Saccharide Sensitive Polyelectrolytes for Charge-based Sensing with a Solid State Nanopore

A thesis submitted in partial satisfaction

of the requirements for the degree of

Bachelor of science

in

Chemistry and Biochemistry

Alexander L. Wollenberg

Spring 2013

Department of Chemistry and Biochemistry, University of California, 1156 High Street, Santa Cruz, CA, 95064

Department of Biomolecular Engineering, University of California, 1156 High Street, Santa Cruz, CA, 95064

The senior thesis of Alexander Wollenberg is approved:

_____

Professor Bakthan Singaram Date

Table of Contents

I. Acknowledgments

I am very grateful for all of the help I have received throughout this project. I would like to take the time to thank everybody that has participated in this project.

- Dr. Boaz Vilozny[2]..... (post doctoral researcher)
- Dr. Paolo Actis[2].....(post doctoral researcher)
- Daniel Hwang[2].....(undergraduate researcher)
- Ace Galermo[1,2].....(undergraduate researcher)
- Charlotte Medina[2].....(summer intern)
- Angel Resendez[1].....(graduate student)
- Singaram Labs[1].....(all members)
- Pourmand Labs[2].....(all members)
- Dr. Bakthan Singaram[1].....(principle investor)
- Dr. Nader Pourmand[2].....(principle investor)

[1]Department of Chemistry and Biochemistry, University of California, 1156 High Street, Santa Cruz, CA, 95064

[2]Department of Biomolecular Engineering, University of California, 1156 High Street, Santa Cruz, CA, 95064

II. Abstract

This work presents the synthesis and characterization of a sugar sensitive polymer (m-PVP-BA) which was incorporated into a nanopore, a quartz conical channel with an opening of 20-40 nm. Combining m-PVP-BA with a solid state pore allowed for the development of a chemosensor used for the detection of monosaccharides. The polymer is based on poly(4-vinylpyridne), and was functionalized with a non-enzymatic sugar receptor known as phenyl boronic acid. Using spectrophotometry, the affinity for the polymer with several dyes was measured in solution. This made it possible to quantify two different variables that affected the affinity of the m-PVP-BA to a substrate: reversible covalent bonds between boronic acid and diols, and electrostatic interactions. By exploiting the properties of the polymer we were capable of designing a sensor that relied on charge-based sensing to induce a change in electrical current. The sensor provided a real time reversible signal that was capable of detecting concentration of fructose lower than 700 μM.

III. Introduction

a) Importance of Saccharides in Biological Systems

The role that saccharides play in biological systems is extremely important in the study of metabolism. Furthering our understanding of metabolism has the potential to lead to many medical breakthroughs. For instance, there is a strong link between the breakdown of D-glucose transporters and the following diseases: renal glycosuria[1,2], cystic fibrosis[3], and diabetes[4,5]. Therefore, it is of paramount importance to design new sensors and probes that can identify different types of saccharides in biological systems.

b) Boronic Acid

Boron is a very versatile element that can form many different types of compounds. Some of which are known as oxygenated organoboron compounds (Figure 1)[6]. The scope of this project will cover only boronic acid, which possesses an alkyl substituent and two hydroxyl groups. Boronic acid is not naturally occurring and was first synthesized in 1860.[7]

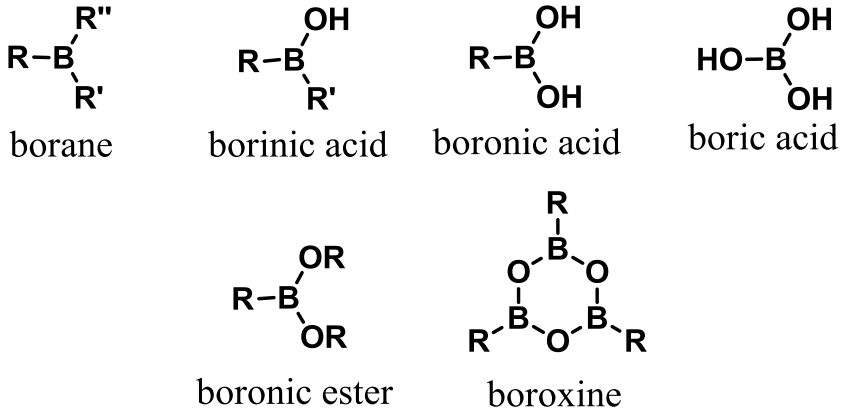
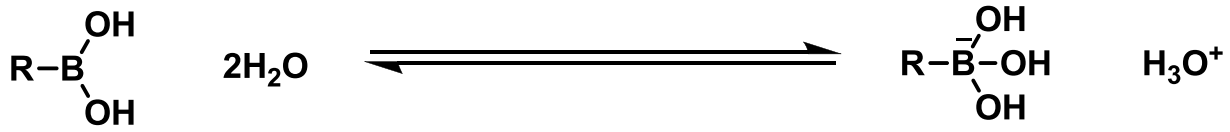


Figure 1: Oxygenated organoboron compounds.

In 1955 Edwards and Morrison showed that in basic conditions, boronic acids have the ability to form an anionic compound known as boronate.[8] The formation of this compound is caused by the lewis acidity of the boron, it has the ability to ionize water and form hydronium ions through a proton transfer (Scheme 1).



Scheme 1: Lewis acidity of boron ionizing water.

A few years later Lorand and Edwards discovered that boronic acids can interact with compounds that contain 1,2-cis diols to form a boronic ester.[9] The formation of this complex increases the electrophilicity of the boron which increases its lewis acidity and allows the boronic ester to shift its equilibrium towards the anionic boronate ester. For example, phenyl boronic acid possesses a pKa of 8.8, but in the presence of glucose and fructose its pKa is lowered to 6.8 and 4.5, respectively.[10,11]. If the pKa of the boronic acid is shifted below the pH of the overall solution it will allow the compound to go from a neutral boronic acid to an anionic boronate ester (Figure 2). This property has proven to be useful in the design of many different

sugar sensors and probes. Singaram has designed a bisboronic acid compound that quenches the fluorescents of a dye. Upon the presents of a monosaccharide the fluorescents is returned.[12,13] Singaram's system has also been applied to a thin film hydrogel[14] where current work is being done to embed it into a fiber optics cable to transmit the fluorescents signal.

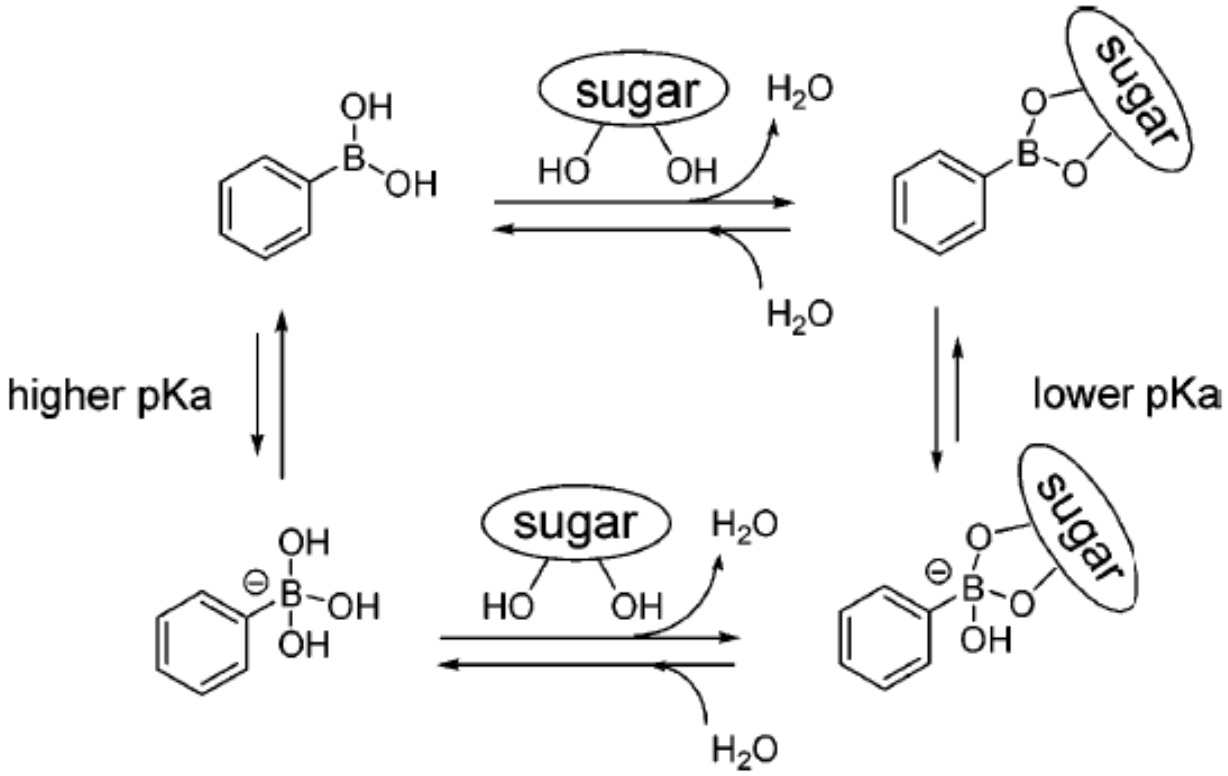


Figure 2: The reversible binding of boronic acid to 1,2-cis diols causes a change in its pKa to favor the anionic boronate ester complex.

c) Nanopores and Their Use as Analytical Devices

Nanopores have become increasingly popular due to their ability to serve as a label-free, real-time biosensor. They are capable of performing single molecule detection of biomolecules, which can lead to high sensitivity[15] Nanopores have recently been modified with different proteins and enzymes in order to detect mono and disaccharides.[16,17,18] There were two recent publications where nanochannels were modified with boronic acids as a sugar receptor.[19,20]

Developing such sensors can be convenient for glucose detection because they are non-enzymatic and they use electrical signal. Most glucose sensors rely on enzymes and a colorimetric test.

A nanopipette is a type of nanopore which can be thought of as a conically shaped tube, that is hollow in the center, and converges to a point with a 30-50 nm pore. Unlike most nanostructures, nanopipette fabrication is very inexpensive, quick, and simple. A popular technique used is laser pulling of a quartz capillary tube using a pipette puller (Figure 3-A). This results in a conically shaped pore with silicon hydroxyl groups on the surface (Figure 3-B). By adjusting the pulling parameters, of the micropipette puller, different sized pores can be achieved. Although this technique is convenient, it is very difficult to reproduce pores with nanometer accuracy. There have been other techniques employed to increase the reproducibility, such as etching[21]

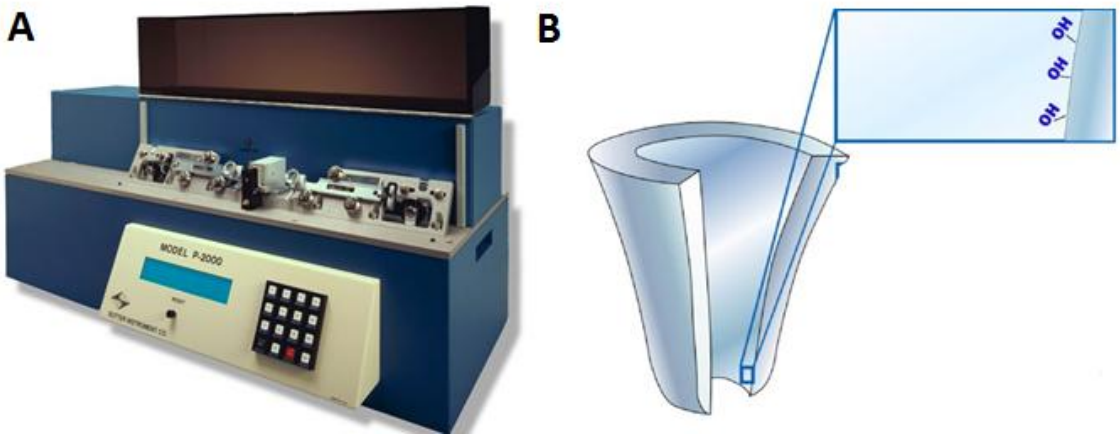


Figure 3: (A) P-2000 laser puller (Sutter Instrument Co.) used for nanopipette fabrication. **(B)** Conically shaped nanopipette with surface hydroxyl groups.

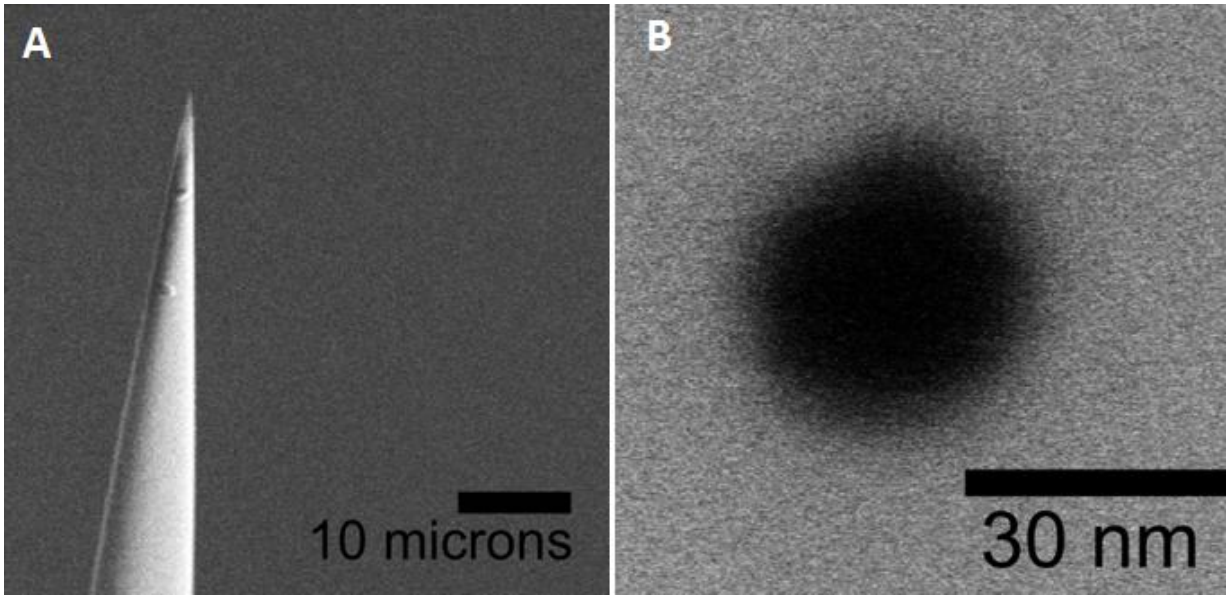


Figure 4: SEM images of **(A)** a conically shaped nanopipette **(B)** with a pore diameter of approximately 30 nm.

As mentioned previously nanopipettes can serve as a biosensor. In order to apply this nanostructure as a sensor, it is used in an electrochemical cell. The nanopipette, acting as the working electrode, is backfilled with the working buffer, and a Ag/AgCl electrode is inserted. Another Ag/AgCl electrode is placed in bulk solution acting as auxiliary/reference electrode. Both electrodes are connected to the Axopatch 700B amplifier with the DigiData 1322A digitizer (Molecular Devices), and a PC equipped with pClamp 10 software (Molecular Devices) (Figure 5).[22]

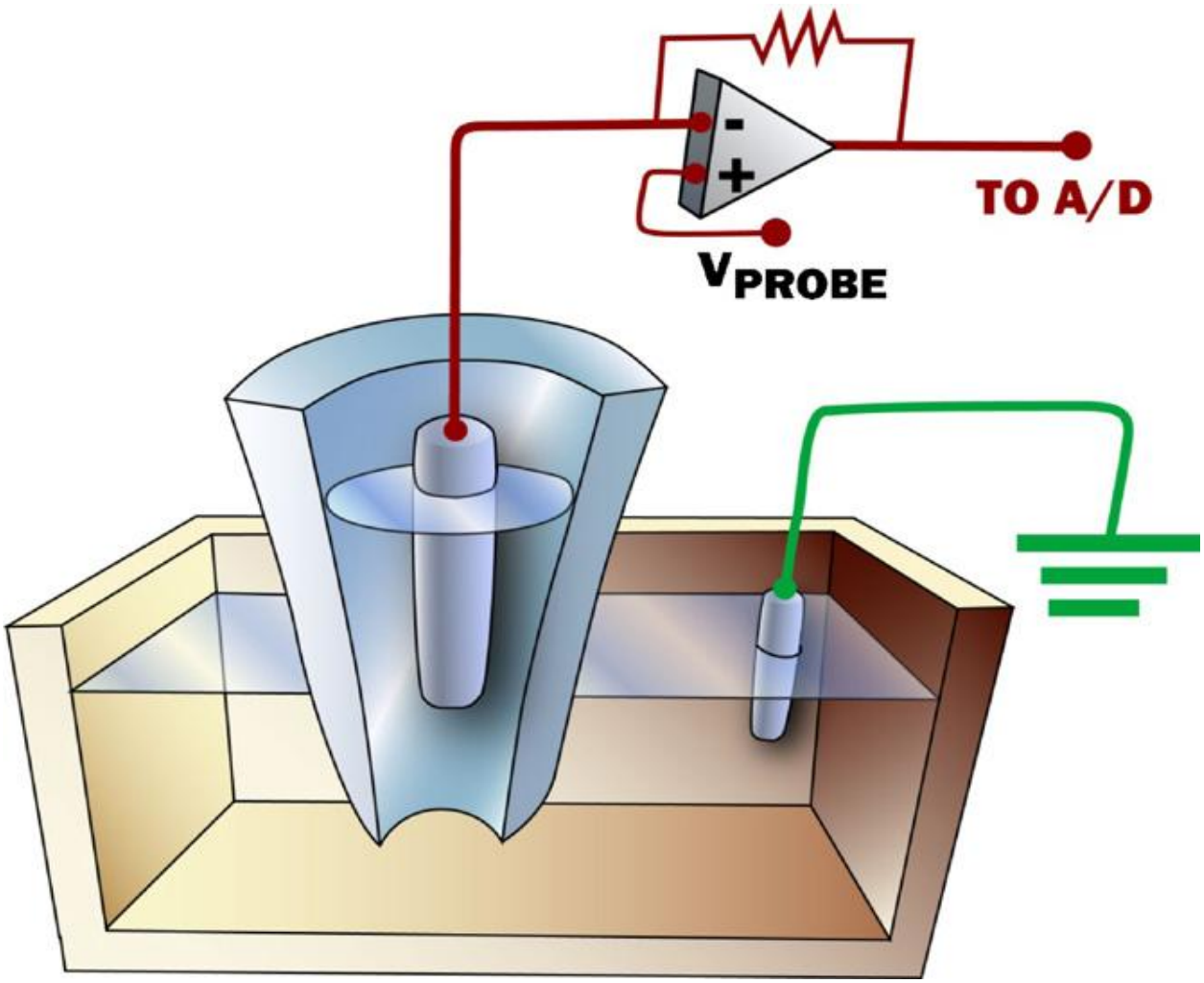


Figure 5: Demonstrating how nanopipettes can be assembled into an electrochemical cell used for measuring electrical signals.

When applying a symmetric amount of voltage to a nanopipette, there is an asymmetric amount of current produced. This asymmetric amount of current produced is known as current rectification. Positive current rectification is when the amount of positive current is greater than the amount of negative current. Similarly, negative current rectification is when there is a larger amount of negative current than positive current.

The effects of asymmetric current is caused by the formation of a diffuse electric double layer, which is represented in Figure 6.[23] The formation of a "double layer" occurs when the

charged surface of the nanopipette causes stacking of oppositely charged ions. The electrostatic interactions between ionic species and surface charge will effect ion transport properties. This asymmetric current effect is only seen on the nano scale. When working with quartz nanopipettes the double layer effect has shown to be affected by electrolyte concentration, pH, and applied voltage.[24,25] A micropipette will not produce the same result because the size of the double layer is insignificant in comparison to the size of the pore.

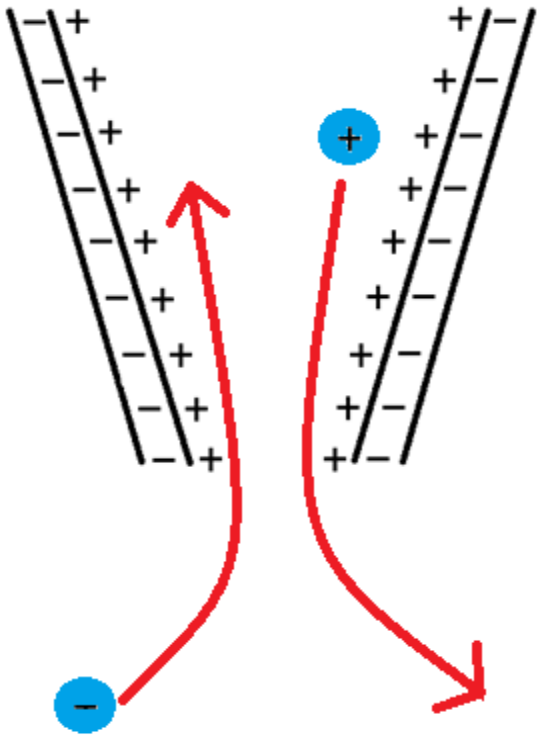


Figure 6: A diffuse electric double layer is formed when the oppositely charged ions stack on the surface of the charged nanopipette. This effect is responsible for current rectification.

It has been shown that the current rectification, of a nanopipette, can be completely switched from negative rectification, to positive rectification by modifying the surface of the pipette with poly-l-lysine, cationic dendrimers, and PEG-modified nanopipettes.[26,27,28] A rule of thumb, when working with nanopipettes, is that a negatively charged surface will lead to

negative rectification, and a positively charges surface will lead to positive rectification. Figure 7 reflects the surface charge compared to the current.

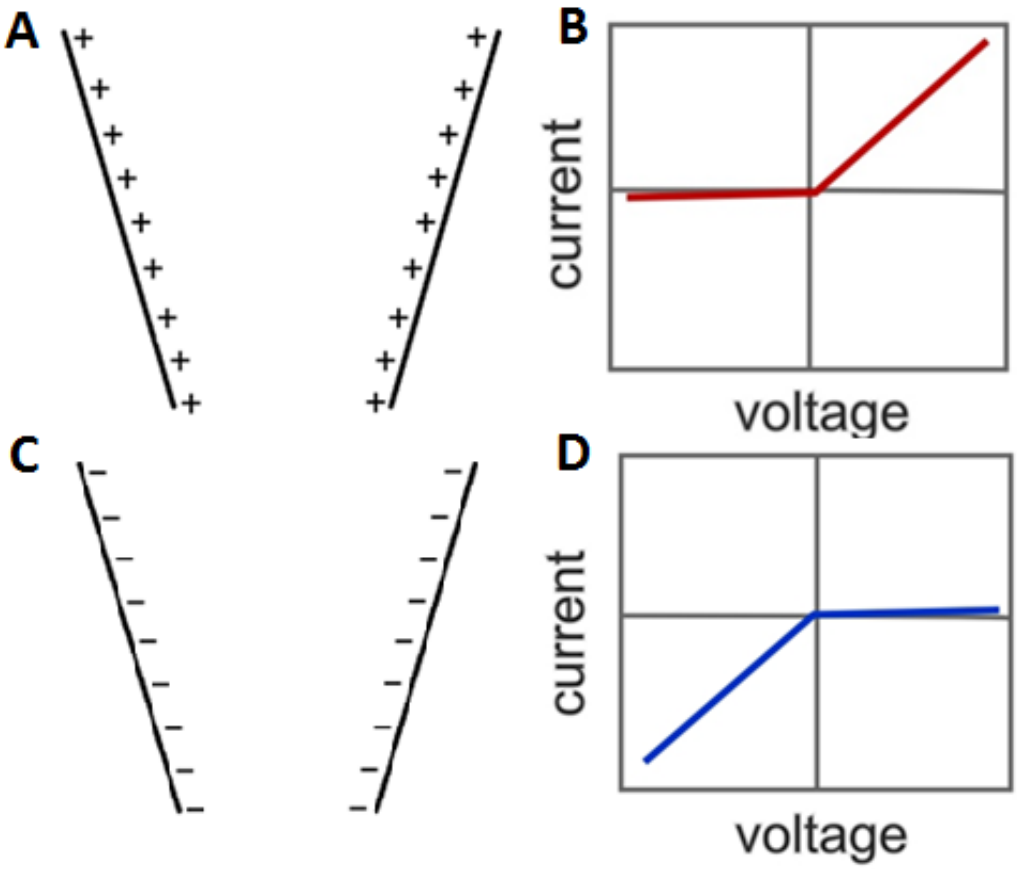
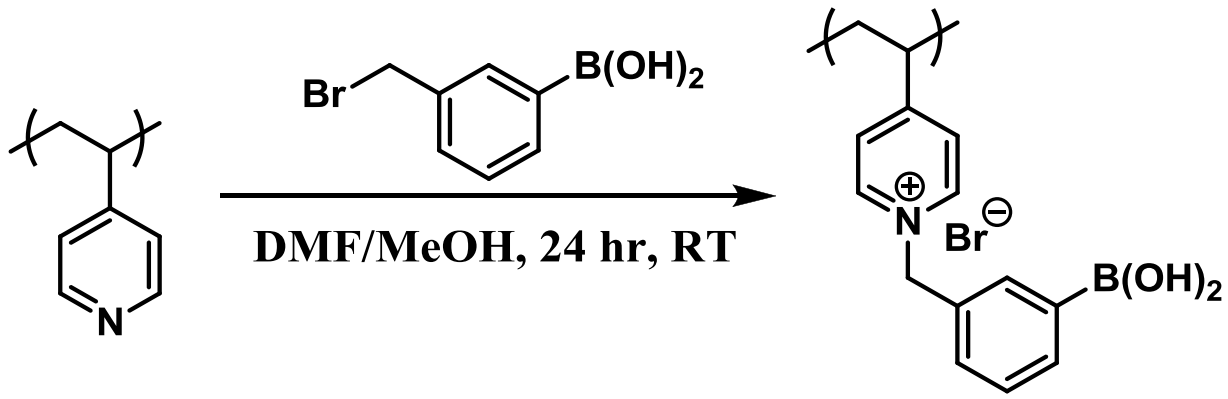


Figure 7: A **(A)** positively charged surface leads to **(B)** positive rectification, and a **(C)** negatively charge surface leads to **(D)** negative rectification.

d) Introduction to the Project

The scope of this project covers the synthesis and characterization of a sugar sensitive polymer (m-PVP-BA). This polymer is based on poly(4-vinylpyridine) which has been covalently modified with a non-enzymatic sugar receptor known as phenyl boronic acid (Scheme 2).



Scheme 2: Synthesis of m-PVP-BA

Using spectrophotometry, the affinity of the polymer with several dyes was measured in solution. This made it possible to quantify two different variables that affected the affinity of the m-PVP-BA to a substrate: reversible covalent bonds between boronic acid and diols, and electrostatic interactions.

After successfully characterizing the properties of m-PVP-BA we incorporated it into a nanopore, a quartz conical channel with an opening of 20-50 nm. Modifying a nanopipette with the polyer allowed for the development of a chemosensor used for the detection of monosaccharides. By exploiting the properties of this polymer, we were capable of using charge-based sensing to produce a change in an electrical signal. This method provided us with real time, reversible detection that was capable of sensing concentration of fructose lower than 700 μM.

IV. Results and Discussion

The synthesis of m-PVP-BA was carried out through a substitution reaction involving Poly(4-Vinylpyridine) (M.W. 60,000) and m-bromomethyl phenylboronic acid. The formation of this product resulted in a cationic polyelectrolyte that has exactly one positive charge for every addition of phenylboronic acid. Polymer m-PVP-BA was synthesized with a 90 % yield and is soluble at low concentrations (1% m/v) in methanol, methanol/water (1:1), acidic, and basic solutions. It was insoluble in other common laboratory solvents.

In order to optimize the reaction conditions and yield, we attempted this reaction in many different solvents. The reaction occurred very rapidly when using a polar aprotic solvent such as DMF. Unfortunately, the reaction would not go to completion because the product would precipitate out of solution. Knowing that the product was more soluble in methanol, we attempted to use it as a reaction solvent. This lead to extremely slow reaction kinetics, even when heat was applied. Therefore, we were able to optimize the reaction conditions by mixing the two solvents. We were capable of getting a complete reaction in a modest amount of time without applying any heat.

Studying the speed of this reaction, in different solvents, gave us insight into the reaction mechanism. We believe the reaction was proceeding by means of a SN_2 mechanism. It is commonly known that polar solvents will solvate the nucleophile which will not allowing it to attach the electrophile.[29] We can eliminate the $_{SN1}$ mechanism because the rate limiting step is dependent upon the leaving group, and not the strength of the nucleophile. The methanol is solvating the pyridine groups on polymer PVP not allowing it to attach the benzyl carbon.

The percent alkylation of phenylboronic acid was determined using ^{1}H-NMR (Figure 8). The broad signals in the ^{1}H-NMR spectra are; aliphatic (2.1 ppm), benzyl (5.8 ppm), and aromatic (7.3 to 8.9 ppm). Assuming 100% alkylation was to occur there would be an integration ratio of 3 to 2 (aliphatic: benzyl). By normalizing these two peaks according to Equation 1 we achieve a percent alkylation of 82%.

$$\frac{\frac{\boldsymbol{Benzyle}}{\mathbf{2}}}{\frac{\boldsymbol{Aliphatic}}{\mathbf{3}}} \times \mathbf{100\%} = \boldsymbol{Percent\ Alkylation} \qquad \textbf{Equation 1}$$

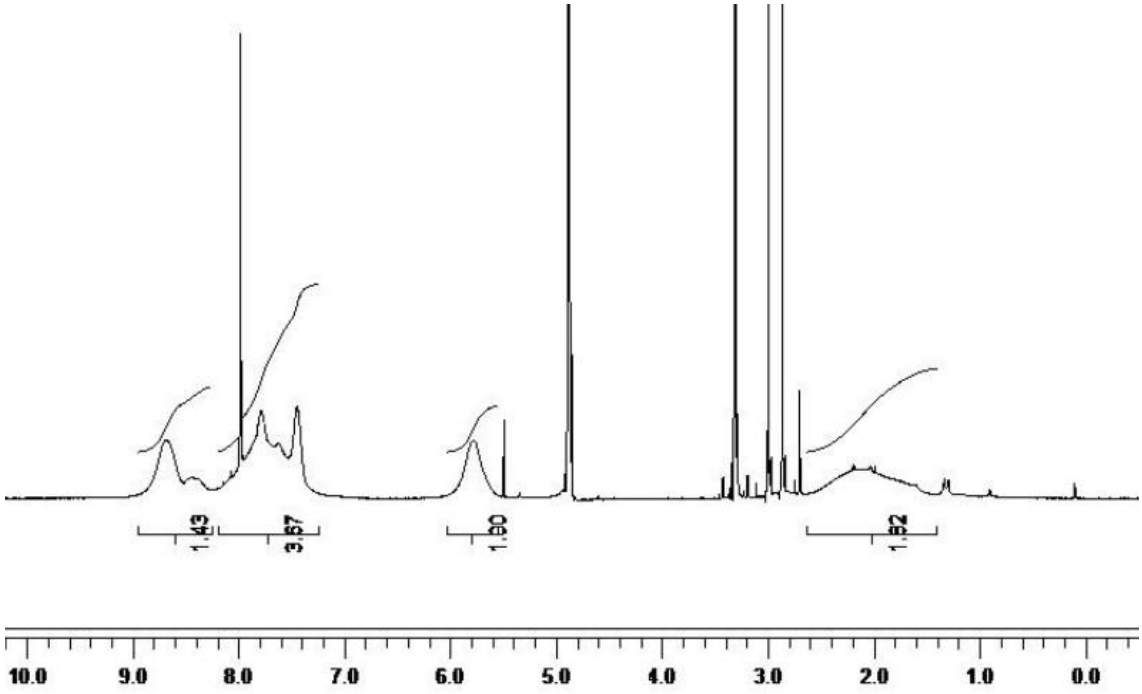
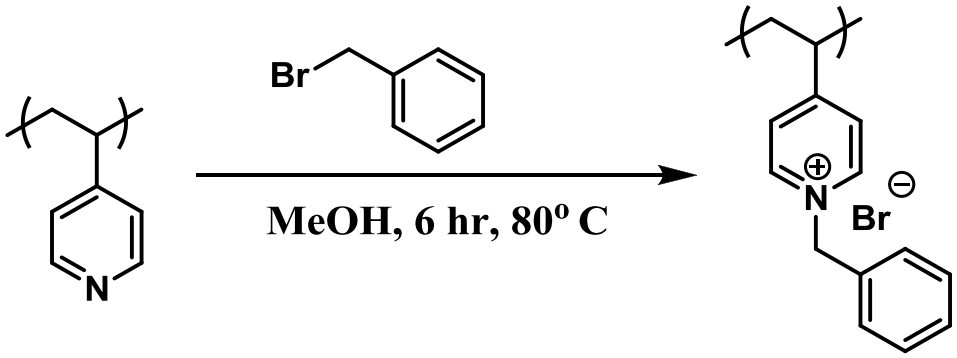


Figure 8: ^{1}H-NMR of m-PVP-BA shows 82% alkylation of phenyl boronic acid. The following peaks are aliphatic (2.1 ppm), benzyl (5.8 ppm), and aromatic (7.3 to 8.9 ppm).

We also synthesized a "control" polymer called poly(4-vinylpyridine) benzyl (PVP-Bn). The synthesis of this polymer was done by performing a substitution reaction between poly(4-vinylpyridine) and benzyl bromide (Scheme 3). This polymer is very similar to m-PVP-BA, but

it lacks the boronic acid group . This polymer will assist us in studying the degree that the boronic acid contributes to the overall interaction between the polymer and analyte.



Scheme 3: Synthesis of the control polymer PVP-Bn.

PVP-Bn was synthesized with a 90% yield. The broad signals in the ^{1}H-NMR were consistent with the aliphatic (2.0 ppm), benzyl (5.5ppm), and aromatic protons (7.0- 9.0 ppm). By integrating these peaks, and applying Equation 1, we calculated a 96% alkylation of the benzyl groups onto the polymer.

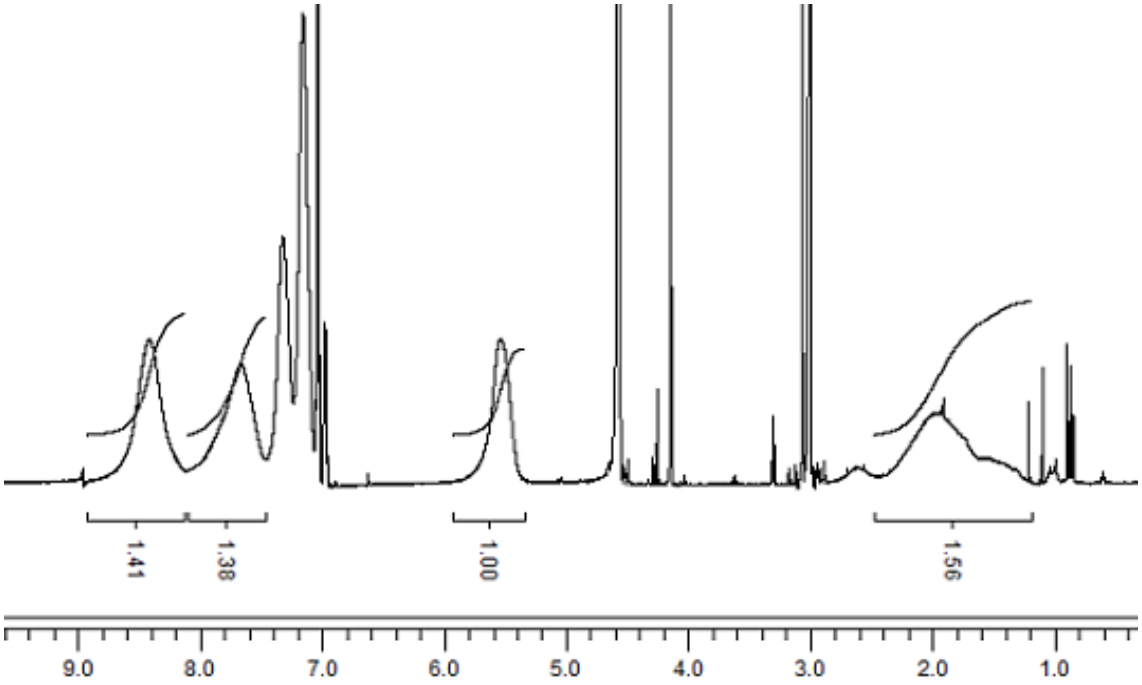


Figure 9: ^{1}H-NMR of PVP-Bn shows 96% alkylation of benzyl. The following peaks are aliphatic (2.0 ppm), benzyl (5.5 ppm), and aromatic (7.0-9.0 ppm).

The solubility of m-PVP-BA is dependent upon pH. At low pH, the polymer is cationic and maintains a "swollen" state, which allows it to be soluble. When the pH of the solution nears the pKa of the boronic acid, the polymer will become zwitterionic (overall neutral) which causes it to form the "collapsed" state and precipitate out of solution (Figure 10).

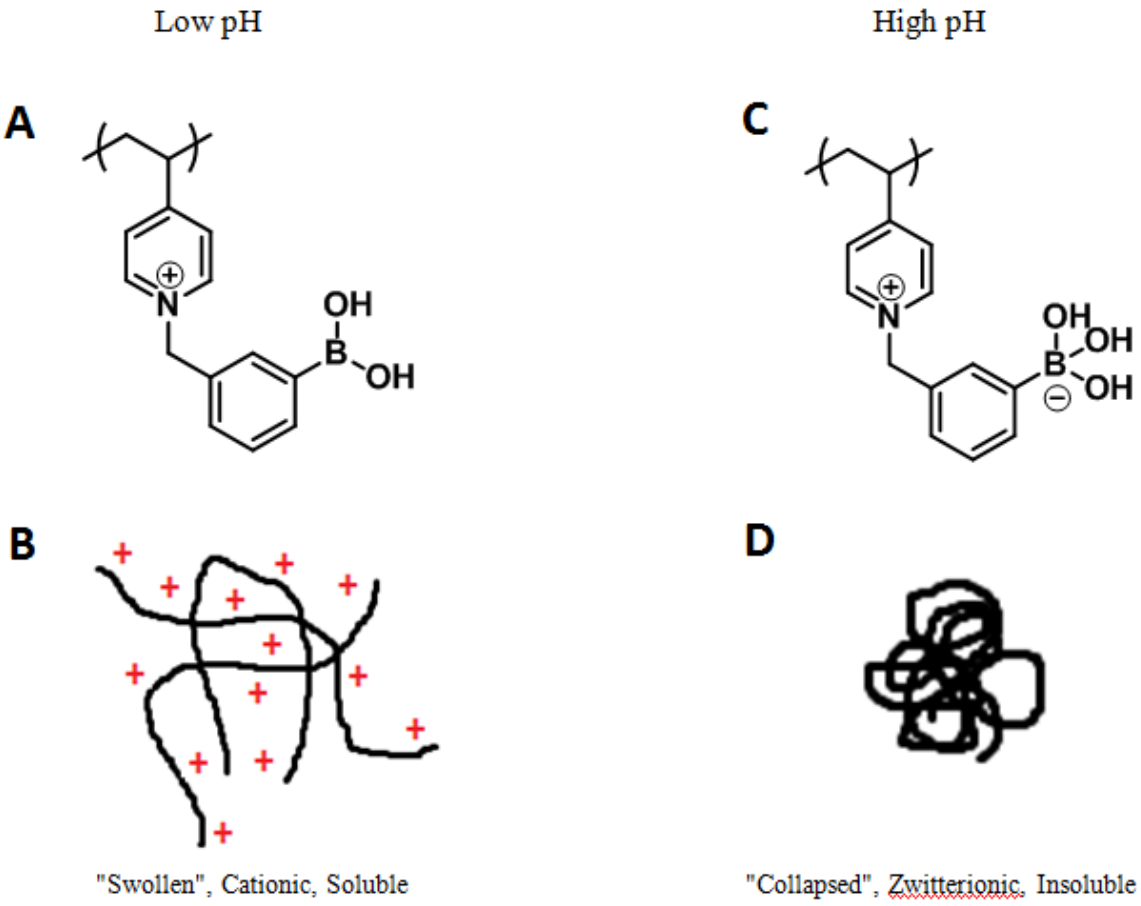


Figure 10: (**A**) At low pH m-PVP-BA is cationic which allows it to remain soluble in the (**B**) "swollen" state. (**C**)When the pH of the solution nears the pKa of the boronic acid, the polymer will become zwitterionic, which allows it to form the (**D**) "collapsed" state and precipitate out of solution.

In order to determine the pKa of the polymer we performed a pH titration. When the pKa of the solution nears the pKa of a phenylboronic acid[30] the polymer precipitates out of solution. This is due to the previous explanation of the polymer going from the "swollen" to "collapsed" state. When repeating this experiment, in triplicate, the precipitation point proved to be highly reproducible.

It is known that the pKa of a boronic ester is lower than the pKa of a boronic acid.[31] By performing this same titration experiment, but this time in the presence of different

monosaccharides, we were able to demonstrate that the precipitation point was dependent upon the pKa of the boronic acid. Phenylboronic acids are known to have a much greater affinity to fructose than glucose. Therefore, the precipitation point of m-PVP-BA in the presents of fructose is expected to occur at a lower pH then when m-PVP-BA is in the presents of glucose. This experiment demonstrates sugar selectivity of the polymer. These results are shown in Figure 11.

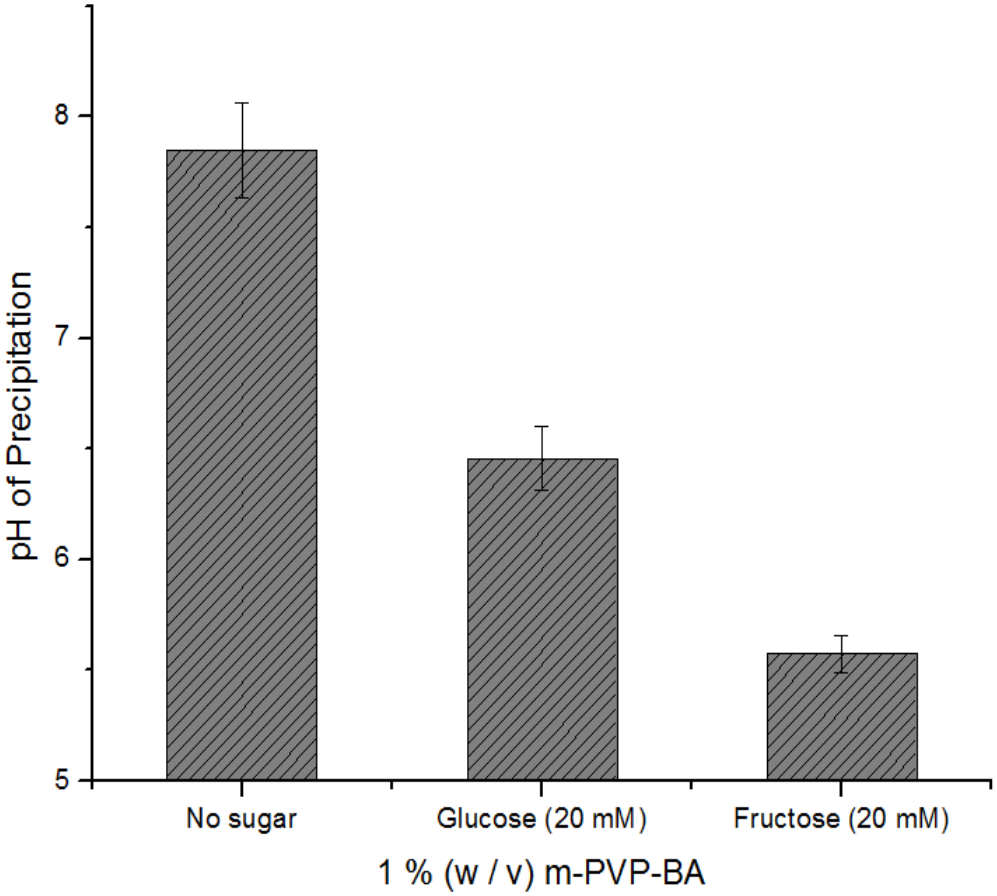


Figure 11: pH titration of 1% m-PVP-BA (w/v) in a solution of methanol/water (1:1). The precipitation point is dependent upon the pH and the pKa of the boronic acid.

By performing UV-Vis spectrophotometry, we were able to determine the attractive forces that influence the affinity that m-PVP-BA had for different dyes. We suspected there would be two forces of attraction; a covalent attraction between boronic acid and diols, and an electrostatic interaction between positive and negative charges. In order to determine this we studied the interactions of m-PVP-BA and PVP-Bn with three different dyes (ARS, HPTS, and Aesculetin).

Alizarin Red S. (ARS) is a dye that contains 1,2-cis diols and one negative charge. In the presents of m-PVP-BA the dye has the potential to interact electro statically and covalently (Figure 13-A). When comparing this dye-polymer complex to the dye by itself, we can see a higher intensity of absorbance which is red shifted (purple line, Figure 12). Because PVP-Bn is lacking boronic acid, we would expect it to interact differently with ARS (Figure 14-A). By analyzing the absorbance data we can see that there was a decrease in intensity (red line Figure 12), which was entirely different from m-PVP-BA.

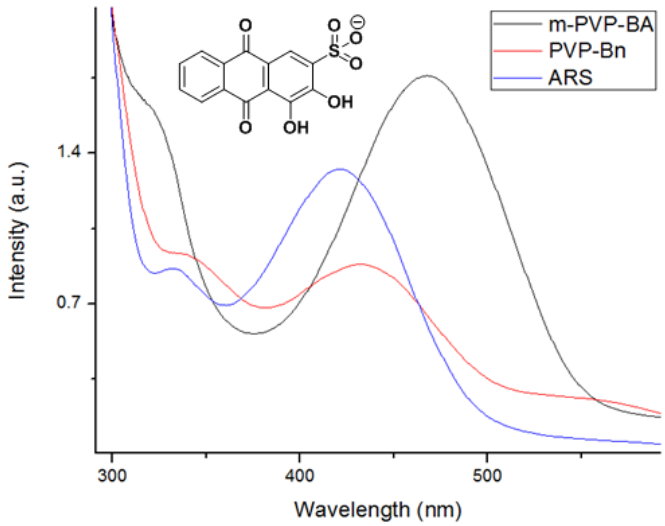


Figure 12: Absorbance spectrum of ARS (blue), ARS with 250 µM m-PVP-BA (purple), and ARS with 250 µM PVP-Bn (red).

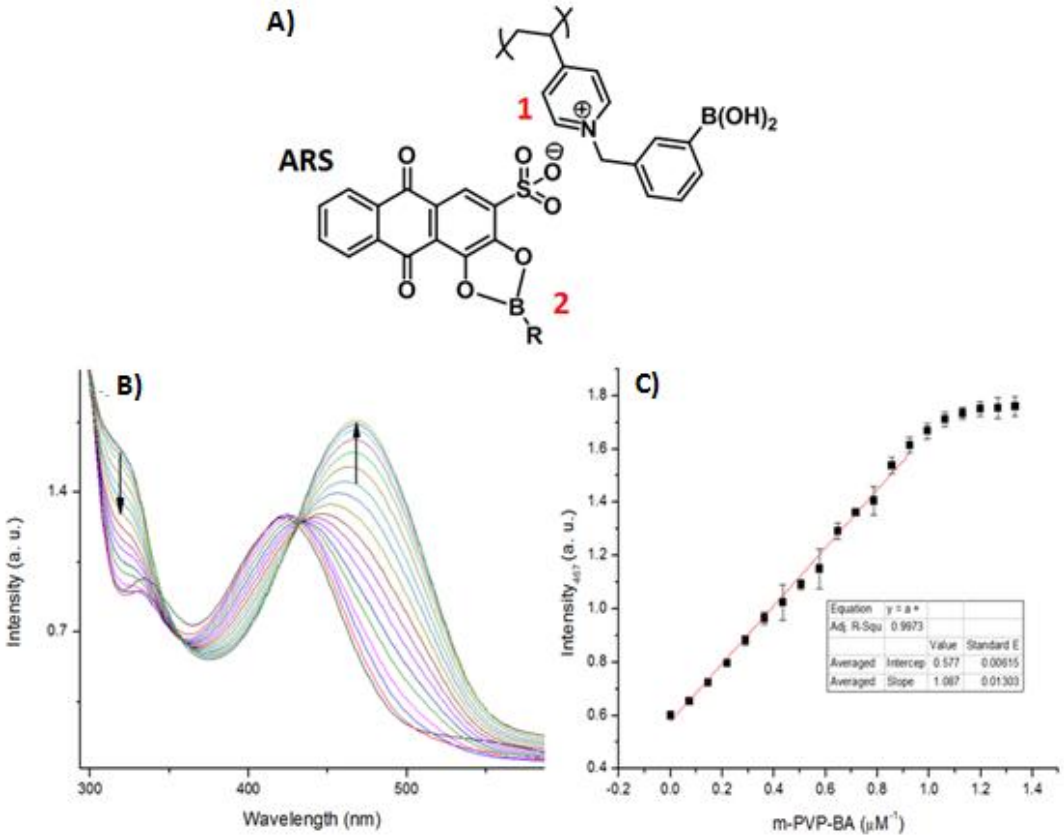


Figure 13: (A) m-PVP-BA can interact with ARS through electrostatics (1) and covalently (2) (R= m-PVP-BA). **(B)** UV-Vis absorbance spectrum of ARS (250 µM) with m-PVP-BA being titrated into cuvette (0.0 - 1.4µM). **(C)** Change in absorbance at 467 nm.

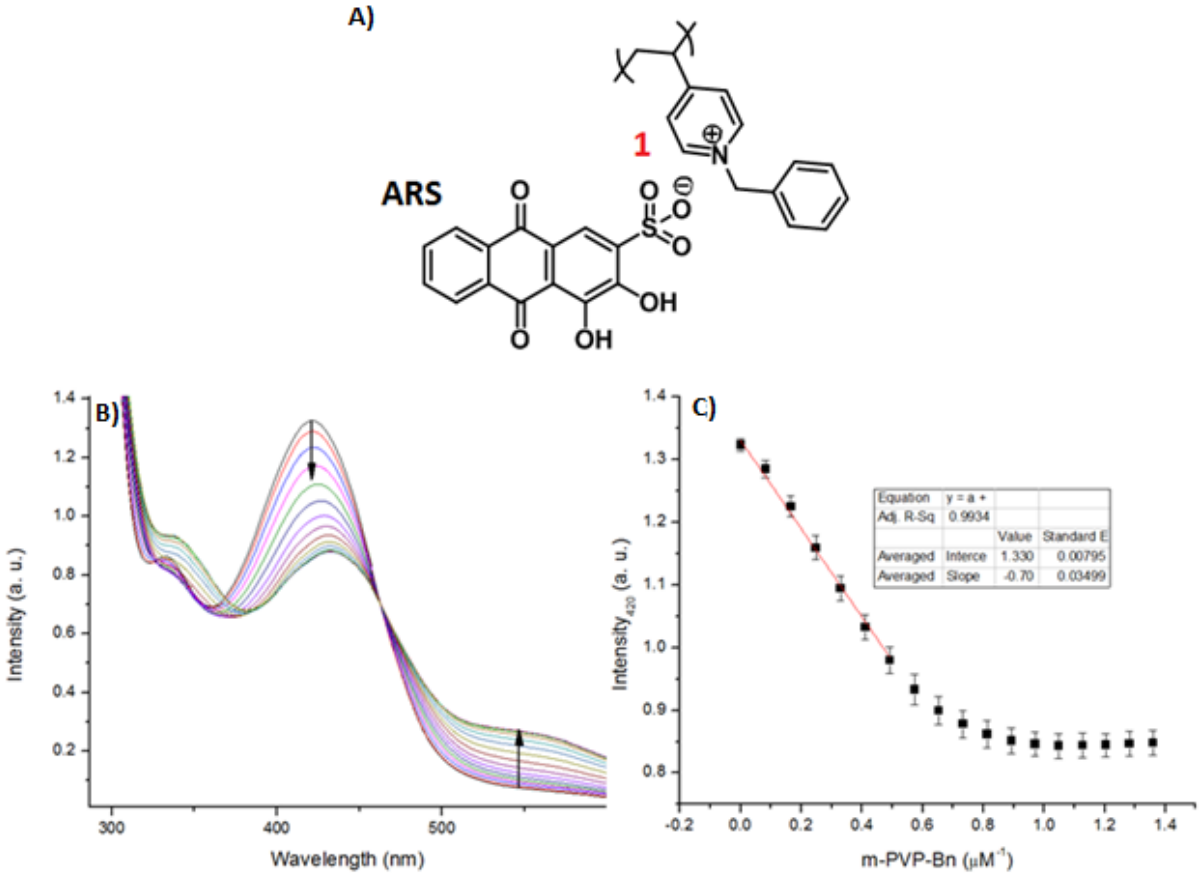


Figure 14: (A) PVP-Bn can interact with ARS electrostatically (1). **(B)** UV-Vis absorbance spectrum of ARS (250 µM) with PVP-Bn being titrated into cuvette (0.0 - 1.4µM). **(C)** Change in absorbance at 420 nm.

The next dye that we used to study the dye-polymer interaction was HPTS. HPTS is an anionic dye that contains three negative charges, but does not posses 1,2-cis diols. This means that HPTS should have a similar electrostatic interaction with m-PVP-BA (Figure 15-A) and PVP-Bn (Figure 16-A). After analyzing the titration curves, (Figure 15-B, 16-B) it is obvious that the change in absorbance, between these two polymers, gives a similar red shift with a decrease in absorbance intensity.

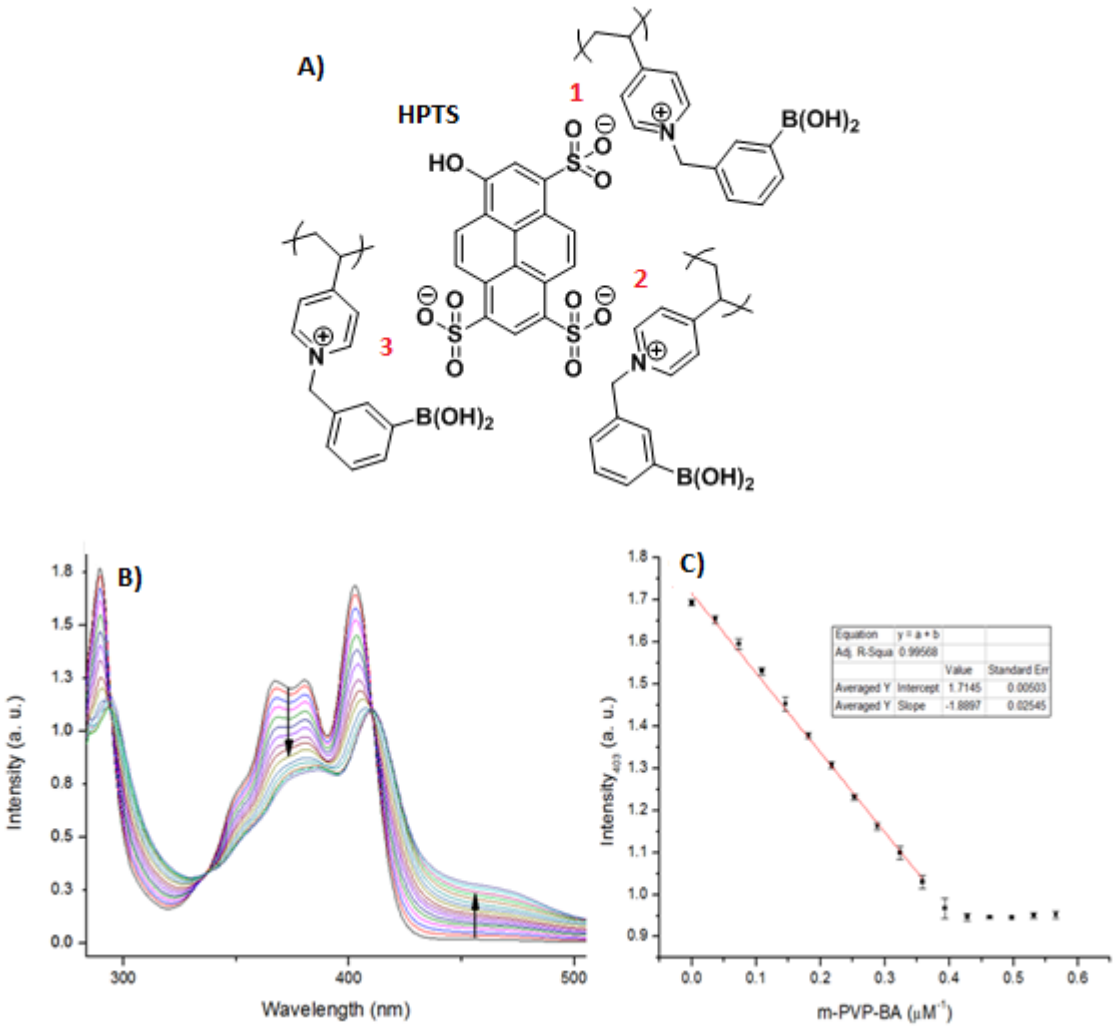


Figure 15: (A) There are three possible electrostatic interactions (1-3) that can occur between HPTS and m-PVP-BA**. (B)** UV-Vis absorbance spectrum of HPTS (80 µM) with m-PVP-BA being titrated into cuvette (0.0 - 0.6µM). **(C)** Change in absorbance at 403 nm.

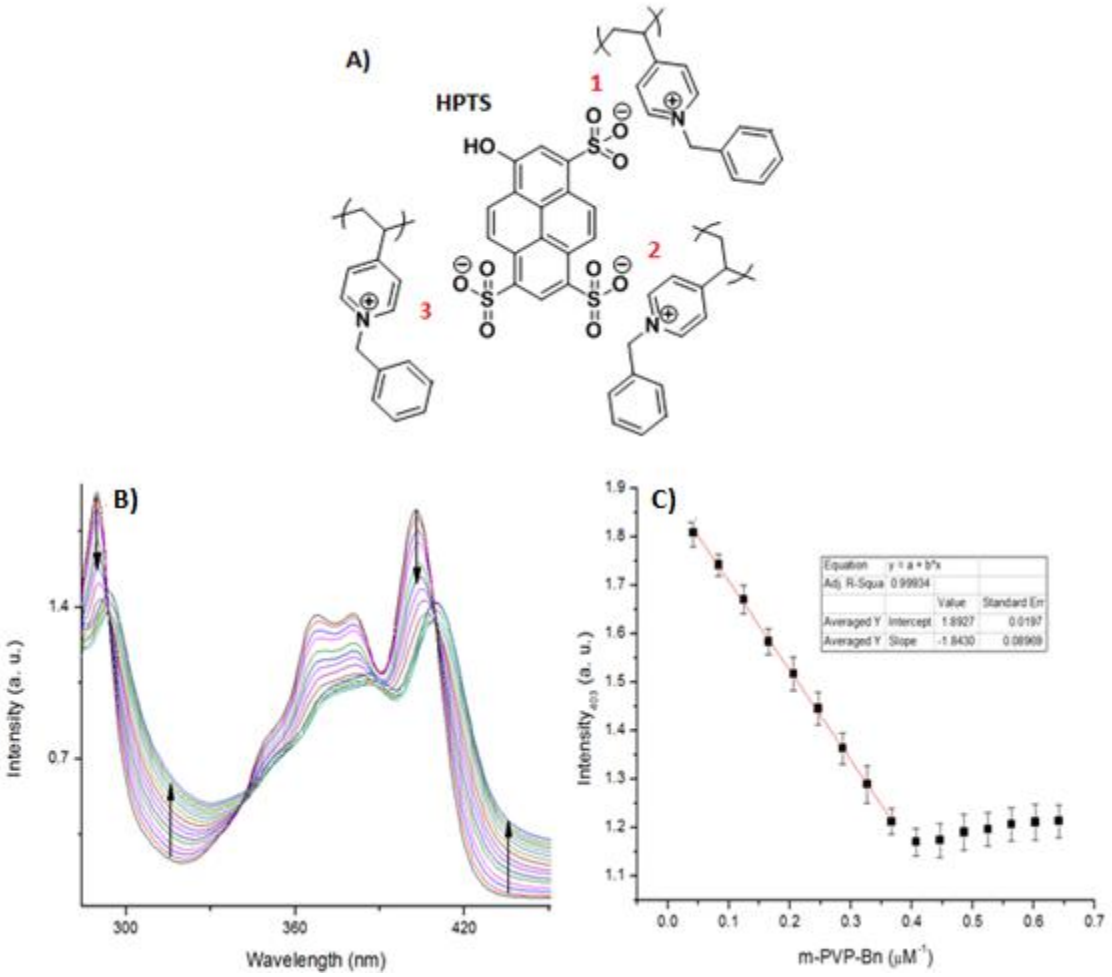


Figure 16: (A) PVP-Bn can interact with HPTS through electrostatics (1-3). **(B)** UV-Vis absorbance spectrum of HPTS (80 µM) with PVP-Bn being titrated into cuvette (0.0 - 0.65µM). **(C)** Change in absorbance at 403 nm.

We also used a dye known as aesculetin. It is a neutrally charged molecule that contains 1,2-cis diols. We expect there to be a covalent interaction between the boronic acid, on m-PVP-BA, and the 1,2-cis diols of aesculetin (Figure 17-A). When viewing the titration data, from Figure 17, it shows a large red shift in the absorbance spectra. Because PVP-Bn does not contain boronic acid, and aesculetin is not anionic, there should not be any polymer-dye interaction (Figure 18-A). After studying the titration data, (Figure 18) it is clear that there is not a significant change in the absorbance of the dye. Note, in Figure 18, there is a slight decrease in

the absorbance as PVP-Bn is titrated in. This could be due to a dilution effect, and could serve as a good approximation the error in this experiment.

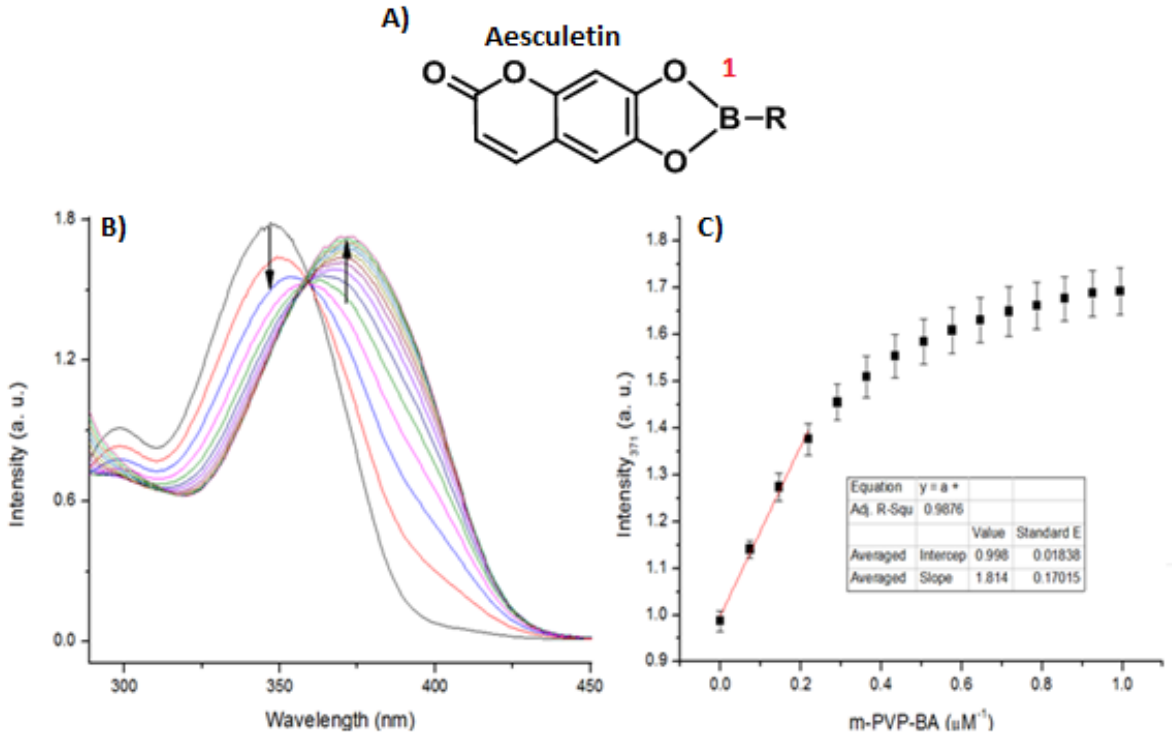


Figure 17: (A) There is one possible covalent interaction (1) between m-PVP-BA and Aesculetin. (R= m-PVP-BA) **(B)** UV-Vis absorbance spectrum of Aesculetin (140 µM) with m-PVP-BA being titrated into cuvette (0.0 - 1.0µM). **(C)** Change in absorbance at 371 nm.

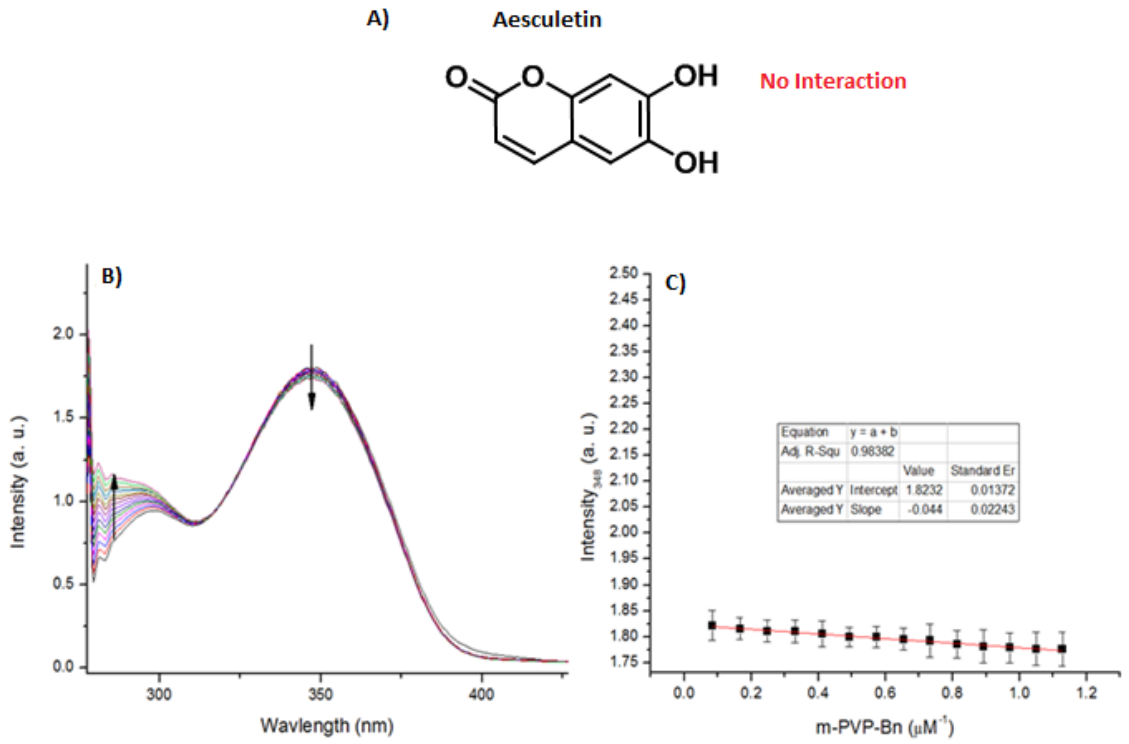


Figure 18: (A) PVP-Bn does not interact with Aesculeting covalently or electro statically. **(B)** UV-Vis absorbance spectrum of Aesculetin (140 µM) with PVP-Bn being titrated into cuvette (0.0 - 1.1µM). **(C)** Change in absorbance at 348 nm.

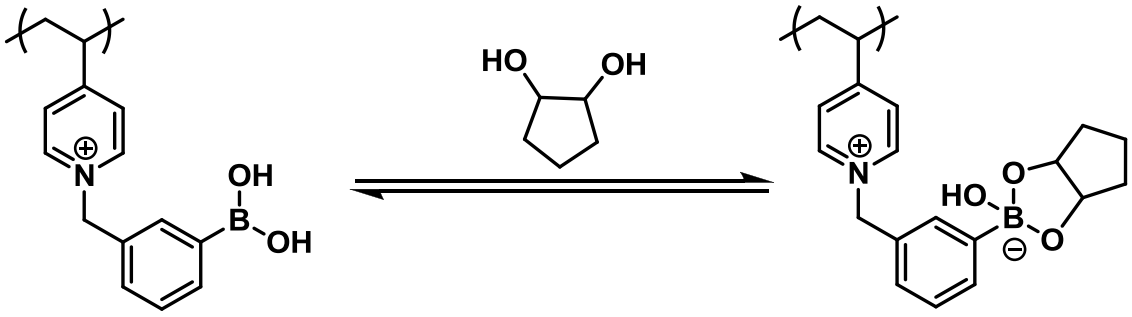
By performing a linear fit to the titration curves (Figure 13-B to 18-B), we were able to obtain relative binding affinities (Table 1). The data, from Table 1, reflected the previous assumptions that we made about the dye-polymer interactions. HPTS had nearly the same affinity with m-PVP-BA as it did with PVP-Bn. Aesculetin had a high affinity with m-PVP-BA, but did not interact with PVP-Bn. Finally, ARS had a higher affinity for m-PVP-BA than PVP-Bn. Note that ARS still had a significant interaction with PVP-Bn.

	m-PVP-BA (M^{-1})	**PVP-Bn (M^{-1})**
HPTS	1.89	1.84
ARS	1.087	0.70
Aesculetin	1.81	.044

Table 1: Relative binding affinity that m-PVP-BA and PVP-Bn have for HPTS, ARS, and Aesculetin.

Knowing how the polymer behaves in solution will allow us to understand its behavior inside of a nanopore. There are many techniques that researchers use to functionalize molecules inside of the pipette. A popular method is through covalent modification, this usually involves a silinization reaction to attach additional functional groups to the surface of the pipette.[32] Usually, these techniques have low success rates and are difficult to characterize.

We will be taking a much less labor intensive approach by sticking the cationic polymer to the negative[33] surface of the pipette. We used I-V plots to determine if the modification of the pore was successful. By immersing an unmodified pipette into a pH 9.5 carbonate buffer, negative current rectification was observed (Figure 19-A). We expect a negative current rectification because of the effects of the diffuse electric double layer discussed in the introduction (negative surface charge leads to negative current rectification). By simply placing the unmodified nanopipette into a methanol solution, containing .03% (w/v) m-PVP-BA, the cationic polymer embeds itself into the negatively charged nanopipette. A successful modification of the pipette is demonstrated by a complete reversal in current rectification (Figure 19-B). The rectification is now positive due to the cationic properties of the polymer.



Scheme 4: In the presence of a sugar, m-PVP-BA will go from a cationic polymer to overall neutral.

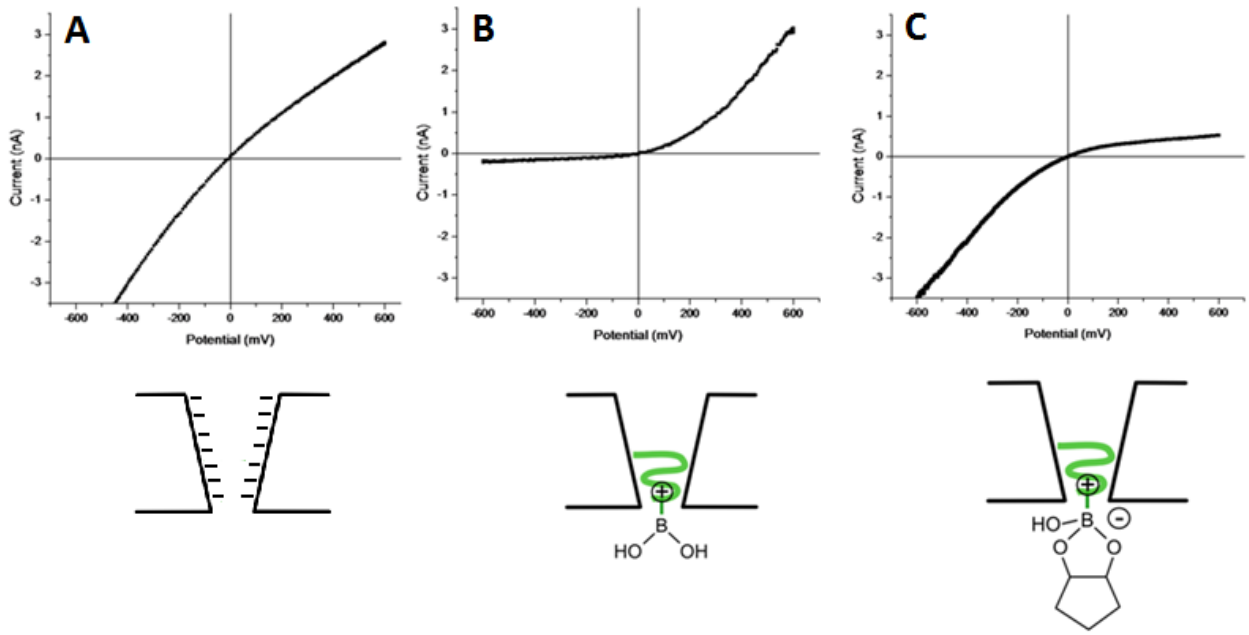


Figure 19: (A) Unmodified pipettes have negatively charged surfaces which lead to negative current rectification. **(B)** By embedding m-PVP-BA into the pipette, the current rectification becomes positive due to cationic properties of the polymer. **(C)** In the presents of 10 mM fructose the polymer become neutral, which causes current rectification to become negative.

As previously mentioned, boronic acid has the ability to bind sugar to form a boronic ester. This binding causes the pKa of the boron to decrease approximately 1-2 values. If the pKa of the boron is lower than the surrounding media, the boronic ester will hydrolyze water to form the negatively charged boronate ester complex. This process causes the previously cationic m-PVP-BA to become overall neutral (Scheme 4). By placing the modified nanopipette in a buffered solution containing 10 mM fructose, this effect is observed. Now that the polymer is overall neutral, the current rectification is dictated by the negatively charges walls of the pipette. This causes the current rectification to returned to its negatively rectified state (Figure 19-C). Figure 19-C shows much less positive current than 19-A. This effect is currently not understood, but might be due to an effect of the fructose attaching onto the polymer.

When developing a sensor it is important to show that the signal is dependent on the concentration of an analyte. After functionalizing a nanopipette with m-PVP-BA, we submersed it into a bath containing a pH 9.5 carbonate buffer. By adding small alliquotes, of a concentrated fructose solution, we were capable of gradually changing the current rectification (Figure 20). As the concentration of fructose increases, there are more negatively charged boronate ester formed (Figure 20-B). We have demonstrated that this sensor can detect fructose concentration lower than 700 µM.

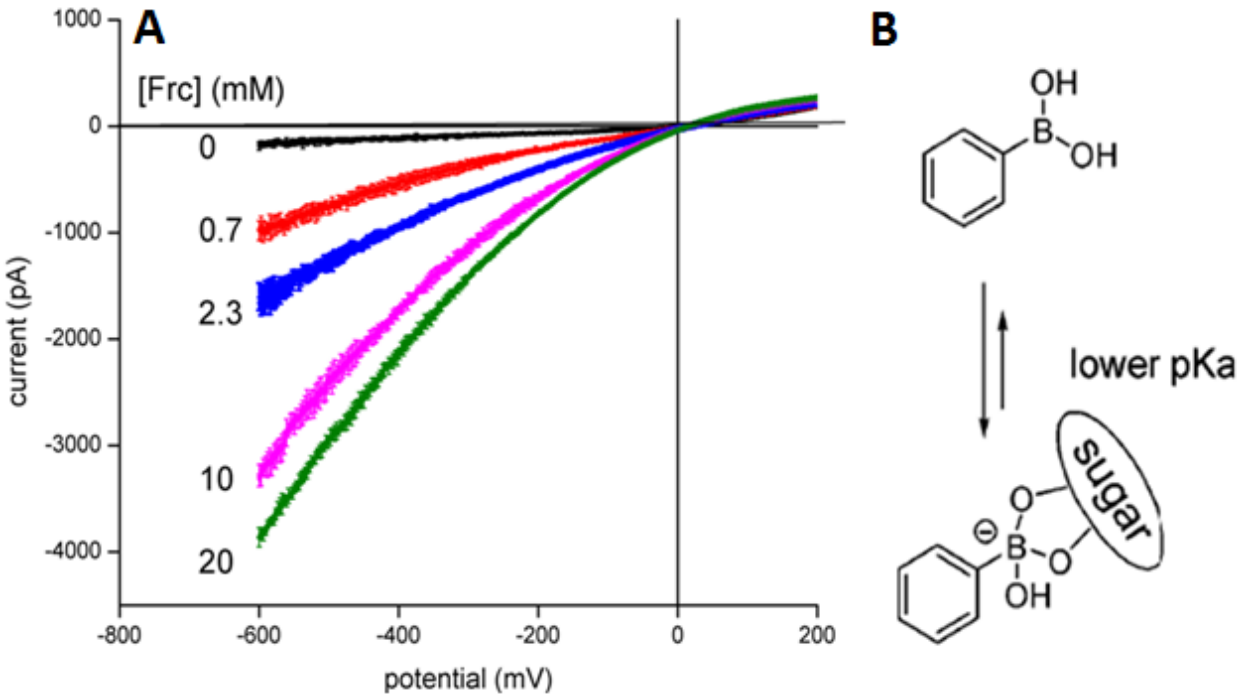


Figure 20: (A) Dependence that current rectification has on a varying fructose concentration in pH 9.5 carbonate buffer. Error bars demonstrate 5 consecutive scans. **(B)** As more fructose is added the boronic acid favors the negative boronate ester.

In order to monitor sugar concentrations continually, it is important to have a sensor that produces a reversible signal. Using boronic acid as a sugar receptor is nice because it has a binding affinity for sugars. If the concentration of sugar is decreased enough, it will dissociate

from the boronic acid which will return the rectification back to its original state. By functionalizing a nanopipette with m-PVP-BA, and placing it back and forth between a media with and without 10 mM fructose, we have demonstrated reversibility (Figure 21). This also provides evidence of how robust the sensor is, and that the previous results were not caused by the polymer washing off the pore.

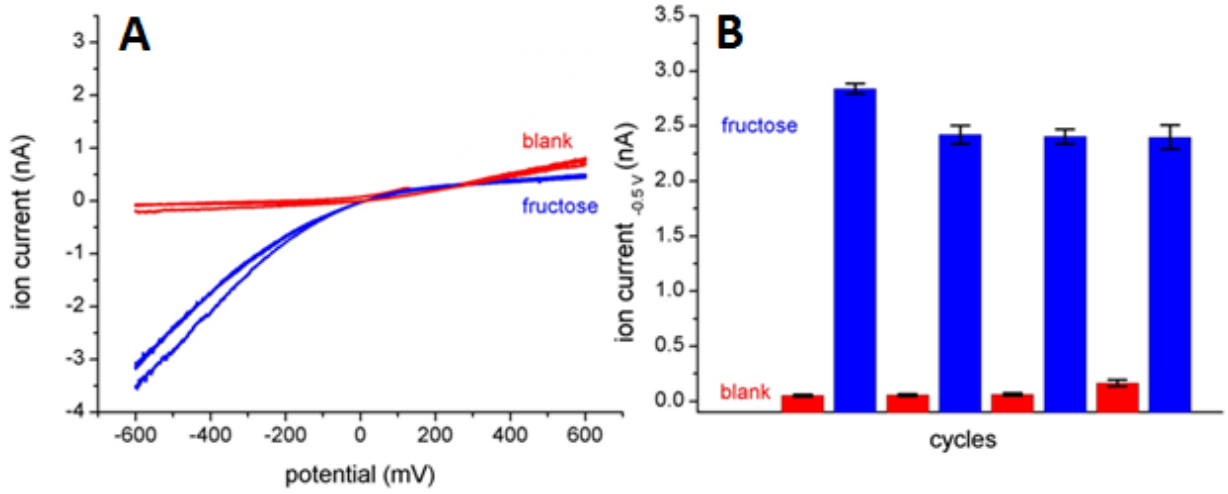


Figure 21: Change in current rectification of a nanopipette functionalized with m-PVP-BA. **(A)** I-V plot of a pipette submersed in a pH 9.5 carbonate buffer without sugar (red) and with 10 mM fructose (blue). **(B)** The change in current at 500 mV. Error bars represent standard deviations from 5 consecutive scans.

V. Experimental

Reagents and Solutions

All of the dyes and sugars: 8 - Hydroxypyrene - 1,3,6 - trisulfonic acid, trisodium salt (HPTS), Alizarin Red S. (ARS), Aesculetin, fructose, glucose, and galactose were purchased from Sigma. Starting materials were also purchased from Sigma: Poly(4-vinylpyridine) (M.W. 60,000), Benzyl Bromide, and various solvents. All stock solutions were prepared using Milli-Q

ultrapure water. All buffers were made using potassium chloride, sodium phosphate (dibasic), sodium carbonate, and sodium bicarbonate. Their pH was then adjusted by adding HCl of NaoH.

Synthesis of Polymer m-PVP-BA. Poly(4-vinylpyridine) (MW 60,000) was purchased from Sigma and used as received. The synthesis of *m*-bromomethylphenylboronic acid was carried out using an established procedure. To a 10 mL round bottom flask containing a magnetic stir bar were added poly(4-vinylpyridine) (0.100 g, .00167 mmols) and m-bromomethyl phenylboronic acid (0.206 g, 0.954 mmols). Then N,N-dimethylformamide (2 mL) and methanol (2 mL) were added to dissolve the reagents. The mixture stirred 24 hours, then was added dropwise to a 50 mL beaker containing ethyl acetate (10 mL) to precipitate the product. The beaker was placed in an ice bath to allow the complete precipitation of the product. The solution was then poured into a two-piece fritted filter with removable top and vacuum-filtered under inert conditions using argon gas. The product was washed with 3 x 15 mL portions of dichloromethane, then left in a vacuum dessicator to dry overnight. Product isolated was 0.257 g (90% yield). As described in the supporting information, ^{1}H-NMR showed 82% alkylation of the polymer.

Synthesis of Polymer PVP-Bn

Poly(4-vinylpyridine) (MW 60,000) and Benzyl Bromide were purchased from Sigma and used as received. To a 10 mL round bottom flask containing a magnetic stir bar were added poly(4-vinylpyridine) (0.100 g, .00167 mmols) and Benzyl Bromide (102µL, 0.864 mmols). Then methanol (4 mL) was added to dissolve the reagents. The mixture refluxed for 6 hours, and was then allowed to cool to room temperature. The reaction mixture was removed via rotary evaporation and then dried overnight in a vacuum dessicator. Product isolated was 0.257 g (90% yield). ^{1}H-NMR (found in "spectra" section) showed 96% alkylation of the polymer.

pH Titrations of m-PVP-BA to a flask (50mL) add a .25 % m-PVP-BA (w/v) dissolved in a 1:1 methanol/water solution (10 mL) and sugar (20 mM) if experiment calls for it. Submerse an pH electrode into the solution and acidify the solution to pH 2.0 with HCl (1.0 mol). Begin titrating aliquots (15µL) of NaOH (0.50 mM) into the beaker while keeping track of the pH. At approximately pH 7-8 m-PVP-BA will precipitate out of the solution.

UV-Vis Spectroscopy (m-PVP-BA &HPTS) Add a mixture of 1:1 methanol/water (2 mL) containing 8 - Hydroxypyrene - 1,3,6 - trisulfonic acid, trisodium salt (HPTS) (80 µM) into a quarts cuvette. Begin titrating aliquots (5 µL) of .25% m-PVP-BA (w/v) into the cuvette. Continue titrating until the dye reaches saturation.

UV-Vis Spectroscopy (m-PVP-BA & Aesculetin) Add a mixture of 1:1 methanol/water (2 mL) containing esculetin (140 µM) into a quarts cuvette. Begin titrating aliquots (5 µL) of .50% m-PVP-BA (w/v) into the cuvette. Continue titrating until the dye reaches saturation.

UV-Vis Spectroscopy (m-PVP-BA & ARS) Add a mixture of 1:1 methanol/water (2 mL) containing alizarin red sulfonate (ARS) (250 µM) into a quarts cuvette. Begin titrating aliquots (5 µL) of .50% m-PVP-BA (w/v) into the cuvette. Continue titrating until the dye reaches saturation.

UV-Vis Spectroscopy (PVP-Bn & HPTS) Add a mixture of 1:1 methanol/water (2 mL) containing 8 - Hydroxypyrene - 1,3,6 - trisulfonic acid, trisodium salt (HPTS) (80 µM) into a quarts cuvette. Begin titrating aliquots (5 µL) of .25% PVP-Bn (w/v) into the cuvette. Continue titrating until the dye reaches saturation.

UV-Vis Spectroscopy (PVP-Bn & Aesculetin) Add a mixture of 1:1 methanol/water (2 mL) containing esculetin (140 µM) into a quarts cuvette. Begin titrating aliquots (5 µL) of .50% PVP-Bn (w/v) into the cuvette. Continue titrating until the dye reaches saturation.

UV-Vis Spectroscopy (PVP-Bn & ARS) Add a mixture of 1:1 methanol/water (2 mL) containing alizarin red sulfonate (250 µM) into a quarts cuvette. Begin titrating aliquots (5 µL) of .50% PVP-Bn (w/v) into the cuvette. Continue titrating until the dye reaches saturation.

Current Measurement with Quartz Nanopipette Electrodes. Nanopipettes were fabricated using a P-2000 laser puller (Sutter Instrument Co.) from quartz capillaries with filaments, with an outer diameter of 1.0 mm and an inner diameter of 0.70 mm (QF100-70-5; Sutter Instrument Co.). Parameters used were: Heat 625, Filament 4, Velocity 60, Delay 170, and Pull 180. To measure ion current, a two electrode setup was used. The nanopipette was backfilled with buffer solution (phosphate/KCl, pH 7) and an Ag/AgCl electrode inserted. Another Ag/AgCl electrode was placed in 0.3 mL bulk solution acting as auxiliary/reference electrode. Both electrodes were connected to an Axopatch 700B amplifier with the DigiData 1322A digitizer (Molecular Devices), and a PC equipped with pClamp 10 software (Molecular Devices). To ensure complete wetting of the nanopipette electrodes, nanopipette tips were immersed in N,N-dimethylformamide for 5-10 seconds after being backfilled with buffer. Positive potential refers to anodic potential applied to the electrode in the barrel of the nanopipette relative to the counter electrode. Experiments were carried out at 24 °C.

Embedding m-PVP-BA in Nanopipettes. Nanopipette barrels were filled with phosphate buffer (pH 7) and immersed in carbonate buffer (pH 9.5) containing the counter electrode. After verifying the nanopipettes displayed negative current rectification, they were briefly immersed in a methanol solution containing 0.03% (w/v) polymer, then returned to the carbonate solution. Successful immobilization of the polymer resulted in complete reversal of current rectification.

Measuring Carbohydrate Response with Polymer-Modified Nanopipettes. Modified nanopipettes were analyzed in 0.30 mL of a carbonate buffer solution (pH 9.5) containing the counter electrode. To the solution were added aliquots of concentrated analyte solutions in pure water. The total volume added did not exceed 15 μL, in order to limit the change in volume to 5%. To measure response in real time, the current was analyzed using a sinusoidal potential at frequency of 0.5 Hz from -500 to +500 mV. After the signal had stabilized following addition of an aliquot, the current was analyzed by sweeping the voltage from -500 to +500 mV at a rate of 0.5 mV/ms. Each measurement consisted of 5 sweeps.

Electrochemical Data Analysis. Ion current measurements recorded with pClamp software (sampling frequency 1000 Hz for voltage sweeps, 200 Hz for sinusoidal function) were imported to OriginPro 8.5 (Origin Labs) for analysis and graphing. To generate I-V curves for each data point, 5 voltage sweeps from -800 to +800 mV at a scan rate of 500 mV s-1 were averaged and the standard deviation calculated for each point. To generate binding isotherms, the current at a fixed potential was plotted as a function of analyte concentration.

VI. Conclusion

This work discusses the synthesize and characterization of m-PVP-BA, which is a cationic polymer that has been functionalized with boronic acid sugar receptors. By performing various spectrophotometry assays, we have determined that there are two factors that dictate the binding affinity of the polymer with a substrate. Due to the cationic properties of the polymer, it was attracted, through electrostatics, to various anionic substrates. The polymer also had a covalent attraction to substrates containing 1,2-cis diols.

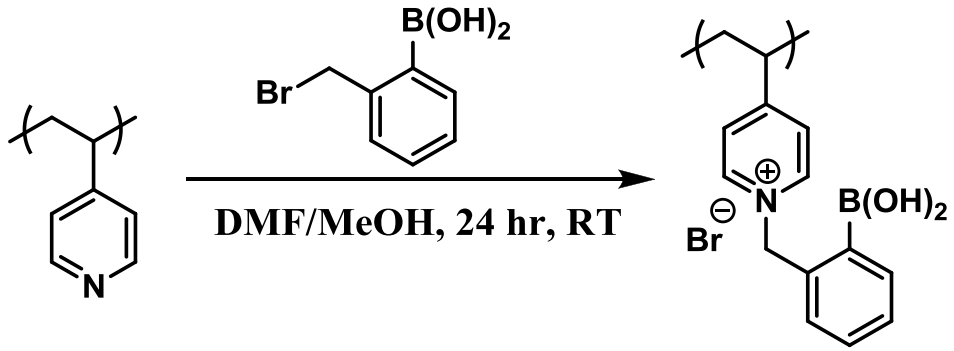
During our research, we discovered a quick and convenient way of functionalizing a solid state nanopore with m-PVP-BA. This was done through an electrostatic interaction between the polymer and the pore. By simply submersing the nanopipette into a solution of dilute polymer it quickly embed itself into the pore. In the presents of saccharides, the polymer will modulate its charge from overall positive, to overall neutral. By using electrical signals we were able to take advantage of this property and perform sugar sensing using charge based detection. This system was capable of detecting fructose lower than 700 μM. The binding affinity of boronic acid to sugar allowed us to produce a reversible signal in real-time.

When designing this system, we ran into a few difficulties. The polymer was not soluble in aqueous solutions, which made it difficult to characterize. We have addressed this problem by attaching different solvating groups onto a number of the monomeric groups of the polymer. The procedures for these compounds, m-PVP-BA-EtOH and m-PVP-BA-NH_3, are located in the "additional experimental" section. These new polymers have given us insight into the relative pKa values of the boronic acid sugar receptor.

It is commonly known that boronic acid has a greater affinity for fructose than glucose. In the past there have been groups that have synthesized boronic acid containing compounds that are more selective for glucose.[34,35,36] In order to adjust the sugar selectivity of the polymer, we have positioned the boronic acid differently around the polymer.[37] These polymers are known as o-PVP-BA and p-PVP-BA. Their sugar selectivity has not been characterized, but their synthetic procedures can be found in the "additional experimental" section.

VII. Additional Experimental

Synthesis of Polymer o-PVP-BA

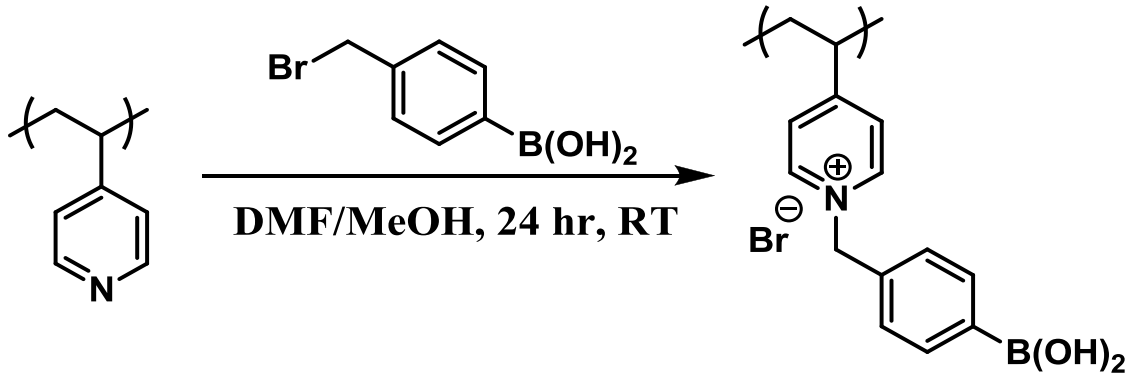


Poly(4-vinylpyridine) (MW 60,000) was purchased from Sigma and used as received. The synthesis of *o*-bromomethylphenylboronic acid was carried out using an established procedure. To a 10 mL round bottom flask containing a magnetic stir bar were added poly(4-vinylpyridine) (0.100 g, .00167 mmols) and o-bromomethyl phenylboronic acid (0.206 g, 0.954 mmols). Then N,N-dimethylformamide (2 mL) and methanol (2 mL) were added to dissolve the reagents. The mixture stirred 24 hours, then was added dropwise to a 50 mL beaker containing ethyl acetate

(10 mL) to precipitate the product. The beaker was placed in an ice bath to allow the complete precipitation of the product. The solution was then poured into a two-piece fritted filter with removable top and vacuum-filtered under inert conditions using argon gas. The product was washed with 3 x 15 mL portions of dichloromethane, then left in a vacuum dessicator to dry overnight. Product isolated was 0.257 g (90% yield). ^{1}H-NMR (found in "spectra" section) showed 100% alkylation of the polymer.

Polymer showed similar solubility properties as m-PVP-BA.

Synthesis of Polymer p-PVP-BA

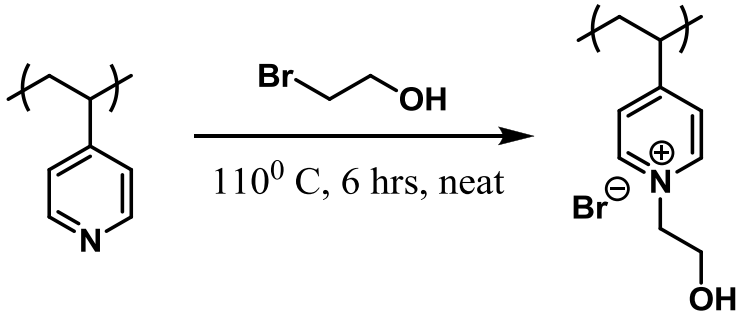


Poly(4-vinylpyridine) (MW 60,000) was purchased from Sigma and used as received. The synthesis of p-bromomethylphenylboronic acid was carried out using an established procedure. To a 10 mL round bottom flask containing a magnetic stir bar were added poly(4-vinylpyridine) (0.100 g, .00167 mmols) and p-bromomethyl phenylboronic acid (0.206 g, 0.954 mmols). Then N,N-dimethylformamide (2 mL) and methanol (2 mL) were added to dissolve the reagents. The mixture stirred 24 hours, then was added dropwise to a 50 mL beaker containing ethyl acetate (10 mL) to precipitate the product. The beaker was placed in an ice bath to allow the complete precipitation of the product. The solution was then poured into a two-piece fritted filter with

removable top and vacuum-filtered under inert conditions using argon gas. The product was washed with 3 x 15 mL portions of dichloromethane, then left in a vacuum dessicator to dry overnight. Product isolated was 0.257 g (86 % yield). ^{1}H-NMR (found in "spectra" section) showed 90% alkylation of the polymer.

Polymer showed similar solubility properties as m-PVP-BA.

Synthesis of Polymer PVP-EtOH

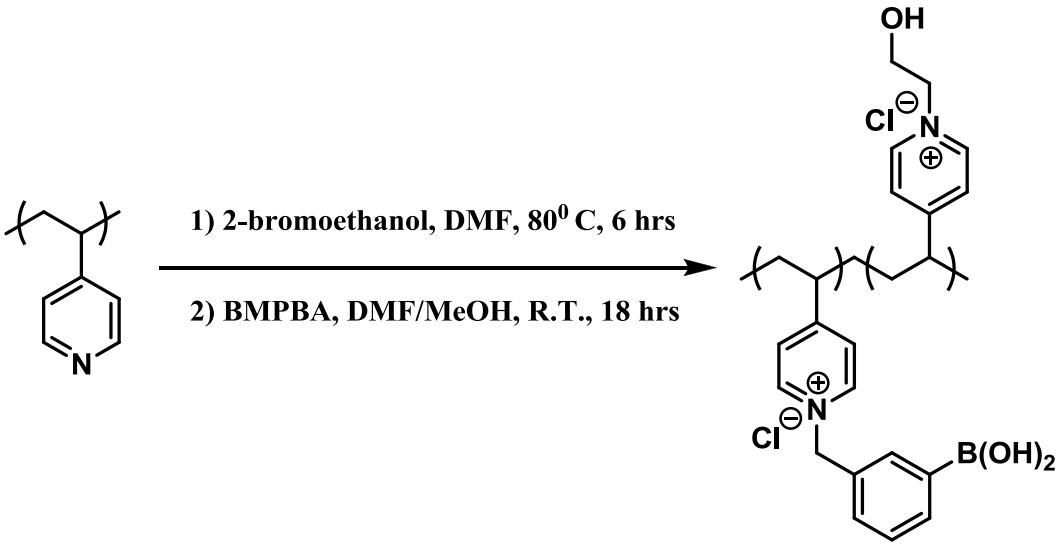


Poly(4-vinylpyridine) (MW 60,000) and 2-bromoethanol were purchased from Sigma and used as received. To a round bottom flask (10 mL) containing a magnetic stir bar were added poly(4-vinylpyridine)(.100 g, .00167 mmols) and 2-bromoethanol (1.5 mL, 21 mmols). While stirring, the reaction was heated to 110^0 C for 6 hours. After the reaction mixture has cooled to room temperature add it, dropwise, to a centrifuge tube containing ethyl acetate (20 mL). Place centrifuge tube into an ice bath for 15 minutes to ensure complete precipitation of product. Decant excess ethyl acetate after centrifugation. Wash the product (2 x 20 mL) with methanol and ethanol to remove excess 2-bromoethanol. Dry under argon and place in a vacuum sealed dessicator over night. ^{1}H-NMR (found in "spectra" section) showed 100% alkylation of ethanol.

The water solubility of this polymer was greatly increased. Polymer m-PVP-BA was unable to be dissolved in pure water. By adding ethanol groups onto PVP, we were able to

dissolve this polymer in pure water. A solution of 1% PVP-EtOH (w/v) in water, caused this polymer to form a hydrogel. This polymer was stable inside a nanopore and allowed for the detection of negatively charged analytes such as DNA.

Synthesis of Polymer m-PVP-BA-EtOH



Poly(4-vinylpyridine) (MW 60,000) and 2-bromoethanol were purchased from Sigma and used as received. The synthesis of m-bromomethyl phenylboronic acid was carried out using an established procedure. To a roundbottom flask (25 mL) containing a magnetic stir bar add 2-bromoethanol (.215 g, 1.7 mmols) and poly(4-vinylpyridine) (.300 g, 2.9 mmols). N,N-dimethylformamide (5 mL) was added to dissolve the reagents. While stirring, the reaction was heated to 80^0 C for 6 hours (product precipitated out of solution in approximately 3 hours). Add methanol (5mL) to the reaction mixture after it has been allowed to cool. This will dissolve the products back into solution. Then add m-bromomethyl phenylboronic acid (.606g, 2.9 mmols) and stir at room temperature for 18 hours. Drip the reaction mixture into a flask (250 mL) containing acetone (200 mL) and concentrated hydrochloric acid (.4 mL). Allow precipitate to settle to the bottom of the flask and decant excess acetone. Centrifuge product and decant

remaining acetone. Dissolve pellet in methanol (20 mL). Repeat isolation step in acetone and again centrifuge off excess acetone. Dry pellet under argon and leave it overnight in a vacuum sealed dessicator. Product isolated was 0.613 g (93 % yield). ^{1}H-NMR (found in "spectra" section) showed 49% addition of phenylboronic acid and 48% addition of ethanol. (integration using computer program was difficult due to unwanted solvent peaks. Therefore, I used integration by mass).

This polymer contains phenyl boronic acids and still remained water soluble. We were capable of perform a pH titration in pure water (Figure 22). By using the Henderson-Hasselbalch equation we were able to determine a relative pKa of phenyl boronic acid (pKa = 6.5-7.5). With the addition of sugar the pKa decreased, this is consistent with what we know about boronic acids.

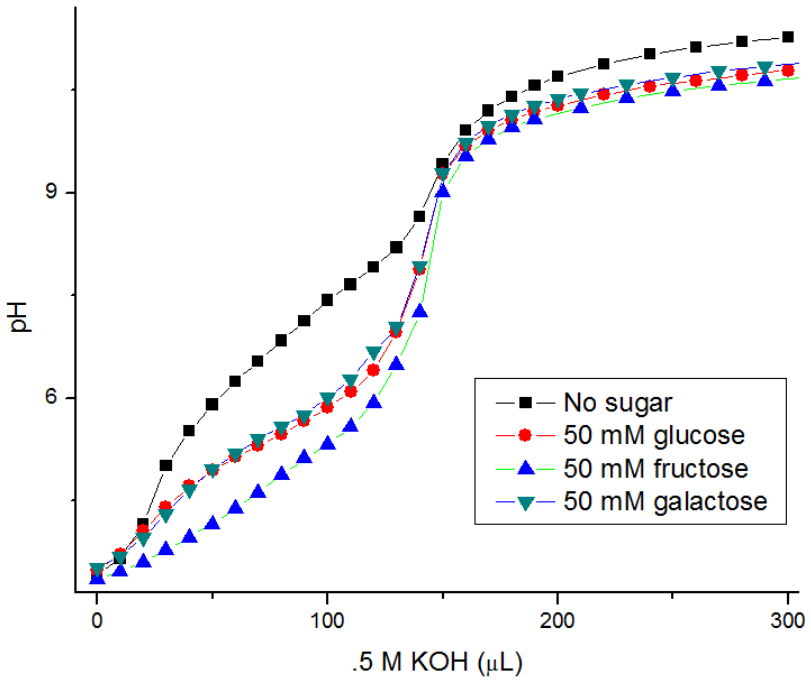
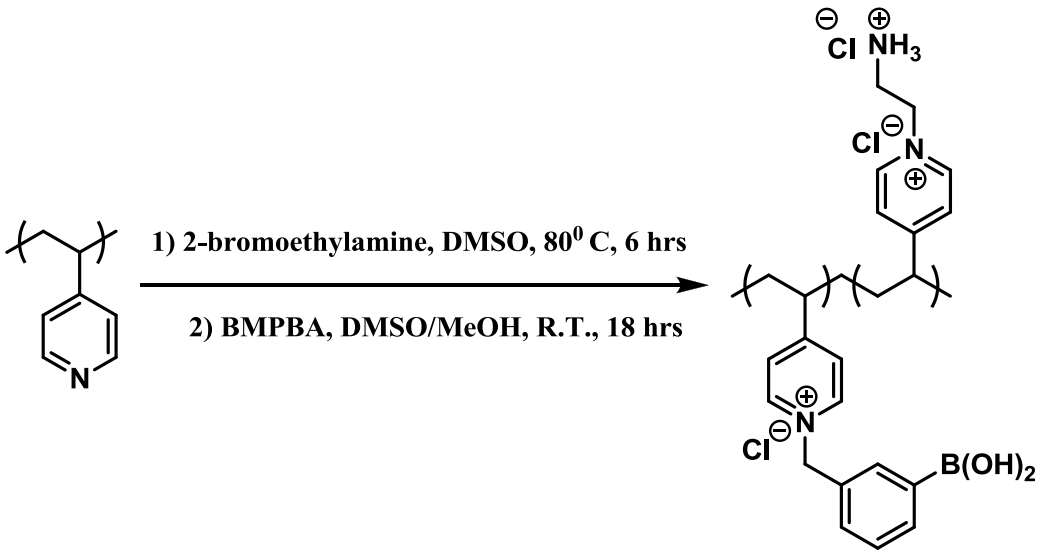


Figure 22: .125% m-PVP-BA-EtOH (w/v) in water. Aliquots of .50 M KOH were added to solution.

Synthesis of Polymer m-PVP-BA-NH_2



Poly(4-vinylpyridine) (MW 60,000) and 2-bromoethanol were purchased from Sigma and used as received. The synthesis of m-bromomethyl phenylboronic acid was carried out using an established procedure. To a roundbottom flask (25 mL) containing a magnetic stir bar add 2-bromoethylamine hydrobromide (.352 g, 1.7 mmols) and poly(4-vinylpyridine) (.300 g, 2.9 mmols). Dimethyl sulfoxide (5 mL) was added to dissolve the reagents. While stirring, the reaction was heated to 80^0 C for 6 hours (solution change colors throughout reaction; green, yellow/orange, and brown). After reaction cooled to room temperature add methanol (5 mL) and m-bromomethyl phenylboronic acid (.606g, 2.9 mmols). Continue stirring at room temperature for 18 hours. Add reaction mixture, dropwise, to a flask (250 mL) containing acetone (200 mL) and concentrated hydrochloric acid (.4 mL). Allow precipitate to settle to the bottom of the flask and decant excess acetone. Centrifuge product to remove remaining acetone. Wash product with methanol (20 mL). Repeat isolation step in acetone and again centrifuge off excess

acetone. Dry pellet under argon and leave it overnight in a vacuum sealed dessicator. Product isolated was 0.544 g (94 % yield). ^{1}H-NMR (found in "spectra" section) showed 45% addition of phenylboronic acid and 33% addition of ethylamine (integration using computer program was difficult due to unwanted solvent peaks. Therefore, I used integration by mass).

This polymer contains phenyl boronic acids and still remains water soluble. We were capable of perform a pH titration in pure water (Figure 23). By using the Henderson-Hasselbalch equation, it is possible to determine a relative pKa. The pKa of amines are similar to boronic acids, therefore, it was difficult to get an accurate pKa. Performing a pH titration in the presents of different sugars lead to an increase in the pKa of the phenyl boronic acid. This is a very interesting effect because it is behaving in the opposite why we would expect.

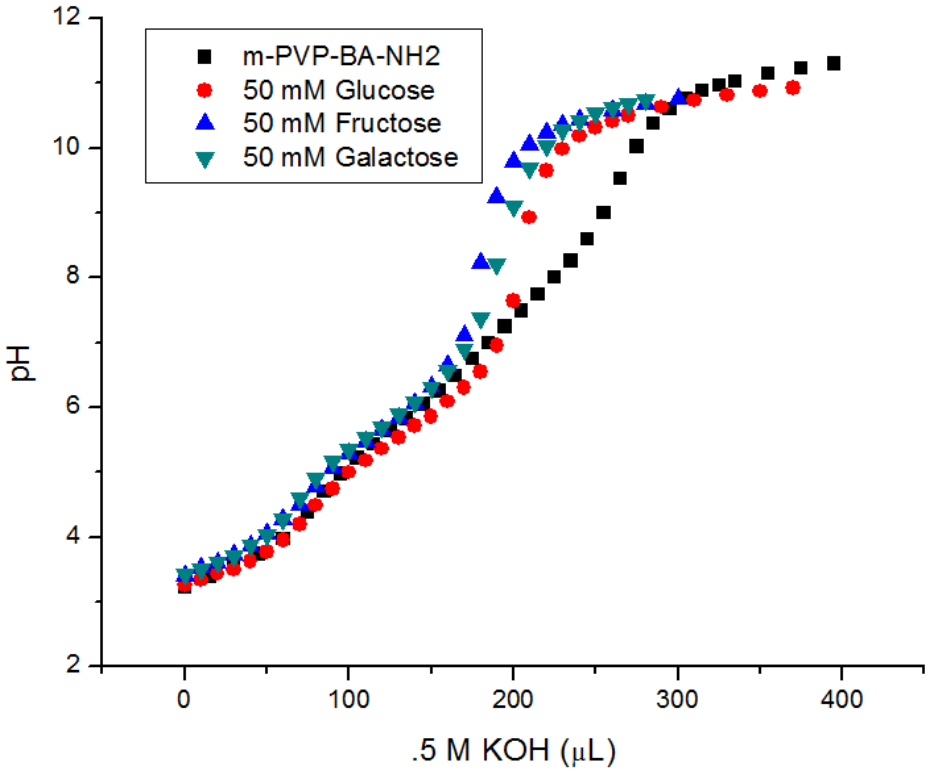
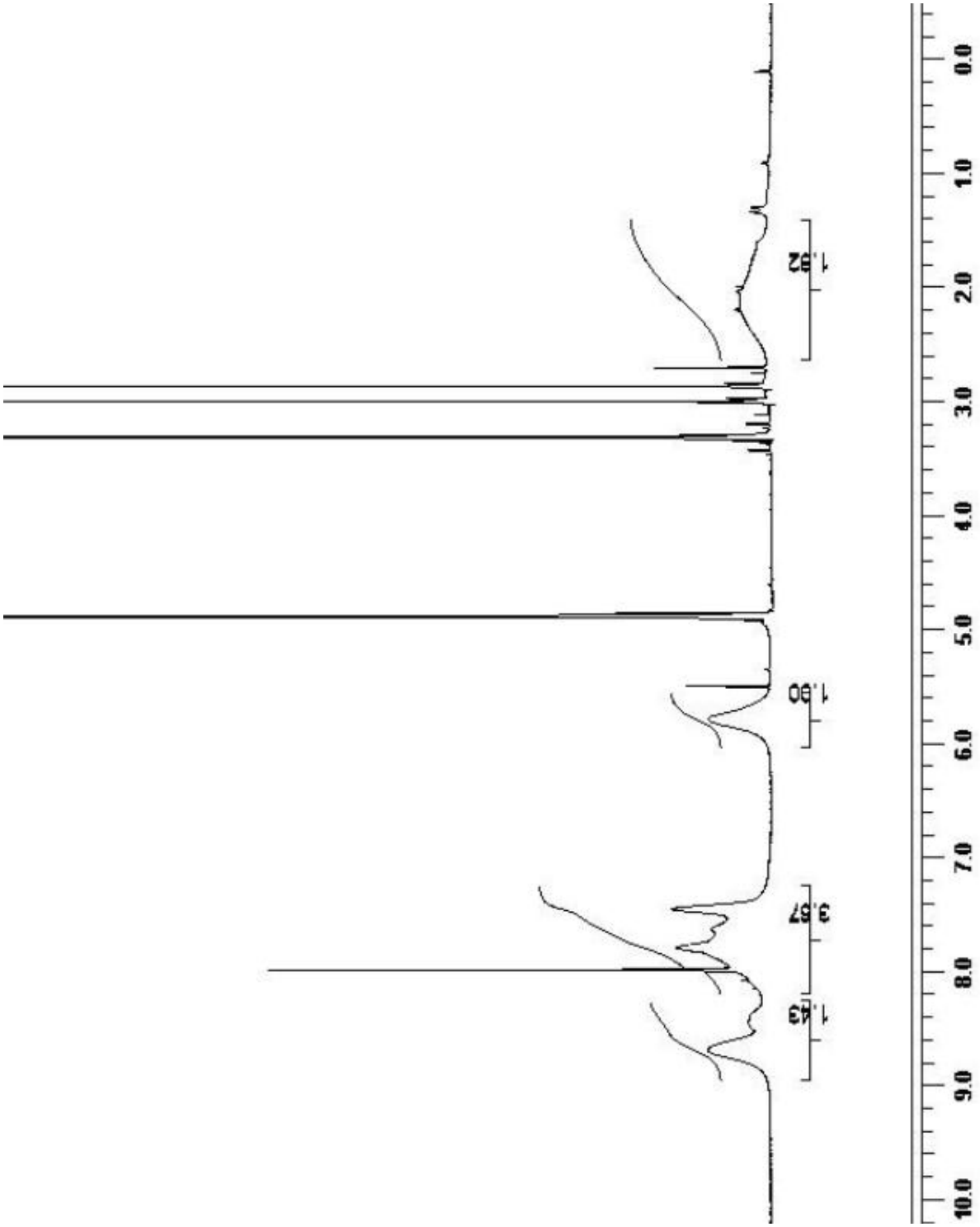


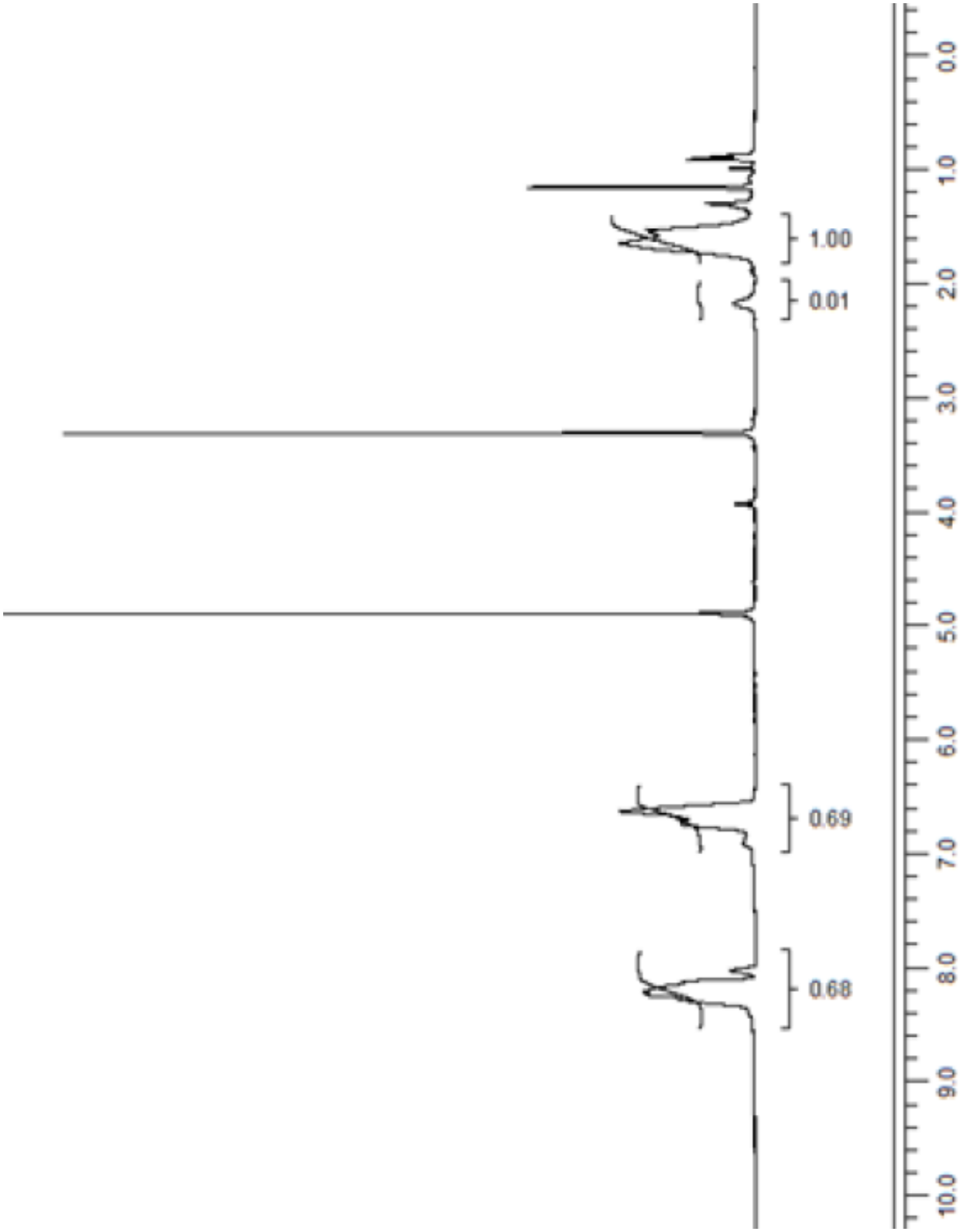
Figure 23: .125% m-PVP-BA-NH_3 (w/v) in water. Aliquots of .50 M KOH were added to solution.

VIII. Spectra

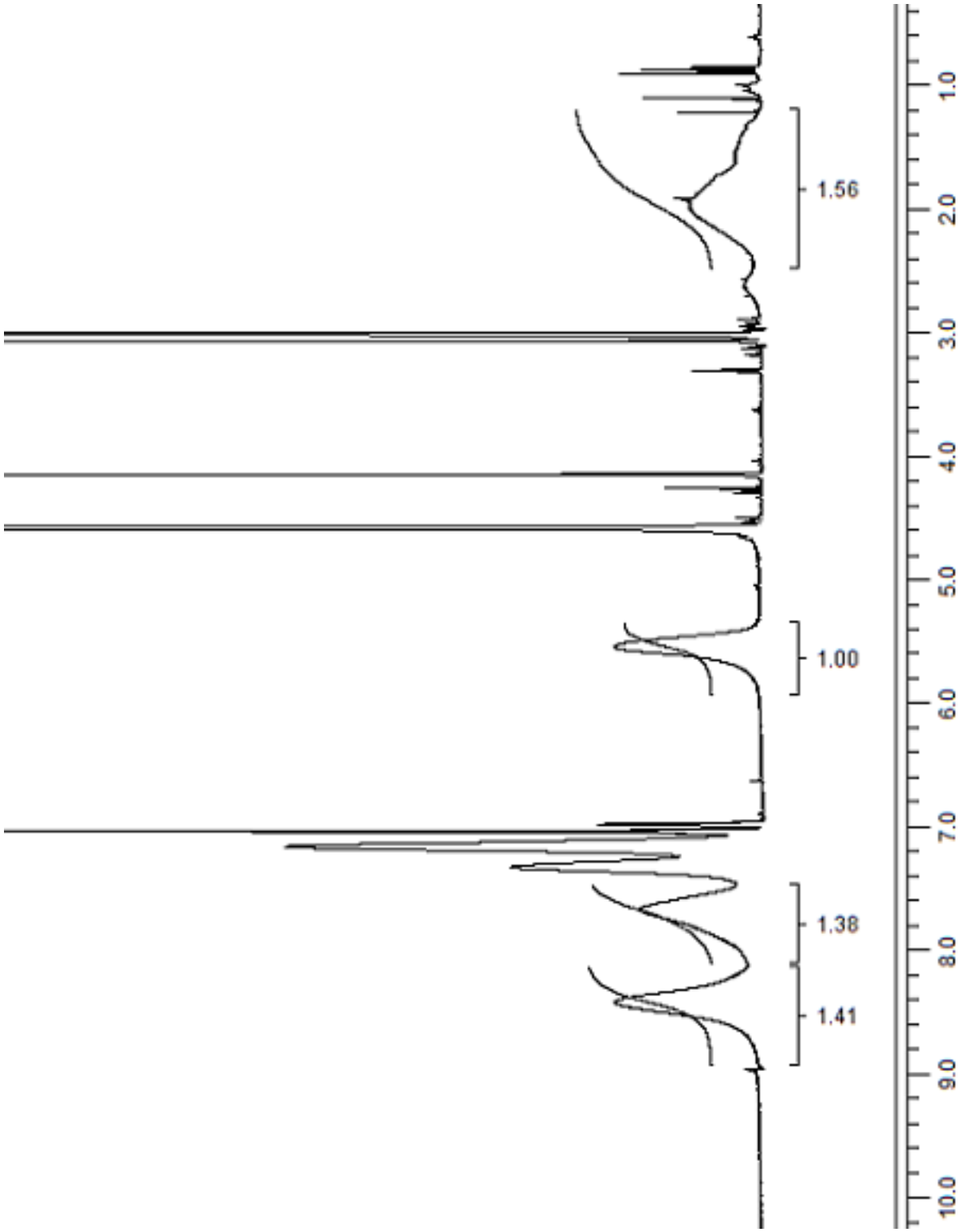
^{1}H-NMR (500 MHz) of m-PVP-BA (CD_3OD)



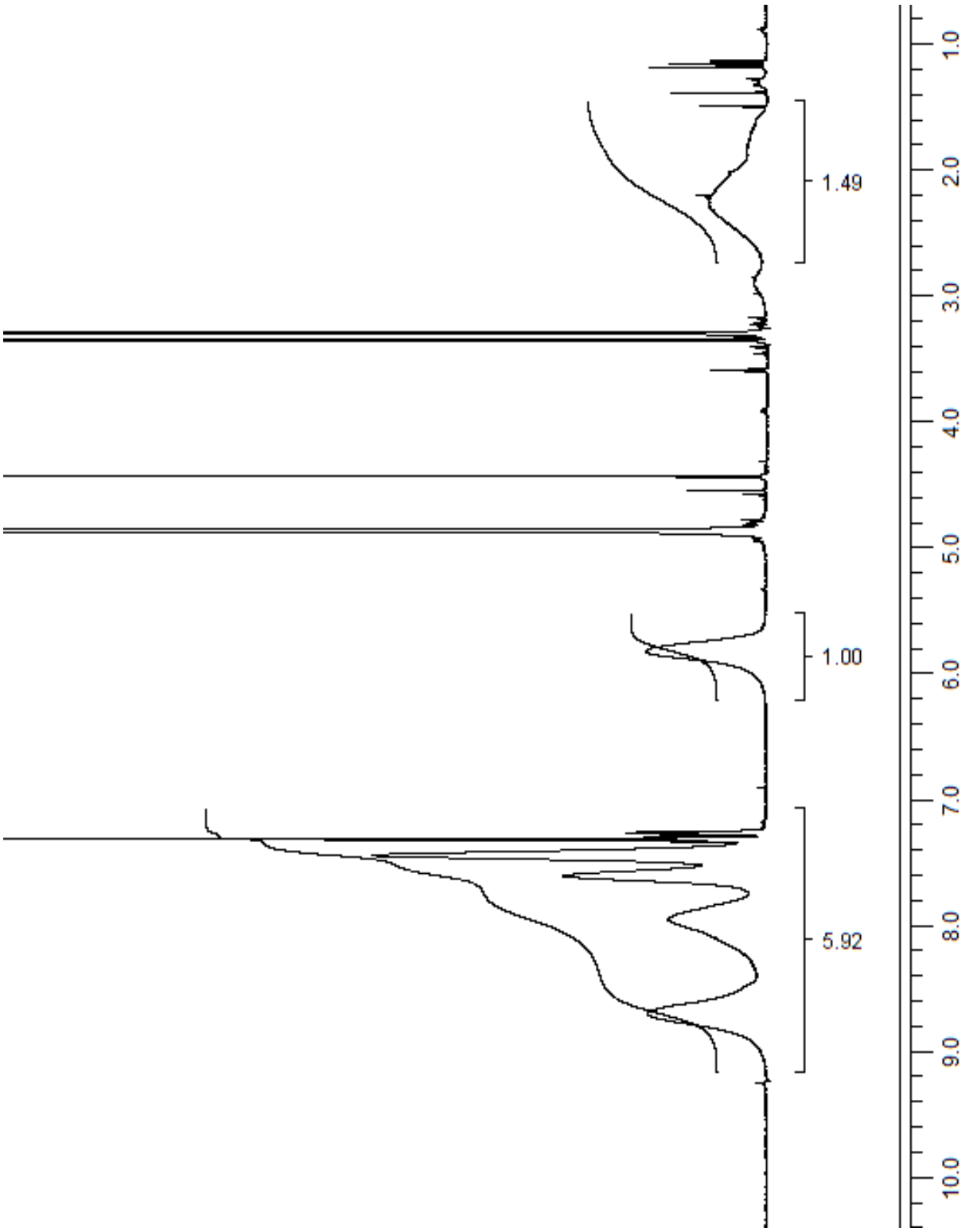
^{1}H-NMR (500 MHz) of Poly(4-Vinylpyridine) (CD_3OD)



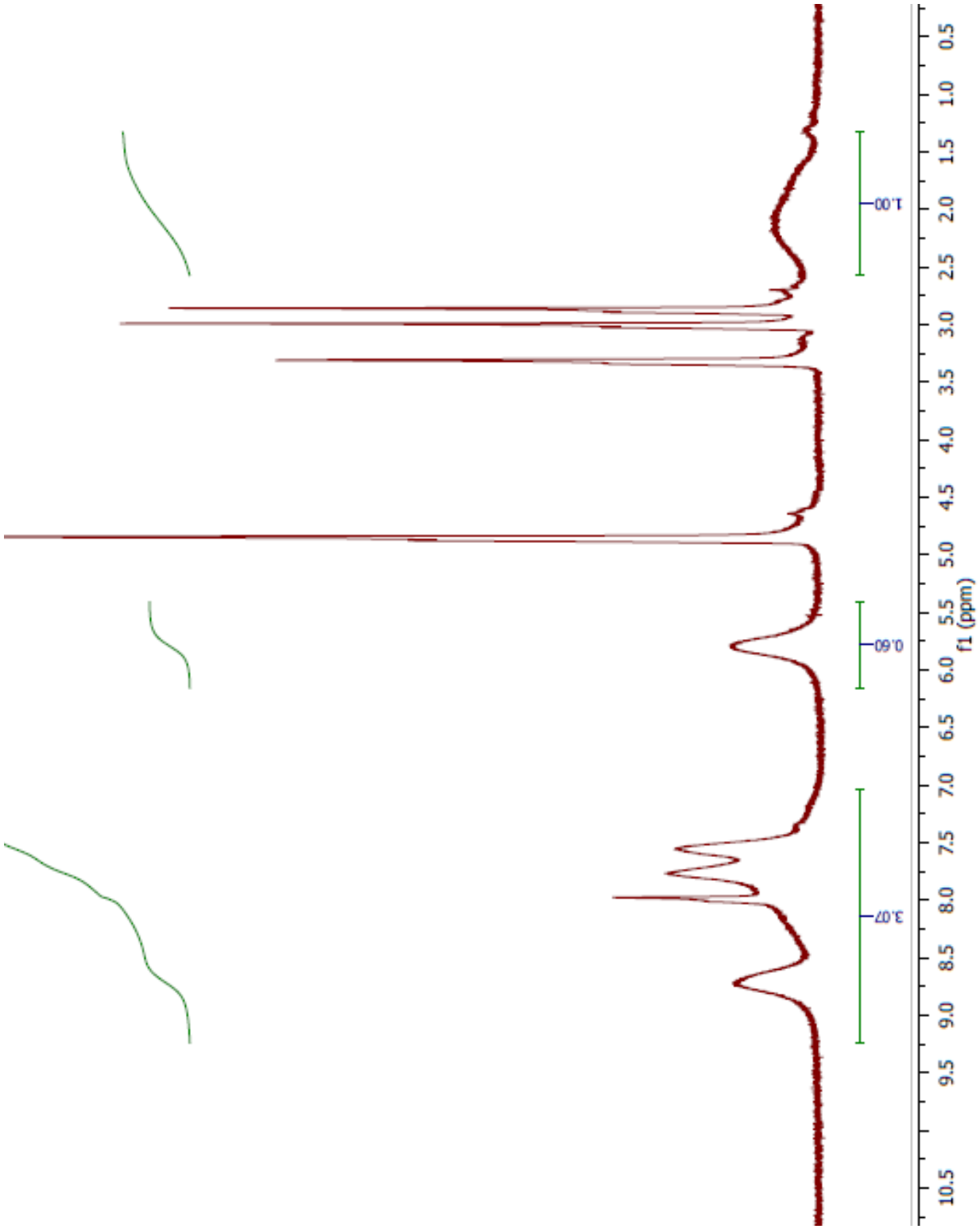
^{1}H-NMR (500 MHz) of PVP-Bn (CD_3OD)



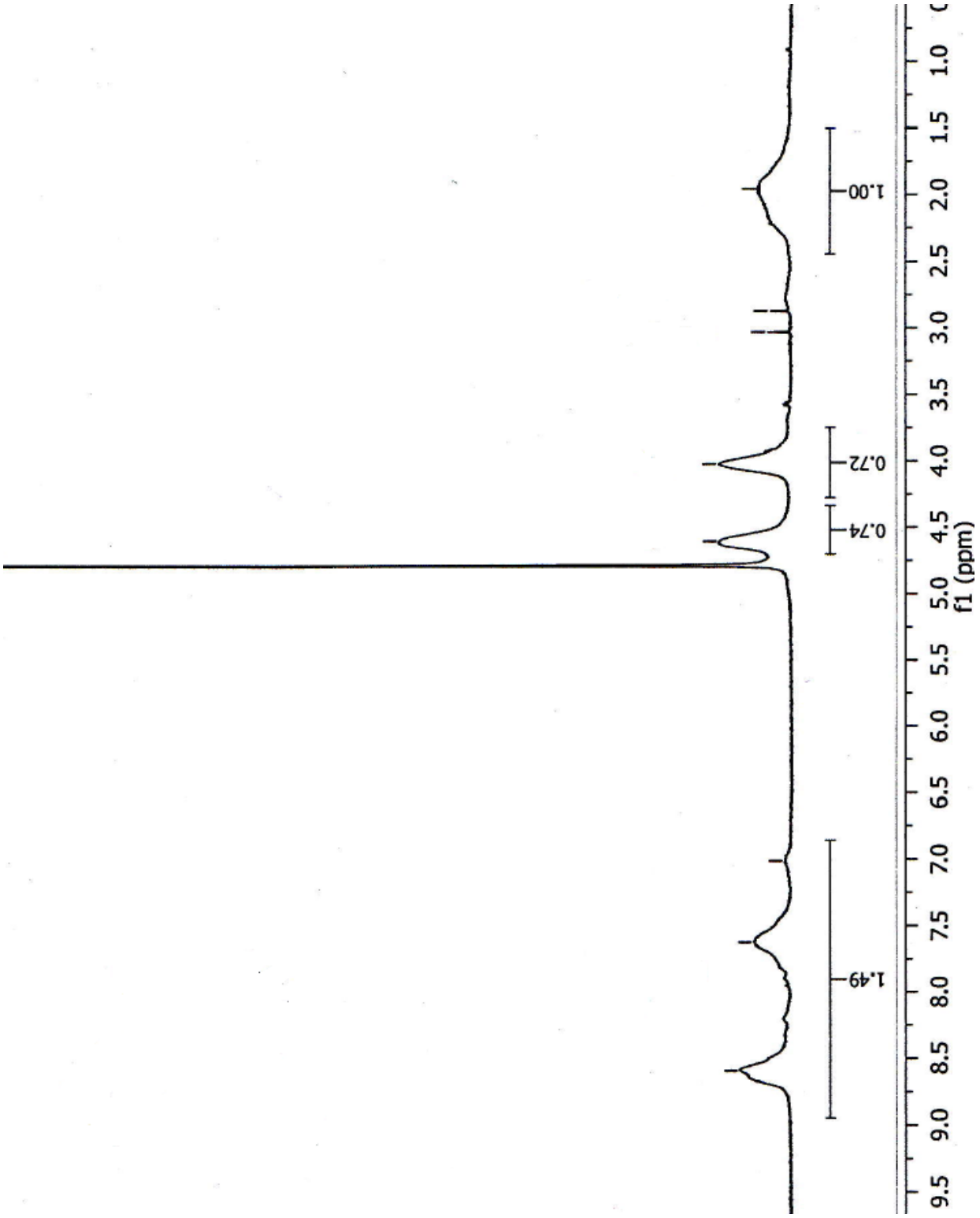
^{1}H-NMR(500 MHz) of o-PVP-BA (CD_3OD)



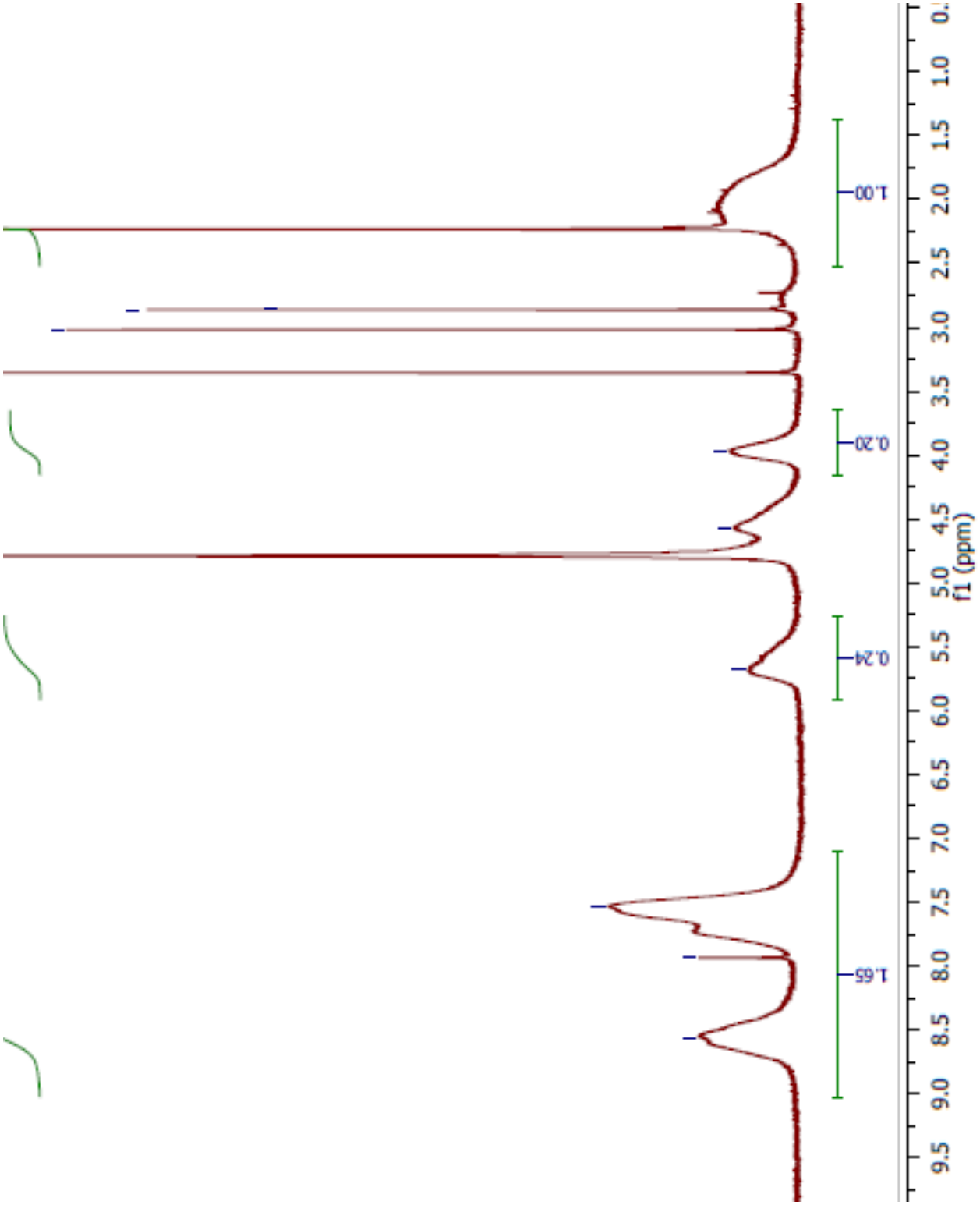
^{1}H-NMR (500 MHz) of p-PVP-BA (CD_3OD)



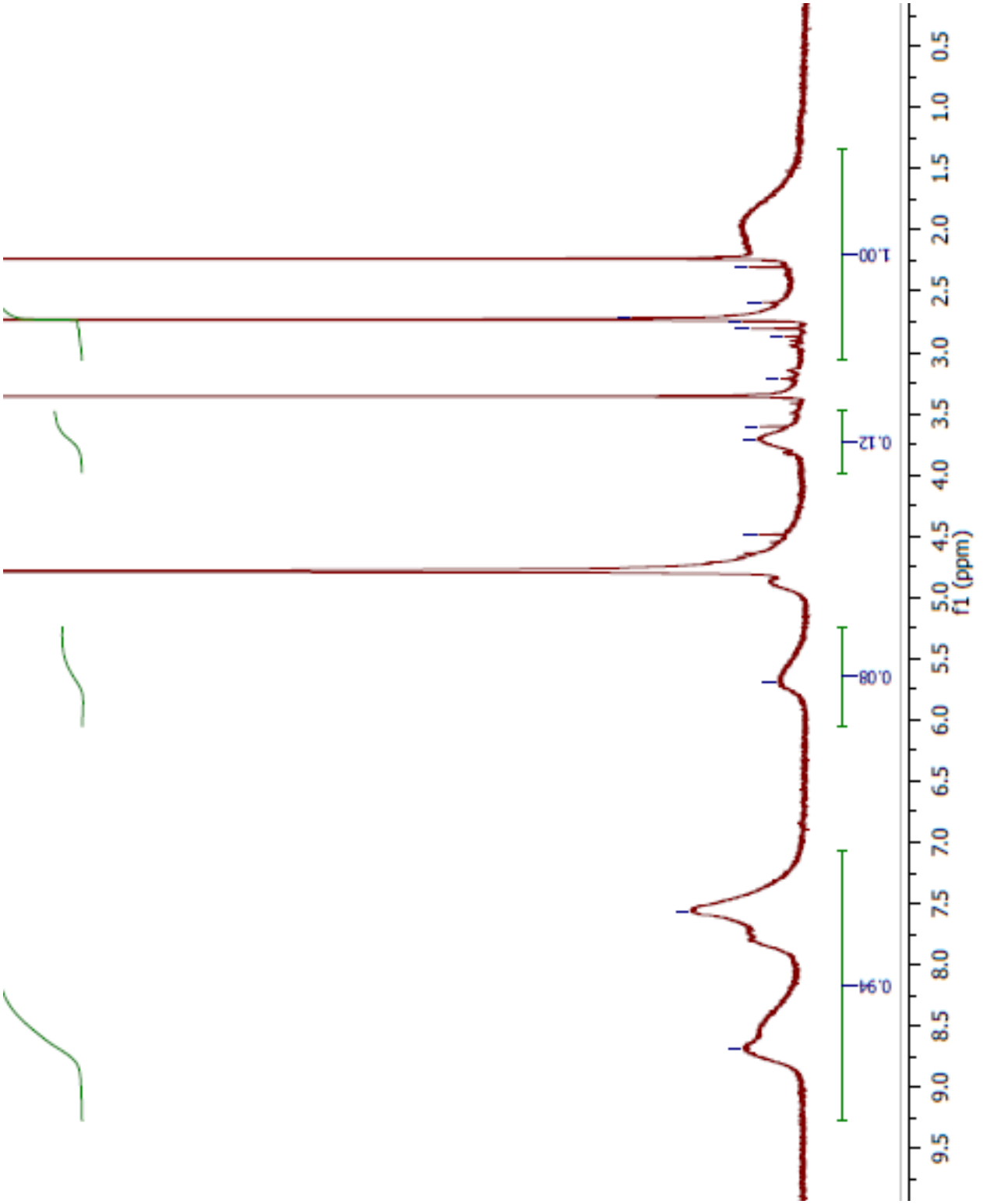
^{1}H-NMR (500 MHz) for PVP-EtOH (D_2O)



^{1}H-NMR (500 MHz) of m-PVP-BA-EtOH (D_2O)



^{1}H-NMR (500 MHz) of m-PVP-BA-NH_2 (D_2O)



IX. References

[1] De Marchi, A; Cecchin, E.; Basile, A.; Proto, G.; Donadon, W.; Jengo, A.; Schinella, D.; Jus, A.; Villalta, D.; De Paoli, P.; Santini, G; Tesio, F., *Am. J. Nephrol.* **1984**, 4, 280.

[2] Elsas, L. J.; Rosenberg, L. E., *J. Clinic. Invest.* **1969**, 48, 1845.

[3] Baxter, P.; Goldhill, J.; Hardcastle, P. T.; Taylor, C. J., *Gut* **1990**, 31, 817.

[4] Fedorak, R. N.; Gershon, M. D.; Field, M., *Gastroenterology* **1989**, 96, 37.

[5] Yasuda, H.; Kurokawa, T.; Fujii, Y.; Yamashita, S.; Ishibashi, S., *Biochim. Biophys. Acta* **1990**, 1020, 114.

[6] Hall, D.G.; Structure, Properties, and Preparation of Boronic Acid Derivatives. *Boronic Acids*, Ed. 2; D. G. Hall;Wiley-VCH Verlag & Co: Weinheim, Germany, 2011, Vol. 1, 2.

[7] Frankland, E.; Duppa, B. F.; *Justus L., Ann. Chem.* **1860**, 115, 319.

[8] Edwards, J. O.; Morrison, G. C.; Ross, V.; Schultz, W., *J. Am. Chem. Soc.* **1955**, 77, 266.

[9] Lorand, J. P.; Edwards, J. O., *J. Org. Chem.* **1959**, 24, 769.

[10] Springsteen, G.; Wang, B., *Tetrahedron*, **2002**, 58, 5291.

[11] Yan, J.; Springsteen, G.; Deeter, S.; Wang, B.; *Tetrahedron* **2004**, 60, 11205.

[12] Suri, J.T.; Cordes, D.B.; Cappuccio, F.E.; Wessling, R.A.; Singaram B.; *Langmuir* **2003**, 19, 5145.

[13] Camara, J.N.; Suri, J.R.; Cappuccio, F.E.; Wessling, R.A.; Singaram, B.; *Tetrahedron Lett.* **2002**, 43, 1139.

[14] Suri, J.T.; Cordes, D.B.; Cappuccio, F.E.; Wessling, R.A.; Singaram, B.; *Angew. Chem.* **2003**, 115, 6037.

[15] Howorka, S.; Siwy, Z., *Chem. Soc. Rev.* **2009,** 38, 2360.

[16] Tripathi, A.; Wang, J.; Luck, L. A.; Suni, I. I., *Anal. Chem.* ***2006,*** 79, 1266.

[17] Fink, D.; Klinkovich, I.; Bukelman, O.; Marks, R. S.; Kiv, A.; Fuks, D.; Fahrner, W. R.; Alfonta, L., *Biosens. Bioelectron.* **2009,** 24, 2702.

[18] Ali, M.; Ramirez, P.; Tahir, M. N.; Mafe, S.; Siwy, Z.; Neumann, R.; Tremel, W.; Ensinger, W., *Nanoscale* **2011,** 3, 1894.

[19] Nguyen, Q. H.; Ali, M.; Neumann, R.; Ensinger, W., *Sensors Actuat. B-Chem.* **2012,** 162, 216.

[20] Sun, Z.; Han, C.; Wen, L.; Tian, D.; Li, H.; Jiang, L., *Chem. Commun. (Cambridge)* **2012,** 48, 3282.

[21] Gao, C.; Ding, S.; Tan, Q.; Gu, L.Q., *Anal. Chem.* **2008**, 81, 80.

[22] Actis, P.; Jejelowo, O.; Pourmandq, N., *Biosensors and bioelectronics,* **2010**, 26, 333.

[23] Wei, C.; Bard, A.J.; Feldberg, S.W., *Anal. Chem.* **1997**, 69, 4627.

[24] Umehara, S.;Pourmand, N.;Webb, C.D.; Davis, R.W.; Yasuda, K.; Karhanek, M., *Nano. Lett.* **2006**, 6, 2486.

[25] Bard AJ, Faulkner LR (eds) (1980) Electrochemical methods: fundamentals and applications. Wiley, New York

[26] Umehara, S.; Pourmand, N.; Webb, C.D.; Davis, R.W.; Yasuda, K.; Karhanek, M., *Nano. Lett.* **2006**, 6, 2486.

[27] Fu, Y.; Tokuhisa, H.; Baker, L. A., *Chem. Commun. (Camb.)* **2009**, 32, 4877.

[28] Sexton, L.T.;Horne, L.P.; Sherrill, S.A.; Bishop, G.W.; Baker, L.A.; Martin, C.R., *J. Am. Chem. Soc.* **2007**, 129, 13144.

[29] A. Streitwieser, *Solvolytic Displacement Reactions*, McGraw-Hill, New York, 1962; J. F. Bunnett, *Annu. Rev. Phys. Chem,*, **14**, 271(1963).

[30] Yan, J.; Springsteen, G.; Deeter, S.; Wang, B.; *Tetrahedron* **2004**, 60, 11205.

[31] Yan, J.; Springsteen, G.; Deeter, S.; Wang, B.; *Tetrahedron* **2004**, 60, 11205.

[32] Zhang, L.X.; Cai, S.L.; Zheng, Y.B.; Cao, X.H.; Li, Y.Q., *Advanced Functional Materials* **2011**, 21, 2103.

[33] Bard AJ, Faulkner LR (eds) (1980) Electrochemical methods: fundamentals and applications. Wiley, New York.

[34] Gamsey, S.; Miller, A.; Olmstead, M. M.; Beavers, C. M.; Hirayama, L. C.; Pradhan, S.; Wessling, R. A.; Singaram, B., *J. Am. Chem. Soc.* **2007**, 129, 1278.

[35] James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S., *Angew. Chem., Int. Ed. Engl.* **1994**, 33, 2207.

[36] Karnati, V. V.; Gao, X.; Gao, S.; Yang, W.; Ni, W.; Sankar, S.; Wang, B., *Bioorg. Med. Chem. Lett.* **2002**, 12, 3373.

[37] Gamsey, S.; Miller, A.; Olmstead, M. M.; Beavers, C. M.; Hirayama, L. C.; Pradhan, S.; Wessling, R. A.; Singaram, B., *J. Am. Chem. Soc.* **2007**, 129, 1278.