A Selective Sensor for Potassium and Ammonium Ions

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Abstract

Recent decades have seen considerable research into the field of ionophores, with a large number of such compounds with high selectivity for certain target ions having been synthesized and studied. However, there are few existing designer ionophores capable of selecting between multiple ions, especially ions of similar size and charge. The goal of this project was to develop such a compound that is capable of sensing both potassium and ammonium ions, depending on pH conditions of the surrounding system.

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1. Introduction

The fields of biotechnology and nanotechnology have expanded rapidly in recent years,[1] and have resulted in the development of many important medical devices.[2,3] These devices range from laser tools, used to aid in and preform surgeries to blood sensor technology, which make use of designer molecules to sense the presence of key ions and molecules in blood.[4]

One important group of medical devices spawned from biotechnology and nanotechnology research is blood sensors. Blood sensors take in a sample of blood and through various methods, return important information about blood chemistry to the user. This technology has been around for some time- there have been blood glucose meters on the market for diabetes patients since the 1980s, however recently there has been greater interest in expanding the technology for further applications.[5]

Figure 1: Handheld blood glucose meter for diabetes patients. An example of blood sensor technology on the market today

Blood sensors that are capable of sensing a greater number of blood borne molecules are of particular interest due to their potential to allow for simple, accurate and cheap information about a patient's blood chemistry.[6]

Current blood sensors work by exposing the blood sample to a piece of glass or plastic coated with molecules designed to sense a particular blood borne compound. In the presence of the specific compound, the molecule undergoes some physical change- some visibly fluoresce, some produce changes in electrochemical properties, or other result in other quantifiable changes, which can be translated directly to usable information about the concentration of the compound in question. By developing additional molecules that can sense additional blood borne compounds, blood sensors are able to return more useful information about blood chemistry. This has the advantage of reducing or eliminating the need for time consuming, costly and potentially inaccurate laboratory blood work, which is a goal of medical professionals.[7]

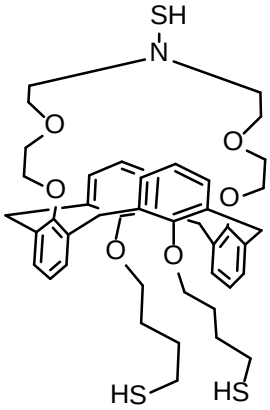

Figure 2: Target fluoroionophore

This aim of this project was to develop and test a fluoroionophore sensor that was capable of selectively sensing between potassium and ammonium ions. The desire to sense potassium and ammonium stems from their relative similarity in terms of charge, size and physiological importance. For this project, a sensor molecule (Figure 2) derived from the compound calix[4]arene was hypothesized, a synthetic scheme for the molecule developed

[1] (Carlson 2006)
[2] (Today, Microfluidic Chip Developed to Stem Flu Outbreaks 2012)
[3] (Today, Laser Accuracy in Surgeries Improved By Groundbreaking Device 2012)
[4] (Benco, Nienabar and McGimpsey, Optical Sensors for Blood Analysis 2001)
[5] (Harsanyi 2001)
[6] (Turner, Karube and Wilson 1987)
[7] (Gallarda and Dragon 1999)

and the synthesis carried out. This synthesis was attempted prior to this Major Qualifying Project, however no usable product was obtained, and therefore no electrochemical characterization could be carried out. This report describes the results of an attempted scale-up of the synthesis with the intent of producing usable product, however at the time of writing of this report, this scale up attempt has not yet produced the target molecule in any quantity.

2. Background

In recent decades, calix[4]arene based molecules have been found to be excellent ionophores, for a multitude of reasons, including ease of synthesis, high selectivity for target ions and good stability under sensing conditions.[8] Calixarenes' usefulness in sensing alkali metals stems from their structure- the presence of a hydrophobic cavity in the molecule makes for an ideal receptor for such ions, as the ionic radii of many alkali metal ions match closely the size of the cavity in the calixarene molecule. This inherent sensitivity to these ions can be further enhanced by attaching functional groups capable of improving upon the base calixarene's ability to electrostatically complex with the target ions.[9] There are many examples of such compounds capable of complexing in such a way with target alkali metal ions such as lithium,[10] sodium,[11] potassium,[12,13,14,15] rubidium[16] and cesium.[17,18,19] There have also been reported ionophores containing calixarene functionality that produce visible color changes when complexed with sodium.[20]

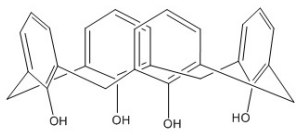

Figure 3: Calix[4]arene, cone conformation. Functionalized calixarenes have been found to make good ionophores

Most of functionalized calixarenes' usefulness as ionophores stems from the thermodynamic properties of their complexation with analyte ions. Calixarenes have a semi-rigid pre-organized structure to begin with, as the component phenyl groups are stable in any conformation they may undertake. This inherent rigidity is useful for ionophores, as it prevents them from deforming when in the presence of non-target ions to better complex with them and reduce selectivity for the analyte ion. An example of this effect can be seen by comparing two well-studied potassium ionophores, the simple 18-crown-6 ether and the naturally occurring antibiotic valinomycin. 18-crown-6 (Figure 5) is a flexible cyclic compound capable of wrapping itself around potassium ions, forming an octahedral cavity which the K^+ ion can occupy. However, this flexibility also allows 18-crown-6 to complex with both smaller and larger ions, due to its ability to contort into a configuration better suited for smaller or larger ions, all though it will not bind as strongly with ions significantly larger or smaller than potassium. In contrast to the flexible 18-crown-6,

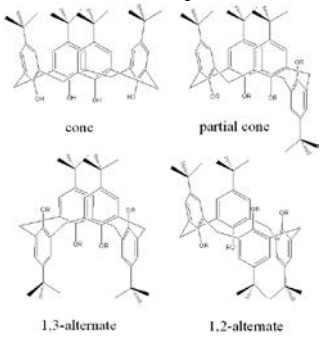

Figure 4: 4 possible conformations of calix[4]arene functionality- cone, partial cone, 1,3-alternate and 1,2-alternate

[8] (Pulla Rao and Jospeh 2011)
[9] (Creaven, Donlon and McGinley 2008)
[10] (Sirit, et al. 2006)
[11] (Rojanathanes, et al. 2005)
[12] (Rojanathanes, et al. 2005)
[13] (Ji, Dabestani and Brown 2000)
[14] (Webber, Cowley and Beer, Calix[4]semitube diquinone: a potassium selective redox-active ionophore 2003)
[15] (He, et al. 2009)
[16] (Webber, Beer, et al. 2003)
[17] (Webber, Beer, et al. 2003)
[18] (Li, et al. 2010)
[19] (He, et al. 2009)
[20] (Liu, et al. 2003)

valinomycin is much more rigid, and its three dimensional complexation pocket changes very little between its complexed and non-complexed forms. Unlike 18-crown-6, valinomycin cannot easily alter its structure in the presence of larger or smaller ions, which prevents it from complexing with such ions, which in turn greatly increases its selectivity for potassium as opposed to other ions, being 38 times more selective for potassium as opposed to sodium than the 18-crown-6 ether.[21] It is in part because of this rigidity present in calixarene compounds that they are such effective and selective ionophores- they are essentially incapable of conforming to and complexing with ions other than the ion(s) for which they were designed to complex with.

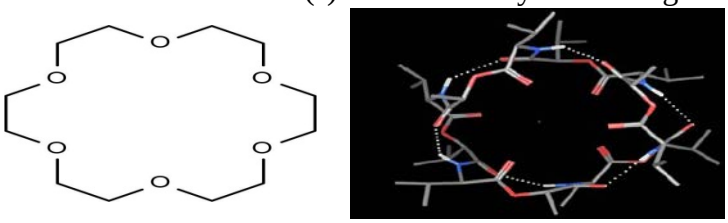

Figure 5: 18-crown-6 and valinomycin, respectively. Valinomycin's rigid 3-dimensional structure makes it a much more effective ionophore for potassium ions.[21]

Another important reason calixarene molecules make good building blocks for highly selective ionophores is their ability to be functionalized in many ways to make them more sensitive and more selective towards the analyte ion. The beginning of this section referenced several ionophores that have been developed in recent years that have shown good or better selectivity for their designated analyte ions, and it is important to note that these compounds share many things in common. As can be seen in Figure 6,[22] many calixarene ionophores reported in the literature combine the inherent ionophoric properties of the calixarene functionality with the ionophoric properties of the crown ether moiety, which allows the molecule to electrostatically complex with the target ion. These electrostatic interactions are what give the complexed ionophore-analyte ion system its stability, and help to determine which ions a particular ionophore will be more selective for- ions too small for the ionophore's cavity will not experience as strong of electrostatic interactions and therefore not be bound as tightly, whereas ions too large for the ionophore's cavity will experience very few, if any electrostatic effects, and will similarly not be complexed as strongly.

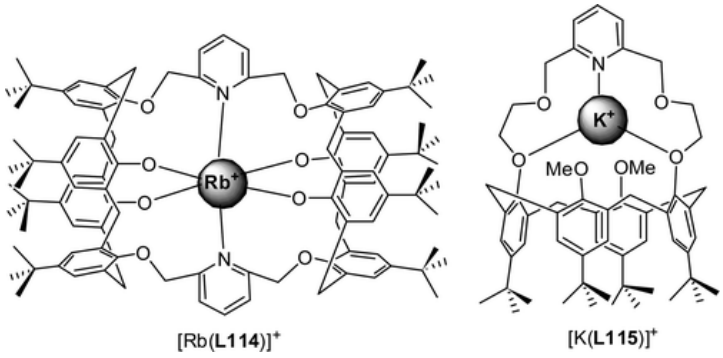

Figure 6: Example of alkali metal ion complexation in functionalized calixarene ionophores.[22] Of note is the crown ether moiety present in many calixarene ionophores.

The final goal of this project is to develop a sensor capable of sensing both potassium and ammonium ions that can be incorporated into clinical devices for use in the medical field. To this

[21] (Benco, The Rational Design and Synthesis of Ionophores and Fluoroionophores for the Selective Detection of Monovalent Cations 2003)
[22] (Marchand, et al. 2000)

end, the target molecule was also designed with functional thiol groups which would ease deposition onto surfaces for both initial testing and ideally final clinical use. Thiol functionality was chosen because it is well known to be capable of binding to gold surfaces,[23] and because it is relatively easy to synthesize. While ionophores capable of complexing any alkali metal ion exist in abundance, compounds that exhibit selectivity for target ions and are capable of being bound to a solid-state sensor are not as well studied and have only been developed much more recently.[24,25] The intent of this project is to eventually attach the target ionophore (see Figure 2, section 1) to gold slides, and test their selectivity for potassium and ammonium using electrochemical methods.

[23] (Nuzzo and Allara 1983)
[24] (Li, et al. 2012)
[25] (Lapresta-Fernandez and Capitan-Vallvey 2011)

3. Synthesis

3.1. Overall Synthesis

The overall synthesis involves 6 total reactions, as outlined below. The first 2 steps follow closely procedures found in the literature.[26,27] The remaining steps were not found in the literature and was developed by Dr. James P. Dittami of the Department of Chemistry and Biochemistry at Worcester Polytechnic Institute.

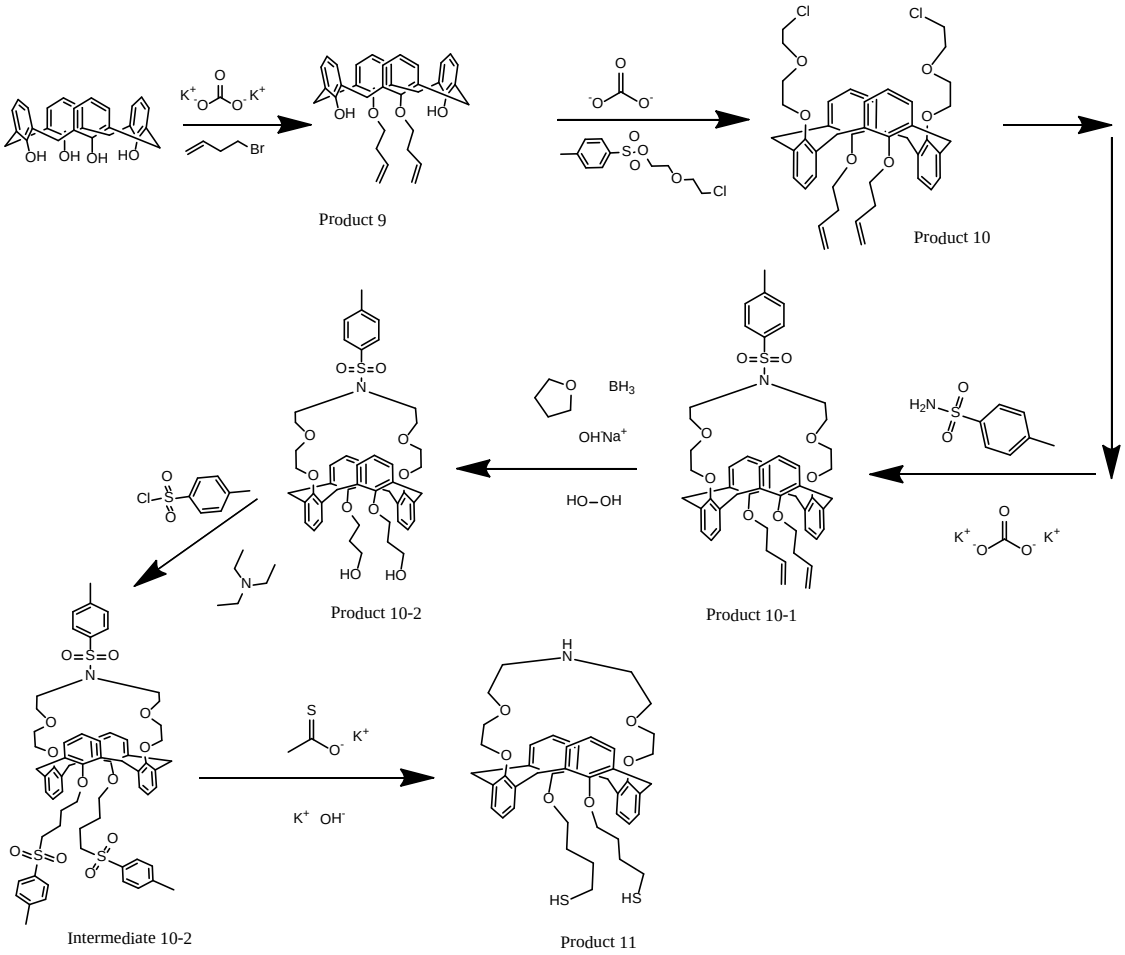

Figure 7: Overall synthetic scheme

[26] (Pitarch, Browne and McKervey 1997)
[27] (Kim, et al. 2000)

3.2. Materials and Methods

All chemicals were reagent grade or better and used as revived from suppliers. 4-bromo-1-butene was purchased from TCI America (Portland, OR, USA). Potassium carbonate was purchased from Aldrich Chemical (St. Louis, MO, USA). All other reagents, chemicals and solvents were from Alfa Aesar (Ward Hill, MA, USA).

^{1}H and ^{13}C NMR spectra were obtained using a 400-MHz Bruker (Billerica, MA) Advance NMR spectrometer and referenced to TMS. Mass spectra were obtained on an Agilent 1200 series LC/ Agilent 6139 mass spectrometer using chemical ionization and a 50/50 acetonitrile/ water carrier solvent and nitrogen curtain gas.

3.3. Synthesis of Product 9

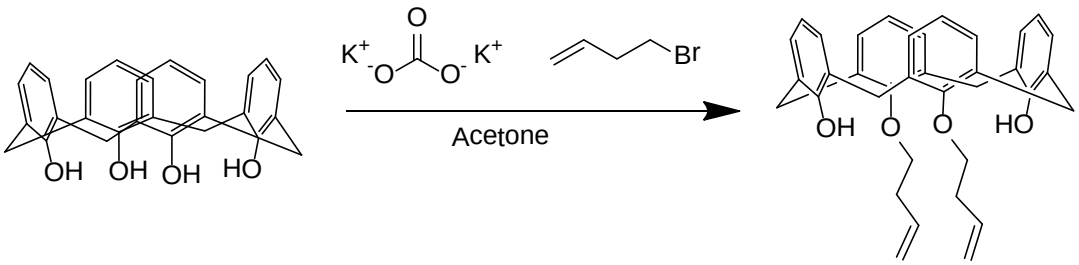

Figure 8: Synthesis of 25,27-bis(1-(3-butenyl)oxy)calix[4]arene (Product 9)

The synthesis of 25,27-bis(1-(3-butenyl)oxy)calix[4]arene (Product 9) was carried out as described in the literature[1]. For the 1g scale reaction calix[4]arene (1.0047 g, 2.367 mmol), potassium carbonate (0.654 g, 4.73 mmol), and 4-bromobut-1-ene (0.721 ml, 7.10 mmol) were added to a 50 mL round bottom flask in Acetone (Volume: 10 ml) to give a colorless solution. The reaction was monitored regularly by TLC using silica plates of calix[4]arene, a co-spot and the reaction mixture to determine reaction progress. The reaction was allowed to reflux for 117.5 hours before TLC indicated that the reaction had gone to completion. The solution was then refrigerated until work-up could be completed.

After reflux, solution was rotovaped to remove acetone solvent. The remaining mixture was then dissolved in dichloromethane and washed with DI water, then separated. Organic layer was washed with saturated NaCl and separated again. Organic layer was then dried over $MgSO_4$, and concentrated under vacuum to give Product 9 (25,27-bis(1-(3-butenyl)oxy)calix[4]arene). 0.5487 g (43.5% yield) Product 9 was obtained.

The desired product was purified by recrystallization instead of column chromatography. Trial of various combinations of methanol and dichloromethane solvents reveal that the simplest and most effective method of recrystallization is as follows. Raw product is suspended in warm methanol, and boiling dichloromethane is added to just barely dissolve the product. The solution is then removed from heat, covered (to keep out dust and airborne contaminations) and allowed to cool to room temperature. When the solution has cooled and purified product has crystallized out, the solid is filtered and collected. The overall yield of the recrystallization can be significantly improved by boiling the mother liquor to drive of dichloromethane and allowing more product to crystallized out, so this was done as well.

This reaction was twice at the 1g scale after several runs at 0.2g trial scale to ensure enough final product could be obtained. The second 1g scale reaction yielded 0.9211g Product 9, for a yield of 73.0%

IUPAC name: *26,28-bis(but-3-en-1-yloxy)pentacyclo[19.3.1.3^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diol*

[1] (Pitarch, Browne and McKervey 1997)

3.4. Synthesis of Product 10

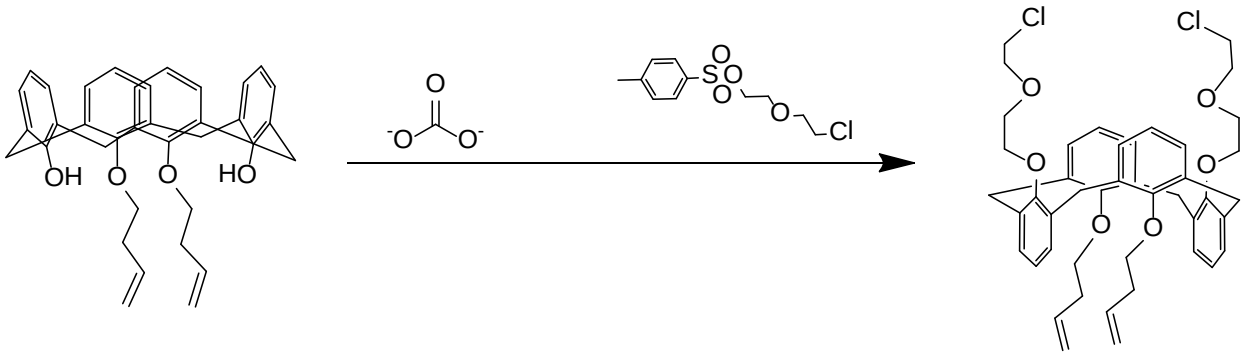

Figure 9: Synthesis of 25,27-bis(1-(3-butenyl)oxy)-26,28-bis(5-chloro-3-oxapentyloxy)calix[4]arene (Product 10)

The synthesis of 25,27-bis(1-(3-butenyl)oxy)-26,28-bis(5-chloro-3-oxapentyloxy)calix[4]arene (Product 10) was carried out using a synthetic reaction for a similar compound described by Kim et all.[28]

In a 50 mL round bottom flask, Product 9 was added with 2 molar equivalents of cesium carbonate ($CsCO_3$). A stir bar was placed in the flask, and acetonitrile was added such that the concentration of Product 9 was ~0.1M. 2 molar equivalents of p-TsCl (see section 3.9 for synthesis of this reagent) was added drop wise via glass syringe. The solution was refluxed under nitrogen for ~26 hours, monitored by TLC in 10:1 (Hex:EA) solution run every 2 hours until only one product spot is present. The author notes that solution turned purple at the beginning of the reaction and was brown in color when the reaction was stopped.

The mixture was allowed to cool to room temperature before 10 mL 10% HCl and 10 ML dichloromethane were added to the mixture. The organic layer was separated from the aqueous layer and dried over $MgSO_4$. The solution was then concentrated under vacuum to yield a brown oil.

The oil was then dissolved in benzene for flash column chromatography and run through a silica gel column. Hexanes was run through the column first to remove the benzene solvent, then a solution of 8:1 (Hex:EA). The 1:8 mixture separates the mixture into two fractions with UV-Vis properties. The first fraction is the desired 1,3 alternate, which yields a white powder upon concentration in vacuo, the second is a mixture of the remaining alternates, which yields a yellow oil upon concentration.

Presence of the desired 1,3 alternate is confirmed by the appearance of a peak at ~36.0 ppm in C13 NMR spectra.[29] ^1H spectra are consistent with published data for similar compounds.

[28] (Kim, et al. 2000)
[29] (Kim, et al. 2000)

IUPAC name: *25,27-bis(but-3-en-1-yloxy)-26-28-bis[2-(2-chloroethoxy)ethoxy]pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3(28),4,6,9(27)10,12,15(26),16,18,21,23-dodecaene*

3.5. Synthesis of Product 10-1

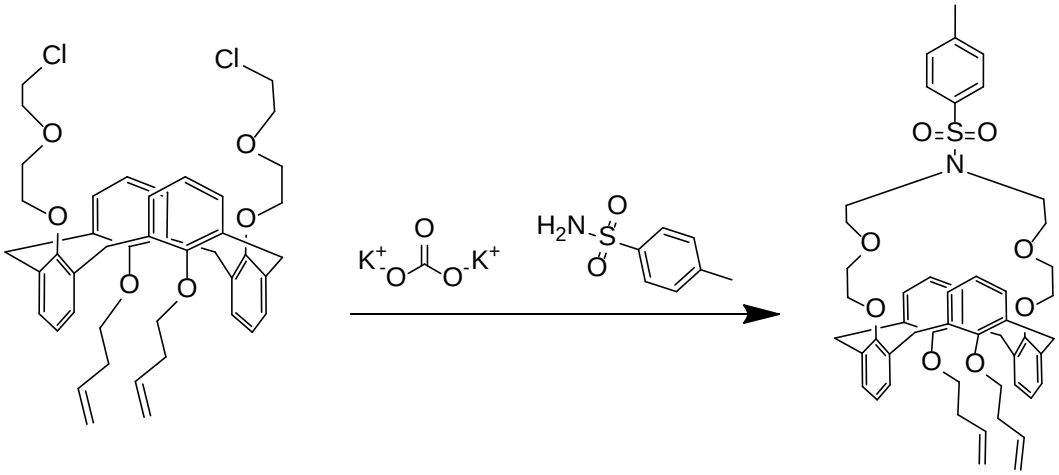

Figure 10: Synthesis of Product 10-1

In a 50 mL round bottom flask, Product 10 will be added to 5 molar equivalents of KCO_3 and one molar equivalent of p-toulenesulfonamide and sufficient DMF such that the concentration of Product 10 is ≥ 0.1M. The solution is refluxed for 22 hours, monitored by silica gel TLC in 10:1 (Hex:EA) solvent.

DFM is removed in vacuo and 10mL 10% aqueous $NaHCO_3$ and 10 mL dichloromethane is added. The organic layer is separated and washed with water (2x 50 mL), dried over magnesium sulfate and filtered using vacuum filtration. Removal of dichloromethane by vacuum should yield a yellow oil, which will be purified by column chromatography (using same chromatography setup from section 3.4) using 8:1 Hex:EA solvents. Two fractions result from purification. The desired product is a white powder, any yellow oil remaining is unreacted tosyl group which is removed at a later step of the synthesis.

IUPAC name: *40,41-bis(but-3-en-1-yloxy)-9-[(4-methylphenyl)sulfonyl]-3,6,12,15-tetraoxa-9-azahexacyclo[15.15.7.1^{23,27}.1^{34,38}.0^{2,29}.0^{16,21}]hentetraconta-1,16,18,20,23(41),24,26,29,31,34(40),35,37-dodecaene* (non-preferred name)

3.6. Synthesis of Product 10-2

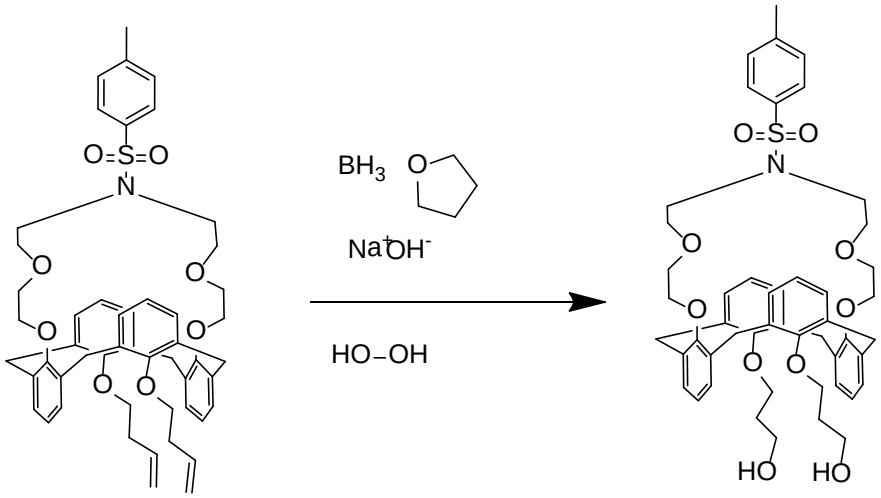

Figure 11: Synthesis of Product 10-2

A 50 mL three-necked, round bottomed flask equipped with a reflux condenser, thermometer, stirring bar, and dropping funnel is assembled. The apparatus is heated while the system was flushed with nitrogen. After adding THF such that the concentration of Product 10-1 is 0.1M, Product 10-1 is added to the flask under an excess flush of nitrogen, recapped and allowed one minute to equilibrate to a positive pressure of nitrogen. This colorless solution is then cooled in an ice bath, while stirring, to 5°C. Once cooled the borane-THF complex is added to the dropping funnel and added to the mixture drop wise at a rate of approximately 1 drop every 30 seconds. The reaction is allowed to stir after the addition of the borane-THF complex for 30 minutes. Excess borane is then destroyed by adding 5 mL of water. This evolves hydrogen gas, so 10 minutes should be allowed to complete this step.

After any hydrogen gas evolved has dissipated, 0.2 mL 1M NaOH is added to the reaction mixture. Hydrogen peroxide (30%) is then added drop wise into the solution via syringe, at slow enough a rate that the temperature of the reaction mixture is kept below 40°C. The mixture is then heated and maintained at 50°C for 1 hour to complete the oxidation.

The desired product (Product 10-2) is isolated by washing the reaction mixture with 20 mL of ethyl ether. The aqueous layer of the mixture is removed and the organic layer is washed with water (2x 25 mL) and brine (1x 25 mL). The organic layer is then separated from the aqueous layer and dried over anhydrous magnesium sulfate. The solvent is then removed in vacuo, yielding a white powder.

IUPAC name: *3,3'-[{9-[(4-methylphenyl)sulfonyl]-3,6,12,15-tetraoxa-9-azahexacyclo[15.15.7.1^{23,27}.1^{34,38}.0^{2,29}.0^{16,21}]hentetraconta1,16,18,20,23(41),24,26,29,31,34(40),35,37-dodecaene* (non-preferred name)

3.7. Synthesis of Intermediate 10-2

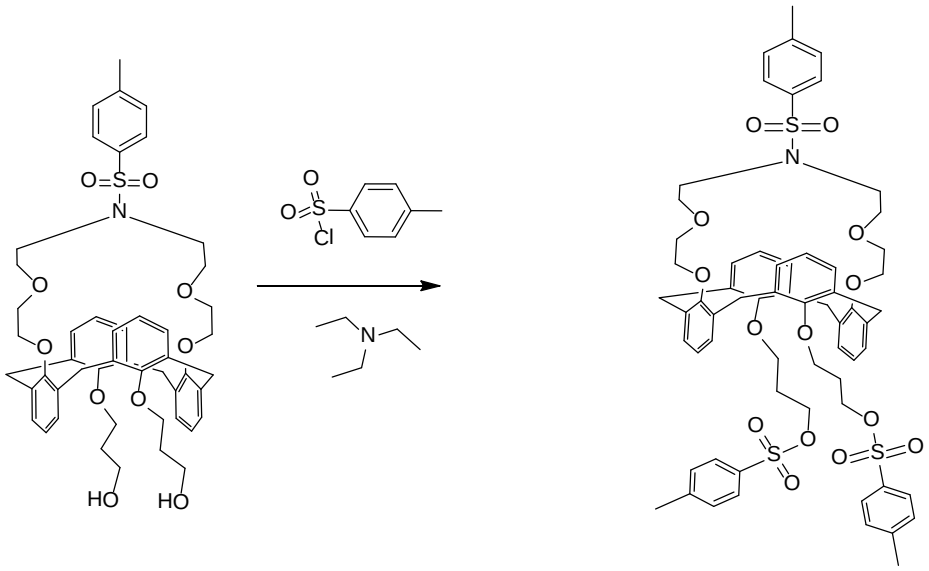

Figure 12: Synthesis of Intermediate 10-2

Product 10-2 is dissolved in methylene chloride such that the concentration of Product 10-2 (the calixarene reactant) is 0.016 M. 2 molar equivalents of triethylamine was then added to the solution prior to addition of 2 molar equivalents of toluenesulfonyl chloride. This order is important since the basic triethylamine is needed to neutralize and acid present in the reaction mixture so that the tosyl chloride will survive in solution. The reaction is allowed to stir at room temperature overnight. Completion of reaction is confirmed by product spot TLC, run in (10:1) Hex:EA eluent.

The product is extracted using (2 x 20mL) water. The aqueous layers are combined and extracted using (2 x 10mL) methylene chloride. These organic layers are then combined and washed with brine (2 x 10mL), dried over $MgSO_4$, and finally the dried product is concentrated under vacuum to give the Intermediate 10-2 product as a yellowish oil.

IUPAC name: *9-[(4-methylphenyl)sulfonyl]-40,41-bis-{3-[(4-methylphenyl)sulfonyl]propoxy}-3,6,12,15-tetraoxa-9-azahexacyclo[15.15.7.1^{23,27}.1^{34,38}.0^{2,29}.0^{16,21}]hentetraconta-1,16,18,20,23(41),24,26,29,31,34(40),35,37-dodecaene* (non-preferred name)

3.8. Synthesis of Product 11

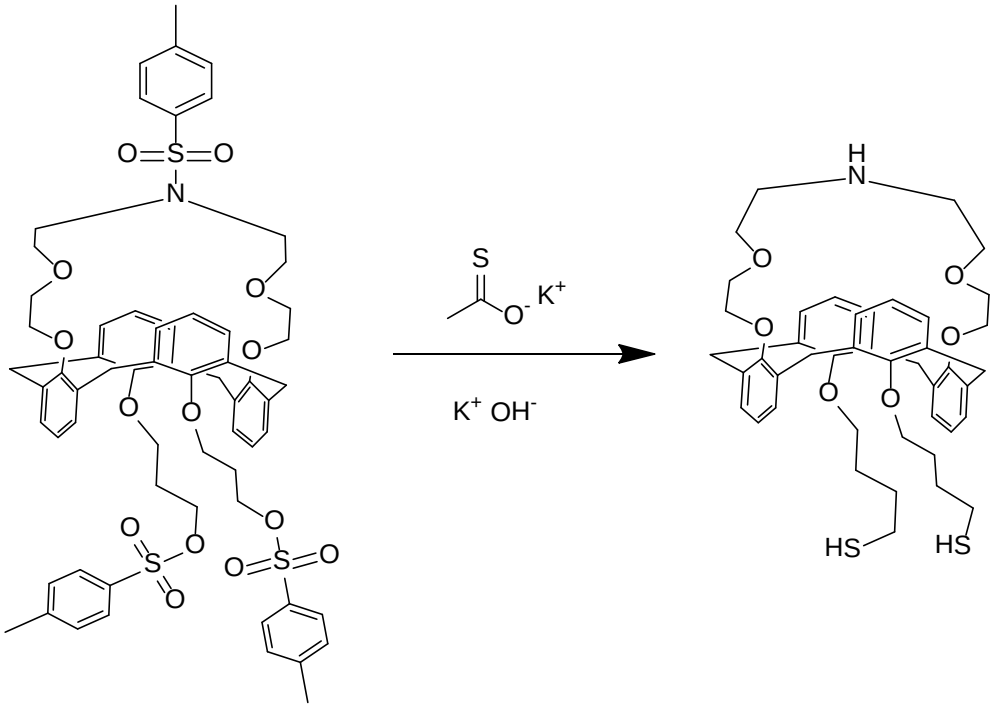

Figure 13: Synthesis of Product 11, target ionophore

What Intermediate 10-2 is available is placed in a round bottom flask of adequate size, and 4 molar equivalents of potassium thioacetate is added. Enough THF solvent is then added to the mixture to completely dissolve the compound. This works out to ~4 mmolar concentration. Finally, 4 molar equivalents of KOH is added as a 3M solution very SLOWLY!!! dropwise to the solution while stirring to remove the tosyl groups, replacing them with hydrogens to give the final target ionophore.

The solvent is then evaporated in vacuo to afford a white powder and yellowish oil mixture. This mixture is then extracted with a 1:1 mixture of THF and DCM where the calixarene product remains in the THF while DCM removes any remaining tosyl group by-product.

IUPAC name: *3,3'-{[9-sulfanyl-3,6,12,15-tetraoxa-9-azahexacyclo[15.15.7.1^{23,27}.1^{34,38}.0^{2,29}.0^{16,21}]hentetraconta-1,16,18,20,23(41),24,26,29,31,34(40),35,37-dodecaene-40,41-diyl]bis(oxy)dipropane-1-thiol* (non-preferred name)

3.9. Synthesis of p-TsCl [2-(2-chloroethoxy)ethanol-p-toluenesulfonate]

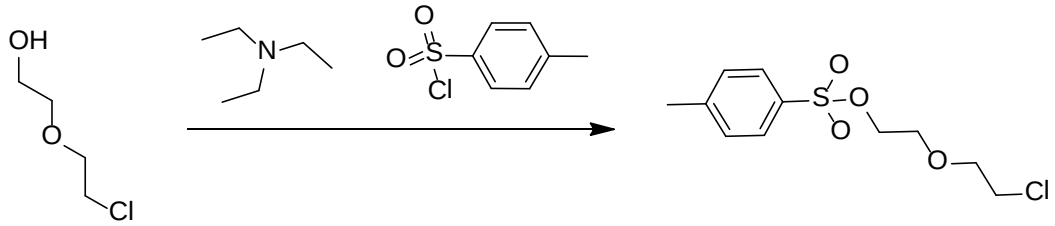

Figure 14: Synthesis of 2-(2-chloroethoxy)ethanol

A 250 mL round bottom flask was dried under vacuum and nitrogen and capped with a septum before reactant addition. To this 250 mL flask, 7.84 g p-TsCl was added, followed by 100 mL dichloromethane solvent. Still under nitrogen, 4.40 mL 2-(2-chloroethoxy)ethanol was added via syringe to the solution. 12 mL dry triethylamine was then added drop wise to the solution via syringe. The reaction apparatus was placed in a salt/ice bath for ~12 hours, keeping the temperature under 2.5°C. The reaction was monitored by TLC in 1:1 Hex:EA solvent.

At room temperature, the solution was transferred to a 250 mL separatory funnel. The TsCl was extracted into 2 x 25mL volumes of dichloromethane and the organic phase were combined. The organic phase was then washed with 2 x 25mL volumes of DI H_2O and 2 x 25mL volumes of brine. The organic phase was then separated from the aqueous phase, dried over $MgSO_4$ and concentrated under vacuum to yield a yellow oil, the desired 2-(2-chloroethoxy)ethanol-p-toluenesulfonate product.

4. Spectra

4.1. Calix[4]arene starting material

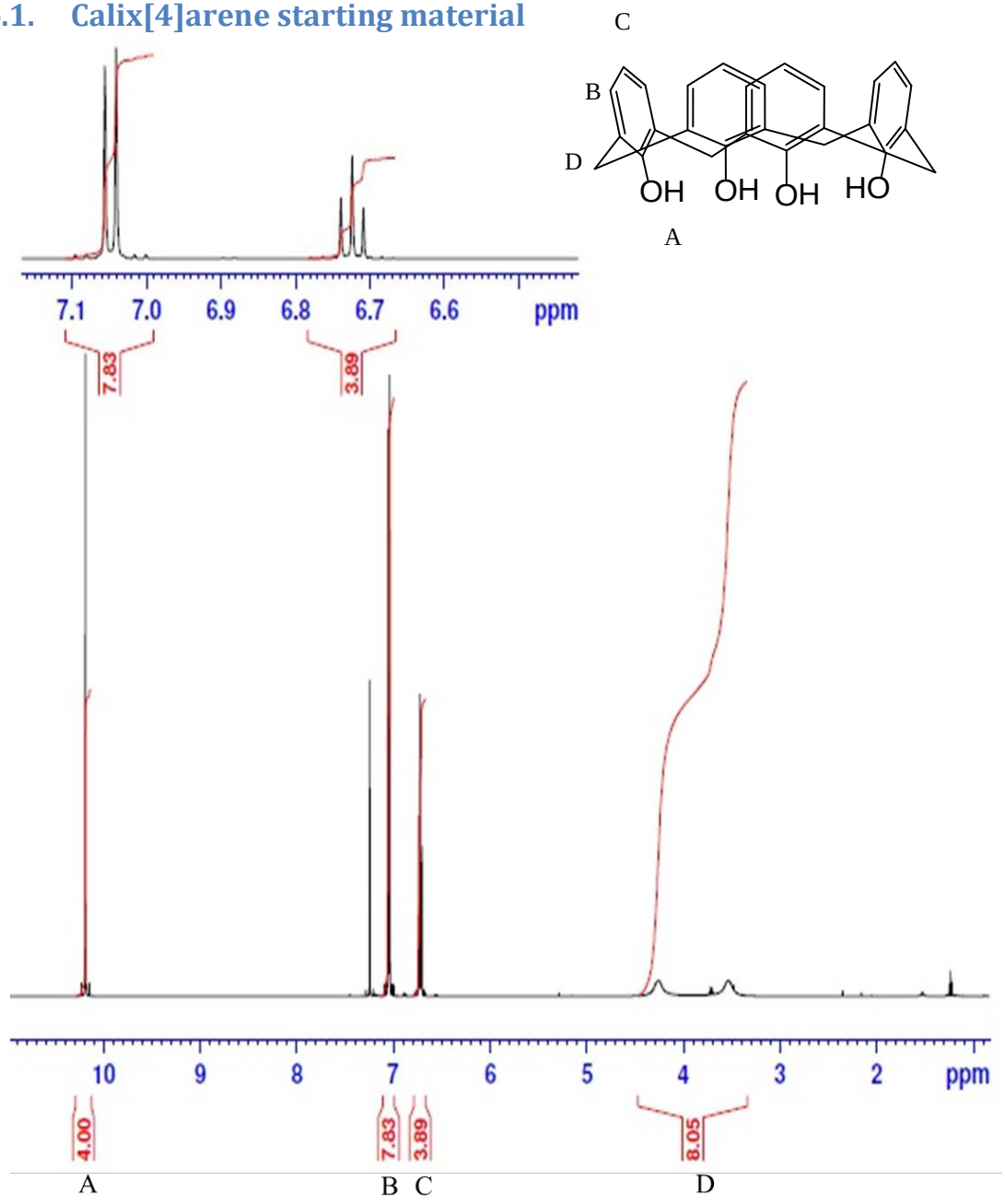

Figure 15: ^{1}H NMR Spectra of Calix[4]arene with labeled peaks corresponding to labeled protons in structure

4.2. Product 9

4.2.1. ^{1}H NMR

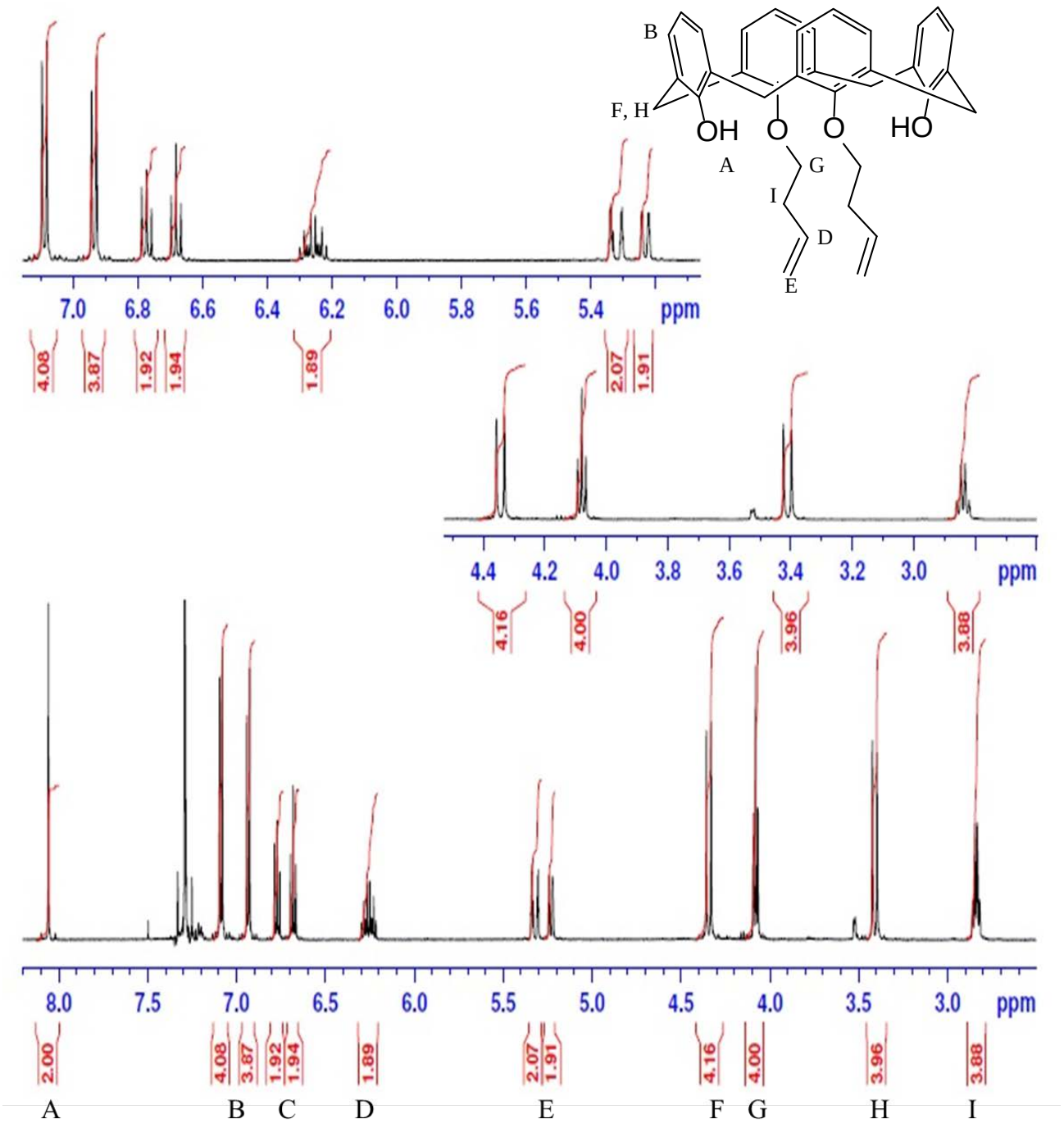

Figure 16: ^{1}H NMR Spectrum of Product 9 with labeled peaks corresponding to labeled peaks in structure. Expansions provided so splitting patterns may be better seen.

4.2.2. Mass Spectrum

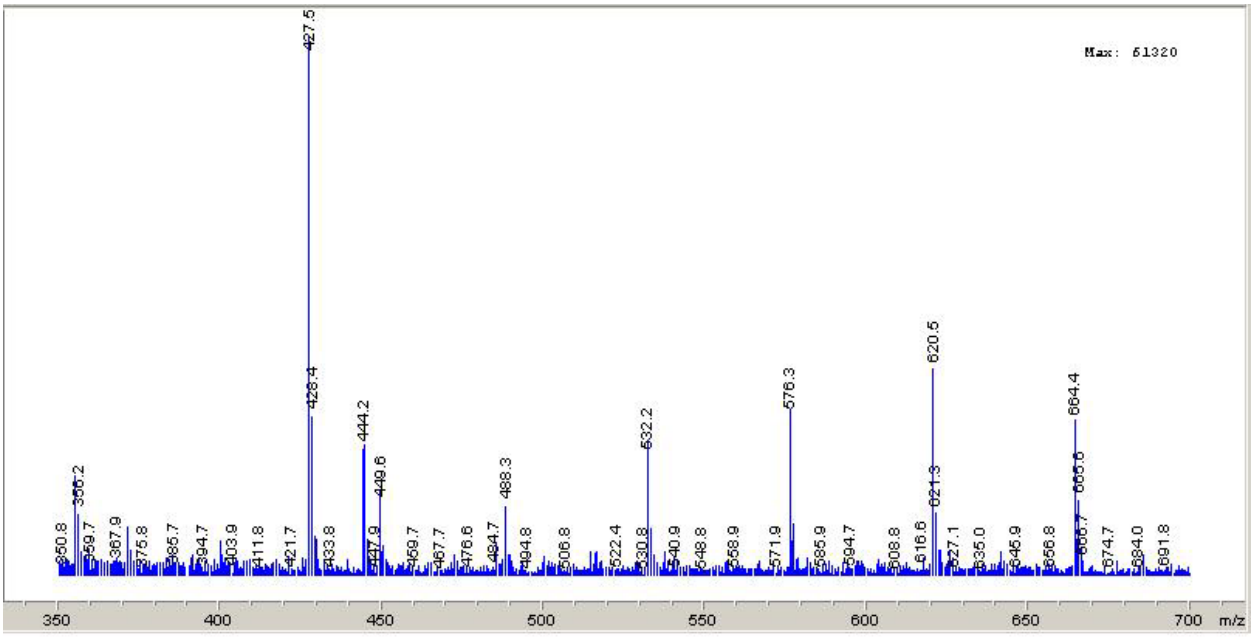

Figure 17: Product 9 mass spectrum. Chemical ionization, m/z = 427

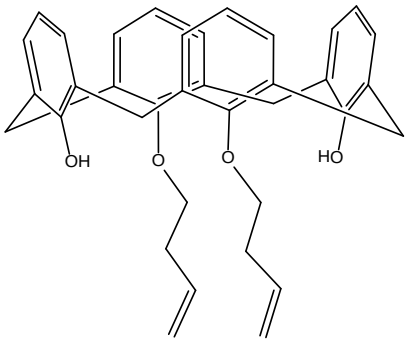

Figure 18: Product 9, molecular mass 426 g/mol

4.3. Product 10

Shown are the ^{1}H NMR, C13 CPD, C13 DEPT-135, DEPT-90, DEPT-45 spectra for Product 10, along with its chemical ionization mass spectrum. It should be noted that the large multiplet in the ^{1}H spectra at ~3.7ppm is believed to be due to 6 different proton groups with similar chemical shifts overlapping. The 6 proton groups believed to be contributing to the multiplet are all labeled as the same peak, for clarity. The author also wishes to note that the proposed peak assignments for the given carbon spectra are strictly hypothetical, and have yet to be reviewed by an advisor, it is therefore not suggested that they be used as standards against which to compare future ^{13}C NMR spectra of this compound.

4.3.1. ^{1}H NMR

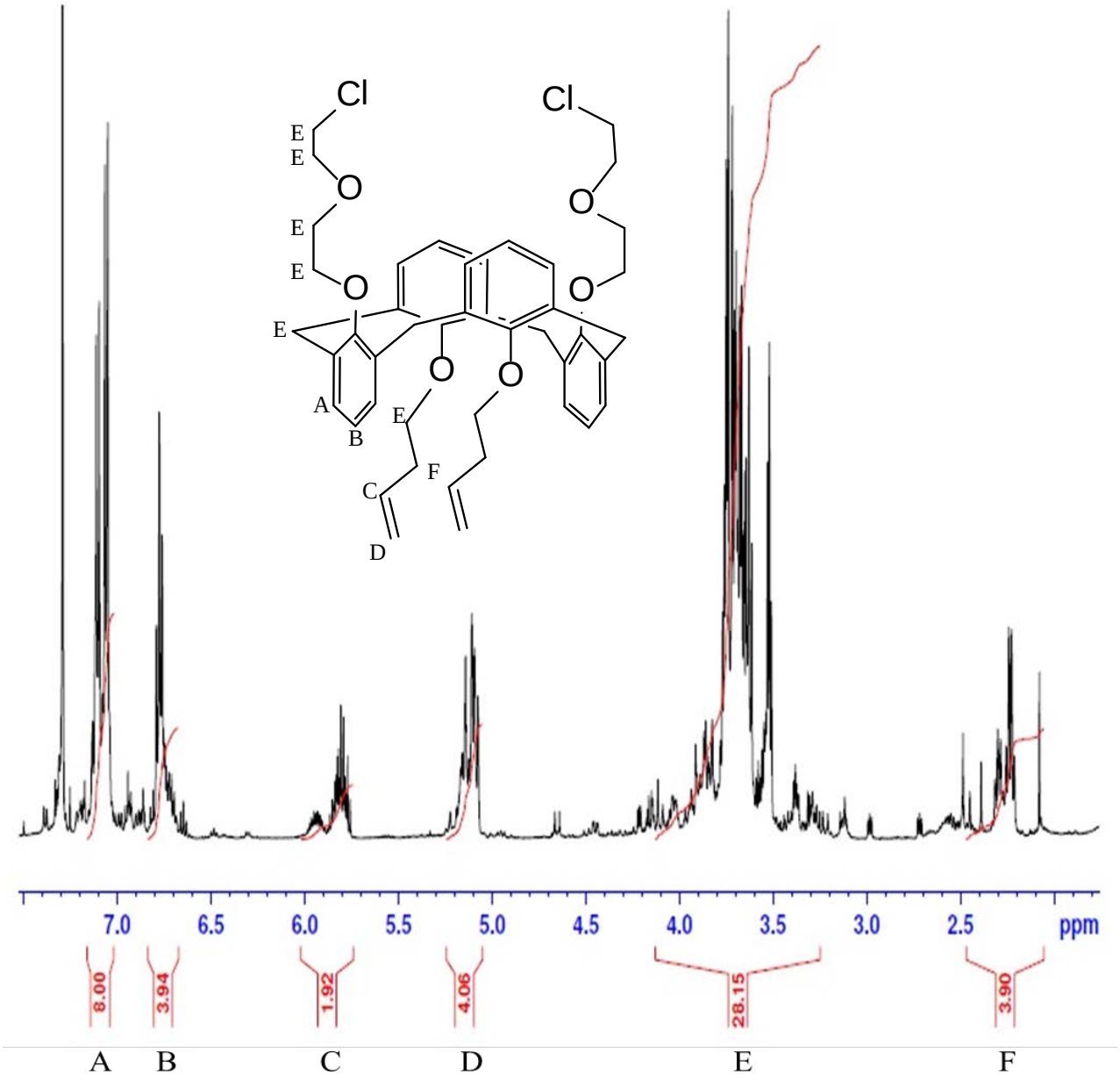

Figure 19: ^{1}H NMR Spectra of Product 10, with labeled peaks corresponding to labeled proton groups in molecule structure.

4.3.2. ^{13}C CPD

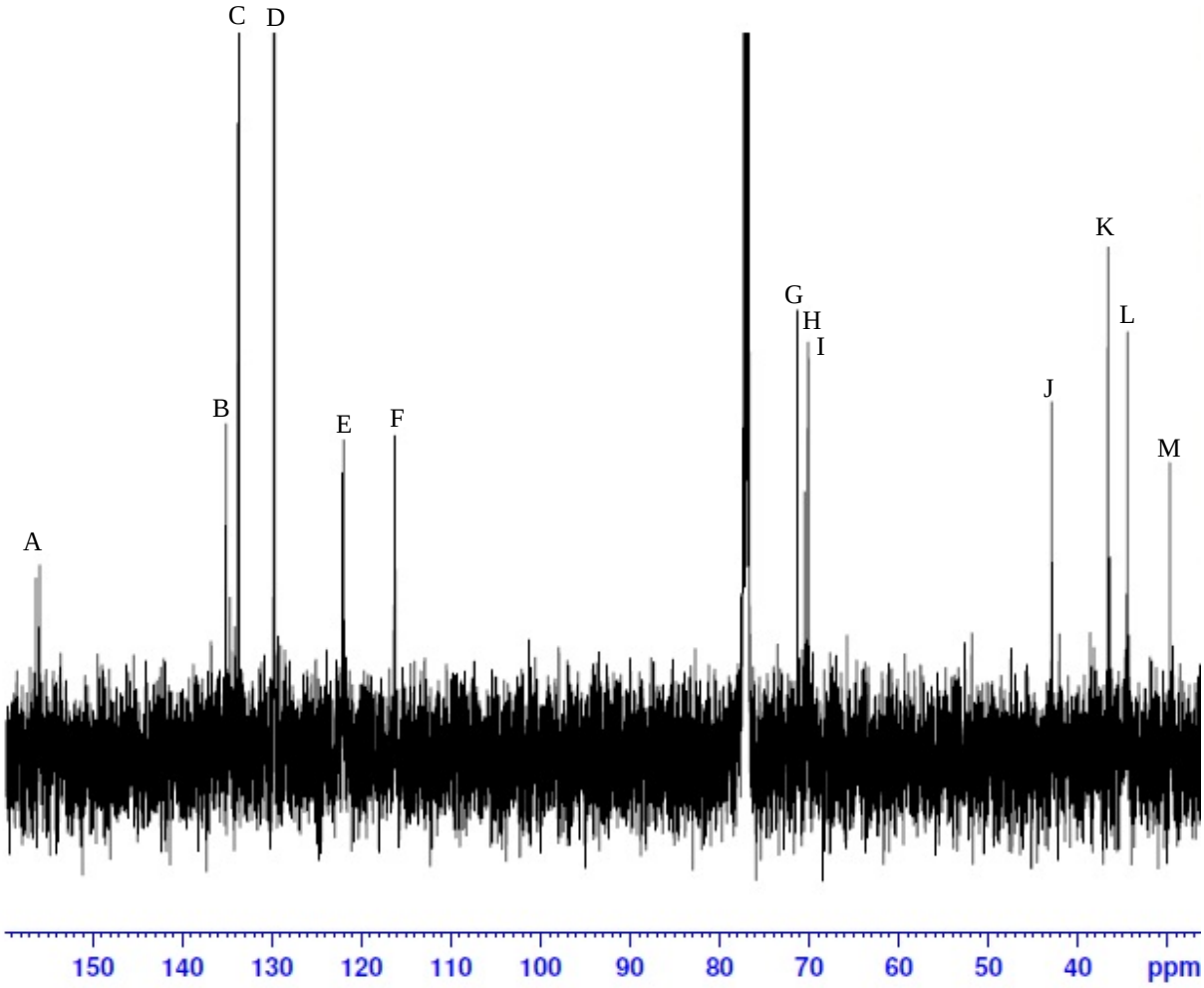

Figure 20: ^{13}C CPD spectrum of Product 10

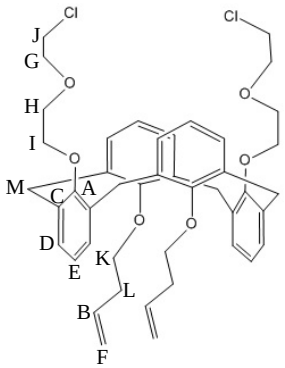

Figure 21: Product 10 structure labeled with corresponding ^{13}C CPD peaks

4.3.3. ^{13}C DEPT 45

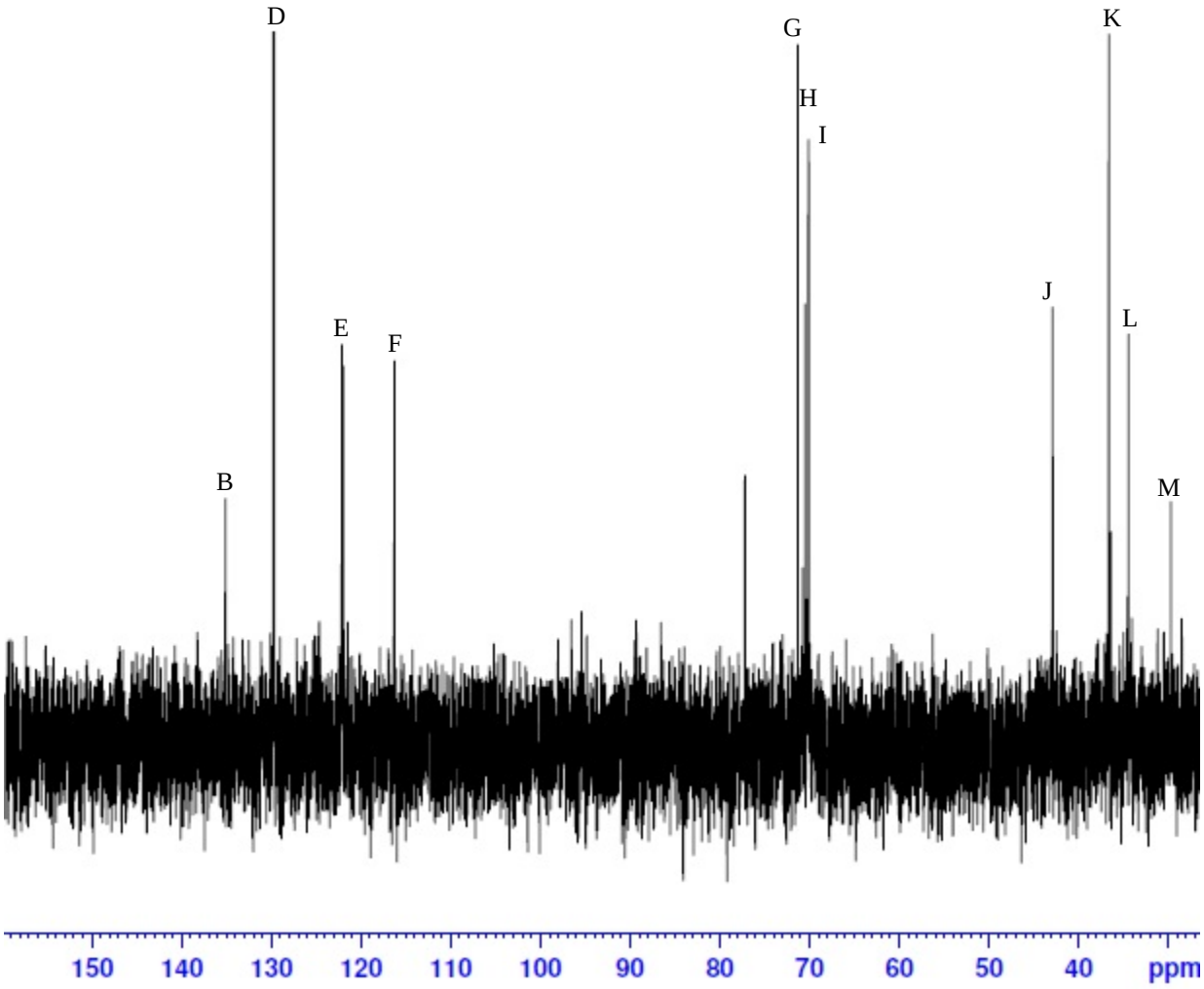

Figure 23: ^{13}C DEPT 45 spectrum of Product 10

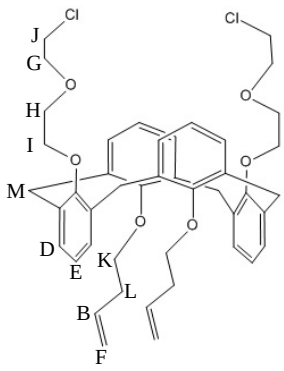

Figure 22: Product 10 structure labeled with corresponding ^{13}C DEPT 45 peaks

4.3.4. ^{13}C DEPT 90

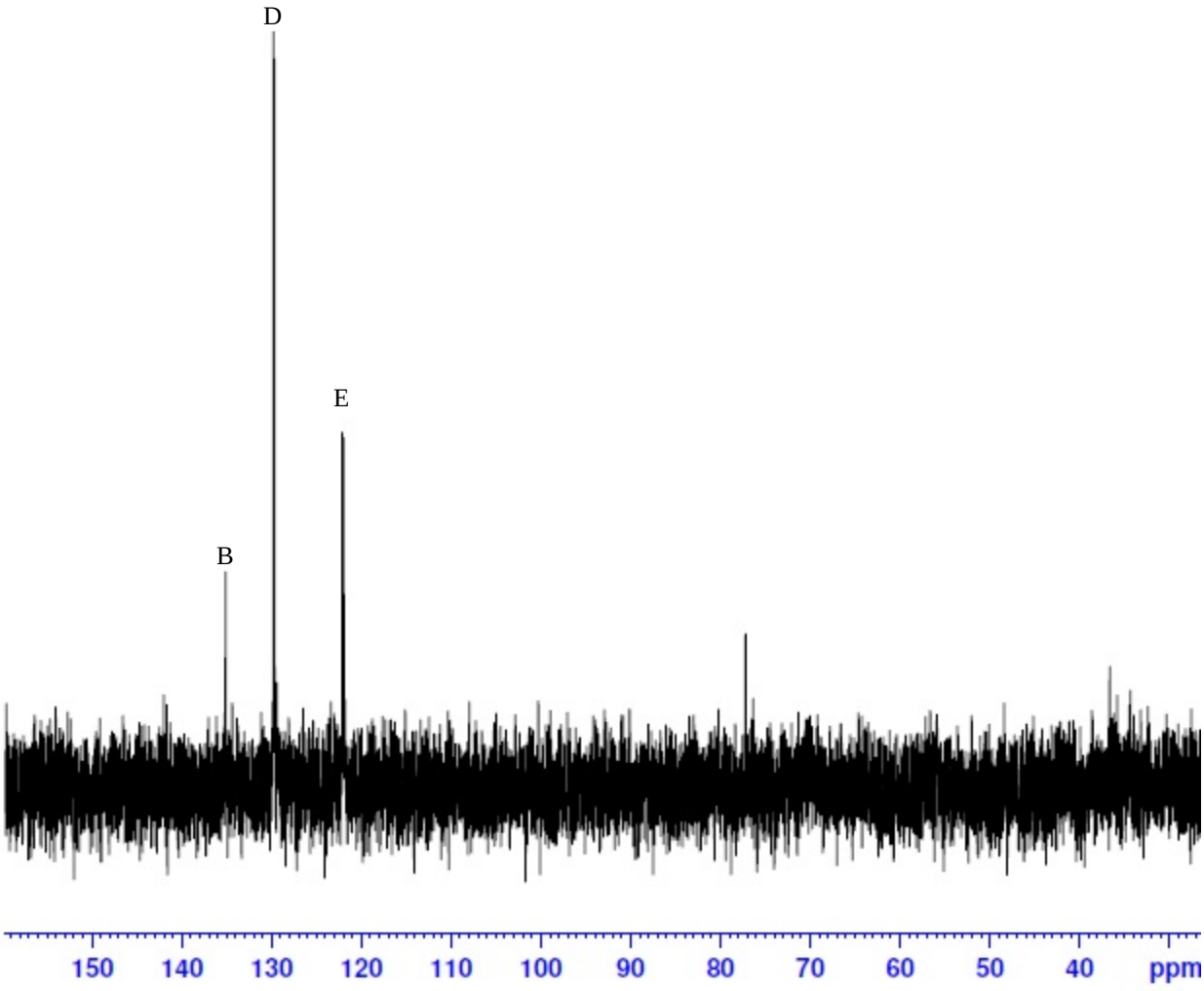

Figure 24: ^{13}C DEPT 90 spectrum of Product 10

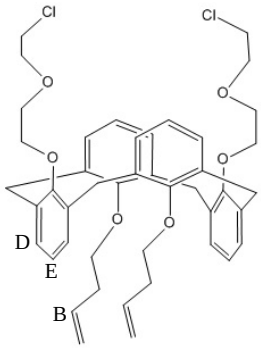

Figure 25: Product 10 structure labeled with corresponding 13C DEPT 90 peaks

4.3.5. ^{13}C DEPT 135

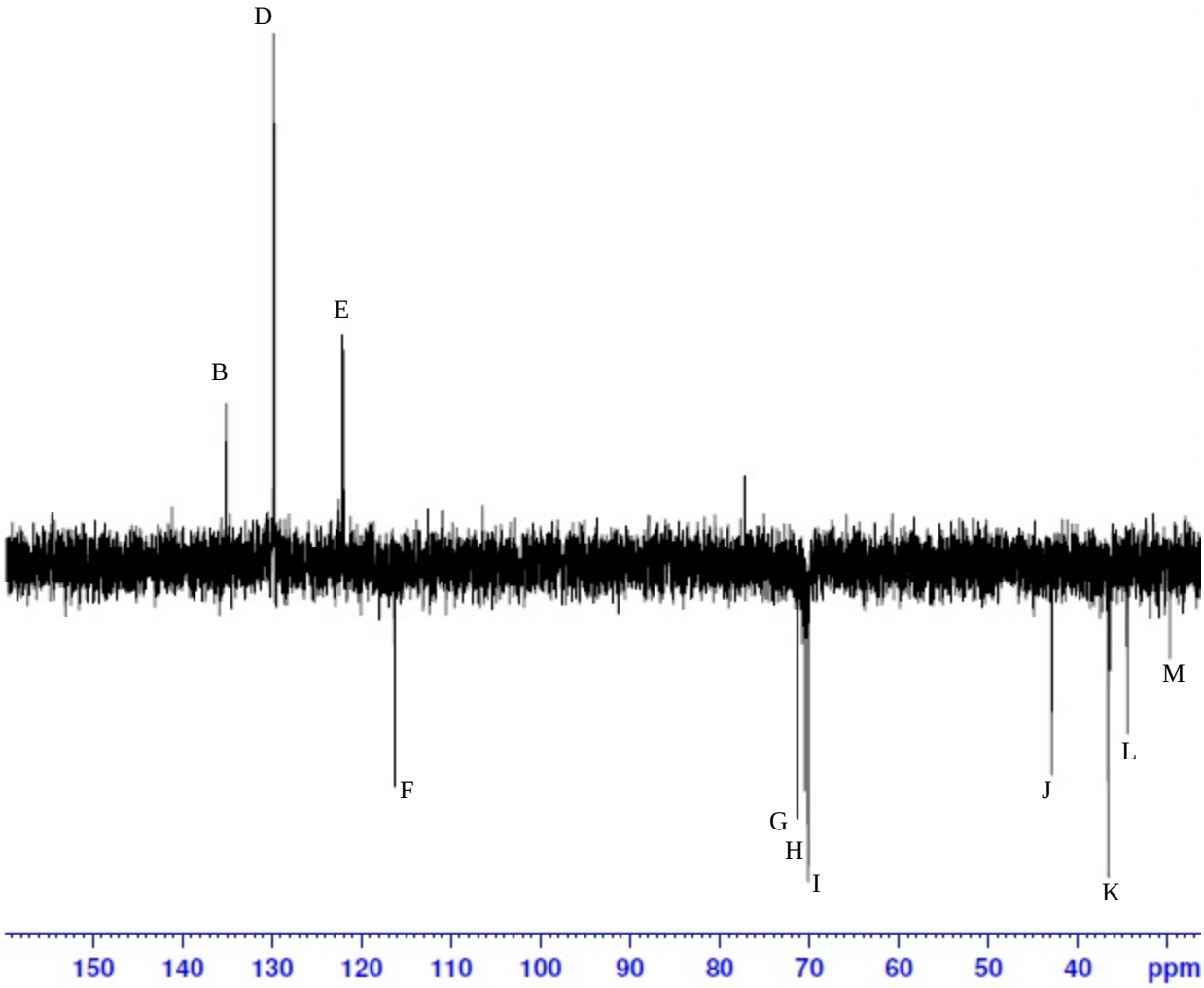

Figure 27: ^{13}C DEPT 135 spectrum of Product 10

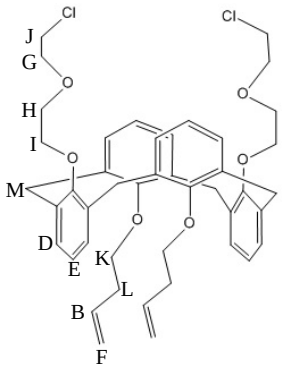

Figure 26: Product 10 structure labeled with corresponding 13C DEPT 135 peaks

4.3.6. Combined ^{13}C spectra

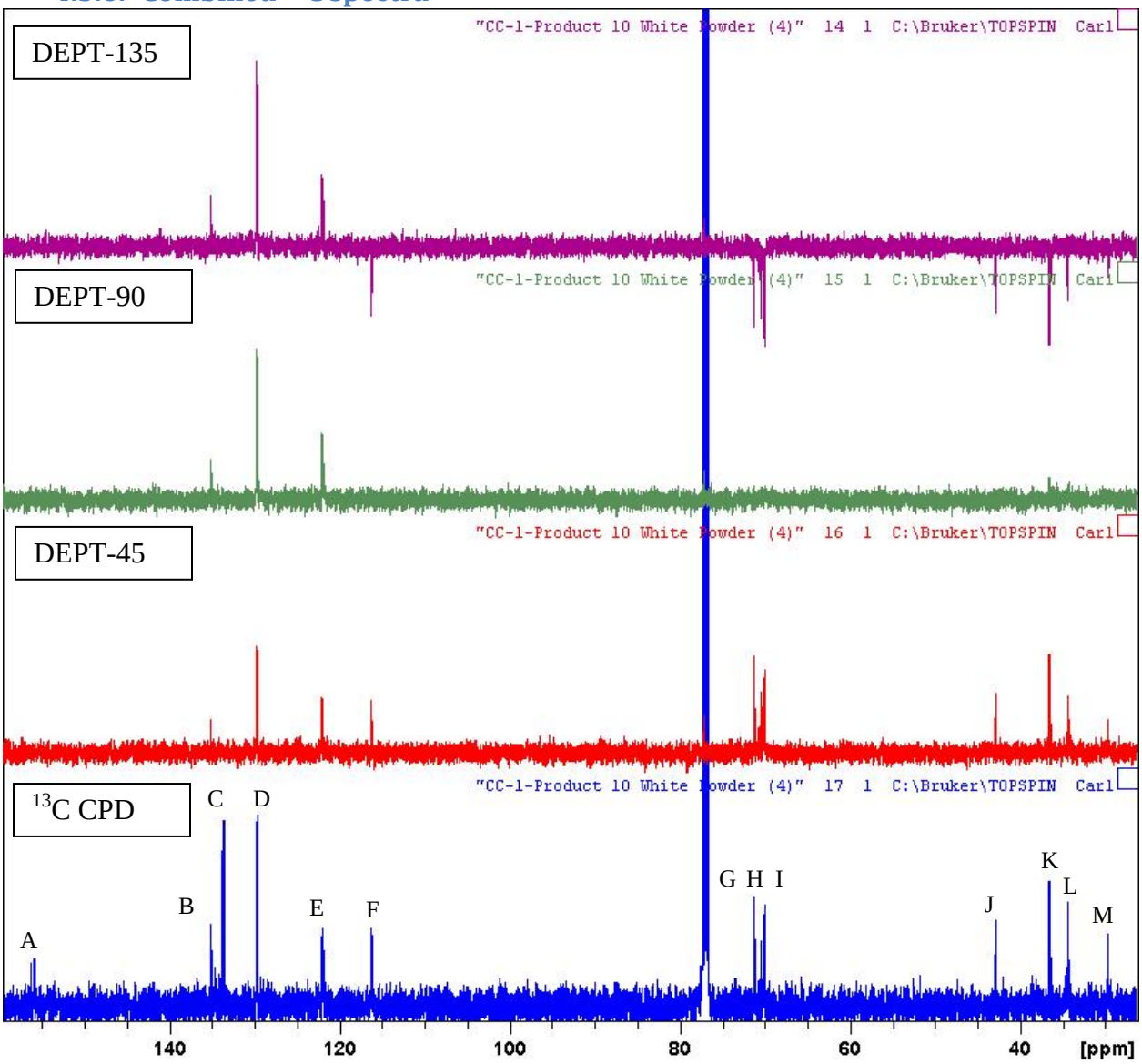

Figure 28: ^{13}C CPD, DEPT 45, 90 & 135 spectra viewed simultaneously, to ease assigning peaks to carbons in molecule.

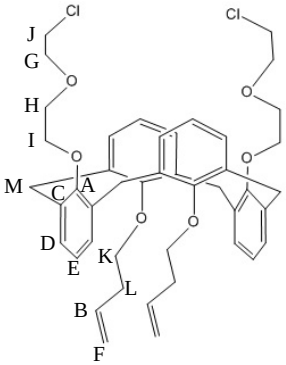

Figure 29: Structure of Product 10 with hypothesized carbon NMR peaks corresponding to ^{13}C NMR data

4.3.7. Mass Spectrum

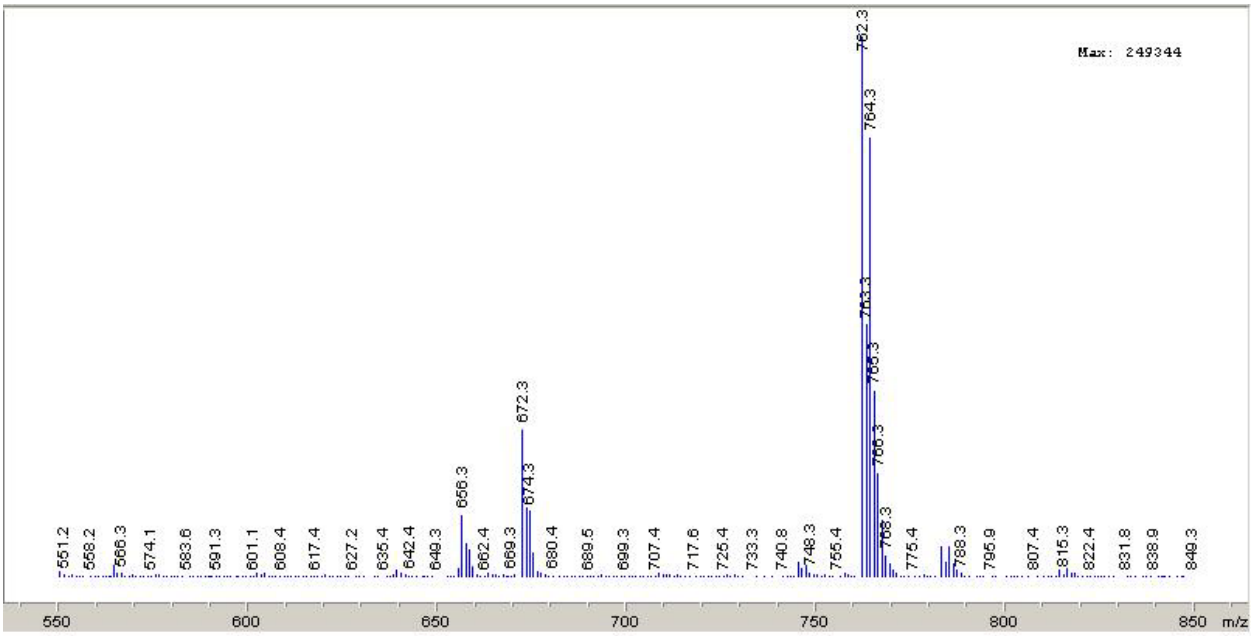

Figure 30: Product 10 mass spectrum. Chemical ionization, m/z = 762

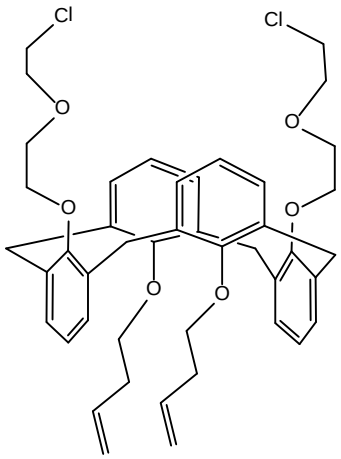

Figure 31: Product 10, molecular mass 745 g/mol

5. Conclusions, Difficulties and Reccomendations

5.1. Conclusions

This report details the synthetic scheme for the synthesis of a target molecule hypothesized to be an ionophore capable of reversibly complexing with K^+ and NH_3^+ ions, depending on the pH of the overall system. The synthesis was carried out, however held up at numerous points by various complications, which will be outlined in section 5.2. As of the submission of this report to the faculty of the Department of Chemistry and Biochemistry at WPI, and to the administration of Worcester Polytechnic Institute in fulfillment of the author's degree requirement, the following has been accomplished.

1.8 g Product 9 successfully synthesized. Product was characterized using 1H NMR spectroscopy and mass spectrometry (see section 4.2. for spectra). As recrystallization was used as the preferred method of purification, an effective recrystallization procedure was developed. The author suggests that future synthesis of this molecule use this recrystallization procedure to purify this compound. The details of the procedure can be found in section 3.2.

280 mg Product 10 has been synthesized. Of that 280 mg Product 10, 20 mg was obtained in the 1st 100 mg scale synthesis and characterized by ^{1}H, ^{13}C CPD, DEPT 45, 90 & 135 NMR spectroscopy, and mass spectrometry. These spectra can be seen in section 4.3. An additional 260 mg is believed to have been obtained from a second synthesis, using 500 mg Product 9 reagent. As of writing, this product has yet to be separated or characterized.

No further syntheses were carried out. The next section will discuss reasons why no further progress was made.

5.2. Difficulties and Recommendations

This section will outline the difficulties the author encountered over the course of the project and potential methods to minimize or eliminate those difficulties for future work. Section 5.2.1 outlines difficulties relating to equipment and instrumentation, while section 5.2.2 outlines difficulties stemming from the actual chemistries and physical properties of the compounds worked with.

5.2.1. Equipment & Instrumental Difficulties

One of the biggest issues the author faced over the course of the project was a lack of effective methods of concentrating and drying product solutions, due to a lack of well-maintained vacuum pumps. As a result, the author was forced to make use of rotary evaporators in other laboratories, which in turn required supervision from graduate students in those labs. Before further work on this project commences, it is highly suggested that vacuum pumps are in good working order so that rotary evaporation may be carried out whenever necessary. This will allow much faster turnaround times between finishing reactions and characterization tests and carrying out subsequent reactions, in turn allowing for more progress to be made.

Another important thing to note is the use of mass spectrometry in the synthesis of the target ionophore. Several runs reveal that the most effective method mass spectrometry technique is the chemical ionization method, as opposed to electron spray ionization. The reason for this is that these functionalized calixarene compounds are not easily ionized, which must occur for compounds to be detected by the detection apparatus in the mass spectrometer. Chemical ionization is a more powerful ionization method, and this method was found to yield ionized compounds which were capable of being detected by the instrument.

5.2.2. Chemistry Difficulties

The first difficulty arising from the chemistry of the calixarene compounds is solubility. These compounds have been found to have low solubility in most common laboratory solvents, forcing the author to run reactions at low concentrations, resulting in much longer reaction times. Furthermore, the necessity of monitoring each reaction with TLC presents many opportunities for human error to hurt the progress of a reaction, when samples are being taken for TLC analysis. The author suggests further research into calixarene chemistry to see if these reactions can be run at higher concentrations, in turn reducing the overall run time and chance for human error to hinder progress. It may also be worth trying running reactions in 2-neck round bottom flasks so that reactions do not need to be stopped to run TLC analysis. However, this consideration must be weighed against the necessity of keeping solvent from boiling off by providing another route for it to escape the reaction setup.

The second difficulty that results from the chemistry of the calixarene compounds occurs in the second step of the overall synthesis, in which the calixarene functionality must be directed to the 1,3 alternate from the cone alternate of the starting material. The method used (see section

3.4) does not go in terribly good yield, only giving about 50% of raw product as the desired 1,3 alternate. More research into published methods of conformational control in calixarenes may reveal better methods by which the desired product in this step can be obtained in good, or better, yield.

An additional difficulty that arises during the second step of the synthesis is separation of the desired 1,3 alternate from additional products that form over the course of the reaction. Column chromatography was used in this step, however the separation achieved is not ideal, as mixtures of desired product and undesired alternates were recovered alongside the desired product, which itself is likely not completely pure, based on ^{1}H NMR analysis (see section 4.3.1). As in the case of selecting between alternates, more research into published methods of separating calixarene compounds which differ only in their conformational pattern may enable higher yields.

Furthermore, low yields in general of many of the reactions also present issues. Because of these low yields, early steps of the synthesis must use larger amounts of material, with the first step in the synthesis requiring large amounts of an expensive starting material. Again, more research into more recently published methods of synthesizing calixarene ionophores may result in higher yield reactions, which will reduce the need for costly starting materials.

The last difficulties that arose during the course of the project were related to the scale of the syntheses carried out. During the 200 mg trial scale syntheses of the first product, Product 9 (see section 3.3), many attempts were made to recrystallize what little product was obtained in an attempt to purify it. This proved to be very difficult considering the high solubility of the product in dichloromethane and small volumes of solvent required for recrystallization to be effective at that scale. Similarly, the 1st synthesis of Product 10, which used 100 mg Product 9 starting material, yielded very little pure product after separation, which has prevented good, clean NMR spectra from being obtained. Running future reactions at larger scale ought to eliminate or minimize these issues, so that is suggested for future work on this project.

6. Acknowledgements

I would like to take this space to extend my sincerest thanks to all who helped me in this project:

To Professor Christopher R. Lambert, thank you for all your guidance over the past few years. Your guidance as both an MQP advisor and employer has been invaluable in helping me to become the student, scientist and person that I am today. I wish you the best of luck in all your future endeavors, both scientific and otherwise.

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