

Targeting *Plasmodium falciparum* IspD in the Methyl-D-Erythritol Phosphate Pathway: Urea-Based Compounds with Nanomolar Potency on Target and Low-Micromolar Whole-cell Activity

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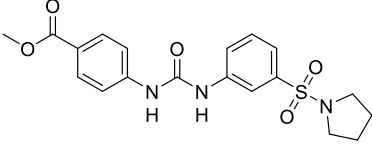
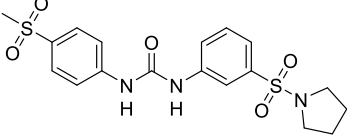
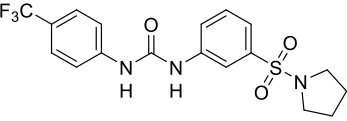
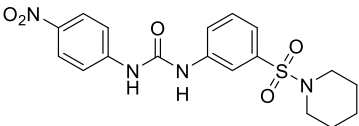
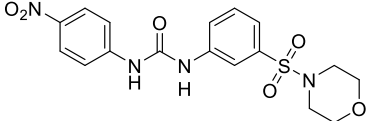
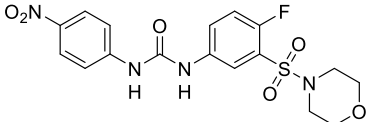
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S1. Activities of selected compounds against auxiliary enzymes

Table S1. Activities of selected compounds against auxiliary enzymes

#	Structure, R=	<i>PflspD</i> (IC ₅₀ nM) ^a	<i>EclspE</i> (IC ₅₀ μM) ^a	PK-LDH (IC ₅₀ μM) ^a
7		170 ± 20	>500	242 ± 72
9		330 ± 40	>500	>500
11		91 ± 19	73 ± 15	73±15
26		225 ± 20	49 ± 7	>500
27		600 ± 110	>500	104 ± 19
31		400 ± 100	75 ± 11	>500

^aAssays were performed in replicate as independent experiments ($n = 2$); values are shown as mean ± SD.

S2. Calibration curve for CDP-ME

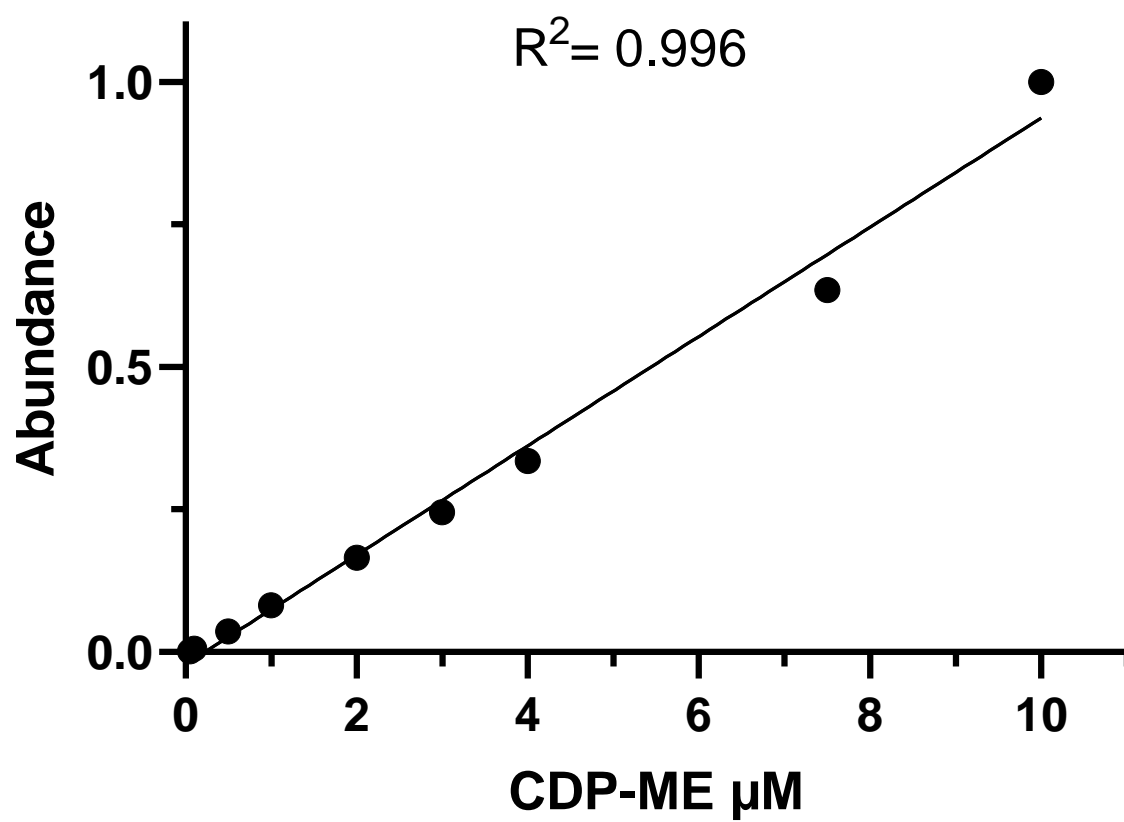


Figure S1. Calibration curve for CDP-ME detection. Concentrations ranging from 50 nM to 10 µM. The calculated area under the curve was converted to values ranging for 0 to 1.

S3. Determination kinetic parameters concerning CTP.

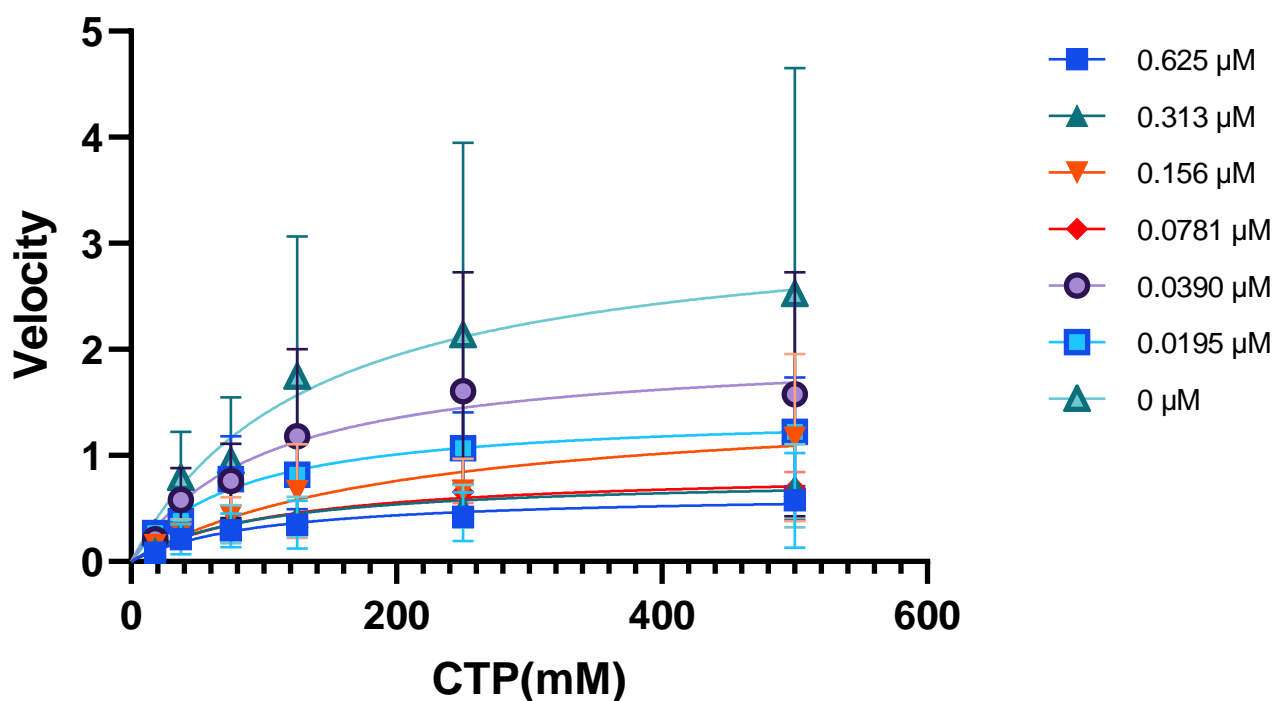


Figure S2. Kinetic parameters were determined concerning the CTP substrate. Each data point reflects the mean \pm SD obtained from a minimum of two-independent experiments.

Table S2. Kinetic parameters at different concentrations of compound 10 at varying CTP concentrations.

Compound 10 (μM)	0.625	0.313	0.156	0.0781	0.0390	0.0195	0.000
V_{max}	0.6496	0.8006	1.537	0.8674	2.021	1.418	3.253
K_m	97.89	97.23	204.4	111.7	98.52	80.79	134.4

S4. Determination kinetic parameters concerning MEP.

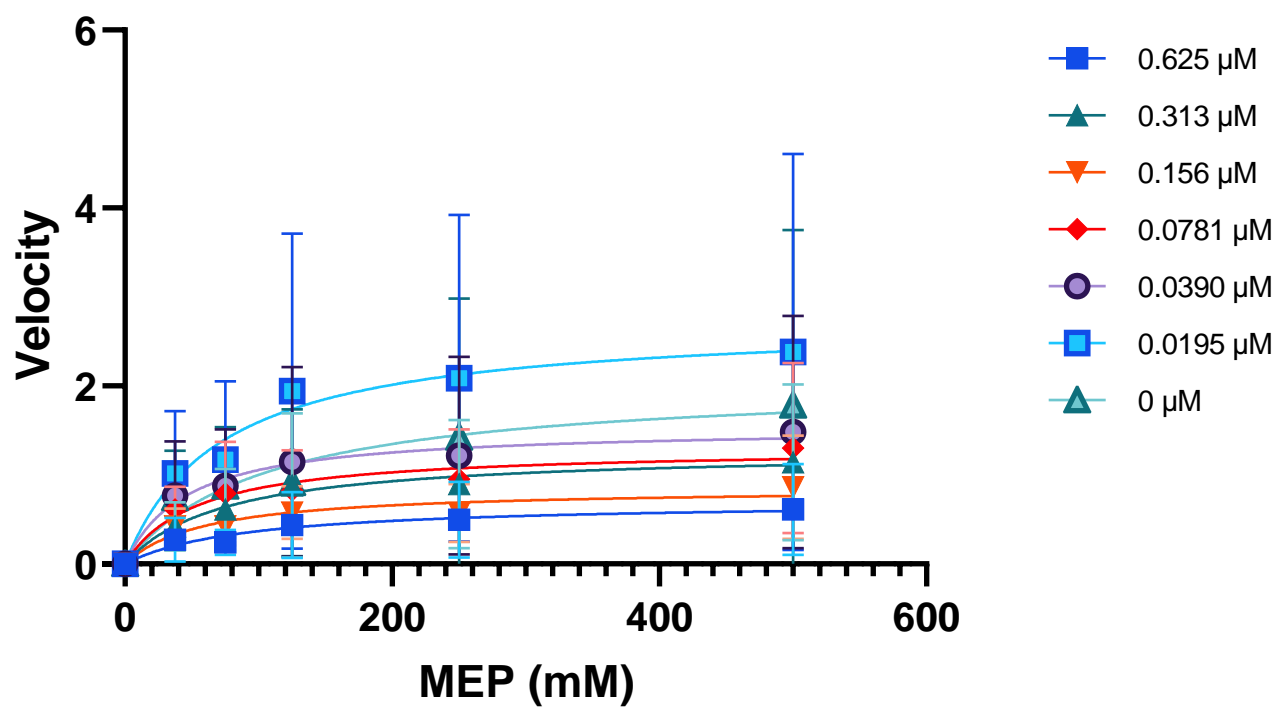


Figure S3. Kinetic parameters were determined concerning the CTP substrate. Each data point reflects the mean \pm SD obtained from a minimum of two-independent experiments.

Table S3. Kinetic parameters at different concentrations of compound 10 at varying MEP concentrations

Compound 10 (μM)	0.625	0.313	0.156	0.0781	0.0390	0.0195	0.000
V_{max}	0.7037	1.277	0.8556	1.305	1.541	2.734	2.064
K_m	90.58	74.61	59.79	53.57	46.05	71.50	106.2

S5. LC-MS chromatograms of MEP, 4-methyl-1-oxo-1-(p-tolylamino)pentane-2-sulfonic acid and CDP-ME respectively

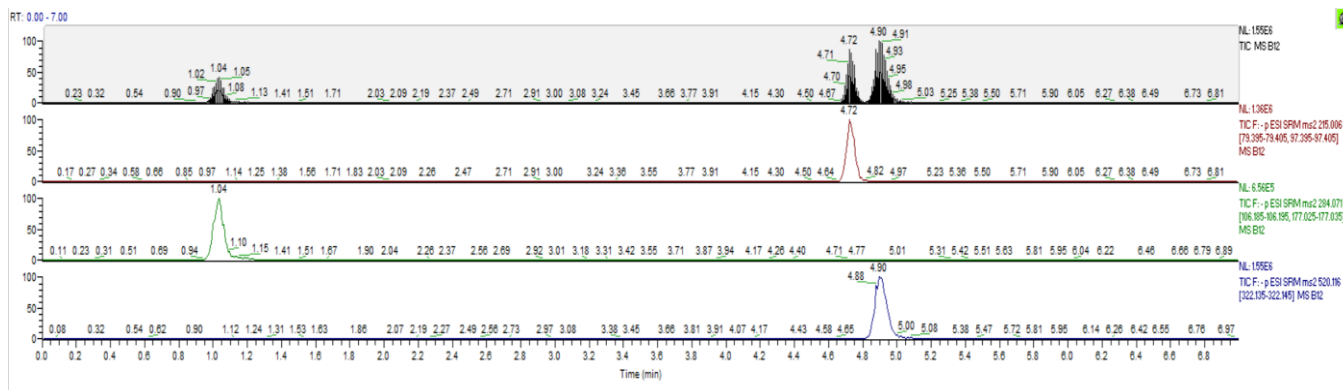


Figure S4. LC-MS chromatogram of MEP (second, red), 4-methyl-1-oxo-1-(p-tolylamino)pentane-2-sulfonic acid (third, green) and CDP-ME (fourth, blue) respectively.

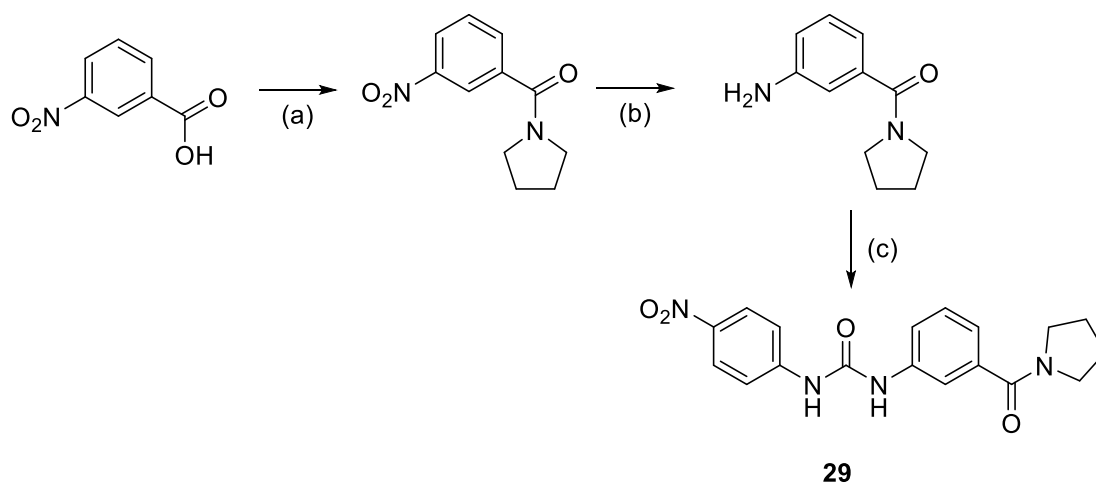
S6. Q1 and Q3 masses for glipizide, 10 and 28

Table S4. Q1 and Q3 masses for glipizide, 10 and 28

ID	Q1 Mass [Da]	Q3 Mass [Da]	time [msec]	CE [volts]	CXP [volts]	DP [volts]
10	371.022	355.0	30	15	20	101
	371.022	253.0	30	25	28	101
10	392.998	23.2	30	69	10	96
		353.9	30	13	20	96
		252.2	30	19	22	96
28	395.952	136.9	30	-16	-11	-40
		231.9	30	-34	-21	-40
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glipizide	443.936	319.100	150	-26	-21	-66
glipizide	443.936	170.100	150	-40	-7	-66

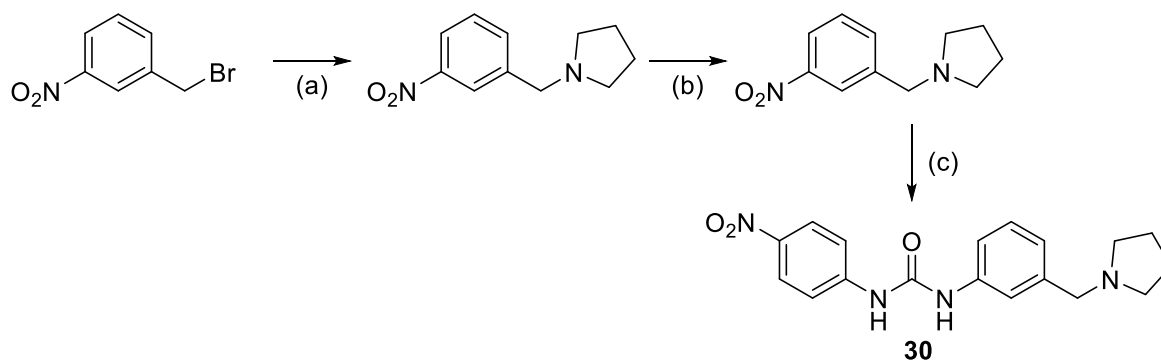
S7. Supplementary reaction schemes

Scheme S1. Synthetic route for the synthesis of 29



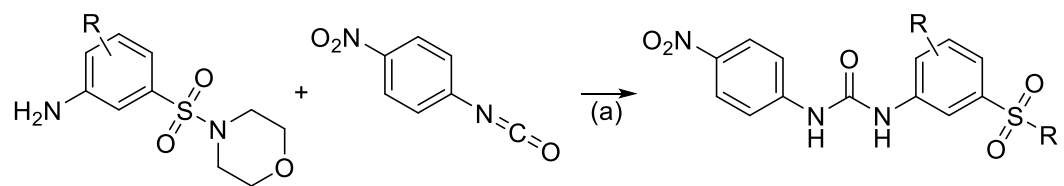
^aReagents and conditions: (a) pyrrolidine, trimethylamine, propanephosphonic acid anhydride, room temperature, 2 h; (b) NH_4Cl (166 mM in water), Fe powder, 80 °C, 2,5 h; (c) 1-isocyanato-4-nitrobenzene, room temperature, 1 h, 9% yield over all steps.

Scheme S2. Synthetic route for the synthesis of 30



^aReagents and conditions: (a) pyrrolidine, trimethylamine, room temperature, 2 h; (b) NH_4Cl (166 mM in water), Fe powder, 80 °C, 2,5 h; (c) 1-isocyanato-4-nitrobenzene, room temperature, 1 h, 9% yield over all steps.

Scheme S3. General reaction scheme for the synthesis of compounds 31–33^a.



^aReagents and reactions conditions: 1-isocyanato-4-nitrobenzene, DMF, room temperature, overnight. 7–23% yield.

S8. Activities of representative compounds against different bacterial pathogens

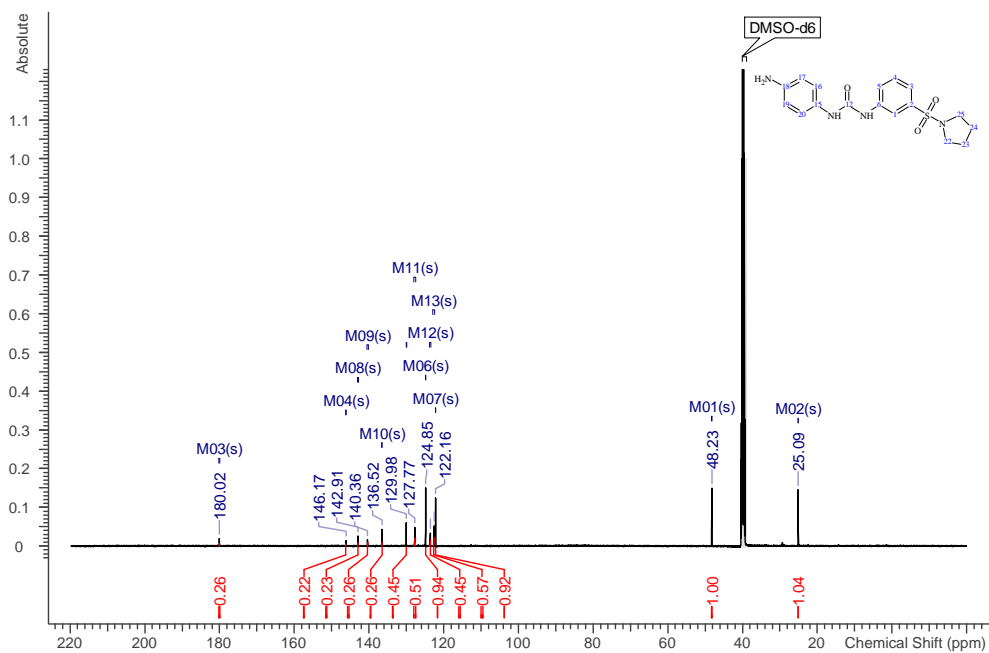
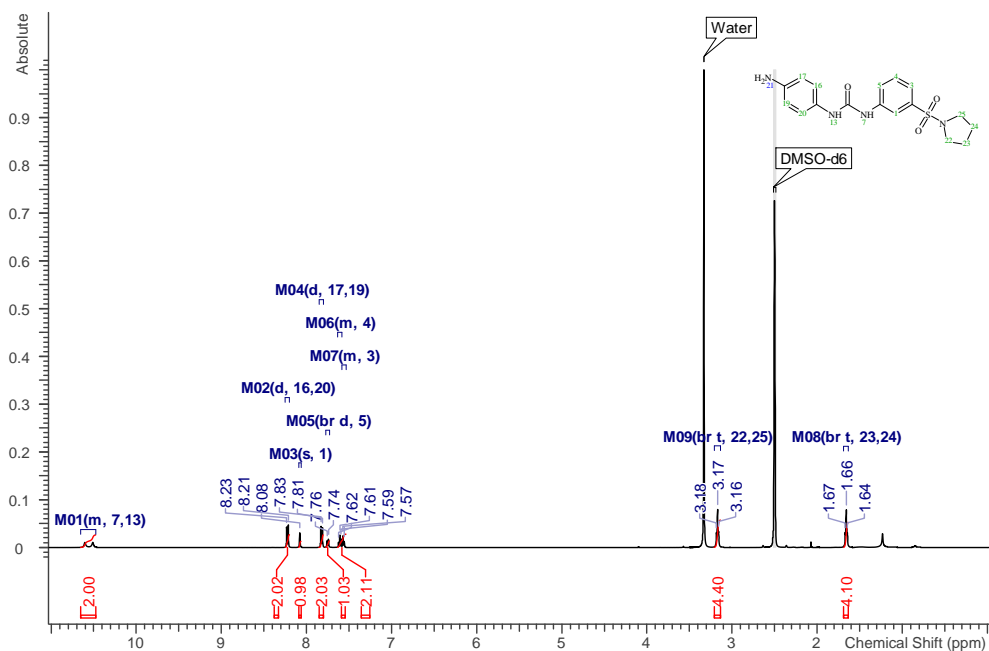
Table S5 Activities of representative compounds against different bacterial pathogens

#	5	8	10	28
Inhibition of <i>E. coli</i> K12 growth (at 100 μ M)	45 \pm 6%	14 \pm 5%	46 \pm 4%	No inhibition
Inhibition of <i>E. coli</i> <i>acrB</i> growth (at 100 μ M)	Full inhibition	71 \pm 6%	Full inhibition	No inhibition
MIC 90 (growth inhibition) of <i>Mtb</i> H37Rv <i>mCherry</i> 10 strain	n.a	n.d	64 μ M	n.d

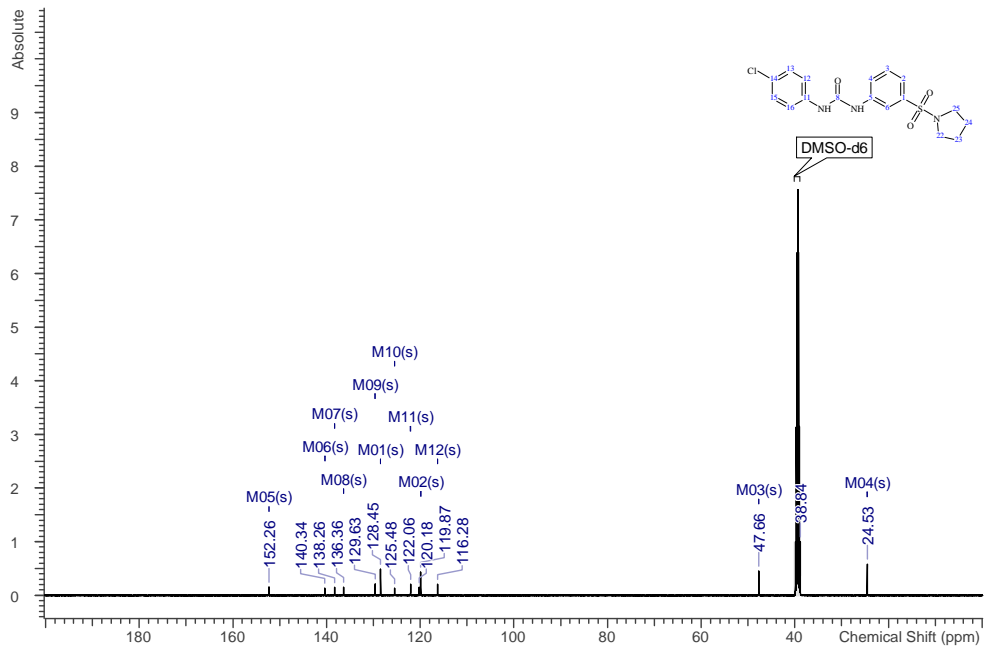
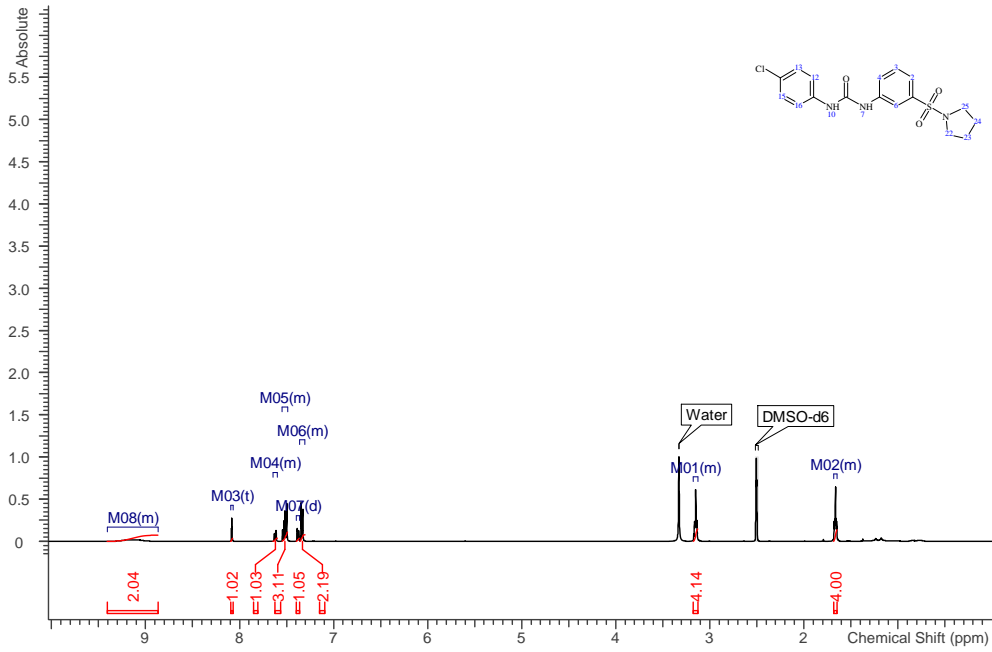
^aAssays were performed in replicate as independent experiments ($n \geq 2$); values are shown as mean \pm SD. values are shown as mean \pm SD. n.a. indicates the absence of activity

S9. ¹H NMR and ¹³C NMR spectra as well as LC-MS and purity traces of Compounds 4–40

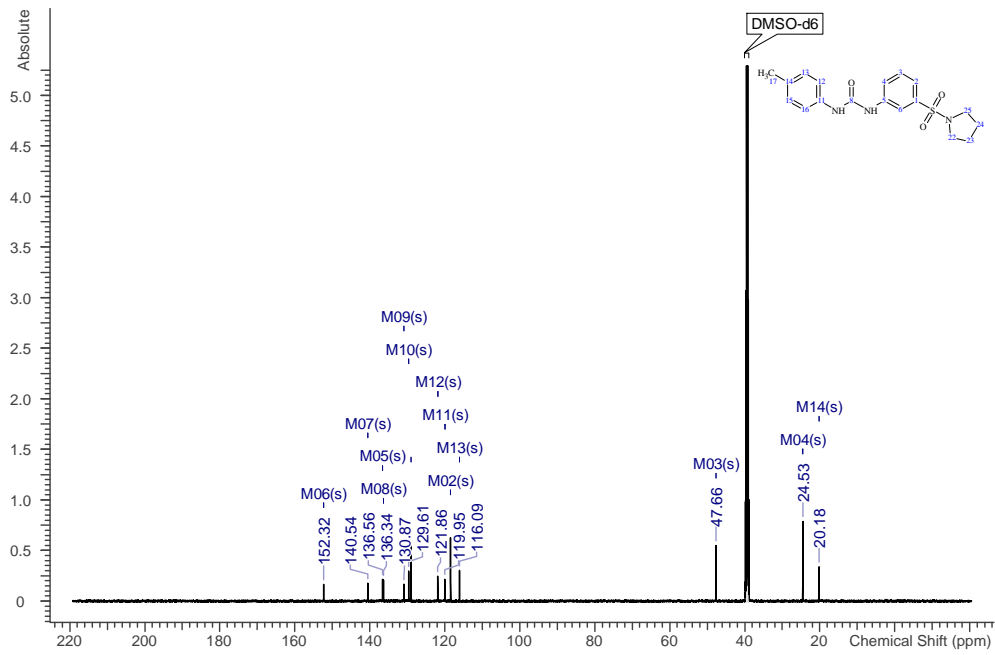
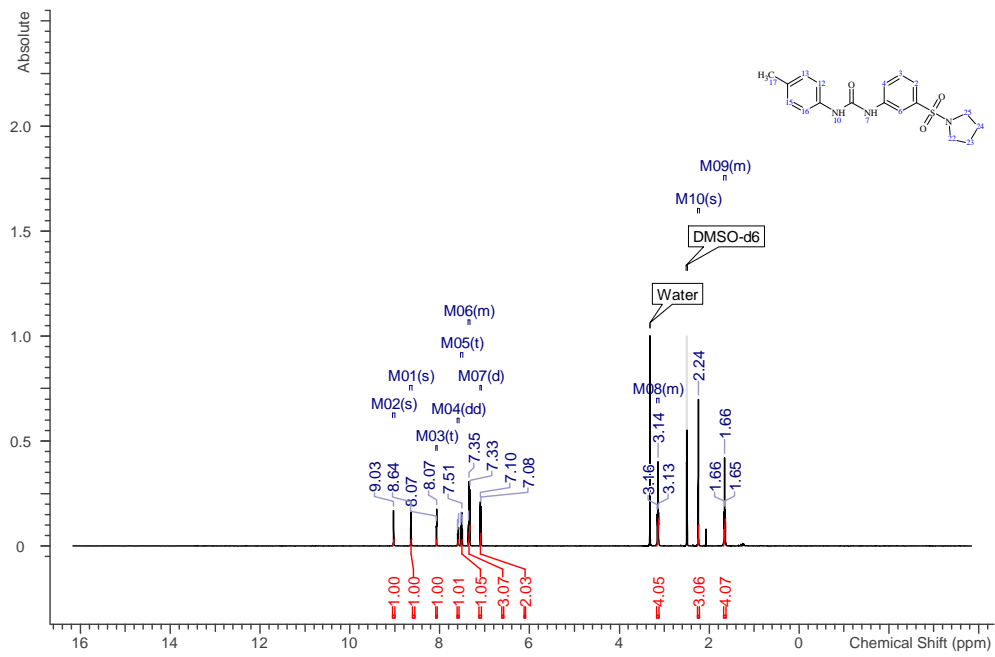
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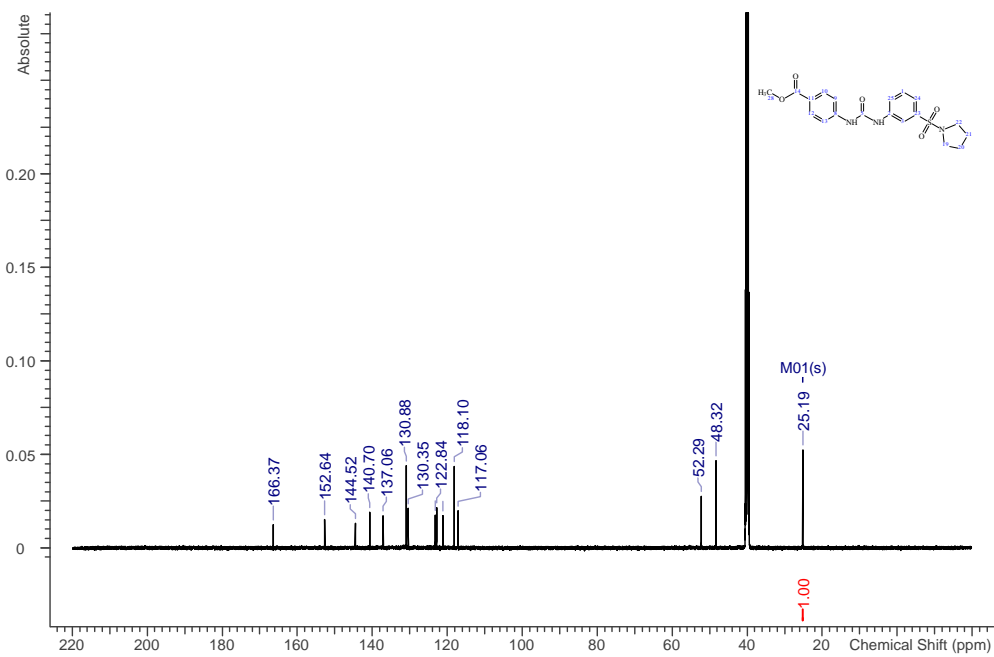
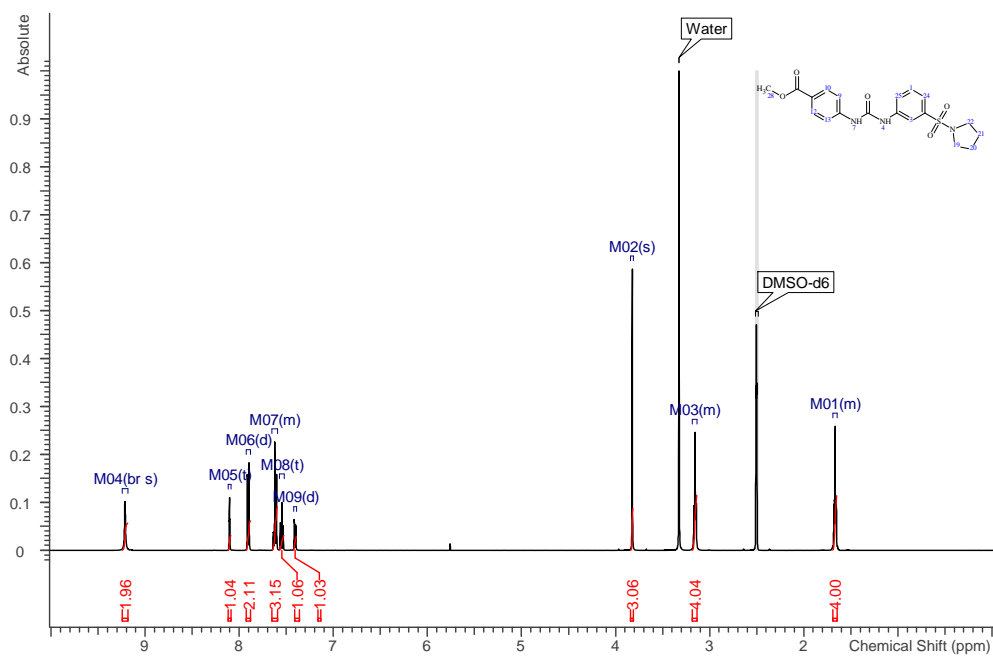
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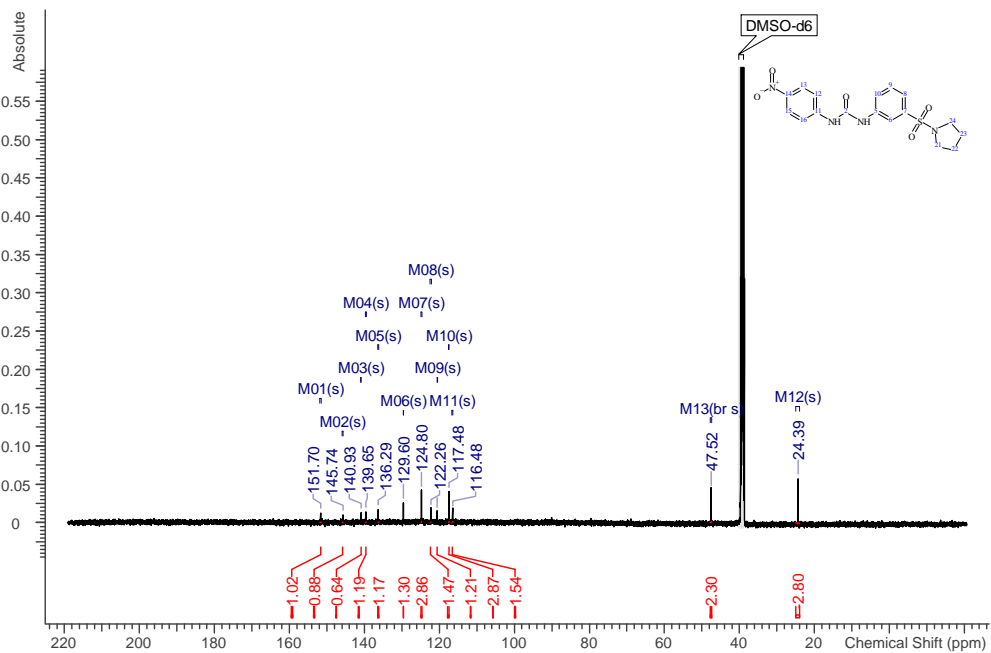
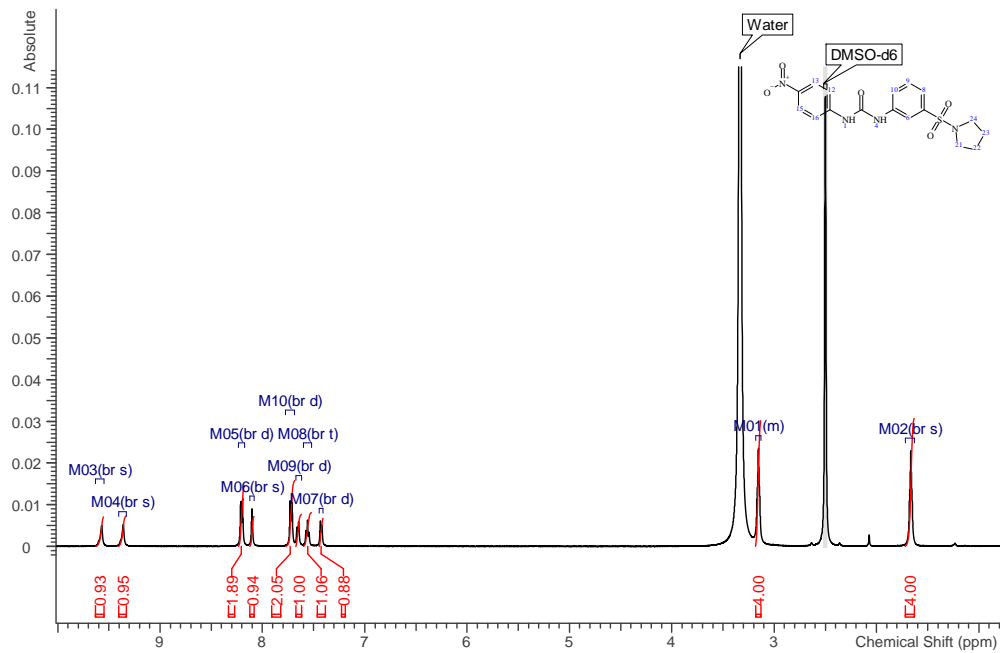


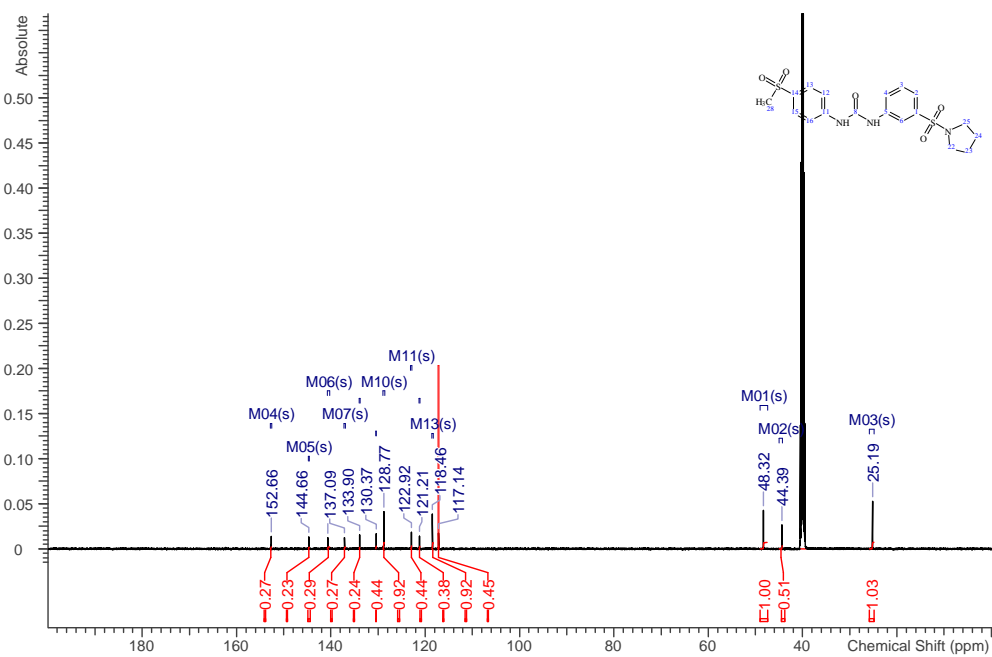
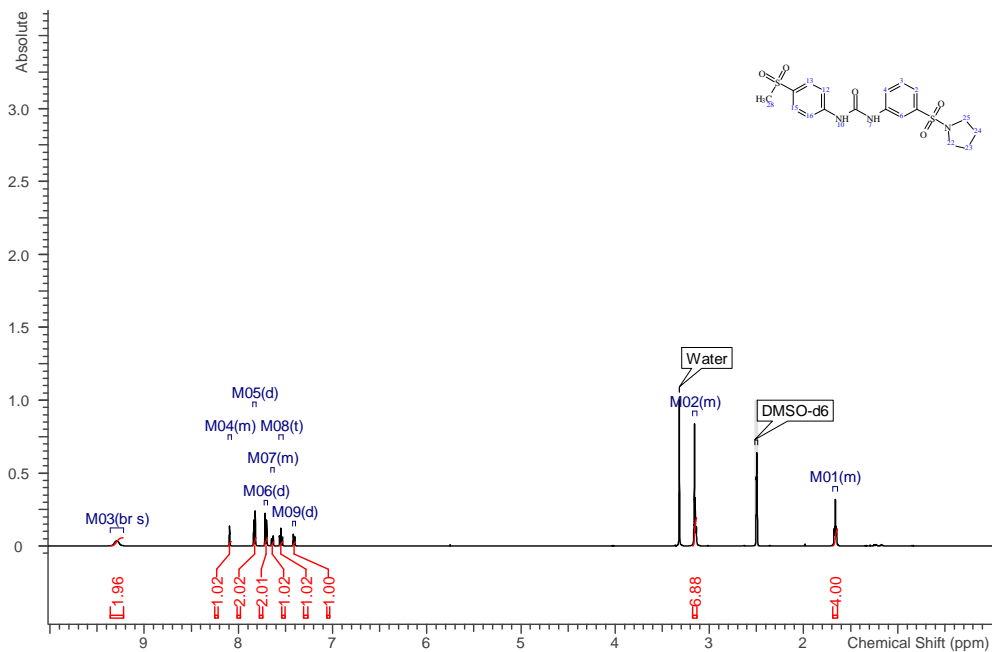
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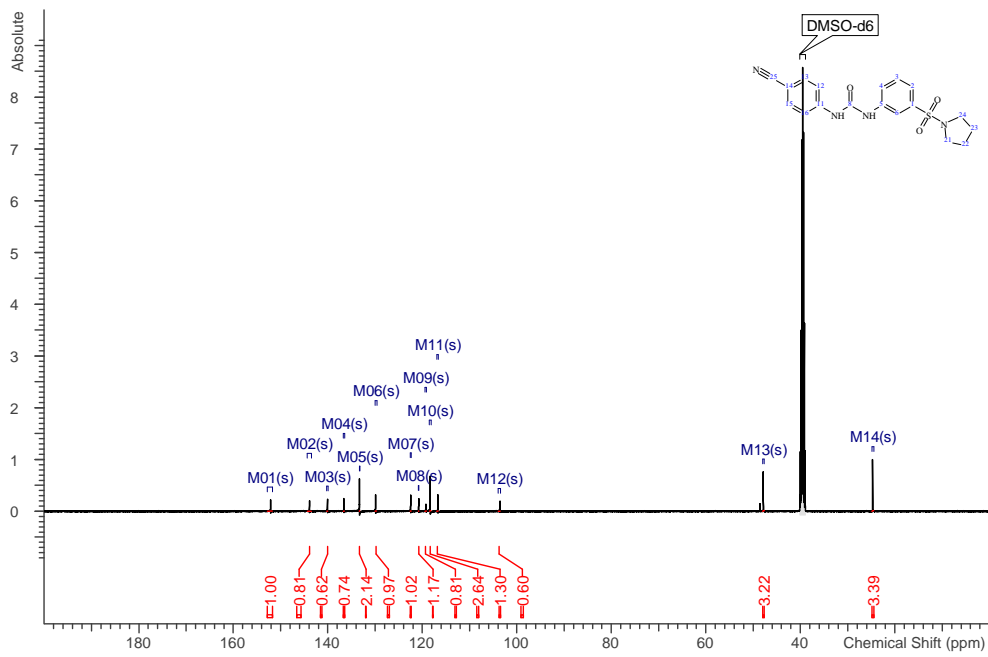
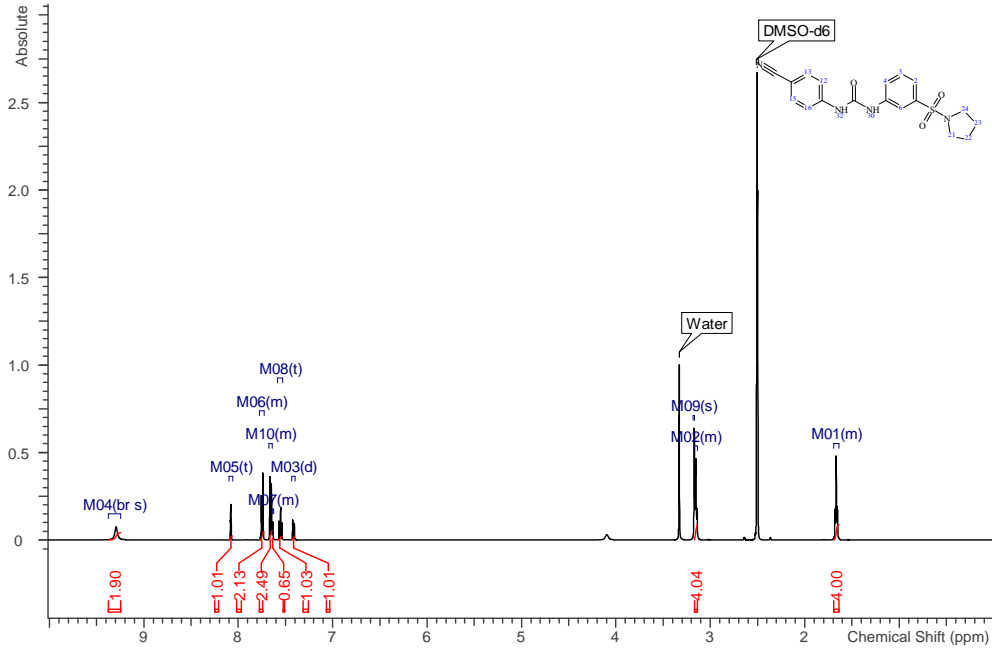
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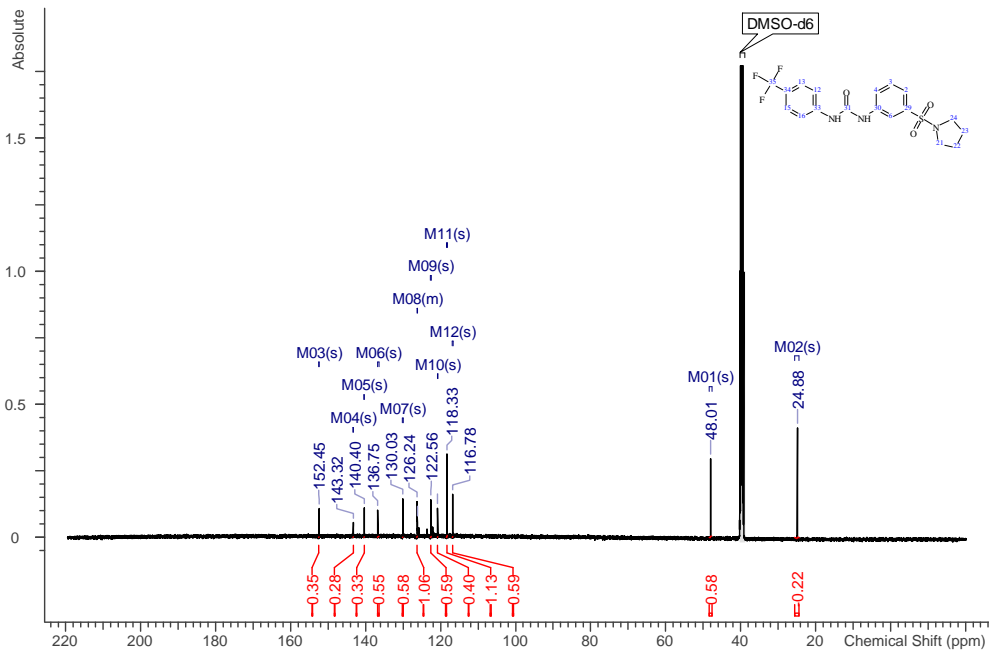
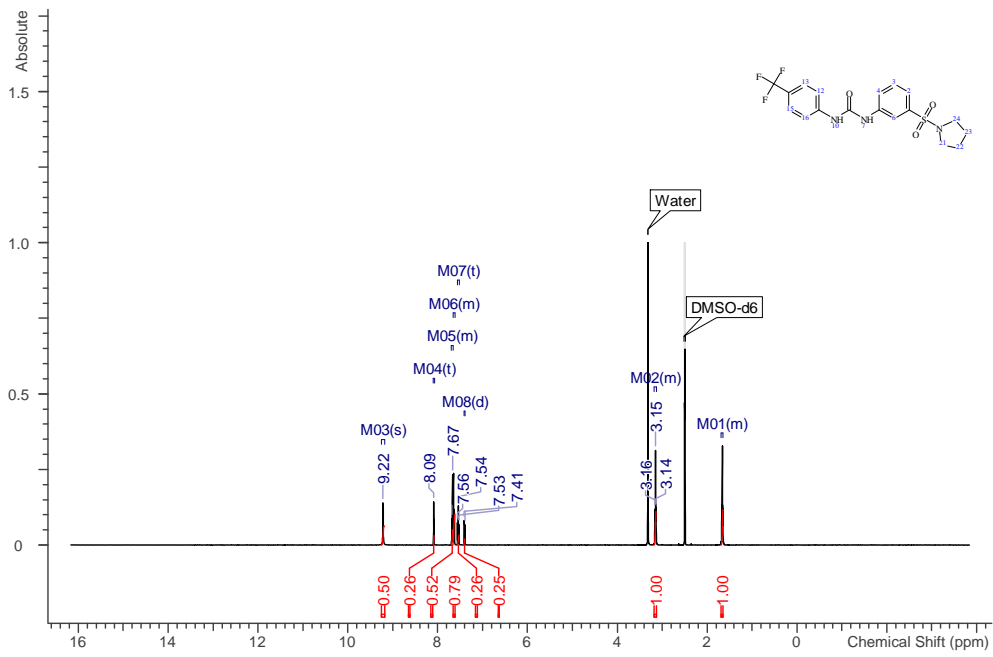




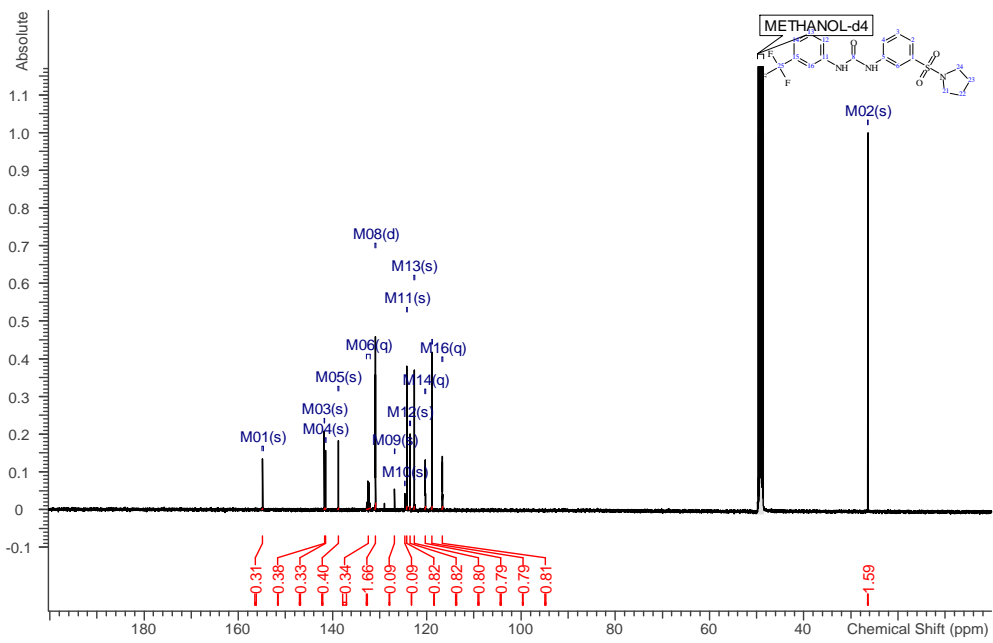
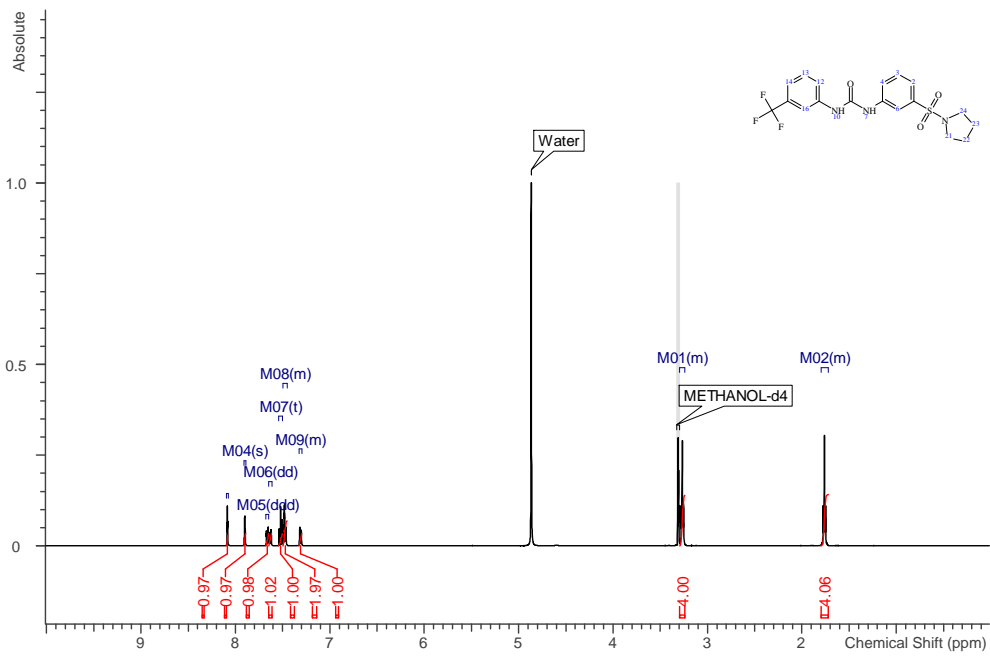
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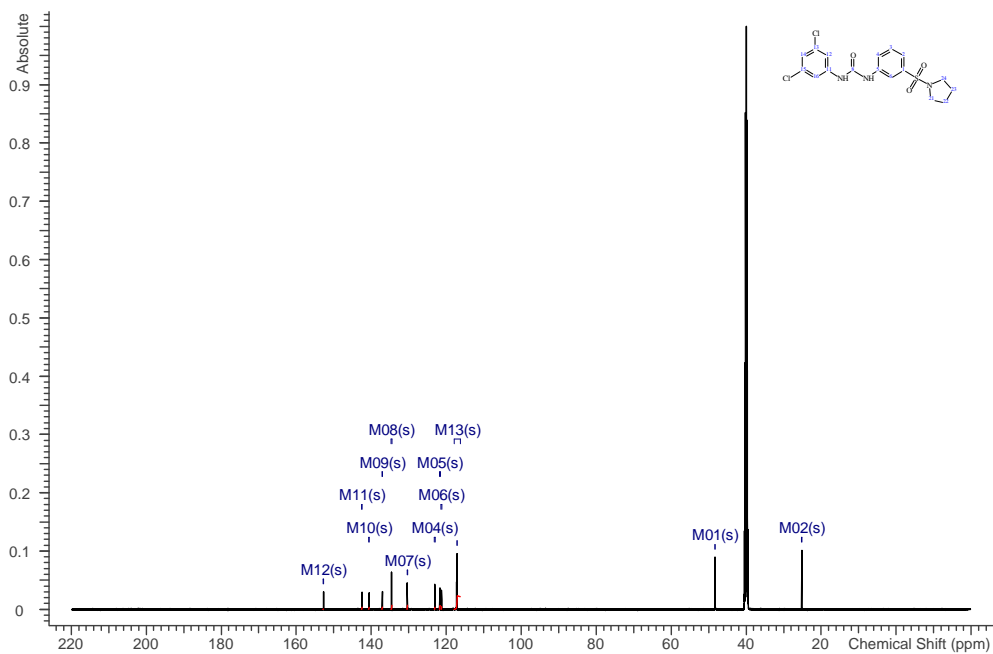
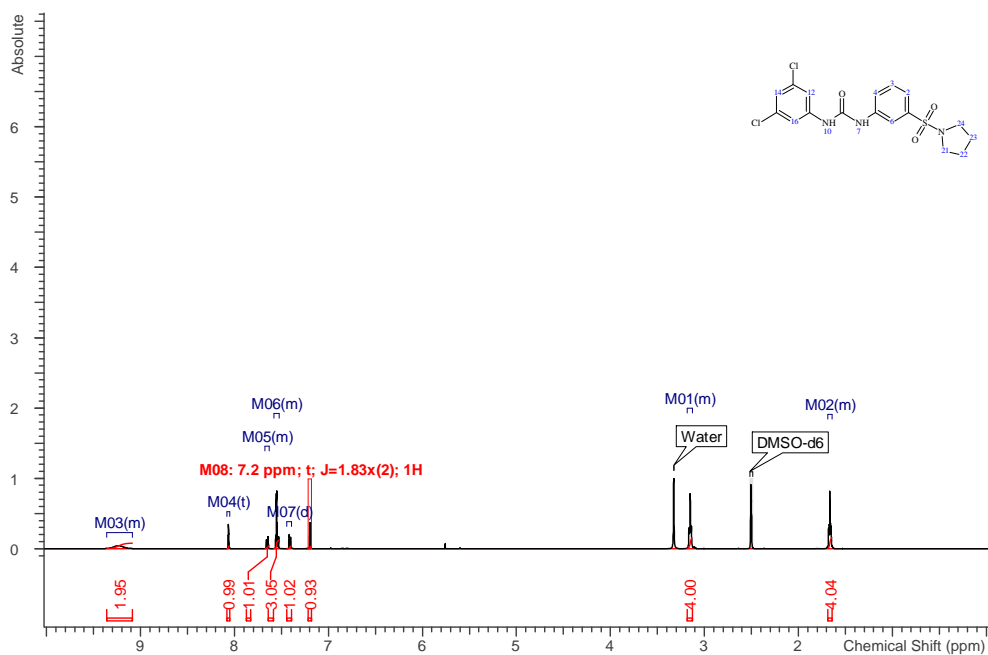
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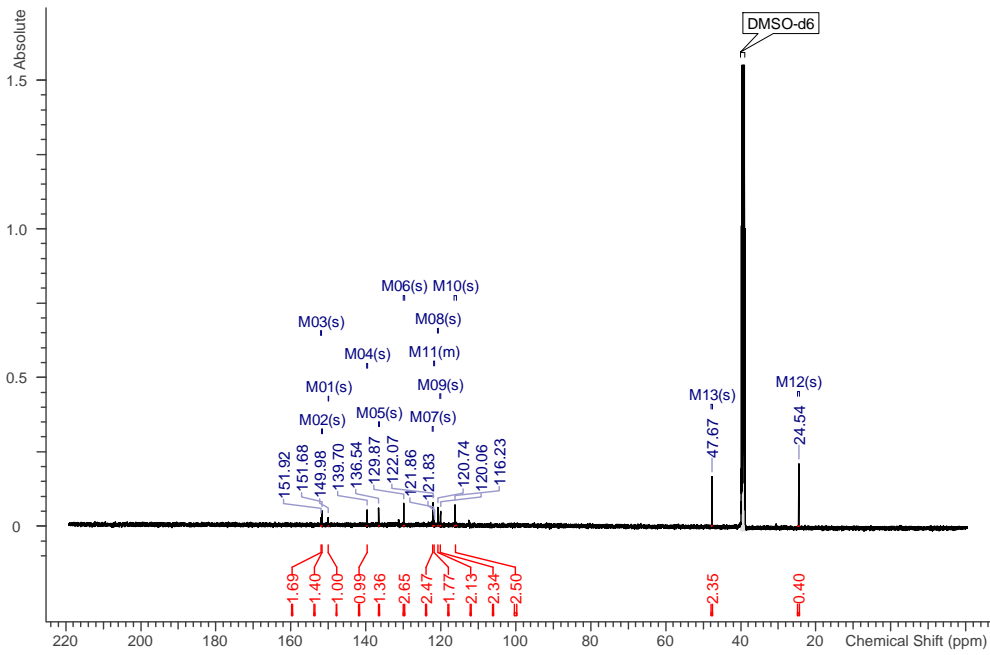
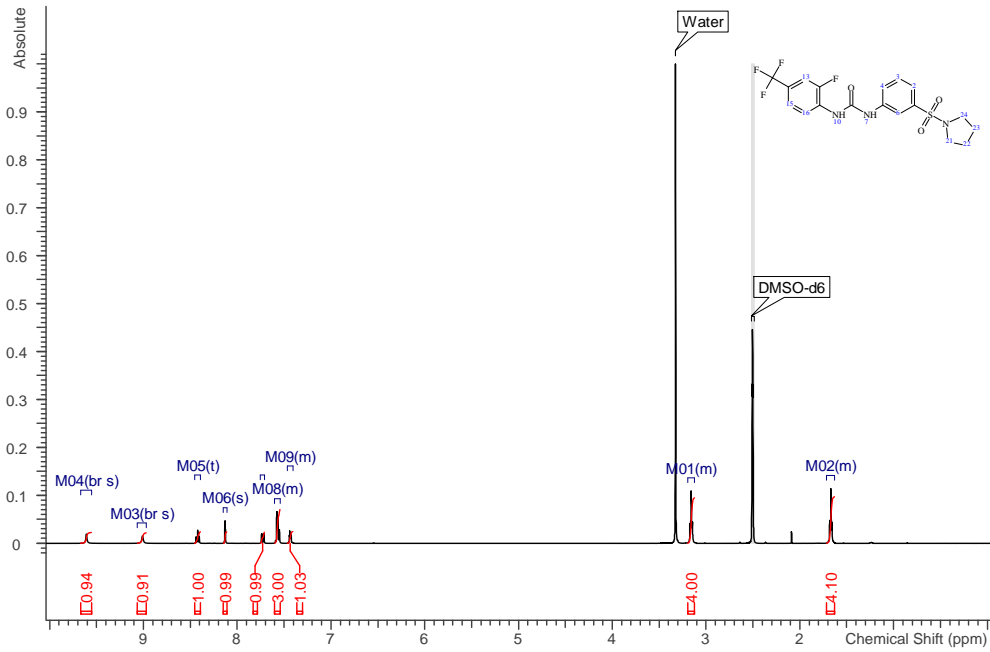


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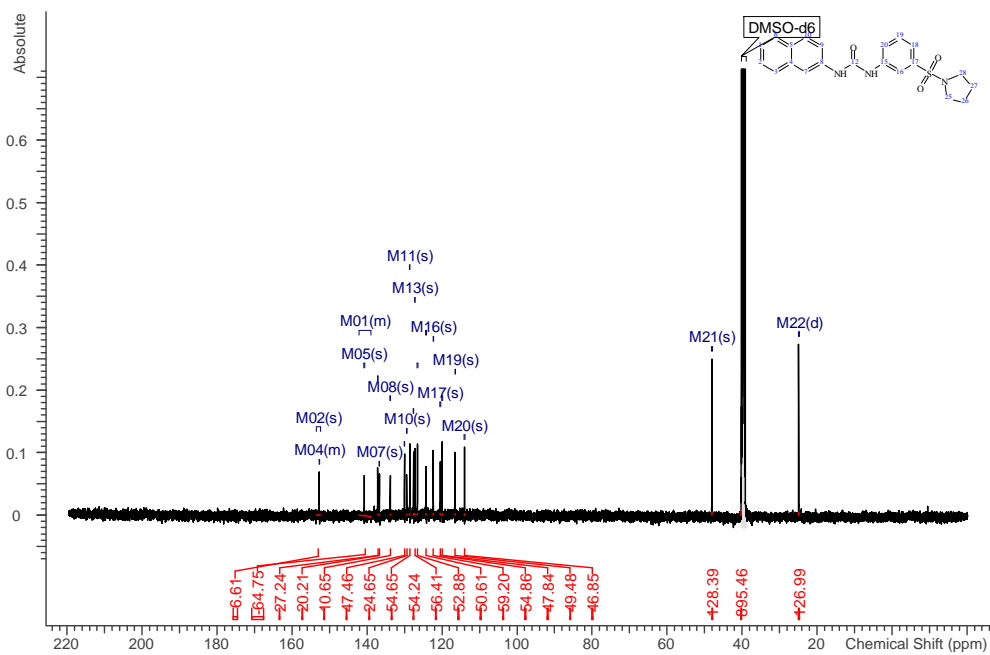
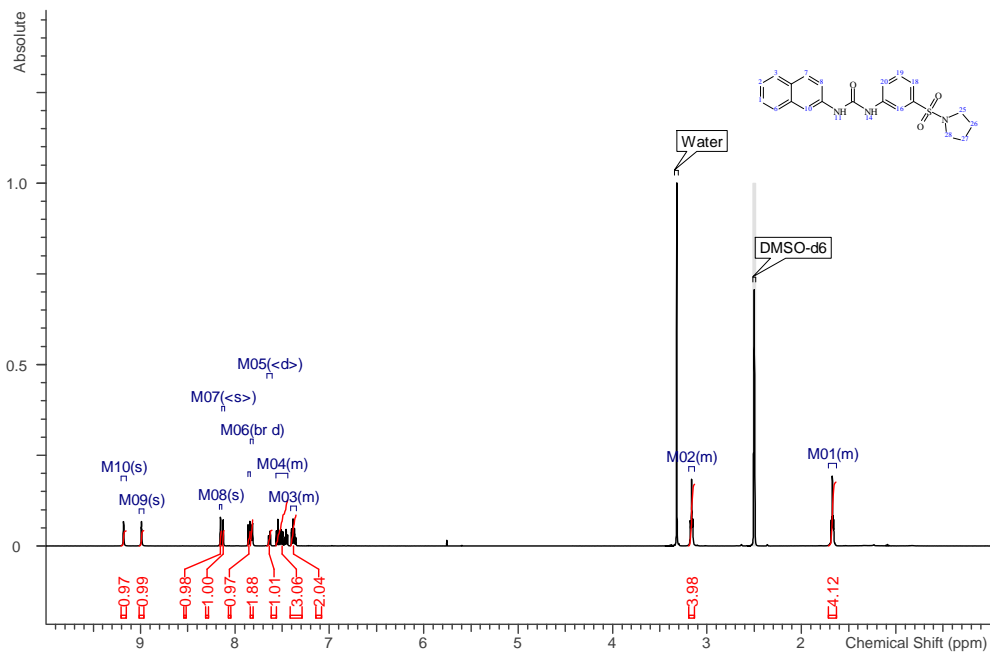


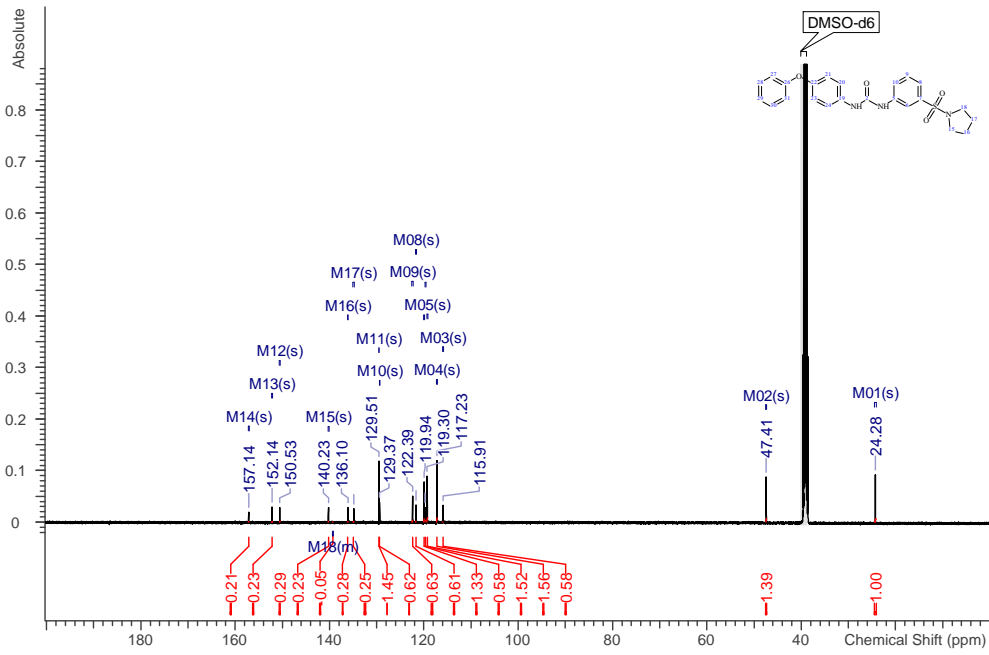
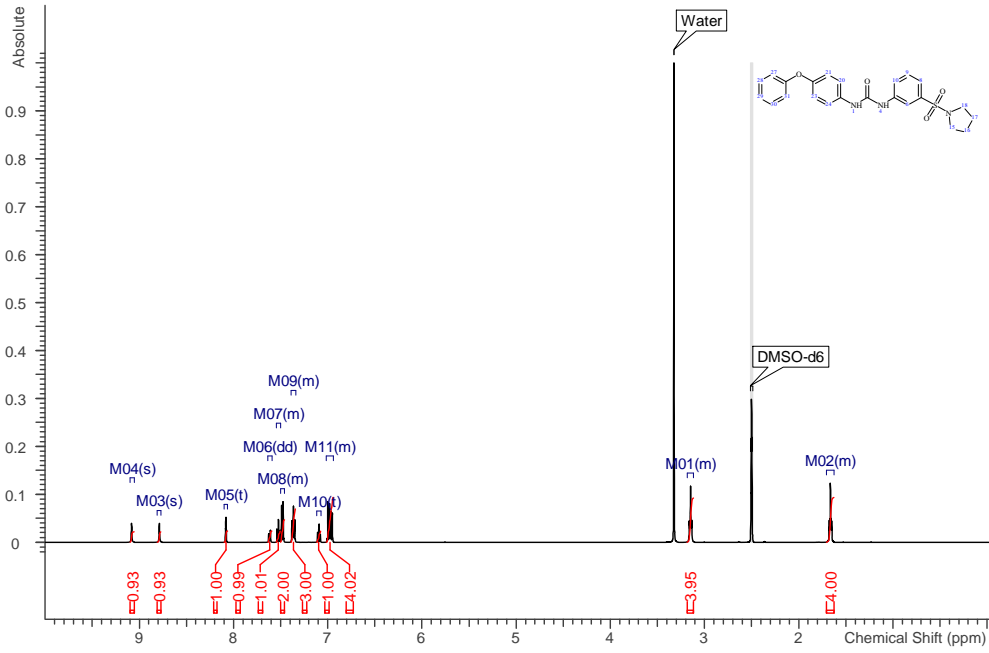
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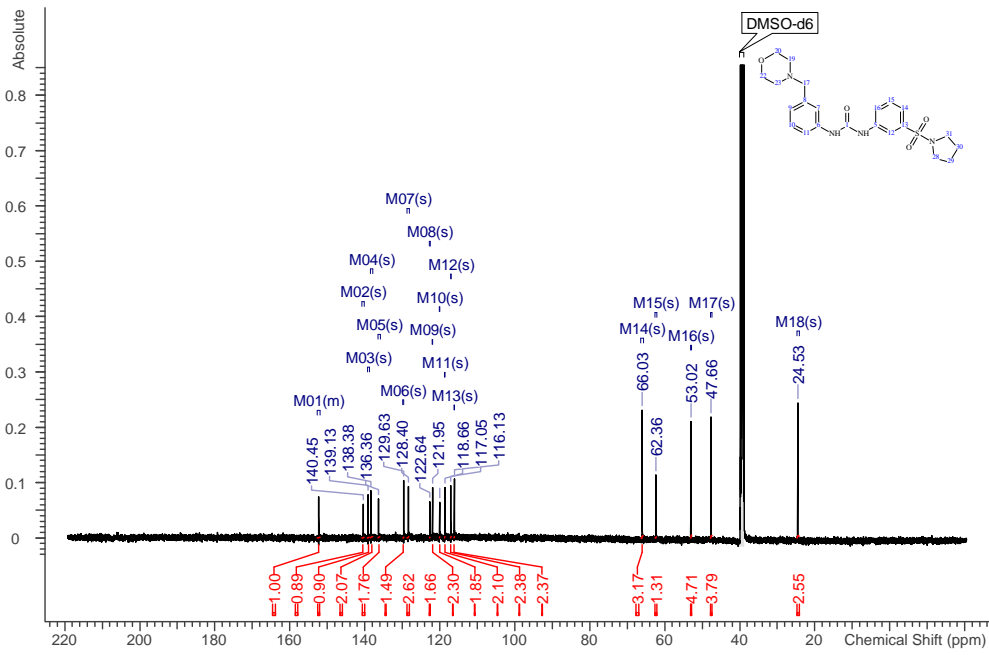
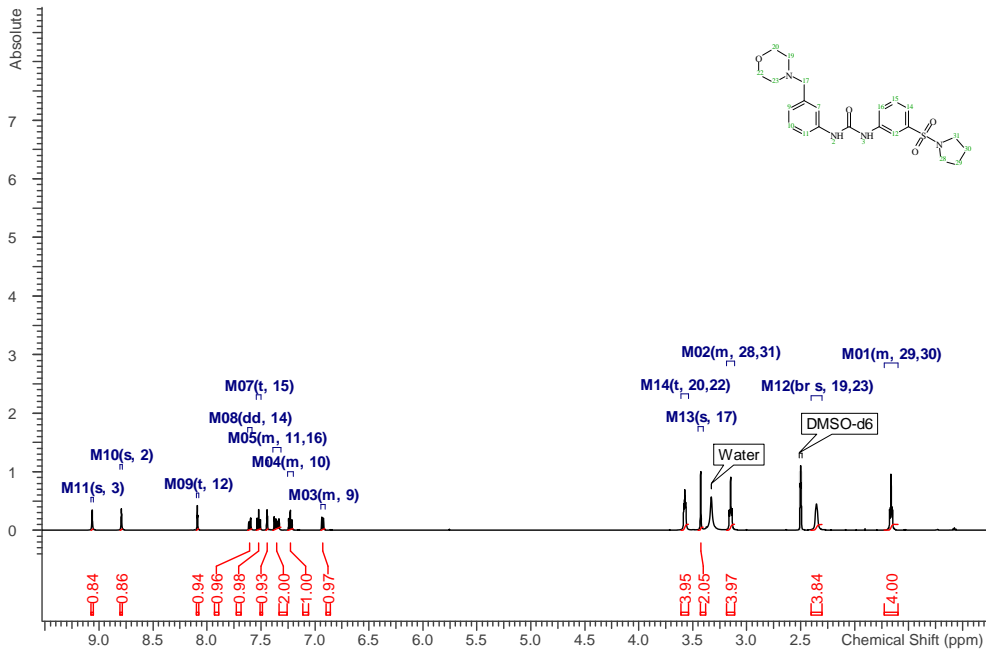




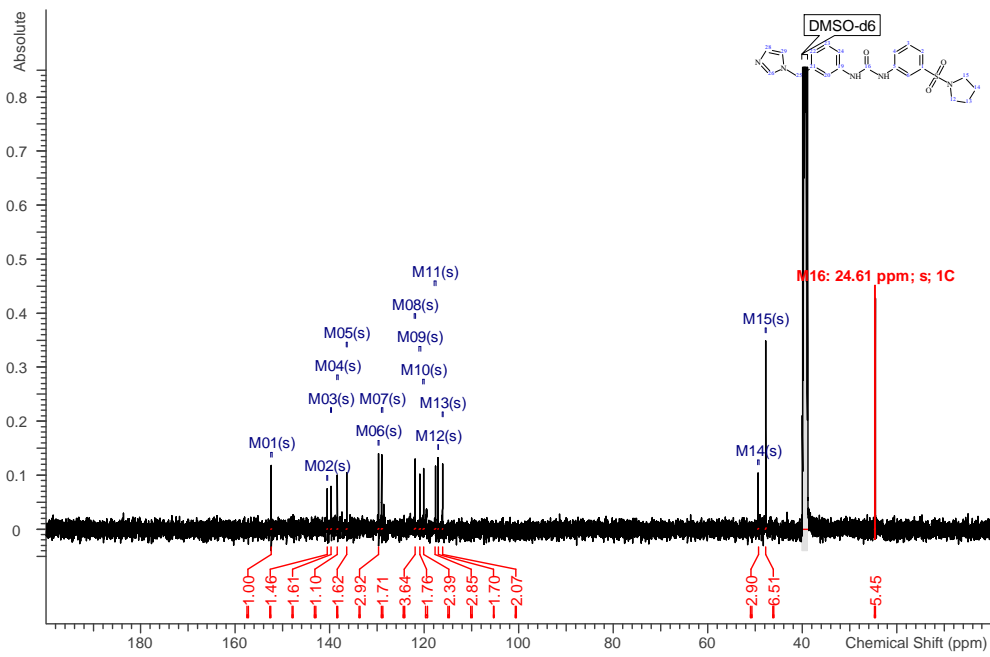
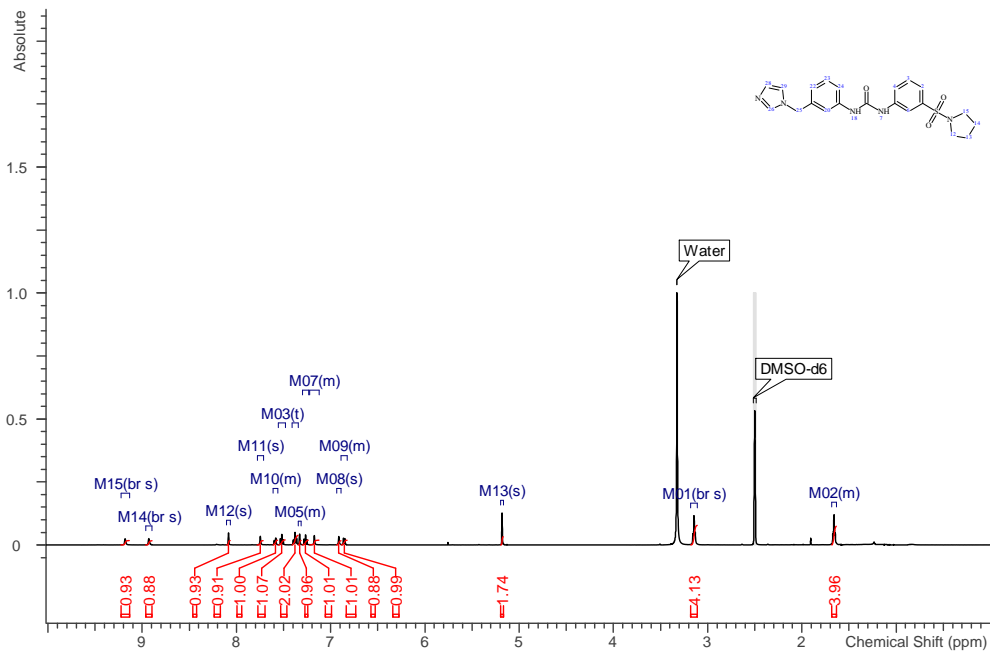
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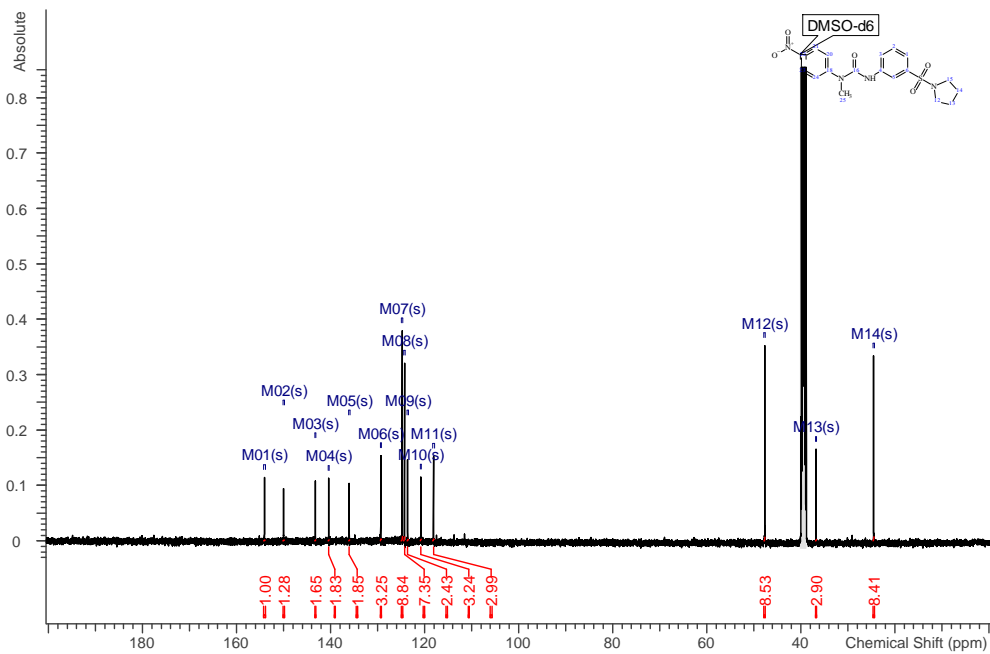
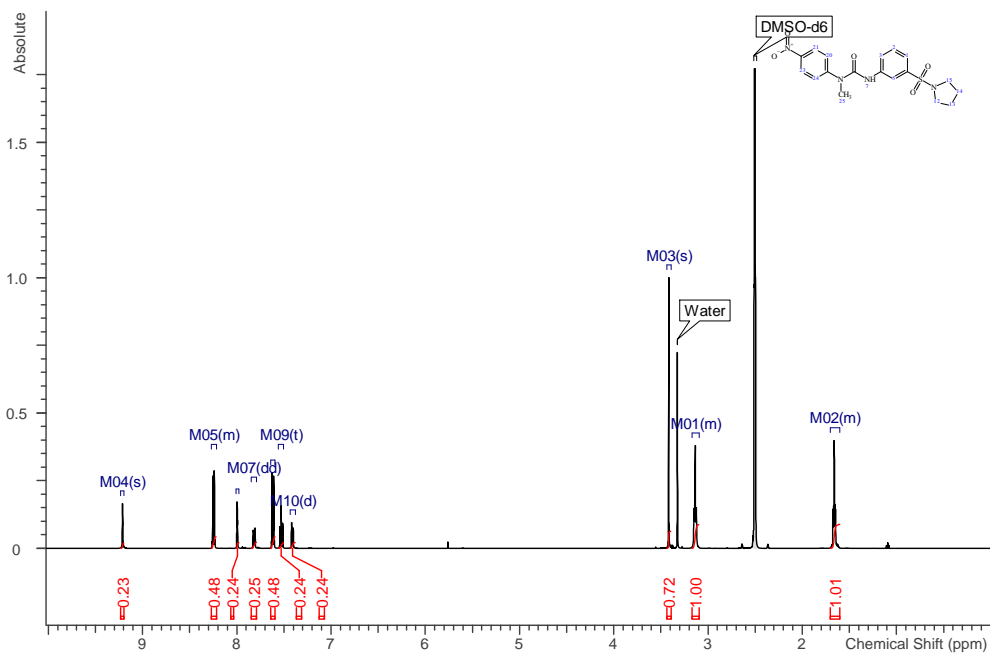




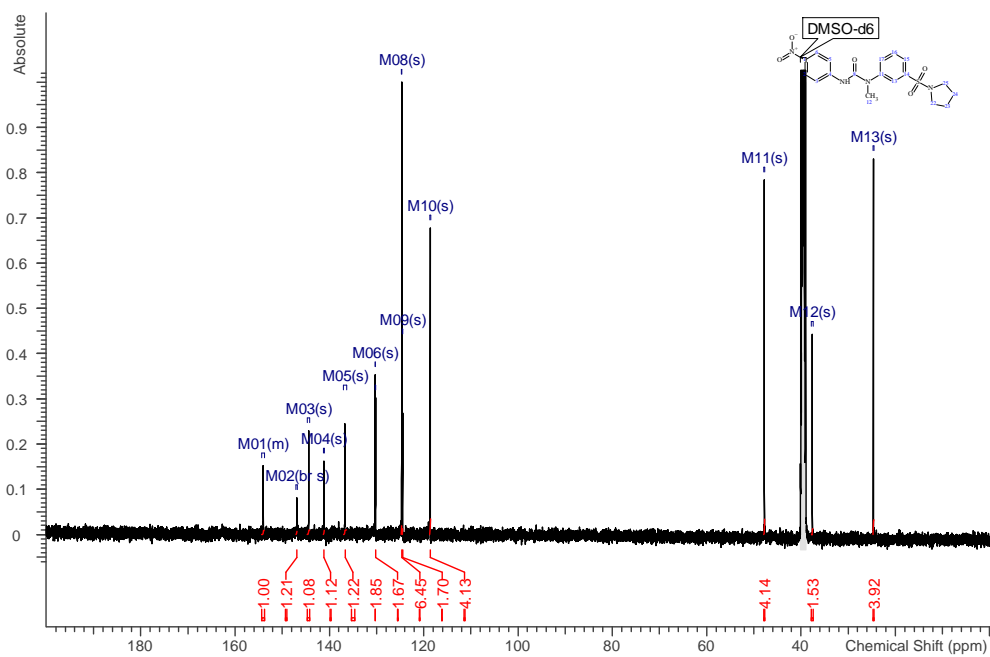
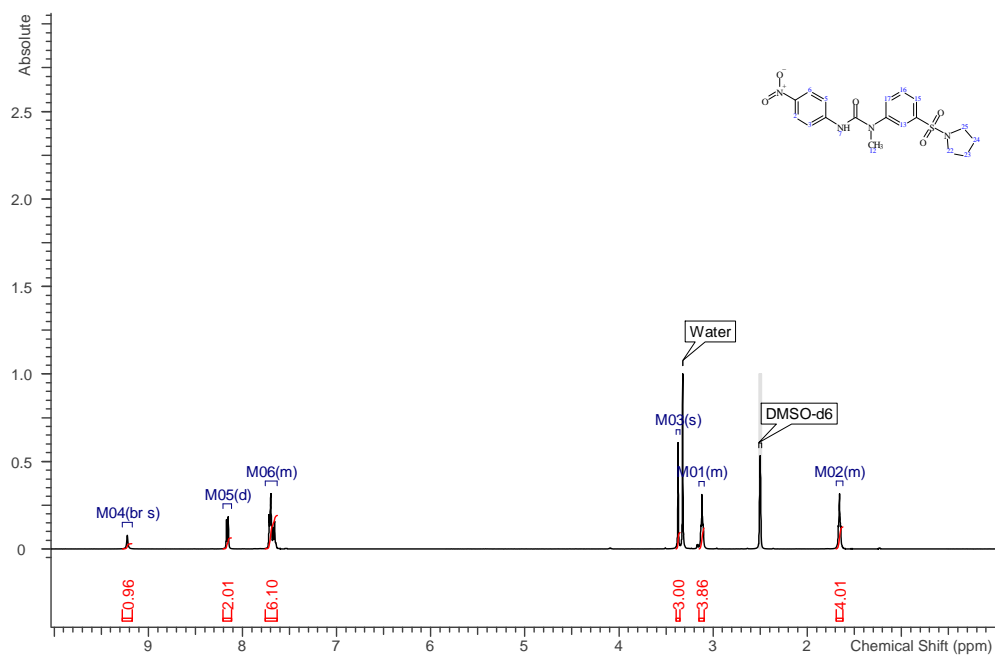
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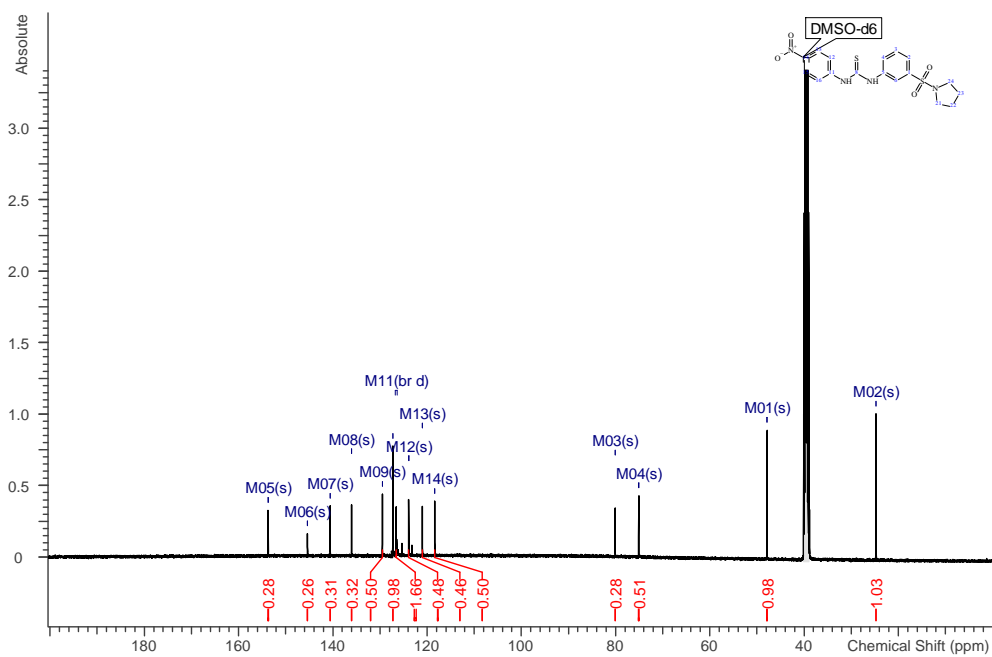
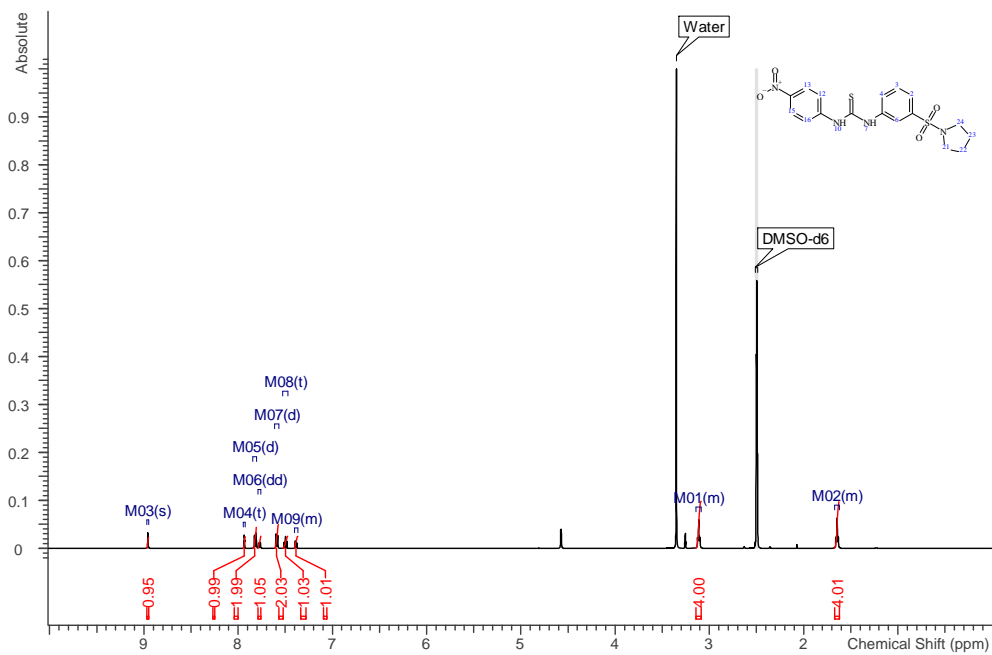


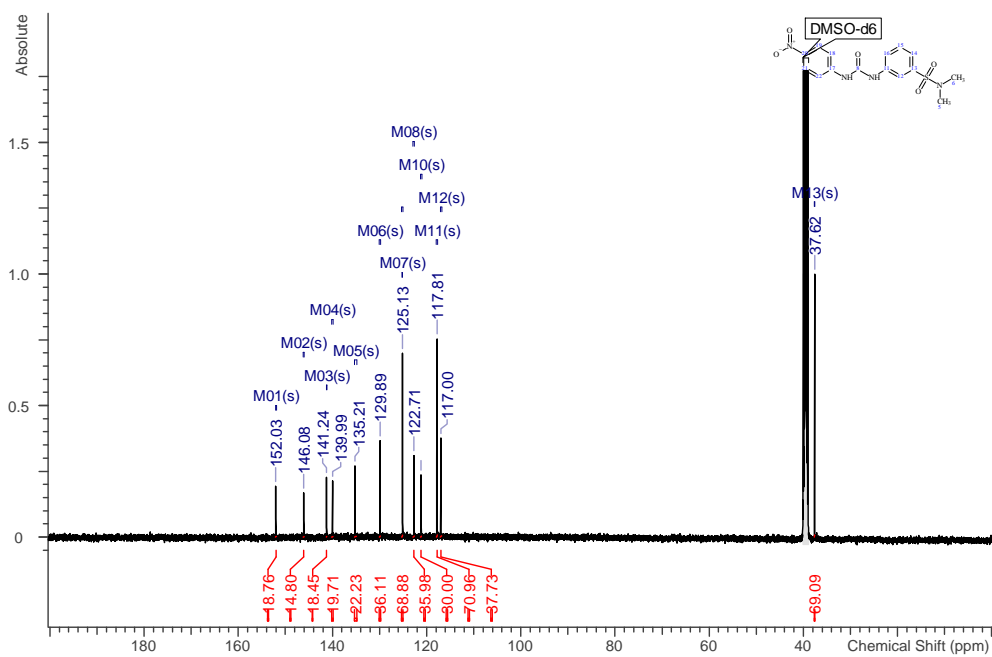
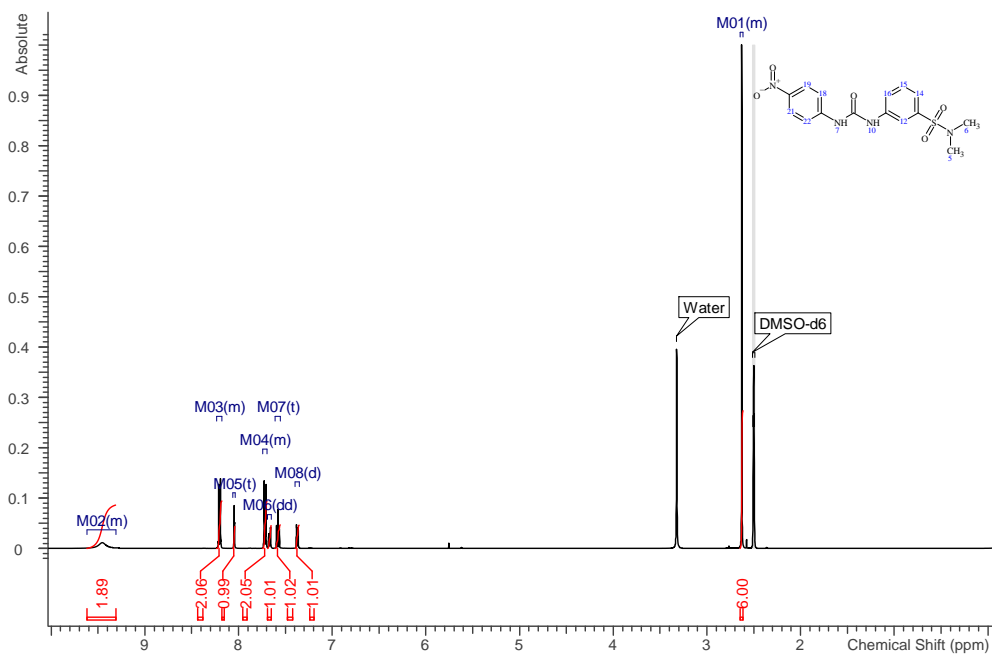
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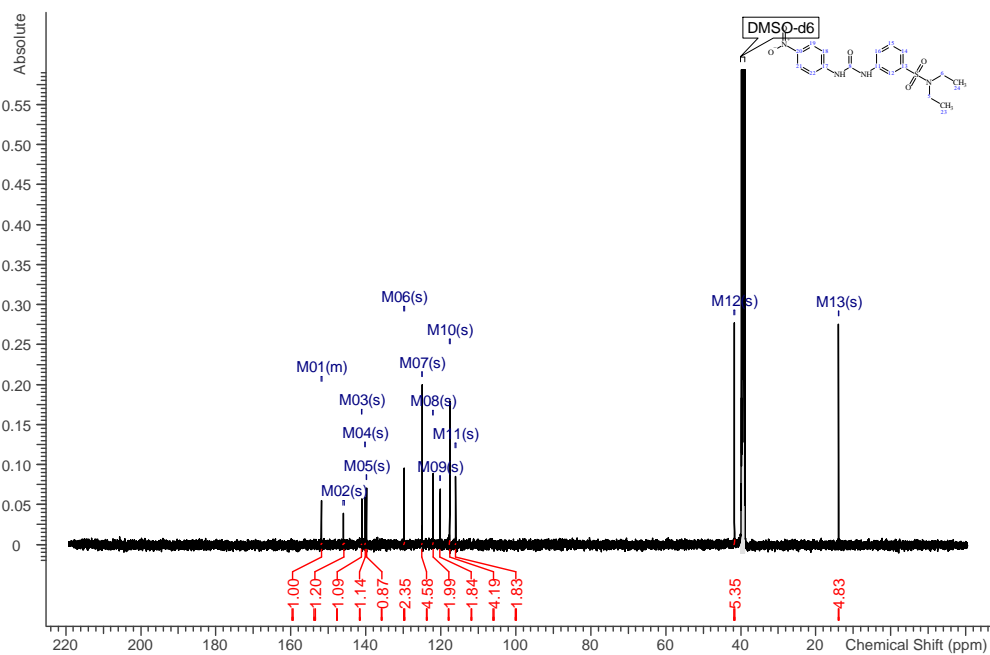
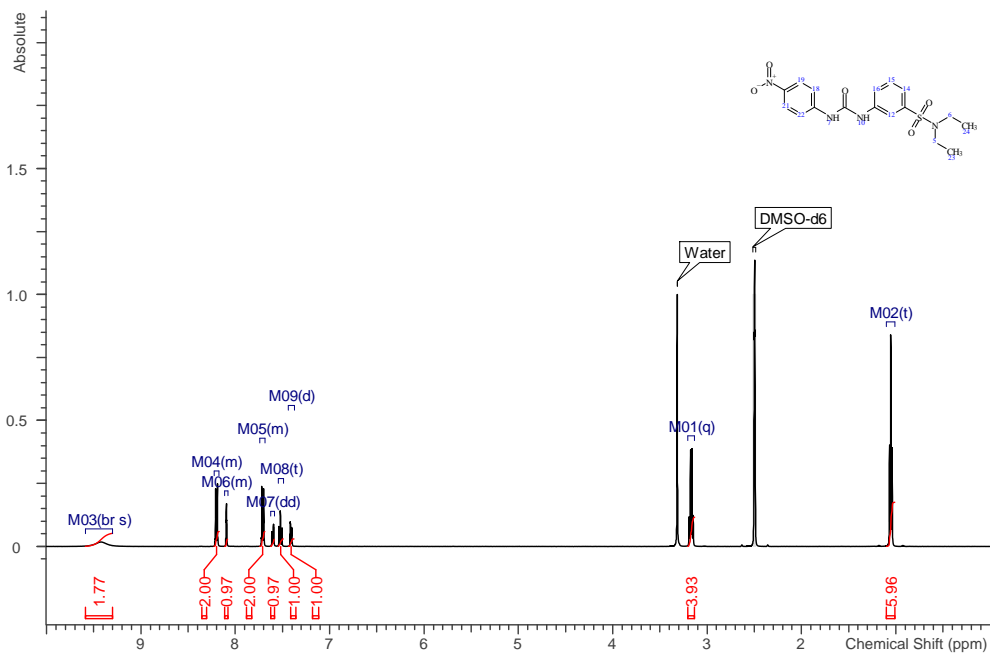
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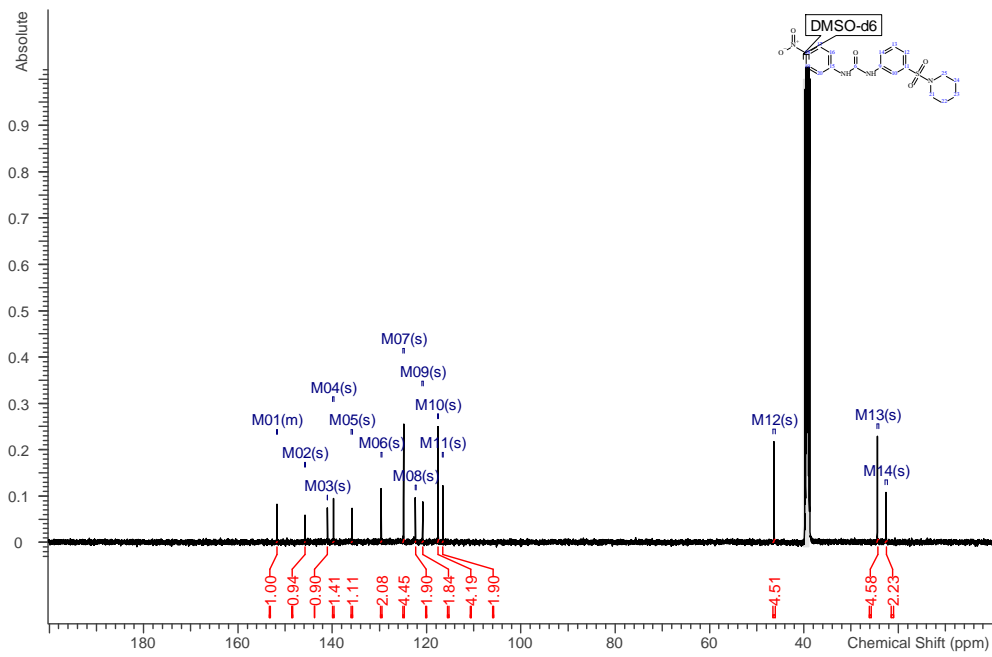
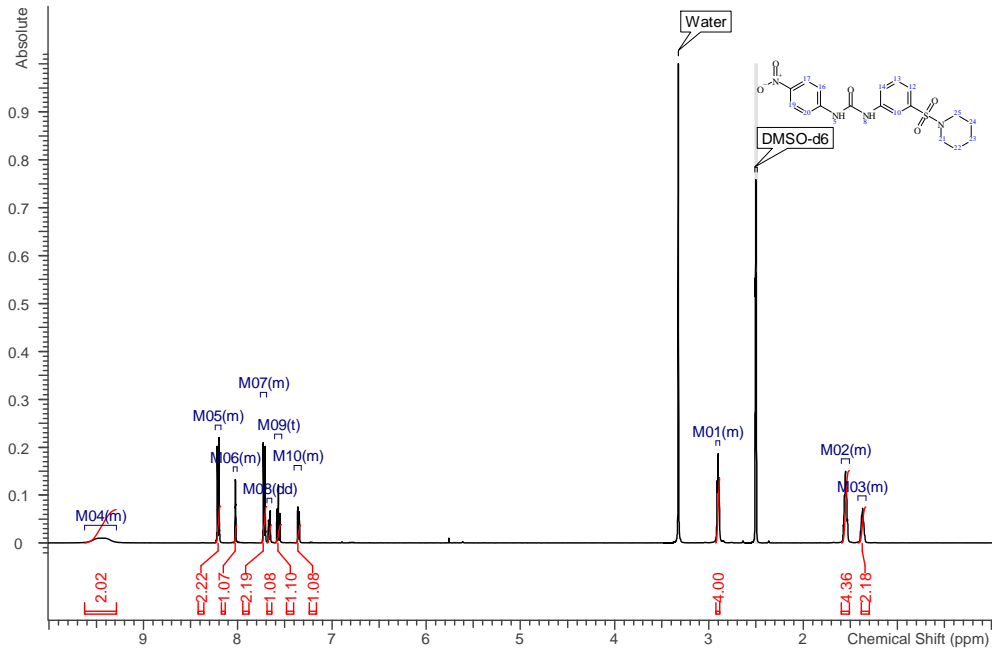


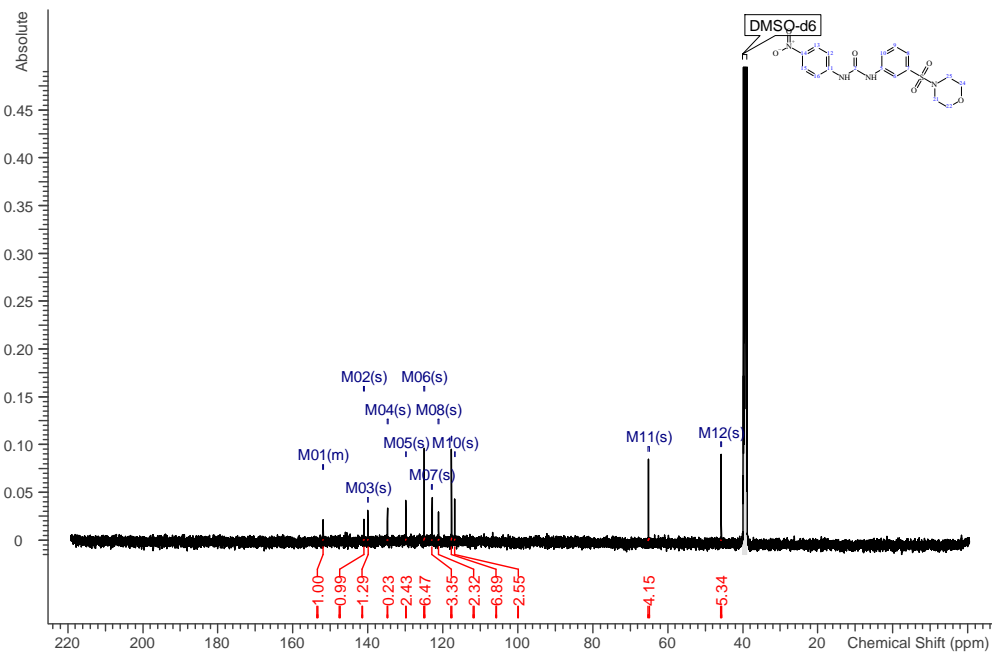
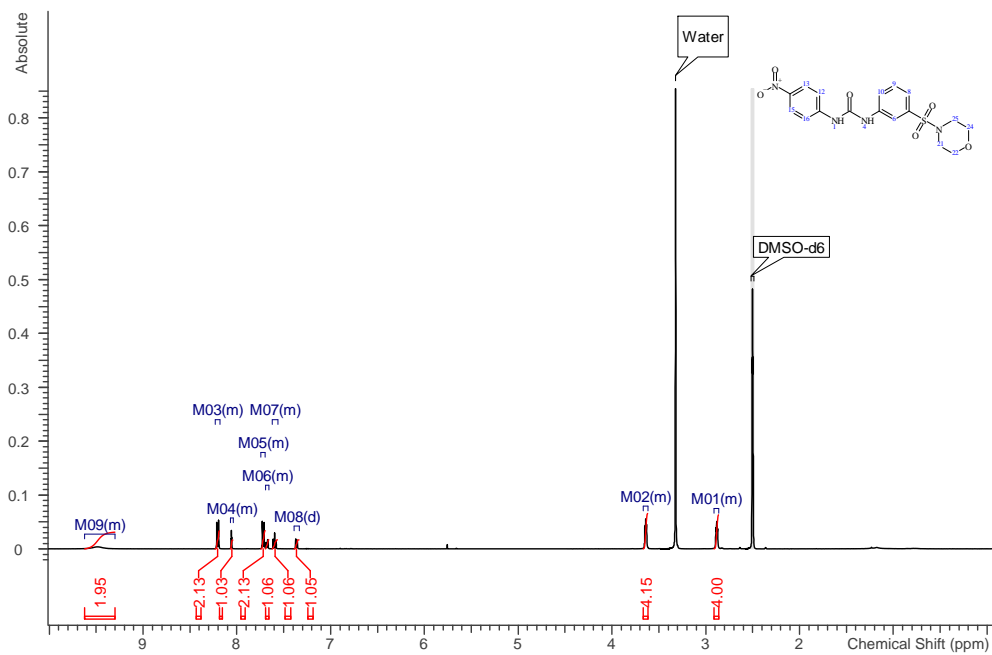


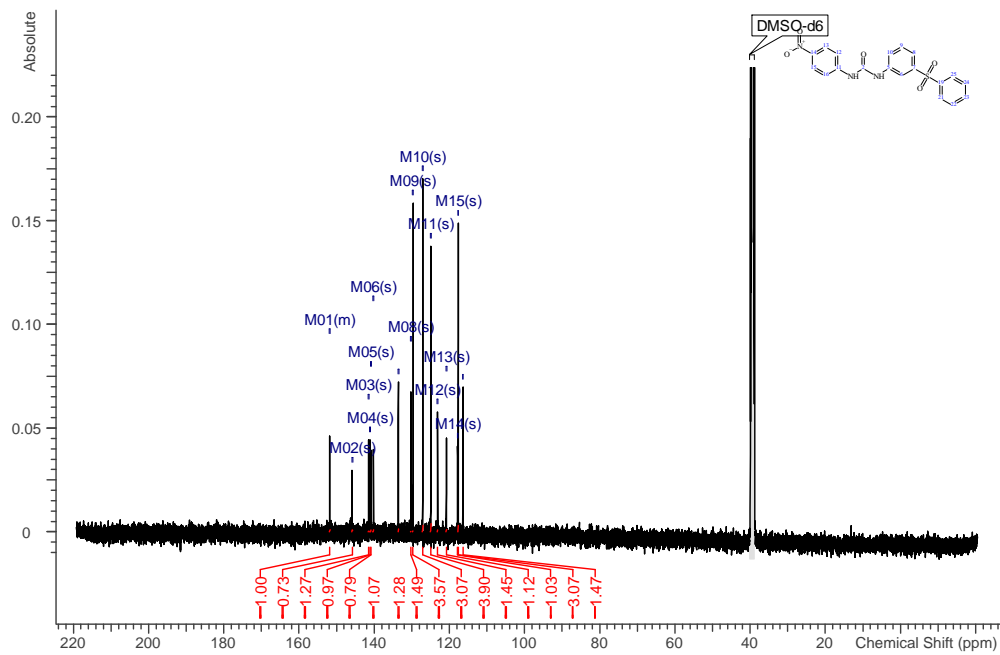
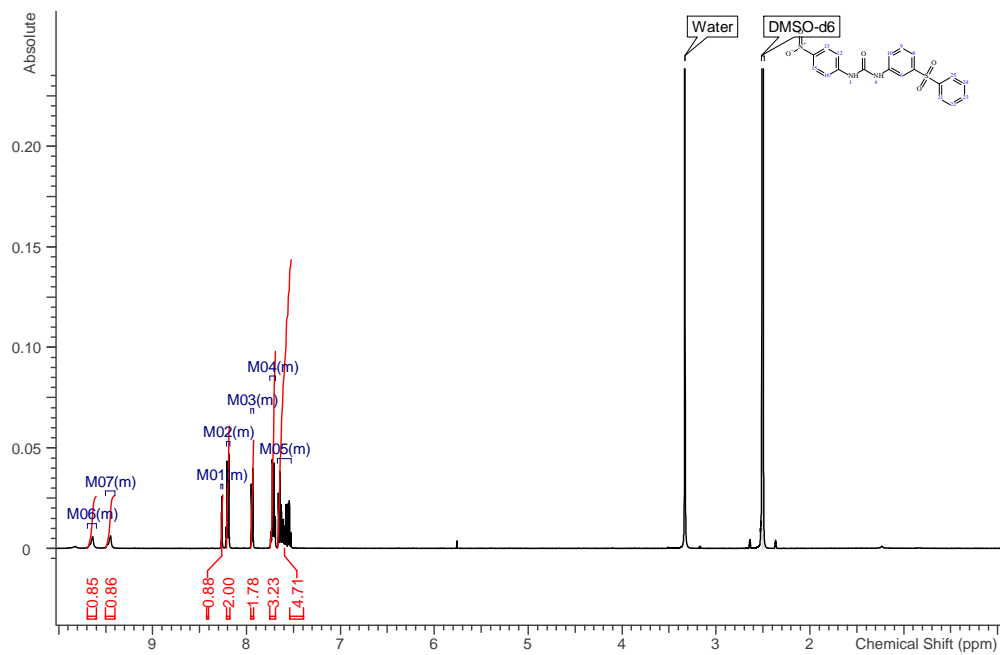


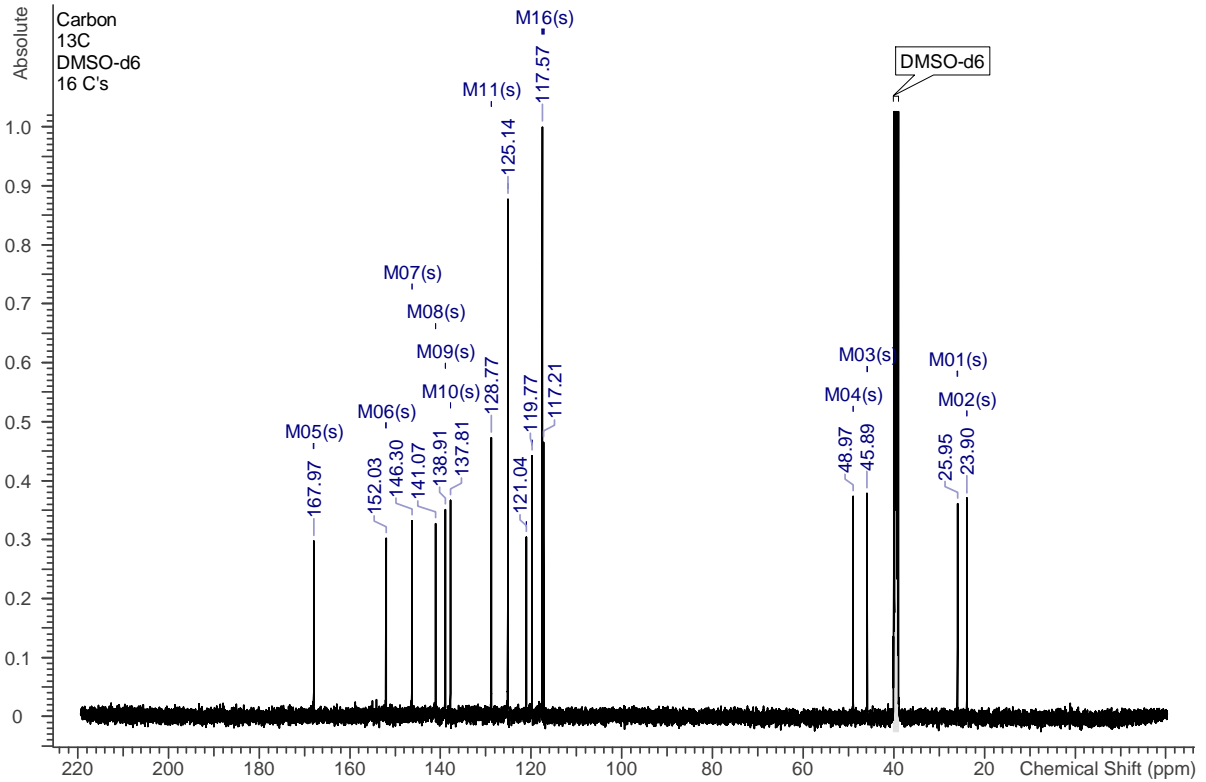
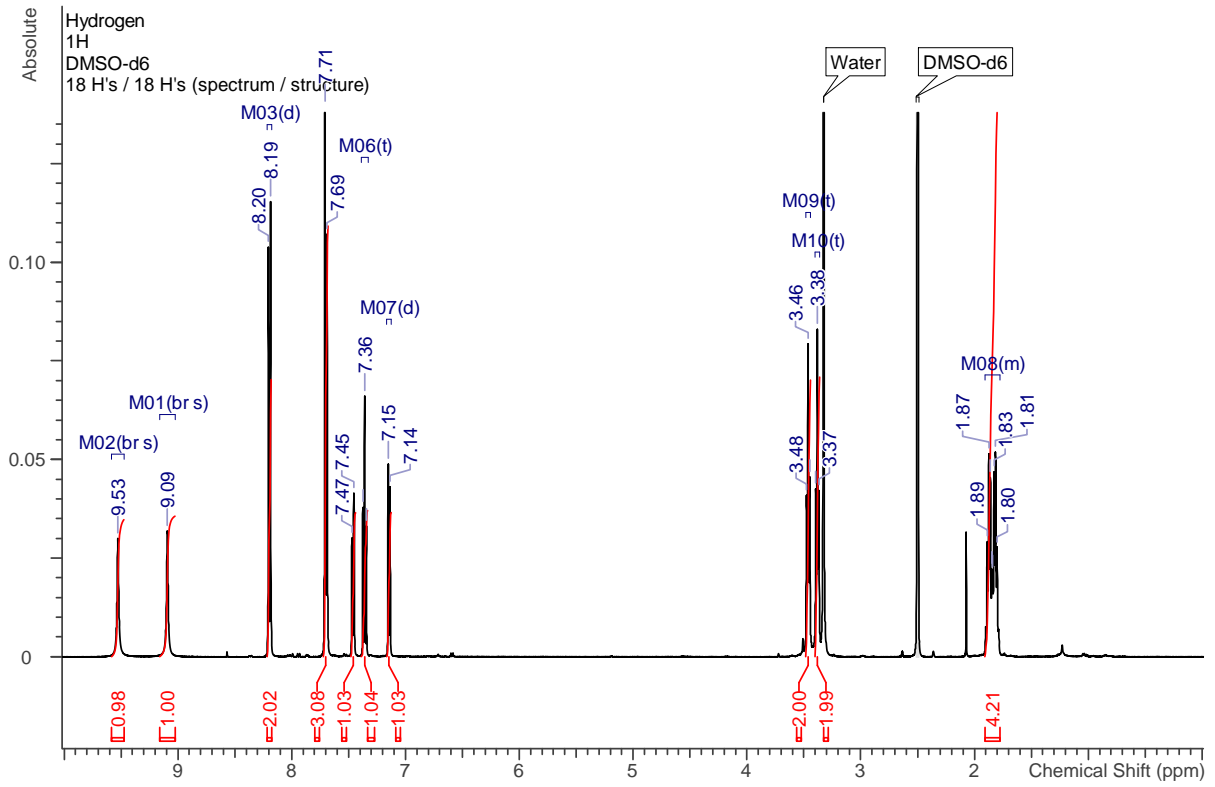
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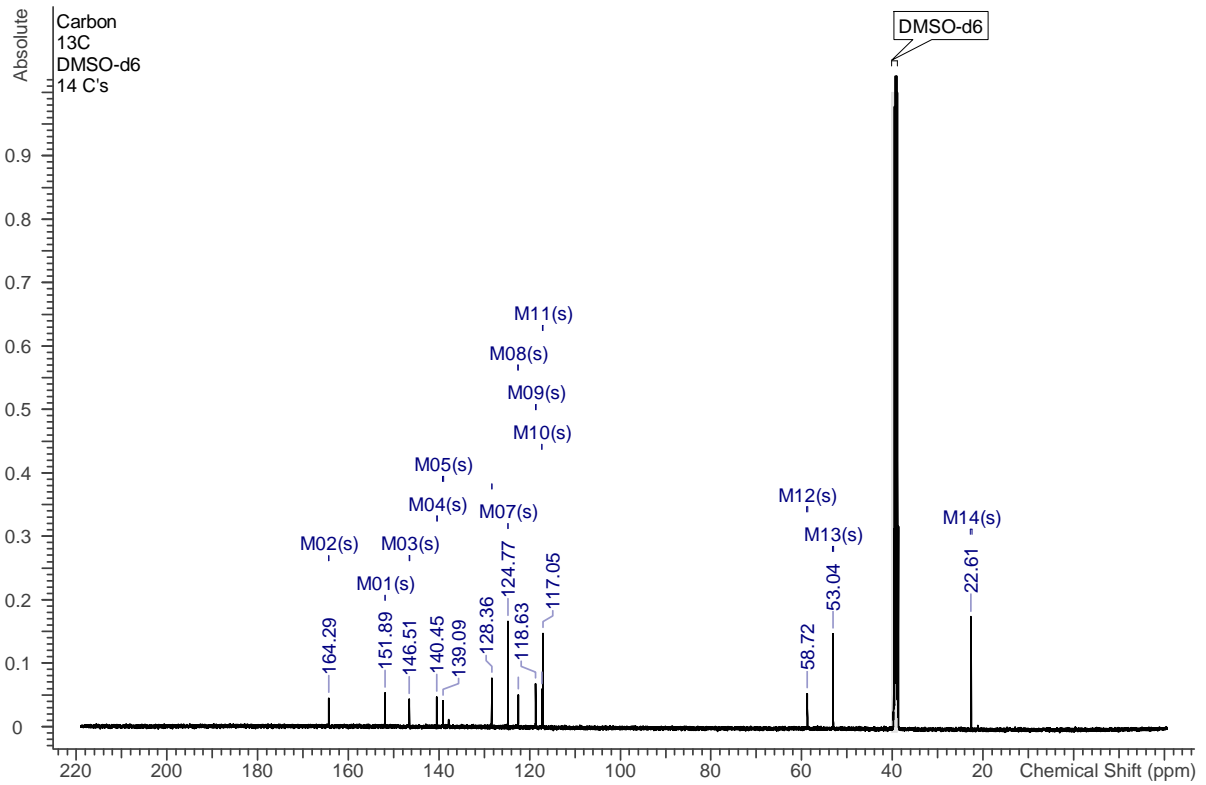
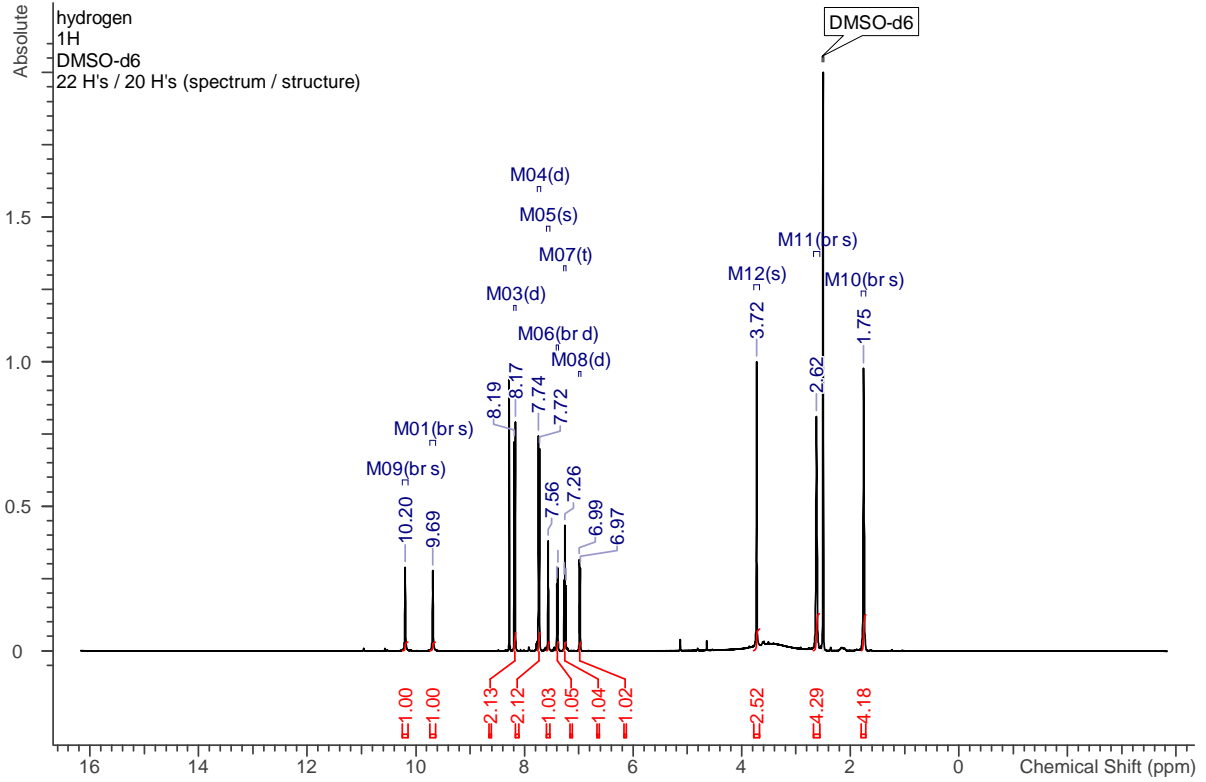




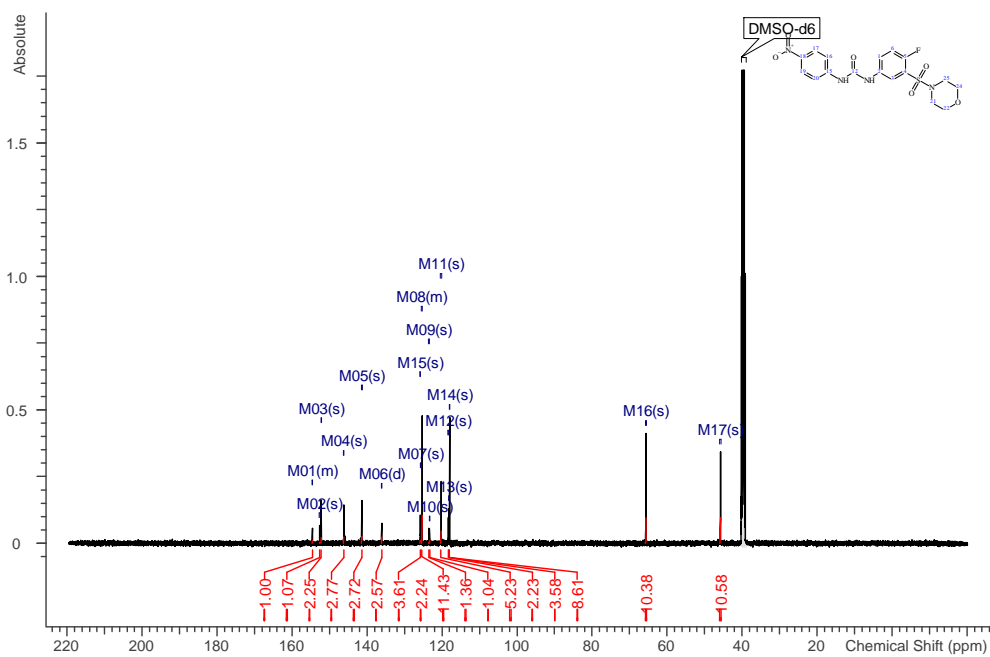
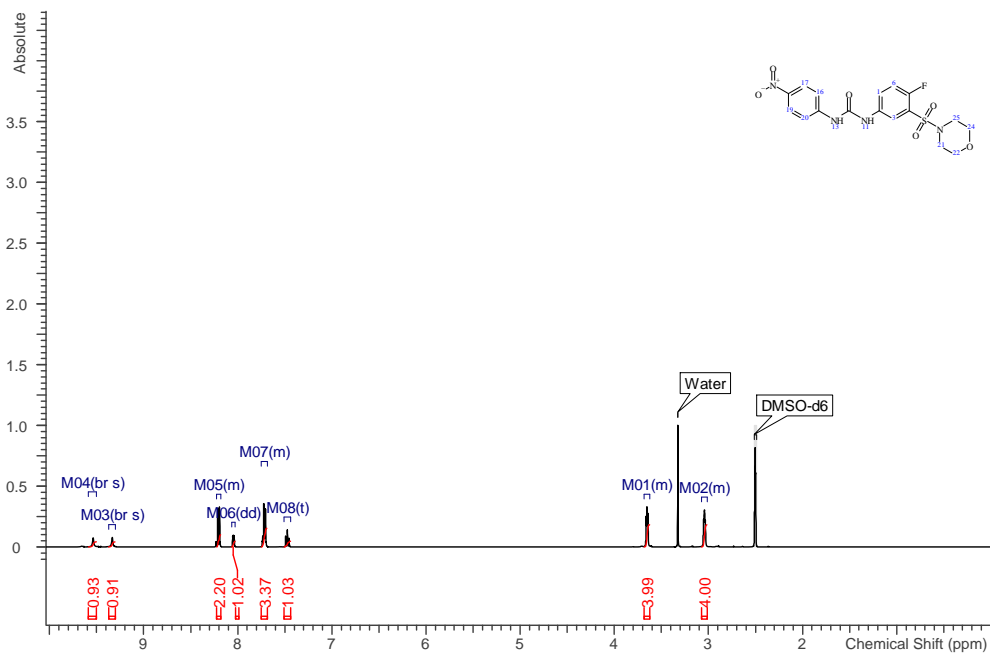




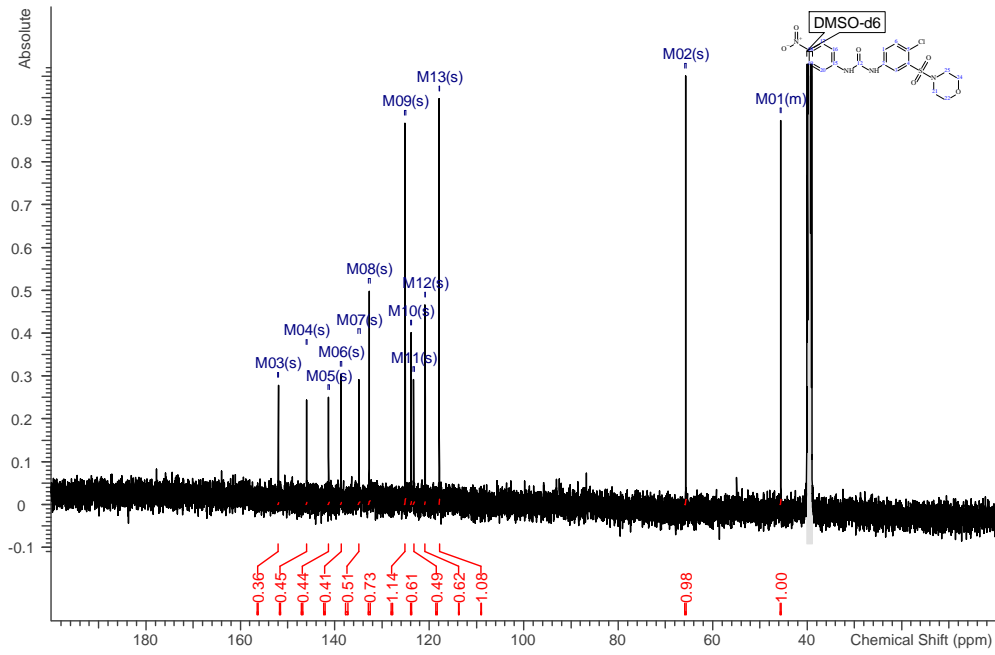
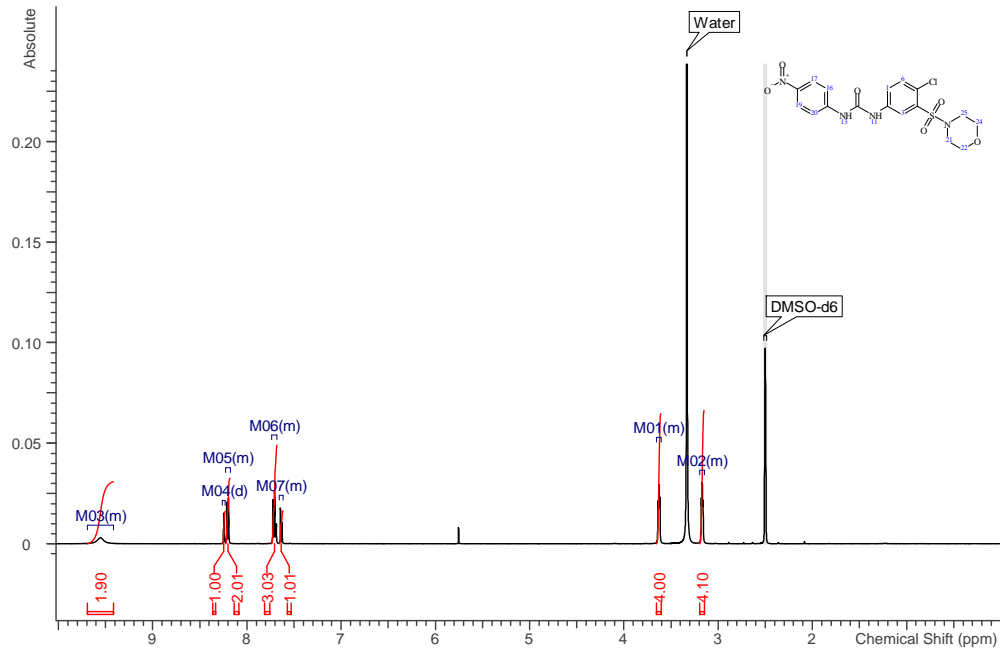
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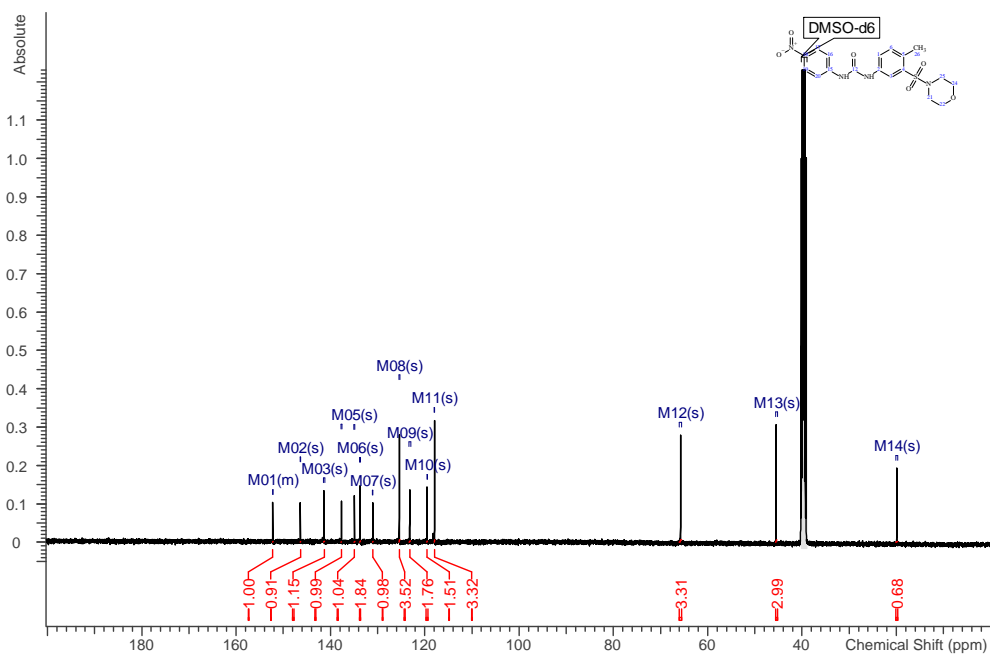
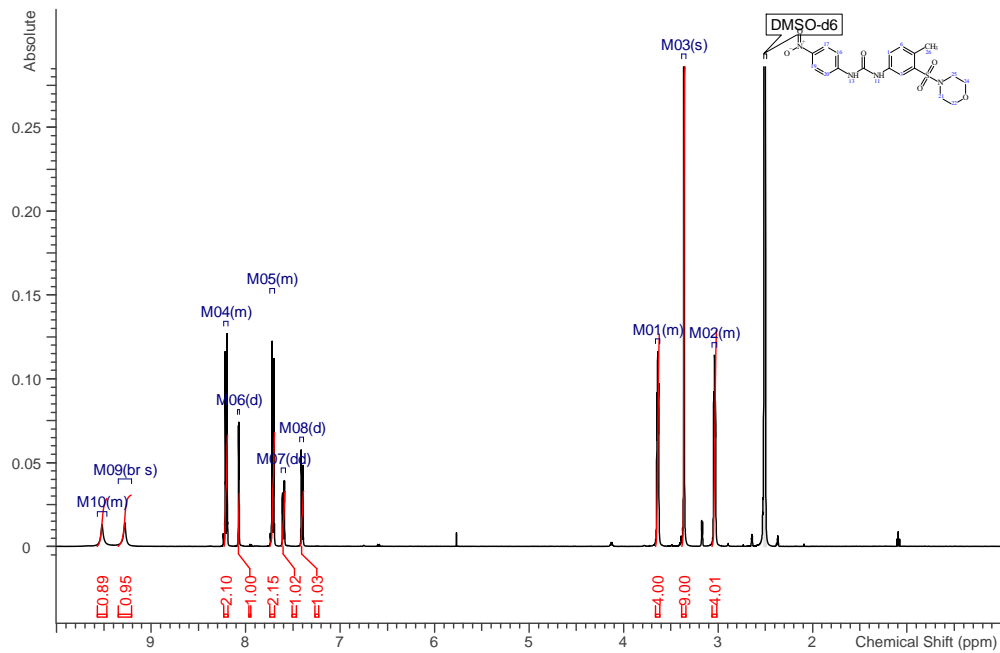


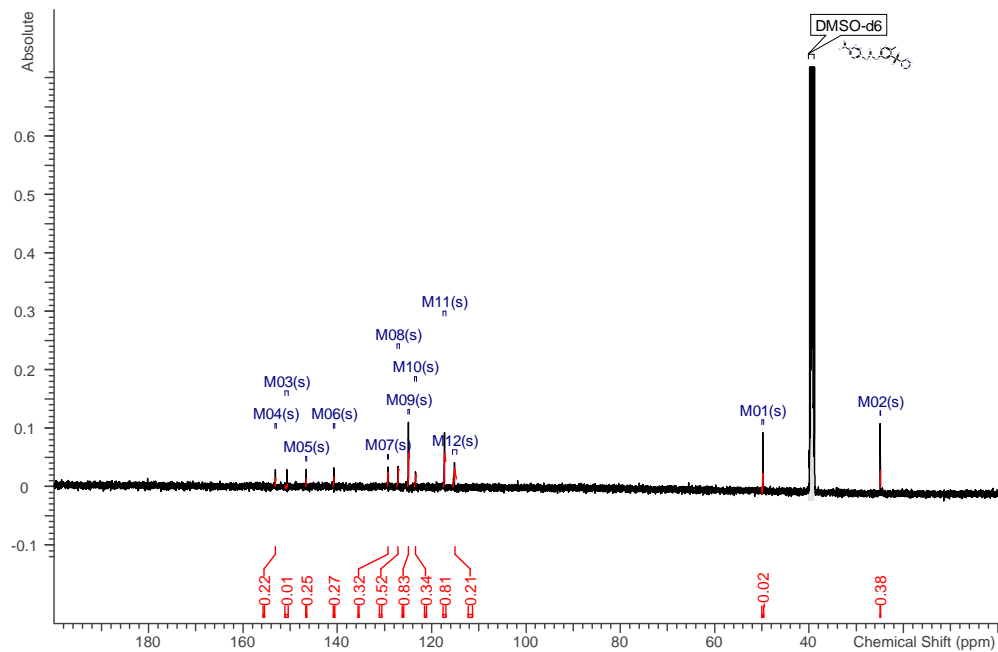
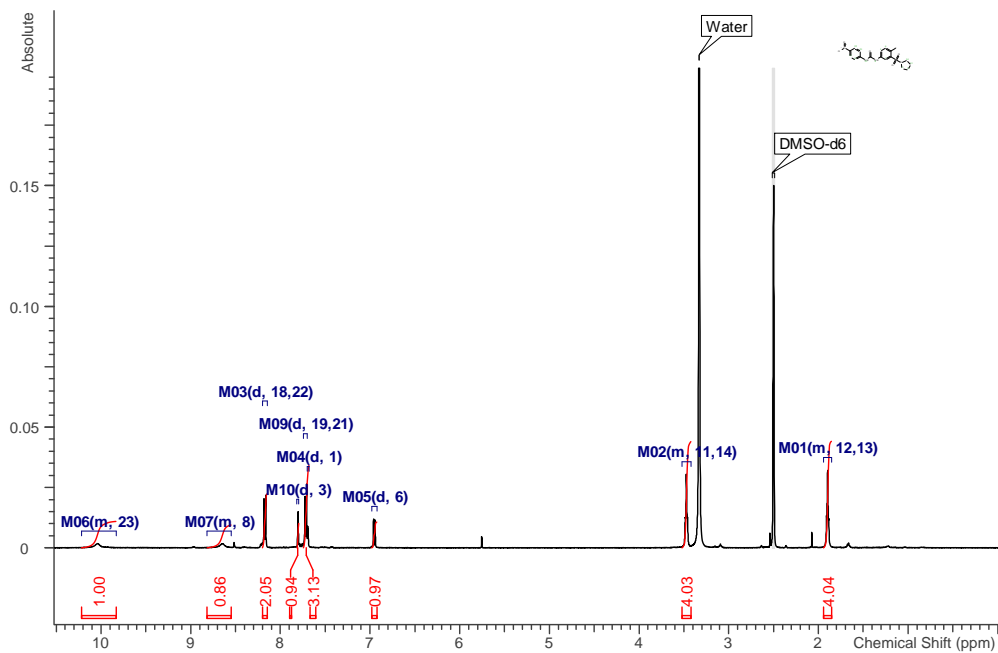
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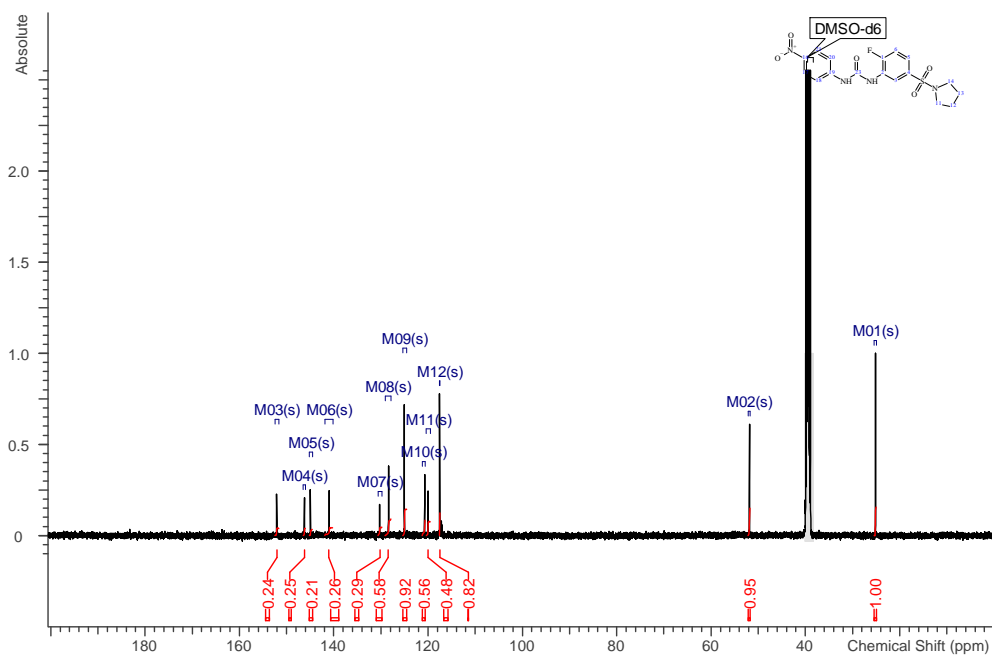
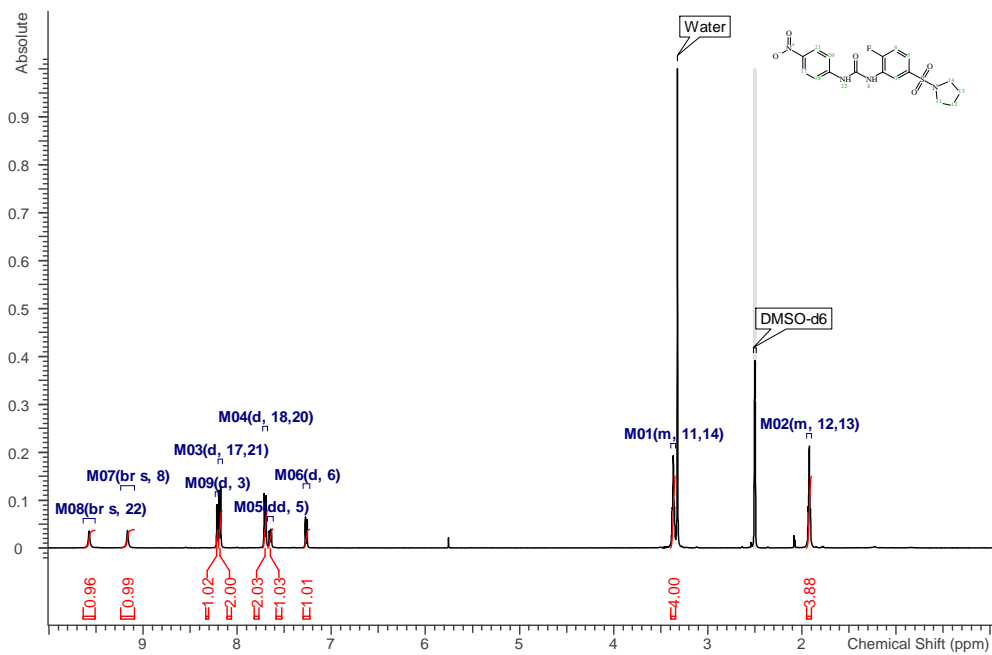


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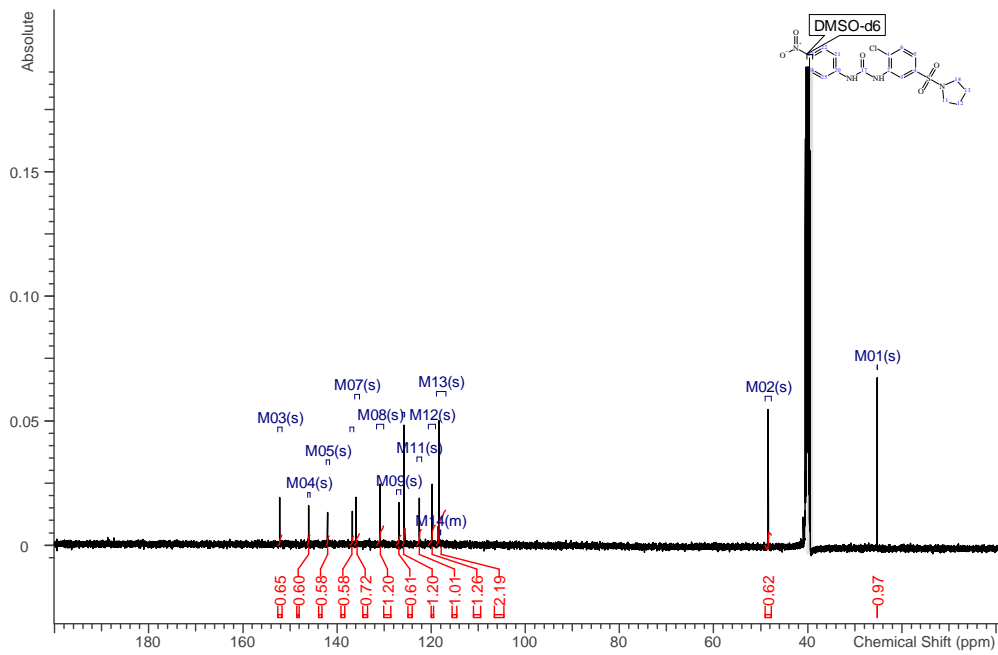
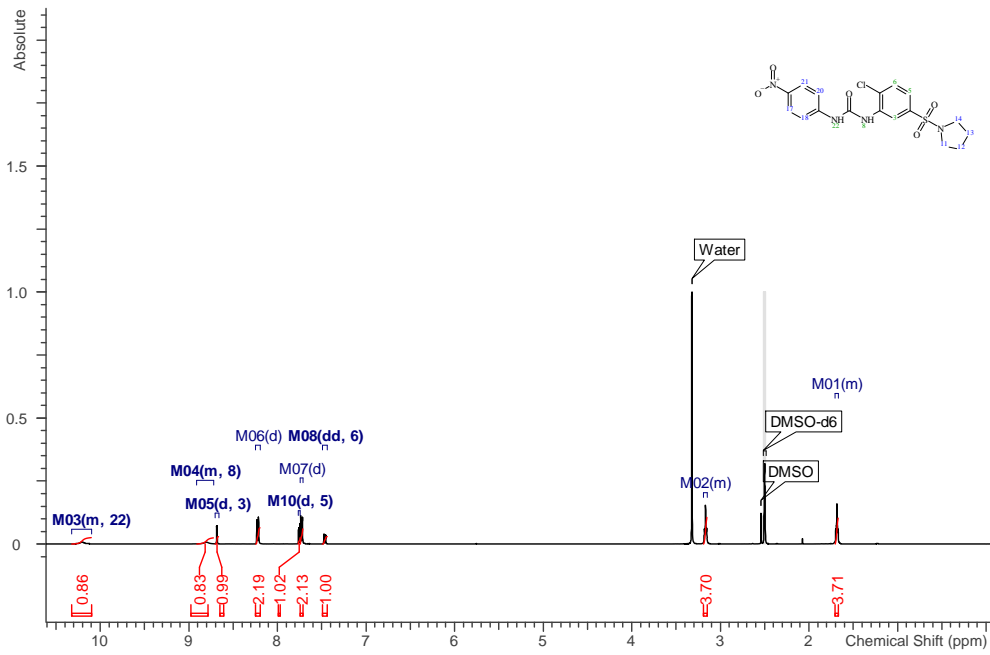


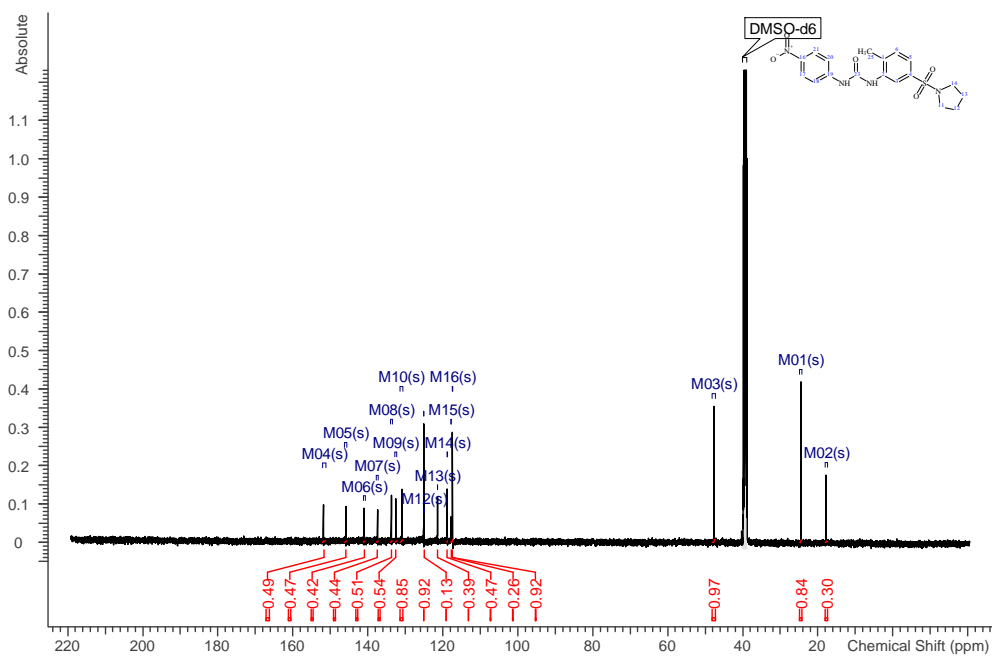
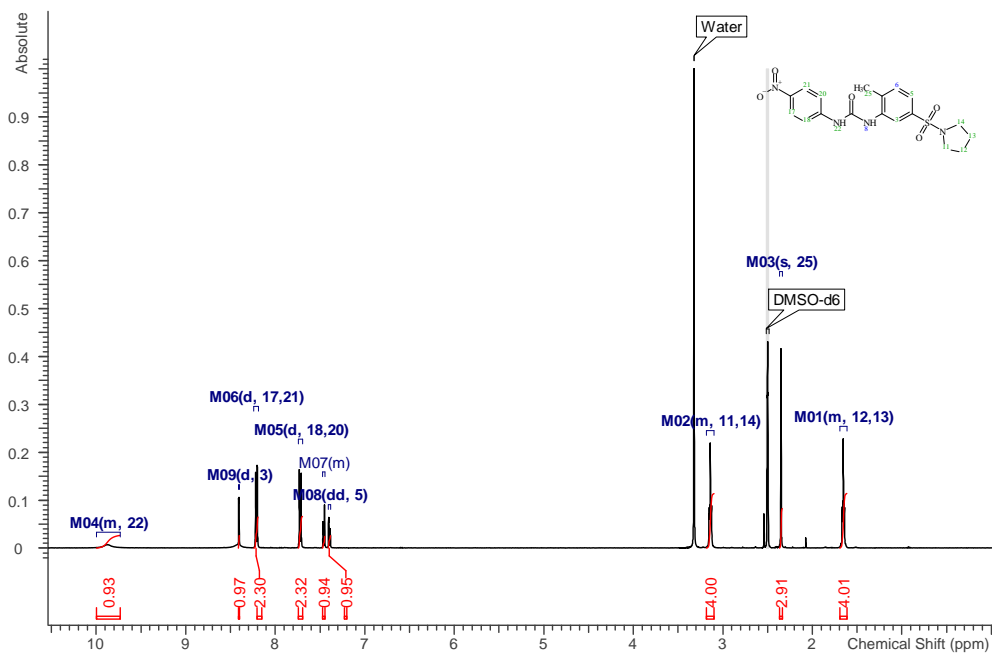




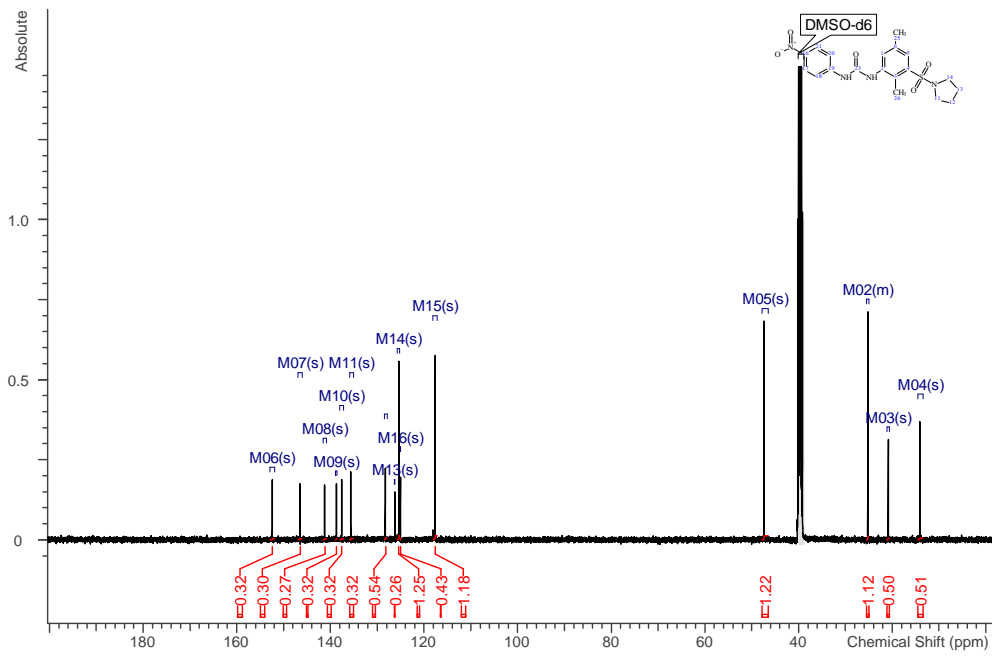
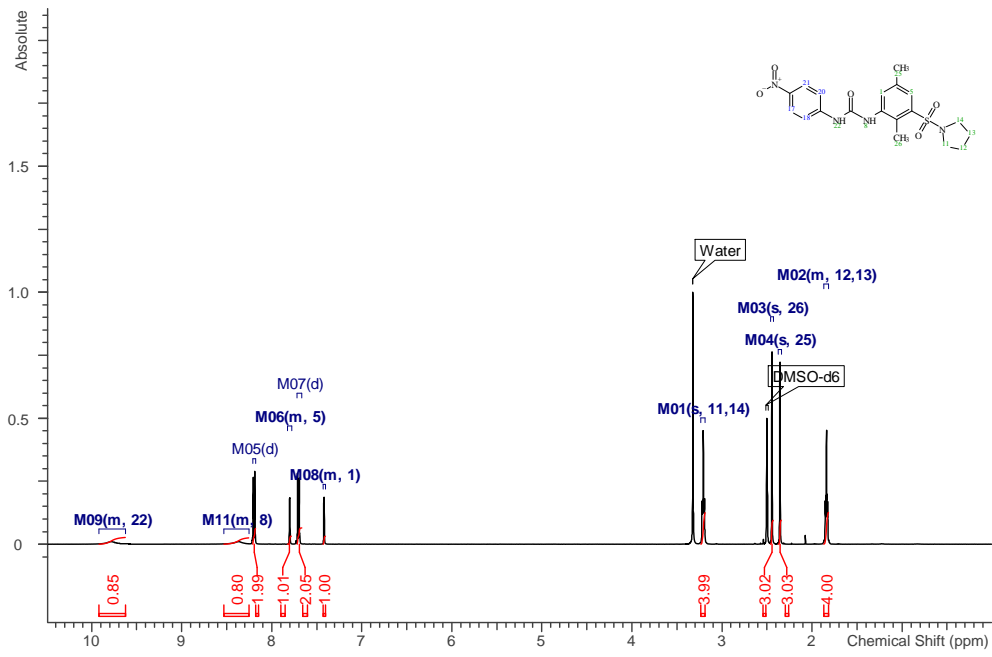


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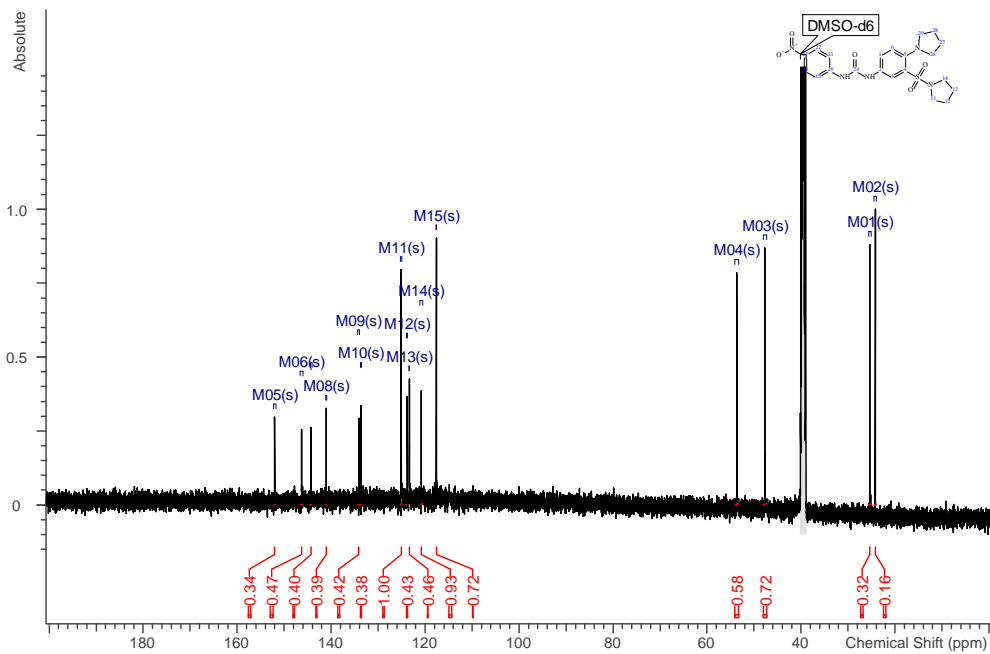
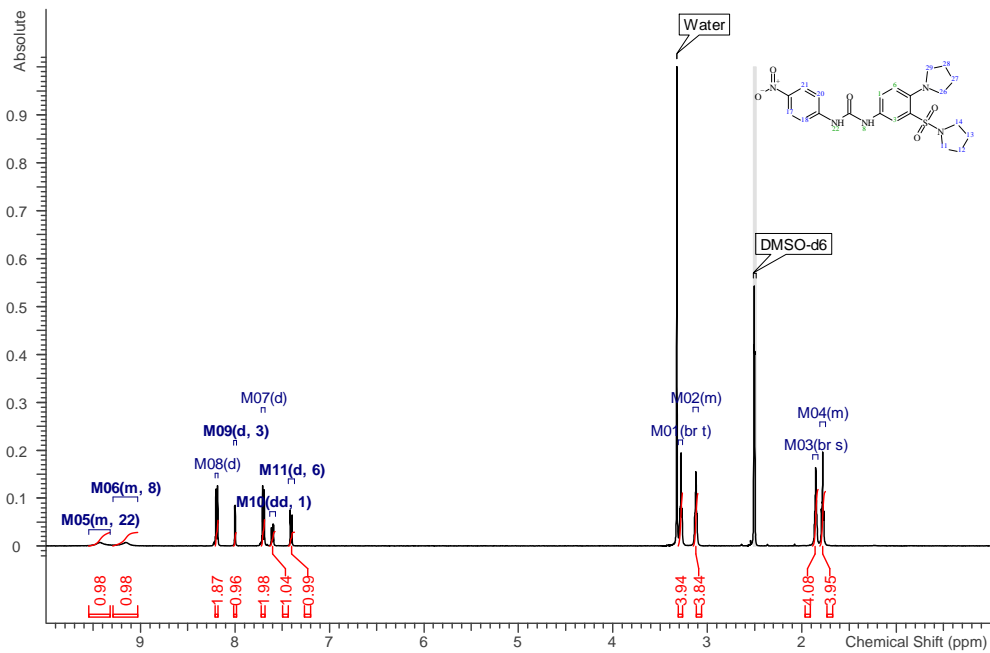


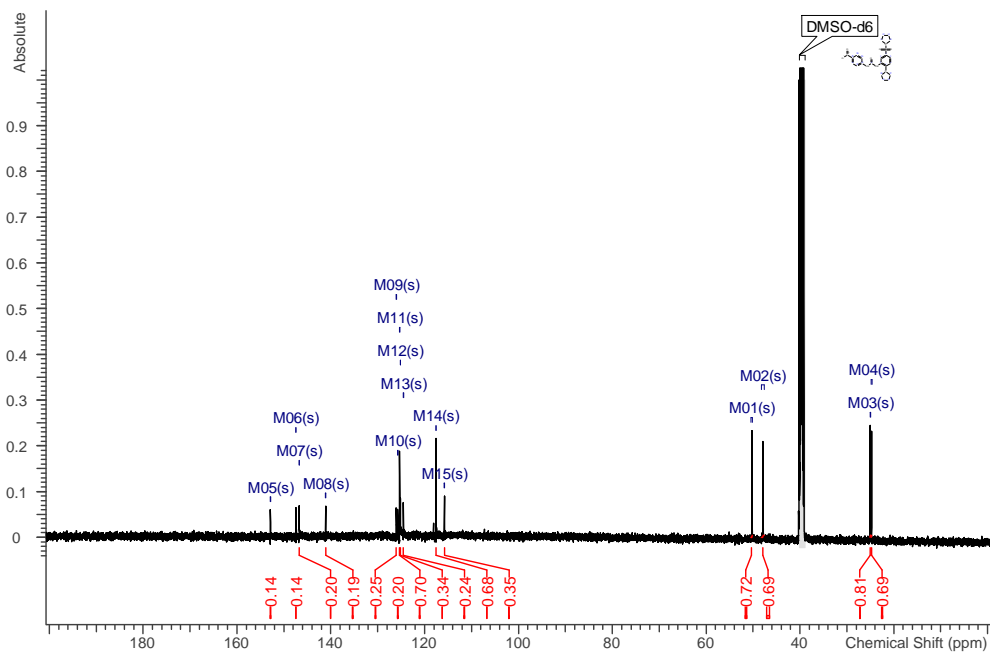
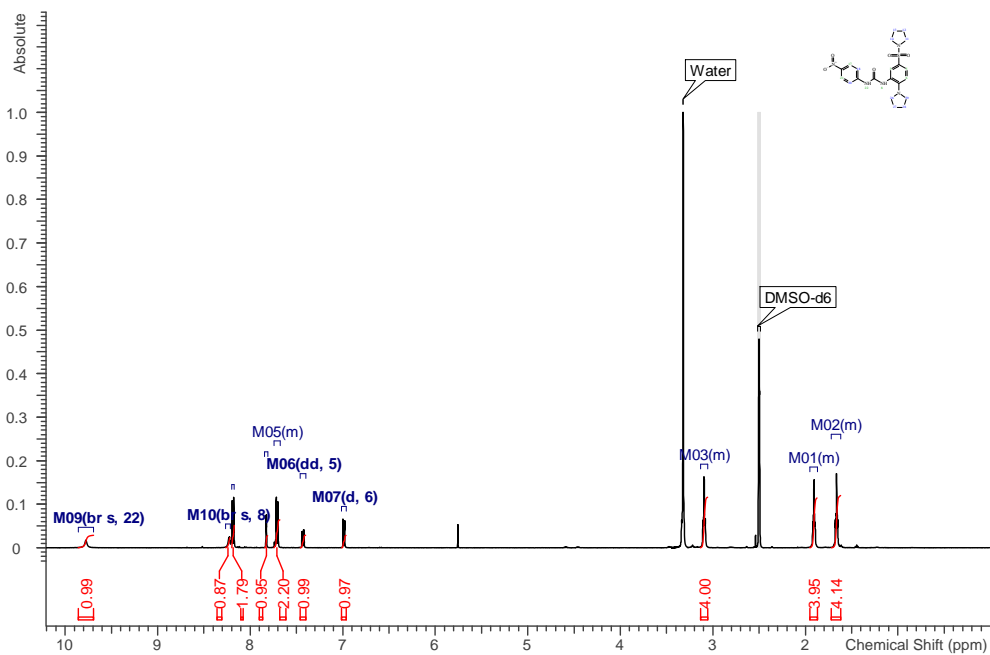


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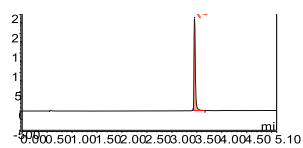


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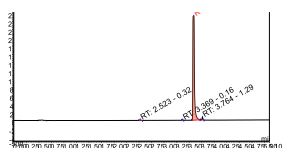




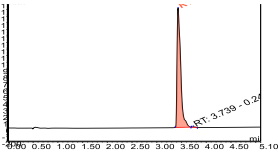
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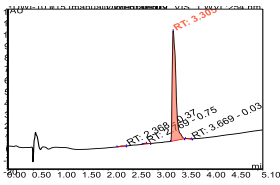
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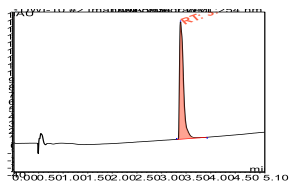
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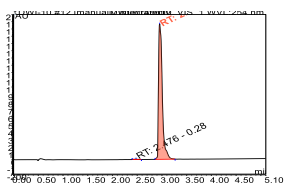
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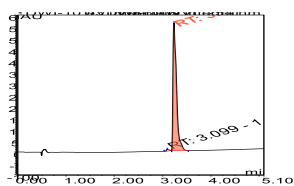
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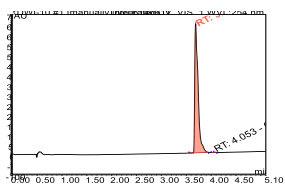
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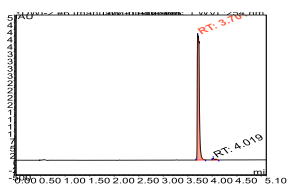
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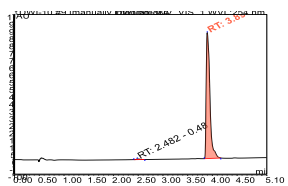
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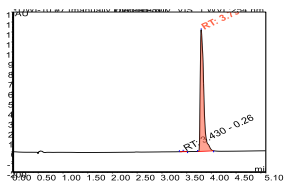
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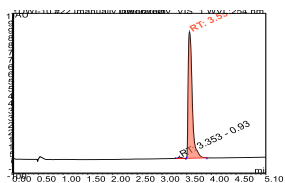
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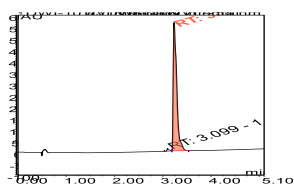
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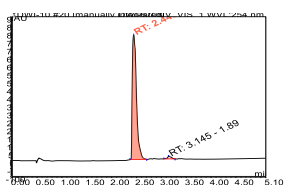
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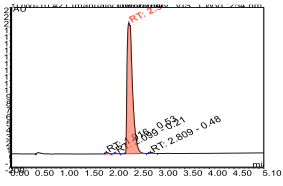
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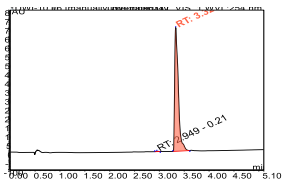
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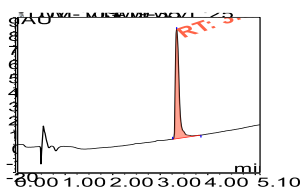
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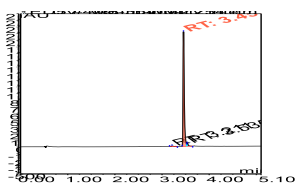
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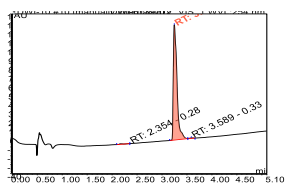
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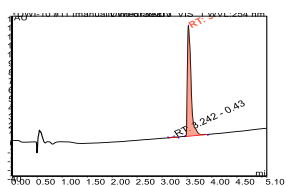
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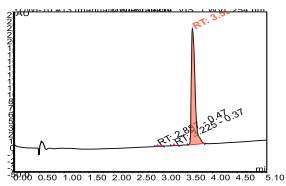
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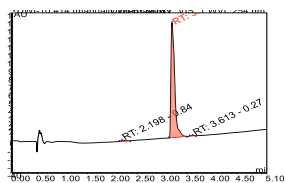
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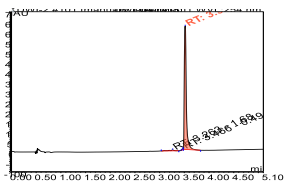
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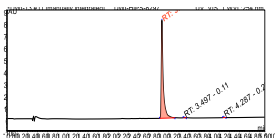
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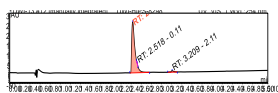
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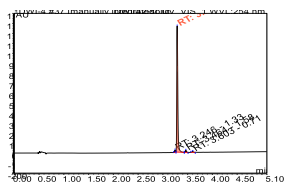
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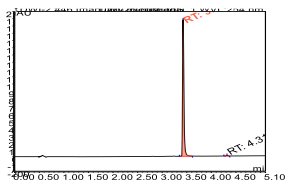
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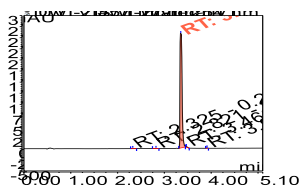
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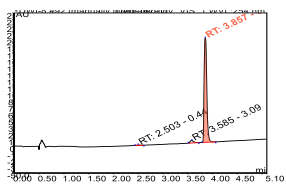
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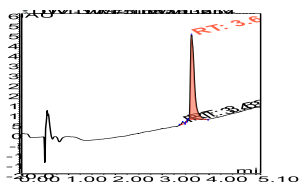
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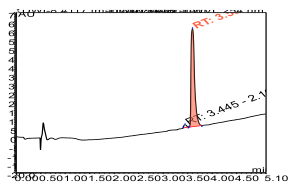
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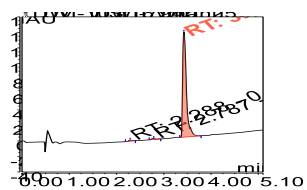
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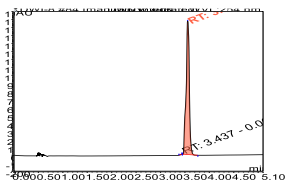
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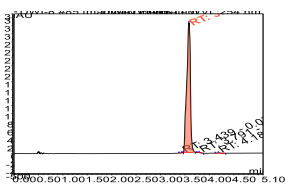
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