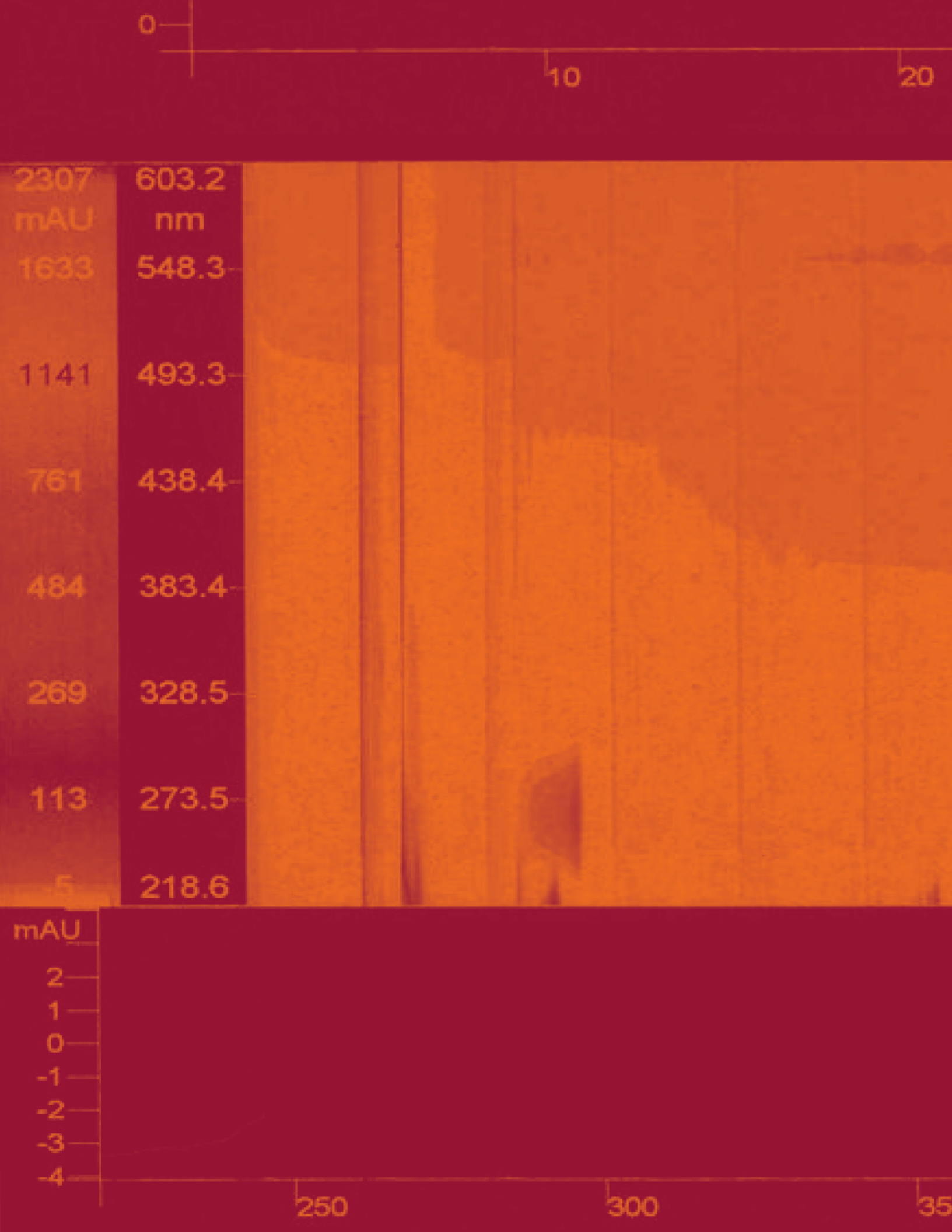
0
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20
2307
mAU
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438.4
484
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269
328.5
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273.5
5
218.6
mAU
2
1
0
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250
300
35

Synthesis of Solvent Reorganization Energy Probes

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Abstract

In many biological systems, ligand-receptor pairs bind to each other via noncovalent interactions such as hydrogen-bonding and Van der Waals forces. This project's method of studying these interactions uses electron transfer theory. When a receptor binds to a redox-modified ligand, there is a change in the reorganization energy of the redox center and a concurrent change in the rate of electron transfer. With the aim of developing a model system to test in future electrochemical studies, an alkane thiol was synthesized that incorporates a ligand, desthiobiotin, and a pyridine for redox modification. The synthesis revealed that the final product is present in two conformations.

Introduction

Various biological reactions involve two molecules that interact to lead to a physiological response. The smaller molecule is usually referred to as the ligand, and the larger molecule is referred to as the receptor. Electron transfer theory can be used to study how other parameters involved with these interactions relate to the rate of electron transfer. In particular, the Marcus equation relates electron transfer rate (k°) to the following parameters: temperature (T), Gibbs free energy (ΔG), electronic coupling (H_{AB}), and reorganization energy (λ).[1]

$$k^{\circ} = \frac{4\pi^2 H_{AB}^{\ 2}}{h\sqrt{4\pi\lambda RT}} \exp\left[-(\Delta G^{\circ} + \lambda)^2 / 4\lambda k_B T\right]$$

The parameter of interest for this research was the outer sphere reorganization energy, which refers to the energy associated with the movement of solvent molecules when electron transfer occurs between two redox centers.[1] The solvent molecules rearrange around the complex to accommodate the new charge. The outer sphere reorganization energy is related to noncovalent interactions, such as hydrogen bonding, dipole-dipole interactions, and hydrophobic interactions.[3] The parameter of interest for this work was the solvent reorganization energy (λ), which is the energy associated with the movement of solvent molecules when electron transfer occurs between two redox centers. This research focuses on the synthesis of a molecular probe, a molecule designed for the purpose of investigating a particular scientific phenomenon. The probe must incorporate the ligand, a metal complex, and an alkane thiol (a series of CH_2 groups with a sulfur atom attached at the end) for the electrochemical measurements. The project focuses on the conversion of a dibromoalkane into an alkane thiol incorporating a pyridine and a ligand.

Background

The development of electron transfer theory can be attributed to the work of Rudolph Marcus, who received the Nobel Prize in chemistry in 1992. One of his most important contributions to the theory of electron transfer is the Marcus equation, which relates electron transfer rate to the solvent reorganization energy. Many scientific studies of solvent reorganization energy use this equation. The model system chosen for this research on solvent reorganization energy is the ligand-receptor pair biotin-avidin. Avidin is a 66 kDa tetrameric protein.[5] Biotin (vitamin H) consists of two heterocycles with a valeric acid substituent attached to a one-ring carbon atom.[5] The avidin-biotin system is an ideal system because of the resistance of avidin to denaturation over a wide range of pH and temperature, the large affinity of avidin for biotin, and the ease with which biotin can be modified.[5] A ruthenium pentaammine complex was chosen because previous studies have shown that the outer sphere reorganization energy is large in such a system, while the inner sphere is low. Monolayers of the biotin modified with ruthenium pentaammine complex were self-assembled on a gold electrode. The electron transfer between the redox center of the complex and the electrode was measured before and after avidin binding. Binding caused a change in the reorganization energy and therefore a change in the rate. To study these interactions, it is crucial to synthesize a suitable molecule to form the complex and the monolayers. For this

Synthesis of Solvent Reorganization Energy Probes *(continued)*

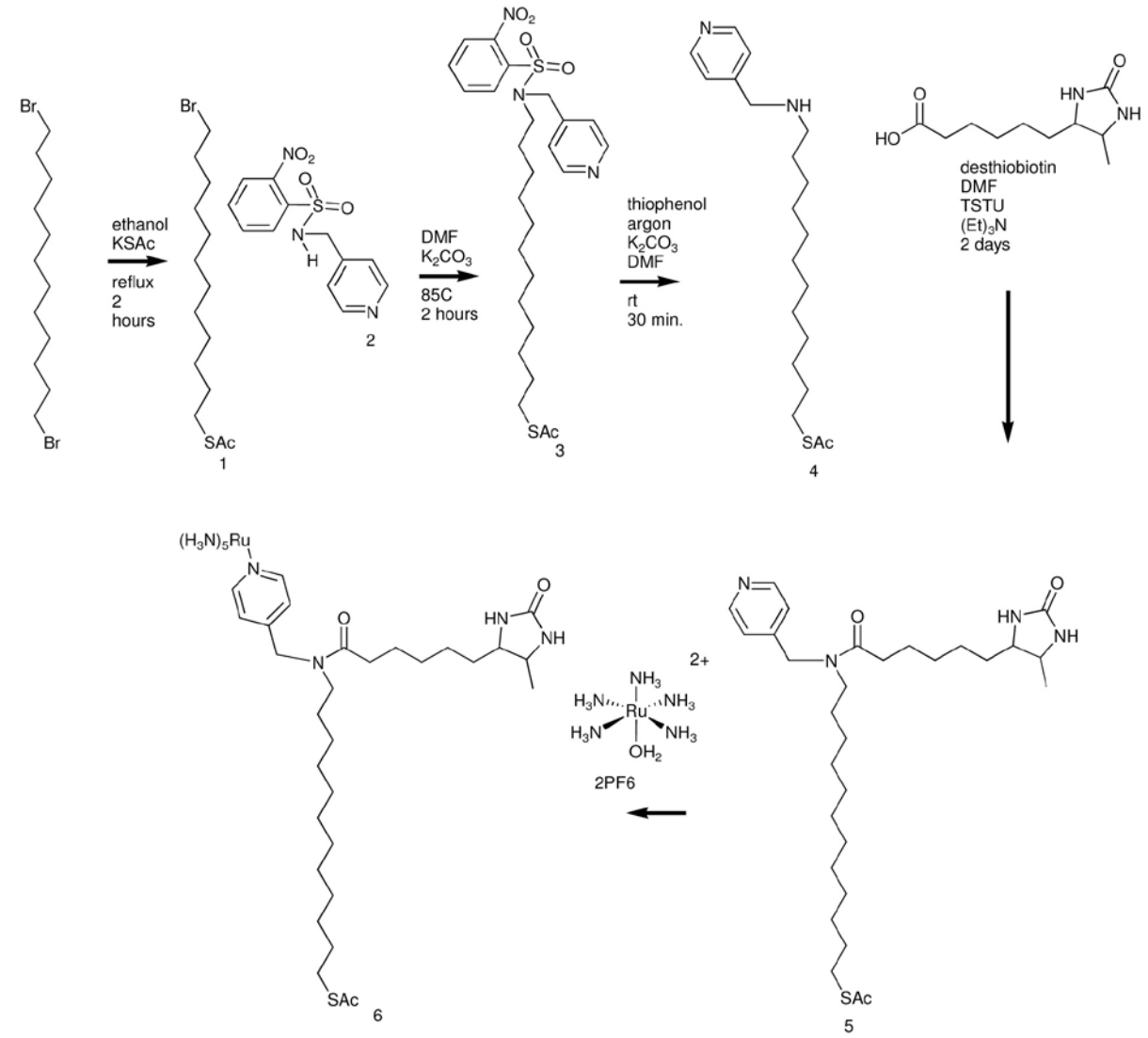

Figure 1. Reaction Scheme

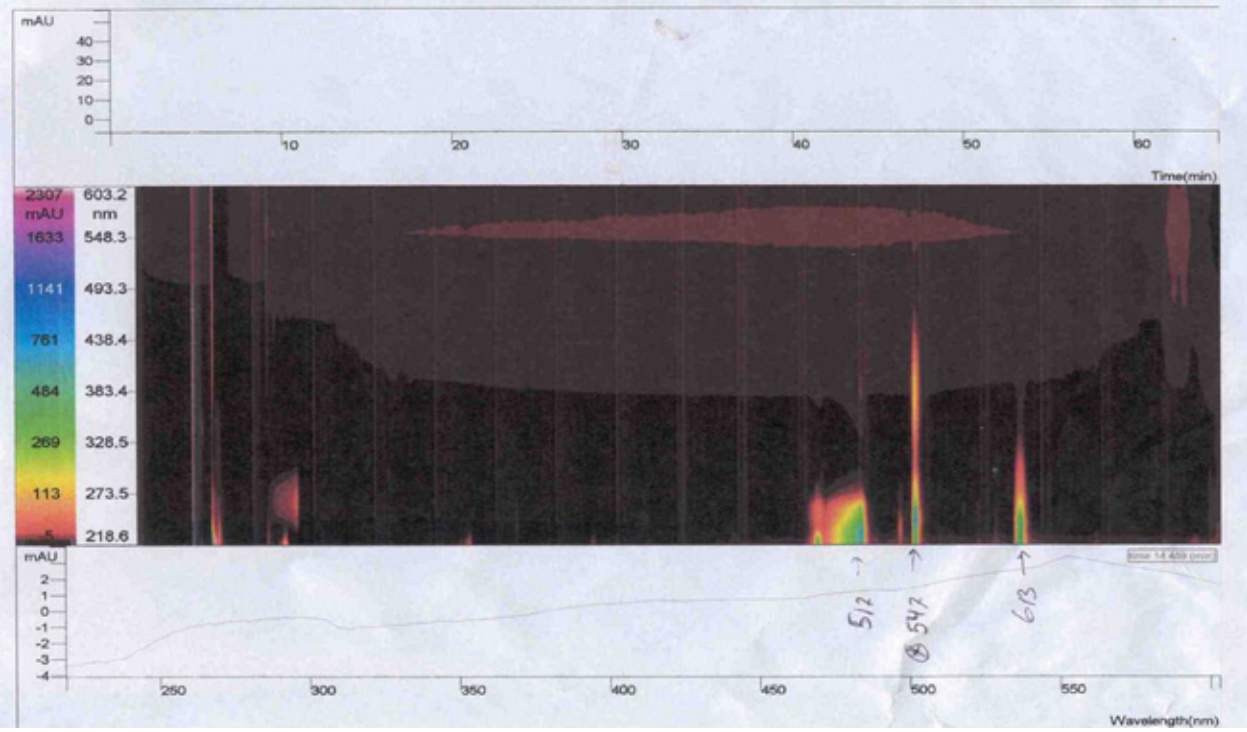

Figure 2. Analytical HPLC chromatogram.

study a synthetic method was chosen that is time efficient and results in pure product.

Approach

A probe was designed to incorporate the ligand, the metal complex, and alkane thiol. The synthesis of **5** was accomplished using four steps (Figure 1). 1,12-dibromododecane was reacted with potassium thioactetate to produce **1**. **1** was then reacted with **2**, and 4-aminomethylpyridine with a para-nitrosulfone protecting group, in order to attach a pyridine and its protecting group to produce **3**. Next, **3** was deprotected by reacting it with thiophenol under an argon atmosphere that yielded **4**. **4** was then reacted with desthiobiotin, a peptide coupling reagent called TSTU, and $(Et)_3N$ in a solution of DMF to afford the final product, **5**.

Results and Discussion

*Synthesis of **1***

1,12-dibromododecane (15.12g, 0.0461 mol) was reacted in a 3:1 mole ratio with potassium thioacetate (1.76g, 0.0154 mol) in a solution of ethanol (80 mL) under reflux at 100° C for 2 hrs. The reaction mixture was vacuum-filtered to remove unreacted potassium thioacetate, dissolved in hexane, and vacuum-filtered once more. The reaction resulted in a mixture of products: unreacted 1,12-dibromododecane, a monosubstituted alkane thiol, and a disubstituted alkane thiol. The mixture was purified using silica gel column chromatography (95% hexane/5% diethyl ether) with the unreacted starting material eluting first, followed by the monosubstituted product, and then the disubstituted product. The solvent was removed by rotary evaporation, and the monosubstituted product was yielded as a light brown powder (0.86 g, 0.00266 mol, 17.3 %). The yield was low due to some unreacted starting material running with

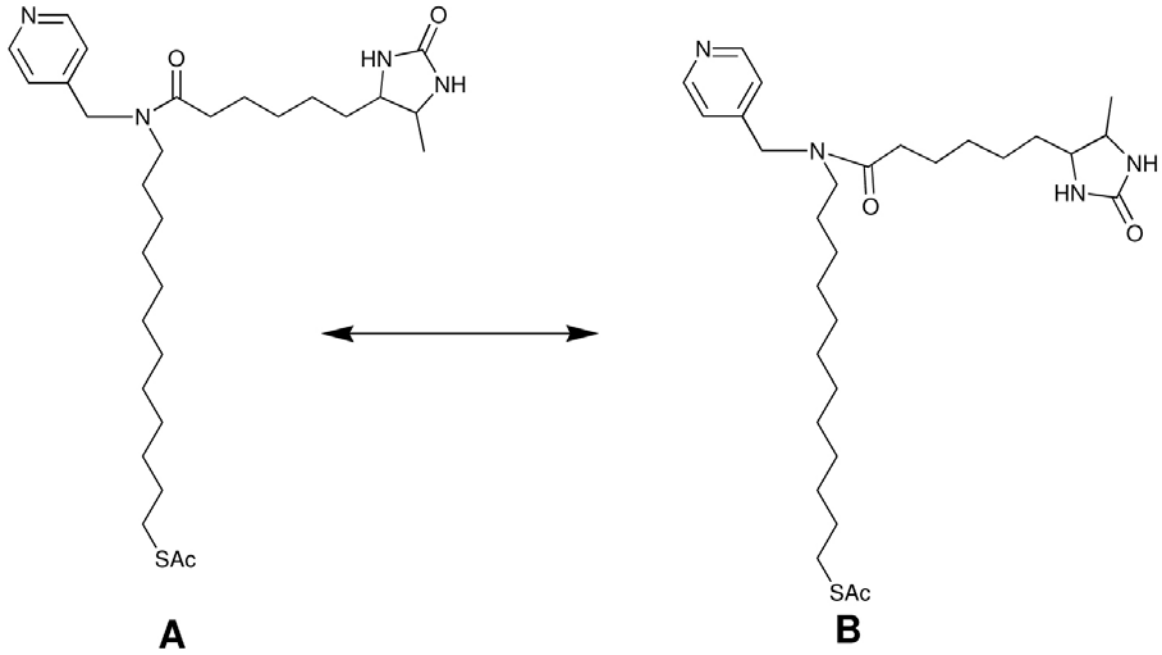

Figure 3. Possible conformations of 5.

the monosubstituted product; this mixture was not purified. The structure of the monosubstituted product was confirmed by mass spectrometry and ^{1}H-NMR spectroscopy. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.26 (m, 16H), 1.56 (m, 2H), 1.85 (m, 2H), 2.33 (s, 3H), 2.86 (t, 2H), 3.41 (t, 2H).

*Synthesis of **3***

1 (0.86 g, 0.00266 mol) was reacted with potassium carbonate (0.368 g, 0.00266 mol) and **2** (0.780 g, 0.00266 mol), which had been prepared previously, in a solution of DMF (40 mL), and then heated to 89° C for 2 hr. **2** contained a protecting group that serves as an attachment point for a ruthenium pentaammine complex. The crude product was purified by a silica gel column (98% dicholormethane/2% methanol), and the solvent was removed in vacuo. **3** was yielded as a dark brown liquid (0.68 g, 0.001269 mol, 47.9 %). The ^{1}H-NMR spectrum was consistent with the structure of **3**, except for a peak that was surmised to be water; however, the mass spectrum indicated the presence of an impurity with an approximate molecular weight of 686 g/mol. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.26 (m, 29H), 2.32 (s, 3H), 2.86 (t, 2H), 3.24 (t, 2H), 4.56 (s, 2H), 7.25 (s, 1H), 7.70 (m, 2H), 8.08 (d, 2H), 8.57 (d, 1H).

*Synthesis of **4***

The protecting group was removed by reacting **3** (0.68 g, 0.001269 mol) with thiophenol (0.287 mL) and potassium carbonate (0.18 g, 0.001302 mol) under an argon atmosphere for 30 min. It was essential to conduct this reaction under an argon atmosphere because thiophenol is easily oxidized in air into diphenyl disulfide. The reaction mixture was purified using a silica gel column (90% chloroform/10% methanol), and excess solvent was removed by rotary evaporation to yield **4** as an orange brown liquid (0.29 g, 8.27×10^{-4} mol, 65.9%). The ^{1}H-NMR and mass spectra differed in their indication of the purity level of **3**. The ^{1}H-NMR spectrum indicated that the product was pure; however, the mass spectrum indicated the presence of an impurity with an approximate molecular weight of 452 g/mol. This discrepancy in the two analytical techniques can be explained by the fact that ^{1}H-NMR has a threshold of 5% when detecting an

impurity. If the impurity is less than 5% of the sample, then there is a chance that it might not be detected by the ^{1}H-NMR. Therefore, the impurity detected by the mass spectrometer was likely less than 5% of the sample. ^{1}H-NMR (400 MHZ, $CDCl_3$): δ 1.47 (m, 22H), 2.33 (s, 3H), 2.61 (t, 2H), 2.86 (t, 2H), 3.81 (s, 2H), 7.26 (s, 2H), 8.54 (s, 2H).

Synthesis of ***5***

In order to incorporate the ligand (desthiobiotin), **4** (0.29 g, $8.27x10^{-4}$ mol) was reacted with d-desthiobiotin (0.18 g, $8.27x10^{-4}$ mol), *O*-(*N*-Succinimidyl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TSTU) (0.27 g, $9.1x10^{-4}$ mol) and triethylamine (0.12 mL) in a solution of DMF (20 mL). The crude product was purified twice by a silica gel column with a gradient (100% chloroform to 96% chloroform/1% methanol to 4% methanol) because thin-layer chromatography (TLC) and mass spectrometry revealed the presence of an impurity. A variety of solvent mixtures were used with TLC, but always resulted in one spot, and no conditions were found to separate the impurity. High-performance liquid chromatography (HPLC) was chosen as an alternative method to purify **5**. The HPLC instrument ran reversed phase, meaning that nonpolar molecules take a longer time to travel down the column. Analytical HPLC was used to determine the composition of the mixture containing **5**, and it revealed the presence of three components (Figure 2).

The sample was then purified by HPLC twice to obtain **5**, which eluted at 41 min. The mass spectrum indicated that the product was pure; however, the ^{1}H-NMR indicated that there may be two conformations of the product. There were two doublet peaks in the aromatic region (8–9 ppm) when only one peak was expected, and there were two singlet peaks between 4–5 ppm when only one peak was expected. The two possible conformations of pyridine substituent attached to the amide nitrogen could explain this discrepancy (Figure 3).

Conclusion

The results of this work could be used to improve the synthesis of solvent reorganization energy probes. The yields must be optimized in order to ensure a sufficient amount of product for addition of the complex and testing with electrochemistry. In particular, the yield of the first step of the synthesis must be increased, which could be done by increasing the amount of 1,12-dibromododecane that is reacted with potassium thioacetate. An impurity seemed to be present in steps 2 to 4 of the synthesis. Instead of using HPLC at the end of step 4, the results indicate that it would have been more prudent to use HPLC at the end of step 2 to remove an impurity that was carried throughout the synthesis. The most challenging task for future study is the separation of the two conformations of product **5** because purity is essential before adding the ruthenium complex. The theory as to why there are two conformations of product **5** is that resonance between the tertiary nitrogen and oxygen results in a structure with a double bond. Two conformations results because there is no rotation around a double bond. Once the synthesis is improved, electrochemical studies can be performed.

This research was supported primarily by the Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award Number ***EEC-0647560.*** *Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect those of the National Science Foundation.*

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