TETRASTYRYL-BODIPY DYES AS POTENTIAL PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

A THESIS

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FOR THE DEGREE OF

MASTER OF SCIENCE

By
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August 2011

I certify that I have read this thesis and that in my opinion it is fully adequate, in scope and in quality, as a thesis of the degree of Master of Science.

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August, 2011

Photodynamic therapy (PDT) is a novel methodology for a wide range of treatments of cancerous and noncancerous diseases. Success of treatment and photodynamic action is highly dependent the performance of PDT agents. Therefore, PDT agents with unique photophysical properties are required in clinical applications. There are several photosensitizers in literature and some are available. However, most of them less than ideal due to limitations such as low molar absorptivity and photo stabilities.

In this study, we synthesized near-IR tetrastyryl-Bodipy derivatives as photosensitizers. These sensitizers have strong absorption in therapeutic region and are good photo-generator of singlet oxygen. They are promising for photodynamic therapy with their favorable properties.

Keywords: Boradiazaindacene (Bodipy), photodynamic therapy (PDT), photosensitizer (PS), singlet oxygen.

ÖZET

FOTODİNAMİK TERAPİ İÇİN POTANSİYEL FOTODUYARLAŞTIRICI OLAN TETRASTİRİL-BORADİAZAİNDASEN

Nisa Yeşilgül

Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. Engin U. Akkaya
Ağustos, 2011

Fotodinamik terapi bir çok kanser tipi ve kanser tipi olmayan hastalık tedavisinde kullanılan bir tedavi yöntemidir. Tedavinin, dolayısıyla fotodinamik uygulamanın başarısı fotoduyarlaştırıcıya bağlıdır. Bu yüzden klinik uygulamalarda kullanılacak fotoduyarlaştırıcıların belirli özelliklerinin olması gereklidir. Çok sayıda yayınlanmış fotoduyarlaştırıcı çeşidi vardır. Fakat çoğu düşük molar absortivite ve foto-kararlılık nedeniyle yetersizdir.

Bu çalışmada, yakın kızıl ötesi absorbansları olan yeni tetrastiril-Bodipy fotoduyarlaştırıcıları sentezlenmiştir. Bu duyarlaştırıcıların teröpatik aralıkta yüksek absorbansı vardır. Bu olumlu özellikleriyle, bunlar fotodinamik terapi için ümit vaadetmektedirler.

Anahtar Kelimeler: Bodipy, fotodinamik terapi, fotoduyarlaştırıcı, singlet oksijen

Dedicated to my father and mother

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisor Prof. Dr. Engin U. Akkaya for his guidance, support, patience and for teaching us how to become a good scientist. I will never forget his support throughout my life.

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Finally, I want to express my gratitude to my family for their love, support, and understanding. I owe them a lot.

LIST OF ABBREVIATIONS

PV: Photovoltaic

DSSC: Dye sensitized solar cell

FTO: Fluorine doped tin oxide

CTO: Conductive and transparent oxide

HTM: Hole transport material

Spiro-OMeTAD: 2,2,7,7-tetrakis(*N*,*N*di-*p*-methoxyphenyl-amine)9,9-spirobifluorene

IPCE: Incident photon to current efficiency

LHE: Light harvesting efficiency

DFT: Density functional theory

BODIPY: Boradiazaindacene

TLC: Thin layer chromatography

NMR: Nuclear magnetic resonance

CHENO: 3a,7a-dihydroxy-5b-cholic acid

CV: Cyclic voltammetry

DMF: Dimethylformamide

THF: Tetrahydrofuran

DDQ: Dichlorodicyanoquinone

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CHAPTER 1

INTRODUCTION

1.1. Photodynamic Therapy

Light has been used in the treatment of certain diseases since old times. However, its popularity of use in medicine has been increased considerably in recent years. [1] Photodynamic therapy (PDT) is a promising treatment for cancer and some other diseases involves a photosensitizer (PS), a light activable chemical, and light at a specific range of wavelength.

In clinical applications, photosensitizer can be applied intravenously or topically and then light is illuminated to the specific tissue. Therefore, photosensitizer is activated by visible or nearly visible light resulting with reactive oxygen species, singlet oxygen (O_2^1) species.[2]

Cell death in the presence of intense light was first reported by Oscar Raab in literature.[3,4] During his study on the effects of acridine on malaria, he observed toxic effects of acridine red molecule on certain bacteria in the presence of light.[5] By these control experiments, requirement of fluorophore for the light induced toxicity was proved. After these discoveries, eosin became the first photosensitizer, which was used for medical purposes by Tappeiner and Jesionek and also essential participation of oxygen is realized by Tappeiner and Jodbauer.[6] However, the role of the oxygen in the mechanism of oxygen-dependent toxicity of photoactive molecules is resolved at 1979 by electron spin resonance technique.[7] By this technique generation of singlet oxygen, which is highly reactive excited state oxygen, is monitored.

The first clinical application of photodynamic therapy was conducted at Roswell Park Cancer Institute in 1978, following this application, photofrin is approved by United States FDA as a photosensitizer in 1980.[8] Since that time a

number of photosensitizers such as HPPH, ALA, Metvix are approved by FDA and reported in literature (Figure 1).

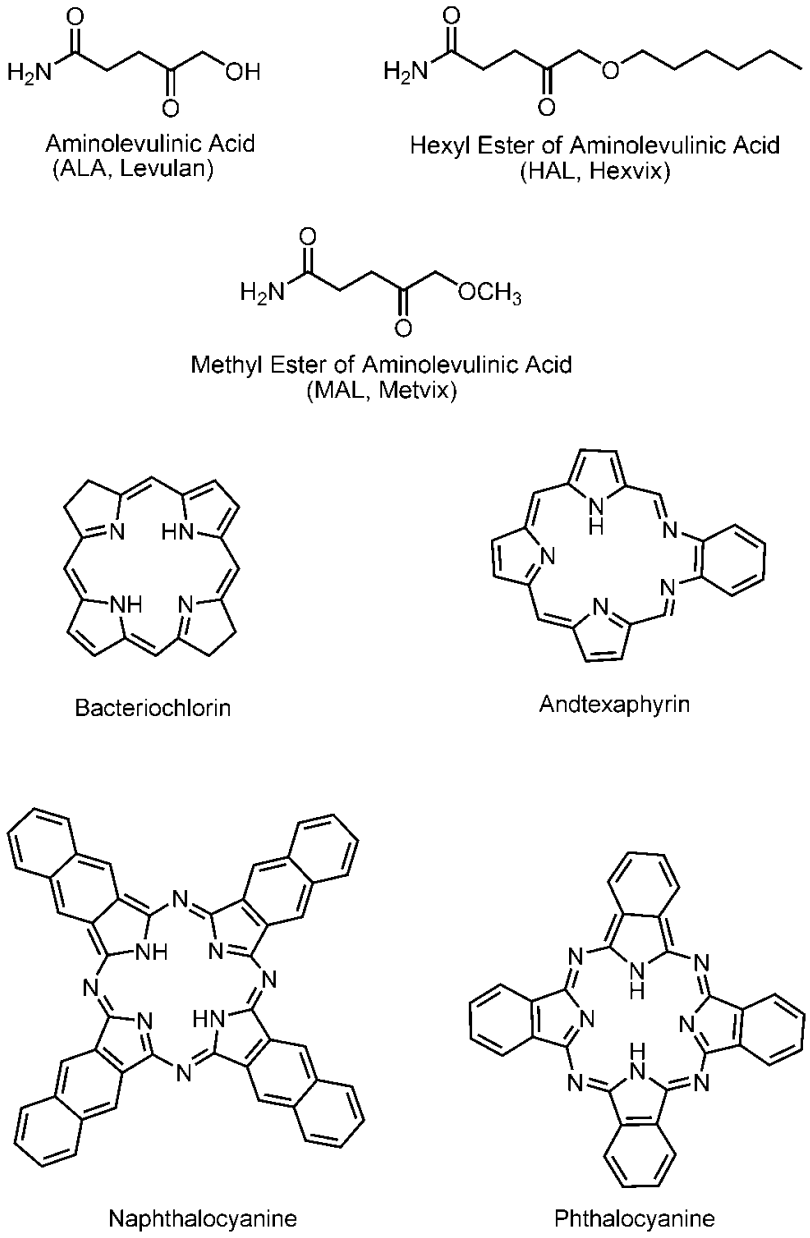


Figure 1. Different type of photosensitizers

1.1.1. Mechanism of Photodynamic Action

The generation of reactive singlet oxygen species is the crucial step of photodynamic action.[9-16] The process start with excitation of photosensitizer via photon at appropriate energy, usually between 600-800 nm, from its ground state to singlet excited state following with relaxation to ground state vibrational level of excited electronic state (Figure 2). There are three possible ways that electron can follow at this point. It can go back to its ground state, nonradiative internal conversion or it can fall back to its ground state, fluorescence, or it can go to triplet excited state, intersystem crossing. Intersystem crossing can result with phosphorescence or in a way of favorable for photodynamic action if energy is transferred to ground state of oxygen resulting with reactive oxygen species.[9]

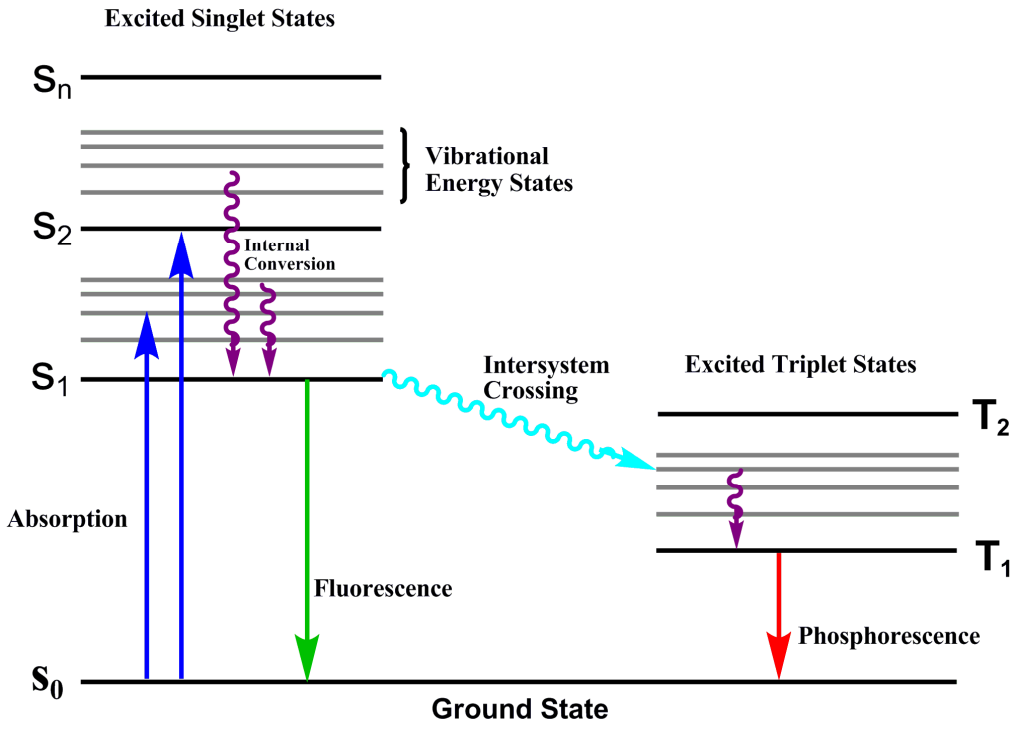


Figure 2. Jablonski energy diagram[16]

Besides singlet oxygen generation from direct energy transfer of excited photosensitizer to oxygen molecule, singlet oxygen can also be generated by energy transfer from photosensitizer to other biomolecules or substrates in the environment.[10] Therefore, formed unstable radicals can react with other biomolecules and substrates or molecular oxygen, as a result singlet oxygen generation is provided.

The interactions of singlet oxygen species with other cells result with cytotoxic effects for both targeted tumor cells and surrounding cells besides these direct cytotoxic effects of photodynamic therapy, they trigger immunological response to tumor regions by preventing vascular supplements to the cancerous tissues.[11] This action triggers two types of cell deaths, apoptosis and necrosis (Figure 3). [12, 13]

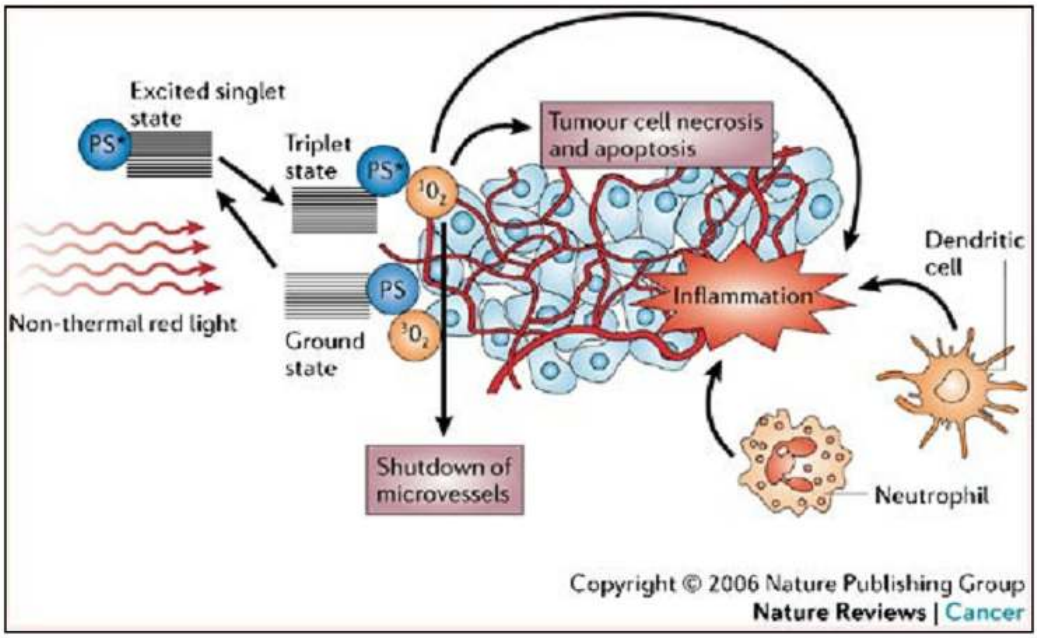


Figure 3. Biological response of photodynamic therapy[11]

Apoptosis is a programmed cell death and it is characterized by cell shrinkage and nuclear fragmentation. Necrosis, on the other hand, is a traumatic cell death and it is characterized by swelling of the cell. In contrast to necrosis, apoptosis produces apoptotic cell fragments that clean the contents of the cell before they are spill out to surrounding tissues.

1.1.2. Light and Photosensitizer

Careful control of light and photosensitizer is very important for the success of photodynamic applications.

1.1.2.1. Ligth

Light interaction with a tissue can result by absorption, scattering, transmission or reflection depending on the light properties such as absorption coefficient and on the optical features of the tissue (Figure 4). Inhomogeneous sides such as membranes and nuclei affect the propagation of light in the tissue by causing scattering. Moreover, existence of water or some endogenous dyes such as melanin in the tissue affect the penetration depth of light.[9]

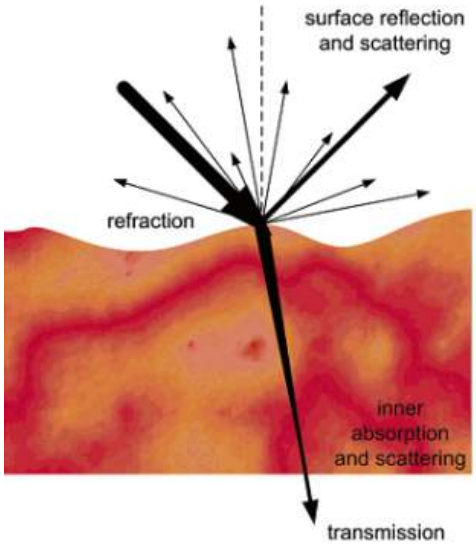


Figure 4. Light interactions on tissue[9]

In a general consideration, however, light in the spectral range of 600-700 nm penetrates 50-200% more according to the light in the spectral range of 400-500 nm.[14] Laser or other sources such as light-emitting diodes (LEDs) can be used as the light sources for photodynamic application.

1.1.2.2. Photosensitizers

In the phototherapeutic window, 620-850 nm, light penetrates in the maximum depth when certain biomolecules are considered such as melanin, collagens, nicotinamide, flavins (Figure 5).[9,15].[16] Photosensitizers activated by visible or near visible light attack surrounding cancer cells stimulate immune system. Besides, absorption of photosensitizers in the 400-600 nm range should be low to avoid prolonged skin sensitivity. As the efficiency of photosensitizer is considered, it should be very pure and stable. Besides these properties photosensitizers should be water-soluble for medical uses and should be ineffective until light is applied (nontoxic in the dark).[1, 9]

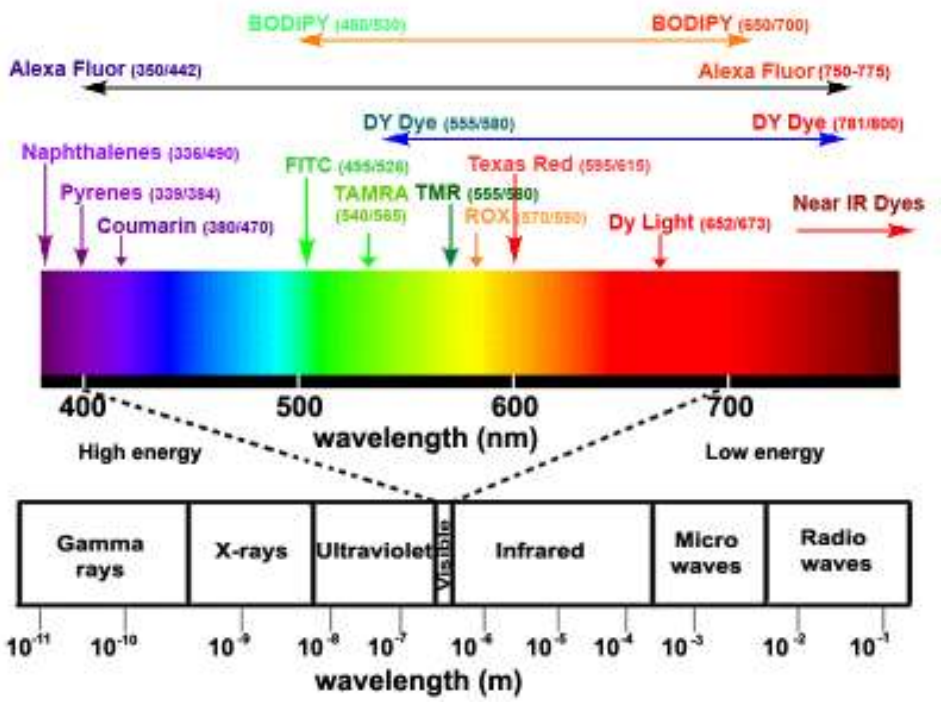


Figure 5. Dye families in the visible region[16]

Photofrin, and hematoporphyrin derivatives are the first generation photosensitizers, however, these type of photosensitizers have low absorption in the phototherapeutic window and also cause 2- 3 months of skin sensitivity (prolonged skin sensitivity).[17-21] The second generation of photosensitizers is based on porphyrin derivatives, which have a better absorption in the phototherapeutic window.[18] In the third generation, photosensitizers linked to biomolecules so that a better selectivity is provided.[19]

Several groups of photosensitizers are studied in clinical applications; bacteriochlorins, phthalocyanines, naphthalocyanines, andtexaphyrins are some of them (Table 1).[11,12, 18]

Photosensitizer	Trade name	Approval	Excitation (nm)	Clearance time	Sites
Porfimer sodium	Photofrin	1998 FDA	630 nm	4-6 weeks	Lung
ALA-PpIX	Levulan Kerastastick	1999 FDA	635 nm	2 days	Actinic Keratosis
Methyl aminolevulate-PpIX	Metvix	2004 FDA	635 nm	2 days	Actinic Keratosis
Hexyl aminolevulate-PpIX	Hexvix	2005 EU	405 nm	2 days	Detection bladder tumors
BPD-MA	Verteporfin, Visudyne	2000 FDA	689 nm	5 days	Choroidal reovascularization (CNV)
mTHPC	Foscan	Phase I trials, 2001 (EU)	652 nm	15 days	Head and neck, prostate, pancreas, esophagus, mesothelioma
Motexafin Lutetium	Mlu, Lutex, Lutrin	Phase I trials	732 nm	3 h	prostate, atherosleros is
Pd-bacteriopheophorbide	Tookad	Phase I trials	762 nm	2 h	Prostate
Taloporfin sodium	LS11	Phase I and II trials	664 nm	1 h	CNV, liver and colorectal
Silicon pthalocyanine 4	PC-4	Phase I trials	672 nm	24-36 h	skin

Table 1. Some photosensitizers are used in clinical applications.[18]

Xanthane derivative (fluorescein, eosin and Rose Bengal) , Cyanine dyes, hypericin, squaraines, texaphyrins, fullerenes, Bodipy derivatives and Perylenediimide can be listed as some of the several sensitizers in the literatures.[20,21] Perylendiimide dyes are promising when their high quantum

yields are considered, besides their absorption maxima can be shifted to the therapeutic window with proper functionalization (Figure 6).[19]

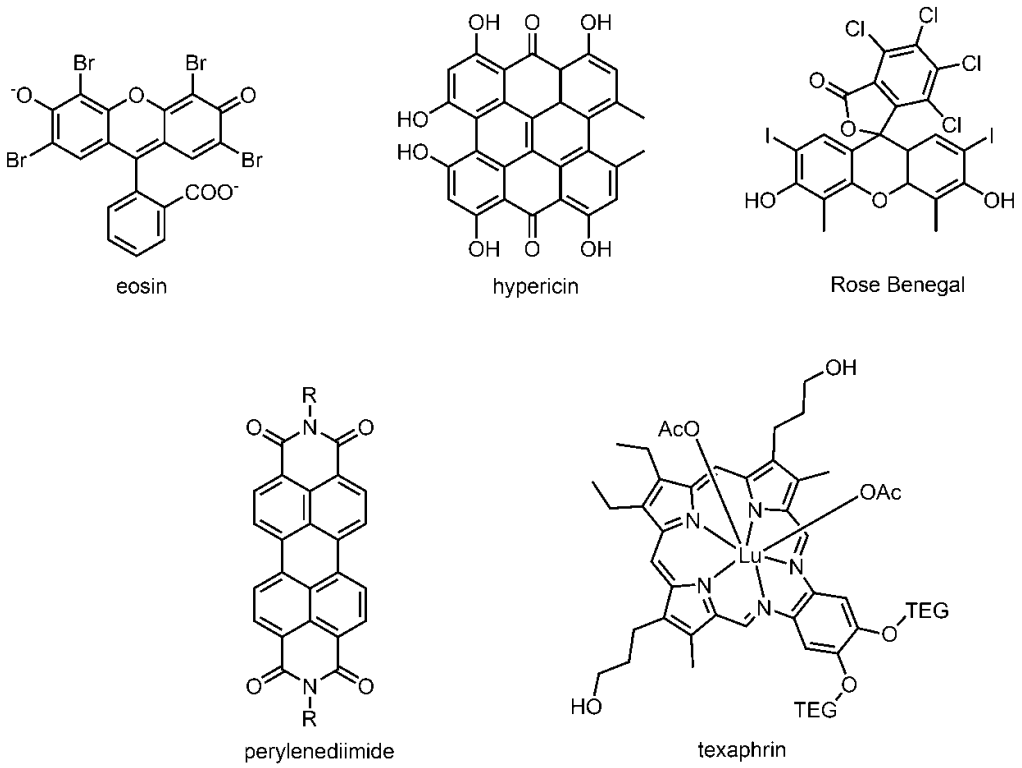


Figure 6. Photosensitizers in literature.

1.2. Bodipy dyes

4,4'-difluoro-4-bora-3a,4a-diaza-s-indacene, abbreviated to Bodipy, dyes have gained considerable attention over several fluorescent organic molecules within the last decade. As bright fluorescent dyes, Bodipy dyes were used in cellular imaging for several years after their first synthesis by Treibs and Kreuzer in 1968. [22,23] However, Bodipy dyes have been used in many applications within the last decade.[24]

1.2.1. Functionalization

The ease of functionalization from different positions is one of the important features of Bodipy dyes make them favorable to use in different areas. There are several literature examples of Bodipy derivatives functionalized from different positions (Figure 7).[27-40]

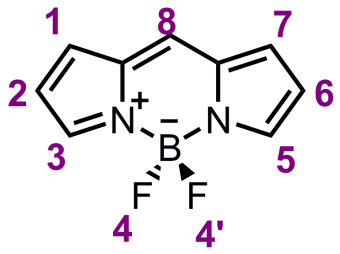


Figure 7. Functionalization of Bodipy dye

The substitution of aryl groups on the *meso* position has little effect on the absorption and emission bands. However, quantum yields of 1, 7-substitution of these Bodipy derivatives are more than the unsubstituted ones because 1, 7-substitutions prevent free rotation of the phenyl ring (Figure 8).[27]

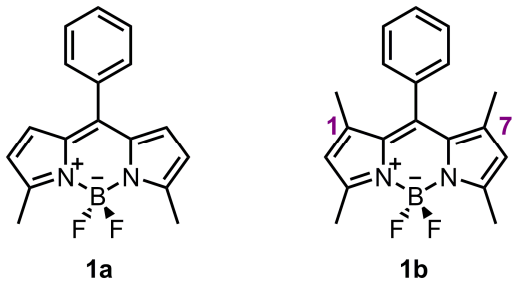


Figure 8. Bodipy derivatives in literature. Quantum yields of compound 1a and 1b; 0.19 and 0.65 in MeOH, respectively.

The 2, 6- positions of Bodipy dyes can be sulfonated by electrophilic reaction with chlorosulfonic acid, as a result, sulfonated Bodipy derivative is more stable than the Bodipy core besides water solubility of functionalized Bodipy derivative are increased (Figure 9).[25,26]

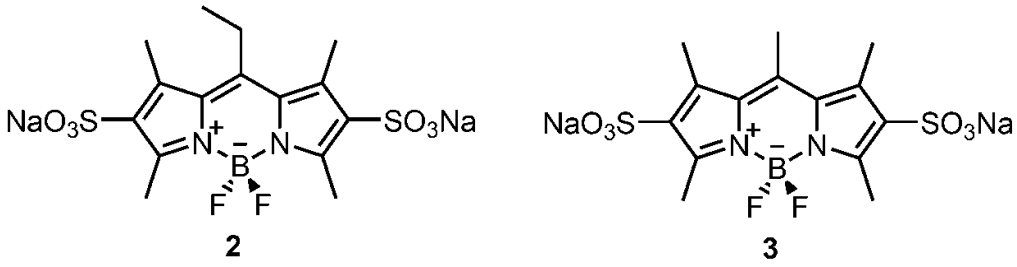


Figure 9. Sulfonated Bodipy derivatives in literature.

Bromination or Iodination of Bodipy core gives dibromination, diiodination at 2, 6-positions. These kind of functionalized Bodipy derivatives have showed a significant red shift of absorption and emission maxima (Figure 10).[27]

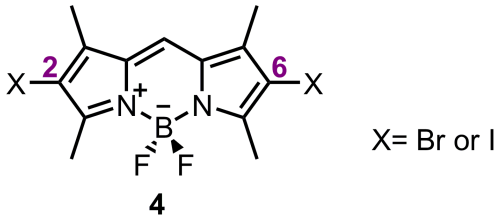


Figure 10. Bromination or Iodination on Bodipy core

The substitution of 3- and 5- positions has an important effect on shifting emission and absorption spectra and on fluorescent quantum yields. The substitution of electron donating groups such as thioalkoxides or amino groups to the 3- and 5- positions creates a significant bathochromic shift of both emission and absorption spectra (Figure 11).[28]

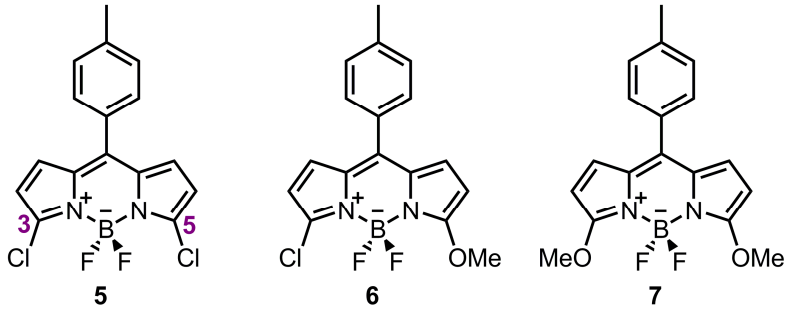


Figure 11. The substitution of 3- and 5- positions on Bodipy core in literature.

The acidic character of methyl groups at 3- and 5- positions of Bodipy derivative gives a high yield with Knoevenagel reactions. Therefore, different styryl substituted Bodipy derivatives can be synthesized by using several aldehydes (Figure 12).[29-33] Functionalization at 3- and 5 positions are also possible with transition metal catalyzed reactions such as Suziki, Sonagashira, Stille and Heck couplings.[30] Therefore, series of Bodipy dyes with extended conjugation and dispersed emission maxima have synthesized.

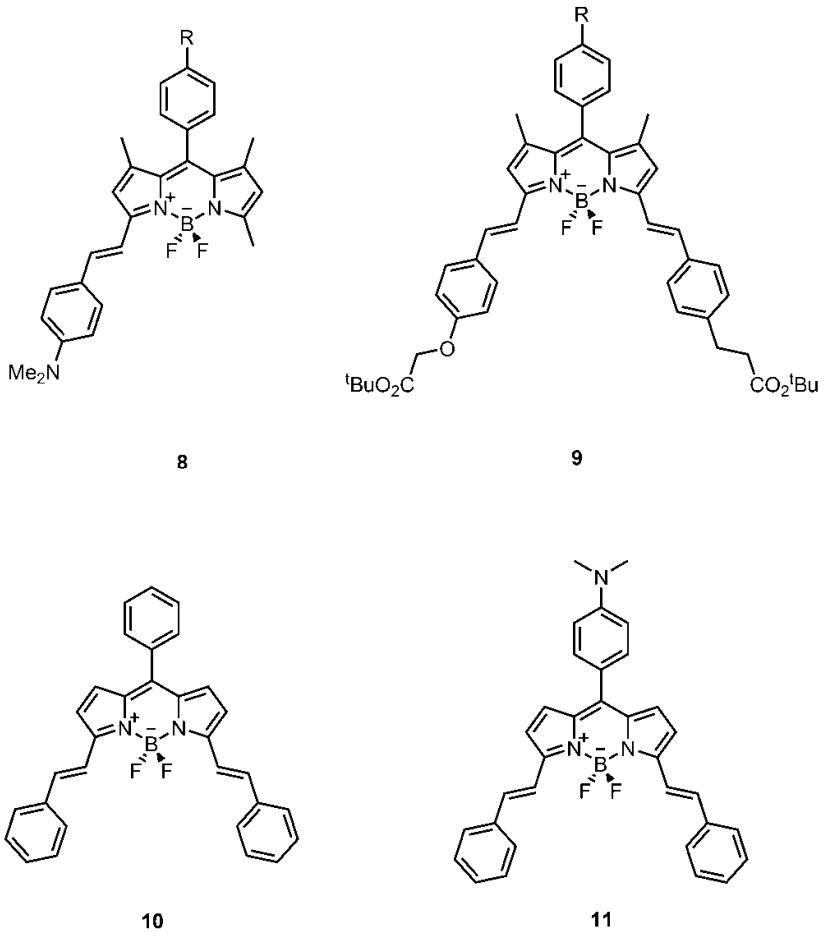


Figure 12. Different Bodipy derivatives substituted on 3, 5- positions in literature.

The functionalization on the positions of fluoride ions of boron center at the Bodipy derivative is also possible (Figure 13). Ziessel and coworkers have replaced fluoride ions by aryl, ethynyl, and ethynylaryl substituents.[31-36]

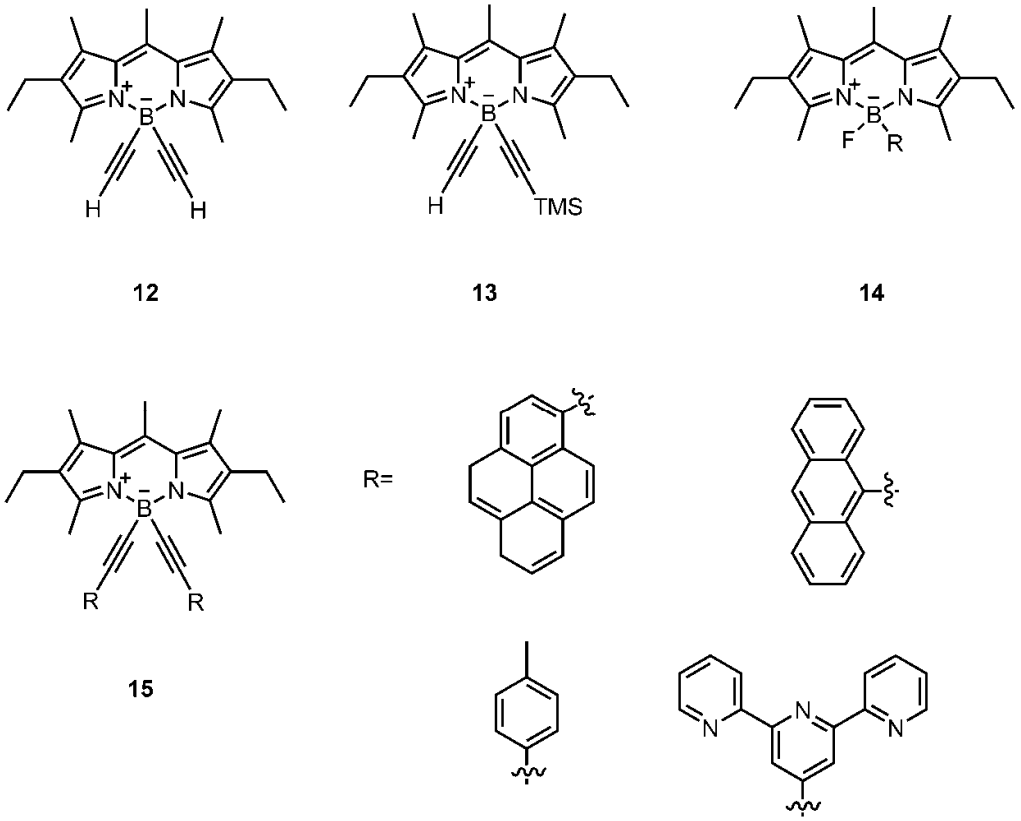


Figure 13. The functionalization on fluoride ions of Bodipy core in literature.

1.2.2. Applications of Bodipy Dyes

Their several distinctive properties such as their high fluorescent quantum yields, narrow emission bands, thermal and photostabilities, solubility and ease of functionalizations make Bodipy dyes convenient and popular in many different applications over commonly used fluorescent organic compounds. They are used as fluorophores in different kind of applications such as energy transfer systems, light emitting devices, mesomorphic materials, biochemical labeling, light harvesting systems, photodynamic therapy applications and solar cell applications.

1.2.2.1. Light harvesting systems and Energy transfer

Light harvesting systems as a mimic of natural systems are made up join of fluorescent units, donors and acceptors, with mutual spectral properties. Spectral overlap of donor emissions and acceptor absorbances and the distance between these units are important for the effectiveness of the energy transfer system. Akkaya *et al.* synthesized a dendritic light harvesting system, which consists of four Bodipy units as donors and a perylendiimide core as acceptor (Figure 14).[45] Because of the increased number of Bodipy dyes on the structure, extinction coefficient of the system at the maximum absorption of Bodipy dye (526 nm) is increased to 240000 $M^{-1}cm^{-1}$ and energy transfer efficiency is found to be 99%.

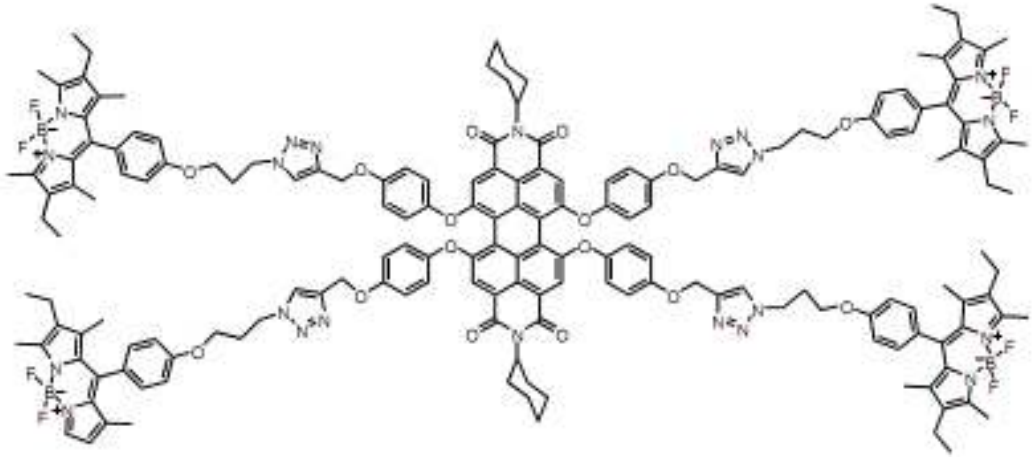


Figure 14. A Bodipy based dendritic light harvesting system.[45]

Here is another example of light harvesting system containing Bodipy dyes as donors. The energy is transferred to zinc porphyrin from Bodipy dye and then it is transferred to a fullerene (Figure 15).[46] In this synthetic system, protein matrix is not required because chromophores are self assembled for coordination.

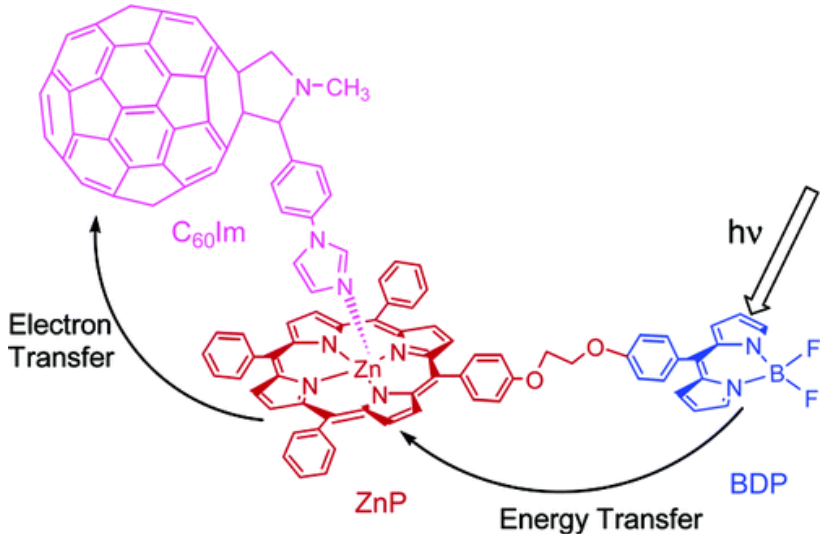


Figure 15. Artificial antenna[46]

1.2.2.2. Solar cells

Dye synthesized solar cells has received a considerable interest as an alternative to the semiconductor based solar cells in recent years due to their low cost. In order to increase the efficiency of the dye synthesized solar cell systems, several groups are incorporated such as a zinc porphyrin Bodipy dyad application to a dye sensitized solar cell (Figure 16).[47]

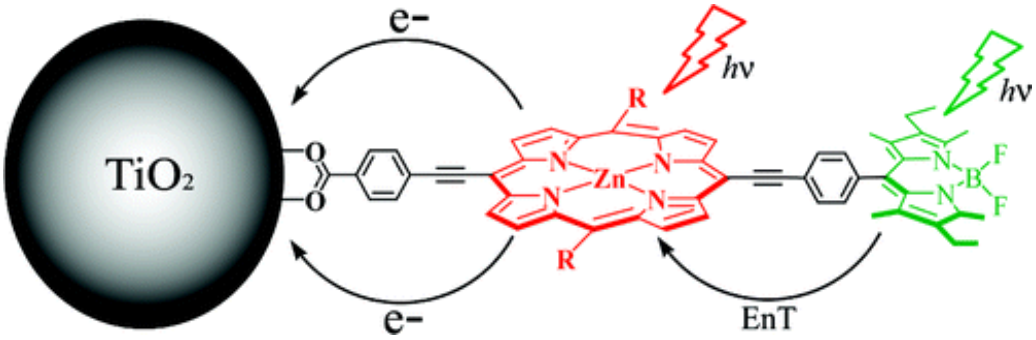
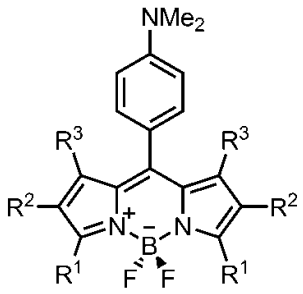


Figure 16. Zinc porphyrin Bodipy dyad.[47]

1.2.2.3. Chemosensors

Fluorescent chemosensors have attracted great interest not only for scientific but also commercial tools for biomedical applications since their first literature examples in 1980 by Tsien. First Bodipy based chemosensors are synthesized by Daub in 1997.[48] Since then Bodipy based chemosensors are used sensing of different kind of cations and anions and as pH indicators (Figure 17).



R^1 = Me, H, CO_2Et,
R^2 = = Et, H,
R^3 = Me

17

Figure 17. Bodipy based chemosensor.

1.3. Photodynamic therapy, Bodipy derivatives as Photosernsitizers

Bodipy dyes have received considerable attention within the last decade due to their sharp fluorescence emissions, high quantum yields and other distinctive properties mentioned before.[49] There are different Bodipy derivatives have emissions between 530 nm and 630 nm.[50]

There are several literature examples of use of Bodipy derivatives as photosensitizers in recent years. Azaboradiazaindacenes, aza- Bodipy derivatives, are studied extensively by O'Shea and coworkers, compound **18**.[51,52] These dyes show a favorable characteristic for photodynamic therapy applications with their sharp absorption at therapeutic window (Figure 18).

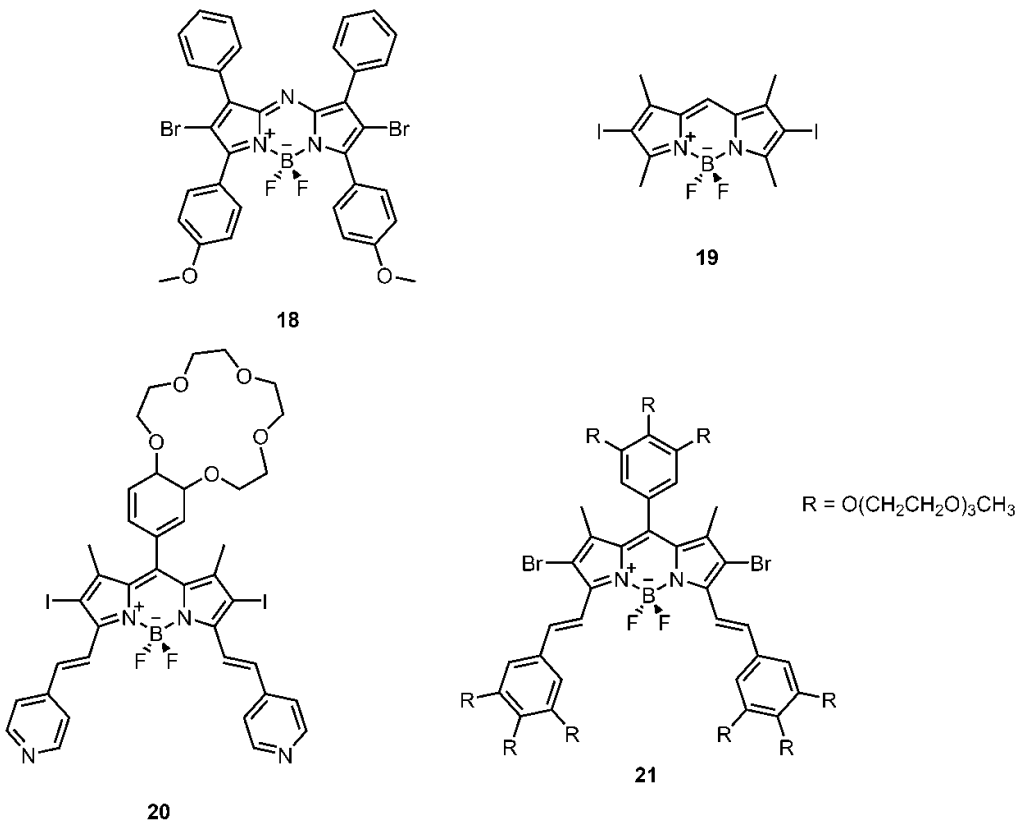


Figure 18. Bodipy based photosensitizers in literature.

Akkaya et al. synthesized a photosensitizer by functionalization of Bodipy dye (Figure 19).[53] The photosensitizer can detect acidity and the sodium concentration of the medium. In the acidic environment, pyridine groups are protonated and this protonation results with a bathachromic shift of the absorbance. These protonated molecules, but not the others, can be activated by light at 660 nm. Therefore, these excited molecules induce the photodynamic action and photo-induced electron transfer (PET) is prevented by sodium binding crown ether moieties. In cancer tissues, pH is low and Na^+ concentration is high. Therefore, when light is applied at 660 nm, the photosensitizer is activated and singlet oxygen generation is provided.

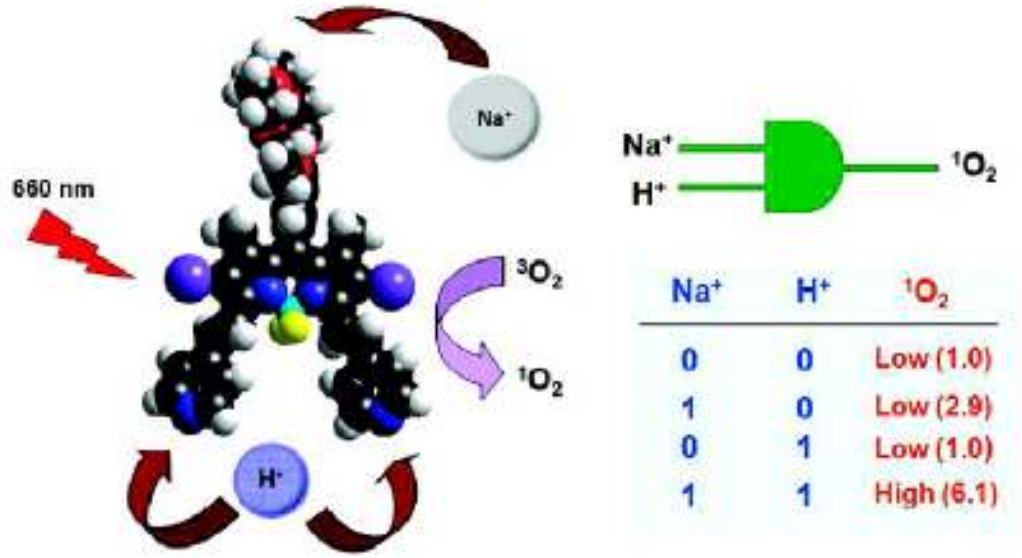


Figure 19. Photosensitezer working according to cancer parameters.[53]

1.3.1. Tetra-styryl Bodipy Dyes

Fluorescent probes in the near-IR region are important for photodynamic therapy. For instance, during intracellular imaging, emissions obscure below approximately 550 nm due to autofluorescence of cells, which makes difficult to visualize *in vivo*, but at longer wavelengths it becomes less. Therefore, Bodipy dyes, which have emission in 530 nm- 630 nm, are suboptimal for tissue imaging.

Extending their absorption and emission maxima to the near-IR region have been achieved by replacement of carbon in the eight position by a nitrogen atom, synthesis of aza-Bodipy derivatives,[54,55] , replacement of pyrole by isoindole[56,57], attachment of some aromatic rings to Bodipy core[58,59], substitution at meso-, 2, 6- and 3, 5- positions of Bodipy core[60]. The substitution with aryl groups at the 8 position on the Bodipy core has little effect on extending absorption and optical properties of Bodipy derivatives unless electron donating or electron withdrawing substituent such as NO_2, CN[61,62].

It is possible to synthesize tetrastyryl Bodipy dyes bearing same or different side arms and with a proper functionalization is possible to generate and to enhance desired chemical properties such as water solubility (Figure 20).[63]

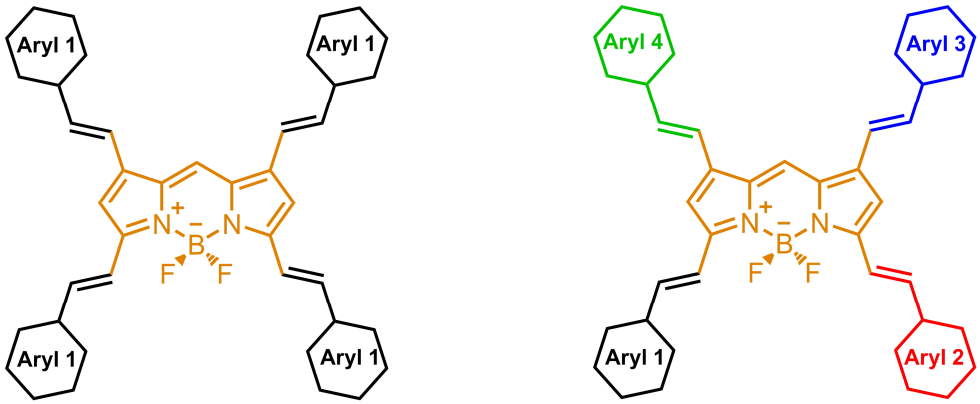


Figure 20. Tetrastyryl-Bodipy dyes bearing same and different side arms

Knoevenagel reaction is a high yielding method for the substitution at the 3- and 5- positions of Bodipy core.[64-66] It is a simple condensation reaction, which is a nucleophilic addition of an active hydrogen compound to a carbonyl compound. Mono-, di-, tri-, and tetrastyryl-Bodipy derivatives can be synthesized in one reaction by stopping reaction at several times.[67-69]

3-, and 5- positions on the Bodipy core yield first in Knoevenagel reaction because these positions are the most acidic ones according to Mulliken-charge analysis on the core carbon atoms of tetramethyl-Bodipy. Mulliken-charge analysis is showed the electron density changes in the following order according to the positions on Bodipy dye: 2, 6>> 1, 7>> 3, 5.[70] ^{1}H NMR spectra also provides additional information about the acidity of methyl substitutents (Figure 21).

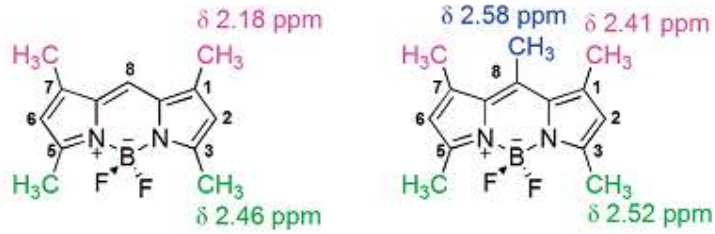


Figure 21. Tetramethyl- and pentamethyl Bodipy derivatives with ^{1}H NMR chemical shifts in $CDCl_3$.[70]

The first example of functionalization on 1, 3, 5, 7- positions on Bodipy core published by Akkaya *et al.* in 2009 although several literature examples of 3, 5-functionalized Bodipy examples exist.[70] Therefore, absorption and emission wavelength of synthesized Bodipy dyes were up to 700 nm before this study. In Akkaya Research Group's study, 1, 3, 5, 7-tetra styryl and 3, 5, 7- tristyryl Bodipy derivatives besides 3- mono styryl and 3, 5- distyryl Bodipy derivatives were synthesized.

With a proper combination of the substitutents on side arms, optical properties of tetrastyryl-Bodipy derivatives can be improved tremendously. Absorption and emission maxima can be tuned and each arm can be sensitive to environment at a pH range.

Zissel *et al.* synthesized tetrastyryl-Bodipy derivatives, bearing same and four different aldehydes in their side arms starting from tetramethyl Bodipy derivative (Figure 22).[71]By using different aldehyhdes in substitution of the side arms, mono-, di-, tri- and tetra- styryl substituted derivatives can be achieved to synthesize.

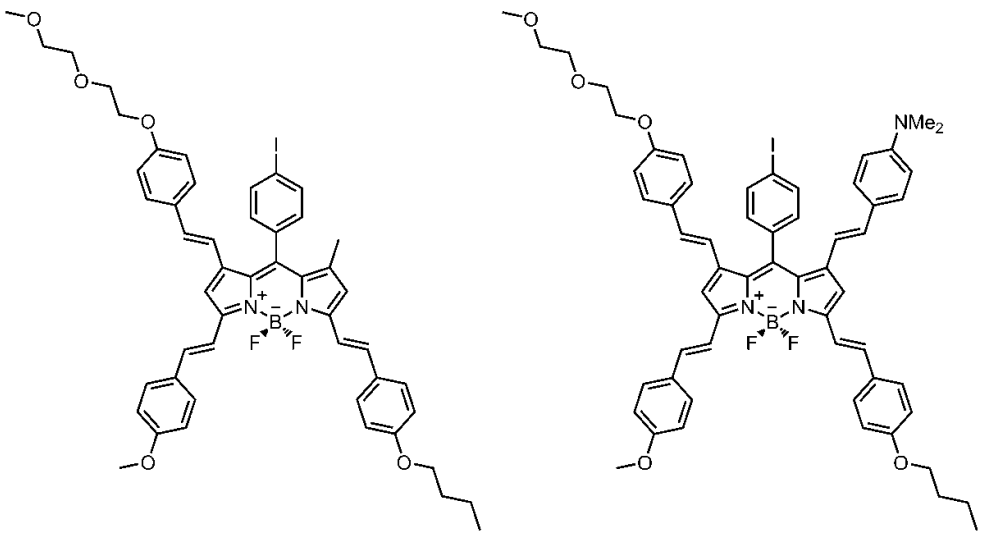


Figure 22. Tetra- styryl substituted Bodipy derivatives from literature[71]

Tetrastyryl-Bodipy dyes have unique peaks belong to trans coupled protons of methyl groups on 1, 7- positions in ^{1}H NMR spectrum, which is used characterization of these dyes. In Figure 23, there are ^{1}H NMR spectra of distyryl- and tetrastyryl Bodipy derivatives. There is a shift to downfield, 8.6 ppm, in both spectra come from protons that are on the side of fluorine atoms due to electron withdrawing ability of fluorine atoms. The other protons, which do not close to fluorine atoms, give peak in the same region, 7.8 ppm. Coupling constants of these protons are 16 MHz because they are trans to each other. In ^{1}H NMR spectrum of tetrastyryl-Bodipy dye, however, there is a upfield shift, 6.05 ppm, come from protons marked in pink due to shielding zone of phenyl ring at the meso position. The other proton (marked in green) is not affected from the phenyl ring on the meso position so it gives a peak in a similar region (7.8 ppm) with trans coupled protons (marked in blue) and again coupling constants are 16 MHz, which show trans positions of these protons.

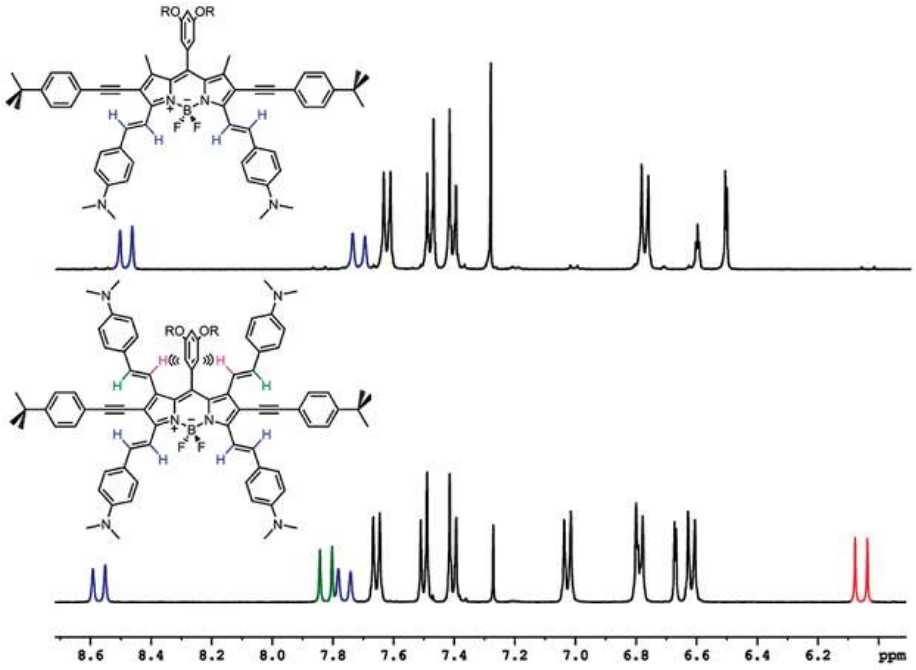


Figure 23. ^{1}H NMR spectra of distyrl- and tetrastyryl- Bodipy dyes.[70]

CHAPTER 2

EXPERIMENTAL

2.1.General

All chemicals and solvents obtained from Sigma-Aldrich were used without further purification. Thin layer chromatography by Merck TLC Silica gel 60 F_{254} was used to monitor reactions. Chromatography on silica gel was used over Merck Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on Bruker DPX-400 (operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Coupling constants (*J values*) are given in Hz and chemical shifts are reported in parts per million (ppm). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and p (pentet). Absorption spectra in solution were acquired using a Varian spectrophotometer. Varian Eclipse spectrofluorometer was used to determine the fluorescence emission spectra. All spectroscopy experiments were performed using spectrophotometric grade solvents. Mass spectroscopy measurements were conducted using MSBQTOF at Bilkent University, UNAM, Mass Spectrometry Facility

2.2. Synthesis Scheme

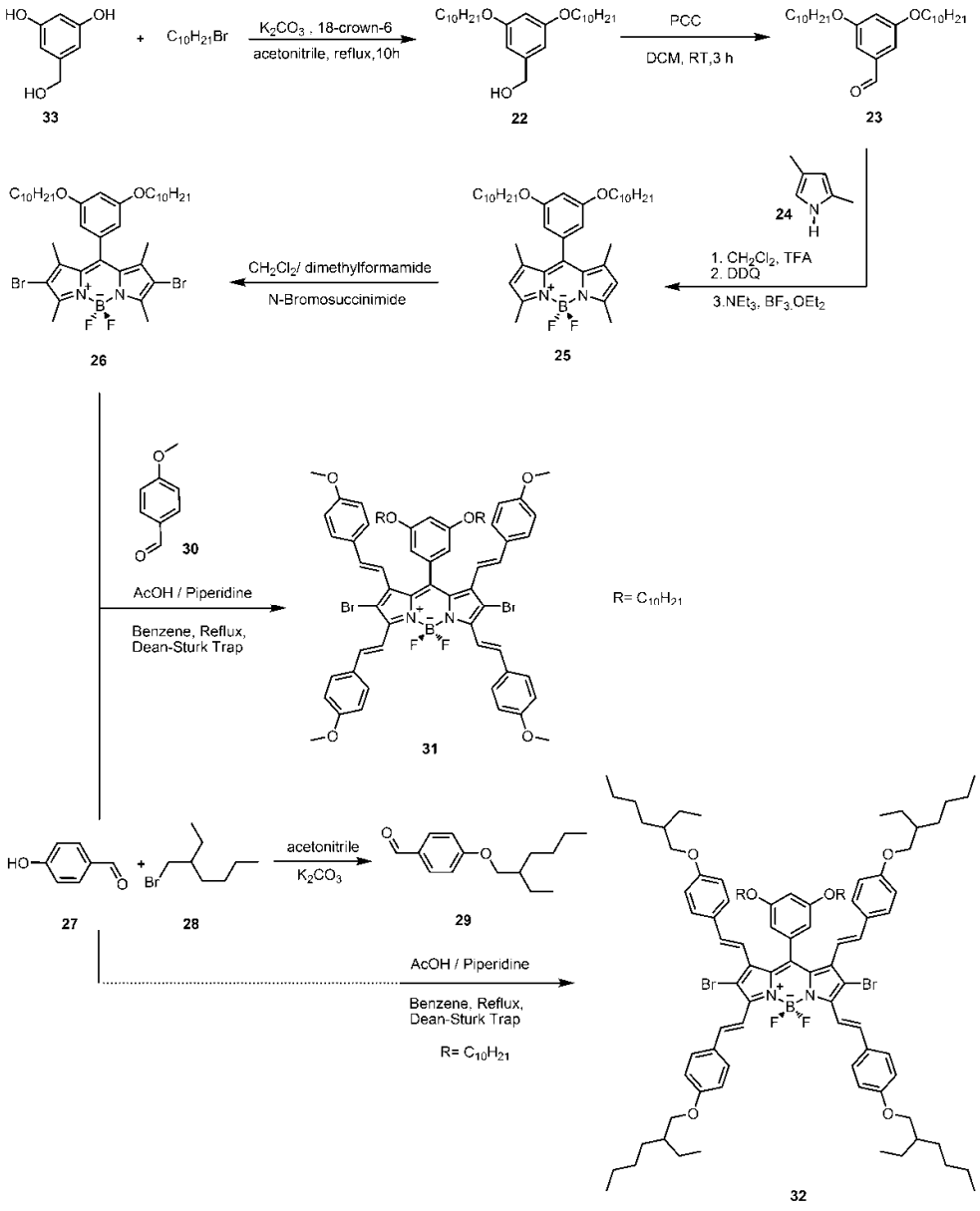


Figure 24. Synthesis Scheme for compound **31** and **32**

2.3. Syntheses

3,5-Bis(decyloxy)benzyl alcohol (compound **22**) and 3,5-Bis(decyloxy)benzaldehyde (compounds **23**) were synthesized according to literature.[70]

2.3.1. Synthesis of Compound 25

Argon gas was degassed through solution of 400 mL CH_2Cl_2. 2,4-dimethyl pyrrole (**29**) (15.8 mmol, 1.5 g) and 3,5-bis(decyloxy)phenylbenzaldehyde (**30**) (7.17 mmol, 3.0 g) were added. Then, 1-2 drops of TFA was added to the reaction mixture. The reaction mixture was stirred under N_2 atmosphere overnight at room temperature. DDQ (7.17 mmol, 1.63 g) is added to the reaction mixture, and stirring was continued for 30 min. Then Et_3N (5 mL) and $BF_3.OEt_2$ (5 mL) were added, respectively. 100 mL of water was added to the reaction mixture and extracted with $CHCl_3$ (3 x 100 mL). Na_2SO_4 was used as a drying agent. The solvent was evaporated and the residue was purified by silica gel column chromatography using 2:1 $CHCl_3$: Hexane as the eluant afforded desired product as red solid (1.381 g, 30%).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.45 (s, 1H, Ar*H*), 6.35 (s, 2H, Ar*H*), 5.90 (s, 2H, Ar*H*), 3.85 (t, 4H, *J* = 6.56 Hz, OC*H*$_2$), 2.47 (s, 6H, C*H*$_3$), 1.70 (m, 4H, C*H*$_2$), 1.49 (s, 6H, C*H*$_3$), 1.35 (m, 4H, C*H*$_2$), 1.20 (s, 24H, C*H*$_2$), 0.80 (t, 6H, *J* = 6.5 Hz, C*H*$_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 161.2, 155.4, 143.2, 136.4, 131.2, 121.0, 106.4, 102.3, 68.4, 31.9, 29.6, 29.5, 29.3, 29.2, 26.0, 22.7, 14.6, 14.2, 14.0 ppm.

MS (TOF- ESI): m/z: : Calcd: 636.4638 $[M-H]^+$, Found: 636.4564 $[M-H]^+$, Δ=11.6 ppm.

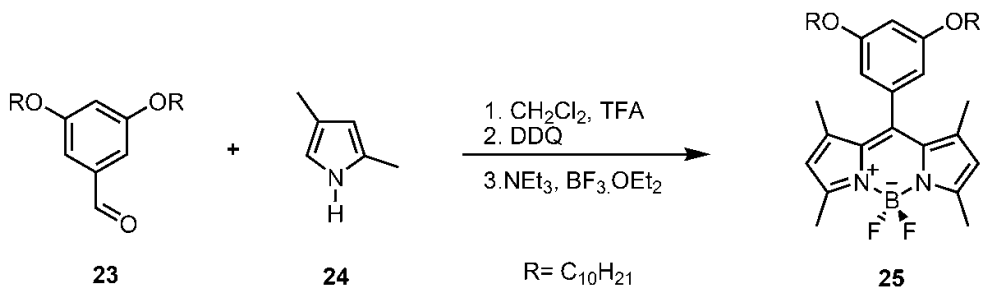


Figure 25. Synthesis of Compound **25**

2.3.2. Synthesis of Compound 26

Compound **25**(0.70 mmol, 420 mg) is dissolved in dichloromethane(15 ml) and dimethylformamide (15 ml) mixture. To the reaction mixture, *N*-Bromosuccinimide (1.96 mmol, 348.8 mg) dissolved in 10 ml dichloromethane is added dropwise. Reaction is monitored by using thin layer chromatography until all the starting material is consumed. 50 mL of water was added to the reaction mixture and extracted with CH_2Cl_2 (3 x 50 mL). Na_2SO_4 was used as a drying agent. The solvent was evaporated and the residue was purified by silica gel column chromatography using 2:1 $CHCl_3$: Hexanes as the eluant afforded desired product as red solid (500 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.60 (s, 1H, ArH), 6.40 (s, 2H, ArH), 3.95 (t, 4H, J = 6.64 Hz, OCH_2), 2.62 (s, 6H, CH_3), 1.79 (m, 4H, CH_2), 1.58 (s, 6H, CH_3), 1.45 (m, 4H, CH_2), 1.30 (s, 24H, CH_2), 0.88 (t, 6H, J = 6.64 Hz, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 161.4, 153.8, 142.1, 140.7, 135.7, 130.1, 106.0, 102.6, 77.3, 77.0, 76.7, 68.5, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 26.0, 22.7, 14.1, 13.7, 13.6 ppm.

MS (TOF- ESI): m/z: : Calcd: 790.2794 $[M-H]^-$, Found: 790.2811 $[M-H]^-$ Δ=2.2 ppm.

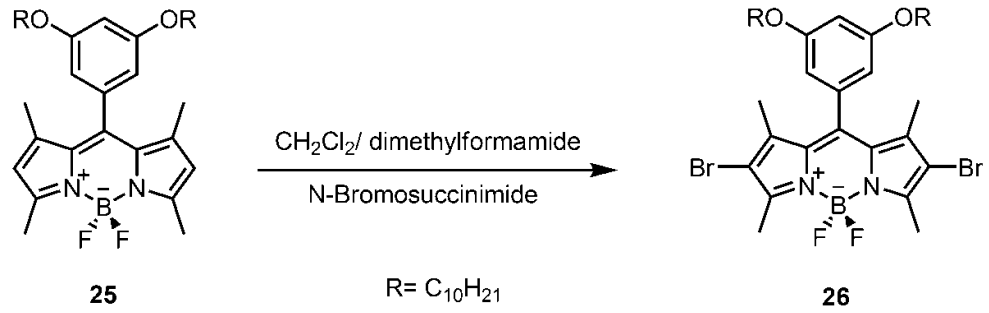


Figure 26. Synthesis of Compound **26**

2.3.3. Synthesis of Compound 29

A mixture of 4-hydroxybenzaldehyde (1.00g, 8.19mmol), 2-ethylhexylbromide (1.58g, 8.19mmol), K_2CO_3 (2.38g, 17.20mmol) and acetonitrile (30mL) was refluxed for 1 day under N_2. Solvent was evaporated and water was added. Reaction product was extracted into CH_2Cl_2. The organic layer was dried over Na_2SO_4 and after the evaporation of the solvent, silica gel column chromatography using $CHCl_3$ as the eluant afforded desired product (1.29g,67%).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 9.90 (1H,s), 7.90 (2H, d, J = 6.64 Hz, ArH), 7.01 (2H, d, J = 8.68 Hz, ArH), 3.96 (d, 2H, J = 5.7 Hz, OCH_2), 1.80 (m, 1H, CH), 1.48 (m,4H, CH_2), 1.32 (m,4H, CH_2), 0.96 (t, 6H, J = 3.88 Hz,CH_3).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 190.8, 164.5, 132.0, 129.7, 114.8, 77.4, 77.0, 76.7, 70.9, 39.3, 30.5, 29.0, 23.8, 23.0, 14.1, 11.1.

MS (TOF- ESI): m/z: : Calcd: 235.1721 $[M-H]^-$, Found: 235.1693 $[M-H]^+$ Δ= -12.01 ppm.

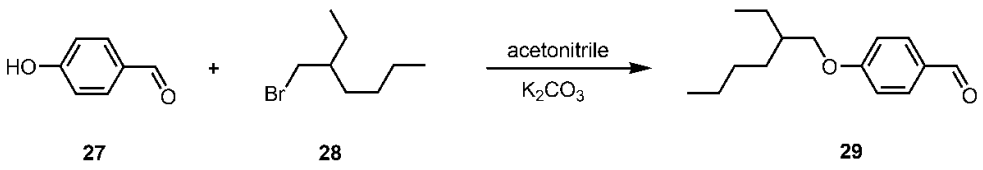


Figure 27. Synthesis of Compound **29**

2.3.4. Synthesis of Compound 31

To the mixture of compound **26** (0.19 mmol, 150 mg), 4- methoxy benzaldehyde (1.33 mmol, 181 mg) and benzene (50 mL) in a 100 mL round bottomed flask, piperidine (0.4 mL) and acetic acid (0.4 mL) were added and then the mixture was reflux by using a Dean Stark trap and reaction was monitored by TLC 3:1, $CHCl_3$: Hexanes until all the starting material had been consumed, the mixture was cooled to room temperature and solvent was evaporated under vacuum. Water (100 mL) was added to the residue and the product was extracted into the chloroform (3 x 100 mL). The organic layer was dried over Na_2SO_4 and after the evaporation of the solvent, silica gel column chromatography using 3:1 $CHCl_3$: Hexanes as the eluant afforded desired product. Deep green solid (70 mg, 29%).

^{1}H NMR (400 MHz, CDCl3): δ = 8.15 (2H, d; J = 16.5 Hz, CH), 7.70 7.60 (6H, m; 2H: CH, and 4H: ArH), 7.05 (2H, d; J = 16.5 Hz, CH), 7.02, 6.92 (8H, m; ArH), 6.78 (4H, d; J = 8.8 Hz, ArH), 6.49 (3H, s; ArH), 5.88 (2H, d; J = 16.5 Hz, CH), 3.89 (6H, s; OCH3), 3.82 (6H, s; OCH_3), 3.67 (4H, t; J = 6.6 Hz, OCH_2), 1.60, 1.50 (4H, m; CH_2), 1.35, 1.15 (28H, m; CH_2), 0.87 (6H, t; J = 6.9 Hz, CH_3);

^{13}C NMR (100 MHz, CDCl3): δ = 161.4, 160.8, 159.8, 149.5, 139.2, 136.0, 134.8, 129.8, 129.3, 128.0, 118.3, 116.1, 114.3, 113.8, 108.1, 103.3, 68.5, 55.4, 55.3, 31.9, 29.6, 29.4, 29.3, 29.0, 25.9, 22.9, 14.1.

MS (TOF ESI): m/z: : Calcd: 1263.4491 [M]+, Found: 1263.4558 [M] , I=5.39 ppm.

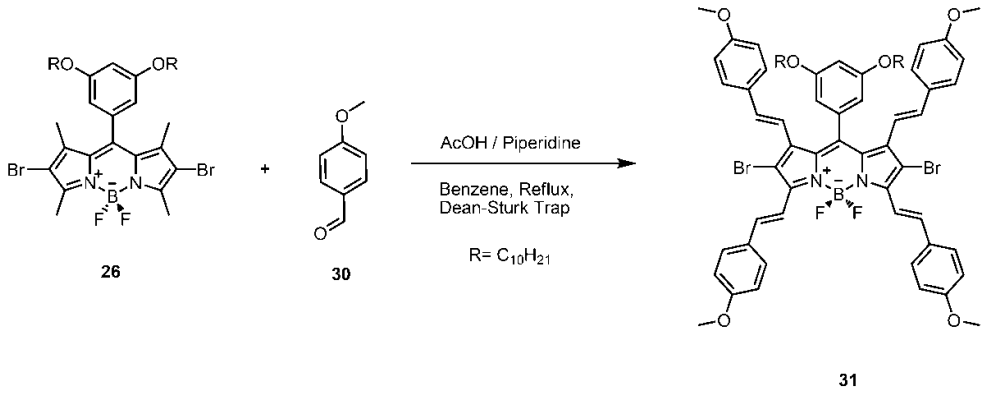


Figure 28. Synthesis of Compound **31**

2.3.5. Synthesis of Compound 32

Compound **26** (0.19 mmol, 150 mg) and compound **29** (1.33 mmol, 311 mg) are added to a 100 mL round bottomed flask containing benzene (50 mL), then piperidine (0.4 mL) and acetic acid (0.4 mL) were added and the mixture was reflux by using a Dean Stark trap and reaction was monitored by TLC 3:1, $CHCl_3$: Hexanes until all the starting material had been consumed, the mixture was cooled to room temperature and solvent was evaporated under vacuum. Water (100 mL) was added to the residue and the product was extracted into the chloroform (3 x 100 mL). Organic phase dried over Na_2SO_4, evaporated and residue was purified by silica gel column chromatography using 3:1 $CHCl_3$: Hexanes as the eluant afforded desired product as green solid (97 mg, 31%).

^{1}H NMR (400 MHz, CDCl3): δ = 8.13 (2H, d; J = 16.7 Hz, CH), 7.66, 7.63 (6H, m; 2H: CH, and 4H: ArH), 7.08 (2H, d; J = 16.5 Hz, CH), 7.01, 6.96 (8H, m; ArH), 6.78 (4H, d; J = 8.8Hz, ArH), 6.49 (3H, s; ArH), 5.89 (2H, d; J = 16.5 Hz, CH), 3.94 (4H, d; OCH_2; J = 5.6Hz), 3.85 (4H, d; OCH_2; J = 5.6Hz), 3.65 (4H, t; J = 6.6 Hz, OCH_2), 1.81- 1.72 (4H, m; CH), 1.54- 1.41 (12H, m; CH_2), 1.39, 1.33(24H, m; CH_2), 1.32-1.19 (28H, m; CH_2), 0.95 (30H, m; CH_3).

^{13}C NMR (100 MHz, CDCl3): δ = 161.5, 160.7, 159.7, 149.5, 140.3, 139.4, 137.0, 136.1, 134.9, 132.4, 129.7, 129.3, 128.0, 118.3, 114.9, 114.4,

108.5, 108.2, 105.8, 70.7, 70.6, 68.5, 39.4, 31.9, 31.6, 30.6, 29.6, 29.4, 29.11, 25.9, 23.8, 23.0, 22.7, 14.1, 11.0 .

MS (TOF ESI): m/z: : Calcd: 1655.8712 [M]+, Found: 1655.8941 [M] , I=13.82 ppm.

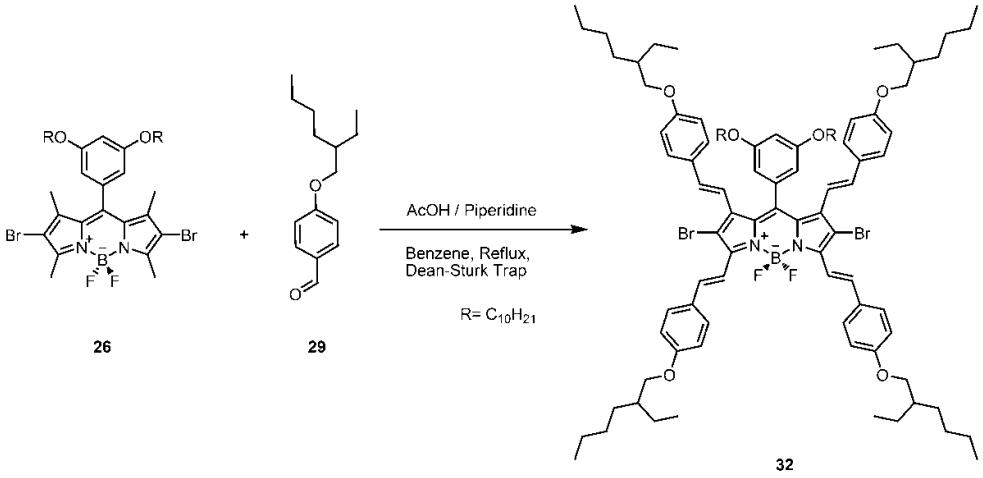


Figure 29. Synthesis of Compound **32**

CHAPTER 3

RESULTS and DISCUSSION

3.1. General Perspective

Photodynamic therapy is a growing field of treatment of cancer and gives promising results in clinical studies because photodynamic action allows local application on targeting areas, which are cancerous and noncancerous tissues, and gives minimum damage in surrounding area in contrast to other conventional treatments such as radiotherapy or chemotherapy. Besides, further selectivity is provided by exposing light to only targeting tissue. Efficiency of photodynamic action is dependent on how sufficiently cytotoxic effects are provided on the targeted tissues by singlet oxygen generation. Therefore, efficient singlet oxygen generation of photosensitizers is the crucial step on photodynamic applications.

Here, we proposed two photodynamic therapy reagents based on Bodipy dye. Bodipy is preferred due to its photostability and it can be modified in a number of regions easily as it is mentioned in introductory part. Bodipy core was synthesized through standard Bodipy reaction. 3,5-bis(decyloxy)benzaldehyde is used as the aldehyde to increase the solubility of Bodipy derivative in organic solvents such as benzene or toluene.

2, 6- positions were decorated with bromines in compound **31** and **32**. Spin orbit perturbation is required for electronic transition between states of different spin multiplicities otherwise electronic transition occurs inefficiently. Spin orbit perturbation can be enhanced by heavy atoms in the environment, external heavy

atom effect, or placing heavy atoms directly into core of molecule, internal heavy atom effect. Heavy atom effect can be defined as the increase of rates of inter system crossing due to influence of heavy atoms such as Br, I onto molecule. The efficiency of spin forbidden electronic transition from a singlet state to a triplet state, intersystem crossing, is highly important for the formation of singlet oxygen so the effectiveness of photosensitizers. We fused bromo substitutents on 2,-6,- positions of Bodipy core. Heavy atom effect on singlet oxygen generation of Bodipy dyes are studied by O'Shea et al.[72]. In their work, they used internal heavy atom effect and so they attached heavy atom at different positions on Bodipy derivative in order to observe the change of spin orbit couplings at different positions. In order to observe singlet oxygen generation efficiency, they synthesized three different aza-Bodipy derivatives. In the first derivative, no heavy atom is used, in the second derivative, heavy atoms, bromine atoms, are attached on the aryl rings not directly on the core of the photosensitizer and in the last one heavy atoms directly attached to photosensitizer core at 2-, 6- positions. Inherent spin orbit coupling of the first molecule does not change. In the second one, increase in intermediate singlet oxygen generation is provided due to intermolecular external effect and in the last Bodipy derivative, singlet oxygen generation is increased tremendously by internal heavy atom effect. The most efficient singlet oxygen generation is provided in the last Bodipy derivative. Therefore, in our design, we took advantage of internal heavy atom effect and we placed bromine atoms at 2,-6-positions of Bodipy core (Figure 30).

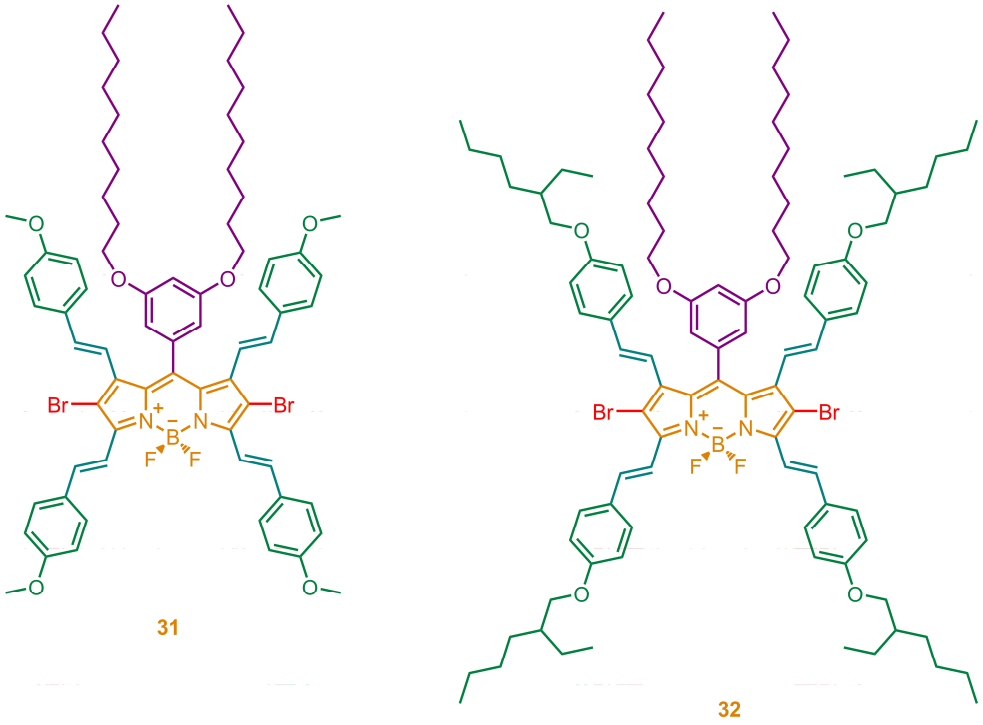


Figure 30. Target photosensitizers

Photosensitizer should also absorb light in the phototherapeutic window, 620-850 nm, because light penetrates in a maximum depth in this range and also autofluorescence of tissues can be avoided. Therefore, photosensitizers that absorb light below 620 nm are not optimal for photodynamic action. Tetrastyryl substitution on Bodipy core was one of the ways to extend absorption and emission maxima of Bodipy based dyes as it is mentioned before. We substituted Bodipy core at 1, 7, 3, 5- positions with 4-methoxybenzaldehyde for compound **31** and 4-(2-ethylhexyloxy)benzaldehyde for compound **32.** Maximum absorbance wavelengths of photosensitizers are determined to be 700 nm and 704 nm in $CHCl_3$ for compounds **31** and **32** respectively (Figure 31 and 32). The resulting wavelengths are very suitable for photodynamic therapy applications since penetration depth of light at this wavelength, light in near IR region, is considerably high.

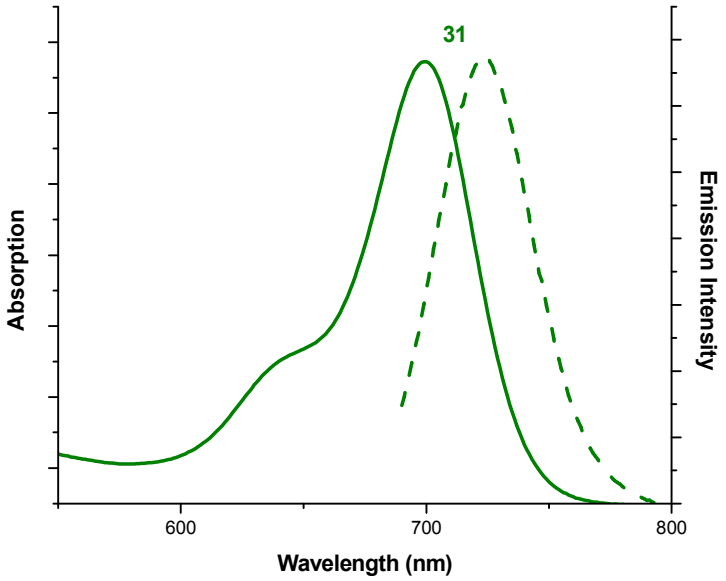


Figure 31. Absorbance (solid line) and Emission (dashed line) spectrum of compound **31** in $CHCl_3$

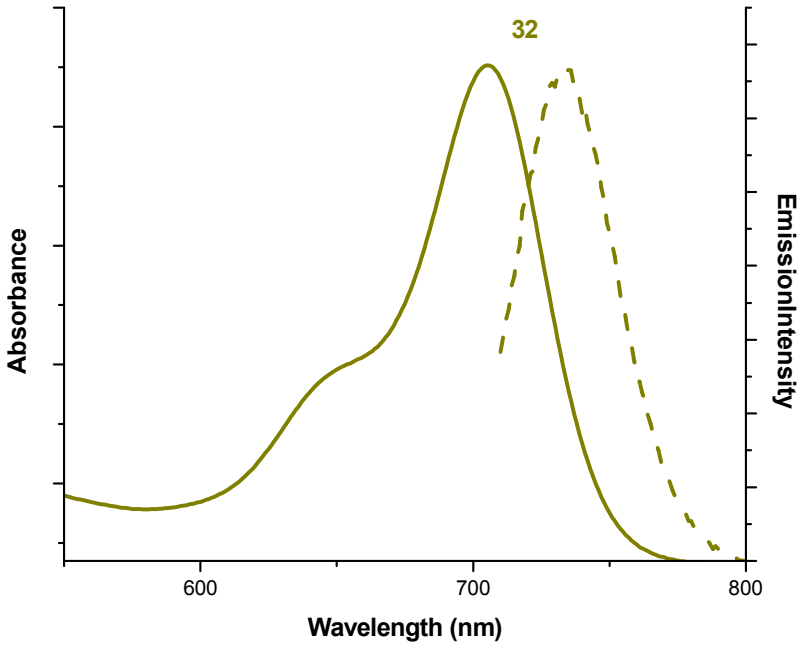


Figure 32. Absorbance (solid line) and Emission (dashed line) spectrum of compound **32** in $CHCl_3$

All the compounds obtained after each reaction were characterized using ^{1}H NMR, ^{13}C NMR spectra and Mass spectrometry analysis. NMR spectra were measured using $CDCl_3$ as solvent. All these data are provided in appendices A and B.

3.2. Singlet Oxygen Generation: Degradation of tetrastyrl-BODIPY dyes and 1,3-diphenyl-isobenzofuran under near-IR light

Singlet oxygen generation capability of compound **31** and **32** were measured by using 1,3-diphenylisobenzofuran (DPBF). Photosensitizers were excited at their maximum absorption wavelength, 700 nm light illumination for compound **31** and 704 nm light illumination for compound **32.** The light intensity was determined to be 0.2 mW/ cm^2.

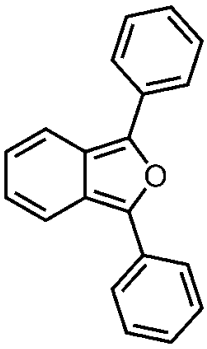


Figure 33. 1,3-diphenylisobenzofuran (DPBF)

DPBF is used as a singlet oxygen trap. Therefore, photobleaching of DPBF is used to monitor singlet oxygen generation capability of synthesized photosensitizers. When singlet oxygen is generated as the excitation of photosensitizers, DPBF reacts with generated singlet oxygen as a result absorbance at 411 nm decreases (Figure 32). DPBF is soluble in isoporopanol so experiments were conducted in this solvent. In control experiment, absorption of 50 μM of DPBF solution was monitored 25 minutes in dark. There is no significant change is observed in absorbance of DPBF.

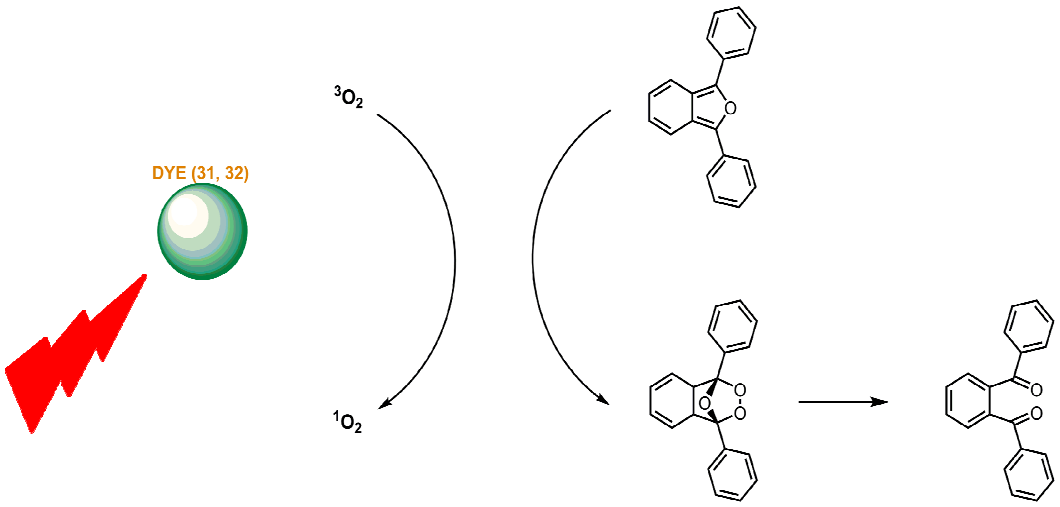


Figure 34. Excitation of photosensitizer with near-IR light, resulting in singlet oxygen generation. Trap molecule reacts with singlet oxygen with a decrease in the absorption wavelength at 411 nm.

The experiment was performed with 50 μM of DPBF and 50 nM of Bodipy dye solutions. There is no change is observed in absorbance of this solution in dark, however, a significant decrease in absorbance at 411 nm is observed when the light is applied to this solution.

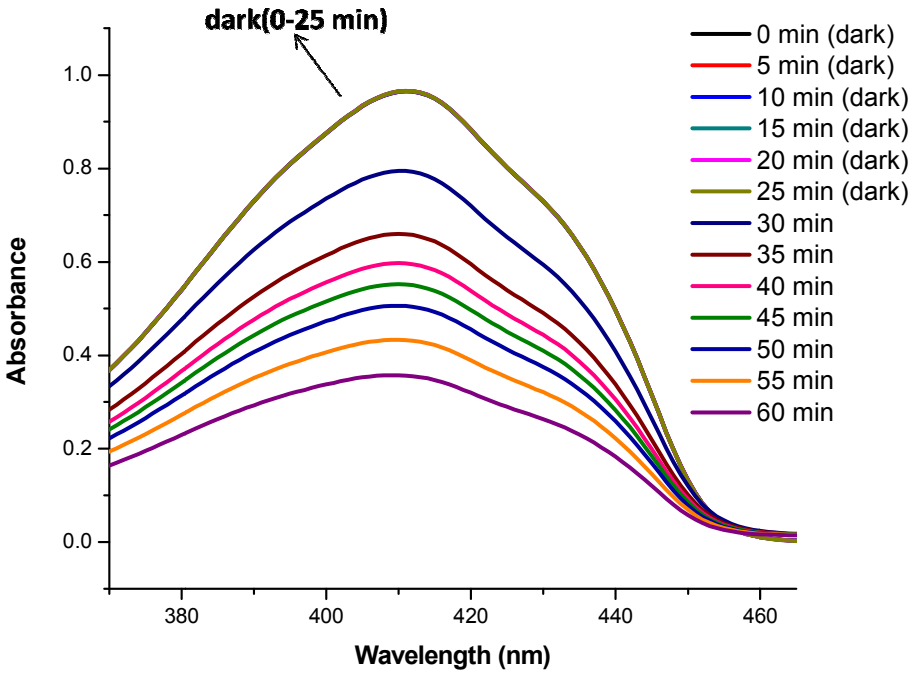


Figure 35. Bleaching of 50 μM DPBF in isopropanol in the presence of 50 nM compound **31**. For the first 25 minutes sample was kept at dark, then irradiated with 700 nm light for 45 minutes. Absorbance was measured in 5 minutes intervals.

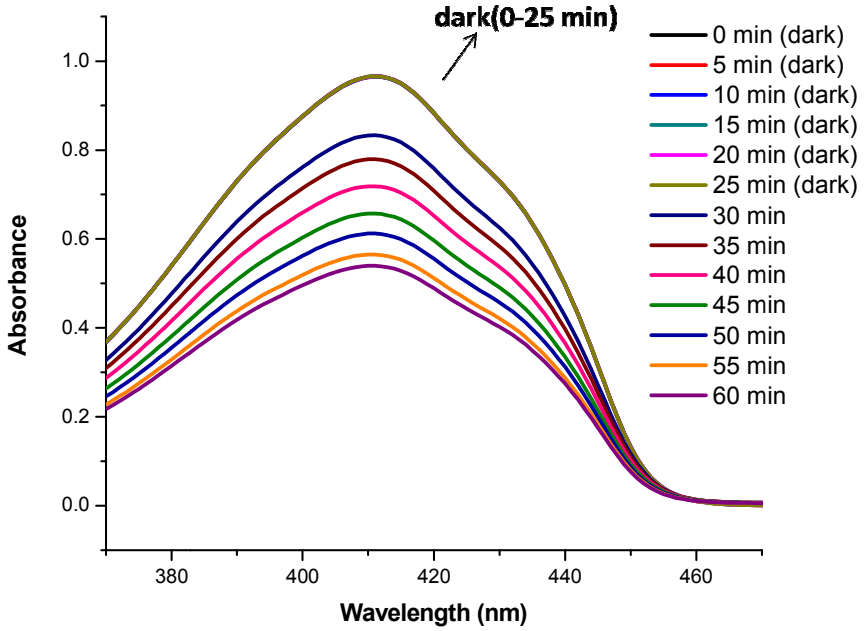


Figure 36. Bleaching of 50 μM DPBF in isopropanol in the presence of 50 nM compound **32**. For the first 25 minutes sample was kept at dark, then irradiated with 704 nm light for 45 minutes. Absorbance was measured in 5 minutes intervals.

To sum up, we have synthesized two different Bodipy based near-IR photosensitizers. These photosensitizers with bromo substituents on Bodipy core are effective singlet oxygen generators. In addition, these dyes are optimal for biomedical applications with their near-IR absorbances and they do not show dark toxicity. The activities of photosensitizers are effective under near-IR light.

CHAPTER 4

CONCLUSION

In this thesis study, two Bodipy based photodynamic therapy agents were synthesized and characterized. Through rational synthesis, photodynamic therapy requirements such as enhanced intersystem crossing and absorption in therapeutic range, near-IR absorption, are provided by bromine substitution on 2,6-positions and tetra-styryl substitution on Bodipy core, respectively. Singlet oxygen generation experiments of synthesized compounds show that both of the compounds are efficient singlet oxygen generators in nanomolar concentrations.

In conclusion, we demonstrated tetrastyryl-Bodipy compounds can be synthesized in such a way that they perform as efficient photosensitizers for use in photodynamic therapy, with near IR excitability and high singlet oxygen generation capacity. This is the first study of this novel class of near IR dyes towards photodynamic therapy applications. Further work in transforming these derivatives to water soluble or amphiphilic compounds may yield novel candidates for photodynamic therapy.

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APPENDIX A

NMR SPECTRA

Figure 37. ^{1}H NMR spectrum of compound 25

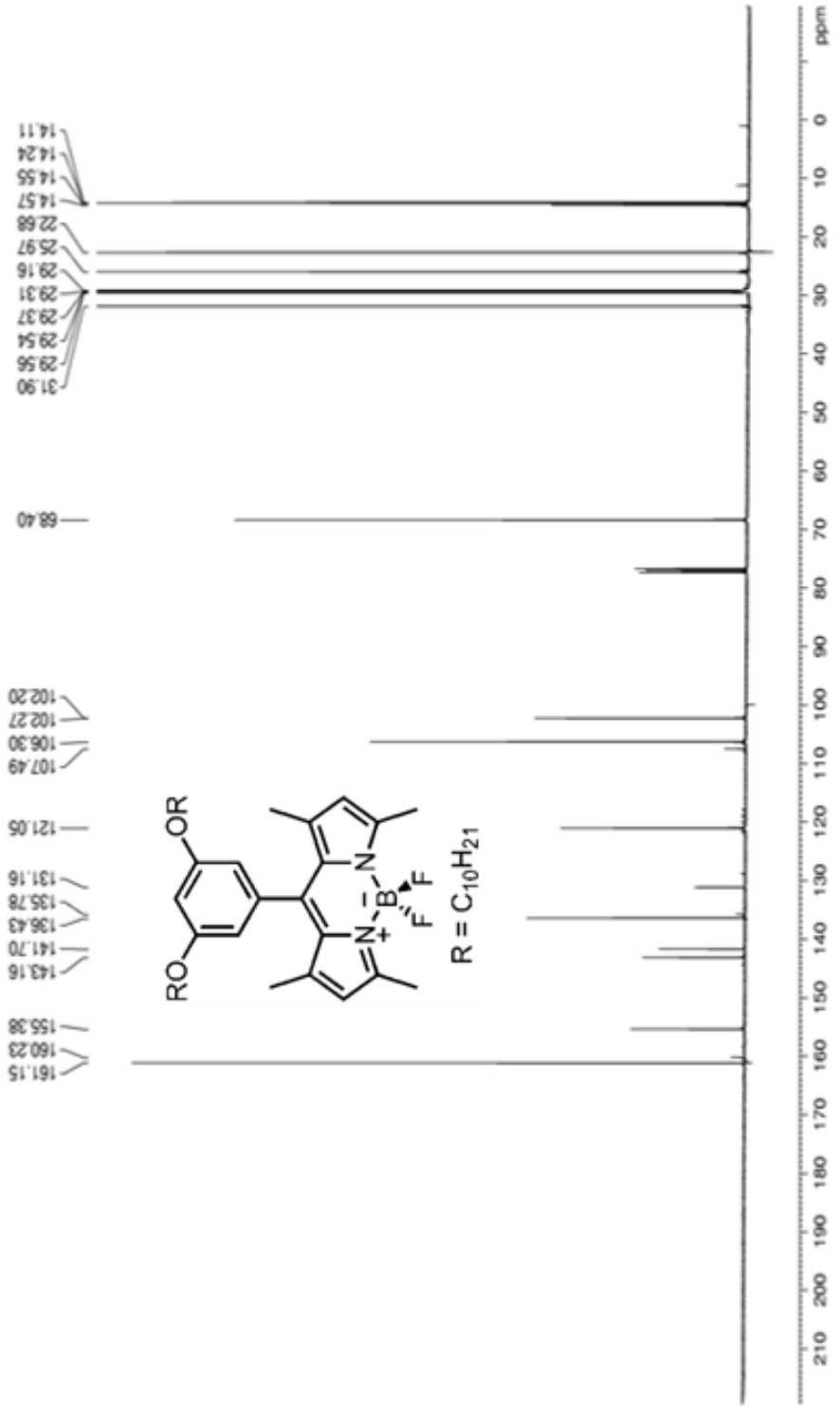

Figure 38. ^{13}NMR spectrum of compound 25

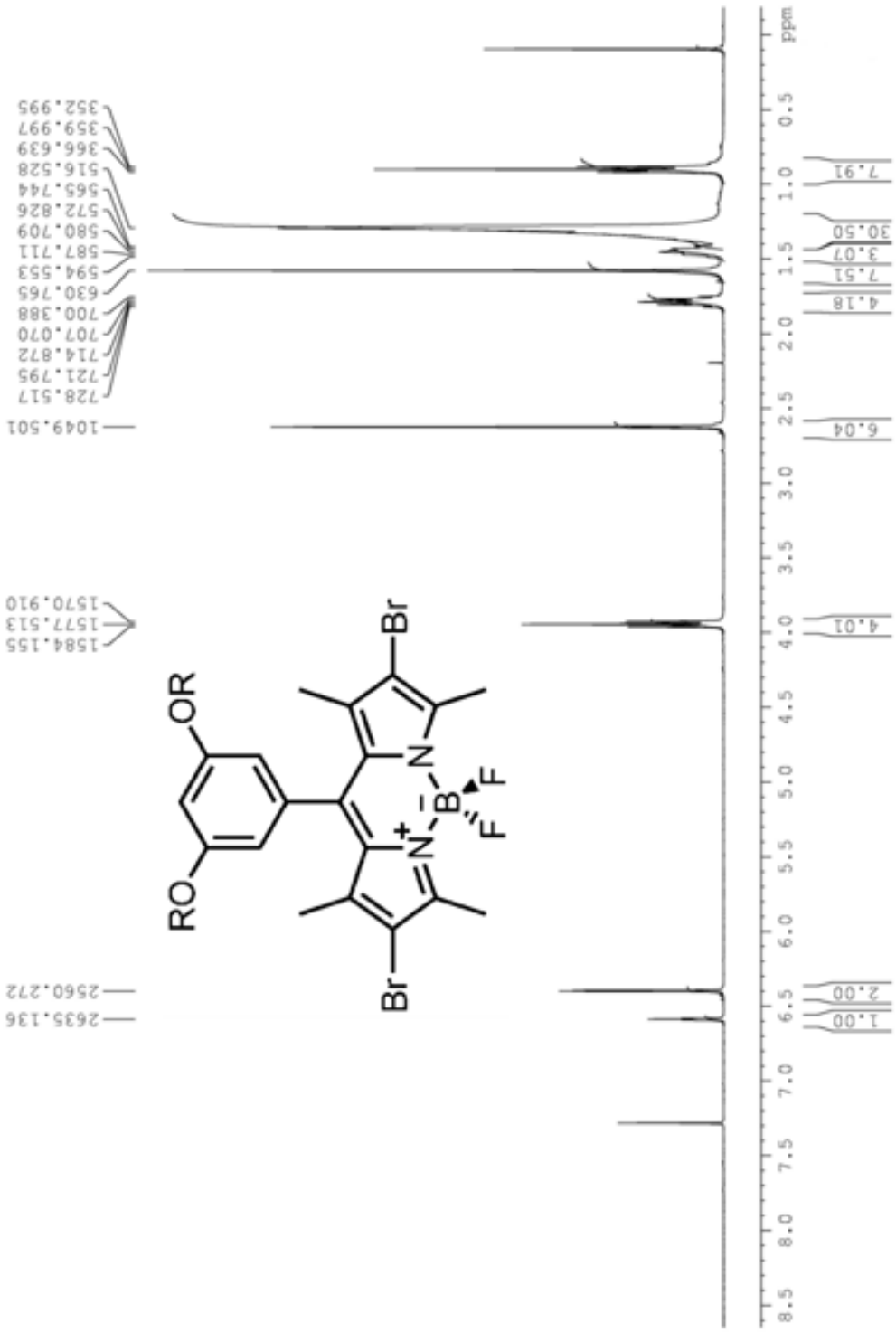

Figure 39. ^{1}H NMR spectrum of compound 26

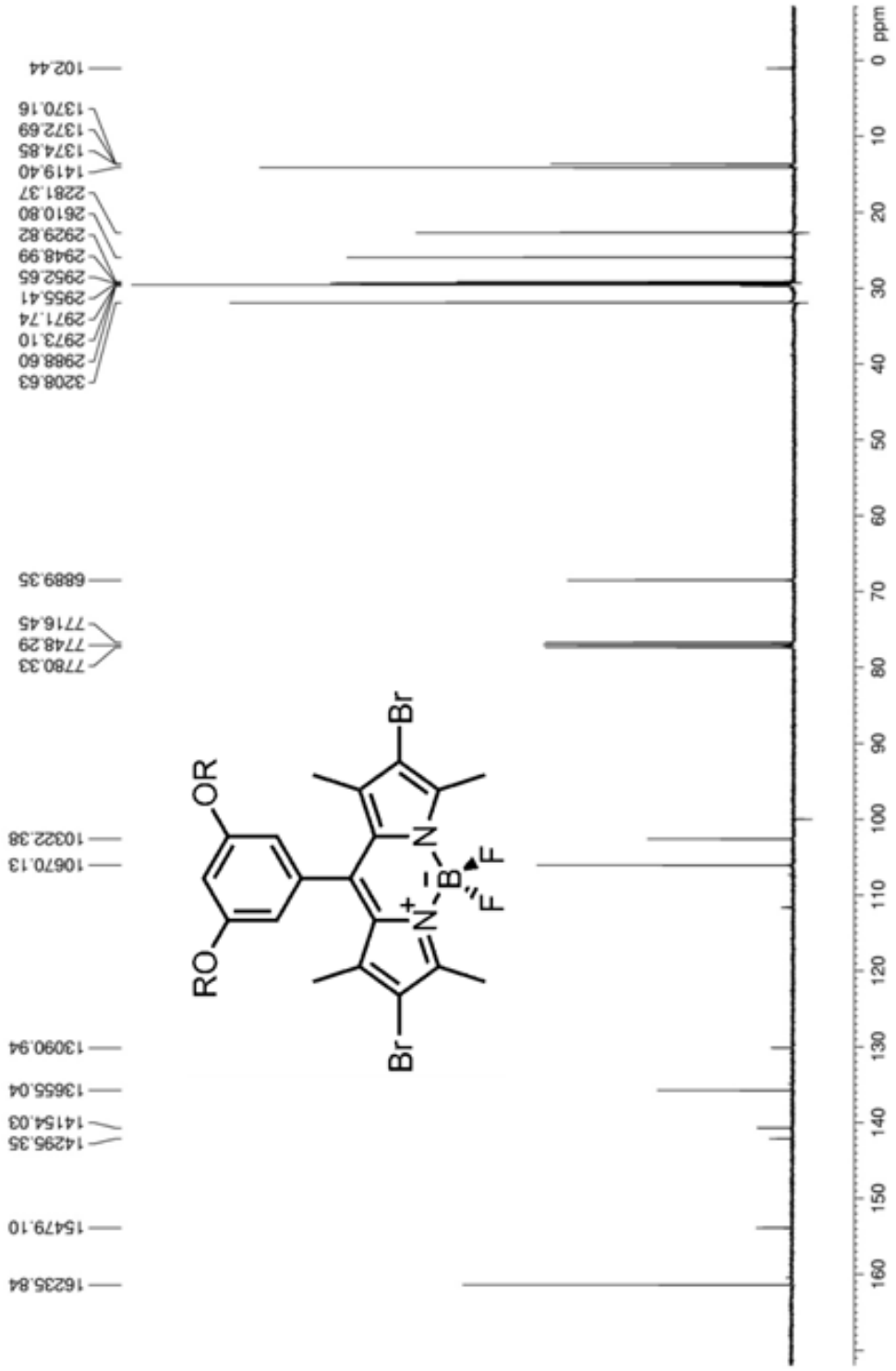

Figure 40. ^{13}NMR spectrum of compound 26

Figure 41. ^{1}H NMR spectrum of compound **29**

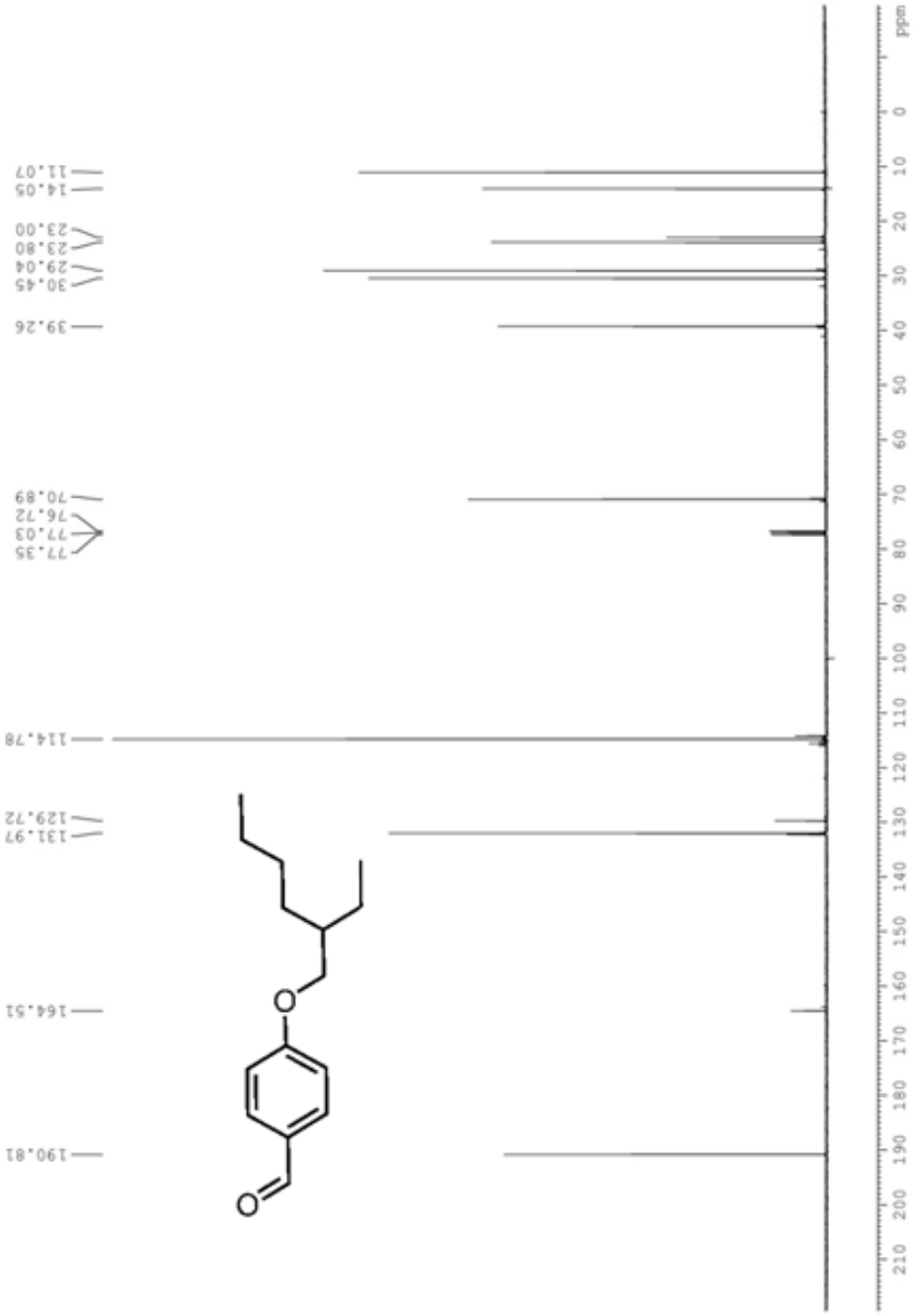

Figure 42. ^{13}NMR spectrum of compound **29**

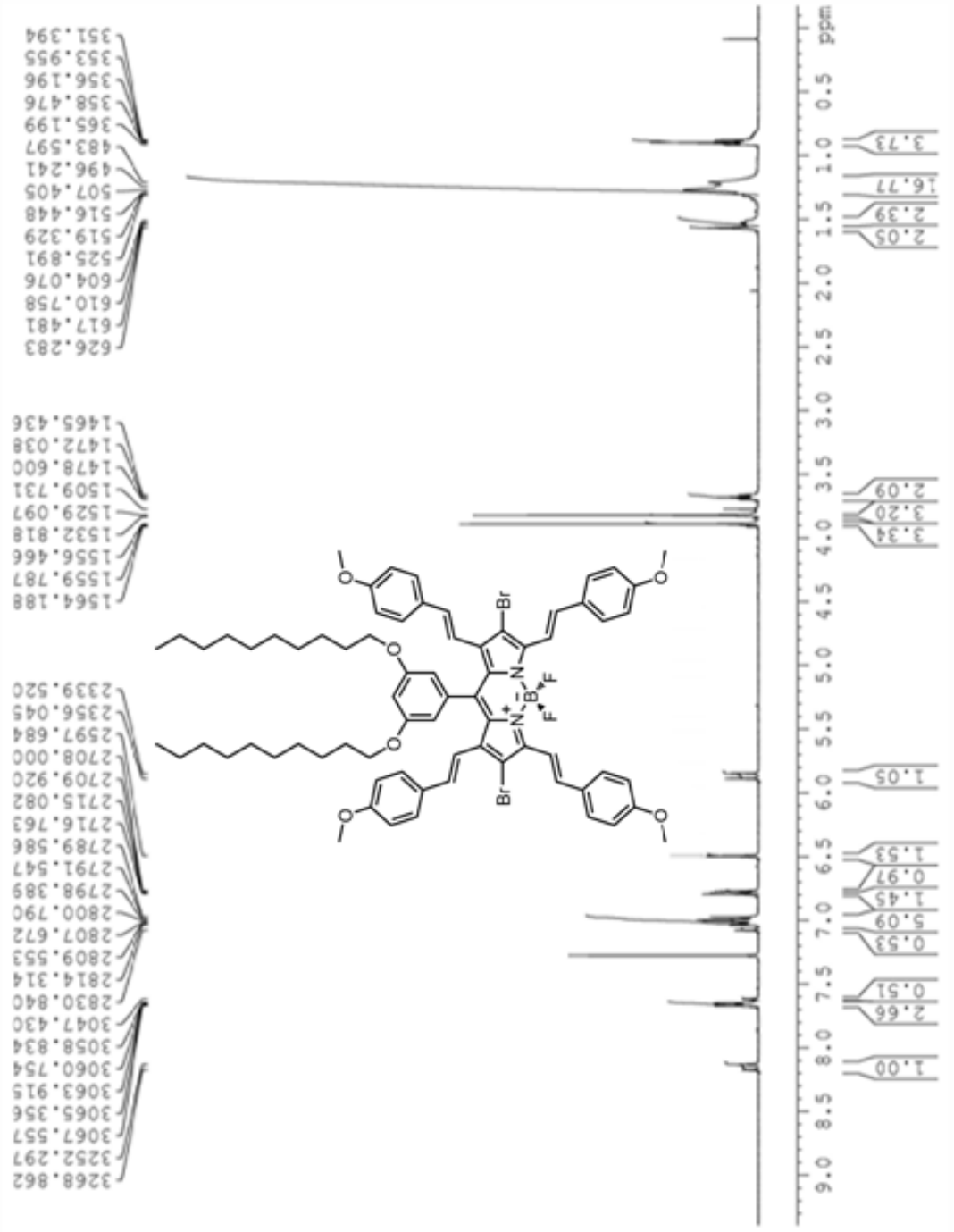

Figure 43. ^{1}H NMR spectrum of compound **31**

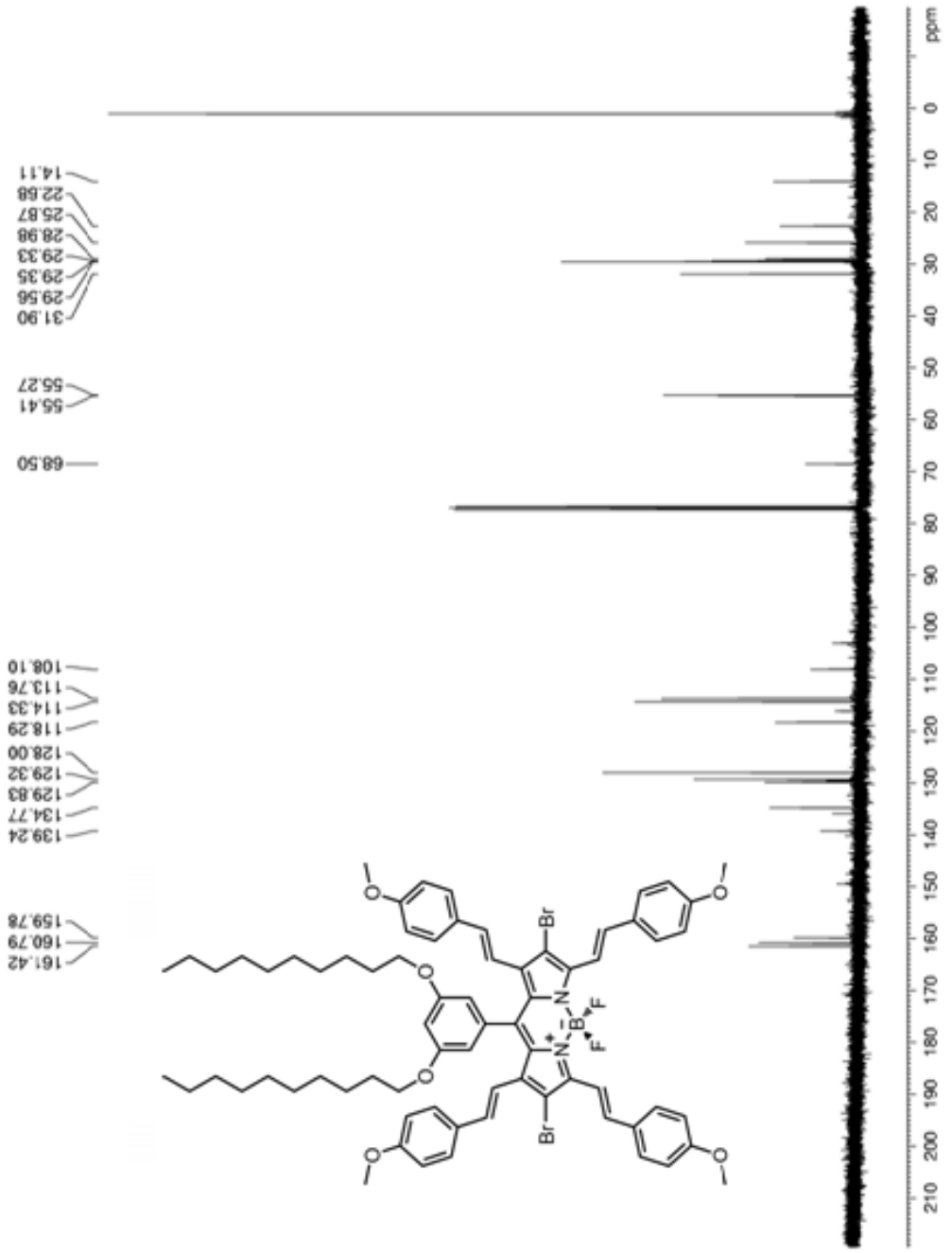

Figure 44. ^{13}NMR spectrum of compound **31**

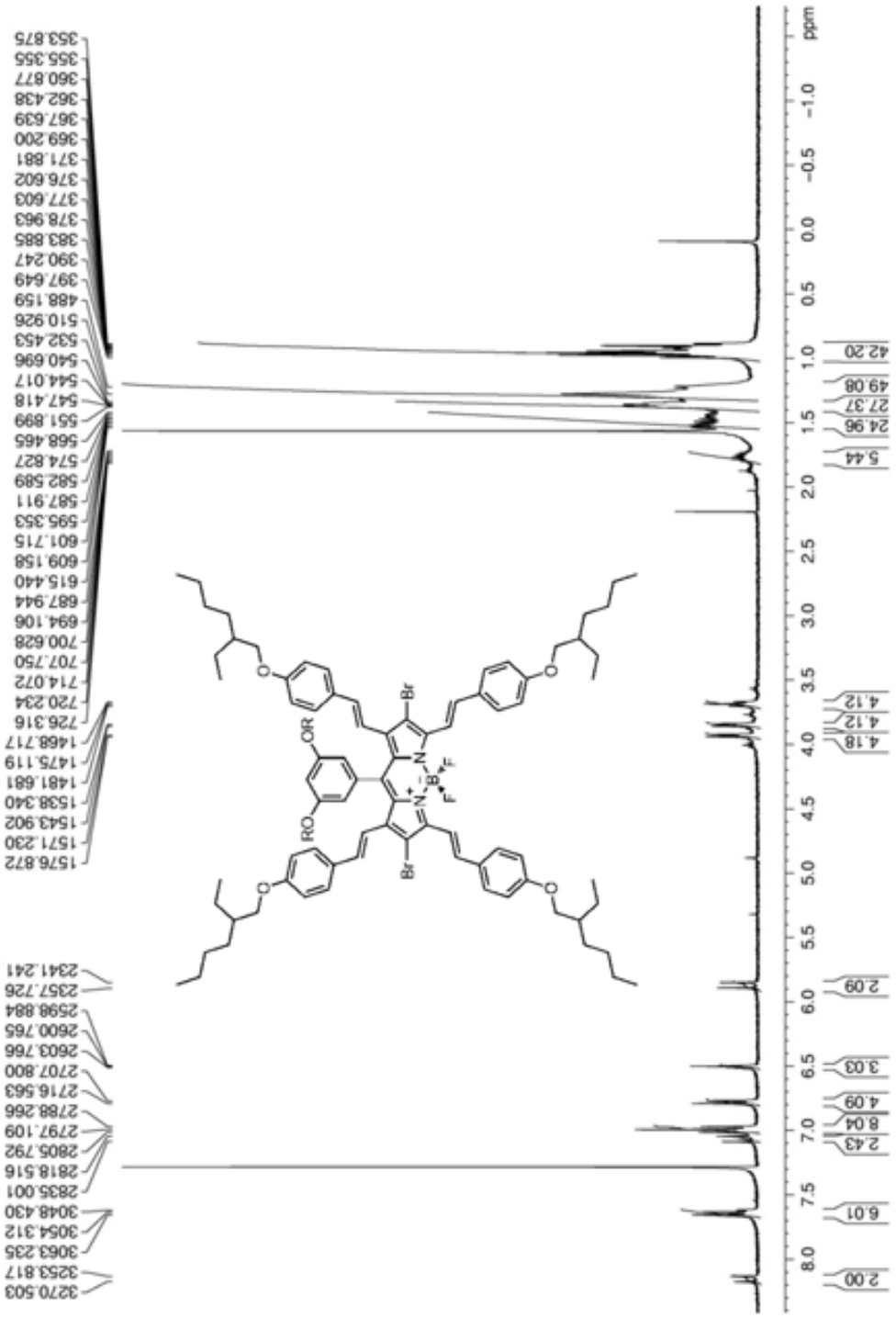

Figure 45. ^{1}H NMR spectrum of compound **32**

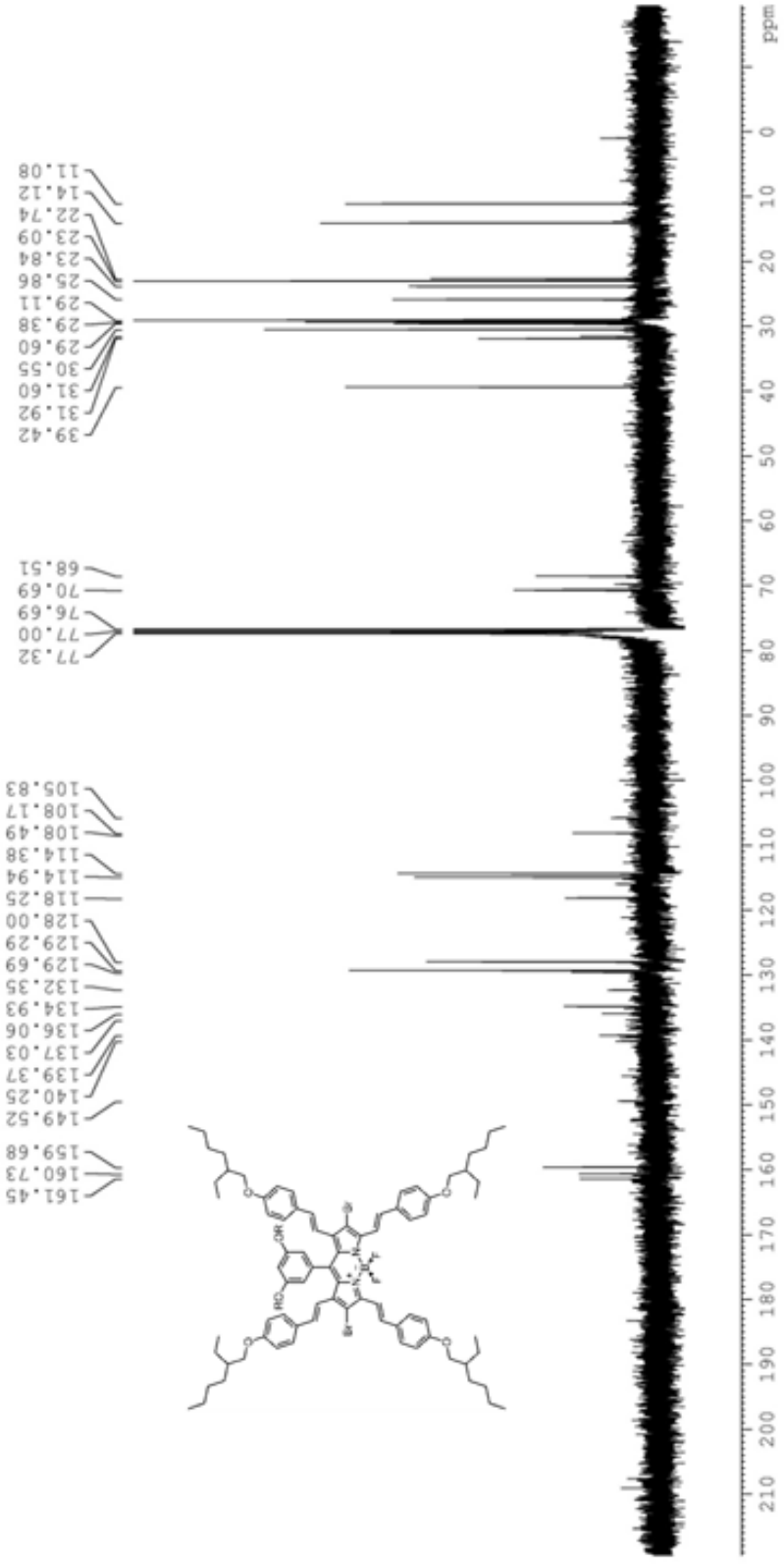

Figure 46. ^{13}NMR spectrum of compound **32**

APPENDIX B

MASS SPECTRA

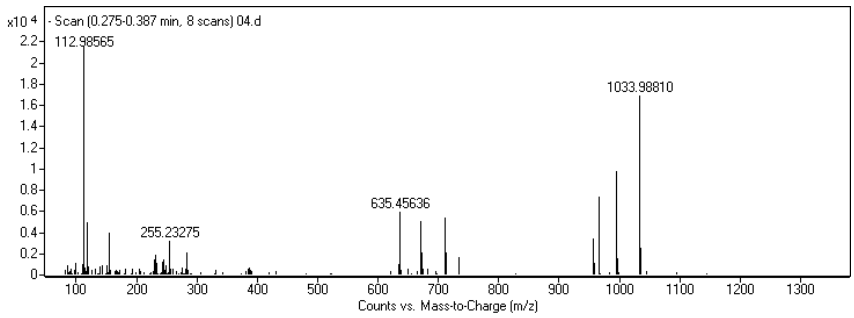

Figure 47. ESI-HRMS of compound **25**

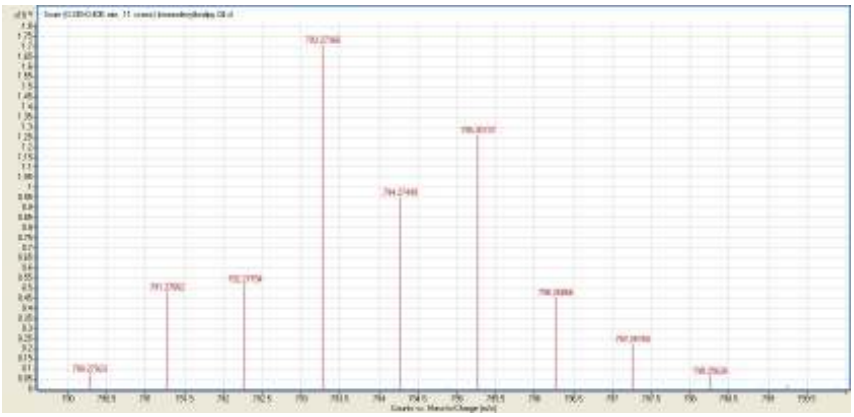

Figure 48. ESI-HRMS of compound **26**

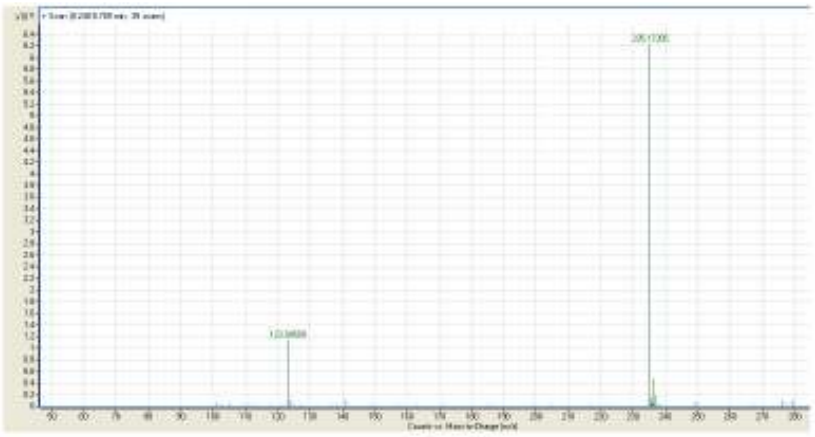

Figure 49. ESI-HRMS of compound **29**

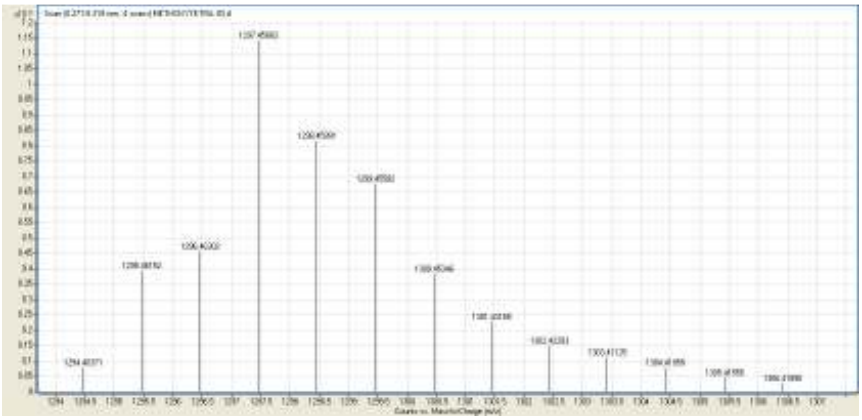

Figure 50. ESI-HRMS of compound **31**

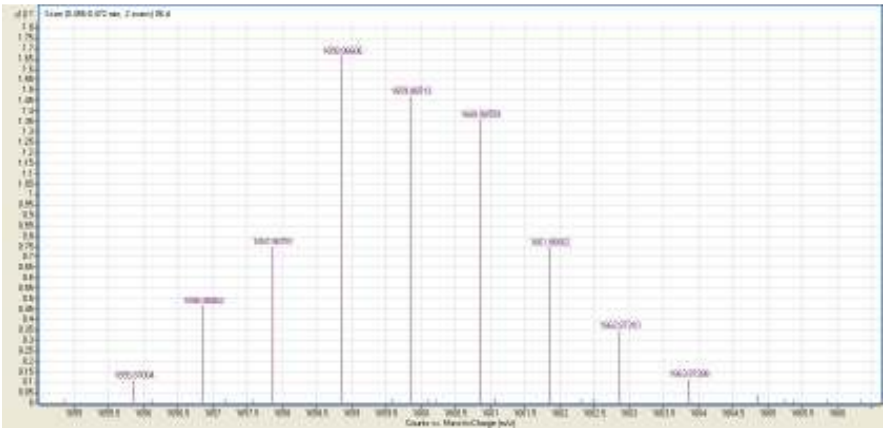

Figure 51. ESI-HRMS of compound **32**