

SYNTHESIS AND CHARACTERIZATION OF BODIPY- DERIVED SINGLET OXYGEN SENSOR

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OXYGEN SENSOR

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

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ABSTRACT

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M. Sc. In Department of chemistry

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Singlet oxygen is a biochemically produced reactive species, which can trigger various cellular responses. It is also produced by photosensitization by the intermediacy of photosensitizer therapy of cancer. Real-time imaging of singlet oxygen is very important to assess the progress of cellular changes leading to apoptosis. We targeted a long wavelength dye, which responds to singlet oxygen with a change in emission color. Color change in emission is particularly important, as it allows internal referencing.

Keywords: Bodipy, Photodynamic therapy, singlet oxygen sensor

ÖZET

BODİPY TÜREVLİ SINGLET OKSİJEN SENSÖRÜNÜN SENTEZİ VE KARAKTERİZASYONU

Yahya Abdelwahed Fikry Ismaiel

Kimya Bölümü, Yüksek Lisans

Tez Danışmanı: Engin Umut Akkaya

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Singlet oksijen biyokimyasal olarak üretilen reaktif bir türdür ve çeşitli hücre sel tepkileri tetikleyebilir. Ayrıca, kanser terapisi sırasında fotoduyarlayıcının etkileşmesiyle de singlet oksijen üretilir. Singlet oksijenin gerçek zamanlı görüntülenmesi, hücre ölümü sırasında gerçekleşen değişimleri incelemek açısından çok önemlidir.

Singlet oksijene emisyon renginde değişimle tepki veren uzun dalga boyuna sahip boya sentezlemeyi hedefledik. Emisyondaki renk değişimi iç referanslama yapılmasına izin vereceği için özellikle önemlidir.

Anahtar Kelimeler: Bodipy, fotodinamik terapi, singlet oksijen sensörü



Dedicated to my father and mother

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LIST OF ABBREVIATIONS

AcOH	: Acetic Acid
BODIPY	: Boradiazaindacene
CHCl₃	: Chloroform
DCM:	: Dichloromethane
PDT	: Photodynamic therapy
TPP:	: Tetraphenylporphyrin
Et₃N	: Triethylamine
MS	: Mass Spectroscopy
NMR	: Nuclear Magnetic Resonance
TFA	: Trifluoroacetic Acid
TLC	: Thin Layer Chromotography
TOF	: Time of Flight

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CHAPTER 1

1. Introduction

1.1. Photodynamic Therapy

Cancer, the disease that have been catching attention in science in an increasingly growing fashion and that is because cancer itself is responsible alone of the deaths of more than 15% of world population and it is expected to increase.¹

There are several different diagnostic and treatment methods that have been developed to diagnose and treat cancer, most of them were invasive and harmful to a certain extent to cancer patients, like chemotherapy and radiotherapy and today as we are more keen into developing new safer and non-invasive methods, Photodynamic therapy showed itself as one of the most promising and still developing technique that can fulfill such a goal.²

Photodynamic therapy is promising new method of treating superficial types of cancer such as bladder cancer , esophageal cancer , skin cancer , endobronchial and certain types of head and neck cancer , and the interest in it was mainly because of its effectiveness and because it is non-invasive and its concept is mainly about the usage of light.³ This therapy is mainly about administering light-sensitive compounds (drugs) either locally on the skin “topically” , or systemically via the oral or the intravenous route.³ The main idea is that this drug will concentrate itself in the tumor cells more than the normal cells and by exploiting the light and its properties towards those cancerous cells , the therapy can be feasible , and also when it comes to work on localizing the drug its self this will depend on several factors like the hydrophobicity and the charge of the photosensitizer (drug).⁴

Photosensitizers is being exploited by using their property that when they are activated they can produce singlet oxygen (O_2^1) among other reactive oxygen species and the singlet oxygen will be capable of exerting oxidative damage to the cells and hence turning those cells either inactive or dead.^{5,6}

1.2. History of Photodynamic Therapy

Using such a concept in its simplistic form preceded our complex understanding of it by thousands of years that we can see its being mentioned in the holy book of India *Athrava-Veda* and also by the Greeks who actually were pioneers in using heliotherapy which its main concept was a full exposure of sunlight. However, it was brought to the scientific community by Oscar Raab's the medical student who was studying the effects of acridine on certain types of bacteria that causes malaria, and he realized how the combination of acridine and light exerted a lethal effect on the bacteria. The term "photodynamic action" however was delivered to us by the scientists Tappeiner and Jesionek who used a combination of eosin and white light to treat skin cancer.^{7, 8} Then by further understanding of the concept Tappeiner and Jodbauer reached a conclusion that oxygen is a crucial part in the "photodynamic therapy" but the mechanism was not clarified until later in 1979. The first clinical application of photodynamic therapy took place in 1978 at 'Roswell Cancer Institute' and following this, the first approved photosensitizer came to light by FDA in 1980 and from this moment forward a lot of other PDT photosensitizer were approved and were used as well.¹⁰

1.3. Mechanism of Photodynamic Therapy

For PDT to work and produce singlet oxygen, reactive oxygen should be generated in its lowest lying excited state and this can be fulfilled by photoactivation of the photosensitizer (fluorophore) and this is considered to be the most important step in this therapy in order to give its desired output. In the figure 1 below as we see is a thorough explanation of the concept. First the fluorophore will be excited upon illuminating enough photon energy and it will go from ground state to the first excited state as shown. Then it will have to go in either of two directions both of them are means of relaxation of the vibrational states, the first is to relax back to its ground state which is called "fluorescence", the second one which is actually the desired pathway in PDT is

intersystem crossing as here spin conversion will occur and if there is molecular oxygen the energy of such conversion will be transferred to it and singlet oxygen will be generated, this know phenomena is usually described by a diagram which is called “Jablonski diagram”. Moving forward, generation of singlet oxygen illicit a biological response as it forms various types of reactive oxygen species which causes in return an oxidative damage of the cells, but due its short life time which about (0.6 us) and its short diffusion distance (0.1 um) the expected damage to the cells is only confined within the vicinity of the photosensitizer so the main goal now in research is going toward synthesizing a more localized and oriented photosensitizer.¹¹

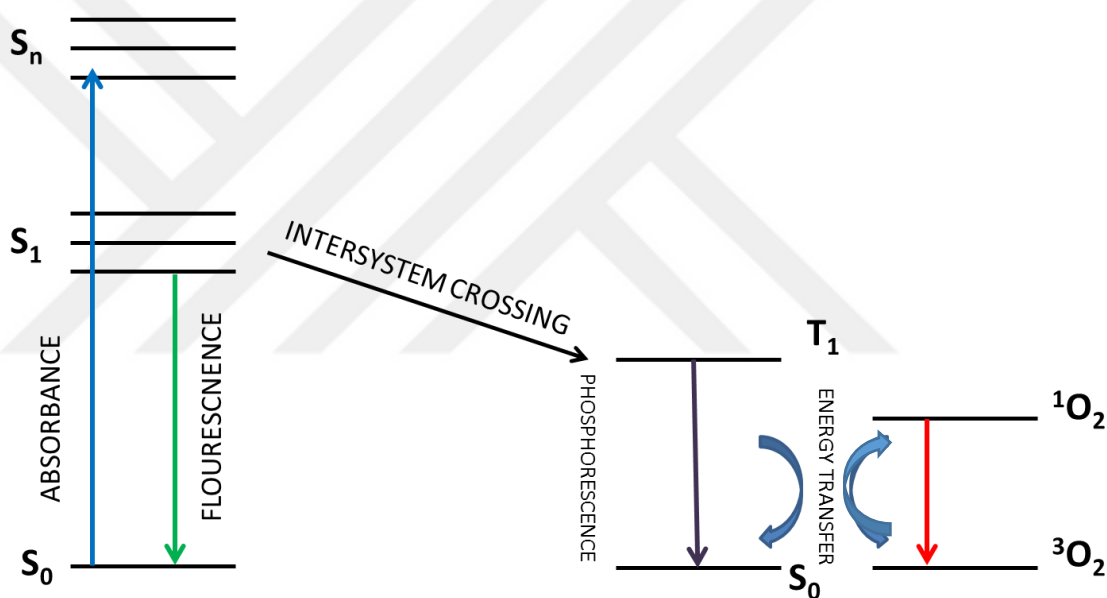


Figure 1. Jablonski energy diagram

1.4. Oxidative damage of singlet oxygen

There are two responses that arises from the effect of photosensitizer, a chemical one and a biological one, the chemical one is related to the “Photosensitizer mediated cytotoxicity” and this has two types of reactions that occurs simultaneously, type-I

reaction occurs when the photosensitizer after its excitation reacts directly with the cellular biomolecules and as a result, radicals will be generated from such a reaction which they will exert a certain damage to the cell. The type-II reaction though, happens when the single oxygen - which has been produced by the photosensitizer-, reacts with the cellular biomolecules. Singlet oxygen as powerful oxidant exerts an oxidative stress to the cell by several ways, by reaction with membrane lipids it can lead to lipid peroxidation which in return releases lipid peroxide which as a result will have cytotoxic effect as well. Furthermore, it had an effect on the membrane proteins in the mitochondria by reacting with the sulfides and thiols in certain amino acids leading to an increase in the mitochondrial permeability resulting in the leakage of its contents, leading to the cell death, as a result. Lastly, singlet oxygen affects the DNA by exerting modification on its dioxy sugars and bases which will lead to DNA mutations and in the process will impair some of the repair enzymes in the DNA, leading to DNA accumulation and cell death.¹²⁻¹⁵

The biological response in PDT can induces two of modes of cell death, apoptosis and necrosis. Apoptosis is “Cell Programmed Death” and PDT can help increase this process by inducing the formation of certain death-inducing signal complexes, because once apoptotic events happens it cannot be reversed and cell death is inevitable. However, necrosis is an unnatural cell death that characterized by swelling of the cell and disrupting intracellular mechanism that maintain the cell intact, resulting eventually in its rupture thus killing it .¹⁶⁻¹⁹

1.5. Requirements of Photosensitizers

The photosensitizer as one of the components in PDT that should acquire certain fundamentals and properties to work efficiently and as needed. Most of the photosensitizers decompose in the presence of light and such common finding is called photobleaching and for a photosensitizer to be efficient and desirable it should be photostable. It should have an absorption coefficient in the range from 600-900 nm and this is because it is the pharmaceutical therapeutic window and the reason behind it is that the depth of penetration of the light in this region is higher than any other wavelength. Another property which is really important is for the photosensitizer to have “High Quantum Triplet Yield” which will result in effective ISC thus generating singlet oxygen as desired. Additionally, the Photosensitizer should maintain biological properties as well, an absence of dark toxicity which by definition is being inactive in the absence of light. It should also acquire an amphiphilic character, in other words having the proper ratio of hydrophobicity/hydrophilicity for it to penetrate the tissues.^{21, 22}

On the other hand, the light source which is used in PDT is a determinant of the efficiency of the whole process. Using blue light for example will limit the efficiency because of its poor penetration compared to red light and near-IR. Choosing the wavelength is also important, as shorter wavelengths are harmful to a lot of tissues and will not be useful upon its arrival to the desired target while using a longer wavelength will not be effective as it will be out of the therapeutic window thus rendering the whole process useless. To summarize we can say that the right dosage with right wavelength with right time of exposure can give us the maximum clinical efficacy needed.^{20, 22, 23}

1.6. Photosensitizers in the literature

1.6.1. First Generation Photosensitizers.

Photofrin[®] and HpD are the first generation, they are well known for their high singlet oxygen quantum yield that gives a high generation of singlet oxygen and they are also safe that they are FDA approved and are being used in different types of cancer like lung and esophageal cancer. However, their drawback resides in them having a low absorption wavelength at 630 nm thus not having a good tissue penetration property also them having a long-term side effect of phototoxicity. Finally their synthesis is not simple.²⁴

1.6.2. Second Generation Photosensitizers.

In this generation most of the previous drawbacks were considered and modified.

1.6.2.1 Porphyrins

From the porphine family, second generation PDT sensitizers some of them showed it to be a potential candidate for treating basal cell carcinoma and some of them were approved for non-oncological PDT treatments of certain diseases.²⁴

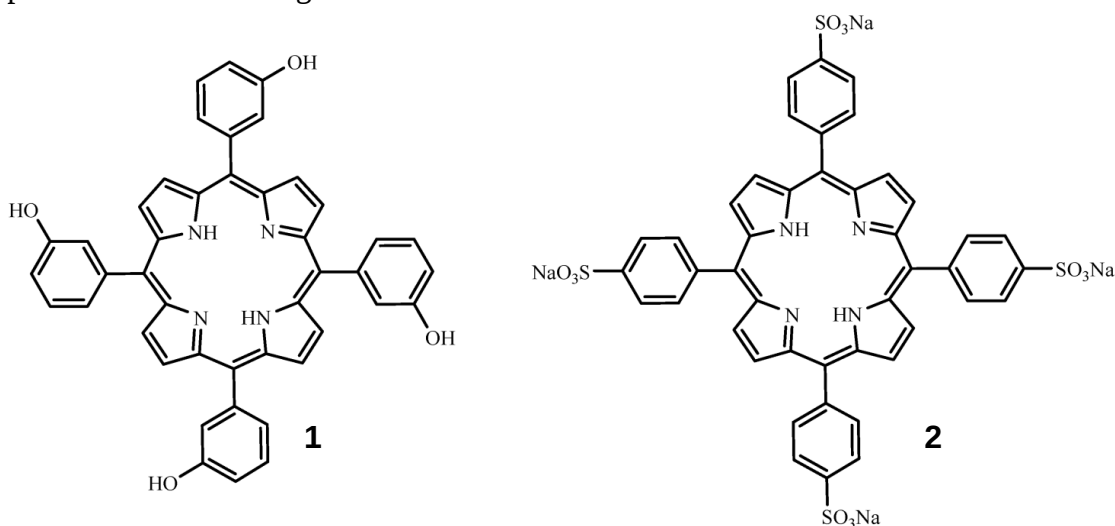


Figure 2. Examples of porphyrin family photosensitizers

1.6.2.2. Chlorines

Chlorines are better than porphyrins in having a longer wavelength and this is due to a small change in the chemical structure that leads to a bathochromic shift. Also phototoxicity was found to be lower than Photofrin[®] and a small dosage requirement compared to the previous generation.²⁴

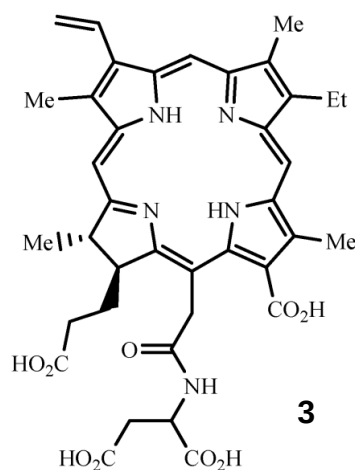
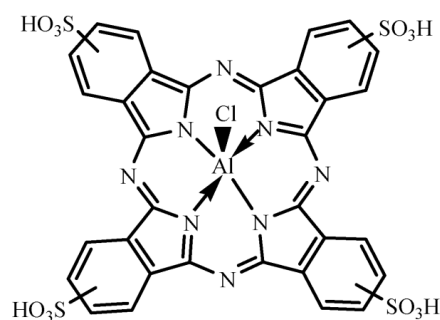


Figure 3. Example of chlorines family photosensitizers

1.6.2.3. Phthalocyanines

Phthalocyanines (Pc) they exhibit PDT properties because of their chemical structure because it has heavy a metal complex formation thus allowing ISC to occur, they have a longer wavelengths compared to the previous porphyrins, its used in Russia to treat oral , breast and stomach cancer but still develop certain skin phototoxicity .There is another Pthalocyanine derivative “Silicon Phthalocyanine” which is still in trials and it was able to be used to treat skin diseases like keratosis and micosis and also in skin cancer as well.²⁵⁻²⁷



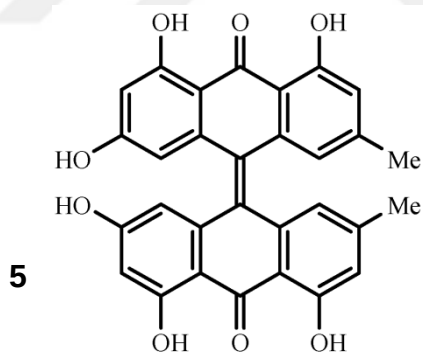
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Figure 4. Example of phthalocyanines photosensitizer

1.6.3. Non-Porphyrin Photosensitizers

1.6.3.1. Anthraquinones

The most famous one in this family is “Hypericin” which is a naturally occurring anthraquinone, it has a light absorption at 590 nm. It aim was in to treating squamous cell carcinoma and basal cell carcinoma but the results from the clinical trial were unsatisfactory.²⁸⁻³¹



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Figure 5. Example of anthraquinone sensitizers

1.6.3.2. Phenothiazines.

Methylene blue one of the most commonly used and well known photosensitizer in PDT, its structure is from phenothiazolium family with light absorbance at 666 nm. Its effect *in vivo* is used to treat basal cell carcinoma and Kaposi's sarcoma that is usually targeting melanomas, however, *in vitro* is still being testing against adenocarcinoma, bladder carcinoma and Hela cervical tumor cells. Another dye of the same family "toluidine blue" is still being tested in clinical trial in treating chronic periodontitis.³²⁻³⁴

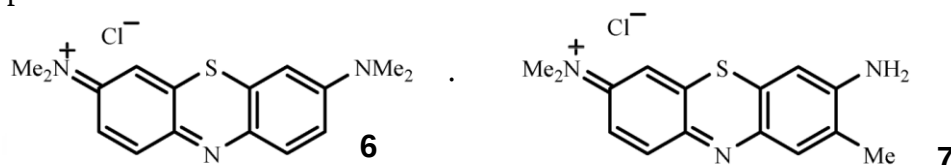


Figure 6. Examples of Phenothiazene photosensitizers.

1.6.3.3. Xanthenes

In this family there is two know members, the first is Rose Bengal it is known for its water solubility and it absorbs light at 549 nm. Experimentally it is used PDT in the treatment of breast carcinoma and metatstatic melanoma. The other members is 4,5-Dibromorhodamine methyl ester its absorb light at 514 , its still being evaluated in PDT treatment for graft-versus-host disease and its known of being to destroy lymphocytes via apoptosis.^{35,36,40}

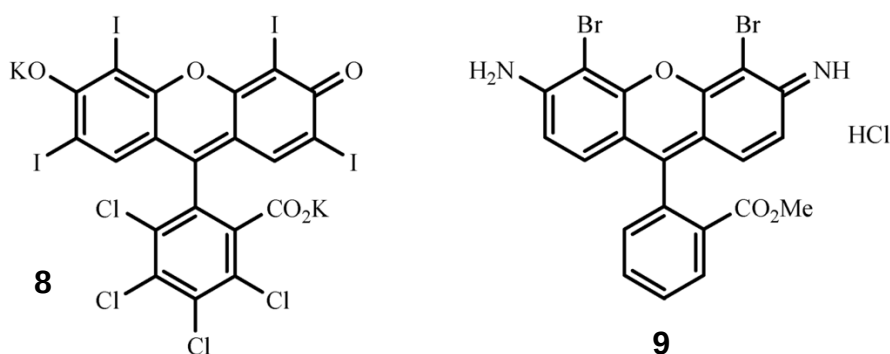


Figure 7. Examples of xanthenes photosensitizer

1.6.3.4. Cyanines

Merocyanine 540 is from the cyanine family absorbs at 556 nm which put it in the desirable therapeutic window and it is usually used In PDT treatment for targeting leukemia and lymphoma cells. This sensitizer is still being investigated in preclinical trials and *in vitro* models for treatment of leukemia and neuroblastoma.^{28, 37, 38}

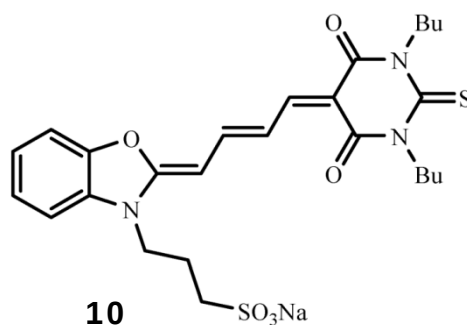


Figure 8. Example of cyanine photosensitizers

1.6.3.4. Curcuminoids

Curcumin is a natural colorant absorbs at 420 nm and has been used via PDT as a disinfectant in oral surgery and as an agent for destroying bacteria.^{28, 38, 39}

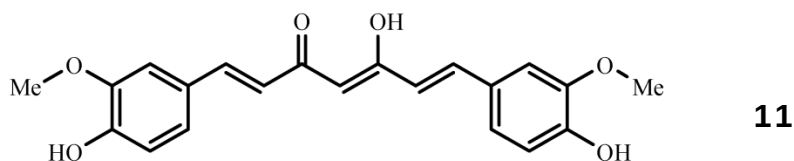


Figure 9. Example of Curcuminoids photosensitizes

1.7. Bodipy dyes

Bodipy a family of compounds that have gained tremendous amount of attention in research area due to their properties. 4, 4'-difluoro-4-bora-3a, 4a-diaza-s-indacene or as to put it in a simple word “Organic fluorescent molecules”, they are used in several applications, solar cells, cellular im

aging, drug delivery etc., they were first discovered in 1968 by Triebs and Kreuzer. Their advantages over other sensitizers can be attributed to their high molar extinction coefficients, high fluorescence quantum yields and also most of them are designed to have high triplet quantum yields. Them being in the desired therapeutic window and proper physiological PH and also able to maintain good solvent polarity is one of the reasons why they really interesting in this field of research.

1.7.1 Functionalization

Bodipy structure has been utilized by allocating different chemical groups through different positions. Burges et al, Negano et al, Rurack et al, Akkaya et al and Ziessel et al. have been exploring such ideas in their research in the last decade. We are going to discuss different substitutions as the structure maintains symmetry.⁴¹⁻⁵²

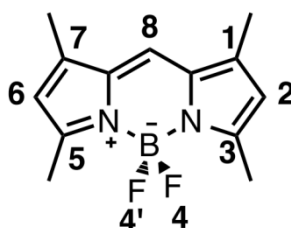


Figure 10. Bodipy main core structure and numbering

2, 6- positions substitutions of Bodipy dyes is utilized via sulfonation, nitration and Halogenation with different properties attributed to each one of those reactions. Sulfonated Bodipy dyes (12 and 13) has an advantage over their parent dyes by being more stable in water and methanol and also them having high fluorescence in water. However, Nitrated Bodipy dyes (15) reduce the fluorescent quantum yield drastically. Regarding the halogenated Bodipy dyes because of the heavy atom effect they exhibit a significant red shift in UV-absorption and fluorescence emission.⁴¹

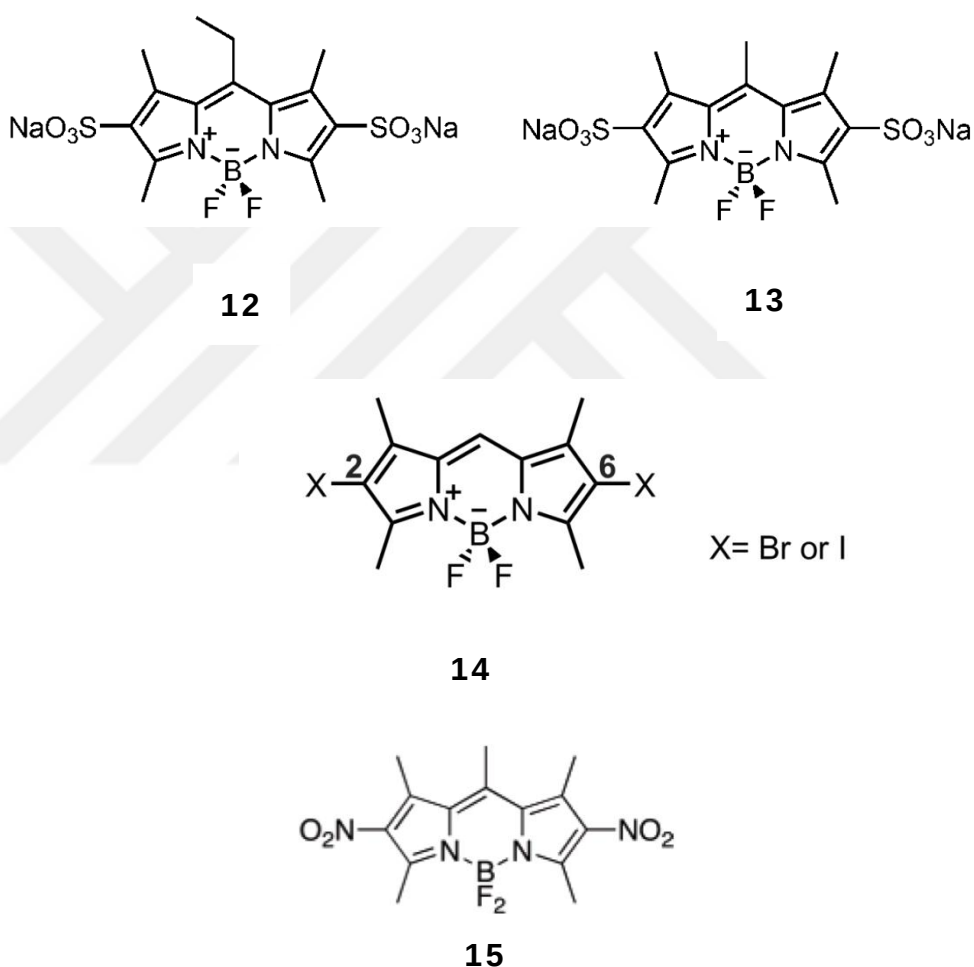


Figure 11. Examples of Bodipy-derived structures in 2, 6-positions.

3 and 5 positions substitution was usually done in order to incorporate electron donating groups which in return benefited us as that sort of attachment created a significant bathochromic shift in both absorption and emission spectra as shown in figure 11.^{42,43}

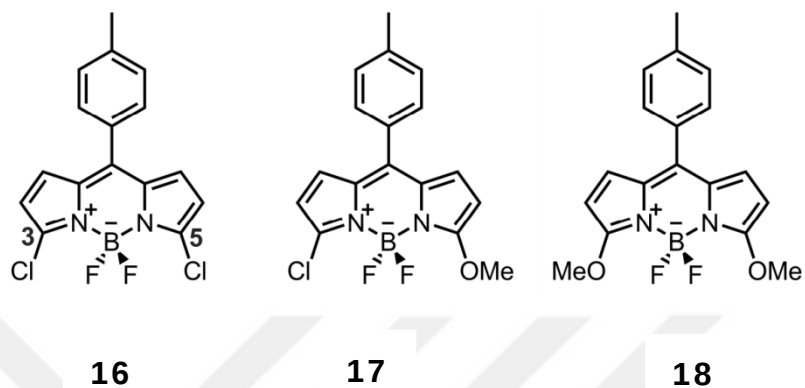
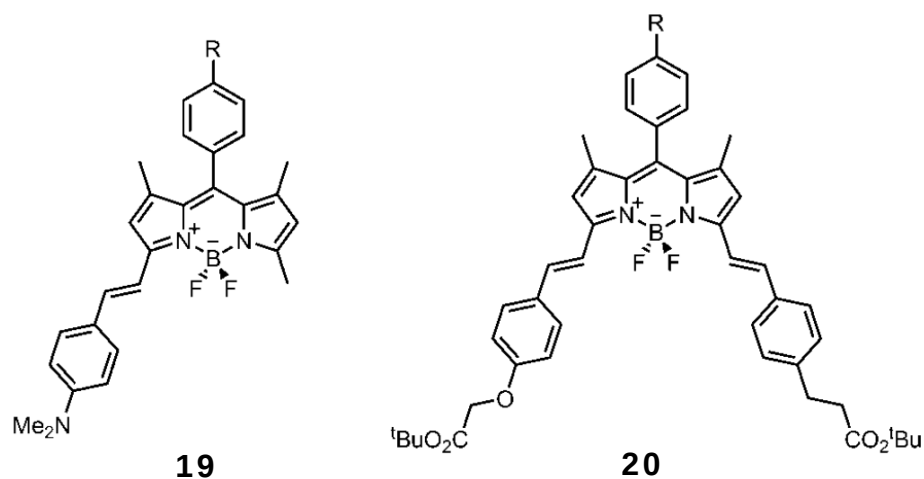


Figure 11. Examples of Bodipy-derived structures in 3,5 positions.

Also 3 and 5 positions were realized to exhibit certain acidic character when attached to methyl groups and this was exploited in a lot of ways specially when dealing with Knoevenagel reactions. Furthermore, different form of styryl substituted Bodipy structures was obtained by exploiting such an advantage and using different aldehyde to achieve the desired Bodipy as shown in figure 12.⁴³⁻⁴⁶



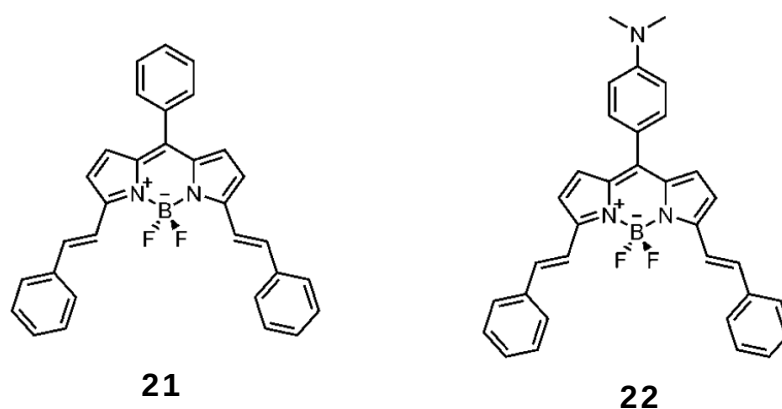


Figure 12. Examples of Bodipy structures in 3, 5 positions in Koevenagel reactions.

Ziessel *et al.* have been able to replace the fluoride ion of boron center by aryl, ethynyl and ethynylaryl substituents as shown in figure 13.⁴¹⁻⁴⁴

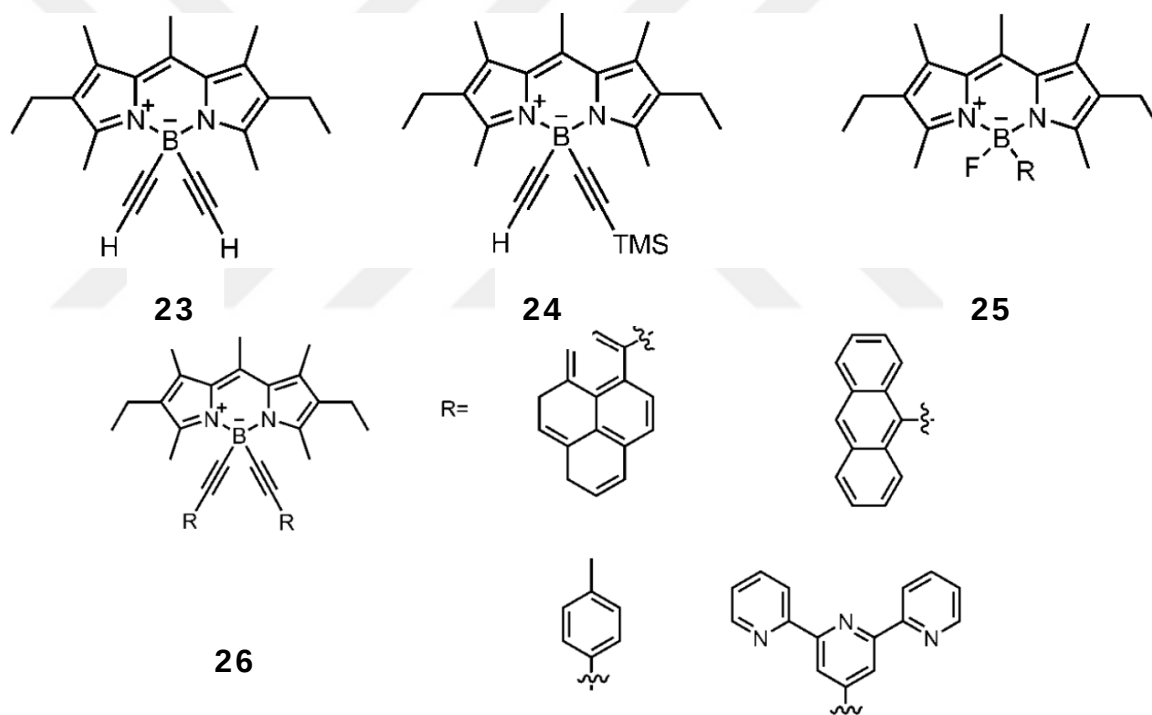


Figure 13. Examples of Bodipy structures that their fluoro groups ion haven been replaced.

Chemical structures of compounds 27 and 28 are shown. Compound 27 is a phenyl-substituted boron-dipyrromethene derivative. Compound 28 is a similar derivative but with additional methyl groups on the pyrrole rings, labeled 1 and 7.

1.8. Applications of Bodipy dyes.

Chemical structures of compounds 29, 30, and 31 are shown. Compound 29 is a macrocyclic boronate ester with a central boron atom coordinated by two fluorine atoms and two nitrogen atoms. The boron atom is also bonded to a phenyl group and a bromine atom. The macrocycle is substituted with two 4,4'-dihydroxy-1,1'-biphenyl-2,2'-diyl units and two 4,4'-dihydroxy-1,1'-biphenyl-2,2'-diyl units. Compound 30 is a macrocyclic boronate ester with a central boron atom coordinated by two fluorine atoms and two nitrogen atoms. The boron atom is also bonded to a phenyl group and a bromine atom. The macrocycle is substituted with two 4,4'-dihydroxy-1,1'-biphenyl-2,2'-diyl units and two 4,4'-dihydroxy-1,1'-biphenyl-2,2'-diyl units. Compound 31 is a macrocyclic boronate ester with a central boron atom coordinated by two fluorine atoms and two nitrogen atoms. The boron atom is also bonded to a phenyl group and a bromine atom. The macrocycle is substituted with two 4,4'-dihydroxy-1,1'-biphenyl-2,2'-diyl units and two 4,4'-dihydroxy-1,1'-biphenyl-2,2'-diyl units.

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1.9. Singlet oxygen Sensors.

Molecular oxygen (O_2) is one of the essential compounds to all aerobic organisms humans included, however, during the vital processes a lot of other compounds that is derived from this molecules is generated and they are called “Reactive oxygen species” ROS , those are involved in physiological and pathological processes ranging from signal transduction to carcinogenesis. ROS are generated normally in cells but the problem comes when they are overly produced as this will cause oxidative stress that they will eventually induce cell death. Due their significance in the areas of pathology and how they work, their involvement of biological functions caught a huge attention in the areas of research. The problem of studying them arose from the fact that ROS have very short life times *in vivo* and their fast destruction by the antioxidants that present in the cells. Singlet oxygen is known biologically to be an activator of gene expression however it is also responsible of irreversibly damaging the cell via oxidation of DNA and various different proteins.

Different probes were synthesized to identify and detect different form of ROS, here we are going to discuss different form of probes that been utilized to detect singlet oxygen.

1.9.1. (DPA) 9, 10-diphenylanthracene.

Most of the singlet oxygen sensors are designed as Anthracene moiety derivatives. Two decades ago DPA shown to have an oxygen sensing ability due to formation of endoperoxide product, this compound displayed low sensitivity of detection of singlet oxygen as it is based on absorption rather than fluorescence.⁶⁵

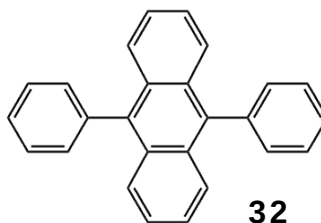


Figure 16. DPA structure

1.9.2. (DPAXs) 9-[2-(3-Carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3H-xanthen-3-ones.

Umezaka *et al.* aim was to modify DPA to make its reactivity based on fluorescence rather than absorption so they fused DPA with a Fluorescein and it was chosen as fluorophore because of its high fluorescence quantum yield in H₂O and its excitation in longer wavelength. “DPAXs were the first chemical traps for ¹O₂ that permitted fluorescence detection”. Their reaction with singlet oxygen produces an endoperoxide product DPAX-EP. By contrast DPAX-EP is highly fluorescent compared to DPAX. However, in acidic conditions fluorescein is known to decrease its fluorescence intensity, so in order to overcome such an obstacle in physiological pH, an electron withdrawing group was incorporated in the 2 and 7 position of the xanthene chromophore [(DPAX-2), (DPAX-3)]. Several studies were performed against other ROS, but the end results showed that DPAXs were only specific to singlet oxygen generators. Furthermore, some other studies were performed to detect DPAX-2 cell permeability and they studied against it DPAX-2 diacetate and the results were the same, showing that DPAX-2 is cell permeable and can be used *in vivo*.^{64,65}

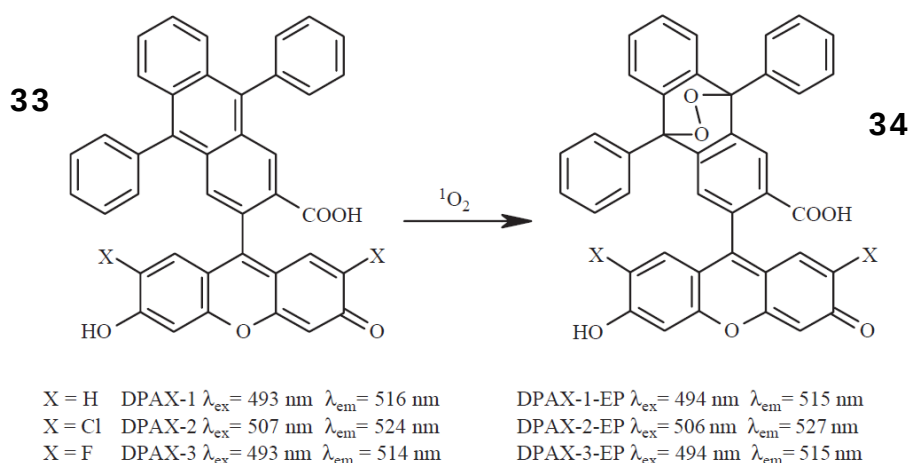


Figure 17. DPAXs and their reactions with singlet oxygen

1.9.3. (DMA) 9,10-Dimethylantracene

DMA showed that it reacts selectively with singlet oxygen, but its main problem was that its poor selectivity *in vivo* as it was not able to reach the desired depth in the intracellular medium.^{64,65}

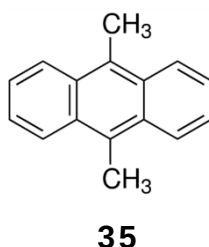


Figure 18. DMA structure

1.9.4. (DMAX) 9-[2-(3-Carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one

Tanaka et al. goal was to synthesize another singlet oxygen probe which will more sensible and stable than DPAX. Their interest arose from the fact that DPA reacts with O_2 rapidly and selectively via forming an endoperoxide product. Using the same concept, DMAX was synthesized by fusing DMA with a Fluorescein. DMAX is practically nonfluorescent, while DMAX-EP presents high fluorescence making this a really useful tool during singlet oxygen experiments. DMAX also showed a concentration dependent behavior with singlet oxygen and this made it possible to use DMAX as a probe for detecting 1O_2 quantitatively. Also via different studies DMAX showed to reacts with 1O_2 more selectively and more properly *in vivo* than DPAX. So DMAX is a “fluorescent probe appropriate for detecting 1O_2 with a potential usefulness for assays in biological systems”.^{64,65}

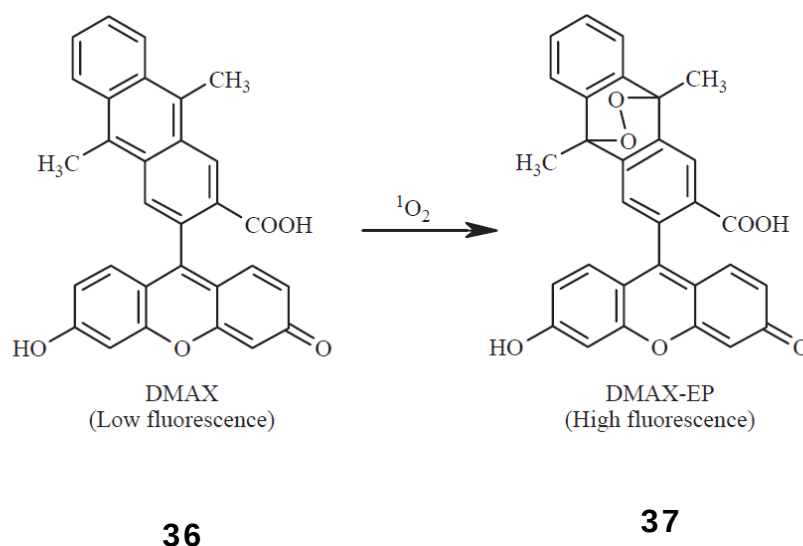


Figure 19. DMAXs and their reactions with singlet oxygen

1.9.5. (ATTA-Eu³⁺) [4'-(9-anthryl)-2,2':6',2''-terpyridine-6,6''-diyl] bis (methylenenitrilo)tetrakis(acetate)-Eu³⁺

Yuan et al. have developed a “Eu³⁺ chelate-based phosphorescence probe” (ATTA-Eu³⁺). The almost non-luminescent chelate showed that it can specifically react with ¹O₂ to yield endoperoxide product (EP-ATTA-Eu³⁺) with a sound increase in its phosphorescence intensity. EP-ATTA-Eu³⁺ luminescence quantum yield is 17 times higher than that of ATTA-Eu³⁺. It showed high stability up for several days and its ability to detect singlet oxygen in very low concentrations. The probe was expected to be useful for detecting and monitoring of ¹O₂ *in vivo*.⁶⁴

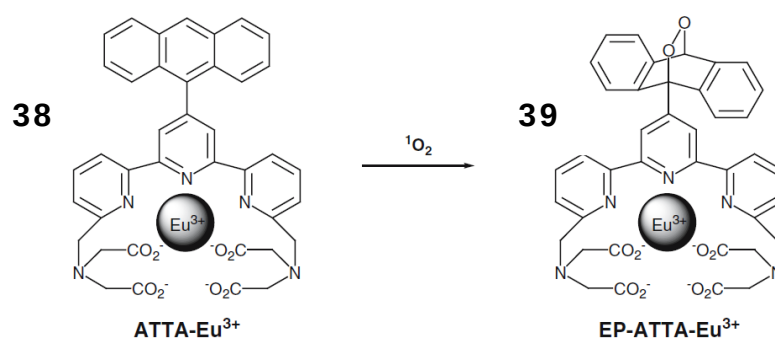


Figure 20. ATTA-Eu³⁺ and its reaction with singlet oxygen

1.9.6. (TTF) 4,4'(5')-Bis[2-(9-anthryloxy)ethylthio]tetrathiafulvalene.

This compound was developed by Ma and Zhang et al.; it consists of a strong electron-donating tetrathiafulvalene (TTF) unit and an anthracene luminophore. This design is based on the fact that $^1\text{O}_2$ has tendency to react with electron-rich organic molecules, and the anthracene moiety reacts specifically with $^1\text{O}_2$. The probe exhibits a highly selective response to singlet oxygen and not to other ROS. It detects Single oxygen concentration at low concentrations as well. It seems that the biological applications of these chemiluminescence kinds of probes have not yet been reported, future development is expected.⁶⁴

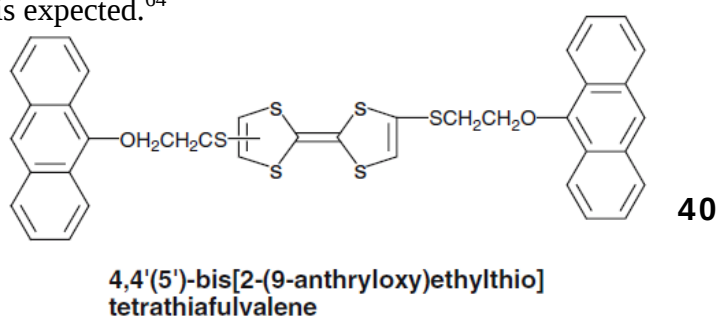


Figure 21. TTF based probe

1.10. Toward a Bodipy-derived singlet oxygen sensor

After reviewing singlet oxygen sensors in the literature, we can see how the singlet oxygen sensors are limited and their research area is still nascent even though it is really important to try to detect singlet oxygen in normal biological systems or an abnormal ones like cancer as it will lead us to a more elaborate understanding of the processes that are involved in creating such molecule. Bodipy as a “fluorescent organic molecules” is not yet been utilized in such field , and it is crucial to exploit such an advantageous molecule as it is been already proven to be working efficiently *in vivo* and it can be excited with proper light and in the right wavelength thus showing its certain therapeutic efficacy and safety and they have shown advantageous properties being used in longer wavelengths and this biologically is favored and by synthesizing such compounds we can expand the realm of singlet oxygen sensing and in the same time expanding the functionality of such important molecule that haven proven efficient in PDT and that is our aim in this study .

Chapter 2

Experimental Procedures

2.1. Methods and materials

All chemicals were purchased from Merck and Sigma-Aldrich and were used without further purification. To monitor the reactions, Merck TLC silica gel 60 F₂₅₄ was used. Merck Silica Gel 60 (particle size: 0.040-0.063 mm, 230-240 mesh ASTM) was used for column chromatography. All ¹H NMR and ¹³C NMR spectra were recorded on Bruker DPX-400 in CDCl₃ with tetramethylsilane as internal standard. Chemical shifts were given in parts per million and the coupling constants (J) were in Hz. Mass spectra were recorded on Agilent Technologies 6224 TOF LC/MS. The absorption spectra were recorded on Varian Cary-100 spectrophotometer and Varian Cary 5000 UV-VIS-NIR absorption spectrophotometer. For fluorescence measurements Varian Eclipse Spectrofluometer was used. TPP (commercial) was used as a singlet oxygen generator in organic medium.

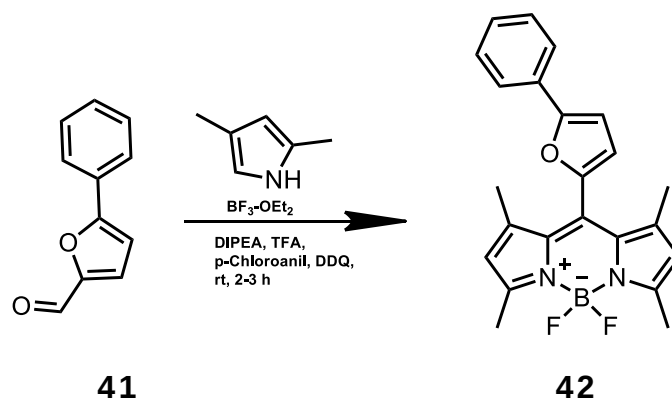


Figure 22. Synthesis of compound 42

2.2. Synthesis of compound 42

To 500 mL round-bottomed flask which contains 250 mL argon-degassed dichloromethane, was added 2,4-dimethylpyrrole (0.925 mL, 6.635 mmol), 5-Phenyl-2-furaldehyde (500mg, 2.930 mmol). Then, trifluoroacetic acid (400 μ L) is added to the reaction mixture and will be left to stir overnight. Then, p-chloranil (785mg, 3.194 mmol) was added and mixed for 1 more hour and $\text{BF}_3\cdot\text{OEt}_2$ (3.5 mL) is added after that and the reaction mixture is left to stir at room temperature for 1h. When the starting material was consumed, water (100 mL) was added and the reaction mixture was extracted with DCM (3x100 mL), evaporated and dried over Na_2SO_4 . The product was purified by silica gel column chromatography using Hexane:Toluene (1:3). ^1H NMR (400 MHz, CDCl_3) δ 7.82 – 7.64 (m, 2H), 7.50 – 7.29 (m, 2H), 6.88 – 6.78 (m, 1H), 6.57 (dd, J = 6.3, 4.1 Hz, 1H), 6.05 (s, 1H), 2.70 – 2.49 (m, 5H), 1.74 (d, J = 8.5 Hz, 5H). MS (TOF-ESI): m/z : Calculated $[\text{M}+\text{H}]^+$: 390.18241 Found $[\text{M}+\text{H}]^+$: 390.18406 Δ = -4,23 ppm

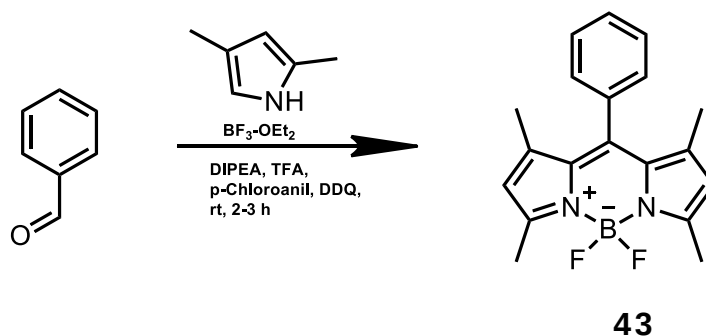


Figure 23. Synthesis of compounds 43

2.3. Synthesis of compound 43

To a 500 mL round-bottomed flask containing 250 mL argon-degassed dichloromethane, 2,4-dimethylpyrrole (0.86 mL, 8.1 mmol), benzoylechloride (500 mg, 3.55 mmol) were added. Then, trifluoroacetic acid (400 μ L) was added to the reaction mixture and left to stir overnight. Then, p-chloranil (926 mg, 3.9 mmol) was added and mixed for 1 additional hour. After that, TEA (5 mL) was added and mixed for 1 additional hour and $\text{BF}_3\cdot\text{OEt}_2$ (4 mL) was added and the reaction mixture was left to stir at room temperature for 1h. When the starting material was consumed, water (100 mL) was added and the reaction mixture was extracted with DCM (3x100 mL), evaporated and dried over Na_2SO_4 . The product was purified by silica gel column chromatography using DCM:Hexane (1:1) as the eluent and the compound was obtained as yellow reddish solid (250 mg, 50 %).

^1H NMR (400 MHz, CDCl_3) δ 7.82 (d, J = 7.7 Hz, 2H), 7.71 (d, J = 16.1 Hz, 1H), 7.53 (d, J = 5.1 Hz, 1H), 7.39 (dd, J = 13.2, 5.1 Hz, 3H), 7.31 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 10.3 Hz, 2H), 7.13 – 7.06 (m, 1H), 6.80 (d, J = 3.4 Hz, 1H), 6.70 (d, J = 3.4 Hz, 1H), 6.63 (s, 1H), 1.46 (d, J = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 129.03 (d, J = 18.9 Hz), 127.97 (s), 121.21 (s), 77.34 (s), 77.02 (s), 76.70 (s), 14.57 (s), 14.31 (s).

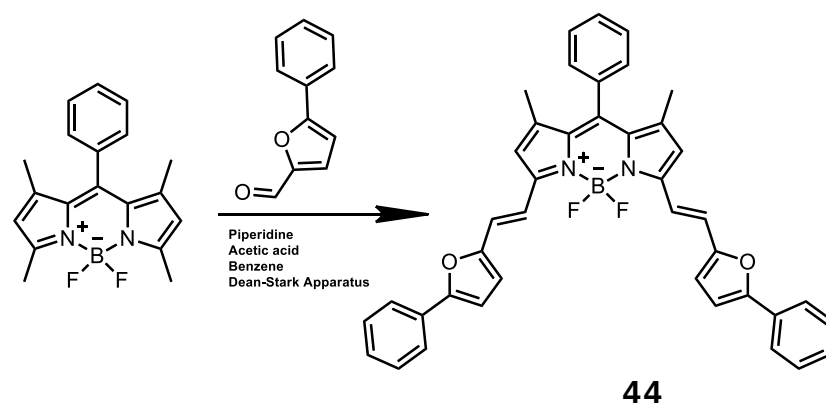


Figure 24. Synthesis of compound 44

2.4. Synthesis of 44

Compound (41) (50 mg, 1.145 mmol) and compound (43) (30 mg, 1.560 mmol) were added to a 100 mL round-bottomed flask containing 50 mL benzene and to this solution, piperidine (270 μ L, 2.73 mmol) and acetic acid (220 μ L, 3.84 mmol) were added. The mixture was heated under reflux at 100 °C overnight by using a Dean Stark trap and it is monitored by TLC. When all the starting material had been consumed, the mixture was cooled to room temperature and the solvent was evaporated. Water (100 mL) was added to the residue and the product was extracted with the DCM (3 x100 mL). The organic phase dried over Na₂SO₄, evaporated. The residue was purified by silica gel column chromatography using 1:10 (EtOAc: Hexane) solution as the eluent and the compound was obtained as blue solid". ¹H NMR (400 MHz, CDCl₃) δ 7.82 (d, *J* = 7.8 Hz, 1H), 7.71 (d, *J* = 16.1 Hz, 1H), 7.53 (d, *J* = 5.2 Hz, 1H), 7.39 (d, *J* = 15.3 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 16.3 Hz, 1H), 6.80 (d, *J* = 3.4 Hz, 1H), 6.70 (d, *J* = 3.5 Hz, 1H), 6.63 (s, 1H), 1.47 (s, 1H).

Chapter 3

Results and Discussion

3.1. General Perspective and objectives

Photodynamic therapy as mentioned in the introduction is a promising field when it comes to treat different types of cancer, and as we have seen that Bodipy was designed and used for different and several approaches and goals that favoring PDT, for example the usage of Bodipy as photosensitizers primarily, and also in logic gates for the purpose to differentiate between normal and malignant cells. Our aim here is to prove that we can extend Bodipy application furthermore into a new realm, to use Bodipy as a singlet oxygen sensor and by this we are exploiting all the advantages this molecule had given us already, given that Bodipy absorption is usually in high wavelengths which makes it optimum in vivo studies and as a sensor it will overcome a lot of problems that were discussed in the introduction, and it will be optimal as its absorption lies in the therapeutic window (600-900 nm) thus it can be used optimally to study and observe biological singlet oxygen generation and chemical one (Photosensitizer). So we tried to prove this principle on two Bodipy compounds, compound **42** and compound **44**, we studied their absorption spectrum after illuminating them with light in different time intervals using TPP as a singlet oxygen generator and we studied their fluorescent properties as well. Our work was corroborated by of Dayoung, et al. paper and their study of fluorescent probes for detection of intracellular singlet oxygen and our expected results was based on this study.⁶⁶

3.2. Compound 42 as potential singlet oxygen sensor.

For this compound we illuminated a green light at 500 nm for 160 minutes with 10 minutes interval and as our expectation is for the compound (42) to react with oxygen that have been generated by TPP in chloroform medium as shown in the figure 25 and the furan ring would open, we used 5 μ L of TPP and from a solution of 5 mmol of compound 42 we used 100 μ L.

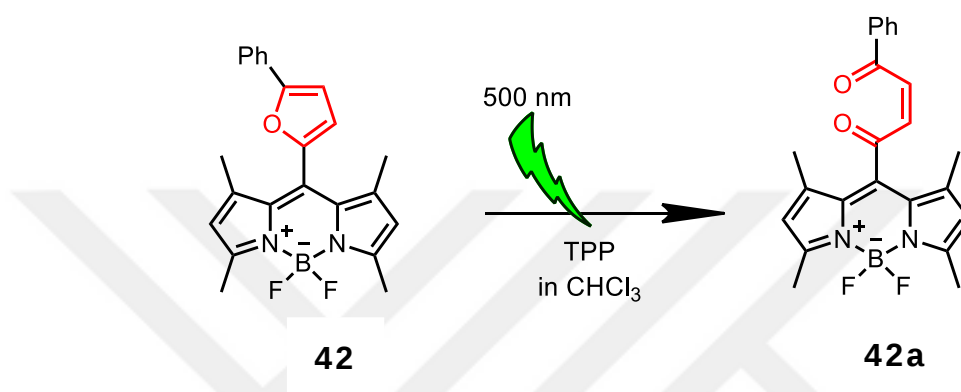


Figure 25. Expected reaction of compound 42 with singlet oxygen

So as we can see the absorption spectrum in Figure 26 below, there is two peaks the first one which the main one is around 530 nm and the other one is around 445 nm, the first one had shown a decrease over the 160 minutes but it is a really slow decrease compared to what we have expected ⁶⁶, the second peak (the one around 445 nm) which is shown better in Figure 27 , we can see it start appearing after 100 minutes from illuminating the light which tells us that the compound has reacted with singlet oxygen and gave compound 42a but to a little extent that can be eventually rendered insignificant because as we can see , the increase in the Y axis is really small compared to the actual concentration of the compound, so as a result this compound even though it showed a promising result , it appeared that it will be impractical if we continued working on this compound with such results.

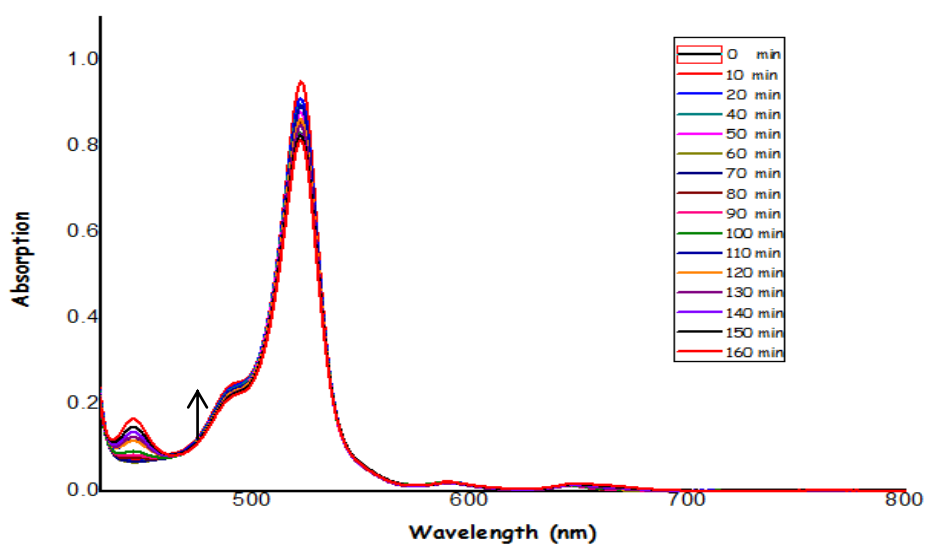


Figure 26. Singlet oxygen generation experiment in CHCl_3 solution. Absorption spectrum of compound **42** is decreasing over 160 minutes at 550 nm and there is a new formed peak at 445 nm after using 5 μL of TPP and 5 mmol of compound **42**.

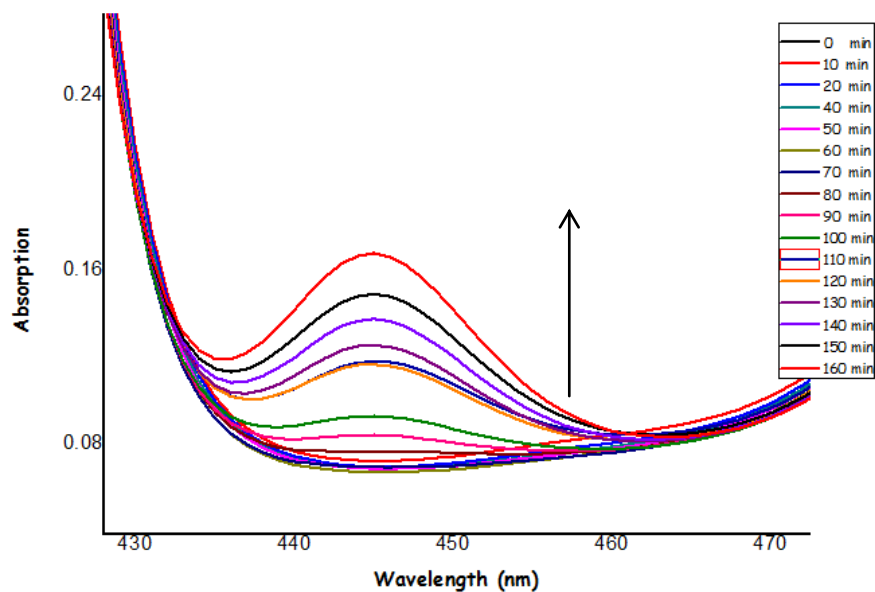


Figure 27. Compound **42a** expected peak in the absorption spectrum of compound **42**

3.3. Compound 44 as a potential singlet oxygen sensor.

After studying the previous compounds we decided to take another approach and to synthesize a Bodipy that is electron rich and in principle can overcome the previous compound problems. For compound 44 we did the same with the previous compound as illuminated a green light at 500 nm for 120 minutes with 10 minutes interval and our expectation for the compound 44 is to react with oxygen that have been generated by TPP in chloroform medium as shown in the figure 28 and the furan ring would open but in two domains fashions, one ring will open first and then the two rings would open, we used 5 μ L of TPP and from a solution of 5 mmol of compound 44 we used 200 μ L.

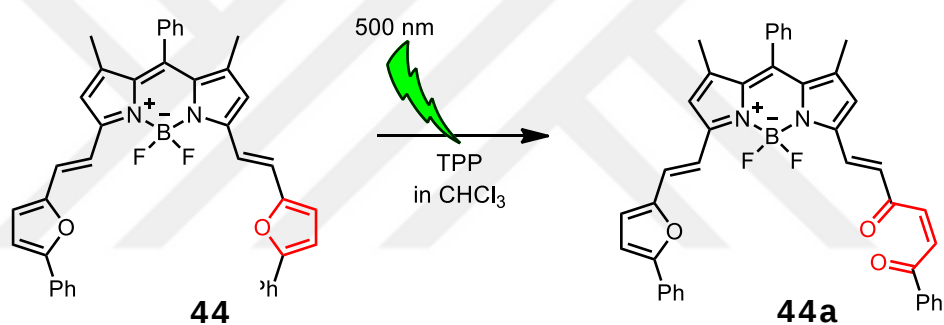


Figure 28a

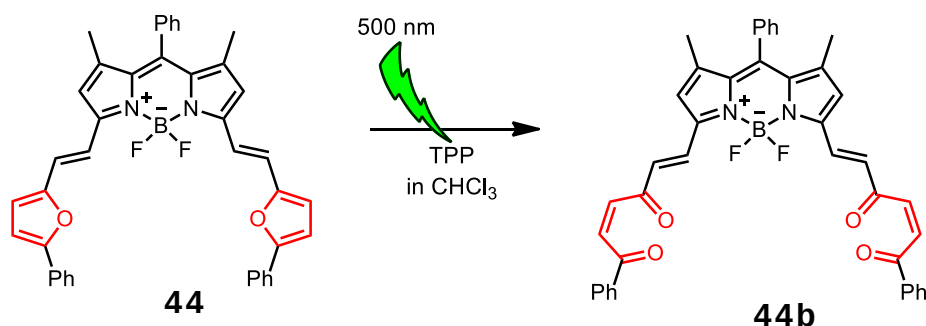


Figure 28b

Figure 28. Expected reaction of compound 44 with singlet oxygen

3.3.1. Absorption spectrum of compound (44)

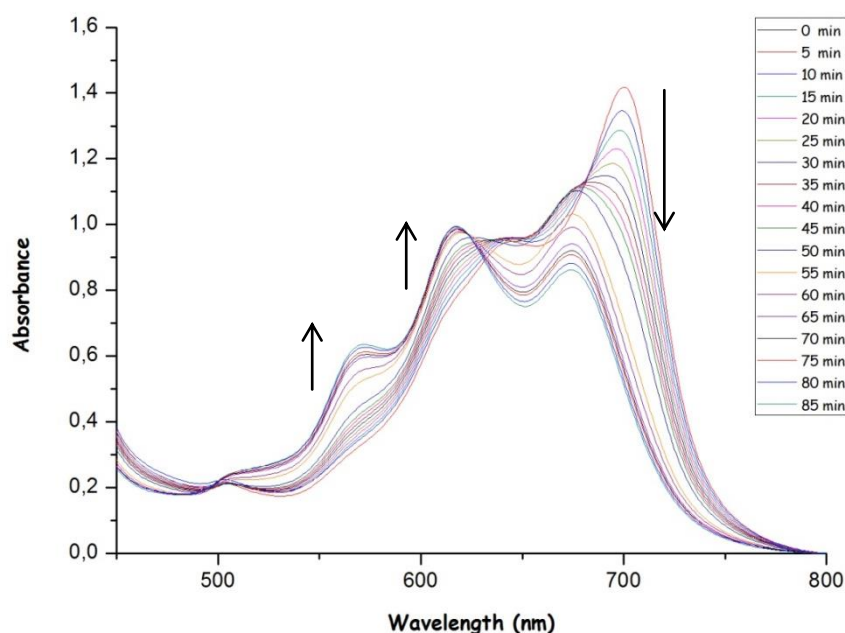


Figure 29. Singlet oxygen generation experiment in CHCl₃ solution. Absorption spectrum of compound 44 is decreasing over 85 minutes at 710 nm and there are new formed peaks at 625 nm and 575 nm after using 5 μ L of TPP and 5 mmol of compound **44**.

In the figure 29 above we can see the absorption spectrum of compound **44**, and it actually fulfilled our expectations. We can see three main peaks, the main absorption peak of the compound at 710 nm and we can observe a significant decrease over the time and a hypsochromic shift that stops at 690 nm but still continue it decreasing over time and as the compound reacts with oxygen we can observe the a second peak forming at 620 nm after 50 minutes and as we expected that this peak is related with the compound **42a** that formed in Figure 28. Finally, we can see a third peak forming after 70 minutes and this peak is related with compound **42b** that we proposed its structure at Figure 28.

3.3.2. Emission spectra of compound **44**.

In principle we are expecting to find three species of compound **44** in the chloroform media thus we are expecting to see three different peaks upon emitting light in different wavelengths, we have done this at two wavelengths one at 500 nm and one at 550 nm and we have observed three different peaks as we assumed we would.

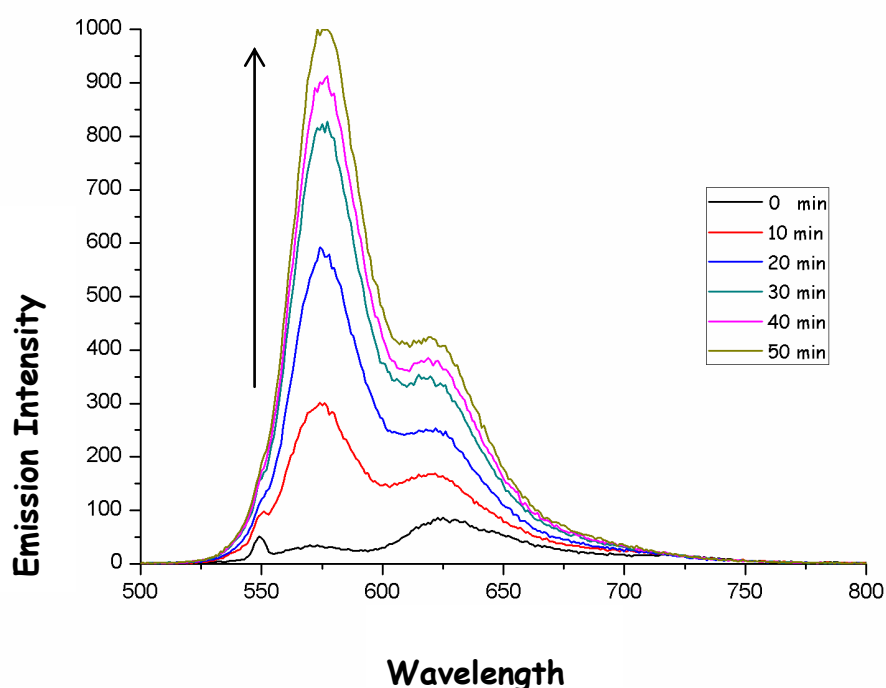


Figure 30. Emission spectrum of compound **44** with excitation at 550 nm in CHCl_3 .

In the figure 30 above we studied the emission spectrum of compound **44** and the light emitted at 550 nm and as we can see the significant increase for existing of two peaks one at 575 nm and the other one at 625 nm, and this is consistent with our expectations as the compound emission intensity will increase with time due to its reaction with light and as we expect there will be two different species in the medium one is the compound itself and one is the new formed compound (**42a** and **42b**), thus we can see two peaks which are adjoined peaks as we can see them increase together.

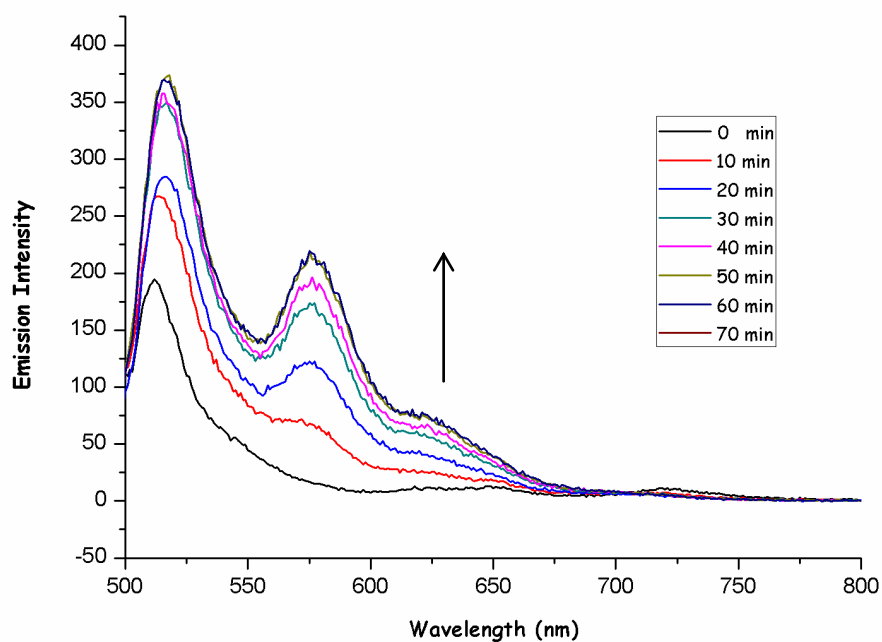


Figure 31. Emission spectrum of compound **44** with excitation at 500 nm in CHCl_3

In the figure 31 we studied the emission spectrum of compound and the light emitted at 500 nm and what matter here is actually the new peak forming which corroborate our finding in figure 30 as this peak is the peak that showing us the resulted compound after its reaction with singlet oxygen.

3.3.3. Color differences in different light environments for compound 44

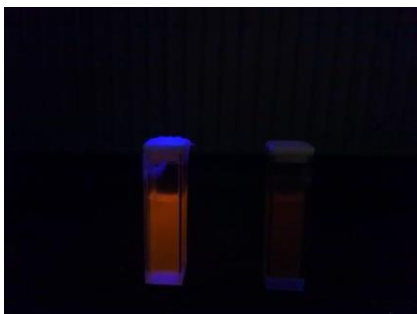


Figure 32A



Figure 32B

Figure 32. Compound **44** colors in different environments , under ambient light and UV-lamp .

In studying the emission spectra of the compound we took pictures for the compound before and after the reaction for both the control cell and the light-emitted cell. As we can see in the figure above in figure 32A cells were put under UV-Lamp and we can see the difference as shown in the picture above the less fluorescent emission was before the reaction and the more fluorescent (orange) is after the reaction, also in figure 32B cells were put under the ambient light and we can see the differences between the color of the cells before (green) and after the reaction (violet).

Chapter 4

Conclusion

In this thesis study, two Bodipy based structures were synthesized and characterized as a potential singlet oxygen sensor, compound **42** did not appeal as a promising singlet oxygen sensor due its long time response to singlet oxygen reaction and as it was obvious that it is a poor sensor while the distyryl-bodipy compound **44** gave the results which were expected and this was corroborated by a previous study that was dealing with fluorescent probes for detection of intracellular singlet oxygen. Compound **44** was also studied and its expected products were shown in long wavelengths and it showed fluorescent changes during the reaction with singlet oxygen which make it a promising new singlet oxygen sensor. It was tested in the dark, as well as in the presence of light and showed no reactivity in the dark as was expected and it showed appealing results upon excitation in the presence of light at different wavelengths. Compound **44** will be tested again in an environment with no light , but has a chemical singlet oxygen generator ability that generates oxygen by other means than light and this will be a positive-control test to ensure that the compound acted according to what we proposed in the beginning and it will also be studied further in vivo, for its potential biological application as a singlet oxygen probe.

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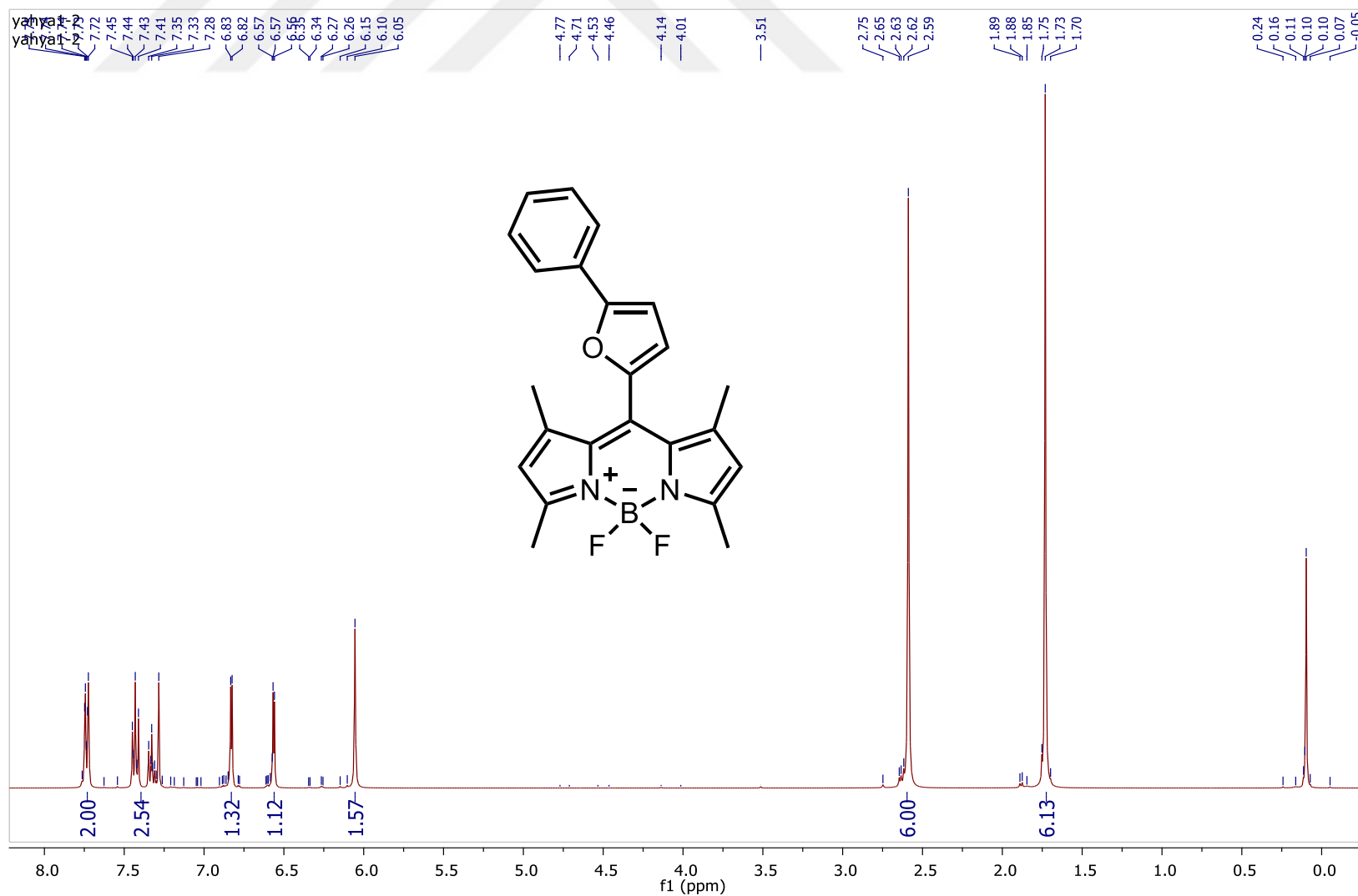
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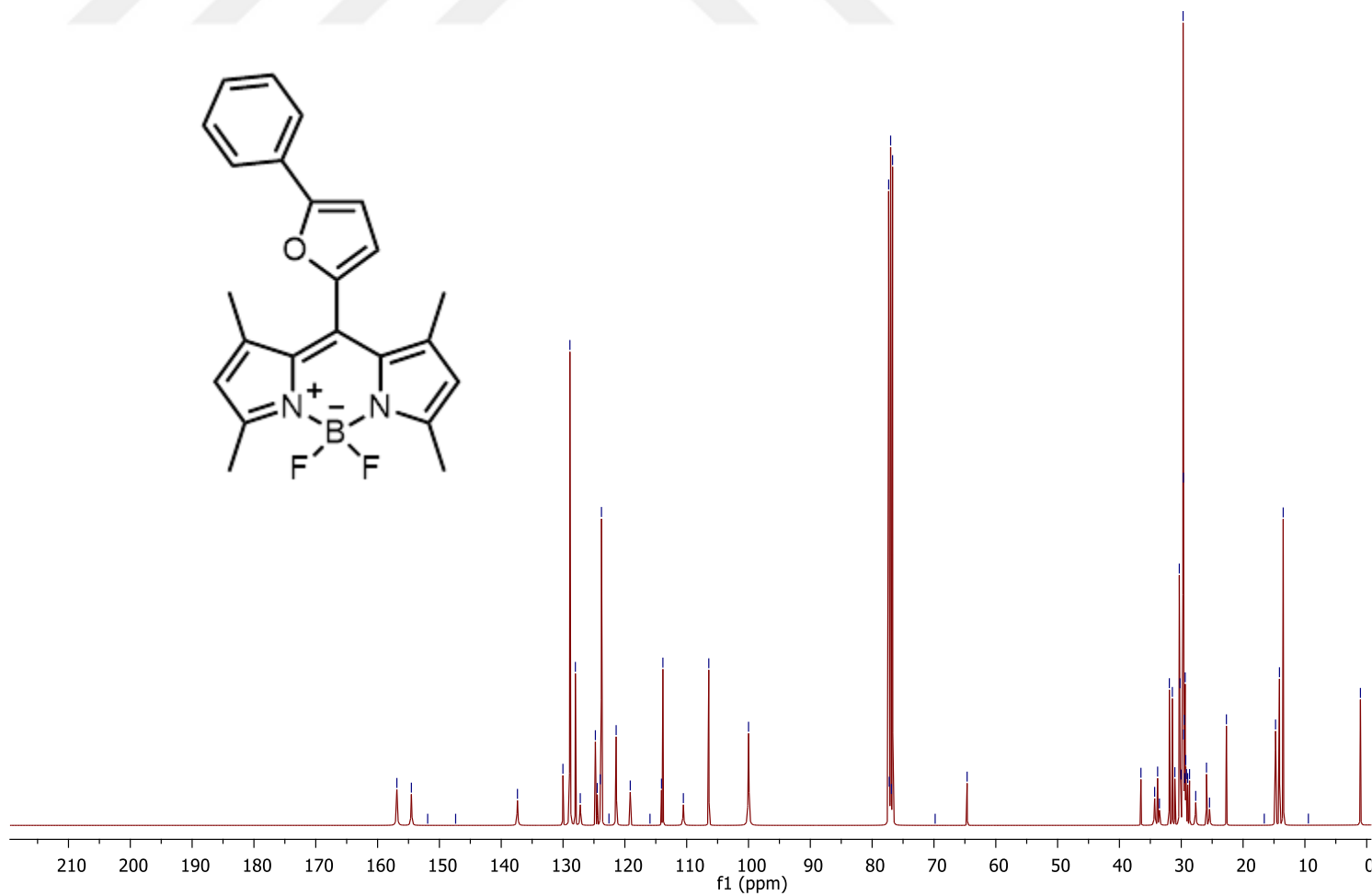
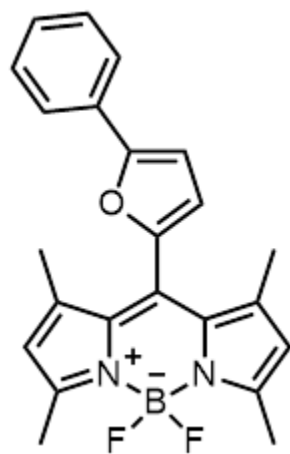
APPENDIX A- NMR SPECTRA

Compound 42 ^1H NMR



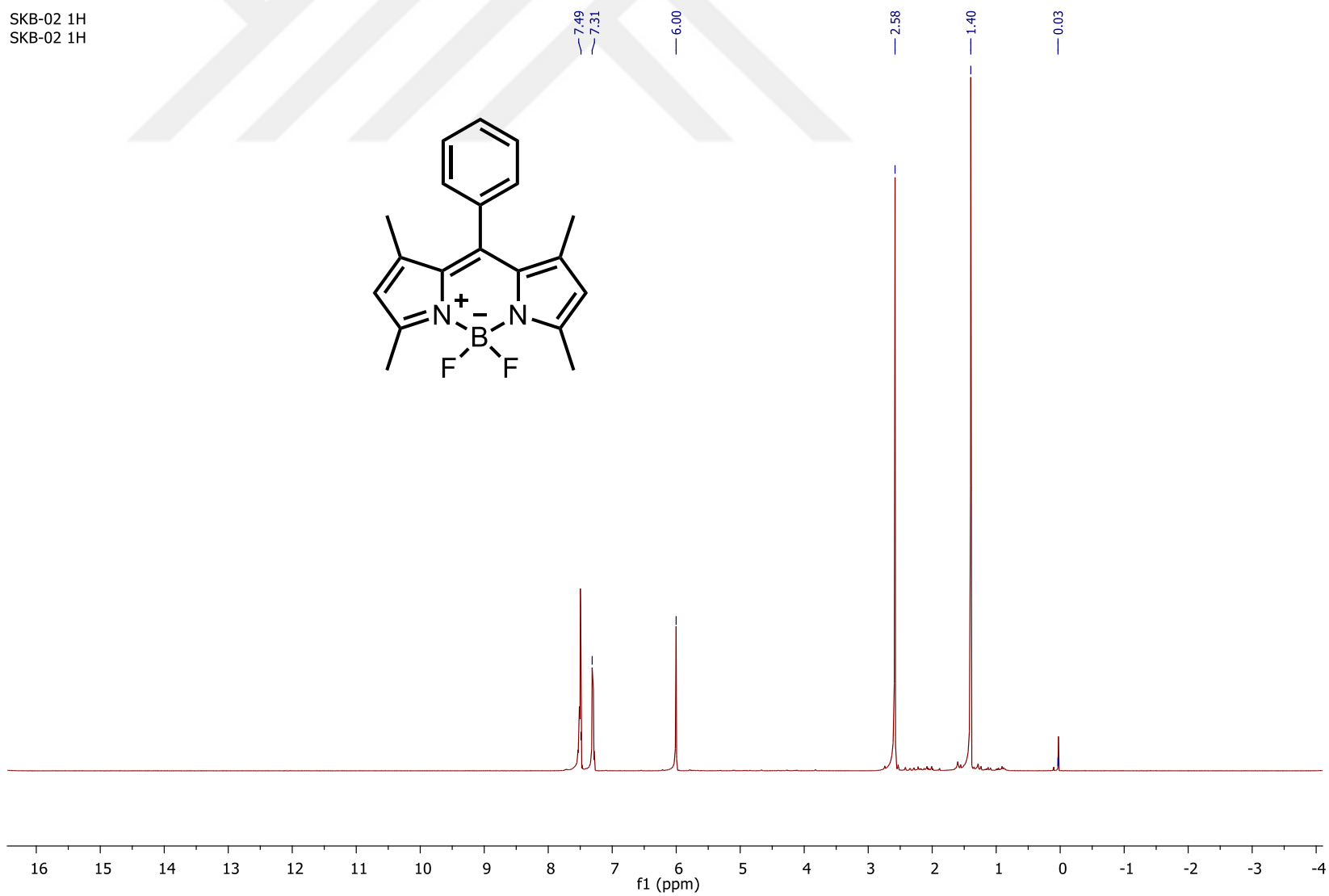
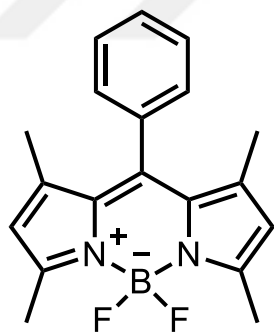
Compound 42 ¹³C NMR

YI Meso 18 10 16 C13
YI Meso 18 10 16 C13



Compound 43 H¹NMR

SKB-02 1H
SKB-02 1H



Compound 43 ¹³C NMR

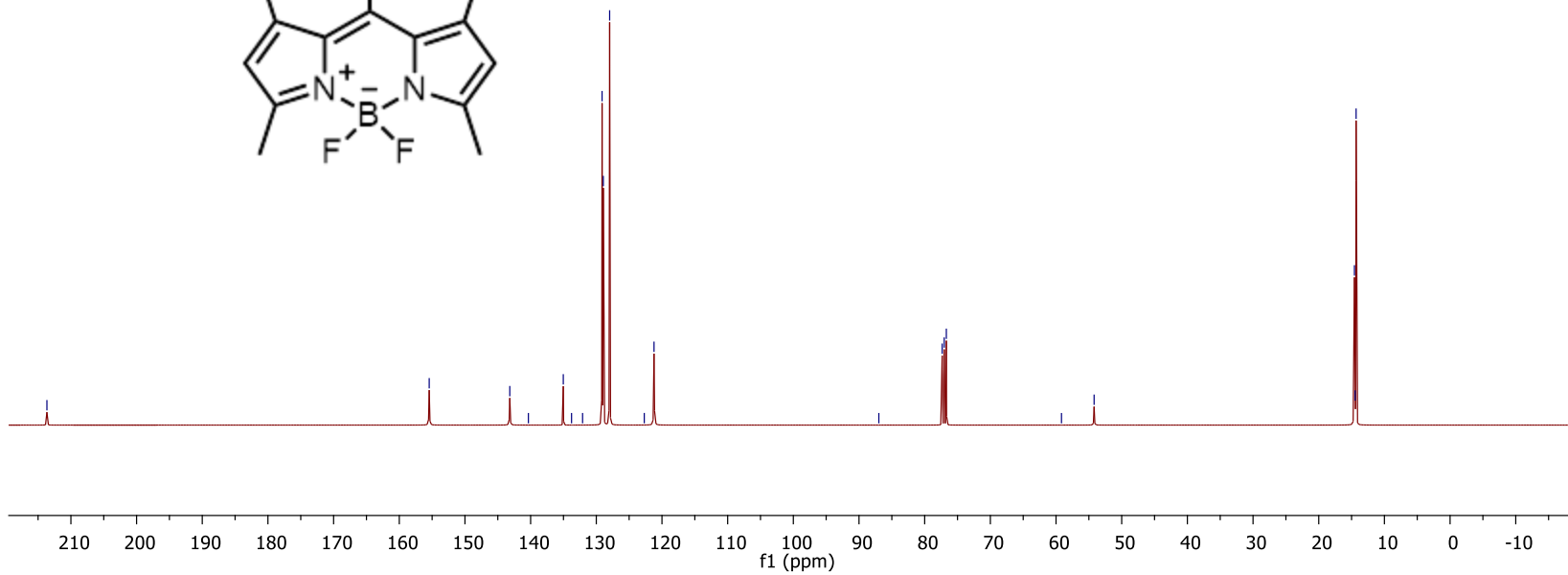
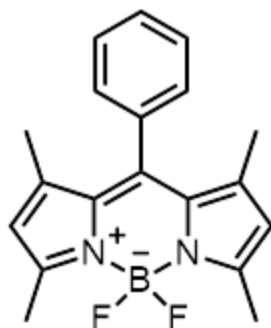
SKB02 13C
SKB02 13C

155.44
143.17
140.34
135.03
133.77
132.09
129.12
128.93
127.97
122.69
121.21

86.98
77.34
77.02
76.70

59.17
54.20

14.56
14.46
14.31

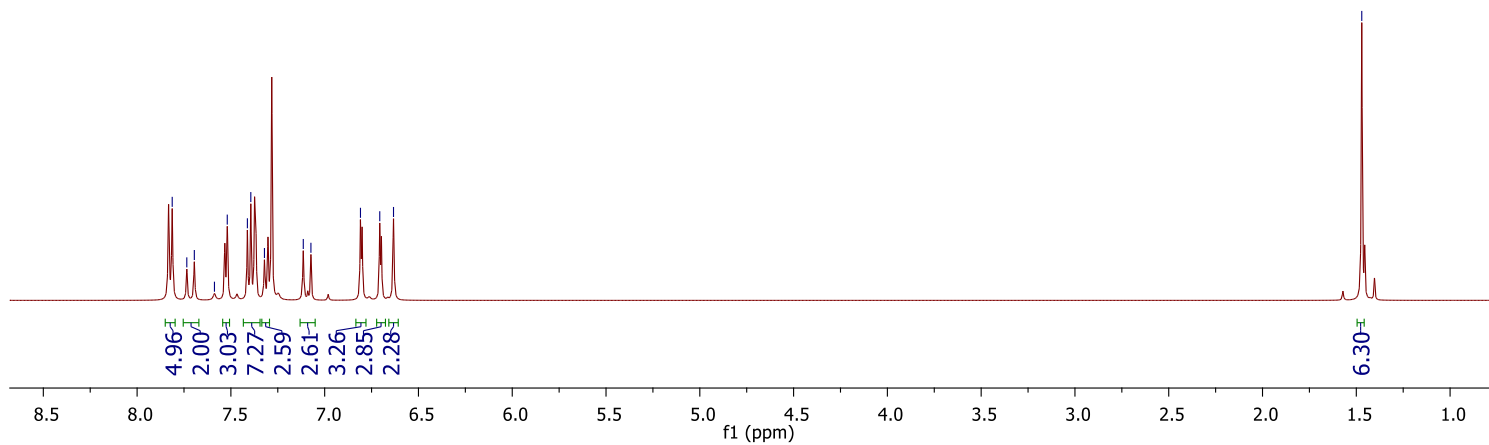
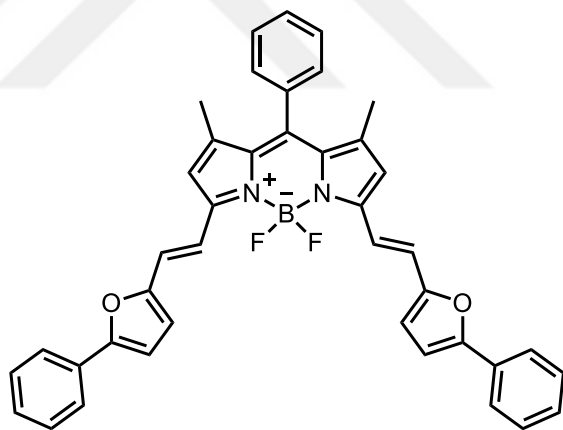


Compound 44 H¹NMR

YI di HNMR
YI di HNMR

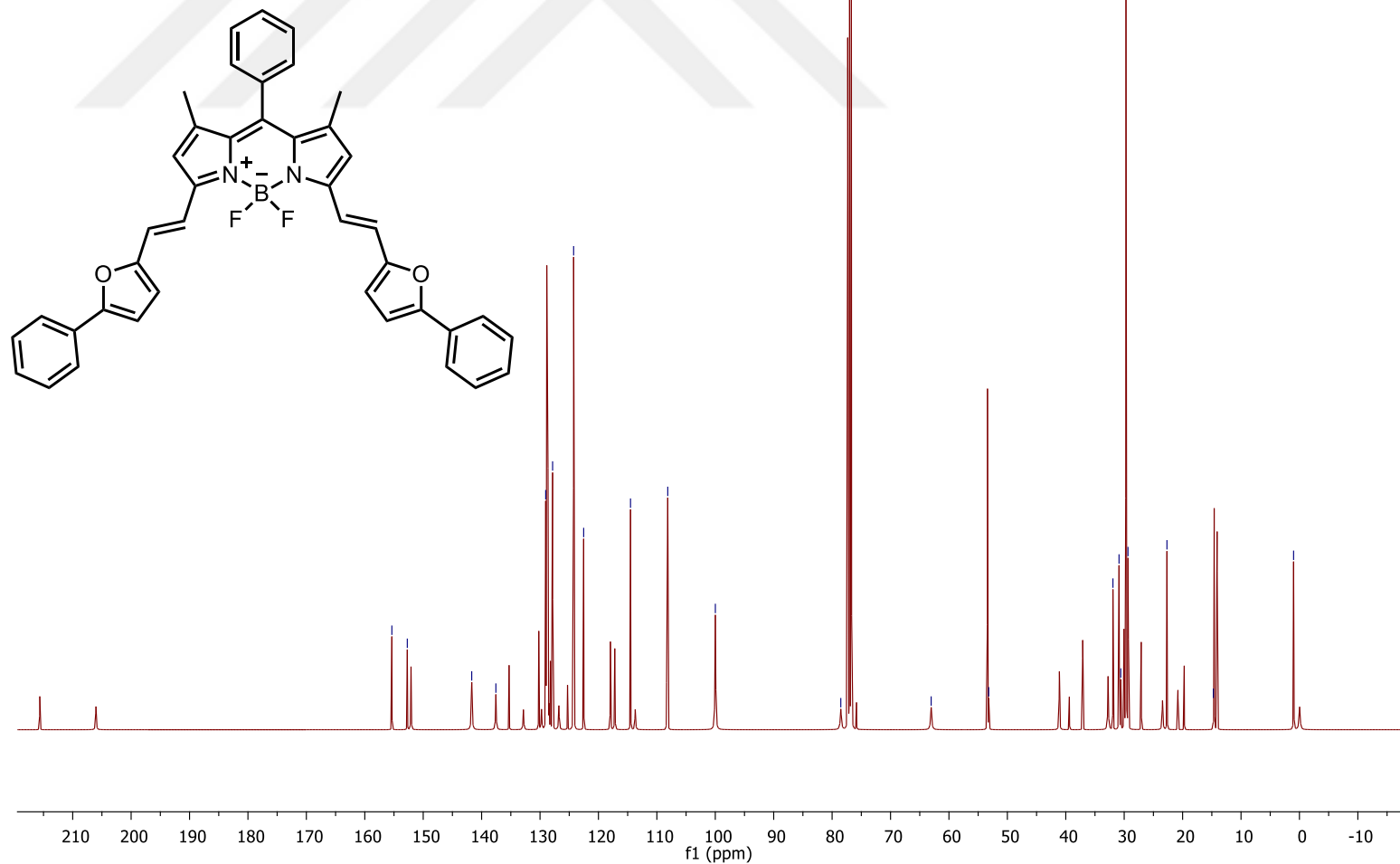
8.88
8.78
8.68
8.58
8.48
7.41
7.39
7.32
7.11
7.07
6.81
6.71
6.63

— 1.47



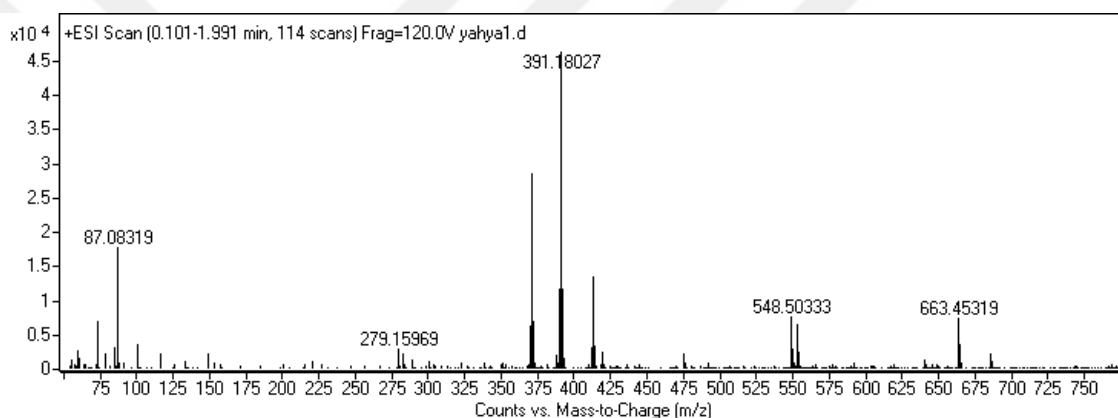
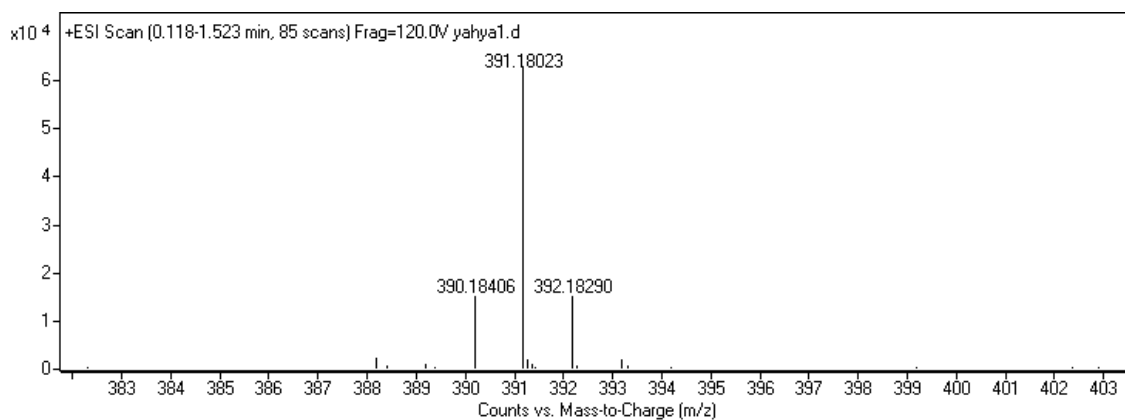
Compound 44 ¹³C NMR

YI 8 21 07 2016 di c13
YI 8 21 07 2016 di c13

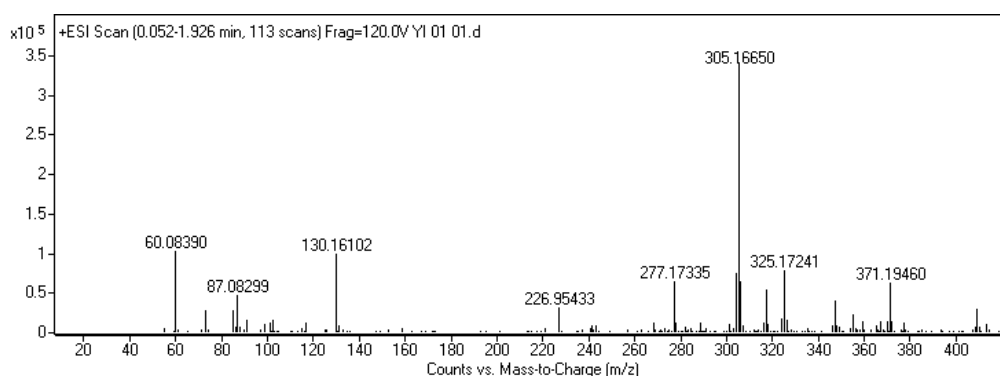


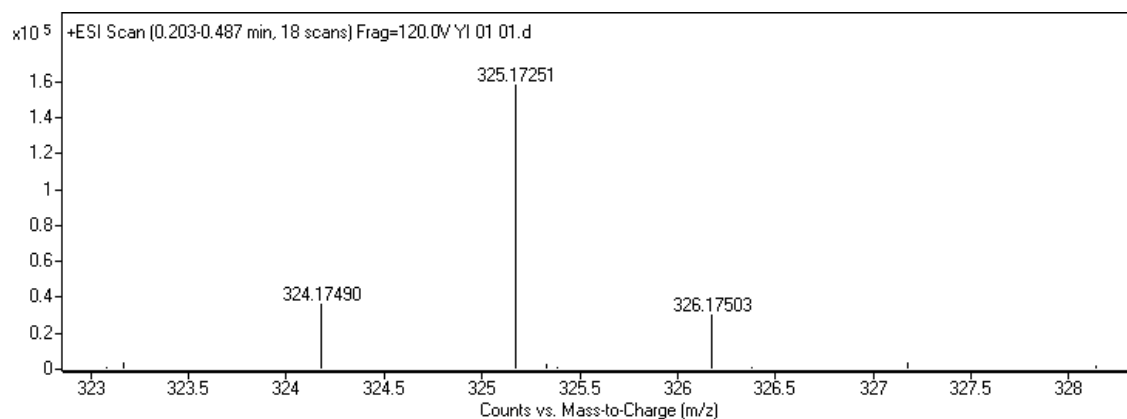
APPENDIX B MASS SPECTRA

Compound 42 Mass spectrum



Compound 43 Mass spectrum





Compound 44 Mass spectrum

