Synthesis of a Boronic Acid-Based Oligosaccharide Receptor Using a Porphyrin Scaffold

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1. Abstract

The multi-step synthesis of a boronic acid-based oligosaccharide receptor is described. The proposed molecule was synthesized with the aim of developing a sensor that could be tuned to bind specific oligosaccharides on the cell surface. This receptor consisted of a *meso*-substituted tetraphenylporphyrin scaffold. 5,15-bis(4-cyanophenyl)-10,20-bis(4-hydroxyphenyl) porphyrin was first coordinated to a zinc ion. Polyethylene glycol chains were then added to the phenols of the porphyrin with hopes of increasing water solubility. The alcohol groups of the PEG chains were then protected from base by dihydropyran, and reduction of the cyano groups with $LiAlH_4$ followed. Subsequent refluxing in acid resulted in the removal of the alcohol-protecting groups and the zinc as well as the oxidation of any reduced porphyrin. The reductive amination of formylphenylboronic acid by the porphyrin resulted in the incorporation of two boronic acid functional groups. Upon purification, this porphyrin will be used in saccharide binding experiments to test the specific porphyrin-sugar interactions. These interactions will possibly be studied through the use of affinity chromatography, utilizing a solid phase such as Sephadex. This study may also ultimately provide a method for purification of the boronic acid porphyrin, which has not yet been easily conducted using conventional column chromatography.

2. Introduction

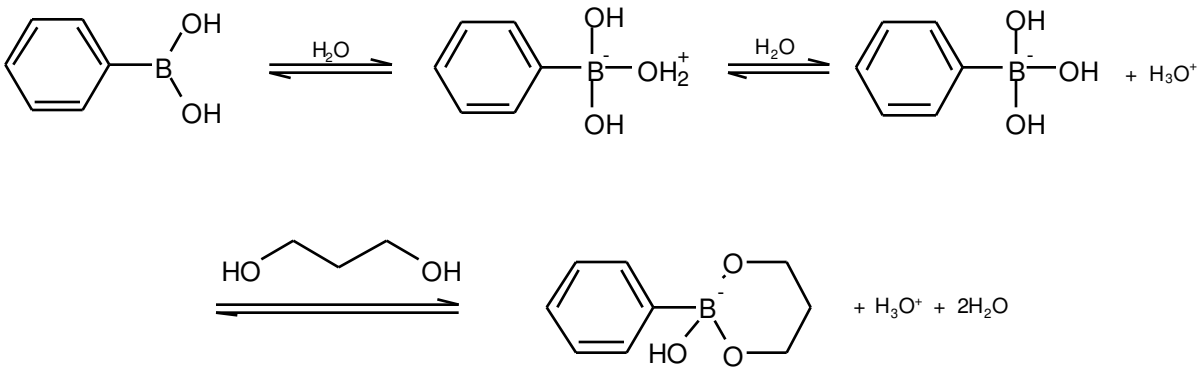
Oligosaccharides on the cell surface play a major part in cell-to-cell communication.[1] These carbohydrates are often associated with specific recognition and signaling processes. The interactions that occur between oligosaccharides and proteins can lead to important cellular functions or disease, and expression of specific oligosaccharides can serve as a marker for cellular abnormalities.[1-6,9] It has been shown that the aberrant expression of glycoproteins and glycolipids plays an important role in diseases such as AIDS, diabetes, rheumatoid arthritis, and cancer.[1-4] In breast cancer, for example, the diversity of the oligosaccharides on the surface of tumor cells has been related to the malignancy of the tumor, with less diversity and higher levels of certain oligosaccharides present in the cells of tumors that have metastasized.[5] Studies have linked such abnormal expressions to mutations in the genes for glycosyltransferases and/or glycosidases, which result in the shortening of glycan chains and ultimately lessen the diversity of the glycocalyx – the extracellular polysaccharide matrix.[7] These mutations result in an increased rate of growth in tumor cells, while reverse mutations have been shown to decrease the rate of metastasis and increase the number of larger oligosaccharides.[6,7]

The ability to detect differences in cell-surface oligosaccharides would be a powerful tool in the world of diagnostic medicine. While differences in certain oligosaccharides of normal and abnormal cells have recently been experimentally determined *in vitro*, detecting differences between the sugar residues *in vivo* poses an even larger challenge.[8] The difficulty in both scenarios results from the nearly infinite possibilities for oligosaccharide structure, which are due to the number of possible positions for linkage between monomers as well as the differing stereochemistry of the bonds.[1,2] These structures differ from amino acid chains, in which the character of the polypeptide only depends on the order in which the monomers were joined. For

example, there is only one dimer that can be formed from two copies of a single amino acid, while two copies of a single monosaccharide can form 11 different dimers.[2] Even subtle differences affect the overall structure and function of the oligosaccharide, making detection by chemical structure very difficult. However, being able to detect specific oligosaccharides would provide invaluable information for predicting cell activity. For example, the binding of these specific carbohydrates by a fluorophore would possibly allow for detection of anomalous cellular structures.

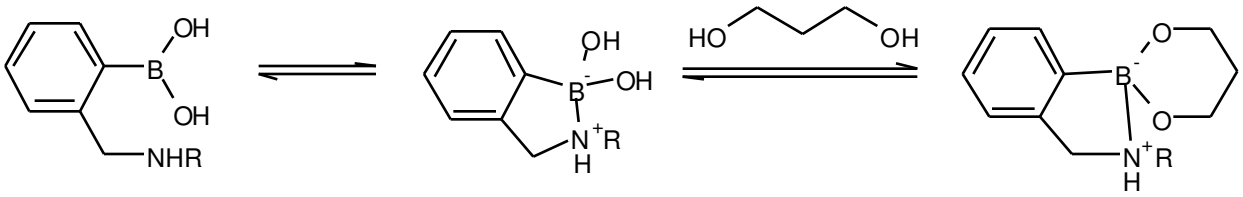
Most of the natural interactions that happen between oligosaccharides and other biological molecules such as lectins are polar attractions (van der Waals and hydrogen bonding) and are relatively weak in aqueous environments, making these molecules ineffective as sensors.[4,10,15] However, the covalent binding that occurs between saccharide diols and boronic acids can be exploited in oligosaccharide sensing when the boronic acid is attached to an easily detectable marker. Diboronic acid receptors have been previously used in the binding and detection of specific oligosaccharides.[11-16] The covalent bonding that occurs between a boronic acid and a *cis* 1,2- or 1,3-diol results in a 5 or 6-membered ring, respectively, according to the following scheme in aqueous media[16]:

Scheme 2.1: Covalent bonding of a phenylboronic acid to a 1,3-diol.



It has been previously shown that this reaction occurs best at high pH.[13] It is desired that this molecule be used in neutral aqueous environments, so these association events would be marked by low formation constants (~10^{-3} M) in the conditions of this experiment.[13-16] In an attempt to overcome this limitation, an amine functionality could be added next to the boronic acid. It has been shown that a secondary amine would form a dative bond with the boronic acid, creating a tetrahedral boron atom that would both stabilize the sensor structure and increase the kinetics of the saccharide binding.[28] The following scheme represents the same diol binding as **Scheme 2.1**, but contains an amine functionality.

Scheme 2.2: Covalent bonding of a phenylboronic acid, with adjacent secondary amine, to a 1,3-diol.



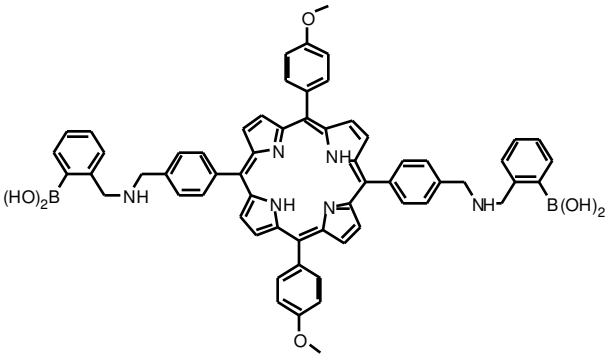
In addition, while this mechanism is rather unselective, selectivity has been previously shown in diboronic acid sensors in which the spacing between the boronic acids was varied.[12-15] However, the sensors in these studies are specific to certain oligosaccharides and can only function in basic or aprotic environments. By varying the distance between boronic acid functional groups and adding amine functionality to the molecule, it is proposed that the structural characteristics of other oligosaccharides in protic media can be targeted.[12]

When considering the structure of the sensing molecule to which boronic acids will be introduced, it is important to take two features into account. First, the molecule should act as a reporter.[12,13] Fluorescence is an invaluable phenomenon in the sensing of specific targets, since fluorescence detectors usually possess very low detection limits. This sensitivity is ideal in our

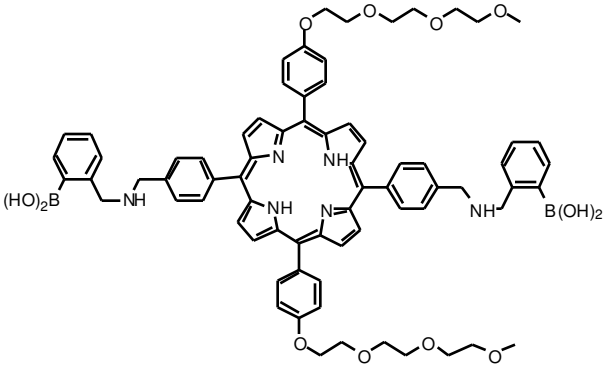
case since binding is marked by low formation constants.[13-16] In addition, a molecule that is rigid, stable, and has many different possible sites for functionalization is ideal.[13,14] The use of a porphyrin scaffold fits this description. Porphyrins have shown many advantages, including strong fluorescence and a high level of substitution possibilities. The twelve positions available for substitution on the aromatic porphyrin system allow for countless possibilities of functional group addition, as well as varying degrees of space between the functionalization.[17,18] This versatility could possibly make the porphyrin system very useful when adapting the molecules to different saccharides.

This project incorporates diboronic acid functionality onto a tetraphenylporphyrin scaffold. The structure of the proposed receptor was based on the requirement that the porphyrin contain boronic acids for covalent binding to saccharide diols as well possess some degree of water solubility for use in aqueous cellular environments. Previous attempts at a water-soluble, diboronic acid porphyrin presented a few problems for which adjustments in the structure of the porphyrin were needed. The initial problem was the solubility of the porphyrin. The following structures represent previously-synthesized porphyrins that showed problems with solubility.

Scheme 2.3: Previously synthesized boronic acid porphyrins.



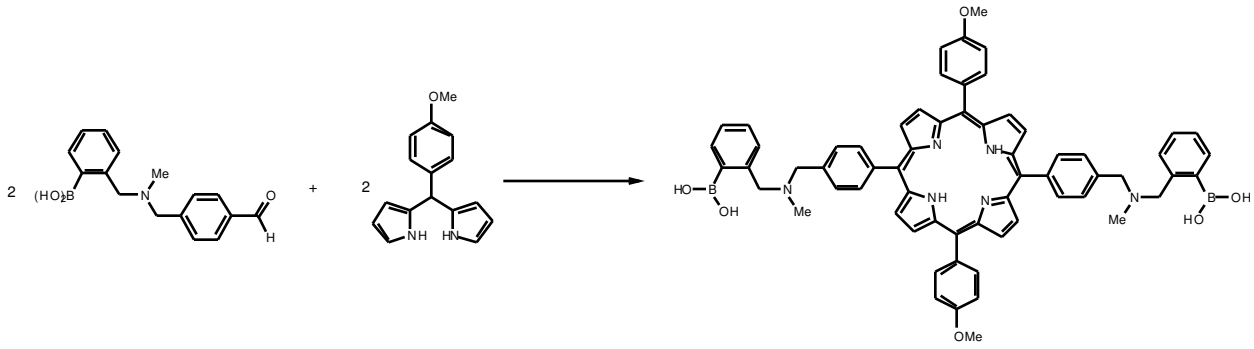
i



ii

Porphyrin **i** showed limited solubility in anything except acidic media, and while porphyrin **ii** showed solubility in aprotic media, it was not soluble enough in protic media to conduct successful saccharide-binding experiments. These two problems led to the idea for incorporation of PEG chains possessing terminal hydroxyl groups for hopes of greater aqueous solubility. Secondly, another previous synthesis went by the following scheme:

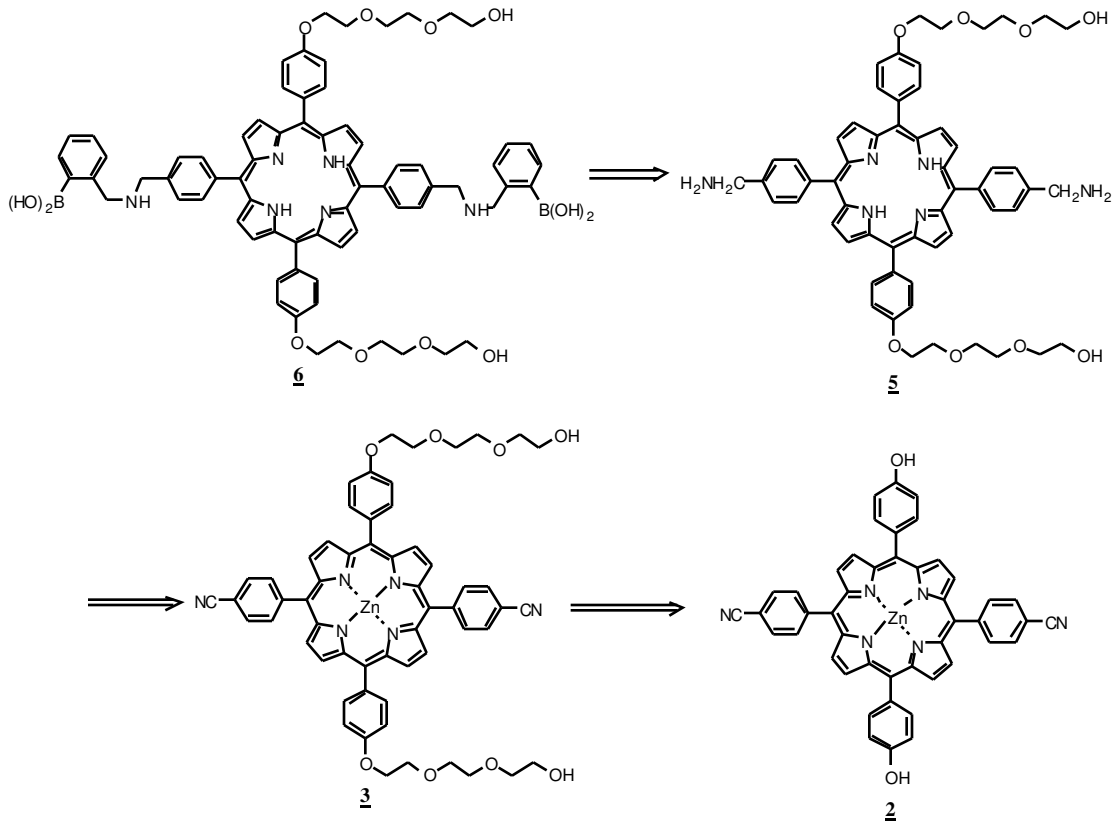
Scheme 2.4: Previous synthesis of diboronic acid porphyrin.



This porphyrin was impossible to separate from oligomeric side-products by chromatography on either silica or basic alumina. Therefore, it was determined that boronic acid functionality should be added *after* initial porphyrin synthesis and purification.

With respect to this project, polyethylene glycol chains were incorporated into the structure of the porphyrin with hopes of achieving a higher degree of water solubility. An amine functionality would stabilize formation of the boronate ion in **Scheme 2.1** through ion pairing and would possibly allow for saccharide binding in protic media. Taking these needs into account, a new scheme for the synthesis of an oligosaccharide-binding, water-soluble, porphyrin-based receptor has been proposed using the following retrosynthesis.

Scheme 2.5: Retrosynthesis of boronic acid-substituted porphyrin



This synthesis will begin with 5,15-bis(4-cyanophenyl)-10,20-bis(4-hydroxyphenyl)porphyrin, to which polyethylene glycol appendages will be added. Reduction of the nitrile groups will produce primary amines to which two 2-formylphenylboronic acid molecules are attached by reductive amination.

Purification of the boronic acid porphyrins that were synthesized in previous experiments has been one of the major problems in their synthesis. Chromatography on silica or alumina either resulted in no movement of the porphyrin through the column or did not allow for successful separation of oligomers. It has been shown that affinity chromatography has been used extensively in the determination of dissociation constants well below 10^{-3} M.[22-24] Using affinity chromatography would aid in studies aiming to provide insight towards the "tunability" of the boronic acid porphyrin to specific oligosaccharides. In addition, affinity chromatography

using a solid phase such as Sephadex could possibly provide a method for the purification of the desired porphyrin product. Sephadex, a chromatographic medium synthesized from dextrin, has been previously used in sugar-protein and protein-DNA studies to not only test the strengths of their interactions, but as a purification method.[25-27] The polysaccharide structure of Sephadex possibly makes it a good solid phase for purification of the boronic acid porphyrin. By partitioning the porphyrin on a Sephadex column and eluting with a solution of the saccharide that exhibited the highest binding constant with the porphyrin, it may be possible to separate the porphyrin from any impurities that may have been impossible to separate out otherwise. This future direction will follow the successful synthesis and characterization of the desired porphyrin.

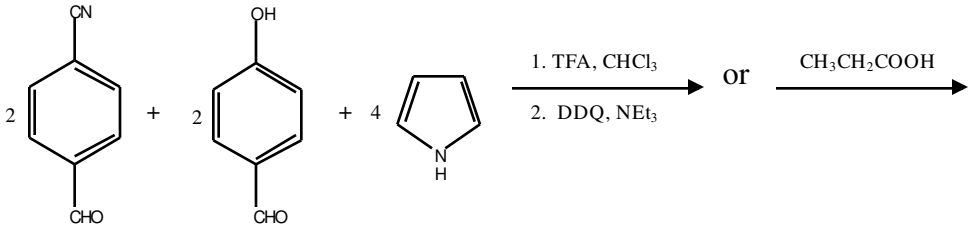
Using **Scheme 2.5**, the formation of 5,15-bis(2-((benzylamino)methyl)phenylboronic acid)-10,20-bis(4-(2-(2-(2-hydroxyethoxy) ethoxy)ethyl)phenyl)porphyrin was attempted in a seven-step synthesis as follows.

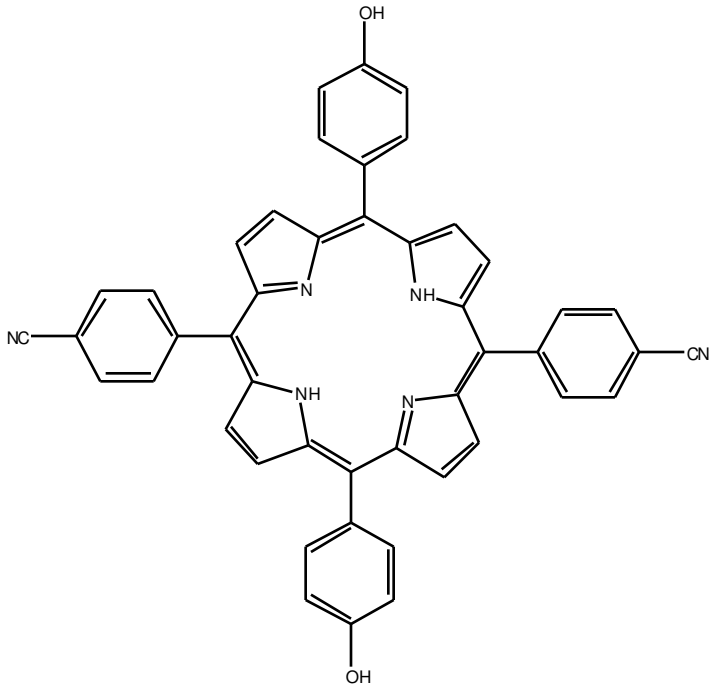
3. Synthesis

3.1. Synthesis of 5,15-bis(4-cyanophenyl)-10,20-bis(4-hydroxyphenyl)porphyrin

To synthesize porphyrin **1**, three different routes were considered. The first two involved porphyrin synthesis from aldehydes and pyrrole, while the third involved synthesis from a dipyrromethane. The first two methods took place according to the following reaction:

Scheme 3.1. Porphyrin synthesis starting from aldehydes and pyrrole – a 2 + 2 + 4 condensation.





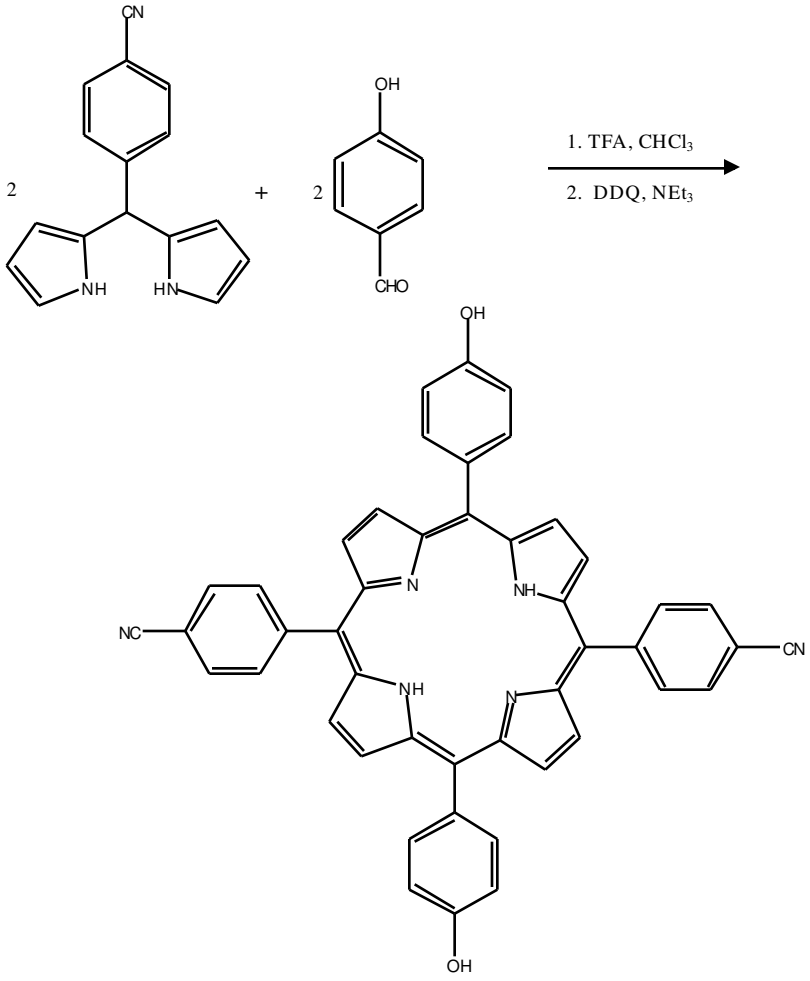
1

The porphyrin synthesis was done in two ways. It was first tried by dissolving the aldehydes (*p*-cyanobenzaldehyde and *p*-hydroxybenzaldehyde) in chloroform and adding pyrrole that had been

purified through basic alumina. Trifluoroacetic acid (TFA) was used as the acid catalyst. After quenching and adding dichloro-dicyano-benzoquinone, column chromatography allowed for purification of the porphyrin. After metallation with zinc, a yield of about 3.5% was calculated for porphyrin **1**.

The second method considered for the reaction above used propionic acid as both the catalyst and the solvent. This method is very fast compared to the previous method and can be done on a very large scale (229 mg of product vs. 87.4 mg); however, the resultant yield was substantially smaller (1.3 % vs. 3.5 %). While this was a fast method for producing large amounts of product, one more reaction was attempted in order to minimize the amount of reactant needed and maximize the yield. The following reaction represents the third method by which the porphyrin was synthesized:

Scheme 3.2. Porphyrin reaction from condensation of 4-(di-1*H*-pyrrol-2-ylmethyl) benzonitrile and *p*-hydroxybenzaldehyde.



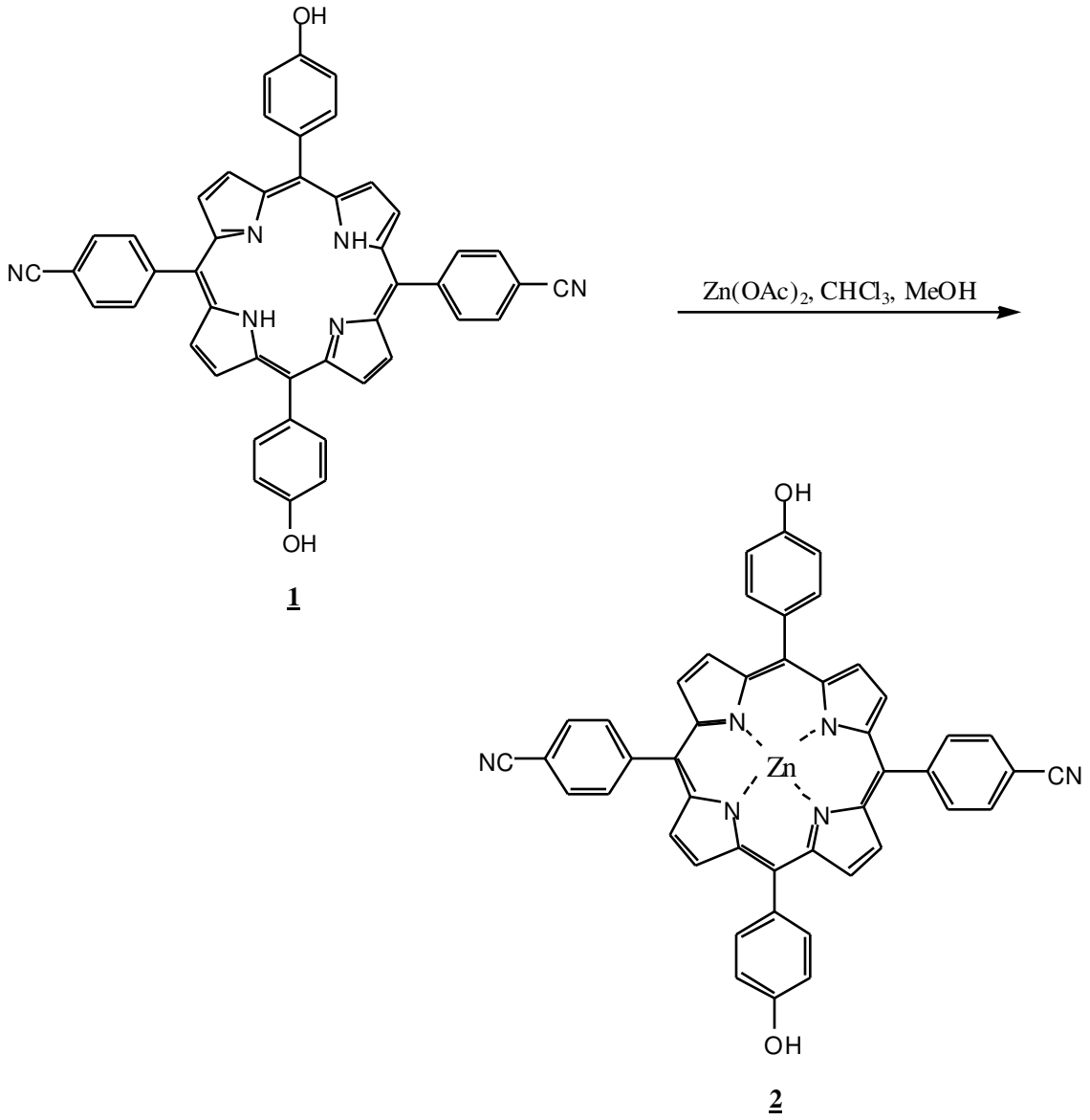
1

In this scheme, a dipyrromethane made from *p*-cyanobenzaldehyde and pyrrole was utilized to increase the probability that the porphyrin of interest was synthesized by decreasing side products. This effect could be seen in the reaction yields, which ranged from 10-19%. Using reactants on the same scale as the first method described above produced amounts of porphyrin on the same scale as the propionic acid method. Therefore, this third synthesis is preferred.

3.2. Zinc coordination by porphyrin 1

The next step in the boronic acid porphyrin synthesis involved zinc coordination. This reaction proceeded as follows:

Scheme 3.3. Zinc ion coordination to form metalloporphyrin **2**.



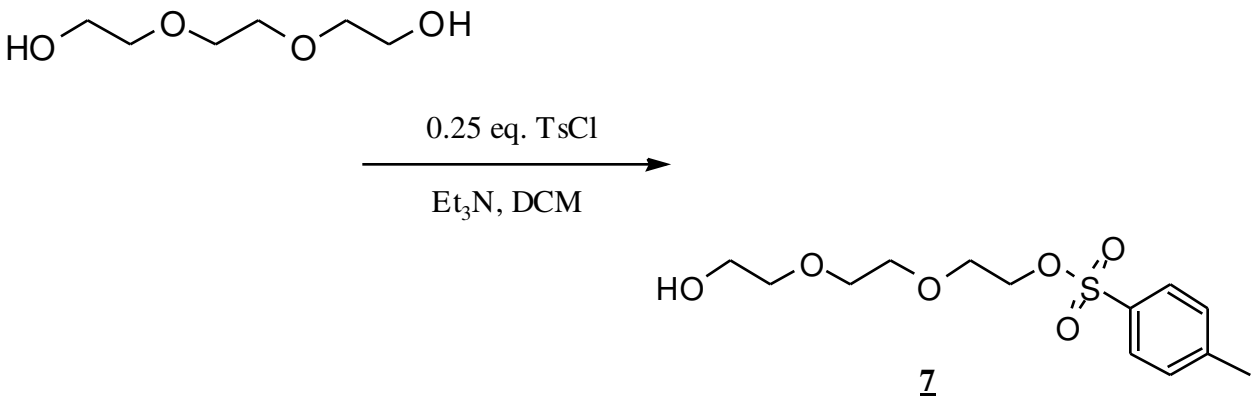
1 **2**

This reaction results in an almost quantitative yield of the metal-coordinated porphyrin (97-99%).

3.3. Synthesis of toluene-4-sulfonic acid 2-[2-(2-hydroxyethoxy)ethoxy]ethyl ester

In an attempt to make the final boronic acid porphyrin product more water soluble, polyethylene glycol (PEG) chains were substituted on two of the porphyrin's phenyl groups. This addition was achieved by an S_N2 reaction of a tosylated PEG chain with the hydroxyl groups on the porphyrin. According to the literature[21], this chain can be synthesized by the following scheme:

Scheme 3.4. General reaction for synthesis of tosylated triethylene glycol, from the literature[21].



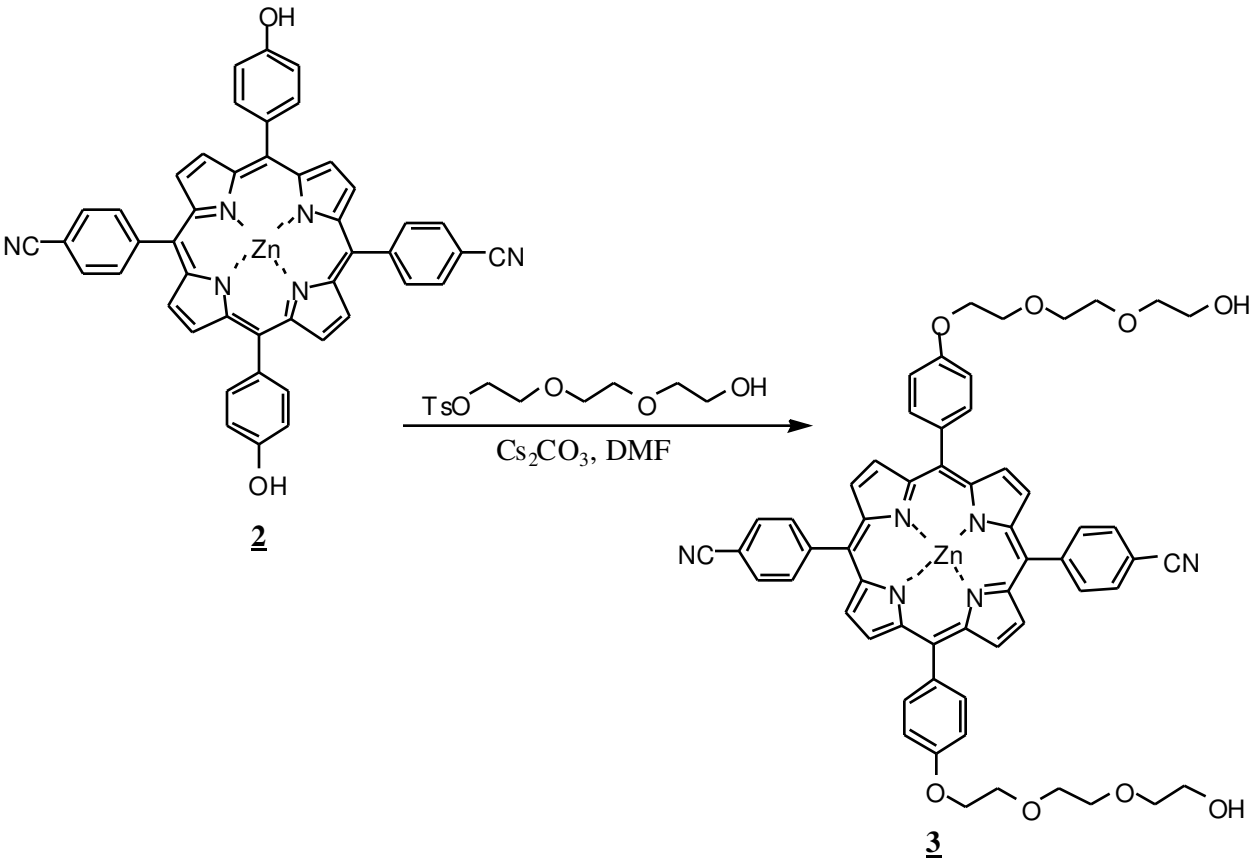
7

This reaction produces two products, since the *p*-toluene sulfonic acid can attack one or both ends of the PEG chain. Therefore, column chromatography was employed to separate the bis-tosylated product from the mono-tosylated product. A yield of about 84.4 % was determined. This reaction can be conducted on a large scale compared to the porphyrin reaction. It was found to be very important that the mono-tosylated PEG product **7** be extremely pure before each use in subsequent reactions to avoid unwanted products. Mixtures of the monotosylated and bistosylated products result in very impure porphyrin products after the next step in the synthesis, since a bis-tosylated chain can link the hydroxyl groups of two different porphyrin molecules, ultimately resulting in the polymerization of porphyrin molecules.

3.4. Synthesis of 5,15-bis(4-cyanophenyl)-10,20-bis(4-(2-(2-(2-hydroxyethoxy) ethoxy)ethyl)phenyl)porphyrin zinc(II)

As stated above, polyethylene glycol chains were incorporated into the structure of the boronic acid porphyrin in an attempt to increase its water solubility. This reaction proceeded by the following scheme using the previously synthesized mono-tosylated PEG product **7**.

Scheme 3.5. Reaction of mono-tosylated PEG **7** with metalloporphyrin **2**.



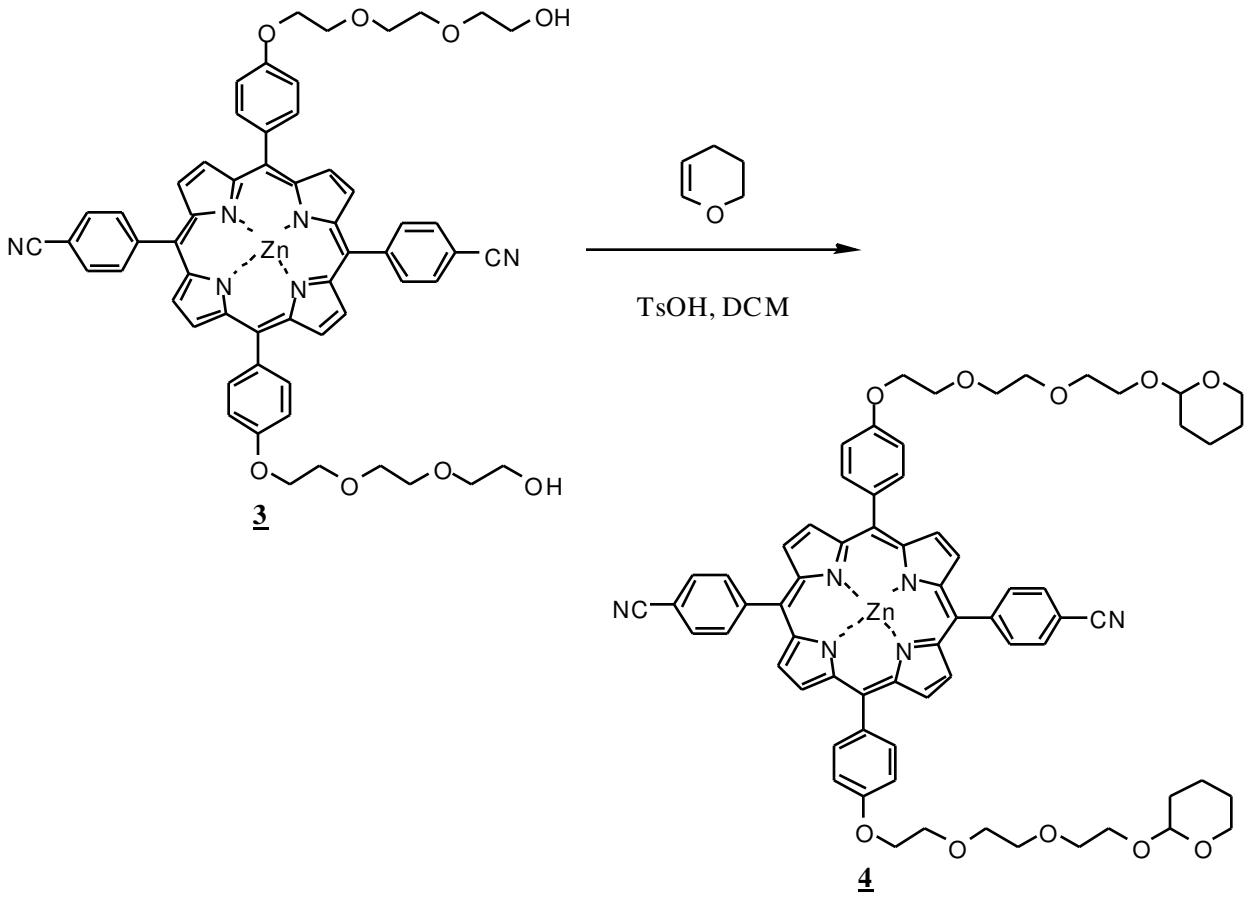
2 **3**

In this reaction, the cesium carbonate acted in deprotonating the hydroxyl groups of the porphyrin. This resulted in an S_N2 reaction between the phenol and **7**. Excess **7** was used in this reaction to ensure that the porphyrin was doubly substituted. To isolate the di-substituted product, the resulting porphyrin was purified by column chromatography. This product was precipitated from $MeOH/CH_2Cl_2$ and hexanes to result in a 57.5-60.0% yield.

3.5. Alcohol protection by dihydropyran: Synthesis of 5,15-bis(4-cyanophenyl)-10,20-bis(4-(2-(2-(2-(tetrahydro-2H-pyran-2-yloxy)ethoxy)ethoxy)ethyl)phenyl) porphyrin zinc(II)

Before the cyano groups of the porphyrin can be reduced with lithium aluminum hydride (LAH), the alcohol groups on the PEG chains must be protected to prevent deprotonation. It was desired that a protecting group be used that is stable to base and reductive conditions but can be easily removed in the presence of acid used to work up the nitrile reduction reaction. Therefore, dihydropyran was used, which was added to the PEG chains by the following scheme.

Scheme 3.6. Protection of alcohol by addition of LAH-stabile dihydropyran.



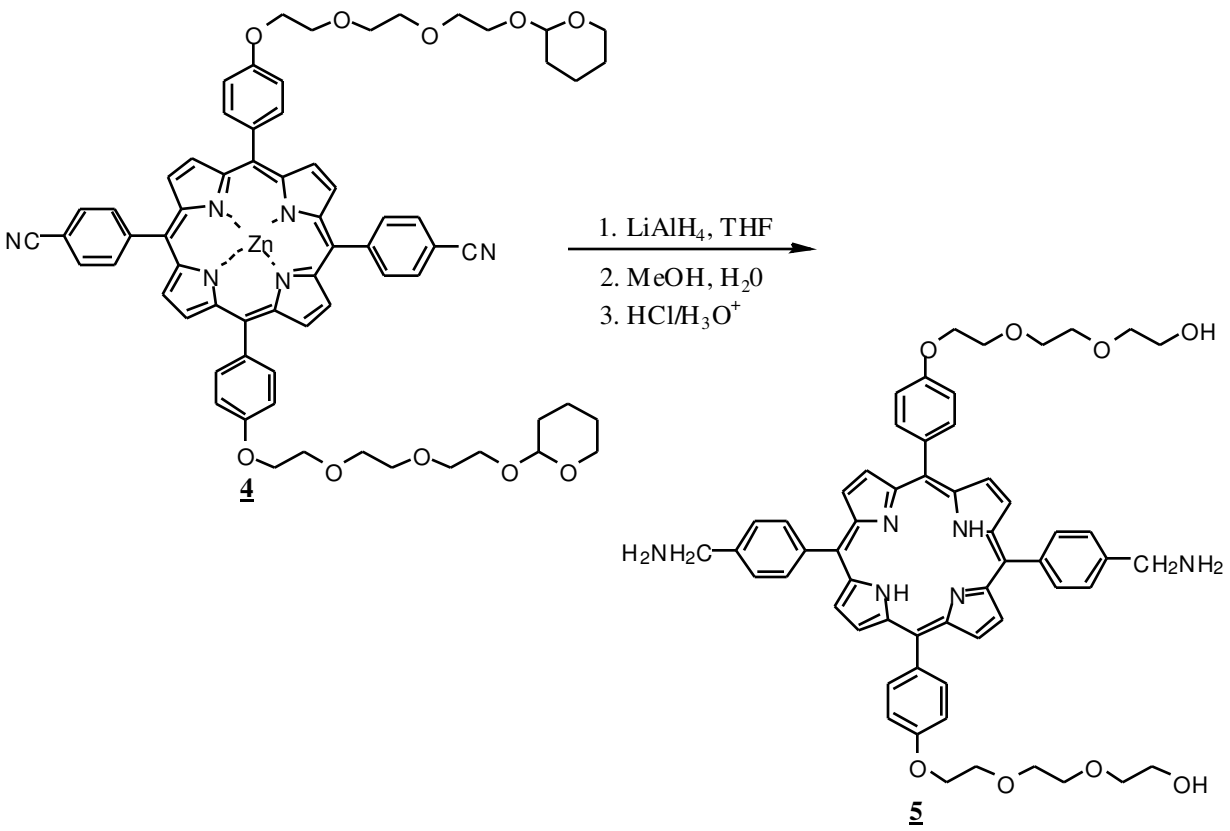
3 **4**

Porphyrin **4** was easily purified. To isolate the porphyrin that was di-substituted, column chromatography was employed to allow for quick separation. Yields of 50 - 60% were observed, indicating that there may have been some impurities in porphyrin **3** or the reagents used.

3.6. Nitrile reduction: Synthesis of 5,15-bis(4-(aminomethyl)phenyl)-10,20-bis(4-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)phenyl)porphyrin

The cyano groups of porphyrin **4** were then reduced using LAH. To do so, the porphyrin was allowed to react with LAH, quenched, and subsequently refluxed in acid, which in turn removed the protecting groups and the coordinated zinc and oxidized any reduced porphyrin.

Scheme 3.7. Nitrile reduction using lithium aluminum hydride.



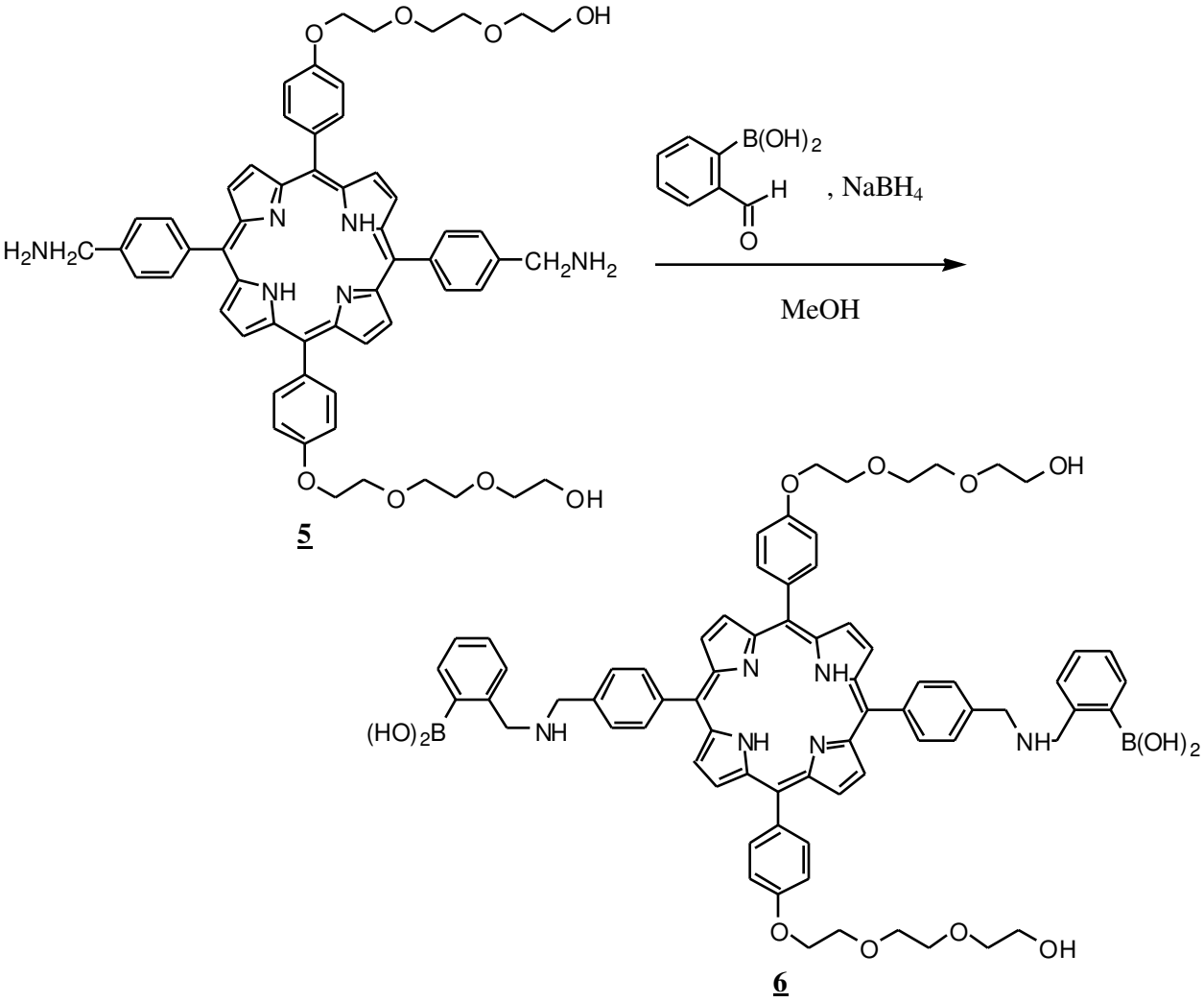
4 **5**

The amine groups of porphyrin **5** made it very hard to purify. Unlike the previous porphyrins in the synthesis, porphyrin **5** could not be easily purified using silica or alumina. However, it was able to be purified with a high level of success by using a disposable C18 solid-phase extraction column. After neutralization, the resulting purple solid was precipitated with MeOH/DCM and hexanes. This purification resulted in a much cleaner NMR than the uncolumned product. The recovered solid measured only 50% of the expected yield.

3.7. Reductive Amination of Formyphenylboronic Acid: Synthesis of 5,15-bis(2-((benzylamino)methyl)phenylboronic acid)-10,20-bis(4-(2-(2-(2-hydroxyethoxy) ethoxy)ethyl)phenyl)porphyrin

To synthesize the final boronic acid porphyrin **6**, a reductive amination of formylphenylboronic acid was conducted by the following scheme.

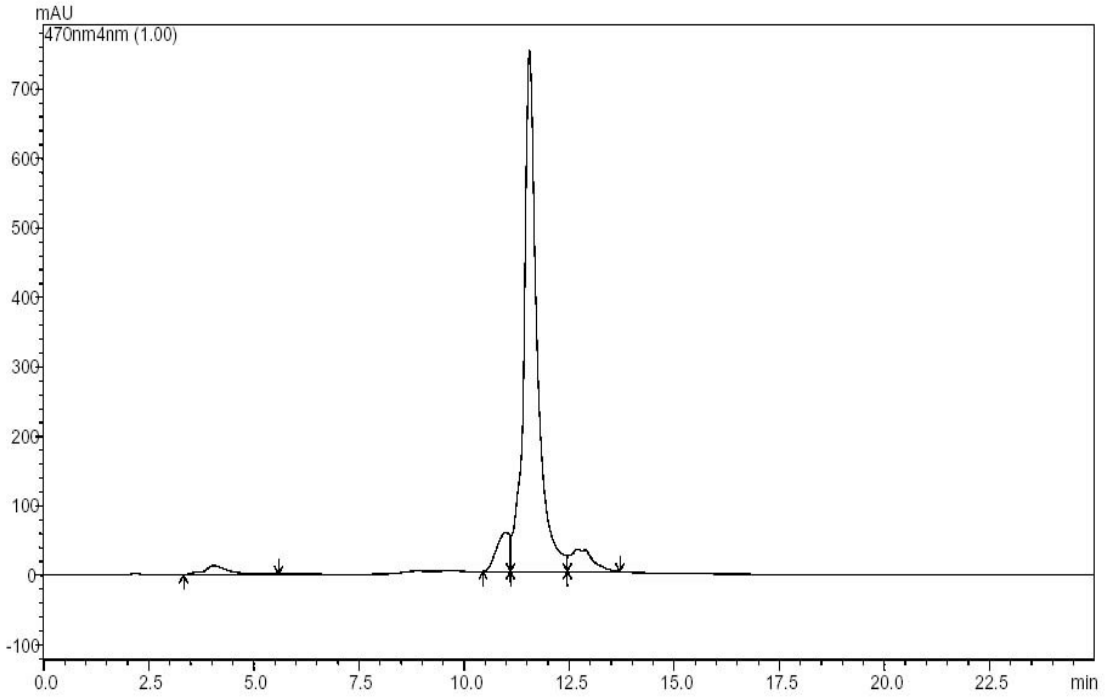
Scheme 3.8. Reductive amination of formylphenylboronic acid to form boronic acid porphyrin **6**.



Like the amine porphyrin in the previous step, this porphyrin could not be successfully purified on either silica or alumina. It was purified by the same method as before, using a disposable C18

solid phase extraction cartridge. The fraction containing **6** was run by HPLC to determine purity using gradient elution (A: 1% TFA/water, B: Acetonitrile).

Figure 3.1. HPLC chromatogram of boronic acid porphyrin using gradient elution.

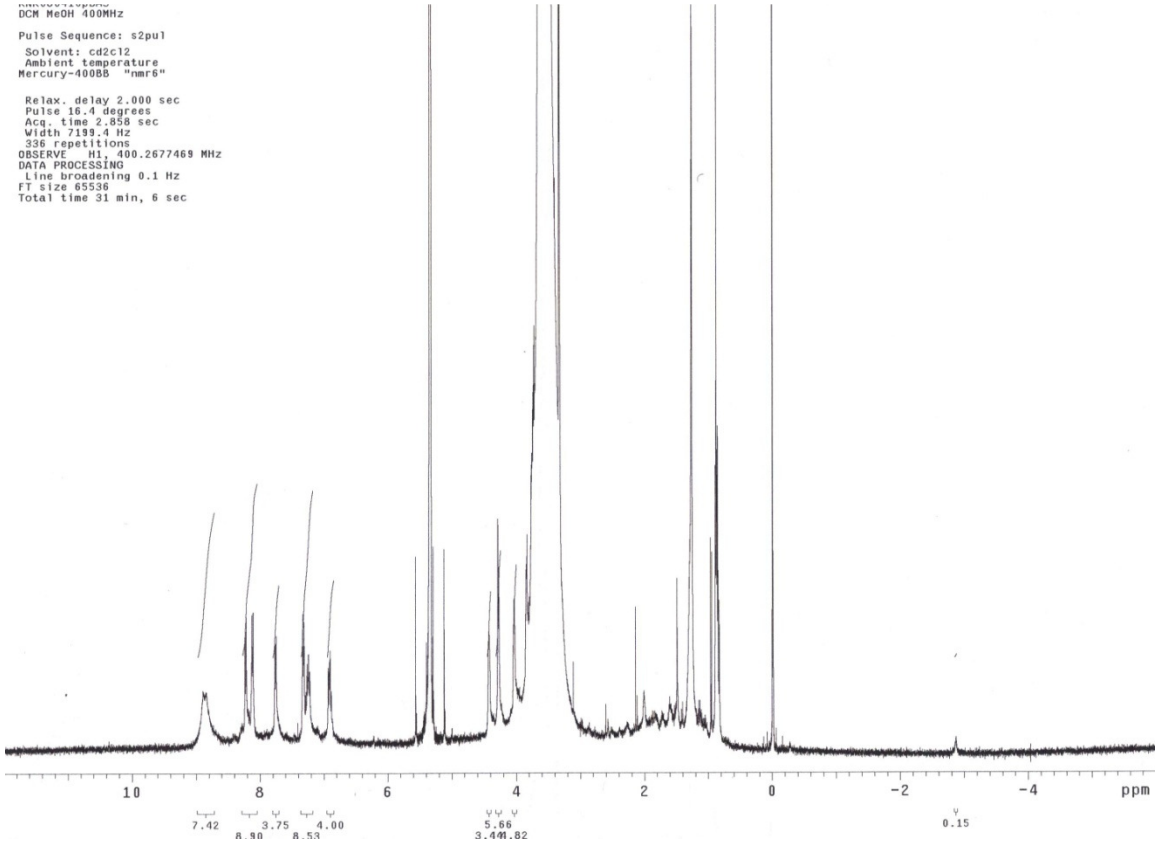


Ret.Time	Area	Height	Peak Start	Peak end	Area %
4.043	525509	13316	3.328	5.589	2.7004
10.992	1220207	56310	10.453	11.104	6.2703
11.554	16534132	749487	11.104	12.459	84.9643
12.696	1180252	32349	12.459	13.707	6.0650

Using a detection wavelength of 415 nm, the resulting chromatogram indicated that this fraction of porphyrin was about 85% pure. This fraction was then brought to neutral pH with ammonium hydroxide and extracted with chloroform. Like porphyrin **5**, this reaction was done on such a small scale that most of the product was stuck to the filter. Therefore, all of the product was used in the NMR sample, which was done by dissolving the porphyrin in CD_2Cl_2 and minimal CD_3OD. While this did not produce the ideal NMR due to the presence of methanol peaks,

previous tries with pure CD_2Cl_2, pure $CDCl_3$, and the combinations of THF/DMSO and CD_2Cl_2/DMSO did not allow for enough dissolution of the porphyrin for a good NMR.

Figure 3.2. ^{1}H-NMR of boronic acid porphyrin.



The ^{1}H-NMR of the final porphyrin, although slightly messy in the methanol region, shows characteristic peaks in the aromatic region (7-9 ppm). The peak at 8.89 ppm represents the 8 protons present at the *beta*-positions of the porphyrin scaffold. Doublets at 8.24, 8.13, 7.77, and 7.35 ppm each represent 4 protons present on the phenyl groups attached to the *meso*-positions of the porphyrin. The multiplets at 7.28 and 6.95 ppm then each represent 4 protons, for a total of 8 protons found on the phenyl-boronic acid groups. In addition, at 4.45 and 4.06 ppm before the large methanol peak, singlet peaks can be seen that represent the methylene groups that bridge the phenyl groups of the porphyrin and the boronic acid to the amine group. A multiplet peak at 4.31 ppm representing 4 of the protons on the PEG chains is also visible.

The product was then analyzed by mass spectrometry. An ESI full scan indicated a base peak of 1237, which corresponds to that of the boronic acid porphyrin, as well as a product with m/z equal to 619.28482[M^{2+}]. Elemental composition of the latter peak indicated a product with formula $C_{72}H_{76}N_6O_{12}B_2^{+2}$ (m/z = 619.2850). This formula matches that of the desired product.

4. Conclusion and Future Direction

A successful synthetic route for the formation of a porphyrin-based boronic acid has been discussed. A few adjustments will be made in future syntheses, such as addition of the triethylene glycol to the 4-hydroxybenzaldehyde before porphyrin synthesis in order to minimize the number of steps required to obtain the final product after the porphyrin scaffold has been constructed. This procedure should both decrease the time of the full boronic acid porphyrin synthesis as well as increase the total yield.

Upon the collective formation of more product, further experiments will be conducted that give insight to the sugar-binding properties of this molecule. Affinity chromatography has been previously used in the determination of binding constants and may help in determining the respective binding constants between the boronic acid and different saccharides. A solution of the saccharide that associates best with the boronic acid porphyrin could then possibly be used in a highly-efficient purification technique at the end of the synthesis. This would eliminate the use of the C18 solid-phase extraction column, which resulted in only 85% purity.

5. Experimental

5.1. Porphyrin 2: 5,15-bis(4-cyanophenyl)-10,20-bis(4-hydroxyphenyl)porphyrin zinc(II)

Porphyrin **1** was synthesized by stirring 3 equivalents of *p*-hydroxybenzaldehyde (1.05 g, 8.61 mmol) in 1.150 L chloroform (5 mM) to dissolve while degassing with N_2. After 45 minutes, 2 equivalents of 4-(di-1H-pyrrol-2-ylmethyl)benzonitrile (1.42 g, 5.74 mmol) were added to the solution. Trifluoroacetic acid was then added to result in a 30 mM solution (2.65 mL, 34.5 mmol). This solution was allowed to stir in the dark at room temperature overnight under argon. After 15 hours, the solution was quenched with triethylamine, after which the solution was allowed to stir for an additional 20 minutes. Three equivalents of DDQ (1.95 g, 8.61 mmol) was then added and the solution was allowed to stir in air for two hours. The resulting solution was then evaporated until no apparent solvent was left. The desired product was purified by column chromatography (0.5% ammonia-sat. methanol in dichloromethane, 450 mL silica). The fraction collected was evaporated down in a round bottom flask, and a 20% yield was then assumed to determine equivalents needed to synthesize porphyrin **2**. A 1:1 solution of methanol and chloroform (57.4 mL) was added to dissolve the product to a concentration of 0.01 M. Ten equivalents of zinc acetate (1.05 g, 5.74 mmol) were then added to the solution, and the solution was allowed to reflux in the dark for thirty minutes. The resulting product was checked against the starting material by TLC (5% MeOH/CH_2Cl_2, silica) to determine completeness of the reaction. The solution was then washed with saturated sodium bicarbonate and back extracted with CH_2Cl_2 until a colorless aqueous layer resulted. The combined organic layers were dried with anhydrous sodium sulfate and gravity filtered, after which the filtrate was evaporated to dryness. The resulting residue was precipitated by dissolving in minimal CH_2Cl_2/MeOH and crashing out with hexanes. The precipitate was rinsed with pentane and allowed to dry, resulting in a purple solid (421.5 mg, 19.1%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.927 (d, 4H), 8.715 (d, 4H), 8.334 (d, 4H), 8.044 (d, 4H), 7.973 (d, 4H), 7.160 (d, 4H). HR-MS (m/z): calculated: 758.1409, found: 758.1407 [M^+].

5.2.: Toluene-4-sulfonic acid 2-[2-(2-hydroxyethoxy)ethoxy]ethyl ester

This reaction was done as conducted by van Ameidje and Liskamp.[27] The NMR peaks of the obtained product closely agreed with those determined in the literature. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, 2H), 7.33 (d, 2H), 7.26 (s, 1H), 4.16 (t, 2H), 3.72-3.68 (m, 4H), 3.60 (s, 4H), 3.56 (t, 2 H).

5.3. Porphyrin 3: 5,15-bis(4-cyanophenyl)-10,20-bis(4-(2-(2-(2-hydroxyethoxy)ethoxy) ethyl)phenyl)porphyrin zinc(II)

Porphyrin **3** was synthesized by dissolving **2** (200 mg, 0.263 mmol) in 14 ml dimethylformamide to make a 0.02 M solution. Ten equivalents of cesium carbonate (857.2 mg, 2.63 mmol) were then added, along with five equivalents of mono-tosylated PEG **7** (400.5 mg, 1.316 mmol). This solution was heated to 75°C and allowed to run overnight. Twenty-six hours later, the resulting solution was washed 3 times with 2M NaOH, and the resulting aqueous solution was back extracted with methylene chloride. The combined organic layers were then washed with deionized water until a colorless aqueous layer resulted. The organic layer was then dried with

sodium sulfate, gravity filtered, and evaporated to dryness. The residue was then dissolved in minimal MeOH/CH_2Cl_2 and the porphyrin was precipitated with hexanes. The precipitate was collected by vacuum filtration and washed with pentanes. The dry product was 155.1 mg of purple solid (57.5 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (d, 4H), 8.68 (d, 4H), 8.27 (d, 4H), 8.02 (d, 4H), 7.98 (d, 4H), 7.22 (d, 4H), 4.39-4.34 (t, 4H), 4.02-3.96 (t, 4H), 3.84-3.78 (t, 4H), 3.63-3.58 (t, 4H), 3.30-3.26 (m, 8H). HR-MS (m/z): calculated: 1023.3060, found: 1023.3066 [M^+].

5.4.: Porphyrin 4: 5,15-bis(4-cyanophenyl)-10,20-bis(4-(2-(2-(2-(tetrahydro-2H-pyran-2-yloxy) ethoxy)ethoxy)ethyl)phenyl)porphyrin zinc(II)

To synthesize porphyrin **4**, porphyrin **3** (153.4 mg, 0.150 mmol) was added to a round-bottom flask and 10 ml of methylene chloride was added. Three equivalents of dihydropyran (37.8 mg, 0.449 mmol) were then added to the flask and the solution was cooled to 0ºC. 0.1 equivalents of *p*-toluene sulfonic acid (2.579 mg, 0.015 mmol) was then added, allowing the solution to stir for 10 minutes before the solution was brought back to room temperature. The solution was checked by TLC (silica, 5% MeOH/ CH_2Cl_2) until the reaction went to completion (about 3 hours). The solution was then washed with saturated sodium bicarbonate, and the aqueous layer was back extracted with methylene chloride until a colorless aqueous layer resulted. The combined organic layers were then dried with Na_2SO_4 and gravity filtered before being evaporated to dryness. The residue was precipitated by dissolving in minimal MeOH/CH_2Cl_2 and precipitating with hexanes. The solid was then washed with pentanes and allowed to dry, which resulted in 100.9 mg purple solid (56.5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.83 (d, 4H), 8.67 (d, 4H), 8.26 (d, 4H), 8.00 (d, 4H), 7.97 (d, 4H), 7.21 (d, 4H), 4.58-4.55 (t, 4H), 4.36-4.32 (t, 4H), 4.00-3.96 (t, 4H), 3.74-3.64 (m, 8H), 3.62-3.54 (m, 4H), 3.28-3.25 (m, 8H). HR-MS (m/z): calculated: 1191.4210, found: 1191.4203 [M^+]. Note: The number of alkyl protons do not add up correctly due to some peaks being hidden by large methanol peaks.

5.5. Porphyrin 5: 5,15-bis(4-(aminomethyl)phenyl)-10,20-bis(4-(2-(2-(2-hydroxyethoxy)ethoxy) ethyl)phenyl)porphyrin

To synthesize porphyrin **5**, porphyrin **4** (110 mg, 0.092 mmol) was added to an oven-dried round-bottom flask and placed under nitrogen. 46.1 ml of distilled THF was then added through the septum with an oven-dried needle to make a 2 mM solution. To the stirring solution, 10 equivalents of lithium aluminum hydride (0.922 ml, 0.922 mmol) were added dropwise to result in a green solution. After 6 hours, 20 ml of a 1:1 water/methanol mixture was slowly added to produce slight bubbling. This solution was allowed to stir for 30 min. The THF and methanol was then evaporated, and 20 ml of concentrated HCl was added to the porphyrin solution and refluxed overnight. The next day, this solution was brought to a pH of 11-12 using ammonium hydroxide. This solution was extracted with methylene chloride, and the organic layer was then washed with 2M NaOH twice. The organic layer was then washed with deionized water until a colorless aqueous layer resulted. The organic layer was dried with sodium sulfate, gravity filtered, and evaporated to dryness. The residue was precipitated from minimal MeOH/CH_2Cl_2 + hexanes, then washed with pentanes and dried to produce 90.4 mg of purple solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.78 (s, 8H), 8.125 (d, 4H), 8.052 (d, 4H), 7.621 (d, 4H), 7.256 (d, 4H), 4.390-

4.372 (t, 4 H), 4.141 (s, 4H), 4.018-4.000 (t, 4H), 3.835-3.811 (m, 4H), 3.746-3.716 (m, 8H), 3.637-3.619 (m, 4H), 3.340-3.327 (m, 4H). HR-MS (m/z): calculated: 969.4551, found: 969.4550 [M^+].

5.6. Porphyrin 6: 5,15-bis(2-((benzylamino)methyl)phenylboronic acid)-10,20-bis(4-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)phenyl)porphyrin

To synthesize porphyrin **6**, porphyrin **5** (80.0 mg, 0.083 mmol) was added to an oven-dried flask with 4-Å molecular sieves under nitrogen. Ten equivalents of 2-formylphenylboronic acid (123.8 mg, 0.826 mmol) were also added to the flask. With an oven dried needle, 41.3 ml of distilled MeOH was added to the flask to make a 2 mM solution. This solution was allowed to stir overnight. Twenty-six hours later, 15 equivalents of sodium borohydride (46.8 mg, 1.24 mmol) were added slowly and the resulting solution was stirred for two hours under nitrogen. Chloroform was then added and this solution was washed with saturated sodium bicarbonate twice. The aqueous layers were back-extracted with chloroform, and the combined organic layers were dried with sodium sulfate, gravity filtered, and evaporated to dryness. The purple residue was then dissolved in methanol, followed by ten times as much 1% TFA/water. The resulting solution was then purified with a disposable C18 solid-phase extraction column by eluting with 50% acetonitrile in 1% TFA/water. The collected fraction was brought to neutral pH with NH_4OH and extracted with CH_2Cl_2. This solution was then dried with sodium sulfate, filtered, and evaporated to dryness. Precipitation was done with CH_2Cl_2 and hexanes. HR-MS (m/z): calculated: 619.2850, found: 619.28482 [M^{2+}].

6. References

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