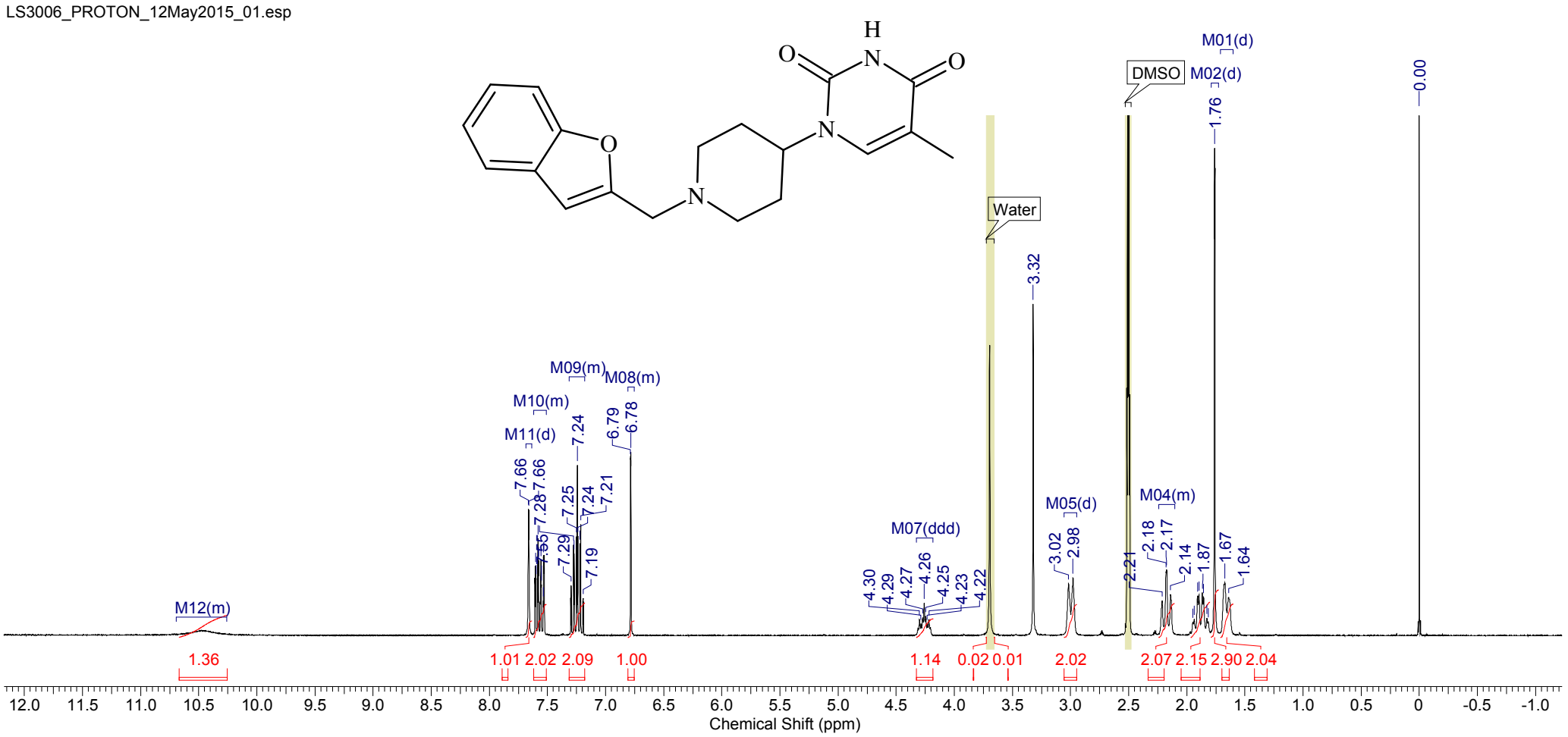
Compound 40

^{13}CNMR 75 MHz DMSO-d_6



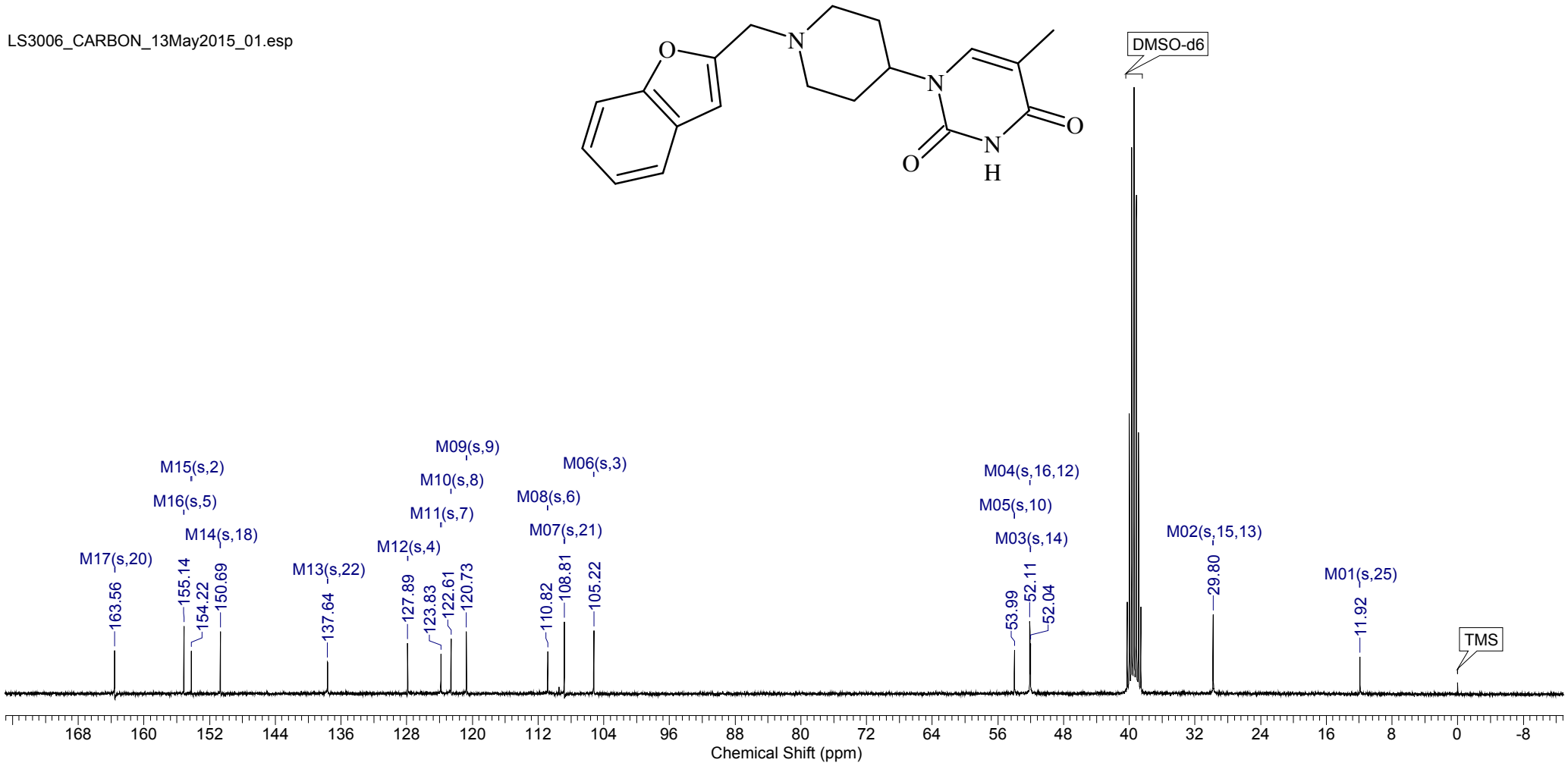
Compound 41

^{1}HNMR 300 MHz DMSO-d_6



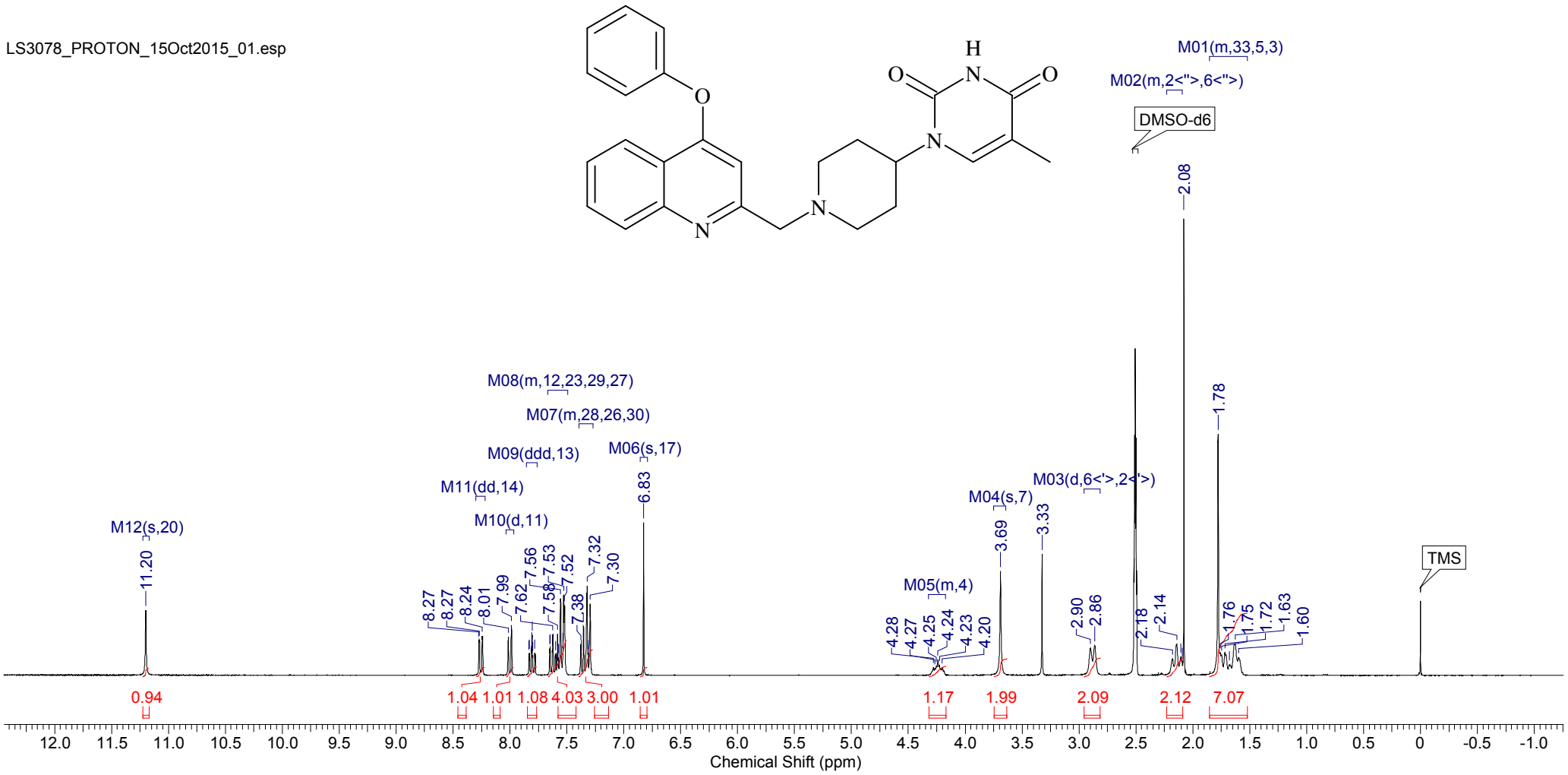
Compound 41

^{13}CNMR 75 MHz DMSO-d_6



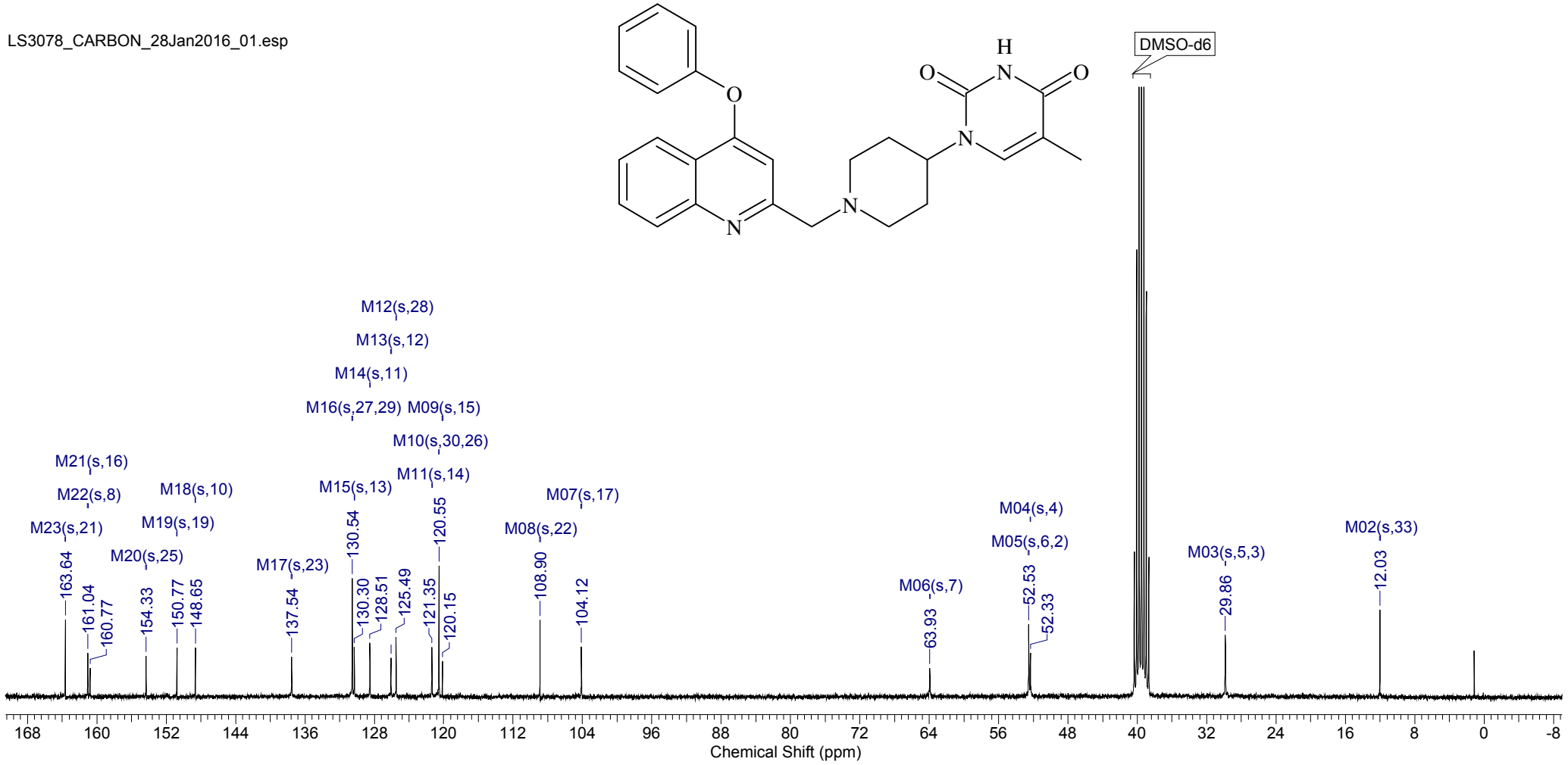
Compound 42

^{1}HNMR 300 MHz DMSO-d_6



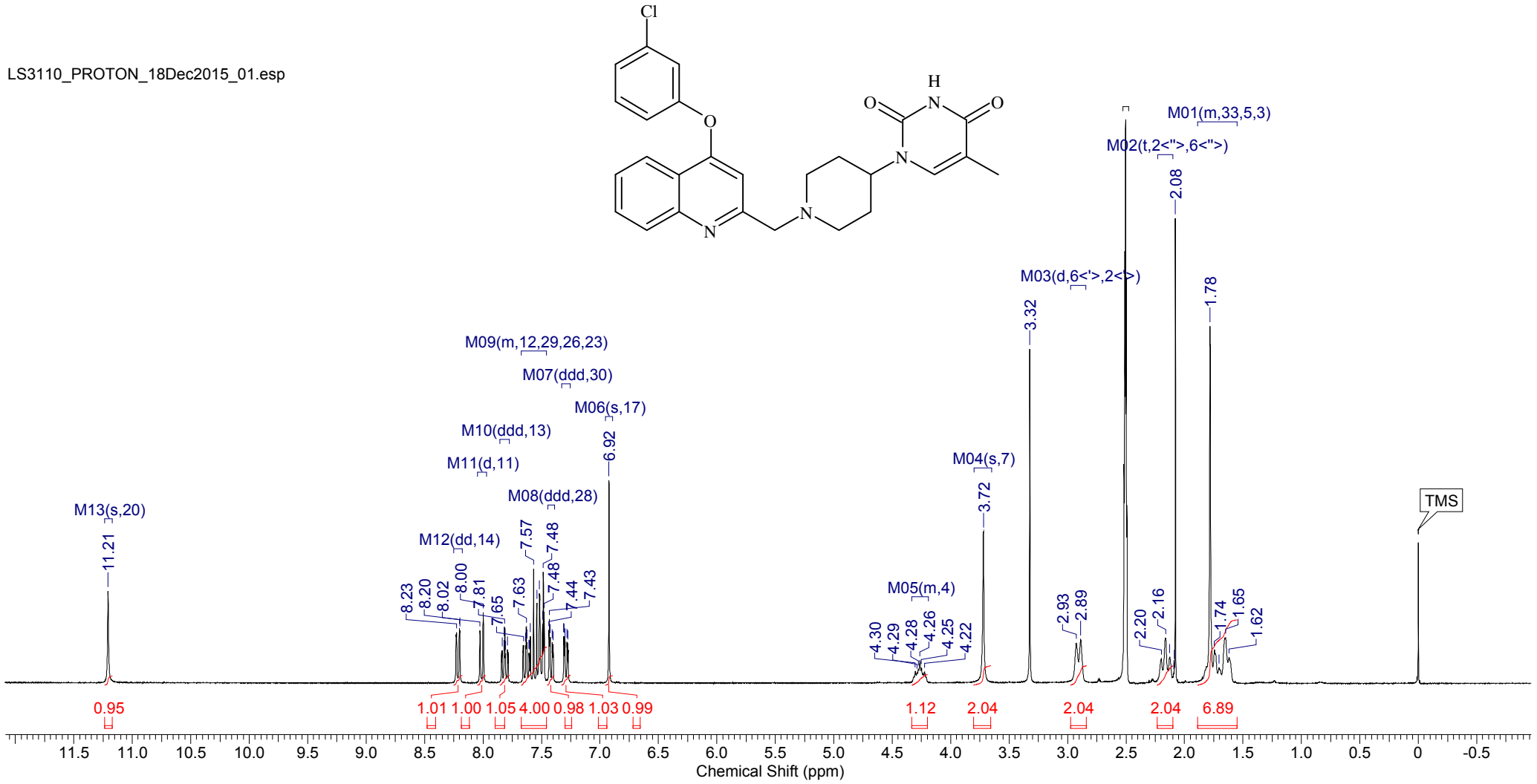
Compound 42

^{13}CNMR 75 MHz DMSO-d_6



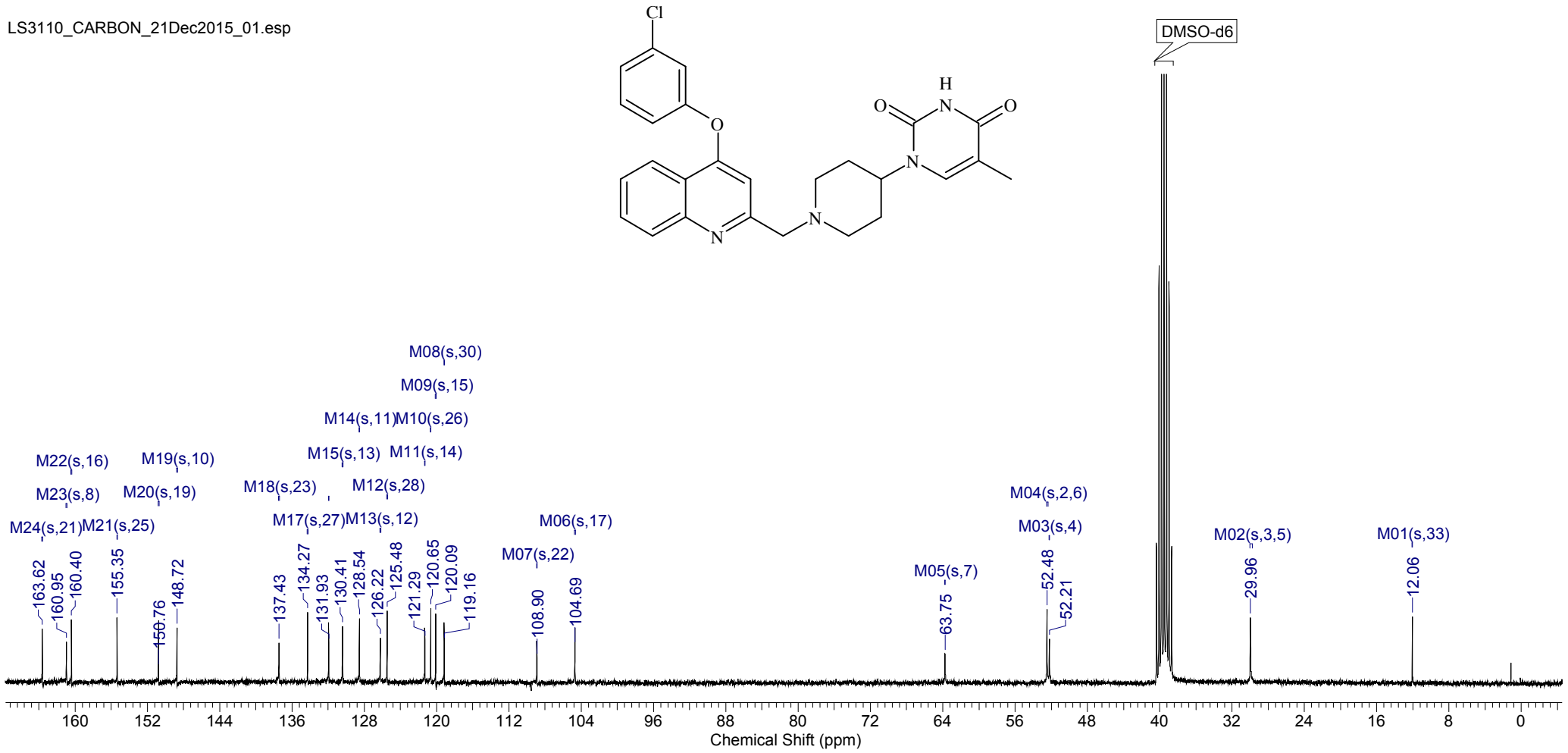
Compound 43

^{1}HNMR 300 MHz DMSO-d_6



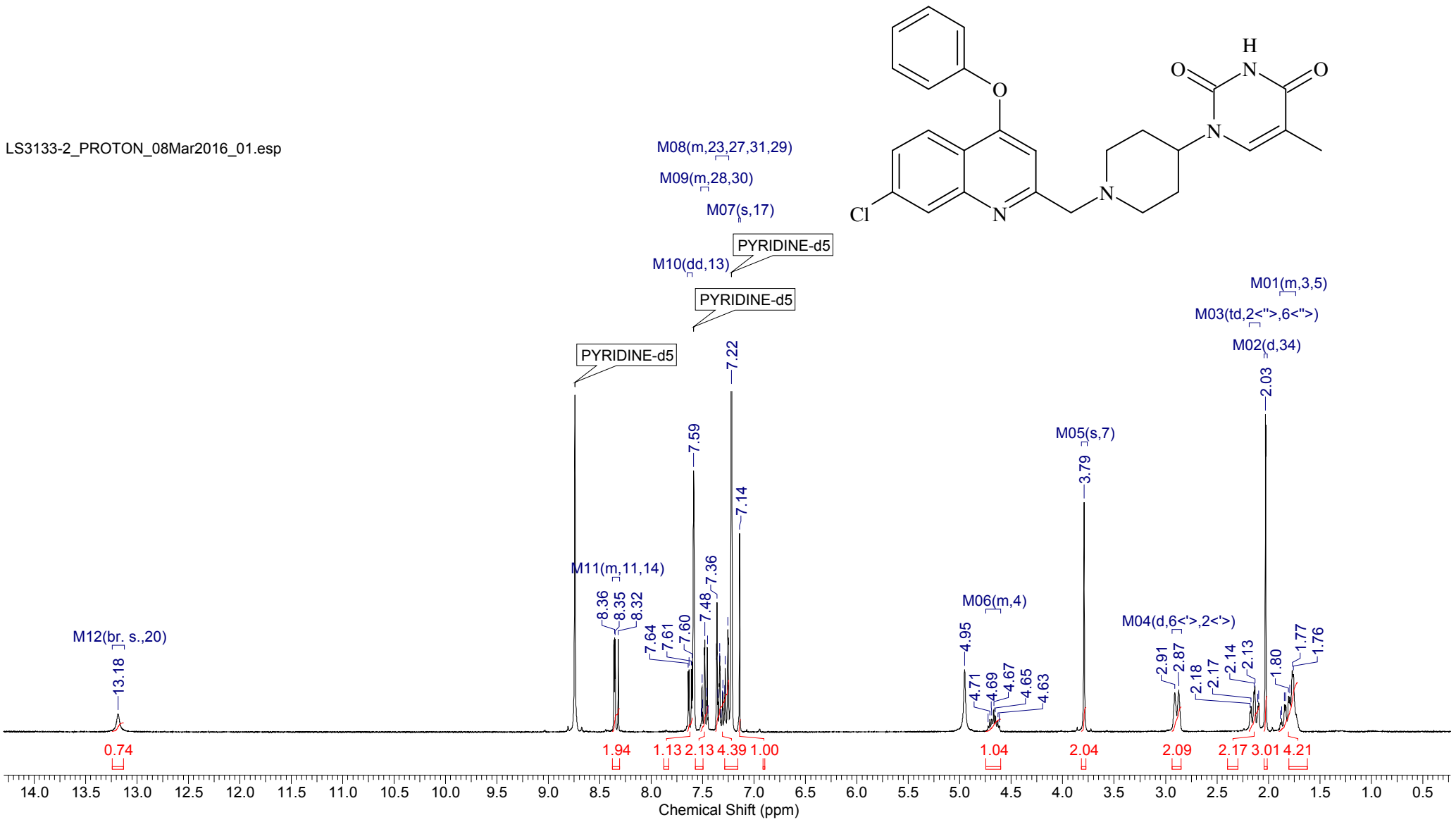
Compound 43

^{13}CNMR 75 MHz DMSO-d_6



Compound 44

^{1}HNMR 300 MHz PYRIDINE-d$_5$



Compound 44

^{13}CNMR 75 MHz PYRIDINE-d_5