Supporting Information

Development of Substituted Phenyl Dihydrouracil as the Novel Achiral Cereblon Ligands for Targeted Protein Degradation

Haibo Xie,[1‡] Chunrong Li,[1‡] Hua Tang,[1] Ira Tandon,[1] Junzhuo Liao,[1] Brett L. Roberts,[1] Yu Zhao[1] and Weiping Tang[1,2].*

1. Lachman Institute for Pharmaceutical Development, School of Pharmacy, University of Wisconsin-Madison, 777 Highland Avenue, Madison, WI 53705 (USA)

E-mail: weiping.tang@wisc.edu

2. Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, WI 53706 (USA)

# These authors contributed equally.

Table of Contents

Structural Modeling of the Binding Poses of 6F

Since the imide moiety of novel achiral ligand **2** is the same as the classic glutarimide ligands, which makes three H-bond contacts with CRBN, and that FP competition binding is successfully performed with a classic ligand probe, it is presumed the binding pocket and core pose will be the same. By comparison, the closest analog with crystal structure (PDB: 7SHH) available also shows a binding pose the same as all other classic imides.

PDB: 4TZ4 was the starting point of the modeling. Missing non-terminal protein residues were restored by Modeller[1] in UCSF Chimera. Ligand **6F** was converted into 3D mol2 format by OBabel[2] from mol format saved from ChemDraw.

The docking was performed by GOLD.[3] The search efficiency was set to 200%. Automatic bond types were on for both protein and ligand. The protein was set to be rigid. Other settings were kept at default with the ChemPLP scoring function being used. From human judgement, the binding pose can be either up or down in regard to the phenyl ring substituents (see SI Figure S1), and the up or down pose is not interconvertible while the ligand is bound due to sterics of rotation, so subsequent modeling by MD refinement will need two separate starting points. Due to the randomness incorporated in the GOLD algorithm, results are different for each run. Most poses were on the downside, and the best scoring one, which also had the correct imide binding pose, was chosen for the down pose. The down pose had an H-Bond with neighboring Histidine 353, and the relevant HID tautomer was used. Docking was run multiple times until a reasonable top pose was generated, which had an even more favorable score than the best chosen down pose.

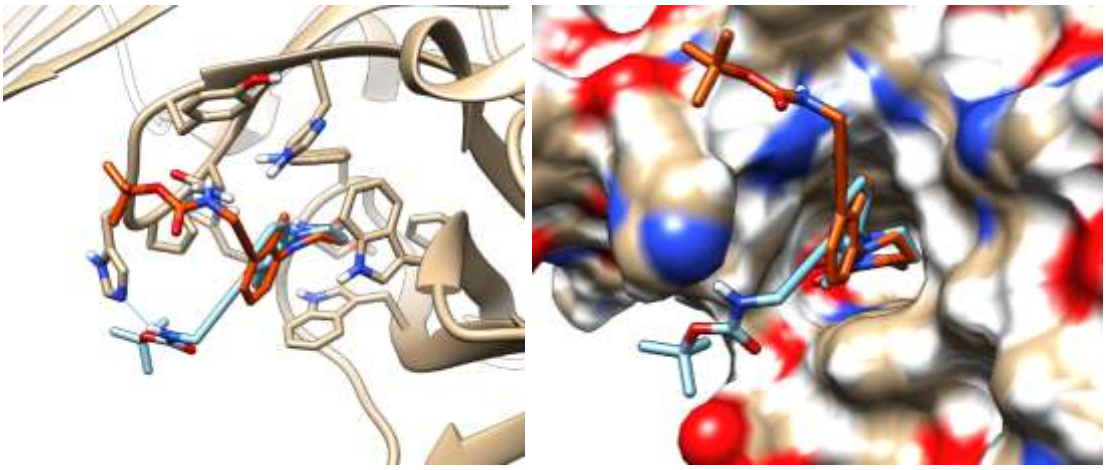


Figure S1. Docking poses to start the MD refinement. Blue is best pose of phenyl ring substituents rotated down, also determined later to be the ideal ligand binding pose. Orange is best pose of phenyl ring substituents rotated up.

The refinement allows both the ligand and protein to fully relax since ligand induced changes may occur on protein side chains. Additionally, ligand binding stabilities can be observed during the refinement process, including filtering out false positive poses. To perform the refinement, in the AMBERTools Suite,[4] the ligand was assigned AM1-BCC partial charges by antechamber, and remaining ligand parameters derived from gaff2 force field with parmchk2. In tleap, the ff19SB force field was used for the protein. An OPC octahedral box of water was set to extend at least 10 Å from any solute atom, and counterions (Na^+, Cl^-) were added to neutralize charges.

Using pmemd.cuda,[5] 5,000 steps of steepest descent minimization was performed with 5 kcal/mol/Å^2 restraints on the heavy atoms. To equilibrate the system, MD simulation was performed with 5 kcal/mol/Å^2 restraints on the heavy atoms for 50,000 steps in the NPT ensemble with a 0.001 ps timestep, followed by 50,000 steps with 1 kcal/mol/Å^2 restraints, and 50,000 steps with no restraints. The Langevin thermostat was used with gamma_ln set to 2.0 ps^{-1}, and the Monte Carlo barostat was used with taup set to 0.5 ps. For the production runs, in the NVT ensemble, the timestep was set to 0.004 ps with hydrogen-mass-repartitioning (HMR) applied to give solute hydrogens 3.024 mass, and MD simulation performed for 4 ns, using the Langevin thermostat with a gamma_ln of 0.01 ps^{-1}. The SHAKE algorithm was applied to restrict bond length changes involving hydrogens, and no forces were calculated for bonds with hydrogens. Snapshots were collected every 0.1 ns. 5 replicate simulation runs were performed.

After the simulation, in cpptraj,[6] solvent and ions were removed and frames aligned based on protein pocket C-α atoms within 10 Å of the ligand. The 5 production runs were combined and clustered based on ligand heavy atoms and protein heavy atoms within 4 Å of the ligand. The DBSCAN algorithm was used and the Epsilon was set to 0.9 Å and minpoints set to 1. No sieving was used so all frames were used to cluster. For each cluster, one representative pose with the lowest cumulative distance to all other frames in cluster was extracted.

Based on the above clustering conditions, **6F** top formed 15 clusters greater than 1 frame and the top cluster was 22%, and second cluster was 2.5%. The ligand moved away from the docked pose. **6F** down formed 4 clusters greater than 1 frame and the top cluster was 57% and all clusters maintained this H-bond with the neighboring Histidine 353. The docked pose is mostly maintained. The distinction is apparent that the down pose is the more stable and likely one. The representative pose from the most populous cluster is shown as the modeled binding pose in Figure 3 of main text.

Human Plasma Stability

Selected compounds were submitted to CRO company (SAI Life Sciences Limited) for human plasma stability.

MATERIALS

Table S1. Consumables and reagents

Materials	Catalog/ Lot No.	Manufacturer
Propantheline bromide hydrochloride	P8891	Sigma, USA
DMSO	D5879	
Human plasma	NA	Poona Research Foundation, India

Table S2. Equipments

Equipments	Manufacturer
Single and multi channel pipettes	Eppendorf, Germany
Refrigerated centrifuge	Kubota, Tokyo, Japan
Incubator 1000	Heidolph Instruments GmbH & Co. KG, Walpersdorfer
LC-MS/MS	Waters ACQUITYTM, ultra performance LC, Canada
API-4000 MDS Sciex	Applied Biosystems, Canada

METHOD

Preparation of DMSO stocks and working solutions

A 20 mM stock solutions of test compounds was prepared by dissolving appropriate amount of compounds in DMSO. These were diluted in DMSO to prepare a stock of 1 mM. A 1 mM stock was further diluted 200-folds in human plasma (pre-warmed at 37 ^{o}C for 15 minutes) to attain a final concentration of 1 µM respectively (0.1 % DMSO).

Table S3. Assay conditions

Compounds concentration	1 µM
Matrix	human plasma
Incubation temperature	37^{o}C
Time Points	0, 60,90 ,120 and 240 min
DMSO	0.1 %
Replicates	2

Procedure

Incubation

Positive controls and test compounds spiked into 400 µL aliquot (n = 2) of human plasma was incubated in a CO_2 incubator at 37 ^{o}C. An aliquot of 50 µL was withdrawn at 0, 60, 90, 120 and 240 min and immediately quenched with 400 µL of ice-cold acetonitrile containing internal standard followed by storing at minus 2-8 ^{o}C.

Preparation of test samples for bio-analysis

All the samples were thawed at room temperature. Samples were vortexed for 5 min followed by centrifugation at 4000 RPM for 15 min at 4 ^{o}C. An aliquot of 100 µL was transferred to 96-well deep plate and submitted for analysis by LC-MS/MS.

Analysis

The analyte area of test compounds and positive control was quantified in the test samples using LC-MS/MS. The LC-MS/MS conditions and MRM chromatogram will be provided as per client's request.

DATA ANALYSIS

The percentage of compounds remaining at each time point was calculated by considering the peak area (PA) ratios (analyte versus internal standard) obtained at 0 min as 100%. The slope of initial linear range of logarithmic curve of percent remaining versus time was used for calculation of half-life ($t_{1/2}$).

$$\text{Elimination rate constant (k)} = (-\text{gradient})$$

$$\text{Half life } (t_{1/2})\ (\text{min}) = \frac{0.693}{k}$$

RESULTS AND CONCLUSIONS

Parent compounds remaining and half-life of positive control Propantheline bromide was used in the experiment is in good agreement with validation data generated in-house (Table 1). All the compounds were stable in human plasma within duration of incubation. Details provided in table (1)

Table S4. Time versus % remaining of control and test compounds in human plasma

Time (min)	Propantheline bromide	Lenalidomide	6F	9	10	11
0	100	100	100	100	100	100
60	3	91	100	79	106	107
90	0	77	111	82	96	104
120	0	83	101	76	86	100
240	0	62	97	81	88	102
$t_{1/2}$ (min)	**27.1**	**>240**	**>240**	**>240**	**>240**	**NC**

NC: Not calculated as compound was stable within duration of incubation

Human Liver Microsome Stability

Selected compounds were submitted to CRO company (SAI Life Sciences Limited) for liver microsome stability

MATERIALS

Table S5. Consumables and reagents

Materials	Catalog No.		Manufacturer
Pooled Human liver microsomes	4521117		Corning,USA
Verapamil hydrochloride	381195		Sigma, USA
Glipizide	G117		
Albendazole	A4643		
Potassium phosphate monobasic	P5655		
Potassium phosphate dibasic	P2222		
DMSO	D5879		
NADPH	2646-71-1		SRL, India
96 well-plates	PCR-96MR-HS-C		Axygen, Union City, California

Table S6. Equipments

Equipment	Manufacturer
Single and multi channel pipettes	Eppendorf, Germany
Incubator 1000	Heidolph Instruments GmbH & Co. KG, Walpersdorfer
Refrigerated centrifuge	Eppendorf, Germany
LC-MS/MS	API 4000 AB Sciex, USA
UPLC	Waters Acquity H Class. USA

METHOD

Preparation of reagents

Preparation of potassium phosphate buffer, 50 mM (pH 7.4)

50 mM Potassium phosphate buffer (pH 7.4) was prepared by adding 0.647g potassium phosphate monobasic (KH_2PO_4) and 3.527g potassium phosphate dibasic (K_2HPO_4) to 400 mL of Milli-Q water. pH of the buffer was adjusted to 7.4 and volume was made up to 500 mL.

Preparation of microsomes

Microsomes (20 mg/mL) were diluted in 50 mM Potassium phosphate buffer (pH 7.4) buffer to prepare a concentration of 0.714 mg/mL.

Preparation of test compounds

A stock solution of the test compounds were prepared in DMSO at a concentration of 1 mM.

Preparation of NADPH solution

A stock solution of 3.33 mM NADPH (3.33X) was prepared by dissolving appropriate amount of NADPH in 50 mM Potassium phosphate buffer (pH 7.4)

Preparation of internal standard solution

1 mg/mL of internal standard glipizide and albendenzole were prepared in DMSO and methanol (1:1). From this stock solution of glipizide (300 ng/mL) and albendenzole (100 ng/mL) prepared in acetonitrile.

Table S7. Assay Conditions

Total Incubation volume	100 µL
Compound concentration	1 µM
Protein Concentration	0.5 mg/mL
NADPH	1 mM
Final DMSO contain	0.1%
Number of replicates	2
Time points	0 and 30 min

Assay

A microsomal mix (microsomes and potassium phosphate buffer pH 7.4) was prepared at concentration of 0.714 mg/mL. A 1 µL (1 mM) of test compounds/positive control was spiked into 699 µL of microsomal working solution (intermediate test compound/positive control concentration is 1.42 µM). After mixing, 70 µL (microsomal mix and compounds) was transferred to 96 well plate for 0 min and 30 min time point (n=2) for with NAPPH and without NADPH and pre-incubated at 37 ºC for 5 min. After pre-incubation, for zero min time point 30 µL of NADPH (3.33 mM) in NAPDH samples and potassium phosphate buffer pH 7.4 in without NAPDH samples was added (Final microsomal mix conc. is 0.5 mg/mL and test compound/positive control conc. is 1 µM) and reaction was stopped immediately using 200 µL of ice-cold acetonitrile containing internal standard. For 30 min time point reaction was initiated by addition of 30 µL of NADPH (3.33 mM) in NAPDH samples and potassium phosphate buffer pH 7.4 in without NAPDH samples was added and incubated at 37 ºC for 30 min, incubation reaction was stopped with 200 µL of ice-cold acetonitrile containing internal standard. A 200 µL of acetonitrile containing internal standard was added into both 0 min and 30 min quenched plates. The plates were centrifuged at 4000 RPM for 10 min and 100 µL supernatant were submitted for analysis by LC-MS/MS.

Bio-Analysis

Samples were monitored for parent compound disappearance in MRM mode using LC-MS/MS. The LC-MS/MS conditions and MRM chromatogram will be provided as per client request.

DATA ANALYSIS

The peak area ratios of analyte versus internal standard were used to calculate the % remaining at the end of 60 minutes in presence NADPH.

RESULTS AND CONCLUSIONS

Percentage remaining of positive control, Verapamil and test compounds in HLM were tabulated in table 2.

Table S8. Metabolic stability of positive control and test compounds in human liver microsomes.

S. No.	Compounds ID	% PCR at 30 min with NADPH	% PCR at 30 min without NADPH
1	VERAPAMIL	9	81
2	Lenalidomide	89	93

3	**6F**	87	90
4	**9**	85	99
5	**10**	100	93
6	**11**	90	102

PCR: Parent Compound Remaining

Glutarimide and Dihydrouracil Hydrolytic Ring Opening Stability

pH 7.4 was 100 mM potassium phosphate buffer. pH 8.8 was 100 mM bicine buffer. pH 1.0 was 100 mM HCl solution. The stability assays were performed at 100 μM and incubated at 37 ºC for indicated time. The percentage of compound remaining was determined by HPLC peak area ratio with internal standard (L-tryptophan).

Fluorescence Polarization Assay

The human recombinant DDB1/CRBN protein (Cat.# E3-500-MTO, Lot 35378320) was purchased from R&D Systems. The CRBN binding fluorescence probe was obtained by fluorescence labelling the Thalidomide 4'-oxyacetamide-alkylC4-amine (Purchased from TOCRIS, Cat.# 6469) by 5(6)-SFX (Purchased from TOCRIS, Cat.# 6488). The assay was carried out in Black Nunc™ 384-Shallow Well Standard Height Polypropylene Microplates (Catalog Number: 267461).
Assay buffer: 50 mM HEPES, 75 mM NaCl, 0.01% Triton X-100, pH7.4; Assay volume: 20μL.
Single concentration binding comparison: DDB1/CRBN: 150 nM, fluorescence probe: 8 nM, compound: 1000 nM;
Dose response for IC_{50}: DDB1/CRBN: 150 nM, fluorescence probe: 8 nM.
After mixing the CRBN protein, fluorescence probe and compound, the plate was incubated at room temperature for 20 minutes. The polarization signals (mP) were acquired by PHERAstar FS Plate Reader (FP 485-520-520nM Optic module). The data was processed, IC_{50} was calculted by GraphPad 9.0.

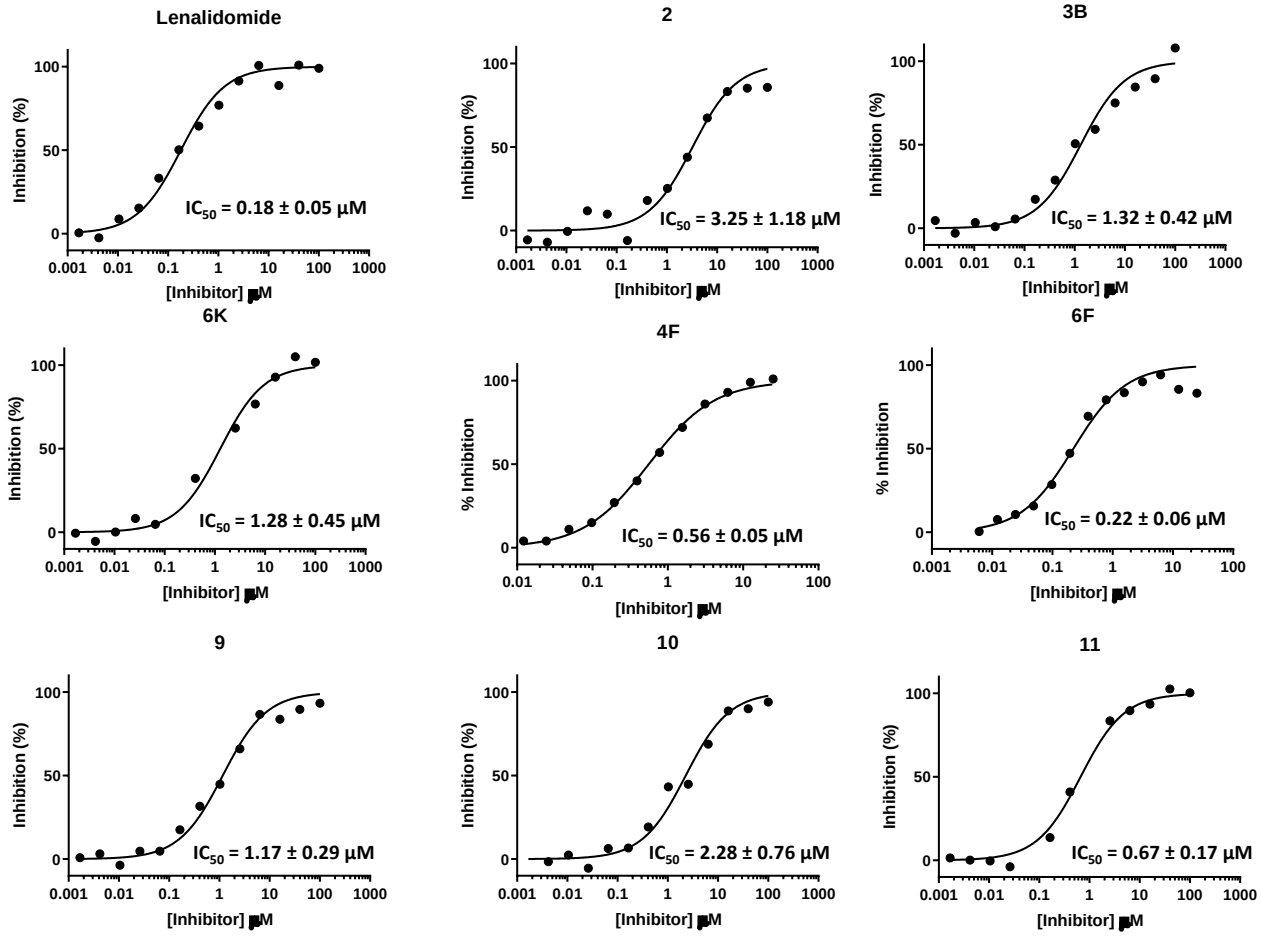


Figure S2. Dose response of selected compound by fluorescence polarization assay

$K_i = IC_{50}/(1 + ([L]/K_D))$,[7]
where
[L] = the concentration of labeled ligand,
K_i = the inhibition constant, defined as the equilibrium concentration of competitive inhibitor that would occupy 50% of receptor sites if no competing labeled ligand was present,
IC_{50} = the concentration of competitive inhibitor that displaces 50% of the specifically bound labeled ligand, and
K_D = the affinity constant, defined as the equilibrium concentration of labeled ligand that occupies 50% of receptor sites in the absence of competition. K_D = 122 nM [8]
$K_d/K_i = IC_{50}/ (1 + 8\ nM/122\ nM) = IC_{50}/1.066$

References

(1) Šali, A.; Blundell, T. L. Comparative Protein Modelling by Satisfaction of Spatial Restraints. *J. Mol. Biol.* **1993**, *234* (3), 779–815. https://doi.org/10.1006/jmbi.1993.1626.

(2) O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open Babel: An Open Chemical Toolbox. *J. Cheminformatics* **2011**, *3* (1), 33. https://doi.org/10.1186/1758-2946-3-33.

(3) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and Validation of a Genetic Algorithm for Flexible Docking11Edited by F. E. Cohen. *J. Mol. Biol.* **1997**, *267* (3), 727–748. https://doi.org/10.1006/jmbi.1996.0897.

(4) D.A. Case, K. Belfon, I.Y. Ben-Shalom, S.R. Brozell, D.S. Cerutti, T.E. Cheatham, III, V.W.D. Cruzeiro, T.A. Darden, R.E. Duke, G. Giambasu, M.K. Gilson, H. Gohlke, A.W. Goetz,R Harris, S. Izadi, S.A. Izmailov, K. Kasavajhala, A. Kovalenko, R. Krasny, T. Kurtzman, T.S. Lee, S. LeGrand, P. Li, C. Lin, J. Liu, T. Luchko, R. Luo, V. Man, K.M. Merz, Y. Miao, O. Mikhailovskii, G. Monard, H. Nguyen, A. Onufriev, F. Pan, S. Pantano, R. Qi, D.R. Roe, A. Roitberg, C. Sagui, S. Schott-Verdugo, J. Shen, C.L. Simmerling, N.R. Skrynnikov, J. Smith, J. Swails, R.C. Walker, J. Wang, L. Wilson, R.M. Wolf, X. Wu, Y. Xiong, Y. Xue, D.M. York and P.A. Kollman (2020), AMBER 2020, University of California, San Francisco.

(5) Salomon-Ferrer, R.; Götz, A. W.; Poole, D.; Le Grand, S.; Walker, R. C. Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 2. Explicit Solvent Particle Mesh Ewald. *J. Chem. Theory Comput.* **2013**, *9* (9), 3878–3888. https://doi.org/10.1021/ct400314y.

(6) Roe, D. R.; Cheatham, T. E. PTRAJ and CPPTRAJ: Software for Processing and Analysis of Molecular Dynamics Trajectory Data. *J. Chem. Theory Comput.* **2013**, *9* (7), 3084–3095. https://doi.org/10.1021/ct400341p.

(7) Newton, P.; Harrison, P.; Clulow, S. A Novel Method for Determination of the Affinity of Protein: Protein Interactions in Homogeneous Assays. *J. Biomol. Screen.* **2008**, *13* (7), 674–682. https://doi.org/10.1177/1087057108321086.

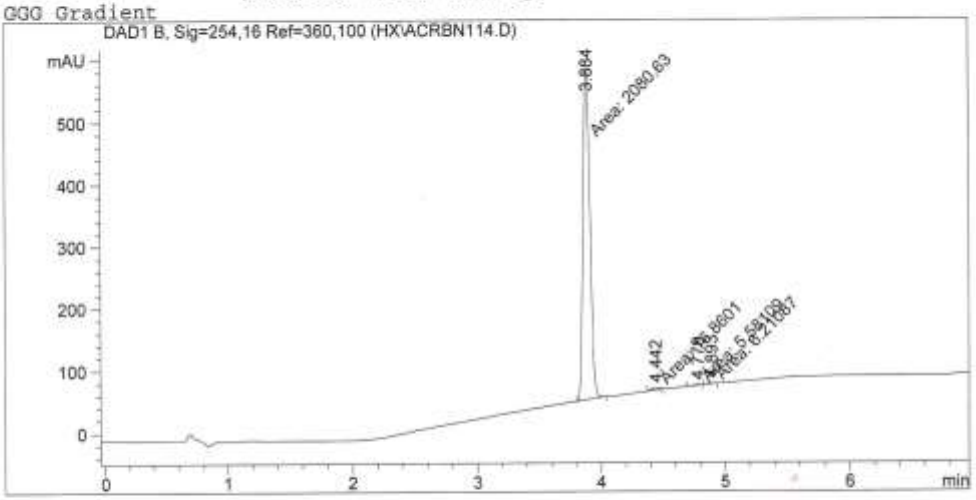
(8) Fischer, E. S.; Böhm, K.; Lydeard, J. R.; Yang, H.; Stadler, M. B.; Cavadini, S.; Nagel, J.; Serluca, F.; Acker, V.; Lingaraju, G. M.; Tichkule, R. B.; Schebesta, M.; Forrester, W. C.; Schirle, M.; Hassiepen, U.; Ottl, J.; Hild, M.; Beckwith, R. E. J.; Harper, J. W.; Jenkins, J. L.; Thomä, N. H. Structure of the DDB1–CRBN E3 Ubiquitin Ligase in Complex with Thalidomide. *Nature* **2014**, *512* (7512), 49. https://doi.org/10.1038/nature13527.

HPLC traces of compounds 6F, 9-11, 12A, 12B, 12C, 13, 13NT and 14

Data File D:\HPCHEM\1\DATA\HX\ACRBN114.D Sample Name: HX4285

6F

```
Injection Date  : 12/11/21 2:05:34 PM          Seq. Line :  14
Sample Name     : HX4285                        Vial :  44
Acq. Operator   : HX                            Inj :  1
                                                Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 12/10/21 1:53:38 PM by HX
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```



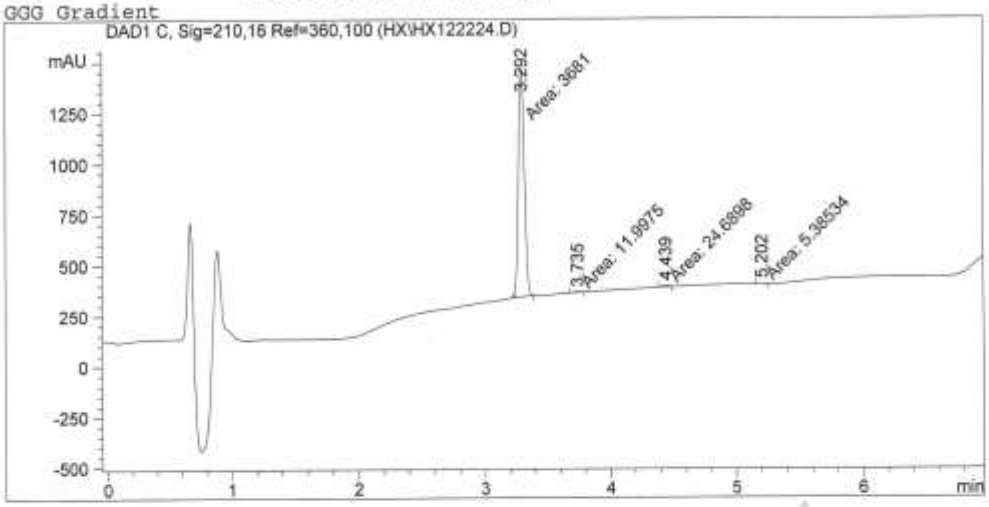

```
                        Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.884 MM    0.0645 2080.63135  537.72906  98.6416
   2   4.442 MM    0.0683   16.86013    4.11413   0.7993
   3   4.779 MM    0.0759    5.58109    1.22478   0.2646
   4   4.893 MM    0.0705    6.21087    1.46868   0.2945

Totals :                  2109.28343  544.53666

 Results obtained with enhanced integrator!

                        Summed Peaks Report

Signal 1: DAD1 B, Sig=254,16 Ref=360,100

                      Final Summed Peaks Report

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
                        *** End of Report ***
```

Data File D:\HPCHEM\1\DATA\HX\HX122224.D

Sample Name: HX5044

9

```
==================================================================
Injection Date  : 12/22/21 2:54:47 PM          Seq. Line :  24
Sample Name     : HX5044                            Vial :  43
Acq. Operator   : HX                                 Inj :   1
                                               Inj Volume : 10 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 12/10/21 1:53:38 PM by HX
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```

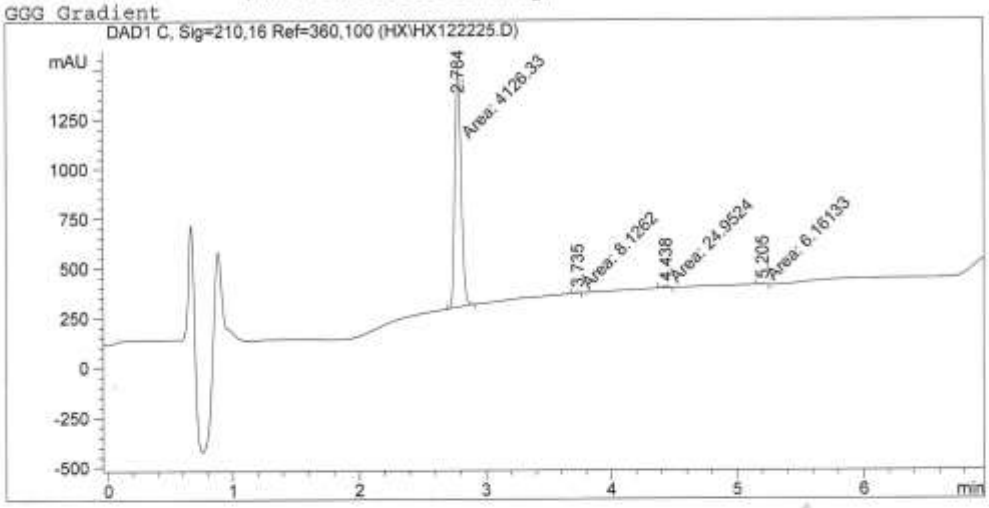



```
==================================================================
                      Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 C, Sig=210,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.292 MM    0.0540 3681.00220 1135.43750  98.8700
   2   3.735 MM    0.0601   11.99747    3.32725   0.3222
   3   4.439 MM    0.0602   24.68976    6.83894   0.6632
   4   5.202 MM    0.0546    5.38534    1.64315   0.1446

Totals :                  3723.07477 1147.24684

 Results obtained with enhanced integrator!
==================================================================
                      Summed Peaks Report
==================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
==================================================================
                    Final Summed Peaks Report
==================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
                      *** End of Report ***
```

Data File D:\HPCHEM\1\DATA\HX\HX122225.D Sample Name: HX5045

10

```
==================================================================
Injection Date  : 12/22/21 3:03:16 PM          Seq. Line :  25
Sample Name     : HX5045                         Vial :  44
Acq. Operator   : HX                              Inj :   1
                                               Inj Volume : 10 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 12/10/21 1:53:38 PM by HX
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```




```
==================================================================
                      Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
```

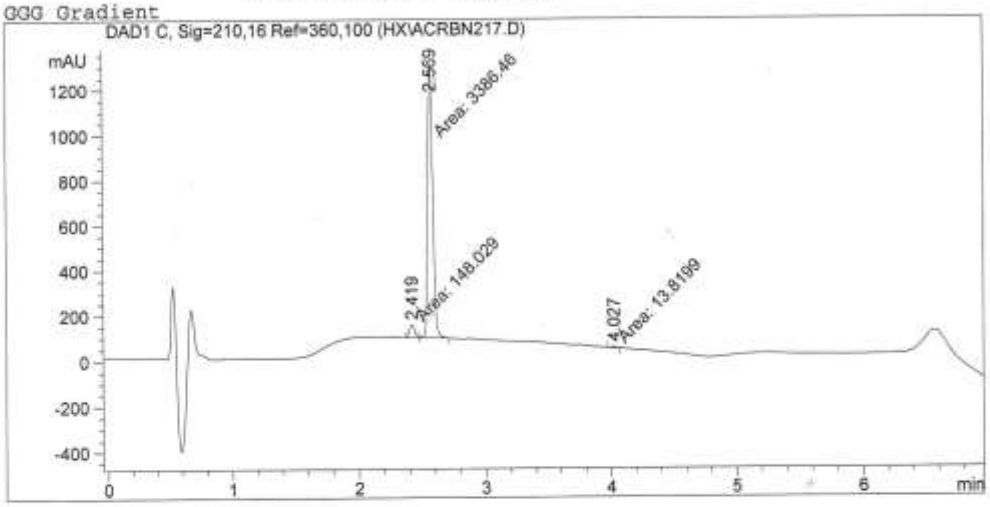
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.784	MM	0.0569	4126.32520	1208.36877	99.0580
2	3.735	MM	0.0452	8.12620	2.99556	0.1951
3	4.438	MM	0.0600	24.95240	6.92864	0.5990
4	5.205	MM	0.0558	6.16133	1.83906	0.1479
Totals :				4165.56513	1220.13204	

```
 Results obtained with enhanced integrator!
==================================================================
                      Summed Peaks Report
==================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
==================================================================
                    Final Summed Peaks Report
==================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
                      *** End of Report ***
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN217.D Sample Name: HX5048

11

```
==========================================================================
Injection Date  : 11/22/22 4:10:37 PM          Seq. Line :  18
Sample Name     : HX5048                            Vial :  17
Acq. Operator   : HX                                 Inj :   1
                                               Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```

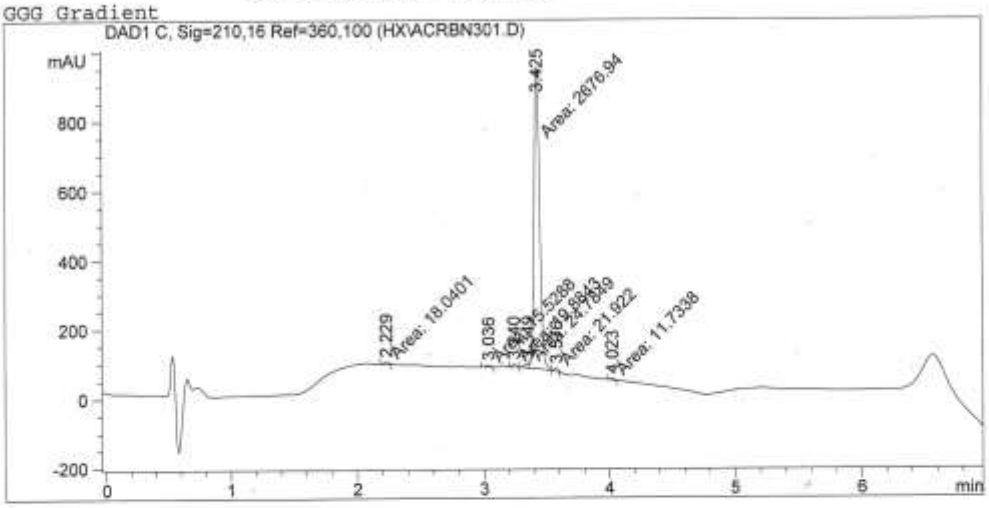



```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000


Signal 1: DAD1 C, Sig=210,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.419 MM    0.0446  148.02930   55.32790   4.1718
   2   2.569 FM    0.0466 3386.45557 1212.02075  95.4387
   3   4.027 MM    0.0468   13.81989    4.91879   0.3895

Totals :                  3548.30475 1272.26744

 Results obtained with enhanced integrator!
==========================================================================
                        Summed Peaks Report
==========================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
==========================================================================
                      Final Summed Peaks Report
==========================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
                        *** End of Report ***
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN301.D Sample Name: HX5022

IZA

```
==========================================================================
Injection Date  : 11/22/22 5:52:23 PM          Seq. Line :   2
Sample Name     : HX5022                            Vial :  23
Acq. Operator   : HX                                 Inj :   1
                                                Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 1 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
```

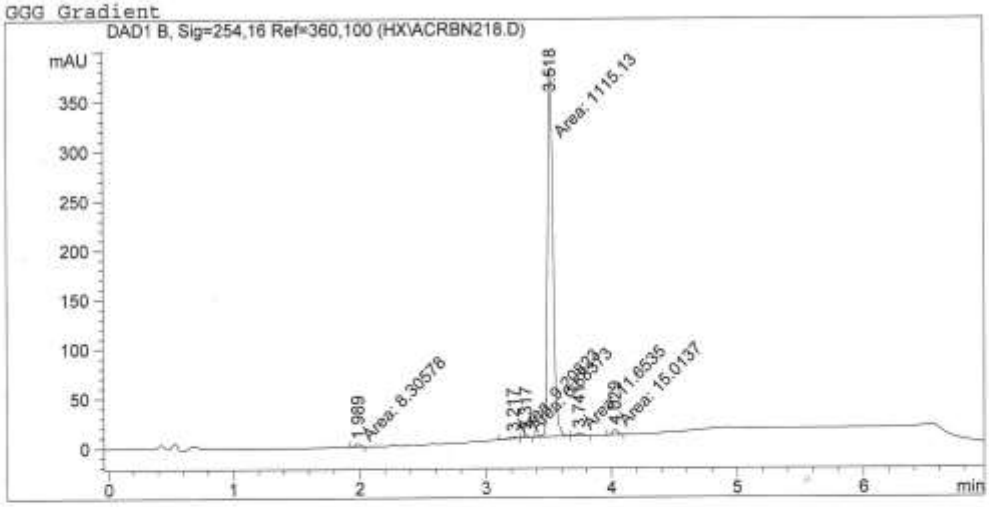
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.229	MM	0.0435	18.04005	6.90549	0.6469
2	3.036	MM	0.0536	15.52878	4.82857	0.5568
3	3.240	MM	0.0439	19.88430	7.54501	0.7130
4	3.349	MF	0.0371	24.78491	11.13879	0.8887
5	3.425	FM	0.0507	2676.93555	879.61127	95.9878
6	3.576	MM	0.0401	21.92204	9.10044	0.7861
7	4.023	MM	0.0424	11.73377	4.61374	0.4207
Totals :				2788.82942	923.74332	

```
 Results obtained with enhanced integrator!
==========================================================================
                        Summed Peaks Report
==========================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
==========================================================================
                     Final Summed Peaks Report
==========================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN218.D Sample Name: HT37

12B

```
==========================================================================
Injection Date  : 11/22/22 4:19:03 PM          Seq. Line :  19
Sample Name     : HT37                             Vial :  18
Acq. Operator   : HX                               Inj :   1
                                               Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.989	MM	0.0477	8.30578	2.90192	0.7123
2	3.217	MM	0.0780	9.20823	1.96638	0.7897
3	3.317	MM	0.0438	6.68373	2.54435	0.5732
4	3.518	MM	0.0491	1115.12622	378.38632	95.6376
5	3.741	MM	0.0659	11.65351	2.94818	0.9995
6	4.029	MM	0.0492	15.01368	5.08525	1.2876
Totals :				1165.99115	393.83240	

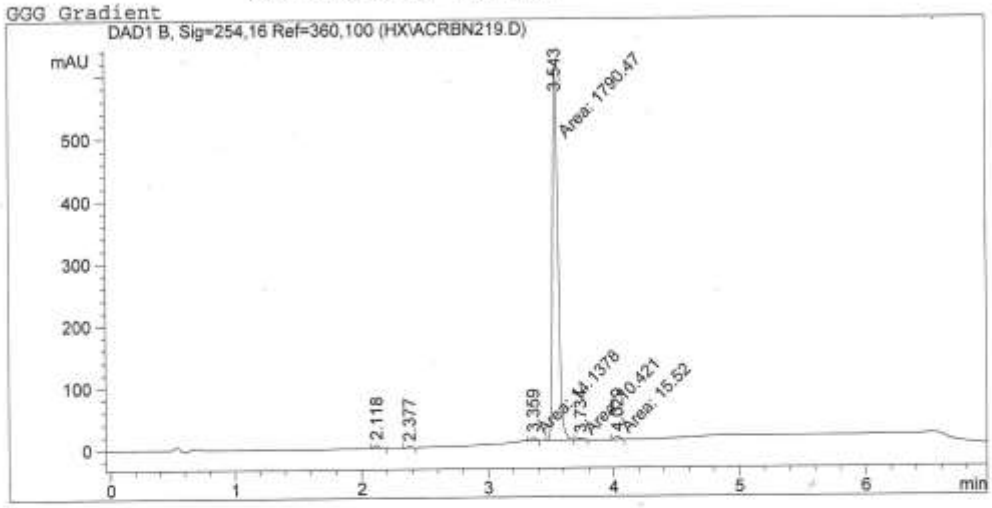
```
 Results obtained with enhanced integrator!
==========================================================================
                        Summed Peaks Report
==========================================================================

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
==========================================================================
                      Final Summed Peaks Report
==========================================================================

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN219.D

Sample Name: HT38

12C

```
==================================================================
Injection Date  : 11/22/22 4:27:36 PM          Seq. Line :  20
Sample Name     : HT38                              Vial :  19
Acq. Operator   : HX                                 Inj :   1
                                                Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```




```
==================================================================
                      Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.118	BP	0.0422	14.92668	5.77318	0.8062
2	2.377	PP	0.0418	6.10328	2.38568	0.3296
3	3.359	MM	0.0402	14.13776	5.85836	0.7636
4	3.543	MM	0.0493	1790.46948	604.77234	96.6996
5	3.734	MM	0.0570	10.42096	3.04821	0.5628
6	4.029	MM	0.0474	15.51996	5.45429	0.8382
Totals :				1851.57812	627.29205	

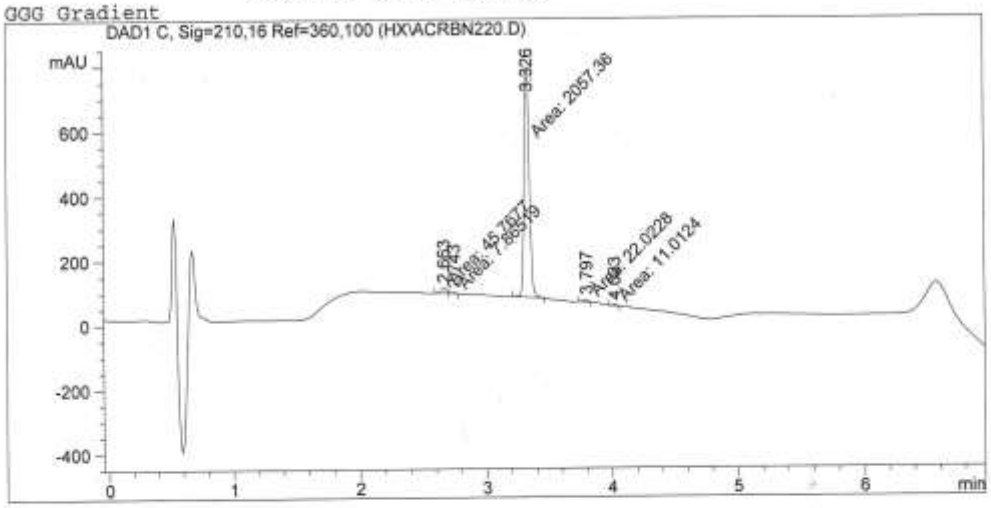
```
 Results obtained with enhanced integrator!
==================================================================
                      Summed Peaks Report
==================================================================

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
==================================================================
                    Final Summed Peaks Report
==================================================================

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN220.D

Sample Name: HX5023

13

```
==================================================================
Injection Date  : 11/22/22 4:36:02 PM          Seq. Line :  21
Sample Name     : HX5023                            Vial :  20
Acq. Operator   : HX                                 Inj :   1
                                                Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```




```
==================================================================
                      Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
```

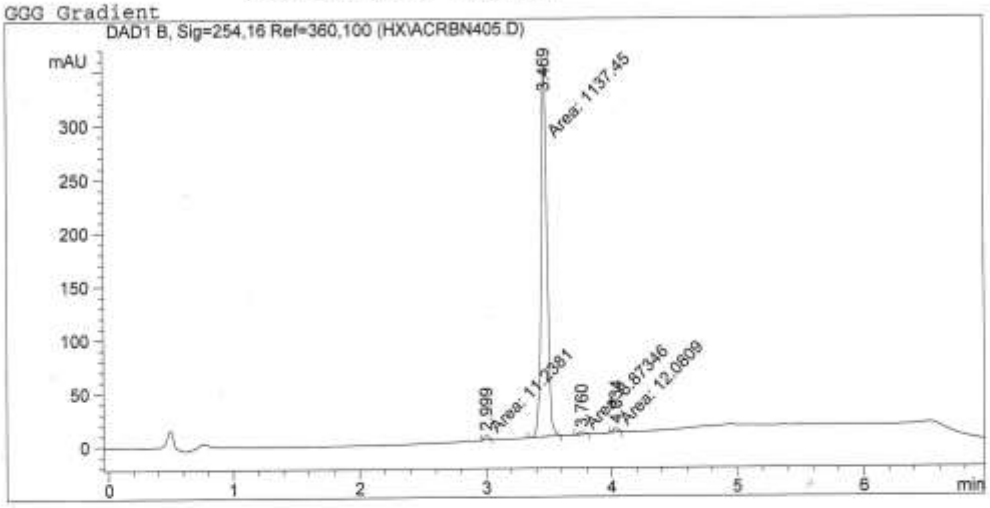
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.663	MM	0.0466	45.76773	16.35418	2.1347
2	2.743	MM	0.0395	7.86519	3.31990	0.3668
3	3.326	MM	0.0475	2057.36060	721.53906	95.9577
4	3.797	MM	0.0467	22.02277	7.86695	1.0272
5	4.023	MM	0.0441	11.01241	4.16222	0.5136
Totals :				2144.02869	753.24232	

```
 Results obtained with enhanced integrator!
==================================================================
                      Summed Peaks Report
==================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
==================================================================
                    Final Summed Peaks Report
==================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
                      *** End of Report ***
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN405.D Sample Name: HX5047

13NT

```
==========================================================================
Injection Date  : 11/23/22 2:44:30 PM          Seq. Line :   2
Sample Name     : HX5047                            Vial :   2
Acq. Operator   : HX                                 Inj :   1
                                                Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 50 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
```

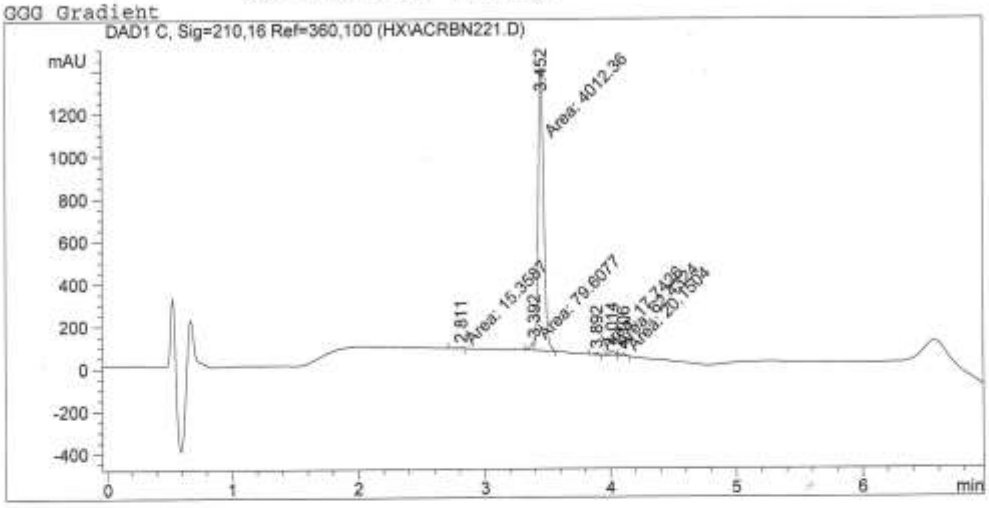
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.999	MM	0.0456	11.23807	4.11089	0.9608
2	3.469	MM	0.0542	1137.44678	349.45285	97.2477
3	3.760	MM	0.0645	8.87346	2.29280	0.7586
4	4.034	MM	0.0493	12.08090	4.08039	1.0329
Totals :				1169.63921	359.93693	

```
 Results obtained with enhanced integrator!
==========================================================================
                        Summed Peaks Report
==========================================================================

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
==========================================================================
                      Final Summed Peaks Report
==========================================================================

Signal 1: DAD1 B, Sig=254,16 Ref=360,100
                          *** End of Report ***
```

Data File D:\HPCHEM\1\DATA\HX\ACRBN221.D Sample Name: HX5025

14

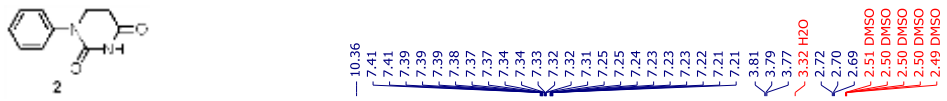
```
==========================================================================
Injection Date  : 11/22/22 4:44:31 PM          Seq. Line :  22
Sample Name     : HX5025                            Vial :  21
Acq. Operator   : HX                                 Inj :   1
                                                Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 9/12/22 1:24:01 PM by HUA
Analysis Method : D:\HPCHEM\1\METHODS\G GRADRP.M
Last changed    : 11/12/22 3:05:14 PM by Chelsi Almodovar
                  (modified after loading)
GGG Gradient
```

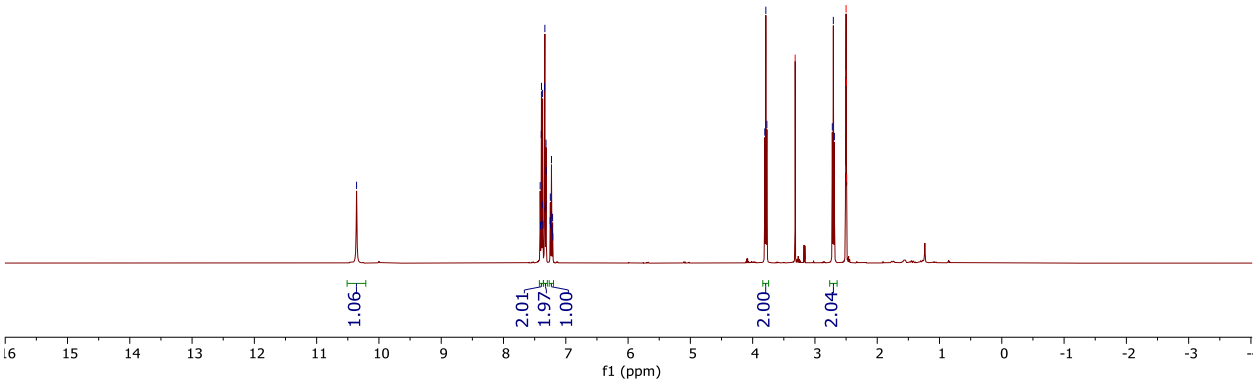


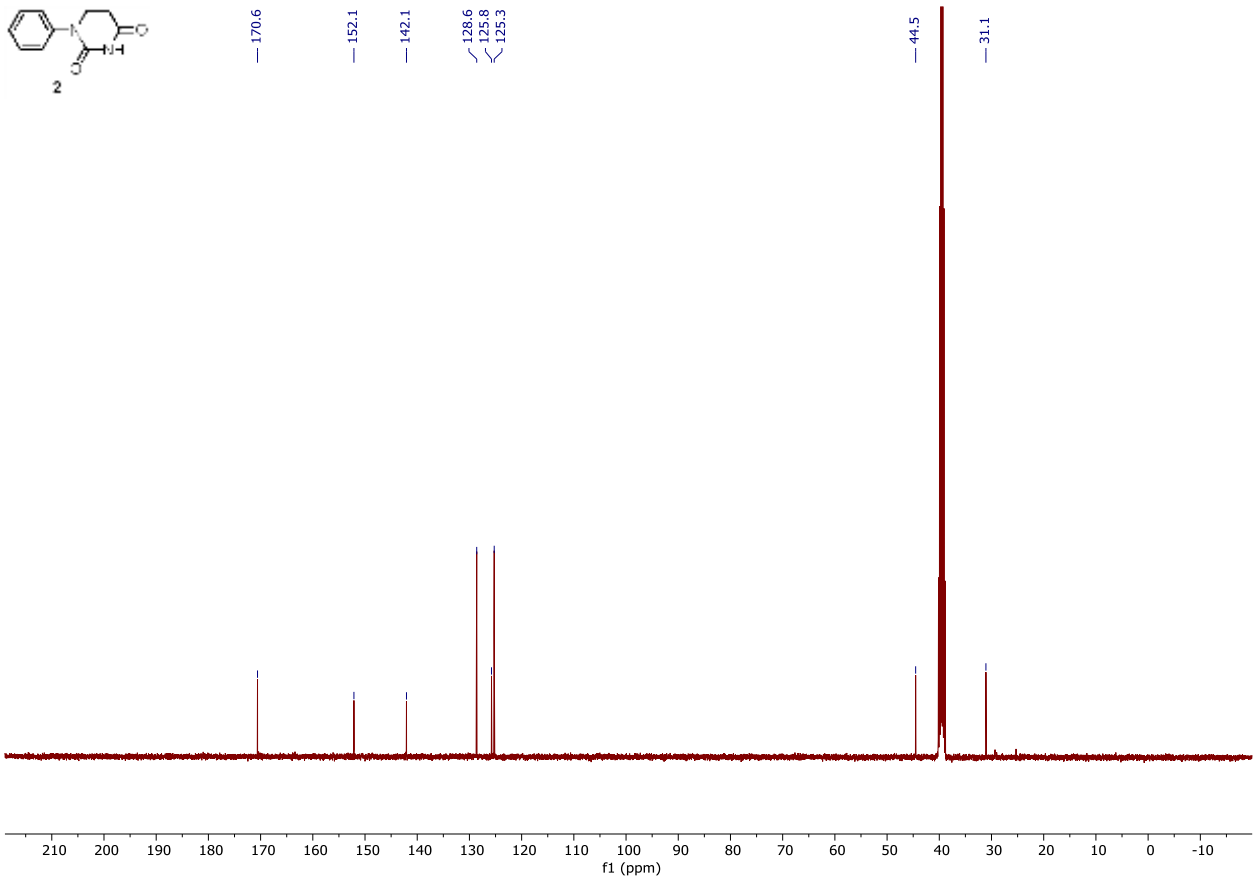

```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 C, Sig=210,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.811 MM    0.0786   15.35868    3.25481   0.3650
   2   3.392 MF    0.0361   79.60774   36.76394   1.8920
   3   3.452 FM    0.0499 4012.36084 1338.80505  95.3586
   4   3.892 MM    0.0540   17.74263    5.48084   0.4217
   5   4.014 MM    0.0496   62.43245   20.99657   1.4838
   6   4.106 MM    0.0460   20.15042    7.29313   0.4789

Totals :                  4207.65276 1412.59434

 Results obtained with enhanced integrator!
==========================================================================
                        Summed Peaks Report
==========================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
==========================================================================
                     Final Summed Peaks Report
==========================================================================

Signal 1: DAD1 C, Sig=210,16 Ref=360,100
```

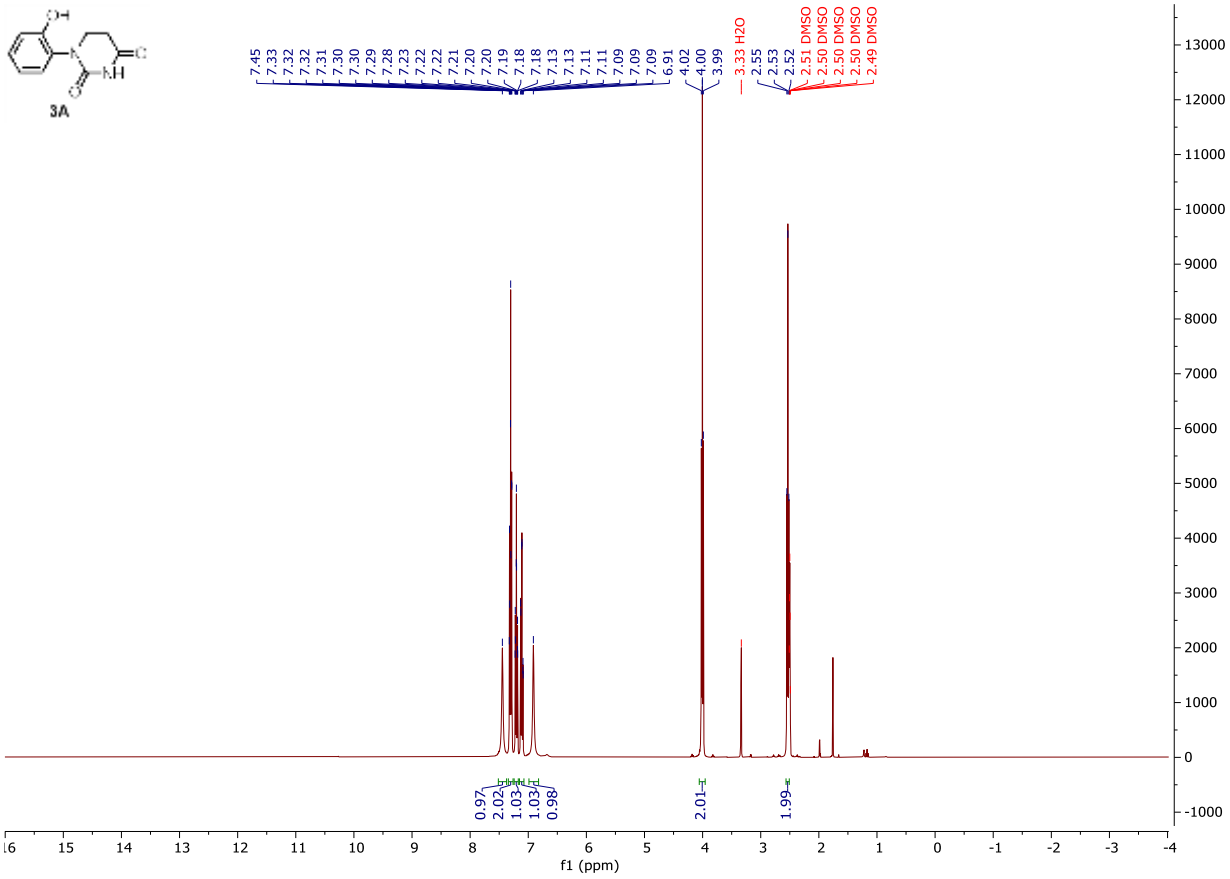
instrument 1 11/22/22 5:29:34 PM Chelsi Almodovar Page 1 of 1

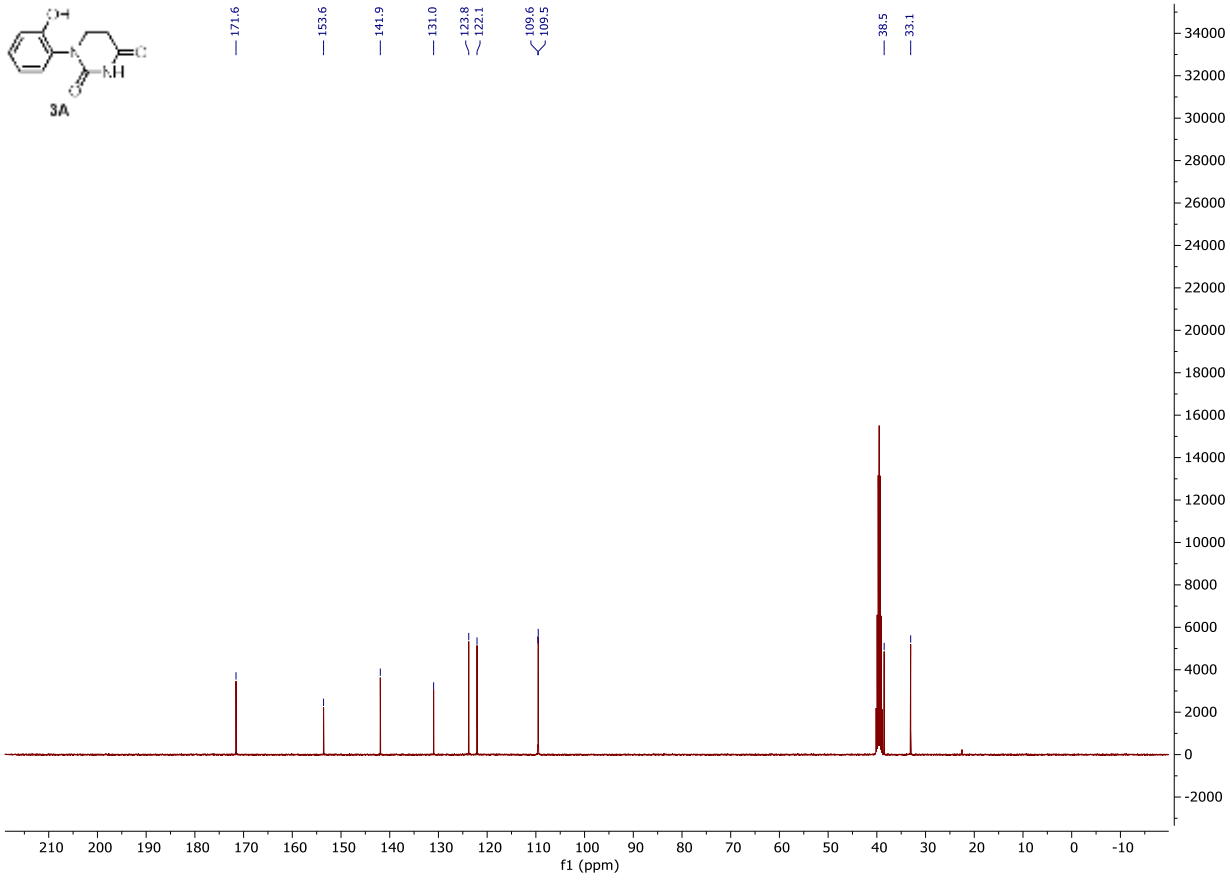
^{1}H and ^{13}C NMR spectrum

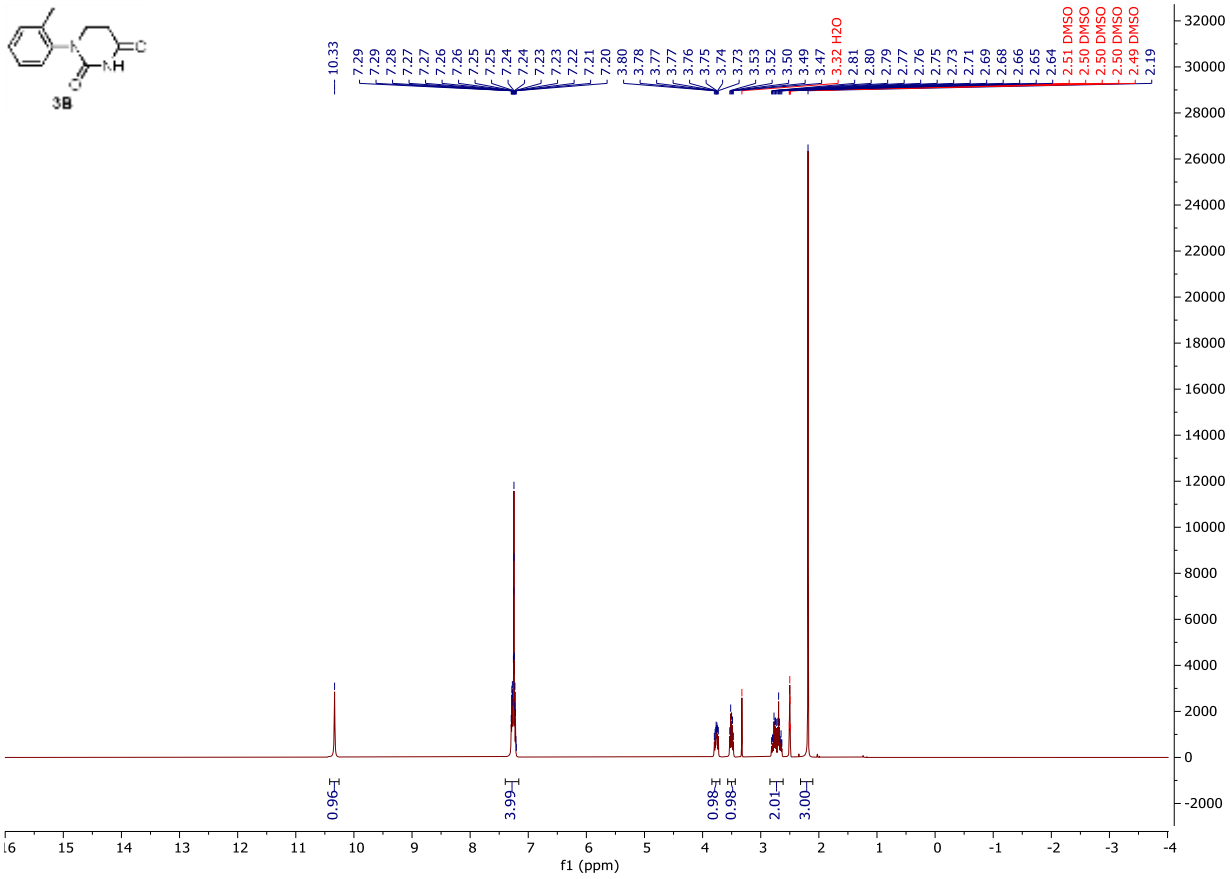


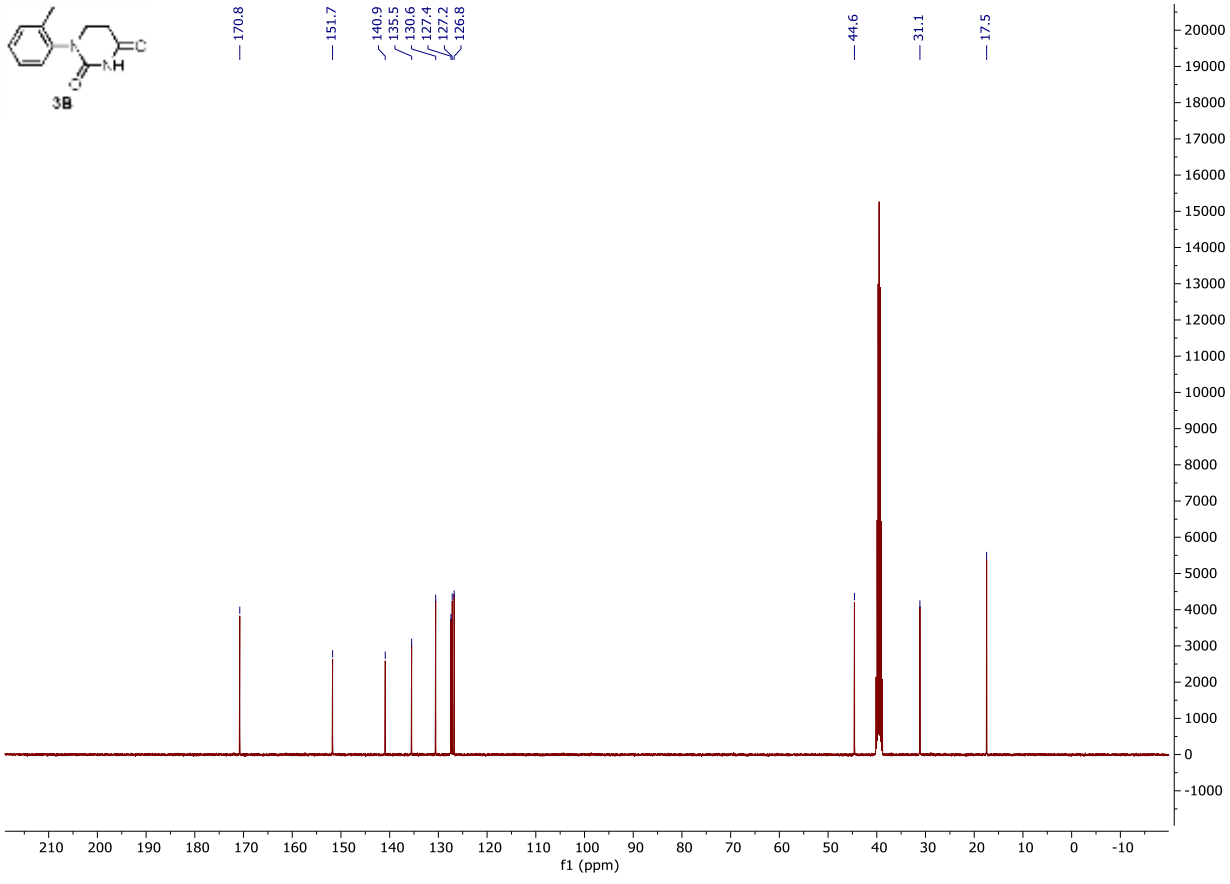


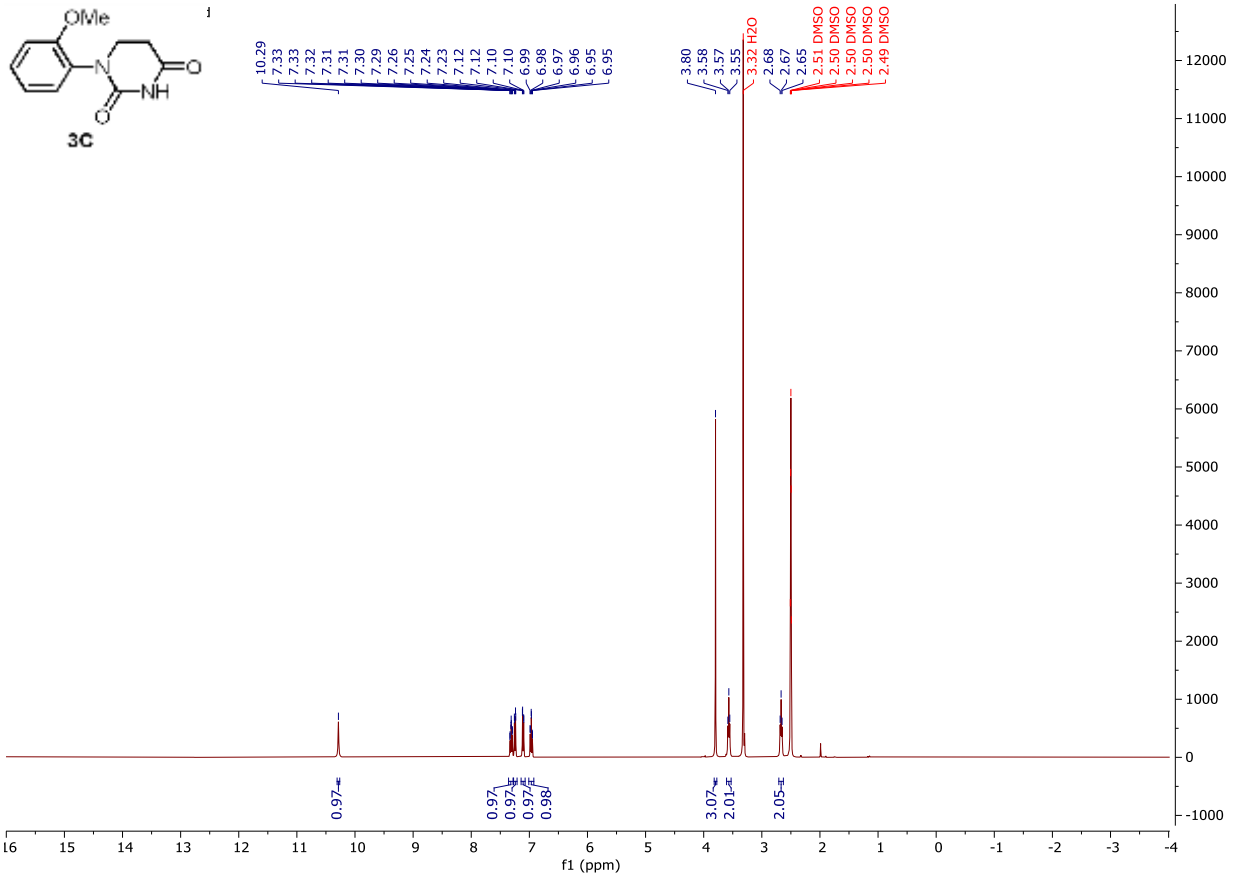


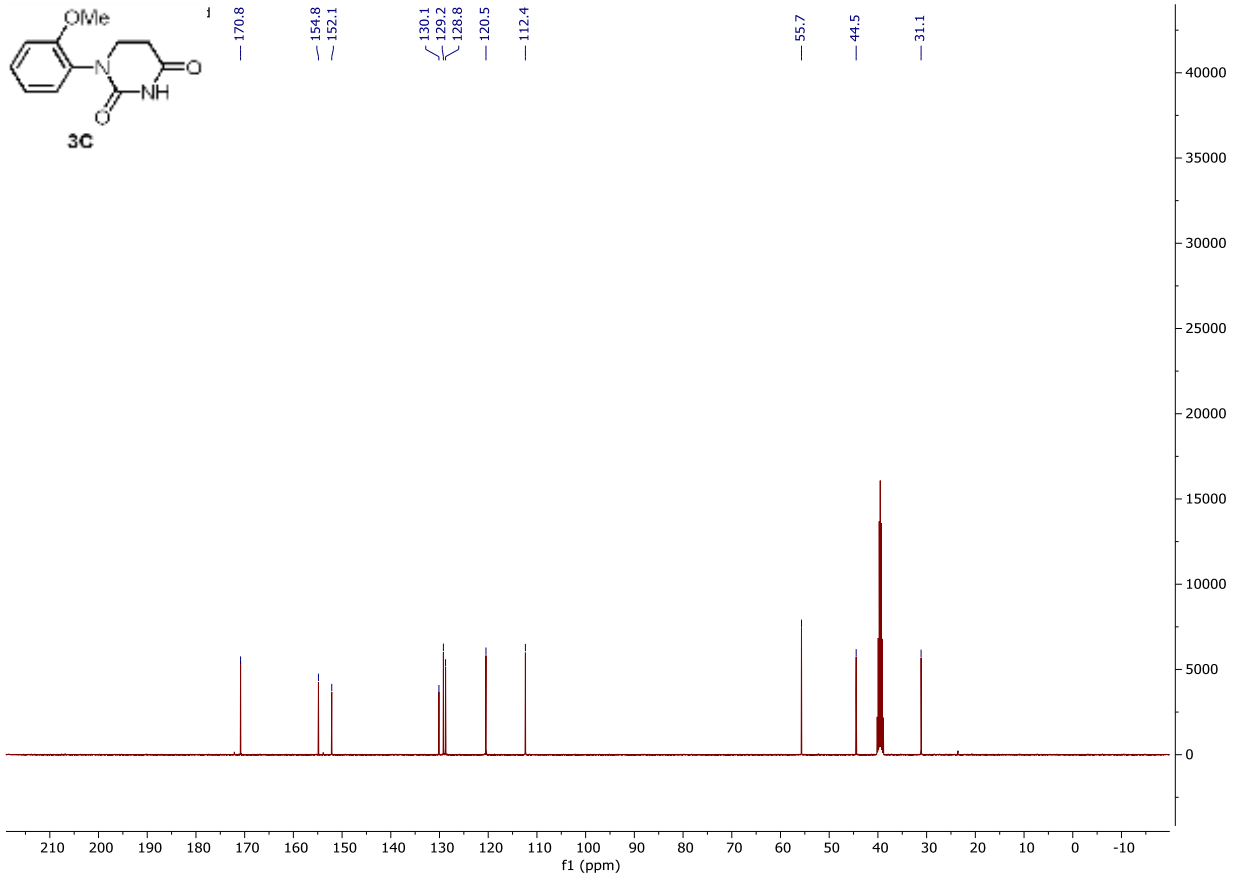

3A
7.45
7.33
7.32
7.32
7.31
7.30
7.30
7.29
7.28
7.23
7.22
7.22
7.21
7.20
7.20
7.19
7.18
7.18
7.13
7.13
7.11
7.11
7.09
7.09
7.09
6.91
4.02
4.00
3.99
3.33 H2O
2.55
2.53
2.52
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
0.97
2.02
1.03
1.03
0.98
2.01
1.99
f1 (ppm)

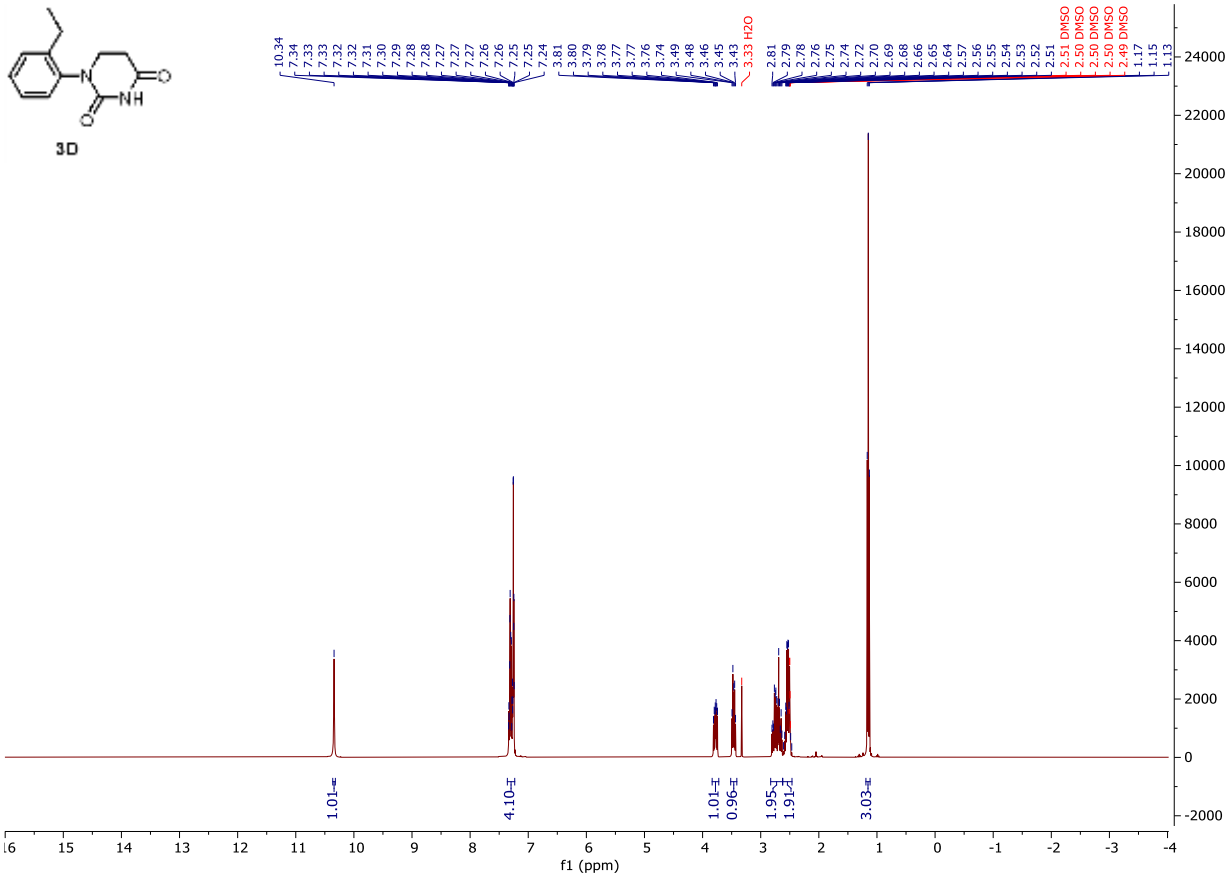

3A
171.6
153.6
141.9
131.0
123.8
122.1
109.6
109.5
38.5
33.1
f1 (ppm)

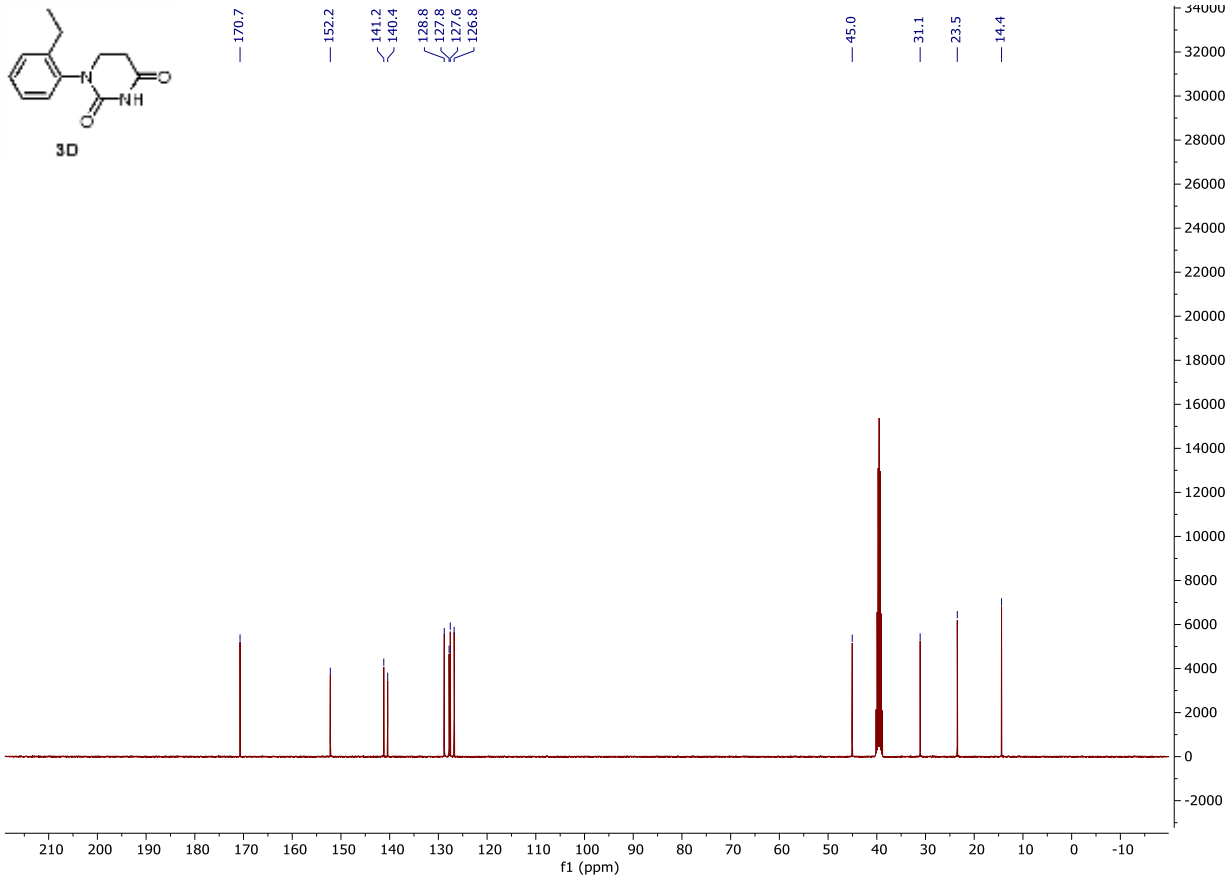

3B
10.33
7.29
7.29
7.28
7.27
7.27
7.26
7.26
7.25
7.25
7.24
7.24
7.23
7.23
7.22
7.21
7.20
3.80
3.78
3.77
3.77
3.76
3.75
3.74
3.73
3.53
3.52
3.50
3.49
3.47
3.32 H2O
2.81
2.80
2.79
2.77
2.76
2.75
2.73
2.71
2.69
2.68
2.66
2.65
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.19
0.96
3.99
0.98
0.98
2.01
3.00
f1 (ppm)

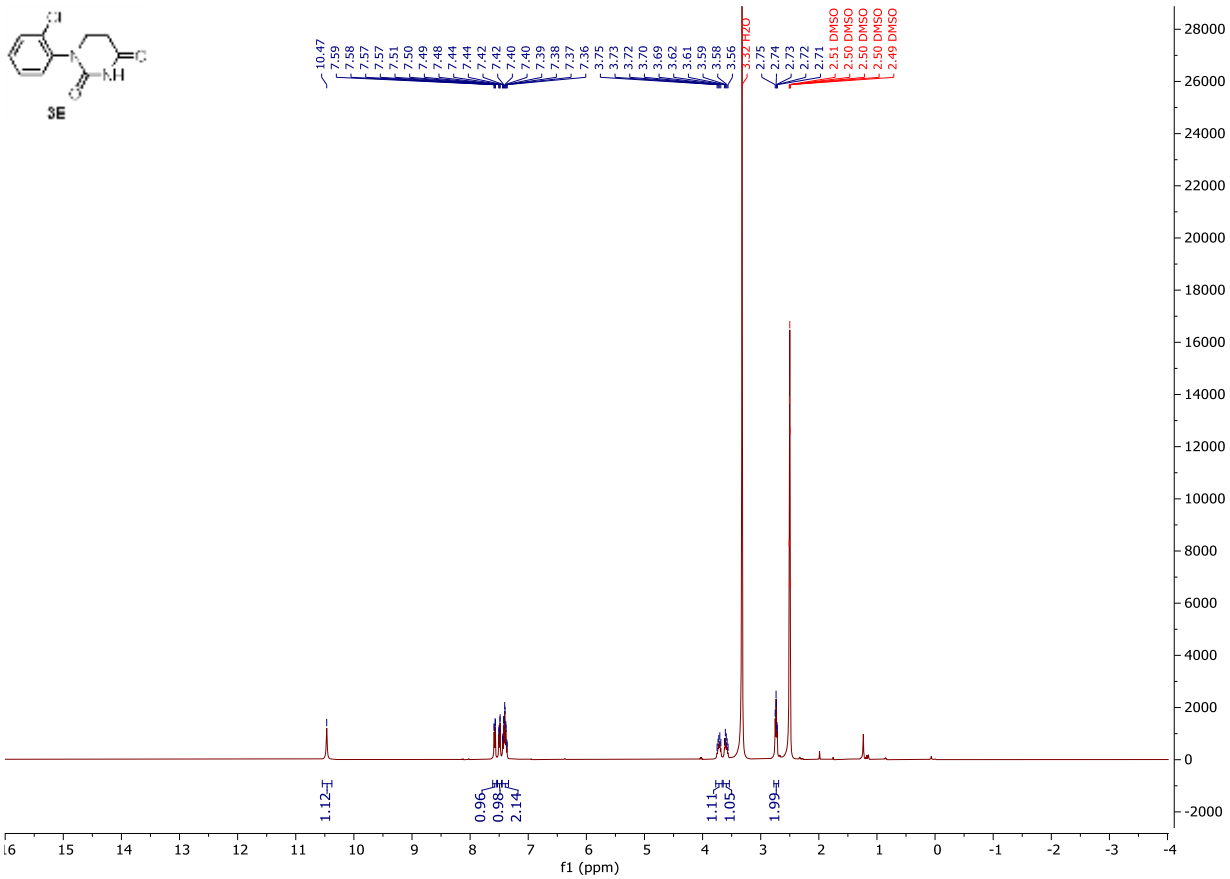

3B
170.8
151.7
140.9
135.5
130.6
127.4
127.2
126.8
44.6
31.1
17.5
f1 (ppm)

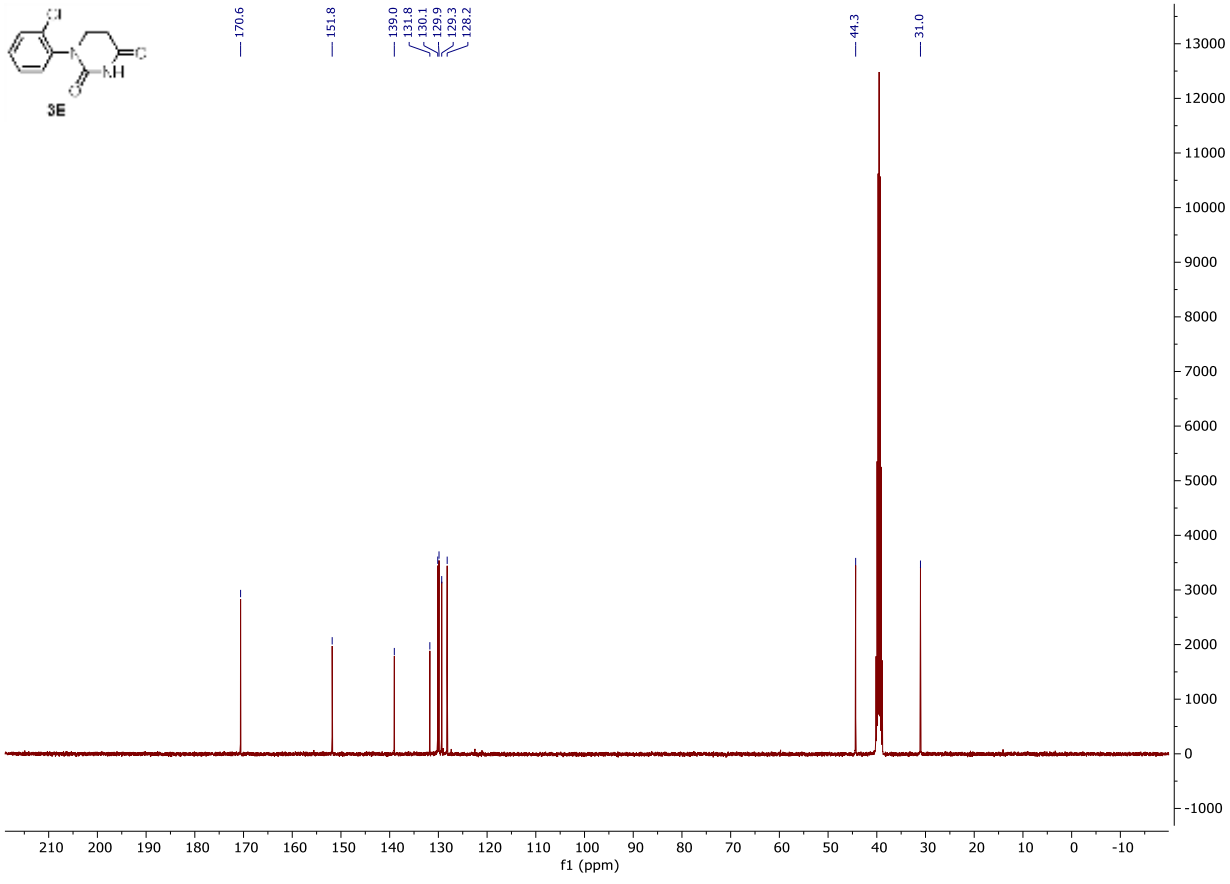

OMe
N
O
NH
O
3C
10.29
7.33
7.33
7.32
7.31
7.31
7.30
7.29
7.26
7.25
7.24
7.23
7.12
7.12
7.10
7.10
6.99
6.98
6.97
6.96
6.96
6.95
6.95
3.80
3.58
3.57
3.55
3.32 H2O
2.68
2.67
2.65
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
0.97
0.97
0.97
0.97
0.98
3.07
2.01
2.05
f1 (ppm)

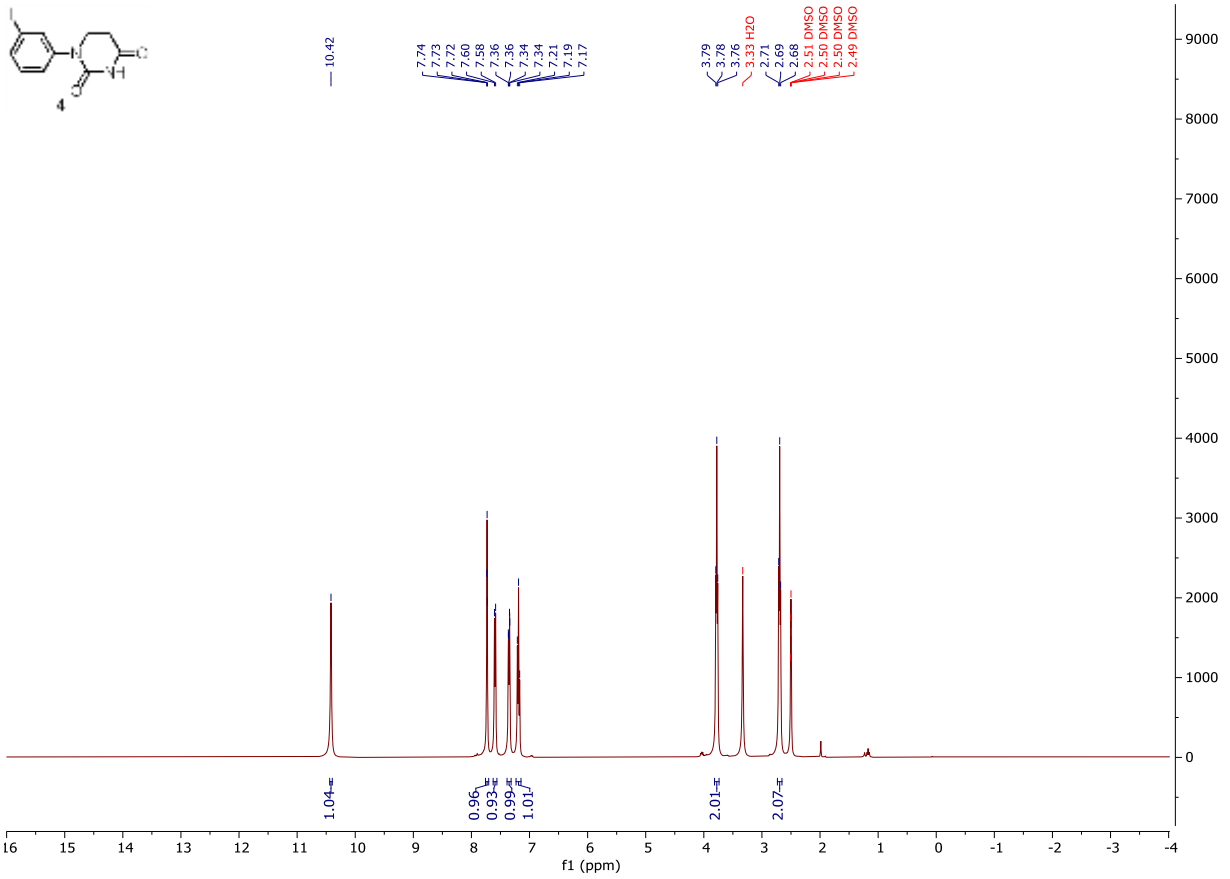

OMe
N
O
NH
O
3C
170.8
154.8
152.1
130.1
129.2
128.8
120.5
112.4
55.7
44.5
31.1
f1 (ppm)

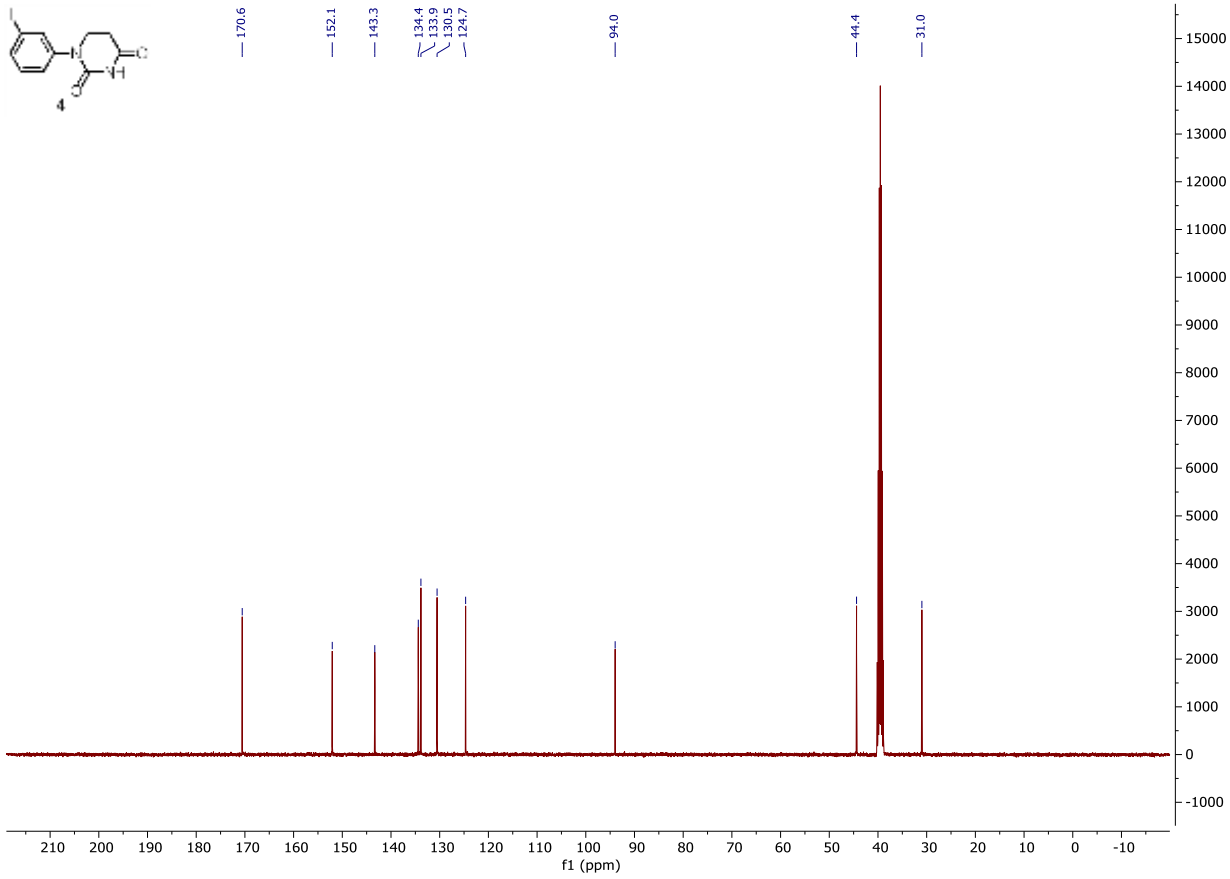

3D
10.34
7.34
7.33
7.33
7.32
7.32
7.31
7.30
7.29
7.28
7.28
7.27
7.27
7.27
7.26
7.26
7.25
7.25
7.24
3.81
3.80
3.79
3.78
3.77
3.77
3.76
3.74
3.49
3.48
3.46
3.45
3.43
3.33 H2O
2.81
2.79
2.78
2.76
2.75
2.74
2.72
2.70
2.69
2.68
2.66
2.65
2.64
2.57
2.56
2.55
2.54
2.53
2.52
2.51
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.17
1.15
1.13
1.01
4.10
1.01
0.96
1.95
1.91
3.03
f1 (ppm)

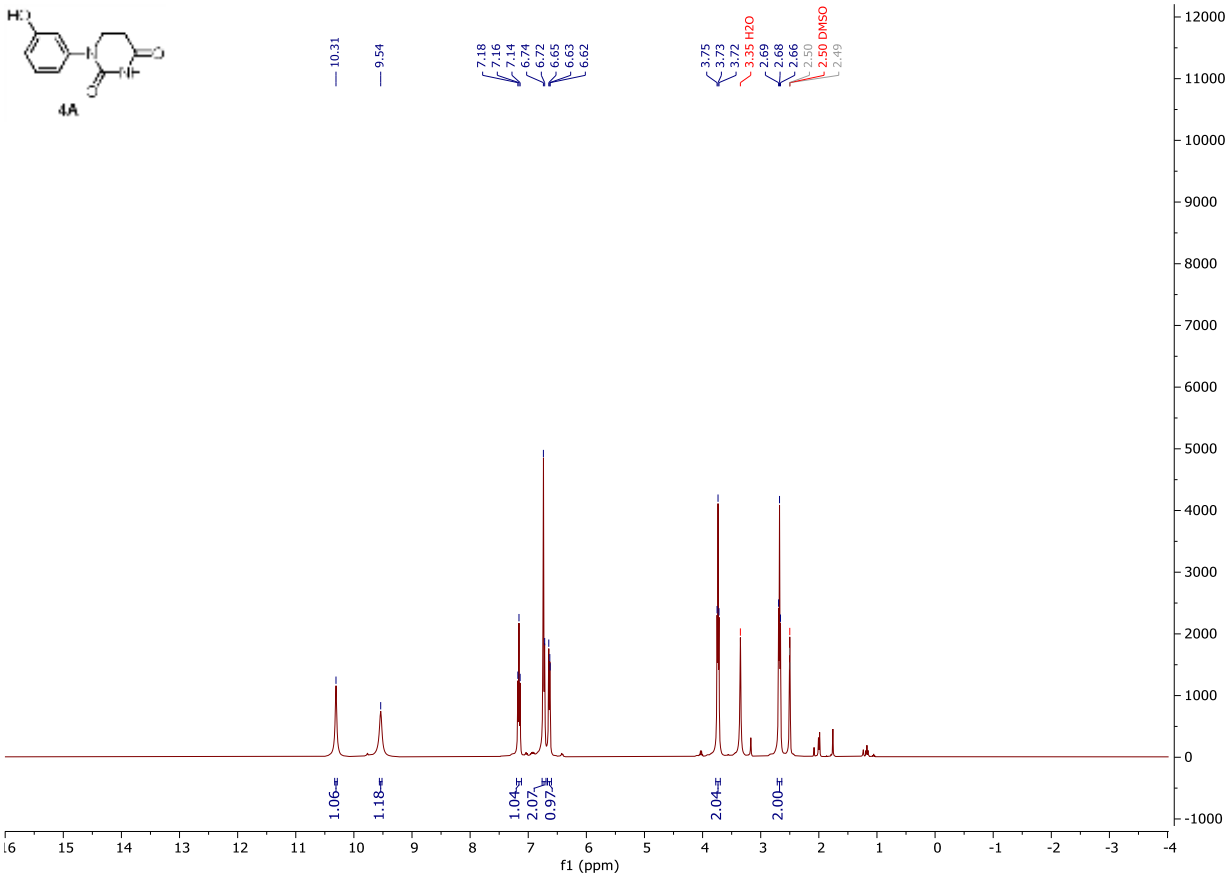

3D
170.7
152.2
141.2
140.4
128.8
127.8
127.6
126.8
45.0
31.1
23.5
14.4
f1 (ppm)

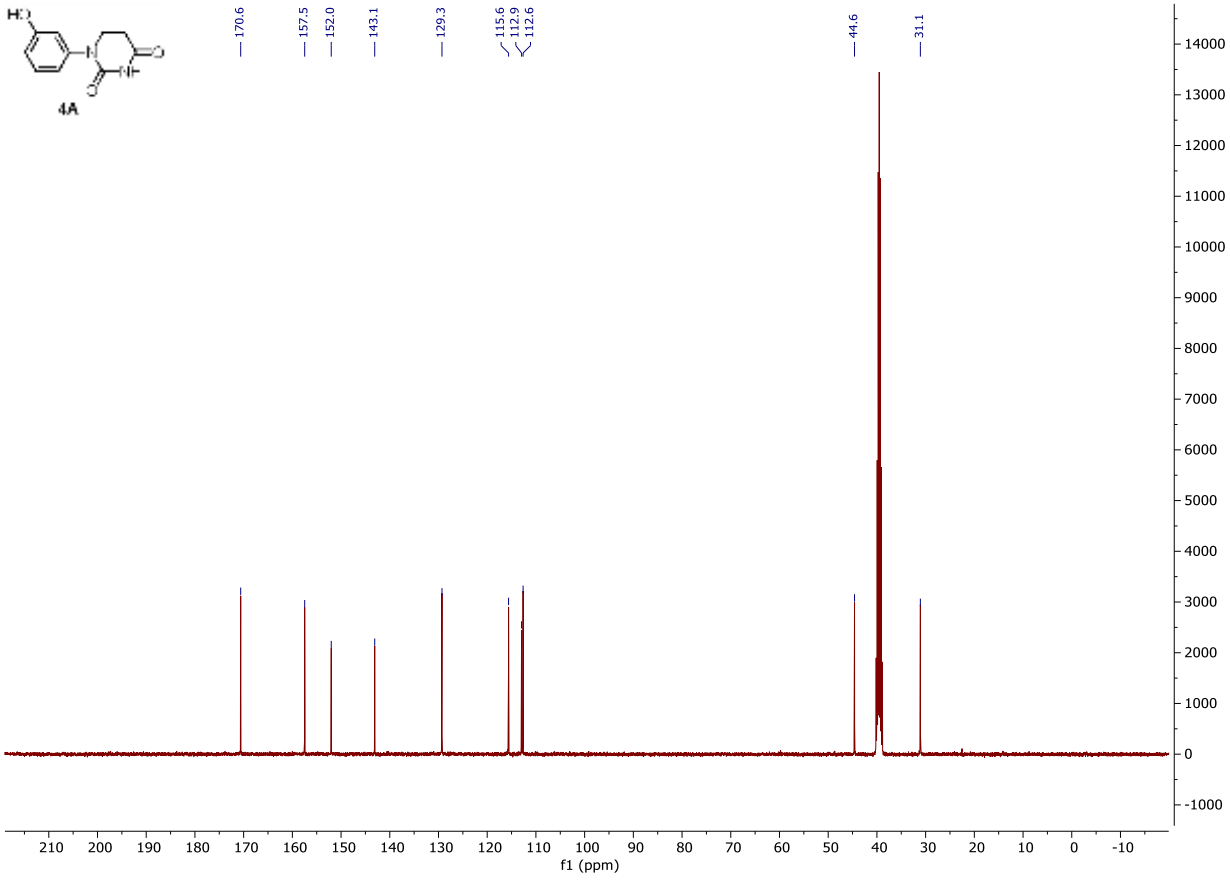

3E
10.47
7.59
7.58
7.57
7.57
7.51
7.50
7.49
7.48
7.44
7.44
7.42
7.42
7.40
7.40
7.39
7.38
7.37
7.36
3.75
3.73
3.72
3.70
3.69
3.62
3.61
3.59
3.58
3.56
3.32 H2O
2.75
2.74
2.73
2.72
2.71
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.12
0.96
0.98
2.14
1.11
1.05
1.99
f1 (ppm)

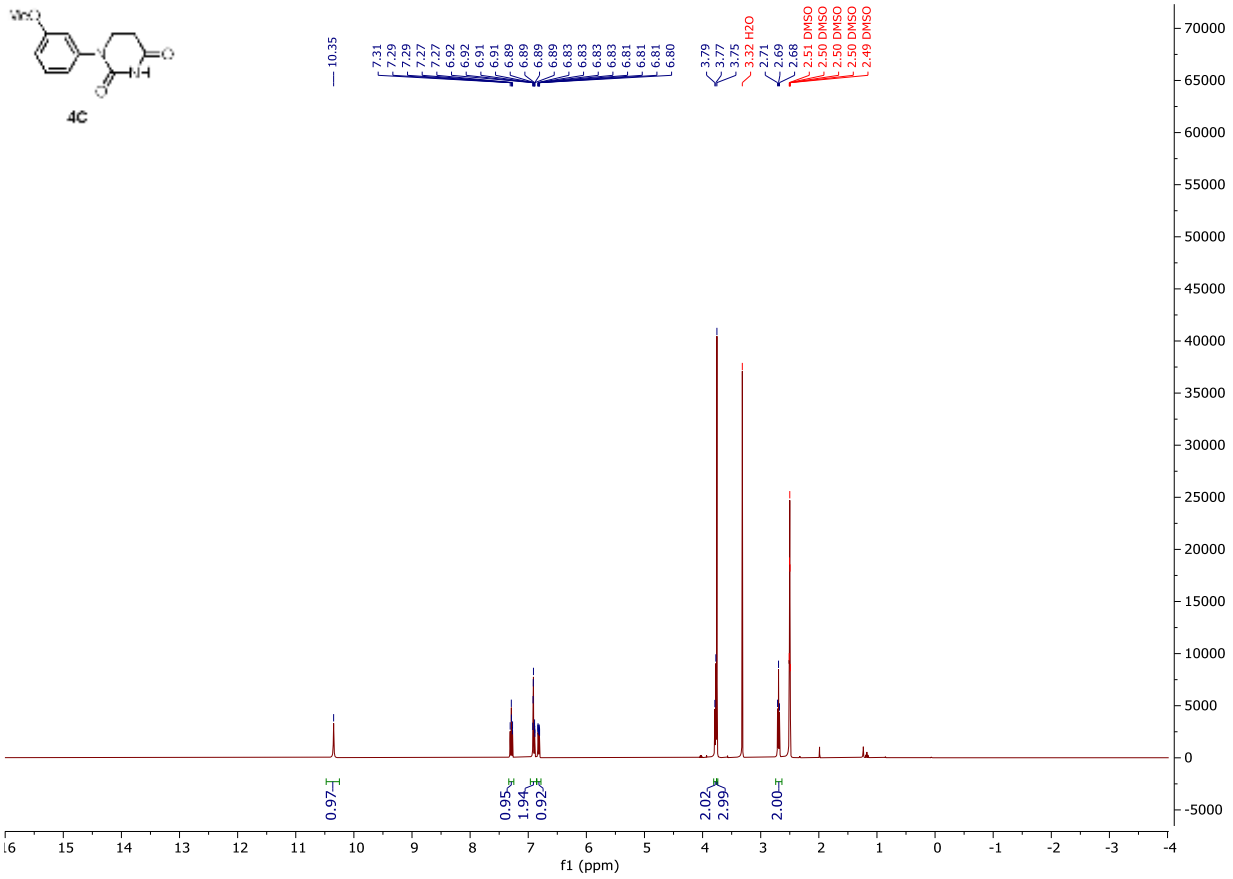

3E
170.6
151.8
139.0
131.8
130.1
129.9
129.3
128.2
44.3
31.0
f1 (ppm)

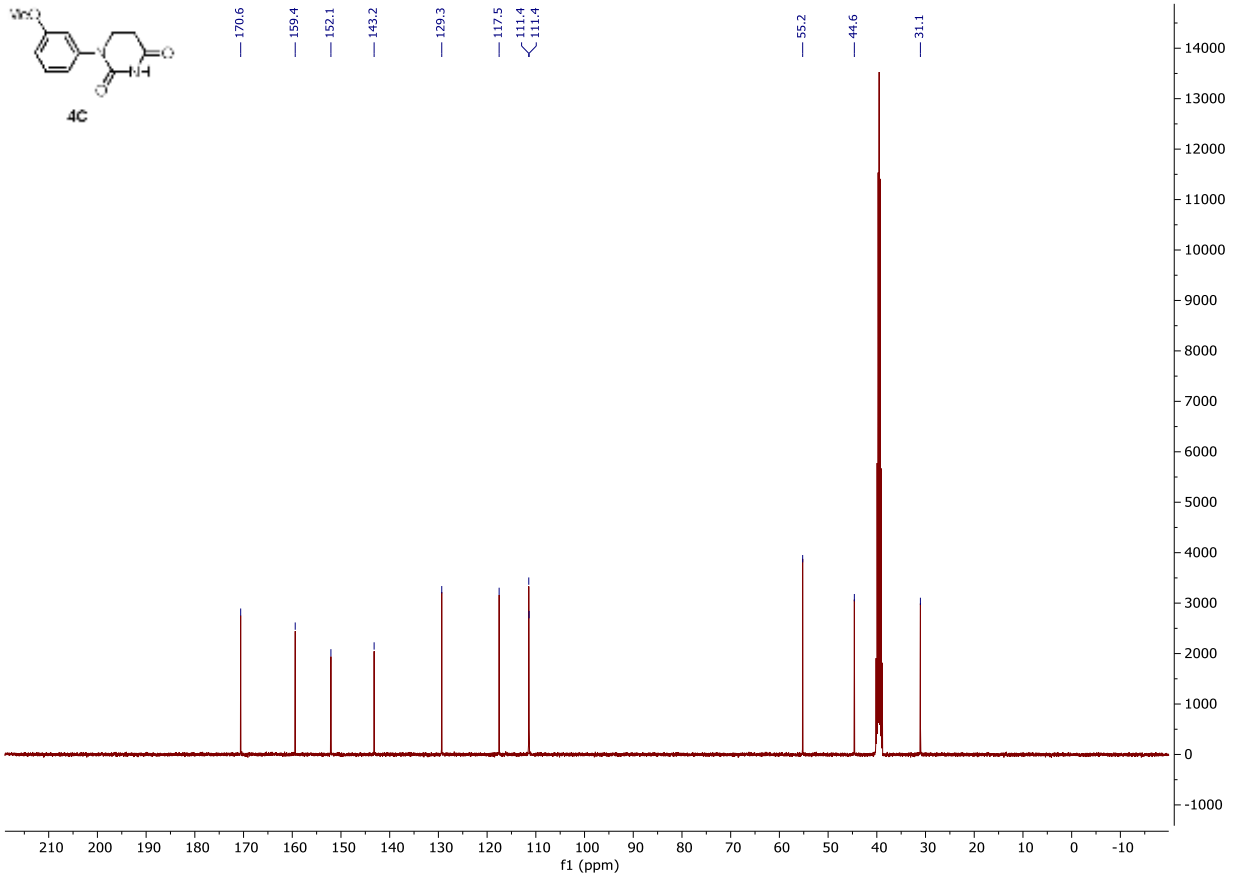

4
10.42
7.74
7.73
7.72
7.60
7.58
7.36
7.36
7.34
7.34
7.21
7.19
7.17
3.79
3.78
3.76
3.33 H2O
2.71
2.69
2.68
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.04
0.96
0.93
0.99
1.01
2.01
2.07
f1 (ppm)

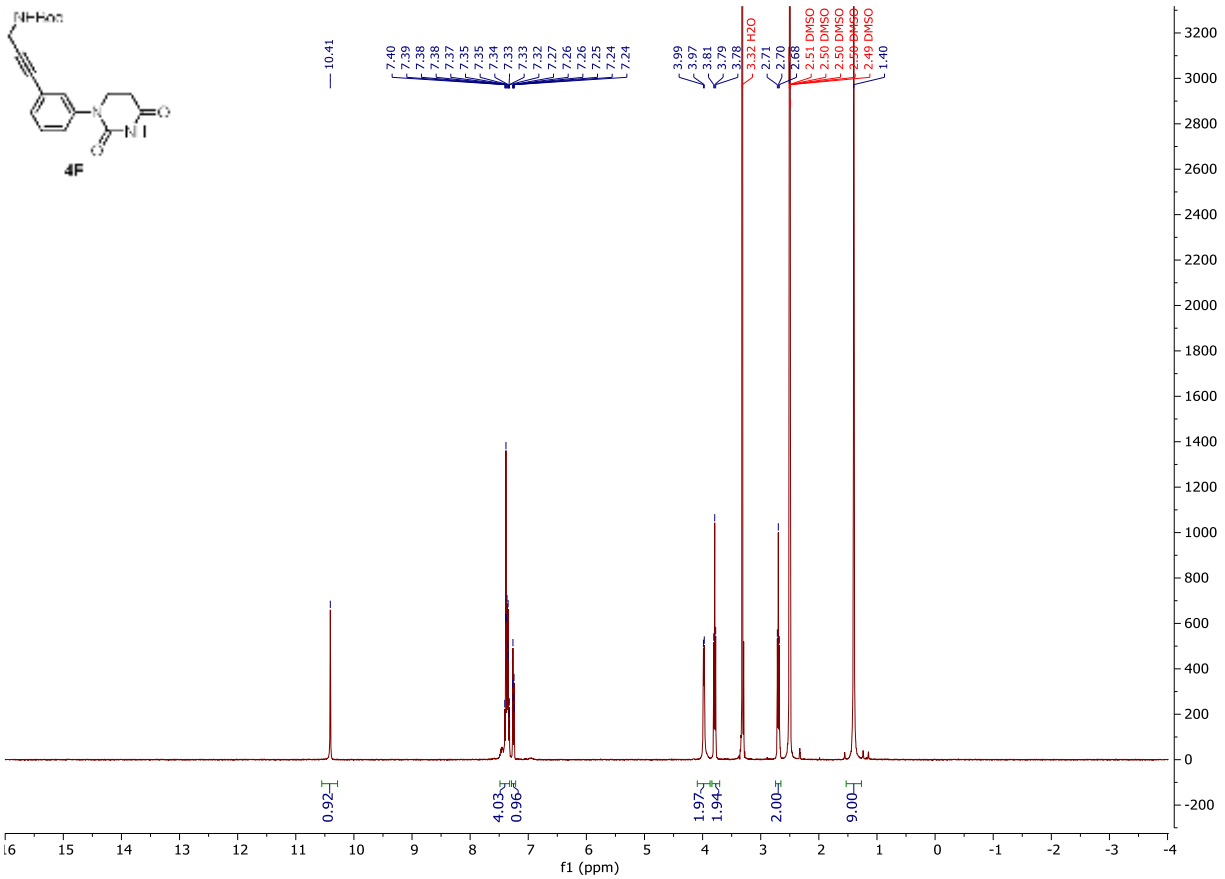

4
170.6
152.1
143.3
134.4
133.9
130.5
124.7
94.0
44.4
31.0
f1 (ppm)

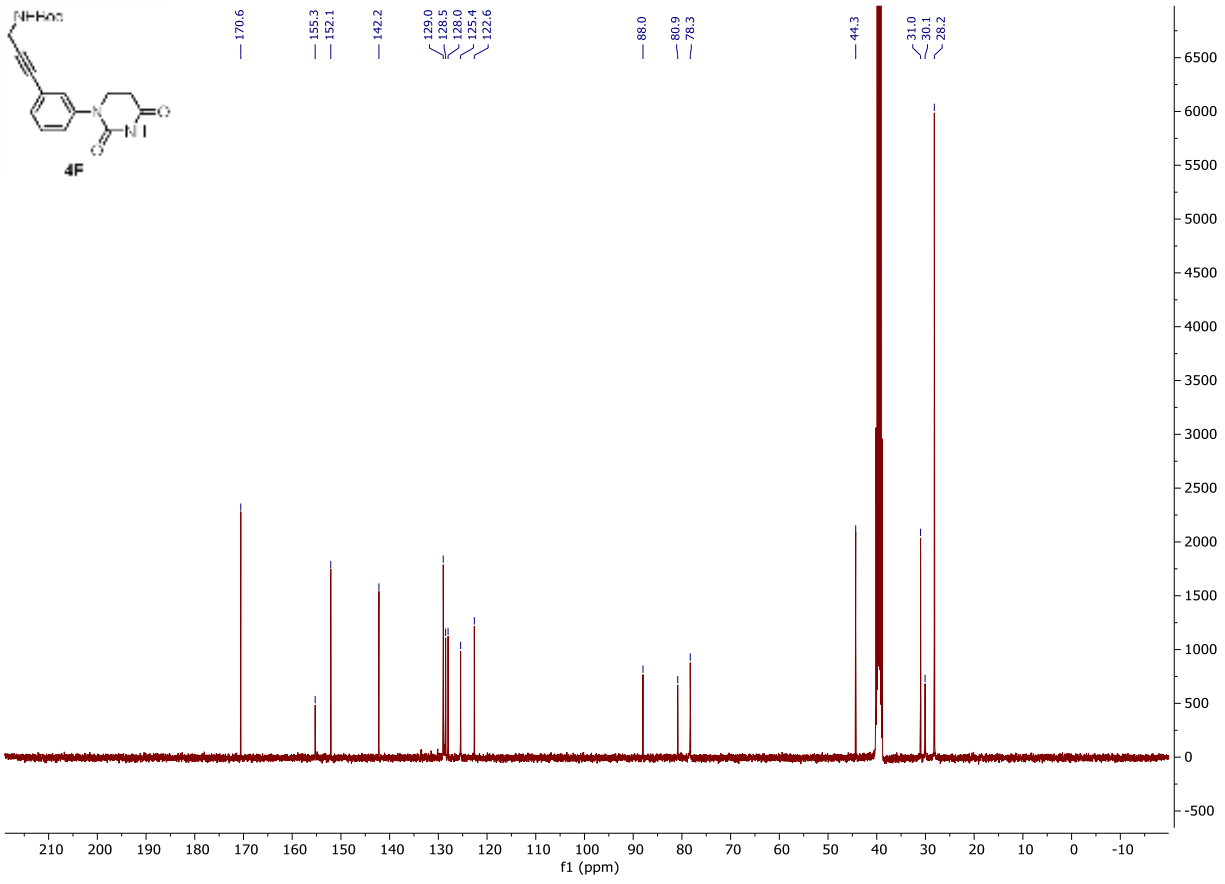

4A
10.31
9.54
7.18
7.16
7.14
6.74
6.72
6.65
6.63
6.62
3.75
3.73
3.72
3.35 H2O
2.69
2.68
2.66
2.50
2.50 DMSO
2.49
1.06
1.18
1.04
2.07
0.97
2.04
2.00
f1 (ppm)

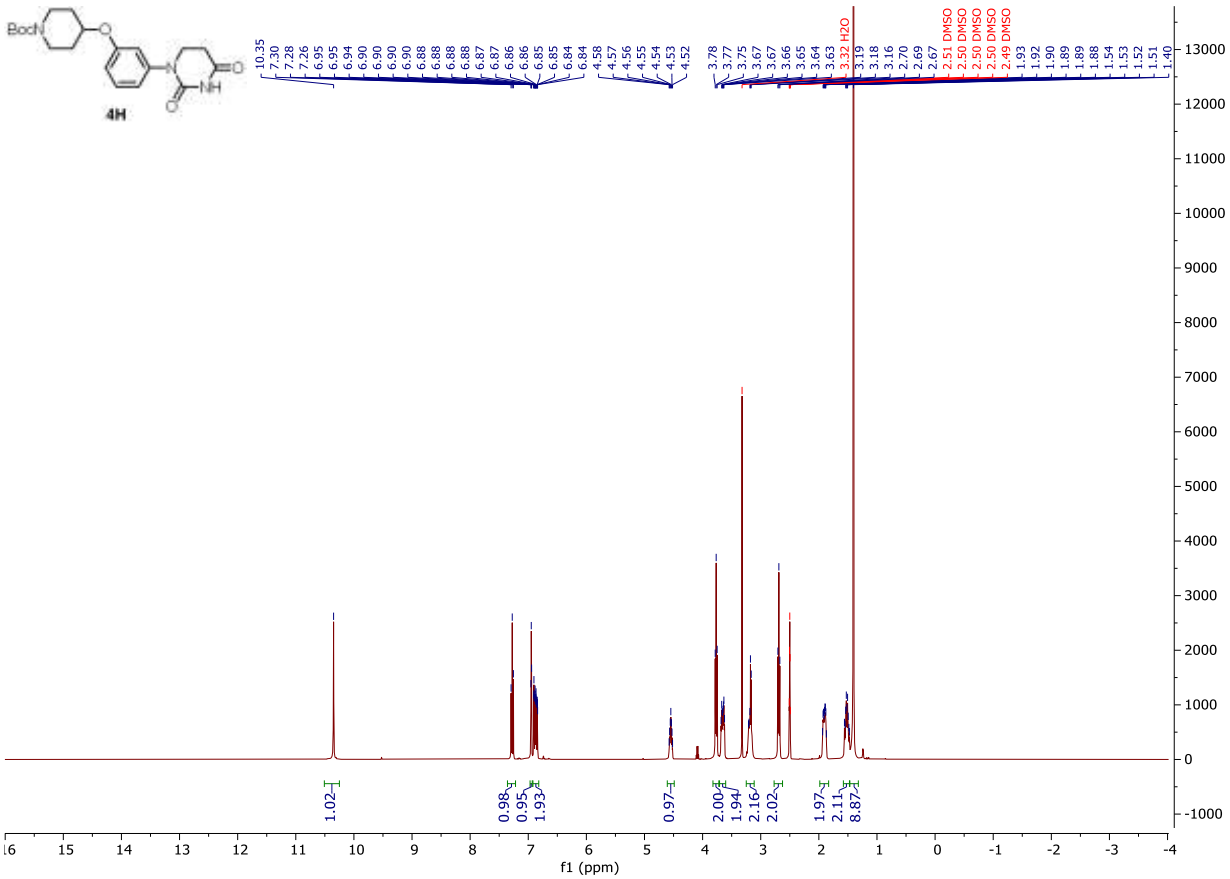

4A
170.6
157.5
152.0
143.1
129.3
115.6
112.9
112.6
44.6
31.1
f1 (ppm)

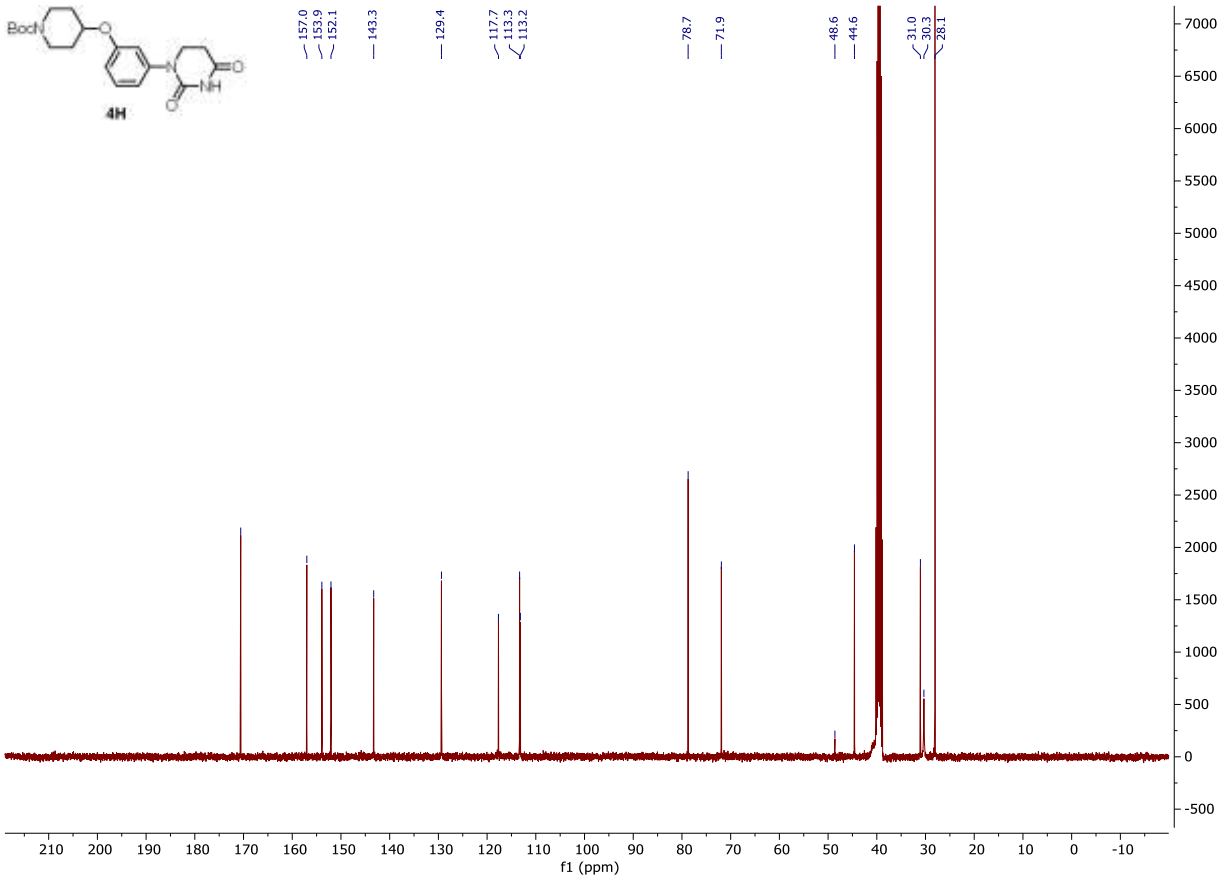

4C
10.35
7.31
7.29
7.29
7.27
7.27
6.92
6.92
6.91
6.91
6.89
6.89
6.89
6.89
6.83
6.83
6.83
6.83
6.81
6.81
6.81
6.80
3.79
3.77
3.75
3.32 H2O
2.71
2.69
2.68
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
0.97
0.95
1.94
0.92
2.02
2.99
2.00
f1 (ppm)

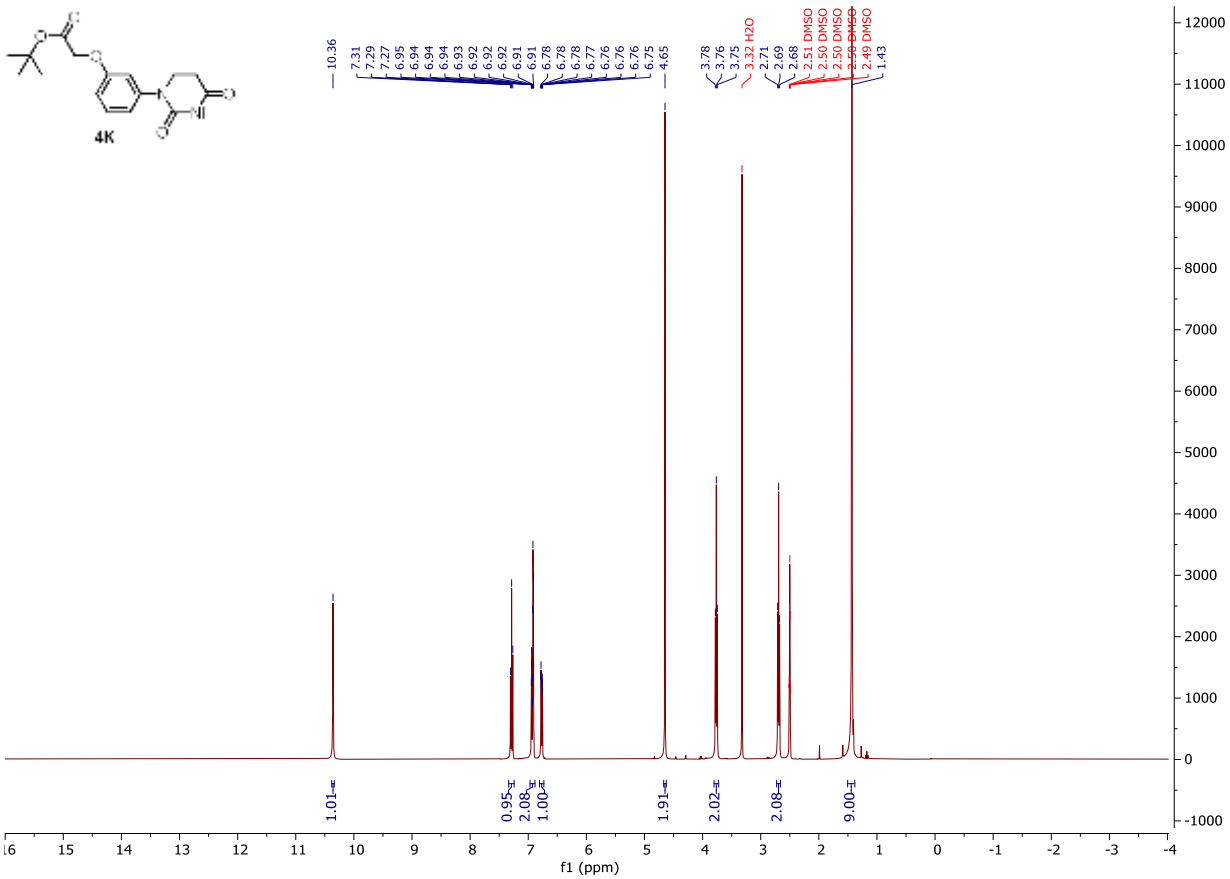

4C
170.6
159.4
152.1
143.2
129.3
117.5
111.4
111.4
55.2
44.6
31.1
f1 (ppm)

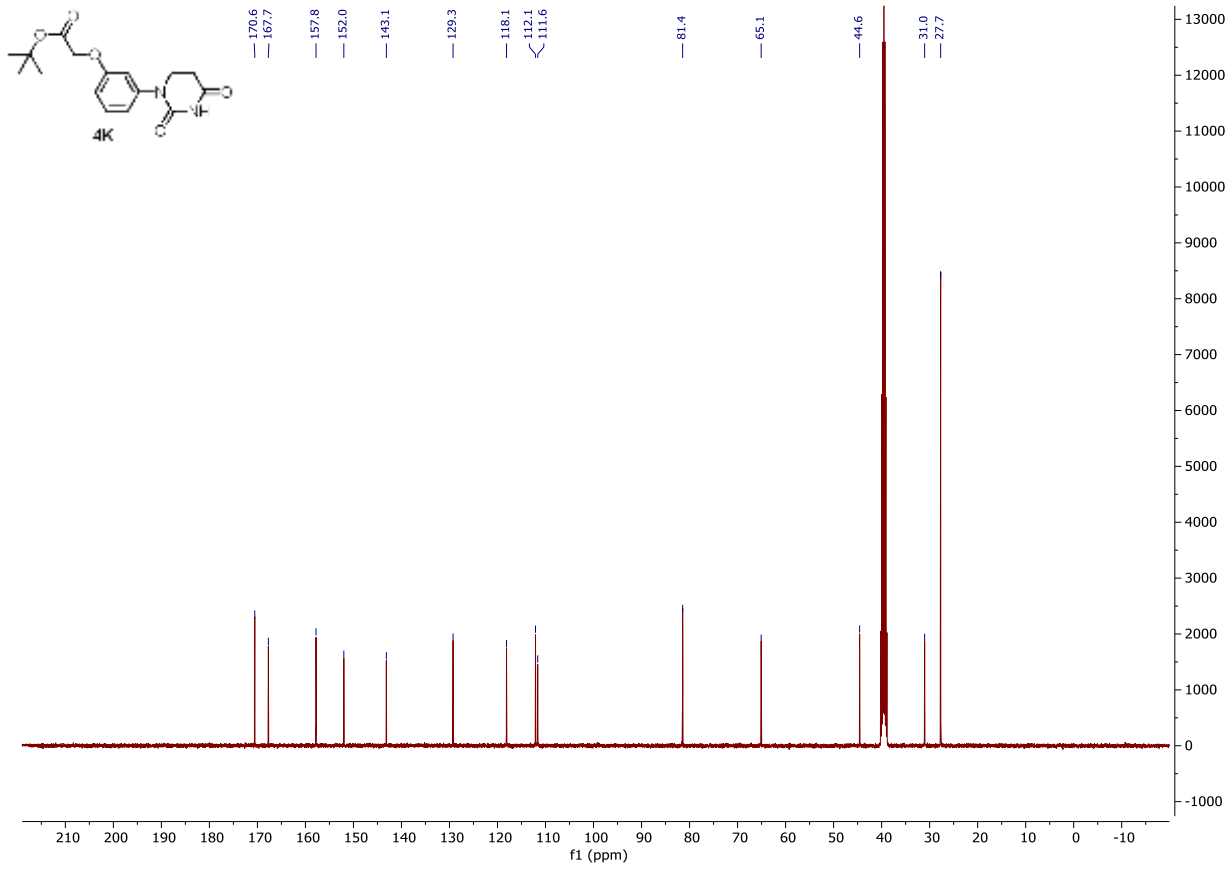

4F
10.41
7.40
7.39
7.38
7.38
7.37
7.35
7.35
7.34
7.33
7.33
7.32
7.27
7.26
7.26
7.25
7.24
7.24
3.99
3.97
3.81
3.79
3.78
3.32 H2O
2.71
2.70
2.68
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.40
0.92
4.03
0.96
1.97
1.94
2.00
9.00
f1 (ppm)

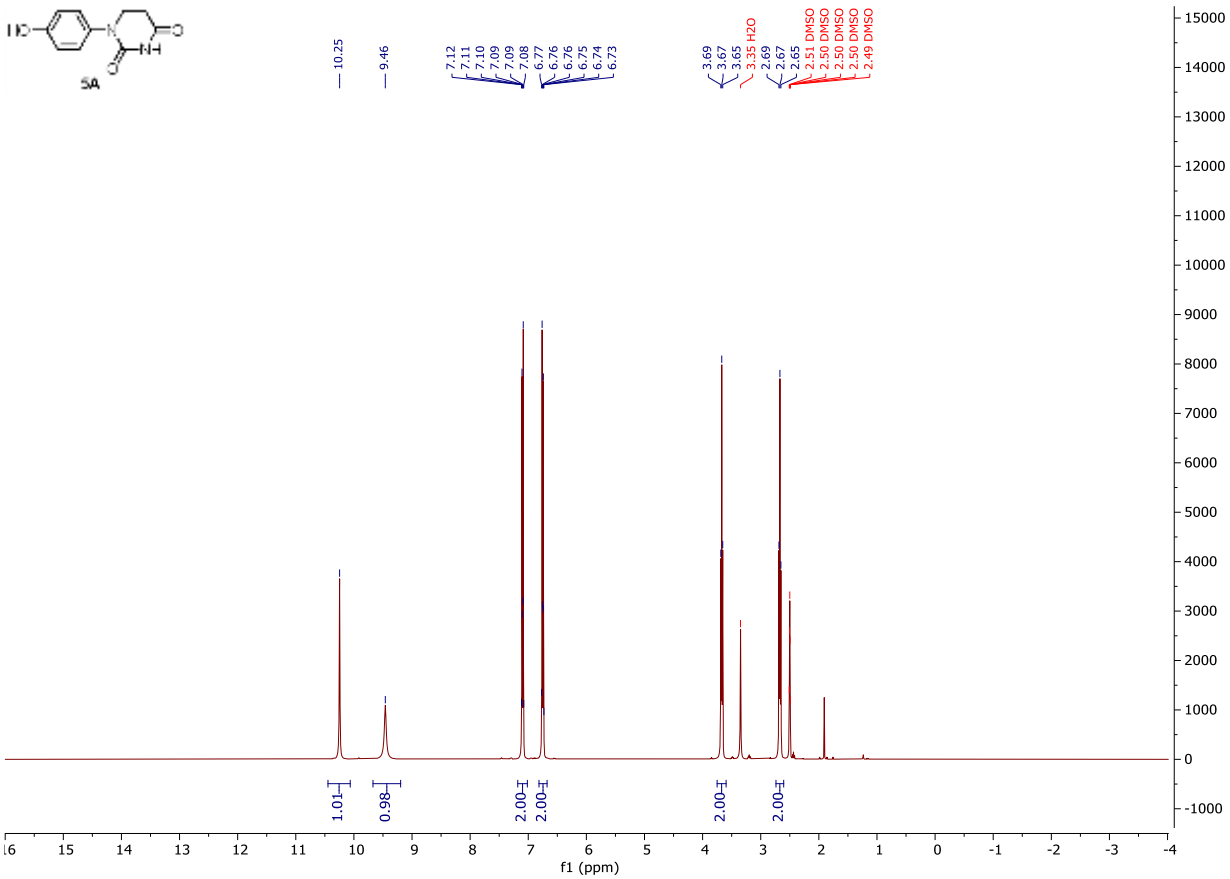

4F
170.6
155.3
152.1
142.2
129.0
128.5
128.0
125.4
122.6
88.0
80.9
78.3
44.3
31.0
30.1
28.2
f1 (ppm)

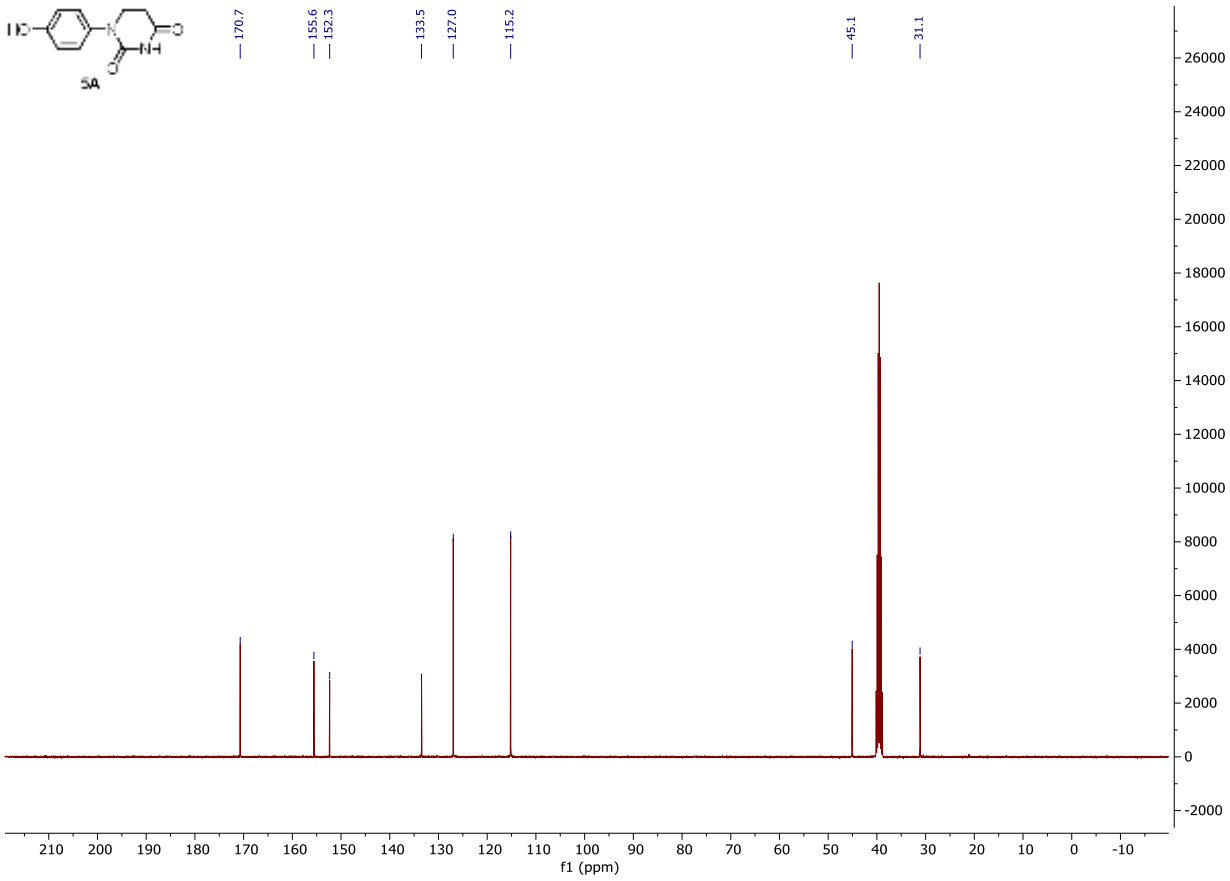

4H
10.35
7.30
7.28
7.26
6.95
6.94
6.90
6.90
6.90
6.90
6.88
6.88
6.88
6.88
6.87
6.87
6.86
6.86
6.85
6.84
6.84
4.58
4.57
4.56
4.55
4.54
4.53
4.52
3.78
3.77
3.75
3.67
3.67
3.66
3.65
3.64
3.63
3.32 H2O
3.19
3.18
3.16
2.70
2.69
2.67
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.93
1.92
1.90
1.89
1.89
1.88
1.54
1.53
1.52
1.51
1.40
1.02
0.98
0.95
1.93
0.97
2.00
1.94
2.16
2.02
1.97
2.11
8.87
f1 (ppm)

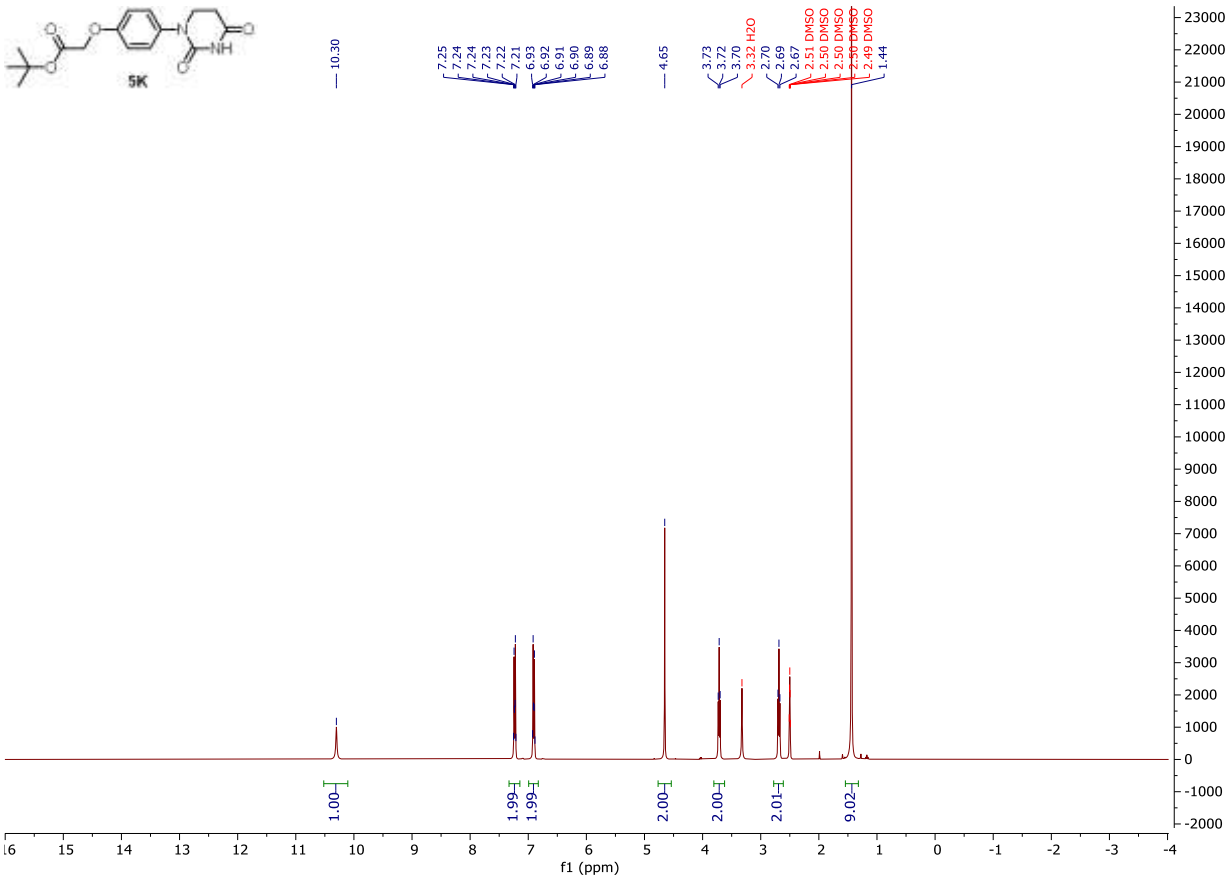

4H
157.0
153.9
152.1
143.3
129.4
117.7
113.3
113.2
78.7
71.9
48.6
44.6
31.0
30.3
28.1
f1 (ppm)

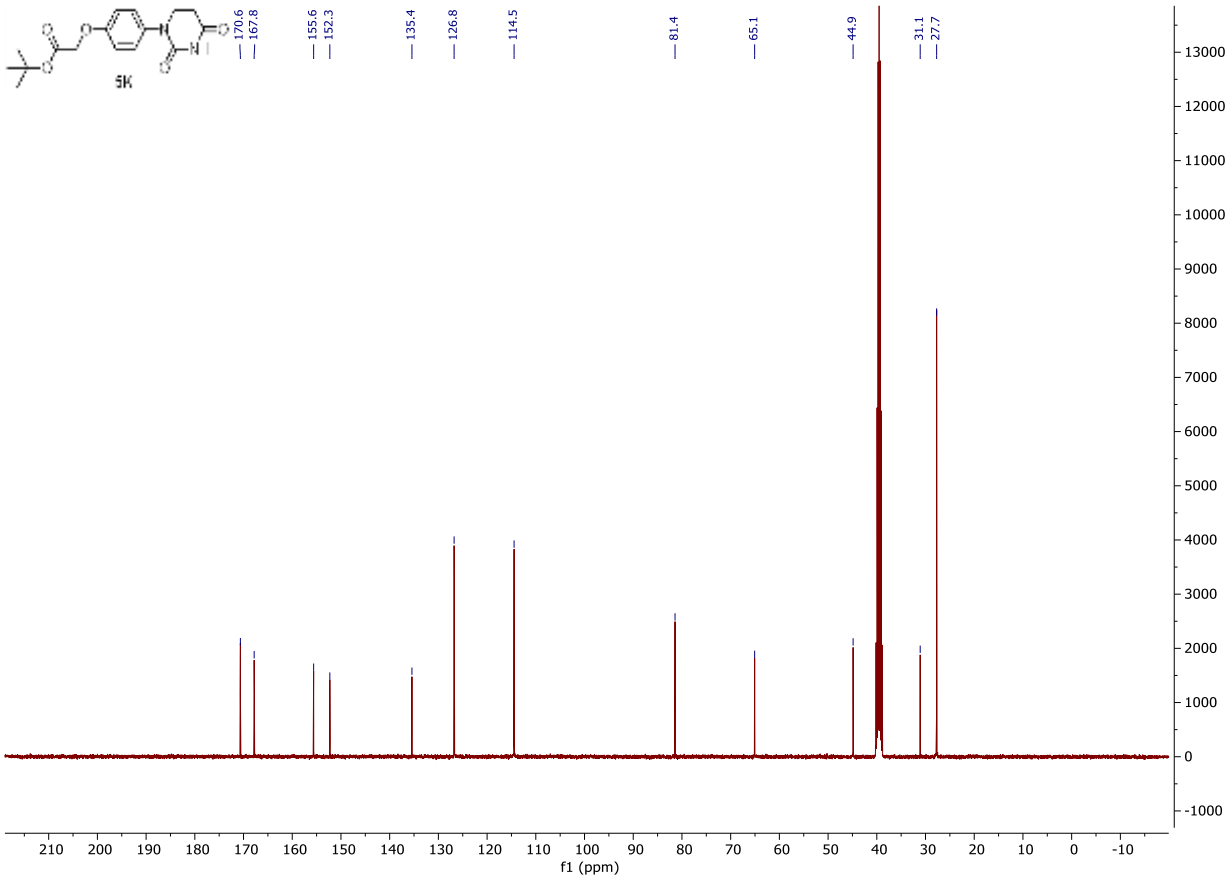

4K
10.36
7.31
7.29
7.27
6.95
6.94
6.94
6.94
6.93
6.92
6.92
6.92
6.91
6.91
6.78
6.78
6.78
6.77
6.76
6.76
6.76
6.75
4.65
3.78
3.76
3.75
3.32 H2O
2.71
2.69
2.68
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.43
1.01
0.95
2.08
1.00
1.91
2.02
2.08
9.00
f1 (ppm)

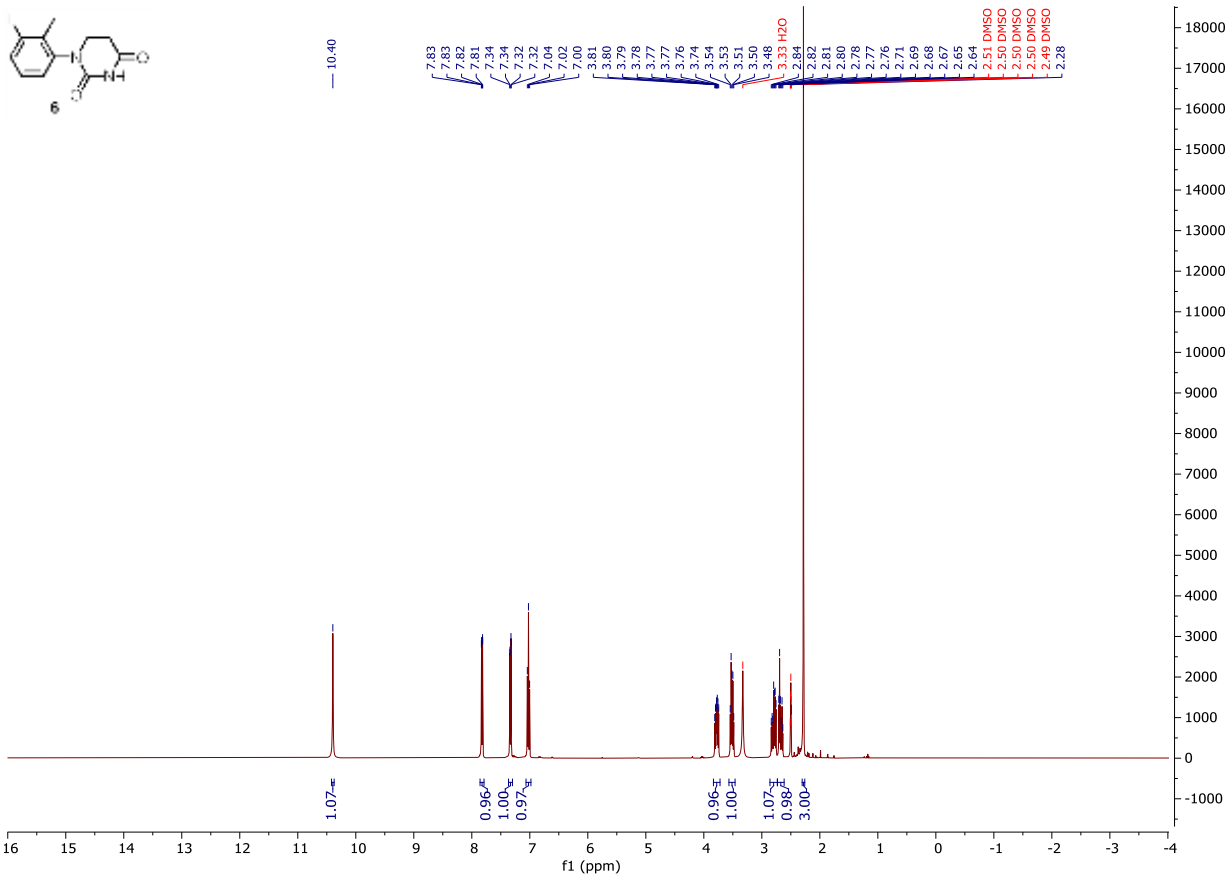

4K
170.6
167.7
157.8
152.0
143.1
129.3
118.1
112.1
111.6
81.4
65.1
44.6
31.0
27.7
f1 (ppm)

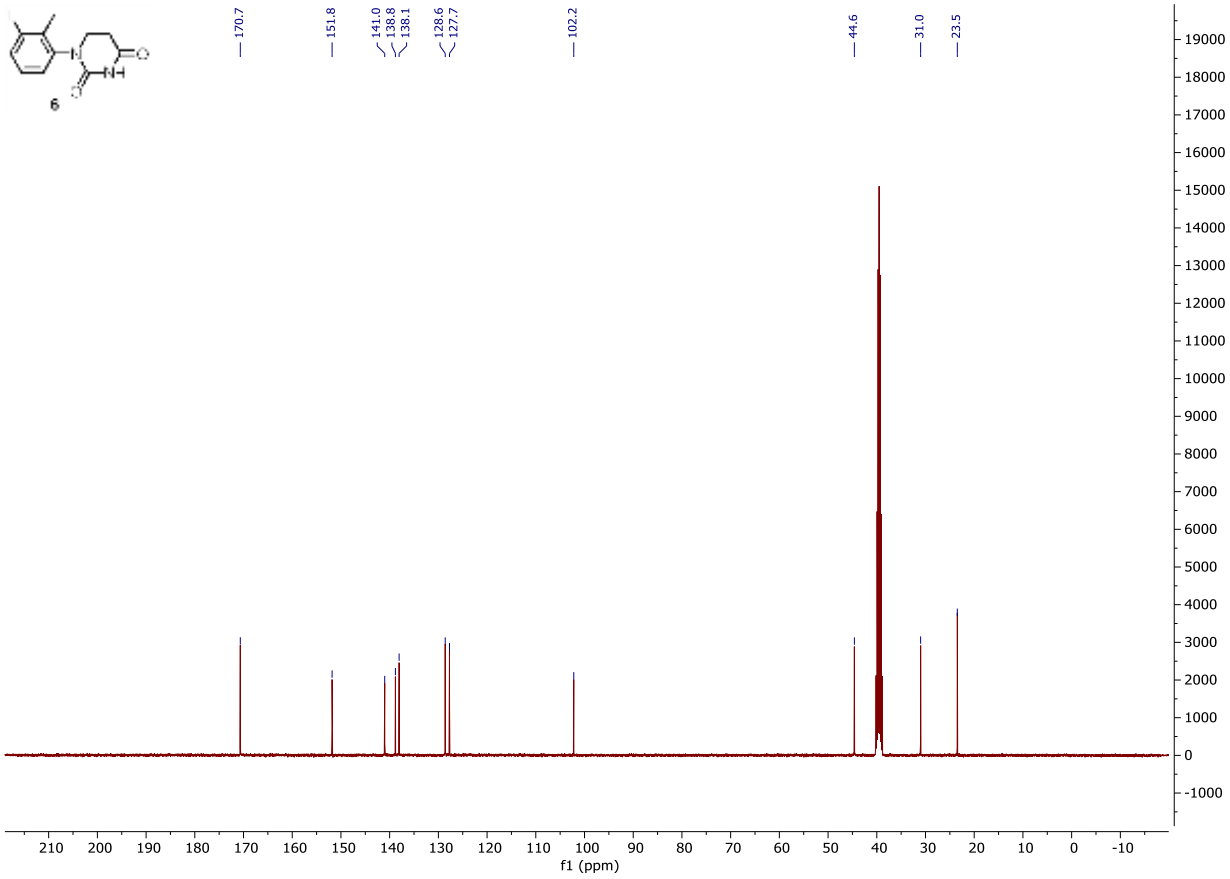

5A
10.25
9.46
7.12
7.11
7.10
7.09
7.09
7.08
6.77
6.76
6.76
6.75
6.74
6.73
3.69
3.67
3.65
3.35 H2O
2.69
2.67
2.65
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.01
0.98
2.00
2.00
2.00
2.00
f1 (ppm)

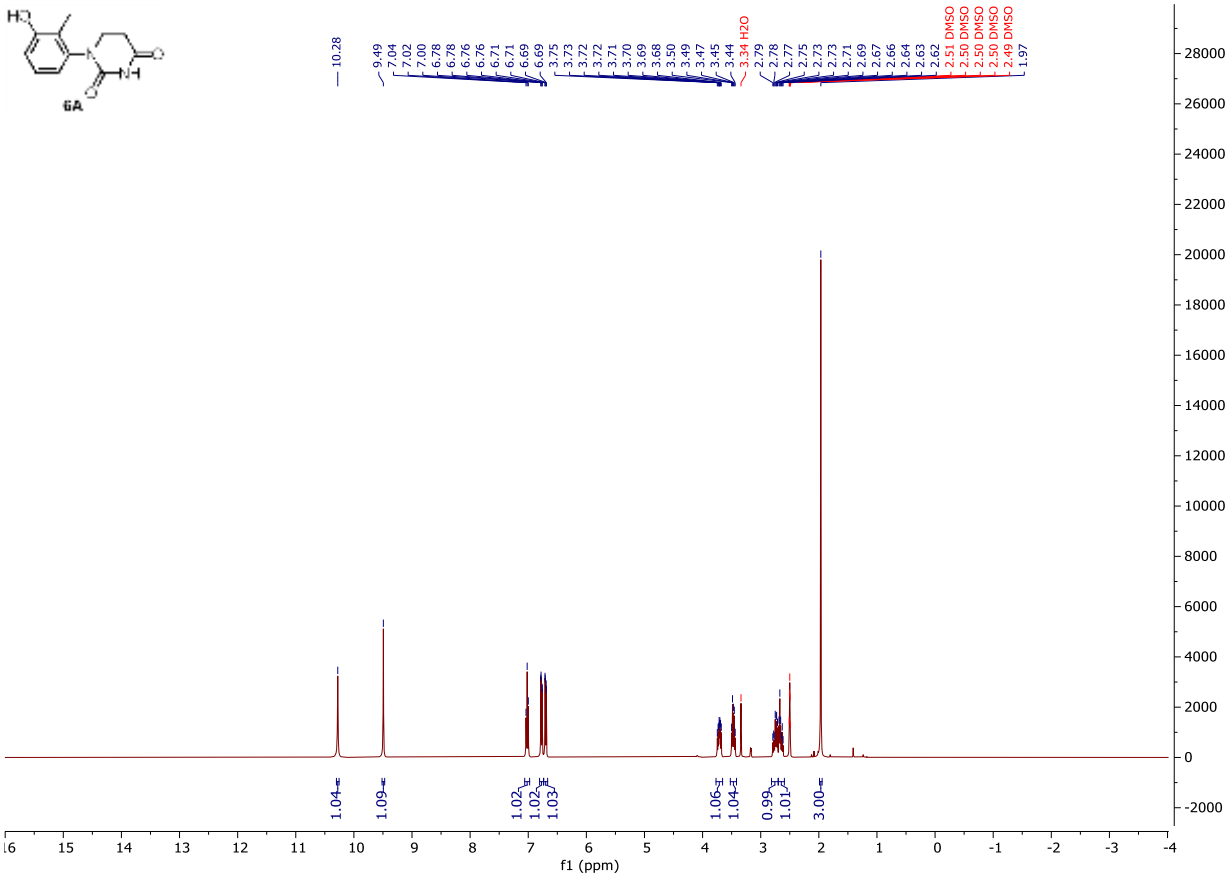

5A
170.7
155.6
152.3
133.5
127.0
115.2
45.1
31.1
f1 (ppm)

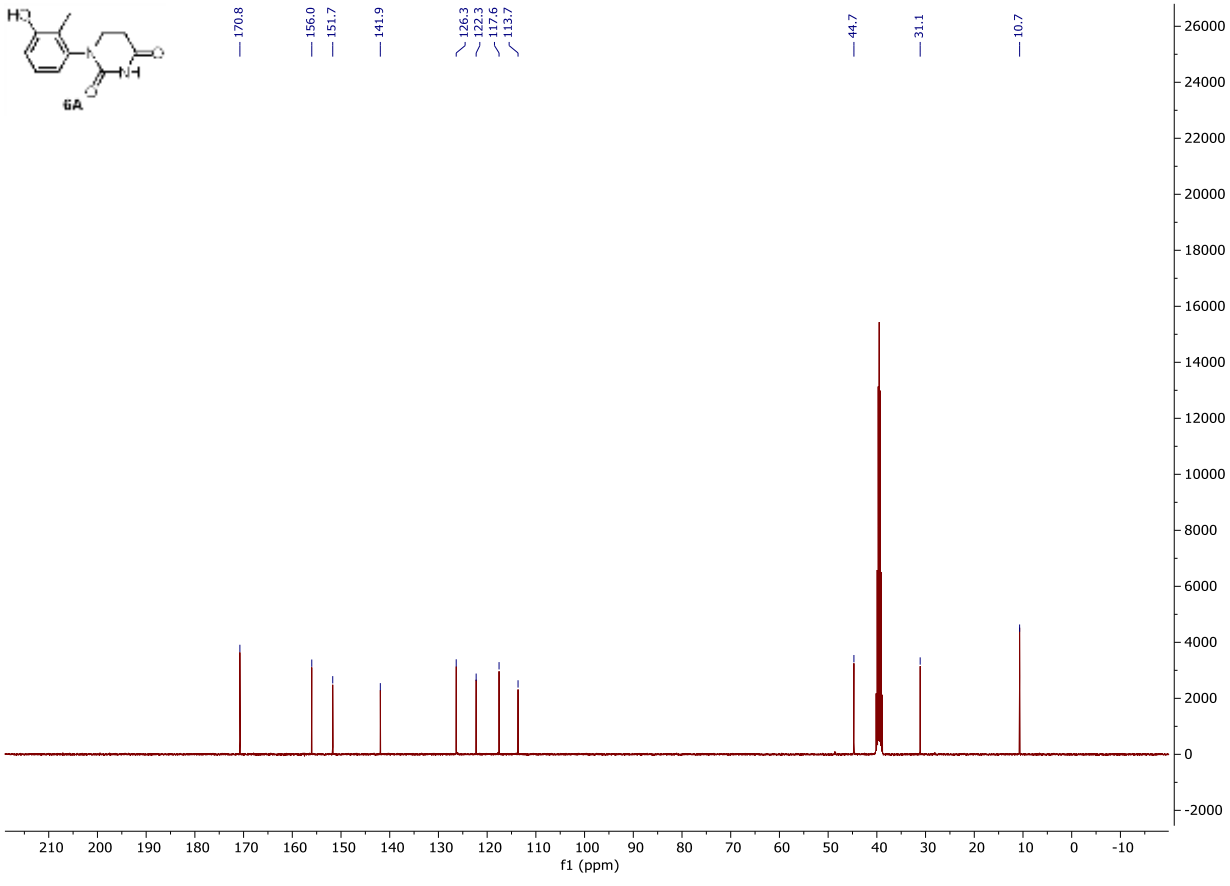

5K
10.30
7.25
7.24
7.24
7.23
7.22
7.21
6.93
6.92
6.91
6.90
6.89
6.88
4.65
3.73
3.72
3.70
3.32 H2O
2.70
2.69
2.67
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.44
1.00
1.99
1.99
2.00
2.00
2.01
9.02
f1 (ppm)

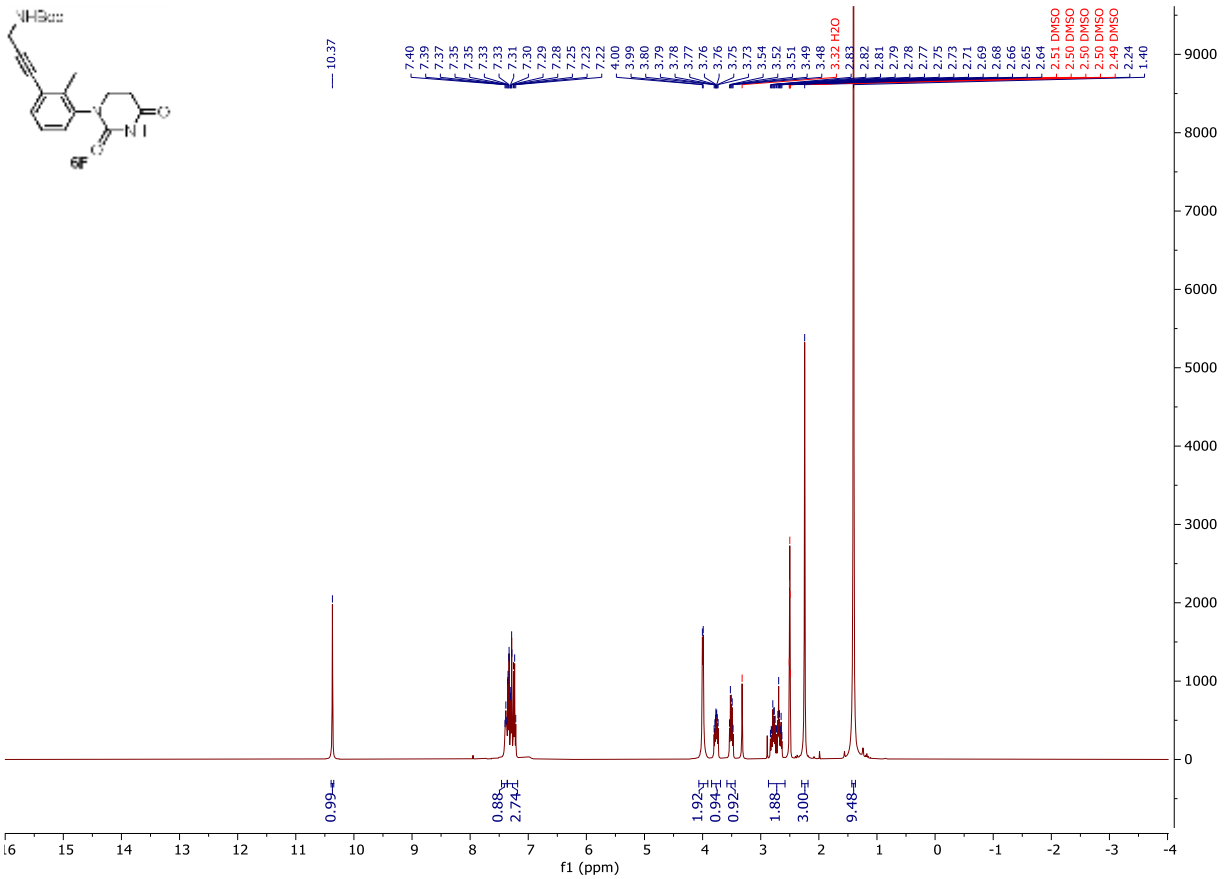

5K
170.6
167.8
155.6
152.3
135.4
126.8
114.5
81.4
65.1
44.9
31.1
27.7
f1 (ppm)

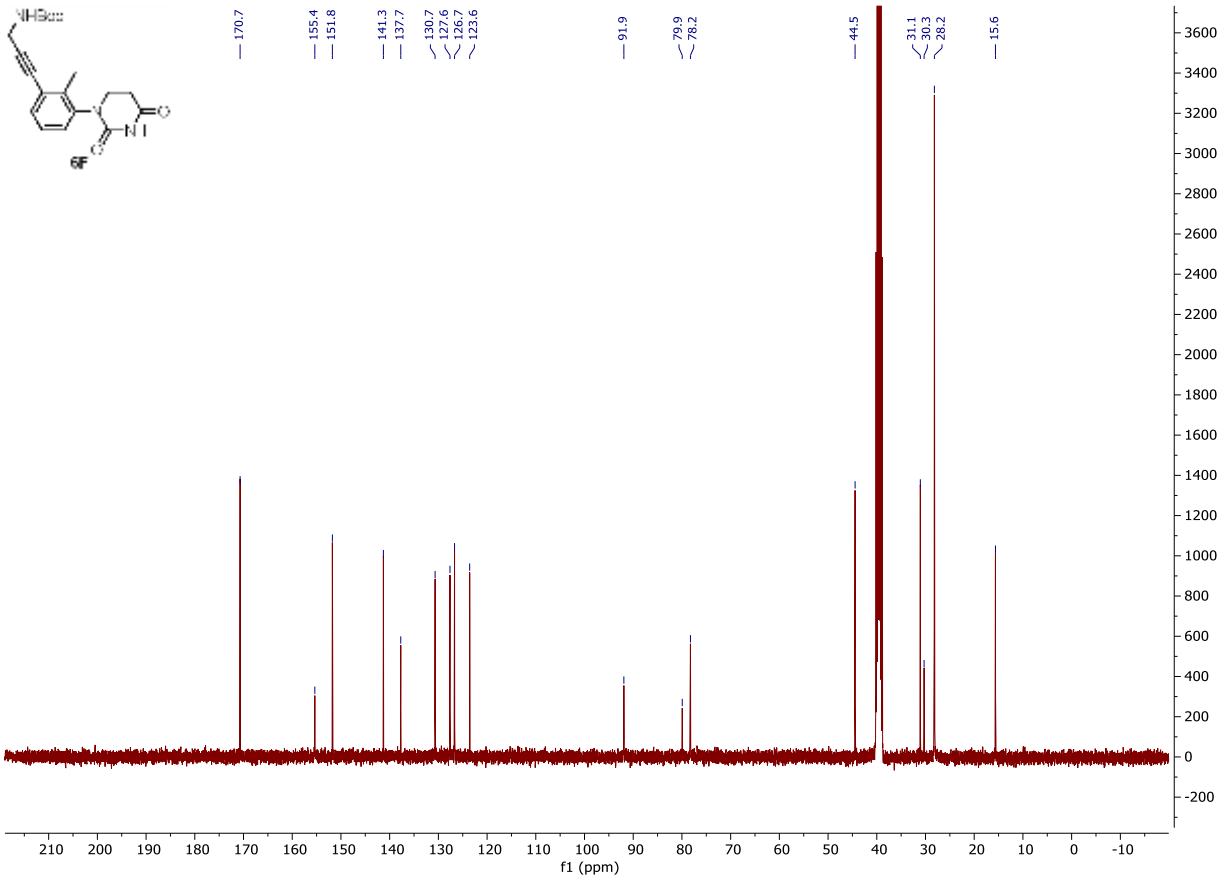

6
10.40
7.83
7.83
7.82
7.81
7.34
7.34
7.32
7.32
7.04
7.02
7.00
3.81
3.80
3.79
3.78
3.77
3.77
3.76
3.74
3.54
3.53
3.51
3.50
3.48
3.33 H2O
2.84
2.82
2.81
2.80
2.78
2.77
2.76
2.71
2.69
2.68
2.67
2.65
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.28
1.07
0.96
1.00
0.97
0.96
1.00
1.07
0.98
3.00
f1 (ppm)

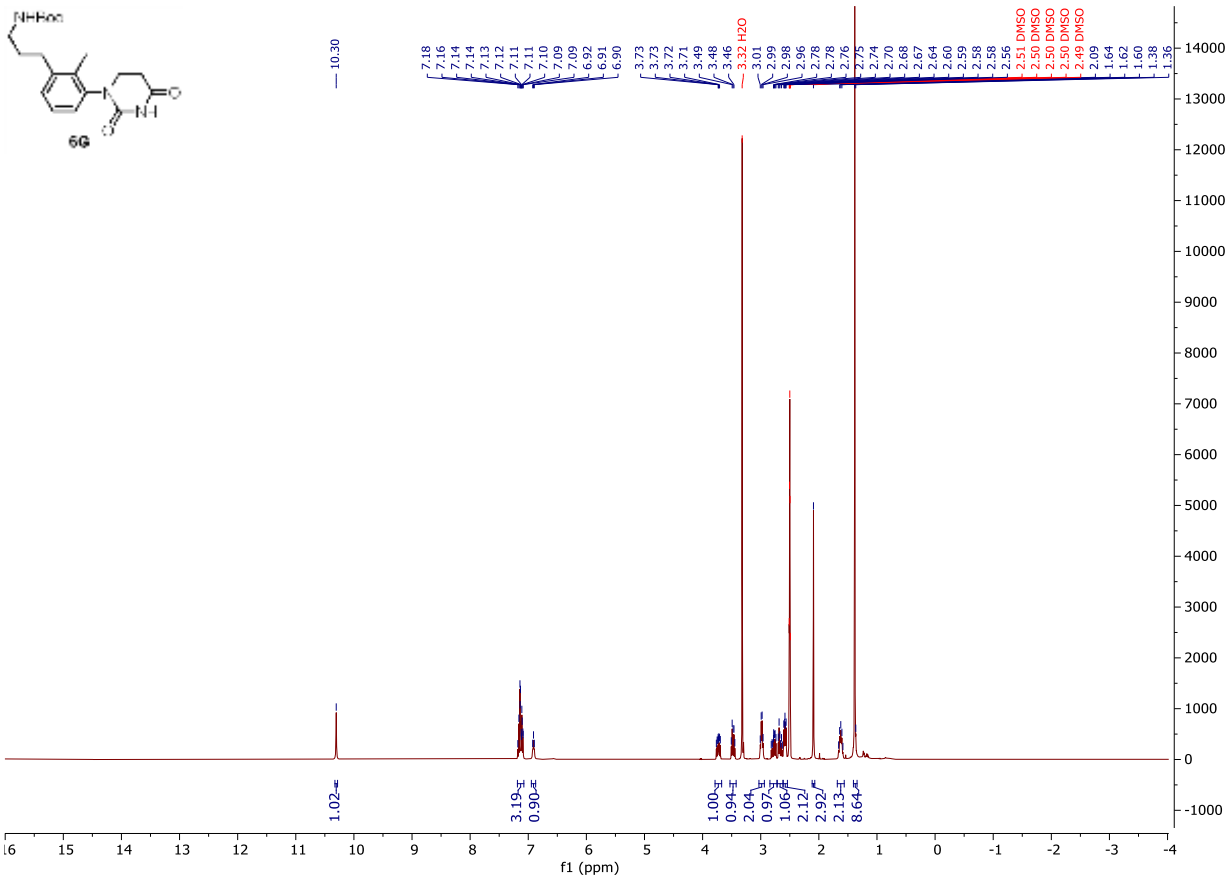

6
170.7
151.8
141.0
138.8
138.1
128.6
127.7
102.2
44.6
31.0
23.5
f1 (ppm)

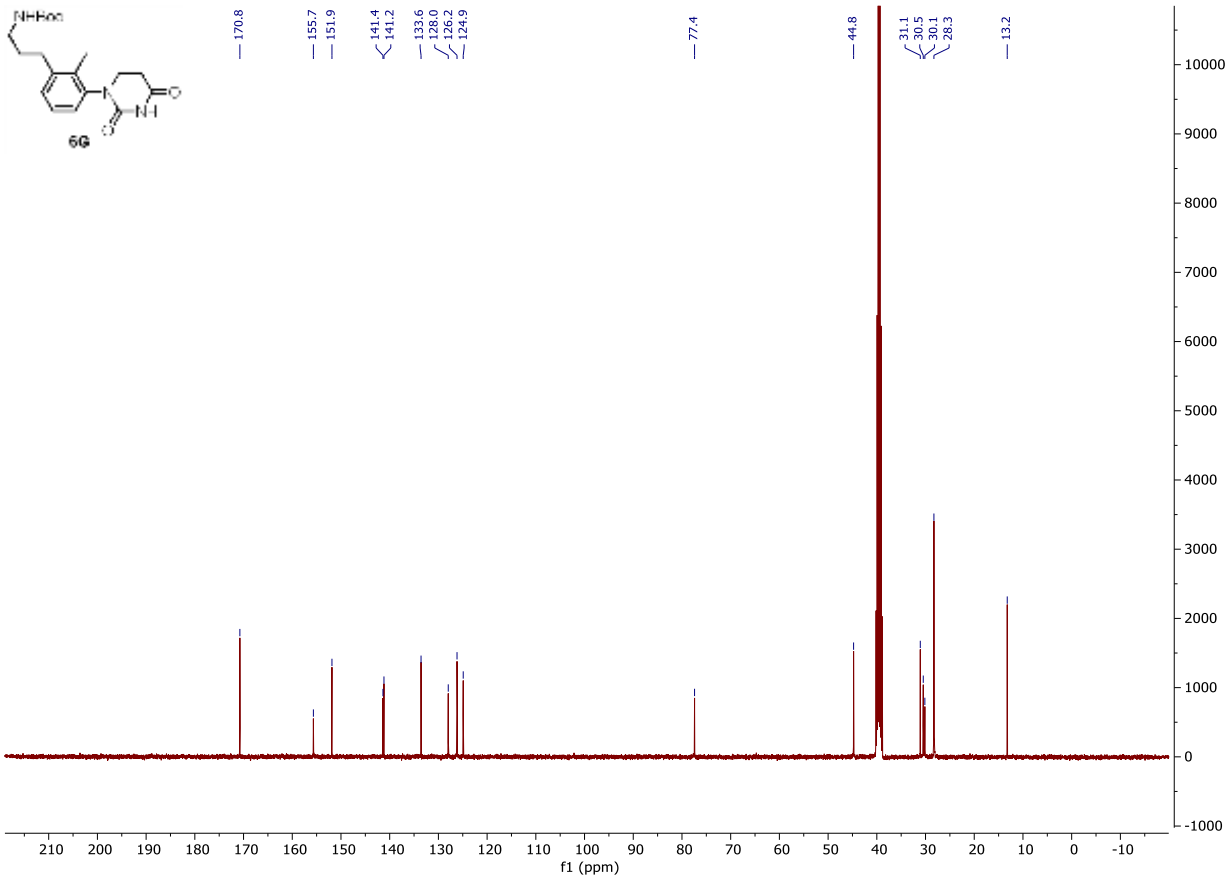

6A
10.28
9.49
7.04
7.02
7.00
6.78
6.78
6.76
6.76
6.71
6.71
6.69
6.69
3.75
3.73
3.72
3.72
3.71
3.70
3.69
3.68
3.50
3.49
3.47
3.45
3.44
3.34 H2O
2.79
2.78
2.77
2.75
2.73
2.73
2.71
2.69
2.67
2.66
2.64
2.63
2.62
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.97
1.04
1.09
1.02
1.02
1.03
1.06
1.04
0.99
1.01
3.00
f1 (ppm)

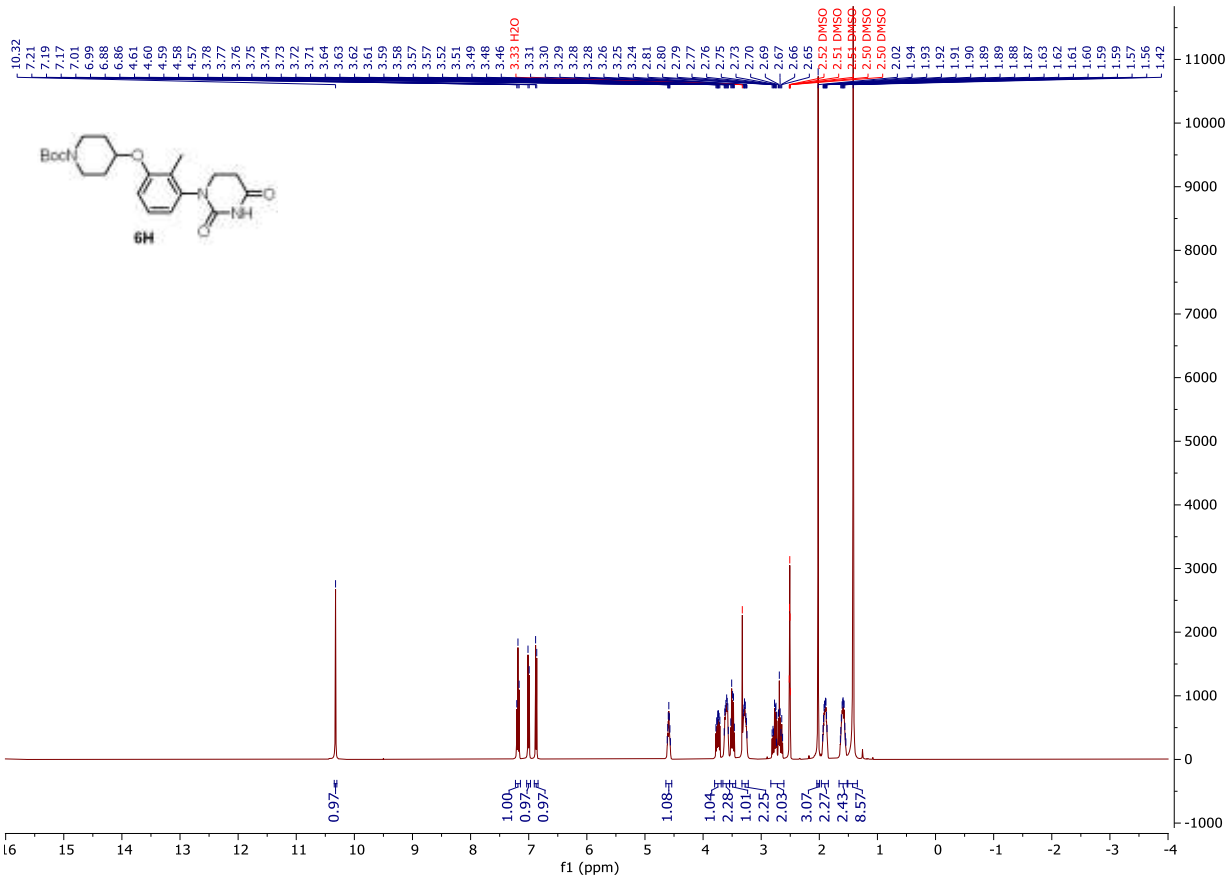

6A
170.8
156.0
151.7
141.9
126.3
122.3
117.6
113.7
44.7
31.1
10.7
f1 (ppm)

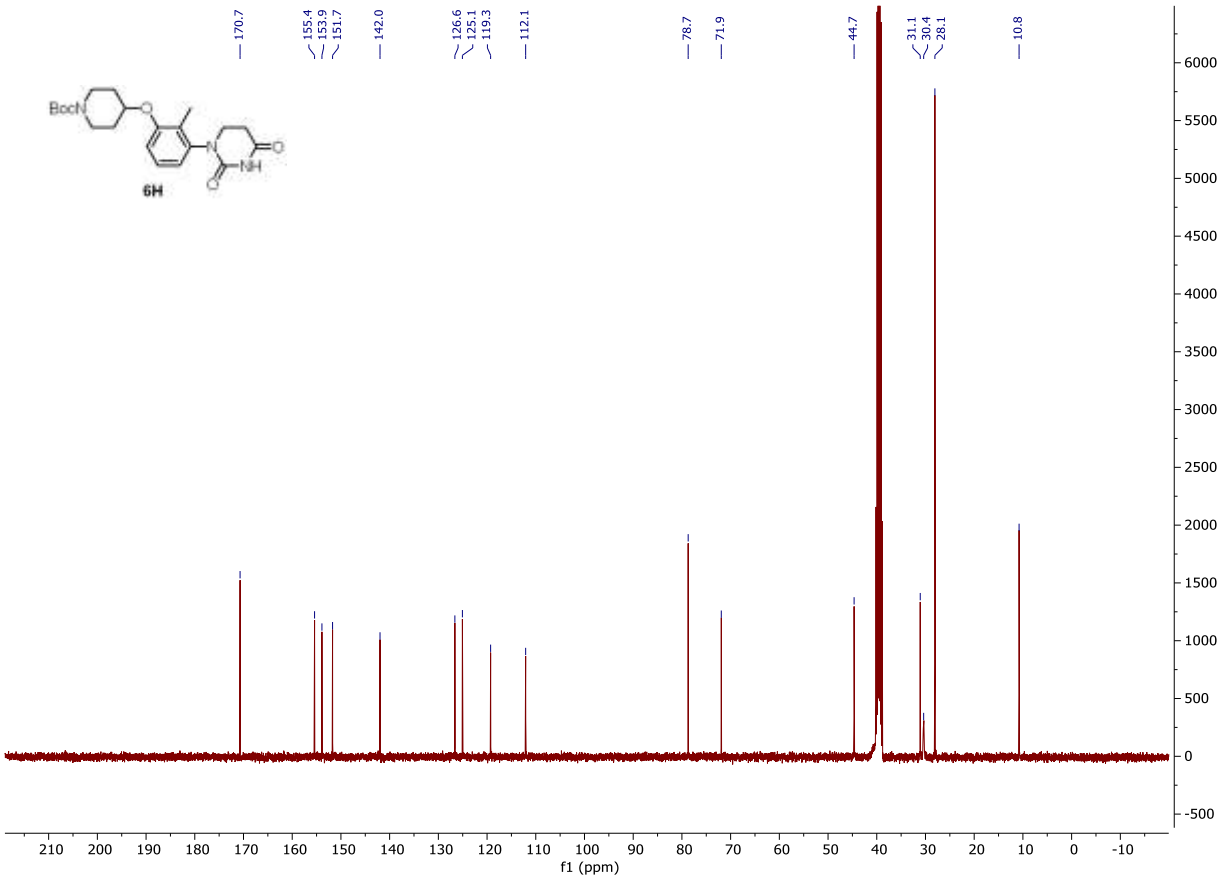

6F
10.37
7.40
7.39
7.37
7.35
7.35
7.33
7.33
7.31
7.30
7.29
7.28
7.25
7.23
7.22
4.00
3.99
3.80
3.79
3.78
3.77
3.76
3.76
3.75
3.73
3.54
3.52
3.51
3.49
3.48
3.32 H2O
2.83
2.82
2.81
2.79
2.78
2.77
2.75
2.73
2.71
2.69
2.68
2.66
2.65
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.24
1.40
0.99
0.88
2.74
1.92
0.94
0.92
1.88
3.00
9.48
f1 (ppm)

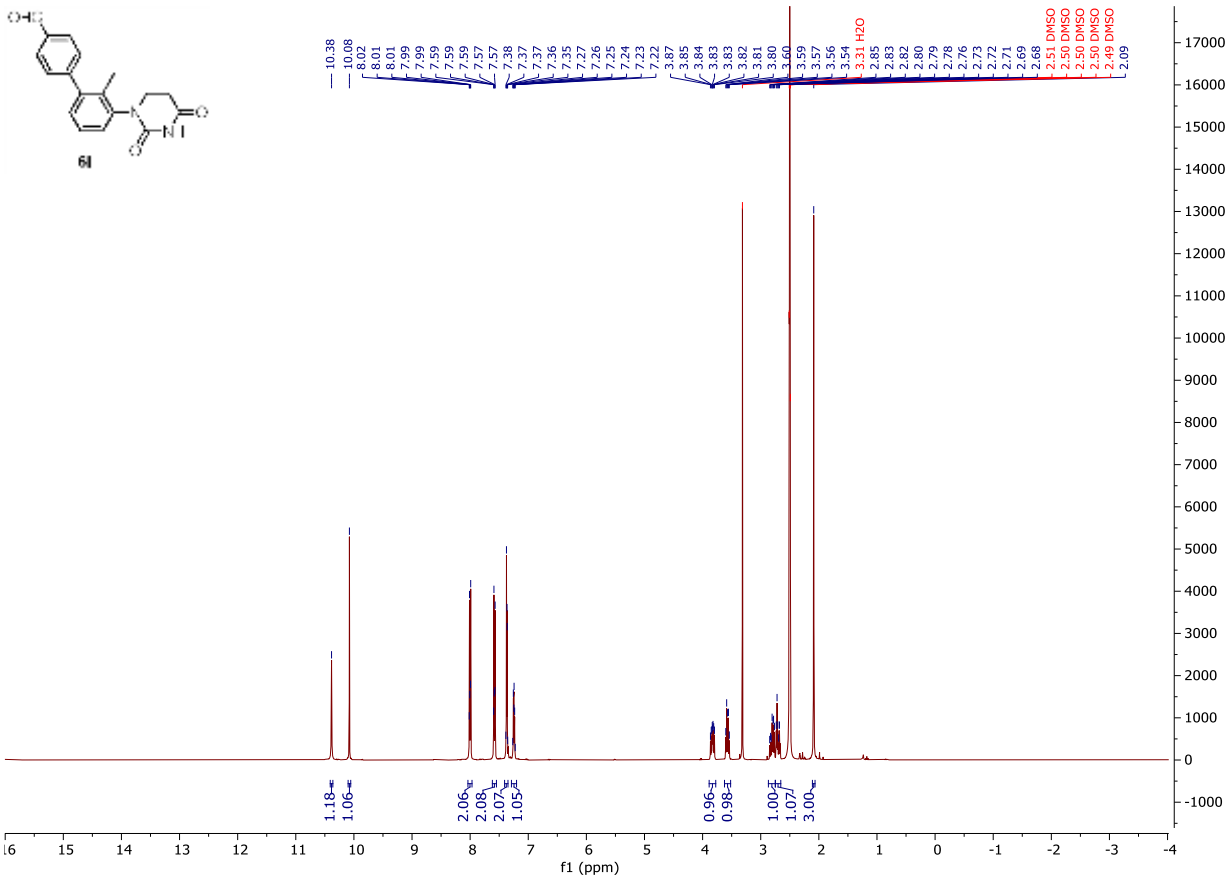

6F
170.7
155.4
151.8
141.3
137.7
130.7
127.6
126.7
123.6
91.9
79.9
78.2
44.5
31.1
30.3
28.2
15.6
f1 (ppm)

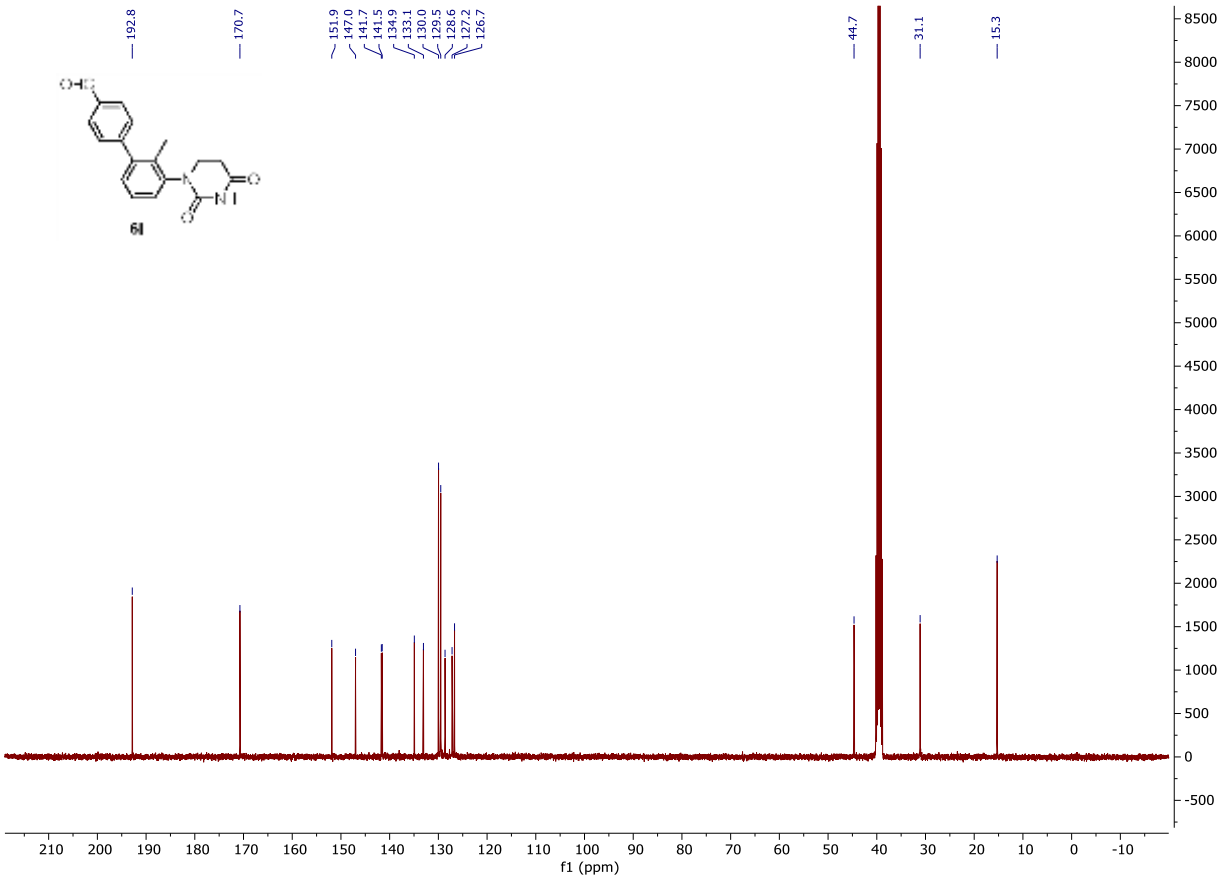

NHBoc
6G
10.30
7.18
7.16
7.14
7.14
7.13
7.12
7.11
7.11
7.10
7.09
7.09
6.92
6.91
6.90
3.73
3.73
3.72
3.71
3.49
3.48
3.46
3.32 H2O
3.01
2.99
2.98
2.96
2.78
2.78
2.76
2.75
2.74
2.70
2.68
2.67
2.64
2.60
2.59
2.58
2.58
2.56
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.09
1.64
1.62
1.60
1.38
1.36
1.02
3.19
0.90
1.00
0.94
2.04
0.97
1.06
2.12
2.92
2.13
8.64
f1 (ppm)

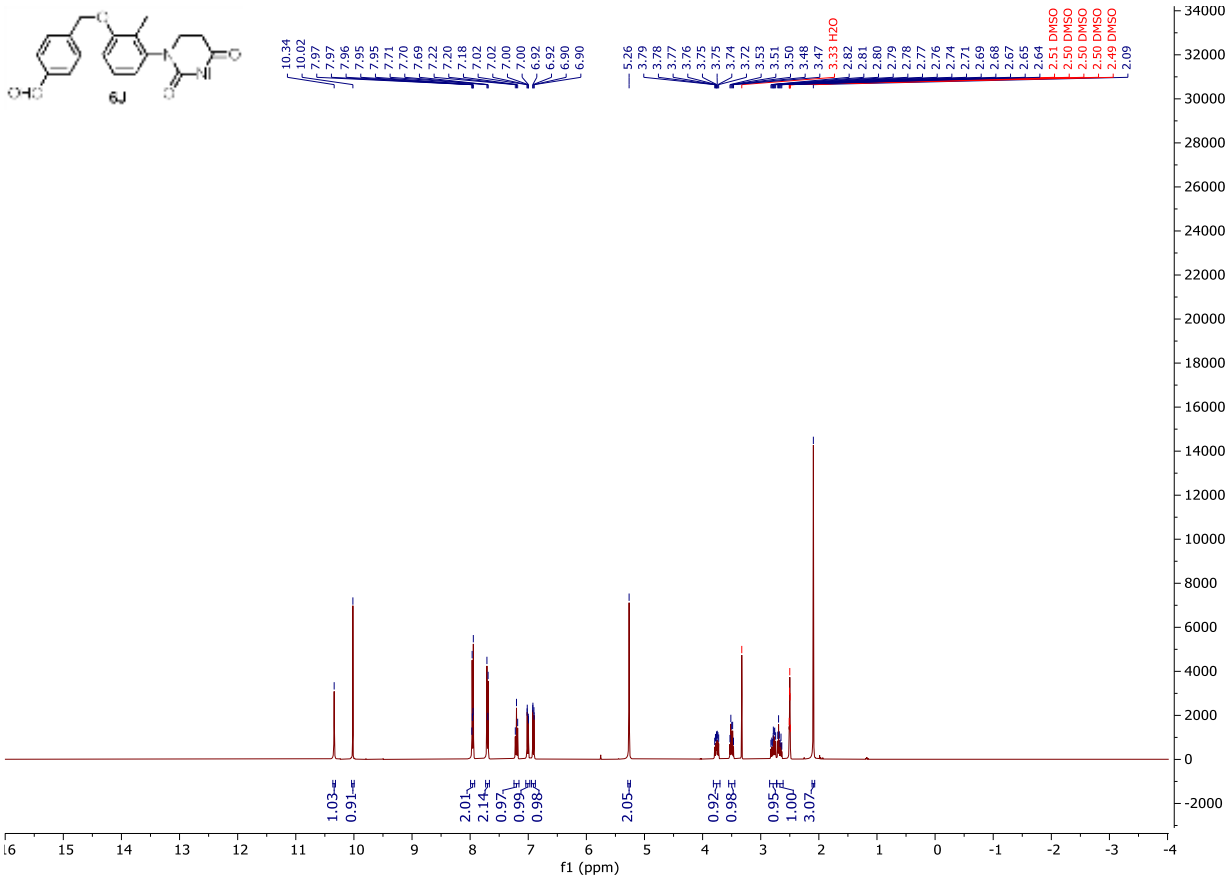

NHBoc
6G
170.8
155.7
151.9
141.4
141.2
133.6
128.0
126.2
124.9
77.4
44.8
31.1
30.5
30.1
28.3
13.2
f1 (ppm)

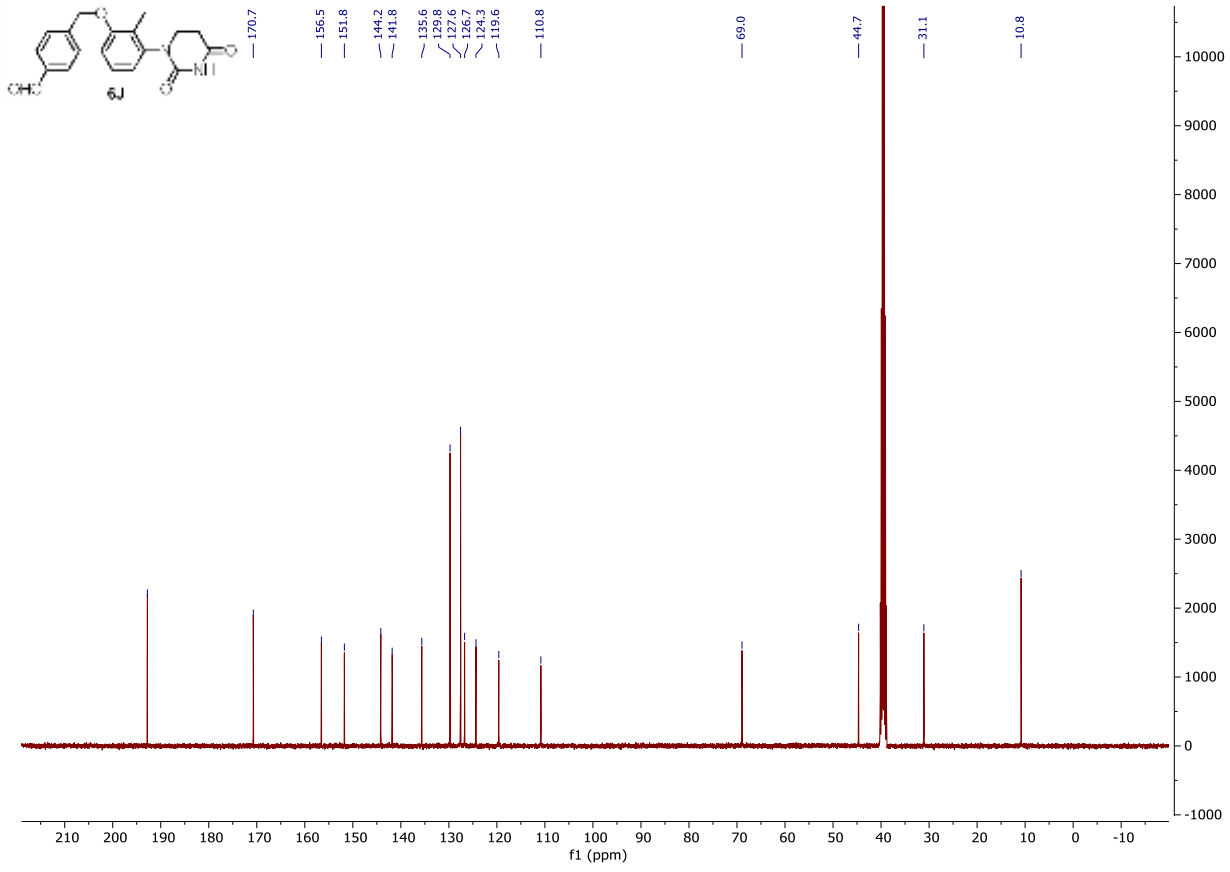

10.32
7.21
7.19
7.17
7.01
6.99
6.88
6.86
4.61
4.60
4.59
4.58
4.57
3.78
3.77
3.76
3.75
3.74
3.73
3.72
3.71
3.64
3.63
3.62
3.61
3.59
3.58
3.57
3.57
3.52
3.51
3.49
3.48
3.46
3.33 H2O
3.31
3.30
3.29
3.28
3.26
3.25
3.24
2.81
2.80
2.79
2.77
2.76
2.75
2.73
2.70
2.69
2.67
2.66
2.65
2.52 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.02
1.94
1.93
1.92
1.91
1.90
1.89
1.89
1.88
1.87
1.63
1.62
1.61
1.60
1.59
1.59
1.57
1.56
1.42
Boc
N
O
NH
O
6H
0.97
1.00
0.97
0.97
1.08
1.04
2.28
1.01
2.25
2.03
3.07
2.27
2.43
8.57
f1 (ppm)

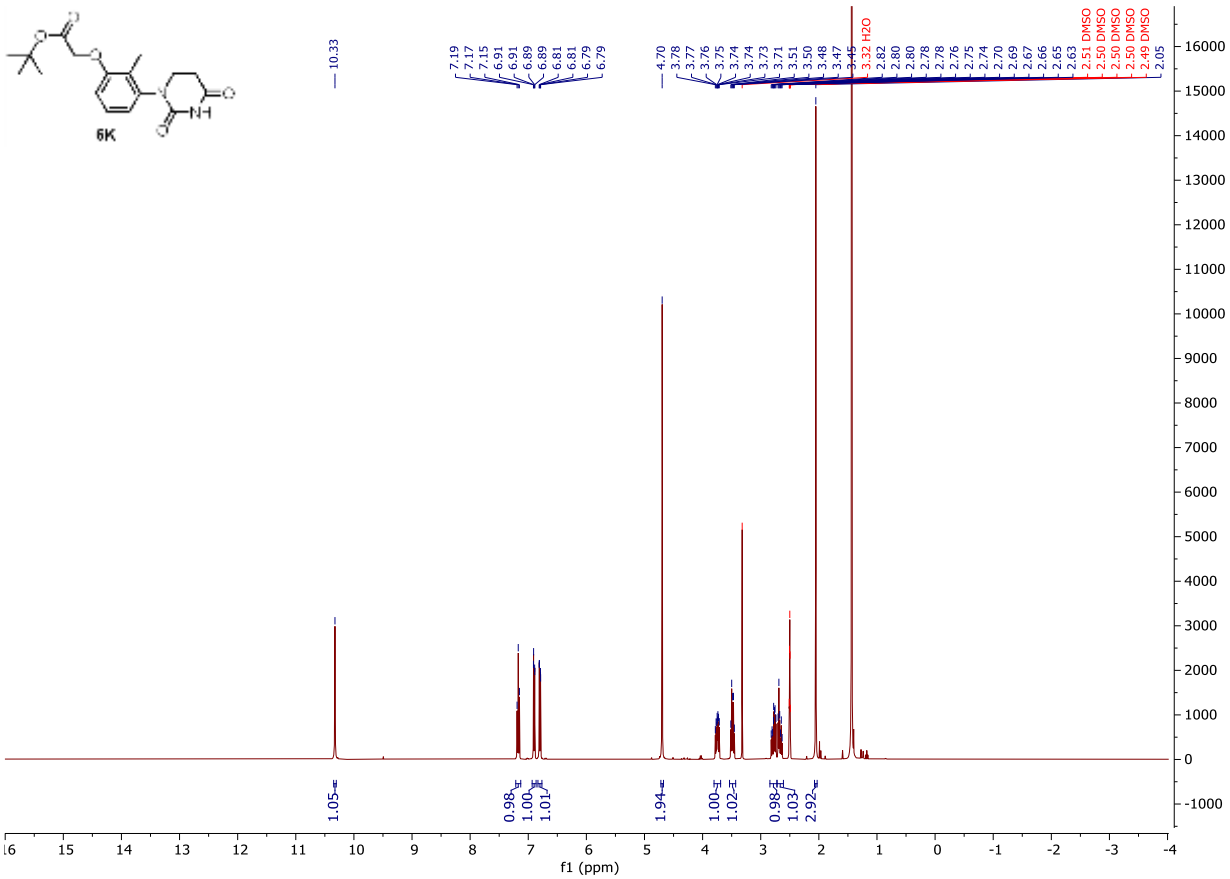

170.7
155.4
153.9
151.7
142.0
126.6
125.1
119.3
112.1
78.7
71.9
44.7
31.1
30.4
28.1
10.8
Boc
N
O
NH
O
6H
f1 (ppm)

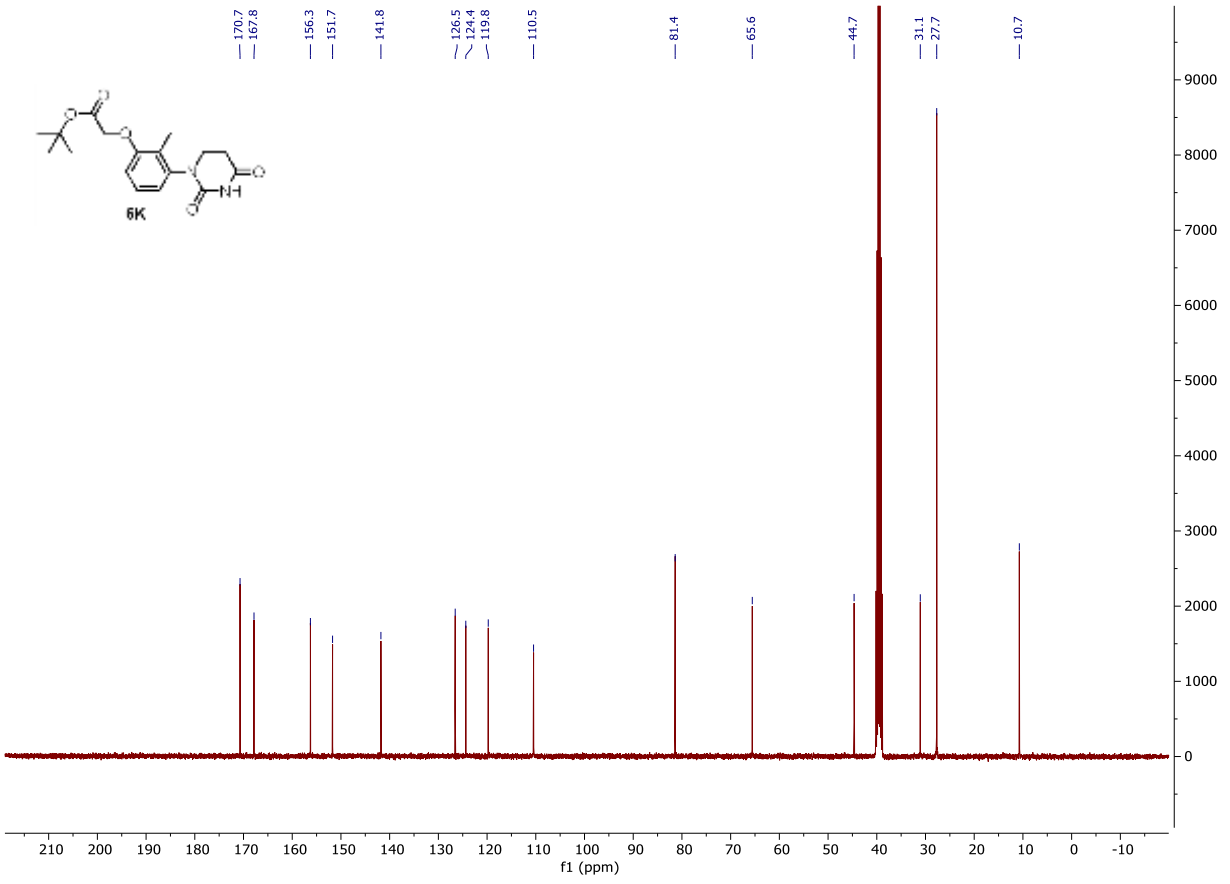

6l
10.38
10.08
8.02
8.01
8.01
7.99
7.99
7.59
7.59
7.59
7.57
7.57
7.38
7.37
7.37
7.36
7.35
7.27
7.26
7.25
7.24
7.23
7.22
3.87
3.85
3.84
3.83
3.83
3.82
3.81
3.80
3.60
3.59
3.57
3.56
3.54
3.31 H2O
2.85
2.83
2.82
2.80
2.79
2.78
2.76
2.73
2.72
2.71
2.69
2.68
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.09
1.18
1.06
2.06
2.08
2.07
1.05
0.96
0.98
1.00
1.07
3.00
f1 (ppm)

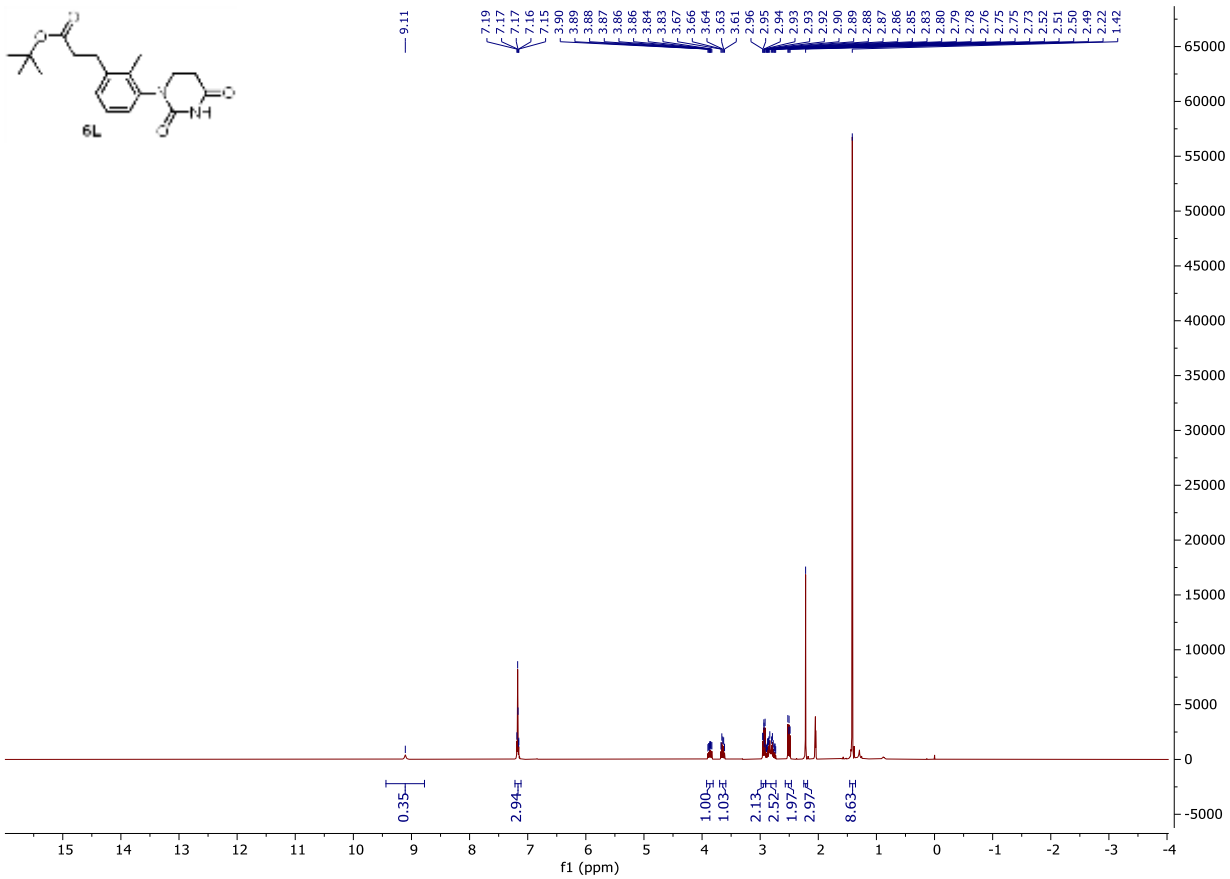

6l
192.8
170.7
151.9
147.0
141.7
141.5
134.9
133.1
130.0
129.5
128.6
127.2
126.7
44.7
31.1
15.3
f1 (ppm)

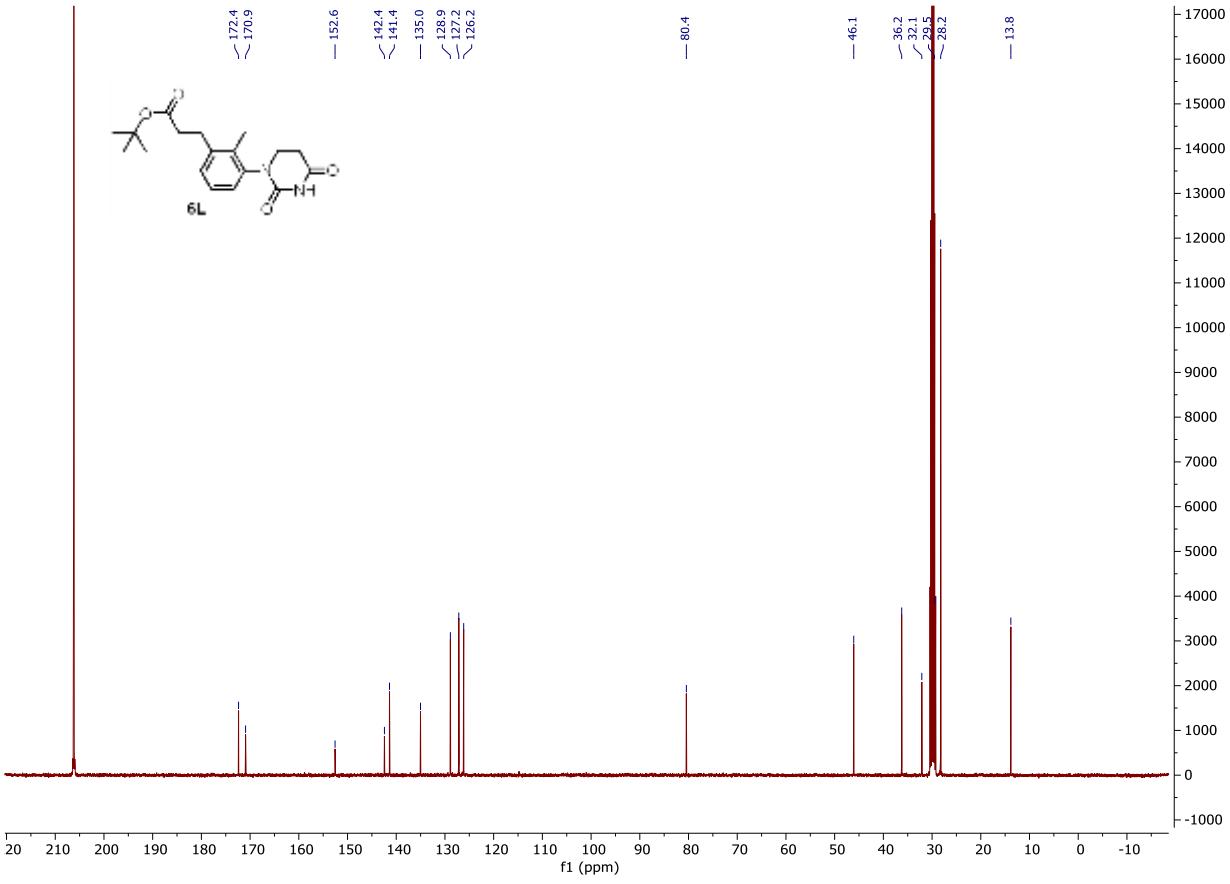

6J
10.34
10.02
7.97
7.97
7.96
7.95
7.95
7.71
7.70
7.69
7.22
7.20
7.18
7.02
7.02
7.00
7.00
6.92
6.92
6.90
6.90
5.26
3.79
3.78
3.77
3.76
3.75
3.75
3.74
3.72
3.53
3.51
3.50
3.48
3.47
3.33 H2O
2.82
2.81
2.80
2.79
2.78
2.77
2.76
2.74
2.71
2.69
2.68
2.67
2.65
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.09
1.03
0.91
2.01
2.14
0.97
0.99
0.98
2.05
0.92
0.98
0.95
1.00
3.07
f1 (ppm)

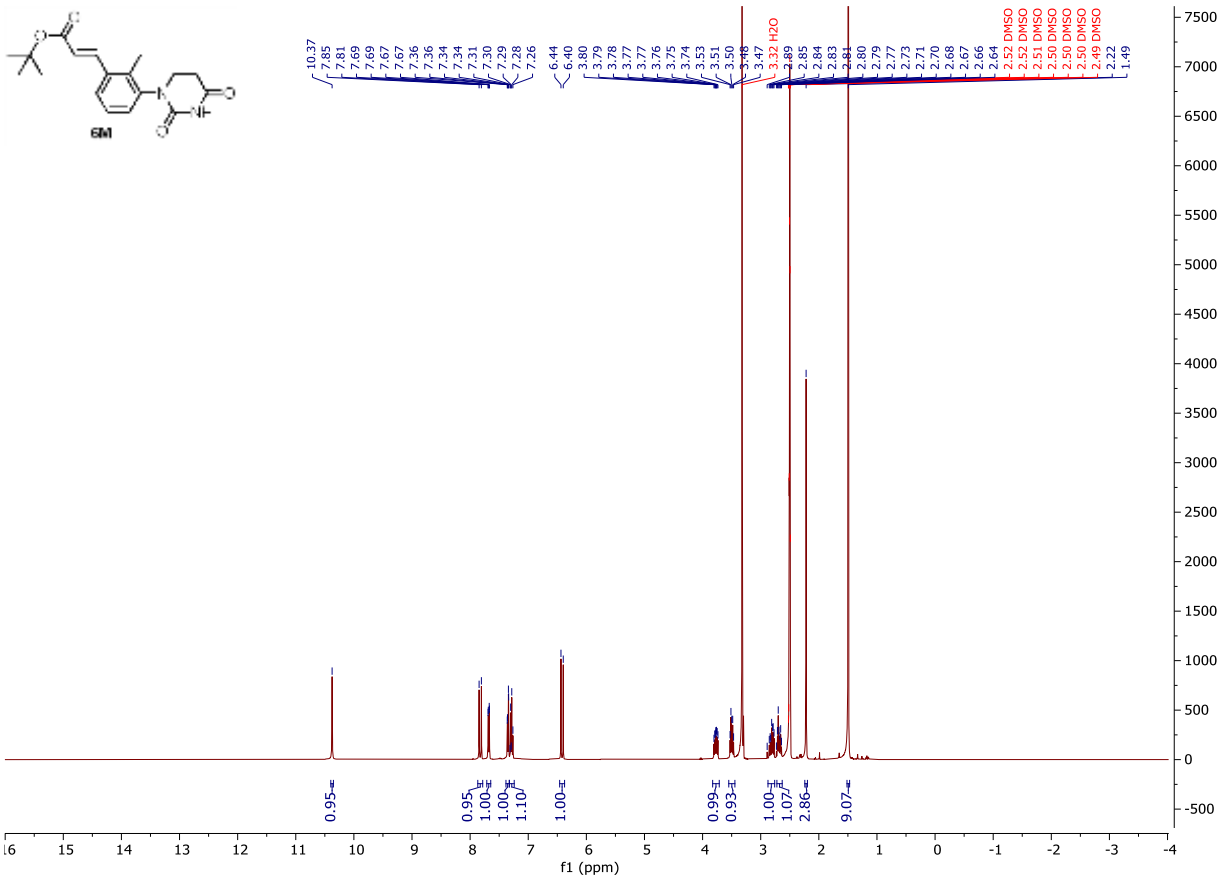

6J
170.7
156.5
151.8
144.2
141.8
135.6
129.8
127.6
126.7
124.3
119.6
110.8
69.0
44.7
31.1
10.8
f1 (ppm)

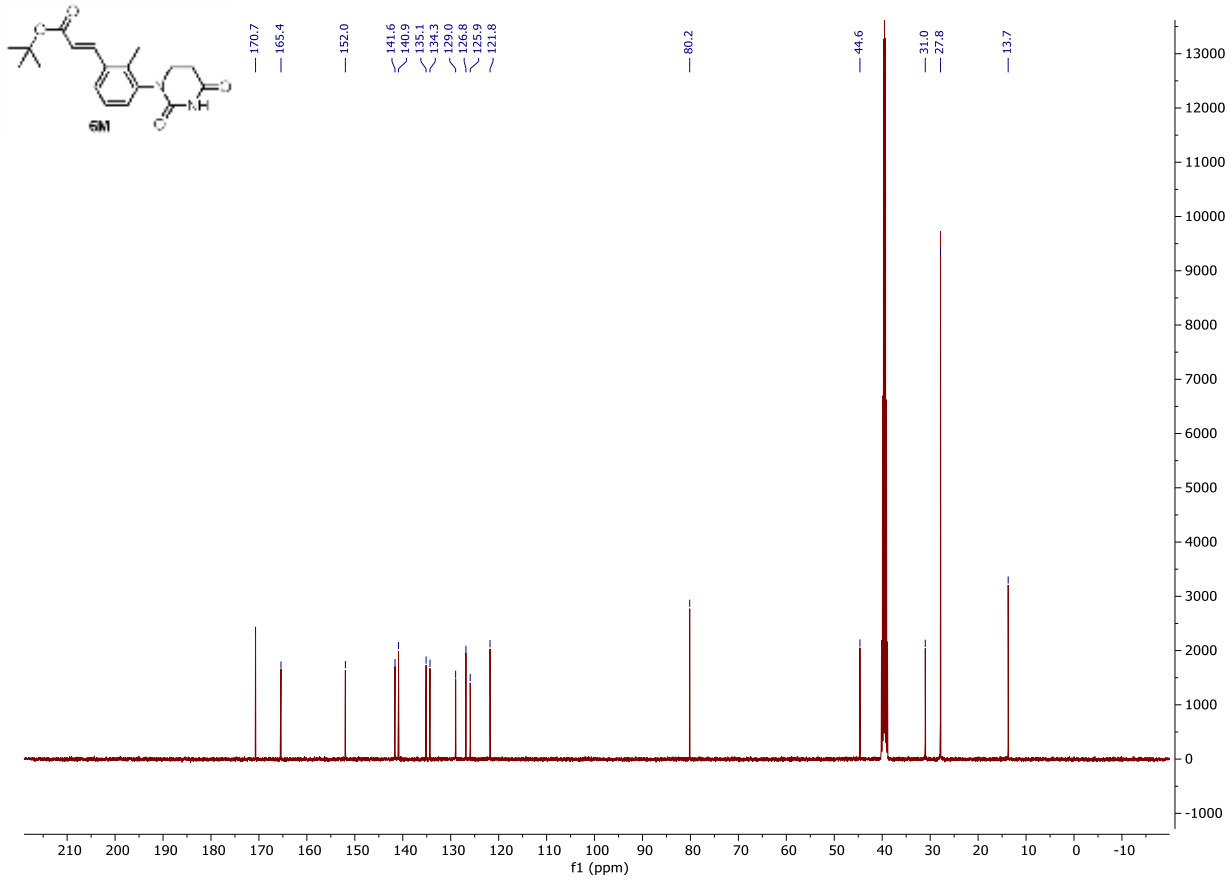

6K
10.33
7.19
7.17
7.15
6.91
6.89
6.89
6.81
6.81
6.79
6.79
4.70
3.78
3.77
3.76
3.75
3.74
3.74
3.73
3.71
3.51
3.50
3.48
3.47
3.45
3.32 H2O
2.82
2.80
2.80
2.78
2.78
2.76
2.75
2.74
2.70
2.69
2.67
2.66
2.65
2.63
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.05
1.05
0.98
1.00
1.01
1.94
1.00
1.02
0.98
1.03
2.92
f1 (ppm)

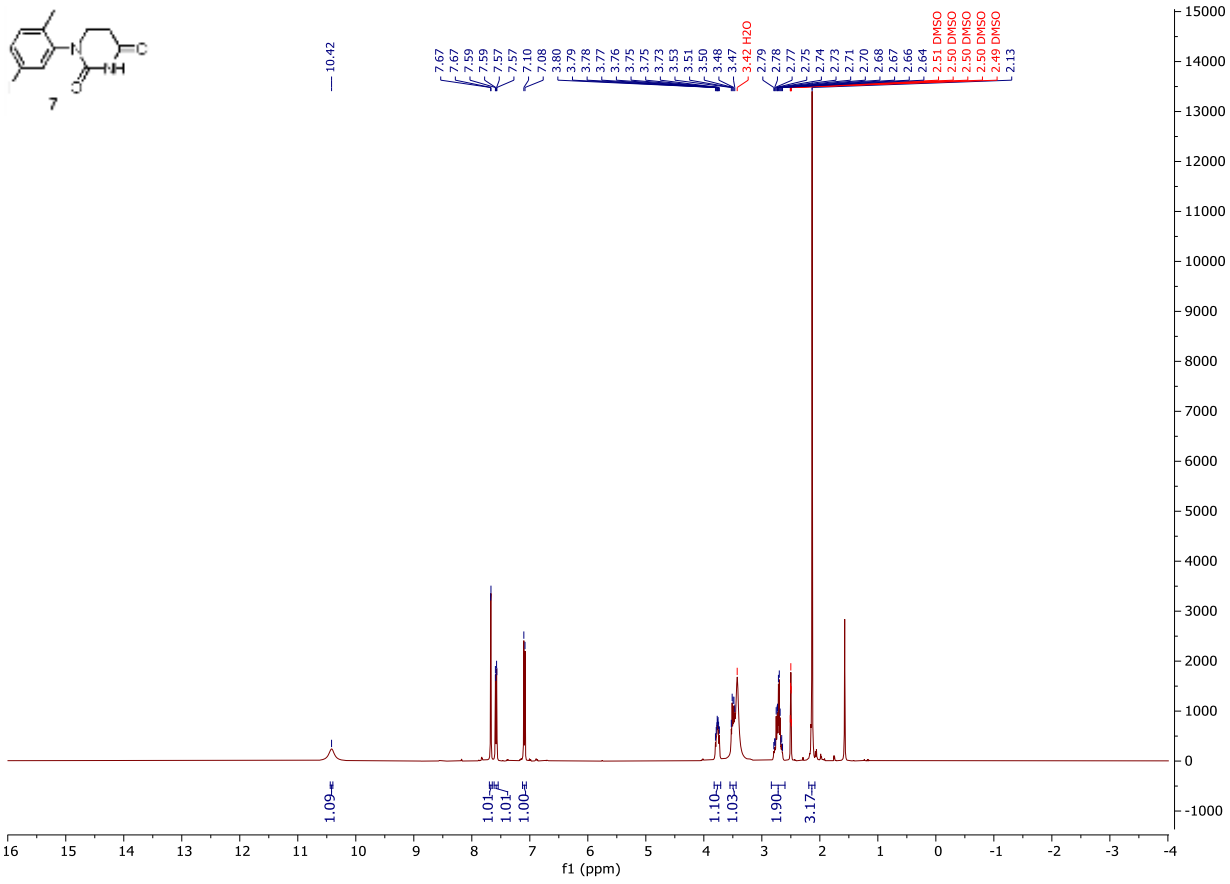

6K
170.7
167.8
156.3
151.7
141.8
126.5
124.4
119.8
110.5
81.4
65.6
44.7
31.1
27.7
10.7
f1 (ppm)

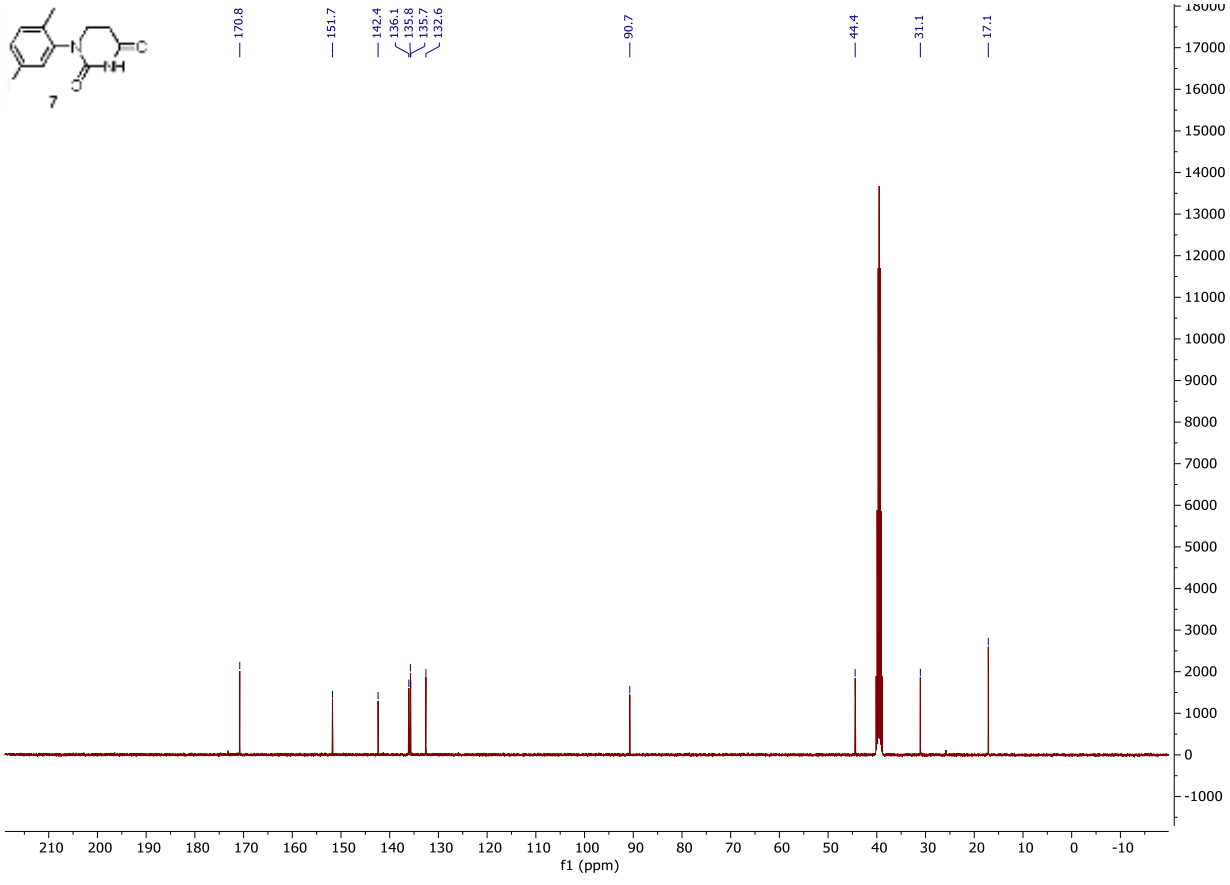

6L
9.11
7.19
7.17
7.17
7.16
7.15
3.90
3.89
3.88
3.87
3.86
3.86
3.84
3.83
3.67
3.66
3.64
3.63
3.61
2.96
2.95
2.94
2.93
2.93
2.92
2.90
2.89
2.88
2.87
2.86
2.85
2.83
2.80
2.79
2.78
2.76
2.75
2.75
2.73
2.52
2.51
2.50
2.49
2.22
1.42
0.35
2.94
1.00
1.03
2.13
2.52
1.97
2.97
8.63
f1 (ppm)

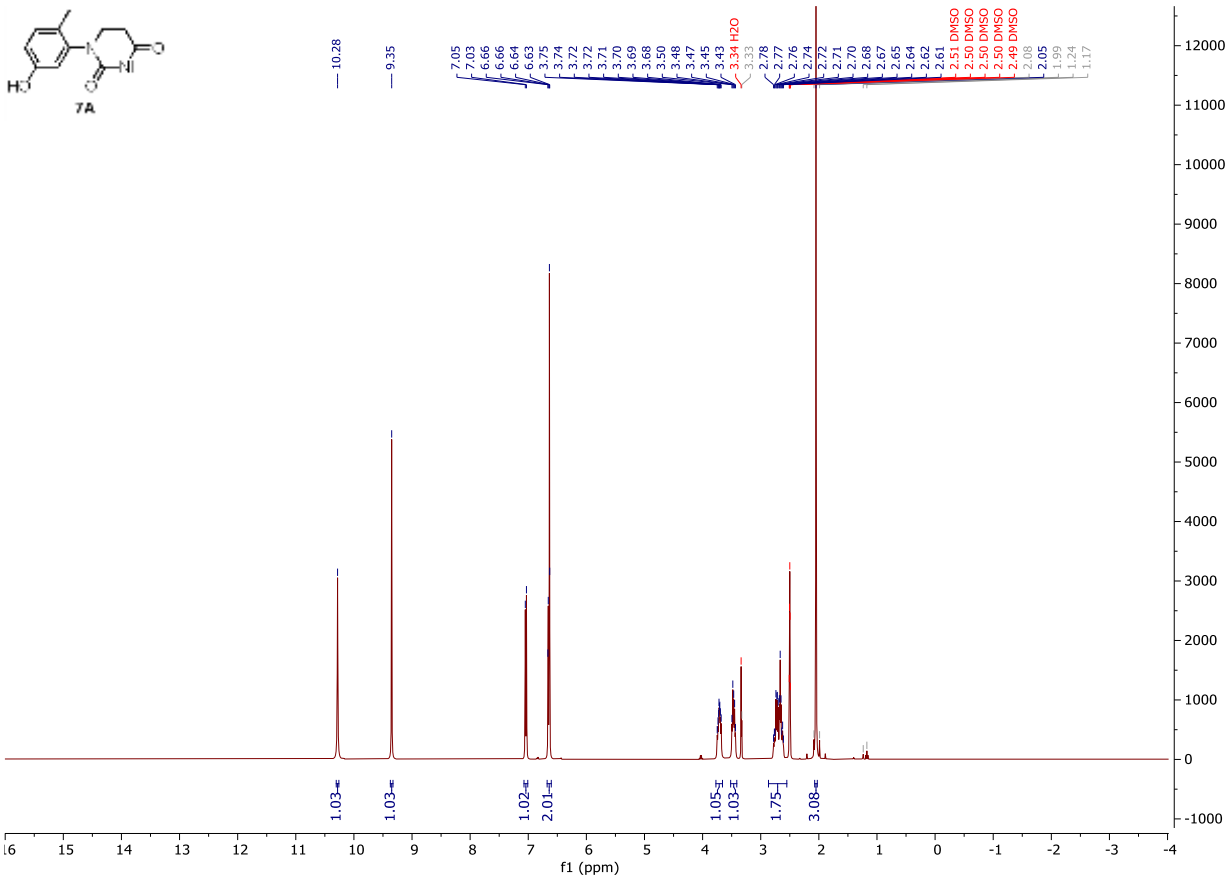

6L
172.4
170.9
152.6
142.4
141.4
135.0
128.9
127.2
126.2
80.4
46.1
36.2
32.1
29.5
28.2
13.8
f1 (ppm)

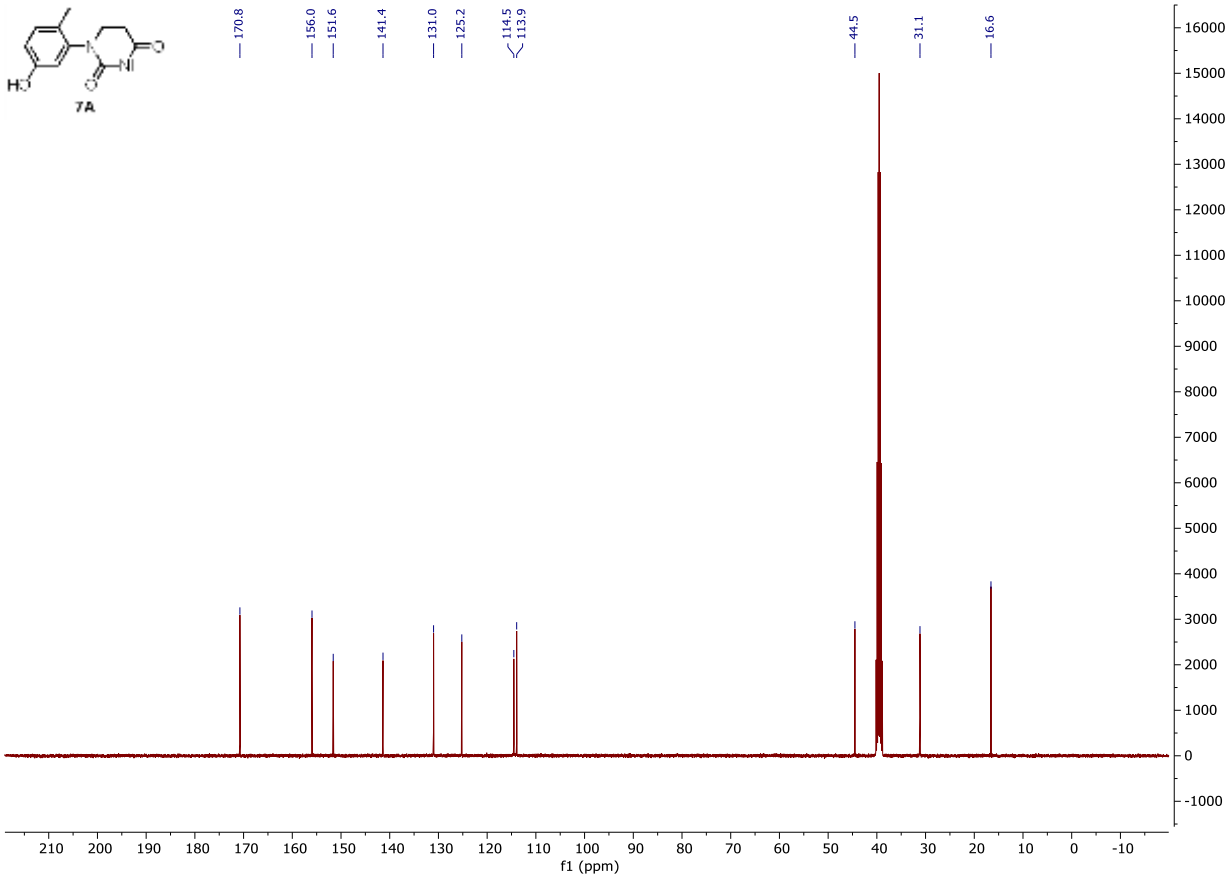

6M
10.37
7.85
7.81
7.69
7.69
7.67
7.67
7.36
7.36
7.34
7.34
7.31
7.30
7.29
7.28
7.26
6.44
6.40
3.80
3.79
3.78
3.77
3.77
3.76
3.75
3.74
3.53
3.51
3.50
3.48
3.47
3.32 H2O
2.89
2.85
2.84
2.83
2.81
2.80
2.79
2.77
2.73
2.71
2.70
2.68
2.67
2.66
2.64
2.52 DMSO
2.52 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.22
1.49
0.95
0.95
1.00
1.00
1.10
1.00
0.99
0.93
1.00
1.07
2.86
9.07
f1 (ppm)

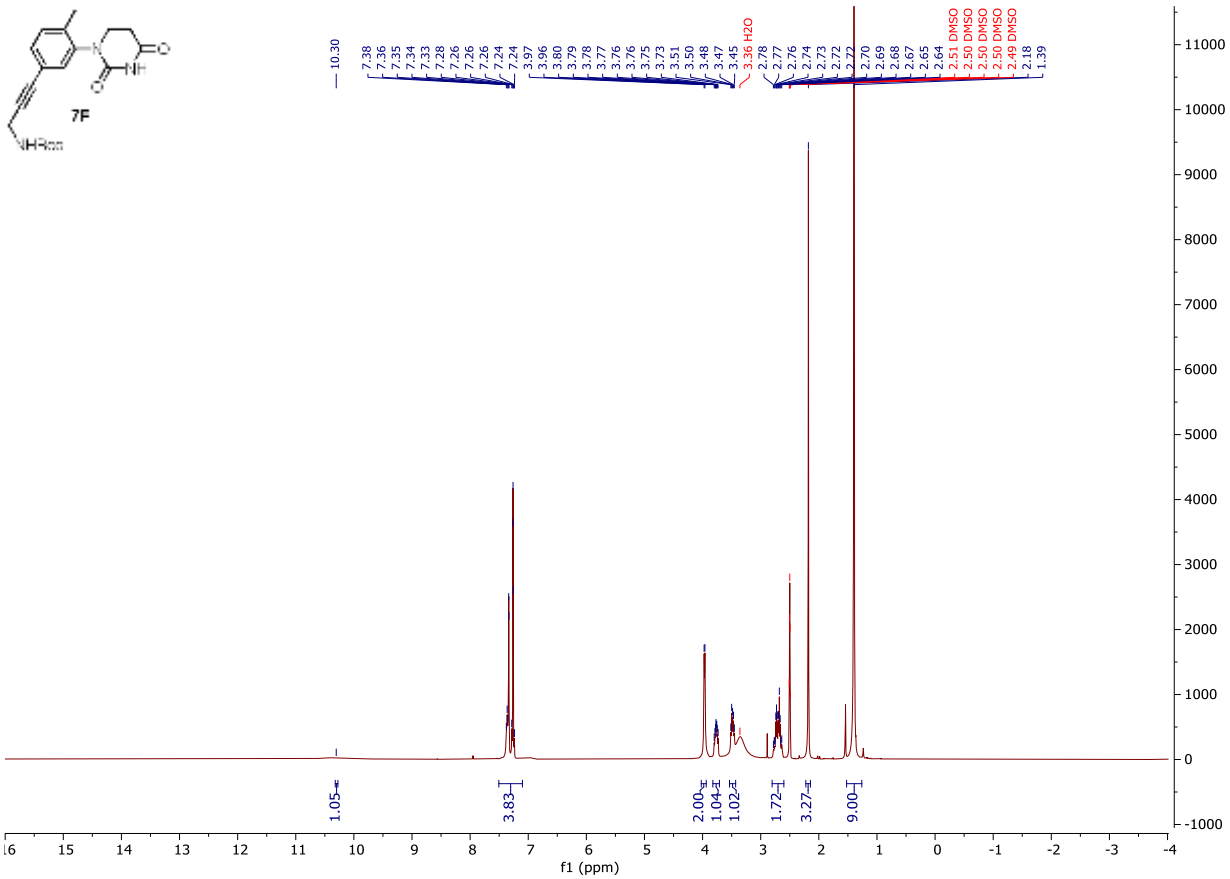

6M
170.7
165.4
152.0
141.6
140.9
135.1
134.3
129.0
126.8
125.9
121.8
80.2
44.6
31.0
27.8
13.7
f1 (ppm)

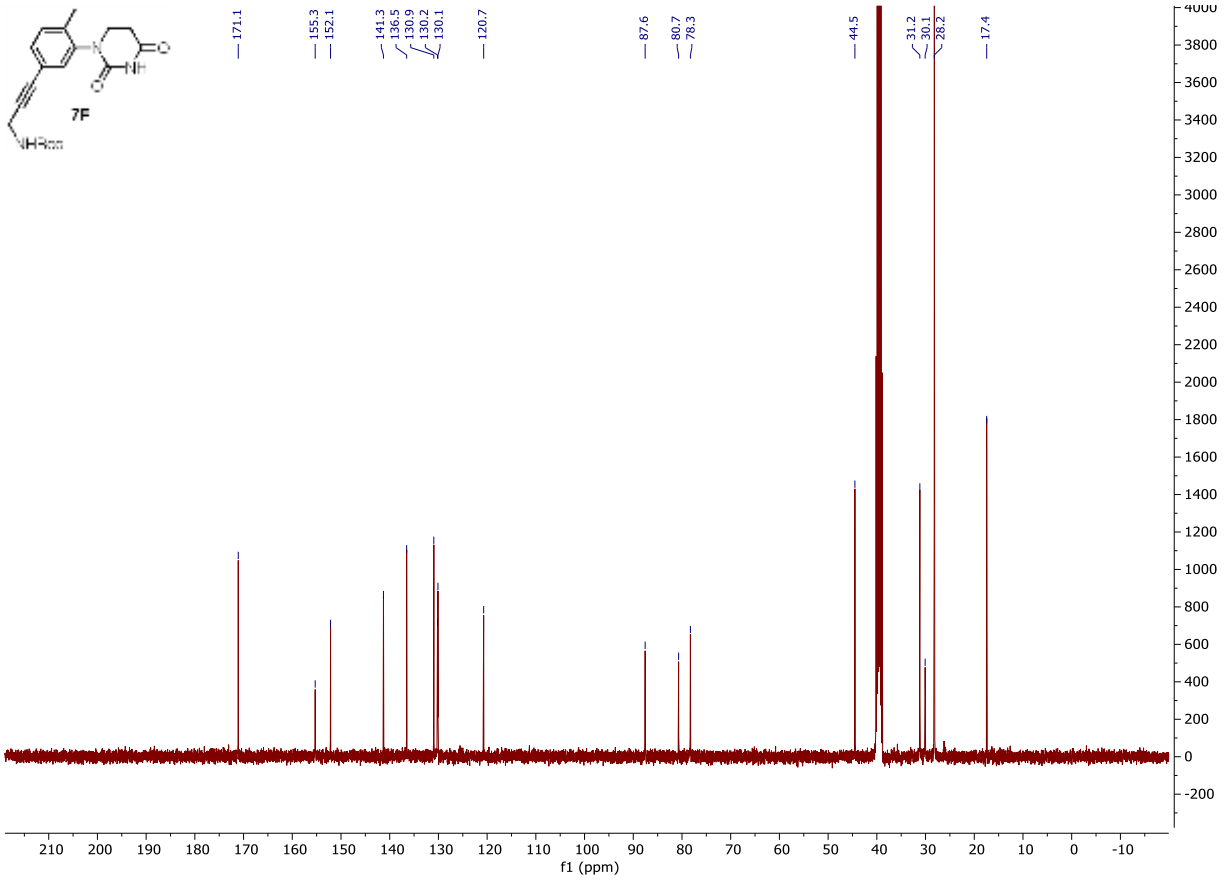

7
10.42
7.67
7.67
7.59
7.59
7.57
7.57
7.10
7.08
3.80
3.79
3.78
3.77
3.76
3.75
3.75
3.73
3.53
3.51
3.50
3.48
3.47
3.42 H2O
2.79
2.78
2.77
2.75
2.74
2.73
2.71
2.70
2.68
2.67
2.66
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.13
1.09
1.01
1.01
1.00
1.10
1.03
1.90
3.17
f1 (ppm)

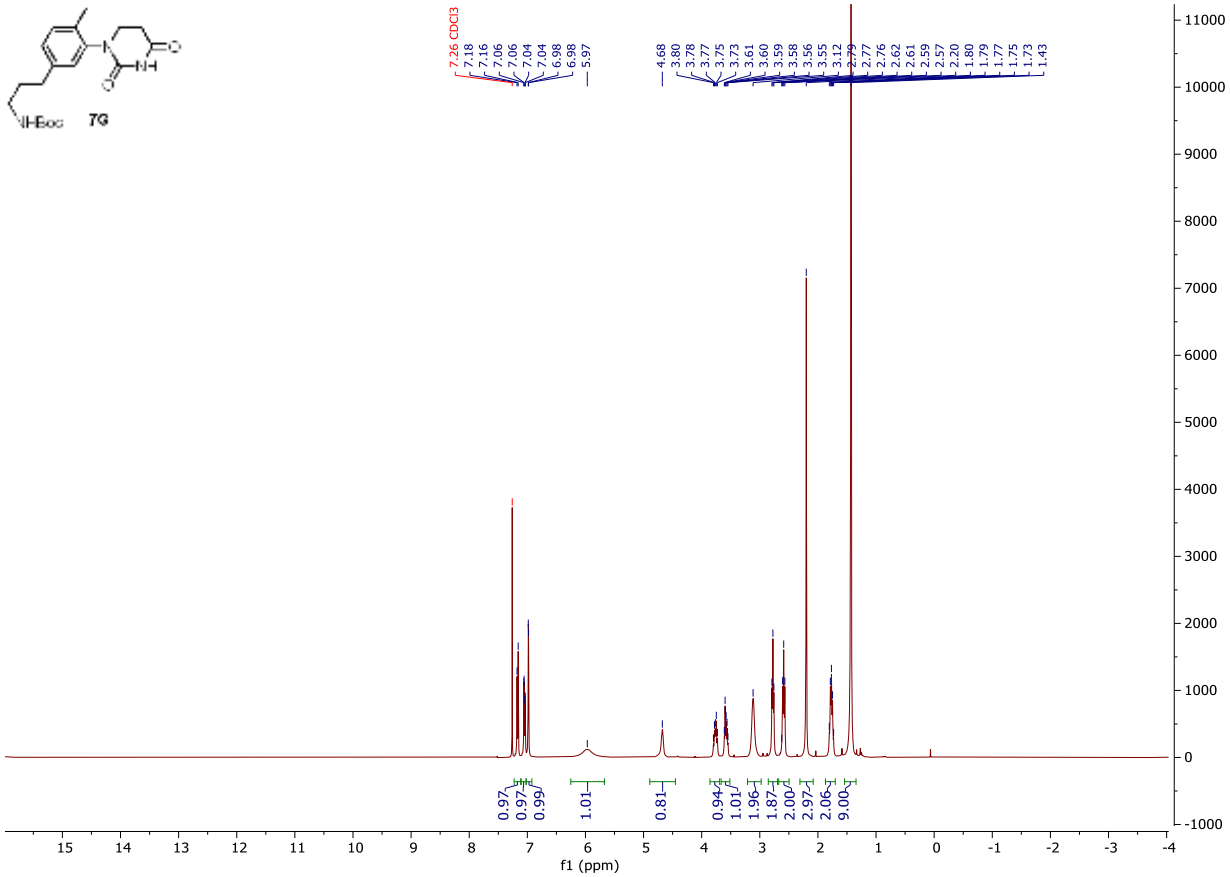

7
170.8
151.7
142.4
136.1
135.8
135.7
132.6
90.7
44.4
31.1
17.1
f1 (ppm)

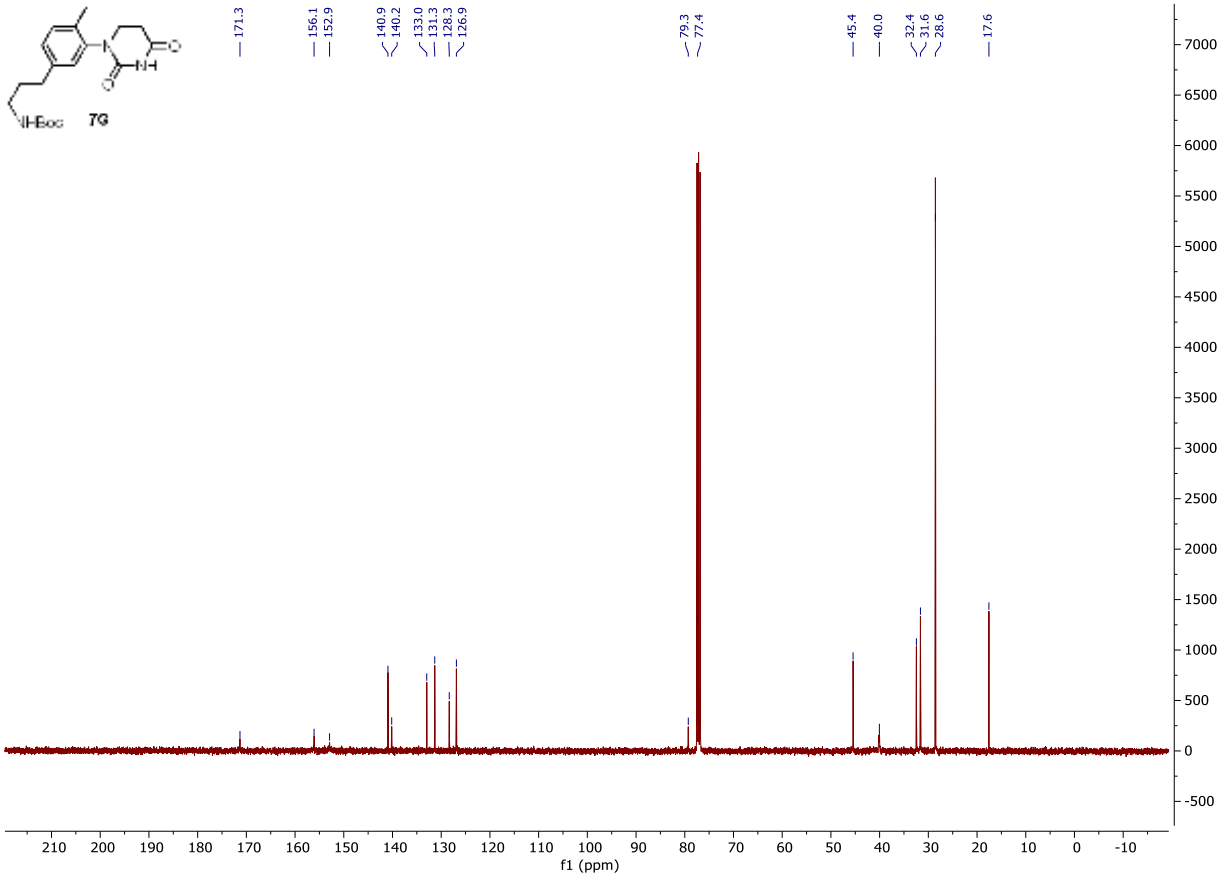

7A
10.28
9.35
7.05
7.03
6.66
6.64
6.63
3.75
3.74
3.72
3.71
3.70
3.69
3.68
3.50
3.48
3.47
3.45
3.43
3.34 H2O
3.33
2.78
2.77
2.76
2.74
2.72
2.71
2.70
2.68
2.67
2.65
2.64
2.62
2.61
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.08
2.05
1.99
1.24
1.17
1.03
1.03
1.02
2.01
1.05
1.03
1.75
3.08
f1 (ppm)

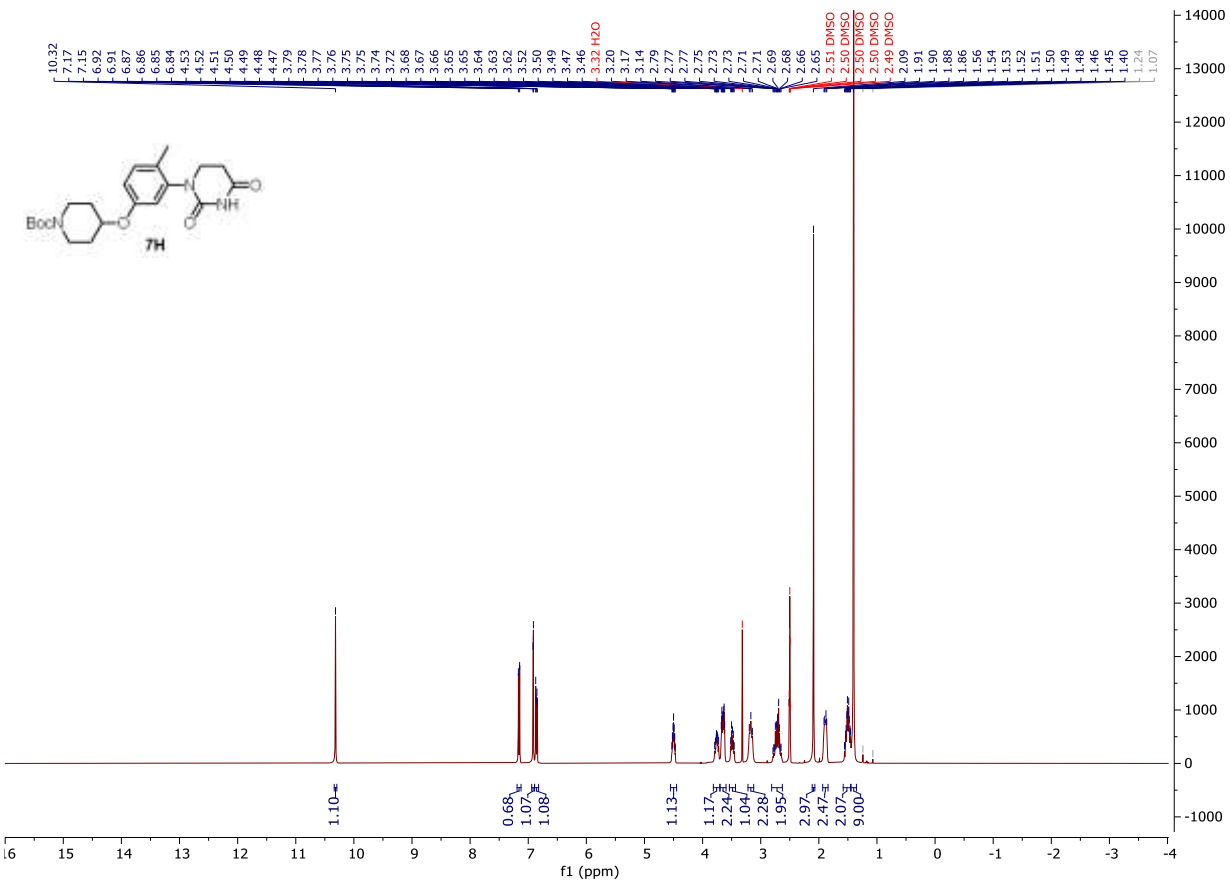

7A
170.8
156.0
151.6
141.4
131.0
125.2
114.5
113.9
44.5
31.1
16.6
f1 (ppm)

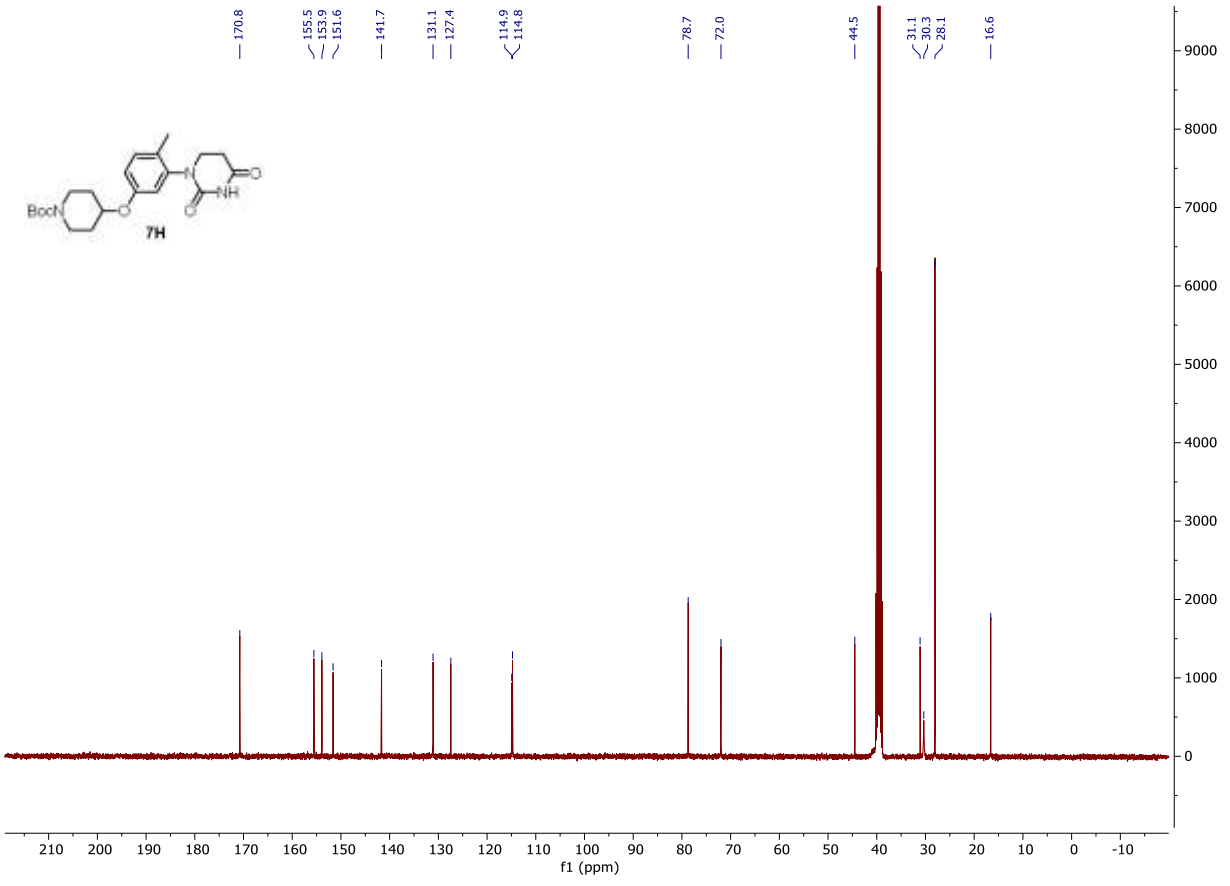

7F
10.30
7.38
7.36
7.35
7.34
7.33
7.28
7.26
7.26
7.26
7.24
7.24
3.97
3.96
3.80
3.79
3.78
3.77
3.76
3.76
3.75
3.73
3.51
3.50
3.48
3.47
3.45
3.36 H2O
2.78
2.77
2.76
2.74
2.73
2.72
2.72
2.70
2.69
2.68
2.67
2.65
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.18
1.39
1.05
3.83
2.00
1.04
1.02
1.72
3.27
9.00
f1 (ppm)

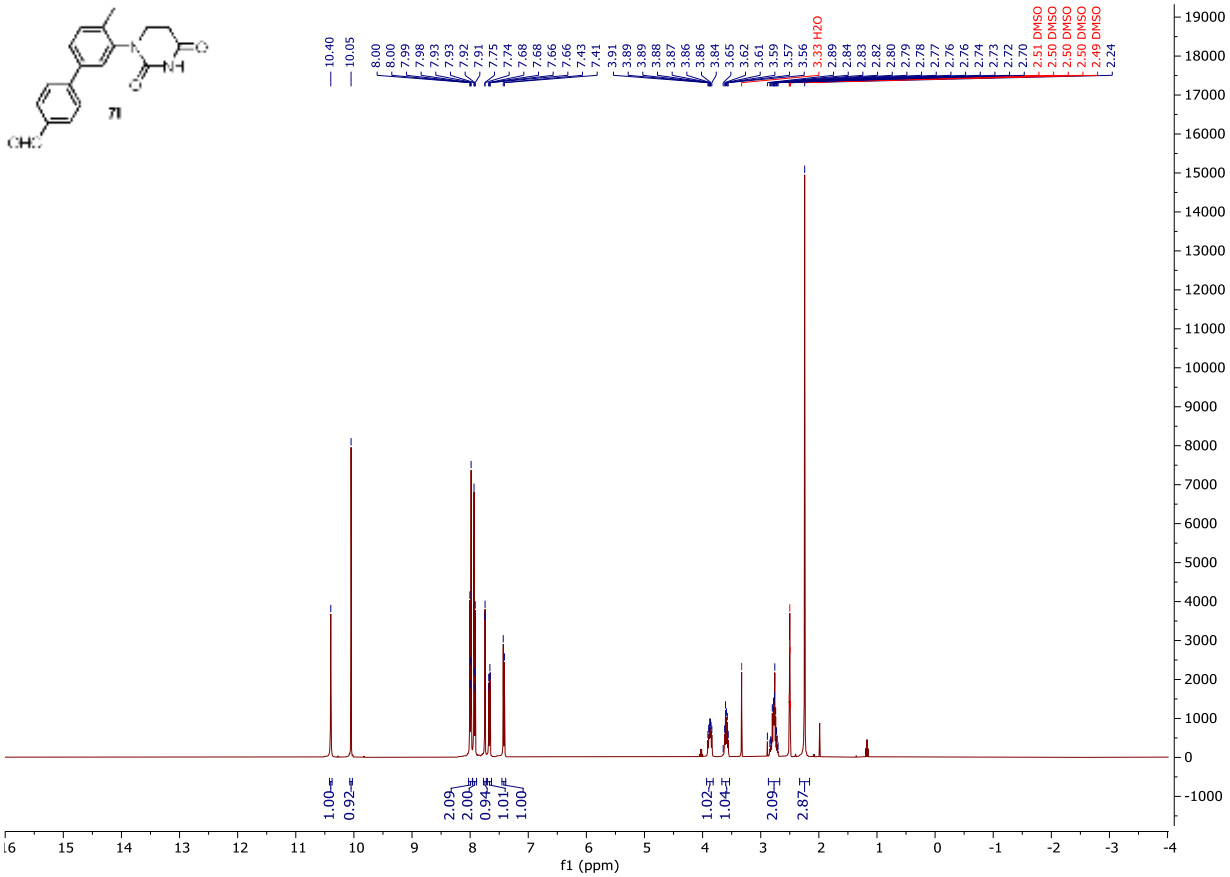

7F
171.1
155.3
152.1
141.3
136.5
130.9
130.2
130.1
120.7
87.6
80.7
78.3
44.5
31.2
30.1
28.2
17.4
f1 (ppm)

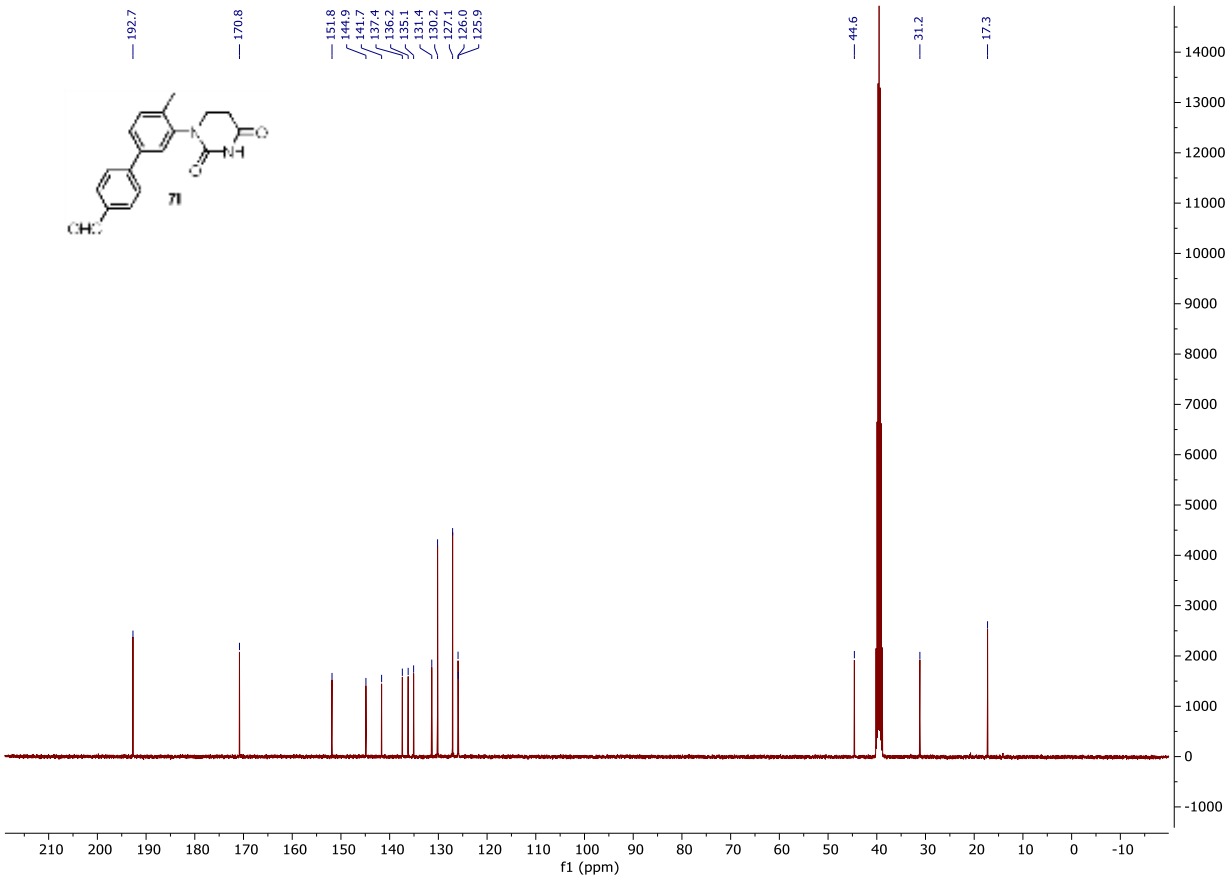

7G
7.26 CDCl3
7.18
7.16
7.06
7.06
7.04
7.04
6.98
6.98
5.97
4.68
3.80
3.78
3.77
3.75
3.73
3.61
3.60
3.59
3.58
3.56
3.55
3.12
2.79
2.77
2.76
2.62
2.61
2.59
2.57
2.20
1.80
1.79
1.77
1.75
1.73
1.43
0.97
0.97
0.99
1.01
0.81
0.94
1.01
1.96
1.87
2.00
2.97
2.06
9.00
f1 (ppm)

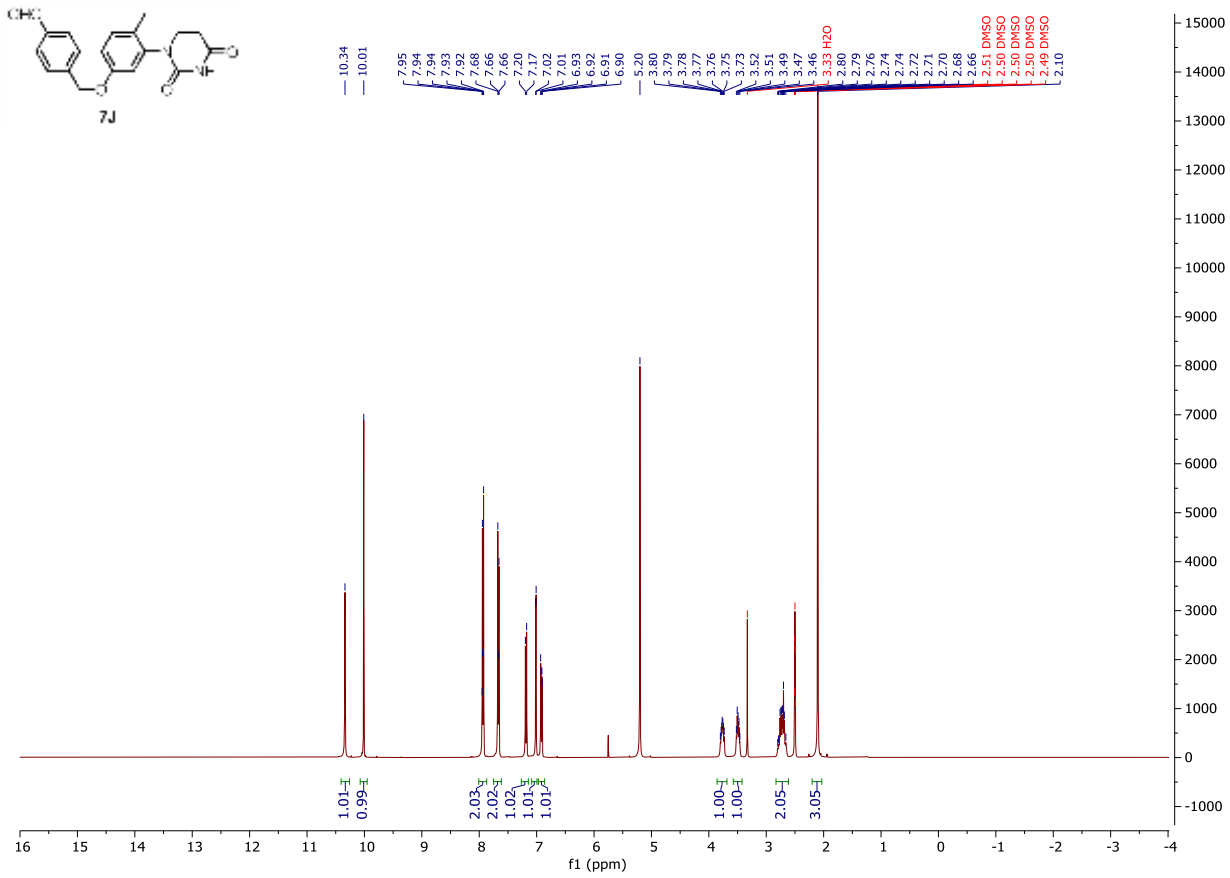

7G
171.3
156.1
152.9
140.9
140.2
133.0
131.3
128.3
126.9
79.3
77.4
45.4
40.0
32.4
31.6
28.6
17.6
f1 (ppm)

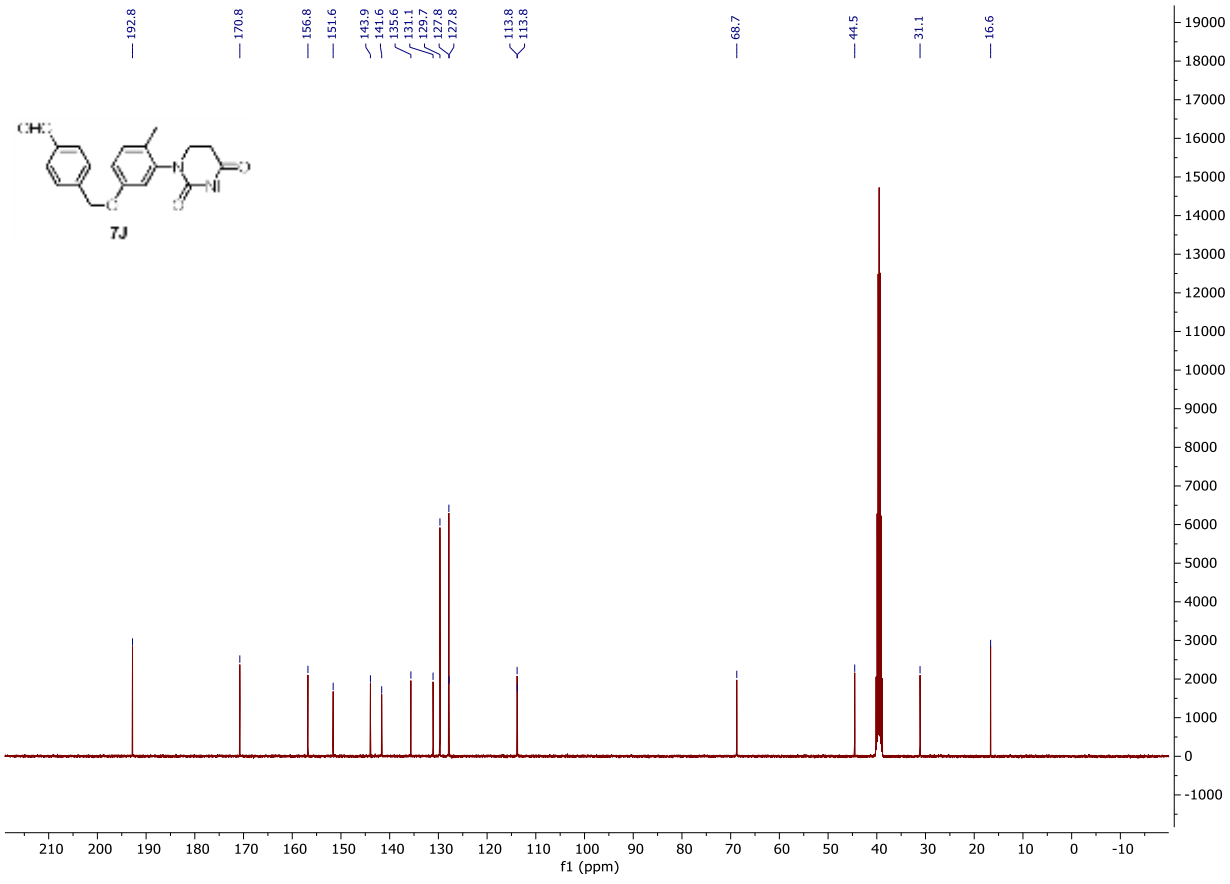

10.32
7.17
7.15
6.92
6.91
6.87
6.86
6.85
6.84
4.53
4.52
4.51
4.50
4.49
4.48
4.47
3.79
3.78
3.77
3.76
3.75
3.74
3.72
3.68
3.67
3.66
3.65
3.64
3.63
3.62
3.52
3.50
3.49
3.47
3.46
3.32 H2O
3.20
3.17
3.14
2.79
2.77
2.77
2.75
2.73
2.73
2.71
2.71
2.69
2.68
2.66
2.65
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.09
1.91
1.90
1.88
1.86
1.56
1.54
1.53
1.52
1.51
1.50
1.49
1.48
1.46
1.45
1.40
1.24
1.07
BocN
NH
7H
1.10
0.68
1.07
1.08
1.13
1.17
2.24
1.04
2.28
1.95
2.97
2.47
2.07
9.00
f1 (ppm)

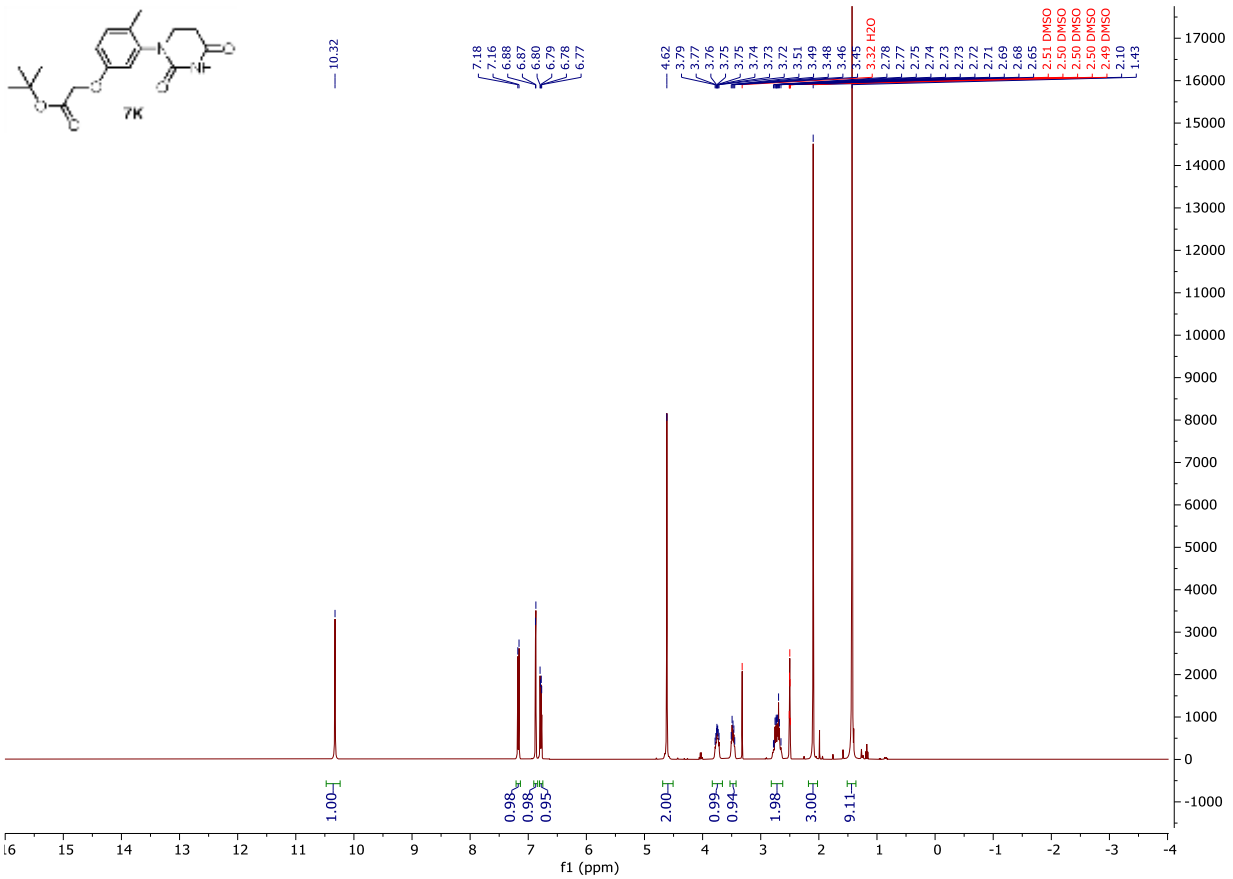

170.8
155.5
153.9
151.6
141.7
131.1
127.4
114.9
114.8
78.7
72.0
44.5
31.1
30.3
28.1
16.6
BocN
NH
7H
f1 (ppm)

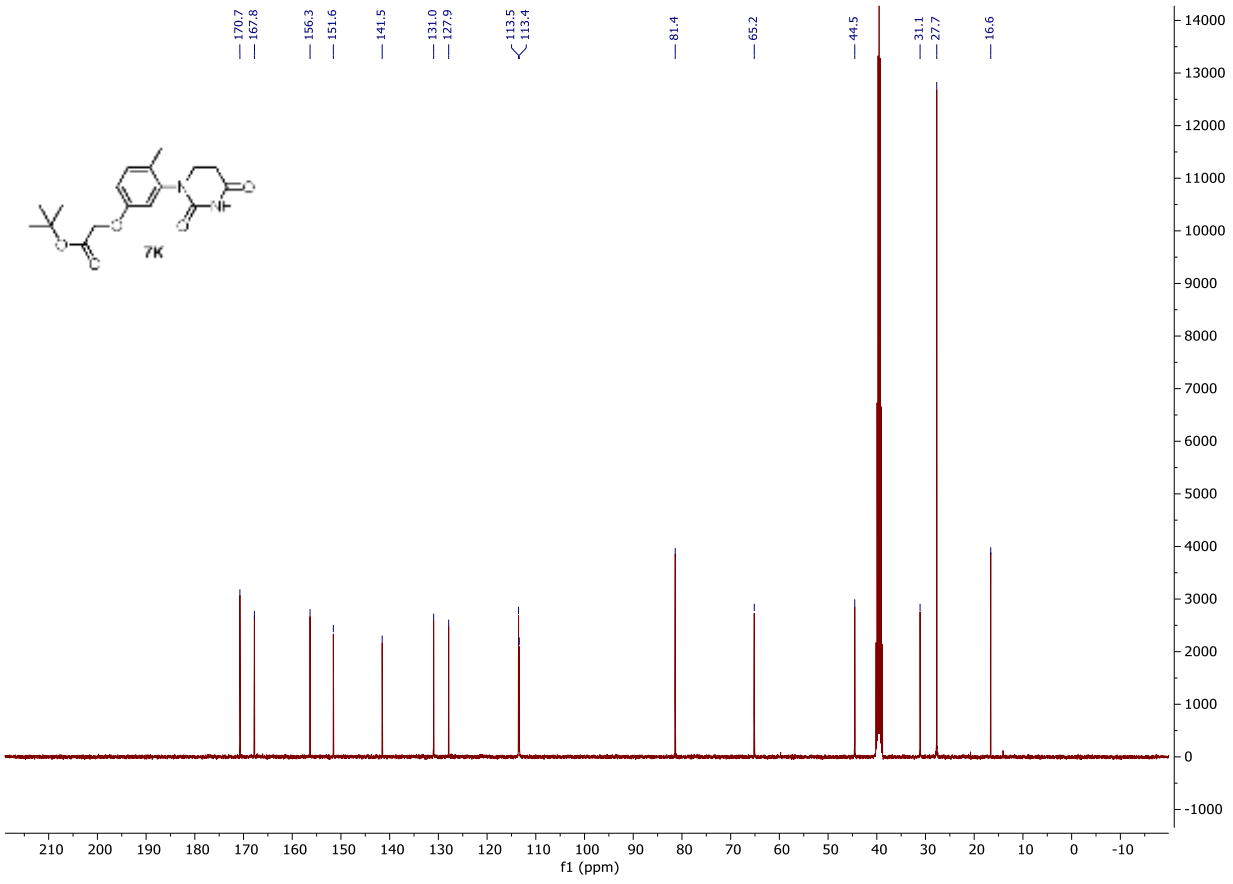

10.40
10.05
8.00
8.00
7.99
7.98
7.93
7.93
7.92
7.91
7.75
7.74
7.68
7.68
7.66
7.66
7.43
7.41
3.91
3.89
3.89
3.88
3.87
3.86
3.86
3.84
3.65
3.62
3.61
3.59
3.57
3.56
3.33 H2O
2.89
2.84
2.83
2.82
2.80
2.79
2.78
2.77
2.76
2.76
2.74
2.73
2.72
2.70
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.24
1.00
0.92
2.09
2.00
0.94
1.01
1.00
1.02
1.04
2.09
2.87
f1 (ppm)

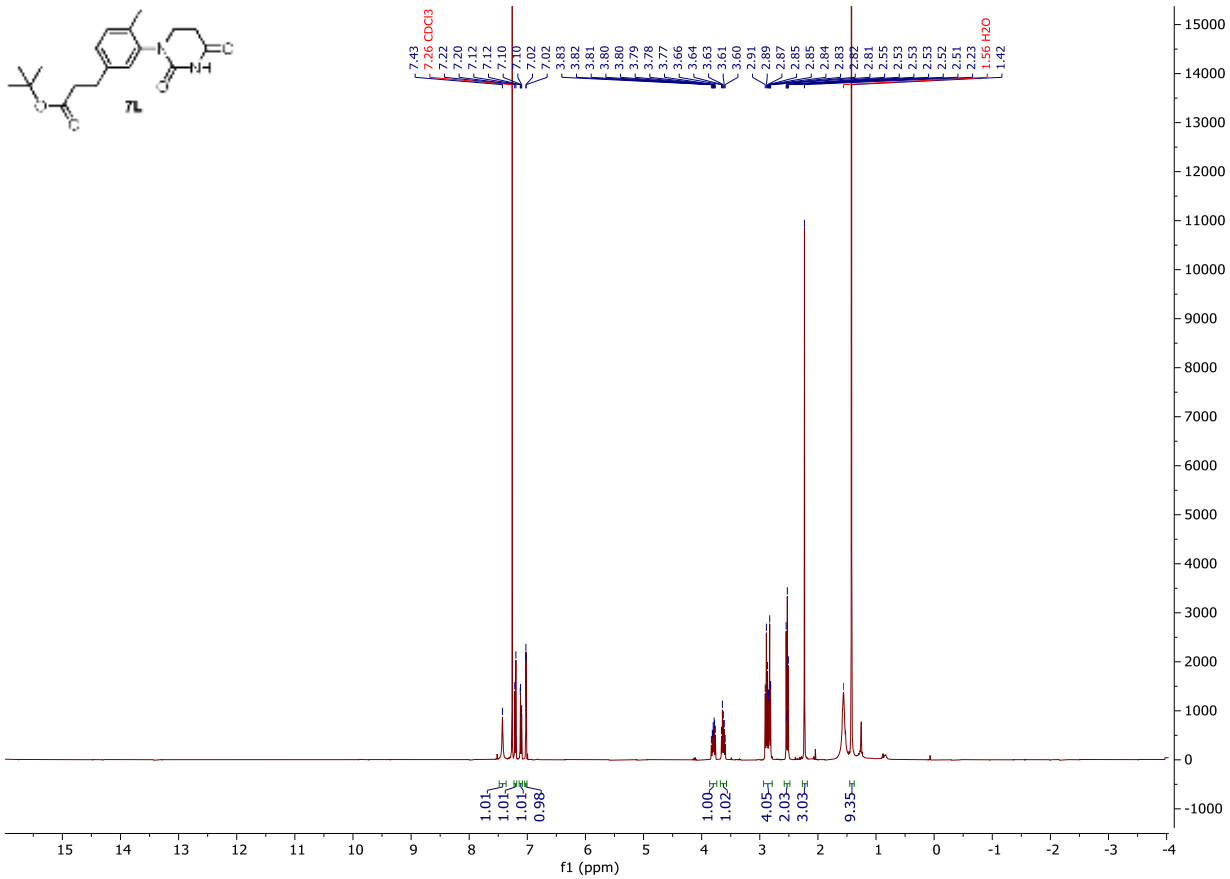

192.7
170.8
151.8
144.9
141.7
137.4
136.2
135.1
131.4
130.2
127.1
126.0
125.9
44.6
31.2
17.3
f1 (ppm)

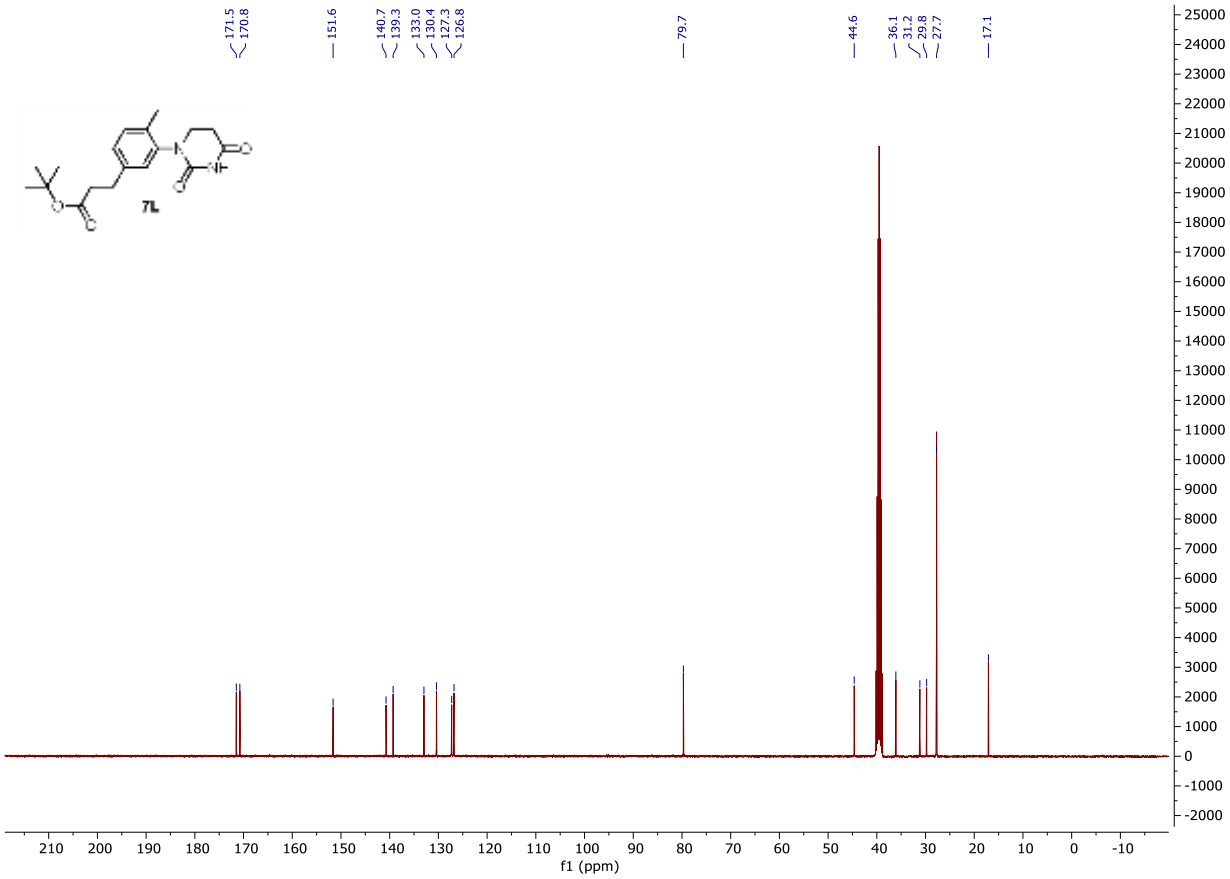

7J
10.34
10.01
7.95
7.94
7.94
7.93
7.92
7.68
7.66
7.66
7.20
7.17
7.02
7.01
6.93
6.92
6.91
6.90
5.20
3.80
3.79
3.78
3.77
3.76
3.75
3.73
3.52
3.51
3.49
3.47
3.46
3.33 H2O
2.80
2.79
2.76
2.74
2.72
2.71
2.70
2.68
2.66
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.10
1.01
0.99
2.03
2.02
1.02
1.01
1.01
1.00
1.00
2.05
3.05
f1 (ppm)

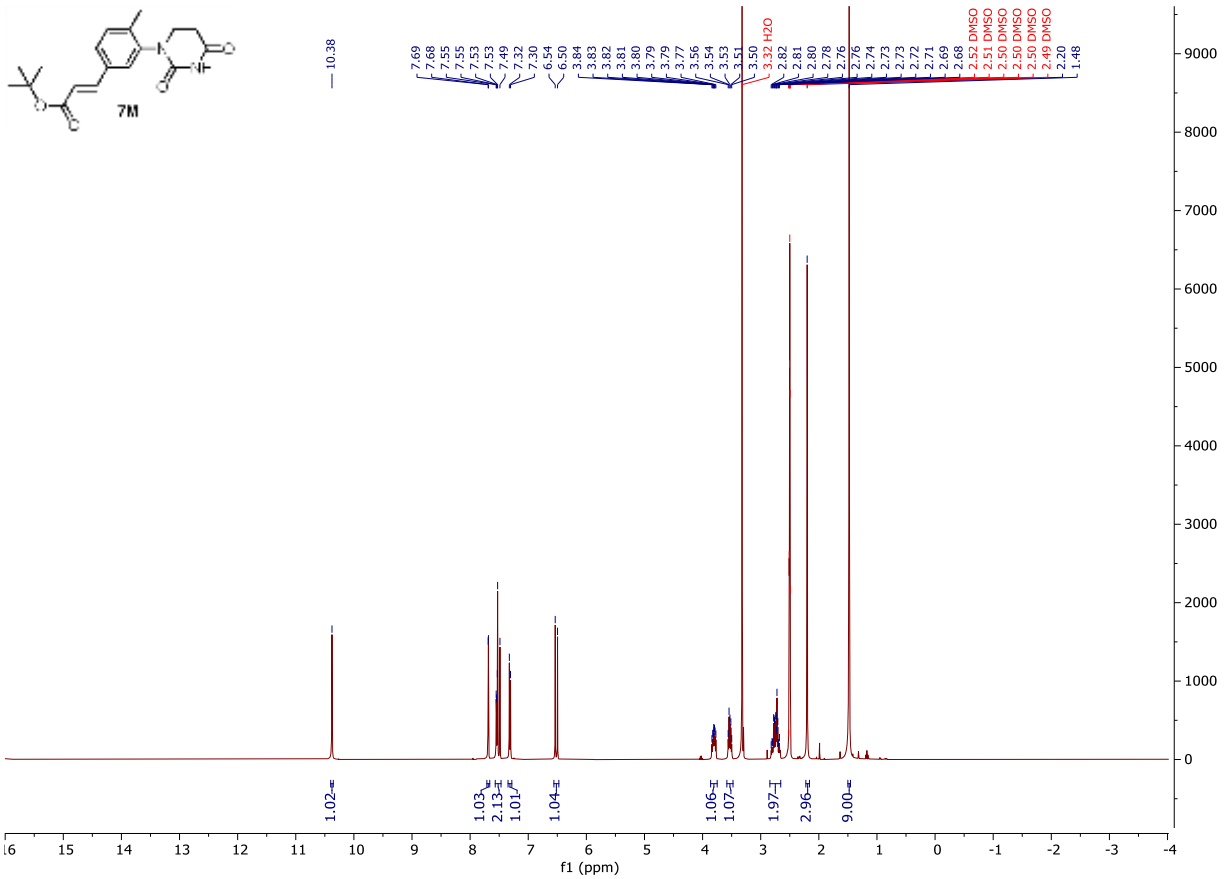

7J
192.8
170.8
156.8
151.6
143.9
141.6
135.6
131.1
129.7
127.8
127.8
113.8
113.8
68.7
44.5
31.1
16.6
f1 (ppm)

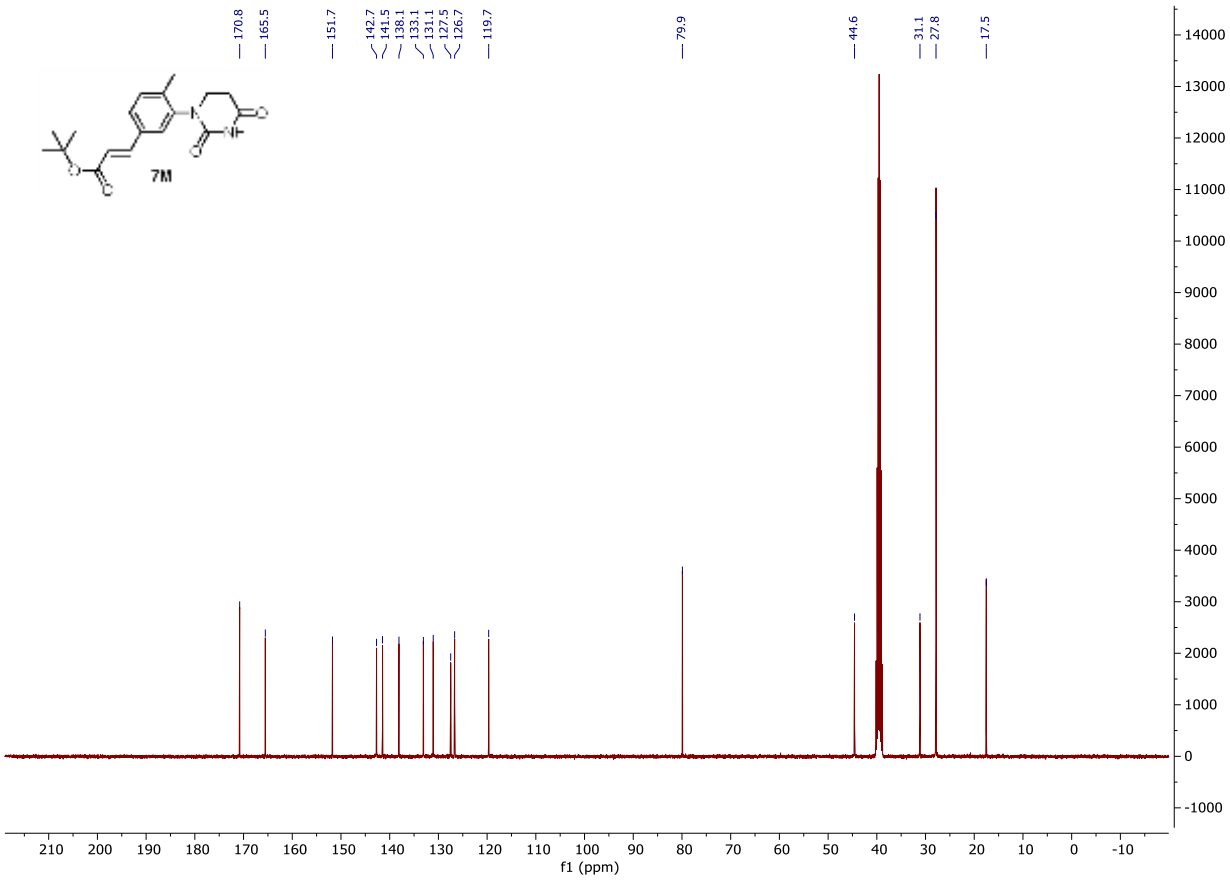

7K
10.32
7.18
7.16
6.88
6.87
6.80
6.79
6.78
6.77
4.62
3.79
3.77
3.76
3.75
3.75
3.74
3.73
3.72
3.51
3.49
3.48
3.46
3.45
3.32 H2O
2.78
2.77
2.75
2.74
2.73
2.73
2.72
2.71
2.69
2.68
2.65
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.10
1.43
1.00
0.98
0.98
0.95
2.00
0.99
0.94
1.98
3.00
9.11
f1 (ppm)

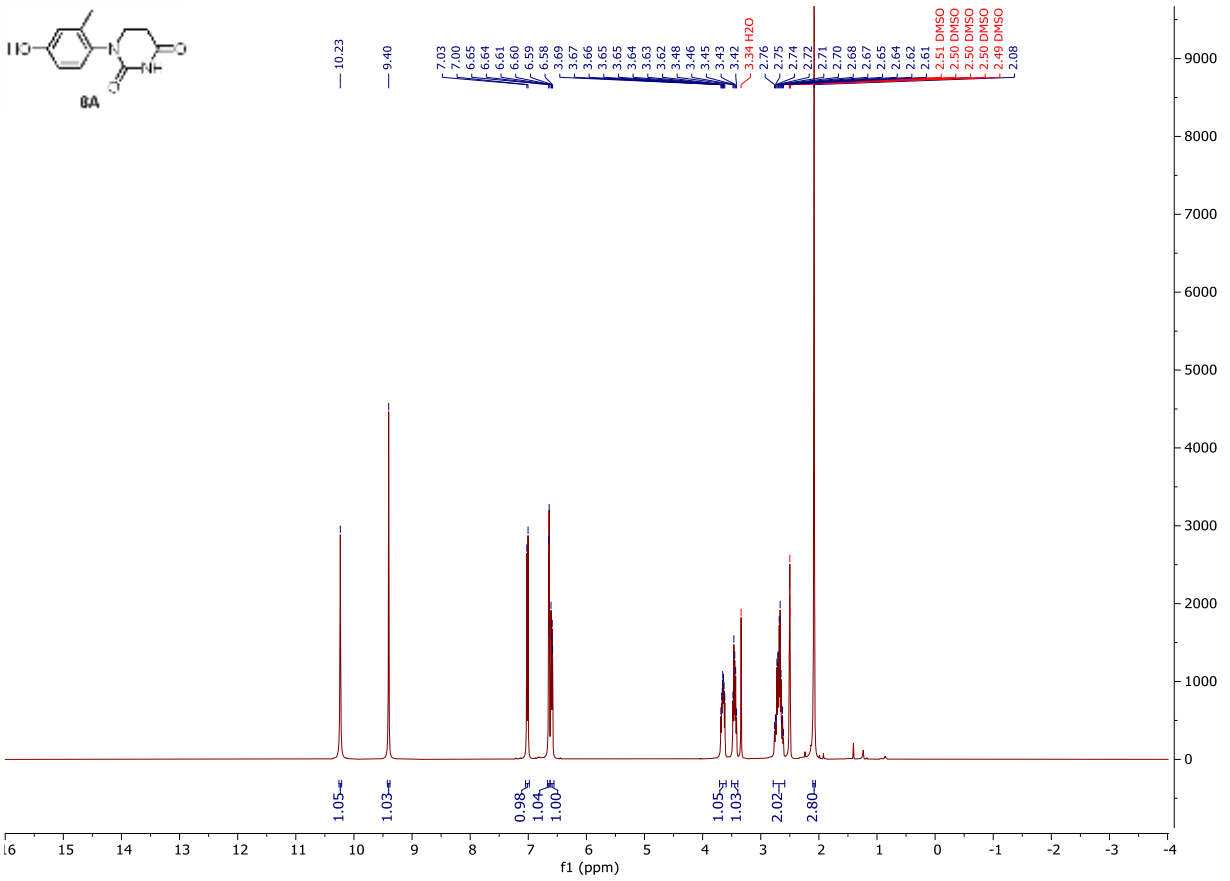

7K
170.7
167.8
156.3
151.6
141.5
131.0
127.9
113.5
113.4
81.4
65.2
44.5
31.1
27.7
16.6
f1 (ppm)

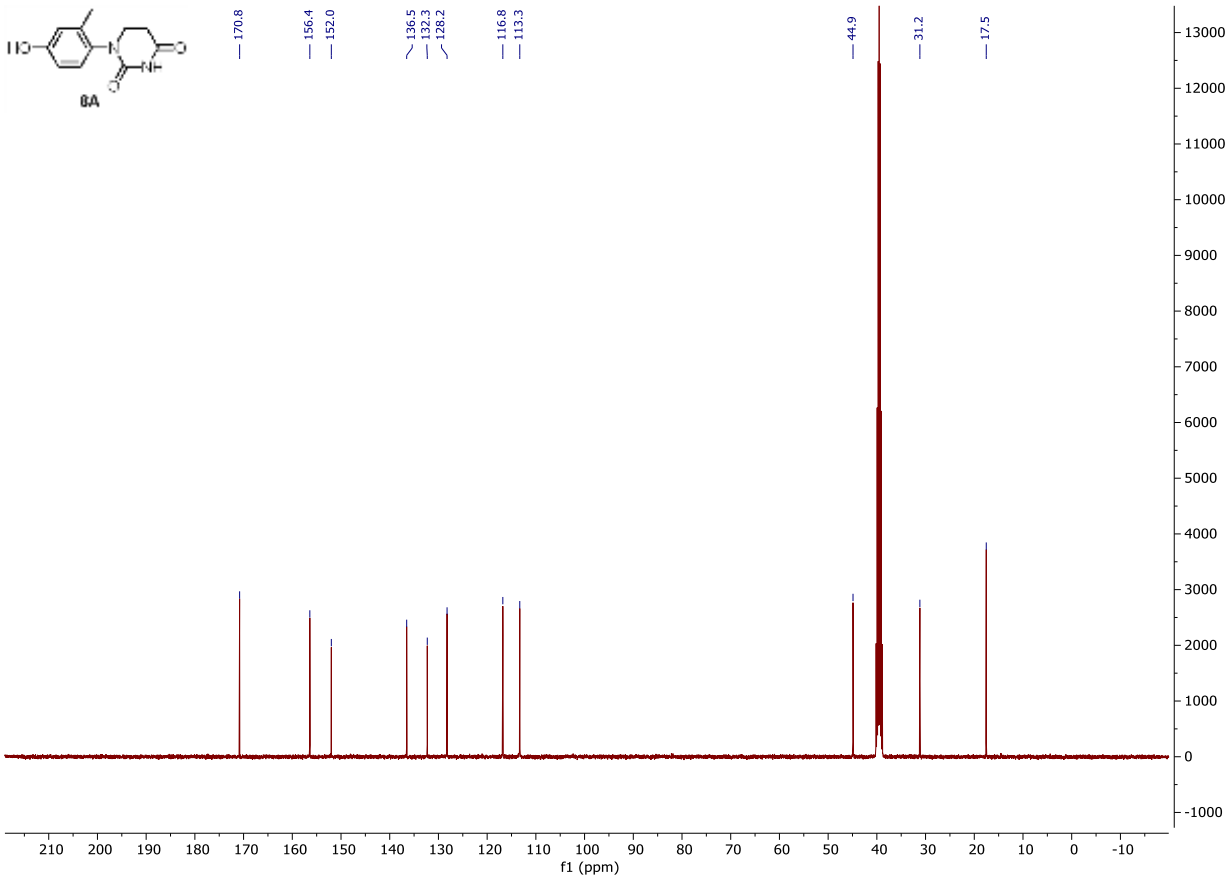

7L
7.43
7.26 CDCl3
7.22
7.20
7.12
7.12
7.10
7.10
7.02
7.02
3.83
3.82
3.81
3.80
3.80
3.79
3.78
3.77
3.66
3.64
3.63
3.61
3.60
2.91
2.89
2.87
2.85
2.85
2.84
2.83
2.82
2.81
2.55
2.53
2.53
2.52
2.51
2.23
1.56 H2O
1.42
1.01
1.01
1.01
0.98
1.00
1.02
4.05
2.03
3.03
9.35
f1 (ppm)

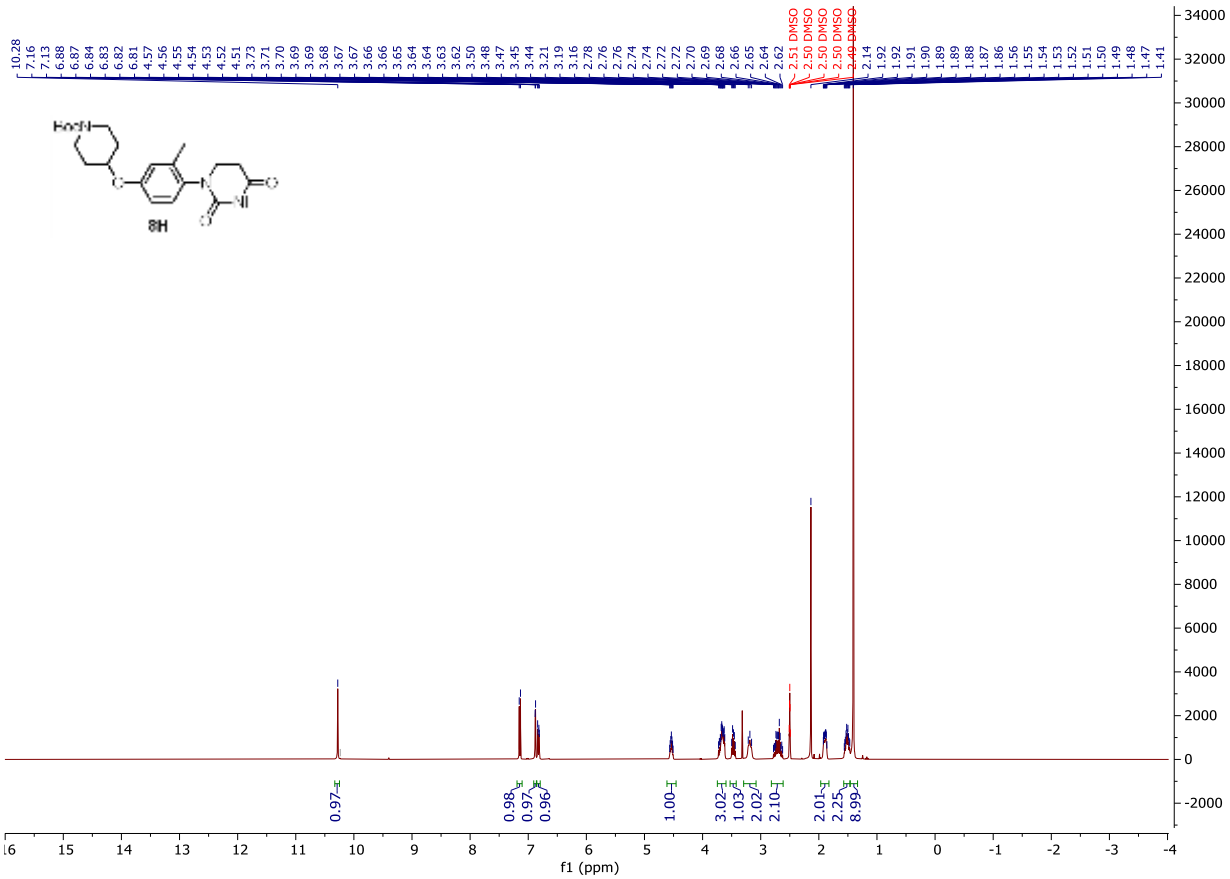

171.5
170.8
151.6
140.7
139.3
133.0
130.4
127.3
126.8
79.7
44.6
36.1
31.2
29.8
27.7
17.1
7L
f1 (ppm)

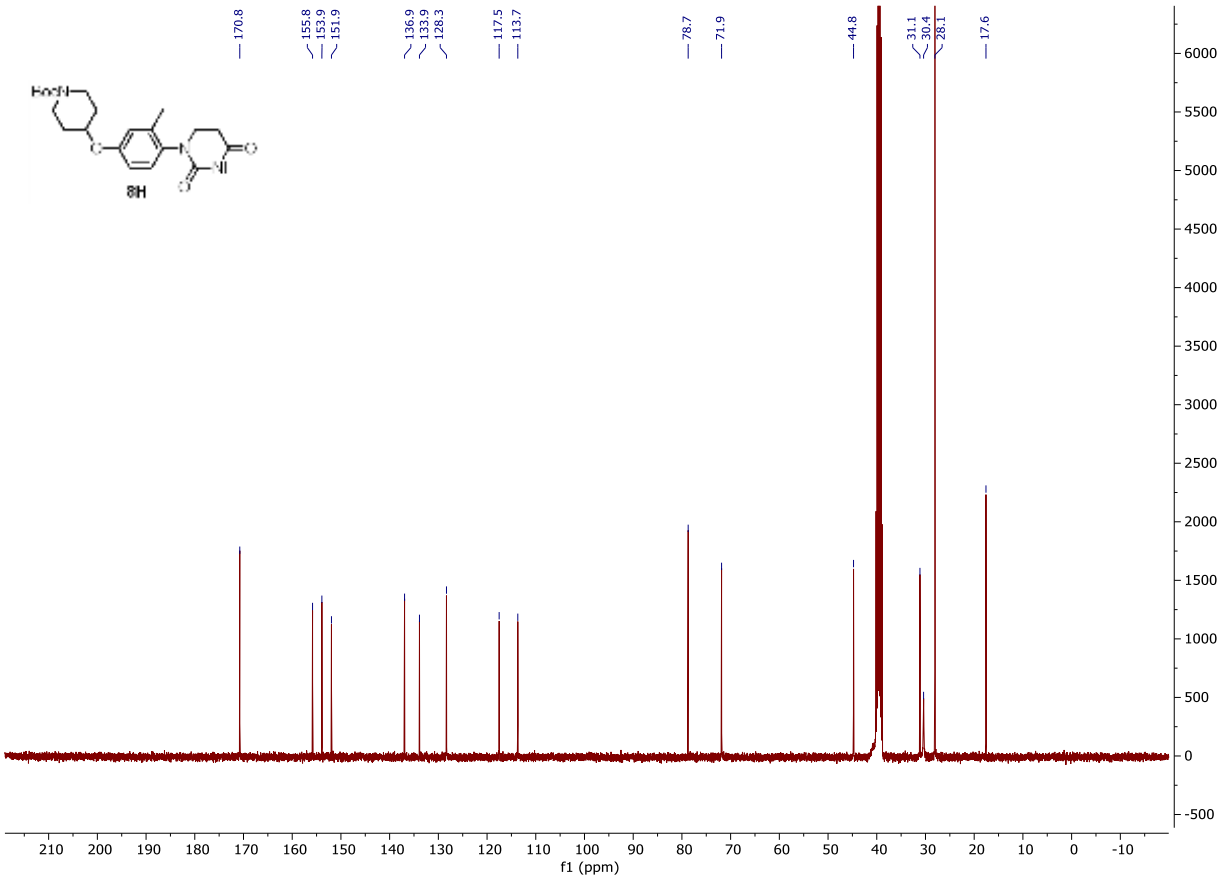

7M
10.38
7.69
7.68
7.55
7.55
7.53
7.53
7.49
7.32
7.30
6.54
6.50
3.84
3.83
3.82
3.81
3.80
3.79
3.79
3.77
3.56
3.54
3.53
3.51
3.50
3.32 H2O
2.82
2.81
2.80
2.78
2.76
2.76
2.74
2.73
2.72
2.71
2.69
2.68
2.52 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.20
1.48
1.02
1.03
2.13
1.01
1.04
1.06
1.07
1.97
2.96
9.00
f1 (ppm)

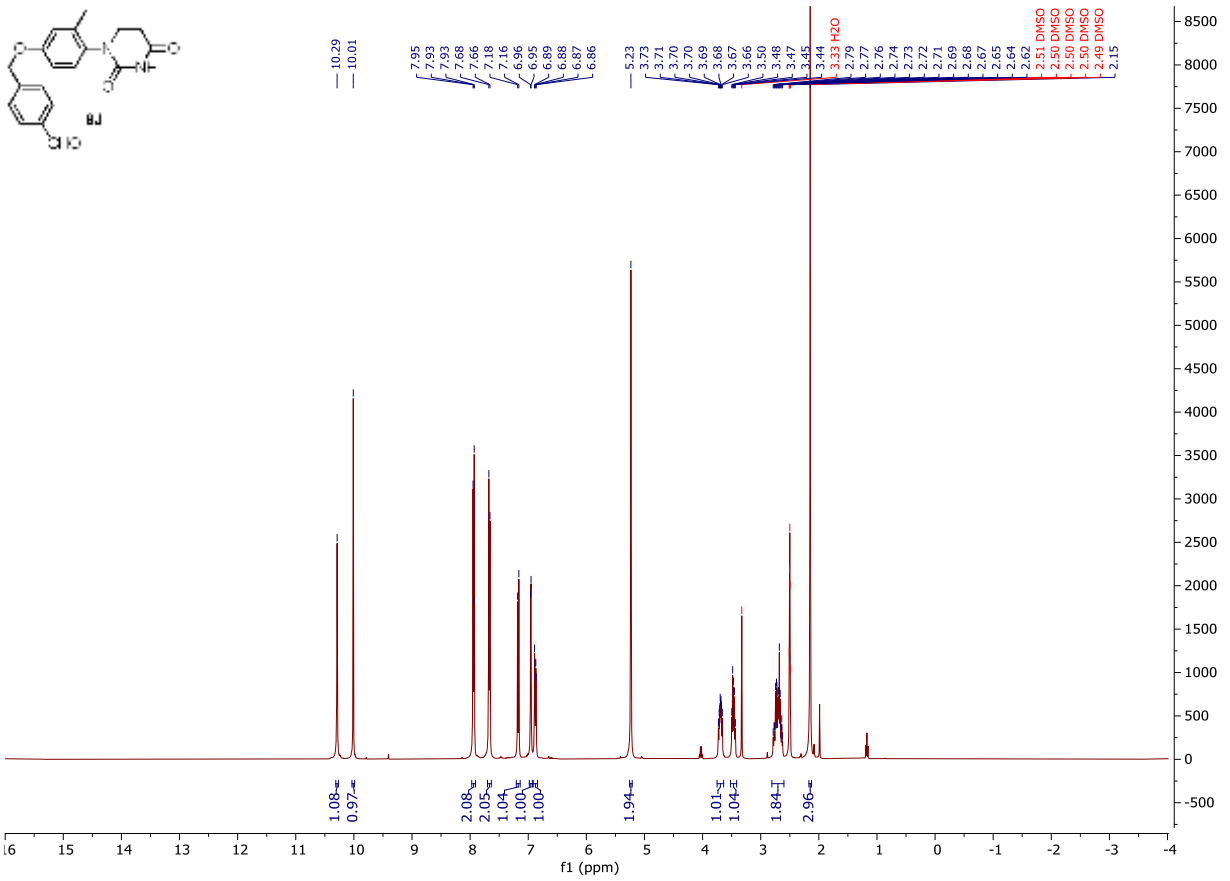

7M
170.8
165.5
151.7
142.7
141.5
138.1
133.1
131.1
127.5
126.7
119.7
79.9
44.6
31.1
27.8
17.5
f1 (ppm)

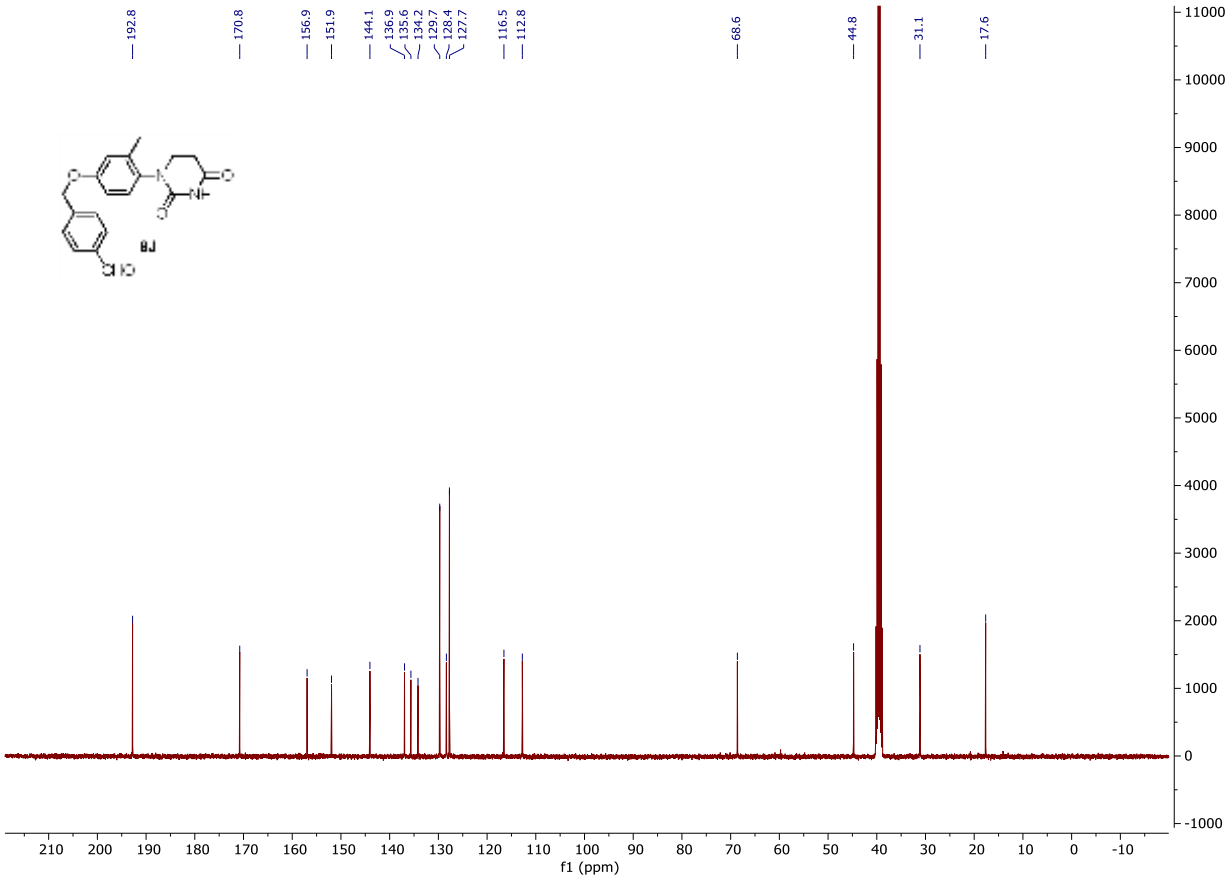

8A
10.23
9.40
7.03
7.00
6.65
6.64
6.61
6.60
6.59
6.58
3.69
3.67
3.66
3.65
3.65
3.64
3.63
3.62
3.48
3.46
3.45
3.43
3.42
3.34 H2O
2.76
2.75
2.74
2.72
2.71
2.70
2.68
2.67
2.65
2.64
2.62
2.61
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.08
1.05
1.03
0.98
1.04
1.00
1.05
1.03
2.02
2.80
f1 (ppm)

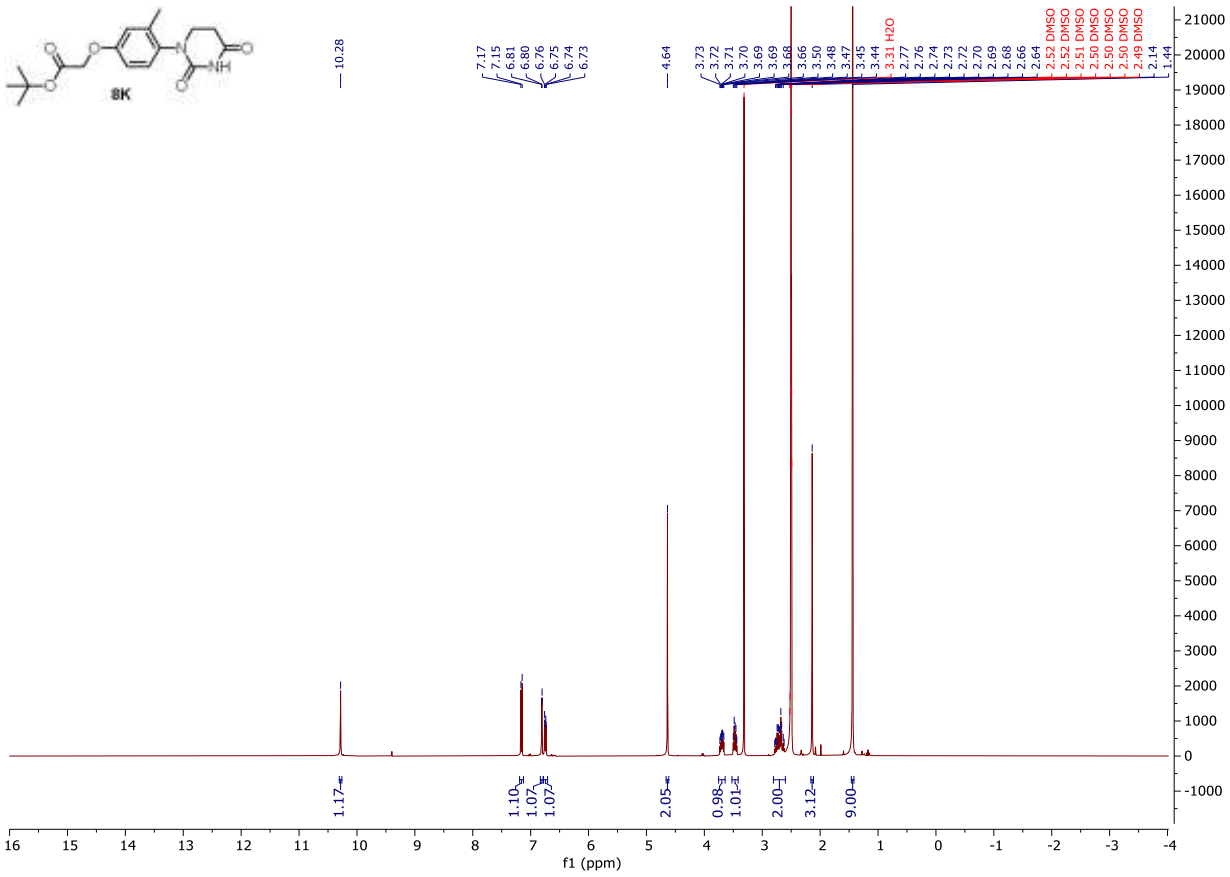

8A
170.8
156.4
152.0
136.5
132.3
128.2
116.8
113.3
44.9
31.2
17.5
f1 (ppm)

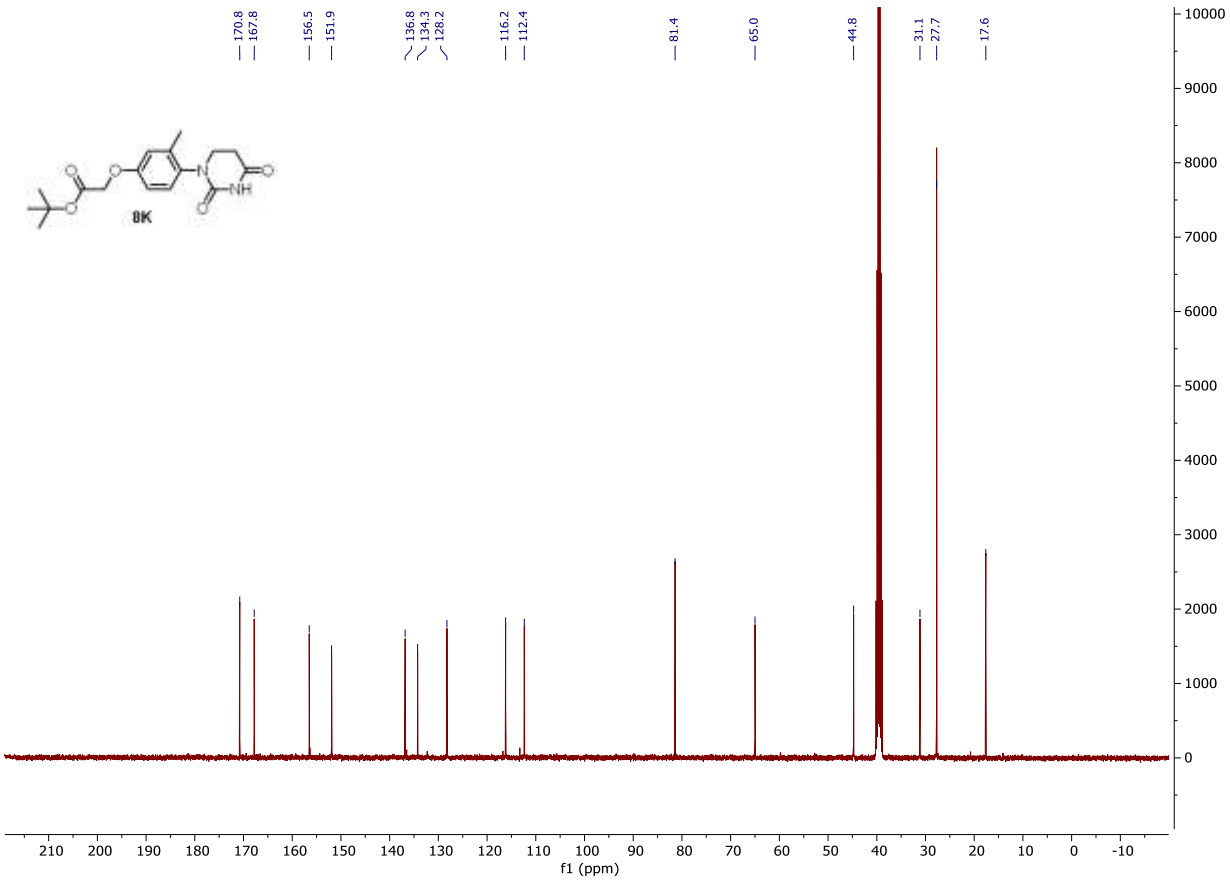

8H
f1 (ppm)

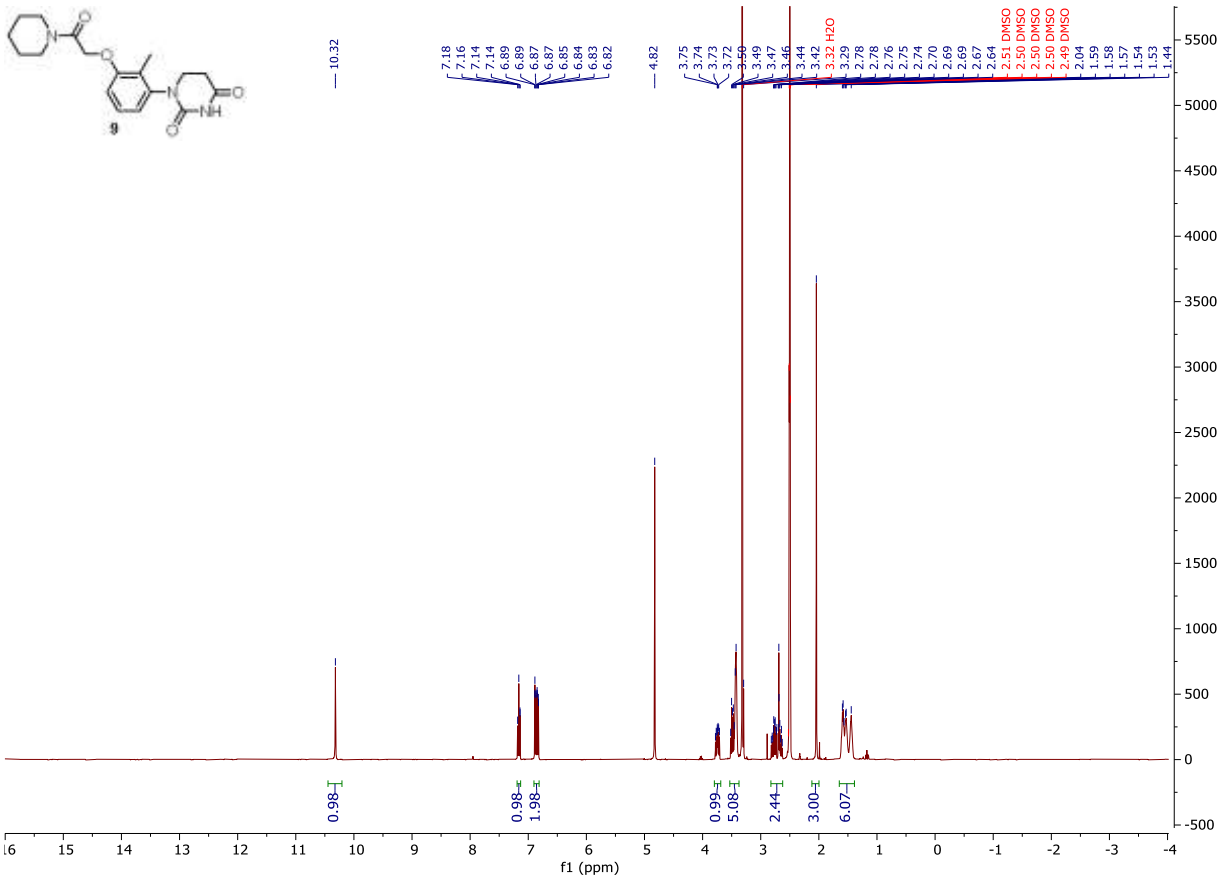

8H
f1 (ppm)

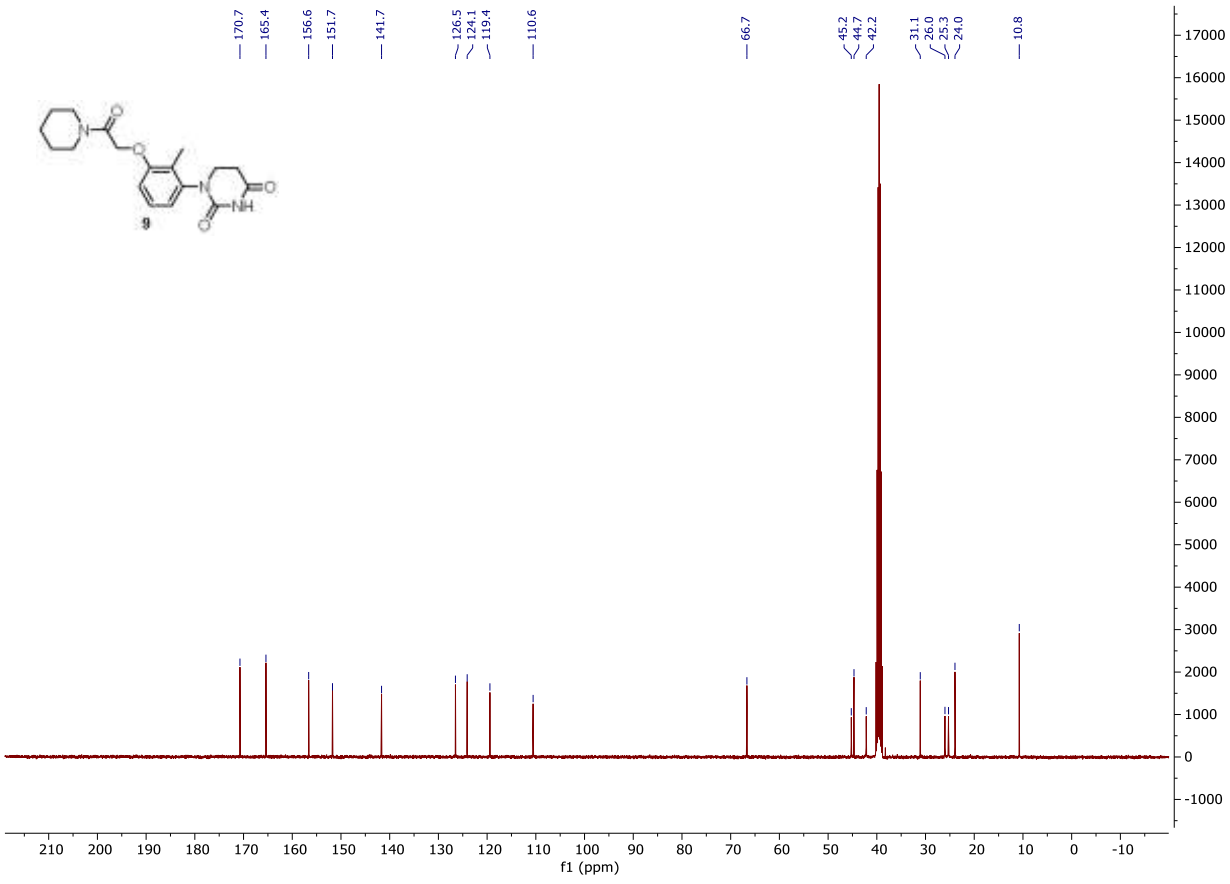

10.29
10.01
7.95
7.93
7.93
7.68
7.66
7.18
7.16
6.96
6.95
6.89
6.88
6.87
6.86
5.23
3.73
3.71
3.70
3.70
3.69
3.68
3.67
3.66
3.50
3.48
3.47
3.45
3.44
3.33 H2O
2.79
2.77
2.76
2.74
2.73
2.72
2.71
2.69
2.68
2.67
2.65
2.64
2.62
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.15
1.08
0.97
2.08
2.05
1.04
1.00
1.00
1.94
1.01
1.04
1.84
2.96
f1 (ppm)

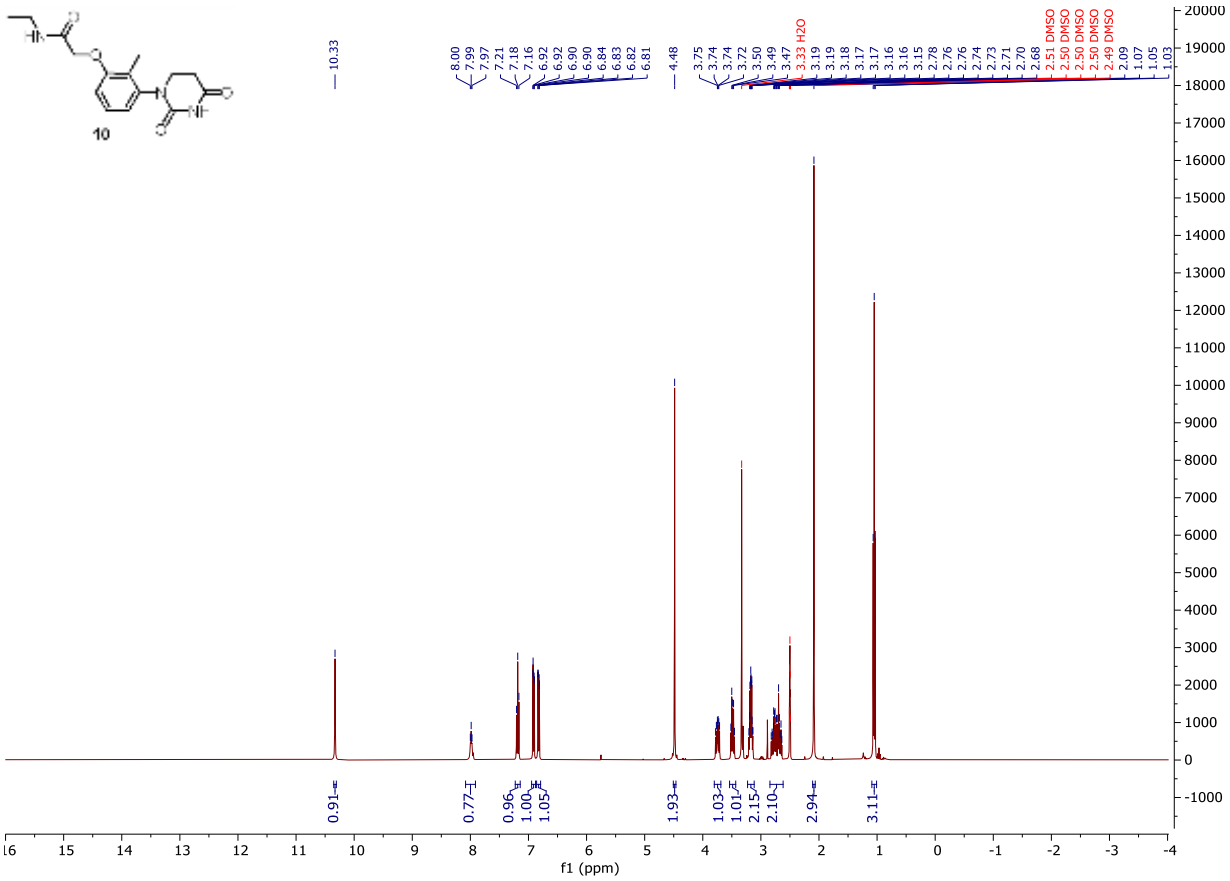

192.8
170.8
156.9
151.9
144.1
136.9
135.6
134.2
129.7
128.4
127.7
116.5
112.8
68.6
44.8
31.1
17.6
f1 (ppm)

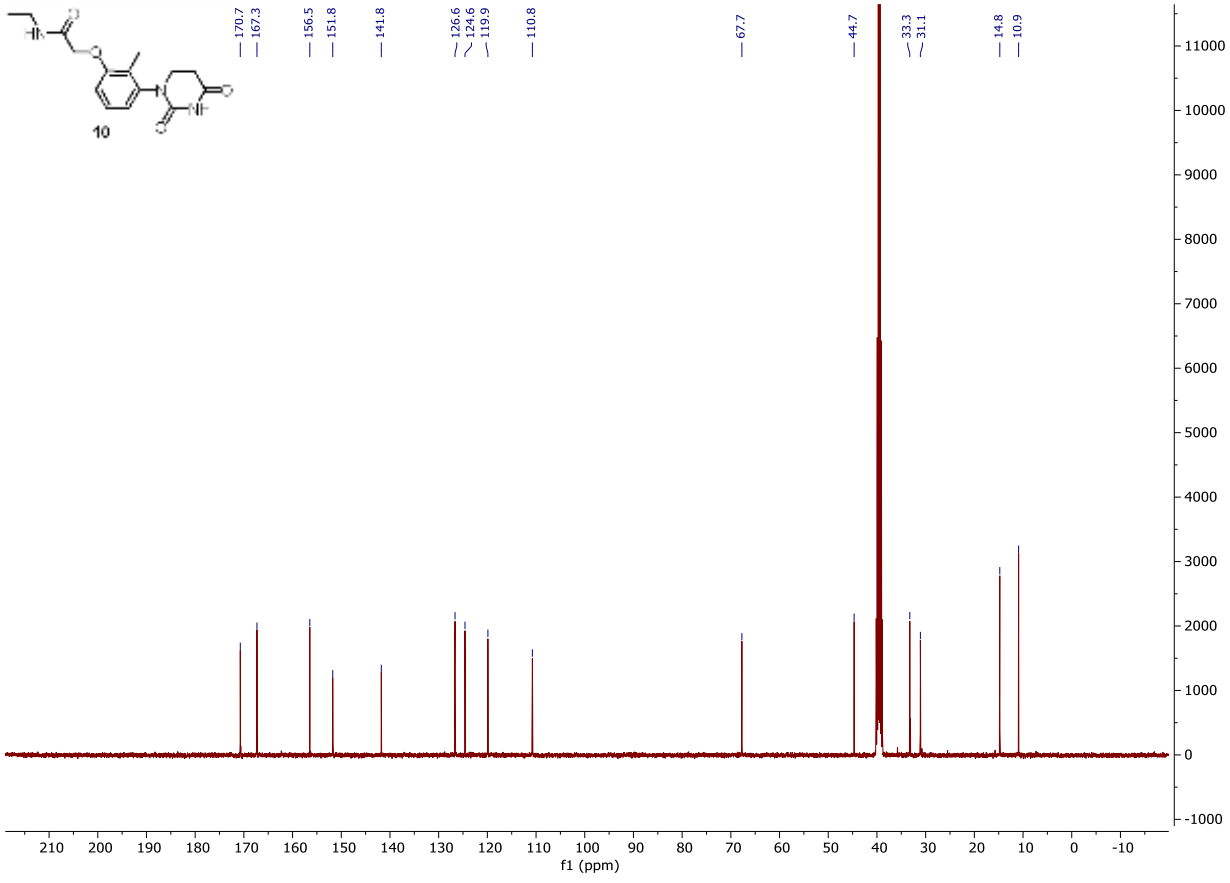

8K
10.28
7.17
7.15
6.81
6.80
6.76
6.75
6.74
6.73
4.64
3.73
3.72
3.71
3.70
3.69
3.68
3.66
3.50
3.48
3.47
3.45
3.44
3.31 H2O
2.77
2.76
2.74
2.73
2.72
2.70
2.69
2.68
2.66
2.64
2.52 DMSO
2.52 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.14
1.44
1.17
1.10
1.07
1.07
2.05
0.98
1.01
2.00
3.12
9.00
f1 (ppm)

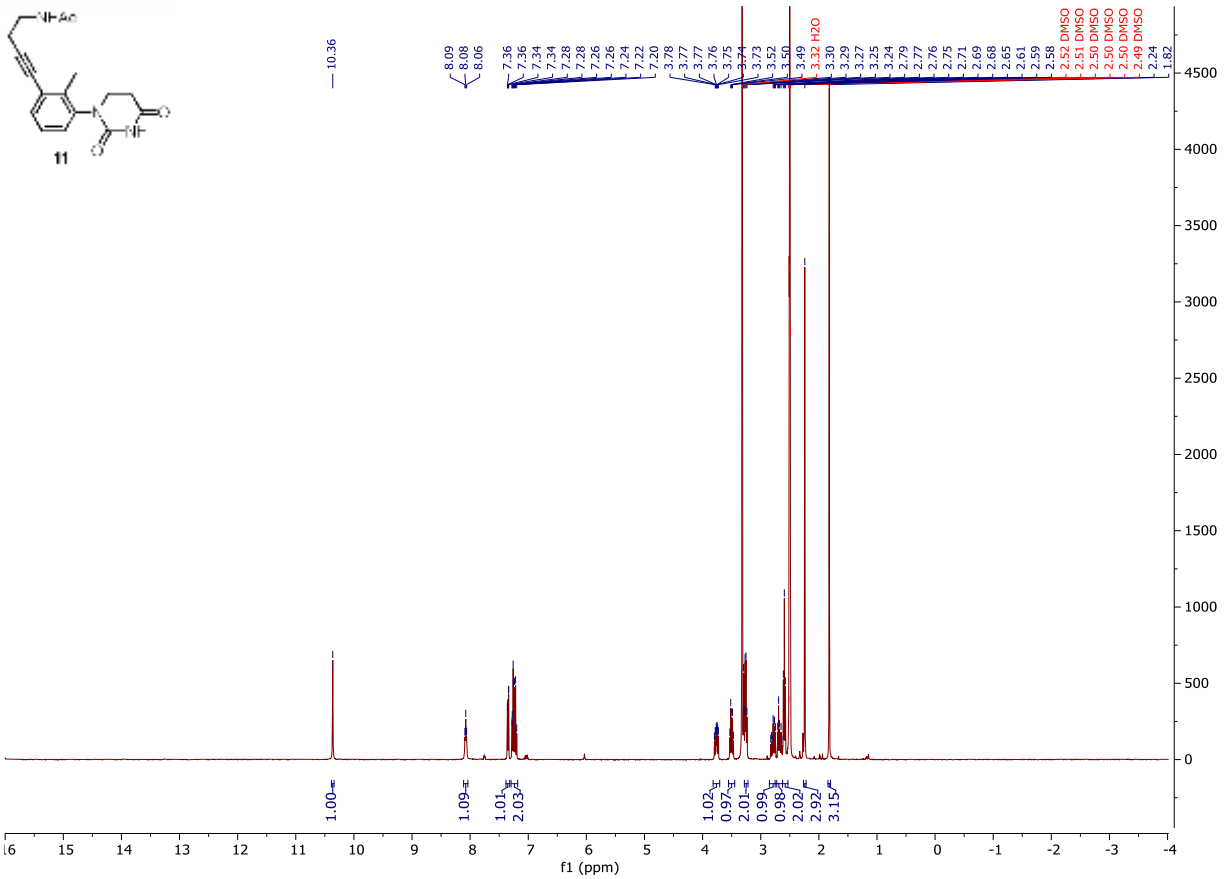

8K
170.8
167.8
156.5
151.9
136.8
134.3
128.2
116.2
112.4
81.4
65.0
44.8
31.1
27.7
17.6
f1 (ppm)

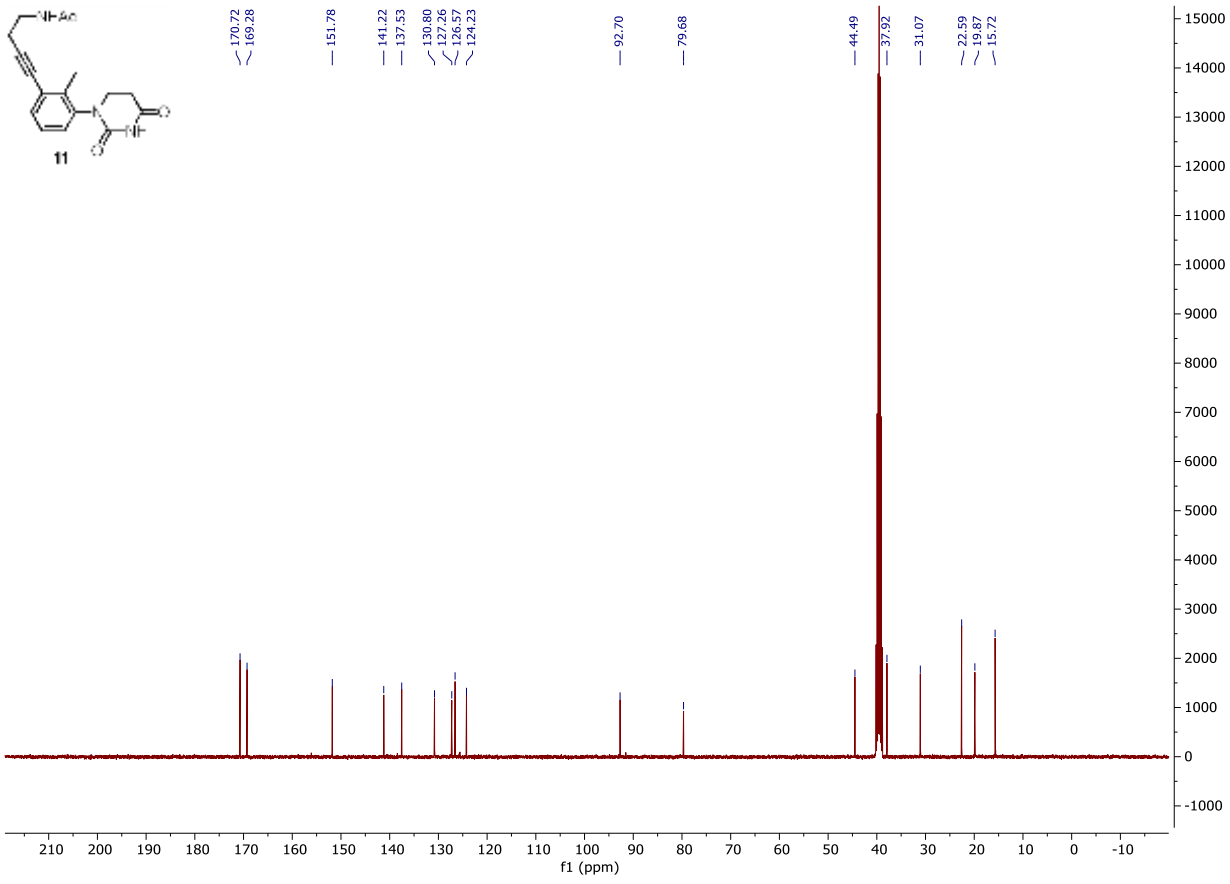

9
10.32
7.18
7.16
7.14
7.14
6.89
6.89
6.87
6.87
6.85
6.84
6.83
6.82
4.82
3.75
3.74
3.73
3.72
3.50
3.49
3.47
3.46
3.44
3.42
3.32 H2O
3.29
2.78
2.78
2.76
2.75
2.74
2.70
2.69
2.69
2.67
2.64
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.04
1.59
1.58
1.57
1.54
1.53
1.44
0.98
0.98
1.98
0.99
5.08
2.44
3.00
6.07
f1 (ppm)

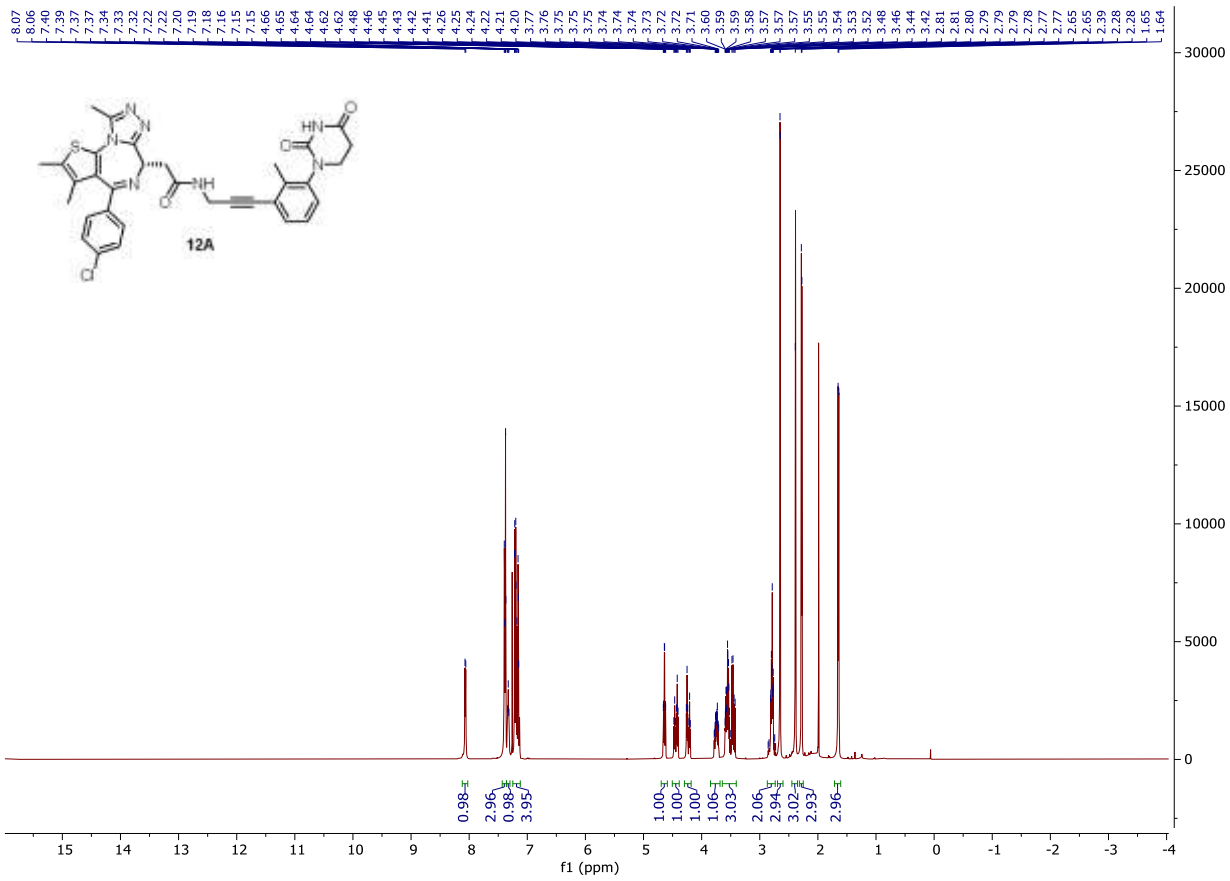

9
170.7
165.4
156.6
151.7
141.7
126.5
124.1
119.4
110.6
66.7
45.2
44.7
42.2
31.1
26.0
25.3
24.0
10.8
f1 (ppm)

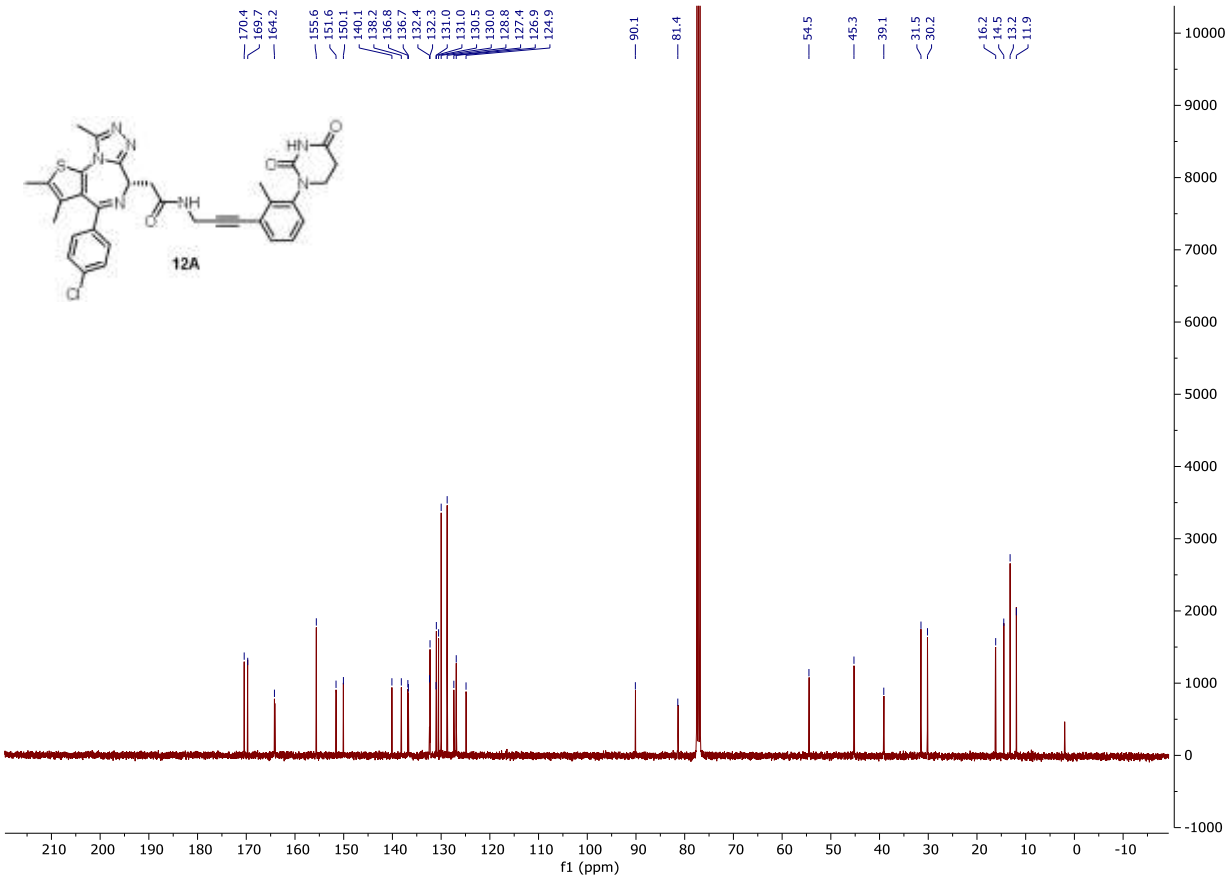

10
10.33
8.00
7.99
7.97
7.21
7.18
7.16
6.92
6.92
6.90
6.90
6.84
6.83
6.82
6.81
4.48
3.75
3.74
3.74
3.72
3.50
3.49
3.47
3.33 H2O
3.19
3.19
3.18
3.17
3.17
3.16
3.16
3.15
2.78
2.76
2.76
2.74
2.73
2.71
2.70
2.68
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.09
1.07
1.05
1.03
0.91
0.77
0.96
1.00
1.05
1.93
1.03
1.01
2.15
2.10
2.94
3.11
f1 (ppm)

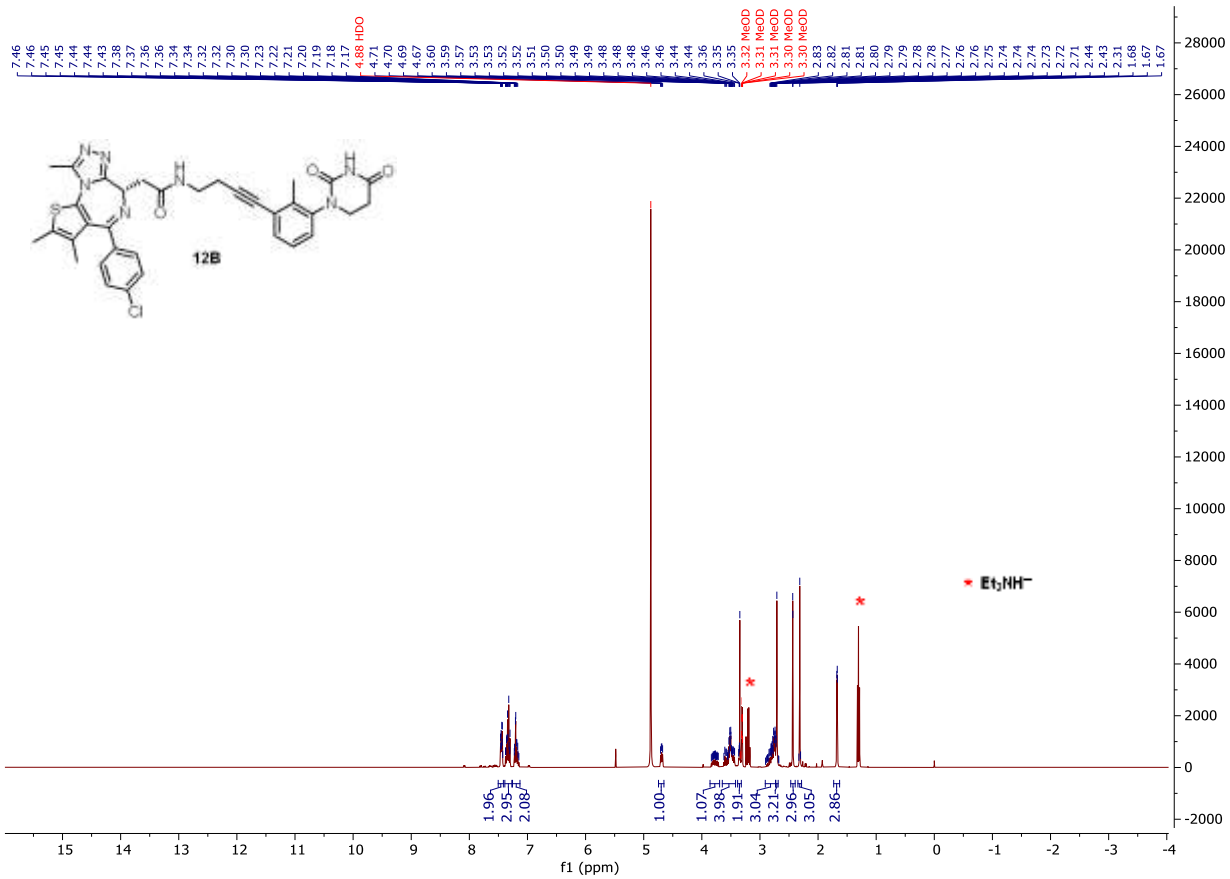

10
170.7
167.3
156.5
151.8
141.8
126.6
124.6
119.9
110.8
67.7
44.7
33.3
31.1
14.8
10.9
f1 (ppm)

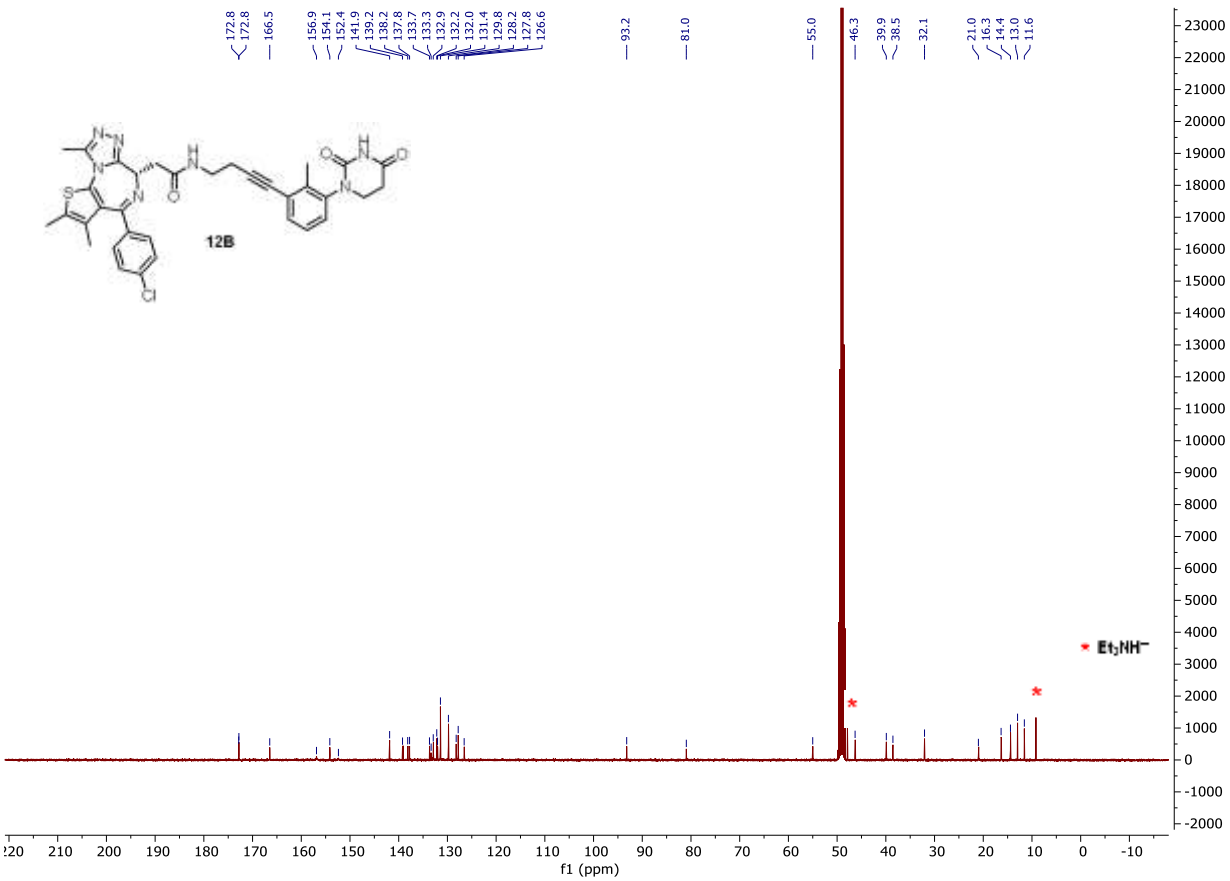

11
10.36
8.09
8.08
8.06
7.36
7.36
7.34
7.34
7.28
7.28
7.26
7.26
7.24
7.22
7.20
3.78
3.77
3.77
3.76
3.75
3.74
3.73
3.52
3.50
3.49
3.32 H2O
3.30
3.29
3.27
3.25
3.24
2.79
2.77
2.76
2.75
2.71
2.69
2.68
2.65
2.61
2.59
2.58
2.52 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.24
1.82
1.00
1.09
1.01
2.03
1.02
0.97
2.01
0.99
0.98
2.02
2.92
3.15
f1 (ppm)

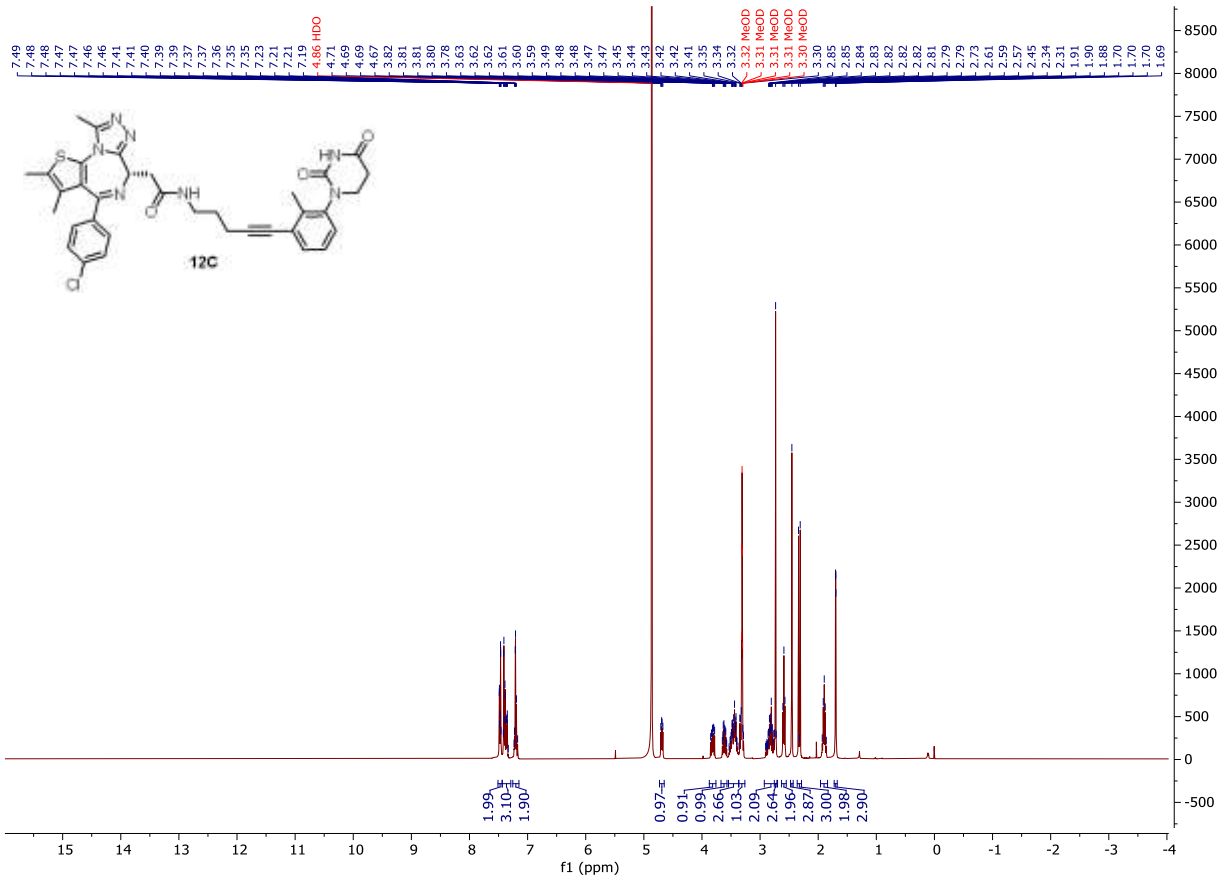

11
170.72
169.28
151.78
141.22
137.53
130.80
127.26
126.57
124.23
92.70
79.68
44.49
37.92
31.07
22.59
19.87
15.72
f1 (ppm)

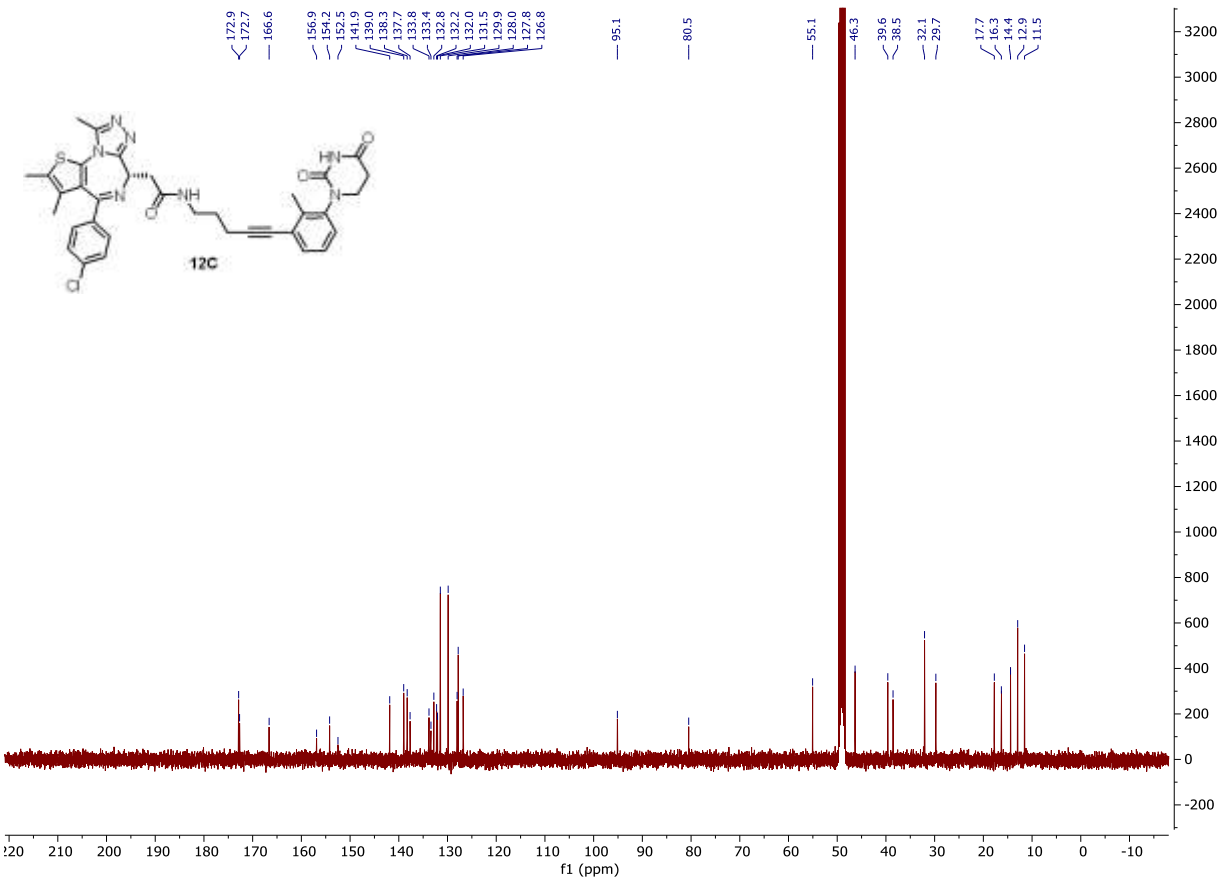

12A
f1 (ppm)

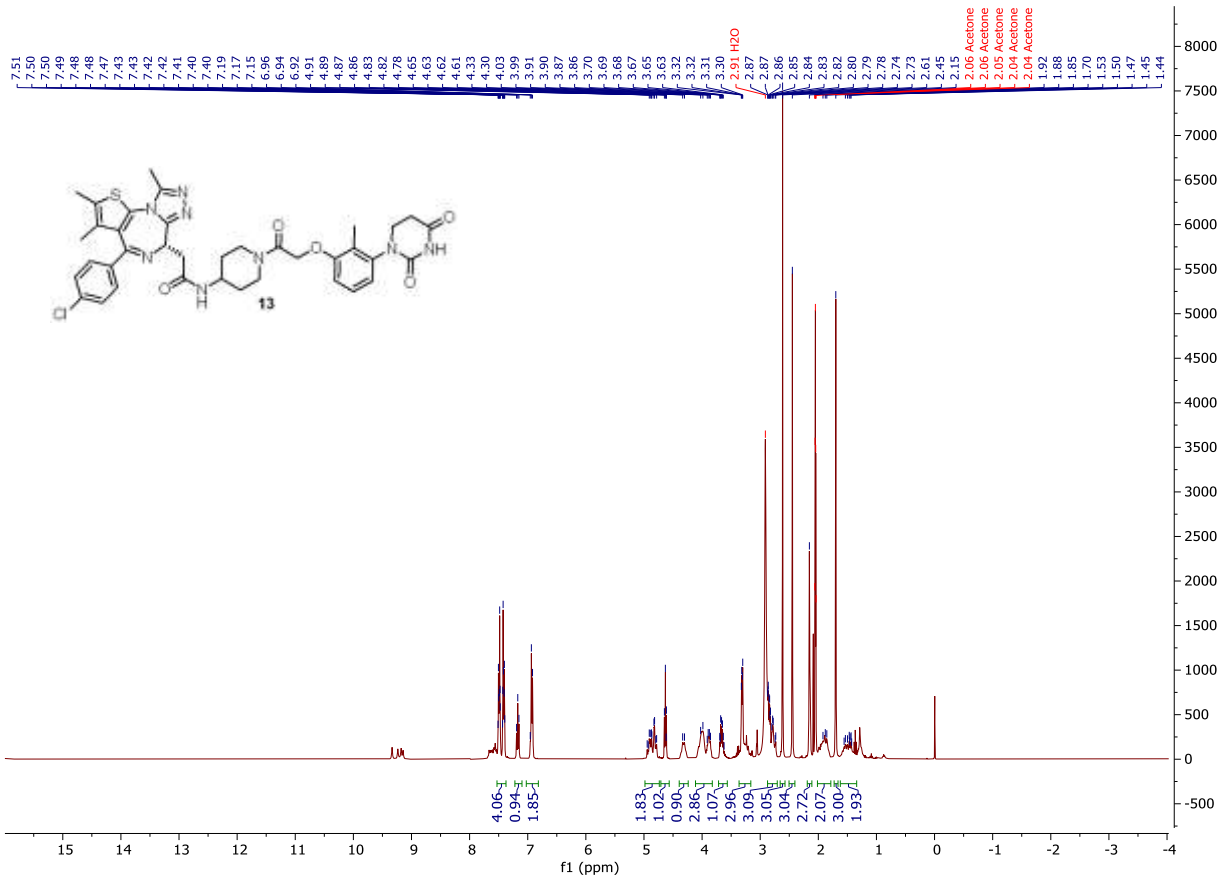

12A
f1 (ppm)

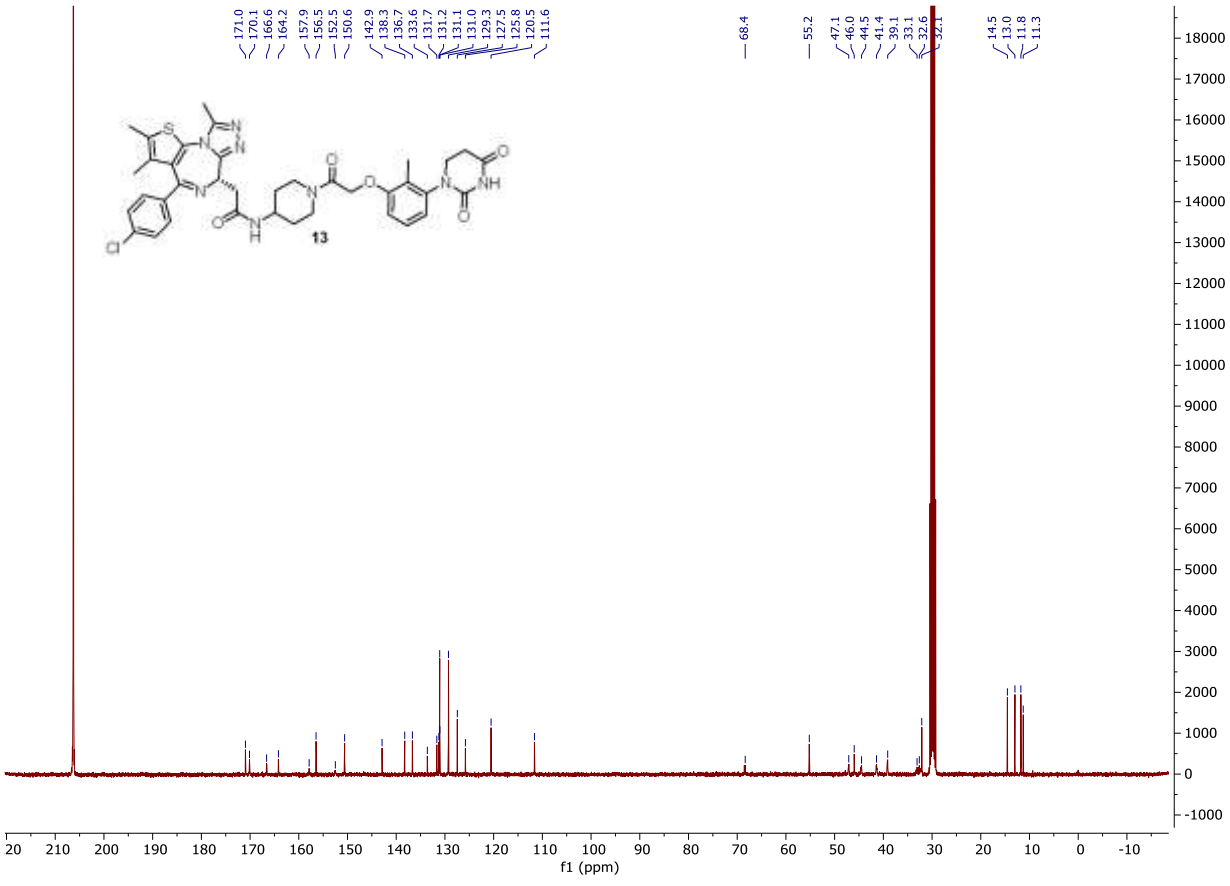

12B
Et3NH+
f1 (ppm)

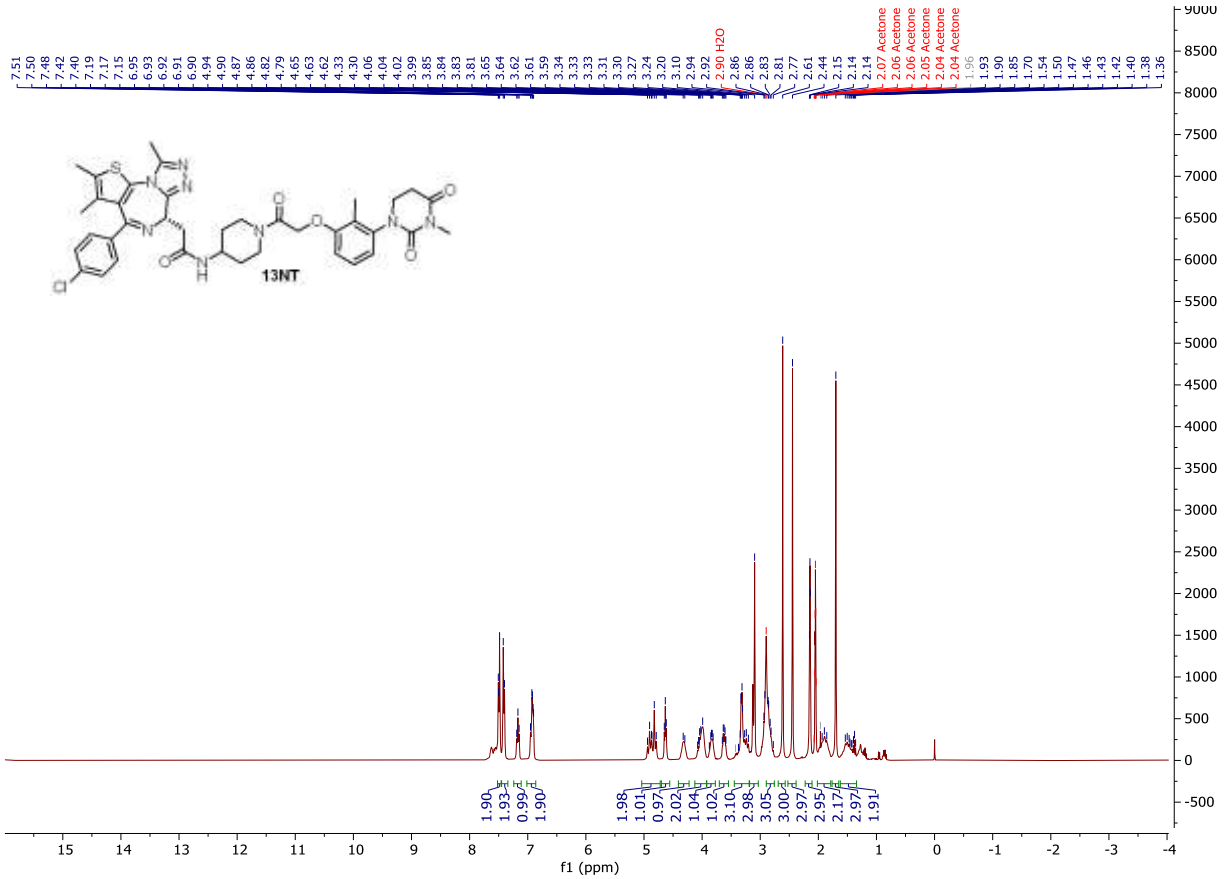

12B
Et3NH+
f1 (ppm)

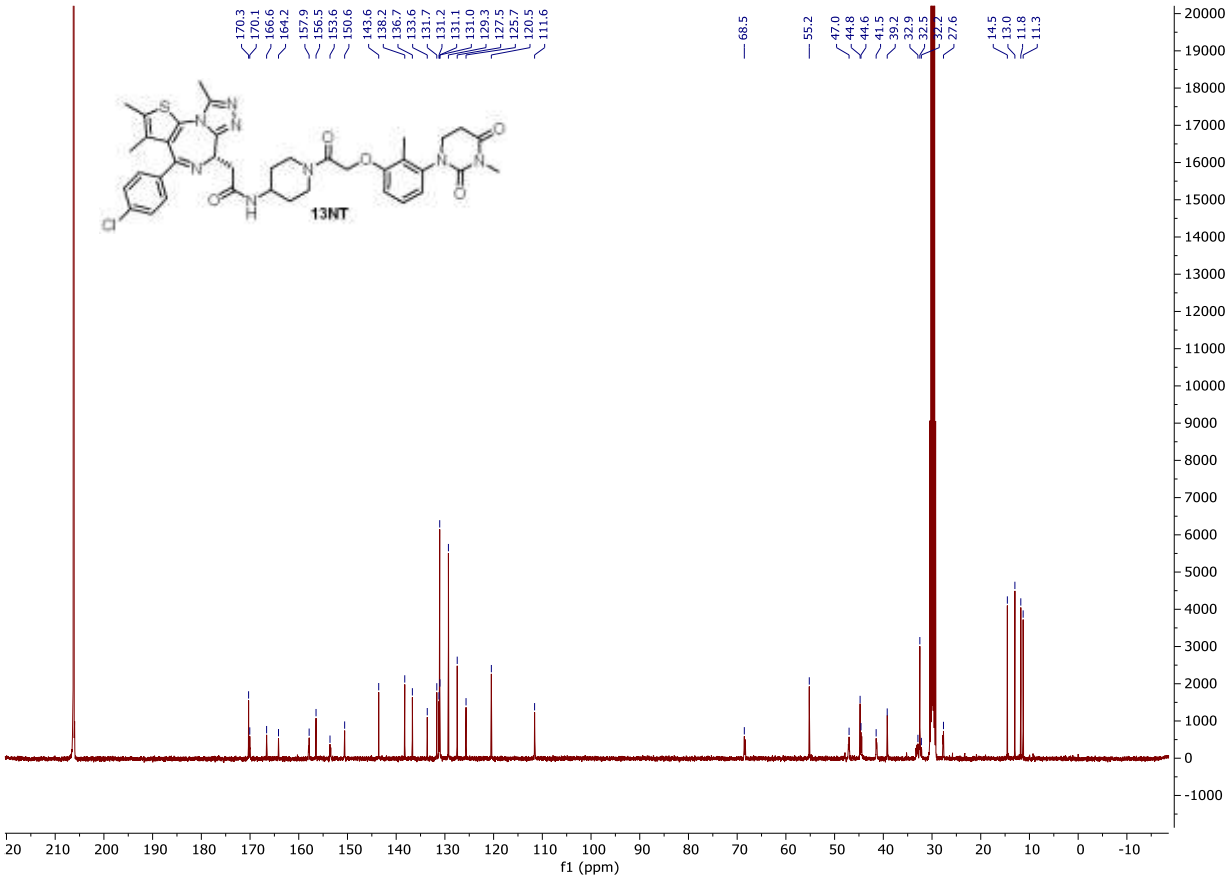

12C
f1 (ppm)

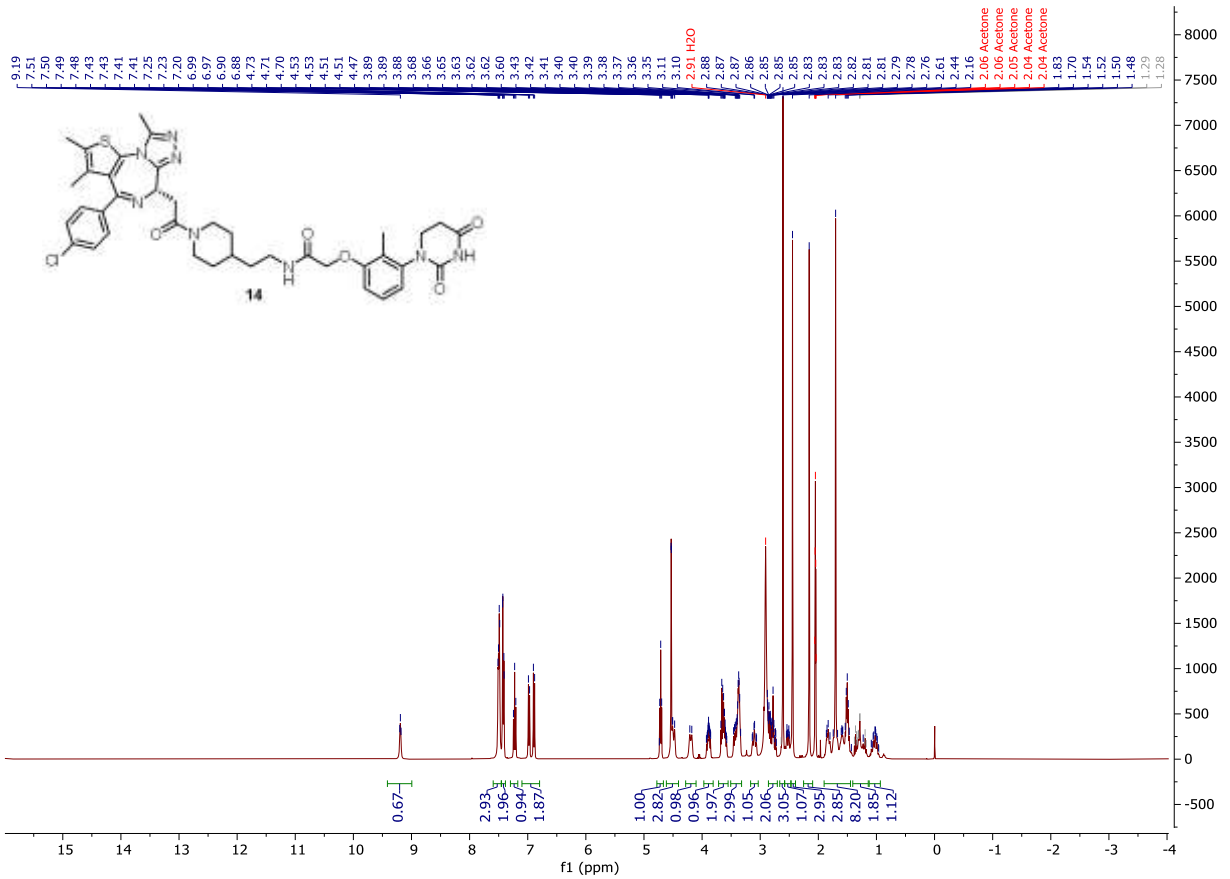

12C
f1 (ppm)

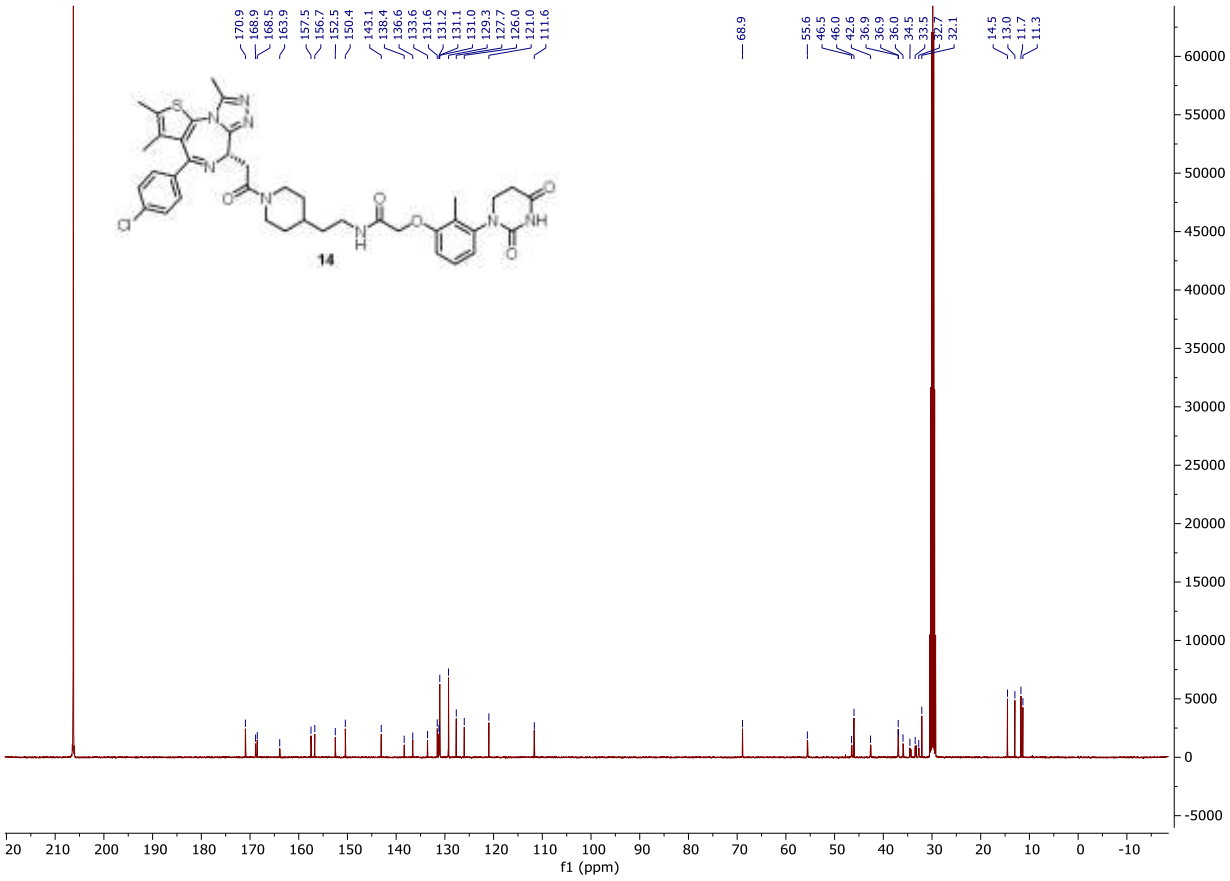

7.51 7.50 7.50 7.49 7.48 7.48 7.47 7.43 7.43 7.42 7.42 7.41 7.40 7.40 7.19 7.17 7.15 6.96 6.94 6.92 4.91 4.89 4.87 4.86 4.83 4.82 4.78 4.65 4.63 4.62 4.61 4.33 4.30 4.03 3.99 3.91 3.90 3.87 3.86 3.70 3.69 3.68 3.67 3.65 3.63 3.32 3.32 3.31 3.31 3.30 2.91 H2O 2.87 2.87 2.86 2.85 2.84 2.83 2.82 2.80 2.79 2.78 2.74 2.73 2.61 2.45 2.15 2.06 Acetone 2.06 Acetone 2.05 Acetone 2.04 Acetone 2.04 Acetone 1.92 1.88 1.85 1.70 1.53 1.50 1.47 1.45 1.44
13
4.06 0.94 1.85 1.83 1.02 0.90 2.86 1.07 2.96 3.09 3.05 3.04 2.72 2.07 3.00 1.93
f1 (ppm)


171.0 170.1 166.6 164.2 157.9 156.5 152.5 150.6 142.9 138.3 136.7 133.6 131.7 131.2 131.1 131.0 129.3 127.5 125.8 120.5 111.6 68.4 55.2 47.1 46.0 44.5 41.4 39.1 33.1 32.6 32.1 14.5 13.0 11.8 11.3
13
f1 (ppm)


13NT
f1 (ppm)


13NT
f1 (ppm)


14
f1 (ppm)


14
f1 (ppm)