DESIGN AND SYNTHESIS OF BODIPY BASED PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

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ABSTRACT

DESIGN AND SYNTHESIS OF BODIPY BASED PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

Photodynamic therapy is a promising modality for the non-invasive treatment of several cancerous and non-cancerous diseases. PDT is more preferable than other therapies due to its low damage to non-targeted tissues and its controllable characteristics. The therapy involves the activation of a photosensitizer under light illumination to generate singlet oxygen which is the cytotoxic agent employed against the cancerous tissues. Thus, there is currently a great effort to develop various photosensitizers. Among these, BODIPY based photosensitizers are distinguished due to certain characteristics, including excellent photostability, high extinction coefficients and high resistance to photobleaching.

In this study, we aimed to synthesize and develop new BODIPY based photosensitizers for the use of photodynamic therapy agents. BODIPY skeleton was devised using the dibromoethylene unit from the 2,6-positions in order to enhance the π-conjugation system for red shift to longer wavelengths resulting in a deep penetration of tissue. Heavy atoms such as bromine were introduced to the BODIPY core to ensure the transition from singlet states to triplet states via intersystem crossing for the generation of singlet oxygen. Photophysical properties and spectroscopic measurements of photosensitizers were performed successfully. Finally the photodynamic activities of photosensitizers in cancerous cells were also investigated.

ÖZET

FOTODİNAMİK TERAPİ İÇİN BODIPY BAZLI IŞIK DUYARLAŞTIRICILARIN TASARIMI VE SENTEZİ

Fotodinamik terapi, kanserli ve kanserli olmayan birçok hastalığın non-invaziv tedavisinde kullanılan umut vaat edici bir yöntemdir. Hedeflenmeyen dokulara minimal oranda zarar vermesinden ve kontrol altına alınabilen özelliklerinden dolayı PDT diğer tedavi yöntemlerine kıyasla daha çok tercih edilmektedir. Terapi, ışık ile uyarılan fotoduyarlaştırıcının aktif hale gelerek, kanserli dokulara karşı sitotoksik bir ajan olan singlet oxygen üretmesine dayanmaktadır. Bu nedenle, günümüzde çeşitli fotoduyarlaştırıcılar geliştirmek için büyük bir çaba harcanmaktadır. Bunların arasında, BODIPY bazlı fotoduyarlaştırıcılar üstün fotostabiliteye, yüksek sönümleme katsayısına ve florışıldama bozunmasına karşı yüksek dirence sahip olması gibi belirli özelliklerinden dolayı daha seçkindir.

Bu çalışmada, fotodinamik terapi ajanı olarak kullanılmak üzere, yeni BODIPY türevli fotoduyarlaştırıcıları tasarlamayı ve sentezlemeyi amaçladık. BODIPY iskeleti, π-konjugasyonunu arttırarak boyanın uzun dalga boylarına kayması sonucu dokulara daha derinden nüfuz etmesi için, 2- ve 6- pozisyonlarından dibromoetilen grubu ile türevlendirilerek tasarlanmıştır. Sistemler arası geçiş yöntemi ile singlet halden triplet hale geçişi sağlamak ve bu sayede singlet oksijen üretmek için BODIPY merkezinde brom gibi agır atomlar kullanılmıştır. Fotoduyarlaştırıcıların, foto fiziksel özellikleri ve spektroskopik ölçümleri başarı ile gerçekleştirilmiştir. Son olarak fotoduyarlaştırıcıların kanserli hücreler üzerindeki fotodinamik etkileri incelenmiştir.

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LIST OF ABBREVIATIONS

PDT	Photodynamic Therapy
PS	Photosensitizer
ROS	Reactive Oxygen Species
ISC	Intersystem Crossing
BODIPY	4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacene
DPBF	1,3-Diphenylisobenzofuran
THF	Tetrahydrofuran
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
$POCl_3$	Phosphorous oxychloride
DCM	Dichloromethane
DCE	1,2-Dichloroethane
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
DMF	Dimethyl formamide
EtOH	Ethanol
DAPI	4',6-diamidino-2-phenylindole
$CdCl_3$	Deutero Chloroform
NMR	Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

1.1. Photodynamic Therapy

PDT is an efficient and confirmed clinical modality for the treatment of a several types of cancers such as; skin cancer, head and neck cancer, pancreatic cancer, breast cancer, lung cancer and basal cell carcinoma. It is also used for the treatment of other malignant and localized diseases such as dermatological and cardiovascular illnesses, brain tumors, localized infections and actinic keratosis (Gomer et al., 1989). PDT which has been under development since the beginning of the twentieth century is a promising treatment method for the future. Compared to traditional cancer therapies, PDT is a less harmless non-invasive method (Triesscheijn et al., 2006).

The working principle of the PDT relies on using light to activate a photosensitizer in order to generate a cytotoxic reactive oxygen species (e.g., singlet oxygen) within cells to promote irreversible cellular damage and cell death by apoptosis and/or necrosis (Belfield et al.,2017, Emrullahoğlu et al., 2017).

Accordingly, there are three main components of effective PDT: light, oxygen and photosensitizer. In PDT, it is important to use long-wavelength light sources such as LEDs (350-1100 nm) because of their ability to penetration the tissues deeply. Thus, it is important to use light sources in therapeutic window during the treatment. Singlet oxygen needed for photodynamic activity occurs in triplet states. When a photosensitizer is exposed to light, it is excited from its ground state to excited state and is then converted to a triplet state from a singlet state via intersystem crossing.

ISC is the transition between electronic states that have different multiplicities. The most common of these is from an excited singlet state to a triplet state (S1-T1), which is a forbidden transition. However introducing heavy metal atoms to the molecule increases spin-orbit coupling and leads to increased spin conversion. Also, using a saturated solution with a paramagnetic species like molecular oxygen increases ISC (Skoog, Holler and Crouch 2007).

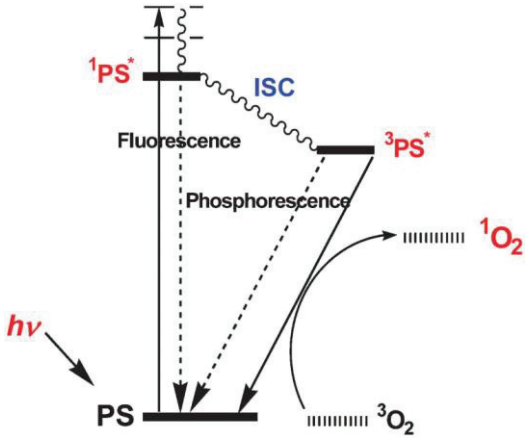


Figure 1.1. ISC and PDT by a simplified Jablonski Diagram

(Source: You and Awauah, 2012)

There are two types of reactions used in order to generate reactive oxygen. In the Type II process, excited PS in a triplet state reacts with molecular oxygen to produce reactive singlet oxygen, which is a short-lived cytotoxic agent in consequence of its reaction between living biomolecules. In the Type I process, excited PS in triplet state, undergoes a chemical reaction with a substrate in order to form radical or radical ions such as superoxide, hydroxyl ions and hydrogen peroxides (Awuah and You 2012, Lee et al., 2018).

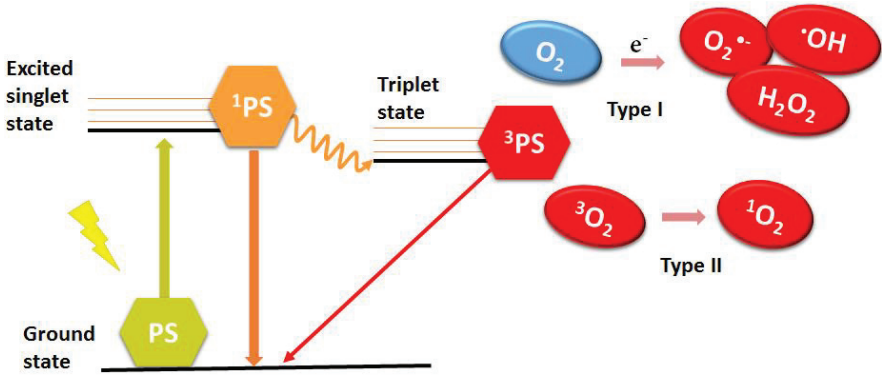


Figure 1.2. Type I and Type II mechanism of ROS generation by photodynamic therapy

(Source: Liu, Qui, Zaat, Breukink, Heger, 2015)

In PDT, since cell death is triggered by singlet oxygen, photosensitizers that work with the Type II mechanism are more preferable. Moreover, as the lifetime of singlet oxygen is very low ($0{,}6x10^{-6}s$)[3] and also its diffusion rate is limited (10-300 nm), the possibility of damaging non-targeted cells are relatively low. For these reasons, a favorable photosensitizer, the critical component of the PDT, should have a high capacity for

singlet oxygen generation, absorption bands within the therapeutic window, and the ability to delocalize in cancer tissue.

1.2. Singlet Oxygen

Singlet oxygen is the major cytotoxic agent in PDT (among the other reactive oxygen species). Therefore, it is important detect the singlet oxygen generation of photosensitizers before conducting biological studies. There are two methods used to accomplish this purpose, which namely direct and indirect measurements (Yoshimura et al., 2016).

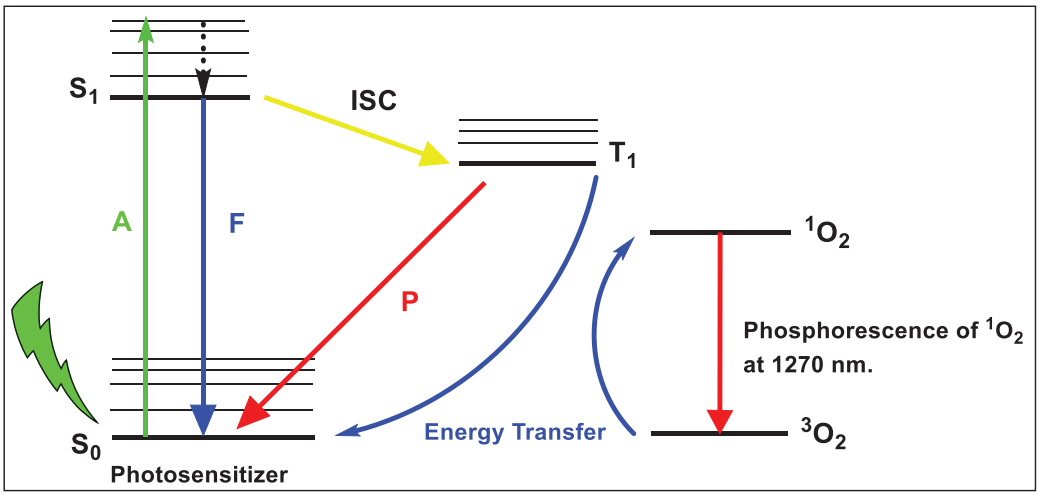


Figure 1.3. Scheme for generation of singlet oxygen

In direct measurements, since the singlet oxygen produces a phosphorescence signal at 1270 nm, detection is performed by looking for steady-state and time resolved phosphorescence measurements with emission signals at 1270 nm. The advantages of this method are the clarity of the signal, the quickly obtained results and its being independent of solvents and insensitive to other species at wavelengths higher than 1000 nm. However, it has some disadvantageous such as, a weak signal, the decreased sensitivity of detectors to shorter wavelengths, and the cost of instrumentation (Crutchley et al., 2002).

Indirect measurements rely on the use of trap molecules whose optical properties (absorbance and/or fluorescence) change with singlet oxygen interaction. Common trap molecules are diene molecules capable of 2,4 cycloaddition to yield endoperoxidase. The upsides of this method are, no instrumentation needed and the ability to detect even small amounts of singlet oxygen. The high possibility of human error and its time-

consuming nature depending on the sample and scavenger are the drawbacks of this method (Yoshimura et al., 2016).

1.3. Photosensitizers

PSs play a crucial role in PDT by generating reactive oxygen species under light irradiation that causes cellular damage. Most photosensitizers investigated in clinical trials are comprised of cyclic tetrapyrrole structures inspired by naturally derived porphyrins as shown in Figure 1.4. (Bugress et al., 2013).

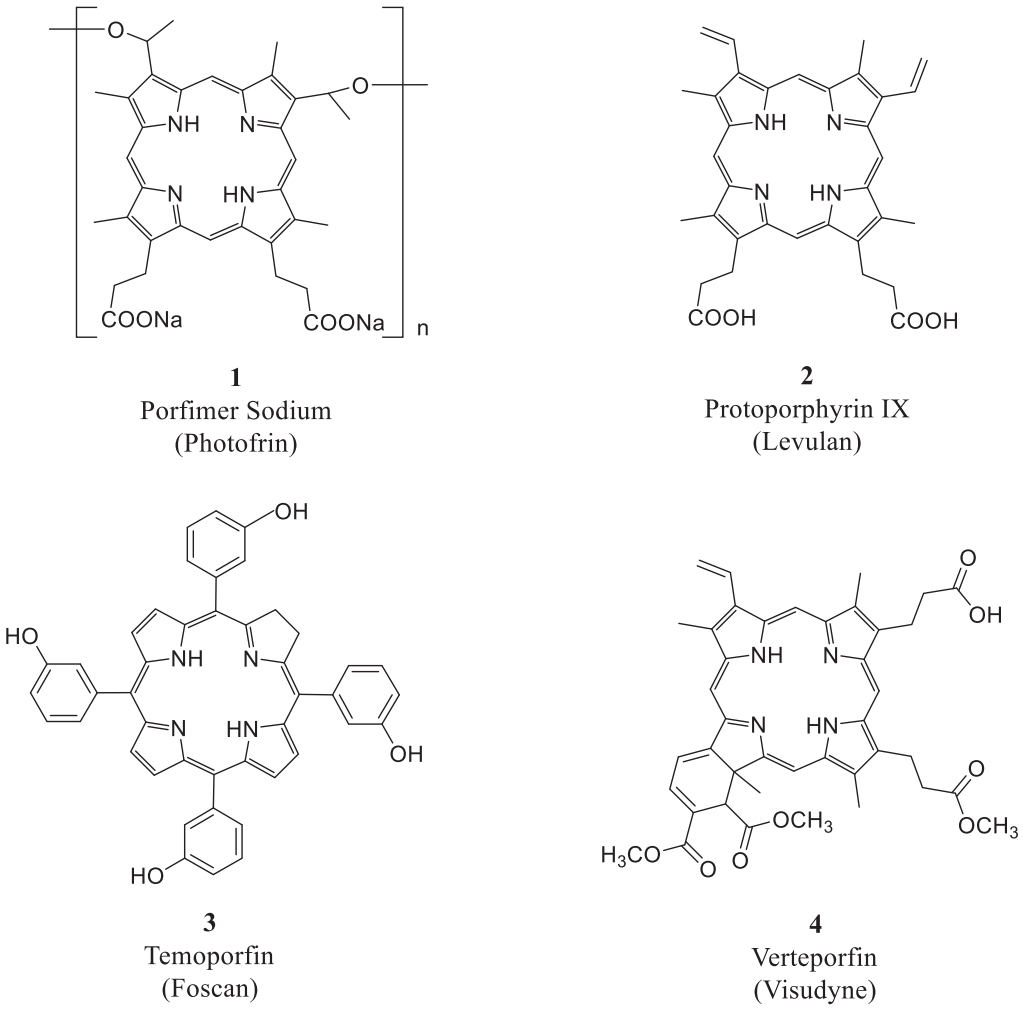


Figure 1.4. Chemical Structures of Porphyrin based PSs

(Source: Burgess, Chung, Kiew, Lee, Lim, Kamkaew, 2013)

Porfimer sodium is one of the oldest PSs used for the treatment of lung cancer, bladder cancer and other diseases in clinical PDT trials. However, it has a low absorption band of 630 nm and therefore needs to be irritated by high energy light sources which cause complications within the body. Other drawbacks of profimer sodium are that it penetrates the surface of the tissue and is not suitable for skin photosensitivity (Burgess, Chung, Kiew, Lee, Lim, Kamkaew, 2013).

Differing from profimer sodium, protophyrin is excreted from the body easily which makes it a suitable agent for skin photosensitivity. However, it is not useful for deep tissue penetration. In addition to these compounds, chlorin-based PSs such as temoporfin and verteporfin are used as clinical PDT agents. Due to their characteristics, they cause some complications in body during the treatment (Bugress et al., 2013).

Although synthetic dyes not of porphyrin origin have also been evaluated for their photosensitizing abilities, they suffer from drawbacks such as chemical complexity, dark cytotoxicity, low absorption bands at 630 nm and photobleaching (Bugress et al., 2013). Besides, in order to make singlet oxygen generation more efficient, a Ps needs to have desirable properties. First, it needs to have high efficiency to support singlet to triplet intersystem crossing.

Additionally, a photosensitizer should have an absorption maxima within the therapeutic window in order to deep tissue penetration. It is important to have a low toxic effect in the lack of light and low side effects such as skin photosensitivity and suffering after light irradiation. Furthermore, an effective photosensitizer should have low quantum yields for bleaching and light resistance. The ease of elimination from the body, the simplicity of synthesis and purification are also desirable properties of photosensitizers (Burgess et al. 2013).

As an alternative class to porphyrin and non-porphyrin based photosensitizers, BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indicane) and its aza-analogue have also been subject to extensive study during clinical PDT trials.

1.4. BODIPY based Photosensitizers

BODIPY dye was synthesized and reported by Treibs and Kreuzer for the first time in 1968 (Treibs and Kreuzer, 1968). According to the IUPAC numbering system, BODIPY dyes are numbered differently from their precursors; however,

dipyrromethane and dipyyromethene but alpha, beta and meso positions are same for both molecules.

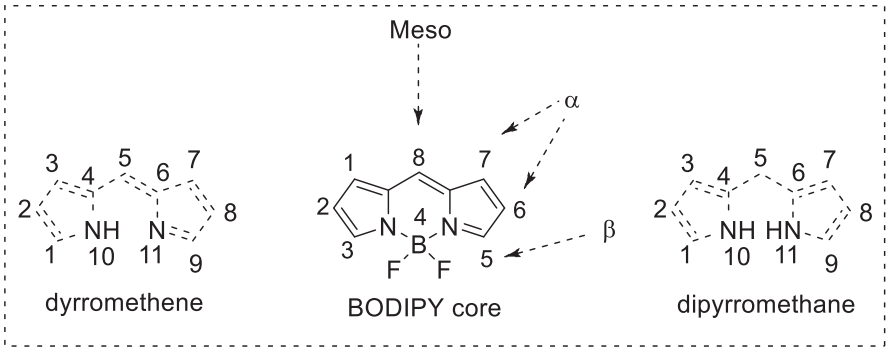


Figure 1.5. IUPAC numbering system of a boron dipyrrin compound

(Source: Wood and Thompson, 2007)

From the acylation reaction of 2,4-dimethylpyrrole with acetic anhydride dipyrromethane was prepared. Then, after the formation of dipyrromethene through the oxidation of dipyrromethane, a highly fluorescent dye molecule was synthesized in the presence of boron trifluoride diethyl etherate complex and a proper base (Treibs and Kreuzer, 1968).

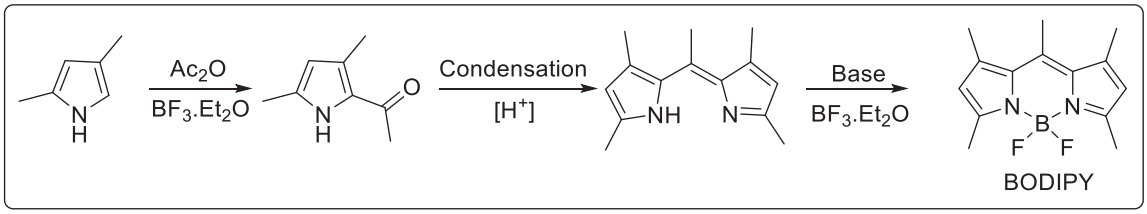


Figure 1.6. Serendipitioous discovery of BODIPY

(Source: Treibs and Kreuzer, 1968)

Due to the very favorable chemical and physical features of BODIPY dyes such as, high fluorescence quantum yields, high extinction coefficients, superior photostability, long emission and absorption bands in the visible region, they have a wide range of use. Mostly, they have been used for bio-imaging applications and chemical sensors as fluorescent probes (Boens et al., 2015; Ulrich et al., 2008). Incorporating nitrogen atom in the meso position of the BODIPY core comprise an aza-BODIPY structure which has long absorption wavelengths. Furthermore, BODIPY structure and its aza-analogue can be easily modified from the different reactive sites.

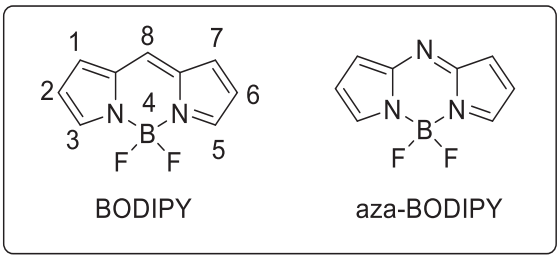


Figure 1.7. Chromophores of BODIPY and aza-BODIPY (Awauah and You, 2012)

Recently, there has been a great effort to synthesize BODIPY based photosensitizers for clinical PDTs. Since they have certain characteristics, including tunable excitation wavelengths, rapid cellular uptake, a robust resistance to light and chemicals, resistance to photobleaching, insensitivity to environments such as the pH and polarity of a given solvent, ease of functionalization and a favorable extinction coefficient. However, deprived of excited triplet states, which represent a key photophysical parameter necessary for generating singlet oxygen from the molecular oxygen, the unmodified BODIPY core remains photoinactive. Consequently, recent studies on BODIPY based photosensitizers have elegantly demonstrated that the controlled modification of a BODIPY core from the different positions could precipitate significance photophysical changes (Burgess et al 2013, Emrullahoğlu et al 2017).

In that context, Nagano, Akkaya, O'Shea and McClenaghan, as well as other researchers, have clearly proved that modifying a BODIPY core by placing heavy atoms (e.g., bromine or iodine) in appropriate positions can promote spin-orbit coupling, which allows for the ISC needed to observe triplet states. As alternatives to heavy atom-bearing BODIPY dyes, orthogonal BODIPY dimers and trimers have been reported by Akkaya et al. and Zhang et al. to be exceptionally effective photosensitizers for singlet oxygen generation. Conversely, Zhao et al, have identified BODIPY-C_{60} as a heavy atom-free organic triplet sensitizer that uses C_{60} as a spin converter.

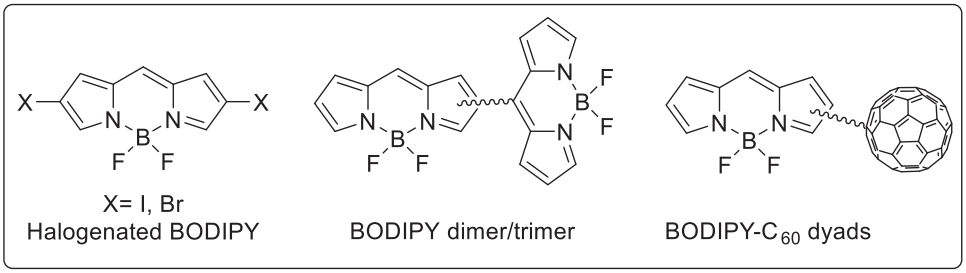


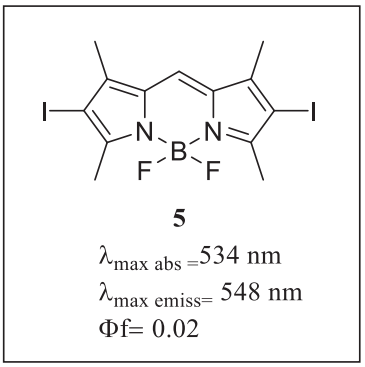
Figure 1.8. BODIPY-based PSs for singlet oxygen generation (Emrullahoğlu, 2017)

1.5. Literature Studies

In this section, literature examples about the BODIPY based photosensitizers for photodynamic therapy will be given.

1.5.1 Halogenated BODIPYs

The first BODIPY based photosensitizer was designed by Nagano et al. in 2005. They proved that the BODIPY fluorophore can be converted into a photosensitizer by introducing heavy atoms directly onto the structure without losing its desirable characteristics. For the observation of singlet oxygen generation, they use 1,3-diphenylisobenzofuran (DPBF) as a $^1O_2$ scavenger whose absorbance band decreases in the presence of singlet oxygen under light irradiation. Compared to Rose Bengal, is a novel photosensitizer, 2I-BODIPY showed high singlet oxygen generation in various environments, a low fluorescence quantum yield and high photostability. They also examined the activity of the compound in cells by using a cell imaging experiment. After a thirty-minute light exposure, 2I-BODIPY showed highlight to dark photocytotoxicity ratios on HeLa cells at low concentrations (Nagano et al., 2005).



5

$\lambda_{max\ abs}$ =534 nm

$\lambda_{max\ emiss}$= 548 nm

Φf= 0.02

Figure 1.9**.** Molecular Structure of diiodo-BODIPY

Arbeloa and his co-workers have designed iodinated BODIPYs by placing iodine atoms not only at 2,6 positions but also at 3,5 positions to increase ISC efficiency by heavy atom effect in 2012. Surprisingly, compound **8** gives rise to an increase in fluorescence. They used flash photolysis experiment to show that these molecules have a high stability under photobleaching resulting from the mono exponential decay of excited states. Additionally, high singlet oxygen quantum yield was observed in compounds (Arbeloa et al., 2012).

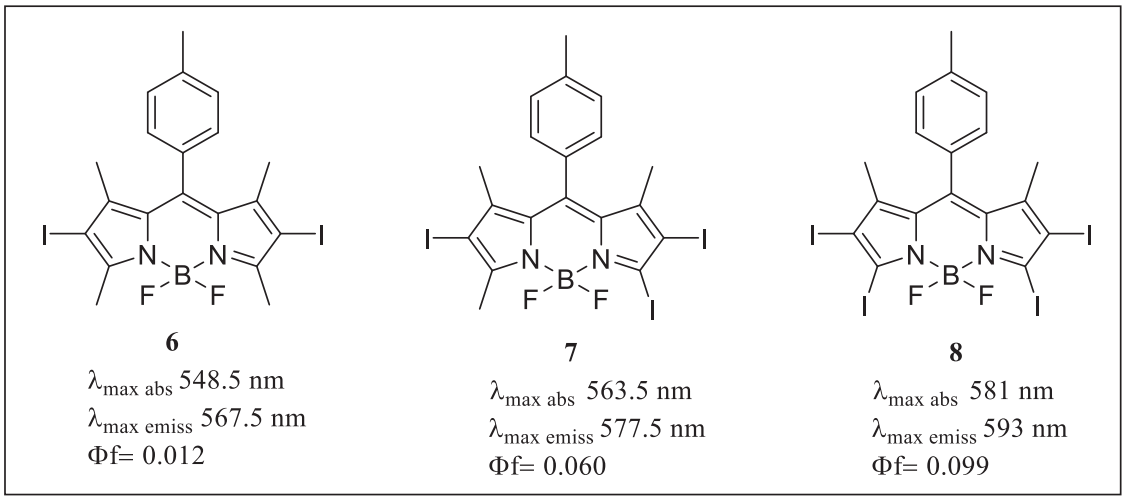


Figure 1.10. Molecular structures of halogenated BODIPYs

In 2017, Belfied and colleagues reported a new-halogenated BODIPY derivative bearing with iodine atoms to increase the transition from singlet state to triplet state. Additionally, they introduced a polyethylene glycol chain to the BODIPY core in order to increase hydrophilicity. The compound showed a low fluorescence quantum yield and a high ability to generate singlet oxygen as a result of direct measurement of near-infrared luminescence. Then they investigated the in vitro activities of the compound in Lewis Lung Carcinoma cells. After the MTT assay experiments, the compound exhibited high photocytotoxicity on living cells with IC_{50}= 10 μM. (Belfield et al., 2017)

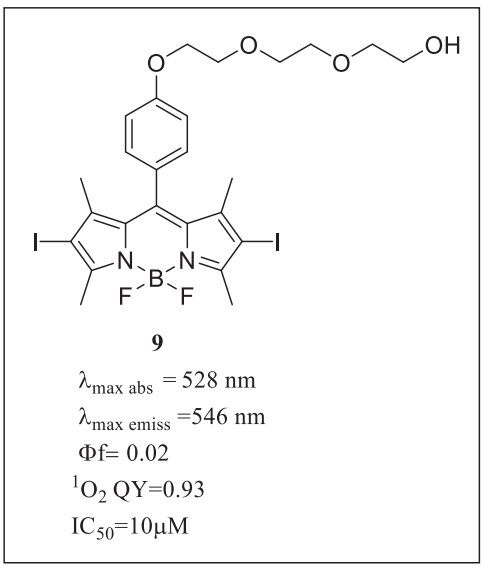
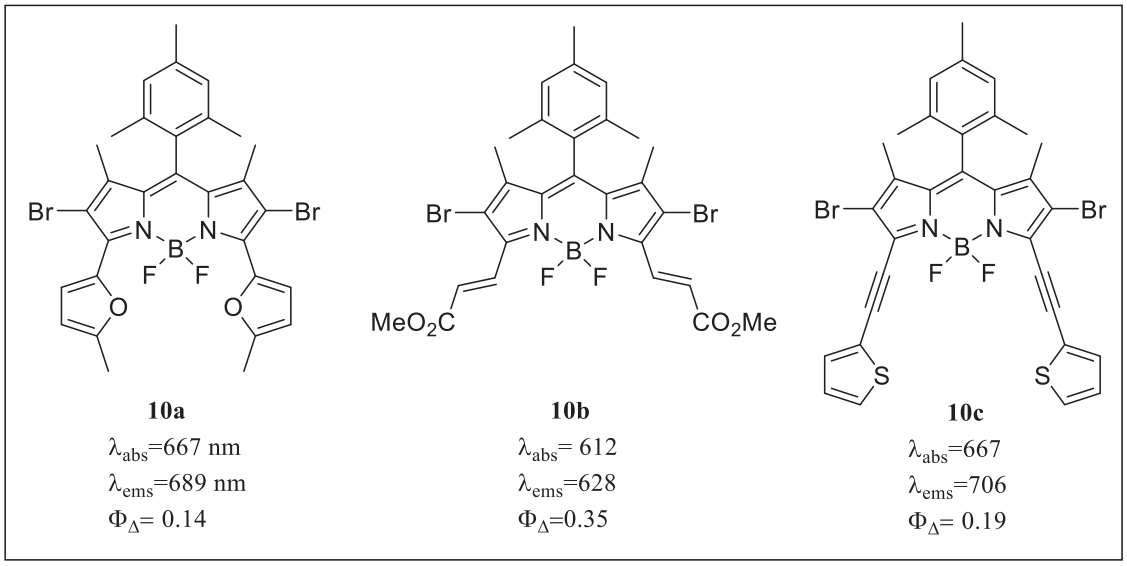


Figure 1.11. Molecular structure of BODIPY-based photosensitizer (9)

In 2016, Hao et al. reported facile, region-selective and stepwise synthesis of functionalized BODIPY dyes from a C-H arylation reaction with Sonagashira, Suzuki and Heck couplings. The resulting compounds displayed longer absorption and emission wavelengths as a result of the high π-conjugation capabilities. Then, in order to investigate the singlet oxygen efficiencies, they performed in-direct measurements by using the trapping method. According to their results the compounds have relatively high singlet oxygen generation based upon the enhanced ISC resulting from the bromine atoms. These dyes also adhered to the previously reported novel bromo- and iodo- containing BODIPYs.



10a
λ_{abs}=667 nm
λ_{ems}=689 nm
Φ_{Δ}= 0.14

10b
λ_{abs}= 612
λ_{ems}=628
Φ_{Δ}=0.35

10c
λ_{abs}=667
λ_{ems}=706
Φ_{Δ}= 0.19

Figure 1.12. Molecular structure of BODIPY-based brominated photosensitizers

An iodine atom which is heavier than bromine is more preferable for designing photosensitizers to increase the efficiency of the heavy atom effect. However, placing heavy atoms on a BODIPY core is not the only factor that increases singlet oxygen generation. To this end, Dong and colleagues designed halogenated BODIPY derivatives by connecting them with a benzene ring. Compound **11a** shows a higher singlet oxygen quantum yield compared to dimeric structures, most likely because of the interaction between the four iodine atoms and their dimeric configuration. Also, compound **11b** exhibits some cytotoxicity in dark environments which makes its IC_{50} value high (Dong et al., 2017).

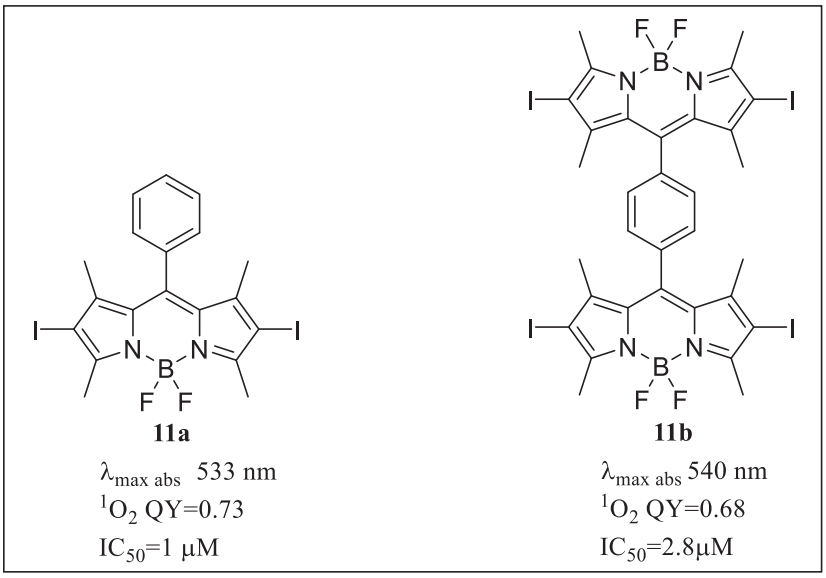


Figure 1.13. Molecular structure of BODIPY-based photosensitizers 11a and 11b

Different from the studies mentioned above, Akkaya and co-workers exhibit a water soluble BODIPY derivative by using a styryl moiety in 2006 (**12**). With the addition of styryl groups at 3 and 5 positions, the absorption band of BODIPY dye shifted to longer wavelength which is desirable for deep tissue penetration in PDT. Besides, placing amphiphilic triethyleneglycol group within a BODIPY core makes it water soluble. The compound shows sufficient singlet oxygen generation and no cytotoxicity in dark conditions. The EC_{50} value of the compound in K562 human erthroleukemia cells reported to be 200 nM (Akkaya et al., 2006).

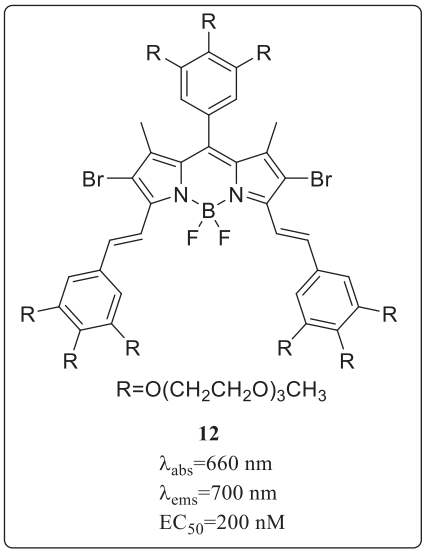


Figure 1.14. Molecular structures of halogenated distyryl-BODIPY

In PDT it is also crucial to use bio-targeting photosensitizers for singlet oxygen generation. For that reason, Wang and colleagues demonstrated two halogenated distyryl BODIPY derivatives whose absorption bands in the NIR region targeted and cleavage of DNA for the first time in 2012. The compounds exhibit low fluorescence quantum yield; however, their singlet oxygen quantum yield values are acceptable. By observing absorption titration, viscosity experiments and circular dichroism, the binding affinity of BODIPY dyes is proven. The compound **13a** generate reactive oxygen species with both Type I and Type II mechanisms which may lead to new ideas for designing more effective photosensitizers.

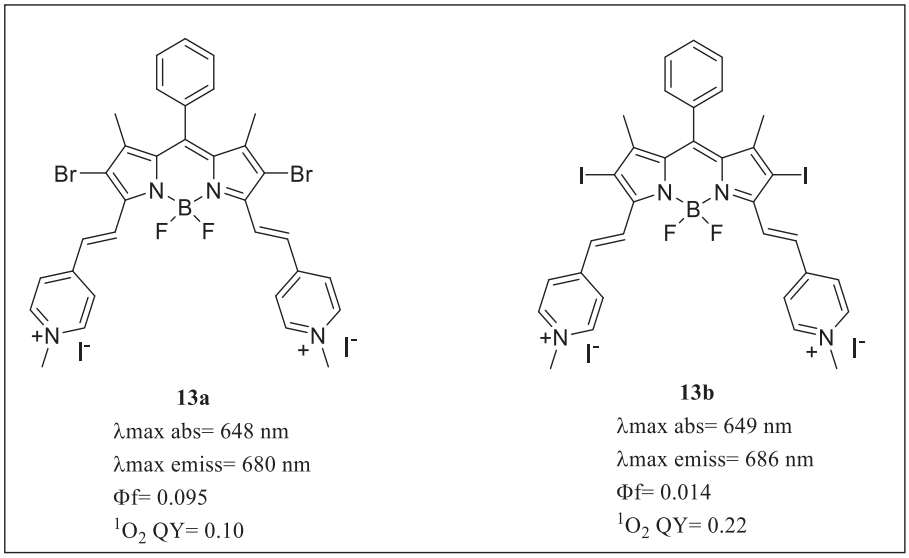


Figure 1.15. Molecular structures of BODIPY based bio-targeting PSs.

There were many studies using different derivatizations of BODIPY structures to increase π-conjugation thus shifting absorption maxima in the NIR region for deep tissue penetration under conditions such as the addition of styryl units in several positions and placing a nitrogen atom in a meso position to form aza-BODIPY.

In that context, Shen and co-workers designed a fused-ring expanded BODIPY dye by using two thienopyrrole units to prolong the π-conjugation system in 2013. Also in order to enhance ISC, bromine atoms were incorporated into the BODIPY structure. In this way, the resulting compound showed an absorption maxima at a longer wavelength of 698 nm. With high singlet oxygen quantum yield, the compound exhibited high cytotoxicity under light irritation and no toxicity in dark medium. An IC50 value was recorded as 7.12 μM in HeLA cells according to MTT assay results. In that case the compound can be used as a potential photosensitizer for PDT.

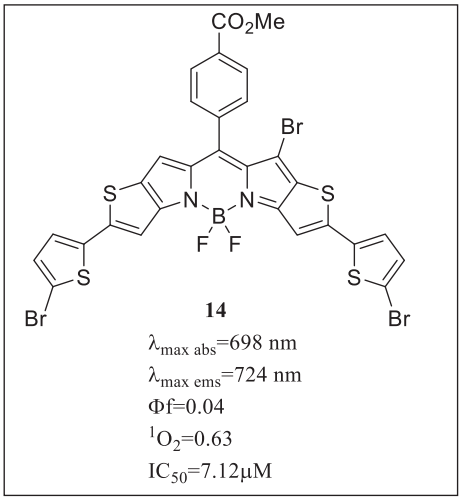


Figure 1.16. Molecular structure of the heteroaryl-fused BODIPY based photosensitizer

In 2017, Cosa and his colleagues reported two halogenated BODIPY based photosensitizers functionalizing with electron-withdrawing acetoxymethyl groups at meso positions in order to enhance the photostability towards singlet oxygen mediated photodegradation. They used both iodine and bromine atoms in order to make a comparison. Acetoxymethyl substitution at meso position improved photostability as relative efficiencies were found, Erel=91 for brominated and Erel=33 for iodinated while Erel=20 for BODIPY structure with hydrogen atom at the meso position. At this stage, the compounds showed both high singlet oxygen quantum yields and excellent photocytotoxicity against HeLa cells. In comparison with iodinated BODIPY, the brominated BODIPY structure displayed higher photostability and better cytotoxicity under light which has more potential for PDT.

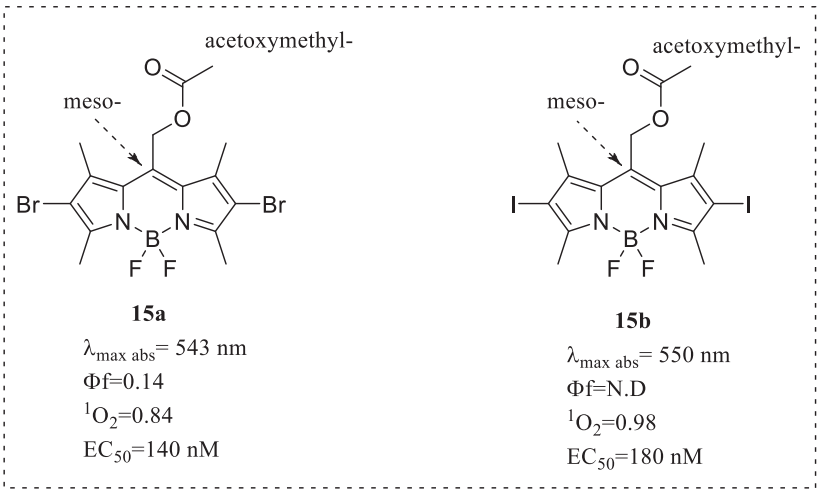
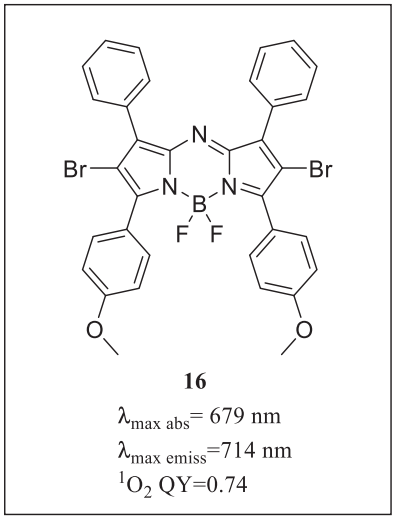


Figure 1.17. Molecular structures of acetoxymethyl-fused BODIPY based photosensitizers

Due to the great absorption bands in the NIR region, Aza-BODIPYs have great potential for designing photosensitizers. In 2004, O'Shea and co-workers synthesized an aza-BODIPY structure bearing two bromine atoms and donating a group para-oriented to the alkene. The compound shifted at longer wavelengths with the help of the aza structure and produced a great heavy atom effect with the help of bromine atoms. The EC_{50} value of the compound was recorded as 63 nM and 37 nM for HeLa and ADPM06 cell lines respectively with no cytotoxicity in the dark.



16

$\lambda_{max\ abs}$= 679 nm

$\lambda_{max\ emiss}$=714 nm

$^1O_2$ QY=0.74

Figure 1.18. Molecular structure of halogenated aza- BODIPY based photosensitizer

1.5.2 Non-halogenated BODIPYs

According to the "degrade" excited state characteristics of orthogonal BODIPY dimers and trimers, they have the ability to generate singlet oxygen via ISC. Since placing heavy atom within the BODIPY core slightly increases its toxicity in dark, orthogonal BODIPY dimers/trimers and BODIPY-C_{60} dyads are used as alternative photosensitizers in clinical PDT.

In 2016, Akkaya et al., reported that, the conversion of BODIPY dyes into approval photosensitizer is accomplishable by composing its orthogonal dimer and trimer derivatives without placing heavy atom to the molecule. Apart from that, connecting the orthogonal dimers to upconverting nanoparticles may shift their excitation wavelengths to 980 nm which increase the singlet oxygen efficiency. In this regard, they synthesized a new enlarged orthogonal BODIPY trimer by incorporating p-tert-butylbenzene group

at meso position. In order to detect singlet oxygen, they employed a trapping method by using DPBF singlet oxygen scavenger. The results showed that, the compound exhibits singlet oxygen generation with a quantum yield of 0.53 which makes it a feasible candidate as a potential photosensitizer.

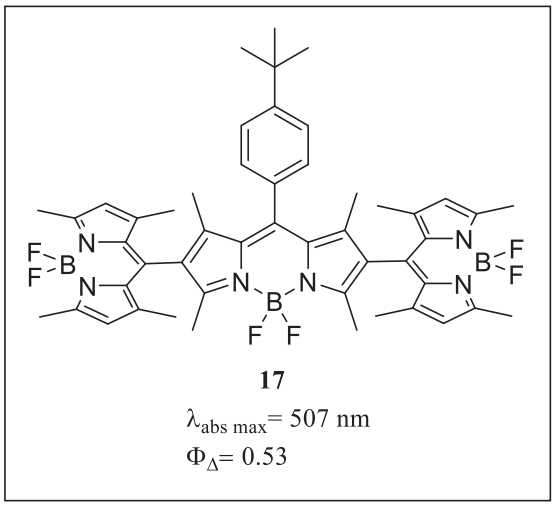


Figure 1.19. The molecular structure of orthogonal BODIPY trimer

In 2017, Ortiz et al. developed new orthogonal BODIPY dimers with different molds in order to observe the effect of solvent polarity on singlet oxygen generation. BODIPY dimer **19a** was designed by covalently linking two identical BODIPY core as the fundamental reference structure for other derivatives. Initially, Ortiz et al. studied the effect of solvent polarity on **19a** by using cyclohexane (as a non-polar solvent), chloroform (as a moderate polar solvent) and acetonitrile (as a polar solvent). The results showed that, compound **19a** have a higher singlet oxygen generation in chloroform. Then, they introduced both an electron donating $–NH_2$ group (**18b**) and an electron withdrawing $–NO_2$ (**18c**) group to the BODIPY-dimer skeleton. While compound **18b** exhibited higher singlet oxygen generation then **18a**, compound **18c** showed lower. In cell studies, due to photoactivity related to the corresponding photophysical parameters such as the strength of absorption at the irradiation wavelength and the ability to generate singlet oxygen species, they conducted their studies with compound **18a** and **18b**. They showed the highly lethal effect on HeLa cells at the same concentrations under light irradiation. However, although compound **18a** has a lower singlet oxygen quantum yield it exhibits higher cytotoxicity as EC_{50} value of 50 nM.

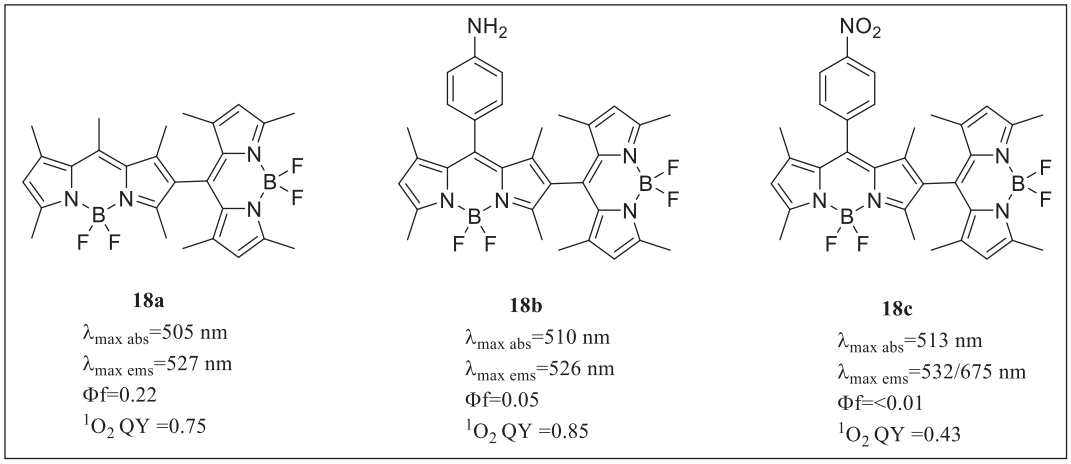


Figure 1.20. Molecular structures of orthogonal BODIPY dimers

In 2017, our research group developed a new chemical construct as an efficient singlet oxygen generator a BODIPY dye bearing with –LAu (I) (L=PPh3) as a potential spin converter (S1-T1). Incorporating this moiety into the alkynyl backbone of a BODIPY core increases the ISC due to the heavy atom effect of gold metal. BOD-Au exhibited high singlet oxygen quantum yield in terms of trapping method by using DBPF to trap singlet oxygen. While the absorbance of DPBF at 414 nm decrease steadily during the generation of singlet oxygen under the light irritation, the absorbance of the **BOD-Au** remains constant thus proving that the compound is photostable. According to these results, the photodynamic activity of the compound was explored in A549 human adenocarcinoma cells. The observations showed that, after the exposure of light, cell viability decreases even at low concentrations of **BOD-Au** with the EC_{50} value as 2.5 nM. Moreover it has no cytotoxicity under dark conditions up to 40 nM. With respect to these results, we have synthesized a potential photosensitizer different from the literature's examples of BODIPY based photosensitizers (Emrullahoğlu, 2017).

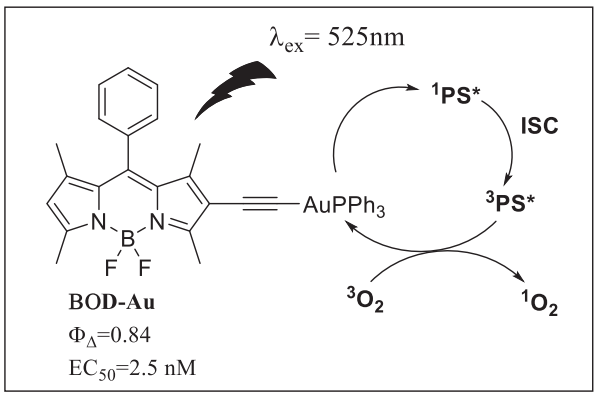


Figure 1.21. Molecular structure of BOD-Au photosensitizer

Another approach for BODIPY based photosensitizers relies on the integration of dyads with fullerene moieties. In this type of compound, BODIPY dye is used as a light harvesting unit and fullerene structure is utilized as a spin converter for the generation of singlet oxygen. In general, fullerene construction has a very low excited singlet state energy however, the help of the intramolecular charge transfer from the chromophore increases its population of S1, and thus hence its population of T1 by the means of its ability to generate ISC.

Thus, Zhao and co-workers designed a BODIPY-fullerene dyad to investigate its photodynamic activity. The styryl BODIPY-C_{60} derivative is designed as an organic triplet photosensitizer without halogens or any other heavy atoms. The compound displayed absorbance maxima at high wavelengths in the red region and long-term triplet excited states via a "ping-pong" intra-molecular energy transfer between the BODIPY structure and C_{60} dyad. Also, the singlet oxygen efficiency of the compound was found to be twenty-fold greater than an Ir(III) complex which is the novel triplet photosensitizer.

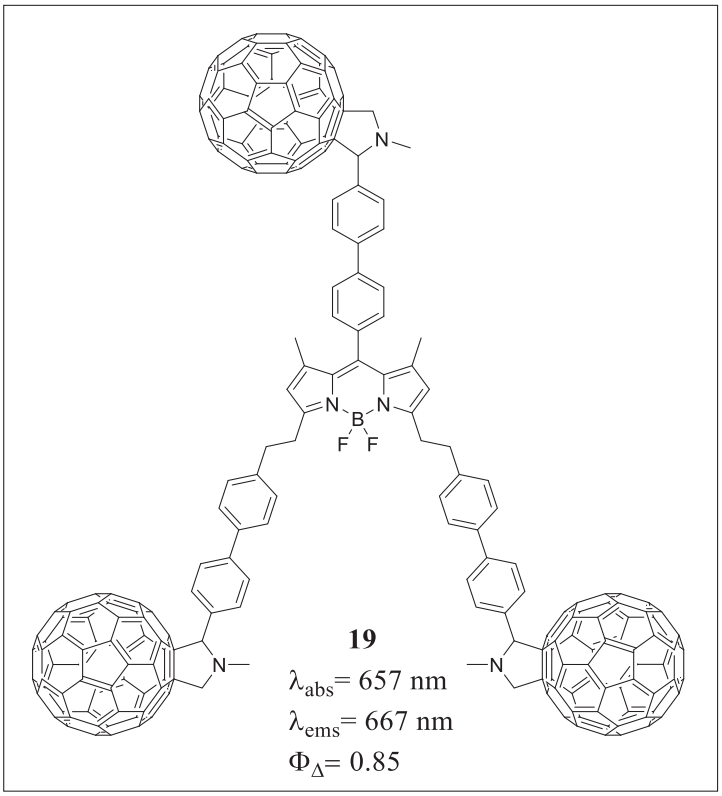


Figure 1.22. Chemical structure of BODIPY-C_{60} dyad

CHAPTER 2

EXPERIMENTAL STUDY

2.1. General Methods

All reagents were purchased from commercial suppliers (Aldrich and Merck) and used without any purification. ^{1}H NMR and ^{13}C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using Varian Cary Eclipse Fluorescence Spectrophotometer and samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). In singlet oxygen measurements 1,3-Diphenylisobenzofuran was used as a singlet oxygen scavenger and was purchased from supplier. Fluorescence lifetimes were measured by a time-correlated single photon counting (TCSPC) system using a Edinburgh FLS920 spectrophotometer with excitation wavelength at 480 nm for **DBBOD-1** and 500 nm for **DBBOD-2** (pulse width: < 200ps). The lifetime values were computed by the F900 software.

2.2. Determination of Quantum Yield

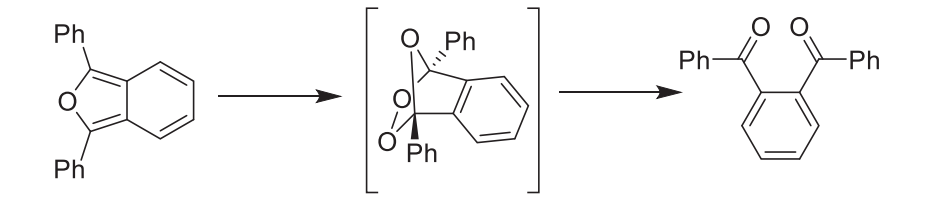
Fluorescence quantum yields of **DBBOD-1** and **DBBOD-2** were determined by using optically matching solutions of Rhodamine 6G (Φ_F=0.95 in ethanol) as a standard. The quantum yield was calculated according to the equation;

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

2.3. Singlet Oxygen Trap Experiments

For singlet oxygen measurements indirect method was performed. 1,3-Diphenylisobenzofuran (**DPBF**) was used as a singlet oxygen trap in DMSO and was purchased from a supplier. In a typical procedure for the detection of singlet oxygen generation by using trap molecules, **DBBO-1** (5 μM) and **DPBF** (25 μM) were mixed in O_2 bubbled CH_2Cl_2. Initially several dark measurements were taken followed by irradiation of the mixture at absorption maximum of a sensitizer. Absorbance decrease of **DPBF** was monitored suggesting singlet oxygen generation in the presence of light and **DBBOD-1**. Same procedure was performed for **DBBOD-2**. Measurements were performed using 525 nm LED (3.3 mW/cm^2) and samples were irradiated with the light source from a 15 cm distance.



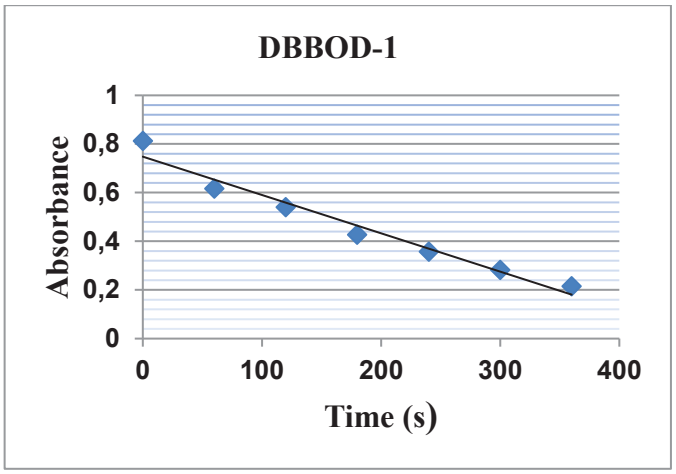
1,3-Diphenylisobenzofuran (DPBF)
λ_{abs}= 414 nm

1,2-dibenzoylbenzene

Figure 2.1. The reaction of DBPF with singlet oxygen

2.4. Singlet Oxygen Quantum Yield Calculations

Calculation of relative singlet oxygen quantum yields was carried out by following the literature . Relative singlet oxygen quantum yields were calculated by using **2I-BOD** as a reference whose singlet oxygen quantum yield is 0.79 in CH_2Cl_2. Singlet oxygen trap molecule (**DPBF**) and photosensitizers (**DBBOD-1 and DBBOD-2**) were placed into cuvette containing oxygen saturated CH_2Cl_2. Solution was kept in dark and mixed by micropipette until absorbance readings were stable. After stabilization, absorbance readings were recorded for 5 minutes by exposing cuvette to light (525 nm) for 60 seconds (from 15 cm distance). Absorbance readings of **DPBF** at its absorbance maxima were recorded against time and recorded graph is given below.



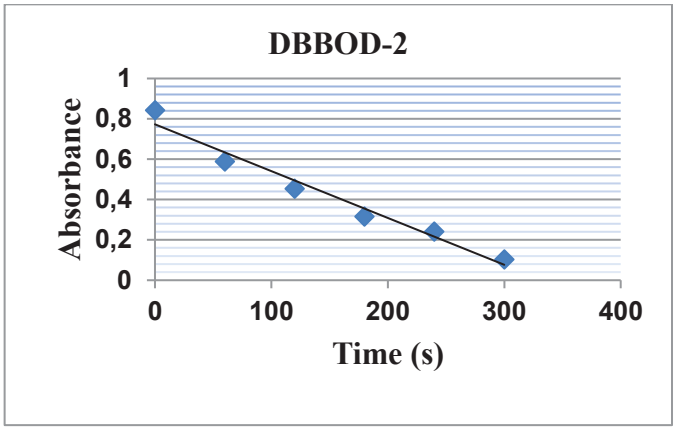


Figure 2.2. Relative singlet oxygen quantum yield experiment. Absorbance decrease of DPBF (25 μM) at absorbance maxima with time in CH_2Cl_2 in the presence of compound **DBBOD-1** (5 μM) and **DBBOD-2** (5 μM) as photosensitizers

Singlet oxygen quantum yields were calculated according to the equation:

ϕ_Δ (PS) = ϕ_Δ(R) x m(PS)/ m(R) x F(R)/ F(PS) x PF(R)/ PF(PS)

where PS and R designate photosensitizers (**DBBOD-1** and **DBBOD-2**) and reference dye (**2I-BOD**) respectively. "m" is the slope of difference in change in absorbance of **DPBF** at absorbance maxima with the irradiation time. F is the absorption correction factor, which is given as $F=1-10^{-OD}$ and PF is absorbed photonic flux.

2.5. Cell Viability

A549 Human Lung Adeno carcinoma cells were seeded in 96-well plates at a density of 1×10^4 cells per well with 100 μL culture and incubated for 24 hours at 37 °C under 5%

CO_2. After the overnight incubation, cell medium was removed and replaced with complete medium supplemented with varying concentration of **DBBOD-1** and **DBBOD-2**. The experimental group of the cells were illuminated with a yellow light source (525 nm, distance between light source and cells: 15 cm) for 60 min. Then cells were replaced new media and incubated another 24 hours. At the end of incubation periods, medium of the cells were removed and cells were washed by pre-warmed phosphate buffered saline (PBS) to remove any trace of compounds and to prevent color interference during optical density (OD) determination. MTT solution (0.5 mg/mL in PBS) was added into each well and incubated for 4 hours. After the incubation time plates were centrifuged at 1800 rpm for 10 minutes at room temperatures to avoid accidental removal of formazan crystals. Crystals were dissolved with 100 µL DMSO. The absorbance was determined at 540 nm.

2.6. Synthesis Section

The synthesis pathway for **DBBOD-1** and **DBBOD-2** were shown in Scheme 1. **BODIPY**, **BODIPY-AL**, **BODIPY-DI-AL**, **DBBOD-1** and **DBBOD-2** were synthesized by using literature procedure.

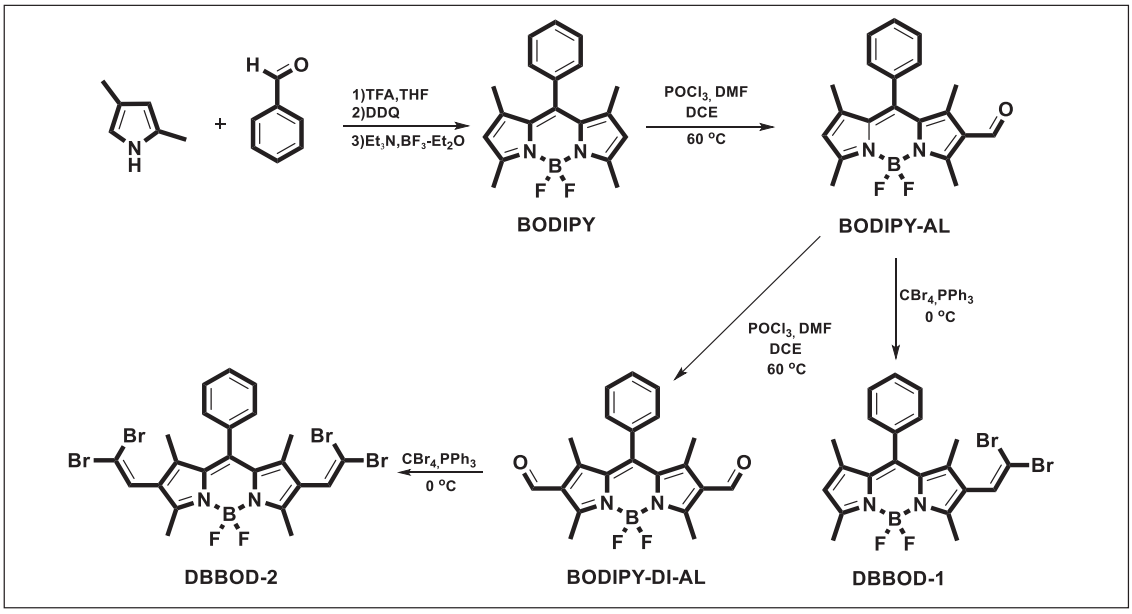
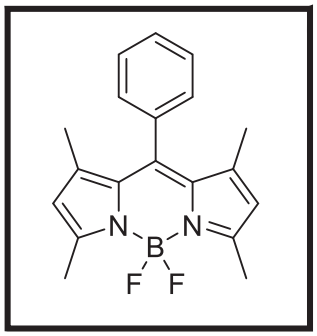


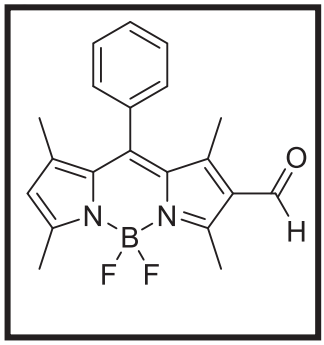
Figure 2.3. Stepwise synthesis of **DBBOD-1** and **DBBOD-2**

2.6.1. Synthesis of BODIPY



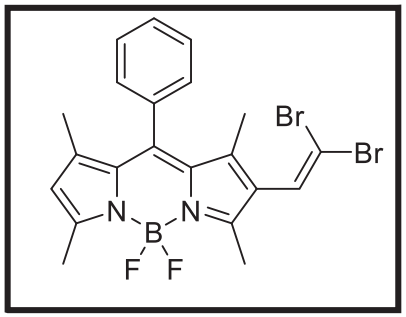
BODIPY dye was synthesis according to literature procedure (Sauer et al., 2012). To a solution of 2,4-dimethylpyrrole (4 mmol, 411 µL) in 25 ml dry THF, benzaldehyde (2 mmol, 200 µL) and 60 µL trifluoroacetic acid were added under argon atmosphere. The color of solution became light brown. After 16 hours, DDQ (2 mmol, 255 mg) was dissolved in 15 ml of dry THF and added to reaction medium by using dropping funnel in ice. The resulting mixture was stirred for 4 hours. Then 12 ml of triethylamine was added dropwise in ice and the reaction was stirred further 45 minutes. Finally, 13 ml of $BF_3.Et_2O$ was added by using dropping funnel in ice and the reaction was left overnight. At the end of reaction solvent was removed under reduced pressure. Then, extracted with dichloromethane (3x30 ml) and dried over $MgSO_4$. Solvent was evaporated in and the resultant compound purified by column chromatography (%40 yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49-7.47 (m, 3H), 7.28-7.26 (m, 2H), 5.98 (s, 2H), 2.55 (s, 6H), 1.37 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.4, 143.2, 141.7, 135.0, 129.1, 128.9, 127.9, 121.29, 15.0, 14.3.

2.6.2. Synthesis of BODIPY-AL



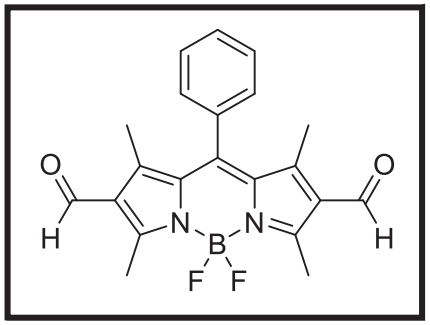
BODIPY-AL was synthesized by using literature procedure (Isik et al., 2013). The first step of reaction includes the formation of Vilsmeier Haack reagent via the dropwise addition of $POCl_3$ (250 µL, 1 mmol) into the dry DMF (250 µL, 3.6 mmol). The mixture was stirred for 40 min at room temperature under argon atmosphere. Then, the solution of **BODIPY** (81 mg, 0.25 mmol) in DCE was added drop by drop and the mixture stirred overnight at 60ºC. The reaction was quenched with the addition of 100 mL of ice cooled-$NaHCO_3$ solution and extracted with CH_2Cl_2. Combined organic phases were dried over $MgSO_4$ and they were purified with column chromatography to afford **BODIPY-AL** as orange solid (60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.0 (s, ^{1}H), 7.53-7.51 (m, 3H), 7.28-7.26 (m, 2H), 6.15 (s, 1H), 2.81 (s, 3H), 2.61 (s, 3H), 1.64 (s, 3H), 1.41 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 185.9, 161.6, 156.4, 147.3, 143.6, 142.9, 134.1, 129.5, 127.7, 126.3, 124.0, 15.1, 14.8, 13.0, 11.6.

2.6.3 Synthesis of DBBOD-1



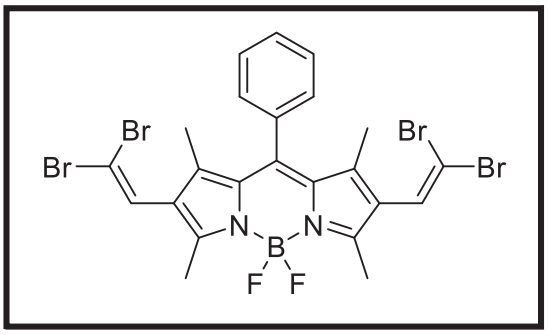
DBBOD-1 was synthesized by using literature procedure (Rao et al., 2015). In the first part of the reaction, to a carbontetrabromide (0.34 mmol, 2 equivalents) in 0.35 ml dry DCM, **BODIPY-AL** (0.17 mmol, 1 equivalent) in 0.35 ml DCM was added dropwise at 0 ^{o}C very slowly. Then, triphenylphosphine (0.68 mmol, 4 equivalents) was dissolved in 0.35 ml dry DCM and added to reaction medium at 0 ^{o}C. After addition, cooling bath was removed and the reaction was stirred at room temperature for 3 hours. Color of the reaction turned from dark orange to dark purple. After the completion of reaction, extraction was done with dichloromethane (3x 30 ml) and dried over $MgSO_4$. Solvent was evaporated in and the resultant compound purified by column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 (s, 3H), 7.28 (s, 2H), 7.11 (s, 1H), 6.01 (s, 1H), 2.56 (s, 3H), 2.52 (s, 3H), 1.37 (s, 3H), 1.29 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ:157.22 , 157.02 , 152.24 , 144.40 , 142.11 , 139.14 , 134.75 , 130.67 , 129.18 , 127.85 , 121.96 , 94.47 , 14.72 , 14.48 , 13.77 , 13.17.

2.6.4 Synthesis of BOD-DI-AL



BODIPY-DI-AL was synthesized by using literature procedure (Yin et al., 2016). The first step of reaction includes the formation of Vilsmeier Haack reagent via the dropwise addition of POCl3 (250 μL, 1 mmol) into the dry DMF (250 μL, 3.6 mmol). The mixture was stirred for 40 min at room temperature under argon atmosphere. Then, the solution of **BODIPY-AL** (81 mg, 0.25 mmol) in 1,2-dichloroethane was added drop by drop and the mixture stirred overnight at 60^{o}C. The reaction was quenched with the addition of 100 ml of ice cooled-$NaHCO_3$ solution and extracted with CH_2Cl_2. Combined organic phases were dried over $MgSO_4$ and they were purified with column chromatography to afford **BODIPY-DI-AL** as orange solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 10.06 (s, 2H), 7.60 (s, 3H), 7.31 (s, 2H), 2.89 (s, 6H), 1.71 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 185.55,160.75, 148.39,147.26, 133.58,133.55, 131,87, 130,18, 130.16, 129.91, 129.88, 128.10, 127.39, 127.36, 13.80, 12.03.

2.6.5 Synthesis of DBBOD-2



DBBOD-2 was synthesized by using literature procedure (Rao et al., 2015). In the first part of the reaction, to a carbon tetrabromide (0.496 mmol, 4equivalents) in 0.35 ml dry DCM, **BODIPY-AL** (0.124mmol, 1 equivalent) in 0.35 ml DCM was added dropwise at 0^{o}C very slowly. Then, triphenylphosphine (0.992 mmol, 8 equivalents) was dissolved in 0.35 ml dry DCM and added to reaction medium at 0 ^{o}C. After addition, cooling bath was removed and the reaction was stirred at room temperature for 3 hours. After the completion of reaction, extraction was done with dichloromethane (3x 30 ml) and dried over $MgSO_4$. Solvent was evaporated in and the resultant compound purified by column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 – 7.50 (m, 3H), 7.32 – 7.27 (m, 2H), 7.11 (s, 2H), 2.53 (s, 6H), 1.29 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.02 , 142.63 , 140.36 , 134.55 , 131.26 , 130.31 , 129.40 , 128.50 , 127.76 , 94.98 , 13.92 , 13.37 , 1.02.

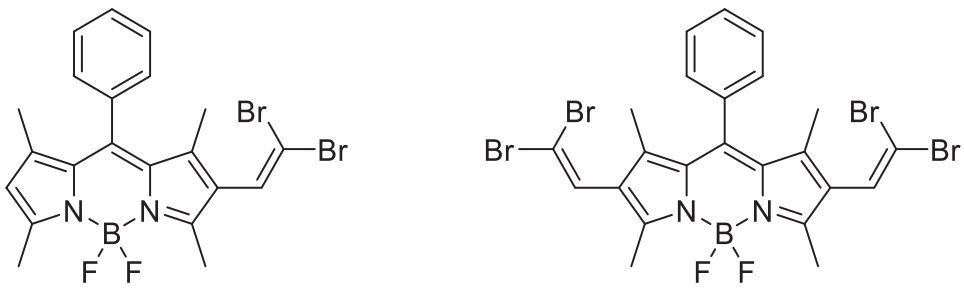
CHAPTER 3

RESULTS AND DISCUSSION

3.1. General Perspective

As mentioned in the first section, PDT is an encouraging treatment method for several types of cancer. In contrast to other therapies, the photodynamic action is more controllable due to its capacity to localize target areas and its lower degree of damage to non-targeted tissues. Furthermore, the selectivity of this method is ensured by exposing only cancerous tissues to this light. The effectiveness of photodynamic action depends on the cytotoxic effects on specific tissues via high singlet oxygen generation. Therefore, the efficiency of singlet oxygen generation is related to the features of the photosensitizers that make them an important component of photodynamic therapy.

In this study, we designed and synthesized new photosensitizers, **DBBOD-1** and **DBBOD-2,** and evaluated their potential for use as cytotoxic agents in PDT. These photosensitizers were constructed by introducing a dibromoethylene unit into the backbone of the BODIPY skeleton, with the expectation of preparing new BODIPY fluorophores with high efficiencies in generating singlet oxygen within the therapeutic window. Heavy atoms such as bromine are then introduced into a BODIPY scaffold in order to promote spin orbital coupling, which allows for the ISC necessary to observe excited triplet states. BODIPY was selected as the light-harvesting chromophore not only for its outstanding photophysical properties but also for its ease of chemical modification.



DBBOD-1 **DBBOD-2**

Figure 3.1. Chemical Structures of **DBBOD-1** and **DBBOD-2**

3.2. Spectroscopic Measurements

We then performed a detailed study by measuring the photophysical and spectroscopic properties of the photosensitizers and investigated their potential as cytotoxic agents in PDT. The performance of **DBBOD-1** and **DBBOD-2** in generating singlet oxygen was systematically compared with a reference photosensitizer (e.g. **2Br-BOD**) previously synthesized and investigated in the literature (Gotor et al, 2018). Furthermore **2Br-BOD** is known to exhibit low singlet oxygen generation, which markedly limits its use as a photosensitizer in PDT.

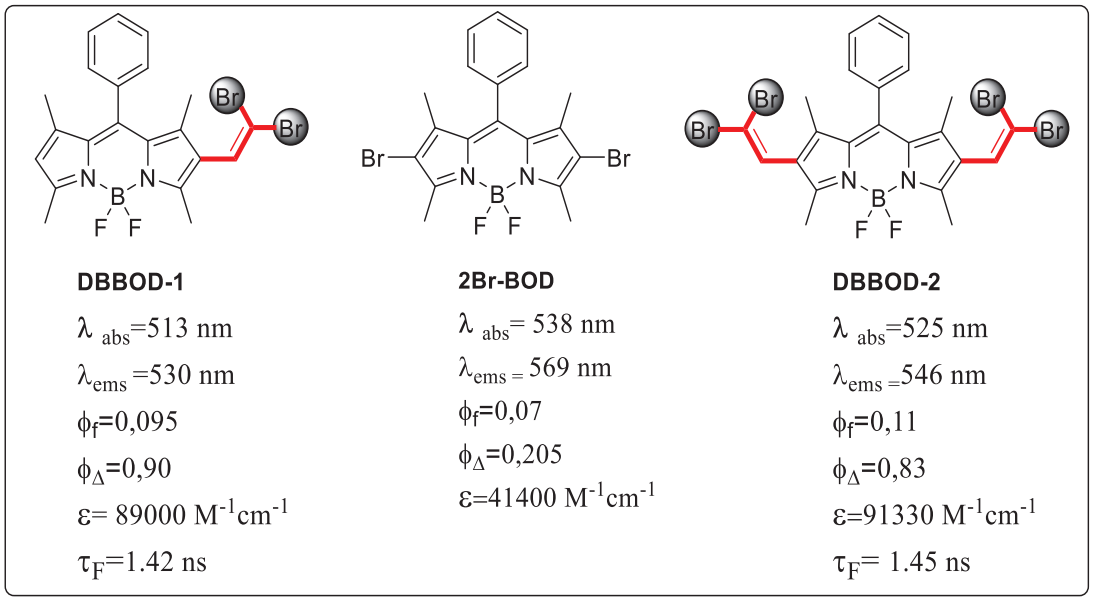


Figure 3.2. Photophysical properties of **DBBOD-1**, **DBBOD-2** and **2Br-BOD**

Figure 3.2 outlines a comparison of the photophysical parameters off the newly synthesized photsensitizers with reference to **2Br-BOD**. As shown, **2Br-BOD** has a relatively low fluorescence quantum yield along with a low efficiency in generating singlet oxygen (e.g. singlet oxygen quantum yield), whereas the compounds that were synthesized displayed remarkably high singlet oxygen quantum yields **DBBOD -1** and **DBBOD-2** (0.90 and 0.83 respectively). Additionally, both of these compounds, despite their low fluorescence quantum yields, have a noticeable fluorescence emissions that also make them also good candidates for in- vivo imaging applications.

The UV-visible spectra of **DBBOD-1** and **DBBOD-2** showed absorption bands in the range of 510-560 nm. The absorption band of **DBBOD-1** with the mono dibromoethylene group was centered at 513 nm. On the other hand, the presence of a

second dibromoethylene group in the BODIPY core induced a moderate bathochromic shift of the absorption band of **DBBOD-2** to 525 nm which clearly indicated an increment of the π-conjugation. Emission spectra of the two BODIPY derivatives **DBBOD-1** and **DBBOD-2** (5 uM in CH_2Cl_2) induced the aspect of recognizable emissions in the 530-555 nm range with low fluorescence quantum yields and brief fluorescence lifetimes (Φ_F= 0.095, τ_F=1.42 for **DBBOD-1** and Φ_F=0.11, τ_F=1.44 for **DBBOD-2**). The small value of fluorescence quantum yields and short time of fluorescence lifetimes could be attributed to ISC efficiency from an excited singlet state to an excited triplet state via a heavy atom effect raised by the halogens linked to the BODIPY structures.

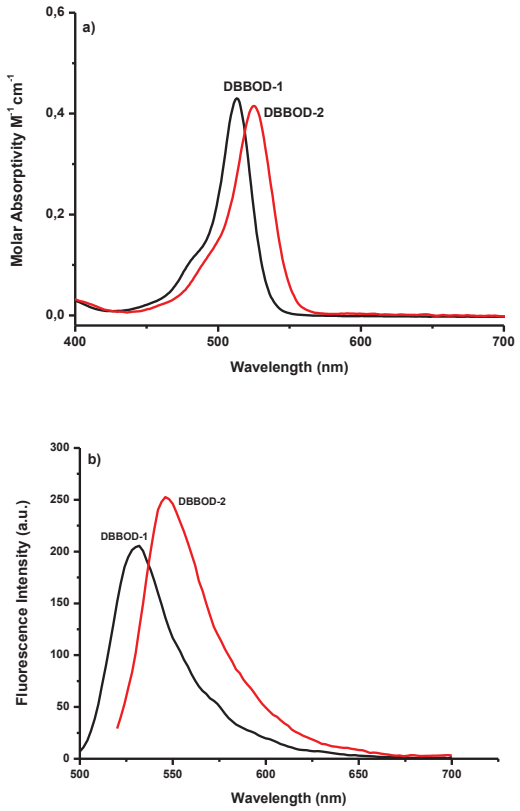


Figure 3.3. a) UV-vis spectra of DBBOD-1 (5μM) and DBBOD-2 (5μM) in CH_2Cl_2;
b) Emission spectra of DBBOD-1 and DBBOD-2 in CH_2Cl_2
(ʎex: 480 nm for DBBOD-1 and ʎex: 500 nm for DBBOD-2)

After spectroscopic measurements, the ability to generate singlet oxygen of **DBBOD-1** and **DBBOD-2** was investigated via the trapping method. For this purpose, 1,3-diphenylisobenzofuran was used as a scavenger of $^1O_2$ whose absorbance spectra at 415

nm decreases gradually in the presence of singlet oxygen resulting from the reaction between singlet oxygen and a trapping molecule, yielding 1,2-dibenzoylbenzene.

In control experiments, the absorption band of a DPBF (25 µM) solution was monitored for 15 minutes under dark conditions and no significant change was observed in the absorbance of DPBF. Thus, measurements were performed in different solvents in order to observe the best result for singlet oxygen generation efficiency. Solutions of compound **DBBOD-1** (5 µM) in DCM, DMF, EtOH and CH_3CN that sensitized dissolved oxygen were prepared and then mixed with DPBF (25 µM). The photo degradation of DPBF was evaluated under light irradiation a distance of 15 cm distance from green LED (525nm, 3.3mW/cm^2) lights. The results showed that, the best decay in absorbance band of DPBF was observed in CH_2Cl_2 among other solvent systems. We observed no signs of photo degradation in the absorption band of the compound under light irradiation whereas the absorbance of DPBF at 415 nm decreased remarkably and disappeared entirely within a couple of minutes which clearly confirms its prolific ability to generate singlet oxygen and its high resistance to photo bleaching.

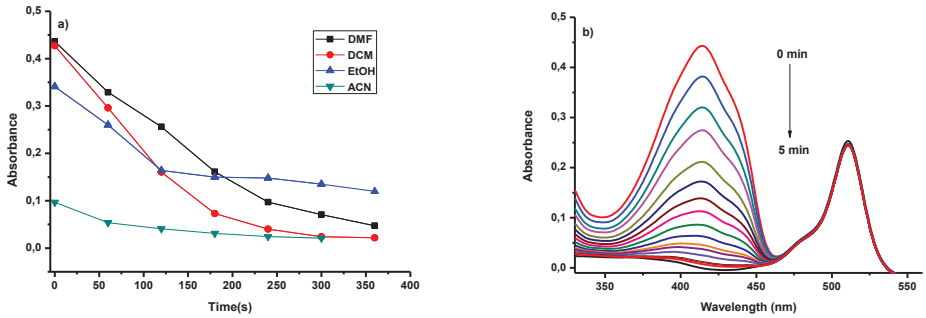


Figure 3.4. a) The absorbance degradation of DPBF (25 µM) in different solvents in the presence of **DBBOD-1** (5µM). b) Singlet oxygen mediated bleaching of DPBF (25 µM) in the presence of **DBBOD-1** (5 µM) in CH_2Cl_2.

Since the structural features of the compounds are similar, in order to verify that **DBBOD-2** can generate T1 states via ISC, the trapping method was repeated in the same solvent system (CH_2Cl_2) with **DBBOD-1**. As shown in Figure 3.5., there were no signs of photodegradation in the absorption band of **DBBOD-2** under light irradiation. However, the absorbance of the trapping molecule reduced remarkably in a short span of time, which clearly indicates that the compound has high singlet oxygen generation efficiency and a high resistance to light.

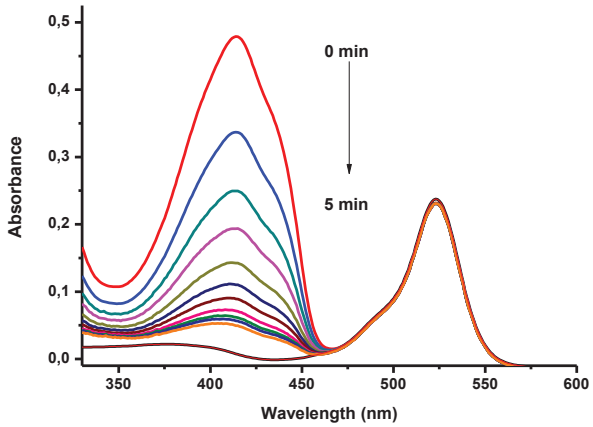


Figure 3.5. Singlet oxygen mediated bleaching of DPBF (25 μM) in the presence of **DBBOD-2** (5 μM) in CH_2Cl_2.

3.3. Cell Studies

Encouraged by these observations, we investigated the photocytotoxic activity of the promising sensitizers **DBBOD-1** and **DBBOD-2** against A549 human lung adenocarcinoma cell lines. An MTT assay which is a colorimetric process includes the reduction of a yellow tetrazole to a purple formazan for measuring the activity of enzymes, was used to evaluate cytotoxicity.

First, the photodynamic activity of **DBBOD-1** was investigated in A549 cells both in the absence and presence of light. First, the cancer cell line was loaded with an increasing doses of **DBBOD-1** (2.5, 5, 20, 40, 80, 160 and 200 nM).One group of cells that was protected from the light throughout the incubation process showed relatively no decrease in viability up to 200 nM, which demonstrates the great cellular biocompatibility of the sensitizer. Another group of cell lines was irritated with green light at 525 nm at a 3.3 mW/ cm^2 fluence rate for 60 minutes. For these cells illuminated by green light, we observed a notable reduction in viability, even at very low concentrations of **DBBOD-1** with an EC_{50} value of 20 nM.

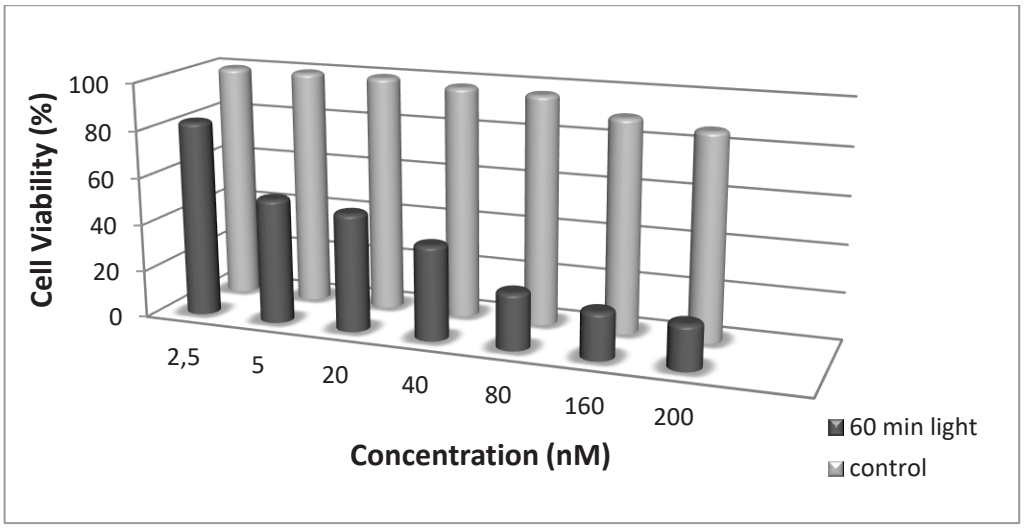


Figure 3.6. Cell viability of A549 cells after treatment with **DBBOD-1** at different concentrations. Control group was incubated only with the cell culture medium.

Furthermore, in order to examine the effect of change in light dosage, cells treated with 80 nM **DBBOD-**1 was exposed to light (green LED, 525 nm, 3,3 W/cm^2) for 30 minutes. Results apparently showed that the extent of light illumination is critical to the photodynamic efficiency of the photosensitizer.

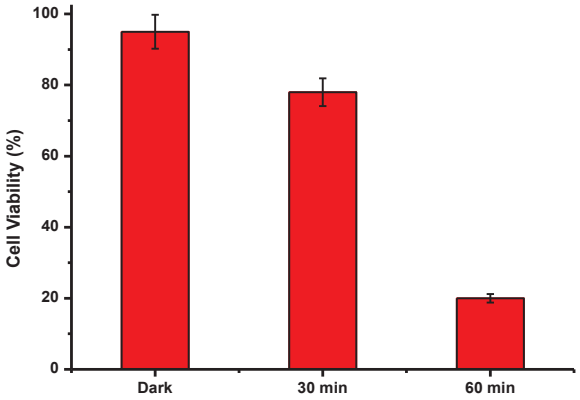


Figure 3.7. Effect of light dose on cell viability (**DBBOD-1**, 80 nM)

The in vitro photodynamic activity of **DBBOD-2** was demonstrated against A549 cells by performing an MTT assay to determine if the compound can be used as a PDT agent. First, dark cytotoxicity with a **DBBOD-2** concentration ranging from 5 nM to 320 nM and photoinduced cytotoxicity of the photosensitizer were assessed with an MTT assay. Cell viability remained over 70 % until 80 nM in darkness and after illumination with a

light dose of 3.3 W/cm^2 for 60 minutes, at which time, approximately 70% of the cells seemed to be killed which indicates the effective light induced cytotoxicity of the sensitizer.

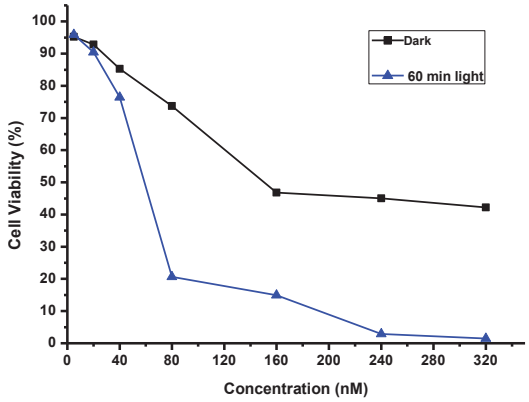


Figure 3.8. Percent cell viability with A549 cells kept in full medium at 37 ºC in an incubator, in the presence of varying concentrations of **DBBOD-2** under 60 min irradiation with green LED

We also examined the effect of light dosage on singlet oxygen generation by exposing cells treated with 80 nM of **DBBOD-2** to light for 30 minutes. As shown in figure 3.9. when the light exposure time increases, cell death increases proportionally. Cytotoxicity studies reveal that although **DBBOD-2** is moderately cytotoxic in the absence of light due to the high number of heavy atoms in its structure, it displays high photocytotoxicity with an EC50 value of 60 nM.

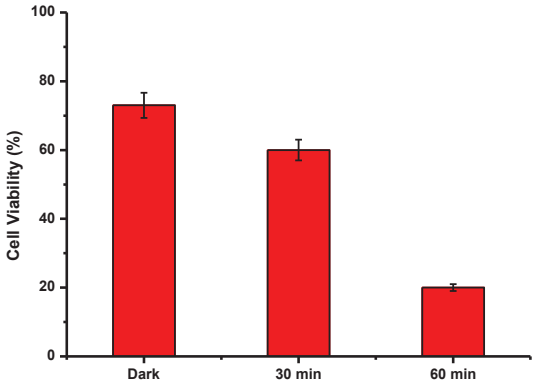


Figure 3.9. Effect of light dose on cell viability (**DBBOD-2**, 80 nM)

CHAPTER 4

CONCLUSION

New photosensitizers comprising a BODIPY dye as a visible light harvesting chromophore were developed as therapeutic agents in photodynamic therapy. By introducing dibromoethylene moiety to the BODIPY skeleton, a photoinactive BODIPY dye was transformed into a highly photo active triplet sensitizer.

Differing from the body of literature, heavy atoms were connected to the BODIPY core by using alkene group as a bridge. The compounds **DBBOD-1** and **DBBOD-2** exhibit remarkably higher singlet oxygen generation with low quantum yields than the novel photosensitizer **2Br-BOD** which makes them potential candidates for photodynamic therapy. The compounds displayed desirable photophysical properties, including high photostability, high extinction coefficients, high molar absorptivity and high singlet oxygen generation efficiencies with singlet oxygen quantum yields as 0.90 and 0.83 respectively.

In keeping with its high singlet oxygen generation efficiency, **DBBOD-1** showed excellent biocompatibility under dark conditions and photocytotoxic activity against cancer cells under light irradiaton with a low EC_{50} value of 20 nM. According to the photophysical properties of **DBBOD-2**, the singlet oxygen measurements showed that too many heavy atoms cannot effectively increase the generation of singlet oxygen, rather, a high dark toxicity of the photosensitizer would be detected on cancer cells. The results were obtained by performing an MTT assay on A549 cells. **DBBOD-2** has low cytotoxicity in dark even at low concentrations. Still it induced a nearly 70 % percent of cell death with an EC_{50} value of 60 nM.

In conclusion, both in vivo and in vitro studies demonstrate that **DBBOD-1** and **DBBOD-2** can effectively cause cell death under light irradiation with no dark cytotoxicity thereby they can be a guide for designing new photosensitizers. **DBBOD-1** and **DBBOD-2** or their derivatives could be feasible therapeutic agents in PDT for the treatment of certain types of cancers in future studies.

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APPENDIX A

^{1}H-NMR AND ^{13}C-NMR SPECTRA OF COMPOUNDS

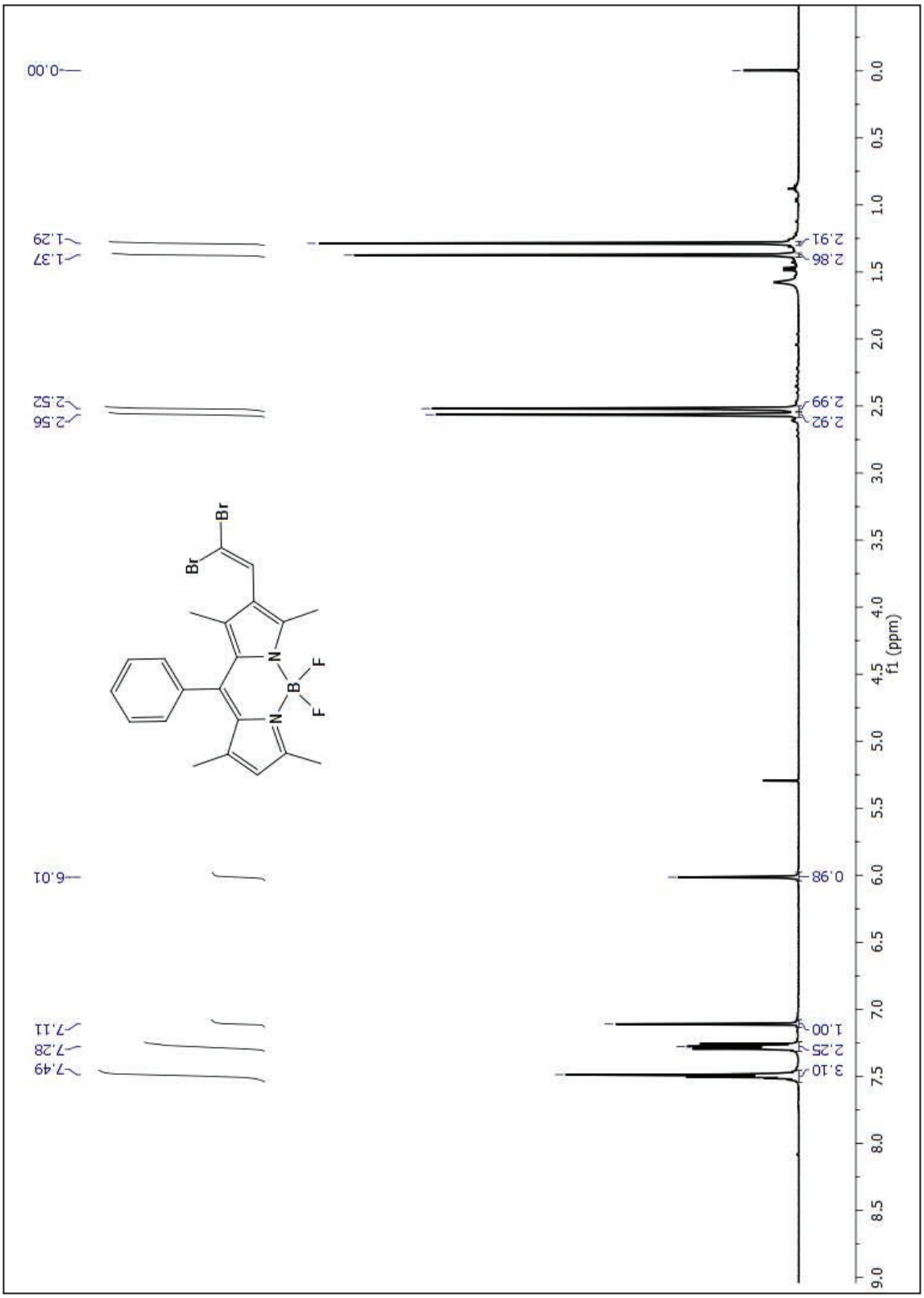

Figure A.1. ^{1}H NMR of **DBBOD-1**

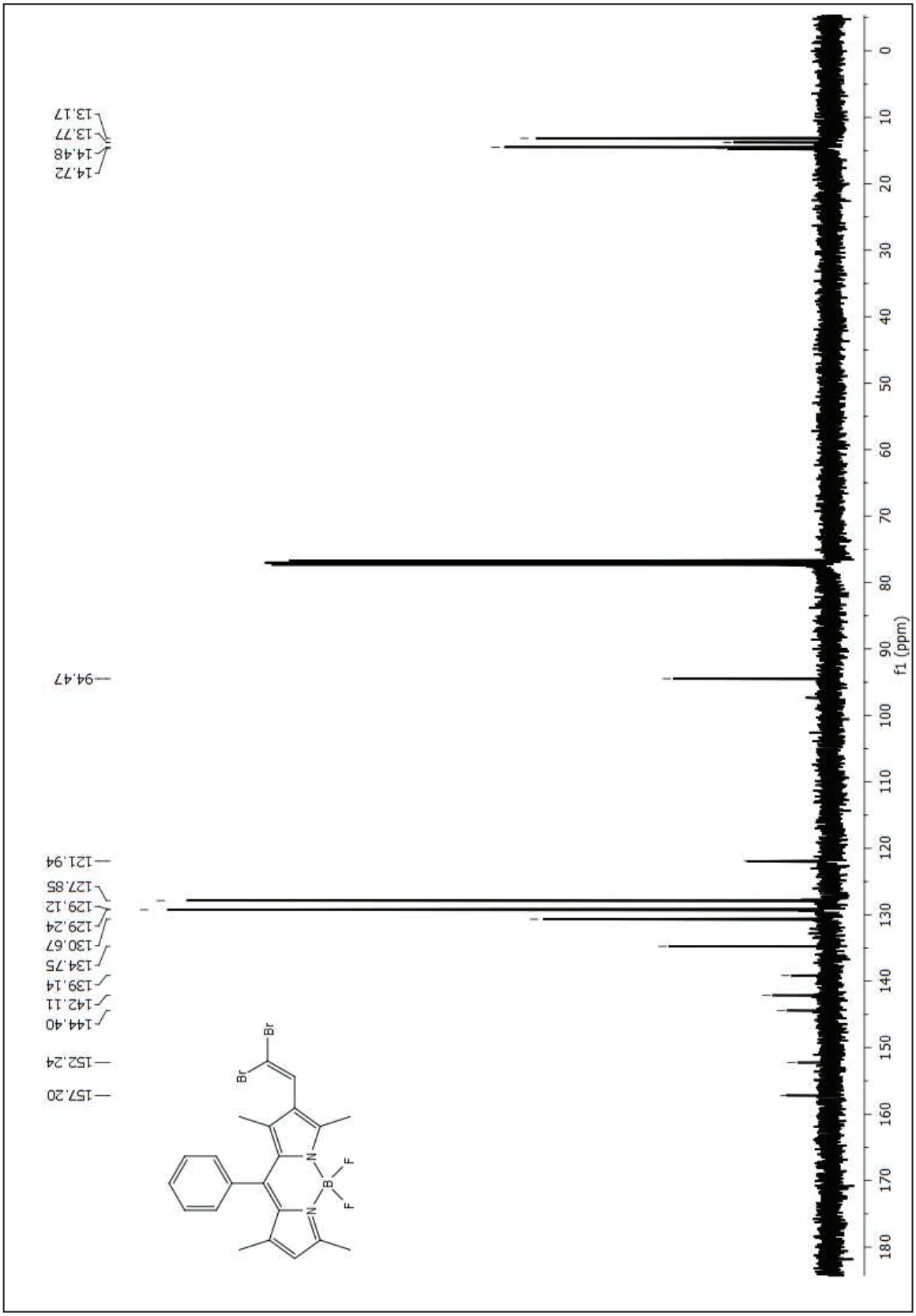

Figure A.2. ^{13}C NMR of **DBBOD-1**

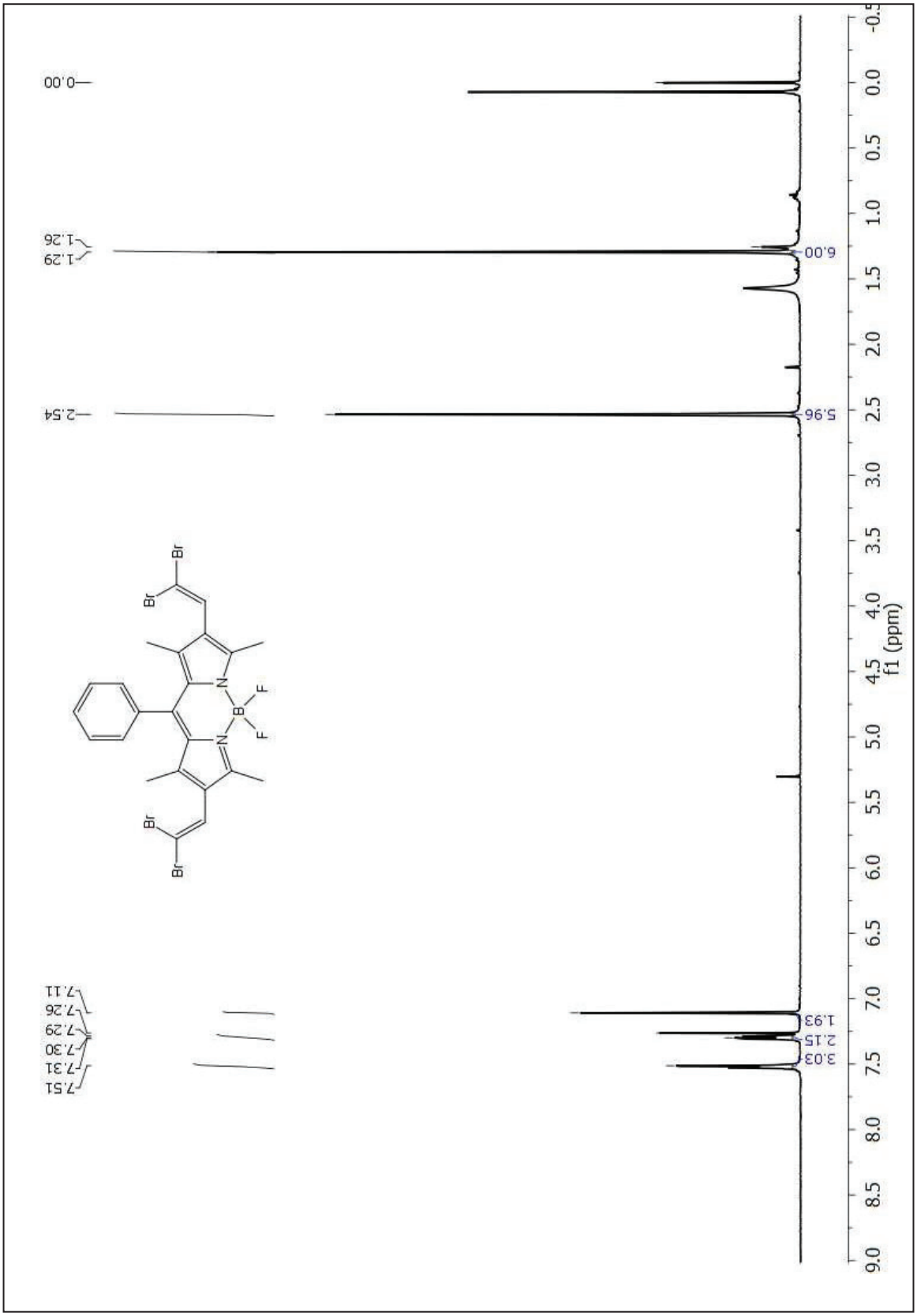

Figure A.3. ^{1}H NMR of **DBBOD-2**

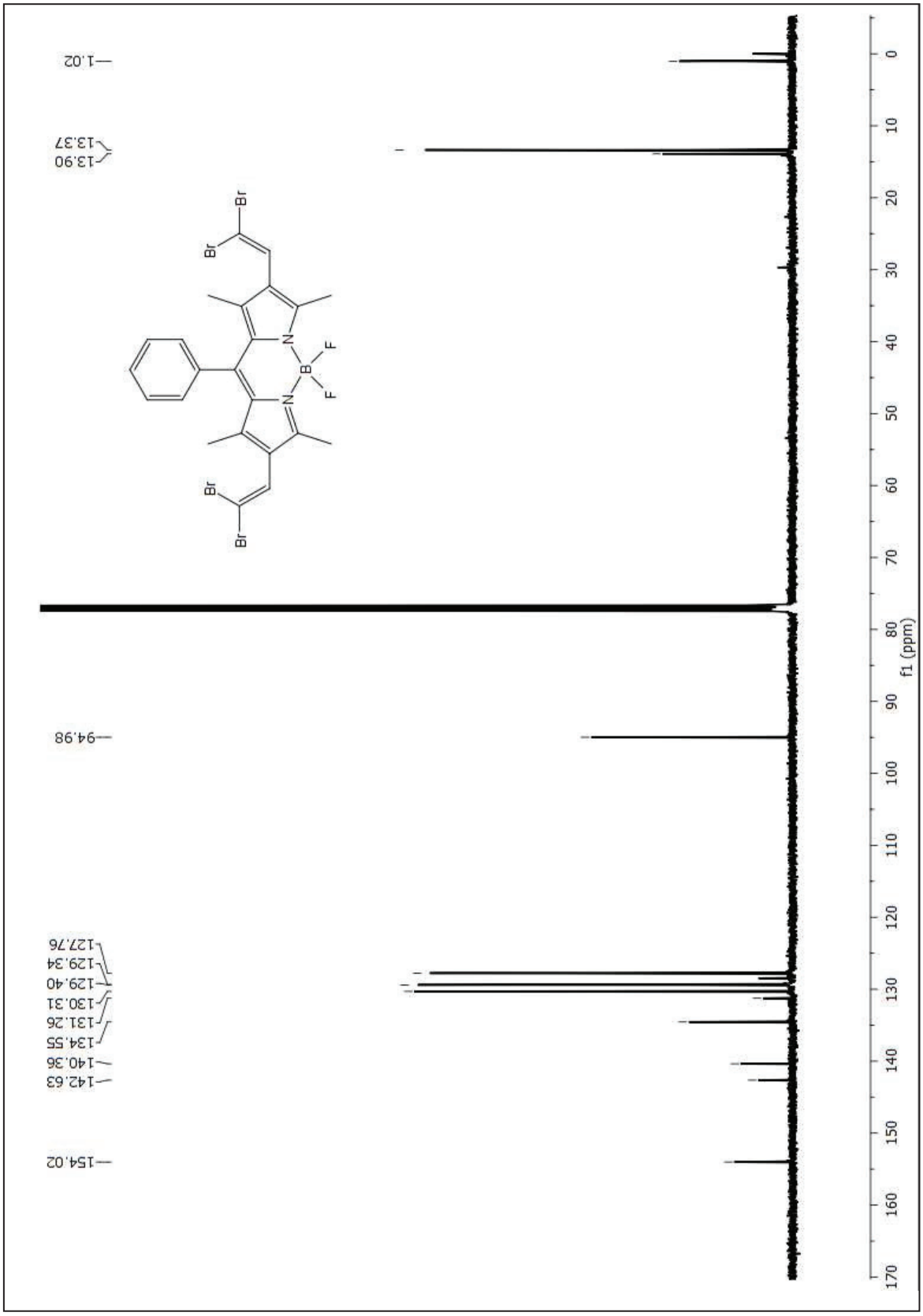

Figure A.4. ^{13}C NMR of **DBBOD-2**