

THE ATTEMPTED SYNTHESIS OF 4,4'-BIS(DIMETHYLAMINO)AZOBENZENE-
3,3'-DISULFONYLCHLORIDE AS A CAP FOR β -CYCLODEXTRIN

A Thesis

Presented to

The Faculty of the Department of Chemistry
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Arts

by

Katherine M. Ross

August 2000

APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Arts

Jessamine Ross

Author

Approved, August 2000

Christopher J. Abelt

Dr. Christopher Abelt

Kathleen Min Morgan

Dr. Kathleen Morgan

Robert J. Hinkle

Dr. Robert Hinkle

Table of Contents

| | |
|---|----|
| Acknowledgements | iv |
| List of Figures | v |
| Abstract | vi |
| Introduction | 2 |
| Background | 7 |
| Cyclodextrins | 7 |
| Capping of β -Cyclodextrin | 12 |
| Aromatic Azo Compounds | 15 |
| Experimental | 17 |
| 2-Acetamido-5-nitrobenzene sulfonic acid | 17 |
| 2-Acetamido-5-aminobenzene sulfonic acid | 18 |
| 3,3'-Diaminoazobenzene-4,4'-disulfonic acid | 19 |
| Results | 20 |
| Discussion | 31 |
| Conclusion | 37 |
| Appendix of Spectra | 38 |
| References | 45 |
| Vita | 47 |

Acknowledgements

The author would like to express her gratitude to Dr. Abelt for the opportunity to do graduate research under his tutelage. His endless patience and knowledge were deeply appreciated, as was his calm and collected way of looking at anything that happened in the lab. She would also like to thank Dr. Morgan and Dr. Hinkle for their critique of this manuscript and Dr. Starnes for her introduction to research as an undergraduate. Finally she would like her friends and colleagues in the Abelt lab, especially those from the Annex who know who they are.

List of Figures

Figure:

| | | |
|-----|--|----|
| 1. | General Features of β -Cyclodextrin | 3 |
| 2. | Symmetric Cap with Sulfonate Ester Linkages | 5 |
| 3. | An α -1,4-Glycosidic Linkage Between Two Glucopyranose Units | 7 |
| 4. | Three Most Widely Characterized Cyclodextrins | 8 |
| 5. | Hydrogen Bonding Between Adjacent Glucopyranose Units | 10 |
| 6. | Three Regioisomers of a Symmetrically Capped β -Cyclodextrin | 12 |
| 7. | Acid-Base Forms of an Azo Dye | 16 |
| 8. | Three Paths to 4,4'-bis(dimethylamino)azobenzene-3,3'-disulfonyl chloride | 20 |
| 9. | First Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene | 21 |
| 10. | Second Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene | 22 |
| 11. | Third Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene | 23 |
| 12. | Fourth Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene | 24 |
| 13. | Fifth Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene | 25 |
| 14. | Synthesis of 4,4'-diaminoazobenzene | 26 |
| 15. | First Methylation Attempt on 4,4'-diaminoazobenzene | 27 |
| 16. | Synthesis of 4,4'-bis(dimethylamino)azobenzene | 27 |
| 17. | Synthesis of 4,4'-bis(dimethylamino)azobenzene-3,3'-disulfonic acid | 29 |
| 18. | First Methylation Attempt on 4,4'-bis(diamino)azobenzene-3,3'-disulfonic acid | 29 |
| 19. | Second Methylation Attempt on 4,4'-bis(diamino)azobenzene-3,3'-disulfonic acid | 30 |
| 20. | Mechanism for Acetylation of Amino Group | 32 |
| 21. | Mechanism for Azo Coupling | 34 |
| 22. | Hydrolysis of Acetyl Group back to an Amino Group | 35 |
| 23. | ^1H NMR of Recrystallized 2-Amino-5-nitrobenzene sulfonic acid | 39 |
| 24. | ^1H NMR of 2-Acetamido-5-nitrobenzene sulfonic acid | 40 |
| 25. | ^1H NMR of 2-Acetamido-5-aminobenzene sulfonic acid | 41 |
| 26. | ^1H NMR of 3,3'-bis(diamino)azobenzene-4,4'-disulfonic acid | 42 |
| 27. | ^1H NMR of 4,4'-Diaminoazobenzene | 43 |
| 28. | ^1H NMR of 4,4'-Dimethylaminoazobenzene | 44 |

Abstract

The synthesis of an organic dye molecule, 4,4'-bis(dimethylamino)azobenzene-3,3'-disulfonic acid, was attempted. This molecule was intended to be a pH sensitive cap for β -cyclodextrin. This cap would induce gross structural changes in the β -cyclodextrin with a change in pH, and thus affect its binding properties in host/guest chemistry. Numerous synthetic pathways were explored. Characterization of all products was by proton NMR spectroscopy. Complications caused by the electron withdrawing character of the sulfonate functionalities hindered development of the synthesis.

THE ATTEMPTED SYNTHESIS OF 4,4'-BIS(DIMETHYLAMINO)-AZOBENZENE-
3,3'-DISULFONYLCHLORIDE AS A CAP FOR β -CYCLODEXTRIN

Introduction

Curiosity into the function of natural enzymes has long existed. Enzymes are proteins with catalytic activity that exhibit both high specificity and rate acceleration. Enzymes possess an active site, the part of the enzyme that binds to a specific substrate enabling the substrate to undergo a reaction at an increased rate. Developing an understanding of how enzymes function at this active site would be useful to those interested in synthetic techniques involving catalysis.

Due to the vast number and size of biological enzymes it is necessary to study their function using model systems. An enzyme can be effectively modeled when the exact nature of the binding subsite has been synthetically mimicked in terms of shape, size and microscopic environment.¹ For the model to completely succeed, numerous aspects of the active site must also be mimicked. These include the identity of functional groups, any stereochemistry involved and the mechanism behind how the enzyme functions. Although all of these criteria present a challenge, synthesizing these enzyme mimics is well documented in the literature.^{2,3,4}

One example of a molecule that can function as an enzyme mimic is cyclodextrin. Cyclodextrins in general are cyclic oligomers of glucose that can form inclusion complexes with a substrate due to their shape. In particular, β -cyclodextrin has been used as a mimic for various specific enzymatic systems. Examples include model systems for ribonuclease⁵, carbonic anhydrase⁶, and chymotrypsin.⁷ Formation of inclusion

complexes with various compounds is one of the most important characteristics of cyclodextrins.¹

β -Cyclodextrin is able to catalyze reactions both covalently and non-covalently. The molecule's structure provides functional groups for covalent catalysis and an interior cavity that is capable of binding in a non-covalent manner (Figure 1).

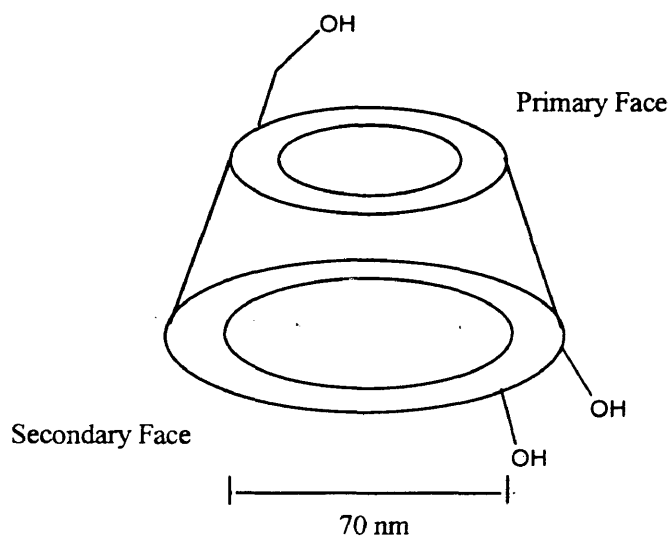


Figure 1: General Features of β -Cyclodextrin

During covalent catalysis, the molecule's numerous hydroxyl groups can act as nucleophiles or can be derivatized to other nucleophilic groups. During non-covalent catalysis, the molecule's interior cavity acts as the active site and binds a substrate with the help of a hydrophobic interaction between the substrate and the aqueous medium.

The relationship between an enzyme and a substrate is a type of host/guest chemistry. In our model, the cyclodextrin plays the part of the host. However, our host lacks an important feature that naturally occurring enzymes possess, namely an ability to adjust based on environmental conditions. This is necessary in biological systems so that an organism can utilize different enzymes when it needs their help for whatever reaction is necessary to keep the system functioning at a specific moment in time. In the cyclodextrin mimic, the so-called “environmental switch” is absent; it can bind a variety of guests within a variety of environmental conditions.

In order to devise an appropriate environmental switch for cyclodextrin, we had to consider the ease with which the environment could be altered. The goal was to allow some aspect of the environment to cue differences in how the host binds its guest. Perhaps one of the most easily controlled conditions in aqueous medium is hydrogen ion concentration. Therefore, the pH of the solution was chosen as the cue in our experiments.

Once the cue was chosen, the question remained as to how to modify cyclodextrin to create a pH dependent host/guest relationship. The benefit of choosing organic indicator dyes as the environmental switches was two-fold. As the pH of the solution changes, the organic molecules can either protonate or deprotonate. This can change the hybridization and therefore the bond angles of the molecule altering its shape and dimensions. If the dye molecule is attached to cyclodextrin with two linkages, then this change in the molecule’s span could affect the host molecule’s ability to form inclusion complexes with guests. In order for the switch to be effective, the host/guest complex would exist at one pH and the guest would be completely excluded at another pH.

A second benefit to the use of the dye molecules is the ability to visually follow a reaction. When the structure of the organic dye is altered in some way it can be accompanied by a color change in the solution. Ideally, a color change in solution would signal that the organic dye molecule structure had changed in some way, thereby confirming or denying the presence of a guest in the host molecule.

The pH indicator chosen for this study was a symmetric molecule consisting of two substituted aniline ring systems that are joined by a diazo linkage. This molecule would be attached to β -cyclodextrin by creating two sulfonate ester bonds at positions where the cyclodextrin contains hydroxyl functionalities (Figure 2).

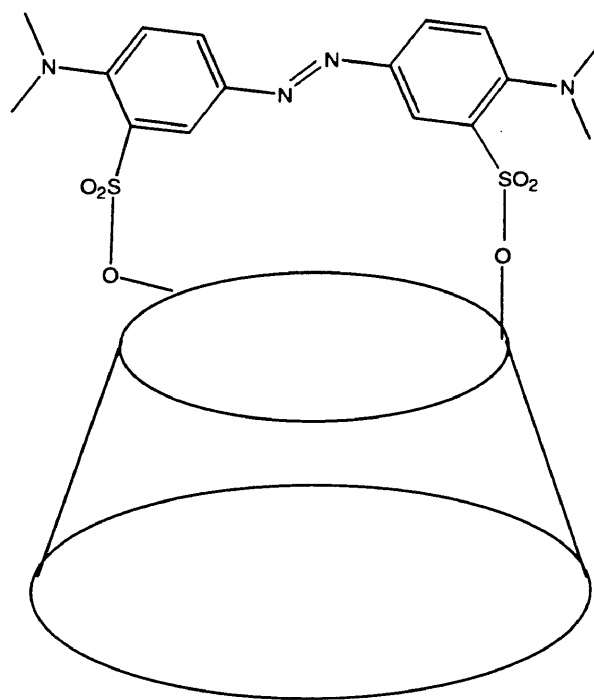


Figure 2: Symmetric Cap with Sulfonate Ester Linkages

This newly altered cyclodextrin system derives its chromophoric behavior from the diazo linkage. As pH changes, protonation at one of the nitrogens of the diazo linkage leads to a difference in the electronics of the diazo bond. This can lead to a change in the structure of the cap molecule and therefore in the structure of cyclodextrin. This change may be signaled by a color change in solution and could confirm or deny an appropriate guest's presence or absence.

Background

Cyclodextrins

Cyclodextrins are cyclic oligosaccharides composed of D(+)-glucopyranose units. These units each reside in a chair conformation and are connected via α -1,4-glycosidic linkages to form a ring structure (Figure 3)⁸.

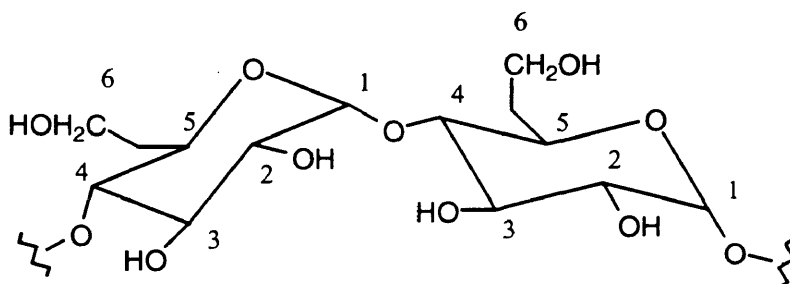


Figure 3: An α -1,4-glycosidic linkage between two glucopyranose units

Villiers first discovered cyclodextrins in 1891 when he grew a culture of *Bacillus amylobacter* on medium that contained starch⁹. He gave the new material the name “cellulosine” because he felt it possessed a distinct similarity to cellulose. It took nearly fifty years before cyclodextrin’s potential became apparent. This occurred with the

discovery of the molecule's structure by Freudenberg during the mid-twentieth century¹⁰.

In recent years, cyclodextrin has enjoyed a wide variety of uses in research and industrial settings. Interest in the molecules by both food and pharmaceutical industries stem from the ability of the body to metabolize them. Cyclodextrins can be used to “mask” unpleasant tastes in certain foods. As a drug delivery agent, cyclodextrins can transport organic molecules that would normally be insoluble in the body. Cyclodextrins can also offer a level of protection against modifying agents such as ultraviolet radiation and oxidants that can cause degradation. Finally, polymer based cyclodextrins are reportedly being used to clean up the water supply by removing contaminants including trichloroethylene among others.¹¹

The best characterized cyclodextrins are those containing 6, 7, and 8 glucose units. These are referred to as α , β , and γ -cyclodextrin respectively (Figure 4).

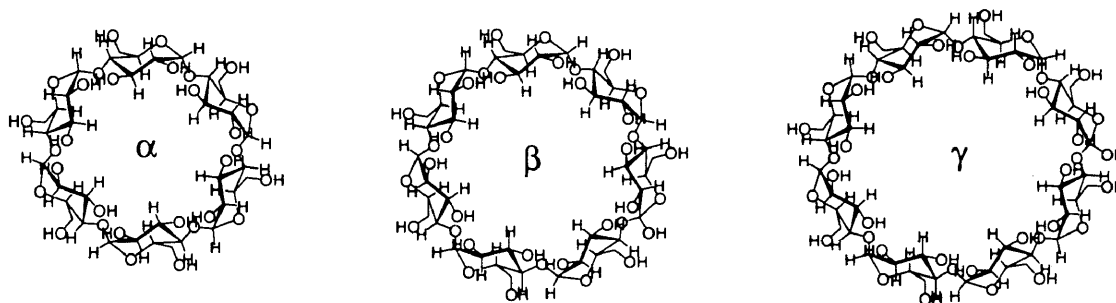


Figure 4: Three Most Widely Characterized Cyclodextrins

Cyclodextrins with less than six glucose units encounter severe steric problems in closing the ring structure⁹. Although cyclodextrins of greater than eight units have been successfully synthesized, they are not as widely studied. This is mainly due to the fact that as the number of units increase, the cyclodextrin will lose its ability to hold a defined shape. This lowers the molecule's utility immensely.

Of the cyclodextrins, β -cyclodextrin is the molecule we focus on as our "host" molecule in this investigation. Spectroscopic techniques such as ¹H NMR spectroscopy and infrared spectroscopy as well as X-ray diffraction data have provided additional structural detail for cyclodextrins since Freudenberg's discovery.⁹ β -cyclodextrin's seven glucose units reside in a toroidal shape. The ring structure has two faces that are referred to as primary and secondary based on the hydroxyl groups present on that face. The primary face contains the C6 primary hydroxyl groups while the secondary face contains the C2 and C3 secondary hydroxyl groups.

The primary and secondary hydroxyl groups in β -cyclodextrin have different characteristics. The C6 hydroxyls are flexible, free to rotate or "flop" around. This gives them the ability to partially cover the smaller primary opening to β -cyclodextrin's center. The C2 and C3 hydroxyl groups are not flexible but are held in place by intramolecular hydrogen bonding that occurs when a C2 hydroxyl on one glucopyranose unit interacts with the C3 hydroxyl of an adjacent unit (Figure 5).

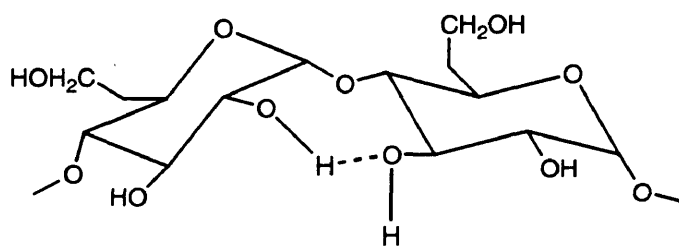


Figure 5: Hydrogen Bonding Between Adjacent Glucopyranose Units

This intramolecular hydrogen bonding is strongest in β -cyclodextrin. The shape of α -cyclodextrin is distorted and γ -cyclodextrin does not hold its shape well enough to have maximum hydrogen bonding. However β -cyclodextrin's shape allows complete hydrogen bonding creating a more rigidly shaped molecule.

The increased rigidity provided by this hydrogen bonding is one of the key features that allows β -cyclodextrin to be a good enzyme model. Due to its toroidal shape, cyclodextrin possesses a well-defined internal cavity that measures 7.0\AA wide by 7.0\AA long. The outside of the molecule is lined with primary and secondary hydroxyl groups, which are hydrophilic enabling the entire molecule to reside in an aqueous environment. On the other hand, non-polar carbon-carbon bonds and only slightly polar carbon-hydrogen bonds and ether linkages characterize the interior of the cavity. With its large diameter and hydrophobic character, cyclodextrins can form inclusion complexes with small organic molecules in a stoichiometric 1:1 manner especially in aqueous solution.¹² By attaching catalytic functional groups to the cyclodextrin, one can mimic an enzyme.

The differing environments of the primary and secondary faces of β -cyclodextrin lead to enzyme mimics with different selectivities.¹³ Because of its larger opening, steric

considerations allow the formation of an inclusion complex more easily there. The hydroxyl groups on the primary face are the most reactive toward electrophilic reagents. This can be attributed to two factors. The C6 hydroxyls are primary so they are less hindered and they also have a pK_a of $\sim 15-16$.¹³ Due to these factors, derivitization of cyclodextrin is easiest at the C6 positions.

Capping of β -Cyclodextrin

Modification of cyclodextrins by attachment of other molecules to the primary face usually occurs through the creation of sulfonate or carboxylic ester bonds at the C6 hydroxyl positions. This modification can be divided into two distinct categories. Linking to one glucopyranose unit is referred to as tethering. Linking to two different glucopyranose units is called capping. Because there are seven glucopyranose units in β -cyclodextrin, more than one regioisomer is possible. Assigning each unit a letter, A through G, and then designating where the derivitization is taking place designates the regiochemistry of a capped β -cyclodextrin (Figure 6). Symmetric caps can give the regioisomers A-B, A-C, and A-D. With an unsymmetric cap, the isomers A-E, A-F, and A-G can also exist.

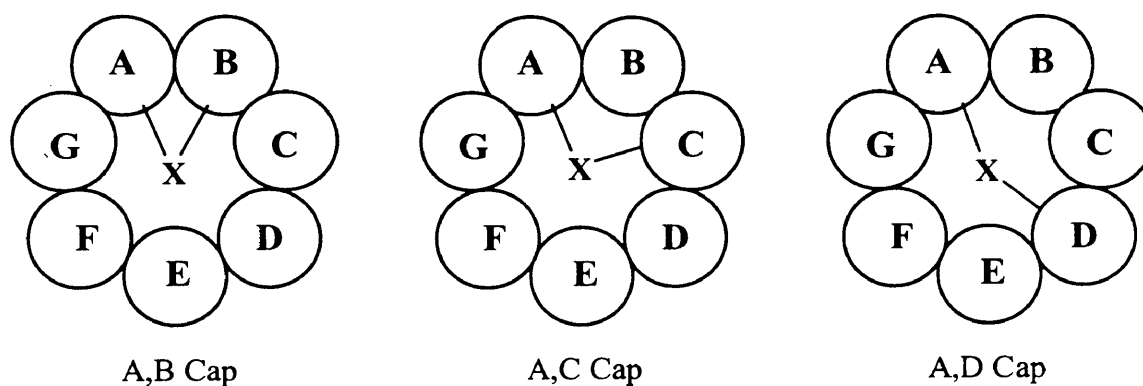


Figure 6: Three Regioisomers of a Symmetrically Capped β -Cyclodextrin

The regioisomer that is formed when capping β -cyclodextrin depends mainly on the geometry and span of the capping molecule. In the “looper’s walk” mechanism of capping, the first linkage to the β -cyclodextrin determines the site of the second connection at the “best fit” position.¹⁴ This model considers the distance across the primary face, the strain in the transition state, and the direction of the approaching entering group. It forms what is referred to as a rigid cap.

To address possible confusion, it should be addressed that the synthesis of capped β -cyclodextrin could be complicated by the possibility of polycapping, where a single cap molecule could bind to two different cyclodextrin molecules.¹⁵ However, this is not a large problem because once a molecule has been tethered to cyclodextrin capping becomes much more likely than crosslinking. Once tethered, the cap will bind to the closest hydroxyl group that it can reach without placing undue strain on itself.

Capping β -cyclodextrin can increase its utility as a model system for an enzyme and its use in host/guest chemistry. It is sometimes difficult for β -cyclodextrin to tightly bind a substrate due to the nature of binding by the cyclodextrin cavity itself.¹⁶ The cylindrical cavity with open ends does not truly immobilize the substrate even with the hydrophobic interaction with the solvent. By essentially blocking one opening via a cap, the cavity can hold a substrate better.

Derivatization of β -cyclodextrin by capping with a molecule that builds in an “environmental switch” has been attempted using various organic molecules as the cap and various conditions as the “switch.” Switching between *cis* and *trans* forms of a cap can be accomplished with azophenyl capped β -cyclodextrin.¹⁷ Although the binding ability was increased significantly in the *cis* form of the molecule, it would eventually

thermally relax to the trans form. Photochemical cis-trans isomerization has been used with stilbene-capped β -cyclodextrin¹⁸, but the cis isomer in this case broke down as well. Other examples of caps for β -cyclodextrin that have been synthesized and attached include anthraquinone-2,6-disulfonate¹⁹ and 9,10-dicyanoanthracene-2,6-disulfonate.²⁰

In this project we want to attach a cap that will be sensitive to hydrogen ion concentration. We propose to cap with an azo dye molecule which when exposed to acidic and basic conditions will change its structure sufficiently to induce gross structural changes in the β -cyclodextrin host molecule.

Aromatic Azo Compounds

In 1858, Peter Griess isolated diazotised picramic acid and recognized it as a member of a previously unknown group of compounds.²¹ This class was given the name diazo due to the belief that two atoms of hydrogen in the aromatic nucleus were replaced by nitrogen.²¹ However, Kekule later showed that the nitrogen atoms were actually attached at the same position.²¹ Since then, azo compounds have been extensively characterized.

The broad class now referred to as azo compounds includes organic molecules containing an $-N=N-$ functionality. Aromatic azo compounds are resonance stabilized which can manifest in the shortening of the C-N bond in trans azobenzene as a result of conjugation.²² Due to the nitrogen-nitrogen double bond in these molecules, they can exist in both the cis and trans forms. In the cis form the benzene rings are rotated out of the plane due to steric factors, while the trans isomer has an almost coplanar structure.²² The cis structure is much less stable however, so we will be concerned with the trans configuration.

Another manifestation of the presence of the $-N=N-$ group is the chromophoric behavior of these molecules. Azo compounds are used in the manufacturing of organic dyes. By attaching various groups to the nitrogens, different colored dyes can be created. This chromophoric tendency of azos, as well as their extensive characterization, are excellent reasons to choose this class of molecules as the environmental switch to build into β -cyclodextrin.

The other major consideration when selecting the environmental switch is how it reacts to the cue that has been selected. Each of the nitrogens in an azo compound has a lone pair of electrons. Protonation at either one of these lone pairs can change the structure of the molecule and an indicator of this transformation will be a color change in the solution. Figure 7 shows the interconversion between the acid and base forms of an azo dye.

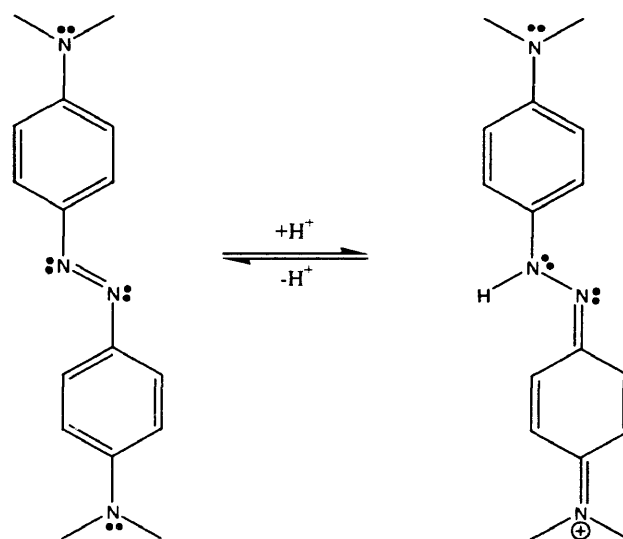


Figure 7: Acid-Base Forms of an Azo Dye

When this interconversion happens, at least one atom undergoes a change in hybridization between sp^2 and sp^3 . The bond angles corresponding to these two hybridizations, 120° and 109.5° respectively, are different enough to change the span of the cap and structure of the β -cyclodextrin. Hopefully the structure of the host will change enough so that the guest will bind at one pH, whereas the guest will be rejected at another pH.

Experimental

Instrumentation

For sample analysis, both a Varian Mercury 400MHz and a Gemini 300MHz spectrometer were used to obtain proton NMR spectra. All samples were analyzed at ambient temperature with deuterated DMSO as the solvent. Distillation under aspirator vacuum was performed using a Buchi Rotovapor. Hydrogenation was done using a Parr Hydrogenator.

Chemicals

All starting materials were commercial products purchased from Acros, Aldrich, Pfaltz and Bauer, and Fisher Scientific that were used as received unless otherwise noted.

Synthesis of 2-Acetamido-5-nitrobenzene sulfonic acid

Due to visible impurities in the proton NMR spectra of the starting material it was recrystallized before use. 50g of 2-amino-5-nitrobenzene sulfonic acid ammonium salt was recrystallized from 50% acetic acid (~300mL). Crystals were allowed to precipitate overnight and then were collected by filtering through a sintered glass funnel. The solid was allowed to air dry and analyzed by NMR spectroscopy (for NMR, see Appendix, Figure 23) prior to beginning the first step.

20g (92 mmol) of recrystallized 2-amino-5-nitrobenzene sulfonic acid ammonium salt was placed in a 250 mL Erlenmeyer flask containing 50 mL acetic anhydride and 10 mL pyridine. The mixture was heated to reflux loosely covered for a period of one hour and then allowed to stand at room temperature overnight. The following day the product was collected using suction through a sintered glass funnel. The yield was ~17 g (~71%). The product had a melting temperature of 310 °C and was verified by NMR spectroscopy (for NMR, see Appendix, Figure 24).

Synthesis of 2-Acetamido-5-aminobenzene sulfonic acid

15 g (58 mmol) of 2-acetamido-5-nitrobenzene sulfonic acid was placed in a 1:1 ethanol:water mixture (~300mL). 1g of 10% Pd/C catalyst was added. After for stirring ~15 minutes, the mixture was hydrogenated overnight at a pressure of 54 psi. The following day, the liquid was filtered with suction through a Buchner funnel and the filtrate retained. (The catalyst was also recovered and could be reused in subsequent hydrogenations.) The filtrate was distilled under vacuum aspiration to obtain a yellow solid. This solid was recrystallized from water and allowed to stand overnight. The product was collected with suction in a sintered glass funnel and checked by NMR (for NMR, see Appendix, Figure 25). Until it was needed for the next step, the product was kept dry in a vacuum oven. The yield was 7.1 g (~53%) and the product had a melting temperature of 289 °C.

Synthesis of 3,3'-Bis(diamino)azobenzene-4,4'-disulfonic acid²³

2g (7.3mmol) of dried 2-acetamido-5-aminobenzene sulfonic acid was added to 100 mL acetic acid at room temperature in a 250 mL round bottom flask. To this mixture was added 6.69g (43mmol) of sodium perborate tetrahydrate and 0.43g (7.0mmol) of boric acid. The flask was then fitted with a reflux condenser and a CaCl₂ drying tube and allowed to stir in a 55°C oil bath for six hours. At this time, the mixture was distilled under aspirator vacuum to remove all of the acetic acid. 50 mL of methanol and 50 mL of 6N HCl were added to the flask and the mixture was heated at reflux overnight. The following day, both the methanol and HCl were removed by distillation under aspirator vacuum. The remaining solid was slurried in acetic acid and filtered with suction through a sintered glass funnel. The acetic acid was removed from the filtrate by rotary evaporation and the remaining solid was placed on the vacuum pump overnight. The solid was dissolved in H₂O and stirred with 15 mL of Amberlite IR-120(plus) ion-exchange resin for 15 minutes. Gravity filtration removed the beads and the H₂O was then removed using rotary evaporation. The solid was dried overnight under high vacuum.

A solution of 100 mL ethanol, 100 mL ethyl acetate and 5 mL acetic acid was made. The compound was dissolved in 20 mL of the solution and placed on top of a layer of silica gel in a sintered glass funnel. The remaining solution was pulled through the silica by applying a vacuum. The filtrate was placed on the rotary evaporator to remove the solvent and then the remaining solid was placed on the pump to dry. The yield was 0.9 g (~27%) and the melting temperature was 148 °C. The dried product was checked by NMR spectroscopy (for NMR, see Appendix, Figure 26).

Results

The synthesis of the desired symmetric diazo compound was challenging. For this reason, it was necessary to explore several synthetic pathways. These different pathways can be divided into three groups based on the starting material used in each one. The structures of the three starting materials (1,2,3) and the desired diazo product (4) can be seen in Figure 8.

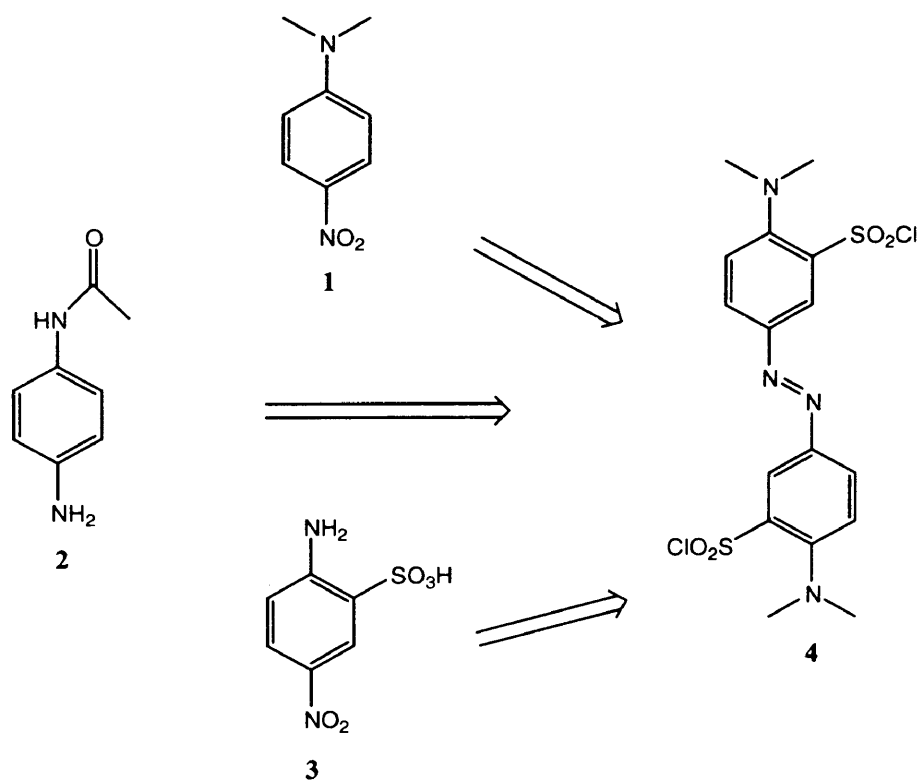


Figure 8: Three Paths to 4,4'-bis(dimethylamino)azobenzene-3,3'-disulfonylchloride

The first starting material we considered was *N,N*-dimethyl-*p*-nitroaniline. With the dimethylamino functionality already in place, formation of the azo connection and sulfonation meta to the azo linkage remained. The azo coupling reaction was done first and was attempted in numerous different ways.

The first attempt to connect two molecules of **1** with an azo bridge to yield **5**, involved reductive dimerization with sodium borohydride in dimethyl sulfoxide (Figure 9).²⁴ This reaction is thought to go through four steps so three products are possible depending on how long the reaction is allowed to proceed. The aromatic nitro compound is first dimerized into an azoxy compound by reduction of one nitro group to a hydroxylamine and reduction of another to a nitroso group. Following this, a compound with the hydroxylamine functionality reacts with the nitroso compound yielding the azoxy compound. The azoxy compound can be converted to the azo compound, and finally the azo compound can be converted to the amine. The difficulty in this method was stopping the process before the azo turned into an amino group. Another consideration was that the authors reported none of the three possible products for similar starting material such as *p*-nitroaniline. Instead their success in synthesizing the azo compound was reported with the highest yield using nitrobenzene. Due to these reasons, the failure of this reaction, as seen by the presence of the peaks for the starting material in the proton NMR, was not totally surprising.

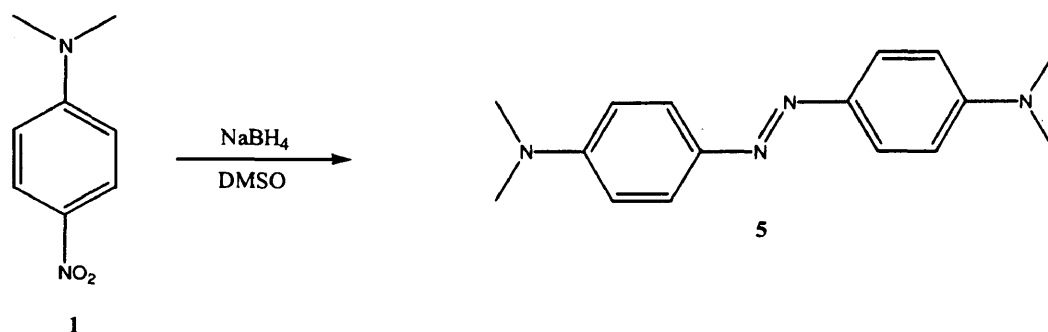


Figure 9: First Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene

The second attempt to azo couple 1 followed a published procedure for the synthesis of azobenzene (Figure 10).²⁵ *N,N*-dimethyl-*p*-nitroaniline was heated at reflux in methanol with aqueous sodium hydroxide and zinc dust. Following a hot filtration, the methanol was removed by distillation. The product was recrystallized from ethanol and dried. TLC showed a new fluorescent purple spot not present in the starting material. Analysis of the NMR spectrum was difficult due to a large impurity peak in the aromatic region. However, the fact that the peaks shifted upfield was promising. It was originally believed that this reaction did in fact partially yield 5 but subsequent attempts to repeat it yielded only starting material so new methods were explored.

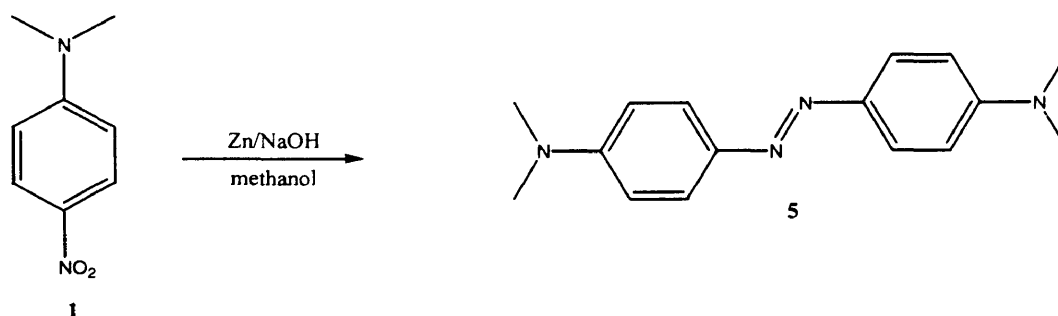


Figure 10: Second Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene

The third, fourth and fifth azo coupling attempts on *N,N*-dimethyl-*p*-nitroaniline were two-step processes. The first step in all three convert the nitro moiety of the starting material, **1**, to an amine by hydrogenation.

In the third reaction, the *N,N*-dimethyl-*p*-aminoaniline, **6**, was reacted with nitrosylsulfuric acid. The nitrosylsulfuric acid can form a nitrosonium ion that can be attacked by the nitrogen lone pair. This reaction should produce a diazonium salt²⁶ that can act as an electrophile in the next step. After formation of the diazonium intermediate, it reacts with *N,N*-dimethylaniline in an electrophilic aromatic substitution (Figure 11). This reaction was done in various solvents including acetic acid, ethanol, and acetonitrile, but never appeared to give the azo product when monitored by TLC and ¹H NMR. Other possible reactions that could have taken place included reaction between the nitrosonium ion and the tertiary amine by either an addition reaction or an electron transfer.

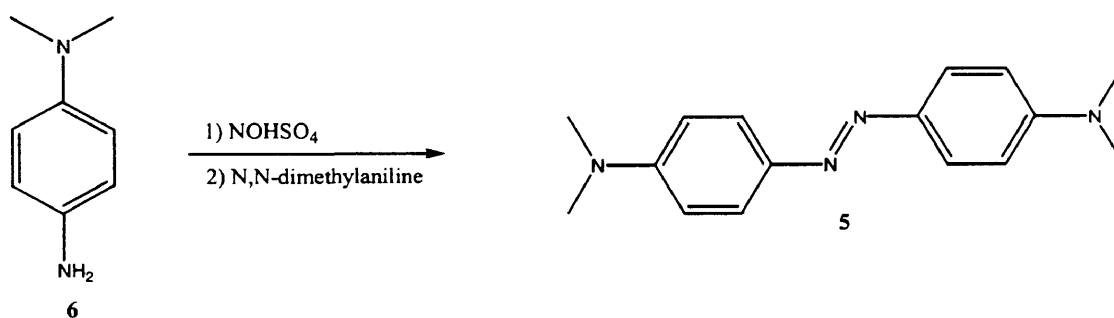


Figure 11: Third Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene

In the fourth attempt, **6** was reacted with sodium perborate and boric acid in acetic acid (Figure 12).²³ The workup of this reaction was tedious and involved attempting to

extract from water with methylene chloride. When the product was dried and analyzed by NMR, the chemical shifts corresponded to the starting material. In the literature, the starting material that was diazotised had an acetamido group in place of the dimethylamino group. This may have been enough of a difference to cause this reaction to fail due to the possibility of reaction between the dimethylamino group and the sodium perborate. This method was consequently used later during the investigation and was successful using the same starting material as the authors (see Discussion).

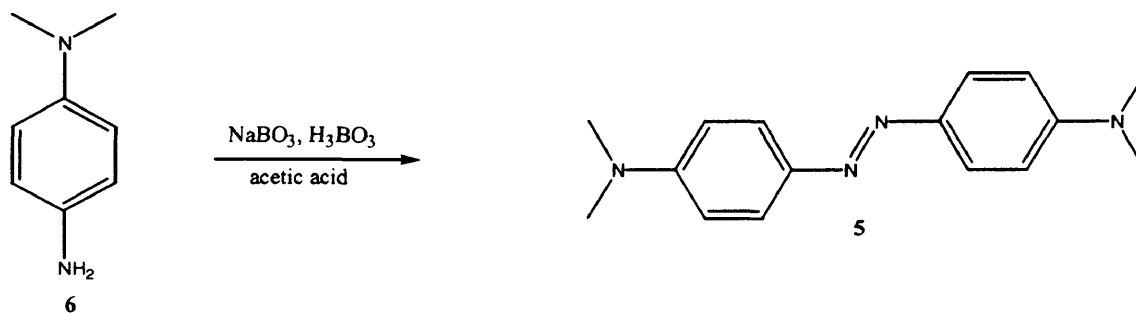


Figure 12: Fourth Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene

The final attempt with **6** involved the preparation of a needed reagent. Barium manganate was prepared using a published method²⁷ where BaH₂O₂·8H₂O is first formed by reacting BaH₂O₂ with water. Next, saturated KMnO₄ is added to synthesize the barium manganate. The *N,N*-dimethyl-*p*-aminoaniline was reacted with barium manganate in dry benzene (Figure 13). When the NMR spectrum for this reaction was analyzed, it was originally believed to have failed. Due to the fact that the reported success of this reaction in the literature is almost exclusively using unsubstituted aniline or aniline substituted with electron withdrawing groups, this reaction was not investigated

any further. Overshadowed by the excitement of successful azo coupling with the next starting material, it was not discovered until much later that this reaction had actually been successful in producing **5**. However, the method with the second starting material was reliable and gave a clean product and a good yield so it was used in place of the barium manganate synthesis. Due to the failed sulfonation attempts on the product compound, the missed success of this synthesis turned out to be irrelevant to synthesis of our desired final product.

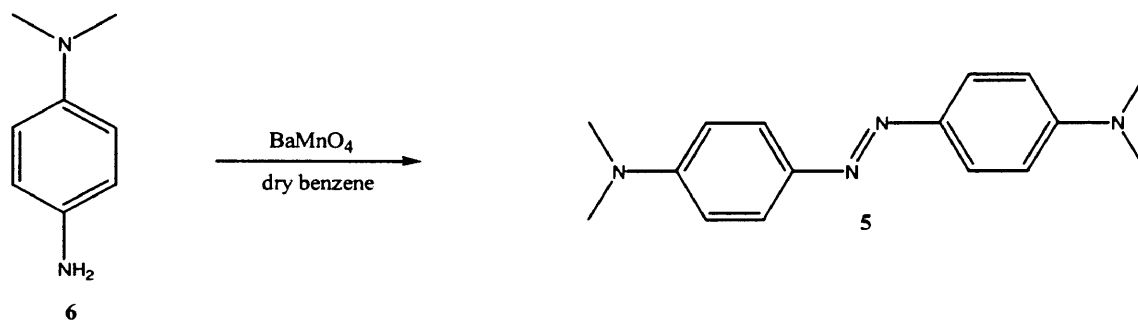


Figure 13: Fifth Attempted Synthesis of 4,4'-bis(dimethylamino)azobenzene

At this point, a decision was made to pursue a new pathway that involved using a different starting material but using a reaction we had used previously. Returning to the published procedure for the synthesis of 4,4'-diaminoazobenzene,²³ we repeated the reaction using 4-aminoacetanilide, **2**. This reaction is a two-step process. In the first step, the azo connection is obtained by reacting the 4-aminoacetanilide with perborate in acetic acid. In the second step, the acetamido groups are hydrolyzed to amino groups (Figure 14). This reaction consistently yielded **7**, which could be checked by NMR by

looking for a 1:1:1 integration ratio for the two aromatic doublets of doublets and the amino hydrogens' singlet. Another indication in the NMR that the reaction was successful was the disappearance of the methyl peak of the acetamido group from the spectrum (for NMR, see Appendix, Figure 27).

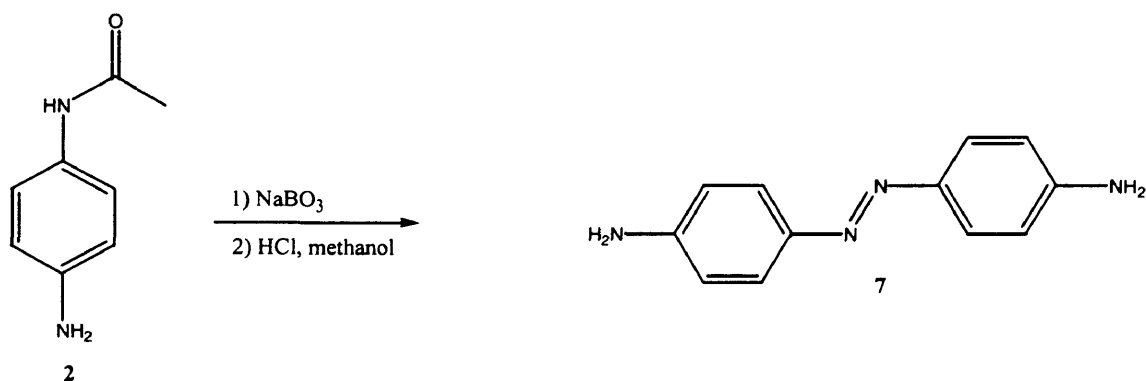


Figure 14: Synthesis of 4,4'-diaminoazobenzene

With the successful synthesis of 4,4'-diaminoazobenzene, attention turned to attaching two methyl groups to each of the amino groups in the molecule. This synthesis of 4,4'-dimethylaminoazobenzene was attempted using two previously published methods.

The first methylation reaction was done by reacting **7** dissolved in ethanol/water with dimethyl sulfate and sodium bicarbonate (Figure 15).²⁸ When the integrations for the proton NMR peaks were analyzed, it was clear that the compound had only partially methylated. Although re-methylation was attempted, each successive reaction resulted in

a loss of product and more impurities. The conditions could never be optimized to ensure complete methylation so other methylation methods were considered.



Figure 15: First Methylation Attempt on 4,4'-diaminoazobenzene

The second methylation method involved adding 7 and sodium borohydride to formaldehyde and sulfuric acid (Figure 16).²⁹ In this reaction, the amino nitrogen can attack the formaldehyde carbon which has a partial positive charge due to protonation at its oxygen. This can happen twice at each amino group and with the loss of four molecules of water, four methyl groups can be added. After adding sodium hydroxide to neutralize the remaining acid, the workup and subsequent analysis revealed that the reaction was successful in yielding 5. The ¹H NMR spectrum showed the characteristic 1:1:3 ratio for the two aromatic peaks and the methyl peak that we expected (for NMR, see Appendix, Figure 28).

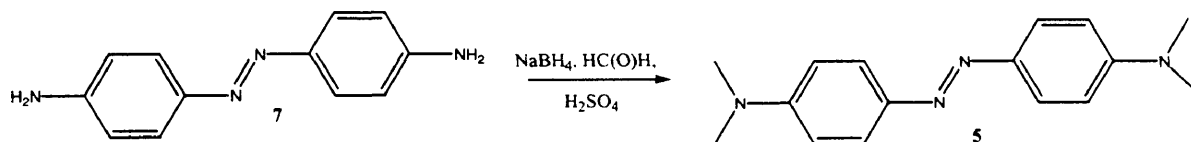


Figure 16: Synthesis of 4,4'-bis(dimethylamino)azobenzene

With the synthesis of **7**, what remains is the introduction of the sulfonyl chloride functionalities that will serve as the links when this molecule is attached to β -cyclodextrin. In order to accomplish this, two sulfonic acid groups must be added to 4,4'-dimethylaminoazobenzene in the two positions meta to the azo bridge. In these sulfonate reactions, different combinations of sulfonating agents and solvents were used. Chlorosulfonic acid and trimethylsilylchlorosulfonic acid were each used with the azo compound in the solvents methylene chloride and chloroform. This reaction never gave the expected product regardless of the combination of reagents and solvents used. Attempts to sulfonate the azo before methylation met with similar failure. No further methods of sulfonation were explored due to the discovery of the third and last starting material.

The last starting material, 2-amino-5-nitrobenzenesulfonic acid ammonium salt, avoided the difficult sulfonation reaction. Before this compound (**3**) could be azo coupled, there were several necessary adjustments to make to its functionalities. After acetylation of the amino group to ensure protection during the azo coupling, the nitro group was hydrogenated to an amino group. Our molecule was now a sulfonic acid derivative of 4-aminoacetanilide, the second starting material. Due to the success of the previous azo coupling of that compound using the perborate method,²⁶ this method was used on the sulfonic acid derivative (Figure 17). After this reaction we obtained the product, 4,4'-bis(diamino)azobenzene-3,3'-sufonic acid (**8**) which was checked by ¹H NMR spectroscopy (for NMR, see Appendix, Figure 26).

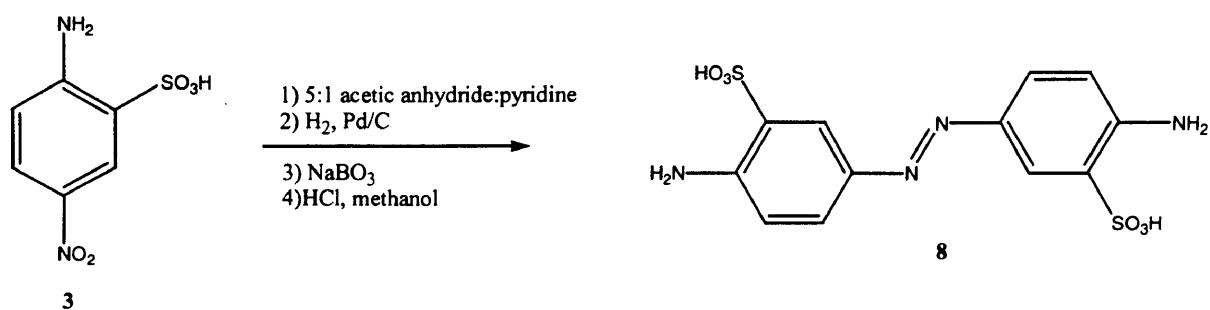


Figure 17: Synthesis of 4,4'-bis(diamino)azobenzene-3,3'-disulfonic acid

Now that the azo connection was in place and the sulfonic acid groups were present and waiting to be converted into sulfonyl chlorides, all that remained was to add four methyl groups to the two amino groups in **8**.

The first methylation attempt involved using dimethyl sulfate in a 10:1 volume to mass ratio to **8** (Figure 18). The mixture was heated and the excess dimethyl sulfate was removed by distillation. The mixture was slurried in acetone to try and crash out the solid product. The solution was filtered and the solid was dried. The product showed no signs of methylation by ¹H NMR spectroscopic analysis.

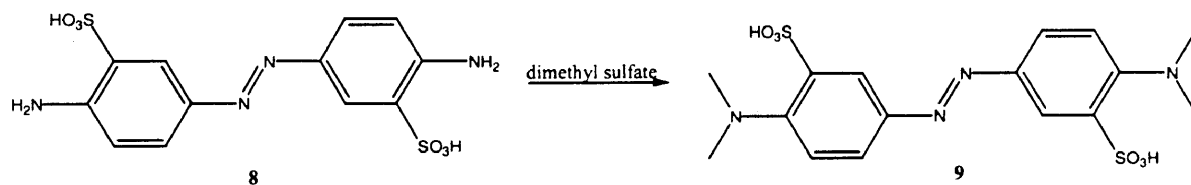


Figure 18: First Methylation Attempt on 4,4'-bis(diamino)azobenzene-3,3'-disulfonic acid

The second methylation attempt on **8** was accomplished using the published procedure that we previously used to successfully methylate 4,4'-diaminoazobenzene.²⁹ Following the reaction with formaldehyde and sulfuric acid in THF (Figure 19), the solvent was removed and the solid product was slurried in acetic acid and subjected to cation exchange in an effort to purify it. The aromatic region of the ¹H NMR spectrum showed no product peaks present and TLC of this compound versus starting material was inconclusive. It was possible that solubility of the starting material in THF may have been partly responsible for the failure of this reaction.

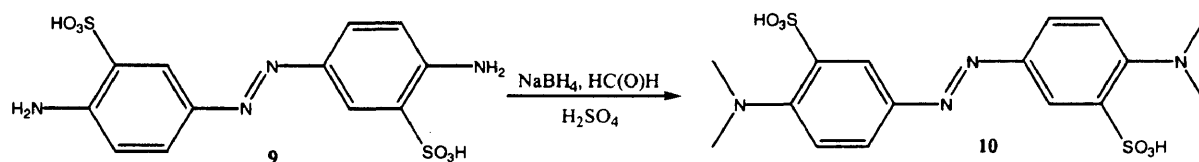


Figure 19: Second Methylation Attempt on 4,4'-bis(diamino)azobenzene-3,3'-disulfonic acid

Further attempts to methylate **9** were not explored. The failure of this compound to methylate was probably due almost completely to the electron withdrawing sulfonate groups ortho to the amino groups we were attempting to methylate. It is not clear whether the next step in the synthesis of our product would be to continue the attempt to methylate or to explore alternative syntheses.

Discussion

There were numerous synthetic hurdles to get past when synthesizing the target molecule in this project. The first pathway was hampered by numerous failures during the azo coupling reaction. Although it was unsuccessful on the first starting material, the procedure that eventually did yield an azo compound was discovered during the work done during the first pathway.

Our second pathway was characterized by several successful reactions including both the azo coupling and the tetramethylation. Unfortunately the failure of all attempts to add sulfonate groups to this molecule created a “dead end.” Addition of the sulfonate group should have occurred ortho to the dimethylamino group because it activates the benzene ring making it more nucleophilic. Other sulfonation methods such as fuming sulfuric acid could not be used, because the acidic conditions would have caused protonation at the dimethylamino group changing it from electron donating to electron withdrawing. This results in a non-nucleophilic hence unreactive benzene ring.

The final starting material used was a trisubstituted benzene ring. Although the functionalities had to be adjusted, they were already located in the correct positions on the aromatic molecule.

The first adjustment made was to acetylate the amino group of **3**. Acetylating the amino group was necessary so that in the azo coupling step, there would exist no confusion concerning which nitrogen would form the azo bridge. The amino group, now converted to an acetamido group, is protected. This protecting group is easily removed in

the workup of the azo coupling step. With deprotection, the amino functionality will return.

The acetylation reaction was done using acetic anhydride. By experiment it was discovered that adding pyridine in a 1:5 ratio relative to acetic anhydride gave a complete conversion to acetylated product **11**. When heated to reflux, the nitrogen lone pair attacks one of the carbonyl carbons of the acetic anhydride. Through movement of bonds and proton transfers an acetyl group becomes attached to the nitrogen. The function of the pyridine is to act as a base and remove protons when needed. The complete mechanism for the reaction is shown in Figure 20.

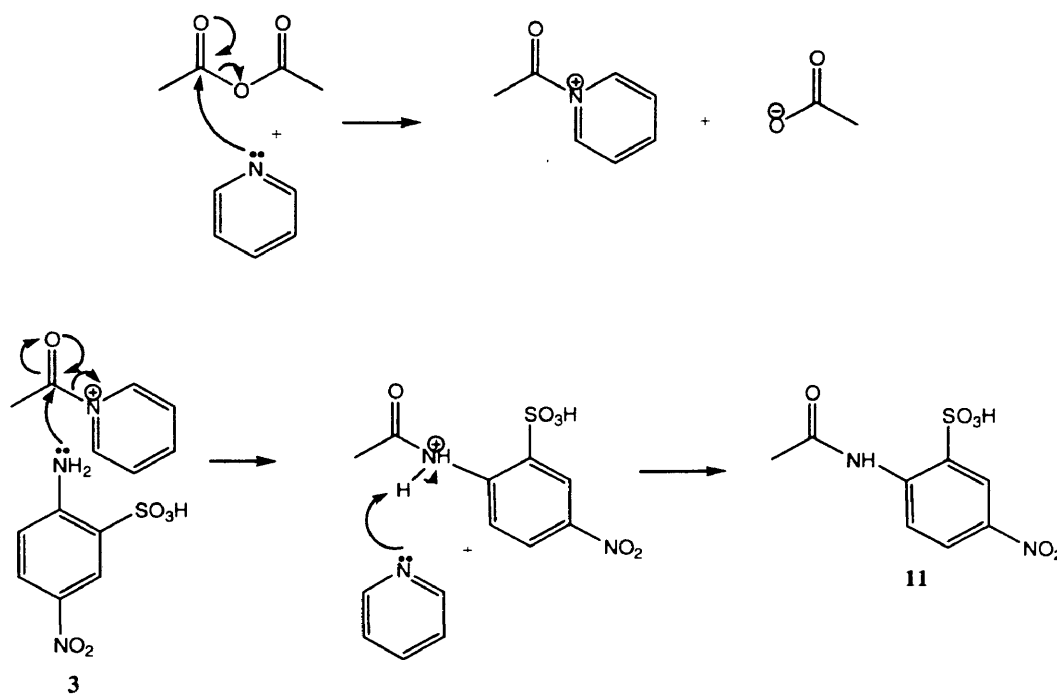


Figure 20: Mechanism for Acetylation of Amino Group

Although the NMR spectrum for this reaction (for NMR, see Appendix, Figure 24) showed the aromatic peaks for **11**, there were three other unassigned peaks in the aromatic region. After obtaining an NMR spectrum of pyridine, these peaks were subsequently assigned to the pyridinium salt that was an additional product formed during the acetylation. At this point steps were not taken to attempt to separate our two products because it was quite easy to separate the two at the end of the next step.

Now that the acetamido protecting group had been created, it was necessary to prepare our molecule for the azo coupling. We wanted two of our molecules to azo couple at the nitro positions on the benzene ring system. Because of the azotisation method chosen, the nitro groups had to be reduced to amino groups.

The following optimum hydrogenation conditions were discovered. The mixture of 2-acetamido-5-nitrobenzene sulfonic acid and pyridinium salts from the first reaction was hydrogenated at high pressure overnight to insure complete conversion to the amino groups. After removal of the solvent, the product **12** was recrystallized from water leaving behind any residual pyridine derivatives in the mother liquor. The product showed a clean ^1H NMR spectrum in the aromatic region with only the expected three peaks assigned to the product (for NMR, see Appendix, Figure 25).

Now that the amino functionality existed at the location where the azo linkage was desired, the diazotisation reaction could proceed. The mechanism for the perborate method of diazotisation proceeded through several steps. The first involved oxidation of the amino group to a hydroxyl amine group by addition of the amino group to a molecule of H_3BO_4 . With the addition of the nitrogen to another molecule of H_3BO_4 and the loss of water, the hydroxyl amine group is converted to a nitroso group. Once the nitroso

group has replaced the amino group on one molecule of **12**, another molecule of starting material with the amino functionality still in place can attack the nitroso. With proton transfers and a loss of water, the azo linkage forms between two molecules of the starting material. The complete mechanism is shown in Figure 21.

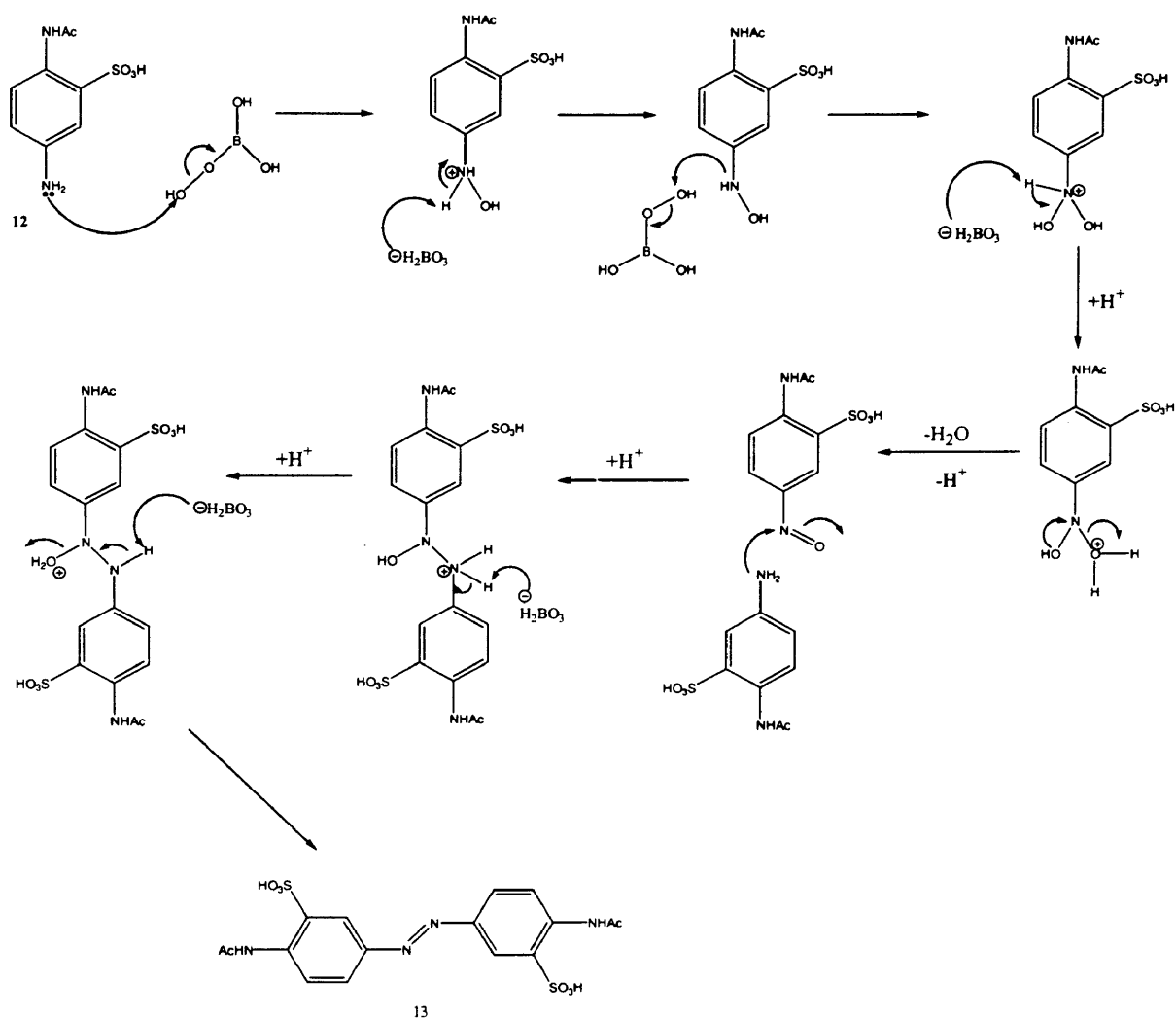


Figure 21: Mechanism for Azo Coupling

Following the azo coupling, an acidic workup is necessary to remove the acetyl protecting groups from **13** and restore the free amines. By refluxing **13** in a combination of methanol and hydrochloric acid, the acetyl group was removed. Methanol attacks the protonated carbonyl carbon of the acetyl group to form a tetrahedral intermediate that can expel the desired free amino group. The mechanism is shown in Figure 22.

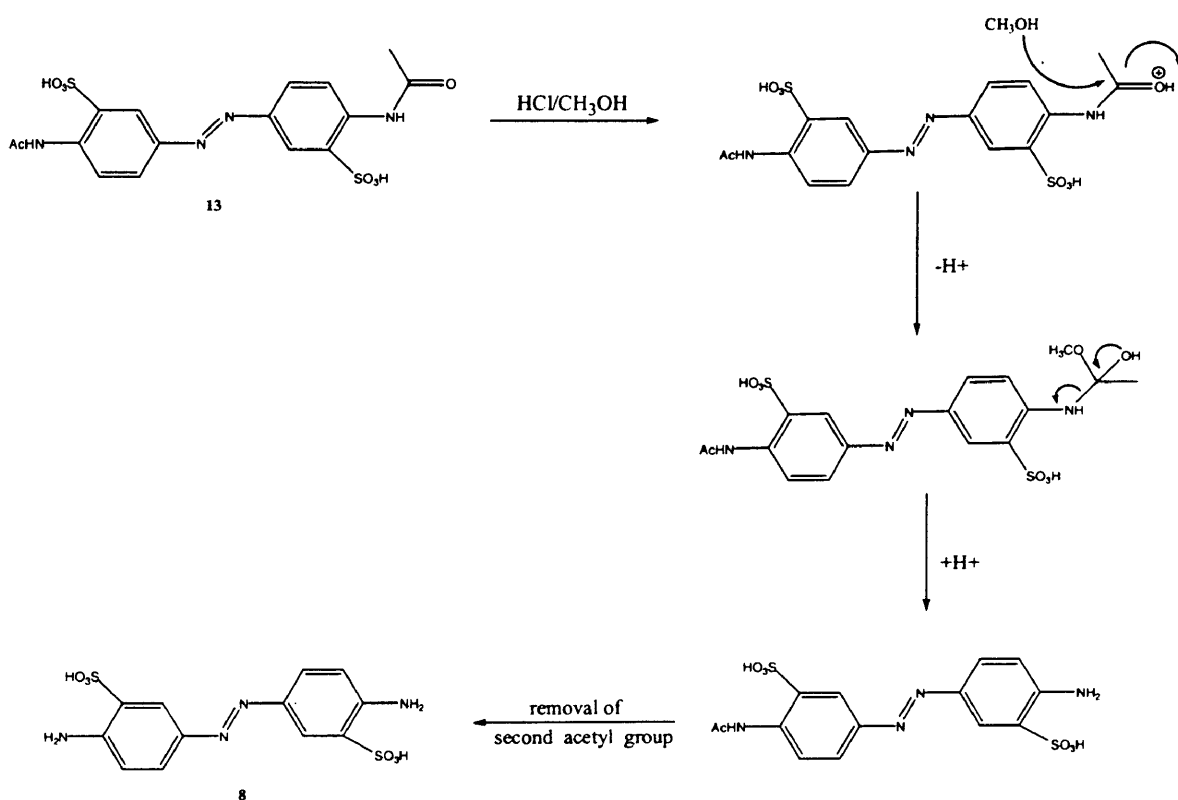


Figure 22: Hydrolysis of Acetyl Group back to an Amino Group

In order to remove excess acid and impurities from the reaction following evaporation of the solvent, the solid is run through silica gel using a 20:20:1 ethanol:ethyl acetate:acetic

acid solvent system. The removal of impurities can be seen in the increased cleanliness of the NMR spectrum for the azo compound (see Appendix, Figure 26).

The final hurdle to overcome prior to chlorination and attachment is to add the four methyl groups to the amino groups now present on the benzene rings para to the azo linkage. Although methylation of a similar compound was achieved earlier in this research, small changes in the molecule may make this methylation more difficult.

In 3,3'-diaminodiazobenzene-4,4'-sulfonic acid (**8**), there is a sulfonic acid group ortho to each of the amino groups. The oxygens on the sulfur in a sulfonic acid group are electron withdrawing. The mechanism of the methylation step relies on the nucleophilicity of the amino nitrogen and its ability to use its lone pair to attack the methylating agent. Having an electron withdrawing group next to it in a benzene system cuts down on its ability to perform its function as a nucleophile and leads to the possibility of partial methylation or even no methylation.

As mentioned previously, following the repeated failure of methylation attempts this pathway was abandoned (see Results). It may be that the electron withdrawing nature of the sulfonate groups makes the synthesis of the product unattainable and that concentration should be shifted to the synthesis of other azo capping molecules.

Conclusion

In order to affect host/guest binding properties in β -cyclodextrin via capping, an appropriate cap needed to be synthesized. The molecule selected was an organic dye molecule sensitive to pH. Many synthetic hurdles thwarted a completely successful synthesis of 4,4'-bis(dimethylamino)azobenzene-3,3'-disulfonic acid. The complexity of the different functionalities present as well as their interactions with one another were probable causes of the difficulty. Related areas of exploration include replacing the sulfonate groups with carboxylic acid groups as well as moving the sulfonate functionality from the position ortho to the dimethylamino group to the meta position.

Appendix of Spectra

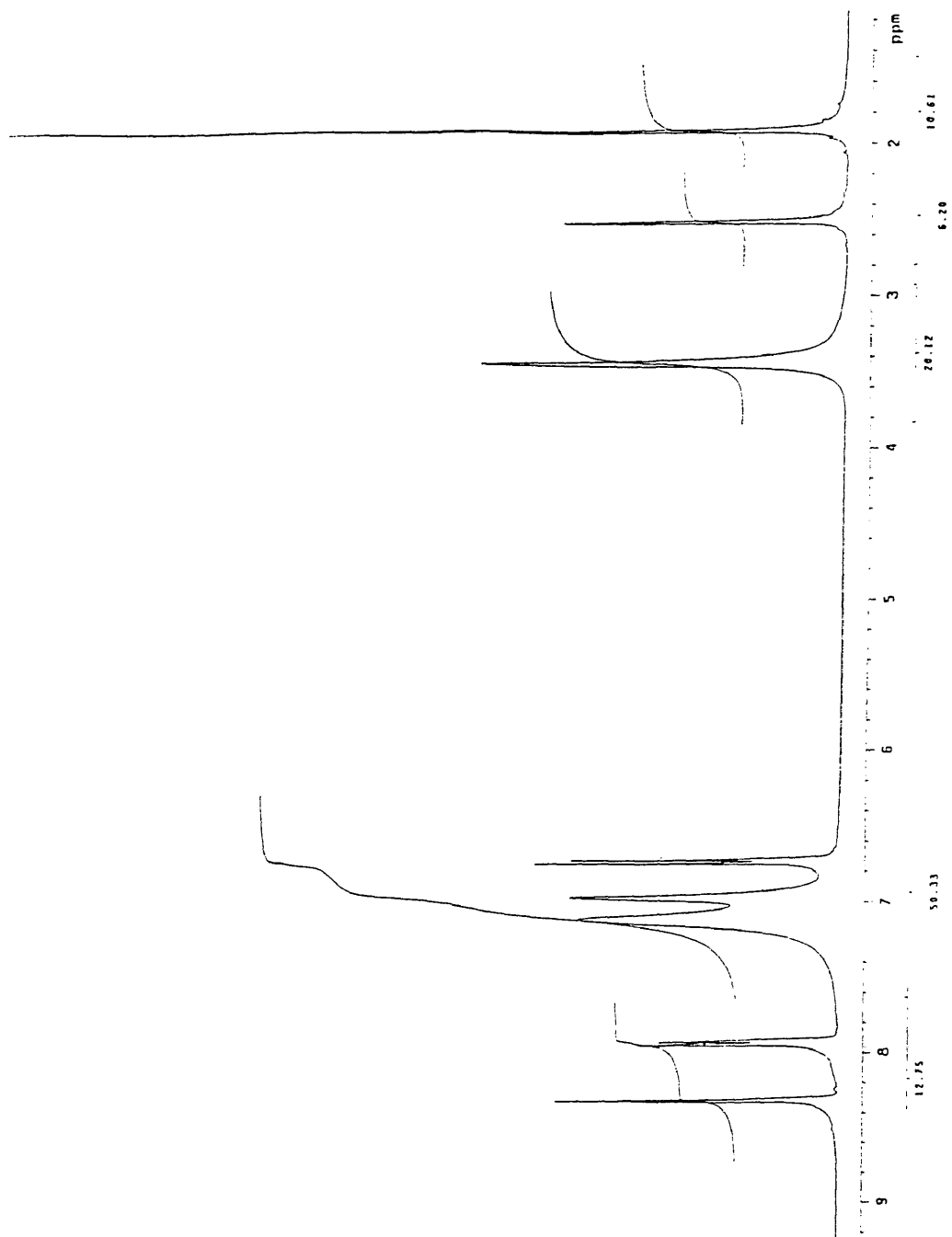
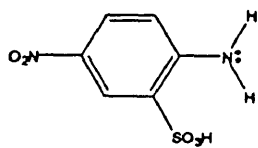


Figure 23: ¹H NMR of Recrystallized 2-Amino-5-nitrobenzene sulfonic acid

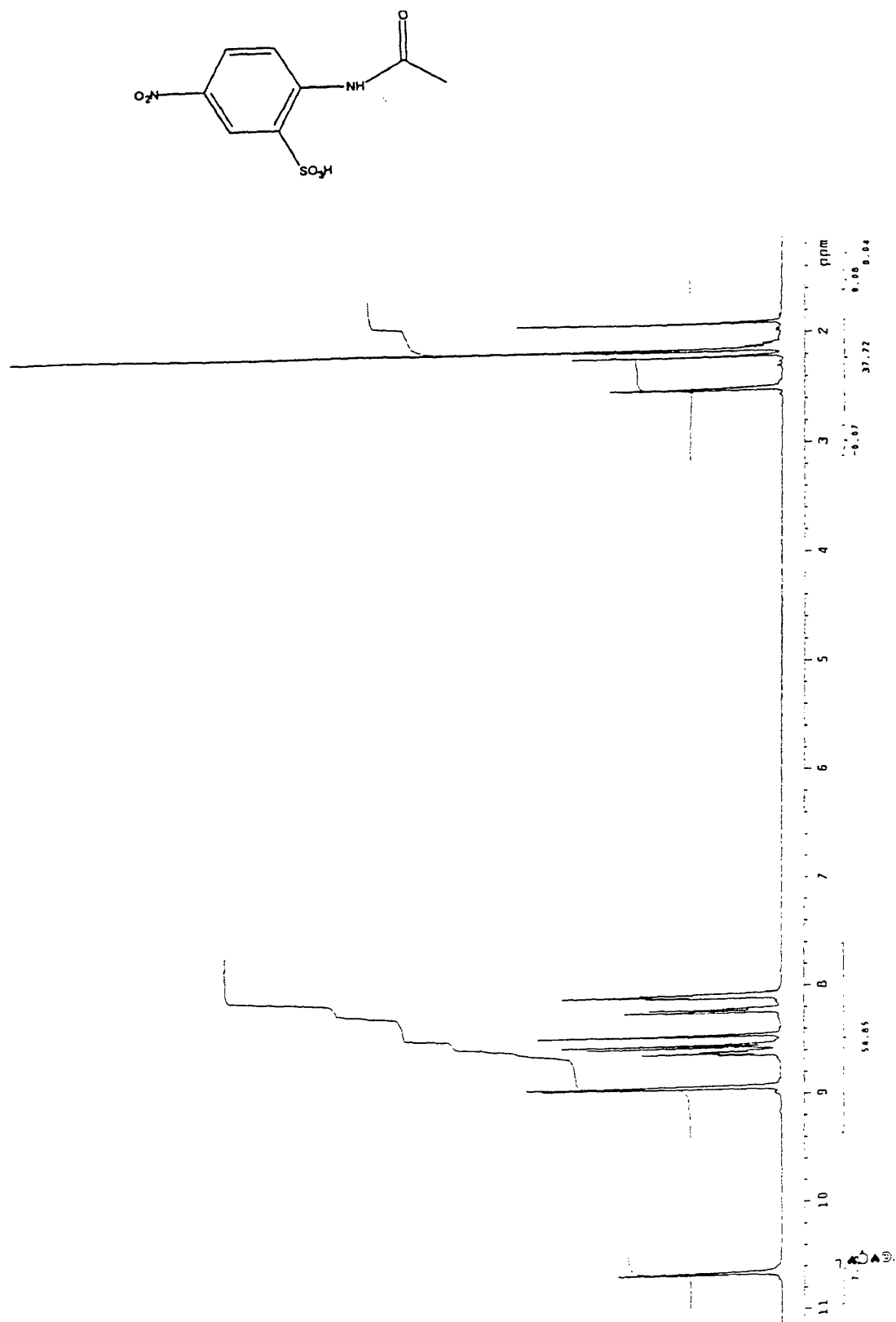


Figure 24: ¹H NMR of 2-Acetamido-5-nitrobenzene sulfonic acid

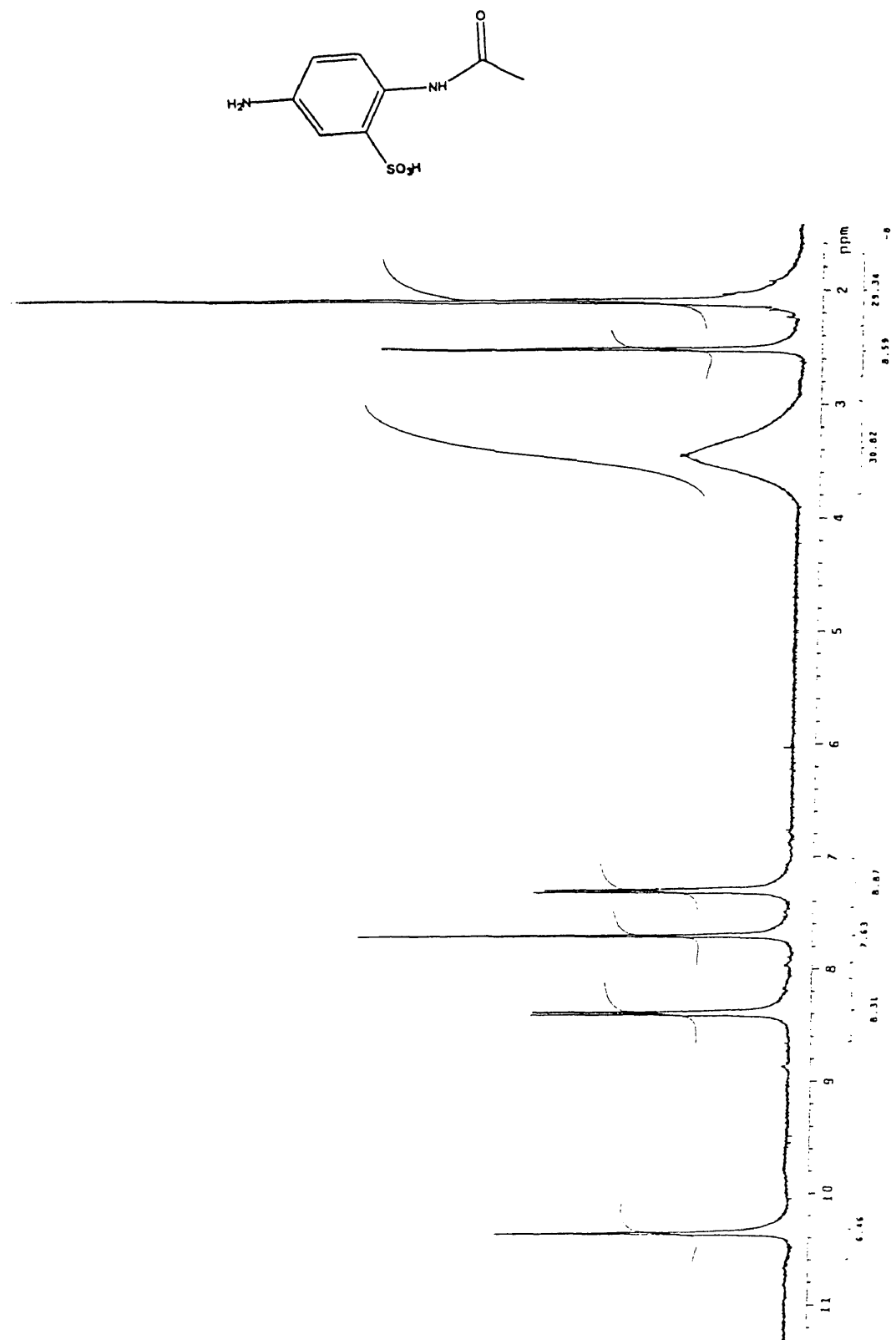


Figure 25: ¹H NMR of 2-Acetamido-5-aminobenzene sulfonic acid

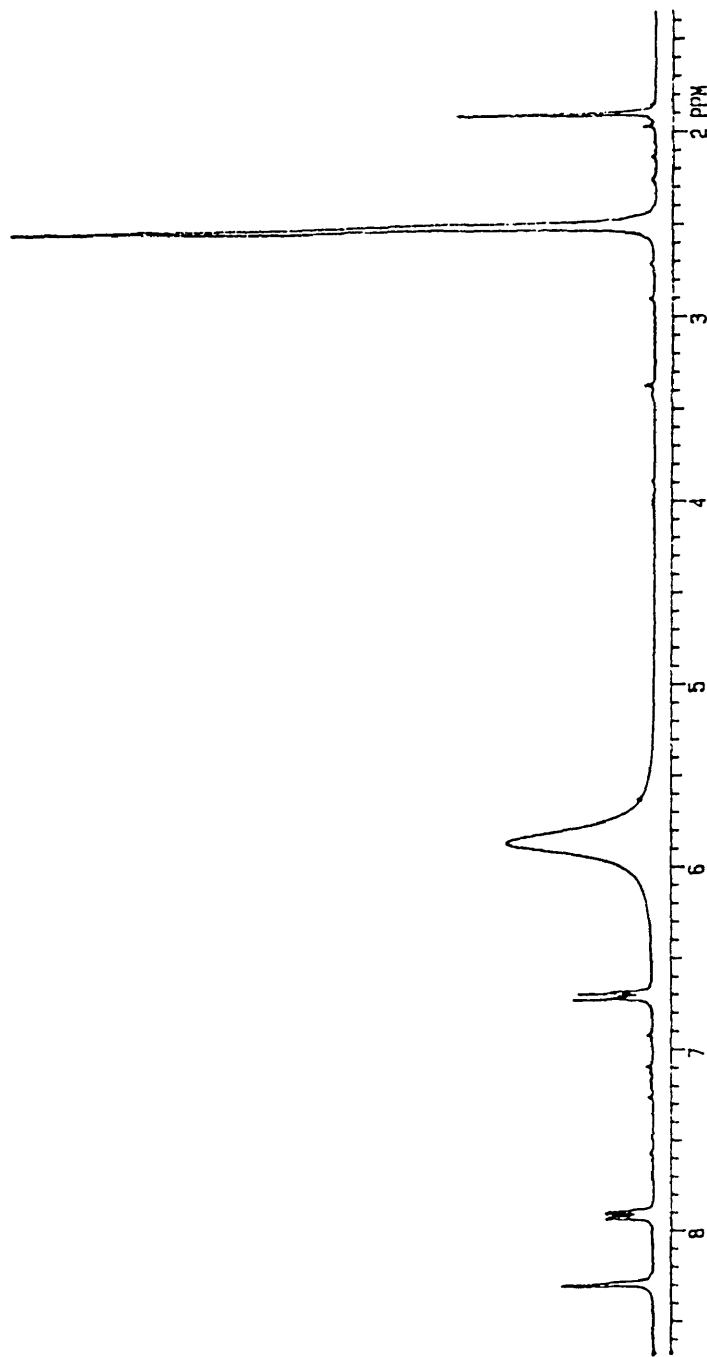
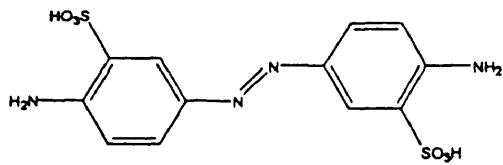


Figure 26: ¹H NMR of 3,3'-Bis(Diamino)azobenzene-4,4'-disulfonic acid

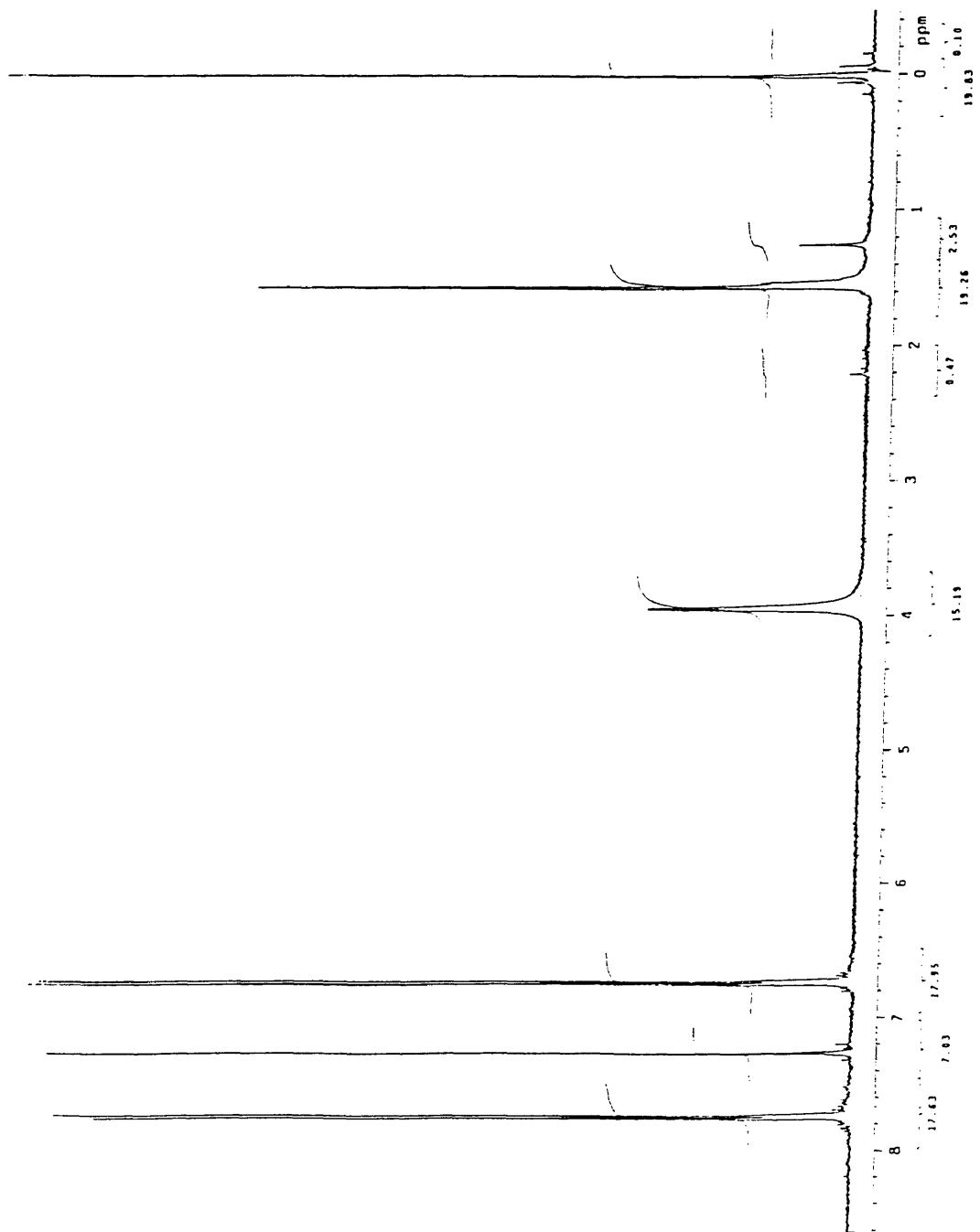
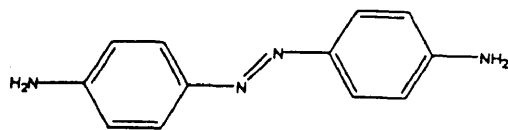


Figure 27: ^1H NMR of 4,4'-Diaminoazobenzene

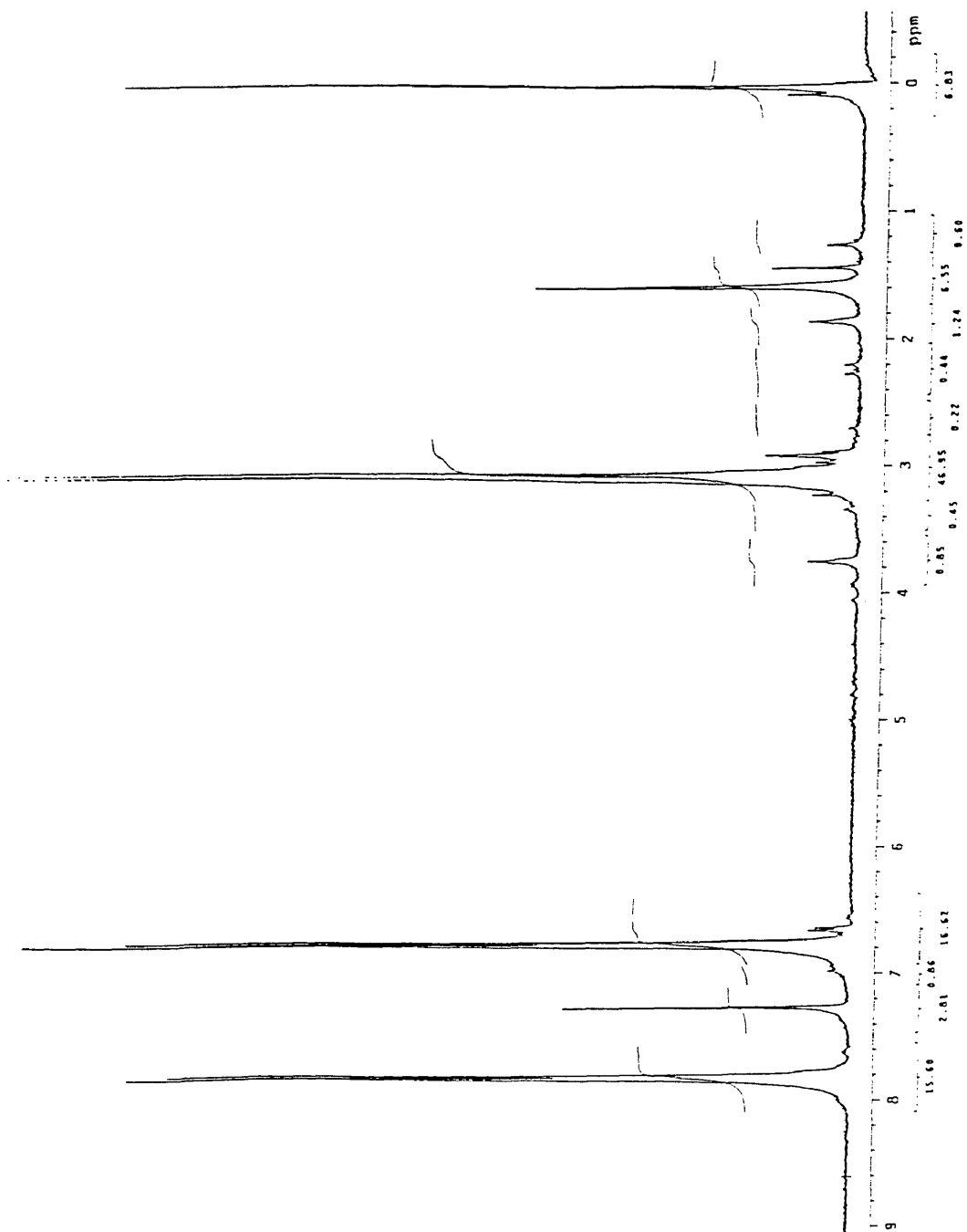
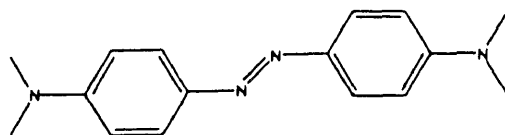


Figure 28: ^1H NMR of 4,4'-Dimethylaminoazobenzene

References

- ¹D'Souza, V.T.; Bender, M.L. *Acc. Chem. Res.* **1987**, *20*, 146-152.
- ²Breslow, R.; Czarnik, A.W. *J. Am. Chem. Soc.* **1983**, *105*, 1390-1391.
- ³Fujita, K.; Tahara, T.; Nagamura, S.; Imoto, T.; Koga, T. *J. Org. Chem.* **1987**, *52*, 636-640.
- ⁴Martin, K.A.; Mortellaro, M.A.; Sweger, R.W.; Fikes, L.E.; Winn, D.T.; Clary, S.; Johnson, M.P.; Czarnik, A.W. *J. Am. Chem. Soc.* **1995**, *117*, 10443-10448.
- ⁵Breslow, R.; Doherty, J.B.; Guillot, G.; Lipsey, C. *J. Am. Chem. Soc.* **1978**, *100*, 3227-3229.
- ⁶Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152-1153.
- ⁷VanEtten, R.L.; Sebastian, J.F.; Clowes, G.A.; Bender, M.L. *J. Am. Chem. Soc.* **1967**, *89*, 3242-3253.
- ⁸Bender, M.L.; Komiyama, M. *Cyclodextrin Chemistry* Springer-Verlag: New York, 1978.
- ⁹Szejtli, J. *Cyclodextrins and their Inclusion Complexes* Akademiai Kiado: Budapest, 1982.
- ¹⁰Szejtli, J. *Cyclodextrin Technology* Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988.
- ¹¹Wilson, E. *C&E News*, **1999**, *77*, 05, 32-33.
- ¹²Bender, M.L. "Enzyme Models-Cyclodextrins" in *Enzyme Mechanisms*, Page, M.I.; Williams, A. Eds. The Royal Society of Chemistry: London, 1987.
- ¹³Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275-4278.
- ¹⁴Tabushi, I.; Kuroda, Y.; Yokota, K.; Yuan, L. C. *J. Am. Chem. Soc.* **1981**, *103*, 711-712.
- ¹⁵Tabushi, I.; Yuan, L.C.; Shimokawa, K.; Yokotak, Mizutani, T.; Kuroda, Y. *Tetrahedron Lett.* **1981**, *22*, 2273-2275.

- ¹⁶ Emert, J.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 670-672.
- ¹⁷ Ueno, A.; Yoshimura, H.; Saka, R.; Osa, T. *J. Am. Chem. Soc.* **1979**, *101*, 2779-2780.
- ¹⁸ Tabushi, I.; Yuan, C. *J. Am. Chem. Soc.* **1981**, *103*, 3574-3575.
- ¹⁹ Aquino, A.M.; Abelt, C.J.; Berger, K.L.; Daragh, C.L.; Kelley, S.E.; Cossette, M.V. *J. Am. Chem. Soc.* **1990**, *112*, 5819-5824.
- ²⁰ Acquavella, M.F.; Evans, M.E.; Farragher, S.W.; Nevoret, C.J.; Abelt, C.J. *J. Chem. Soc. Perkin Trans. 2* **1995**, 385-388.
- ²¹ Allen, K. H.; Saunders, R. L. M. *Aromatic Diazo Compounds*. 3rd ed. London: Edward Arnold Publishers Ltd. 1985. pp. 1-3.
- ²² Krueger, P.J. "Basicity, hydrogen bonding and complex formation involving hydrazo, azo and azoxy groups" in *The Chemistry of the Hydrazo, Azo, and Azoxy Groups, Part I*, Patai, S. Ed. John Wiley & Sons: New York, 1975.
- ²³ Santurri, P.; Robbins, F.; Stubbings, R. *Organic Syntheses* **1973**, *5*, 341-343.
- ²⁴ Hutchins, R.O.; Lamson, D.W.; Rua, L.; Milewski, C.; Maryanoff, B. *J. Org. Chem.* **1971**, *36*, 803-807.
- ²⁵ Bigelow, H.E.; Robinson, D.B. *Organic Syntheses* **1955**, *3*, 103-104.
- ²⁶ Olah, G.A.; Surya Prakesh, G.K.; Wang, Q.; Li, X. "Nitrosyl Sulfuric Acid" in *Encyclopedia of Reagents for Organic Synthesis*, 6, Paquette, L.A. Ed. John Wiley & Sons: New York, 1995.
- ²⁷ Firouzabadi, H.; Mostafavipoor, Z. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 914-917.
- ²⁸ Hunig, S.; Quast, H.; Brenninger, W.; Frankenfield, E. *Organic Syntheses* **1973**, *5*, 1018-1021.

Vita

Katherine Meyerdierks Ross

Born in Washington, D.C. on April 13, 1977. Graduated magna cum laude with a concentration in chemistry from The College of William and Mary in Williamsburg, VA in May, 1999. Masters of Arts candidate, The College of William and Mary, 1999-2000, with a concentration in chemistry. The course requirements for this degree have been completed, but not the thesis: The Attempted Synthesis of 4,4'-Bis(dimethylamino) azobenzene-3,3'-disulfonyl chloride as a Cap for β -Cyclodextrin.