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

I would like to thank Professor Kevin Burgess for his support and advice through out this project. I am very thankful to him for all the help and for providing a conducive environment for carrying out independent research. Thanks to Professors Coran M H Watanabe, and Gregory D. Reinhart for serving on my graduate advisory committee. Thanks to Jing Liu for her valuable help in analytical reverse phase HPLC and all others in the Burgess group for their friendship. Thanks to Jill Rutledge for assistance with plenty of office related work. Thanks to Lauren Kulpa for help with Endnote and reference collection.

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LIST OF ABBREVIATIONS

DCM	dichloromethane
INP	isonipecotic acid
DMF	*N, N*-dimethylformamide
EtOAc	ethyl acetate
EtOH	ethanol
HCl	hydrochloric acid
MeOH	methanol
Et_3N	triethylamine
THF	tetrahydrofuran

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]

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:

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

Where Q_D = quantum yield of donor in the absence of the acceptor κ^2 = orientation factor (range from 0 to 4, usually assumed to be 0.67 for dynamic random averaging). κ^2 = 4 if the transition dipoles of the donor and acceptor are perfectly parallel and 0 when they are orthogonal. τ_D = lifetime of donor in the absence of the acceptor. N = 6.02 x 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

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad \text{.....(ii)}$$

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 λ. 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

$$R_0 = \left[\frac{9000(\ln 10)Q_D\kappa_2}{128\pi_5 Nn^4}\right]\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad \text{.....(iii)}$$

R_0 is typically in the range of 20 to 60 A^o 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 retrons 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.

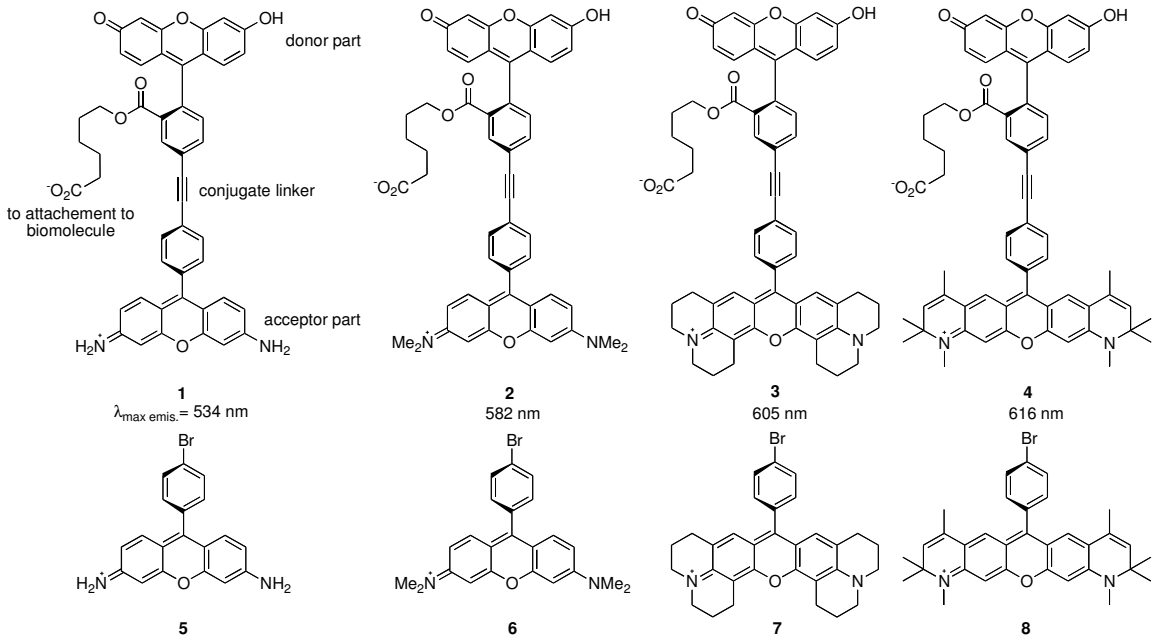


Figure 1.1. The first generation through-bond energy transfer cassettes and acceptor synthons.

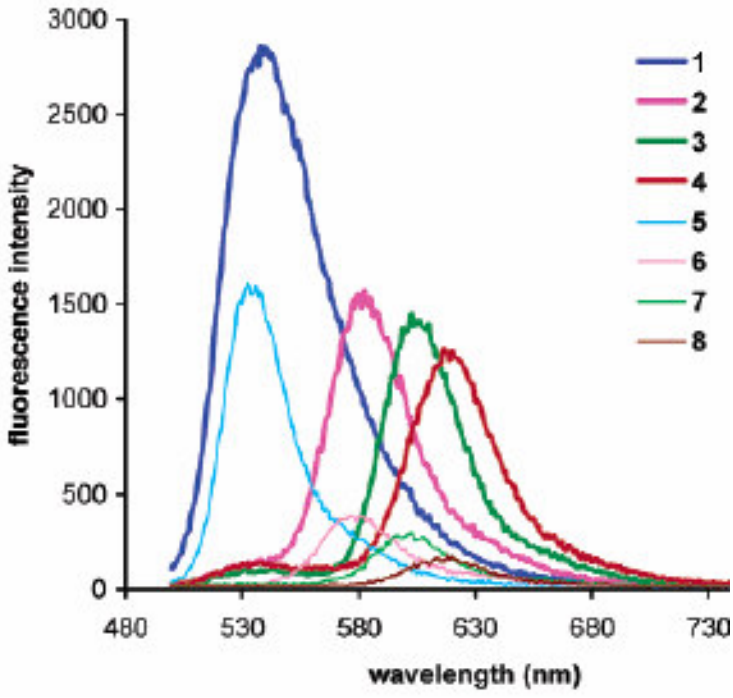


Figure 1.2. Fluorescence of equimolar EtOH solutions of **1-8** excited at 488 nm.

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**.

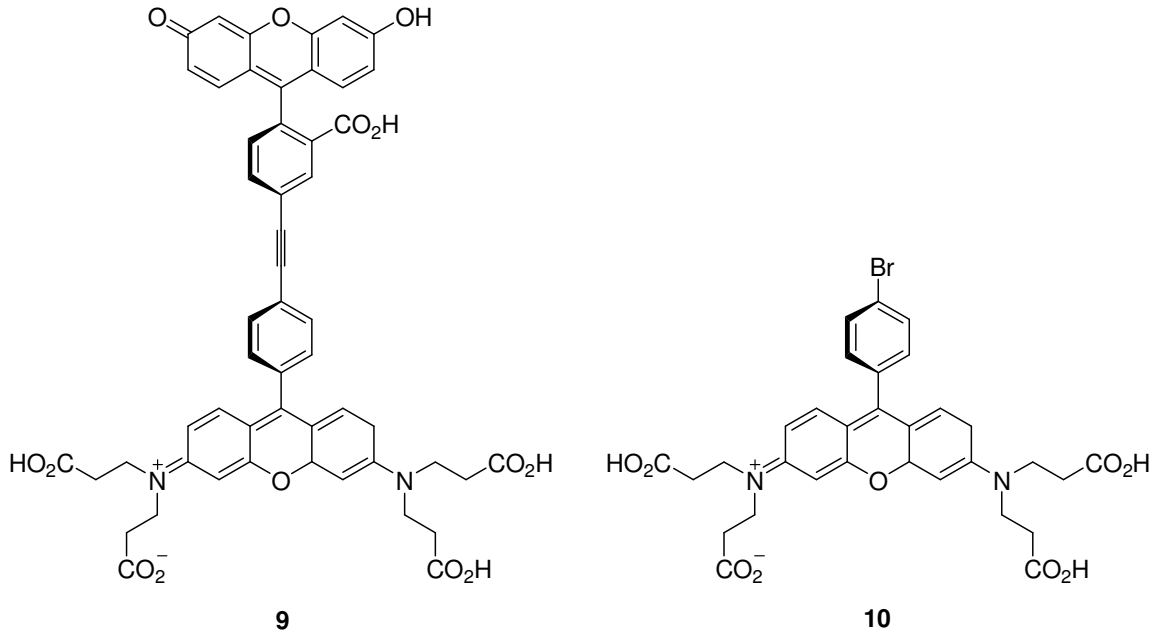


Figure 1.3. The second generation through-bond energy transfer cassette **9** and acceptor synthon.

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.

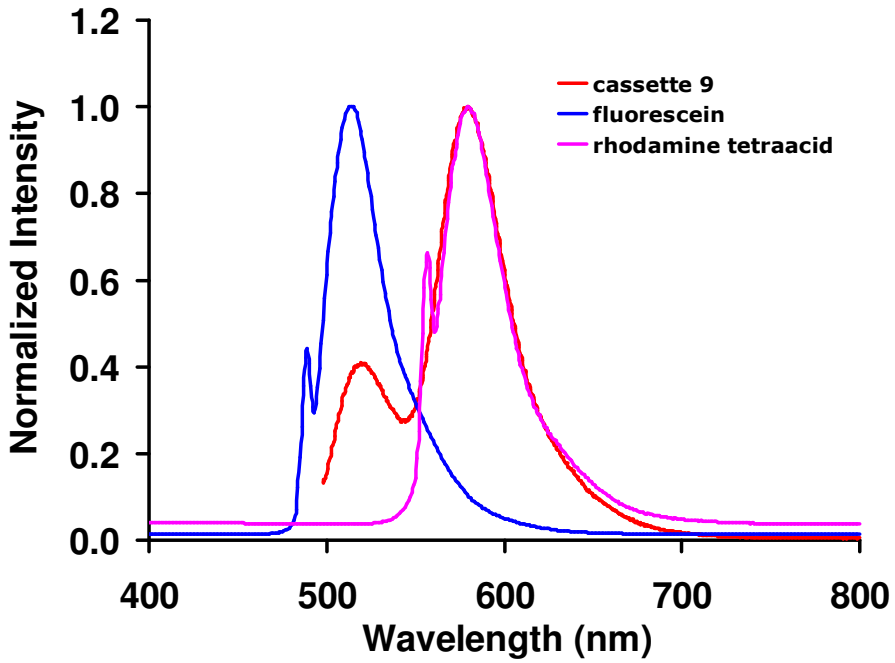


Figure 1.4. Comparison of the fluorescence peak of cassette **9** in pH 8 phosphate buffer with fluorescein and a tetraacid rhodamine derivative **10** under the same conditions.

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 BODPYs 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.

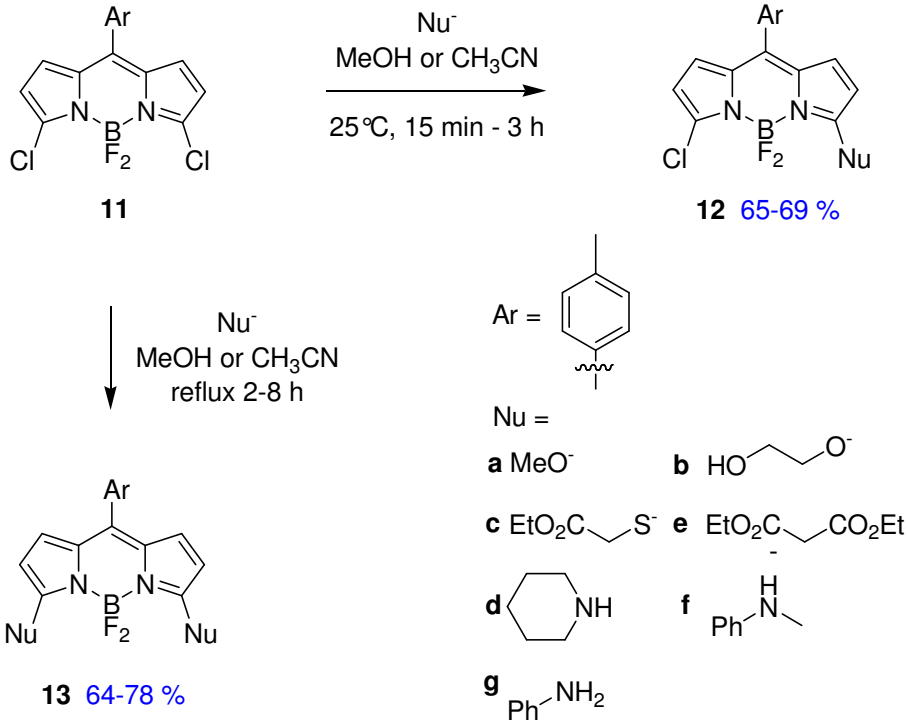
CHAPTER II

LIPOPHILIC BODIPY DERIVATIVES

2.1 S_NAr 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.

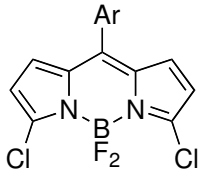
Several nitrogen-centered nucleophiles were tried also. Piperidine gave mono-substitution of **11** without additional base at room temperature yielding **12d**. Again, heating at reflux temperature (in acetonitrile) with excess amine lead to the disubstituted BODIPY derivative **13d**. The primary amine,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

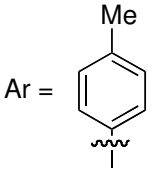
Diethyl malonate with sodium hydride as base was used as a carbon nucleophile to afford either mono- or di-substituted BODIPY derivatives,

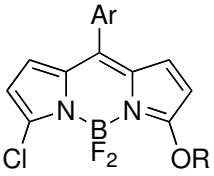
The optical data are given for the S_NAr reaction products (Figure 2.1). Compound **11** absorbs at 508 nm in methanol and fluoresces at 519 nm. The quantum yield is 0.27. The mono- and di-substituted BODIPYs with alkoxides and secondary amine gave very low quantum yields in methanol and cyclohexane. 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.

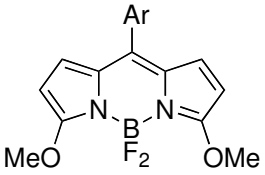


3,5-dichloroBODIPY
in MeOH; Φ 0.27
$\lambda_{max\ abs}$ 508 nm
$\lambda_{max\ emiss}$ 519 nm

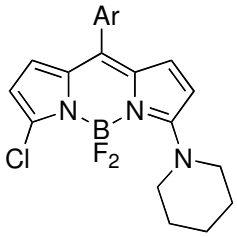




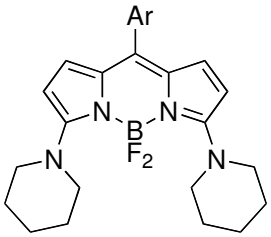
in MeOH; Φ 0.062-0.083
$\lambda_{max\ abs}$ 500 nm
$\lambda_{max\ emiss}$ 515 nm



in MeOH; Φ 0.20
$\lambda_{max\ abs}$ 510 nm
$\lambda_{max\ emiss}$ 523 nm

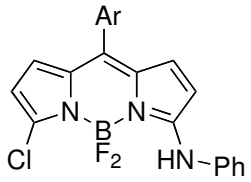


in MeOH; Φ 0.002
$\lambda_{max\ abs}$ 479 nm
$\lambda_{max\ emiss}$ 562 nm

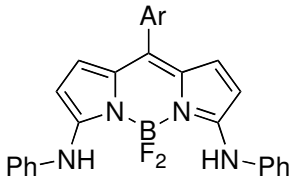


in MeOH; Φ 0.011
$\lambda_{max\ abs}$ 572 nm
$\lambda_{max\ emiss}$ 612 nm

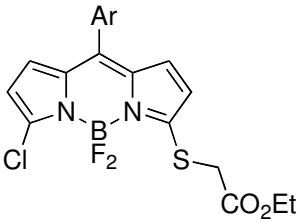
Figure 2.1. Spectroscopic data for some BODIPYs formed by S_NAr reactions.



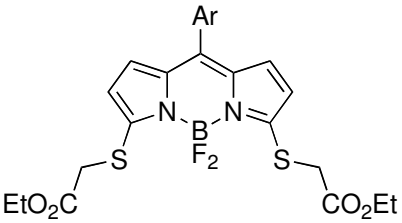
in MeOH; Φ 0.003
$\lambda_{max\ abs}$ 498 nm
$\lambda_{max\ emiss}$ 566 nm



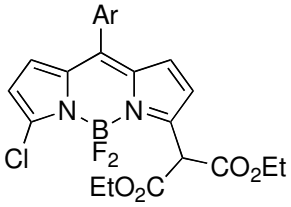
in MeOH; Φ 0.45
$\lambda_{max\ abs}$ 588 nm
$\lambda_{max\ emiss}$ 613 nm



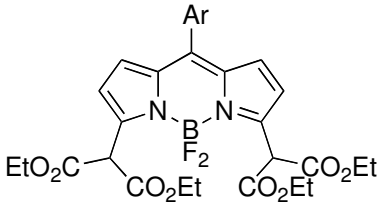
in MeOH; Φ 0.26
$\lambda_{max\ abs}$ 536 nm
$\lambda_{max\ emiss}$ 550 nm



in MeOH; Φ 0.50
$\lambda_{max\ abs}$ 564 nm
$\lambda_{max\ emiss}$ 579 nm



in MeOH; Φ 0.28
$\lambda_{max\ abs}$ 508 nm
$\lambda_{max\ emiss}$ 522 nm



in MeOH; Φ 0.35
$\lambda_{max\ abs}$ 509 nm
$\lambda_{max\ emiss}$ 522 nm

Figure 2.1. Continued.

2.2 Results and Discussion

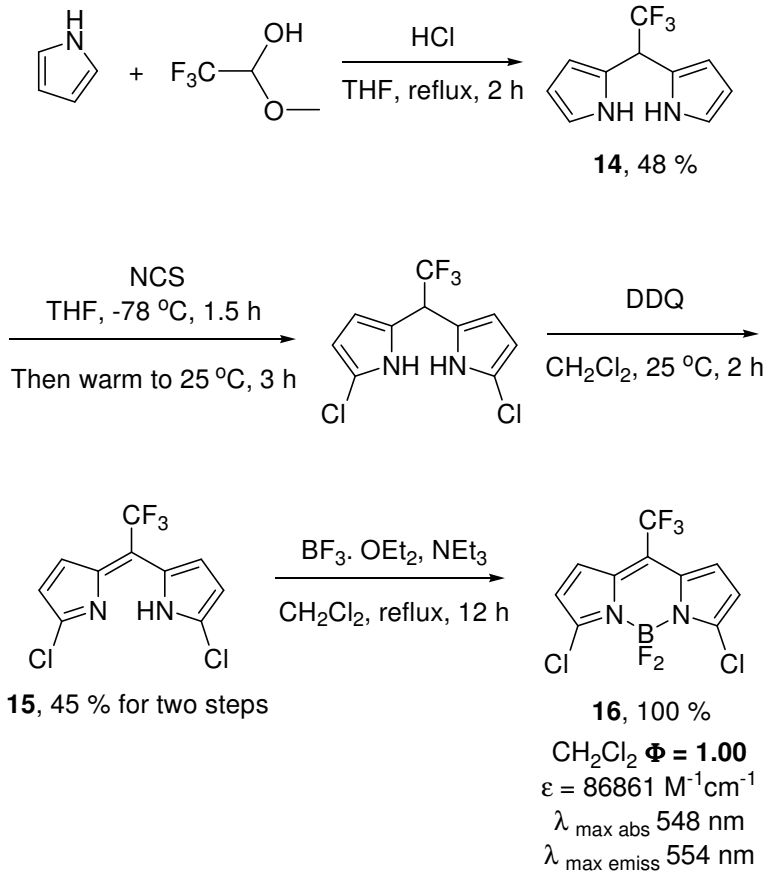
New dichloroBODIPYs were synthesized in our group which showed increased reactivity toward S_NAr reactions. Trifluoromethyl or bromophenyl groups were used for the *meso*-sub in the new BODIPYs.

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**,[8] which was followed by

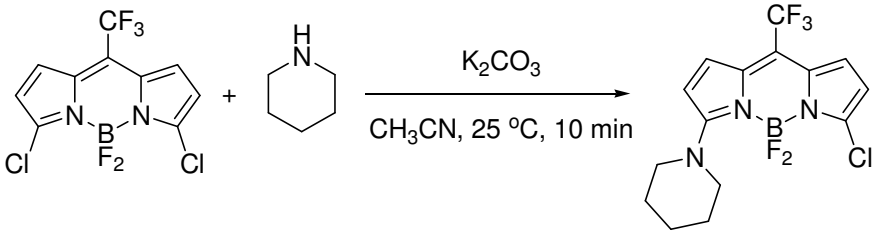
chlorination[9] using N-chlorosuccinimide reagent in THF at -78 ^{o}C to form CF_3-dichloro dipyrromethane. Then DDQ was used to oxidize dipyrromethane to CF_3-dichloro dipyrromethene **15**, which was then chelated with BF_2 at reflux temperature (in CH_2Cl_2) to give the target CF_3-dichloro BODIPY **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.[10, 11] 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 CF_3-dichloroBODIPY **16**.



S_NAr reactions can be processed easily on compound **16** with really good yields; it may be due to the strong electron withdrawing group CF_3.

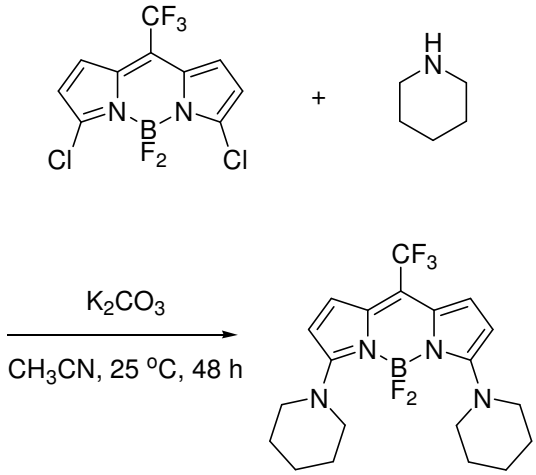
Scheme 2.3. Mono-substitution of compound **16**.



17, 100 %

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**.

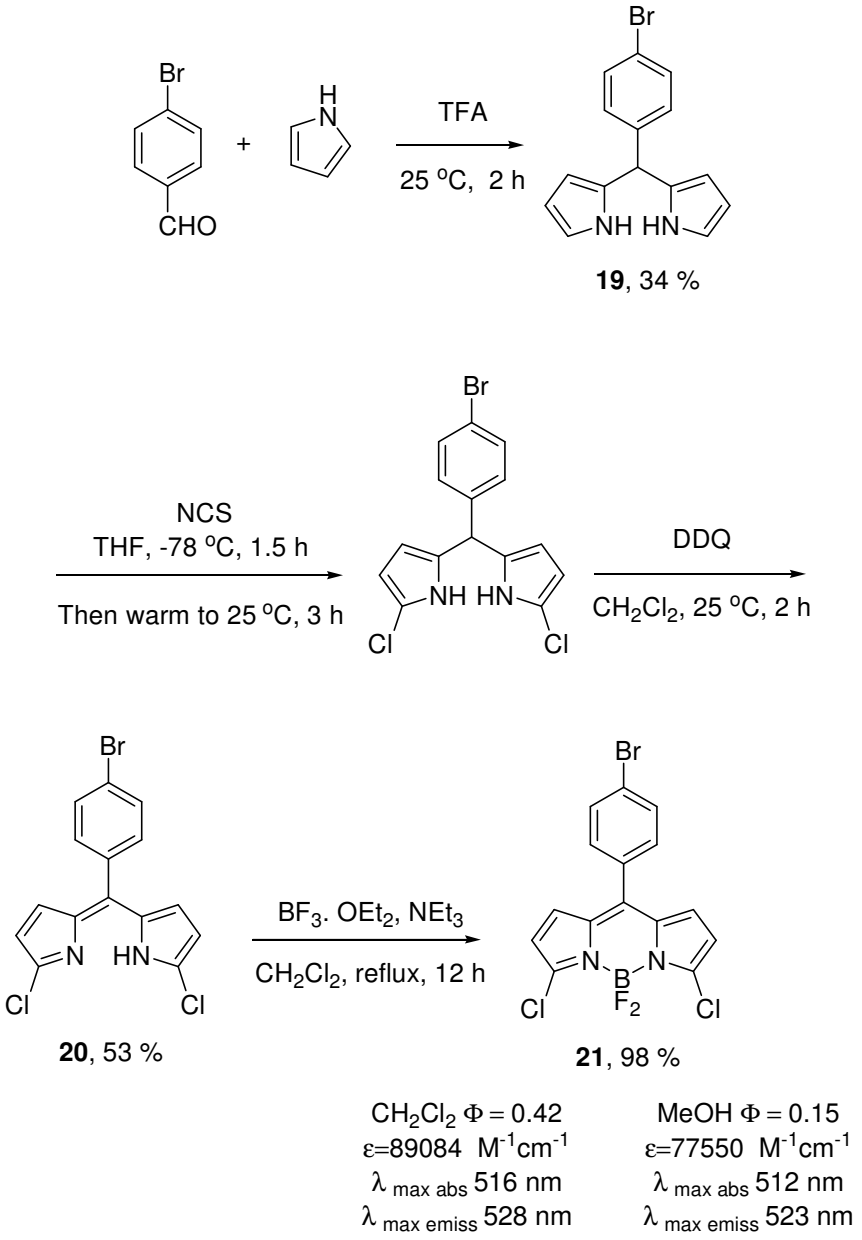


18, 93 %

2.2.2 Syntheses of Br-DichloroBODIPY and Its Derivatives

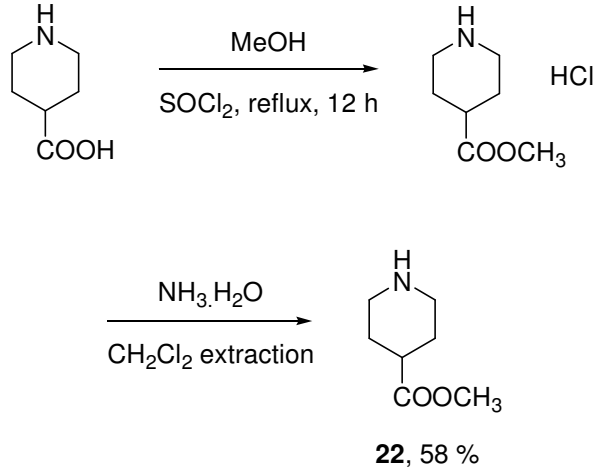
S_NAr reactions can be easily applied on Br-dichloroBODIPY **21** as well. It is easy to synthesize tens of grams compound **21**, since bromo-dipyrromethane can be crystallized from the dichloromethane/hexane mixture for the very first step.[12] The key step for the synthesis is to use excess pyrrole (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 quantitive yield.[13] 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.

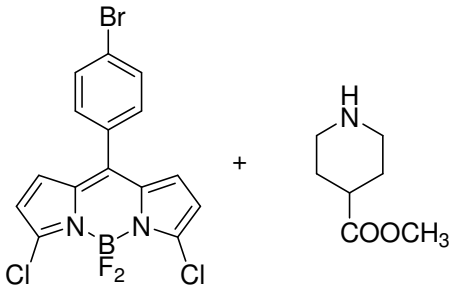


22, 58 %

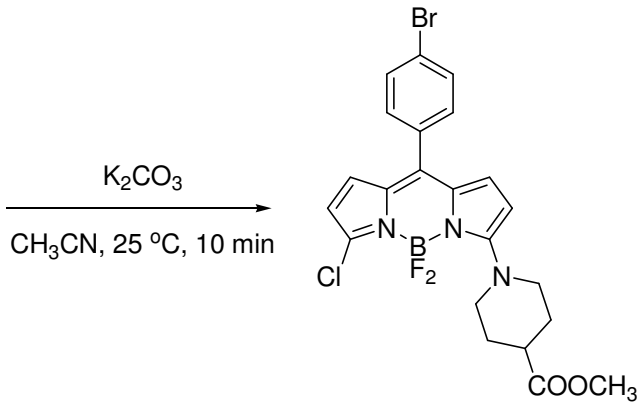
Displacement of the first of the two chlorines in BODIPY **21** with INP methyl ester occurred rapidly. The second chlorine can be displaced using extended reaction times at elevated temperature. The di-substituted product **24** can be modified to be a potential acceptor, but one consideration is its low quantum yield. [6]

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

a

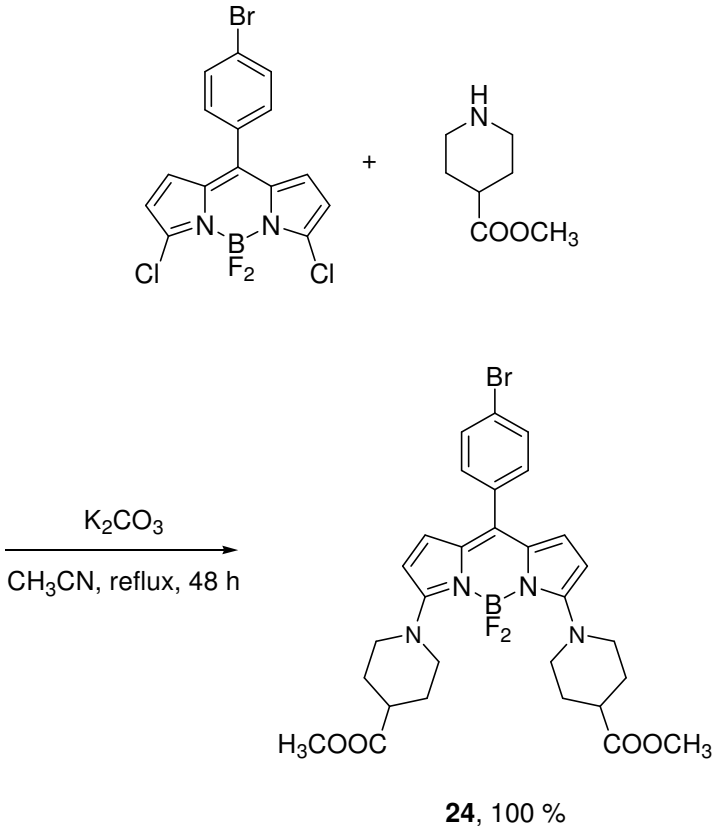


Scheme 2.7. Continued.



23, 98 %

b

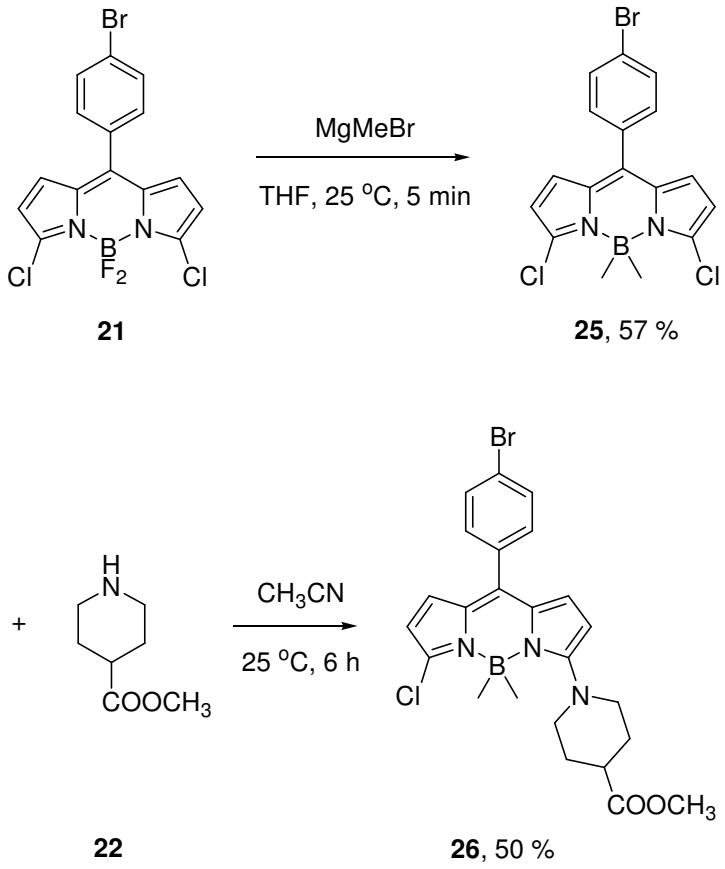


24, 100 %

It is known that fluorine atom can be substituted by alkyl groups,[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**.

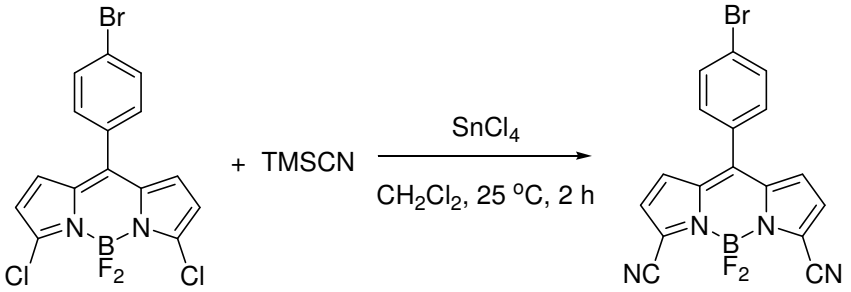


Since alkoxides, amines, thioalkoxides and the diethyl malonate anion were tried as the nucleophiles for S_NAr reactions, cyanide anion should be also easy to attack the electron deficient carbons.[14-16] Unfortunately, none of those conditions gave me the desired product when compound **21** was treated with sodium cyanide in methanol, acetonitrile or DMSO. Then the organic cyanide anion source TMSCN was tried instead and gave good results.[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.[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.

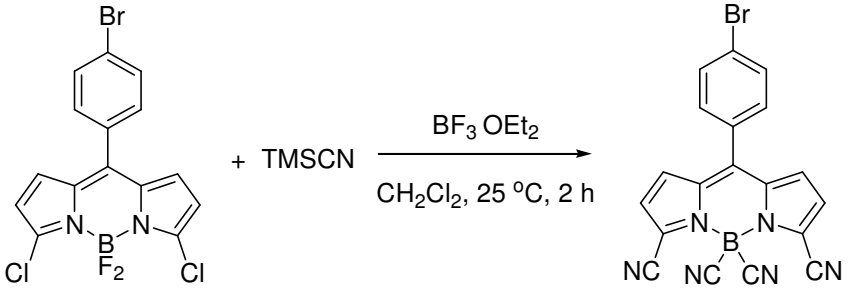
Scheme 2.9. a) Synthesis of compound **27**; **b)** synthesis of compound **28**.

a



21 **27**, 100 %

b

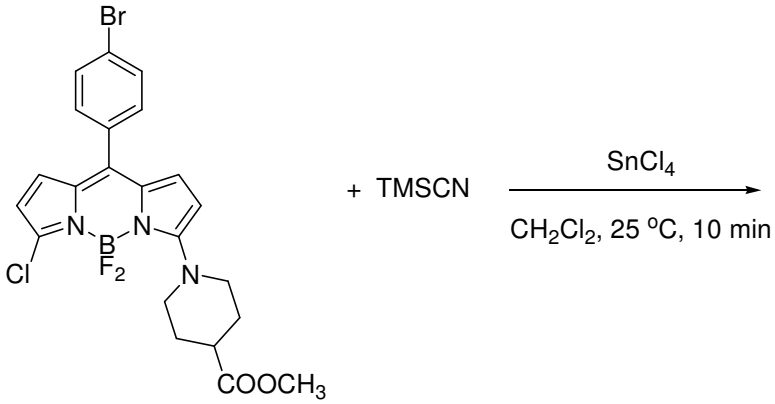


21 **28**, 71 %

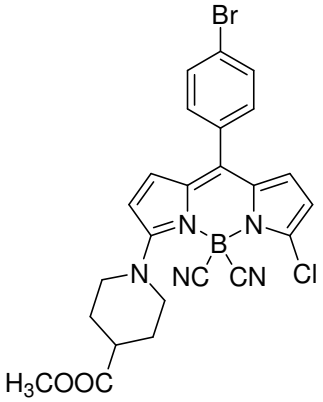
Cyanide anion displacement reactions were also tried on compound **23** to see if the only chlorine atom remaining would be displaced, but when the same condition that was used

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**.



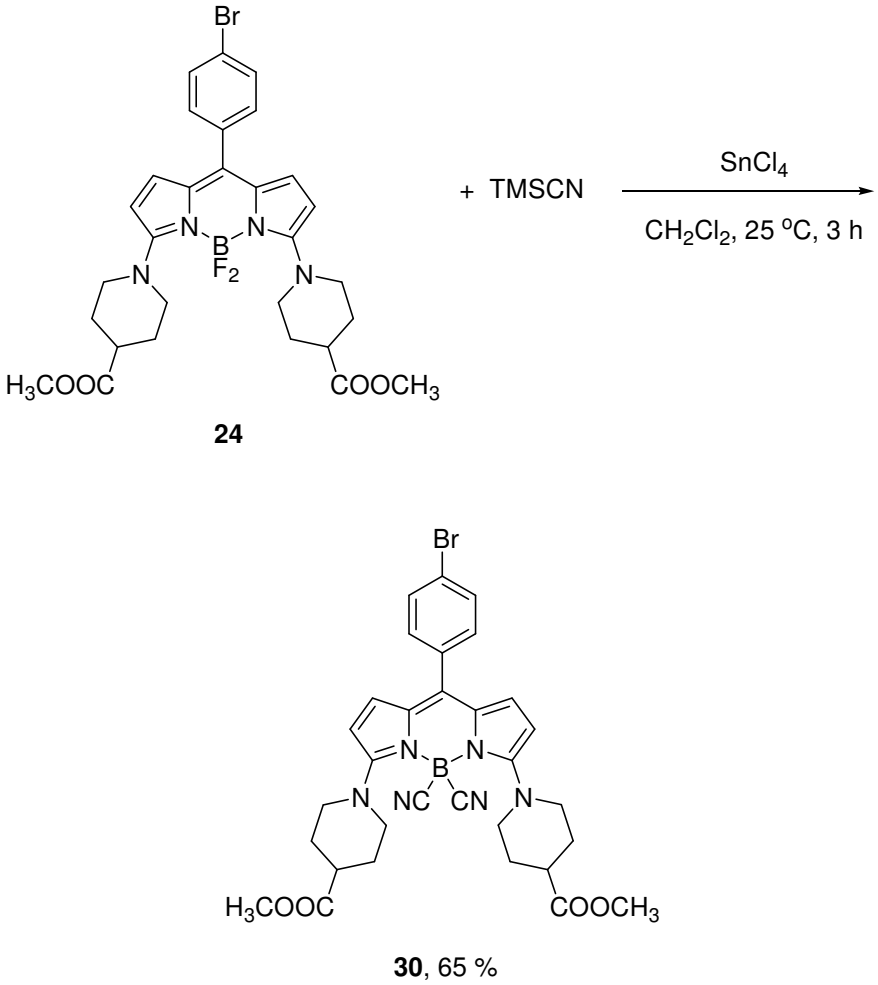
23



29, 93 %

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**.

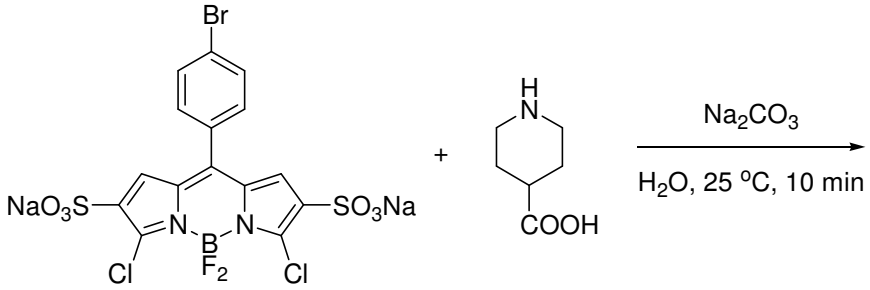


24

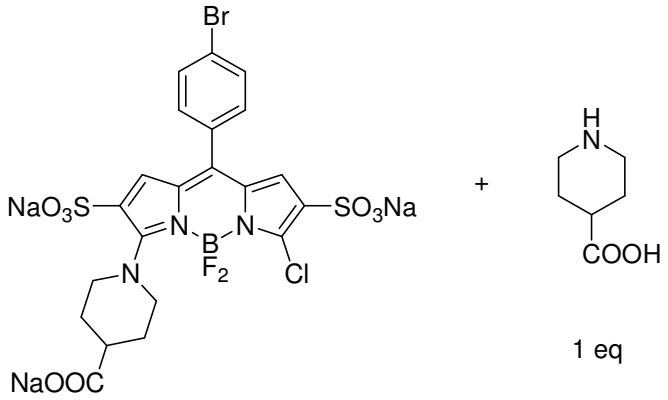
30, 65 %

The water-soluble dichloroBODIPY **53** (synthesis described in Chapter IV) was also reacted with INP to test if the sulfonated group can accelerate S_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 ^1H 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_NAr reaction much faster.

Scheme 2.12. Syntheses of water-soluble mono- and di-substituted compounds **59** and **60**.

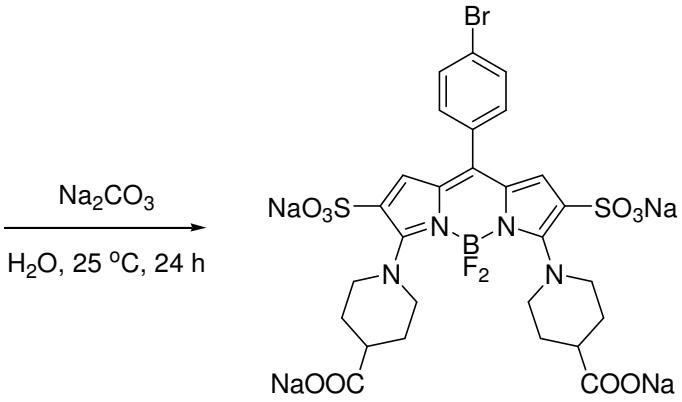


53 1 eq



1 eq

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 maximam of CF_3-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.

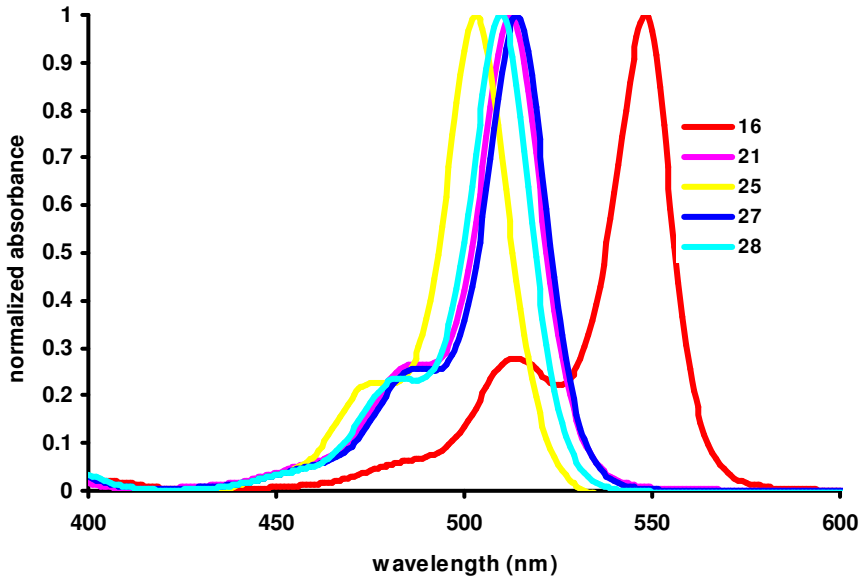
Quantum yields (Table **2.1**) for the non-substituted BODIPYs are very good, from 0.13 to 1.0. Compound **21** and **25** have the relatively low quantum yields, 0.15 and 0.13 respectively. Compounds **27** and **28** have really good quantum yields even though they both have the phenyl ring, which 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.

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

[a] In CH_2Cl_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.

a



b

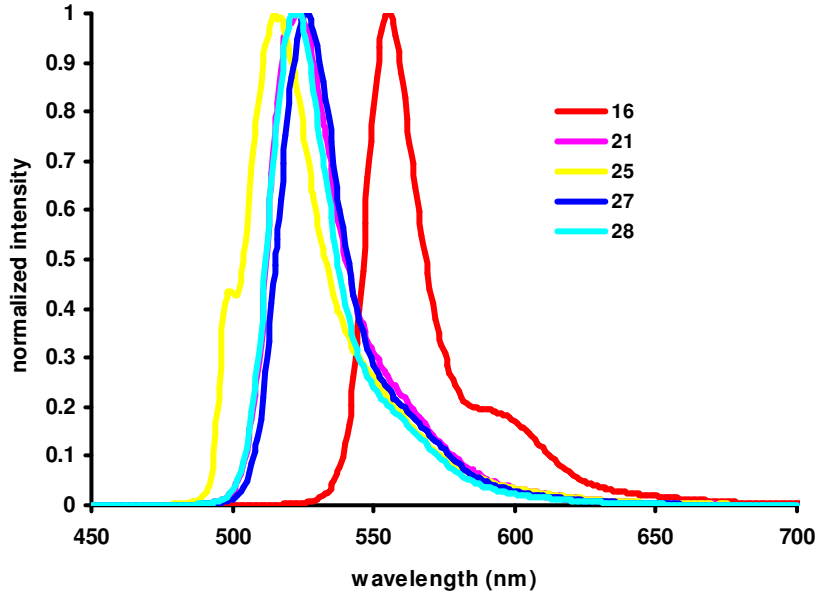
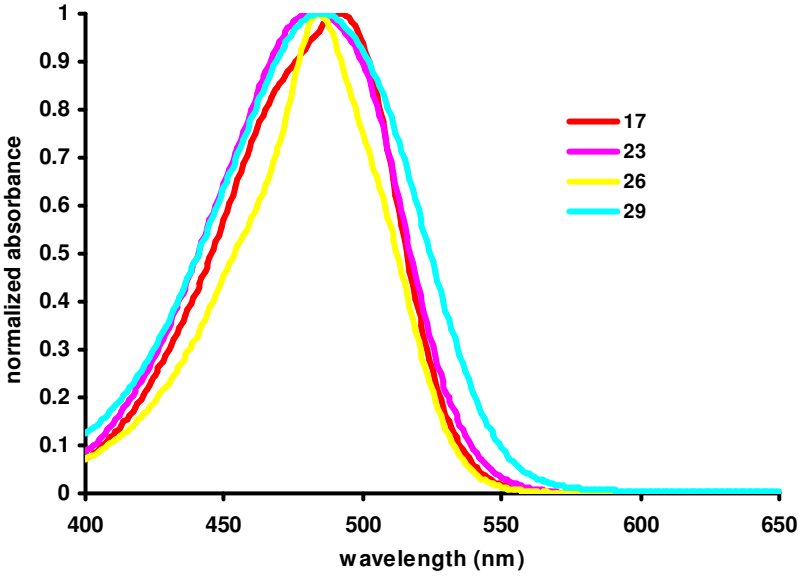


Figure 2.2. a) UV absorption, and **b)** fluorescence: spectra for non-substitued BODIPYs.

c



d

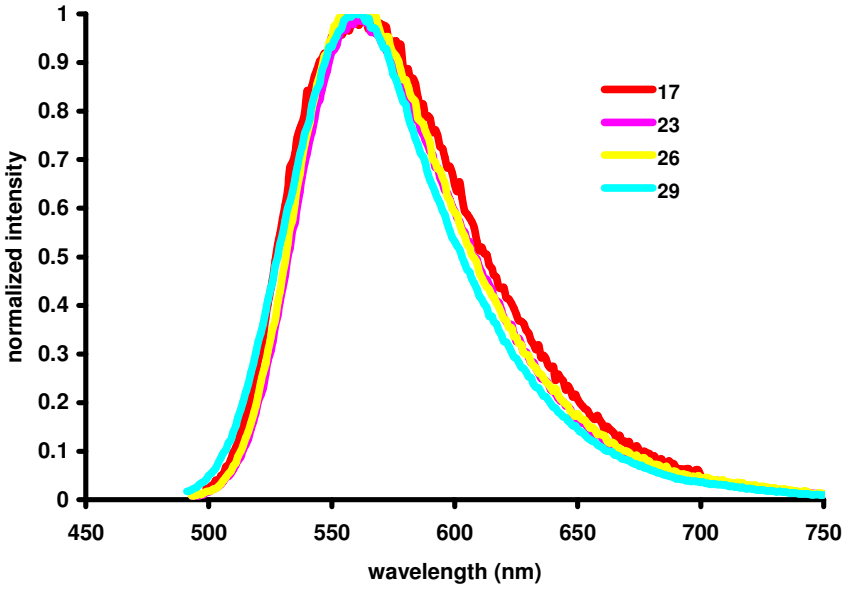
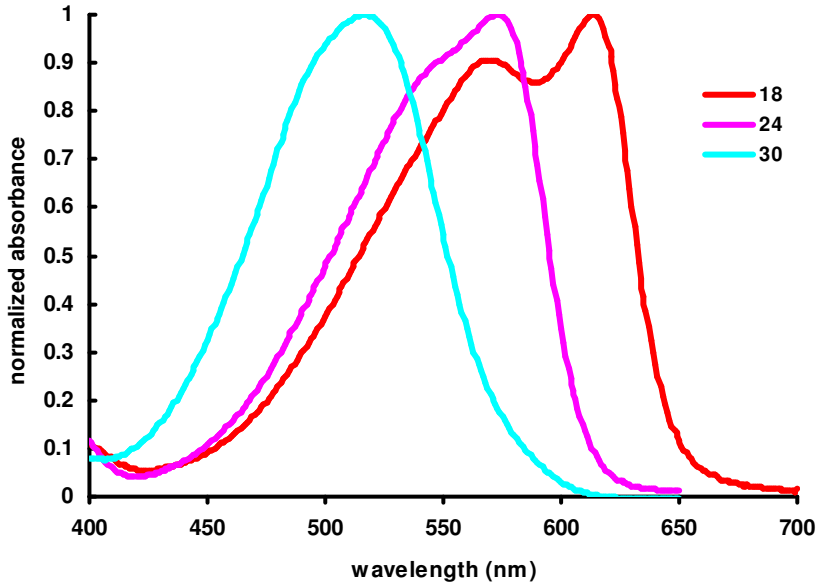


Figure 2.2. Continued. **c)** UV absorption, and **d)** fluorescence: spectra for mono-substituted BODIPYs.

e



f

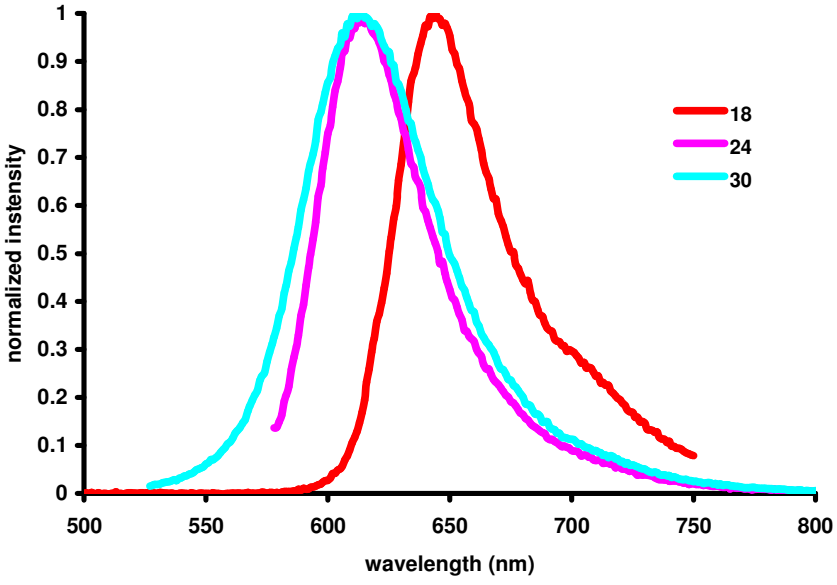


Figure 2.2. Continued. **e)** UV absorption, and **f)** fluorescence: spectra for di-substitued BODIPYs.

2.3 Coupling with Protein

*2.3.1 Synthesis of **16-Avidin***

As mentioned above, the S_NAr reaction works well on CF_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.

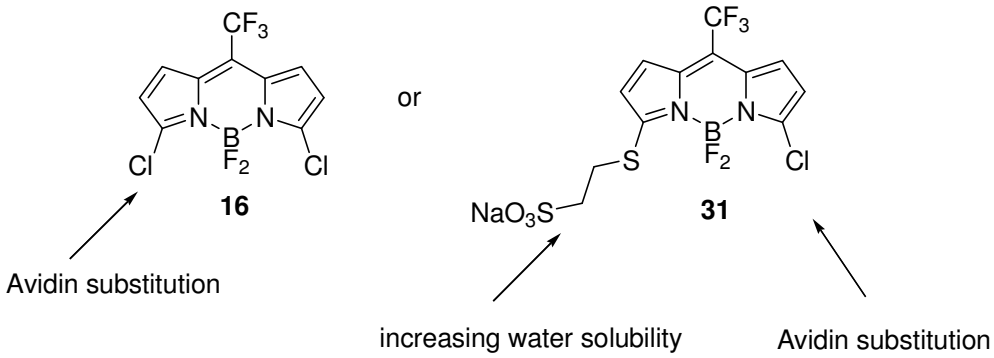
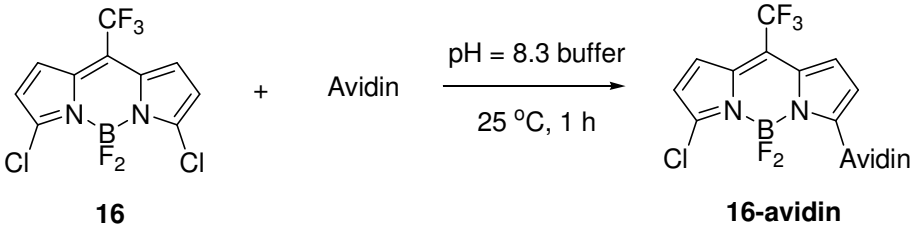


Figure 2.3. Proposed dyes which can be used to label proteins.

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] ε_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.

$$\frac{C_d}{C_p} = \frac{A_d}{A_p} \times \frac{\varepsilon_p}{\varepsilon_d} \quad \text{.....(1)}$$

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

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

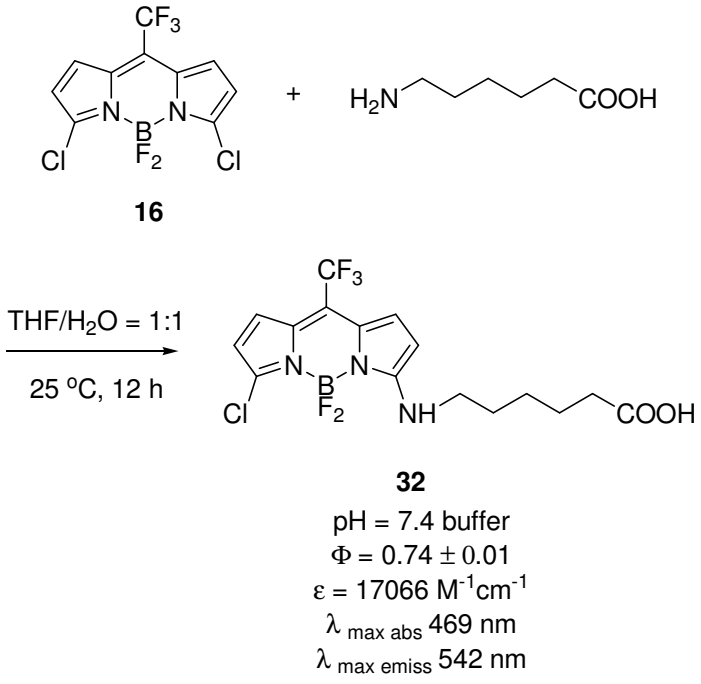
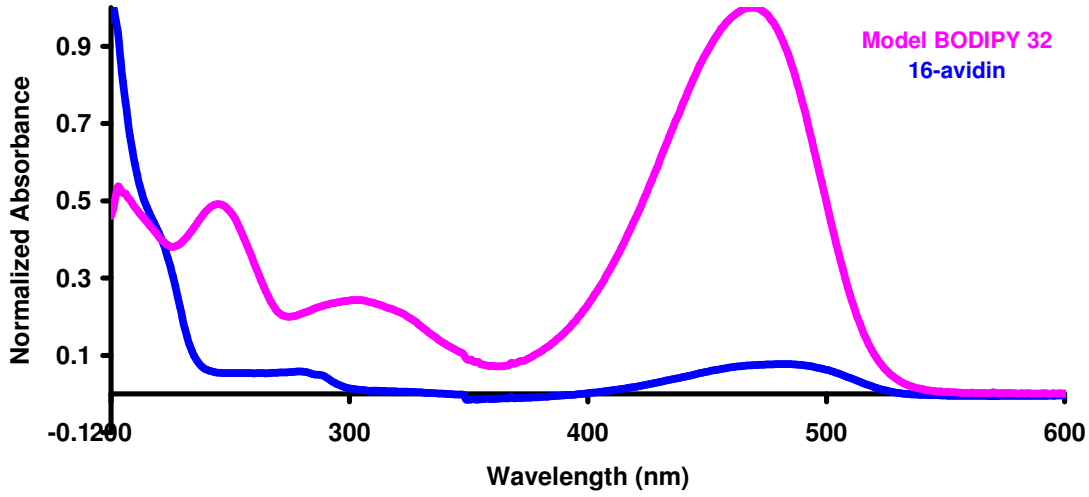


Figure 2.4 **a** and **b** show the absorption and emission peaks for the model BODIPY **32** and compound **16-avidin**. There is only 13 nm difference in absorption maximum wavelength between these two (469 for **32**, and 481 for **16-avidin**).

a



b

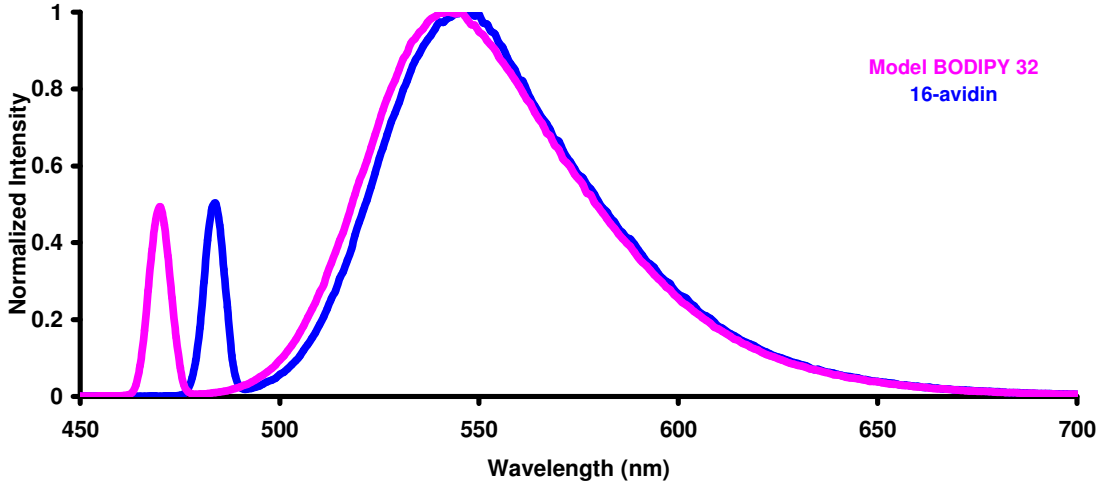
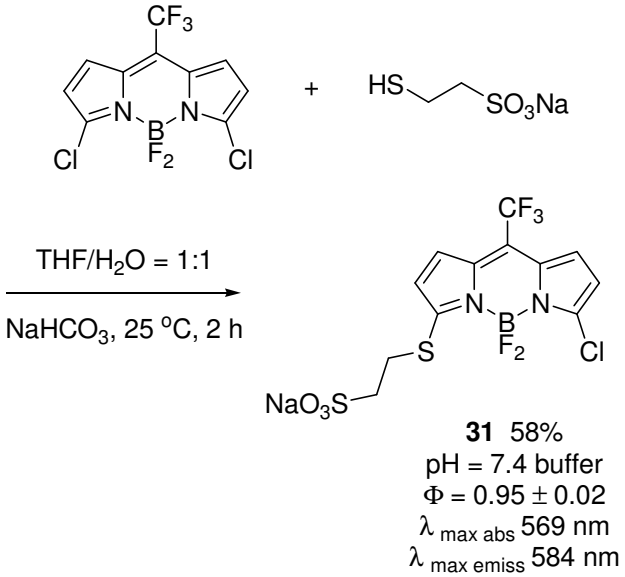


Figure 2.4. a) UV Absorption and **b)** fluorescence: spectra for model study **32** and **16-avidin**.

*2.3.3 Synthesis of **31-Avidin***

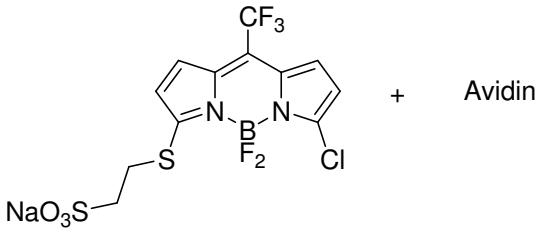
Sodium 2-mercaptoethanesulfonate can be used as a sulfur nucleophile to displace one chlorine of BODIPY **16** and yield water-soluble BODIPY **31** which absorbs at 569 nm and emits at 584 nm in the buffer (0.1 M lithium phosphate, pH 7.4).

Scheme 2.15. Synthesis of water soluble BODIPY **31**.

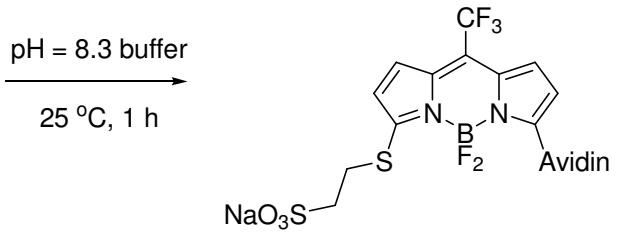


Avidin can be considered as the second nucleophile to react with BODIPY **31** and then compound **31-avidin** was formed after the reaction was shaken in the dark for one hour at room temperature in the buffer (0.1 M sodium bicarbonate, pH = 8.3).

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



Scheme 2.16. Continued.

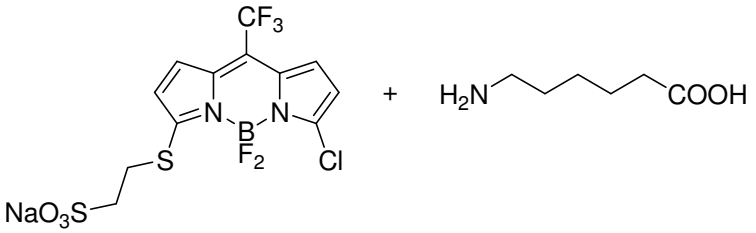


31-avidin

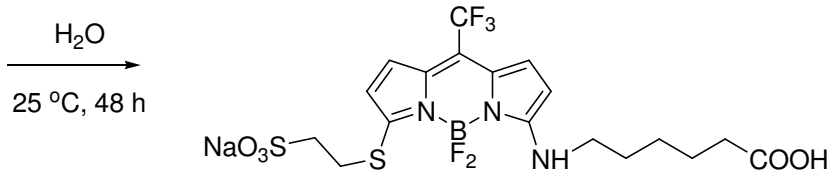
2.3.4 Calculation of Dye-Protein Ratio

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.



33
pH = 7.4 buffer
Φ = 0.70 ± 0.01
ε = 41810 M-1cm-1
λabs 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.

a

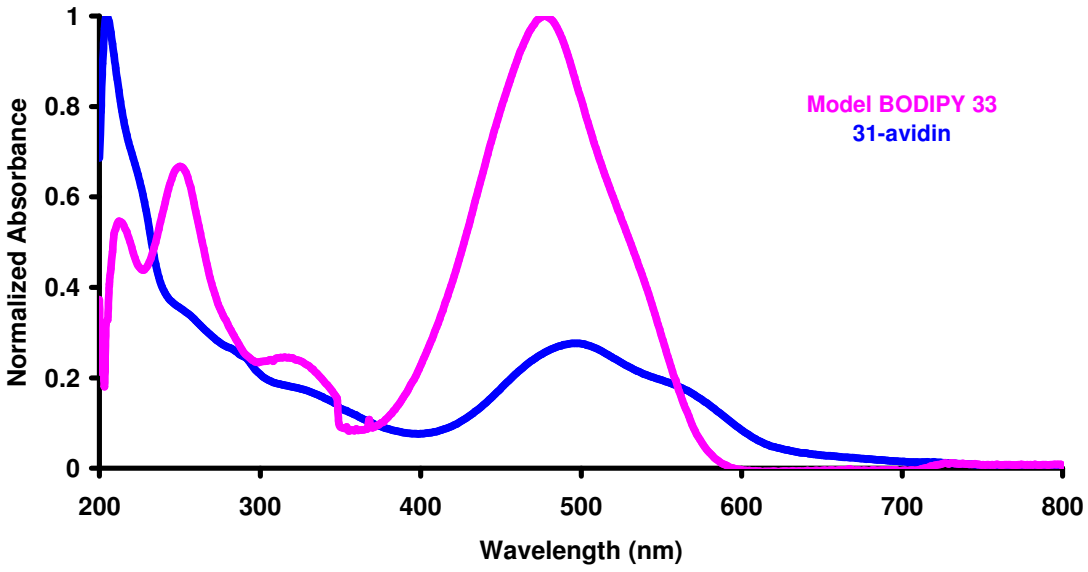


Figure 2.5. a) UV absorption and **b)** fluorescence: spectra for model study **33** and **31-avidin**.

b

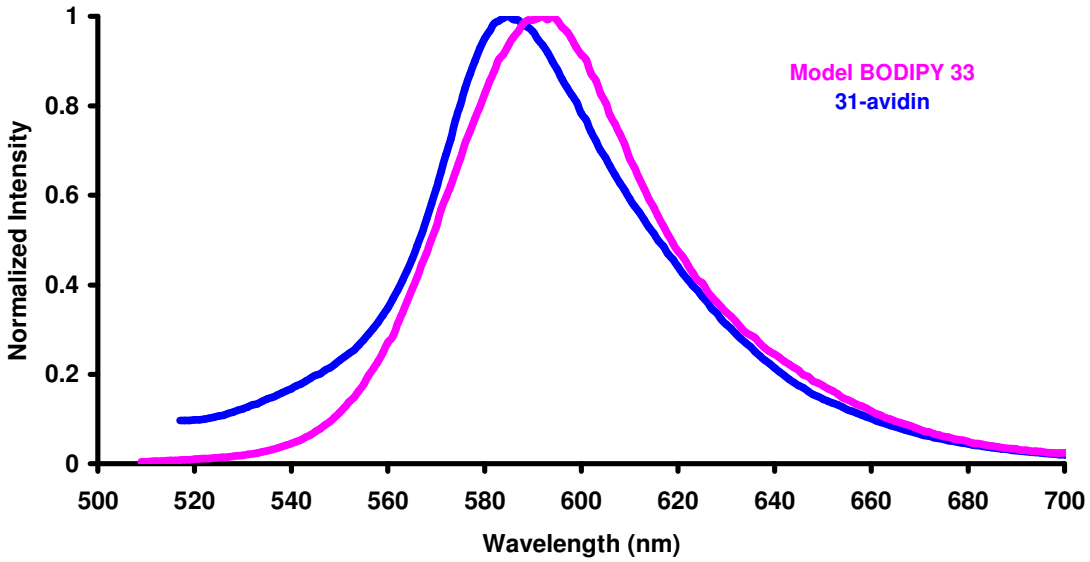


Figure 2.5. Continued.

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_NAr 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_NAr 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[4] and superexchange.[5] 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)

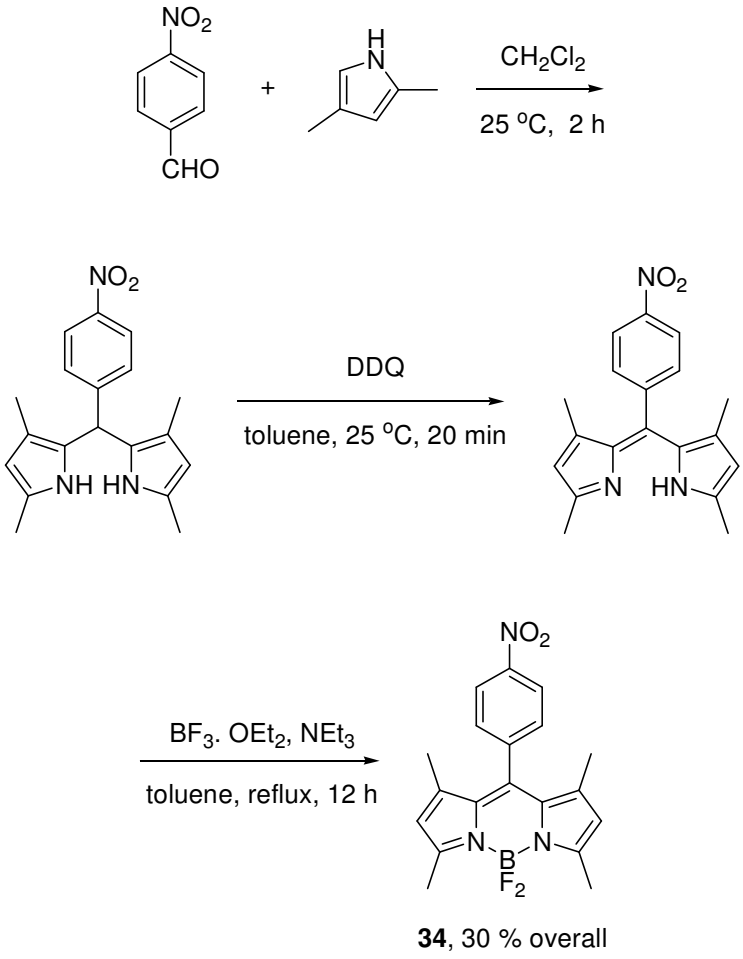
Lipophilic BODIPY derivatives which emit around 520 nm potentially can be used as donors for cassettes. Those BODIPYs emitting at longer wavelength can be used as acceptors. This chapter describes two new lipophilic through-bond energy transfer cassettes.

*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 eletrons to the substituent LUMO then oxidative-PeT, d-PeT, occurs ("d" for donor).

Scheme 3.1. Synthesis of tetramethyl NO_2-BODIPY **34**.

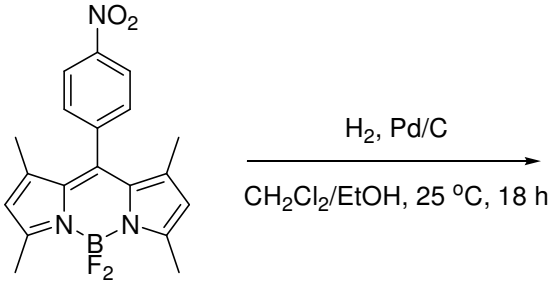


34, 30 % overall

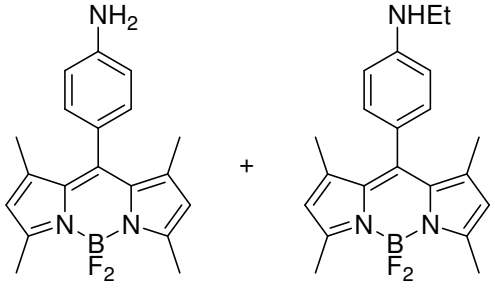
Since the NO_2 group can't be used to couple with any acceptor, it was modified to an NH_2 group in compound **35**, which can be transformed to N_3-tetramethyl BODIPY and then used for "click" chemistry. When Pd/C and H_2 was used to reduce the nitro compounds **34**.[22, 23] Formation of by-product **36** could not be avoided[24] 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.[21] The only drawback for this method is that NH_2NH_2 H_2O 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.



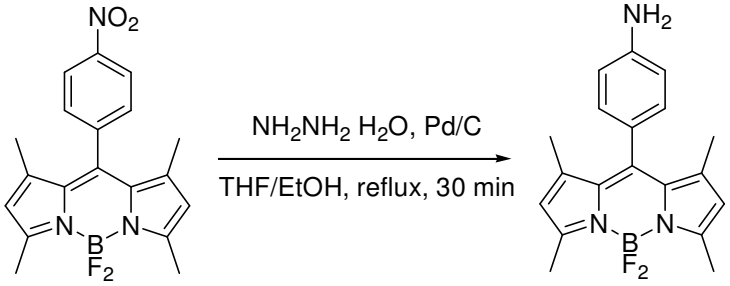
34



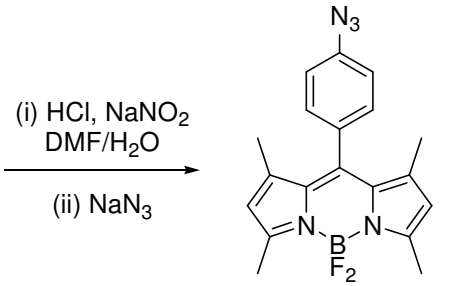
35 **36**

Compound **35** can be treated with 2 M HCl and $NaNO_2$ in the mixture of DMF and H_2O 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, $\Phi = 0.92$ in ethanol).

Scheme 3.3. Synthesis of amino- and azido-BODIPYs **35** and **37**.



34 **35**, 96 %



37, 77 %
CH_2Cl_2 $\Phi = 0.48$
ε=98591 $M^{-1}cm^{-1}$
$\lambda_{max\ abs}$ 503 nm
$\lambda_{max\ emiss}$ 513 nm

Copper mediated azide-alkyne cycloaddition of N_3-tetramethyl BODIPY **37** with suitable alkynes were envisaged to gave through-bond energy transfer cassettes. Scheme 3.4 shows N_3-BODIPY **37** coupled with Nile Red to form the lipophilic cassette **38**.[25, 26] The reaction was carried out in 4:1 THF/H_2O 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.**

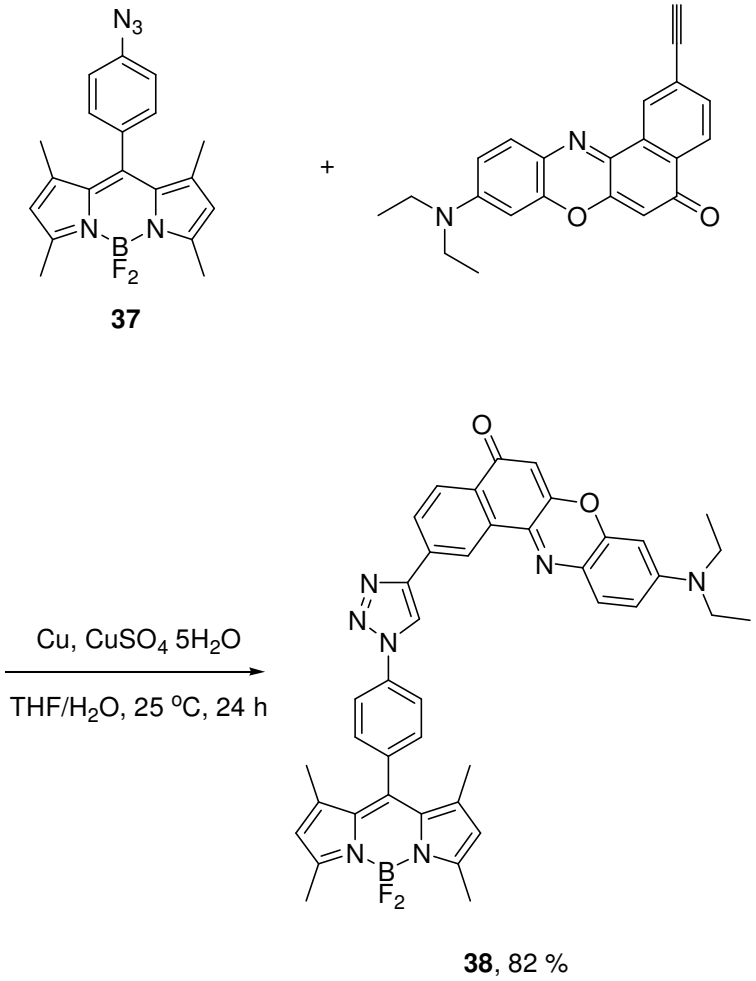
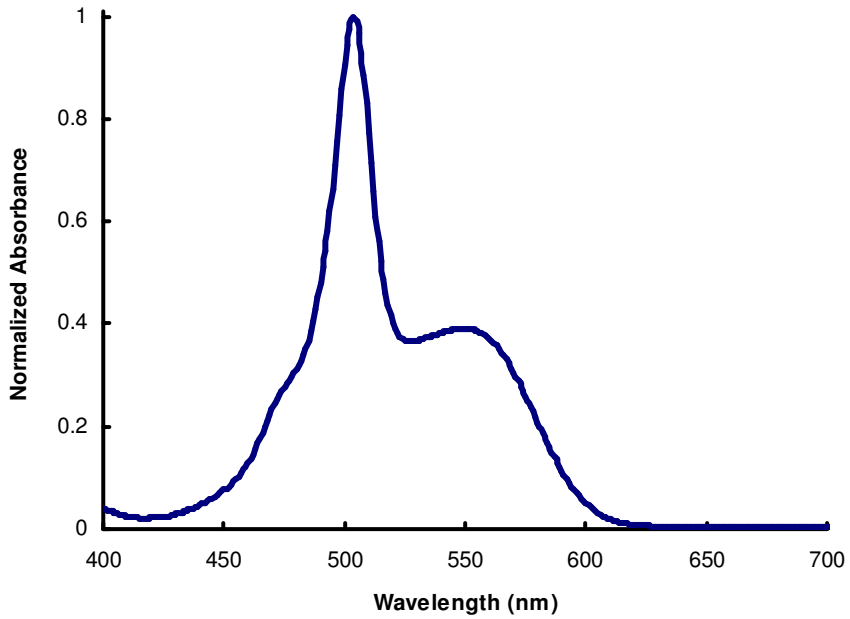


Figure 3.1 shows the absorption of cassette **38** in dichloromethane. This spectrum shows two peaks: one from the donor N_3-BODIPY **37** at 504 nm and the other from the acceptor, Nile Red at 549 nm. The peak from the acceptor Nile Red is broader and has the lower intensity, because of the smaller 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.

a



b

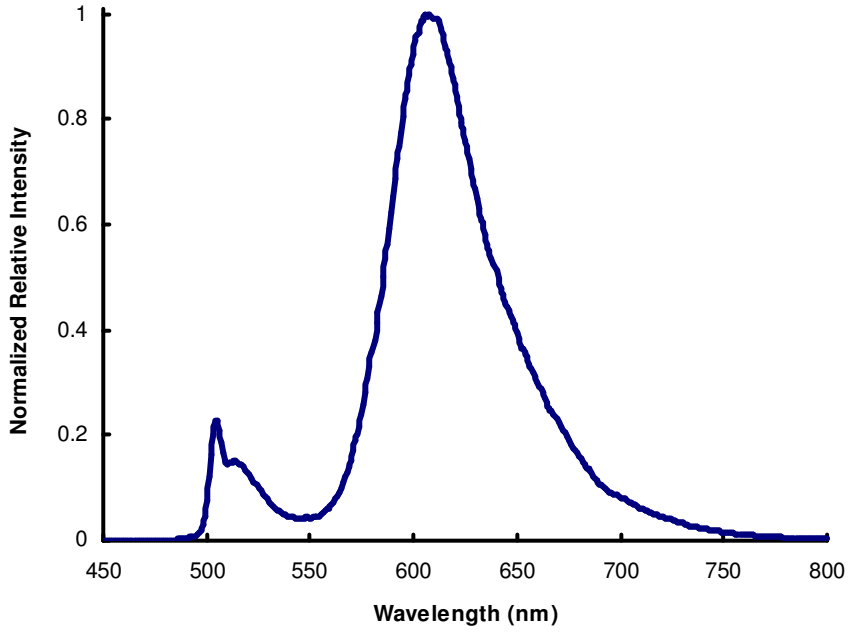
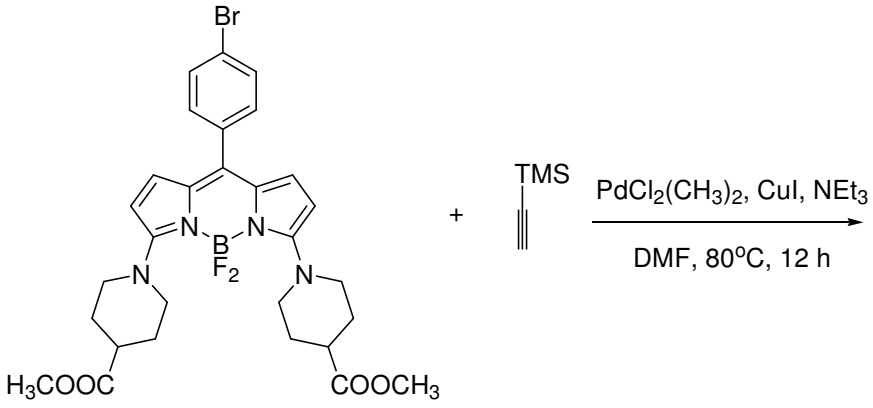


Figure 3.1. a) UV absorption and **b)** fluorescence： spectra for cassette **38** (10^{-5} M in dicloromethane).

*3.2.2 Cassette **41** with BODIPYs Acceptor and Donor*

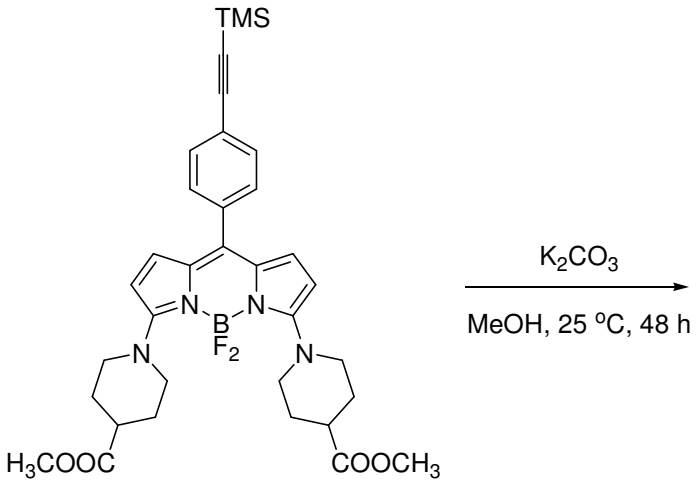
BODIPY **24** is a potential acceptor if its bromophenyl group can be converted to ethynyl phenyl. Thus the Sonogashira reaction was performed to give TMS-ethynyl BODIPY **39** in 76% yield following a literature procedure for a similar compound.[27] Deprotection of TMS was achieved using potassium carbonate 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,[6] 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**.

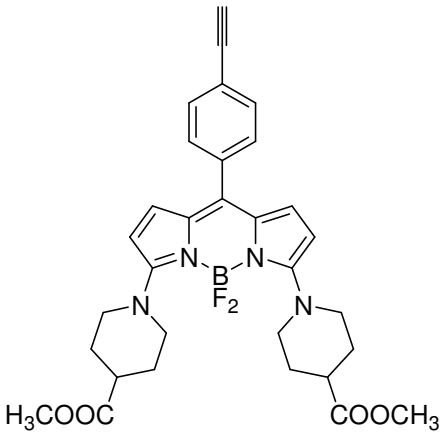


24

Scheme 3.5. Continued.



39, 76 %

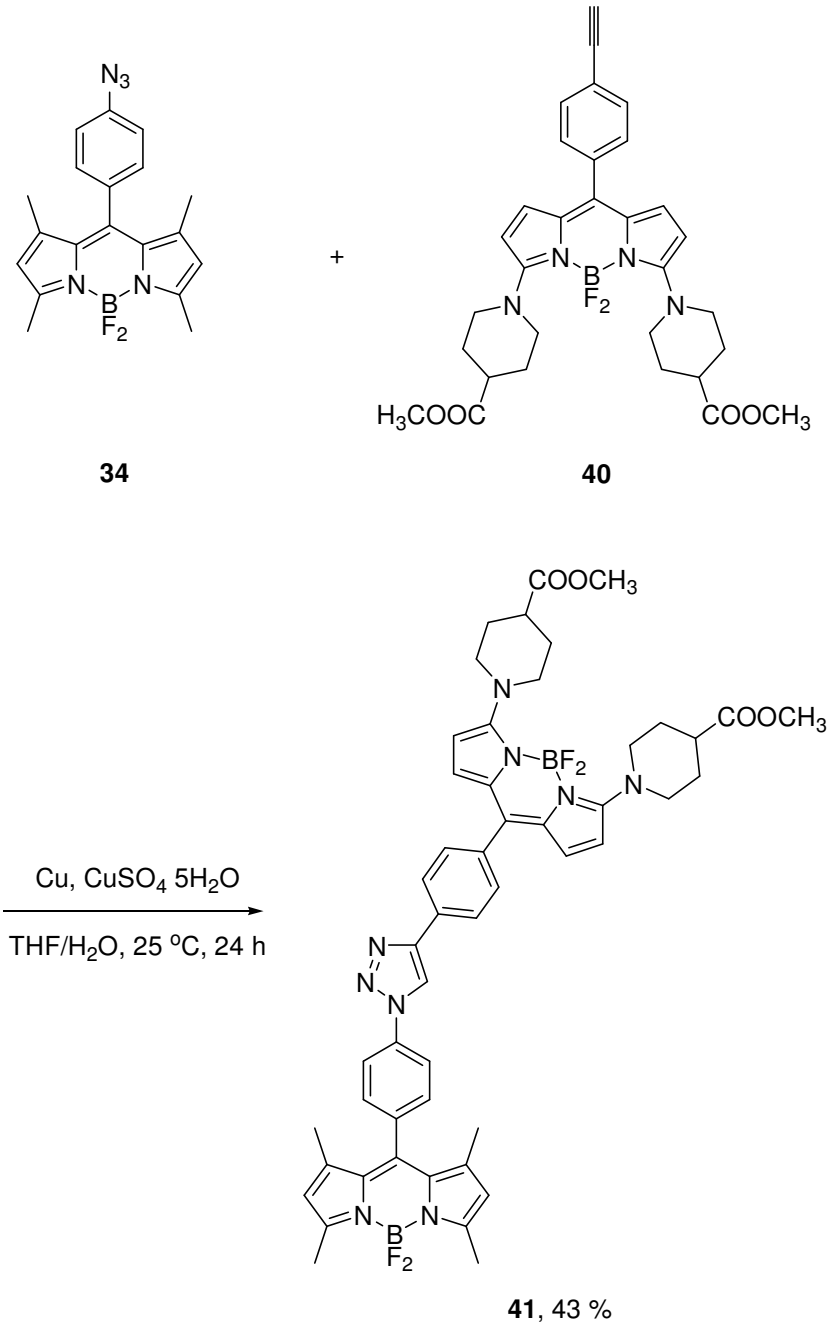


40, 94 %
CH_2Cl_2
$\lambda_{max\ abs}$ 582 nm
$\lambda_{max\ emiss}$ 627 nm
Φ = 0.15 (MeOH)

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%.

a

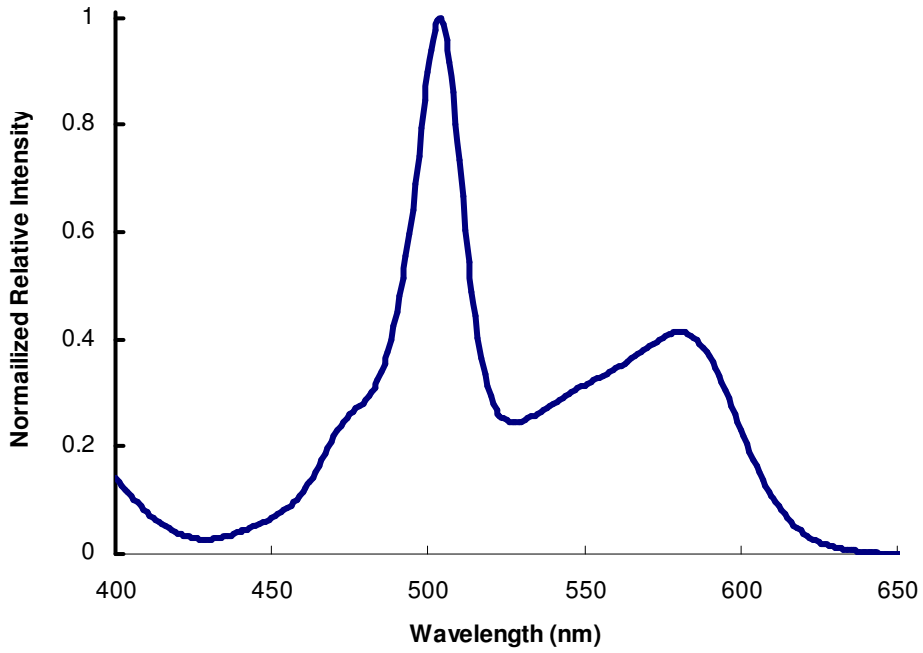


Figure 3.2. a) UV absorption and **b)** fluorescence: spectra for cassette **41** (10^{-5} M in dichloromethane)

b

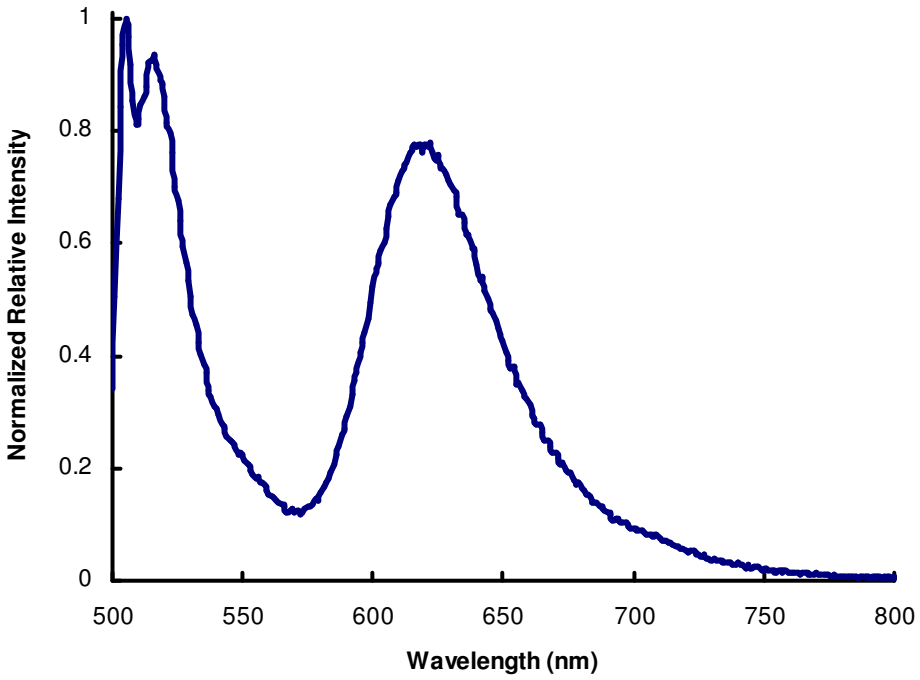


Figure 3.2. Continued.

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[28-31] 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;[32] 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 BOIDPY 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.[33-36] 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**[37, 38]and several closely related oligoethylene-glycol-containing systems, of which **E**[39] is illustrative (Figure 4.1**a**).

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 ($NaHCO_3$). 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_2O, 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 4.1**b**). 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_NAr reactions.[6, 40] 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,[41, 42] 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 scenerios for labeling biological molecules.

a

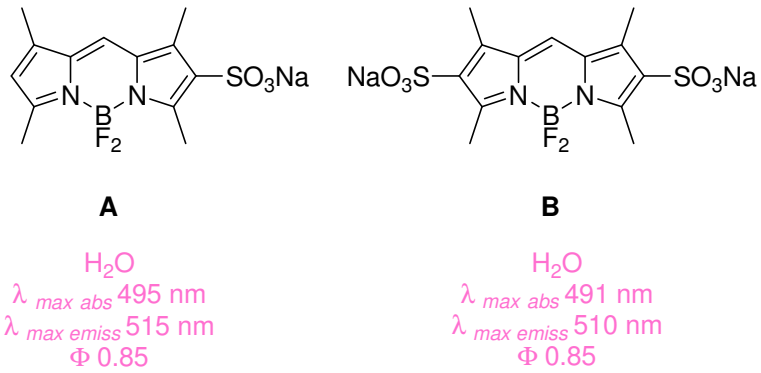
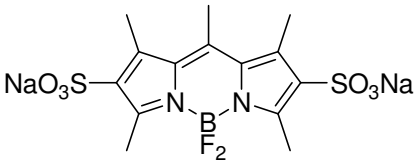
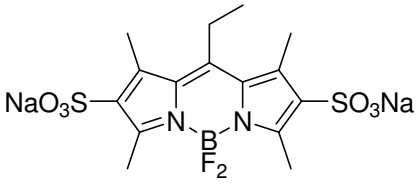


Figure 4.1. a) Previously known water-soluble BODIPY systems; and, **b)** compounds prepared in this work.



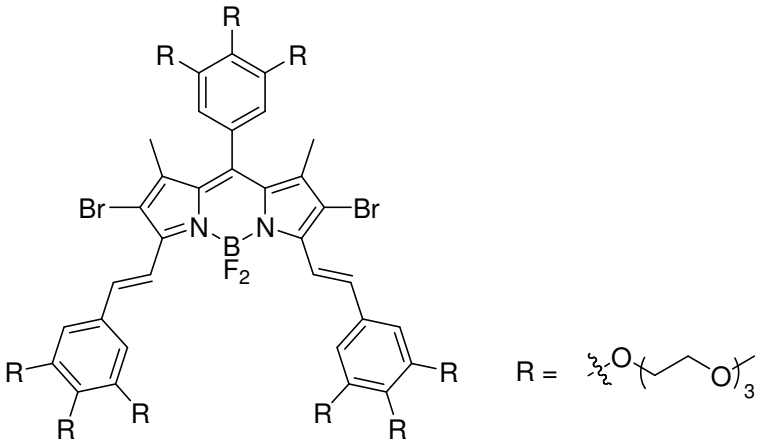
C

MeOH
λ *max abs* 492 nm
λ *max emiss* 533 nm
Φ 0.73 (H_2O)



D

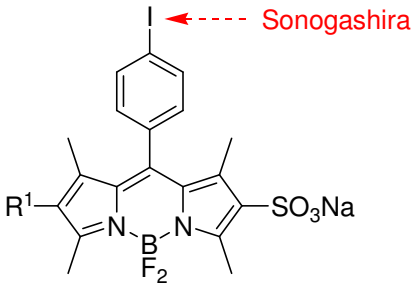
MeOH
λ *max abs* 498 nm
λ *max emiss* 530 nm
Φ 0.44 (EtOH)



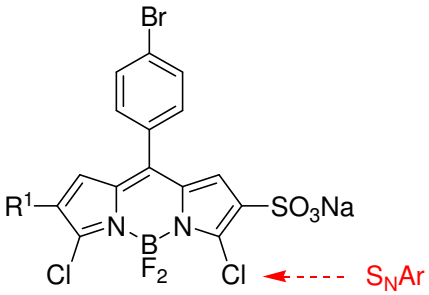
E

EtOH
λ *max abs* 660 nm
λ *max emiss* 690 nm

b



R^1 = H, **49**; R^1 = SO_3Na,**50**



R^1 = H, **52**; R^1 = SO_3Na, **53**

Figure 4.1. Continued.

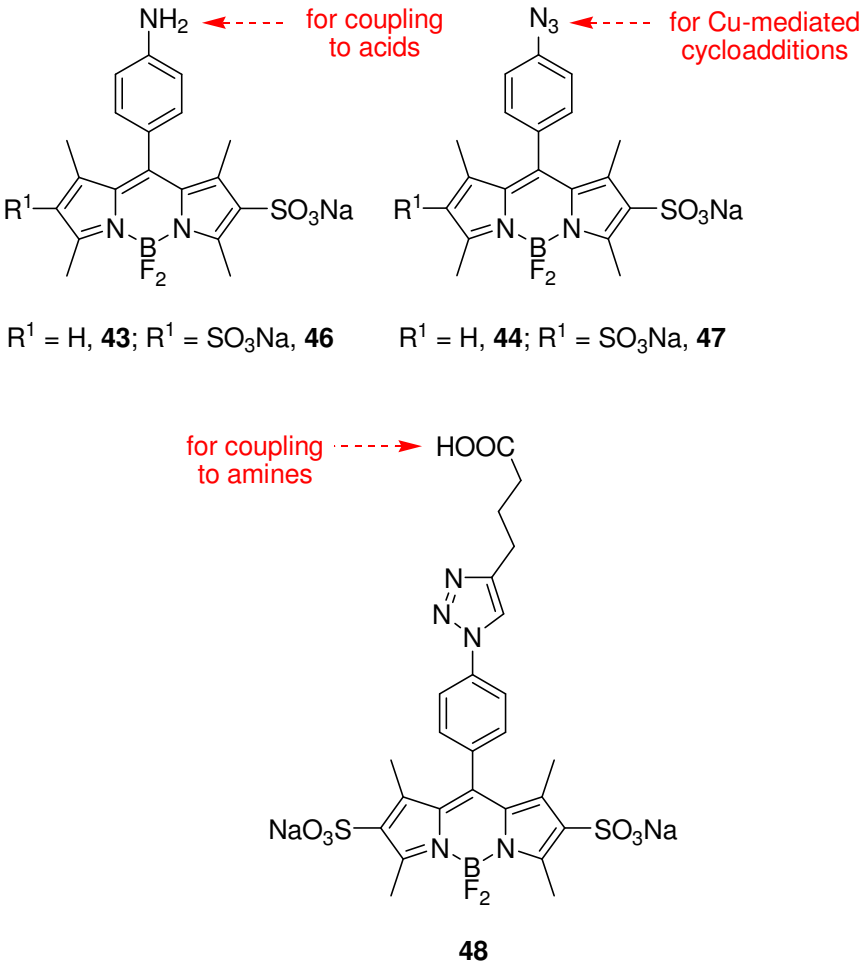


Figure 4.1. Continued.

4.2 Results and Discussion (Syntheses and Spectral Studies)

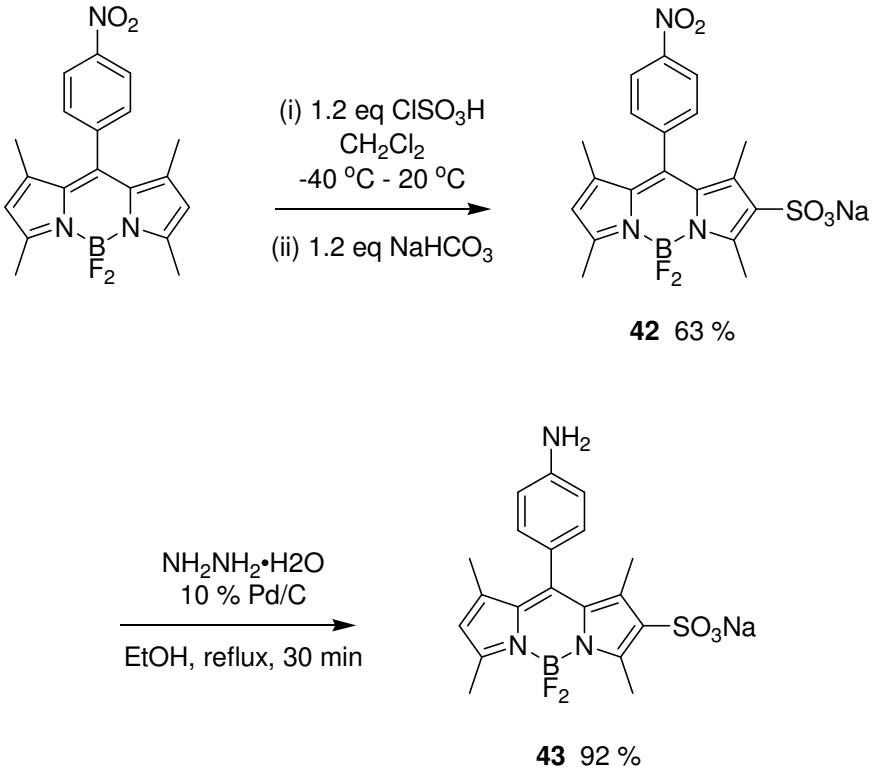
The following sections describe the preparation of the unusual BOIDPY starting materials, the pivotal sulfonation reactions, and reactions of the sulfonated products to further transform them into useful probes. Finally, the spectral properties of the target molecules are discussed.

4.2.1 Syntheses

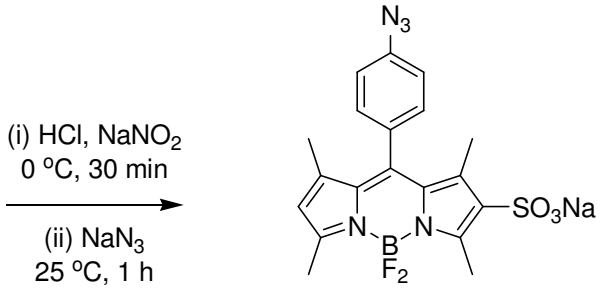
Scheme 4.1 shows the mono-sulfonation on tetramethyl nitroBODIPY **34** with 1.2 equivalent chlorosulfonic acid at -40°C to afford **42** with 63 % yield. Flash chromatography on silica was needed to purify the product. Nitro group can be

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 NO_2-BODIPY.



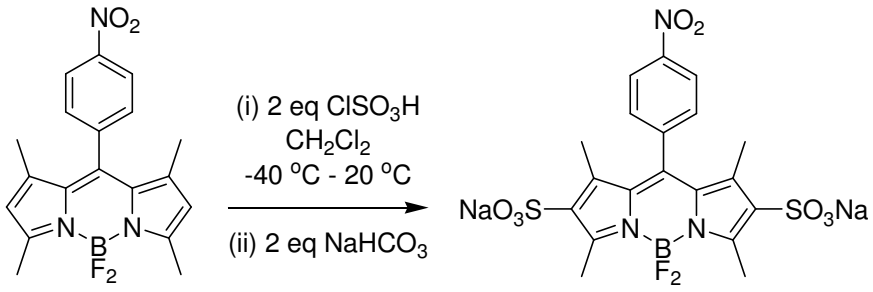
Scheme 4.1. Continued.



44 75 %

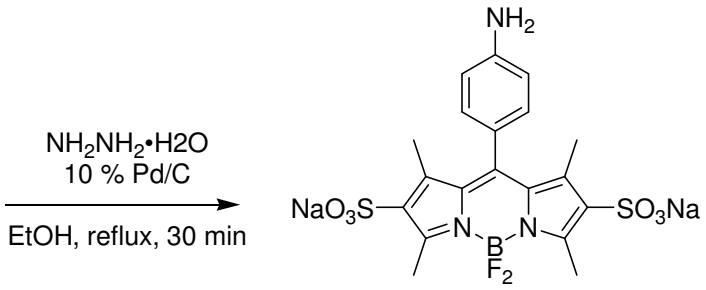
The di-sulfonated BODIPY **45** can also be obtained when 2 equivalent chlorosulfonic acid was used. Separation in this case is relatively easy because the di-sulfonic acids precipitate from the dichloromethane solution after 20 min at room temperature. The products were collected by filtration, dissolved in a small amount of aqueous $NaHCO_3$, 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 NO_2-BODIPY.

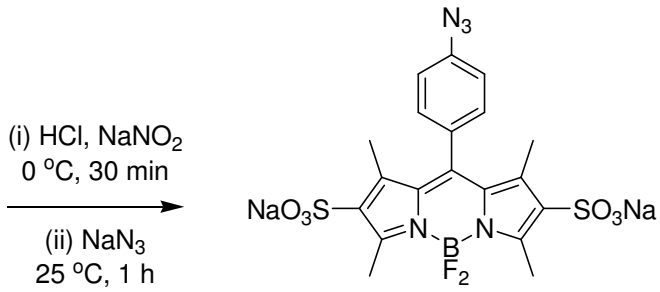


45 100 %

Scheme 4.2. Continued.



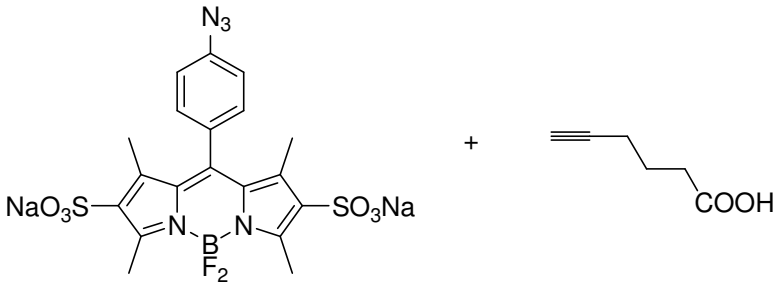
46 70 %



47 77 %

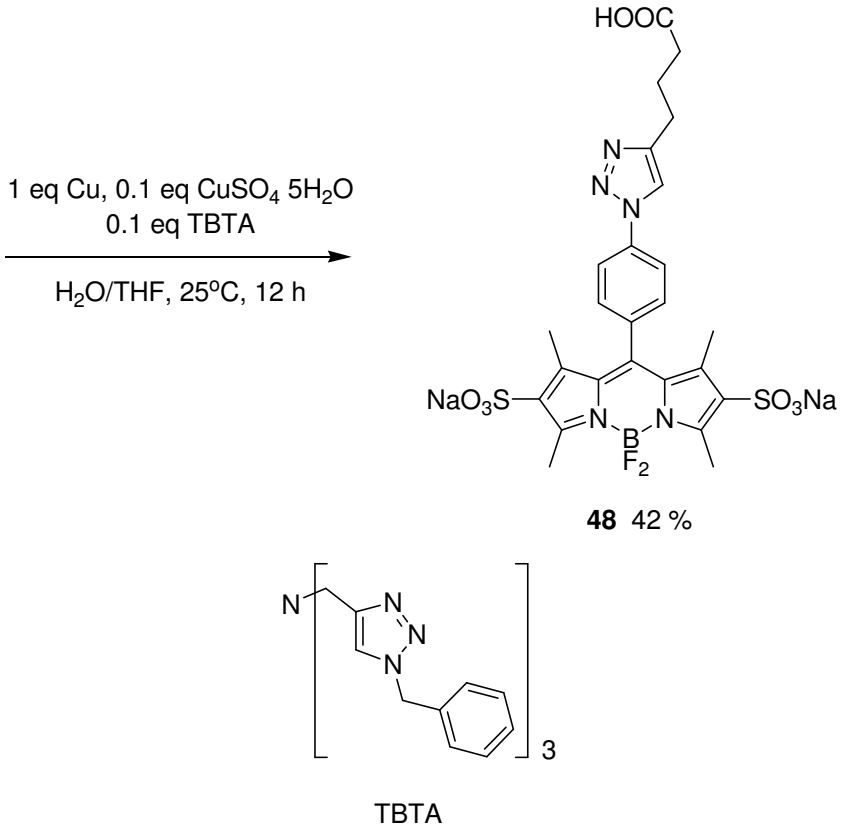
Scheme 4.3 shows one 'click' reaction between azidoBODIPY **47** and hexynoic acid. This reaction can be completed in 12 hours at room temperature and yield compound **48**; the ligand *tris*-(benzyltriazolylmethyl)amine (TBTA)[43] was needed for this reaction. Compound **48** is freely water-soluble and contains an easily accessible carboxylic acid for activation and conjugation to biomolecules.

Scheme 4.3. Synthesis of water-soluble BODIPY **48** with carboxylic aicd.



47

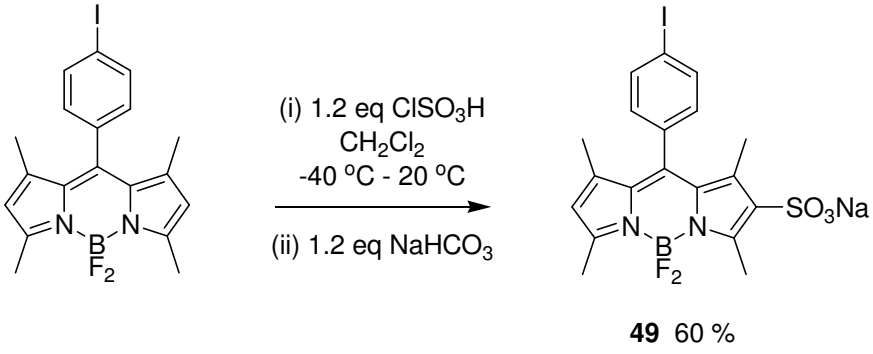
Scheme 4.3. Continued.



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.

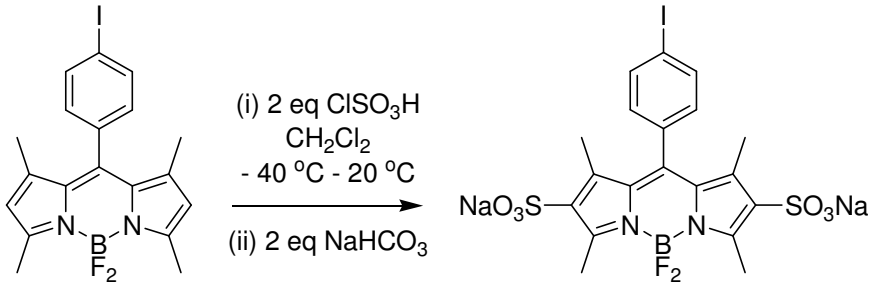
Scheme 4.4. a) Mono-sulfonation; and **b**) di-sulfonation on tetramethyl iodoBODIPY.

a



Scheme 4.4. Continued.

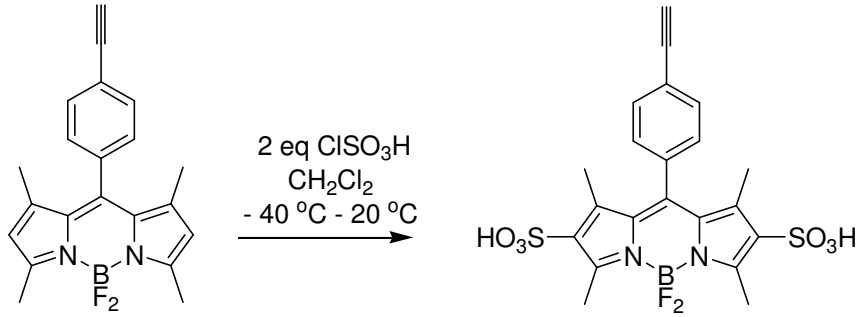
b



50 88 %

Modified conditions for the sulfonation were not suitable for the alkyne-functionalized BODIPY.[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**.

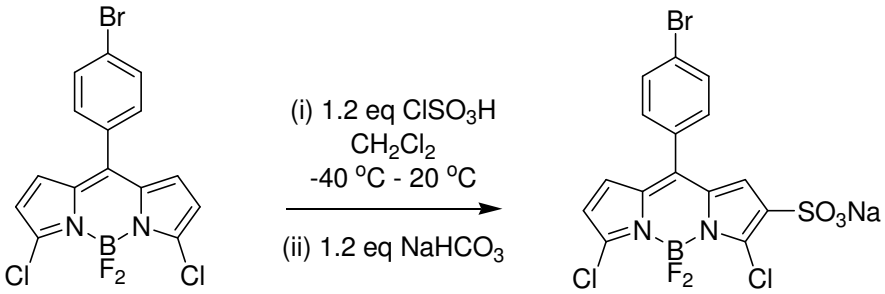


51 64 %

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.

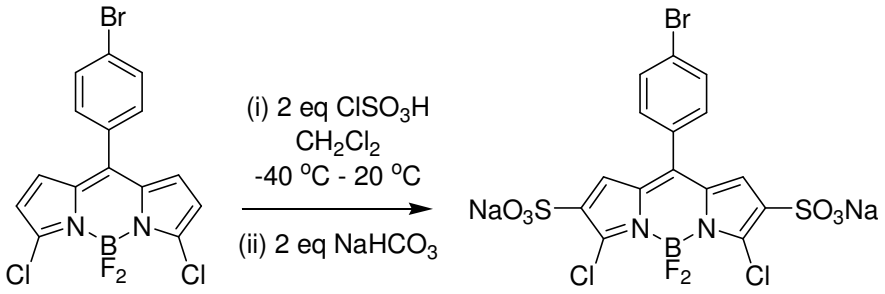
Scheme 4.6. a) Mono-sulfonation; and **b)** di-sulfonation on dichloroBODIPY **21**.

a



21 **52** 92 %

b

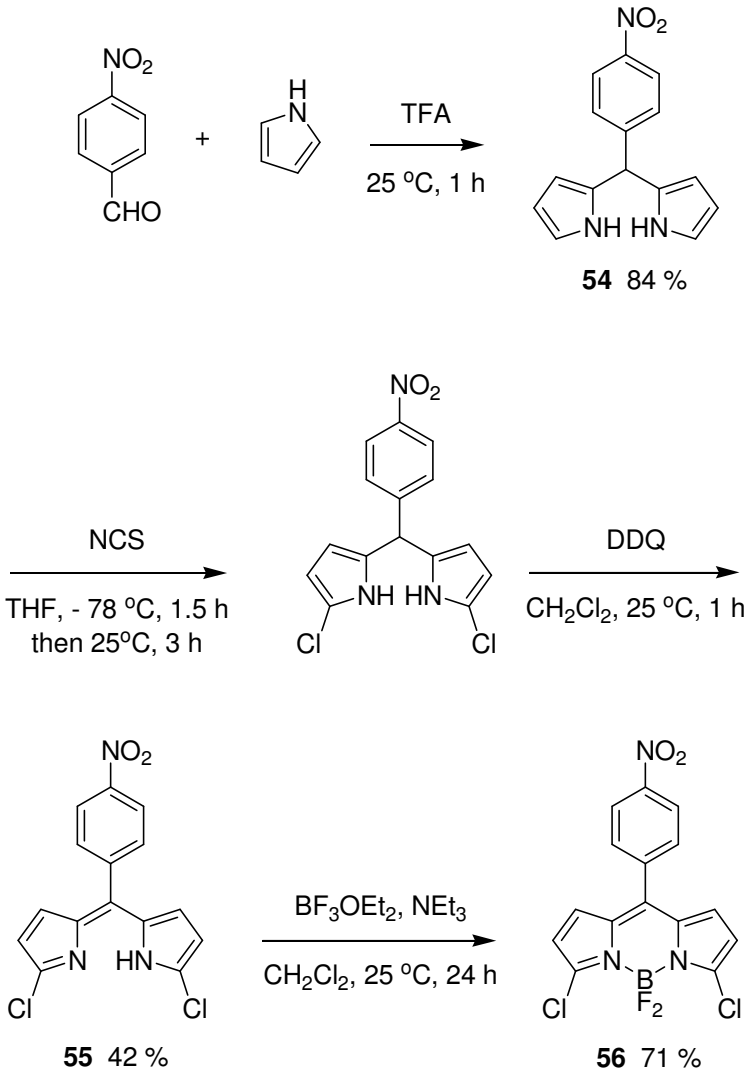


21 **53** 85 %

The dichloroBODIPYs can be used not only for the S_NAr reaction, but also for the Sonagashira, Suzuki, Stille and Heck reactions.[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.

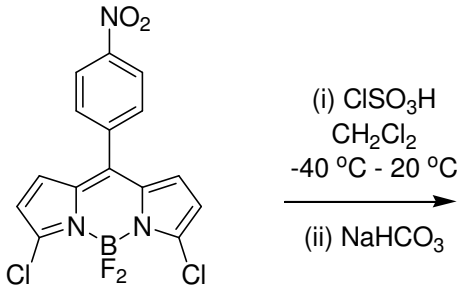
The Scheme 4.7 shows the synthetic route to nitro-dichloroBODIPY **56**, which is similar to compound **21**. The synthesis can be scaled up to tens of grams in an overall yield of 24%.[21]

Scheme 4.7. Syntheis of NO_2-dichloroBODIPY **56**.

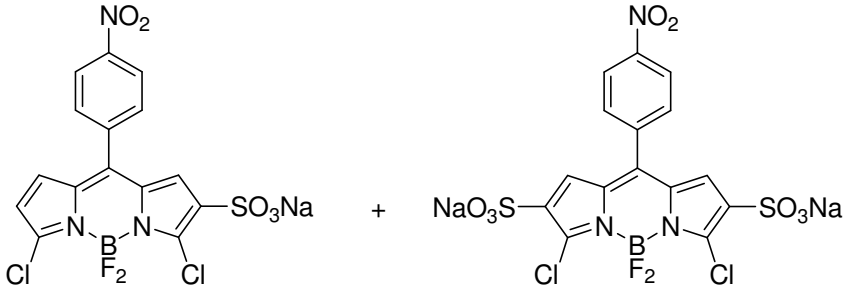


Scheme **4.8.** shows a sulfonation of the relatively electron-poor BODIPY system **56** with varying equivalents of chlorosulfonic acid. A mixture of mono- **57** and di-sulfonation **58** products formed if less than 3.5 equivalents of the sulfonating agents were used, and neither of these materials 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.



56



57 **58**

amount of $ClSO_3H$ (eq)	yield of **57** (%)	yield of **58** (%)
1.2	90	0
2.0	68	21
3.0	22	74
3.5	0	97

4.2.2 Spectroscopic Studies

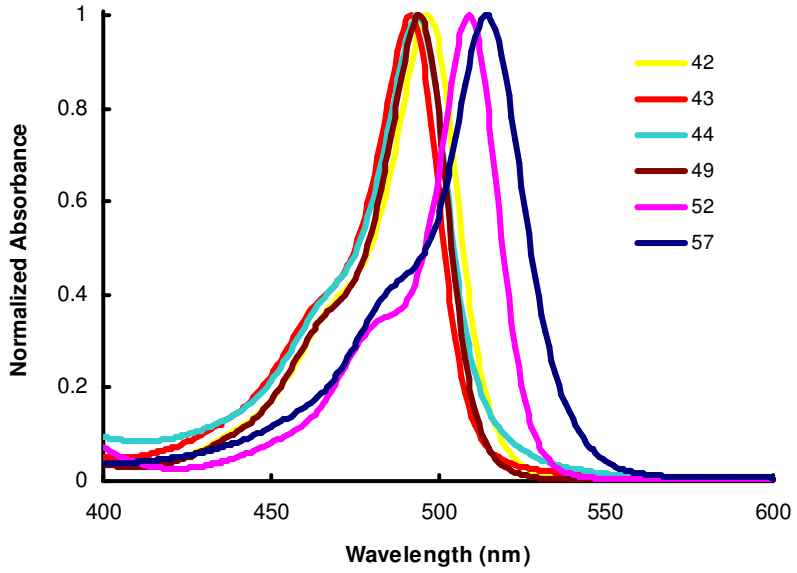
Absorption and emission spectra for all the BODIPYs were recorded in deionized water. All the compounds shown in Table 4.1 have absorption maxima in the range 492 – 518 nm, and their extinction coefficients are high (5.7 – 14.9 x 10^4 $M^{-1}cm^{-1}$), as is characteristic of BODIPY dyes in general. 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.

a



b

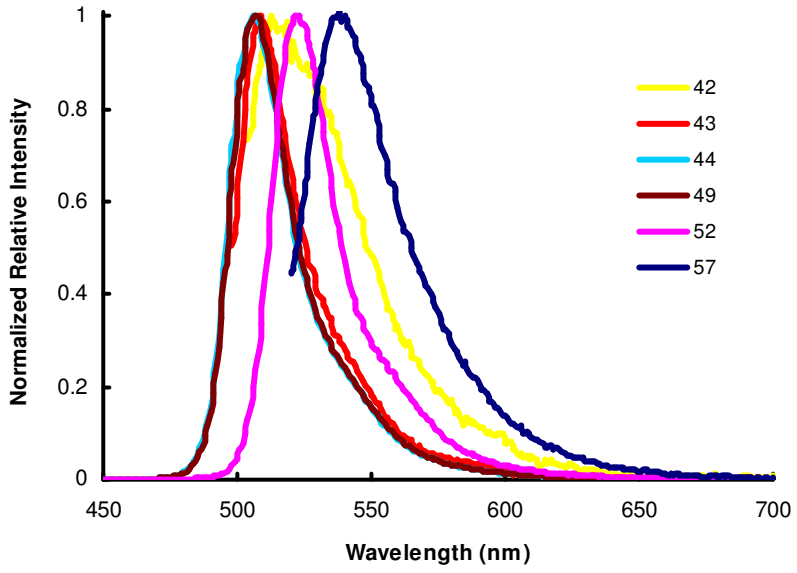
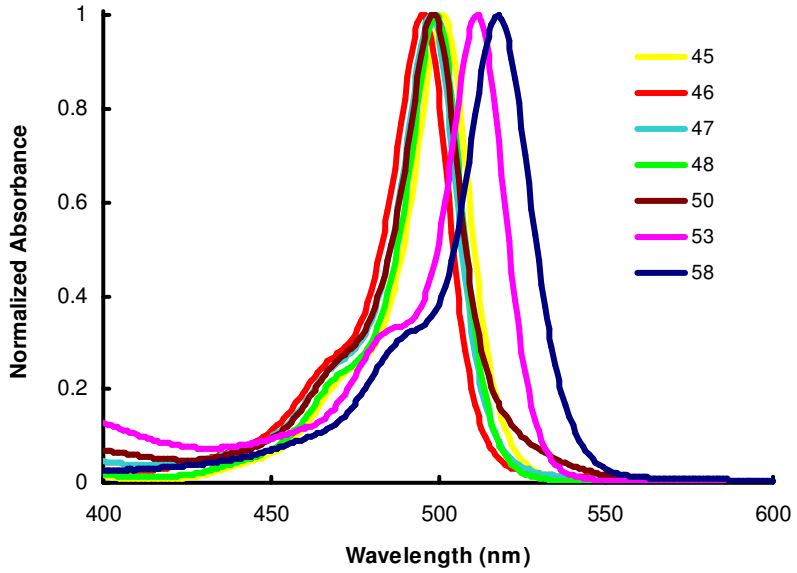


Figure 4.2. a) UV absorption, and **b)** fluorescence: spectra for the mono-sulfonated BODIPYs. All these spectra were recorded in deionized water at concentrations of approximately 10^{-6} M for the UV spectra 10^{-7} to 10^{-6} M for the fluorescence, then normalized.

c



d

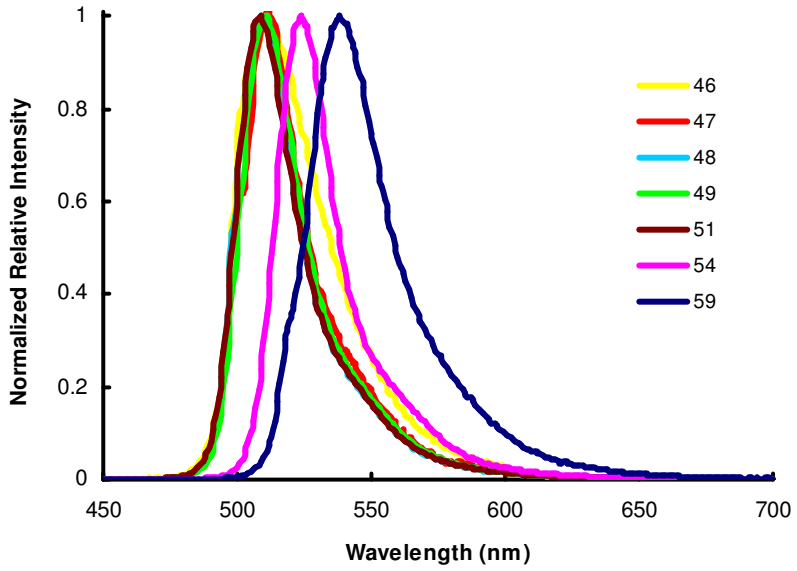


Figure 4.2. Continued. **c)** UV absorption, and **d)** fluorescence: spectra for the di-sulfonated BODIPYs. All these spectra were recorded in deionized water at concentrations of approximately 10^{-6} M for the UV spectra 10^{-7} to 10^{-6} M for the fluorescence, then normalized.

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 Φ_{st} is the reported quantum yield of the standard, I is the integrated emission spectrum, A is the absorbance at the excitation wavelength and η is the refractive index of the solvents used. X subscript denotes unknown, and st denotes standard. Fluorescein (Φ = 0.92 in 0.1 M $NaOH_{aq}$) and Rhodamine 6G (Φ = 0.94 in ethanol) were used as standards.[47]

Table 4.1. Special characteristics of dyes in H_2O

dye	λ_{abs} [a] (nm)	ε [a] ($M^{-1}cm^{-1}$)	$\lambda_{emi.}$ [a] (nm)	fwhm [a] (nm)	Φ [a]
42	497	58130	513	48	≤ 0.001 [b]
43	492	85340	507	58	≤ 0.001 [b]
44	494	69840	507	26	0.34 ± 0.03 [b]
45	501	92030	511	36	≤ 0.002 [b]
46	496	114820	511	25	≤ 0.001 [b]
47	498	77360	509	46	0.15 ± 0.01 [b]
48	498	80290	511	27	0.49 ± 0.05 [b]
49	494	149640	507	27	0.47 ± 0.05 [b]
50	498	99640	509	27	0.34 ± 0.04 [b]
52	509	73280	523	28	0.27 ± 0.03 [c]
53	512	78300	524	25	0.41 ± 0.04 [c]
57	514	66340	540	42	≤ 0.002 [c]
58	518	57000	538	35	≤ 0.008 [c]

[a] In H_2O. [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.

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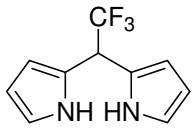
APPENDIX A

EXPERIMENTAL DATA FOR CHAPTER II

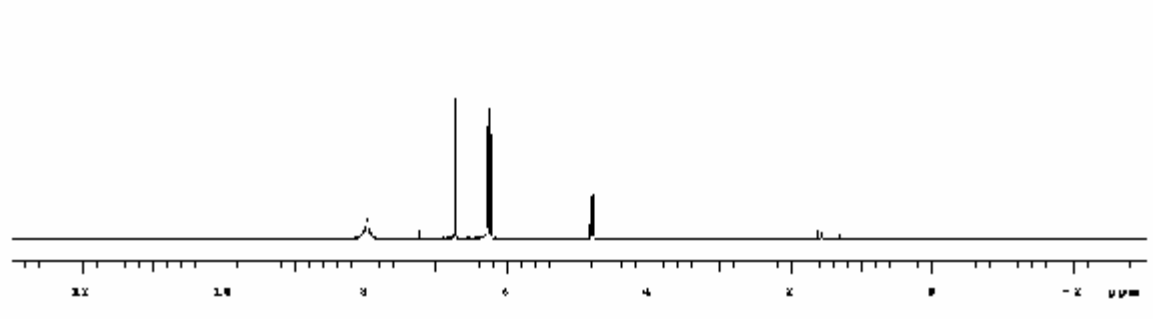
General Experimental Procedures. All chemicals were obtained from commercial suppliers and used without further purification. Chromatography on silica gel was performed using a forced flow of the indicated solvent on EM reagents silica gel 60 (230-400 mesh). ^{1}H NMR spectra were recorded at room temperature and chemical shifts are reports in ppm from the solvent resonance ($CDCl_3$ 7.24 ppm and CD_3OD 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_3OD 49.0 ppm). Mass spectra were measured under ESI condition.

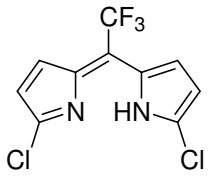


14

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_2Cl_2 was added to the residue. After washing with sat. sodium bicarbonate aqueous (2 x 100 ml) and H_2O (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_2Cl_2/hexane to afford white solid (4.34 g, 48 %), which should be stored at 0 ^oC. R_f = 0.5 (2:1 CH_2Cl_2/hexane). ^1H 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, $^3J_{HF}$ = 9.0 Hz).

^1H NMR



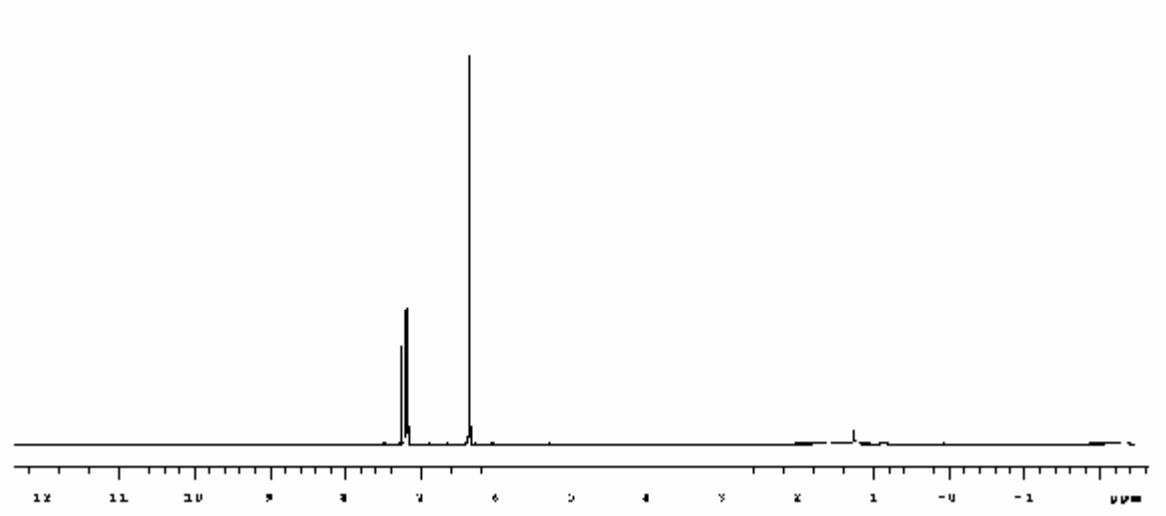


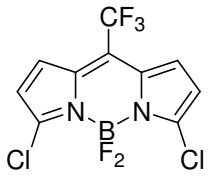
15

A solution of **14** (4.34 g, 20.3 mmol) in 150 ml dry THF was purged with N_2 and cooled to -78°C. A suspension of N-chlorosuccinimide (5.7 g, 42.6 mml) 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. H_2O (50 ml) was added to the mixture. After extraction with CH_2Cl_2 (3 x 100 ml), the combined organic layers were dried over anhydrous Na_2SO_4, 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 CH_2Cl_2. 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). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (m, 2H), 6.35 (d, 2H, J = 4.4 Hz).

^{1}H NMR

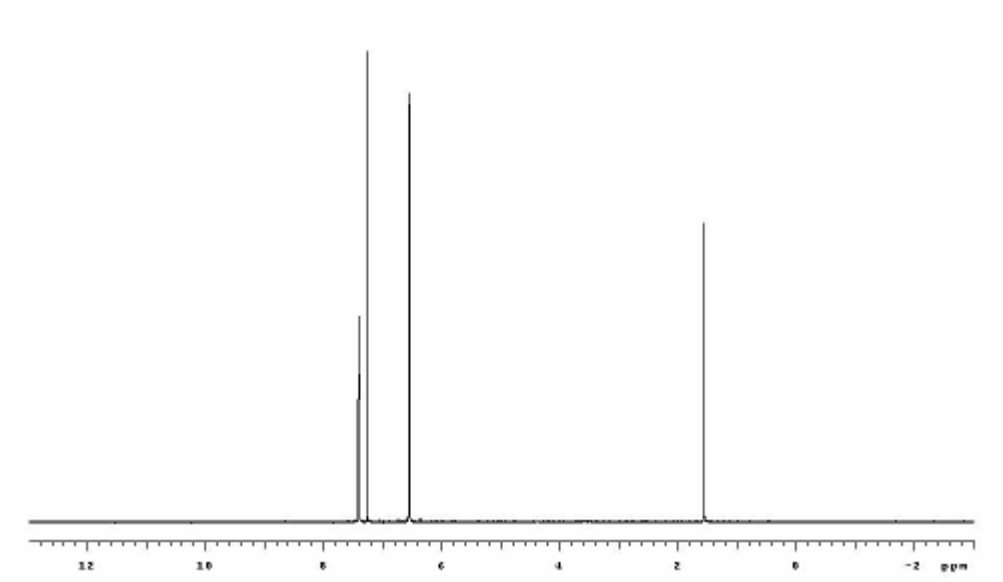




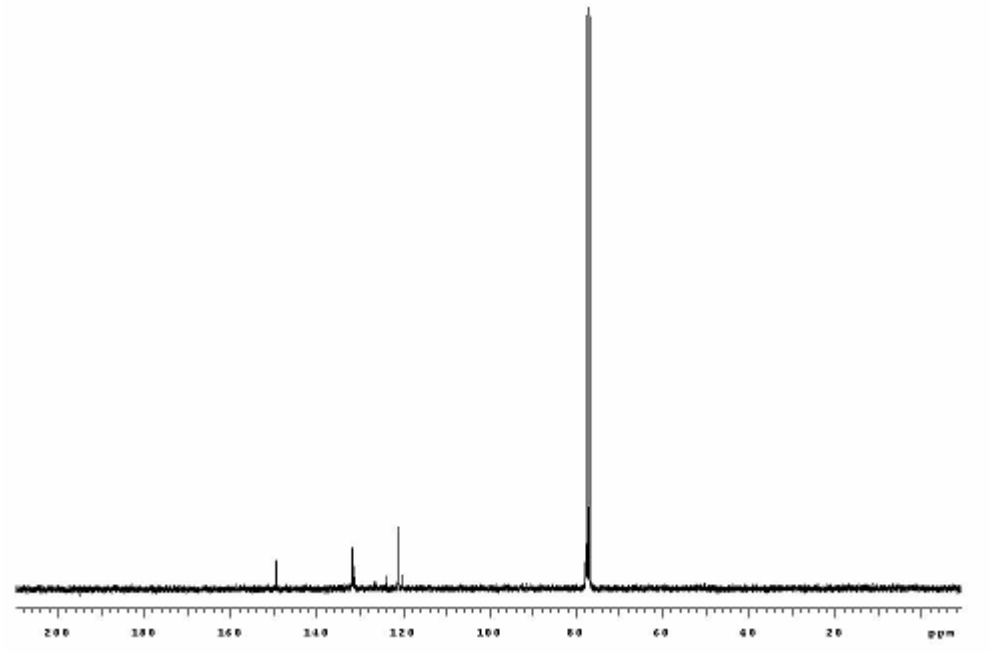
16

A solution of **15** (2.54 g, 9 mmol) and triethylamine (7.6 ml, 6 eq) in 150 ml dry CH_2Cl_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_2SO_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 $C_{10}H_4BCl_2F_5N_2^+$ (M^+) 327.9765 found 327.9766; IR (thin film) 3177, 2928, 1572, 1394, 1279, 1220, 1122, 1104, 986, 773, 725 cm^{-1}.

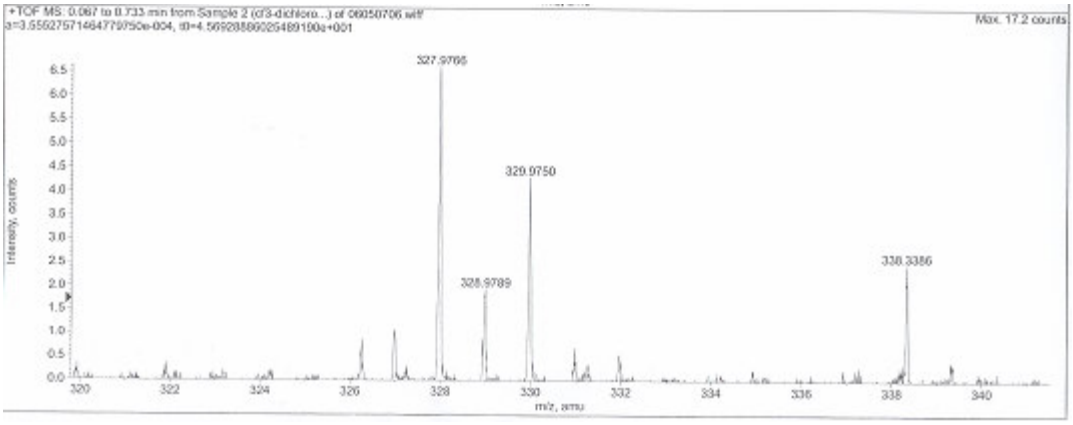
^{1}H NMR

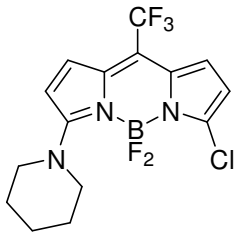


^{13}C NMR



Mass spectrum

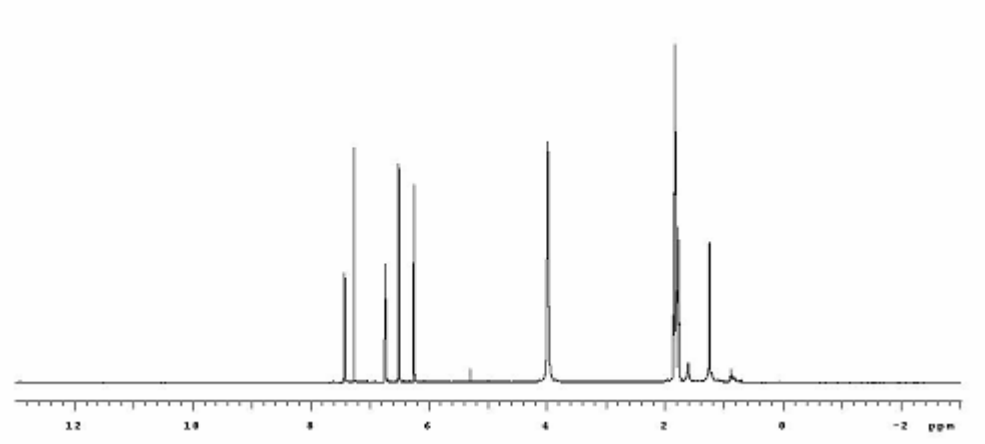




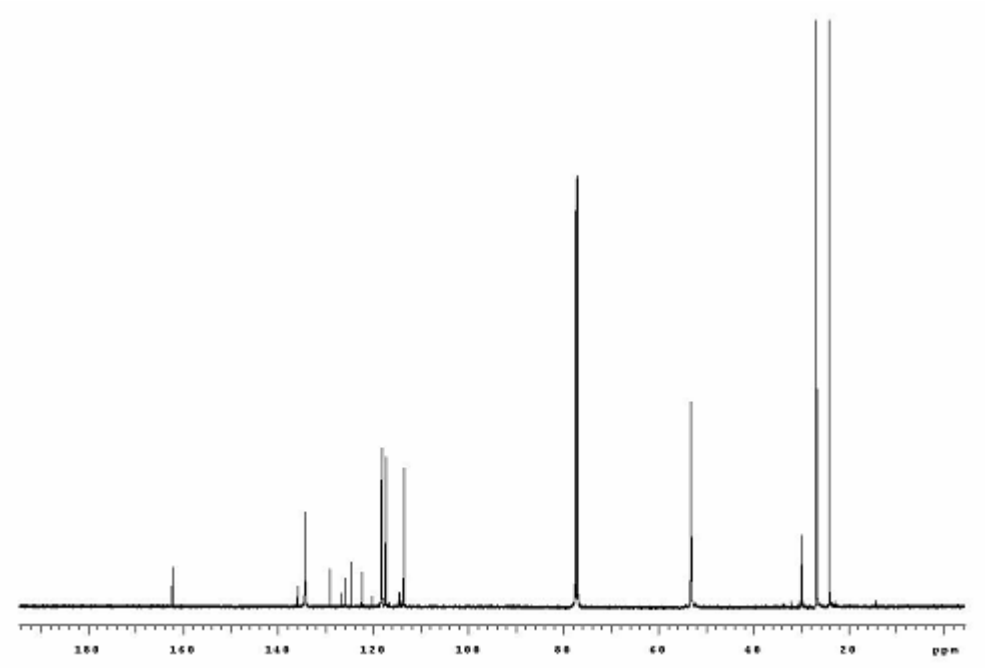
17

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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 122.48 (s), 43.01 (q). MS (ESI) calcd for $C_{15}H_{15}BClF_5N_3^+$ $(M + H)^+$ 378.0968 found 378.1056.

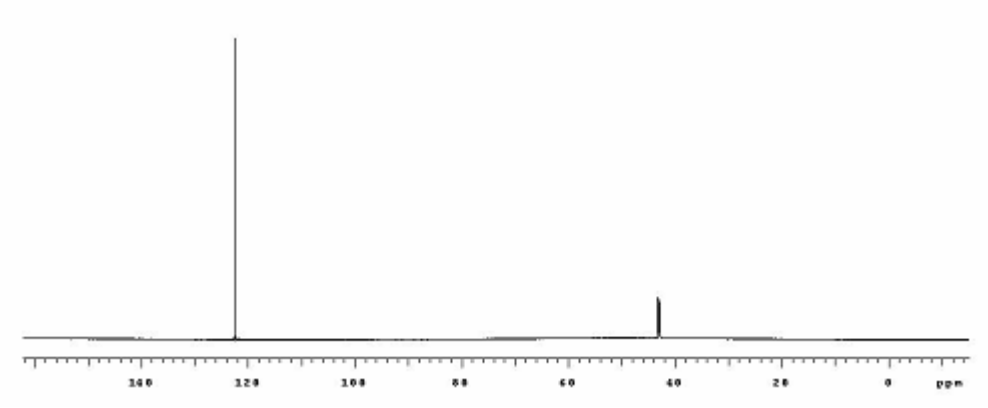
^{1}H NMR



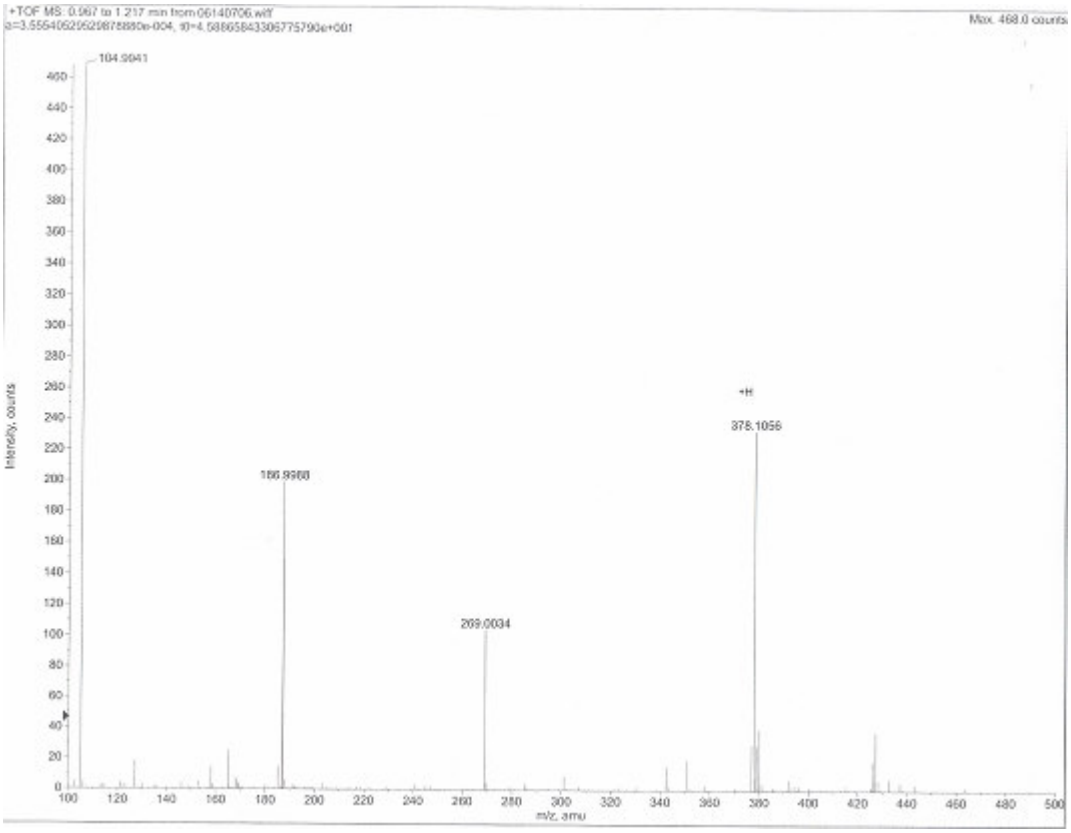
^{13} C NMR

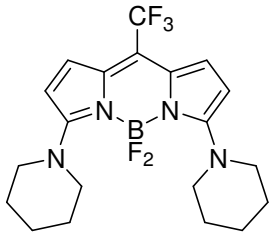


^{19}F NMR



Mass spectrum

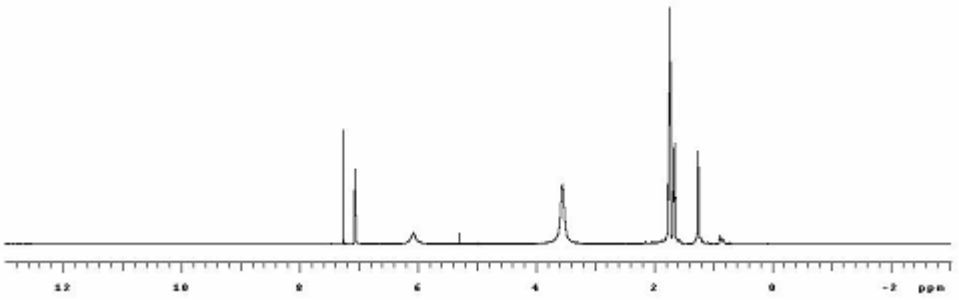




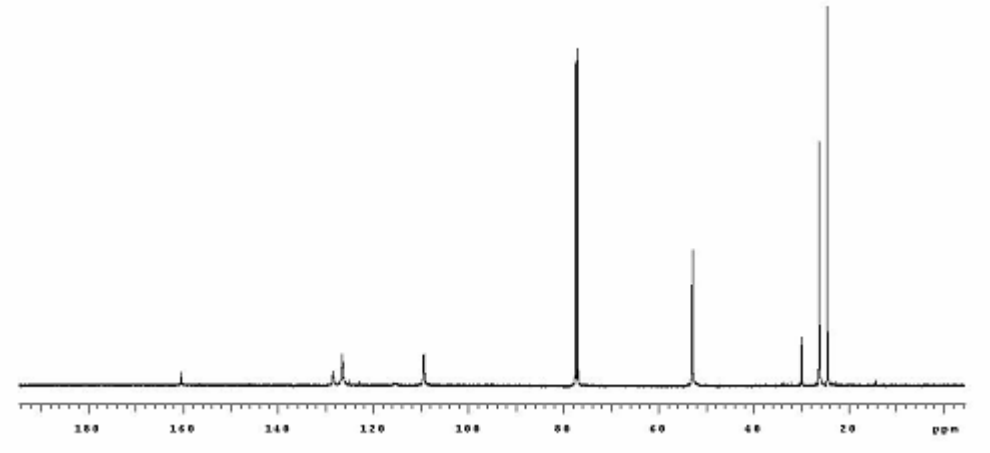
18

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, $CDCl_3$) δ 7.07 (m, 2H), 6.08 (br, 2H), 3.55 (br, 8H), 1.81-1.61 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.5, 128.6, 126.5, 109.5, 52.8, 26.2, 24.5; MS (ESI) calcd for $C_{20}H_{24}BF_5N_4^+$ (M^+) 426.20 found 426.21.

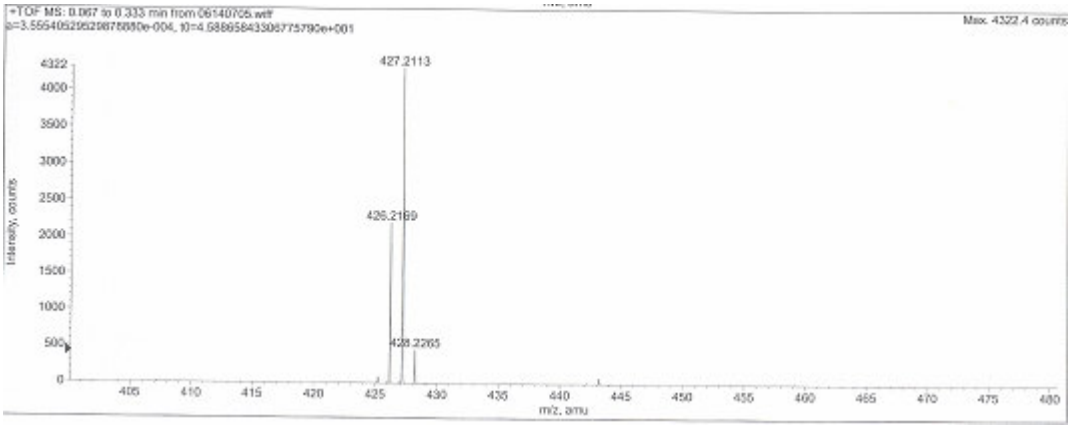
^{1}H NMR

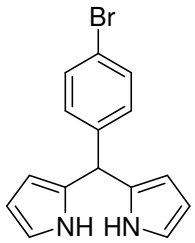


^{13}C NMR



Mass spectrum

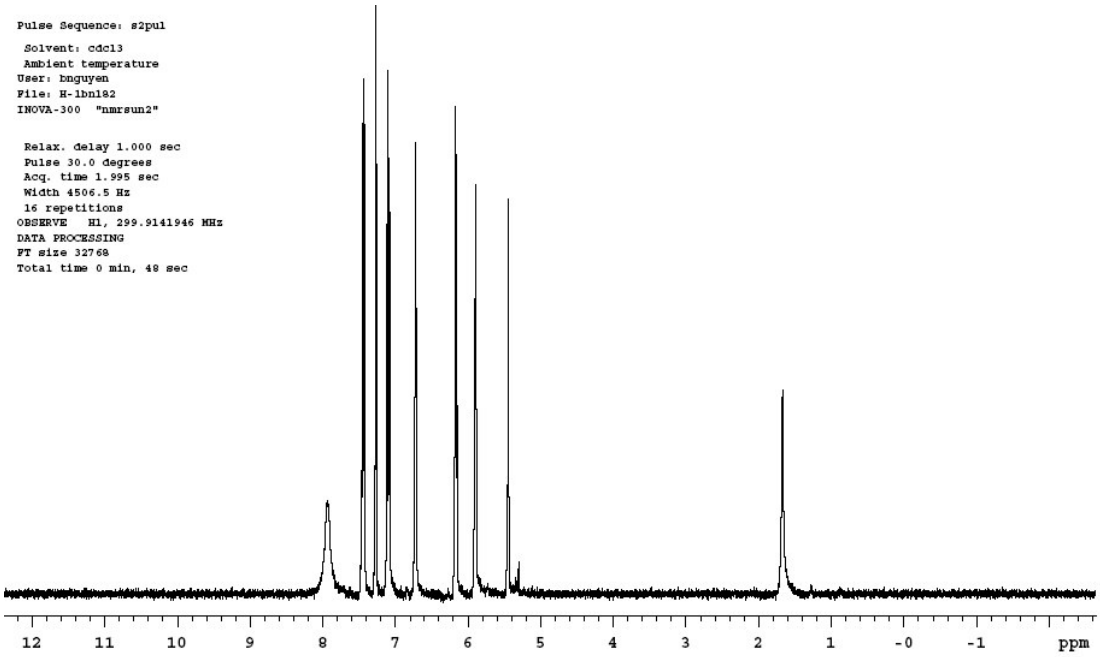




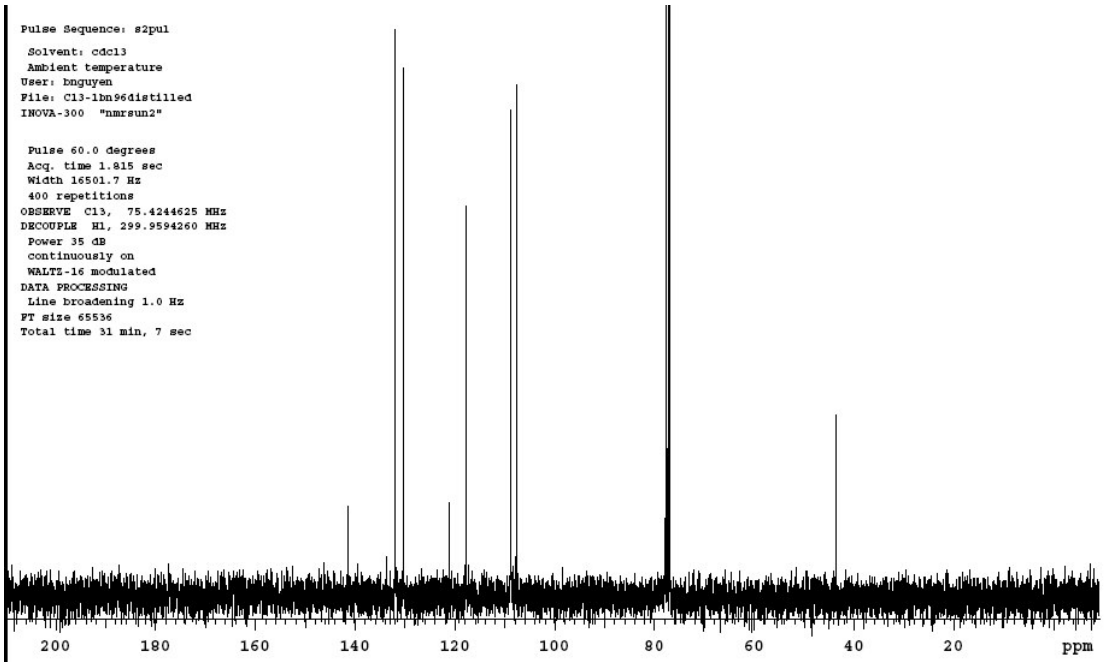
19

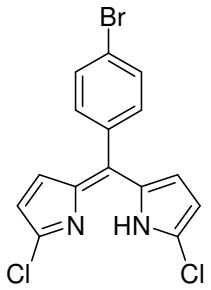
Pyrrole (25 eq) and 4-bromobenzaldehyde (21.4 g, 116 mmol) were added to a 500 ml dry round-bottomed flask and degassed with a stream of N_2 for 5 min. TFA (0.1 ml) was then added. The solution was stirred under N2 at room temperature for 1 h and then excess pyrrole was removed under reduced pressure. The residue was recrystallized in the minimal hexane and dichloromethane mixture to give a white solid (11.7 g, 34%). ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.3, 132.0, 131.8, 130.3, 121.0, 117.6, 108.7, 107.6, 43.6; MS (ESI) calcd for $C_{15}H_{14}BrN_2^+$ $(M+H)^+$ 301.03 found 301.02.

^{1}HNMR



^{13}C NMR



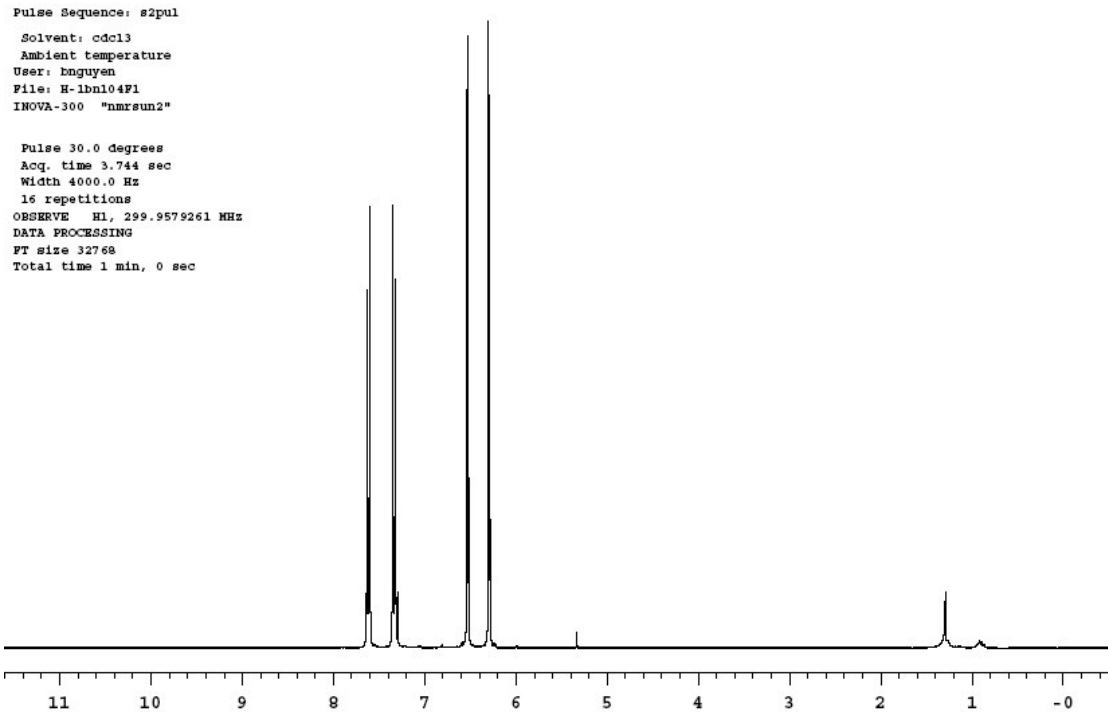


20

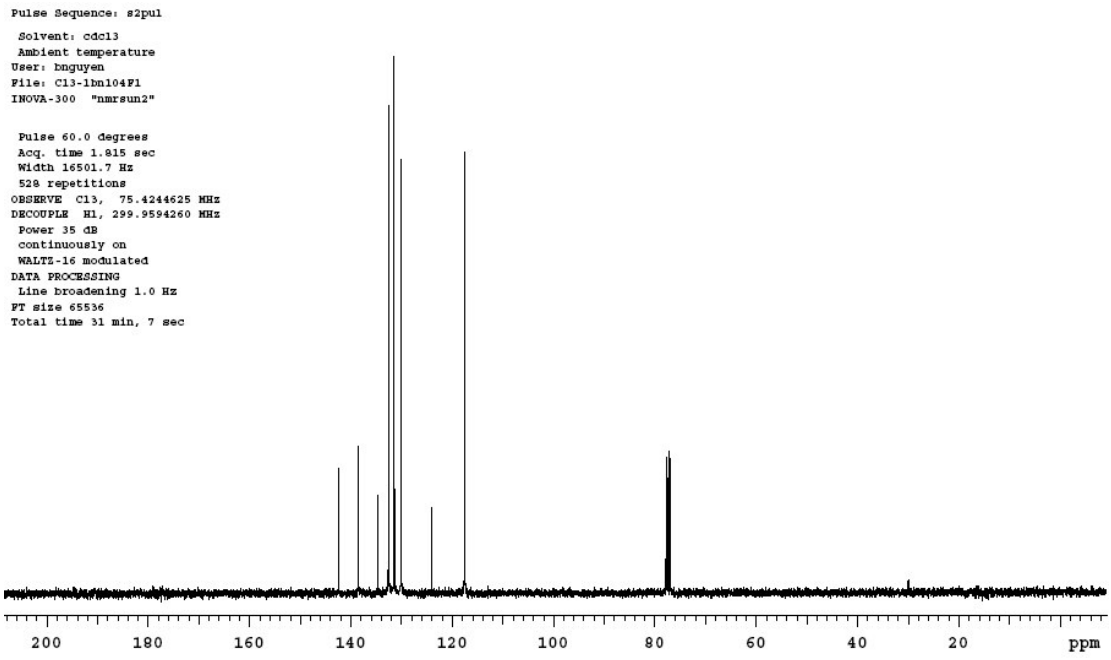
A solution of **19** (7.84 g, 26 mmol) in 200 ml dry THF was purged with N_2 and cooled to -78^{o}C. A suspension of N-chlorosuccinimide (2.2 eq) in 60 ml THF was added to the cooled solution. The reaction mixture was stirred at -78^{o}C for 1.5 h, then warmed to room temperature and stirred for additional 3 h. H_2O (100 ml)was added to the mixture. After extraction with CH_2Cl_2 (3 x 100 ml), the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solution was evaporated to dryness. The residue was used for oxidation immediately without further purification.

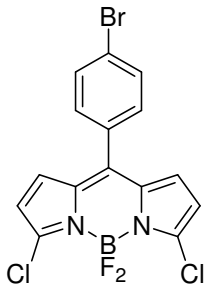
DDQ (2.8 g, 12.3 mmol) was added to the solution of dichloro-dipyrromethane as generated above in 250 ml CH_2Cl_2. The mixture was stirred at the room temperature for 1 h. After evaporation the solvent, the residue was applied to a silica gel flash column using hexane to afford the orange powder (5.08 g, 53 % for 2 steps). R_f = 0.7 (20% EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.2, 138.4, 138.3, 134.5, 132.4, 131.3, 129.9, 123.9, 117.4; MS (ESI) calcd for $C_{15}H_{10}BrCl_2N_2^+$ $(M+H)^+$ 366.9404 found 366.9403.

^{1}H NMR



^{13}C NMR

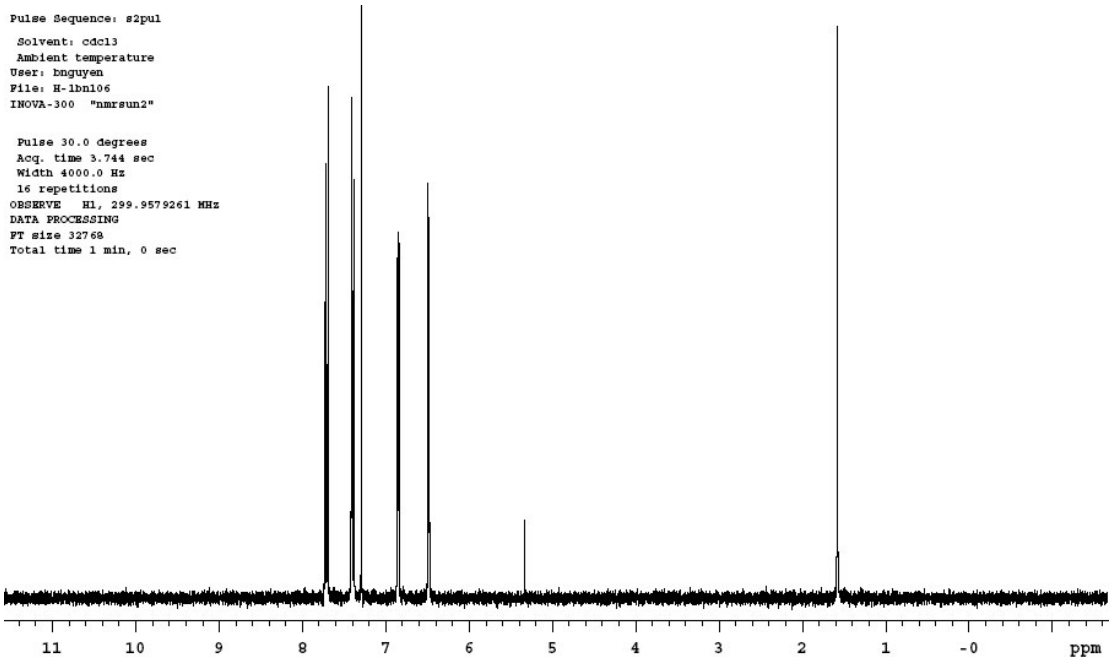




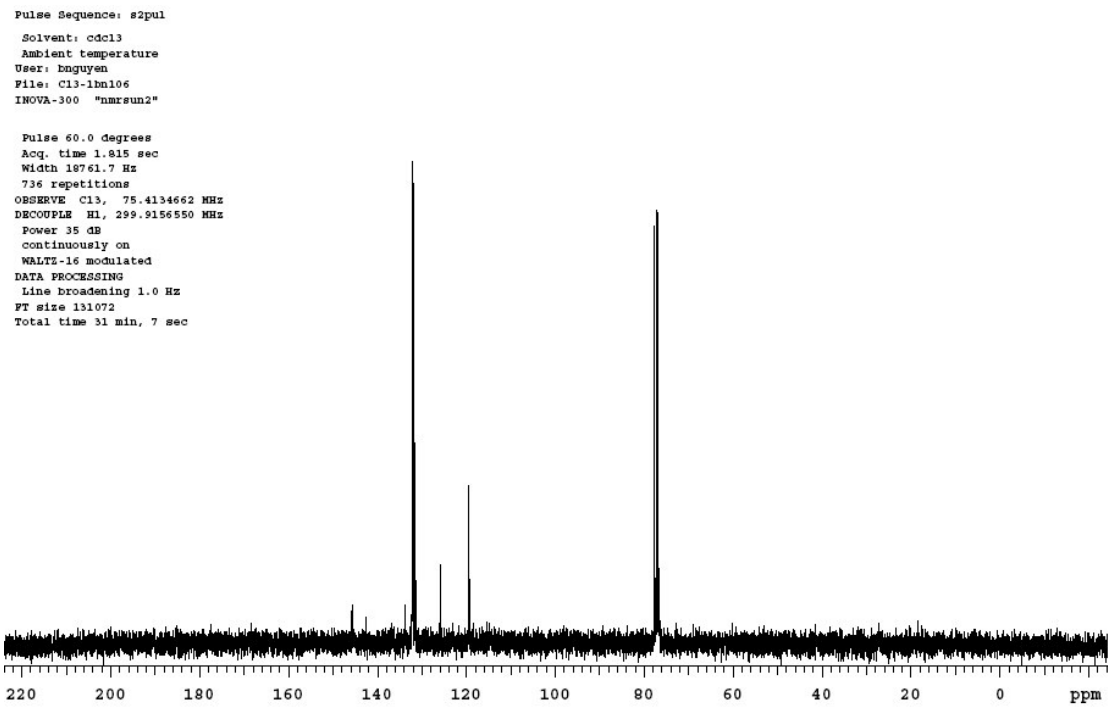
21

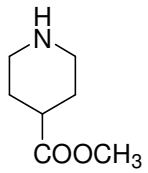
A solution of **20** (4.73 g, 13 mmol) and triethylamine (2.2 eq) in 120 ml dry CH_2Cl_2 was stirred at the room temperature for 10 min. Boron trifluoride etherate (4.3 eq) was added slowly over 10 min. After 24 h stirring, the resulting solution was washed with water (3 x 100 ml), dried over anhydrous Na_2SO_4, 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 hexance/dichloromethane as red crystals (5.21 g, 98 %). ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.6, 142.4, 133.6, 132.1, 131.9, 131.3, 125.8, 119.3; MS (ESI) calcd for $C_{15}H_9BBrCl_2F_2N_2^+$ $(M+H)^+$ 414.9838 found 414.9407. IR (thin film) 3135, 1569, 1542, 1391, 1261, 1199, 1107, 983, 728 cm^{-1}.

^{1}H NMR



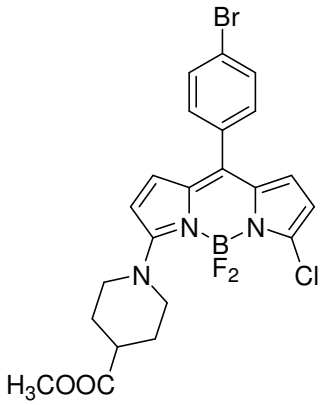
^{13}C NMR





22

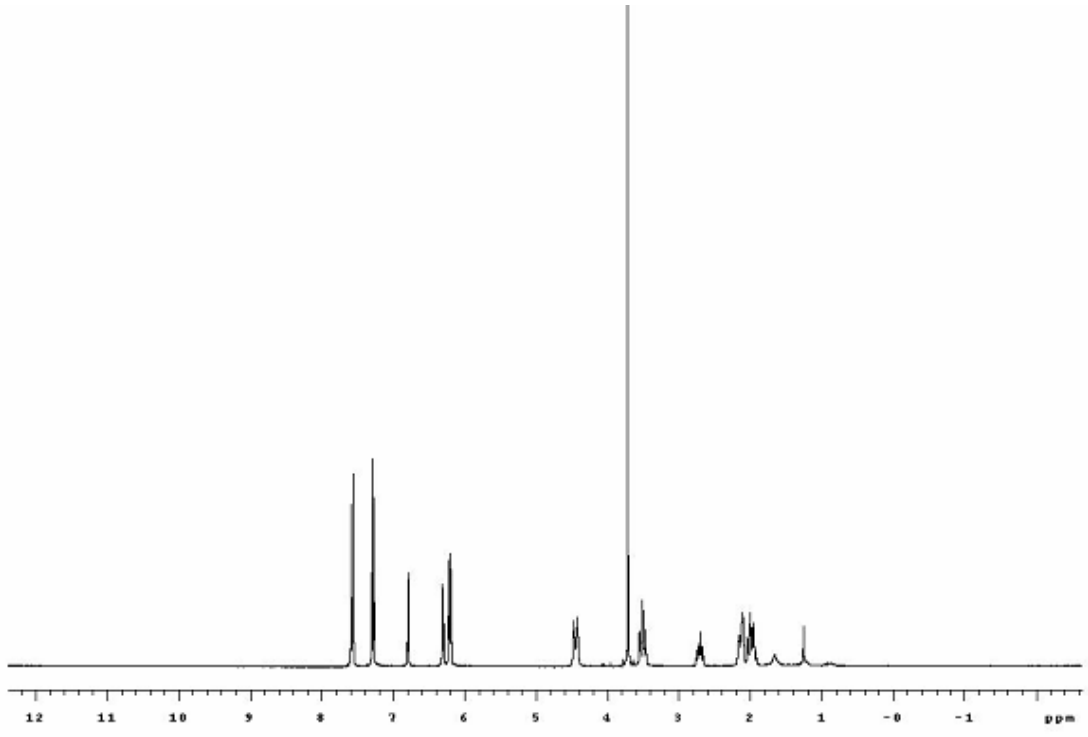
Thionyl chloride (4.22 ml, 57.9 mmol) was dropwise added to a solution of piperidine-4-carboxylic acid (5 g, 38.6 mmol) in MeOH (70 ml) and solution was heated to reflux for 12 hours. After cooling to 25 ^{0}C, the mixture was concentrated and 50 ml ether was added to solidify the salt. Dissolved the salt in 50 ml dichloromethane and washed with ammonium hydroxide (3 x 50 ml), 100 ml saturated NaCl, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a clear oil (3.2 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) 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.



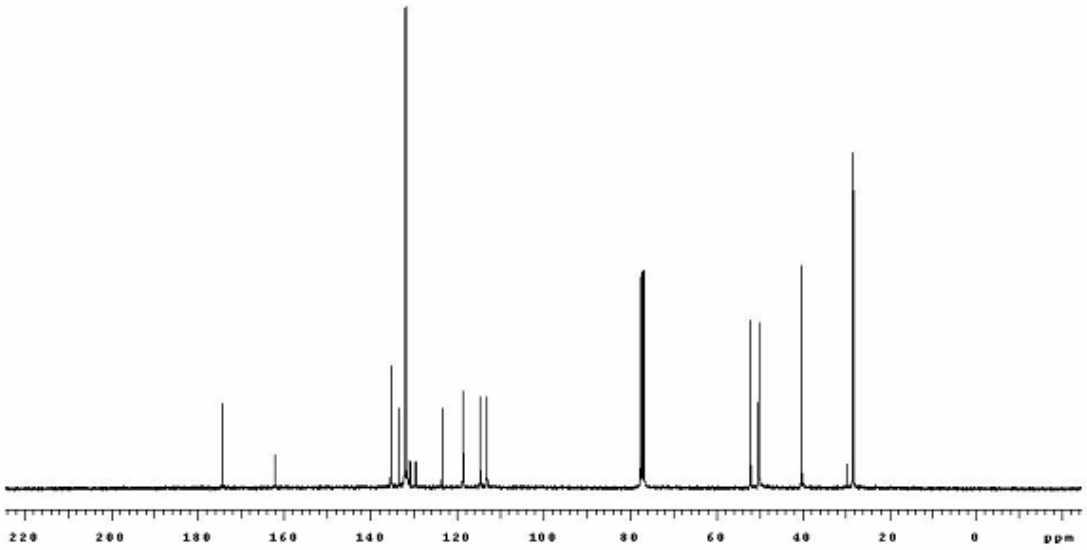
23

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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{22}H_{21}BBrClF_2N_3O_2^+$ $(M+H)^+$ 522.06 found 522.09.

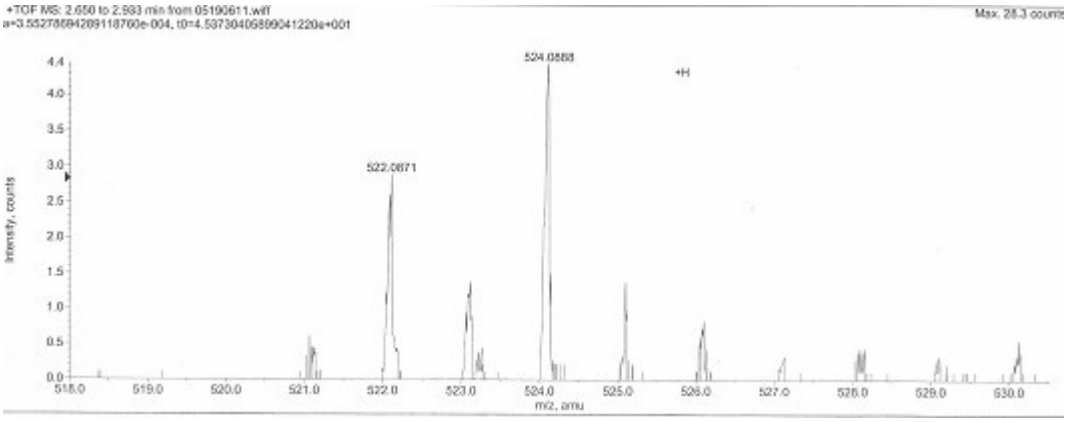
^{1}H NMR

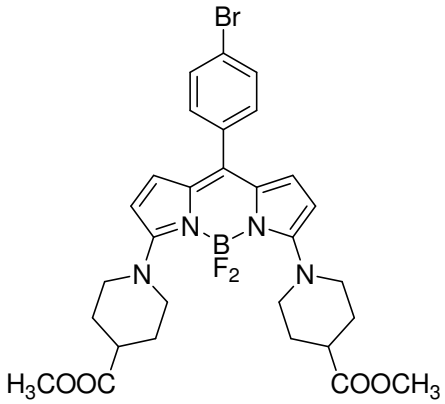


^{13}C NMR



Mass spectrum

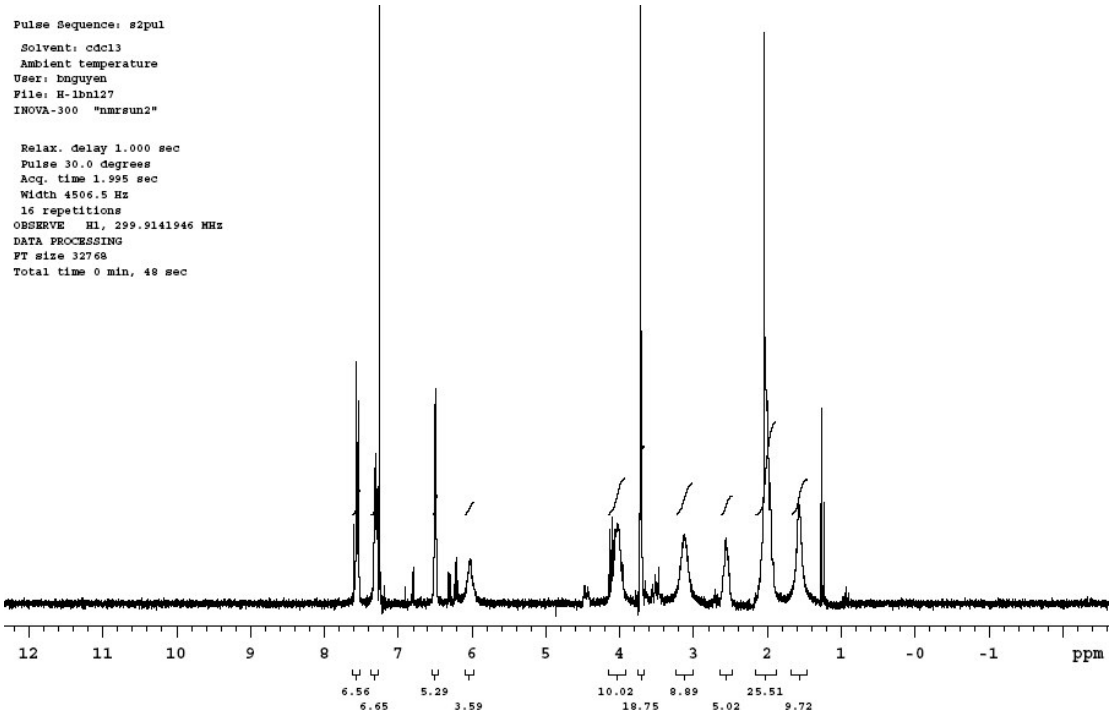




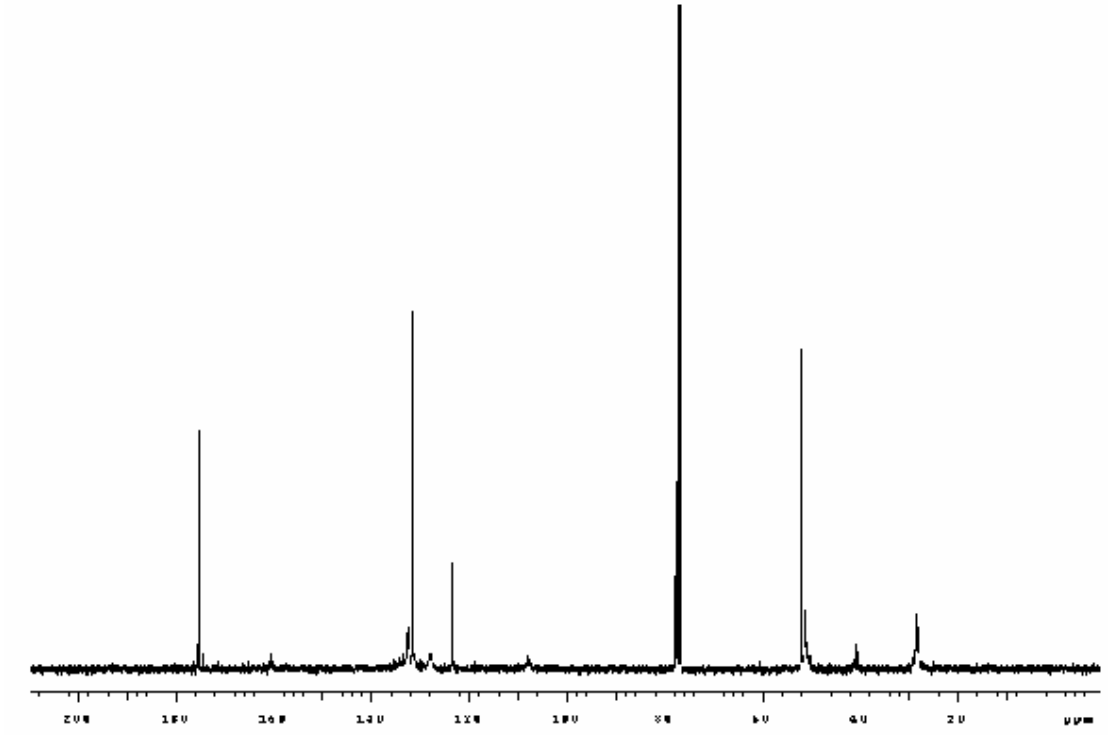
24

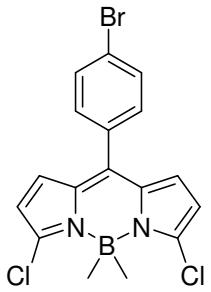
Compounds **21** (3.15 g, 7.6 mmol) and **22** (4 eq) were dissolved in dry MeCN in a dry round-bottom flask equipped with a condenser. The mixture was heated to reflux for 12 hours to produce a dark purple solution. The solvent was removed under reduced pressure and the residue was passed through a 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, 2 H, 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



^{13}C NMR

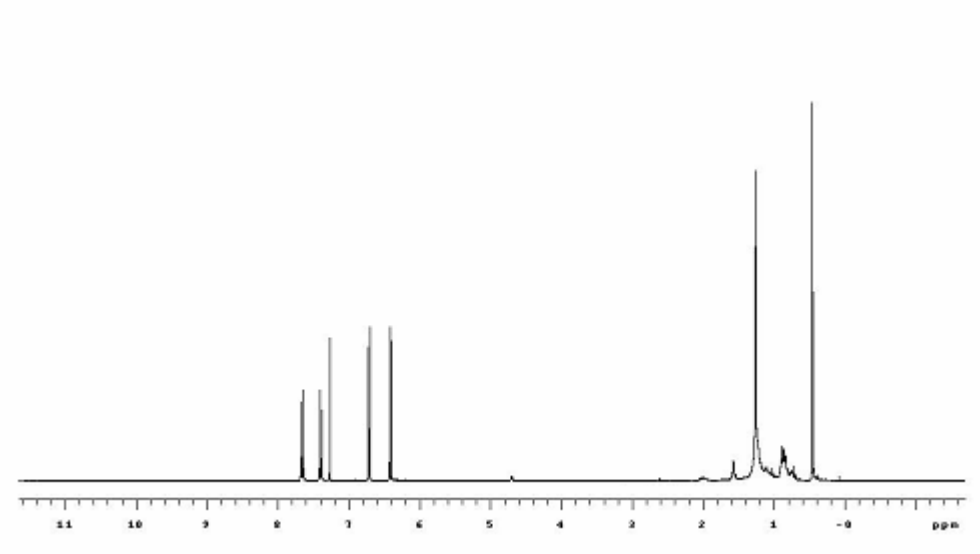




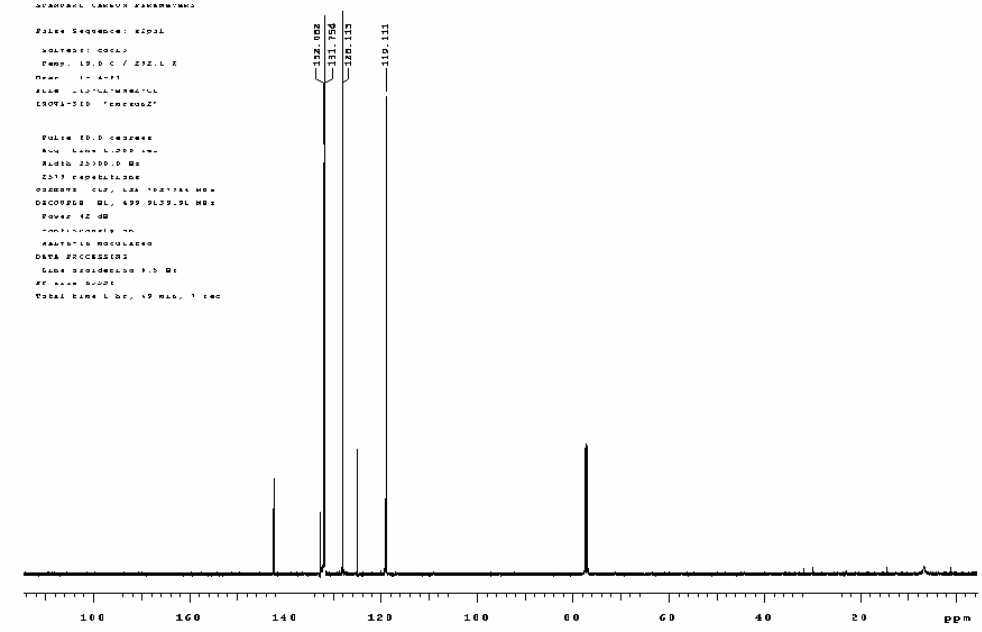
25

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 N_2. 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). ^{1}H NMR (300 MHz, $CDCl_3$) 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). ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.7, 142.1, 132.7, 132.1, 131.8, 128.1, 124.8, 119.1, 6.7. ^{19}F NMR (300 MHz, $CDCl_3$) showed no peaks at all; MS (ESI) calcd for $C_{16}H_{11}BBrCl_2N_2^+$ $(M\text{-}CH_3)^+$ 390.9576 found 390.9631.

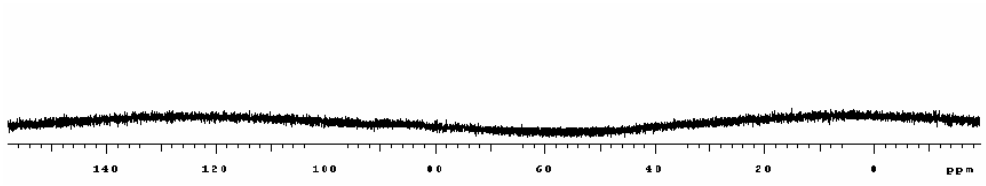
^{1}H NMR



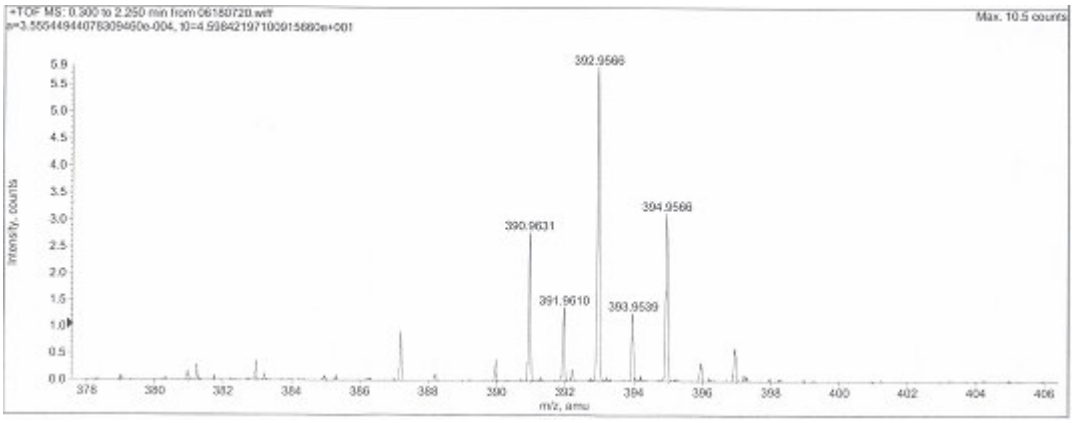
^{13}C NMR

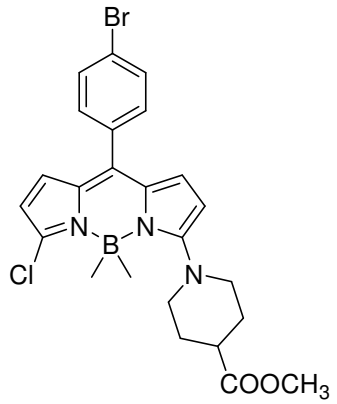


^{19}F NMR



Mass spectrum

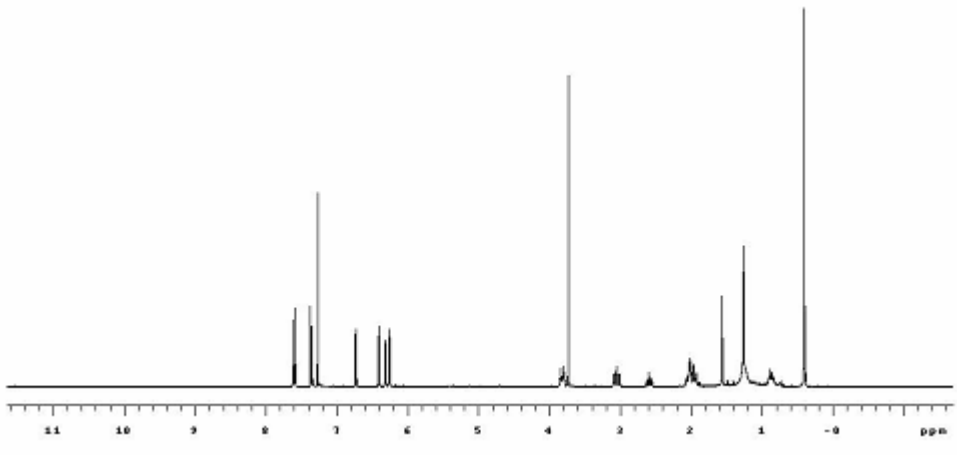




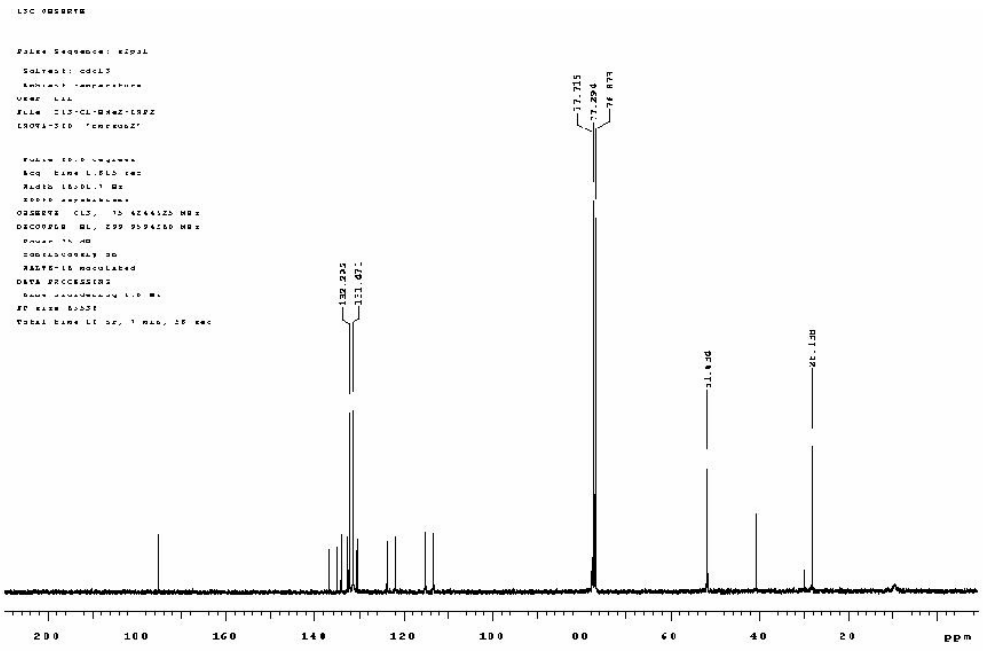
26

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, $CDCl_3$) δ 7.60 (d, 2H, J = 8.6 Hz), 7.36 (d, 2H, J = 8.6 Hz), 6.73 (d, 2H, J = 4.8 Hz), 6.41 (d, 2H, J = 4.3 Hz), 6.31 (d, 2H, J = 4.8 Hz), 6.26 (d, 2H, J = 4.3 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, $CDCl_3$) δ 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 $C_{24}H_{27}BBrClN_3O_2^+$ $(M+H)^+$ 514.1068 found 514.1118.

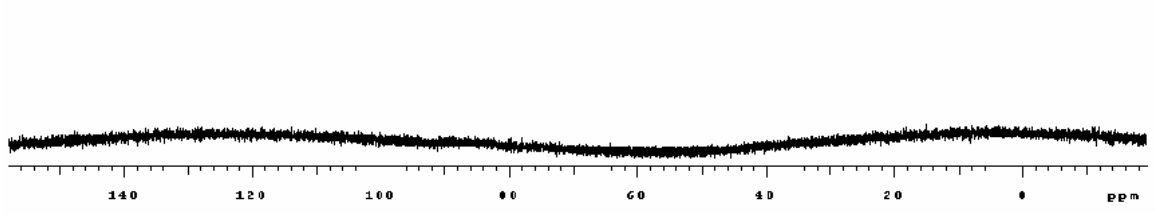
^{1}H NMR



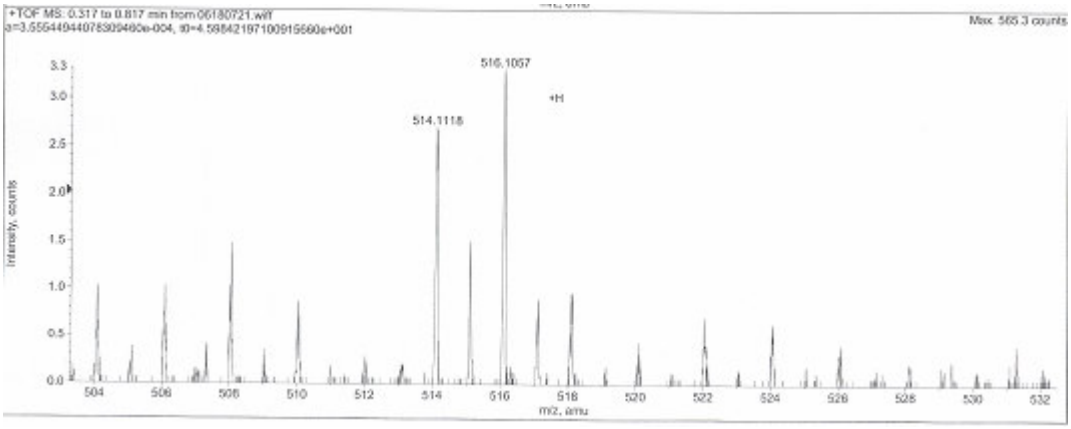
^{13}C NMR

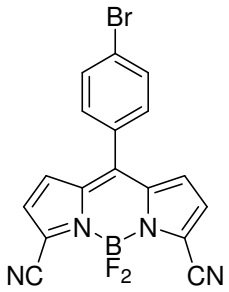


^{19}F NMR



Mass spectrum

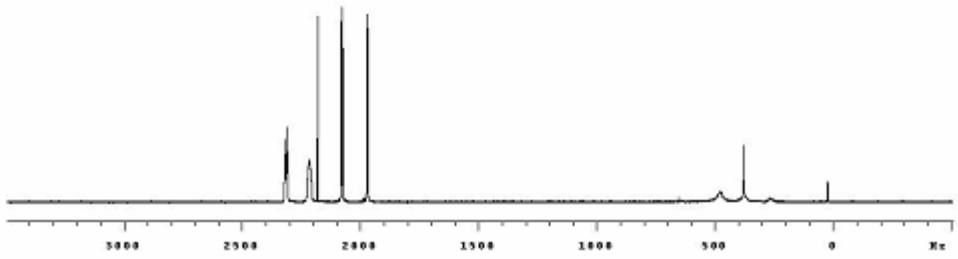




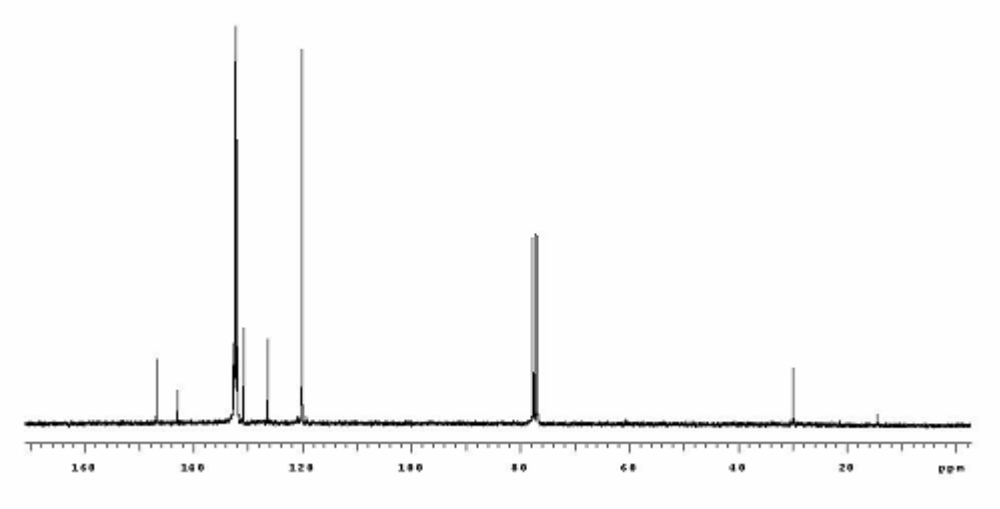
27

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 $C_{17}H_9BBrF_2N_4^+$ $(M+H)^+$ 397.01 found 396.95.

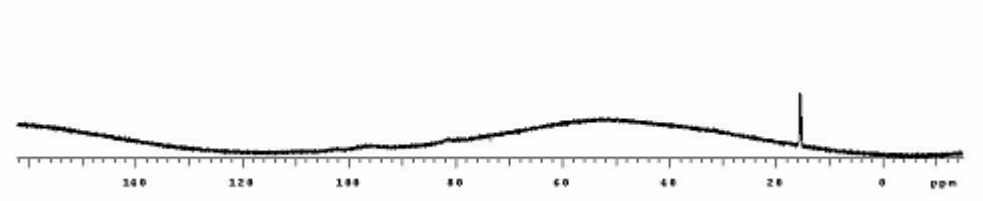
^{1}H NMR



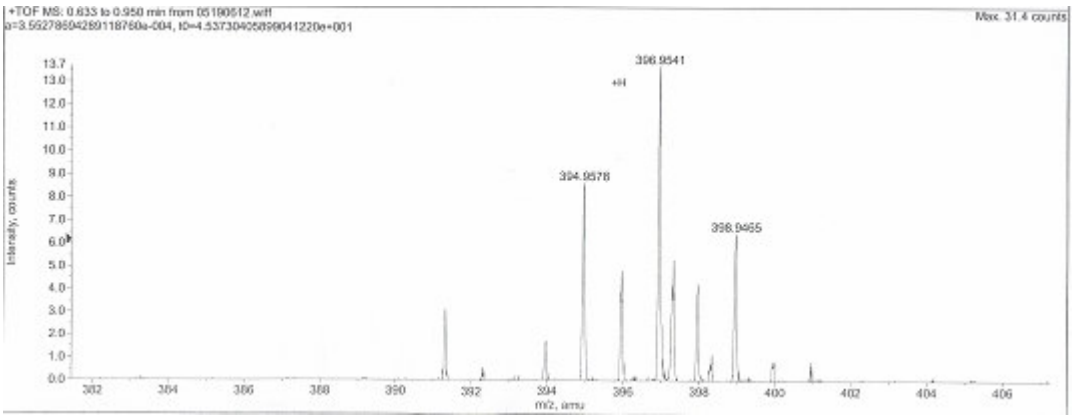
^{13}C NMR

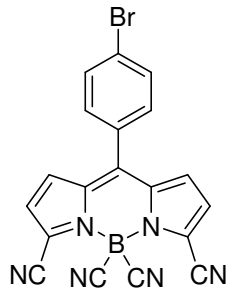


^{19}F NMR



Mass spectrum

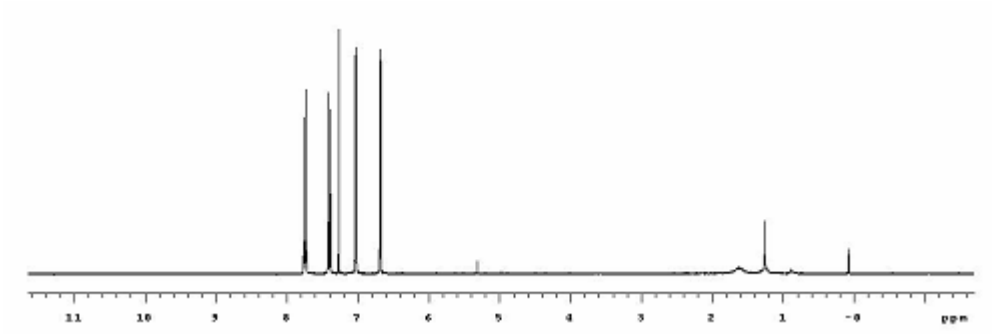




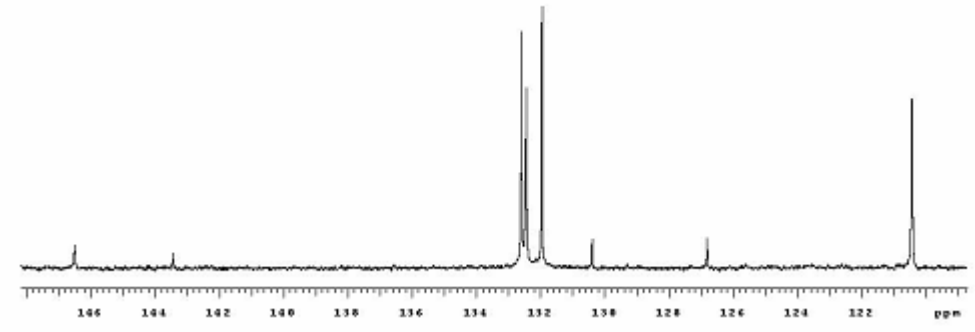
28

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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{19}H_9BBrN_6^+$ $(M+H)^+$ 411.02 found 411.00.

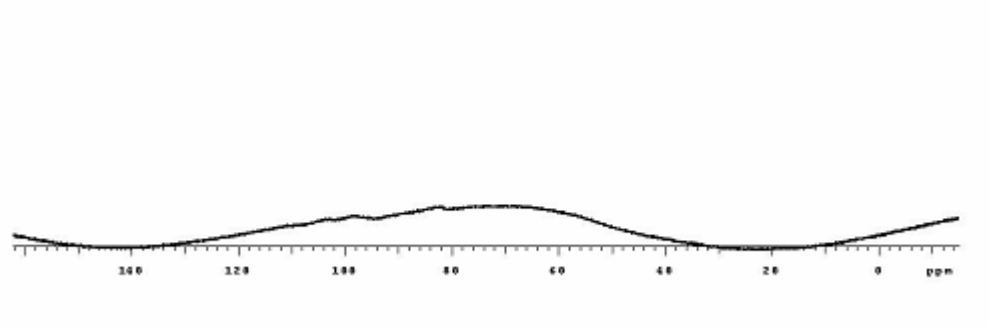
^{1}H NMR



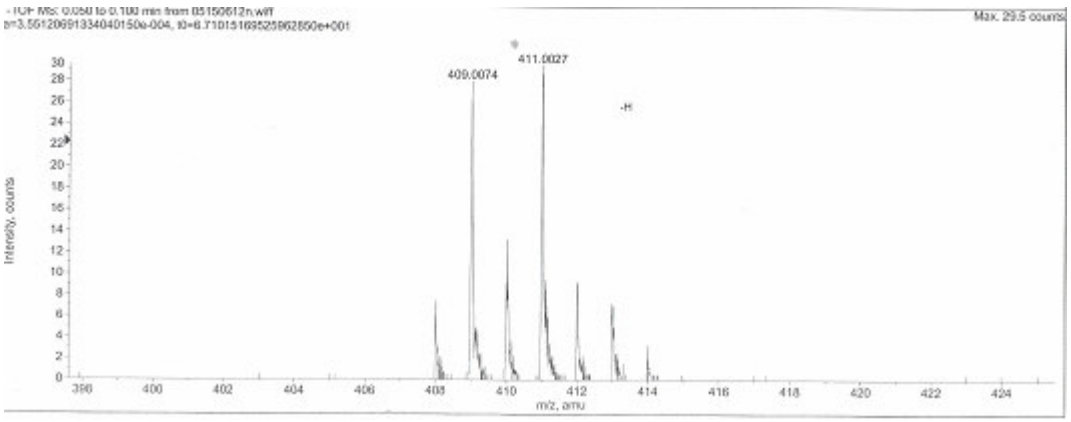
^{13}C NMR

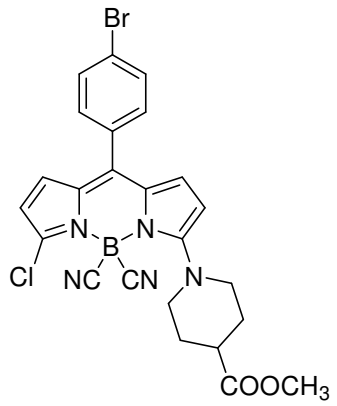


^{19}F NMR



Mass spectrum

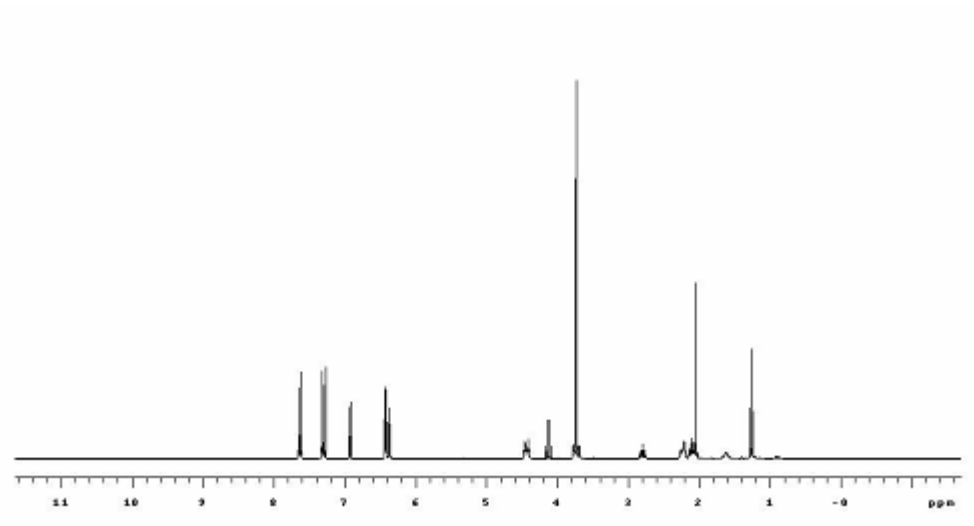




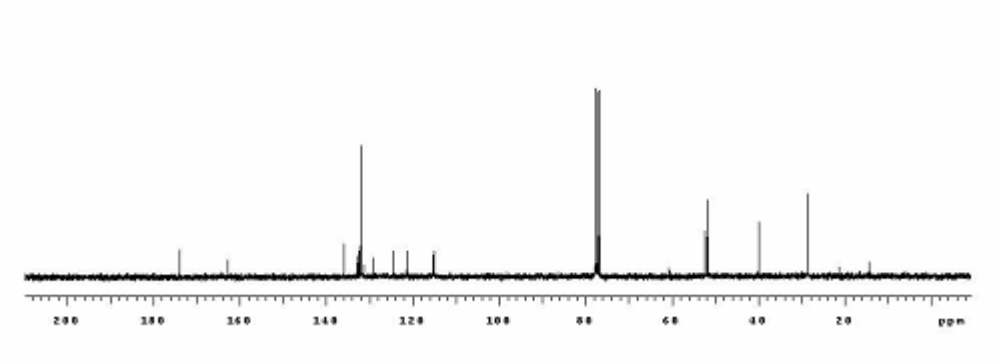
29

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/gexane 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 $C_{24}H_{20}BBrClN_5O_2Li^+$ $(M+Li)^+$ 542.07 found 542.06.

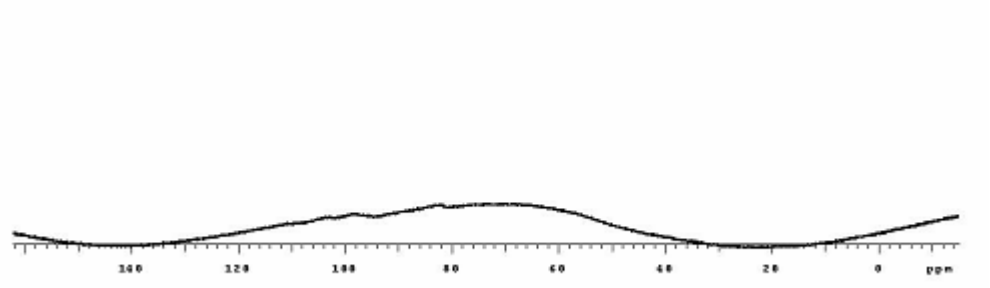
^{1}H NMR



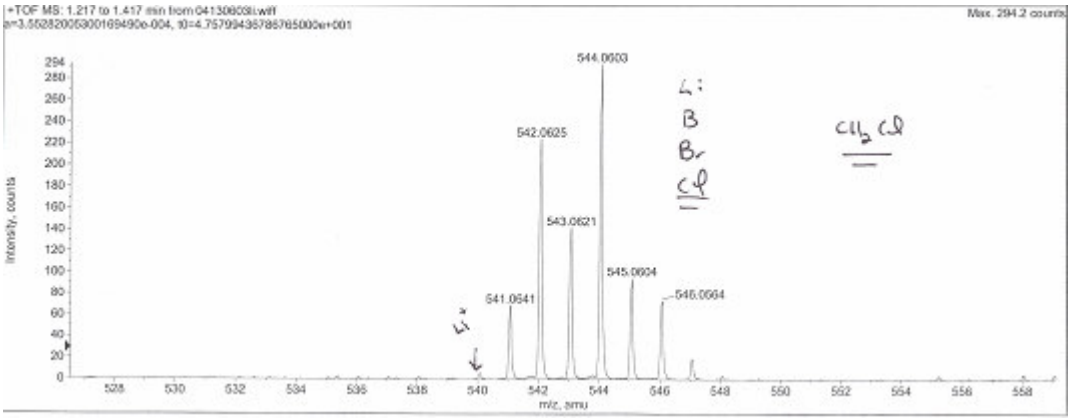
^{13}C NMR

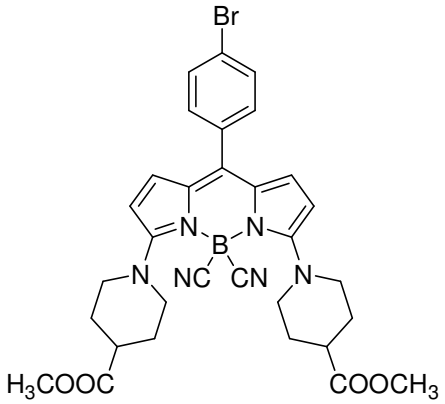


^{19}F NMR



Mass spectrum

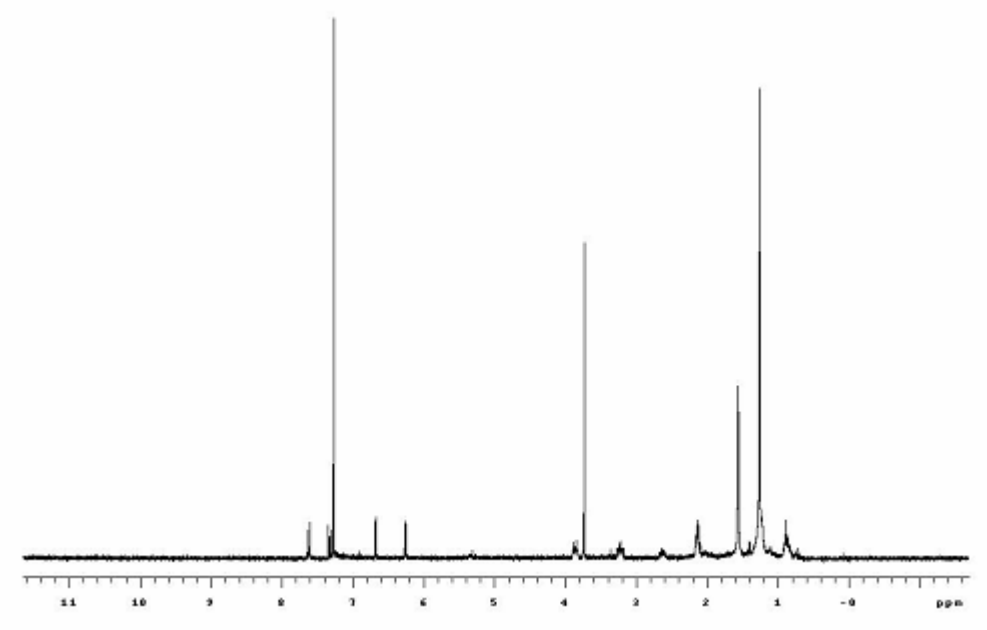




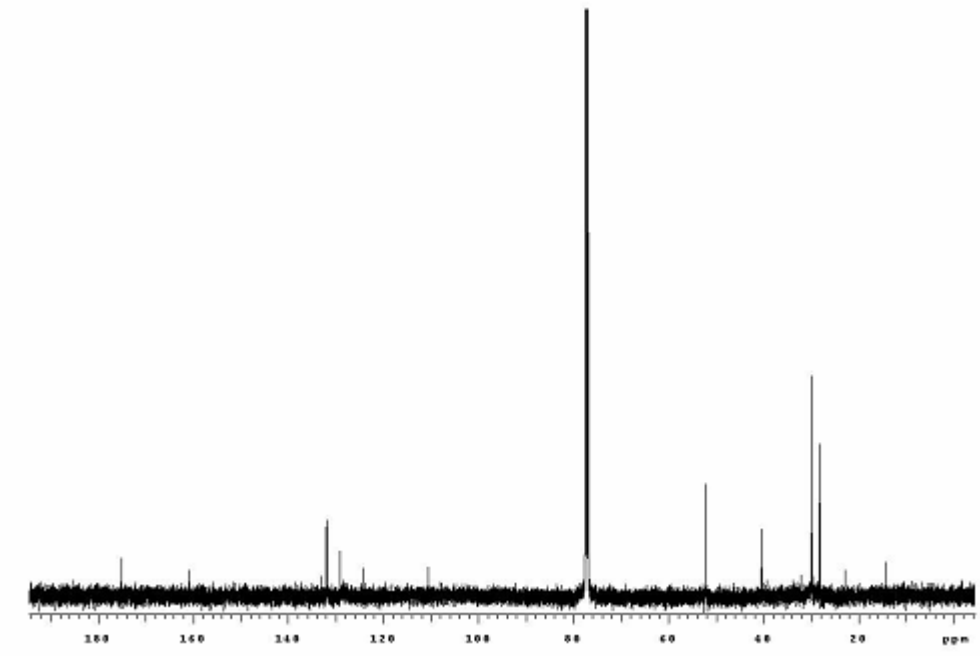
30

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 $C_{31}H_{33}BBrN_6O_4^+$ $(M+H)^+$ 643.1840 found 643.1380.

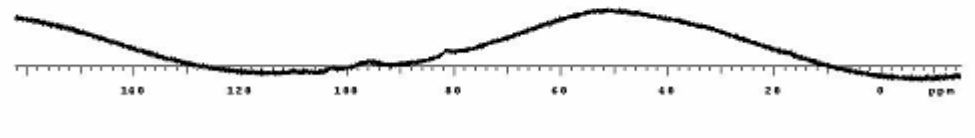
^{1}H NMR



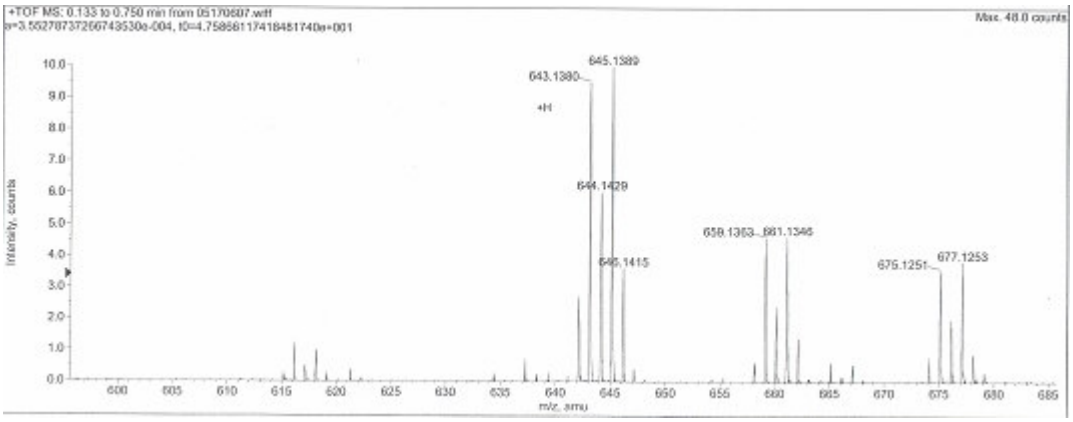
^{13}C NMR

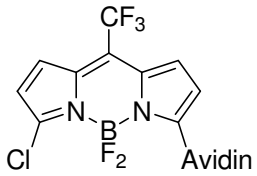


^{19}F NMR



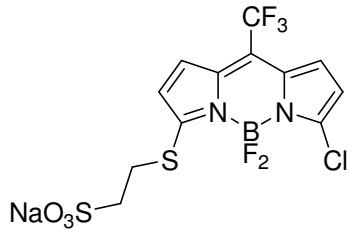
Mass spectrum





16-avidin

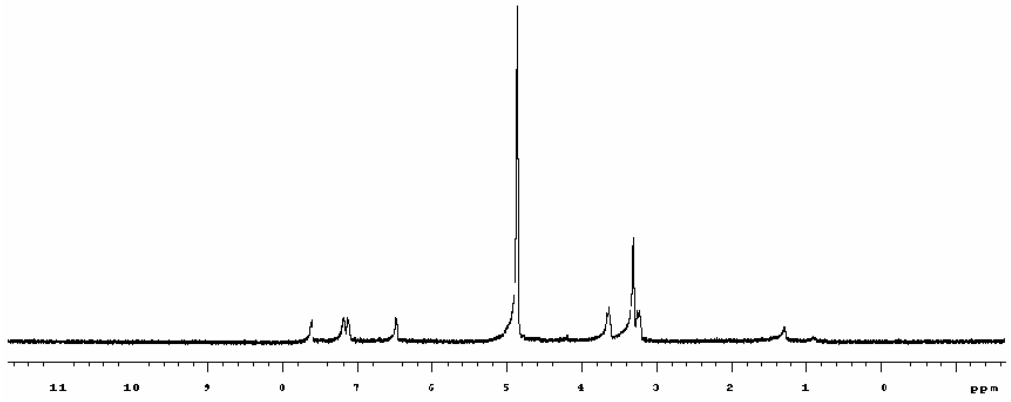
A solution of compound **16** (10 eq) in THF (20 μl) was added into a solution of avidin (2 mg) in 0.1 N $NaHCO_3$ buffer (pH = 8.3, 0.5ml). The solution was shaken in the dark for 1 h at room temperature. Then the residue was purified by PD10 desalting column.



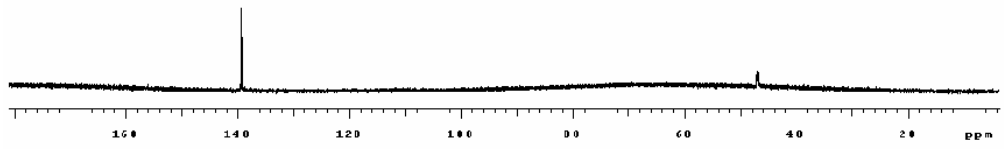
31

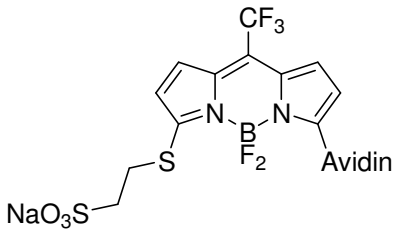
Sodium bicarbonate (2eq) was added to a solution of sodium 2-mercaptoethanesulfonate (50 mg) and compound **16** in THF/H_2O (2:1, 10 ml). The mixture was stirred at room temperature for 6 h. After removal the solvents, the residue was applied to a silica gel flash column using 10 % MeOH/CH_2Cl_2 to yield purple solid (80 mg, 58%). R_f = 0.3 (20% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, CD_3OD) δ 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_3OD) 139.17 (s), 46.89 (q, J_{BF} = 28.2 Hz). MS (ESI) calcd for $C_{12}H_8BClF_5N_2O_3S_2^-$ $(M\text{-}Na)^-$ 432.97 found 432.93.

^{1}H NMR



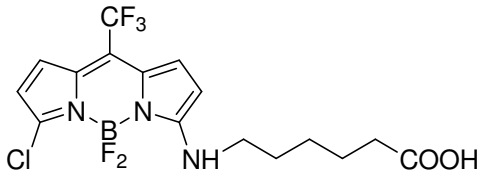
^{19}F NMR





31-avidin

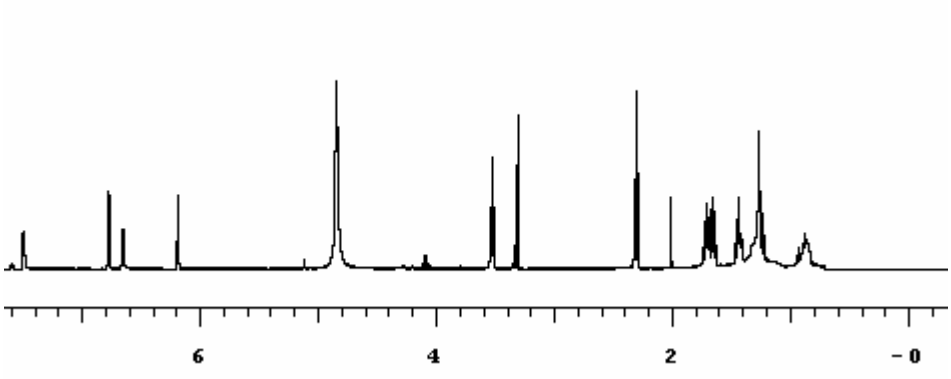
A solution of compound **31** (10 eq) in 0.1 N $NaHCO_3$ buffer (pH = 8.3, 20 μl) was added into a solution of avidin (2 mg) in the same buffer (0.5ml). The solution was shaken in the dark for 1 h at room temperature. Then the residue was purified by PD10 desalting column.



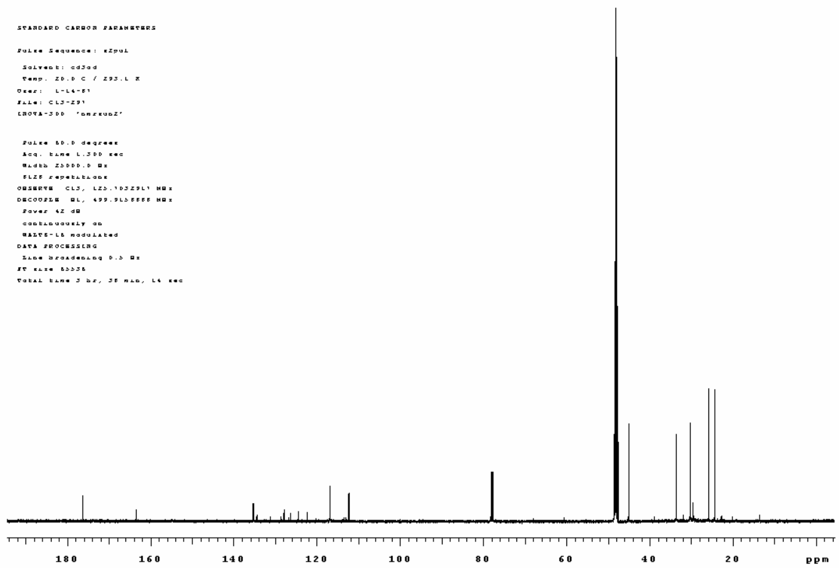
32

6-Aminohexanoic acid (24 mg) and compound **16** (30 mg) were dissolved in the co-solvent THF/H_2O (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_3OD) δ 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_3OD) δ 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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Solvent: cd3od
DATA PROCESSING
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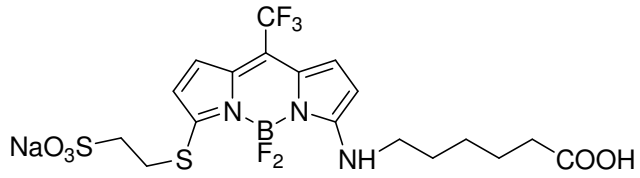
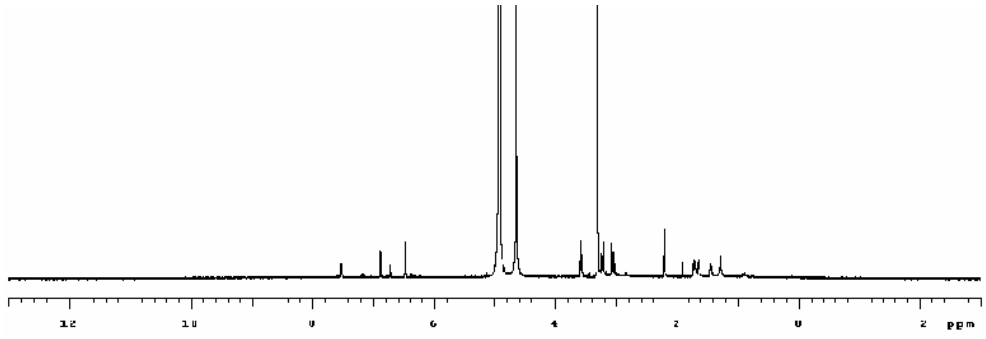
20
ppm



33

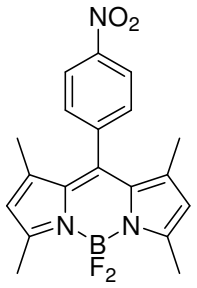
6-Aminohexanoic acid (3 eq) was added to a solution of compound **31** (20 mg) in 5 ml water. The solution was stirred at room temperature for 48 h. After removal the solvents, the residue was applied to a silica gel flash column 10 % MeOH/CH_2Cl_2. However, it was really hard to separate the desired product, only 2 mg yield a purple solid was obtained. R_f = 0.2 (20% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, CD_3OD) δ 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); MS (ESI) calcd for $C_{18}H_{20}BF_5N_3O_5S_2^-$ $(M\text{-}Na)^-$ 528.09 found 528.02.

^{1}H NMR



APPENDIX B
EXPERIMENTAL DATA FOR CHAPTER III

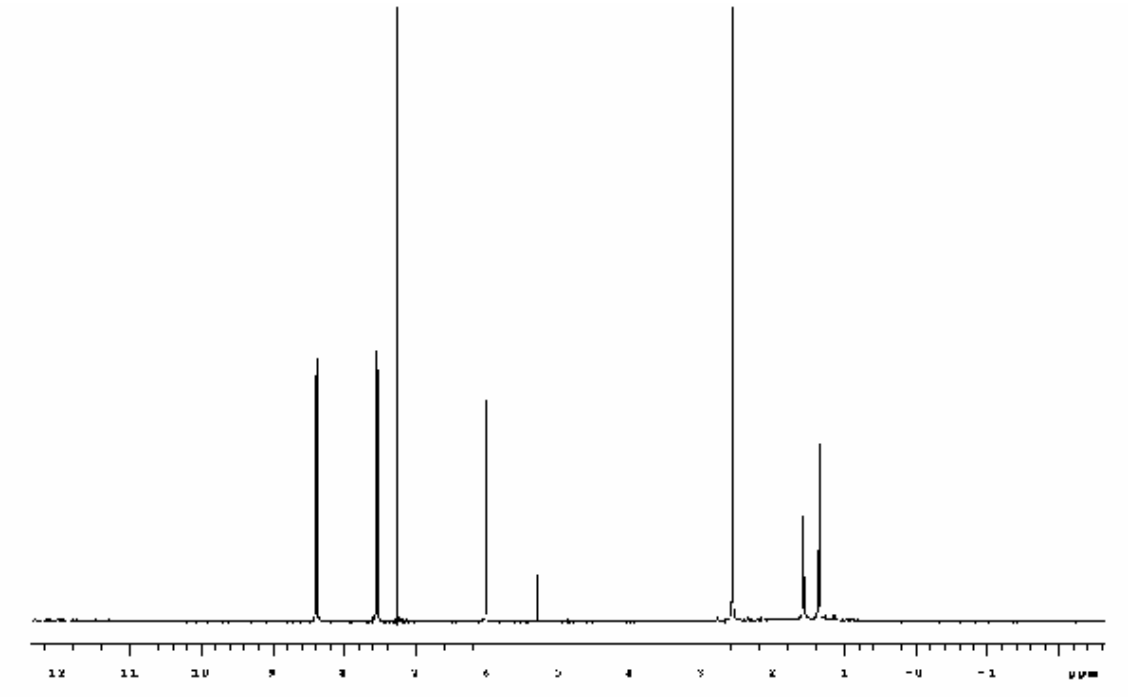
General Experimental Procedures. All chemicals were obtained from commercial suppliers and used without further purification. Chromatography on silica gel was performed using a forced flow of the indicated solvent on EM reagents silica gel 60 (230-400 mesh). ^{1}H NMR spectra were recorded at room temperature and chemical shifts are reports in ppm from the solvent resonance ($CDCl_3$ 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 tetramethylsiliane resonance ($CDCl_3$ 77.2 ppm). Mass spectra were measured under ESI condition.



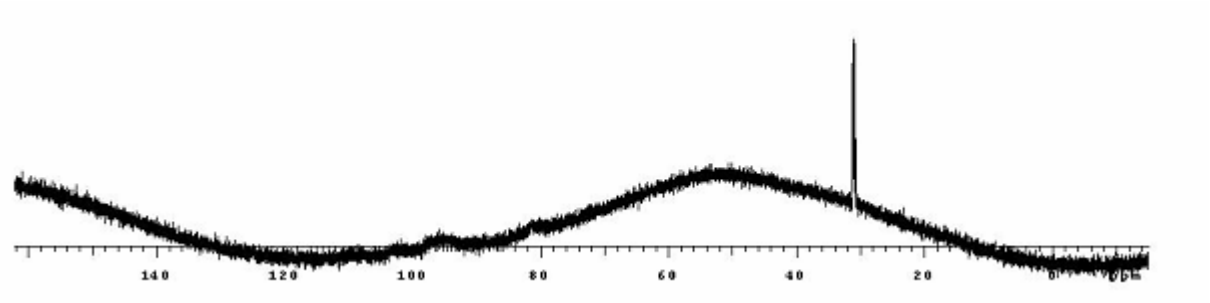
34

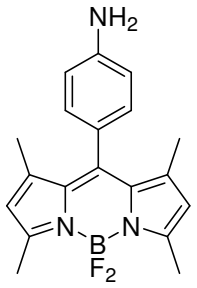
A solution of 4-nitrobenzaldehyde (1.8 g, 12 mmol) and 2,4-dimethylpyrrole (2.46 ml, 24 mmol) in dry CH_2Cl_2 (200 ml) was purged with N_2 for 30 min at room temperature. 0.1 ml TFA was added to initiate the condensation. After 2 h, the resulting solution was washed with 0.1 M NaOH (2 x 100 ml) and then water (2 x 100 ml), dried over anhydrous Na_2SO_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_2SO_4, filtered, and the solution was evaporated to dryness. The residue was applied to a silica gel flash column. Elution with 1:1 CH_2Cl_2/hexane yielded an orange crystal (1.2 g, 30 %). R_f = 0.7 (2:1 CH_2Cl_2/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 30.96 (q).

^{1}H NMR



^{19}F NMR

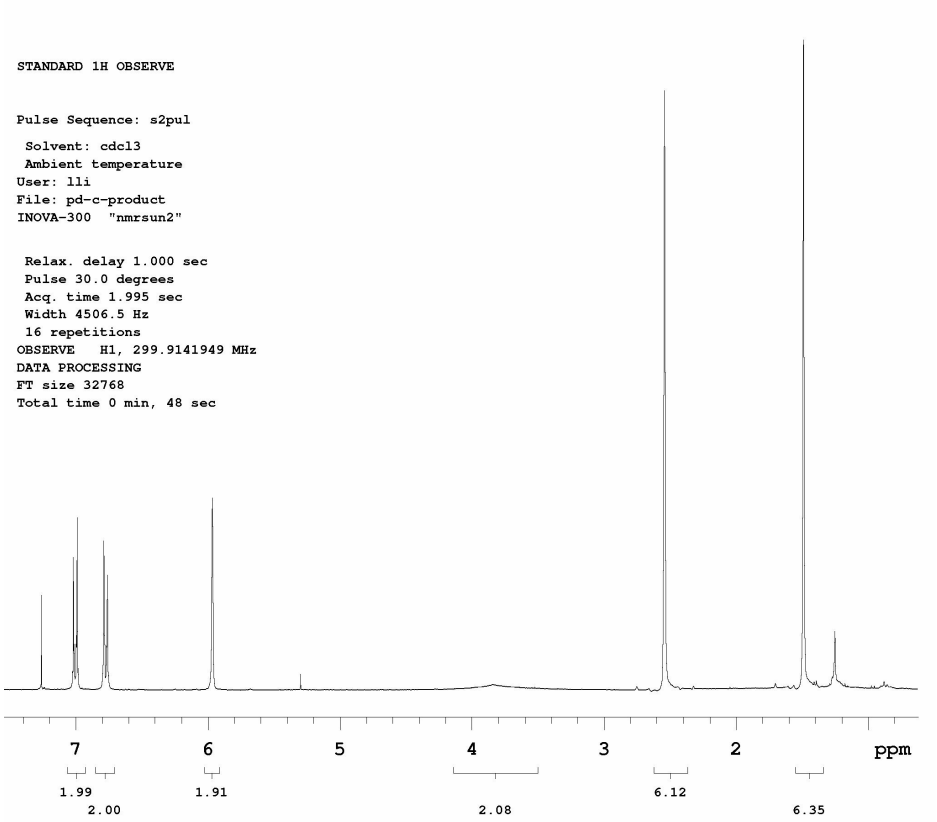




35

A solution of **34** (500 mg, 1.36 mmol) in 1:1 THF/EtOH (50 ml) was purged with N_2 for 10 min. 10% Pd/C (144 mg, 0.14 mmol) and 1 ml hydrazine were added. The solution was stirred at reflux under N_2 for 30 min. Cooled to the room temperature and poured into 50 ml H_2O. The aqueous mixture was extracted with CH_2Cl_2 (2 x 50 ml). The combined organic layers were extract dried over anhydrous Na_2SO_4, 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_f = 0.2 (20% EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) 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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User: lli
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Pulse 30.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions
OBSERVE H1, 299.9141949 MHz
DATA PROCESSING
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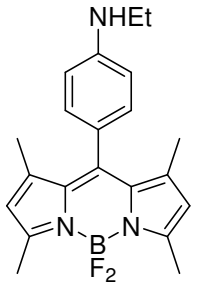
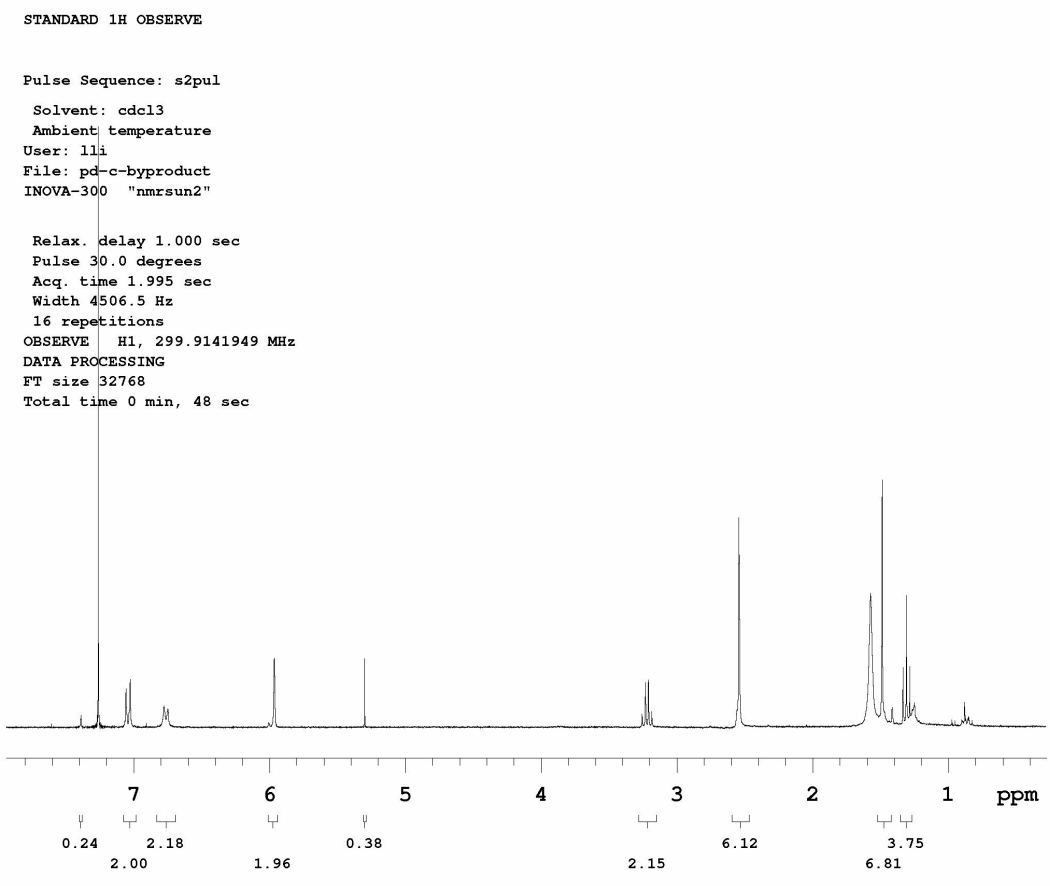
Total time 0 min, 48 sec
7
6
5
4
3
2
ppm
1.99
2.00
1.91
2.08
6.12
6.35



36

A solution of **34** (200 mg) in 20 ml 1:1 CH_2Cl_2/EtOH was hydrogenated over 10% Pd/C and bubbled with H_2 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 CH_2Cl_2/hexane to afford the orange crystals **36** (R_f = 0.3) and then eluting with 2:1 CH_2Cl_2/hexane to yield **35** (R_f = 0.2) (2:1 CH_2Cl_2/hexane). Longer reaction time will give higher yield. ^{1}H NMR (300 MHz, $CDCl_3$) 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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Ambient temperature
User: lli
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OBSERVE H1, 299.9141949 MHz
DATA PROCESSING
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7
6
5
4
3
2
1
ppm
0.24
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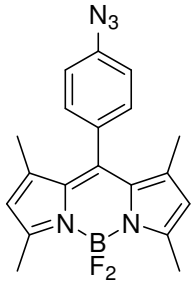
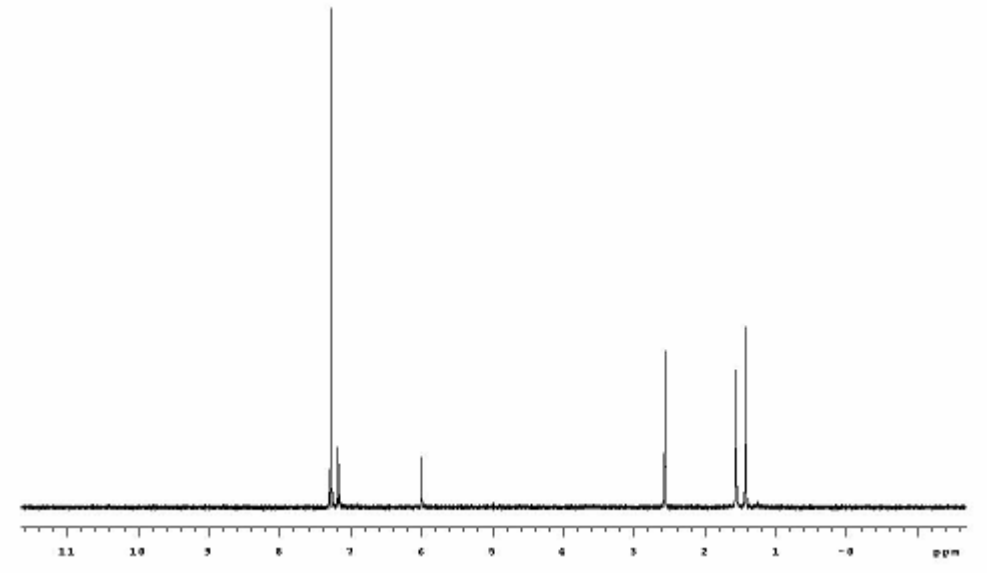
0.38
6.12
3.75
2.00
1.96
2.15
6.81



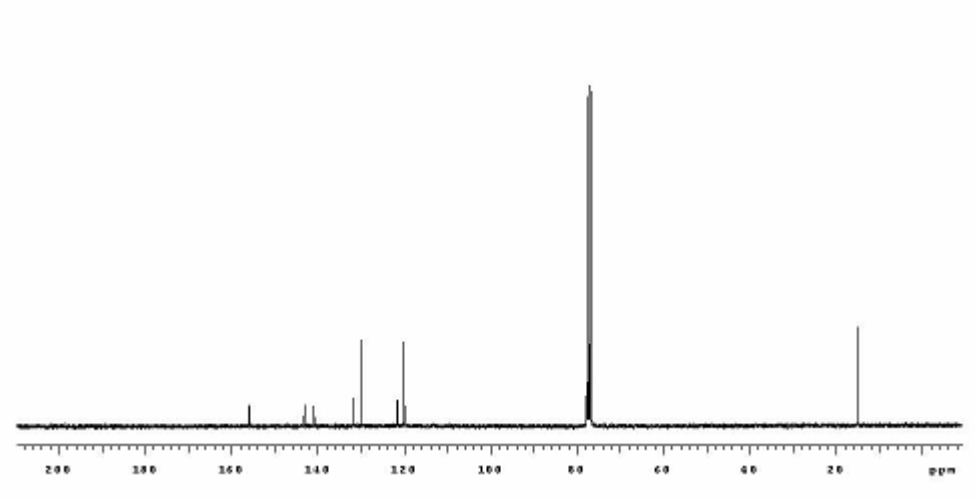
37

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 $NaNO_2$ (15.3 mg, 0.22mmol) in 2 ml H_2O was added slowly and then the mixture was kept at 0°C for 30 min. NaN_3 (29 mg, 0.45 mmol) in 2 ml H_2O 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%). R_f = 0.5 (20% EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.0, 143.2, 141.3, 140.8, 131.8, 129.9, 121.7, 120.1, 14.9 (2); MS (ESI) calcd for $C_{19}H_{18}BF_2N_5^+$ (M^+) 365.1623 found 365.1563; IR (thin film) 2126, 2096, 1542, 1510, 1309, 1193, 1081, 980, 832, 761 cm^{-1}.

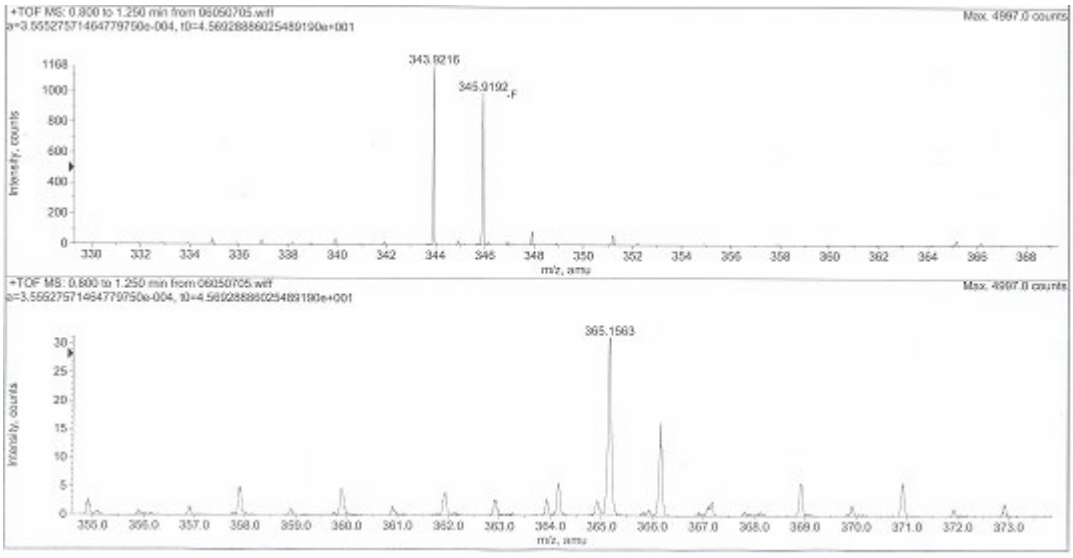
^{1}H NMR

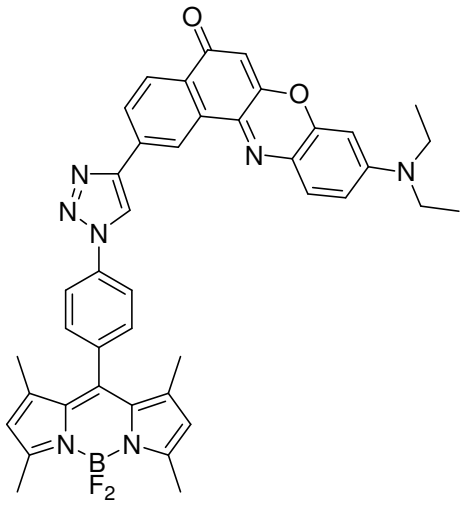


^{13}C NMR



Mass spectrum

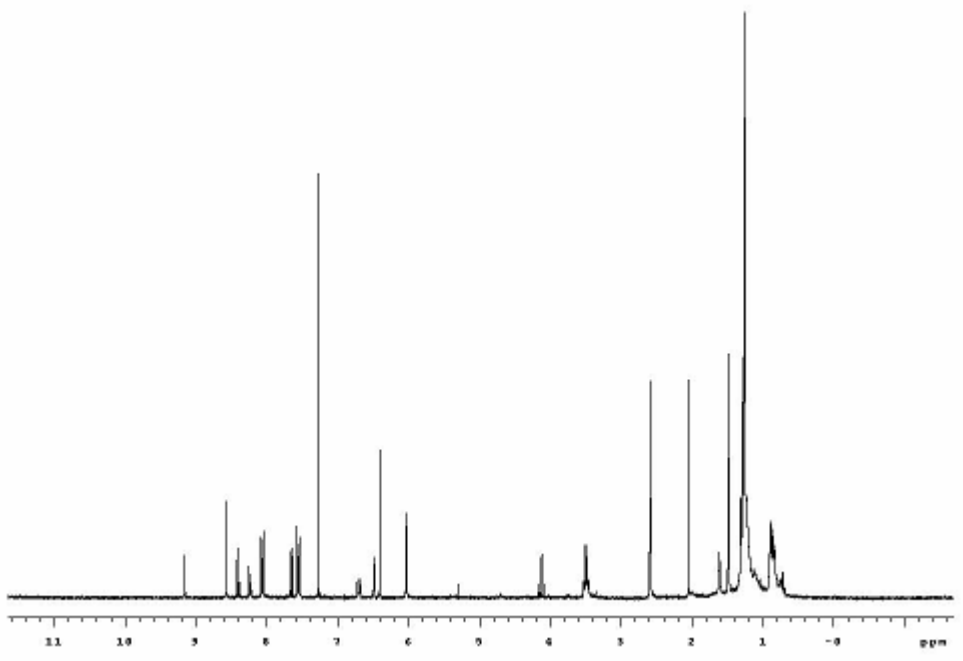




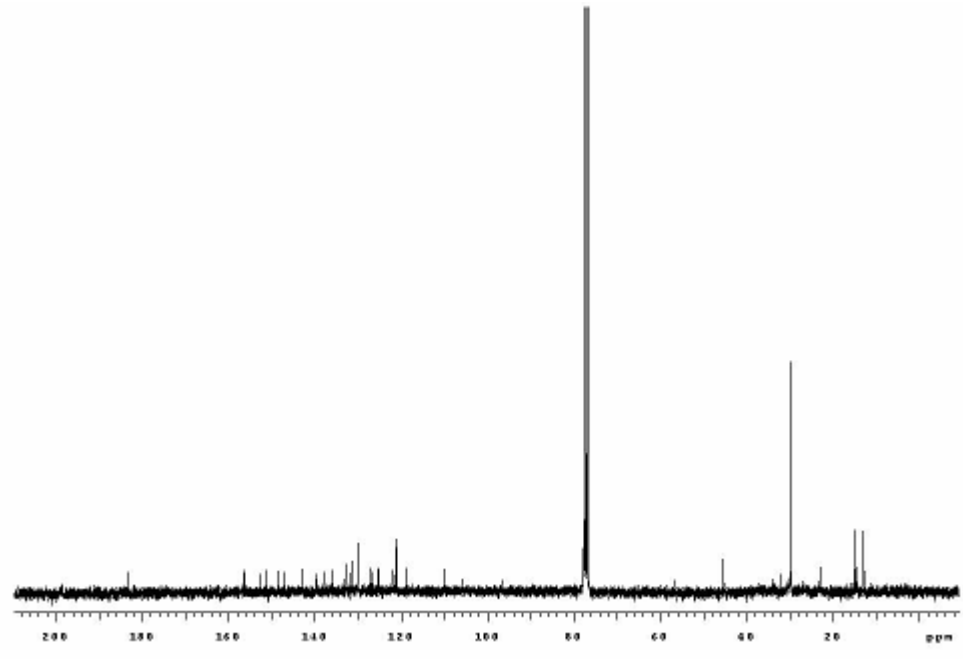
38

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). ^{1}H 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); ^{13}C 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; ^{19}F NMR (300 MHz, $CDCl_3$) δ 31.01 (q); MS (ESI) calcd for $C_{41}H_{37}BF_2N_7O_2^+$ $(M+H)^+$ 708.3070 found 708.3074.

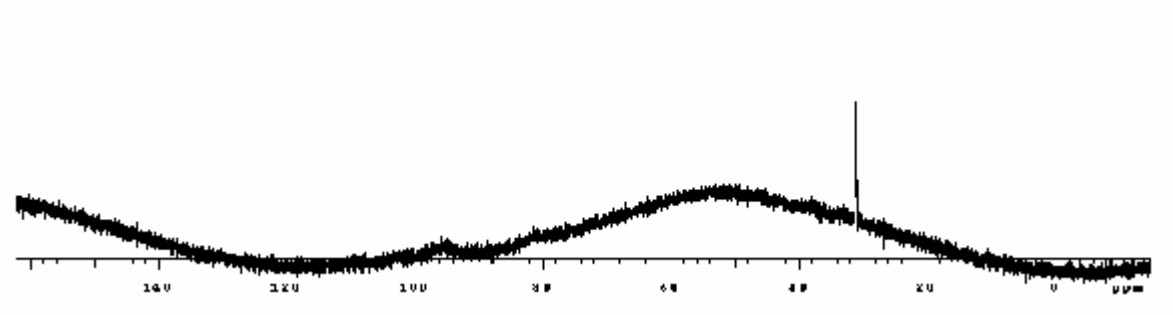
^{1}H NMR



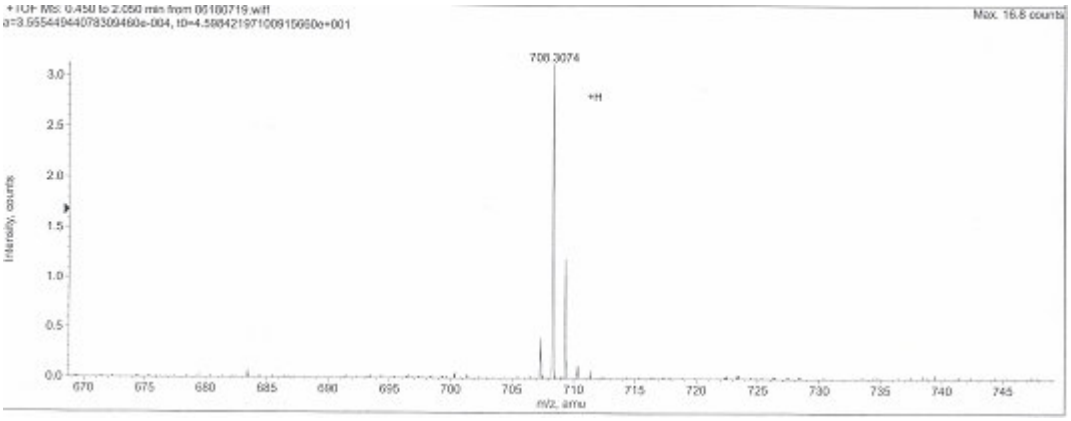
^{13}C NMR

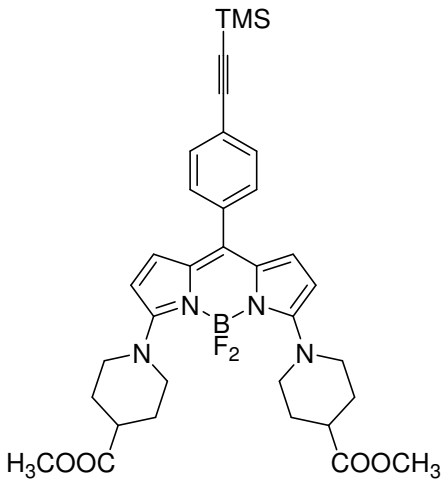


^{19}F NMR



Mass spectrum

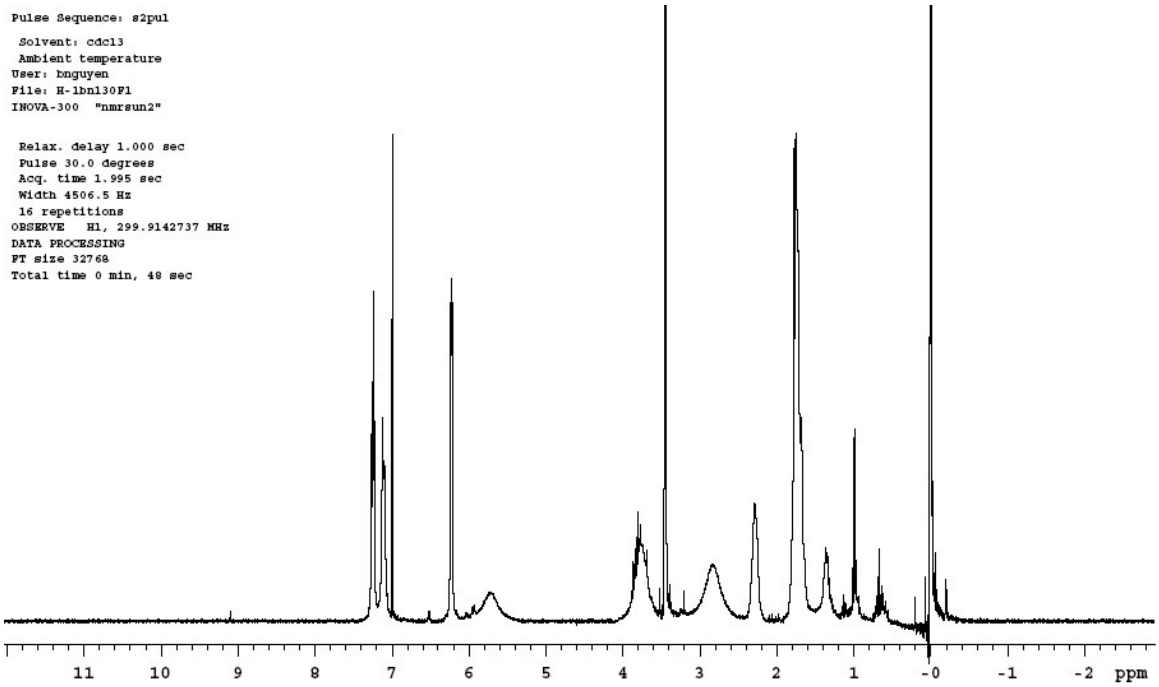




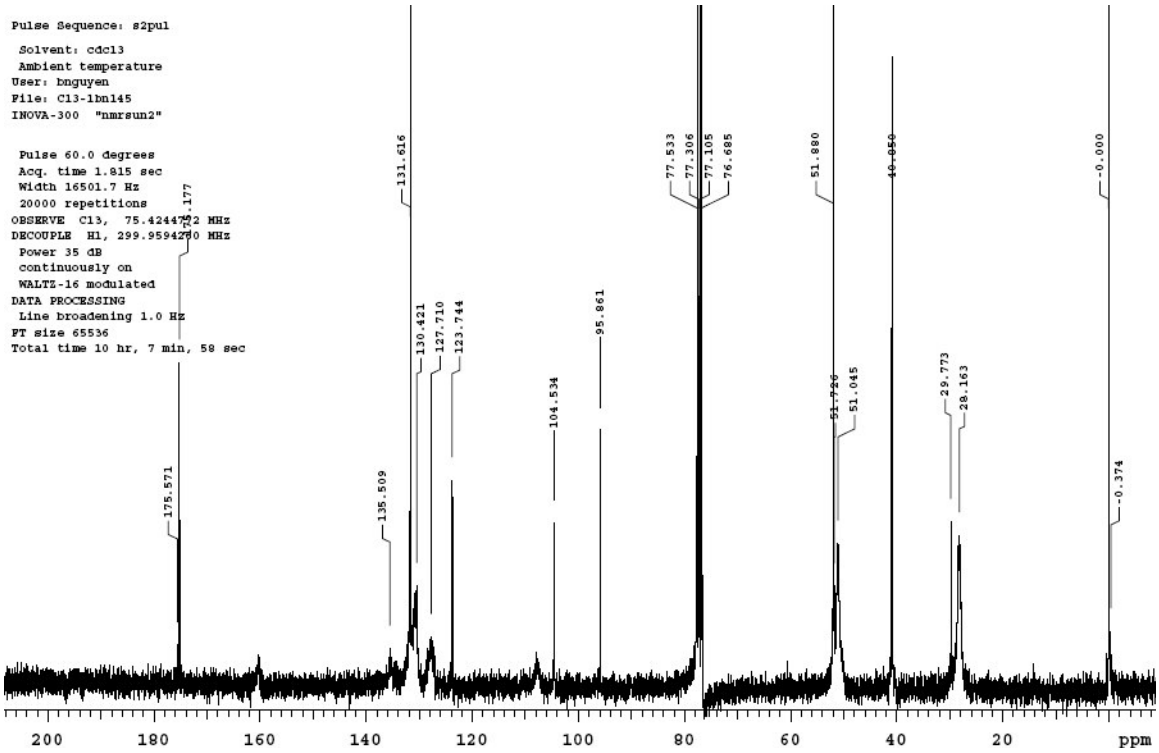
39

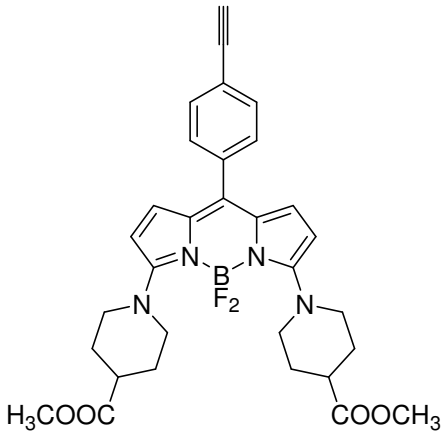
CuI (1.17 mmol), $PdCl_2(PPh_3)_2$ (0.62 mmol), and Et_3N (100 mmol) were added to a solution of compound **24** (3.26 g, 5.12 mmol) in dry DMF (120 ml) into a dry sealed tube. The mixture was heated to 80 ^oC for 12 hours. The DMF was removed under reduced pressure. The residue was dissolved in dichloromethane (150 ml), washed with water (2 x 50 ml), saturated NaCl (50 ml), dried over Na_2SO_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%). ^1H 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 $C_{34}H_{42}BF_2N_4O_4Si^+$ $(M+H)^+$ 647.3036, found 647.3048.

^{1}H NMR



^{13}C NMR

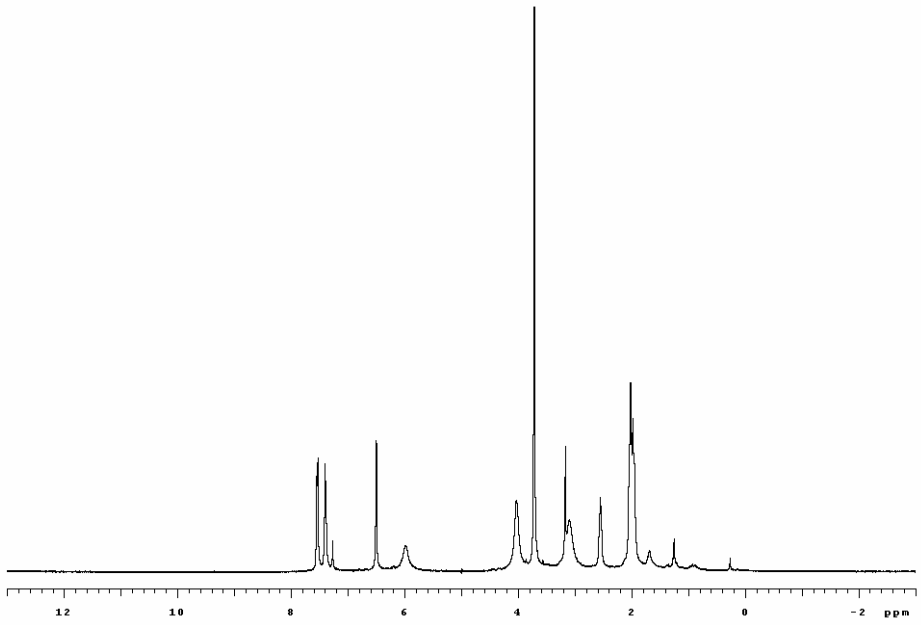




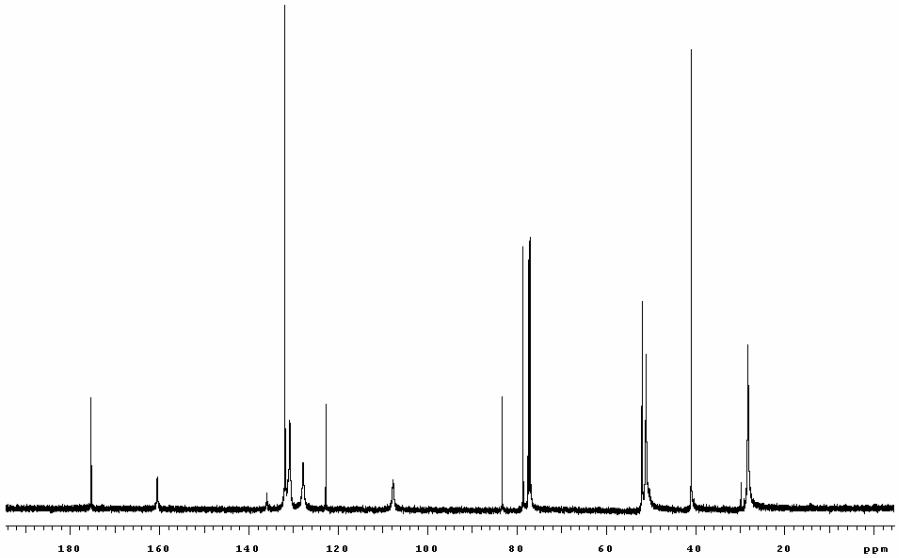
40

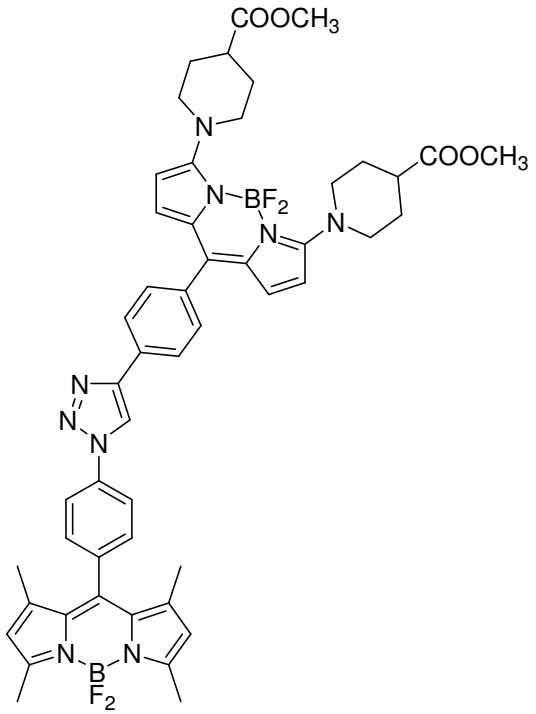
K_2CO_3 (1.1 eq) was added to a solution of compound **39** (0.12 g, 0.18 mmol) in MeOH (15 ml, not dry). The mixture was stirred at 25 ^{0}C for 48 hours. The reaction was quenched with 30 ml water, and extracted with dichloromethane (2 x 20 ml). The extracted organic layers were washed with saturated NaCl (20 ml), dried over Na_2SO_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 $C_{31}H_{34}BF_2N_4O_4^+$ $(M+H)^+$ 575.2641, found 575.2655.

^{1}H NMR



^{13}C NMR

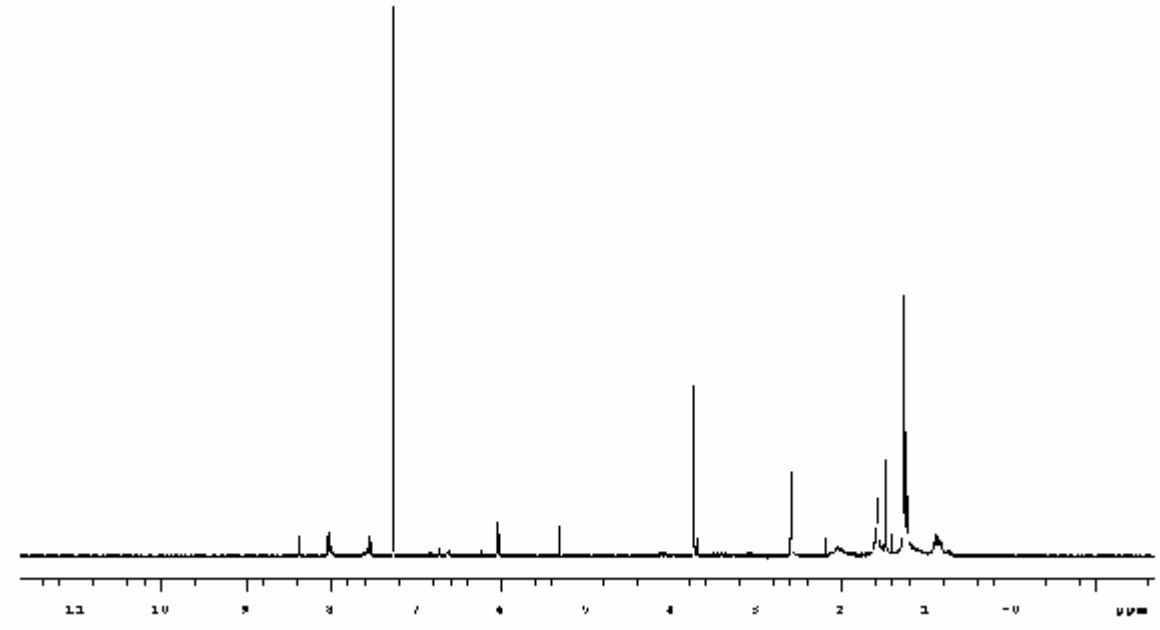




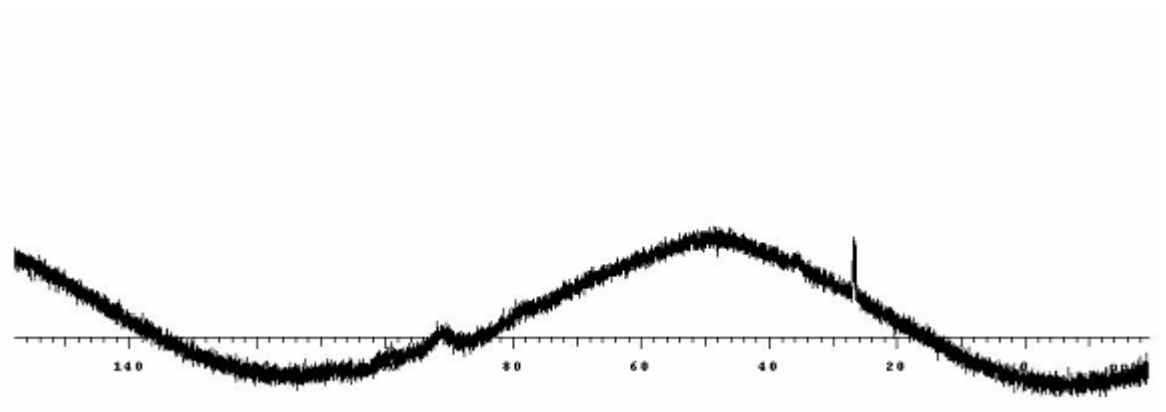
41

Cu (2 mg) and $CuSO_4$ $5H_2O$ (1 mg) was added to a solution of compound **37** (10 mg) and **40** (31 mg) in 5 ml 3:1 THF/H_2O. 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, $CDCl_3$) δ 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.

^{1}H NMR



^{19}F NMR



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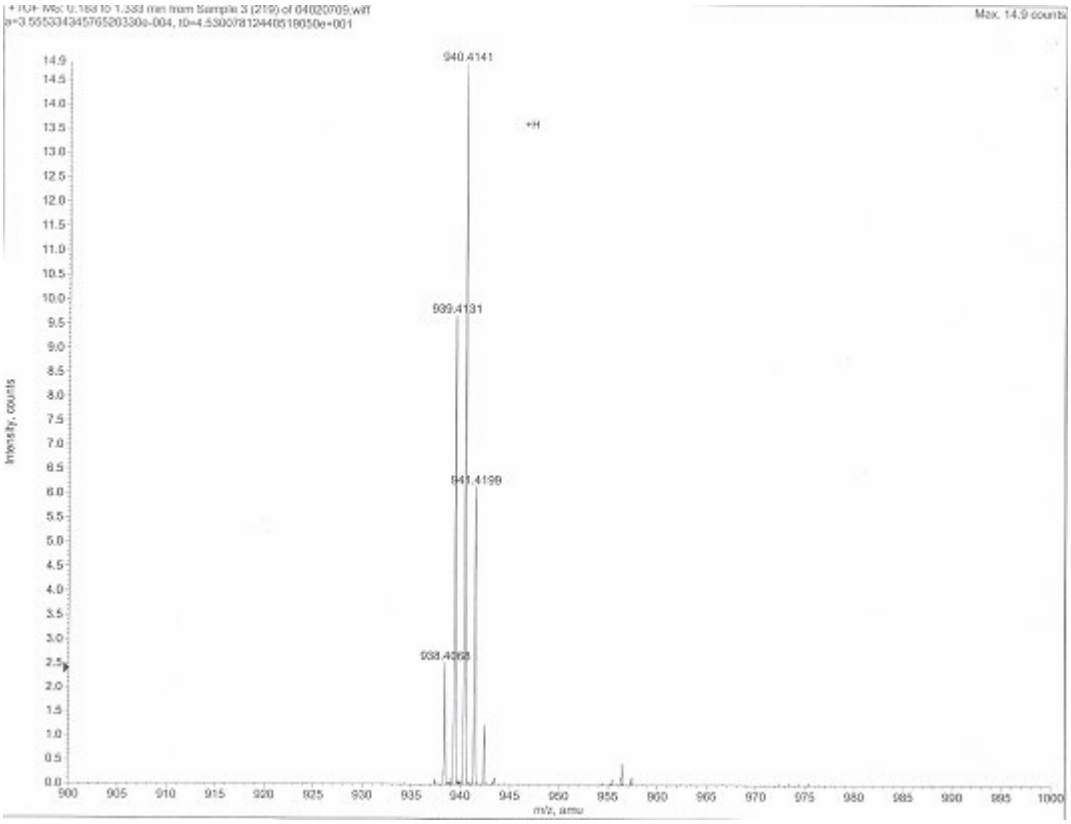
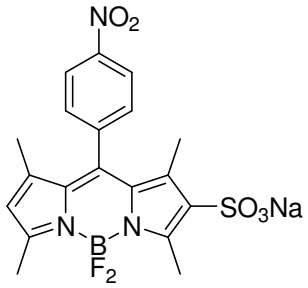


APPENDIX C
EXPERIMENTAL DATA FOR CHAPTER IV

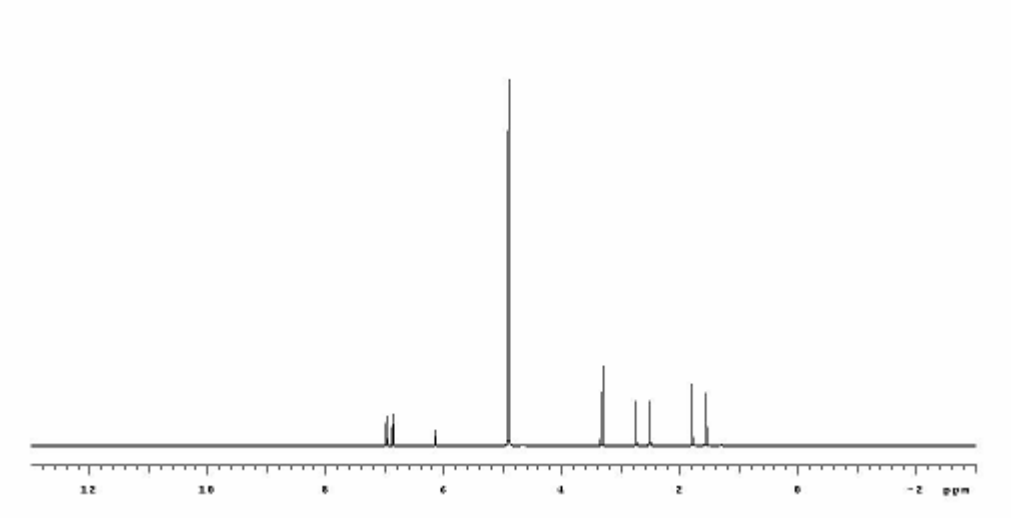
General Experimental Procedures. All chemicals were obtained from commercial suppliers and used without further purification. Chromatography on silica gel was performed using a forced flow of the indicated solvent on EM reagents silica gel 60 (230-400 mesh). ^{1}H NMR spectra were recorded at room temperature and chemical shifts are reports in ppm from the solvent resonance ($CDCl_3$ 7.24 ppm, DMSO-d_6 2.50 ppm, CD_3OD 3.31 ppm, D_2O 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 tetramethylsiliane resonance ($CDCl_3$ 77.2 ppm, DMSO-d_6 39.5 ppm, CD_3OD 49.1 ppm). Mass spectra were measured under ESI condition.



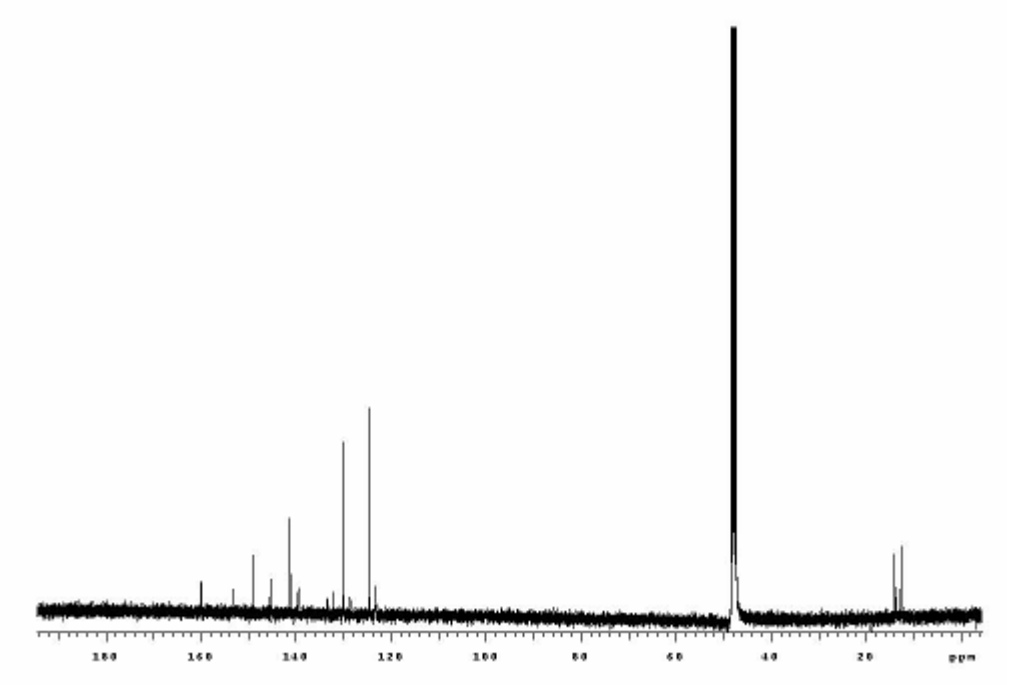
42

A solution of chlorosulfonic acid (22 μl, 0.33 mmol) in CH_2Cl_2 (2 ml) was added dropwise to a solution of BODIPY **34** (100 mg, 0.27 mmol) in CH_2Cl_2 (25 ml) over 10 min at -40 ^oC. 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_2Cl_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_2Cl_2 to afford the orange powder (80 mg, 63%). R_f = 0.4 (20% MeOH/CH_2Cl_2). ^1H NMR (500 MHz, CD_3OD) 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); ^{13}C NMR (125 MHz, CD_3OD) 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 $C_{19}H_{17}BF_2N_3NaO_5S^-$ $(M\text{-}Na)^-$ 448.0950 found 447.9834; IR (thin film) 1513, 1343, 1200, 1086, 988, 806 cm^{-1}.

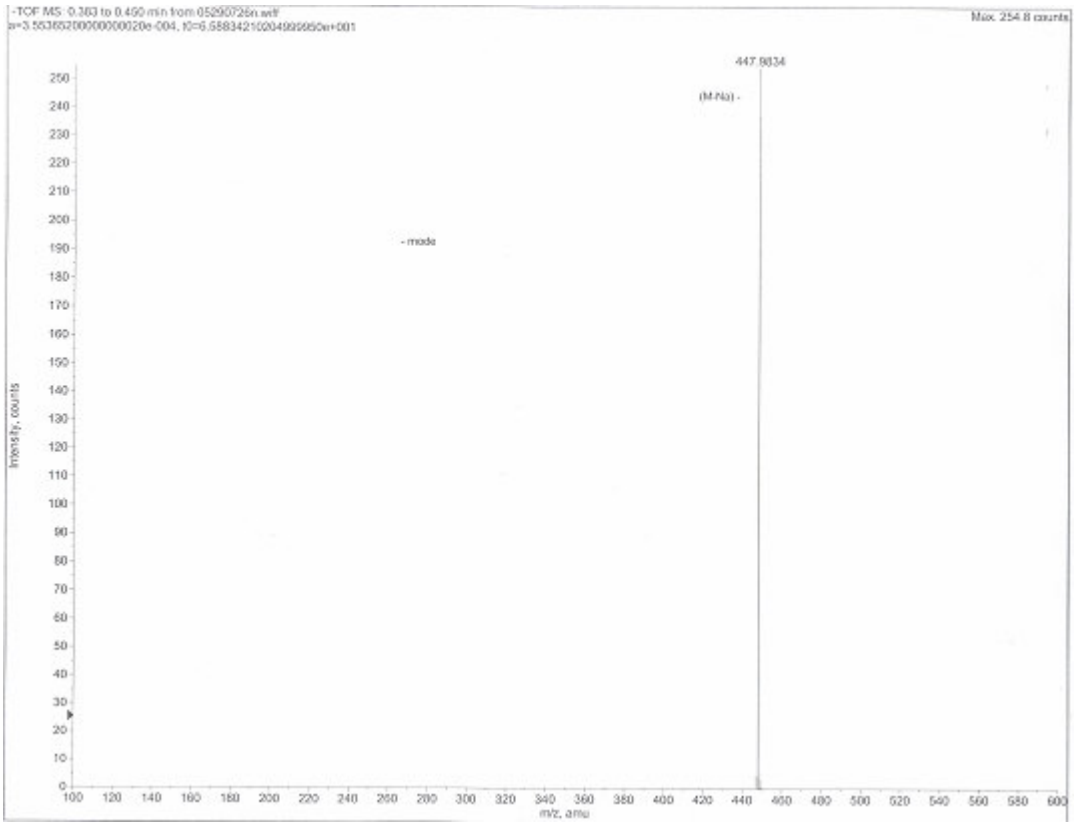
^{1}H NMR

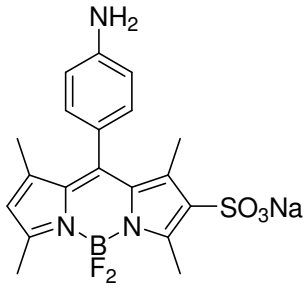


^{13}C NMR



Mass spectrum

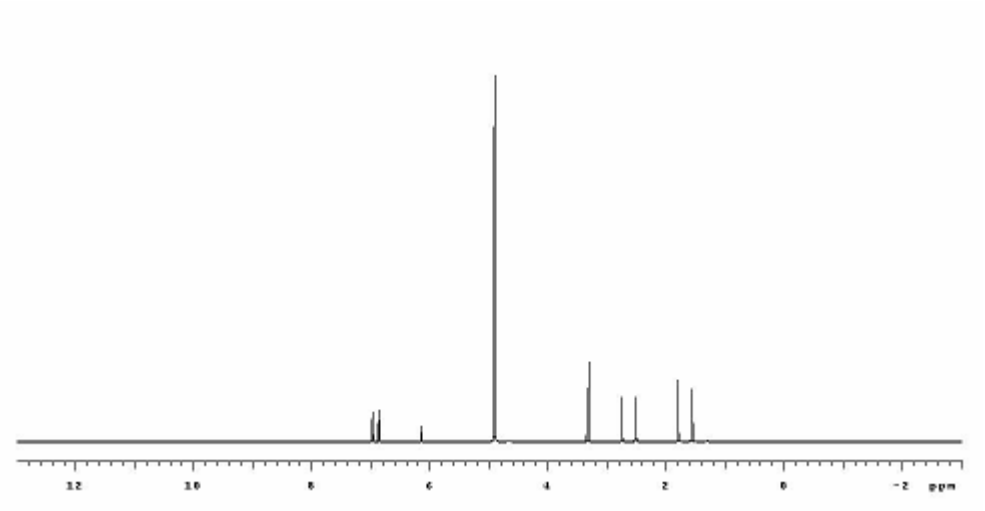




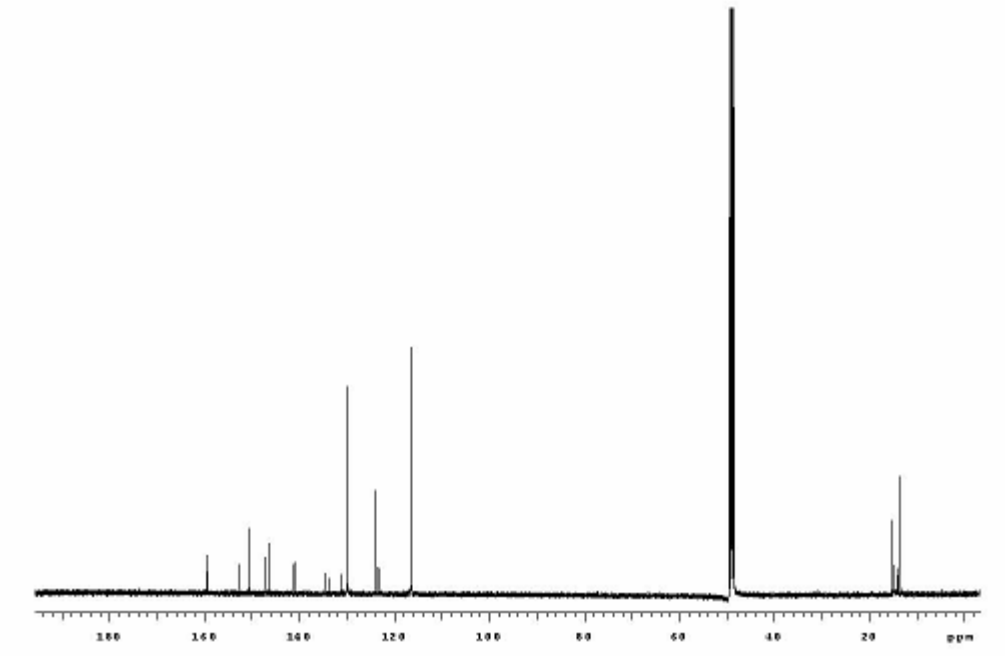
43

A solution of **42** (35 mg, 0.07 mmol) in EtOH (10 ml) was purged with N_2 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 N_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 15% MeOH/CH_2Cl_2 to afford the orange solid (30 mg, 92%). R_f = 0.3 (20% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, CD_3OD) δ 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); ^{13}C NMR (125 MHz, CD_3OD) δ 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 $C_{19}H_{19}BF_2N_3O_3S^-$ $(M\text{-}Na)^-$ 418.1208 found 418.0397; IR (thin film) 3414, 2922, 1608, 1540, 1519, 1196, 1036, 684 cm^{-1}.

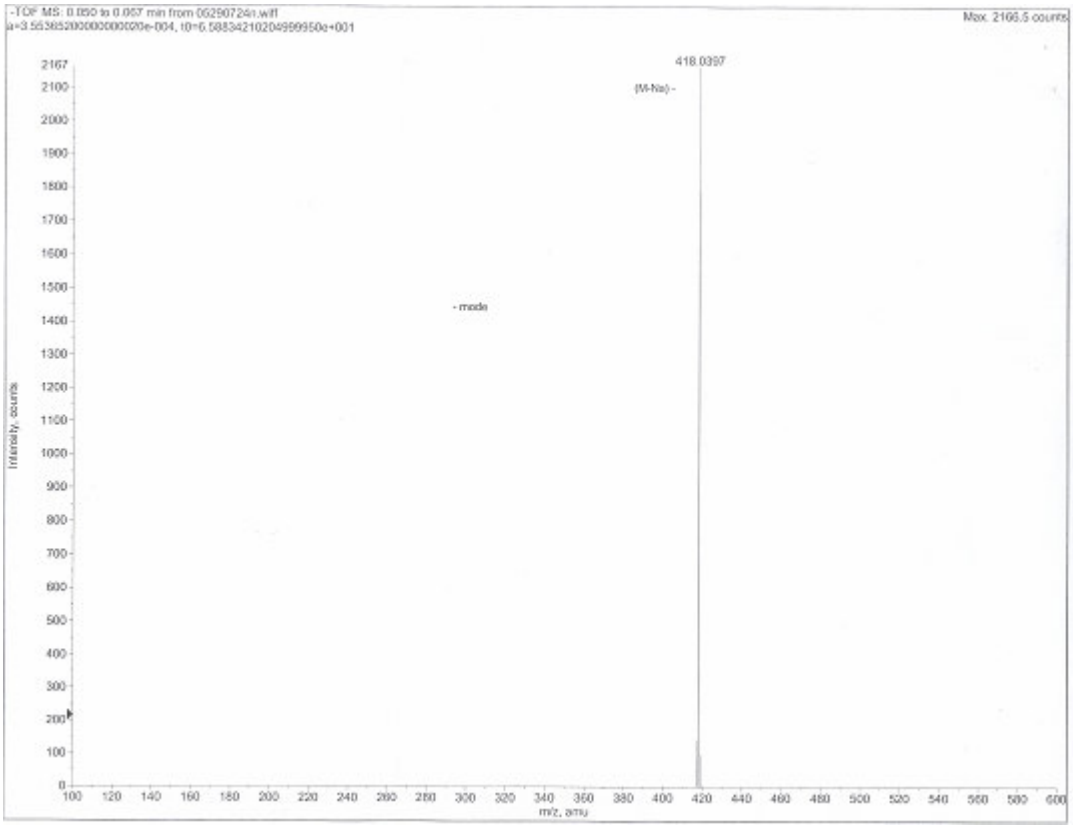
^{1}H NMR

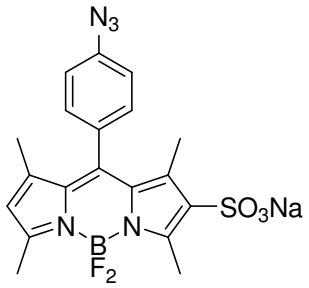


^{13}C NMR



Mass spectrum

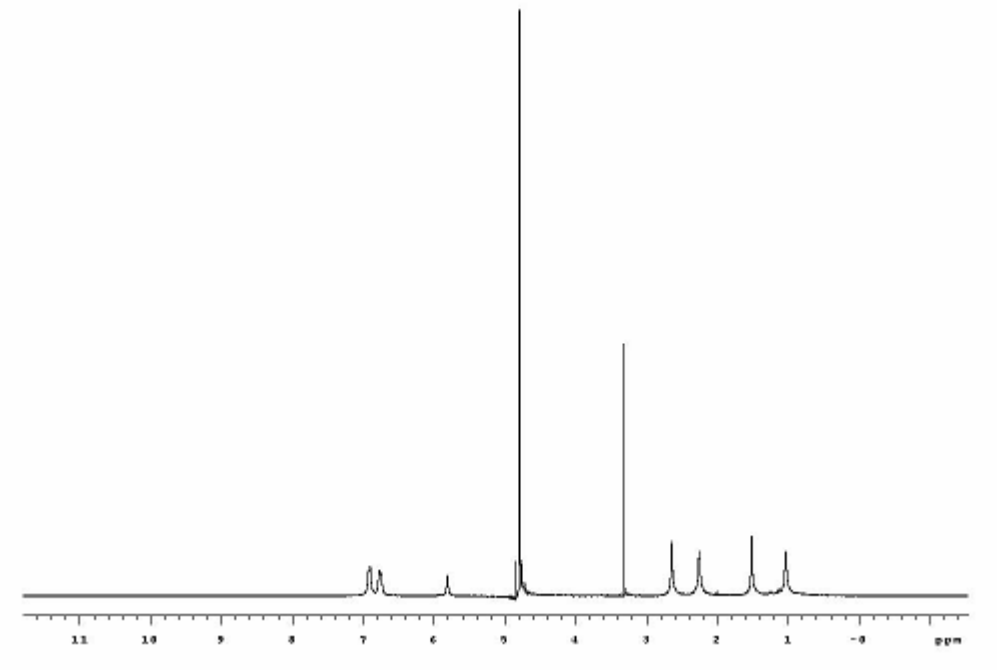




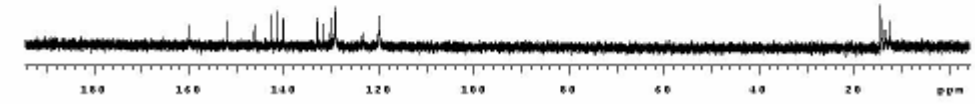
44

A solution of **43** (29 mg, 0.07 mmol) in 2 ml H_2O and 5 ml 2 M HCl was cooled to 0°C. The solution of $NaNO_2$ (11.3 mg, 0.16mmol) in 2 ml H2O was added slowly and then the mixture was kept at 0°C for 30 min. NaN_3 (22 mg, 0.33 mmol) in 2 ml H_2O 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_2O and the residue was applied to a silica gel flash column chromatography using 15% MeOH/CH_2Cl_2 to afford the orange solid (23 mg, 75%). R_f = 0.3 (20% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, D_2O) 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) ; ^{13}C NMR (125 MHz, D_2O) 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 $C_{19}H_{17}BF_2N_5O_3S^-$ (M-Na)$^-$ 444.1113 found 444.0220; IR (thin film) 2128, 2105, 1541, 1304, 1192, 1023, 686 cm^{-1}.

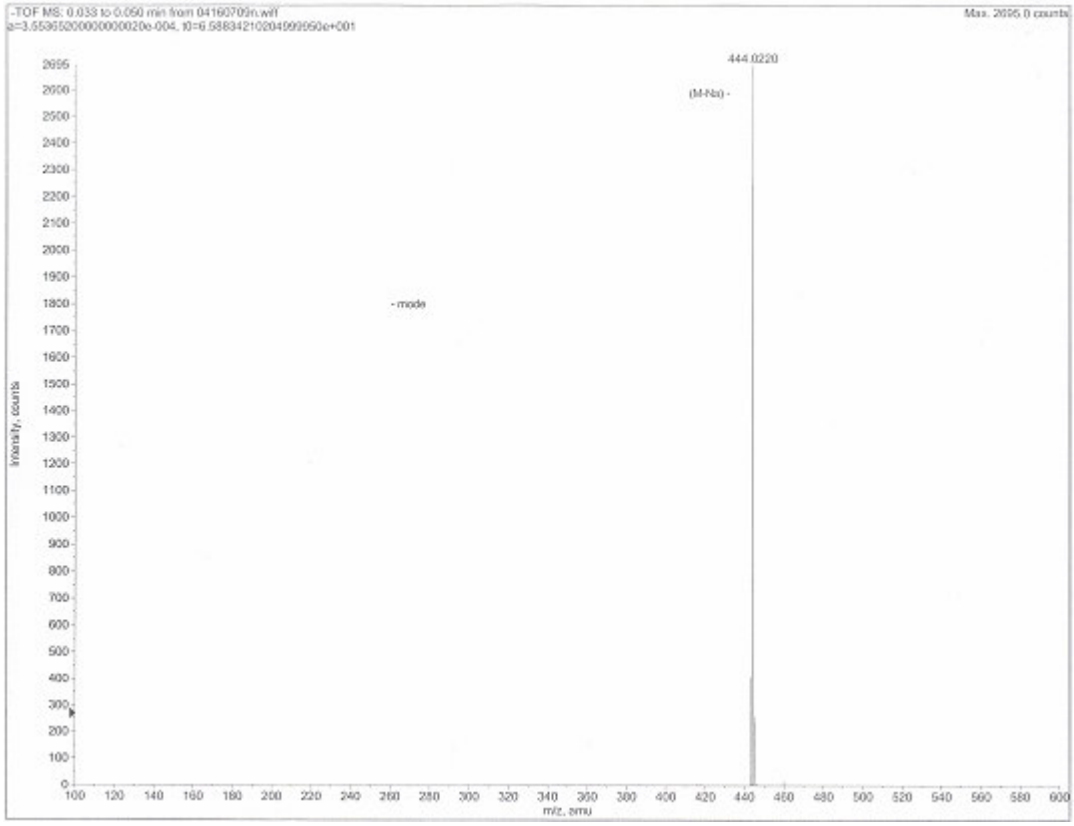
^{1}H NMR

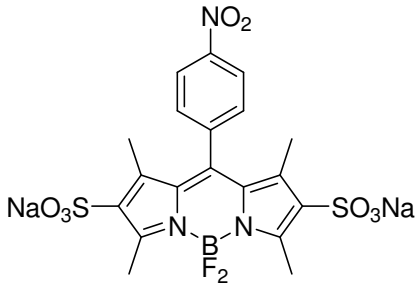


^{13}C NMR



Mass spectrum

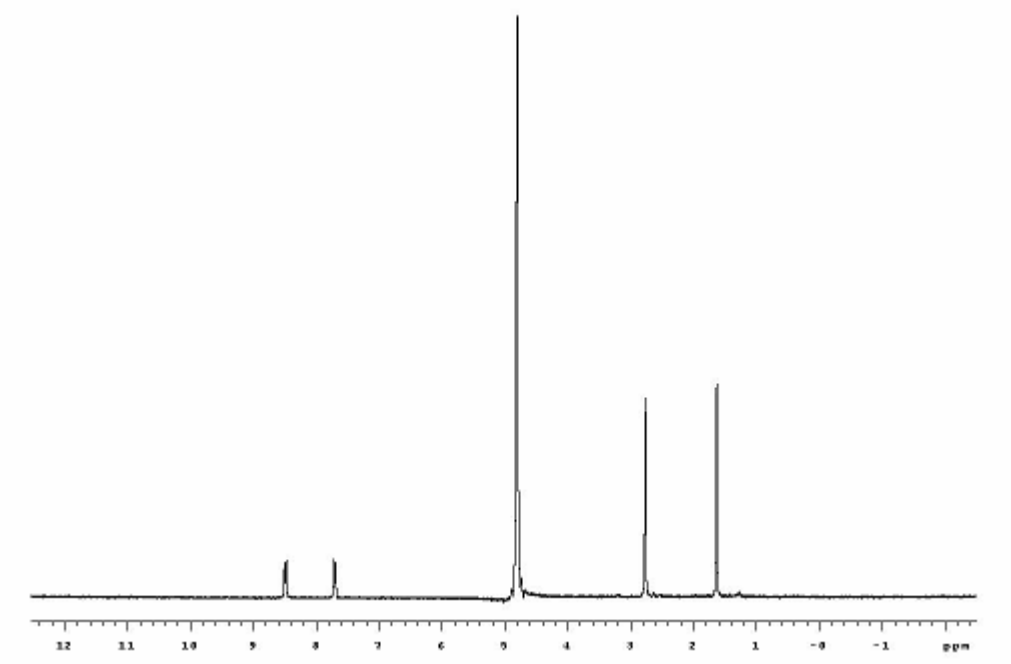




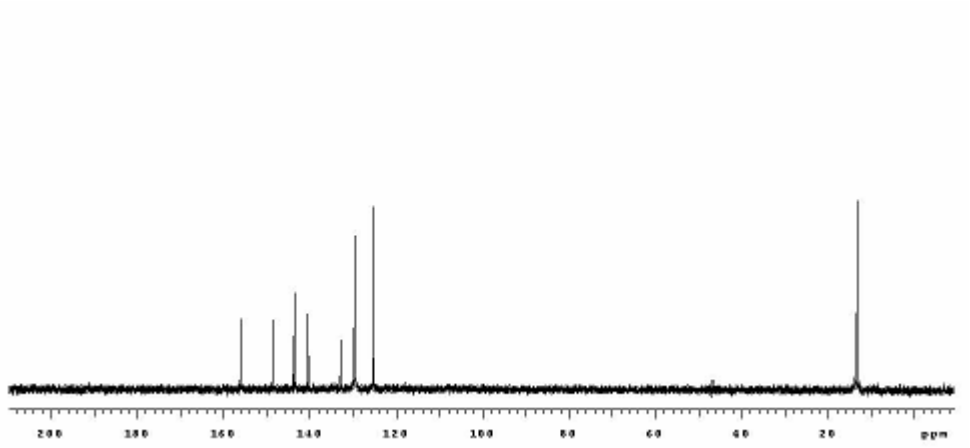
45

A solution of chlorosulfonic acid (144 µl, 2.16 mmol) in CH_2Cl_2 was added dropwise to a solution of BODIPY **34** (400 mg, 1.08 mmol) in CH_2Cl_2 over 10 min at -40 ^oC. 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 $NaHCO_3$ (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, D_2O) 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); ^{13}C NMR (75 MHz, D_2O) 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 $C_{19}H_{16}BF_2N_3O_8S_2^{2-}$ $(M-2Na)^{2-}$ 263.5220 found 263.4547; IR (thin film) 1522, 1347, 1190, 1004, 853, 669 cm^{-1}.

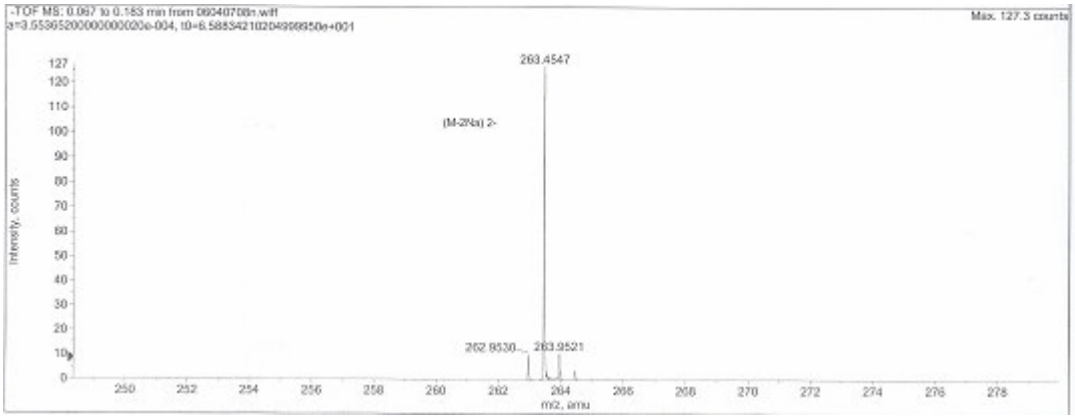
^{1}H NMR

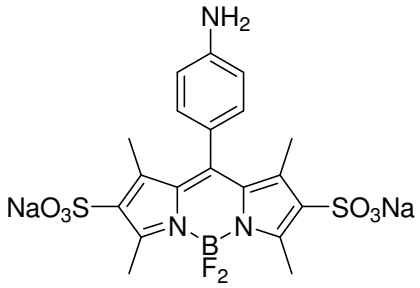


^{13}C NMR



Mass spectrum

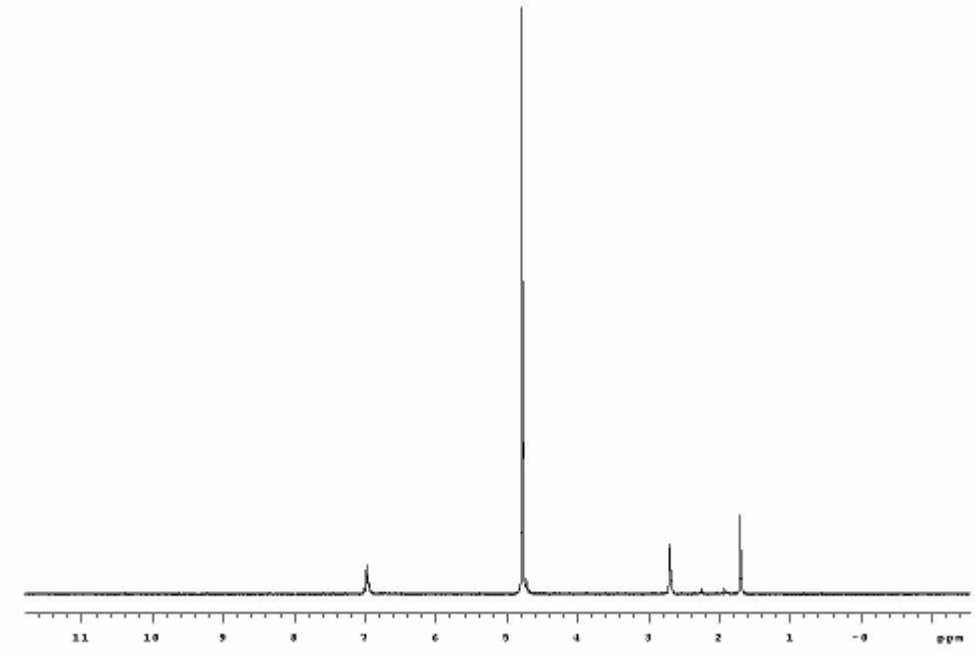




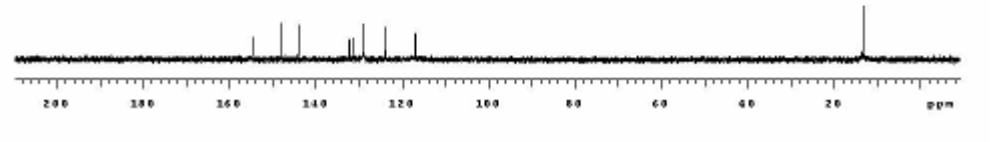
46

A solution of **45** (200 mg, 0.35 mmol) in EtOH (10 ml) was purged with N_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_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/CH_2Cl_2 to afford the orange solid (133 mg, 70%). R_f = 0.2 (30% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, D_2O) δ 7.02-6.94 (m, 4H), 2.70 (s, 6H), 1.70 (s, 6H); ^{13}C NMR (75 MHz, D_2O) δ 154.7, 148.2, 144.1, 132.3, 131.2, 130.0, 123.9, 117.1, 117.0, 13.0 (2); MS (ESI) $C_{19}H_{19}BF_2N_3Na_2O_6S_2^+$ $(M+H)^+$ 544.0572 found 544.0557; IR (thin film) 3346, 2854, 1608, 1519,1197, 1032, 655 cm^{-1}.

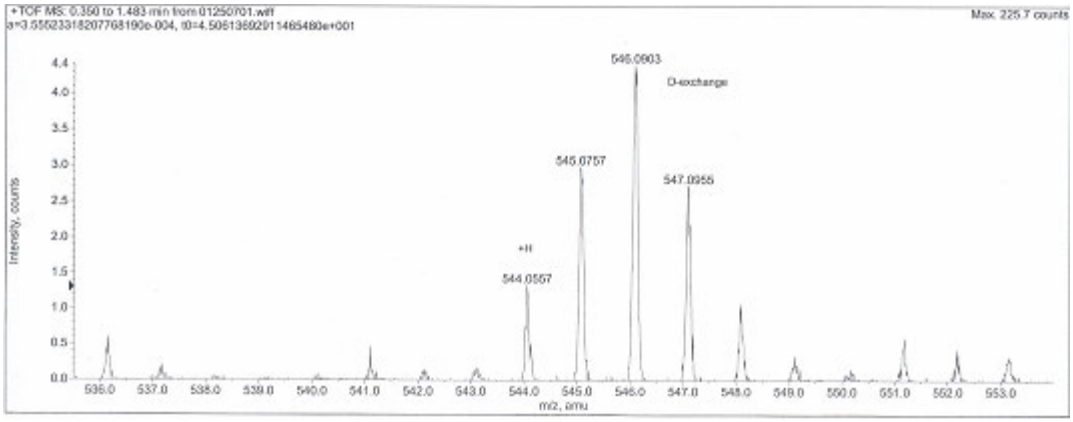
^{1}H NMR

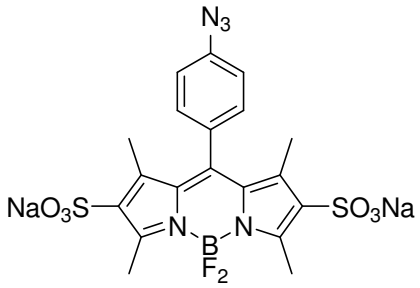


^{13}C NMR



Mass spectrum

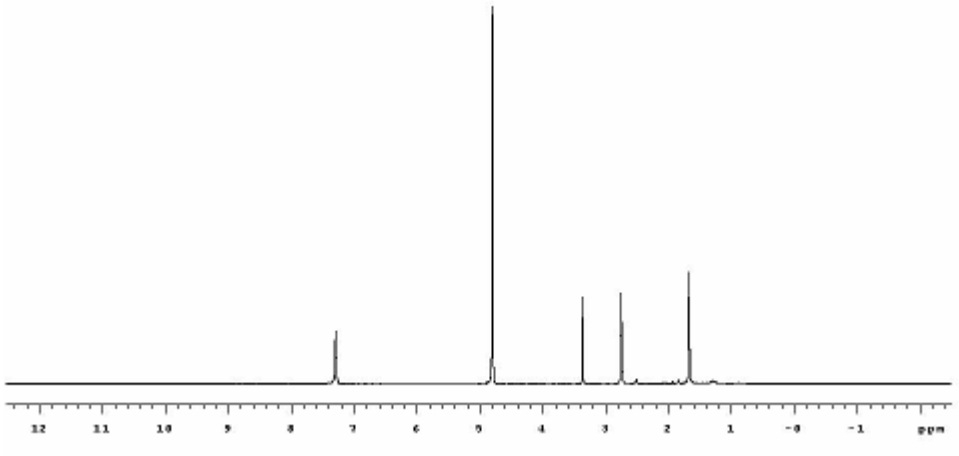




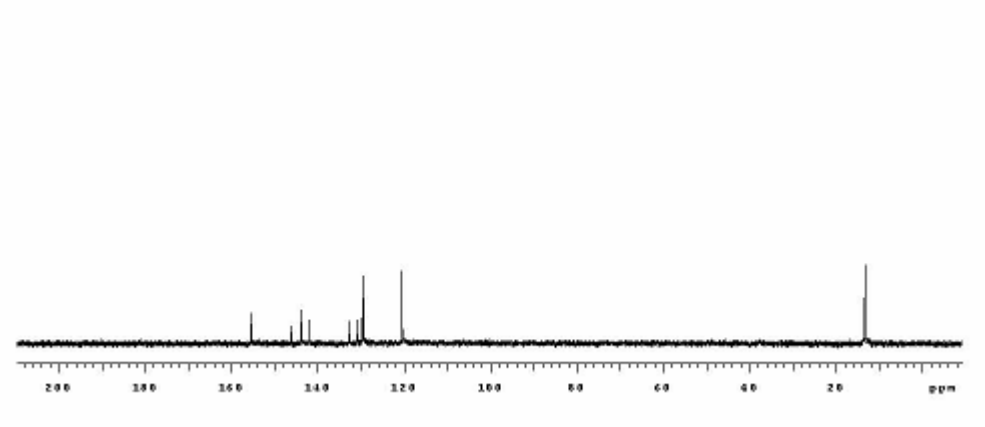
47

A solution of **46** (100 mg, 0.18 mmol) in 5 ml H_2O and 20 ml 2 M HCl was cooled to 0°C. The solution of $NaNO_2$ (32 mg, 0.46mmol) in 3 ml H_2O 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_2O 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_2O and the residue was applied to a silica gel flash column chromatography using 30% MeOH/CH_2Cl_2 to afford the orange solid (88 mg, 77%). R_f = 0.2 (30% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, D_2O) δ 7.33-7.26 (m, 4H), 2.75 (s, 6H), 1.67 (s, 6H); ^{13}C NMR (75 MHz, D_2O) δ 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 $C_{19}H_{17}BF_2IN_2O_6S_2^-$ $(M-2Na+H)^-$ 608.9634 found 608.9776; IR (thin film) 2130, 1549, 1295, 1038, 667 cm^{-1}.

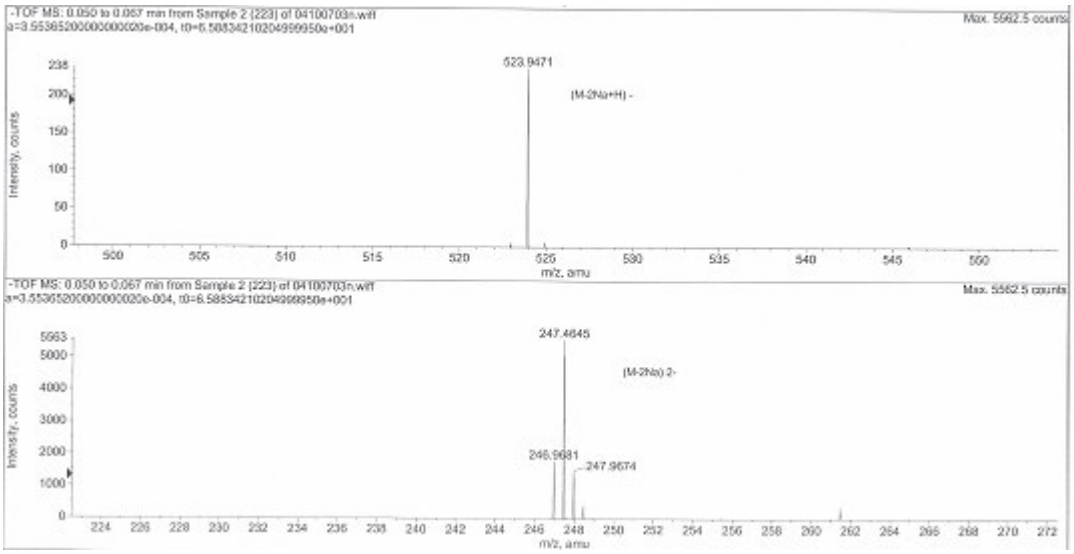
^{1}H NMR

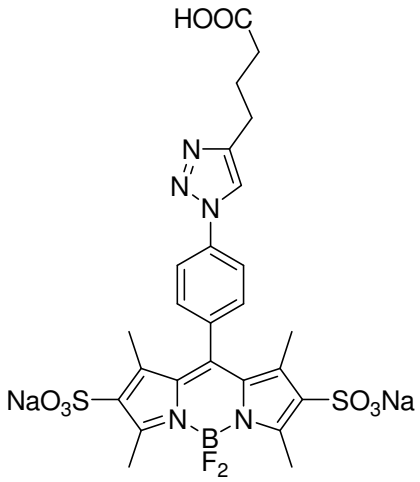


^{13}C NMR



Mass spectrum

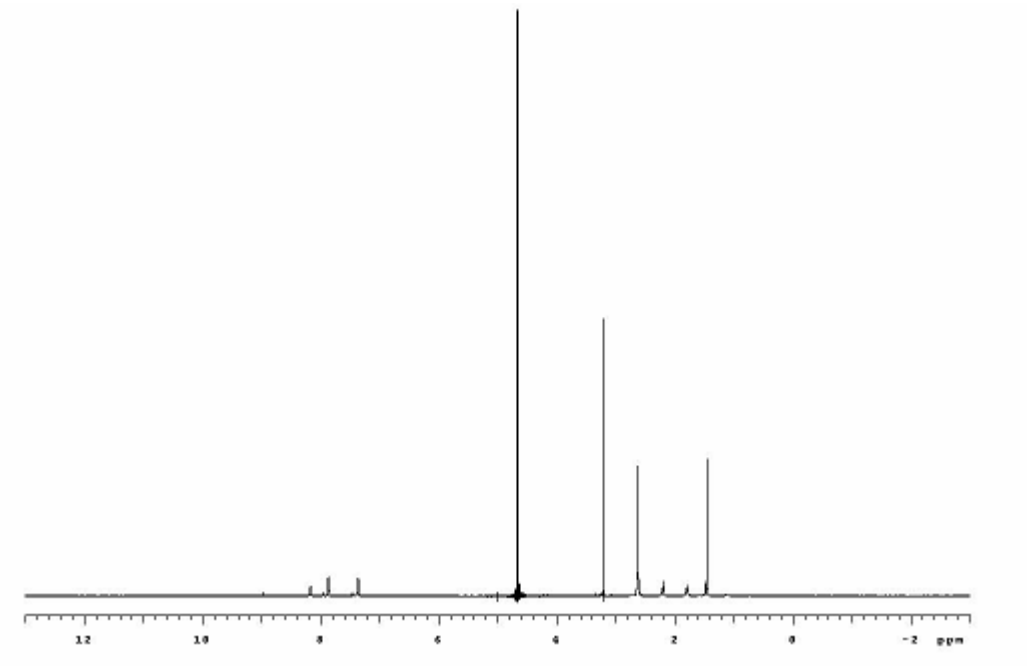




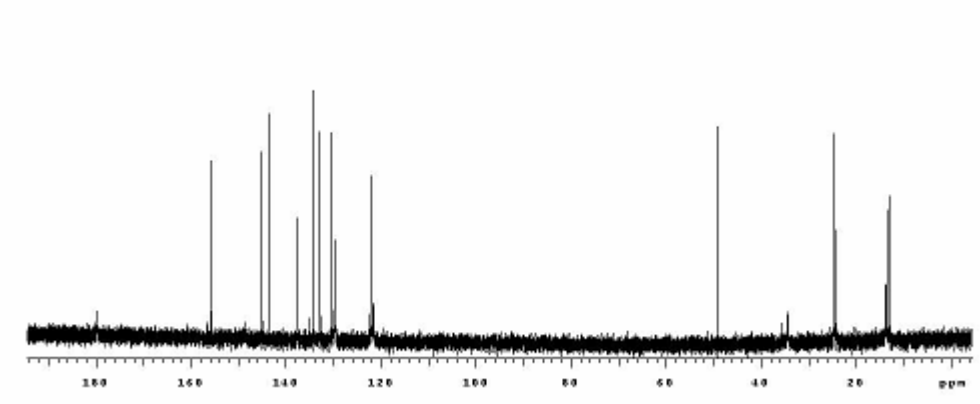
48

To a solution of **47** (40 mg) and hexynoic acid (2 eq) in 5 ml 1:1 THF/H_2O was added Cu (1 eq), $CuSO_4$•H_2O (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/CH_2Cl_2 to afford the orange solid (20 mg, 42%). R_f = 0.1 (30% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, D_2O) δ 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, D_2O) δ 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 $C_{25}H_{24}BF_2N_5O_8S_2^{2-}$ $(M-2Na)^{2-}$ 317.5564 found 317.4960.

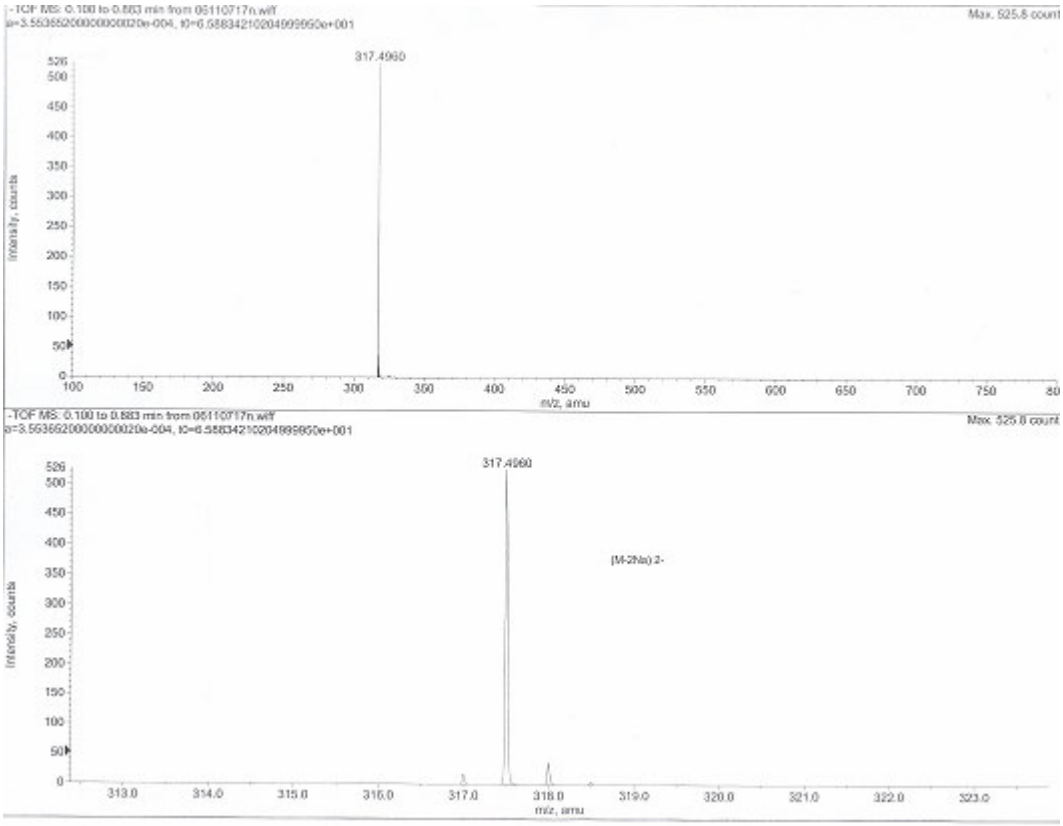
^{1}H NMR

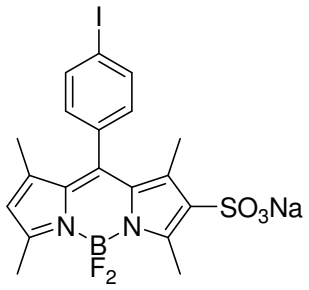


^{13}C NMR



Mass spectrum

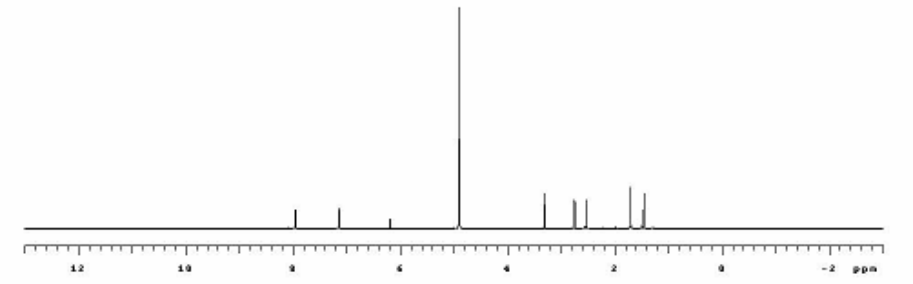




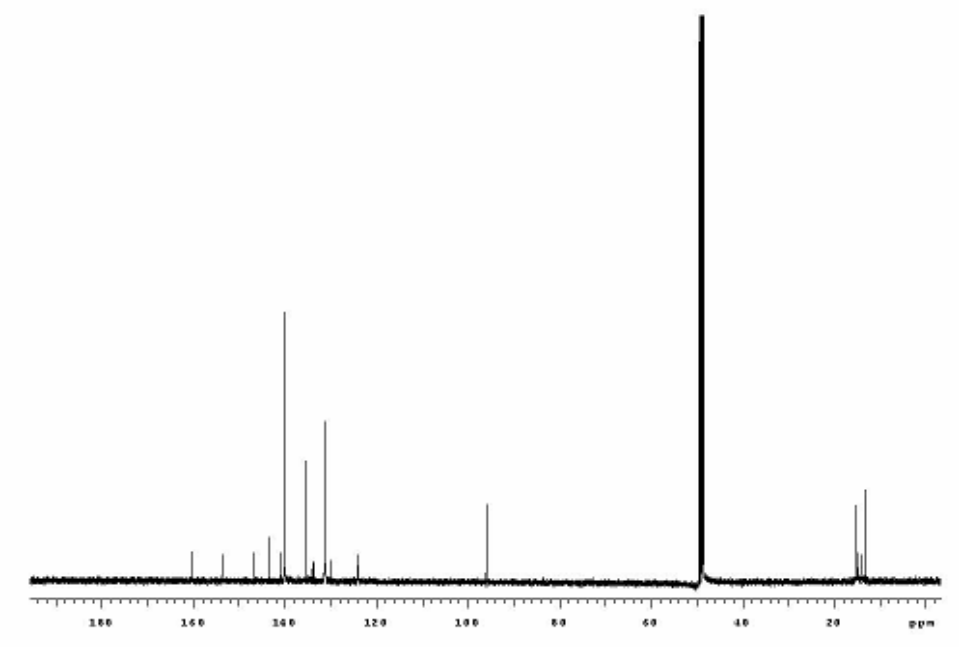
49

A solution of chlorosulfonic acid (18 μl, 0.27 mmol) in CH_2Cl_2 (2 ml) was added dropwise to a solution of tetramethyl iodoBODIPY (100 mg, 0.22 mmol) in CH_2Cl_2 (25 ml) over 10 min at -40 ^oC. 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_2Cl_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_2Cl_2 to afford the orange powder (74 mg, 60%). R_f = 0.4 (20% MeOH/CH_2Cl_2). ^1H NMR (500 MHz, CD_3OD) 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); ^{13}C NMR (125 MHz, CD_3OD) 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 $C_{19}H_{17}BF_2IN_2O_3S^-$ $(M\text{-}Na)^-$ 529.0066 found 528.8784; IR (thin film) 2922, 1717, 1540, 1312, 1193, 1033, 1006, 678 cm^{-1}.

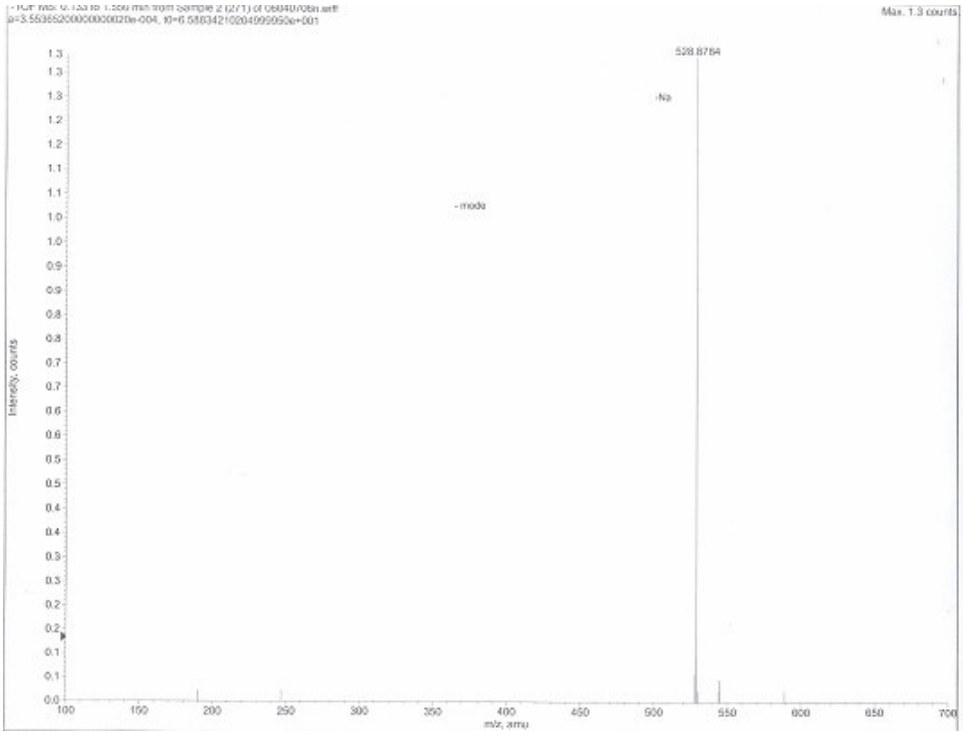
^1H NMR

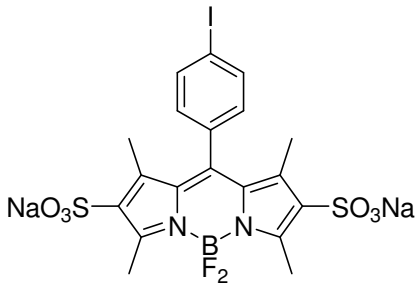


^{13}C NMR



Mass spectrum

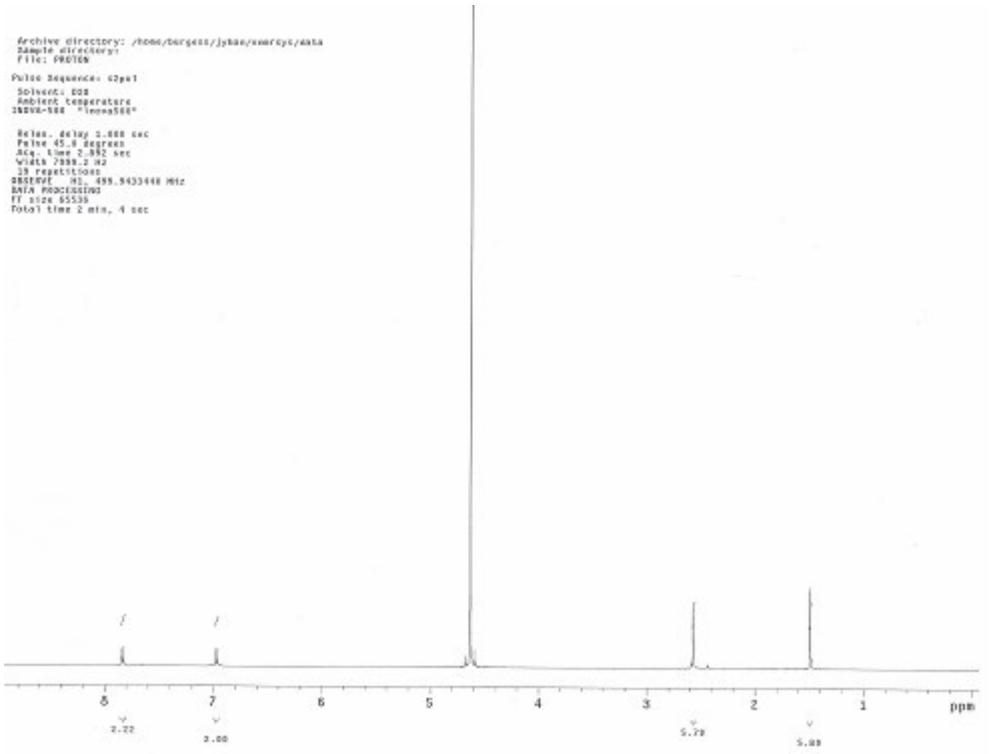




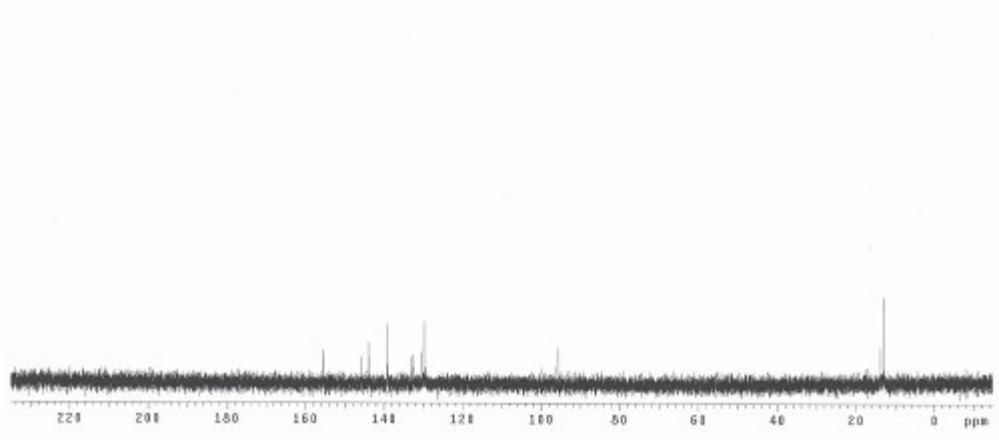
50

A solution of chlorosulfonic acid (16 µl, 0.236 mmol) in CH_2Cl_2 was added dropwise to a solution of tetramethyl iodoBODIPY (53 mg, 0.118 mmol) in CH_2Cl_2 over 10 min at -40 ^oC. 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 $NaHCO_3$ (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, D_2O) 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); ^{13}C NMR (75 MHz, D_2O) 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 $C_{19}H_{17}BF_2IN_2O_6S_2^-$ $(M-2Na+H)^-$ 608.9634 found 608.9776.

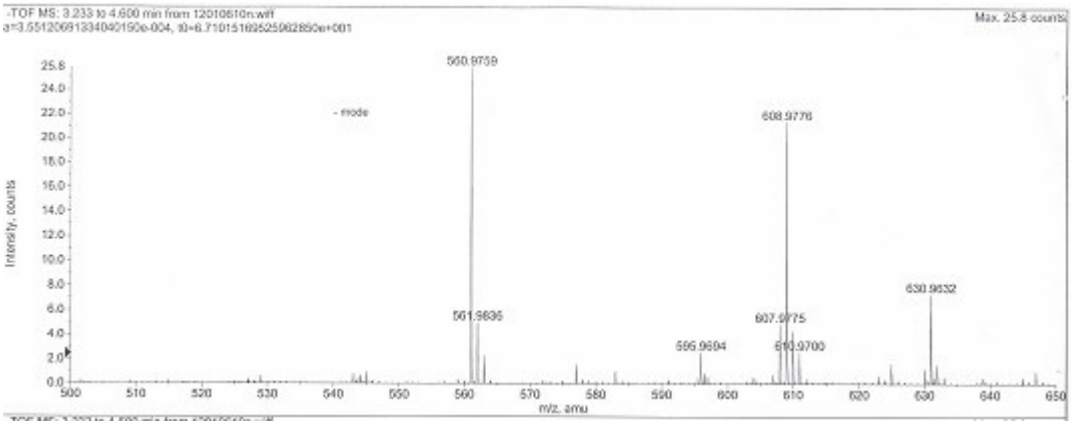
^{1}H NMR

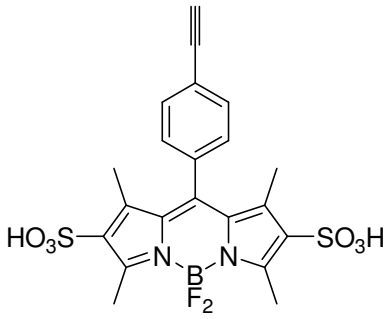


^{13}C NMR



Mass spectrum

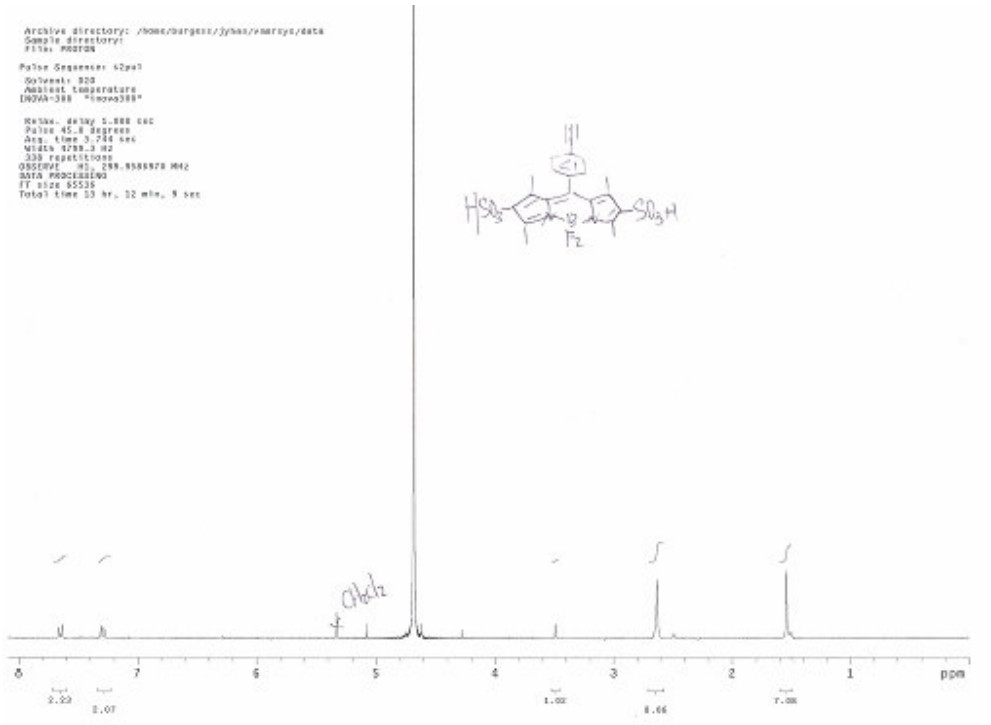




51

A solution of chlorosulfonic acid (19 µl, 0.276 mmol) in CH_2Cl_2 was added dropwise to a solution of tetramethyl ethynylBODIPY (48 mg, 0.138 mmol) in CH_2Cl_2 over 10 min at -40 ^oC. 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, D_2O) 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 $C_{21}H_{18}BF_2N_2O_6S_2^-$ $(M-H)^-$ 507.0667 found 507.0815.

^{1}H NMR



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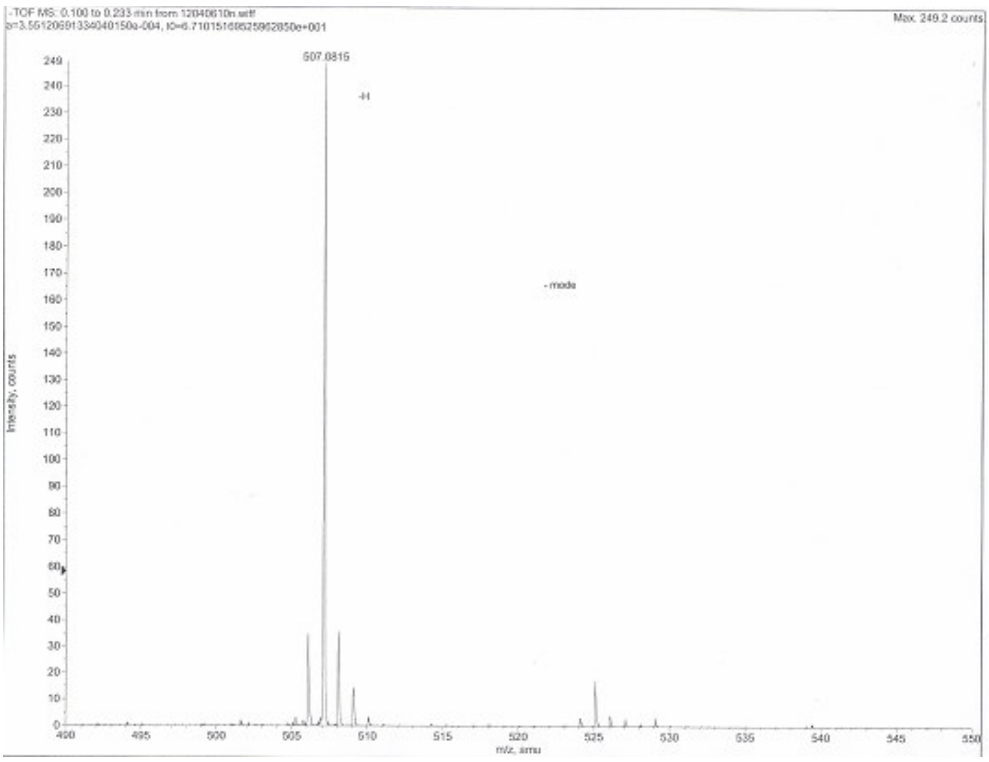
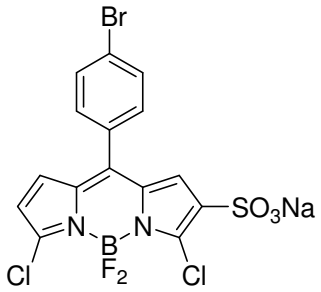
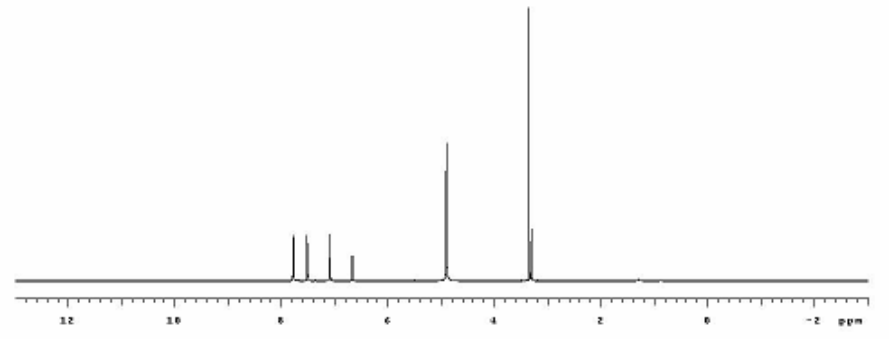




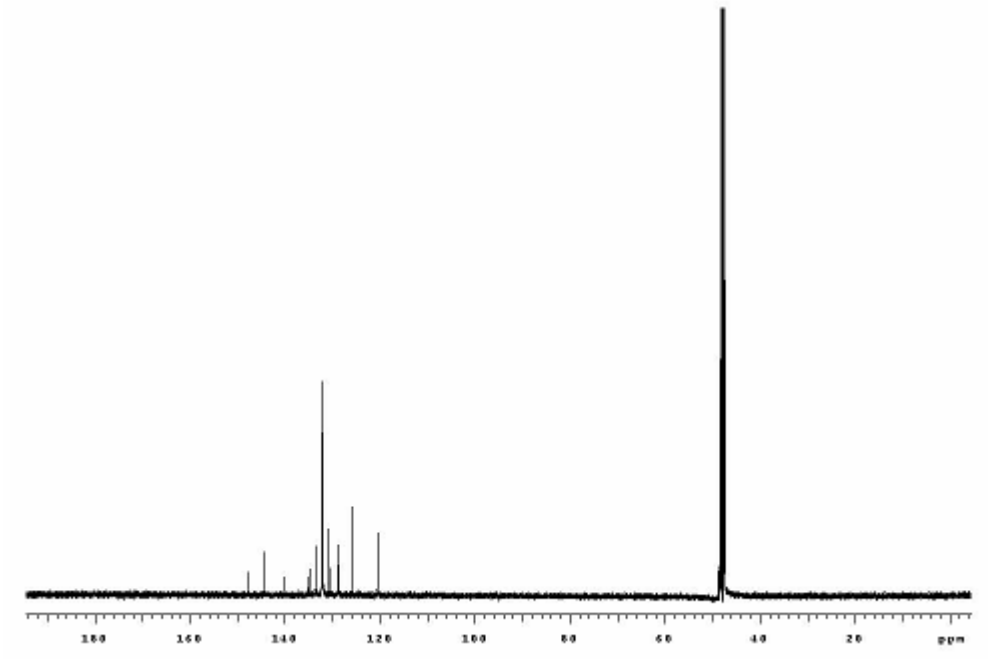
52

A solution of chlorosulfonic acid (19.2 μl, 0.29 mmol) in CH_2Cl_2 (2 ml) was added dropwise to a solution of BODIPY **21** (100 mg, 0.24 mmol) in CH_2Cl_2 (25 ml) over 10 min at -40 ^oC. 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_2Cl_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_2Cl_2 to afford the orange powder (114 mg, 92%). R_f = 0.4 (20% MeOH/CH_2Cl_2). ^1H NMR (500 MHz, CD_3OD) δ 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_3OD) δ 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 $C_{15}H_7BBrCl_2F_2N_2O_3S^-$ $(M\text{-}Na)^-$ 492.8799 found 492.7563; IR (thin film) 1572, 1379, 1259, 1198, 1119, 1055, 667 cm^{-1}.

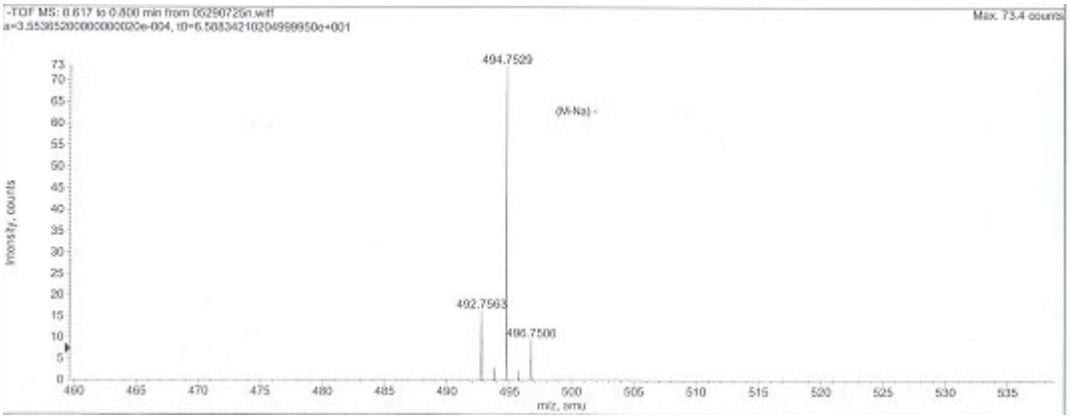
^1H NMR

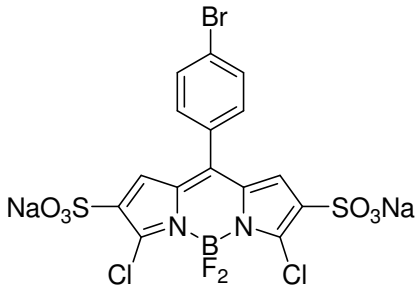


^{13}C NMR



Mass spectrum

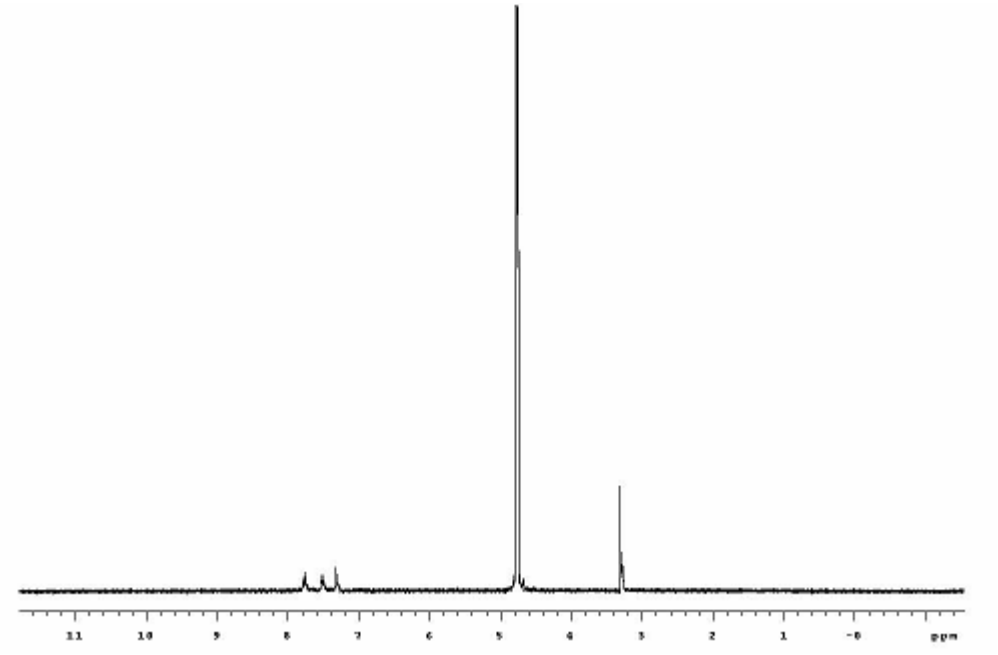




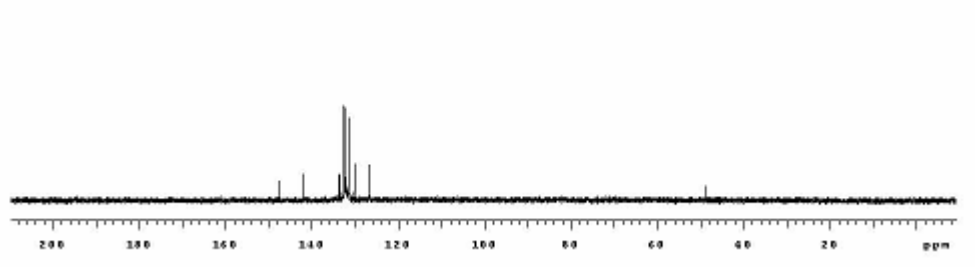
53

A solution of chlorosulfonic acid (160 μl, 2.4 mmol) in CH_2Cl_2 was added dropwise to a solution of BODIPY **21** (500 mg, 1.2 mmol) in CH_2Cl_2 over 10 min at -40 ^oC. 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 $NaHCO_3$ (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%). ^1H NMR (500 MHz, D_2O) 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, D_2O) δ 147.6, 141.9, 133.7, 132.6, 132.3, 131.8, 131.5, 130.0, 126.7; MS (ESI) calcd for $C_{15}H_6BBrCl_2F_2N_2Na_2O_6S_2^{2-}$ $(M-2Na)^{2-}$ 285.9135 found 285.8405; IR (thin film) 2968, 1572, 1382, 1206, 1033, 650 cm^{-1}.

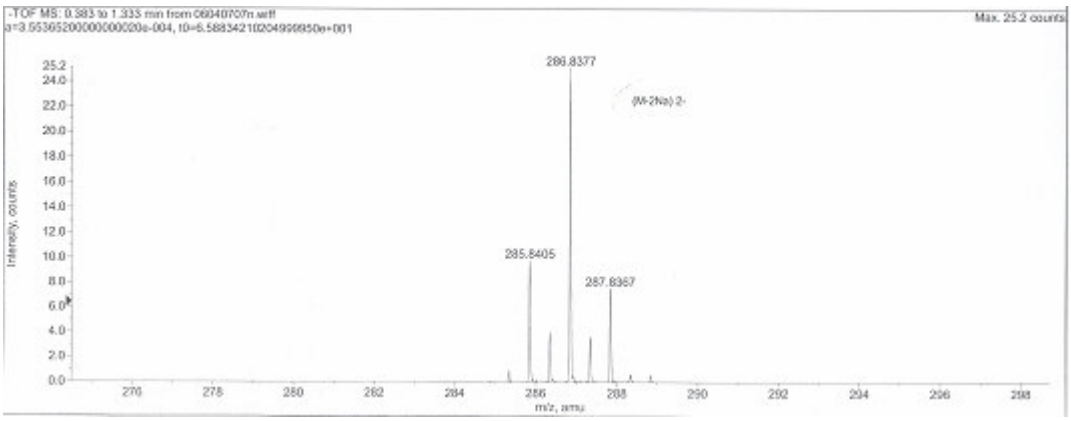
^{1}H NMR

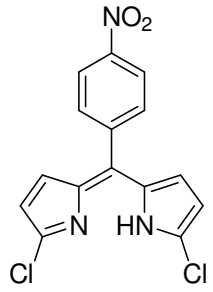


^{13}C NMR



Mass spectrum

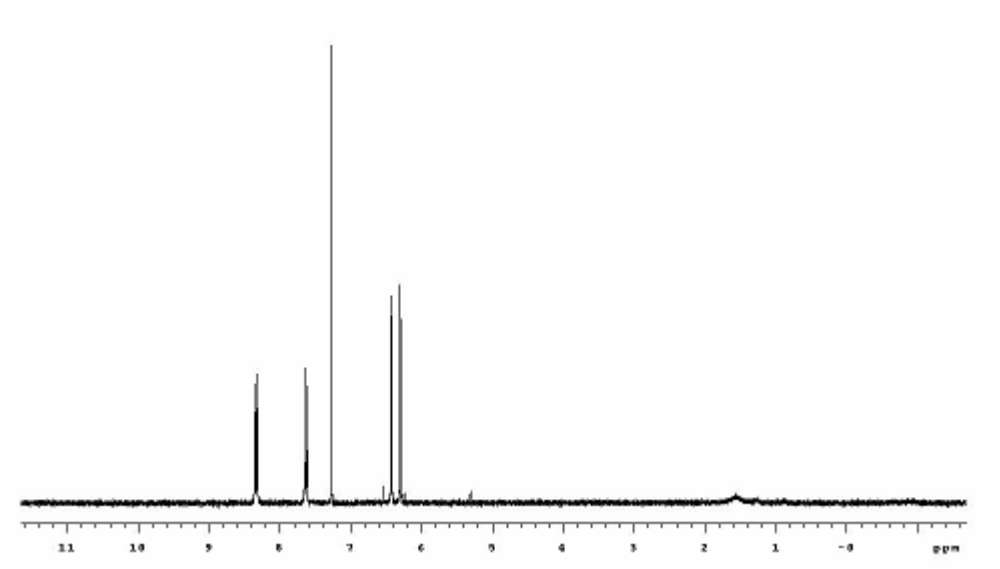




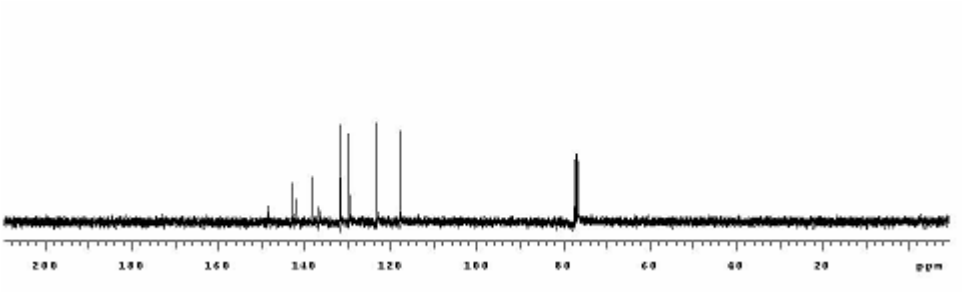
55

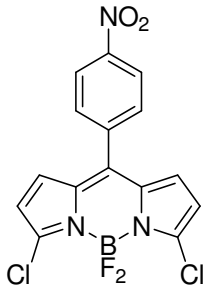
Pyrrole (25 ml, 370 mmol) and 4-nitrobenzaldehyde (2.23 g, 14.8 mmol) were added to a dry round-bottomed flask and degassed with a stream of N_2 for 5 min. TFA (0.1 ml) was then added, and the solution was stirred under N_2 at room temperature for 1 h and then quenched with 0.1 M NaOH. Ethyl acetate was then added. The organic phase was washed with water (3 x 50 ml) and dried over anhydrous Na_2SO_4, 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



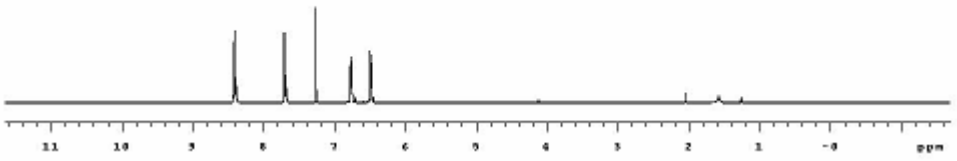


56

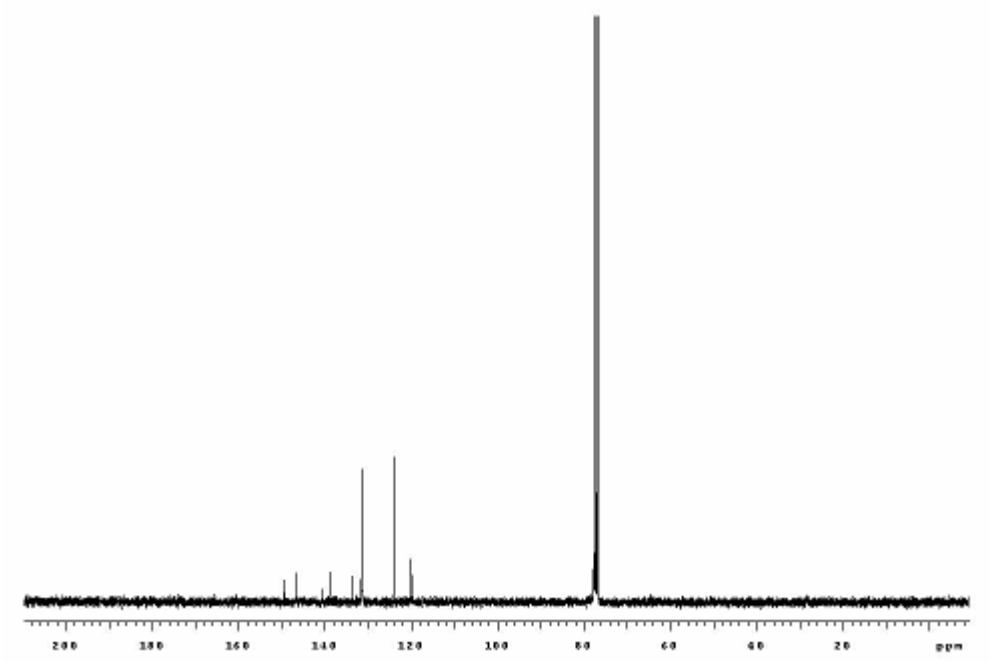
A solution of **55** (3.3 g, 12.3 mmol) in 100 ml dry THF was purged with N_2 and cooled to -78°C. To the cooled solution, a suspension of N-chlorosuccinimide (3.5 g, 25.9 mml) in 40 ml THF was added. The reaction mixture was stirred at -78°C for 1.5 h, then warmed up to the room temperature and stirred for additional 3 h. 50 ml H_2O was added to the mixture. After extraction with CH_2Cl_2 (3 x 100 ml), the combined organic layers were dried over anhydrous Na_2SO_4, 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 CH_2Cl_2. 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, $CDCl_3$) δ 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, $CDCl_3$) δ 148.5, 143.1, 142.1, 138.1, 136.7, 131.8, 129.7, 123.3, 118.0.

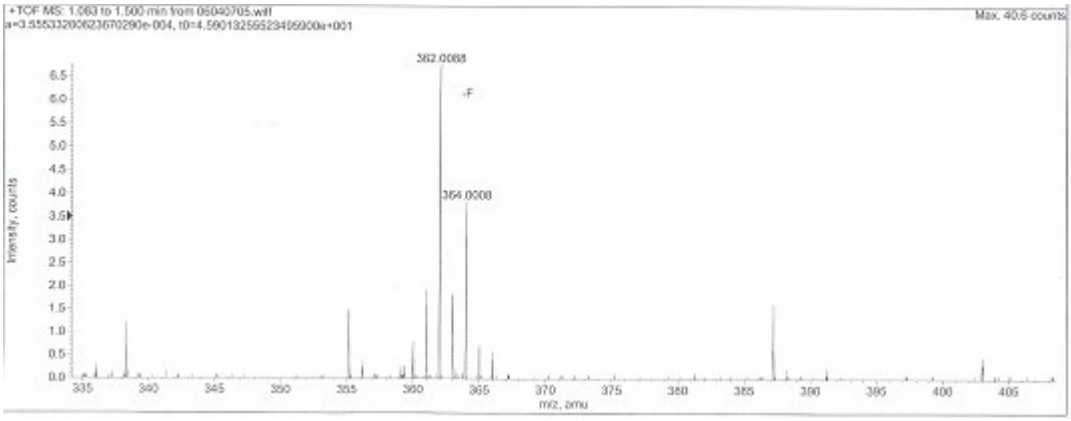
^{1}H NMR

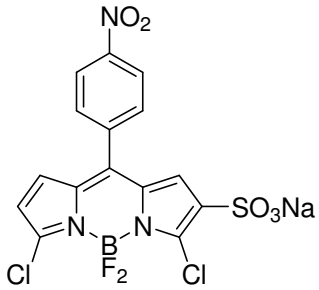


^{13}C NMR



Mass spectrum

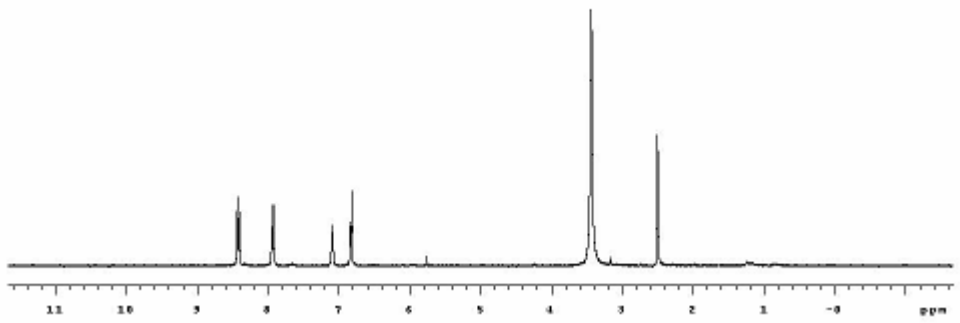




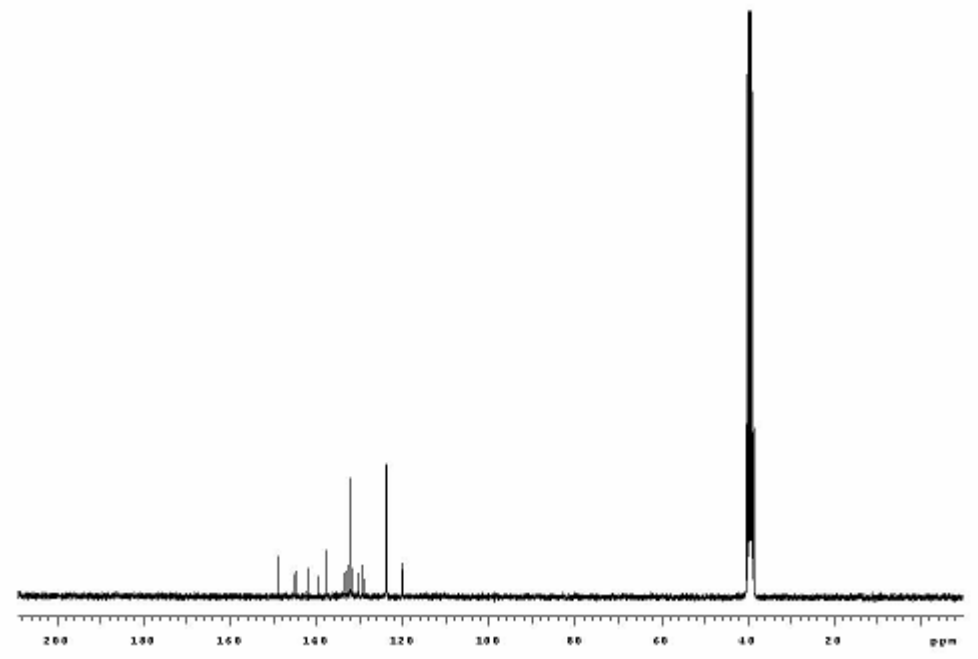
57

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 $NaHCO_3$ (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/CH_2Cl_2 to afford the monosulfonated sodium salt **57** as the orange powder (115 mg, 90 %). R_f = 0.7 (20% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 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) δ 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 $C_{15}H_7BCl_2F_2N_3O_5S^-$ $(M\text{-}Na)^-$ 459.9545 found 459.8544; IR (thin film) 2982, 1558, 1390, 1348, 1197, 1030, 667 cm^{-1}.

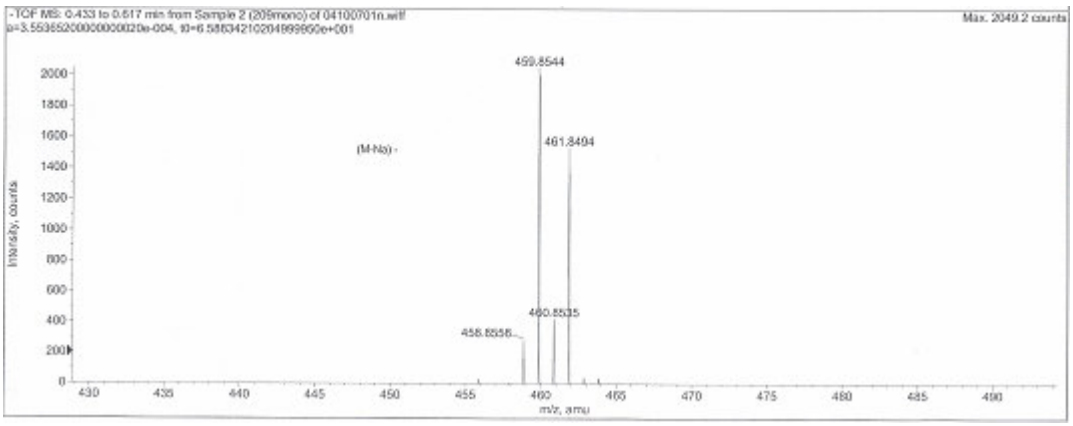
^{1}H NMR

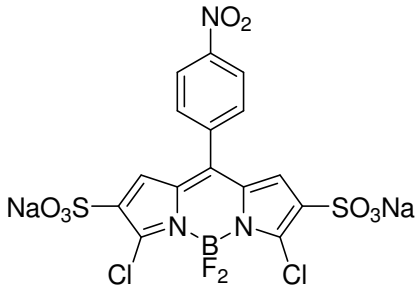


^{13}C NMR



Mass spectrum

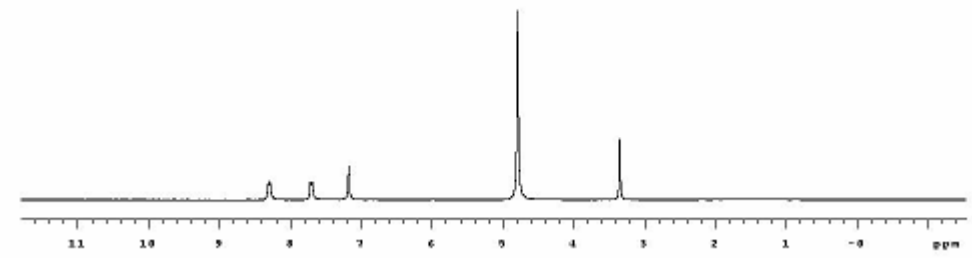




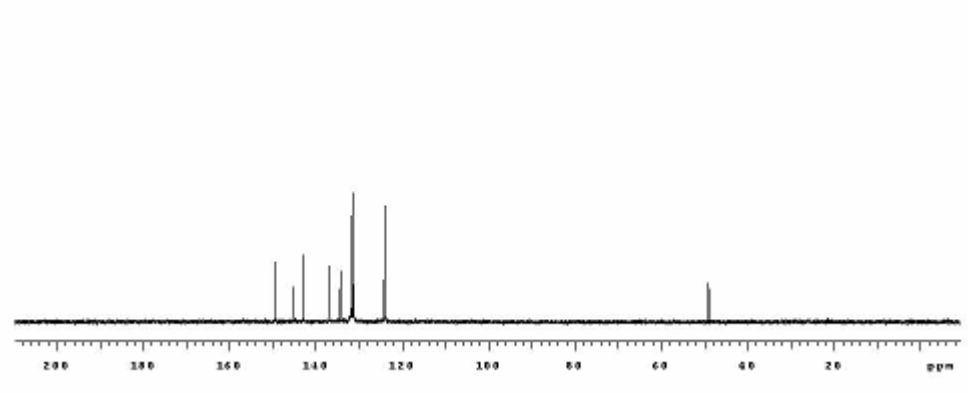
58

A solution of chlorosulfonic acid (61 µl, 0.91 mmol) in CH_2Cl_2 was added dropwise to a solution of BODIPY **56** (100 mg, 0.26 mmol) in CH_2Cl_2 over 10 min at -40 ^oC. 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 $NaHCO_3$ (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/CH_2Cl_2). ^1H NMR (300 MHz, D_2O) δ 8.30 (d, 2H, *J* = 7.5 Hz), 7.70 (d, 2H, *J* = 7.5 Hz), 7.18 (s, 2H); ^{13}C NMR (75 MHz, D_2O) δ 149.4, 145.2, 143.1, 136.8, 134.5, 132.0, 131.3, 124.1; MS (ESI) calcd for $C_{15}H_6BCl_2F_2N_3O_8S_2^{2-}$ $(M-2Na)^{2-}$ 269.4515 found 269.3838; IR (thin film) 3113, 1519, 1379, 1348, 1200, 1030, 848, 692, 680, 664 cm^{-1}.

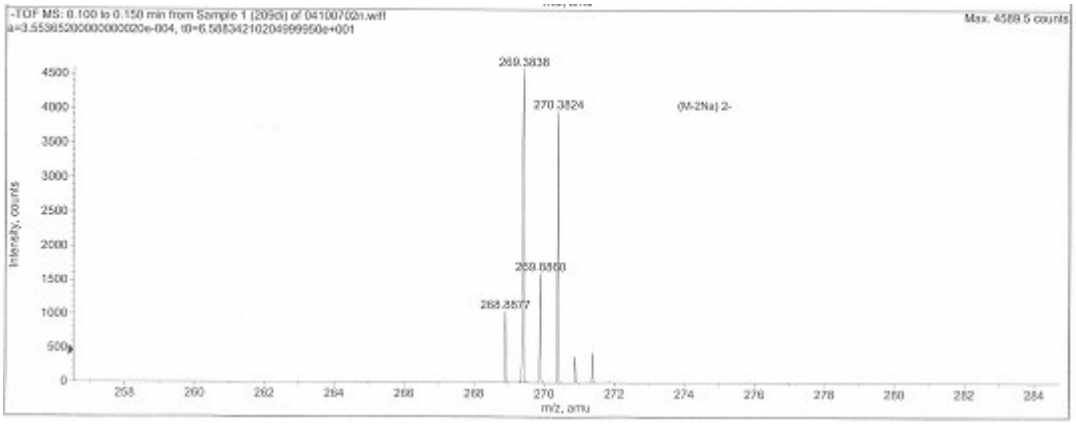
^1H NMR

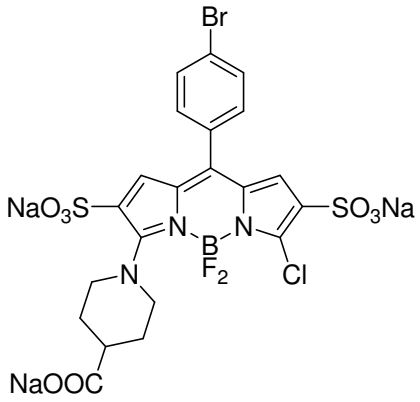


^{13}C NMR



Mass spectrum

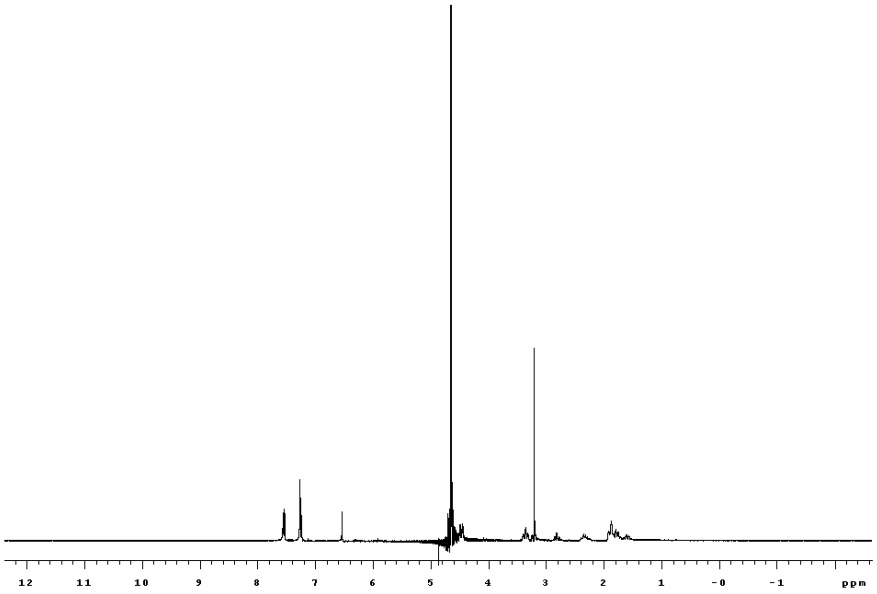


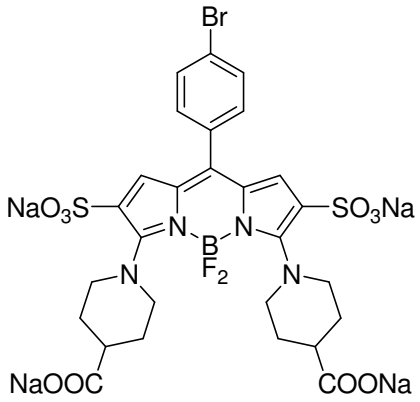


59

Sodium bicarbonate (3 eq) was added to a solution of compounds **22** (2.1 mg) and **53** (10 mg) in D_2O (2 ml). The solution changed color to dark red immediately. ^{1}H NMR (300 MHz, D_2O) 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

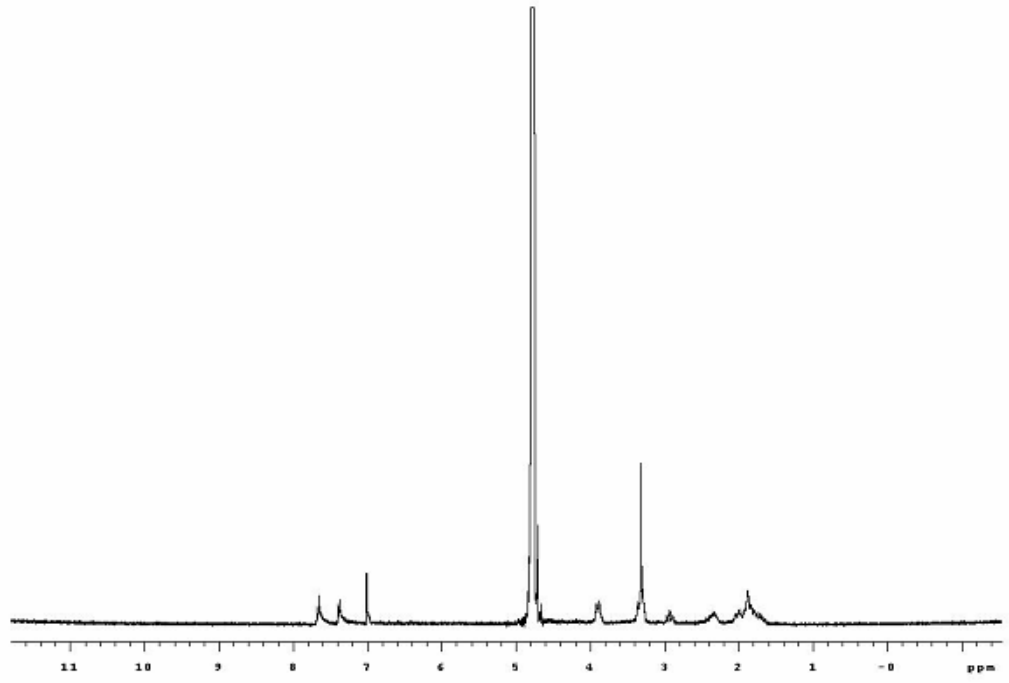




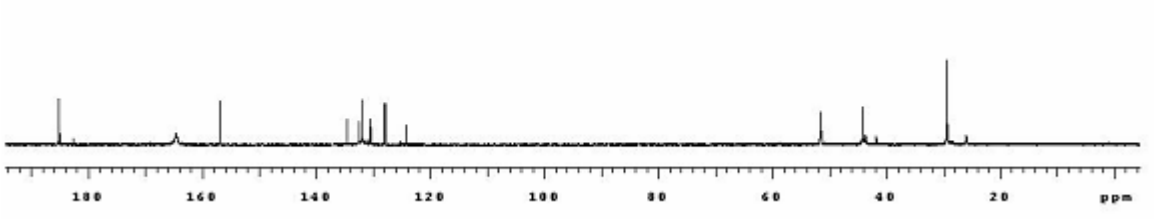
60

Another 3 eq of sodium bicarbonate and 1 eq compound **22** (2.1 mg) was added to a solution of **59** in D_2O. The mixture was stirred at room temperature for 24 h. ^{1}H NMR (300 MHz, D_2O) 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_2O) 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}C NMR



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