AN APPROACH TO THERMODYNAMICALLY CONTROLLED SUPRAMOLECULAR ASSEMBLY IN WATER

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Abstract

Supramolecular chemistry focuses on the formation of multi-molecular complexes held together by non-covalent interactions. One prominent sub-field of study within supramolecular chemistry is the use of thermodynamic favorability to drive the self-assembly of complex structures, such as rotaxanes. Rotaxanes are a type of supramolecular complex in which a linear 'guest' molecule with bulky ends is threaded through a large ring-shaped 'host' molecule. Rotaxanes are important for several reasons such as their use as molecular shuttles and synthetic dye stabilizers.

Many rotaxane synthesis techniques rely solely on the thermodynamic favorability of non-covalent interactions such as π-rich and π-poor aromatic unit interactions. A recent study created a synthesis method that used reversible carbonyl condensation chemistry to form the linear guest molecule within the host macrocycle much like assembling a ship piece by piece within a bottle.[1] Then they used an irreversible dehydration step to remove the formation reversibility and 'trap' the guest molecule within the host molecule, creating a stable rotaxane.

This research project examined whether rotaxanes could be formed in water through the addition of sodium bisulfite to augment the method used in the previous study. This project tested the premise that the hydrophilicity of the water combined with the favorable non-covalent interactions would then drive rotaxane formation. It was theorized that this addition should increase the yield of rotaxane formation while simultaneously decreasing the complexity of the process.

However, modified experiments focused on guest molecule formation in water produced much lower product yields than expected.

Additional experimentation determined the cause for this drop in yield to be an unexpected reaction between the sodium bisulfite and the unadded bulky end groups of the guest molecule. This sodium bisulfite reaction was irreversible in the presence of aqueous base and thus removed much of the sodium bisulfite and the bulky end molecules from the reaction. Several attempted bypass reactions prove ineffective. Due to the overactivity of sodium bisulfite, future experiments could focus on testing other possible water solubilizing agents such as amino acids in imine formation reactions.

Chapter 1: Introduction

Supramolecular chemistry is a field of study that researches complexes of two or more molecules held together by non-covalent interactions. These complexes consist of two types of molecules: receptors and substrates.[2] A receptor, or host, is a molecule that contains a cavity that can hold or bind to another smaller molecule. A substrate, or guest, is a molecule that is held within a receptor's cavity.

Research into supramolecules began with the study of molecular recognition, which is the selective formation of host-guest complexes.[3] Studying these complexes resulted in a better understanding and predictability of non-covalent interactions. Chemists used this new-found understanding to make molecular subunits that would self-assemble into supramolecular superstructures. Then, pairing this self-assembly with covalent modifications enabled scientists to produce an even wider range of complex structures that could be used to mimic and study biological complexes.

1.1: The Foundations of Supramolecular Chemistry

Research into supramolecular chemistry began with Charles Pedersen in 1960.[4] At the time, Pedersen was working to develop a series of compounds that would bind to metal ions and suppress their catalytic activity. One day he found that the synthesis of one of these compounds also produced a low yield of white crystals. Further research showed that, when present, sodium cations were electrostatically binding to the oxygens within the polyether ring of the metal

deactivator.[3] These polyether rings, such as shown in Figure 1A, became known as a class of cation acceptors called crown ethers.

Over several years, the production of many varying sizes of crown ethers led to the discovery of geometrical selectivity[4]. In other words, researchers discovered a relationship between cavity size and cationic radius that influenced the thermodynamic stability of the complexes. This showed that specific crown ether - cation complexes could be selectively produced.

While researching the binding capabilities of crown ethers in 1969, Jean-Marie Lehn discovered a type of molecule that more stably bound group 1 and 2 metal cations.[3] Unlike crown ethers, these were cage-like molecules, as shown in Figure 1B, that used nitrogen atoms as well as oxygen atoms to bind cations inside the cavity. These types of cation acceptors were named cryptands. Much like crown ethers, further research showed that cryptands could also be specifically adapted to fit the desired cation.

Crown ethers and cryptands were able to relieve the electron-electron repulsion in their cavities via one of two methods.[5] The first method was by folding inward and filling the potential cavity to separate the lone electron pairs on the oxygen atoms as much as possible. The second method was by forming a complex where the unshared electron pairs on the oxygen atoms would interact with a metal ion.

With this in mind, Donald Cram researched possible receptor molecules, such as shown in Figure 1C, that had an enforced cavity.[5] The lack of flexibility would ensure that only spherical entities, such as metal ions, could fill the cavity.

Cram believed this would increase the likelihood of formation as well as the stability of the complex. Cram named this new class of cation acceptors spherands.

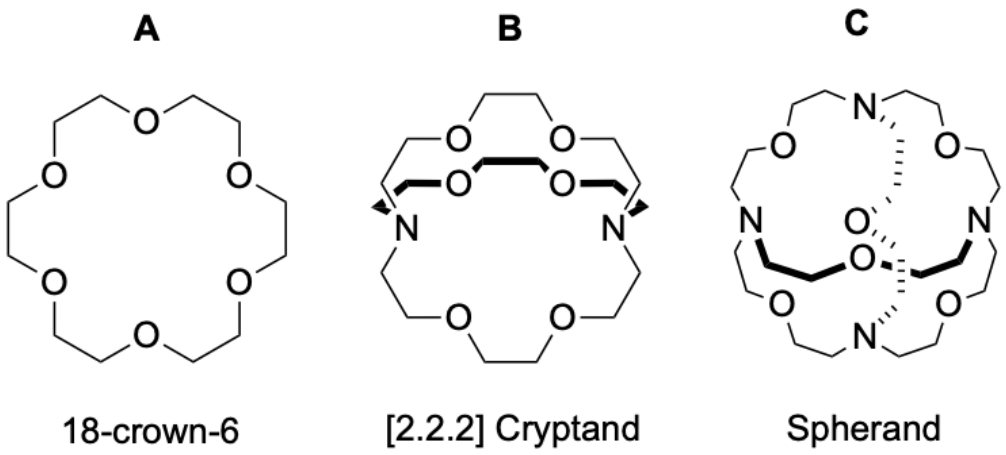

Figure 1. Example structures of a crown ether (A), a cryptand (B), and a spherand (C).

For their ground-breaking work, Pedersen, Lehn, and Cram were jointly awarded the 1987 Nobel Prize in chemistry.[3] Following this, the newly recognized field of molecular recognition grew rapidly. Chemists developed receptors that could specifically bind anions using only electrostatic ion-ion interactions, only hydrogen bonds, or both. Similar avenues of research led to hosts that could specifically bind neutral guests using complementary hydrogen bond arrays and the hydrophobic effect. All of this research led to the production of complex biomolecules, such as functional synthetic antibodies.

Similarly, researchers began to direct their attention toward better understanding and using the processes of biological systems for inspiration.[3] They recognized that many biological actions, such as DNA double helix formation, ribosome complex construction, and protein folding, used self-assembly. This was

a process in which non-covalent interactions drove the spontaneous and reversible organization of pre-existing components into ordered structures.

Chemists recognized that they could use their knowledge of non-covalent interactions to design or identify simple subunits that could self-assemble into a larger and more complicated structure.[3] Adding further modifications to this approach produced an ever wider variety of complex structures, such as catenanes, molecular knots, and rotaxanes.

1.2: Rotaxanes

Rotaxanes are a type of molecular complex where a linear dumbbell-shaped molecule is threaded through a macrocycle as shown by Figure 2A. Rotaxanes are commonly synthesized in one of three ways: slipping, clipping, or capping.

The slipping method, as shown by Figure 2B, involves reversibly threading an appropriately sized dumbbell molecule through the macrocycle.[6] This process requires an external energy source, such as high temperatures, to 'override' the kinetically unfavorable action of slipping the bulky dumbbell ends through the macrocycle. Removing this external energy source then 'traps' the guest molecule within the host molecule, forming a stable rotaxane.

Clipping, as shown in Figure 2C, is a method where a partial macrocycle is used to wrap around the linear portion of the guest molecule.[7] The partial macrocycle is then closed into a full ring resulting in a fully formed rotaxane.

The capping method, as shown in Figure 2D, involves thermodynamically threading the linear center of the guest molecule, or thread, through a

macrocycle.[8] This creates a pseudorotaxane that can be converted into a rotaxane by reacting the ends of the threaded guest with large bulky groups.

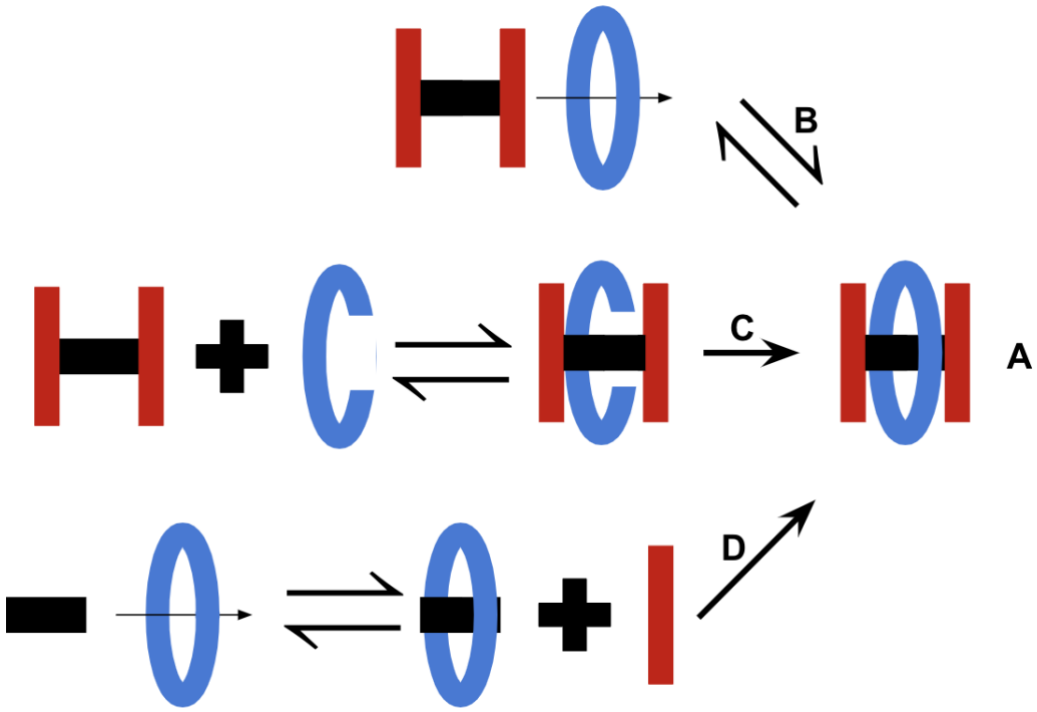

Figure 2. Schematic of the three methods of rotaxane (A) synthesis: slipping (B), clipping (C), and capping (D).

1.3: Carbonyl Condensation Chemistry Approach to Rotaxane Formation

In 2008, Cagulada et al. proposed a carbonyl condensation chemistry approach to capping a pseudorotaxane. The assembly method utilized reversible carbonyl chemistry followed by an irreversible dehydration. The non-covalent interaction of π-rich and π-poor aromatic units provided the driving force behind this rotaxane formation.

The first step was the creation or identification of a suitable thread with aromatic aldehyde termini.[1] To demonstrate this, as shown in Figure 3, naphthalene diimide-diol (Compound **A**) was selected as a suitable center thread

component. The addition of 4-formylbenzoic acid (Compound **B**) to this center component produced the desired dialdehyde thread (Compound **C**).

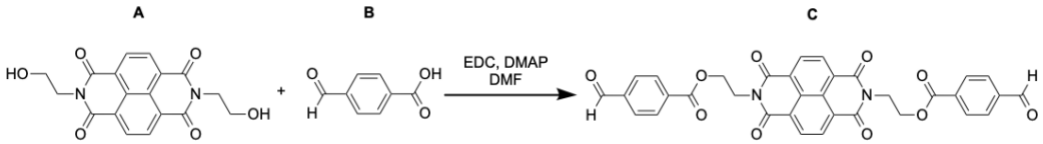

Figure 3. The synthesis of a suitable dialdehyde thread (Compound **C**) using naphthalene diimide-diol (Compound **A**) and 4-formylbenzoic acid (Compound **B**).

The next step was the insertion of this thread into a macrocycle.[1] As shown in Figure 4, dinaphtho crown macrocycle (Compound **D**) was introduced to the newly formed dialdehyde thread (Compound **C**). Compound **D** was selected for its electron rich aromatic diethers that would interact well with the electron deficient naphthalene diimides of the dialdehyde thread. These two compounds then produced a pseudorotaxane complex (Compound **E**).

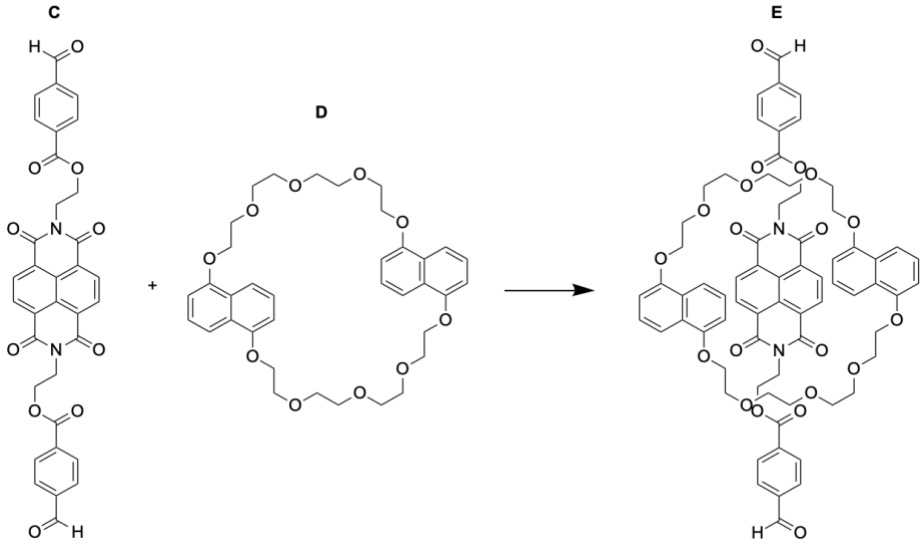

Figure 4. The synthesis of a pseudorotaxane (Compound **E**) from the insertion of a dialdehyde thread (Compound **C**) into a dinaphtho crown macrocycle (Compound **D**).

Following that step, the thread was reversibly capped.[1] Spirodiketone (Compound **F**) was chosen as a sufficiently bulky compound that would easily

react with the aldehyde ends of the ringed thread. Compound **F** was added via base catalysis to the aromatic dialdehyde ends of the pseudorotaxane complex (Compound **E**). As shown in Figure 5, the addition of the first equivalent of Compound **F** produced a small intermediate (Compound **G**) that could easily be threaded into or dethreaded out of the macrocycle. The addition of a second equivalent of Compound **F**, however, produced a large reversibly formed addition product (Compound **H**) that could not be threaded or unthreaded. The reversibility of this second addition allowed time for the favorable interaction of π-rich and π-poor aromatic units to drive Compound **G** into the macrocycle, if it wasn't there already, before the second addition of Compound **F**.

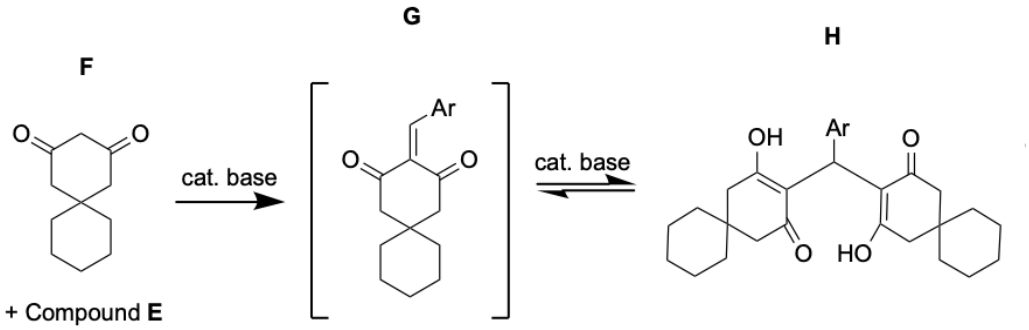

Figure 5. The reversible capping of the dialdehyde thread of the pseudorotaxane (Compound **E**) with spirodiketone (Compound **F**) to form a large addition product (Compound **H**).

Once the capping step was completed, the reversibility of the thread formation was 'turned off.'[1] More specifically, the reaction underwent an irreversible acid-catalyzed dehydration, as shown in Figure 6. This formed a bulky polycyclic system (Compound **I**) that fixed the assembled topology of the rotaxane permanently in place.

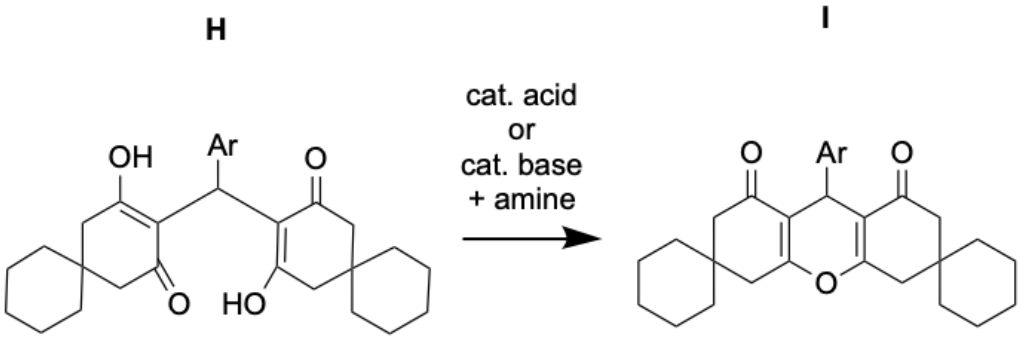

Figure 6. The synthesis of a fixed rotaxane assembly (Compound **I**) from the large addition product (Compound **H**) via acid-catalyzed dehydration.

1.4: Bisulfite Addition Compounds

There are many instances where water insoluble reactions and compounds would be useful in water-based environments such as biological systems. For example, dapsone is an antileprosy drug that is water insoluble but would be most useful as an oral drug.[9] Oral drugs are the most easily accessible to those with leprosy, but must be soluble in water to be absorbed into the bloodstream. Fortunately, it was discovered that adding a formaldehyde bisulfite adduct to dapsone, as shown in Figure 7, would make it temporarily water soluble.

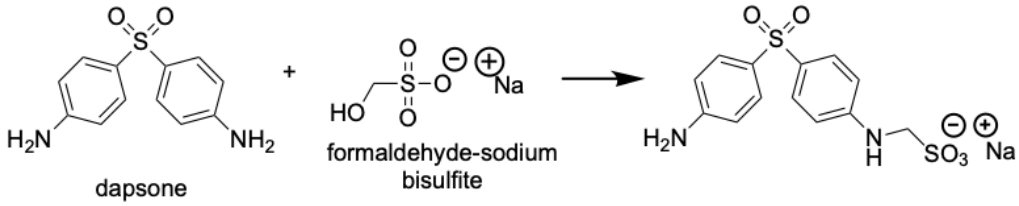

Figure 7. The formation of a temporarily water soluble version of the anti-leprosy drug dapsone using formaldehyde sodium bisulfite.

Similarly, sodium bisulfite adds to aldehydes and forms a bisulfite addition compound as shown in Figure 8. This newly formed compound is water soluble and can be hydrolysed back into the aldehyde with the addition of dilute

aqueous acid or base. This reversibility makes bisulfite addition compounds particularly useful as intermediates in the synthesis of other compounds.

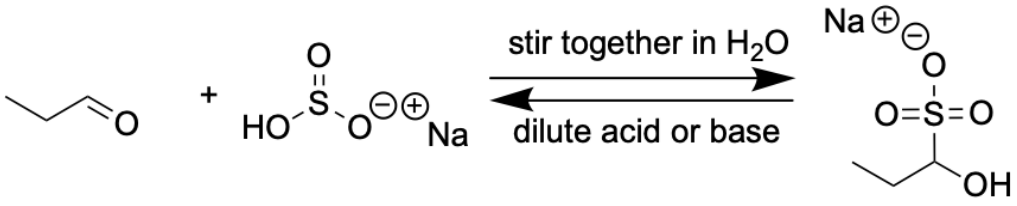

Figure 8. The reversible synthesis of soluble bisulfite addition compounds from the addition of sodium bisulfite to an aldehyde containing molecule.

1.5: The Thermodynamics of Water

Despite its importance and abundance on Earth, water is a complex material. Water molecules have a bent structure that gives them an electric dipole moment making them polar. This polarity and water's composition enables it to hydrogen bond with up to four other water molecules. These very strong hydrogen bonds bind the water molecules together, which gives water its high surface tension and boiling point.

The addition of hydrophobic non-organic compounds, however, interrupts these hydrogen bonds. This disruption leads to water molecules forming new hydrogen bonds in a cage-like structure around the hydrophobic compound. This added structure decreases the total entropy and only slightly increases the total enthalpy of the system. According to the equation for Gibbs free energy, this slight favorable increase in enthalpy does not compensate for the unfavorable decrease in entropy. As such, the ordering of water molecules is highly unfavorable, making the mixing of water and hydrophobic molecules thermodynamically unfavorable.

However, one way to decrease this thermodynamic unfavourability is to decrease the number of water molecules that are ordered. The most effective way to do this is to decrease the total hydrophobic surface exposed to the water molecules. In order to decrease total hydrophobic surface, the hydrophobic molecules spontaneously come together and interact with each other.

1.6: Project Overview

Rotaxanes have several possible applications. One is their use as molecular machines, such as molecular shuttles.[10] Movement of the ring or threaded dumbbell of rotaxanes also enables them to act like molecular switches. Another application is the formation of rotaxane dyes. Forming rotaxanes from dyes has been shown to increase the stability and fluorescence of those dyes.[11]

Chemists constantly work to improve techniques and increase the yields of useful compounds like rotaxanes. One possible way to further increase the yield of an approach such as the one described by Cagulada et al. would be to increase the thermodynamic drive of the reaction. The addition of sodium bisulfite to the aldehyde thread would make the compound temporarily soluble in water. Thermodynamically favorable π-rich and π-poor aromatic unit interactions as well as hydrophobic interactions would then drive this thread into the macrocycle. From there the rotaxane synthesis could occur as originally proposed and the yield of rotaxane synthesized should be increased. In this manuscript, efforts toward synthesizing rotaxanes in an aqueous system will be discussed.

Chapter 2: Materials and Methods

2.1: Materials

Methyl 4-formylbenzoate (MW = 164.15 g/mol and MP = 59 - 63℃), 5,5-Dimethyl-1,3-cyclohexanedione (MW = 140.18 g/mol, MP = 146℃, and 95% hydrolyzed), and Piperidine (MW = 85.15 g/mol, biotech grade, and 99.5+ % hydrolyzed), were purchased from Sigma-Aldrich. Sodium metabisulfite (MW = 190.10 g/mol and 97+ % hydrolyzed) was purchased from Acros Organics. Sodium bicarbonate (MW = 84.01 g/mol) was purchased from FischerChemical. A large batch of Compound **1** (MW = 408.46 g/mol and MP = 171 - 173℃) was produced by and collected from a laboratory session for one of Mount Holyoke College's Organic Chemistry 2 classes. Isopropyl ether and ethanol (95%) were purchased from Pharmco.

2.2: Instrumentation

All reactions were done in 20 mL screw-cap vials or 10 - 25 mL round bottom flasks. Most heated reactions were done imprecisely on a hot plate. The precisely heated reactions were done in a temperature controlled oil bath. A Bückner filter flask and a 15mL PYREX ASTM 4-5.5 filter was used for vacuum filtration. A scale was used to determine product yield and a melting point apparatus was used to determine approximate purity by comparison with authentic samples.

2.3: Filtration Collection Techniques

2.3.1: Precipitate Collection

This method was used to collect the precipitates formed in or remaining from a reaction. The reaction solution was carefully poured into a standard vacuum filtration set-up with a fine filter. The vacuum pump was turned on just enough to allow the solution to slowly flow through the filter. The filter and any collected precipitate was then washed with a few milliliters of the appropriate solvent. The precipitate was then left to thoroughly dry for a period of time with the vacuum pump still turned on. Once dry, the precipitate was carefully collected and weighed.

2.3.2: Filtrate Collection

This method was used to collect solid samples of the soluble chemicals that ran through the filter. As explained in the section above, the reaction solution was carefully vacuum filtered. As with the precipitate, the liquid filtrate was left to evaporate for a period of time with the vacuum pump still on to accelerate the process. Once fully evaporated, the solid filtrate was carefully collected and weighed.

2.4: Experimental Procedures

2.4.1: Testing Unedited Capabilities

For Reactions 1, 2, and 3, methyl 4-formylbenzoate (0.22g, 1.3mmol) was stirred in the reaction-specific solvent (Table 1). To the stirred solution, 5,5-dimethyl-1,3-cyclohexanedione (2.1 equiv.; 0.46g, 3.3mmol) and catalytic piperidine (3 drops, ~0.035g, ~0.41mmol) were added. The solution was stirred at

75 ℃ for 2 hours in an oil bath before being allowed to cool to room temperature. Once cooled to room temperature, the solution was refrigerated for three days. The solution was then vacuum filtered, washed as specified in Table 1, and left to dry for an hour before being weighed and the melting point recorded.

Table 1. Solvent and filter wash specifications for Reactions 1, 2, and 3.

Reaction	Solvent	Filter Wash
1 & 2	Ethanol	Ethanol (3mL) & Water (5mL)
3	Water	Water (5mL)

2.4.2: Adding Sodium Bisulfite

For Reaction 4, sodium metabisulfite (0.6 equiv.; 0.14g, 0.74mmol) was stirred in deionized water (5mL). Methyl 4-formylbenzoate (0.21g, 1.3mmol) was added to the stirred solution. The solution was left to stir for five hours. The solution was then heated to 65-70℃ for five minutes or until fully clear. Once clear, the solution was allowed to cool to room temperature and left unstirred overnight.

For Reactions 5, 6, and 7, methyl 4-formylbenzoate (0.21g, 1.3mmol) was added to a stirred solution of sodium metabisulfite (0.6 equiv.; 0.15g, 0.79mmol) and deionized water (5mL). The solution was heated to 65-70℃ for five minutes before being allowed to cool to room temperature. For Reaction 5, once at room temperature, aqueous base was added as specified in Table 2 and the solution was stirred for three days. For Reactions 6 and 7, after the aqueous base was added, 5,5-dimethyl-1,3-cyclohexanedione (2.1 equiv.; 0.37g, 2.6mmol) was also added. The solution was then stirred at room temperature for three days. For Reactions 6

and 7, the stirred solution was then vacuum filtered, washed with water (5mL), and left to dry for three days. Once dry, the product was weighed and a melting point was taken.

Table 2. Base type and amount specifications for Reactions 5, 6, and 7.

Reaction	Base Type	Base Amount
5	Sodium Bicarbonate	1.2 equiv. 0.13g 1.5mmol
6 & 7	Piperidine	3 drops ~0.035g ~0.41mmol

2.4.3: Adjusting Base Quantity and Type

For Reactions 8 - 12, methyl 4-formylbenzoate (0.21g, 1.3mmol) was added to a stirred solution of sodium metabisulfite (0.6 equiv.; 0.15g, 0.79mmol) and deionized water (5mL). The solution was heated to 65-70℃ for five minutes before being allowed to cool to room temperature. Once at room temperature, 5,5-dimethyl-1,3-cyclohexanedione (2.1 equiv.; 0.37g, 2.6mmol) and aqueous base, as specified in Table 3, were added. The solution was then stirred at room temperature for three days. The stirred solution was then vacuum filtered, washed with water (5mL), and left to dry for three days. Once dry, the product was weighed and a melting point was taken.

Table 3. Base type and amount specifications for Reactions 8-12.

Reaction	Base Type	Base Amount
8	Piperidine	6 drops ~0.07g ~0.82mmol
9 & 10		1.2 equiv. 0.15mL ~0.13g ~1.50mmol
11		0.9 equiv. 0.11mL ~0.09g ~1.13mmol
12	Sodium Bicarbonate	1.2 equiv. 0.13g 1.5mmol

2.4.4: Reacting Sodium Bisulfite and 5,5-Dimethyl-1,3-cyclohexanedione

For Reactions 13 - 21, sodium metabisulfite, as specified in Table 4, was stirred in deionized water and 5,5-dimethyl-1,3-cyclohexanedione was added. For Reaction 20, the 5,5-dimethyl-1,3-cyclohexanedione was added in batches and each batch was added after the previous was judged to be dissolved. Batch 2 was added after 30 minutes and batch 3 was added after approximately 18 hours. The solution was stirred either in an oil bath at 75°C or at room temperature for three days, with the exception of Reactions 17 and 18 which were only stirred for 30 minutes. The solutions were then vacuum filtered and washed with water (5mL). The filtered products were then dried for three days and both the precipitates and filtrates were weighed and their melting points were measured.

Table 4. Specifications of the solvent, sodium metabisulfite, and 5,5-dimethyl-1,3-cyclohexanedione amounts for Reactions 13 - 21 as well as specifications as to whether or not they were heated to 75°C in an oil bath.

<table>
<tr><th>Reaction</th><th>Solvent Amount (mL)</th><th>Sodium Metabisulfite Amount</th><th>5,5-Dimethyl-1,3-cyclohexanedione</th><th>Heated?</th></tr>
<tr><td>13, 14, & 15</td><td rowspan="2">5</td><td rowspan="5">0.6 equiv.
0.16g
0.86mmol</td><td rowspan="4">0.2g
1.4mmol</td><td>No</td></tr>
<tr><td>16</td><td rowspan="2">Yes</td></tr>
<tr><td>17 & 18</td><td rowspan="2">20</td></tr>
<tr><td>19</td><td rowspan="3">No</td></tr>
<tr><td>20</td><td rowspan="2">5</td><td>0.05g
0.35mmol
batches</td></tr>
<tr><td>21</td><td>1.2 equiv.
0.32g
1.72mmol</td><td>0.2g
1.4mmol</td></tr>
</table>

2.4.5: Recrystallization

For Reaction 22, 5,5-dimethyl-1,3-cyclohexanedione (0.05g, 0.35mmol) was stirred in a solution of sodium metabisulfite (0.16g, 0.86mmol) in deionized water (5mL). The solution was then left to stir until fully dissolved (approximately 30 minutes). Once fully dissolved, sodium bicarbonate (0.15g, 1.79mmol) was added to the solution and it was left to stir for three days.

For Reaction 23, 24, and 25, the filtered product from a reaction specified in Table 5 (~0.05g in each vial) was added to four extra small vials of deionized water (~½ mL in each vial). The solutions were then heated to 65-70℃ until fully dissolved. Once fully dissolved, the solutions were allowed to slowly cool to room temperature. Once cooled, the solutions were carefully pipetted through a filter, made from a glass pipette with a small wad of cotton, into four new and carefully scratched extra small vials. The four extra small vials were then each covered with parafilm, which had four small holes poked into it with a needle. The newly covered extra small vials were then carefully placed inside of slightly larger vials containing isopropyl ether (~1mL). Two of the vial sets were then left at room temperature and the other two were refrigerated and left untouched for two weeks.

Table 5. Specifications of the original reactions for the filtrates used in Reactions 23, 24, and 25.

Reaction	Filtrate's Originating Reaction
23	Reaction 15
24	Reaction 18
25	Reaction 21

2.4.6: Increasing 5,5-Dimethyl-1,3-cyclohexanedione Addition

For Reaction 26, 27, and 28, methyl 4-formylbenzoate (0.21g, 1.3mmol) was added to a stirred solution of sodium metabisulfite (0.6 equiv.; 0.15g, 0.79mmol) in deionized water (5mL) and heated to 65-70℃ for about five minutes. The solution was then cooled to room temperature and 5,5-dimethyl-1,3-cyclohexanedione and aqueous base were added as specified in Table 6. The solution was then stirred at room temperature for approximately three days before being vacuum filtered, washed with water (5mL), and left to dry for three days. Once dry, the product was weighed and its melting point was measured.

Table 6. Specifications of the base type and amount as well as the amount of 5,5-dimethyl-1,3-cyclohexanedione added in Reactions 26, 27, and 28.

Reaction	**Base Type**	**Base Amount**	**5,5-Dimethyl-1,3-cyclohexanedione**
26	Piperidine	0.9 equiv. 0.11mL ~0.09g ~1.13mmol	3.3 equiv. 0.58g 4.1mmol
27	Sodium Bicarbonate	1.2 equiv. 0.13g 1.50mmol	
28			10 equiv. 1.77g 12.6mmol

*2.4.7: Compound **1** and 5,5-Dimethyl-1,3-cyclohexanedione Solubilities*

For Reaction 29, 5,5-dimethyl-1,3-cyclohexanedione (0.5g, 3.57mmol) was stirred in a solution of deionized water (5mL) and 95% ethanol (5mL).

For Reaction 30, Compound **1** (0.2g, 0.49mmol) was stirred in a solution of deionized water (2.5mL) and 95% ethanol (2.5mL) overnight. The solution was then vacuum filtered and washed with a solution of 95% ethanol (2.5mL) and

deionized water (2.5mL). The solution was then left to dry for three days before measurements of its mass and melting point were taken.

2.4.8: Testing Solvent-based Purification

For Reaction 31, 5,5-dimethyl-1,3-cyclohexanedione (10 equiv.; 1.76g, 12.56mmol) and compound **1** (0.51g, 1.25mmol) were stirred in a 95% ethanol (15mL) and deionized water (15mL) solution. The solution was left to stir for three days before being vacuum filtered, washed with a 95% ethanol (2.5mL) and deionized water (2.5mL) solution, and left to dry for three days. Once dry, the precipitate and filtrate were weighed and their melting points were measured.

For Reaction 32, 5,5-dimethyl-1,3-cyclohexanedione (0.5g, 3.75mmol) was stirred in a 95% ethanol (5mL) and deionized water (5mL) solution. Once fully dissolved (after 3 minutes), another batch of 5,5-dimethyl-1,3-cyclohexanedione (0.1g, 0.71mmol) was added. Once fully dissolved (after 2 minutes), another batch of 5,5-dimethyl-1,3-cyclohexanedione (0.1g, 0.71mmol) was added. Once fully dissolved (after 5 minutes), another batch of 5,5-dimethyl-1,3-cyclohexanedione (0.1g, 0.71mmol) was added. Once fully dissolved (after 2 minutes), another batch of 5,5-dimethyl-1,3-cyclohexanedione (0.1g, 0.71mmol) was added. Once fully dissolved (after 5 minutes), another batch of 5,5-dimethyl-1,3-cyclohexanedione (0.1g, 0.71mmol) was added.

For Reaction 33, Compound **1** (0.51g, 1.25mmol) was added to a stirred solution of 5,5-dimethyl-1,3-cyclohexanedione (10 equiv.; 1.76g, 12.56 mmol) in 95% ethanol (10mL) and deionized water (10mL). After three days of stirring, the solution was vacuum filtered and washed with a solution of 95% ethanol (2.5mL)

and deionized water (2.5mL). The solution then dried for three days before the filtrate and precipitates were weighed and tested for their melting points.

2.4.9: Excess 5,5-Dimethyl-1,3-cyclohexanedione Product Purification

For Reactions 34, 35, and 36, the precipitate from a reaction specified in Table 7 was stirred in a 95% ethanol and deionized water solution. The solution was then left to stir for three days before being filtered, washed with 95% ethanol and deionized water solution, and left to dry for three days. Once dry, the precipitate was weighed and had its melting point measured.

Table 7. Specifications of the original reactions for the filtrates, their original masses, and the amount of water and ethanol in which they were purified in Reactions 34, 35, and 36.

Reaction	Product's Originating Reaction	Product's Original Mass (g)	Ethanol Amount (mL)	Water Amount (mL)
34	Reaction 26	0.250	2	2
35	Reaction 27	0.258		
36	Reaction 28	1.396	15	15

2.4.10: Adding 5,5-Dimethyl-1,3-cyclohexanedione in Batches

For Reaction 37, sodium metabisulfite (0.6 equiv.; 0.15g, 0.79mmol) was stirred in deionized water (5mL). Methyl 4-formylbenzoate (0.21g, 1.3mmol) was added and the stirred solution was heated to 65-70℃ for five minutes. Once cooled to room temperature, sodium bicarbonate (1.2 equiv.; 0.13g, 1.5mmol) was added. Then, approximately 0.1g of 5,5-dimethyl-1,3-cyclohexanedione (3.3 equiv.; 0.58g, 4.1mmol) was added every five minutes. Once all of the 5,5-dimethyl-1,3-cyclohexanedione was added, the solution was left to stir for three

days before being filtered, washed with deionized water (5mL), and left to dry. The dried precipitate was then weighed and had its melting point measured.

For Reaction 38, the precipitate from Reaction 37 (0.200g) was stirred in a solution of 95% ethanol (4mL) and deionized water (4mL) for three days. The solution was then vacuum filtered with a 95% ethanol (2mL) and deionized water (2mL) wash before being left to dry for three days. Once dry, the precipitate was weighed and its melting point was measured.

2.4.11: Halving Sodium Metabisulfite Addition

For Reaction 39, a solution of sodium metabisulfite (0.3 equiv.; 0.07g, 0.4mmol), methyl 4-formylbenzoate (0.21g, 1.3mmol), and deionized water (5mL) was stirred and heated to 65-70℃ for five minutes. Once cooled, 5,5-dimethyl-1,3-cyclohexanedione (3.3 equiv.; 0.58g, 4.1mmol) was added and the solution was left to stir. After three days, the solution was filtered, washed with deionized water (5mL), and left to dry. The dried precipitate was then weighed and had its melting point measured.

Chapter 3: Results and Discussion

*3.1: Compound **1** Production in Ethanol and Water*

The protocol for Compound **1**, as shown in Figure 9, was selected from Cagulada et al.'s collection of partial thread synthesis reactions, which model the formation of a full thread. Compound **1** was selected as a suitable template reaction partly because its foundational components are inexpensive and readily available. It was also selected because both the reactants and the product were insoluble in water at room temperature and would effectively show how the addition of sodium bisulfite would affect the thread synthesis step in rotaxane formation.

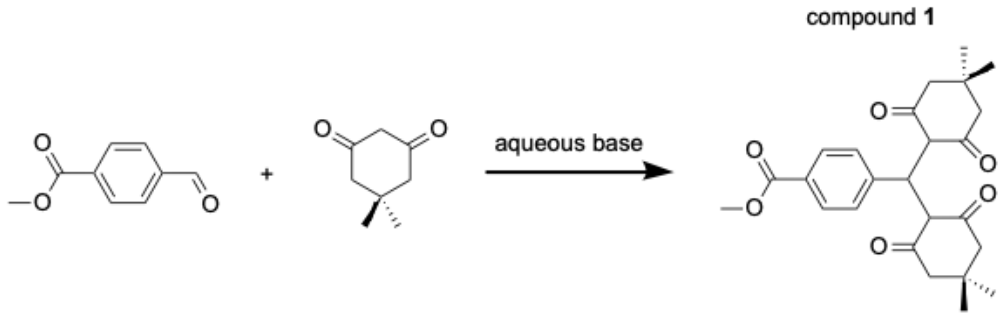

Figure 9. The basic mechanism for Cagulada et al.'s Compound **1** production.

To start off this project, the chosen prototype reaction was done as published to better understand the procedure and confirm Cagulada et al.'s results in this setting. The same reaction was then done in water to test the reaction's unmodified capabilities in the desired solvent. Table 8 shows measurements of the masses and melting points of the dried products as well as calculations of their percent yields. The melting point of the expected product, Compound **1**, was recorded in the literature as 171 - 173 °C.[8] Since the experimental melting points

closely aligned with the literature values it was confirmed that the precipitated solids from Reactions 1, 2, and 3 were Compound **1**.

Table 8. Measurements of the masses and melting points as well as calculations of their yields of the products produced in ethanol or water.

Reaction	Solvents	Mass (g)	Yield (%)	Melting Point (°C)
1	Ethanol (#1)	0.204	37	168.7 - 169.2
2	Ethanol (#2)	0.250	45	167.0 - 168.0
3	Water	0.620	112	168.7 - 170.7

During Reaction 3, 5,5-dimethyl-1,3-cyclohexanedione (dimedone) and methyl 4-formylbenzoate were both found to be largely insoluble in water. However, the melting point of the resulting product suggested that small portions of the reactants could move into solution for short periods of time. This enabled the two compounds to interact within the water and produce the desired product. Given enough time, the reaction in water produced a very high yield of Compound **1.** The over one hundred percent yield may have been due to the collected product not being fully dry. However, even fully dry, the yield would have still been much larger than both the experimental and literature yields in ethanol. This supported the theory that the reactants would be more likely to interact in water and produce a higher yield of product due to the hydrophobic effect. Despite this, the limited water solubility of these compounds and their intermediates vastly decreased the likelihood of synthesis inside a water soluble macrocycle. Such a reaction would more than likely take extensive time and produce a low yield of the desired rotaxane.

3.2: Methyl 4-Formylbenzoate Solubilization

It was theorized, however, that sodium bisulfite would nucleophilically attack the carbonyl group on the methyl 4-formylbenzoate and form a water soluble bisulfite addition compound (Compound **J**), as shown in Figure 10. The hydrophobicity of water would drive the remaining hydrophobic regions of the aldehyde thread to interact with the similarly hydrophobic regions inside the macrocycle. Both the increased thread solubility and the hydrophobic effect would increase the likelihood of the thread forming inside of the macrocycle. Such a reaction would also occur quickly and produce a high yield of the desired rotaxane.

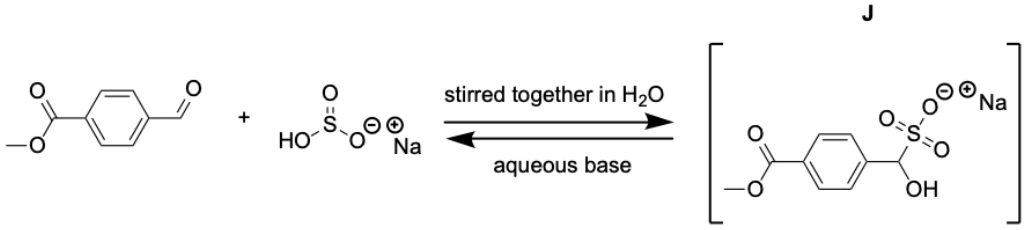

Figure 10. The addition of sodium bisulfite to methyl 4-formylbenzoate in water forming the reversible intermediate bisulfite addition compound (Compound **J**).

Instead of aqueous sodium bisulfite, solid sodium metabisulfite was used to simplify the process. This was because sodium metabisulfite was easily purchased in a solid state and, when dissolved in water, it produces two equivalents of sodium bisulfite. However, when methyl 4-formylbenzoate was added to the clear solution of sodium metabisulfite in water during Reaction 4, the solution became cloudy and remained cloudy after stirring for five hours at room temperature. It was suspected that this was because the unmodified methyl 4-formylbenzoate, which was only slightly soluble in water as explained previously,

still had to go into solution to interact with the sodium bisulfite. As such, it was probable that the reaction rate was very slow and not all of the methyl 4-formylbenzoate was able to react with the sodium bisulfite within that time frame.

To accelerate the reaction rate, the solution of methyl 4-formylbenzoate and sodium metabisulfite in water was heated to 65 - 70 ℃, which resulted in the expected clear solution within five minutes. This heat, however, would also act as a catalyst for the dethreading of the compound from within the macrocycle. With this in mind, Compound **J** needed to remain soluble in water at room temperature. Fortunately, Reaction 4 confirmed that, even after being left at room temperature unstirred overnight, Compound **J** did remain dissolved in solution.

Once threaded inside the macrocycle, the solubility conferred by reaction with sodium bisulfite would no longer be necessary because its removal would 'trap' the methyl 4-formylbenzoate inside of the macrocycle. The hydrophobic regions of the two compounds interacting would be the most thermodynamically favorable configuration in water. The presence of the sodium sulfite group would also make the methyl 4-formylbenzoate unavailable for the addition of two equivalents of dimedone.

The literature suggested that the addition of a base, such as piperidine or sodium bicarbonate, would reverse the reaction, as shown in Figure 10. The base would hydrolyse Compound **J** back into methyl 4-formylbenzoate. To confirm this reverse in solubility, the aqueous base sodium bicarbonate was added in Reaction 5. This caused a white precipitate to form, indicating that the insoluble methyl 4-formylbenzoate was reformed.

With the removal of the sodium bisulfite, the revealed aldehyde end could be capped. Accordingly, two equivalents of dimedone were added to this solution alongside the aqueous base in Reactions 6 and 7. Masses and melting points of the products were measured and the yields were calculated as recorded in Table 9. Since the recorded melting points aligned with the expected values, it was confirmed that the precipitated solid was indeed Compound **1**.

Table 9. Measurements of the masses and melting points as well as yield calculations of the products produced with the addition of 2.1 equivalents of dimedone and 3 drops of piperidine to the solution of Compound **J** in water.

Reaction	Conditions	Mass (g)	Yield (%)	Melting Point (°C)
6	Added Dimedone and Piperidine	0.170	33	169.9 - 170.2
7	Added Dimedone and Piperidine	0.241	47	168.9 - 170.2

As explained above, the aqueous base would have reversed the sodium bisulfite addition reaction resulting in reformed methyl 4-formylbenzoate. The first equivalent of dimedone would have then nucleophilically attacked the carbonyl group on the reformed methyl 4-formylbenzoate. This process would have formed an intermediate (Compound **K**) as shown in Figure 11.

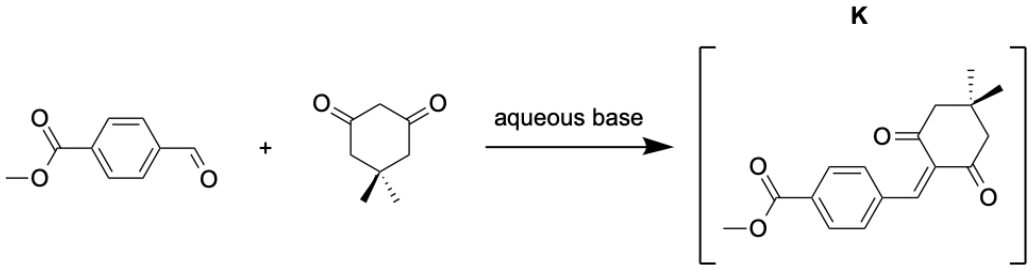

Figure 11. The addition of the first equivalent of 5,5-dimethyl-1,3-cyclohexanedione to methyl 4-formylbenzoate in water forming an intermediate (Compound **K**).

From this point, the reaction could have proceeded two ways. The first and most likely path was that the second equivalent of the added dimedone would have nucleophilically attacked Compound **K**. This would have formed the desired Compound **1** as shown in Figure 12.

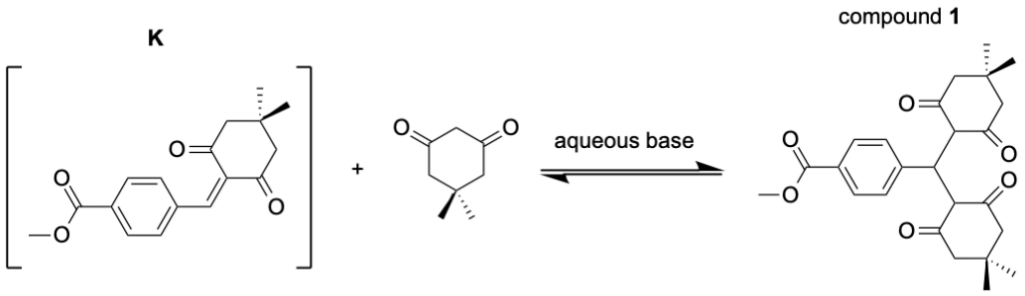

Figure 12. The addition of the second equivalent of 5,5-dimethyl-1,3-cyclohexanedione to Compound **K** in water forming Compound **1**.

The second possible path was that the newly released sodium bisulfite would have been able to react with Compound **K**. This would have formed a soluble sodium bisulfite addition compound (Compound **L**), as shown in Figure 13. Then, the continued presence of dilute base would have once again reversed the sodium bisulfite addition reaction, resulting in the reformed Compound **K**. As explained above, this compound would have then been available for nucleophilic attack by dimedone to create the desired Compound **1.**

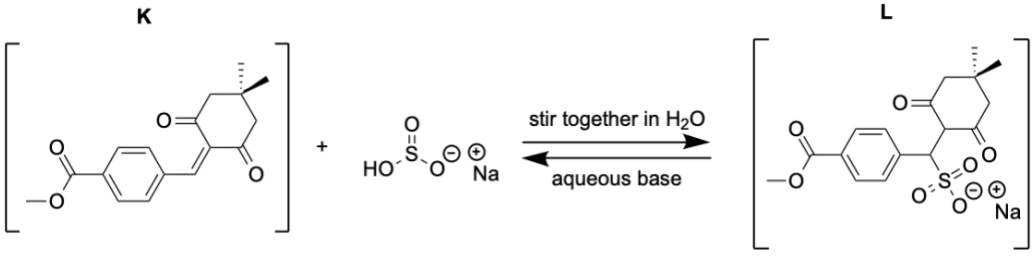

Figure 13. The addition of sodium bisulfite to Compound **K** in water forming the reversible intermediate bisulfite addition compound (Compound **L**).

However, the yields produced from this reaction were much lower than expected. One possibility was that the small amount of aqueous base piperidine

limited the amount of product formed within three days. The original procedure in the literature only used piperidine as a catalytic base to increase the reaction rate between methyl 4-formylbenzoate and dimedone. In the modified procedure, however, the piperidine was also hydrolyzing the sodium bisulfite compounds. To increase the amount and rate of this hydrolyzation, stoichiometric amounts of piperidine were possibly necessary.

3.3: Base Effectivity

Various amounts of piperidine were tried and the masses and melting points of the products were measured and their yields were calculated, recorded in Table 10. Reaction 8 showed that increased quantities of piperidine might have increased the yield of the product slightly, as predicted. However, when amounts of piperidine in excess of stoichiometric amounts were used in Reactions 9 and 10, the percent yields of the product appeared to dramatically decrease.

Table 10. Measurements of the masses and melting points as well as yield calculations of the products produced with varying amounts of piperidine.

Reaction	Conditions	Mass (g)	Yield (%)	Melting Point (°C)
8	6 Drops Piperidine	0.226	44	168.8 - 170.4
9	1.2 eq. Piperidine (#1)	0.148	29	168.9 - 170.2
10	1.2 eq. Piperidine (#2)	0.141	27	169.4 - 170.2

These much larger amounts of a reasonably strong base, such as piperidine, may have caused more deprotonation to occur than expected. To increase the product yield while decreasing the likelihood of excessive deprotonation, slightly less than stoichiometric amounts of piperidine were tested.

Additionally, a high amount of a weaker base, such as sodium bicarbonate, was tested because it would potentially achieve the same goal.

These two methods were tried, and the products were analyzed for their masses and melting points before their yields were calculated, as shown in Table 11. The product yields from Reactions 11 and 12 were much higher than those from Reactions 9 and 10. However, they were still lower than those of Reactions 7 and 8 and much lower than the expected yield.

Table 11. Measurements of the masses and melting points as well as yield calculations of the products produced with less than stoichiometric piperidine and more than stoichiometric sodium bicarbonate.

Reaction	Conditions	Mass (g)	Yield (%)	Melting Point (°C)
11	0.9 eq. Piperidine	0.188	37	169.4 - 170.2
12	1.2 eq. Sodium Bicarbonate	0.164	32	169.4 - 170.2

*3.4: Compound **2** and Compound **3** Production*

Another possibility was that the sodium bisulfite was reacting with both the methyl 4-formylbenzoate and the dimedone. This possibility seemed unlikely because no literature referencing dimedone mentioned the possibility of a reaction with sodium bisulfite. However, when dimedone was added to a solution of sodium bisulfite, some of it appeared to have dissolved over time. With this in mind, the filtrates were carefully collected and the solvent was evaporated over time at room temperature with the help of a constant air flow from the vacuum. Masses and melting points of the dry filtrates and precipitates were taken as shown in Table 12.

Table 12. Measurements of the masses and melting points from the filtrates and precipitates collected after the combination of 5,5-dimethyl-1,3-cyclohexanedione and sodium bisulfite in water.

Reaction	Conditions	Product	Mass (g)	Melting Point (℃)
13	Dimedone + 1.2 eq. $NaHSO_3$ (#1)	Precipitate	0.042	147.4 - 148.5
		Filtrate	0.255	>250
14	Dimedone + 1.2 eq. $NaHSO_3$ (#2)	Precipitate	0.056	146.3 - 148.5
		Filtrate	0.236	>250
15	Dimedone + 1.2 eq. $NaHSO_3$ (#3)	Precipitate	0.036	146.8 - 148.3
		Filtrate	0.195	>250

In the literature, the melting point of the expected precipitate, dimedone, is recorded as 147 - 150 ℃. Because the experimental melting points of the precipitates approximately aligned with the values in the literature, it was very likely that the precipitated compounds were dimedone. Meanwhile, the decomposition point of the expected filtrate, sodium bisulfite, was recorded in the literature as 150 ℃, which did not align with the experimental melting points of the filtrates. The filtrate compounds also turned a vibrant red when heated, as shown in Figure 14. Both this color change and the unexpectedly high melting points meant that the filtrated compounds were neither dimedone nor sodium bisulfite.

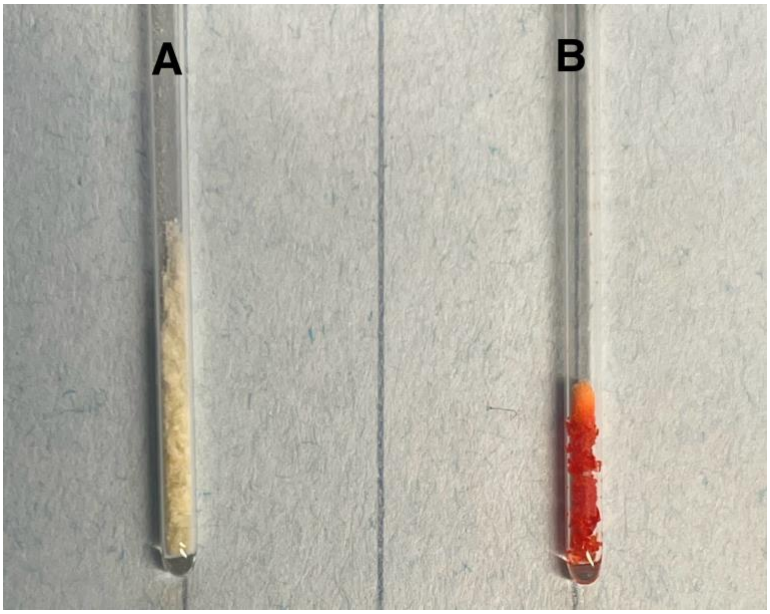

Figure 14. Picture of the unknown product (Compound **2**) from the reaction (#13) between sodium bisulfite and 5,5-dimethyl-1,3-cyclohexanedione at (A) room temperature and (B) after being heated to 150.0℃.

Further research showed that certain ketones could react with sodium bisulfite in much the same way as aldehydes[12]. Dimedone, however, was not tested in the literature and none of the ketones specified had a similar structure to that of dimedone. As such, the identity of Compound **2,** formed from the reaction between the sodium bisulfite and dimedone, was still unclear.

As shown by the precipitate masses in Table 12, not all of the dimedone appeared to react with the sodium bisulfite. This may have been because more time or a catalyst were necessary for the dimedone to react fully with the sodium bisulfite. With this in mind, a solution of dimedone and sodium bisulfite was heated to try to catalyze the reaction. The precipitate and filtrate were then weighed and their melting points were measured as recorded in Table 13.

Table 13. Measurements of the masses and melting points from the filtrates and precipitates collected from the reaction between 5,5-dimethyl-1,3-cyclohexanedione and sodium bisulfite in water at 75℃.

Reaction	**Conditions**	**Product**	**Mass (g)**	**Melting Point (℃)**
16	Dimedone + 1.2 eq. $NaHSO_3$ + heat	Precipitate	0.094	144.2 - 146.3
		Filtrate	0.145	211.3 - >250

However, the filtrate's melting point did not align with that expected of Compound **2**. The new compound also turned a light orange rather than a vibrant red when heated, as shown in Figure 15. Both the change in color and the different melting point suggested that the heat catalyzed the formation of another new compound, Compound **3**, from the reaction between sodium bisulfite and dimedone. This meant that the reaction between sodium bisulfite and dimedone could form either a kinetic or a thermodynamic product dependent upon the conditions.

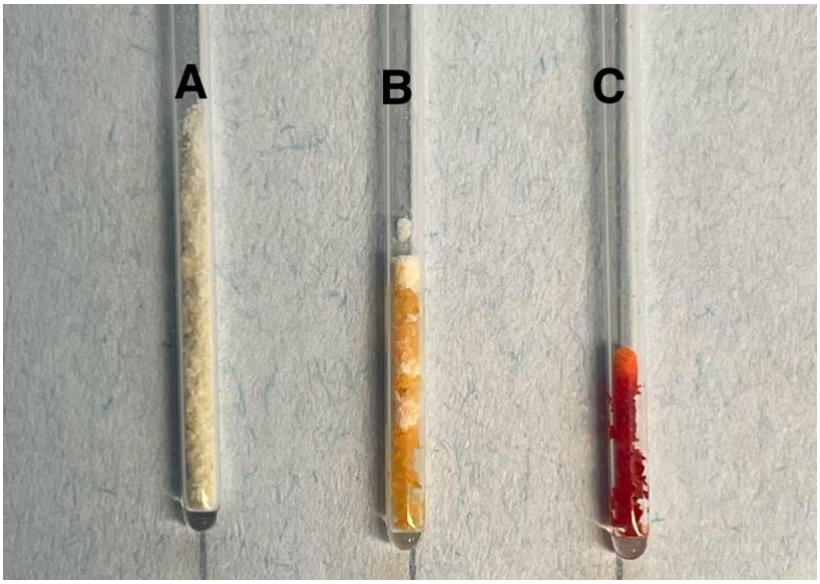

Figure 15. Picture of the unknown product (Compound **3**) from the heated reaction (#16) between sodium bisulfite and 5,5-dimethyl-1,3-cyclohexanedione at (A) room temperature and (B) after being heated to 150.0℃ with (C) Compound **2** from the reaction (#13) after being heated to 150.0 ℃ as a comparison.

The melting point of the precipitate formed from Reaction 16, however, was aligned with that expected of dimedone. This meant that the dimedone was still not reacting fully with the sodium bisulfite. Another possible explanation was that the small solvent volume limited the amount of Compound **2** and Compound **3** that could go into solution and thus limited the amount of these products that could be produced. To test this, dimedone was reacted with sodium bisulfite in a larger volume of solvent at the two varying temperatures. Measurements of the dried precipitates and filtrates' masses and melting points were taken and recorded in Table 14.

Table 14. Measurements of the masses and melting points from the filtrates and precipitates collected from the reactions between 5,5-dimethyl-1,3-cyclohexanedione and sodium bisulfite in excess water at room temperature and heated to 75℃.

Reaction	Conditions	Product	Mass (g)	Melting Point (℃)
17	Dimedone + 1.2 eq. $NaHSO_3$ + heat + excess H_2O (#1)	Precipitate	0.0	N/A
		Filtrate	0.267	220 - >250
18	Dimedone + 1.2 eq. $NaHSO_3$ + heat + excess H_2O (#2)	Precipitate	0.0	N/A
		Filtrate	0.245	218 - >250
19	Dimedone + 1.2 eq. $NaHSO_3$ + excess H_2O	Precipitate	0.007	146.8 - 148.3
		Filtrate	0.257	136.8 - >250

Both Reaction 17 and 18's filtrates had melting points that matched those of Compound **3**. Also, no precipitates were collected from these reactions, which suggested that the volume increase did enable more dimedone to react with the sodium bisulfite to produce more of Compound **3**. Moreover, this suggested that one equivalent of sodium bisulfite was reacting with each molecule of dimedone as shown in Figure 16. Although, this would need to be confirmed using more specific and accurate analysis methods such as X-ray diffraction.

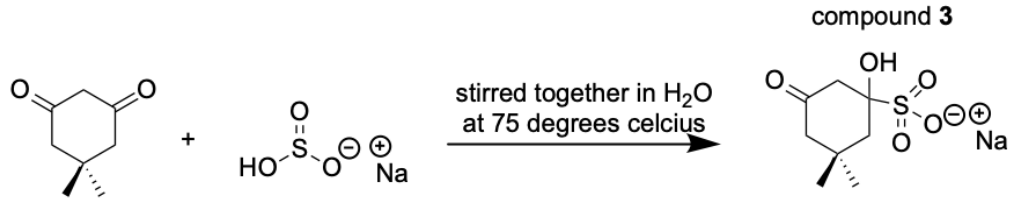

Figure 16. A proposed mechanism for the formation of Compound **3** from the heated reaction of sodium bisulfite with 5,5-dimethyl-1,3-cyclohexanedione.

Similarly, less precipitate, with a melting point similar to that of dimedone, was collected from Reaction 19 than that collected from Reactions 14, 15, and 16. On the other hand, Reaction 19's filtrate had a lower initial melting point. Even so, some of the compound remained unmelted and turned a vibrant red color, which was similar to Compound **2**. This suggested that the lower precipitate yield was due to more dimedone going into solution unreacted rather than more dimedone reacting with the sodium bisulfite.

The next probable explanation was that more than one molecule of the sodium bisulfite was reacting with each molecule of dimedone. In order to approximate this ratio small batches of dimedone were added to a solution of sodium bisulfite in water over time. The masses and melting points of the precipitate and filtrate were measured, as recorded in Table 15. The precipitate's melting point aligned with that of dimedone and the filtrate's melting point aligned with that of Compound **2**. It also appeared that only 0.121 grams of dimedone reacted with the sodium bisulfite to make Compound **2**. This meant that it was likely that two molecules of sodium bisulfite were reacting with each molecule of dimedone.

Table 15. Measurements of the masses and melting points from the filtrate and precipitate collected from the reaction between sodium bisulfite in room temperature water and batches of 5,5-dimethyl-1,3-cyclohexanedione added over time.

Reaction	**Conditions**	**Product**	**Mass (g)**	**Melting Point (°C)**
20	Dimedone (in 25% Batches) + 1.2 eq. $NaHSO_3$	Precipitate	0.029	147.5 - 148.5
		Filtrate	0.227	>250

To further test this ratio, dimedone was added to a solution of water with two times the amount of sodium bisulfite previously used. The dried precipitate and filtrate's masses and melting points were measured and recorded in Table 16. Similar to Reaction 19, the amount of precipitate, with a melting point matching that of dimedone, was less than what was collected from Reactions 14, 15, and 16.

Table 16. Measurements of the masses and melting points from the filtrate and precipitate collected from the reaction between 5,5-dimethyl-1,3-cyclohexanedione and two times the previous amounts of sodium bisulfite in room temperature water.

Reaction	Conditions	Product	Mass (g)	Melting Point (°C)
21	Dimedone + 2.4 eq. $NaHSO_3$	Precipitate	0.014	146.8 - 148.3
		Filtrate	0.419	>250

Unlike Reaction 19, Reaction 21's filtrate had a melting point that matched that of Compound **2**. Additionally, the amount of Compound **2** produced was nearly double that of Reactions 12, 13, and 14. Both of these factors further suggested that two equivalents of sodium bisulfite were reacting with the two ketones on one equivalent of dimedone, such as shown in Figure 17. Although, as with Compound **3**, this would need to be confirmed using more specific and accurate analysis methods such as X-ray diffraction.

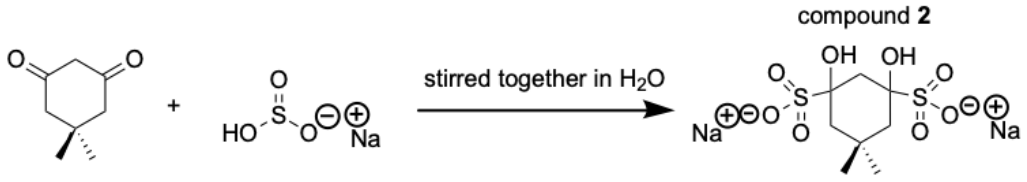

Figure 17. A proposed mechanism for the formation of Compound **2** from the reaction of sodium bisulfite with 5,5-dimethyl-1,3-cyclohexanedione.

Unlike the reaction between sodium bisulfite and methyl 4-formylbenzoate, this reaction between dimedone and sodium bisulfite might have also been irreversible in the presence of aqueous base. This irreversibility would have made both the sodium bisulfite and the dimedone unavailable for a reaction with methyl 4-formylbenzoate. To confirm this irreversibility sodium bicarbonate was added to a clear solution of dimedone with excess sodium bisulfite. Even after Reaction 22 sat for three days unstirred, no precipitate came out of solution. This meant that it was likely that the reaction was irreversible in the presence of aqueous base and this was what was reducing the production of Compound **1**.

3.5: Recrystallization

The definite identities and structures of Compounds **2** and **3** were still unknown. The best way to solve this mystery was to recrystallize the two compounds and analyze them with X-ray diffraction. The recrystallization for Reaction 23 was done with the filtrate from Reaction 15 and was separated into two vial sets that were left at room temperature and two vial sets that were refrigerated. Reactions 24 and 25 were done in the same way as Reaction 23 but with the filtrates from Reaction 18 and 21 respectively. However, Reaction 24 was the only reaction that produced a crystal, as shown in Figure 18, that might have the quality necessary for x-ray diffraction. As such, the sample was carefully collected and stored to be sent off for analysis when possible.

Figure 18. A picture of the crystal formed from Reaction 24.

3.6: Excess Dimedone

Since the formation of Compound **2** was taking the dimedone out of reaction, it was theorized that adding excessive amounts of dimedone might bypass this reaction and increase the yield of Compound **1**. In other words, it would increase the likelihood that the methyl 4-formylbenzoate bisulfite addition compound would react with the dimedone before the sodium bisulfite. This method was tested with two varying amounts of excess dimedone and the resulting precipitates had their masses and melting points measured before their percent yields were calculated, as shown in Table 17.

Table 17. Measurements of the masses and melting points from the precipitate collected from the addition of excess 5,5-dimethyl-1,3-cyclohexanedione.

Reaction	**Conditions**	**Mass (g)**	**Yield (%)**	**Melting Point (°C)**
26	3.3 eq. Dimedone + 0.9 eq. Piperidine	0.250	49	145.9 - 152
27	3.3 eq. Dimedone + 1.2 eq. Sodium Bicarbonate	0.258	51	155.5 - 158.3
28	10 eq. Dimedone + 1.2 eq. Sodium Bicarbonate	1.396	>100	139.6 - 142.2

The percent yields of the reactions were higher than those in previous reactions. However, the very low melting points showed that the precipitates collected were impure compounds of both unreacted dimedone and Compound **1**. In order to get a more accurate representation of the Compound **1** yields, a purification process needed to be developed.

3.7: Solvent-Based Purification

In this case, the easiest purification method was a solvent mixture where only one of the compounds was dissolved in solution. The literature suggested that dimedone was very soluble in ethanol at room temperature while Compound **1** was only partially soluble in ethanol at room temperature. This suggested that dimedone would remain fully soluble in water diluted with ethanol, but Compound **1** would remain largely undissolved. As a trial, two vials of a half ethanol and half deionized water solution were prepped. For Reaction 29, dimedone was added to one of the vials. After five minutes of stirring, the dimedone appeared to fully dissolve. For Reaction 30, Compound **1** was added to the second vial. Even after being left to stir overnight, Compound **1** remained largely undissolved. To confirm this, the precipitate was collected and weighed and a percent yield was calculated as shown in Table 18.

Table 18. Measurements of the masses and calculated yield from the precipitate collected from Compound **1** stirred in a ½ ethanol (EtOH) and ½ water solution.

Reaction	Conditions	Mass (g)	Yield (%)
30	Compound **1** + ½ EtOH and ½ H_2O solution	0.184	92

Reactions 29 and 30 confirmed that dimedone was fully soluble in the mixed solvent while Compound **1** remained mostly undissolved. However, to further test the reliability of this possible purification method, the expected experimental equivalents of dimedone and Compound **1** were added to a large volume of half ethanol and half deionized water solution. Measurements of the dried precipitate and filtrate's masses and melting points were taken as shown in Table 19.

Table 19. Measurements of the masses, melting points, and calculated yields from the precipitate and filtrate collected from Compound **1** and excess 5,5-dimethyl-1,3-cyclohexanedione stirred in a ½ ethanol (EtOH) and ½ water solution.

Reaction	**Conditions**	**Product**	**Mass (g)**	**Yield (%)**	**Melting Point (°C)**
31	Dimedone and Compound **1** + ½ EtOH and ½ H_2O solution	Precipitate	0.447	88	166.6 - 170.5
		Filtrate	1.631	108	144.7 - 146.4

The melting point of the precipitate was aligned with the expected melting point for Compound **1** while the melting point of the filtrate was aligned with that of dimedone. This meant that the purification method was successful in separating the dimedone from Compound **1.** However, the yield of the precipitate showed that at least some of Compound **1** was being lost in solution. A reduced amount of solvent used was expected to cause the solution to become too saturated with dimedone for Compound **1** to be able to dissolve, which would increase the percent yield.

For Reaction 32, dimedone was added to the mixed solvent in batches over time. This revealed that about 0.9 grams of dimedone dissolved in every ten

milliliters of solvent. Following this, a purification test was done with the minimum amount of solvent and the precipitate and filtrate's masses and melting points were measured and recorded in Table 20. The higher precipitate yield suggested a better recovery of Compound 1, however, the lower melting point also showed that the purity decreased as a consequence of the increased yield. The results for both Reactions 31 and 32 suggested that using an amount of the mixed solvent at least slightly above the minimum would yield the best purification results.

Table 20. Measurements of the masses, melting points, and calculated yields from the precipitate and filtrate collected from Compound **1** and excess 5,5-dimethyl-1,3-cyclohexanedione stirred in a minimum amount of ½ ethanol (EtOH) and ½ water solvent.

Reaction	Conditions	Product	Mass (g)	Yield (%)	Melting Point (°C)
33	Dimedone and Compound **1** + ½ EtOH and ½ H_2O solution	Precipitate	0.511	101	139.5 - 149.5
		Filtrate	1.489	84	146.2 - 148.7

3.8: Purified Excess Dimedone Product

The purification process specified in the previous section was used to get more accurate yields of the excess dimedone reactions. The precipitates from these reactions were weighed and tested for their melting points before new percent yields were calculated, recorded in Table 21. The melting points aligned better with that expected of Compound **1**. However, the yield did not increase as much as was expected. This may have been because the increase in dimedone also

increased the likelihood of the released sodium bisulfite reacting with dimedone rather than the methyl 4-formylbenzoate.

Table 21. Measurements of the masses, melting points, and calculated yields of the purified precipitates from the addition of excess 5,5-dimethyl-1,3-cyclohexanedione.

Reaction	Conditions	Mass (g)	Yield (%)	Melting Point (°C)
34	3.3 eq. Dimedone + 0.9 eq. Piperidine	0.168	33	164.0 - 165.8
35	3.3 eq. Dimedone + 1.2 eq. Sodium Bicarbonate	0.142	28	164.0 - 165.8
36	10 eq. Dimedone + 1.2 eq. Sodium Bicarbonate	0.252	49	165.5 - 167.2

3.9: Slowed Dimedone Addition

Another possibility explored was that extending the period of time over which the dimedone was added could increase the yield of Compound **1**. This was based on the idea that if there was less dimedone available, compared to the amount of methyl 4-formylbenzoate in solution, then the sodium bisulfite would be more likely to react with the methyl 4-formylbenzoate and produce Compound **1**. As such, small batches of an excess overall amount dimedone were added over the course of approximately 30 minutes. Measurements of the precipitate's mass and melting point were taken as shown in Table 22.

Table 22. Measurements of the mass, melting point, and calculated yield of the precipitate from the slow addition of excess 5,5-dimethyl-1,3-cyclohexanedione.

Reaction	Conditions	Mass(g)	Yield (%)	Melting Point (°C)
37	Dimedone Batches	0.200	39	148.3 - 159.8

As with Reactions 24, 25, and 26, the melting point of the product was quite low compared to the expected melting point of Compound **1**. So, the product was purified and measurements of the precipitate's mass and melting point were taken and recorded in Table 23. The low yield showed that the slow addition of dimedone did not have the desired effect on the yield of Compound **1**. This might have been because there was more sodium bisulfite in solution than methyl 4-formylbenzoate. This would have made it more likely for the dimedone to react with the sodium bisulfite rather than the methyl 4-formylbenzoate.

Table 23. Measurements of the mass, melting point, and calculated yield of the purified precipitate from the slow addition of excess 5,5-dimethyl-1,3-cyclohexanedione.

Reaction	Conditions	Mass (g)	Yield (%)	Melting Point (°C)
38	Dimedone Batches + ½ ethanol and ½ H_2O solution	0.133	26	166.4 - 168.0

3.10: Reduced Sodium Bisulfite Addition

The sodium bisulfite seemed to be outcompeting the methyl 4-formylbenzoate. This led to a supposition that lowering the amount of sodium bisulfite added would decrease Compound **2** production and, as a result, increase Compound **1** production. This was especially probable because the sodium

bisulfite reversibly reacted with the methyl 4-formylbenzoate, which meant that one molecule of sodium bisulfite could react with multiple methyl 4-formylbenzoate molecules in a consecutive pattern.

Therefore, Reaction 39 was done with half the amount of sodium bisulfite. The precipitate's mass and melting point were measured as shown in Table 24. The yield and the purity of this experiment were both very high, however, the reduced amount of sodium bisulfite meant that not all of the methyl 4-formylbenzoate went into solution. With this in mind, it was determined that, despite increasing the yield of this experimental model compound, the reduction in sodium bisulfite would also reduce the likelihood of the thread compound forming inside of the macrocycle.

Table 24. Measurements of the mass, melting point, and calculated yield of the precipitate from the addition of half the normal amount of sodium bisulfite.

Reaction	Conditions	Mass (g)	Yield (%)	Melting Point (°C)
39	0.6 eq. Sodium Bisulfite	0.360	70	169.5 - 170.3

Chapter 4: Conclusion

This project's purpose was to provide a foundation for the creation of a more effective and efficient method for rotaxane synthesis using the thermodynamics of water. Cagulada et al. wrote that their protocol for Compound **1** synthesis in ethanol should result in a yield of 70%. This project shows that using water as a solvent instead of ethanol greatly increases Compound **1** production. This suggests that the thermodynamic favorability of hydrophobic interactions in water could also be used to increase the synthesis and stability of rotaxanes.

However, to use this thermodynamic favorability to its fullest potential, the typically water insoluble aldehyde containing center component of the thread should be made water soluble. As the literature suggests, sodium bisulfite is an effective solubilizing agent for aldehyde compounds. This study also shows that the distinction between sodium bisulfite reactive and unreactive ketones is still unclear. This lack of distinction makes sodium bisulfite an unreliable solubilizing agent for the purpose of rotaxane synthesis.

Given these results, future researchers should further explore the reaction between sodium bisulfite and dimedone as well as the resulting products. Further research should also be done on alternative methods for aldehyde compound solubilization. The use of amino acids in imine formation is an alternative worth testing.

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