

Synthesis and Study of Ligands in Photocage Complexes

A Major Qualifying Project Report

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Project Advisor

Abstract

Caged complexes are metal ion chelators that release analytes when exposed to light of a specific wavelength. When irradiated, the complex undergoes a structural change that weakens the metal-ligand interaction and shifts the binding equilibrium to free metal ions. This chemistry is then studied and applied to biological research in areas such as metal ion homeostasis and metal-based neurotransmission. This project continued the research of photochemistry through the synthesis and characterization of carboxylic acid derivative ligands that can bind to various metals and create photocage complexes. The properties and photochemistry of the complexes were then analyzed and used to determine new ligand syntheses.

Acknowledgements

I would like to thank Professor Shawn C. Burdette for his guidance as advisor of this project. I would also like to thank Prem Basa, Chelsea Barr, Xiaomeng Liang, and Jingjing Yan for their assistance in the lab.

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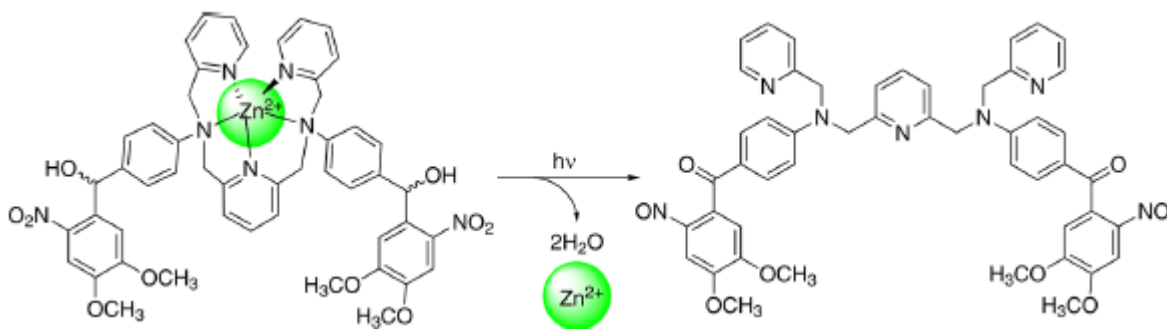
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Introduction

Photocage complexes are an important area of study that can be applied to many different areas of chemistry and biology. These complexes can be used to explain the signaling pathways of metal ions or small molecules and can be used to manipulate bioactive species with great precision. When exposed to light, these metal cage complexes undergo a structural change that weakens the ligand-metal interaction which then releases the central metal ion. This light-induced release of the metal ion helps to control the time and delivery location. Zn^{2+} is an ion that is currently being studied for its functionality as a neural signaling agent in healthy individuals as well as those with neurological diseases. Previous research showed that Zn^{2+} creates a photocage with ligands that contain multiple nitrobenzyl groups as shown in the figure below.

Figure 1: Uncaging Mechanism of Nitrobenzyl Cage

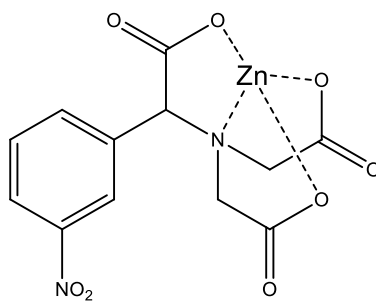


Picture from C. Gwizdala and P.N. Basa and J.C. MacDonald and S.C. Burdette

Inorg. Chem. 2013, 52, 8483-8494

After studying the mechanism above and determining the ability for Zn^{2+} to bind to the nitrobenzyl groups, new ligand designs were speculated. This project worked mostly with the synthesis of ligands with carboxylic acid groups to form a cage like the one shown below.

Figure 2: NTA Cage Complex



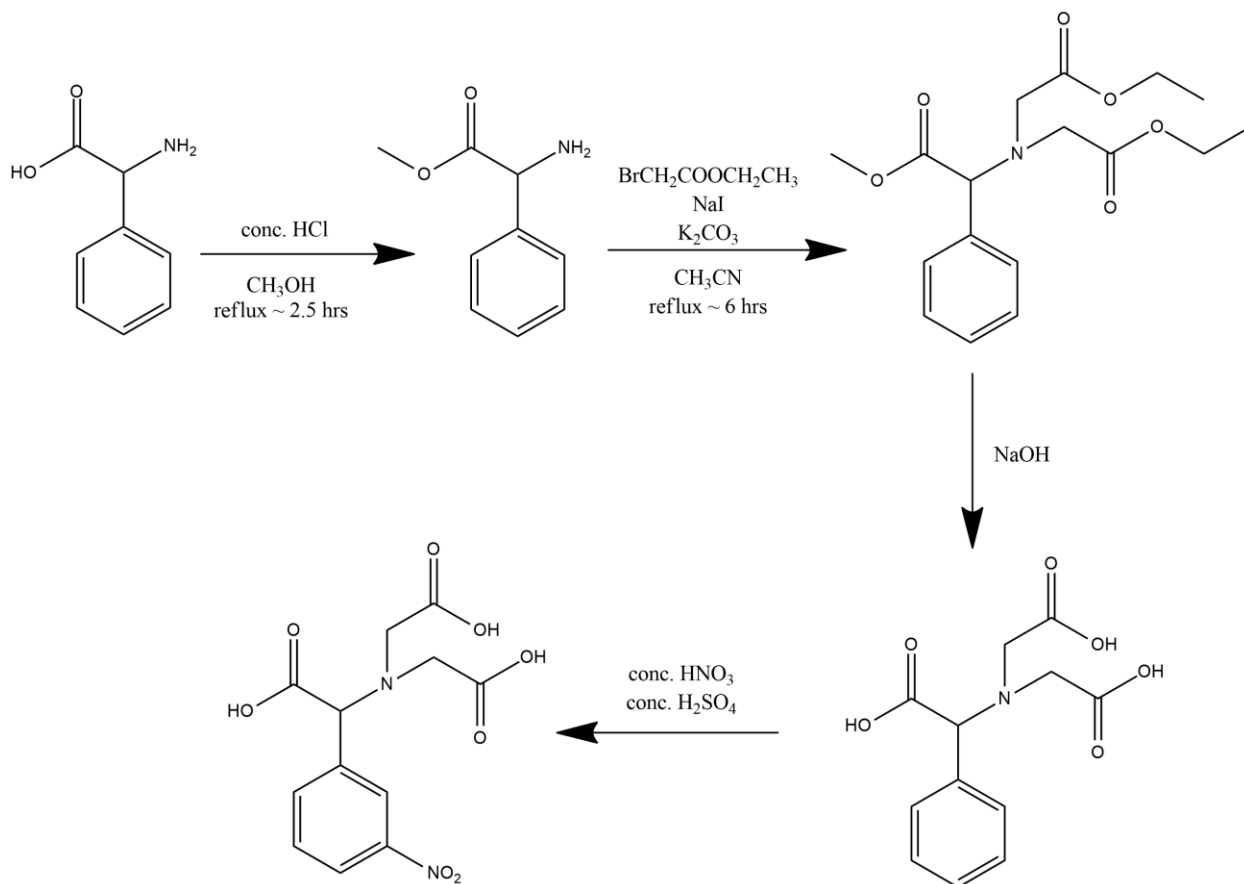
Predicted binding of Zn²⁺ by Prem Basa

Syntheses of ligands with multiple carboxylic acid groups as well as combinations of nitrobenzyl and carboxylic acid groups were performed to determine the effectiveness of this new direction.

Experimental

NTA Cage Synthesis

Figure 3: Pathway for Synthesis 1: NTA Cage



- 1.0g of L – (+) – 2 – phenylglycine was mixed with 20.0mL of methanol and added to a two-necked flask. 5.0mL of 6M HCl was added dropwise and the mixture was refluxed under nitrogen for approximately 2.5 hours. The methanol was rotary evaporated off and the pH adjusted to 9 with ammonium hydroxide. The product was extracted with dichloromethane and saturated sodium chloride multiple times, sodium sulfate was added

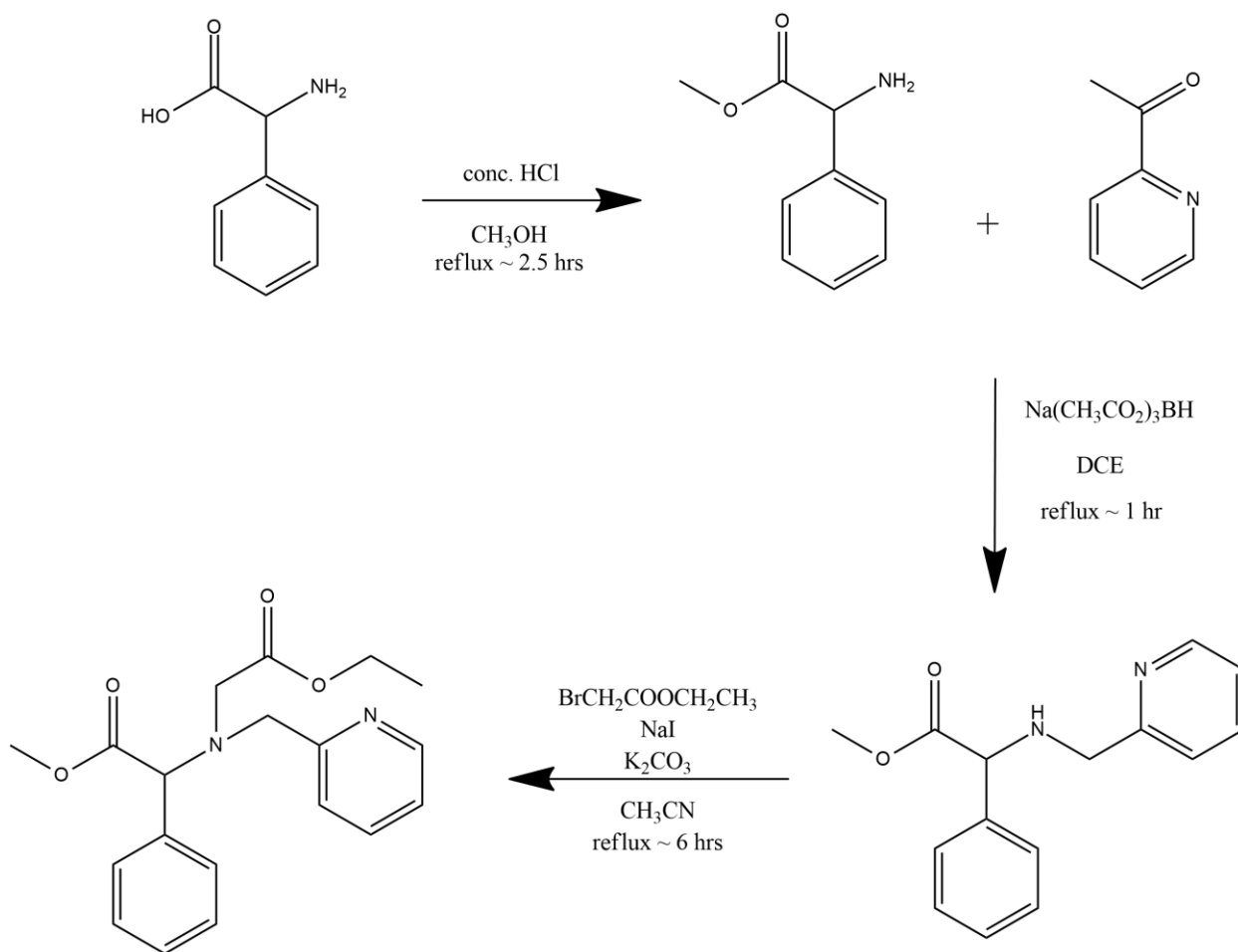
to remove excess water and the mixture was filtered. Rotary evaporation was used to isolate the product and $^1\text{HNMR}$ was taken and is shown in Appendix A.

- 500mg of the product and 30mL of acetonitrile was then added to a two-necked flask with 3 equivalents of potassium carbonate and a 0.1 equivalents of sodium iodide. 1.5 equivalents of ethylbromoacetate was added dropwise and the mixture was refluxed under nitrogen for approximately 6 hours. Rotary evaporation was used to remove the solvent and extractions were performed with dichloromethane and saturated sodium chloride. Sodium sulfate was added to remove excess water and then the mixture was filtered and the product was isolated using rotary evaporation.
- The product was purified using a silica gel column that was packed with a 50:50 hexanes and dichloromethane mixture. $^1\text{HNMR}$ was run again and is shown in Appendix B.
- The triester product was dissolved in 5.0mL of methanol and added to 10 equivalents of sodium hydroxide. This mixture was placed in an ice bath and stirred for approximately 10 minutes before adding 500 μL of water. The mixture continued to stir for another 10 minutes and then removed from the ice bath and stirred overnight. The methanol was rotary evaporated off and the pH adjusted to 2 using hydrochloric acid. Extractions were performed with ethyl acetate and saturated sodium chloride. Sodium sulfate was added to remove excess water and the mixture was filtered. Rotary evaporation was used to remove solvent and isolate the product. ^1HMR is shown in Appendix C.
- The product was dissolved in 1.5mL of concentrated H_2SO_4 and 100 μL of concentrated HNO_3 was added dropwise. The solution was stirred for 1 hour in an ice bath in the dark. In order to quench the reaction, the solution was pipetted on ice and washed with ethyl acetate and saturated sodium chloride twice. After rotary evaporation, 2mL of hexanes

and 2mL of ether were added to precipitate the product. Rotary evaporation was used to isolate the product. ^1HMR was performed and is shown in Appendix D.

Synthesis of Diester Pathway 1

Figure 4: Pathway for Synthesis 2



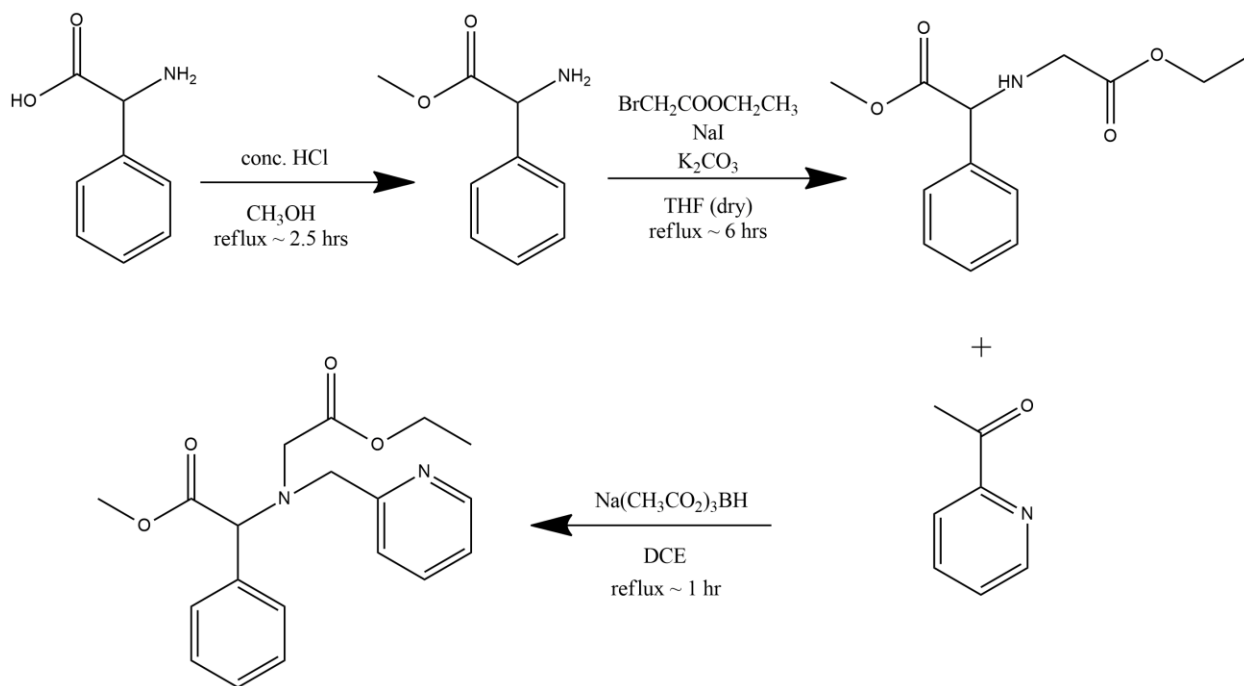
- 6.0g of L – (+) – 2 – phenylglycine was mixed with 80.0mL of methanol and added to a two-necked flask. 20.0mL of 6M HCl was added dropwise and the mixture was refluxed under nitrogen for approximately 2.5 hours. The methanol was rotary evaporated off and

the pH adjusted to 9 with ammonium hydroxide. The product was extracted with dichloromethane and saturated sodium chloride multiple times, sodium sulfate was added to remove excess water and the mixture was filtered. Rotary evaporation was used to isolate the product and ¹HNMR was taken and is shown in Appendix E.

- 1.05g of the product was dissolved in 30mL of dichloroethane and added to a two-necked flask. 600μL of 2-formylpyridine was added dropwise and the solution was refluxed under nitrogen for approximately 1 hour. The solution was cooled to room temperature and 1.36g of sodium triacetoxyborohydride was added. The solution stirred at room temperature overnight.
- Purification was performed using a silica gel column packed with diethyl ether. Dichloromethane and methanol were used as solvents. ¹HMR showed the product was formed and is shown in Appendix F.
- 1.0g of the product and 50mL of acetonitrile was then added to a two-necked flask with 3 equivalents of potassium carbonate and a 0.1 equivalents of sodium iodide. 1.5 equivalents of ethylbromoacetate was added dropwise and the mixture was refluxed under nitrogen for approximately 6 hours. Rotary evaporation was used to remove the solvent and extractions were performed with dichloromethane and saturated sodium chloride. Sodium sulfate was added to remove excess water and then the mixture was filtered and the product was isolated using rotary evaporation.
- The product was purified using a silica gel column and a 50:50 hexanes to dichloromethane solvent mixture. ¹HNMR was taken and is shown in Appendix G.

Synthesis of Diester Pathway 2

Figure 5: Pathway for Synthesis 3



- 6.0g of L – (+) – 2 – phenylglycine was mixed with 80.0mL of methanol and added to a two-necked flask. 20.0mL of 6M HCl was added dropwise and the mixture was refluxed under nitrogen for approximately 2.5 hours. The methanol was rotary evaporated off and the pH adjusted to 9 with ammonium hydroxide. The product was extracted with dichloromethane and saturated sodium chloride multiple times, sodium sulfate was added to remove excess water and the mixture was filtered. Rotary evaporation was used to isolate the product and ¹HNMR was taken and is shown in Appendix H.
- 1.0g of the product and 50mL of dry tetrahydrofuran was then added to a two-necked flask with 3 equivalents of potassium carbonate and a 0.1 equivalents of sodium iodide.

1.5 equivalents of ethylbromoacetate was added dropwise and the mixture was refluxed under nitrogen for approximately 6 hours. Rotary evaporation was used to remove the solvent and extractions were performed with dichloromethane and saturated sodium chloride. Sodium sulfate was added to remove excess water and then the mixture was filtered and the product was isolated using rotary evaporation. ¹H NMR was taken and is shown in Appendix I.

- 150mg of the product was dissolved in 25mL of dichloroethane and added to 1.5 equivalents of 2-formylpyridine and 2 equivalents of sodium triacetoxyborohydride. This mixture was refluxed under nitrogen for approximately 1 hour and then stirred overnight.
- ¹NMR showed no evidence of the product as shown in Appendix J.
- This synthesis was also performed using dry acetonitrile which also gave no product as shown in the ¹NMR in Appendix K.

Results and Discussion

The first synthesis was overall a successful pathway. The reaction steps could easily be carried out and the final product was easily isolated in yields of approximately 60%. This ligand was then studied to determine how well metals such as Zn^{2+} could bind to it.

The second synthesis was a generally successful pathway. The problems that arose with this approach were that the product often had many impurities that needed to be removed using column chromatography. Silica gel and alumina columns were both tried and with each purification a portion of the product was lost in this step. Not only was this pathway more time consuming but the overall yield of the product was fairly low. The product was successfully made and with some adjustments to the purification process, this reaction could be more effective overall in creating the diester complex.

The third synthesis was an attempt to make the same diester but this time performing the bromination step first and then attaching the pyridine group. This approach proved to be less effective than the other. Many trials with different equivalents of 2-formylpyridine and sodium triacetoxyborohydride as well changing from dry acetonitrile to dry tetrahydrofuran all gave no evidence of the desired product. The amount of time the reaction was refluxed was also monitored by TLC but still no product was formed.

Conclusion

This project encompassed the synthesis of ligands that could then be used to make caged complexes that when exposed to light would undergo a structural change that would release the central metal ion. After running all three reaction pathways several times, the first synthesis proved to be the most effective. If time had permitted, caged complexes would have been created with metals such as zinc or iron. Future research will be able to determine the ability of these metals to bind to this NTA Cage ligand.

The second synthesis did successfully give the desired product, however purification and overall yield of the product could be improved. This combination of carboxylic acid and nitrobenzyl groups can give new insight to these caged complexes and research of the ability for metals to bind to this ligand is another area the group may study in the future.

The last synthesis that gave no desired product proved to be a direction that was not worth exploring. The potential to make this diester ligand is greater in the second synthesis where the imine is created before the bromination step.

This project and its research can be used in the ongoing research of photochemistry that can then be applied to biological applications such as metal ion homeostasis, metal neurotransmission and disease pathology.

References

C. Gwizdala and P.N. Basa and J.C. MacDonald and S.C. Burdette *Inorg. Chem.* 2013, 52, 8483-8494

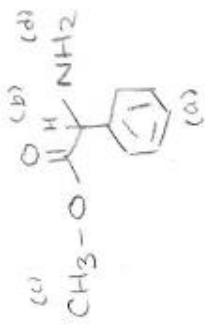
C. Gwizdala and D.P. Kennedy and S.C. Burdette *Chem. Commun.* 2009, 6967-6969

H.M.D. Bandara, D.P. Kennedy, E. Akin, C.D. Incarvito and S.C. Burdette *Inorg. Chem.* 2009, 48, 8445-8455

Appendix A

①

ADVISOR Burdette
NTA-2
Elizabeth Miloscia
PROTON



7.397
7.394
7.381
7.380
7.364
7.343
7.330
7.292

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4.649

Chiral
Singlet
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CDCl3

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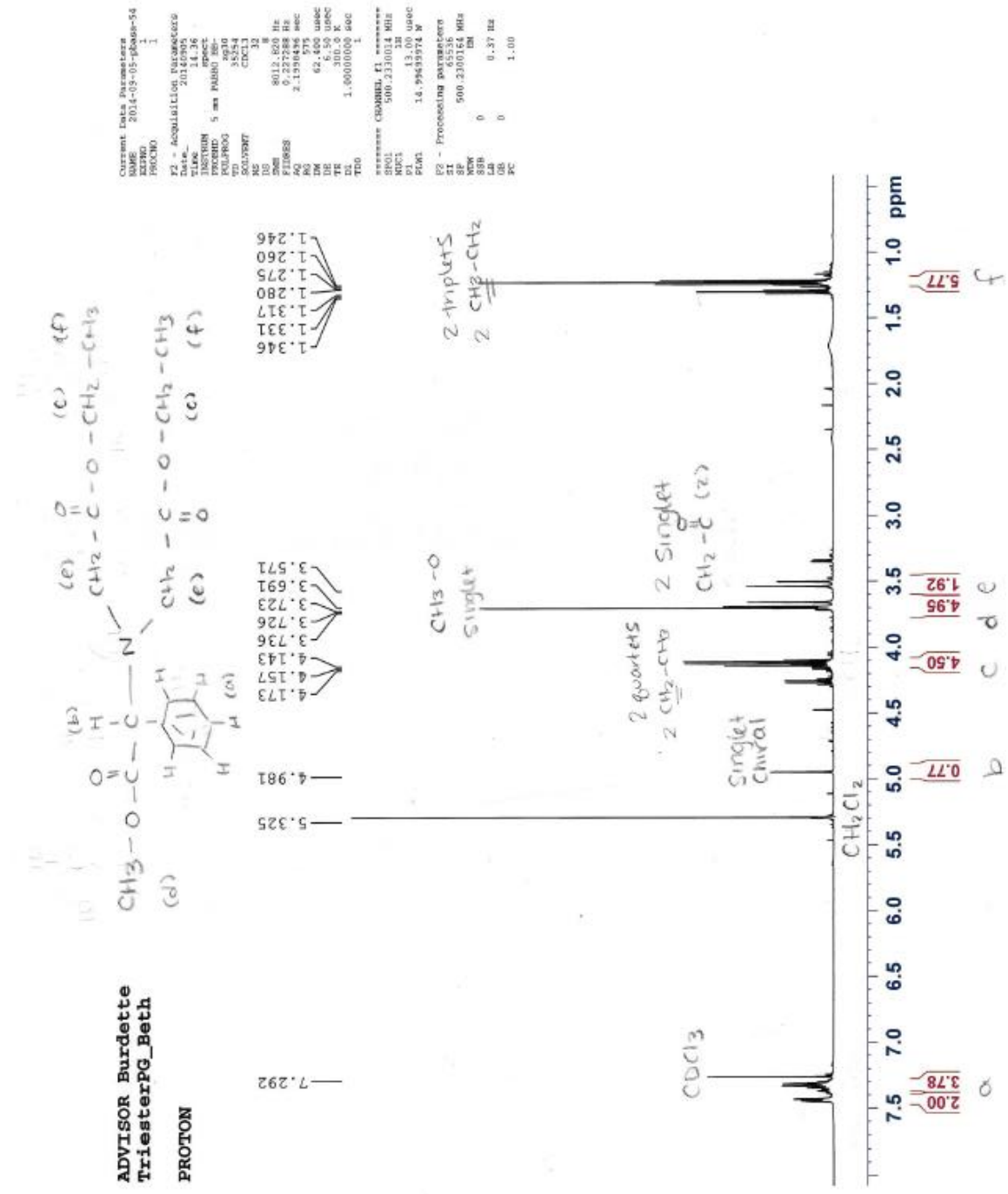
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a
b
c
d

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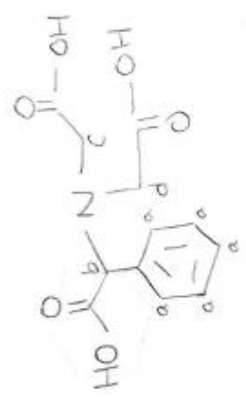
Appendix B



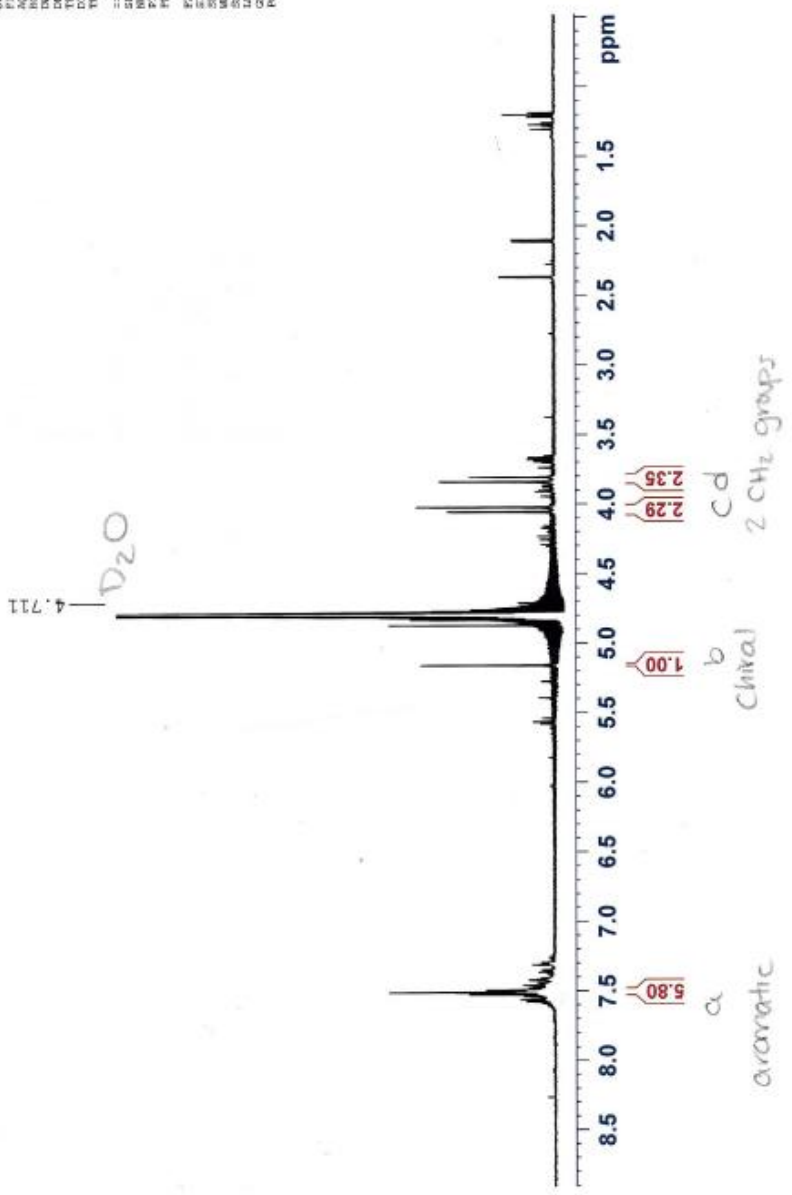
Appendix C

3

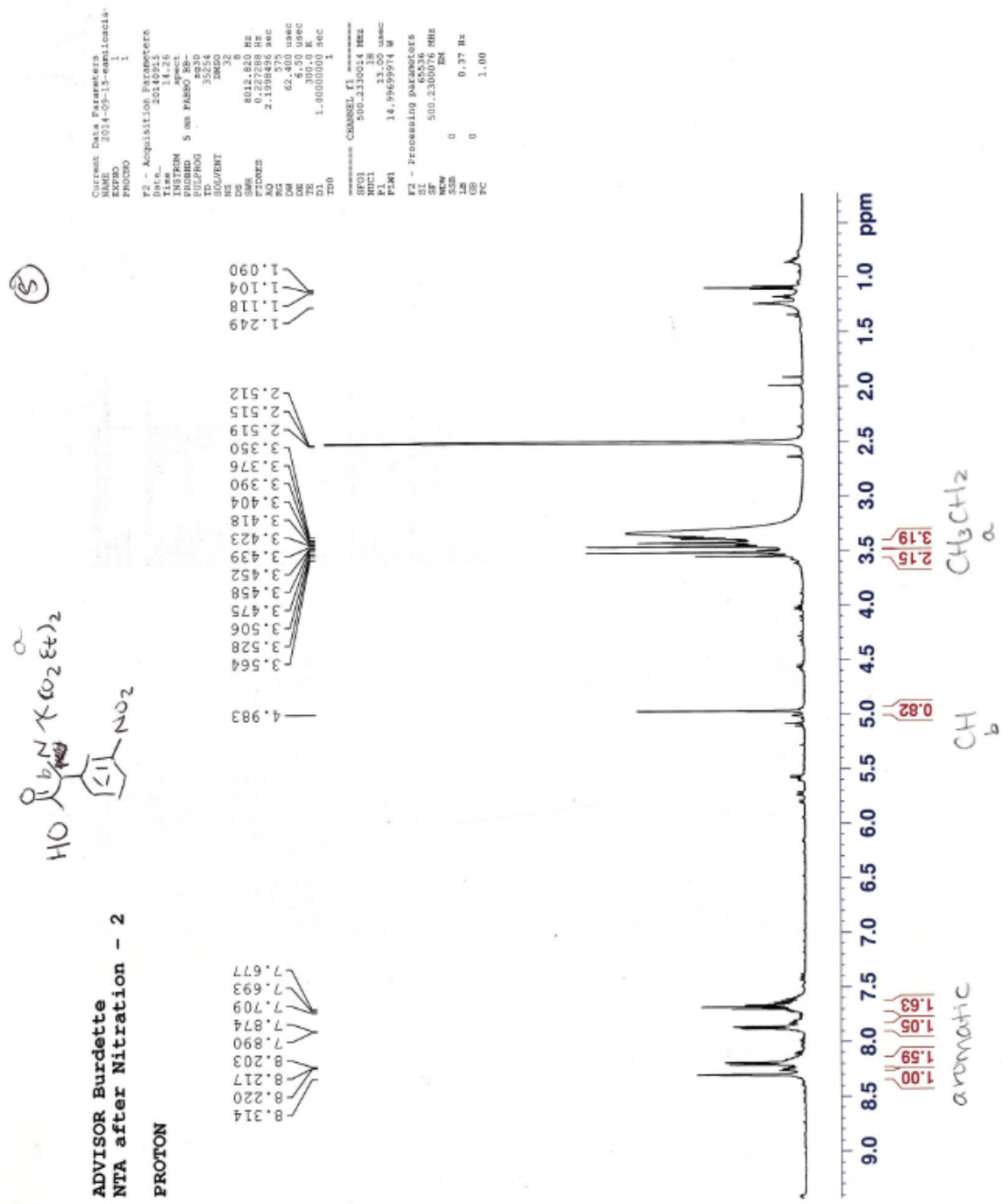
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NTA
PROTON



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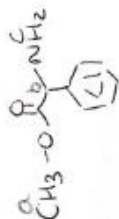
Appendix D



Appendix E

9

ADVISOR Burdette
Starting Material
PROTON



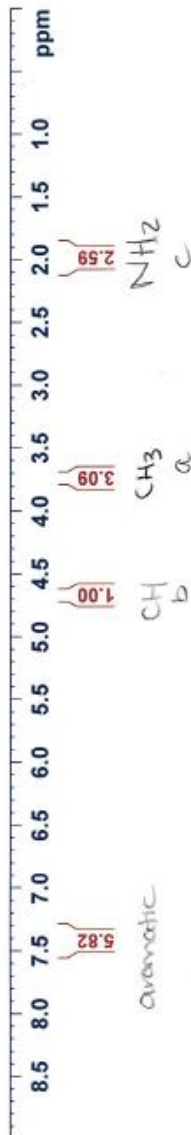
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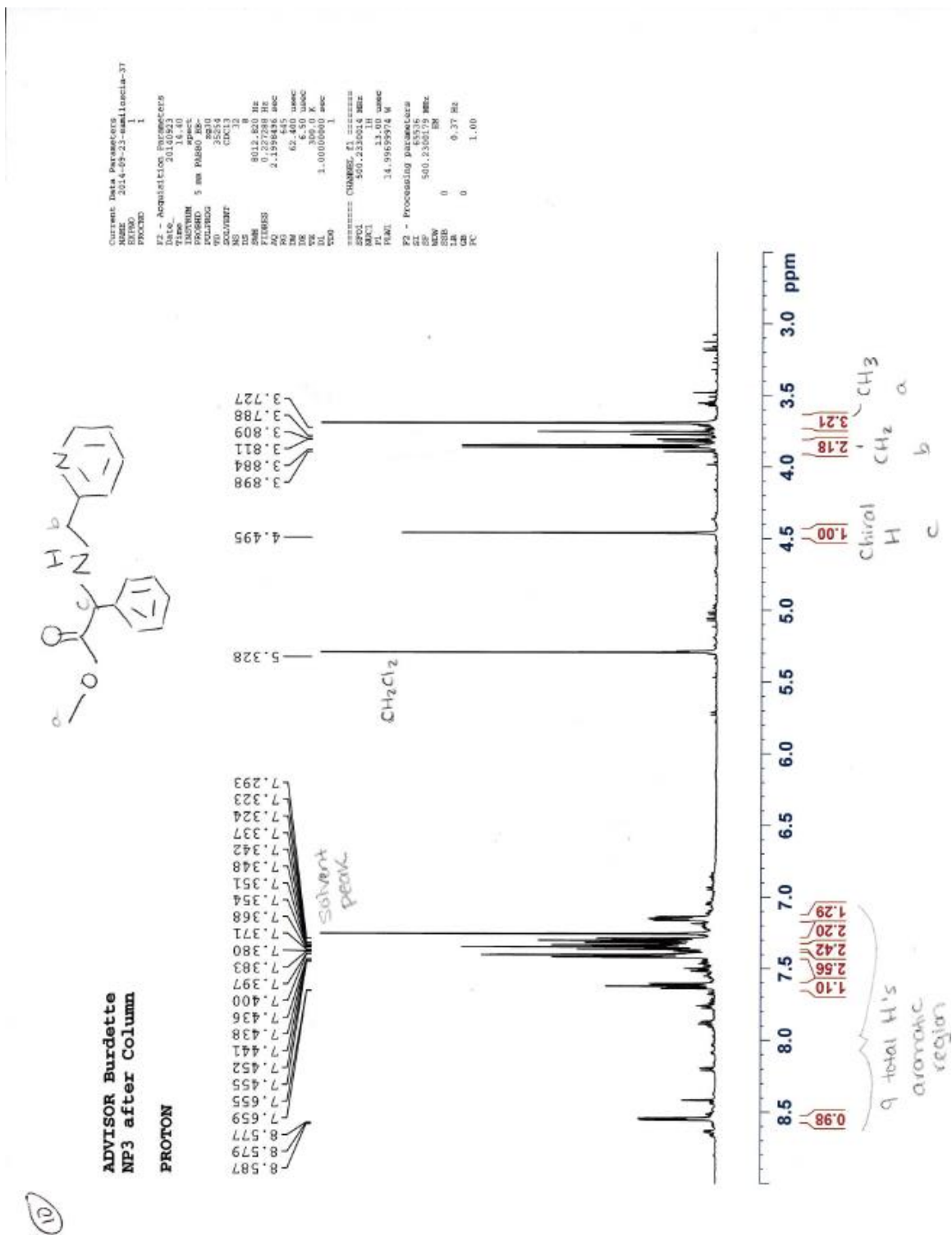
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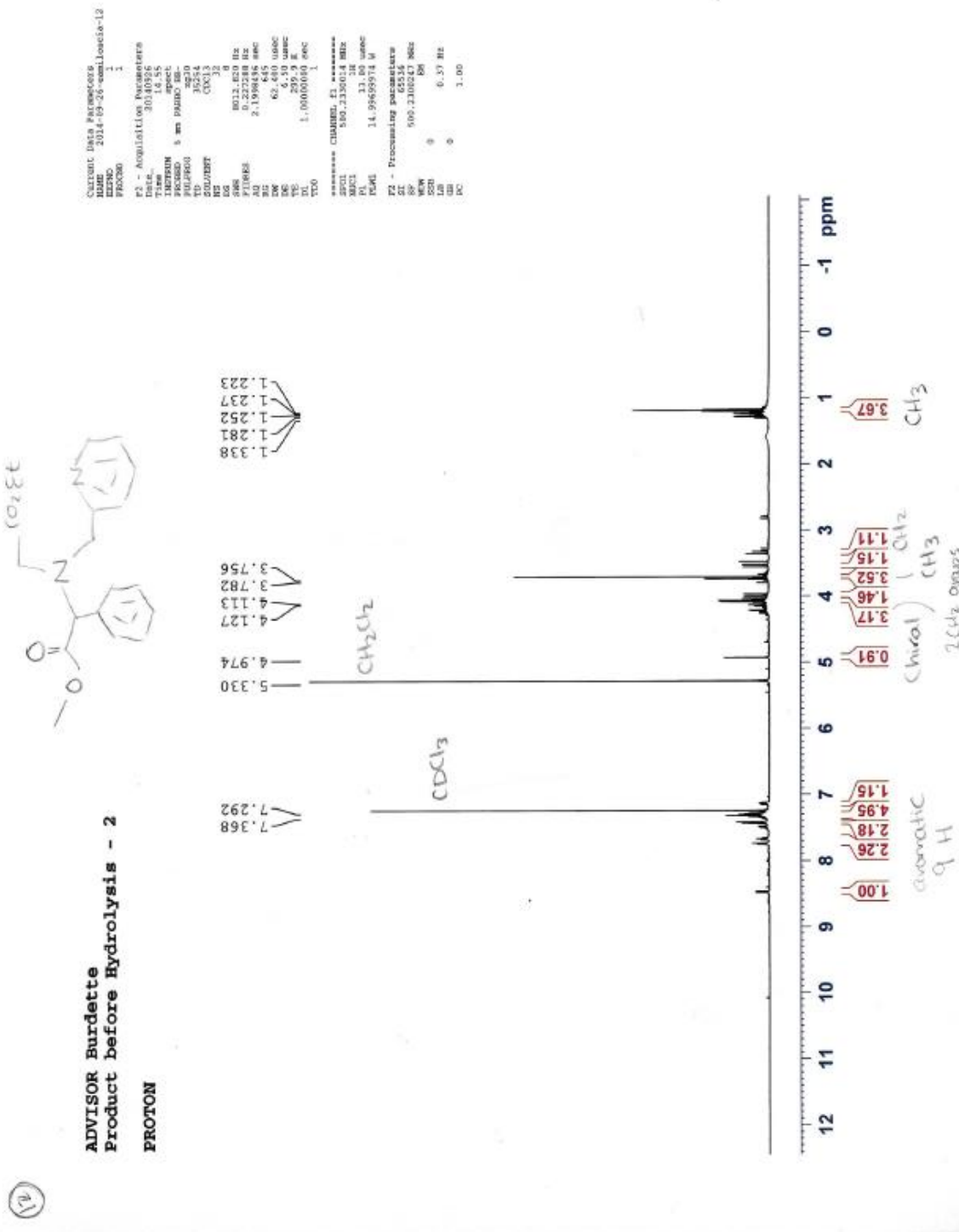
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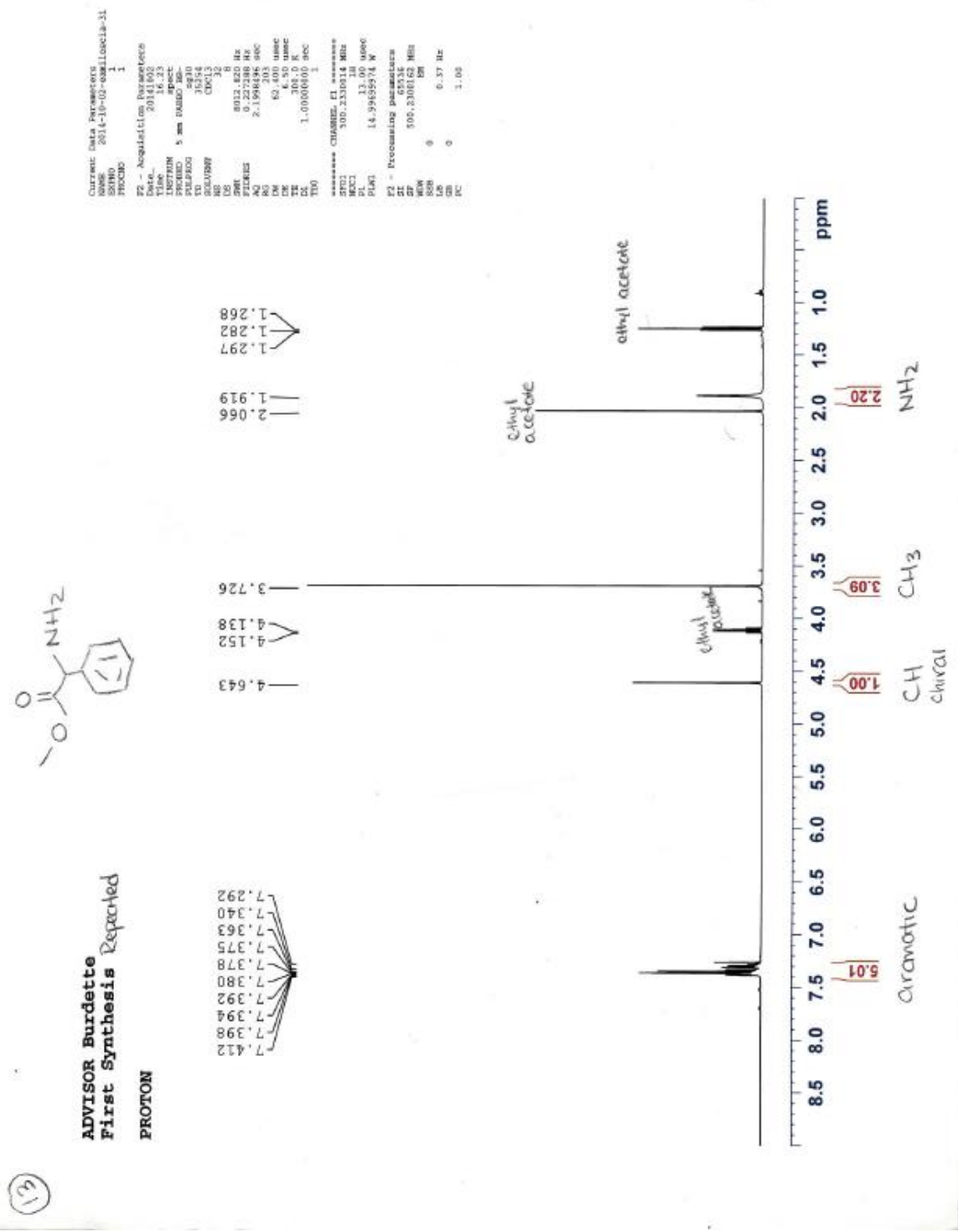
Appendix F



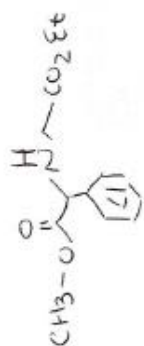
Appendix G



Appendix H

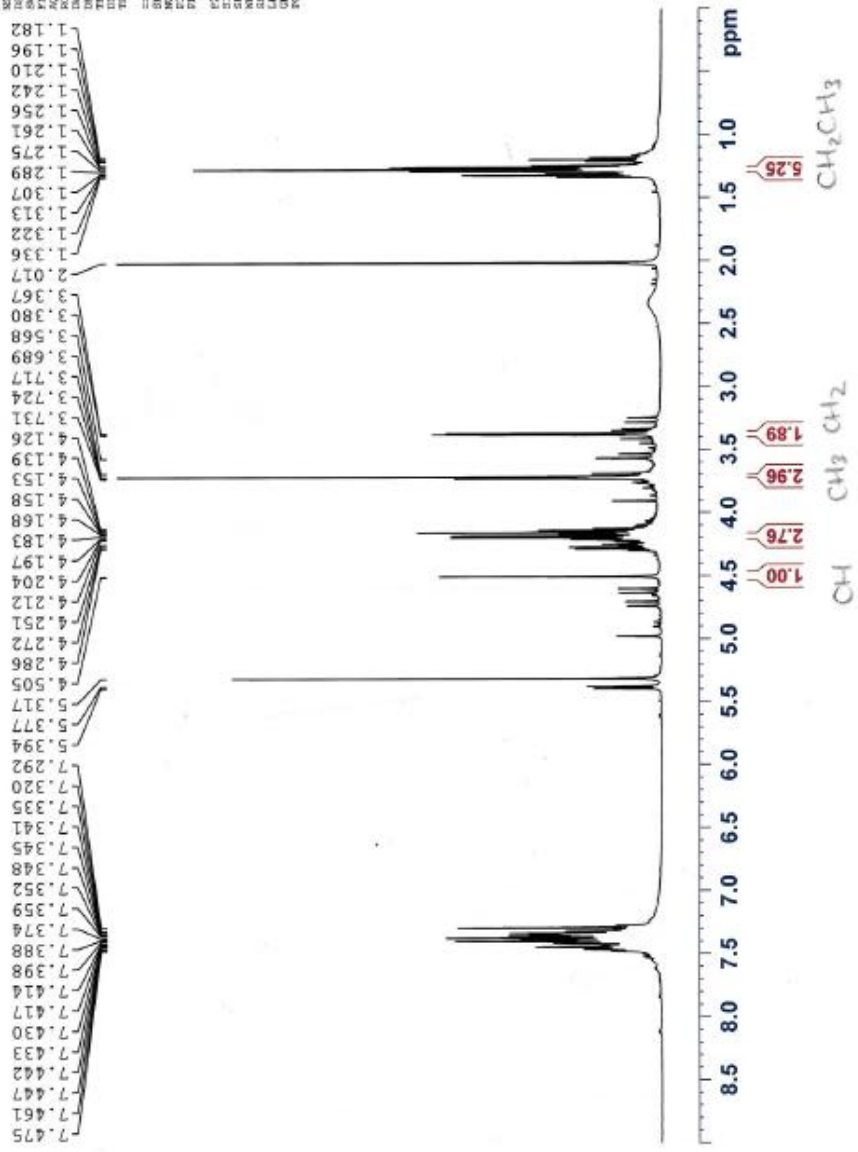


Appendix I



ADVISOR Burdette
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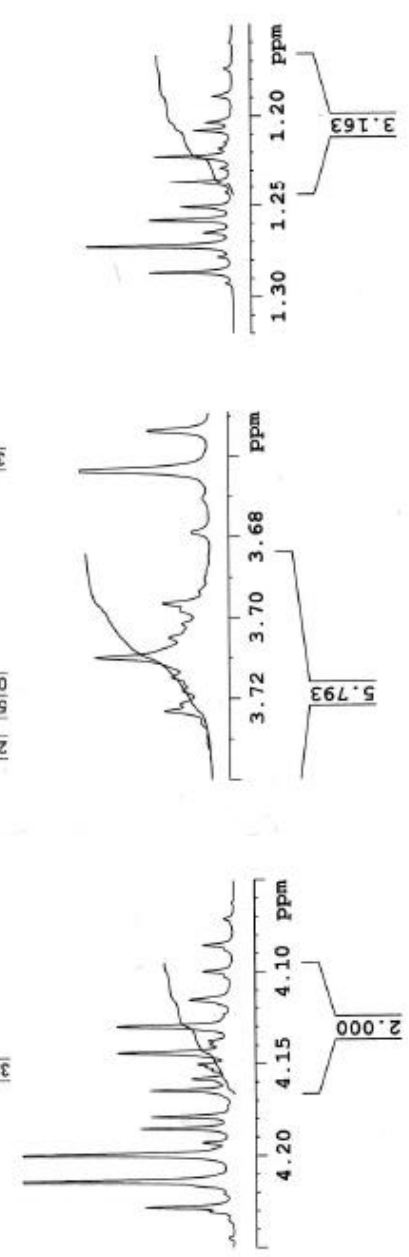
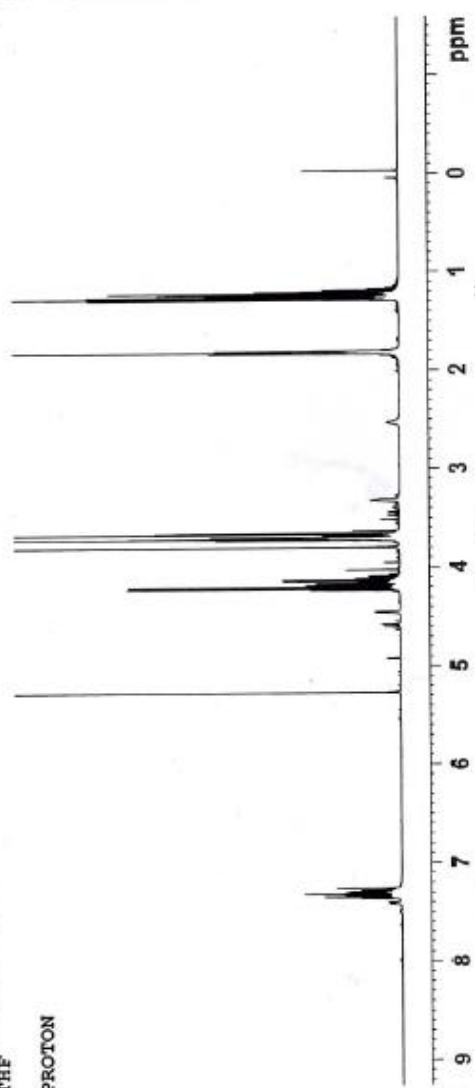


Appendix J

ADVISOR Burdette
THF
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Appendix K

ADVISOR Burdette
ACN
PROTON

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LB: 0.37 Hz
GB: 0
PC: 1.00

===== CHANNEL f1 =====
NUC1: 13C
P1: 13.00 usec
PL1: 0 dB
PL12: 14.99629774 W
===== CHANNEL f2 =====
F2 - Processing parameters
SI: 65536
SF: 500.136090 MHz
WDW: EM
SSB: 0
LB: 0.37 Hz
GB: 0
PC: 1.00
  
```

