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Synthesis and Functionalization of BODIPY Dyes for Applications in the Detection of Colorectal Cancer

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by

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Undergraduate honors thesis under the direction of

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I. Abstract

The development of specific dyes for the selective labeling of cancerous tissues has been proposed as a more efficient means of detecting colorectal cancer. In recent years BODIPY (boron dipyrromethene difluoride) molecules have emerged as beneficial chromophores for biomolecular labeling and other fluorescent imaging applications. When used in combination with certain peptide ligands, it is hypothesized that BODIPY dyes will be able to selectively bind to and fluorescently image cancerous tissues.

BODIPY molecules were created using multiple synthetic routes, and various methods of functionalization were explored. BODIPYs **1** and **3** were synthesized *de novo* from pyrrole and various anhydrides using a simplistic one-pot method that generated a free carboxylic acid group. Synthesis was followed by activation and purification, as well as spectral analysis. Yields for both BODIPYs remained low due to difficulties encountered in purification. Reaction optimization and further improvement of purification methods will be required. BODIPY **5** was successfully synthesized from 2,4-dimethylpyrrole and benzaldehyde and was iodinated with high yields (>70%). Synthetic routes were proposed for the functionalization of BODIPY **5** at the 2 and 6 positions with amino and alkoxy groups. Nucleophilic aromatic substitution and palladium-catalyzed substitution reactions with alkoxide ions proved unsuccessful, and attempted nitration was likewise ineffective. Future routes for the successful addition of amino and alkoxy groups are being explored; such ligands will eventually serve as coupling sites for specific peptides, functioning similarly to the carboxylic acids generated in BODIPYs **1** and **3**. In addition, Suzuki couplings were investigated as a means of forming new carbon-carbon bonds and extending the conjugation of the BODIPY chromophore. BODIPY **6a** successfully

underwent a Suzuki coupling reaction with phenylboronic acid. Other metal-catalyzed coupling routes are being explored as approaches to adding aromatic carbon groups to synthesized BODIPYs.

II. Acknowledgments

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III. List of Abbreviations

ACN	acetonitrile
amu	atomic mass unit
BODIPY	boron dipyrromethene difluoride
CRC	colorectal cancer
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ESI	electrospray ionization
HRMS	high resolution mass spectrometry
MALDI	matrix-assisted laser desorption/ionization
MHz	Mega Hertz
MS	mass spectrometry
NBS	N-bromosuccinimide
NIR	near infrared
nm	nanometer
NMR	nuclear magnetic resonance
TLC	thin layer chromatography
TOF	time of flight

IV. Introduction

IV.a. Colorectal Cancer

Colorectal cancer is currently the second leading cause of cancer-related deaths in the United States.[1] According to recent studies, over 136,000 new cases of colorectal cancer (CRC) will be detected in the United States in 2014, with over 50,000 of these cases resulting in death.[1] However, it is estimated that nine out of ten cases of CRC can be successfully treated if detected early.[2] Cancerous cells are characterized by an overexpression of a cell surface protein known as epidermal growth factor receptor (EGFR). Epidermal growth factor (EGF) is a relatively short, 53 amino acid peptide sequence that serves as the natural binding ligand of EGFR.[3] Upon binding to EGFR, EGF stimulates mitogenic activity within the cell.

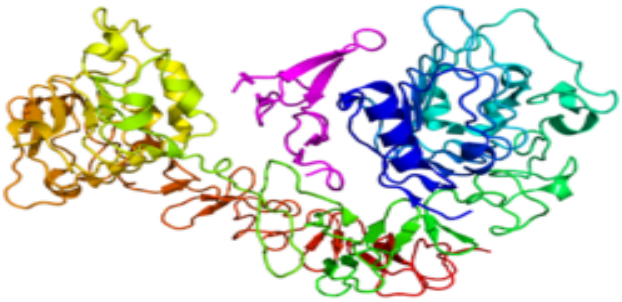


Figure 1. EGFR in complex with its natural ligand, EGF.[4] The amino acid sequence of EGF is NSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRDLKWWELR.[5]

IV.b. Detection of CRC

Current detection methods for CRC include the use of non-specific synthesized dyes as fluorescent imaging tools. Such dyes are applied in solution to intestinal tissues and enable fluorescent imaging of the entire area. Current dyes used for detection are fluorescein, acriflavine, and cresyl violet; however, each of these dyes emits at wavelengths

below 630 nm.[6] These wavelengths are often difficult to distinguish from the background emissions of biological molecules in blood and plasma tissues, which range from 280-630 nm.[7] The synthesis of dyes that emit at longer wavelengths, specifically near-infrared (NIR), would provide better contrast in imaging and would enable earlier and more accurate detection of CRC.

Another problem encountered with current dyes is their lack of specificity. Current dyes simply provide a fluorescent map of tissues, while specific dyes would enable the selective labeling of only cancerous cells. Such methods would provide a unique advantage in the efficiency and specificity of both detection and treatment.

IV.c. EGFR-L1 and EGFR-L2

In order to construct a specific detection dye, it is ideal to take advantage of the high affinity of EGFR for EGF, its natural binding ligand. Because of EGFR's overexpression on the exterior of cancer cells, ligands mimicking EGF can be used to selectively label these cells. The use of synthesized imitation ligands is necessary because the natural binding ligand, EGF, induces mitogenic activity upon binding to cancer cells. Replacement ligands have been designed to effectively bind to EGFR without stimulating mitosis within the cancer cell. Two mimic ligands, EGFR-**L1** and EGFR-**L2**, have been previously synthesized, and have demonstrated comparable binding affinity to that of EGF. These two peptide ligands can be conjugated to fluorescent agents to enable selective binding of dyes to cancerous cells.

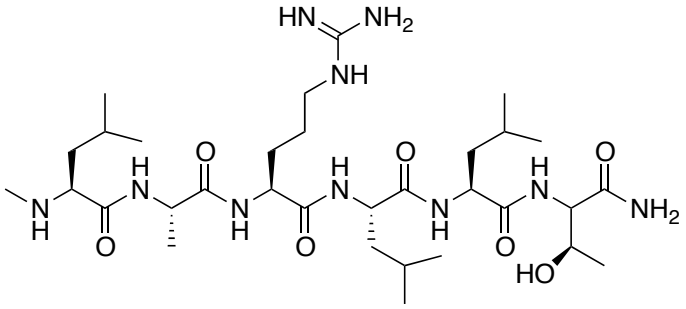


Figure 2. The amino acid sequence of EGFR-**L1** is LARLLT.

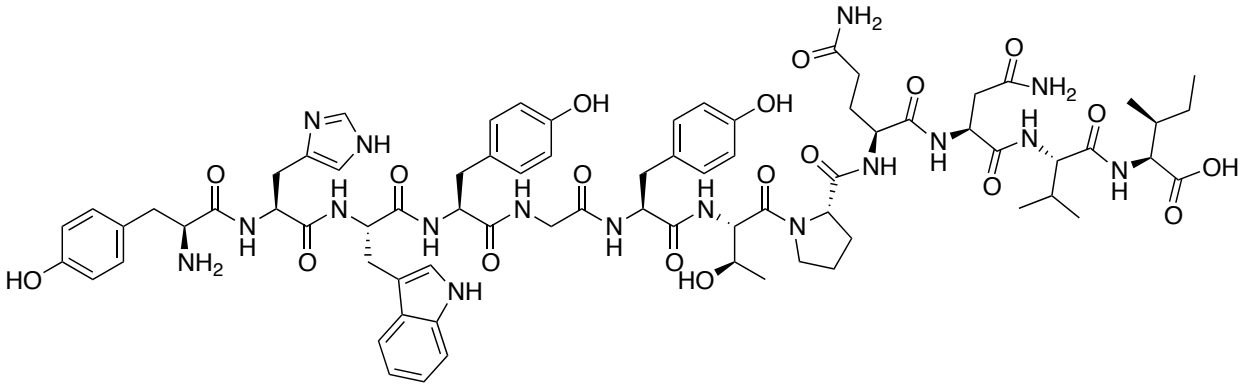


Figure 3. The amino acid sequence of EGFR-**L2** is YHWYGYTPQNVI.

IV.d. BODIPY Dyes

In recent years the use of boron dipyrromethene difluorides, or BODIPYs, as a biologically stable fluorescent dye has become increasingly widespread.[8] BODIPY molecules, frequently called "semi-porphyrins," provide many advantages. One such advantage is a low sensitivity to the polarity and pH of their environment; this characteristic makes them considerably stable in physiological settings, and ideal for use inside the human body.[9] BODIPYs are advantageous chromophores due to their overall stability, high quantum yields, tunable near infrared properties, and relatively low aggregation in various environments.[10] In addition, BODIPYs have been proven to be responsive to a variety of structural modifications.[8] BODIPY molecules contain seven carbons that are available for functionalization, enabling the addition of aromatic groups

which can extend the conjugation of the molecule. Such extensions of conjugation can be used to induce red shifts in BODIPY emission, and the addition of other functional groups can further alter emission patterns. This adaptability of structure also welcomes the possibility of functionalizing BODIPYs with groups that can serve as platforms for future peptide coupling reactions. The versatility and potential for modification exhibited by BODIPYs makes them ideal candidates for applications in biomolecular labeling.

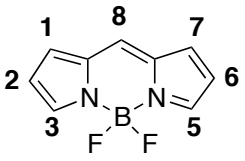


Figure 4. Structure of a BODIPY chromophore with labeled carbons.[11]

IV.e. BODIPY Synthesis and Functionalization

Several common methods of BODIPY synthesis exist, two of which are featured in our current research. The first synthetic method involves the reaction of a dicarboxylic acid anhydride with two equivalents of pyrrole.[10] In this method, a free carboxylic acid group is directly released during BODIPY formation. This route is advantageous because the free carboxyl group produced can later act as a site for peptide coupling; it also employs a simplistic one-pot procedure.[10] A second possible route achieves synthesis through the condensation of acyl chlorides with pyrroles; this method includes the formation of a dipyrromethene hydrochloride salt intermediate.[10] BODIPYs can also be constructed by the reaction of pyrroles with aldehydes, which is followed by oxidation and the formation of a complex with a dihedral boron. [10] In this case aromatic aldehydes are typically used, producing *meso*-substituted BODIPY products. Asymmetric dyes can be created through a

fourth method: the condensation of ketopyrrole intermediates with pyrroles using a Lewis acid.[1]

A wide range of *aryl-, alkenyl-,* and *alkynyl-* substituted BODIPYs can be synthesized by using their respective pyrrole derivatives; in addition, many other functionalizations are possible once synthesis is complete. BODIPYs are commonly functionalized at the *meso*- and 1-7 positions, as well as at the BF_2 group. Mesomeric structures of the BODIPY core demonstrate that the 2- and 6- BODIPY positions are the least positively charged, suggesting that these positions are most susceptible to electrophilic attack.

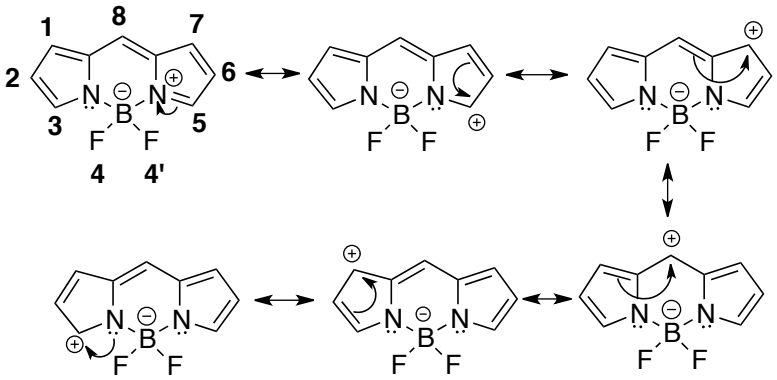


Figure 5. Resonance structures of the BODIPY core.

However, because the aromatic structure of BODIPY dyes is so intrinsically electron rich, other positions are frequently blocked with pyrrole substituents preceding electrophilic substitution reactions. Common electrophilic substitution reactions include sulfonation, nitration, and halogenation; these reactions are typically carried out under acidic conditions.[10] Nucleophilic attack can be used to functionalize the *meso*-position with cyano groups, and the inclusion of other *meso*-groups into the BODIPY structure can be accomplished during synthesis. Examples include *aryl-*, *sulfur-*, and *anilino-* BODIPY derivatives.[10]

Introducing substituents at the 1, 3, 5 and 7 positions is usually accomplished *de novo* using substituted pyrroles; however, this can also be achieved through performing nucleophilic substitution reactions on 1-, 3-, 5-, or 7-halogenated BODIPYs. Possible nucleophiles include amines, alkoxides, and thioalkoxides.[12] Halogenation reactions are commonly used to precede substitutions, as halogen substituents provide good leaving groups or ideal sites for metal-mediated coupling reactions. Halogenation can be accomplished via a simple, one-pot reaction, and stepwise halogenation has been shown to occur first at the 2,6-positions, then at the 3,5 and 1,7 positions.[12] This can be attributed to the enhanced nucleophilic character of the 2,6-positions.

IV.f. Carbon-Carbon Bond Formation

Transition metal-catalyzed carbon-carbon coupling reactions have also been used as an effective means of functionalizing BODIPY molecules and extending their conjugation.[10] Stille, Suzuki, Sonogashira, and Heck reactions have been used to functionalize the 3,5-positions of BODIPYs with aryl, ethenylaryl and ethynylaryl moieties.[13] Halogenated BODIPYs are typically used as starting material for these metal-catalyzed reactions. Products are shown to have high fluorescence capabilities with emission and excitation spectra that span from green to infrared wavelengths.[13] Because the addition of aromatic carbon groups results in a substantial alteration of the photophysical properties of BODIPYs, this method is also a promising means of producing fluorescent agents with tunable emission wavelengths.

III.g. BODIPYs as Detection Agents

For use as detection dyes, it is ideal to construct molecules with functional groups that can easily be conjugated to cell-binding peptides. Because peptides include an amino terminus and a carboxylic acid terminus, ligands mimicking these groups are ideal BODIPY substituents. For our purposes, BODIPYs including these groups can later undergo peptide-coupling reactions to produce EGFR-L1-BODIPY and EGFR-L2-BODIPY conjugates. To accomplish this a particularly beneficial synthetic route is *de novo* synthesis from two equivalents of pyrrole and one equivalent of a dicarboxylic acid anhydride. This method overcomes the problems presented when attempting to synthesize asymmetrical dyes from two different pyrroles, one of which contains a carboxyl group.[8] A variety of anhydrides can be used, and potential for BODIPY diversity is further increased by the use of variously-substituted pyrroles during synthesis. This simplistic one-pot method directly releases a carboxyl group during the formation of the BODIPY dye.[8]

Another method of equipping BODIPYs with the potential for peptide coupling is by attaching oxygen and nitrogen nucleophiles to the synthesized BODIPY core. Ideal nucleophiles for this purpose include amino or alkoxy groups. Halogenation reactions are frequently used to precede substitution.[11] Following halogenation, a variety of methods, including nucleophilic substitution at various temperatures and palladium-catalyzed coupling, can be used as an attempt to add nucleophilic groups to the BODIPY.[12] The addition of amino groups to BODIPYs can also be accomplished through nitration followed by reduction.[14]

Carbon-carbon coupling reactions can be used to further tune the emission wavelengths of constructed detection dyes. These metal-catalyzed coupling reactions can

accomplish the addition of aromatic groups and extension of the π-system of the chromophore. Such extensions induce red shifts in emission and further distinguish dye fluorescence from that of body tissues.

IV.h. Project Goals

In order to construct optimal dyes for the detection of colorectal cancer, several of the methods previously discussed will be explored. The synthesis of BODIPYs from dicarboxylic acid anhydrides will be investigated, as well as activation reactions and potential for future peptide coupling. Reaction optimization for these methods also requires detailed consideration and will be addressed. A variety of BODIPY substitution reactions will be examined with specific emphasis on those involving the use of nitrogen and oxygen nucleophiles. Previous research has been conducted substituting BODIPYs with oxygen and nitrogen nucleophiles at the 1, 7, 3, 5, and *meso*-positions.[11] These substitutions have demonstrated significant photophysical effects; however, to date little progress has been made in performing nucleophilic substitutions at the 2,6-positions.[11] In order to better understand the potential for substitution at the 2 and 6 positions, a variety of conditions will be explored.

Due to the advantageous effects of carbon-carbon coupling reactions when applied to dye synthesis, the work herein will also expand upon existing methods of metal-catalyzed carbon coupling. Little investigation has been done in achieving these substitutions at the 2 and 6 positions, so knowledge of the potential of these reactions is thus far limited. This essay will detail the development of methodology for 2,6-functionalization of BODIPYs with various carbon groups using metal-catalyzed reactions.

V. Materials and Methods

V.a. Specifications

All reagents and solvents were obtained from Sigma Aldrich as reagent grade and used without further purification. Reactions were monitored by TLC using 0.2 mm silica with UV indicator (UV254). Column chromatography was performed using Sorbent Technologies 60Å silica gel (230 - 400 mesh). All ^{1}H NMR spectra were obtained using a Bruker AV-400 spectrometer (400 MHz) in deuterated chloroform as solvent with trimethylsilane as an internal indicator. Chemical shifts (δ) are reported in ppm with $CDCl_3$ (^{1}H: 7.27 ppm) used as reference. Coupling constants (*J*) are reported in Hertz (Hz). Peak multiplicities are noted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). High-resolution ESI and MALDI mass spectra were obtained using an Agilent Technologies 6210 ESI-TOF Mass Spectrometer or a Bruker UltrafleXtreme MALDI-TOF/TOF.

V.b. Synthesis of BODIPYs 1 and 2 Using Phthalic Anhydride[8]

In a three-necked flask phthalic anhydride (0.889 g, 6.0 mmol) was dissolved in dry ACN (60 mL). Next pyrrole (or pyrrole derivatives) (12.0 mmol) and $BF_3{\cdot}OEt_2$ (1.704 g, 12.0 mmol) were added sequentially. The reaction mixture was heated to reflux under nitrogen and monitored for 8 h. The mixture was then cooled to room temperature and Et_3N (3.642 g, 36.0 mmol) was added, followed by the slow addition of $BF_3{\cdot}OEt_2$ (6.814 g, 48.0 mmol). The mixture was then stirred for 4 h at 50° C. After stirring, the reaction mixture was washed with water and extracted with DCM. Following extraction the organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed by rotary evaporation. The residue was purified by silica gel (hexane/EtOAc/methanol, 100:30:3). The synthesis of

BODIPYs **1** and **2** can be seen in Scheme 1 in the Results and Discussion section. Yield of BODIPY **1**: 0.42%. BODIPY **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (dd, 1H, 7.8 Hz), 7.66 (dd, 1H, 7.4 Hz), 7.56 (d, 1H, 7.8 Hz), 7.32 (d, 1H, 7.7 Hz), 5.93 (s, 2H, β-pyrrole H), 2.52 (s, 6H, -CH_3), 1.31 (s, 6H, -CH_3), MS (ESI-TOF) *m/z* calculated for $C_{20}H_{19}BF_2N_2O_2$: 368.1508, found $[M+H]^+$ 369.1597.

V.c. Synthesis of BODIPYs 3 and 4 Using Glutaric Anhydride[8]

In a three-necked flask were combined sequentially glutaric anhydride (0.888 g, 5.99 mmol), dry THF (100 mL), pyrrole (or pyrrole derivatives) (9.99 mmol), and $BF_3{\cdot}OEt_2$ (1.69 g, 11.98 mmol). The reaction mixture was heated to reflux for 8 h under nitrogen. Afterwards, the mixture was cooled to room temperature, and Et_3N (4.04 g, 39.96 mmol) then $BF_3{\cdot}OEt_2$ (4.26 g, 30 mmol) were added slowly. The mixture was stirred at 50° C for 4 h. Once complete, the reaction was washed with water then extracted with DCM. Anhydrous Na_2SO_4 was used to dry the organic fraction then solvent was removed under vacuum. The product residue was purified by silica gel (hexane/EtOAc/HOAc, 80:40:1). The synthesis route for BODIPYs **3** and **4** can be seen in Scheme 2. Yield of BODIPY **3**: 3.34%. BODIPY **3**: ^{1}H NMR (400MHz, $CDCl_3$) δ 6.06 (s, 2H, β-pyrrole H), 3.21 (m, 2H, -CH_2, 6.9 Hz), 3.02 (m, 2H, -CH_2), 2.53 (s, 6H, -CH_3), 2.42 (s, 6H, -CH_3), 1.97 (m, 2H, -CH_2); MS (ESI-TOF) *m/z* calculated for $C_{17}H_{21}BF_2N_2O_2$: 334.1664, found $[M+ Na]^+$ 357.1566.

V.d. Activation of BODIPYs 1 and 3[8]

Synthesized BODIPY with a free carboxylic acid (0.3 mmol) was dissolved in anhydrous acetonitrile (10 mL). N-Hydroxy succinimide (0.041 g, 0.36 mmol) and

dicyclohexylcarbodiimide (0.154 g, 0.75 mmol) were added to the reaction mixture. The mixture was stirred under nitrogen for 12-24 h at room temperature. TLC was used to monitor the mixture until all starting material was consumed. The workup procedure was as follows: the compound was washed with water and extracted with DCM. The resulting mixture was dried over $MgSO_4$ then filtered and dried under vacuum. The product was purified with silica gel (DCM/EtOAc, 12:1 to 8:1) and impure fractions were further purified with prep TLC. The activation reactions can be seen in Scheme 3. Yield BODIPY **1a**: 84.1%. Yield BODIPY **3a**: 10.9%. BODIPY **1a**: ^{1}H NMR (400MHz, $CDCl_3$), δ 7.12 (d, 2H, 8.3 Hz), 6.81 (d, 2H, 8.0 Hz), 6.06 (s, 2H, β-pyrrole H), 2.53 (s, 6H, -CH_3), 2.43 (s, 4H, -CH_2), 1.25 (s, 6H, -CH_3); MS (MALDI-TOF) *m/z* calculated for $C_{25}H_{23}BF_2N_2O_4$: 464.17, found $[M-H]^+$ 465.656. BODIPY **3a**: MS (ESI-TOF) *m/z* calculated for $C_{21}H_{24}BF_2N_3O_4$: 431.18, found $[M-H]^+$ 430.1756.

V.e. Synthesis of BODIPY 5[15]

2,4-dimethylpyrrole (0.999 g, 10.5 mmol), benzaldehyde (0.530 g, 5.0 mmol), and dichloromethane (300mL) were combined in an oven-dried flask. This was followed by the dropwise addition of $BF_3{\cdot}OEt_2$ (0.15 mL). The solution was set to stir under nitrogen at room temperature for 48 h, or until the aldehyde no longer remained; TLC was used to monitor reaction progression. After the completion of 48 h, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (1.1578 g, 5.1 mmol) was added to the solution. The solution was then stirred for 1 h, followed by the addition of treithylamine (7.24 mL, 37.5 mmol). The mixture was stirred for 30 min. BF_3OEt_2 (6.17 mL, 50 mmol) was then added to the reaction, and it was set to stir for an additional 3 h. Following stirring, the reaction mixture was added to

water. The organic layer was collected and passed over anhydrous Na_2SO_4, and the solvent was removed under vacuum. Silica gel was used to purify the crude product (DCM/hexanes, 50:50). The synthesis for BODIPY **5** can be seen in Scheme 4 in the Results and Discussion section. Yield**:** 90%. BODIPY **5**: ^{1}H NMR (400MHz, $CDCl_3$), δ 7.48 (m, 3H, *o,m*-phenyl H), 7.29 (m, 2H, *p*-phenyl H), 5.99 (s, 2H, β-pyrrole H), 2.57 (s, 6H, 3,5-CH_3), 1.38(s, 6H, 1,7-CH_3); HRMS (ESI-TOF) *m/z* calculated for $C_{19}H_{20}BF_2N_2$: 325.1688, found $[M]^+$ 325.1709.

V.f. Iodination of BODIPY 5[15]

In a minimal volume of water was dissolved iodic acid (0.109 g, 0.620 mmol). The resulting solution was added drop-wise to the solution of BODIPY **5** (0.100 g, 0.308 mmol) and iodine (0.195 g, 0.771 mmol) in a solvent mixture of 50:50 ethanol/DCM (100 mL). The reaction mixture was stirred for 2 h at 60° C. Once the solution was cooled, the solvent was removed under vacuum. The resulting residue was purified using a silica gel chromatography column (DCM/hexanes, 50:50). The iodination reaction can be seen in Scheme 4. Yield BODIPY **5a**: 91%. BODIPY **5a**: ^{1}H NMR (400MHz, $CDCl_3$), δ 7.54 (m, 3H, *o,p*-phenyl H), 7.27 (m, 2H, *m*-phenyl H), 2.66 (s, 6H, 3,5-CH_3), 1.39 (s, 6H, 1,7-CH_3); HRMS (ESI-TOF) *m/z* calculated for $C_{19}H_{17}BF_2I_2N_2$: 575.9545, found $[M]^+$ 575.9461.

V.g. Nucleophilic Aromatic Substitution of BODIPY 5a[9]

BODIPY **5a** (0.012 g, 0.021 mmol) was dissolved in ACN (30 mL) in a round-bottomed flask. Sodium alkoxide (0.0412 mmol) and potassium carbonate were added to the mixture, and the reaction was left stirring. TLC was used to monitor reaction progress.

After disappearance of the starting material, the potassium carbonate was removed by filtration, and the reaction was washed with ACN. The solvent was removed under vacuum, and the crude product was purified using column chromatography (DCM).

V.h. Palladium-Catalyzed Substitution of BODIPY 5a[16]

BODIPY **5a** (0.010 g, 0.017 mmol) was combined with $Pd(PPh_3)_4$ (0.001 g, 0.0009 mmol), diphenyl phosphine (0.005 g, 0.002 mmol), and K_2CO_3 (0.010 g, 0.069 mmol) in a round-bottomed flask and the mixture was placed under nitrogen. Toluene (1.74 mL) was added through a rubber septum, followed by MeOH (0.0012 mL). The reaction was allowed to run for 17 h at 80° C. An outline of this reaction can be seen in Scheme 6.

V.i. Nitration of BODIPY 5 Using N-bromosuccinimide[17]

A mixture of NBS (0.021 g, 0.12 mmol) in ACN (5 mL) was set to stir at reflux. $AgNO_3$ (0.020 g, 0.12 mmol) and BODIPY **5** (0.020g, 0.06 mmol) were added to the mixture. After 1.5 h the reaction was monitored using TLC. Once complete, AgBr was removed from the mixture by filtration and the solvent was evaporated under vacuum. The residue was dissolved in DCM (10 mL) and washed with aqueous 4% $NaHCO_3$ (2 x 5 mL). The aqueous phase was separated, and the organic phase was dried and concentrated. The organic residue was then purified using column chromatography (hexanes/EtOAc, 4:1). The attempted nitration reaction is depicted in Scheme 7.

V.j. Suzuki Coupling of BODIPY 6a with Phenylboronic Acid[12]

BODIPY **6a** (0.010 g, 0.016 mmol) was combined in a small flask with phenylboronic acid (0.008 g, 0.064 mmol) and palladium catalyst (10 mol%), respectively, over molecular

sieves. Toluene (2 mL) was added, followed by 1 M sodium carbonate (0.25 mL). The reaction was monitored by TLC and set to reflux overnight. Once complete, the reaction was quenched with water and extracted with DCM. The organic layer was dried over sodium sulfate. The product was purified using preparative thin layer chromatography (hexanes/EtOAc, 8:1). Scheme 8 below shows details of the coupling reaction. Yield: 122%. BODIPY **6b**: ^{1}H NMR (400MHz, $CDCl_3$), δ 7.41 (m, 4H, *m*-2,6-phenyl H), 7.32 (m, 2H, *p*-2,6-phenyl H), 7.19 (m, 4H, *o*-2,6-phenyl H), 6.55 (m, 3H, *o,p*-meso-phenyl H), 3.81 (s, 6H, *meso*-phenyl-OCH_3), 2.55 (s, 6H, 3,5-CH_3), 1.50 (s, 6H, 1,7-CH_3); HRMS (ESI-TOF) *m/z* calculated for $C_{33}H_{31}BF_2N_2O_2$: 536.2447, found $[M]^+$ 536.2491.

V.k. Suzuki Coupling of BODIPY 6a with Ethylboronic Acid[12]

BODIPY **6a** (0.010 g, 0.016 mmol) was combined in a round bottom flask with ethylboronic acid (0.005 g, 0.064 mmol) and $Pd(PPh_3)_4$ (10 mol%), respectively, over molecular sieves. Toluene (2 mL) was added, followed by 1 M sodium carbonate (0.25 mL). The reaction was monitored by TLC and set to reflux overnight. Once complete, the reaction was quenched with water and extracted with DCM. The organic layer was dried over sodium sulfate.

VI. Results and Discussion

VI.a. Synthesis and Characterization of BODIPYs 1-4

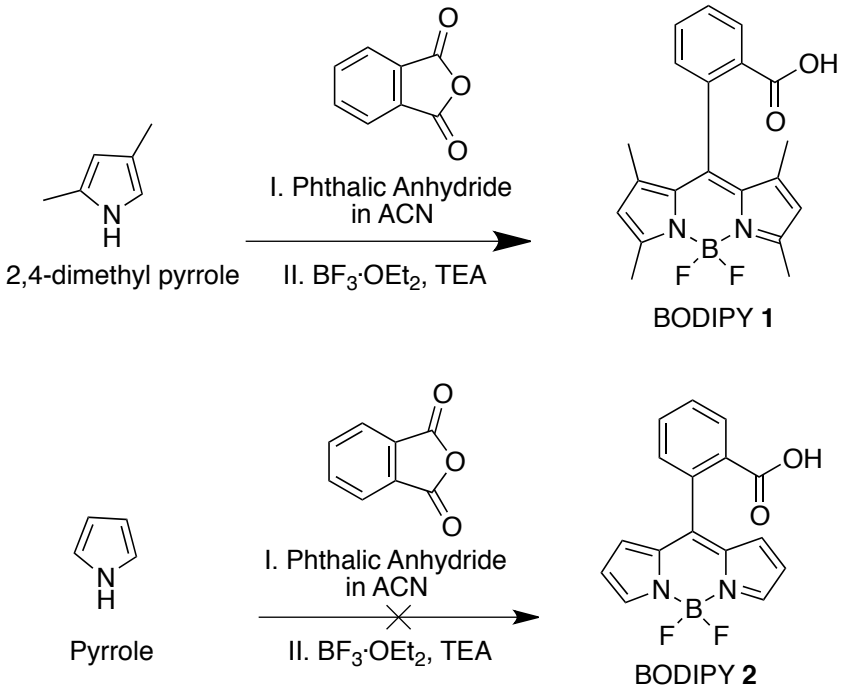
The synthesis of BODIPYs **1** and **2** from phthalic anhydride can be seen in Scheme 1 below, while the synthesis of BODIPYs **3** and **4** is shown in Scheme 2. Purification of the products for each of the four reactions proved difficult, and the extreme polarity of the

carboxylic acid group caused a significant amount of product to be lost within the silica. Mass spectrometry and ^{1}H-NMR were used to analyze each of the purified BODIPYs **1** and **3** were confirmed. HRMS of BODIPY **1** showed the presence of the $[M+H]^{+}$ peak at 369.1597 amu, and ^{1}H-NMR results showed the presence of β-pyrrole hydrogens (5.93 ppm), 3,5-methyl and 1,7-methyl hydrogens (2.52 ppm and 1.31 ppm, respectively), and the carboxylic acid hydrogen (8.17 ppm). HRMS analysis of BODIPY **3** displayed an $[M-H]^{+}$ peak at 333.1580 amu, and ^{1}H-NMR evidenced the presence of β-pyrrole hydrogens (5.82 ppm), 3,5-methyl and 1,7-methyl hydrogens (2.34 ppm and 2.24 ppm, respectively), and hydrogens from the *meso*-alkane chain (2.80 ppm, 2.47 ppm, and 2.06 ppm). However, the post-purification yields for these reactions were still extremely low (<5%). The presence of BODIPYs **2** and **4** could not be confirmed by mass spectrometry, indicating that these reactions were most likely unsuccessful. It was suspected that these results were due to the lower selectivity of the unsubstituted pyrrole starting material when compared with the 2,4-dimethylpyrrole. Due to its two available α-positions, it is likely that the bare pyrrole polymerized instead of successfully producing the BODIPYs **2** and **4.**

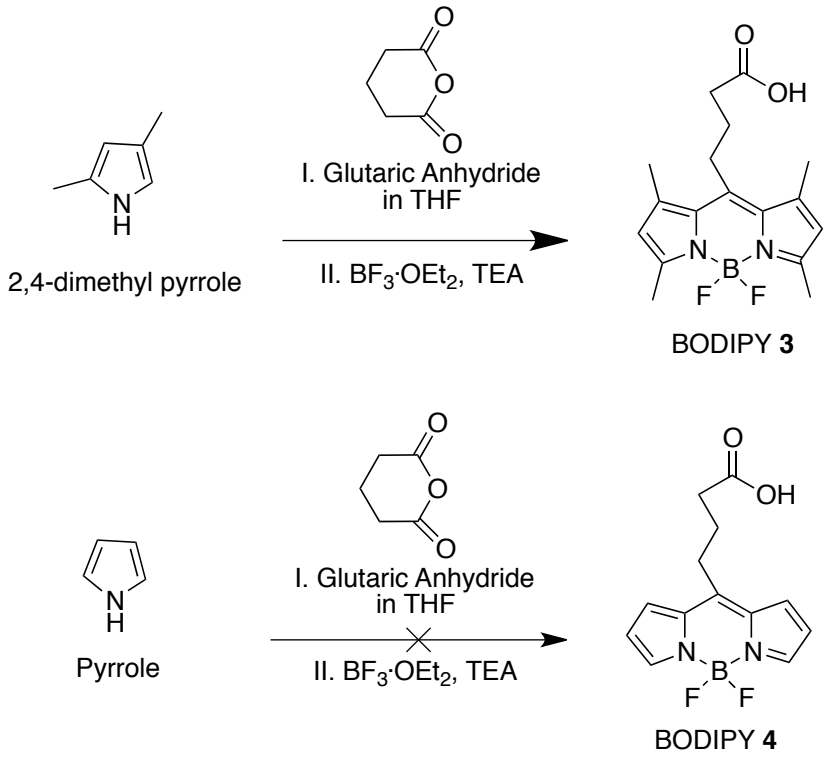
It was discovered that the use of dry solvents was critical for the success of the BODIPY synthesis reactions. Preceding the reactions, ACN was dried with benzophenone and sodium cubes. Molecular sieves were also applied to each reaction vessel to aid in water removal. The purity of the pyrrole starting material also had a significant effect on reaction success, so pyrrole was distilled prior to use to ensure optimal purity. It was also found that the BODIPY products degraded easily, and a large amount of product was lost due to degradation on the silica gel. In order to improve purification yields, it was suggested that products should undergo activation prior to silica purification to eliminate

the difficulty produced by the strong affinity of the carboxylic acid group for the silica gel. It was furthermore noted that the efficiency of purification was improved by the use of chilled solvents.

In addition to improving purification, it would also be beneficial to explore methods of reaction optimization. Routes for increasing the specificity of the reaction and decreasing side product formation include the possible use of catalysts and reduction of temperature. Potential catalysts include metal compounds, such as Grignard reagent.



Scheme 1. Synthesis of BODIPYs **1** and **2** with an available carboxylic acid from phthalic anhydride.[8]

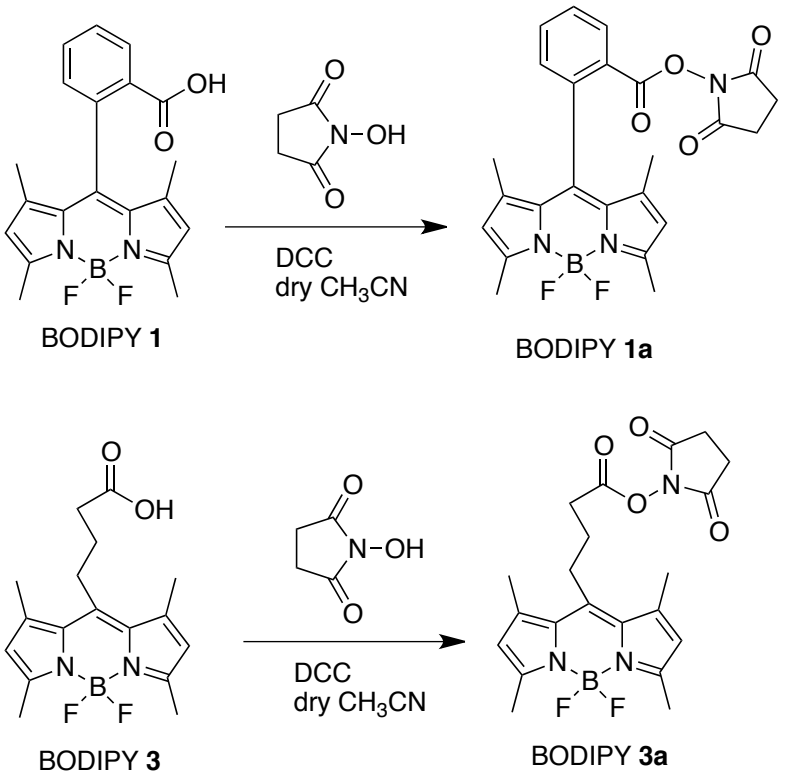


Scheme 2. Synthesis of BODIPYs **3** and **4** with an available carboxylic acid from glutaric anhydride.[8]

VI.b. Activation of BODIPYs 1 and 3 and Product Characterization

The activation reactions performed can be seen detailed below in Scheme 3. After the completion of synthesis and purification, the products of the activation reactions were characterized using mass spectrometry. Mass spectra confirmed the successful synthesis and isolation of both products; however, yeilds remained somewhat low. The $[M-H]^+$ peak for BODIPY **1a** appeared at 465.656 amu, and the $[M-H]^+$ peak for BODIPY **3a** was visible at 430.1756 amu. The successful synthesis of BODIPY **1a** was also supported by ^{1}H-NMR data; the four hydrogens of the succinimide group were visible in a peak that occurred at 2.43 ppm along with the previously identified BODIPY **1** ^{1}H-NMR peaks. It was hypothesized that low yields were the result of continued product interaction with the silica or could be

due to the formation of side products during reaction. Consideration is being given to the future use of buffered silica or alternate silica gels.

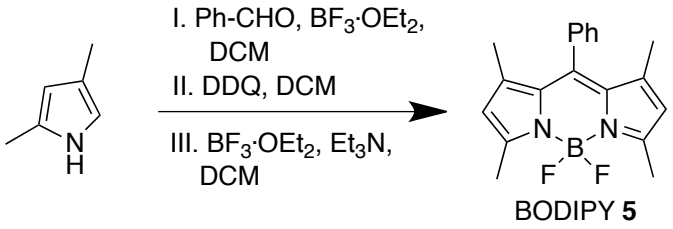


Scheme 3. Activation of BODIPYs **1** and **3** using N-hydroxysuccinimide in dicyclohexylcarbodiimide (DCC).[8]

VI.c. Synthesis and Characterization of BODIPY 5

The synthesis of *meso*-phenyl-1,3,5,7-tetramethyl BODIPY, or BODIPY **5**, was successfully completed and is shown in Scheme 4. HRMS of BODIPY **5** showed the $[M+H]^+$ peak at 325.1709 amu, in agreement with literature values. ^{1}H-NMR peaks were identified for *ortho*- and *meta*-hydrogens on the *meso*-phenyl group (7.48 ppm), the *para*-hydrogens of the *meso*-phenyl group (7.29 ppm), β-pyrrole hydrogens (5.99 ppm), as well as for the 3,5- and 1,7-methyl hydrogens (2.57 ppm and 1.38 ppm, respectively). These results closely agreed with literature values; the ^{1}H-NMR spectrum obtained for purified BODIPY **5**

is shown below in Figure 5.[15] The reaction had an overall yield of 90.5%. Photophysical data on synthesized BODIPY **5** has been published and is available for viewing.[15] The isolated product was then used as starting material for the iodination reaction to follow.



Scheme 4. Synthesis of BODIPY **5** from two equivalents of 2.4-dimethylpyrrole.[15]

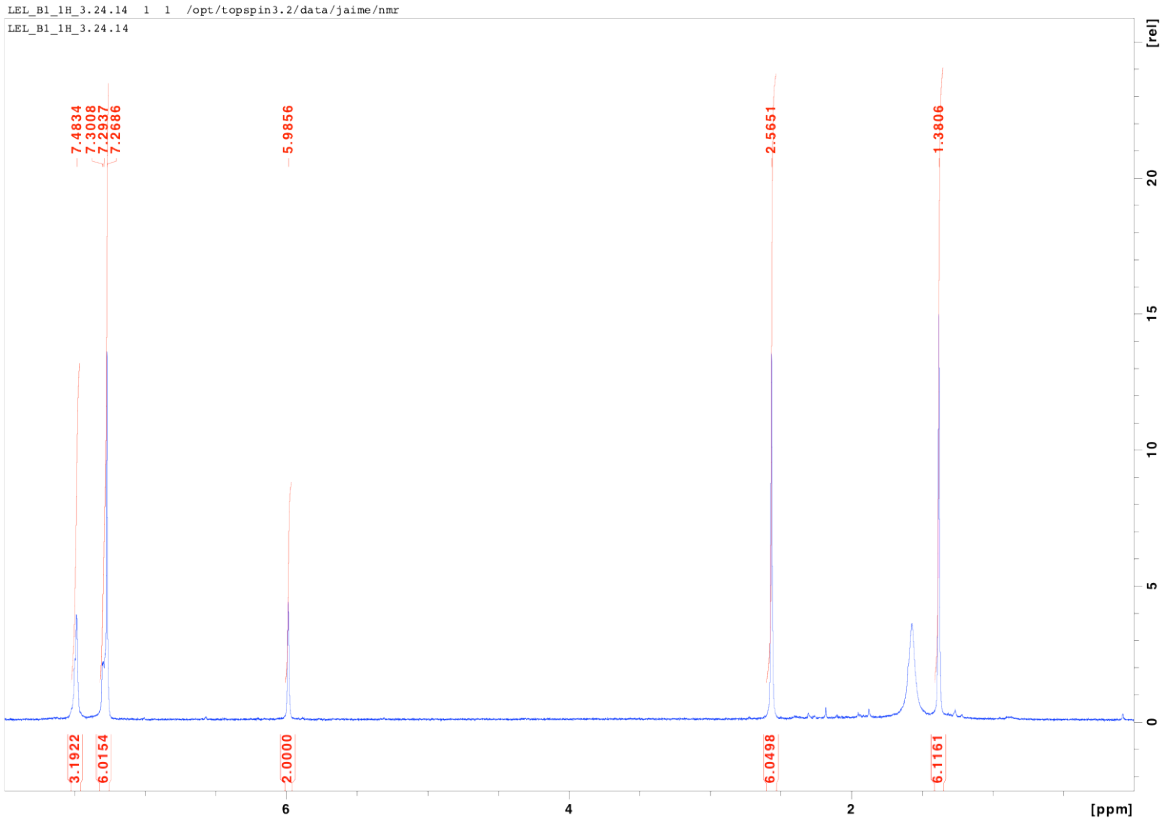
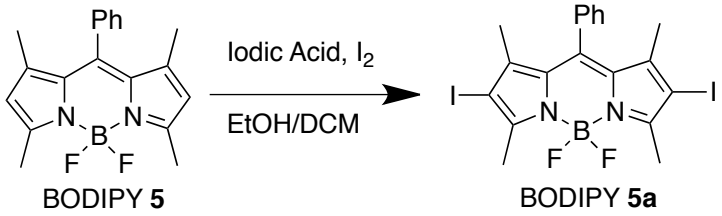


Figure 5. ^{1}H-NMR spectrum of BODIPY **5** in $CDCl_3$ at 400MHz.[15]

VI.d. Iodination of BODIPY 5 and Product Characterization

The iodination reaction performed on BODIPY **5** can be seen in Scheme 5 below. The reaction was successfully carried out, and the BODIPY **5a** product was purified using column chromatography. HRMS was performed and showed the presence of the $[M]^+$ ion at an *m/z* value of 575.9543 amu. ^{1}H-NMR analysis further supported the successful synthesis of BODIPY **5a,** with results that corresponded to literature values.[15] Peaks were identified for the *ortho*- and *para*-hydrogens of the *meso*-phenyl group (7.54 ppm), the *meta*-hydrogens of the *meso*-phenyl group (7.27 ppm), as well as for the hydrogens of the 3,5- and 1,7-methyl groups (2.66 ppm and 1.39 ppm, respectively). The ^{1}H-NMR spectrum obtained can be seen in Figure 6 below. The reaction had an overall yield of 73.4%. The addition of the iodine at the 2,6-positions caused a 33 nm shift in absorption, from 501 nm to 534 nm, as well as a shift in the emission wavelength from 511 nm to 550 nm.[15] The product was used as starting material for a number of substitution reactions detailed herein, including metal-catalyzed couplings.



BODIPY **5** BODIPY **5a**

Scheme 5. Electrophilic addition of iodine to BODIPY **5** at the 2,6-positions under acidic conditions.[15]

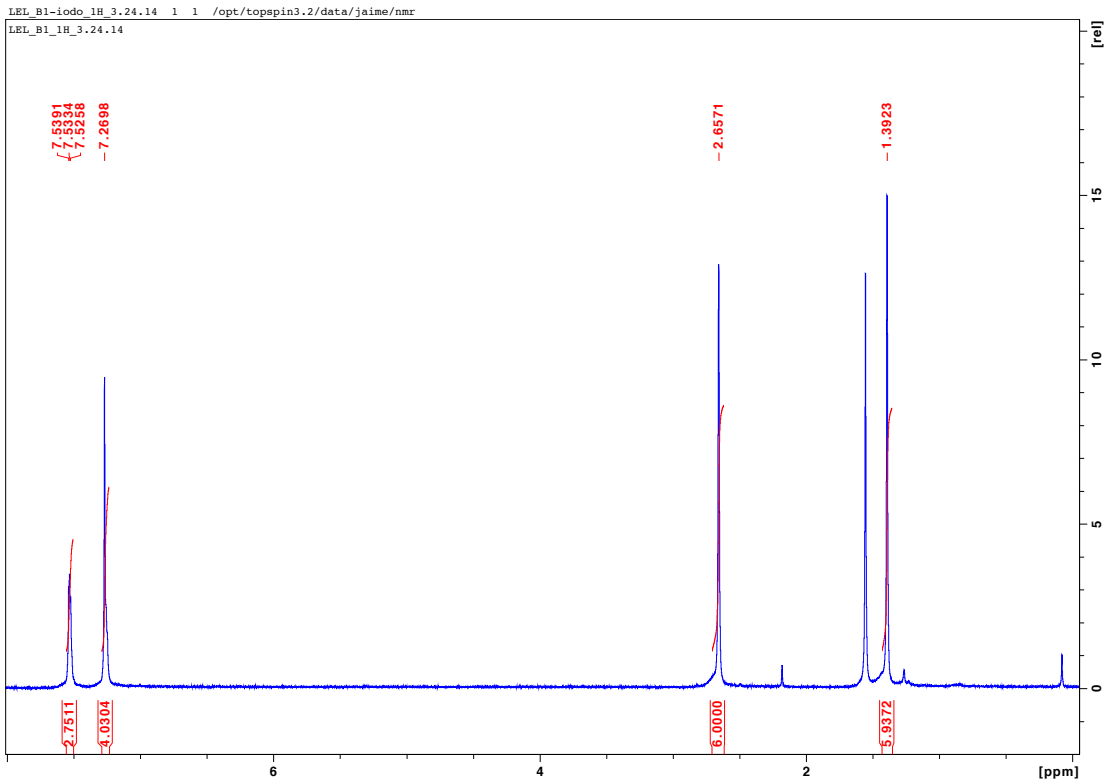


Figure 6. ^{1}H-NMR spectrum of BODIPY **5a** in $CDCl_3$ at 400MHz.[15]

VI.e. Nucleophilic Aromatic Substitution of BODIPY 5a with Alkoxy Ligands

Multiple sets of conditions were used in an effort to accomplish the nucleophilic aromatic substitution of BODIPY **5a**. The first attempted reaction was performed at room temperature using sodium methoxide as the nucleophile, and was allowed to run for one hour. TLC of the reaction mixture showed disappearance of the starting material and the formation of product. However, after the product was isolated using silica gel, ^{1}H-NMR and mass spectrometry data could not confirm the presence of the desired substitution product. The identity of the compound formed was unable to be determined.

The substitution reaction was performed a second time using sodium ethoxide as the nucleophile; it was predicted that the additional protons in the ethoxide ligands would

provide better visualization in ^{1}H-NMR spectra and easier product identification. The reaction was run at a lower temperature, -12°C, in an attempt to slow the reaction and increase selectivity for the 2,6-positions. Although mass spectrometry analysis showed the presence of the product $[M+H]^{+}$ peak at m/z 413.2680 amu, crystallography data indicated that the iodine ligands were still present in their original positions in the bulk of the crystalline product. The X-ray crystal structure obtained for the product can be seen below in Figure 7. It was concluded that both nucleophilic aromatic substitution reactions were unsuccessful in substituting at the 2,6-positions. In addition, no *m/z* peak indicated the presence of product with substitution at the boron center.

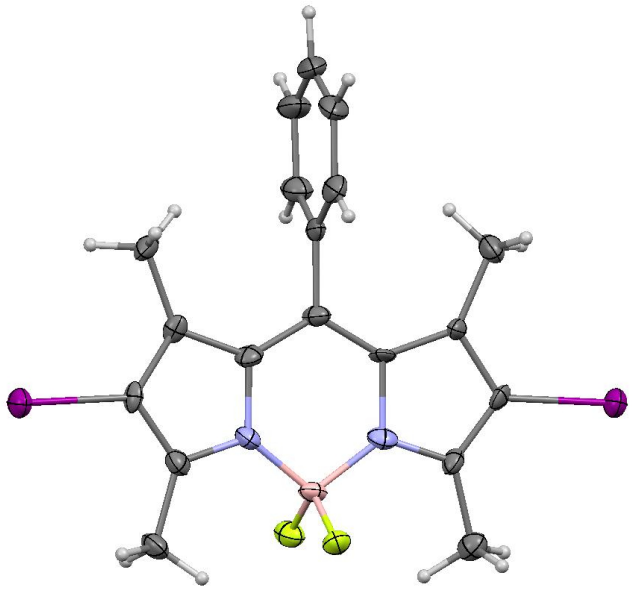
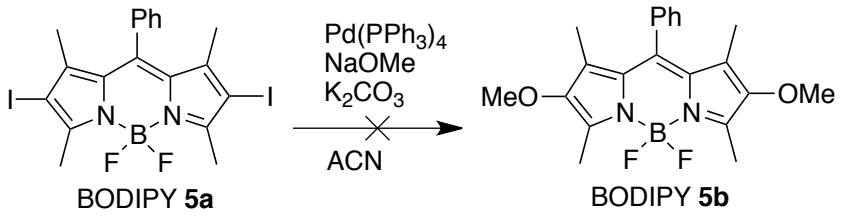


Figure 7. X-ray structure of BODIPY **5a**, ellipsoids are drawn at 50% probability level.[15]

VI.f. Palladium-Catalyzed Substitution of BODIPY 5a

The attempted palladium-catalyzed substitution of BODIPY **5a** with alkoxy ligands can be seen below in Scheme 6. It was supposed that the use of a palladium catalyst might provide increased selectivity for the 2 and 6 positions. However, the reaction was allowed

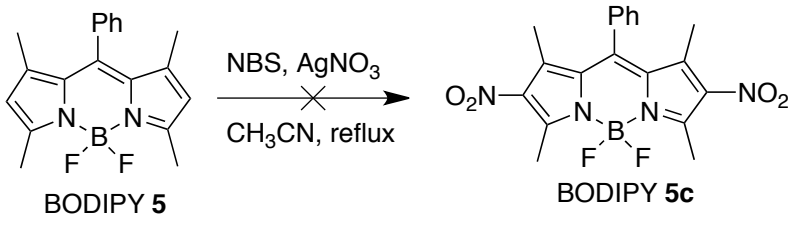
to run overnight, and TLC of the mixture showed no product formation after this time. This reaction was considered unsuccessful.



Scheme 6. Attempted palladium-catalyzed substitution of BODIPY **5a** at the 2,6-positions using methoxy ligands.[16]

VI. g. Nitration of BODIPY 5 Using N-bromosuccinimide

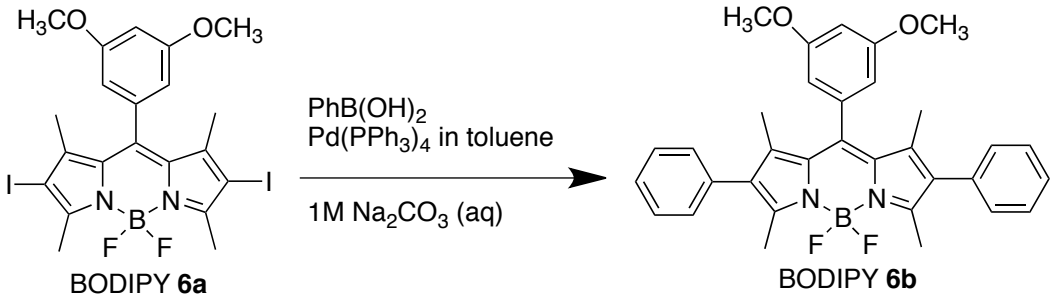
In an effort to establish a means of nitrating BODIPYs under neutral conditions, a nitration reaction was attempted using N-bromosuccinimide and silver nitrate. Previous literature had shown multiple successful nitration reactions at the 2 and 6 positions; however, yields for these reactions were relatively low due to the harsh acidic conditions used.[14, 18] The proposed nitration reaction can be seen below in Scheme 7. The reaction was allowed to run for three hours, and TLC showed the disappearance of starting material. However, the presence of the desired nitration product was not evidenced by ^{1}H-NMR or mass spectrometry data. The reaction was unsuccessful in nitrating the 2,6-positions; it is possible that nitration occurred at the *meso*-phenyl group rather than at the 2,6-positions.



Scheme 7. Attempted nitration of BODIPY **5** under neutral conditions.[17]

VI. h. Suzuki Coupling of BODIPY 6a with Phenylboronic Acid

The Suzuki coupling performed is shown below in Scheme 8. Once complete, the reaction mixture was purified using preparative thin layer chromatography. Other methods were shown to provide poor separation and were not sufficient to isolate the product. The purified product was submitted for both ^{1}H-NMR and mass spectrometry analysis, and results confirmed the presence of the predicted product. A *m/*z peak representing the $[M]^+$ ion was present at 536.2491 amu. The ^{1}H-NMR spectrum of BODIPY **6b** can be seen in Figure 8 below. ^{1}H-NMR peaks representing *meta-*, *para-,* and *ortho*-hydrogens of the successfully installed phenyl groups are visible at 7.41 ppm, 7.32 ppm, and 7.19 ppm, respectively. However, the calculated yield was greater than 100%, indicating that impurities still exist within the product.



Scheme 8. Suzuki coupling of BODIPY **6a** under basic conditions with phenylboronic acid.[12]

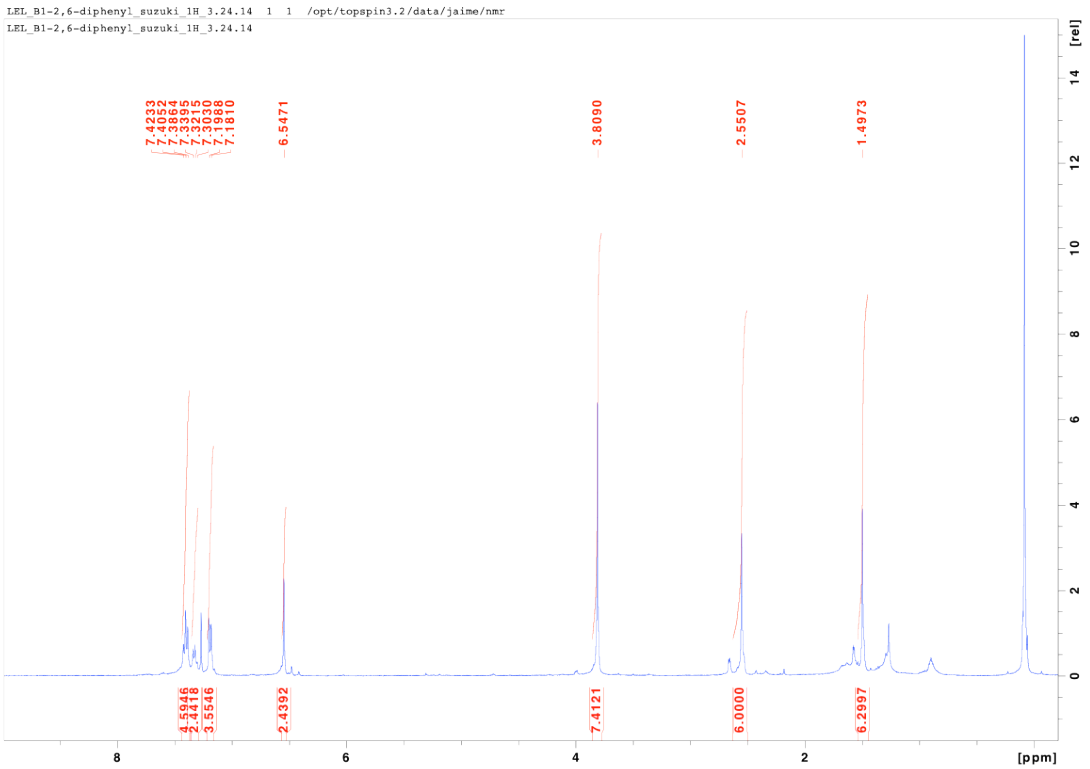


Figure 8. ^{1}H-NMR of BODIPY **6b** in $CDCl_3$ at 400MHz.

VI.i. Suzuki Coupling of BODIPY 6a with Ethylboronic Acid

A second Suzuki coupling reaction was attempted using ethylboronic acid and the same conditions used for the previous pheylboronic acid coupling. After the reaction had proceeded overnight, TLC showed the continued presence of starting material. The reaction was quenched, and the solvent was removed; the residue obtained was used as starting material for a second coupling reaction. The second reaction utilized the same conditions as above, but the aqueous Na_2CO_3 was substituted instead with three equivalents of solid K_2CO_3. The reaction was allowed to run overnight, but no further reaction took place.

A final attempt of the Suzuki coupling reaction was performed using ethylboronic acid and three equivalents of the base Cs_2CO_3. In the third trial, the solvent used was tetrahydrofuran. TLC showed the continued presence of starting material in the reaction mixture, and the reaction was deemed unsuccessful.

VII. Conclusions

In order to develop more ideal dyes for CRC detection, two BODIPYs containing carboxylic acid groups ready for coupling were successfully synthesized from glutaric and phthalic anhydride. Mass spectrometry and ^{1}H-NMR data were used to confirm the successful synthesis and activation of BODIPYs **1** and **3**; however, yields remained low for both products. It has been suggested that neutral silica could be used to decrease product loss during purification, or a metal catalyst could be used to increase the yield of the reaction.

BODIPY **5** was successfully synthesized and iodinated at the 2,6-positions, with both of these reactions producing large yields (>70%). In efforts to functionalize BODIPYs at the 2 and 6 positions, it was shown that these positions were largely unreactive to nucleophilic attack under a variety of conditions. Attempts to perform aromatic substitution of BODIPY **5a** with alkoxide nucleophiles were unsuccessful, as was the attempted palladium-catalyzed substitution. These results are most likely due to the somewhat nucleophilic character of the 2, 6 BODIPY positions. However, it has been hypothesized that the electron withdrawing effects of the nitrogen atoms within the BODIPY may allow substitution at these positions if the correct conditions are discovered. Future work to achieve 2,6-substitution could include the synthesis of BODIPYs with electron-withdrawing

groups at the 1,7-positions. These electron-withdrawing groups could likely increase the susceptibility of the 2,6-positions to nucleophilic attack, enabling the addition of alkoxy or amino groups.

The nitration reaction performed with BODIPY **5** under neutral conditions was unsuccessful. Other possible routes to be considered for nitration include the use of copper nitrate, sodium nitrate, or nitronium tetrafluoroborate.[19-21] These reagents have proven successful in the nitration of other aromatic chromophores, such as porphyrins.[19-21]

Once the yields of BODIPYs **1** and **3** are improved or the successful synthesis of amino- and alkoxy-substituted BODIPYs is achieved, future work could include spectroscopic analysis and BODIPY-peptide coupling. Promising conjugates should be further analyzed in order to determine their overall biological efficacy. Time dependent cellular uptake, intracellular localization, and cytotoxicity studies should be performed on HT29 cells via *in vitro*, with the best conjugates advancing to *in vivo* studies.

A Suzuki coupling using phenylboronic acid was successfully performed on BODIPY **6a**. Further research will be conducted into 2,6 BODIPY functionalization with aromatic carbon groups using metal catalysts. Potential reactions include Sonogashira coupling using ethynyl trimethylsilane, ethynyl benzene, and 2-ethynyl thiophene, as well as Stille couplings using tin reagents to install other aromatic moieties.

VIII. References

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