

CHIRAL BODIPY DYES & PHOTSENSITIZERS FOR
PHOTODYNAMIC THERAPY AND DYE SENSITIZED SOLAR
CELLS

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DOCTOR OF PHILOSOPHY

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February, 2013

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ABSTRACT

CHIRAL BODIPY DYES & PHOTSENSITIZERS FOR PHOTODYNAMIC THERAPY AND DYE-SENSITIZED SOLAR CELLS

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PhD in Materials Science and Nanotechnology

Supervisor: Prof. Dr. Engin U. Akkaya

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Bodipy is a molecule with many superior properties. After its discovery in 1968, most of the features were not recognized until mid 1990s. Thereafter, many research papers and patents have been produced and the number of publications and citations is still on the rise today. An important fraction of the research done with this fluorophore is in chemosensing field to probe various analytes including anions, cations and even biomolecules. However, in this research we have focused on different areas of subjects and tried to find novel applications for these dyes. First, we designed orthogonal bodipy dimers for efficient triplet photosensitization without heavy atoms in contrast to most other sensitizers and efficient singlet oxygen generation was achieved ($\Phi_{\Delta}=0.51$). In the second project, calix[4]arene molecules were designed and synthesized as carriers for photodynamic therapy, potentially behaving as a molecular basket carrying the agents to the tumor tissues. Later, we focused on obtaining axial chiral molecules by using solely bodipy dyes, and we were able to obtain enantiopure fragments were separated by using chiral HPLC. These rare molecules are desirable for modern biological labeling and advanced optoelectronic devices. Finally, we designed bodipy dyes for dye sensitized solar cells by adapting relevant functional groups, and following synthesis work, we constructed cells to assess the design parameters via measuring the electrical output results.

Keywords: Photodynamic therapy, triplet photosensitization, axial chirality, dye sensitized solar cell, singlet oxygen

ÖZET

KİRAL BODIPY BOYALARI & FOTODİNAMİK TERAPİ VE BOYA DUYARLILAŞTIRILMIŞ GÜNEŞ PİLLERİ İÇİN FOTODUYARLILAŞTIRICILAR

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Bodipy üstün özelliklere sahip olan bir moleküldür. 1968 yılında keşfedilmesinden sonra 1990'lı yılların ortalarına kadar özelliklerinin büyük bir kısmı bilinmiyordu. Sonrasında ise, oldukça fazla sayıda bilimsel makale ve patent üretilmiştir, bugün bile bu boya ile ilgili makale sayısı ve atıf sayısı hızlı artmaktadır. Bu madde ile yapılan çalışmaların büyük kısmı anyonları, katyonları ve hatta biyomolekülleri algılama da kullanılmaktadır. Fakat, bu araştırmada biz bodipy molekülünün en iyi olmadığı fakat iyi olma potansiyeli olan diğer alanlara yoğunlaştık ve bodipy'yi bu alanlara uygulamaya çalıştık. İlk olarak bir çoğunda olduğunun aksine ağır atom içermeyen dik bodipy dimerleri dizayn ettik ve etkili singlet oksijen üretimi elde ettik ($\Phi_{\Delta}=0.51$). Bu konu altındaki ikinci projede kaliks[4]aren moleküllerini, fotodinamik terapi ajanları taşıyacak ve moleküler sepet gibi davranan hedefli bir ünite olarak tasarladık. Sonra, sadece bodipy moleküllerinden oluşan eksensel kiralite özelliği olan moleküllere yoğunlaştık ve enantiyosaf parçaları kiral HPLC kullanarak ayırmayı başardık. Bu nadir görülen maddeler modern biyolojik etiketlemede ve gelişmiş optoelektronik aygıtlar için talep edilmektedir. Son olarak, başarısı kanıtlanmış fonksiyonel grupları bodipy molekülüne uygulayarak boya duyarlılaştırılmış güneş pilleri için boyar madde tasarladık ve sentez ve pil yapımı aşamalarından sonra elektrik çıktı sonuçlarını ölçerek dizayn parametrelerini değerlendirdik.

Anahtar kelimeler: Fotodinamik terapi, triplet fotoduyarlılaştırma, eksensel kiralite, boya duyarlılaştırılmış güneş pili, singlet oksijen

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LIST OF ABBREVIATIONS

Bodipy	: 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
CASSCF	: Complete active-space self consistent field methodology
cheno	: Chenodeoxycholic acid
DSSC	: Dye-sensitized solar cell
DS-TR	: Doubly Substituted-Tetra radicalic
DFT	: Density functional theory
ISC	: Intersystem crossing
FRET	: Förster Resonance Energy Transfer
HOMO	: Highest Occupied Molecular Orbital
LUMO	: Lowest Unoccupied Molecular Orbital
MALDI	: Matrix-Assisted Laser Desorption/Ionization
MS	: Mass Spectrometry
NMR	: Nuclear Magnetic Resonance
NOON	: Natural Orbitals and Occupation Numbers
PDT	: Photodynamic Therapy
PS	: Photosensitizer
SS	: Singly substituted
SOMO	: Singly occupied molecular orbital
TLC	: Thin Layer Chromotography

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CHAPTER 1

1 INTRODUCTION

It is very well known that bodipy dye is an important tool with wide application areas in biology, chemistry and physics and intersection of these sciences. When searched bodipy as a topic totally 6385 articles is found in Web of Science Citation report. The first publications have been published in 1990 and the second one in 1993, and now the publication number and the citation number increases efficiently. Hence, it means that this type of dyes will be popular also in the future studies at least a few decades.

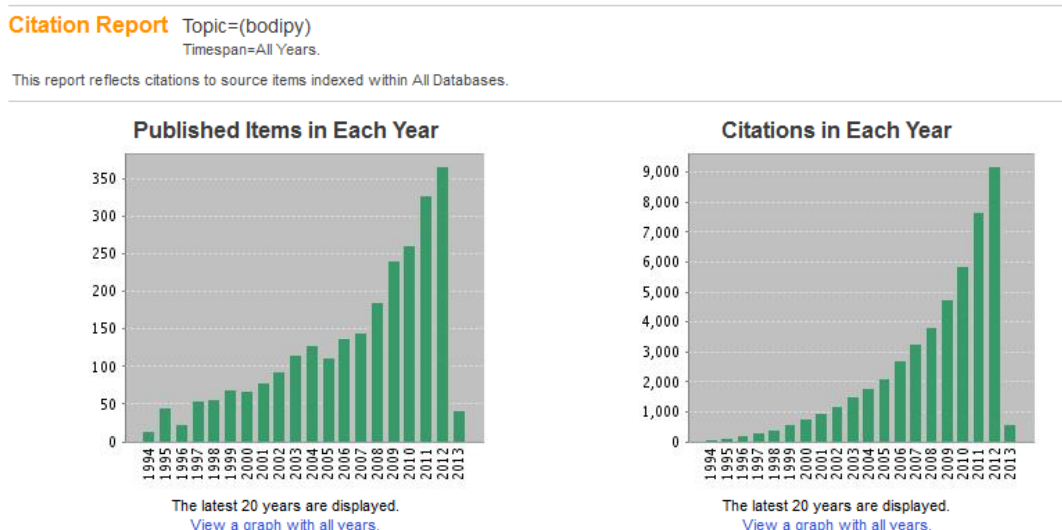


Figure 1. Web citation report of the number of research papers and the citations when searched in the topic part “Bodipy”.

Although the first bodipy molecule synthesized is back to 1968 by Treibs and Kreuzer after this date to mid 1990s the significance has not been recognized. After this date there has been a dramatical increase in the patent number also with the increase in the number of research fields that bodipy has been applied to. These include; biological labeling, electroluminescent devices, tunable laser dyes, candidate for solid-state solar concentrators, fluorescent switches and fluorophores

in sensors and labels. In addition, in recent years, these dyes were studied in photodynamic therapy and dye sensitized or polymer solar cell concepts too. These latter subjects have been studied after 2005 and our group has been working on these extensively.

What make these dyes special are their superior properties compared to its analogues. The properties are robustness against light and chemicals, high molar extinction coefficients, high fluorescence quantum yields, low triplet-state formation, high emission peak intensities with narrow bands, good solubility in common organic solvents, resistance towards self-aggregation in solution, visible region excitation and emission, and nanosecond fluorescence lifetime.¹ Another important thing is its ease of functionalization, can be tuned well according to the type of application. For instance, low triplet state yield can be increased by incorporation of heavy atoms. Due to its rich chemistry around the core, many research groups have been studying the use of these dyes for the purpose of their application. Our aim is parallel to this thought and we aimed to employ this excellent dye accordingly in three fields of research; in triplet-state photosensitization and photodynamic therapy (PDT), in atropisomeric chiral dyes and in dye-sensitizer-solar-cell fields.

To begin with by starting from the Chapter 3, we tried to put some novelty to the triplet-state photosensitizers (PS) used up to now. Triplet state PSs was mainly used in photodynamic therapy concept but it has found application also in other fields like photoinduced catalytic H₂ production from water. It has been known that attaching heavy atoms like bromine and iodine to a molecule increases its spin-orbit coupling and leads to increased population of triplet states and this strategy has been employed in various bodipy dyes also and successful results has been obtained. On the other hand, mechanism of triplet state population is an attractive field since it has not been exploited much before (e.g. the mechanism of singlet oxygen production of metal-free porphyrin is not known yet, although it is a biomolecule). Therefore, we have designed a molecule and inspected its photophysical properties including

triplet-state population efficiency and observed that orthogonally arranged two bodipy molecules can produce singlet oxygen upon reaction with ground state triplet oxygen upon irradiation with light through population of its triplet excited state without having a heavy atom! A series of dimeric orthogonal bodipys has been synthesized, their mechanism of intersystem crossing has been evaluated, and their successes in producing singlet oxygen hence killing the malignant cancerous cells *in vitro* were studied thanks to the collaboration with the active scientists in the field.² In the second study in this field, we designed a photosensitizer this time synthesized with heavy atoms, where calix[4]arene molecule has been used as a carrier scaffold. Although, we have not utilized this molecule *in vitro* assays, the mode of design and novelty in the synthesis of the molecule is thought to be useful in more elegant PSs including a targeting group for the malignant tissues in the body.

In Chapter 4, I have been focused on the axial chirality concept which has been studied very limited with bodipy dyes. In this project, we have been developed the first atropisomeric bodipy dyes formed solely from bodipy units. Fluoregenic chiral molecules have been desired for applications such as the determination of the enantiomeric purity of chiral biomolecules like amines, amino acids, and alcohols. In these assays, binaphthol based compounds have been used in real applications and in order to widen the range of the compounds used and shifting the working wavelength from UV to visible region, Bodipy fluorophores have been employed. In an elegant study by Ziessel *et al.*, asymmetric chiral molecules have been synthesized by employing four different ligands around the boron atom.³ In our project, we achieved several chiral molecules and two of them have been studied extensively by separating them in chiral high-performance liquid chromatography, and we could observed sets of enantiomers that are able to rotate the direction of the incoming polarized light to different directions in circular dichroism spectroscopy. The detailed scope of this project has been given in Chapter 4.

In the last project, I have been involved in dye-sensitized solar cell (DSSC) concept. The first DSSC has been proposed in 1991 and up to now it has been

studied extensively by several research labs including large global companies but the desired efficiency has not been attained compared to the efficiencies of silicon based solar cells. Due to the high cost of production and difficult purification steps silicon solar cells' proliferation seems not probable in the future. Hence, as cheap and easy synthetic methods make DSSCs or polymer solar cells an important candidate for future energy platforms in condition that the overall efficiency has been increased to closer values for monocrystalline solar cells. During my research period, I have been involved in DSSC applications by using bodipy as a photosensitizer dye and we achieved the highest efficiency in DSSC concept with bodipy dyes with 2.46%.⁴ Although this value is low compared to the recently found efficiency of 12.3% with porphyrin dyes co-sensitized with another dye⁵, bodipy dyes believed to be a future prospecting dye due to its advanced properties stated above. In addition, we should consider the progress in porphyrins because in the first application of these molecules on DSSCs in 1993 the efficiency have been found to be 2.6% and any significant development could be done to 2005 and after this date it was increased up to 7.1% in 2007.⁶ In the project I have been involved recently, we attached thiophene units as anchoring groups to TiO_2 and used novel highly bulky donor groups in order to inhibit dye aggregation on semiconductor TiO_2 surface which is known to decrease the of photon-to-electricity conversion efficiency. Thiophene molecule is used frequently in DSSC concept due to its high π electron conjugation ability and conductivity so it was attached to the 8- position of the bodipy which is known to be the most convenient position for electron injection to the semiconductor oxide conduction band.

CHAPTER 2

2 BACKGROUND

2.1 *Photodynamic Therapy*

2.1.1 *General Information*

Photodynamic therapy (PDT) is a method which has been found about a century ago in order to cure carcinogenic tissues. It is an alternative method to traditional chemotherapy and radiotherapy. It is well-known that latter two methods are highly toxic and result in side effects throughout the body. Photodynamic therapy, however, is a method which uses harmless components while curing. In PDT, there are three components: light, photosensitizer and singlet oxygen. Unlike to radiotherapy in which highly energetic light is used, undistruptive low energetic visible/near infrared is used. The second component is the photosensitizer (PS), this is an organic molecule and it is an important parameter of the method. PS is used to form singlet oxygen from the ground state triplet oxygen available in the region of interest which causes cell damage in the region of illumination by reacting with. Hence oxygen is the other component. During therapy, photosensitizer is excited with light and an electron passes to the forbidden triplet state in some special conditions (intersystem crossing) and interacts and transfers energy to ground state molecular triplet oxygen to yield singlet oxygen (Figure 2, details of this mechanism is in section 2.2).

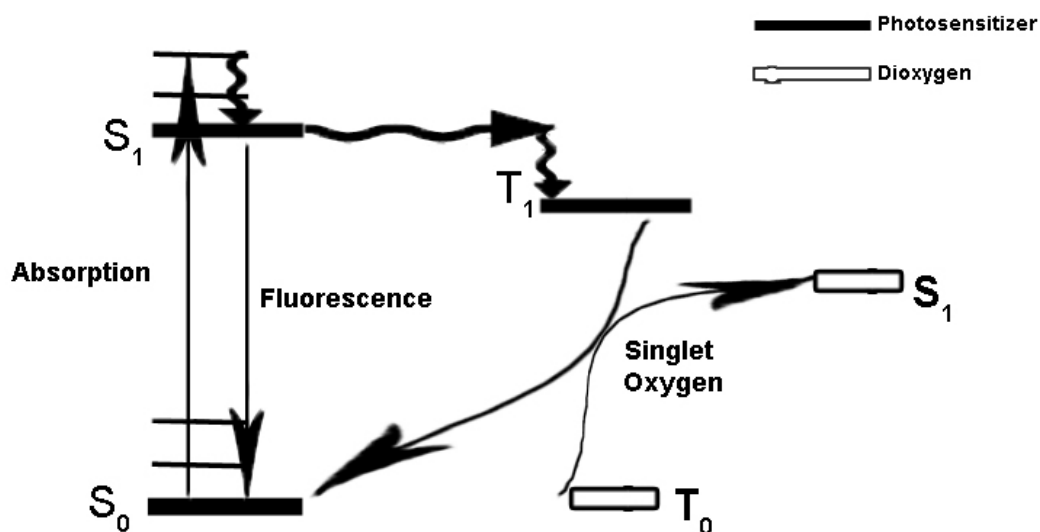


Figure 2. Modified Jablonski diagram for singlet oxygen production.

2.1.2 Development of PDT

The history of photodynamic therapy goes to more than 100 years ago; researchers found that light and certain chemicals can induce cell death. In 1900, Oscar Raab, a medical student found that in the presence of acridine and light has a killing effect to infusoria, a small aquatic creature.⁷ Three years later, Herman Von Tappeiner and A. Jezionek used eosin and light and named this action as *photodynamic action*. Today, the most accepted, widespread photosensitizer is porphyrin and W. Hausmann reported one of the first studies with and used haematoporphyrin (See Figure 2) and light on paramecium and red blood cells and saw that it killed both.⁸ In 1913, Friedrich Meyer-Betz used porphyrins on his own body to see the activity and in light exposed areas, he reported pain and swelling.⁹

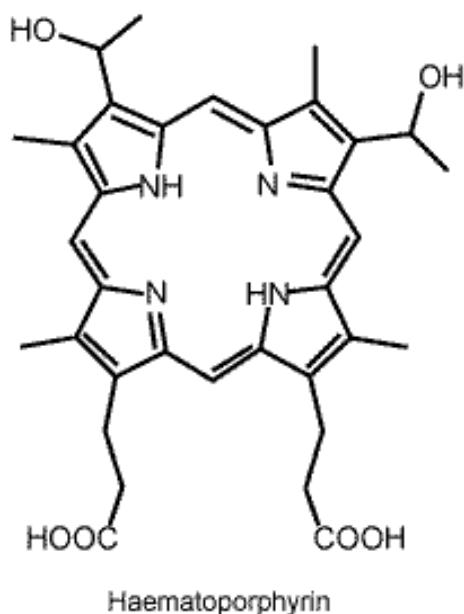


Figure 3. Structure of Haematoporphyrin.

After a long period without any crucial developments, modern studies begun in Mayo Clinic, US in 1960s. Samuel Schwartz developed haematoporphyrin derivative (HPD), and Lipson and Baldes reported that this compound localizes in tumor tissues and fluoresces.⁷ One advantage of using this compound is that it is used in much smaller amount than the crude haematoporphyrin.

The tumor localization and tumor phototoxic effect of porphyrins was thought to be used killing cancer cells in 1972 by Diamond and his colleagues.¹⁰ It was observed that PDT has delayed the growth of gliomas that were implanted to rats during in vivo studies. The growth stopped for about 20 days but later on growing starts again in deeper regions. In 1975 two important progress came true. First, experiments in mammary cells show that tumor cell growth stopped in mice completely in the laboratory of Thomas Dougherty and co-workers.¹¹ Secondly, Kelly and co-workers stated that by using HPD bladder carcinoma was eliminated in mice.¹²

After those studies, first human trials were started. Kelly in 1976 used HPD to diagnose 5 patients and in 1 patient treatment was done to cure bladder

carcinoma.¹³ It was shown that tumor growth delayed and also tumor necrosis observed in this patient. In addition, Dougherty used PDT in 25 patients with total 113 primary or secondary tumors and 98 of them were eliminated by using HPD again as a photosensitizer.¹⁴ After these two types of carcinogenic tumors, Y. Hayata and co-workers tried PDT on lung tumors and observed tumor growth delay in most of the patients, however total curing was observed in one of fourteen patients.¹⁵

Growth of PDT action to other cancers continued in 1984, in which McCaughan and colleagues treated patients with oesophageal cancer¹⁶, Balchum and co-workers used to cure lung cancer.¹⁷ Hayata and his colleagues tried to cure gastric carcinoma one year later. After all these preliminary trials of PDT, it was noted that this method is efficient in *early-stage patients* that is inoperable due to other reasons. Besides the cancer types noted, many other carcinogenic tissues have been tested with PDT action. These are breast cancer, gynaecological tumors, intraocular tumors, brain tumors, head and neck tumors, colorectal cancer, cutaneous malignancies, intraperitoneal tumors, mesothelioma, cholangiocarcinoma and pancreatic cancer. After all these experiments it was noted that PDT has only *limited success* due to the reasons such as specificity and the effectiveness of the photosensitizer. Hence, more powerful in terms of both specificity and strength should have been developed and under clinical trials.

2.1.3 ***The Mechanism and Methods of PDT***

It is told before that PDT is a method applied only in the region of interest e.g. cancerous tissue. And it is not harmful to the other parts of the body because half life of the singlet oxygen in biological systems is $<0.04 \mu\text{s}$ and the singlet oxygen is active only in the region of a radius of $<0.02 \mu\text{m}$.¹⁸ The photodynamic action may be controllable, it may depend on various factors; type of the photosensitizer, its intracellular and extracellular localization, the amount of PS introduced, light's exposure amount and its fluence rate, the amount of oxygen in the region and finally the amount of time between the introduction of the drug to the body and exposing the light.⁷

The mechanism of photodynamic action is very important and it has been investigated deeply in order to broaden the applicability and to increase the respect and universality of the method. Up to date, it has been proposed three ways of killing of tumor cells; direct killing (*necrosis*), shutting down the tumor vasculature so that tumor infarction yields and finally opening ways of immune system to facilitate killing of tumor cells (*apoptosis*).⁷ These three ways should not be thought as independent since each of them may influence the other and the importance and the way that affect the other will probably be seen in future studies.

The first way of killing tumor cells is direct killing by photodynamic activity. It is observed that number of tumor cells decrease with this way, however, it is not enough always to wipe out all the illness. M. Korbelik and his colleagues observed that PS accumulation may vary in tumor tissues.¹⁹ Intravenously injected PS may not always go to the parts of the cancerous cells which are distant from the vasculature. Another issue related with the direct killing of cells is the amount of oxygen needed during therapy. The level of oxygen amount is not always enough may be owing to two reasons; the first reason is during therapy oxygen is consumed and secondly, because of the effect of PDT on the microvasculature fresh oxygen cannot be reached to the region anymore. Both of these effects have been reported.^{20,21} Although shutting down the microvessels have an affirmative effect in long term, it may affect direct killing. A solution to this problem may be decreasing the fluence rate of the light and applying therapy in different times with decreased dose in order to keep the oxygen amount on a tolerable level.⁷

One type of indirect killing of tumor cells is by inducing vascular damage via PDT. Increasing amount of tumor cells is dependent on the available nutrients, and so targeting the vascular system to the tumor tissue is an important tool to utilize. There has been enough number of studies showing PDT causes vascular damage^{22,23,24}, resulting in delay of tumor growth by *hypoxia* and *anoxia*^{25,26,27} which both refer to the reduced level of oxygen where the latter is an extreme version. After PDT treatment, it has been shown in various *in vivo* studies that, first

vasoconstriction takes place and after a period of three hours thrombus formation occurs, which results in tumor growth depletion. In 1989, Henderson and colleagues showed that by using *Photofrin* (an approved photosensitizer being used in clinic) tumor cells eradicated by oxygen depletion in tumor tissues by shutting down tumor vasculature in a fibrosarcoma mouse model.²⁸ Hence, by targeting tumor vasculature it is possible to delay tumor growth.

Another indirect treatment of PDT is calling out the immune system elements (lymphocytes, leukocytes and macrophages) on work into the treated tissue. The inflammation starts with various components such as clotting cascades, acute-phase proteins, proteinases, peroxidases, reactive oxygen species.²⁹ W. deVree observed neutrophil attraction to the PDT activated tissue so that tumor growth was delayed.³⁰ Korbelik and colleagues in 1996 compared the tumor growth in normal tissue and in immunodeficient tissue in order to understand the long term effects of PDT.³¹ In short term they observed that PDT effect is similar, however, in long period of time tumor growing is very fast in immunodeficient tissue compared to the regular one. Therefore, immune response is an important parameter while fighting with cancerous cells.

There is an interesting study on immunology studies after PDT; in 1999 Korbelik and colleagues have shown that tumor-sensitized immune cells have been found on lymphoid sites which are far to the region where PDT applied.³² Another related study was performed by Henderson group; they recovered the tumor-cell lysate from the PDT applied tissue and they used this as a *vaccine* and applied to a mice, and showed that tumor growing prevention.³³ This study is important for the topic of induction of tumor-specific immunity. It has been postulated that this type of vaccination is more effective than producing lysates from tumors that were irradiated with UV or ionizing irradiation. These studies designate that PDT may have a potential to be used as a *systemic immune therapy*. The studies related with anti-tumor immunity and PDT have been reviewed recently³⁴ and it has been emphasized that while other methods like chemotherapy, surgery and radiotherapy

are mostly immunosuppressive, PDT can cause acute inflammation, express heat-shock proteins, calling leukocytes to tumor region and increase the presentation of tumor-derived antigens to T cells. Studies related with the immunogenic properties of PDT is ongoing and it forms as an important part of the study.

In order to PDT to be effective tumor localization is an important issue. Hence, photosensitizers are under study and a PS named MV6401 (a pyropheophorbide derivative) has been developed which especially localizes in tumor vasculature.³⁵ Delivery of the PS depends on vascular permeability and interstitial diffusion into the cell which may change with size, charge, configuration, hydrophilic and hydrophobic properties of the molecule and also to the physiological properties of the blood vessels.

Another issue which affects the PDT efficacy is the time between introduction of the drug to the body and irradiation. If the interval is short like 15 minutes drug generally holds on the vasculature, however in longer times like 4 hours drug localizes on the tumor tissues by using PS MV6401. If irradiation starts in a short time vascular stasis and thrombus formation starts, hence indirect killing of tumors achieved. With most cancer types and PS in the market, generally longer period of times (24-72 hours) have been waited in order to accumulate in extravascular compartment. These indicate that application of light in different time periods affects the mechanisms of PDT.

In 2002, a different approach has been applied and found to be superior to the methods above. The drug has been given to the body more than one time at different time intervals. By this way both vasculatures and tumor cells were activated. This fractionated dose of PSs enabled to obtain long term tumor growth control.^{24b}

Targeting the tumor region is one of the important task in PDT and increased concentration of photosensitizers in tumor cells have been observed and called *passive targeting*. However, the exact reason for this complex phenomenon is not

completely understood. It is reported that probably it is related to high vascular permeability of PS, and their affinity for proliferating endothelium and minimum lymphatic drainage in tumor cells. In order to attain an effective delivery agent, conjugated antibodies have also been utilized in order to direct these to tumor associated antigens or vascular antigens (e.g. ED-B domain).³⁶ In 1990s some photosensitizers have been developed for targeting mitochondria, plasma membrane, nuclei and lysosomes.³⁷ It has been reported that mitochondria is an important organelle to target for PDT^{38,39} and these type of delivery agents are important when combining the strengths of two major areas; PDT and therapeutics studying on targeting subcellular regions.

The efforts for the commercialization of PDT have resulted partially in 1993 for Photofrin in Canada for the first time for the prophylactic treatment of the bladder cancer. This drug is the partially purified version of the haematoporphyrin (HPD) which was utilized in 1970s. However, Photofrin is not very pure; it contains more than 60 types of porphyrin derivatives. This impurity also complicates its reproduction easily. The absorption maximum of this compound is 630 nm which is tolerable but it has low extinction coefficient ($1,170 \text{ M}^{-1} \text{ cm}^{-1}$). Due to this high drug dose and fluence rate should be used during therapy. Moreover, it has a long lasting skin photosensitivity.⁷ According to the studies reported by Orenstein and colleagues, Photofrin has low normal to tumor cell accumulation in C26 colon carcinoma bearing mice.⁴⁰ Hence, patients should avoid sunlight for 4-6 weeks after therapy in order to prevent skin photosensitization. Still, Photofrin is the most widespread drug used in PDT. It has been approved in the Netherlands and France for lung cancer, in Germany for early-stage lung cancer, in the United States for advanced oesophageal cancer and in Japan for early-stage oesophageal, gastric and cervical cancer (Table 1).

Table 1. spectroscopic and physicochemical properties of clinically approved photosensitizers⁴¹.

<i>Photosensitizer</i>	$\lambda_{\text{max abs}}$ (nm)	ϵ (M ⁻¹ cm ⁻¹)	$\lambda_{\text{max emiss}}$ (nm)	Φ_{FL}	Φ_{Δ}	LogP _{o/w}
Porfirmer Sodium (Photofrin [®])	630	3000	NA	NA	0.25	3.96
Protoporphyrin IX(Levulan [®])	635	5000	630	0.011	0.54	NA
Temoporfin (Foscan [®])	650(EtOH)	39000	655	NA	0.31	9.24
Verteporfin (Visudyne [®])	692(PBS)	31200	694	0.049	0.82	7.76
Talaporphin(Laserphyrin [®])	654(PBS)	40000	660	NA	0.77	-1.92
Ce6 (Photolon [®])	663(PBS)	38000	662	0.18	0.75	0.78

The limitation with Photofrin makes scientists to work on more efficient photosensitizers. More ideally the new PS should absorb at longer wavelength in order to achieve deeper skin penetration of light, more target specific and less skin photosensitive. In this way, a drug called *Foscan* has been developed which is very efficient compared to previous and approved for head and neck cancers. This chlorine derivative PS requires using very low dosage (0.1 mg/ kg of body weight).⁴² However, due to its being reactive, some complications have been reported.⁴³ Also, for actinic keratosis and basal-cell carcinoma 5-aminolevulinic acid and 5-ALA methyl ester have been approved. The structure of 5-ALA is unusual since it is not an absorbing dye, but it is an endogenous porphyrin which is the first compound in porphyrin synthesis pathway in the body for synthesis of *heme* in mammals and *chlorophyll* in plants (Figure 4).

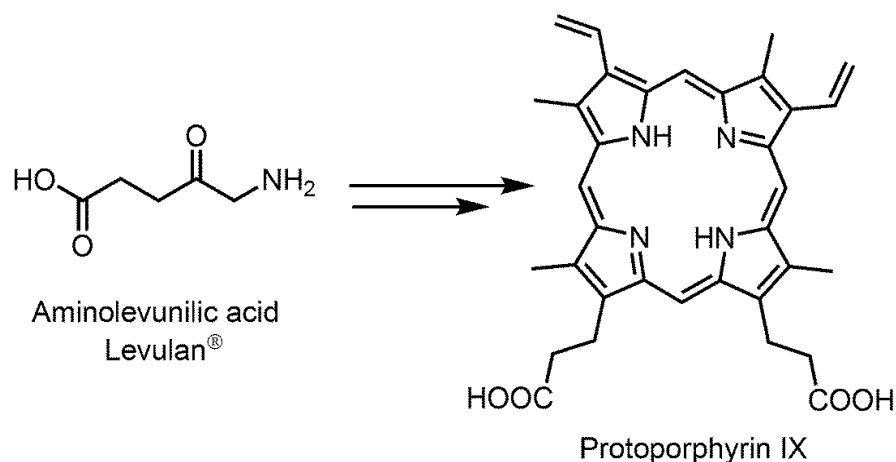


Figure 4. Biosynthesis of protoporphyrin in mammals starting from aminolevulinic acid

Other efforts to increase the efficiency of the photosensitizers are to utilize carrier-mediated delivery in order to accumulate the PSs in tumor regions. Using carriers therefore broaden the scope of the therapy to the many parts of body and it also make light to be applied in an easier way, one should not be very precise while irradiating. As a delivery agents, monoclonal antibodies have been tried in preclinical models for recognizing tumor antigens.^{44,45,46} Also, some ligands which are synthesized in tumor region may also help PS to enter the cell. For example, low-density lipoprotein (LDL) is expressed in tumor cells known to able to internalize a LDL coupled photosensitizer.^{47,48,49} And, another thing to use may be targeting the PS to hormones like peripheral benzodiazepine receptor or oestrogen receptor for hormone dependant tumors.⁵⁰ At last, as a targeting method liposomes or immunoliposomes may be counted.^{51,52} Of course, one should be careful about the problems that may arise like spatially and temporally heterogeneous blood flow and vascular permeability.

In PDT, one may also monitor the region of tumor by tracking the fluorescence of the photosensitizers. However, not all PSs fluoresce due to the intersystem crossing, a study in 1955 with HPD by Rasmussen-Taxdal and colleagues reported that observation of tumors is possible.⁵³ Also, recent studies show that image of the photosensitizer accumulation might be possible in BODIPY

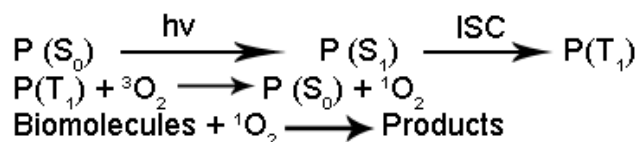
based system by conjugating PS with a fluorophore.⁵⁴ An alternative way of employing PDT is to deliver membrane-impermeable drugs to the cytosol by a method called photochemical internalization.⁵⁵

Besides curing cancerous diseases PDT also employed in other disorders. The most popular ones are age related macular degeneration and other eye problems related to the neo-vascularization.⁵⁶ Verteporfin has been approved starting from 1999 in many countries in order to cure this type of problems. Also, PDT has been used to treat arteries with intimal hyperplasia⁵⁷ and after stent implantation in cardiovascular part of medicine.⁵⁸ In dermatology field, psoriasis and scleroderma have been treated with PDT.^{59,60} In rheumatology area, arthritis has been tested.⁶¹ Finally, PDT has been used to kill microorganisms also.^{62,63}

2.1.4 ***Mechanism of Singlet Oxygen Production***⁶⁴

Biochemical reactions in living things which take place with light can be splitted to two main categories. The first is the photosynthesis and vision processes. These have been a nearly totally investigated topic and known very well since they are both vital topics of living on earth. The second thing is the photodynamic effect. In this topic, a photosensitizer is used and this is not a biologically known object and in the body, and other biological things may behave to these differently. Just because of this its mechanism is an interesting subject and have been investigated by different disciplines. The mechanism of photodynamic effect can be investigated in four parts: *In vivo* interactions (1), excitation (2), reaction of excited species (3) and reactions of reactive secondary products *in vivo* (4). In the *in vivo* interactions case pharmacokinetics (i.e. photosensitizer localization in tissues and cells, targeting and clearance rate) and structure of the photosensitizer and activity relationship play an important role. For the excitation case several photophysical characteristics (i.e. absorption properties, energies, quantum yields, lifetimes and singlet oxygen quantum yields) are investigated. Upon excitation with light, three main reactions of excited species occur: electron transfer (TYPE I, results in radicalic reactions like crosslinking of proteins), energy transfer (TYPE II; singlet oxygen produced) and

photobleaching. For these three pathways of reaction the most important way of cell damage is the TYPE II energy transfer process; in which singlet oxygen is produced. The mechanism of singlet oxygen production can be simplified with following:



The process of singlet oxygen production can also be illustrated in modified Jablonski diagram (Fig. 2)

It has been verified that some biomolecules can react with singlet oxygen and since these biomolecules are important parts of biological systems have a potential to cause to vascular shutdown. Some important biomolecules' reactions have been summarized below in the Figure 5.

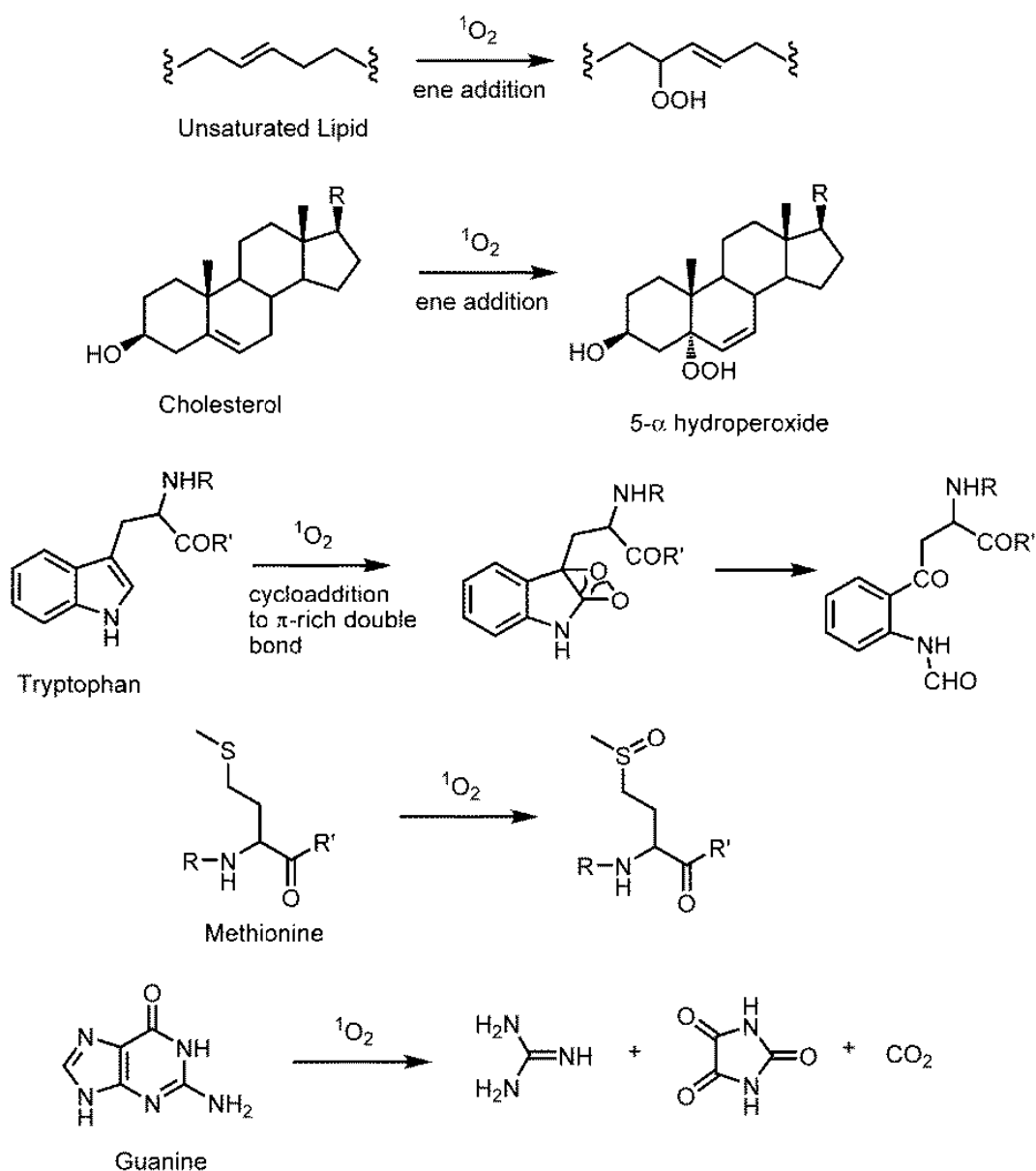


Figure 5. Some biomolecules' reaction with singlet oxygen and its products⁶⁴.

2.1.5 Molecular Orbital Diagram of Triplet and Singlet Molecular Dioxygen⁶⁵

Dioxygen molecule is an exception to the most of the molecules because of its being in triplet state, while most others are singlet. This is possible in molecular orbital theory when degenerate MOs exist. The lowest energy configuration of dioxygen molecule is $[\text{N}_2] (\pi^*)^2$, where $[\text{N}_2]$ is the electronic configuration of the N_2

molecule. Hund's rule application to this molecule yields a triplet due to energy minimization and electrons are in parallel. The representation is shown in Figure 6.

As the name implies, there are three sublevels which are equal in energy. Two of them can be viewed easily, the first one is two up parallel arrows $\alpha(1)\alpha(2)$ as in the Figure 6. Other one is the two parallel down arrows $\beta(1)\beta(2)$, while the arrow indicates each electron's spin projection along a given fixed axis. The third triplet level is difficult to represent since it is the combination of two states. And both of these states have zero spin angular momentum, but the total spin angular momentum is non zero for the triplet shown as: $\alpha(1)\beta(2) + \beta(1)\alpha(2)$. A similar combination stands for singlet when combination is $\alpha(1)\beta(2) - \beta(1)\alpha(2)$.

Hence, the ground triplet state oxygen molecule is denoted as $^3\Sigma$ and according to the Pauli's exclusion principle the two electrons in the HOMO should be in different orbitals as in the Figure 6. The corresponding triplet sublevels are $\alpha(1)\alpha(2)$, $\beta(1)\beta(2)$ and $\alpha(1)\beta(2) + \beta(1)\alpha(2)$. The excited states of oxygen is also shown with the configuration of $[N_2](\pi^*)^2$, however in this case with increased energy. The first excited state is $^1\Delta_g$ and often called as singlet oxygen, where two electrons are in the same orbital in different spins. In this case we have a double degenerate singlet states and it is denoted as $\alpha(1)\beta(2) - \beta(1)\alpha(2)$.^{*} In the second excited state which is higher in energy ($^1\Sigma_g$) corresponds to two electrons with different spins and in different orbitals with again same combination $\alpha(1)\beta(2) - \beta(1)\alpha(2)$. As a result, the absorption and emission take place in oxygen molecule is a singlet-triplet or triplet-singlet transition.

In general, most of the illustrations of the excited state electron transitions are shown as two orbitals have been involved in the process, namely HOMO and LUMO. However, in reality this is not the case because many orbitals may involve

^{*} In this case the spin functions should be thought not strictly as p_x^* and p_y^* . It should thought as linear combinations of these two which have well-defined orbital angular momenta.

in excitation, deexcitation process, and excited states are described as the superposition of these configurations. In some cases different phenomena like double excitations* may contribute.⁶⁵

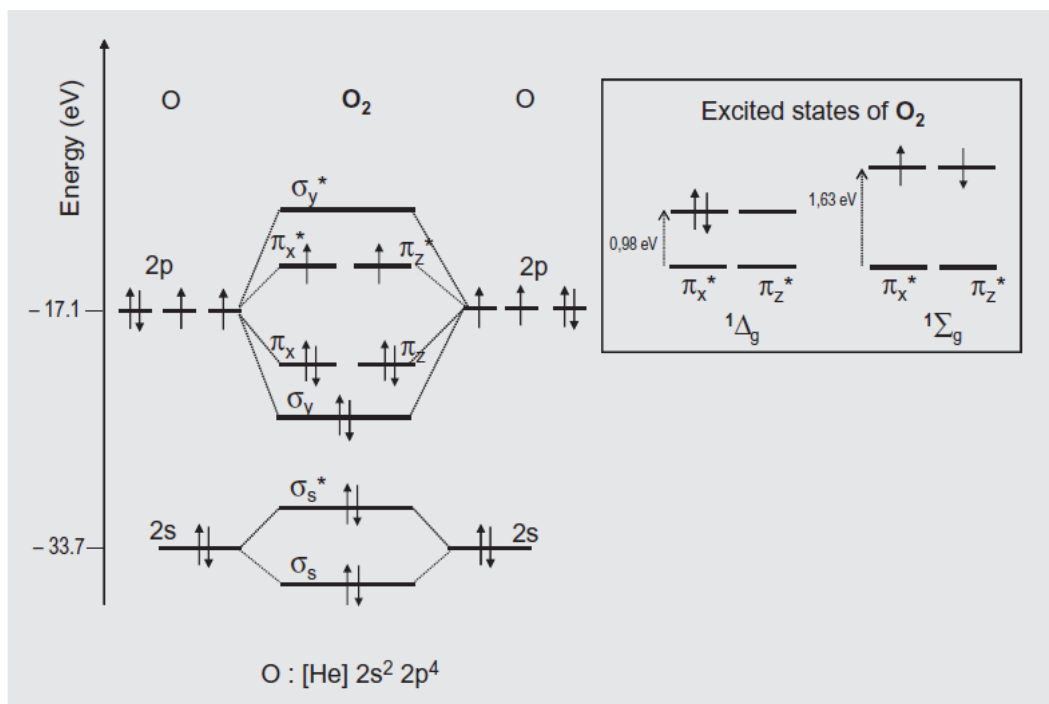


Figure 6. Molecular orbital diagram of molecular dioxygen with its ground and excited states⁶⁵.

2.2 Triplet Photosensitization

When a fluorophore absorbs light and it excites to singlet excited state, and in general case it deactivates via S₁ to S₀ transition (fluorescence).⁶⁵ However, sometimes with ISC triplet state can be populated (S₁ to T_n). So, in order to get successful candidates for triplet PSs, efficient ISC should be attained. The number of studies with triplet photosensitizers and the factors and relationship between the molecular structure and efficiency is limited.⁶⁶ The application areas with triplet PSs is diverse, e.g. electroluminescence, phosphorescent bioimaging,⁶⁷ PDT⁶⁸,

* Simultaneous excitations of two electrons

photoinitiated polymerization and photoredox reactions.⁶⁹ Fluorescent molecules generally used for their luminescence property, however, triplet PSs generally used for initiating another reaction, and the product is used for purpose.⁷⁰ The two main reactions carried with triplet PSs are singlet oxygen production from ground state stable triplet molecular oxygen and triplet-triplet annihilation upconversion reaction. In the latter subject triplet PSs are used for triplet-triplet energy transfer *donor*.

Designing a triplet PS is known to be a challenge especially it does not contain a heavy atom. The main reason for this fact is the covered and unexplored facts of *intersystem crossing*. For example, the photophysical difference between anthracene and 9,10-diphenylanthracene is interesting. Anthracene has a ϕ_F of 0.27 in EtOH however, DPA has 0.95 in same conditions. Similarly, anthracene has a ϕ_{ISC} of 0.73 which is smaller than 0.05 in DPA.⁷¹

One reason for the rare examples of molecules that undergo ISC is because of their including quantum mechanically forbidden steps. However, in some case ISC is allowed. When a chromophore is excited, S_1 state is populated then a non-radiative transition from S_1 state to T_n ($n \geq 1$) should take place. For this transition to occur two spin states should involve in the process. Magnetic torques should be exerted on the magnetic moment (μ_s) vector of an electron spin, or as a result of the spin angular momentum (μ_s) coupling to the orbital angular momentum (μ_L). In organic molecules in most of the time coupling of the electron spin with the orbital angular momentum (i.e. spin-orbit coupling) is the case for ISC. Spin-orbit coupling is defined and can be understood by thinking the motion of the electron around the nucleus in a Bohr-like orbit. There are two types of rotations and these cause a magnetic field. First, electron rotates in its own spin and the second it rotates around the nucleus. These two types of magnetic fields interact with each other yielding spin-orbit coupling.⁶⁵

2.2.1 *Heavy Atom Effect on Triplet Photosensitization*

Atoms with a high number of atomic weights can effect the spin-orbit coupling in order to increase intersystem crossing. Spin-orbit coupling efficiency has a dependence of Z^4 .^{*} The effect of internal heavy atom can be explained with the electron's movement around the nuclei. The movement around the nuclei is accelerated when it gets close to the more positively charged nuclei, inducing increased orbital magnetic torque (μ_L). As a result, the coupling of the spin (μ_S) and orbital (μ_L) angular momentum increases. Hence, ISC is increased with the heavy atoms when they are close to the center of molecule. This strategy is an important task when designing a triplet photosensitizer.⁷² Some heavy atoms used are Ir, Pt, Ru, Os, Re, Rh, I and Br. Various metal complexes are also used for triplet PSs but they have generally have low absorbance in the visible region which limits their utilization.⁷²

2.2.2 *Triplet PSs with low-lying $n-\pi^*$ transitions*

For a transition to occur from singlet state to the triplet state there are some restrictions. It is similar to the selection rules of electronic transitions. El Sayed's rule is generally accepted with these restrictions.⁷² El Sayed's rule is related with the magnitude of rate constant of intersystem crossing denoted as $^1k_{ISC}$. This rate is increases when there is an orbital change between transitions. For example transition from $^1(n-\pi^*)$ orbital to $^3(\pi-\pi^*)$ orbital is faster than $^1(\pi-\pi^*) - ^3(\pi-\pi^*)$ transition. Because of these statements most aromatic chromophores do not display intersystem crossing behavior and show intense fluorescence.

^{*} Where Z is the atomic weight

2.2.3 *Exciton Coupling Behaviour of Chromophores*

Exciton coupling is a way of populating to triplet state from singlet excited state. It occurs when almost similar chromophores link together without having a π conjugation, and their singlet orbitals split into two and the one in lower energy has increased probability to pass to the triplet state because of minimized energy difference between states.⁷³

2.2.4 *Utilizing Spin Converter for Triplet State Photosensitization*

Designing and synthesizing a triplet photosensitizer without using heavy atom is a difficult task. The principles of ISC and organic molecular structure is not a very well known subject. Hence, all the studies done in this topic without using a heavy atom, especially in the visible region is highly important. One strategy used in this context is using a spin converter. In literature the only molecule used as a spin converter is the fullerene.⁷⁴ It is an intrinsic triplet state photosensitizer. In all the cases within this topic, fullerene molecule is used as an excitation energy transfer (EET) acceptor molecule and another molecule is used as a donor. Upon excitation from the donor molecule* intersystem crossing takes place with the help of fullerene moiety.

2.2.5 *Methods to Detect Triplet Excited States*

Detecting a triplet state of a molecule is not a straightforward process like in singlet excited states. Some of the triplet PSs are phosphorescent but some of them not. Time-resolved transient absorption (TA) spectroscopy method is used to get information about triplet population.⁷²

* Which is a poor triplet photosensitizer

This method is based on the pulse-probe (or pump probe) method. There are two light sources have been used, the first is the typical xenon lamp for tracking absorption change and the other is a pulsed laser source for pump purpose.

2.2.6 *Triplet PSs recently studied*

In the literature it is possible to see a growing publication rate of synthesized triplet PSs, However the rate is not as much as fluorescent molecules. The PSs studied can be broadly separated into two parts, the ones with heavy atom and without heavy atom. It is generally accepted that designing a PS without heavy atoms is much difficult.⁷² The PSs with heavy atom can also be divided into two parts: (i) transition metal complexes (ii) iodo or bromo substituted organic chromophores. Both of these have many examples in the literature, and due to the limitations of transition metal complexes halogenated organic molecules have been used more frequently. The most famous of these include xanthene based ones and bodipys. Recently, studies on naphthalene diimides were also conducted. Rose Bengal, Eosin Blue, Erythrosin B and Eosin Yellow are xanthene based PSs (Figure 7).⁷⁵ These molecules have been known for a long time mainly for staining in microscopy. However, one drawback for these types of structures is their limited functionalization leads to restricting the absorption wavelength to visible region. Due to this problem derivatizable organic fluorophores have been studied more in this topic. Most successful candidates are bodipy and perylene which was already noted.

Bodipy derivatives have the advantage of ease functionalization and many PSs in the range of 500-800 nm absorbances have been synthesized. Upon halogenation high singlet oxygen quantum yields have been attained(see Figure 6).⁷⁶

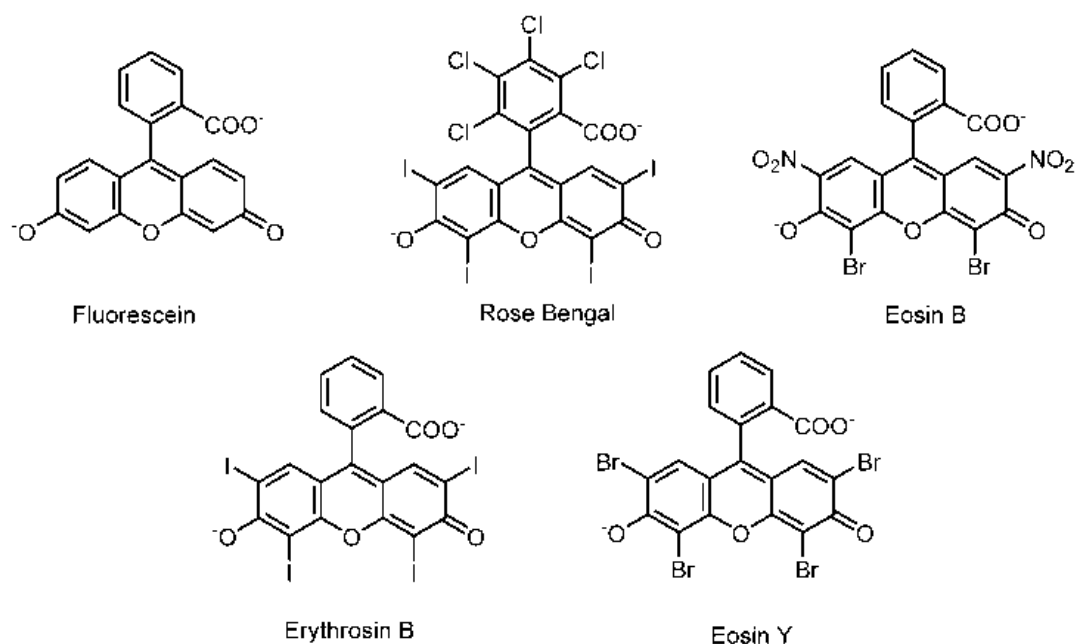


Figure 7. Fluorescein and its halogenated derivatives as triplet PSs⁷⁵.

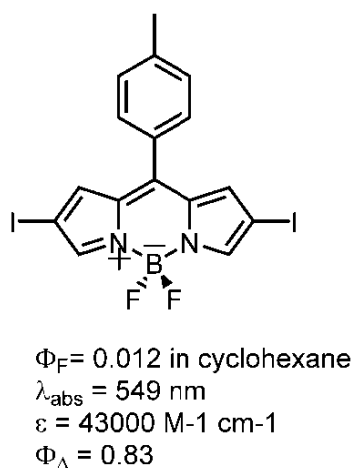


Figure 8. Diiodinated Bodipy for PDT⁷⁶.

Since absorbance around 500 nm is not enough for PDT application due to the limited penetration depth of light at this wavelength, near-IR absorbing bodipy molecules are more important. Recently, Ramaiah *et al.* have synthesized an azabodipy derivative (Figure 7) absorbing at 666 nm.⁷⁷ Their characterization of Φ_Δ and Φ_T showed that these molecules can be used for PDT applications with values 0.70 and 0.78, respectively. In order to learn more about the excited state properties

of these dyes they have analyzed with nanosecond flash photolysis (transient absorption). They observed two transient absorption peaks at 320 and 510 nm with excitation with 355 nm laser pulses. The band at 510 nm is suspected for triplet excited state formation since its quenching with dissolved oxygen (see Figure 9). The triplet quantum yield of this dye was determined using triplet-triplet energy transfer to β -carotene using a tris(bipyridyl)ruthenium(II) complex as a reference. For this calculation transient absorption at 320 nm was tracked and 0.78 yield with at lifetime of 1.6 μ s. This observed triplet quantum yield has been the highest reported for aza-bodipy derivatives so far at the year it was published.

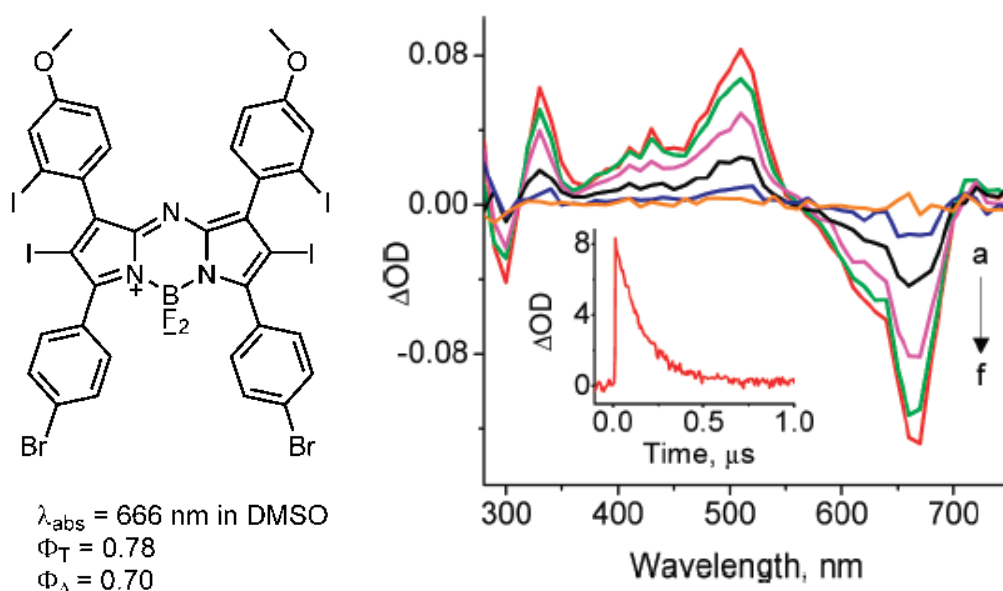
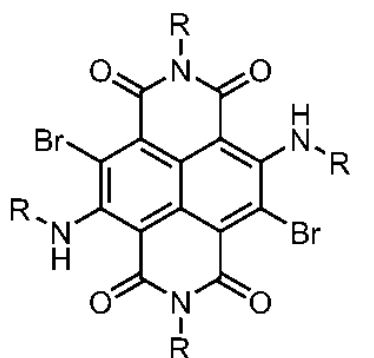


Figure 9. Aza BODIPY derivative for PDT application and its transient absorption spectrum. There are two transient absorption peaks at 320 and 510 nm. The inset graphic shows the first-order decay of the 510 nm absorption.⁷⁸

In the recent study by Zhao group⁷⁹ triplet excited state studies was conducted with naphthalene diimide (NDI) molecule. Since these compounds mainly absorb in the UV range, electron donating amino groups was attached and absorbance maxima at 526 nm was observed. Although this wavelength is not sufficient for PDT, it may have a potential to be used in triplet-triplet annihilation upconversion studies or in photocatalysis reactions. Actually, long triplet state

lifetime has been recorded ($\tau_T = 51.7 \mu s$) and high upconversion quantum yield has been observed (see Figure 10).



$$\lambda_{abs} = 526 \text{ nm (in toluene)}$$

$$\Phi_{UC} = 0.185$$

Figure 10. Naphtalene diimide molecule used for triplet photosensitization.⁷⁹

2.2.7 *Triplet PSs without Heavy Atom*

It was already noted that designing a PS without a heavy atom is a complicated process. One way of realizing this strategy is to use spin convertors. Spin convertor is such a molecule that has a high quantum yield of intersystem crossing without any heavy atom. In the literature rare examples of spin convertors have been reported one of which is the C_{60} fullerene molecule.⁷⁴ This molecule has an absorption maximum at 335 nm and has with very low molar absorptivity in the visible range and near IR range (around $700 \text{ M}^{-1} \text{ cm}^{-1}$) and almost quantitative intersystem crossing quantum yield. Moreover, it has a low S_1 state energy level (1.72 eV) so that a wide range of organic chromophores can make energy transfer to C_{60} molecule*. To use fullerene molecule as a spin convertor dyes should be attached around the fullerene and energy transfer from dye to spin convertor yields intersystem crossing. Actually, the term energy transfer should be used with care in this concept because in typical Forster energy transfer, emission wavelength from

* Dyes have an absorption maxima from UV range to about 700 nm are convenient

the donor should be compatible with the absorption of the acceptor moiety (spectral overlap). A simplified version of Jablonski diagram for C₆₀-chromophore triplet sensitizers is shown below (Figure 9).⁸⁰

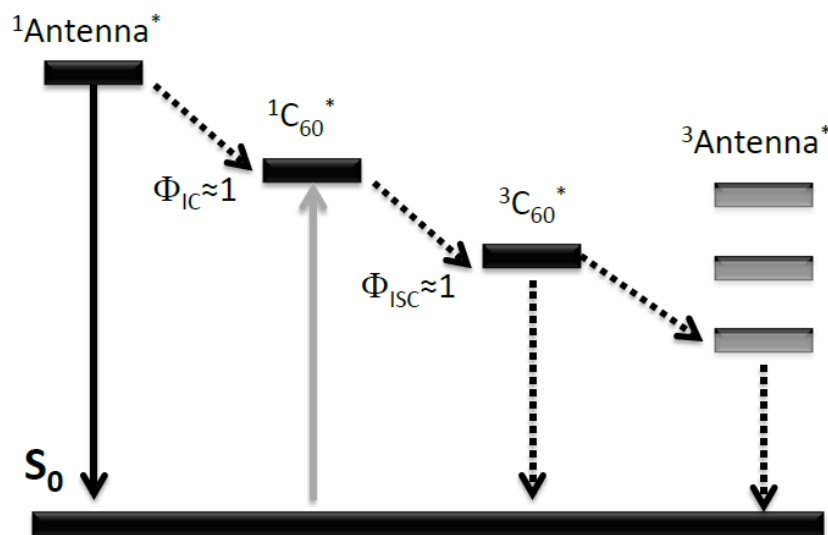


Figure 11. Simplified Jablonski diagram showing the photophysics of C₆₀-chromophore hybrids as heavy atom free triplet PSs.

One successful example to fullerene-chromophore triplet PS is by using the bodipy chromophore.⁸⁰ In this study bodipy units absorbing at 600-650 nm range have been attached to the C₆₀ units, and with the help of transient absorption spectroscopy they have observed *ping-pong* type reaction. First, bodipy core has been excited around 600 nm and energy transfer occurs to the fullerene and intersystem crossing to the triplet state of fullerene took place and this time triplet-triplet EnT from fullerene to antenna bodipy realized. As a result long-lived triplet state lifetime and high singlet oxygen quantum yield have been observed ($\tau_T = 123.2$ ms and $\Phi_\Delta = 0.85$). The studies with this strategy to use triplet PSs are new and expected to be utilized more in the future.

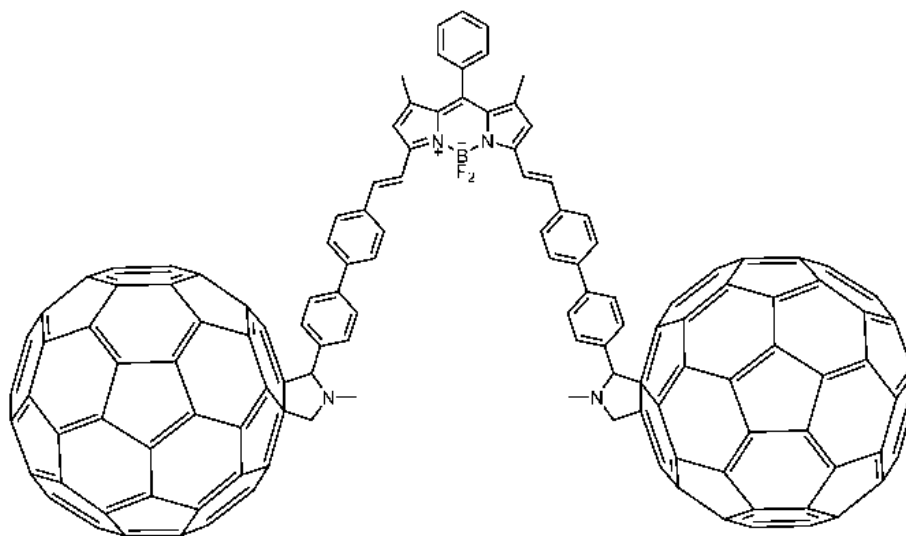


Figure 12. Fullerene-bodipy dyads as efficient heavy-atom-free organic triplet photosensitizers.⁸⁰

Other than spin converters some small organic molecules absorbing at UV region also do sensitize to triplet state. Since their absorbance are at UV region their treatment to applications such as PDT is not seem possible, but their mechanism of ISC is an important issue while designing triplet photosensitizers. A list of several molecules of this type have been show in Figure 13. One if them is pyrene molecule which has been used widely for fluorescence with a fluorescence quantum yield of 0.65, however one cannot discount its intersystem crossing quantum yield of 0.35. In the pyrene, there is a special occasion of delayed fluorescence. It take place when luminescence rate is very slow. In delayed fluorescence T_1 to S_1 state reverse intersystem crossing take place due to this abnormal situation lifetime of emission is very long (e.g. $\tau_F = 450$ ns in cyclohexane which is ten folds of typical bodipy fluorophore's). As in other small organic triplet PSs their modification in the core results diminished ISC rates. For example, when pyrene's core is substituted with π framework, the emission is turned to be a prompt fluorescence with τ_F of a few nanoseconds.⁸¹

Some aromatic ketones shown in Figure 13 also shows high intersystem crossing quantum yields. As it was already noted, low lying $^1(n-\pi^*)$ transition to $^3(\pi-\pi^*)$ transition is fast, so this effect can be explained by obeying this rule.

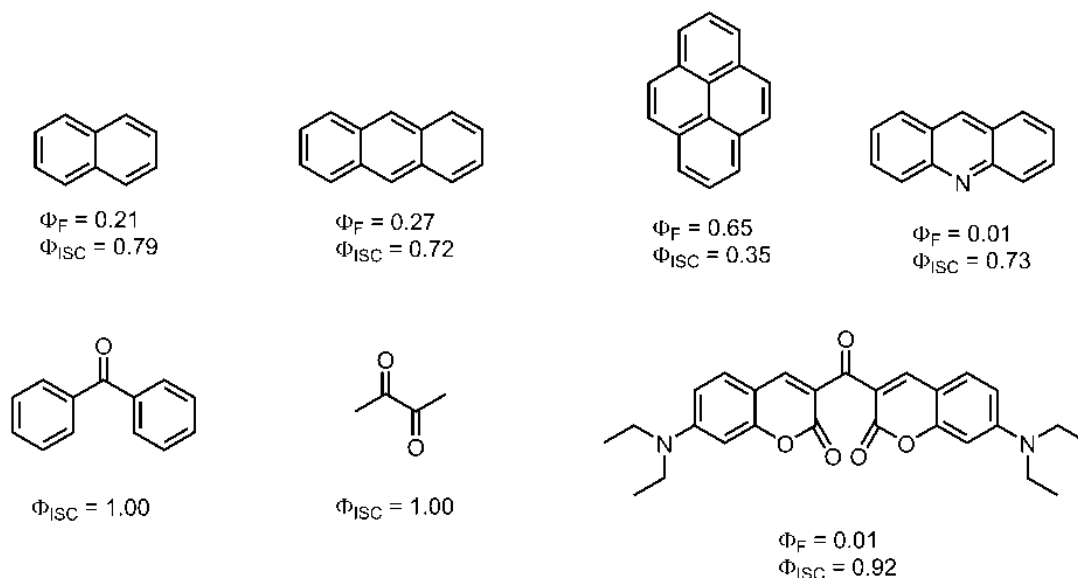


Figure 13. Heavy atom free small organic molecules as triplet PSs absorbing at UV region.

Along with these heavy atom free triplet photosensitizers, another important class of organic molecules, also very important for life on earth, have been utilized for the same function; porphyrins. Their usage in this concept is very early however, their mechanism of triplet photosensitization has not been explained up to now very well. In a study reported, it has been suggested that vibration of the molecule with excited state intramolecular proton transfer aids for intersystem crossing.⁸² Still porphyrins absorbing strongly (e.g. high molar absorptivity) in the near IR region are needed for their wider utilization in PDT.

One of rare examples of triplet PSs without heavy atoms is the di(perylenebisimide)s. It has been synthesized recently and their singlet oxygen efficiencies were investigated with Φ_Δ of 0.67 and 0.59 for two types molecules.⁸³ The near IR absorbances are 671 and 655 nm. The reason for successful intersystem

crossing is thought to be due to the distorted energy minimized structure which enhances the spin-orbit coupling (see Figure 13). The N-linked substitution in the molecule leads to the out-of-plane distortion and resulting X shaped molecule is responsible for breakdown of the σ - π orbital separation which leads to increase in spin-orbit coupling.

Another type of molecules called Bis(BF₂)-2,2'-bidipyrins have also been synthesized and show intersystem crossing property.⁸⁴ Actually, these molecules are the bodipy dimers showing interesting properties. First, the absorption spectra of these compounds split into two bands at around 490 and 560 nm (see Figure 15), which corresponds to an exciton splitting of *ca.* 2600 cm⁻¹.

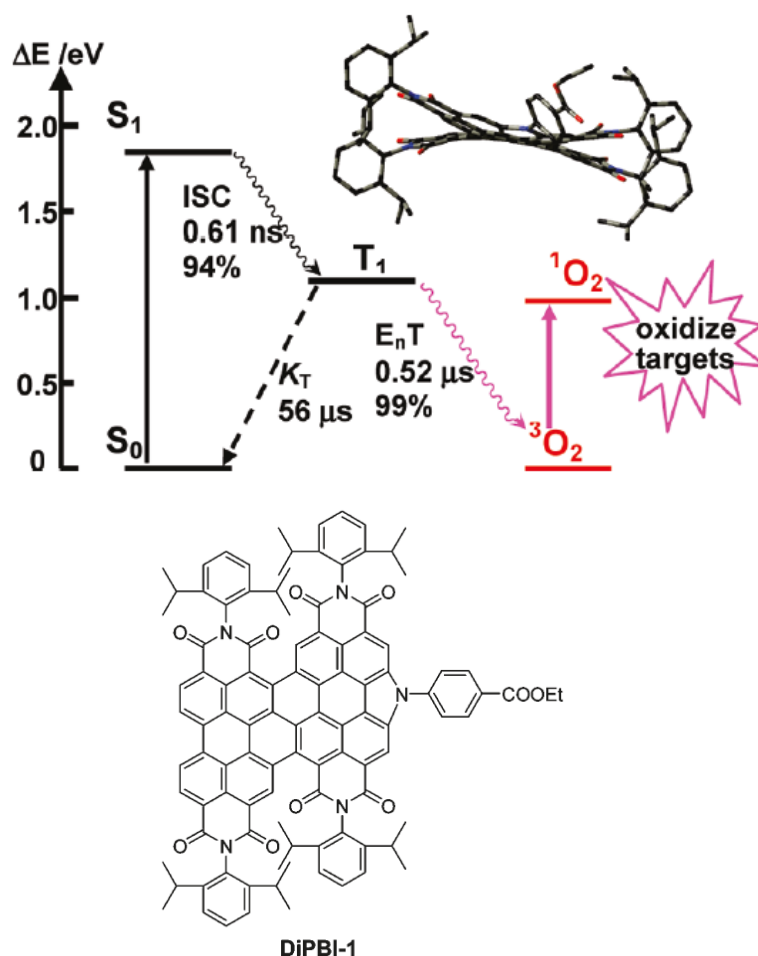


Figure 14. di(Perylene Bisimide)s as PSs for singlet oxygen generation.⁸³

The fluorescence spectra of these compounds are highly solvent dependant with Φ_f of 0.7 in toluene and less than 0.1 in acetonitrile. As it was noted before exciton splitting can facilitate triplet state population of electrons. This phenomena is explained as follows; when two molecules (*e.g.* chromophores) with the same structure come to near, their excited states split into two. The lower exciton state is now closer to triplet state; as a result population of triplet state is easier. Triplet state properties were investigated using flash photolysis apparatus and they could see transient absorption at around 800-900 nm in dimers, however they could not see in monomers. Also, singlet oxygen luminescence was also observed at 1268 nm in

aerated solution upon excitation at 490 nm. The singlet oxygen quantum yield of these dimer dyes have been quantified by using the singlet oxygen emission and found at most 0.4 in toluene, 0.5 in dichloromethane and $\ll 0.1$ in acetonitrile.

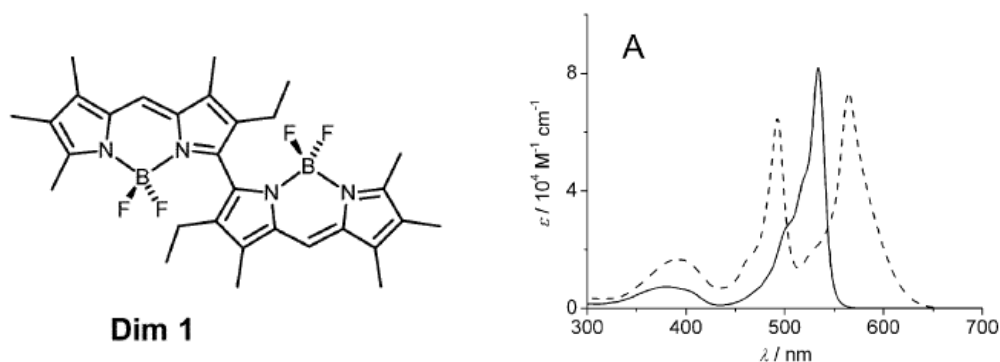


Figure 15. Exciton coupling behaviour of bis-bodipy triplet photosensitizers. The graph on the right shows absorbance of monomer (solid line) and dimer (dashed).⁸⁴

2.2.8 Employment of Triplet PSs

Triplet PSs have been used in various applications including electroluminescence, photodynamic therapy, photoinduced catalytic H_2 production, triplet-triplet annihilation upconversion, photoredox synthetic organic reactions and luminescent oxygen sensing. Transition metal based PSs have been used generally for electroluminescence and will not be discussed here more. And PDT also will not be discussed here since enough information has been given in the early part of background.

2.2.8.1 Photoinduced catalytic H_2 production

Hydrogen gas production from water (water splitting) is thought to be an important research topic for years.⁸⁵ In this topic there are three main components; photosensitizer, sacrificial reductant (SR) and water reduction catalyst (WRC).⁸⁶ The mechanism of water splitting is shown in Figure 16. First, it is needed a PS with a visible light harvesting ability and triplet excited state lifetime is also important. In

the mechanism, first PS is excited with light, and electron transfer occurs from SR to PS. After, PS transfers its electron to WRC to reduce water to obtain H₂.*

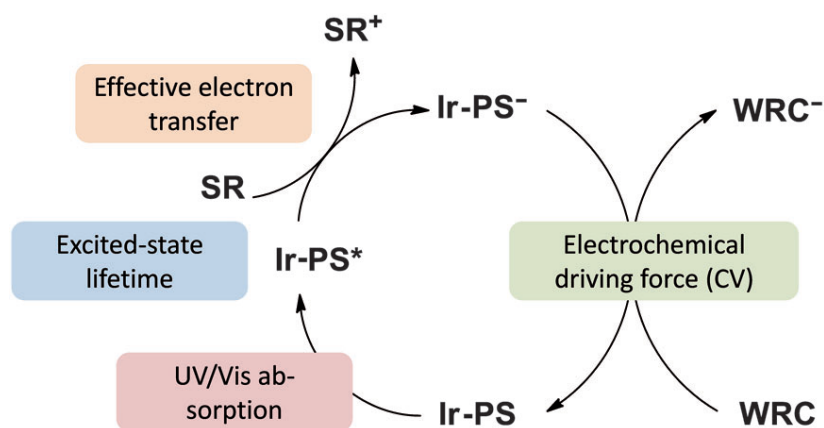


Figure 16. Mechanism for the water splitting to produce H₂. PS is for photosensitizer, SR is for sacrificial reductant and WRC stands for water reduction catalyst.⁸⁶

In an example to this research, Castellano and co-workers designed a platinum complex as a photosensitizer and they observed H₂ production from three different types of series.⁸⁷ They have used triethanolamine as a sacrificial reductant and cobaloxime as a catalyst for water reduction. The structures of platinum PSs are shown below in Figure 17 and the rate of H₂ production is larger as the length of R group is longer due to the increased visible light harvesting ability of the extended π unit.

* H⁺ is reduced to H₂

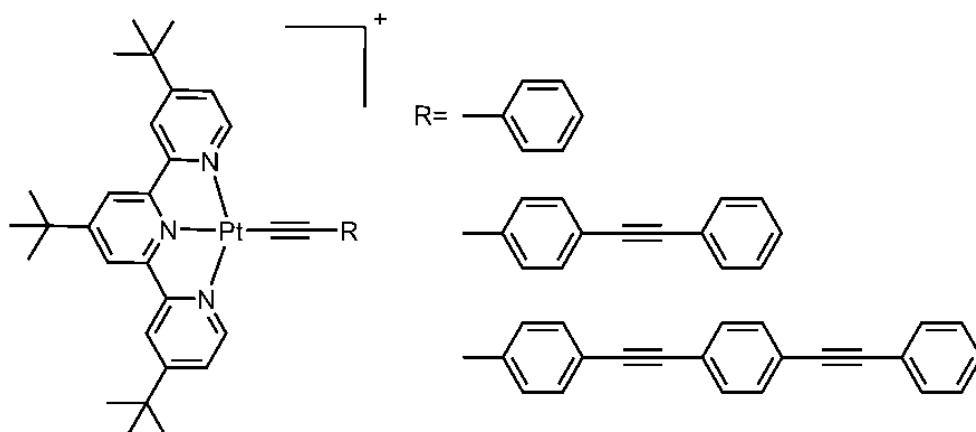


Figure 17. Molecular Structures of the Platinum(II) Terpyridyl π -Conjugated Arylacetylide PSs.⁸⁷

2.2.8.2 *Triplet Triplet Annihilation Upconversion(TTA UC)*

Due to its potential application in photovoltaics, photocatalysis and optical materials etc. upconversion is a hot topic.⁸⁸ Some of the techniques used in upconversion are by using rare earth metal nanoparticles, two-photon absorbing dyes and TTA UC. TTA UC is based on a triplet photosensitizer and a triplet acceptor often in a fluid matrix.⁸⁹ First, a PS is excited with light and singlet excited state is populated and via intersystem crossing triplet excited state of PS is populated, then a triplet acceptor molecule accepts energy transfer from the excited triplet of PS*, finally by annihilation singlet excited state of the triplet acceptor is populated; as a result fluorescence emission from the triplet acceptor's singlet excited state is observed (see Figure 18).⁷⁰ In order for this process take place triplet states of donor and acceptor should be matched to each other.

In literature a recent study exemplifies this type of upconversion where well-known bodipy molecule with heavy atoms is used as a triplet PS and perylene molecule for triplet acceptor.⁹⁰ As a result of the study, fluorescence emission form

* To which is called triplet triplet energy transfer

perylene unit at 445-518 nm range has been observed upon excitation with 532 nm laser; hence laser emission is upconverted. The long lived triplet excited state of bodipy ($\tau_T = 95.2 \mu s$) is a desired property for increased quantum yield of upconversion, where they could observe 0.165. The structure of the PS, triplet acceptor and upconverted emission is also shown in Figure 19.

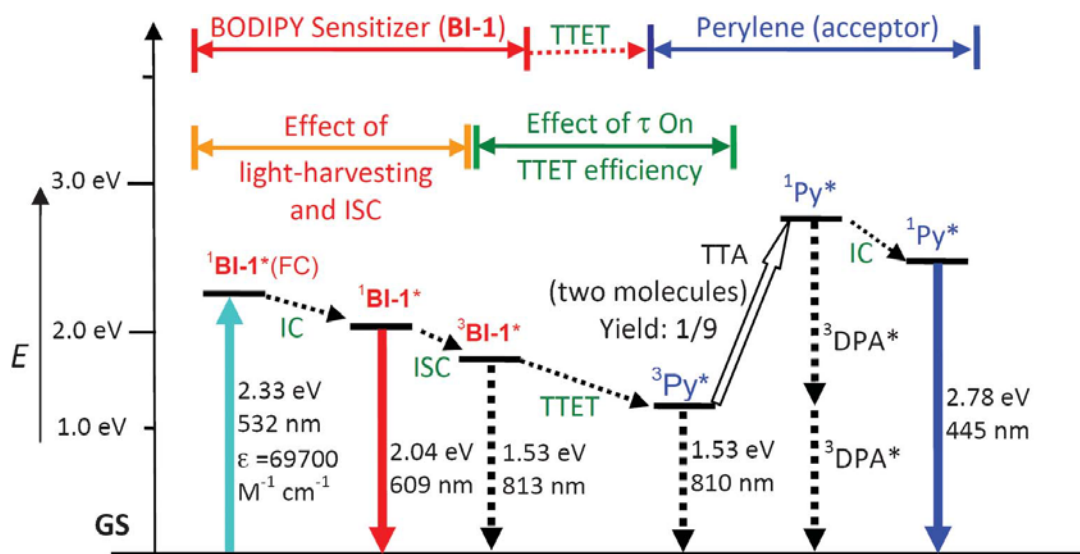


Figure 18. TTA upconversion process in modified Jablonski diagram for bodipy photosensitizer (BI-1) and perylene acceptor (Py). $^1\text{BI-1}^*(\text{FC})$ is the Franck-Condon state of excited singlet state of bodipy. $^1\text{BI-1}^*$ is the excited singlet state of bodipy. $^3\text{BI-1}^*$ triplet excited state of bodipy. Other states are similar to this. IC is internal conversion and ISC is intersystem crossing.⁹⁰

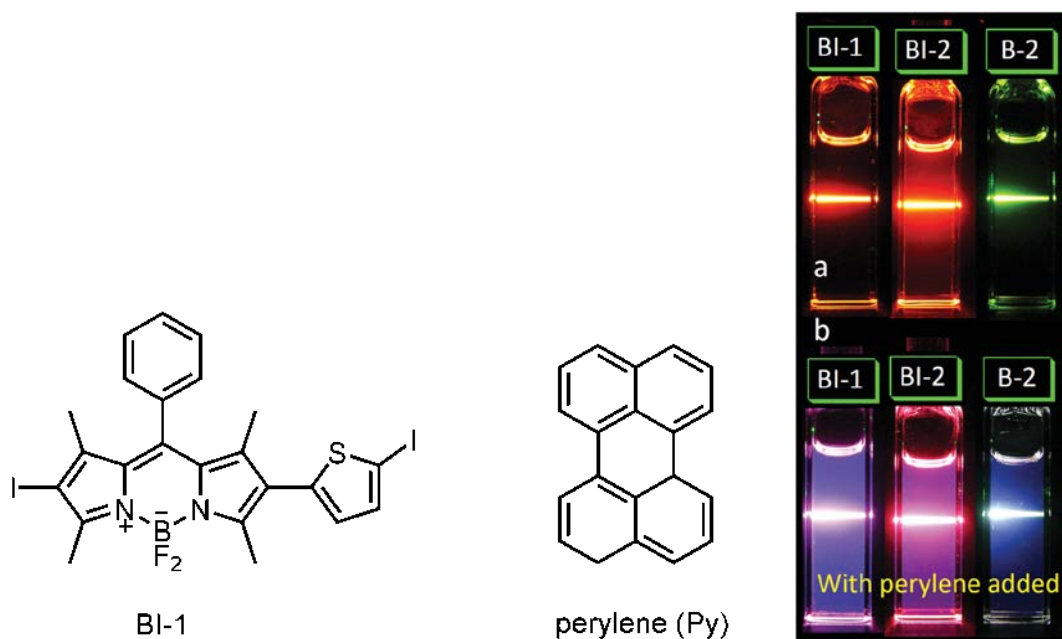


Figure 19. Structure of the bodipy PS, perylene triplet acceptor. Right emission photographs (a) sensitizers alone (b) when triplet acceptor perylene added, upconversion emission is seen. In toluene excited at 532 nm.⁹⁰

2.2.8.3 PSs in Photoredox reactions

To find green and sustainable methods for effective synthesis of important chemicals is an important task in research area.⁹¹ Visible-light-induced photoredox catalysis is one of them and due to high natural abundance, cleanliness, sustainability and environmental friendly it is an important reaction.

Recently emerged this type of reaction has been accomplished for aerobic oxidative hydroxylation of arylboronic acids to aryl alcohols (Fig. 20).⁹² They have utilized Ruthenium complex as a catalyst and in combination with visible light and air (as the source of oxygen) they could achieve excellent yields. Besides, a nonmetal organic catalyst, Acid Red 87 was also used. In the reaction first PS is excited with visible light and this excited PS converts oxygen in the air to superoxide radical anion which reacts with the arylboronic acid to yield arylalcohol.

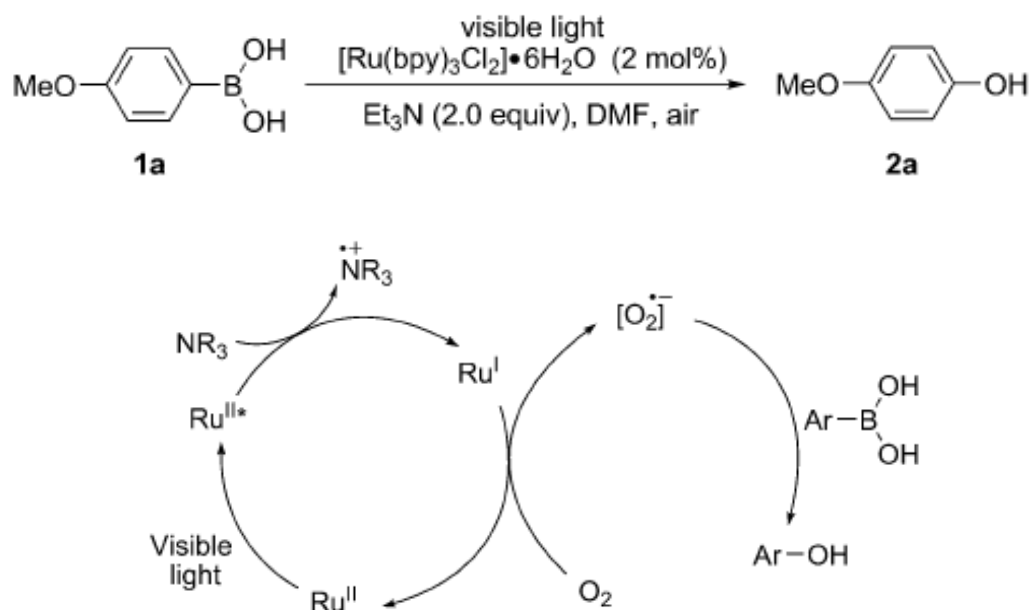


Figure 20. Proposed reaction mechanism of visible-light-induced aerobic hydroxylation reaction⁹²

Visible light induced redox reactions is not limited to this reaction, other reactions include intermolecular [3+2] cycloaddition of cyclopropylamines with olefins⁹³, C-C bond formation by aerobic oxidation⁹⁴ or aerobic cross dehydrogenative coupling reactions⁹⁵.

2.2.8.4 O_2 sensing

O_2 sensing is important for biological and environmental science but also for chemical science. Hence research in this subject has been developed and mainly transition metal complexes have been used. The reason for their utilization is their phosphorescence is quenched with O_2 hence the presence of oxygen can be tracked in that way. Conventional O_2 sensing complexes are Pt(II) or Pd(II) porphyrin based ones.

Other type of sensing strategy of oxygen is using ratiometric sensing. In this route, one fluorescent and one phosphorescent group is used separately or in one molecule. In the presence of oxygen phosphorescence is quenched however, fluorescence is not so that the amount of oxygen concentration can be tracked in that

way. For sensing to be effective, the fluorophor's emission and phosphor's absorbance should not be too close to each other (minimum spectral overlap), so that the singlet energy transfer not be very efficient. In total energy transfer case, fluorescence cannot be seen.

To exemplify, a study has been conducted utilizing a coumarin group as a fluorophor and Ir complex as a phosphorescent group (Fig. 21).⁹⁶ Coumarin group is excited at 405 nm, and due to the low absorptivity of Ir complex at visible region, coumarin make singlet energy transfer* to the Ir center, hence fluorescence is observed and phosphorescence is quenched by oxygen molecule. This ratiometric sensor is also used in living cells.

* Not total but partial

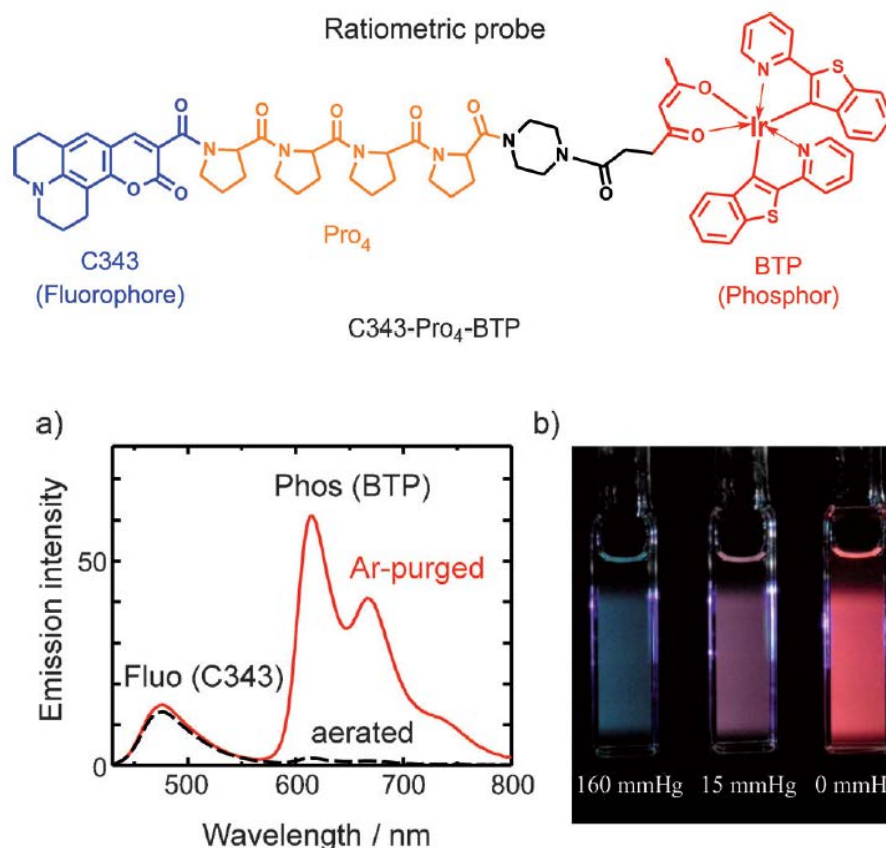


Figure 21. Ratiometric O_2 sensor (on top) a) Emission Intensity of aerated (black dash) and Argon purged (red line) in CH_3CN b) the different emission colors due to the different O_2 partial pressure.⁹⁶

2.3 Asymmetry in Luminophores

Asymmetry is an important topic in biology. For example knowing the stereochemical structure of the potential drug is essential because the potency, selectivity of one enantiomer can be different from the other enantiomer. Solution based sensor systems are important while learning these information during drug-receptor interactions.

Basically, isomers are divided into two main categories and stereoisomers is one of them. The other type structural isomerism is out of our topic. Stereoisomers also has two parts; enantiomers and diastereomers. Enantiomers are characterized by their nonsuperimposability, while diastereomers are not related with their mirror

images. For instance, enantiomers are their mirror images and they are not superimposable to each other, however, diastereomers are not mirror images of each other.

The simplest source of chirality in an organic molecule is the carbon atom with four different substituents. Lactic acid is the oldest example of chirality.⁹⁷ Both enantiomers have been found in nature. (–)-lactic acid is found in certain fermentation products of sugars, in some species mixed with its enantiomer. (+)-lactic acid is found in muscle fluid and it forms after the biochemical reaction of muscle work. Most properties of matter do not change with reflection, so two enantiomers' are identical in many aspects like solubility, density, refractive index, IR, Raman, UV, NMR and very similar X-ray diffraction pattern. Only their chiroptical properties such as optical rotation, optical rotatory dispersion and circular dichroism different in terms of sign but not magnitude.⁹⁷ Some of the fermentation products of lactic acid are mixture of two enantiomers, so they are (±)- or racemic lactic acid. However, in nature these racemic mixtures are unusual since most of the chiral products are either single enantiomer or one enantiomer is predominant.

2.3.1 *Optical Spectroscopy*

Optical spectroscopy is mainly based on the interaction between the atoms or molecules with incident electromagnetic radiation.⁹⁸ In 1860, it was postulated by Scottish physicist James Clerk Maxwell that oscillating electric field generates an oscillating magnetic field and reverse is also true. So, a sinusoidal electromagnetic field takes place when electric field and magnetic field oscillate perpendicular to each other and to the direction of propagation. It was described by Albert Einstein that electromagnetic radiation has both wave and particle properties and in quantum mechanical terms as a particle, referred as a photon which has no mass or charge. In the long wavelength region of the electromagnetic spectrum which is greater than 1000 nm, an oscillating electromagnetic wave interacts with nuclei and causes molecular vibrations and detected by infrared spectroscopy. In the shorter wavelengths region, e.g. UV-Visible, the electromagnetic radiation can no more

interact with the nuclei significantly, however, its oscillation can still affect the surrounding electrons which are polarized to the direction of oscillating electron field, resulting in an electronic transition from ground state configuration to the excited state configuration. Information about this process is measured by using UV-Visible absorption spectroscopy.

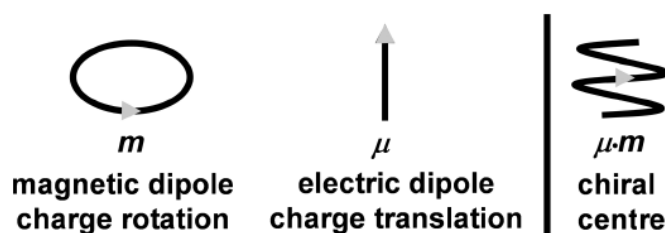


Figure 22. The combined effect of electronic and magnetic transition moments, which is the origin of chirality.⁹⁸

In organic molecules, an absorption phenomenon is often related with the type of bond or a structural unit in the molecule. For example, for the case of saturated organic compounds wavelengths shorter than 200 nm is needed to cause electronic transitions. Current UV-vis spectrometers which are working at room temperature are generally restricted to above 200 nm because in lower wavelengths strong absorption takes place by oxygen and ozone formation. Due to this limitation, this type of spectroscopy mainly applied to π bond including organic molecules or inorganic metal complexes.⁹⁸

2.3.2 CD Spectroscopy

Optical data is the analysis of absorption or emission of a photon of light with specific wavelength.⁹⁸ During analysis redistribution of charge in a molecule takes place which is based on a transition moment coupling with the ground and excited electronic states and as a result a new stationary excited state occurs. When electromagnetic radiation is exerted on a molecule the electric field of

electromagnetic radiation cause linear displacement of the electronic charge^{*} and magnetic field part cause a circulation of charge.[†] When there is no mirror plane or centre of symmetry these two motions combine to cause a helical redistribution of the electron density. The incident photons are chiral because they are composed of left and right circularly polarized light, hence their interaction with chiral molecules are different in terms of different electronic excitation of electronic transitions. For organic molecules, carbon atom with four different substituents is said to be chiral centre. Stereoisomers of a chiral molecule will prefer to absorb either left circularly polarized light or right circularly polarized light according to its handedness of helical electronic redistribution for a particular transition. After this absorption a differential absorption take place due to unequal absorption of left or right circularly polarized light and this affect is often called *Cotton effect* which forms the basis of *CD spectroscopy*.⁹⁸

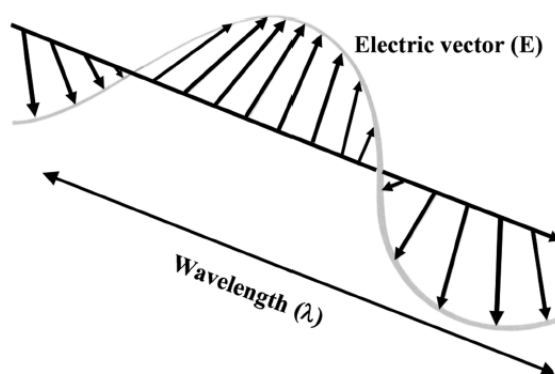


Figure 23. The electric vector of left circularly polarized light following an anticlockwise path. It rotates per wavelength along the axis of light propagation⁹⁸

^{*} which is called electric dipole transition moment

[†] Called magnetic dipole transition moment

Today, CD intensity is usually given as the differential absorbance of left (A_L) and right circularly polarized (A_R) light and it can be expressed as:

$$\Delta A = A_L - A_R$$

The absorbance value can also be given as molar absorptivity:

$$\Delta \epsilon = \epsilon_L - \epsilon_R = \Delta A / cl$$

Where ϵ_L and ϵ_R are molar extinction coefficients, c is the molar concentration and l is the pathlength in cm.

Chemists and biochemists often use absorbance and molar extinction coefficients, however, due to the historical background of CD spectroscopy a new term ellipticity (θ) is used.

Plane polarized light can be thought as a superposition of left and right circularly polarized light with equal amplitude and phase, then a projection of these combined amplitudes of these two components which are perpendicular to the axis of light propagation yields a straight line. If this linear polarized beam passes through an optically active sample, the amplitude of the more strongly absorbed component will be smaller than the other component which is absorbed less. As a result, an ellipsoid figure compose when the amplitudes of these combined. *The degree of ellipticity* is defined as the tangent of the ratio of the minor to major elliptical axes. Linearly polarized light has 0° of ellipticity, the full left and right circularly polarized light has $+45^\circ$ and -45° ellipticity, respectively.

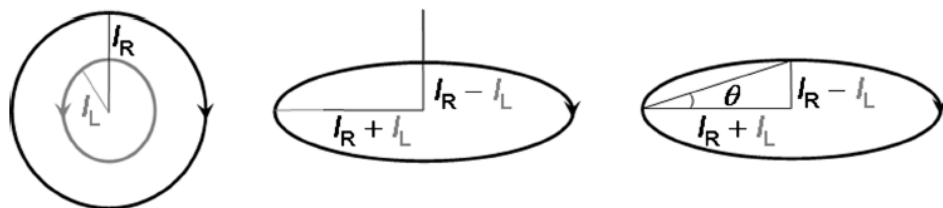


Figure 24. When left and right circularly polarized light have different intensities: I_R and I_L , they combine to form an ellipsoid not an linearly polarized beam. The ellipticity, θ is the angle between the long and short axes of the ellipse.⁹⁸

The conversion between ellipticity and relative absorbance is as follows;

$$\Delta A = \theta / 32.982$$

Molar ellipticity $[\theta]_M$ can be derived similar to the molar absorptivity calculations according to the Beer-Lambert law. The units of molar ellipticity are $\text{deg.cm}^2.\text{dmol}^{-1}$ or $\text{deg.M}^{-1}.\text{m}^{-1}$ and it is calculated as:

$$[\theta]_M = 100\theta / cl$$

And finally relationship between molar extinction coefficient and ellipticity is:

$$\Delta \varepsilon = [\theta]_M / 3298.2$$

2.3.3 Atropisomerism

As told before most of the chiral molecules have a chiral stereogenic center. Carbon atom is the most abundant atom which induces chirality to the molecules. However, nitrogen atom and boron atom based chirality can also be found in literature. Another type of chirality can be found in some molecules without having a chiral center as told. These are called axial chirality and can be defined as the type of stereoisomerism resulting from the non-planar arrangement of four groups in pairs about a chirality axis. The examples of these types of structures are allenes and atropisomerism of *ortho*-substituted biphenyls. The two different configurations of

this type of chirality can be specified by R_a and S_a (or by P or M). These two types of structures are shown below (Figure 25).

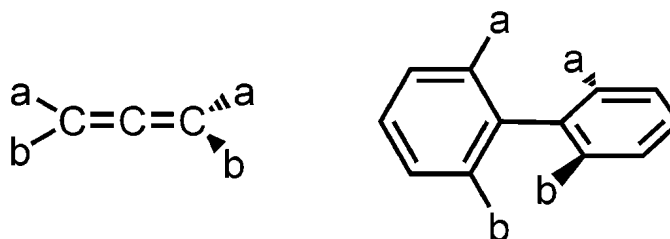


Figure 25. Axial chiral molecules.

In allenes, the chiral axis is sustained by high barrier to rotation of a double bond. However, in atropisomeric molecules the chiral axis whose helical sense is maintained by hindered rotation about single bonds.⁹⁷ So, the hindrance is due to the steric effect of the substituents. Biphenyl example is a very classical example to this type of molecule and very few examples have been reported to date. And in biphenyls $a \neq b$ and the steric interaction between a and b and a and a or b and b should be large so that the planar arrangement is not favorable. If the interconversion to the planar arrangement is slow the enantiomers can be resolved.* This type of stereoisomerism is first found by Christie and Kenner in 1922 with 6,6'-dinitro-2,2'-diphenic acid[†] and they could be able to separate it. In 1933, Kuhn called this type “atropisomerism” (in Greek, *a* means not and *tropos* mean turn).

1,1'-Binaphthyl dimer, oligomer and polymers are important and have been utilized in molecular recognition, asymmetric catalysis and as novel molecules in different systems. These compounds are also used to do analysis in CD spectroscopy, when they are attached to another chromophore. Since binaphthyl

* e.g. separated or isolated

[†] Biphenyl in Figure 23, where $a = \text{NO}_2$ and $b = \text{COOH}$

moiety is not absorbing in the visible region, the chromophore attached should strongly absorb at that region. The other properties that this chromophore should have are large, symmetrical and achiral. In an example phthalocyanines have been used to get use this molecule in chirality concept (Figure 26).⁹⁹

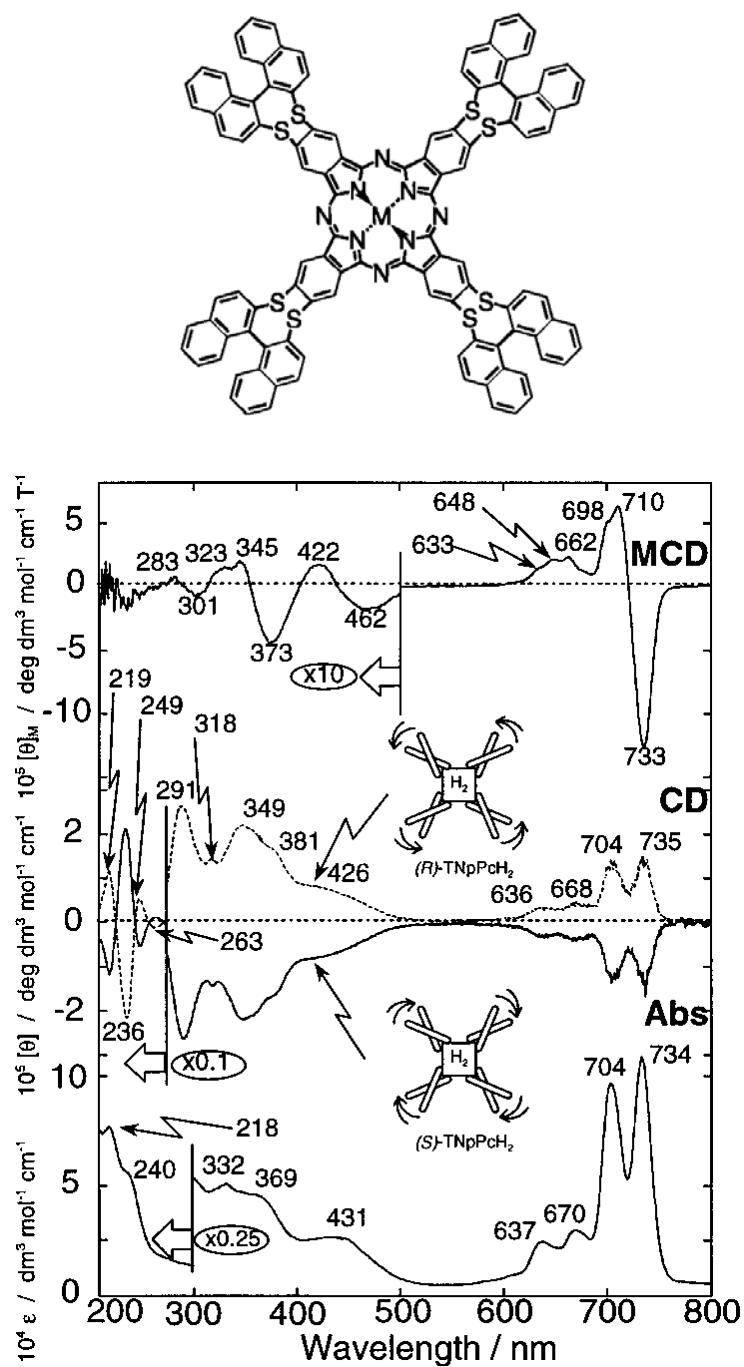


Figure 26. Chirality induced to phthalocyanine molecule by binaphthyl.⁹⁹

As can be seen in Fig. 26 that the CD signal at 709 nm is splitted into two from different enantiomers and originated from phthalocyanine molecule.

These types of molecules are important in terms of distinguishing between enantiomers. Since chiral compounds are everywhere in nature, this type of discrimination is crucial for designing new materials, such as drugs. In this way, there has been published a study that differentiation between two types of chiral compounds has been accomplished, 1-phenylethylamine, which is a chiral compound and the stereogenic center is the nitrogen atom with four different substituents and one of them is the lone pair electrons.¹⁰⁰ In order to discriminate the chiral amine, an organic compound which is also chiral and fluorescent is needed in order to get a quantitative data such as emission spectrum. They have used again binaphthol unit with bodipy chromophore. Since bodipy chromophore has the ability to absorb strongly in visible region and has a high fluorescence quantum yield, incorporation of this dye results in a new compound which has dual property; chirality and sensing. It is known before that 1,1'-bi-2-naphthol can selectively recognize optically active amines or amino acids, hence it is chosen for a chiral receptor unit. The chiral compounds (R-enantiomers) have been obtained by enantiomerically pure binaphthol derivative reaction with pyrrole.

It has been shown that in earlier studies that deprotonation of the phenol-appended-bodipy molecule causes fluorescence quenching.¹⁰⁰ This is due to the photoinduced electron transfer process from phenoxide unit to the bodipy core. In this study Daub et al. wanted to benefit from this fact and they synthesized two compounds in Fig. 27, in one compound hydroxy unit exists in the other methoxy groups exist. Upon addition of achiral diisopropylethylamine they have observed fluorescence quenching in hydroxyl including compound, while no difference take place in methoxy including compound.

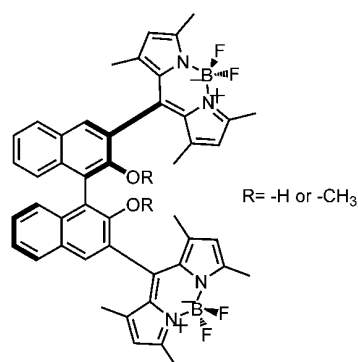


Figure 27. Structure of optically active fluorescent binaphthol appended bodipy¹⁰⁰

In the second and the important part of the study, they have added two enantiomers of 1-phenylethylamine in a solution of compound 1 in Fig. 27 in acetonitrile. Upon addition of both amines, quenching was observed, however, their quenching rate is different and they could get a data as in Fig. 28, showing clearly, which authors argue that this compound can be used as a chiral discriminator. The detailed mechanism of quenching process has not been studied, but it is told that *R*- and *S*- isomer of amine binding can result different structural reorientation of the bodipy units.

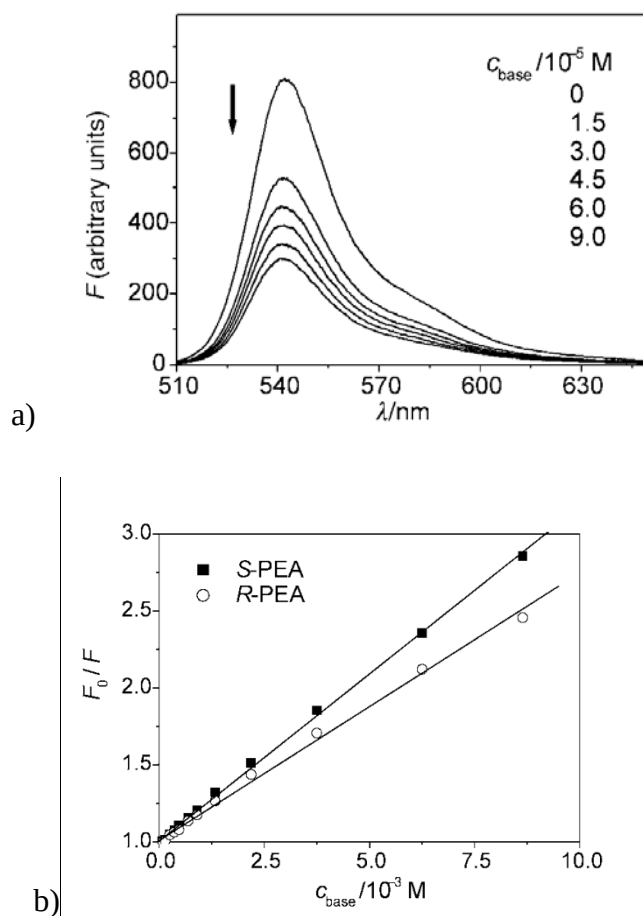


Figure 28. a) decrease of bodipy fluorescence with the addition of diisopropylethylamine in acetonitrile. b) Plots for the quenching of chiral hydroxyl binaphthol bodipy with S- and R- Phenylethylamine (PEA) in acetonitrile.¹⁰⁰

2.3.4 Bodipy Asymmetry

There is a demand of luminophores for the use in modern biological labeling and advanced optoelectronic devices, therefore chemists has an intention to design and engineer new type of organic dyes to use in these applications.¹⁰¹ Bodipy is an important luminophore and it is used frequently in advanced applications including sensors¹⁰², energy transfer cassettes¹⁰³, biological labeling¹⁰⁴, or photovoltaics⁴. Its core is also open to modification which is possible to modify its optical and physical properties. It was notified before that most known chiral organic fluorophores are binaphthol modules and they have used in chiral recognition processes like determining enantiomeric purity of chiral amino acids, amines or alcohols.¹⁰⁵

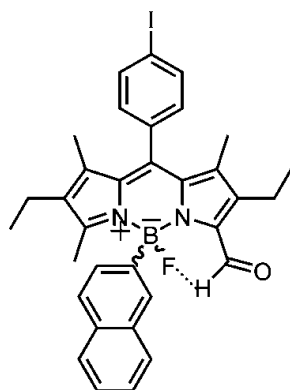


Figure 29. Boron asymmetry in bodipy molecule.³

In order to utilize bodipy core for these applications Ziessel et al. have engineered asymmetric bodipy units by modifying the boron center, e.g. the stereogenic center is the boron atom (Figure 29).³ The number of boron complexes which are stable and asymmetric is less due to the ease of dissociation and inversion processes. The stability of boron is ensured with N-donor chelate which was expressed that it is related to the N-B bond strength.¹⁰⁶ The few examples of enantiomerically pure bodipys known were obtained with decoration of the central core with asymmetric carbon atom^{100, 107} and this research is the first decoration of the boron center.

During designing process following requirements were needed; (i) lateral differentiation of the dipyrromethene core; (ii) attaching a polar group in order to associate intramolecularly with BF unit; (iii) using polyaromatic units for inhibiting rotation around boron atom and easing resolution in chiral HPLC. The antisymmetric core has been accomplished by oxidizing one of the methyl units to aldehyde with DDQ. By this way, it was also made possible that aldehyde proton associate with F atom, so that rotation of F is not possible. Reacting with naphthylmagnesium bromide also led to structure sterically hindered for rotation. The nature of F-H association has also been verified with X-ray crystallography. As a result, optically active bodipy is synthesized and separated with chiral HPLC and

they could analyze the enantiomers in CD successfully (Figure 30). This study is the first example of a fluorophore bearing chiral boron center and may serve as a model compound for similar studies in the way of fluorimetric sensing of chiral biological compounds.

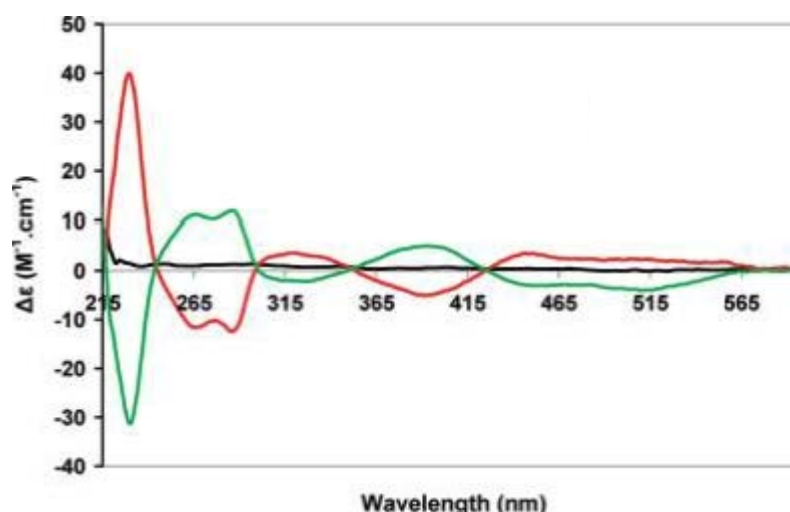


Figure 30. Circular Dichroism (CD) spectra of enantiomeric bodipy molecules; racemic mixture (black), enantiomers (red and green).³

2.4 Dye Sensitized Solar Cell

2.4.1 General Information

Dye sensitized solar cells are a type of solar cell which is based on an organic molecule (e.g. a photosensitizer) is adsorbed on a semiconductor oxide electrodes* and it is one of the important research topics around the world due to its being a sustainable source of energy, low cost of production and high incident-solar-light-to-electricity conversion efficiency.¹⁰⁸ Conventional solar cells are silicon based and their energy conversion efficiencies are reasonable in 15-25% range. On the other hand, the thing that limits the wide-spread use of these in our life is their

* Such as TiO₂, ZnO or NiO

requirement of high-purity silicon and skilled manufacturing techniques, which means that they need high amount of electricity produced from fossil fuels.¹⁰⁸

The first publication of dye sensitized solar cells has been published by Gratzel and O'Regan by using polypyridyl ruthenium (II) complex dyes adsorbed on a nanocrystalline n-type TiO₂ electrode.¹⁰⁹ Most DSSCs are constructed with a dye adsorbed on a wide band gap of a semicrystalline oxide, a liquid electrolyte usually contains I^-/I_3^- and a platinum-coated counter electrode. To date highest conversion efficiencies with Ru dyes have been obtained with N3¹¹⁰, N719¹¹¹ and Black dye¹¹², owing to their intense and wide range of absorption of sunlight. The efficiency measurements have been done under air mass AM 1.5 simulated sunlight (100 mW cm⁻²).

Like ruthenium based DSSCs, organic dye based DSSCs have also been studied intensely. Their advantages are (i) prepared and purified with lower cost, (ii) higher molar extinction coefficients than ruthenium dyes and have wide range of absorption capability (iii) many alternative structural groups can be used, so that control of photophysical, electrochemical and three-dimensional structure on semiconductor is possible (iv) no resource limitation since the structure is not based on rare metals such as ruthenium.¹¹³ Some of the dye types synthesized up to date to be used in DSSC concept are coumarin dyes, polyene dyes, hemicyanine dyes, bodipy dyes, perylene and porphyrin dyes.

There is much effort to get high conversion efficiencies with organic dye DSSCs but still they remain inferior compared to Ru dyes. Some reasons for this situation are; (i) relatively narrow absorption bands of visible light (ii) tendency to p-stack on the semiconductor surface of these dyes which leads to decrease in the amount of injected electrons from dye to the conduction band of semiconductor due to intermolecular energy transfer (iii) relatively low open-circuit photovoltage (V_{oc}) values due to the faster charge recombination between the injected electrons in

semiconductor and triiodide (I_3^-)^{*} (iv) the stability of organic dyes are generally lower, which may be due to the radicals formed upon strong irradiation prone to decomposition.¹¹³

2.4.2 *Main types of organic dye based DSSCs*

Although many studies have been done in this concept because of the importance of energy is well-known, in recent years improvement of cell efficiencies slowed down. In order surpass this situation scientists try new solar cell types and up to now following cell types have been studied:¹¹³

- [1] n-type semiconductor TiO_2 -based Type-I DSSC
- [2] n-type semiconductor TiO_2 -based Type-II DSSC
- [3] new TiO_2 DSSCs with a pyridine group as the anchoring group to semiconductor
- [4] n-type semiconductor ZnO -based Type-I DSSC
- [5] p-type semiconductor NiO -based Type-I DSSC
- [6] tandem DSSCs with TiO_2 as the photoanode and NiO as the photocathode

Among these cell types most studied one is the first type and highest efficiency with 10.1% have been achieved.¹¹⁴ Hence, focus will be on this type.

The detailed mechanism, differences and operational principles of these cells can be found in the recent informative review by Ooyama and Harima.¹¹³ However, basic information about n-type TiO_2 based Type-I DSSC will be outlined below.

2.4.3 *n-Type Semiconductor TiO_2 -based Type-I DSSCs*

^{*} Related to the electron lifetime which is smaller in organic based DSSCs

Schematic representation of n-TiO₂-based type I DSSC is shown below and it is mainly composed of a photoanode and a cathode* which sandwiches an electrolyte solution. It is composed of five materials: 1) Fluorine-doped tin oxide (FTO) 2) a nanocrystalline TiO₂ thin film as a semiconductor 3) an organic dye 4) a redox mediator electrolyte solution 5) platinum-coated glass substrate.

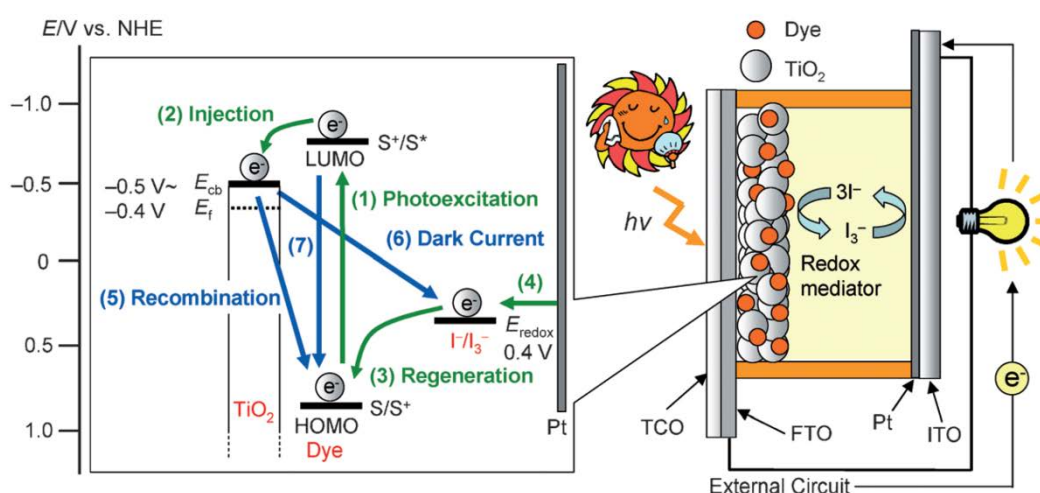


Figure 31. Working scheme and principles of n-type TiO₂ DSSC (Type I)¹¹³

From three main parts photoanode is prepared first by applying nanocrystalline TiO₂ particles to the FTO glass then this material is dipped into the solution of dye to adsorb to the TiO₂ surface. Cathode part is composed from platinum-coated glass substrate and electrolyte is usually a solution of I⁻/I₃⁻.

The generation of photocurrent is occur as follows; first organic dye[†] is excited with sunlight and electron from HOMO of the dye excited to the LUMO, which injects to the conduction band of TiO₂[‡], an electron from the I⁻ in the electrolyte solution go to the cationic dye's HOMO so that cationic dye returns to its neutral state and the injected electron move through interconnected TiO₂

* e.g. a photoinert counter electrode

[†] also called photosensitizer

[‡] or ZnO depends on the semiconductor type used

nanoparticles and reaches to the FTO glass and then to the counter electrode through external circuit (Pt-coated glass). Afterwards, the electron reduces I_3^- to regenerate I^- and the circuit is completed then. The mechanism of these processes is shown below in Fig. 32.

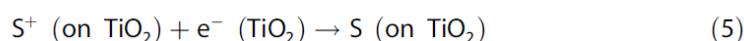
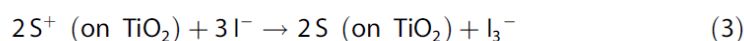
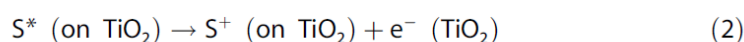
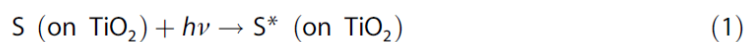


Figure 32. Equations showing the mechanism of photocurrent via n-type TiO₂ based DSSC (Type I)¹¹³

In the mechanism told above there are some problematic issues to deal with. These undesirable side processes originate from (i) when electron injected from LUMO of the dye to the conduction band of TiO₂ it reduces the oxidized dye again to its neutral state (*recombination*) or I_3^- to yield I^- (*dark current*). Also, radiationless relaxation from excited state of the dye to the ground state also takes place. In order to get a high photocurrent value electron injection and regeneration should be kinetically more favorable than dark current and recombination processes.

2.4.4 Solar Cell Photovoltaic Parameters¹¹³

Mainly the performances of DSSCs have been evaluated according to six parameters: IPCE, photocurrent/voltage curves (J/V curves), open circuit voltage (V_{oc}), Short circuit photocurrent density (J_{sc}), fill factor (ff), solar energy-to-electricity conversion yield (η). In addition to these, dye stability under solar illumination is also an important factor. These six important parameters will be explained below.

2.4.4.1 Incident-Photon-to-Current Conversion Efficiency (IPCE)

IPCE is also called as external quantum efficiency (EQE) and the definition is the number of electrons flowing through the external circuit from anode to cathode which is divided by the number of incident photons from sun light. The equation is:

$$\text{IPCE (\%)} = \frac{1240 \text{ (eV nm)} J_{ph} \text{ (mA cm}^{-2}\text{)}}{\lambda \text{ (nm)} I \text{ (mW cm}^{-2}\text{)}}$$

where J_{ph} is the short circuit photocurrent density which is generated by the monochromatic light, λ is the wavelength of the monochromatic light and I is the intensity of the monochromatic light.

Another method for determining the IPCE value is according to the following equation:

$$\text{IPCE}(\lambda) = \text{LHE}(\lambda) \cdot \Phi_{inj} \cdot \eta_{coll}$$

In this equation LHE is the light harvesting efficiency of the dye at wavelength λ and it is determined by

$$\text{LHE}(\lambda) = 1 - 10^{-A}$$

Quantum efficiency of the injected electrons to the TiO_2 is also an important parameter, and η_{coll} is the collection of the injected electrons in the FTO glass.

As a result, IPCE is directly related to the absorption assets of the dye, loading of the adsorbed dye amount on TiO_2 , electron injection efficiency of the dye LUMO to the conduction band of TiO_2 and efficiency of the collected electrons in the external circuit. The maximum efficiency of the IPCE (%) is 80-85 % and the total efficiency is not achievable due to the reflection and absorption losses of the FTO glass. IPCE of the DSSC is generally given as a spectrum of IPCE versus excitation wavelength.

2.4.4.2 **Photocurrent/Voltage Curves (*J/V* curves)**

Under standard AM 1.5 sunlight simulation (100 mW.cm^{-2}) measuring the DSSC performance is easily analyzed with *J/V* curve. And four imperative parameters are V_{oc} , J_{sc} , ff and η which are frequently used to analyze the DSSC performance will be explicated below.

2.4.4.3 **Open-Circuit Photovoltage (*Voc*)**

The definition of this important parameter is the difference between the electrical potentials of the two compartments of the cell upon illumination with sunlight when the circuit is open. The maximum value of this parameter is the difference between the energy level of the conduction band of TiO_2 (E_{cb})^{*} and the redox potential of the electrolyte E_r (I^-/I_3^-)[†]. This corresponds to a value approximately 0.8-0.9 V. However, this value decreases in real applications because of the dark current and recombination events.

2.4.4.4 **Short-Circuit Photocurrent Density (*J_{sc}*)**

This value corresponds to the photocurrent per unit area (mA.cm^{-2}) when the DSSC under irradiation is short circuited. Open circuit voltage (V_{oc}) is the value between the E_f of the electron in TiO_2 and the redox potential of the electrolyte. However, J_{sc} is related with the communication between TiO_2 and dye adsorbed and the absorption capability of the dye. As a result, J_{sc} value is dependant to (i) ability of the dye molecules absorb sunlight over a wide range of the electromagnetic spectrum (ii) electron-injection ability of the dye from HOMO to the CB of the semiconductor oxide (iii) reduction efficiency of the cationic dye molecules by I^- after injection. Consequently, photophysical and electrochemical properties of the dye molecules and molecular structures of the dye are highly important in order to get high J_{sc} value. Hence, the interaction between dye molecules and TiO_2 surface is

^{*} It is -0.5 to -0.4 V vs. NHE (normal hydrogen electrode)

[†] 0.4 V vs. NHE

also important and orientation of the dyes on semiconductor is also crucial. J_{sc} value can be found by integrating the IPCE spectrum according to the following equation:

$$J_{sc} = e \int IPCE(\lambda).I_s(\lambda).d\lambda$$

Here $I_s(\lambda)$ is the photon flux at wavelength λ while irradiated with AM 1.5.

2.4.4.5 **Fill Factor (ff)**

This term is defined as the maximum power output ($J_{mp}V_{mp}$) divided by the product of J_{sc} and V_{oc} . The ff is determined according to the J/V curve and it is found according to the maximum area of the rectangle. So, it is obvious that maximum value of fill factor is unity but various resistances in the cell like electron transport resistance at the cathode reduce this value.

2.4.4.6 **Solar Energy-to-Electricity Conversion Yield (η)**

This term is defined as the ratio of the maximum output electrical power of the DSSC to the energy of incident sunlight (I_0) and it is calculated according to the following equation:

$$\eta(\%) = \frac{J_{sc}(mA.cm^{-2}).V_{oc}(V).ff}{I_0(mW.cm^{-2})}$$

In order to get a high value of η V_{oc} , J_{sc} and ff of the cell should be optimized. This can be attained by optimization of the electrochemical and photophysical properties of the dye, and control of the dye molecules' orientation and arrangement on the semiconductor layer.

CHAPTER 3

3 NOVEL SENSITIZATION IN PDT WITH BODIPY DYES

First part of this chapter was published in the following publications:

Cakmak, Y.; Kolemen, S.; Duman, S.; Dede, Y.; Dolen, Y.; Kilic, B.; Kostereli, Z.; Yildirim, L. T.; Dogan, A. L.; Guc, D.; Akkaya, E. U. *Angew Chem Int Ed* **2011**, 50, 11937-11941.

Duman, S.; Cakmak, Y.; Kolemen, S.; Akkaya, E. U.; Dede, Y. *J Org Chem* **2012**, 77 (10), 4516-4527 (*Featured*).

3.1 *Theory-Guided Access to Efficient Photosensitizers by Heavy Atom Free Orthogonal Bodipy Dimers*

3.1.1 *Introduction*

Photodynamic therapy (PDT) is a treatment modality for certain malignant (skin, head and neck, gastrointestinal, gynecological cancers), premalignant (actinic keratosis), and nonmalignant (psoriasis) indications.¹¹⁵ Broader acceptance by the medical community and applicability is hampered, at least in part, by the less than optimal photophysical characteristics of the porphyrin derivatives. This situation sparked a worldwide search for novel sensitizers leading to new compounds, some holding more promise than others.¹¹⁶ The primary cytotoxic agent involved in the photodynamic action is singlet oxygen ($^1\Delta_g$), the efficient generation of which is linked invariably to the intersystem crossing (ISC) efficiency of the excited state of the sensitizer. Most organic dyes have low triplet quantum yields, and in many recent candidates for photodynamic sensitizers, heavy atoms are incorporated into the structure as a strategy to improve spin-orbit coupling leading to facilitated intersystem crossing.⁷⁸ While this approach seems fail-safe, incorporation of heavy atoms such as bromine, iodine, selenium, and certain lanthanides very often leads to increased “dark toxicity”.¹¹⁷ Unlike traditional chemotherapy agents, in principle,

photodynamic therapy sensitizers themselves can be nontoxic, either at cellular or organ levels, even at relatively high concentrations. We have been interested in trying to find alternative ways of achieving increased intersystem crossing without the use of heavy atoms to minimize dark toxicity and to investigate the intersystem crossing mechanism, turning our attention to the excited state properties of the sensitizers.

3.1.2 *Theoretical Investigation*

Designing efficient photoinduced $^1\text{O}_2$ generators requires that any existing operative fluorescence cycle of the fluorophore, which is through the $S_0 \rightarrow S_1 \rightarrow S_0$ states, has to be perturbed so as to minimize or shut down the $S_1 \rightarrow S_0$ deactivation, and switch to the triplet surface once S_1 is accessed (intersystem crossing). A general design principle for a favorable $S_1 \rightarrow T_1$ transition from an electronic structure viewpoint would in principle require the structural and electronic compatibility of the S_1 and T_1 states to surpass that of the S_1 – S_0 pair.

Once multiple electronic states come into play, quantum mechanical calculations providing a detailed understanding of the electronic structure are extremely helpful. Multi-configurational self-consistent field (MCSCF) techniques are the state-of-the-art computational chemistry approaches, when near degeneracies and excited states are considered. These methods are not accurate for ± 2 – 3 kcal mol^{-1} , but they are very helpful while understanding the photophysics taking place. Therefore, a popular variant of MCSCF techniques was used; the complete active space SCF (CASSCF) method in combination with relatively large basis sets and different active spaces. Details of CASSCF calculations are provided in the computational methods part (Section 3.1.5.8).

Calculations on the parent Bodipy (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene, Fig. 33) showed that natural orbital occupancies of S_1 state do describe an open-shell singlet with essentially double (>1.9) or zero (<0.1) electrons for all orbitals except HOMO and LUMO that are singly occupied (simple single electron excitation taking place in a fluorophore upon excitation). Since most of the

fluorophores have an excited state comprising of only two orbitals with single occupancy leads to low triplet quantum yields, one should design a dye which is different than the simple HOMO $\rightarrow$ LUMO transition. Among multiply excited configurations, doubly substituted ones are particularly important in enhancing S_1 - T_1 coupling as shown by the seminal work of Salem and Rowland¹¹⁸ and the following work by Michl.¹¹⁹ Thus, the substitutions should invoke simultaneous two electron excitation from the ground state, guiding us to search for molecules with a pair of degenerate or near-degenerate occupied frontier orbitals correlating with a similar virtual (unoccupied) pair and pointing out a design principle of orthogonal dimeric chromophores. This foundation has two important features: (i) Orthogonal placement prevents mixing of the π -systems of the two subunits leaving us with two essentially undisturbed chromophore cores. (ii) Upon irradiation, both cores are almost equally likely to undergo a HOMO $\rightarrow$ LUMO electron transfer (referring to the original monomer MOs) yielding an excited state mainly comprised of double substitutions.

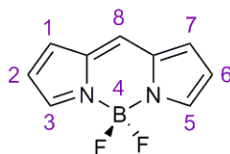


Figure 33. Structure of Bodipy (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene)

3.1.3 *Design Principles*

Our previous involvement with Bodipy dyes led us to seek ways to implement these design considerations using these dyes. Bodipy dyes are exceptional fluorophores with amazing versatility¹²⁰ and rich chemistry.¹²¹ Heavy atom functionalized Bodipy dyes showed some promise as potential sensitizers for photodynamic activity.¹²² It is also interesting to note that a few years ago, dimeric (albeit non-orthogonal) Bodipy derivatives were reported¹²³ with peculiar properties linked to exciton coupling between the chromophores. We believe orthogonality to be an important distinction in our design. The calculations on the orthogonal 8-2' dimer **OB3** clearly show (Fig. 34) the essentially unperturbed Bodipy HOMO and LUMO as a result of lack of mixing of orthogonal π -frameworks.

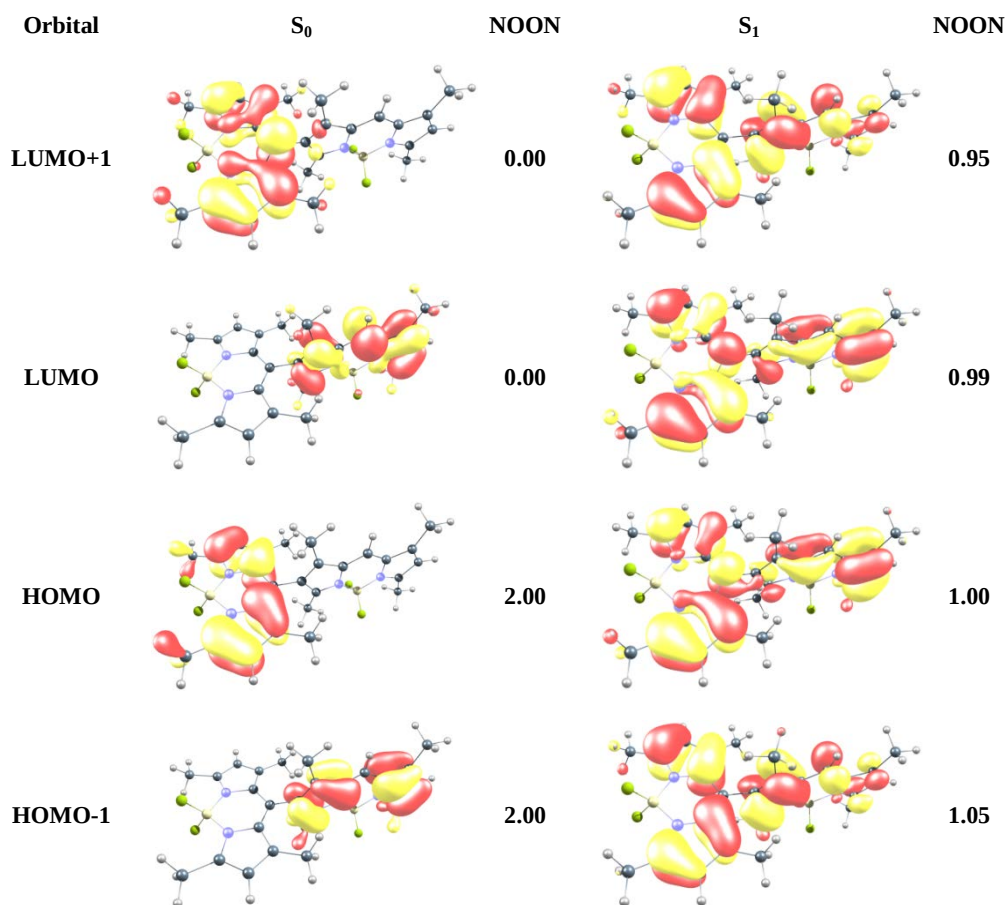


Figure 34. Frontier orbital plots and natural orbital occupation numbers (NOON) for the dimer **OB3**. a) S_0 state. b) S_1 state. a, b, Calculations were done at CAS(6e in 6o)/cc-pVDZ level. Surfaces are plotted at 0.2 a.u. All four orbitals in both states retain characteristics of Bodipy frontier orbitals and NOONs prove tetraradicalic character of S_1 .

Orthogonality is secured by the strategic placement of the methyl substituents, resulting in a dihedral angle very close to 90° for the two Bodipy cores. Orthogonal arrangement of the Bodipy units was also experimentally verified by the X-ray diffraction structure of the compound **OB3** (Figure 38a). The calculated energy differences (2.96, 2.97 eV) remaining very close to HOMO – LUMO gap of Bodipy core (3.10 eV) further support our claim of Bodipy cores remaining essentially unperturbed in the dimeric form. Thus the orthogonal dimer should possess the desired features sought in the excitation process. CASSCF calculations

on the orthogonal dimer **OB3** show that there is a huge amount of electronic reorganization upon $S_0 \rightarrow S_1$ transition. S_1 with an equilibrium geometry very close to S_0 lies 80 kcal/mol above the ground state.

Computations of the $S_0 \leftrightarrow S_1$ vertical transitions show that only 15 kcal/mol of this difference is due to structural deformation, whereas electronic reorganization is more than four times as costly (65 kcal/mol). Thus, interestingly electron movements are more important than the structural change upon excitation. This implies that the electronic structure of S_1 is rather unusual. Natural orbital occupation numbers (NOON) show four odd electrons in four very similar orbitals (Fig. 34). This situation from now on called as tetra-radicalic. Thus, on excitation, both Bodipy units are essentially equally likely to undergo the inherent (Bodipy) HOMO $\rightarrow$ LUMO transition from HOMO-1 to LUMO and HOMO to LUMO+1 of Figure 34. In addition, orbitals optimized for S_1 state support the involvement of both cores via their clearly visible delocalized character. Thus S_1 state could be predicted to be comprised of a linear combination of doubly substituted configurations (with respect to the closed shell reference) and in line with this expectation the CASSCF wavefunction is almost totally made up of doubly substituted configurations, an unexpectedly large value for any chromophore (Fig. 35, and following discussion). And one of five doubly substituted excitations cause tetra radicalic state formation.

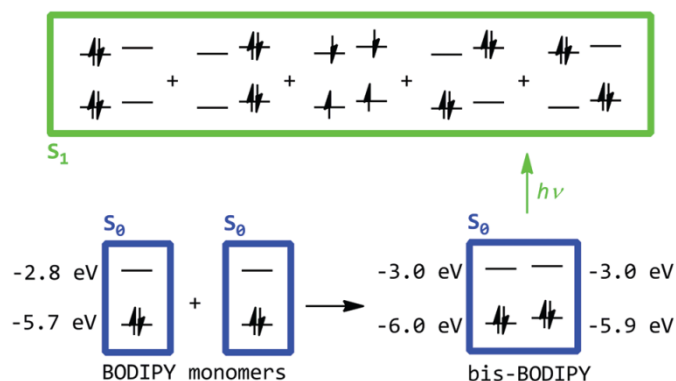


Figure 35. Conceptual frontier MO diagram describing the building up of S_1 states of the dimers **OB3** and **OB6** and dominance of doubly substituted configurations. Only the leading configurations are shown and minus signs to restrain antisymmetry in the S_1 states are intentionally excluded for clarity. As a result of spatial orthogonal arrangement HOMO and LUMO of both Bodipy cores are essentially kept in their original form (only slightly modified) in the dimers. Thus upon irradiation all the five configurations are almost equally probable and this picture is verified by CASSCF calculations. Each of the five configurations making up S_1 are termed as doubly substituted (DS) with respect to the closed shell reference S_0 state of the dimer.

The dominance of the S_1 wavefunction by double substitutions is in stark contrast with S_0 of orthogonal dimer as the latter is *ca.* 90% dominated by the reference configuration. These findings suggest a severe mismatch between the S_1 and S_0 states and an enhanced hop to the T_1 state lying 1.7 eV lower (at 1.8 eV with respect to the ground state). Essentially the same electronic structure fingerprints are observed for 8,8' dimer **OB6** (Fig. 36). Repeated calculations with different active space–basis set combinations without exception, showed a picture dominated by doubly substituted configurations and four singly occupied molecular orbitals in the S_1 state.

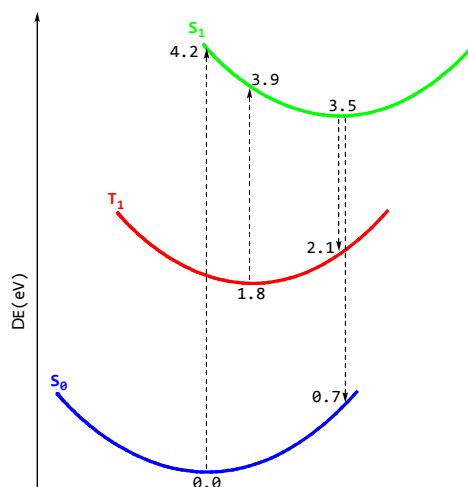


Figure 36. Relative energies (eV) of equilibrium structures of S_0 , S_1 , and T_1 states of **OB3**. Vertical transitions computed at CAS(6e in 6o)/cc-pVDZ level.

3.1.4 *Synthesis Methodology*

As a result, functionalization of the Bodipy core orthogonally, by a second Bodipy is the key to switching to the triplet manifold. Based on these clearly encouraging computational results, we were highly motivated towards the synthesis of a series of orthogonally linked dimeric Bodipys. The synthesis procedure for compound **OB3** is quite straightforward, especially in view of recent contribution to Bodipy chemistry by Jiao and coworkers.¹²⁴ The parent compound **OB1** was formylated through Vilsmeier reaction (Fig. 37). The Bodipy framework was then constructed around the formyl carbon using standard Bodipy chemistry. The absorption spectrum of the product showed a single band centered around 506 nm, not much different than the parent compound. We were fortunate to be able grow crystals appropriate for X-ray crystallographic analysis with slow evaporation of the dichloromethane solutions. The crystal structure obtained was in full agreement with our design expectations; the dihedral angle between the two Bodipy units was very close to 90° (Fig. 38), which was also predicted by the computational studies. Organic solutions of the orange colored compound was noticeably lacking detectable fluorescence emission under ambient or hand-held UV lamp irradiation, an indication of competing excited state process(es) in action.

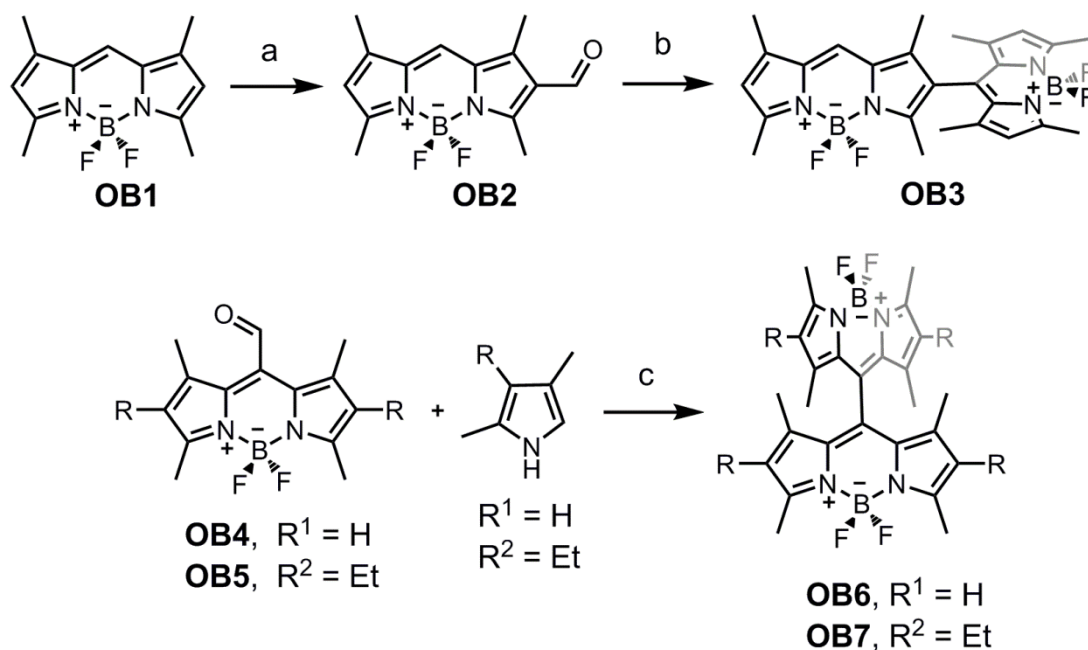


Figure 37. Synthesis of the target photosensitizers. a) POCl_3 , DMF, $\text{ClCH}_2\text{CH}_2\text{Cl}$, 50°C b) 2,4-Dimethylpyrrole, TFA, CH_2Cl_2 , p-chloranil, Et_3N , $\text{BF}_3\cdot\text{OEt}_2$ c) TFA, CH_2Cl_2 , p-chloranil, Et_3N , $\text{BF}_3\cdot\text{OEt}_2$.

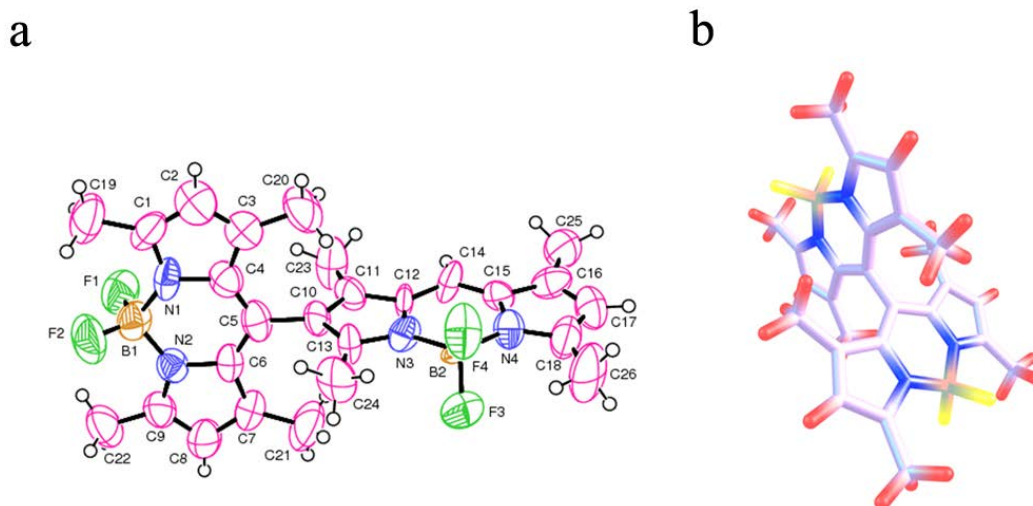


Figure 38. Structures of the dimeric Bodipys. a) X-ray diffraction structure of the orthogonal 8,2' dimer **OB3**. b) Optimized geometry of orthogonal 8,8' dimer **OB6** at CAS(6e in 6o)/cc-pVDZ level. Dihedral angle between the two Bodipy units is very close ($\pm 0.5^\circ$) to 90° in both dimers.

Recent progress in the derivatization of Bodipy dyes also allowed us to synthesize two different and more symmetrical, 8,8' orthogonal dimers as well. Thus, recently reported¹²⁵ 8-formyl-Bodipy derivative was transformed into orthogonal dimers **OB6** and **OB7**, using standard protocols (Fig. 37).

3.1.5 *Singlet Oxygen Production*

We surmised that a comparative study of compounds **OB1**, **OB3**, **OB6** and **OB7** would be instructive. First, we attempted to detect singlet oxygen phosphorescence at 1270 nm in chloroform for all four compounds excited with xenon-arc source at their respective absorption peaks, detection was done with a near IR sensitive detector. When excited at equal absorptivity concentrations for all compounds, dimer **OB3** yielded the strongest singlet oxygen phosphorescence emission (Fig. 39) at the signature wavelengths (peaking around 1270 nm), dimers **OB6** and **OB7** also showed phosphorescence peaks, but with somewhat reduced intensity. On the other hand, no such emission was observed with compound **OB1** (Fig. 39). Non-halogenated Bodipy dyes having low intersystem crossing efficiency, is a well established fact, indeed that is at least part of the reason for their bright fluorescence and photostability. A list of relevant photophysical parameters for the sensitizers is placed in Table 2. It is interesting to note that for the dimers **OB6** and **OB7**, significant fluorescence emission quantum yield is preserved, opening the possibility for a dual use as therapeutic and imaging agents.

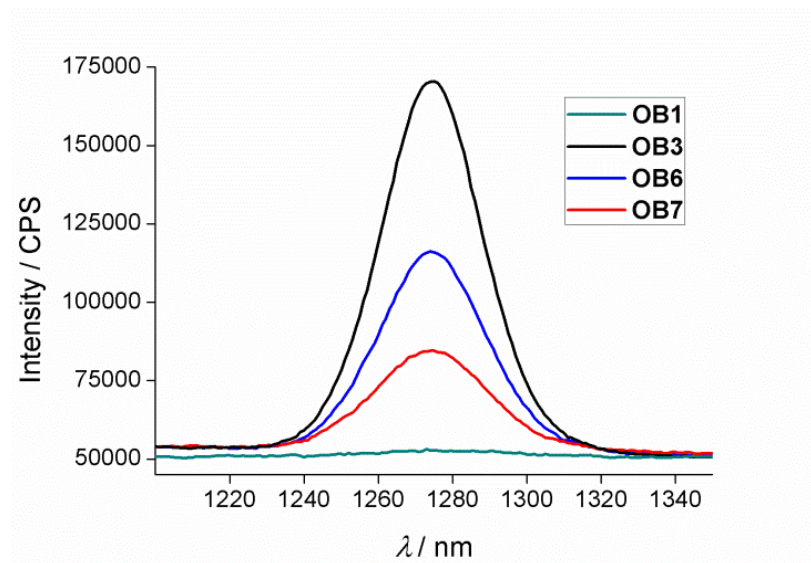


Figure 39. Singlet oxygen phosphorescence with sensitization from Bodipy derivatives: **OB3** (blue), **OB6** (black) and **OB7** (red), **OB1** (green) in CHCl_3 at equal absorbances (0.2) at the peak wavelength of their respective absorbances.

While integral areas under the phosphorescence emission peak is a measure of singlet oxygen quantum yield, we opted for more quantitative assessment of singlet oxygen quantum yields using 1,3-diphenylisobenzofuran (DPBF) as a trap molecule and with Methylene Blue (MB) as the reference compound (Methylene Blue has a singlet oxygen quantum yield of 0.57 under the conditions of the study in dichloromethane¹²⁶). Excitation of the dyes was carried out by irradiation at the respective absorbance peak wavelengths with monochromatized light source, at a fluence rate around $30 \mu\text{W}/\text{cm}^2$. The quantum yield for singlet oxygen generation for **OB3** is 0.51 in dichloromethane, much higher than all non-halogenated Bodipy dyes and many other organic chromophores and photosensitizers, under comparable conditions. The other Bodipy dimers **OB6** and **OB7** also showed respectable singlet oxygen quantum yields of 0.46 and 0.21, respectively (See experimental part for details).

Table 2. Comparative spectroscopic properties of Bodipy compounds.

Compound	$\lambda_{\text{abs}}^{[a]}$ [nm]	$\lambda_{\text{ems}}^{[a]}$ [nm]	Φ_f	$\tau_f^{[a]}$ [ns]	$\Phi_{\Delta}^{[e]}$
OB1	509	514	0.80 ^[d]	5.2	-
OB3	514	527	0.03 ^[a,b]	2.5	0.51
OB6	515	588	0.31 ^[a,c]	10.9	0.46
OB7	542	605	0.49 ^[a,c]	5.0	0.21

[a] in CHCl₃, [b] in reference to Fluorescein in 0.1 M NaOH solution excited at 496 nm, [c] in reference to Rhodamine 6G in EtOH excited at 480 nm, [d] in EtOH [e] Singlet oxygen quantum yield was determined with respect to Methylene Blue (0.57 in Dichloromethane)¹²⁶.

3.1.6 Cytotoxicity Experiments

For further demonstration of singlet generation capacity and photocytotoxicity of the most active dimeric dye (compound **OB3**), we carried out a cell culture assay with cancer cell lines. To that end, we prepared a micellar formulation of the 8,2'-orthogonal dimeric dye using Cremophor-EL (experimental section).¹²² The size distribution of the micelles was determined using electrophoretic light scattering (Appendix part for graph). The size distribution reveals a median size of 100 nm for the micellar constructs. Micelle embedded dye retained high levels of singlet oxygen generation capacity as revealed by another singlet oxygen trapping experiment, this time in aqueous media, using a water soluble anthracene derivative (2,2'-(Anthracene-9,10-diylbis(methylene))dimalonic acid (For the structure of this compound and its use see Section 3.2).

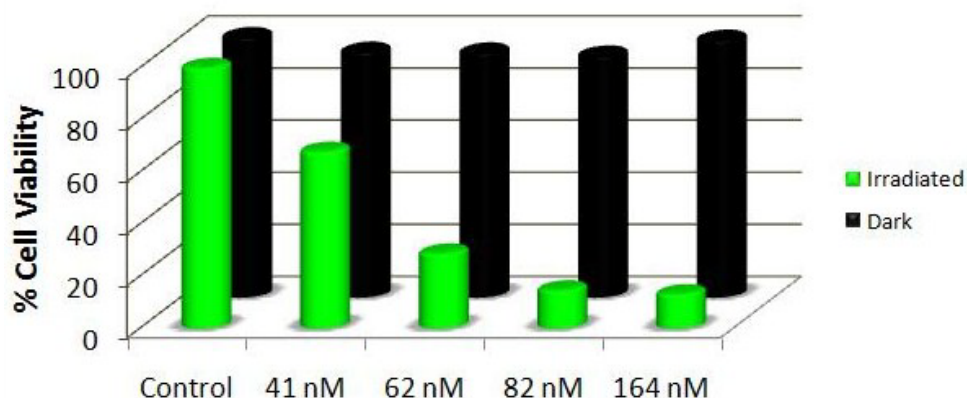


Figure 40. Photocytotoxicity of the sensitizer **OB3** as demonstrated by MTT assay. Cell suspensions (K562, human erythroleukemia cells) were seeded in 96-well flat-bottom plates and varying concentrations of the sensitizers were added into each well. Cells were kept either in dark, or under illumination with a green (520 nm) LED array at 2.5 mW/cm² fluence rate for a period of 4 h at 37°C in a humidified incubator containing 5% CO₂.

Experimental verification of photocytotoxicity was carried out as follows: varying concentrations of the dimeric dye in Cremophor-EL micelles were incubated with K562 human erythroleukemia cells within a standard culture medium at 37 °C in a humidified incubator containing 5% CO₂. Cells were irradiated with the green LED source for 4 hours, followed 44 hours of incubation in dark. The control group was incubated in dark, under otherwise identical conditions. The cell viabilities were determined using standard MTT assay. The results are shown in Figure 40. Even at very low concentrations of dyes (as embedded within micelles) significant decrease of cell viability was observed (green bars in Fig. 40) with a remarkable EC₅₀ of 50 nM. No statistically significant change was observed when the cells were kept in dark, in the presence of same concentration of the photosensitizer **OB3** (black bars in Fig. 40). Photocytotoxicity was also revealed (Fig. 41) using confocal microscopy with two fluorescent probes of cell viability (acridine orange and propidium iodide). In Figure 41-a, the cells were incubated in dark for 24 h without our PS, **OB3**; as a result any phototoxic effect took place and the cells were stained

with acridine orange only (merge-green), the indication of cells are alive. In b, **OB3** was added to the well and incubated for 24h in dark, again any cytotoxicity fulfilled. In c row in Figure 41, the effect of light to cells was investigated without addition of **OB3**, again resulted with survival of cells. However, in d cells were incubated with **OB3** for 20h after illumination with light for 4h and after 24h, the phototoxic affect was revealed since the cells were stained with propidium iodide (merge-red). Thus, using confocal microscopy also, the activity of our micellar PS working well has been proved.

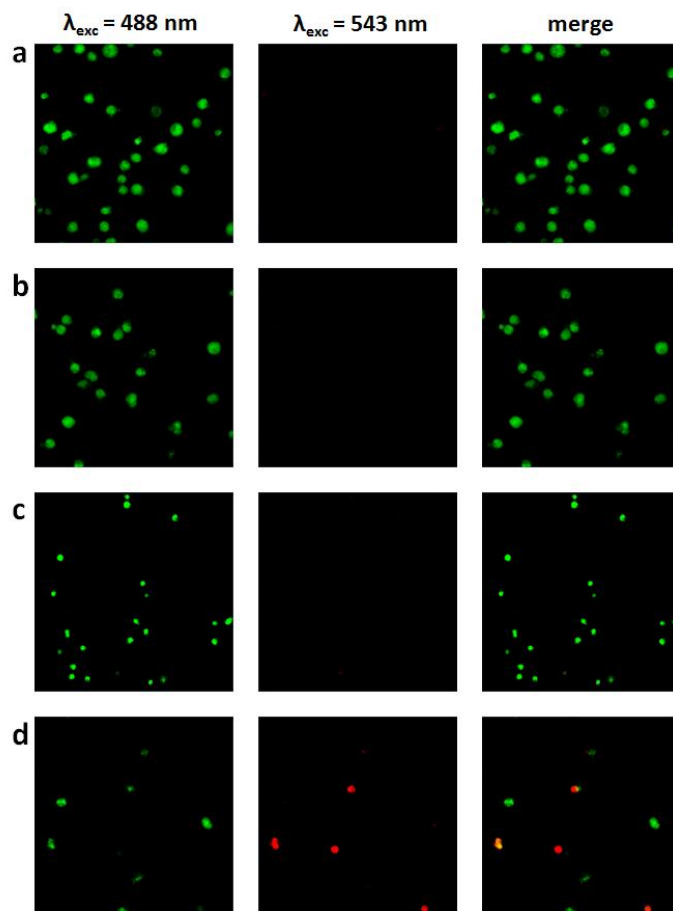


Figure 41. Photocytotoxic activity of the dimeric Bodipy **OB3** visualized via confocal microscopy. Cell suspensions (K562, human erythroleukemia cells) were seeded in 24-well plates. a) Cells in Control 1 (upper plates) wells were incubated in dark for 24 hours; b) Cells in Control 2 wells (second row plates) were incubated with 500 μ l/well Cremophor-EL solubilized sensitizer **OB3** (at a final concentration of 164 nM) and kept in dark for 24 hours in the same incubator, c) Cells in Control 3 well were illuminated for 4 h without the sensitizer and incubated for a further 20 h in dark at the incubator. d) Cells in Control 4 (bottom row of plates) well were illuminated for 4 h after addition of 164 nM dye **OB3** and incubated for a further 20 h in dark at the incubator. Cells in wells were collected after 24 h and imaged at 40x magnification. Live cells are preferentially stained with acridine orange (AO, green) (a), whereas dead cells are preferentially stained with the dye propidium iodide (PI, red) due to increased cellular permeability (b).

3.1.7 *Extended Conjugation of an Bis-BODIPY Derivative*

The above results were motivating, particularly for utilizing many chromophores in heavy atom free ISC by only dimerizing them in an orthogonal

fashion. Consequently, we aimed to surpass the accomplishments of **OB3** and **OB6** by synthesizing modified orthogonal bis-BODIPYs that will operate at the red end of the spectrum and, hence, potentially be better suited for photodynamic applications. The well-known modification to red shift λ_{abs} , i.e., decrease the energy gap between frontier orbitals is, extending¹²⁷ the π -system of the chromophore core. For this purpose, synthesis of bis-8,2-distyrylBODIPY (**OB8**) derivative shown in Figure 42 was performed.

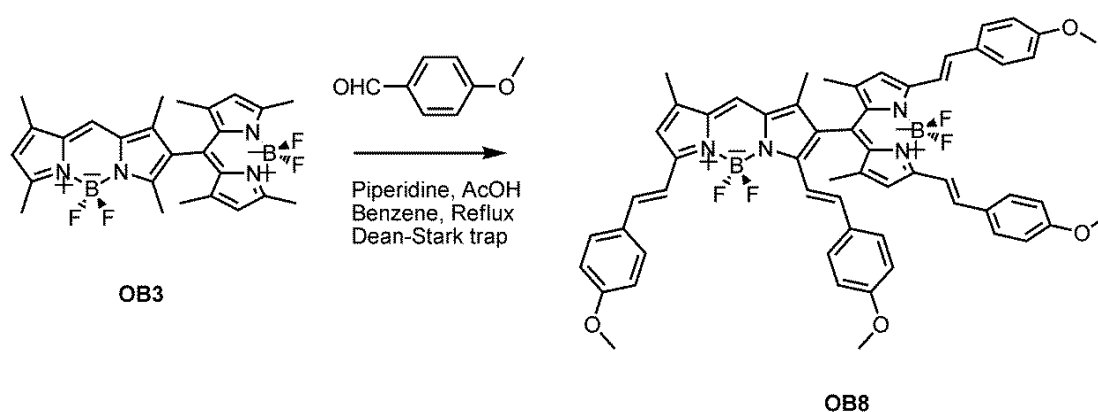


Figure 42. Synthesis scheme of **OB8**

Spectroscopic properties of **OB8** was investigated, and an emission at 680 nm together with a negligible triplet quantum yield were surprisingly observed, contrary to the expectations followed from the computed structural orthogonality (interplane angle = 90.1°). $^1\text{O}_2$ phosphorescence measurements (Table 3) around 1270 nm also confirmed that this red-absorbing dye essentially did not undergo the presumed ISC to the triplet manifold.

Table 3. Photophysical properties of the synthesized Bodipy compounds

Compound	$\lambda_{\text{abs}}^{\text{a}}$ / nm	$\epsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}$	$\lambda_{\text{ems}}^{\text{a}}$ / nm	Φ_{f}	τ^{a} / ns	Φ_{Δ}
OB1	509	89,200	514	0.80 ^d	5.2	-
OB3	514	119,400	527	0.025 ^{a,b}	2.5	0.51
OB8	661	125,300	680	0.06 ^{a,c}	2.2	0.06

a) in CHCl_3 b) with respect to Fluorescein in 0.1 M NaOH solution excited at 496 nm c) with respect to cresyl violet perchlorate in MeOH excited at 610 nm d) in EtOH

These results either suggest that excitation characteristics established for **OB3** and **OB6** by the CASSCF calculations are not complete descriptors for ISC or orthogonality alone does not afford the excited states bearing the features of interest. The former seems questionable in light of the reports on facile nonadiabatic reactions of related singlet states.¹²⁸ The latter hypothesis, on additional requirements to generate DS-TR S_1 states besides orthogonality, was tested via CASSCF calculations on the S_1 state of **OB8**. Interestingly, the S_1 CASSCF wave function for **OB8** was of SS nature and clearly described a HOMO→LUMO-type excitation. The same type of excitation characteristics were obtained for the 8,8' analogue of **OB8** (*vide infra*). Hence, it is not possible to expect these dyes to be used as photodynamic therapy reagent. Spectroscopic measurements on **OB8** lend support to the safe use of DS-TR S_1 states in assessing ISC performance, since our multireference calculations clearly show the SS nature of the S_1 state. From a different viewpoint, however, the SS openshell singlet electronic structure of **OB8** challenges the link between orthogonality and DS-TR S_1 . This may suggest the existence of other requirements for a DS-TR S_1 state; however, there may also be a very specific feature for **OB8** only. To better understand this complex issue, low-lying electronic states of numerous bis-BODIPYs were analyzed by our collaborator (Figure 43).

Since the disappearance of DS-TR character was apparently a result of extending the π framework, it is desirable to analyze the effect of increasing the size of the π -system with a smooth transition from **OB3** or **OB6** to **OB8**. This will enable us to monitor the cause of the drastic change in excitation characteristics from DS-TR to SS. Therefore, first it was generated a set of computational models where BODIPYs bear only ethenyl/ethynyl substituents on various positions of the core. Further extension of the π -system was performed until SS- S_1 character persisted in the dimers. Also important was searching for any correlations between the characteristics of S_1 states of the bis-BODIPYs and the monomer electronic structure. Consequently, the set of monomers given in Figure 43 and their 8,8' and/or 8,2' orthogonal dimers were studied.

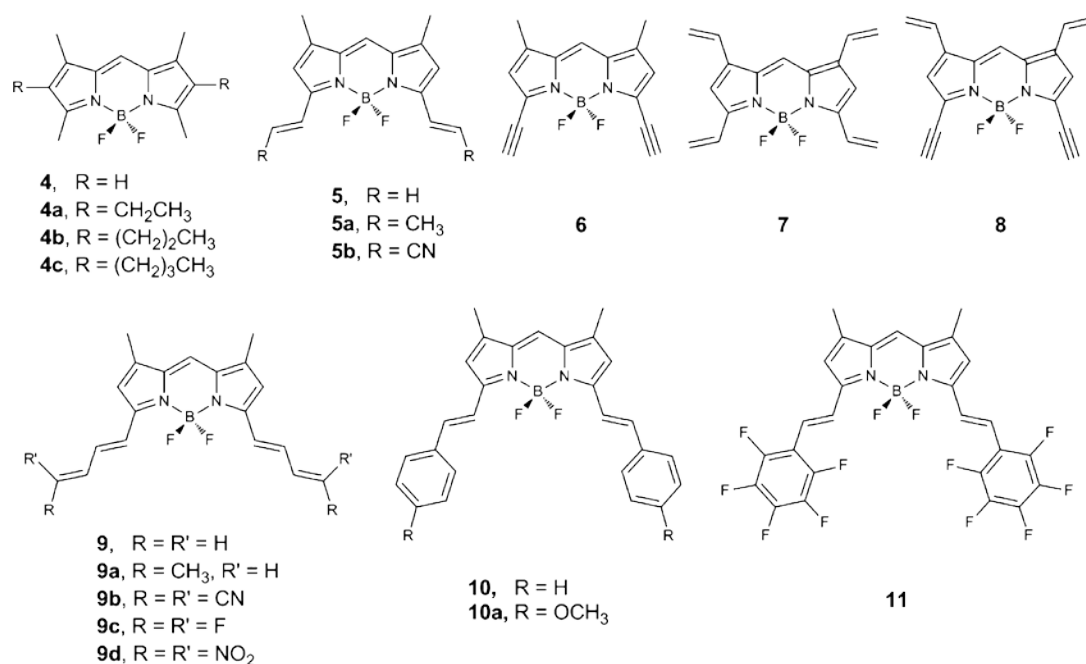


Figure 43. Bodipy monomers and dimers used for the computational calculations.

Extending the conjugated π -systems of the monomers yielded four major sets of BODIPYs possessing the following substituents neighboring the core shown in Figure 43; any substituents (**4**); ethenyl and/or ethynyl (**5** to **8**); butadienyl (**9**); and styryl (**10**, **11**). All these models preserved an essentially planar geometry within the monomer, and their dimers were computed to be spatially orthogonal. Table 4 shows frontier orbital energies of ground states of all the monomers and dimers as well as the relative energies of S₁ and T₁ states of the orthogonal dimers. Also, the information about their excited state character (doubly substituted or singly substituted) was also given.

Table 4. Selected Parameters for Modified BODIPY Cores and Associated Orthogonal Bis-BODIPYs. ^a Prime (') indicates 8,2' coupling motif of the monomers, and absence of the prime (') shows 8,8 binding. ^b DS-TR: Doubly substituted tetraradical. SS: Singly substituted HOMO→LUMO-type open-shell singlet state. ^c Relative energies of T₁ and S₁ states with respect to the ground state S₀ of the dimer at the CAS(6,6) level.

species ^a	monomer				dimer								S ₁ ^b	E(T ₁) ^c (eV)	E(S ₁) ^c (eV)
	E _H (eV)	E _L (eV)	ΔE _{H-L} (eV)	ΔE _{H-L} (nm)	E _{H-1} (eV)	E _H (eV)	E _L (eV)	E _{L+1} (eV)	ΔE _{L-(H-1)} (eV)	ΔE _{(L+1)-H} (eV)					
4	-5.69	-2.78	2.91	426	-6.01	-5.92	-3.04	-3.04	2.97	2.88	DS-TR	1.79	3.69		
4'					-6.04	-5.69	-3.11	-2.76	2.93	2.93	DS-TR	2.35	4.16		
4a	-5.47	-2.60	2.87	432	-5.71	-5.62	-2.84	-2.81	2.87	2.81	DS-TR	2.28	3.90		
4b	-5.46	-2.60	2.86	433	-5.70	-5.60	-2.82	-2.80	2.88	2.80	DS-TR	1.69	3.45		
4c	-5.45	-2.59	2.86	433	-5.67	-5.58	-2.81	-2.77	2.86	2.81	DS-TR	1.69	3.44		
5	-5.51	-3.03	2.48	499	-5.82	-5.75	-3.39	-3.23	2.44	2.52	DS-TR	1.62	3.77		
5'					-5.84	-5.52	-3.33	-3.02	2.51	2.50	DS-TR	2.14	3.81		
5a	-5.22	-2.78	2.43	509	-5.46	-5.39	-3.07	-2.91	2.39	2.48	DS-TR	1.62	3.33		
5a'					-5.46	-5.21	-3.01	-2.75	2.45	2.46	DS-TR	1.69	3.33		
5b	-6.30	-3.97	2.33	532	-6.85	-6.79	-4.49	-4.34	2.36	2.45	DS-TR	1.57	3.68		
5b'					-6.81	-6.50	-4.44	-4.16	2.36	2.34	DS-TR	1.83	3.51		
6	-5.86	-3.31	2.55	487	-6.39	-6.33	-3.79	-3.62	2.60	2.72	DS-TR	1.66	3.43		
6'					-6.44	-5.84	-3.76	-3.29	2.68	2.55	DS-TR	1.71	3.41		
7	-5.65	-3.30	2.35	527	-6.02	-5.87	-3.53	-3.46	2.49	2.41	DS-TR	1.34	2.71		
7'					-5.90	-5.62	-3.56	-3.30	2.34	2.33	DS-TR	1.58	3.18		
8	-5.95	-3.55	2.40	517	-6.42	-6.31	-3.89	-3.82	2.53	2.49	DS-TR	1.22	2.87		
8'					-6.25	-5.88	-3.86	-3.51	2.38	2.37	DS-TR	1.63	3.30		
9	-5.19	-3.03	2.16	574	-5.40	-5.35	-3.24	-3.24	2.16	2.10	SS	1.51	3.48		
9'					-5.39	-5.20	-3.23	-3.02	2.16	2.17	SS	1.60	3.18		
9a	-4.95	-2.83	2.12	584	-5.10	-5.05	-2.99	-2.98	2.12	2.07	SS	1.46	3.17		
9a'					-5.09	-4.93	-2.98	-2.79	2.11	2.14	SS	1.60	3.17		
9b	-6.44	-4.49	1.95	636	-6.92	-6.87	-4.83	-4.83	2.09	2.05	SS	1.47	3.18		
9b'					-6.90	-6.54	-4.90	-4.57	1.99	1.96	SS	1.50	3.06		
9c	-5.59	-3.42	2.17	570	-5.93	-5.86	-3.74	-3.72	2.19	2.14	SS	1.50	3.37		
9c'					-5.91	-5.65	-3.72	-3.46	2.19	2.19	SS	1.59	3.20		
9d	-6.91	-5.03	1.88	659	-7.60	-7.47	-5.67	-5.40	1.93	2.07	SS	1.38	3.19		
9d'					-7.45	-7.05	-5.50	-5.14	1.95	1.91	SS	1.51	3.10		
10	-5.09	-2.97	2.12	584	-5.28	-5.23	-3.16	-3.14	2.12	2.09	SS	1.54	3.26		
10'					-5.25	-5.09	-3.13	-2.95	2.12	2.15	SS	1.61	3.17		
10a	-4.84	-2.78	2.05	604	-4.91	-4.85	-2.92	-2.88	1.99	1.97	SS	1.55	2.76		
10a'					-4.92	-4.82	-2.89	-2.75	2.04	2.08	SS	1.59	3.14		
11	-5.98	-3.81	2.18	570	-6.30	-6.25	-4.12	-4.10	2.17	2.15	SS	1.53	2.56		
11'					-6.35	-6.09	-4.16	-3.90	2.19	2.19	SS	1.54	2.52		

Excitation characteristics of the S₁ state (DS-TR or SS) of bis-BODIPYs are also shown. It is clear from the frontier orbital energy differences that, orthogonality dictates formation of two pairs of near degenerate orbitals in the dimer that effectively preserve monomeric character, as supported by orbital isodensity plots. Extension of the π-system lowered the HOMO–LUMO gaps as anticipated, and interestingly, beyond the very first double bond added to the chromophore core; i.e., for set (**9** to **11**), the DS-TR character of S₁ state disappears, hence population of triplet state is not possible. In such cases, S₁ MOs were no longer delocalized on both chromophore cores (Scheme 44), which is also the case for **OB8**; as a result they

have a simple HOMO-LUMO transition character as found in most organic fluorophores.

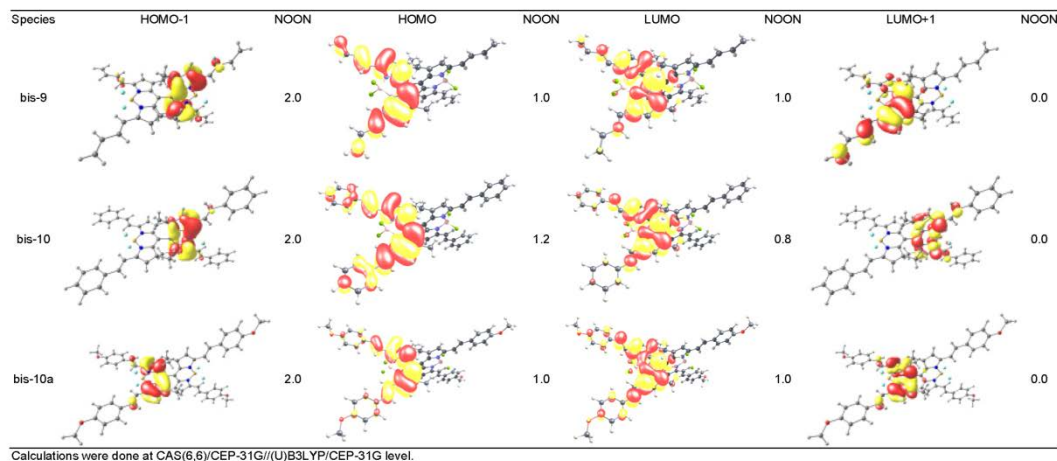


Figure 44. Selected natural orbitals and occupation numbers for **bis-9**, **bis-10** and **bis-10a**

The lowering of the HOMO-LUMO gap and disappearance of DS-TR character of S_1 do correlate; however, it is illusory to conclude that DS-TR fades with decreased HOMO-LUMO gaps since the energy gap decrease itself is caused by a structural perturbation that extends the π -system. Thus, the fundamental reason of switching from DS-TR to SS type S_1 states needs further investigation (*vide infra*). Inspection of the relative energetic positioning of T_1 and S_1 states does not provide an answer since T_1 is roughly equally separated from S_0 and S_1 states for all species and there is no significant pattern in favor of the DS-TR- S_1 or SS- S_1 possessing species. Incorporation of functional groups in order to donate or withdraw electron density as in the series of species **5**, **9**, **10**, and **11** do result in deviations (from core BODIPY) in the anticipated directions for frontier MO levels but do not switch the excitation characteristics of S_1 . Extension of the π -system shutting down the DS-TR S_1 state seems to be one net conclusion that could be drawn, despite the survival of near-degenerate monomer based orbitals for all the dimers considered. Nonetheless, from the four sets of models with increasing sizes of conjugated π -systems, the only groups with a DS-TR S_1 state were (**4** and **5** to **8**), whose π -system extension is

truncated at the very first C=C double bond. Apparently, one is only allowed to slightly modify S_0 – S_1 energy difference without losing the DS-type excitation motif, and unfortunately, this does not result in the desired amount of HOMO–LUMO gap lowering needed for PDT to be applied due to skin penetration reasons. It is important to note that relative energies with the CASSCF method may not be quantitatively reliable and perturbation treatments on the CASSCF wave function are necessary for energy differences of satisfactory quality. Unfortunately, MP2 corrections to this size of molecules are unfeasible and the scope of the current work is identifying the key electronic structure details of the orthogonal dimers that apparently undergo ISC, not a quantitatively accurate prediction of relative energies. Such a high level treatment may be available in the future, yet we were able to complete CAS-MP2 calculations for bis-4 and bis-4' which are the species of utmost interest. CAS-MP2 calculations also suggest that the DS-TR states are above the closed shell S_0 at 2.60 and 2.85 eV and the next singlet state is at 4.58 and 4.68 eV for bis-4 and bis-4', respectively. Although CAS-MP2 treatment decreases the relative spacing between higher excited states with respect to CASSCF, the DS-TR S_1 state is still well separated from S_0 and S_2 . Thus, there is little doubt that DS-TR character is the correct nature of the first excited singlet state. The issue with quantitative reliability may be even more critical depending on the system of interest as nicely demonstrated by the recent work of Martinez *et al.*^{128e} Fortunately, our experiments show that probing for DS-TR or SS character in the S_1 state is successfully working for the systems under investigation in assessment of ISC performance. Our TD-DFT calculations suggest that **8**, the species bearing the lowest HOMO–LUMO gap with a DS-TR S_1 for the dimer, may have an absorption maximum close to but lower than 600 nm when estimated errors for TD-DFT are considered. Thus, it is questionable that π -extension would carry the features of bis-BODIPYs, desired for effective ISC, to longer wavelengths.

3.1.8 Conclusion

Detailed multireference quantum chemical analyses were performed by our collaborator on several electronic states of orthogonal bis-BODIPYs. These parameters are highly important for us before synthesizing new compounds for PDT applications. These studies show how important to go with the successful computational methods. Essentially isolated monomeric states in the dimers dictated formation of a pair of (near) degenerate S_0 frontier orbitals, which yielded four SOMOs (singly occupied molecular orbitals) in the S_1 state. Domination of S_1 wave function by doubly substituted configurations was utilized as an electronic structure descriptor in assessing ISC efficiency. Formation of DS-TR S_1 states was not only tied to orthogonality but also to a certain degree of truncation in the π -framework of the substituents. Bis-BODIPYs with extended π -systems possess destabilized DS-TR states, as *exchange coupling* among unpaired electrons of same spin is significant only at *small distances*. An increase in S_1-T_1 spin orbit coupling constant from monomer to dimer correlates with presence of the DS-TR motif in S_1 state of dimer. Comparisons with iodine incorporated bis-BODIPYs revealed that facile $^1O_2$ production by orthogonal bis-BODIPYs without heavy atoms is via the specific electronic structure fingerprint, DS configurations, that are closely related to zwitterionic configurations which are well known to effectively couple with triplets.¹²⁹ Spectroscopic properties of a newly synthesized near-IR absorbing bis-BODIPY are in excellent agreement with the results of CASSCF calculations. Shifting the absorption wavelength to the red region is achieved with π -extension of bis-BODIPYs; however, ISC properties are adversely affected for such dimers. Modifications in the chromophore cores are likely to be a more promising research direction. It is concluded that DS-TR is a valuable descriptor in assessing the ISC performance of S_1 states for heavy-atom-free bis-chromophores. Calculated DS-TR motif present in the S_1 wave function correlates with, if not explains, the ability of the orthogonal bis-BODIPY systems to photosensitize $^1O_2$. Quantitatively more reliable quantum chemical/dynamical calculations can provide more insight into the

behavior of the excited states. Principles to design DS-TR S_1 states in other bis-chromophores should be established to get desired PS.

Thus, our work demonstrated for the first time a practical example of excited state design leading to efficient cytotoxic singlet oxygen generation with a potential in photodynamic therapy. With a sound theoretical framework in design, it is only natural to expect other photosensitizers to emerge with similar line of reasoning. In this particular proof-of-principle work, excitation outside the therapeutic window is needed, but that issue can be easily addressed by the use of upconverting or persistent luminescence nanoparticles as secondary excitation sources.

3.1.9 *Experimental Section*

3.1.9.1 *Cremophor-EL solubilisation*

Photosensitizer **OB3** (5×10^{-5} moles) was dissolved in THF (1mL) treated with Cremophor EL (CrEL, 150 mg) and sonicated for 30 mins. THF was removed under reduced pressure, the remaining oil was dissolved in phosphate-buffered saline (PBS) (5mL), filtered through a 0.2 μm membrane filter and made up to 10 mL with PBS (1X, pH= 7.4).

3.1.9.2 *Cell Culture and MTT Assay*

K562 human erythroleukemia cells (ATCC) were cultured in 25 cm^3 culture flasks containing RPMI 1640 medium supplemented with heat inactivated 10 % fetal bovine serum, 2 mM L-glutamine, 100 units. mL^{-1} penicillin G and 100 $\mu\text{g}.\text{mL}^{-1}$ streptomycin at 37 $^\circ\text{C}$ in a humidified incubator containing 5% CO_2 . Micellar preparation of the sensitizer was dissolved in RPMI 1640 medium and test concentrations were prepared daily. The methythiazolyltetrazolium (MTT) assay was used to evaluate cell viability. Briefly, 50 μL cell suspensions containing 4×10^4 K562 cells were seeded in 96-well flat-bottom plates (Costar, Cambridge, MA) and varying concentrations of Cremophor-EL solubilized dye **OB3** (Final concentrations= 41 nM - 164 nM) were added in 50 μL into each well. All dye concentrations were tested in triplicate for three times. Then, cells were kept either

in dark or under illumination with a green (520 nm) LED array at 2.5 mW/cm² fluence rate for a period of 4 h at 370°C in a humidified incubator containing 5% CO₂. In order to evaluate the cytotoxicity of dye, 4 h irradiated plates were kept in dark for a further 44 h and then MTT solution was added for measuring cell viability. In addition, plates kept in dark for 4 h were also incubated for an additional 44 h for control. After 48 h, 25 µL of MTT solution (1.0 mg/mL final concentration) (Sigma Chemical Co., St. Louis, MO) were added to each well, and the plates were incubated for a further 4 h. The formazan precipitate was solubilized by adding 80 µL lysing buffer (pH=4.7) composed of 23% SDS (sodium dodecyl sulfate) dissolved in a solution of 45% N,N- dimethylformamide. After an overnight incubation at 370°C, the absorbances were read at 570 nm using a microplate reader (Spectramax Plus, Molecular Devices, Sunnyvale, California, USA). Cells incubated in culture medium alone served as a control for cell viability (nontreated wells) either in irradiated plate or in plate that was kept in dark. Cell viability (%) was calculated as OD of treated wells/OD of nontreated cells x 100.

3.1.9.3 *Preparation of stock solutions for fluorescence microscopy*

A stock solution of propidium iodide (PI) (Sigma P-4170) was prepared in distilled water, and used at a concentration of 0.5 mg/mL. Acridine orange (AO) was dissolved in phosphate buffered saline at a concentration of 100 µg/mL. One tablet of phosphate buffered saline (PBS) (Amresco) was dissolved in the distilled water to yield a pH=7.4 buffered solution.

3.1.9.4 *Confocal microscopy*

Confocal microscopic analysis was also performed to evaluate cell viability. Briefly, 1500 µL cell suspensions containing 2×10^5 K562 cells/well were seeded in 24-well plates. Cells in Control 1 well were incubated in dark for 24 hours at 370°C in a humidified incubator containing 5% CO₂. Cells in Control 2 well were incubated with 500 µL/well Cremophor-EL solubilized dye **OB3** (Final dye concentration = 164 nM) and kept in dark for 24 hours in the same incubator. Cells in Control 3 well were illuminated for 4 h without dye and incubated for a further 20

h in dark at the incubator. Cells in Control 4 well were illuminated for 4 h after addition of 164 nM dye **OB3** and incubated for a further 20 h in dark at the incubator. All conditions were studied in duplicate. Cells in wells were collected after 24 h and confocal microscopy images were acquired.

3.1.9.5 *General Experiment Details*

General: ^1H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 (operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) in CDCl_3 solvent with tetramethylsilane as internal standard. All spectra were recorded at 25°C and coupling constants (J values) are given in Hz. Chemical shifts are given in parts per million (ppm). Absorption spectra were performed by using a Varian Cary-100 and Varian Cary 5000 UV-VIS-NIR absorption spectrophotometer. Fluorescence measurements were conducted on a Varian Eclipse spectrofluorometer. The Fluorescence decay measurements were carried out with the Horiba Jobin-Yvon Time-Resolved Fluorometer, Fluorolog FL-1057. The instrument response function was measured with an aqueous Ludox solution. Singlet oxygen phosphorescence around 1270 nm was determined by using Horiba Jobin-Yvon Fluoremeter with Hamamatsu NIR PMT module, model H-10330-75. The decays were analyzed with a multiexponential fitting function by iterative reconvolution and chi-square minimization. Mass spectra were recorded with Agilent Technologies 6224 TOF LC/MS and 6530 Accurate Mass Q-TOF LC/MS. Irradiation of photosensitizers **OB3**, **OB6**, **OB7** and Methylene Blue (MB) was accomplished by monochromatic light system composed of Spectral Products CM 110 1/8m monochromator, ASB-XE-175 Xenon light source, Newport Multi Function Optical Meter model 1835-C. Electrophoretic Light Scattering was performed with Malvern NanoZS zeta potential. Reactions were monitored by thin layer chromatography using Merck TLC Silica gel 60 F254. Silica gel column chromatography was performed over Merck Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). 4,4-Difluoro-8-formyl-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**OB4**)¹²⁵ and 4,4-Difluoro-8-formyl-2,6-diethyl-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**OB5**)¹²⁵ were synthesized according to literature. All other reagents and solvents

were purchased from suppliers and used without further purification. Micelle has been prepared with compound **OB3** according to the literature.¹²² In singlet oxygen measurements 1,3-Diphenylisobenzofuran was used as a singlet oxygen trap in organic solvent measurements and was purchased from supplier. 2,2'-(Anthracene-9,10-diylbis(methylene))dimalonic acid was used a singlet oxygen trap in aqueous solvent and was synthesized according to the literature.¹³⁰

3.1.9.6 *Synthetic Details*

Compound OB1

250 mL 1,2-dichloroethane was degassed with N₂. 2,4-dimethyl pyrrole (1 mL, 11.37 mmol), triethylorthoformate (0.95 mL, 5.69 mmol) and POCl₃ (0.58 mL, 6.25 mmol) were added to degassed solvent. Reaction was allowed to stir for 2 hours at room temperature. Then 11.55 mL NEt₃ and 11.55 mL BF₃.OEt₂ were added. After 1 hour the reaction was washed with water (3x250 mL), the organic layer separated, dried on NaSO₄ and evaporated in vacuo. Column chromatography with CHCl₃ yielded the pure product as reddish solid (400 mg, 28 %). ¹H NMR (400 MHz, CDCl₃): δ_H 7.09 (1H, s, ArH), 6.10 (2H, s, ArH), 2.60 (6H, s, CH₃), 2.29 (6H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_C 156.8, 141.3, 133.5, 120.2, 119.1, 14.8, 11.2 ppm.

Compound OB2

4 mL of DMF and 4 mL of POCl₃ was stirred in an ice bath for 5 min under argon. Then warmed to room temperature and waited for 30 minutes. To this mixture compound **OB1** (100 mg, 0.328 mmol) was added in dichloroethane (40 mL). The temperature raised to 50 °C and stirred for 2 hours. The reaction medium was cooled and poured into 150 mL NaHCO₃ solution slowly under cool conditions. After cooling it was stirred for further 30 min, then washed with water 3 times. The organic layers was combined and dried over NaSO₄ and evaporated to dryness. The compound has been purified by column chromatography by using chloroform as an eluant and the compound has been got pure as reddish solid (95 %). ¹H NMR (400

MHz, CDCl₃): δ_H 10.02 (1H, s, CHO), 7.20 (1H, s, ArH), 6.20 (1H, s, ArH), 2.77 (3H, s, CH₃), 2.58 (3H, s, CH₃), 2.48 (3H, s, CH₃), 2.28 ppm (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_C 185.5, 163.5, 157.2, 145.4, 140.6, 136.4, 131.1, 125.8, 122.0, 121.5, 15.2, 12.9, 11.5, 10.4 ppm; MS HRMS (TOF- ESI): m/z calcd for C₁₄H₁₅BF₂N₂O⁺: 275.1246 [M+H]⁺; found: 275.1212[M+H]⁺, Δ = 12.3 ppm.

Compound OB3

In N₂ bubbled 250 mL dichloromethane, 2,4-Dimethylpyrrole (50 μ L, 0.478 mmol) and **OB2** (60 mg, 0.217 mmol) were mixed. 1 drop of trifluoroacetic acid has been added. The reaction mixture stirred at room temperature overnight. Then p-chloranil (49.2 mg, 0.217 mmol) was added to the reaction. After stirring 1 hour at room temperature, NEt₃ (2 mL) and BF₃.OEt₂ (2 mL) was added and stirred again for 1 hour. The reaction was finished by extracting with water (3x250 mL). Column chromatography with CHCl₃ yielded the pure product (20 %). ¹H NMR (400 MHz, CDCl₃): δ_H 7.18 (1H, s, ArH), 6.15 (1H, s, ArH), 6.02 (2H, s, ArH), 2.61 (3H, s, CH₃), 2.59 (3H, s, CH₃), 2.57 (3H, s, CH₃), 2.54 (3H, s, CH₃), 2.40 (3H, s, CH₃), 2.30 (3H, s, CH₃), 2.12 (3H, s, CH₃), 1.72 ppm (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_C 160.3, 155.8, 151.5, 143.7, 142.5, 136.6, 134.7, 133.4, 132.3, 131.7, 124.2, 121.3, 120.7, 120.5, 14.9, 14.7, 13.9, 12.7, 9.8, 8.7 ppm; MS HRMS (TOF- ESI): m/z calcd for C₂₆H₂₈B₂F₄N₄⁺: 493.2436 [M+H]⁺; found: 493.2417 [M+H]⁺, Δ = 3.9 ppm. Extinction coefficient (ϵ); 119,400 cm⁻¹mol⁻¹L in CHCl₃.

Compound OB6

In N₂ bubbled 100 mL dichloromethane 2,4-Dimethylpyrrole (16.2 μ L, 0.12 mmol) and **OB4** (20 mg, 0.06 mmol) were mixed. 1 drop of trifluoroacetic acid has been added. The reaction mixture stirred at room temperature overnight. Then p-chloranil (14.8 mg, 0.06 mmol) was added to the reaction. After stirring 1 hour at room temperature, NEt₃ (1 mL) and BF₃.OEt₂ (1 mL) was added and stirred again for 1 hour. The reaction was finished by extracting with water (3x100 mL). Column chromatography with CHCl₃ yielded the pure product (16 %). ¹H NMR (400 MHz, CDCl₃): δ_H 6.05 (4H, s, ArH), 2.61 (12H, s, CH₃), 1.60 (3H, s, CH₃); ¹³C NMR (100

MHz, CDCl₃): δ_C 157.2, 142.6, 121.6, 14.8, 14.3 ppm; MS HRMS (TOF-APCI): m/z calcd for C₂₆H₂₈B₂F₄N₄: 493.2358 [M-H]⁻; 493.2385 found: [M-H]⁻, Δ = 5.5 ppm.

Compound OB7

In N₂ bubbled 100 mL dichloromethane 2,4-Dimethylpyrrole (14.8 μ L, 0.143 mmol) and **OB5** (18 mg, 0.065 mmol) were mixed. 1 drop of trifluoroacetic acid has been added. The reaction mixture stirred at room temperature overnight. Then p-chloranil (16 mg, 0.065 mmol) was added to the reaction. After stirring 1 hour at room temperature, NEt₃ (1 mL) and BF₃.OEt₂ (1 mL) was added and stirred again for 1 hour. The reaction was finished by extracting with water (3x100 mL). Column chromatography with CHCl₃ yielded the pure product (9 %). ¹H NMR (400 MHz, CDCl₃): δ_H 2.54 (12H, s, CH₃), 2.36 (8H, q, J = 11.3 Hz, CH₂), 1.82 (12H, s, CH₃), 1.10 (12H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_C 155.1, 137.8, 134.8, 133.2, 129.9, 17.1, 14.7, 12.8, 11.6 ppm; MS HRMS (TOF-APCI): m/z calcd for C₃₄H₄₃B₂F₄N₄: 605.3610 [M-H]⁻; found: 605.3618 [M-H]⁻, Δ = 1.3 ppm.

Compound OB8

Compound **OB3** (0.121 mmol, 60.0 mg) and 4-methoxybenzaldehyde (0.728 mmol, 99.2 mg) were added to a 100 mL round-bottomed flask containing 50 mL of benzene, and to this solution were added piperidine (0.6 mL) and acetic acid (0.6 mL). The mixture was heated under reflux using a Dean–Stark trap, and the reaction was monitored by TLC (CHCl₃). When all of the starting material had been consumed, the mixture was cooled to room temperature and solvent was evaporated. Water (100 mL) was added to the residue, and the product was extracted into the chloroform (3 $\times$ 100 mL). The organic phase dried over Na₂SO₄ and evaporated, and the residue was purified by silica gel column chromatography using CHCl₃ as the eluant. Product was separated as a blue solid in 30% yield (35 mg, 0.0363 mmol). ¹H NMR (400 MHz, CDCl₃): δ_H 7.68 (3H, d, J = 18.8 Hz), 7.65–7.55 (m, 8H), 7.40 (d, 2H, J = 8.9 Hz), 7.35 (d, 1H, J = 15.2 Hz), 7.22 (d, 2H, J = 15.2 Hz), 7.10 (s, 1H), 7.02–6.93 (m, 6H), 6.86–6.77 (m, 3H), 6.66 (s, 2H), 3.95–3.85 (9H, m), 3.80 (3H,

s), 2.37 (s, 3H), 2.16(s, 3H), 1.85 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3): δ_{C} 160.4, 153.0, 141.5, 141.3, 138.0, 137.2, 136.5, 136.1, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 117.6, 117.3, 116.9, 116.5, 116.2, 114.5, 114.3, 114.2, 55.4, 55.3, 14.2, 11.5, 9.6 ppm. MS HRMS (TOF-ESI): m/z calcd for $\text{C}_{58}\text{H}_{53}\text{B}_2\text{F}_4\text{N}_4\text{O}_4$ + 967.41891 $[\text{M} + \text{H}]^+$, found 967.41898 $[\text{M} + \text{H}]^+$, $\Delta = 0.1$ ppm. Extinction coefficient (ϵ): 125300 $\text{cm}^{-1} \text{mol}^{-1} \text{L}$ in CHCl_3 .

3.1.9.7 *Singlet oxygen measurements:*

Singlet oxygen quantum yields were calculated according to the literature.^{78, 126} The relative quantum yields were calculated with reference to Methylene Blue (MB) in dichloromethane as 0.57.¹²⁶ First, dichloromethane was bubbled with air for 5 minutes. In UV cuvette absorbance of DPBF was adjusted around 1.0 using air bubbled dichloromethane. Then, photosensitizer was added to cuvette and photosensitizer's absorbance was adjusted around 0.2-0.3. After, taking some measurements in dark, we took cuvette to monochromatic light system for irradiation at peak wavelength for 20 seconds. Absorbance was measured for several times after each irradiation. The graphics recorded are shown below; Figures 45 to 49. Then, slope of absorbance maxima of DPBF at 414 nm versus time graph for each photosensitizer were calculated. Singlet oxygen quantum yield were calculated according to the equation:

$$\phi_{\Delta}(\text{bod}) = \phi_{\Delta}(\text{ref}) \times \frac{m(\text{bod})}{m(\text{ref})} \times \frac{F(\text{ref})}{F(\text{bod})} \times \frac{PF(\text{ref})}{PF(\text{bod})}$$

where *bod* and *ref* designate the “orthogonal bodipy photosensitizer” and “MB” respectively. *m* is the slope of difference in change in absorbance of DPBF (414 nm) with the irradiation time, *F* is the absorption correction factor, which is given by $F = 1 - 10^{-\text{OD}}$ (OD at the irradiation wavelength), and *PF* is absorbed photonic flux ($\mu\text{Einstein dm}^{-3} \text{s}^{-1}$).

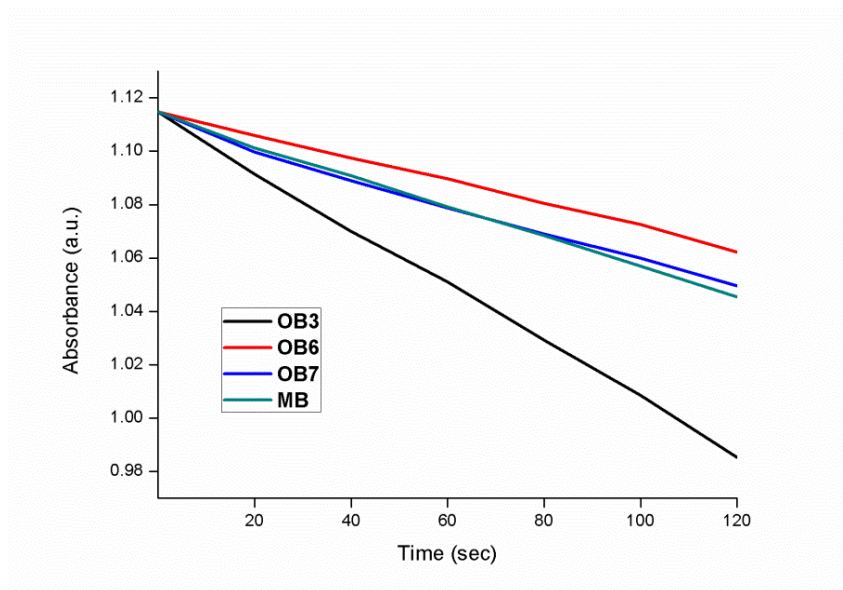


Figure 45. Comparative singlet oxygen generation experiment. Absorbance decrease of DPBF at 414 nm with time in dichloromethane in the presence of Bodipy photosensitizers: **OB3** (black), **OB6** (blue), **OB7** (red) and reference photosensitizer methylene blue (green).

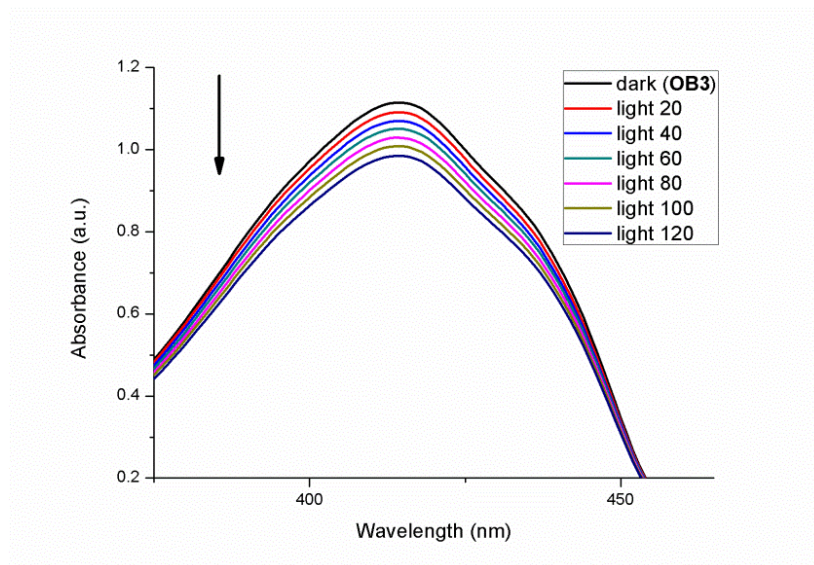


Figure 46. Decrease in absorbance of DPBF in dichloromethane in the presence of compound **OB3** in medium. Details are given in singlet oxygen measurements part.

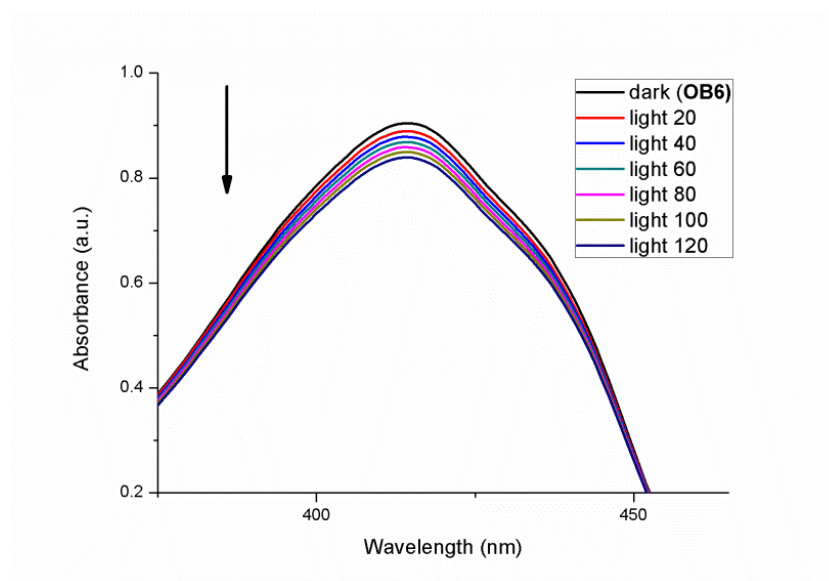


Figure 47. Decrease in absorbance of DPBF in dichloromethane in the presence of compound **OB6** in medium.

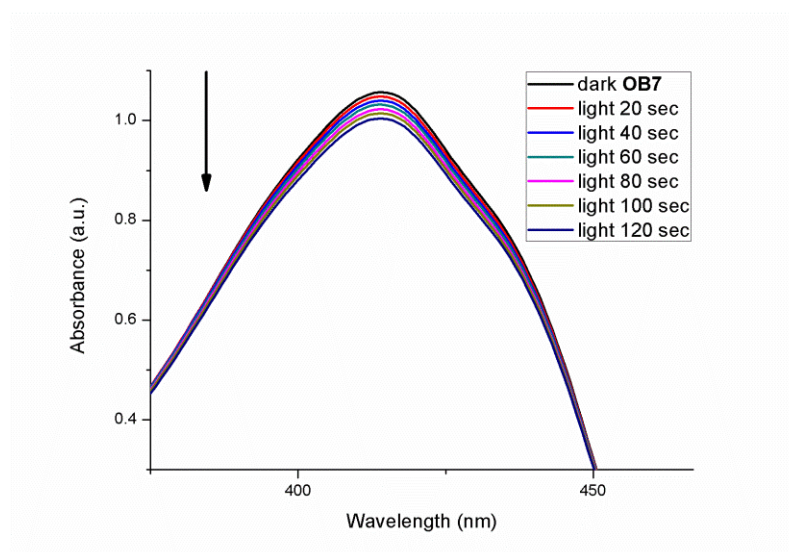


Figure 48. Decrease in absorbance of DPBF in dichloromethane in the presence of compound **OB7** in medium.

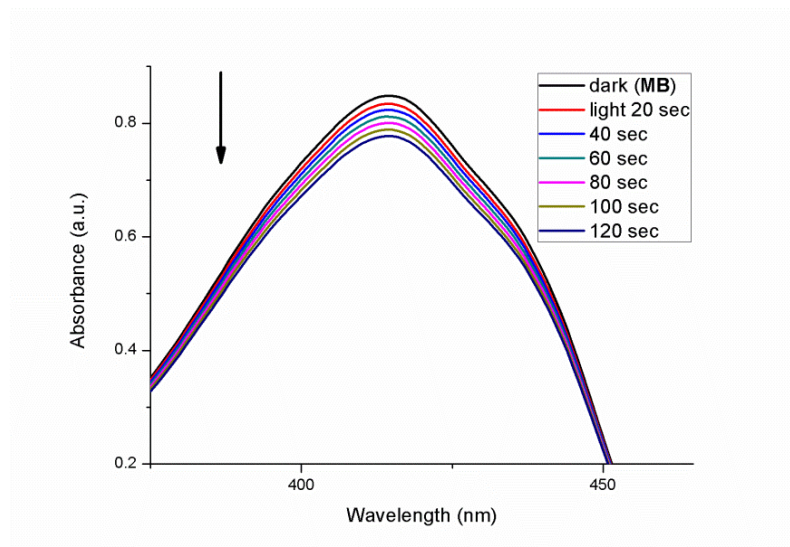


Figure 49. Decrease in absorbance of DPBF in dichloromethane in the presence of compound **methylene blue** in medium.

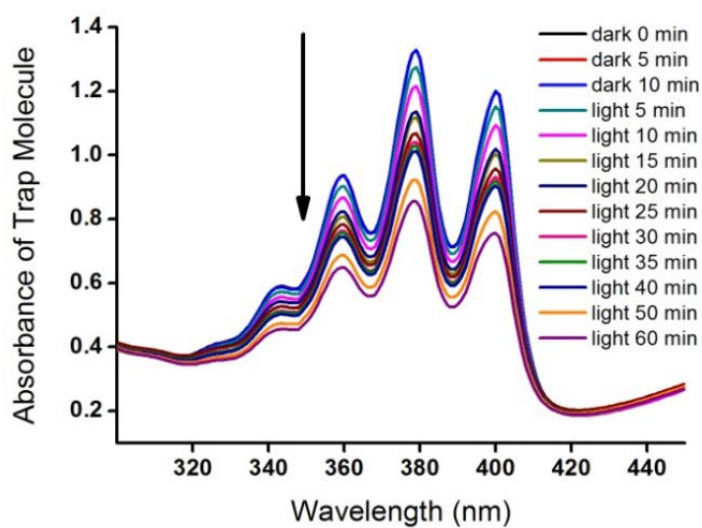


Figure 50. Singlet oxygen generation experiment in aqueous solution. Decrease in Absorbance spectrum of trap molecule (anthracene derivative) in the presence of $1.69 \mu\text{M}$ compound **OB3** in phosphate buffer saline (PBS).

3.1.9.8 Computational Details

Geometries were fully optimized with the *ab initio* Complete Active Space Self Consistent Field (CASSCF) methodology and B3LYP Density Functional employing CEP-31G (SBKJC) and cc-pVDZ basis sets. Harmonic vibrational frequencies were computed to assure that the species did not possess any imaginary frequencies and hence correspond to true minima. After a number of trial and error the active space in the CASSCF calculations were established as 6 electrons distributed over 6 valence orbitals (6,6) for the 8,2' dimer **OB3** and 4 electrons distributed over 4 valence orbitals (4,4) for the 8,8' dimer **OB6** and. Enlarged active spaces up to 12,12 also reproduced the key features presented in the main text. For the sake of consistency a 6 electrons in 6 orbitals CAS is used for both dimers. Geometries of excited states T_1 and S_1 were also fully optimized. Natural orbitals of the CASSCF wavefunction and configuration state function coefficients were analyzed to understand the nature of the electronic state as being a single electron HOMO $\rightarrow$ LUMO type or multiply substituted type. Whereas Bodipy core S_1 is a clean HOMO $\rightarrow$ LUMO dominated $\pi\pi^*$ state, S_1 states of **OB3** and **OB6** are drastically different and showed a large degree of multi-reference character. Gaussian 03 suite was mainly used for the DFT calculations, CASSCF calculations were mainly performed with MOLCAS, and GAMESS-US programs (References for these computational can be found at ref. 129).

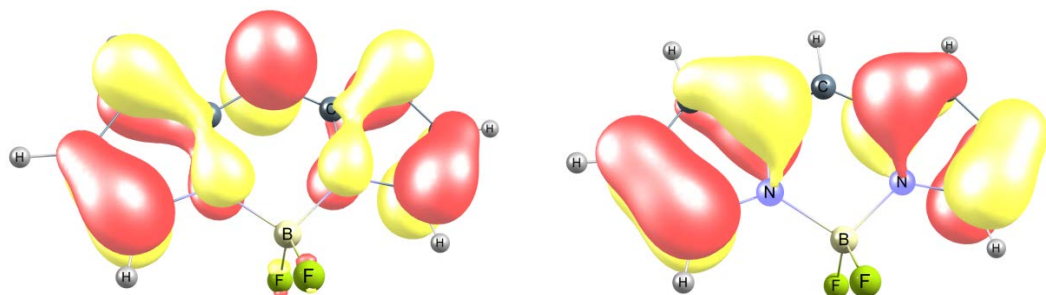


Figure 51. Frontier molecular orbitals. HOMO (left) LUMO (right), of the parent Bodipy core at B3LYP/6-31G(d,p) level of theory. Surfaces are plotted at 0.2 a.u.

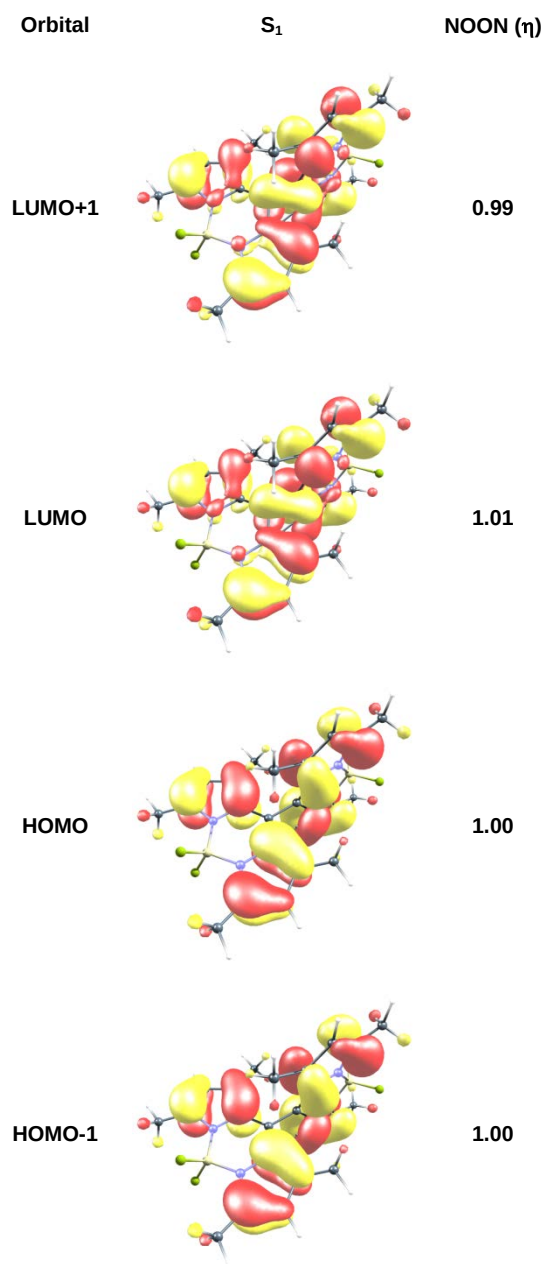


Figure 52. Frontier orbital plots and natural orbital occupation numbers (NOON) of S_1 of **OB6** at CAS(6e in 6o)/cc-pVDZ level. Surfaces are plotted at 0.2 a.u. It is clear that all the four orbitals in both states retain characteristics of BODIPY frontier orbitals and S_1 is a tetra-radical.

3.2 *PEGylated Calix[4]arene as a Carrier for a Bodipy-based Photosensitizer*

3.2.1 *Introduction*

Photodynamic therapy (PDT) is a non-invasive or moderately invasive alternative to more traditional therapeutic methodologies such as surgery, chemotherapy and radiation therapy.⁷ Photodynamic therapy has a built in selectivity as the cytotoxic action is contingent upon the presence of the three components at the same spatiotemporal coordinates, namely, light, sensitizer and dissolved oxygen. Consequently, by controlling the region of irradiation, some selectivity can be achieved.

Recent years witnessed a growing interest in the out-of-the-box proposals for generating and controlling the photodynamic action.^{2, 131} At the same time, novel chromophore families were introduced as potential sensitizers.⁸⁰ Bodipy dyes emerged as a promising class in part, due to our contribution highlighting the chemistry and singlet oxygen generation capacity of these compounds.^{129, 132} Bodipy dyes were previously recognized as chemically and photochemically stable chromophores, and part of this stability was ascribed to the inaccessibility of the triplet manifold, thus reducing the chances of potential degradative interactions with ambient oxygen. However, Bodipys can be transformed into efficient photosensitizers by the incorporation of heavy atoms or by increasing the degeneracy of the excited state frontier orbitals.^{2, 133}

Another matter of concern is the wavelength of irradiation. Current practice of PDT makes use of porphyrins or closely related derivatives.¹³⁴ These dyes have relatively weak absorptions in the red end of the visible spectrum where mammalian tissues are most transparent. Therefore, the ever existing drive to obtain dyes with strong absorptions in the red and near IR region of the spectrum is understandable. The absorption bands of the Bodipy dyes are highly tunable. Especially with styryl substitution, one can move the absorption peak anywhere from 500 nm to 850 nm.¹³⁵

By utilizing this strong property of this dye we come up with a new type of scaffold carrying bodipy units and also enabling it to absorb near IR light.

On the other hand, calix[4]arene is a useful tool for organic chemists due to its hydrophobic core and its intrinsic geometry and shape. Up to date, there is a few example of calix[4]arene molecule have been used with bodipy. In one of the example, where Akkaya *et al.* have synthesized¹³⁶ a calixarene bodipy unit for pH sensing purpose by taking advantage of the photoinduced electron transfer (PeT) process very well known for bodipy type of structures. In this molecule, authors simply monoformylated tetrahydroxycalix[4]arene and bodipy is synthesized around the formyl unit and they could obtained the one bodipy unit is attached to one calix[4]arene unit. It is also important to note that since phenyl of calix[4]arene is orthogonal to the bodipy core, π conjugation is faded, hence the absorption spectrum of the bodipy is not altered. Our synthesis is the improved version of this study in terms of nearly everything; absorbance, application, synthetic route and number of bodipy units attached to the calix[4]arene scaffold.

3.2.2 *Design and Synthesis*

The basic principle in our design is to incorporate maximum number of bodipy units to the calix[4]arene backbone. While achieving this we aimed to put forward a novel type of molecule which has not been synthesized before in order to stimulate for other potential applications.

In the design of our PS (Fig. 54), we have judiciously attached two bodipy chromophores to the calix[4]arene scaffold. The number of bodipy units designed is an indication of our aim to enhance phototoxic effect. The second important parameter of PDT is the absorption wavelength of the PS as told before. Hence, we have thought the bond between calix[4]arene and bodipy should have a π -bond character, therefore we benefit from this strategy to extend the π -conjugation of the bodipy core which causes bathochromic shift of the absorbance wavelength of it. And this is the first time that this strategy has been applied for bodipy with any type

of calixarene. Third, we have designed to attach four decyl units to the lower rim of the calix[4]arene. The aim for this type of attachment is to increase their solubility in common organic solvents and hence to facilitate the synthesis and to make the one side of the molecule hydrophobic for molecule to go inside the cells by diffusing through cell membrane by interacting with hydrophobic moieties there. The fourth design parameter is to make the molecule water soluble, in order to accomplish this, we have attached two poly(ethyleneglycol) polymers to the bodipy. By this way, the other important parameter for this type of applications has been accomplished; amphiphilicity. As a fifth property, in order to increase the π - conjugation further, ethenyl-4-(N,N)-dimethylaminobenzene units were attached to the bodipy core. By this way, maximum absorbance wavelength of around 700 nm is secured. Finally, appropriate to the application we have been trying this molecule to use it for; we have attached heavy iodine atoms to the periphery of the bodipy core. Heavy atom induced spin-orbit coupling causes increase in the rate of intersystem crossing for efficient production of singlet oxygen, one of the basic necessity of PDT.

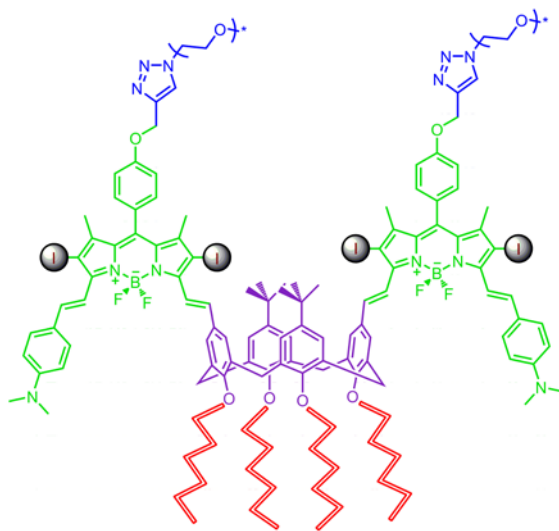


Figure 53. Design of the final CB10 molecule.*

* Note that image is schematic and red alkyl chains are decyl groups

The synthesis of the final compound have been accomplished in eleven steps and eight of it is consecutive and the synthesis scheme is shown in Figures 55-57. In order to get dialdehyde calix[4]arene **CB6** molecule, we needed to go for five steps (Fig. 55). In the first step **CB1** was reacted with benzoyl chloride to get **CB2** as a protecting group for de-*tert*-butylation step. By using AlCl₃ and toluene we successfully de-*tert*-butylated two of the four *tert*-butyl groups of calix[4]arene **CB2** to yield **CB3**. Then, for deprotection of the calixarene from benzoyl groups, we needed to do hydrolysis with NaOH and EtOH. Then, alkylation reaction with 1-bromodecane to yield, **CB5**. In the fifth step, by using hexamethylenetetraamine and TFA at elevated temperature diformylation of the **CB5** is accomplished to get **CB6**, which is then reacted with pre-prepared diiodobodipy to get **CB8**.

CB7 was prepared in three steps (Fig. 56). In the first step propargyl bromide was reacted with 4-hydroxybenzaldehyde. Product was reacted with 2,4-dimethylpyrrole with typical bodipy procedure yielded alkynyl bodipy derivative, which then diiodinated with iodine and iodic acid to get **CB7**. With typical Knoevenagel reaction procedure **CB8** is achieved (Fig. 57), and this compound is purified without performing column chromatography, but precipitated with hexane after dissolved in a minimum amount of CHCl₃. Then a second Knoevenagel reaction was performed on **CB8** to get **CB9**. Finally, with pre-prepared PEG-N₃ click reaction was carried out to yield **CB10**, and purified with column chromatography.

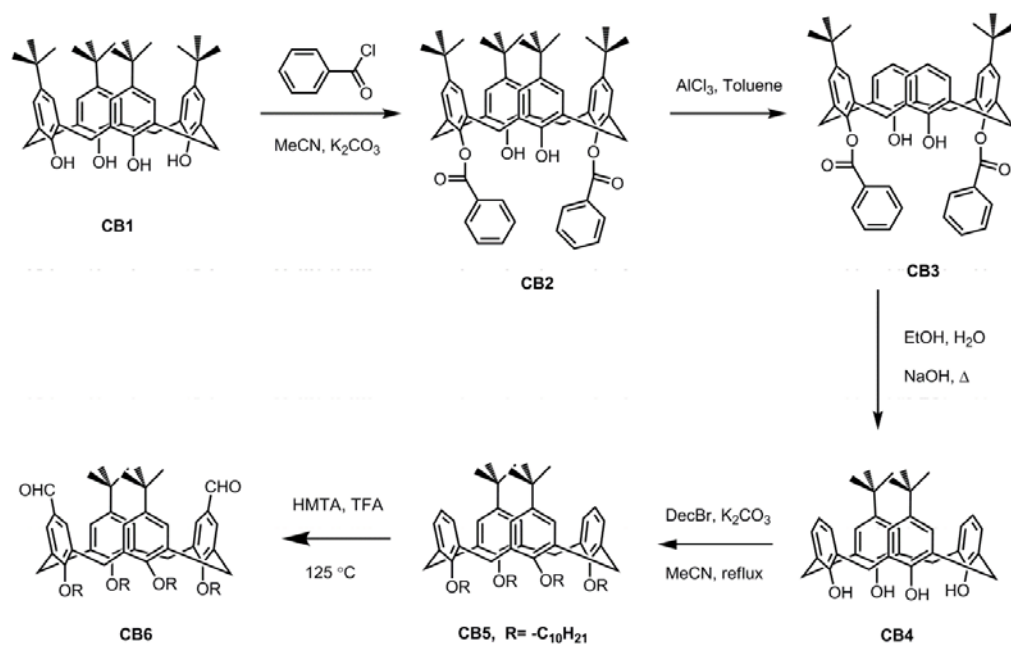


Figure 54. Synthesis of the calix[4]arene derivative to be reacted with Bodipy.

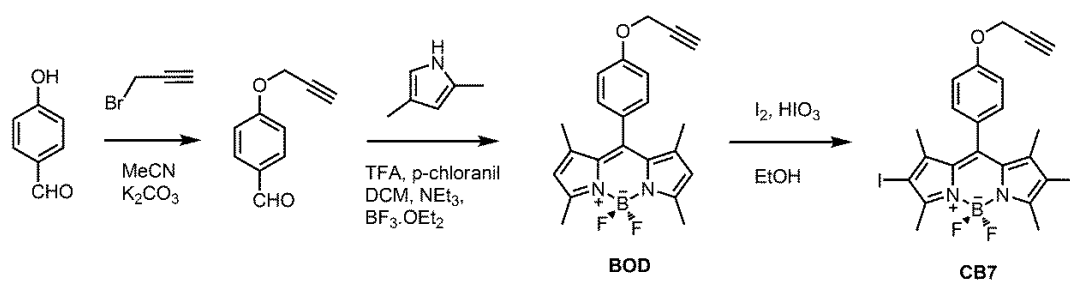


Figure 55. Synthesis of the diiodinated Bodipy derivative.

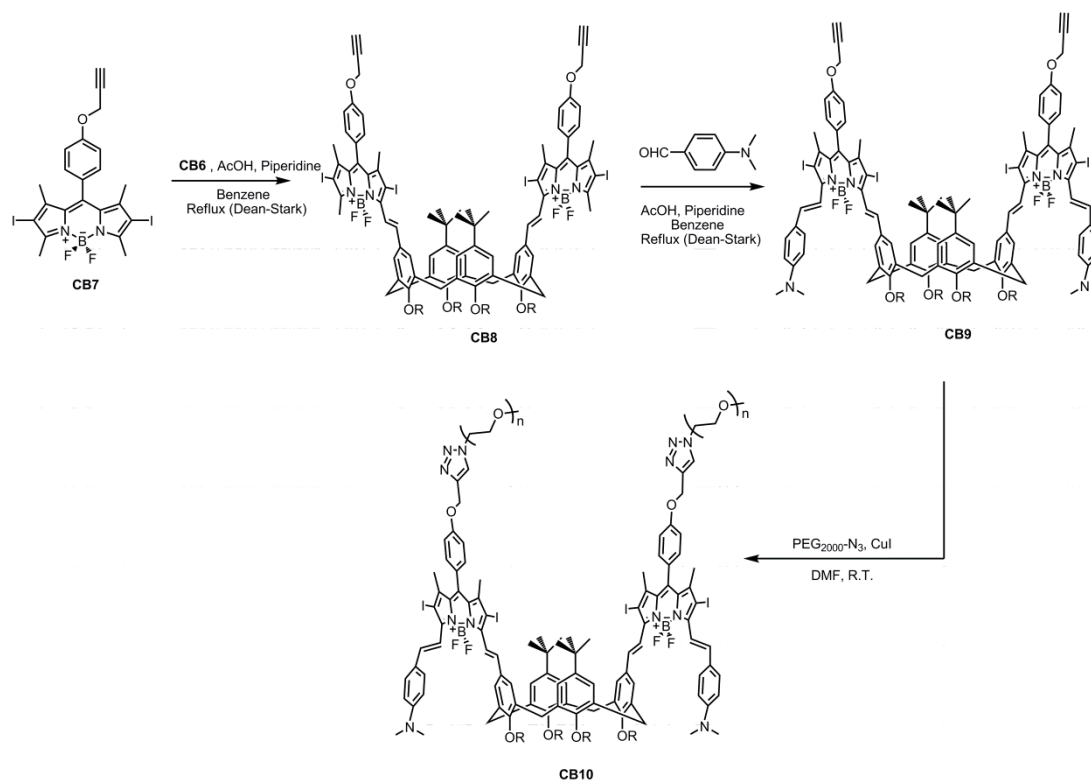


Figure 56. Synthesis scheme of the final **CB10** photosensitizer

The characterization of the compounds has been made by ^1H NMR, ^{13}C NMR and ESI or MALDI mass spectroscopy. Due to the large molecular weights of compounds **CB9** and **CB10** the characterization was hard to get it done. But, by comparative ^1H NMR analysis, the structure of the final compound **CB10** was verified (Fig. 58).

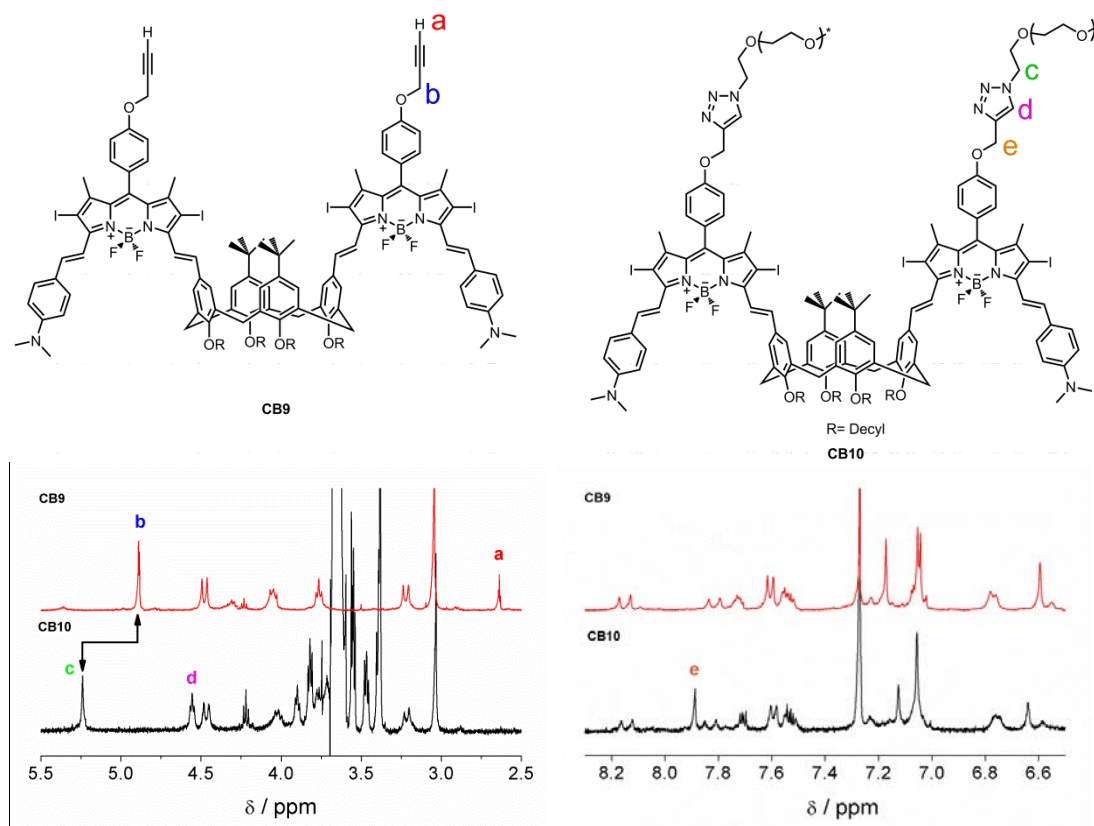


Figure 57. Comparative ^1H NMR Study of the final compound **CB10** and **CB9**.

^1H NMR spectrum of compound **CB10** with respect to **CB9** has been shown in Fig. 58. After final click reaction some alterations took place in limited amounts of nuclei, these will be shown schematically in Figures 58 and will be explained (*vide infra*). In aliphatic region there are three differences between molecules **CB9** and **CB10** which is the proof of the success of the final click reaction. First, disappearance of the acetylenic proton at 2.61 ppm is due to the click reaction which indicates that click reaction is accomplished (proton *a* in Fig. 58). Second, the appearance of the methylene proton of PEG group linked directly to the triazole at 4.58 ppm is verified (proton *d*). And third, shifting of the methylene bridge protons which connects the triazole unit to phenyl group of Bodipy 4.89 ppm to 5.25 ppm is another indication (shift of *b* to *c* in Fig 58). This kind of peak shift is also observed in a similar compound synthesized by Erbas *et al.*¹³³ In the aromatic region

of the comparison (Fig. 58-right) appearance of triazole proton at 7.90 ppm is easily seen (proton *e*). The other protons in this region do not differentiate much since electronic structure and geometric shape of the compound changes a little or not.

3.2.3 *Results and Discussion*

Subsequent to synthesis of the final compound **CB10** we devoted our time to establish the photodynamic activity experiments. At this time, since our compound is amphiphilic we designed two experiments in organic and in aqueous media. As a universal singlet oxygen scavenger compound for studies in organic media we used 1,3-diphenylisobenzofuran molecule (DPBF). This compound has a maximum absorbance at around 410 nm in various solvents and ahead of reacting with singlet oxygen which is produced by the PS in action, the intensity of the absorbance peak decreases, and a new peak in UV region starts to appear. And we focused on the decrease of the intensity at 410 nm.

Initially, we performed control experiments with DPBF, light and bubbled air (5 min) in isopropanol as a solvent, but not add our PS; **CB10**. The photodynamic experiments have been started with recording absorbance spectrum for 15 min in dark by withdrawing light irradiation. Subsequently, for 60 min irradiation with LED light array of 725 nm for 5 min time intervals have been done. The black line in Fig.x successfully shows that there is no decrease in the absorbance of trap compound, specify that no cytotoxic $^1\text{O}_2$ formation.

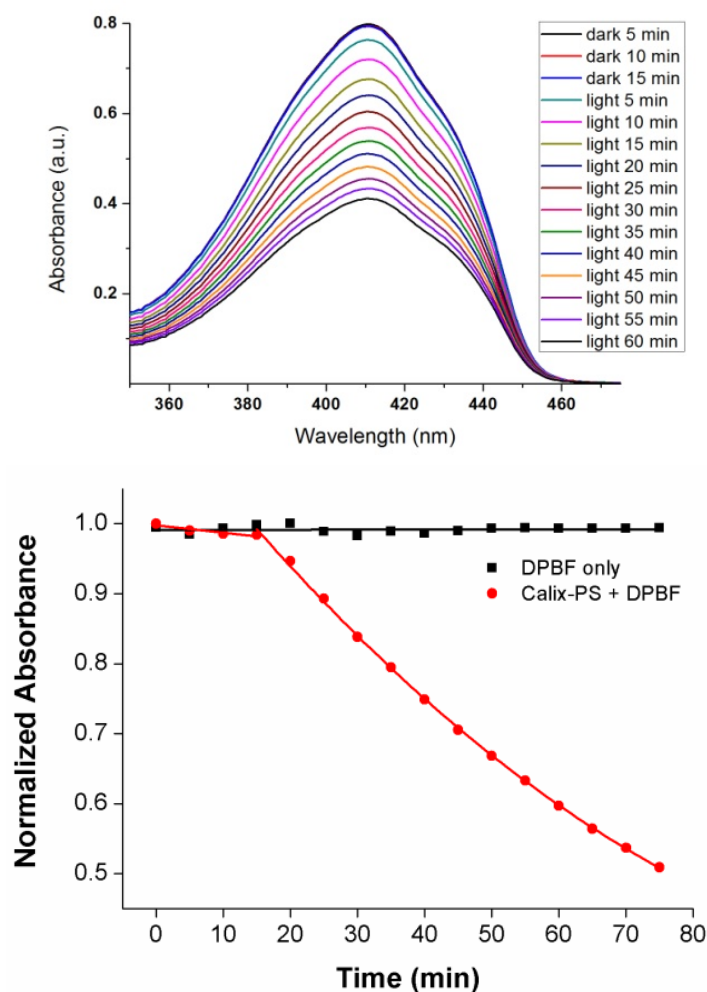


Figure 58. Change in absorbance spectrum of 1,3-Diphenylisobenzofuran (DPBF) in the presence of 46 nM **CB10** in IPA; first 15 min dark then 60 min irradiation with 725 nm LED array (left). Normalized absorbance vs. time graph of DPBF; control experiment without (black line) and with (red line) **CB10** (right).

In the second experiment in isopropanol, we have now **CB10** (46 nM) besides the other reagents in control experiment. In this case, also 15 min dark toxicity experiment has been held, and there is no $^1\text{O}_2$ formation and the absorbance remains the same (red curve in Fig. 59). Afterward, the photodynamic activity has been tested by irradiating with 725 nm light for 60 min with 5 min time intervals again. Successful decrease of the absorbance of scavenger with time is the evidence for $^1\text{O}_2$ formation.

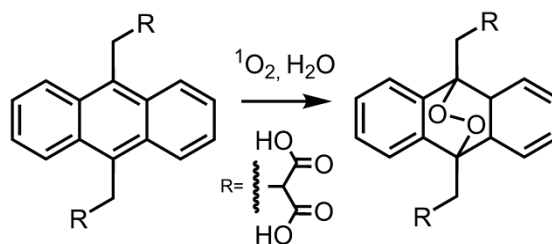


Figure 59. Structure of 2'-(Anthracene-9,10-diylbis(methylene))dimalonic acid (ADMDA) which is used to track $^1\text{O}_2$ production in aqueous media.

Later on, we proceeded to the singlet oxygen ability in aqueous medium. Nonetheless, in this solvent we need a water soluble trap molecule since DPBF is not soluble in highly polar solvents. And we synthesized an anthracene derivative substituted with malonic acid groups* (Figure 60) to track the $^1\text{O}_2$ formation. Through reaction with $^1\text{O}_2$ dioxygen bridge forms and the absorbance signal around 380 nm of the original compound decreases. As a control experiment in PBS solution (with pH 7.4), we recorded absorbance of the trap first with dark (15 min) and 60 min irradiation As it is seen well (Figure 61) (right, black curve) there is a minimum decrease in the absorbance.

* Entire name: 2,2'-(anthracene-9,10-diylbis(methylene))dimalonic acid

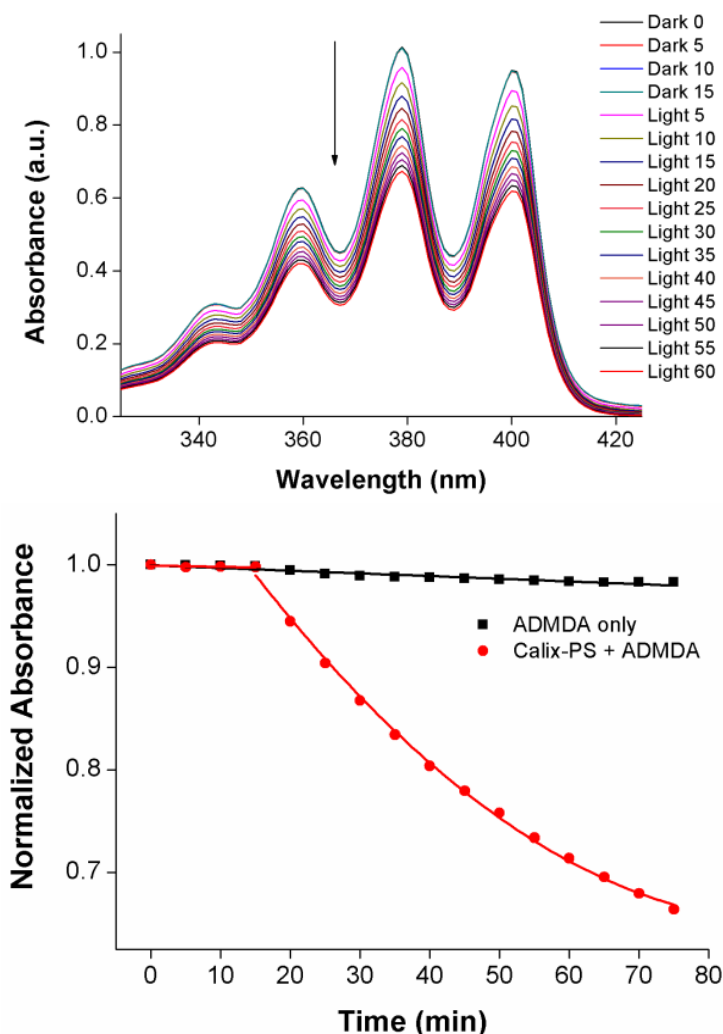


Figure 60. Change in absorbance spectrum of 2'-(Anthracene-9,10-diylbis(methylene))dimalonic acid (ADMDA) in the presence of $2.3\ \mu\text{M}$ of **CB10** in PBS with pH 7.4; first 15 min dark then 60 min irradiation with 725 nm LED array (left). Normalized absorbance vs. time graph of ADMDA; control experiment without (black line) and with (red line) **CB10** (right).

After successful control experiment we prepared a PBS solution with a pH 7.4 and tried to dissolve **CB10**. At this time, the compound has not been dissolved despite the polar ethylene glycol units. However, after dissolving in tiny amount of EtOH and then studying in PBS works well with any precipitation. In this

photoactivity experiment in PBS, 2.3 μM **CB10** was used. Due to short lifetime of $^1\text{O}_2$ in aqua (2 μs) we were obliged to use 50 fold of **CB10** in organic media. The same protocol has also been applied to this experiment; 15 min dark absorbance has been recorded with no change in absorbance and 60 min irradiation with 725 nm LED resulted in successful decrease in the absorbance (Figure 61).

As a result, benefiting from the amphiphilic nature of the calix-bodipy-PS we could successfully observed the singlet oxygen formation and photodynamic activity in both organic (isopropyl alcohol) and aqueous (10% EtOH in PBS) media.

3.2.4 *Conclusion*

At the very beginning of this project, our aim is to get a carrier molecule (e.g. calix[4]arene) substituted with four bodipy units. Moreover, we thought to link this molecule to an antibody of a cancer tissue to recognize it and behave as a targeting group. However, it is obvious that it is difficult to characterize the complex structure of the antibody and react it with our designed PS. In addition, the synthetic difficulties also valid for the calix[4]arene molecule and we had difficulties to functionalize the core unit to yield multiple functional groups (e.g. aldehyde) to react with bodipy. In the end, we come up with decyl units. Although its selectivity in biological media is low, it is very well known that its hydrophobic nature is useful for interacting with cell membrane and diffuse into cells for strong photodynamic effect since nucleus are the primary site of cell destruction. In addition, amphiphilic nature of the PS imitates the biological molecules. As a result, an accessible molecule which is made of facile synthetic routes is the truth of life, at least in an organic chemistry laboratory.

Another problematic part of this study was the low yields that hampered our aim to use this photosensitizer *in vitro* and/or *in vivo* cytotoxicity experiments. Specifically, the two Knoevenagel condensation reactions prior to final reaction was completed with low yields (10-20%). In addition, eight consecutive totally eleven

step reactions sequence is another factor that limits the amount of compound synthesized.

To conclude, though a variety of troubles we faced, we could finally come up with an amphiphilic photosensitizer with dual photoactivity in both organic and aqueous media and the novel type of directly π -conjugated bodipy calix[4]arene scaffold and its unique structure may have a potential to be used for other applications.

3.2.5 *Experimental Details*

3.2.5.1 *General:*

^1H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 (operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) in CDCl_3 solvent with tetramethylsilane as internal standard. All spectra were recorded at 25°C and coupling constants (J values) are given in Hz. Chemical shifts are given in parts per million (ppm). Absorption spectra were performed by using a Varian Cary-100 and Varian Cary 5000 UV-VIS-NIR absorption spectrophotometers. Fluorescence measurements were conducted on a Varian Eclipse spectrofluorometer. Mass spectra were recorded with Agilent Technologies 6224 TOF LC/MS and 6530 Accurate Mass Q-TOF LC/MS. And MALDI spectrums were recorded in Izmir Institute of Technology, Chemistry Department with Bruker MALDI-TOF-TOF. Reactions were monitored by thin layer chromatography using Merck TLC Silica gel 60 F_{254} . Silica gel column chromatography was performed over Merck Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). All other reagents and solvents were purchased from suppliers and used without further purification. In singlet oxygen measurements 1,3-Diphenylisobenzofuran was used as a singlet oxygen trap in organic solvent measurements and was purchased from supplier. 2,2'-(Anthracene-9,10-diylbis(methylene))dimalonic acid was used a singlet oxygen trap in aqueous solvent and was synthesized according to the literature.¹³⁰ Compound **CB4**¹³⁷ and **CB7**¹³³ were synthesized according to the literature.

3.2.5.2 *Synthesis of Compounds*

Calix[4]arene-25,26,27,28-tetradecyloxy (CB5)

NaH (60% dispersion in oil – 2.23 g, 55.7 mmol) was washed with hexane and filtrate was added to the dry DMF (40 mL) with care. Then Calix[4]arene-OH (**CB4**) (1.79 g, 3.34 mmol) and 1-Bromodecane (7.38 mL, 33.4 mmol) were added to the solution. The reaction mixture was heated at 60° C for 12 hours. After reaction it was allowed to cool down. Ice/water was added (100 mL) and the mixture was extracted with dichloromethane (3×50 mL). Organic layer was washed with water (3×50 mL), aqueous ammonium chloride solution (2×50 mL) and brine (50 mL) and dried with K₂CO₃. Then organic layer was evaporated in low pressure and yellow solution was obtained. Upon waiting overnight the compound precipitated in MeOH. The compound was filtrated and washed with MeOH to get the pure product as a white solid (2.4 g, 60%). ¹H NMR : 7.09 (s, 4H; ArH), 6.21 (t, 2H, *J* = 7.5 Hz; ArH), 6.09 (d, 4H, *J* = 7.5 Hz; ArH), 4.45 (d, 4H, *J* = 13.2 Hz; CH₂), 4.05 (t, 4H, *J* = 8.3; OCH₂), 3.72 (t, 4H, *J* = 6.5; OCH₂), 3.11 (d, 4H; *J* = 13.3), 1.95-2.01 (m, 4H; CH₂), 1.81-1.95 (m, 4H; CH₂), 1.51-1.65 (m, 4H; CH₂), 1.15-1.50 (m, 70H; CH₂, CH₃), 1.00-0.85 (m, 12H; CH₂CH₃); ¹³C NMR : 155.5, 155.3, 144.2, 135.9, 133.6, 127.2, 125.6, 122.0, 75.1, 75.0, 34.1, 32.0, 31.8, 31.3, 30.6, 30.4, 30.2, 29.9, 29.8, 29.5, 26.7, 26.1, 22.8, 14.1. HRMS (TOF-ESI): *m/z* calcd for C₇₆H₁₂₀O₄Na: 1119.9084 [M+Na]⁺; found: 1119.9138 [M+Na]⁺, Δ = 4.82 ppm.

Calix[4]arene-11-23-dialdehyde-25,26,27,28-tetradecyloxy (CB6)

A mixture of **CB5** (1 g, 0.91 mmol), hexamethylenetetraamine (4.52 g, 27.34 mmol) and Trifluoroacetic acid (20 mL) was stirred at 125° C for 4 h in a screw-capped vial, then the reaction mixture was cooled to room temperature. Diluted with aqueous 1 M HCl (150 mL) and CH₂Cl₂ (50 mL) and it was stirred for 3 h vigorously. The organic layer was taken and extracted with dichloromethane (50 mL). Then dried with K₂CO₃ and evaporated in vacuo. The compound 3 was obtained pure after column chromatography with chloroform (1.044 g, 99%). ¹H

NMR: 9.15 (s, 1H; CHO), 7.11 (s, 4H; ArH), 6.59 (s, 4H; ArH), 4.49 (d, 4H, $J = 13.4$; CH₂), 4.10 (t, 4H, $J = 8.3$; OCH₂), 3.75 (t, 4H, $J = 6.5$; OCH₂), 3.18 (d, 4H, $J = 13.6$, CH₂), 1.80-2.00 (m, 8H; CH₂), 1.50-1.62 (m, 4H; CH₂), 1.41 (s, 18H; CH₃), 1.18-1.41 (m, 52H; CH₂), 0.88-0.98 (m, 12H; CH₃). ¹³C NMR : 191.5, 160.7, 155.0, 145.3, 135.4, 134.9, 131.1, 129.1, 126.1, 75.3, 75.0, 34.2, 32.0, 31.7, 31.2, 30.4, 30.2, 30.1, 29.8, 29.7, 29.5, 29.4, 26.6, 26.0, 22.7, 14.1. HRMS (TOF-ESI): m/z calcd for C₇₈H₁₂₁O₆: 1153.9163 [M+H]⁺; found: 1153.9107 [M+H]⁺, $\Delta = 4.85$ ppm.

Calix[4]arene-11-23-diethenylBODIPY-25,26,27,28-tetradecyloxy (CB8)

CB6 (100 mg, 86×10⁻³) and Diiodobodipy **CB7** (173×10⁻³) were dissolved in benzene (50 mL). Piperidine (0.3 mL) and then acetic acid (0.3 mL) was added to the solution. The reaction mixture was heated to reflux in Dean-Stark apparatus. When most of the solvent has been evaporated the compound was started to compose and the product was tracked by TLC with CHCl₃ as an eluent. After product formed in majority EtOAc (20 mL) was added and extracted with H₂O (3×30 mL). Organic layer was dried with Na₂SO₄, and evaporated in vacuo. In order to eliminate polar impurities evaporated mixture a short column chromatography done by using chloroform as an eluent. The product then recrystallized with CHCl₃ and Hexanes. The product precipitates in hexane which was filtrated to yield the pure product as blue solid (22 mg, 11 %). ¹H NMR: 7.80 (d, 2H, $J = 16.0$; CH), 7.11-7.21 (m, 6H; CH and ArH), 6.98-7.10 (m, 8H; ArH); 6.51 (s, 4H; ArH), 4.85 (s, 4H; OCH₂), 4.48 (d, 4H, $J = 12.9$ Hz; CH₂), 4.05 (t, 4H, $J = 7.9$ Hz; OCH₂), 3.73 (t, 4H, $J = 6.0$ Hz; OCH₂), 3.20 (d, 4H, $J = 12.9$ Hz; CH₂), 2.60-2.68 (m, 8H, ArCH₃ and CH), 2.00-2.10 (m, 4H, CH₂), 1.82-1.95 (m, 4H, CH₂), 1.50-1.61 (m, 4H, CH₂), 1.41 (s, 6H, ArCH₃), 1.35 (s, 18H, CH₃), 1.10 (s, 6H, ArCH₃), 0.80-1.00 (m, 12H, CH₃). ¹³C NMR : 158.2, 157.1, 155.1, 145.8, 145.2, 140.7, 135.4, 134.0, 132.4, 131.4, 130.5, 129.5, 128.4, 127.3, 126.0, 116.3, 115.7, 78.0, 76.2, 75.5, 75.2, 56.2, 34.2, 32.0, 31.7, 31.0, 30.5, 30.3, 30.2, 29.9, 29.8, 29.5, 29.4, 26.6, 26.2, 22.7, 17.7, 16.9, 14.1. MALDI 2483.722 measured as 2483.558.

**Calix[4]arene-11-23-di(ethenyldimethylaminobenzene)ethenylBODIPY-
25,26,27,28-tetradecyloxy (CB9)**

CB8 (50 mg, 0.021 mmol) and N,N-Dimethylaminobenzaldehyde (7.46 mg, 0.05 mmol) were dissolved in Benzene (50 mL). Piperidine (0.3 mL) and then acetic acid (0.3 mL) was added to the solution. Then the reaction mixture was heated to reflux in Dean-Stark apparatus. When most of the solvent has been evaporated the compound was started to form and tracked by TLC and CHCl_3 used as an eluent. After product formed in majority EtOAc (20 mL) was added and extracted with H_2O (3×30 mL). Organic layer was dried with Na_2SO_4 , and evaporated in vacuo. The mixture was obtained as a pure product after column chromatography with CHCl_3 : Hexane (3:1) as a green solid (17 mg, 31%). ^1H NMR: 8.15 (d, 2H, $J = 16.7$ Hz; CH), 7.81 (d, 2H, $J = 16.6$ Hz; CH), 7.59 (d, 4H, $J = 8.8$ Hz; ArH), 7.53 (d, 2H, $J = 16.8$ Hz; CH), 7.15-7.24 (m, 6H; ArH and CH), 7.00-7.09 (m, 8H; ArH), 6.77 (d, 4H, $J = 8.1$ Hz; ArH), 6.53-6.62 (m, 4H; ArH), 4.89 (d, 4H, $J = 2.4$ Hz; CH_2), 4.48 (d, 4H, $J = 12.8$ Hz; CH_2), 4.05 (t, 4H, $J = 8.1$ Hz; CH_2), 3.75 (t, 4H, $J = 6.6$ Hz; CH_2), 3.21 (d, 4H, $J = 12.9$ Hz; CH_2), 3.01 (s, 12H; $\text{N}(\text{CH}_3)_2$), 2.61 (t, 2H, $J = 2.4$ Hz; CH), 2.00-2.10 (m, 4H; CH_2), 1.82-1.95 (m, 4H; CH_2), 1.51-1.65 (m, 4H; CH_2), 1.48 (s, 6H; ArCH_3), 1.20-1.42 (m, 70H; CH_3 and CH_2), 1.18 (s, 6H; ArCH_3), 0.80-0.97 (m, 12H; CH_3). ^{13}C NMR : 158.1, 156.9, 154.9, 145.4, 135.3, 133.9, 130.9, 129.8, 129.3, 128.8, 127.4, 125.9, 115.7, 112.2, 78.1, 76.0, 75.5, 75.2, 68.2, 56.2, 40.4, 38.8, 37.1, 34.1, 32.0, 31.9, 31.8, 31.7, 31.0, 30.5, 30.4, 30.2, 30.1, 30.0, 29.9, 29.8, 29.7, 29.5, 29.4, 26.6, 26.2, 23.8, 22.7, 17.7, 17.6, 14.1. MALDI 2639.972 measured as 2639.604.

Calix[4]arene-Photosensitizer (CB10)

CB9 (17 mg, 6.44×10^{-3} mmol) and $\text{PEG}_{2000}\text{-N}_3$ (25.76 mg, 0.0129 mmol) were dissolved in dry DMF (5 mL). Argon was bubbled through the solution for 10 minutes and CuI (2.94 mg, 15.4×10^{-3} mmol) was added to the deaerated solution. The reaction mixture was stirred at 60° C overnight. Then 5 mL of CHCl_3 was added and CuI was filtrated. Then solution was evaporated and compound was separated

through column chromatography in MeOH: CHCl₃ (7:93) as green solid. ¹H NMR: 8.15 (d, 2H, *J* = 16.2 Hz; CH), 7.90 (s, 2H; CH), 7.85 (d, 2H, *J* = 16.6 Hz; CH), 7.61 (d, 4H, *J* = 8.1 Hz; ArH), 7.53 (d, 2H, *J* = 15.6 Hz ; CH), 7.21 (d, 2H, *J* = 17.1 Hz, CH), 7.11-7.17 (m, 4H, ArH), 6.72-6.81 (m, 4H, ArH), 6.58-6.70 (m, 4H, ArH), 5.22-5.28 (m, 4H; OCH₂), 4.58 (t, 4H, *J* = 3.8 Hz; NCH₂), 4.48 (d, 4H, *J* = 12.0 Hz; CH₂), 4.03 (t, 4H, *J* = 7.9 Hz; OCH₂), 3.91 (t, 4H, *J* = 4.9 Hz; OCH₂), 3.35-3.88 (m, 364 H, OCH₂), 3.23 (d, 4H, *J* = 11.0 Hz; CH₂), 3.05 (s, 12H; N(CH₃)₂), 1.83-2.11 (m, 8H; CH₂), 1.51-1.63 (m, 4H; CH₂), 1.47 (s, 6H; ArCH₃), 1.23-1.43 (m, 70 H; CH₂), 0.87-0.95 (m, 12H, CH₃). MALDI calculated as 6736.998, found 6728.428.

CHAPTER 4

4 AXIAL CHIRAL BODIPYS

4.1 *Introduction*

Chirality is an important tool for organic chemists especially studying on biology related topics. Because most biological compounds including amino acids and sugars are chiral, and the importance of this topic has been understood very recently compared to the human life on earth. In 20th century the importance of drugs and their role in human health has been recognized in almost all of the developed societies, and a huge amount of money has been invested to pharmaceutical industry. Each year numerous drugs have been put up for sale and most of these are chiral. The effect of racemic mixtures of drugs on health was seen to be lethal by awful examples. Taking into account of the significance, it is evident that chiral organic dyes are extremely vital in several purposes.

Fluorescence enantioselective sensing is one of the most up-to-date and improvable topic among the optical sensing studies due to its potential as a simple detection tool in chiral assays. This detection is quite important for drug discovery, biological labeling, catalysts, and understanding the mechanism of molecular recognition in biological systems.¹³⁸ There are number of fluorescent sensors for recognition of chiral amines, carboxylic acids, alcohols and amino acids.¹³⁸ Specially biologically important hydroxyacids and amino acids have been attracting much interest.¹³⁹ Fluorescence enantioselective sensing can be mainly achieved by two designs. One is the fluorophore-spacer-receptor type probe (PET type) and the second is the fluorophore-receptor integrated probe (internal charge transfer (ICT) type). In both design, one should introduce chirality *on receptor* (binding site) and fluorescence enhancement or quenching have to be seen only with one enantiopure derivative of a chiral molecule. Accordingly, there are many fluorophores have been employed for this purpose such as binaphthol, cyclodextrin, calixarene, cyclic urea

and crown ethers.^{139a} Most widely applied chirality in these compounds is atropisomerism. Atropisomeric compounds are chiral molecules devoid of chiral centers. In this class of compounds, there is a plane or axis of chirality in which chirality exists due to the restricted rotation about single bond. Allenes, cumulenes and biphenyls are good example for atropisomeric molecules.

Bodipy compounds are now important elements of luminophores family due to their excellent properties two of which are ease functionalization and tunability of the absorbance and emission wavelengths. Several attempts to use these compounds in the topic of chirality have been done.^{3, 100, 107} In these examples, a stereogenic center with carbon or boron center exists or bodipy unit is attached to a chiral binaphtol. In the binaphtol case chirality is induced by the binaphtol due to its axial chirality and bodipy is attached as a fluorescent probe, and this compound have been employed for demonstrating different fluorescence characteristics with one enantiomer of a chiral amine^{*} compared to the other one.¹⁰⁰ In this project, we have designed and synthesized various orthogonal bodipy oligomers some of which showing atropisomeric character, hence chiral and investigated their enantiomers by separating using chiral HPLC and verified their helicity using circular dichroism spectra. This study is believed to be used in the chiral assays in the future as the first atropisomeric molecules comprise of solely bodipy dyes.

4.2 *Design and Synthesis*

Previously, we have synthesized a series of dimer bodipy compounds through 2,8' and 8,8' positions, which are shown to exhibit efficient intersystem crossing thus they are efficient singlet oxygen generators. Then, we could use these molecules in PDT concept to kill cancerous cells in vitro in small concentrations upon irradiation with light and in the presence of oxygen. Afterwards, we went further in the synthesis utilizing the same strategy to yield a set of orthogonal bodipy

^{*} specifically 1-phenylethylamine.

oligomers and recognized that some of these structures have a potential to be used as chiral indicators through universal reputation of the bodipy chromophore.

In the first step we formylated 8H Bodipy synthesized from 2,4-dimethylpyrrole, by using classical formulating agents POCl₃ and DMF in 1,2-dichloroethane to get **AB2** in Figure 62. Then typical Bodipy reaction has been accomplished to get **AB3** in 20% to 30% yield. In this set of bodipy oligomers only these two reactions have been done and the yields are around 25% in bodipy reactions and above 90% in formylation steps. In the third step, after formylation we have two types of structures which can be separated easily by using column chromatography, **AB4** and **AB5**. **AB5** is the monoformylated product and **AB4** is the diformylated product. At some stage after monoformylation of the **AB3**, there are two sites for the second formylation and POCl₃-DMF complex picks the bodipy which is not formylated, due to the electron poorer structure of the formylated bodipy and yielding **AB4**. This type of picking has also been verified by the study of Zhu *et al.* since they could synthesize the 2,6-diformyl bodipy.¹⁴⁰ They could not diformylate the bodipy core in one step, but after monoformylation they isolated and consecutive formylation gave the di- product. **AB4** then converted into orthogonal tetramer **AB6** in one step. Monoformyl **AB5** then first converted to chiral **AB7** and then to **AB8** after monoformylation.

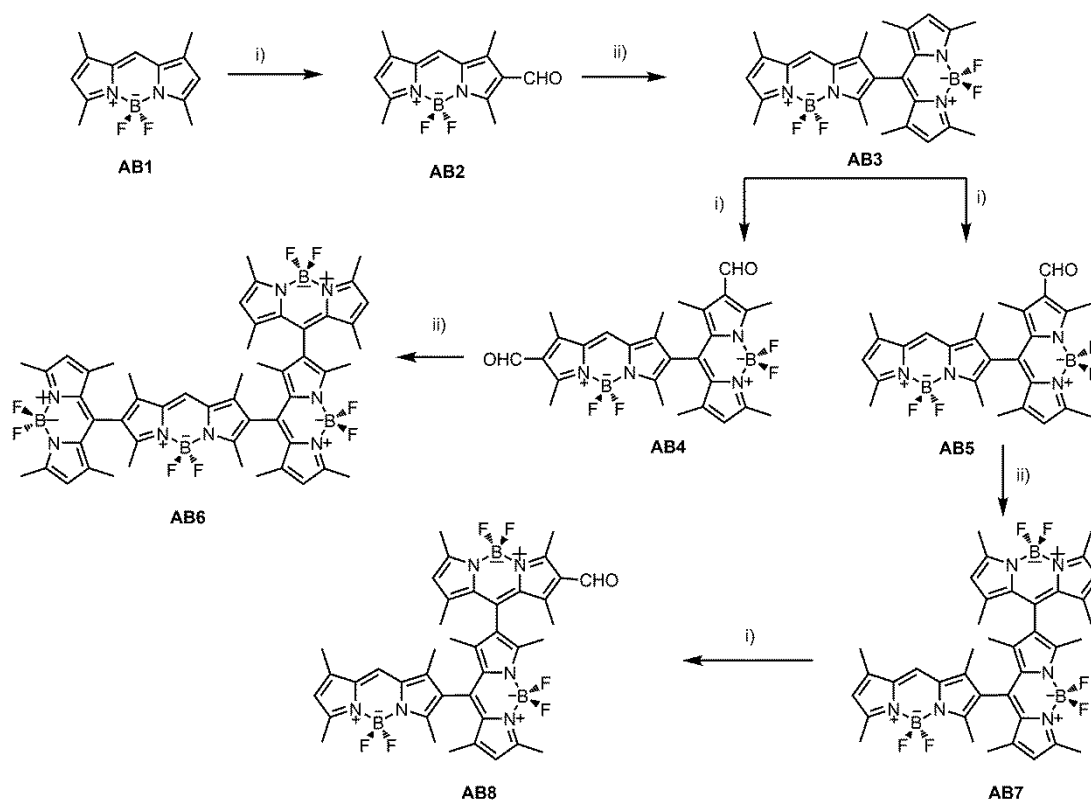


Figure 61. Synthesis scheme for Orthogonal Bodipy Oligomers. i) POCl_3 , DMF, $\text{ClCH}_2\text{CH}_2\text{Cl}$; ii) 2,4-Dimethylpyrrole, TFA, DDQ, DCM, NEt_3 , $\text{BF}_3\cdot\text{OEt}_2$.

Upon mono formylation of the 2,8'-dimer bodipy we achieved **AB5** which ensures one of the chirality requirements of the compound that was synthesized by Ziessel *et al.*; lateral differentiation of the bodipy core.³ After, 3D analysis this compound's comprising a chiral axis has been verified (Figure 63). It is seen explicitly that the angle between two bodipy units is almost 90° . **AB7** which consists of three bodipy units also has a chiral axis (Figure 63-right). Hence, these compounds have a potential to be used as chiral indicators. It is essential to note that two novel compounds, **AB5** and **AB7** are 50%-50% racemic mixture of enantiomers. And it is impossible to separate them using classical column chromatography. Hence, we have performed HPLC separation by using cellulose based chiral columns.

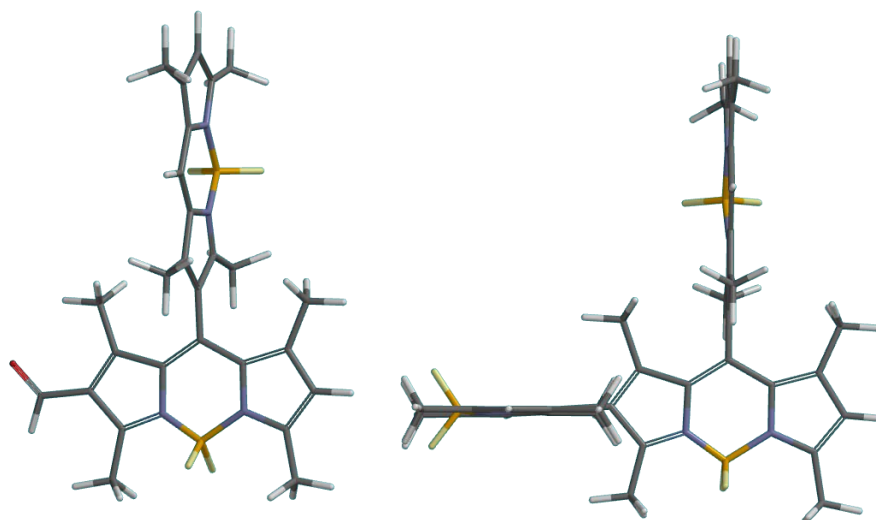


Figure 62. Structures of **AB5** (left) and **AB7** (right).

4.3 *Seperation And Photophysical & Circular Dichroism Characterization Of Enantiomers*

We have synthesized five novel orthogonal bodipy oligomers; two dimeric, two trimeric and one tetrameric. After analysis of their 3D structures we realized that four of these five molecules have a potential to be used as chiral indicators. Only compound **AB8** is a diastereomeric structure. Photophysical of these compounds also examined and it is seen that they have very similar absorption and emission characteristics. All of the synthesized compounds absorb in 499-508 nm region in chloroform. And as expected they all have low fluorescence quantum yield due to the enhanced intersystem crossing property of this type of structures verified in our previous publication.² The normalized absorbance and emission spectra of **AB5** (dimer) and **AB7** (trimer) have been shown in Figure x. The absorbance spectra of these two compounds are very similar and the maxima are 504 and 515 nm for dimer and trimer, respectively. However, their fluorescence spectra have some differences but the common point is their fluorescence quantum yields, both are low; 0.078 and 0.075 for dimer and trimer respectively. Other photophysical parameters like fluorescence lifetime and molar absorptivity have been demonstrated in the Table 5.

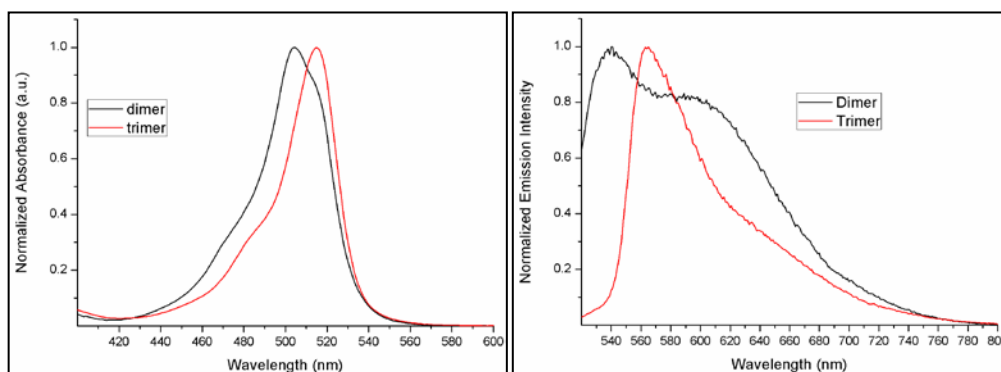


Figure 63. Absorbance and Emission Spectra of the dimer **AB5** and trimer **AB7**.

Table 5. Photophysical Properties of **AB5** and **AB7**.

Dye	λ_{abs} [nm]	ϵ_{max} [$\text{M}^{-1}\text{cm}^{-1}$]	λ_{ems} [nm]	Φ_{f} [%]	τ_{f} [ns]
AB5	504	78000	541	0.78	3.14
AB7	515	162000	565	0.75	3.72

All of the chiral compounds are synthesized as 50%-50% racemic mixtures. And in order to analyze the enantiomers we need to separate with HPLC by using chiral column. We focused on two compounds; **AB5** and **AB7** and we used a cellulose based chiral column. The separation of dimer aldehyde **AB5** is straightforward by using 5% isopropylalcohol in heptane and recorded the HPLC spectrum is shown in Figure 64 and the integrated area of two peaks are equivalent, thus the enantiomers have been separated successfully.

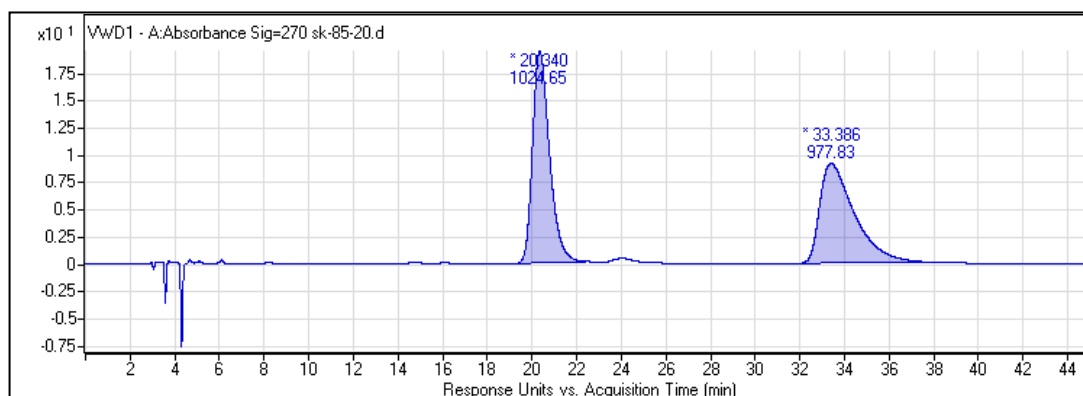


Figure 64. HPLC chromatogram of dimer **AB5**.

After separation in analytical HPLC, we performed preparative HPLC to separate the enantiomers in large amount to analyze. The separated enantiomers have been studied in circular dichroism spectroscopy, and as seen in Figure 65 we got successfully synthesized enantiomers. The peaks around 220 nm corresponds to the aldehyde peaks which are important while tracking the absorbance in HPLC separation. And as seen the absorbance peaks of the two enantiomers around 520 nm converted the incoming polarized light to different directions.

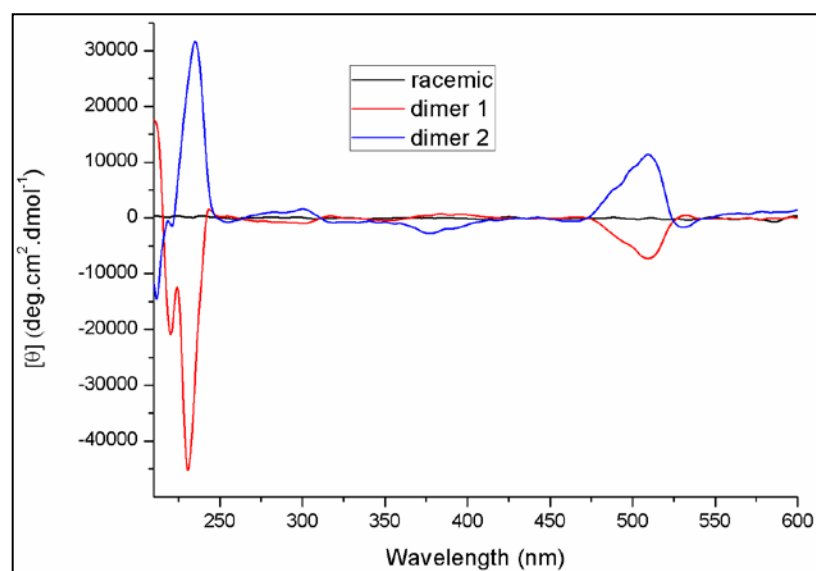


Figure 65. CD spectrum of dimer **AB5**.

After analysis of the dimer **AB5**, we went through to inspect trimer **AB7**. The HPLC separation of this molecule was complicated; the peaks were not

separated 100% though we performed nonpolar solvent system (2% IPA in heptanes - Fig. 66). The rationale behind this fact is the absence of a polar group (e.g. aldehyde) which facilitates the separation. However, we could pick the most pure fractions of the two peaks and analyzed the CD spectrum. This time aldehyde peak around 220 nm has been disappeared and it is unambiguous that the two enantiomers also have been separated well and we could see the absorbances of the two enantiomers around 520 nm which also rotate the incoming polarized light to different directions.

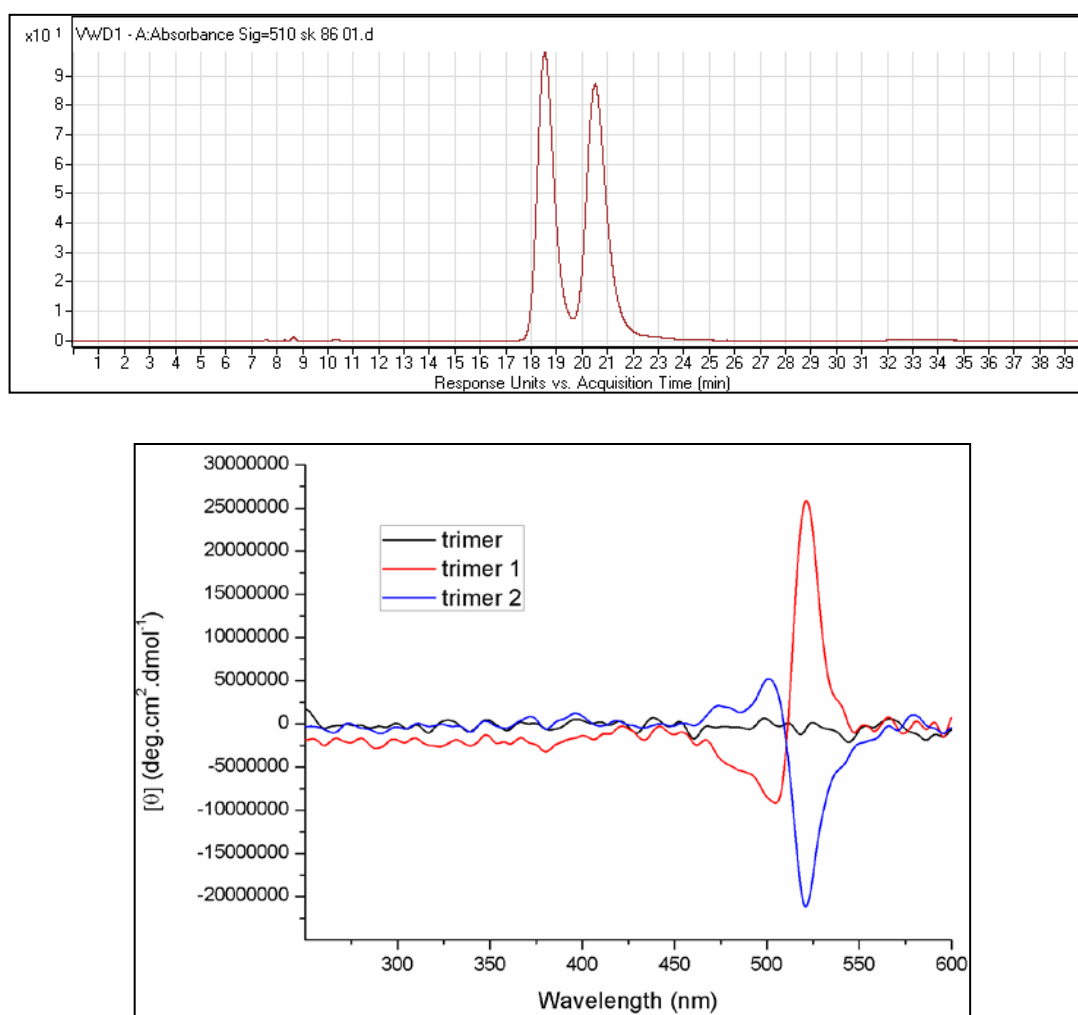


Figure 66. HPLC chromatogram (up) and CD spectrum (below) of trimer **AB7**.

4.4 *Conclusion*

Orthogonal bodipys synthesized for chiral organic fluorophore applications are essential in three ways. First, their structure have a chiral axis which make them chiral molecules without having a chiral stereogenic center and since this type of atropisomeric compounds are very rare and most of these type of structures are binaphtol based, bodipy based chiral assays may have a potential to be used in wide application areas. More, this study is the first of atropisomeric bodipy comprises of solely bodipy structure and nearly all of atropisomeric organic molecules are based on biphenyls. Second, the synthesis is straightforward with two types of reactions and facile purification steps it is possible to get chiral dyes. Third, since binaphtol based compound generally absorb in UV region, these compounds have a substantial gain since they absorb in the visible region. Although their fluorescence quantum yields are low, several chiral organic and/or bioorganic compounds can be probed by tracking the absorbance maxima, in condition that the reaction take place in the or near to the bodipy core (e.g. aldehyde group in **AB5**). We believe these building blocks will find a convenient application area in modern biological labeling and advanced optoelectronic devices.

4.5 *Experimental details*

General: ^1H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 (operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) in CDCl_3 with tetramethylsilane (TMS) as internal standard. All spectra were recorded at 25°C and coupling constants (J values) are given in *Hz*. Chemical shifts were reported in parts per million (ppm). Absorption spectra were performed by using a Varian Cary-100. Fluorescence measurements were conducted on a Varian Eclipse Spectrofluometer. The fluorescence decay measurements were carried out with the Horiba Jobin-Yvon Time-Resolved Fluorometer, Fluorolog FL-1057. The instrument response function was measured with an aqueous ludox solution. Mass spectra were recorded with Agilent Technologies 6224 TOF LC/MS and 6530 Accurate Mass Q-TOF LC/MS.

High performance liquid chromatography (HPLC) separations were performed by using Agilent Technologies HPLC-1200 Series with multi-wavelength detector (MWD) and Agilent Technologies Preparative HPLC-1200 Series with diode array detector (DAD). Circular dichroism (CD) measurements were conducted with Jasco J-815 CD Spectrometer. Reactions were monitored by thin layer chromatography using Merck TLC Silica gel 60 F₂₅₄. Silica gel column chromatography was performed over Merck Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). Compounds (**AB1**), (**AB2**), and (**AB3**) were synthesized according to our pervious study.² All other reagents and solvents were purchased from suppliers and used without further purification.

Synthesis of compound **AB4** and **AB5**

1 mL of DMF and 1 mL of POCl₃ was stirred in an ice bath for 5 min under argon. Then warmed to room temperature and waited for 30 minutes. To this mixture compound (**AB3**) (200 mg, 0.405 mmol) was added in dichloroethane (60 mL). The temperature was raised to 50⁰C and stirred for 2 hours. The reaction medium was cooled and poured into 150 mL of ice cooled NaHCO₃ solution slowly. After that it was stirred for further 30 min, then washed with water 3 times. The organic layers was combined and dried over Na₂SO₄ and evaporated to dryness. **AB5** and **AB4** has been purified by column chromatography by using 2% MeOH in CHCl₃ as an eluent and the compounds has been got pure as reddish solid (70% **AB5** and 25% **AB4**).

AB5:

¹H NMR (400 MHz, CDCl₃): δ_H 10.05 (1H, s, CHO), 7.21 (1H, s, ArH), 6.20 (1H, d, *J*= 3.64 Hz, ArH), 2.83 (3H, s, CH₃), 2.62 (3H, s, CH₃), 2.60 (3H, s, CH₃), 2.41 (3H, s, CH₃), 2.35 (3H, s, CH₃), 2.12 (3H, s, CH₃), 2.02 (3H, s, CH₃), 1.75 (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_C 185.8, 161.9, 161.6, 156.7, 150.1, 146.6, 144.5, 142.2, 135.8, 135.6, 135.2, 134.7, 132.1, 129.9, 126.4, 124.2, 122.9, 121.0, 120.8, 15.1, 14.9, 14.4, 13.1, 12.6, 11.2, 10.8, 9.8 ppm. MS HRMS (TOF-ESI): *m/z* calcd: 522.239; found: 522.6327 [M-H]⁻, Δ = 4.4 ppm.

AB4:

^1H NMR (400 MHz, CDCl_3): δ_{H} 10.11 (1H, s, CHO), 10.06 (1H, s, CHO), 7.45 (1H, s, ArH), 6.23 (1H, s, ArH), 2.86 (3H, s, CH_3), 2.82 (3H, s, CH_3), 2.66 (3H, s, CH_3), 2.61 (3H, s, CH_3), 2.47 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.03 (3H, s, CH_3), 1.76 (3H, s, CH_3); MS HRMS (TOF-ESI): m/z calcd: 549.2262; found: 549.2330 $[\text{M-H}]^-$, $\Delta = 12.4$ ppm.

Synthesis of compound AB7

In N_2 bubbled 250 mL dichloromethane, 2,4-Dimethylpyrrole (145 μL , 1.408 mmol) and (**AB5**) (336.6 mg, 0.646 mmol) were mixed. 2 drops of trifluoroacetic acid has been added. The reaction mixture stirred at room temperature overnight. Then p-chloranil (159 mg, 0.646 mmol) was added to the reaction. After stirring 1 hour at room temperature, NEt_3 (5 mL) and $\text{BF}_3\cdot\text{OEt}_2$ (5 mL) were added and stirred again for additional 1 hour. The reaction was finished by extracting with water (3x250 mL). Column chromatography with CH_2Cl_2 yielded the pure product (20 %). ^1H NMR (400 MHz, CDCl_3): δ_{H} 7.18 (1H, s, ArH), 6.18 (1H, s, ArH), 6.12 (1H, s, ArH), 6.00-6.02 (2H, m, ArH), 2.53 (3H, s, CH_3), 2.52 (3H, s, CH_3), 2.51 (6H, s, CH_3), 2.41 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.25 (3H, s, CH_3), 2.13 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.71 (6H, s, CH_3), 1.53 (3H, s, CH_3); ^{13}C NMR (100 MHz, CDCl_3): δ_{C} 161.4, 159.0, 156.1, 155.5, 150.9, 150.3, 144.8, 144.2, 142.5, 142.0, 137.6, 135.6, 135.1, 134.4, 133.5, 133.0, 132.1, 131.9, 131.7, 131.0, 130.8, 128.8, 125.7, 123.4, 122.6, 121.5, 121.1, 120.8, 120.7, 31.9, 29.7, 22.7, 15.0, 14.8, 14.6, 14.1, 13.8, 13.7, 12.7, 12.5, 11.5, 11.3, 9.7 ppm; MS HRMS (TOF-ESI): m/z calcd: 740.358; found: 740.3585 $[\text{M-H}]^-$, $\Delta = 0.68$ ppm.

Synthesis of compound AB6

In N_2 bubbled 250 mL dichloromethane, 2,4-Dimethylpyrrole (99 μL , 0.56 mmol) and (**AB4**) (120 mg, 0.218 mmol) were mixed. 2 drops of trifluoroacetic acid has been added. The reaction mixture stirred at room temperature overnight. Then p-chloranil (107 mg, 0.436 mmol) was added to the reaction. After stirring 1 hour at

room temperature, NEt₃ (2 mL) and BF₃.OEt₂ (2 mL) were added and stirred again for additional 1 hour. The reaction was finished by extracting with water (3x100 mL). Column chromatography with 2% MeOH in CHCl₃ yielded the pure product (20 %). ¹H NMR (400 MHz, CDCl₃): δ_H 7.29 (1H, s, ArH), 6.15 (1H, s, ArH), 6.05 (1H, s, ArH), 6.04 (1H, s, ArH), 6.02 (1H, s, ArH), 5.99 (1H, s, ArH), 2.62 (3H, s, CH₃), 2.57 (6H, s, CH₃), 2.55 (6H, s, CH₃), 2.43 (9H, s, CH₃), 2.17 (6H, s, CH₃), 1.81 (3H, s, CH₃), 1.76 (3H, s, CH₃), 1.71 (9H, s, CH₃), 1.60 (3H, s, CH₃); MS HRMS (TOF-ESI): *m/z* calcd: 985.4643; found: 985.4712.3585 [M-H]⁺, Δ = 7.0 ppm.

Synthesis of compound AB8

3 mL of DMF and 3 mL of POCl₃ was stirred in an ice bath for 5 min under argon. Then warmed to room temperature and waited for 30 minutes. To this mixture compound (**AB7**) (70 mg, 0.094 mmol) was added in dichloroethane (30 mL). The temperature was raised to 50⁰C and stirred for 2 hours. The reaction medium was cooled and poured into 150 mL of ice cooled NaHCO₃ solution slowly. After that it was stirred for further 30 min, then washed with water 3 times. The organic layers was combined and dried over Na₂SO₄ and evaporated to dryness. **AB8** was purified by column chromatography by using 2% MeOH in CHCl₃ as an eluent two times due to separate from other diastereomer and the compounds has been got pure as reddish solid with 11% yield(7.7 mg, 0.01 mmol).

¹H NMR (400 MHz, CDCl₃): δ_H 10.05 (1H, s, CHO), 7.18 (1H, s, ArH), 6.10-6.20 (3H, m, ArH), 2.83(3H, s, CH₃), 2.63(3H, s, CH₃), 2.62(3H, s, CH₃), 2.59(3H, s, CH₃), 2.42(3H, s, CH₃), 2.40(3H, s, CH₃), 2.30(3H, s, CH₃), 2.12(3H, s, CH₃), 2.04(3H, s, CH₃), 1.76(3H, s, CH₃), 1.75(3H, s, CH₃), 1.55(3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_C 186.2, 161.7, 161.6, 160.3, 157.0, 150.2, 149.5, 146.1, 145.7, 144.4, 142.4, 136.7, 136.0, 135.3, 135.2, 134.7, 133.5, 132.0, 124.0, 123.1, 121.0, 120.6, 15.1, 15.0, 14.4, 14.2, 14.1, 13.1, 12.7, 12.5, 11.5, 11.4, 10.7, 9.7 ppm; MS HRMS (TOF-ESI): *m/z* calcd: 985.4643; found: 985.4712.3585 [M-H]⁺, Δ = 7.0 ppm.

CHAPTER 5

The discussions on this chapter are partially based on the following publication:

Kolemen, S.; Bozdemir, O. A.; Cakmak, Y.; Barin, G.; Erten-Ela, S.; Marszalek, M.; Yum, J. H.; Zakeeruddin, S. M.; Nazeeruddin, M. K.; Gratzel, M.; Akkaya, E. U., *Chem Sci* **2011**, 2 (5), 949-954.

5 THIOPHENE BASED PS'S FOR DSSC

5.1 *Introduction*

The Great East Japan Earthquake happened on March 11, 2011 and it caused a disfunction of the nuclear power plant and made people to search for alternative source of energy in most of the countries in the world. The dye sensitized solar cell (DSSC) concept is a viable alternative to traditional semiconductor based photovoltaic constructs.¹⁰⁹ Practical potential is also being realized in the form of rapid commercialization.¹⁴¹ One of the primary issues, which may benefit from rational design, is the choice of the sensitizer dye. Near IR sensitizability and panchromaticity are important and challenging goals and these can be attained in principle by designing appropriate organic dyes.¹⁴² We are particularly interested in a class of dyes known as Bodipy dyes.^{120, 143} Bodipy dyes are important fluorophores with a multitude of potential applications. Recent developments in Bodipy chemistry allow diverse modification on the core structure.¹⁴⁴ Through these modifications, many characteristics of the parent chromophore can be altered in the desired direction, for example, it is possible to shift absorption wavelength from 500 nm to 800 nm with simple chemical transformations.¹⁴⁵ Solubility and aggregation characteristics of the dyes can also be modulated as needed.¹⁴⁴ These interesting characteristics of bodipy made people direct to itself in many fields. One of those fields is the DSSC concept, or more generally solar cells, since polymer solar cells (bulk heterojunction) also started to construct an important brick of the wall.¹⁴⁶ In a

fresh study by Ziessel et al. they could observe an efficiency of 4.7% by using these types of cells using thiophene appended bodipy structures.

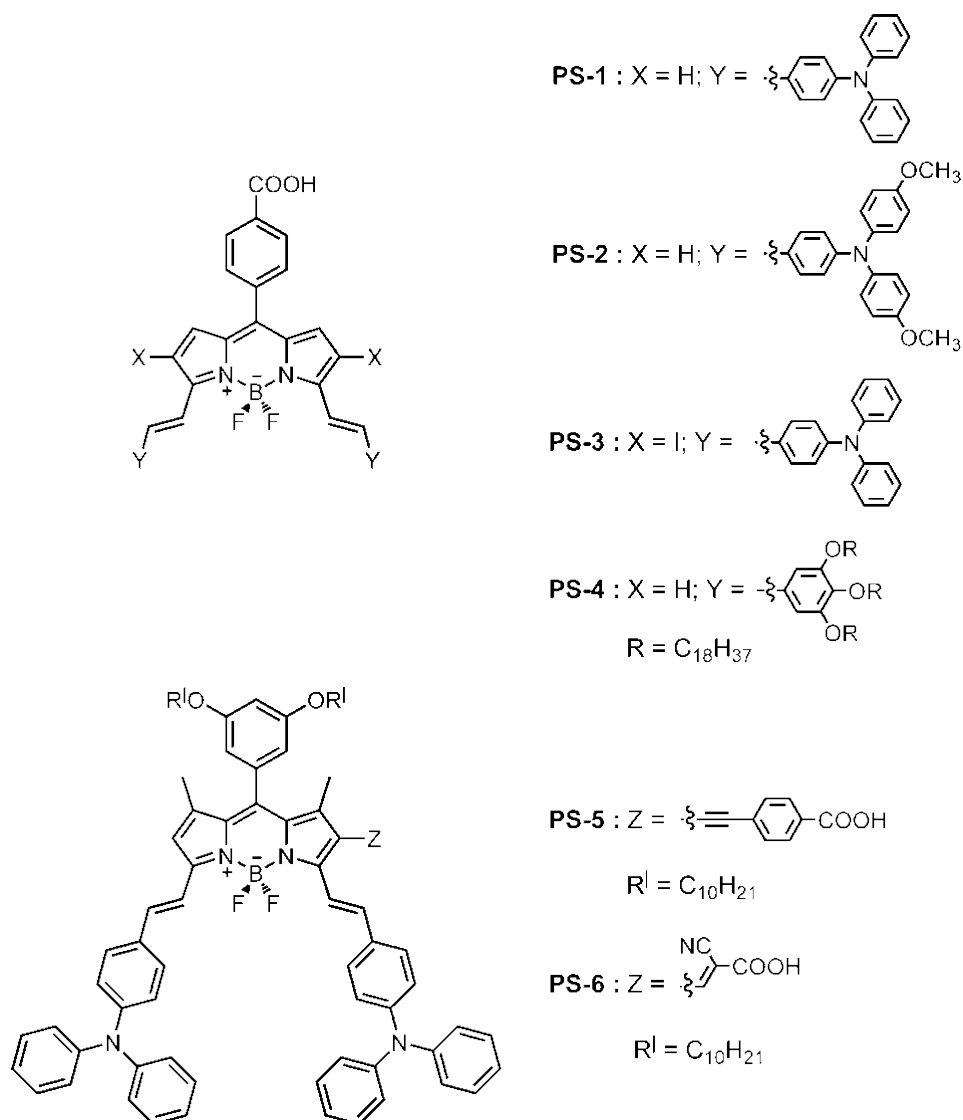


Figure 67. Some of bodipy PSs synthesized recently in literature⁴

The first rationally designed example of a Bodipy based sensitizer was reported a few years ago,¹⁴⁷ followed by a few more recent articles including both liquid electrolyte and solid state based DSSCs.^{4, 148} It is clear that optimal solar cell performance of a photosensitizer is dependent on a large number of parameters;

however, absorption range, anchoring groups and the direction of electronic reorganization on excitation should be among the most important ones. Taking into account of these parameters we have designed and studied seven different bodipy based compounds and constructed cells and evaluated the outputs.⁴ Six of these compounds are in Figure 68. In each one of them, we tried to investigate the role of at least one parameter in solar cell performance. The most successful candidate for a solar cell is the **PS1**; it is formed from 2-methylpyrrole in case of 2,4-dimethylpyrrole so that electron localization to the phenyl ring in the meso position is more feasible. However, we should mention that a different type of cell was constructed special to this dye. In order to inhibit aggregation cheno moiety was used (see abbreviations). Other strategies like putting the anchoring group to 2-position of the dye or incorporating the dye core with iodine atoms did not work. Hence, in our design here we set up novel DSSC candidates (Fig. 69) where we have used novel donor group and a novel anchoring group which have been successful in other DSSC systems but not tried with Bodipy.

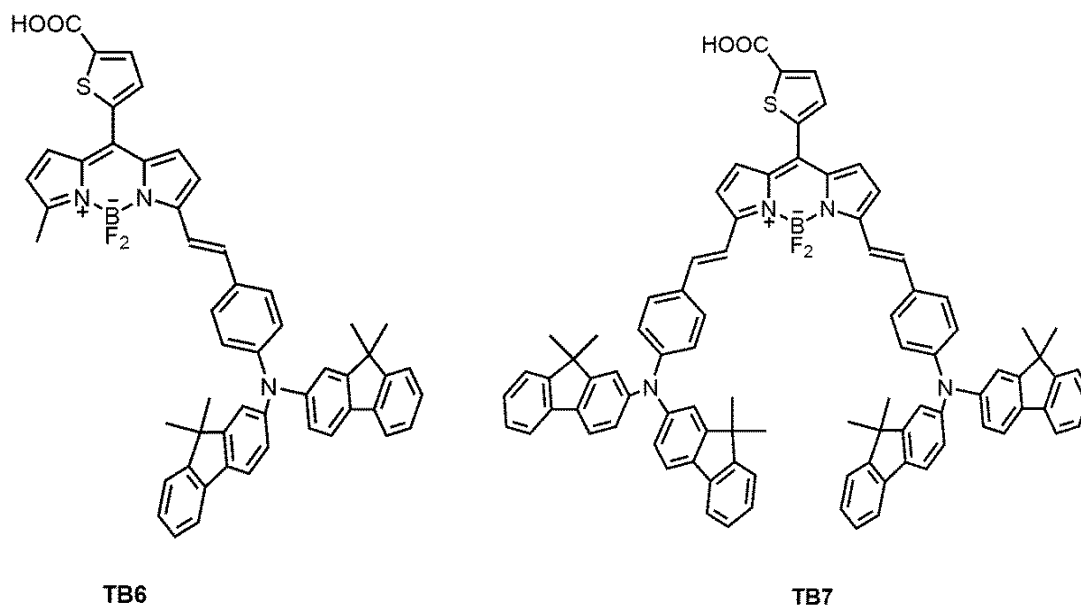


Figure 68. Novel designed PSs for liquid electrolyte DSSC in this research.

5.2 Design and Synthesis:

Thiophene is known to be used frequently in DSSC concept because of its electron transfer ability. Since electron delocalization through the p system is a very important factor in dye sensitized solar cells we designed a bodipy dye, thiophene carboxylic acid unit is attached to the *meso* (8-) position of the bodipy core, where it was calculated before that electron injection is more feasible.¹⁴⁹ Directly attaching of the thiophene to the core also thought to impart efficiency of electron injection, also. As in the successful DSSC application in **PS1** in Figure 68, we have used 2-methylpyrrole instead of 2,4-dimethylpyrrole to increase p electron conjugation from bodipy to thiophene to anchoring unit. As an anchoring group carboxylic acid was used due to the synthetic problems we faced while trying to synthesize cyanoacetic acid moiety, which is also known for efficient binding to the TiO₂ surface and increases electron withdrawing ability to the electron injecting unit. Electron donor part is also an important parameter in DSSC concept, efficiently using these groups have a substantial role in increased cell efficiencies. In this design, we altered our mostly used diphenylaminophenyl group with bis-dimethylfluorenyl amino group. This group has been utilized in some literature reports and due to its bulky structure it has been widely proposed as donor groups. It was also suggested that using bulky structures increases stability of oxidized dye molecules. Hence, first time in bodipy dyes we have designed and synthesized bis-dimethylfluorenyl amino donor groups for DSSC application.

In the synthesis part (Fig. 70), we first iodinated the fluorene molecule to get **TB1** in 90% yield. In the second reaction we methylate the 2-iodofluorene by using a modified procedure from literature with MeI and KOMe in DMF. Then, in the third step we synthesized N,N-Bis(9,9-dimethylfluoren-2-yl)aniline according to the Ullman reaction conditions by using a variety of reagents at high temperatures. Then, in order to synthesize **TB4** aldehyde which is needed for Knoevenagel reaction we performed typical Vilsmeier formylation reaction in CHCl₃. After synthesis of the donor part we moved for synthesis of the bodipy dye core. 5-formyl-

2-thiophenecarboxylic acid was obtained from suppliers and reacted with 2-methylpyrrole to get **TB5** and upon reaction with the synthesized aldehyde **TB4** in 1.3 equivalent we obtained both **TB6** and **TB7** which will be used as photosensitizers in DSSC (Fig. 71).

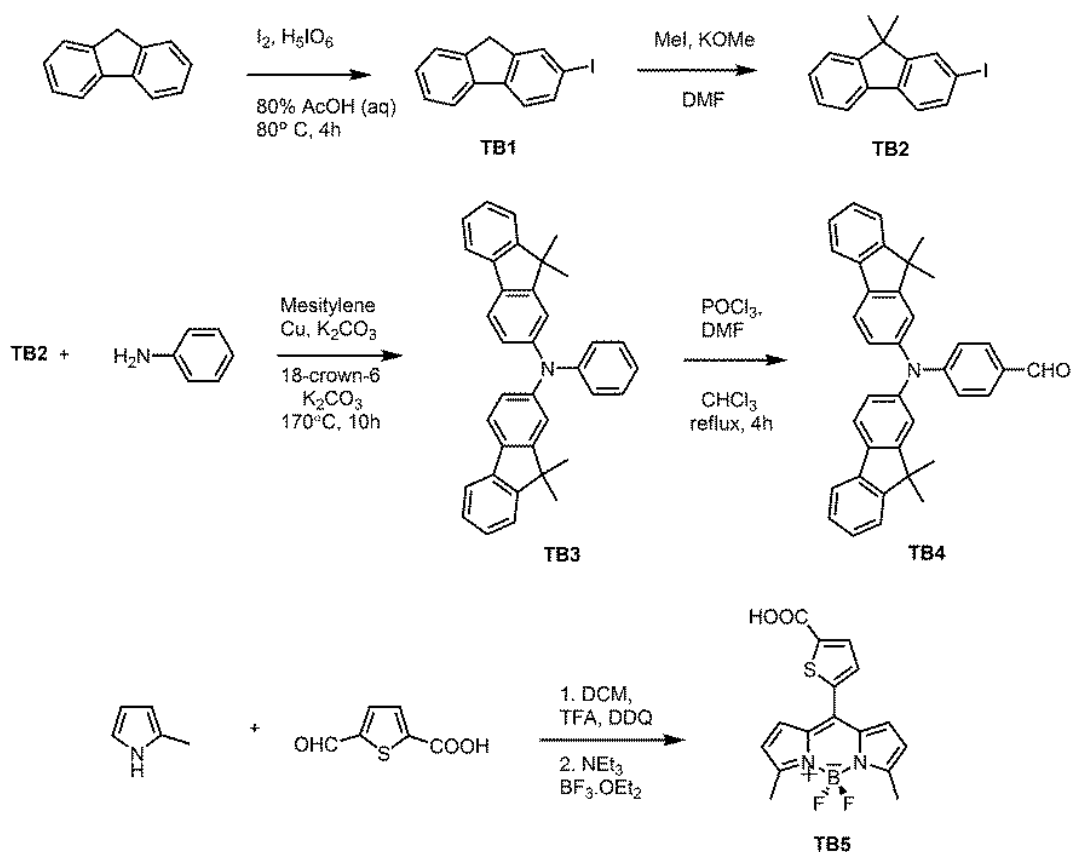


Figure 69. Synthesis scheme of the precursor molecules

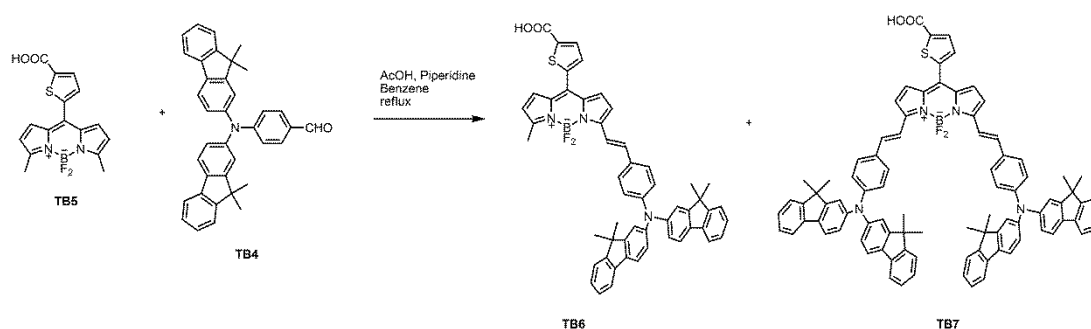


Figure 70. Synthesis of the final PSs **TB6** and **TB7**

5.3 *Results and Discussion*

After synthesis of the PSs **TB6** and **TB7** along with the sole Bodipy **TB5** we have performed photophysical analysis and CV measurements in order to determine if these units are suitable for constructing a cell with TiO_2 and redox couple I^-/I_3^- . Absorbance spectra of these dyes have been investigated in Figure 72, and found that **TB5** has a narrow absorption spectrum with λ_{max} at 520 nm which is characteristic for bodipys and molar absorptivity of this compound is 43700 in MeOH. This compound is barely soluble in DCM or CHCl_3 . **TB6** has a broader absorption spectrum with absorption maximum at 665 nm in DCM, also fluorene moieties also absorb strongly in UV region of the spectrum at around 370 nm. Molar absorptivity of this compound is 38800. Due to fluorene moieties which inhibit π - π stacking of the compound increases solubility, we could dissolve it in DCM. Last PS, **TB7** has a strongly red shifted absorbance maximum at 773 nm in DCM. This compound is promising in this respect because PSs absorbing in the near IR end of the spectrum is highly desired due to strong sunlight illumination at those wavelength region. For example, **PS1** in Figure 68, which has the highest efficiency in Bodipy DSSC has an absorption maximum at 724 nm. The molar absorptivity of **TB7** compound is 53700 at its maximum. All the three compounds have very weak emission which is due to the charge transfer events caused mainly from thiophenecarboxylic acid group.

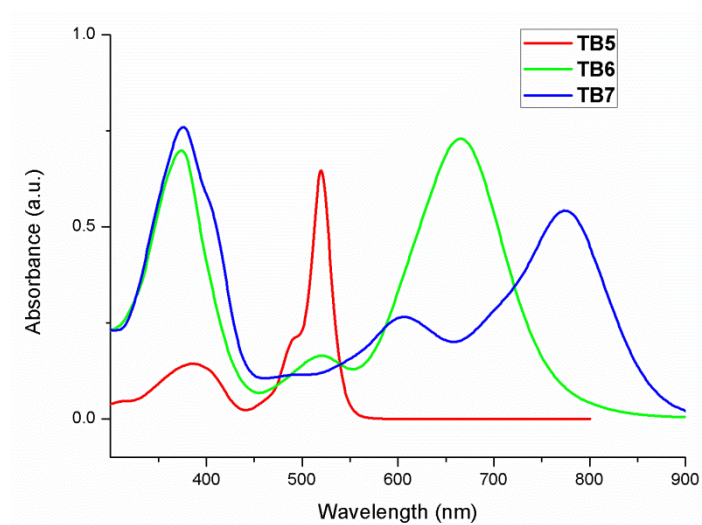


Figure 71. Absorbance Spectra of the compounds synthesized. **TB5** in MeOH, **TB6** and **TB7** in DCM.

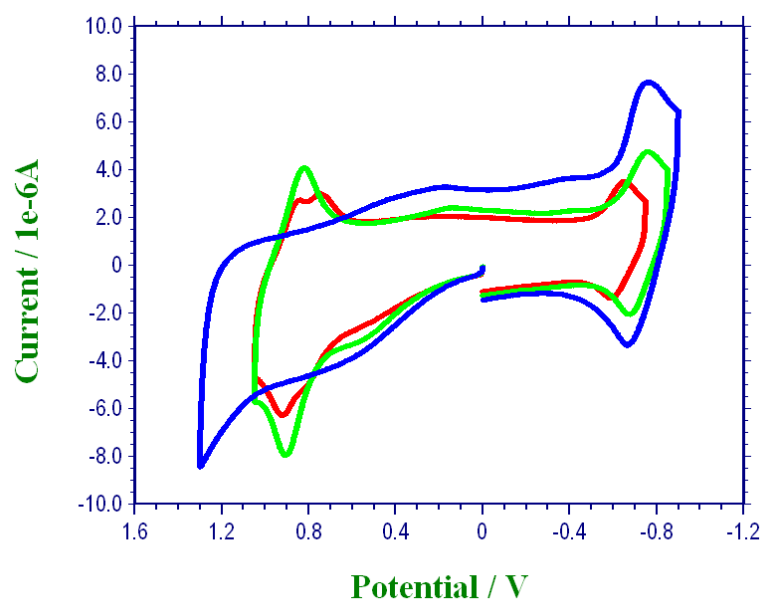


Figure 72. Cyclic Voltammetry Measurements of the **TB5** (red), **TB6** (green) and **TB7** (blue).

Table 6. Redox Potentials and E_{HOMO} and E_{LUMO} Levels of Bodipy Derivatives.

	E _{oxidation} ^a (Volt)	E _{reduction} ^b (Volt)	E _{ferrocene} ^c (Volt)	E _{HOMO} ^d (eV)	E _{LUMO} ^e (eV)	E _{Band Gap} ^f
TB5	0.54	-0.70	0.58	4.84	3.52	1.32
TB6	0.87	-0.72	0.57	5.10	3.51	1.59
TB7	0.83	-0.63	0.61	5.02	3.56	1.46

^a Oxidation potential of Bodipy Derivatives, ^bReduction potential of Bodipy Derivatives, ^cPotential of ferrocene, internal reference electrode ^dHOMO energy level of Bodipy Derivatives, ^eLUMO energy level of Bodipy Derivatives ^fEnergy Band Gap of Bodipy Derivatives

Cyclic Voltammetry of the recently synthesized compounds have been shown in Figure 73. And the table formed from this data has been given also in Table 6 shows explicitly the oxidation reduction bands of these compounds and the HOMO-LUMO band levels and the band gaps. Energy level of the HOMO and LUMO is very important in DSSC concept. It has been given information about this topic in Background part. To summarize upon photoexcitation of the dye molecule an electron excited from HOMO electron orbital to the LUMO and then electron injection from LUMO to the conduction band of the TiO₂ happens. Hence, LUMO energy level should be higher in energy than the conduction band of TiO₂. The energy level of the conduction band is given 4.2 eV⁴. In all three PSs; **TB5**, **TB6**, **TB7**, their LUMO level are convenient for electron injection with 3.51-3.56 eV values which are higher in energy according to the Figure 74. HOMO levels of the bodipy dyes should be also considered since their energy level should be lower than the redox couple energy level (I⁻/ I₃⁻) which is given also as 4.9 eV. As we look at the Table 6, we observe that only **TB5** is not convenient for reducing of the bodipy dye by the redox couple. On the other hand, **TB6** and **TB7** are fit to the desired energy level with 5.10 and 5.02 eV, respectively. However, it was emphasized in a recent review by Ooyama and Harima that, the energy level difference between these levels should be at least 0.2-0.3 eV for efficient electron regeneration.¹¹³ In our

molecules it is 0.2 and 0.12 eV for **TB6** and **TB7**, respectively. Although, these values seem appropriate larger gap would be better suited.

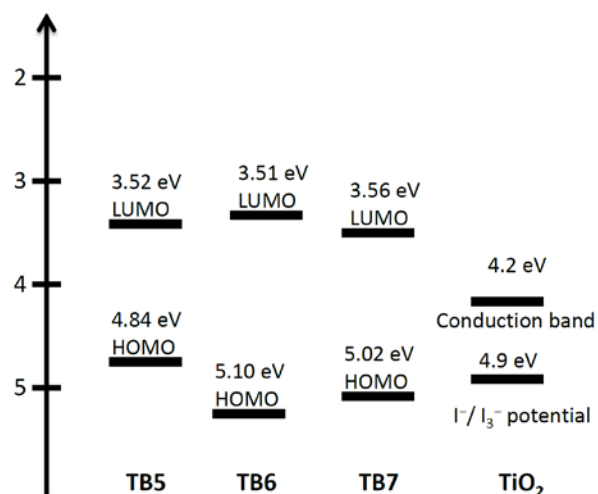


Figure 73. Energy Level Diagrams of **TB5**, **TB6** and **TB7** from the electrochemical data in Table 6.

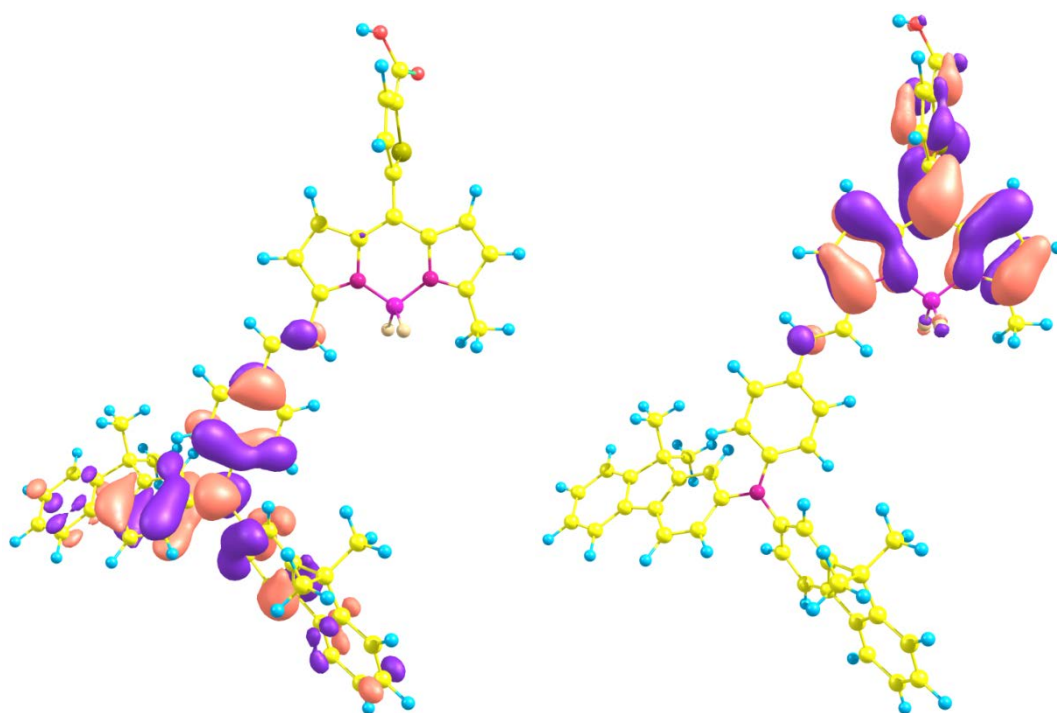


Figure 74. HOMO (left) and LUMO (right) orbitals of the dye **TB6**

The HOMO-LUMO molecular orbitals of **TB6** is shown in Fig. 75 and it seen that in the HOMO molecular orbital electron density is on the diarylphenylamino group and the LUMO electron density is on the bodipy and the thiophene unit. It is apparent that upon photoexcitation electrons move from diarylaminophenyl unit to the anchoring thiophene unit with π - π^* type excitation. However, in the LUMO orbital π electron localization on the bodipy core is not a desired thing which may lower the electron injection efficiency. When looking the optimized geometry of the molecule the degree between the bodipy plane and the thiophene carboxylic acid unit plane is 63° . Although this angle seems large it is a smaller value when compared to the 8-aryl bodipy molecules synthesized from 2,4-dimethylpyrrole due to the steric effect of extra methyl units and in this case the π electron conjugation is limited.

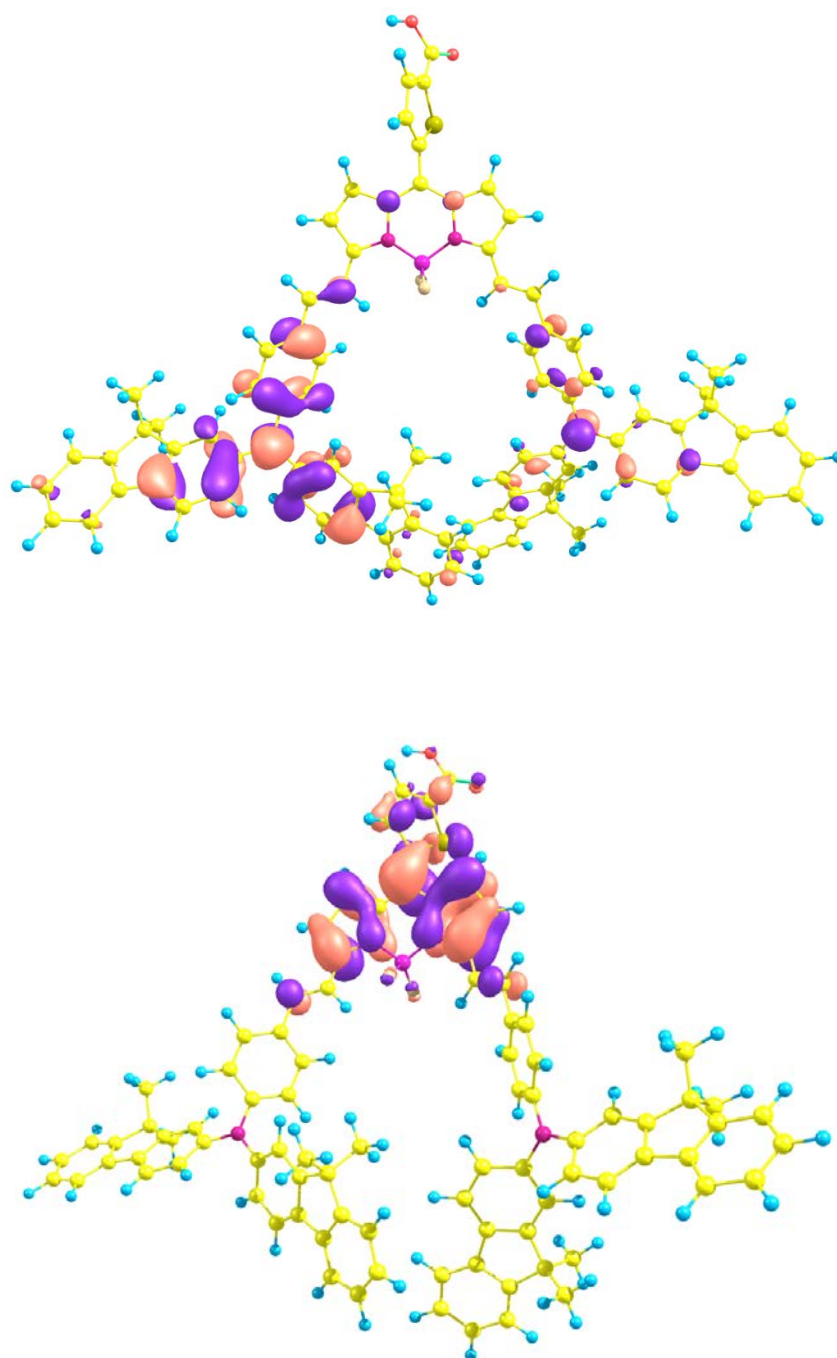


Figure 75. HOMO (up) and LUMO (down) orbitals of the dye **TB7**

In the HOMO-LUMO molecular orbitals of the dye **TB7** (Fig. 76), there is a strange electron localization in the HOMO orbital because the electrons are mainly in one of the diarylaminophenyl unit. This may imply that one of the electron donor groups has a low contribution to electron excitation upon sunlight irradiation. This

may be due to the angle between the styryl plane and the aminophenyl plane. The degrees are 20° and 11° , and in high angle case p electron contribution is low. When looking at the LUMO orbital we see a similar picture compared to the **TB6**. The angle between the bodipy plane and thiophene plane is also same with 63° , and the localization is both on the bodipy and the thiophene. Also, these optimized geometries tell us that in the di-Knoevenagel product **TB7**, large donor groups (difluorenylamino phenyl) affect their orientation and π electron conjugation is diminishes, this may lead to steric congestion which may destabilize the molecule. the design of **TB6** is seems more convenient in terms of three dimensional orientation on TiO_2 surface. On the other hand, aggregation is inhibited more efficiently in **TB7**. Hence, more elegant design may be by utilizing one of this steric donor groups and attaching long alkyl chains to the other side of the molecule.

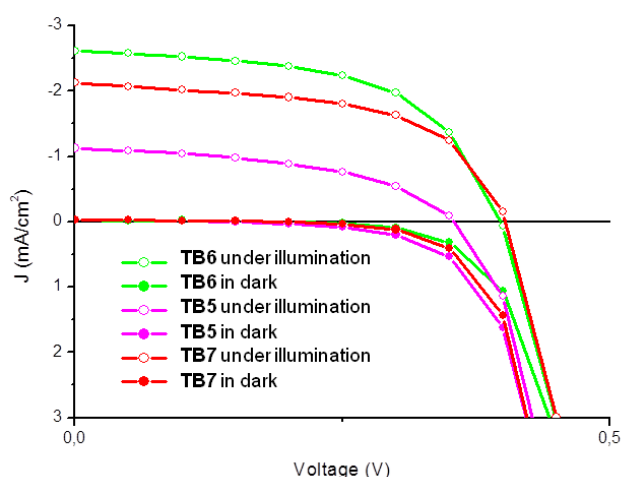


Figure 76. Current vs. voltage graphs of the photosensitizers.

The results indicate above that **TB6** molecule seems more convenient in order to get higher efficiency. After investigation the CV results and computational calculations of valence molecular orbital energies and electron densities we constructed the cells by using typical methods as told in experimental section. And we measured the short-circuit photocurrent density (J_{sc}), open-circuit photovoltage (V_{oc}), photocurrent/voltage curve (J/V curve), fill factor (ff) and incident-photon-to-current conversion efficiency (IPCE) and by the help of these parameters we

calculated the solar energy-to-electricity conversion yield (η). Figure 77 shows the J/V curve of the synthesized three dyes, **TB5**, **TB6** and **TB7** and parallel with our thoughts from CV and computational results **TB6** produces the more photocurrent with 2.61 mAcm^{-2} under AM 1.5 simulated sunlight (100 mWcm^{-2}). And the cell constructed from **TB6** has open-circuit voltage of 400 mV with fill factor of 0.57. And the calculated total sunlight-to-electricity conversion yield (η) is 0.59 (Table 7). This makes this dye finer compared to **TB5** and **TB7**. Also, in the incident-photon-to-current conversion efficiency (IPCE) spectrum also give us information about the response of the dye molecules upon illumination with the monochromatic light in a large range of wavelengths (Figure 78). This spectrum resembles the absorbance of the dyes, however we see that especially in **TB6** and **TB7** there is a big amount of photocurrent produced in the range between 300-400 nm. However, since the irradiation of sunlight in this range is not strong its reflection to the solarlight photocurrent is not so important. For **TB6** we see effective photocurrent in the range between 600-700 nm (Fig. 78).

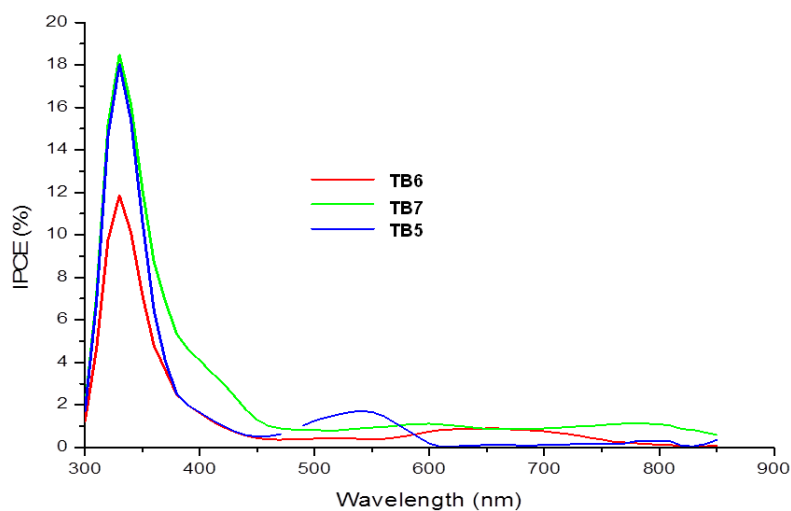


Figure 77. Incident photon to current conversion efficiencies as a function of wavelength for the liquid electrolyte based DSSCs.

Here we constructed the cell of **TB5** as a comparison and we reached low solar cell parameter values with this dye. For the **TB7** DSSC, we acquired similar values to **TB6**. Here, the critical parameter is the highly panchromatic nature of IPCE spectrum (Fig. 78). Although the photocurrent is low it is generated between 500-850 nm. The open-circuit voltage (V_{oc}) is 400 mV, the photocurrent density is 2.12 mAcm^{-2} and the fill factor is 0.58. The overall sunlight energy-to-electricity conversion yield (η) is 0.49.

By evaluating the results it should be kept in mind that the optimization of the cell is an important issue (e.g. electrolyte type, additive (cheno) used) and these are not the final results for the cells constructed with the dyes **TB6** and **TB7**. The photocurrent density is expected to increase after improving the cell construction parameters.

Table 7. Photovoltaic Performance of TiO_2 based Dye Sensitized Solar Cells.

	$J_{sc} \text{ (mAcm}^{-2}\text{)}$	$V_{oc} \text{ (mV)}$	FF	$\eta \text{ (\%)}$
TB5	1.12	370	0.48	0.19
TB6	2.61	400	0.57	0.59
TB7	2.12	400	0.58	0.49

5.4 Conclusion

After constructing the cells from the dye we synthesized overall efficiency (η) of 0.59 for **AB6** and 0.49 for **AB7** has been achieved. However, these results are under evaluation by optimizing the cell parameters and may increase. The novelty in this design compared to the publications from our group is using different donor group and the π electron linker.^{4, 148-149} As a donor group we have used bis-dimethylfluorenyl amino groups in the design. This group is the first time used with bodipy dyes and due to its sterically bulkiness it inhibits the aggregation of the dyes on the TiO_2 nanocrystalline surface. In our two types of dyes these groups sit well for the **TB6**, however in **TB7** by using these two bulky donor groups make one of

the ethenylphenylamino group connected to the bodipy core out-of-plane and electron density in highest occupied molecular orbital (HOMO) is mainly on one of the donor groups only. The second parameter we have used in these molecules is the π electron linker unit, thiophene. This group widely used in various DSSCs due to their high π electron localization ability. And when we inspected the lowest-unoccupied molecular orbitals (LUMO) we observed that electron density is moving successfully from donor groups to the acceptor unit. The main obstacle may be the electron density is not fully transferred to the acceptor and there is some residual density is on the bodipy core. This complication may be solved by using oligothiophene units as a π electron linker for more efficient electron move.

5.5 *Experimental Details*

All chemicals and solvents obtained from suppliers were used without further purification. Reactions were monitored by thin layer chromatography using Merck TLC Silica gel 60 F₂₅₄. Chromatography on silica gel was performed over Merck Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). ¹H NMR and ¹³C NMR spectra were recorded at room temperature on Bruker DPX-400 (operating at 400 MHz for ¹H NMR and 100 MHz for ¹³C NMR) in CDCl₃ or DMSO-*d*₆ with tetramethylsilane (TMS) as internal standard. Coupling constants (*J* values) are given in Hz and chemical shifts are reported in parts per million (ppm). Absorption spectra in solution were acquired using a Varian Cary-100 spectrophotometer. Mass spectra were recorded on Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. Spectrophotometric grade solvents were used for spectroscopy experiments. Compound **TB1**, **TB2**, **TB3** and **TB4** were synthesized according to literature.¹⁵⁰ CV measurement was taken by using CH-Instrument 660 B Model Potentiostat equipment. Solution was prepared in chloroform (10⁻³ M). A three electrode cell was used consisting of Glassy carbon working electrode, Pt wire counter electrode and Ag/AgCl reference electrode, all placed in a glass vessel. Tetrabutylammonium hexafluorophosphate (TBAPF₆), 0.1 M, was used as supporting electrolyte. Ferrocene was used as internal reference electrode. E_{LUMO} level was calculated by

using the formula of $E_{\text{LUMO}} = -e(E_{1/2}(\text{red}) - E_{\text{fer.}} + 4.8)$. The conduction band of TiO_2 layer, $E(\text{TiO}_2\text{-CB}) = -4.2 \text{ eV}$.

5.5.1 **Device Fabrication:**

The construction of the dye sensitized solar cell device requires first cleaning of the fluorine doped tin oxide (FTO) coated glass substrates in acetone and isopropanol by using an ultrasonic bath. FTO ($\text{SnO}_2\text{:F}$, Pilkington TEC-15; Rsheet:150/Ω), electrically conductive oxide-coated glasses were used as transparent electrodes. TiO_2 electrodes consist of an adsorbent mesoporous layer with 20 nm anatase TiO_2 particle size in 7 mm thicknesses and a second light scattering layer with 400 nm anatase TiO_2 particle size of 5 mm thicknesses. Dye solutions were prepared in chlorobenzene-methanol (1:1) mixture with a concentration of 0.5 mM BODIPY. TiO_2 -coated electrodes, after sintering at 450°C for 30 min and cooling to 100°C , were kept overnight in BODIPY solutions for adsorption. BODIPY adsorbed TiO_2 -coated glasses were washed with pure chlorobenzene and dried *in vacuo*. Platinized FTO glasses were used as counter electrode. Platinization of counter electrodes were done by coating of FTO glasses with 1% solution of hydrogen hexachloroplatinate (Aldrich) in 2-propanol and annealing at 400°C for 30 min. Cells were prepared in sandwich geometry. Surlyn-1702 (DuPont) frame was used as a spacer and a thermoplastic sealant between the two electrodes. Cells prepared in this way were then sealed by heating at 100°C . Electrolyte was filled into the space created by Surlyn-1702 between the electrodes under vacuum using a small hole pre-drilled on the counter electrode with the help of a diamond drill. After filling the electrolyte, a small hole was sealed again using a piece of Surlyn-1702 and a piece of cover glass. The electrolyte A6986 consisted of 0.6M N-methyl-N-butyl-imidazolium iodide (BMII) + 0.1 M LiI + 0.05 M I_2 + 0.5 M 4-*tert*-butylpyridine (TBP) in acetonitrile/valeronitrile (85/15 v/v). On the other hand electrolyte Z1040 consisted of 1M LiI, 0.044M I_2 , 0.25M TBP in acetonitrile/valeronitrile (85/15 v/v). Active areas of the cells were adjusted to 0.159 cm^2 with a special mask. Dye sensitized solar cells were characterized by current-

voltage ($J - V$) measurement. All current-voltage measurements ($J - V$) were done under 100 mW/cm^2 light intensity and AM 1.5 conditions. 450 W Xenon light source (Oriel) was used to give an irradiance of various intensities. The spectral output of the lamp was matched in the region of 350–700 nm with the aid of Schott K113 Tempax sunlight filter. $J - V$ data collection was made by using Keithley 2400 Source-Meter and LabView data acquisition software.

5.5.2 *Synthesis*

Compound TB5

400 mL argon-degassed CH_2Cl_2 were added 2-methyl pyrrole (11.5 mmol, 0.93 g) and 5-formyl-2-thiophenecarboxylic acid (5.46 mmol, 0.85 g). One drop of TFA was added and the solution was stirred under N_2 at room temperature for 1 day. After addition of p-chloranil (5.46 mmol, 1.34 g) to the reaction mixture, stirring was continued for 30 min. 5 mL of Et_3N and 5 mL of $\text{BF}_3 \cdot \text{OEt}_2$ were successively added and after 30 min, the reaction mixture was washed three times with water (3 x 100 mL) which was then extracted into the DCM (3 x 100 mL) and dried over anhydrous Na_2SO_4 . The solvent was evaporated and the residue was purified by silica gel column chromatography using (DCM: MeOH 80:20) as the eluant. Red solid (0.75 g, 40%). ^1H NMR (400 MHz, MeOD-d_4): δ_{H} 7.79 (1H, d, $J = 3.8 \text{ Hz}$, ArH), 7.50 (1H, d, $J = 3.9 \text{ Hz}$, ArH), 7.12 (2H, d, $J = 4.1 \text{ Hz}$, ArH), 6.44 (2H, d, $J = 4.4 \text{ Hz}$, ArH), 2.62 (6H, s, CH_3). MS (TOF- ESI): m/z : Calcd: 301.0788, Found: 301.0798 $[\text{M-COOH}]^-$, $\Delta = 3.3 \text{ ppm}$. $\epsilon_{\text{abs}} = 43700$ (in MeOH)

Compounds TB6 and TB7

TB5 (0.29 mmol, 0.10 g) and **TB4** (0.38 mmol, 0.19 g) were added to a 100 mL round-bottomed flask containing 50 mL benzene and to this solution was added piperidine (0.3 mL) and acetic acid (0.3 mL). The mixture was heated under reflux by using a Dean Stark trap and reaction was monitored by TLC (DCM : MeOH 80:20). When all the starting material had been consumed, the mixture is

concentrated and directly added to column chromatography by using DCM:MeOH/ 80:20 and TB7 was separated as a pure product in green color in 52% yield (0.2 mg, 0.152 mmol). To separate the mono Knoevenagel product **TB6** a second column chromatography was done with DCM:MeOH/ 90:10 and **TB6** was separated as a pure product in 15% yield in deep blue color (35 mg, 0.04 mmol).

TB6:

^1H NMR (400 MHz, DMSO- d_6): δH 7.80 (2H, d, J = 8.0 Hz, ArH), 7.77 (2H, d, J = 7.3 Hz, ArH), 7.72 (1H, d, J = 16.3 Hz, CH), 7.62 (1H, d, J = 3.8 Hz, ArH), 7.49-7.60 (5H, m, ArH), 7.43 (1H, d, J =16.3 Hz, CH), 7.25-7.38 (9H, m, ArH), 7.05-7.15 (4H, m, ArH), 6.48 (2H, d, ArH), 2.56 (3H, s, ArCH₃), 1.38 (12H, s, CCH₃). ^{13}C NMR (100 MHz, DMSO- d_6): δC 163.6, 156.7, 155.4, 153.8, 149.4, 146.4, 139.1, 138.6, 135.2, 133.6, 133.0, 131.3, 129.8, 129.4, 127.6, 127.4, 124.4, 123.2, 122.4, 121.7, 120.2, 119.8, 118.5, 116.2, 47.0, 30.1, 27.1 ppm. MS (TOF- ESI): m/z : Calcd: 833.3059, Found: 833.2955 [M], Δ =12.5 ppm. ϵ_{abs} = 38800 (in DCM)

TB7:

^1H NMR (400 MHz, DMSO- d_6): δH 7.76 (4H, d, J = 8.0 Hz, ArH), 7.72 (4H, d, J = 6.8 Hz, ArH), 7.65 (2H, d, J = 16.6 Hz, CH), 7.40-7.50 (12H, m, ArH; CH), 7.20-7.35 (16H, m, ArH), 7.07 (8H, m, ArH), 1.34 (24H, s, CCH₃). ^{13}C NMR (100 MHz, DMSO- d_6): δC 163.4, 155.4, 154.8, 153.7, 149.2, 146.3, 138.5, 137.8, 135.3, 135.2, 132.6, 131.8, 130.0, 129.7, 129.2, 129.0, 127.5, 127.4, 124.3, 123.1, 122.3, 121.7, 120.1, 119.7, 117.9, 116.6, 46.9, 27.1 ppm. MS (TOF-ESI): m/z : Calcd: 1320.5359, Found: 1320.5085 [M], Δ =20.7 ppm. ϵ_{abs} = 53700 (in DCM)

CHAPTER 6

6 CONCLUSION

Investigation of novel materials is an important tool to respond the problems like eradicating the cancerous tissues or to solve the energy problem by finding environmentally friendly devices. In order to achieve these goals the principles of the materials on earth should be inspected carefully. Chemists with the help of other fundamental sciences try to develop methods to observe, learn, and apply them to the materials they study. This thesis is one drop in that ocean which aimed to increase the wisdom of the people studied to make it done. In this thesis included three main titles; photodynamic therapy, axial chirality and dye-sensitized solar cell, and four different research projects.

In the first title of PDT, novel sensitization through intersystem crossing of the dimer bodipy molecule has been achieved. The novelty lies in the theoretical design of the excited states and then applying this to a real example of converting ground state triplet oxygen to the reactive singlet oxygen species with the aim of destroying malignant tissues. In the early study a bodipy dimer absorbing around 500 nm was synthesized and due to the wavelength limitation of the therapy, shifting the wavelength to longer part was thought and applied. However, our study failed in this way because doubly-substituted state forming upon excitation started to disappear. Hence, it was proposed that an adjustment is needed to realize this thought e.g. changing the boron difluoride bridge in the core.

Second study in PDT title was about proposing a nanomaterial acting as a holder for carrying a pharmaceutical to the target. This pharmaceutical is a well-known bodipy unit with heavy atoms and the essential water soluble units and long alkyl chains for cell membrane permission. Using both hydrophilic and hydrophobic structures make these materials amphiphilic which is convenient for studying *in vivo*. Due to synthetic complications our first aim has not been totally came true, but

the original design and employing calix[4]arene unit with bodipy in a π -conjugated manner made this project a future prospecting one.

For the concept of axial chirality, we designed oligobodipy molecules which were attached to each other in an orthogonal fashion. Molecules possessing this property has been desired mainly in advanced optoelectronic devices and biomolecule assays. Our molecules forms the first example of atropisomeric type chiral compounds in which a chiral axis exist around a single bond. A great percentage of these compounds used are formed from biphenyls and when considering the superior properties of bodipy dyes, these compounds have a chance to be utilized in advancing the probing units used.

The final topic was about the DSSCs formed by utilizing the recently synthesized bodipy dyes for developing the efficiency of the cells constructed before. Modifying the p electron linker and the donor molecules seems not working well at least for now. Meso position of the bodipy core is reported to be the most convenient place for attaching the electron withdrawing and anchoring unit. In our previous studies we used phenyl units attached to that position, however in this case thiophene units have been incorporated with carboxylic acid. The angle between the thiophene and bodipy is 63° which is larger than the phenyl attached one. This may be one reason for the low efficiency. Bis-dimethylfluorenyl amino groups are widely used in DSSC due to its steric effect which hampers the aggregation. Using these dyes in **TB7** did not work well because in this molecule this donor groups attached both 3 and 5 position of the bodipy core due to steric congestion in one of the donor p electron conjugation seems hampered as it is understood from the HOMO orbitals. Total solar energy-to-electricity conversion yield (η) was acquired 0.59 for **AB6** and 0.49 for **AB7**, however, these values expected to increase since cell optimization is still proceeding by our collaborator.

To conclude, with these topics studied it is apparent that bodipy is not a sole fluorophore but due to its rich chemistry its applications are expected to spread to most other topic as well.

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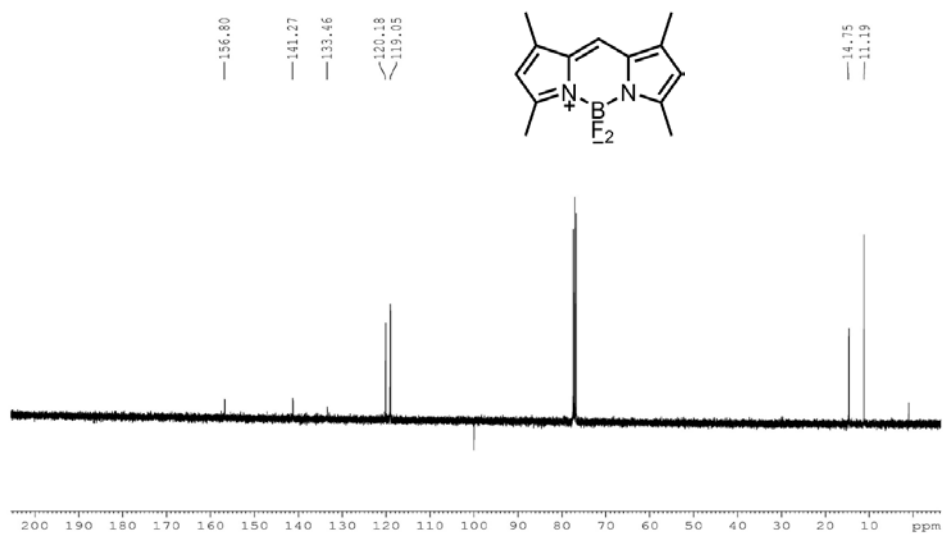
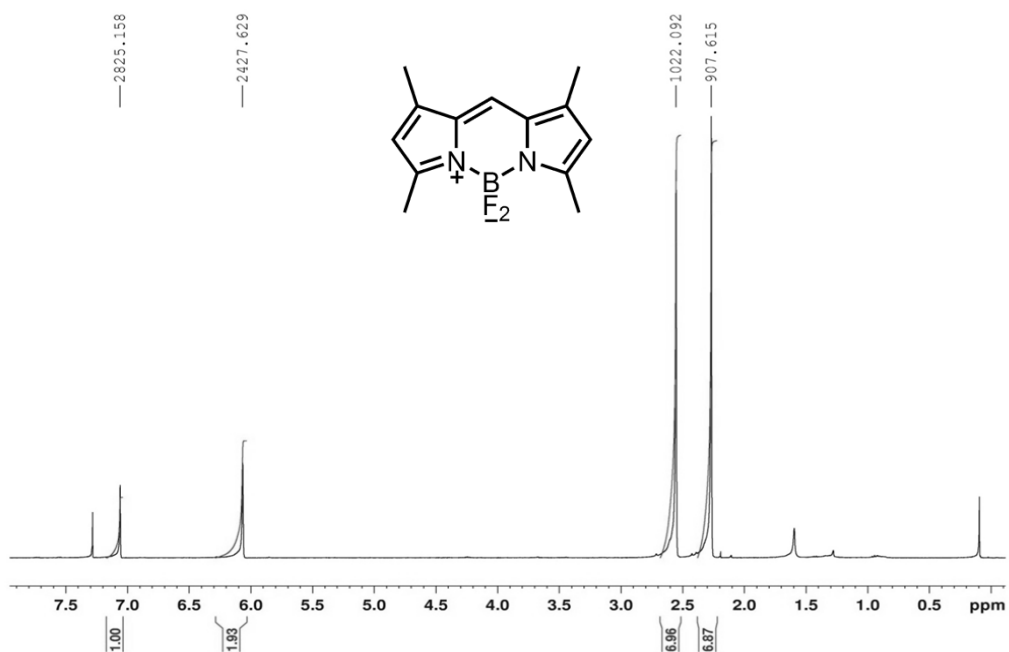
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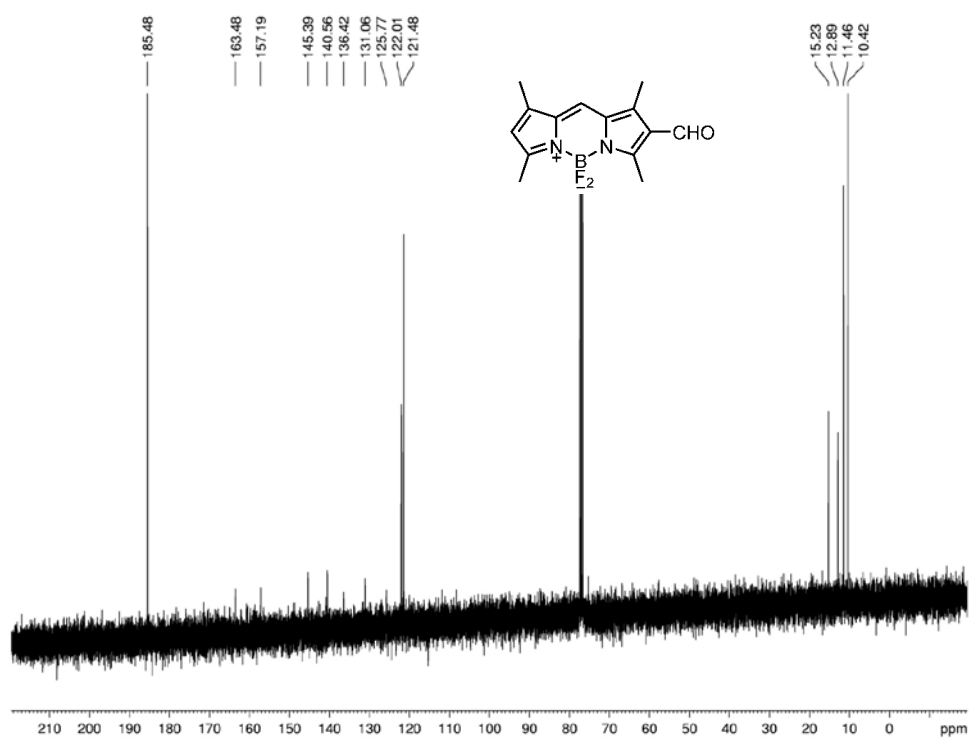
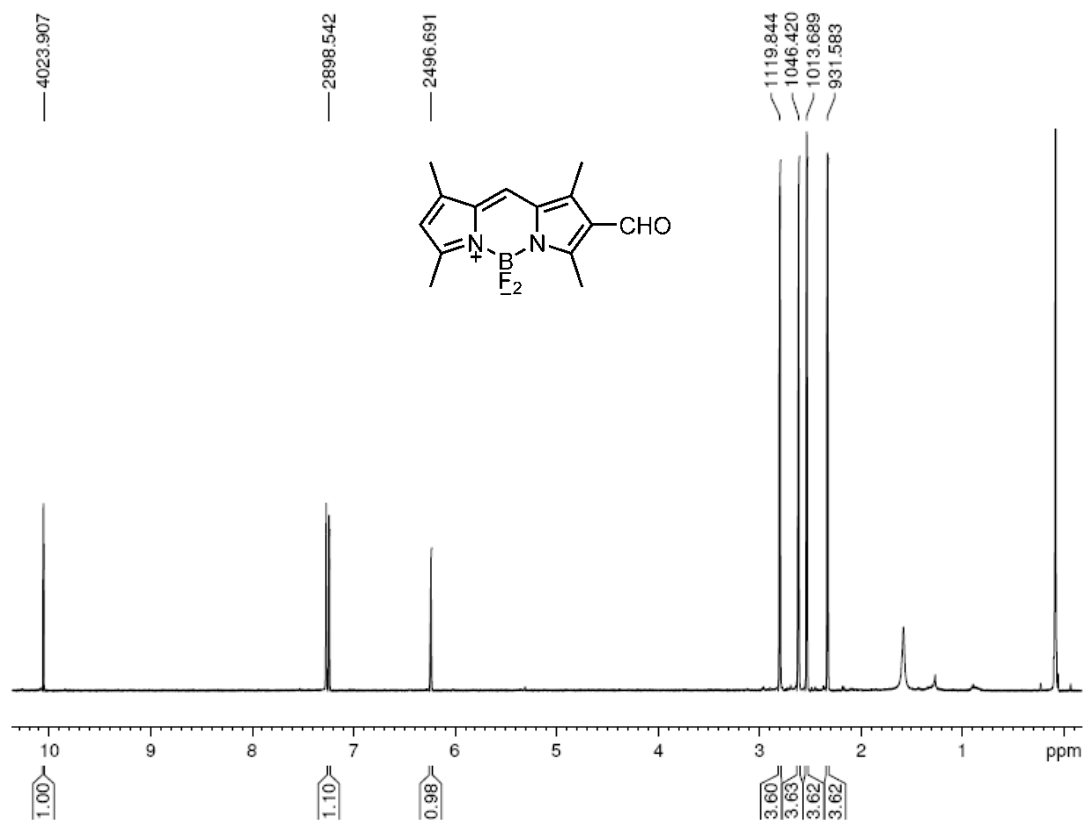
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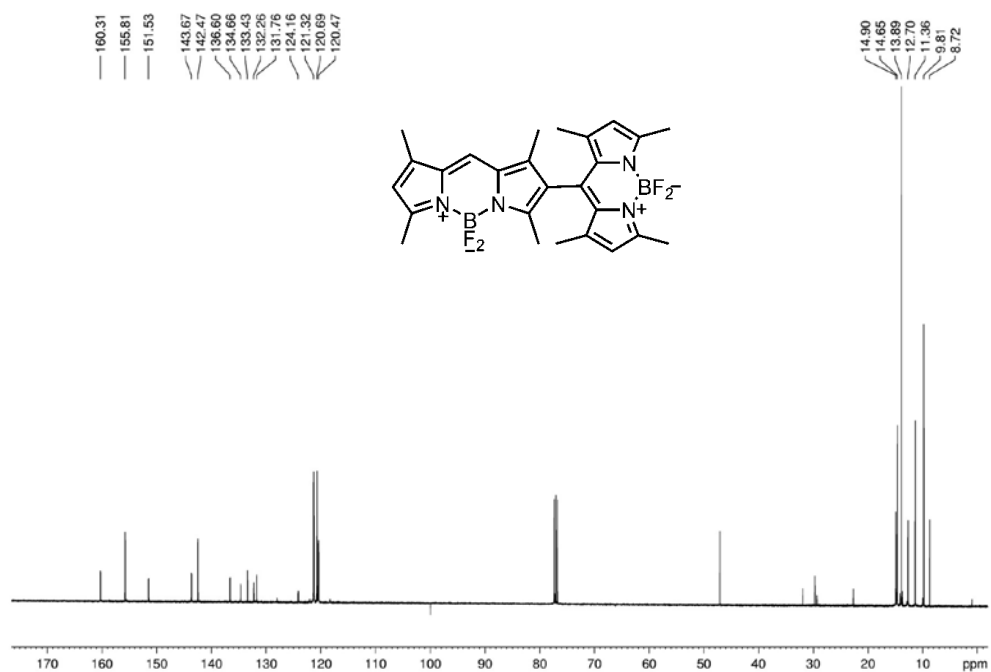
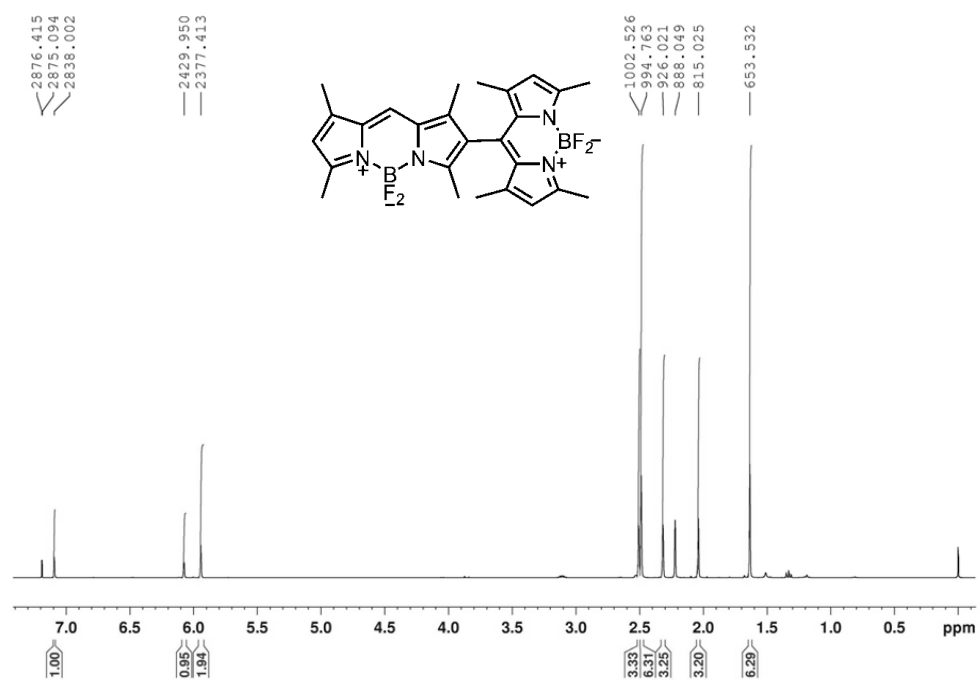
7 APPENDIX

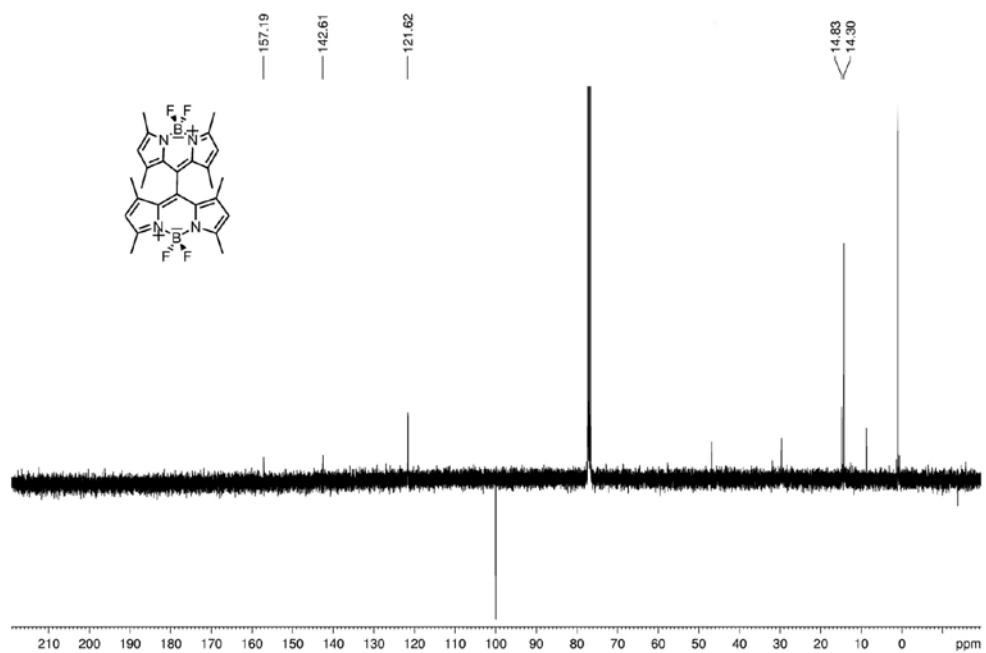
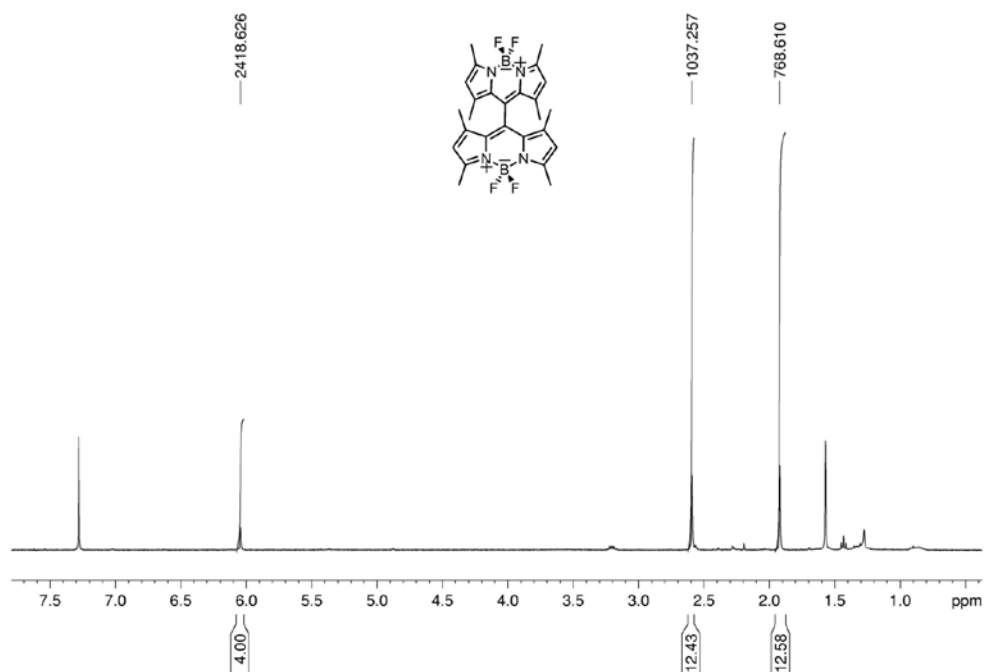
7.1 *Theory-Guided Access to Efficient Photosensitizers by Heavy Atom Free Orthogonal Bodipy Dimers*

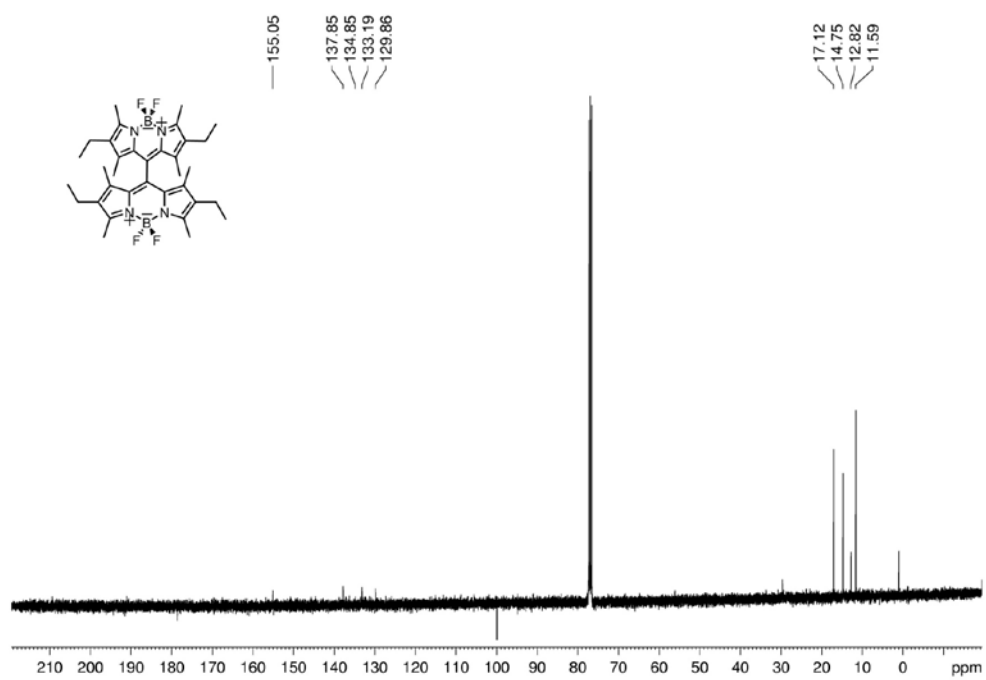
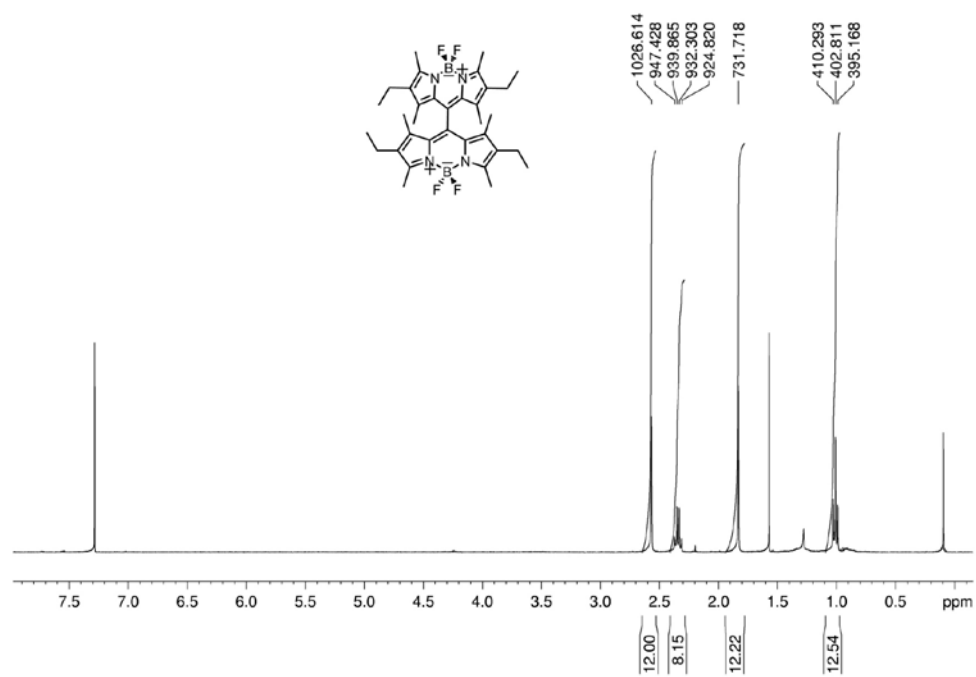
7.1.1 ^1H and ^{13}C NMR Spectra



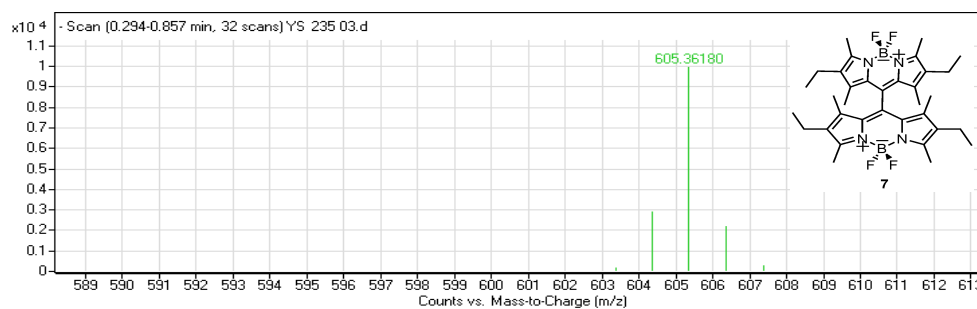
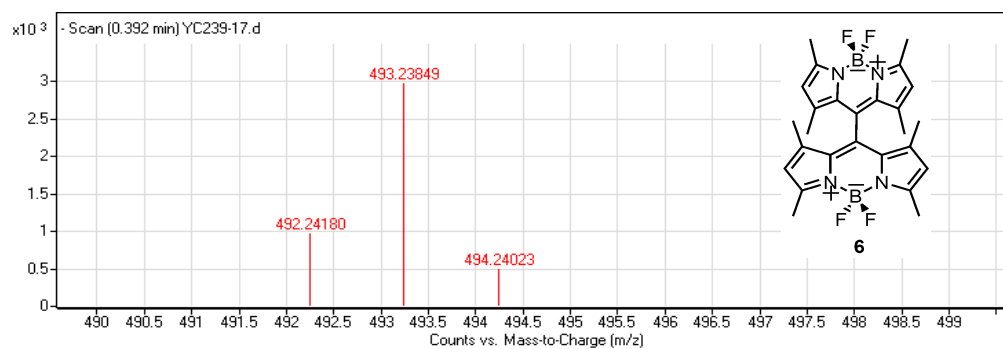
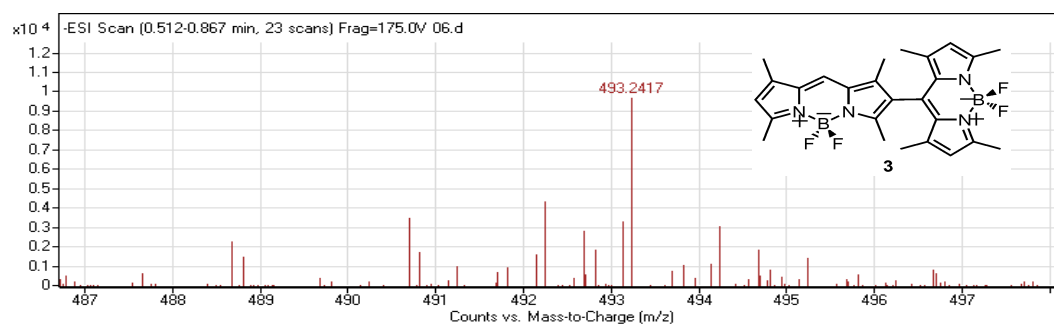
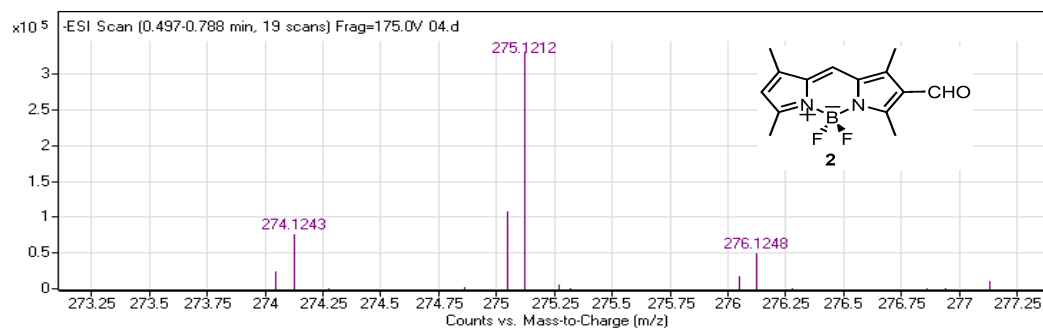


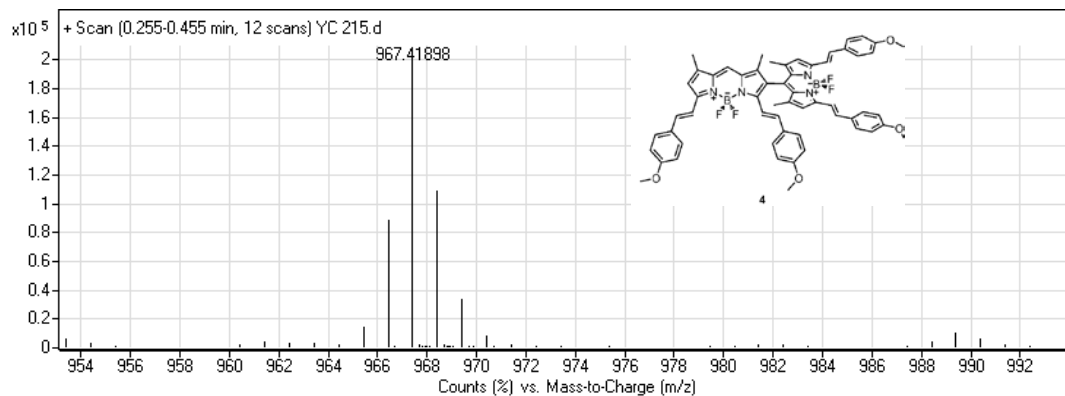






7.1.2 Mass Spectra





7.1.3 Size distribution analysis

Results

	Diam. (nm)	% Number	Width (nm)
Z-Average (d.nm): 225,0	Peak 1: 89,91	100,0	49,15
Pdl: 0,455	Peak 2: 0,000	0,0	0,000
Intercept: 0,617	Peak 3: 0,000	0,0	0,000
Result quality Good			

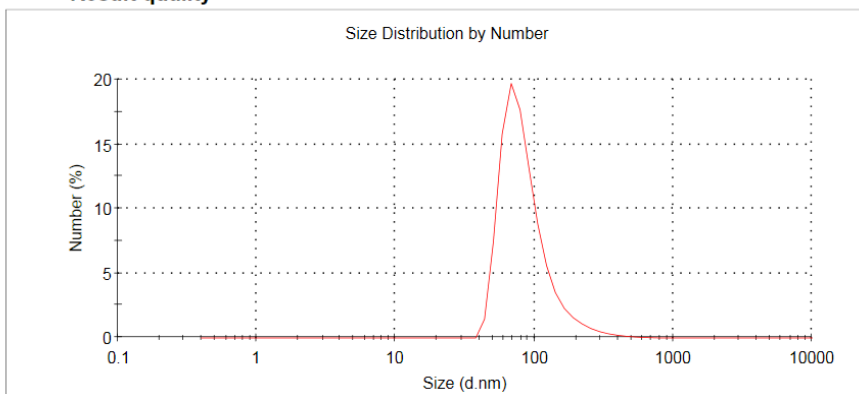


Figure 78. Size distribution of compound micellar compound **3**. Cremophor EL was used for embedding the dye. Other information related with instrument is given in Experimental Details.

7.1.4 Photophysical Measurements

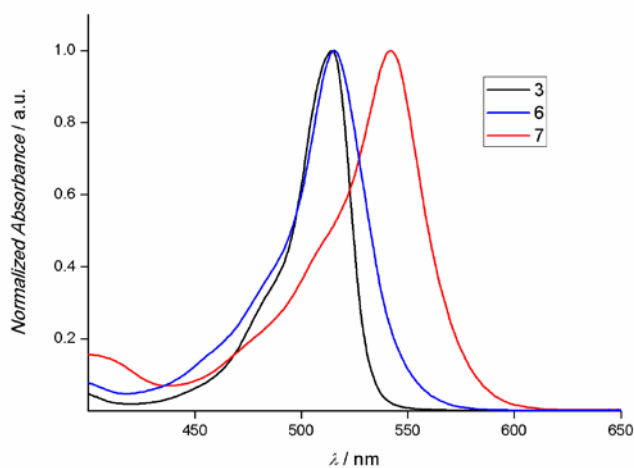


Figure 79. Absorbance spectra of the synthesized molecules **OB3**, **OB6** and **OB7** in CHCl_3 .

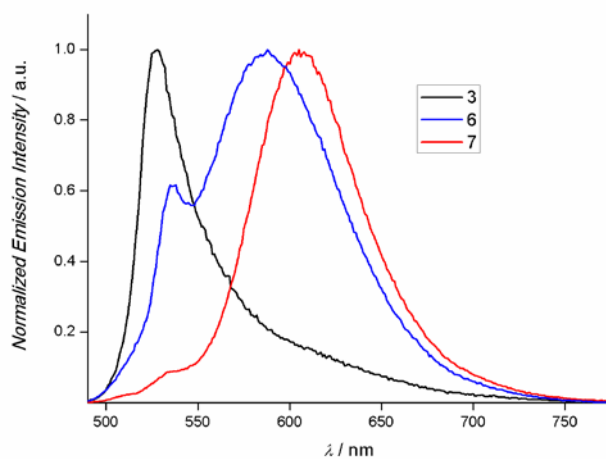


Figure 80. Fluorescence Spectra of the synthesized molecules in CHCl_3 . Compounds **OB3**, **OB6** and **OB7** were excited at maximum absorbances of compounds in Table 1. λ_{abs} (3) = 514 nm, λ_{abs} (6) = 515 nm, λ_{abs} (7) = 542 nm.

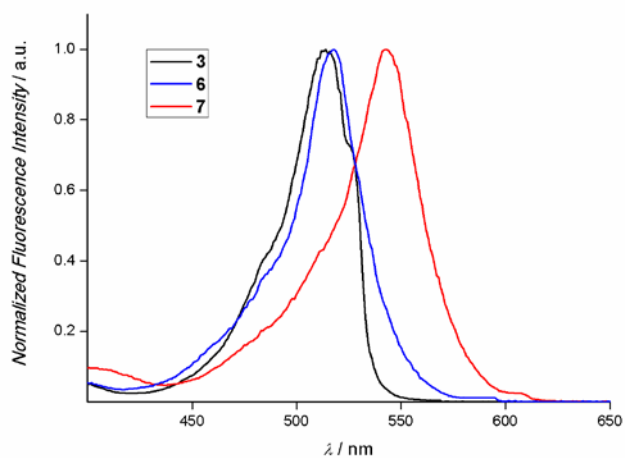
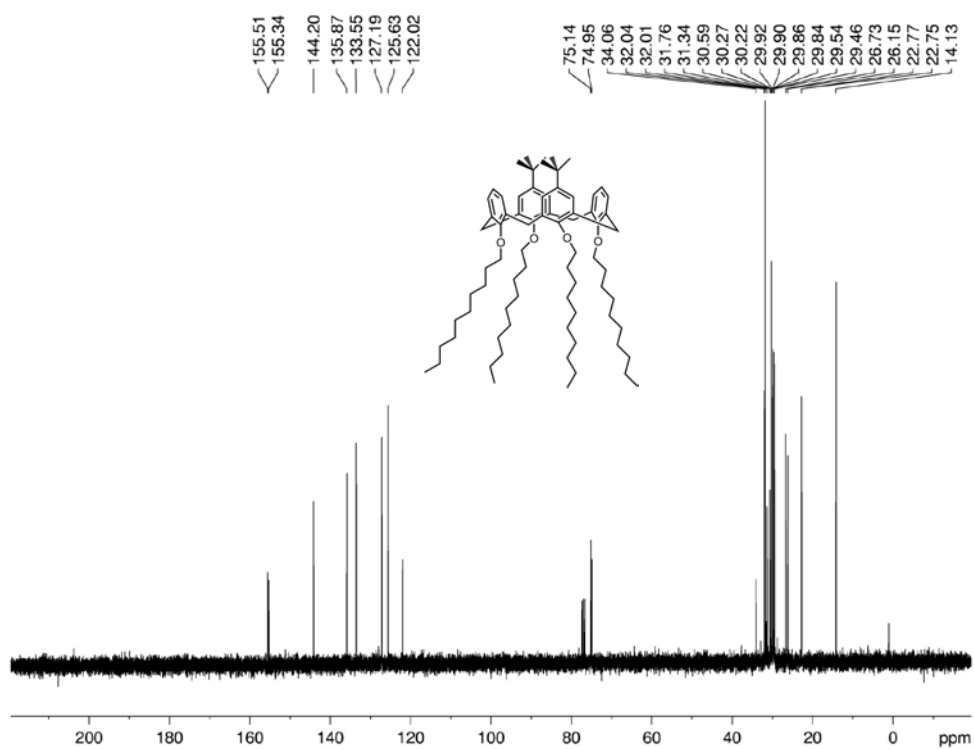
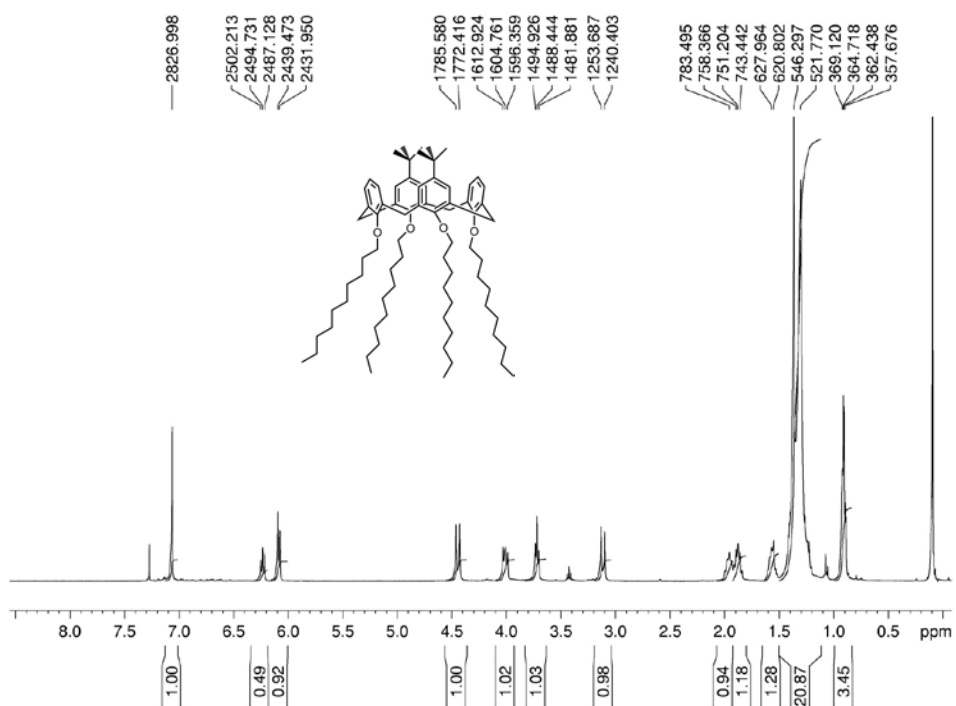
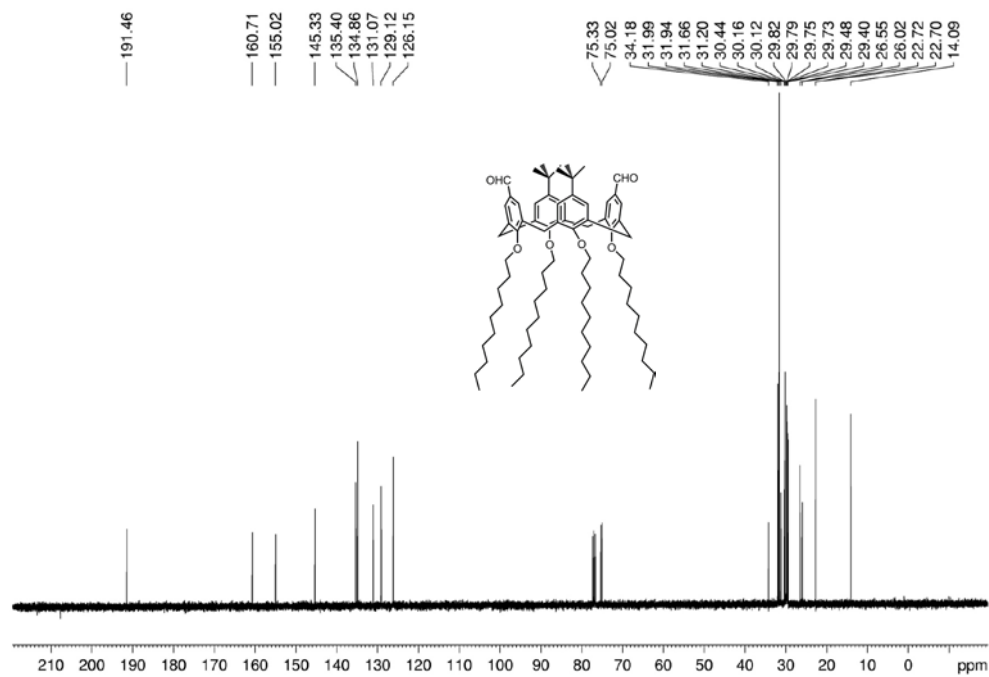
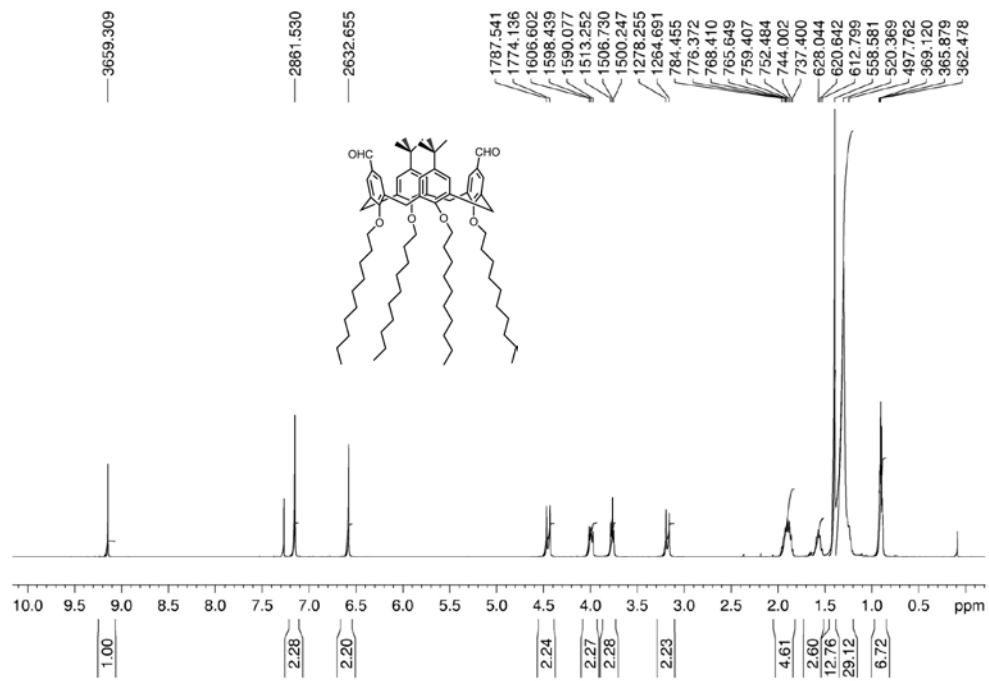


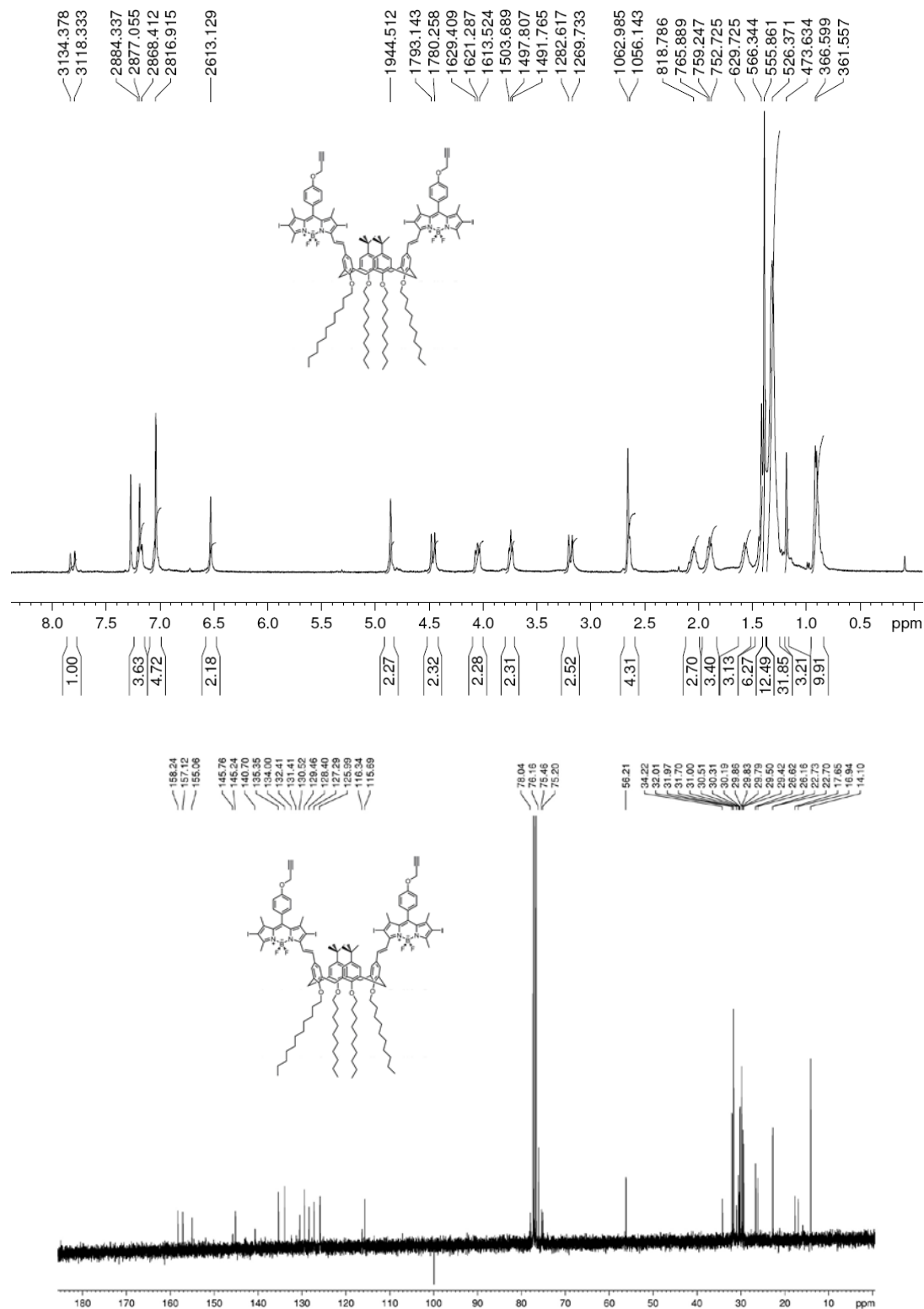
Figure 81. Excitation Spectra of compounds **OB3**, **OB6** and **OB7** in CHCl_3 . Emission data were collected at 527 nm for **OB3**, at 588 nm for **OB6** and 605 nm for **OB7**.

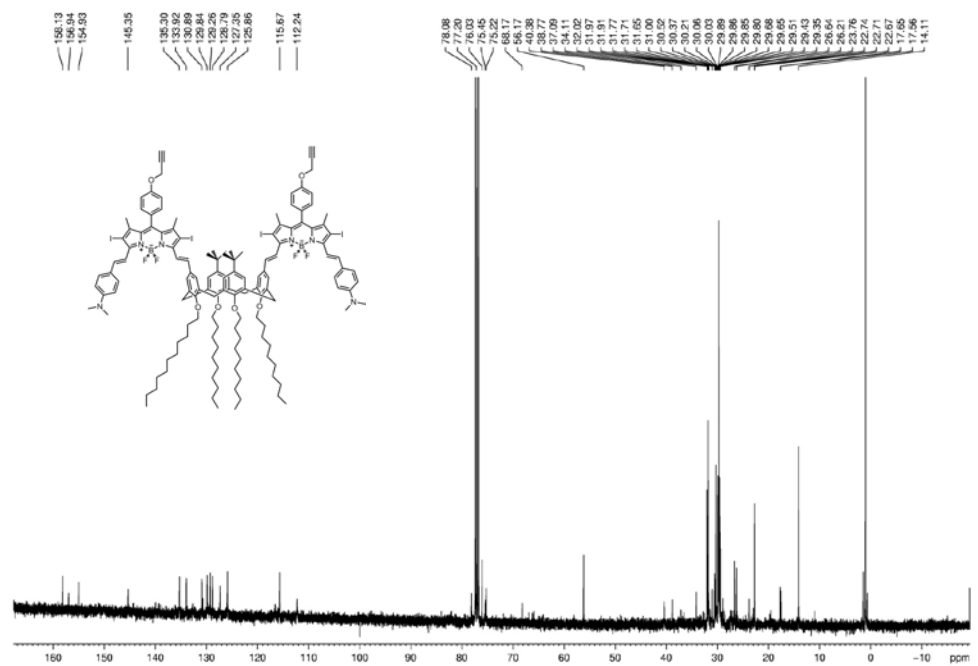
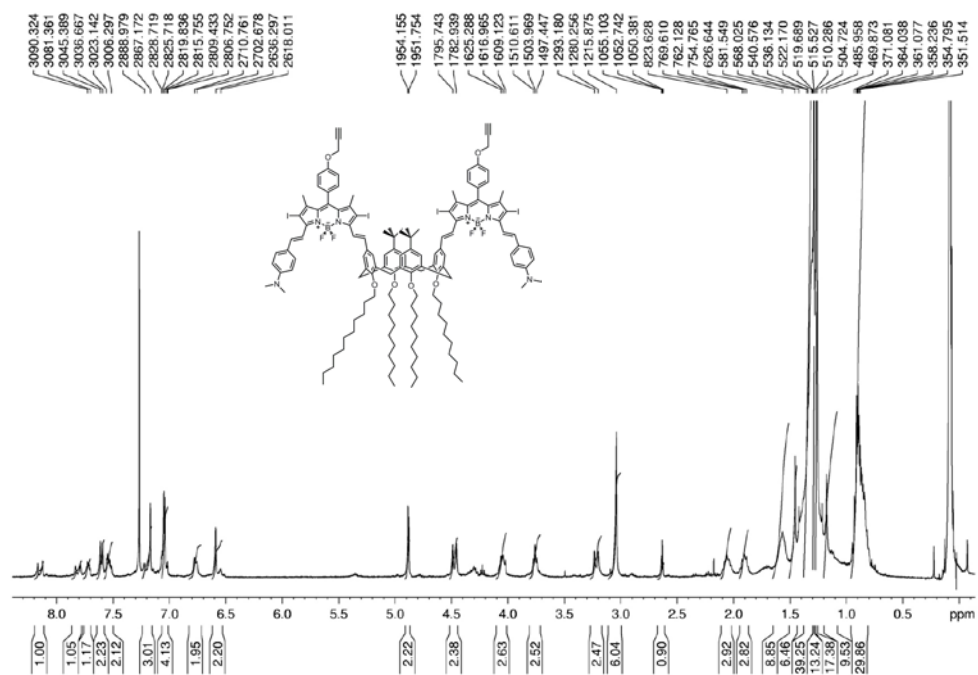
7.2 *PEGylated Calix[4]arene as a Carrier for a Bodipy-based Photosensitizer*

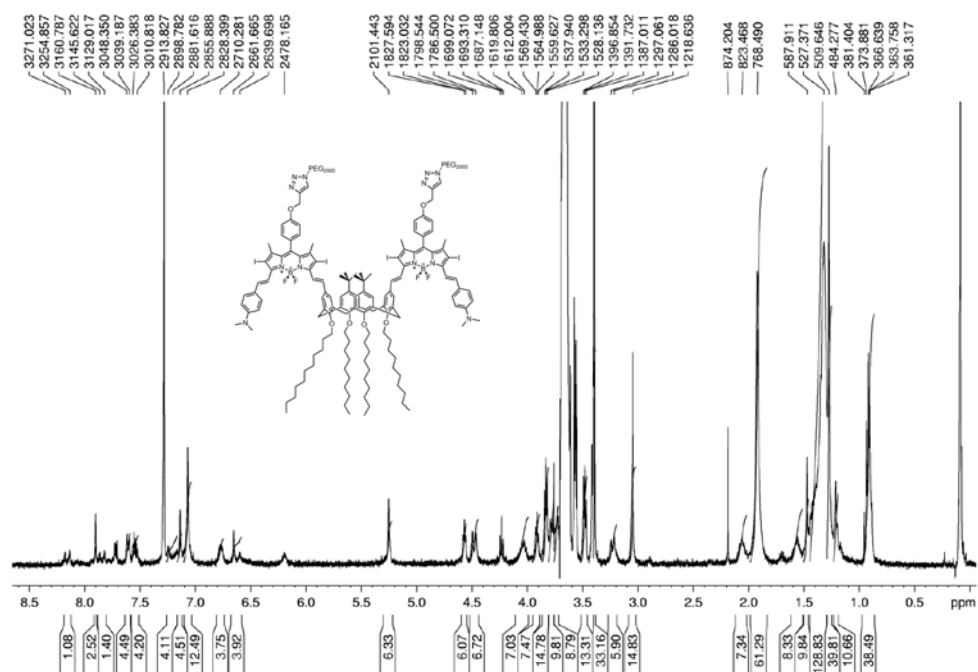
7.2.1 ^1H and ^{13}C NMR Spectra



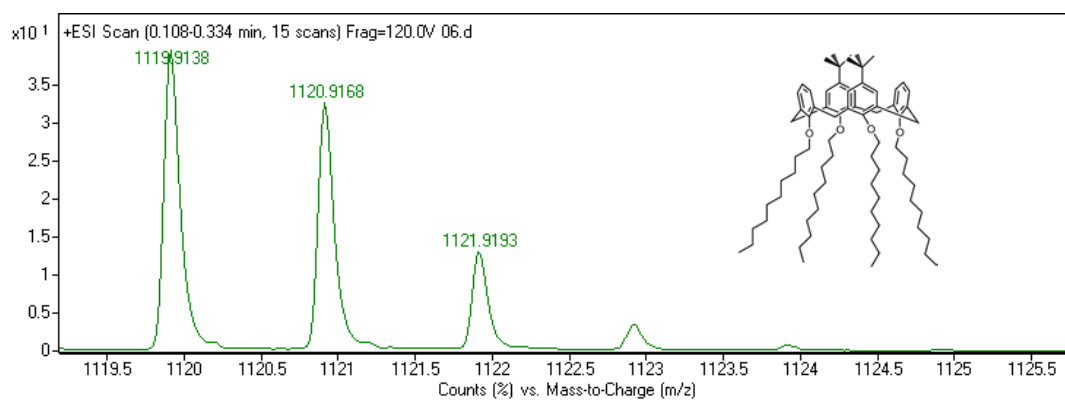


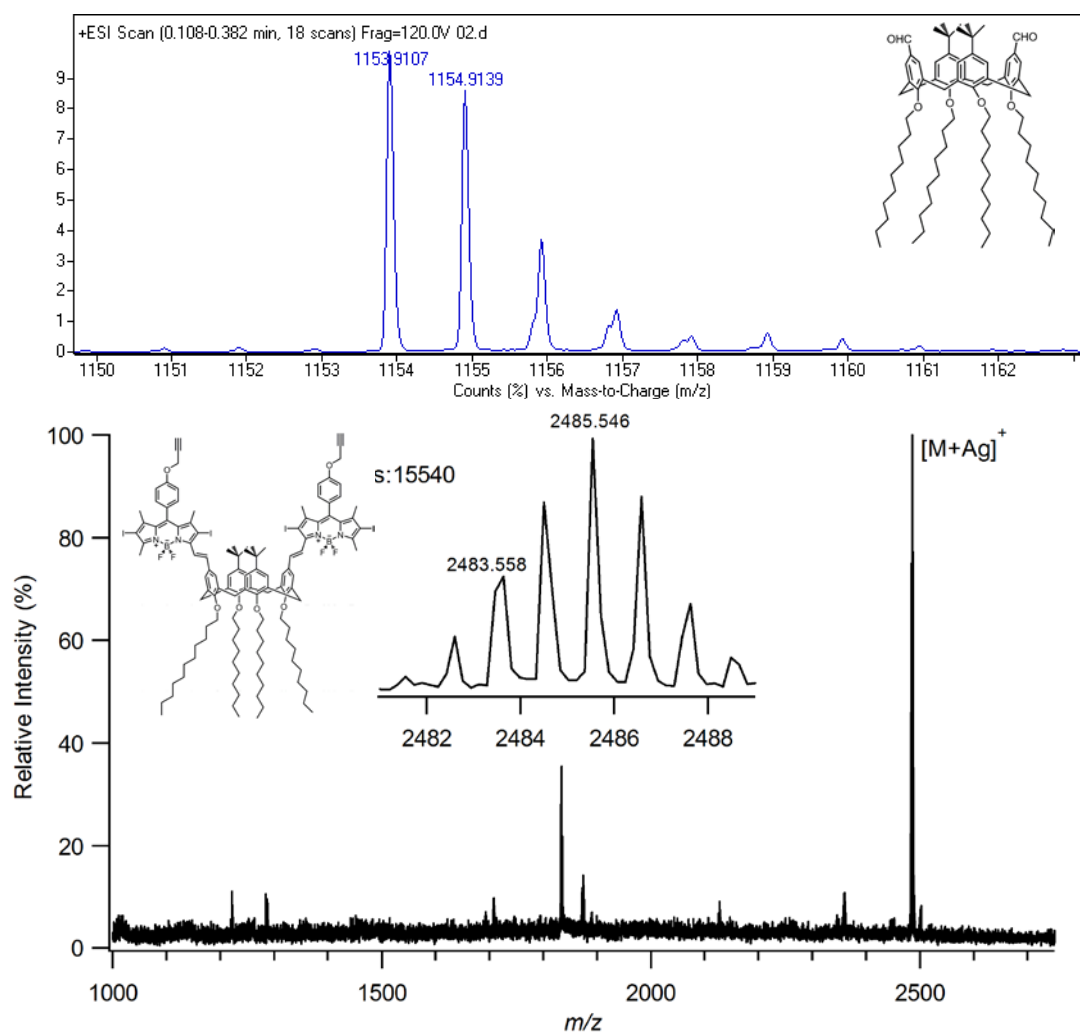


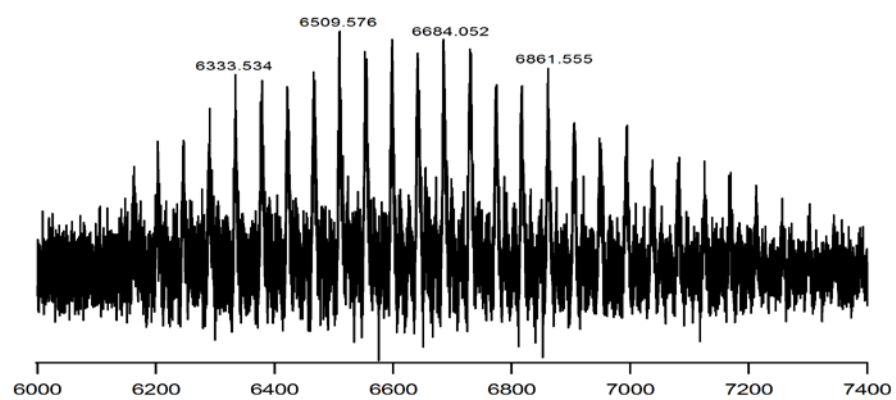




7.2.2 Mass Spectra

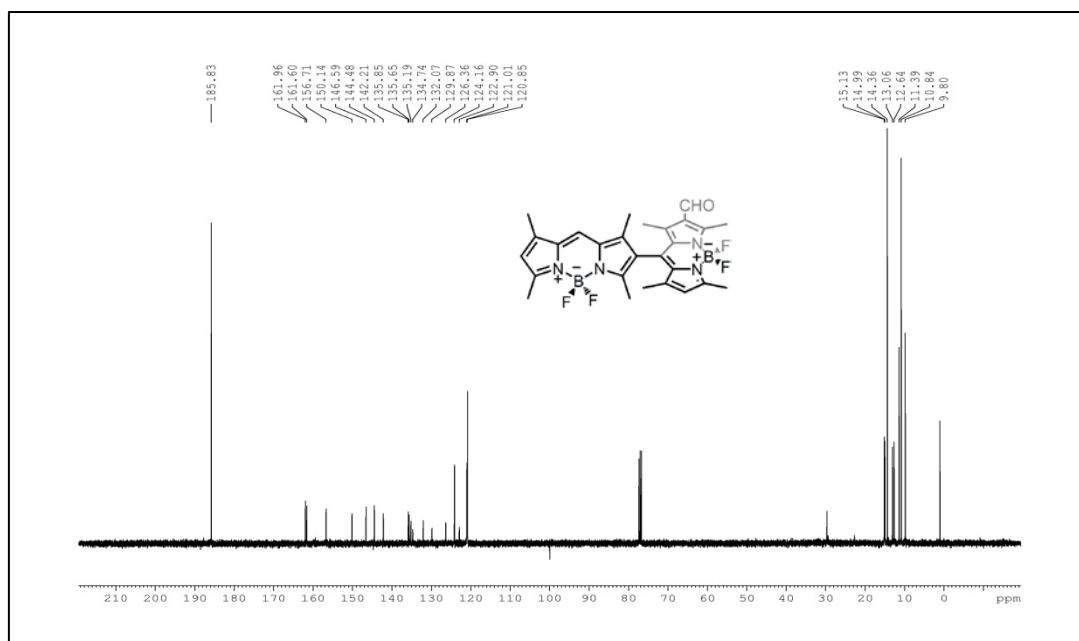
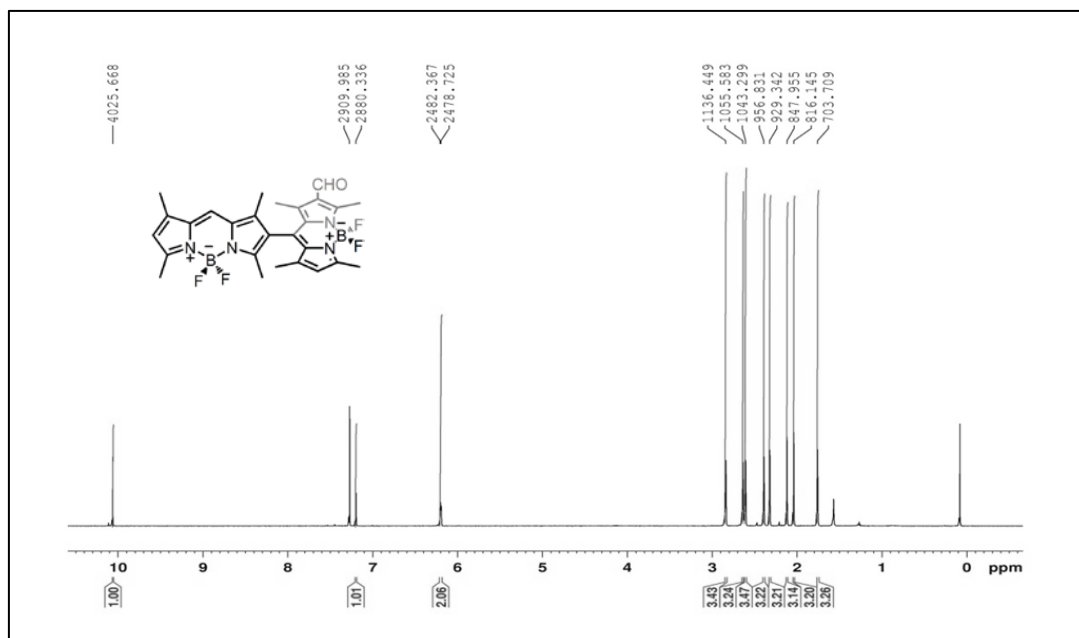


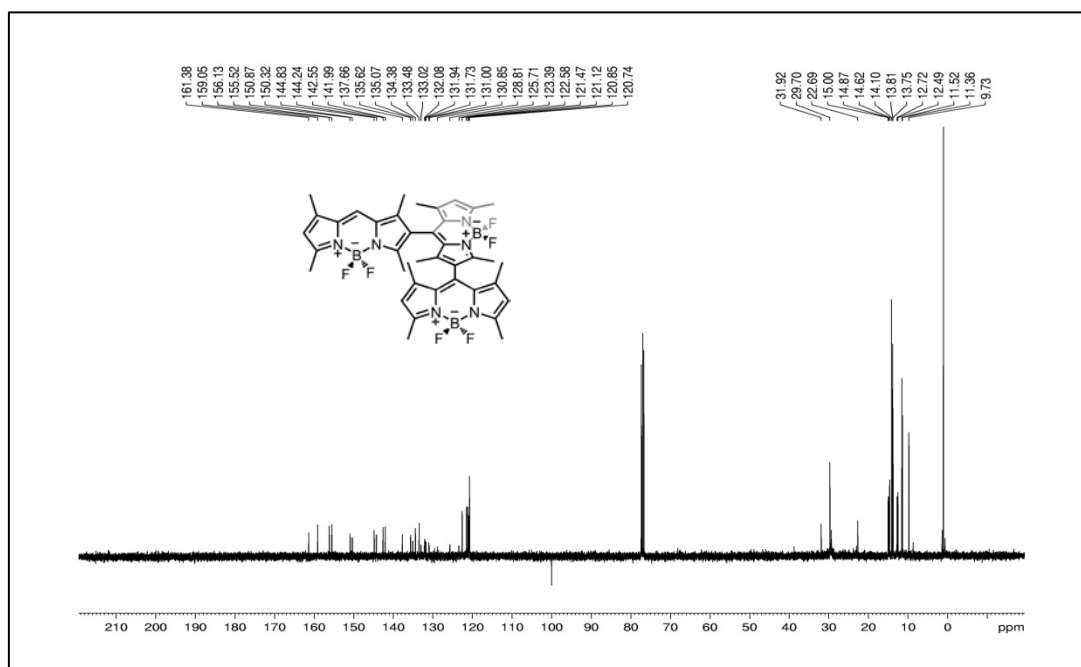
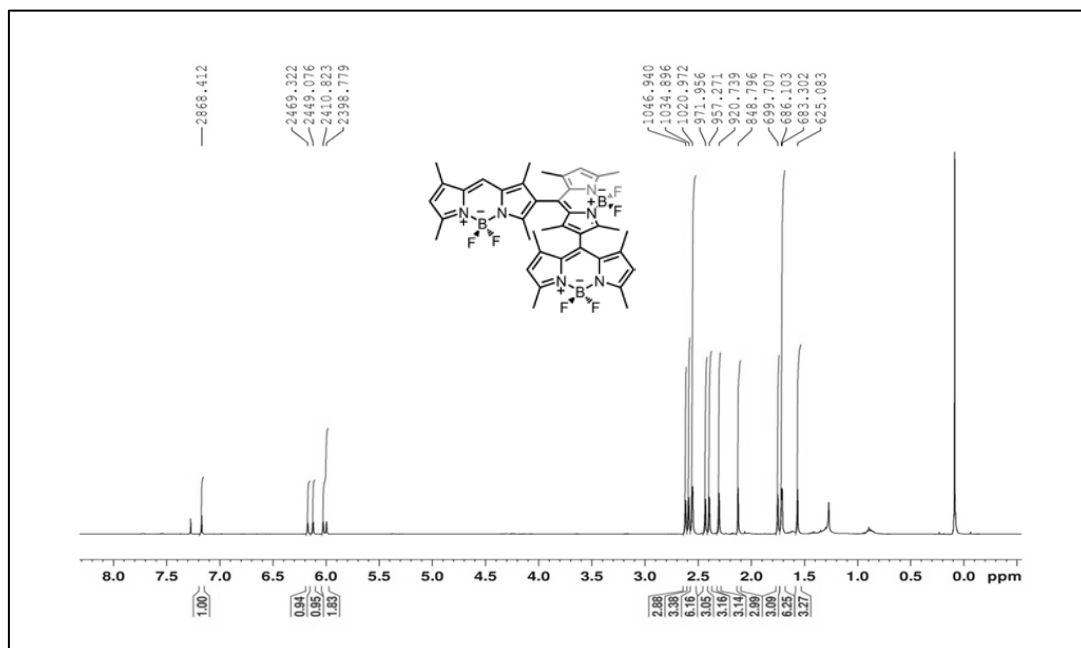


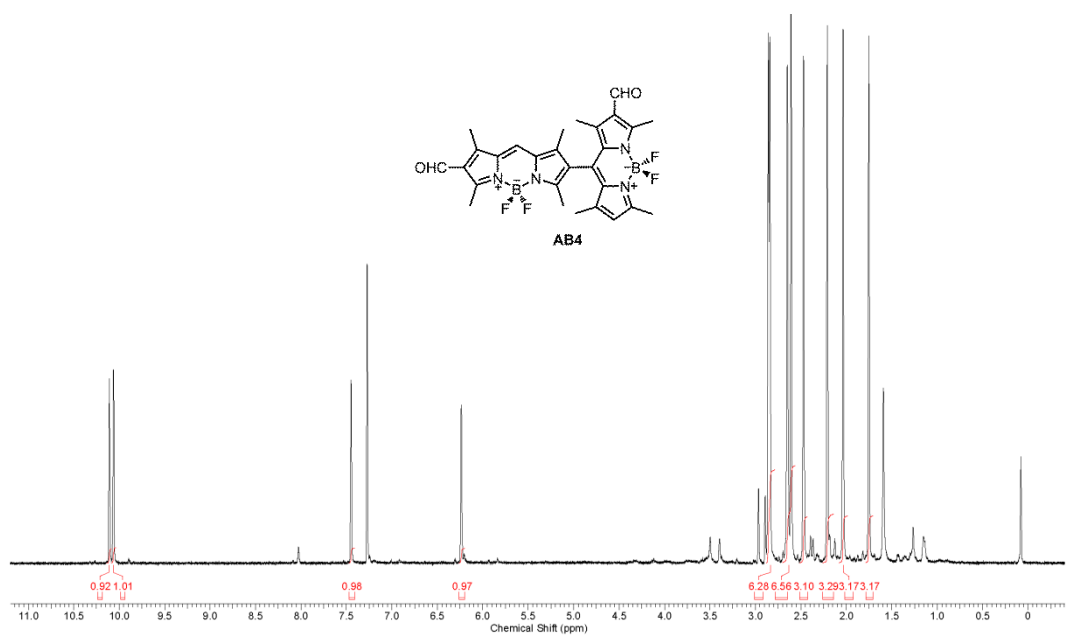


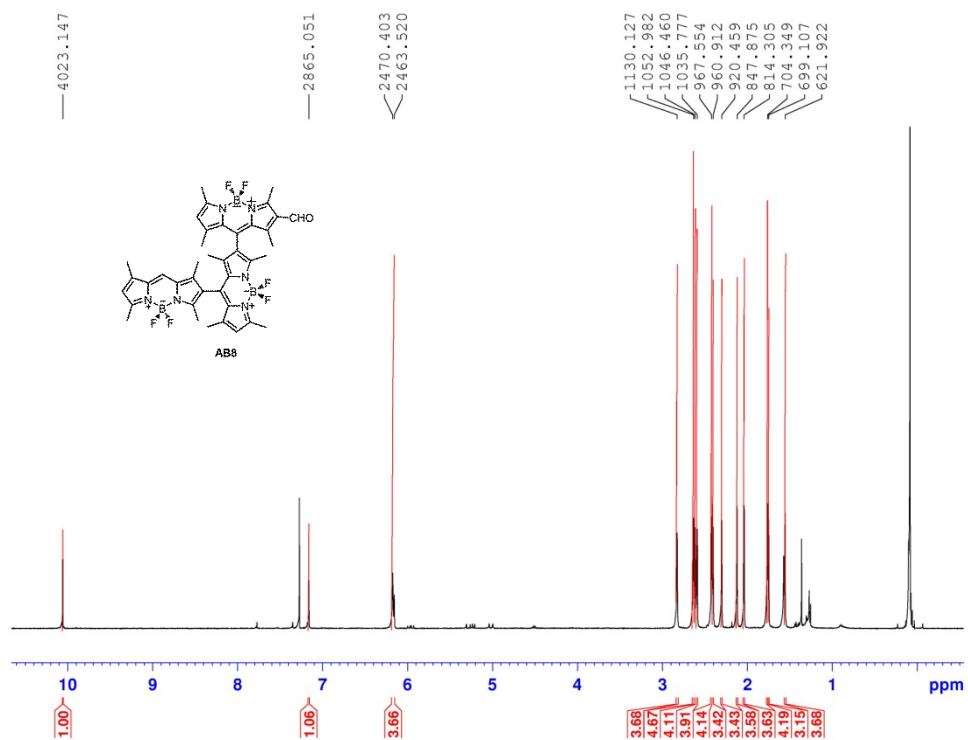
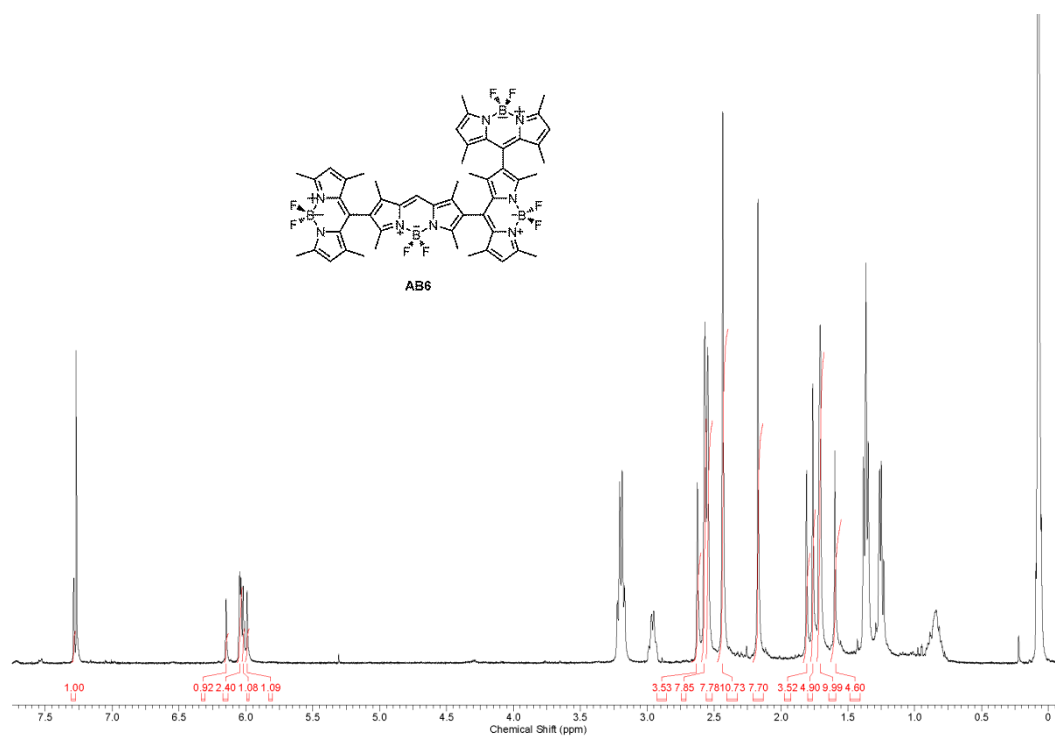
7.3 AXIAL CHIRAL BODIPYS

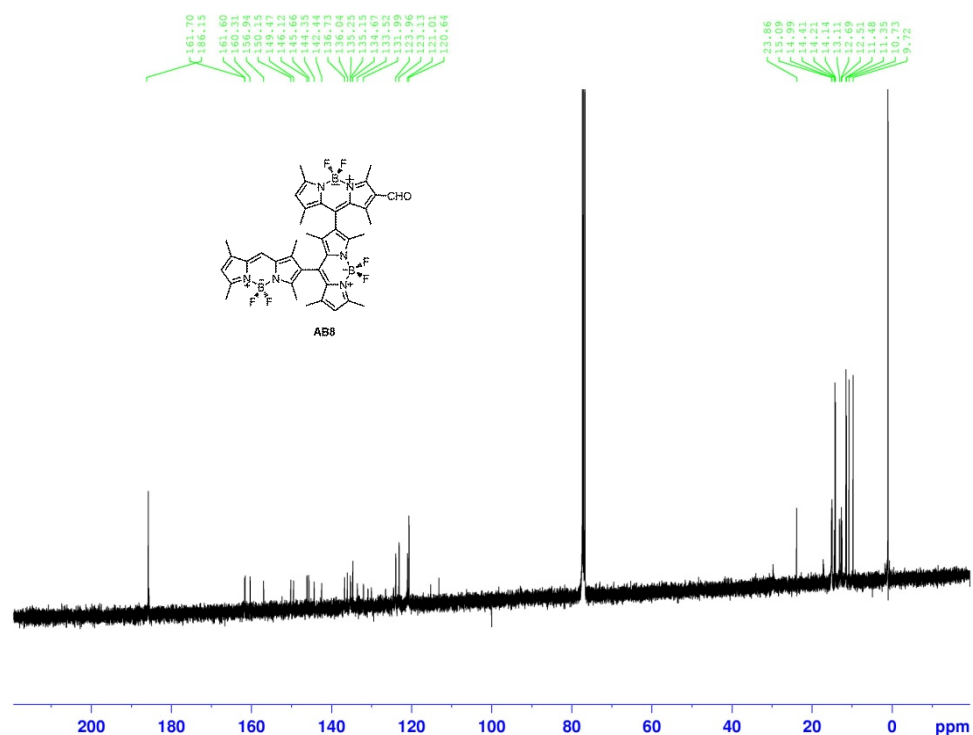
7.3.1 ^1H and ^{13}C NMR Spectra



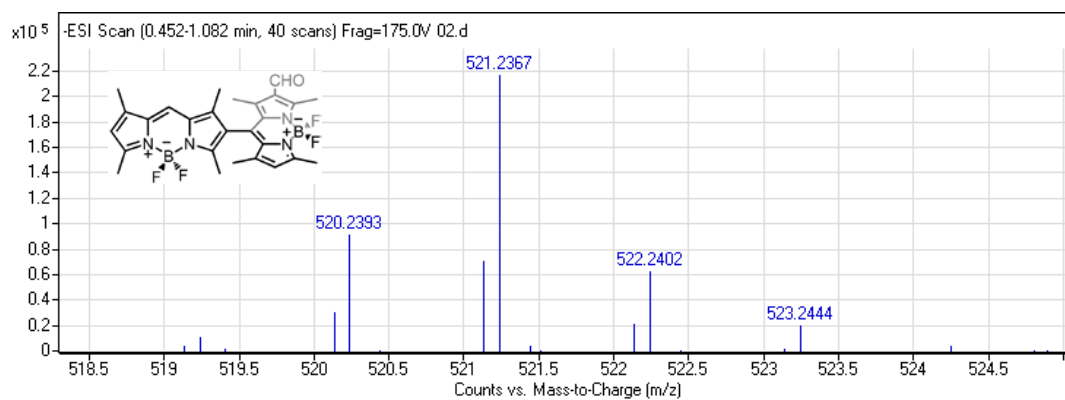


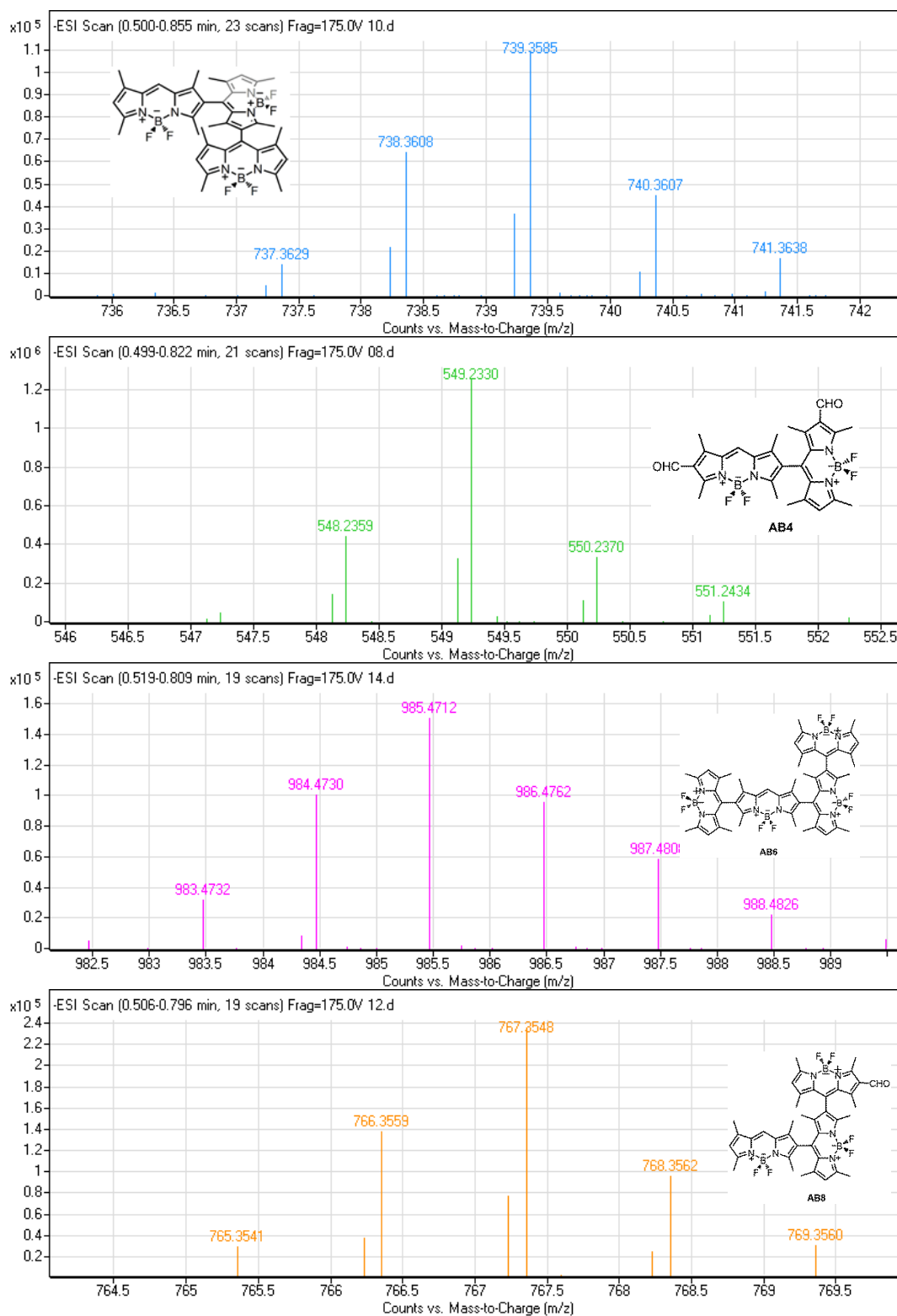




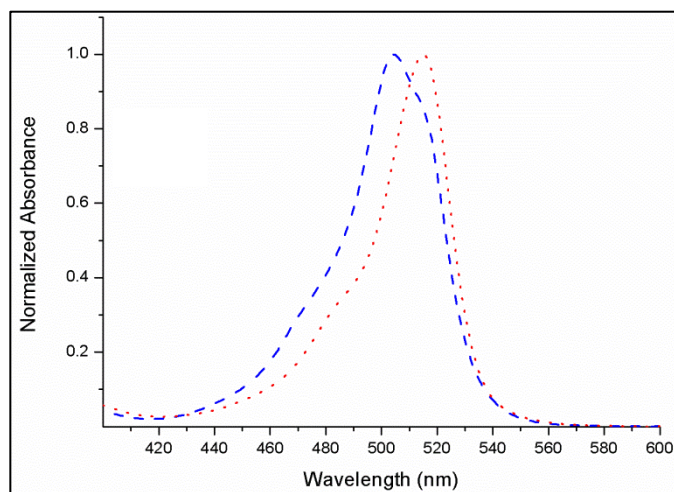


7.3.2 Mass Spectra

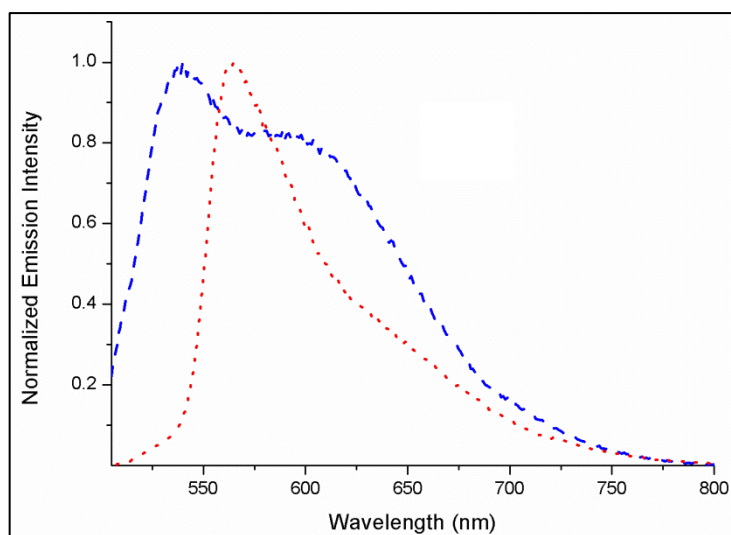




7.3.3 Absorbance and Emission Spectra



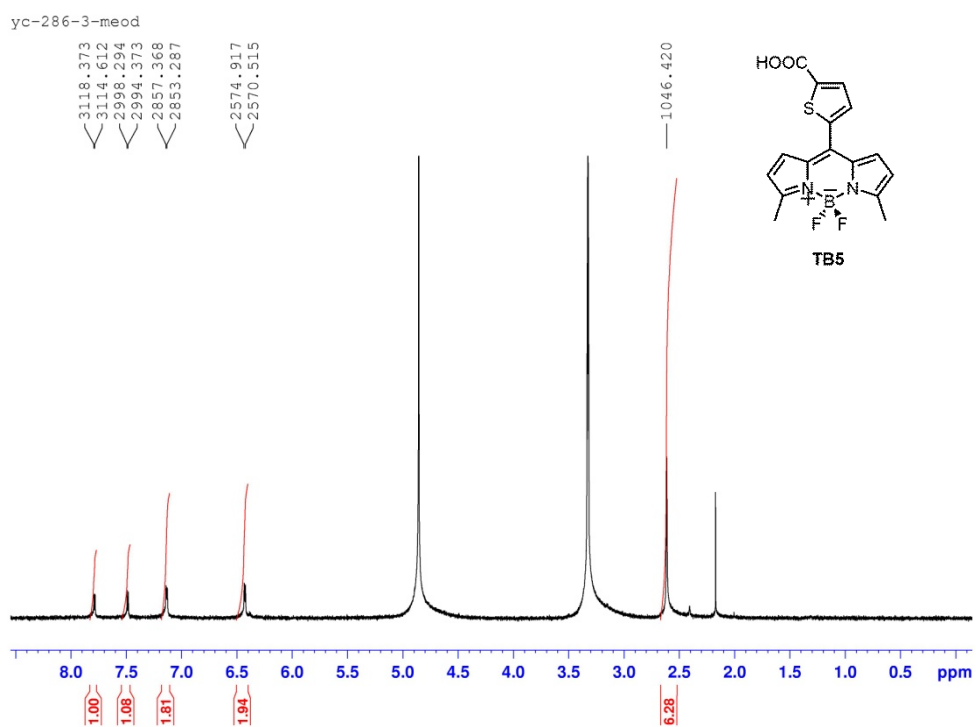
Absorbance spectrum of **AB5** (blue) and **AB7** (red)

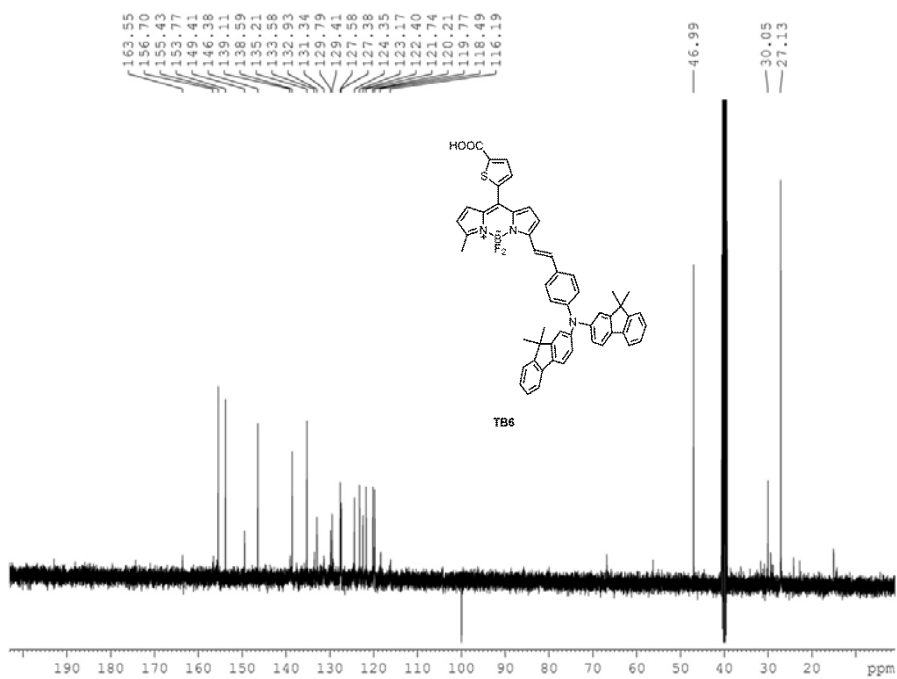
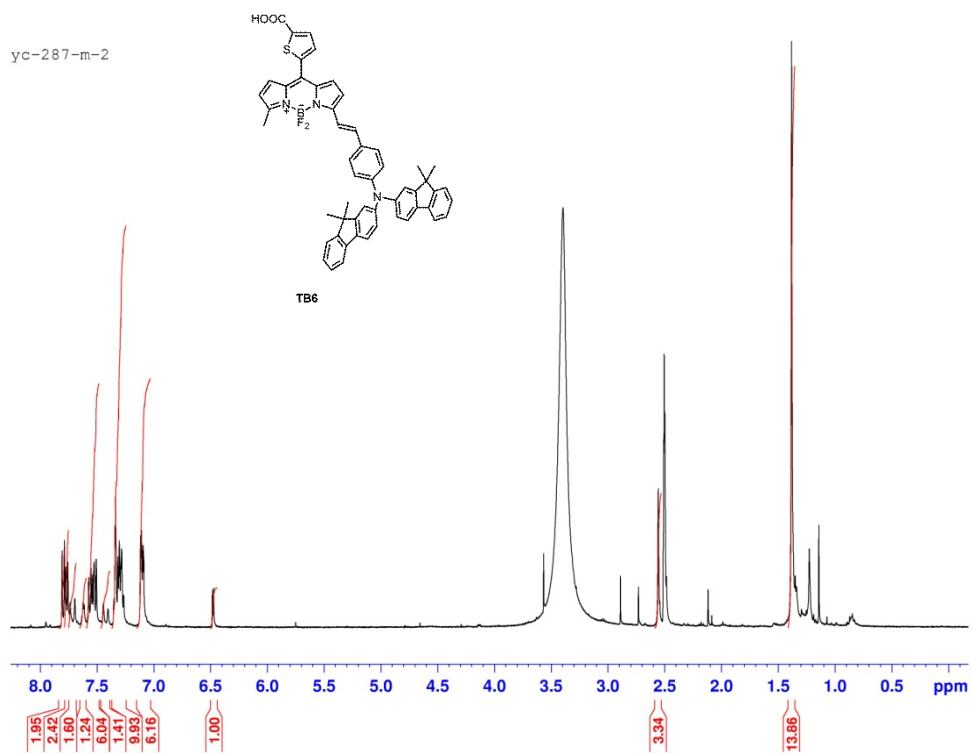


Fluorescence spectrum of **AB5** (blue) and **AB7** (red)

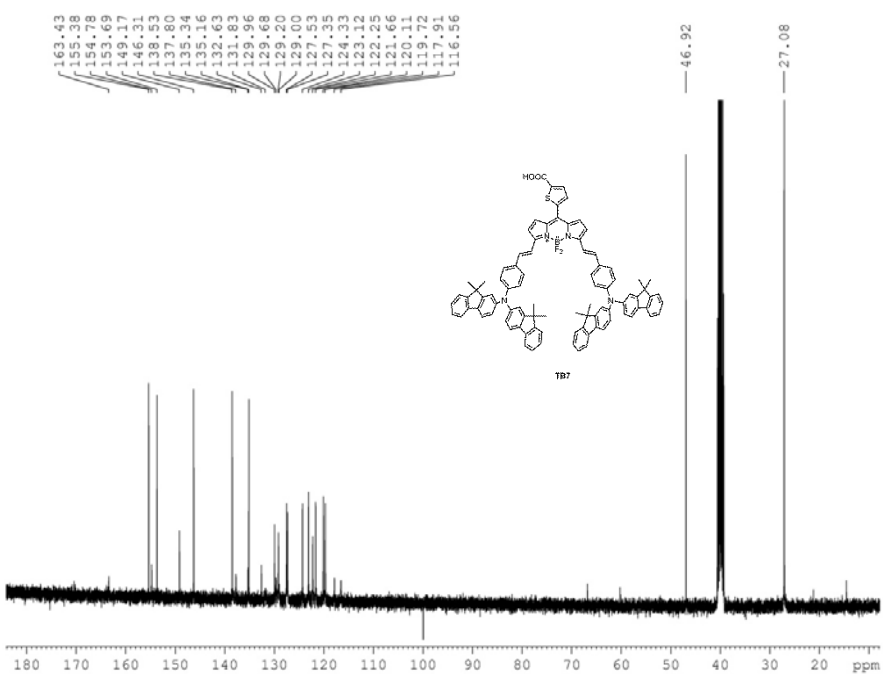
7.4 THIOPHENE BASED PS'S FOR DSSC

7.4.1 ^1H and ^{13}C NMR Spectra





Chemical structure of 1b7:

CC1(C)C2=CC=CC=C2C3=CC=CC=C3C4=CC=CC=C4C5=CC=CC=C5C6=CC=CC=C6C7=CC=CC=C7C8=CC=CC=C8C9=CC=CC=C9C10=CC=CC=C10C11=CC=CC=C11C12=CC=CC=C12C13=CC=CC=C13C14=CC=CC=C14C15=CC=CC=C15C16=CC=CC=C16C17=CC=CC=C17C18=CC=CC=C18C19=CC=CC=C19C20=CC=CC=C20C21=CC=CC=C21C22=CC=CC=C22C23=CC=CC=C23C24=CC=CC=C24C25=CC=CC=C25C26=CC=CC=C26C27=CC=CC=C27C28=CC=CC=C28C29=CC=CC=C29C30=CC=CC=C30C31=CC=CC=C31C32=CC=CC=C32C33=CC=CC=C33C34=CC=CC=C34C35=CC=CC=C35C36=CC=CC=C36C37=CC=CC=C37C38=CC=CC=C38C39=CC=CC=C39C40=CC=CC=C40C41=CC=CC=C41C42=CC=CC=C42C43=CC=CC=C43C44=CC=CC=C44C45=CC=CC=C45C46=CC=CC=C46C47=CC=CC=C47C48=CC=CC=C48C49=CC=CC=C49C50=CC=CC=C50C51=CC=CC=C51C52=CC=CC=C52C53=CC=CC=C53C54=CC=CC=C54C55=CC=CC=C55C56=CC=CC=C56C57=CC=CC=C57C58=CC=CC=C58C59=CC=CC=C59C60=CC=CC=C60C61=CC=CC=C61C62=CC=CC=C62C63=CC=CC=C63C64=CC=CC=C64C65=CC=CC=C65C66=CC=CC=C66C67=CC=CC=C67C68=CC=CC=C68C69=CC=CC=C69C70=CC=CC=C70C71=CC=CC=C71C72=CC=CC=C72C73=CC=CC=C73C74=CC=CC=C74C75=CC=CC=C75C76=CC=CC=C76C77=CC=CC=C77C78=CC=CC=C78C79=CC=CC=C79C80=CC=CC=C80C81=CC=CC=C81C82=CC=CC=C82C83=CC=CC=C83C84=CC=CC=C84C85=CC=CC=C85C86=CC=CC=C86C87=CC=CC=C87C88=CC=CC=C88C89=CC=CC=C89C90=CC=CC=C90C91=CC=CC=C91C92=CC=CC=C92C93=CC=CC=C93C94=CC=CC=C94C95=CC=CC=C95C96=CC=CC=C96C97=CC=CC=C97C98=CC=CC=C98C99=CC=CC=C99C100=CC=CC=C100C101=CC=CC=C101C102=CC=CC=C102C103=CC=CC=C103C104=CC=CC=C104C105=CC=CC=C105C106=CC=CC=C106C107=CC=CC=C107C108=CC=CC=C108C109=CC=CC=C109C110=CC=CC=C110C111=CC=CC=C111C112=CC=CC=C112C113=CC=CC=C113C114=CC=CC=C114C115=CC=CC=C115C116=CC=CC=C116C117=CC=CC=C117C118=CC=CC=C118C119=CC=CC=C119C120=CC=CC=C120C121=CC=CC=C121C122=CC=CC=C122C123=CC=CC=C123C124=CC=CC=C124C125=CC=CC=C125C126=CC=CC=C126C127=CC=CC=C127C128=CC=CC=C128C129=CC=CC=C129C130=CC=CC=C130C131=CC=CC=C131C132=CC=CC=C132C133=CC=CC=C133C134=CC=CC=C134C135=CC=CC=C135C136=CC=CC=C136C137=CC=CC=C137C138=CC=CC=C138C139=CC=CC=C139C140=CC=CC=C140C141=CC=CC=C141C142=CC=CC=C142C143=CC=CC=C143C144=CC=CC=C144C145=CC=CC=C145C146=CC=CC=C146C147=CC=CC=C147C148=CC=CC=C148C149=CC=CC=C149C150=CC=CC=C150C151=CC=CC=C151C152=CC=CC=C152C153=CC=CC=C153C154=CC=CC=C154C155=CC=CC=C155C156=CC=CC=C156C157=CC=CC=C157C158=CC=CC=C158C159=CC=CC=C159C160=CC=CC=C160C161=CC=CC=C161C162=CC=CC=C162C163=CC=CC=C163C164=CC=CC=C164C165=CC=CC=C165C166=CC=CC=C166C167=CC=CC=C167C168=CC=CC=C168C169=CC=CC=C169C170=CC=CC=C170C171=CC=CC=C171C172=CC=CC=C172C173=CC=CC=C173C174=CC=CC=C174C175=CC=CC=C175C176=CC=CC=C176C177=CC=CC=C177C178=CC=CC=C178C179=CC=CC=C179C180=CC=CC=C180C181=CC=CC=C181C182=CC=CC=C182C183=CC=CC=C183C184=CC=CC=C184C185=CC=CC=C185C186=CC=CC=C186C187=CC=CC=C187C188=CC=CC=C188C189=CC=CC=C189C190=CC=CC=C190C191=CC=CC=C191C192=CC=CC=C192C193=CC=CC=C193C194=CC=CC=C194C195=CC=CC=C195C196=CC=CC=C196C197=CC=CC=C197C198=CC=CC=C198C199=CC=CC=C199C200=CC=CC=C200C201=CC=CC=C201C202=CC=CC=C202C203=CC=CC=C203C204=CC=CC=C204C205=CC=CC=C205C206=CC=CC=C206C207=CC=CC=C207C208=CC=CC=C208C209=CC=CC=C209C210=CC=CC=C210C211=CC=CC=C211C212=CC=CC=C212C213=CC=CC=C213C214=CC=CC=C214C215=CC=CC=C215C216=CC=CC=C216C217=CC=CC=C217C218=CC=CC=C218C219=CC=CC=C219C220=CC=CC=C220C221=CC=CC=C221C222=CC=CC=C222C223=CC=CC=C223C224=CC=CC=C224C225=CC=CC=C225C226=CC=CC=C226C227=CC=CC=C227C228=CC=CC=C228C229=CC=CC=C229C230=CC=CC=C230C231=CC=CC=C231C232=CC=CC=C232C233=CC=CC=C233C234=CC=CC=C234C235=CC=CC=C235C236=CC=CC=C236C237=CC=CC=C237C238=CC=CC=C238C239=CC=CC=C239C240=CC=CC=C240C241=CC=CC=C241C242=CC=CC=C242C243=CC=CC=C243C244=CC=CC=C244C245=CC=CC=C245C246=CC=CC=C246C247=CC=CC=C247C248=CC=CC=C248C249=CC=CC=C249C250=CC=CC=C250C251=CC=CC=C251C252=CC=CC=C252C253=CC=CC=C253C254=CC=CC=C254C255=CC=CC=C255C256=CC=CC=C256C257=CC=CC=C257C258=CC=CC=C258C259=CC=CC=C259C260=CC=CC=C260C261=CC=CC=C261C262=CC=CC=C262C263=CC=CC=C263C264=CC=CC=C264C265=CC=CC=C265C266=CC=CC=C266C267=CC=CC=C267C268=CC=CC=C268C269=CC=CC=C269C270=CC=CC=C270C271=CC=CC=C271C272=CC=CC=C272C273=CC=CC=C273C274=CC=CC=C274C275=CC=CC=C275C276=CC=CC=C276C277=CC=CC=C277C278=CC=CC=C278C279=CC=CC=C279C280=CC=CC=C280C281=CC=CC=C281C282=CC=CC=C282C283=CC=CC=C283C284=CC=CC=C284C285=CC=CC=C285C286=CC=CC=C286C287=CC=CC=C287C288=CC=CC=C288C289=CC=CC=C289C290=CC=CC=C290C291=CC=CC=C291C292=CC=CC=C292C293=CC=CC=C293C294=CC=CC=C294C295=CC=CC=C295C296=CC=CC=C296C297=CC=CC=C297C298=CC=CC=C298C299=CC=CC=C299C300=CC=CC=C300C301=CC=CC=C301C302=CC=CC=C302C303=CC=CC=C303C304=CC=CC=C304C305=CC=CC=C305C306=CC=CC=C306C307=CC=CC=C307C308=CC=CC=C308C309=CC=CC=C309C310=CC=CC=C310C311=CC=CC=C311C312=CC=CC=C312C313=CC=CC=C313C314=CC=CC=C314C315=CC=CC=C315C316=CC=CC=C316C317=CC=CC=C317C318=CC=CC=C318C319=CC=CC=C319C320=CC=CC=C320C321=CC=CC=C321C322=CC=CC=C322C323=CC=CC=C323C324=CC=CC=C324C325=CC=CC=C325C326=CC=CC=C326C327=CC=CC=C327C328=CC=CC=C328C329=CC=CC=C329C330=CC=CC=C330C331=CC=CC=C331C332=CC=CC=C332C333=CC=CC=C333C334=CC=CC=C334C335=CC=CC=C335C336=CC=CC=C336C337=CC=CC=C337C338=CC=CC=C338C339=CC=CC=C339C340=CC=CC=C340C341=CC=CC=C341C342=CC=CC=C342C343=CC=CC=C343C344=CC=CC=C344C345=CC=CC=C345C346=CC=CC=C346C347=CC=CC=C347C348=CC=CC=C348C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7.4.2 Mass Spectra

