ANTIBACTERIAL DRUG DESIGN AND DISCOVERY VIA VITAMIN B_1 BIOSYNTHETIC PATHWAY

by

ABU AFZAL MOHAMMAD SHAKAR

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN CHEMISTRY

THE UNIVERSITY OF TEXAS AT ARLINGTON

AUGUST 2016

Acknowledgements

At first, I would like to thank Dr. Frank Foss Jr. for giving me the opportunity to work in the field of medicinal chemistry of organic chemistry division, as I was a pharmacist and had a strong desire to work in drug design and discovery arena. He is a man with strong personality, excellent teacher and has powerful knowledge of chemistry. He led me to run our project and gave me rational advices to improve our experiments. With his guidance, I have learned the field of drug synthesis. Without his instruction, this thesis could not have been completed.

I would like to thank my committee members, Dr. Carl J. Lovely, Dr. Junha Jeon and Dr. Subhrangsu S. Mandal for their support and suggestions. I would also like to thank other organic chemistry faculty member Dr. Alejandro Bugarin, who have taught me chemistry and other faculty members Dr. Kayunta Jhonson-Winters, Dr. Saiful Chowdhury and Dr. Brad Pierce for thought me Biochemistry, Mass spectrometry and Inorganic chemistry respectively.

I would like to thank my group members Dr. Mohammad Shawkat Hossain, Dr. Diego Lopez, Dr. Sumit Bhawal, Pawan Thapa, Abu Esba, Caitlynn Marie, Esai and Trevor for their teamwork, support and knowledge sharing.

I would like to remember my first supervisor Dr. Selim Reza for his encouragement and for teaching me some valuable lessons in Drug formulation and delivery system. Also I would like to remember my colleagues of drug formulation department of ACI pharmaceuticals limited and express my heartfelt thanks for their help and support.

I would like to thank my family who has given me eternal support and love. They have given me the opportunity to the United State of America to pursue my higher education.

July 28, 2016.

Abstract

ANTIBACTERIAL DRUG DESIGN AND DISCOVERY VIA VITAMIN B_1 BIOSYNTHETIC PATHWAY

Abu Afzal Mohammad Shakar, M.S.

The University of Texas at Arlington, 2016

Supervising Professor: Frank W. Foss, Jr.

Antibiotics play a vital role in fighting infectious disease caused by bacterial species. Antimicrobial resistance has been shown for every current antibiotic and poses a major health risk. To combat these growing threats to human health; new molecules must be investigated to treat bacterial infection. Chemical inhibition of the vitamin B_1 biosynthetic pathway in the bacterial cell was hypothesized to inhibit the survival of bacteria. A series of synthetic molecules, which resemble metabolites of the vitamin B_1 pathway, exhibit the inhibition of specific enzymes within this pathway and display initial activity against bacterial growth and survival. The beneficial activity of these new molecules was interpreted both experimentally and computationally. By investigating the relationship between molecular structure and biological function, a second generation of molecules was designed and a number of them were synthesized to be more potent inhibitors of bacterial growth. This iterative process - involving structural design, chemical synthesis and biological evaluation - reveals the vitamin B_1 biosynthetic pathway to be a potential new avenue for antibiotic development to treat human infection.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF ABBREVIREATIONS

FDA	Food and drug administration
MRSA	Methicillin-resistant *Staphylococcus aureus*
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
PK	Pyruvate kinase
LDH	Lactose dehydrogenase
NADH	Nicotinamide adenine dinucleotide
Boc	N-*tert*-butoxycarbonyl
DTT	Dithiothreitol
IPTG	Isopropyl β-*D*-1-thiogalactopyranoside
IMAC	Immobilized metal ion affinity chromatography
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Na	Sodium
NaOH	Sodium hydroxide
KOH	Potassium Hydroxide
Cs_2CO_3	Cesium Carbonate
$LiAlH_4$	Lithium aluminum hydride
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride
HOBt	Hydroxybenzotriazole
DMF	Dimethylformamide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DIAD	Diisopropyl azodicarboxylate

Ph	Phenyl
PPh_3	Triphenylphosphine
UV	Ultraviolet
NMR	Nuclear magnetic resonance
°C	Degrees Celsius
rt	Room temperature
g	Gram(s)
Hz	Hertz (cycles per second)
J	Coupling constant (NMR)
mol	Mole(s)
mmol	millimole
ppm	Parts per million

Chapter 1

Introduction

Antimicrobial Resistance (AMR) can be defined as retaliation from infectious disease-causing microbes including bacteria, viruses, parasites, and fungi–against antimicrobial drugs that were once efficient in curing the disease caused by that specific microbe.[1]

There are four leading mechanisms of AMR. (1) Enzymes degrade the drug. For example, resistance to penicillin caused by β-lactamases, which hydrolytically cleave the core β-lactam ring that is characteristic of the class and essential to antibiotic action.[2] (2) Drug targets are modified. For example, resistance to vancomycin is caused by a change in the *D*-alanine-*D*-alanine part of the peptide in peptidoglycan to *D*-alanine-*D*-lactate, resulting in an incapability of vancomycin to bind.[3] (3) Drugs experience reduced cellular permeability. For example,

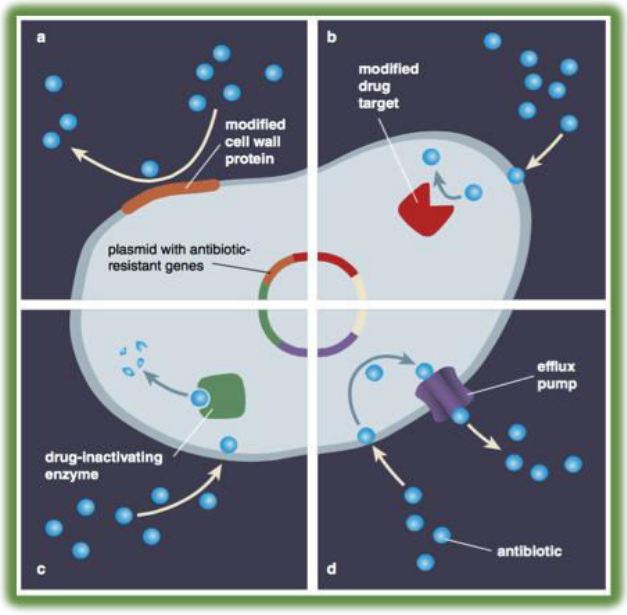


Figure 1.1: Mechanisms of Antimicrobial Resistance (AMR)[4]

Tetracycline resistance is often caused by reduced permeability of the drug.[5] (4) Drug concentration is reduced by an active export of the drug. For example, resistance to sulfonamides is due primarily to plasmid-encoded enzymes that actively export the drug from the bacterium.[6]

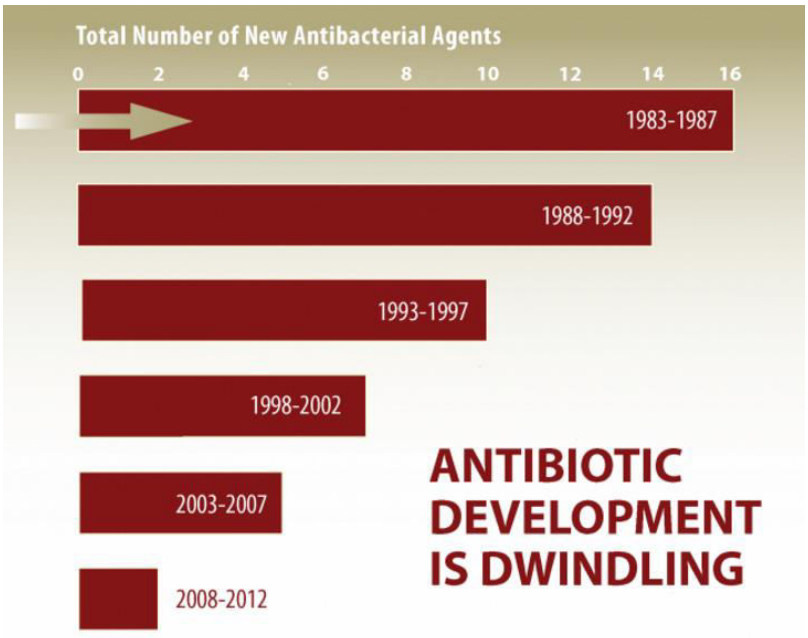


Figure 1.2: New systemic antibacterial agents approved by the FDA, through 2012 [7]

The pressure for new antimicrobial agents is greater than ever for the sake of human well-being amplified by the emergence of multidrug resistance in common pathogens and the rapid emergence of new infections. At the same time, some major pharmaceutical companies have indicated that they are downsizing anti-infective research programs.[8] Large pharmaceutical companies make better profits producing drugs for non-infectious diseases, so it has been left up to small companies and academic institutions to fill the need for new antibiotic drugs. Both private and public health experts warn that the American pharmaceutical industry is failing to produce new

antibiotics for the bacteria of the near future. FDA approved only two new antibiotics, telavancin and ceftaroline fosamil since 2009, which imply that antibiotic development continuing to shrink. Because of this decline in antibiotic research and development, the Infectious Diseases Society of America anticipates to impulse the development of 10 new antibiotics by 2020.[7] To trigger antibiotic research and development, President Obama signed the Generating Antibiotic Incentives Now (GAIN) Act, in July 2012.[9] In recent years, progress has been made, in 2014; researchers at St. Jude Children's Research Hospital discovered a new class of antibiotics, spectinamides, for the treatment of drug-resistant tuberculosis. In that same year, researchers at the University of Notre Dame discovered oxadiazoles, which have been shown to help treat MRSA in mice.[10]

Antibacterial drug discovery is challenged by the continual emergence of multidrug resistant strains and limited new classes of compounds that treat an infection. Coupled with unfavorable economics, this has created the need for academia to investigate new strategies (new targets or mechanisms of action) in concert with expanding the scope of existing approaches.[11]

In response to these antibacterial needs, Dr. Foss's research group has synthesized modified thiamine precursors that have demonstrated bactericidal effects in preliminary tests. We are synthesizing some rational compounds which might act as inhibitors of our desired enzyme system on the basis of preliminary compounds which may act as inhibitors of our desired enzyme system, on the basis of this preliminary structure-activity relationship (SAR).

Chapter 2

Research Background

Our work is an endeavor to investigate the active site of an important enzyme, 4-amino-5-hydroxymethyl-2-methylpyrimidine kinase (HMPK) within the thiamine (vitamin B_1) biosynthetic pathway (Figure-2.1).[12]

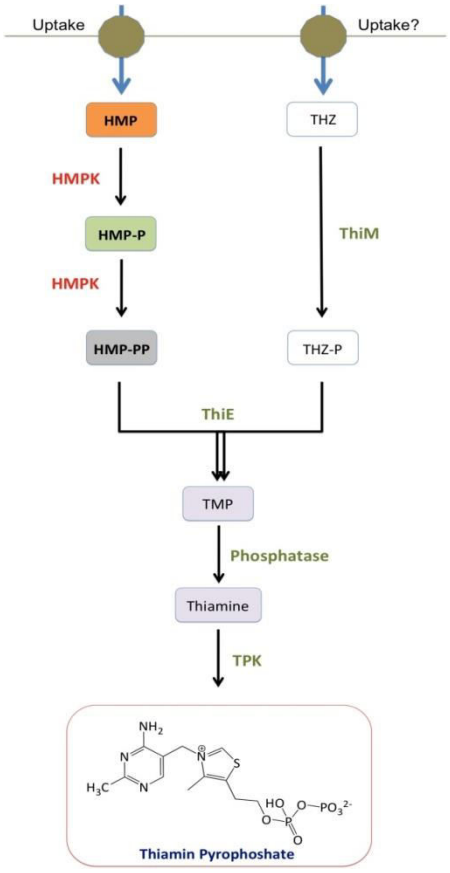


Figure 2.1: Thiamine pathway in *Plasmodium falciparum*

Within the living cell, thiamine or thiamine phosphate is converted to its biologically active form, thiamine pyrophosphate, which acts as an essential cofactor for a wide range of enzymes like pyruvate dehydrogenase complex, branched chain amino

acid dehydrogenase complex, transketolase and alpha-ketoglutarate dehydrogenase (Figure-2.2). [13]

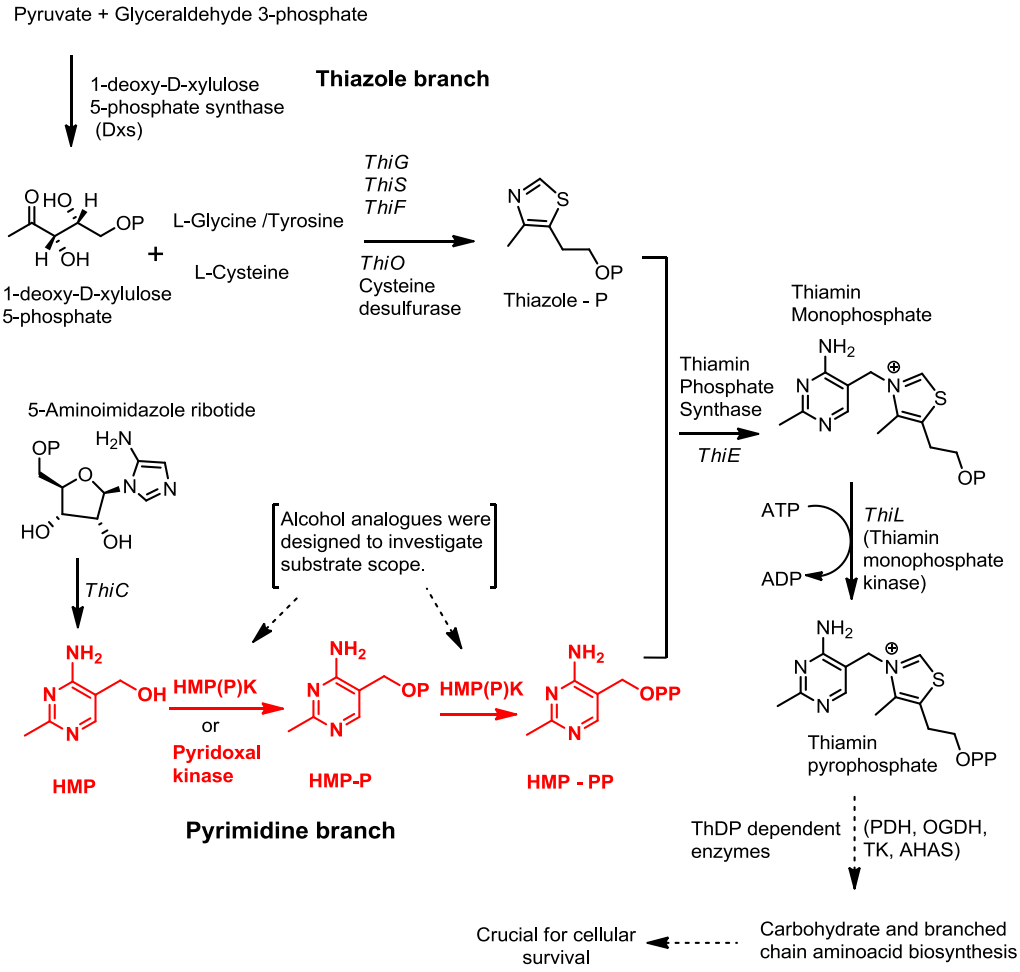


Figure 2.2: General scheme for thiamine biosynthesis. AHAS (aceto-hydroxy acid synthase), OGDH (2-oxoglutarate dehydrogenase), P (phosphate), PDH (pyruvate dehydrogenase), PP (pyrophosphate) TK (transketolase). [13]

Therefore, it is an important metabolic pathway for most bacterial survival, while mammals obtain thiamine only through dietary means. [14]

HMP Kinase possesses several intriguing features, which make it an interesting target for antibiotic discovery, specifically: (I) HMP kinase is a member of the ribokinase family, which are known to accept wide range of substrates, including carbohydrates and aromatic small molecules like 4-amino-5-β–hydroxymethyl-2-methylpyrimidine (HMP) and 4-methyl-5-hydroxyethylthiazole (THZ), all of which are phosphorylated at a hydroxymethyl group.[15] (II) This enzyme is responsible for two consecutive phosphorylation.

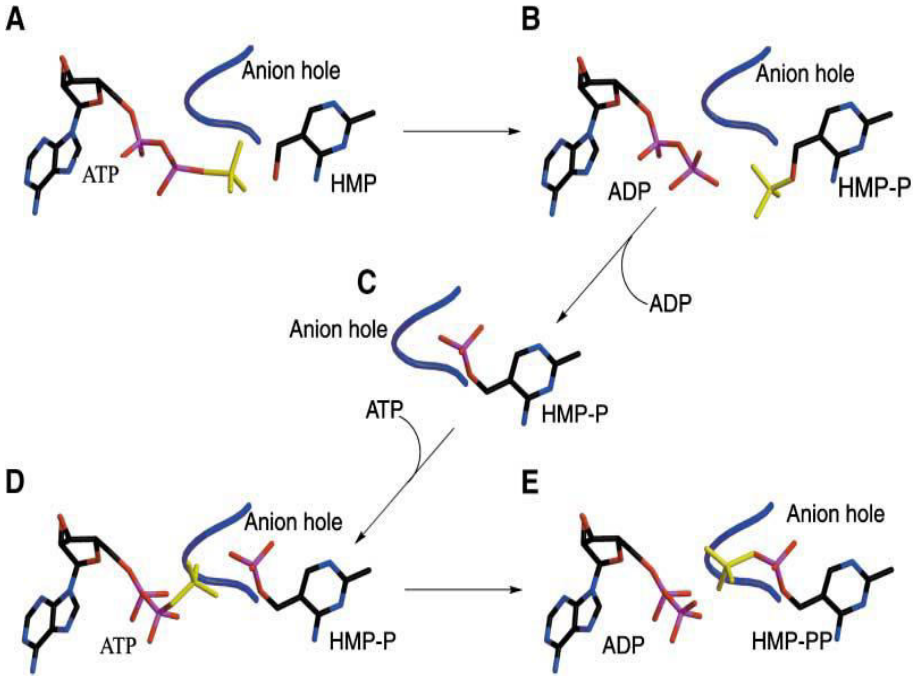


Figure 2.3: Mechanism of consecutive phosphorylation of HMP Kinase where (A) Enzyme-substrate complex for the first phosphorylation reaction (B) Products for the first phosphorylation reaction (C) Phosphate group is repositioned for the second phosphorylation reaction. (D) Enzyme-substrate complex for the second phosphorylation reaction (E) Products for the second phosphorylation reaction. [15]

(III) It is possible to study by a prodrug approach where the bacteria might produce non-natural thiamine analogues selectively, which would be toxic to bacterial cells as they synthesize thiamine *de novo*.

(IV) Amino acid sequence of HMP kinase conserved across different gram stain, gram positive and gram negative bacterial species (Table 2.1). [16]

Table 2.1: Bacterial strains available for whole cell assays and their HMP kinase amino acid sequence homology compared to *S. typhimurium.*

Species	**Gram (+)/(-)**	**HMP Kinase Similarity**
Staphylococcus aureus NCTC 8325	(+)	*97%*
Mycobacterium smegmatis	(+)	*93%*
Enterococcus faecalis	(+)	*95%*
Streptococcus pneumonia	(+)	*89%*
Clostridium difficile	(+)	*95%*
Klebsiella pneumonia	(-)	*95%*
Pseudomonas aeruginosa	(-)	*91%*
Excherichia coli K-12 / TolC	(-)	*95%*
Plasmodium falciparum	N/A	*94%*

The crystal structure of HMP kinase and its complex with its substrate HMP for *Salmonella typhimurium* has reported by Ealick et al. (Figure-2.4).[15] HMP Kinase is a homodimer in a crystal structure. Each monomer possesses one self-contained active

site, which is separated by approximately 20-25 Å. Each active site contains one HMP binding site and ATP binding site. The ATP binding site was not co-crystallized with ATP. However, it is highly conserved with members of the ribokinase family. The active site forms a binding pocket that buries the substrate completely where side chain of Ala18, Val42, Met80 and Val107 provide hydrophobic interactions. HMP's 4-amino group is hydrogen bonded to Glu44. N1 of pyrimidine ring is hydrogen bonded to a water molecule which in turn hydrogen bonded to Gly11 and Met80. A second water molecule situated beside the first water molecule which is positioned near the side chains of Asp23 and Cys213.

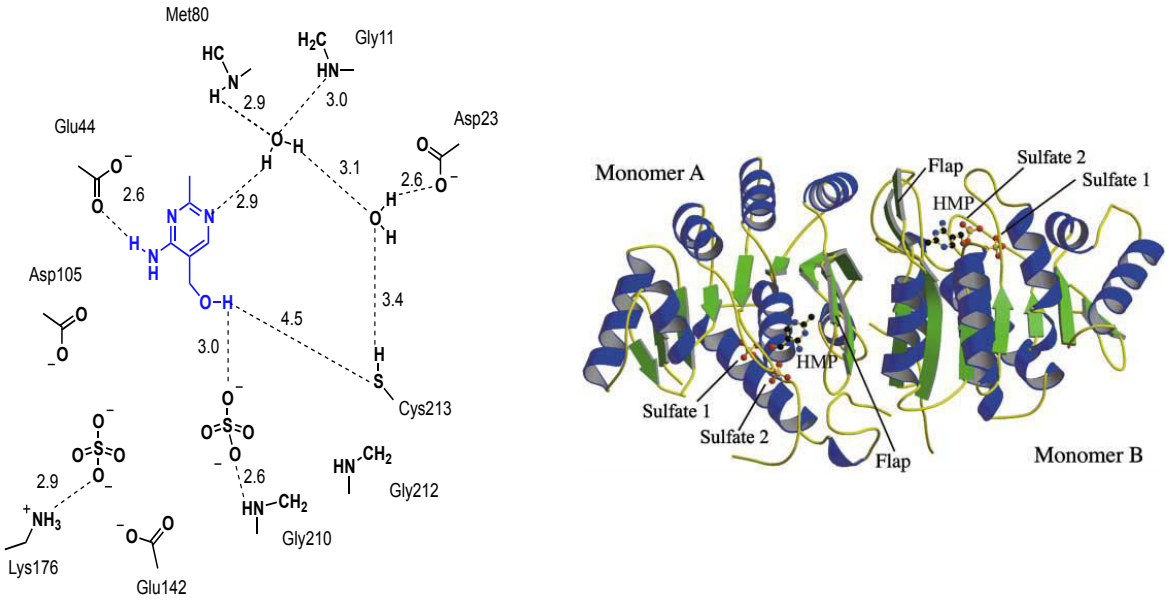


Figure 2.4: A schematic drawing of the active site. Key hydrogen bonds are indicated by dashed lines (Left), Ribbon diagram of HMP kinase dimer viewed along the 2-fold axis (Right).

In our current work, we have investigated the nature of the active site and enzyme function of HMP kinase through a structure-activity relationship study within thiamine biosynthetic pathway using molecular docking study. [17] Thiamine (vitamin B_1) is an essential cofactor for all organisms in its active form, but bacteria synthesize thiamine *de novo*, while humans obtain it through dietary means. Thus humans lack the necessary enzymes for its synthesis, which offers a window of selectivity for any suitable target.

Chapter 3

Previous Studies

3.1. Structure-activity relationship

Various classes of HMP analogues were successfully synthesized in Dr. Foss's lab. Coupled enzyme assay, luminescent kinase (ADP-Glo assay),[18] HPLC assay and whole cell assay were utilized to evaluate an initial substrate scope and inhibitor pharmacophore with synthetic HMP. The substrate scope analysis revealed valuable information regarding binding constraints and flexibility of the binding pocket (Figure-3.1).

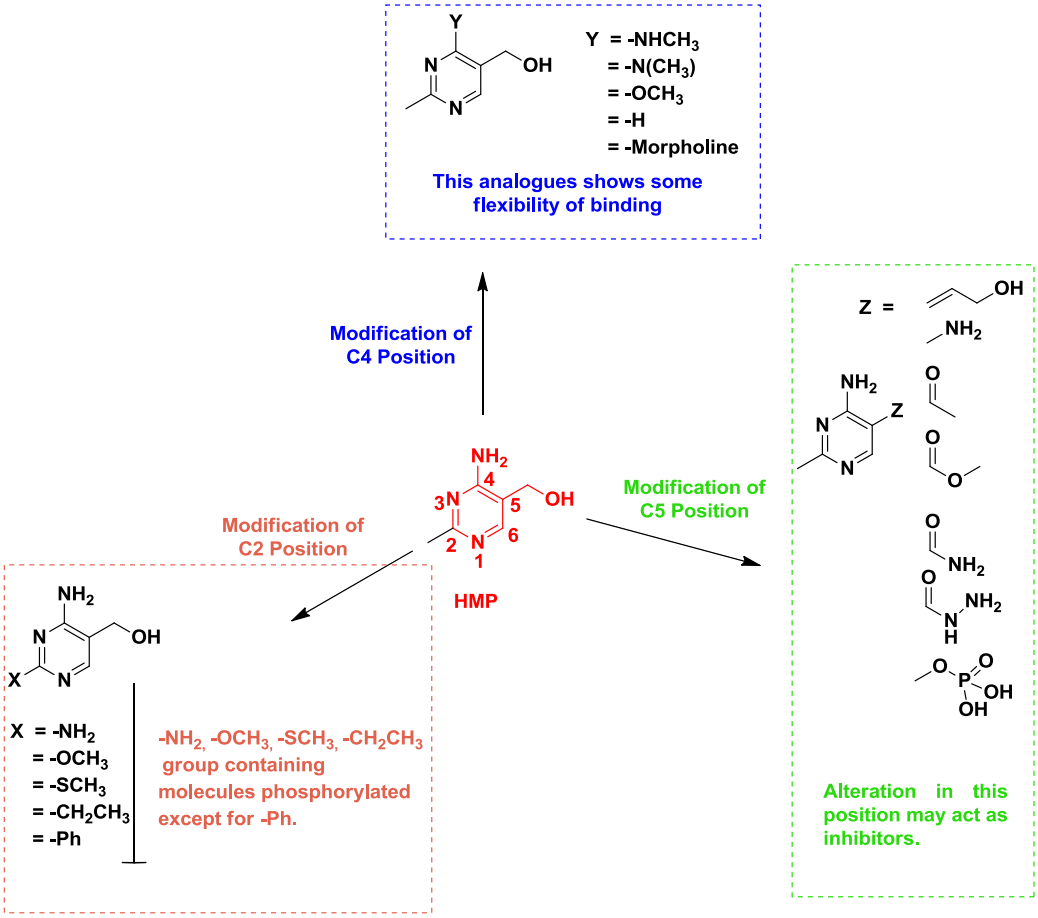


Figure 3.1: Structure-activity relationship [19]

Through inhibition studies, conformational restricted analogues and analogues having different functional groups instead of hydroxymethyl group at C5 position showed their potentiality as an inhibitor of HMP Kinase; and each class had certain structural components in common that may be crucial to exploring further inhibitors through rational drug design.[19]

3.2. Preliminary Results

After running coupled enzyme assay, our group got some potential results on HMP analogues functional group screening.

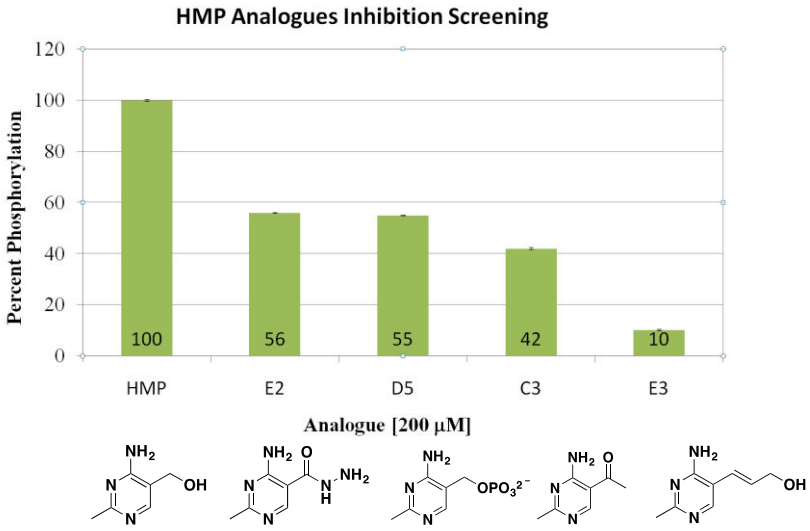


Figure 3.2: HMP analogues inhibition screening [19]

Being inspired by the result of functional group screening from previous work (Figure-3.2), our group focused on the synthesis of few compounds, having a different functional group in the C5 position of pyrimidine moiety, might act as an inhibitor of HMP kinase enzyme system.

Chapter 4

Synthesis of Inhibitors of HMP kinase enzyme system

4.1. Synthesis of Natural Substrate

The natural substrate (4-amino-5-hydroxy methyl-2-methylpyrimidine) HMP **1.4** was synthesized using a condensation reaction to obtain the nitrile **1.1** [20] followed by base hydrolysis to the acid **1.2**, [21] a modified Fischer esterification adopted for the synthesis of ester **1.3** and $LiAlH_4$ reduction to obtain the desired natural substrate HMP **1.4**, (Scheme-1). Our purpose of synthesizing HMP was to compare the inhibitory effect of various synthesized inhibitors on HMP kinase enzyme system in the presence of natural substrate.

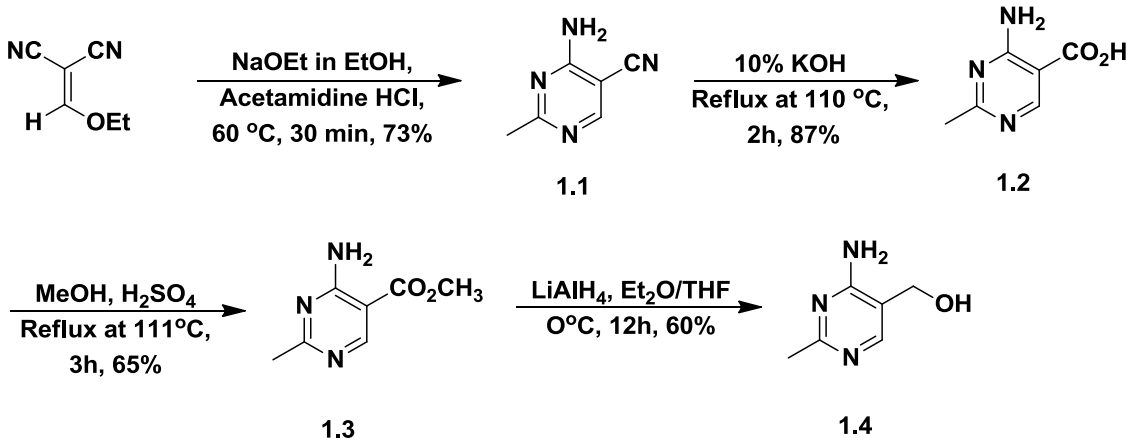
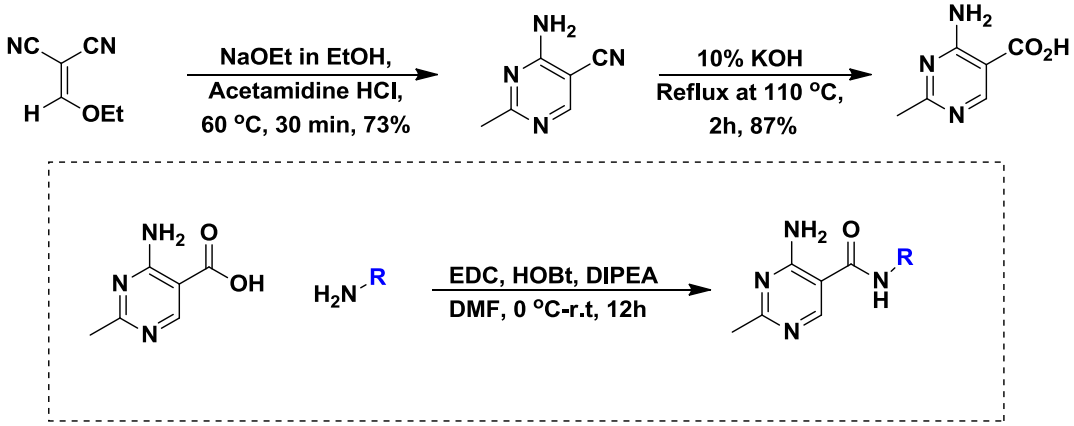


Figure 4.1: Synthesis of Natural Substrate

4.2. Synthesis of HMP amide with non-amino acid derivatives

According to the previous studies shown in Figure 3.2, varieties of functional group attached to the lead pyrimidine moiety such as amide compound (**E2**), Monophosphate (**D5**), ketone (**C3**), allylic alcohol (**E3**) had proved potential as inhibitors



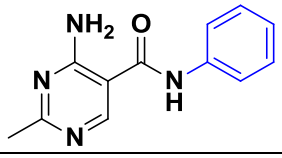
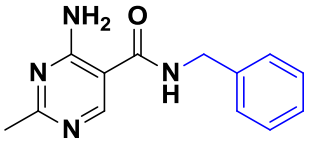
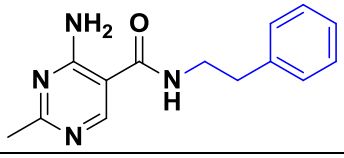
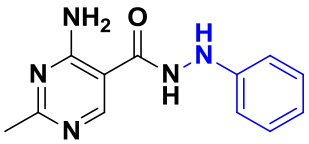
Compound Name	Non-amino acid Starting material	Final Compound with R	% Yield
2.1	Aniline		81
2.2	Benzylamine		79
2.3	Phenethylamine		77
2.4	Phenyl Hydrazine		63

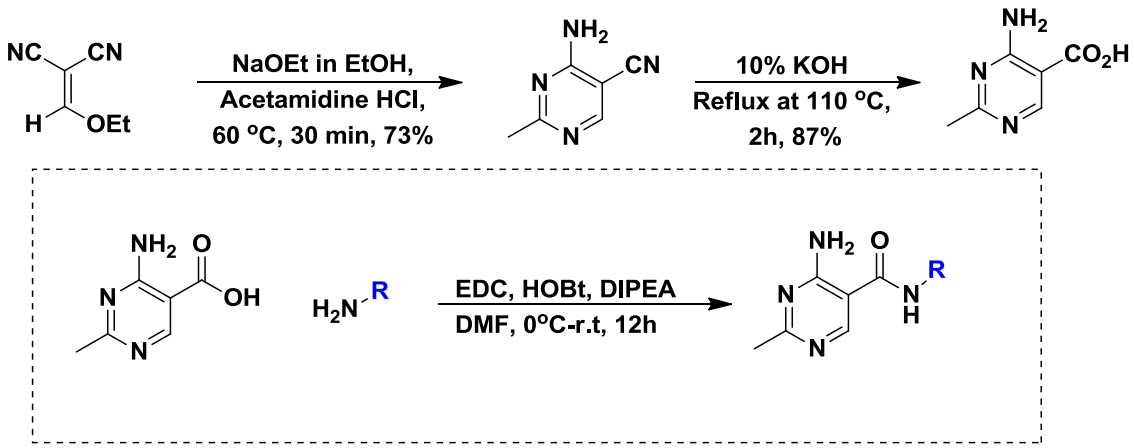
Figure 4.2: Synthesis of HMP amide with non-amino acid derivatives

Through coupled enzyme assay. All of the compounds showed a high binding affinity (via computational docking, showed in Figure-5.4) due to the possibility of hydrogen bonding donor and acceptor atom.[22] Inspired by the previous result and computational study, few amide compounds which were similar to compound **E2** as the first target, were synthesized. Compound **2.1** to **2.3** were amide compounds with an elongated chain with the phenyl ring.

The motivation behind to synthesize those is to increase nonpolar interaction with a nonpolar region of the binding site. Compound **2.4** was including a phenyl ring compares to compound **E2**. All of these compounds synthesize by EDC coupling reaction. [23] Carboxylic acid **1.2** was activated by EDC and HOBt in which amine compound and DIPEA were added that led to a condensation reaction, converted amide compound. (Figure-4.2)

4.3 Synthesis of HMP amide with amino acid derivatives

Compounds **2.5, 2.7 2.9, 2.11** have different amino acid attached to the lead pyrimidine moiety. The motivation behind the synthesis of those compounds was to observe the effect of the side chain of various amino acids to the binding site. These compounds were synthesized by EDC coupling reactions.[23] The amino acid was deprotonated by DIPEA and added to the pre-activated carboxylic acid **1.2** (Figure-4.3).



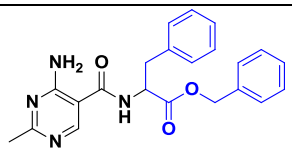
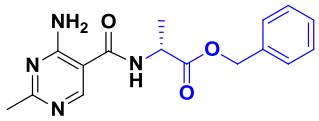
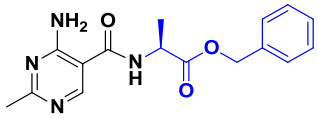
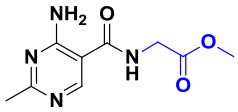
Compound Name	Amino acid Starting material	Final Compound with R	% Yield
2.5	*L*-Phenylalanine benzyl ester hydrochloride salt		75
2.7	*D*-Alanine benzyl ester trifluroacetate salt (2.7c)		82
2.9	*L*-Alanine benzyl ester *4p*-toluene sulfonate		84
2.11	Glycine methyl ester hydrochloride salt		77

Figure 4.3: Synthesis of HMP amide with amino acid derivatives

4.4. Synthesis of HMP amide with *D* or *L*-alanine

Compounds **2.7** and **2.9** are enatiomers with *R* and *S* configuration respectively. These molecules were prepared to observe the effect of stereoisomers within the HMP binding site. (Figure-4.4). Compound **2.9** was

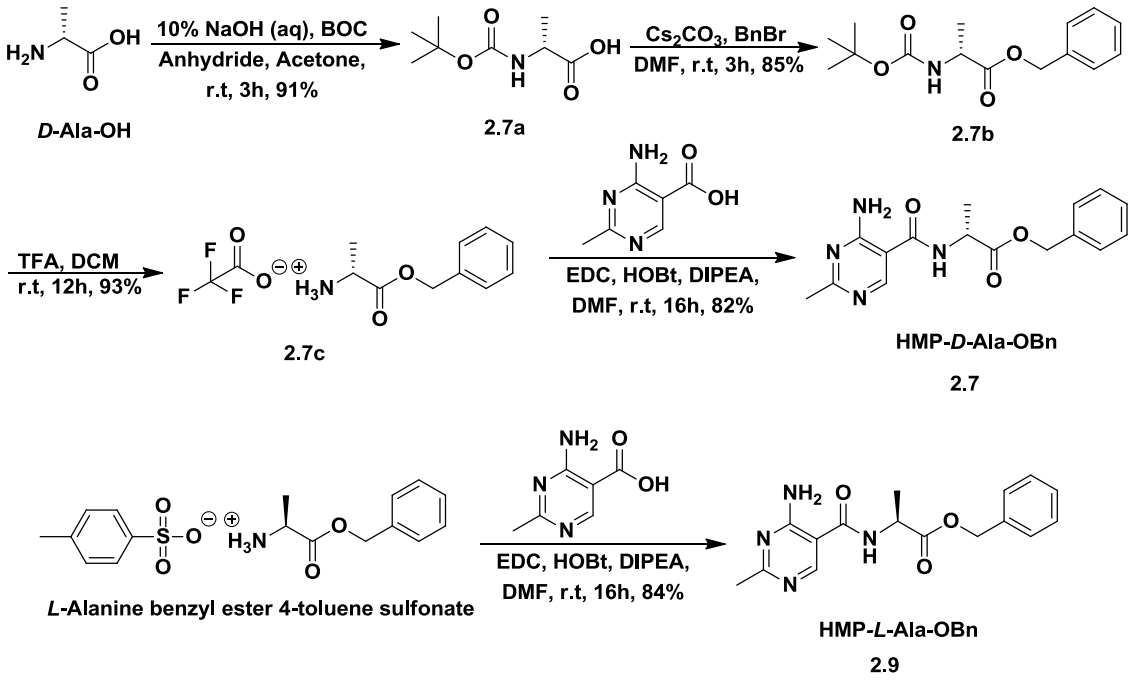


Figure 4.4: Synthesis of HMP amide with *D* or *L*-alanine (Stereospecific HMP analogues)

synthesized by EDC coupling reaction with commercially available *L*-Alanine benzyl ester 4-toluenesulfonate. Compound **2.7** was synthesized from available *D*-Ala-OH. At first compound **2.7a** was synthesized by protection of *D*-alanine by BOC anhydride [24] followed by synthesis of **2.7b** through benzyl protection[25] with benzyl bromide in

presence of Cs_2CO_3. Finally BOC-deprotection was completed by TFA, giving compound **2.7c** which was coupled by EDC conditions with carboxylic acid **1.2** to form compound **2.7** (Figure-4.4)

4.5. Synthesis of HMP phosphate mimics

Compound **2.5**, **2.7** and **2.9** were converted to free carboxylic acids **2.6**, **2.8** and **2.10** respectively by hydrogenolysis reaction in the presence of 10 % palladium on charcoal catalyst.[26] These compounds were prepared because of their resemblance to **D5**, which is a HMP-monophosphate. The carboxylic acids are bioisosteric which

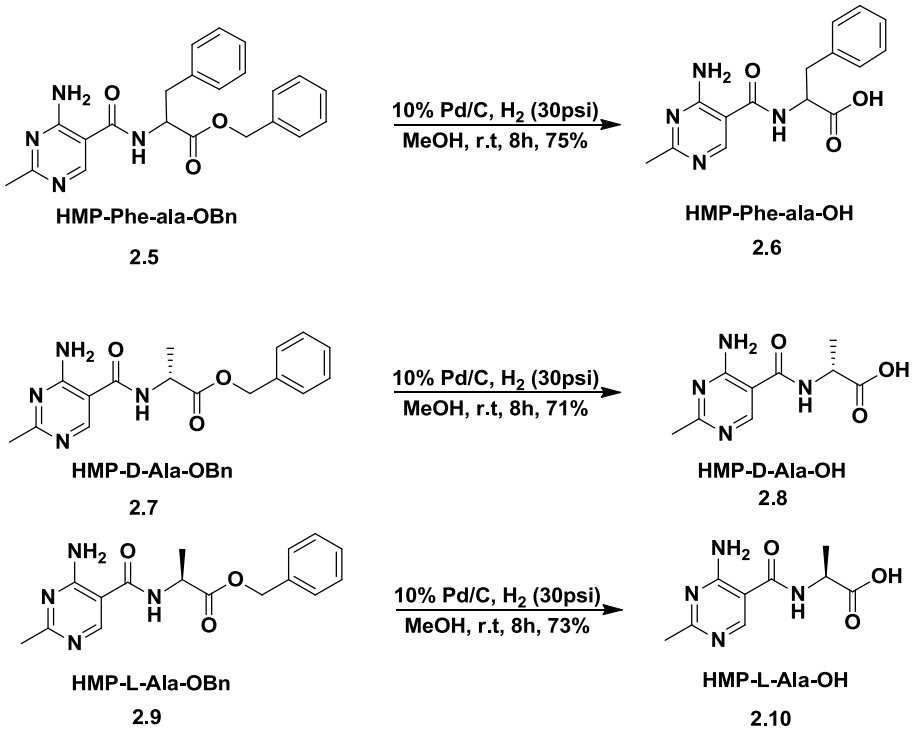


Figure 4.5: Synthesis of HMP phosphate mimics (Transition State analogues)

maintain the phosphate group's negative charge under biological conditions, resulting in potential binding to the anion hole of HMP kinase. As compound **D5** shows an inhibitory effect, the bioisosteric modification could make a better analogue by replacing phosphate group with carboxylic acids. [27]

Chapter 5

Screening of HMP Inhibitors

5.1. Coupled enzyme assay

In an endeavor to evaluate inhibition effect of all synthesized compound on HMP kinase enzyme, Dr. Foss's group monitored HMP kinase activity through coupled enzyme assay.[28] By this assay, it is possible to determine a substrate or enzyme activity by coupling of one enzymatic reaction with another, more easily detectable, reaction. The product of the first reaction is the substrate for the second.

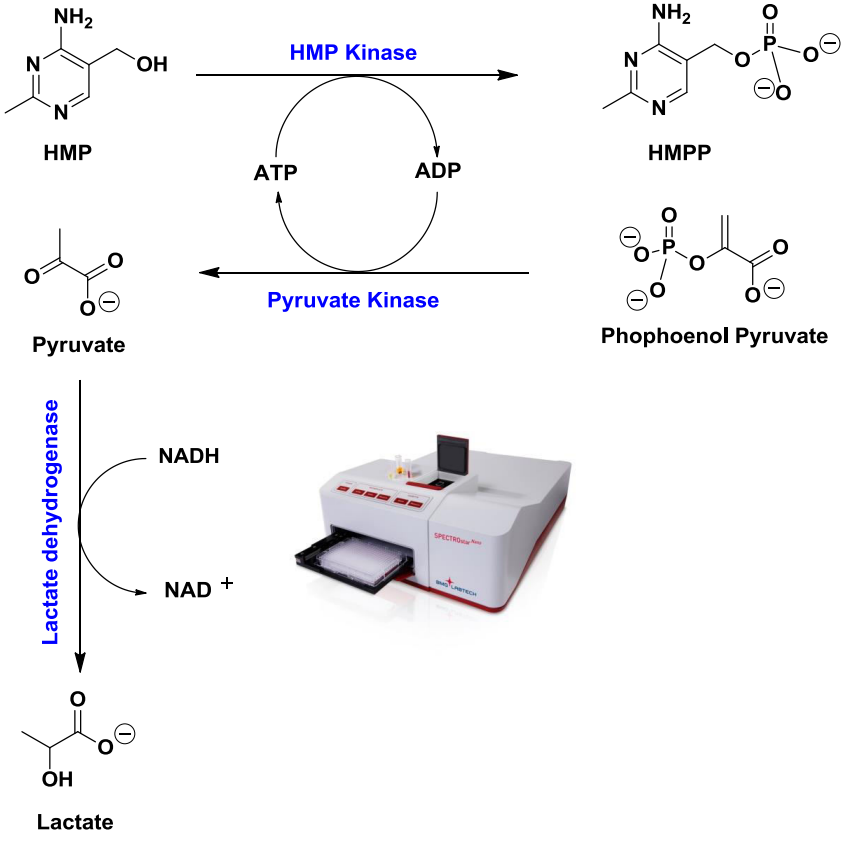


Figure 5.1: Coupled enzyme assay

These assays are often designed to generate or consume a reduced nicotinamide-containing nucleotide, which is measurable by its ultraviolet absorption (Figure-5.1). According to this assay, HMP kinase produces ADP, which is consumed by pyruvate kinase/lactate dehydrogenase enzyme system, leading to depletion of NADH in a directly proportional relationship to ADP production. NADH can be measured at 340 nm by spectrophotometric devices.

The inhibitory effect was measured spectrophotometrically at 25 °C by the pyruvate kinase/lactate dehydrogenase coupled enzyme system. The ADP-dependent

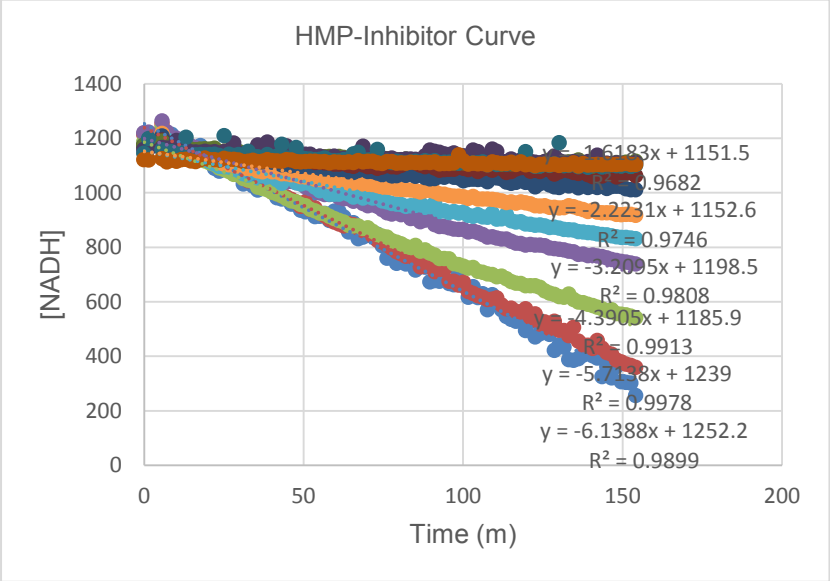
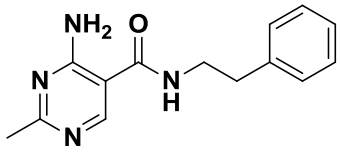
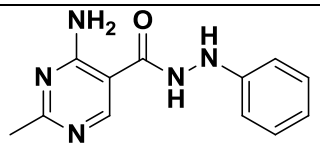
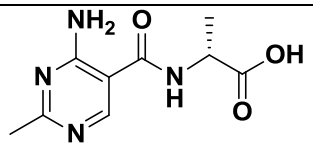
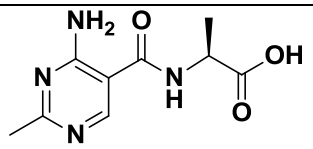


Figure 5.2: HMP Inhibitor curve [29]

consumption of NADH was monitored continuously at 340 nm using a SPECTROstar Nanoplate reader (BMG LabTech). The concentration of a various component in per well of 96 multiwell plates was ATP 200 µM, Phosphoenol pyruvate 200 µM, NADH 200 µM, HMP 20 µM, Inhibitor 200 µM (10 times compare to HMP concentration), HMP kinase 1 µM, 6 U pyruvate kinase, 10 U lactate dehydrogenase. Tris buffer (pH 7.4) including 50 mM Tris-HCl, 50 mM KCl, 5 mM $MgCl_2$ used as media. All of the components except

HMP and ATP mixed in Tris buffer system and made triplicate for each inhibitor. The reaction was incubated for 10 minutes at 25 °C and the cascade initiated by the addition of ATP and HMP. Depletion of NADH is monitored continuously at 340 nm using a SPECTROstar Nanoplate reader (BMG LabTech) which give us negative slope curve in a concentration of NADH vs time plot (Figure-5.2). In the presence of a good inhibitor, HMP will phosphorylated less which will produce less amount of ADP leads to less consumption of NADH. So, we assume in absence of inhibitor, NADH depletion curve slope will be highest and normalize as 100%, whereas in presence of inhibitor NADH depletion curve slope will be decreased.[28-29]

Table 5.1: Results obtained from coupled enzyme assay:

Compound Name	Compound Structure	Percent of Inhibition
2.3		**10**
2.4		**13**
2.8		**11**
2.10		**10**

Discussion: According to the result in Table 5.1, compound **2.3, 2.4, 2.8 and 2.10** showed potential as an inhibitor. For example, HMP phosphorylation could be inhibited by 13 percent in presence of compound **2.4**.

5.2. In vitro whole cell assay:

Cultures of *E. coli* K12 *and S. aureus* NCTC 8325 were inoculated in Mueller Hinton Broth (Becton Dickingson, Franklin Lakes, NJ) and grown overnight at 37 ^{o}C with medium agitation. The overnight cultures were then diluted (1:1000) into Difco^{TM} M9 minimal media (BD, Franklin Lakes, NJ) prepared according to manufacturing recommendations with glucose as the only carbon source. The inocula were transferred into a 96-well plate containing the compounds to be tested. Bacterial growth was monitored spectrophotometrically (OD_{600}) with a SPECTROstar Nanoplate reader (BMG LabTech, Cary, NC). A ten-fold serial dilution of each compound was tested in duplicate. Negative controls were run without the presence of any analogue for each bacterial strain. Isoniazid used as positive control.[29]

The substrate analogs of HMP resulted successfully towards the growth inhibition of the bacterial strains. It is worthy to mention that the compounds of interest were inactive in rich media such as LB or Mueller-Hinton broths. However, when the bacteria strains are grown in minimal media, the effect of HMP analogs are detrimental to the survival of the cell. The results are outlined in Figure-5.3

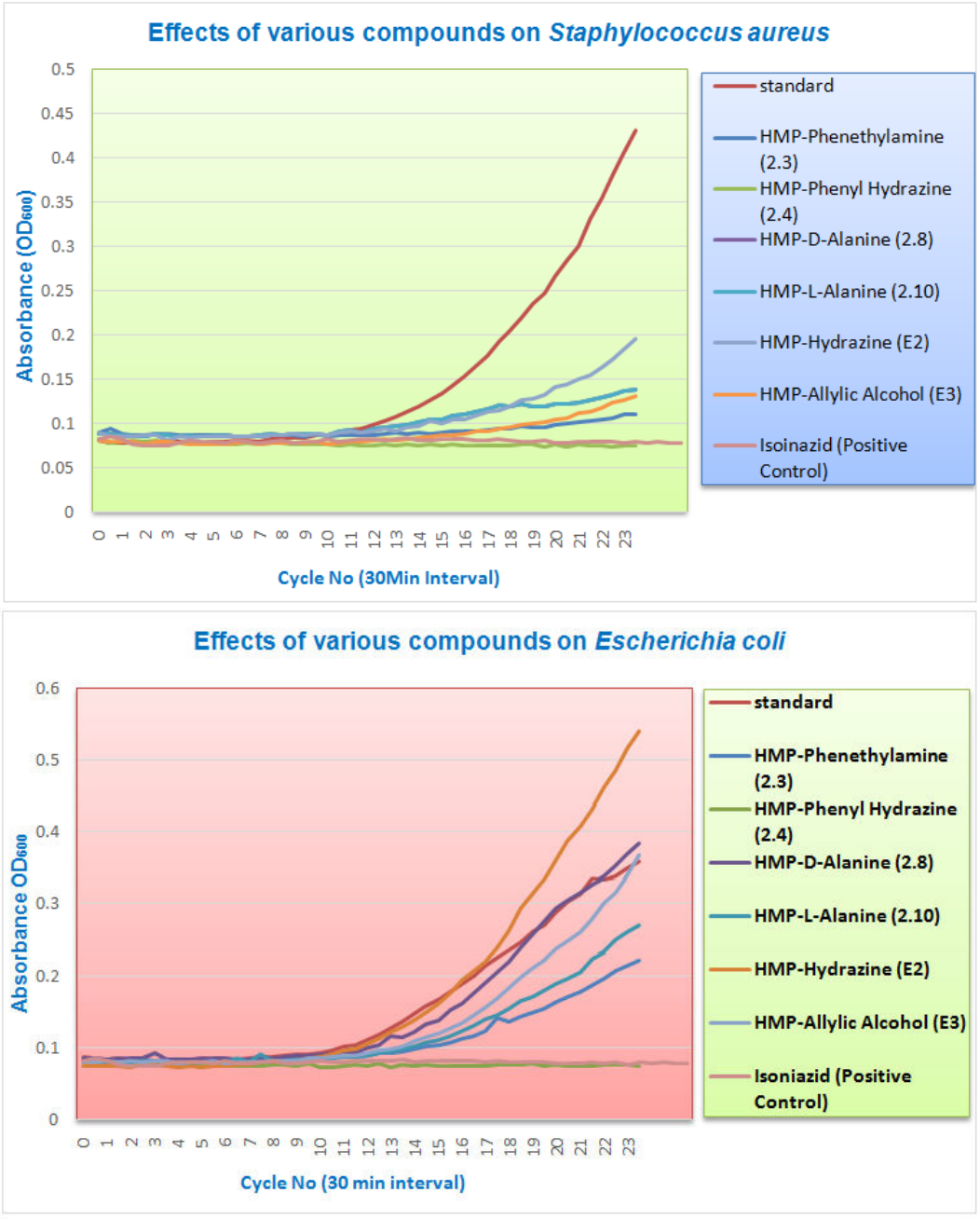


Figure 5.3. Whole cell assay of various inhibitors on gram-positive bacteria *S. aureus (Top)* and gram negative bacteria *E. coli (*Bottom) in 1mM concentration

According to the result among all the synthesized compounds, compound **2.4, 2.3, 2.8** and **2.10** show activity towards growth inhibition in gram-positive species and compound **2.4, 2.3** effective against both gram positive and gram and negative species. The efficiency trend is as follows: **2.8 < 2.10 < 2.3 < 2.4**. The other analogues of HMP kinase showed no growth inhibition in the whole-cell system.

5.3. HMP Kinase Enzyme Purification:

Approximately 10 g of cell paste was suspended in 20 mL of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 15 mM imidazole, 5 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, pH 8.0), and thawed in an ice bath with 5 mg/mL each of lysozyme, deoxyribonuclease I, and ribonuclease with gentle stirring for 30 min. The cell suspension was sonicated in a 10 s on/off pulse cycle for a total of 2 min. The resulting cell-free extract was centrifuged (JA-10.1 rotor) at 15000x g for 30 min. at 4 °C. The supernatant was loaded onto a Ni^{2+} charged HiTrap IMAC HP column (GE Healthcare, Pittsburgh, PA) pre-equilibrated in lysis buffer. The protein was eluted with a linear imidazole gradient (50 mM to 400 mM imidazole in 50 mM NaH_2PO_4, 300 mM NaCl, and pH 8.0). The fractions containing *ThiD* protein, determined by SDS-PAGE, were concentrated via VivaSpin Turbo 15 with 10 kDa MWCO (Sartorius, Bohemia, NY) and dialyzed overnight to remove imidazole from solution. Protein concentration was determined via UV-Vis spectroscopy and standard Coomassie Plus assay (Thermo Fisher Scientific INC. Rockford, IL) [28]

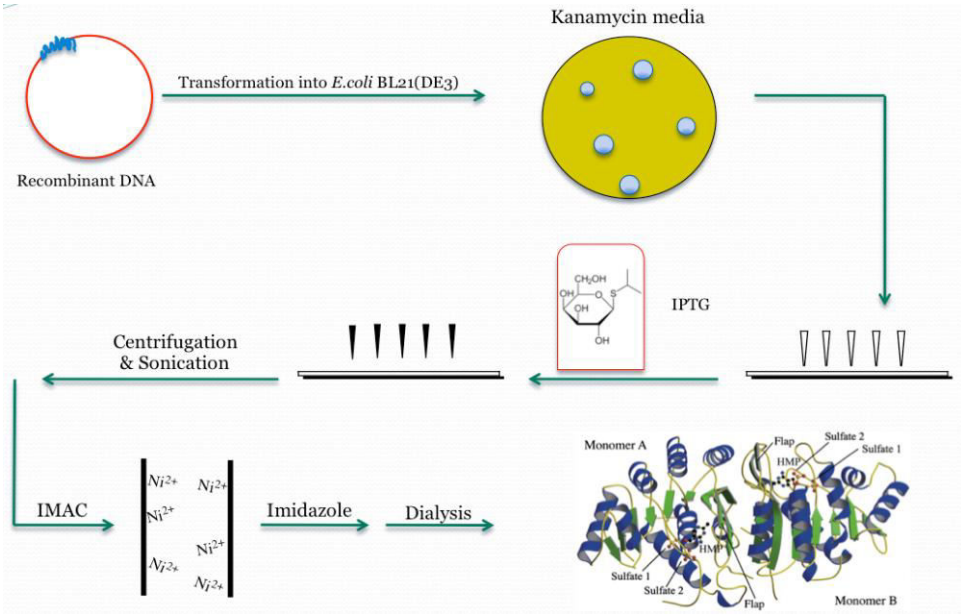


Figure 5.4: Purification of HMP kinase Enzyme

5.4. Molecular Docking Analysis

In silico docking was performed using Autodock Vina run through PyRx and the results visualized through discovery studio (Version 4.5). ChemBioDraw 3D were used to generate 3D structure of Ligands, followed by an MM2 minimization and transferred into Autodock tools 1.5.6 in order to assign Gasteiger charges, nonpolar hydrogen, and setting up torsional restrictions. HMP kinase crystal structure (PDB: 1JXI) was prepared using Discovery Studio 4.5 in order to remove the co-crystallized natural substrate, sulfate ions, and water molecules. A 15-20-Å cubic search space was placed on the HMP's binding pocket and the calculations ran with an exhaustiveness of 16. The binding poses were listed according to their binding affinities (BA).[30] Docking results showed in Table **5.2**. Potential inhibitors found in coupled enzyme assay and whole cell assay

(compound **2.3, 2.4, 2.8** and **2.10**) have a better binding affinity towards binding site than natural substrate HMP, which looks promising.

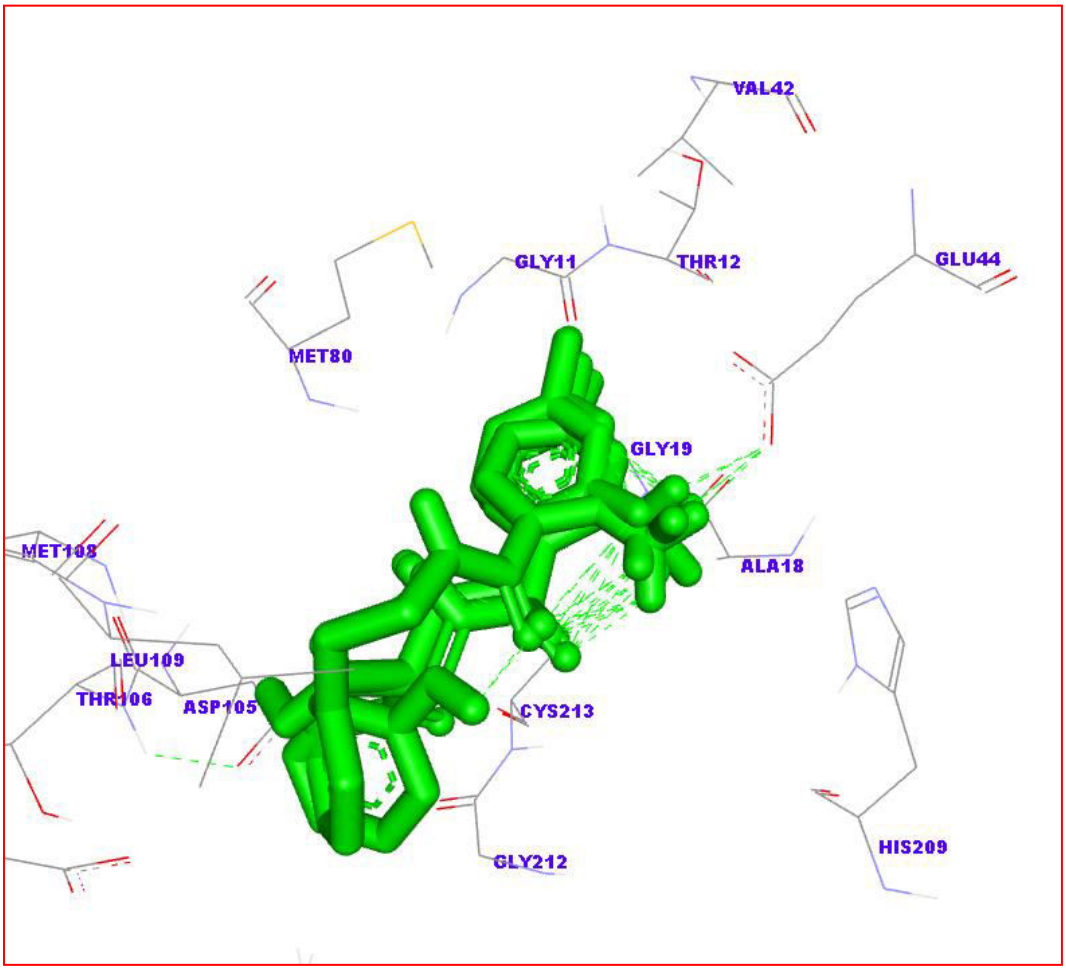


Figure 5.5: Molecular docking of compound **2.3, 2.4, 2.8** and **2.10** by Autodock

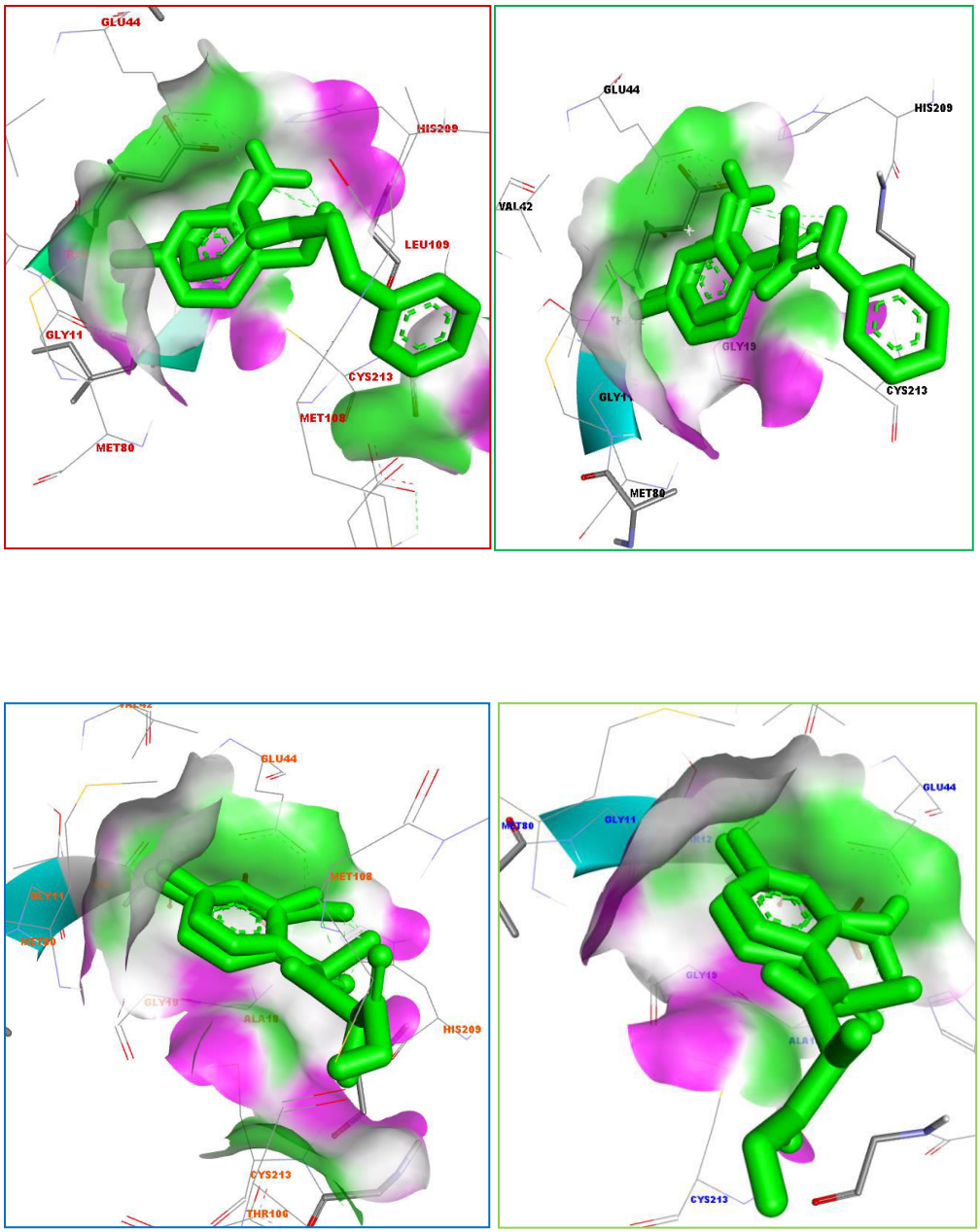
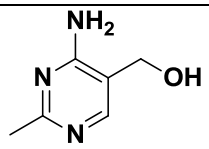
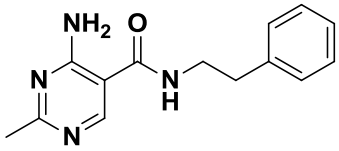
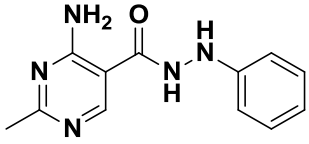
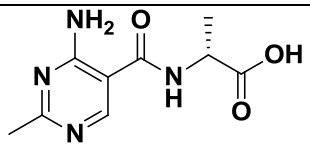
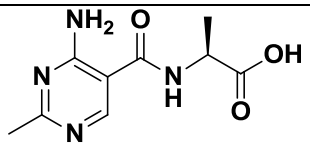


Figure 5.6: Molecular docking of compound **2.3, 2.4, 2.8** and **2.10** by Autodock separately. Compound **2.3** (top left), compound **2.4** (top right), compound **2.8** (bottom left) and compound **2.10** (bottom right). In binding pocket, green region is Hydrogen bond acceptor and red region is hydrogen bond donor.

Table 5.2: Molecular docking by Autodock Vina

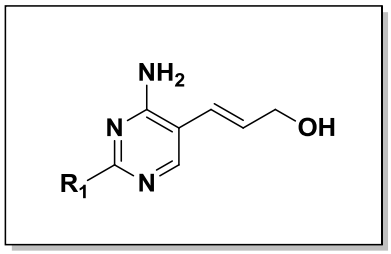
Compound Name	Compound Structure	Docking Score
1.4		**- 4.5 kcal/mol**
2.3		**-6.0 kcal/mol**
2.4		**-6.4 kcal/mol**
2.8		**-5.2 kcal/mol**
2.10		**-5.4 kcal/mol**

Chapter 6

Future Scope

6.1. Conformation-restricted Inhibitor Design (Allylic Alcohol Derivatives)

Allylic alcohol compound **E3** showed significant inhibitory effect (Figure-3.2). So, modification of allylic alcohol in the C2 position of pyrimidine ring might act as a potential inhibitor.

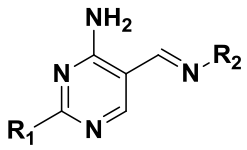


R_1
$-NH_2$
$-OCH_3$
-OH
$-SCH_3$
$-CF_3$

Figure 6.1: Conformation-restricted Inhibitor Design (Allylic Alcohol Derivatives)

6.2. Conformation-restricted Inhibitor Design (Imines Derivatives)

Another future scope would be to adopt the bioisosteric concept - that is replacing a group in the lead compound with an isostere (a group having the same valence) makes it easier to determine whether a particular property such as hydrogen bonding or any other noncovalent interaction is important. So, we can modify carbon-carbon double bond by carbon-nitrogen double bond that is formation of imines in C5 side chain (Scheme-6.3).[31]



R_1	R_2
$-CH_3$	-OH
$-NH_2$	$-NHNH_2$
$-OCH_3$	$-CH_2CF_3$
-OH	$-CH_3$
$-SCH_3$	-Ph
$-CF_3$	$-CH_2COOH$

Figure 6.2: Conformation-restricted Inhibitor Design (Imines Derivatives)

6.3. Transition state Analogues Design

HMP kinase enzyme is responsible for two consecutive phosphorylation events, which is rare. This allows freedom of designing molecules for two much related binding conformations. It facilitates to utilize the concept of transition state analogues, that are compounds having structure resembles the transition state of a substrate molecule in enzyme-catalyzed chemical reaction. Theory suggests that enzyme inhibitors, which resembled the transition state structure, would bind more tightly to the enzyme than the

actual substrate. So, they (Figure-6.3) might act as inhibitors in enzyme-catalyzed reactions. [32]

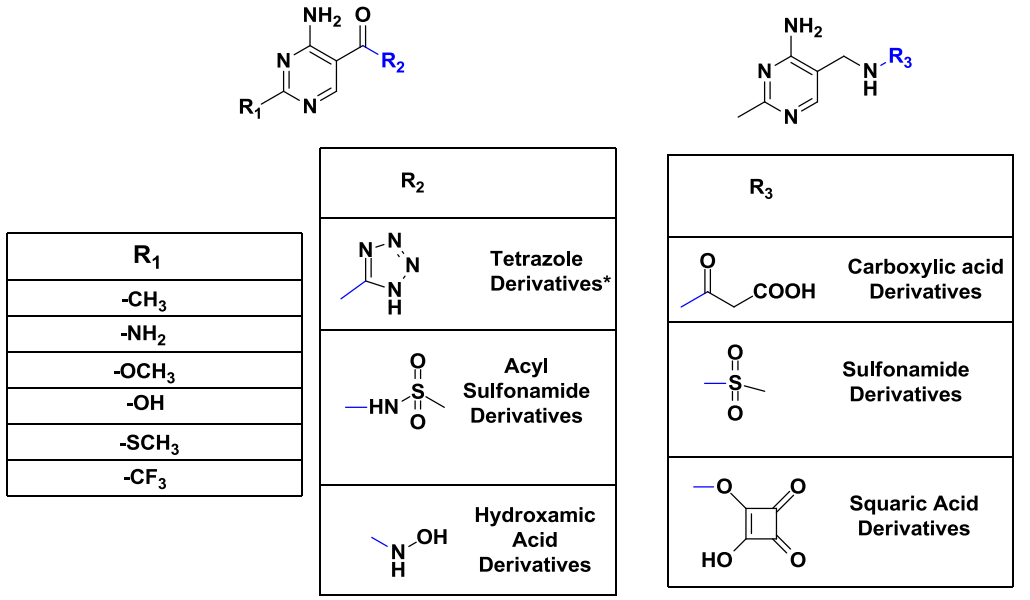


Figure 6.3: Transition state Analogues Design

Chapter 7

Conclusion

We need to focus on more extensive testing to confirm inhibitors responsible for killing microorganism as well as the need to determine the exact mechanism by which these molecules cause cell death. Kinetic enzyme assays are needed in order to determine if HMP analogues are competitive inhibitors of the enzyme HMP kinase. Minimum inhibitory concentrations also need to be determined for the molecules that are confirmed to have antibiotic activity. At the very end, I wanted to turn everyone's attention towards deadly diseases tuberculosis (TB), which is becoming increasingly antibiotic-resistant. Approximately one-third of people living in the world today have tuberculosis. In the United States, there were 91 reported cases of multiple drug resistant TB in 2015 according to the Centers for Disease Control (CDC). The cost of a multiple drug resistant TB infection in developing nations is simply overwhelming and is often not treated effectively. The urgency for new, cost-effective antibiotics both in the United States and abroad is really a crying need. HMP inhibitors and their role in inhibition of thiamine synthesis may be a step toward meeting this exigency.[33] Particularly in bacterial species like *Mycobacterium tuberculosis*, which lack salvage pathways and known transporters of thiamine, potentially eliminating uptake from the environment.[34] In 1961, John F. Kennedy declared to congress "This nation should commit itself to achieving the goal, before the decade is out, of landing a man on the moon and returning him safely to the earth." [35] Whether many thought the statement was only political and impossible, but less ten years, Kennedy's dream was possible in 1969 by human first step on moon. Likewise, it will be possible for a scientist to combat bacterial species that used to employ smart mechanistic pathways, which nullify all scientific endeavors through antimicrobial resistance.

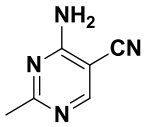
Chapter 8

Experimental section

General Experimental Information

Compounds were purchased in their purest commercial quality. Anhydrous solvents were purchased from EMD drisolv series of solvents through VWR. All materials were used as obtained, unless and otherwise indicated. 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR experiments were performed on JEOL ECX 300 instrument; 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR experiments were performed on a JEOL Eclipse plus 500 instruments. Chemical shifts were recorded in reference to residual solvent peaks (DMSO-d6 residue = 2.50 ppm, $CDCl_3$ residue = 7.26 ppm, D_2O = 4.79, CD_3OD = 3.31). The following abbreviations were used to describe NMR peaks: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, quin = quintet, bs = broad singlet. TLC experiments were performed on EMD Merck F_{254}, 250 µm thickness. *Rf* reported refers to flash chromatography conditions unless otherwise noted. Chromatography was performed on P60 silica gel (mesh 230-400). High-resolution mass spectroscopy was performed at Shimadzu Center for Advanced Analytical Chemistry, using Shimadzu LCMS IT-TOF Mass Spectrometer. IR experiments were recorded with neat samples on a Bruker Alpha instruments fitted with diamond ATR sample plate.

Analysis and characterization of synthesized compounds:

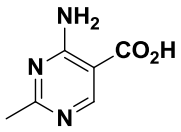


1.1

4-Amino-2-methylpyrimidine-5-carbonitrile (1.1): Acetamidine hydrochloride (5 g, 52.89 mmol) was slowly added to a (~2.3 M) solution of sodium ethoxide, prepared by

adding sodium (1.34 g) to dry ethanol (30 mL) at 0 °C. The solution was filtered through Celite and the filtrate mixed with ethoxy methylene malononitrile (7.75 g, 53.47 mmol). The dense yellow precipitate which formed immediately, (slightly exothermic) was swirled for 20 min to produce a paste, which was filtered and washed with cold methanol followed by ether to obtain a yellow solid (5.17 g, 73%) after drying. The material could be recrystallized from hot ethanol.

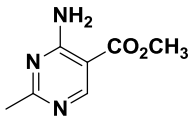
m.p. 247-248 °C; IR (neat, cm^{-1}) 3381, 3334, 3017, 2978, 2849, 2757, 2666, 2224, 1674, 1281, 1240, 958; ^{1}H NMR (500 MHz, DMSO-D6) δ 8.73 (s, 1H), 8.00 (bs, 2H), 2.60 (s, 3H). ^{13}C NMR (125 MHz, DMSO-D6) δ 170.86, 163.13, 161.89, 116.49, 87.44, 26.69; HRMS (ESI-TOF) *m/z* calculated for $C_6H_7N_4$ $[M+H]^+$ 135.0665, found 135.0659.



1.2

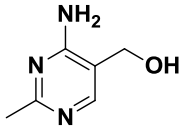
4-Amino-5-carboxy-2-methylpyrimidine (1.2): 4-Amino-2-methylpyrimidine-5-carbonitrile **1.1** (2.5 g, 18.66 mmol) was refluxed for 2 h in 20 mL of 10% KOH (aq.) and the solution filtered hot and acidified with glacial acetic acid resulted into a precipitate on cooling. The precipitate was filtered and washed with cold water and dried under vacuum to afford the acid as light yellow needles (2.48 g, 87%). Recrystallized from water to give fine needles.

m.p. 272-273 °C; IR (neat, cm^{-1}) 3224, 3079, 2351, 1960, 1661,1567, 1316; ^{1}H NMR (300 MHz, Deuterium Oxide) δ 8.31 (d, *J* = 1.3 Hz, 1H), 2.41 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) δ 168.72, 163.87, 162.67, 145.85, 109.35, 20.88.; HRMS (ESI-TOF) *m/z* calculated for $C_6H_8N_3O_2$ $[M+H]^+$ 152.0466, found 152.0467.



1.3

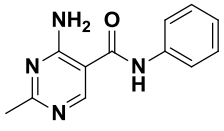
Methyl-5-carbomethyoxy-6-aminopyrimidine (1.3): The amino acid **1.2** (5.0 g, 33 mmol) was dissolved in 15 g of concentrated sulfuric acid and was warmed gently at 50 °C. A mixture of methanol (20 mL) and sulfuric acid (4 g) was added. The resulting solution was refluxed at around 110 °C until it turned clear (~ 30-45 min). Methanol (20 mL) was again added and refluxed for an additional 45 min. The resulting dark yellow/ brownish mixture was cooled, poured into ice-water and neutralized with Na_2CO_3 until the pH~7-8. The precipitated ester was filtered and washed with cold water (120 mL) and dried to afford (3.59 g, 65%) of faint yellow solid; m. p. 175-177 °C; IR (neat, cm^{-1}) 3434, 3264, 3022, 2951, 2851, 1688, 1630, 1439, 1234, 1102; ^{1}H NMR (300 MHz, DMSO-D6) δ 8.61 (s, 1H), 7.90 (s, 1H), 7.50 (s, 1H), 3.78 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 171.06, 166.65, 162.55, 159.49, 101.93, 52.42, 26.24.; HRMS (ESI-TOF) *m/z* calculated for $C_7H_{10}N_3O_2$ $[M+H]^+$ 168.0768, found 168.0756.



1.4

Amino-5-hydroxymethyl-2-methylpyrimidine (1.4, HMP): Diethyl ether (200 mL) was added to (1.5 g, 40 mmol) of lithium aluminum hydride at 0 °C. Then (5.0 g, 30 mmol) of 2-methyl-4-aminopyrimidinecarboxylate **1.3** was dissolved in THF (100 mL) was added slowly. The reaction was allowed to attain room temperature and stirring was continued for 12 h. The reaction was quenched with 2-3 mL of NH_4Cl at 0 °C and stirred for 30 min, following which the slurry was filtered using Celite. After washing the filter bed with 50 mL

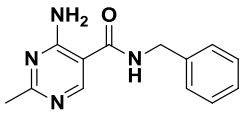
of a mixture of ethyl acetate and methanol (2:1), the solvents were removed under reduced pressure. The crude material was purified by flash silica gel chromatography with hexane/ethyl acetate/methanol (50:40:10), *Rf* = 0.35, to obtain the product as a faint yellow semisolid mass. The product then crystallized out as a white powder, (2.5 g, 60%) from a mixture of methanol and ethyl acetate (~1:9); m.p 191-193 °C (lit63 193-194 °C); IR (neat, cm^{-1}) 3427, 3384, 3286, 3198, 3005, 2971, 2872, 2729, 2682, 1687, 1599, 1000; ^{1}H NMR (500 MHz, DMSO-D6) δ 7.88 (s, 1H), 6.47 (bs, 1H), 5.06 (t, *J* = 5.5 Hz, 1H), 4.26 (d, *J* = 5.5 Hz, 2H), 2.25 (s, 3H); ^{13}C NMR (125 MHz, DMSO-D6) δ 165.87, 161.98, 153.39, 114.23, 58.26, 25.72.; HRMS (ESI-TOF) *m/z* calculated for $C_6H_{10}N_3O$ $[M+H]^+$ 140.0818, found 140.0814.



2.1

4-amino-2-methyl-*N*-phenylpyrimidine-5-carboxamide (2.1): The amino acid **1.2** (0.50 g, 3.27 mmol, 1 equiv), EDC (0.94 g, 4.91 mmol, 1.5 equiv), and HOBt (0.49 g, 3.60 mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, Aniline (0.33 ml, 3.27 mmol, 1 equiv) and *N, N*-diisopropyl ethyl amine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) were mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added drop by drop to the pre-activated acid and stirred for 12 hours. When the reaction was completed (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25 mL), deionized water (2 x 25 mL) and saturated NaCl (2 x 25 mL), and dried with anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the slight brownish compound (0.60 g, 81%).

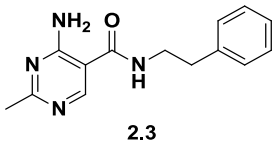
m.p 248-250 °C. IR (neat, cm^{-1}) 3348, 3272, 3127, 3042, 1657, 1629, 1577, 1538; ^{1}H NMR (301 MHz, DMSO-D6) δ 10.18 (s, 1H), 8.67 (s, 1H), 7.79 – 7.53 (m, 4H), 7.32 (t, *J* = 7.9 Hz, 2H), 7.08 (t, *J* = 7.3 Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 169.44, 165.72, 162.51, 156.44, 139.18, 129.15, 124.42, 121.23, 106.38, 26.14; HRMS (ESI-TOF) *m/z* calculated for $C_{12}H_{12}N_4O$ $[M-H]^-$ 227.0938, found 227.0927



2.2

4-amino-*N*-benzyl-2-methylpyrimidine-5-carboxamide (2.2): The amino acid **1.2** (0.50 g, 3.27 mmol, 1 equiv), EDC (0.94 g, 4.91 mmol, 1.5 equiv), and HOBt (0.49 g, 3.60 mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, Benzyl amine (0.35 ml, 3.27 mmol, 1 equiv) and *N, N*-diisopropyl ethyl amine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) were mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added drop by drop to the pre-activated acid and stirred for 12 hours. When the reaction was completed (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25 mL), deionized water (2 x 25 mL) and saturated NaCl (2 x 25 mL), and dried with anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the white compound (0.63 g, 79%).

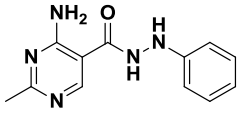
m.p 224-226 °C. IR (neat, cm^{-1}) 3327, 3269, 3135, 3027, 1655, 1583, 1536; ^{1}H NMR (300 MHz, DMSO-D6) δ 9.06 (t, *J* = 6.0 Hz, 1H), 8.62 (s, 1H), 7.67 (bs, 2H), 7.36 – 7.16 (m, 5H), 4.41 (d, *J* = 6.0 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 169.25, 166.76, 162.62, 155.81, 139.91, 128.87, 127.75, 127.35, 105.53, 42.73, 26.06. ; HRMS (ESI-TOF) *m/z* calculated for $C_{13}H_{14}N_4O$ $[M-H]^-$ 241.1095, found 241.1087.



2.3

4-amino-2-methyl-*N*-phenethylpyrimidine-5-carboxamide (2.3): The amino acid **1.2** (0.50 g, 3.27 mmol, 1 equiv), EDC (0.94 g, 4.91 mmol, 1.5 equiv), and HOBt (0.49 g, 3.60 mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, Phenethylamine (0.40 ml, 3.27 mmol, 1 equiv) and *N, N*-diisopropyl ethyl amine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) were mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added drop by drop to the pre-activated acid and stirred for 12 hours. When the reaction was completed (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25 mL), deionized water (2 x 25mL) and saturated NaCl (2 x 25mL), and dried with anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the slight yellowish compound, which was then washed with cold diethyl ether to remove hydrophobic impurities. Flash chromatography could then be performed (95:5 DCM/methanol) to obtain purified desired compound (0.64 g, 77%) as white solid.

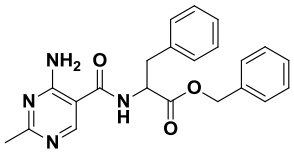
m.p 204-206 °C. IR (neat, cm^{-1}) 3335, 3264, 3135, 3053, 2964, 1654, 1539; ^{1}H NMR (300 MHz, DMSO-D6) δ 8.57 (s, 1H), 8.46 (s, 1H), 7.69 (bs, 2H), 7.33 – 7.10 (m, 5H), 3.49 – 3.35 (m, 2H), 2.79 (t, *J* = 7.4 Hz, 2H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 169.09, 166.67, 162.54, 155.59, 139.95, 129.22, 128.90, 126.68, 105.82, 41.05, 35.51, 26.05; HRMS (ESI-TOF) *m/z* calculated for $C_{14}H_{16}N_4O$ $[M-H]^-$ 255.1251, found 255.1273



2.4

4-amino-2-methyl-*N'*-phenylpyrimidine-5-carbohydrazide (2.4): The amino acid **1.2** (0.50 g, 3.27 mmol, 1 equiv), EDC (0.94 g, 4.91 mmol, 1.5 equiv), and HOBt (0.49 g, 3.60 mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, Phenyl Hydrazine (0.35 ml, 3.27 mmol, 1 equiv) and *N, N*-diisopropyl ethyl amine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) were mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added drop by drop to the pre-activated acid and stirred for 12 hours. When the reaction was completed (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25 mL), deionized water (2 x 25 mL) and saturated NaCl (2 x 25 mL), and dried with anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the yellowish compound, which was then washed with cold diethyl ether to remove hydrophobic impurities. Flash chromatography could then be performed (95:5 DCM/methanol) to obtain purified desired compound (0.51 g, 63%) as slight yellowish solid.

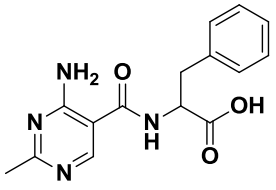
m.p 190-192 °C. IR (neat, cm^{-1}) 3387, 3263, 3200, 3137, 3023, 1600, 1493; ^{1}H NMR (300 MHz, DMSO-D6) δ 10.34 (s, 1H), 8.69 (s, 1H), 7.84 (s, 1H), 7.66 (bs, 2H), 7.29 – 6.96 (m, 2H), 6.91 – 6.47 (m, 3H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 169.60, 166.92, 162.51, 155.78, 149.94, 129.33, 119.28, 112.82, 104.34, 26.14.; HRMS (ESI-TOF) *m/z* calculated for $C_{12}H_{13}N_5O$ $[M-H]^-$ 242.1047, found 242.1040.



2.5

Benzyl 2-(4-amino-2-methylpyrimidine-5-carboxamido)-3-phenylpropanoate (2.5): The amino acid **1.2** (0.50 g, 3.27 mmol, 1 equiv), EDC (0.94 g, 4.91 mmol, 1.5 equiv), and HOBt (0.49 g, 3.60 mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, *L* Phenylalanine benzyl ester hydrochloride (0.95 g, 3.27 mmol, 1 equiv) and *N, N*-diisopropyl ethylamine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) was mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added drop by drop to the pre-activated acid and stirred for 12 hours. When the reaction was completed (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25 mL), deionized water (2 x 25mL) and saturated NaCl (2 x 25mL), and dried with anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the slight yellowish compound, which was then washed with cold diethyl ether to remove hydrophobic impurities. Flash chromatography could then be performed (95:5 DCM/methanol) to obtain purified desired compound (0.96 g, 75%) as white solid.

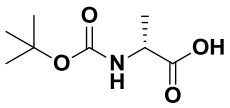
m.p 112-114 °C. IR (neat, cm^{-1}) 3360, 3270, 3177, 3329, 1737, 1644, 1578, 1540; ^{1}H NMR (300 MHz, DMSO-D6) δ 8.89 (d, *J* = 7.6 Hz, 1H), 8.51 (s, 1H), 7.60 (bs, 2H), 7.40 – 7.08 (m, 10H), 5.23 – 4.97 (m, 2H), 4.76 – 4.50 (m, 1H), 3.10 (m, 2H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 171.97, 169.53, 167.04, 162.42, 156.15, 137.97, 136.36, 129.66, 128.92, 128.84, 128.58, 128.31, 127.12, 105.00, 66.61, 54.68, 36.66, 26.09.; HRMS (ESI-TOF) *m/z* calculated for $C_{22}H_{22}N_4O_3$ $[M-H]^-$ 389.1619, found 389.1612



2.6

2-(4-amino-2-methylpyrimidine-5-carboxamido)-3-phenylpropanoic acid (2.6): Compound **2.5** (0.1 g, 0.26 mmol) was dissolved into 10 mL methanol, followed by 0.01 g of 10% Pd/C was added and the reaction mixture subjected to hydrogenation at 30 psi for 8h at room temperature. After completion of the reaction, it subjected to celite filtration. The filtrate was concentrated under reduced pressure to obtain (0.59 g, 75%) as the white compound.

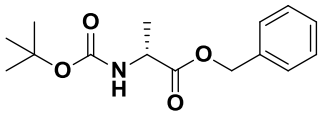
m.p 250-252 °C. IR (neat, cm^{-1}) 3417, 3307, 3202, 3026, 1633, 1535, 1436; ^{1}H NMR (300 MHz, DMSO-D6) δ 8.89 (d, *J* = 7.6 Hz, 1H), 8.51 (s, 1H), 7.60 (bs, 2H), 7.40 – 7.08 (m, 10H), 5.23 – 4.97 (m, 2H), 4.76 – 4.50 (m, 1H), 3.10 (m, 2H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 173.73, 169.28, 166.70, 162.43, 155.92, 138.84, 129.63, 128.70, 126.86, 105.45, 54.71, 36.89, 26.06.; HRMS (ESI-TOF) *m/z* calculated for $C_{15}H_{16}N_4O_3$ $[M-H]^-$ 299.1150, found 299.1179



2.7a

(*R*)-2-((tert-butoxycarbonyl) amino) propanoic acid (2.7a): To a solution of *D*-alanine (5 g, 56.12 mmol) in water (35 mL), NaOH (2.4 g, 56.12 mmol), in 20 mL water, solution was added and cooled to 0°C. BOC anhydride (12.25 g, 56.12 mmol) was diluted in

acetone (25 mL) and added drop wise to the reaction mixture at 0°C. Reaction mixture stirred at room temperature for 3 h. Acetone was removed under reduced pressure and refill with equal volume of ethyl acetate. Cool to 0°C and acidify (pH 2.5-3.0) with $KHSO_4$ when compound transfer to ethyl acetate layer. The organic layer was then washed with deionized water and saturated NaCl, and dried with anhydrous sodium sulfate and concentrated under reduced pressure to obtain white solid desired product (9.66 g, 91%) m.p 80-82 °C. IR (neat, cm^{-1}) 3385, 3236, 3056, 1610, 1510; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.09 (d, *J* = 7.5 Hz, 1H), 3.96 – 3.74 (m, 1H), 1.35 (s, 9H), 1.18 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 175.23, 155.79, 78.45, 49.33, 40.85, 40.57, 40.30, 40.02, 39.74, 39.46, 39.19, 28.74, 17.57.

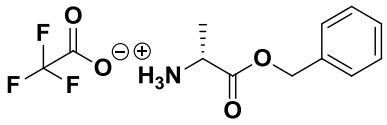


2.7b

(*R*)-benzyl 2-((tert-butoxycarbonyl)amino) propanoate (2.7b): Boc protected *D*-alanine (5.0 g, 26.43 mmol) and Cs_2CO_3 (8.61 g, 26.43 mmol) were dissolved in DMF (40 mL). Benzyl bromide (3.77ml, 31.71 mmol) was added. The mixture was stirred at the room temperature for overnight. The mixture was diluted with ethyl acetate (50-100 mL) and washed with saturated aqueous $NaHCO_3$, brine, dried with anhydrous sodium sulfate. The filtrate was concentrated to give the crude product, which was purified via column chromatography eluting with EtOAc/hexane and obtain liquid oily product (6.28 g, 85%)

IR (neat, cm^{-1}) 3367, 2978, 2934, 2882, 1708, 1498; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 – 7.01 (m, 6H), 5.12 (d, J = 12.7 Hz, 1H), 5.04 (d, *J* = 12.7 Hz, 1H), 4.19 – 3.82 (m, 1H), 1.34 (s, 9H), 1.23 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-D6) δ 173.54,

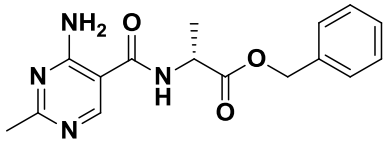
155.83, 136.60, 128.91, 128.50, 128.20, 78.69, 66.27, 49.66, 40.38, 40.21, 40.04, 39.87, 39.71, 39.54, 28.69, 17.31.



2.7c

(*R*)-1-(benzyloxy)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate (2.7c) : Dissolve compound (**2.7b**) (2.5 g, 8.95 mmol), in 10 mL anhydrous DCM. Add drop wise 3.42 mL of TFA (5 equiv), at 0 ^{o}C. The reaction was allowed to stir for 14 hours at room temperature Excess TFA was removed in vacuo multiple times with toluene, ethyl acetate and diethyl ether. The compound was obtained as viscous oil (2.44 g, 93%)

IR (neat, cm^{-1}) 3034, 2951, 1745, 1666, 1130; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (s, 3H), 7.47 – 7.25 (m, 5H), 5.19 (s, 2H), 4.14 (q, *J* = 7.2 Hz, 1H), 4.05 – 3.84 (m, 1H), 1.41 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 170.41, 135.82, 128.99, 128.81, 128.51, 67.43, 48.43, 40.38, 40.10, 39.82, 39.55, 39.27, 16.16.

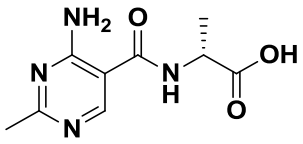


2.7

(*R*)-Benzyl 2-(4-amino-2-methylpyrimidine-5-carboxamido) propanoate (2.7): The amino acid **1.2** (0.50 g, 3.27 mmol, 1 equiv), EDC (0.94 g, 4.91 mmol, 1.5 equiv), and HOBt (0.49 g, 3.60 mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, *D* Alanine benzyl ester salt (0.96 g, 3.27 mmol, 1 equiv) and *N*, *N*-diisopropyl ethylamine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) was mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added drop by drop to the pre-activated acid and

stirred for 12 hours. When the reaction was completed (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25 mL), deionized water (2 x 25mL) and saturated NaCl (2 x 25mL), and dried with andhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the slight yellowish compound, which was then washed with cold diethyl ether to remove hydrophobic impurities. Flash chromatography could then be performed (95:5 DCM/methanol) to obtain purified desired compound (0.84 g, 82%) as white solid.

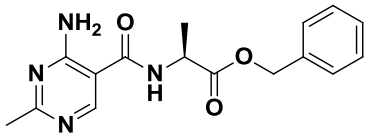
m.p 126-128 °C. IR (neat, cm^{-1}) 3326, 3159, 3044, 2936, 2797, 1746, 1668, 1552; ^1H NMR (300 MHz, DMSO-D6) δ 8.81 (d, *J* = 6.7 Hz, 1H), 8.60 (s, 1H), 7.67 (bs, 2H), 7.40 – 7.18 (m, 5H), 5.19 – 5.01 (m, 2H), 4.50 – 4.36 (m, 1H), 2.33 (s, 3H), 1.38 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 173.00, 169.48, 166.99, 162.51, 156.27, 136.57, 128.96, 128.56, 128.23, 105.01, 66.43, 48.72, 26.10, 17.04.; HRMS (ESI-TOF) *m/z* calculated for $C_{16}H_{18}N_4O_3$ $[M-H]^-$ 313.1306, found 313.1302.



2.8

(*R*)-2-(4-amino-2-methylpyrimidine-5-carboxamido)propanoic acid (2.8): Compound **2.7** (0.1 g, 0.32 mmol) was dissolved into 10 mL methanol, followed by 0.01 g of 10% Pd/C was added and the reaction mixture subjected to hydrogenolysis at 30 psi for 8h at room temperature. After completion of the reaction, it subjected to celite filtration. The filtrate was concentrated under reduced pressure to obtain (0.051 g, 71%) as the slight yellowish compound.

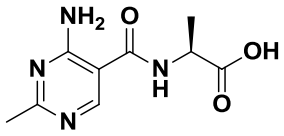
m.p 252-254 °C. IR (neat, cm^{-1}) 3382, 3236, 3062, 1620, 1410; ^{1}H NMR (300 MHz, DMSO-D6) δ 8.63 (s, 1H), 8.59 (s, 1H), 7.68 (bs, 2H), 4.45 – 4.17 (m, 1H), 2.33 (s, 3H), 1.34 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 174.77, 169.27, 166.64, 162.53, 156.08, 105.39, 48.59, 40.85, 40.58, 40.30, 40.02, 39.74, 39.47, 39.19, 26.07, 17.43.; HRMS (ESI-TOF) *m/z* calculated for $C_9H_{12}N_4O_3$ $[M-H]^-$ 223.0837, found 223.0842.



2.9

(*S*)-Benzyl 2-(4-amino-2-methylpyrimidine-5-carboxamido) propanoate (2.9): Carboxylic acid 1.2 (0.50g, 3.27mmol, 1 equiv), EDC (0.94 g, 4.91mmol, 1.5 equiv), and HOBt (0.49g, 3.60mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 mL) for 30 minutes. In a separate vial, H-Ala-Obzl.Tos (1.15 g, 3.27 mmol, 1 equiv) and *N*, *N*-diisopropyl ethylamine (DIPEA) (2.85 ml, 16.35 mmol, 5 equiv) was mixed and stirred at 0 ^{o}C for 30 minutes. The amine was then added slowly to the pre-activated acid and stirred for 12 hours. When the reaction was complete (monitored by TLC, 90:10 DCM/methanol), ethyl acetate (25-50 mL) was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl (3 x 25mL), deionized water (2 x 25mL) and saturated NaCl (2 x 25mL), and dried with andhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain the slight yellowish compound, which was then washed with cold diethyl ether to remove hydrophobic impurities. Flash chromatography could then be performed (95:5 DCM/methanol) to obtain purified desired compound (0.86 g, 84%) as white solid.

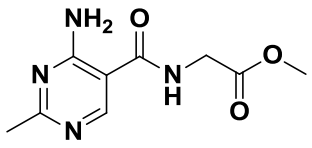
m.p 132-134 °C. IR (neat, cm^{-1}) 3330, 3152, 3043, 2933, 2792, 1750, 1658, 1550, ^{1}H NMR (300 MHz, DMSO-D6) δ 8.81 (d, *J* = 6.7 Hz, 1H), 8.60 (s, 1H), 7.67 (bs, 2H), 7.32 (s, 5H), 5.30 – 4.85 (m, 2H), 4.62 – 4.22 (m, 1H), 2.33 (s, 3H), 1.38 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 173.00, 169.48, 166.99, 162.51, 156.26, 136.57, 128.95, 128.56, 128.23, 105.01, 66.43, 48.73, 26.10, 17.04.; HRMS (ESI-TOF) *m/z* calculated for $C_{16}H_{18}N_4O_3$ $[M-H]^-$ 313.1306, found 313.1302.



2.10

(*S*)-2-(4-amino-2-methylpyrimidine-5-carboxamido) propanoic acid (2.10): Compound **2.9** (0.1 g, 0.32 mmol) was dissolved into 10 mL methanol, followed by 0.01 g of 10% Pd/C was added and the reaction mixture subjected to hydrogenolysis at 30 psi for 8h at room temperature. After completion of the reaction, it subjected to celite filtration. The filtrate was concentrated under reduced pressure to obtain (0.052 g, 73%) as the slight yellowish compound.

m.p 256-258 °C. IR (neat, cm^{-1}) 3386, 3233, 3062, 1613, 1417; ^{1}H NMR (500 MHz, DMSO-D6) δ 8.60 (d, *J* = 7.2 Hz, 1H), 8.58 (s, 1H), 7.67 (bs, 2H), 4.46 – 4.14 (m, 1H), 2.32 (s, 3H), 1.33 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-D6) δ 174.77, 169.25, 166.60, 162.52, 156.04, 105.44, 48.63, 40.53, 40.36, 40.29, 40.20, 40.03, 39.86, 39.69, 39.53.; HRMS (ESI-TOF) *m/z* calculated for $C_9H_{12}N_4O_3$ $[M-H]^-$ 223.0837, found 223.0834.



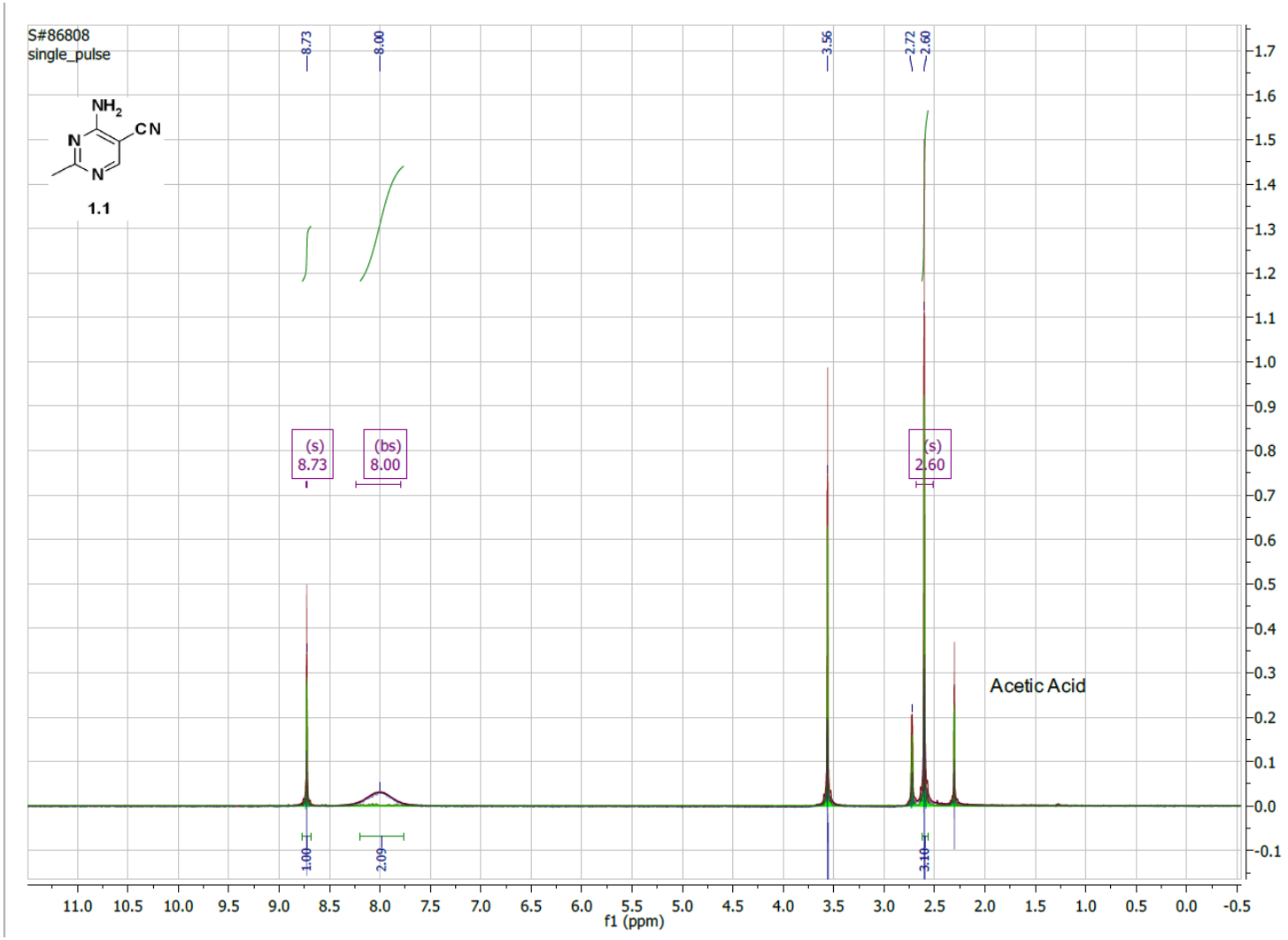
2.11

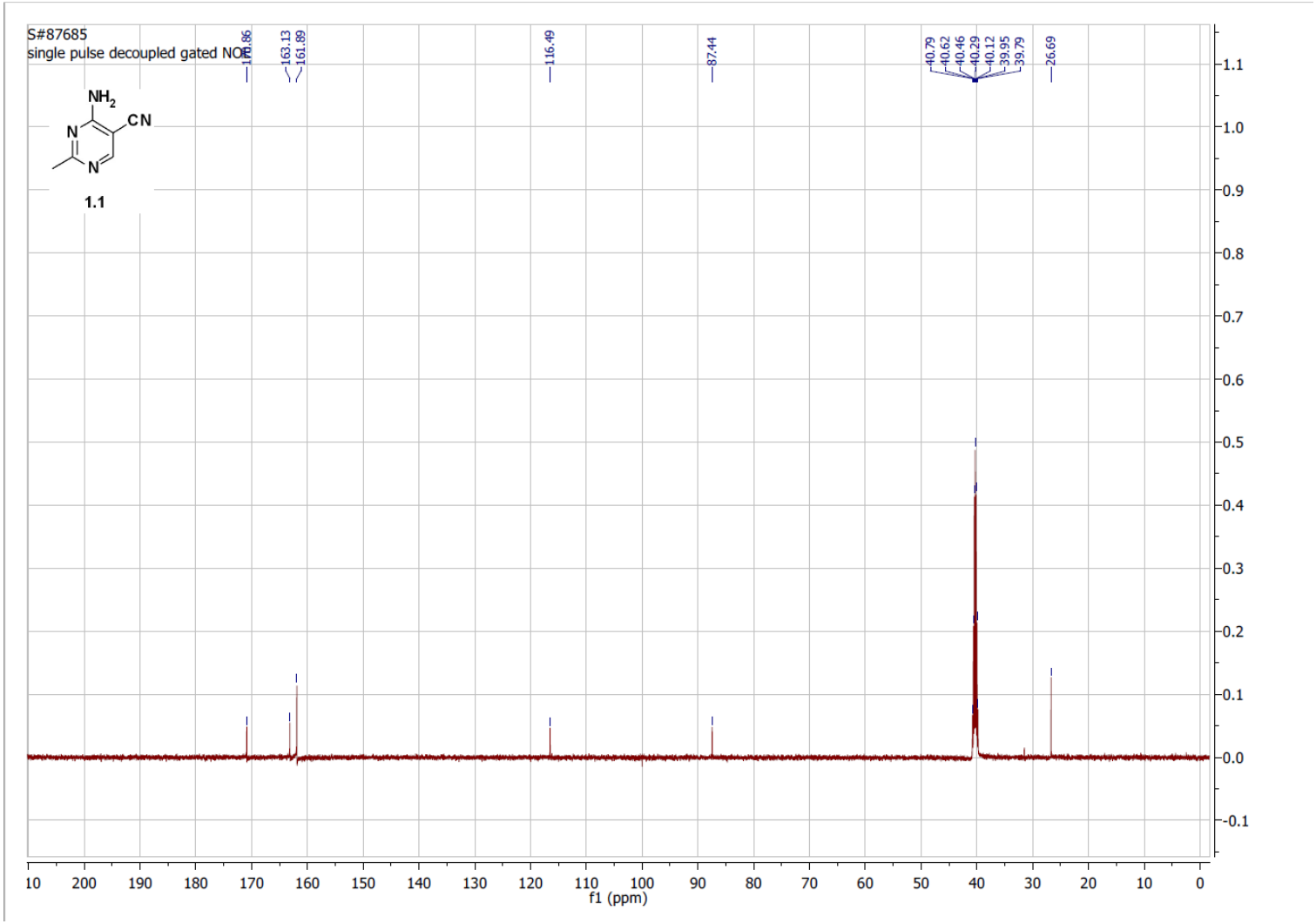
Methyl 2-(4-amino-2-methylpyrimidine-5-carboxamido) acetate (2.11): Carboxylic acid 1.2 (0.50g, 3.27mmol, 1 equiv), EDC (0.94 g, 4.91mmol, 1.5 equiv), and HOBt (0.49g, 3.60mmol, 1.1 equiv) were stirred at 0 ^{o}C in DMF (20 ml) for 30 minutes. A mixture of Glycine methyl ester hydrochloride (0.41 g, 3.27mmol, 1 equiv) and *N, N*-diisopropyl ethylamine (DIPEA) (2.85ml, 16.35mmol, 5 equiv) was then added slowly to the pre-activated acid and stirred for 12 hours. After completion of the reaction, ethyl acetate was added to the reaction mixture. The organic layer was then washed with saturated NH_4Cl, deionized water and saturated NaCl, and dried with anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to obtain white compound (0.56 g, 77%).

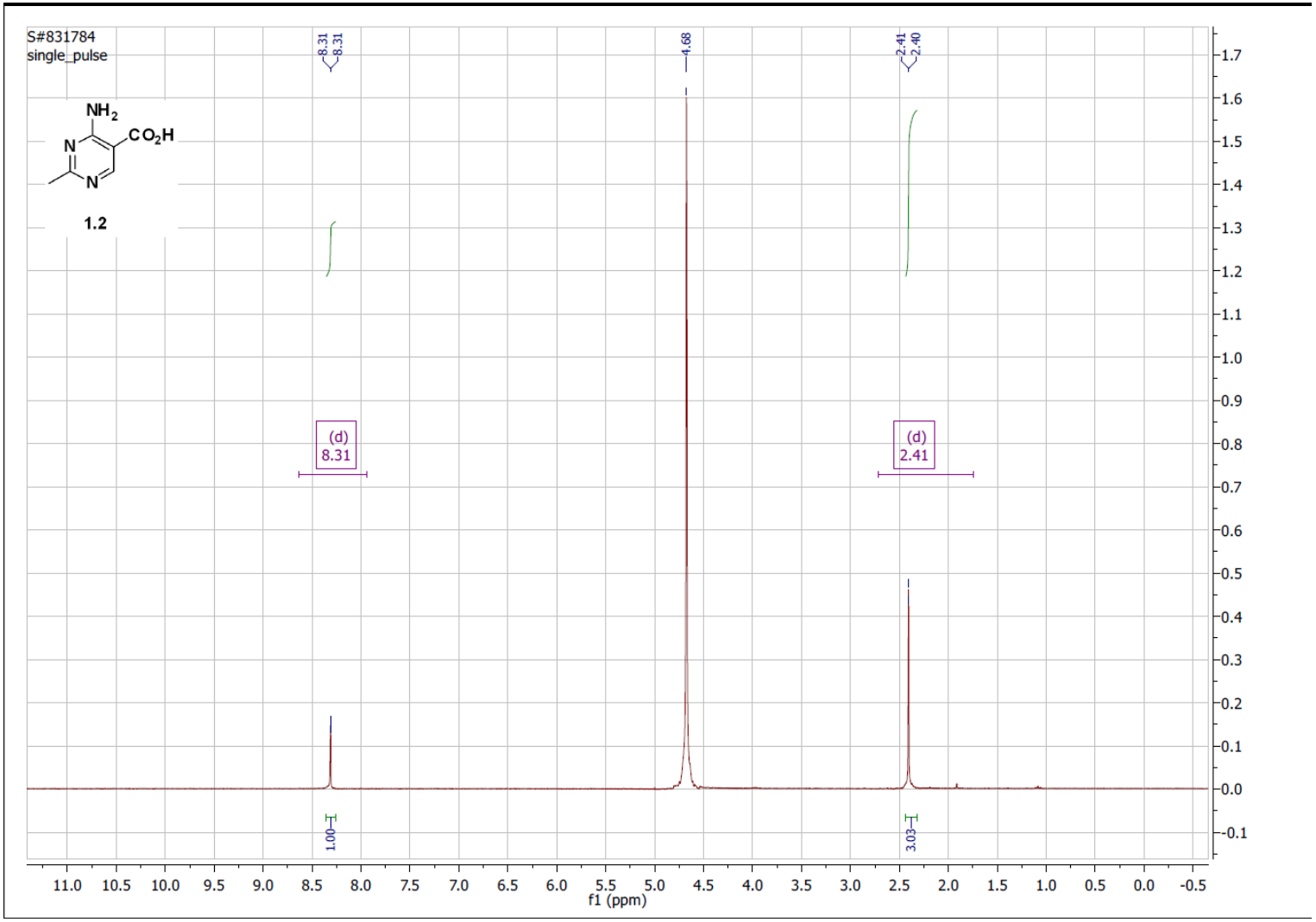
m.p 226-228 °C. IR (neat, cm^{-1}) 3378, 3256, 3098, 2949, 2856, 1654, 1721, 1645, 1519; ^{1}H NMR (300 MHz, DMSO-D6) δ 8.98 (t, *J* = 5.5 Hz, 1H), 8.57 (s, 1H), 7.72 (bs, 2H), 3.95 (d, *J* = 5.7 Hz, 2H), 3.63 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (75 MHz, DMSO-D6) δ 170.83, 169.54, 167.30, 162.53, 156.05, 104.95, 52.35, 41.37, 26.09.; HRMS (ESI-TOF) *m/z* calculated for $C_9H_{12}N_4O_3$ $[M-H]^-$ 223.0837, found 223.0830.

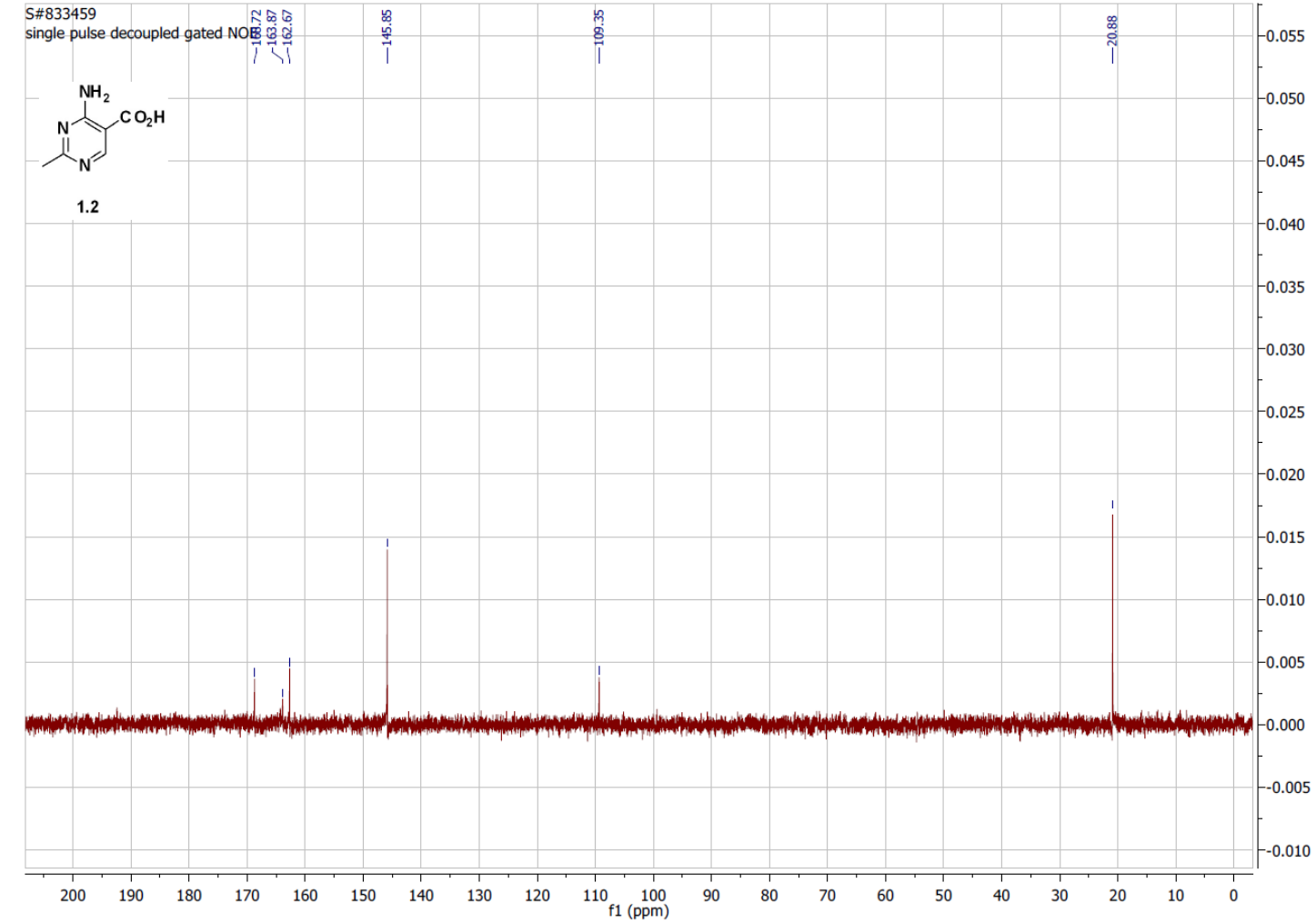
Appendix A

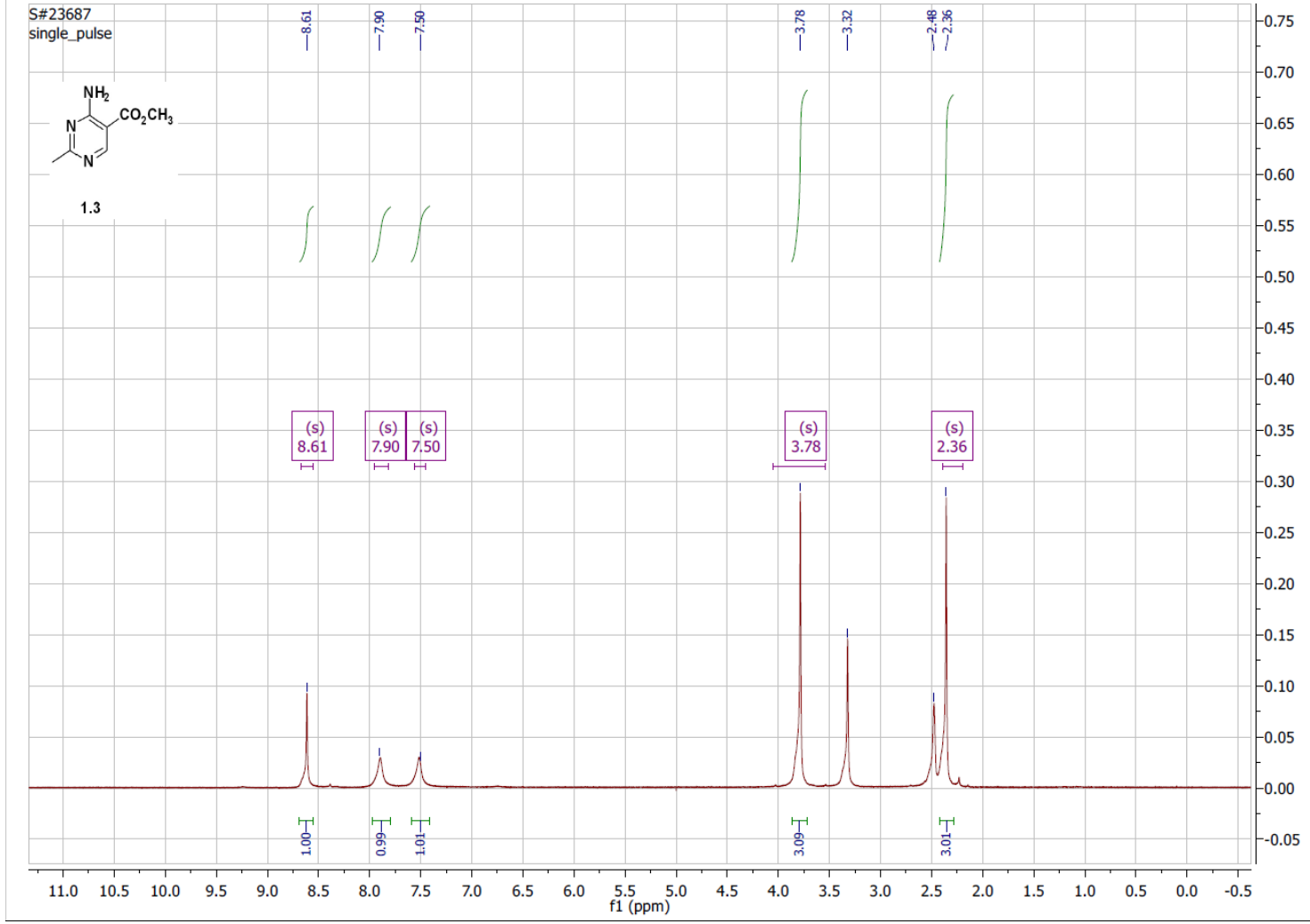
NMR spectrum

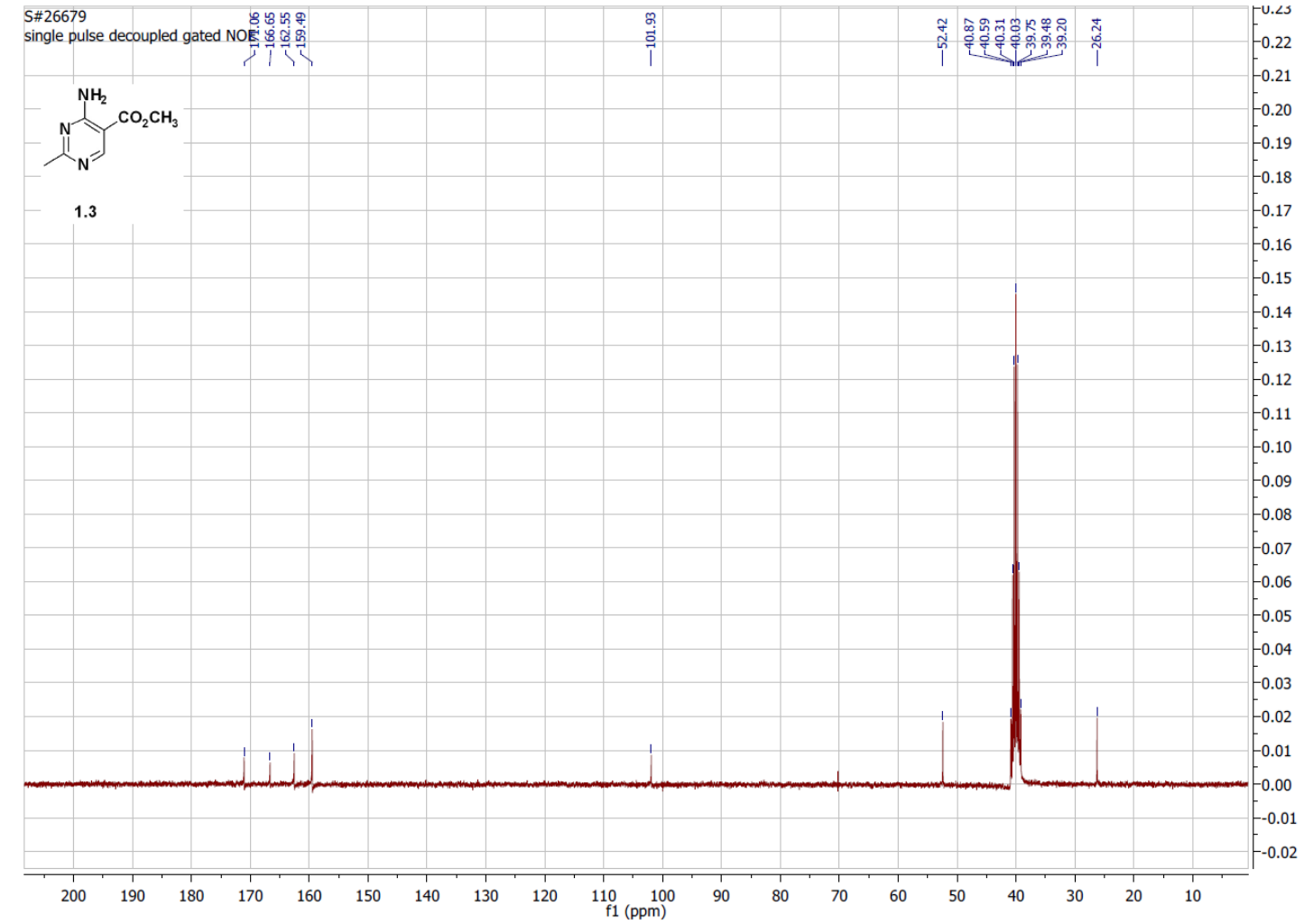

S#86808
single_pulse
NH2
CN
N
N
1.1
8.73
8.00
3.56
2.72
2.60
(s)
8.73
(bs)
8.00
(s)
2.60
Acetic Acid
1.00
2.09
3.10
f1 (ppm)

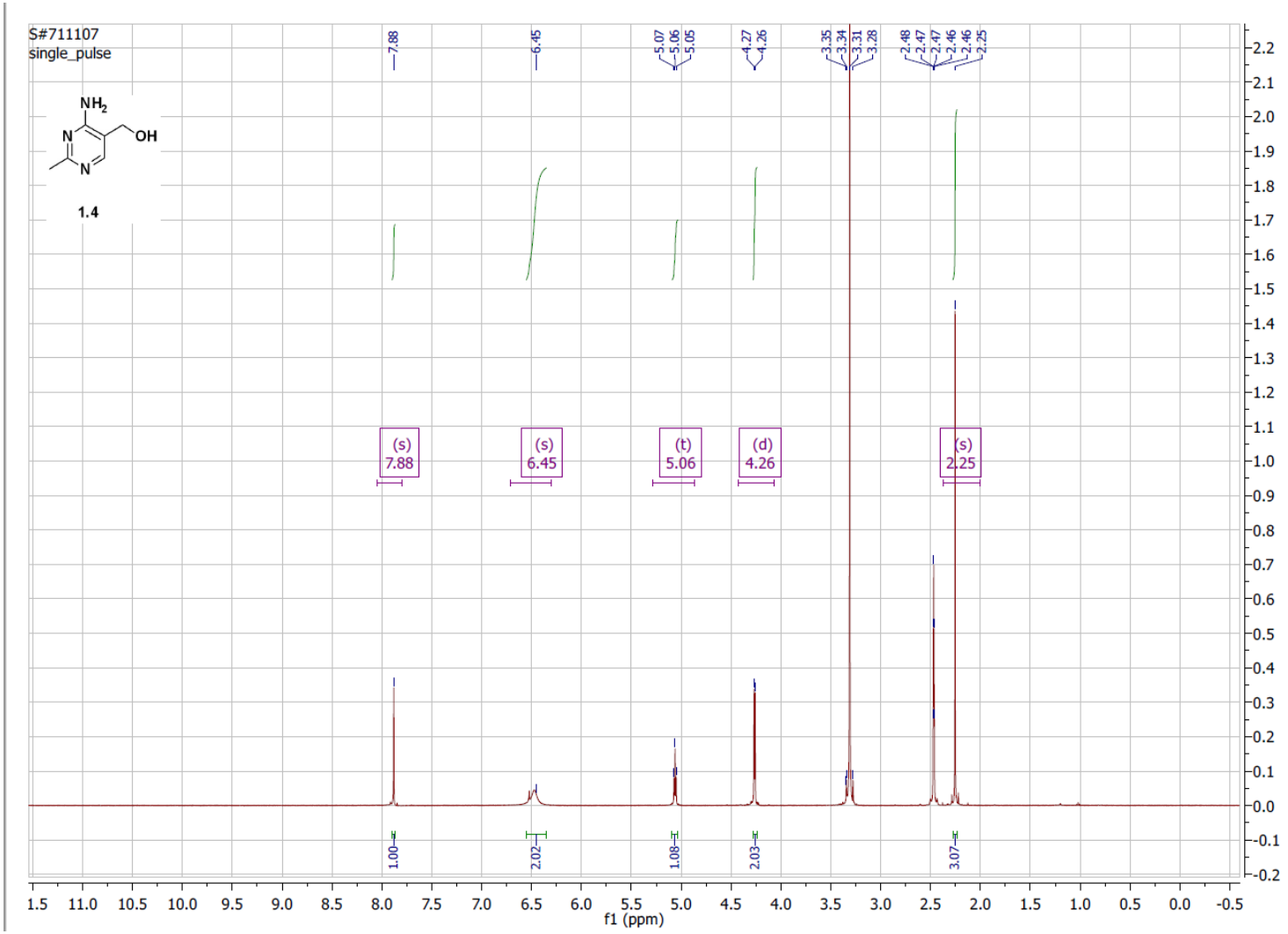

S#87685
single pulse decoupled gated NOE
NH2
CN
N
N
1.1
169.86
163.13
161.89
116.49
87.44
40.79
40.62
40.46
40.29
40.12
39.95
39.79
26.69
f1 (ppm)


S#831784
single_pulse
NH2
CO2H
N
N
1.2
8.31
8.31
4.68
2.41
2.40
(d)
8.31
(d)
2.41
1.00
3.03
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0 -0.1

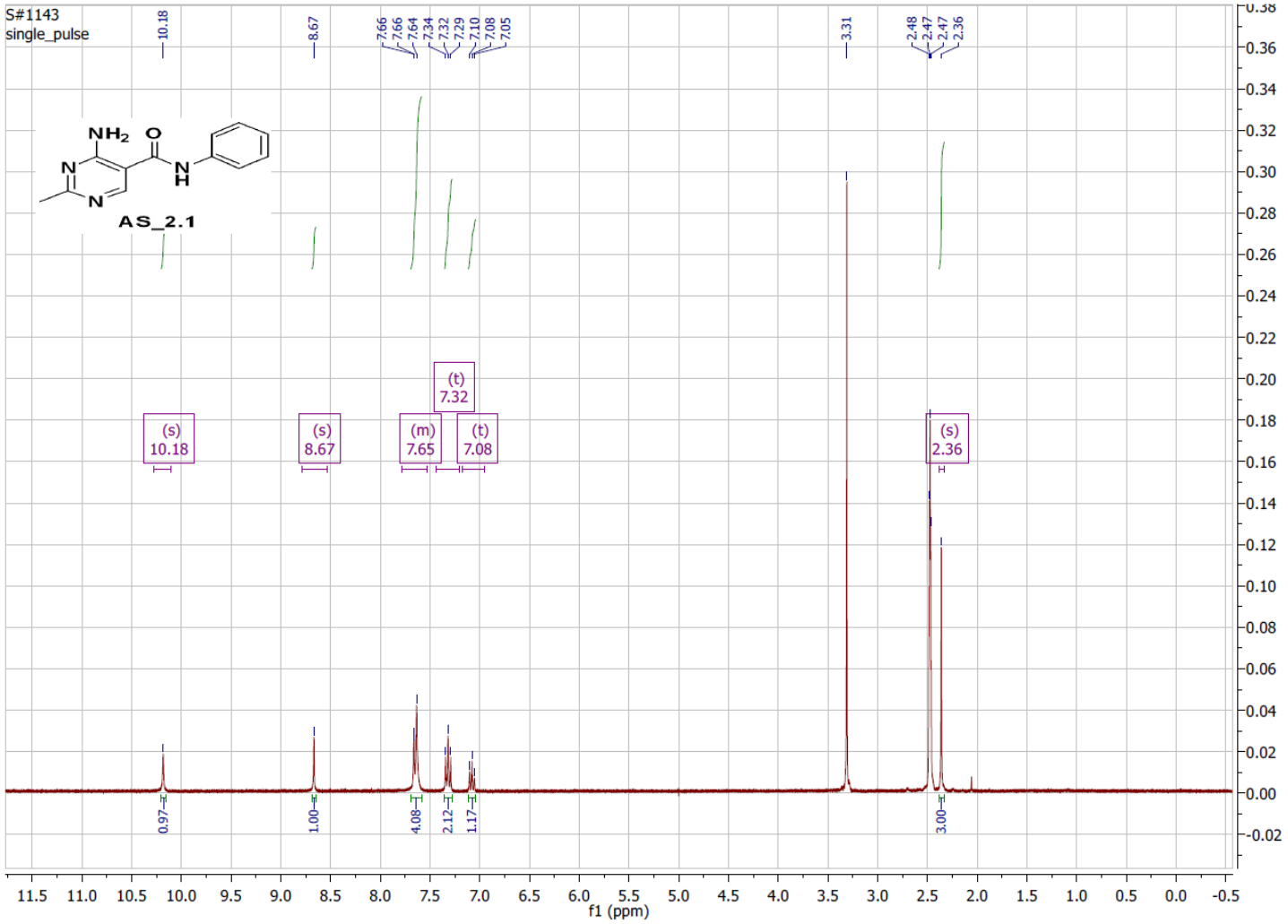

S#833459
single pulse decoupled gated NOE
168.72
163.87
162.67
145.85
109.35
20.88
NH2
CO2H
N
N
1.2
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

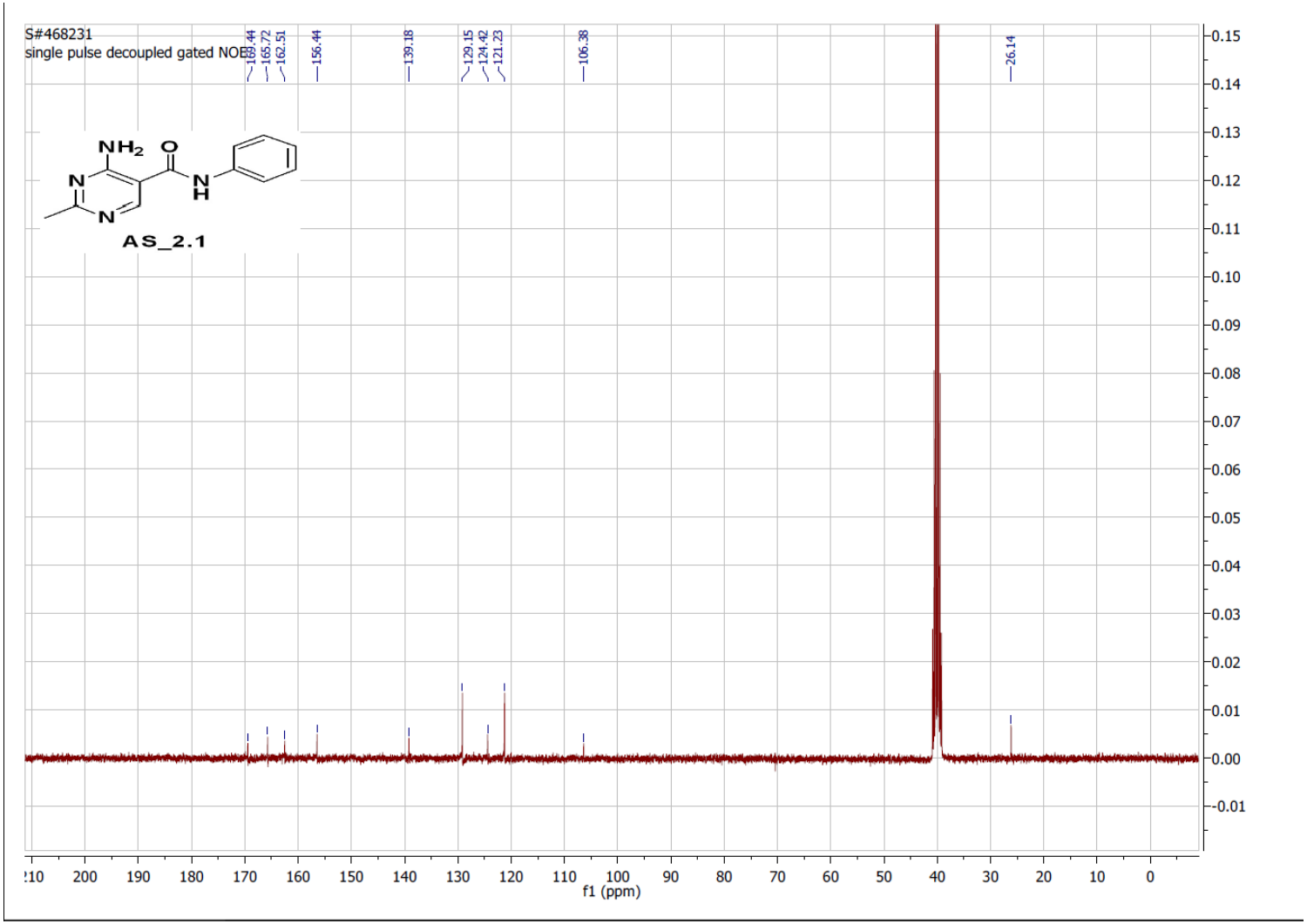

S#23687
single_pulse
8.61
7.90
7.50
3.78
3.32
2.48
2.36
NH2
CO2CH3
N
N
1.3
(s)
8.61
(s)
7.90
(s)
7.50
(s)
3.78
(s)
2.36
1.00
0.99
1.01
3.09
3.01
f1 (ppm)

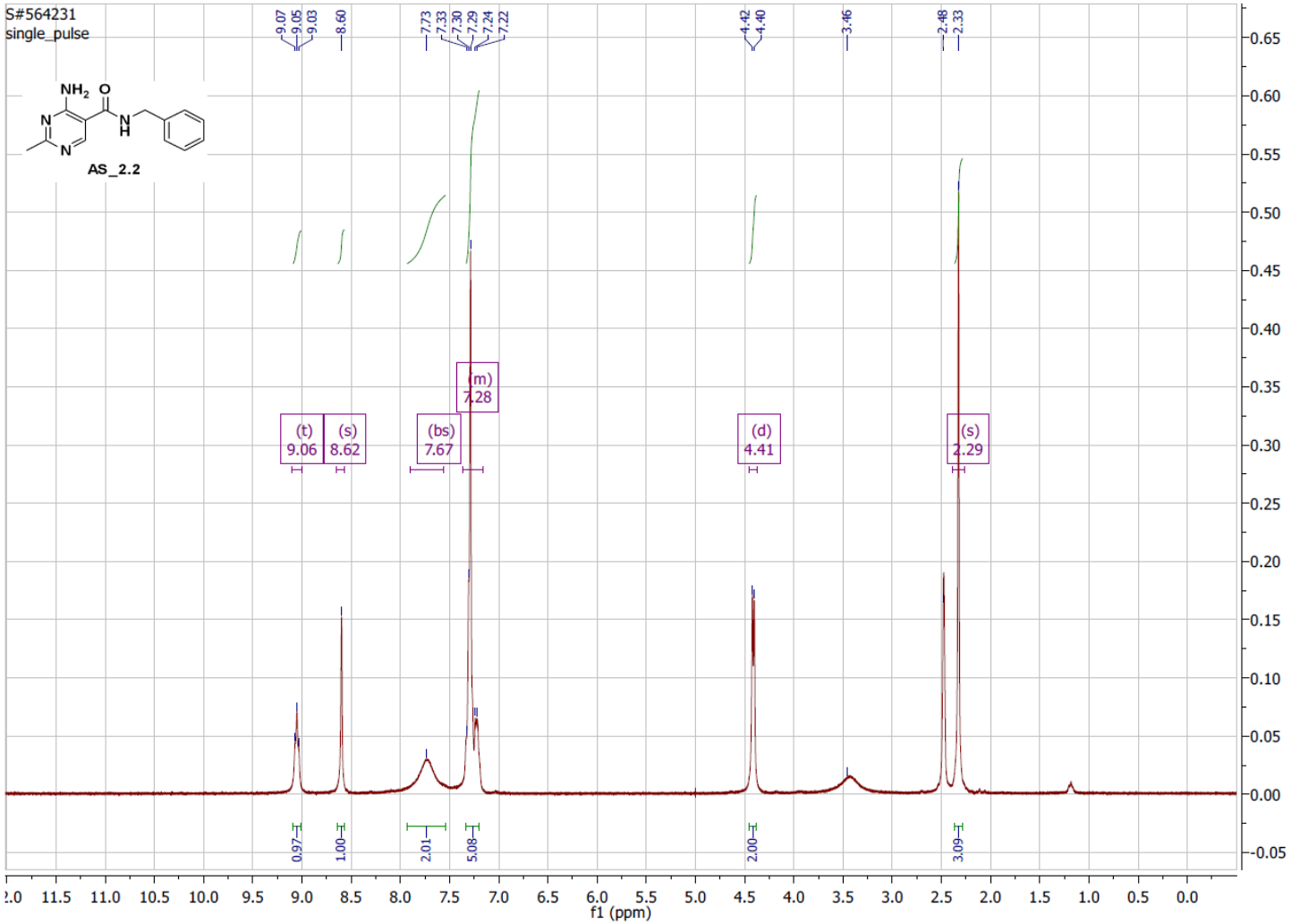

S#26679
single pulse decoupled gated NOE
171.06
166.65
162.55
159.49
101.93
52.42
40.87
40.59
40.31
40.03
39.75
39.48
39.20
26.24
NH2
CO2CH3
N
N
1.3
f1 (ppm)

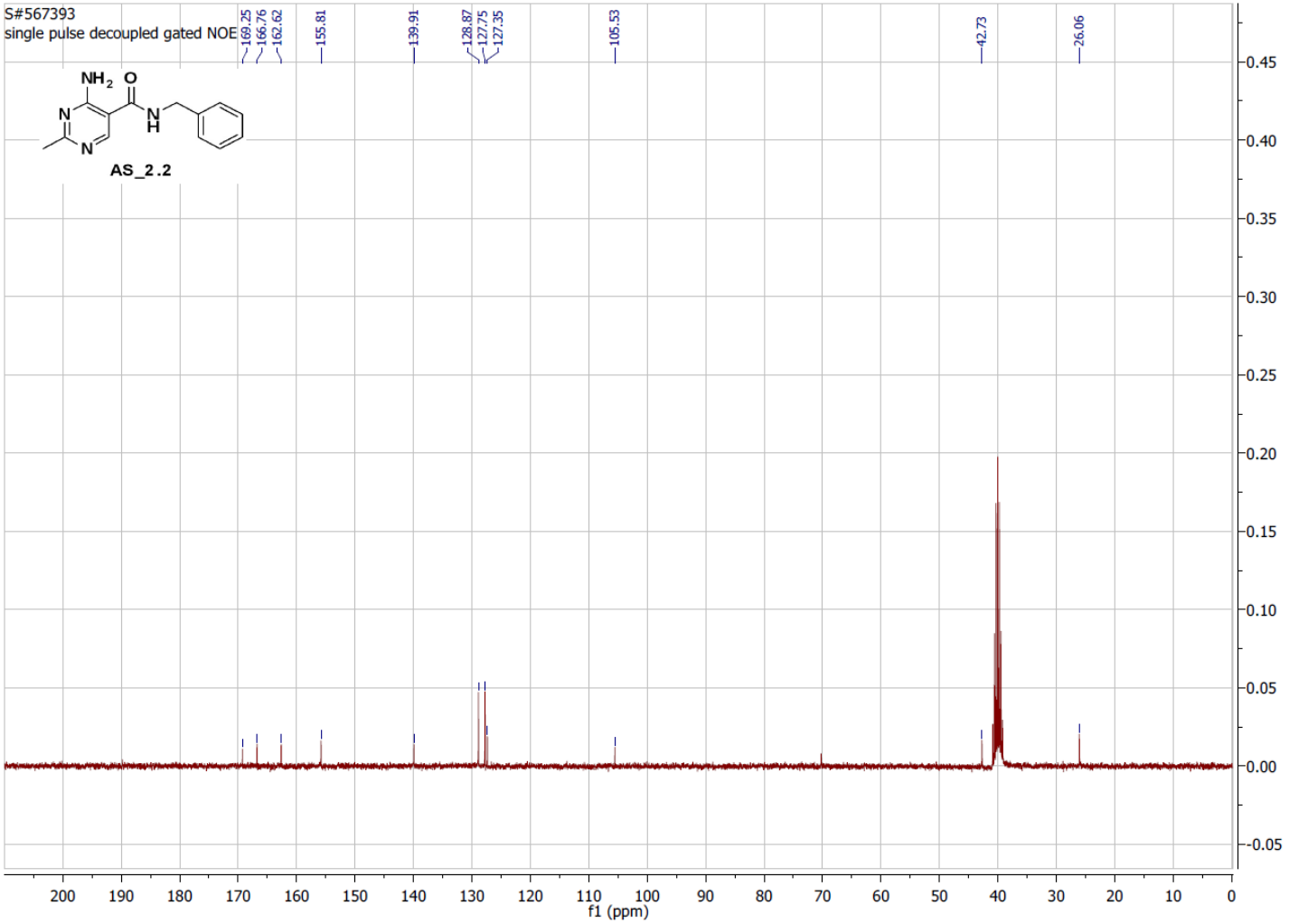

S#711107
single_pulse
NH2
N
N
OH
1.4
7.88
6.45
5.07
5.06
5.05
4.27
4.26
3.35
3.34
3.31
3.28
2.48
2.47
2.47
2.46
2.46
2.25
(s)
7.88
(s)
6.45
(t)
5.06
(d)
4.26
(s)
2.25
1.00
2.02
1.08
2.03
3.07
f1 (ppm)

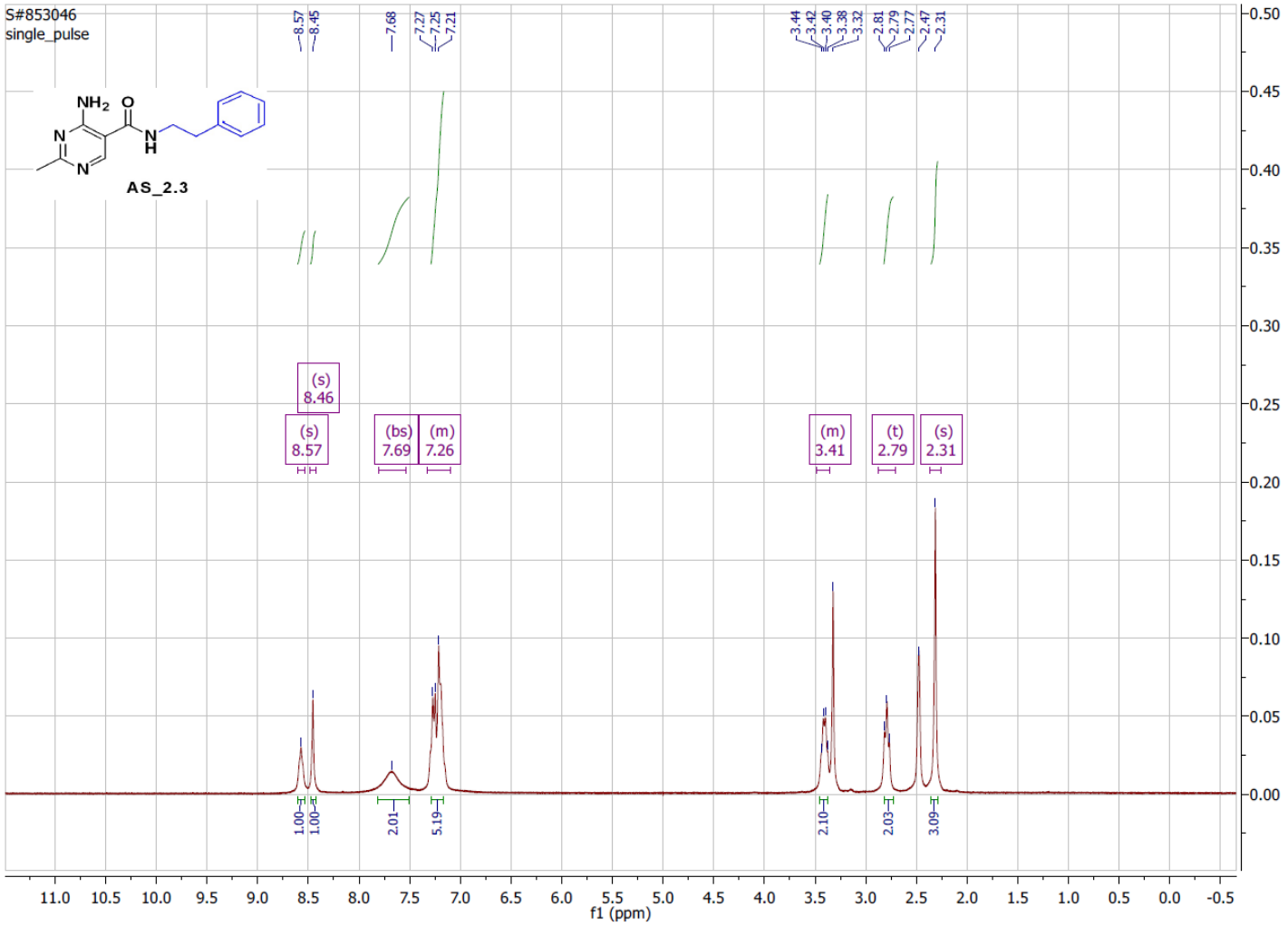

S#712008
single pulse decoupled gated NOE
1.4
NH2
OH
N
N
165.87
161.98
153.39
114.23
58.26
40.54
40.37
40.21
40.04
39.87
39.71
39.54
25.72
f1 (ppm)

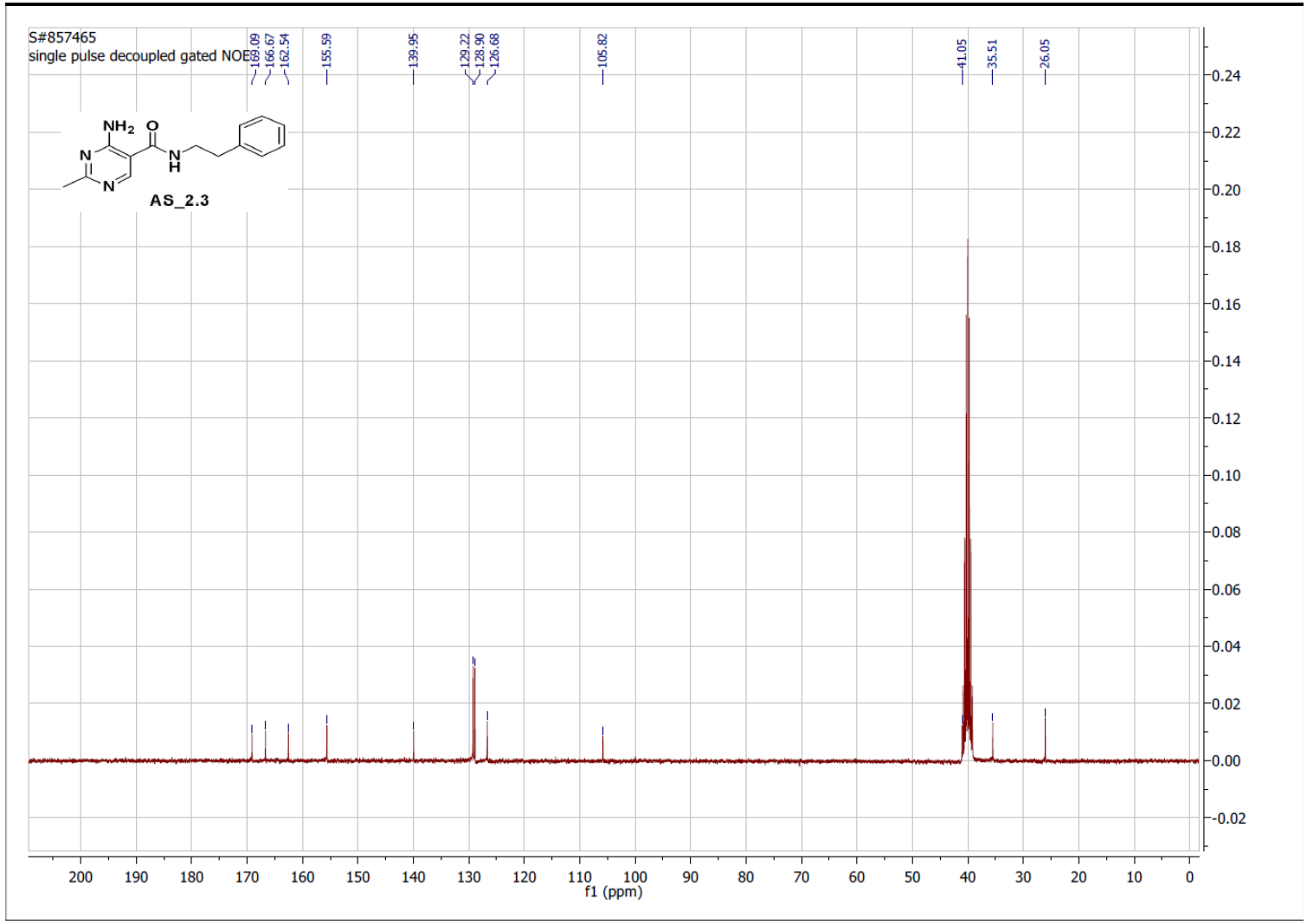

S#1143
single_pulse
10.18
8.67
7.66
7.66
7.64
7.34
7.32
7.29
7.10
7.08
7.05
3.31
2.48
2.47
2.47
2.36
NH2
O
N
N
N
H
AS_2.1
(s)
10.18
(s)
8.67
(m)
7.65
(t)
7.32
(t)
7.08
(s)
2.36
0.97
1.00
4.08
2.12
1.17
3.00
f1 (ppm)

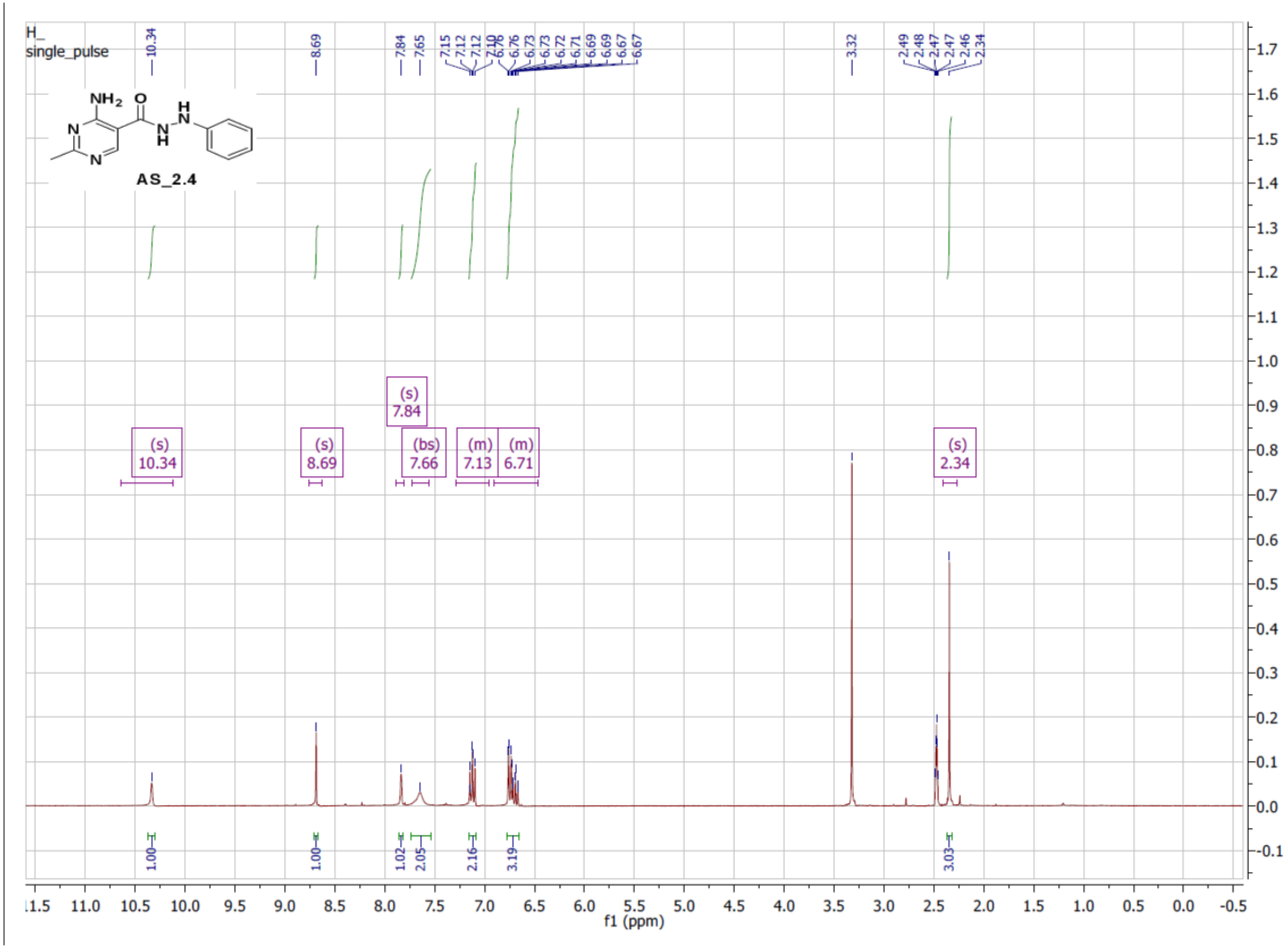

S#468231
single pulse decoupled gated NOE
169.44
165.72
162.51
156.44
139.18
129.15
124.42
121.23
106.38
26.14
AS_2.1
f1 (ppm)

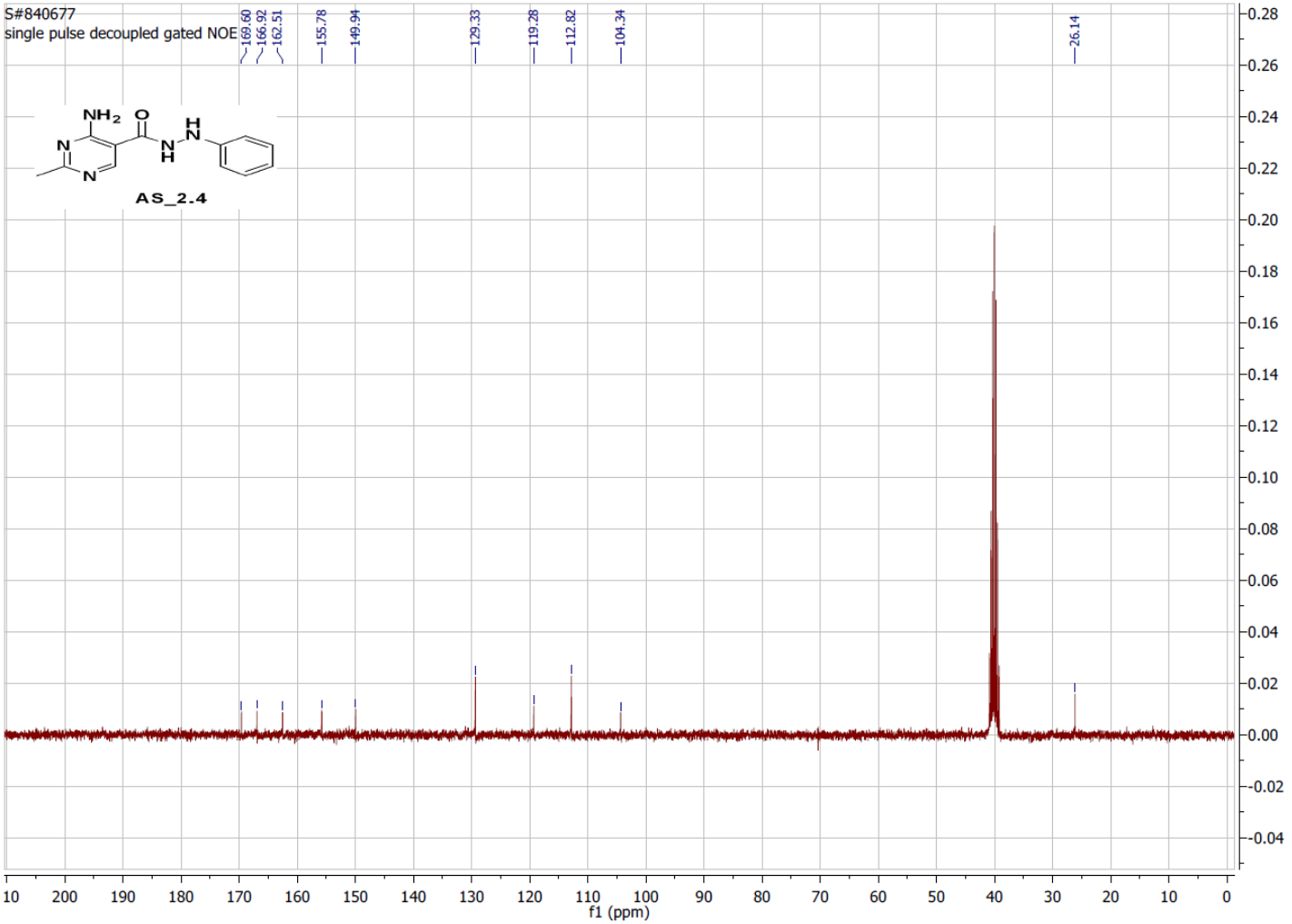

S#564231
single_pulse
AS_2.2
9.07
9.05
9.03
8.60
7.73
7.33
7.30
7.29
7.24
7.22
4.42
4.40
3.46
2.48
2.33
(t)
9.06
(s)
8.62
(bs)
7.67
(m)
7.28
(d)
4.41
(s)
2.29
0.97
1.00
2.01
5.08
2.00
3.09
f1 (ppm)

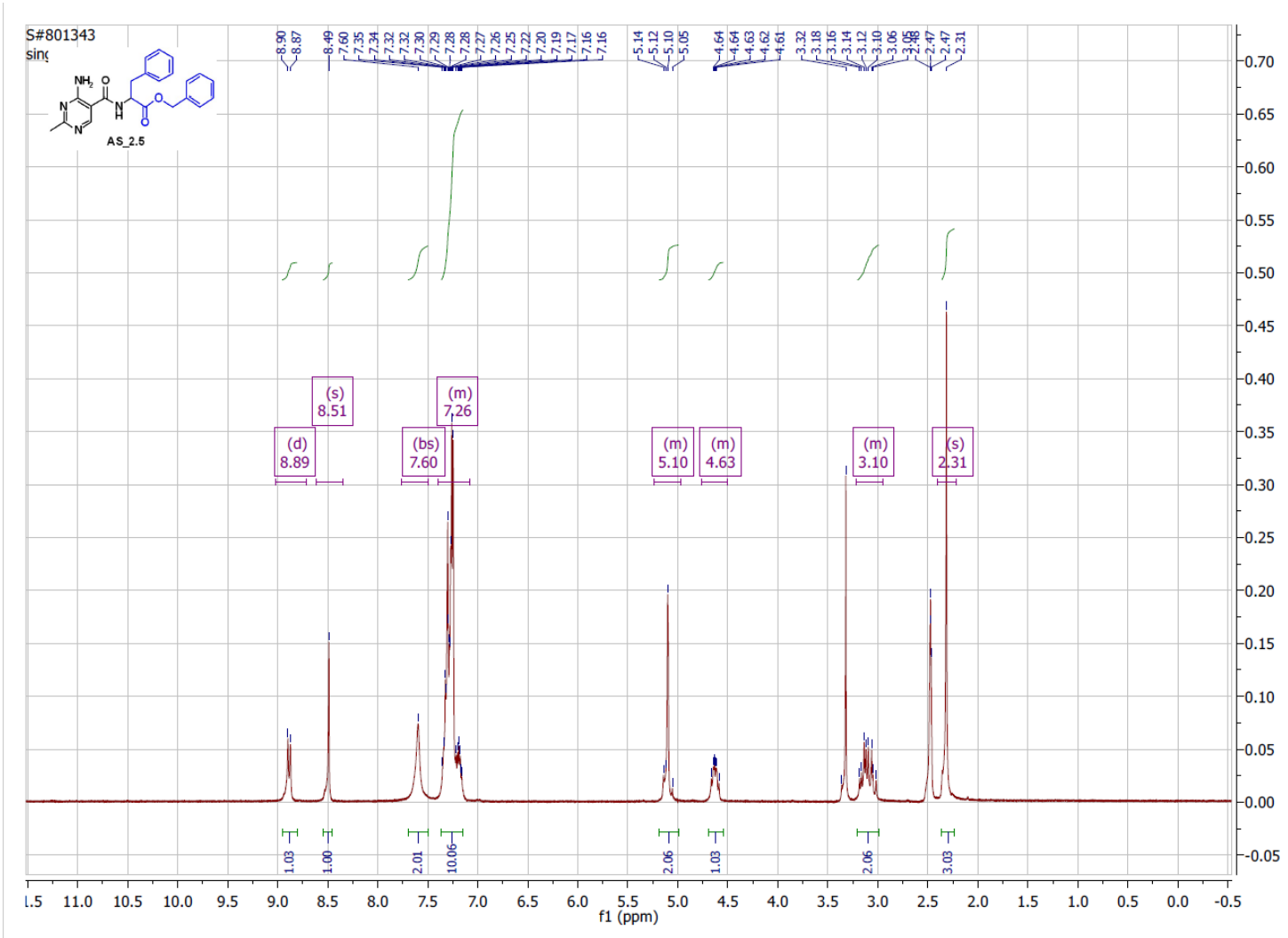

S#567393
single pulse decoupled gated NOE
169.25
166.76
162.62
155.81
139.91
128.87
127.75
127.35
105.53
42.73
26.06
AS_2.2
f1 (ppm)

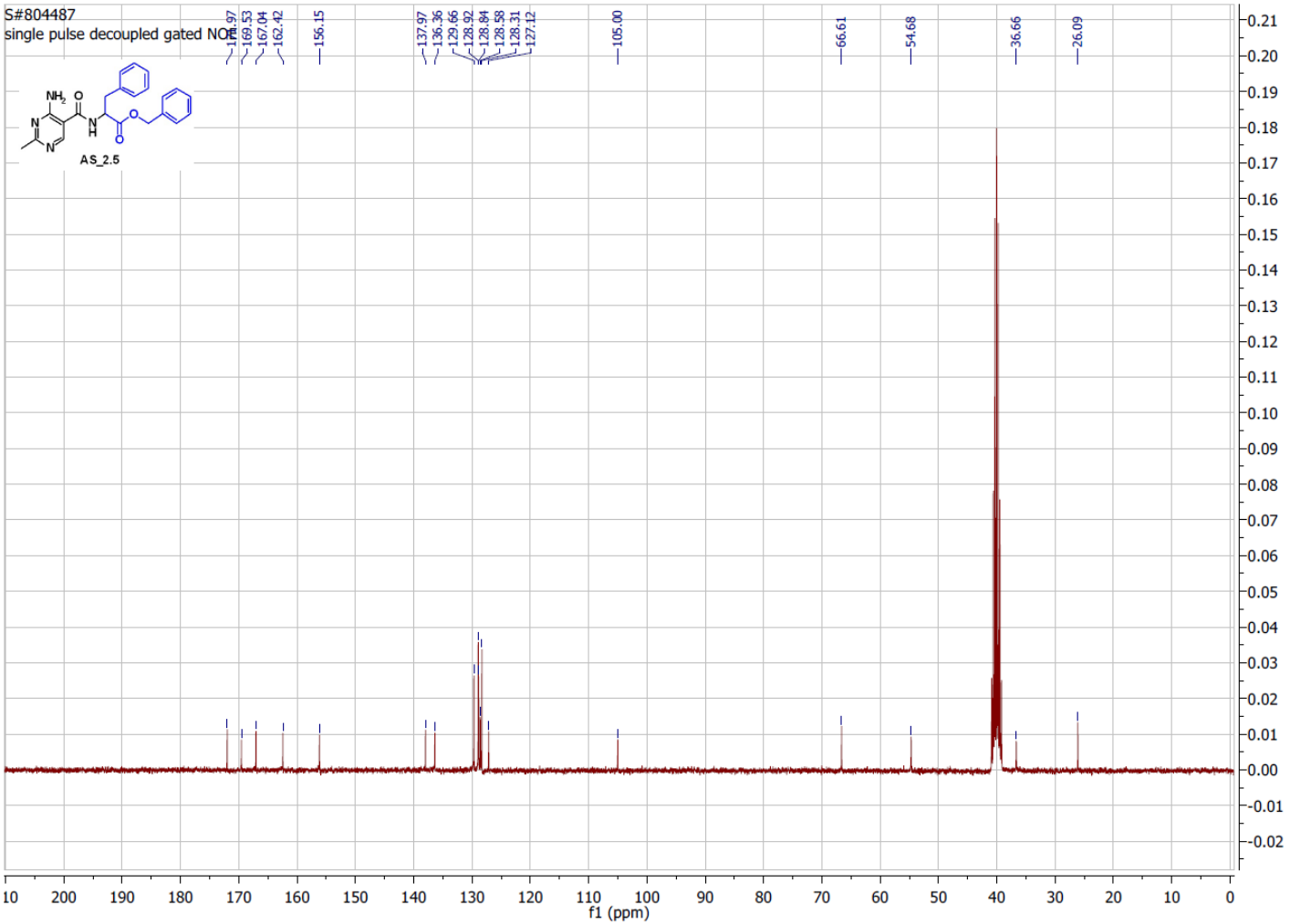

S#853046
single_pulse
AS_2.3
8.57
8.45
7.68
7.27
7.25
7.21
3.44
3.42
3.40
3.38
3.32
2.81
2.79
2.77
2.47
2.31
(s) 8.46
(s) 8.57
(bs) 7.69
(m) 7.26
(m) 3.41
(t) 2.79
(s) 2.31
1.00
1.00
2.01
5.19
2.10
2.03
3.09
f1 (ppm)

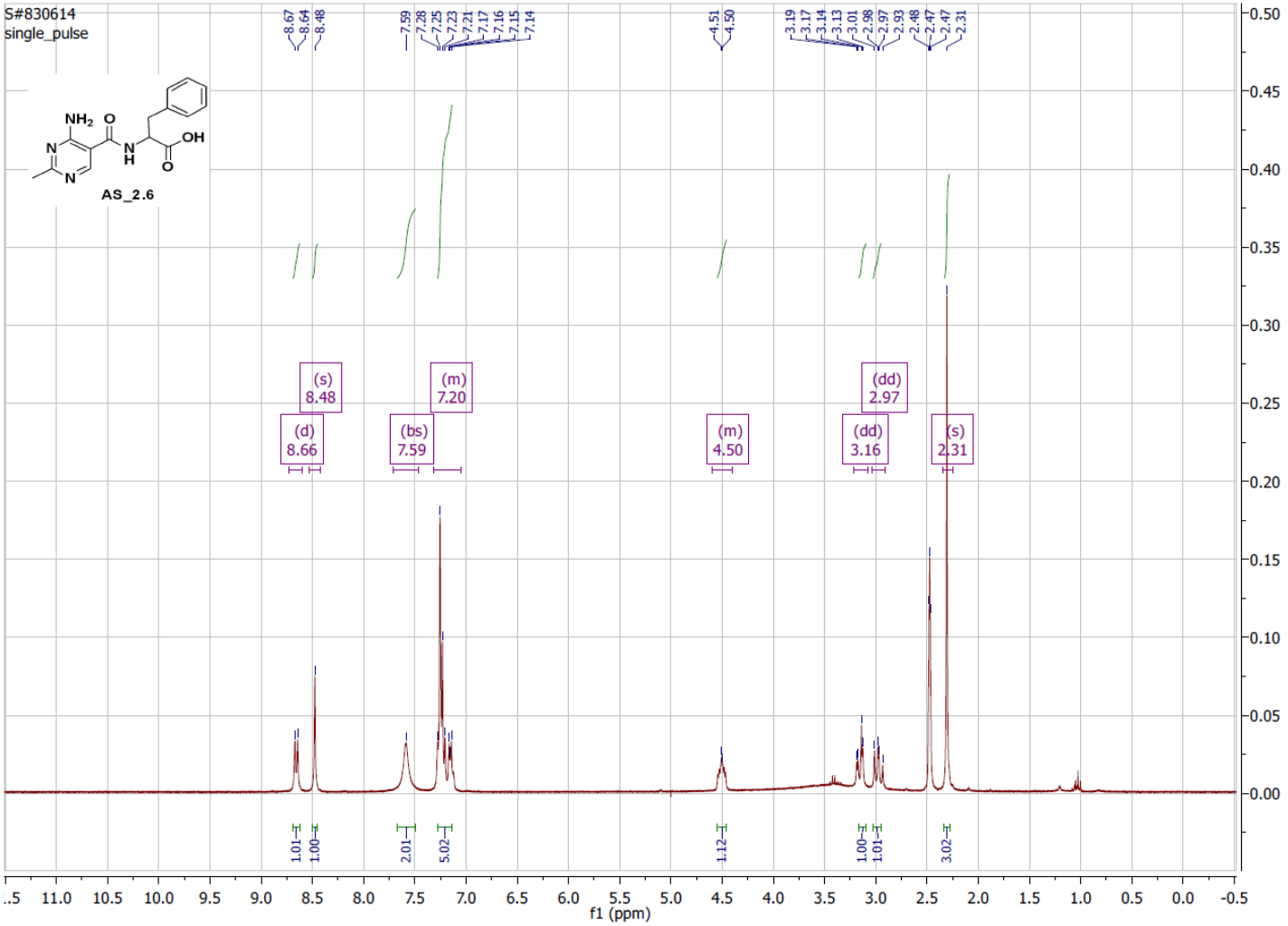

S#857465
single pulse decoupled gated NOE
169.09
166.67
162.54
155.59
139.95
129.22
128.90
126.68
105.82
41.05
35.51
26.05
AS_2.3
f1 (ppm)

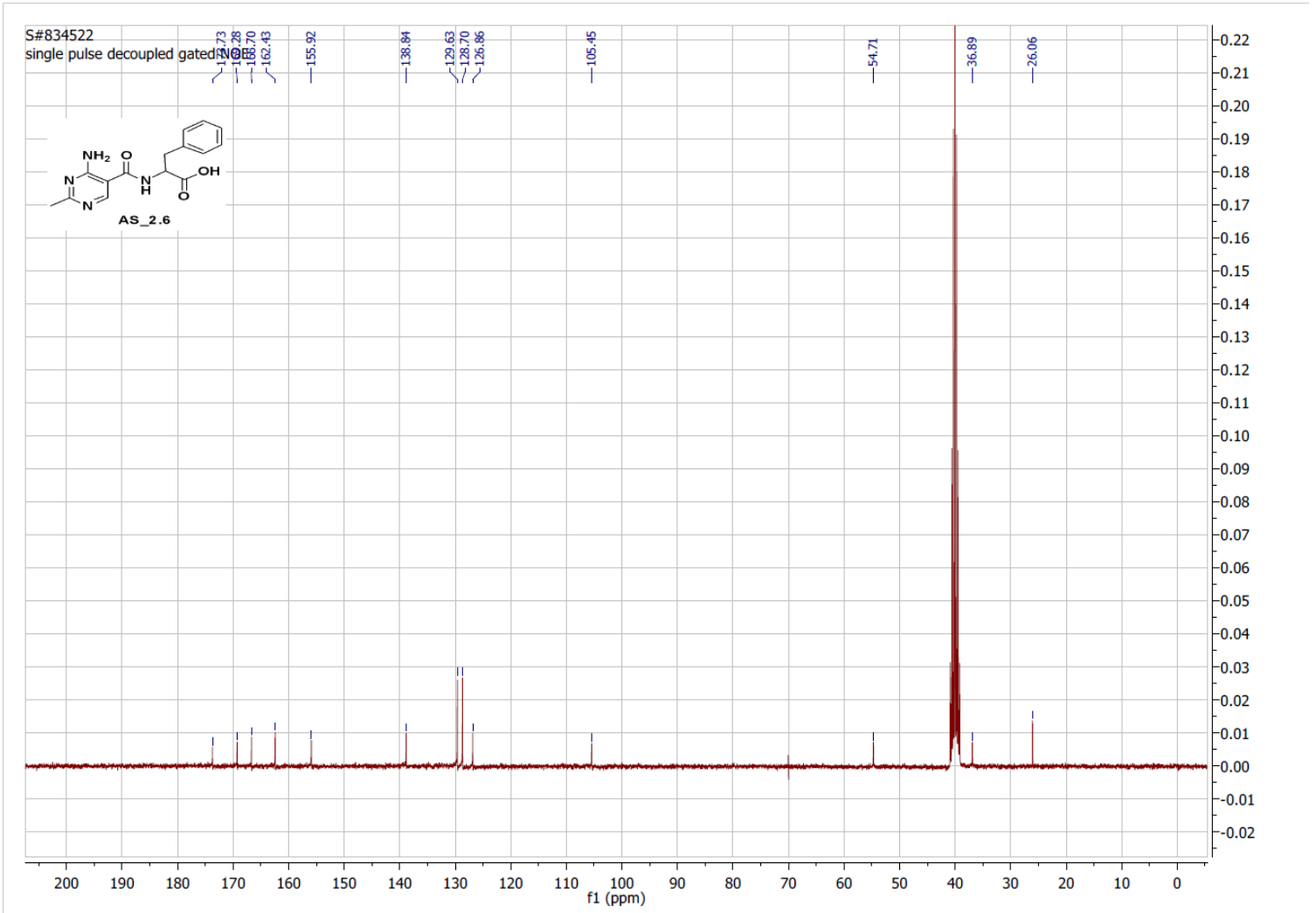

H_
single_pulse
AS_2.4
(s) 10.34
(s) 8.69
(s) 7.84
(bs) 7.66
(m) 7.13
(m) 6.71
(s) 2.34
f1 (ppm)


S#840677
single pulse decoupled gated NOE
169.60
166.92
162.51
155.78
149.94
129.33
119.28
112.82
104.34
26.14
AS_2.4
f1 (ppm)

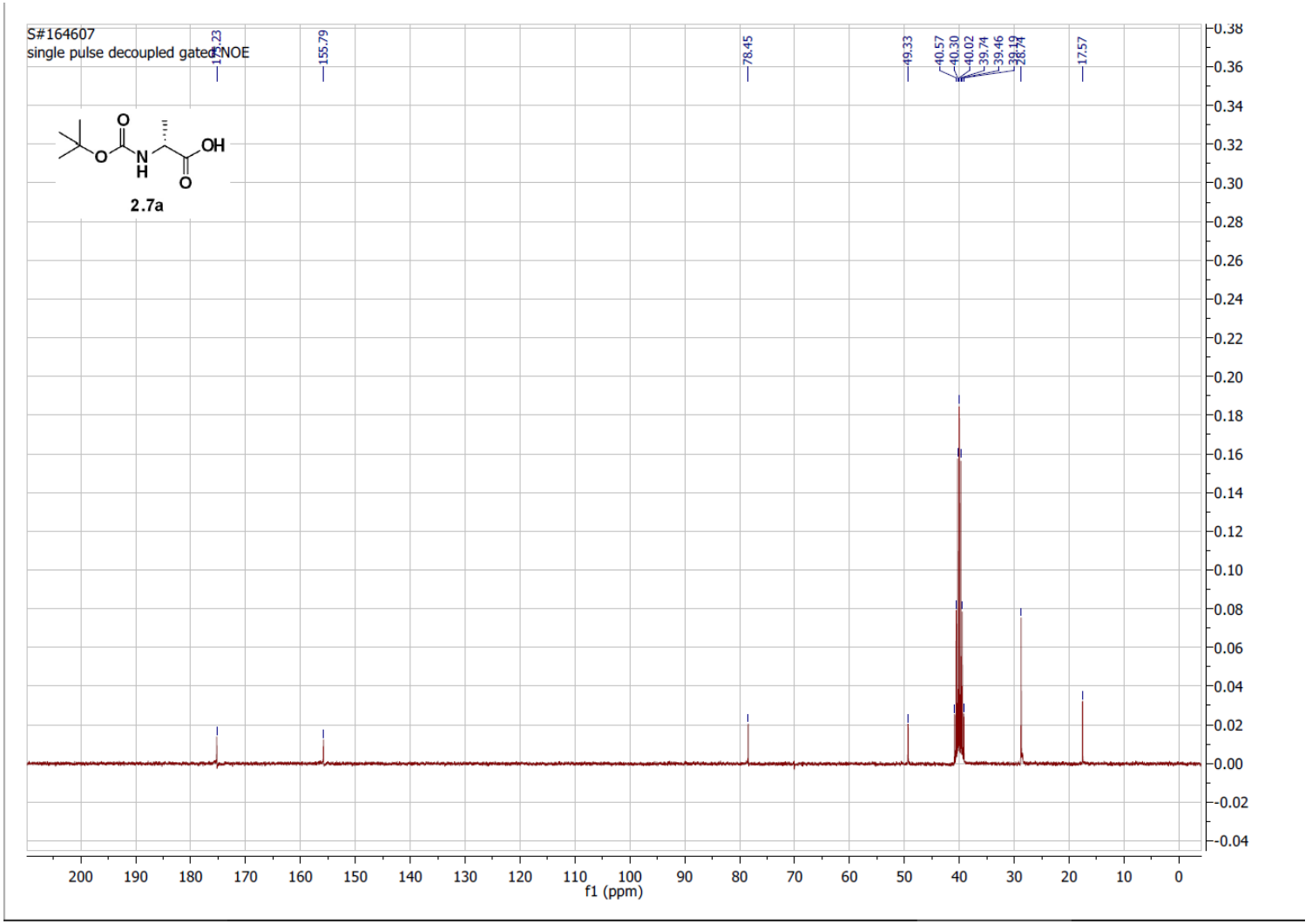

S#801343
AS_2.5
(d) 8.89
(s) 8.51
(bs) 7.60
(m) 7.26
(m) 5.10
(m) 4.63
(m) 3.10
(s) 2.31
1.03
1.00
2.01
10.06
2.06
1.03
2.06
3.03
f1 (ppm)

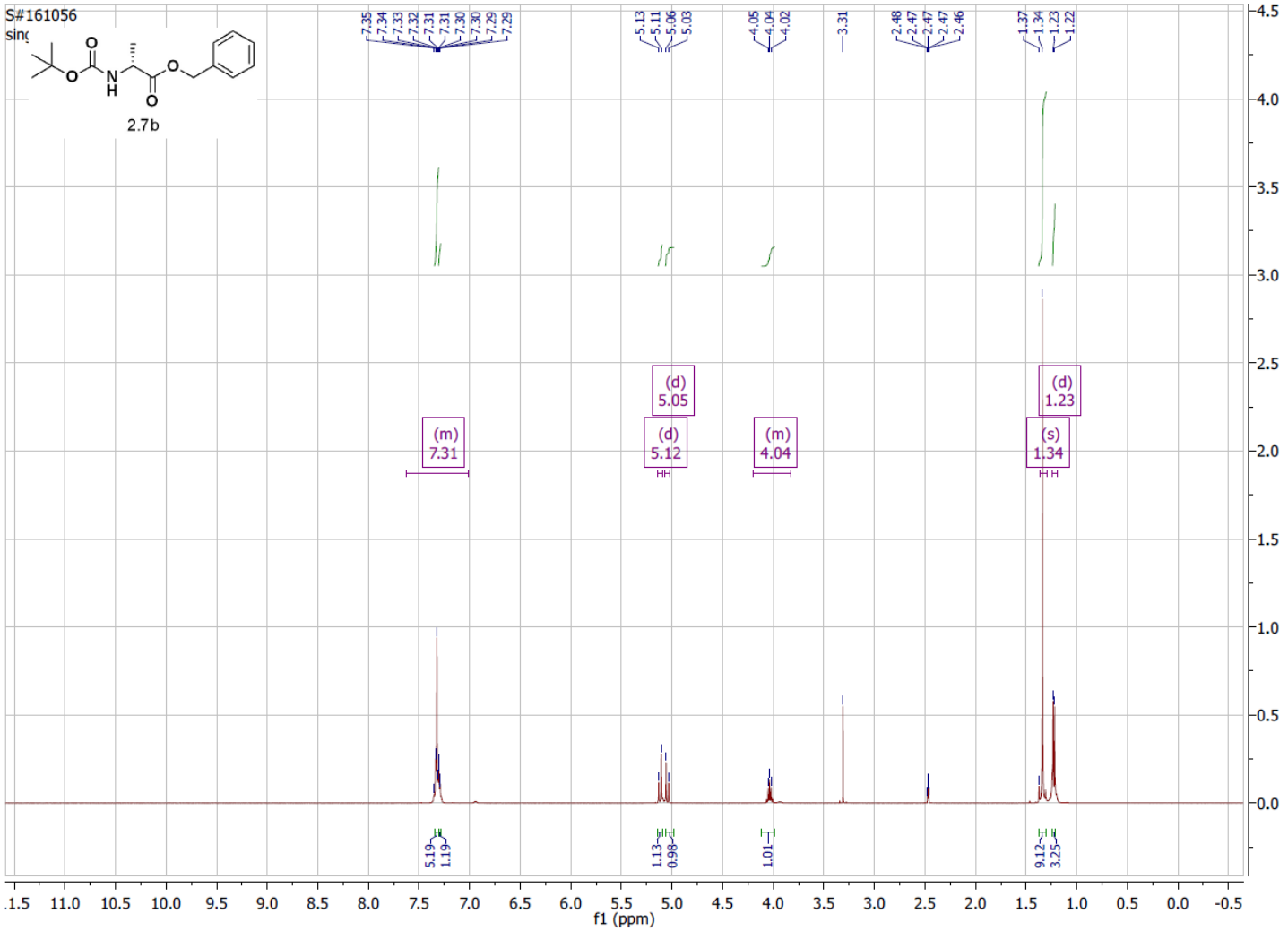

S#804487
single pulse decoupled gated NOE
AS_2.5
168.97
169.53
167.04
162.42
156.15
137.97
136.36
129.66
128.92
128.84
128.58
128.31
127.12
105.00
66.61
54.68
36.66
26.09
f1 (ppm)

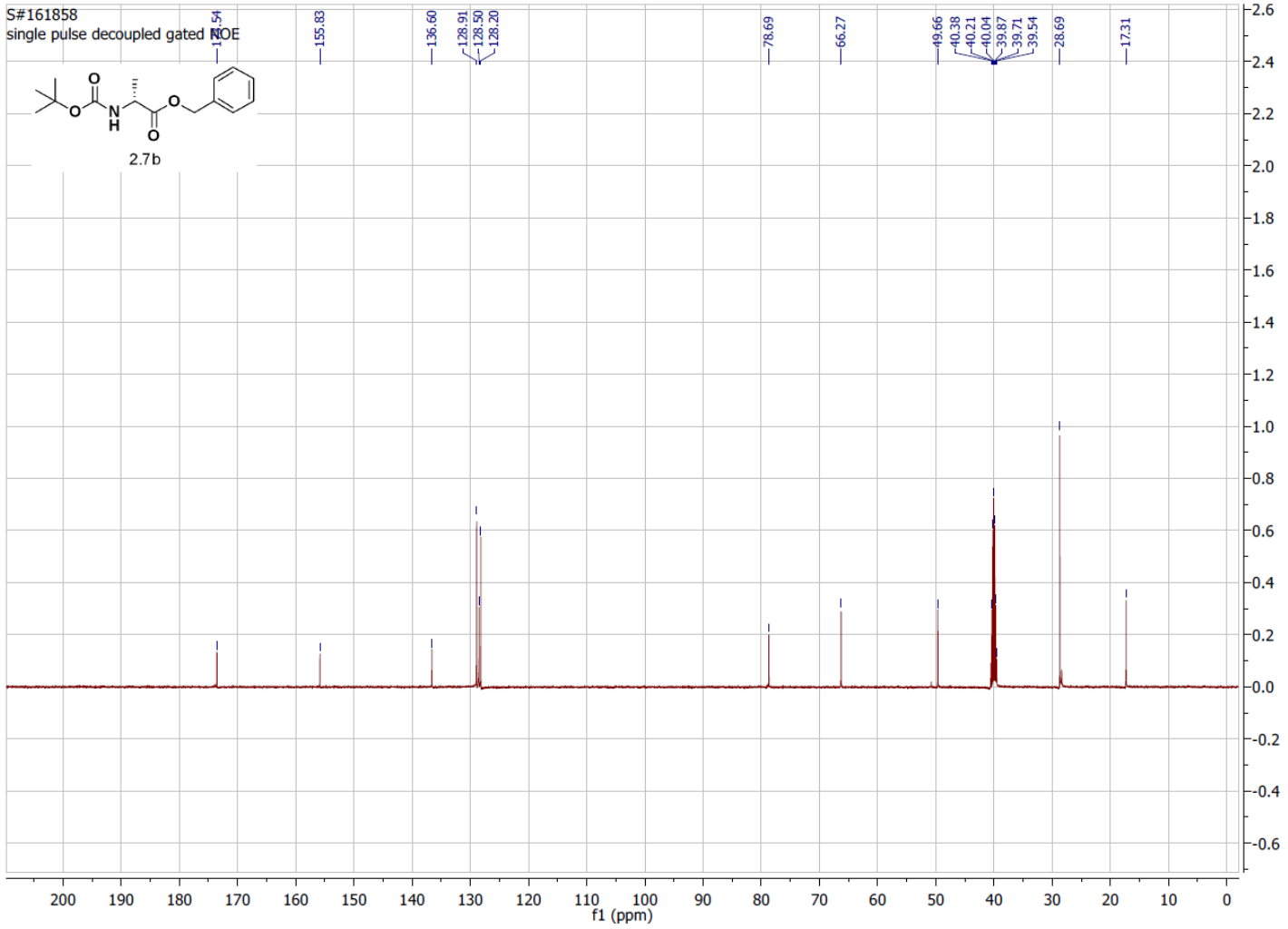

S#830614
single_pulse
AS_2.6
(s)
8.48
(d)
8.66
(bs)
7.59
(m)
7.20
(m)
4.50
(dd)
2.97
(dd)
3.16
(s)
2.31
f1 (ppm)

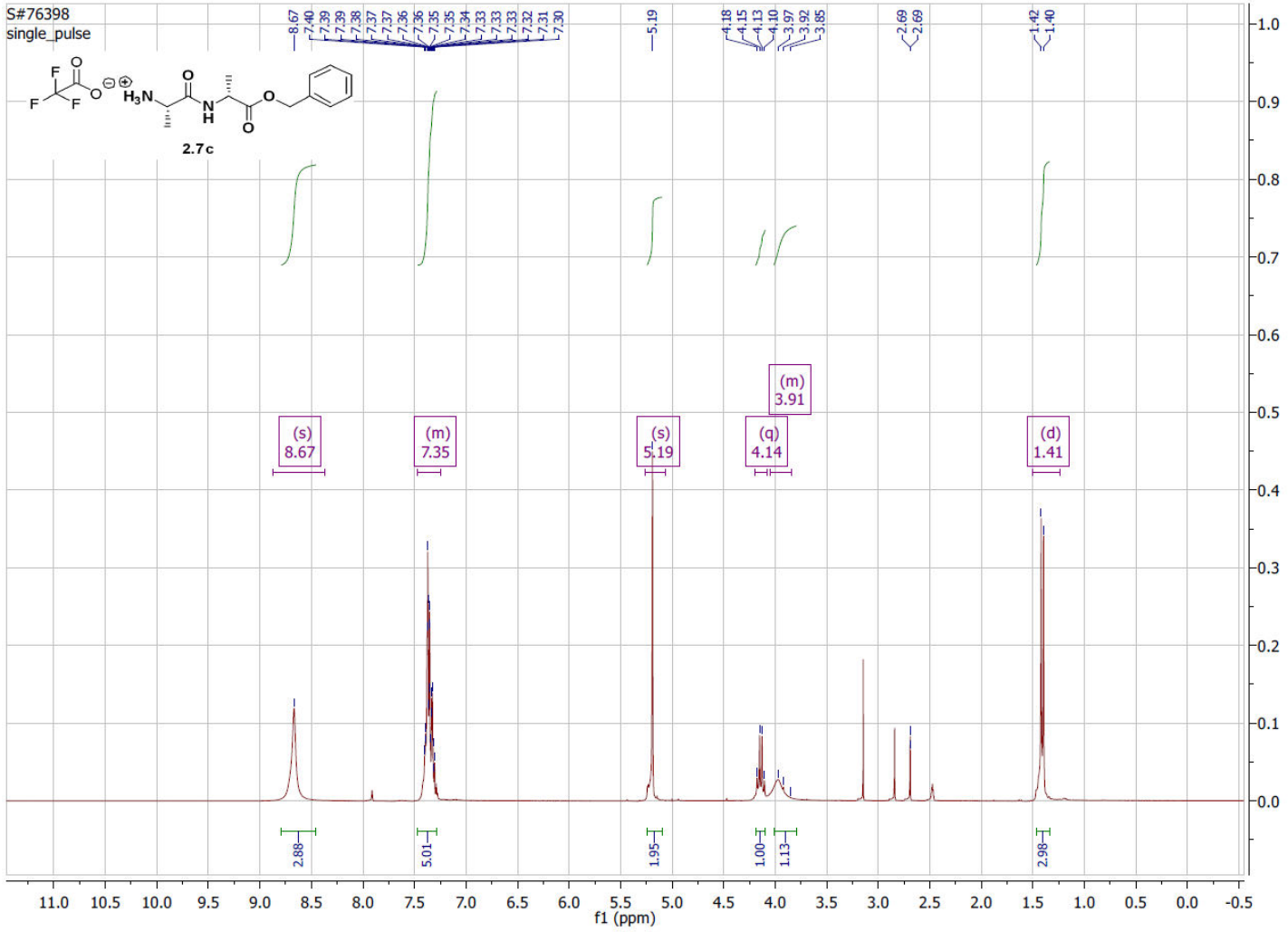

S#834522
single pulse decoupled gated NOE
AS_2.6
155.92
138.84
129.63
128.70
126.86
105.45
54.71
36.89
26.06
f1 (ppm)

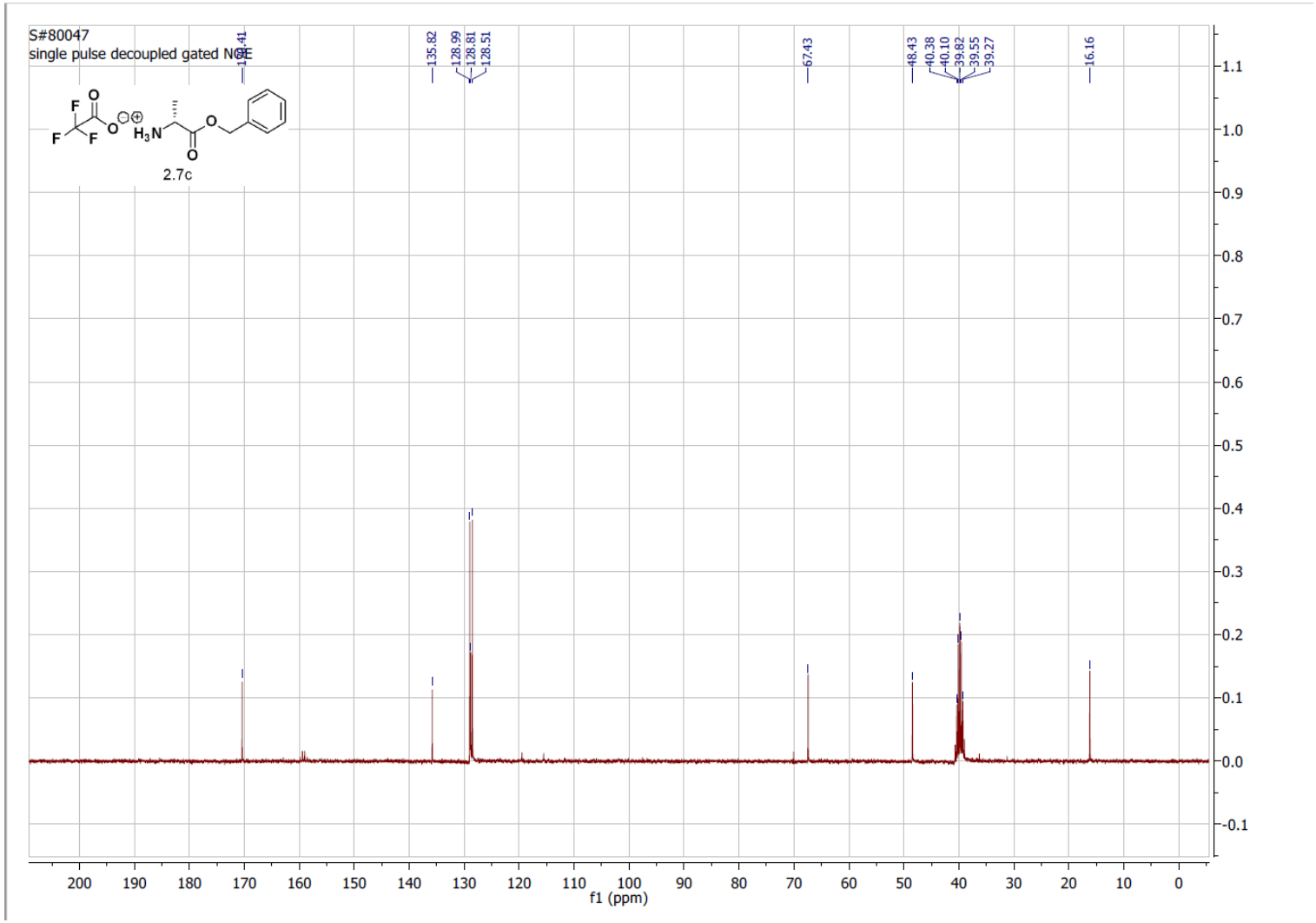

S#161030
single_pulse
2.7a
7.10
7.08
3.91
3.88
3.86
3.83
1.35
1.32
1.20
1.17
(d)
7.09
(m)
3.87
(s)
1.35
(d)
1.18
1.00
1.14
9.00
3.18
f1 (ppm)

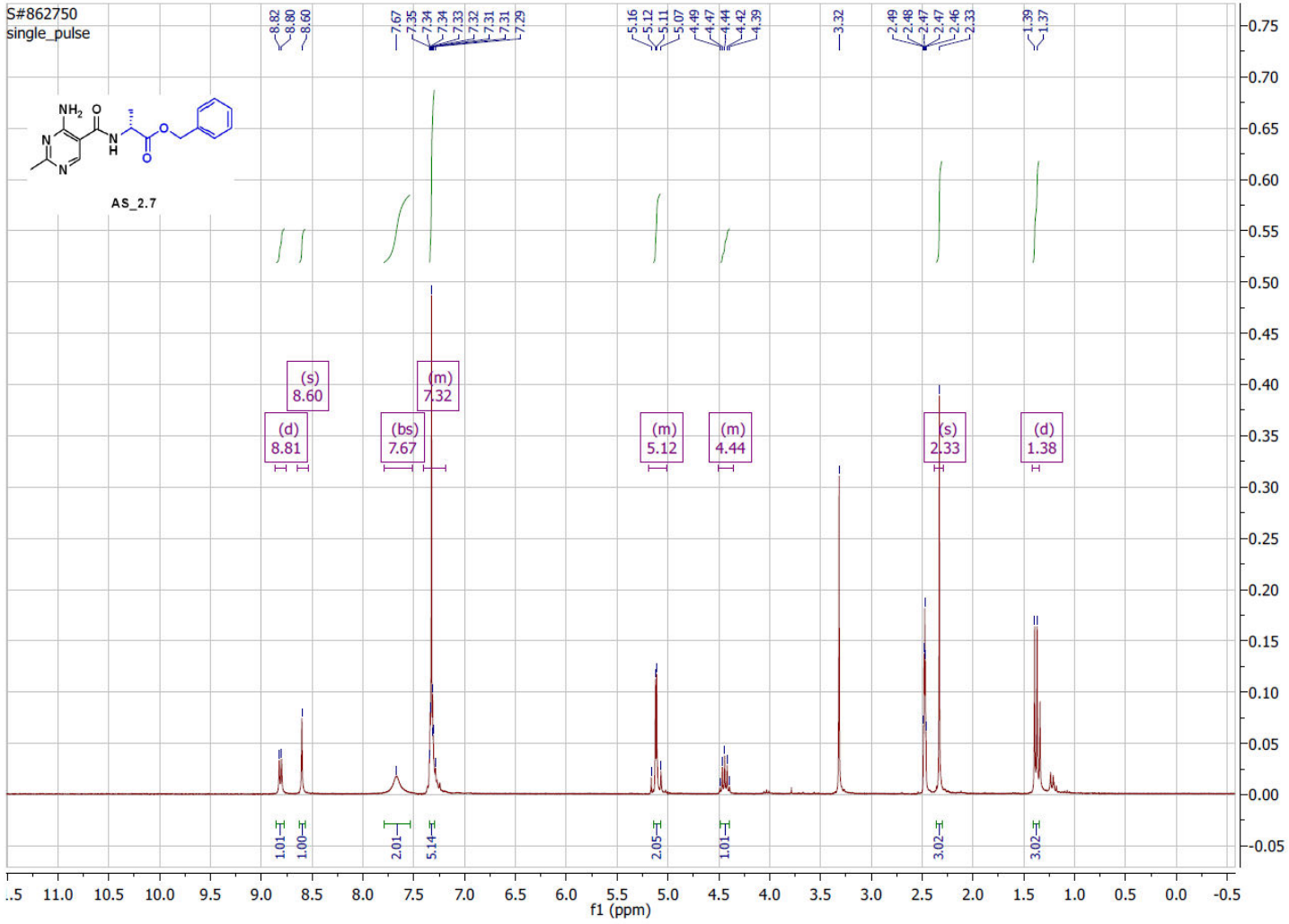

S#164607
single pulse decoupled gated NOE
2.7a
175.23
155.79
78.45
49.33
40.57
40.30
40.02
39.74
39.46
39.19
28.74
17.57
f1 (ppm)

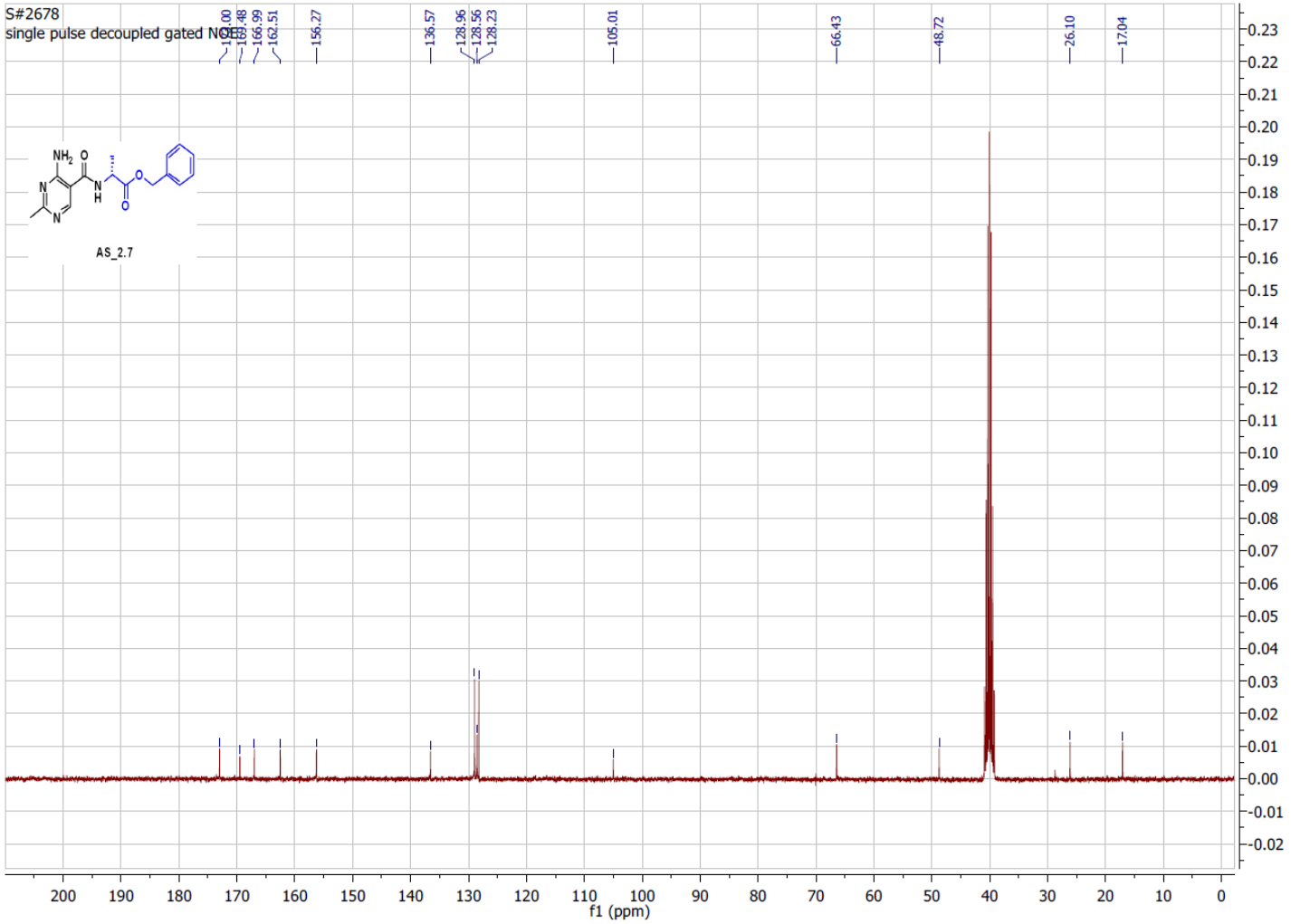

S#161056
2.7b
7.35
7.34
7.33
7.32
7.31
7.31
7.30
7.30
7.29
7.29
5.13
5.11
5.06
5.03
4.05
4.04
4.02
3.31
2.48
2.47
2.47
2.47
2.46
1.37
1.34
1.23
1.22
(m)
7.31
(d)
5.05
(d)
5.12
(m)
4.04
(d)
1.23
(s)
1.34
5.19
1.19
1.13
0.98
1.01
9.12
3.25
f1 (ppm)

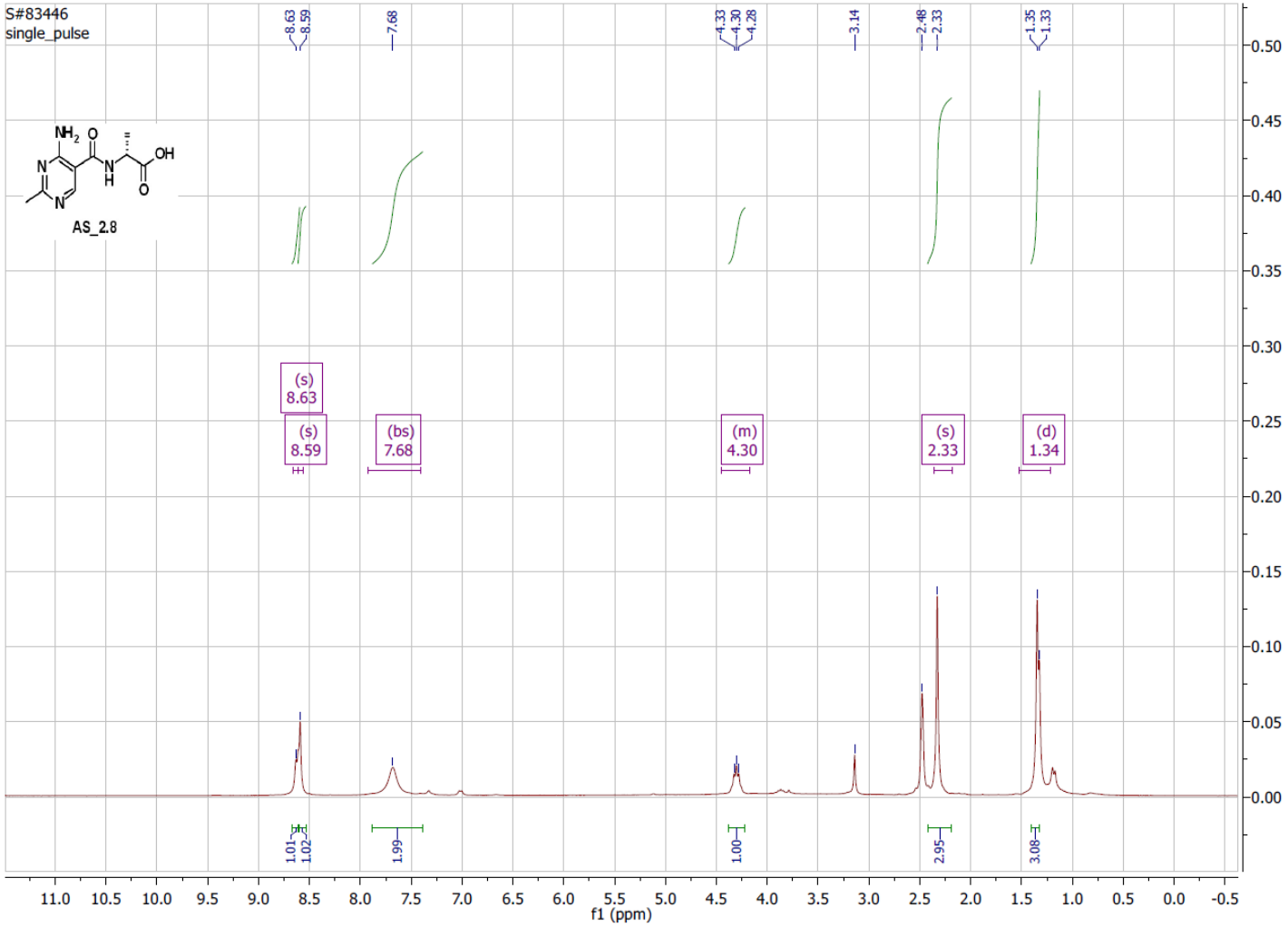

S#161858
single pulse decoupled gated NOE
2.7b
173.54
155.83
136.60
128.91
128.50
128.20
78.69
66.27
49.66
40.38
40.21
40.04
39.87
39.71
39.54
28.69
17.31
f1 (ppm)

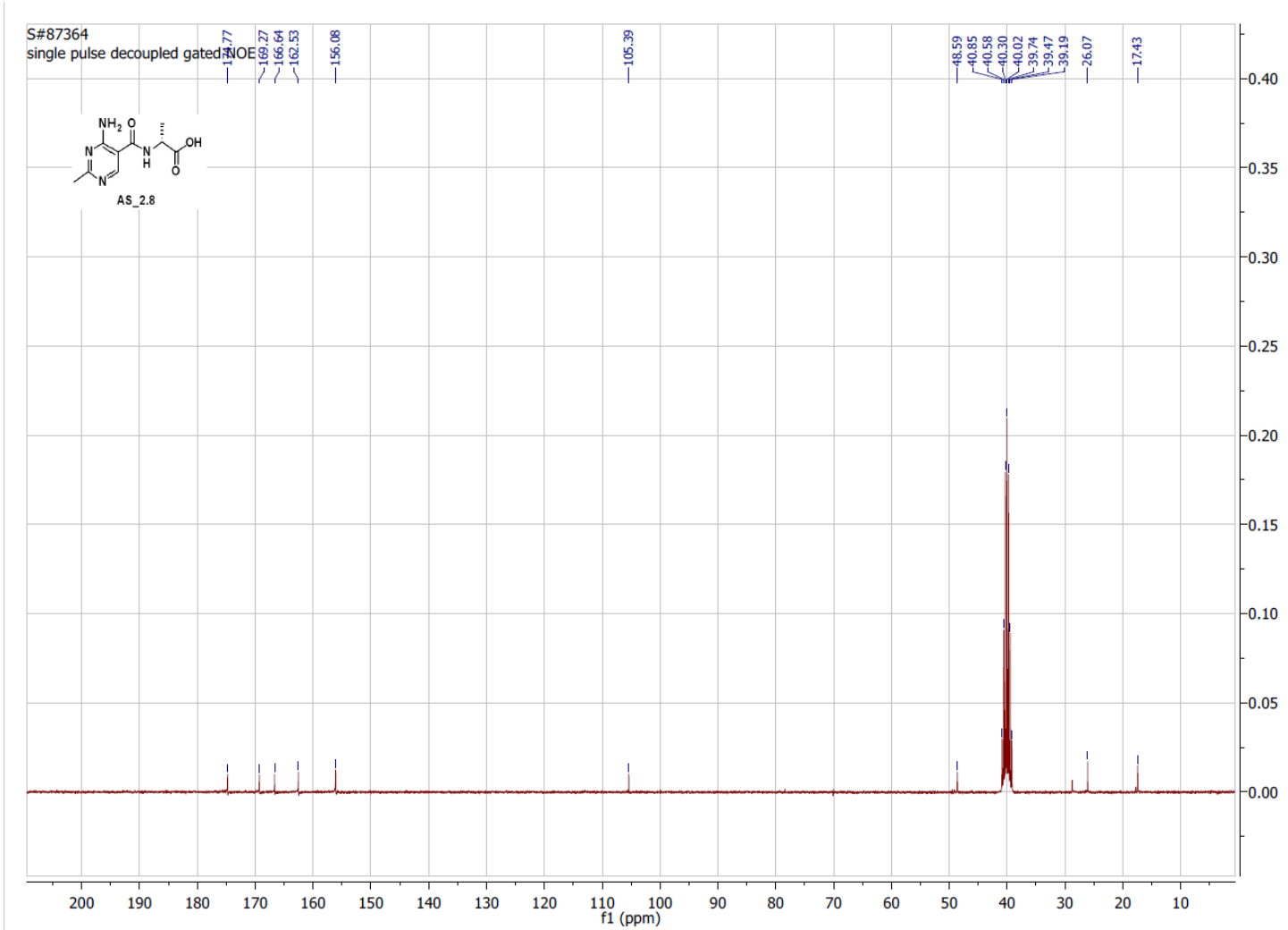

S#76398
single_pulse
2.7c
8.67
7.40
7.39
7.39
7.38
7.37
7.37
7.36
7.36
7.35
7.35
7.34
7.33
7.33
7.32
7.31
7.30
5.19
4.18
4.15
4.13
4.10
3.97
3.92
3.85
2.69
2.69
1.42
1.40
(s) 8.67
(m) 7.35
(s) 5.19
(q) 4.14
(m) 3.91
(d) 1.41
2.88
5.01
1.95
1.00
1.13
2.98
f1 (ppm)

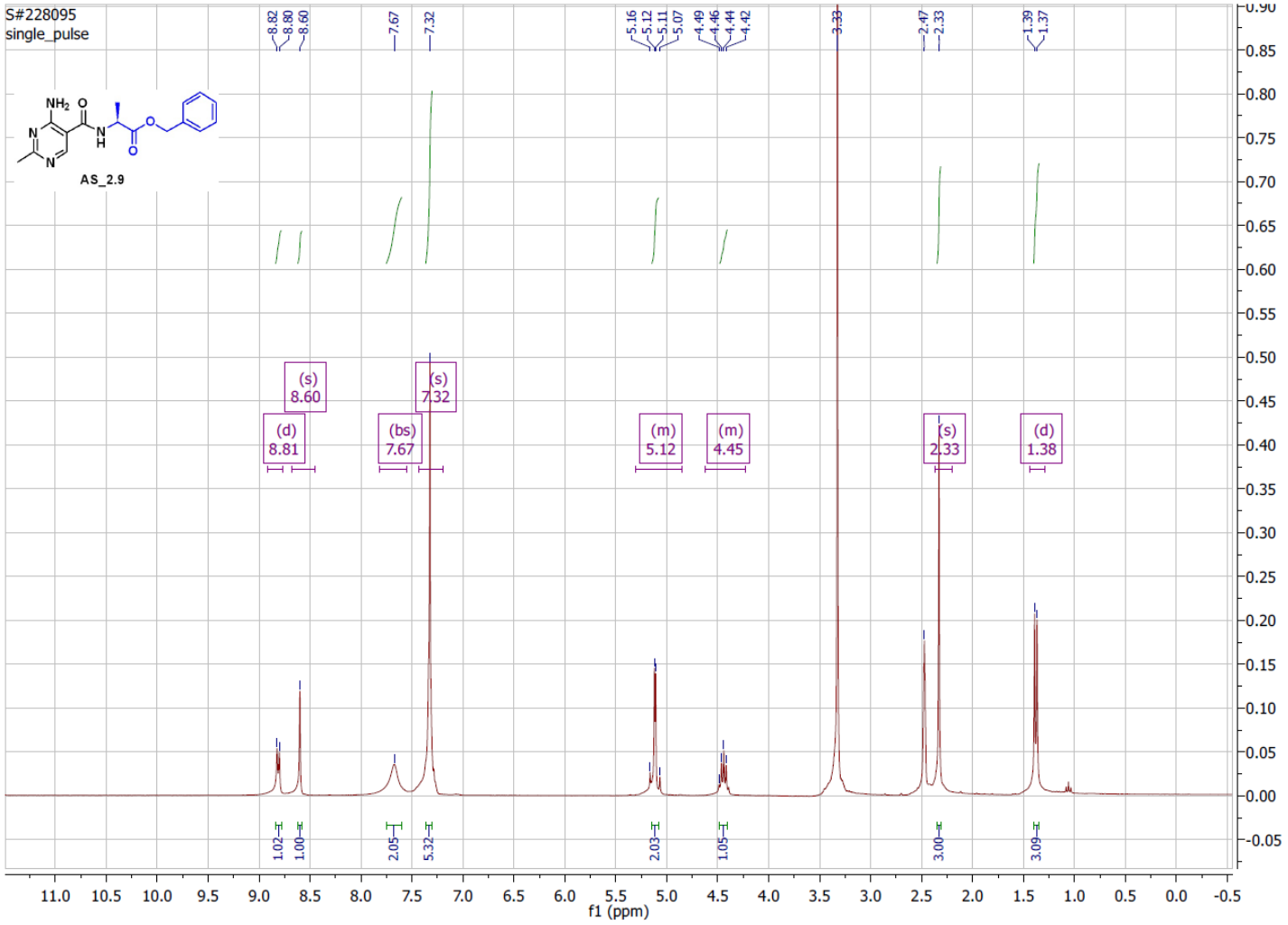

S#80047
single pulse decoupled gated NOE
169.41
135.82
128.99
128.81
128.51
67.43
48.43
40.38
40.10
39.82
39.55
39.27
16.16
2.7c
f1 (ppm)

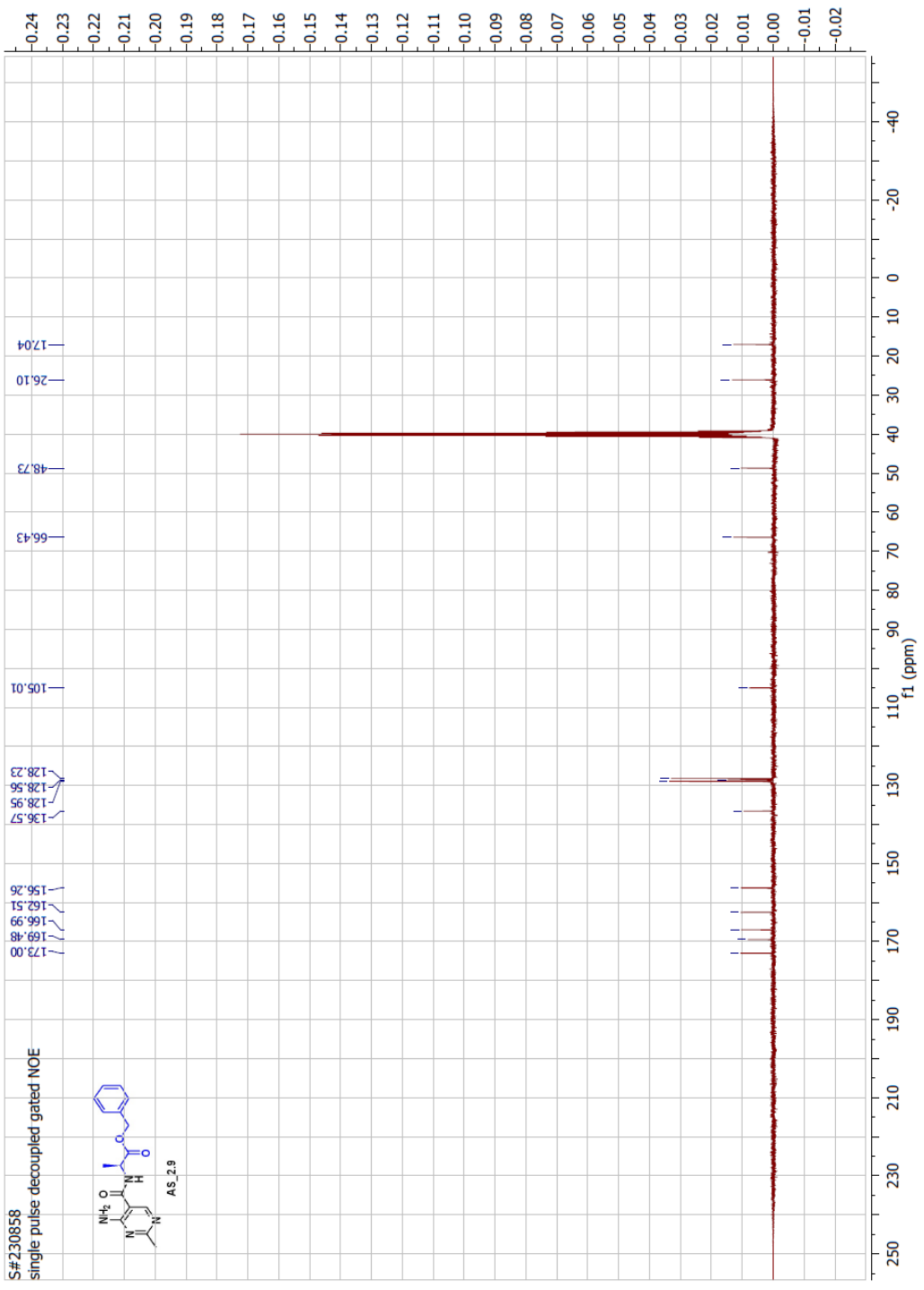

S#862750
single_pulse
AS_2.7
(s)
8.60
(d)
8.81
(m)
7.32
(bs)
7.67
(m)
5.12
(m)
4.44
(s)
2.33
(d)
1.38
f1 (ppm)

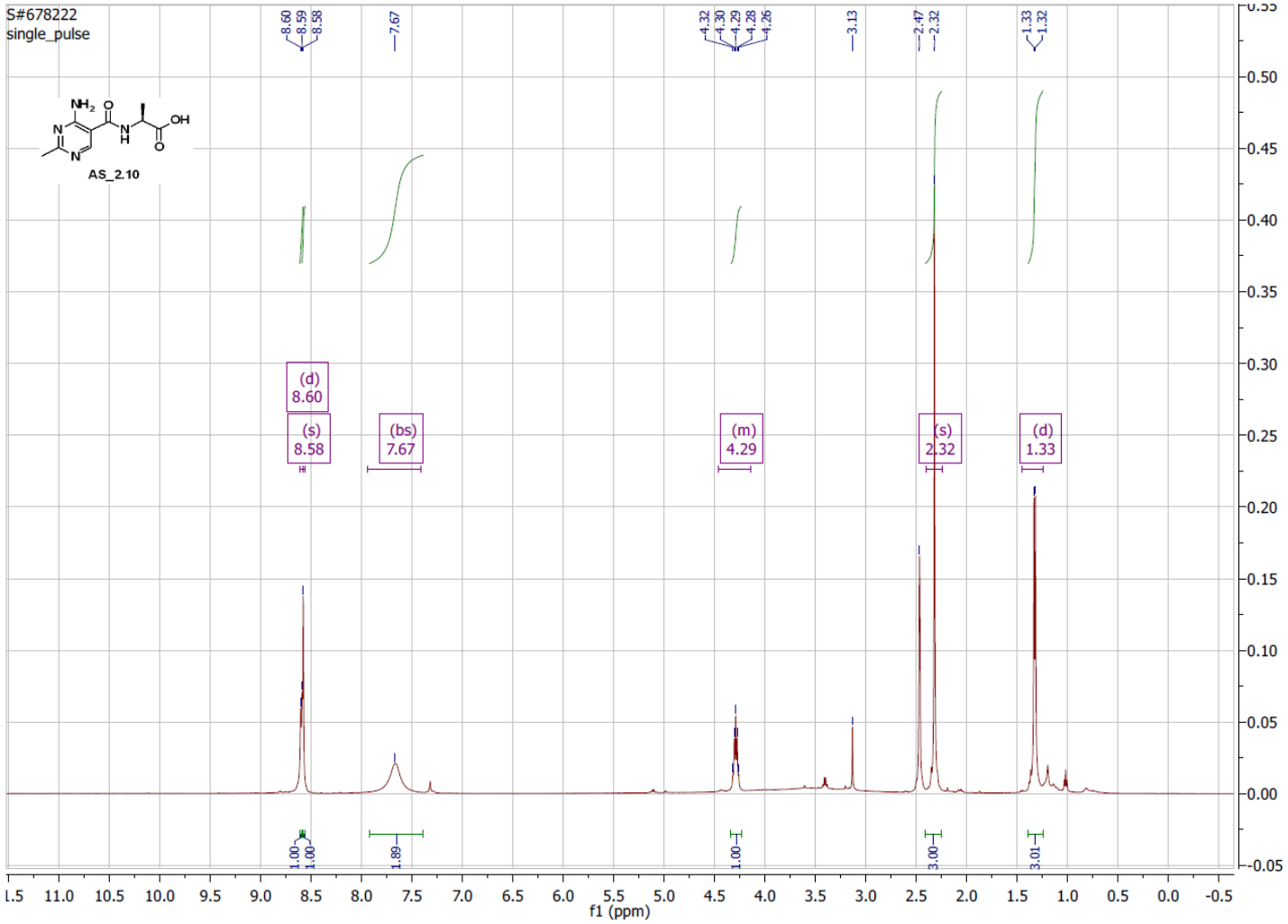

S#2678
single pulse decoupled gated NOE
169.00
168.48
166.99
162.51
156.27
136.57
128.96
128.56
128.23
105.01
66.43
48.72
26.10
17.04
AS_2.7
f1 (ppm)

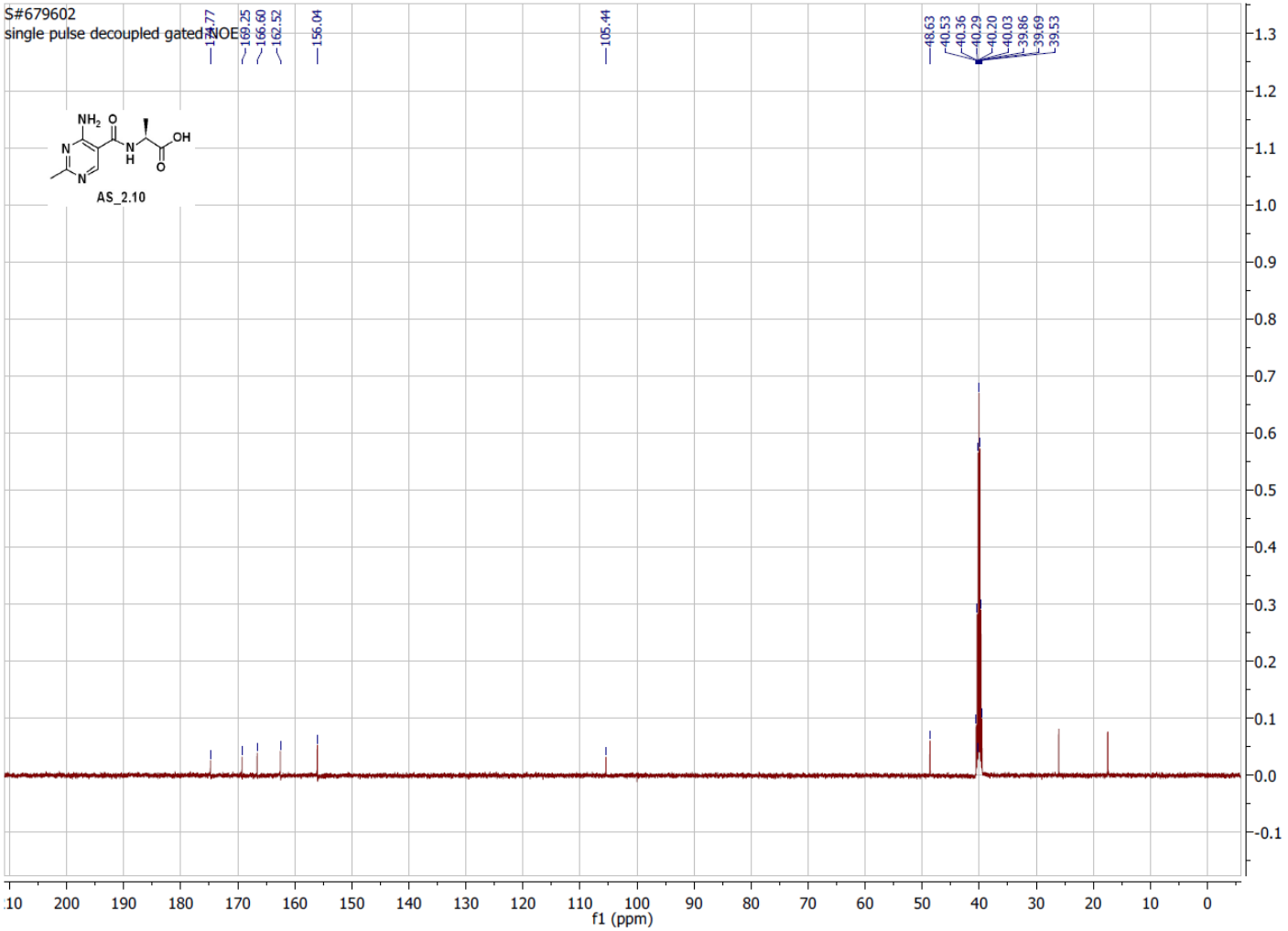

S#83446
single_pulse
AS_2.8
8.63
8.59
7.68
4.33
4.30
4.28
3.14
2.48
2.33
1.35
1.33
(s)
8.63
(s)
8.59
(bs)
7.68
(m)
4.30
(s)
2.33
(d)
1.34
1.01
1.02
1.99
1.00
2.95
3.08
f1 (ppm)

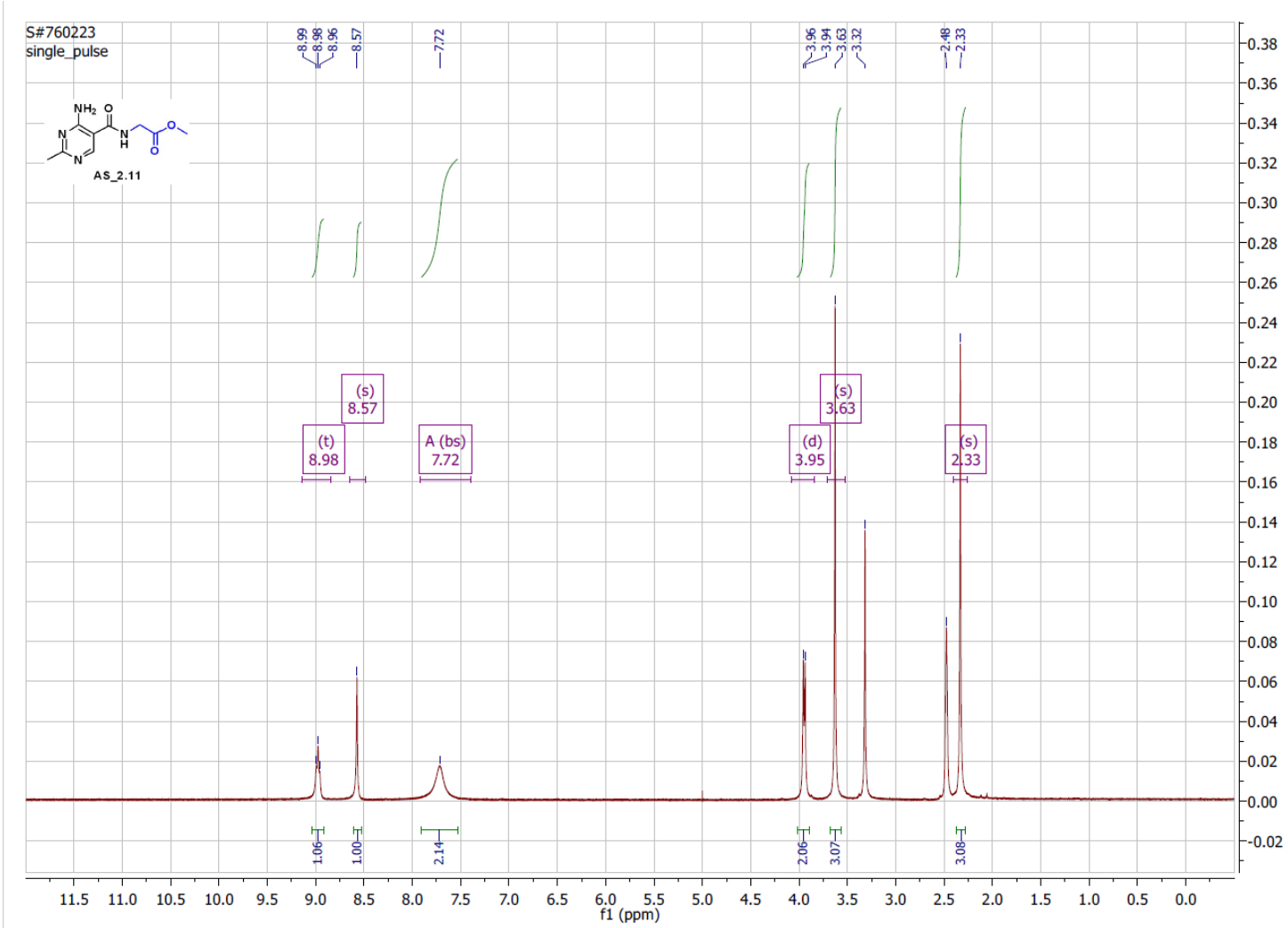

S#87364
single pulse decoupled gated NOE
AS_2.8
174.77
169.27
166.64
162.53
156.08
105.39
48.59
40.85
40.58
40.30
40.02
39.74
39.47
39.19
26.07
17.43
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

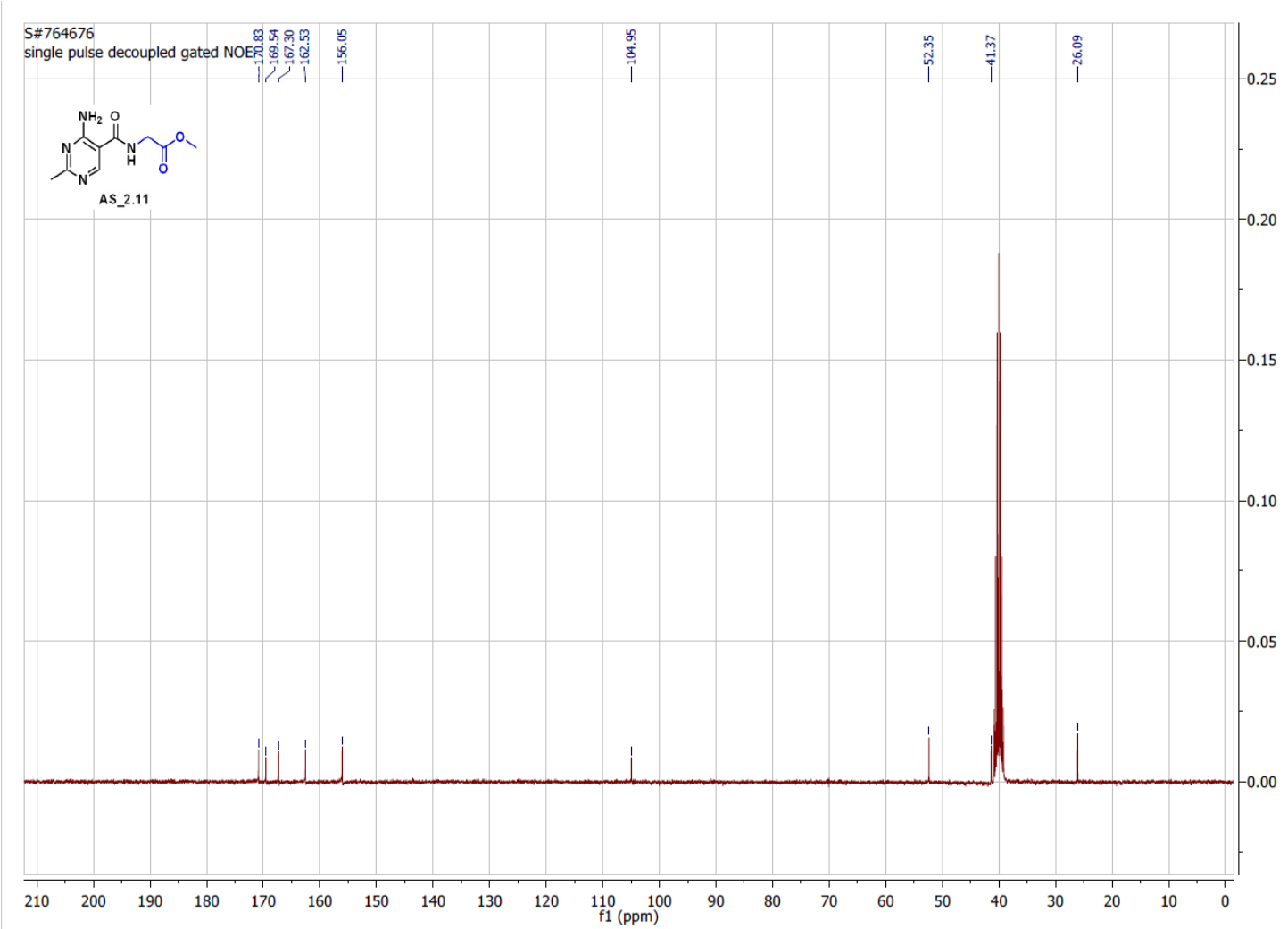

S#228095
single_pulse
AS_2.9
8.82
8.80
8.60
7.67
7.32
5.16
5.12
5.11
5.07
4.49
4.46
4.44
4.42
3.33
2.47
2.33
1.39
1.37
(s) 8.60
(d) 8.81
(bs) 7.67
(s) 7.32
(m) 5.12
(m) 4.45
(s) 2.33
(d) 1.38
1.02
1.00
2.05
5.32
2.03
1.05
3.00
3.09
f1 (ppm)


S#230858
single pulse decoupled gated NOE
AS_2.9
173.00
169.48
166.99
162.51
156.26
136.57
128.95
128.56
128.23
105.01
66.43
48.73
26.10
17.04
f1 (ppm)


S#678222
single_pulse
AS_2.10
8.60
8.59
8.58
7.67
4.32
4.30
4.29
4.28
4.26
3.13
2.47
2.32
1.33
1.32
(d)
8.60
(s)
8.58
(bs)
7.67
(m)
4.29
(s)
2.32
(d)
1.33
1.00
1.00
1.89
1.00
3.00
3.01
f1 (ppm)


S#679602
single pulse decoupled gated NOE
174.77
169.25
166.60
162.52
156.04
105.44
48.63
40.53
40.36
40.29
40.20
40.03
39.86
39.69
39.53
AS_2.10
f1 (ppm)


S#760223
single_pulse
AS_2.11
8.99
8.98
8.96
8.57
7.72
3.96
3.94
3.63
3.32
2.48
2.33
(s)
8.57
(t)
8.98
A (bs)
7.72
(s)
3.63
(d)
3.95
(s)
2.33
1.06
1.00
2.14
2.06
3.07
3.08
f1 (ppm)


S#764676
single pulse decoupled gated NOE
170.83
169.54
167.30
162.53
156.05
104.95
52.35
41.37
26.09
AS_2.11
0.25
0.20
0.15
0.10
0.05
0.00
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

References

1. Levy, S. B., *The Journal of Antimicrobial Chemotherapy* **2002,** *49* (1), 25-30.

2. Abraham, E. P.; Chain, E., *Nature* **1940,** *146* (3713), 837.

3. Wright, G. D.; Walsh, C. T., *Accounts of Chemical Research* **1992,** *25* (10), 468-473.

4. Dantas, G.; Sommer, M. O., *The Scientific Research Society* **2014,** *102*, 42-51.

5. Nikaido, H., *Science-AAAS-Weekly Paper Edition-including Guide to Scientific Information* **1994,** *264* (5157), 382-387.

6. Ugboko, H.; De, N., *Int. J. Curr. Microbiol. App. Sci* **2014,** *3* (12), 461-476.

7. Policy, I. P., *Clinical Infectious Diseases* **2010,** *50*, 1081-1083.

8. Spellberg, B.; Powers, J. H.; Brass, E. P.; Miller, L. G.; Edwards, J. E., *Clinical Infectious Diseases* **2004,** *38* (9), 1279-1286.

9. Forsyth, C., *JL Tech. & Arts* **2013,** *9*, 1.

10. Spink, E.; Ding, D.; Peng, Z.; Boudreau, M. A.; Leemans, E.; Lastochkin, E.; Song, W.; Lichtenwalter, K.; O'Daniel, P. I.; Testero, S. A., *Journal of Medicinal Chemistry* **2015,** *58* (3), 1380-1389.

11. Coates, A.; Hu, Y., *British Journal of Pharmacology* **2007,** *152* (8), 1147-1154.

12. Jurgenson, C. T.; Begley, T. P.; Ealick, S. E., *Annual Review of Biochemistry* **2009,** *78*, 569-603.

13. Bunik, V. I.; Tylicki, A.; Lukashev, N. V., *FEBS Journal* **2013,** *280* (24), 6412-6442.

14. Begley, T. P.; Downs, D. M.; Ealick, S. E.; McLafferty, F. W.; Van Loon, A. P.; Taylor, S.; Campobasso, N.; Chiu, H.-J.; Kinsland, C.; Reddick, J. J., *Archives of Microbiology* **1999,** *171* (5), 293-300.

15. Cheng, G.; Bennett, E. M.; Begley, T. P.; Ealick, S. E., *Structure* **2002,** *10* (2), 225-235.

16. Petersen, L. A.; Downs, D. M., *Journal of Bacteriology* **1997,** *179* (15), 4894-4900.

17. Meng, X.-Y.; Zhang, H.-X.; Mezei, M.; Cui, M., *Current computer-aided Drug Design* **2011,** *7* (2), 146-157.

18. Sanghera, J.; Li, R.; Yan, J., *Assay and Drug Development Technologies* **2009,** *7* (6), 615-622.

19. Bhawal, S. PhD. Dissertation, University of Texas at Arlington, **2014**.

20. BryanáHartley, A.; Henry, W., *Journal of the Chemical Society, Perkin Transactions 1* **1990,** (11), 2963-2966.

21. Price, D.; May, E. L.; Pickel, F. D., *Journal of the American Chemical Society* **1940,** *62* (10), 2818-2820.

22. Abraham, M. H.; Duce, P. P.; Prior, D. V.; Barratt, D. G.; Morris, J. J.; Taylor, P. J., *Journal of the Chemical Society, Perkin Transactions 2* **1989,** (10), 1355-1375.

23. Sheehan, J.; Cruickshank, P.; Boshart, G., *The Journal of Organic Chemistry* **1961,** *26* (7), 2525-2528.

24. Nieto, M.; Perkins, H., *Biochemical Journal* **1971,** *123* (5), 789-803.

25. Li, L.; Xiang, F.; Greer, T.; Frost, D.; Liang, Z., *PCT int. Appl*. US 20130078728 A1, Mar 28, **2013**.

26. Yanting H. PhD. Dissertation, University of Alberta, **1997**.

27. Lassalas, P.; Gay, B.; Lasfargeas, C.; James, M. J.; Tran, V.; Vijayendran, K. G.; Brunden, K. R.; Kozlowski, M. C.; Thomas, C. J.; Smith III, A. B., *Journal of Medicinal Chemistry* **2016,** *59* (7), 3183-3203.

28. Reddick, J. J.; Kinsland, C.; Nicewonger, R.; Christian, T.; Downs, D. M.; Winkler, M. E.; Begley, T. P., *Tetrahedron* **1998,** *54* (52), 15983-15991.

29. Lopez, D. A. PhD. Dissertation, University of Texas at Arlington, **2016**.

30. Trott, O.; Olson, A. J., *Journal of Computational Chemistry* **2010,** *31* (2), 455-461.

31. Meanwell, N. A., *Journal of Medicinal Chemistry* **2011,** *54* (8), 2529-2591.

32. Patani, G. A.; LaVoie, E. J., *Chemical Reviews* **1996,** *96* (8), 3147-3176.

33. Du, Q.; Wang, H.; Xie, J., *International Journal of Biological Sciences* **2011,** *7* (1), 41-52.

34. Khare, G.; Kar, R.; Tyagi, A. K., *PloS One* **2011,** *6* (7), e22441.

35. Collins, J. C.; Porras, J. I., *California Management Review* **1991,** *34* (1), 30-52.

Biographical Information

Abu Afzal Mohammad Shakar was born in Dhaka, Bangladesh. He obtained his B.S and M.S in Pharmaceutical Science from University of Dhaka in 2009 and 2010 respectively. After his graduation, he joined to product manufacturing department in Sanofi Aventis Bangladesh Ltd. He worked for 6 months to pharmaceutical product manufacturing of various world-class drugs of this multinational company. After that he joined to R & D department of Advanced Chemical Industry (ACI) Ltd and he formulated around 30 novel drug formulations and improved around 20 existing drug formulations. He obtained his M.S. in organic chemistry from the University of Texas at Arlington in 2016. During his graduate studies, he worked with Dr. Frank Foss in the field of Medicinal chemistry. He worked on synthesis and evaluation of various inhibitors of 4-amino-5-hydroxymethyl-2-methylpyrimidine kinase (HMPK) enzyme, one important enzyme in vitamin B_1 biosynthesis pathway.