Stony Brook University

I - Discovery and Optimization of 2,5,6- Trisubstituted Benzimidazole Leads for Tuberculosis Chemotherapy

II - Resynthesis of Hit 2,5,7- Trisubstituted Benzimidazole Final Compounds

A Thesis Presented

By

Lucy Li

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Abstract of the Thesis

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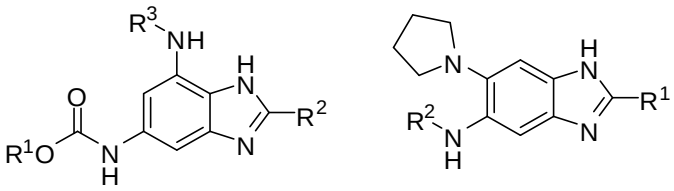
Tuberculosis affects one-third of the world's population and is caused by the bacteria *Mycobacterium tuberculosis* (Mtb). Due to the rise of multi-drug resistant strains of the bacteria, a new antibiotic needs to be developed. FtsZ, a tubulin homologue, plays an essential role in bacterial cell division therefore making it a novel anti-microbial target in drug resistant strains of *Mtb*. Previous works from our laboratory have shown that a number of 2,5,6- and 2,5,7-trisubstituted benzimidazoles possess significant activity against *Mtb* by interfering with filamental temperature sensitive protein Z (FtsZ) polymerization. Libraries of novel trisubstituted benzimidazoles were created through rational design. Based upon previous work, it was noted that two 2,5,6-trisubstituted benzimidazoles containing a pyrrolidine moiety at the 5 position exhibited excellent activity against both drug sensitive and drug resistant strains of *Mtb*. Therefore, a library of 240 2,5,6-trisubstituted benzimidazoles retaining the pyrrolidine moiety

was synthesized, with functionalization of the 2 position with a furoyl, thiophene, tetrahydropyran, or benzyl moiety.

In addition, several 2,5,7-trisubstituted benzimidazoles from a previously synthesized library containing a ethyl carbamate moiety at the 5 position also exhibited good activity against Mtb. In order to obtain more accurate MIC_{99} values, intermediates of these compounds were also synthesized in preparation for the resynthesis of the hit compounds.

Synthesis of the trisubstituted benzimidazoles starts with nucleophilic aromatic substitution of different dinitroaromatic starting materials in the presence of an amine. Subsequent introduction of the desired moieties at the 2 or 5 position via acylation or Curtius rearrangement followed by reduction and cyclocondensation affords the desired final intermediates.



Trisubstituted Benzimidazoles

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Introduction

Tuberculosis (TB) is a highly infectious disease estimated to affect one-third of the world's population and is caused by the bacteria *Mycobacterium tuberculosis* (*Mtb*). It primarily attacks the lungs, but is known to spread to other parts of the body.[1] Most infections are asymptomatic and are classified as latent TB (LTBI), and only about 10% of those infected will develop the disease, although this risk increases dramatically in people who are also infected with HIV. The World Health Organization (WHO) estimated 9.4 million new cases of TB in 2009, of which 1.1 million were also co-infected with HIV.[1] Within the same year, 1.7 million deaths were caused by TB. With the emergence of HIV in the last few decades, TB has also become the most opportunistic infection for AIDS patients, where 380,000 of the 1.7 million deaths were also infected with HIV, about 22%.[2]

Tuberculosis is extremely difficult to treat as current TB treatment involves a cocktail of antibiotics administered over the course of six months to two years, depending on the strain of *Mtb*. The lengthy treatment time is due to the slow growth of the bacteria, where *Mtb* divides once every 12 to 15 hours.[3] Due to the extensive treatment time as well as inadequate resources, most patients do not finish their treatment and as a result, drug resistant forms of the bacteria have emerged and have been classified into two categories: multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB).[2] Current antibiotics work by disrupting essential cellular activities during replication such as cell wall synthesis, DNA/RNA synthesis, or protein synthesis. The primary drugs used are isoniazid, rifampicin, ethambutol, and pyrazinamide, which have to be taken for about six months. If there is resistance to at least the first two drugs, then the disease is classified as MDR-TB and second line drugs such as

thioamides, cyclopeptides, para-aminosalicylic acid, or fluoroquinolones are used and treatment will last about a year. If *Mtb* is also resistant to any fluoroquinolone as well as any injectable second-line drug, such as kanamycin, then it is classified as XDR-TB and treatment lasts about two years. These strains may also be resistant to additional drugs, further complicating treatment.[1,2] Thus, there are requirements for the development of new antituberculosis drugs that can treat both drug sensitive and drug resistant forms of *Mtb*.

To that end, our lab has been focusing on a prokaryotic homologue of tubulin called filamental temperature sensitive protein Z (FtsZ). Previous research has shown that FtsZ plays an essential role in bacterial cell division, making it a novel anti-microbial target in drug resistant strains of *Mtb*.[4] Due to the conservation of amino acids located in the GTP binding pocket, FtsZ polymerization is similar to that of microtubules as both polymerize in the presence of GTP and depolymerize when GTP is hydrolyzed to GDP.[5,6] When the bacterium is ready to divide, FtsZ monomers assemble at the mid-point of the cell and polymerize in the presence of GTP, extending bilaterally to form the Z-ring structure. Recruitment of other cell division proteins leads to Z-ring contraction, resulting in septum formation. Like tubulin, formation of the Z-ring and subsequent septum is finely controlled by the constant and careful polymerization and depolymerization of FtsZ molecules (Figure 1).[6]

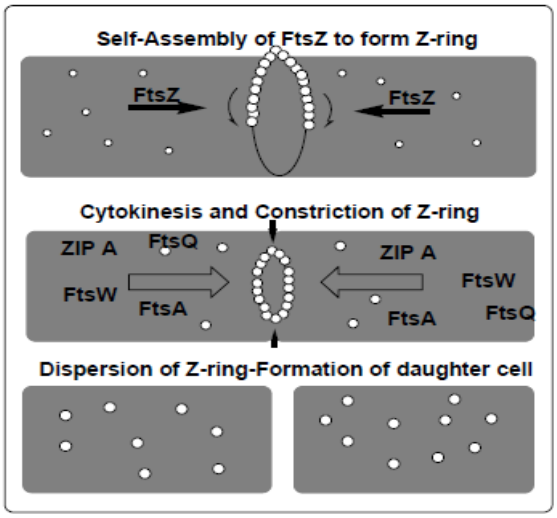


Figure 1. Overview of bacterial cell division.
Z ring formation at site of cytokinesis with the recruitment of other cell division proteins, resulting in septum formation.[6]

Although FtsZ and tubulin are homologous in structure and function, they only share about 10% sequence similarity thus affording the opportunity to discover FtsZ specific compounds that have limited cytotoxicity to eukaryotic cells.

Because FtsZ and tubulin have similar protein folds and GTPase activity, inhibitors of tubulin polymerization may also inhibit FtsZ. Therefore, taxanes, a class of compounds known to destabilize tubulin and used as anti-cancer drugs, were also tested for inhibitory activity against *Mtb* using real time polymerization chain reaction (RT-PCR).[9] These taxanes are efficiently cytotoxic due to its distinct mode of action. It over-stabilizes tubulin[10] and acts as a multidrug-resistance reversal agent (TRA) that inhibits the efflux pumps of ATP-binding cassette (ABC) transporters such as P-glycoprotein.[11] These efflux pumps play an important role in the survival of cancer cells as they actively export cytotoxic compounds out of the cell. An initial 120 taxanes were screened against H37Rv and a few showed promise, with the C-seco derivative

of one particular compound SB-RA-2001 exhibiting low cytotoxicity with MIC_{99} values of 1.25 µM against drug sensitive *Mtb* H37Rv and 2.5 µM against drug resistant strain IMCJ946K2.

Sarcina and Mullineaux showed that albendazole and thiabendazole, known tubulin inhibitors, caused cell elongation of *E. coli* and cyanobacteria and later on Slayden *et al* determined the minimum inhibitory concentration (MIC_{99}) of the two compounds against the drug sensitive *Mtb* cell line, H37Rv (Figure 2).[4,7]

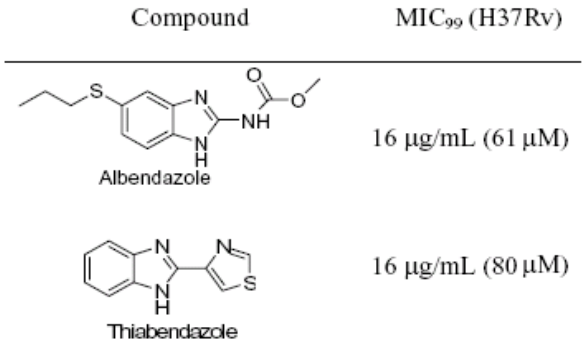


Figure 2. FtsZ polymerization inhibitors.

Based on these results, a library of 272 2,5,6-trisubstituted benzimidazoles were synthesized in our lab by Dr. Seung-Yub Lee and tested against *Mtb* and seven showed promising antibacterial activity against the H37RV strain (Figure 3).[8] In addition, out of 77 2,5,7-trisubstituted benzimidazoles synthesized, one compound in particular, SB-P5C1, showed high inhibitory activity against the *Mtb* strain H37RV (Figure 3).

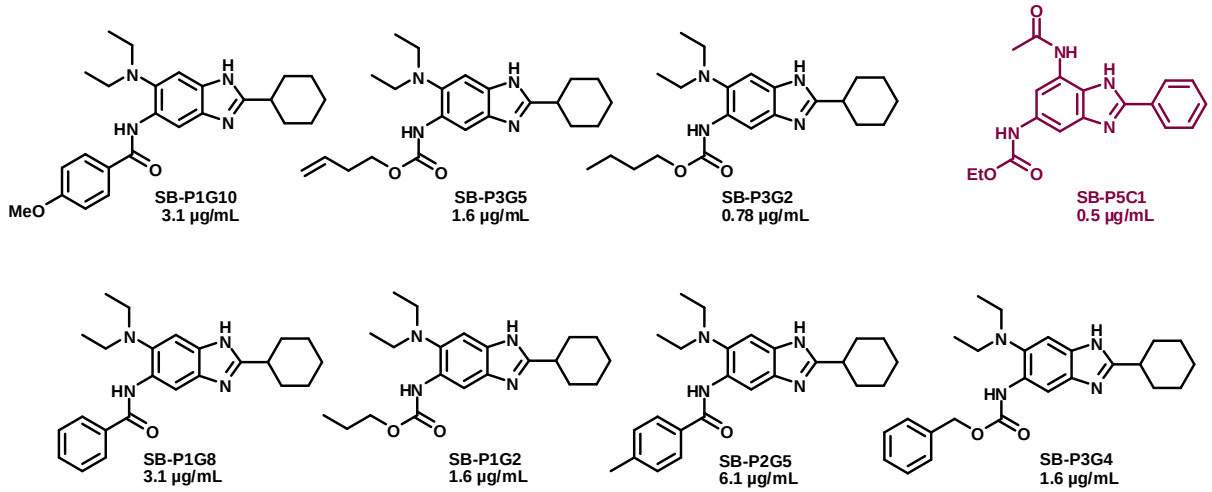


Figure 3. Hit 2,5,6- and 2,5,7-trisubstituted benzimidazoles with corresponding MIC_{99} values.

To further the hypothesis that the anti-microbial activity of benzimidazoles is due to its interaction with FtsZ polymerization, light scattering assays were performed in our lab in which FtsZ incubated in a polymerization buffer was treated with inhibitors SB-P3G5 and SB-P1G2 at various concentrations.[12] A measurement of light scattering as a function of benzimidazole concentration showed inhibition of polymerization in a dose dependent manner (Figure 4).

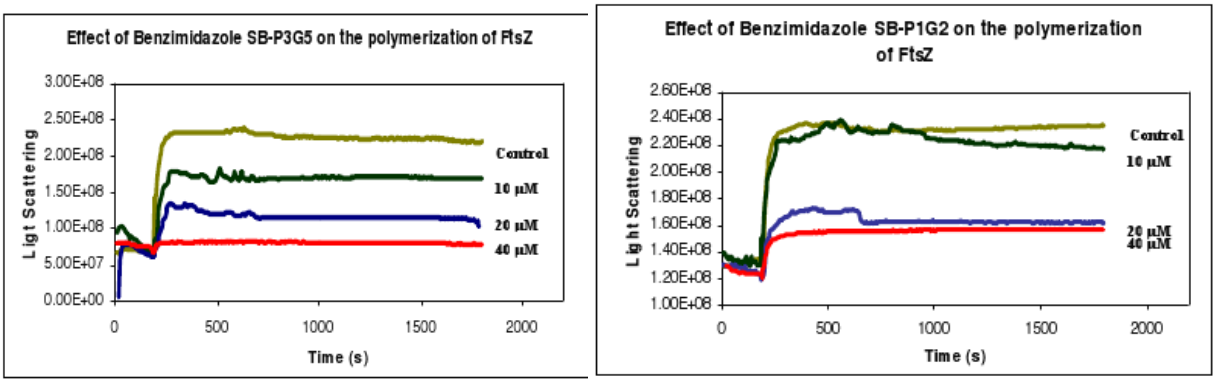


Figure 4. Effect of benzimidazoles on FtsZ polymerization.

In addition, GTPase activity was monitored using Malachite Green assay and shown to be enhanced by SB-P3G2 (blue) and SB-P3G5 (red) in a dose dependent manner (Figure 5). Taken together, these results strongly imply that inhibition of FtsZ polymerization is due to the increased GTPase activity.

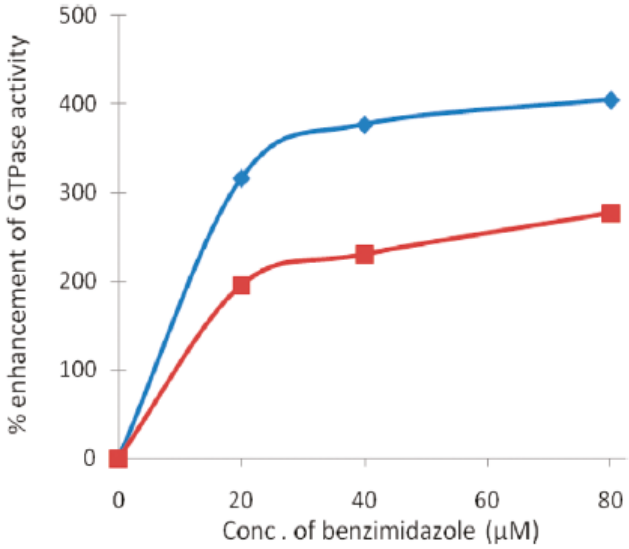


Figure 5. Enhancement of GTPase activity by lead benzimidazoles.

Due to the common benzimidazole backbone, a preliminary structure-activity relationship (SAR) study was done for 2,5,6-trisubstituted benzimidazoles which showed that a diethylamino group at the 6 position and a cyclohexyl group at the 2 position appear to be contributing factors for the compound's good anti-TB activity (Figure 6).

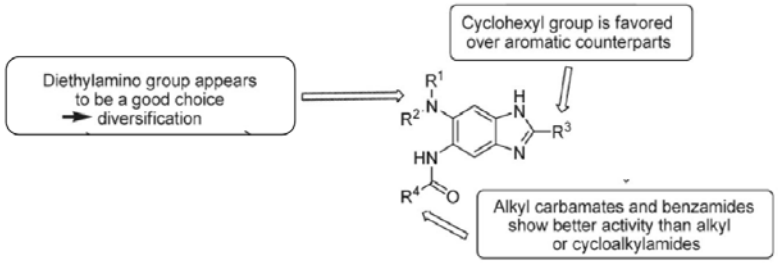


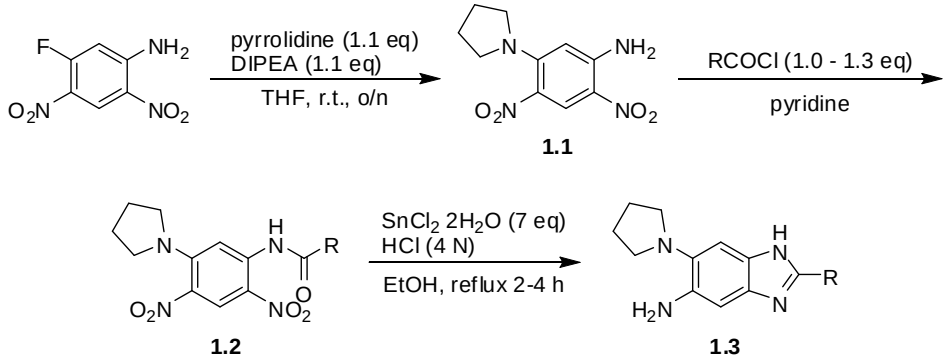
Figure 6. Preliminary SAR study of initial 2,5,6-trisubstituted benzimidazole hit compounds.

Accordingly, another library of 238 compounds was synthesized using different aminoalkyl groups while retaining the cyclohexyl group. From this library, two compounds containing a pyrrolidine at the 6 position showed excellent activity, with MIC_{99} values in the range of 0.39 – 1.56 μg/mL against both drug sensitive and drug resistant strains of *Mtb*.[12] Therefore, 2,5,6-trisubstituted benzimidazoles libraries containing the pyrrolidine moiety were synthesized to continue the effort of developing the next generation of antimicrobials that can eliminate both drug sensitive and drug resistant strains.

Morever, from a library of 2,5,7-trisubstituted benzimidazoles synthesized, several hit compounds were discovered that had MIC_{99} values around 1 μg/mL. Resynthesis of these final compounds is required to determine more accurate MIC_{99} values and further biological testing.

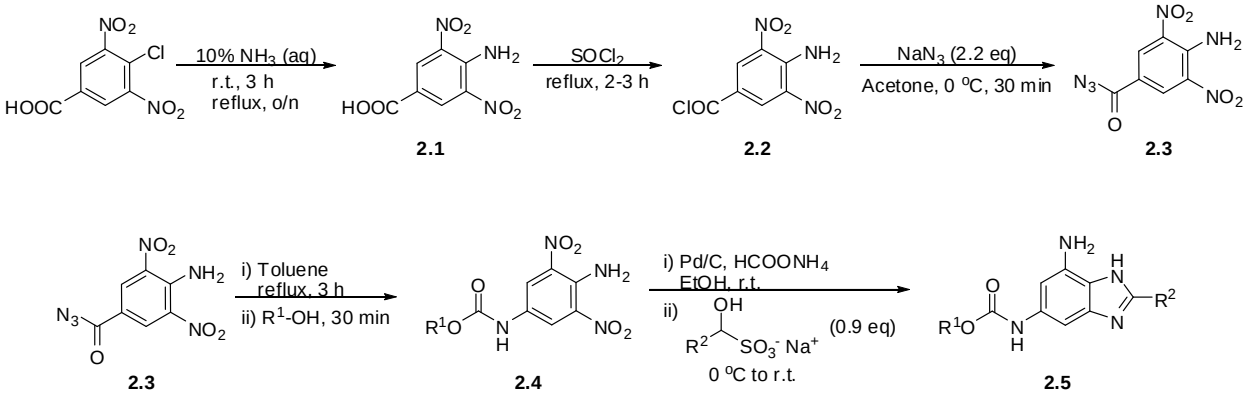
Results and Discussion

The synthesis of 2,5,6-trisubstituted benzimidazoles begins with modifications made on a benzene core. These modifications start with the nucleophilic aromatic substitution of 2,4-dinitro-5-fluoroaniline in the presence of pyrrolidine. The aniline amine is then converted into an amide with treatment of an acid chloride, followed by reduction and cyclization using tin (II) chloride under harsh acidic conditions (Scheme 1).



Scheme 1. Synthesis of 2,5,6-Trisubstituted Benzimidazoles

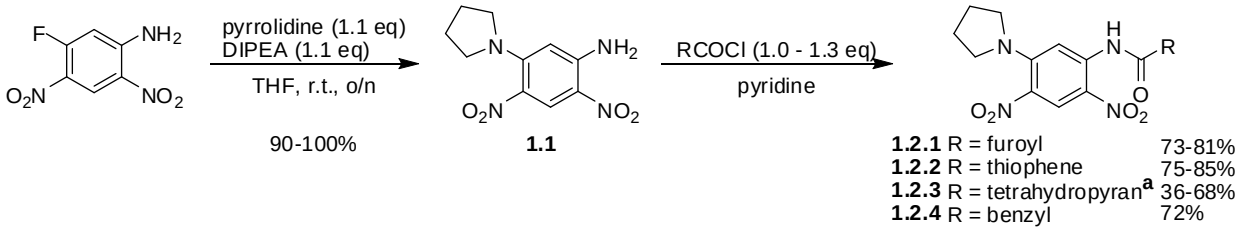
The resynthesis of 2,5,7-trisubstituted benzimidazole also begins with nucleophilic aromatic substitution. 4-Chloro-3,5-dinitrobenzoic acid undergoes nucleophilic aromatic substitution in aqueous ammonia. The carboxylic acid is then converted to the acyl azide via acid chloride intermediate, which then undergoes Curtius rearrangement. The isocyanate is reacted with an alcohol to functionalize the 5 position and consequently reduced to afford an aromatic triamine that reacts with a bisulfite salt of the desired 2 position moiety to produce the final intermediates (Scheme 2).[13]



Scheme 2. Synthesis of 2,5,7-trisubstituted benzimdiazoles

I. Synthesis of 2,5,6-Trisubstituted Benzimidazole Libraries

Aromatic nucleophilic substitution of the commercially available 2,4-dinitro-5-fluoroaniline with pyrrolidine proceeds cleanly to afford 5-diethylaminodinitroaniline **1.1** in quantitative yield. Depending on the functionality desired, acylation was done under various conditions and the *N*-acylanilines were obtained in moderately good yields.



Scheme 3. Synthesis of *N*-acylanilines.
[a]Yield is a 2-step yield calculated from 1.2.3a.

The acylation reaction using 2-furoylcarbonyl chloride or 2-thiophenecarbonyl chloride was done in the microwave at 100 ^{o}C for 1 h using pyridine as both a solvent and base. A slight excess of acid chloride was used to ensure all starting material was consumed. Upon completion of the reaction, the pyridine was evaporated as much as possible. It was observed that the product was not soluble in methanol, therefore excess methanol was added and the product collected by vacuum filtration and washed with cold methanol to remove any remaining pyridine and other impurities to obtain intermediates **1.2.1** and **1.2.2** in 73-85% yield as a yellow-green solid and yellow solid respectively. Ethanol was later discovered to remove pyridine efficiently under rotary evapration.

Acylation with phenylacetyl chloride, however, was not clean as 3 spots were seen on thin layer chromatography (TLC) corresponding to the starting material, desired product, and the diacylated product. This was surprising as the aniline is already highly deactivated due to the two nitro groups ortho and para to the amine. Purification proved to be difficult as both products

had similar solubility properties. They were both insoluble in methanol and ethanol but very soluble in methylene chloride and slightly soluble in ethyl acetate. Attempts at recrystallization using these solvents proved to be futile and a column was attempted using silica. Although the R_f difference between the desired (R_f = 0.66, 1:1/EA:Hex) and undesired product (R_f = 0.79, 1:1/EA:Hex) is large, isolation of the product was not obtained. This was due to the undesired compound sticking to the silica, thus contaminating all fractions containing the desired **1.2.4**. The difficulty of purification led to a desire to optimize this reaction. Model reactions were monitored by TLC (Table 1).

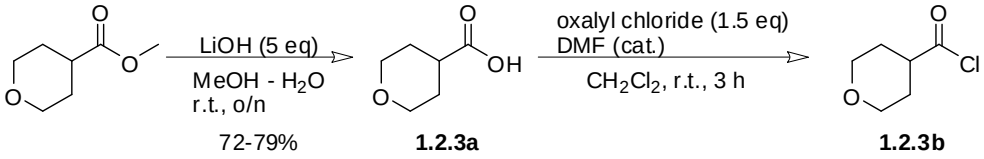
Table 1. Conditions of acylation reaction with phenylacetyl chloride.

Solvent	Base	Temperature	Time	Results
Pyridine	Pyridine	Reflux @ 125 ^{o}C	24 h	1 spot corresponding to starting material
Pyridine	Pyridine	MW @ 60 ^{o}C	1 h	3 spots
Pyridine	Pyridine	MW @ 30 ^{o}C	1 h	3 spots
THF	Pyridine (1 eq)	MW @ 100 ^{o}C	1 h	1 spot

Reflux conditions were tested since a large amount of energy is not being put into the reaction at once, it was thought that the reactivity of the aniline will allow for selectivity. However, after refluxing overnight, very little product was observed so the reaction was quenched and the starting material recovered. The acylation was then attempted using microwave conditions at 60 ºC and 30 ºC and results were the same. Although the pKa of an amide is around 20 and that of pyridine is around 5, the ortho and para substitution pattern of the nitro groups would decrease the pKa due to their electron withdrawing effects. It was suspected that excess pyridine was deprotonating the amide and due to the methylene next to the carbonyl,

a second acylation can occur because steric hindrance of the phenyl group can be avoided due to the rotatable methylenes of the two amides. Therefore, dilute conditions were opted for using dry THF as the solvent. Intermediate **1.1**, phenylacetyl chloride, and pyridine were added in equivalence, sequentially, and placed in the microwave using the original microwave conditions of 100 ºC for 1 h. This greatly reduced the amount of byproduct and starting material and intermediate **1.2.4** was obtained in 72% yield. It was observed that if ethanol was added to the yellow slurry that results after most of the solvent was evaporated, the solid that is obtained is much cleaner.

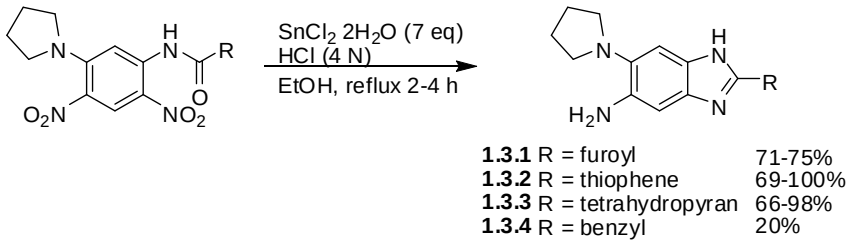
For the synthesis of intermediate **1.2.3**, the acid chloride had to be synthesized from methyl tetrahydro-2H-pyran-4-carboxylate, starting with the hydrolysis of the methyl ester and treating the resulting acid with oxalyl chloride (Scheme 4).



Scheme 4. Synthesis of acid chloride 1.2.3b

The hydrolysis gives the desired acid **1.2.3a** in moderately good yields. The acid is isolated via acid-base extraction. This extraction process resulted in the loss of some product primarily due to human error and solubility issues of **1.2.3a** in organic solutions. The conversion to the acid chloride was done in the presence of oxalyl chloride and dimethylformamide. DMF was added as a catalyst and the evolution of gas was observed due to the release of CO and CO_2 as the catalyst reacts with oxalyl chloride to form the iminium intermediate. This particular acylation was achieved under reflux conditions using THF as the solvent and the amide species **1.2.3** was obtained in poor to moderately good yields.

Once the 2 and 4 positions of the benzene core have been set, reduction of the nitro moieties followed by cyclization was achieved in a 2 step 1 pot reaction using tin(II) chloride dihydrate under harsh acidic conditions (Scheme 5).



Scheme 5. Reduction and cyclization for 2,5,6-trisubstitued benzimidazoles

The reaction proceeds smoothly, however purification over silica gel resulted in decomposition of the desired product. To prevent this decomposition, basification should be done in an ice-water bath as the heat generated was decomposing the product and purification for the 2,5,6-trisubstituted benzimidazole series should be done using a column packed with neutral alumina. Using these modifications, the final intermediates **1.3.1**, **1.3.2**, and **1.3.3** were obtained in good to excellent yield. In addition, the reactions were very clean and it was found that purification using a plug made of alumina to remove any remaining tin(II) chloride or tin byproducts. For the synthesis of **1.3.4**, monitoring the reaction by TLC proved to be quite unreliable. An aliquot of the reaction was worked up and the TLC showed one major spot. However, upon work up of the entire reaction, impurities were seen that possibly could have been the partially reduced or uncyclized intermediates, accounting for the low yield obtained. After purification, the product was obtained as a yellow solid in 20% yield.

Once all final intermediates were obtained, the synthesis of benzimidazole libraries began using acid chlorides, chloroformates, isocyanates, and anhydrides as the acylating agents (Figures 7-9). All intermediates were dissolved in dry methylene chloride and transferred to 96-

well plates. 72 acylating agents were added to **1.3.1-4** and reacted overnight at room temperature on a shaker. Aminomethylated polystyrene resin was added the next day to scavenge excess or unreacted acylating agents. After gentle shaking overnight, the resin was filtered to afford 240 2,5,6-trisubstituted benzimidazoles **1.1**. Plates with 5 µl aliquots of each well were set aside for LC-MS for analysis at a future date.

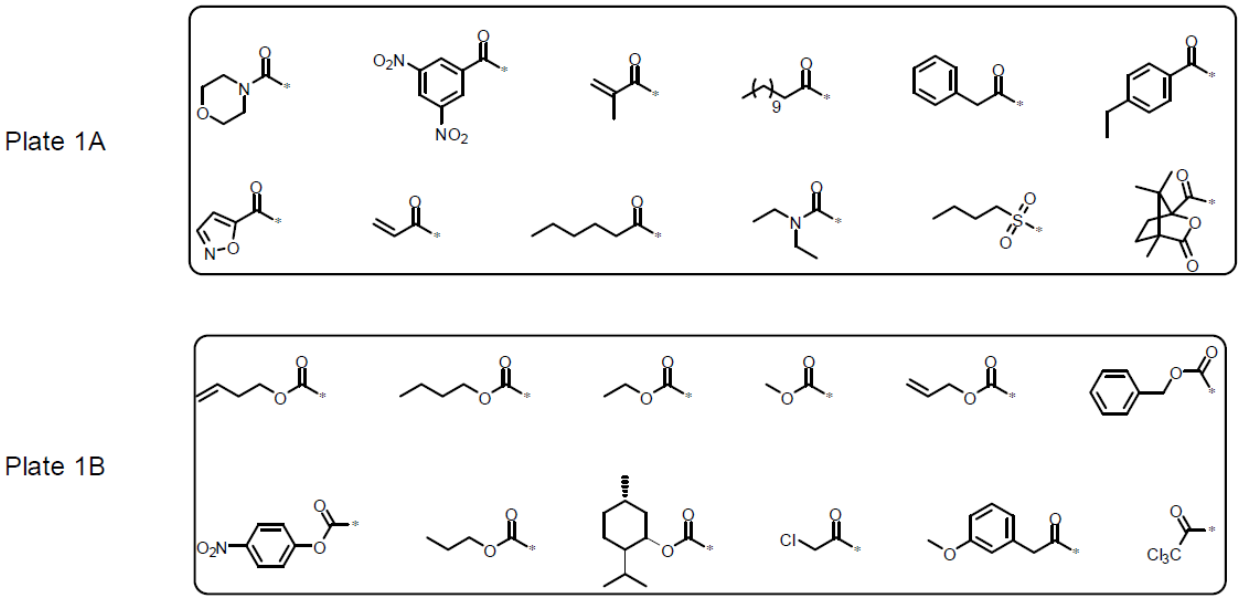


Figure 7. Reagents used for Plate 1

Plate 2A

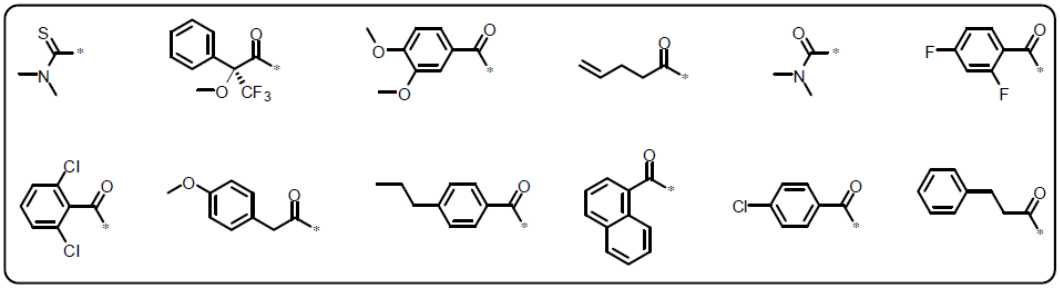


Plate 2B

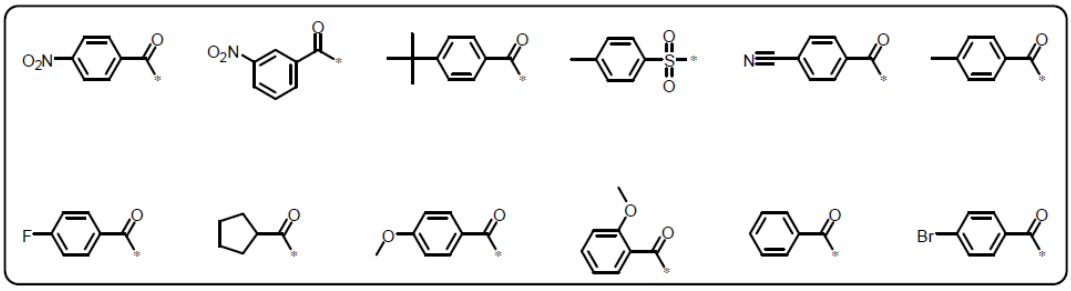


Figure 8. Reagents used for Plate 2

Plate 3A

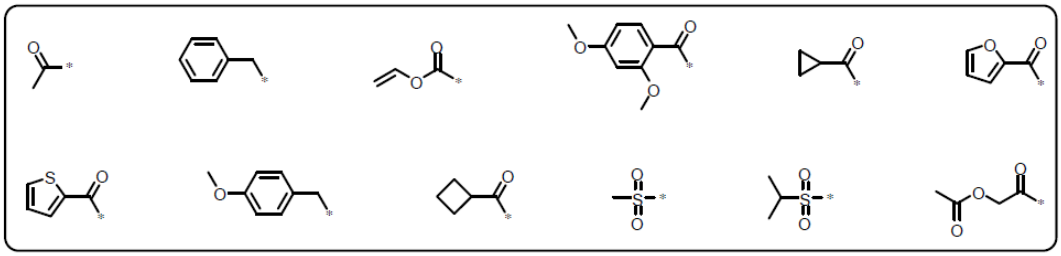


Plate 3B

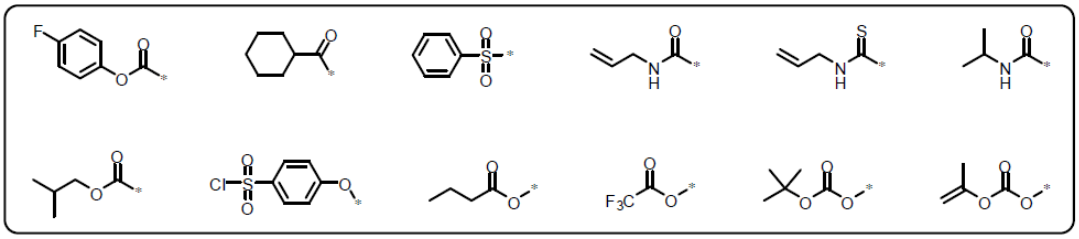
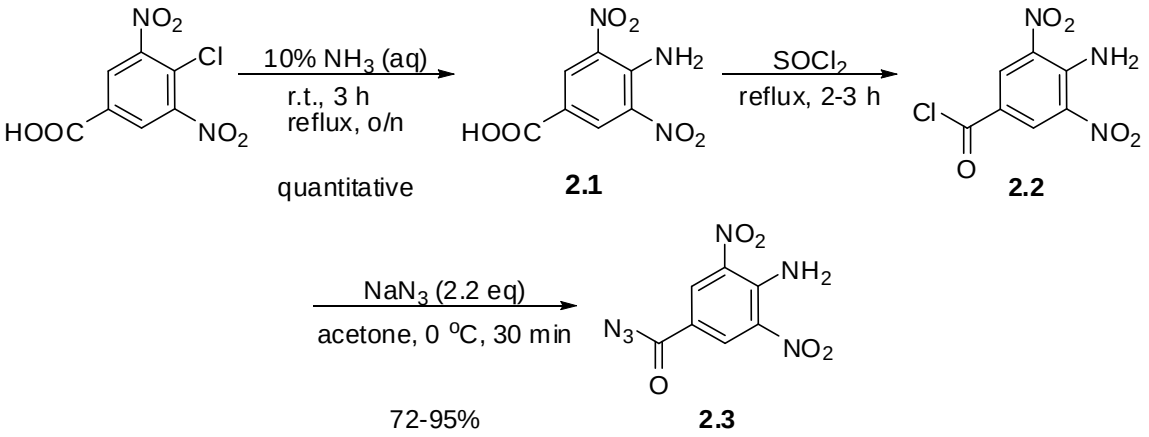


Figure 9. Reagents used for Plate 3

II. Resynthesis of 2,5,7-Trisubstituted Benzimidazole Final Intermediates

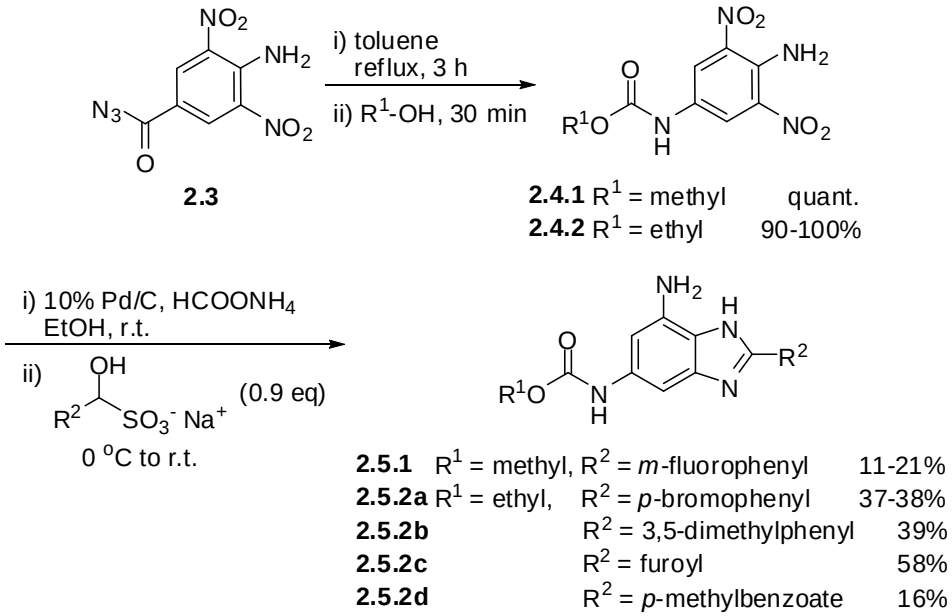
The commercially available starting material 4-chloro-3,5-dinitrobenzoic acid undergoes nucleophilic aromatic substitution in aqueous ammonia. The carboxylic acid is then converted to the acyl azide via formation of the acid chloride (Scheme 6).



Scheme 6. Synthesis of the azide

It was observed that when a solution of NH_3 in methanol was used, the reaction did not go to completion. This is most likely due to the decreased solubility of NH_3 in methanol.[14] However, the reaction proceeds cleanly in NH_4OH and the amine substituted dinitrobenzoic acid **2.1** was obtained in quantitative yield. Conversion of the benzoic acid species to the corresponding acid chloride was done under reflux using neat thionyl chloride to ensure full conversion and prevent the reverse reaction. Once the reaction was complete, thionyl chloride was removed as much as possible by distillation. The crude acid chloride was immediately converted to the acyl azide. Purification consisted of vacuum filtration and the product washed with copious amounts of acetone to remove any excess sodium azide to obtain the product in good to excellent yields. Due to the hazards associated with thionyl chloride, the reaction was also tried in methylene chloride using stoichiometric amounts of thionyl chloride but starting

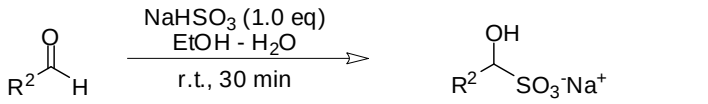
material was observed mixed in with the acyl azide. This is probably because the acetone used contained moisture and the amount of thionyl chloride left over after distillation was enough to remove the water through the generation of HCl and SO_2. Once the acyl azide was obtained, functionality of the 5 position was introduced via Curtius rearrangement (Scheme 7).



Scheme 7. Functionalization of the 2 and 5 positions

The azide intermediate **2.3** was refluxed in toluene to obtain the isocyanate. Methanol or ethanol was added in excess to form the desired carbamate species. The solvent was evaporated and the resulting solid filtered then washed with the alcohol used. The dinitro compound was then reduced using 10% Pd/C and ammonium formate and monitored by mass spectrometry. Upon completion, the palladium was filtered and washed with copious amounts of ethanol. Nucleophilic attack of the amine to the bisulfite salt, synthesized earlier from the corresponding aldehyde (Scheme 8), followed by intramolecular cyclization produces the desired final benzimidazole intermediate. Except in the synthesis of **2.5.1**, purification was achieved via flash

or gradient chromatography to obtain the benzimidazole final intermediate in poor to moderate yields. Due to solubility issues, the crude product was loaded as a solid deposit.



R^2 = *m*-fluorophenyl	quant.
R^2 = *p*-bromophenyl	86-95%
R^2 = 3,5-dimethylphenyl	92%
R^2 = furoyl	89%
R^2 = *p*-methylbenzoate	86%

Scheme 8. Synthesis of bisulfite salts of the corresponding aldehyde

Initially, reduction was set up under N_2 using 10 mol% of 10% Pd/C and took 6-24 h to complete. Increasing the amount of Pd/C to 15% decreased reaction time to 4-6 h. This was still too long as aromatic amines, especially triaminobenzenes, are known to oxidize easily. It was suspected that much of the H_2 generated *in situ* was escaping before oxidation addition could occur with the metal, so the set up was switched to a closed system filled with H_2, reducing hydrogenation time to 1-2 h. Completion of cyclocondensation after addition of the bisulfite salt depended upon the functionality introduced.

Purification of the cyclized benzimidazole over silica gel was difficult due to the polarity of the compounds, such as **2.5.1**, which had an R_f of 0.55 in 75% EtOAc. Decomposition over silica gel was also suspected to contribute to the poor yields, such as for those of intermediates **2.5.1** and **2.5.1d**, due to the appearance of a shadowy spot observed above or underneath the product collected in later fractions as well as the appearance of colored bands not seen on crude TLC. Therefore, purification had to be done quickly and columns packed to be as short as possible. This also posed a problem as some intermediates such as **2.5.2a** contained impurities with a R_f difference of 0.05 and a solvent gradient had to be used, thus increasing the amount of time that the product is in contact with silica gel. During the purification of **2.5.2b**, it was

observed that product could be recrystallized from impure fractions using hot organic solvents. However, recrystallization does not occur in fractions that also contained the baseline impurity, observed on the crude TLC of the related reduction cyclization reactions. Due to this observation, purification was attempted by extracting with ether and filtered through a plug to remove baseline impurities. This method was tried with intermediates **2.5.2c** and **2.5.1** and attempts to recrystallize the product resulted in either full decomposition or slight improvement in yield. During workup of the crude reaction mixture of **2.5.2c**, significant decomposition was observed when the organic layer was concentrated down on the rotovapor. Due to this observation, purification of this particular intermediate was therefore achieved by immediate formation of the solid deposit upon reaction completion, followed by flash chromatography using 85% EtOAc and a moderate improvement in yield was observed.

Conclusion

Four novel 2,5,6-trisubstituted benzimidazole and five known 2,5,7-trisubstituted benzimidazole final intermediates were obtained. The final intermediates for the 2,5,6-trisubstituted benzimidazoles were fully characterized and for the 2,5,7-trisubstituted benzimidazoles, ^{1}H NMR and FIA were taken to verify the correct compound was obtained. In addition, analysis for the two obtained batches of intermediate **2.5.2a** determined the purities to be at 95% and 99%. A library of 240 2,5,6-trisubstituted benzimidazoles was synthesized and awaiting analysis. One acylation reaction was optimized. Resynthesis of known 2,5,7-trisubstituted benzimidazole final compounds was not done due to time constraints, although a sufficient quantity of the desired final intermediate was obtained.

Experimental

Methods. ^{1}H NMR spectra were measured on a Varian 300 NMR spectrometer and ^{13}C NMR measured on a Varian 400 NMR spectrometer. Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel 60 or neutral alumina. High-resolution mass spectra were obtained from Mass Spectrometry Laboratory, University of Illinois at Urbana;Champaign, Urbana, IL.

Materials. Solvents and chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA) and Aldrich Co., Synquest Inc., and Sigma. Tetrahydrofuran was freshly distilled from sodium metal and benzophenone. Dichloromethane was also immediately prior to use under nitrogen from calcium hydride. Aminomethylated polystyrene resin EHL (200-400 mesh) 2% DVB was purchased from Novagen-biochem.

2,4-Dinitro-5-(pyrrolidin-1-yl)aniline (1.1):
2,4-dinitro-5-fluoroaniline (2.26 g, 11.25 mmol) was dissolved in dry THF (40 ml) and stirred under N_2. Pyrrolidine (1.02 ml, 1.1 eq.) and DIPEA (2.16 ml, 1.1 eq) were added sequentially and the reaction allowed to stir at room temperature overnight. The solvent was then removed by rotovapor and the solid dissolved in methylene chloride and washed three times with water then once with brine. The organic layer was then dried over Na_2SO_4 and removed *in vacuo* to obtain the product **1.1** as a yellow solid in quantitative yield: mp 170-171 ºC; ^{1}H NMR ($CDCl_3$) δ 2.05-2.12 (m, 4 H), δ 3.35 (t, 4 H, J = 6.6 Hz), δ 5.98 (s, 1 H), δ 6.37 (s, 2 H), δ 8.81 (s, 1 H); ESI MS *m/z* 253.1 $[M+H]^+$.

General Procedure for the Synthesis of 1.2.1 and 1.2.2:
Compound **1.1** (1.0 eq.) dissolved in pyridine (1.5 ml/mmol) was placed in a microwave tube with a stir bar and the acyl chloride (1.2 eq) added. The reaction was heated in the microwave at 100 ºC for 1 h. The solution was then transferred to a flask and the pyridine removed under pressure. Methanol or ethanol was added and the resultant precipitate filtered and washed with cold methanol and allowed to dry overnight.

***N*-[2,4-Dinitro-5-(pyrrolidin-1-yl)phenyl]furan-2-carboxamide (1.2.1):**
Green-yellow solid, 81% yield : mp > 200 ºC; ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 4 H), δ 3.41 (t, 4 H, J=6.6 Hz), δ 6.62 (m, 1 H), δ 7.32 (m, 1 H), δ 7.64 (dd, 1 H, J=1.5 Hz), δ 8.59 (s, 1 H), δ 8.84 (s, 1 H), δ 11.94 (s, 1 H); ESI MS *m/z* 347.1 $[M+H]^+$.

***N*-[2,4-Dinitro-5-(pyrrolidin-1-yl)phenyl]thiophene-2-carboxamide (1.2.2):**
Yellow solid, 85% yield: mp > 200 ºC; ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 4 H), δ 3.41 (t, 4 H, J=6.6 Hz), δ 7.18 (dd, 1 H), δ 7.75 (d, 1 H, J=6 Hz), δ 7.79 (d, 1 H, J=4.8 Hz), δ 8.59 (s, 1 H), δ 8.84 (s, 1 H), δ 11.94 (s, 1 H); ESI MS *m/z* 363.1 $[M+H]^+$.

Tetrahydro-2*H*-pyran-4-carboxylic acid (1.2.3a):
Methyl tetrahydro-2H-pyran-4-carboxylate (1.08 g, 7.55 mmol) dissolved in methanol (30 ml) was added to a stirring solution of LiOH (5 eq.) in water (15 ml) and methanol (30 ml) and allowed to stir overnight. After the solvent was reduced under pressure, excess water was added and extracted with methylene chloride to remove any unreacted carboxylate. The aqueous layer was then acidified with 37% HCl until the pH was 2, then extracted again with CH_2Cl_2. The organic layers were combined, washed with brine then dried over sodium sulfate and reduced under pressure to afford the carboxylic acid **1.2.3a** as an off white solid (775 mg, 79%): ^{1}H NMR ($CDCl_3$) δ 1.89 (m, 4 H), δ 2.66 (m, 1 H), δ 3.50 (m, 2 H), δ 4.06 (m, 2 H); ESI MS *m/z* 131.1 $[M+H]^+$.

***N*-[2,4-Dinitro-5-(pyrrolidin-1-yl)phenyl]tetrahydro-2*H*-pyran-4-carboxamide (1.2.3):**
Compound **1.2.3a** (330 mg, 2.56 mmol) was dissolved in methylene chloride (5 ml) and stirred under N_2. 2 M Oxalyl chloride (1.92 ml, 1.5 eq) was added along with 2 drops of DMF (cat.). The reaction was stirred for 3 h then placed on the rotovapor to remove solvent and afford the acid chloride **1.2.3b** as a clear yellow liquid that was then immediately diluted in THF (10 ml). **1.1** (645 mg, 1.0 eq.) and pyridine (3 ml) were added sequentially and the reaction refluxed overnight. Methanol was added after the solvents were removed under reduced pressure and the product filtered as a bright yellow solid (635 mg, 68% over 2 steps): ^{1}H NMR (CD_3OD) δ 1.95 (m, 8 H), δ 3.02 (m, 5 H), δ 3.50 (m, 3 H), δ 4.05 (m, 2 H), δ 6.91 (s, 1 H), δ 7.18 (s, 1 H); ESI MS *m/z* 365.1 $[M+H]^+$.

***N*-[2,4-dinitro-5-(pyrrolidin-1-yl)phenyl]-2-phenylacetamide (1.2.4):**
Compound **1.1** (1.19 g, 4.72 mmol) was dissolved in THF (20 ml) and stirred in a microwave tube with pyridine (0.38 ml, 1.0 eq.). Phenylacetyl chloride (0.625 ml, 1.0 eq.) was added and the tube placed in the microwave at 100 ºC for 1 h. Using ethanol, work up is the same as in the previous procedure and the product was filtered as a bright yellow solid (1.16 g, 72%): ^{1}H NMR ($CDCl_3$) δ 2.02 (p, 4 H, J=6.6 Hz), δ 3.35 (t, 4 H, J=6.6 Hz), δ 3.83 (s, 2 H), δ 7.40 (m, 5 H), δ 8.46 (s, 1 H), δ 8.74 (s, 1 H), δ 10.87 (s, 1 H)); ESI MS *m/z* 371.1 $[M+H]^+$.

General Procedure for the Synthesis of 1.3.1-4:
The acylated intermediate (1.0 eq) and $SnCl_2 \cdot 2H_2O$ (7.0 eq) was stirred in ethanol (9 ml/mmol). 12 N HCl was added to obtain a final concentration of 4 N. The reaction was refluxed for 2-4 h and monitored by FIA or TLC. Once completed, the reaction was cooled to room temperature, placed in an ice water bath and quenched with 30% NaOH until the pH was 12-14. The aqueous layer was then extracted with Methylene chloride and purified by either flash chromatography (1% MeOH in CH_2Cl_2) or plug using neutral Alumina.

2-(furan-2-yl)-6-(pyrrolidin-1-yl)-1*H*-benzimidazol-5-amine (1.3.1):
Tan solid, 71-75% yield: mp 143-145 ºC; ^{1}H NMR ($CDCl_3$) δ 1.91 (m, 4 H), δ 3.01 (m, 4 H), δ 6.49 (q, 1 H, J=3.45 Hz), δ 6.84 (s, 1 H), δ 7.04 (d, 1 H, J=3.6 Hz), δ 7.27 (s, 1 H), δ 7.38 (d, 1 H, J=1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 23.95, 51.74, 98.72, 106.10, 109.41, 112.17, 133.23, 134.10, 139.29, 142.28, 143.01, 143.05, 145.72. HRMS (ESI) *m/z* calcd for $C_{15}H_{17}N_4O$ 269.1402 $[M+H]^+$. Found 269.1404.

6-(pyrrolidin-1-yl)-2-(thiophen-2-yl)-1*H*-benzimidazol-5-amine (1.3.2):
Yellow solid, 69% to quant. yield: mp 173-175 ºC; ^{1}H NMR (CD_3OD) δ 1.85 (m, 4 H), δ 2.93 (m, 4 H), δ 6.81 (s, 1 H), δ 7.04 (q, 1 H, J=4.95), δ 7.10 (s, 1 H), δ 7.40 (q, 1 H, J=4.95), δ 7.50 (q, 1 H, J=3.75 Hz); ^{13}C NMR ($CDCl_3$) δ 23.99, 51.81, 98.58, 106.32, 125.50, 125.54, 126.91, 127.85, 133.90, 134.58, 135.96, 139.18, 145.75. HRMS (ESI) *m/z* calcd for $C_{15}H_{17}N_4S$ 285.1174 $[M+H]^+$. Found 285.1172.

6-(pyrrolidin-1-yl)-2-(tetrahydro-2*H*-pyran-4-yl)-1*H*-benzimidazol-5-amine (1.3.3):
White solid, 66-98% yield: mp > 200 ºC; ^{1}H NMR (CD_3OD) δ 1.90 (m, 8 H), δ 3.01 (m, 5 H), δ 3.55 (m, 2 H), δ 4.00 (m, 2 H), δ 6.91 (s, 1 H), δ 7.18 (s, 1 H); ^{13}C NMR (CD_3OD) δ 25.12, 32.65, 36.94, 53.21, 68.75, 100.77, 106.33, 134.04, 135.53, 136.94, 140.04, 157.35. HRMS (ESI) *m/z* calcd for $C_{16}H_{23}N_4O$ 287.1872 $[M+H]^+$. Found 287.1872.

2-benzyl-6-(pyrrolidin-1-yl)-1*H*-benzimidazol-5-amine (1.3.4):
Yellow solid, 20% yield: ^{1}H NMR ($CDCl_3$) δ 1.89 (t, 4 H, J=4.65 Hz), δ 2.96 (s, 4 H), δ 4.12 (s, 2 H), δ 6.71 (s, 1 H), δ 7.26 (m, 7 H); ^{13}C NMR ($CDCl_3$) δ 24.01, 35.73, 51.87, 98.80, 106.05, 126.93, 128.79, 128.91, 133.20, 134.28, 135.09, 136.94, 138.54, 151.45; HRMS (ESI) *m/z* calcd for $C_{18}H_{21}N_4$ 293.1766 $[M+H]^+$. Found 293.1768.

Procedure and Calculations for the Synthesis of Libraries on 96-well Plates:
Intermediates **1.3.1** to **1.3.3** was plated twice in each 8x12 plate and **1.3.4** plated once on two plates. The amount of final intermediates needed for three plates was calculated such that each well contains 0.01 mmol of compound that would react with 12 different acylating agents. The general formula to calculate the amount in milligrams ($m_{(th)}$) needed for each row is

$$m(th) = MW(inter.) \times 0.01\, mmol \times (n+1), \quad \textbf{(1)}$$

where n is the number of wells, and adding 1 to adjust for pipetting errors. After the intermediates are weighed out, the amount of solvent needed in milliliters to dissolve each intermediate is calculated using the formula

$$\frac{m(th) \times 0.1\, ml \times (n+1)}{m(weighed)}, \quad \textbf{(2)}$$

so that each well contained 0.1 ml (100 µl) of solution. The same equations were used for calculations of the acylating reagents, except in the case of chloroformates, where 0.009 mmol was calculated instead.
After all acylating agents were weighed out, intermediates and reagents were dissolved in the calculated amount of solvent and aliquoted. Methylene chloride was used and in some cases of insolubility, a few drops of DMF had to be added. An extra 100 µl of solvent was added so that each well contained a total of 300 µl. The plates were then covered and sealed with parafilm and placed on the shaker at low speed overnight. The following day, to each well was added 150 µl of solvent and 1 mg of resin. The aminomethylated polystyrene resin 302 EHL (200-400 mesh) 2% DVB was purchased from Novagen-303biochem. The plates were again covered, sealed with parafilm, and shaken overnight. The plates were filtered the next day using a special vacuum pump attachment to remove the resin. Each well was washed with solvent three times and filtered onto a new plate. 5 µl of solution from each well was then transferred to another plate to await LC-MS. The solvent was allowed to evaporate and the plates covered, sealed in parafilm, and placed in the refrigerator at -20 ºC.

4-amino-3,5-dinitrobenzoic acid (2.1):
4-chloro-3,5-dinitrobenzoic acid (20.0 g, 8.13 mmol) was dissolved in MeOH (50 ml). 10% aq NH_3 (250 ml) was added and the solution changed from clear yellow to orange. After stirring at room temperature for 2 h, the reaction was refluxed overnight. The solution was then allowed to cool to room temperature and the solvent removed by rotovapor. Additional water was added and the precipitated product was then vacuum filtered and allowed to air dry to afford **2.1** as a yellow solid (18.8 g, quant.): ^{1}H NMR (CD_3OD) δ 8.94 (s, 2 H). ESI MS *m/z* 228.1 $[M+H]^+$.

4-amino-3,5-dinitrobenzoyl azide (2.3):
Intermediate **2.1** (1.03 g, 4.54 mmol) was refluxed in neat $SOCl_2$ (4 ml) for 3 h. The $SOCl_2$ was then distilled then placed on the rotovapor to remove as much of the acid as possible. The resulting acid chloride **2.2** was then dissolved in acetone (12 ml) and stirred in an ice-water bath. NaN_3 (2.2 eq, 670 mg) dissolved in H_2O (3 ml) was added dropwise to the solution and the reaction allowed to stir for 30 min, upon which an additional 10 ml of water was added and the resulting azide extracted with methylene chloride (3x). The organic layer was then combined and washed with brine, dried over $MgSO_4$, and the solvent removed by rotovapor to afford the product **2.3** as a yellow solid (1.09 g, 95%): decomposition at 145-146 ºC; ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 1 H), δ 8.89 (s, 2 H), δ 9.14 (s, 2 H).

Methyl (4-amino-3,5-dinitrophenyl)carbamate (2.4.1):
Intermediate **2.3** (1.29 g, 5.11 mmol) was refluxed in toluene (40 ml) for 2 h. The reaction was allowed to cool to room temperature and dry MeOH (5 ml) was added. The reaction was allowed to stir at room temperature for 1 h then placed on the rotovapor to remove most of the solvent. The resulting precipitate was vacuum filtered to afford red crystals as intermediate **2.4.1** (1.31 g, quant.): ^{1}H NMR (CD_3OD) δ 3.63 (s, 4 H). δ 8.58 (s, 2 H); ESI MS *m/z* 255.0 $[M+H]^+$.

Ethyl (4-amino-3,5-dinitrophenyl)carbamate (2.4.2):
The azide **2.3** (1.00 g, 3.97 mmol) was refluxed in toluene (25 ml) for 2.5 h. The reaction was allowed to cool down to room temperature and dry EtOH (10 ml) was added. The reaction was

allowed to stir at room temperature for 30 min. All solvent was then removed by rotovapor then vacuum pump to afford **2.4.2** as red crystals (1.13 g, quant.): mp 163-165 ºC; ^{1}H NMR (CD_3OD) δ 1.30 (t, 3 H, J = 7.2 MHz), 4.21 (q, 2 H, J = 7.2 MHz); ESI MS *m/z* 255.0 $[M+H]^+$.

General Procedure for the Synthesis of 2.5.1-2d:
To a solution of the dinitrophenyl carbamate intermediate (1.0 eq) stirred in dry ethanol (54 ml/mmol) under N_2 was added Pd/C (0.15 eq) followed by ammonium formate (30 eq) and the atmosphere switched to H_2. The reaction was monitored by FIA and upon completion, Pd/C was filtered through celite and washed with an additional 100 ml of ethanol. The filtrate was stirred in an ice-water bath and the bisulfite salt of the aldehyde (Scheme 8) dissolved in the minimum amount of water was added dropwise. Upon completion of the reaction, the product was purified over silica gel.

Methyl [7-amino-2-(3-fluorophenyl)-1*H*-benzimidazol-5-yl]carbamate (2.5.1):
Off white solid; 11-21% yield; mp 149-151 ^{o}C; ^{1}H NMR (CD_3OD) δ 3.61 (s, 3 H), 7.11 (m, 2 H), 7.437-7.45 (m, 1 H), 7.71 (m, 2 H); ESI MS *m/z* 301.1 $[M+H]^+$.

Ethyl [7-amino-2-(4-bromophenyl)-1*H*-benzimidazol-5-yl]carbamate (2.5.2a):
Brown solid; 37-38% yield; mp > 200 ^{o}C; ^{1}H NMR (CD_3OD) δ 1.31 (t, 3 H, J=6.9 Hz), 4.18 (q, 2 H, J=13.8 Hz), 6.10 (s, 1 H), 7.35 (dd, 4 H); ESI MS *m/z* 375.1 $[M+H]^+$.

Ethyl [7-amino-2-(3,5-dimethylphenyl)-1*H*-benzimidazol-5-yl]carbamate (2.5.2b):
Yellow solid; 39% yield; mp 176-178 ^{o}C; ^{1}H NMR (CD_3OD) δ 1.18 (t, 4 H, J=6.6 Hz), 2.27 (s, 6 H), 4.06 (q, 2 H, J=14.1 Hz), 6.38 (s, 1 H), 6.99 (s, 1 H), 7.44 (s, 1 H), 7.52 (s, 2 H); ESI MS *m/z* 325.1 $[M+H]^+$.

Ethyl [7-amino-2-(furan-2-yl)-1*H*-benzimidazol-5-yl]carbamate (2.5.2c):
Off white solid; 58% yield; mp 143-145 ^{o}C; ^{1}H NMR (CD_3OD) δ 1.18 (t, 4 H, J=6.6 Hz), 2.27 (s, 6 H), 4.06 (q, 2 H, J=14.1 Hz), 6.38 (s, 1 H), 6.99 (s, 1 H), 7.44 (s, 1 H), 7.52 (s, 2 H); ESI MS *m/z* 287.1 $[M+H]^+$.

Methyl 4-{7-amino-5-[(ethoxycarbonyl)amino]-1*H*-benzimidazol-2-yl}benzoate (2.5.2d):
Yellow solid; 16% yield; mp > 200 ^{o}C; ^{1}H NMR (CD_3OD) δ 1.18 (t, 4 H, J=6.6 Hz), 2.27 (s, 6 H), 4.06 (q, 2 H, J=14.1 Hz), 6.38 (s, 1 H), 6.99 (s, 1 H), 7.44 (s, 1 H), 7.52 (s, 2 H); ESI MS *m/z* 325.1 $[M+H]^+$.

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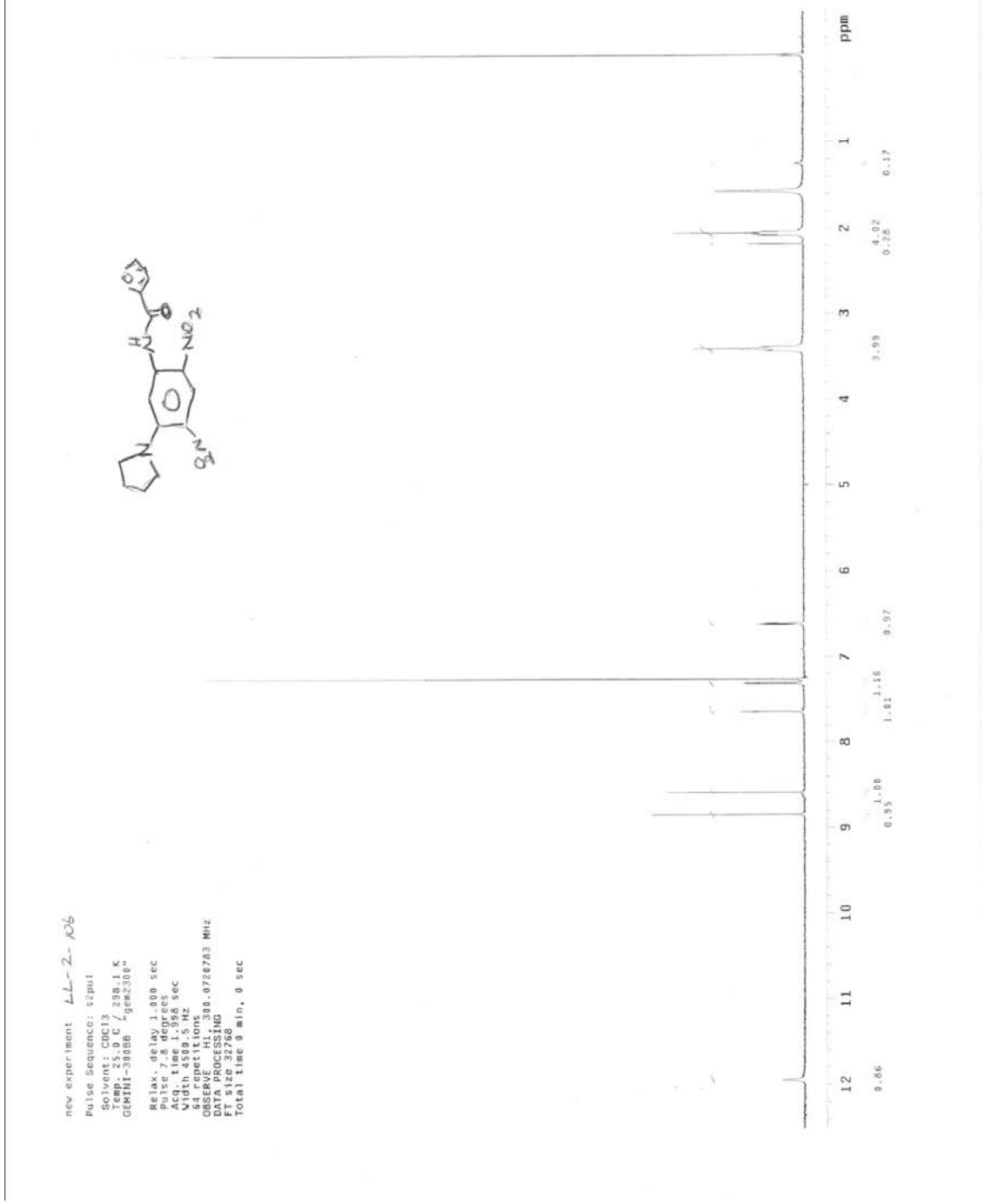
Appendix – Spectra




new experiment 11-2-109
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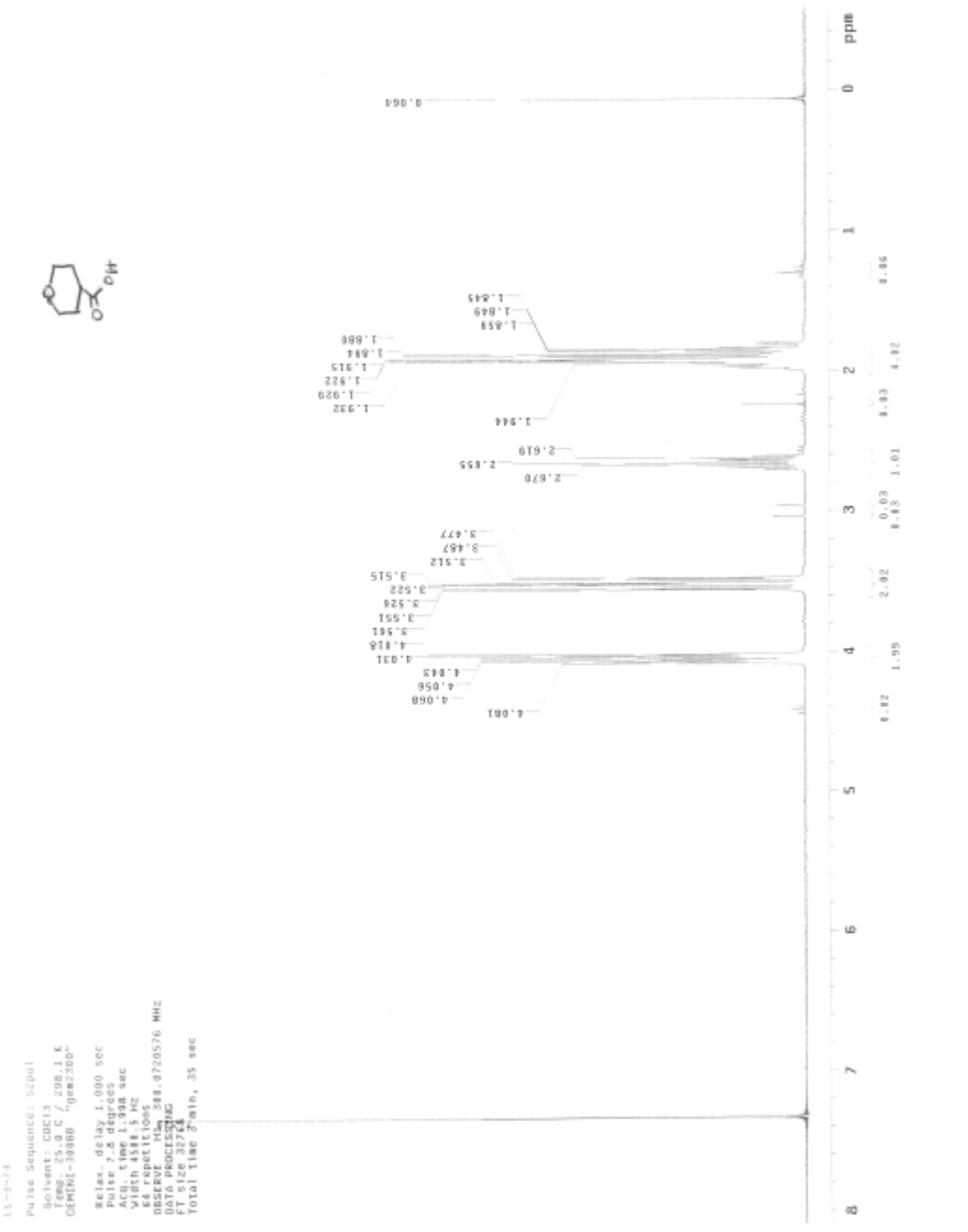
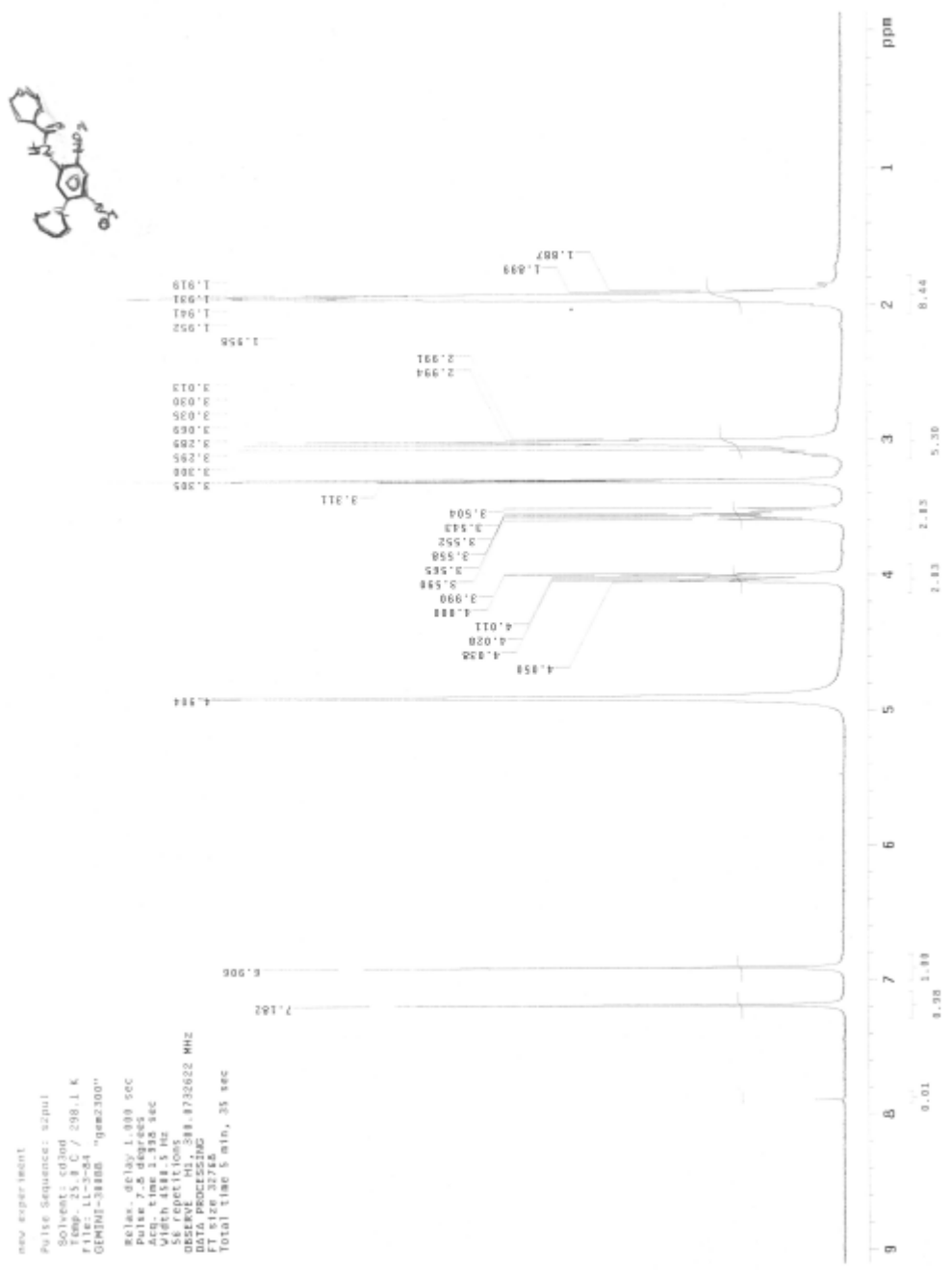
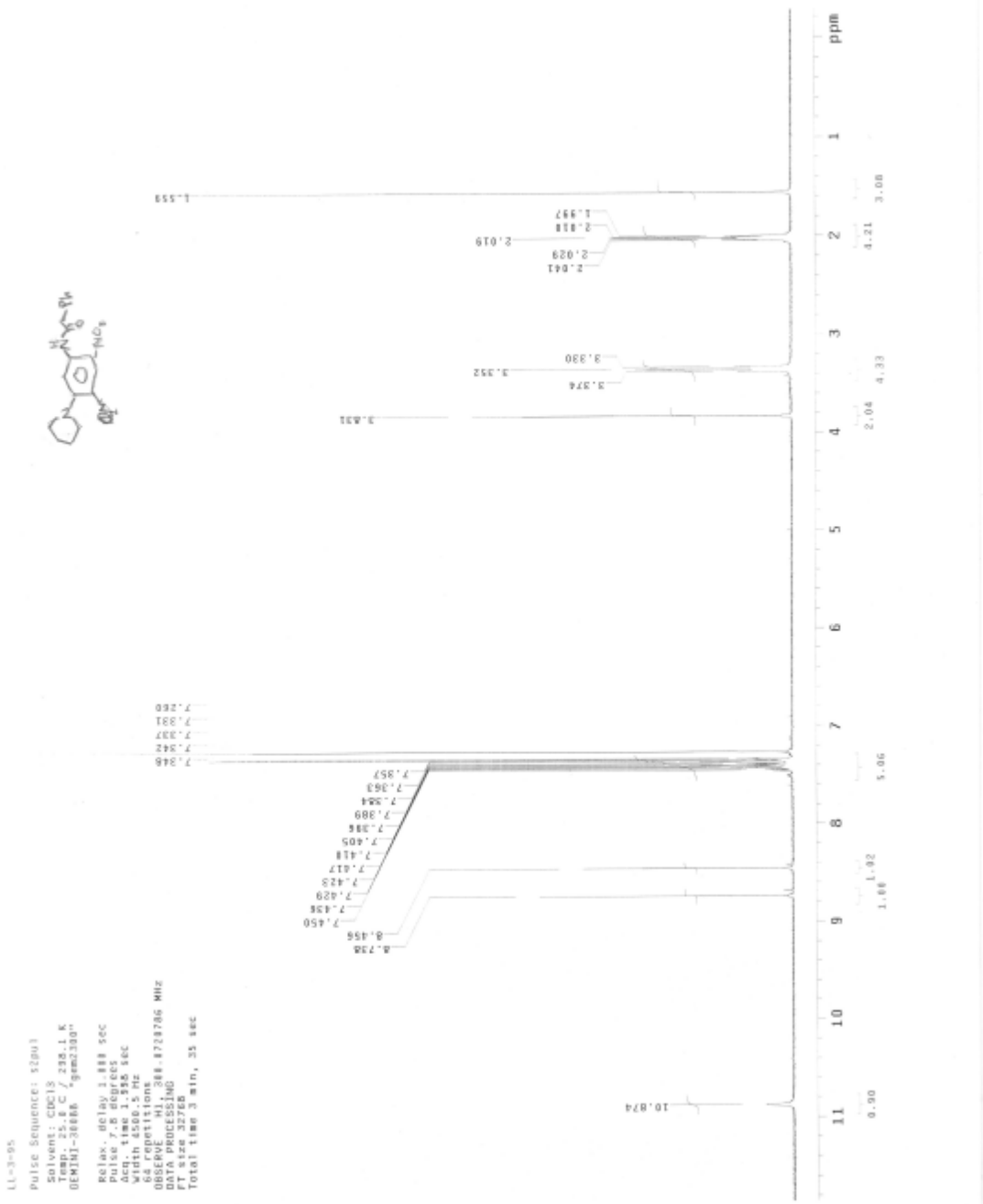
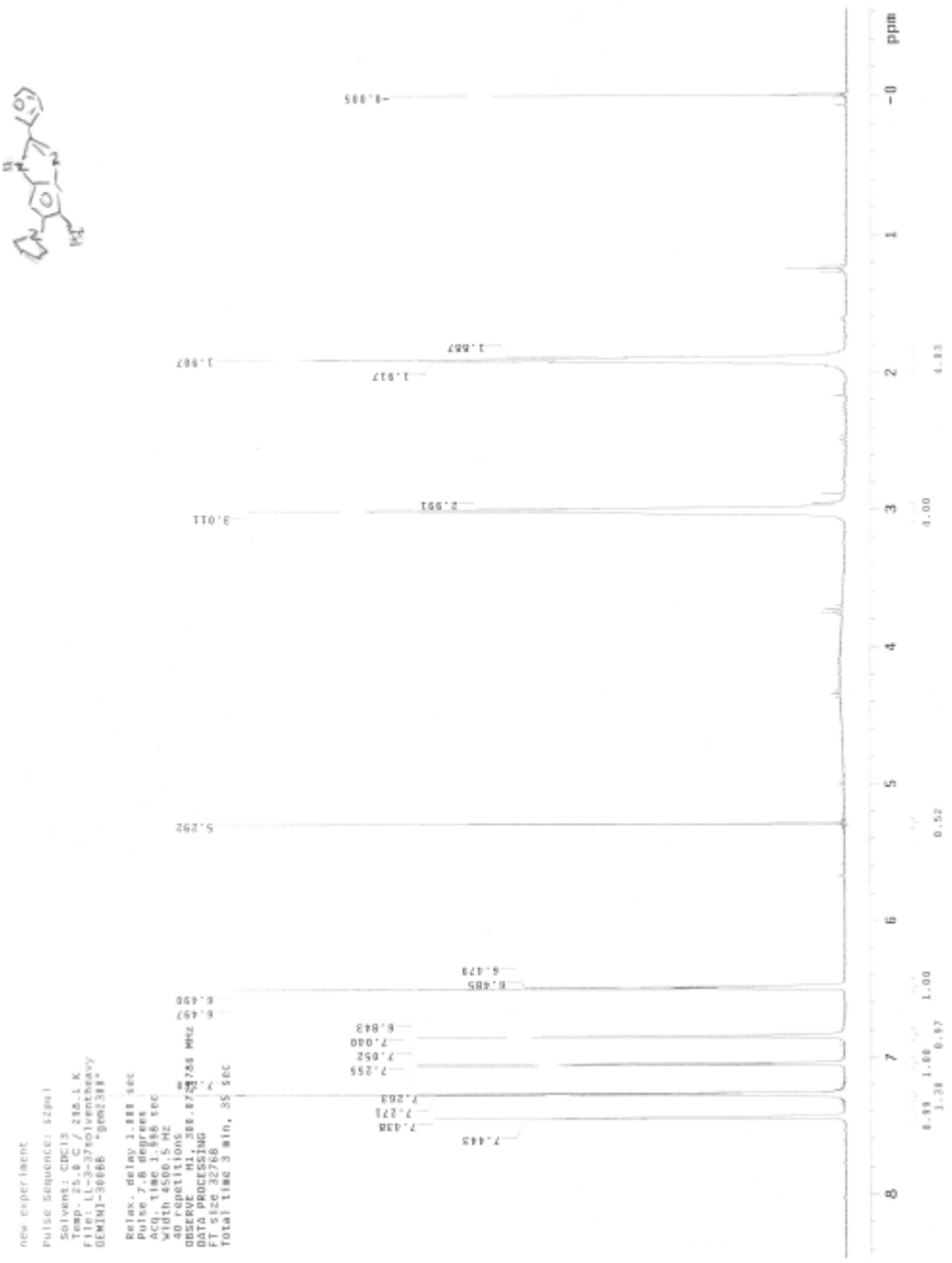
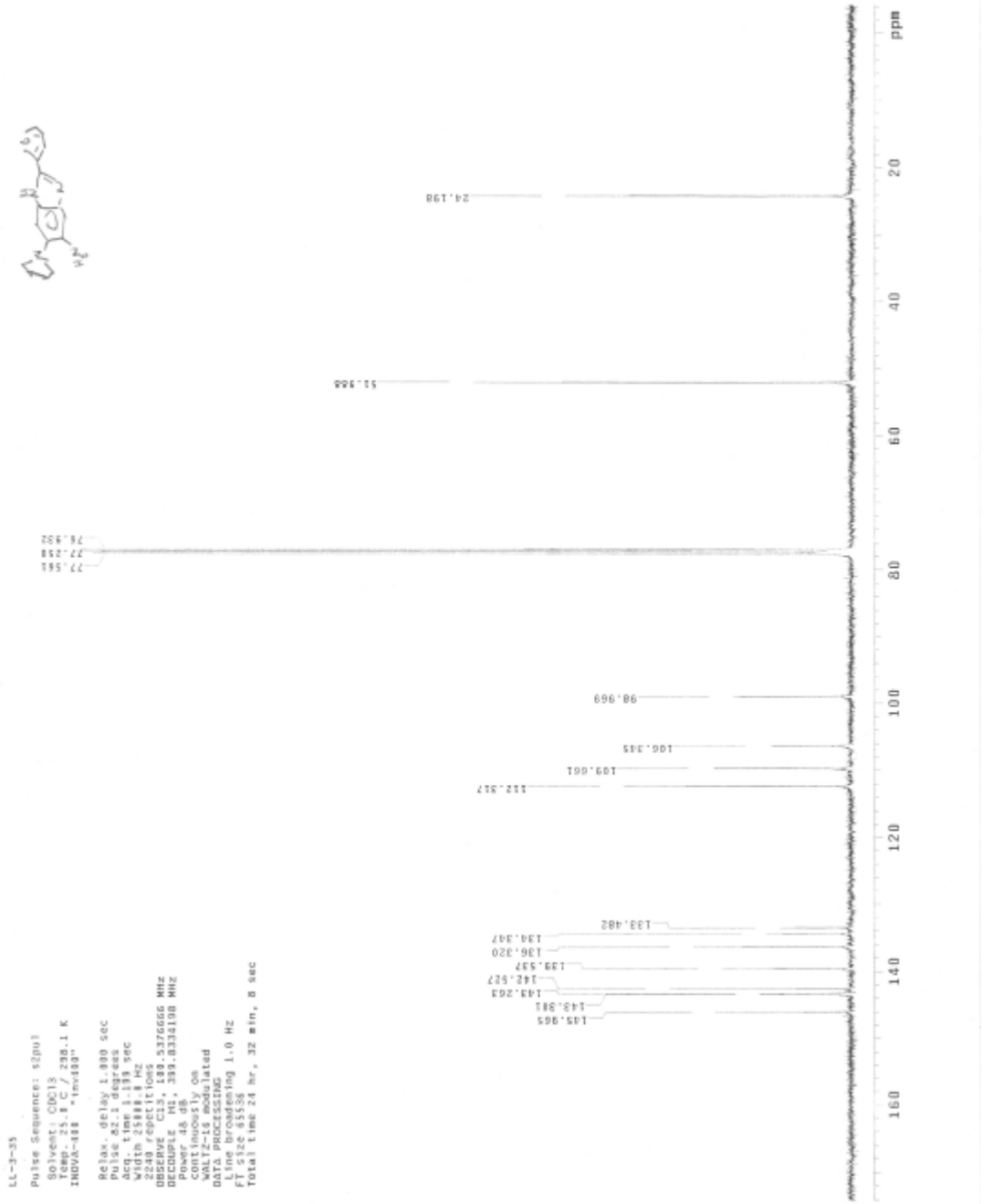
Solvent: CDCl3
Temp. 25.8 C / 298.1 K
GEMINI-300BB "gem2300"
Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.915 sec
Width 4500.5 Hz
40 repetitions
OBSERVE H1, 300.0720783 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 4 sec
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.83
1.00
1.01
1.04
1.18
1.01
4.25
4.27
2.88
0.24

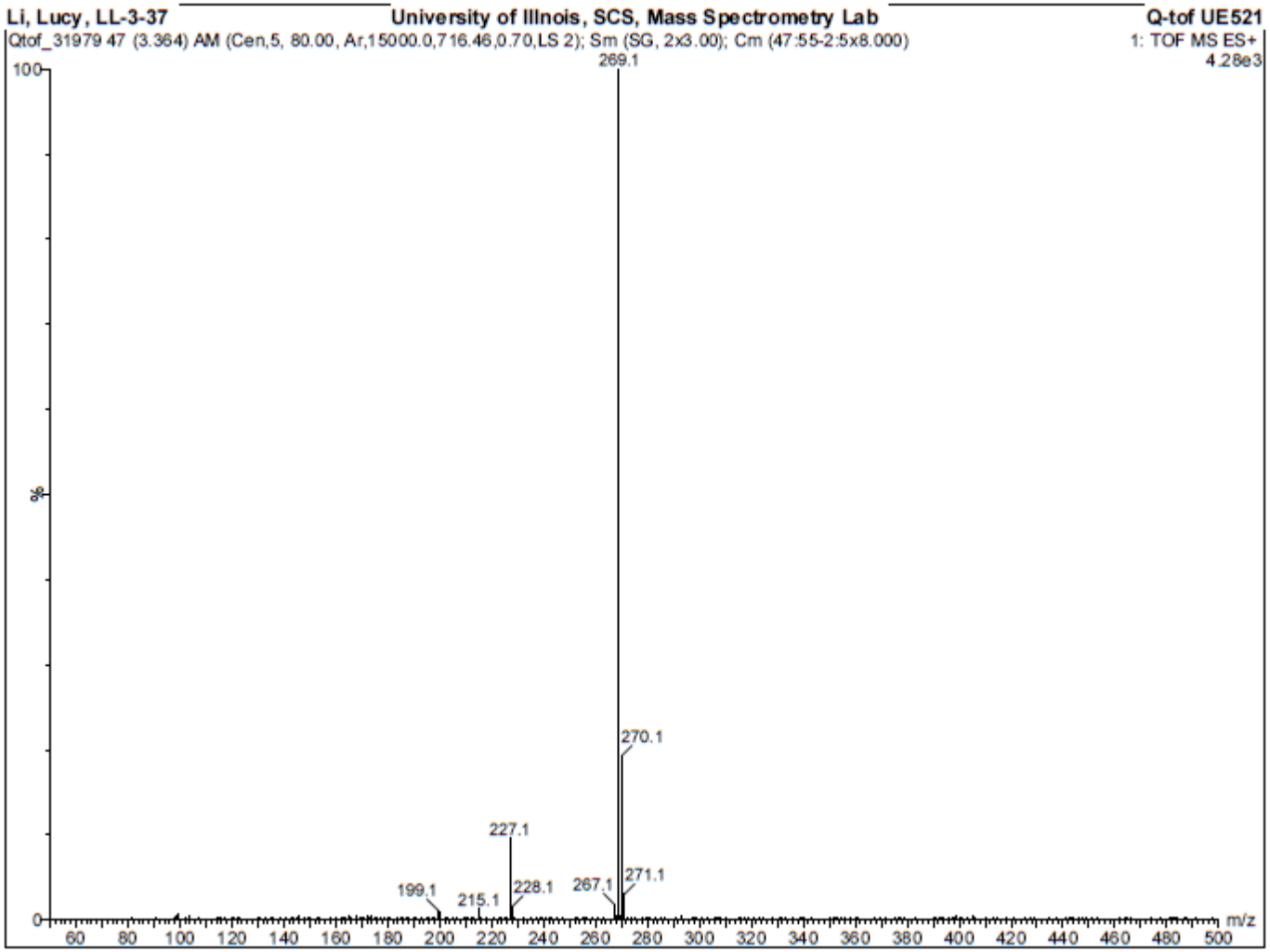

LL-3-74
Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.0 C / 298.1 K
GEMINI-300BB "gem300"
Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 5.798 sec
Width 4813.4 Hz
64 repetitions
OBSERVE H1, 300.0720576 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
0.064
1.845
1.849
1.859
1.880
1.884
1.915
1.922
1.929
1.932
1.944
2.619
2.655
2.670
3.477
3.487
3.512
3.515
3.522
3.526
3.551
3.561
4.018
4.031
4.043
4.056
4.068
4.081
ppm
8.02
1.99
2.02
0.03
0.85
1.01
8.03
4.02
8.06


new experiment
Pulse Sequence: s2pul
Solvent: cd3od
Temp: 25.0 / 298.1 K
File: LL-3-84
GEMINI-38888 "gem2300"
Relax. delay 1.000 sec
Pulse 7.5 degrees
Acq. time 1.398 sec
Width 4598.5 Hz
56 repetitions
OBSERVE H1, 388.8732622 MHz
DATA PROCESSING
FT size 32768
Total time 5 min, 35 sec
ppm
1.887
1.899
1.919
1.931
1.941
1.952
1.956
2.991
2.994
3.013
3.030
3.035
3.069
3.289
3.295
3.308
3.385
3.811
3.504
3.543
3.552
3.558
3.565
3.598
3.990
4.888
4.011
4.020
4.038
4.858
4.984
6.906
7.182


LL-3-95
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
DEMIN-30000 "gem2300"
Relax. delay 1.000 sec
Pulse 7.0 degrees
Acq. time 1.595 sec
Width 4500.5 Hz
64 repetitions
OBSERVE H1, 300.0728706 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
10.874
8.738
8.456
7.650
7.636
7.629
7.623
7.617
7.418
7.405
7.386
7.389
7.384
7.363
7.357
7.348
7.342
7.337
7.331
7.260
3.831
3.374
3.352
3.330
2.061
2.029
2.019
1.997
1.818
1.559
ppm


new experiment
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
File: LL-3-37nolventheavy
DEMIMI-30066 *@pm2300*
Relax. delay 1.000 sec
Pulse 7.0 degrees
acq. time 1.998 sec
width 4500.5 Hz
40 repetitions
OBSERVE H1, 300.0732785 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
8.485
3.887
3.917
1.987
2.991
3.011
5.292
6.485
6.479
8.690
8.697
8.643
7.440
7.052
7.255
7.263
7.271
7.438
7.443
ppm
4.00
0.52
1.00
0.97
1.66
1.38
4.83


LL-3-35
Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.1°C / 298.1 K
INOVA-400 "rmr400"
Relax. delay 1.000 sec
Pulse 23.0 degrees
Acq. time 1.193 sec
Width 25000.8 Hz
2240 repetitions
OBSERVE C13, 100.5376666 MHz
DECOUPLE H1, 399.8334198 MHz
Power 46 dB
continuously ON
WALTZ-16 modulated
DATA processing
Line broadening 1.0 Hz
FT size 65536
Total time 24 hr, 32 min, 8 sec
24.198
51.986
76.932
77.258
77.582
90.969
106.345
109.661
112.317
133.482
134.347
136.320
139.537
142.527
143.263
143.381
145.965
ppm

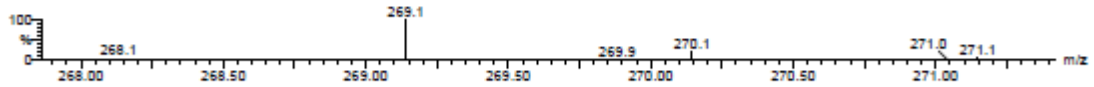

Li, Lucy, LL-3-37
University of Illnois, SCS, Mass Spectrometry Lab
Q-tof UE521
Qtof_31979 47 (3.364) AM (Cen,5, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (47:55-2:5x8.000)
1: TOF MS ES+
4.28e3
100
%
0
269.1
270.1
227.1
199.1
215.1
228.1
267.1
271.1
m/z
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 150.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

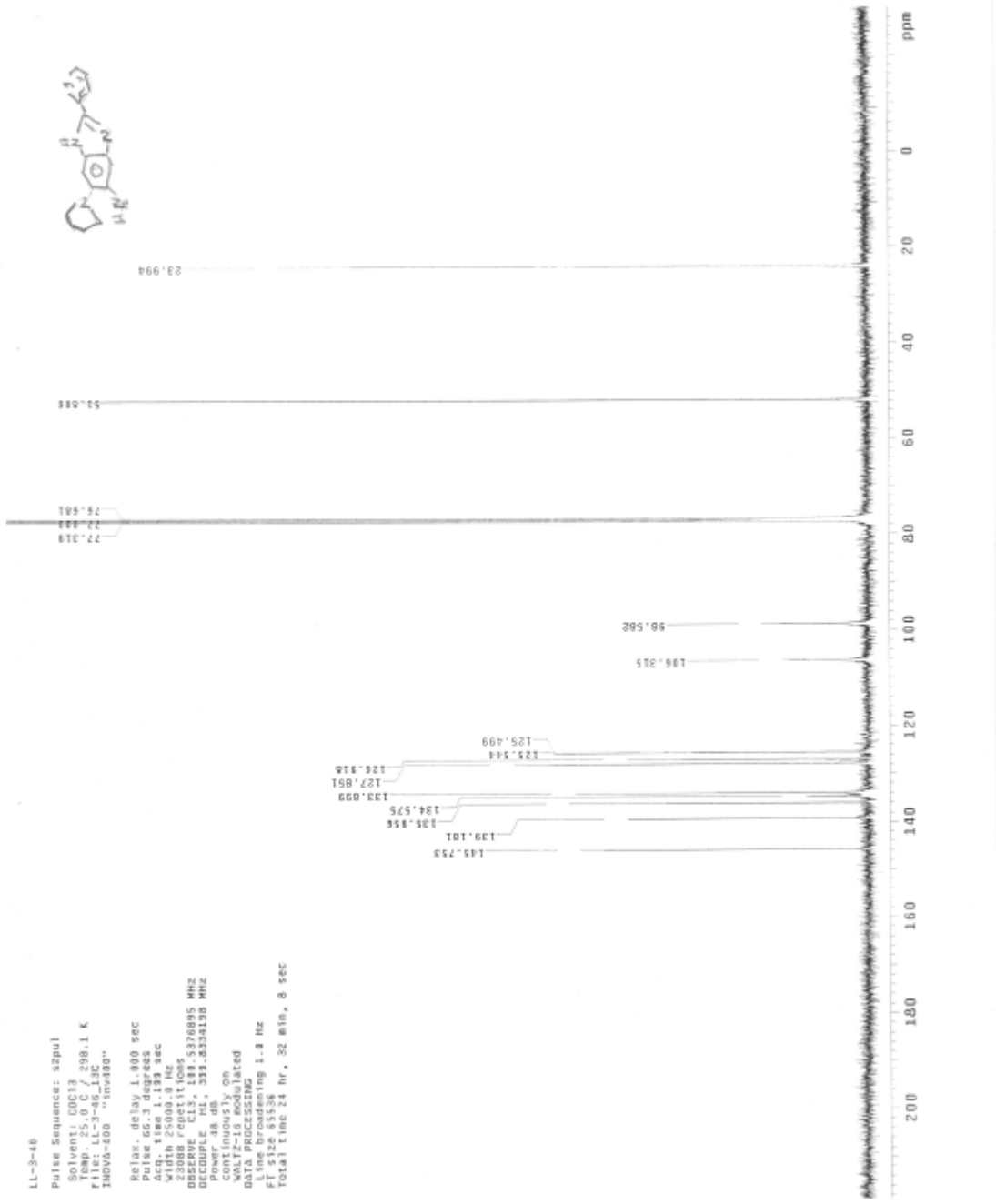
Monoisotopic Mass, Even Electron Ions
145 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 N: 0-5 O: 0-6

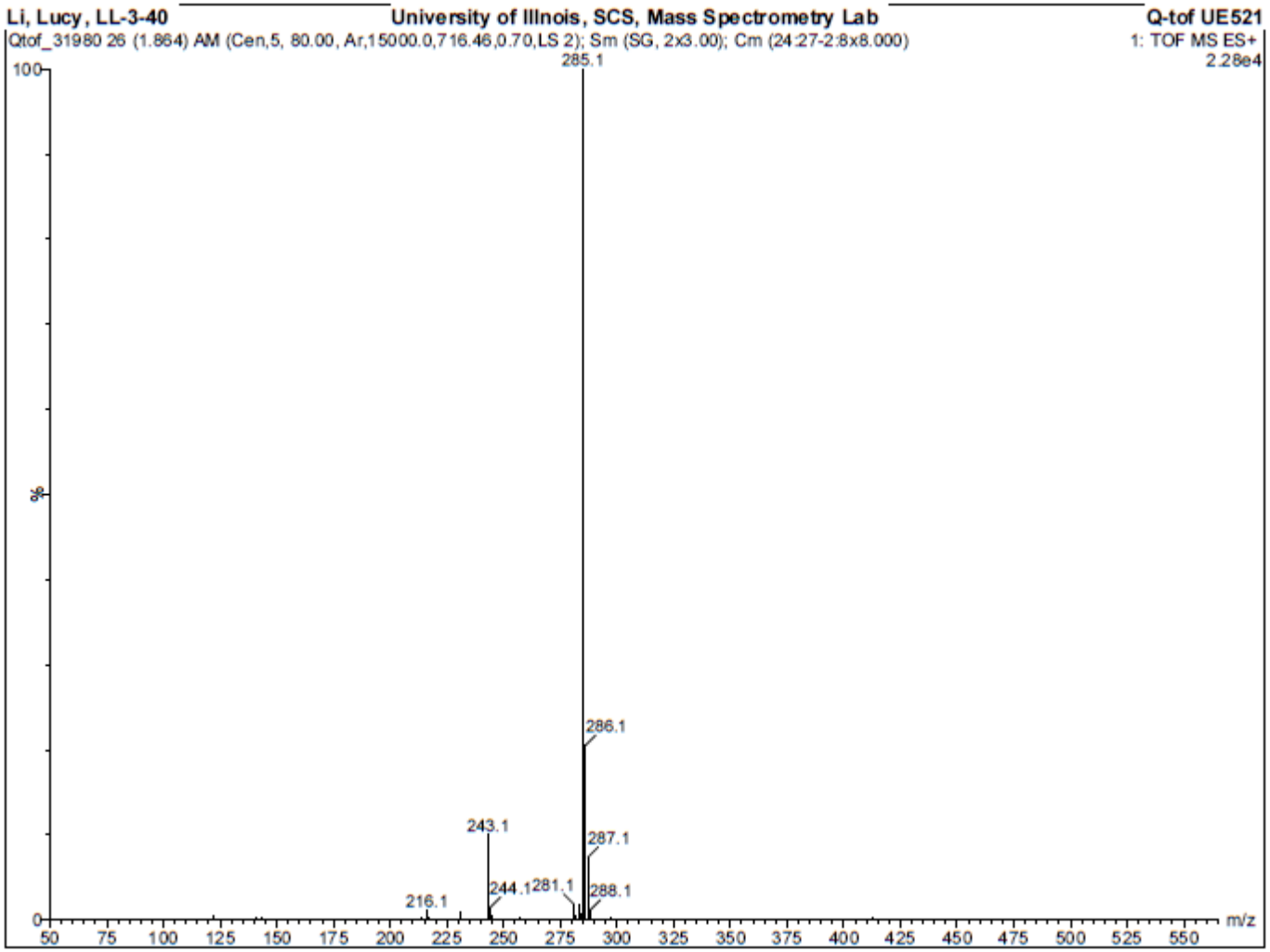
Li, Lucy, LL-3-37 University of Illinois, SCS, Mass Spectrometry Lab Q-tof UE521
Qtof_31979 55 (3.936) AM (Cen,5, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (55:56) 1: TOF MS ES+
1.64e+003



Minimum:				-1.5		
Maximum:		5.0	10.0	150.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
269.1404	269.1402	0.2	0.7	9.5	0.1	C15 H17 N4 O
	269.1389	1.5	5.6	4.5	3.8	C14 H21 O5

LL-5-80
Pulse Sequence: s2pul
Solvent: cdcl3
Temp. 25.0 C / 298.1 K
GEMINI-300BB "gem300"
Relax. delay 1.000 sec
Pulse 90 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
64 repetitions
OBSERVE H1, 300.0732977 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
7.779
7.517
7.513
7.504
7.381
7.415
7.411
7.386
7.385
7.102
7.043
7.031
7.027
7.014
6.989
1.826
1.836
1.848
1.860
1.865
1.870
2.915
2.919
2.923
2.929
2.937
2.935
2.951
2.955
2.959
3.173
3.176
3.182
3.187
3.193
4.758
ppm
0.11
0.61
0.72
0.63
0.69
0.51
3.00
3.04


LL-3-40
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
File: LL3-40.fid
INOVA-600 "inova600"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.193 sec
Width 25000.0 Hz
23000 repetitions
OBSERVE C13, 150.5376895 MHz
DECOUPLE H1, 599.8334198 MHz
Power 46 dB
continuously on
WALTZ-16 modulated
Data processing
Line broadening 1.0 Hz
FT size 65536
Total time 24 hr, 32 min, 8 sec
23.994
53.688
77.319
76.681
98.582
106.315
125.499
125.544
126.926
127.851
133.099
134.575
135.956
139.181
145.753
ppm

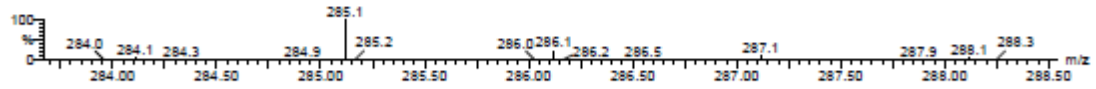

Li, Lucy, LL-3-40
University of Illnois, SCS, Mass Spectrometry Lab
Q-tof UE521
Qtof_31980 26 (1.864) AM (Cen,5, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (24:27-2:8x8.000)
1: TOF MS ES+
2.28e4
100
%
0
285.1
286.1
243.1
287.1
244.1
281.1
288.1
216.1
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 150.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

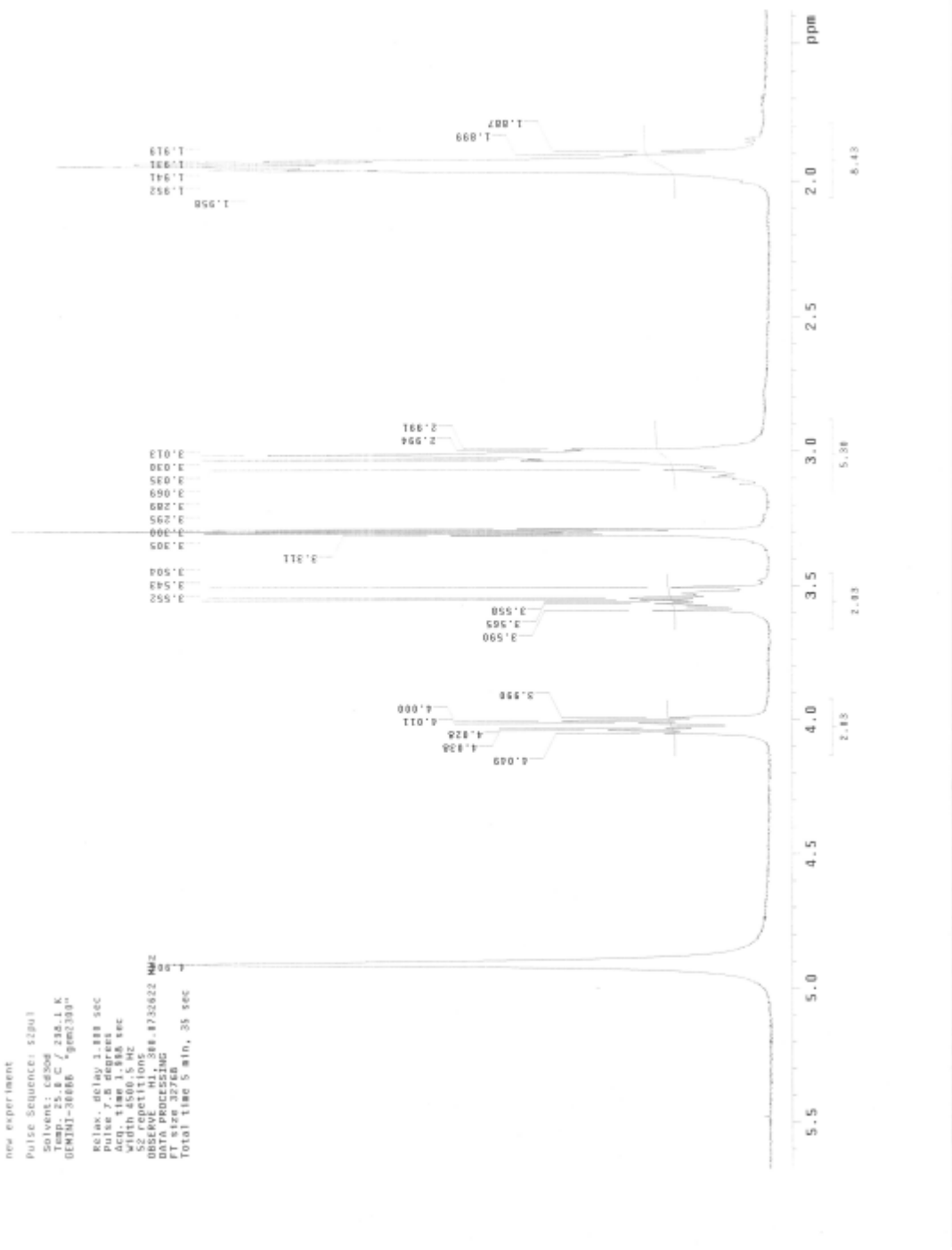
Monoisotopic Mass, Even Electron Ions
137 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 N: 0-5 O: 0-6 S: 1-1

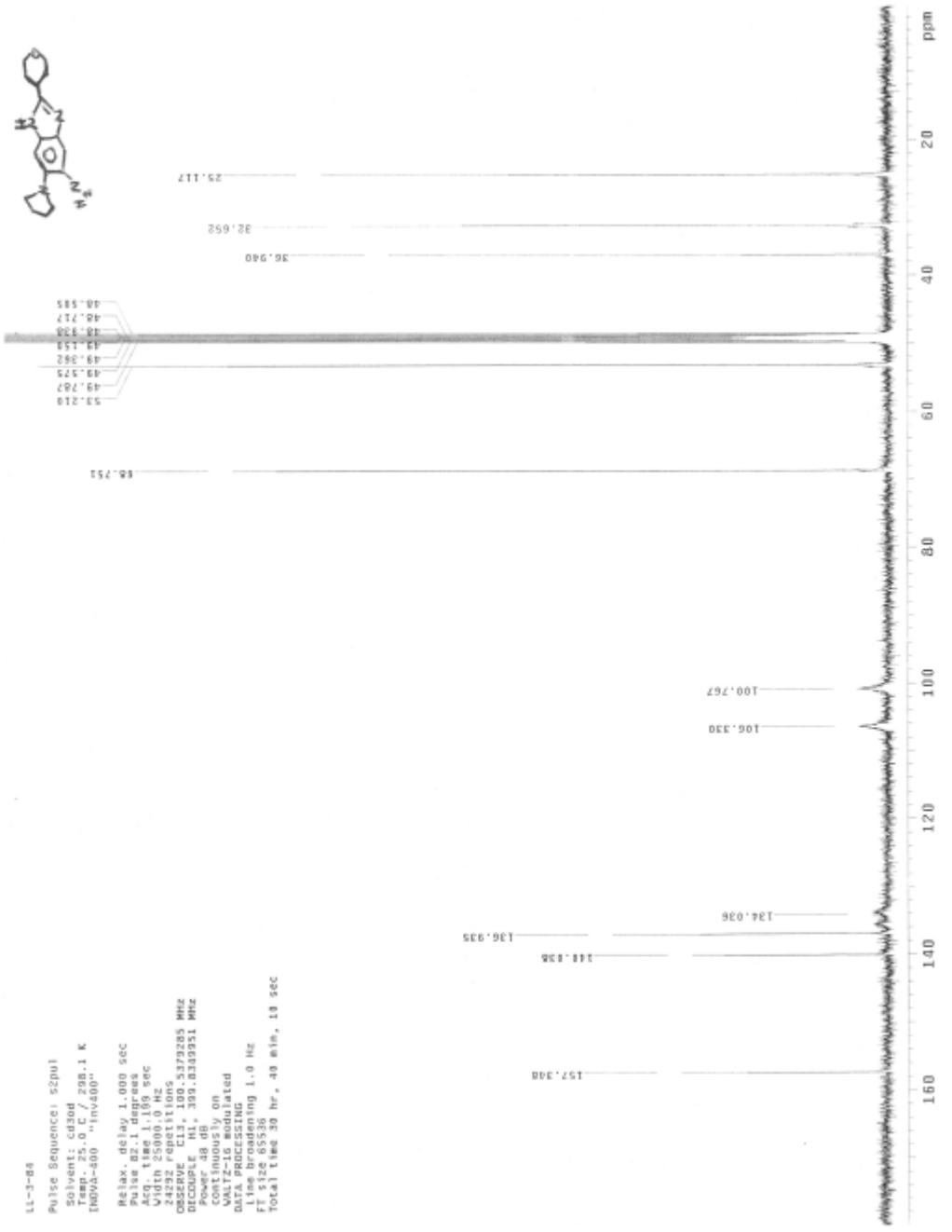
Li, Lucy, LL-3-40 University of Illinois, SCS, Mass Spectrometry Lab Q-tof UE521
Qtof_31980 49 (3.507) AM (Cen,5, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (49:54) 1: TOF MS ES+
2.73e+003

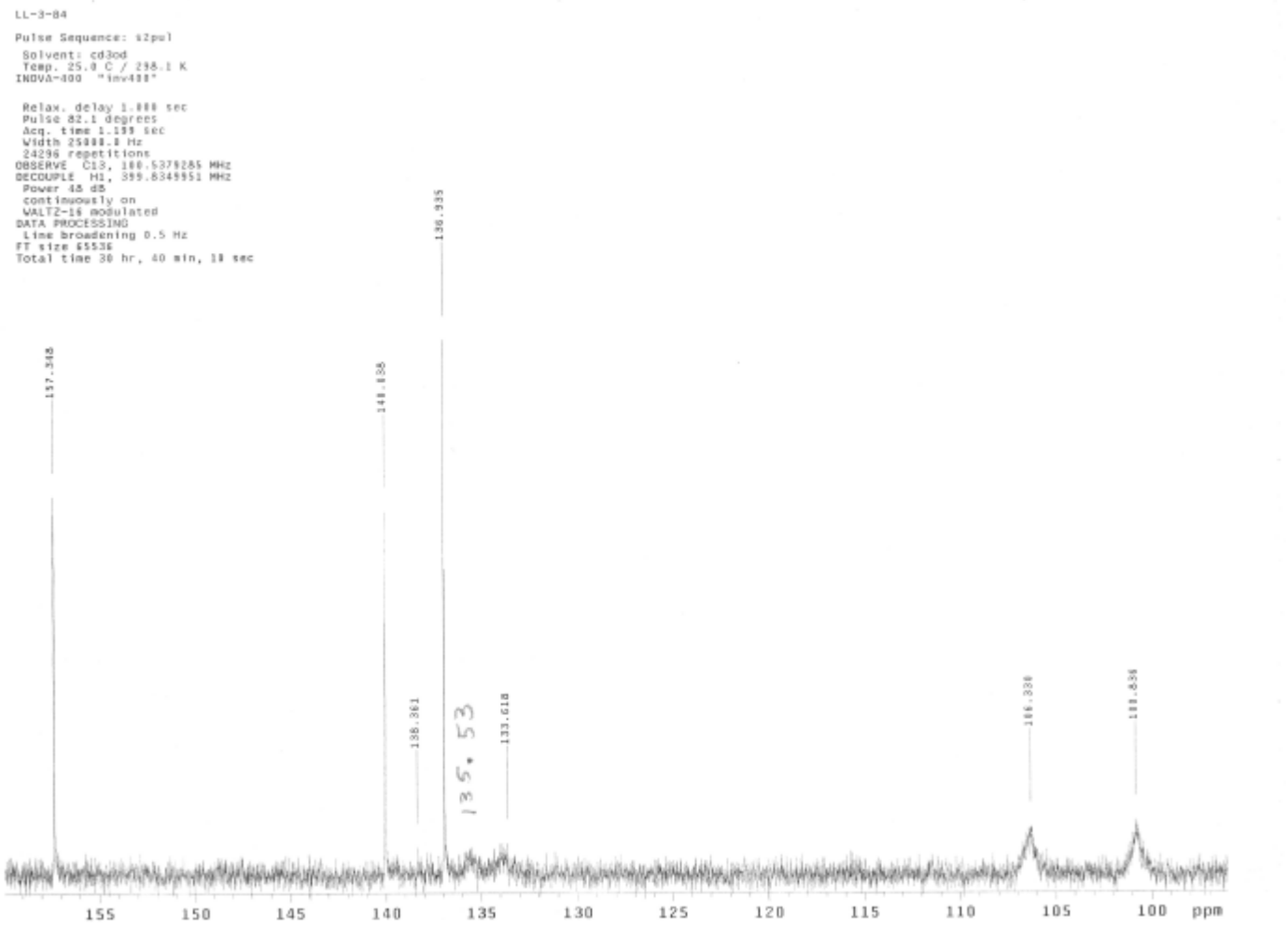


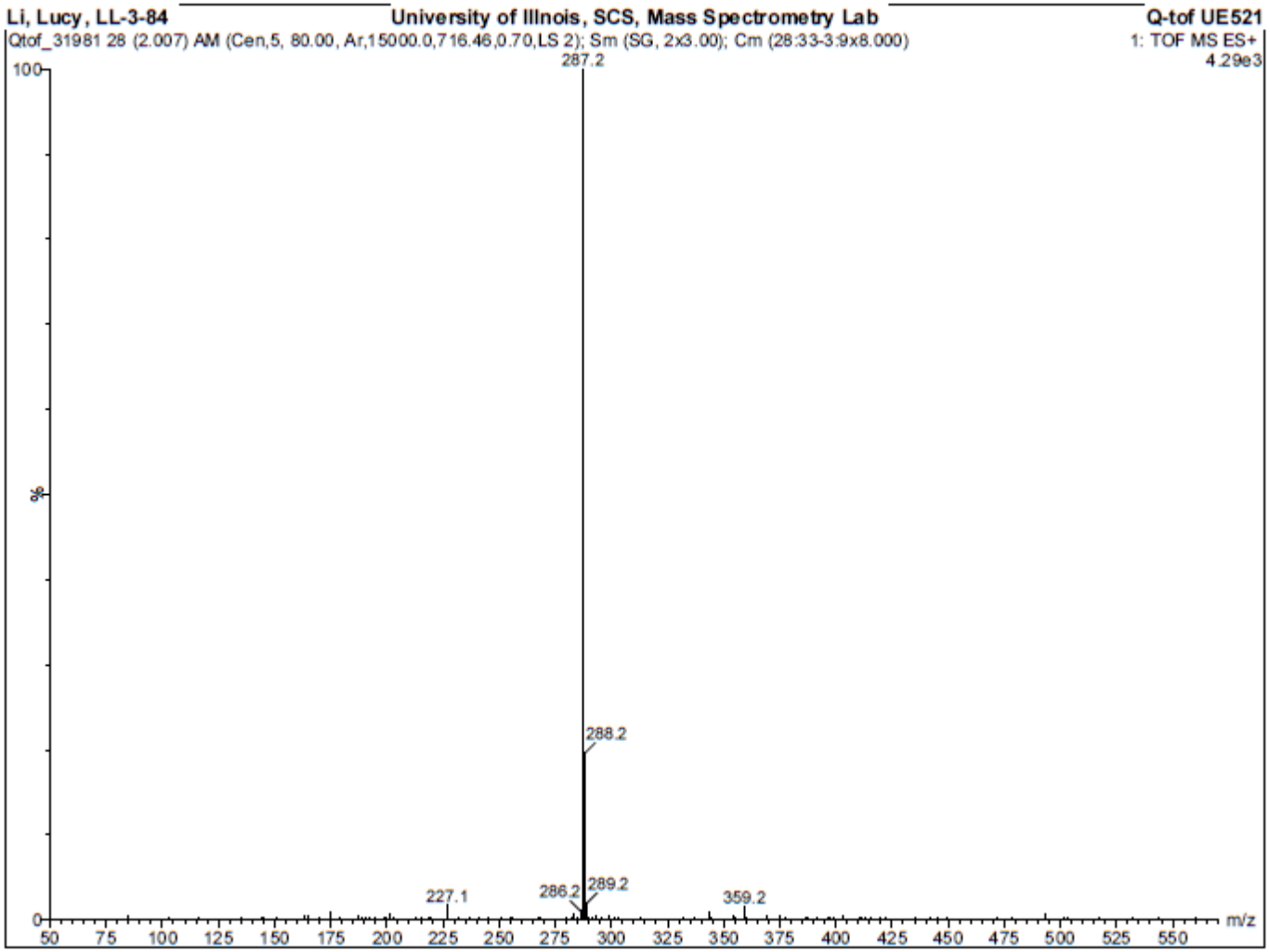
Minimum:				-1.5		
Maximum:		5.0	10.0	150.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
285.1172	285.1174	-0.2	-0.7	9.5	1.8	C15 H17 N4 S
	285.1161	1.1	3.9	4.5	9.9	C14 H21 O4 S

11-3-BH
new experiment
Pulse Sequence: s2pul
Solvent: cdcl3od
Temp. 25.0 C / 298.1 K
GEMINI-300B "gem2300"
Relax. delay 1.000 sec
Pulse 76 degrees
Acq. time 1.998 sec
Width 4588.5 Hz
44 repetitions
OBSERVE H1, 300.0732622 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
ppm


new experiment
Pulse Sequence: s2pul
Solvent: cdcl3d
Temp. 25.0 C / 298.1 K
DEMIN-30686 gem230ar
Relax. delay 1.000 sec
Pulse 45 degrees
Acq. time 1.816 sec
width 4500.5 Hz
52 repetitions
OBSERVE H1, 300.0732622 MHz
DATA PROCESSING
FT size 32768
Total time 5 min, 35 sec
ppm
1.000
1.952
1.941
1.931
1.919
1.899
1.887
1.558
2.994
2.981
3.013
3.030
3.035
3.069
3.289
3.295
3.300
3.305
3.311
3.504
3.543
3.552
3.558
3.565
3.590
3.998
4.000
4.011
4.828
4.838
4.849
5.38
2.03
2.03
8.43


29.117
32.652
36.940
48.585
48.717
48.930
49.158
49.382
49.575
49.767
53.210
68.751
100.767
106.330
134.038
136.935
148.838
157.348
ppm
20
40
60
80
100
120
140
160
Ll-3-84
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.0 C / 298.1 K
INOVA-400 "inv400"
Relax. delay 1.000 sec
Pulse 82.1 degrees
Acq. time 1.190 sec
Width 25000.0 Hz
74292 repetitions
OBSERVE C13, 100.5379285 MHz
DECOUPLE H1, 399.8349951 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 30 hr, 40 min, 18 sec


LL-3-84
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.0 C / 298.1 K
INOVA-400 "inv400"
Relax. delay 1.000 sec
Pulse 82.1 degrees
Acq. time 1.199 sec
Width 25000.1 Hz
24296 repetitions
OBSERVE C13, 100.5379285 MHz
DECOUPLE H1, 399.8349551 MHz
Power 48 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 30 hr, 40 min, 18 sec
157.348
148.838
138.361
136.935
135.53
133.618
116.338
111.838
155
150
145
140
135
130
125
120
115
110
105
100
ppm

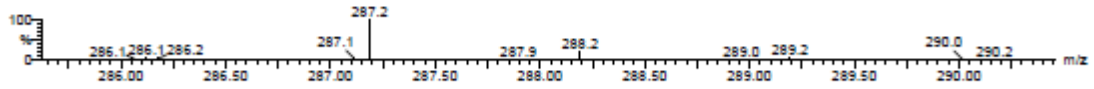

Li, Lucy, LL-3-84
University of Illnois, SCS, Mass Spectrometry Lab
Q-tof UE521
Qtof_31981 28 (2.007) AM (Cen,5, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (28:33-3:9x8.000)
1: TOF MS ES+
4.29e3
100
%
0
287.2
288.2
227.1
286.2
289.2
359.2
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550

Elemental Composition Report **Page 1**

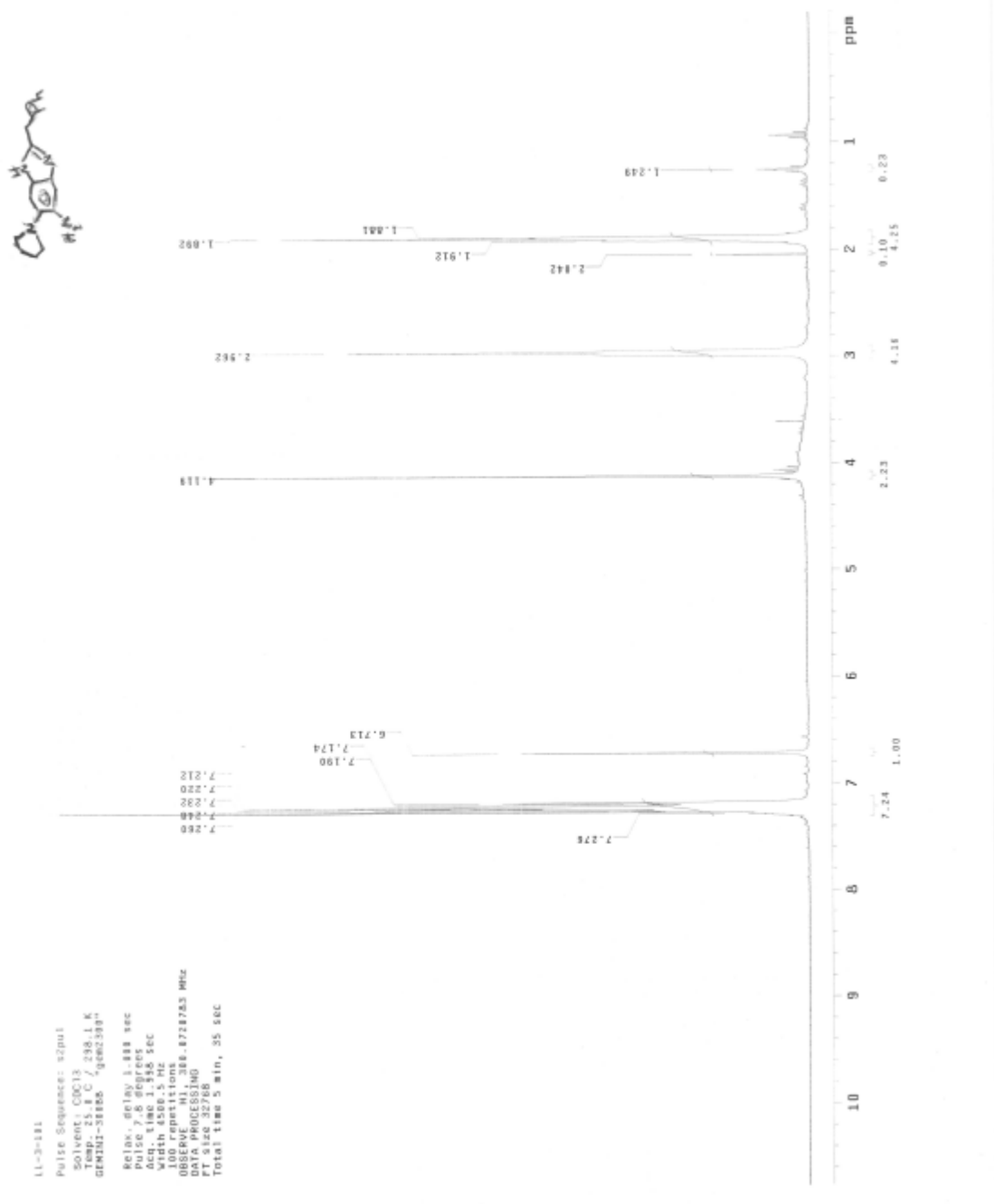
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 150.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

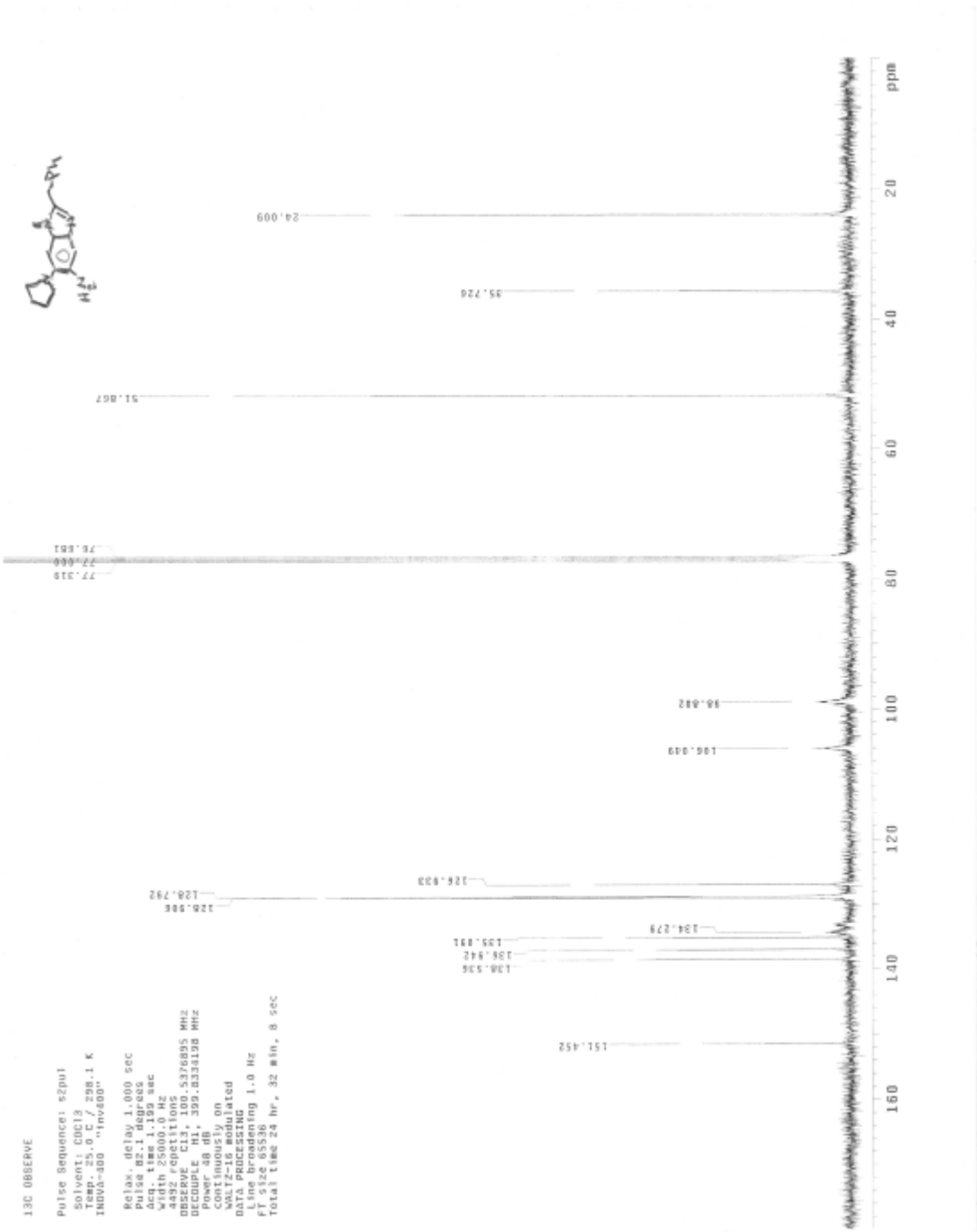
Monoisotopic Mass, Even Electron Ions
154 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 N: 0-5 O: 0-6

Li, Lucy, LL-3-84 University of Illinois, SCS, Mass Spectrometry Lab Q-tof UE521
Qtof_31981 36 (2.578) AM (Cen,5, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (35:41) 1: TOF MS ES+
1.22e+003

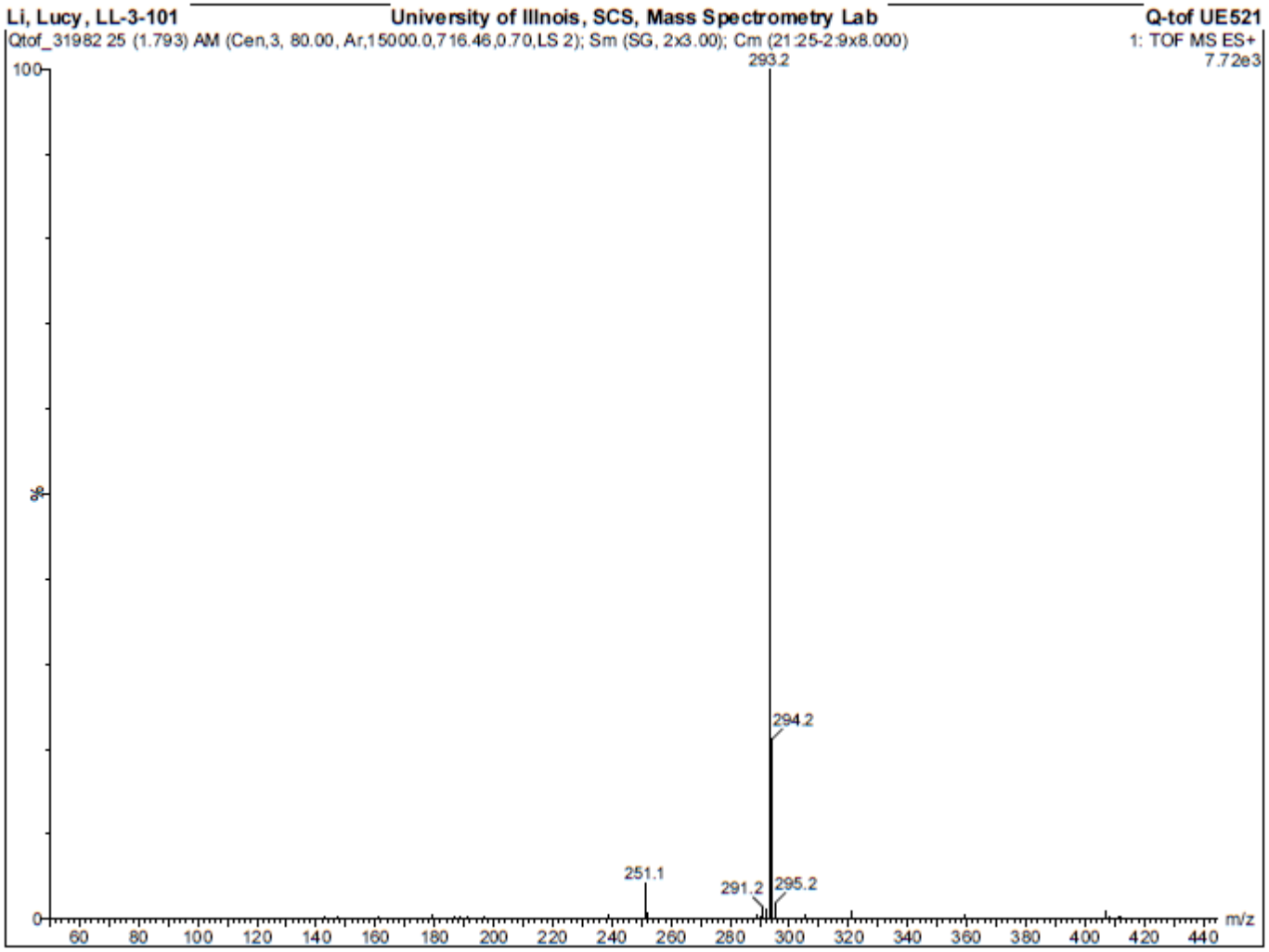


Minimum:				-1.5		
Maximum:		5.0	10.0	150.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
287.1873	287.1872	0.1	0.3	7.5	2.4	C16 H23 N4 O
	287.1858	1.5	5.2	2.5	0.2	C15 H27 O5


LL-3-181
Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.0 C / 298.1 K
GEMINI-300BB "gem300"
Relax. delay 1.000 sec
Pulse 78.6 degrees
Acq. time 4.255 sec
Width 4808.5 Hz
100 repetitions
OBSERVE H1, 300.0728783 MHz
DATA PROCESSING
FT size 32768
Total time 5 min, 35 sec
7.276
7.280
7.248
7.232
7.220
7.212
7.190
7.174
6.713
4.119
2.962
1.892
1.841
1.912
2.842
1.249
ppm
7.24
1.00
4.19
2.23
0.10
4.25
0.23


24.009
35.726
51.867
76.681
77.000
77.319
86.682
106.049
126.933
128.792
128.986
134.279
135.891
136.942
136.536
151.452
ppm
20
40
60
80
100
120
140
160

13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.0 C / 298.1 K
INOVA-400 "inv400"
Relax. delay 1.000 sec
Pulse 82.1 degrees
Acq. time 1.109 sec
Width 25000.0 Hz
4500 repetitions
OBSERVE C13, 100.5376895 MHz
DECOUPLE H1, 399.8331938 MHz
Power 48 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 24 hr, 32 min, 8 sec
151.452
136.596
136.042
135.883
134.229
133.20
128.988
128.792
128.833
106.049
90.802
ppm

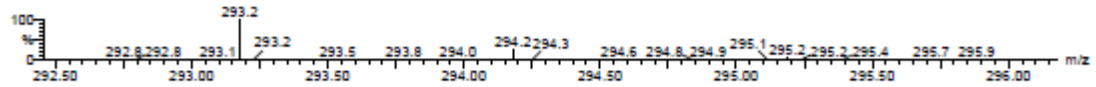

Li, Lucy, LL-3-101
University of Illnois, SCS, Mass Spectrometry Lab
Q-tof UE521
Qtof_31982 25 (1.793) AM (Cen,3, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (21:25-2:9x8.000)
1: TOF MS ES+
7.72e3
100
%
0
293.2
294.2
251.1
291.2
295.2
m/z
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440

Elemental Composition Report **Page 1**

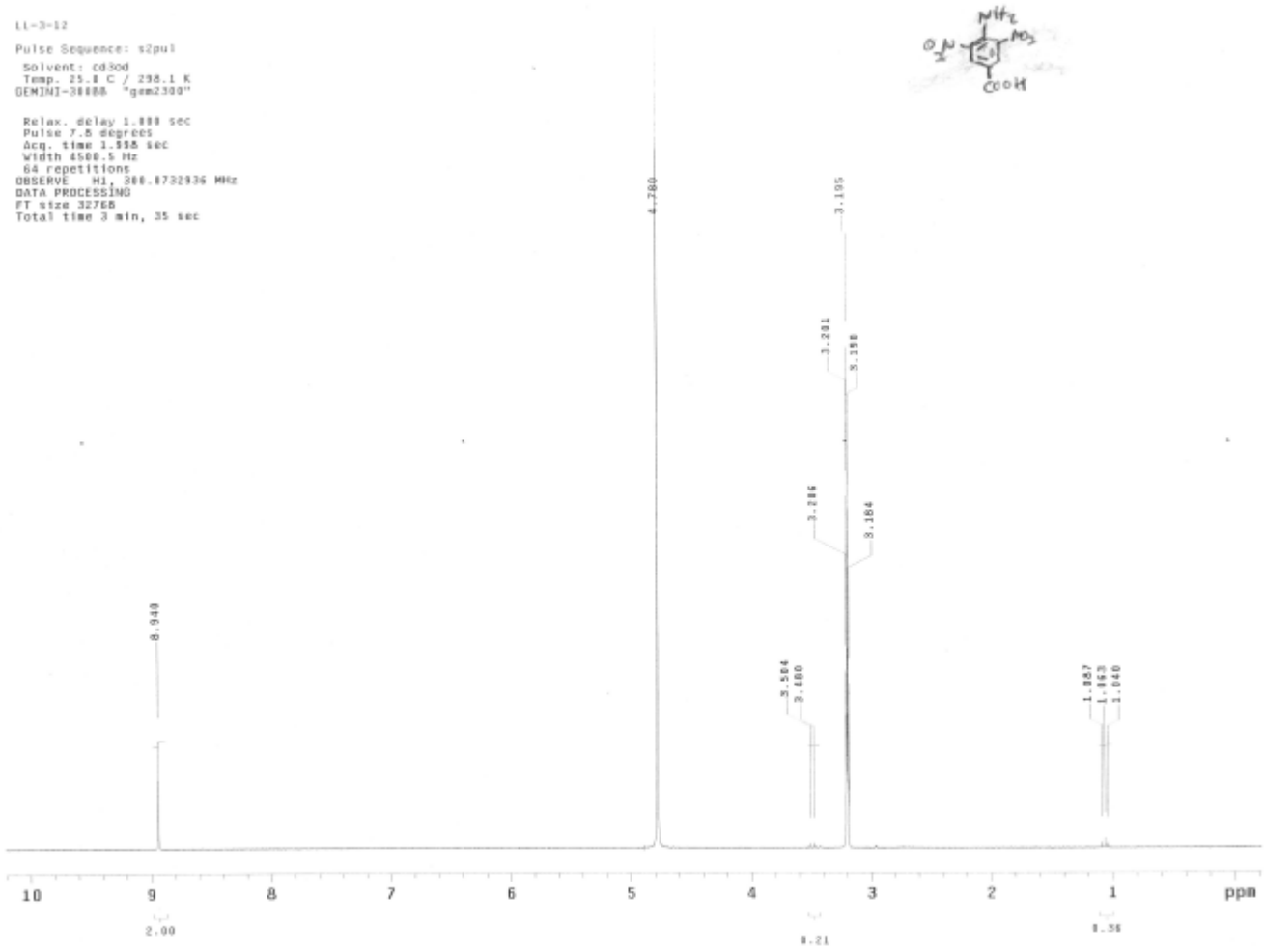
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 150.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
157 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 N: 0-5 O: 0-6

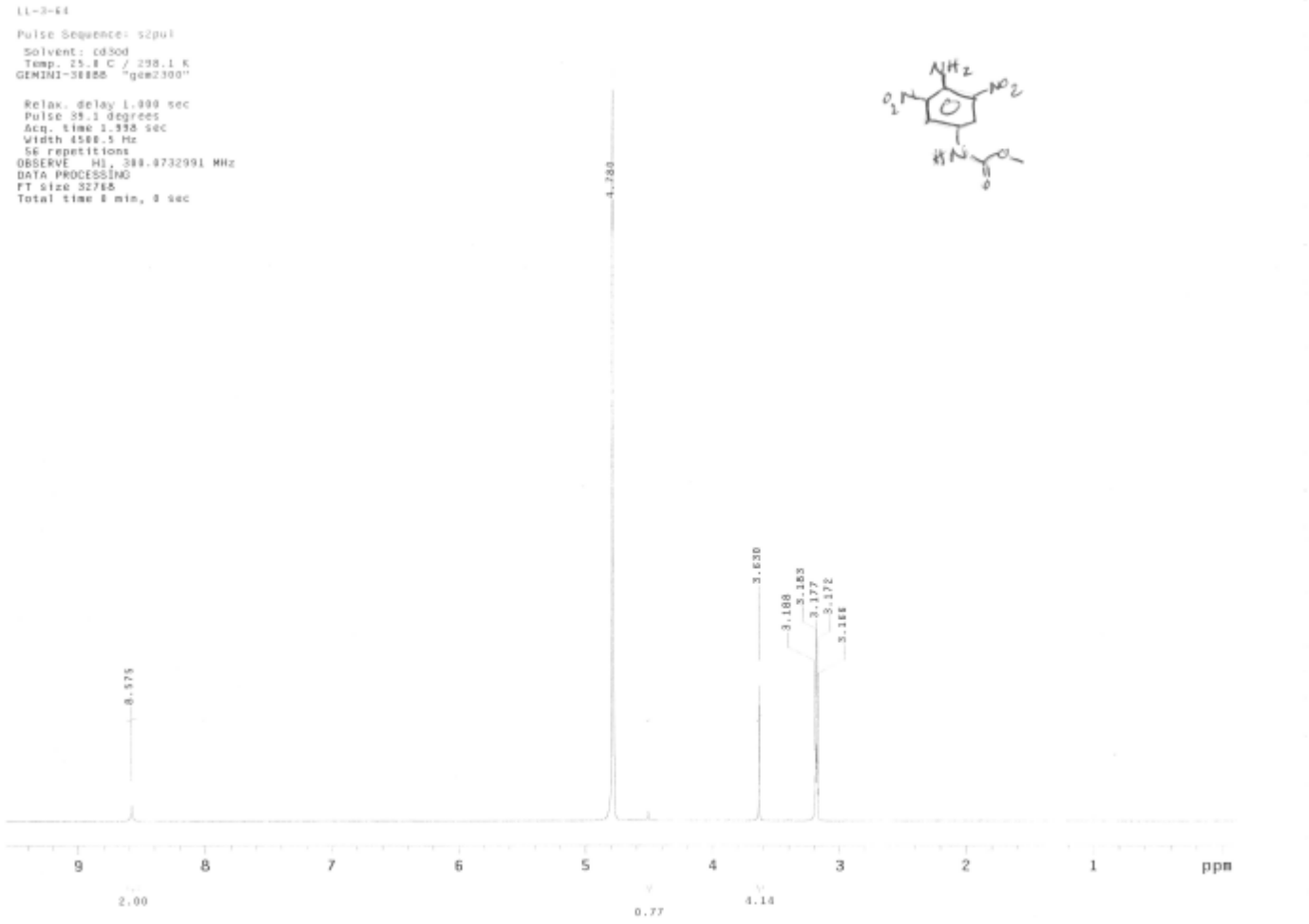
Li, Lucy, LL-3-101 University of Illinois, SCS, Mass Spectrometry Lab Q-tof UE521
Qtof_31982 33 (2.364) AM (Cen,3, 80.00, Ar,15000.0,716.46,0.70,LS 2); Sm (SG, 2x3.00); Cm (33:36) 1: TOF MS ES+
2.27e+003

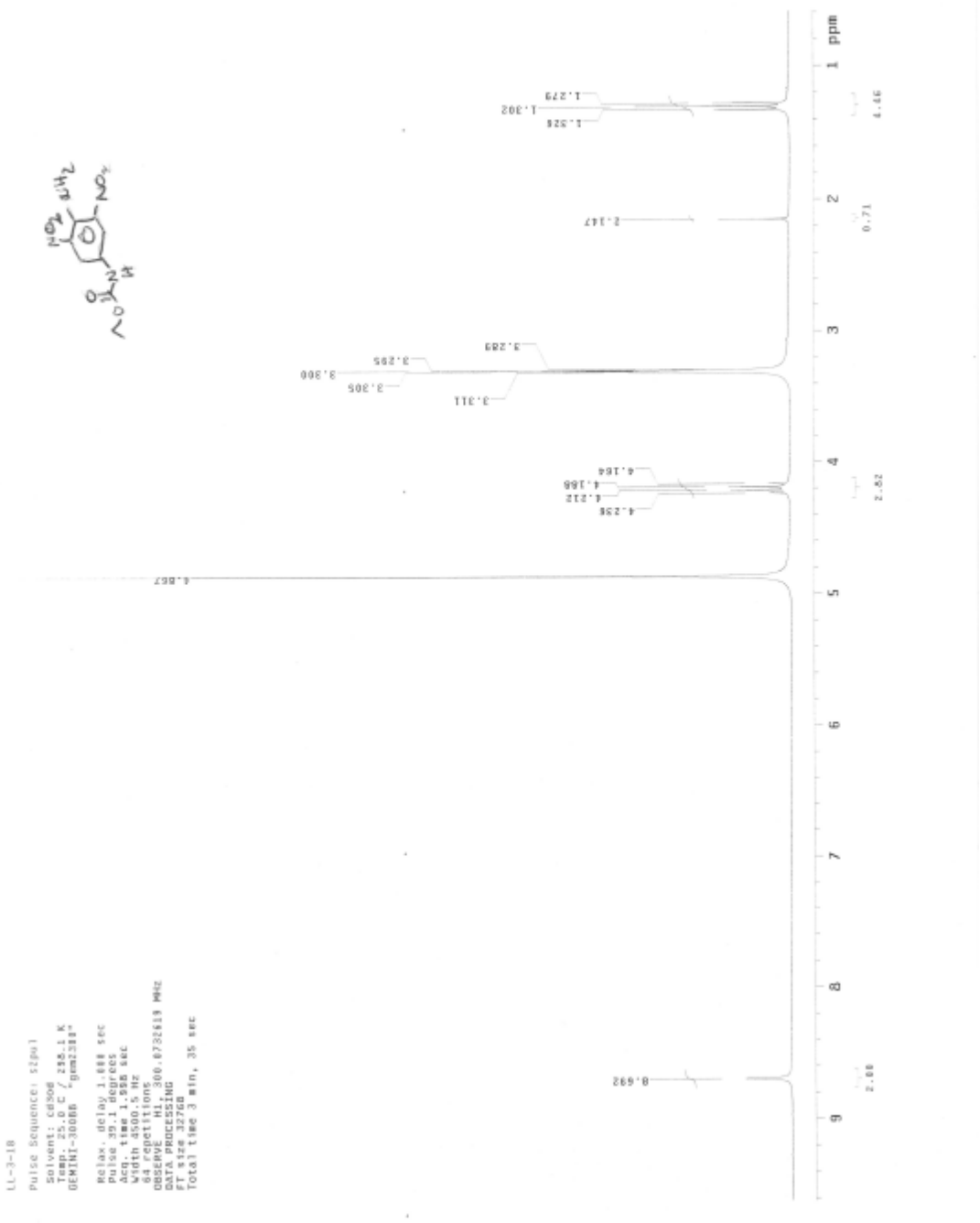


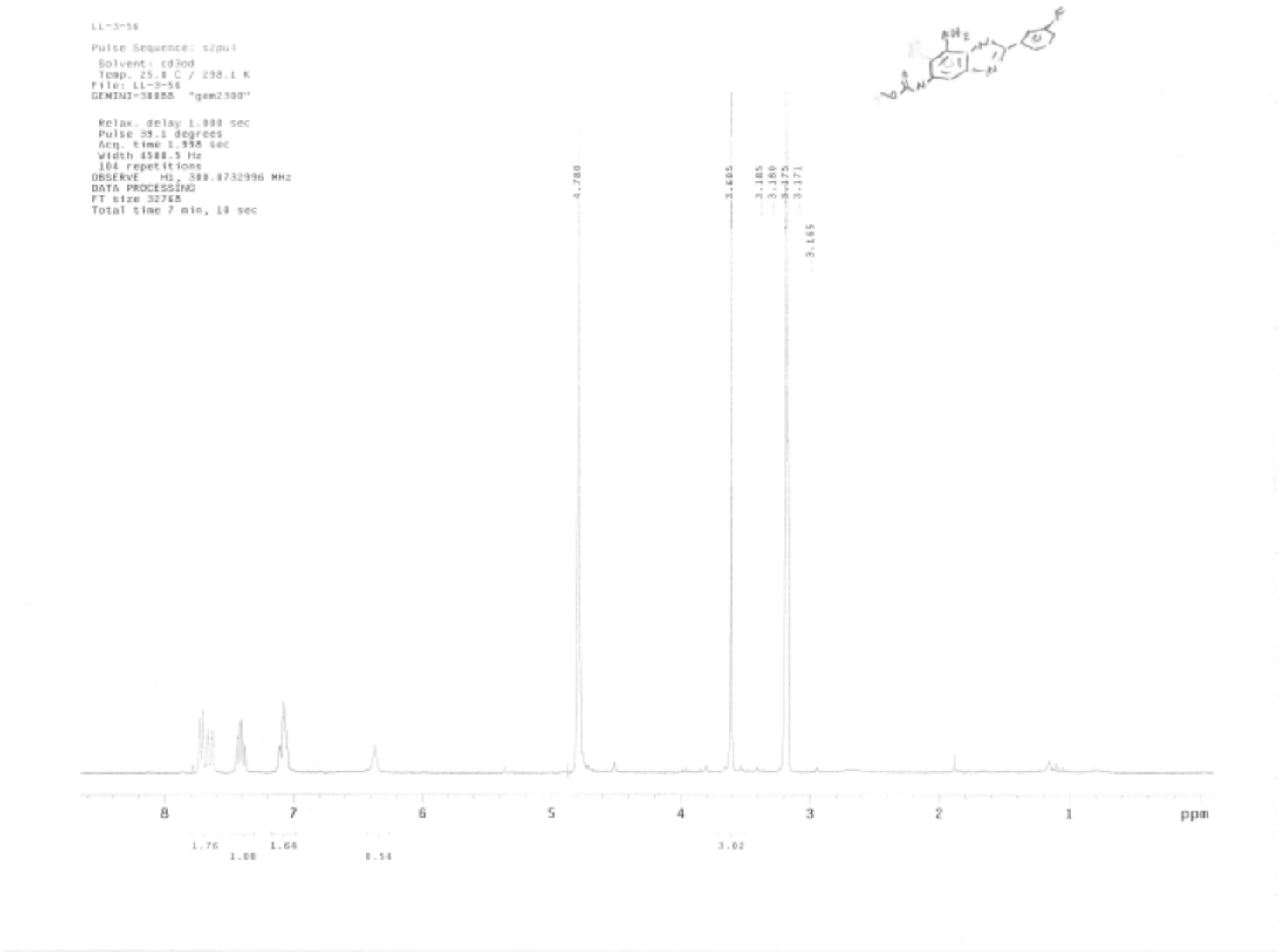
Minimum:				-1.5		
Maximum:		5.0	10.0	150.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
293.1768	293.1766	0.2	0.7	10.5	0.6	C18 H21 N4
	293.1753	1.5	5.1	5.5	6.5	C17 H25 O4


LL-3-12
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.8 C / 298.1 K
GEMINI-300BB "gem2300"
Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
64 repetitions
OBSERVE H1, 300.0732936 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
NH2
NO2
COOH
8.949
4.280
3.565
3.480
3.291
3.195
3.186
3.286
3.184
1.987
1.963
1.940
10
9
8
7
6
5
4
3
2
1
ppm
2.00
1.21
1.38

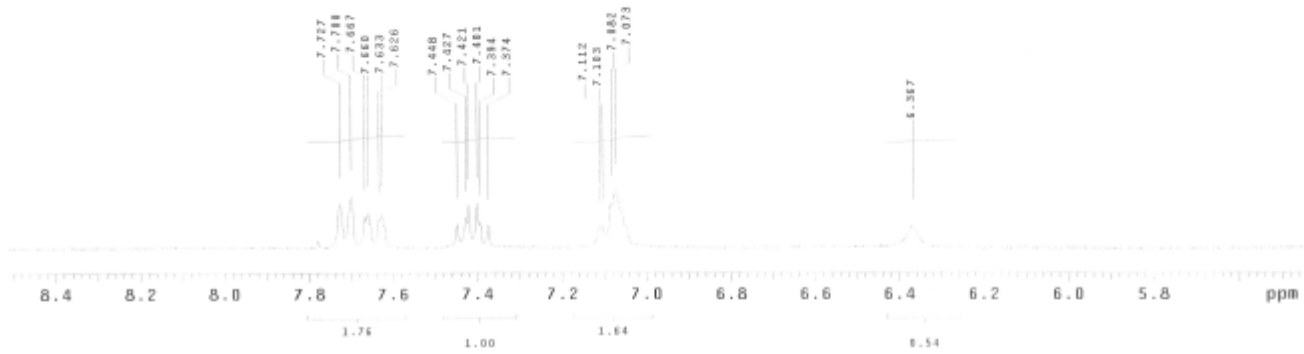
LL-3-17
Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.0 C / 298.1 K
GEMINI-300BB "gem298"
Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.708 sec
Width 4800.5 Hz
8 repetitions
OBSERVE H1, 300.0726775MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
7.268
8.694
1.550
ppm
0.67
0.66
2.89
2.21

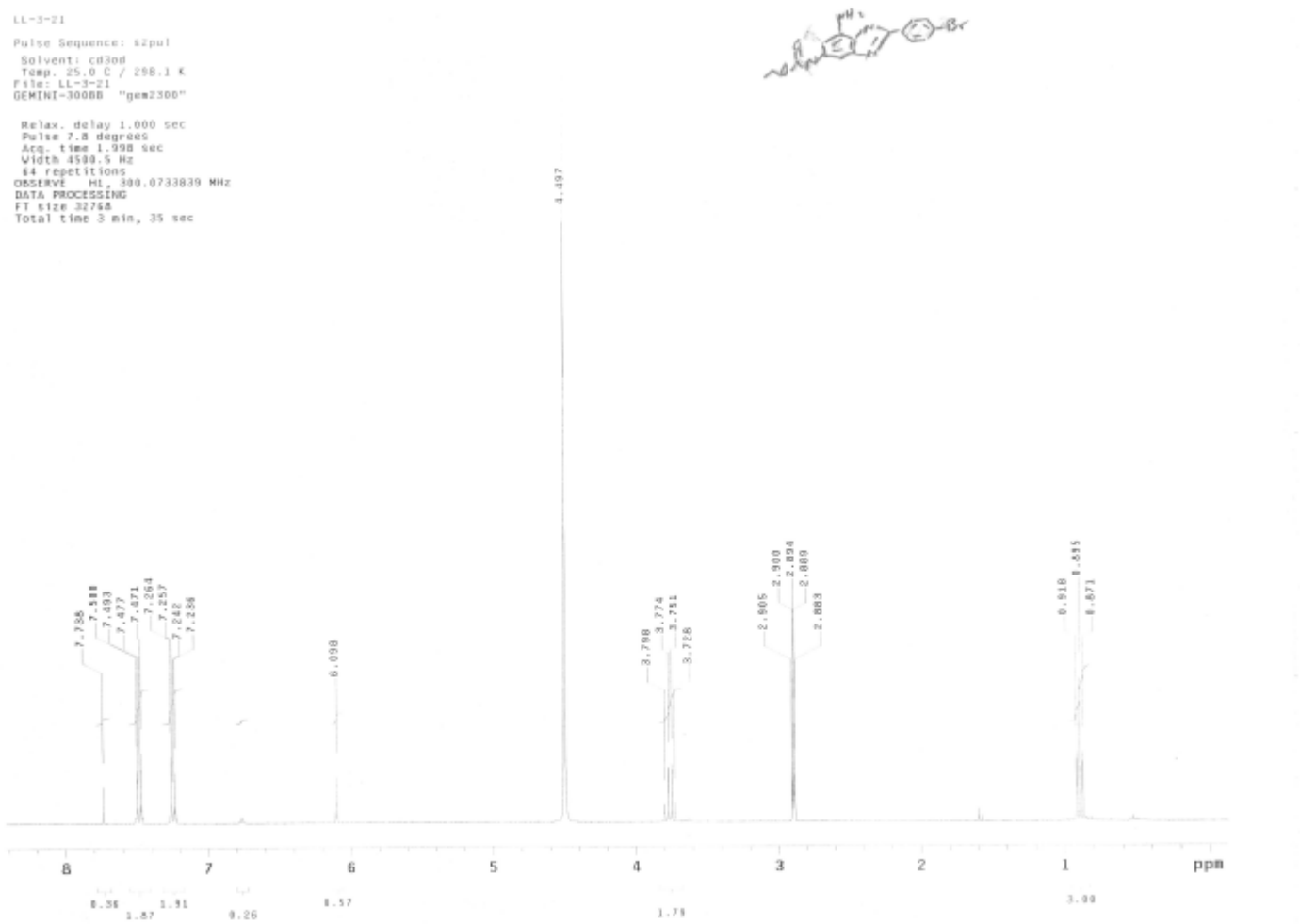

LL-3-44
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.8 C / 298.1 K
GEMINI-38858 "gem2300"
Relax. delay 1.000 sec
Pulse 39.1 degrees
Acq. time 1.998 sec
Width 4508.5 Hz
56 repetitions
OBSERVE H1, 388.8732991 MHz
DATA PROCESSING
FT size 32768
Total time 8 min, 8 sec
8.575
4.780
3.630
3.188
3.183
3.177
3.172
3.166
9
8
7
6
5
4
3
2
1
ppm
2.00
0.77
4.14


LL-3-18
Pulse Sequence: s2pul
Solvent: cdcl3d
Temp: 25.0 C / 298.1 K
DEMINI-300BB "gem2ss"
Relax. delay 1.688 sec
Pulse 39.1 degrees
Acq. time 1.656 sec
Width 4500.5 Hz
64 repetitions
OBSERVE H1, 300.6732619 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
8.692
6.867
4.258
4.212
4.188
4.164
3.311
3.305
3.300
3.285
3.269
2.147
1.329
1.302
1.279
1 ppm

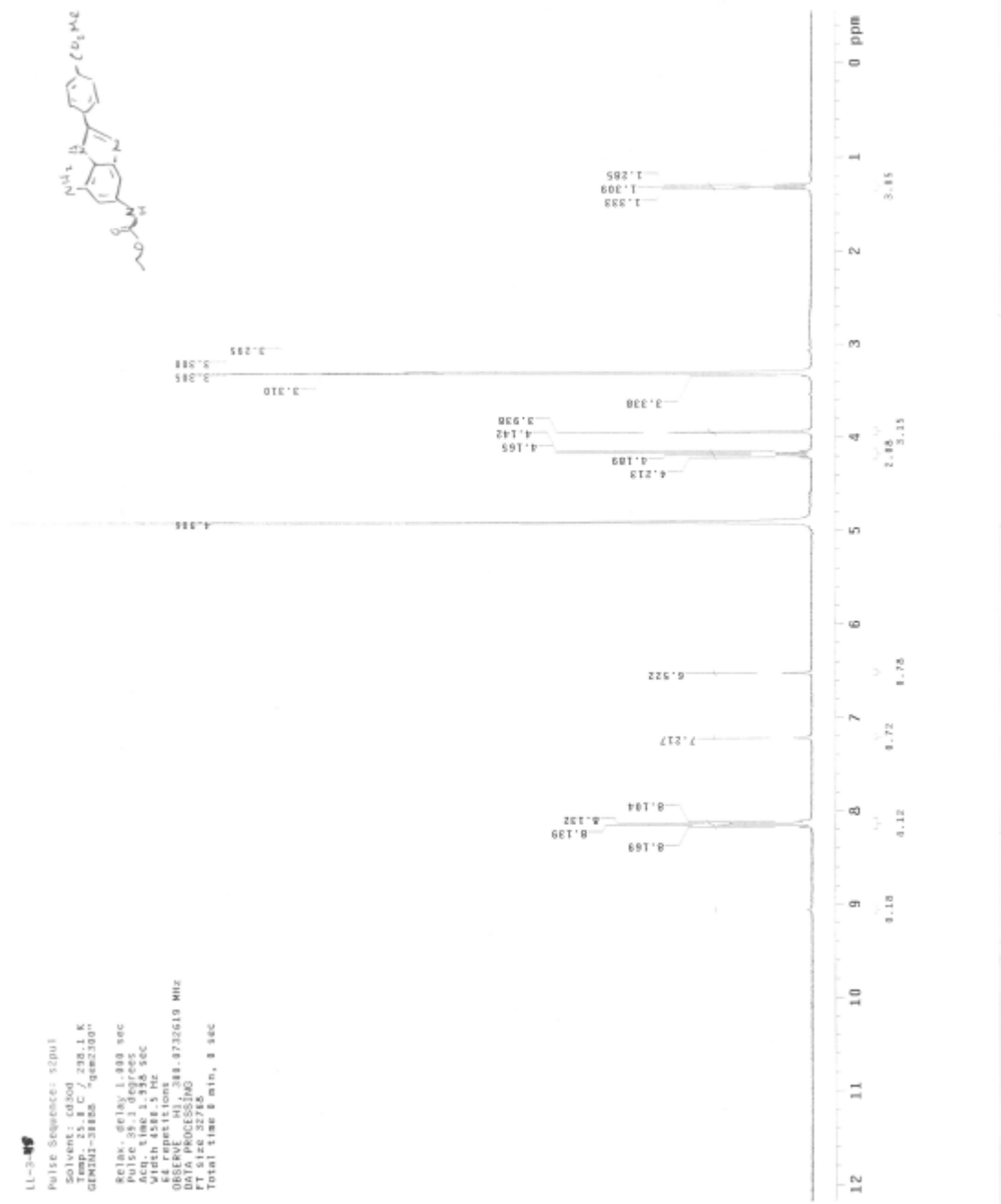

LL-3-56
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.8 C / 298.1 K
File: LL-3-56
GEMINI-38888 "gem2300"
Relax. delay 1.000 sec
Pulse 89.1 degrees
Acq. time 1.998 sec
Width 4588.5 Hz
104 repetitions
OBSERVE H1, 388.8732996 MHz
DATA PROCESSING
FT size 32768
Total time 7 min, 18 sec
4.780
3.605
3.185
3.180
3.175
3.171
3.165
8
7
6
5
4
3
2
1
ppm
1.76
1.00
1.64
8.54
3.02

```
new experiment

Pulse Sequence: s2pul

 Solvent: cd3od
 Temp. 25.0 C / 298.1 K
GEMINI-300BB  "gem2300"

 Relax. delay 1.000 sec
 Pulse 39.1 degrees
 Acq. time 1.998 sec
 Width 4588.5 Hz
 184 repetitions
OBSERVE    H1, 300.0732996 MHz
DATA PROCESSING
FT size 32768
Total time 7 min, 10 sec
```




LL-3-21
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.0 C / 298.1 K
File: LL-3-21
GEMINI-300BB "gem2300"
Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
64 repetitions
OBSERVE H1, 300.0733839 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
7.788
7.588
7.493
7.477
7.471
7.264
7.257
7.242
7.238
6.098
4.497
3.798
3.774
3.751
3.728
2.905
2.900
2.894
2.889
2.883
0.918
0.855
0.671
8
7
6
5
4
3
2
1
ppm
0.56
1.87
1.91
0.26
0.57
1.79
3.00

LL-3-57
Pulse Sequence: s2pul
Solvent: cdcl3
Temp: 25.0 C / 298.1 K
GEMINI-300B "gem300"
Relax. delay 1.000 sec
Pulse 39.1 degrees
Acq. time 1.000 sec
Width 4800.1 Hz
64 repetitions
OBSERVE H1, 300.0732991 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 35 sec
7.516
7.506
7.044
6.992
6.880
6.875
4.783
4.083
4.068
4.036
4.012
3.188
3.185
3.177
3.172
3.166
2.271
1.285
1.181
1.156
ppm
1.85
1.67
0.72
8.38
2.02
6.27
3.00


LL-3-49
Pulse Sequence: s2pul
Solvent: cdcl3
Temp. 18.0 C / 291.1 K
GEMINI-300BB "gem300"
Relax. delay 1.000 sec
Pulse 90.0 degrees
Acq. time 3.998 sec
Width 4508.5 Hz
64 repetitions
OBSERVE H1, 300.0732619 MHz
DATA PROCESSING
FT size 32768
Total time 8 min, 8 sec
1.285
1.309
1.333
3.285
3.310
3.330
3.538
3.585
3.588
3.938
4.142
4.165
4.189
4.213
4.986
6.322
7.217
8.139
8.152
8.169
8.184
0 ppm