INVESTIGATING THE SPONTANEOUS RESOLUTION OF

AN ABIOTIC METALLOFOLDAMER USING SOLID-STATE

CIRCULAR DICHROISM SPECTROSCOPY

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Honors Bachelor of Science in Chemistry with Distinction.

Spring 2009

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ACKNOWLEDGMENTS

I would like to acknowledge the support and guidance of Dr. Joseph Fox, the generosity of Dr. Joel Schneider, the assistance of James Plampin, and the funding I have received from the Howard Hughes Medical Institute and the Donald W. Harward Fellows Award.

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ABSTRACT

Crystals of an abiotic metallofoldamer were used to investigate the viability of solid-state circular dichroism spectroscopy (solid-state CD) as an alternative to X-ray diffraction studies in spontaneous resolution analysis. The metallofoldamer is devoid of chiral centers and the molecules assemble into secondary structures with helical chirality. Although the metallofoldamer complexes racemize in solution, they produce a single enantiomerically pure crystal when subjected to vapor diffusion in a crystallization experiment. CD spectra of microcrystalline samples were obtained by scanning pellets of each crushed crystal mixed with KBr. The reliability, efficiency, and effectiveness of solid-state CD in spontaneous resolution analysis were established. Thus, it was demonstrated that an enantiomerically pure helical molecule can be produced from a dynamic macromolecule without any element of central chirality. A hypothesis for the evolutionary origin of chirality is discussed. Interestingly, all seven crystals characterized thus far have been left-handed. The probability of such an occurrence is 1 in 64. It is plausible that a chiral contaminant serves as a nucleation site for crystallization.

Chapter 1

INTRODUCTION

1.1 Introduction to Metallofoldamers (A Background)

The central aim of this project was to determine the absolute helicity of an abiotic metallofoldamer that spontaneously resolves upon crystallization. An abiotic metallofoldamer is a specific kind of foldamer. Foldamers are "unnatural molecules that fold into compact secondary structures. [They] have been broadly classified as mimics of peptides and oligonucleotides and the abiotic analogues of each.[1]"

As discussed by Moore and coworkers, there are two kinds of abiotic peptide analogs.[2-5] The first comprises "molecules in which the metal serves as a template for a helical secondary structure.[1]" In such molecules, "the metal coordination sphere is inherently helical.[1]" The second group consists of abiotic metallofoldamers. In abiotic metallofoldamers, "metal coordination nucleates the formation of a nonbiological single-stranded helix.[1] Unlike the metal coordination sphere in an abiotic peptide analogue with a templated secondary structure, the metal coordination sphere in an abiotic metallofoldamer is not intrinsically helical, but rather enables "a series of cooperative, noncovalent interactions that ultimately result in a folded structure.[1]" The two kinds of abiotic peptide analogues are depicted in Figure 1.

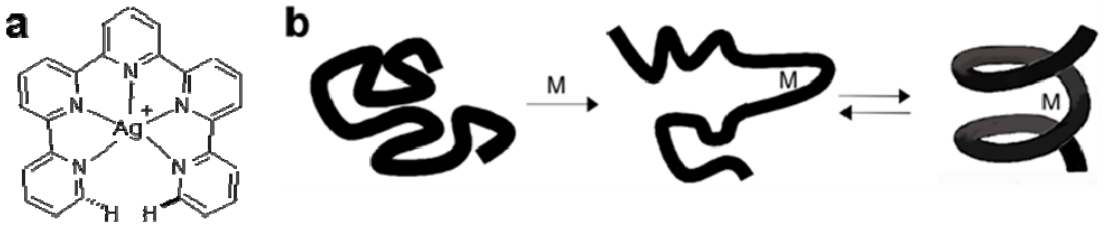

Figure 1 **Juxtaposition of a) templated abiotic analogues of peptides and b) abiotic analogues of peptides with nucleated secondary structures (reprinted[1])**

Members of the Fox Research Group study abiotic metallofoldamers modeled off of salen and salophen ligands. Salen and salophen form complexes with metal ions, as shown in Figure 2.

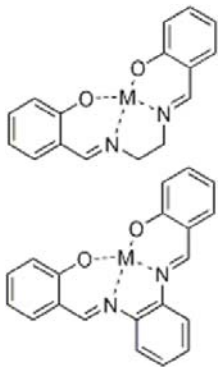

Figure 2 **Structures of metal complexes of salen (top) and salophen (bottom)**

Identical substituents on the benzene rings of both salicylidene pieces create symmetrical arms. When the metallofoldamer ligand forms a complex with a metal ion, the arms are oriented so as to create a single-stranded helix. If the left arm is on top of the right arm, a left-handed helix is formed, and vice versa.

Of note, metallofoldamers derived from chiral salens and salophens have potential applications in organic synthesis. Metallofoldamers based off of chiral salen serve as catalysts in a wide variety of asymmetric reactions.

1.2 Previous Work with Metallofoldamers in the Fox Group

The first metallofoldamer project conducted in the Fox Group involved the synthesis of salen- and salophen-based complexes containing Ni(II) and Cu(II). X-ray diffraction studies and chemical shift anisotropies measured by NMR spectroscopy provided evidence that the free ligands did not fold into distinct structures, and only the complexes were helical. The principal salophen-based metallofoldamers investigated, shown in Figure 3, exhibited surprising crystallization behavior. Although they produced racemic solutions, 2a and 2b formed single, enantiomerically pure crystals upon vapor diffusion in each crystallization experiment. Crystals of 2a were grown from $CHCl_3/CH_3CN$ and those of 2b were grown from hexane/$CHCl_3$.

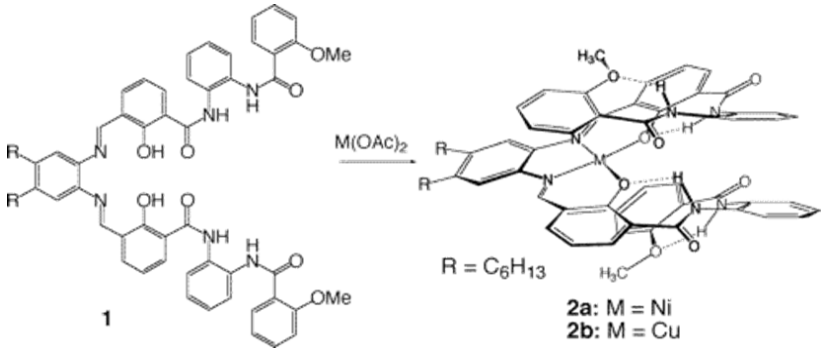

Figure 3 Structures of the free ligand and Ni(II)- and Cu(II)-containing complexes of the main salophen-based metallofoldamers studied (reprinted[1])

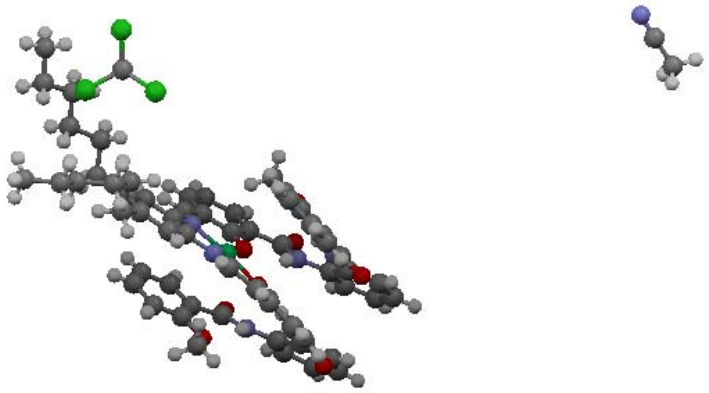

Figure 4 **X-ray crystal structure of 2a with solvent molecules (reprinted[1])**

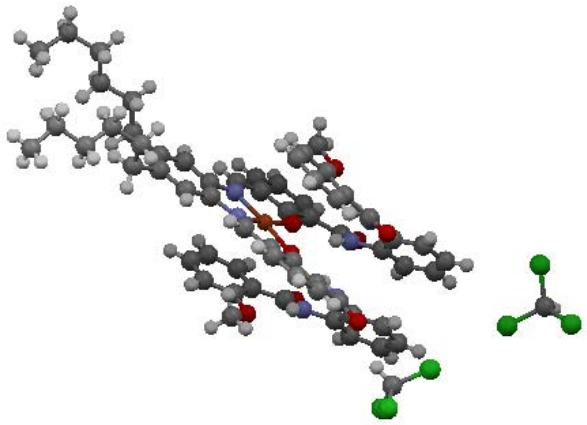

Figure 5 **X-ray structure of 2b with solvent molecules (reprinted[1])**

Building upon the findings of this project, the effect of peripheral stereocenters on the helicity of salophen-derived metallofoldamers was studied in the following manner. The structure of a salophen-based metallofoldamer with chiral end-groups, pictured in Figure 6, was determined by X-ray diffraction studies. It was expected that the complex would be right-handed, since this would allow the methyl substituents on the peripheral benzofuran rings to point away from the helix. Instead, the crystalline compound was left-handed because of "~180° rotation[s] of the outermost arene-nitrogen bonds.[6]" This rotation caused the peripheral carbonyls to point towards the inside of the helix, unlike in the free ligand, and directed the methyl groups away from the helix. In solution, both the anticipated right-handed form and the left-handed form of the complex were in equilibrium, with a slight excess of the left-handed form. Variable temperature ^{1}H NMR studies demonstrated that the barrier to interconversion for the (M)- and (P)-helical forms of this foldamer was ~13 kcal/mol. Therefore, interconversion was rapid at room temperature. It is assumed that the barrier for racemization of 2a is similar to that of this complex.

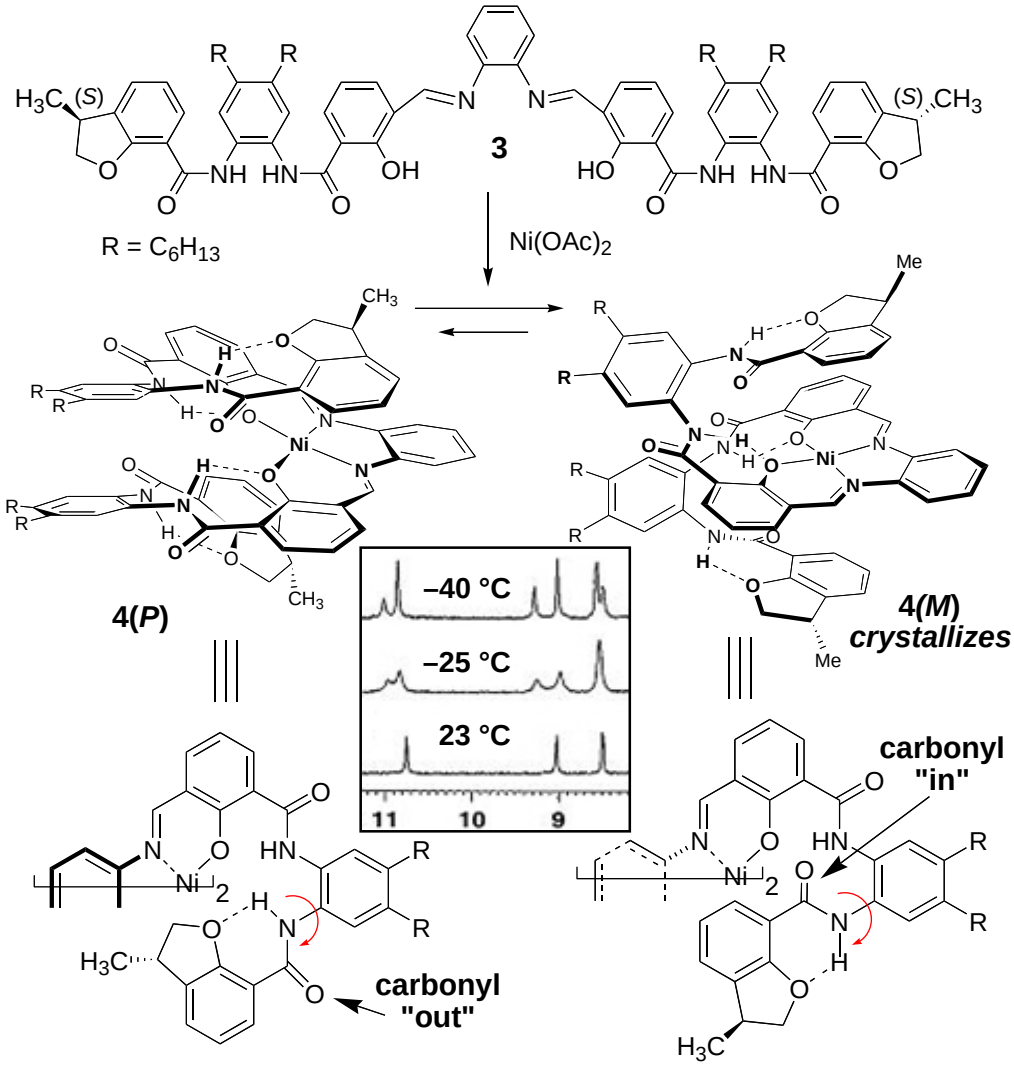

Figure 6 **Conformational behavior of the salophen-based metallofoldamer with chiral methyl groups (reprinted[6])**

Using this information, the metallofoldamer shown in Figure 7 was designed.

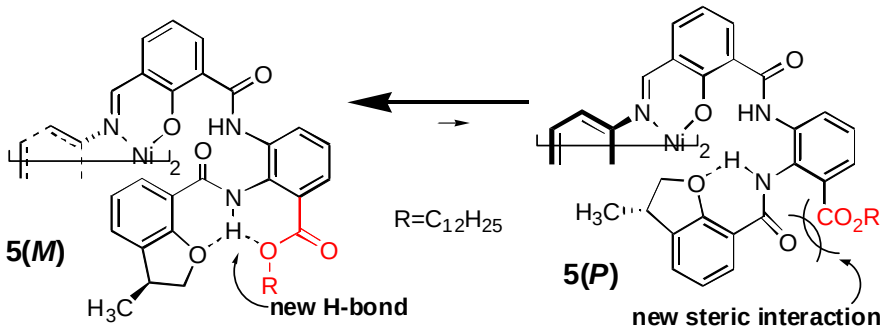

Figure 7 Metallofoldamer designed to strongly favor a left-handed helix (reprinted[6])

The metallofoldamer in Figure 7 was similar to that in Figure 6 except for the presence of ester functions, which created a bias for left-handed complexes. The ester groups caused the formation of stabilizing three-center hydrogen bonds in the left-handed helix and unfavorable steric interference with the amide carbonyls in the right-handed helix. Both in solution and in the solid state, this foldamer was predominantly (M)-helical. Thus, this project showed that peripheral stereocenters can successfully direct the absolute helicity of salophen-derived metallofoldamers.

1.3 Goals and Hypotheses of my Project

Intrigued by the unique crystallization behavior of 2a, I sought to develop a spectroscopic technique to measure whether 2a exhibits an enantiomeric preference upon crystallization. Studies herein have been carried out with material synthesized by Fan Zhang, a graduate student who previously worked on this project. However, I am synthesizing more for further studies. In fact, at this point in time I have made and purified the metallofoldamer on a small scale.

As this is the sole metallofoldamer upon which my project focuses, it is pertinent to describe its behavior in further detail. The complex's single-stranded helical shape results from six-membered-ring hydrogen bonds between amide and phenolic regions which form upon metal complexation. The helix is further reinforced by π-stacking interactions between every fourth aromatic ring, as evidenced in Figure 8.

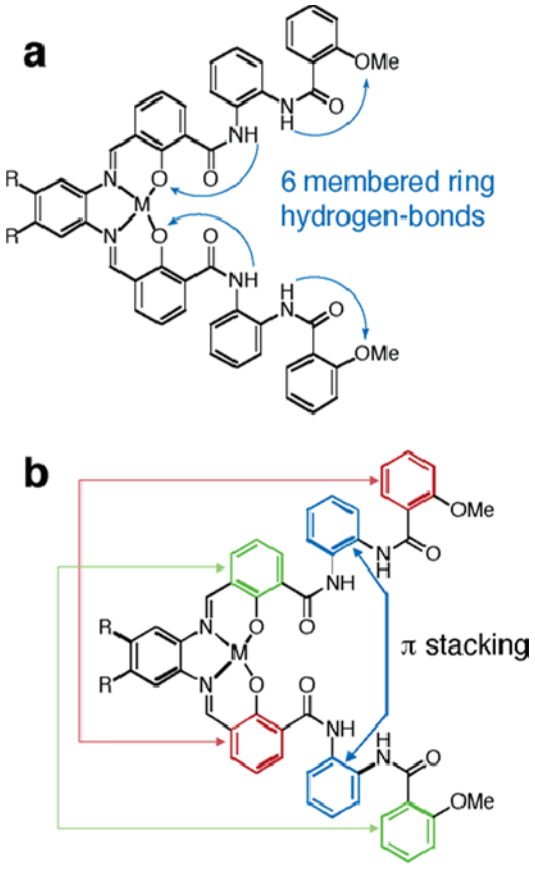

Figure 8 Depiction of six-membered-ring hydrogen bonds and π-stacking (reprinted[1])

In solution, the metallofoldamer constantly folds and unfolds, repeatedly changing from one enantiomer to the other. Equilibrium is rapidly established between the

left- and right-handed helical forms, yielding a racemic solution. However, as mentioned previously, when vapor diffusion occurs in a crystallization experiment, a single enantiomerically pure crystal is produced. Such behavior is very unusual because the ~5-10% of racemates which are able to crystallize normally form many enantiopure crystals per crystallization trial.[7] For 2a, it is hypothesized that nucleation of crystal growth is the rate-determining step. Once crystallization nucleates, the growth of that crystal is relatively fast. Since only one crystal forms, only one enantiomer is produced. In this manner, all of the material funnels into a single helical enantiomer. The absolute helicity for the one crystal that forms should be determined by chance.

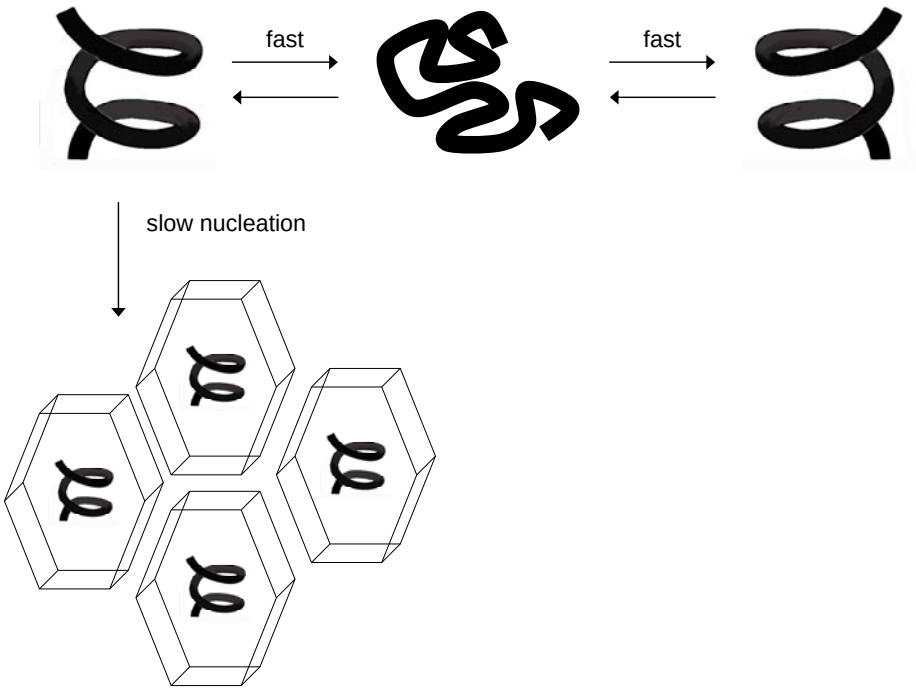

Figure 9 Illustration of crystallization behavior

Crystals of 2a were previously grown by Fan Zhang. Four of them were characterized by X-ray diffraction studies which revealed that all four were left-handed. I sought to utilize solid-state circular dichroism spectroscopy (solid-state CD) to characterize both the remaining crystals and those which I would grow after synthesizing more of the metallofoldamer. Therefore, in addition to gaining a better understanding of an as-yet-unstudied aspect of metallofoldamer behavior, I would also be able to test out the viability of solid-state CD in the context of spontaneous resolution analysis. If solid-state CD were determined to be an effective alternative to X-ray diffraction studies, it would provide a useful spectroscopic verification of the crystallographic result.

Chapter 2

EXPERIMENTAL METHODS

2.1 Synthesis Scheme

The metallofoldamer was made according to a multi-step synthesis scheme, following the original procedure of Fan Zhang. As before, the ligand was constructed from three key components which were combined at different points in time during the synthesis. These three molecules were benzofuran-7-carboxylic acid, N-(2-anisoyl)-1,2-phenylenediamine, and 1,2-diamino-4,5-dihexylbenzene, as shown in Figure 10.

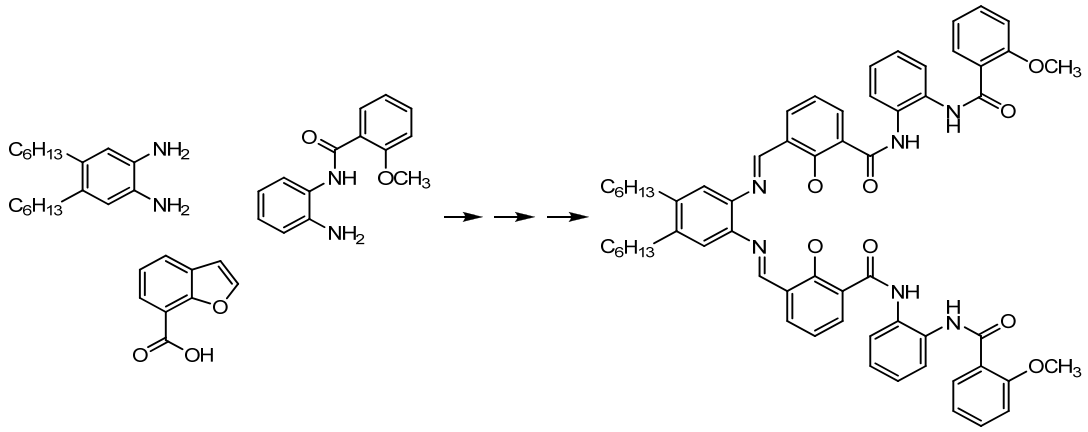

Figure 10 Depiction of three main pieces which compose 2a

A step-by-step description of the procedure followed and optimal achievable percent yields can be found by referring to the Supporting Information for the publication

Abiotic Metallofoldamers as Electrochemically Responsive Materials.[1] The few, minor deviations that were introduced for ease of synthesis are shown below.

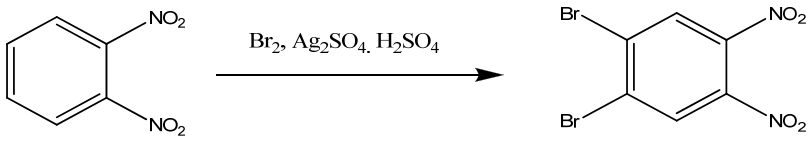

Figure 11 Bromination reaction

For the reaction depicted in Figure 11, 2.5 grams (14.88 mmol) of 1,2-dinitrobenzene, 2.30 mL (44.64 mmol) of Br_2, and 9.29 grams (29.76 mmol) of Ag_2SO_4 were transferred to a 100 mL round-bottomed flask at 0°C (ice bath). Next, 25 mL of H_2SO_4 was added so as to achieve a 0.6 M solution of 1,2-dinitrobenzene in H_2SO_4. Tygon tubing was used to provide a means for the HBr side product to escape into the back of the hood. The reaction mixture was heated from 0°C - 155°C over 30 minutes and then kept at 155°C for 10 minutes. Afterwards, it was cooled to room temperature, poured over a beaker of ice, extracted with CH_2Cl_2 (20 mL • 3), dried over Na_2SO_4, vacuum filtered with CH_2Cl_2, concentrated, and chromatographed (0-6% EtOAc in hexanes over 120 minutes using ISCO).

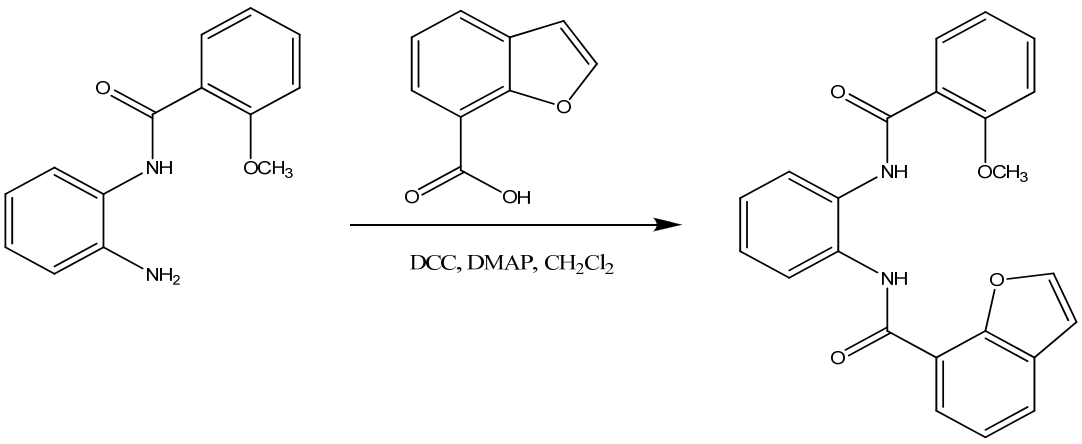

Figure 12 Formation of N-{2-[(2-methoxybenzoyl)amino)phenyl}-1-benzofuran-7-carboxamide

For the reaction shown in Figure 12, 0.3950 grams (2.19 mmol) of benzofuran-7-carboxylic acid, 0.6128 grams (2.53 mmol) of N-(2-anisoyl)-1,2-phenylenediamine, 1.805 grams (8.76 mmol) of DCC, and 0.534 grams (4.38 mmol) of DMAP were combined in a 25 mL round-bottomed flask. A 7.3 mL aliquot of methylene chloride was added so as to achieve a 0.3 M solution of benzofuran-7-carboxylic acid in CH_2Cl_2. The reaction mixture was allowed to reflux at 60°C overnight under N_2. Afterwards, the reaction mixture was cooled, concentrated, and chromatographed (30% EtOAc in hexanes).

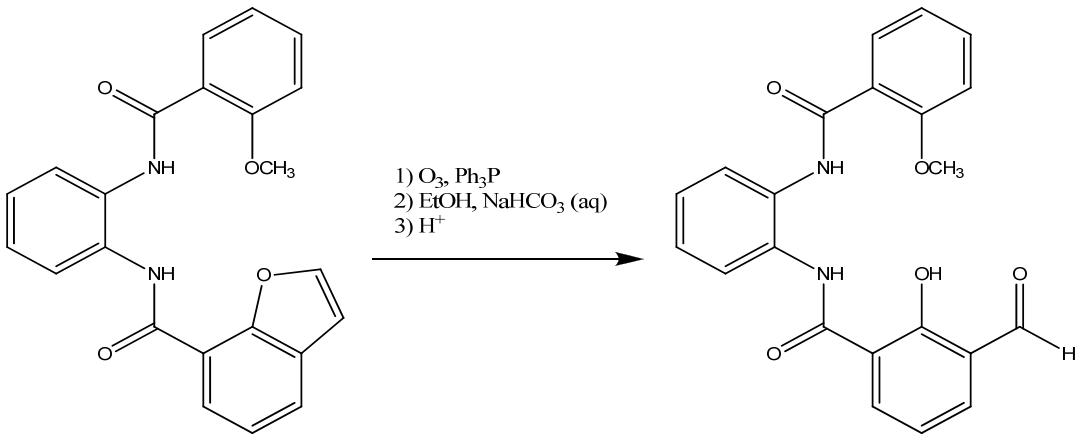

Figure 13 **Formation of N-(2-anisoyl)-N'-(3-formyl-2-hydroxybenzoyl)-1,2-phenylenediamine**

A 100 mL round-bottomed flask containing 0.8326 grams (2.15 mmol) of N-{2-[(2-methoxybenzoyl)amino)phenyl}-1-benzofuran-7-carboxamide in 43 mL of methylene chloride, to achieve a 0.05 M solution, was sparged with N_2 and cooled to -78°C, using a dry-ice/acetone bath. Ozone was bubbled into the reaction for ~15 minutes (the reaction mixture turned blue). Next, N_2 was bubbled into the system until a yellow hue was observed. 2.2532 grams (8.6 mmol) of triphenylphosphine were added and the solution was allowed to reach room temperature and stir for 1 hour. After concentration, 21 mL of EtOH and 36 mL of 1% $NaHCO_3$ (aq) were added. The contents were allowed to reflux (at 100°C) for 2 hours and then cool to room temperature. 10% HCl (aq) was used to change the pH of the reaction mixture to 2. After this, the material was extracted with CH_2Cl_2 (20 mL • 3), dried over Na_2SO_4, vacuum filtered with CH_2Cl_2, concentrated, and chromatographed (0-20% EtOAc in hexanes by 5% increments of 100 mL each).

As mentioned previously, at this point in time, I have synthesized and purified the metallofoldamer on a small scale.

2.2 Crystallization Procedure

Upon learning that the six crystals grown but not characterized by Fan Zhang had become desolvated, I sought to regrow them. I made a ~0.012 M solution of the metallofoldamer by pooling the six crystals and dissolving this material in approximately 10 mL of $CHCl_3$. Then, I filtered this solution through a piece of Kimwipe that was wedged into a Pasteur pipette. Roughly, 1 mL aliquots of this solution were added to 10 Pasteur pipettes whose bottom ends had been sealed using a Bunsen burner. These bottom-sealed pipettes were carefully placed into capable 20 mL test tubes to which about 8 mL CH_3CN had been added. It was necessary that the level of CH_3CN be well below the top of the bottom-sealed pipette so that it would not spill into the metallofoldamer mixture. The test tubes were capped, placed into a test tube holder, and left undisturbed on a cabinet shelf. The crystallization experiment setup used is illustrated in Figure 14.

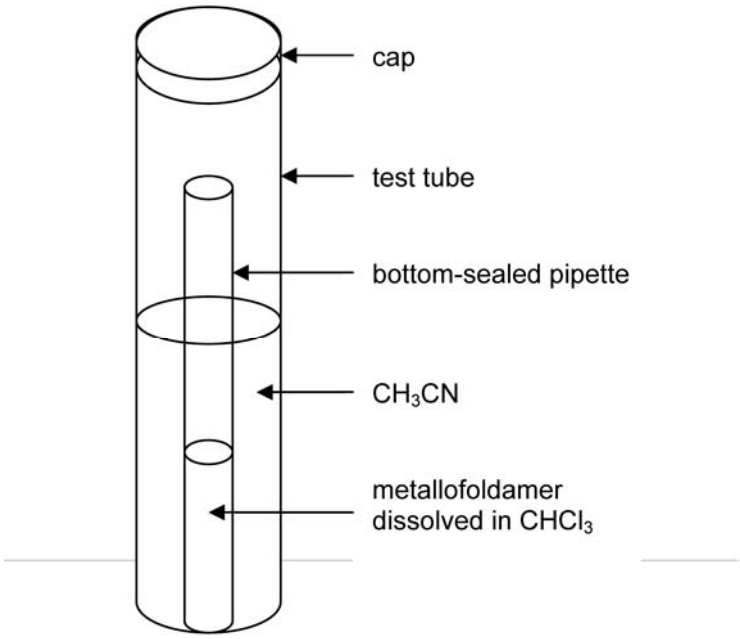

Figure 14 Illustration of crystallization setup

Vapor diffusion resulted in crystal growth because, over time, $CHCl_3$ vapor diffused out of the bottom-sealed pipettes and CH_3CN vapor diffused into them. Thus, the percent of CH_3CN in the metallofoldamer solution gradually increased. Since the metallofoldamer is poorly soluble in CH_3CN, this caused crystals of the complex to form.

2.3 Collection of Solid-state CD Data

Once crystals were grown, they were characterized by solid-state circular dichroism (or CD) spectroscopy. In CD spectroscopy, circularly polarized light is shone through an optically active, or chiral, sample. The difference between an enantiomer's electronic absorption spectra for left- and right-handed circularly polarized light is the circular dichroism, $\Delta\varepsilon$, with units of L/(mol*cm). The CD spectra of enantiomers differ only in sign for salophen-based metallofoldamers. A negative CD spectrum corresponds to a left-

handed helix and a positive reading indicates the presence of a right-handed helix. Thus, it is possible to determine the handedness of molecules by chiroptical means.

There are two techniques by which one can obtain CD spectra: solution and solid-state CD. Solution CD measures the Δε of a solution containing an optically active compound, while solid-state CD measures the Δε of a crystallized compound. Solution CD is a very popular technique, in part because a reliable, standard procedure for data collection has been developed. In contrast, solid-state CD is rarely used "because of the experimental difficulties and theoretical complexities" encountered.[8] The two types of solid-state CD - single-crystal CD of uniaxial crystals and "microcrystalline CD of. . . crystal system[s] in either KBr disk or nujol mull form[8]" - both have drawbacks. The former often suffers from birefringence, hindering meaningful data collection. Additionally, the latter, in which "microcrystals are dispersed randomly either in nujol or in a KBr microcrystalline matrix,[8]" can experience significant light beam depolarization "due to reflection and refraction at the grain boundaries.[8]" Although one can attempt to make homogenous and sufficiently translucent samples, it is extremely difficult to avoid depolarization.

Although solution CD is much easier to deal with, it was necessary to use solid-state CD to determine whether 2a was enantiomerically enriched, since the complex racemizes in solution. Specifically, microcrystalline CD of samples in KBr matrices were chosen upon consideration of the type of crystal system formed. Since solid-state CD is very rarely used for spontaneous resolution analysis, there is no standard way to collect data. I had to develop my own method and provide evidence that it would yield trustworthy and consistent data.

After much experimentation, I developed the following microcrystalline technique. First, Glenn Yap, the X-ray crystallographer, examined the quality of each crystal.

He used thin glass fibers whose ends were dipped into viscous oil to remove the crystals from the walls of the test tubes, as this was the most efficient and appropriate way to extract crystals without running the risk of damaging them. Using a microscope, he double-checked whether the crystals were properly formed. If a crystal contained a second crystal inside of it, it was deemed unusable. Additionally, in the rare occurrence of two crystals that were joined at one face, he carefully cut them apart so that they could be scanned individually.

After this was accomplished, the crystal was weighed and crushed with a mortar and pestle. Small increments of a sample of KBr (weighed so as to achieve a mass ratio of ~1.6 mg metallofoldamer per g KBr, for 2a) were slowly added to the metallofoldamer and crushed as well. Roughly 200 mg of the mixture was transferred to a KBr pellet press, shown in Figure 15. The sample container's vacuum adapter was hooked up to a vacuum pump to help form the pellet in addition to aiding in the removal of any moisture the KBr in the pellet might have picked up and preventing any further water absorbance during compression.

Next, I subjected the KBr pellet press to a pressure of roughly 17,000 psi for approximately 7 minutes, using a hydraulic press. Then, I carefully removed the pellet and measured its width and diameter with calipers. Subsequently, I loaded the pellet into a special clip, shown in Figure 15, which could easily be placed into the sample slot in the JASCO J-810 spectropolarimeter, which was used to collect the CD spectra. In order to obtain a smoother, more well-defined signal, I averaged about 10 scans of each pellet. Some of the equipment used is pictured in Figure 15.

Figure 15 The KBr pellet press, pellet clip, and mortar and pestle used to make pellets

After some testing, I found the optimum pellet concentration to be about $3*10^{-3}$ M (using the original concentration determination technique, discussed below), which corresponded to a mass ratio of roughly 1.6 milligrams of crystal to one gram of KBr for 2a, as mentioned previously. Around this concentration, I observed that samples were sufficiently concentrated to yield unambiguous spectra but not too concentrated so as to create insufficiently translucent pellets, causing poor readings. As a rule of thumb, readings are considered to be unreliable when the HT (high tension) voltage value, a measure of disorder in the system that relates to the amount of light scattering, exceeds 800. Pellets which contain opaque regions can be associated with high HT values because light that cannot easily pass through the pellet is scattered within the instrument.

The raw readout of the J-810 spectropolarimeter is observed ellipticity angle in millidegrees as a function of wavelength. I converted the y-axes of my spectra to the more useful value of molar circular dichroism, $\Delta\varepsilon$, using the following equation (where θ is the observed ellipticity angle in millidegrees, c is the molar concentration of the solute, and l is the cell length (in this case pellet thickness) in centimeters).

$$\Delta\varepsilon = \frac{\theta}{33{,}000 * c * l} \qquad 1$$

Originally, I obtained the concentrations of the pellets in the following manner: from the masses of the crystals, I calculated the moles of metallofoldamer and from the diameters and thicknesses of the pellets I calculated the pellet volumes. I divided the moles of metallofoldamer by the volumes to get the concentrations of the pellets.

Later, it was suggested that I determine pellet concentrations from UV-Vis absorbance spectra, in hopes of obtaining more accurate values. Using Fan Zhang's UV-Vis spectrum of 2a at a known concentration[1], I calculated the extinction coefficient at 382 nm to be 34,732 $M^{-1}cm^{-1}$. To determine the concentrations of the pellets, I substituted the extinction coefficient into the following equation, a rearrangement of the Beer-Lambert law (where A_{382} is the UV-Vis absorbance at 382 nm and b is the pellet thickness in centimeters) .

$$c = \frac{A_{382}}{34{,}732 M^{-1} cm^{-1} * b} \qquad 2$$

Finally, the concentrations thus obtained were substituted into Equation 1 to calculate the circular dichroism values. Interestingly, the original method and the UV-Vis technique yielded substantially different concentration values. A likely explanation for this discrepancy is discussed in Section 3.2.

Chapter 3

RESULTS

3.1 Establishing the Reliability of Solid-state CD

As mentioned previously, before characterizing crystals of 2a, it was necessary to establish the reliability of the solid-state CD technique. To accomplish this, I compared the solution and solid-state CD spectra of a nonracemizing metallofoldamer, M3, shown in Figure 16.

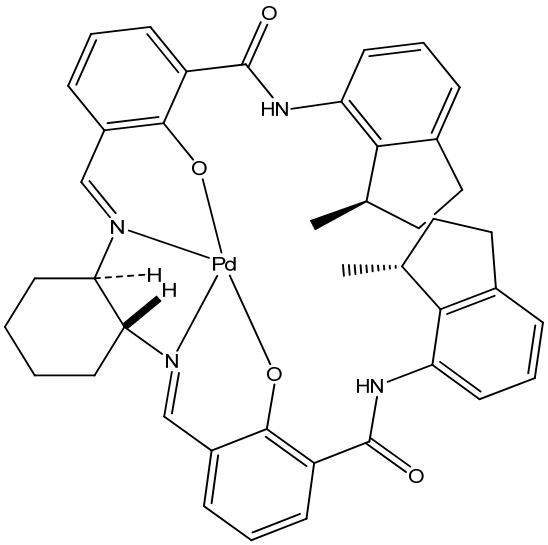

Figure 16 Structure of salen-based palladium metallofoldamer, M3

Both the chiral diamine and chiral end-groups bias for the left-handed helix, and the chiroptical data[9] were consistent with a predominantly (M)-helical structure in solution. Therefore, it was reasoned that M3 could serve as a benchmark for solid-state studies on 2a.

The spectra for the solution and solid-state samples are presented in Figures 17 and 18. Of note, the solid-state pellet was made from a 200 mg portion of a mixture of 0.56 mg metallofoldamer in 561.25 mg KBr.

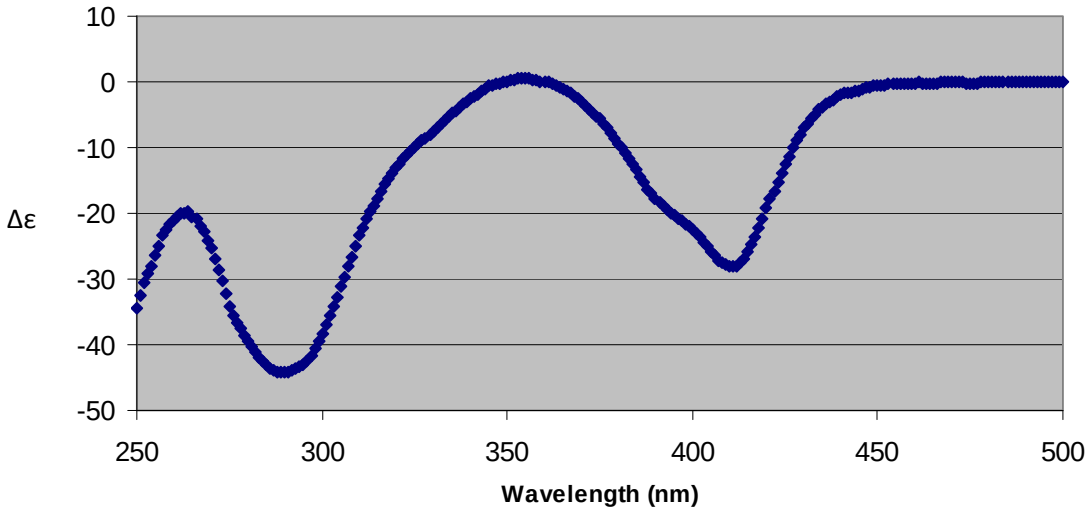

Figure 17 Solution CD spectrum of M3 at a concentration of $9.22*10^{-4}$ M

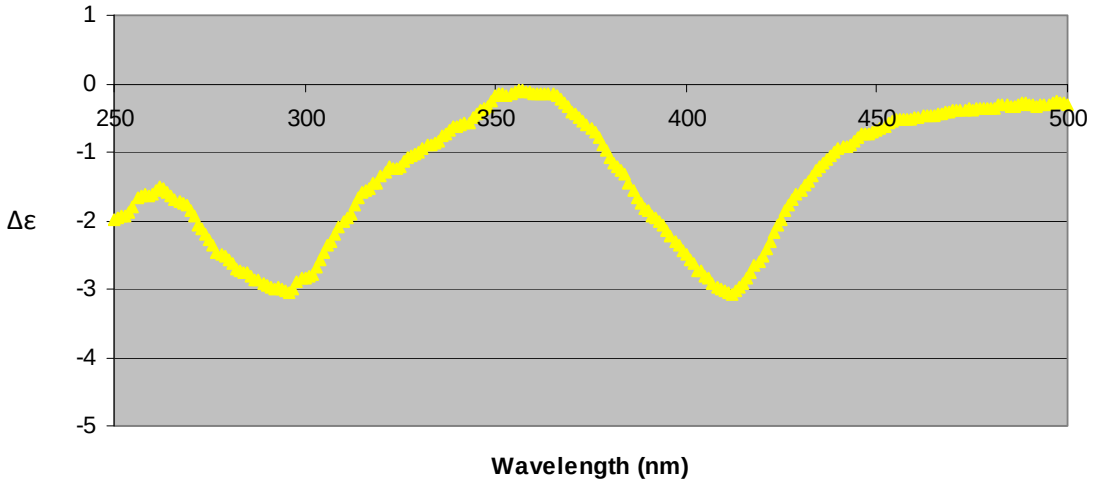

Figure 18 **Solid-state CD spectrum of M3 at a concentration of $3.35*10^{-3}$ M (this concentration value was determined by the original technique discussed in Section 2.3)**

As evidenced in Figures 17 and 18, the solution and solid-state spectra were in favorable agreement. The negative dichroisms were at essentially the same wavelengths and the shapes of the spectra were very similar. The intensity of the solid-state CD spectrum was less than that of the solution spectrum by an order of magnitude. This was presumably due to the phenomenon of absorption flattening. Absorption flattening is a reduction in spectrum intensity of a solid-state CD reading due to light scattering caused by pellet opaqueness and inhomogeneity. Thus, this spectrum comparison exercise showed that I could depend upon my solid-state CD technique, but I should anticipate the occurrence of significant absorption flattening.

3.2 Solid-state CD Spectra of 2a

Once I established the reliability of my solid-state CD technique, I sought to characterize the remaining six crystals which had previously been grown but not structurally

determined. However, as mentioned earlier, I discovered that the crystals had become desolvated and had to regrow them. Desolvation was problematic because the $CHCl_3$ and CH_3CN solvent molecules stabilize the crystalline structures of the metallofoldamer complexes.

Crystallization by vapor diffusion is a very slow process. So far, three crystals have formed. Table 1 specifies the masses of crystal and KBr in the mixtures from which the pellets were made. For the first pellet, ~200 mg of mixture was used. For the other two pellets, ~150 mg of mixture were used.

Table 1 Masses of crystal and KBr composing the mixtures from which the pellets of 2a were made

Identity	Mass of crystal (mg)	Mass of KBr (mg)
1st crystal of 2a	1.01	1276
2nd crystal of 2a	0.88	475.58
3rd crystal of 2a	0.39	243

The spectra of the three crystals, using concentration values determined by the original technique discussed in Section 2.3, are displayed in Figures 19-21.

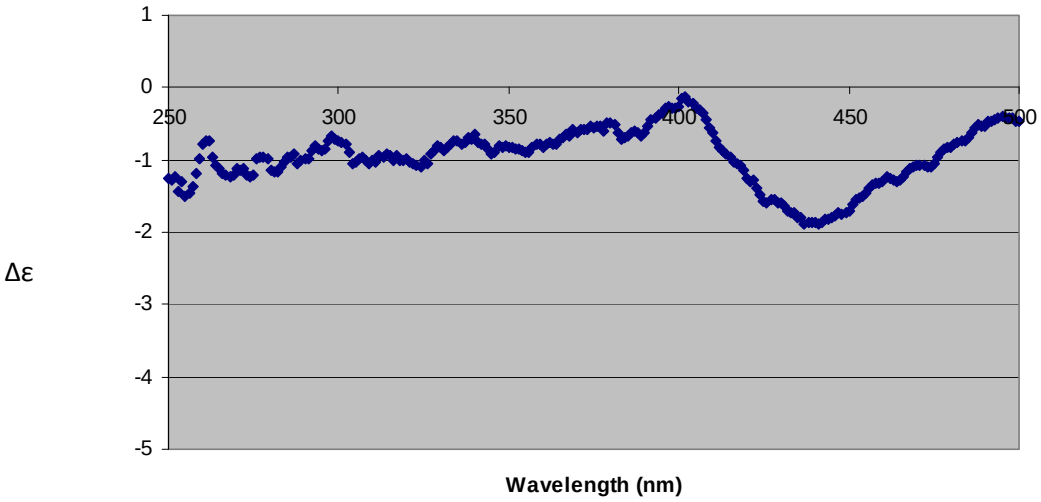

Figure 19 **Solid-state CD spectrum corresponding to the first crystal, at a pellet concentration of $1.90*10^{-3}$ M**

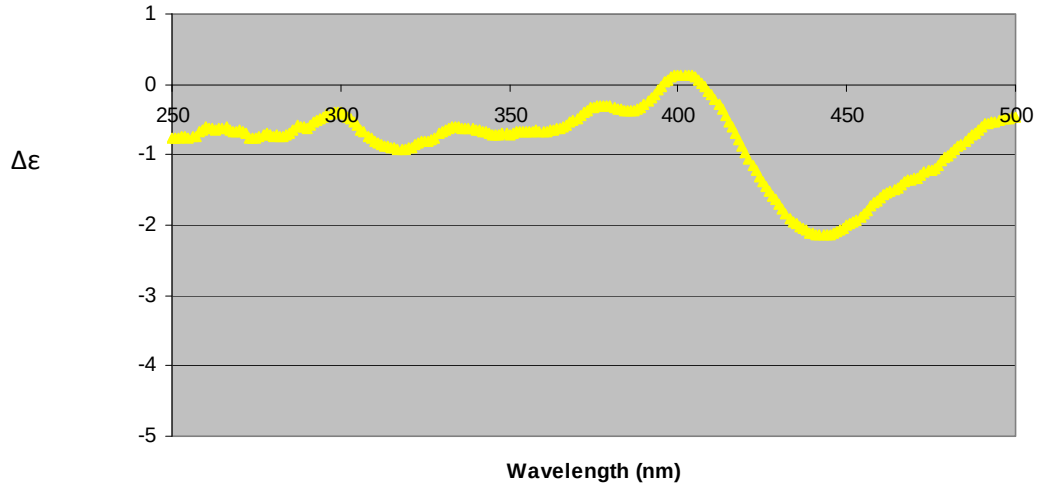

Figure 20 **Solid-state CD spectrum corresponding to the second crystal, at a pellet concentration of $3.83*10^{-3}$ M**

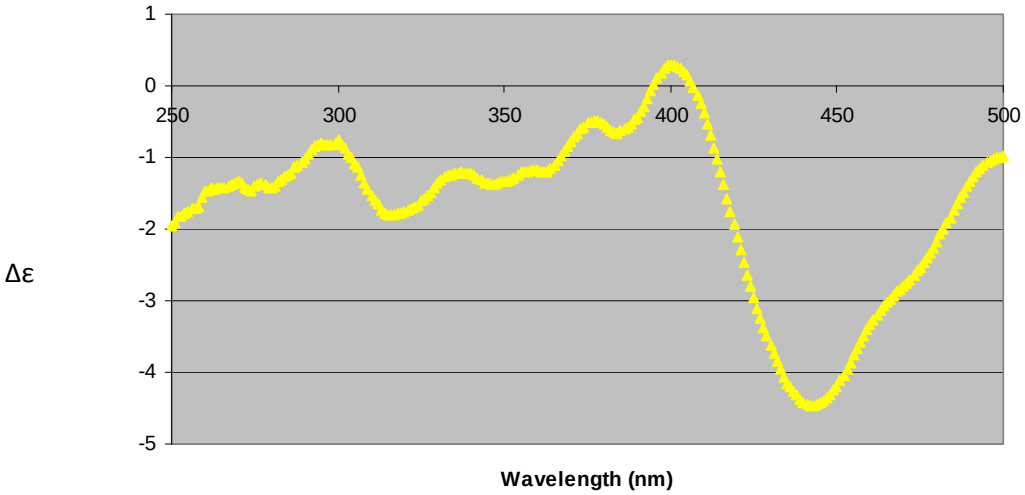

Figure 21 **Solid-state CD spectrum corresponding to the third crystal, at a pellet concentration of $3.85*10^{-3}$ M**

The UV-Vis and CD spectra for the first and second pellets, using the alternative technique for concentration determination discussed in Section 2.3, are displayed in Figures 22-25. As mentioned previously, utilizable spectra were only obtained for the first two pellets.

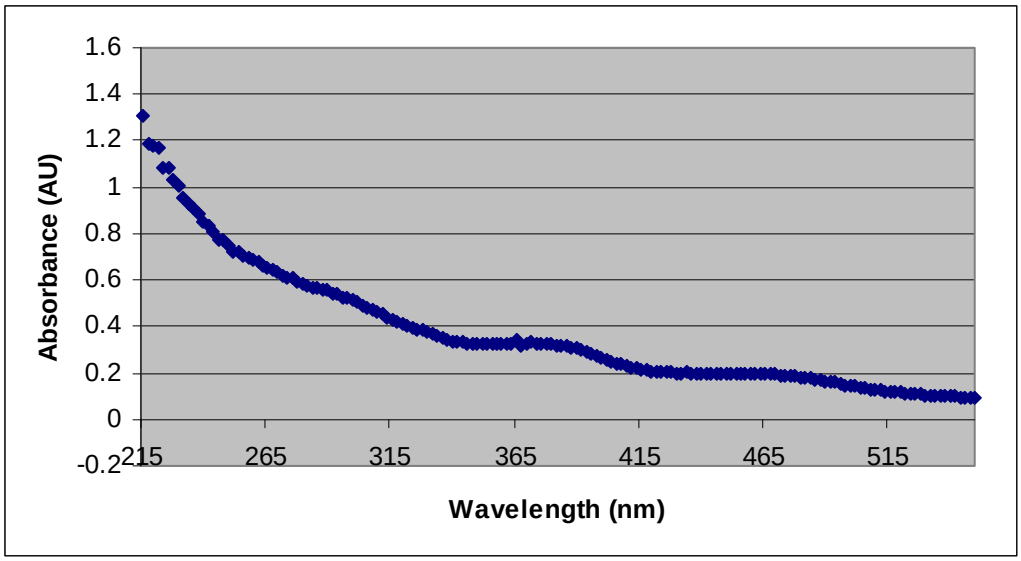

Figure 22 **UV-Vis absorbance spectrum of the first pellet**

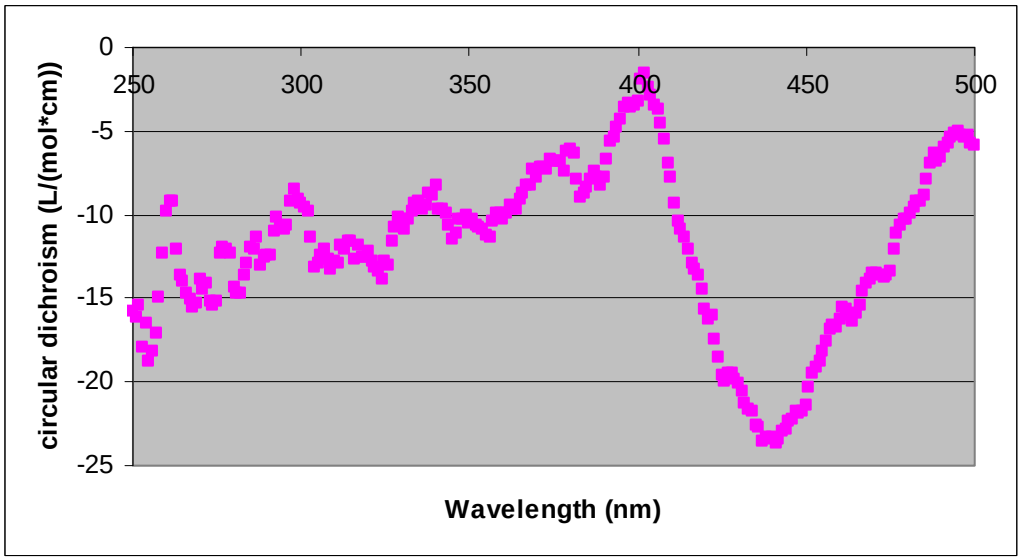

Figure 23 **Solid-state CD spectrum of first pellet, using the UV-Vis technique for concentration determination**

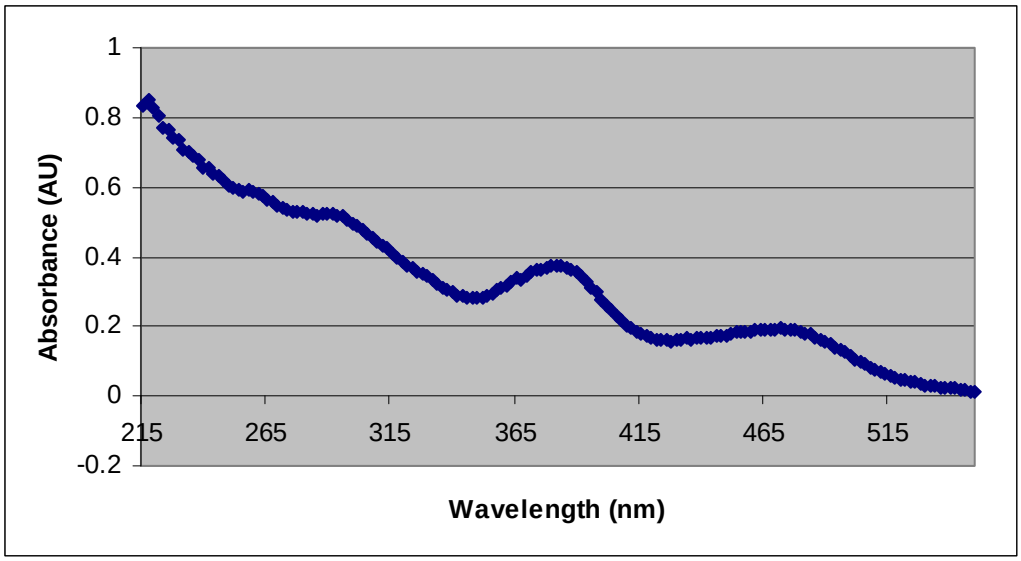

Figure 24 **UV-Vis absorbance spectrum of second pellet**

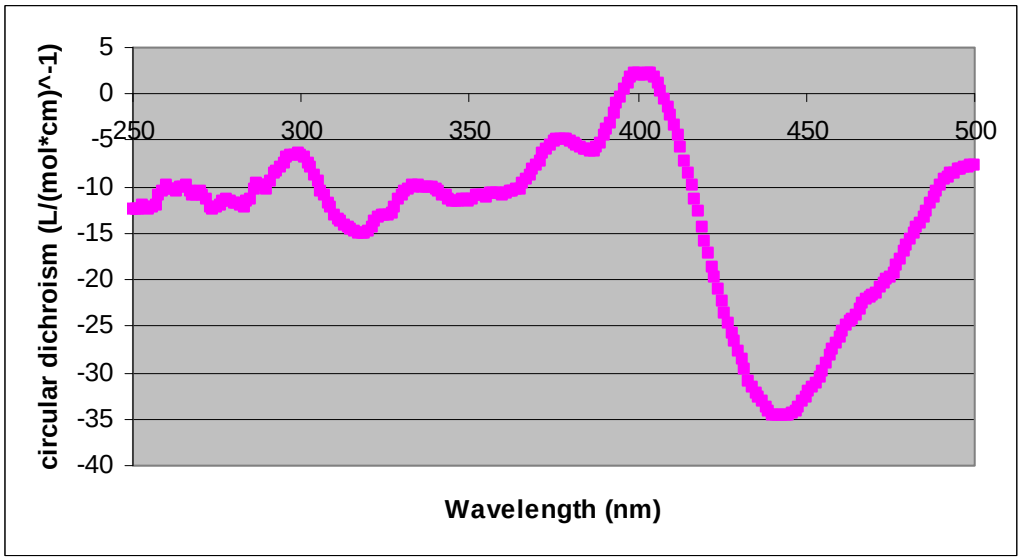

Figure 25 **Solid-state CD spectrum of second pellet, using the UV-Vis technique for concentration determination**

For Figures 19-21, the intensities of these three CD spectra were similar to that of the solid-state CD spectrum for M3. Since the signals in the spectra were negative, all three crystals were left-handed. Although, the signal intensities of the first and second pellets were very close, the intensity of the third pellet was roughly twice as great. Also, the intensity of the spectrum in Figure 25 is roughly 1.5 times as great as that in Figure 23. Since $\Delta\varepsilon$ is uniquely dependent upon the identity of the sample, one would anticipate that the intensities of all spectra should be approximately the same.

The readings between pellets using the same method are probably off because of varying amounts of residual viscous oil on the crystals. As mentioned previously, viscous oil was used to remove the crystals from the crystallization experiment setup. Although weighing paper was employed to gently scrape off the oil, this technique was unable to remove all the oil or even leave the same amount of oil on each crystal. Thus, it is very likely that the masses of oil left on the crystals differed for each crystal. Since the crystal weights were so small (refer to Table 1), this would have had a significant effect on the mass values. Thus, skewed crystal masses would have been entered into the denominator of Equation 1, yielding circular dichroism values which differed slightly for the three pellets.

Additionally, results could have been affected by significant light scattering due to cracks and cloudiness in the pellet and/or partial racemization because of humidity. It is essentially impossible to create a perfectly homogeneous pellet. Some pellets have more cracks and cloudiness than others. Such pellets would yield poor-quality data of lower intensity due to hindrance of the light passing through the sample and light scattering. If the first two pellets were of poor quality, the above scenario would furnish a reasonable explanation for the lower signal intensity.

Furthermore, it is possible that differences in humidity between days could have caused slight differences in the signal intensities of the CD spectra. Although the handedness of the majority of metallofoldamer complexes in a pellet on a humid day would remain unchanged, a small number of molecules might racemize in areas where KBr picked up water. Such a situation could have generated lower signal intensities for the first two pellets.

Also, as evidenced in Figures 19-25, the circular dichroism values for the second approach were greater than those for the original method by more than a order of magnitude. The dissimilarity between the concentrations determined by the original and UV-Vis methods probably result from the fact that the original method can be greatly affected by the presence of viscous oil residue, unlike the UV-Vis method. As discussed in Section 2.3, the original method relies upon the mass of the oil-covered crystal to determine metallofoldamer concentration. However, UV-Vis absorbance spectroscopy measures the concentration of analyte directly and thus circumvents such inaccuracy in metallofoldamer concentration. Thus, for the original technique, artificially high concentrations would be obtained, yielding much lower circular dichroism values than for the UV-Vis technique, as observed.

To clarify, solid-state CD spectroscopy does yield reliable data concerning the spatial orientation of optically active molecules. The above spectra were clearly negative and so unmistakably indicated the handedness of the complexes in the crystal. There are several plausible explanations for the unanticipated discrepancies among the spectra obtained.

Chapter 4

DISCUSSION

4.1 Apparent Preference for the Left-handed Enantiomer

As discussed in the previous section, all three crystals analyzed were left-handed. Since the four crystals whose structures were solved by X-ray diffraction studies were also all left-handed, this means that all seven crystals of 2a characterized thus far shared the same handedness. This was an unexpected result because the macromolecule lacks any stereocenters or other sources of chirality. One would have anticipated an equal chance of forming either enantiomer.

If one were to assume that the likelihood of forming either helical structure was 0.5, the probability of observing seven left-handed crystals in a row would be ~0.016. This small value reflects the very low probability of obtaining seven crystals of one handedness in a row in the absence of an enantiomeric bias. Although I cannot yet rule out the possibility that the consistent formation of the (M)-helix was by chance, it is possible that a preference for the left-handed form did indeed exist.

One possible explanation which could lead to a left-handed bias might have been contamination by an adventitious, chiral material that seeded crystallization. Both Fan Zhang and I used Kimwipes to filter the metallofoldamer solution before setting up crystallization trials. To do so, we firmly lodged a plug of Kimwipe into a pipette and added the metallofoldamer solution to the top of the pipette, washed with $CHCl_3$, and collected the filtered solution. Filtering solutions in this manner is a common research practice. Normally,

it is considered to be an efficient way to remove contaminants. However, since Kimwipe fibers are made of cellulose, it is plausible that they provide a chiral surface upon which crystallization begins. This might cause preferential seeding of crystals made of (M)-helices. Accordingly, a Millipore filter will be used to purify the metallofoldamer I synthesize and it will be observed whether a left-handed preference is still exhibited when crystals form.

4.2 Implications of Metallofoldamer Behavior for the Study of the Origin of Chirality

The origin of central chirality is a subject of great scientific interest. Many theories have been developed to explain how molecules came to possess chiral centers. We hypothesize that central chirality may have developed from chirality not associated with stereocenters. If this were the case, products possessing stereocenters could have been formed by reactions involving helical molecules, perhaps serving as catalysts. It is possible that the first chiral molecules may have come to exist through the crystallization of a macromolecule that was devoid of stereocenters. If the macromolecule were dynamic in solution, but static in the crystalline state, then an enantiomeric bias could have existed, so long as the nucleation of the crystallization event was slower than crystal growth. If a non-infinite number of crystals were formed, then chance would have had to bias for the formation of one enantiomer over the other. These studies on 2a serve as a dramatic proof of this principle, with the extreme case where only one, enantiomerically pure crystal was formed.

As explained previously, salen-based metallofoldamers are well-known asymmetric catalysts. So, perhaps salophen-based metallofoldamers might also be able to serve as catalysts. If, in future work, 2a could be used in surface catalysis experiments, it would be very interesting and informative to observe whether molecules with stereocenters could be produced. If so, this would lead to the first example in which stereocenters are

produced in enantiomeric excess from materials that are completely achiral. Such an experiment, if successful, would lend support to the idea that the first enantiomerically enriched stereocenters emerged from an enantioselective reaction of an achiral or prochiral molecule and an enantiomerically enriched, insoluble macromolecule that was devoid of stereocenters.

Chapter 5

CONCLUSIONS

This research established the efficiency and effectiveness of solid-state CD in spontaneous resolution analysis. Despite the occurrence of absorption flattening and slight differences in signal intensity, which can easily be explained, the solid-state CD technique I devised can be trusted to yield unambiguous structural information. Consequently, solid-state CD can provide an effective and efficient alternative to X-ray diffraction studies. Metallofoldamer 2a appears to have a preference for the left-handed enantiomer. A possible reason for this unexpected behavior is contamination by a trace chiral impurity which seeds crystal growth. Finally, it is possible that future study on the behavior of the metallofoldamer may yield insight into the origin of chirality.

REFERENCES

1 Zhang, F.; Bai, S.; Yap, G. P. A.; Tarwade, V; Fox, J. M. J. Am. Chem. Soc. [Online] 2005, 127, 10590-10599.

2 Moore, J. S. Acc. Chem. Res. [Online] 1997, 30, 402-413.

3 Moore, J. S. J. Am. Chem. Soc. [Online] 1999, 121, 2643-2644.

4 Stone, M. T.; Fox, J. M.; Moore, J. S. Org. Lett. [Online] 2004, 6, 3317-3320.

5 Prince, R. B.; Okada, T.; Moore, J. S. Angew. Chem., Int. Ed. [Online] 1999, 38, 233-236.

6 Dong, Z.; Karpowicz, R. J., Jr.; Bai, S.; Yap, G. P. A.; Fox, J. M. J. Am. Chem. Soc. [Online] 2006, 128, 14242-14243.

7 Wikipedia. Chiral Resolution. http://en.wikipedia.org/wiki/Chiral_resolution (accessed Apr 2009).

8 Berova, N.; Nakanishi, K.; Woody, R. W; et al. Circular Dichroism: Principles and Applications, Second Edition; Wiley & Sons: New York, 2000.

9 Dong, Zhenzhen. Synthesis of Two-dimensional Scaffolds and Metallofoldamers. Ph.D. Thesis, University of Delaware, Newark, DE, 2007.

10 Palca, Joe. How the Primordial Soup Took a Left Turn. http://www.npr.org/templates/story/story.php?storyId=5448305 (accessed Apr 2009).

11 Levine, M.; Kenesky, C. S.; Mazori, D.; Breslow, R. Org. Lett. [Online] 2008, 10, 2433-2436.

12 Harada, N.; Nakanishi, K. Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry; University Science Books: California, 1983.

13 Castiglioni, Ettore; Abbate, Sergio; Longhi, Giovanna; Gangemi, Roberto. Chirality. [Online] 2007, 19, 491-496.