WATER-SOLUBLE BODIPYS: SYNTHESES,
DERIVATIZATION AND PHOTOPHYSICAL STUDIES

A Thesis
by
LINGLING LI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2007

Major Subject: Chemistry
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Approved by:

Chair of Committee, Kevin Burgess
Committee Members, Gregory D. Reinhart
Coran M H Watanabe
Head of Department, David H Russell

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ABSTRACT

Water-soluble BODIPYs: Syntheses, Derivatization and Photophysical Studies.

(December 2007)

Lingling Li, B.E., University of Science & Technology of China

Chair of Advisory Committee: Dr. Kevin Burgess

A set of water-soluble 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) derivatives, has been prepared and their spectroscopic properties examined. These dyes can be used as either donor or acceptor in synthesis of through-bond energy transfer cassettes. Sulfonation conditions were developed for several BODIPY dyes to give the mono-sulfonated and di-sulfonated products. Compounds with an aryl iodide could be used for organometallic couplings. Similarly, BODIPYs with an aromatic bromide, but also two chlorine atoms could be replaced via S_NAr reactions. The amine sulfonated BODIPY is amenable to couple to biomolecules via acylation reactions. A diazotization/azide reaction sequence was used to convert the amines into azides; the latter may be functionalized via click reactions. Spectral data for these materials indicates they are highly fluorescent probes in aqueous environments.

We have also prepared some lipophilic BODIPY derivatives, which can be used for S_NAr reactions and make some through-bond, energy transfer cassettes. DichloroBODIPYs can also be used for labeling proteins successfully.
ACKNOWLEDGMENTS

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>Cell Imaging</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Fluorescence Resonance Energy Transfer (FRET)</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>Through-Bond Energy Transfer</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>LIPOPHILIC BODIPY DERIVATIVES</td>
<td>8</td>
</tr>
<tr>
<td>2.1</td>
<td>$S_{N}Ar$ Reaction on BODIPY Substrates</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Results and Discussion</td>
<td>11</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Syntheses of CF$_3$-DichloroBODIPY and Its Derivatives</td>
<td>11</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Syntheses of Br-DichloroBODIPY and Its Derivatives</td>
<td>13</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Spectroscopic Studies</td>
<td>22</td>
</tr>
<tr>
<td>2.3</td>
<td>Coupling with Protein</td>
<td>27</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Synthesis of 16-Avidin</td>
<td>27</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Calculation of Dye-Protein Ratio</td>
<td>28</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS (cont’d)

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.3</td>
<td>Synthesis of 31-Avidin ................................................................. 30</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Calculation of Dye-Protein Ratio ................................................... 31</td>
</tr>
<tr>
<td>2.4</td>
<td>Conclusion ........................................................................................ 33</td>
</tr>
<tr>
<td>III</td>
<td>THROUGH-BOND ENERGY TRANSFER CASSETTES ........................................ 35</td>
</tr>
<tr>
<td>3.1</td>
<td>Introduction .................................................................................... 35</td>
</tr>
<tr>
<td>3.2</td>
<td>Results and Discussion (Syntheses and Spectroscopic Studies) .......... 35</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Cassette 38 with Nile-Red Acceptor, BODIPY Donor ....................... 35</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Cassette 41 with BODIPY Acceptor and Donor .................................. 41</td>
</tr>
<tr>
<td>3.3</td>
<td>Conclusion ........................................................................................ 45</td>
</tr>
<tr>
<td>IV</td>
<td>WATER-SOLUBLE BODIPY DERIVATIVES AND CONCLUSION ......................... 46</td>
</tr>
<tr>
<td>4.1</td>
<td>Introduction .................................................................................... 46</td>
</tr>
<tr>
<td>4.2</td>
<td>Results and Discussion (Syntheses and Spectroscopic Studies) .......... 49</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Syntheses ....................................................................................... 49</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Spectroscopic Studies ...................................................................... 57</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Determination of Quantum Yields .................................................... 61</td>
</tr>
<tr>
<td>4.3</td>
<td>Conclusion ........................................................................................ 62</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>................................................................................................. 64</td>
</tr>
<tr>
<td>APPENDIX A EXPERIMENTAL DATA FOR CHAPTER II ......................... 67</td>
<td></td>
</tr>
<tr>
<td>APPENDIX B EXPERIMENTAL DATA FOR CHAPTER III .......................... 111</td>
<td></td>
</tr>
<tr>
<td>APPENDIX C EXPERIMENTAL DATA FOR CHAPTER IV ............................ 130</td>
<td></td>
</tr>
<tr>
<td>VITA</td>
<td>................................................................................................. 174</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The first generation through-bond energy transfer cassettes and acceptor synthons</td>
<td>5</td>
</tr>
<tr>
<td>1.2</td>
<td>Fluorescence of equimolar EtOH solutions of 1-8 excited at 488 nm</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>The second generation through-bond energy transfer cassette 9 and acceptor synthon</td>
<td>6</td>
</tr>
<tr>
<td>1.4</td>
<td>Comparison of the fluorescence peak of cassette 9 in pH 8 phosphate buffer with fluorescein and a tetraacid rhodamine derivative 10 under the same conditions</td>
<td>7</td>
</tr>
<tr>
<td>2.1</td>
<td>Spectroscopic data for some BODIPYs formed by S$_{N}$Ar reactions</td>
<td>10</td>
</tr>
<tr>
<td>2.2</td>
<td>a) UV absorption, and b) fluorescence:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spectra for non-substitued BODIPYs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) UV absorption, and d) fluorescence:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spectra for mono-substitued BODIPYs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>e) UV absorption, and f) fluorescence:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spectra for di-substitued BODIPYs</td>
<td>24</td>
</tr>
<tr>
<td>2.3</td>
<td>Proposed dyes which can be used to label proteins</td>
<td>27</td>
</tr>
<tr>
<td>2.4</td>
<td>a) UV absorption, and b) fluorescence:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spectra for model study 31 and 16-avidin</td>
<td>29</td>
</tr>
</tbody>
</table>
LIST OF FIGURES (cont’d)

Figure 2.5 a) UV absorption, and b) fluorescence:
   spectra for model study 33 and 31-avidin .............................................. 32

Figure 3.1 a) UV absorption, and b) fluorescence: spectra for cassette 38 ............ 40

Figure 3.2 a) UV absorption, and b) fluorescence: spectra for cassette 41 ............ 44

Figure 4.1 a) Previously known water-soluble BODIPY systems; and
   b) compounds prepared in this work....................................................... 47

Figure 4.2 a) UV absorption, and b) fluorescence:
   spectra for mono-sulfonated BODIOYs
   c) UV absorption, and d) fluorescence:
   spectra for di-sulfonated BODIOYs ....................................................... 59
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Spectral characteristics of dyes in MeOH</td>
<td>23</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Spectral characteristics of dyes in H$_2$O</td>
<td>62</td>
</tr>
</tbody>
</table>
## LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme 2.1. Mono- and di-substitution of Compound 11</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 2.2. Synthesis of CF$_3$-dichloroBODIPY 16</td>
<td>12</td>
</tr>
<tr>
<td>Scheme 2.3. Mono-substitution of compound 16</td>
<td>13</td>
</tr>
<tr>
<td>Scheme 2.4. Di-substitution of compound 16</td>
<td>13</td>
</tr>
<tr>
<td>Scheme 2.5. Synthesis of dichloroBODIPY 21</td>
<td>14</td>
</tr>
<tr>
<td>Scheme 2.6. Synthesis of INP methyl ester</td>
<td>15</td>
</tr>
<tr>
<td>Scheme 2.7. a) Mono-subsitution of compound 21;</td>
<td></td>
</tr>
<tr>
<td>b) di-substitution of compound 21</td>
<td>15</td>
</tr>
<tr>
<td>Scheme 2.8. Syntheses of compounds 25 and 26</td>
<td>17</td>
</tr>
<tr>
<td>Scheme 2.9. a) Synthesis of cyano-compound 27;</td>
<td></td>
</tr>
<tr>
<td>b) Synthesis of cyano-compound 28</td>
<td>18</td>
</tr>
<tr>
<td>Scheme 2.10. Syntheses of compound 29</td>
<td>19</td>
</tr>
<tr>
<td>Scheme 2.11. Synthesis of compound 30</td>
<td>20</td>
</tr>
<tr>
<td>Scheme 2.12. Syntheses of water-soluble mono- and</td>
<td></td>
</tr>
<tr>
<td>di-substituted compounds 59 and 60</td>
<td>21</td>
</tr>
<tr>
<td>Scheme 2.13. Synthesis of compound 16-avidin</td>
<td>27</td>
</tr>
<tr>
<td>Scheme 2.14. Synthesis of Model BODIPY 32</td>
<td></td>
</tr>
<tr>
<td>for measuring the extinction coefficient</td>
<td>28</td>
</tr>
</tbody>
</table>
# LIST OF SCHEMES (cont’d)

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.15</td>
<td>Synthesis of water-soluble BODIPY 31</td>
<td>30</td>
</tr>
<tr>
<td>2.16</td>
<td>Synthesis of compounds 31-avidin</td>
<td>30</td>
</tr>
<tr>
<td>2.17</td>
<td>Synthesis of Model BODIPY 33</td>
<td>31</td>
</tr>
<tr>
<td>3.1</td>
<td>Syntheses of tetramethyl NO2-BODIPY 34</td>
<td>36</td>
</tr>
<tr>
<td>3.2</td>
<td>Reduction with H₂ and Pd/C</td>
<td>37</td>
</tr>
<tr>
<td>3.3</td>
<td>Synthesis of amino- and azido-BODIPYs 35 and 37</td>
<td>38</td>
</tr>
<tr>
<td>3.4</td>
<td>Synthesis of Nile Red containing cassette 38</td>
<td>39</td>
</tr>
<tr>
<td>3.5</td>
<td>Synthesis of ethynyl-BODIPY 40</td>
<td>41</td>
</tr>
<tr>
<td>3.6</td>
<td>Synthesis of cassette 41 with BODIPY</td>
<td>43</td>
</tr>
<tr>
<td>4.1</td>
<td>Syntheses of mono-sulfonated BODIPYs 42-44 from tetramethyl NO2-BODIPY</td>
<td>50</td>
</tr>
<tr>
<td>4.2</td>
<td>Syntheses of di-sulfonated BODIPYs 45-47 from tetramethyl NO2-BODIPY</td>
<td>51</td>
</tr>
<tr>
<td>4.3</td>
<td>Synthesis of water-soluble BODIPY 48 with carboxylic acid</td>
<td>52</td>
</tr>
<tr>
<td>4.4</td>
<td>a) Mono-sulfonation; and b) di-sulfonation on tetramethyl iodoBODIPY</td>
<td>53</td>
</tr>
<tr>
<td>4.5</td>
<td>Synthesis of di-sulfonic acid 51</td>
<td>54</td>
</tr>
<tr>
<td>4.6</td>
<td>a) Mono-sulfonation; and b) di-sulfonation on dichloroBODIPY 21</td>
<td>55</td>
</tr>
<tr>
<td>Scheme</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>Scheme 4.7</td>
<td>Synthesis of NO$_2$-dichloroBODIPY 56</td>
<td>56</td>
</tr>
<tr>
<td>Scheme 4.8</td>
<td>Sulfonation on dichloroBODIPY 56 with various equivalent chlorosulfonic acid</td>
<td>57</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>INP</td>
<td>isonipecotic acid</td>
</tr>
<tr>
<td>DMF</td>
<td>$N, N$-dimethylformamide</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
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<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>Et$_3$N</td>
<td>triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

1.1 Cell Imaging
Cell imaging can be defined as a multidisciplinary discipline that detects and analyzes cellular macromolecules with the help of microscopy and computer programming. Over the past decades, advances in living cell imaging have dramatically transformed the biological sciences. Cell imaging investigates functional and molecular changes in cells as well as morphological changes during the development of diseases or during the therapy. Cell imaging provides a direct evidence of cell type-specific and subcellular information of a certain biomolecule, whereas traditional molecular biology techniques cannot due to the homogenization of cells. Beginning with the invention of confocal microscopy and more recent advances such as Nipkow dual-disk technology, today's live cell imagers offer the resolution required to image living cells without destroying them. Developments in fluorescent dyes and proteins have further facilitated the study of complex cellular processes using fluorescent staining or labeling of various proteins, ions and lipids in living cells.

In fluorescence imaging, the energy from an external source of light is absorbed and almost immediately re-emitted at a longer, low-energy wavelength. Irrespective of the mode of signal generation, systems suitable for use in vivo are those that employ compounds with high quantum yields that emit in the NIR region, because hemoglobin, water and lipids have their lowest absorption coefficient in the NIR region of around 650-900 nm. Imaging in the NIR region also has the added advantage of minimizing tissue autofluorescence, which can further improve “target/background ratios”.¹

¹ This thesis follows the style of the Journal of Organic Chemistry.
1.2 Fluorescence Resonance Energy Transfer (FRET)

The excitation transfer process, when applied to fluorescent molecules can be defined as exciting a fluorescent molecule which is in proximity to a second fluorophore, results in emission occurring from the second fluorophore with minimal or almost no fluorescence seen from the excited molecule. This phenomenon is termed as Fluorescence Resonance Energy Transfer (FRET), which was first reported by Professor Theodor Förster in 1946.² FRET occurs via a dipole-dipole mechanism and does not involve the emission and reabsorption of a photon as one might initially assume. The excited fluorophore can be termed as a donor and the molecule emitting light can be termed as an acceptor. The energy transfer which takes place between the donor and acceptor which is separated by a distance \( r \), is given by:

\[
K_T(r) = \frac{Q_D \kappa^2}{\tau_D r^6} \left[ \frac{9000(\ln 10)}{128\pi^5 N n^4} \right] \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda \tag{i}
\]

Where \( Q_D \) = quantum yield of donor in the absence of the acceptor, \( \kappa^2 \) = orientation factor (range from 0 to 4, usually assumed to be 0.67 for dynamic random averaging). \( \kappa^2 = 4 \) if the transition dipoles of the donor and acceptor are perfectly parallel and 0 when they are orthogonal. \( \tau_D \) = lifetime of donor in the absence of the acceptor. \( N = 6.02 \times 10^{23} \), \( n \) = refractive index of the medium (usually assumed to be 1.4 for biomolecules in aqueous solution). The rate of energy transfer is inversely proportional to the sixth power of the distance, \( r \), between the donor and the acceptor. The overlap integral \( J(\lambda) \) can be given as

\[
J(\lambda) = \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda \tag{ii}
\]

The extent of overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor is given by above equation. \( F_D(\lambda) \) is the normalized emission spectrum of the donor. \( \varepsilon_A(\lambda) \) is the extinction coefficient of the acceptor at wavelength \( \lambda \). The Förster radius, \( R_0 \), is the distance \( r \), at which the rate of energy transfer is equal to the rate of decay of the donor (1/\( \tau_D \)) in the absence of the acceptor.
$R_0$ is the distance at which FRET is 50 % efficient. At $r = R_0$, $K_T = (1/\tau_D)$. Equation (i) can be written as

$$R_0 = \left[ \frac{9000 (\ln 10) Q_D \kappa_2}{128 \pi_N n^4} \right] \int_0^\infty F_D(\lambda) E_A(\lambda) \lambda^4 d\lambda \quad \text{...(iii)}$$

$R_0$ is typically in the range of 20 to 60 Å for organic fluorophores.

Knowing $R_0$, one can calculate the ET rate by:

$$k_T = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6 \quad \text{...(iv)}$$

The efficiency of energy transfer, $E$ is the fractions of photons absorbed by the donor that are transferred to the acceptor. $E$ is given by:

$$E = \frac{k_T}{\tau_D^{-1} + k_T} \quad \text{...(v)}$$

which is the ratio of the energy transfer rate to the total decay rate of the donor. $E$ can also be expressed as:

$$E = \frac{R^6}{R^6 + r^6} = 1 - \frac{\tau_{DA}}{\tau_D} = 1 - \frac{F_{DA}}{F_D} \quad \text{...(vi)}$$

Hence the efficiency of energy transfer can be calculated from the emission intensity of the donor in the absence and the presence of the acceptor or from the lifetime of the excited donor in the presence and absence of the acceptor.$^3$

### 1.3 Through-Bond Energy Transfer

In contrast to through-space energy transfer cassettes, donor and acceptor units connected by conjugated linker fragments may transfer energy via through bond, which does not require the emission spectrum of the donor to overlap with the lowest energy excited state of the acceptor. There are two mechanisms proposed for the observed energy transfer. Dexter$^4$ and superexchange energy transfer.$^5$ As compared to Förster energy transfer, Dexter energy transfer is a short range phenomenon and requires the interaction between excited donor orbital with the orbital of acceptor in ground state. Superexchange energy
transfer can take place over a longer distance since energy is relayed through bonds connecting the donor and the acceptor.

 Appropriately designed through-bond energy transfer cassettes could absorb photons via a donor part and transfer the energy rapidly through the conjugate linker to the acceptor fragment that emits at a longer wavelength. There is no known constraint on the difference between the donor absorption and acceptor emission wavelength in this Scheme since no overlap is required for energy transfer to occur. Thus it is possible to design dyes that can absorb strongly at short wavelength and emit brightly at longer wavelength. In summary, through bond energy transfer cassettes have the potential to increase the resolution and fluorescence intensities obtained from several probes excited by laser source operating at single wavelength.

Requirements for good through-bond energy transfer cassettes are:
• donor components should have strong absorbance
• acceptor components that fluoresce strongly
• functional groups that allow labeling of biomolecules, also to enhance hydrophilicity
• suitable conjugate linker that can prevent donor and acceptor retron from becoming planar

Our group has been working on such through-bond energy transfer cassettes for years. The first generation cassettes 1-4 (Figure 1.1) were made in 2003. Figure 1.2 shows their photophysical properties in ethanol. Excitation of the cassettes at 488 nm produces fluorescence characteristic of only the acceptor component, that is, 100% energy transfer efficiency between the donor and the acceptor. The comparison of the intensities of fluorescence shows the cassettes fluoresce more brightly than the corresponding acceptor components 5-8 irradiated at 488 nm.
Figure 1.1. The first generation through-bond energy transfer cassettes and acceptor synthons.

Figure 1.2. Fluorescence of equimolar EtOH solutions of 1-8 excited at 488 nm.
The second generation cassette (Figure 1.3) was water-soluble through-bond energy transfer. Cassette 9 was assembled by coupling the fluorescein alkyn and the bromorhodamine derivative 10.

![Figure 1.3](image-url)

Figure 1.3. The second generation through-bond energy transfer cassette 9 and acceptor synthon.

Figure 1.4 shows the fluorescence of cassette 9, donor fluorescein and acceptor 10 in pH 8 phosphate buffer. The energy transfer efficiency was not 100% because some of the fluorescence leaks from the fluorescein donor rather than being transferred to the acceptor.
Figure 1.4. Comparison of the fluorescence peak of cassette 9 in pH 8 phosphate buffer with fluorescein and a tetraacid rhodamine derivative 10 under the same conditions.

BODIPYs (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) were chosen in our group to work with and displace fluorescein and rhodamines for labeling with proteins.

Some advantages for BODIPYs are: they can
- absorb UV radiation efficiently
- emit relatively sharp fluorescence peaks
- have high quantum yields
- are insensitive to solvent polarity and pH

A detailed study of these dyes and our effort towards its modification for our specific purpose is illustrated in the chapters II-IV.
CHAPTER II

LIPOPHILIC BODIPY DERIVATIVES

2.1 $S_N$Ar Reactions of BODIPY Substrates

The most common approach to introduce substituents on 3- and 5-positions of BODIPYs is to start with appropriately substituted pyrroles. However an exciting recent development reaches the same goal via nucleophilic substitution on the 3,5-dichloro-BODIPY.$^6,^7$

Scheme 2.1. Mono- and di-substitution of compound 11.

The nucleophiles used so far include alkoxides, amines, thioalkoxides, and the diethyl malonate anion. These reactions can be stopped at the mono-substitution stage or forced
to the disubstitution product, hence they are useful for access to asymmetric 12 and symmetric 13, hetero-substituted, BODIPY dyes.

Oxygen-centered nucleophile was tried first; two equivalents of methoxide (in methanol) at room temperature gave the mono-substituted product 12a in good yield. Under more forcing conditions, four equivalents of methoxide (in methanol) at reflux temperature gave the di-substituted derivative 13a. Ethylene glycol with sodium hydride in acetonitrile at room temperature reacted with dichloroBODIPY 11 to afford the mono-substitued 12b. However reaction with excess ethylene glycol/sodium hydride at reflux temperature did not give the di-substituted derivative.

Several nitrogen-centered nucleophiles were tried also. Piperidine gave mono-substitution of 11 without additional base at room temperature yielding 12d. Again, heating at reflux temperature (in acetonitrile) with excess amine lead to the disubstituted BODIPY derivative 13d. The primary amine, aniline was also tried and gave the mono and disubstituted compounds successfully without any additional base.

Ethyl 2-thioacetate with triethylamine as base was used to demonstrate the reactivity of sulfur-based nucleophiles. Again reaction at room temperature yielded the mono-substituted derivative, while di-substitution was possible at reflux temperature (in acetonitrile).

Diethyl malonate with sodium hydride as base was used as a carbon nucleophile to afford either mono- or di-substituted BODIPY derivatives.

The optical data are given for the S_NAr reaction products (Figure 2.1). Compound 11 absorbs at 508 nm in methanol and fluoresces at 519 nm. The quantum yield is 0.27. The mono- and di-substituted BODIPYs with alkoxides and secondary amine gave very low quantum yields in methanol and cyclohexane. The product 12g from mono-substitution with primary amine also gave low quantum yield in methanol (0.003), but a better one in
cyclohexane (0.28). The product 13g from di-substitution gave very good quantum yield in both methanol (0.45) and cyclohexane (0.86). In addition, the mono- and di-substituted BODIPYs with thioalkoxides and diethyl malonate anion gave good quantum yields (0.24-0.62). It was also found that quantum yields of di-substituted compounds are much higher than the mono-substituted.

![Chemical structures](image)

**Figure 2.1.** Spectroscopic data for some BODIPYs formed by $S_{N}Ar$ reactions.
2.2 Results and Discussion

New dichloroBODIPYs were synthesized in our group which showed increased reactivity toward S_N_Ar reactions. Trifluoromethyl or bromophenyl groups were used for the meso-sub in the new BODIPYs.

2.2.1 Syntheses of CF_3-DichloroBODIPY and Its Derivatives

Pyrrole (1.8 eq) can be condensed with trifluoacetaldehyde methyl hemiacetal (90% technology grade) to give the CF_3-dipyrromethane 14, which was followed by
chlorination using N-chlorosuccinimide reagent in THF at -78 °C to form CF₃-dichloro dipyrrromethane. Then DDQ was used to oxidize dipyrrromethane to CF₃-dichloro dipyrrmethene 15, which was then chelated with BF₂ at reflux temperature (in CH₂Cl₂) to give the target CF₃-dichloro BODIPY 16. Compound 16 has very high quantum yield which is 1.0 in dichloromethane; this may be attributed to removal of a pathway for non-radiative decay. Absorption maximum of BODIPY 16 is 548 nm, at least 20 nm red-shifted compared to BODIPY 11, but the Stoke’s shift is very small, 6nm.

Scheme 2.2. Synthesis of CF₃-dichloroBODIPY 16.

SₙAr reactions can be processed easily on compound 16 with really good yields; it may be due to the strong electron withdrawing group CF₃.
Scheme 2.3. Mono-substitution of compound 16.

The mono-substitution of compound 16 with piperidine was much faster than the di-substitution. It took 48 h for the second substitution to be completed.

Scheme 2.4. Di-substitution of compound 16.

2.2.2 Syntheses of Br-DichloroBODIPY and Its Derivatives

S_NAr reactions can be easily applied on Br-dichloroBODIPY 21 as well. It is easy to synthesize tens of grams compound 21, since bromo-dipyrromethane can be crystallized from the dichloromethane/hexane mixture for the very first step.12 The key step for the synthesis is to use excess pyrrole (at least 25 eq) which could be recovered at the end by the distillation. The quantum yield of compound 21 is lower than CF_3-dichloro BODIPY 16, but still quite good, which is 0.42 in dichloromethane and 0.15 in methanol by using Rhodimine 6G as standard (Φ = 0.94 in ethanol).
Scheme 2.5. Synthesis of dichloroBODIPY 21.

S_NAr reaction can also happen between Br-dichloro BODIPY 21 and INP (Isonipecotic Acid) methyl ester 22. The INP methyl ester salt was very easily made with almost quantitative yield. When it was treated with ammonia hydroxide to remove HCl, the yield was drastically reduced (Scheme 2.6).
Scheme 2.6. Synthesis of INP methyl ester.

Displacement of the first of the two chlorines in BODIPY 21 with INP methyl ester occurred rapidly. The second chlorine can be displaced using extended reaction times at elevated temperature. The di-substituted product 24 can be modified to be a potential acceptor, but one consideration is its low quantum yield. 

Scheme 2.7. a) Mono- and b) di-substitution of compound 21.

a
Scheme 2.7. Continued.

It is known that fluorine atom can be substituted by alkyl groups,\textsuperscript{11} so compound 25 can be easily formed when Br-dichloroBODIPY 21 was treated with magnesium methyl bromide in dry THF. Compound 25 also shows a strong green fluorescence. The
mono-substitution of compound 25 with INP methyl ester was not as easy as BODIPY 21. Six equivalents of INP methyl ester 22 and much longer reaction time were needed; one hypothesis for that could be methyl groups on boron make BODIPY 25 more electron rich and unreactive.

Scheme 2.8. Syntheses of compounds 25 and 26.

Since alkoxides, amines, thioalkoxides and the diethyl malonate anion were tried as the nucleophiles for S\textsubscript{N}Ar reactions, cyanide anion should be also easy to attack the electron deficient carbons.\textsuperscript{14-16} Unfortunately, none of those conditions gave me the desired product when compound 21 was treated with sodium cyanide in methanol, acetonitrile or DMSO. Then the organic cyanide anion source TMSCN was tried instead and gave good results.\textsuperscript{17} Displacement was achieved using Lewis acids to active the reaction. Compound 27 was obtained with a high yield when the reaction was stirred in dry dichloromethane at
room temperature for 2h using tin tetrachloride as catalyst.\textsuperscript{18, 19} However, when boron trifluoride etherate was used as Lewis acid, tetracyanoBODIPY 28 was formed instead of dicyanoBODIPY 27. The difference is cyanide anion also substituted the fluorine atoms on boron. Evidence for this assignment is $^{19}$F NMR showed a quartet for 27, but no peaks at all for 28. Other conditions were also tried to see whether mono-substitution of chloride with cyanide would happen with milder Lewis acids (MgCl$_2$) or just one equivalent of trimethyl silyl cyanide. However, only di-substituted compound was formed. That means the second substitution is a lot faster than the first one.

**Scheme 2.9. a) Synthesis of compound 27; b) synthesis of compound 28.**

a

\[
\begin{align*}
\text{Br} & \quad \text{N} \quad \text{Cl} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \quad \text{Br} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2, 25 \degree C, 2 \text{ h} & \quad \text{Br} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \quad \text{CN} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2, 25 \degree C, 2 \text{ h} & \quad 27, 100 \% \\
\end{align*}
\]

b

\[
\begin{align*}
\text{Br} & \quad \text{N} \quad \text{Cl} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \quad \text{Br} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2, 25 \degree C, 2 \text{ h} & \quad \text{Br} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \quad \text{CN} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2 \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{B} \quad \text{F}_2, 25 \degree C, 2 \text{ h} & \quad 28, 71 \% \\
\end{align*}
\]

Cyanide anion displacement reactions were also tried on compound 23 to see if the only chlorine atom remaining would be displaced, but when the same condition that was used
for compound 27 was employed, only fluorine atoms were substituted by the cyanide anion. Thus compound 29 was formed; nothing happened on the carbon bonding with chlorine, even when a stronger Lewis acid, boron trifluoride etherate was used to catalyze the reaction. $^{19}$F NMR showed no fluorine existed.

Scheme 2.10. Synthesis of compound 29.

BODIPY 24 that was substituted with INP methyl ester, was treated with trimethyl silyl cyanide and tin tetrachloride. The same result as compound 29 was achieved; compound 30 was formed as evidenced by fluorine atoms disappearing on $^{19}$F NMR.
Scheme 2.11. Synthesis of compound 30.

\[
\begin{align*}
\text{Br} & \quad + \quad \text{TMSCN} \\
\text{SnCl}_4 & \quad \text{CH}_2\text{Cl}_2, \quad 25^\circ\text{C}, \quad 3\ h
\end{align*}
\]

The water-soluble dichloroBODIPY 53 (synthesis described in Chapter IV) was also reacted with INP to test if the sulfonated group can accelerate $S_{\text{NAr}}$ reactions. Deuterated water was used as the solvent to facilitate NMR monitoring. One equivalent of INP without any protecting groups and three equivalents of sodium bicarbonate were added to the solution. The mono-substitution was extremely fast. The reaction was almost instantaneous and gave one product by TLC and $^1\text{H}$ NMR. When one more equivalent of INP and three more equivalents of sodium bicarbonate were added, the second displacement was complete in 24 h at room temperature and gave the di-substituted product 60. In summary, the sulfonated-dichloroBODIPY processed the $S_{\text{NAr}}$ reaction much faster.
Scheme 2.12. Syntheses of water-soluble mono- and di-substituted compounds 59 and 60.

\[
\begin{align*}
\text{Br} & \quad \text{Na}_2\text{CO}_3 \\
\text{H}_2\text{O}, 25^\circ\text{C}, 10 \text{ min} & \\
& \\
\text{Cl} & \\
\end{align*}
\]

\[
\begin{align*}
\text{Br} & \quad \text{Na}_2\text{CO}_3 \\
\text{H}_2\text{O}, 25^\circ\text{C}, 24 \text{ h} & \\
& \\
\text{Cl} & \\
& \\
\end{align*}
\]

59, 100 % conversion

60, 100 % conversion
2.2.3 Spectroscopic Studies

Figure 2.2. a and b show sharp peaks for the absorption and fluorescence of BODIPYs 21, 25, 27 and 28 (in methanol) and 16 (in dichloromethane). The absorption and emission maximum of CF₃-BODIPY 16 were about 30 nm red-shifted. On the other hand, the absorption maximum of the dimethylated BODIPY 25 is only 503 nm, about 10 nm blue-shifted compared with the other three BODIPYs in methanol.

Surprisingly, Figure c shows blue-shifted and broadened peaks for the absorption of mono-substituted compounds 17, 23, 26 and 29 compared with the non-substituted BODIPYs. It can be seen from Figure d all the mono-substituted compounds have very similar maximum wavelengths of emission (about 560 nm in MeOH), and fwhms are broad, in the range of 75-85 nm.

Figure e and f show several differences to di-substituted compounds 18, 24, 30. Compound 18 is at least 30 nm red-shifted for both of the absorption and emission. The most interesting thing is the boron dicyanide BODIPY 30 absorbs only at 517nm, at least 50 nm blue shifted compared with the other two di-substituted BODIPYs. Fwhms for the di-substituted BODIPYs are not as broad as mono-derivatives; they are in the range of 48-61 nm.

Quantum yields (Table 2.1) for the non-substituted BODIPYs are very good, from 0.13 to 1.0. Compound 21 and 25 have the relatively low quantum yields, 0.15 and 0.13 respectively. Compounds 27 and 28 have really good quantum yields even though they both have the phenyl ring, which can rotate and is supposed to reduce the quantum yield. Mono-substituted BODIPYs have bad quantum yields, from 0.001 to 0.006, worse than di-substituted derivatives (0.008~0.03).
Table 2.1. Spectral characteristics of dyes in MeOH.

<table>
<thead>
<tr>
<th>dye</th>
<th>$\lambda_{abs}$ (nm)</th>
<th>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</th>
<th>$\lambda_{emi.}$ (nm)</th>
<th>fwhm (nm)</th>
<th>$\Phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>16$^a$</td>
<td>548</td>
<td>86860</td>
<td>554</td>
<td>22</td>
<td>$1.00 \pm 0.1^b$</td>
</tr>
<tr>
<td>17</td>
<td>492</td>
<td>58640</td>
<td>560</td>
<td>85</td>
<td>$\leq 0.001^c$</td>
</tr>
<tr>
<td>18</td>
<td>614</td>
<td>32100</td>
<td>643</td>
<td>48</td>
<td>$\leq 0.03^d$</td>
</tr>
<tr>
<td>21</td>
<td>512</td>
<td>77550</td>
<td>523</td>
<td>27</td>
<td>$0.15 \pm 0.01^e$</td>
</tr>
<tr>
<td>23</td>
<td>482</td>
<td>68440</td>
<td>562</td>
<td>78</td>
<td>$\leq 0.003^c$</td>
</tr>
<tr>
<td>24</td>
<td>573</td>
<td>39860</td>
<td>614</td>
<td>52</td>
<td>$\leq 0.01^d$</td>
</tr>
<tr>
<td>25</td>
<td>503</td>
<td>134310</td>
<td>516</td>
<td>30</td>
<td>$0.13 \pm 0.01^c$</td>
</tr>
<tr>
<td>26</td>
<td>483</td>
<td>61770</td>
<td>564</td>
<td>84</td>
<td>$\leq 0.002^c$</td>
</tr>
<tr>
<td>27</td>
<td>514</td>
<td>127350</td>
<td>526</td>
<td>25</td>
<td>$0.66 \pm 0.07^e$</td>
</tr>
<tr>
<td>28</td>
<td>510</td>
<td>36500</td>
<td>523</td>
<td>25</td>
<td>$0.80 \pm 0.08^e$</td>
</tr>
<tr>
<td>29</td>
<td>485</td>
<td>33660</td>
<td>560</td>
<td>75</td>
<td>$\leq 0.006^c$</td>
</tr>
<tr>
<td>30</td>
<td>517</td>
<td>26050</td>
<td>613</td>
<td>61</td>
<td>$\leq 0.008^e$</td>
</tr>
</tbody>
</table>

$^a$ In CH$_2$Cl$_2$. $^b$ Rhodamine B was used as a standard ($\Phi = 0.73$ in EtOH). $^c$ Fluorescein was used as a standard ($\Phi = 0.92$ in 0.1 M NaOH$_{aq}$). $^d$ Rhodamine 101 was used as a standard ($\Phi = 1.00$ in EtOH). $^e$ Rhodamine 6G was used as a standard ($\Phi = 0.94$ in EtOH). For each compound, it was excited at the same wavelength as standard.
Figure 2.2. a) UV absorption, and b) fluorescence: spectra for non-substituted BODIPYs.
Figure 2.2. Continued. c) UV absorption, and d) fluorescence: spectra for mono-substitued BODIPYs.
Figure 2.2. Continued. e) UV absorption, and f) fluorescence: spectra for di-substituted BODIPYs.
2.3 Coupling with Protein

2.3.1 Synthesis of 16-Avidin

As mentioned above, the S\textsubscript{N}Ar reaction works well on CF\textsubscript{3}-dichloroBODIPY 16 with piperidine as a nucleophile, thus we wondered if the protein could be used also to displace chlorine atoms, since it has lysines or cystines, which can be considered as nitrogen and sulfur centered nucleophiles (Figure 2.3). Compound 31 was also made as a target to react with protein. The advantage for 31 is increasing the water solubility.

![Figure 2.3. Proposed dyes which can be used to label proteins.](image)

Scheme 2.13 shows that avidin (4mg/ml 0.1 M sodium bicarbonate buffer, pH 8.3) reacted with compound 16 (10 eq in 10μl THF) after 1 h stirring at room temperature to give 16-avidin. After the PD10 desalting column, only one fraction was obtained and it was assumed that all 10 eq of BODIPY 16 reacted. Extinction coefficients were also needed to calculate the dye/protein ratio and prove this assumption.

**Scheme 2.13.** Synthesis of compound 16-avidin.
2.3.2 Calculation of Dye-Protein Ratio

Compound 32 was made as a model to estimate the extinction coefficient for the dye on protein; this amine was chosen because the nucleophile for the substitution would be likely the lysine. The quantum yield for this model BODIPY 32 was measured as 0.74 in the 0.1 M lithium phosphate buffer (pH 7.4) by using fluorescein as standard. Extinction coefficient of the model 32 was measured as 17066 M\(^{-1}\)cm\(^{-1}\) (in the same buffer). Equation 1 was used to calculate the dye protein ratio.\(^{20}\) \(\varepsilon_p\) is 101640 M\(^{-1}\)cm\(^{-1}\). \(A_p\) represents the absorbance of avidin at 280 nm, which equals \(A_{280} - CF \times A_d\). \(A_{280}\) is the absorbance of 16-avidin at 280 nm. \(CF\) means the ration of absorbances for the model dye 32 at 280 and 469 nm. \(A_d\) means the absorbance of compound 16-avidin at 469 nm.

\[
\frac{C_d}{C_p} = \frac{A_d \times \varepsilon_p}{A_p \times \varepsilon_d}
\]

Finally, \(C_d/C_p\) was calculated to be approximately 10:1.

**Scheme 2.14.** Synthesis of Model BODIPY 32 for measuring extinction coefficient.

![Scheme 2.14](image)
Figure 2.4 a and b show the absorption and emission peaks for the model BODIPY 32 and compound 16-avidin. There is only 13 nm difference in absorption maximum wavelength between these two (469 for 32, and 481 for 16-avidin).

Figure 2.4. a) UV Absorption and b) fluorescence: spectra for model study 32 and 16-avidin.
2.3.3 Synthesis of 31-Avidin

Sodium 2-mercaptoethanesulfonate can be used as a sulfur nucleophile to displace one chlorine of BODIPY 16 and yield water-soluble BODIPY 31 which absorbs at 569 nm and emits at 584 nm in the buffer (0.1 M lithium phosphate, pH 7.4).

Scheme 2.15. Synthesis of water soluble BODIPY 31.

Avidin can be considered as the second nucleophile to react with BODIPY 31 and then compound 31-avidin was formed after the reaction was shaken in the dark for one hour at room temperature in the buffer (0.1 M sodium bicarbonate, pH = 8.3).

Scheme 2.16. Synthesis of compound 31-avidin.
Scheme 2.16. Continued.

\[
\text{pH = 8.3 buffer} \\
25^\circ C, 1 \text{ h}
\]

2.3.4 Calculation of Dye-Protein Ratio

In order to calculate the dye-protein ratio, the model 33 was synthesized. It took 2 days to achieve the second substitution. Purification of compound 33 was really hard because of its high polarity. Only 1/5 of the desired pure product could be separated from the silica gel column. The quantum yield for this model BODIPY was measured as 0.70 in the lithium phosphate buffer (pH 7.4) by using fluorescein as standard. Extinction coefficient for the model BODIPY 33 was measured as 41810 M\(^{-1}\)cm\(^{-1}\) in the same buffer. The equation 1 was again used to calculate the dye/protein ratio. Finally, \(C_d/C_p\) was calculated to be approximately 3:1.

Scheme 2.17. Synthesis of Model BODIPY 33.
Scheme 2.17. Continued.

\[
\begin{align*}
\text{H}_2\text{O} \\
25 \degree \text{C}, 48 \text{ h}
\end{align*}
\]

\[
\begin{align*}
\text{NaO}_3\text{S} & \rightarrow \\
\text{CF}_3 & \\
\text{NaO}_3\text{S} & \\
\text{F}_2 & \\
\text{N} & \\
\text{B} & \\
\text{N} & \\
\text{NH} & \\
\text{COOH} & \\
\end{align*}
\]

33

- pH = 7.4 buffer
- Φ = 0.70 ± 0.01
- ε = 41810 M⁻¹cm⁻¹
- λ_{abs} 477 nm
- λ_{emiss} 584 nm

Figure 2.5 a and b show the absorption and emission peaks for the model BODIPY 33 and compound 31-avidin. There is a little difference in maximum wavelength between these two. Compound 32 absorbs at 477 nm and emits at 584 nm, whereas 31-avidin absorbs at 492 nm and fluoresces at 592 nm.

\[
\begin{align*}
f_{\text{abs}} & = 41810 \text{ M}^{-1}\text{cm}^{-1} \\
f_{\text{emiss}} & = 584 \text{ nm} \\
\end{align*}
\]

Figure 2.5. a) UV absorption and b) fluorescence: spectra for model study 33 and 31-avidin.
2.4 Conclusion

The easily obtained 3,5-dichloroBODIPYs can be substituted with a wide range of oxygen, nitrogen, sulfur and carbon centered nucleophiles and the reaction conditions can be adjusted to have either mono- or di-substitution. These nucleophilic addition-elimination substitution reactions of the 3,5-dichloroBODIPY core happen to be a very successful approach for preparing a variety of symmetric and asymmetric BODIPY compounds. The new more reactive dichloroBODIPYs 16 and 21 were synthesized. They have relatively red shifted absorption and emission compared with 1,3,5,7-tetramethyl BODIPY. Cyanide anion can not only substitute chlorine atoms, but also fluorine atoms. The interesting thing is compound 25 can also process the $S_N$Ar reaction, but much more unreactive, so the longer reaction time was needed.

BODIPY 16 and 31 were used to label protein successfully based on $S_N$Ar reaction. Model 32 was synthesized to prove the mono-substitution with avidin. Extinction coefficients for models were measured to calculate the dye/protein ratio. Both of model
BODIPYs gave very good quantum yields in the 0.1 M lithium phosphate buffer (pH 7.4).
CHAPTER III

THROUGH-BOND ENERGY TRANSFER CASSETTES

3.1 Introduction
Two fluorescent entities can be joined in the same molecule to give a ‘cassette’. One of them, the donor can collect radiation at the excitation wavelength and transfer energy to the second fluorescent moiety that emits at a longer wavelength. Donor and acceptor units connected by conjugated linker fragments may transfer energy via through bonds. There are two mechanisms proposed for this energy transfer: Dexter and superexchange.\(^4\) As compared to Förster energy transfer, Dexter energy transfer is a short range phenomenon and requires interaction between excited donor orbital with the orbital of the acceptor in ground state. Superexchange energy transfer can take place over a longer distance since energy is relayed through bonds connecting the donor and the acceptor.

3.2 Results and Discussion (Syntheses and Spectroscopic Studies)
Lipophilic BODIPY derivatives which emit around 520 nm potentially can be used as donors for cassettes. Those BODIPYs emitting at longer wavelength can be used as acceptors. This chapter describes two new lipophilic through-bond energy transfer cassettes.

3.2.1 Cassette 38 with Nile Red Acceptor, BODIPY Donor
The NO\(_2\)-tetramethyl BODIPY 34 was made via the procedure in the literature in an overall yield of 30%.\(^{21}\) The whole synthesis is performed in one-pot without any purification of intermediates. The quantum yield of this nitroBODIPY is very low due to d-PeT (photoinduced electron transfer). D-PeT dictates that the energy states are such that
the exited state of the fluorescent group can donate electrons to the substituent LUMO then oxidative-PeT, d-PeT, occurs (“d” for donor).

**Scheme 3.1.** Synthesis of tetramethyl NO$_2$-BODIPY 34.

Since the NO$_2$ group can’t be used to couple with any acceptor, it was modified to an NH$_2$ group in compound 35, which can be transformed to N$_3$-tetramethyl BODIPY and then used for “click” chemistry. When Pd/C and H$_2$ was used to reduce the nitro compounds 34, Formation of by-product 36 could not be avoided and found in quite significant yield if reaction time was extended. Hydrazine monohydrate was therefore tried. After 30 min heating at reflux in THF/EtOH, this gave a very clean reaction and much higher yield. The only drawback for this method is that NH$_2$NH$_2$ H$_2$O is very
explosive, so the reaction should be carried on very carefully. The NH$_2$ group of 35 didn’t significantly quench the fluorescence, and its quantum yield was determined to be much higher than the NO$_2$- BODIPY.

**Scheme 3.2.** Reduction with H$_2$ and Pd/C.

![Scheme 3.2](image)

Compound 35 can be treated with 2 M HCl and NaNO$_2$ in the mixture of DMF and H$_2$O to form the corresponding diazo-compound. BODIPYs are not stable with strong acid or base, so HCl should be relatively diluted. Then sodium azide solution was added slowly to the mixture. Gas (presumably N$_2$) was evolved and a precipitate was generated during the reaction. Purification of this precipitate gave green strongly fluorescent N$_3$-tetramethyl BODIPY 37. This azide fluoresced with a high quantum yield, 0.48 in dichloromethane (fluorescein as standard, Φ = 0.92 in ethanol).
Scheme 3.3. Synthesis of amino- and azido-BODIPYs 35 and 37.

Copper mediated azide-alkyne cycloaddition of N₃-tetramethyl BODIPY 37 with suitable alkynes were envisaged to gave through-bond energy transfer cassettes. Scheme 3.4 shows N₃-BODIPY 37 coupled with Nile Red to form the lipophilic cassette 38. The reaction was carried out in 4:1 THF/H₂O and stirred at room temperature for 24 h to gave an 82% yield of the product.
**Scheme 3.4.** Synthesis of Nile Red containing cassette 38.

![Scheme 3.4](image)

Figure 3.1 shows the absorption of cassette 38 in dichloromethane. This spectrum shows two peaks: one from the donor N$_3$-BODIPY 37 at 504 nm and the other from the acceptor, Nile Red at 549 nm. The peak from the acceptor Nile Red is broader and has the lower intensity, because of the smaller extinction coefficient than the donor 37.

When cassette 38 was excited at 504 nm (the absorption maximum of N$_3$-BODIPY 37), two emission peaks were observed: one from the donor N$_3$-BODIPY 37 at 514 nm and another one from the acceptor Nile red at 606 nm. The emission peak from the donor is much smaller than the one from the acceptor. The energy transfer for this cassette is above 90% in dichloromethane as calculated by the peak area.
Figure 3.1. a) UV absorption and b) fluorescence: spectra for cassette 38 (10⁻⁵ M in dicloromethane).
3.2.2 Cassette 41 with BODIPYs Acceptor and Donor

BODIPY 24 is a potential acceptor if its bromophenyl group can be converted to ethynyl phenyl. Thus the Sonogashira reaction was performed to give TMS-ethynyl BODIPY 39 in 76% yield following a literature procedure for a similar compound. Deprotection of TMS was achieved using potassium carbonate in methanol. The reaction time was long, but a clean product was formed. Surprisingly, the ethynyl compound 40 was stable; it was stored for several months at room temperature without significant decomposition. However BODIPY 40 has a very low quantum yield, only 0.02 in ethanol, so it is not an ideal acceptor. It can be seen that absorption and emission don’t change much when compared with 24.

Scheme 3.5. Synthesis of ethynyl-BODIPY 40.
Scheme 3.5. Continued.

Scheme 3.6 shows the N$_3$-BODIPY 37 coupled with ethynyl BODIPY 40 to give another lipophilic through-bond energy transfer cassette 41. Same conditions used for cassette 38 were employed for cassette 41. Cassette 41 might have highly desirable characteristics if only hydrolysis of the ester group could be achieved cleanly. However, cassette 41 was not as stable as cassette 38. It was easily decomposed to a non-fluorescent compound.
even when it was stored in the freezer over a period of one day. Some hydrolysis of BODIPY 40 was observed when it was treated with potassium hydroxide (1 M), but the corresponding carboxylic acid was even less stable.

**Scheme 3.6.** Synthesis of cassette 41 with BODIPY.
The absorption spectrum of cassette 41 in dichloromethane corresponded to N$_3$-BODIPY at 504 nm and ethynyl BODIPY 40 at 581 nm. The peak from the acceptor is broader and has the lower intensity than the peak from the donor (Figure 3.2).

When the cassette 41 was excited at the absorption maximum of the donor N$_3$-BODIPY 37, 504 nm, two emission peaks were observed: one from the donor at 516 nm and another one from the acceptor at 622 nm. Although the excitation peak is combined with the emission peak of the donor part, it shows that the energy transfer for cassette 41 is not very good, about 70%.

![Figure 3.2.](image)  
Figure 3.2. **a)** UV absorption and **b)** fluorescence: spectra for cassette 41 (10$^{-5}$ M in dichloromethane)
Figure 3.2. Continued.

3.3 Conclusion
Two new lipophilic through-bond energy transfer cassettes were synthesized via “click” chemistry. The cassette 38 was somewhat stable at room temperature and gave a very good energy transfer in the organic solvent. However, the cassette 41 was less stable, even at reduced temperature and did not give a good energy transfer in dichloromethane.

The cassette 38 is a starting place to develop similar water soluble through-bond energy transfer cassettes.
CHAPTER IV

WATER-SOLUBLE BODIPY DERIVATIVES AND CONCLUSION

4.1 Introduction
The core of BODIPY dyes is hydrophobic, and does not contain any functionality to attach the probes to proteins. Both these obstacles can be overcome via synthetic modifications. For instance, there are many BODIPY dyes with carboxylic acid functional groups that can be activated then linked to amino groups on proteins or DNA-derivatives. Further, such carboxylic acids can be activated using sulfonated succinimide reagents; this makes the hydrophobic dyes more water-soluble enabling them to be dissolved in aqueous media for coupling to various water-soluble biomolecules. Once hydrophobic BODIPY dyes are conjugated to biomolecules then they tend to embed into hydrophobic pockets, or even create micellular-like environments via aggregation effects. This is not always disadvantageous; indeed, variations of BODIPY fluorescence with the polarity of their immediate environment can be useful. However, in other cases it is definitely advantageous to have water-soluble BODIPY dyes that can be conjugated easily, and that will tend to exist in the aqueous environment that surrounds a biomolecule without perturbing it.

Despite the obvious practical value of water-soluble BODIPY dyes, very few have been reported in the open literature. Indeed, the sum total of synthetic procedures to obtain BODIPY dyes includes only the four sulfonated derivatives A–D and several closely related oligoethylene-glycol-containing systems, of which E is illustrative (Figure 4.1a).

A handful of sulfonated BODIPY dyes A-D were obtained from tetra-, or penta-substituted BODIPYs via treatment with chlorosulfonic acid, then neutralization with a base (NaHCO₃). Monosulfonated systems can be obtained when only one equivalent chlorosulfonic acid is used. All of these BODIPYs have high quantum yields
in polar solvents (H$_2$O, MeOH or EtOH). The UV-Vis and fluorescence spectra are virtually superimposable on those of regular simple BODIPYs in organic solvents, indicating that sulfonato group does not disturb the electronic properties of the BODIPY system.

This chapter describes several procedures for the preparation of several sulfonated, water-soluble BODIPY systems (Figure 4.1b). Mono- and di-substituted tetramethyl-BODIPYs 49 and 50 have a 4-iodo-benzene substituent at the meso-position to enable further functionalization via organometallic cross coupling reactions. The bromo compounds 52 and 53 can be similarly derivatized, but they are also potentially reactive towards nucleophiles in $S_N$Ar reactions. Compounds 43 and 46 are valuable since they can be coupled to active carbonyl groups, the azides 44 and 47 are amenable to copper-mediated cycloadditions to alkynes, and the di-sulfonate 48 can be activated and coupled to amino groups on biomolecules. Thus the end-products of this work have potential uses in many different scenarios for labeling biological molecules.

![Figure 4.1. a) Previously known water-soluble BODIPY systems; and, b) compounds prepared in this work.](image-url)
Figure 4.1. Continued.
The following sections describe the preparation of the unusual BOIDPY starting materials, the pivotal sulfonation reactions, and reactions of the sulfonated products to further transform them into useful probes. Finally, the spectral properties of the target molecules are discussed.

### 4.2 Results and Discussion (Syntheses and Spectral Studies)

The following sections describe the preparation of the unusual BOIDPY starting materials, the pivotal sulfonation reactions, and reactions of the sulfonated products to further transform them into useful probes. Finally, the spectral properties of the target molecules are discussed.

#### 4.2.1 Syntheses

Scheme 4.1 shows the mono-sulfonation on tetramethyl nitroBODIPY 34 with 1.2 equivalent chlorosulfonic acid at -40°C to afford 42 with 63 % yield. Flash chromatography on silica was needed to purify the product. Nitro group can be
functionalized to a useful functional group. When treated with hydrazine hydroxide, compound 42 can be reduced to 43 with a really high yield. Both of compounds 42 and 43 have low quantum yields in water, because nitro and amino groups quench fluorescence a lot, but when 43 is treated with the acid, the LUMO of meso substituent becomes higher, d-PeT is decreased and strong green fluorescence shows back. However, the strong fluorescence disappeared again when diazonium salt was formed after sodium nitrite was added to the acid form of 43, which can generate compound 44 when treated with sodium azide.

Scheme 4.1. Syntheses of mono-sulfonated BODIPYs 42-44 from tetramethyl NO₂-BODIPY.

\[
\begin{align*}
\text{(i) } & 1.2 \text{ eq } \text{ClSO}_3\text{H} \\
\text{CH}_2\text{Cl}_2 & -40 \, ^\circ\text{C} - 20 \, ^\circ\text{C} \\
\text{(ii) } & 1.2 \text{ eq } \text{NaHCO}_3 \\
& 42 \, 63 \% \\
\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O} & 10 \, % \text{ Pd/C} \\
\text{EtOH, reflux, 30 min} & 43 \, 92 \%
\end{align*}
\]
Scheme 4.1. Continued.

The di-sulfonated BODIPY 45 can also be obtained when 2 equivalent chlorosulfonic acid was used. Separation in this case is relatively easy because the di-sulfonic acids precipitate from the dichloromethane solution after 20 min at room temperature. The products were collected by filtration, dissolved in a small amount of aqueous NaHCO₃, evaporated to dryness, then reprecipitated from brine to give essentially pure products. No chromatography is involved, so the procedure is convenient and amenable to scale up. The BODIPY 45 can also be functionalized to amino BODIPY 46, and then azido BODIPY 47.

Scheme 4.2. Syntheses of di-sulfonated BODIPYs 45-47 from tetramethyl NO₂-BODIPY.
Scheme 4.2. Continued.

Scheme 4.3 shows one ‘click’ reaction between azidoBODIPY 47 and hexynoic acid. This reaction can be completed in 12 hours at room temperature and yield compound 48; the ligand tris-(benzyltriazolylmethyl)amine (TBTA) was needed for this reaction. Compound 48 is freely water-soluble and contains an easily accessible carboxylic acid for activation and conjugation to biomolecules.

**Scheme 4.3.** Continued.

1 eq Cu, 0.1 eq CuSO₄ 5H₂O
0.1 eq TBTA

H₂O/THF, 25°C, 12 h

Scheme 4.4 shows mono- and di-sulfonation reactions on tetramethyl iodoBODIPY under the same conditions that were used in Scheme 4.1 to give desired products 49 and 50 with good yields. These compounds can be applied on the Sonogashira reaction.

**Scheme 4.4.**

a) Mono-sulfonation; and b) di-sulfonation on tetramethyl iodoBODIPY.
Scheme 4.4. Continued.

Modified conditions for the sulfonation were not suitable for the alkyne-functionalized BODIPY.\textsuperscript{44, 45} When two equivalents of chlorosulfonic acid was added to the tetramethyl ethynylBODIPY, some orange precipitate 51 was formed just like the other di-sulfonates 45 and 50. The di-sulfonic acid 51 is not very stable in water because the ethynyl group can be easily hydrolyzed to a ketone. For that reason, compound 51 is not a particularly useful building block.

Scheme 4.5. Synthesis of di-sulfonic acid 51.

Sulfonation can also be applied on the DichloroBODIPYs under the same conditions. Scheme 4.6 shows that mono- and di-sulfonated compounds 52 and 53 were formed with good yields.
Scheme 4.6. a) Mono-sulfonation; and b) di-sulfonation on dichloroBODIPY 21.

a

\[ \text{Br} \]
\[ \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{F}_2 \\
\text{F}_2 \\
\text{N} \\
\text{B} \\
\text{N} \\
\end{array} \]
\[ \text{H} \\
\text{CH}_2 \\
\text{Cl} \\
\text{21} \]
\[ \text{Br} \]
\[ \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{F}_2 \\
\text{F}_2 \\
\text{N} \\
\text{B} \\
\text{N} \\
\end{array} \]
\[ \text{SO}_3 \text{Na} \]
\[ \text{SO}_3 \text{Na} \]
\[ \text{52} \text{ 92%} \]

(i) 1.2 eq \text{ClSO}_3\text{H} \\text{CH}_2\text{Cl}_2 \quad \text{-40 °C - 20 °C}

(ii) 1.2 eq \text{NaHCO}_3

b

\[ \text{Br} \]
\[ \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{F}_2 \\
\text{F}_2 \\
\text{N} \\
\text{B} \\
\text{N} \\
\end{array} \]
\[ \text{H} \\
\text{CH}_2 \\
\text{Cl} \\
\text{21} \]
\[ \text{Br} \]
\[ \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{F}_2 \\
\text{F}_2 \\
\text{N} \\
\text{B} \\
\text{N} \\
\end{array} \]
\[ \text{NaO}_3 \text{S} \]
\[ \text{NaO}_3 \text{S} \]
\[ \text{53} \text{ 85%} \]

(i) 2 eq \text{ClSO}_3\text{H} \\text{CH}_2\text{Cl}_2 \quad \text{-40 °C - 20 °C}

(ii) 2 eq \text{NaHCO}_3

The dichloroBODIPYs can be used not only for the S\text{N}Ar reaction, but also for the Sonagashira, Suzuki, Stille and Heck reactions.\textsuperscript{40} Compounds 52 and 53 have the bromo functional group besides the chlorines. Too many active groups will raise regioselective isomers, so compounds 57-59 were considered to be made, and the nitro group could be transformed to amino and azido groups later.

The Scheme 4.7 shows the synthetic route to nitro-dichloroBODIPY 56, which is similar to compound 21. The synthesis can be scaled up to tens of grams in an overall yield of 24%.\textsuperscript{21}
Scheme 4.7. Synthesis of NO$_2$-dichloroBODIPY 56.

\[
\text{NO}_2 + \text{CHO} \xrightarrow{\text{TFA, } 25 \degree C, 1 \text{ h}} \begin{array}{c}
\text{NO}_2 \\
\text{54 84 \%}
\end{array}
\]

\[
\text{THF, -78 \degree C, 1.5 h then } 25 \degree C, 3 \text{ h} \xrightarrow{\text{NCS}} \begin{array}{c}
\text{Cl} \\
\text{55 42 \%}
\end{array}
\]

\[
\text{CH}_2\text{Cl}_2, 25 \degree C, 1 \text{ h} \xrightarrow{\text{DDQ}} \begin{array}{c}
\text{Cl} \\
\text{56 71 \%}
\end{array}
\]

Scheme 4.8. shows a sulfonation of the relatively electron-poor BODIPY system 56 with varying equivalents of chlorosulfonic acid. A mixture of mono- 57 and di-sulfonation 58 products formed if less than 3.5 equivalents of the sulfonating agents were used, and neither of these materials precipitated from the solution; it was, however, possible to obtain the yields indicated via flash chromatography. Clean di-sulfonation was obtained when 3.5 equivalents of chlorosulfonic acid were used and, under those conditions, the product 57 precipitated in a relatively pure form and the sample could be further purified by re-precipitation from brine.
Scheme 4.8. Sulfonation on dichloroBODIPY 56 with various equivalent chlorosulfonic acid.

4.2.2 Spectroscopic Studies

Absorption and emission spectra for all the BODIPYs were recorded in deionized water. All the compounds shown in Table 4.1 have absorption maxima in the range 492 – 518 nm, and their extinction coefficients are high \((5.7 - 14.9 \times 10^4 \text{ M}^{-1}\text{cm}^{-1})\), as is characteristic of BODIPY dyes in general. All the mono-sulfonated compounds are not as soluble in water as the di-sulfonated BODIPYs, especially 57 has the worst solubility. Throughout, there are insignificant differences between the emission maxima of the
mono- and di-sulfonated forms; in fact, these differences are all less than 4 nm. DichloroBODIPYs 52, 53 and 57, 58 are more interesting because they are red-shifted for both of the absorption and emission compared with the other sulfonated tetramethyl BODIPYs. Further more, the more electronic withdrawing group in the phenyl ring makes the dichloroBODIPY shift to the longer wavelength. The absorption of 52 and 53 is 2-6 nm blue shifted compared with 57 and 58.

The sulfonated BODIPYs have the sharp emission peaks also just like the unsulfonated regular BODIPYs. The full width at half maximum height (fwhm) of compound 43 is the biggest one, which is 58 nm. Compounds 42, 46, 48, 49, 50, 52 and 53 are favorable with fwhm, which are about 25-28 nm; to calibrate, a series of water-soluble Nile Red derivatives were recently reported to have fwhm values for their fluorescence emission of between 56 – 70 nm.46

Quantum yields for the target compounds 44, 47, 48, 49, 50, 52 and 53 were all acceptably high for fluorescent probes (0.15 – 0.49). Compounds 43 and 46 have a 4-aminobenzene meso-substituent; this electron rich aromatic ring probably quenches the fluorescence of the BODIPY core via photoinduced electron transfer (PeT) in which the excited state of the BODIPY is reduced via contribution of electron density form the relatively high-lying HOMO of the meso-substituent. The low quantum yield observed is not a concern if the amine group is transformed into an amide in the bioconjugation process, because that will adjust the oxidation potential of the meso-substituent, bringing down its HOMO level, and restoring the fluorescence.
Figure 4.2. a) UV absorption, and b) fluorescence: spectra for the mono-sulfonated BODIPYs. All these spectra were recorded in deionized water at concentrations of approximately $10^{-6}$ M for the UV spectra $10^{-7}$ to $10^{-6}$ M for the fluorescence, then normalized.
Figure 4.2. Continued. c) UV absorption, and d) fluorescence: spectra for the di-sulfonated BODIPYs. All these spectra were recorded in deionized water at concentrations of approximately $10^{-6}$ M for the UV spectra $10^{-7}$ to $10^{-6}$ M for the fluorescence, then normalized.
4.2.3 Determination of Quantum Yields

Since the purpose for this work is to improve the water solubility for BODIPYs, all the relative quantum yields are measured in deionized water.

Fluorescence quantum yields measurements were performed on a Cary Eclipse Spectrofluorometer. The slit width was 5 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectrum. The following equation was used to calculate quantum yield.

\[
\Phi_x = \Phi_{st} \frac{I_x}{I_{st}} \frac{A_{st}}{A_x} \frac{\eta_x^2}{\eta_{st}^2}
\]

Where \(\Phi_{st}\) is the reported quantum yield of the standard, \(I\) is the integrated emission spectrum, \(A\) is the absorbance at the excitation wavelength and \(\eta\) is the refractive index of the solvents used. \(X\) subscript denotes unknown, and \(st\) denotes standard. Fluorescein (\(\Phi = 0.92\) in 0.1 M NaOH) and Rhodamine 6G (\(\Phi = 0.94\) in ethanol) were used as standards.\(^47\)
Table 4.1. Special characteristics of dyes in H$_2$O

<table>
<thead>
<tr>
<th>dye</th>
<th>$\lambda_{\text{abs}}^a$ (nm)</th>
<th>$\varepsilon^a$ (M$^{-1}$ cm$^{-1}$)</th>
<th>$\lambda_{\text{emi.}}^a$ (nm)</th>
<th>fwhm$^a$ (nm)</th>
<th>$\Phi^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>497</td>
<td>58130</td>
<td>513</td>
<td>48</td>
<td>$\leq 0.001^b$</td>
</tr>
<tr>
<td>43</td>
<td>492</td>
<td>85340</td>
<td>507</td>
<td>58</td>
<td>$\leq 0.001^b$</td>
</tr>
<tr>
<td>44</td>
<td>494</td>
<td>69840</td>
<td>507</td>
<td>26</td>
<td>$0.34 \pm 0.03^b$</td>
</tr>
<tr>
<td>45</td>
<td>501</td>
<td>92030</td>
<td>511</td>
<td>36</td>
<td>$\leq 0.002^b$</td>
</tr>
<tr>
<td>46</td>
<td>496</td>
<td>114820</td>
<td>511</td>
<td>25</td>
<td>$\leq 0.001^b$</td>
</tr>
<tr>
<td>47</td>
<td>498</td>
<td>77360</td>
<td>509</td>
<td>46</td>
<td>$0.15 \pm 0.01^b$</td>
</tr>
<tr>
<td>48</td>
<td>498</td>
<td>80290</td>
<td>511</td>
<td>27</td>
<td>$0.49 \pm 0.05^b$</td>
</tr>
<tr>
<td>49</td>
<td>494</td>
<td>149640</td>
<td>507</td>
<td>27</td>
<td>$0.47 \pm 0.05^b$</td>
</tr>
<tr>
<td>50</td>
<td>498</td>
<td>99640</td>
<td>509</td>
<td>27</td>
<td>$0.34 \pm 0.04^b$</td>
</tr>
<tr>
<td>52</td>
<td>509</td>
<td>73280</td>
<td>523</td>
<td>28</td>
<td>$0.27 \pm 0.03^c$</td>
</tr>
<tr>
<td>53</td>
<td>512</td>
<td>78300</td>
<td>524</td>
<td>25</td>
<td>$0.41 \pm 0.04^c$</td>
</tr>
<tr>
<td>57</td>
<td>514</td>
<td>66340</td>
<td>540</td>
<td>42</td>
<td>$\leq 0.002^c$</td>
</tr>
<tr>
<td>58</td>
<td>518</td>
<td>57000</td>
<td>538</td>
<td>35</td>
<td>$\leq 0.008^c$</td>
</tr>
</tbody>
</table>

$^a$ In H$_2$O. $^b$ Fluorescein was used as a standard ($\Phi = 0.92$ in 0.1 N NaOH$_{aq})$. $^c$ Rhodamine 6G was used as a standard ($\Phi = 0.94$ in EtOH). For each compound, it was excited at the same wavelength as standard.

4.3 Conclusion

Sulfonation reactions of BODIPY derivatives are hard to develop into useful synthetic procedures for two reasons: (i) inappropriate conditions give mixtures of products; and (ii) sulfonic acid derivatives of BODIPYs can be hard to purify. The sulfonation reactions
shown in Scheme 4.1 – 4.6 tend to give predominantly one product, and Scheme 4.8 give essentially binary mixtures that are easily separated by flash chromatography. Conjugation of the target materials to biomolecules could be achieved via amide bond formation to amines or acids, or “click” chemistry. Further, some of the dyes presented here can be derivatized via organometallic couplings to the organic halide functionalities, and, in the case of the chlorinated derivatives 52, 53, 57 and 58 via S_NAr reactions.
REFERENCES


APPENDIX A

EXPERIMENTAL DATA FOR CHAPTER II

General Experimental Procedures. All chemicals were obtained from commercial suppliers and used without further purification. Chromatography on silica gel was performed using a forced flow of the indicated solvent on EM reagents silica gel 60 (230-400 mesh). $^1$H NMR spectra were recorded at room temperature and chemical shifts are reported in ppm from the solvent resonance (CDCl$_3$ 7.24 ppm and CD$_3$OD 3.31 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), number of protons, and coupling of constants. Proton decoupled $^{13}$C NMR spectra were also reported at room temperature. Chemical shifts are reported in ppm from tetramethylsilane resonance (CDCl$_3$ 77.2 ppm and CD$_3$OD 49.0 ppm). Mass spectra were measured under ESI condition.
Concentrated HCl (4 ml) was added to a solution of pyrrole (5.8 ml, 83.5 mmol) and trifluoroacetaldehyde methyl hemiacetal (4.4 ml, 46.1 mmol) in 200 ml THF. The solution was refluxed for 2h, then 100 ml CH$_2$Cl$_2$ was added to the residue. After washing with sat. sodium bicarbonate aqueous ( 2 x 100 ml) and H$_2$O (2 x 100 ml), the combined organic layers were dried over anhydrous sodium sulfate and concentrated to dryness. The residue was then applied to a silica gel flash column using 1:1 CH$_2$Cl$_2$/hexane to afford white solid (4.34 g, 48 %), which should be stored at 0 °C. $R_f = 0.5$ (2:1 CH$_2$Cl$_2$/hexane). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.97 (br, 2H), 6.73 (m, 2H), 6.27 (br, 2H), 6.24 (m, 2H), 4.80 (q, 1H, $^3$J$_{HF}$ = 9.0 Hz).

$^1$H NMR
A solution of 14 (4.34 g, 20.3 mmol) in 150 ml dry THF was purged with N₂ and cooled to -78°C. A suspension of N-chlorosuccinimide (5.7 g, 42.6 mmol) in 80 ml THF was added to the cooled solution. The reaction mixture was stirred at -78°C for 1.5 h, then warmed to room temperature and stirred for additional 3 h. H₂O (50 ml) was added to the mixture. After extraction with CH₂Cl₂ (3 x 100 ml), the combined organic layers were dried over anhydrous Na₂SO₄, filtered, and the solution was evaporated to dryness. The residue was used for oxidation immediately without further purification.

DDQ (4.6 g, 20.3 mmol) was added to the solution of dichloro-dipyrromethane as generated above in 150 ml CH₂Cl₂. The mixture was stirred at the room temperature for 1 h. After evaporation the solvent, the residue was applied to a silica gel flash column using hexane to afford the orange powder (2.54 g, 45 % for 2 steps). R_f = 0.7 (20% EtOAc/hexane). ¹H NMR (300 MHz, CDCl₃) δ 7.19 (m, 2H), 6.35 (d, 2H, J = 4.4 Hz).
A solution of 15 (2.54 g, 9 mmol) and triethylamine (7.6 ml, 6 eq) in 150 ml dry CH$_2$Cl$_2$ was stirred at room temperature for 10 min. Boron trifluoride etherate (9 ml, 8 eq) was added slowly over 10 min. After 12 h stirring at reflux, the resulting solution was washed with water (3 x 100 ml), dried over anhydrous Na$_2$SO$_4$, filtered, and the solution was rotary evaporated. The residue was passed through a short silica gel flash column with EtOAc. After removing the solvent in vacuo, the product 3 was recrystallized from EtOAc as green crystals (3 g, 100%). $^1$H NMR (300 MHz, CDCl$_3$) δ 7.40 (d, 2H, $J$ = 4.6 Hz), 6.55 (d, 2H, $J$ = 4.6 Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 149.5, 131.9, 131.4, 124.0, 121.3, 120.3. MS (ESI) calcd for C$_{10}$H$_4$BCl$_2$F$_5$N$_2^+$ (M$^+$) 327.9765 found 327.9766; IR (thin film) 3177, 2928, 1572, 1394, 1279, 1220, 1122, 1104, 986, 773, 725 cm$^{-1}$.

$^1$H NMR
$^{13}$C NMR

Mass spectrum
Potassium carbonate (63 mg, 0.45 mmol) was added to a solution of 16 (50 mg, 0.15 mmol) and piperidine (15.7 µl, 0.15 mmol) in 5 ml acetonitrile. The mixture was stirred at room temperature for 10 min. The residue was filtered and concentrated and then was applied to a silica gel flash column using 20% EtOAc/hexane to yield an orange solid (58 mg, 100%). $R_f = 0.2$ (20% EtOAc/hexane). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.43 (m, 1H), 6.73 (m, 1H), 6.51 (d, 2H, $J = 5.6$ Hz), 6.25 (d, 2H, $J = 5.6$ Hz), 3.98 (br, 4H), 1.85-1.74 (m, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 162.3, 135.9, 134.4, 129.1, 126.0, 124.7, 122.5, 118.2, 117.5, 114.6 (q), 113.7, 53.3, 26.8, 24.2; F NMR (300 MHz, CDCl$_3$) $\delta$ 122.48 (s), 43.01 (q). MS (ESI) calcd for C$_{15}$H$_{15}$BClF$_5$N$_3^+$ (M + H)$^+$ 378.0968 found 378.1056.

$^1$H NMR
$^{13}$C NMR

$^{19}$F NMR
Mass spectrum
Potassium carbonate (50 mg, 0.36 mmol) was added to a solution of 16 (20 mg, 0.06 mmol) and piperidine (30.0 µl, 0.30 mmol) in 5 ml acetonitrile. The mixture was stirred at room temperature for 48 h. The residue was filtered and concentrated and then was applied to a silica gel flash column using 5 % EtOAc/Hexane to yield a purple solid (24 mg, 93%). $R_f = 0.4$ (20% EtOAc/hexane). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.07 (m, 2H), 6.08 (br, 2H), 3.55 (br, 8H), 1.81-1.61 (m, 12H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 160.5, 128.6, 126.5, 109.5, 52.8, 26.2, 24.5; MS (ESI) calcd for C$_{20}$H$_{24}$BF$_5$N$_4$ $^+$ (M$^+$) 426.20 found 426.21.

$^1$H NMR
$^{13}$C NMR

Mass spectrum
Pyrrole (25 eq) and 4-bromobenzaldehyde (21.4 g, 116 mmol) were added to a 500 ml dry round-bottomed flask and degassed with a stream of N₂ for 5 min. TFA (0.1 ml) was then added. The solution was stirred under N₂ at room temperature for 1 h and then excess pyrrole was removed under reduced pressure. The residue was recrystallized in the minimal hexane and dichloromethane mixture to give a white solid (11.7 g, 34%). ¹H NMR (300 MHz, CDCl₃) δ 7.93 (br, 2 H), 7.43 (d, 2H, J = 8.62 Hz), 7.11 (d, 2H, J = 8.62 Hz), 6.17 (m, 2H), 5.89 (s, 2H), 5.45 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 141.3, 132.0, 131.8, 130.3, 121.0, 117.6, 108.7, 107.6, 43.6; MS (ESI) calcd for C₁₅H₁₄BrN₂⁺ (M+H)⁺ 301.03 found 301.02.
$^1$HNMR

Pulse sequence: signal

Detector: 400 MHz

Sample temperature

Field: 400 MHz

400 MHz 3D "chemical"

Delay: 0.6 sec

Pulse 50.0 degrees

Avg. time 1.09 sec

Width 465.4 Hz

$^13$C NMR

Pulse sequence: signal

Detector: 400 MHz

Sample temperature

Field: 400 MHz

400 MHz 3D "chemical"

Delay: 0.6 sec

Pulse 50.0 degrees

Avg. time 1.09 sec

Width 465.4 Hz
A solution of 19 (7.84 g, 26 mmol) in 200 ml dry THF was purged with N₂ and cooled to -78°C. A suspension of N-chlorosuccinimide (2.2 eq) in 60 ml THF was added to the cooled solution. The reaction mixture was stirred at -78°C for 1.5 h, then warmed to room temperature and stirred for additional 3 h. H₂O (100 ml) was added to the mixture. After extraction with CH₂Cl₂ (3 x 100 ml), the combined organic layers were dried over anhydrous Na₂SO₄, filtered, and the solution was evaporated to dryness. The residue was used for oxidation immediately without further purification.

DDQ (2.8 g, 12.3 mmol) was added to the solution of dichloro-dipyrrromethane as generated above in 250 ml CH₂Cl₂. The mixture was stirred at the room temperature for 1 h. After evaporation the solvent, the residue was applied to a silica gel flash column using hexane to afford the orange powder (5.08 g, 53 % for 2 steps). R_f = 0.7 (20% EtOAc/hexane). ¹H NMR (300 MHz, CDCl₃) δ 7.63 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 6.52 (d, 2H, J = 4.3 Hz), 6.30 (d, 2H, J = 4.3 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 142.2, 138.4, 138.3, 134.5, 132.4, 131.3, 129.9, 123.9, 117.4; MS (ESI) calcd for C₁₅H₁₀BrCl₂N₂⁺ (M+H)⁺ 366.9404 found 366.9403.
$^{1}$H NMR

Pulse sequence: zspul
Solvent: cdcl3
Ambient temperature
User: baggs
File: H-bold91
INLWA-300 "Nanomix"

Pulse 90.0 degrees
Acq. time 3.744 sec
Width 4000.0 Hz
16 repetitions

Spectrum 11, 298.5796251 MHz
Data processing
FFT size 4097
Total time 1 min. 0 sec

$^{13}$C NMR

Pulse sequence: ts-pul
Solvent: cdcl3
Ambient temperature
User: baggs
File: C12-1010897
INLWA-300 "Nanomix"

Pulse 90.0 degrees
Acq. time 1.816 sec
Width 15000.7 Hz
163 repetitions

Spectrum 11, 75.414663 MHz
Decoupled H, 199.854860 MHz
Power 75 dB
Continuous on
Multi-16 modulated
Data processing
Line broadening 1.0 Hz
FF size 4097
Total time 31 min. 7 sec
A solution of 20 (4.73 g, 13 mmol) and triethylamine (2.2 eq) in 120 ml dry CH₂Cl₂ was stirred at the room temperature for 10 min. Boron trifluoride etherate (4.3 eq) was added slowly over 10 min. After 24 h stirring, the resulting solution was washed with water (3 x 100 ml), dried over anhydrous Na₂SO₄, filtered, and the solution was evaporated to dryness. The residue was passed through a short silica gel flash column with EtOAc. After removing the solvent in vacuo, the product 21 was recrystallized from hexane/dichloromethane as red crystals (5.21 g, 98 %). ¹H NMR (300 MHz, CDCl₃) δ 7.72 (d, 2H, J = 8.7 Hz), 7.41 (d, 2H, J = 8.7 Hz), 6.84 (d, 2H, J = 4.4 Hz), 6.49 (d, 2H, J = 4.4 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 145.6, 142.4, 133.6, 132.1, 131.9, 131.3, 125.8, 119.3; MS (ESI) calcd for C₁₅H₉BBrCl₂F₂N₂⁺ (M+H)⁺ 414.9838 found 414.9407. IR (thin film) 3135, 1569, 1542, 1391, 1261, 1199, 1107, 983, 728 cm⁻¹.
$^1$H NMR

Pulse Sequence: zgpol
Solvent: ncl3
Ambient temperature
Time: 20.4s
File: NMR-200

Pulse 90.0 degrees
Acq. time 0.819 s/s
NMR 4500.0 Hz
80 repetitions

$^{13}$C NMR

Pulse Sequence: zgpol
Solvent: ncl3
Ambient temperature
Time: 20.4s
File: NMR-200

Pulse 90.0 degrees
Acq. time 0.819 s/s
NMR 4500.0 Hz
80 repetitions

Data Processing
Time 20.4s
Thionyl chloride (4.22 ml, 57.9 mmol) was dropwise added to a solution of piperidine-4-carboxylic acid (5 g, 38.6 mmol) in MeOH (70 ml) and solution was heated to reflux for 12 hours. After cooling to 25 °C, the mixture was concentrated and 50 ml ether was added to solidify the salt. Dissolved the salt in 50 ml dichloromethane and washed with ammonium hydroxide (3 x 50 ml), 100 ml saturated NaCl, dried over Na₂SO₄ and the solvent was removed under reduced pressure to give a clear oil (3.2 g, 58%). ¹H NMR (300 MHz, CDCl₃) δ 3.21 (s, 3H), 2.60 (m, 2H), 2.19 (m, 2H), 1.98 (m, 1H), 1.50 (s, 1H), 1.41 (m, 2H), 1.18 (m, 2H). The compound was used without further purification and characterization.
A solution of 21 (20 mg, 0.048 mmol) and 22 (13.77 mg, 0.096 mmol) in 5 ml acetonitrile was stirred at room temperature for 10 min. The residue was concentrated and then applied to a silica gel flash column using 20 % EtOAc/hexane to yield an orange solid (24.7 mg, 98%). \( R_f = 0.2 \) (20% EtOAc/hexane). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.57 (d, 2H, \( J = 8.5 \) Hz), 7.28 (d, 2H, \( J = 8.5 \) Hz), 6.79 (d, 1H, \( J = 5.2 \) Hz), 6.30 (d, 1H, \( J = 5.2 \) Hz), 6.21 (m, 1H), 4.45 (m, 2H), 3.72 (s, 3H), 3.51 (m, 2H), 2.71 (m, 1H), 2.81-1.91 (m, 4H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 174.4, 162.2, 135.3, 133.4, 132.1, 131.6, 130.9, 129.8, 129.4, 123.5, 118.6, 114.6, 113.2, 52.1, 50.2, 40.3, 28.4; MS (ESI) calcd for \( \text{C}_{22}\text{H}_{21}\text{BBrClF}_2\text{N}_3\text{O}_2^+ \) (M+H\(^+\)) 522.06 found 522.09.
$^1$H NMR

$^{13}$C NMR
Mass spectrum
Compounds 21 (3.15 g, 7.6 mmol) and 22 (4 eq) were dissolved in dry MeCN in a dry round-bottom flask equipped with a condenser. The mixture was heated to reflux for 12 hours to produce a dark purple solution. The solvent was removed under reduced pressure and the residue was passed through a silica gel column with hexane:EtOAc (2:1) to give a purple solid (3.91 g, 82%).

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.57 (d, 2H, $J = 8.31$ Hz), 7.32 (d, 2H, $J = 6.96$ Hz), 6.50 (d, 2H, $J = 4.30$ Hz), 6.02 (br, 2H), 4.02 (br, 4H), 3.71 (s, 6H), 3.13 (br, 4H), 2.56 (br, 2H), 2.04 (m, 8H);

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 175.3, 160.5, 132.3, 131.6, 131.4, 127.8, 123.2, 107.8, 52.0, 51.1, 41.0, 28.4;

MS (ESI) calcd (M+H)$^+$ 629.1746 found 629.1741.
$^{1}H$ NMR

Pulse sequence: zgptl
Solvent: cdCl$_3$
Residual temperature: poor
Data: sage
Films: N$_2$-sublimed
1300-300 MHz "commercial"

Delay: delay 1.000 sec
Pulse 90.6 degrees
Rec. time 2.999 sec
Width 4166.6 Hz
2500 spectra
GSR/NMR: 21.1, 21.031, 144 MHz
Data processing
FF size 32768
Total time 6 min. 40 sec

$^{13}C$ NMR

[Graph showing NMR spectrum with peaks at specified ppm values]
Methylmagnesium bromide (48 μl, 0.144 mmol) in diethyl ether was added to a solution of 21 (20 mg, 0.048 mmol) in 3 ml dry THF purged with N₂. The solution was complete at room temperature only in 2 min and then quenched with ammonium chloride aqueous. The product was extracted with dichloromethane (2 x 10 ml) and washed with sodium bicarbonate (2 x 10 ml) and water (2 x 10 ml). Then the combined organic layers were dried over anhydrous sodium sulfate, concentrated and applied to a silica gel flash column using hexane to yield an orange solid (11.2 mg, 57%). R_f = 0.7 (20 % EtOAc/Hexane). ^1H NMR (300 MHz, CDCl₃) δ 7.66 (d, 2H, J = 8.4 Hz), 7.39 (d, 2H, J = 8.4 Hz), 6.71 (d, 2H, J = 4.4 Hz), 6.41 (d, 2H, J = 8.4 Hz), 0.43 (s, 6H). ^13C NMR (125 MHz, CDCl₃) δ 142.7, 142.1, 132.7, 132.1, 131.8, 128.1, 124.8, 119.1, 6.7. ^19F NMR (300 MHz, CDCl₃) showed no peaks at all; MS (ESI) calcd for C₁₀H₁₁BBrCl₂N₂⁺ (M-CH₃)⁺ 390.9576 found 390.9631.
19F NMR

Mass spectrum
A solution of 25 (11.2 mg, 0.027 mmol) and 22 (26 mg, 0.11 mmol) in 5 ml acetonitrile was stirred at room temperature for 6 h. The residue was concentrated and then applied to a silica gel flash column using 5% EtOAc/hexane to yield a red solid (7 mg, 50%). \( R_f = 0.2 \) (10% EtOAc/hexane). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.60 (d, 2H, \( J = 8.6 \text{ Hz} \)), 7.36 (d, 2H, \( J = 8.6 \text{ Hz} \)), 6.73 (d, 2H, \( J = 4.8 \text{ Hz} \)), 6.41 (d, 2H, \( J = 4.3 \text{ Hz} \)), 6.31 (d, 2H, \( J = 4.8 \text{ Hz} \)), 6.26 (d, 2H, \( J = 4.3 \text{ Hz} \)), 3.82 (m, 2H), 3.73 (s, 3H), 3.05 (m, 2H), 2.59 (m, 1H), 2.08-1.88 (m, 4H), 0.41 (s, 6H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 175.1, 163.7, 136.8, 134.9, 134.2, 132.6, 132.3, 131.5, 130.5, 123.7, 122.0, 115.3, 113.4, 52.2, 51.7, 40.8, 28.2, 9.6. \(^{19}\)F NMR (300 MHz, CDCl\(_3\)) showed no peaks at all; MS (ESI) calcd for C\(_{24}\)H\(_{27}\)BBrClN\(_3\)O\(_2\)\(^+\) (M+H\(^+\)) 514.1068 found 514.1118.
$^1$H NMR

$^{13}$C NMR
\[ ^{19}\text{F NMR} \]

Mass spectrum
Tin tetrachloride (0.1 ml) in dichloromethane was added to a solution of 21 (20 mg, 0.048 mmol) and trimethylsilyl cyanide (0.1 ml) in 2 ml dry dichloromethane. The reaction was stirred at room temperature for 2 h and then concentrated. The residue was applied to a silica gel flash column using 10% EtOAc/hexane to yield orange solid (20 mg, 100%). $R_f = 0.2$ (15% EtOAc/hexane). $^1$H NMR (300 MHz, CDCl$_3$) δ 7.72 (d, 2H, $J = 8.2$ Hz), 7.39 (d, 2H, $J = 8.2$ Hz), 6.93 (d, 2H, $J = 4.4$ Hz), 6.57 (d, 2H, $J = 4.4$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 146.8, 143.0, 132.7, 132.4, 132.2, 132.0, 130.8, 126.4, 120.1; $^{19}$F NMR (300 MHz, CDCl$_3$) δ 15.50 (q); MS (ESI) calcd for C$_{17}$H$_9$BrF$_2$N$_4$ (M+H)$^+$ 397.01 found 396.95.
Boron trifluoride etherate (0.1 ml) was added to a solution of 21 (20 mg, 0.048 mmol) and trimethylsilyl cyanide (0.1 ml) in 2 ml dry dichloromethane. The reaction was stirred at room temperature for 2 h and then concentrated. The residue was applied to a silica gel flash column using 15 % EtOAc/hexane to yield an orange solid (20 mg, 100%). $R_f = 0.1$ (15% EtOAc/hexane). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.74 (d, 2H, $J = 8.4$ Hz), 7.40 (d, 2H, $J = 8.4$ Hz), 7.03 (d, 2H, $J = 4.4$ Hz), 6.68 (d, 2H, $J = 4.4$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 146.5, 143.4, 132.6, 132.5, 132.0, 130.4, 126.9, 120.5; $^{19}$F NMR (300 MHz, CDCl$_3$) showed no peaks at all; MS (ESI) calcd for C$_{19}$H$_9$BBrN$_6$+ (M+H)$^+$ 411.02 found 411.00.

$^1$H NMR
$^{13}$C NMR

$^{19}$F NMR

Mass spectrum
Tin tetrachloride (50 µl, 6 eq) in dichloromethane was added to a solution of 23 (25 mg, 0.047 mmol) and trimethylsilyl cyanide (40 µl, 6 eq) in 2 ml dry dichloromethane. The reaction was stirred at room temperature for 10 min and then concentrated. The residue was applied to a silica gel flash column using 35 % EtOAc/hexane to yield an orange solid (23.5 mg, 93%). $R_f = 0.2$ (40% EtOAc/hexane). $^1$H NMR (300 MHz, CDCl$_3$) δ 7.63 (d, 2H, $J = 8.7$ Hz), 7.30 (d, 2H, $J = 8.7$ Hz), 6.93 (d, 1H, $J = 5.4$ Hz), 6.42 (dd, 2H, $J = 4.4$, 3.9 Hz), 6.36 (d, 1H, $J = 3.9$ Hz) 4.44 (m, 2H), 3.74 (m, 2H), 3.74 (s, 3H), 2.80 (m, 1H), 2.28-2.06 (m, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 173.9, 162.7, 135.9, 132.8, 132.4, 132.0 (2), 131.2, 129.2, 124.4, 121.3, 115.2, 114.9, 52.3, 51.7, 39.9, 28.5; $^{19}$F NMR (300 MHz, CDCl$_3$) showed no peaks at all; MS (ESI) calcd for C$_{24}$H$_{20}$BBrClN$_5$O$_2$Li$^+$ (M+Li)$^+$ 542.07 found 542.06.
\(^1\)H NMR

\(^{13}\)C NMR

\(^{19}\)F NMR
Mass spectrum
Tin tetrachloride (12 μl, 0.5 eq) in dichloromethane was added to a solution of 24 (15 mg, 0.024 mmol) and trimethylsilyl cyanide (16 μl, 5 eq) in 2 ml dry dichloromethane. The reaction was stirred at room temperature for 3 h and then concentrated. The residue was applied to a silica gel flash column using 30 % EtOAc/hexane to yield a purple solid (23.5 mg, 93%). $R_f = 0.15$ (40% EtOAc/hexane). $^1$H NMR (300 MHz, CDCl$_3$) δ 7.62 (d, 2H, $J = 8.6$ Hz), 7.33 (d, 2H, $J = 8.6$ Hz), 6.68 (d, 2H, $J = 4.6$ Hz), 6.26 (d, 2H, $J = 4.6$ Hz), 3.86 (m, 4H), 3.74 (s, 6H), 3.23 (m, 4H), 2.64 (m, 2H), 2.14 (m, 8H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 175.1, 160.9, 133.2, 132.2, 131.9, 129.2, 129.1, 124.3, 110.7, 52.2, 40.5, 28.3, 23.0; $^{19}$F NMR (300 MHz, CDCl$_3$) showed no peaks at all; MS (ESI) calcd for C$_{31}$H$_{33}$BBrN$_6$O$_4$\(^+\) (M+H)$^+$ 643.1840 found 643.1380.
$^{1}H$ NMR

$^{13}C$ NMR
$^{19}$F NMR

Mass spectrum
A solution of compound 16 (10 eq) in THF (20 μl) was added into a solution of avidin (2 mg) in 0.1 N NaHCO₃ buffer (pH = 8.3, 0.5ml). The solution was shaken in the dark for 1 h at room temperature. Then the residue was purified by PD10 desalting column.
Sodium bicarbonate (2eq) was added to a solution of sodium 2-mercaptoethanesulfonate (50 mg) and compound 16 in THF/H$_2$O (2:1, 10 ml). The mixture was stirred at room temperature for 6 h. After removal the solvents, the residue was applied to a silica gel flash column using 10 \% MeOH/CH$_2$Cl$_2$ to yield purple solid (80 mg, 58\%). $R_f$ = 0.3 (20\% MeOH/CH$_2$Cl$_2$). $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 7.62 (m, 1H), 7.19 (br, 1H), 7.12 (d, 1H, $J$ = 4.2 Hz), 6.49 (d, 1H, $J$ = 3.6 Hz), 3.64 (m, 2H) 3.24 (m, 2H); $^{19}$F NMR (300 MHz, CD$_3$OD) 139.17 (s), 46.89 (q, $J_{BF}$ = 28.2 Hz). MS (ESI) calcd for C$_{12}$H$_8$BCl$_5$N$_2$O$_3$S$_2^-$ (M-Na)$^-$ 432.97 found 432.93.

$^1$H NMR

$^{19}$F NMR
A solution of compound 31 (10 eq) in 0.1 N NaHCO₃ buffer (pH = 8.3, 20 μl) was added into a solution of avidin (2 mg) in the same buffer (0.5ml). The solution was shaken in the dark for 1 h at room temperature. Then the residue was purified by PD10 desalting column.
6-Aminohexanoic acid (24 mg) and compound 16 (30 mg) were dissolved in the co-solvent THF/H$_2$O (2:1, 5ml). The solution was stirred at room temperature for 12 h. After removal the solvents, the residue was applied to a silica gel flash column using 40% EtOAc/hexane to yield a yellow solid (28 mg, 72%). $R_f = 0.3$ (50% EtOAc/hexane). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.50 (m, 1H), 6.78 (d, 1H, $J = 5.5$ Hz), 6.66 (br, 1H), 6.19 (d, 1H, $J = 3.9$ Hz), 3.53 (t, 2H, $J = 7.1$ Hz) 2.31 (t, 2H, $J = 7.3$ Hz), 1.73-1.62 (m, 4H), 1.43 (m, 2H); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 176.3, 163.5, 135.3, 128.0, 126.4, 124.6, 122.4, 117.0, 116.9, 113.3 (q, $J = 33.4$ Hz), 112.4, 45.1, 33.7, 30.3, 25.9, 24.5;

$^1$H NMR
$^{13}$C NMR
6-Aminohexanoic acid (3 eq) was added to a solution of compound 31 (20 mg) in 5 ml water. The solution was stirred at room temperature for 48 h. After removal the solvents, the residue was applied to a silica gel flash column 10 % MeOH/CH₂Cl₂. However, it was really hard to separate the desired product, only 2 mg yield a purple solid was obtained. 

\[ R_f = 0.2 \ (20\% \ MeOH/CH₂Cl₂). \]

\[ ^1H \text{ NMR (500 MHz, CD₃OD)} \delta 7.53 \ (m, 1H), 6.87 \ (d, 1H, J = 5.5 \ Hz), 6.71 \ (br, 1H), 6.47 \ (d, 1H, J = 3.9 \ Hz), 3.58 \ (t, 2H, J = 6.51 \ Hz) 3.23 \ (m, 2H), 3.07 \ (m, 2H), 2.21 \ (t, 2H, J = 7.0 \ Hz), 1.74-1.62 \ (m, 4H), 1.44 \ (m, 2H); \text{MS (ESI) calcd for } C_{18}H_{20}BF_3N_3O_5S_2^{-} (M-Na)^{-} 528.09 \text{ found 528.02.} \]

\[ ^1H \text{ NMR} \]
APPENDIX B
EXPERIMENTAL DATA FOR CHAPTER III

**General Experimental Procedures.** All chemicals were obtained from commercial suppliers and used without further purification. Chromatography on silica gel was performed using a forced flow of the indicated solvent on EM reagents silica gel 60 (230-400 mesh). $^1$H NMR spectra were recorded at room temperature and chemical shifts are reported in ppm from the solvent resonance ($\text{CDCl}_3$ 7.24 ppm). Data are reported as follows: chemical shift, multiplicity ($s =$ singlet, $d =$ doublet, $t =$ triplet, $q =$ quartet, $br =$ broad, $m =$ multiplet), number of protons, and coupling of constants. Proton decoupled $^{13}$C NMR spectra were also reported at room temperature. Chemical shifts are reported in ppm from tetramethylsilane resonance ($\text{CDCl}_3$ 77.2 ppm). Mass spectra were measured under ESI condition.
A solution of 4-nitrobenzaldehyde (1.8 g, 12 mmol) and 2,4-dimethylpyrrole (2.46 ml, 24 mmol) in dry CH$_2$Cl$_2$ (200 ml) was purged with N$_2$ for 30 min at room temperature. 0.1 ml TFA was added to initiate the condensation. After 2 h, the resulting solution was washed with 0.1 M NaOH (2 x 100 ml) and then water (2 x 100 ml), dried over anhydrous Na$_2$SO$_4$, filtered, and the solution was rotary evaporated. The resultant product was used immediately. The product was dissolved in toluene (75 ml) and DDQ (2.7 g, 12 mmol) was added in the solution. After 20 min, triethylamine (6 ml, 43 mmol) and Boron trifluoride etherate (7 ml, 56 mmol) were added. After stirred for 1.5 h at room temperature, the mixture was washed with water (3 x 100 ml), dried over anhydrous Na$_2$SO$_4$, filtered, and the solution was evaporated to dryness. The residue was applied to a silica gel flash column. Elution with 1:1 CH$_2$Cl$_2$/hexane yielded an orange crystal (1.2 g, 30 %). $R_f = 0.7$ (2:1 CH$_2$Cl$_2$/hexane). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.39 (d, 2H, $J = 8.8$ Hz), 7.54 (d, 2H, $J = 8.8$ Hz), 6.02 (s, 2H), 2.57 (s, 6H), 1.36 (s, 6H). $^{19}$F NMR (300 MHz, CDCl$_3$) $\delta$ 30.96 (q).
\(^1\text{H NMR}\)

\[^{19}\text{F NMR}\]
A solution of 34 (500 mg, 1.36 mmol) in 1:1 THF/EtOH (50 ml) was purged with N₂ for 10 min. 10% Pd/C (144 mg, 0.14 mmol) and 1 ml hydrazine were added. The solution was stirred at reflux under N₂ for 30 min. Cooled to the room temperature and poured into 50 ml H₂O. The aqueous mixture was extracted with CH₂Cl₂ (2 x 50 ml). The combined organic layers were extract dried over anhydrous Na₂SO₄, filtered, and the solution was rotary evaporated. The residue was applied to a silica gel flash column using 20% EtOAc/hexane to afford an orange crystal (450 mg, 98%). R<sub>f</sub> = 0.2 (20% EtOAc/hexane). <sup>1</sup>H NMR (300 MHz, CDCl₃) δ 7.00 (d, 2H, J = 8.8 Hz), 6.77 (d, 2H, J = 8.8 Hz), 5.97 (s, 2H), 3.85 (br, 2H), 2.54 (s, 6H), 1.49 (s, 6H).
$^1$H NMR

STANDARD IN OBSERVE

Pulse Sequence: s1pul
Solvent: dca/d3
Ambient temperature
User: lli
File: pɛ-ɛ-product
INova-300 "nrexn2"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.995 sec
Width 4506.3 Hz
16 repetitions
OBSERVE H1, 299.9141345 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 49 sec
A solution of 34 (200 mg) in 20 ml 1:1 CH₂Cl₂/EtOH was hydrogenated over 10% Pd/C and bubbled with H₂ balloon at room temperature for 18 h. The mixture was filtered through celite and concentrated. The residue was applied to a silica gel flash column using 1:1 CH₂Cl₂/hexane to afford the orange crystals 36 (Rᵣ = 0.3) and then eluting with 2:1 CH₂Cl₂/hexane to yield 35 (Rᵣ = 0.2) (2:1 CH₂Cl₂/hexane). Longer reaction time will give higher yield. ¹H NMR (300 MHz, CDCl₃) δ 7.04 (d, 2H, J = 8.8 Hz), 6.76 (d, 2H, J = 8.8 Hz), 5.97 (s, 2H), 3.22 (q, 2H, J = 7.2 Hz), 2.54 (s, 6H), 1.49 (s, 6H), 1.31 (t, 3H, J = 8.8 Hz).
$^1$H NMR

STANDARD $^1$H OBSERVE

Pulse Sequence: a2pul
Solvent: cdc13
Ambient temperature
User: lli
File: pd-c-byproduct
INOVA-300 "nmxsun2"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions
OBSERVE H1 299.9141949 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 48 sec
A solution of 35 (30 mg, 0.09 mmol) in 2 ml DMF and 5 ml 2 M HCl was cooled to 0°C. The solution of NaNO₂ (15.3 mg, 0.22 mmol) in 2 ml H₂O was added slowly and then the mixture was kept at 0°C for 30 min. NaN₃ (29 mg, 0.45 mmol) in 2 ml H₂O was dropwise added to the mixture. Some red precipitant was formed after 1 h, filtered and dried under vacuum. The residue was applied to a silica gel flash column 5% EtOAc/hexane to afford an orange powder. (22 mg, 70%). Rₛ = 0.5 (20% EtOAc/hexane). ¹H NMR (300 MHz, CDCl₃) δ 7.29 (d, 2H, J = 8.8 Hz), 7.17 (d, 2H, J = 8.8 Hz), 6.00 (s, 2H), 2.56 (s, 6H), 1.43 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 156.0, 143.2, 141.3, 140.8, 131.8, 129.9, 121.7, 120.1, 14.9 (2); MS (ESI) calcd for C₁₉H₁₈BF₂N₅⁺ (M⁺) 365.1623 found 365.1563; IR (thin film) 2126, 2096, 1542, 1510, 1309, 1193, 1081, 980, 832, 761 cm⁻¹. ¹H NMR
$^{13}$C NMR

Mass spectrum
To a solution of 37 (10 mg) and Nile red (12 mg) in 5 ml 4:1 THF/H_2O was added Cu (4 mg) and CuSO_4 5H_2O (1 mg). The mixture was stirred at room temperature for 24 h. After filtration, the residue was concentrated and applied to a silica gel flash column chromatography using 40-50% EtOAc/hexane to afford an purple solid (16 mg, 82%). R_f = 0.15 (40% EtOAc/hexane). ^1H NMR (300 MHz, CDCl_3) δ 9.16 (d, 2H, J = 1.8 Hz), 8.57 (s, 1H), 8.41 (d, 1H, J = 8.3 Hz), 8.24 (dd, 1H, J = 8.3, 1.8 Hz), 8.07 (d, 2H, J = 8.6 Hz), 7.66 (d, 1H, J = 9.0 Hz), 7.56 (d, 2H, J = 8.6 Hz), 6.70 (dd, 1H, J = 9.0, 2.8 Hz), 6.49 (d, 1H, J = 2.8 Hz), 6.41 (s, 1H), 6.03 (s, 2H), 3.49 (q, 4H, J = 7.1 Hz), 2.59 (s, 6H), 1.49 (s, 6H), 1.29 (t, 6H, J = 7.1 Hz); ^13C NMR (75 MHz, CDCl_3) δ 183.4, 156.4, 152.5, 151.2, 148.3, 147.1, 143.0, 139.7, 139.6, 137.6, 136.0, 132.9 (2), 131.6, 131.4, 130.2, 127.2, 126.9, 125.3, 121.8 (2), 121.1, 121.0, 118.7, 110.1, 106.0, 96.5, 45.4, 15.0, 14.9, 12.8; ^19F NMR (300 MHz, CDCl_3) δ 31.01 (q); MS (ESI) calcd for C_{41}H_{37}BF_{2}N_{7}O_{2}^+ (M+H)^+ 708.3070 found 708.3074.
$^{19}$F NMR

Mass spectrum
CuI (1.17 mmol), PdCl$_2$(PPh$_3$)$_2$ (0.62 mmol), and Et$_3$N (100 mmol) were added to a solution of compound 24 (3.26 g, 5.12 mmol) in dry DMF (120 ml) into a dry sealed tube. The mixture was heated to 80 °C for 12 hours. The DMF was removed under reduced pressure. The residue was dissolved in dichloromethane (150 ml), washed with water (2 x 50 ml), saturated NaCl (50 ml), dried over Na$_2$SO$_4$, and concentrated. The residue was passed through a silica gel column with hexane:EtOAc (3:1 to 2:1) to yield a dark purple solid (2.55 g, 76%). $^1$H NMR (300 MHz, CDCl$_3$) δ 7.26 (d, 2H, $J = 7.7$ Hz), 7.12 (d, 2H, $J = 7.7$ Hz), 6.23 (d, 2H, $J = 4.10$ Hz), 5.73 (br, 2 H), 3.75 (br, 4H), 3.44 (s, 6H), 2.83 (br, 4H), 2.28 (s, 2H), 1.76 (m, 8H), 0.00 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) 175.5, 175.1, 135.5, 131.6, 130.4, 127.7, 123.7, 104.5, 95.8, 76.6, 51.8, 51.7, 51.0, 29.7, 28.1, 0.0; MS (ESI) calcd for C$_{34}$H$_{42}$BF$_2$N$_4$O$_4$Si$^+$ (M+H)$^+$ 647.3036, found 647.3048.
1H NMR

Pulse Sequence: n1pl1
Solvent: cdcl3
Ambient Temperature
User: Jogygen
File: H1-100504
INSTR: 300 "Mariner"

B000, decay 1.069 000
Pulse 90.0 degree
Avg. time 1.865 sec
Width 4160.6 Hz
14 repetitions
OBSERVED NAT. 79.08210779 MHz
DATA PROCESSING
PT size 1024
Total time 0 min, 40 sec

13C NMR

Pulse Sequence: n1pl1
Solvent: cdcl3
Ambient Temperature
User: Jogygen
File: C13-100503
INSTR: 300 "Mariner"

Pulse 60.0 degree
Avg. time 1.815 sec
Width 5000.17 Hz
5000 repetitions
OBSERVED NAT. 75.0842232 MHz
DECOUPL 50.295667 MHz
Power 55 dB
continuously on
WALTZ-16 Modulated
DATA PROCESSING
Inte broadening 1.0 Hz
PT size circle
Total time 50 min, 7 min, 90 sec
K$_2$CO$_3$ (1.1 eq) was added to a solution of compound 39 (0.12 g, 0.18 mmol) in MeOH (15 ml, not dry). The mixture was stirred at 25 °C for 48 hours. The reaction was quenched with 30 ml water, and extracted with dichloromethane (2 x 20 ml). The extracted organic layers were washed with saturated NaCl (20 ml), dried over Na$_2$SO$_4$, and concentrated. The residue was passed through a silica gel column with hexane:EtOAc (3:1 to 2:1) to give a dark purple solid (90.3 mg, 94 %). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.54 (d, 2H, $J = 7.5$ Hz), 7.39 (d, 2H, $J = 7.5$ Hz), 6.50 (d, 2H, $J = 3.8$ Hz), 5.99 (br, 2H), 4.03 (br, 4H), 3.72 (s, 6H), 3.17 (s, 1H), 3.10 (br, 4H), 2.56 (br, 2H), 2.10-1.91 (m, 8H); $^{13}$C NMR (125 MHz, CDCl$_3$) 175.3, 160.6, 136.1, 131.8, 130.8, 127.9, 122.8, 107.7, 83.3, 78.6, 52.0, 51.1, 41.0, 28.3; MS (ESI) calcd for C$_{31}$H$_{34}$BF$_2$N$_4$O$_4^+$ (M+H)$^+$ 575.2641, found 575.2655.
$^1$H NMR

$^{13}$C NMR
Cu (2 mg) and CuSO₄ 5H₂O (1 mg) was added to a solution of compound 37 (10 mg) and 40 (31 mg) in 5 ml 3:1 THF/H₂O. The mixture was stirred at room temperature for 24 h. After filtration, the residue was concentrated and applied to a silica gel column with 20 % EtOAc/hexane to give a dark purple solid (11 mg, 43 %). $R_f = 0.15$ (20 % EtOAc/hexane). $^1$H NMR (300 MHz, CDCl₃) $\delta$ 8.37 (s, 1H), 8.03 (d, 4H, $J = 8.5$ Hz), 7.54 (d, 4H, $J = 8.5$ Hz), 6.62 (br, 2H), 6.04 (s, 4H), 3.73 (s, 6H), 2.59 (s, 6H), 2.04 (br, 8H), 1.87 (m, 2H), 1.48 (s, 6H), 1.23 (br, 8H); MS (ESI) calcd for $C_{50}H_{52}B_2F_4N_9O_4^+$ (M+H)$^+$ 940.43, found 940.41.
Mass spectrum
APPENDIX C
EXPERIMENTAL DATA FOR CHAPTER IV

General Experimental Procedures. All chemicals were obtained from commercial suppliers and used without further purification. Chromatography on silica gel was performed using a forced flow of the indicated solvent on EM reagents silica gel 60 (230-400 mesh). $^1$H NMR spectra were recorded at room temperature and chemical shifts are reported in ppm from the solvent resonance (CDCl$_3$ 7.24 ppm, DMSO-$d_6$ 2.50 ppm, CD$_3$OD 3.31 ppm, D$_2$O 4.79 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), number of protons, and coupling of constants. Proton decoupled $^{13}$C NMR spectra were also reported at room temperature. Chemical shifts are reported in ppm from tetramethylsililane resonance (CDCl$_3$ 77.2 ppm, DMSO-$d_6$ 39.5 ppm, CD$_3$OD 49.1 ppm). Mass spectra were measured under ESI condition.
A solution of chlorosulfonic acid (22 µl, 0.33 mmol) in CH₂Cl₂ (2 ml) was added dropwise to a solution of BODIPY 34 (100 mg, 0.27 mmol) in CH₂Cl₂ (25 ml) over 10 min at -40 °C. Then the resulting solution was slowly warmed up to room temperature. After 20 min, TLC showed all of start material was consumed and NaHCO₃ aqueous (1.2 eq) was added to neutralize the solution and extracted the desired product from CH₂Cl₂. The aqueous layer was concentrated under rotary evaporated. The residue was applied to a silica gel flash column chromatography (dry load) using 15% MeOH/CH₂Cl₂ to afford the orange powder (80 mg, 63%). Rf = 0.4 (20% MeOH/CH₂Cl₂). 

1H NMR (500 MHz, CD₃OD) δ 8.46 (d, 2H, J = 8.8 Hz), 7.69 (d, 2H, J = 8.8 Hz), 6.22 (s, 1H), 2.77 (s, 3H), 2.54 (s, 3H), 1.66 (s, 3H), 1.42 (s, 3H); 13C NMR (125 MHz, CD₃OD) δ 161.2, 154.4, 150.0, 146.6, 142.7, 142.1, 140.8, 134.6, 133.4, 131.3, 129.8, 125.7, 124.3, 15.2, 14.9, 14.2, 13.5; MS (ESI) calcd for C₁₉H₁₇BF₂N₃NaO₃S⁻ (M-Na)⁻ 448.0950 found 447.9834; IR (thin film) 1513, 1343, 1200, 1086, 988, 806 cm⁻¹.
$^1$H NMR

$^{13}$C NMR
Mass spectrum

TOF MS: 3.363 to 0.410 min from 0.52935 to 0.816 amu
Max. 254.8 counts

[Graph showing mass spectrum]
A solution of 42 (35 mg, 0.07 mmol) in EtOH (10 ml) was purged with N₂ for 10 min. 10% Pd/C (7.9 mg, 0.1 eq) and 0.05 ml hydrazine were added. The solution was stirred at reflux under N₂ for 30 min. Cooled to the room temperature, removed Pd/C under vacuum filtration and evaporated the solvent. The residue was applied to a silica gel flash column chromatography using 15% MeOH/CH₂Cl₂ to afford the orange solid (30 mg, 92%). \( R_f = 0.3 \) (20% MeOH/CH₂Cl₂). ¹H NMR (500 MHz, CD₃OD) \( \delta \) 6.97 (d, 2H, \( J = 8.5 \) Hz), 6.86 (d, 2H, \( J = 8.5 \) Hz), 6.15 (s, 1H), 2.74 (s, 3H), 2.50 (s, 3H), 1.79 (s, 3H), 1.55 (s, 3H); ¹³C NMR (125 MHz, CD₃OD) \( \delta \) 159.5, 152.7, 150.7, 147.2, 146.5, 141.2, 134.8, 133.7, 131.1, 130.0, 124.2, 123.5, 116.6, 15.2, 14.8, 14.1, 13.4; MS (ESI) calcd for C₁₉H₁₉BF₂N₃O₃S⁻ (M-Na)⁻ 418.1208 found 418.0397; IR (thin film) 3414, 2922, 1608, 1540, 1519, 1196, 1036, 684 cm⁻¹.
$^1$H NMR

$^{13}$C NMR
Mass spectrum
A solution of 43 (29 mg, 0.07 mmol) in 2 ml H₂O and 5 ml 2 M HCl was cooled to 0°C. The solution of NaNO₂ (11.3 mg, 0.16 mmol) in 2 ml H₂O was added slowly and then the mixture was kept at 0°C for 30 min. NaN₃ (22 mg, 0.33 mmol) in 2 ml H₂O was dropwise added to the mixture. Strong green fluorescence showed up after 1 h stirring at room temperature. The solution residue was then neutralized with NaHCO₃. Decanted H₂O and the residue was applied to a silica gel flash column chromatography using 15% MeOH/CH₂Cl₂ to afford the orange solid (23 mg, 75%). R_f = 0.3 (20% MeOH/CH₂Cl₂).

¹H NMR (300 MHz, D₂O) δ 6.92 (d, 2H, J = 7.2 Hz), 6.76 (d, 2H, J = 7.2 Hz), 5.81 (s, 1H), 2.65 (s, 3H), 2.26 (s, 3H), 1.52 (s, 3H), 1.04 (s, 3H); ¹³C NMR (125 MHz, D₂O) δ 160.0, 152.0, 146.3, 142.7, 141.5, 140.2, 133.0, 131.9, 130.3, 129.3, 129.1, 123.4, 120.1, 14.4, 14.3, 13.5, 12.6; MS (ESI) calcd for C₁₉H₁₇BF₂N₅O₅S⁻ (M-Na⁻) 444.1113 found 444.0220; IR (thin film) 2128, 2105, 1541, 1304, 1192, 1023, 686 cm⁻¹.
Mass spectrum
A solution of chlorosulfonic acid (144 μl, 2.16 mmol) in CH₂Cl₂ was added dropwise to a solution of BODIPY 34 (400 mg, 1.08 mmol) in CH₂Cl₂ over 10 min at -40 °C. An orange precipitate was formed as the solution mixture warmed slowly to the room temperature. The disulfonic acid was isolated by the vacuum filtration and treated with water. The aqueous solution was neutralized with NaHCO₃ (2 eq). The solution was concentrated to 5 ml and treated with brine. The desired product was precipitated afterwards to afford an orange solid. (630 mg, quat. yield). ¹H NMR (300 MHz, D₂O) δ 8.49 (d, 2H, J = 8.5 Hz), 7.70 (d, 2H, J = 8.5 Hz), 2.77 (s, 6H), 1.63 (s, 6H); ¹³C NMR (75 MHz, D₂O) δ 156.1, 148.8, 144.0, 143.6, 140.5, 132.9, 130.2, 129.6, 125.3, 13.8, 13.0; MS (ESI) calcd for C₁₉H₁₆BF₂N₃O₈S₂₂⁻ (M-2Na)²⁻ 263.5220 found 263.4547; IR (thin film) 1522, 1347, 1190, 1004, 853, 669 cm⁻¹.
$^1$H NMR

$^{13}$C NMR
Mass spectrum
A solution of 45 (200 mg, 0.35 mmol) in EtOH (10 ml) was purged with N\textsubscript{2} for 10 min. 10\% Pd/C (37.1 mg, 0.1 eq) and 0.2 ml hydrazine were added. The solution was stirred at reflux under N\textsubscript{2} for 30 min. Cooled to the room temperature, removed Pd/C under vacuum filtration and evaporated the solvent. The residue was applied to a silica gel flash column chromatography using 30\% MeOH/\text{CH}_{2}\text{Cl}_{2} to afford the orange solid (133 mg, 70\%). \textit{R}\textsubscript{f} = 0.2 (30\% MeOH/\text{CH}_{2}\text{Cl}_{2}). \textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) \textit{\delta} 7.02-6.94 (m, 4H), 2.70 (s, 6H), 1.70 (s, 6H); \textsuperscript{13}C NMR (75 MHz, D\textsubscript{2}O) \textit{\delta} 154.7, 148.2, 144.1, 132.3, 131.2, 130.0, 123.9, 117.1, 117.0, 13.0 (2); MS (ESI) \textsubscript{C}_{19}\textsubscript{H}_{19}\textsubscript{BF}_{2}\textsubscript{N}_{3}\textsubscript{Na}_{2}\textsubscript{O}_{6}\textsubscript{S}_{2}^{+}(M+H)^{+} 544.0572 found 544.0557; IR (thin film) 3346, 2854, 1608, 1519, 1197, 1032, 655 cm\textsuperscript{-1}.

\textsuperscript{1}H NMR
$^{13}$C NMR

Mass spectrum
A solution of 46 (100 mg, 0.18 mmol) in 5 ml H$_2$O and 20 ml 2 M HCl was cooled to 0°C. The solution of NaNO$_2$ (32 mg, 0.46 mmol) in 3 ml H$_2$O was added slowly and then the mixture was kept at 0°C for 30 min. NaN$_3$ (60 mg, 0.92 mmol) in 3 ml H$_2$O was dropwise added to the mixture. Strong green fluorescence showed up after 1 h stirring at room temperature. The solution residue was then neutralized with NaHCO$_3$. Decanted H$_2$O and the residue was applied to a silica gel flash column chromatography using 30% MeOH/CH$_2$Cl$_2$ to afford the orange solid (88 mg, 77%). $R_f = 0.2$ (30% MeOH/CH$_2$Cl$_2$).

$^1$H NMR (300 MHz, D$_2$O) $\delta$ 7.33-7.26 (m, 4H), 2.75 (s, 6H), 1.67 (s, 6H); $^1^3$C NMR (75 MHz, D$_2$O) $\delta$ 155.5, 146.2, 143.9, 142.0, 132.7, 130.9, 129.9, 129.5, 120.5, 13.7, 13.0;

MS (ESI) calcd for C$_{19}$H$_{17}$BF$_2$IN$_2$O$_6$S$_2$\(^-\) (M-2Na+H)$^-$ 608.9634 found 608.9776; IR (thin film) 2130, 1549, 1295, 1038, 667 cm$^{-1}$.

$^1$H NMR
\(^{13}\text{C} \text{NMR}\)

Mass spectrum
To a solution of 47 (40 mg) and hexynoic acid (2 eq) in 5 ml 1:1 THF/H₂O was added Cu (1 eq), CuSO₄•H₂O (0.1 eq) and TBTA (0.1 eq). The reaction was stirred at room temperature for 24 h and the solvent was removed in vacuo. The residue was applied to a silica gel flash column chromatography using 40% MeOH/CH₂Cl₂ to afford the orange solid (20 mg, 42%). \( R_f = 0.1 \) (30% MeOH/CH₂Cl₂). \(^1\)H NMR (500 MHz, D₂O) \( \delta \) 8.30 (s, 1H), 8.00 (d, 2H, \( J = 8.3 \) Hz), 7.49 (d, 2H, \( J = 8.3 \) Hz), 2.75 (br, 8H), 2.33 (t, 2H, \( J = 7.2 \) Hz), 1.95 (m, 2H), 1.58 (s, 6H); \(^{13}\)C NMR (125 MHz, D₂O) \( \delta \) 180.0, 155.8, 145.1, 143.8, 137.7, 134.3, 132.9, 130.6, 129.8, 122.5, 122.0, 121.7, 39.7, 24.6, 24.2, 13.8, 13.1; MS (ESI) calcd for C₂₅H₂₄BF₂N₅O₈S₂⁻ (M-2Na)⁻ 317.5564 found 317.4960.
$^{1}H$ NMR

$^{13}C$ NMR
Mass spectrum
A solution of chlorosulfonic acid (18 µl, 0.27 mmol) in CH₂Cl₂ (2 ml) was added dropwise to a solution of tetramethyl iodoBODIPY (100 mg, 0.22 mmol) in CH₂Cl₂ (25 ml) over 10 min at -40 °C. Then the resulting solution was slowly warmed up to room temperature. After 20 min, TLC showed all of start material was consumed and NaHCO₃ aqueous (1.2 eq) was added to neutralize the solution and extracted the desired product from CH₂Cl₂. The aqueous layer was concentrated under rotary evaporated. The residue was applied to a silica gel flash column chromatography (dry load) using 15% MeOH/CH₂Cl₂ to afford the orange powder (74 mg, 60%). Rᶠ = 0.4 (20% MeOH/CH₂Cl₂).

¹H NMR (500 MHz, CD₃OD) δ 7.95 (d, 2H, J = 8.3 Hz), 7.14 (d, 2H, J = 8.3 Hz), 6.19 (s, 1H), 2.75 (s, 3H), 2.52 (s, 3H), 1.70 (s, 3H), 1.46 (s, 3H); ¹³C NMR (125 MHz, CD₃OD) δ 159.3, 152.7, 145.7, 142.3, 139.8, 138.8, 134.4, 133.2, 132.7, 130.2, 129.0, 122.8, 94.8, 14.0, 13.6, 13.0, 12.2; MS (ESI) calcd for C₁₉H₁₇BF₂IN₂O₃S⁻ (M-Na)⁻ 529.0066 found 528.8784; IR (thin film) 2922, 1717, 1540, 1312, 1193, 1033, 1006, 678 cm⁻¹.

¹H NMR
$^{13}$C NMR

Mass spectrum
A solution of chlorosulfonic acid (16 µl, 0.236 mmol) in CH₂Cl₂ was added dropwise to a solution of tetramethyl iodoBODIPY (53 mg, 0.118 mmol) in CH₂Cl₂ over 10 min at -40 °C. An orange precipitate was formed as the solution mixture warmed slowly to the room temperature. The disulfonic acid was isolated by the vacuum filtration and treated with water. The aqueous solution was neutralized with NaHCO₃ (2 eq). The solution was concentrated to 5 ml and treated with brine. The desired product was precipitated afterwards to afford an orange solid (68 mg, 88%). ¹H NMR (500 MHz, D₂O) δ 7.84 (d, 2H, J = 8.0 Hz), 6.97 (d, 2H, J = 8.0 Hz), 2.57 (s, 6H), 1.49 (s, 6H); ¹³C NMR (75 MHz, D₂O) δ 155.5, 145.7, 144.0, 139.2, 133.1, 132.7, 130.6, 129.7, 95.7, 13.7, 13.0; MS (ESI) calcd for C₁₉H₁₇BF₂IN₆O₆S₂⁻ (M-2Na+H)⁻ 608.9634 found 608.9776.
$^1$H NMR

$^{13}$C NMR
Mass spectrum
A solution of chlorosulfonic acid (19 μl, 0.276 mmol) in CH₂Cl₂ was added dropwise to a solution of tetramethyl ethynylBODIPY (48 mg, 0.138 mmol) in CH₂Cl₂ over 10 min at -40 °C. An orange precipitate was formed as the solution mixture warmed slowly to the room temperature. The disulfonic acid was isolated by the vacuum filtration to afford an orange solid (42 mg, 60%). ¹H NMR (300 MHz, D₂O) δ 7.66 (d, 2H, J = 8.8 Hz), 7.29 (d, 2H, J = 8.5 Hz), 3.48 (s, 1H), 2.63 (s, 6H), 1.54 (s, 6H); MS (ESI) calcd for C₂₁H₁₅BF₂N₂O₆S₂ (M-H)⁻ 507.0667 found 507.0815.
$^1$H NMR

Mass spectrum
A solution of chlorosulfonic acid (19.2 μl, 0.29 mmol) in CH$_2$Cl$_2$ (2 ml) was added dropwise to a solution of BODIPY 21 (100 mg, 0.24 mmol) in CH$_2$Cl$_2$ (25 ml) over 10 min at -40 °C. Then the resulting solution was slowly warmed up to room temperature. After 20 min, TLC showed all of start material was consumed and NaHCO$_3$ aqueous (1.2 eq) was added to neutralize the solution and extracted the desired product from CH$_2$Cl$_2$. The aqueous layer was concentrated under rotary evaporated. The residue was applied to a silica gel flash column chromatography (dry load) using 15% MeOH/CH$_2$Cl$_2$ to afford the orange powder (114 mg, 92%). $R_f = 0.4$ (20% MeOH/CH$_2$Cl$_2$). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.78 (d, 2H, $J = 8.5$ Hz), 7.51 (d, 2H, $J = 8.5$ Hz), 7.10 (s, 1H), 7.08 (s, 1H), 6.67 (d, 1H, $J = 4.63$ Hz); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 148.9, 145.5, 141.4, 136.3, 135.7, 134.7, 133.5, 133.2, 132.1, 131.6, 130.0, 127.0, 121.7; MS (ESI) calcd for C$_{15}$H$_7$BBrCl$_2$F$_2$N$_2$O$_3$S$^-$ (M-Na)$^-$ 492.8799 found 492.7563; IR (thin film) 1572, 1379, 1259, 1198, 1119, 1055, 667 cm$^{-1}$.

$^1$H NMR
$^{13}$C NMR

Mass spectrum
A solution of chlorosulfonic acid (160 µl, 2.4 mmol) in CH₂Cl₂ was added dropwise to a solution of BODIPY 21 (500 mg, 1.2 mmol) in CH₂Cl₂ over 10 min at -40 °C. An orange precipitate was formed as the solution mixture warmed slowly to the room temperature. The disulfonic acid was isolated by the vacuum filtration and treated with water. The aqueous solution was neutralized with NaHCO₃ (2 eq). The solution was concentrated to 5 ml and treated with brine. The desired product was precipitated afterwards to afford an orange solid (624 mg, 85%).

$^1$H NMR (500 MHz, D₂O) δ 7.73 (d, 2H, $J = 8.4$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 7.27 (s, 2H); $^{13}$C NMR (75 MHz, D₂O) δ 147.6, 141.9, 133.7, 132.6, 132.3, 131.8, 131.5, 130.0, 126.7; MS (ESI) calcd for C₁₅H₆BrCl₂F₂N₂Na₂O₆S₂⁻ (M-2Na)⁻ 285.9135 found 285.8405; IR (thin film) 2968, 1572, 1382, 1206, 1033, 650 cm⁻¹.
$^{1}H$ NMR

$^{13}C$ NMR
Mass spectrum
Pyrrole (25 ml, 370 mmol) and 4-nitrobenzaldehyde (2.23 g, 14.8 mmol) were added to a dry round-bottomed flask and degassed with a stream of N₂ for 5 min. TFA (0.1 ml) was then added, and the solution was stirred under N₂ at room temperature for 1 h and then quenched with 0.1 M NaOH. Ethyl acetate was then added. The organic phase was washed with water (3 x 50 ml) and dried over anhydrous Na₂SO₄, filtered, and the solution was rotary evaporated. The crude (95% pure) product 54 was solidified from EtOAc/hexane as the green powder (3.3 g, 84 % crude yield). It was used to synthesize 55 directly without any further purification.
$^1$H NMR

$^{13}$C NMR
A solution of **55** (3.3 g, 12.3 mmol) in 100 ml dry THF was purged with N₂ and cooled to -78°C. To the cooled solution, a suspension of N-chlorosuccinimide (3.5 g, 25.9 mmol) in 40 ml THF was added. The reaction mixture was stirred at -78°C for 1.5 h, then warmed up to the room temperature and stirred for additional 3 h. 50 ml H₂O was added to the mixture. After extraction with CH₂Cl₂ (3 x 100 ml), the combined organic layers were dried over anhydrous Na₂SO₄, filtered, and the solution was rotary evaporated. The residue was used for oxidation immediately without further purification.

DDQ (2.8 g, 12.3 mmol) was added to the solution of dichloro-dipyrromethane in 150 ml CH₂Cl₂. The mixture was stirred at the room temperature for 1 h. After evaporation the solvent, the residue was applied to a silica gel flash column chromatography using 20% EtOAc/Hexane to afford the orange powder (1.7 g, 42 % for 2 steps). \( R_f = 0.7 \) (20% EtOAc/hexane). \(^1\)H NMR (300 MHz, CDCl₃) \( \delta 8.34 \) (d, 2H, \( J = 8.8 \) Hz), \( 7.63 \) (d, 2H, \( J = 8.8 \) Hz), \( 6.43 \) (d, 2H, \( J = 4.3 \) Hz), \( 6.30 \) (d, 2H, \( J = 4.3 \) Hz), 1.56 (br, 1H); \(^{13}\)C NMR (75 MHz, CDCl₃) \( \delta 148.5, 143.1, 142.1, 138.1, 136.7, 131.8, 129.7, 123.3, 118.0. \)

\(^1\)H NMR
$^{13}$C NMR

Mass spectrum
Compound 56 (100 mg, 0.26 mmol) and chlorosulfonic acid (21 µl, 0.31 mmol) were reacted by the general procedure. However, the crude product didn’t precipitate from the solution. The residue was treated with 10 ml water after dichloromethane was evaporated. Then the solution was neutralized with NaHCO₃ (66 mg, 0.78 mmol). After evaporation the solvent, the residue was applied to a silica gel flash column chromatography (dry load) using 10% MeOH/CH₂Cl₂ to afford the monosulfonated sodium salt 57 as the orange powder (115 mg, 90 %). \( R_f = 0.7 \) (20% MeOH/CH₂Cl₂). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \( \delta \) 8.42 (d, 2H, \( J = 8.7 \) Hz), 7.93 (d, 2H, \( J = 8.8 \) Hz), 7.09 (d, 1H, \( J = 4.5 \) Hz), 6.84 (d, 1H, \( J = 4.5 \) Hz), 6.82 (s, 1H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \( \delta \) 149.6, 145.5, 142.6, 140.1, 138.2, 134.1, 133.4, 132.7, 132.3, 130.8, 130.0, 124.5, 120.8; MS (ESI) calcd for \( C_{15}H_7BCl_2F_2N_3O_5S^- \) (M-Na) 459.9545 found 459.8544; IR (thin film) 2982, 1558, 1390, 1348, 1197, 1030, 667 cm\(^{-1}\).
$^1$H NMR

$^{13}$C NMR
Mass spectrum
A solution of chlorosulfonic acid (61 µl, 0.91 mmol) in CH₂Cl₂ was added dropwise to a solution of BODIPY 56 (100 mg, 0.26 mmol) in CH₂Cl₂ over 10 min at -40 °C. An orange precipitate was formed as the solution mixture warmed slowly to the room temperature. The disulfonic acid was isolated by the vacuum filtration and treated with water. The aqueous solution was neutralized with NaHCO₃ (3.5 eq). The solution was concentrated to 5 ml and treated with brine. The desired product was precipitated afterwards to afford an orange solid (151 mg, 97 %). R_f = 0.1 (20% MeOH/CH₂Cl₂). ^1H NMR (300 MHz, D₂O) δ 8.30 (d, 2H, J = 7.5 Hz), 7.70 (d, 2H, J = 7.5 Hz), 7.18 (s, 2H); ^13C NMR (75 MHz, D₂O) δ 149.4, 145.2, 143.1, 136.8, 134.5, 132.0, 131.3, 124.1; MS (ESI) calcd for C₁₅H₆BCl₂F₂N₃O₈S₂²⁻ (M-2Na)²⁻ 269.4515 found 269.3838; IR (thin film) 3113, 1519, 1379, 1348, 1200, 1030, 848, 692, 680, 664 cm⁻¹.

^1H NMR
$^{13}$C NMR

Mass spectrum
Sodium bicarbonate (3 eq) was added to a solution of compounds 22 (2.1 mg) and 53 (10 mg) in D$_2$O (2 ml). The solution changed color to dark red immediately. $^1$H NMR (300 MHz, D$_2$O) δ 7.55 (d, 2H, $J = 8.4$ Hz), 7.27 (s, 1H), 7.25 (d, 2H, $J = 8.4$ Hz), 6.54 (s, 1H), 4.47 (m, 2H), 3.36 (m, 2H), 2.81 (m, 1H), 1.93-1.71 (m, 4H).

$^1$H NMR
Another 3 eq of sodium bicarbonate and 1 eq compound 22 (2.1 mg) was added to a solution of 59 in D$_2$O. The mixture was stirred at room temperature for 24 h. $^1$H NMR (300 MHz, D$_2$O) δ 7.67 (d, 2H, $J = 8.3$ Hz), 7.38 (d, 2H, $J = 8.3$ Hz), 7.01 (s, 2H), 3.90 (m, 4H), 3.33 (m, 4H), 2.93 (m, 2H), 2.05-1.75 (m, 8H); $^{13}$C NMR (125 MHz, D$_2$O) δ 185.3, 164.7, 157.1, 134.6, 132.5, 131.9, 130.5, 128.0, 127.9, 124.3, 51.6, 44.2, 29.4.

$^1$H NMR
$^{13}\text{C}\text{ NMR}$
VITA

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