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Synthesis of a Potential FRET-Based Continuous Glucose Sensor Molecule

A Thesis Presented

by

Steven Qizhi Gao

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Master of Science

in

Chemistry

Stony Brook University

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The Graduate School

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Abstract of the Thesis

Synthesis of a Potential FRET-Based Continuous Glucose Sensor Molecule

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For decades, millions of people have been suffering from diabetes. Common methods to monitor blood glucose levels include the use of finger stick glucose meters and test strips. Finger prick to draw blood sample is required regularly and can be painful and annoying. Continuous blood glucose monitors (CGM) are commercially available however they still require calibration with a traditional blood glucose measurement and test results lag behind actual blood glucose values. The objective of this project is to develop an indicator molecule that can be potentially used for fluorescence-based continuous glucose sensors once inserted under skin. A scaffold structure was previously designed using the computer program CAVEAT. A simple structure corresponding to this scaffold was prepared that showed some selectivity for glucose and affinity in the proper range. Incorporation of donor and acceptor flurophores for fluorescence signaling derived the final structure. Once being bound to glucose, this structure would be locked in a conformation that holds the fluorophores about 20 Å apart. The extent of FRET between

fluorophores would be greatly decreased hence providing a fluorescence signal for glucose binding. The synthesis of original designed FRET-based glucose sensor **7** encountered great difficulties. It was modified to **35** to make the synthesis easier. The synthesis of the modified FRET-based glucose sensor is under investigation.

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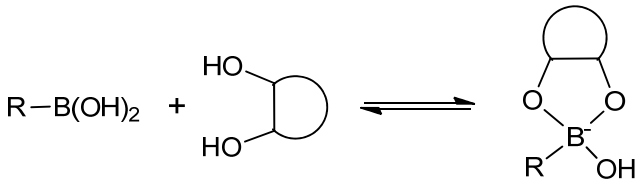
I. Introduction

For many decades, millions of people had been suffering from diabetes. Just in the United States, 5.8 million children and adults in the United States, that is 8.3% of the population, have diabetes according to statistics.[1] Patients with diabetes are required to measure their blood glucose level twice or more a day. Currently, some common methods to detect the blood glucose level include the use of finger stick glucose meters and test strips. A finger prick is required to take a drop of blood and have the glucose meters or test strips to take the reading. This method is painful and inconvenient and must be done daily for diabetes patients. There are also many continuous blood glucose monitors (CGM) available commercially. The principles are similar. A needle is inserted under the skin to draw interstitial fluid while the sensor takes the glucose level measurements continuously and later transmits the signal to a pager like receiver to display the reading. However, they still require calibrations with a traditional blood glucose measurement (the finger prick method) and test results lag behind actual blood glucose values (often more than 6 minutes)[2]. A minimally invasive, efficient, accurate, and consistent continuous glucose monitor is crucial in treatment of diabetes.

Boronic acids are best known for its ability to reversibly bind to diol groups of various kinds of sugars and form stable covalent-bonded complexes in aqueous solution (Scheme 1). We utilized such characteristics to design a glucose sensor molecule. Our current research focuses on the development of a fluorescence-based continuous glucose sensors. The principle is to use a glucose-sensitive fluorophore to reveal blood glucose concentration once it is inserted into the skin, maybe something like a tattoo. The molecule contains boronic acid groups which can

covalently bond 1,2 or 1,3 diols to form five or six membered rings. Two properly spaced boronic acid sites allow glucoses to be bound to form a 1:1 complex. Attached fluorophores allow it to show fluorescence under visible light, hence the glucose concentrations can be determined by measuring the fluorescence intensity.

Scheme 1. Binding of Boronic Acid to Glucose

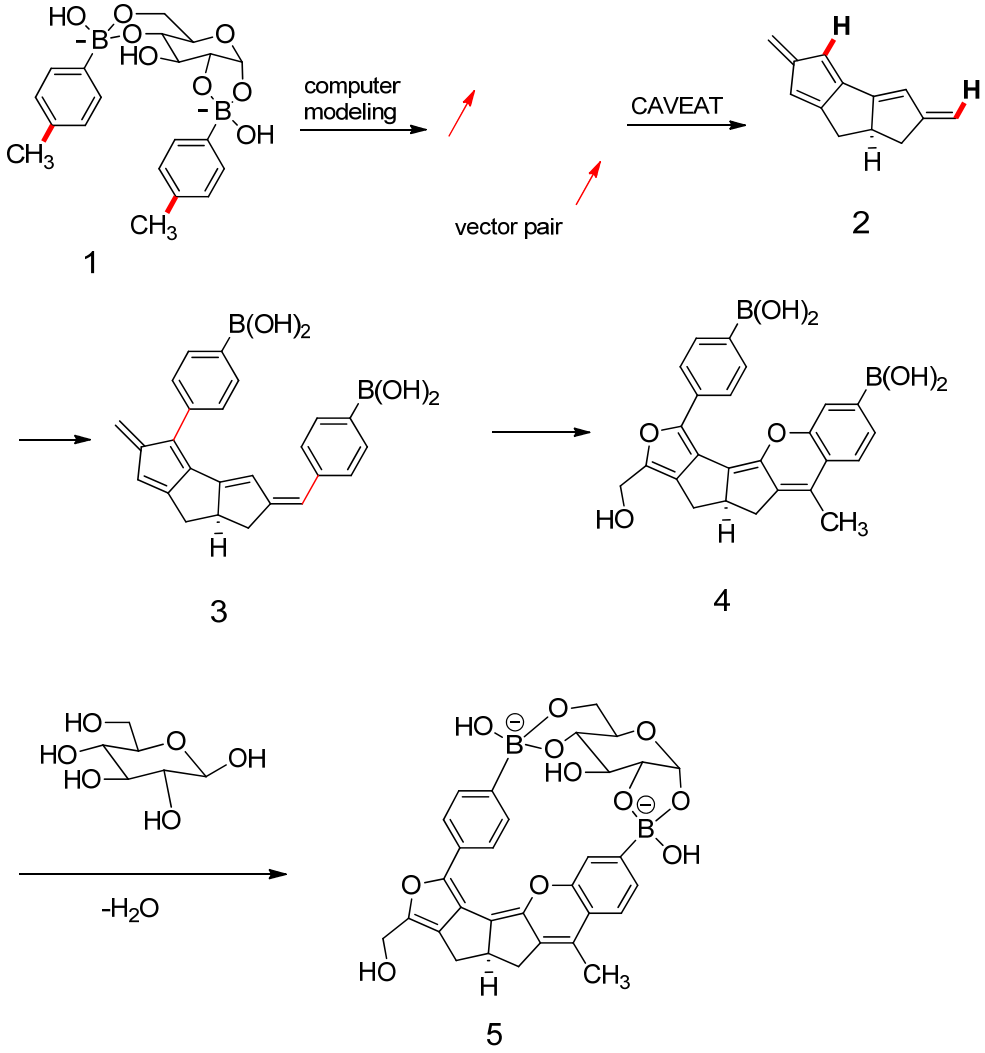


There are some important criteria that the glucose sensor molecule has to meet to be useful for continuous glucose monitoring. First, it must bind selectively to glucose. Second, it must have proper affinity to glucose at physiological glucose levels and pH. Third, the formation of the 1:1 glucose complex must be rapid and reversible to reflect accurate real time glucose concentration. Fourth, it must absorb and show fluorescence under visible light to permit measurement through the skin. Finally, fluorescence difference at different glucose concentration must be distinguishable for accurate measurements. After the glucose sensor molecule is synthesized, it will be examined to see if it meets criteria mentioned above so as to determine whether it is suitable for continuous glucose sensing.

II. FRET-Based Glucose Sensor

1. Preliminary Studies

Scheme 2. Molecular Design of an Arylboronic Acid-Based Glucose Sensor



Previously in the Drueckhammer Group, the glucose receptor **4** was designed and prepared. Computer modeling of the glucose complex **1** defined the vector pair shown in Scheme 2. The computer program CAVEAT was used to identify scaffold **2** by searching a database to identify molecules having a pair of bonds that match the defined vectors. Attachment of the

phenylboronic acid groups to scaffold **2** gave **3**, which was further modified to arrive at **4**. Computer modeling predicted that compound **4** should bind to glucose and form 1:1 glucose complex **5** with little or no change in conformation**.** The proposed structure **4** was synthesized and studied for its glucose binding affinity and fluorescence properties.

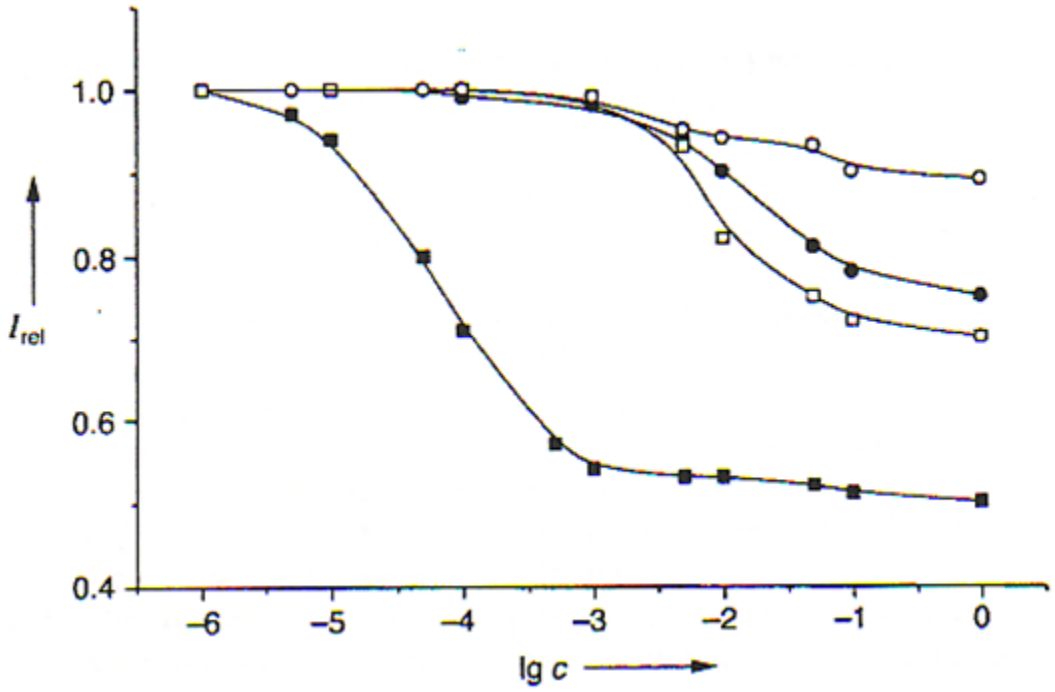


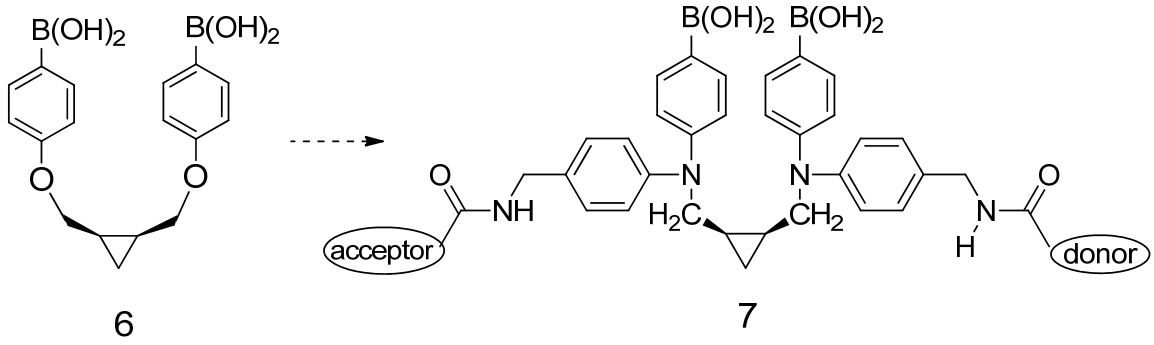
Figure 1. Relative fluorescen intensity I_{rel} of 6 as a function of the saccharide concentrations lg c at 25ºC with 1.0 x 10^{-5} M of 6 in 30% MeOH/aqueous phosphate buffer at pH 7.5. λ_{ex}=375 nm, λ_{em}=447 nm. ■ D-glucose, □ D-galactose, ● D-mannose, ○ D-fructose.

Compound **4** showed affinity to glucose that was more than 100-fold greater than its affinity for galactose, mannose, and fructose. As the saccharide concentration increased, complex formation resulted in decrease in fluorescence intensity (Figure 2). In a healthy adult, the blood glucose level is expected to range from 3.6 mM to 5.8 mM.[2] The apparent affinity of compound **4** for glucose is 100-fold too high to be useful for physiological monitoring of glucose

because it would exist completely in its complex form at the relevant glucose concentration and pH. Its absorbance is too low and fluorescence wavelengths are also too short. Thus while compound **4** gave the desired high selectivity for glucose, it could never be used as a continuous glucose sensor molecule. Therefore, further design work was pursued to find another scaffold on which to build a practical glucose sensor.

2. Design of the FRET-Based Glucose Sensor

Scheme 3. Design of the FRET-Based Glucose Sensor



A simple structure **6** was previously designed and prepared in the group that shows some selectivity for glucose and has affinity in the proper range. Incorporation of donor and acceptor fluorophores for fluorescence signaling gave structure **7** (Scheme3). The computer model of the glucose complex of compound **7** is shown in Figure 2. The cyclopropane in the middle secures the structural conformation of **6**. Two benzene rings at each end (Figure 2) represent the donor and acceptor fluorophores. When the compound is free to rotate, the FRET (Förster resonance energy transfer) will be at its maximum as the two fluorophores can achieve a minimum distance. When the compound is bound to glucose, the structure is locked in a conformation that holds the

fluorophores about 20 Å apart, the extent of FRET will be less and the ratio of donor to acceptor emission is expected to increase. This is expected to provide a fluorescence signal for glucose binding making compound **7** a promising glucose receptor for continuous glucose monitoring.

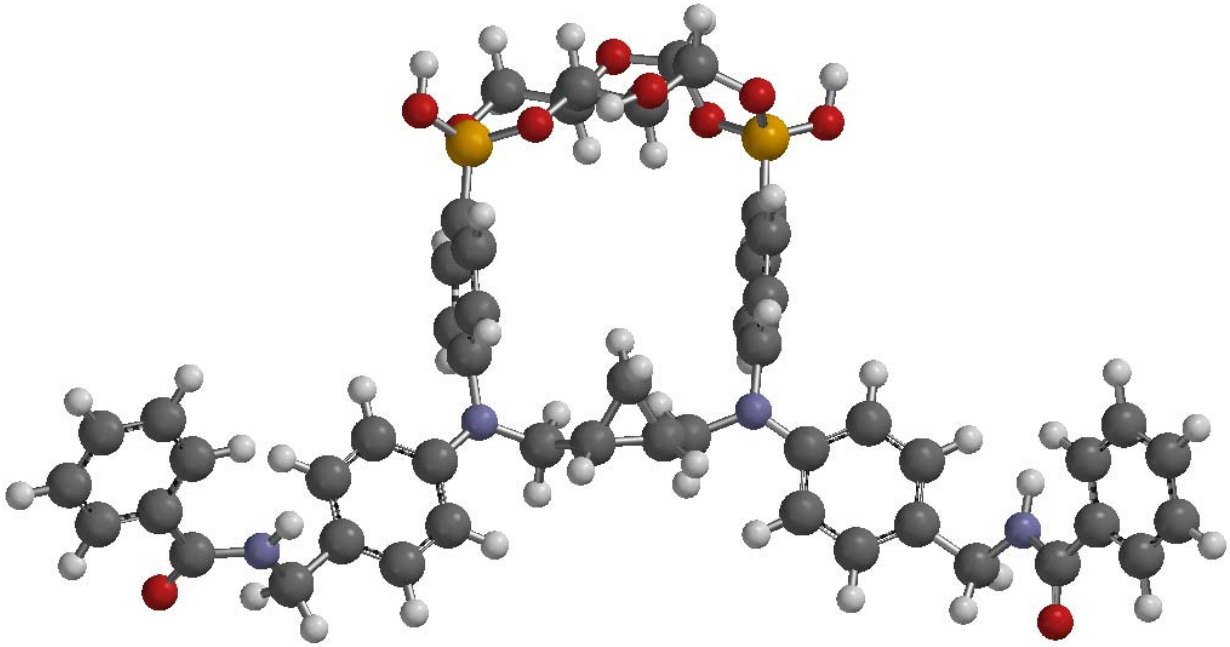
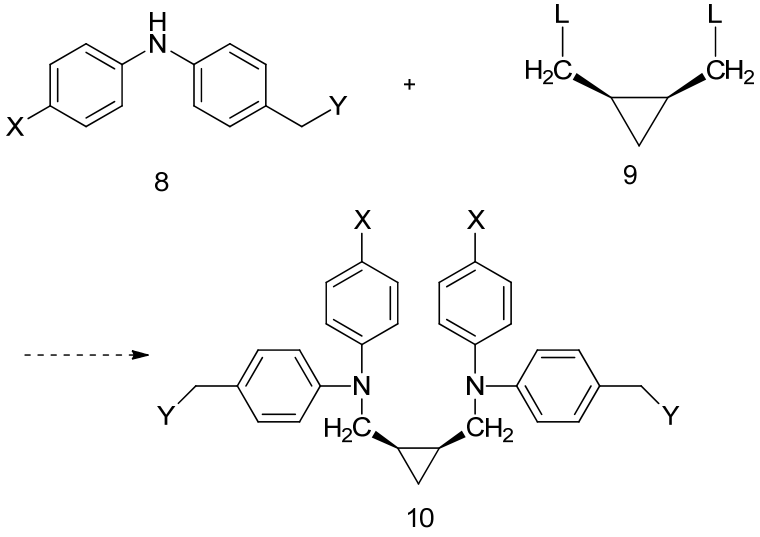


Figure 2. Computer model of the glucose complex of **7**. The donor and acceptor fluorophores are represented by benzene rings.

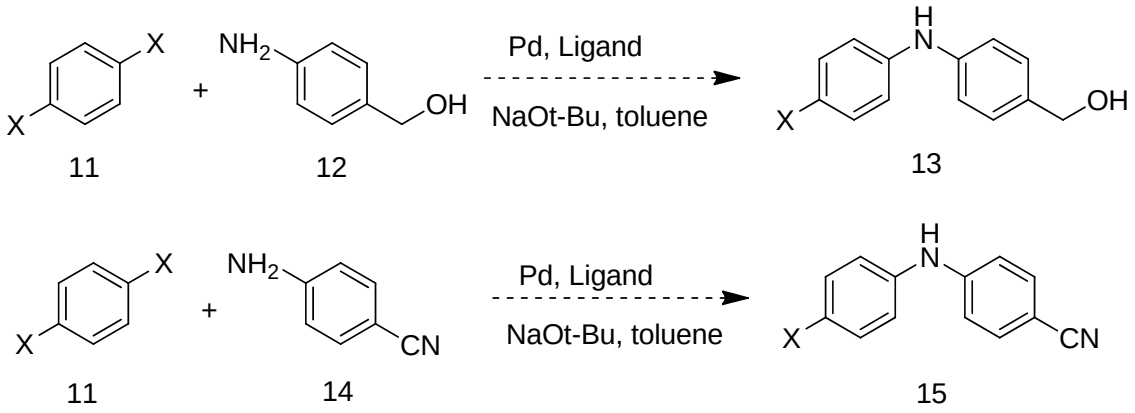
3. Synthesis of the Glucose Sensor

The general approach to synthesis of **7** is shown in Scheme 4. The diarylamine **8** would substitute the leaving group in structure **9** to arrive structure **10**. Group X would be converted to boronic acids and Y would be attached with fluorophores to arrive at the final structure **7**.

Scheme 4. General synthesis route of 10, X=Br, I, L=leaving group



Scheme 5. Attempted synthesis of general structure 8

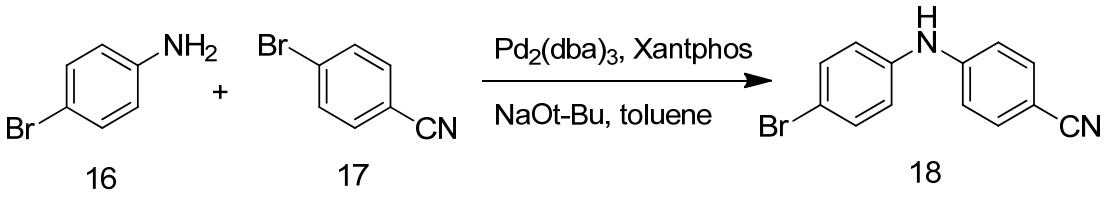


X=I, Br
Pd-catalyst: $Pd_2(dba)_2$
Ligand: BrettPhos, BINAP, Xantphos,dppf, or DPPE.

For structure **8**, aryl halide to aryl amine coupling reactions were initially attempted by reacting 1,4-diiodobenzene (or 1,4-dibromobenzene) (**11**)and 4- aminobenzyl alcohol (**12**) (or aminobenzonitrile (**14**)) with tris(dibenzylideneacetone)dipalladium(0) catalyst in combination

with different ligands including Chloro{[BrettPhos][2-(2-aminoethylphenyl]palladium(II)]}/[BrettPhos]admixture, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene, 1,1"-bis(diphenylphosphino)ferrocene and 1,2-bis(diphenylphosphino)ethane.[3] These reactions were done under nitrogen at about 110 ºC. The literature suggested that the monoamination of dihalogenated benzenes should work, however none of these reactions went to completion even after several days.[4] The reactions all gave little or no product based on TLC results. Other combinations of reactants were thus studied.

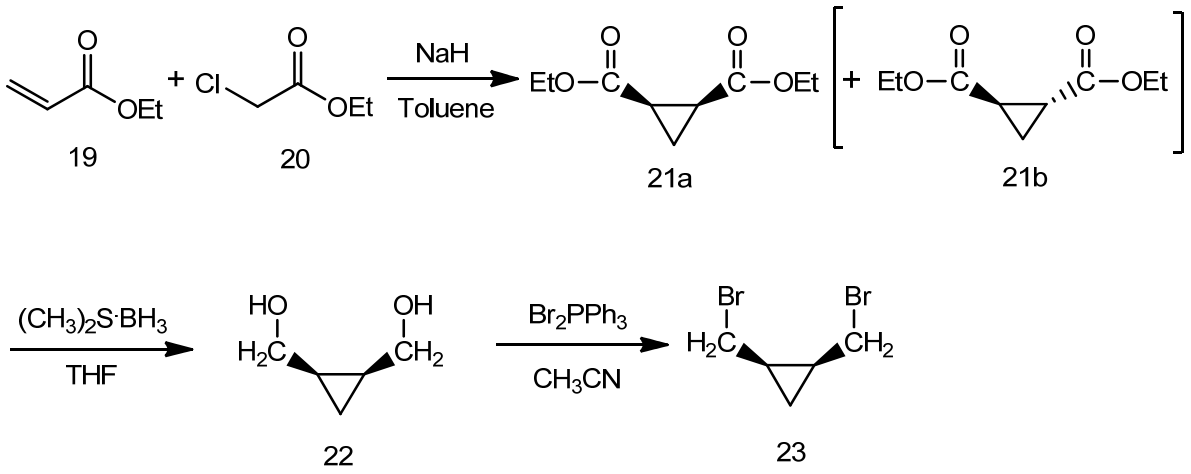
Scheme 6. Synthesis of Compound 18



16 17 18

The reactions of 4-bromoaniline (**16**) and 4-bromobenzonitrile (**17**) with different combinations of Pd-catalyst and ligands were successful based on TLC results. ^{1}H-NMR spectra for each sample were then taken to determine the reaction conditions that gave the best conversion of starting material to products. The reaction in Scheme 6 was chosen and was originally run overnight, however it was found that running the reaction for one hour gave the best yield. Hydrochloric acid (1 M) was used to neutralize the base sodium t-butoxide (NaOt-Bu) and wash the catalyst and ligands. The desired product **18**, which corresponds to general structure **8** was purified by column chromatography. The yield was about 50 to70%.

Scheme 7. Synthesis of Compound 23



The cyclopropane core structure was prepared following literature procedures as shown in Scheme 7. Ethyl acrylate (**19**) and ethyl chloroacetate (**20**) were allowed to react with sodium hydride to yield a mixture of cis and trans diesters (**21a** and **21b**).[5] Temperature control in this step was extremely crucial. The reaction generally started at room temperature, bubbling can be observed due to H_2 evolution. The exothermic reaction automatically accelerated as more and more heat was produced. Without intervention, vigorous foaming would inevitably take place. The resulting product consisted of mostly trans product and little cis product as the trans isomer is favored at high temperature while the cis isomer os favored at low temperature (<40ºC).[6] Therefore, the reaction flask must be immersed in an ice bath or use liquid nitrogen if necessary once H_2 evolution occurs.

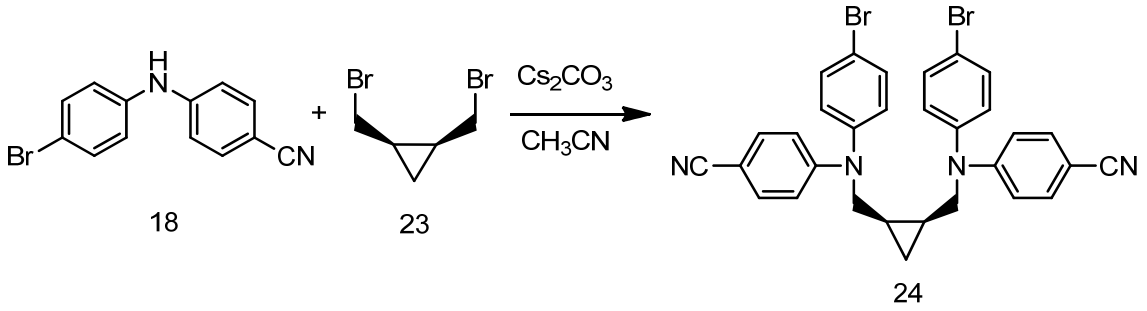
Lithium aluminum hydride as the reducing agent was initially attempted to reduce the diesters to the diols. However, it was found that the diol products were very soluble in water such that after quenching the reaction with water, it became too difficult to extract the diol from the aqueous layer even with continuous ether extraction for days. As a result, the cis diol **22** was

synthesized through an alternative route in which borane dimethyl sulfide complex (BMS) was used as the reducing agent to avoid the use of water.[7]

Initially, the crude diesters were directly converted to the diols and then the desired cis diol **22** was purified by column chromatography. Because the diols have no UV abbsorbance, the TLC plate must be treated with p-anisaldehyde to visualize the spots. This method was not convenient due to the large number of individual TLCs and straining required. It was also a waste of material to reduce the trans diester **21b** with BMS. The diesters showed some UV absorption at 210 nm, therefore it was more convenient to first purify the cis diester, and then converted it to the cis diols **22**, which was used directly in the next step without purification.

By adding bromine dropwise to suspension of triphenylphosphine in dry acetonitrile at 0ºC, Br_2PPh_3 was formed. As bromine was added dropwise over one hour, the reaction mixture formed a yellow suspension. The suspension turned clear orange upon addition of **22**. After the reaction was completed, the solvent was evaporated by vacuum.[6] The residue was washed with hexane or pentane and filtered to remove triphenylphosphine oxide.[8] Evaporation of the solvent gave pure **23**.

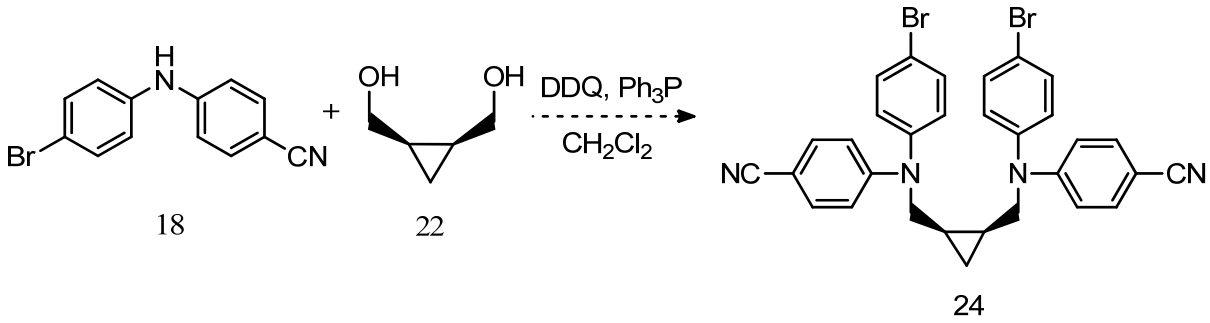
Scheme 8. Synthesis of compound 24



18 23 24

Coupling reaction between amine **18** and dibromide compound **23** gave dinitrile compound **24**.[9] When column chromatography to obtain purified **24**, mono-nitrile compound and unreacted amine **18** could also be collected for future use to conserve materials.

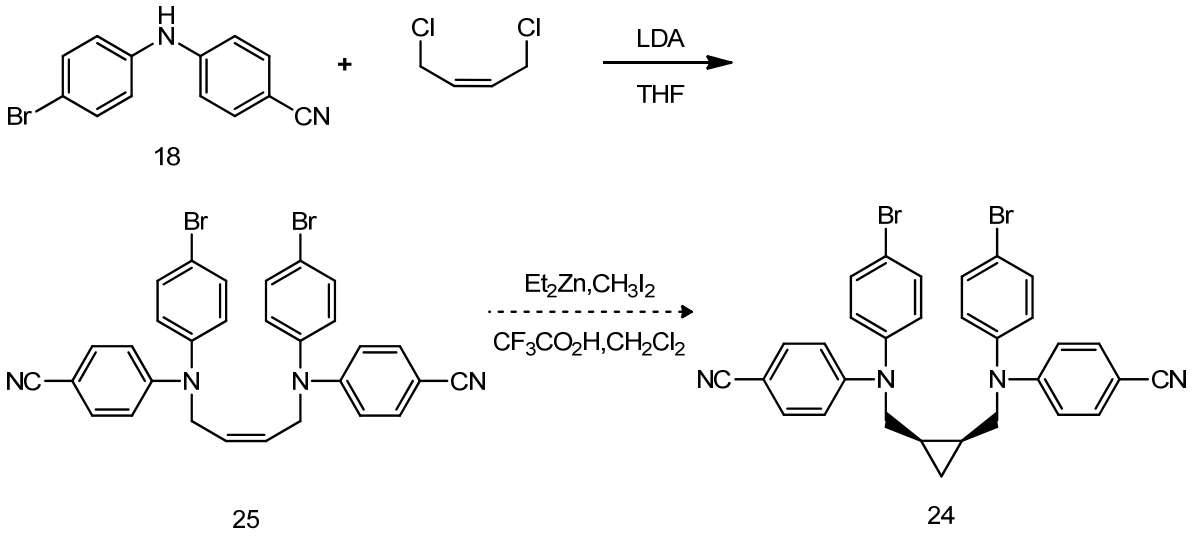
Scheme 9. Attempted alternative synthesis of 24



18 22 24

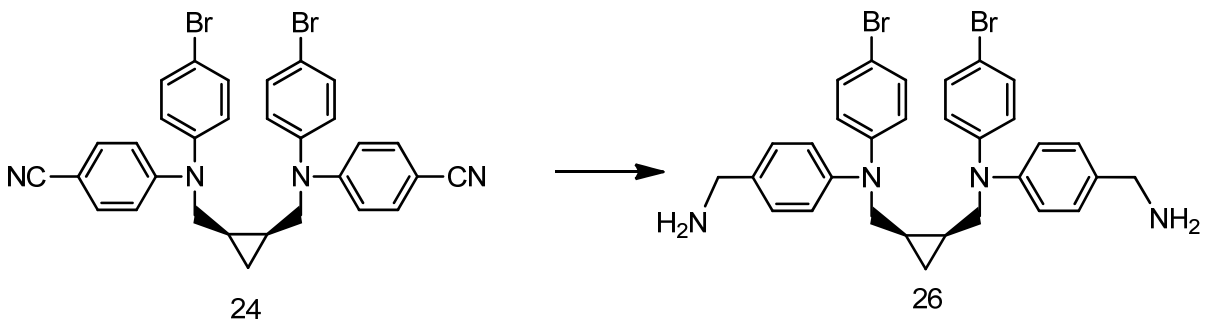
Synthesis of compound **24** by directly reacting **18** and the cis-diol **22** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and triphenylphosphine (Ph_3P) in dry dichloromethane was attempted.[10] Peaks from two geminal protons of the cyclopropyl group were not observed from the NMR spectrum indicating that the reaction was not successful. It was concluded that the alcohol groups were not sufficiently activated under these conditions hence conversion to bromide groups was necessary.

Scheme 10. Another Attempted alternative synthesis of 24



Another alternate route to synthesize **24** was attempted (Scheme 10). Alkene **25** was obtained through coupling reactions between amine **18** and cis-1,4-dichloro-2-butene. Ideally, cyclopropanation of the alkene would also give compound **24** via a Simmons-Smith reaction. The Simmons-Smith reaction required oxygen-free conditions and proper Lewis acids as activators. CF_3CO_2H was chosen to accelerate the cyclopropanation reaction because it generally gives the best result in the literatures.[11] Allylbenzene and cyclohexene were tested in standard conditions; they both yield the desired product. Compound **25** under the same conditions, however, remained unreacted. cis-1,4-Diacetoxy-2-butene was tested under standard condition and remained unreacted as well suggesting that electron withdrawing groups might hinder the Simmons-Smith reaction. Heating the reaction mixture at reflux for 2 days did not help. As a result, the synthesis route through cyclopropanation of the alkene was abandoned.

Scheme 11. Synthesis of Compound 26



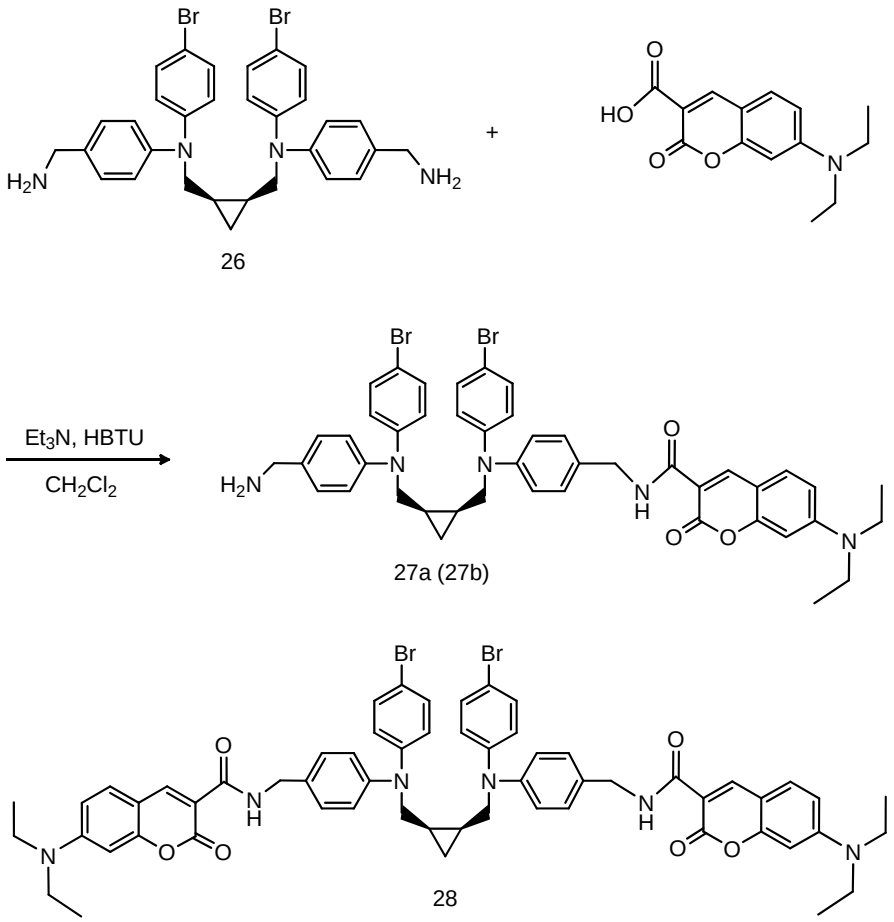
To attach amine-reactive fluorophores, compound **24** must be further modified into **26** shown in Scheme 11. Several conditions were considered and investigated (Table 1). The best result was obtained when the reaction was performed in dry tetrahydrofuran with $LiAlH_4$ as the reducing agent at reflux temperature.

Table 1. Conditions in Scheme 11

Entry	Conditions	Time (h)	Yield (%)
1	1.BMS; 2. HCl, H_2O; 3. NaOH[12]	>24	0
2	$NiCl_2$, $NaBH_4$, dry EtOH[13]	>24	0
3	$LiBH_4$, DGM, MeOH[14]	>24	0
4	1.$LiAlH_4$, 15-Crown-5, Benzene; 2.H_2O[15]	>24	0
5	1.$LiAlH_4$, dry diethyl ether; 2.H_2O[16, 17]	>24	67[a]
6	1.$LiAlH_4$, dry THF, 80 ºC; 2.H_2O[16,17]	4	>99[a] , 81.6-91.8[b]

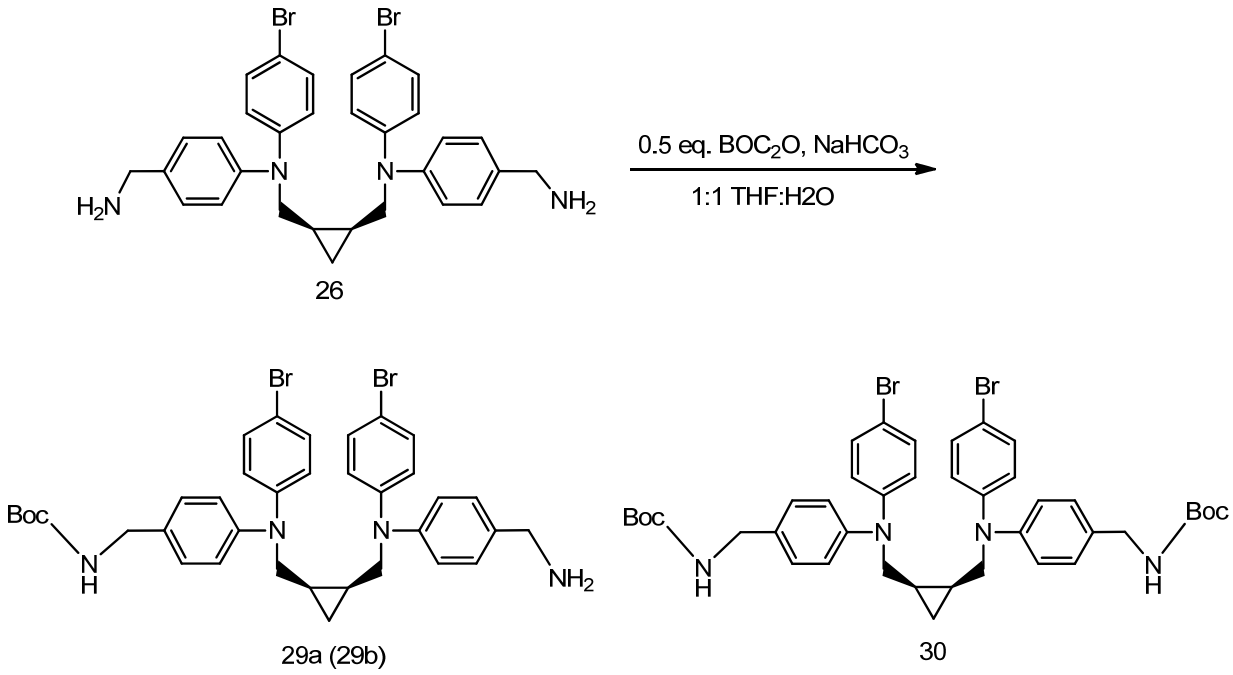
[a] Yield base on H-NMR integrals. [b] Yield of the isolated product.

Scheme 12. Attempted Synthesis of Racemic Compound 27a and 27b



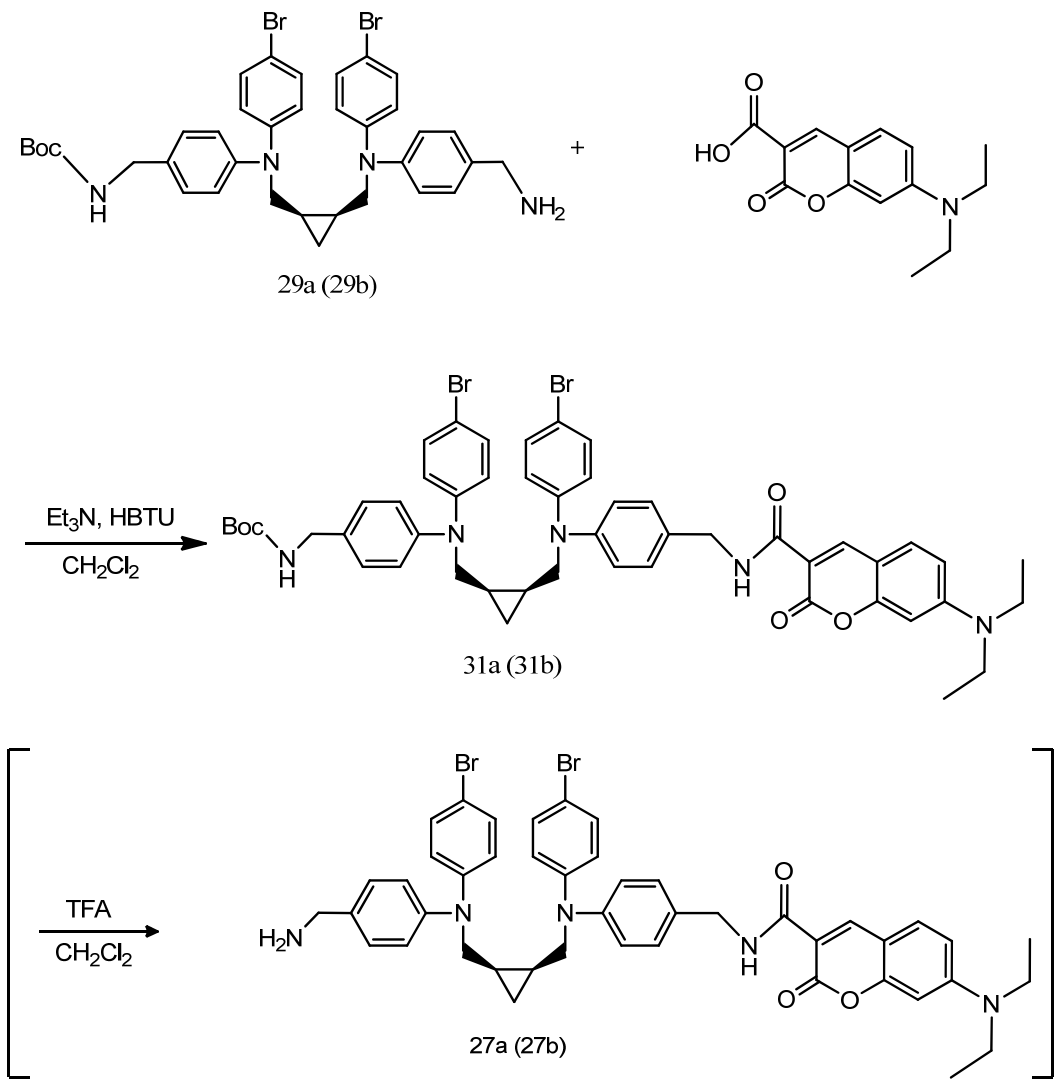
With two free amine groups, 7-(diethylamino)coumarin-3-carboxylic acid, our first designated coumarin fluorophore, can be readily attached to either side.[18, 19] In principle, by reacting **26** and the fluorophore in 1:1 ratio, the desired racemic mixture **27a** and **27b** with only one fluorophore attached can be obtained as well as unwanted di-substituted compound **28** and unreacted **26** (Scheme 11). However, in our experiment, even when the fluorophore was reduced to 1/3 equivalent related to **26**, the product was consisted mostly **28** and unknown byproducts. We failed to isolate out any **27a** and **27b**.

Scheme 13. Synthesis of Racemic Compound 29a and 29b



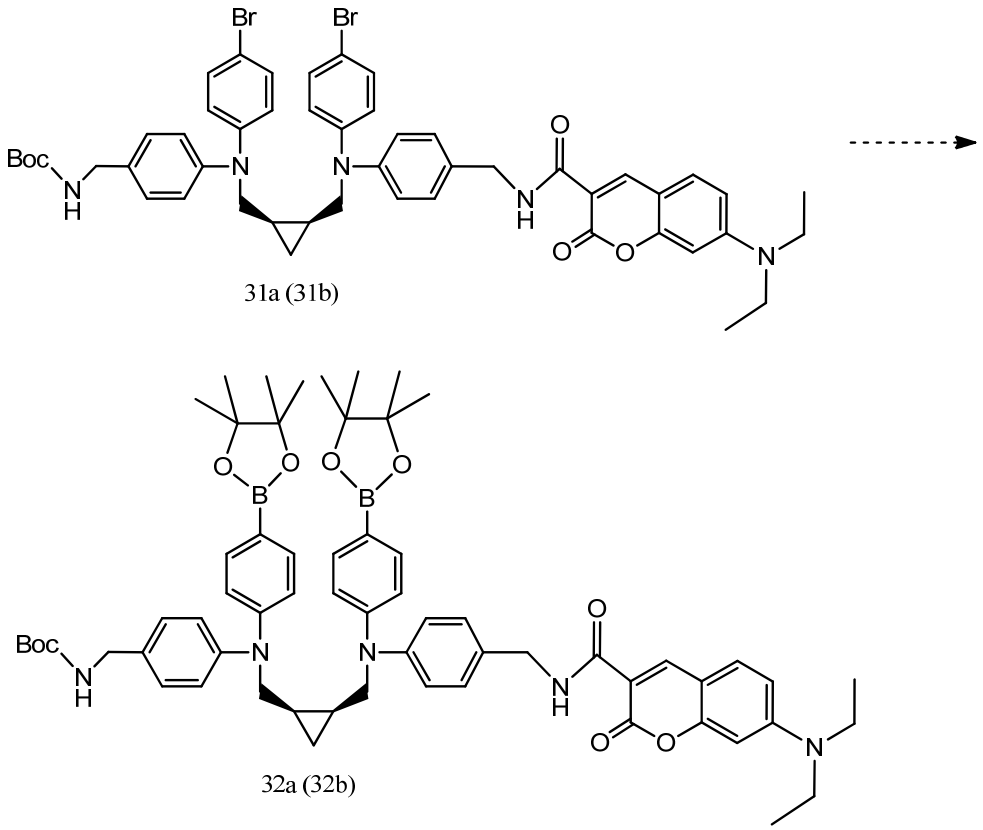
To avoid compound **28**, we decided to specifically protect one amine group with BOC group while leaving the other one open for fluorophore attachment as shown in Scheme 13.[20, 21] The fluorophores can then be readily attached to racemic compounds **29a** and **29b** to give the desired racemic mixture **31a** and **31b** as well as diprotected adduct **30** (Scheme 13). By cleaving the t-BOC group with CF_3COOH (TFA), **30** can be converted back to **26** for future use so that the overall yield is improved.[20, 22]

Scheme 14. Synthesis of 31a and 31b



With only one available amine group, racemic mixtures **29a** and **29b** were allowed to react with 1.5 equivalents of courmarin fluorophores to give **31a** and **31b** with ease as shown in Scheme 14. Compound **27a** and **27b** were also successfully synthesized through the TFA/CH_2Cl_2 method to confirm that cleaving the BOC group had no effect on the attached fluorophore on the other side.[19]

Scheme 15. Attempted Synthesis of 32a and 32b

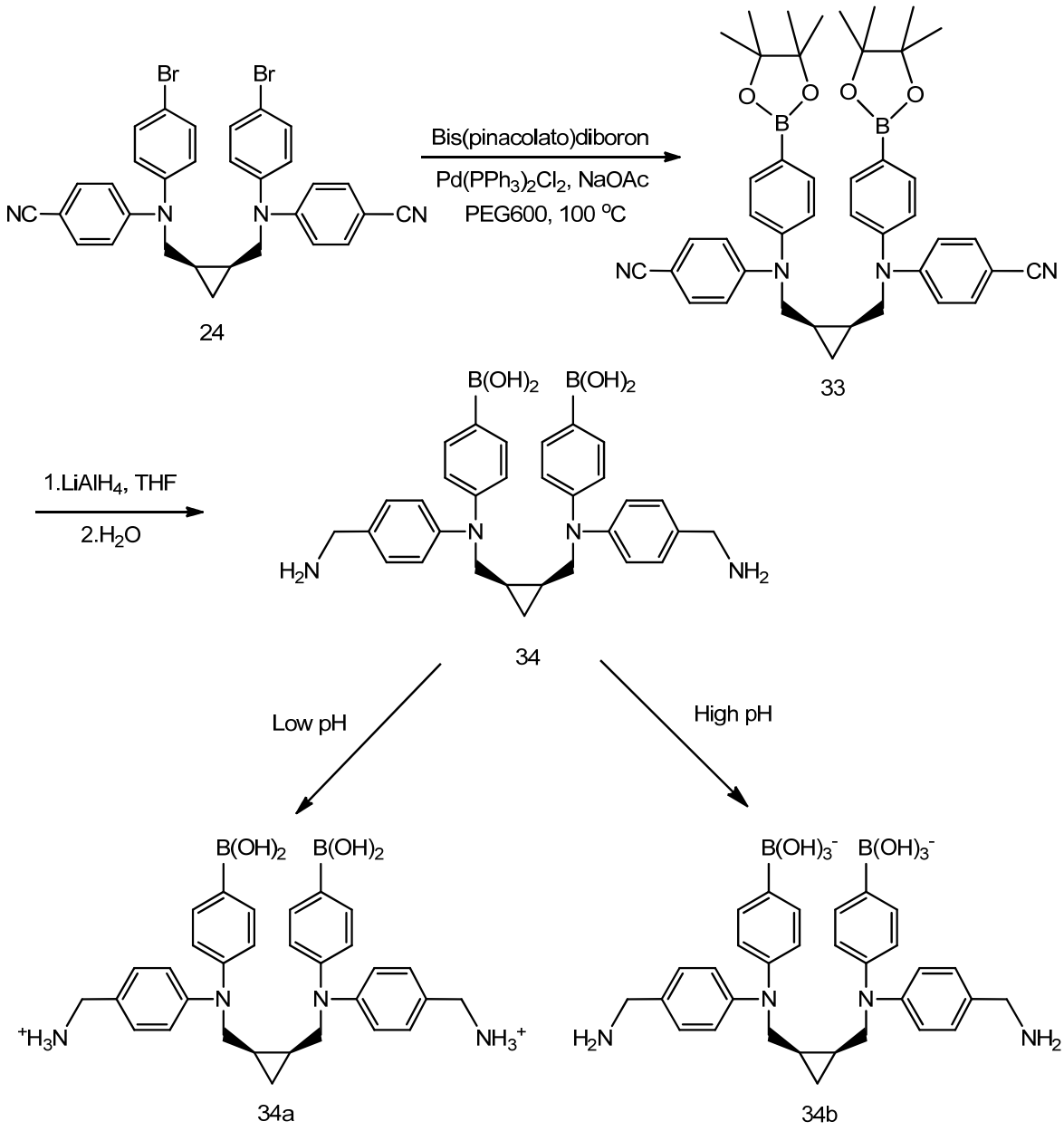


At this point, we decided to convert the bromide groups into boronic ester groups, which would become boronic acid groups in the end. Because **32a** and **32b** were precious, compound **30** was used as the model compound to test three different conditions.[23, 24, 25] None of the three conditions worked however, and gave only recovered starting material. Electron donating groups are known to decrease reactivity in this type of reactions, thus donating effect of the central amine groups may be the problem. Borylation of the diamine **26** was also attempted with no success. Bromobenzene was tested as a model compound and it was found that entry 4c in Table 2 worked the best. When the same condition (entry 5b) was applied to dinitrile **24**, the conversion was very good. Apparently the inductive effect of the nitrile groups sufficiently diminished the electron donating effect of the central amine groups.

Table 2. Conditions for Borylation reactions				
Entry	**Aryl halide**	**Conditions**	**Time (h)**	**Yield (%)**
1a	**30**	Pinacol Borane 4% $PdCl_2(CH_3CN)_2$:SPhos (Pd: Phoshpine =1:4), Et_3N 1,4–Dioxane (dry), 110ºC	>40	0[a]
1b	**30**	Bis(pinacolato)diboron 6% $PdCl_2$(dppf), KOAc DMSO (dry), 140 ºC	>40	0[a]
1c	**30**	Bis(pinacolato)diboron 10% $Pd(PPh_3)_2Cl_2$, NaOAc PEG600, 100 ºC	>40	0[a]
2	**31a (31b)**	Bis(pinacolato)diboron 6% $PdCl_2$(dppf), KOAc DMSO (dry), 140 ºC	>40	0[a]
3	**26**	Bis(pinacolato)diboron 10% $Pd(PPh_3)_2Cl_2$, NaOAc PEG600, 100 ºC	>40	0[a]
4a	Bromobenzene	Pinacol Borane 2% $PdCl_2(CH_3CN)_2$:SPhos (Pd: Phoshpine =1:4), Et_3N 1,4–Dioxane (dry), 110ºC	>40	0[a]
4b	Bromobenzene	Bis(pinacolato)diboron 3% $PdCl_2$(dppf), KOAc DMSO (dry), 140 ºC	18	50[a]
4c	Bromobenzene	Bis(pinacolato)diboron 5% $Pd(PPh_3)_2Cl_2$, NaOAc PEG600, 100 ºC	18	>90[a]
5a	**24**	Bis(pinacolato)diboron 6% $PdCl_2$(dppf), KOAc DMSO (dry), 140 ºC	18	29[a]
5b	**24**	Bis(pinacolato)diboron 10% $Pd(PPh_3)_2Cl_2$, NaOAc PEG600, 100 ºC	18	>90[a], 73[b]

[a] Yield base on H-NMR integrals. [b] Yield of the isolated product.

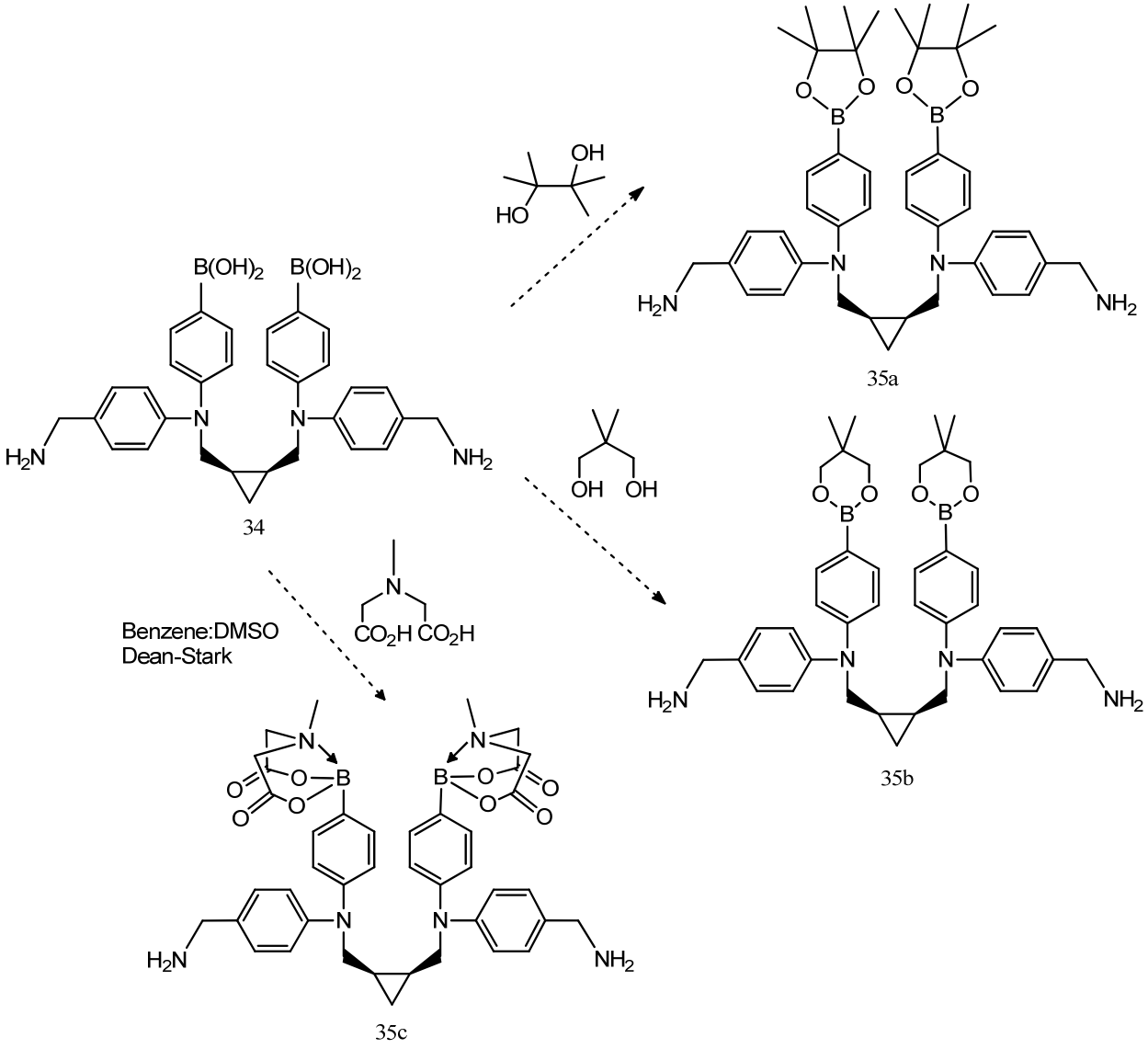
Scheme 16. Synthesis of Compound 33 and 34



After the borylation of **124,** compound **33** must be converted to **34** to allow fluorophore attachments. Based on Table 1, it seemed $LiAlH_4$ was the only choice to reduce the nitriles to primary amines. As shown in scheme 16, $LiAlH_4$ was expected to not only reduce the nitriles to amines but also to reduce the boronic esters to aryl boranes which would hydrolyze to boronic

acids upon aqueous workup.[26] After the reaction, **34** is expected to exist in its basic form **34b** while at low pH, it should exist in its acidic form **34a**. Neither **34a** nor **34b** are expected to be extracted from the aqueous phase. Therefore, the pH was adjusted to maximize the neutral form **30**. The pKa of benzylamine is 9.35 and that of the boronic acid is similar, so we set the pH at 9.4 - 9.5. However extraction with ethyl acetate gave no product. To make **30** easier to be extracted from the aqueous phase, conditions shown in scheme 17 were examined.

Scheme 17. Assisted Extractions of Compound 34



In scheme 17, pinacol or neopentyl glycol were first dissolved in diethyl ether, the organic mixture were then used for extraction. Compound **31a** or **31b** were expected to form by equilibrium.[27] For Compound **31c**, an aqueous sample containing **30** and N-methyliminodiacetic acid were refluxed with stirring in benzene and DMSO with Dean-Stark trap attached for two days. NMR data of the extracted product under these three conditions showed very little aromatic peaks suggesting that these experiments were not successful. The products were reluctant to be extracted from the aqueous layer. Neither **30a** nor **30b** would be extracted by organic solvents easily. It was very difficult know whether we had successfully neutralized the boronic ester groups without protonating the amine groups.

To retrieve **30** from the aqueous solution, the cation exchange method was investigated. The aqueous mixture containing the desired product was acidified with HCl to dissolve all visible precipitate. A column of DOWEX-50WX8-40 resin was washed with 1M LiOH solution then deionized water to make it strongly basic such that the proton in $-SO_3^-H^+$ group is substituted with Li^+ ions to form $-SO_3^-Li^+$. The acidic aqueous mixture containing the amine product **30**[a] was applied to the column. The column was washed with methanol to get rid of the water. Finally, the column was eluted with Et_3N/MeOH solution to retrieve the amine product. Evaporation of the eluent gave no material indicating that the cation exchange method did not work. After all known methods to retrieve compound **30** failed, we concluded that modification of the final molecule was needed to avoid troubles encountered here.

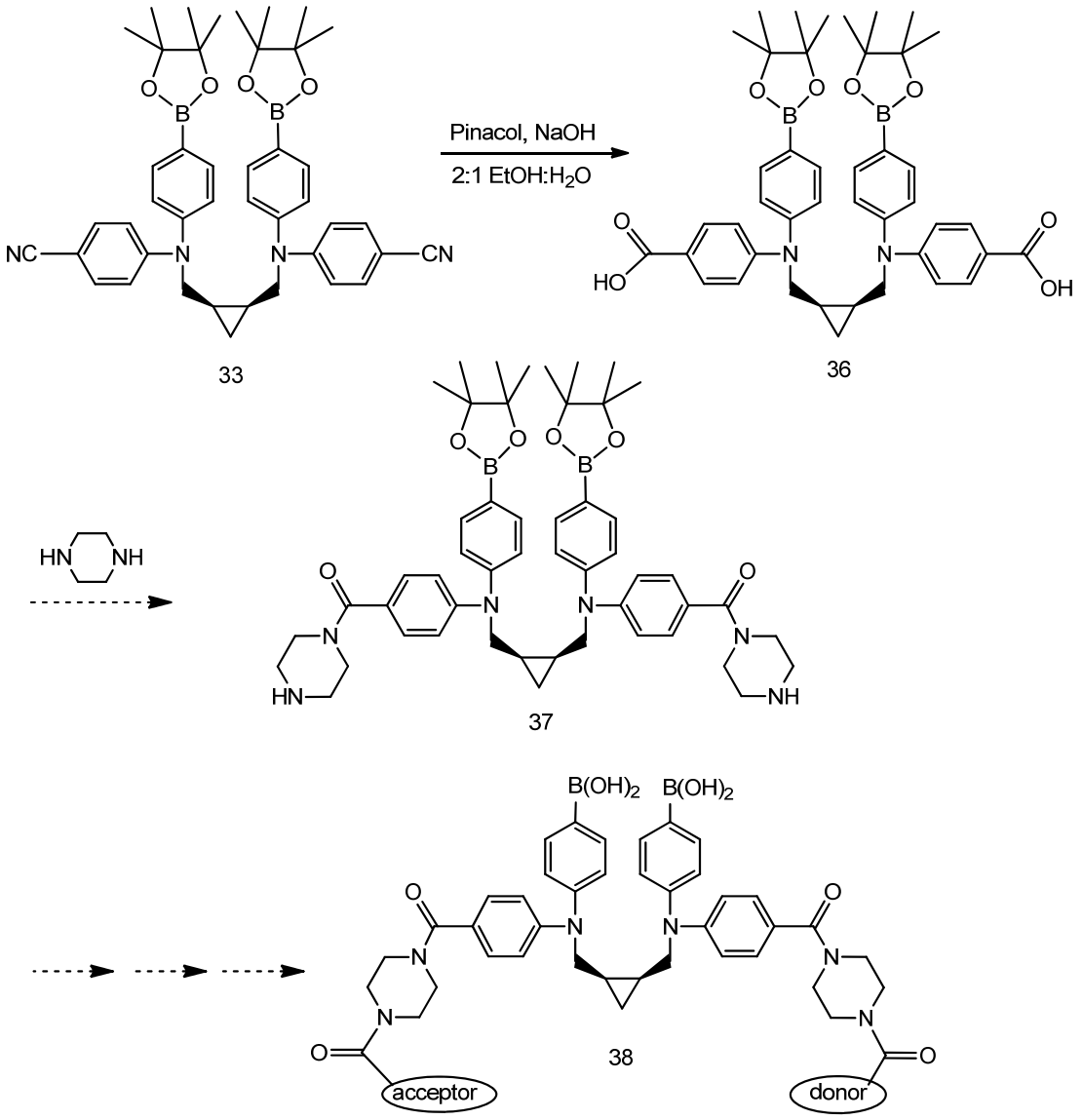
III. Modified FRET-Based Glucose Sensor

1. Modification of the FRET-Based Glucose Sensor

Since extractions became extremely difficult when both boronic ester and amine groups were present, Scheme 18 shows an alternative approach. Compound **33** was dissolved in EtOH/H_2O solution to which NaOH was added. The mixture was refluxed at 100 ºC overnight before removing the EtOH by vacuum evaporation. Pinacol was added and the resulting residue was acidified with HCl to pH 4-5 to allow better extraction with CH_2Cl_2. Evaporation of the organic layer gave compound **36**.[28]

At this point, we envisioned either using carboxylic acid reactive fluorophores to replace our previously planned amine reactive fluorophores, or coupling with piperazine to give **37** which allow attachment of the amine reactive fluorophores.The latter approach would form the modified sensor molecule **38**. We found that the yield in conversion of **33** to **36** was low, and thus chose to develop an alternate route to **38**.

Scheme 18. Modification of the FRET-Based Glucose Sensor

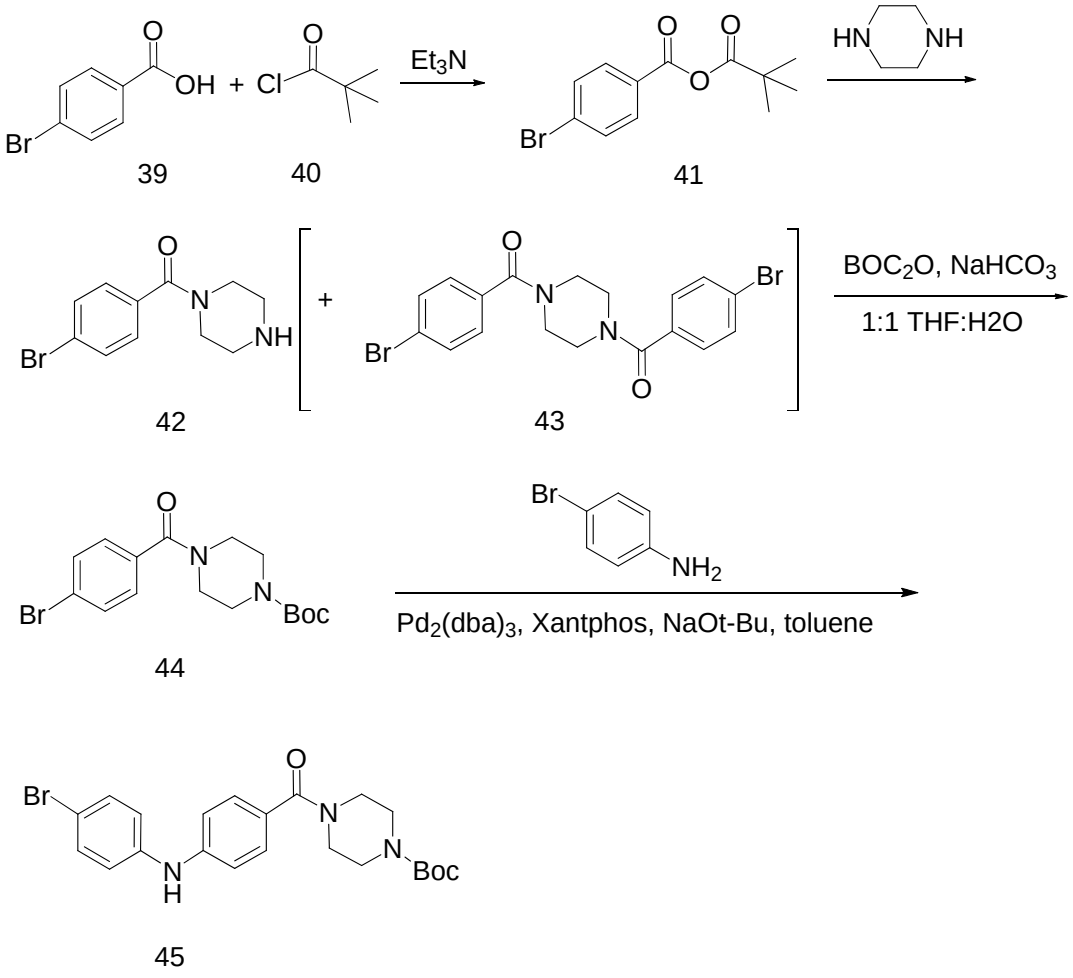


2. Synthesis Route of the Modified FRET-Based Glucose Sensor 38

To synthesize **37** more efficiently, another synthesis route was attempted. The general approach was the same as shown in Scheme 4. As shown in Scheme 19, excess Et3N were added

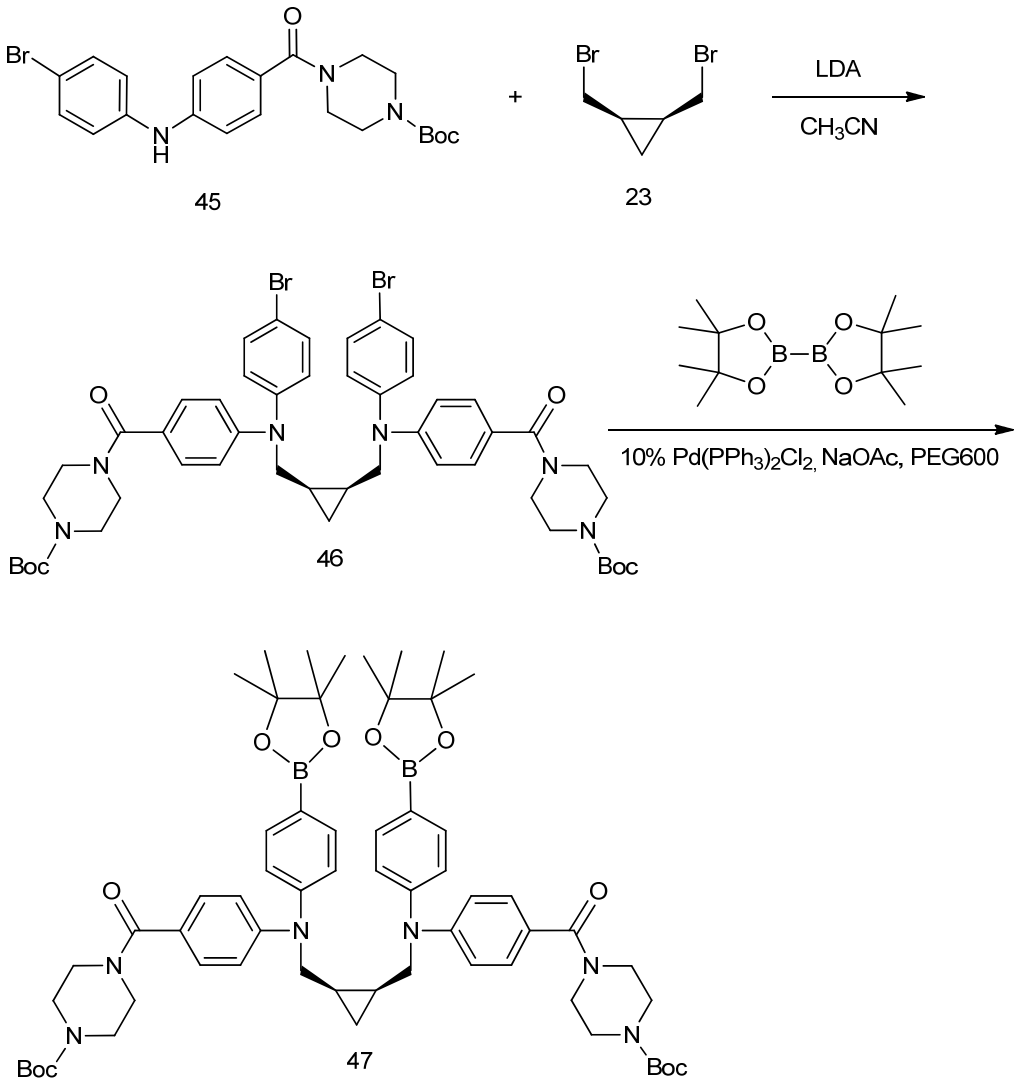
to a mixture of 4-bromobenzoic acid (**39**) and trimethylacetyl chloride (**40**) in dry CH_2Cl_2 to form intermediate **41**. Addition of piperazine in EtOH to the reaction mixture gave **42** and bisadduct **43**. A few steps of acidic and basic extraction gave relatively pure **42**.[29] Without further purification, **42** was allowed to react with di-tert-butyl dicarbonate to protected the amine group. Palladium-catalyzed coupling reactions between **44** and 4-bromoaniline gave **45**. Extraction with HCl resulted in a greenish foam would form that was insoluble in either ether or the aqueous layer and thus this extraction step was omitted.

Scheme 19. Synthesis of Compound 45



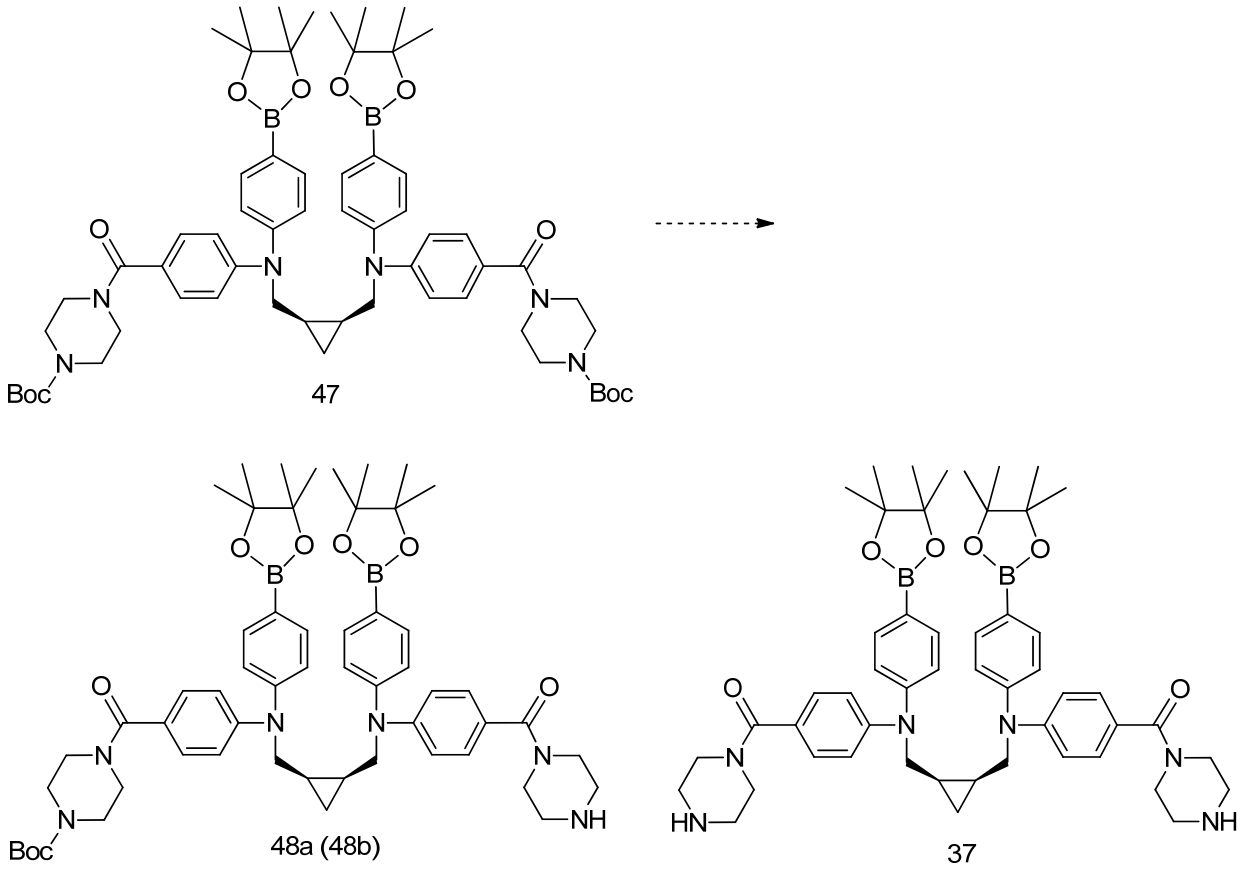
39 40 41 42 43 44 45

Scheme 20. Synthesis of Compound 47



As shown in scheme 20, 2.2 equivalent of **45** was allowed to couple with **23** to form **47**. Cesium carbonate gave poor yield as conversion was less than 50% based on the ^{1}H-NMR data. LDA was used as the base instead, the conversion was greatly improved and the yield of the isolated product was 55%. With the presence of the electron-withdrawing aryl carbonyl groups, borylation of **47** was very successful. The yield for **47** was 94%.

Scheme 21. Synthesis of Compound 48a, 48b and 37



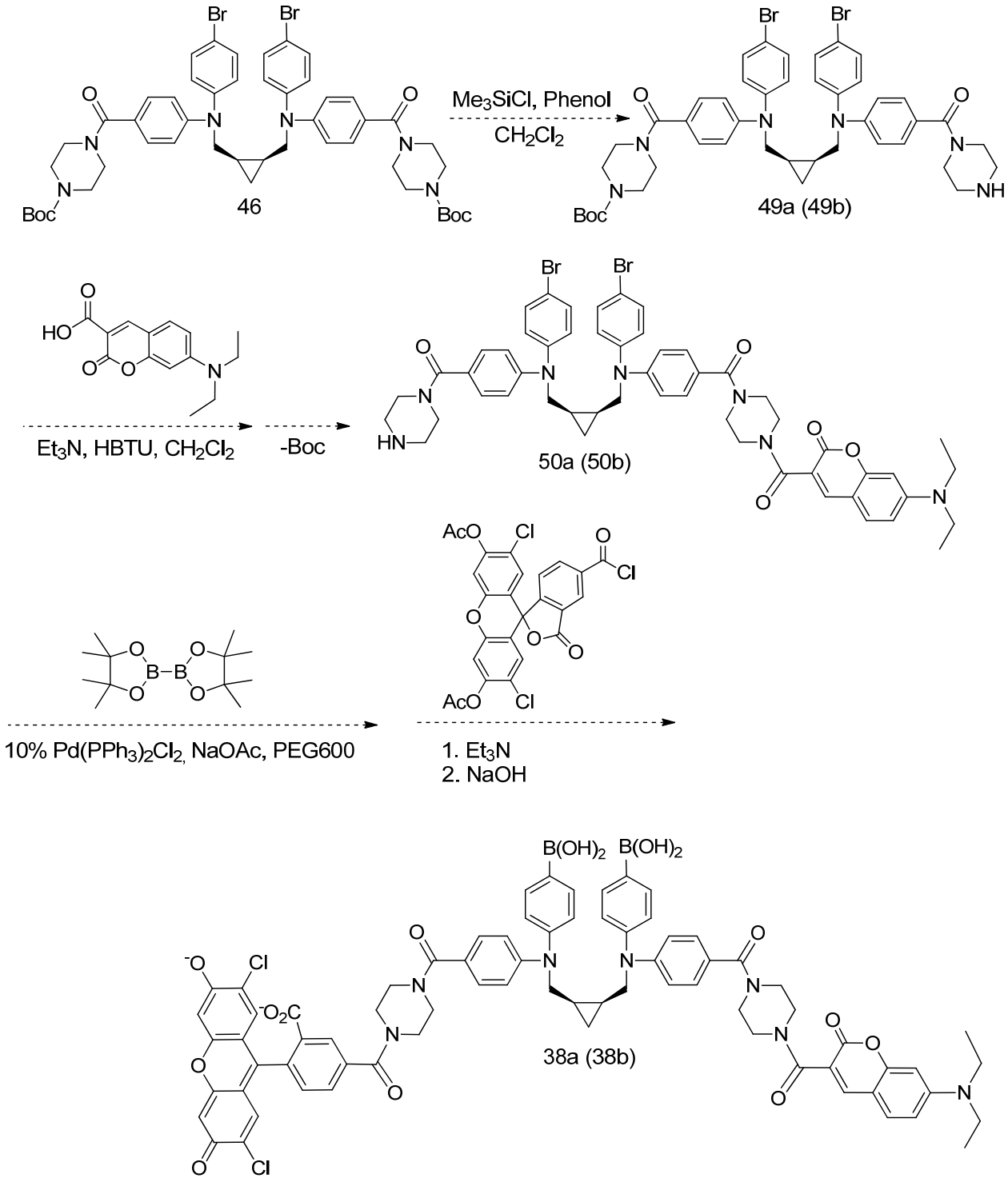
Cleaving the BOC groups on **47** using the TFA/CH_2Cl_2 method was attempted (Scheme 22). Ideally, by using 1 equivalent of TFA to **47**, the product would consist mostly **48a** and **48b** which allow only one fluorophore attached. However, NMR data indicated that TFA not only cleaved the BOC groups but it also cleaved the boronic ester groups. The alternative HCl/EtOAc method has also been tried but only the starting material was recovered.

Another attempt to cleaving the BOC groups was made by adding **47** to Me_3SiCl and phenol in CH_2Cl_2. The reaction mixture was stirred at r.t. for 6 h and then transferred to a separatory funnel where the organic layer was extracted with saturated NaOH solution.[30,31] NMR data suggested that both BOC groups and boronic ester groups were cleaved.

3. Future Work

In order to avoid the problem when both boronic ester and BOC groups are present, the synthesis sequence must be rearranged. As shown in Scheme 22, compound **46** will be allowed to react with Me_3SiCl to cleave one of the BOC groups to form **49a** and **49b.** Attachment of the coumarin fluorophore and subsequent cleavage of the remaining BOC group will give **50a** and **50b**. Boronic ester groups will be attached by using bis(pinacolato)diboron. Attachment of the last fluorophore will be done in a two-step reaction in which the last step will also hydrolyze the boronic esters to bornoinc acids resulting in **38a** and **38b** which represent the modified final structure **38** in Scheme 19. The proposed molecule will be tested in aqueous environment to see if it matches all five important criteria of continuous glucose sensors.

Scheme 22. Final Synthesis of the Glucose Sensor 38a and 38b



IV. Experimental Section

4-(4-Bromophenylamino)benzonitrile (18): A solution of 4- bromobenzonitrile (1.82 g, 10 mmol), 4-bromoaniline (1.72 g, 10 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.114 g, 0.125 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (0.145g, 0.25 mmol), and sodium t-butoxide (1.153 g, 12 mmol) in toluene (40 ml) was heated under reflux and nitrogen for 1 h. Ethyl acetate (40ml) was added and the solution was extracted with 1 M HCl (3 x 40 ml). The solvent was removed. The residue was purified by column chromatography (heptane/acetone 1:2). Yield 50-70%. ^{1}H NMR ($CDCl_3$): 6.94-7.12 (4H, m), 7.39-7.58 (4H, m)

1,2-dicarboethoxycyclopropane (21a and 21b): A solution of ethyl chloroacetate (53.3 ml, 0.5 mol) and ethyl acrylate (54.5 ml, 0.5 mol) was added with stirring to a suspension of 60% dispersion of sodium hydride in mineral oil (25.1 g, 0.625 mol) and toluene (125 ml) under nitrogen. When the mixture was starting to bubbles due H_2 evolution, the flask was immediately cooled in an ice bath to maintain the temperature below 35 ºC. The mixture was stirred until its color transformed into orange and there was no more bubbling. Water (250 ml) and diethyl ether (150ml) were added. The aqueous phase was extracted with diethyl ether (3 x 100ml). Evaporation of the organic solvent in vacuo resulted a mixture of cis and trans products which was purified by column chromatography (heptane/acetone 1:1).[5] The yield is 50-60%. 1H NMR ($CDCl_3$) for the cis product: 1.219-1.267 (7H, m), 1.650 (1H, m), 2.035 (2H, dd), 4.118 (4H, q).

cis-1,2-Dihydroxymethylcyclopropane (22): cis 1,2-dicarboethoxycyclopropane (9.31 g, 0.05 mol) was added with stirring to borane-methyl sulfide complex 2M (50 ml, 0.10 mol) and THF (35 ml). The solution was heated for 3 hours with distillation of dimethyl sulfide and THF then was allowed to cool to room temperature. To the solution was added methanol (85ml) and stirred

for an hour. The solvent was removed. Addition of methanol and evaporation of volatiles were repeated (3x 42ml) to give the desire product. p-Anisaldehyde was used for TLC staining. ^{1}H NMR ($CDCl_3$): 0.21 (1H, dd), 0.74-0.85 (1H, m) 1.19-1.37(2H, m), 3.19-3.28 (2H, m), 4.06-4.13 (2H, m). Literature ^{1}H NMR ($CDCl_3$): 0.19 (1H, dd), 0.72-0.83 (1H, m), 1.19-1.38 (2H, m), 3.12 (2H, s, OH), 3.16-3.27 (2H, m), 4.03-4.12 (2H, m).[6]

cis-1,2-Dibromomethylcyclpropane (23): Bromine (4.507 g, 1,453 ml, 28.2 mmol) was added dropwise with stirring to a suspension of triphenylphosphine (7.397 g, 8.2 mmol) in dry acetonitrile (40 ml) in ice water bath. The ice water bath was subsequently removed and a solution cis-1,2-dihydroxymethylcyclopropane (1.43 g, 14.02 mmol) in dry acetonitrile (10 ml) was added dropwise to the reaction mixture which was then stirred under nitrogen overnight. The solvent was evaporated. The residue was finely dispersed in hexane (100 ml) and filtered to remove the triphenylphosphine oxide. The residue was washed with hexane or pentane (3 x 50ml). The combined organic solvent was removed in vacuo to give the desired compound. The yield is 90-93%. ^{1}H NMR ($CDCl_3$): 0.40 (1H, dd), 1.09-1.22(1H, m), 1.57-1.67 (2H, m), 3.40-3.51 (4H, m). Literature ^{1}H NMR ($CDCl_3$): 0.41 (1H, dd), 1.09-1.21(1H, m), 1.57-1.70 (2H, m), 3.39-3.55 (4H, m).[6]

Compound 24: A 500 ml round bottom flask equipped with a magnetic stir bar and reflux apparatus was connected to the nitrogen line. Amine **18** (6.556 g, 24 mmol) and cesium carbonate (9.774 g, 30 mmol) were added and dry acetonitrile (120 ml) was added under N_2. A solution of compound **23** (2.278 g, 10 mmol) in dry acetonitrile (10 ml) was added. The reaction mixture was refluxed overnight. Cesium carbonate was removed by filtration and washed with ethyl acetate. The combined organic mixture was concentrated and purified by column chromatography (heptane/acetone 1:3). The yield is 55%. ^{1}H NMR ($CDCl_3$): -0.040 (1H, m),

0.853 (1H, m), 1.180 (2H, m), 3.400 (4H, m), 6.606 (4H, d), 7.059 (4H, d), 7.392 (4H, d), 7. 587 (4H, d).

Compound 25: To a 100 ml round bottom flask equipped with a magnetic stir bar under nitrogen, amine **18** (3.278 g 12 mmol) in dry tetrahydrofuran (30ml) was added at 0°C. Lithium diisopropylamide 2M (18 mmol, 9 ml) was added dropwise. The ice bath was removed after one hour and cis-1,4-dichloro-2-butene (0.658 g, 5 mmol) in dry THF (10 ml) was added. The reaction mixture was heated to reflux for 20 minutes and then transferred to a separatory funnel. Ethyl acetate (100 ml) and water (50ml) were added. The aqueous phase was extracted with ethyl ether (3 x 50 ml). The organic phases were combined and concentrated in vacuum. Purification by column chromatography (Heptane/Acetone 1:3) gave the desired product (yellow crystals). The yield is 50%. ^{1}H NMR ($CDCl_3$): 4.10 (4H, d), 5.70 (2H, m), 6.55 (4H, d), 6.97 (4H, d), 7.34 (4H, d), 7.51 (4H, d).

Compound 26 (Converting Nitriles to Primary Amines): Di-nitrile **24** (0.612 g, 1 mmol) and lithium aluminum hydride (0.320 g, 4 mmol) were placed a round bottom flask equipped with a reflux apparatus. Under nitrogen atmosphere, dry THF (10ml) was added. The reaction mixture was heated to reflux for at least 3 hr. The flask was cooled in an ice bath and water (50 ml) was added dropwise to decompose the excess hydride. During this process, gelatinous white precipitate formed in the aqueous solution. The precipitate was removed by filtration and washed with ethyl acetate (50 ml). After separating the organic layer in a separatory funnel, the aqueous layer was extracted with ethyl acetate (2 x 25 ml). The combined organic fractions were concentrated under vacuum to give the product (orange oil). The yield is 81.6-91.8. ^{1}H NMR ($CDCl_3$): -0.040 (1H, m), 0.735 (1H, m), 1.160 (2H, m), 3.415 (4H, m), 3.814 (4H, s), 6.881-6.934 (8H, m), 7.200-7.265 (8H, m).

Compound 29a and 29b (Attachment of the BOC Groups): At room temperature, to a stirred solution of amine **26** (5.40 g, 8.71 mmol) and $NaHCO_3$ (2.184 g, 26 mmol) in THF (50 mL) and water (50 mL) were added di-tert-butyl dicarbonate (1.901 g, 8.71 mmol). The solution was stirred at room temperature overnight and then concentrated under vacuum. The resulting residue was washed with CH_2Cl_2 (50 ml) and water (50 ml) and transferred to a separatory funnel where the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 ml). Removal of the solvent under vacuum gave the crude product. After purification through column chromatography (ethyl acetate/methanol 1:9), di-BOC product **25**, mono-BOC product **29a** and **29b** and unreacted amine **26** were collected. The yield for **29a** and **29b** is 20-30%. ^{1}H NMR ($CDCl_3$) for **29a** and **29b**: 0.001 (1H, m), 0.752 (1H, m), 1.189 (2H, m), 1.508 (9H, s), 3.456 (4H, m), 3.835 (2H, s), 4.280 (2H, d), 6.918-7.035 (8H, m), 7.188-7.305 (8H, m). ^{1}H NMR ($CDCl_3$) for **25**: 0.001 (1H, m), 0.752 (1H, m), 1.189 (2H, m), 1.508 (9H, s), 3.456 (4H, m), 4.280 (4H, d), 6.918-7.035 (8H, m), 7.188-7.305 (8H, m).

Compound 31a and 31b (Attachment of the Coumarin Fluorophore): 7-(Diethylamino)coumarin-3-carboxylic acid (0.235 g, 0.9 mmol)was dissolved in CH_2Cl_2 (25 ml) at 0°C. Triethylamine (0.183 g, 1.8 mmol) and O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU, 0.683 g, 0.4 mmol) were added sequentially. The mixture was stirred for 20 min before **29a** and **29b** (0.441g, 0.6 mmol) in CH_2Cl_2 (5 ml) was added. The solution was stirred at room temperature overnight. After removal of the solvent under vacuum, the residue was re-dissolved in ethyl acetate (50ml) and extracted with saturated $NaHCO_3$ (2 x 50 ml) and brine (50 ml). Removal of solvent gave the crude product which was purified by column chromatography (hexane/ethyl acetate 1:3). ^{1}H NMR ($CDCl_3$): 0.040 (1H, m), 0.750 (1H, m), 1.208 (2H, m), 1.418 (9H, s), 3.350-3.550 (8H, m), 4.230 (2H, d), 4.565 (2H, d),

6.480 (1H, d), 6.650 (1H, dd), 6.860-6.927 (8H, m), 7.210-7.265(8H, m), 7.420 (1H, d), 8.729 (1H, s), 9.100 (1H, m).

Compound 27a and 27b (Cleavage of the BOC group): To a mixture of **31a** and **31b** (0.8.19 g, 0.6 mmol) in CH_2Cl_2 (8 ml) was added trifluoroacetic acid (TFA, 8ml). After 2 hr, TFA was evaporated off. The residue was extracted with ethyl acetate and saturated $NaHCO_3$. Evaporation of the organic layer gave the desired products (black oil). ^{1}H NMR ($CDCl_3$): 0.040 (1H, m), 0.750 (1H, m), 1.208 (2H, m), 3.350-3.550 (8H, m), 3.920 (2H, s), 4.530 (2H, m), 6.480 (1H, d), 6.650 (1H, dd), 6.860-6.927 (8H, m), 7.210-7.265(8H, m), 7.420 (1H, d), 8.729 (1H, s), 9.100 (1H, m).

Compound 33 (Attachment of the boronic ester): At 80°C under nitrogen, a mixture of **24** (0.612 g, 1 mmol), bis(pinacolato)diboron (0.762 g, 3 mmol), NaOAc (0.656 g, 8 mmol), $Pd(PPh_3)_3Cl_2$ (0.070 g, 0.10 mmol), in PEG 600 (5 ml) was stirred overnight. The reaction mixture was cooled to room temperature and transferred to a separatory funnel where CH_2Cl_2 (50 ml) and water (50 ml) were added. The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 ml). The organic fractions were combined and concentrated under vacuum to give the crude product which was further purified by column chromatography (heptane/acetone 1:3). ^{1}H NMR ($CDCl_3$): -0.083 (1H, m), 0.75 (1H, m), 1.12 (1H , m), 3.26-3.44 (4H, m), 6.58 (4H, d), 7.14 (4H, d), 7.36 (4H, d), 7.87 (4H, d).

Compound 36 (Converting nitriles to carboxylic Acid): At 100°C, compound **33** (0.434 g, 0.61 mmol) and NaOH (0.976 g, 25 mmol) in EtOH (10 ml) and H_2O (5 ml) were stirred overnight. The reaction mixture was adjusted to pH 4.5 then transferred to a separatory funnel where the aqueous layer was extracted with CH_2Cl_2 (3 x 50 ml). The organic layers were

combined and concentrated under vacuum to give the crude product. ^{1}H NMR ($CDCl_3$): -0.084 (1H, m), 0.71 (1H, m), 1.12 (2H, m), 1.32 (24H, s), 3.38-3.50 (4H, m), 6.73 (4H, d), 7.13 (4H, d), 7.87 (4H, m).

Compound 42: 4-Bromobenzoic acid (4.426g, 22 mmol) in CH_2Cl_2 (100 ml) and trimethylacetyl chloride (2.412 g, 20 mmol) were added to a round bottom flask under nitrogen. Excess of Et_3N (3.06 g, 30 mmol) was added and the mixture became clear almost instantly. The resulting mixture was stirred at r.t. for 30 minutes. Piperazine (7.04 g, 80 mmol) and EtOH (100 ml) were added and the mixture was further stirred for 3 h. Concentrated HCl (4 ml) was added to lower the pH to 2 and the resulting mixture was extracted with CH_2Cl_2 (3 x 50 ml) to get rid of the bisadduct **43** and unreacted materials. NaOH was added to the aqueous solution to increase the pH to 10 and then extracted with CH_2Cl_2 (3 x 50 ml). The organic extract was concentrated to yield the desired product **42**. The yield is 50-75%. ^{1}H NMR ($CDCl_3$): 2.80 (2H, br s), 2.90 (2H, br s), 3.39 (2H, br s), 3.70 (2H, br s), 7. 24 (2H, d), 7.38 (2H, d). Literature ^{1}H NMR ($CDCl_3$): 2.65 (2H, br s), 2.78 (2H, br s), 3.25 (2H, br s), 3.40 (2H, br s), 7.15 (2H, d), 7.43 (2H, d).

Compound 44: 42 (9.20 g, 34.2 mmol) in THF (100 mL) and water (100 mL) were added di-tert-butyl dicarbonate (11.196g g, 51.3 mmol). The mixture was stirred for 4 h then extracted with CH_2Cl_2 (3 x 50 ml). Evaporation of the organic layers gave white crystals which were further purified by column chromatography (heptane/acetone 1:3). The yield is 89%. ^{1}H NMR ($CDCl_3$): 1.46 (9H, s), 3.40 (2H, br s), 3.70 (2H, br s), 7.28 (2H, d), 7.56 (2H, d).

Compound 45: A solution of **44** (11.99 g, 32.2 mmol), 4-bromoaniline (5.873 g, 34.2 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.114 g, 0.125 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (0.145g, 0.25 mmol), and sodium t-butoxide (3.940 g, 41 mmol) in toluene

(180 ml) was heated under reflux and nitrogen for 6 h. Ethyl acetate (100ml) was added and the solution was extracted with water (3 x 50 ml). Evaporation of the organic extract gave the crude product which purified by column chromatography (heptane/acetone 1:3). The yield is 50-70%. ^{1}H NMR ($CDCl_3$): 1.45 (9H, s), 3.40 (2H, br s), 3.70 (2H, br s), 6.96 (4H, d), 7.28 (4H, m).

Compound 46: To a round bottom flask in ice bath under nitrogen condition, **45** (2.300g, 5 mmol) in dry THF (20 ml) was added LDA 2M (6 ml, 12 mmol) and the reaction was stirred for 1 hr. The ice bath was removed, **23** (0.501 g, 2.2 mmol) was added and the reaction was heated at reflux for 6 h. Water (50 ml) was added and the solution was extracted with ethyl acetate (3x 50 ml). Evaporation of the organic layer gave the crude product, which was purified by column chromatography (heptane/acetate 1:3). The yield is 50-68%. ^{1}H NMR ($CDCl_3$): -0.04 (1H, m), 0.79 (1H, m), 1.13 (2H, m), 1.29 (24H, s), 1.43 (2H, s), 3.39-3.60 (20H, br m), 6.73 (4H, d), 6.97 (4H, d), 7.27 (4H, d), 7.44 (4H, d).

Compound 47: 46 (2.672 g, 2.7 mmol), bis(pinacolato)diboron (2.057 g, 8.1 mmol), NaOAc (1.968 g, 24 mmol), $Pd(PPh_3)_3Cl_2$ (0.190 g, 0.27 mmol) in PEG 600 (15 ml) were refluxed for 3 h at 100 ºC under nitrogen. The reaction mixture was transferred to a separatory funnel where CH_2Cl_2 (50 ml) and water (50 ml) were added. The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 ml). The organic fractions were combined and concentrated under vacuum to give the crude product which was further purified by column chromatography (heptane/acetone 1:4). The yield is 73%. ^{1}H NMR ($CDCl_3$): -0.04 (1H, m), 0.72 (1H, m), 1.21 (2H, m), 1.29 (24H, s), 1.43 (2H, s), 3.39-3.60 (20H, br m), 6.84 (4H, d), 6.97 (4H, d), 7.27 (4H, d), 7.73 (4H, d).

V. Reference

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