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KAHRAMANMARAŞ ST İMAM UNIVERSITY
GRADUATE SCHOOL of NATURAL and APPLIED SCIENCES

**SYNTHESIS of BODIPY COMPOUNDS and THEIR
FLUORESCENCE SENSOR PROPERTIES in
DETECTION of β -AMYLOID PLAQUES**

HİLAL KIRPIK

Ph.D. THESIS

DEPARTMENT of CHEMISTRY

KAHRAMANMARAŞ 2023

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CURRICULUM VITAE		Hata! Yer işareti tanımlanmamış.

DECLARATION

I hereby declare that all information in the thesis has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all the material and results that are not original to this work.

Hilal KIRPIK

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DEDICATION

Dedicated to all girls in the world. Wishing that girls who are happy, powerful, educated, and not restricted who run to their dreams, be raised.



“I love to see a young girl go out and grab the world by the lapels. Life's a bitch. You've got to go out and kick ass.”

— Maya Angelou

SYNTHESIS of BODIPY COMPOUNDS AND THEIR FLUORESCENCE SENSOR PROPERTIES in DETECTION of β -AMYLOID PLAQUES

(Ph.D. THESIS)

HİLAL KIRPIK

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease that is usually observed in old age and is the most common cause of dementia. Two histopathological markers of Alzheimer's disease are β -amyloid plaques ($A\beta$ plaques) and the formation of neurofibrillary tangles. Currently, no treatment can definitively stop the course of Alzheimer's disease. The drugs available in the market relieve the symptoms of the disease and do not have the feature of curing the disease. Studies indicate that $A\beta$ plaques form years before the onset of dementia (dementia). Detection of β -amyloid plaques in Alzheimer's disease is critical for early diagnosis. In this thesis study, BODIPY-based compounds were synthesized to investigate the fluorescence sensor properties against $A\beta$ plaques for the early diagnosis of Alzheimer's disease. Synthesized compounds were characterized and their photophysical properties were investigated. The structures of **BODIPY-5b**, **BODIPY-5d**, **BODIPY-5f**, **BODIPY-5g**, **BODIPY-5i**, and **BODIPY-Sq** were determined by X-ray diffraction technique. The fluorescence sensor properties of the synthesized compounds against $A\beta$ plaques were investigated. Compound BODIPY-5b exhibited quenching in the emission band when incubated with $A\beta_{1-42}$ aggregates. On the other hand, when **BODIPY-Sq** interacted with $A\beta_{1-42}$ aggregates, an enhancement of its fluorescence intensity was observed. Results indicated that BODIPY-Sq is a promising compound as a fluorescence sensor for $A\beta$ plaques.

Keywords: Alzheimer's Disease, $A\beta$ plaques, BODIPY derivatives, Optical imaging, Fluorescence probe, Fluorescence sensor

Kahramanmaraş Sütçü Imam University

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**BODIPY BİLEŞİKLERİNİN SENTEZİ VE β -AMİLOİD PLAKLARININ
SAPTANMASINDA FLORESANS SENSÖR ÖZELLİKLERİ
(DOKTORA TEZİ)
HİLAL KIRPIK**

ÖZET

Alzheimer hastalığı genellikle ileri yaşlarda ortaya çıkan nörodejeneratif bir hastalıktır ve demansın (bunamanın) en sık rastlanan sebebidir. Alzheimer hastalığının iki histopatolojik belirteci beta amiloid plakları ($A\beta$ plakları) ve nörofibriler yumakların oluşumudur. Günümüzde Alzheimer hastalığının seyrini kesin olarak durdurabilecek bir tedavi mevcut değildir. Piyasada mevcut olan ilaçlar hastalığın semptomlarını giderici olup hastalığı tedavi edici özelliğe sahip değildirler. Günümüzde Alzheimer hastalığının tanısı bilişsel testlerle, beyin görüntüleme teknikleri kullanılarak ve beyin omurilik sıvısında $A\beta$ ve tau protein seviyelerine bakılarak yapılmaktadır. Bu tekniklerin zaman alma, pahalı olma ve radyoaktiviteye maruz kalma gibi bazı dezavantajları söz konusudur. Bu noktada floresan sensörler ile optik görüntüleme Alzheimer hastalığının tanısında alternatif bir teknik olarak ortaya çıkmaktadır. Ayrıca yapılan araştırmalar $A\beta$ plaklarının demans (bunama) başlangıcından yıllar önce oluştuğuna işaret etmektedir. Alzheimer hastalığında $A\beta$ plaklarının tespiti erken teşhis açısından kritik bir öneme sahiptir. Bu tez çalışmasında Alzheimer hastalığının erken tanısı için $A\beta$ plaklarına karşı floresans sensör özellikleri incelenmek üzere BODIPY temelli bileşikler sentezlenmiştir. **BODIPY-Bg1** bileşiğinin *mezo* konumunda bulunan fenil halkası bir biguanid olan disiyandiamid grubu içermektedir. Çeşitli aldehitlerin **BODIPY 2** bileşiği ile reaksiyonundan *mezo*-aril Schiff bazları olan BODIPY imin bileşikleri (BODIPY-5a/5i) elde edilmiştir. **BODIPY-Sq** bileşiğinde ise *mezo*-pozisyonundaki fenil halkası bir dietil squarat grubu içermektedir. Sentezlenen BODIPY bileşikler DCM, ACN, THF, DMSO gibi çözücülerde çözünmektedir. BODIPY-Bg1 bileşiği ise bu çözücülerden farklı olarak suda çözünmektedir. Sentezlenen BODIPY bileşiklerinin karakterizasyonu yapılarak foto fiziksel özellikleri incelenmiştir. **BODIPY-5b**, **BODIPY-5d**, **BODIPY-5f**, **BODIPY-5g**, **BODIPY-5i** ve **BODIPY-Sq** bileşiklerinin yapıları X-ışını kırınımı tekniği ile aydınlatılmıştır. Sentezlenen bileşiklerin $A\beta$ plaklarına karşı floresans sensör özellikleri *in vitro* koşullarda incelenmiştir. **BODIPY-Bg1** bileşiği sulu ortamda çözünme gibi büyük bir avantaj sunmasına karşın bileşik $A\beta_{1-42}$ agregatları ile inkübe edildiğinde emisyon bandında kayda değer bir değişim sergilememiştir. **BODIPY-5b** bileşiği $A\beta_{1-42}$ agregatları ile inkübe edildiğinde emisyon bandında sönümlenme sergilerken **BODIPY-**

Sq bileşigi $A\beta_{1-42}$ agregatları ile etkileşime girdiğinde floresans şiddetinde bir artış gözlemlenmiştir. **BODIPY-Sq** bileşigi sığır serum albümin (BSA) ile inkübe edildiğinde emisyon bandında kayda değer bir değişim gözlenmemiştir. Bu sonuç **BODIPY-Sq** bileşiginin $A\beta_{1-42}$ agregatlarına karşı seçicilik gösterdiğine işaret etmektedir. **BODIPY-Sq** bileşiginin $A\beta_{1-42}$ agregatları için hesaplanan K_d değeri 11.7 μM olarak hesaplanmıştır. Sonuçlar, BODIPY-Sq bileşiginin $A\beta$ plakları için bir floresan sensörü olarak umut verici bir bileşik olduğu yönündedir.

Anahtar Kelimeler: Alzheimer Hastalığı, $A\beta$ plakları, BODIPY türevleri, Optik görüntüleme, Floresan probu, Floresan sensörü.

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Hilal Kırpık

ABBREVIATIONS

°C	Degree Celsius
¹³ C	Carbon-13
¹ H	Hydrogen-1
AD	Alzheimer's Disease
ATR	Attenuated total reflection
Aβ	β-amyloid peptide
BF ₃ ·O(C ₂ H ₅) ₂	Boron trifluoride diethyl etherate
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
BSA	Bovine serum albumin
CHCl ₃	Chloroform
cm	Centimetre
CSF	Cerebrospinal fluid
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublet
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMSO	Dimethylsulfoxide
eq.	Equivalent
ESI-Q-TOF	Electrospray-ionisation quadrupole time-of-flight
Et ₃ N	Triethylamine
EtOH	Ethanol
FRET	Förster resonance energy transfer
FTIR	Fourier-transform infrared spectroscopy
g	Gram
h	Hour
HRMS	High-resolution mass spectrometry
Hz	Hertz
ISC	Intersystem crossing
J	Joule
J	Coupling constant in Hz
L	Litre
M	Molarity
m	Multiplet
m/z	Mass-to-charge ratio
M ⁺	Molecular ion
MeCN	Acetonitrile
MeOH	Methanol
mg	Milligram
MHz	Megahertz
min	Minute
mL	Millilitre
mM	Millimolar
mmol	Millimole
mol	Mole
NIA-AA	National Institute on Aging and Alzheimer's Association

nM	Nanomolar
nm	Nanometer
NMR	Nuclear Magnetic Resonance
PBS	Phosphate buffered saline
PeT	Photoinduced Electron Transfer
PET	Positron Emission Tomography
ppm	Part per million
P-tau	Phosphorylated tau
q	Quartet
rpm	Revolutions per minute
s	second
s	Singlet
t	Triplet
TEA	Triethylamine
TFA	Trifluoroacetic acid
Tg2576	Transgenic Mause
THF	Tetrahydrofuran
ThT	Thioflavin T
TLC	Thin layer chromatography
UV-Vis	ultraviolet-visible
v/v	Volume-to-volume ratio
δ	Chemical shift
ϵ	Molar extinction coefficient
λ	Wavelength
λ_{abs}	Absorption wavelength
λ_{em}	Emission wavelength
λ_{ex}	Excitation wavelength
μL	Microliter
μM	Micromolar
ΦF	Fluorescence quantum yield

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CHAPTER 1 BACKGROUND

1.1 Introduction

1.1.1 Alzheimer's Disease (AD)

Alzheimer's disease (AD) is the most common neurodegenerative disorder in the elderly people and its symptoms include cognitive decline, memory loss, and behavioural disability (Vaz & Silvestre, 2020a). Alzheimer's Disease (AD) was first described by German Bavarian psychiatrist and neurologist Alois Alzheimer in 1907 (Goedert & Spillantini). A patient of Alois Alzheimer who was a 51-year-old woman named Auguste Deter, was suffering from loss of memory, language, disorientation, and hallucinations before dying (Breijyeh & Karaman, 2020). When Auguste Deter died, Alois Alzheimer investigated her brain microscopically and he observed amyloid plaques, neurofibrillary tangles, and a massive loss of neurons (Goedert & Spillantini). After this observations Alois Alzheimer described the condition as a serious disease of the cerebral cortex and then Emil Kraepelin named this medical condition Alzheimer's disease for the first time in his 8th edition psychiatry handbook "Handbook of Psychiatry". Although the pathogenesis and mechanism of AD progression remain unclear, extracellular accumulation of the protein beta-amyloid ($A\beta$) in the form of plaques outside neurons and the accumulation of Tau protein in the form of neurofibrillary tangles (NFT) inside neurons are two main histopathological hallmarks of AD. Other brain changes observed in AD include inflammation and atrophy (decreased brain volume). The physiological structures of the brain and neurons in healthy and AD brains are shown in **Figure 1.1**.

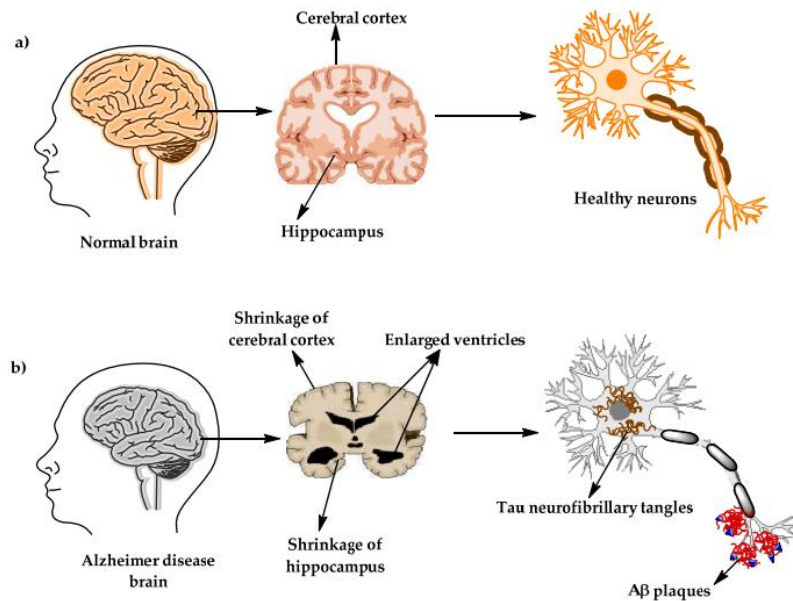


Figure 1.1 The physiological structure of the brain and neurons in (a) healthy brain and (b) AD brain [3]

1.1.2 The stage of Alzheimer's Disease

Alzheimer's disease can be classified into three broad phases depending on the cognitive impairment: preclinical, mild and dementia. The Alzheimer's dementia phase is further divided into three: mild, moderate, and severe dementia (**Figure 1.2**).

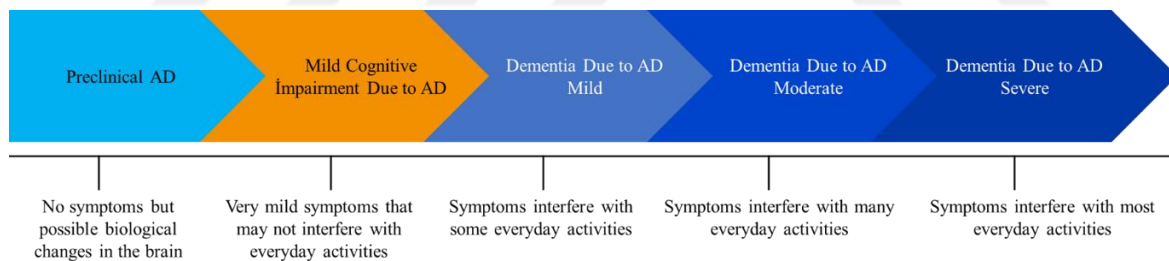


Figure 1.2 Stage of AD (2022 Alzheimer's Disease Facts and Figures)

The preclinical AD can last for several years or more and individuals with preclinical AD have no cognitive symptoms such as memory loss, but they already may have measurable brain changes indicating the earliest pathological signs of Alzheimer's disease (biomarkers). However, it is important to realize that not all individuals having Alzheimer-related brain changes go to develop symptoms of Alzheimer's mild cognitive impairment or dementia phases (Bennett et al., 2006; Knopman et al., 2003). For example, beta-amyloid plaques have been observed in the brain of some individuals at death, but these individuals did not have cognitive problems such as memory and thinking in their lifetime (Scheltens et al., 2016). In MCI phase, individuals with Alzheimer's disease have new understated symptoms including

cognitive problems such as language, thinking and memory in addition to preclinical AD symptoms. These cognitive symptoms emerge because the brain can no longer atone for the neuronal damage and death induced by AD. Individuals with MCI may not experience trouble in the daily life activities. Among individuals with MCI, about one-third develop dementia within five years (Knopman et al., 2003). However, some of them do not experience additional cognitive problems or they revert to normal cognitive conditions. Most individuals with the mild stage of Alzheimer's Dementia can function independently in many fields for example driving and working but they are likely to need help because symptoms interfere with some daily activities such as paying bills. Moreover, they need more time to finish their daily task. The longest stage of Alzheimer's disease is the moderate dementia stage where symptoms are more significant (Wattmo, Minthon, & Wallin, 2016). Completing the daily task including bathing or dressing is more challenging for individuals with moderate dementia because they experience more memory and language problems. They are more likely to feel confused and may become incontinent at times and may develop personality and behavioural changes such as suspiciousness (Wattmo et al., 2016). Moreover, they may also begin to have recognizing problems, such as not knowing their loved ones. In the severe stage of AD dementia, individuals have progressive cognitive and functional impairment, and the disease has spread to the whole cortex. Individuals with AD dementia cannot recognize their family members anymore and they need around-the-clock care and become bedridden (Breijyeh & Karaman, 2020). They may have trouble with control of swallowing which may result in their death because foods may go into the windpipe instead of the food pipe. Moreover, when they become bedridden, further several problems occur such as skin infections, blood clots, and sepsis (Apostolova). Eventually, all these complications lead to their death.

1.1.3 Causes and Risk Factors of Alzheimer disease

AD has been linked to several risk factors but among these factors, the most important one is advanced age carrying APOE ϵ 4 allele, especially older than 65 years (S. J. van der Lee et al., 2018). Moreover, AD can be broken down into types based on age: early-onset and late-onset. The first one is a rare form, and it is observed in about 1-6% of cases in which most of them comprise familial AD. Familial AD is when there is more than one family member in at least two generations with AD, aged 30-60 or 65 (Khan et al., 2022). In addition to the age factor when we evaluated according to the gender factor, women are more likely to develop Alzheimer's disease, particularly after the age of 80 years than men (International, -a). The other risk factors for AD include vascular diseases, lifestyle, genetic factors, infections, head

injuries, and environmental factors (heavy metals and others). All the risk factors for AD are presented in **Figure 1.3**.

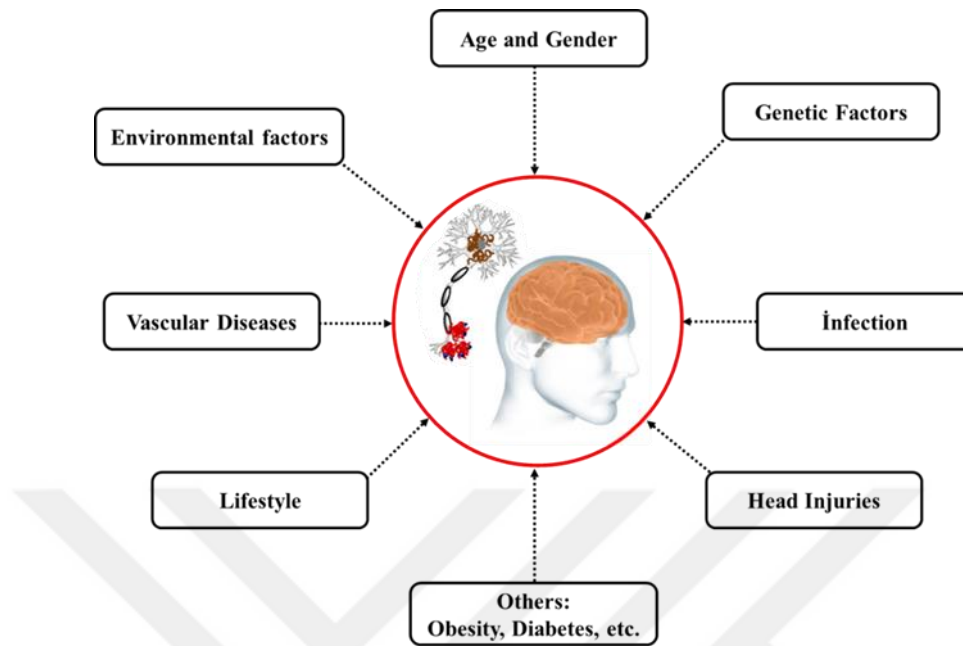


Figure 1.3 The Risk Factors of AD (Breijyeh & Karaman, 2020)

Several independent hypotheses such as oxidative stress, Metal ion, Cholinergic, Tau, and A β cascade have been proposed for the cause of AD, but the accurate mechanism of the disease remains unclear (Srivastava, Ahmad, & Khare, 2021). Among the proposed hypotheses, the A β cascade hypotheses are believed to be the main cause of AD, although none of them or other hypotheses alone is insufficient to explain the pathological abnormalities of the disease. Summary of pathophysiology of AD are shown in **Figure 1.4**.

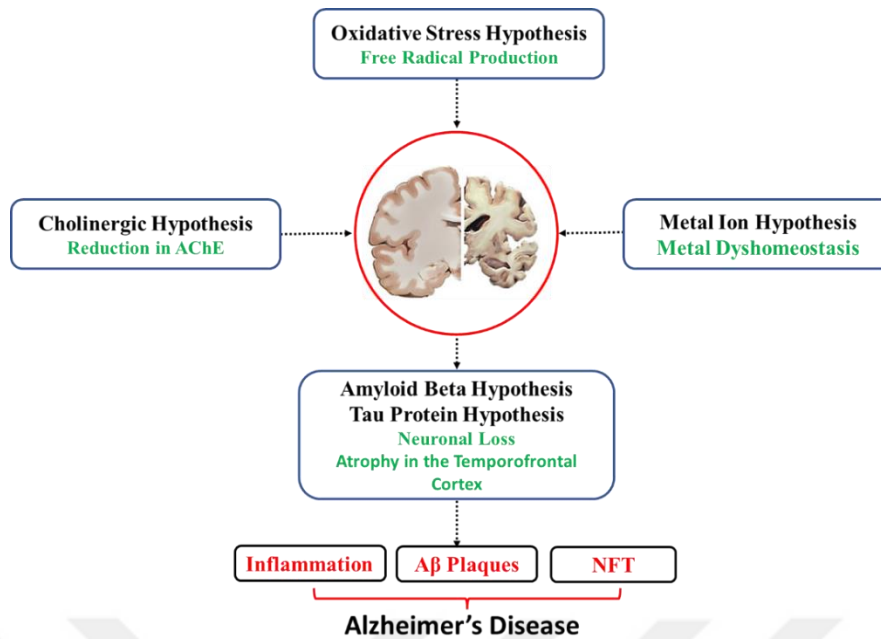


Figure 1.4 Hypothesis for pathophysiology of AD

1.1.3.1 Amyloid Cascade Hypothesis

The amyloid cascade hypothesis, which was formulated in 1992 by Hardy and Higgins, is still the most accepted model to explain the pathogenesis of inherited Alzheimer's disease (J. A. Hardy & Higgins, 1992). According to the amyloid cascade hypothesis, the aggregation and accumulation of A β peptides in the brain as A β plaques is a critical process that causes Alzheimer's disease (J. A. Hardy & Higgins, 1992; J. Hardy & Selkoe, 2002; Karran, Mercken, & Strooper, 2011). Monomeric A β peptides (39–43 amino acids in length) are generated via the cleavage of amyloid precursor protein (APP), a transmembrane glycoprotein, by β -secretases and γ -secretase (J. Lee et al., 2008). It has been found that APP is generated in large quantities in neurons and is metabolized very rapidly. There are two pathways for processing of APP: non-amyloidogenic pathway and amyloidogenic pathway (**Figure 1.5**). In non-amyloidogenic pathway, APP is cleaved sequentially by the enzymes α -secretase and γ -secretase, producing a soluble peptide without a tendency to aggregate. When APP is cleaved by β -secretase and γ -secretase, the neurotoxic monomeric A β peptide is formed (amyloidogenic pathway).

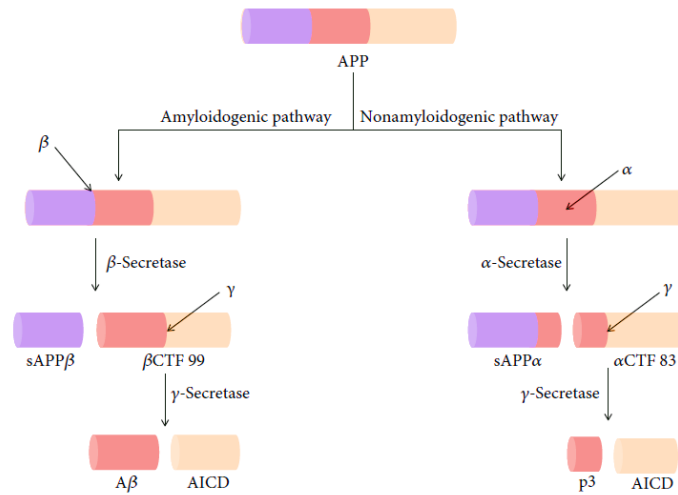


Figure 1.5 The amyloidogenic and nonamyloidogenic pathways of amyloid precursor protein processing (Uddin et al., 2020)

Monomeric A β peptides are comprised of a hydrophilic N-terminal domain (1–28) and a hydrophobic C-terminal domain (29–39/43) (J. Hardy & Selkoe, 2002; Seubert et al., 1992). The most abundant fragments of A β peptides are A β _{1–40} and A β _{1–42}, the latter is the predominant component in senile plaques and more toxic than A β _{1–40} (J. Hardy & Selkoe, 2002; Seubert et al., 1992). The monomeric A β peptides are capable of aggregation mainly through hydrophobic interactions. According to the amyloid cascade hypothesis, an increased A β peptides production and the accumulation of these peptides via aggregation first lead to the formation of A β oligomers than to protofibrils and ultimately to fibrils which are the main components of A β plaques (**Figure 1.6**) (Klein, Stine, & Teplow, 2004). These structures cause a disruption of synapses and neuronal death.

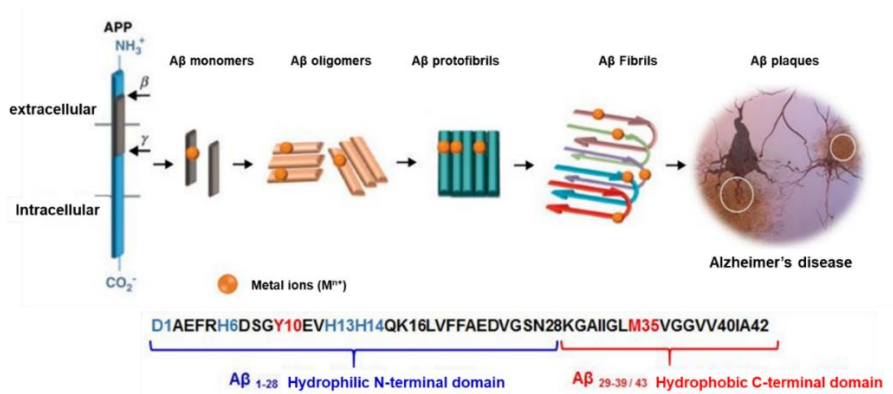


Figure 1.6 Scheme of the generation of amyloid- β species and the interaction of metal ions with A β peptides (up). The amino acid sequence of the A β _{1–42} (down)

1.1.4 Current diagnosis and treatment

The criteria for diagnosing AD published by the National Institute on Neurological and Communicative Disorder and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) have been widely utilized by clinicians to distinguish between the symptoms present in “probable,” “possible,” or “definite” AD (Reitz, 2012). After the diagnostic criteria for AD were released in 1984, they were updated in 2011 by the National Institute on Aging and Alzheimer's Association (NIA-AA) due to the advancement of knowledge regarding AD pathogenesis and considerable advancements in modern clinical, imaging, and research technologies (**Figure 1.7**). Imaging techniques and CSF biomarkers were identified tools as valid diagnostic in the 2018 revision of the NIA-AA diagnostic criteria. (J. C. Lee, Kim, Hong, & Kim, 2019).

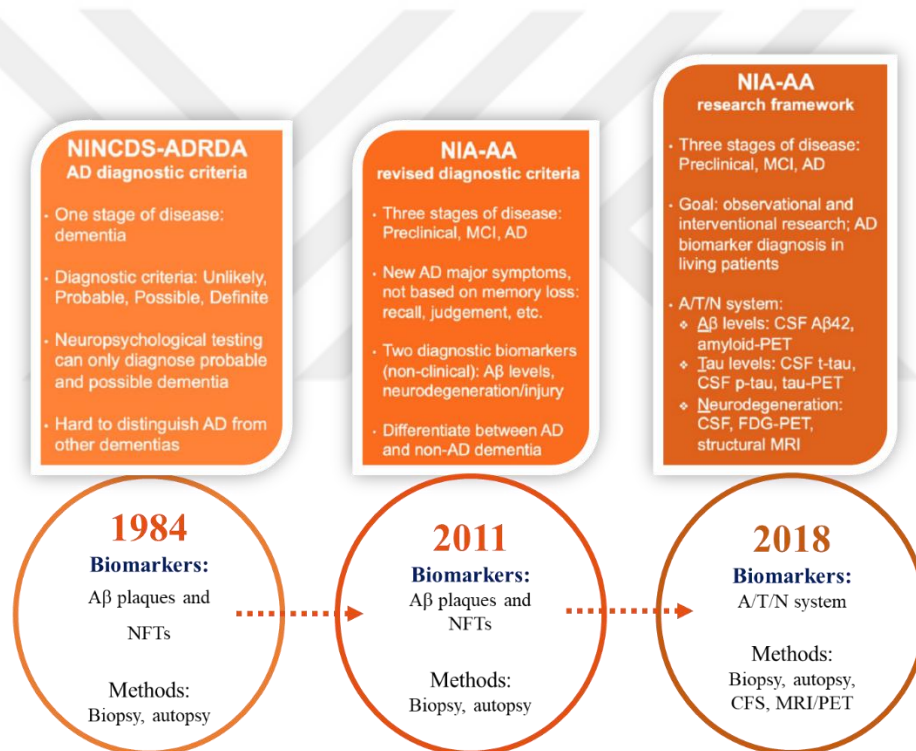


Figure 1.7 Evolution of AD diagnostic criteria (J. C. Lee et al., 2019)

Presently, AD cannot be definitively diagnosed until post-mortem neuropathological examination where the presence of A β plaques and NFTs in the brain can be revealed (Deture & Dickson, 2019). The traditional diagnosis of Alzheimer's disease relies on behavioural history assessments, physical examinations, neuropsychiatric tests (i.g., the Mini-Mental State Examination, MMSE), laboratory tests and neuroimaging techniques. To date, the best techniques for diagnosis of AD include cerebrospinal fluid (CSF) measures of A β and tau, structural MRI, functional MRI, ^{18}F -2-fluoro-2-deoxy-D-glucose (FDG) PET, and amyloid-

PET. Structural or compositional abnormalities (hippocampal atrophy) can be monitored with MRI scans, while FDG-PET monitors glucose metabolism mechanisms to identify areas of decreased brain activity. The amyloid-PET can characterize A β aggregates in the brain by using tracer [Pittsburgh Compound B (PIB)]. In the CSF measurements, potential AD biomarkers can be identified without the need for autopsy or biopsy, and concentrations of A β and tau isoforms are analyzed independently or in comparison with one another (J. C. Lee et al., 2019). Currently, only four drugs are approved by the Food and Drug Administration (FDA) for the treatment of AD. Three of these are inhibitors of the acetylcholinesterase enzyme (AChE) (donepezil, galantamine and rivastigmine) and one is a low affinity N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) for moderate to severe AD (Vaz & Silvestre, 2020b). However, these drugs only contribute to modest benefits in symptoms management and do not treat the underlying causes of the disease (Karran et al., 2011). Moreover, they do not prevent neuronal loss, brain atrophy and, consequently, the progressive deterioration of cognition (“2020 Alzheimer’s disease facts and figures,” 2020).

1.1.5 Why We Need Early Diagnosis of AD?

AD is the most common cause of dementia, and it is estimated that more than 50 million people worldwide have dementia, nowadays. This number is expected to reach over 150 million by 2050 (International, -b). None of current methods by themselves or in combination provide for early detection or yield high accuracy. The low sensitivity of MRI probes, the high cost of PET probes, and the radioactivity of SPECT probes limit the clinical applications of these techniques (Xu, Ren, Tang, Hu, & Zhang, 2016a). The cost of a PET examination is approximately \$1000, which is high for an average family living in a developing country (Xu et al., 2016a). There are still no effective treatments to prevent, halt, or reverse AD. Common medications are only symptomatic and aim to restore the disrupted neurotransmission between the degenerated neurons (Vaz & Silvestre, 2020b). A great deal of research focuses on the search for blood-borne biomarkers indicative of AD pathology, but most attempts have met only limited success. (Nagele, Han, DeMarshall, Belinka, & Nagele, 2011). Currently various therapeutic approaches are under investigation including inhibition of A β production; inhibition of A β aggregation, anti-inflammation, cholesterol homeostasis modulation; and metal chelation . Studies have shown that β -amyloid plaques can form in the brain maybe 20 years or more before symptoms appear (Bateman et al., 2012; Gordon et al., 2018; Villemagne & Rowe, 2010). At this point, optical imaging using a fluorescence sensor (probe) for the detection of A β plaques emerges as an alternative technique that offers a non-

radioactive, high-resolution, fast, and inexpensive imaging system for the early diagnosis of Alzheimer's disease (Ntziachristos, 2006; Ntziachristos, Ripoll, Wang, & Weissleder, 2005).

1.2 Fluorescence

Luminescence, also known as fluorescence or phosphorescence, is a process which occurs when photons of electromagnetic radiation are absorbed by molecules, raising them to some excited state, and then, on returning to a lower energy state, the molecules emit radiation or rather luminesce. The luminescence process is depicted using a Jablonski diagram (**Figure 1.8**) Fluorescence is the emission of light when an electron in a singlet excited state relaxes back to the ground state, whereas phosphorescence is the emission of light when the electron in its triplet excited state returns to the ground state. The major difference between fluorescence and phosphorescence is their emission rates: fluorescence – fast (10^8 s^{-1}), phosphorescence – slow (10^3 s^{-1}) (Joseph R. Lakowicz, 2011). The absorption of light from a fluorophore, results in its excitation to a higher vibrational level, either S_1 or S_2 . In the case of fluorescence, all of the excited vibrational states quickly relax to the lowest vibrational level of S_1 (10^{-12} s), which is known as internal conversion (IC). The electron in the excited orbital is paired to the second electron (with an opposite spin) in the ground state orbital therefore the subsequent transition to the ground state is spin allowed and the emission of light is fast. However, molecules in the S_1 can undergo a spin conversion to the first triplet state T_1 (intersystem crossing). This transition from T_1 to the singlet ground state is forbidden, therefore the emission (phosphorescence) is at a longer wavelength (lower energy) and the emission rate is considerably slower than for fluorescence. In this thesis, we will be solely focusing on fluorescence as a method to detect specific analytes and their application in diagnostic systems (Joseph R. Lakowicz, 2011).

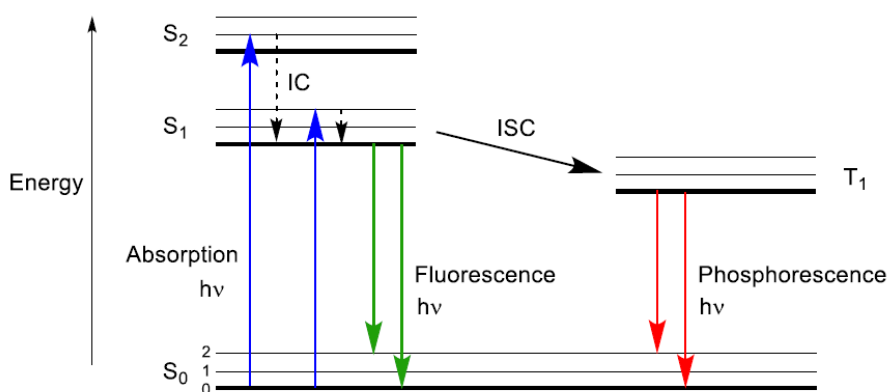


Figure 1.8 A typical Jablonski diagram. S_0 is the electronic ground state. S_1 and S_2 are the singlet excited states and T_1 is the first excited triplet state. IC is internal conversion and ISC is intersystem crossing (Joseph R. Lakowicz, 2011).

The utility of fluorescence originates with the difference between the excitation and emission wavelengths. Absorption occurs at lower wavelength than emission because energy of absorption is higher than energy of emission due to the loss of energy in vibrational levels and this phenomenon called “Stokes shift” (Fu & Finney, 2018) (**Figure 1.9**).

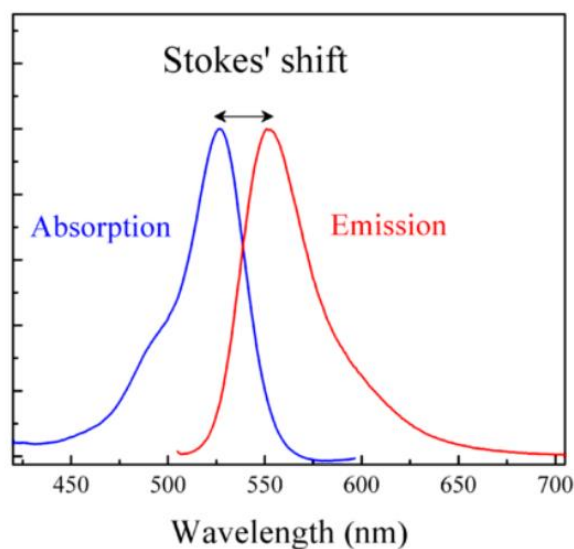


Figure 1.9 Representation of Stokes shift

Fluorescence Spectroscopy has several advantages over the traditional detection methods including HPLC, GC-MS. Therefore, it has been extensively used as a method for the detection of a range of different chemical species using small molecule fluorescent probes (Yang, Zhao, Feng, & Li, 2013). In this technique, the binding action of a target to the analyte is converted into a molecular-level readable optical signal. This technique also allows for

studying living biological samples in real time because it uses fluorescence phenomena offering a non-invasive approach. A schematic representation of a fluorescence probe is given in **Figure 1.10**.

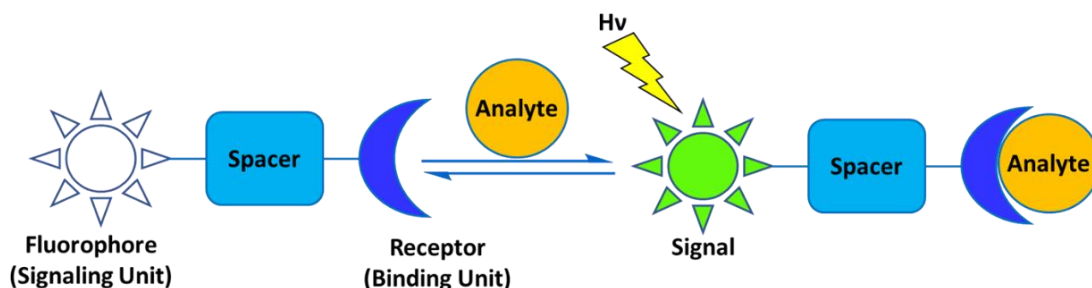


Figure 1.10 Schematic presentation of fluorescence probes

Designing fluorescent probes that can selectively interact with a single target analyte and exhibit high selectivity and sensitivity to that analyte allows the detection of a single target analyte in a complex biological environment. Moreover, fluorescence is a versatile technique, and thanks to this, it is used to study molecular interactions in a range of different media and interfaces (Yang et al., 2013). Consequently, fluorescent probes have been used for a broad range of applications such as clinical diagnostics and the detection of environmental, agricultural, and industrial pollutants. In the design of a fluorescent probe, we need to use specific host-guest interactions, or a specific chemical reaction to induce a change in the fluorescent properties of the system. When the interaction of the host-guest is non-covalent and reversible, the fluorescence probe is referred to as a chemo sensor. If an irreversible chemical reaction occurs from the interaction of the host-guest, the fluorescence probe is referred to as a chemidosimeter (Yang et al., 2013). On the other hand, over the years, these two definitions and “fluorescence probe” have been used interchangeably and ambiguously (Wu et al., 2017) When designing a fluorescent probe, we must first select a suitable reporter (fluorophore). A suitable fluorophore is selected considering many factors. For example, the fluorophore should be non-toxic at the concentration used, and the excitation and emission wavelength of the fluorophore for biological applications should be in the near-infrared (NIR) region (Tang, Pei, Lu, Fan, & Huang, 2019). This provides a non-invasive method for *in vivo* visualization of mammalian tissues with minimal light damage to the biological sample, minimal interference from background autofluorescence, and acceptable penetration through biological tissues (Yuan, Lin, Zheng, He, & Huang, 2013). In addition, the balance of hydrophobicity and hydrophilicity must be considered to provide properties such as suitable cell permeability and water solubility. Finally, high optical brightness is important to

minimize the amount of fluorescent probe required, which prevents interference from the biological sample. Some of fluorophores which has been widely used as reporter molecule are shown in **Figure 1.11**. In design of a fluorescent probe, the next step is the choice of receptor. The receptor is usually covalently linked to the reporter directly or linked by a spacer to make up a whole sensing unit. The aim of the receptor is to interact with the target in a chemo selective manner, which then relays a response to the reporter that provides the signal that is observed by the fluorimeter. Finally, in the design of a fluorescent probe, the change in the fluorescence intensity needs to be considered. It is generally, preferred that upon binding to the target analyte, an increase in fluorescence emission intensity called “turn on” is observed, rather than a decrease in fluorescence emission intensity called “turn off”.

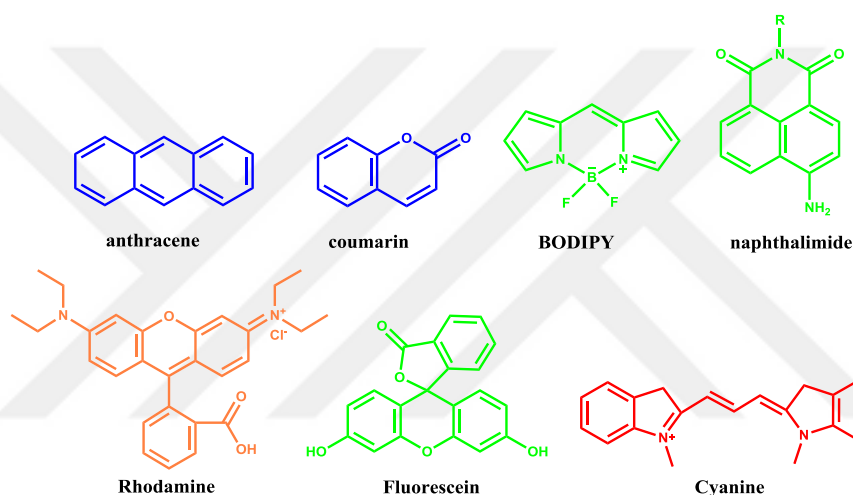


Figure 1.11 A representative set of common fluorophores used in design of fluorescence probe

1.2.1 Fluorescence Mechanism

There are three classic fluorescence mechanisms that can be used to generate a fluorescent response, these are PeT (Photoinduced Electron Transfer), FRET (Förster Resonance Energy Transfer) and ICT (Internal Charge Transfer).

1.2.1.1 Photoinduced Electron Transfer (PeT)

Photoinduced electron transfer (PeT) is the most used methods the design of fluorescent probes. PeT can result two-way, fluorescence quenching or an increase in fluorescence intensity. A requirement is that energetically the receptor electron's energy must lie between the energies of the π and π^* orbitals of the fluorophore. In this condition, an electron transfer from receptor to excited state of a fluorophore resulting in a fluorescence quenching. On the other hand, when receptor interacts with the target analyte, the receptor HOMO energy level

become lower than the fluorophore blocking PeT process thus resulting in fluorescence (**Figure 1.12**).

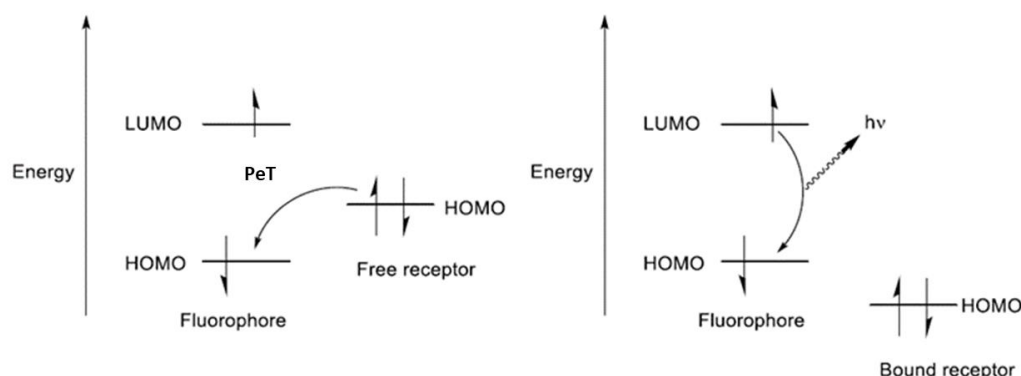


Figure 1.12 Schematic representation of PeT process

Several PET fluorescent probes have been designed and synthesised for the sensing of a various analytes. Benzylic nitrogen atoms are the most used donors for the PeT process. PeT resulting from the N atom lone pair quenches fluorescence (Wu et al., 2017). Metal ion coordination or protonation turns the lone pair into a σ -bonding electron pair, which is far lower than the energy of π -orbital. PeT is blocked, and fluorescence is taken place. Ren *et al.* were ported the development of the first PeT probe (1) to directly locate A β aggregates (A β plaques) in the brain without the need of post-washing procedures (**Figure 1.13**) (Ren et al., 2018a).

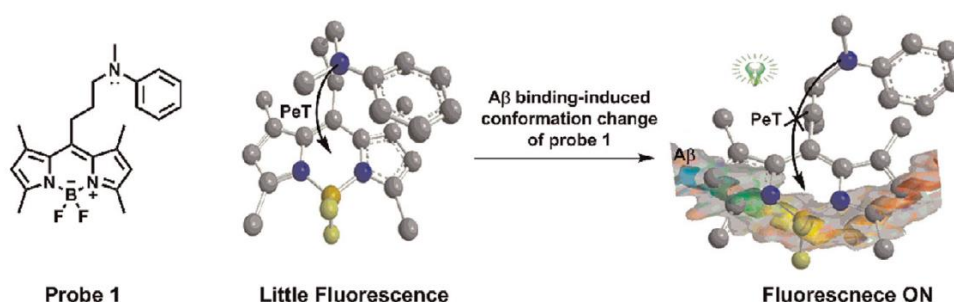


Figure 1.13 Schematic representation of a PeT based fluorescent probe for the detection A β plaques (Ren et al., 2018a)

1.2.1.2 Intramolecular charge transfer (ICT)

Intramolecular charge transfer (ICT) is one of the most common strategies used for design in fluorescent probe. When the fluorophore possesses an electron donating group such as amino, methoxy conjugated to an electron-withdrawing group often referred to as a ‘push-pull’ system. In some cases, the fluorophore itself serves as the donor or the acceptor. That is to

say, the fluorophore bears just a donor or an acceptor group. When ICT fluorophore is excited, electron density is redistributed resulting in a substantial dipole. At this situation, the binding of a target analyte to integrated receptor led to an interaction with this excited dipole and it results in a shift in the emission and excitation spectra to give a ratiometric response. ICT excited states are very sensitive to the presence of electric fields and therefore are affected by solvents, cations and hydrogen bonding (Prasanna De Silva et al., 1997). A relevant example is shown **Figure 1.14**. In that example, the initial ICT state is non-radiative, due to conjugation to the aldehyde. In the initial state, the BODIPY donor and the aldehyde acceptor represent a very strong D–A system. In a strong D–A system, the S_0/S_1 energy gap can become so small that internal conversion (IC) becomes very efficient, and fluorescence is quenched. When the probe reacts with H_2S , ICT is eliminated, and the fluorescence of the BODIPY is turn on (Chung, Srikun, Lim, Chang, & Cho, 2011).

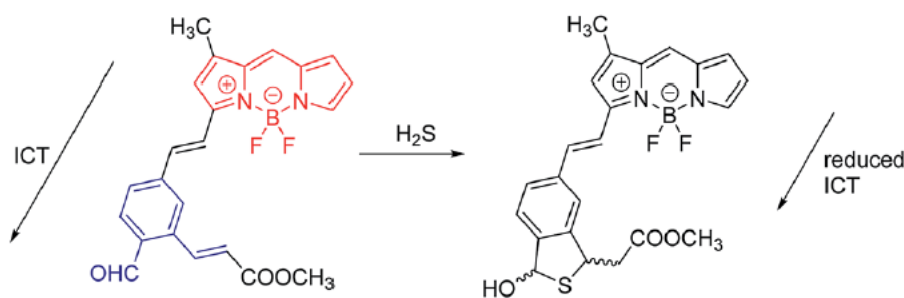


Figure 1.14 An BODIPY based ICT probe for H_2S in cells (Chung et al., 2011)

1.2.1.3 Förster resonance energy transfer (FRET)

Förster resonance energy transfer (FRET) is not a photon absorption process, it is a non-radiative process in which two or more fluorophores with similar excited state energies can exchange their energy due to long range dipole-dipole resonance interactions. But this process depends strongly on the proximity between the fluorophores, with the distance having to be within 1 – 10 nm. The FRET process also requires that the absorption spectrum of the acceptor fluorophore should overlap with the emission spectrum of the donor fluorophore. The requirements for FRET are illustrated below in **Figure 1.15**. FRET and intramolecular charged transfer (ICT) systems are the most exploited systems for the design of ratiometric fluorescent probes, with a number of reviews available on FRET based fluorescent probes (Fan, Hu, Zhan, & Peng, 2013; Yuan, Lin, Zheng, & Zhu, 2013).

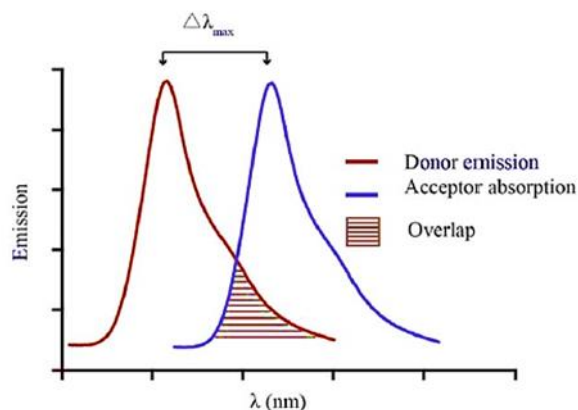


Figure 1.15 Requirements for the FRET process to take place (Fu & Finney, 2018)

1.3 An ideal fluorescence probe for the detection of A β plaques.

In principle, an ideal fluorescence probe for the detection of A β plaques should have the following properties (Ametamey, Honer, & Schubiger, 2008; Chang et al., 2011; Chongzhao et al., 2009; Nesterov et al., 2005; Xu, Ren, Tang, Hu, & Zhang, 2016b).

- A high selectivity and binding affinity (K_d , K_i , IC_{50}).
- A high quantum yield.
- A significant change in fluorescence properties upon binding to A β (i.e., fluorescence intensity, fluorescence lifetime, emission wavelength, and quantum yield).
- A suitable emission wavelength greater than 450 nm to minimize background fluorescence from brain tissue, ideally between 650-900 nm for *in vivo* detection.
- The ability to rapidly cross the blood-brain barrier (BBB) with reasonable lipophilicity (logP values between 2 and 3.5, or clogP < 5.0 are considered).
- A high metabolic stability, low-affinity binding with bovine serum albumin (BSA)
- A molecular mass less than 600 Da.
- Fast washout kinetics from normal brain regions.
- Low toxicity
- Straightforward synthesis.

To date, a variety of fluorescence probes based on Thioflavin-T, curcumin, stilbene, benzophenoxazine, thiophene, and BODIPY have been reported for the *in vitro* and *in vivo* detection of β -amyloid plaques. These probes are given in sections **1.3.1** and **1.4.1**.

1.3.1 Fluorescence sensors for the detection of A β plaques

Currently, the probes reported for A β plaques have a common push-pull structure and are highly sensitive to solvent polarity. They are generally non-fluorescent in aqueous solutions, but highly fluorescent in solutions with nonpolar solvents or upon binding to A β plaques. First introduced in 1959, the fluorescent dye thioflavin-T (ThT) has become a widely used “gold standard” for selectively staining and identifying A β plaques both *in vivo* and *in vitro* (see **Figure 1.16**). ThT is a planar molecule and contains electron acceptor (benzothiazole) and donor (aniline) groups that allow it to rotate freely around the C-C bond in the unbound state. When ThT binds to A β plaques, the free rotation hindrance between the acceptor and donor groups suppresses quenching, and the process results in a high quantum yield. Despite its widespread use, ThT has some important disadvantages. In the last decade, a wide variety of fluorescence sensors based on curcumin, stilbene, benzo phenoxazine, thiophene, and BODIPY have been designed and synthesized for the *in vitro* and *in vivo* detection of β -amyloid plaques. These fluorescence sensors show binding selectivity and affinity to hydrophobic grooves on the β -sheet surface along with the A β fibril axis and exhibit variations in the intensity of emission, maximum wavelength shifts, or lifetimes when they interact with A β (**Figure 1.16 and 1.17**).

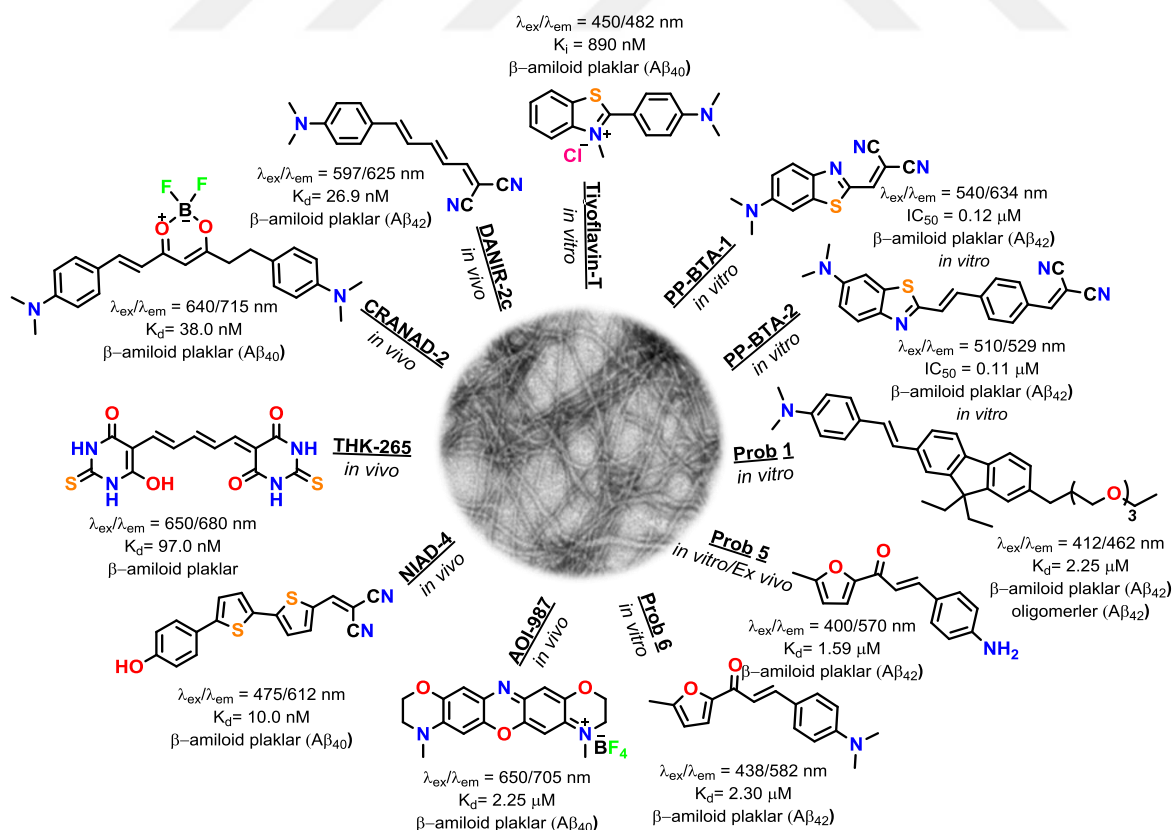


Figure 1.16 Various fluorescence probes for the detection of A β plaques

Hintersteiner *et al.* synthesized a fluorescence probe, AOI-987, which is an oxazine derivative. They reported that the AOI-987 probe is capable of binding to the A β plaques and can cross to BBB. However, the signal contrast is not meaningful between wild-type with transgenic mice (Hintersteiner *et al.*, 2005).

Nesterov *et al.*, designed and synthesized a thiophene-based fluorescence probe, NIAD-4 (**see Figure 1.16**). It is reported that the probe strongly binds to β -amyloid plaques and detects both A β plaques and cerebrovascular amyloid angiopathies (SAA) by crossing to the blood-brain barrier in the living brain tissue after intravenous (intravenous) injection (Nesterov *et al.*, 2005).

Ran *et al.* designed and synthesized a fluorescence probe (CRANAD-2, **see Figure 1.16**) based on curcumin derivative (Chongzhao *et al.*, 2009). They reported that when CRANAD-2 interacts with A β plaques, several changes such as a 70-fold enhancement of the fluorescence intensity, 90 nm of a blue shift in emission wavelength, and a large enhancement of quantum yield were observed. Also, CRANAD-2 shows high selectivity to β -amyloid plaques and exhibits an acceptable lipophilicity value (Log P = 3). The only factor limiting the *in vivo* use of the probe is its slow brainwashing (Chongzhao *et al.*, 2009).

Ono *et al.* synthesized two thioflavin-based fluorescence probes (PP-BTA-1 and PP-BTA-2) for the detection of A β plaques (**see Figure 1.16**) (Ono *et al.*, 2009). Both probes show high selectivity for A β plaques. Also, it is reported that synthesized probes stained β -amyloid plaques in both transgenic mice and Alzheimer's patients (Ono *et al.*, 2009).

Okumara *et al.* developed a near-infrared (NIRF) probe, THK-265, for the *in vivo* detection of A β plaques (**see Figure 1.16**) (Okamura *et al.*, 2011). THK-265 has a wavelength greater than 650 nm, and it exhibits high selectivity against β -amyloid plaques with high quantum yield and molar absorption coefficient. THK-265 transparently stained A β plaques in the neocortical brain section and it has a moderate lipophilicity value, Log P = 1.8. It is reported that the probe THK-265 is a potential NIRF probe for the *in vivo* detection of the A β plaques (Okamura *et al.*, 2011).

Lee *et al.* designed a fluorescence probe which was a stilbene derivative, Probe 1 (**see Figure 1.16**). Probe 1 detects A β plaques and water-soluble oligomeric A β peptides (Y. O. Lee *et al.*, 2014). When the probe binds to A β plaques, it exhibits increased disaggregation-induced fluorescence (DIEE). The probe functions with a different mechanism but its mechanism is affected by solvent polarity (Xu *et al.*, 2016b).

Cui *et al.* designed and synthesized a NIRF probe, DANIR-2c (see **Figure 1.15**). It is reported that DANIR-2c shows high selectivity for the A β plaques *in vitro* studies, and it stains the A β plaques in both Alzheimer's patients and transgenic mouse brain sections (Cui et al., 2014). However, when the probe binds to A β plaques, its emission wavelength shifts to blue. This blue shift limits its use as a A β NIRF probe in the diagnosis of Alzheimer's disease.

Jung *et al.* designed and synthesized two new stilbene-based fluorescence probes for the detection of A β plaques. Probes 5 and 6 (See **Figure 1.16**) detect the A β plaques in APP/PSI transgenic mouse brain section (Jung et al., 2015). They indicated that they selected Probe 5 for the ex vivo studies because it exhibited better results in vitro studies. Also, they confirmed that Probe 5 stains A β plaques by crossing to BBB in 14-month-old APP/PSI transgenic mice.

1.4 BODIPY dyes

Among the many classes of fluorophor, Boron-dipyrromethene (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) (**Figure 1.17**), BODIPY-based class perhaps shows the highest potential, and their popularity has increased dramatically.

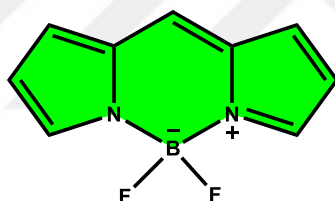


Figure 1.17 Structure of BODIPY core

The structure of a BODIPY core formally consists of a complex of a monoanionic dipyrromethene ligand and a disubstituted electron-deficient boron, generally a cationic boron difluoride (BF₂) group. Although BODIPY core has similarities with dipyrromethenes, the IUPAC name and the numbering system of BODIPY core are based on the *s*-indacene (Wood & Thompson, 2007) (See **Figure 1.17**). The 3,5-positions of BODIPY core are commonly specified by α , while the 1,7- and 2,6-positions are referred to as the β -positions. The central 8-position of BODIPY is often called the *meso*-position originating from porphyrin. In the structure of BODIPY core the formal negative charge is on the boron atom and the formal positive charge is delocalized over the ring structure (See **Figure 1.17**). Because of the relatively nonpolar nature of BODIPY core, its structure is usually depicted without formal charges as in **Figure 1.18**.

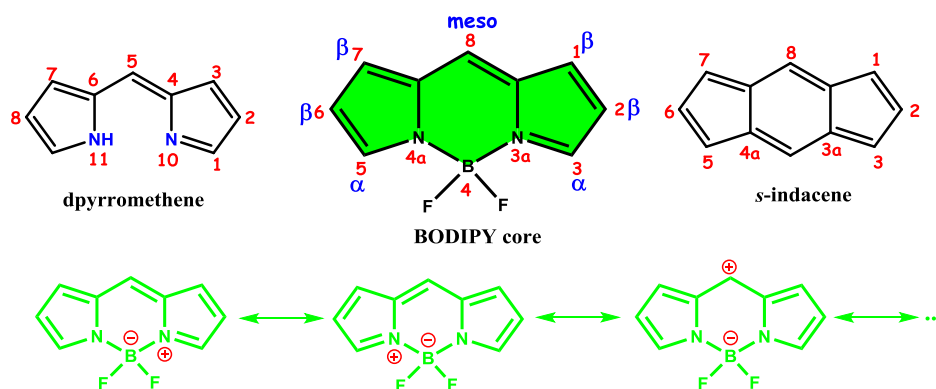


Figure 1.18 Structure and IUPAC numbering of the dipyrrromethene, boron dipyrryn or BODIPY core, and s-indacene frameworks. Three of the seven possible delocalized structures of unsubstituted BODIPY core.

Treibs and Kreuzer synthesized the first member of the BODIPY compounds in 1968 but these compounds received relatively little attention until the late 1980s (**Figure 1.19**) (Alfred Treibs & Kreuzer, 1968; Berendsen et al., -a; Worries et al.). Later, the potential usage of this dye for labelling in the biological application was discovered and various BODIPY-based dyes were designed and commercialized. The application of BODIPY-based compounds as electrochemiluminescence devices, tuneable laser dyes, and fluorophores for fluorescence sensors reached in the mid-1990s. As a result, since then, the number of research articles and patents has increased dramatically as more researchers enter the field. Today, BODIPY dyes can find various application are which are given **Figure 1.20**.

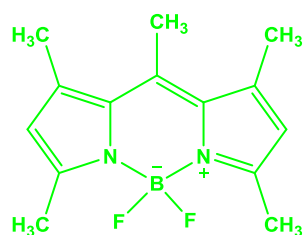


Figure 1.19 First BODIPY compound synthesised by Treibs and Kreuzer

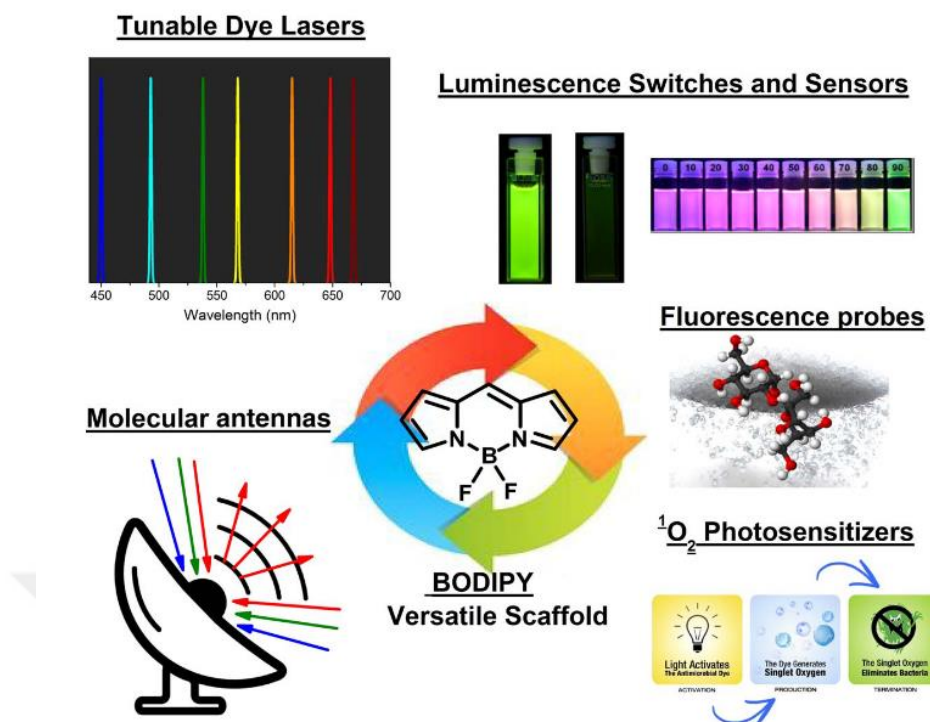


Figure 1.20 The main application fields of BODIPY dyes

The increasing interest in the BODIPY fluorophore can be explained by the excellent features given below.

- The robustness against light and chemicals.
- The relatively high molar absorption coefficients, fluorescence quantum yields, negligible triplet-state formation, and narrow emission bandwidths with high peak intensities.
- Excitation/emission wavelengths in the visible spectral region (≥ 500 nm), and fluorescence lifetimes in the nanosecond range.
- Good solubility, and resistance towards self-aggregation in solution.

All these properties contribute to the appeal of these interesting compounds and make them an important group of compounds in the development of fluorescent probes for optical imaging [59–61]. Moreover, one of the most attractive properties of the BODIPY group is their rich chemistry enabling the spectroscopic and photophysical properties of BODIPY can be tuneable by introducing new groups at any suitable position of the BODIPY core (α , β , and *meso*-substitutions) [62]. The rich chemistry of BODIPY core offers to obtain blue-emitting BODIPY derivatives such as *meso*-amino-BODIPY to red-emitting BODIPY derivatives by

with extended conjugation with substitution of peripheral phenylacetylene or styryls groups, at positions 2 and 6, or 3 and 5, respectively (Navale et al., 2015) (**Figure 1.21**).

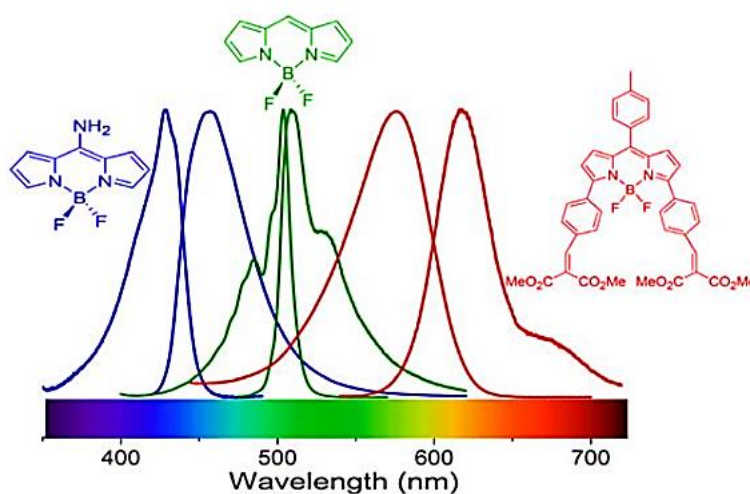


Figure 1.21 BODIPY dyes with tuneable absorption and emission wavelengths (Bañuelos, 2016)

1.4.1 BODIPY-based fluorescence sensor for the detection of A β species

BODIPY-based fluorescence probes used in the detection of the A β plaques are given in **Figure 1.24**. The first BODIPY-based fluorescence probes for the detection of A β plaques were synthesized by Pahri *et al.* (Parhi, Kung, Ploessl, & Kung, 2008) but the probes were only chemically characterized, *in vitro* binding constant and the other biological properties did not investigate in their study. Smith *et al.* designed and synthesized BODIPY compounds that can discriminate secondary structure conformation of the soluble A β_{1-42} oligomers. It was observed that the compounds exhibit different fluorescence enhancement in the presence of A β_{1-42} oligomers (Smith, Alonso, Brown, & Dzyuba, 2010). However, they did not study the application of the compounds as fluorescence imaging probes for A β peptides.

Ono *et al.* (Ono et al., 2010) developed BODIPY-based bifunctional SPECT/fluorescence probes (**See Figure 1.24**). It is reported that the probe, BODIPY-7, labelled with ^{125}I , exhibits good selectivity against the A β plaques with $K_i = 108$ nM value. The probe has a high quantum yield and detects A β plaques in the brain section tissue of transgenic mice (**Figure 1.22**), but it shows low uptake in the brain and high uptake in the liver. Additional modification in the structure is needed to enhance brain uptake.

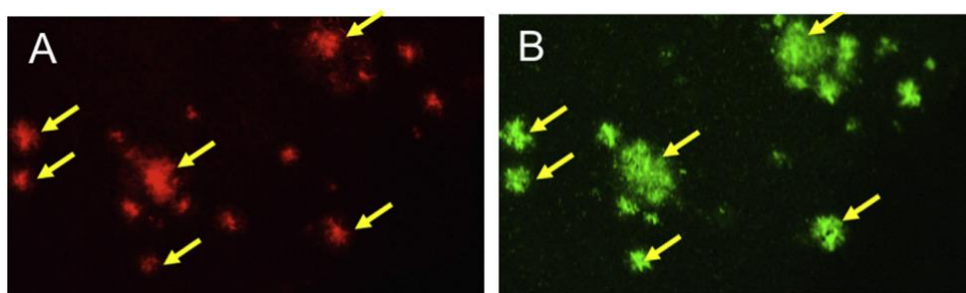


Figure 1.22 Neuropathological staining of BODIPY 7 (A) in Tg2576 mouse brain. Labeled plaques were confirmed by staining of the adjacent section with thioflavin S (B).

Ono *et al.* (Ono, Watanabe, Kimura, & Saji, 2012) designed and synthesized a BODIPY-based fluorescence probe, BAB-1, to detection of A β plaques (see **Figure 1.24**). BAB-1 probe shows high selectivity towards amyloid- β plaques *in vitro* experiments and visualizes A β plaques in the brain of transgenic mice. Results of the study show that BAB-1 probe can use in Alzheimer's disease patients with additional modifications for imaging the A β plaques. The same research group synthesized new BODIPY-based NIRF probes (BAPS) for the detection of A β plaques in 2013 by making substitution changes on the BAP-1 probe (see **Figure 1.24**) (Watanabe et al., 2013). *In vitro* binding constants of synthesized probes were determined for the A β plaques. The binding constants of the probes BAP-2, BAP-3, BAP-4, and BAP-5 were determined as 55, 149, 27, and 18 nM, respectively. The probes visualize A β plaques in the transgenic mice brain (Tg2576). It is reported that one of these probes, BAP-2, showed ideal brain kinetic and was chosen for the *ex vivo* experiments. BAP-2 shows selectivity against A β plaques in *ex vivo* studies (**Figure 1.23**). However, it cannot differentiate between transgenic and normal-type mice *in vivo* and show higher accumulation in the scalp than in the brain. Results of the study show that these BODIPY-based compounds need modification for their use *in vivo* applications (Watanabe et al., 2013).

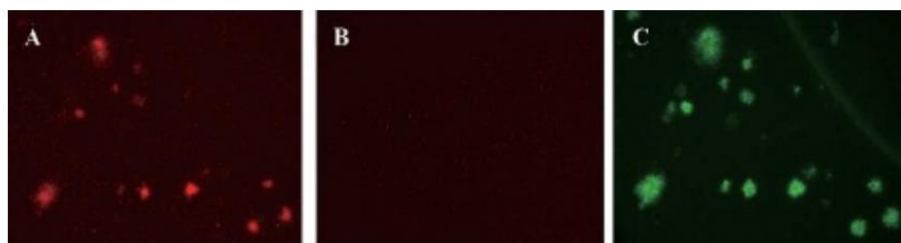


Figure 1.23 *Ex vivo* fluorescence observation of brain sections from a Tg2576 mouse (A) and a wild-type mouse (B) after injection of BAP-2. The presence of Ab plaques in the section from Tg2576 mouse was confirmed with thioflavin S (C).

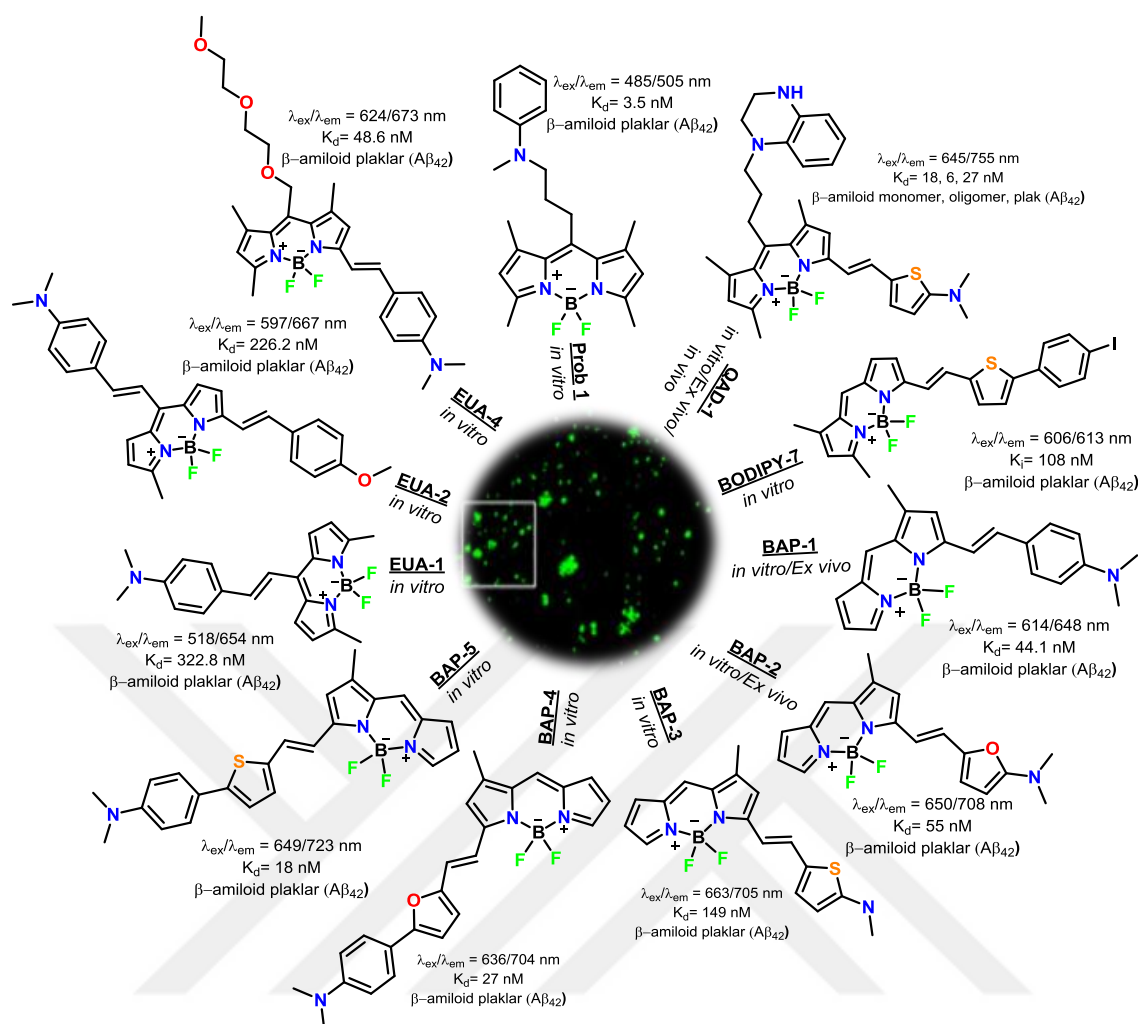


Figure 1.24 Various BODIPY-based fluorescence probes for the detection of β -amyloid species

Sözmen *et al.* (Sozmen et al., 2014) designed and synthesized a series of BODIPY-based compounds for fluorescence imaging of A β plaques (EUA1-5) (see **Figure 1.24**). The synthesized probes show emission wavelengths in the range of 654-763 nm. The measured K_d values of EUA1-5 probes for A β plaques were determined as 320, 230, 320, 48.6, and 97 nM, respectively. The probes, EUA-1, EUA-2, and EUA-4, stained amyloid- β plaques in the brain section of transgenic mice. It is reported that the EUA-4 probe, which has the highest selectivity and the best performance in fluorescence imaging, may be a promising fluorescence probe for β -amyloid plaques in the diagnosis of Alzheimer's disease with a further modification.

Ren *et al.* (Ren et al., 2016) designed and synthesized a fluorescence probe, Probe 1, for the in vitro detection of β -amyloid plaques (see **Figure 1.24**). Synthesized Probe 1 is the first PET probe, and it detects A β plaques in the brain. It is reported that Probe 1 shows high selectivity

against A β plaques ($K_d = 3.5$ nM). While the probe itself shows a slight emission, when it selectively binds to A β plaques, it exhibits an enhancement in the emission intensity. Moreover, it is reported that the probe does not require the post-wash procedure and visualizes A β plaques in the section of transgenic mice (**Figure 1.21**).

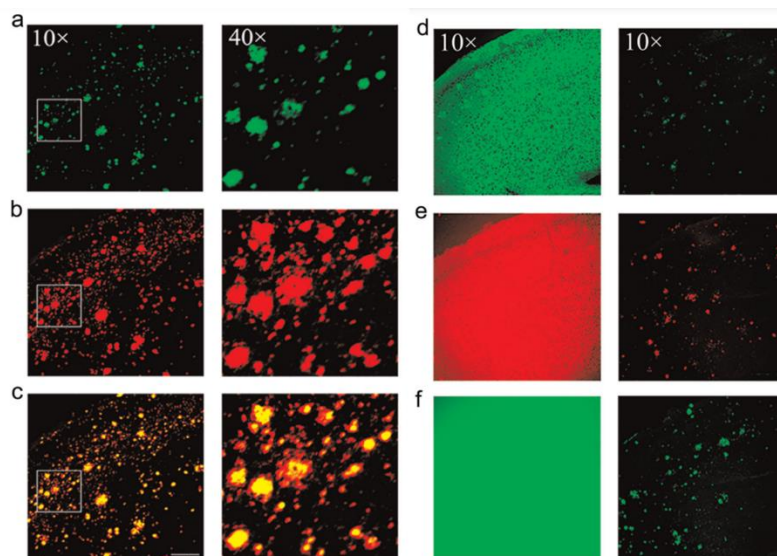


Figure 1.25 Fluorescent staining of A β plaques in transgenic brain sections (cerebral cortex) from double transgenic mice (APPsw/PS1De9, 12 months old, male) by laser confocal microscope. (a) Staining with probe 1 without washing; (b) Staining primary with A β antibody ab2454 then secondary antibody with fluorophore Alexa 546; (c) Merged images of a and b (d) Staining with ThS by washing (right) and no washing (left) assays; (e) Staining with CRANAD-58 by washing (right) and no washing (left) assays; (f) Staining with BODIPY acid 2 by washing (right) and no washing (left) assays.

Ren et al. (Ren et al., 2018b) synthesized a fluorescence probe, QAD-1 (see **Figure 1.20**). The probe QAD-1 is the first NIR off-on fluorescence probe for *in vivo* detection of A β plaques. The probe shows fluorescence enhancement when it binds to both water-soluble A β species and A β plaques. The researchers performed both *in vitro* and *in vivo* experiments and it is reported that the QAD-1 fluorescence probe is a potential probe for the *in vivo* and *in vitro* detection of β -amyloid species for early diagnosis of Alzheimer's disease.

CHAPTER 2 Results and Discussion

Within the scope of this thesis, BODIPY-based compounds were designed and synthesized. The structures of the compounds whose synthesis and characterization were completed are given in the **Figure 2.1**.

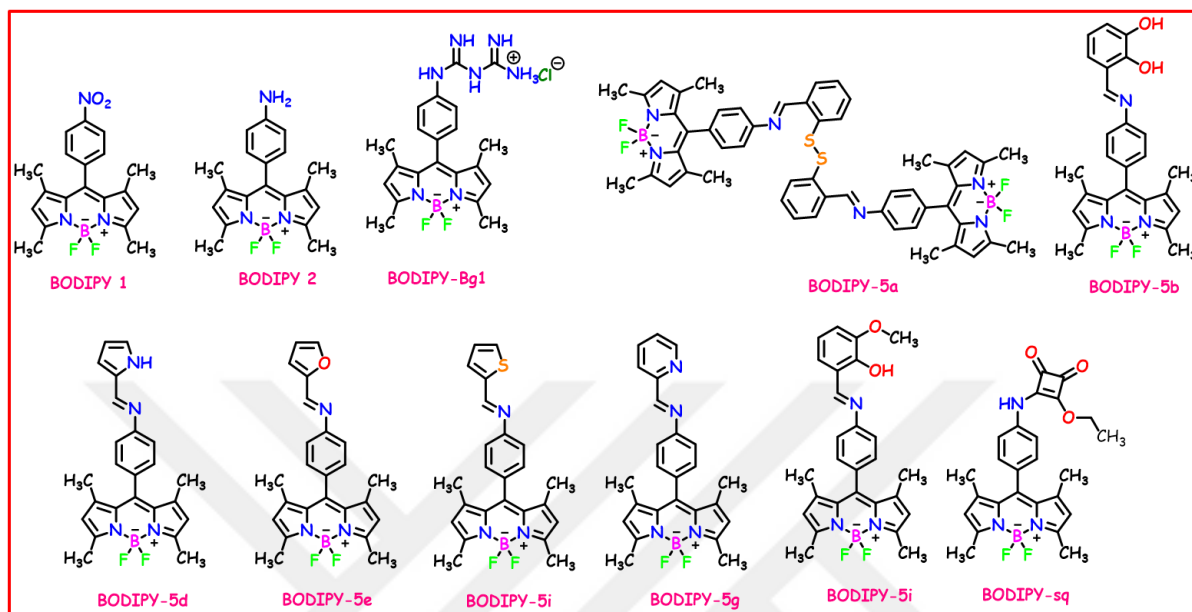
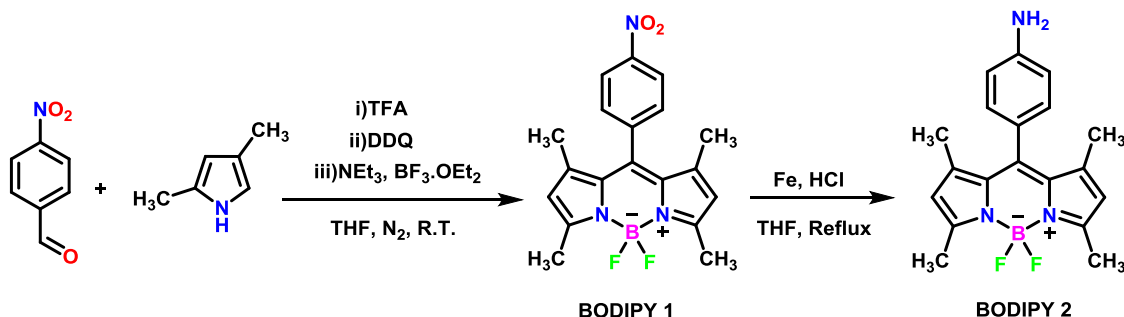


Figure 2.1 BODIPY-based compound synthesised within the scope of this thesis

The target compounds **BODIPY 1** and **BODIPY 2** were synthesized according to the methods given in the literature [77,78]. **BODIPY 1** was obtained from the acid-catalysed reaction of 4-nitro benzaldehyde with 2,4-dimethylpyrrole in a one-pot reaction (**Scheme 2.1**). To obtain **BODIPY 2** compound, the nitro group of **BODIPY 1** was reduced to the amino group using Bechamp Reduction (**Scheme 2.1**). Experimental details are given below. The synthesized compounds **BODIPY 1** and **BODIPY 2** were fully characterised by ^1H NMR, ^{13}C NMR, HRMS and FTIR spectroscopy at RT.



Scheme 2.1 Synthetic pathway to **BODIPY 1** and **BODIPY 2**

The ^1H NMR spectra of **BODIPY 1** and **BODIPY 2** were measured in $\text{DMSO-}d_6$. The spectral data are presented in the experimental section and spectra are given in **Figure 2.2** and **Figure 2.3**. In the spectrum of **BODIPY 1**, the *meso*-aryl group protons appeared as doublet signals at 8.41 and 7.76 ppm. The pyrrolic protons of **BODIPY 1** were observed as singlet signal at 6.24 ppm. In the ^1H spectrum of **BODIPY 1**, aliphatic protons appeared as singlet signals at 2.48 and 1.35 ppm. A new singlet signal observed at 5.46 ppm in the spectrum of **BODIPY 2** was assigned to amino group protons. This observation confirmed the reduction of the nitro group to the amino group. The pyrrolic protons were observed at 6.15 ppm as a singlet signal in the spectrum of **BODIPY 2**. In the spectrum of **BODIPY 2**, doublet signals observed at 6.71 and 6.91 were assigned to the *meso*-aryl group protons. The aliphatic protons appeared as singlet signals at 2.44 and 1.50 ppm in the spectrum of **BODIPY 2**. In the ^{13}C NMR spectrum of **BODIPY 1**, CH_3 groups signals were observed at 14.77 ppm (**Figure 2.4**). These $-\text{CH}_3$ groups signals appeared at 14.8 and 14.6 ppm in the spectrum of **BODIPY 2** (**Figure 2.5**). The signal observed at 156.17 ppm in the spectrum of **BODIPY 1** was assigned to the $=\text{CH}-$ group carbon signal in positions 4 and 5. The other $=\text{CH}-$ group carbons signals were observed in the range of 122.30-130.48 ppm in the spectrum of **BODIPY 1**. In ^{13}C NMR spectrum of **BODIPY 1**, carbon signals observed in the range of 139.74-148.51 ppm were assigned to the *meso*-aryl group carbon atoms. These signals were observed in the range of 114.6-154.4 ppm in **BODIPY 2**. ESI-Q-TOF mass spectrum of **BODIPY 2** was measured in methanol. The peaks at m/z 320.1813 and 340.1893 were assigned to the molecular ions $[\text{M-F}]^+$ and $[\text{M+H}]^+$, respectively (**Figure 2.6**).

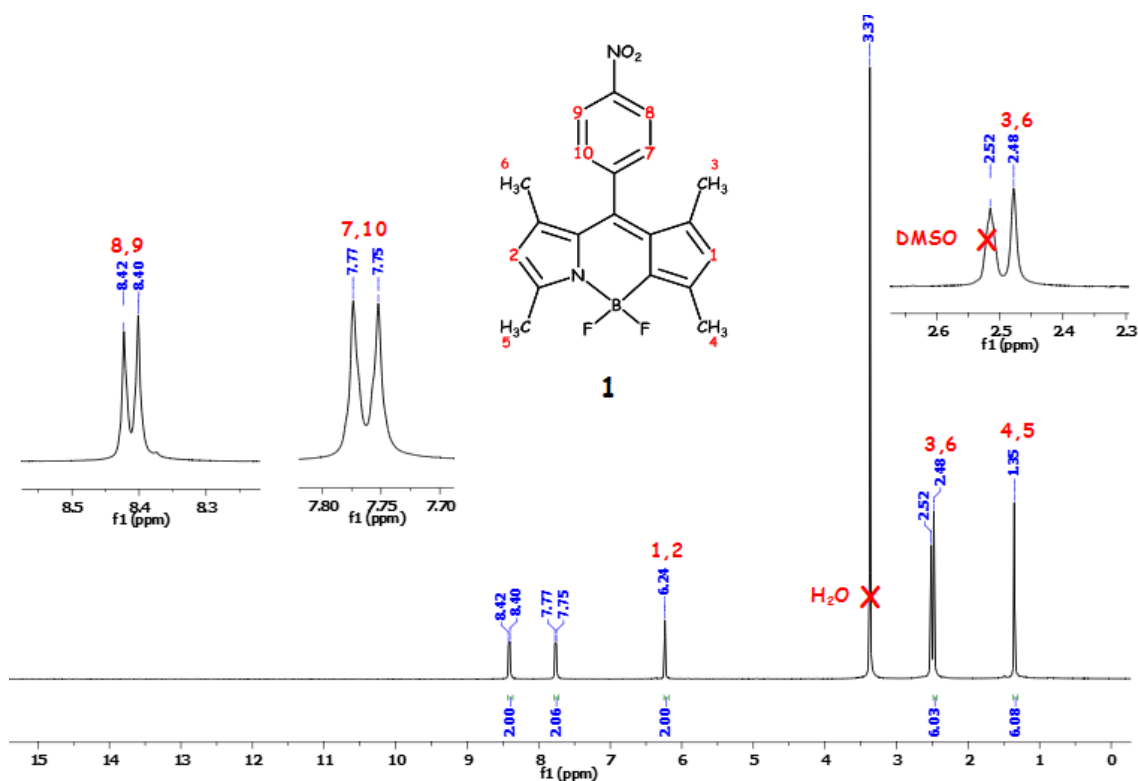


Figure 2.2 The ^1H NMR spectrum of compound **BODIPY 1** at RT.

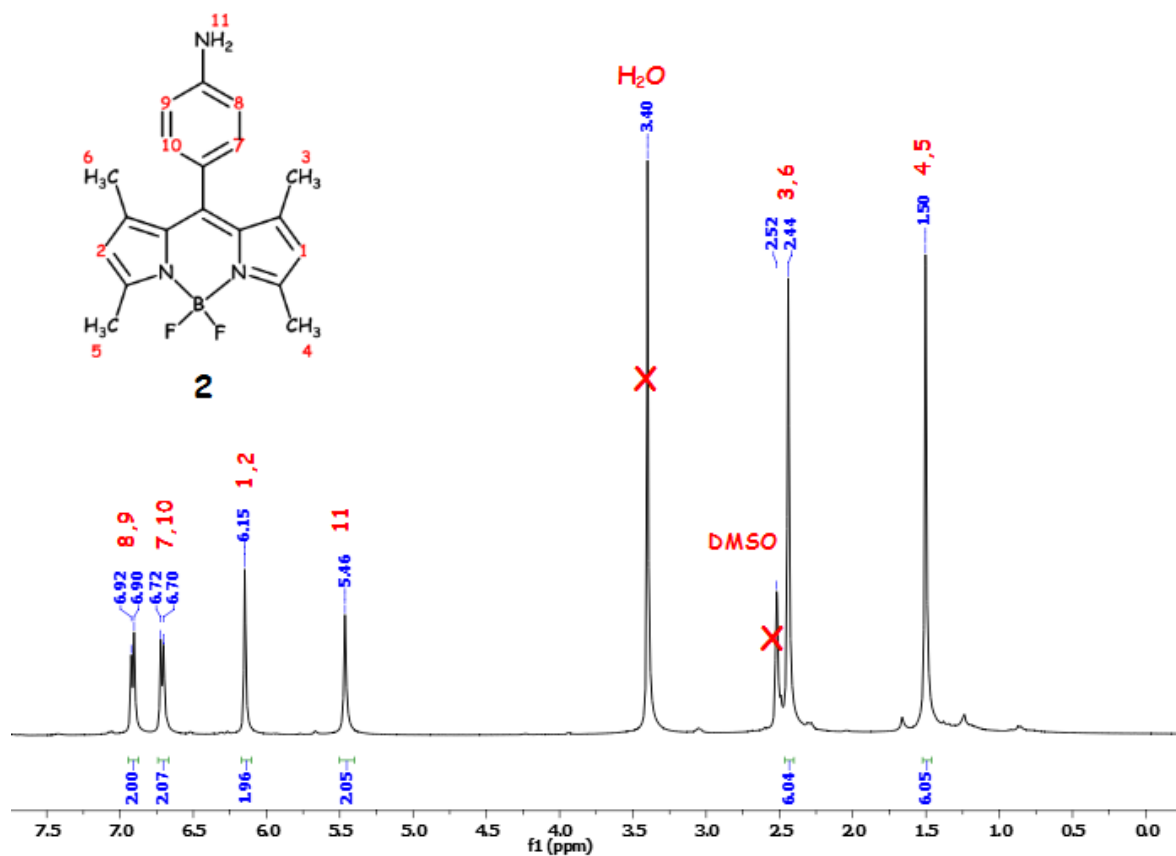


Figure 2.3 The ^1H NMR spectrum of compound **BODIPY 2** at RT.

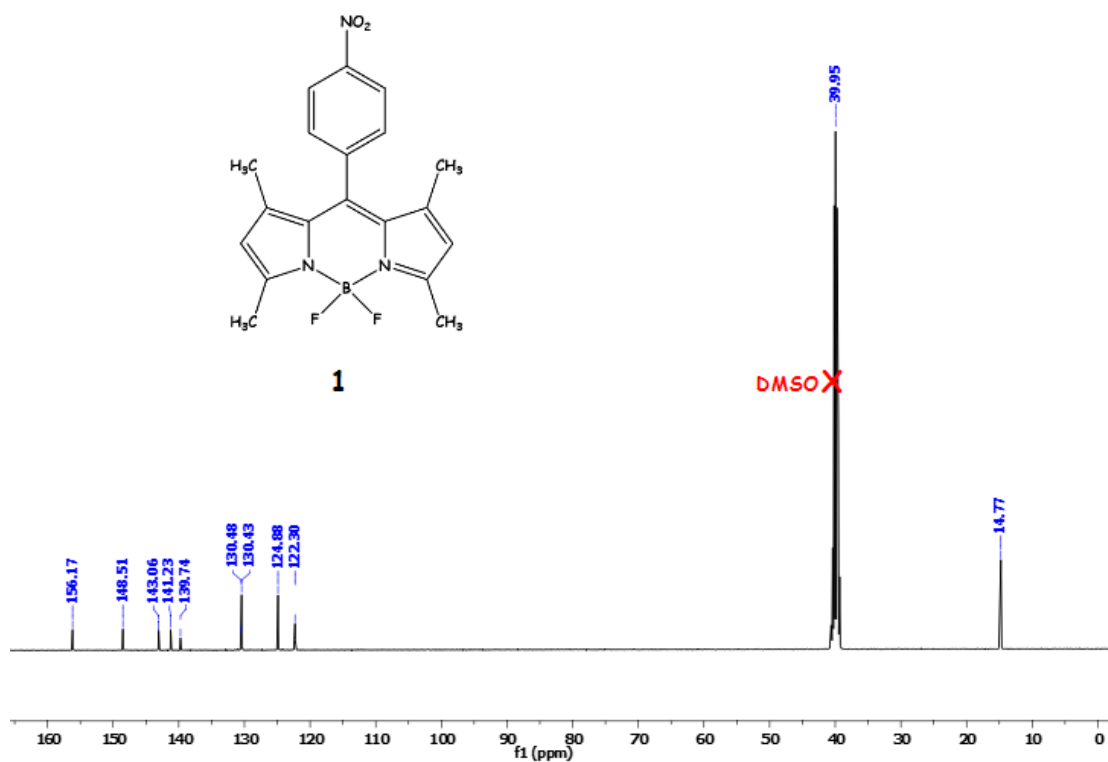


Figure 2.4 The ^{13}C NMR spectrum of compound **BODIPY 1** at RT.

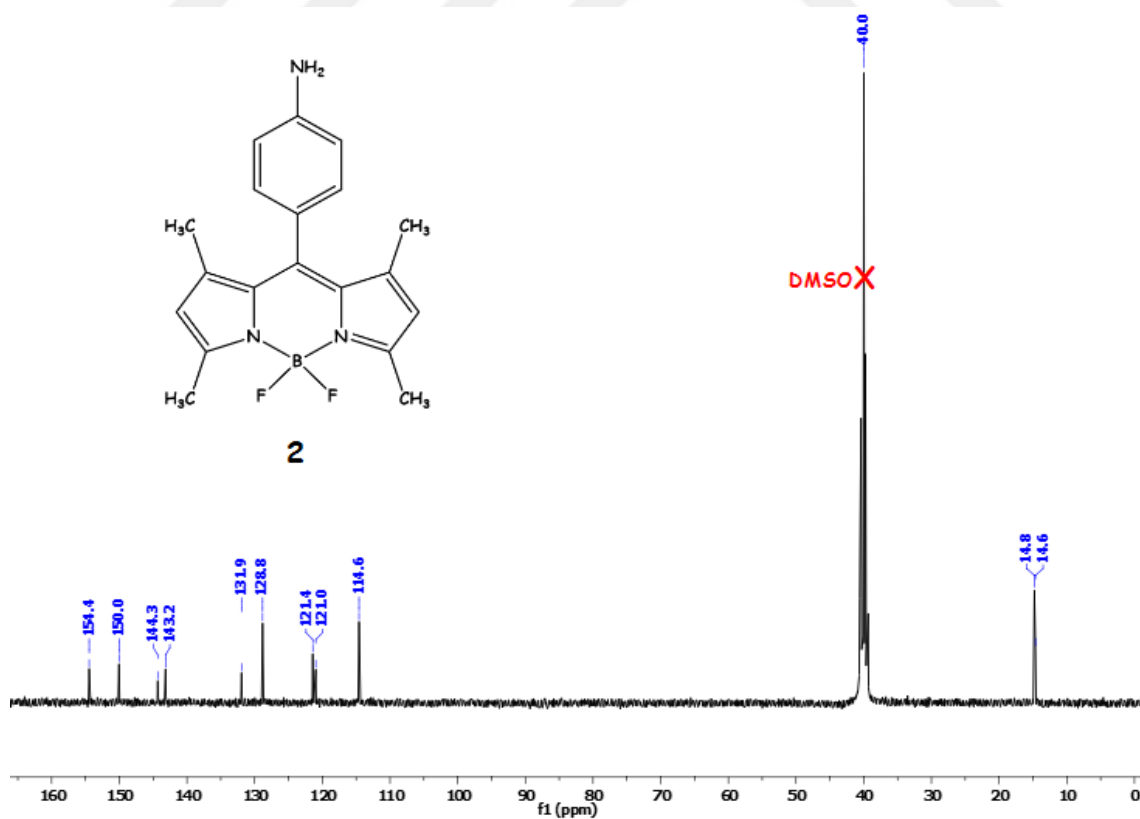


Figure 2.5 The ^{13}C NMR spectrum of compound **BODIPY 2** at RT.

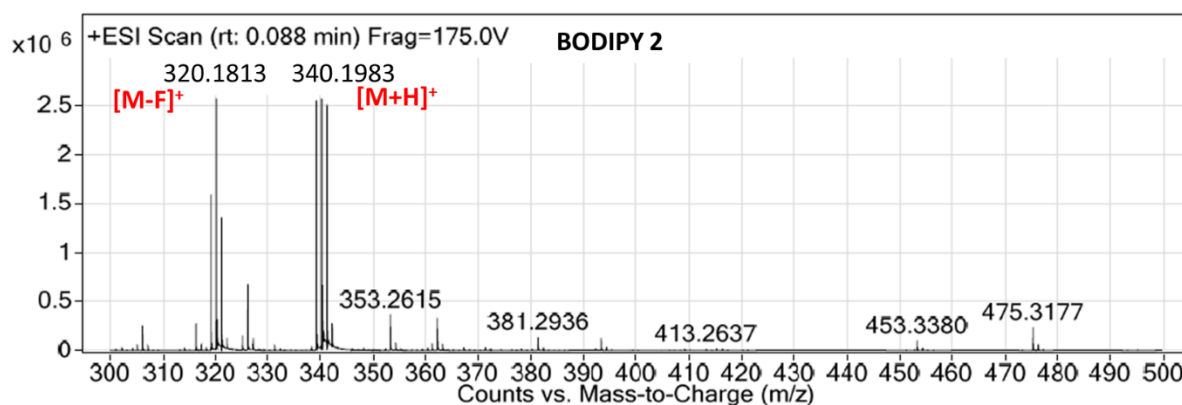


Figure 2.6 ESI-Q-TOF mass spectrum of **BODIPY 2**

The ^1H NMR spectra of **BODIPY-Bg1** was measured in $\text{DMSO-}d_6$. The spectral data are presented in the experimental section and spectra are given **Figure 2.6**. Compared to the spectrum of **BODIPY 2** (see **Figure 2.3**), in the spectrum of **BODIPY-Bg1**, several new proton signals appeared (**Figure 2.7**). Also, the amino group proton signals observed at 5.46 ppm disappeared in the spectrum of **BODIPY-Bg1**. These observations support the formation of desired **BODIPY-Bg1** compound. In the spectrum of **BODIPY-Bg1** compound, the aliphatic protons appeared as singlet signals at 2.37 and 1.71 ppm. The pyrrolic protons were observed at 6.37 ppm as a singlet signal in the spectrum of **BODIPY-Bg1**. In the spectrum of **BODIPY-Bg1**, doublet signal observed at 6.77 was assigned to the *meso*-aryl group protons. The *meso*-aryl -NH proton was observed as a broad singlet signal at 11.79 ppm. The singlet signal observed at 10.51 ppm was assigned to the -NH group proton of the dicyandiamide unit. The proton signals observed between 7.13 and 8.27 ppm in the ^1H NMR spectrum of **BODIPY-bg1** could not be fully characterized, which may be due to free rotation of the *meso*-phenyl group giving rise to rotamers in solution. The ^{13}C NMR spectrum of **BODIPY-Bg1** compound was measured in $\text{DMSO-}d_6$. The spectral data are presented in the experimental section and spectrum is given in **Figure 2.8**. Compared to the spectrum of **BODIPY 2** (see **Figure 2.4**), in the spectrum of **BODIPY-Bg1**, several new carbon signals appeared. The aliphatic carbon signals appeared 13.97 and 13.81 ppm. The other carbon atom signals were observed in the range of 114-159 ppm. The chemical shifts observed in the ^{13}C NMR spectra are in accordance with the chemical structures of **BODIPY-Bg1** compound. Also, ESI-Q-TOF mass spectrum of **BODIPY-Bg1** was measured in methanol. In the ESI-Q-TOF mass spectrum of **BODIPY-Bg1**, we observed a molecular ion peak at m/z 398.2717 (**Figure 2.9**).

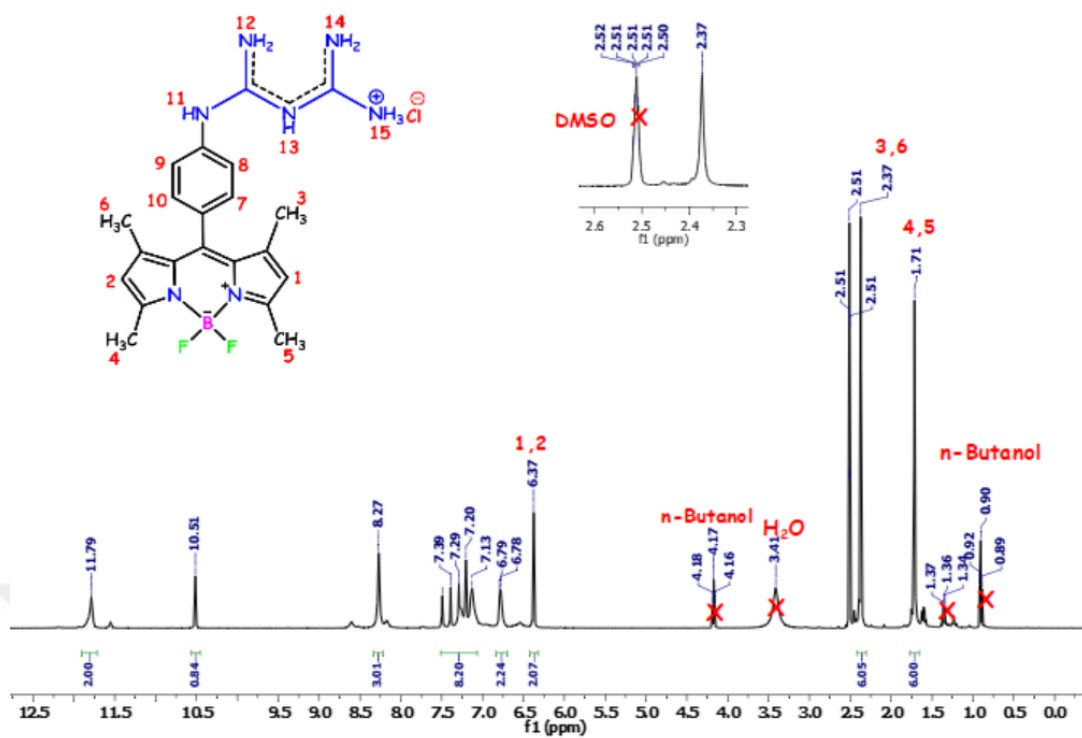


Figure 2.7 ¹H spectrum of BODIPY-Bg1

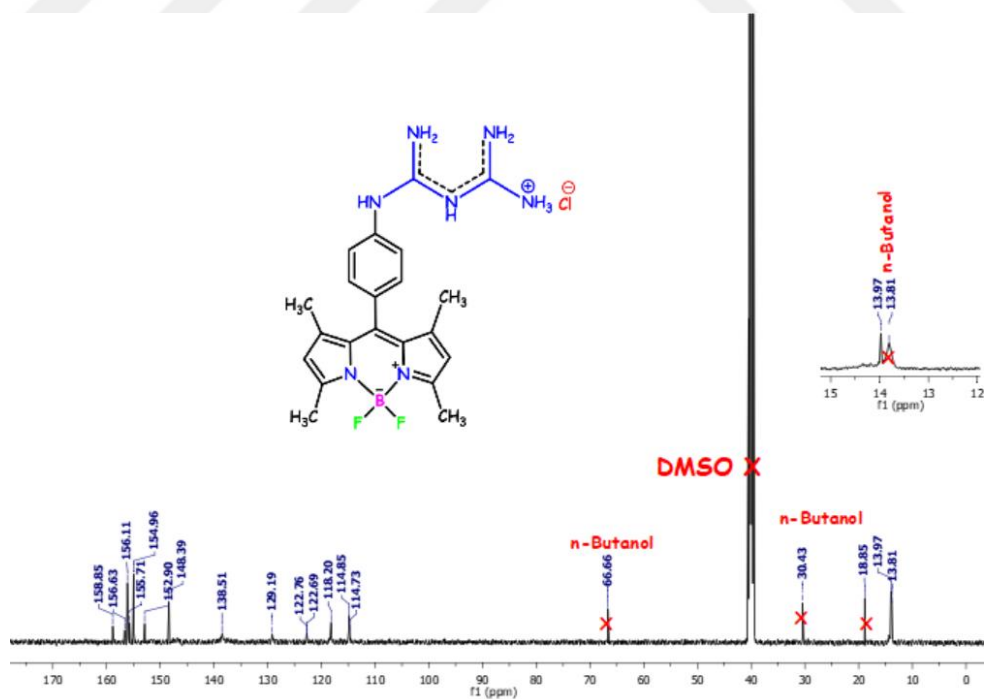


Figure 2.8 ¹³C NMR spectrum of BODIPY-Bg1

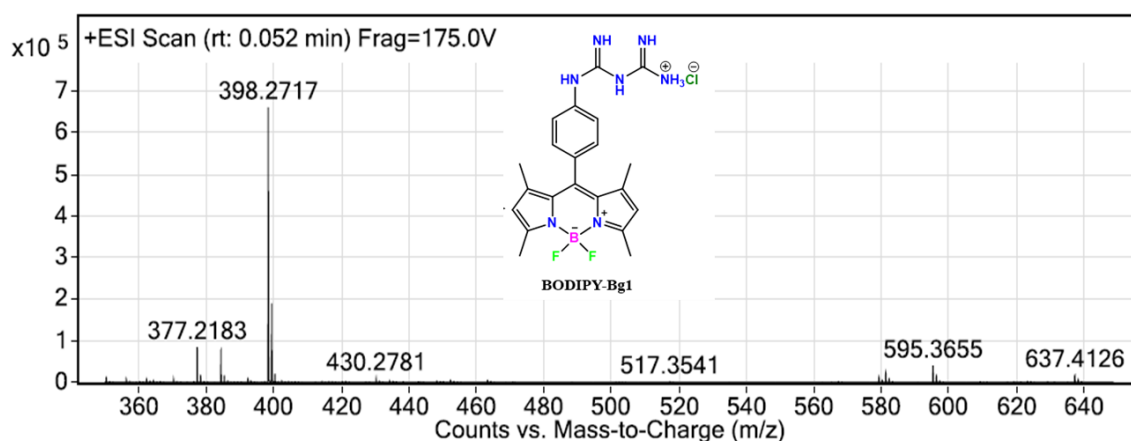


Figure 2.9 ESI-Q-TOF mass spectrum of **BODIPY-Bg1**

The ^1H NMR spectra of BODIPY-imine compounds (**BODIPY-5a-i**) except **BODIPY-5g** (CDCl_3) were measured in $\text{DMSO}-d_6$. The spectral data are presented in the experimental section and spectra are given in **Figure A. 1-8**. Compared to the spectrum of **BODIPY 2** (see **Figure 2.3**), in the spectrum of BODIPY-imine compounds several new proton signals appeared. Also, the amino group proton signals observed at 5.46 ppm disappeared in the spectrum of BODIPY-imine compounds. All these observations demonstrated the formation of desired BODIPY-imine compounds.

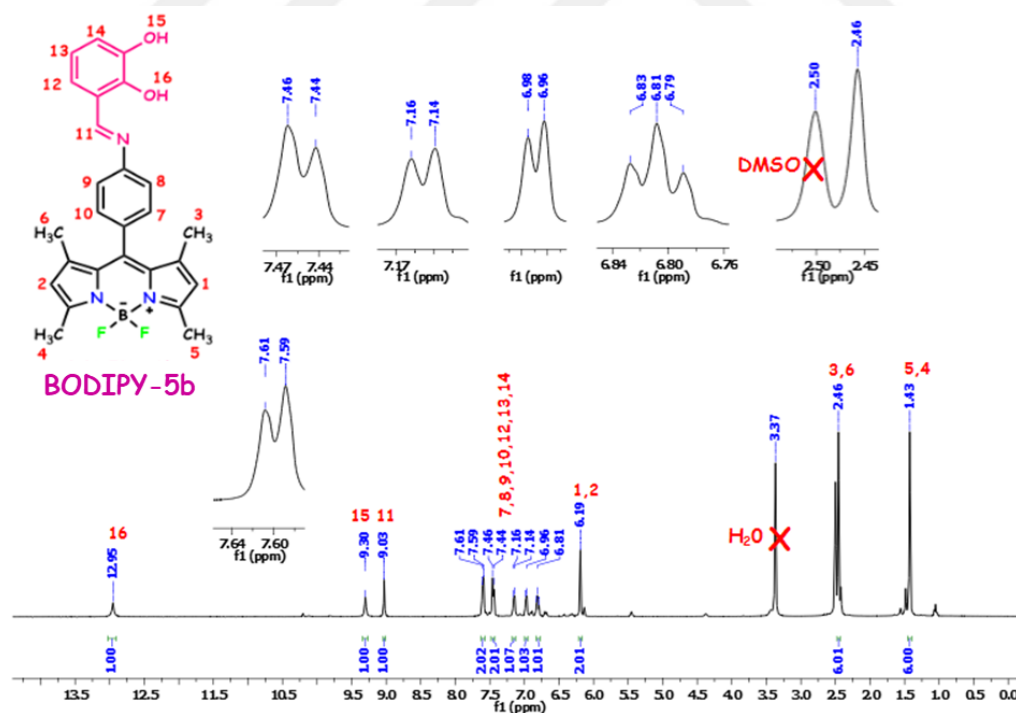


Figure 2.10 ^1H NMR spectrum of **BODIPY-5b**

In the ^1H spectrum of **BODIPY-5b**, -OH group protons were observed at 12.95 and 9.30 ppm as singlet signals (**Figure 2.10**). The imine group proton appeared as a singlet signal at 9.03 ppm. In the spectrum of **BODIPY-5b**, the aryl groups protons were observed between 7.60-

6.81 ppm. The pyrrolic protons appeared at 6.19 ppm as a singlet signal in the spectrum of **BODIPY-5b**. The aliphatic protons of **BODIPY-5b** were observed at 2.46 and 1.43 ppm as singlet signals.

The ^{13}C NMR spectra of BODIPY-imine compounds (BODIPY-5a-i) except BODIPY-5g (CDCl_3) were measured in $\text{DMSO}-d_6$. The spectral data are presented in the experimental section and spectrum is given in **Figure A.9-15**. Furthermore, ESI-Q-TOF mass spectrum of BODIPY-imine compounds were measured in methanol. BODIPY-imine compounds show the $[\text{M}+\text{H}]^+$ molecular ions peaks. The spectral data are presented in the experimental section and spectra are given in **Figure A. 16-23**.

FTIR spectra of **BODIPY 1**, **BODIPY 2** and **BODIPY-imine** compounds were recorded in the $4000\text{--}400\text{ cm}^{-1}$ region. The FTIR spectrum of **BODIPY 1** shows stretching peaks around 3096 cm^{-1} assigned to the aromatic C-H stretching's (**Figure 2.11**). The aliphatic C-H vibrations of **BODIPY 2** were observed in the range of $2855\text{--}2959\text{ cm}^{-1}$. The new peaks were observed $3479\text{--}3386\text{ cm}^{-1}$ in the FT-IR spectrum of **BODIPY 2** and they were assigned to the N-H stretching resulting from the reduction of the nitro group to amino(Cui et al., 2014) group (**Figure 2.11**) (Berendsen et al., -b).

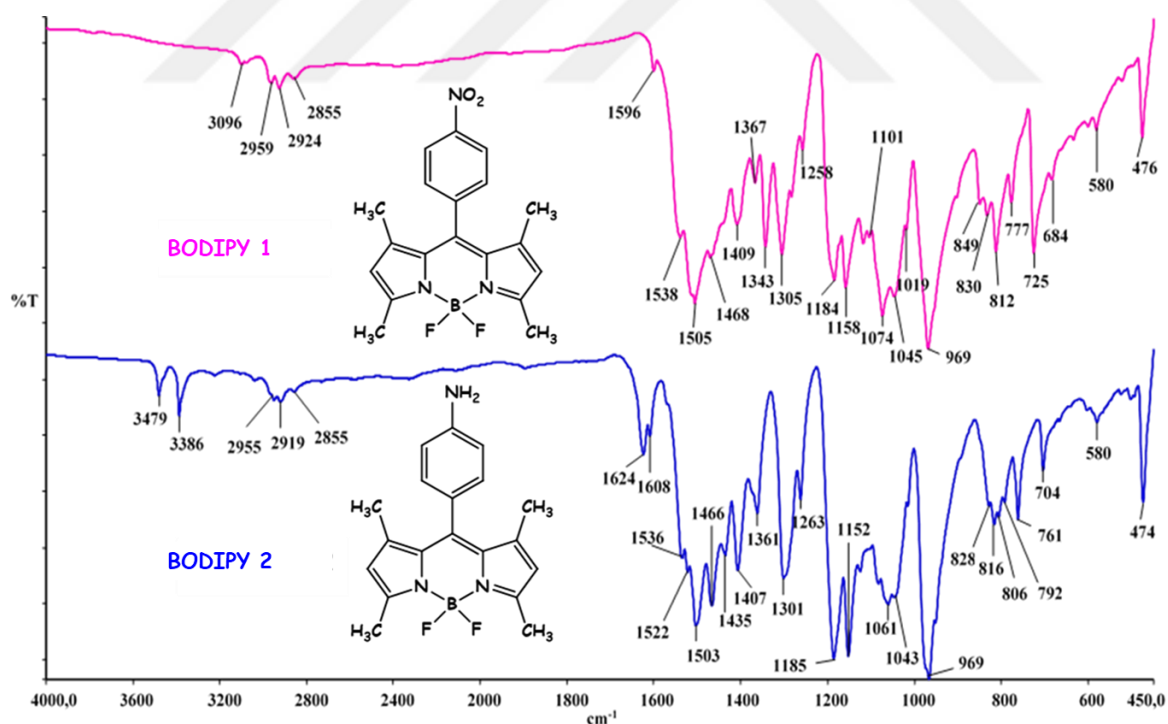


Figure 2.11 FT-IR spectra of BODIPY 1 and 2.

Generally, there is intramolecular hydrogen bonding exist in a six-membered ring system, the O-H stretching band is observed $3550\text{--}3200\text{ cm}^{-1}$ region (Dabbagh, Teimouri, Najafi Chermahini, & Shahraki, 2008; Pan, Zhou, & Luo, 2014a). In the FT-IR spectrum of

BODIPY-5b, a sharp peak was observed at 3466 cm^{-1} due to the O-H vibrations. Imine (C=N) bond stretching vibration was observed at 1619 cm^{-1} in the spectrum of **BODIPY-5b** (Figure 2.12). The spectral data of other BODIPY-imine compounds are presented in the experimental section and spectra are given in Figure A. 24-29.

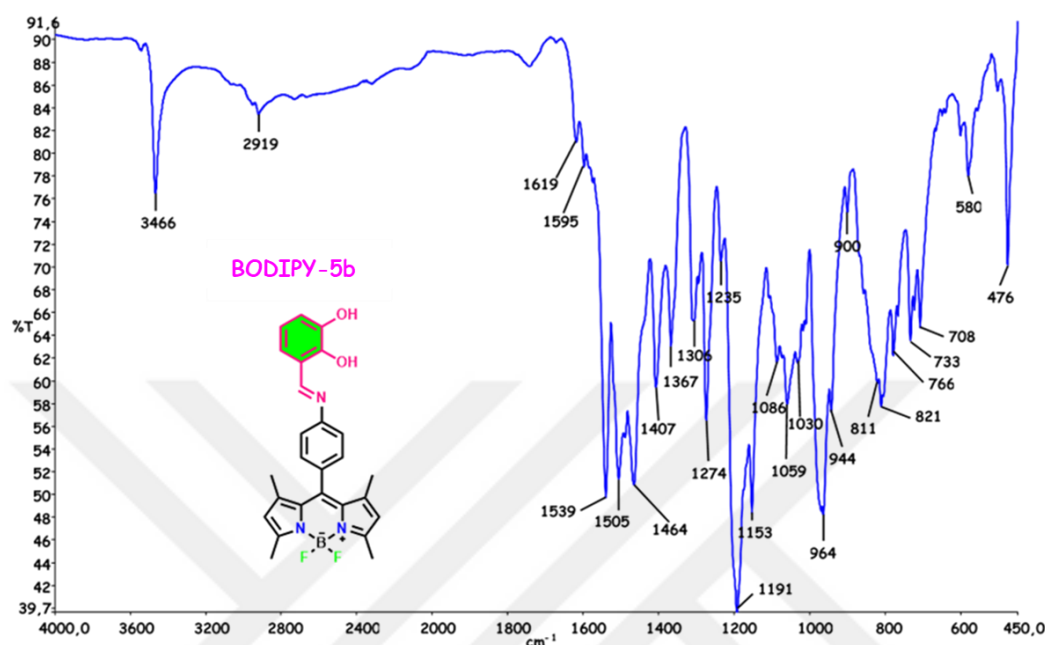


Figure 2.12 FT-IR spectrum of BODIPY-5b

Synthesized BODIPY compound are soluble in common solvent such as DCM, CHCl_3 , CH_2Cl_2 , THF, CH_3CN and DMSO. Furthermore, **BODIPY-Bg1** compound is soluble in water.

2.1 Photophysical characterisation of the synthesized compounds

Starting compounds **BODIPY 1** and **BODIPY 2** already have been synthesised in literature. We recorded the UV-vis. absorption and emission spectra of **BODIPY 2** and the other target compounds to observe any possible changes in the absorption and emission bands.

2.1.1 BODIPY-Bg1

The UV-vis. absorption and emission spectra of **BODIPY 2** were measured in different solvents (ACN, DCM, DMSO) (Figure 2.13). The electronic absorption maxima of BODIPY 2 are centred at 496, 501 and 502 nm (in ACN, DCM and DMSO respectively) assigned to the $S_0 \rightarrow S_1$ ($\pi-\pi^*$) electronic transition localized on the BODIPY core. In the absorption spectrum of BODIPY 2, broadband centred at 321 nm was observed in the high energy region, and it

was assigned to S_0-S_2 ($\pi - \pi^*$) electronic transition. BODIPY 2 have high molar absorption coefficients with 4.92 log ϵ value in DMSO.

BODIPY-Bg1 exhibits a broad band in the range of 300-600 nm and absorption maxima slightly blue shifted at 491, 491 and 498 nm (in ACN, DCM and DMSO respectively) compared to compound **BODIPY 2** (**Figure 2.14**). Furthermore, the S_0-S_2 ($\pi - \pi^*$) electronic transition band almost disappeared in the absorption spectra of **BODIPY-Bg1** compound. **BODIPY-Bg1** compound is soluble in water, and it show a absorption maximum at 491 nm in water with high extinction coefficient value (log $\epsilon_{\max}$ = 4.82) as shown in **Figure 2.15**.

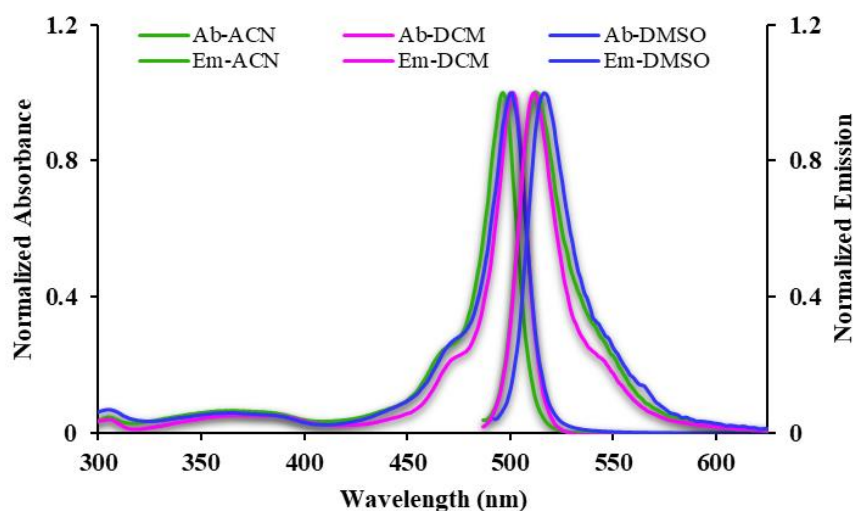


Figure 2.13 Normalized absorption and emission spectra of BODIPY 2 in different solvents (10 μ M)

The emission spectrum of **BODIPY 2** showed a maximum intensity at 496, 500 and 501 nm in ACN, DCM and DMSO, respectively, with small Stokes Shifts. **BODIPY-Bg1** compound showed a very weak emission, almost it is not fluorescent, when excited at 490 nm (**Figure 2.16**). Also, the emission band of **BODIPY-Bg1** was redshifted compared to **BODIPY 2** compound. **BODIPY-Bg1** showed bigger Stokes shifts than **BODIPY 2** compound. The absorption and emission spectra of **BODIPY-Bg1** show that the attachment of the biguanide fragment to the *meso*-position of BODIPY resulted in some changes in its optical properties. The spectroscopic data of **BODIPY 2** and **BODIPY-Bg1** are summarized in **Table 2.1** and **Table 2.2**.

The absorption and emission bands of the compounds **BODIPY 2** and **BODIPY-Bg1** were slightly affected by the solvent polarity, the absorption and emission maxima red-shifted as the solvent polarity increased. This red shift is consistent with the general behaviour of BODIPY dyes (Kee et al., 2005; Kollmannsberger, Rurack, Resch-Genger, & Daub, 1998;

Qin, Baruah, Van der Auweraer, De Schryver, & Boens, 2005) and represent the polarizability of the solvent (Benniston, Clift, & Harriman, 2011).

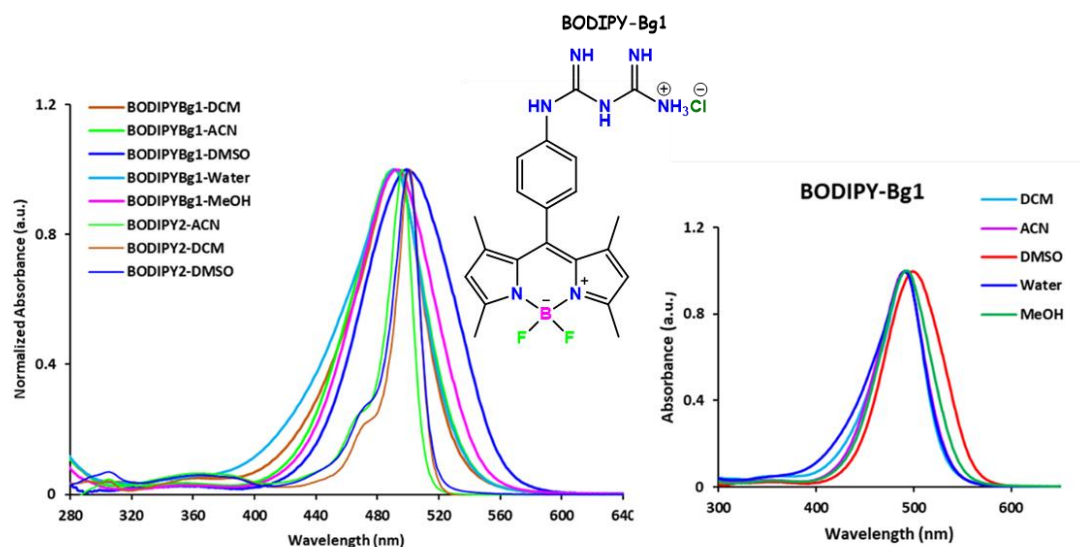


Figure 2.14 Normalized absorption spectra of BODIPY-Bg1 in different solvents (10 μ M)

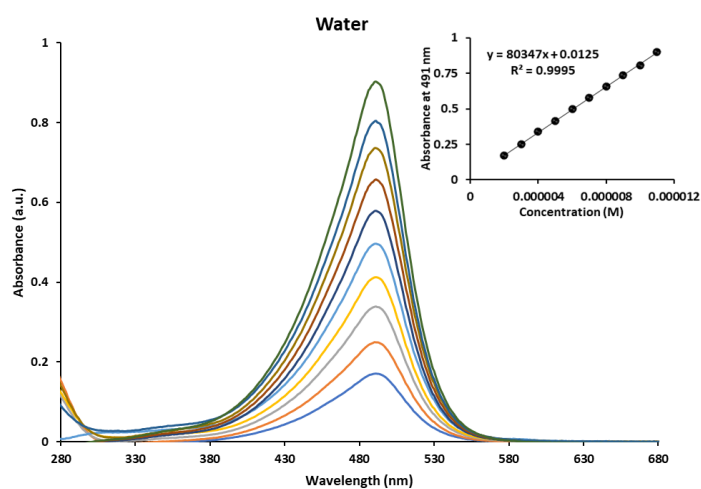


Figure 2.15 UV/Visible spectra of BODIPY-Bg1, upon increasing concentration in Water (0 – 11 μ M). Inset: Plot of absorbance at 491 nm as a function of increasing concentration of BODIPY-Bg1

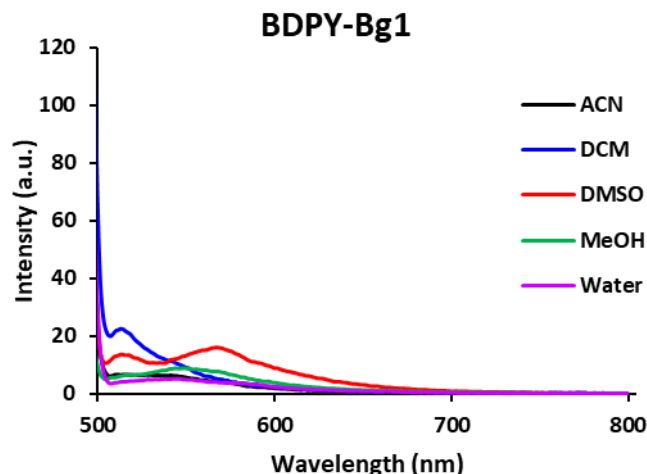


Figure 2.16 Emission spectra of BODIPY-Bg1 in different solvent (10 μ M).

Table 2.1 The spectroscopic data of BODIPY 2 in various solvents

BODIPY 2				
Solvent	λ_{abs} max(nm)	λ_{em} max(nm)	Stokes Shift (nm)	ΦF
ACN	496	512	16	0.004 (Pan,
DCM	501	512	11	0.30 (Pan,
DMSO	500	517	17	-

Table 2.2 The spectroscopic data of BODIPY-Bg1 in various solvents

BODIPY-Bg1				
Solvent	λ_{abs} max(nm)	λ_{em} max(nm)	Stokes Shift (nm)	$\log \epsilon_{\text{max}}$
ACN	491	542	51	-
DCM	491	512	21	-
DMSO	498	567	69	-
Water	491	540	49	4.82
MeOH	492	548	56	-

2.1.2 BODIPY-imine compounds (BODIPY-5a-5i)

The UV-vis. absorption and emission spectra of **BODIPY 2** were measured in different solvents (ACN, DCM, DMSO). We calculated the fluorescence quantum yield of the compounds in ACN and DCM solvent. BODIPY-imine compounds have high molar extinction coefficients ($\log \epsilon_{\text{max}}$) in DMSO (see **Table 2.3**, **Figure A.30-36**). Also, the fluorescence lifetime (τ_{Fl}) of BODIPY-imine compounds were determined using Time-Correlated Single Photon Counting (TCSPC) technique (In DMSO solution, 10 μ M). The spectroscopic data of BODIPY-imine compound are summarized in **Table 2.3**. Normalized

absorption and emission spectra of the compounds are shown in **Figure 2.17-2.23**, respectively. BODIPY-imine compounds, exhibit an intense absorption band centred in the range of at 498-503 nm which is due to the $S_0 - S_1 (\pi-\pi^*)$ electronic transition localized on the BODIPY core. In the absorption spectra of the compounds, a broadband centred at about 350 nm was observed in the high energy region, and it was assigned to $S_0-S_2 (\pi - \pi^*)$ electronic transition. The fluorescence emission spectrum of BODIPY-imine compounds shows a maximum intensity at 512-520 nm range with small Stokes shifts. The absorption and emission spectra of BODIPY-imine compounds are like that of most BODIPY fluorescent dyes. In comparison to **BODIPY 2**, remarkable changes were not observed in the absorption and emission spectrum of the compounds. This suggested the attachment of the imine group fragment to the *meso*-position of BODIPY core resulted in minimal changes in its optical properties. However, an increase in fluorescence quantum yields of BODIPY-imine compounds was observed when compared to **BODIPY 2** compound. It was reported that **BODIPY 2** exhibits quenched emission in ACN with $\Phi_f < 1\%$ (Pan, Luo, et al., 2014b) and this quenching phenomenon has all the hallmarks of photoinduced electron transfer (PET) from the phenylamino unit to the BODIPY core. Upon condensation of **BODIPY 2** with aldehyde derivatives to form BODIPY-imine compounds, the reduction potential of the nonbonding electron pair on the nitrogen atom decreased due to the formation of an imine functional group. In other words, electron transfer from the electron-deficient imine moiety to the electron-rich BODIPY fluorophore became less feasible. Thereby preventing PET quenching of the emission, resulting in the fluorescence quantum yield varying between 0.017 and 0.33 in BODIPY-imine compounds (**Table 2.3**). The fluorescence lifetimes of BODIPY-imine compounds were determined in ns region. This case consistent with the general behaviour of BODIPY dyes, they tend to be in the sub ns region because the radiative rate constants do not vary greatly (Benniston & Copley, 2009). The fluorescence decay curves of BODIPY-imine compounds are presented in **Figure A. 37-43**.

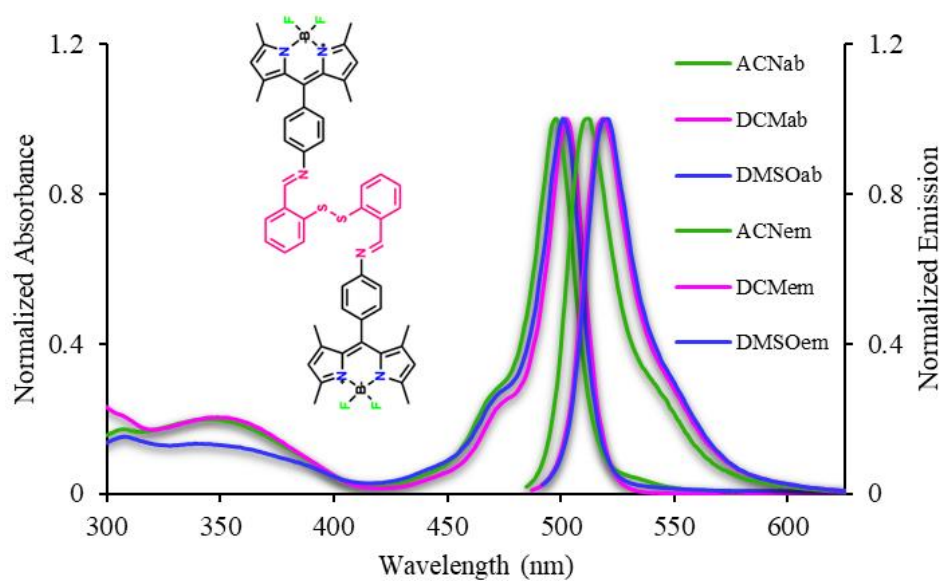


Figure 2.17 Normalized absorption and emission spectra of **BODIPY-5a**

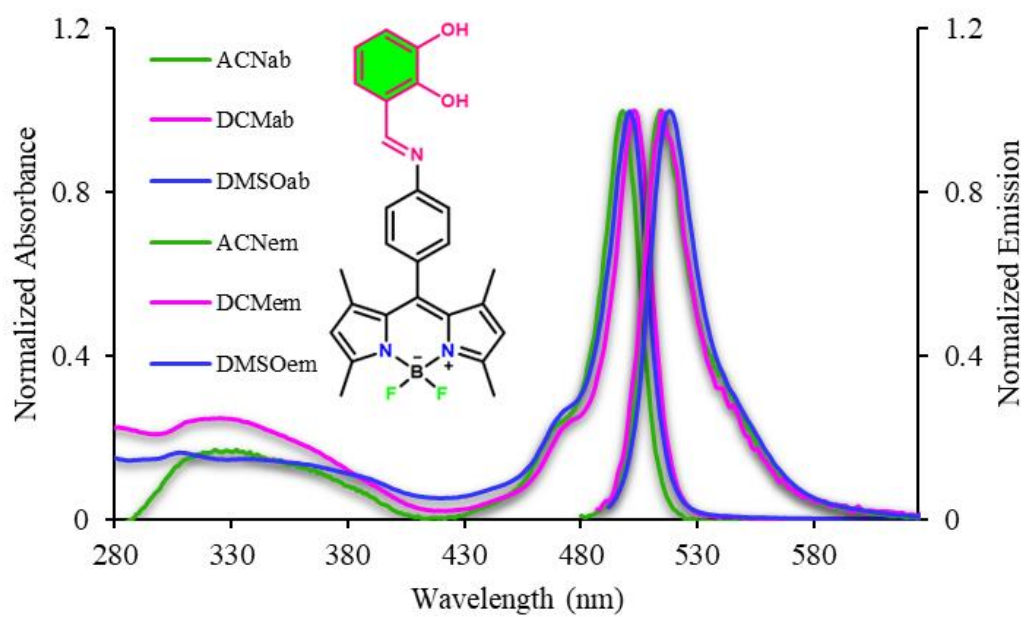


Figure 2.18 Normalized absorption and emission spectra of **BODIPY-5b**

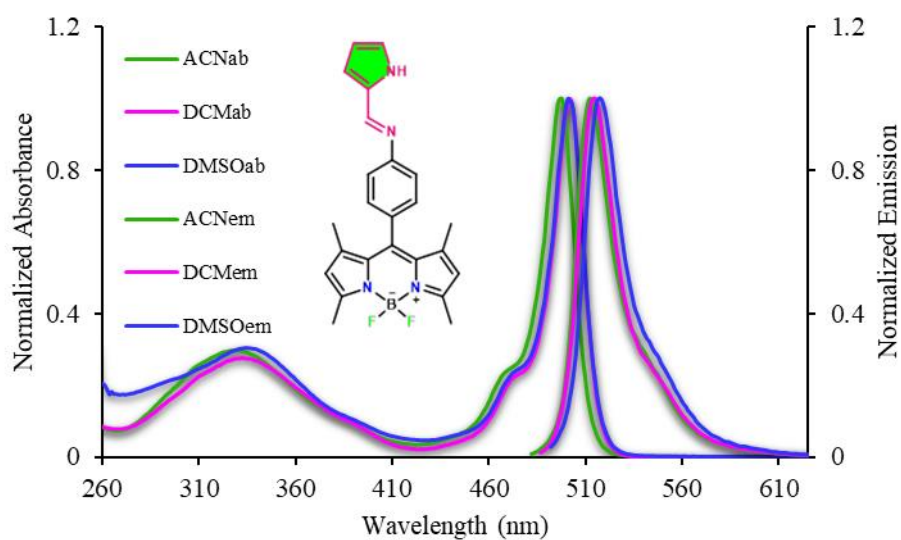


Figure 2.19 Normalized absorption and emission spectra of **BODIPY-5d**

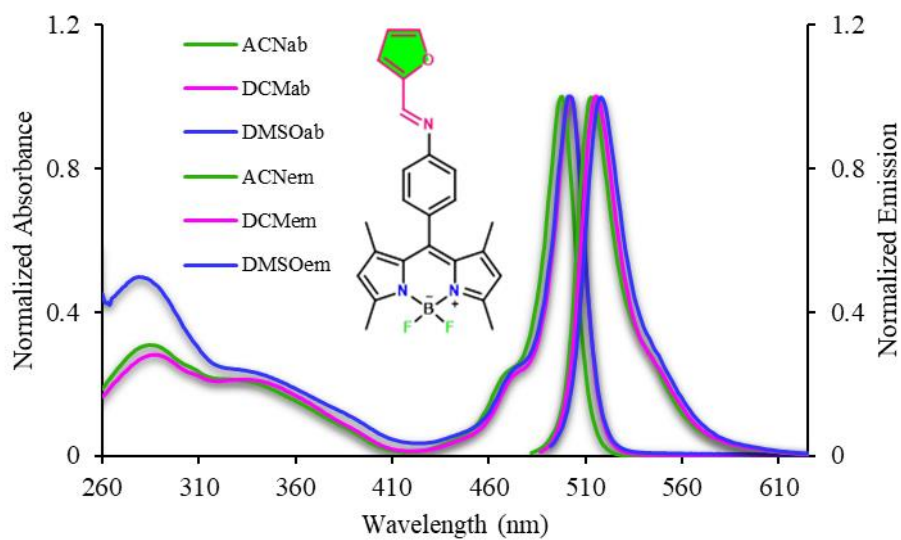


Figure 2.20 Normalized absorption and emission spectra of **BODIPY-5e**

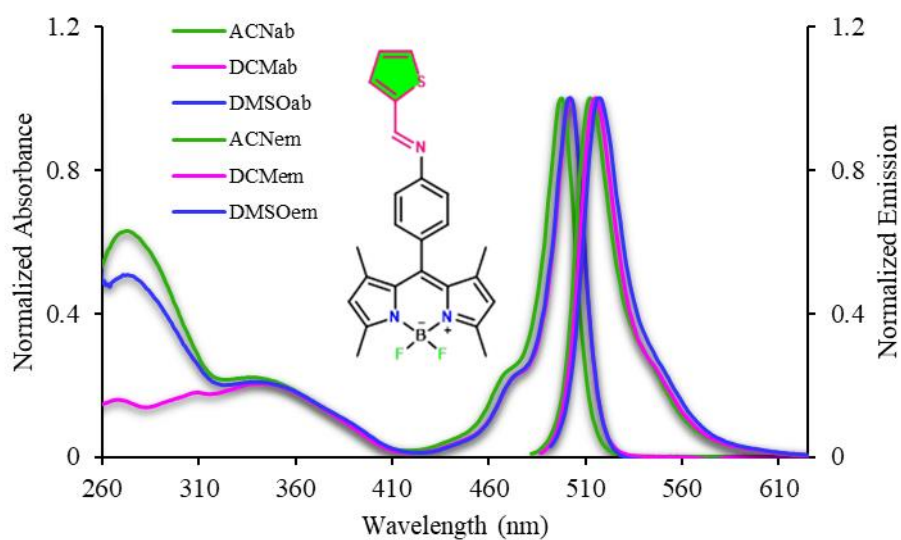


Figure 2.21 Normalized absorption and emission spectra of **BODIPY-5f**

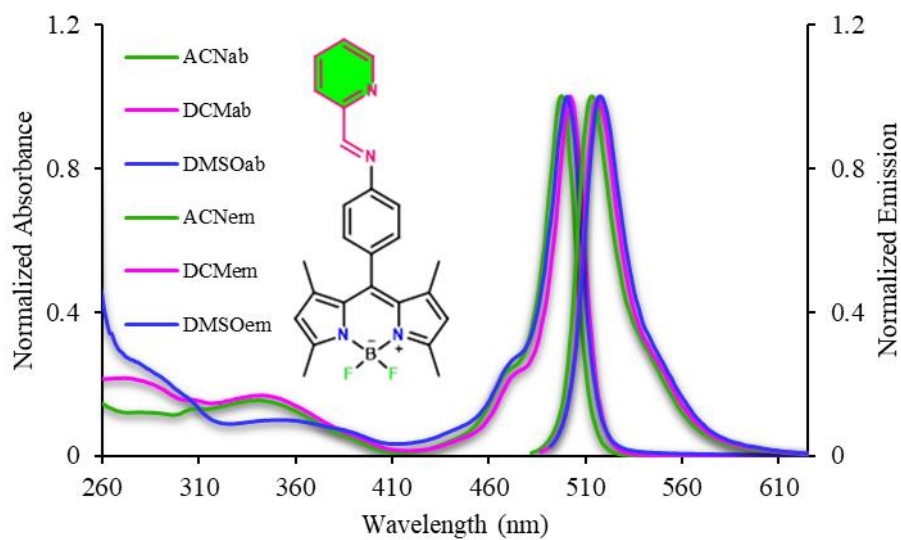


Figure 2.22 Normalized absorption and emission spectra of **BODIPY-5g**

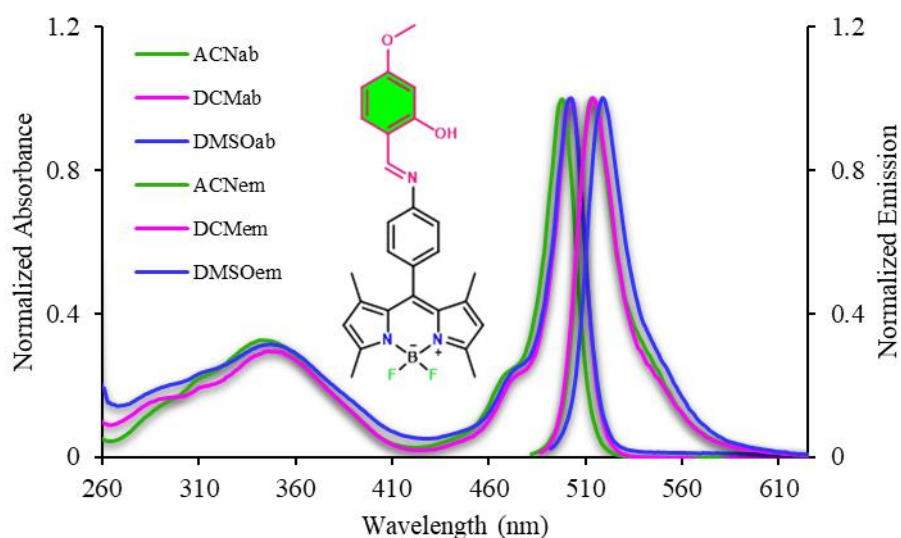


Figure 2.23 Normalized absorption and emission spectra of **BODIPY-5i**

Table 2.3 The spectroscopic data of BODIPY imine compounds

Compound	Solvent	λ_{abs} max(nm)	λ_{em} max(nm)	$\Delta\nu_{max}$ (cm^{-1})	$\log\epsilon_{max}$	Φ_f	τ_{Fl}/ns
<i>BODIPY-5a</i>	DCM	502	518	615	-	0.40	-
	ACN	498	512	549	-	0.25	-
	DMSO	501	520	729	4.93	-	2.60
<i>BODIPY-5b</i>	DCM	501	515	543	-	0.026	-
	ACN	498	514	625	-	0.017	-
	DMSO	501	518	655	4.94	-	4.12
<i>BODIPY-5d</i>	DCM	502	515	503	-	0.046	-
	ACN	497	512	589	-	0.033	-
	DMSO	501	517	618	5.01	-	0.40
<i>BODIPY-5e</i>	DCM	502	516	540	-	0.41	-
	ACN	498	513	587	-	0.30	-
	DMSO	501	518	655	5.06	-	2.84
<i>BODIPY-5f</i>	DCM	502	515	503	-	0.53	-
	ACN	498	512	549	-	0.32	-
	DMSO	502	518	615	4.92	-	2.98
<i>BODIPY-5g</i>	DCM	502	517	578	-	0.46	-
	ACN	498	513	587	-	0.33	-
	DMSO	501	519	692	4.99	-	2.71
<i>BODIPY-5i</i>	DCM	503	514	425	-	0.45	-
	ACN	498	513	587	-	0.22	-
	DMSO	503	517	538	5.04	-	0.75

2.1.3 BODIPY-Sq

The UV-vis. absorption and emission spectra of BODIPY-sq were measured in DCM. BODIPY-Sq exhibits an intense absorption band centred in the range of at 503 nm which is due to the $S_0 - S_1$ ($\pi-\pi^*$) electronic transition localized on the BODIPY core. (**Figure 2.24**). BODIPY-Sq compound have a 4.82 $\log \epsilon_{\max}$ in DMSO/PBS solven mixture. The fluorescence emission spectrum of BODIPY-Sq shows a maximum intensity at 517 nm with a small Stokes shift (15 nm). The absorption and emission spectra of BODIPY-Sq compounds are like that of most BODIPY fluorescent dyes. In comparison to **BODIPY 2**, remarkable changes were not observed in the absorption and emission spectrum of the compounds. This suggested the attachment of the diethyl squarate unit to the *meso*-position of BODIPY resulted in minimal changes in its optical properties. Moreover, Φ_F of **BODIPY-Sq** was calculated 0.58 in DCM.

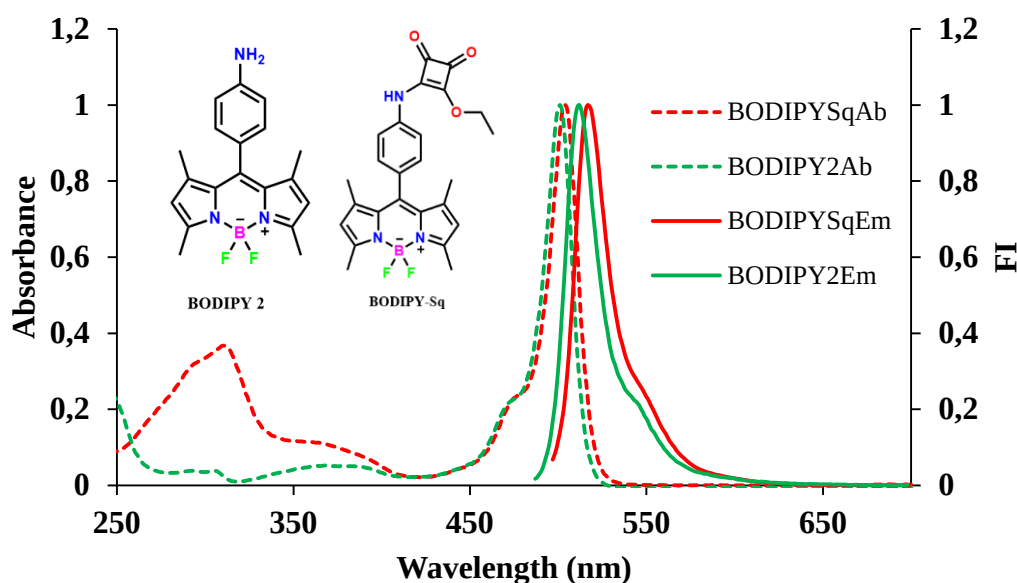


Figure 2.24 Normalized absorption and emission spectra of **BODIPY 2** and **BODIPY-Sq** in DCM (10 μ M)

2.2 Fluorescence sensor properties of synthesised compounds for A β aggregates

We studied the molecular interactions of synthesized BODIPY-based compounds in the absence and presence of A β_{42} aggregates through the fluorescence titrations in DMSO/PBS buffer (10 mM, pH = 7.4). As shown in **Figure 2.26** BODIPY-Bg1 (8 μ M) exhibited very weak fluorescence in PBS (pH 7.4). First, we wondered whether the emission band of **BODIPY-Bg1** was affected or not at different pH values. So, emission spectra of the compound at pH 6 and pH 8 were recorded. However, no obvious changes were observed in emission spectra with these pH values (biological area: pH: 6-8) (**Figure 2.25**).

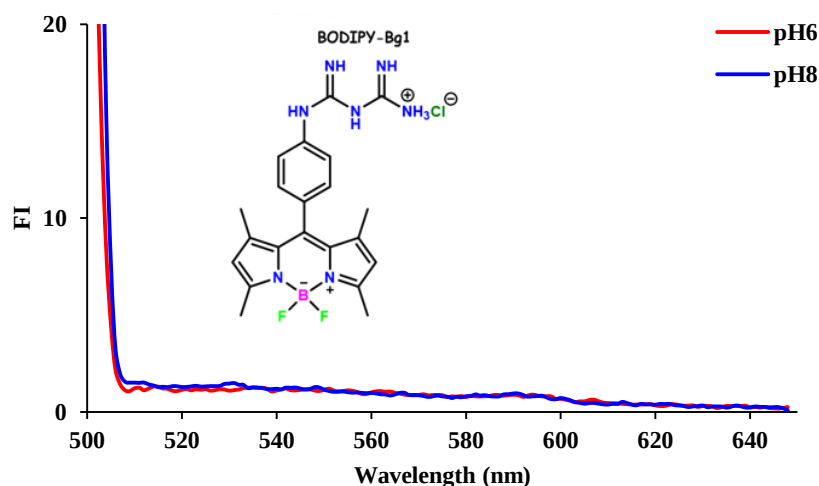


Figure 2.25 The emission spectra of BODIPY-Bg1 at pH 6 and pH 8 (Final concentration: 5 μ M, λ_{Ex} : 495 nm, Slit width: Ex/Em: 5/10 nm)

When $A\beta_{1-42}$ aggregates (final concentration: 4 μ M) were added to the **BODIPY-Bg1** solution, unfortunately, we observed little increase in fluorescence. Moreover, **BODIPY-Bg1** incubated with BSA (10 μ M), the emission band of BODIPY-Bg1 disappeared indicating **BODIPY-Bg1** may interact with BSA. The fluorescence intensity changes of **BODIPY-Bg1** upon incubation with $A\beta_{42}$ and BSA are given in **Figure 2.26**.

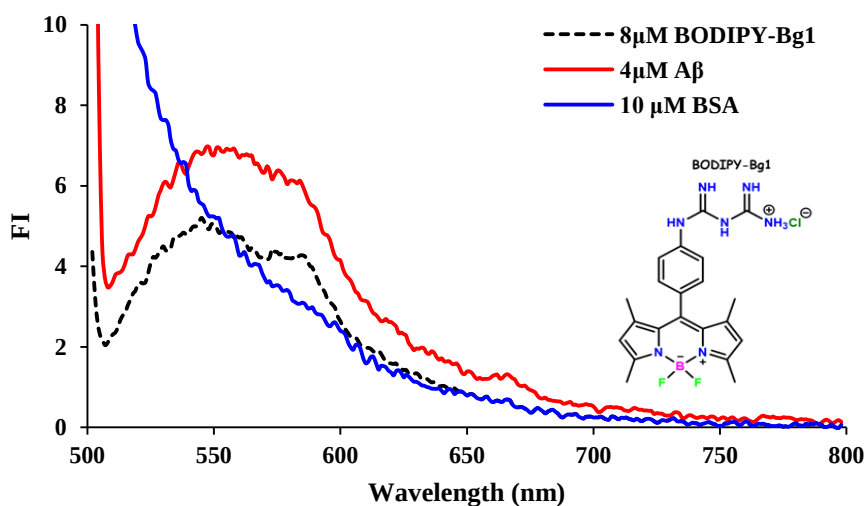


Figure 2.26 Fluorescence intensity of **BODIPY-Bg1** upon interaction with $A\beta_{1-42}$ aggregates (4 μ M) and BSA (10 μ M) in PBS (10 mM, pH: 7.4). (λ_{Ex} : 495 nm, Slit width: Ex/Em: 5/10 nm)

Interaction with BODIPY-5a were studied DMSO: PBS (v/v: 7/3, 10 mM; pH: 7.4). The absorption band of BODIPY-5a at 500 nm followed the Lambert-Beer law (**Figure 2.27**), indicating that BODIPY-5a compound is not significantly aggregated in this solvent system.

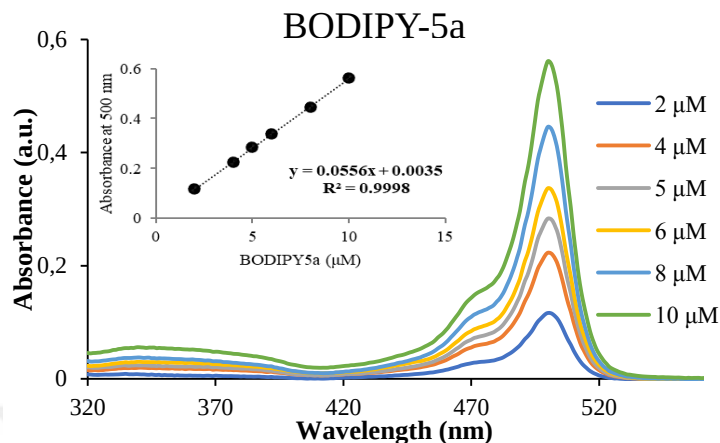


Figure 2.27 Electronic absorption spectra of BODIPY-5a in DMSO/PBS (7:3 v/v) at different concentrations. Inset plot is the absorbance at 500 nm vs. the concentration of BODIPY-5a.

When we incubated to **BODIPY-5a** with A β ₁₋₄₂ aggregates (Final concentration are 2, 4 and 6 μ M), its emission band quenched. On the other hand, no significant change was observed when BODIPY-5a incubated with BSA indicating **BODIPY-5a** could bind to A β aggregates selectively (**Figure 2.28**). Next, increasing concentration of BODIPY-5a (0–4.94 μ M) was titrated against a fixed concentration of A β ₁₋₄₂ aggregates (2 μ M) and fluorescence intensity at 515 nm was recorded. Moreover, we recorded emission spectra of **BODIPY-5a** in the absence of A β ₁₋₄₂ aggregates in the same concentration as a blank. (**Figure 2.39**)

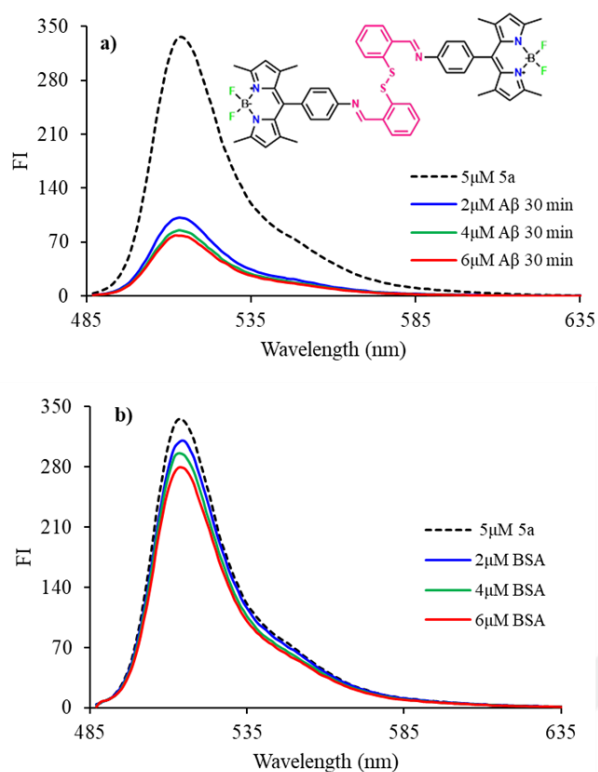


Figure 2.28 Fluorescence intensity of **BODIPY-5a** upon interaction with different concentrations **a)** Aβ₁₋₄₂ aggregates (final concentration: 2, 4 and 6 μM) and **b)** BSA (final concentration: 2, 4 and 6 μM) in DMSO/PBS (v/v: 7/3; 10 mM, pH: 7.4). (λ_{Ex}: 480 nm, Slid width: Ex/Em: 2.5/5 nm)

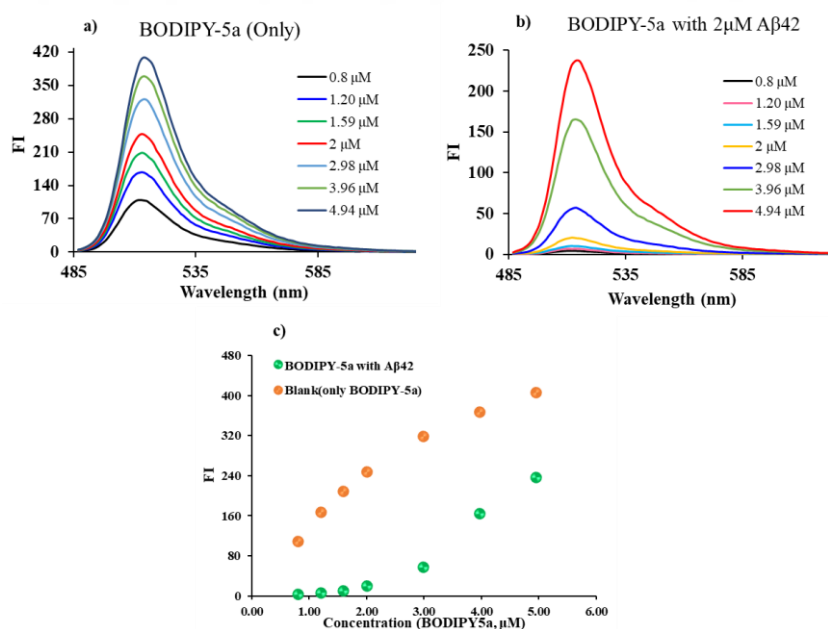


Figure 2.29 Titration data of increasing concentration of **BODIPY-5a** **a)** in the absence of Aβ₁₋₄₂ aggregates and **b)** in the presence of Aβ₁₋₄₂ aggregates (final concentration: 2, 4 and 6 μM) **c)** Comparison of a) and b)

After the addition of $A\beta_{1-42}$ aggregates to **BODIPY-5b**, we observed a little change in the emission spectra. The emission band of **BODIPY-5b** is quenched when we added both $A\beta_{42}$ and BSA indicating BODIPY-5b is not show selectivity for $A\beta_{1-42}$ (**Figure A. 44**). Among the other synthesized BODIPY-imine compounds, **BODIPY-5d**, **BODIPY-5e**, and **BODIPY-5f** did not show a remarkable change in their emission bands upon both the addition of $A\beta_{1-42}$ and BSA (**Figure A. 45-47**). We incubated to **BODIPY-5g** and **BODIPY-5i** with $A\beta_{42}$ aggregates no obvious change was observed while their emission band showed a little quenching when incubated with BSA (**Figure A. 48-49**).

Interaction with **BODIPY-Sq** were studied DMSO: PBS (v/v: 4/1, 10 mM; pH: 7.4). The absorption band of BODIPY-Sq at 502 nm followed the Lambert-Beer law (**Figure 2.30**), showing that BODIPY-Sq compound is not significantly aggregated in this solvent system. When we incubated to BODIPY-Sq with $A\beta_{1-42}$ aggregates (Final concentration is 6 μ M), BODIPY-Sq showed an increase in emission band. However, no significant change was observed when BODIPY-Sq incubated with BSA indicating compound BODIPY-Sq could bind to $A\beta_{1-42}$ aggregates selectively (**Figure 2.31**). Next, we calculated the affinity of **BODIPY-Sq** for $A\beta_{1-42}$ aggregates by studying the fluorescence response with increasing concentrations of **BODIPY-Sq** (0-24.5 μ M) against a fixed concentration of $A\beta_{1-42}$ aggregates. (2 μ M) **Figure 2.36, b**. We also recorded emission spectra of BODIPY-Sq in the absence of $A\beta_{42}$ aggregates in the same concentrations as a blank (**Figure 2.32**) The obtained standard saturation curve was fitted to a single-binding site, which gave a dissociation constant K_d of 11.7 μ M (**Figure 2.33**). Lipophilicity and molecular weight are considered to be important factors for BBB permeability. In the final step, we theoretically calculated the lipophilicity value by using SwissADME website. The results show that BODIPY-Sq have 4.32 Log P value and it can cross to BBB (**Figure A. 50**). Also, we applied the Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) which is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules to predict BODIPY-Sq can cross BBB or not (Daina & Zoete, 2016). According to the method, the presence of the compound in the yolk region of the egg indicates that it can cross the BBB. Accordingly, the study showed that BODIPY-Sq compound can cross to BBB (**Figure A. 51**).

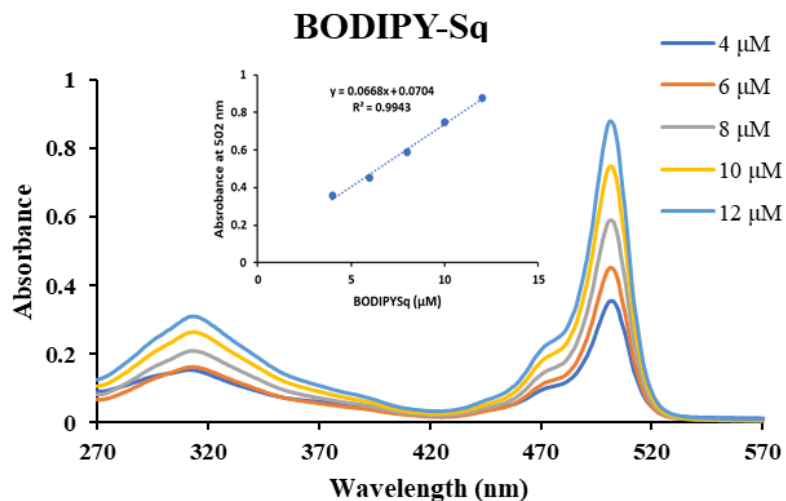


Figure 2.30 Electronic absorption spectra of BODIPY-Sq in DMSO/PBS (4:1 v/v) at different concentrations. Inset plot is the absorbance at 502 nm vs. the concentration of BODIPY-Sq.

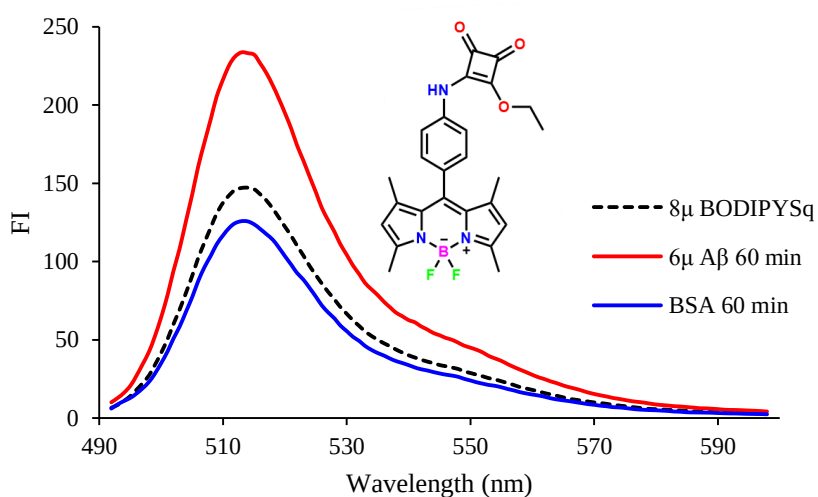


Figure 2.31 Fluorescence intensity of BODIPY-Sq upon interaction with A β_{1-42} aggregates (final concentration: 2 μ M) and **b**) BSA (10 μ M) in DMSO/PBS (v/v: 4/1; 10 mM, pH: 7.4). (λ_{EX} : 485 nm, Slid width: Ex/Em: 2.5/5 nm)

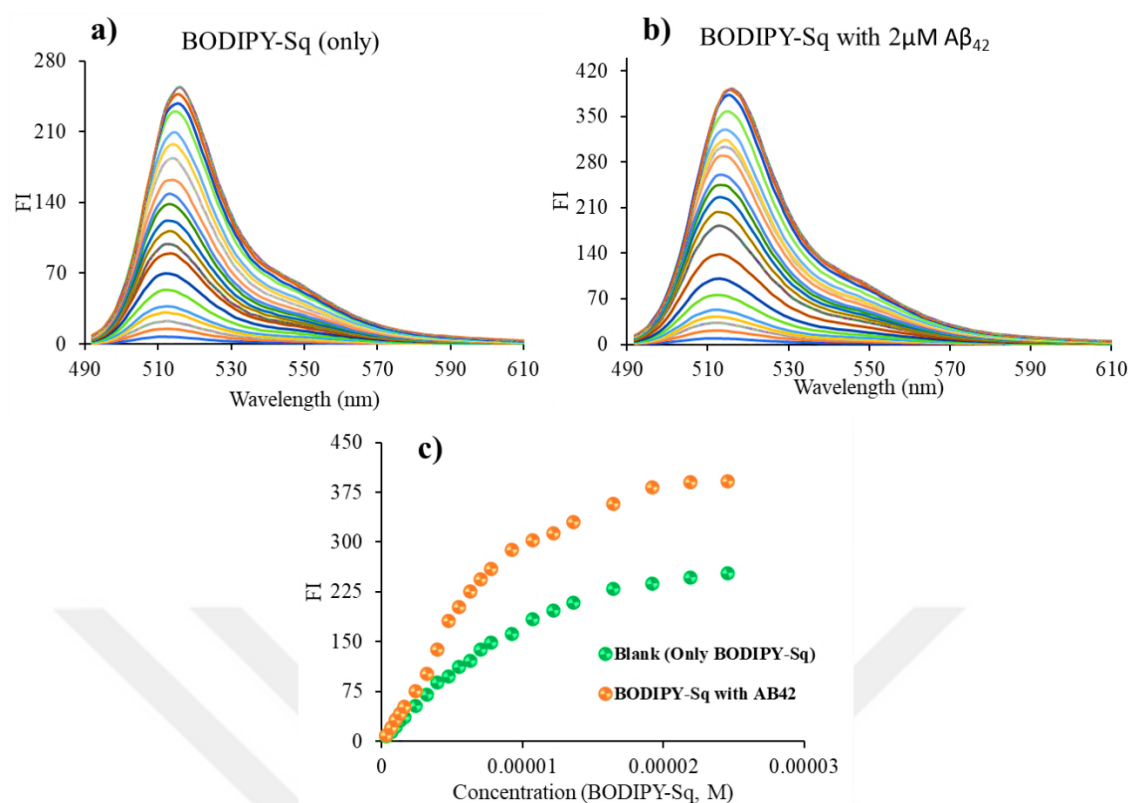


Figure 2.32 Titration data of increasing concentration of BODIPY-Sq **a)** in the absence of Aβ₄₂ aggregates and **b)** in the presence of Aβ₄₂ aggregates (final concentration: 2 μM) **c)** Comparison of a) and b)

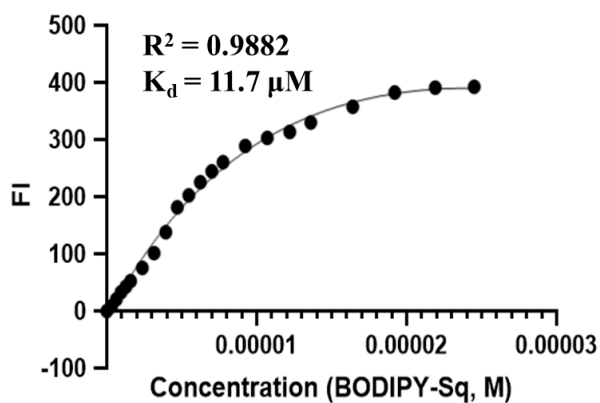


Figure 2.33 Binding curves of BODIPY-Sq to Aβ₄₂ aggregates: 2 μM; in DMSO/PBS (v/v: 7/3; 10 mM, pH: 7.4).

2.3 X-Ray crystallography

2.3.1 Crystal structure of compound BODIPY-5b

Single crystals of the BODIPY-Schiff base compound were obtained slow evaporation of a THF solution of the compound. The crystal structure of the compound was then determined by single crystal XRD analysis. Structure of the compound was solved in triclinic unit cell and P-1 space group. The asymmetric unit contains a BODIPY-Schiff base compound and a THF solvent of crystallization. The structure of the compound is shown in **Figure 2.34**. The bond lengths and angles in the BODIPY core as well as the Schiff base fragment are comparable to those similar structures reported in literature (Kushwah et al., 2019; Pan, Zhou, & Luo, 2014b). In the structure of the compound, the geometry around boron atom in the BODIPY core $B(N_2F_2)$ is approximately tetrahedron with the average bond angle of 109.47° around the boron atom. In the BODIPY unit, the central six-membered and two five-membered rings are almost co-planar with small deviations by some atoms. As seen in similar structures (Kushwah et al., 2019; Pan, Zhou, et al., 2014b), the π -electron delocalization in the BODIPY core is interrupted by two B-N bonds. The B1-N1 and B1-N2 distances are 1.551(6) and 1.550(6) Å, respectively. The methyl groups at C3 and C7 atoms of the BODIPY core prevents the free rotation of the phenyl ring at the meso position. The meso-phenyl ring is almost perpendicular to BODIPY moiety with the dihedral angle of 84.64° . The phenyl and phenol rings in the Schiff base moiety are tilted with respect to each other with the dihedral angle of 27.00° .

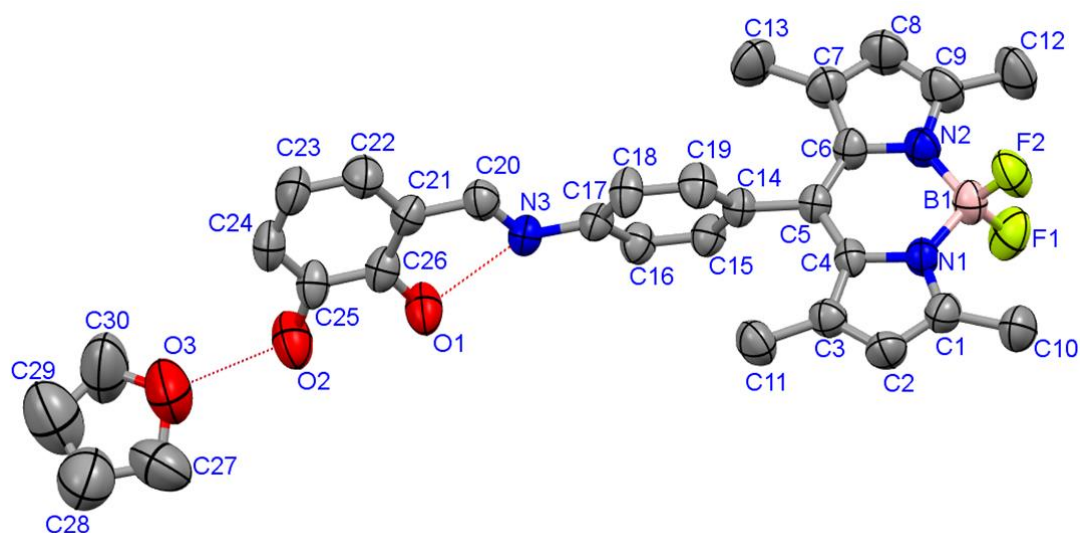


Figure 2.34 Thermal ellipsoid (50% probability) plot of BODIPY-5b with a THF solvent of crystallization

o-Hydroxy Schiff base compounds can exist in two tautomeric forms (enol-imine and keto-amine) due to the proton transfer from the phenolic oxygen to the imine nitrogen atom. The existence of the tautomeric forms in the crystalline state can be examined by both phenolic C-O and imine C=N bond distances. The single crystal XRD showed the preference of the enol-imine form in the Schiff base moiety for the compound as indicated by the bond distances [N3-C20 = 1.275(5) Å and C26-O1 = 1.354(5) Å]. The enol-imine and keto-amine tautomeric forms can also be inferred by the harmonic oscillator model aromaticity (HOMA) index (Kruszewski & Krygowski; Krygowski & Cyrański, 2001). HOMA indices in the range of 0.900–0.990 and 0.500–0.800 are assigned to the aromatic and the non-aromatic rings, respectively. The HOMA indices for C14/C19 and C21/C26 in the Schiff base moiety are 0.968 and 0.992, respectively. These values reveal the aromatic character for both rings and confirming the enol-imine tautomeric form in the crystalline form.

In the structure of the compound, there is an expected phenol-imine hydrogen bond (O1-H····N3). The second phenolic group in the structure makes a hydrogen bond with the THF solvent (O2-H····O3). The THF solvent molecules links the BODIPY-Schiff base molecules via C-H····F and C-H····O interactions forming 1D supramolecular chain (**Figure 2.35a**). There are also π - π edge to edge stackings in the structure. The C20/C22 edge of one of the molecules is stacked with the same section of an adjacent molecule (symmetry operation of 1-x, 1-y, 1-z) (**Figure 2.35b**).

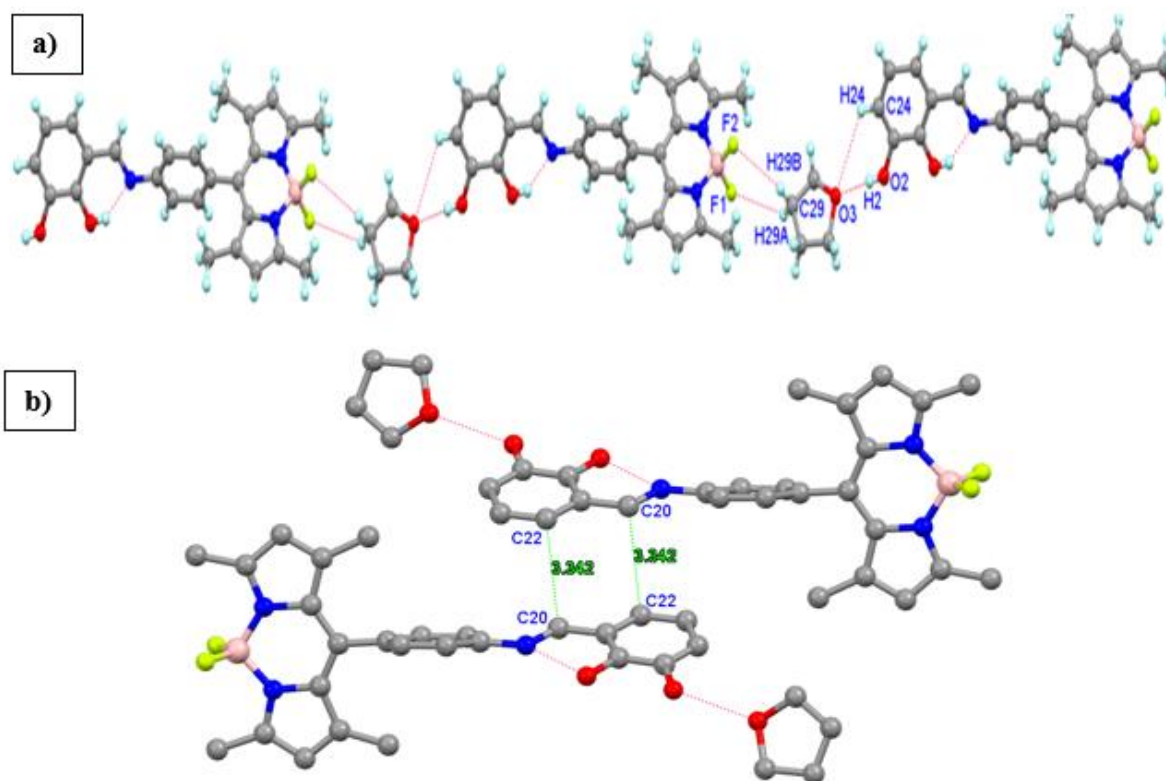


Figure 2.35 a) 1D supramolecular chain via C-H $\cdots$ F and C-H $\cdots$ O interactions in the structure of the compound. b) π - π edge to edge stackings in the structure

2.3.2 Crystal structure of BODIPY-5i

Crystal structure of compound **BODIPY-5i** was solved in triclinic unit cell and P-1 space group. The thermal ellipsoid plot of the compound with 50% probability is depicted in **Figure 2.36**. The structure shows no crystallographically imposed symmetry. The structure of the compound contains a BODIPY core unit and a Schiff base moiety. The bond distances and angles in the BODIPY are comparable to that of compound **BODIPY-5i**. The BODIPY unit contains an imine bearing phenyl ring at the *meso*-position. The phenyl ring C14/C19 is almost perpendicular to the planar BODIPY core with the dihedral angle of 76.24°. The salicylidene (C21/C26) and phenyl(C14/C19) rings are not coplanar with the twist angle of 27.48°. The imine bond (C20-N3) and C-O distances is 1.263(3) and 1.336(3) Å, respectively. These bond distances suggest the phenol-imine tautomeric form in the crystalline state. As expected, molecules show a phenol-imine intramolecular hydrogen bond (O1-H $\cdots$ N3). In the structure of the compound, the salicylidene unit of the molecule is π - π stacked with the same section of an adjacent molecule (head to tail) and these π - π stacking are extended to their respective planes to form 1D supramolecular structure. The π - π stacking interactions are

shown in **Figure 2.37**. The fluoride atoms (F1 and F2) are involved in intermolecular C-H $\cdots$ F interaction which stabilise the crystal structure (**Figure 2.38**).

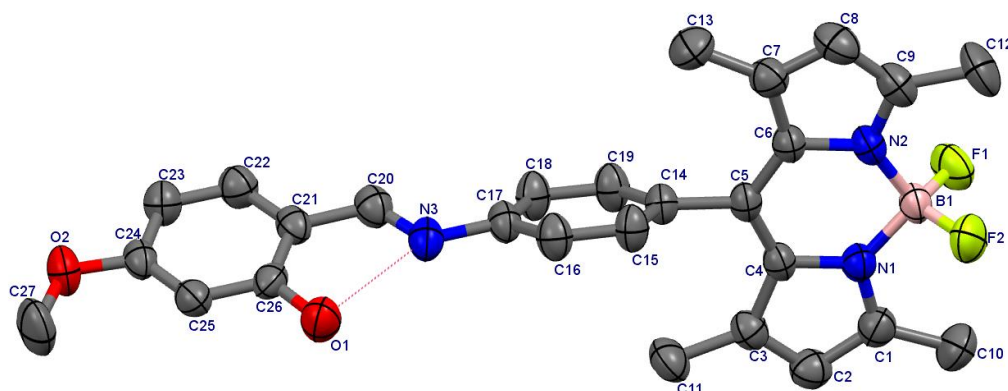


Figure 2.36 Crystal structure of **BODIPY-5i** with atom numbering. Hydrogen atoms are omitted for clarity

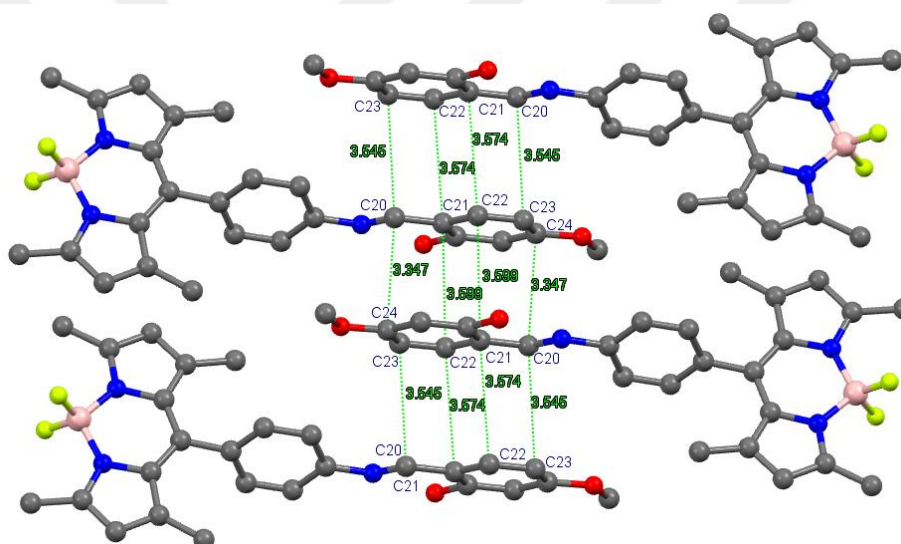


Figure 2.37 The π - π stacking interactions in the structure of **BODIPY-5i**

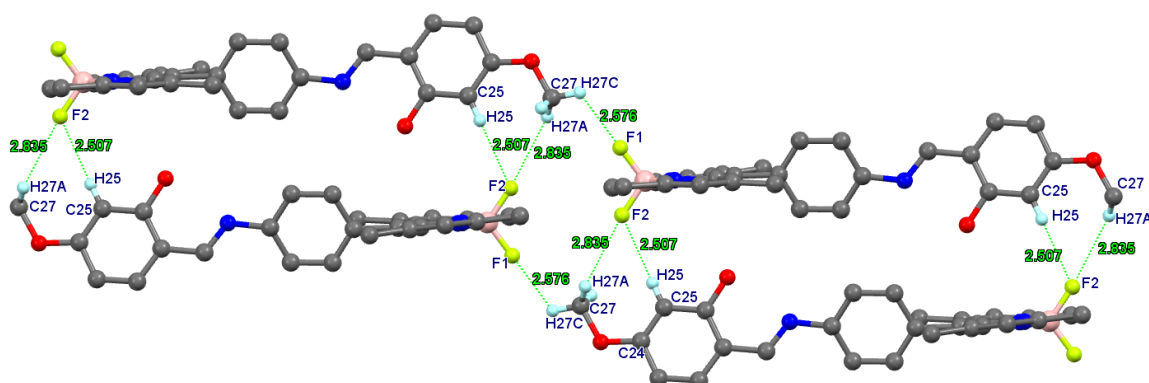


Figure 2.38 The C-H $\cdots$ F contacts in the structure of **BODIPY-5i**

2.3.3 Crystal structure of BODIPY-5d and BODIPY-5f

The compounds **BODIPY-5d** and **BODIPY-5f** were found to crystallise in monoclinic unit cell and P21/c space group with similar unit cell parameters. The crystal structures of the compounds are given in **Figure 2.39**. The structures of the compounds are closely similar differing the 5-membered heterocyclic unit (pyrrole ring for BODIPY-3d and thiophen ring for BODIPY-3f) attached to the phenyl ring (C14/C19) via an imine bond. Bond distances and angles in the BODIPY core unit of both molecules are similar to those of closely related molecules. The imine bond distances (C20-N3) for compounds BODIPY-3d and BODIPY-3f are 1.264(3) and 1.262(6) Å, respectively. These values are within the range of C=N double distances. For both compounds, the phenyl ring (C14/C19) at the meso position are twisted considerably due to the methyl groups located at the C3 and C7 atoms. The dihedral angles between the planar BODIPY core unit and phenyl ring (C14/C19) are 86.17° for BODIPY-3d and 79.33° for BODIPY-3f. In BODIPY-3f, the phenyl and thiophen rings are almost coplanar with the dihedral angle of 3.24°. However, in compound BODIPY-3f, the phenyl and pyrrole rings are considerably twisted with the dihedral angle of 36.90°. This reflects the different intermolecular interactions for the compounds. In BODIPY-3d, the pyrrole nitrogen atom (N4) is involved intermolecular hydrogen bonding with the fluoride (F2) atom of a neighbouring molecule [N4-H····F2]. Moreover, molecules of BODIPY-3d are also linked by C-H····F interactions forming a 2D supramolecular synthon (Figure 2.44). In BODIPY-3d, molecules are also linked by C-H····F interactions (Figure 2.45). No π - π stacking interactions are observed in the structures of both compounds.

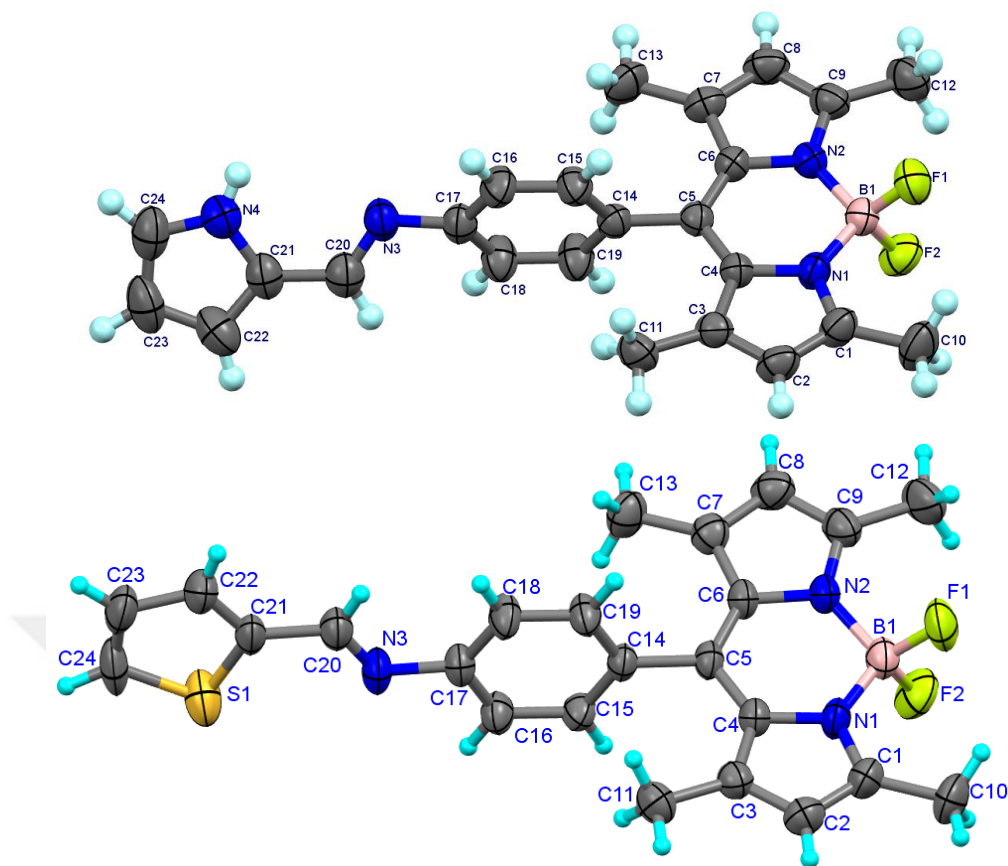


Figure 2.39 Crystal structure of **BODIPY-5d** (up) and **BODIPY-5f** (down) (thermal ellipsoid 50% probability) with atom numbering.

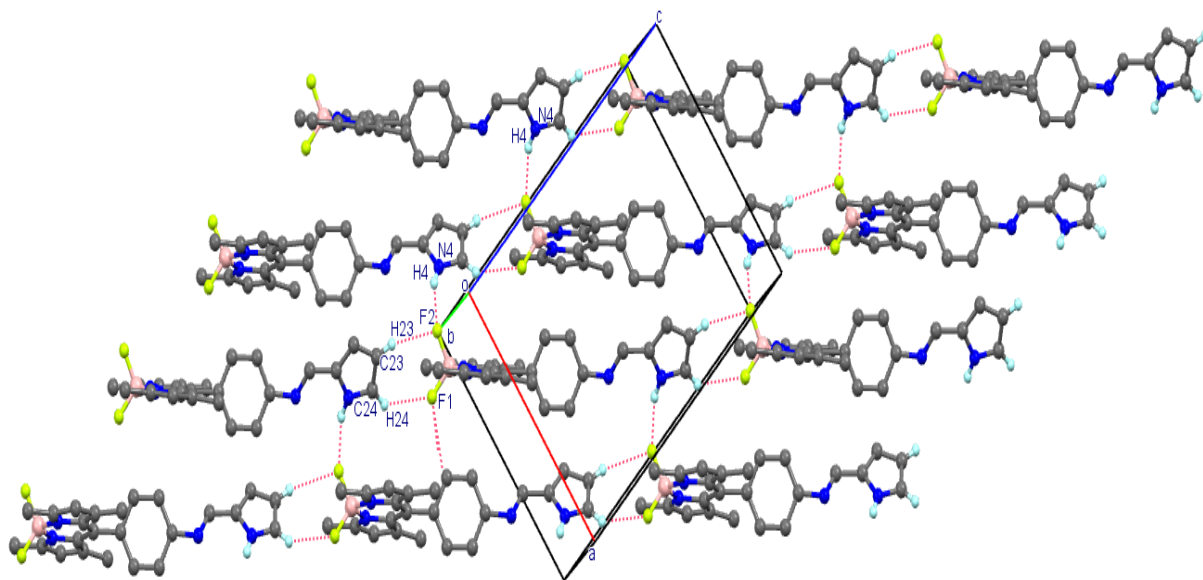


Figure 2.40 The C-H $\cdots$ F contacts in the structure of **BODIPY-5d**

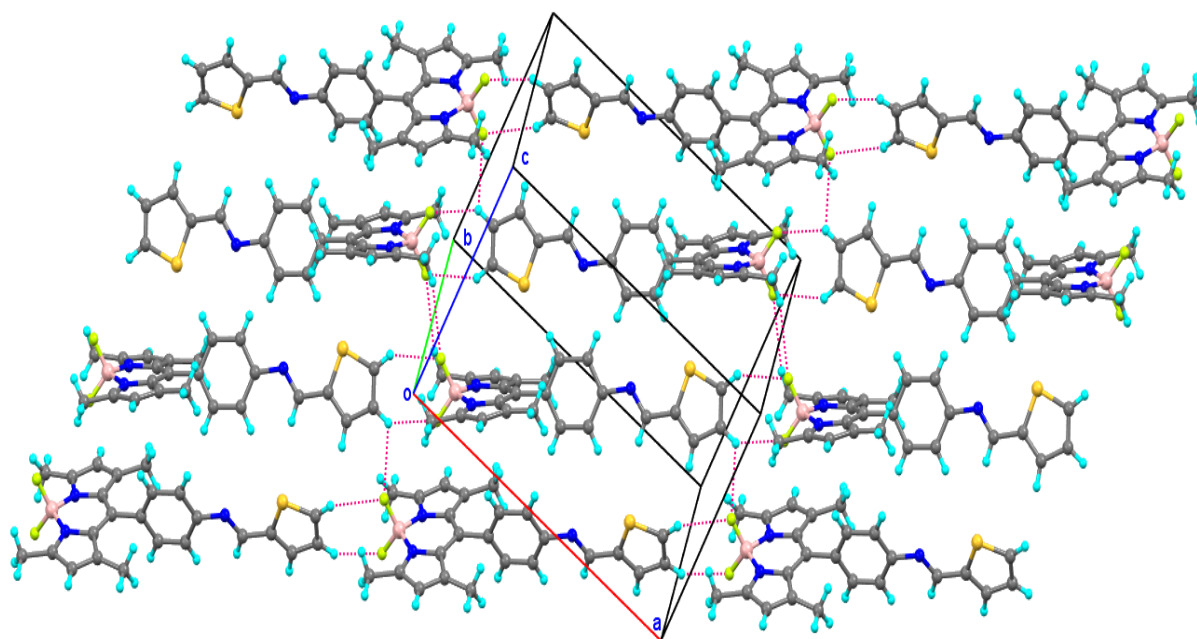


Figure 2.41 The C-H····F contacts in the structure of **BODIPY-5f**

2.3.4 Crystal structure of **BODIPY-3g**

Crystal structure of the compound was solved in triclinic unit cell and P-1 space group. Thermal ellipsoid plot of the compound with 50% probability is depicted in **Figure 2.46**. The structure of the compound contains N-phenyl-1-(pyridin-2-yl) methanimine moiety at the *meso* position of a BODIPY core unit. The bond distances and angles within the BODIPY unit are within the range of expected values. The C20-N3 bond distance is 1.2625(19) Å showing C=N double bond character. The phenyl ring (C15/C19) at the meso position is almost perpendicular to the BODIPY unit with the twist angle of 80.55°. Additionally, the phenyl (C15/C19) and pyridine rings (N4/C25) are inclined with the dihedral angle of 50.23°. In the structure of the compound, two molecules are linked by two concordant C-H····F contacts as shown **Figure 2.47** Moreover, BODIPY moiety of two compounds are stacked by C····F contacts (head to tail fashion) under symmetry operation of 2-x, -y, 1-z. The C5····F2 distance is 3.074 Å.

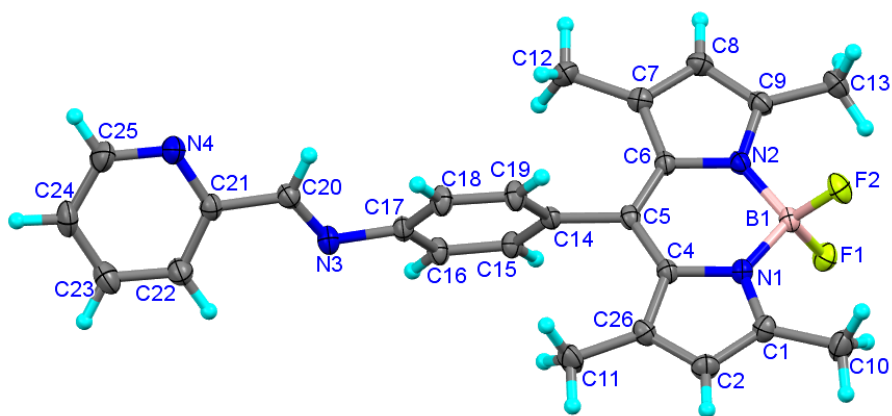


Figure 2.42 Crystal structure of BODIPY-5g (thermal ellipsoid 50% probability) with atom numbering.

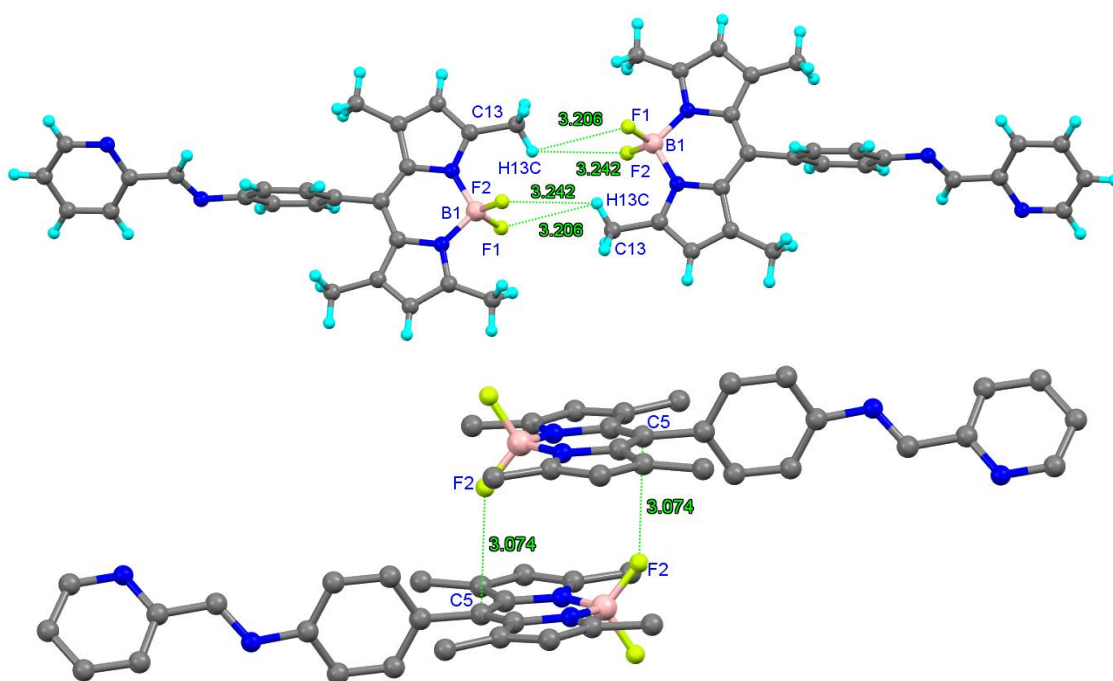


Figure 2.43 The C-H $\cdots$ F and C $\cdots$ F contacts in the structure of BODIPY-5g

2.3.5 Crystal structure of BODIPY-Sq

X-ray crystallographic data showed that the compound crystallised in triclinic unit cell and P-1 space group. The asymmetric unit contains four independent molecules with similar bond lengths and angles differing in the dihedral angles between the squaric ester and phenyl rings. The structure of one of the four independent molecules (thermal ellipsoid plot 50% probability) with atom numbering is given in **Figure 2.48**. The squaric ester unit is attached to the BODIPY core unit *via* an amide bond. The structural parameters in the BODIPY core unit are comparable to those discussed earlier in this thesis. The C21-O1 and C22-O2 bond distances of 1.219(3) and 1.217(2) Å, respectively, exhibit C=O double bond character. In the

structure, two molecules are dimerised by concordant hydrogen bonds [N-H····O] (**Figure 2.49**). Molecules are further linked by C-H····O/F contacts.

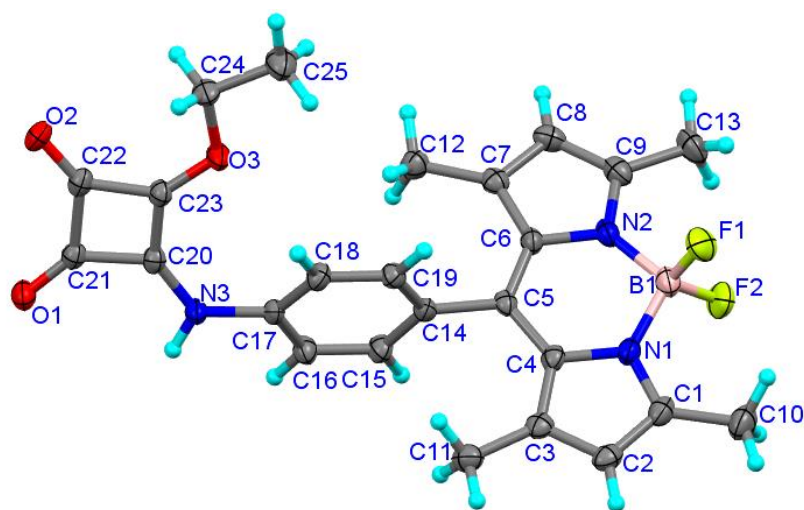


Figure 2.44 Crystal structure of BODIPY-sq (thermal ellipsoid 50% probability) with atom numbering.

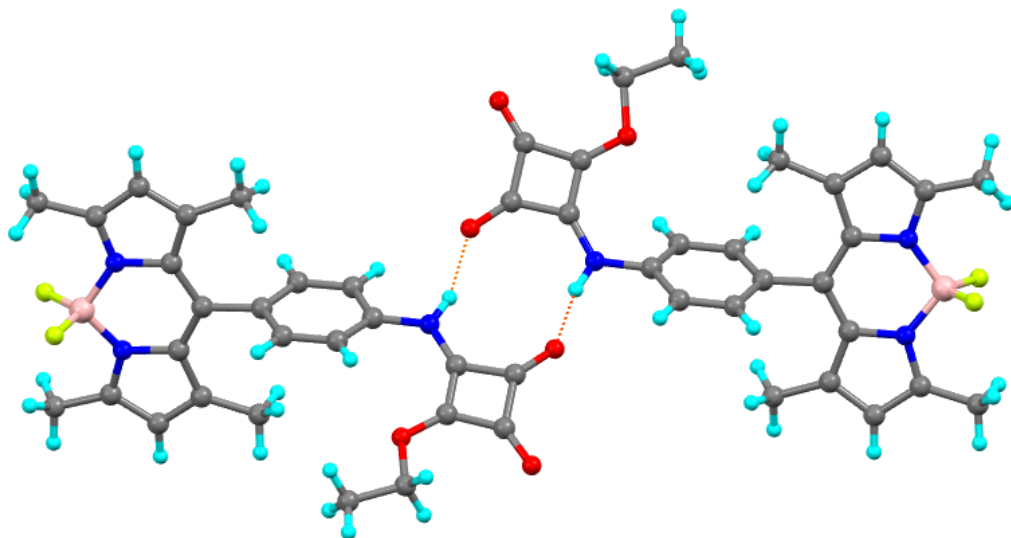


Figure 2.45 Hydrogen bond dimer in the structure BODIPY-sq

Table 2.4 X-ray crystallographic data and refinement details

	BODIPY-5g	BODIPY-Sq
Empirical formula	C ₂₅ H ₂₃ BF ₂ N ₄	C ₂₅ H ₂₄ BF ₂ N ₃ O ₃
Formula weight	428.28	463.28
Temperature/K	150.00	150.00
Crystal system	Triclinic	Triclinic
Space group	P-1	P-1
a/Å	8.7693(3)	14.4665(5)
b/Å	10.9097(4)	18.0299(7)
c/Å	11.9916(4)	18.9794(7)
α/°	74.3120(10)	84.6900(10)
β/°	85.7330(10)	70.1450(10)
γ/°	72.3600(10)	76.3170(10)
Volume/Å ³	1052.54(6)	4523.6(3)
Z	2	8
ρ _{calc} /g/cm ³	1.351	1.361
μ/mm ⁻¹	0.093	0.101
Crystal size/mm ³	0.27 × 0.23 × 0.16	0.25 × 0.21 × 0.18
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2θ range for data collection/°	5.818 to 58.328	4.98 to 53.424
Reflections collected	24034	76997
Independent reflections	5636 [R _{int} = 0.0345]	19103 [R _{int} = 0.0719]
Goodness-of-fit on F ²	1.023	1.014
Final R indexes [I>=2σ (I)]	R ₁ = 0.0465, wR ₂ = 0.1102	R ₁ = 0.0549, wR ₂ = 0.1034
Final R indexes [all data]	R ₁ = 0.0652, wR ₂ = 0.1211	R ₁ = 0.1156, wR ₂ = 0.1239

Table 2.5 X-ray crystallographic data and refinement details

	BODIPY-5b	BODIPY -5i	BODIPY-5d	BODIPY-5f
Empirical formula	C ₃₀ H ₃₂ N ₃ O ₃ F ₂ B	C ₂₇ H ₂₆ N ₃ O ₂ BF ₂	C ₂₄ H ₂₃ BN ₄ F ₂	C ₂₄ H ₂₂ N ₃ BSF ₂
Formula weight	531.39	473.32	416.27	433.31
Temperature/K	293(2)	293(2)	293(2)	293(2)
Crystal system	Triclinic	Triclinic	Monoclinic	Monoclinic
Space group	P-1	P-1	P2 ₁ /c	P2 ₁ /c
a/Å	9.7381(10)	7.1589(5)	10.8173(6)	15.0478(10)
b/Å	11.1513(11)	12.0801(8)	14.1658(8)	13.7940(19)
c/Å	13.8657(14)	14.0864(7)	13.7404(7)	10.2477(8)
α/°	108.610(9)	85.938(5)	90	90
β/°	104.420(9)	85.817(5)	92.841(4)	96.335(7)
γ/°	90.153(8)	75.138(6)	90	90
Volume/Å ³	1376.5(3)	1172.59(13)	2102.9(2)	2114.1(4)
Z	2	2	4	4
ρ _{calc} /g/cm ³	1.282	1.341	1.315	1.361
μ/mm ⁻¹	0.092	0.096	0.091	0.187
Crystal size/mm ³	0.12 × 0.09 × 0.08	0.17 × 0.14 × 0.09	0.12 × 0.11 × 0.1	0.14 × 0.12 × 0.08
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2θ range for data collection/°	6.74 to 57.812	6.616 to 58.046	6.598 to 58.15	7.044 to 58.16
Reflections collected	10191	9488	9325	9416
Independent reflections	6067 [R _{int} = 0.0460]	5323 [R _{int} = 0.0226]	4789 [R _{int} = 0.0285]	4865 [R _{int} = 0.0646]
Goodness-of-fit on F ²	0.983	1.030	1.041	1.050
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0842, wR ₂ = 0.2049	R ₁ = 0.0622, wR ₂ = 0.1395	R ₁ = 0.0665, wR ₂ = 0.1333	R ₁ = 0.0958, wR ₂ = 0.2578
Final R indexes [all data]	R ₁ = 0.2233, wR ₂ = 0.2839	R ₁ = 0.1094, wR ₂ = 0.1658	R ₁ = 0.1300, wR ₂ = 0.1677	R ₁ = 0.1493, wR ₂ = 0.2981

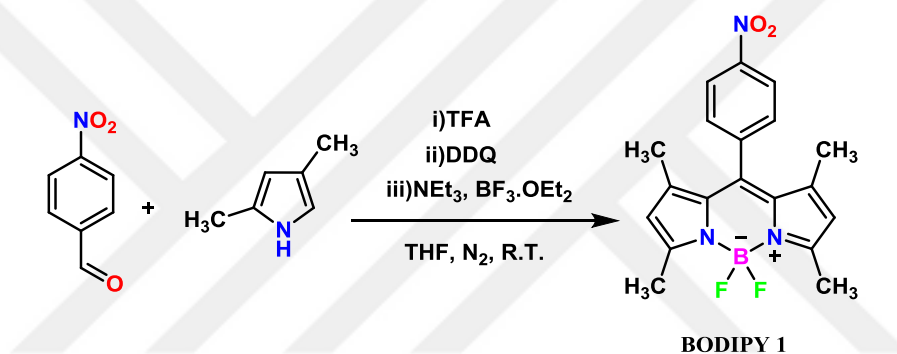
CHAPTER 3 Experimental Section

3.1 General

DCM was distilled over calcium hydride. THF was distilled under a nitrogen atmosphere using metallic sodium (Na) and benzophenone. All other solvents and reagents were of reagent grade and used as received. Analytical TLC was performed using pre-coated silica gel plates (Merck Kieselgel 60 F254). Chromatographic purification was performed using Merck Kieselgel 60 silica gel as the stationary phase.

3.2 Synthesis

3.2.1 Synthesis of 4,4-difluoro-8-[4-nitro]-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY 1)

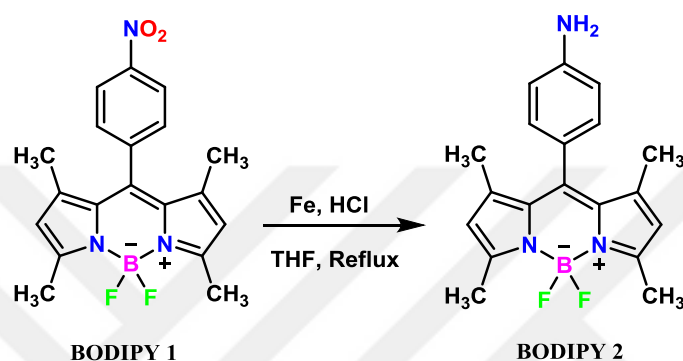


Scheme 3.1 Synthesis scheme of BODIPY 1

2,4-dimethylpyrrole (630 mg, 6.6 mmol) and 4-nitrobenzaldehyde (453 mg, 3.0 mmol) were dissolved in dry THF and stirred about 1 hour under N₂ atmosphere at room temperature. To the reaction mixture, 10 drops of trifluoroacetic acid were added and then the reaction mixture was stirred in the dark for 24 h under an N₂ atmosphere at room temperature. After TLC plate showed disappearance of 4-nitrobenzaldehyde, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (680 mg, 3.0 mmol) was dissolved in 10 mL of dry THF and then slowly added dropwise to the reaction mixture. When the reaction mixture was stirred for 4-5 hours, it was transferred to the ice bath. Triethylamine (18 mL) and BF₃.OEt₂ (18 mL) were added slowly to the reaction mixture using a constant pressure dropping funnel. After the reaction mixture was further stirred for 12 h at room temperature, the reaction was stopped. The reaction mixture was repeatedly extracted twice with dichloromethane and water. The combined organic layer dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography using DCM/petrol (2:1) as an eluent to obtain compound 2 as an orange-red solid.

BODIPY 1: C₁₉H₁₈BF₂N₃O₂, MW: 369.18.g/mol. Yield: 40 % (442 mg). FT-IR (ATR, cm⁻¹): 3100 (Ar C-H), 2963-2851 (aliphatic C-H), 1598, 1538, 1505, 1468, 1409, 1343, 1184, 1158 (C-N-C), 1074, 1045, 969, 816. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 8.41 (d, *J* = 8.6 Hz, 2H, ArCH), 7.76 (d, *J* = 8.6 Hz, 2H, ArCH), 6.24 (s, 2H, =CH), 2.48 (s, 6H, CH₃), 1.35 (s, 6H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆, δ ppm): 156.17, 148.51, 143.06, 141.23, 139.74, 130.48, 130.43, 124.88, 122.30, 14.77.

3.2.2 Synthesis of the 4,4-difluoro-8-[4-amino]-1,3,5,7-tetramethyl-4-bora-3a,4adiaza-s-indacene (BODIPY 2)

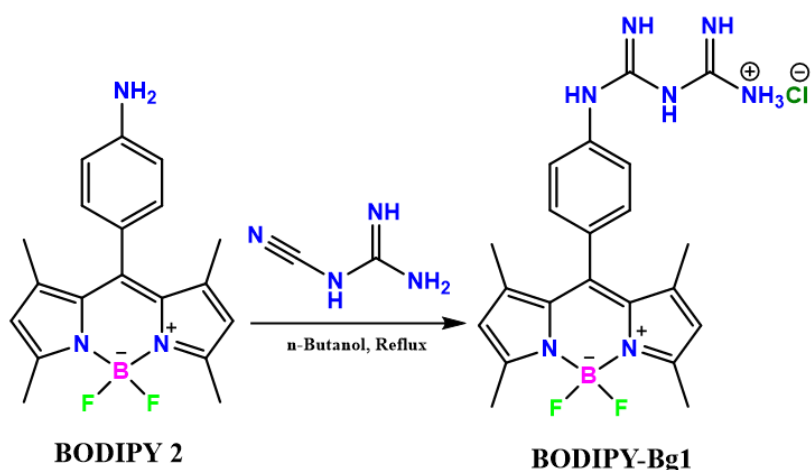


Scheme 3.2 Synthesis scheme of **BODIPY 2**

BODIPY 1 (1.23 g, 3.3 mmol) was dissolved in THF (75 mL) and then iron powder (3.53 g, 63.3 mmol) 0.5 M methanolic HCl (20 mL), and water (5 mL) were added to the reaction mixture. The reaction mixture refluxed for 2 h. Then the reaction mixture was concentrated under reduced pressure, the residue was redissolved in DCM (100 mL), filtered, and concentrated again. The crude product was purified by silica-gel column chromatography using DCM/hexane (1 : 1) as eluent to obtain **BODIPY 2** as an orange solid.

BODIPY 2: C₁₉H₂₀BF₂N₃ MW: 339.20 g/mol, Yield: 76 % (0.85 g), FT-IR (ATR, cm⁻¹): 3479, 3386 (NH₂), 2955-2855 (aliphatic C-H), 1624, 1608, 1503, 1301, 1185, 1152 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ= 6.91 (d, *J* = 8.0 Hz, 2H, ArCH), 6.71 (d, *J* = 8.1 Hz, 2H, ArCH), 6.15 (s, 2H, =CH), 5.46 (s, 2H, NH₂), 2.44 (s, 6H, CH₃), 1.50 (s, 6H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆, δ ppm): 154.45, 150.05, 144.32, 143.16, 131.94, 128.83, 121.41, 120.97, 114.58, 14.79, 14.63. ESI-Q-TOF_(m/z): 340.1983 [M+H]⁺, 320.1813 [M-F]⁺.

3.2.3 Synthesis of BODIPY-Bg1



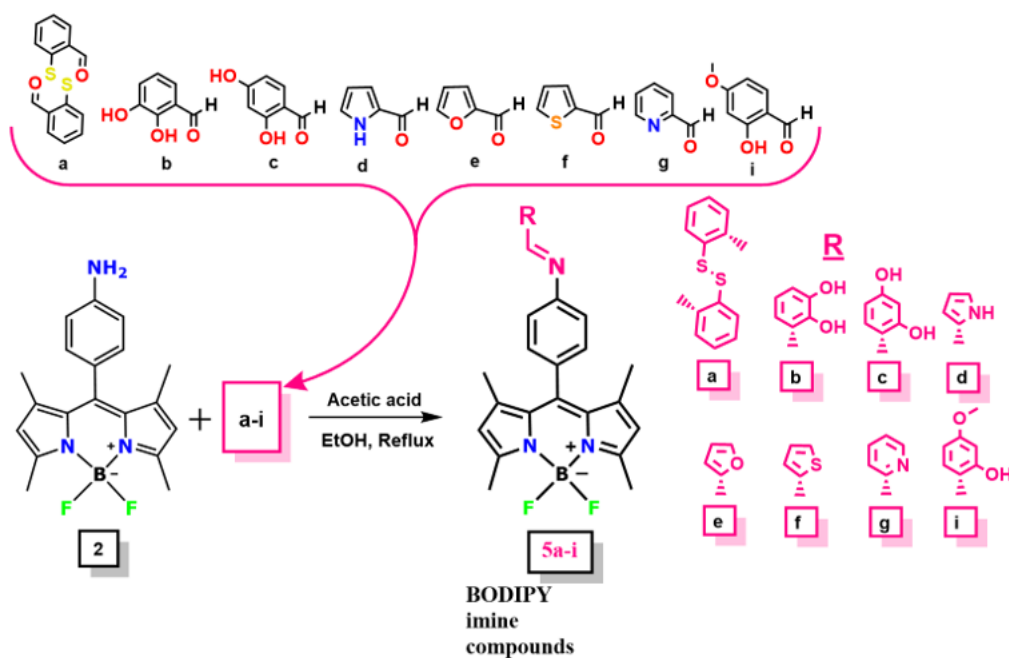
Scheme 3.3 Synthesis scheme of BODIPY-biguanide compound (BODIPY-Bg1)

BODIPY 2 (0.25 mmol, 0.0848 g) was dissolved in *n*-butanol. After the addition of one drop of hydrochloric acid (aq., %37'lik), dicyandiamide (0.275 mmol, 0.0231 g) was added to the reaction mixture. The reaction mixture was refluxed until the complete consumption of **BODIPY 2** (monitored by TLC). After the evaporation of the solvent, the desired compound, **BODIPY-Bg1**, was obtained as a green-reddish solid.

BODIPY-Bg1: MW: 459.74 g/mol, Yield: 65 % (0.075 g). ^1H NMR (500 MHz, DMSO) δ 11.79 (s, 2H), 10.51 (s, 1H), 8.27 (s, 3H), 7.51 – 7.06 (m, 8H), 6.78 (d, $J = 6.4$ Hz, 2H), 6.37 (s, 2H), 2.37 (s, 6H), 1.71 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 158.85, 156.63, 156.11, 155.71, 154.96, 152.90, 148.39, 138.51, 129.19, 122.76, 122.69, 118.20, 114.85, 114.73, 13.97.

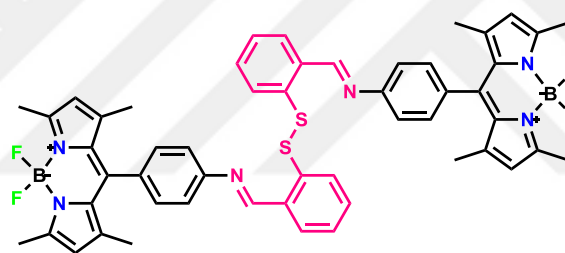
3.2.4 General procedure for synthesis of BODIPY-imine compounds

A drop of acetic acid was added to a mixture of **BODIPY 2** (0.3 mmol, 102 mg) and various aldehyde derivatives (**b-i**) (0.3 mmol) in anhydrous ethanol and the reaction mixtures were refluxed for about 4 hours. During this time precipitations were observed in the reaction mixtures. The reaction mixtures were cooled to room temperature and filtered. The resulting solids were washed several times with cold ethanol and dried to yield BODIPY-imine compounds (**5b-i**). For compound 5a, 0.3 mmol of **BODIPY 2** and 0.15 mmol of aldehyde were used. The synthetic pathway for BODIPY-imine compound is given in Scheme 3.4 and characterization data of synthesized compounds are given below.



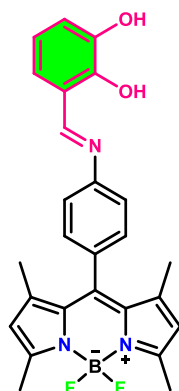
Scheme 3.4 Synthetic pathway for BODIPY-imine compound.

BODIPY-5a



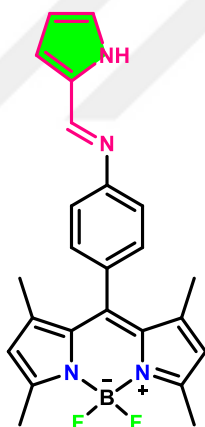
BODIPY-5a: $C_{26}H_{24}BF_2N_3O_2$, MW: 916.72 g/mol. Yield: 66% (0.093 g). FT-IR (ATR, cm^{-1}): 2971, 2922, 1625, 1542, 1505, 1469, 1434, 1408, 1371, 1260, 1187, 1155, 1078, 1048, 974, 838, 767, 718, 705, 639, 581, 476 cm^{-1} . ^1H NMR (400 MHz, DMSO) δ 9.02 (d, J = 4.1 Hz, 2H), 8.03 (d, J = 6.8 Hz, 2H), 7.86 (d, J = 8.0 Hz, 1H), 7.60 – 7.40 (m, 11H), 6.92 (d, J = 8.3 Hz, 1H), 6.72 (d, J = 8.1 Hz, 1H), 6.18 (t, J = 12.7 Hz, 4H), 2.46 (d, J = 13.9 Hz, 12H), 1.54 – 1.41 (m, 12H).

BODIPY-5b



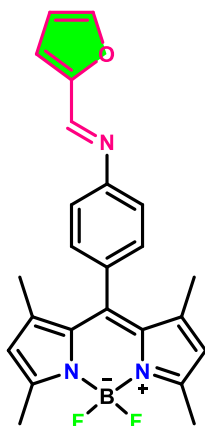
BODIPY-5b: C₂₆H₂₄BF₂N₃O₂, MW: 459.30 g/mol. Yield: 79.1 % (0.1090 g). FT-IR (ATR, cm⁻¹): 3466, 2919, 1619, 1595, 1539, 1505, 1464, 1407, 1367, 1306, 1274, 1191, 1153, 1059, 964, 811 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm) 12.95 (s, 1H, Ar-OH), 9.30 (s, 1H, Ar-OH), 9.03 (s, 1H, HC=N-), 7.60 (d, *J* = 7.9 Hz, 2H, ArH), 7.45 (d, *J* = 7.9 Hz, 2H, ArH), 7.15 (d, *J* = 7.5 Hz, 1H, ArH), 6.97 (d, *J* = 7.6 Hz, 1H, ArH), 6.81 (t, *J* = 7.6 Hz, 1H, ArH), 6.19 (s, 2H, =CH- pyrrole), 2.46 (s, 6H, CH₃), 1.43 (s, 6H, CH₃). ESI-Q-TOF_(m/z): 460.2039 [M+H]⁺

BODIPY-5d



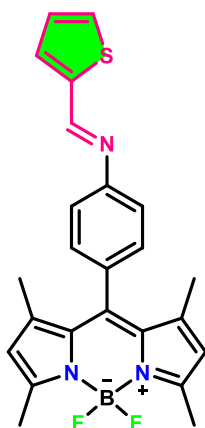
BODIPY-5d: C₂₄H₂₃BF₂N₄, MK: 416.28 g/mol. Yield: 62.5 % (0.0781 g). FT-IR (ATR, cm⁻¹): 3564, 3390, 2961, 2922, 2879, 2853, 1621, 1590, 1539, 1505, 1469, 1403, 1303, 1188, 1154, 1034, 974, 962, 834, 817, 755 cm⁻¹. ¹H NMR (400 MHz, DMSO) δ 11.81 (s, 1H), 8.40 (d, *J* = 3.0 Hz, 1H), 7.33 (d, *J* = 3.1 Hz, 4H), 7.07 (s, 1H), 6.76 (s, 1H), 6.23 – 6.12 (m, 3H), 2.43 (d, *J* = 11.5 Hz, 6H), 1.42 (s, 6H). ¹³C NMR (101 MHz, DMSO) δ 155.22, 153.26, 151.66, 143.19, 142.53, 131.36, 130.99, 130.74, 129.18, 124.78, 122.10, 121.81, 117.50, 114.55, 110.37, 14.68, 14.59. ESI-Q-TOF_(m/z): 417.2094 [M+H]⁺

BODIPY-5e



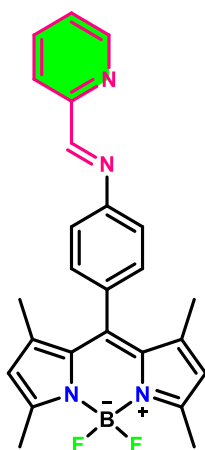
BODIPY-5e: $C_{24}H_{22}BF_2N_3O$, MW: 417.27 g/mol. Yield: 77.4 % (0.968 g). FT-IR (ATR, cm^{-1}): 3142, 3030, 2969, 2920, 1621, 1593, 1539, 1508, 1468, 1405, 1368, 1304, 1186, 1155, 1070, 976, 834, 824, 764, 708, 595, 476 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6 , δ ppm): 8.53 (s, 1H, HC=N-), 7.98 (s, 1H), 7.39 (dd, J = 19.3, 7.4 Hz, 4H), 7.21 (s, 1H), 6.74 (d, J = 1.6 Hz, 1H), 6.18 (s, 2H), 2.45 (s, 6H), 1.41 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 155.32, 154.43, 152.25, 150.05, 149.75, 149.71, 147.29, 144.29, 143.16, 131.91, 129.31, 128.81, 122.38, 121.85, 121.41, 120.90, 118.40, 114.55, 113.41, 113.12, 14.81, 14.63. ESI-Q-TOF(m/z): 418.2094 $[M+H]^+$

BODIPY-5f



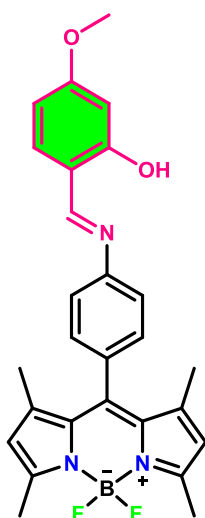
BODIPY-5f: $C_{24}H_{22}BF_2N_3S$, MW: 433.33 g/mol. Yield: 80.8 % (0.1050 g). FT-IR (ATR, cm^{-1}): 3038, 2923, 1615, 1590, 1539, 1505, 1406, 1304, 1158, 1188, 1077, 1048, 972, 839, 828, 714, 583, 475 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6 , δ ppm) δ 8.88 (s, 1H, HC=N-), 7.85 (d, J = 4.9 Hz, 1H), 7.75 (d, J = 3.6 Hz, 1H), 7.42 (q, J = 8.3 Hz, 4H), 7.25 (dd, J = 4.8, 3.8 Hz, 1H), 6.19 (s, 2H, =CH), 2.47 (s, 6H, CH_3), 1.45 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 155.41, 155.35, 151.98, 143.21, 142.72, 134.71, 132.15, 131.95, 131.32, 131.31, 129.35, 128.83, 122.46, 121.86, 14.72, 14.64. ESI-Q-TOF(m/z): 434.1692 $[M+H]^+$

BODIPY-5g



BODIPY-5g: C₂₅H₂₃BF₂N₄, MW: 428.29 g/mol. Yield: 65.1 % (0.0836 g). FT-IR (ATR, cm⁻¹): 2958, 2921, 1738, 1539, 1510, 1467, 1436, 1405, 1365, 1309, 1259, 1198, 1157, 1114, 1073, 1042, 978, 968, 875, 805, 777, 737, 709, 476 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 8.76 (d, *J* = 4.8 Hz, 1H), 8.68 (s, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 7.90 – 7.82 (m, 1H), 7.45 – 7.40 (m, 3H), 7.34 (d, *J* = 8.2 Hz, 2H), 6.01 (s, 2H), 2.57 (s, 6H), 1.47 (s, 6H). ¹³C NMR (101 MHz, DMSO) δ 157.02, 150.81, 149.45, 146.97, 145.49, 145.14, 138.37, 136.49, 132.36, 132.08, 128.54, 126.74, 124.30, 124.16, 123.17, 120.76, 117.29, 117.15, 117.00, 116.53, 110.66, 72.32, 9.93, 9.85. ESI-Q-TOF_(m/z): 429.2105 [M+H]⁺

BODIPY-5i

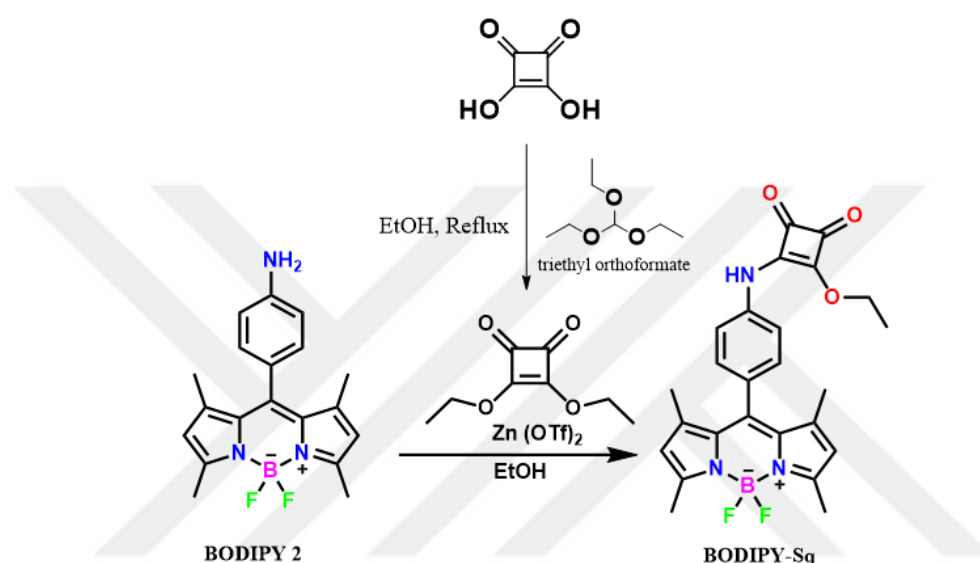


BODIPY-5i: C₂₇H₂₆BF₂N₃O₂, MW: 473.33 g/mol. Yield: %93.7 (0.1330 g). FT-IR (ATR, cm⁻¹): 2961, 2916, 1617, 1593, 1567, 1535, 1505, 1462, 1402, 1346, 1304, 1288, 1190, 1156, 1071, 1026, 966, 832, 798, 706, 537, 476 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 13.49 (s, 1H, Ar-OH), 8.98 (d, *J* = 4.8 Hz, 1H, HC=N), 7.57 (td, *J* = 8.4, 5.1 Hz, 3H, ArH), 7.42 (dd, *J* = 8.0, 4.8 Hz, 2H, ArH), 6.58 (m, 1H, ArH), 6.52 (m, 1H, ArH), 6.18 (d, *J* = 4.0 Hz,

2H, -CH=pyrrol), 3.81 (d, $J = 5.0$ Hz, 3H), 2.46 (s, 6H, CH₃), 1.42 (d, $J = 3.8$ Hz, 6H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆, δ ppm) 164.41, 163.88, 163.82, 163.61, 155.38, 149.21, 143.18, 142.06, 134.75, 132.36, 131.27, 129.56, 122.50, 121.88, 113.49, 107.48, 101.88, 101.32, 101.25, 55.97, 14.69, 14.66. ESI-Q-TOF(m/z): 474.2196 [M+H]⁺

3.2.5 Synthesis of BODIPY-Sq

2-diethoxy-1-cyclobutene-3,4-dione (diethylsquarate) was synthesised previously reported (Liu, Tomooka, & Moore, 1997).

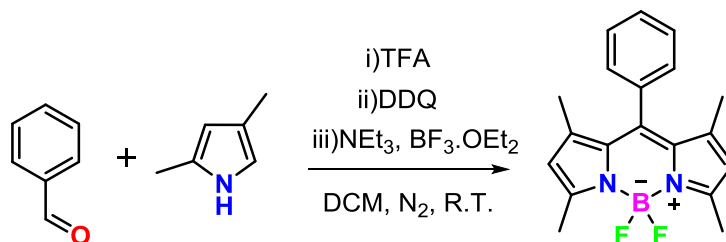


Scheme 3.5 Synthesis scheme of BODIPY-Sq compound

A solution of BODIPY 2 (0.2 g, 0.59 mmol, 1 eqv) in EtOH (10 ml) was added to a mixture of diethylsquarate (0.131 mL, 0.885 mmol, 1.5 eqv) and Zn(OTf)₂ (0.043 g, 0.118 mmol) in EtOH (5 mL). the reaction mixture was stirred at room temperature for 48 h. The precipitate was formed and filtered to yield red product.

BODIPY-Sq: C₂₅H₂₄BF₂N₃O₃, MW: 463.29 g/mol. Yield: %84.25 (0.230 g). ¹H NMR (500 MHz, DMSO) δ 10.94 (s, 1H, H-C=N-), 7.61 (d, $J = 6.7$ Hz, 2H, Phenyl), 7.40 – 7.31 (m, 2H, Phenyl), 6.19 (s, 2H), 4.79 (q, $J = 7.1$ Hz, 2H, -CH₂-), 2.45 (s, 6H, -CH₃), 1.52-1.36 (m, -CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆, δ ppm) 170.12, 155.31, 131.34, 129.27, 121.85, 120.39, 121.85, 120.39, 70.20, 16.07, 14.70.

3.2.6 Synthesis of 8-Phenyl-4,4-difluoro-1,3,5,7-tetramethyl 4-bora-3a,4a-diaza-s-indacene (Phenyl-BODIPY)



Scheme 3.6 Synthesis scheme of **phenyl-BODIPY** compound

8-Phenyl-4,4-difluoro-1,3,5,7-tetramethyl 4-bora-3a,4a-diazas indacene (Phenyl-BODIPY) was synthesized according to the literature report (Pan, Luo, et al., 2014c). Synthetic pathway is given **Scheme 3.6**. The phenyl-BODIPY compound was regularly checked for stability and purity because it was used as a reference dye for the determination of ΦF .

Phenyl-BODIPY: C₁₉H₁₉BF₂N₂, MW: 324.18 g/mol. Yield: 45.00 % (0.292 mg). ¹H NMR (500 MHz, CDCl₃) δ 7.52-7.44 (m, 3H, phenyl), 7.29-7.25 (m, 2H, phenyl), 5.98 (s, 2H), 2.56 (s, 6H, CH₃), 1.37 (s, 6H, CH₃). ¹³C NMR (126 MHz, CDCl₃, δ ppm) 155.45, 143.18, 141.74, 135.01, 131.44, 129.14, 128.95, 127.96, 121.22, 14.60, 14.35.

3.3 X-ray crystallography

Single crystal X-ray diffraction data for compounds **HLM-3b**, **HLM-3i**, **HLM-3d** and **HLM-3f** were collected at ambient temperature on a Agilent Supernova diffractometer using Mo-K α radiation ($\lambda = 0.71073\text{\AA}$). Using Olex2 (Dolomanov, Bourhis, Gildea, Howard, & Puschmann, 2009), the structure was solved with the SHELXT (Sheldrick, 2015b), structure solution program using Intrinsic Phasing and refined with the SHELXL (Sheldrick, 2015a) refinement package using Least Squares minimisation. The structure was solved by direct method and refined on F^2 using all the reflections. All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters and hydrogen atoms bonded to carbon and oxygen atoms were located at calculated positions using a riding model. X-ray crystallographic data and refinement details are given in Table 2.4. Bond distances and angles as well as hydrogen bond parameters for all compounds are provided in Appendix (**Table A. 1-Table A. 18**).

3.4 Photophysical Characterisation

The photophysical properties of the synthesized compounds within the scope of the project were investigated by UV-vis. absorption and molecular fluorescence spectroscopy techniques. UV-vis. data was recorded using a Varian Cary 50 UV-Vis Spectrophotometer. The absorbance was recorded from 200 nm to 700 nm. Emission spectra of synthesised compounds was recorded using a Varian Cary Eclipse fluorimeter.

3.4.1 Determination of the fluorescence quantum yield

In this work, fluorescence quantum yield (Φ_F) was determined employing the comparative method based on a reference compound with known Φ_F (Rhys Williams, Winfield, & Miller, 1983). Φ_F is then determined using the followed Equation:

$$\Phi_s = \Phi_r (m_s/m_r)(n_s/n_r)^2$$

Where r and s refer to reference and sample, respectively. m is gradient of the plots of integrated fluorescence intensities against absorbances, and n is the refractive index of solvent. Rhodamine 6G or Phenly-BODIPY was used as a reference ($\Phi_F = 0.95$ in water) (Kubin & Fletcher, 1982). Absorption and emission spectra of reference and sample were measured three different concentrations with absorbance values < 0.1 under identical conditions. The reference and the sample were excited at the same wavelength.

3.4.2 Fluorescence lifetime measurements

Time-Correlated Single Photon Counting (TCSPC) technique was used to determine the fluorescence lifetime (τ_{FI}). The fuorescence decay measurements were carried out with the Horiba Jobin-Yvon Time-Resolved Fluorometer, Fluorolog FL-1057. The instrument was equipped with a nanoLED-458 as the excitation source. The instrument response function (IRF) was measured with an aqueous Ludox solution.

3.5 Preparation of A β ₁₋₄₂ aggregates

A β ₁₋₄₂ human peptide was purchased from thermos scientific (Cas no:J66387, LOT: T081012). A β ₁₋₄₂ peptide (1 mg) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and aliquoted to 10 samples (Yu et al., 2020). The samples were placed at room temperature for 12 h until HFIP evaporated. Then they were dried up in vacuum for 2 h and stored at -20°C. A 50 μ M stock solution was made by redissolving the peptide (0.1mg) with 11 μ L DMSO, followed by 432 μ L phosphate buffer solution (PBS, pH:7.4) and A β aggregates were

prepared by shaking gently these solutions for 7 days at room temperature. The formation of A β aggregates were confirmed by ThT assay (Rajasekhar et al., 2016).

3.6 Preparation of ThT assay

ThT (Sigma, product no. T3516) was dissolved in PBS buffer pH :7.4 and was filtered through a 0.2 μ m syringe filter. Then the concentration of thioflavin T was determined using an extinction coefficient of 36 mM⁻¹cm⁻¹ at 412 nm (Xue, Lin, Chang, & Guo, 2017)

3.7 Preparation of solution of Bovine Serum Albumin (BSA)

BSA was purchased from Sigma Aldrich (Cas No: 9048-46-8). The BSA stock solution was prepared by dissolving 225 μ g BSA in 1 mL PBS (pH: 7.4) and then the concentration of BSA was determined using an extinction coefficient of 45.540 M⁻¹cm⁻¹ at 278 nm (Tatarets et al., 2006)

3.8 Determination of the binding constant of BODIPY-Sq for A β ₄₂ aggregates

Increasing concentration of compounds was titrated against a fixed concentration of A β ₄₂ aggregates and fluorescence intensity was recorded. The K_d binding curve was generated by GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA) by using below equation, where X is concentration of fluorescence probe and Y is change in fluorescence intensity

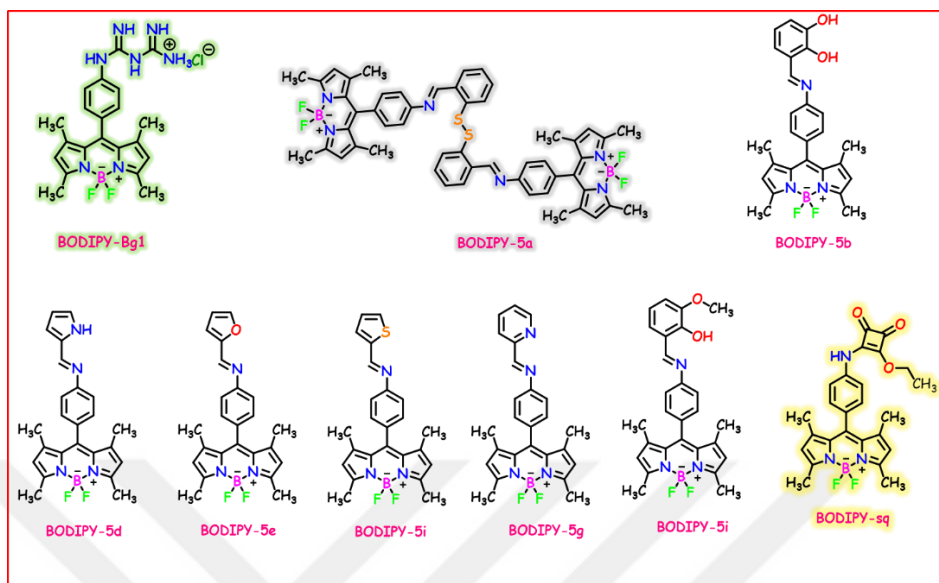
$$Y = B_{\max} * X / (K_d + X)$$

B_{max} is the maximum specific binding has the same units as Y.

K_d is the equilibrium binding constant.

CHAPTER 4 General Conclusion and Future Perspective

The compounds synthesized within the scope of this thesis are given below.



BODIPY-Bg1 contains the dicyanamide group, which is a biguanide at the *meso*-position. From the reaction of various aldehydes with the starting material **BODIPY 2**, *meso*-aryl Schiff bases substitute BODIPY compounds (**BODIPY-5a/5i**) were obtained. Finally, **BODIPY-Sq** contains a diethyl squarate group substituted at the *meso*-phenyl group of the compound. Photophysical properties of synthesised compounds were investigated. The structures of the compounds **BODIPY-5b**, **BODIPY-5d**, **BODIPY-5f**, **BODIPY-5g**, **BODIPY-5i**, and **BODIPY-Sq** were determined by X-ray diffraction studies. Fluorescence sensor properties of compounds against A β aggregates for early detection of Alzheimer's disease were investigated *in vitro*. **BODIPY-Bg1** was very important for us as it is the only water-soluble compound, but it did not show a significant change in emission intensity when incubated with A β aggregates. In **BODIPY-5a** compound, one of BODIPY-imine compounds, quenching was observed in the emission band after addition A β aggregates whereas **BODIPY-Sq** compound showed a promising effect. When **BODIPY-Sq** is incubated with A β aggregates its fluorescence intensity shows an increase. **BODIPY-Sq** displayed low affinity ($K_d = 11.4 \mu\text{M}$) to A β aggregates. On the other hand, BOILED-Egg method which is used to predict the lipophilicity and permeability of BBB indicated that **BODIPY-Sq** can cross to BBB. In this perspective **BODIPY-Sq** whether could stain or not A β plaques *in vitro* would be very helpful for further studies. Furthermore, it would be interesting to try to increase the selectivity and/or lipophilicity with additional modifications to the compounds.

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APPENDIX

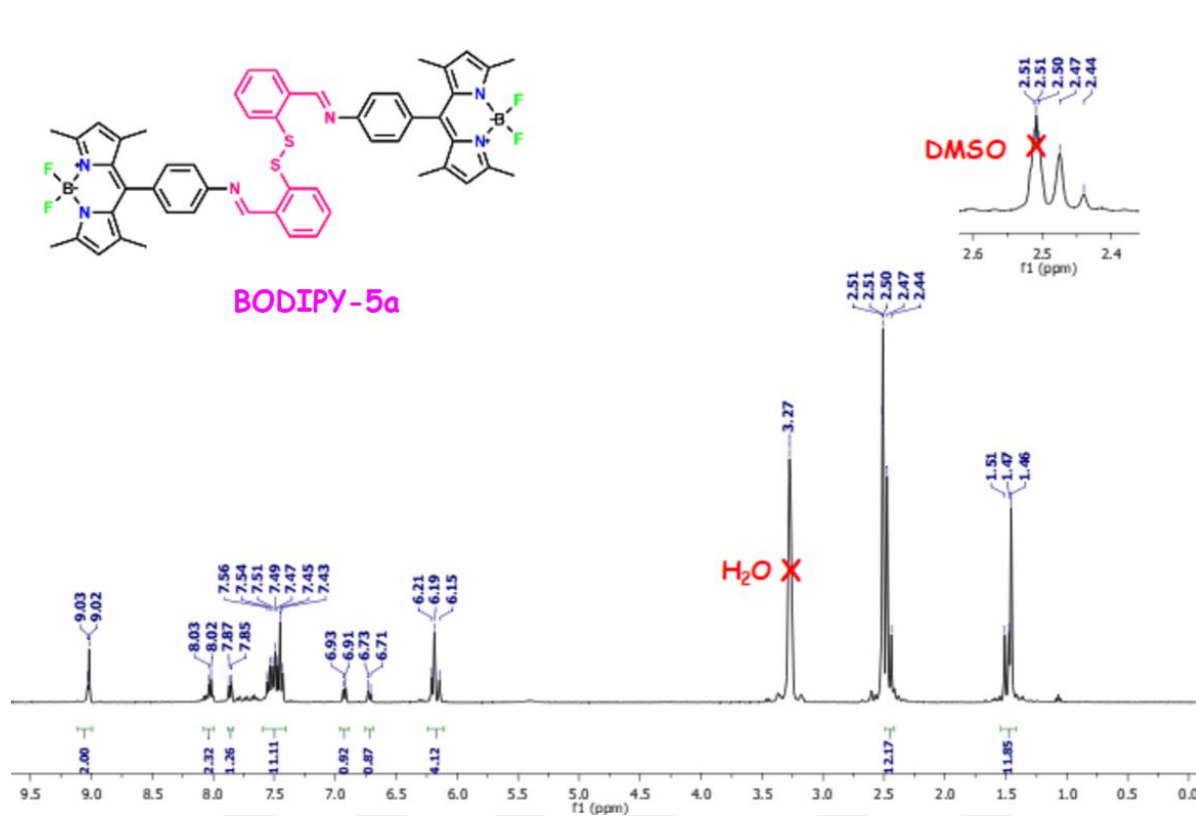


Figure A. 1 ^1H NMR spectrum of BODIPY-5a

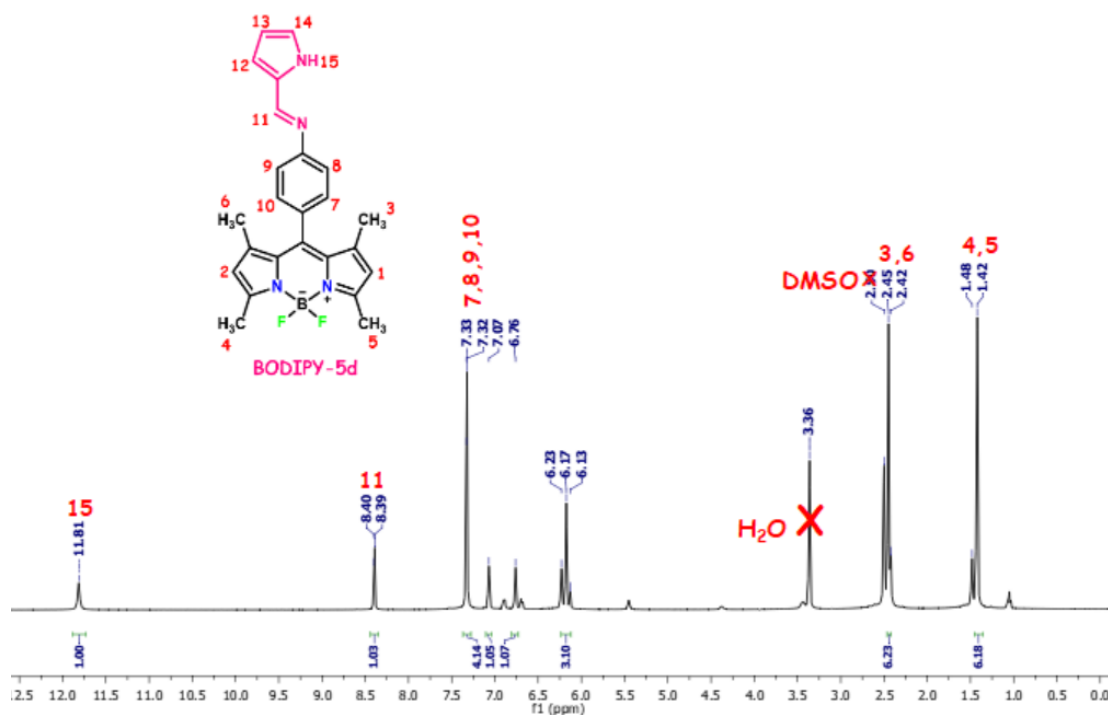


Figure A. 2 ^1H NMR spectrum of BODIPY-5d

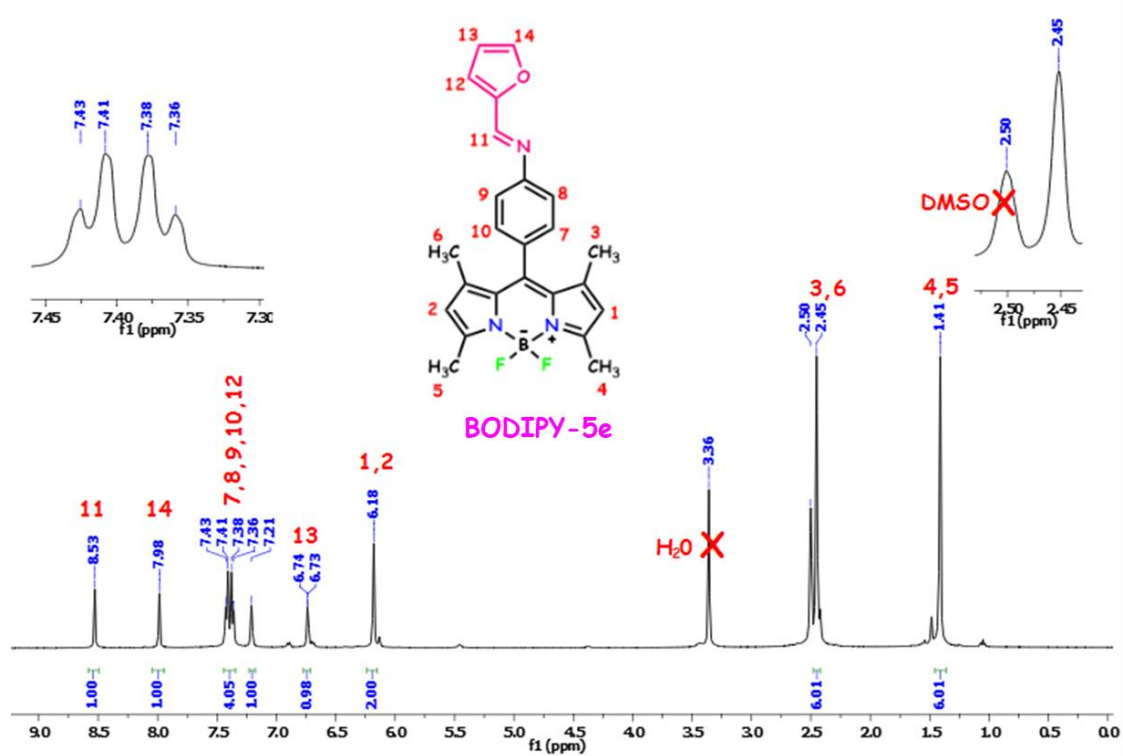


Figure A. 3 ^1H NMR spectrum of BODIPY-5e

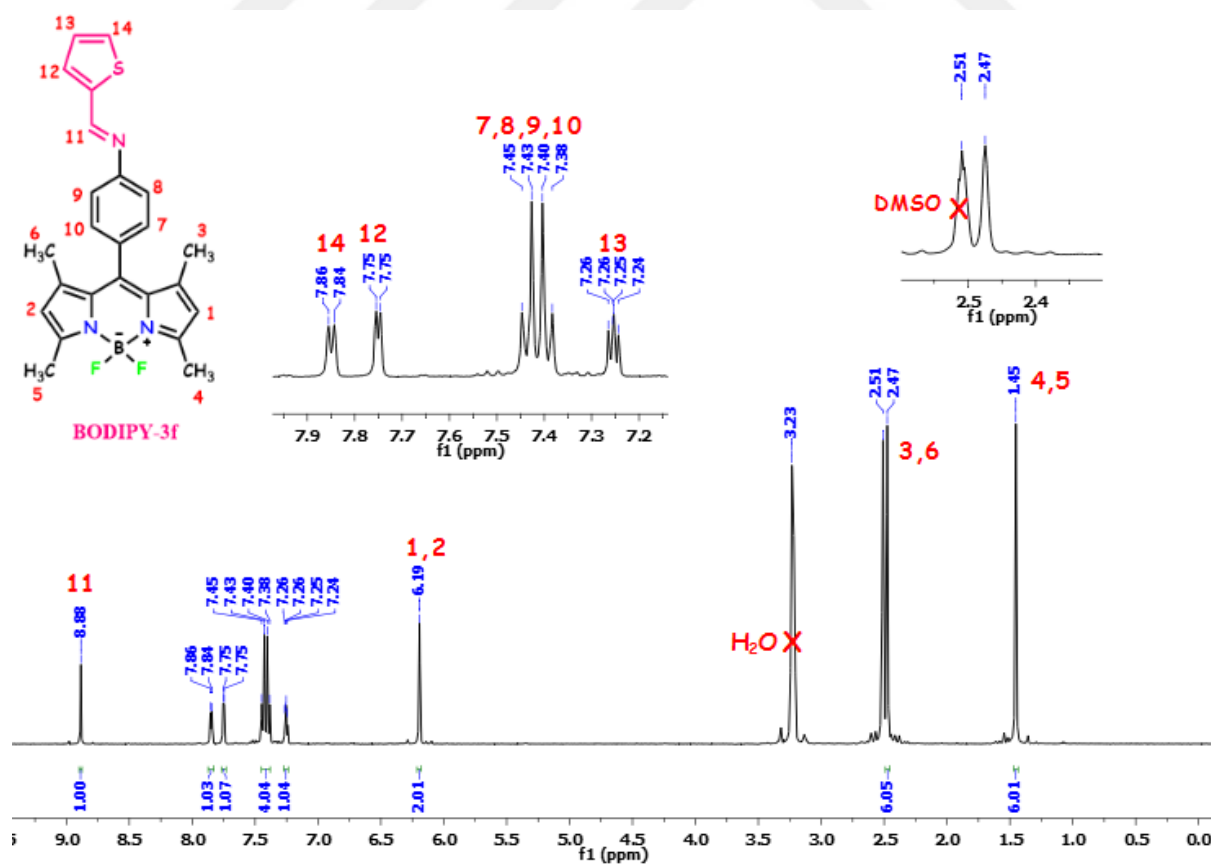
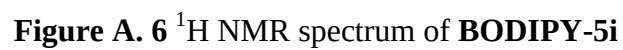


Figure A. 4 ^1H NMR spectrum of BODIPY-5f



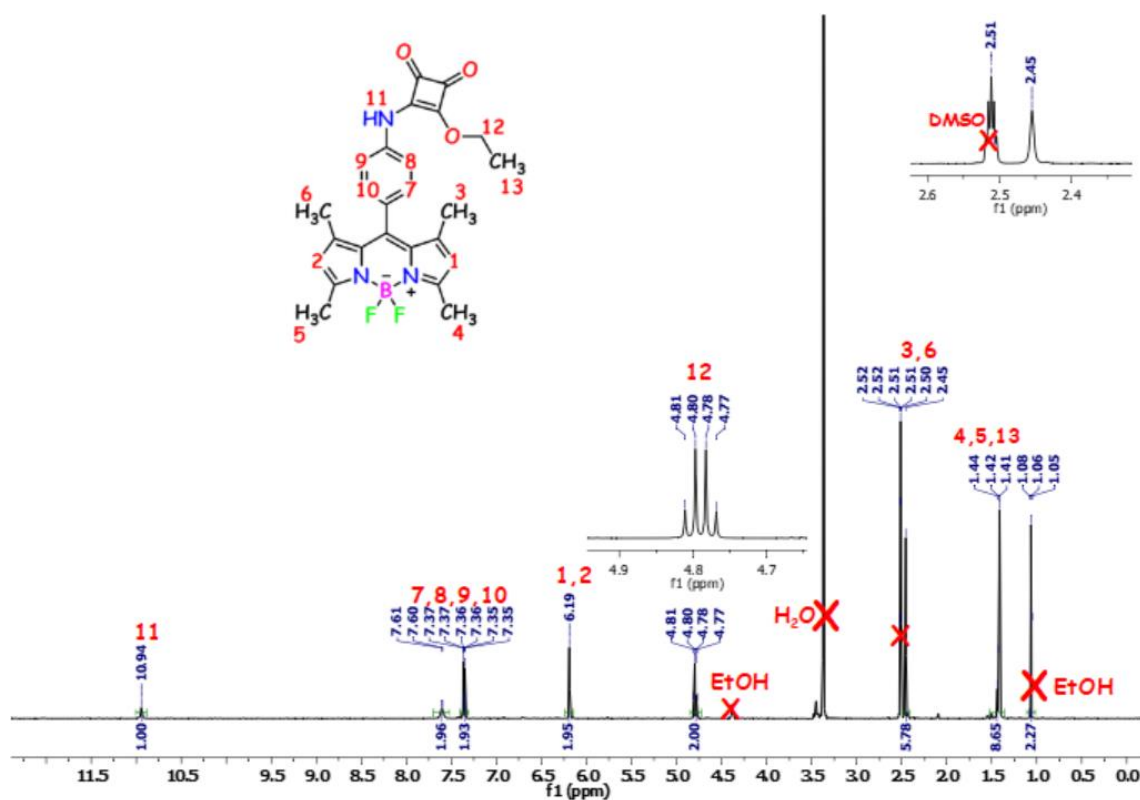


Figure A. 7 ^1H NMR spectrum of BODIPY-Sq

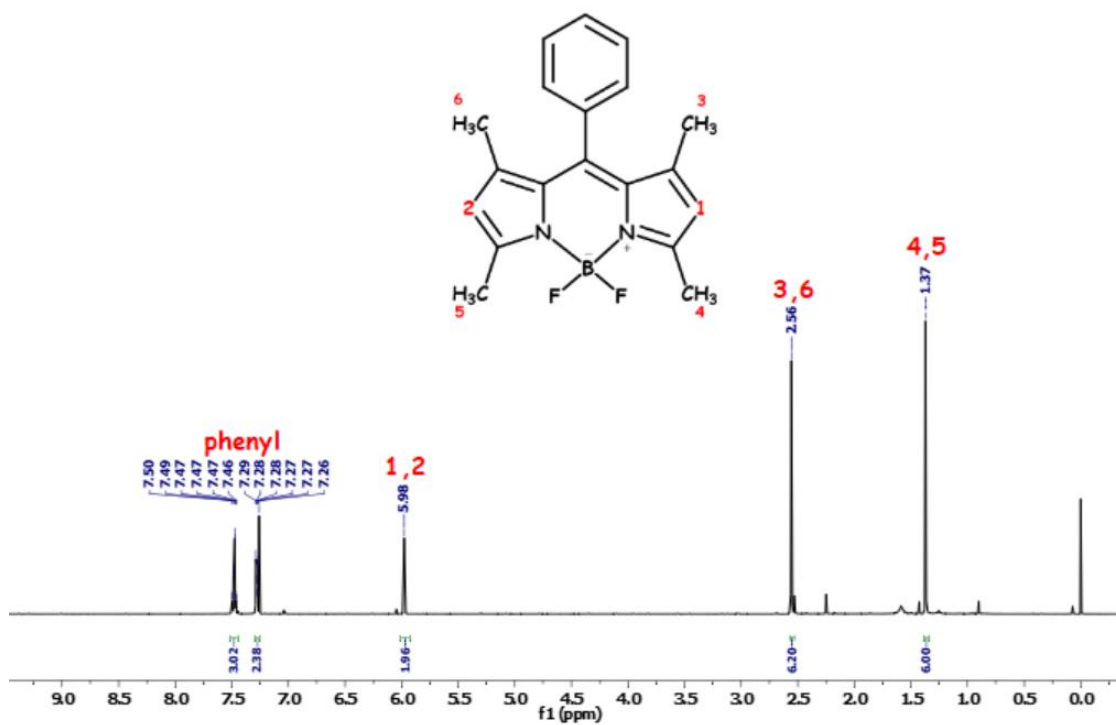


Figure A. 8 ^1H NMR spectrum of Phenyl-BODIPY

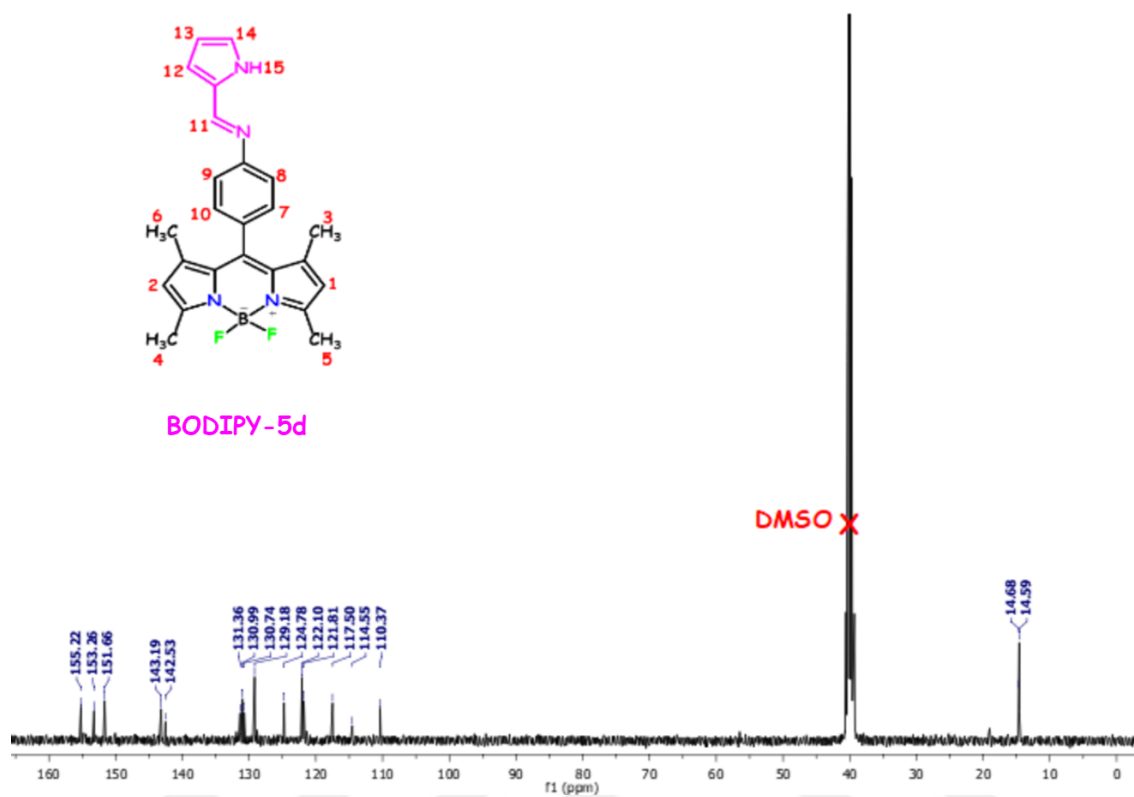


Figure A. 9 ^{13}C NMR spectrum of **BODIPY-5d**

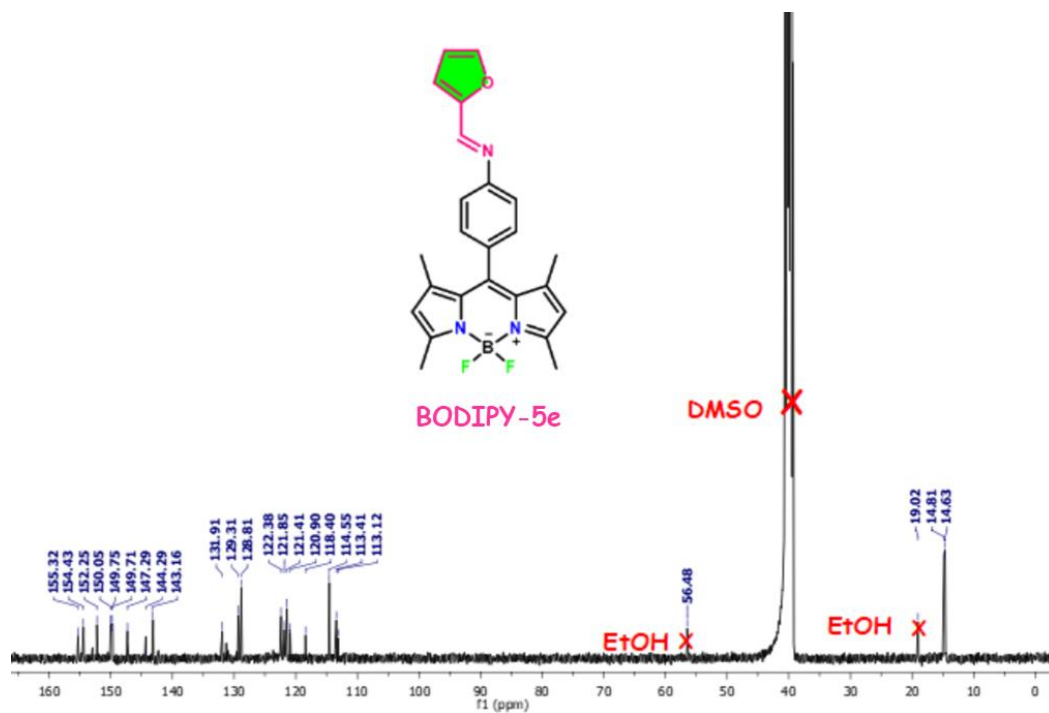


Figure A. 10 ^{13}C NMR spectrum of **BODIPY-5e**

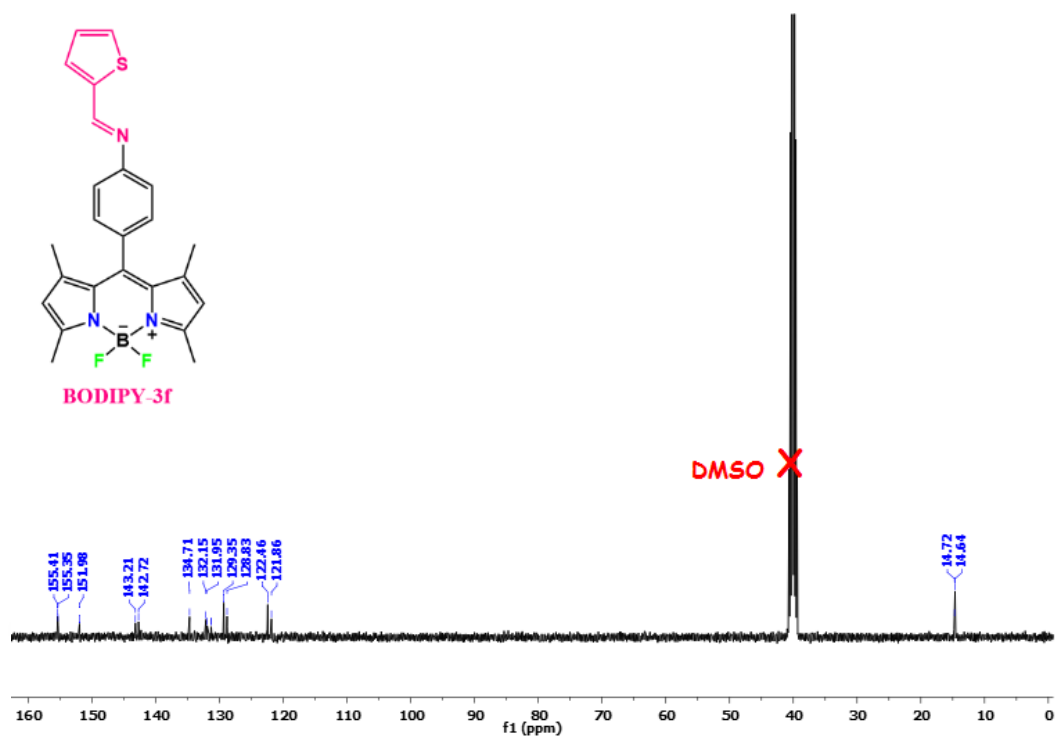


Figure A. 11 ^{13}C NMR spectrum of BODIPY-5f

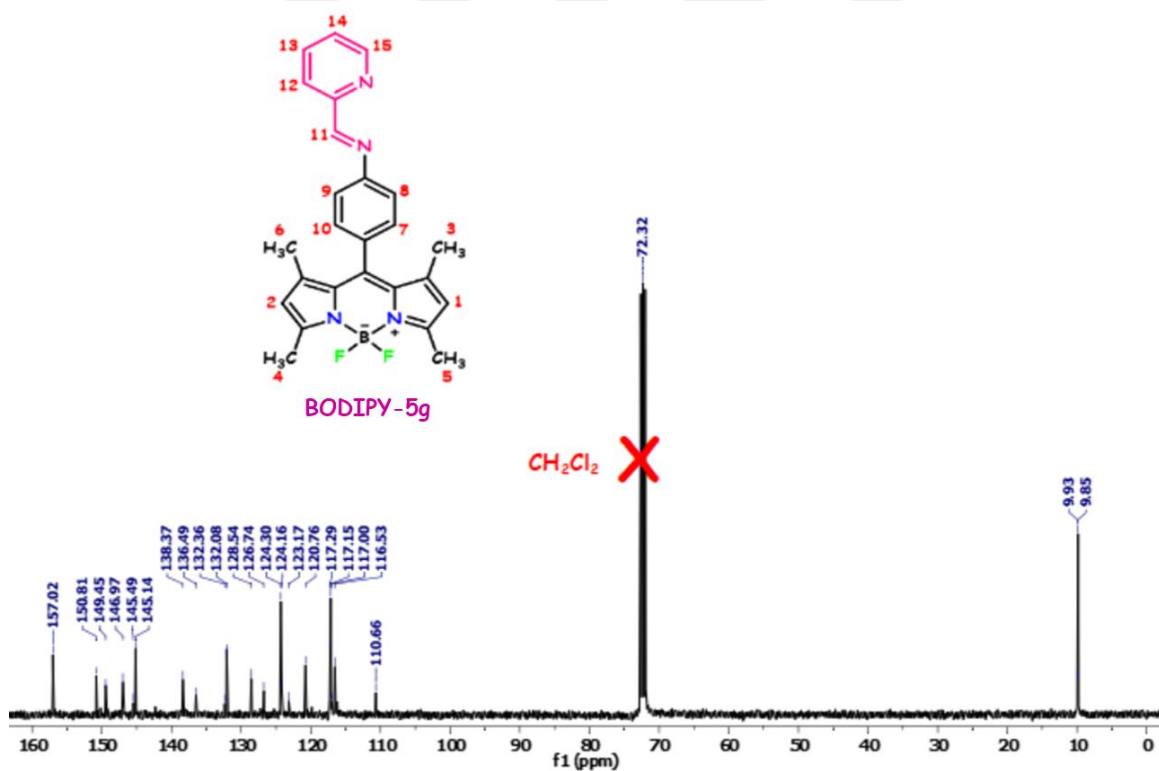


Figure A. 12 ^{13}C NMR spectrum of BODIPY-5g

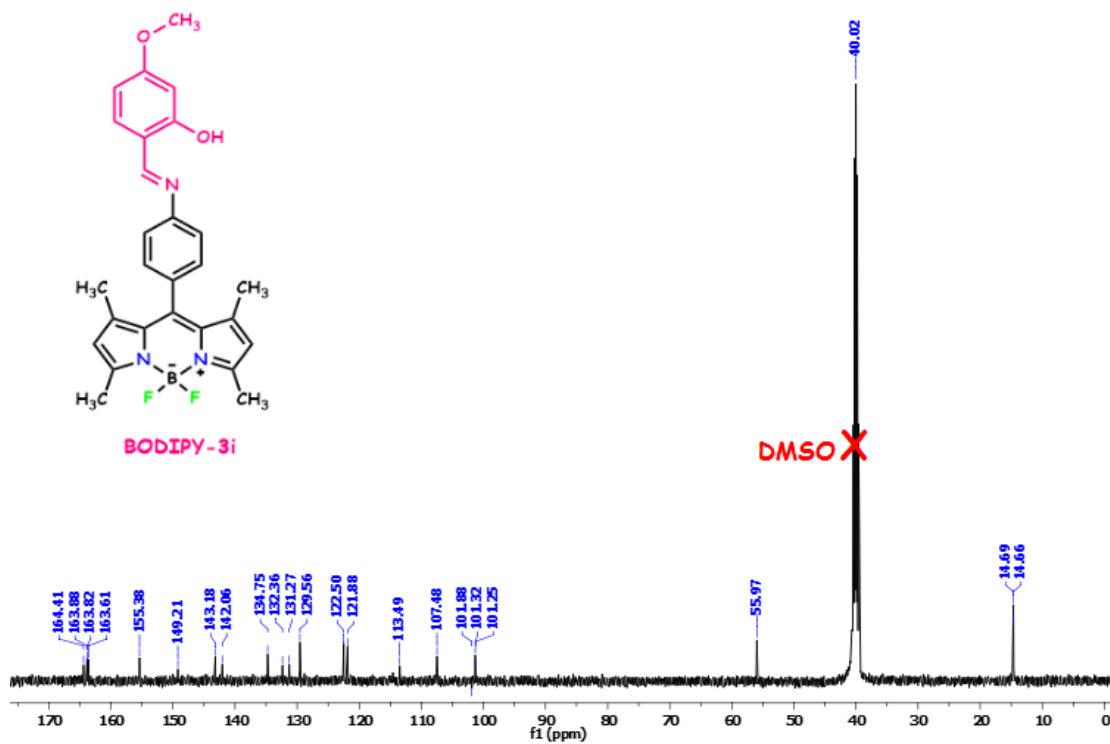


Figure A. 13 ^{13}C NMR spectrum of **BODIPY-5i**

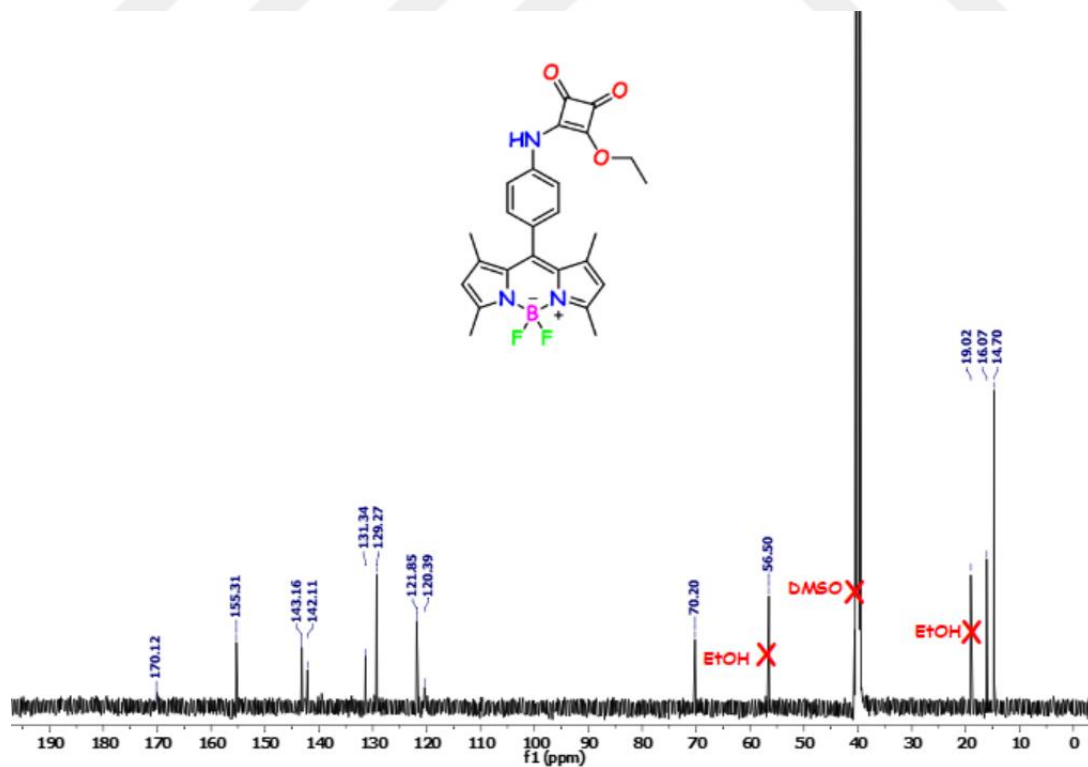


Figure A. 14 ^{13}C NMR spectrum of **BODIPY-Sq**

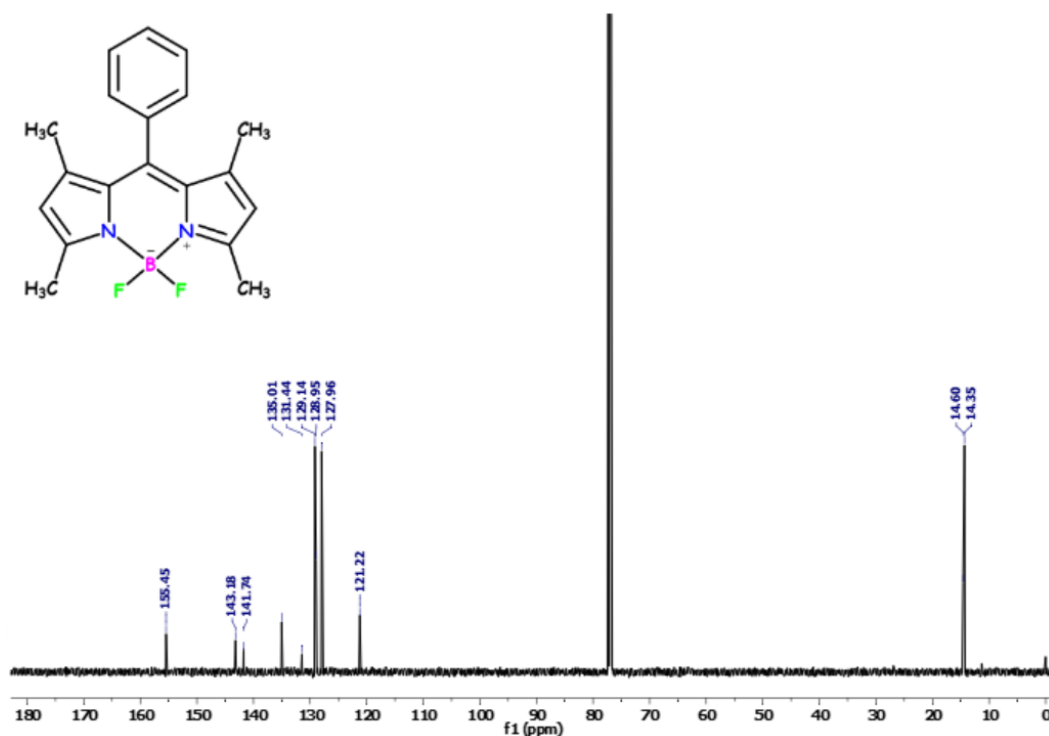


Figure A. 15 ^{13}C NMR spectrum of Phenyl-BODIPY

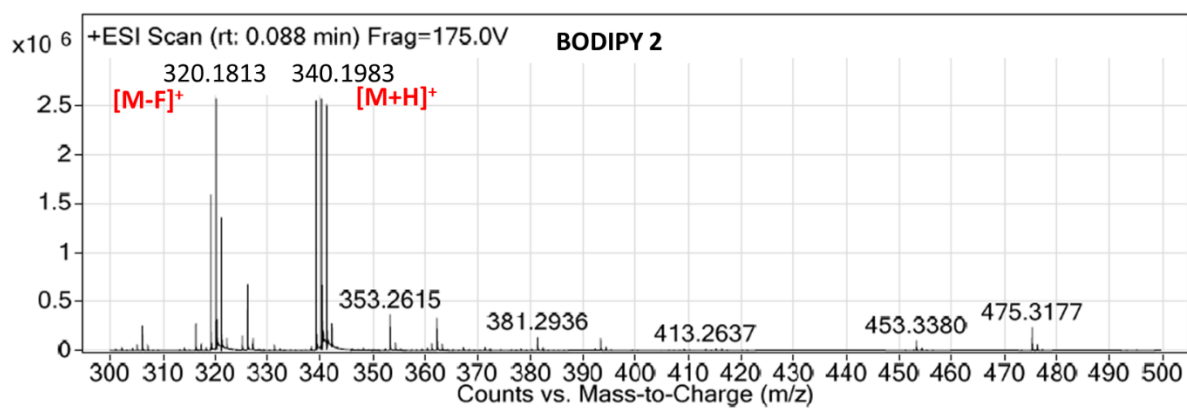


Figure A. 16 ESI-Q-TOF mass spectrum of BODIPY 2

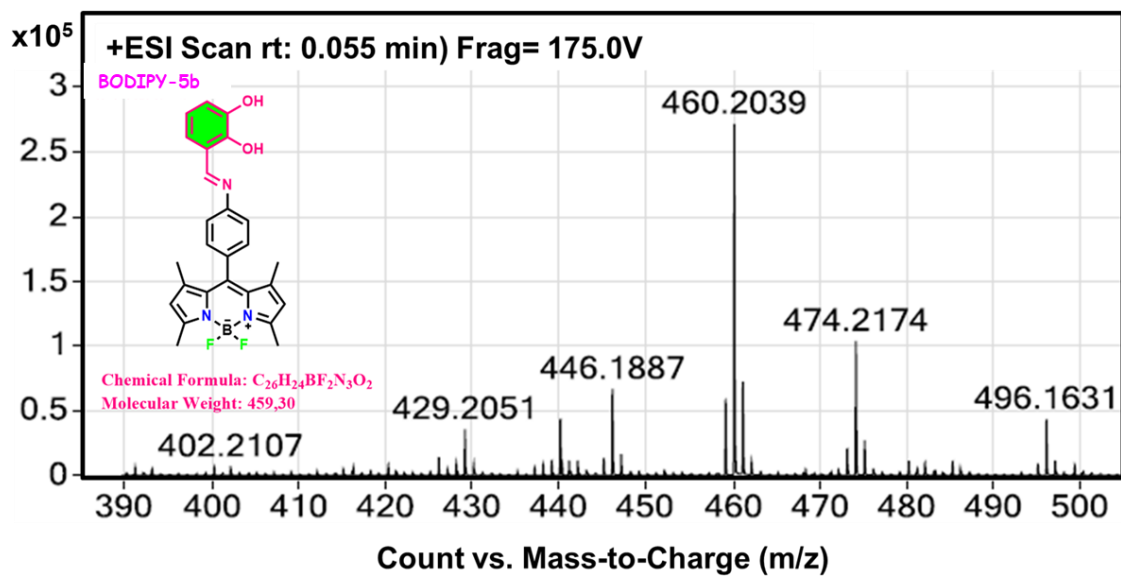


Figure A. 17 ESI-Q-TOF mass spectrum of **BODIPY-5b**

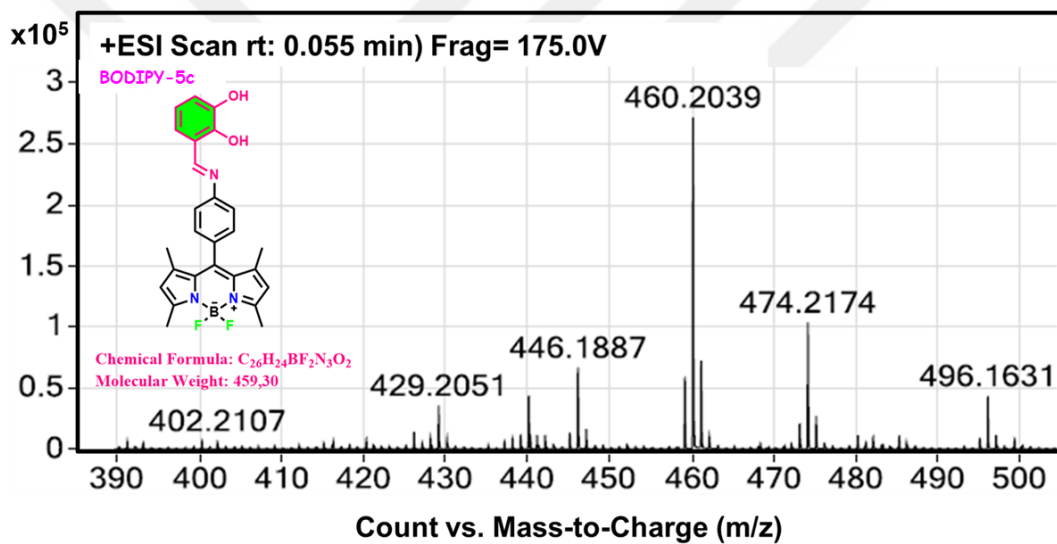


Figure A. 18 ESI-Q-TOF mass spectrum of **BODIPY-5c**

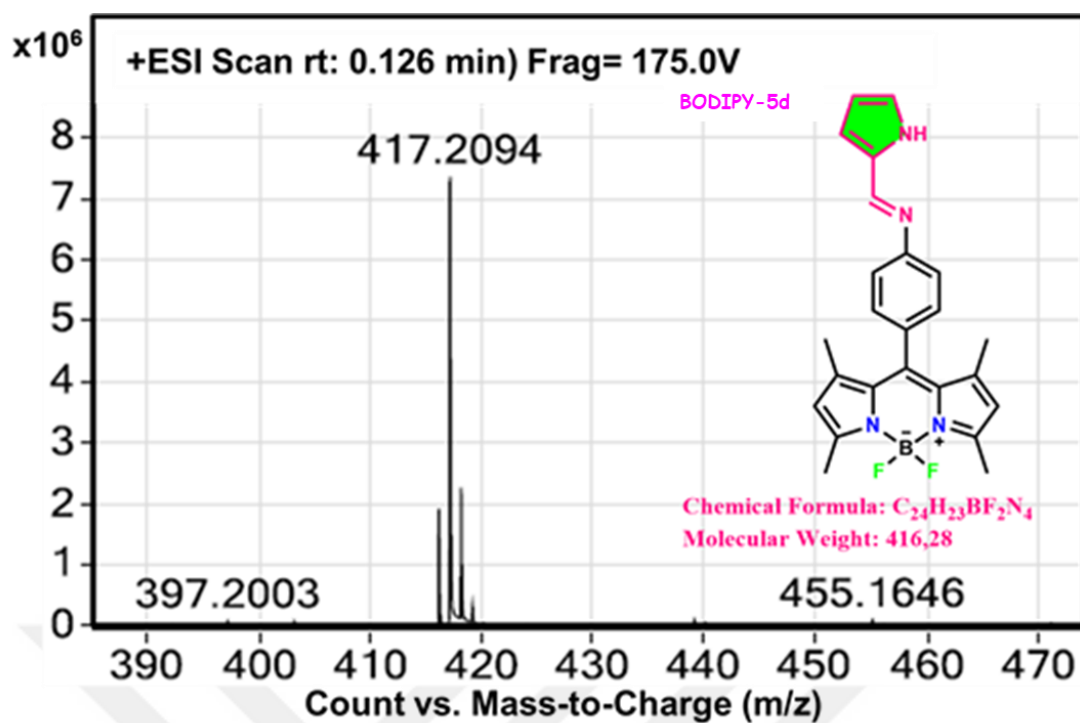


Figure A. 19 ESI-Q-TOF mass spectrum of BODIPY-5d

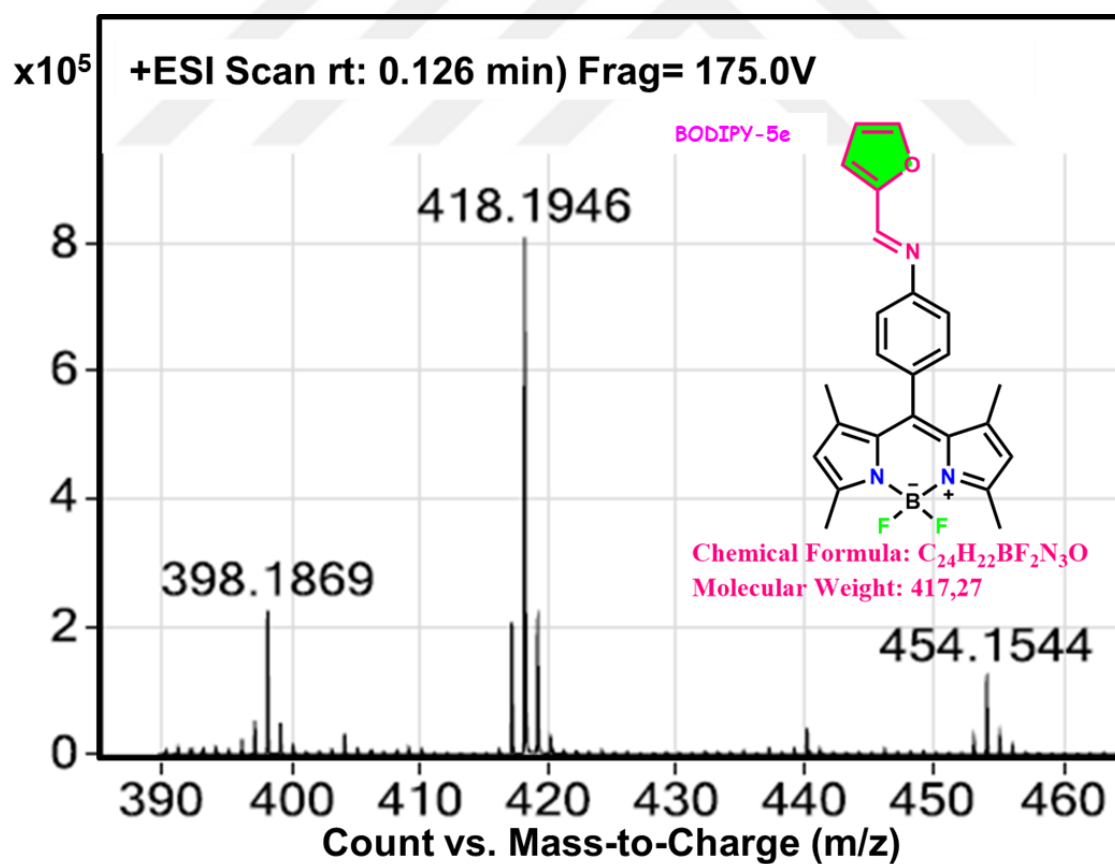


Figure A. 20 ESI-Q-TOF mass spectrum of BODIPY-5e

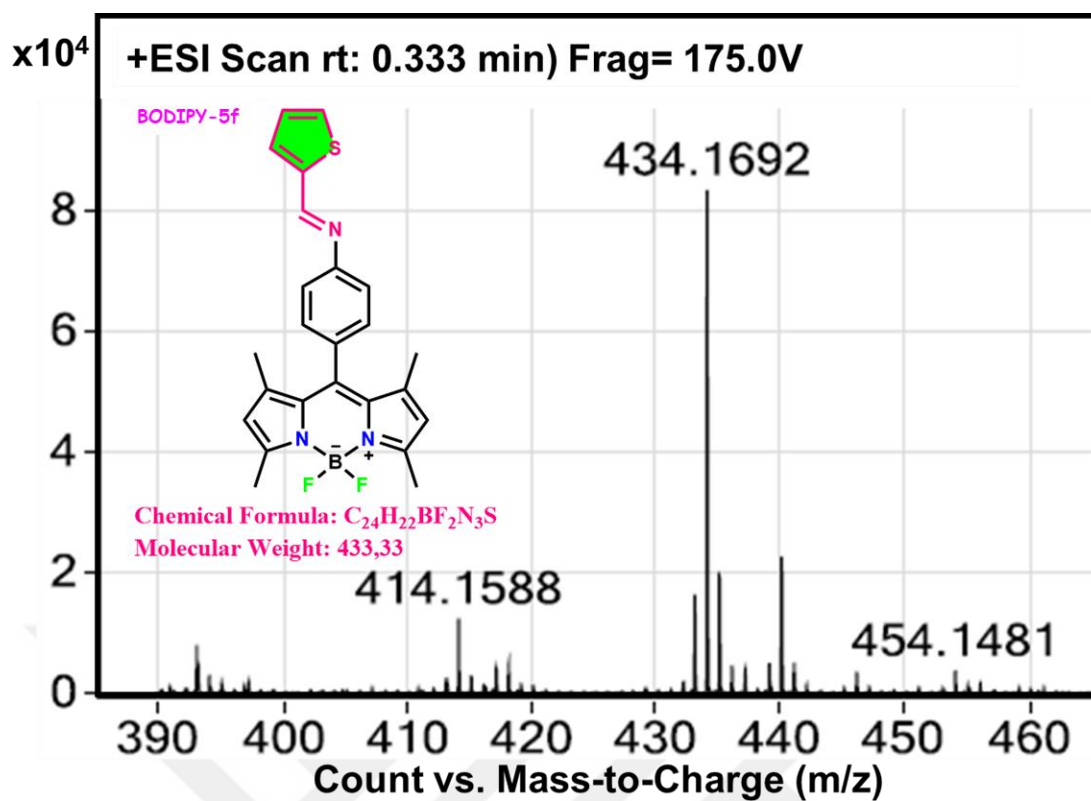


Figure A. 21 ESI-Q-TOF mass spectrum of **BODIPY-5f**

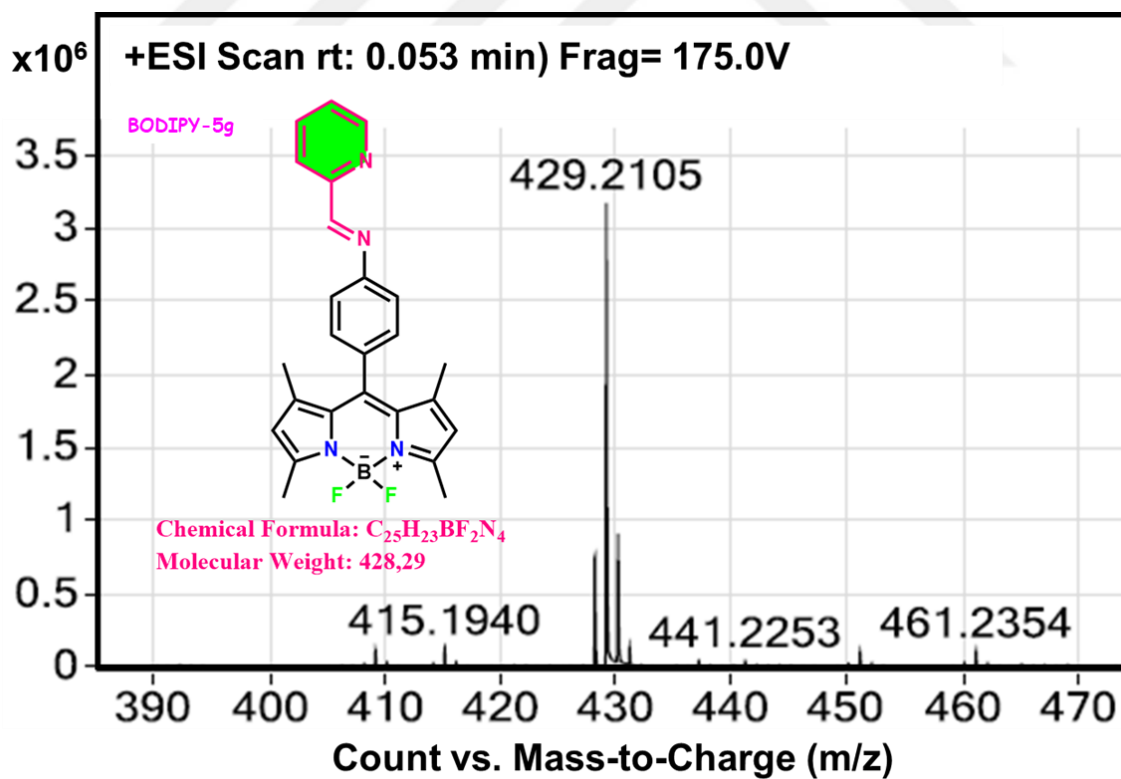


Figure A. 22 ESI-Q-TOF mass spectrum of **BODIPY-5g**

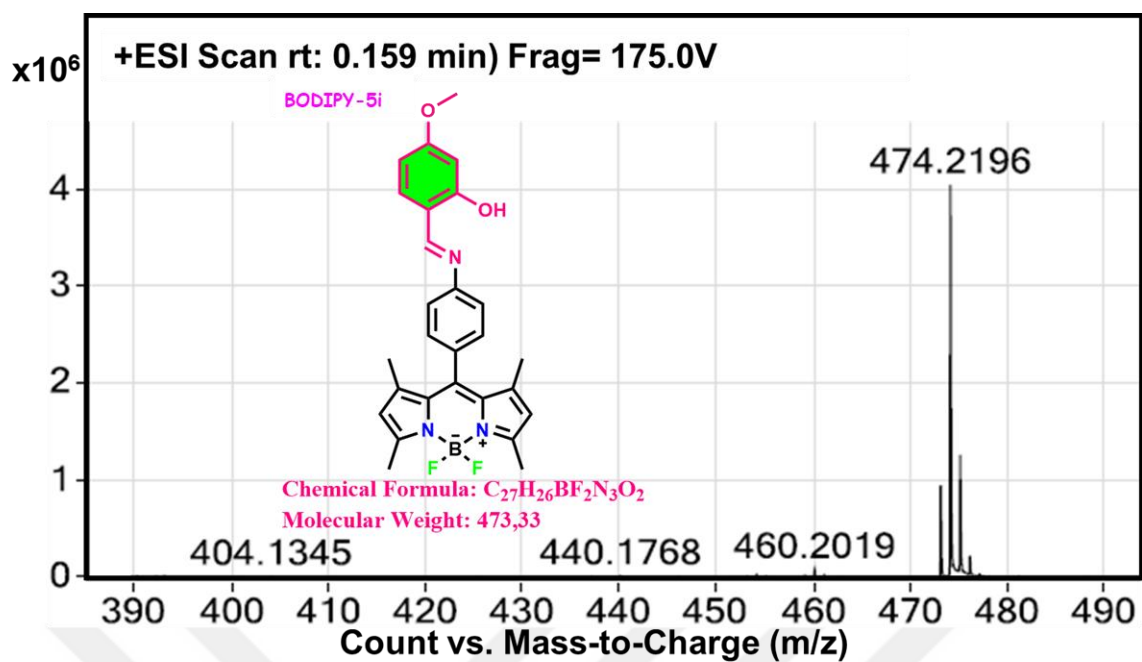


Figure A. 23 ESI-Q-TOF mass spectrum of **BODIPY-5i**

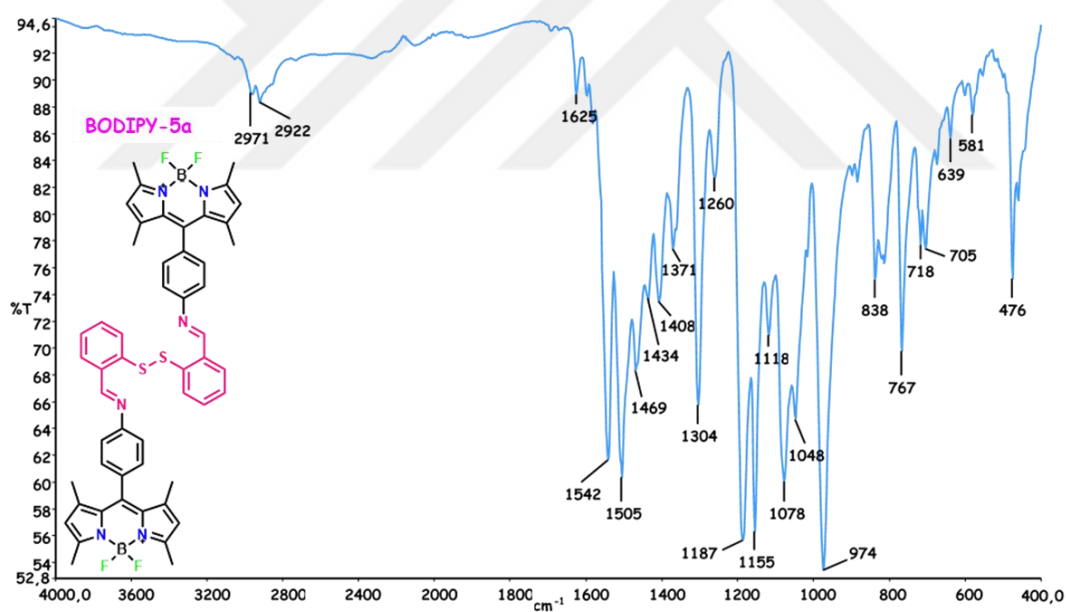


Figure A. 24 FTIR spectrum of **BODIPY-5a**

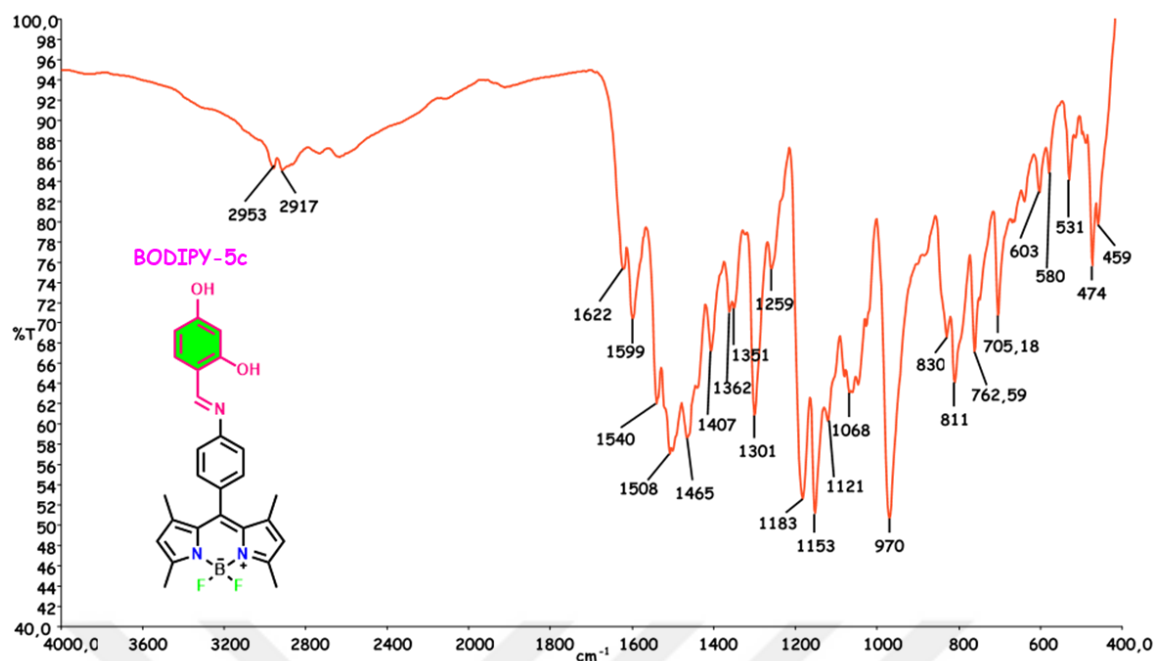


Figure A. 25 FTIR spectrum of **BODIPY-5c**

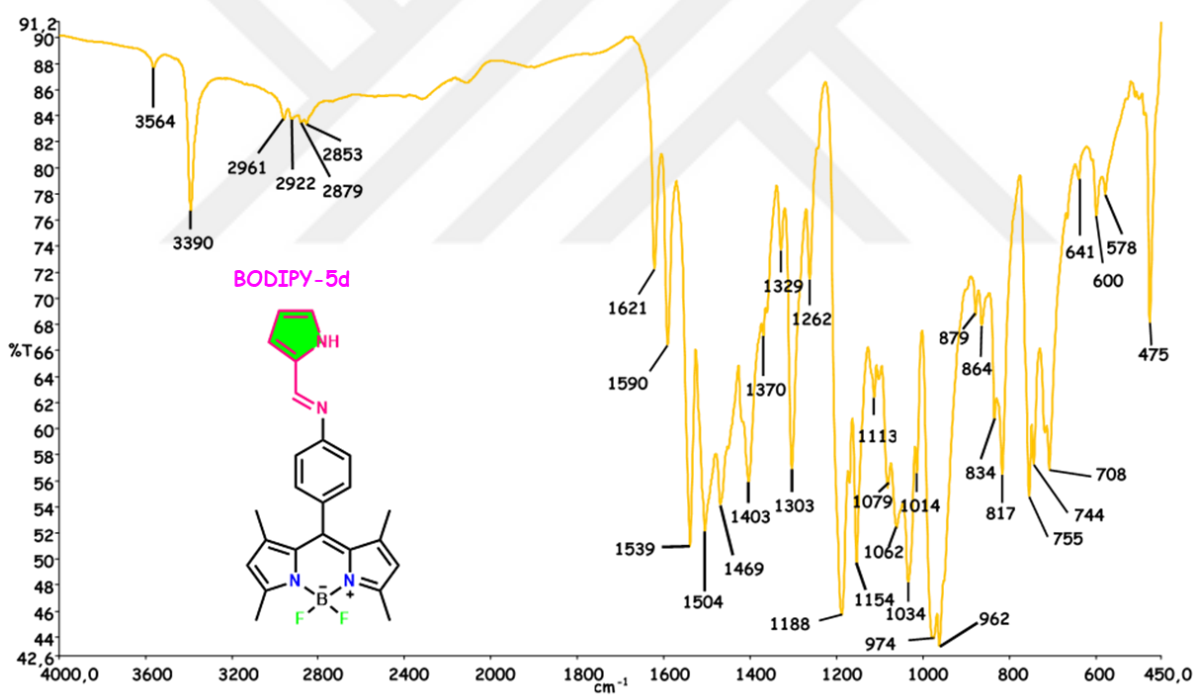


Figure A. 26 FTIR spectrum of **BODIPY-5d**

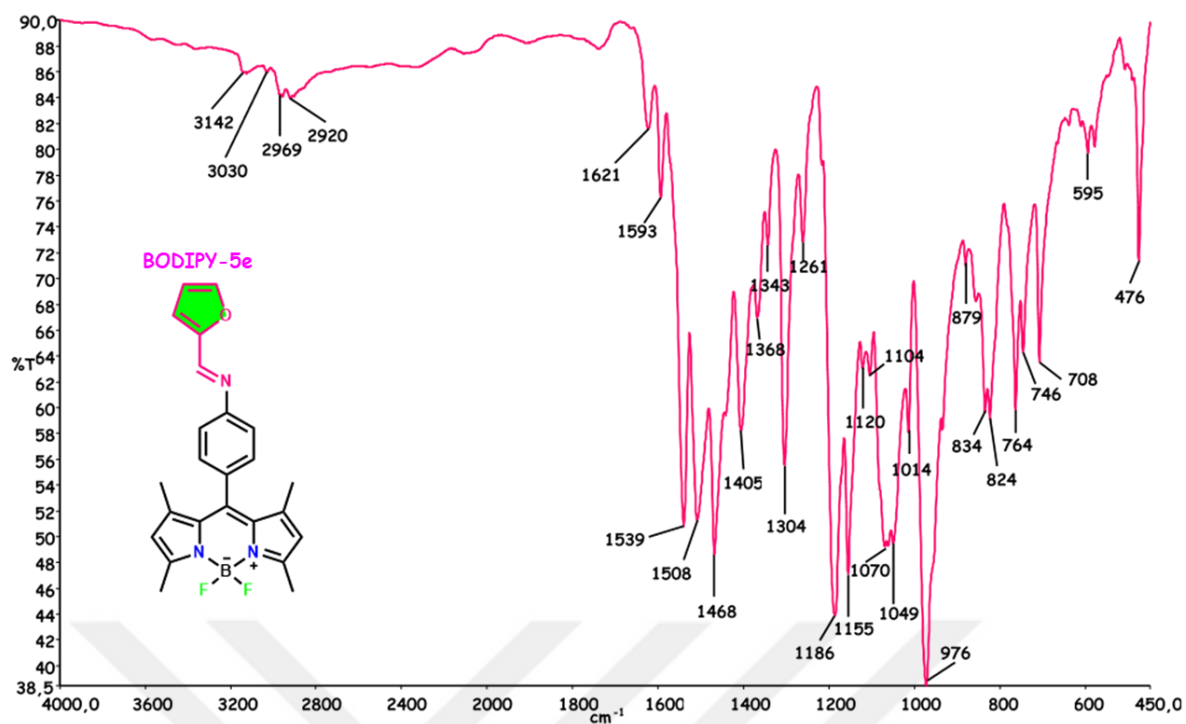


Figure A. 27 FTIR spectrum of **BODIPY-5e**

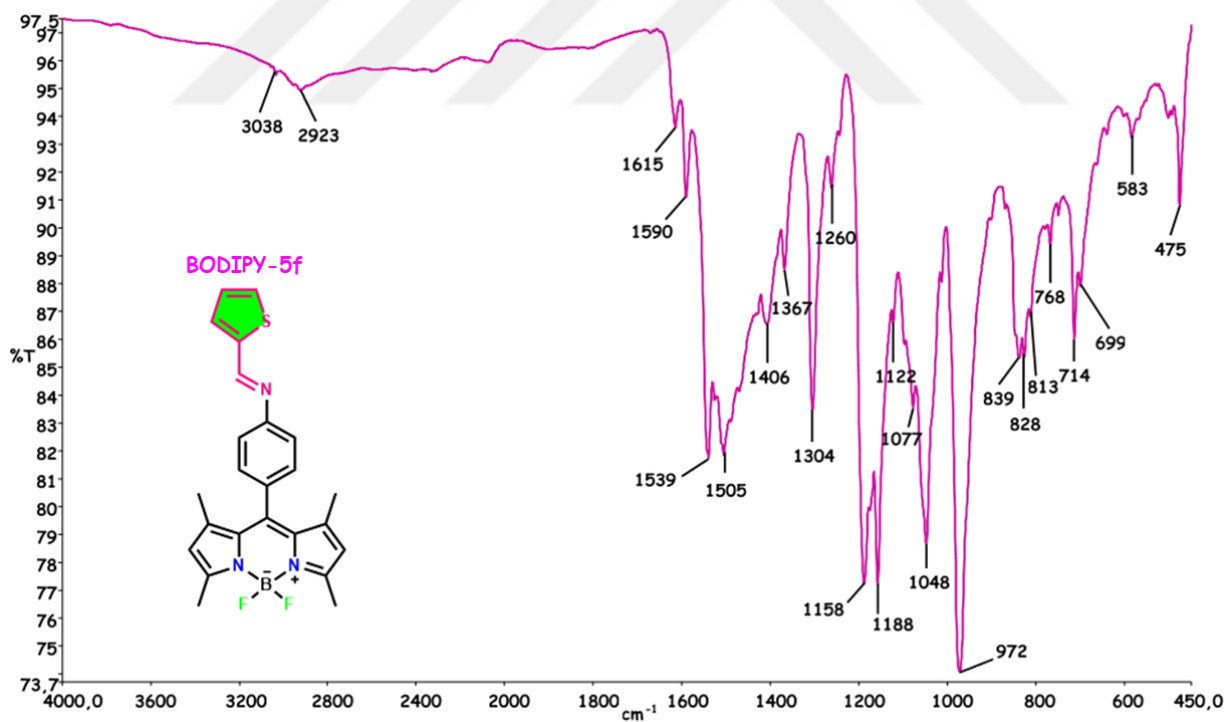


Figure A. 28 FTIR spectrum of **BODIPY-5f**

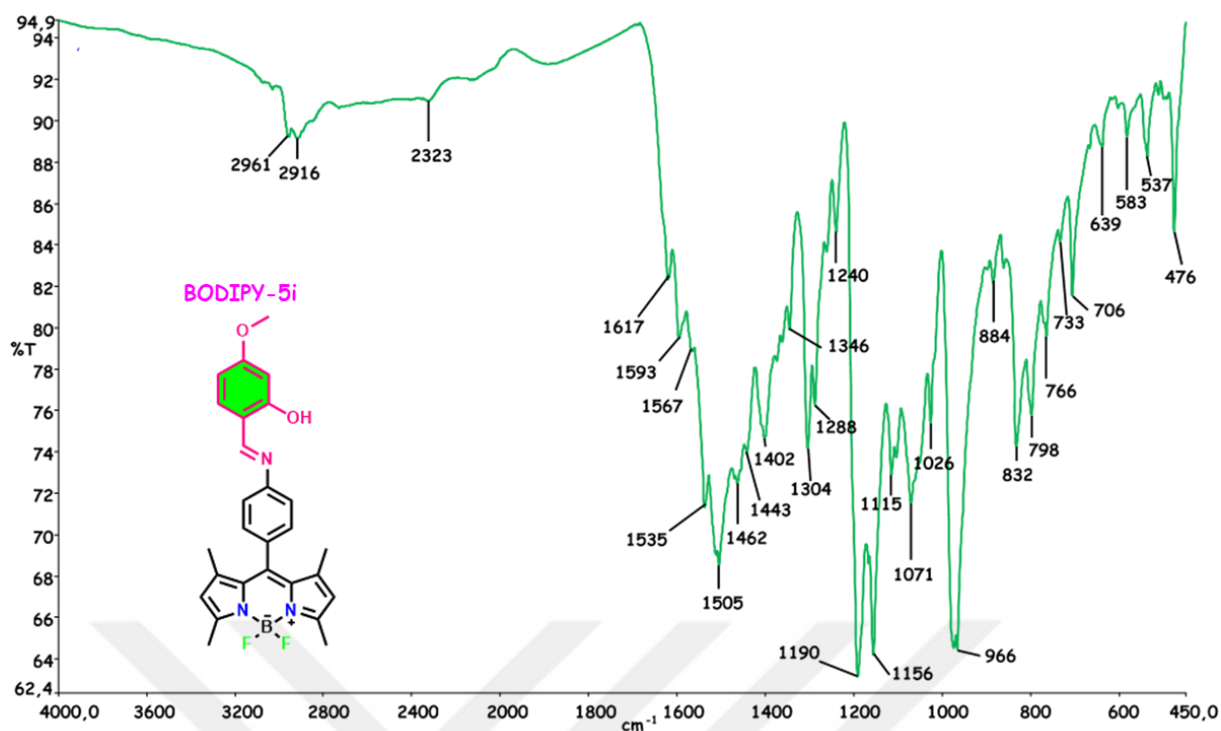


Figure A. 29 FTIR spectrum of **BODIPY-5i**

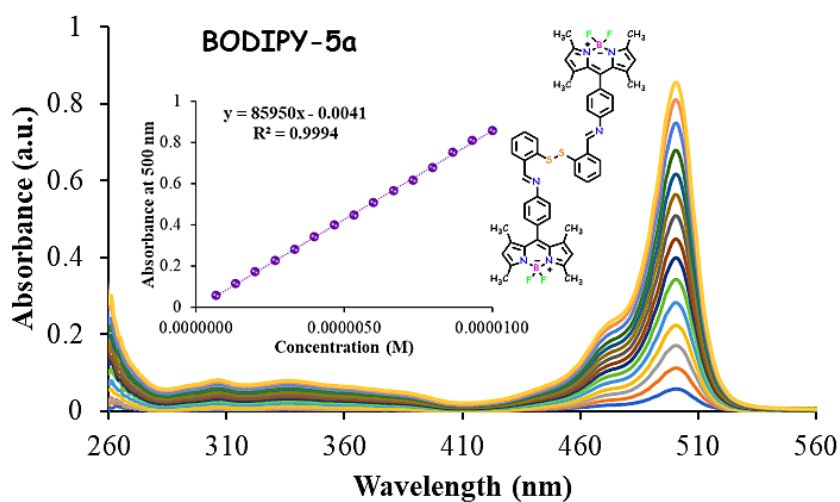


Figure A. 30 Absorption spectra upon the increasing concentration of **BODIPY-5a** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 500 nm versus concentration giving a molar extinction coefficient for **BODIPY-5a** in DMSO.

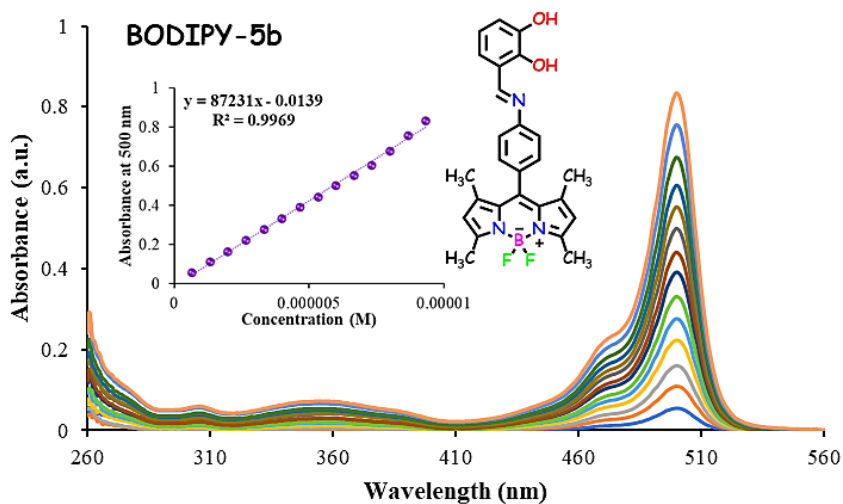


Figure A. 31 Absorption spectra upon the increasing concentration of **BODIPY-5b** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 500 nm versus concentration giving a molar extinction coefficient for **BODIPY-5b** in DMSO.

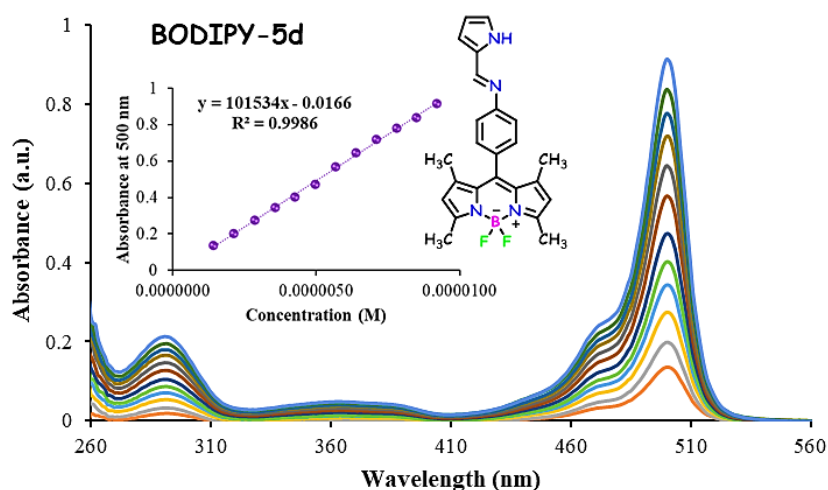


Figure A. 32 Absorption spectra upon the increasing concentration of **BODIPY-5d** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 500 nm versus concentration giving a molar extinction coefficient for **BODIPY-5d** in DMSO.

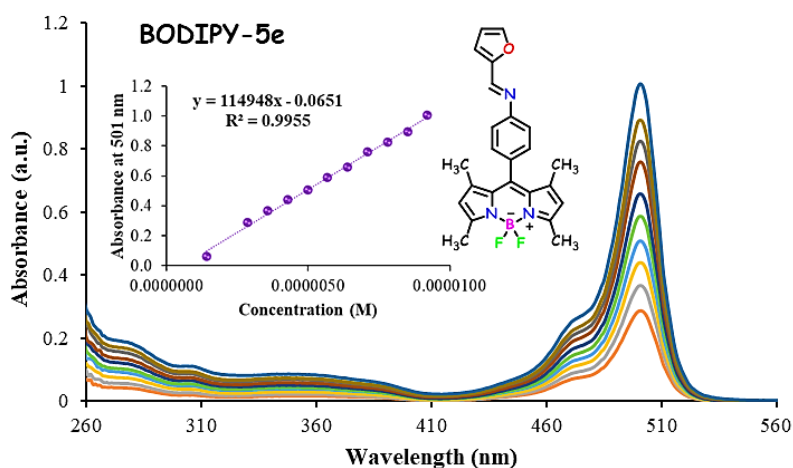


Figure A. 33 Absorption spectra upon the increasing concentration of **BODIPY-5e** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 501 nm versus concentration giving a molar extinction coefficient for **BODIPY-5e** in DMSO.

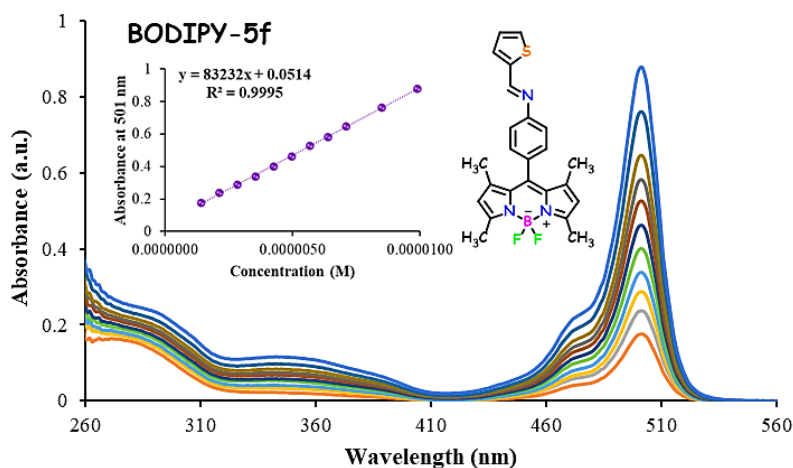


Figure A. 34 Absorption spectra upon the increasing concentration of **BODIPY-5f** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 501 nm versus concentration giving a molar extinction coefficient for **BODIPY-5f** in DMSO.

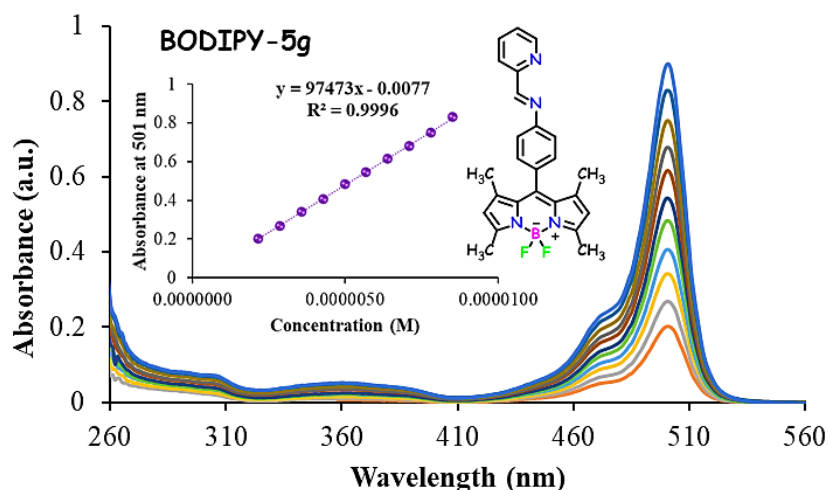


Figure A. 35 Absorption spectra upon the increasing concentration of **BODIPY-5g** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 501 nm versus concentration giving a molar extinction coefficient for **BODIPY-5g** in DMSO.

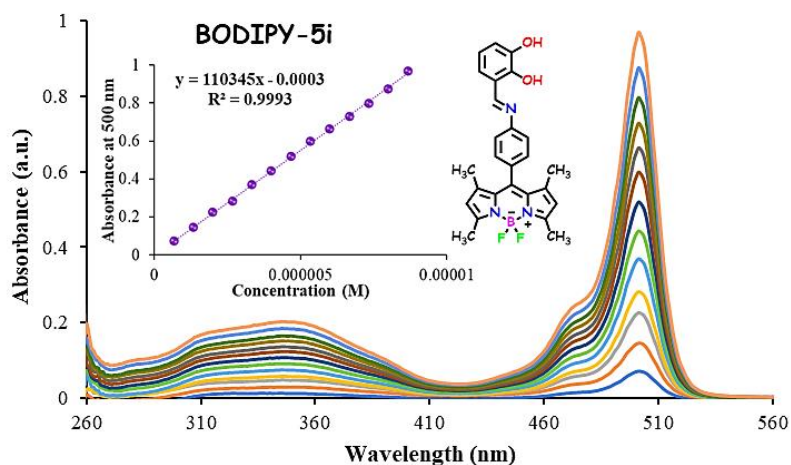


Figure A. 36 Absorption spectra upon the increasing concentration of **BODIPY-5i** in DMSO (0 – 10 μ M). **Inset:** Calibration graph of absorbance at 500 nm versus concentration giving a molar extinction coefficient for **BODIPY-5i** in DMSO.

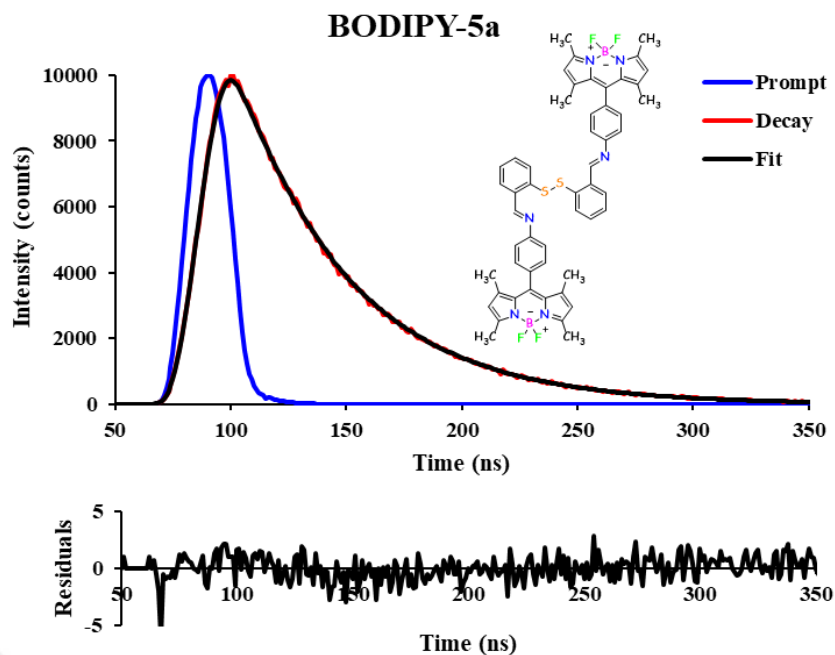


Figure A. 37 Fluorescence decay curve and residuals of fit to a mono-exponential function of **BODIPY-5a** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

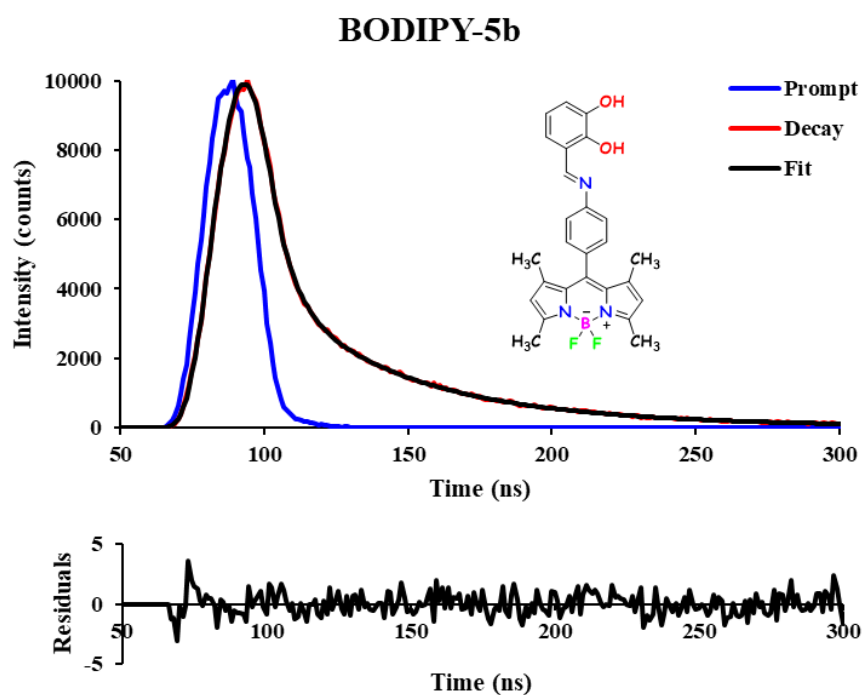


Figure A. 38 Fluorescence decay curve and residuals of fit to a three-exponential function of **BODIPY-5b** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

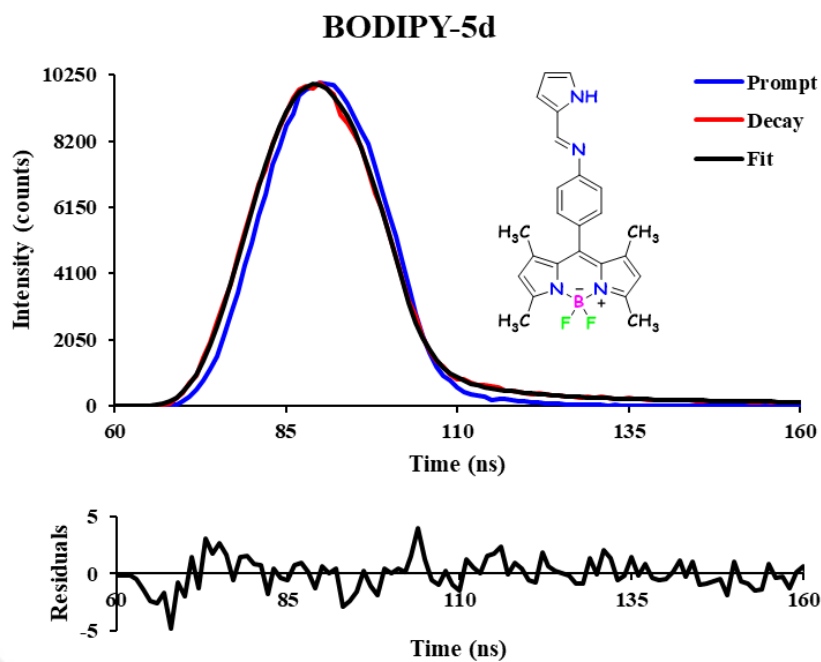


Figure A. 39 Fluorescence decay curve and residuals of fit to a bi-exponential function of **BODIPY-5d** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

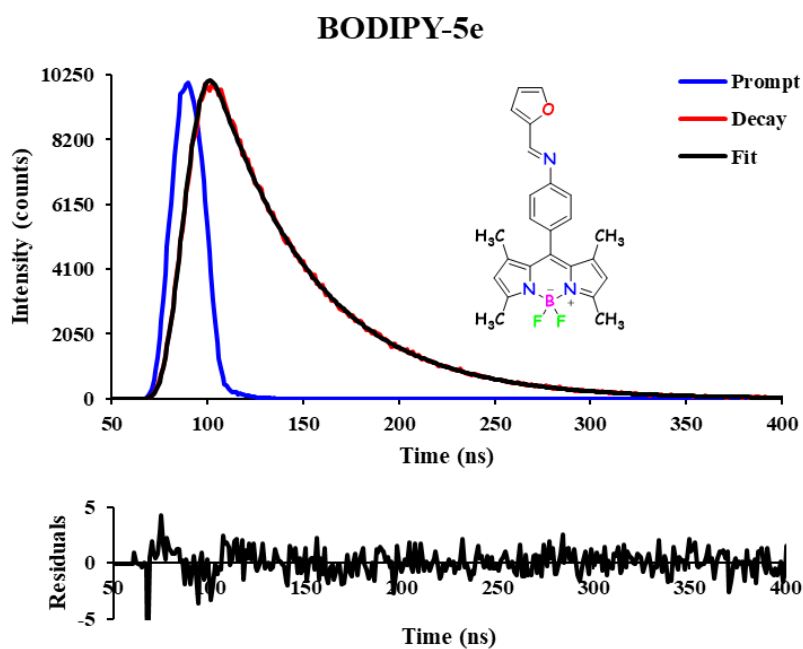


Figure A. 40 Fluorescence decay curve and residuals of fit to a mono-exponential function of **BODIPY-5e** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

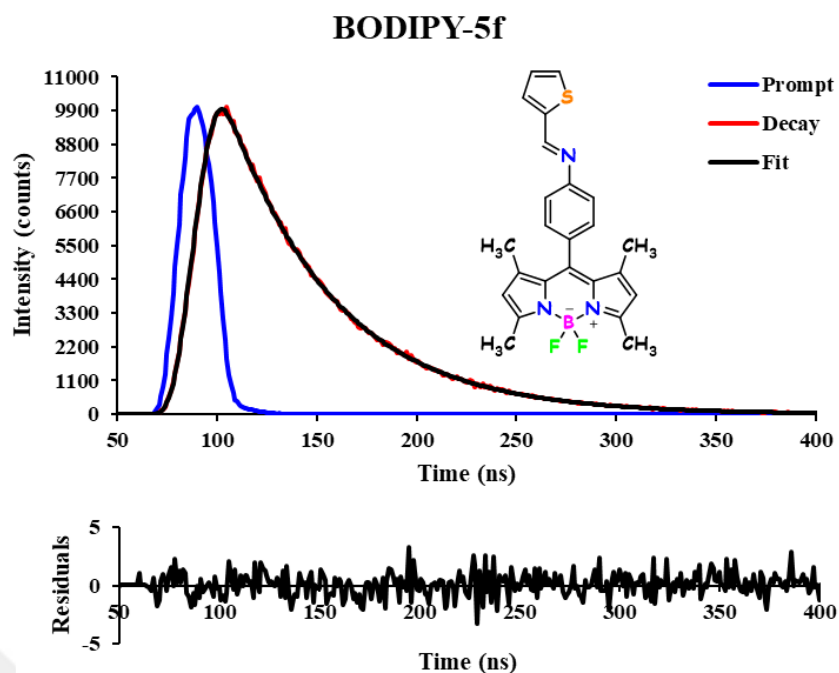


Figure A. 41 Fluorescence decay curve and residuals of fit to a mono-exponential function of **BODIPY-5f** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

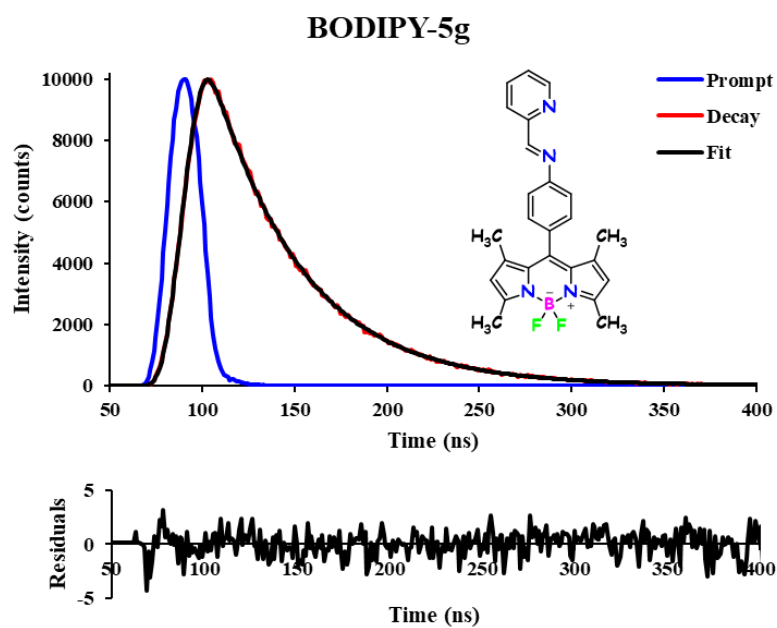


Figure A. 42 Fluorescence decay curve and residuals of fit to a mono-exponential function of **BODIPY-5g** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

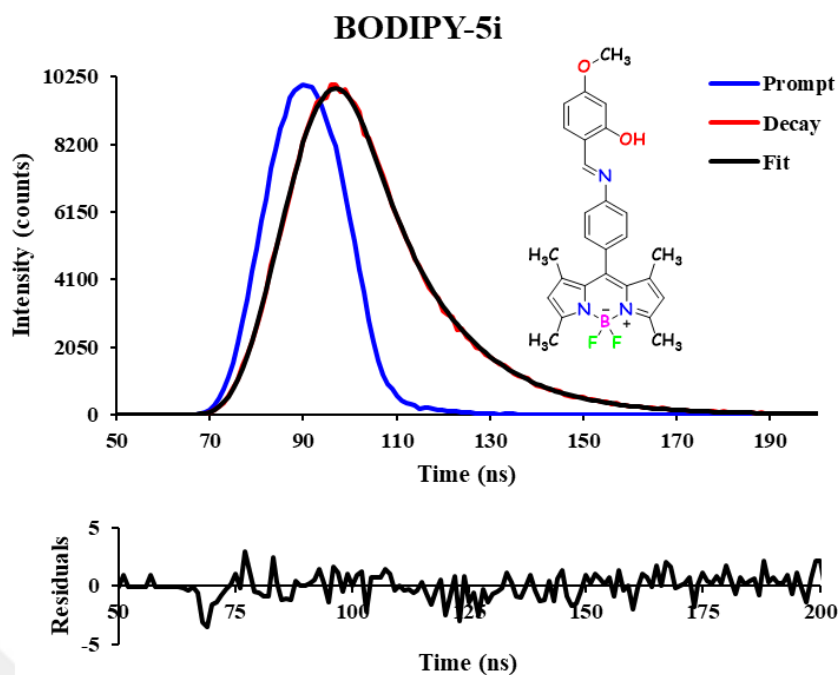


Figure A. 43 Fluorescence decay curve and residuals of fit to a mono-exponential function of **BODIPY-5i** obtained at ambient temperature in DMSO (λ_{ex} : 458 nm)

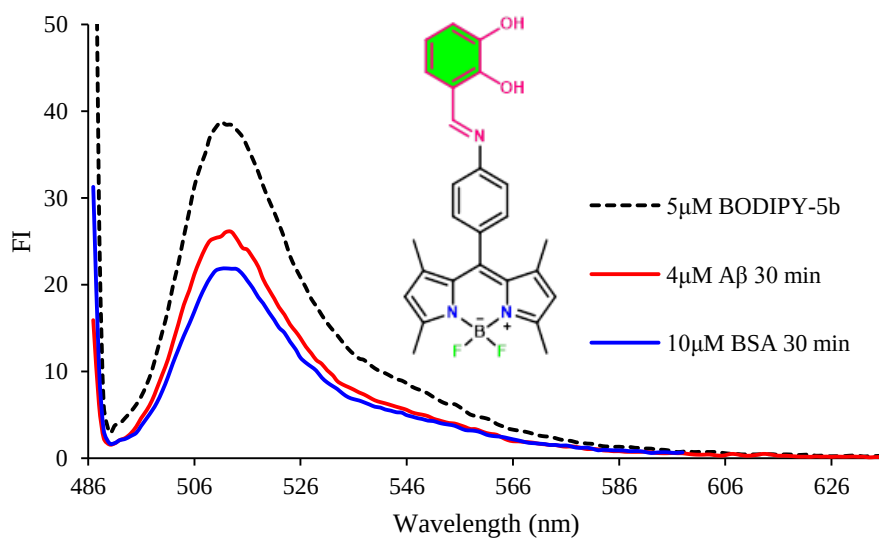


Figure A. 44 Fluorescence response of **BODIPY-5b** upon interaction with A β_{1-42} aggregates (5 μM) and BSA (10 μM) in DMSO/PBS mixture (v/v: 1/1; 10 mM, pH: 7.4). (λ_{Ex} : 480 nm, Slit width: Ex/Em: 2.5/5 nm)

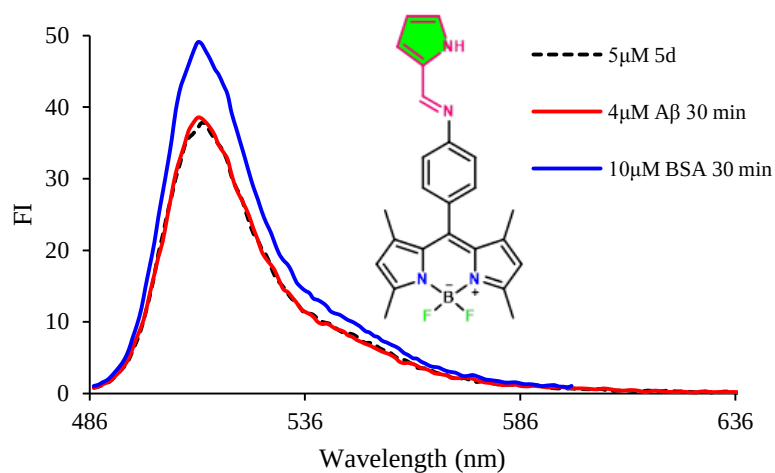


Figure A. 45 Fluorescence response of **BODIPY-5d** upon interaction with A β ₁₋₄₂ aggregates (5 μ M) and BSA (10 μ M) in DMSO/PBS mixture (v/v: 1/1; 10 mM, pH: 7.4). (λ_{Ex} : 480 nm, Slit width: Ex/Em: 2.5/5 nm)

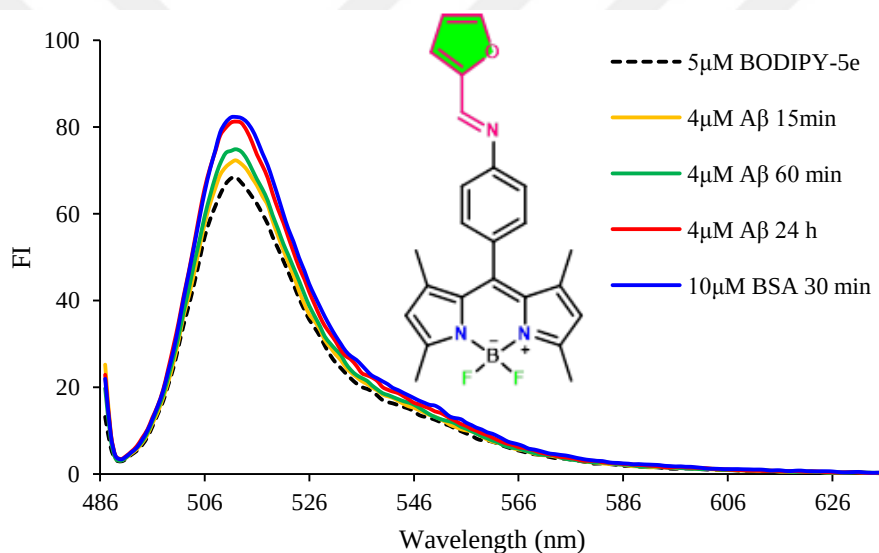


Figure A. 46 Fluorescence response of **BODIPY-5e** upon interaction with A β ₁₋₄₂ aggregates (5 μ M) and BSA (10 μ M) in DMSO/PBS mixture (v/v: 1/1; 10 mM, pH: 7.4). (λ_{Ex} : 480 nm, Slit width: Ex/Em: 5/5 nm)

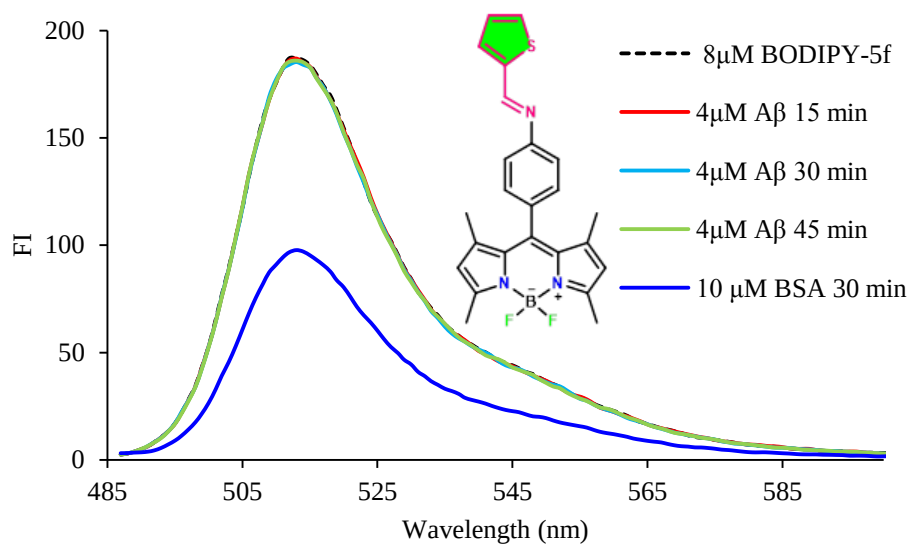


Figure A. 47 Fluorescence response of **BODIPY-5f** upon interaction with A β ₁₋₄₂ aggregates (8 μ M) and BSA (10 μ M) in DMSO/PBS mixture (v/v: 3/1; 10 mM, pH: 7.4). (λ_{Ex} : 480 nm, Slit width: Ex/Em: 2.5/2.5 nm)

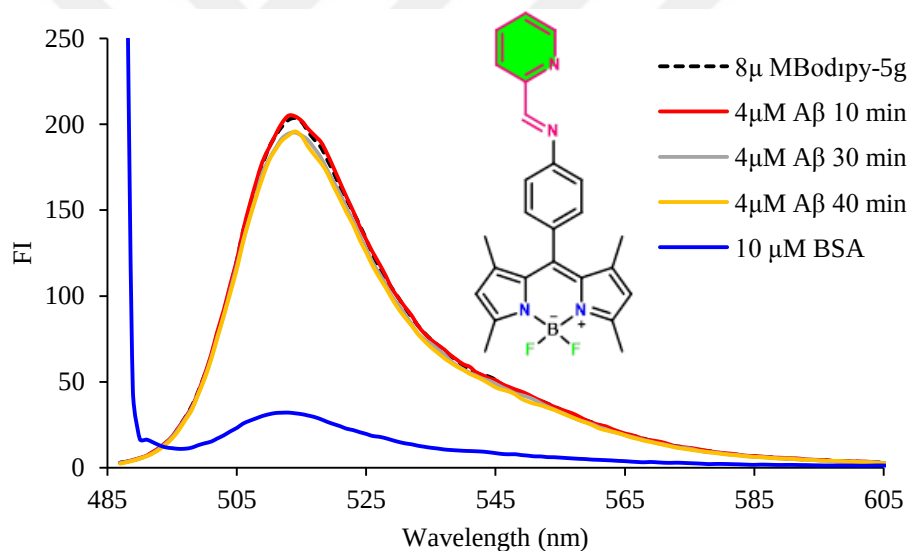


Figure A. 48 Fluorescence response of **BODIPY-5g** upon interaction with A β ₁₋₄₂ aggregates (8 μ M) and BSA (10 μ M) in DMSO/PBS mixture (v/v: 3/1; 10 mM, pH: 7.4). (λ_{Ex} : 480 nm, Slit width: Ex/Em: 2.5/2.5 nm)

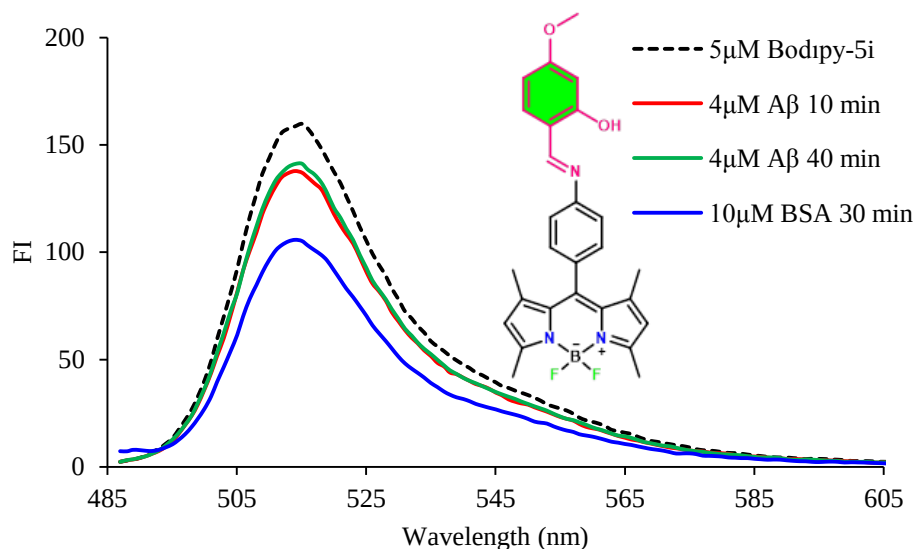


Figure A. 49 Fluorescence response of **BODIPY-5i** upon interaction with A β_{1-42} aggregates (5 μ M) and BSA (10 μ M) in DMSO/PBS mixture (v/v: 3/1; 10 mM, pH: 7.4). (λ_{Ex} : 480 nm, Slid width: Ex/Em: 2.5/5 nm)

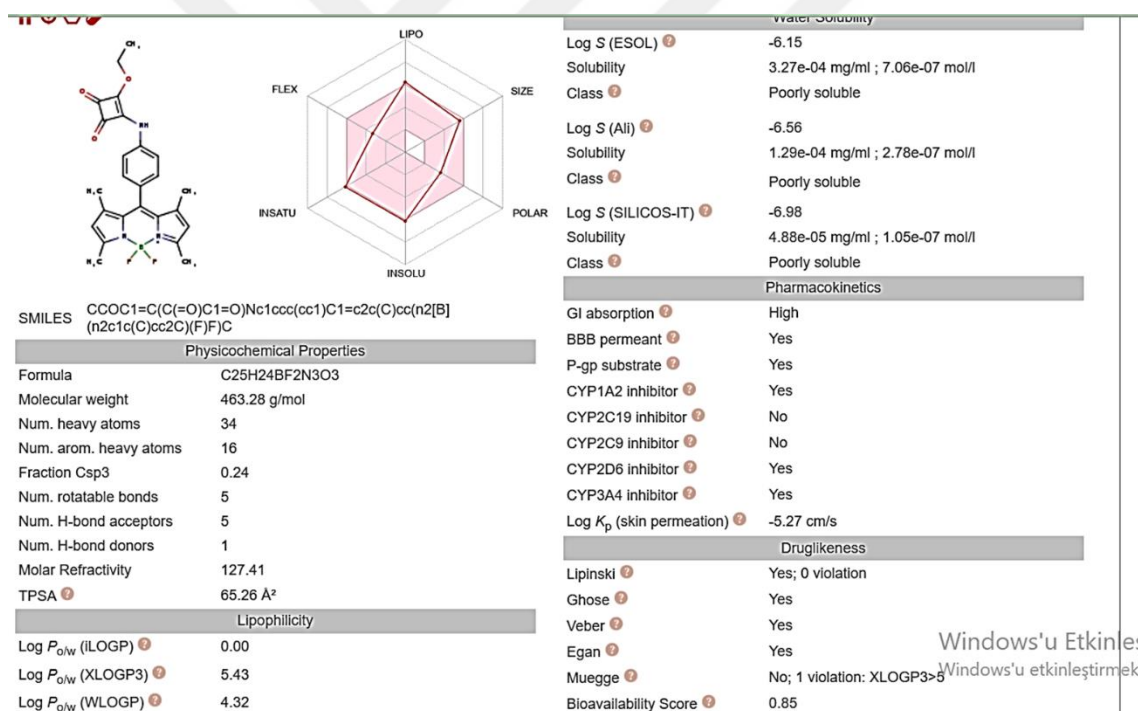


Figure A. 50 Calculation of the lipophilicity of the BODIPY-Sq compound by using the SwissADME website

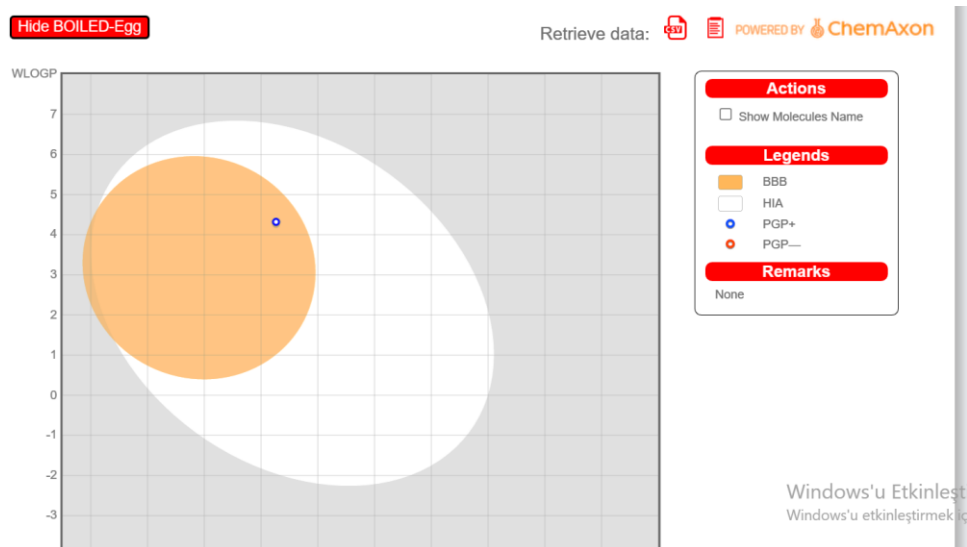


Figure A. 51 BOILED-Egg method applied to predict blood brain barrier permeability of **BODIPY-Sq**

Table A. 1 Bond lengths(Å) for **BODIPY-5b**

F(1)	B(1)	1.382(6)	C(7)	C(13)	1.520(6)
F(2)	B(1)	1.376(6)	C(8)	C(9)	1.401(6)
O(1)	C(26)	1.354(5)	C(9)	C(12)	1.490(6)
O(2)	C(25)	1.384(6)	C(14)	C(15)	1.380(6)
N(1)	C(1)	1.348(5)	C(14)	C(19)	1.379(6)
N(1)	C(4)	1.405(5)	C(15)	C(16)	1.382(5)
N(1)	B(1)	1.551(6)	C(16)	C(17)	1.377(6)
N(2)	C(6)	1.399(5)	C(17)	C(18)	1.385(6)
N(2)	C(9)	1.346(5)	C(18)	C(19)	1.394(6)
N(2)	B(1)	1.550(6)	C(20)	C(21)	1.458(6)
N(3)	C(17)	1.417(5)	C(21)	C(22)	1.383(6)
N(3)	C(20)	1.275(5)	C(21)	C(26)	1.392(6)
C(1)	C(2)	1.380(6)	C(22)	C(23)	1.386(6)
C(1)	C(10)	1.507(6)	C(23)	C(24)	1.381(7)
C(2)	C(3)	1.365(6)	C(24)	C(25)	1.346(6)
C(3)	C(4)	1.429(6)	C(25)	C(26)	1.385(6)
C(3)	C(11)	1.496(6)	O(3)	C(27)	1.456(7)
C(4)	C(5)	1.401(5)	O(3)	C(30)	1.449(7)
C(5)	C(6)	1.388(5)	C(27)	C(28)	1.471(9)
C(5)	C(14)	1.480(5)	C(28)	C(29)	1.447(9)
C(6)	C(7)	1.437(6)	C(29)	C(30)	1.401(9)
C(7)	C(8)	1.368(6)			

Table A. 2 Bond angles(°) for **BODIPY-5b**

C(1)	N(1)	C(4)	107.9(3)	C(19)	C(14)	C(15)	118.0(4)
C(1)	N(1)	B(1)	125.8(3)	C(14)	C(15)	C(16)	120.9(4)
C(4)	N(1)	B(1)	126.3(3)	C(17)	C(16)	C(15)	121.0(4)
C(6)	N(2)	B(1)	125.2(3)	C(16)	C(17)	N(3)	117.4(4)
C(9)	N(2)	C(6)	108.8(3)	C(16)	C(17)	C(18)	118.8(4)
C(9)	N(2)	B(1)	126.0(4)	C(18)	C(17)	N(3)	123.7(4)
C(20)	N(3)	C(17)	120.9(4)	C(17)	C(18)	C(19)	119.6(4)
N(1)	C(1)	C(2)	109.1(4)	C(14)	C(19)	C(18)	121.6(4)
N(1)	C(1)	C(10)	121.9(4)	N(3)	C(20)	C(21)	122.0(5)
C(2)	C(1)	C(10)	129.0(4)	C(22)	C(21)	C(20)	120.2(5)
C(3)	C(2)	C(1)	109.6(4)	C(22)	C(21)	C(26)	118.7(4)
C(2)	C(3)	C(4)	106.1(4)	C(26)	C(21)	C(20)	121.2(4)
C(2)	C(3)	C(11)	125.4(4)	C(21)	C(22)	C(23)	121.0(5)
C(4)	C(3)	C(11)	128.4(4)	C(24)	C(23)	C(22)	119.0(5)
N(1)	C(4)	C(3)	107.1(3)	C(25)	C(24)	C(23)	120.5(4)
C(5)	C(4)	N(1)	119.8(3)	O(2)	C(25)	C(26)	115.6(4)
C(5)	C(4)	C(3)	133.1(3)	C(24)	C(25)	O(2)	123.1(4)
C(4)	C(5)	C(14)	118.5(3)	C(24)	C(25)	C(26)	121.2(5)
C(6)	C(5)	C(4)	120.9(3)	O(1)	C(26)	C(21)	121.8(4)
C(6)	C(5)	C(14)	120.5(3)	O(1)	C(26)	C(25)	118.7(5)
N(2)	C(6)	C(7)	107.2(4)	C(25)	C(26)	C(21)	119.4(5)
C(5)	C(6)	N(2)	121.5(3)	F(1)	B(1)	N(1)	109.2(4)
C(5)	C(6)	C(7)	131.4(4)	F(1)	B(1)	N(2)	110.4(4)
C(6)	C(7)	C(13)	128.7(4)	F(2)	B(1)	F(1)	110.2(4)
C(8)	C(7)	C(6)	106.1(4)	F(2)	B(1)	N(1)	109.8(4)
C(8)	C(7)	C(13)	125.2(5)	F(2)	B(1)	N(2)	111.0(4)
C(7)	C(8)	C(9)	109.4(4)	N(2)	B(1)	N(1)	106.2(3)
N(2)	C(9)	C(8)	108.5(4)	C(30)	O(3)	C(27)	105.4(6)
N(2)	C(9)	C(12)	123.3(4)	O(3)	C(27)	C(28)	106.6(6)
C(8)	C(9)	C(12)	128.2(5)	C(29)	C(28)	C(27)	106.2(8)
C(15)	C(14)	C(5)	120.3(4)	C(30)	C(29)	C(28)	108.7(8)
C(19)	C(14)	C(5)	121.7(4)	C(29)	C(30)	O(3)	108.9(7)

Table A. 3 Hydrogen bond parameters for **BODIPY-5b**

D-H·····A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O(1)-H(1)·····N(3)	0.82	1.87	2.598(5)	147.1
O(2)-H(2)·····O(3)	0.82	1.84	2.648(6)	166.2
C(10)-H(10A)·····F(1)	0.96	2.63	3.137(6)	113.3
C(12)-H(12A)·····F(2)	0.96	2.63	3.170(6)	116.2
C(15)-H(15)·····O(2)*	0.93	2.58	3.271(6)	131.8
C(16)-H(16)·····O(2)*	0.93	2.85	3.406(6)	119.3
C(29)-H(29A)·····F(1)**	0.97	2.84	3.387(10)	116.7

* 2-x,1-y,1-z; ** +x,2+y,1+z

Table A. 4 Bond lengths(Å) for **BODIPY-5i**

F(1)	B(1)	1.384(3)	C(5)	C(14)	1.486(3)
F(2)	B(1)	1.382(3)	C(6)	C(7)	1.420(3)
O(1)	C(26)	1.336(3)	C(7)	C(8)	1.377(3)
O(2)	C(24)	1.356(3)	C(7)	C(13)	1.492(4)
O(2)	C(27)	1.423(3)	C(8)	C(9)	1.383(4)
N(1)	C(1)	1.346(3)	C(9)	C(12)	1.496(3)
N(1)	C(4)	1.406(3)	C(14)	C(15)	1.389(3)
N(1)	B(1)	1.539(3)	C(14)	C(19)	1.375(3)
N(2)	C(6)	1.407(3)	C(15)	C(16)	1.375(3)
N(2)	C(9)	1.345(3)	C(16)	C(17)	1.375(3)
N(2)	B(1)	1.538(4)	C(17)	C(18)	1.390(3)
N(3)	C(17)	1.426(3)	C(18)	C(19)	1.395(3)
N(3)	C(20)	1.263(3)	C(20)	C(21)	1.455(3)
C(1)	C(2)	1.393(3)	C(21)	C(22)	1.377(3)
C(1)	C(10)	1.486(3)	C(21)	C(26)	1.403(3)
C(2)	C(3)	1.375(3)	C(22)	C(23)	1.364(3)
C(3)	C(4)	1.428(3)	C(23)	C(24)	1.398(3)
C(3)	C(11)	1.497(3)	C(24)	C(25)	1.371(3)
C(4)	C(5)	1.389(3)	C(25)	C(26)	1.394(3)
C(5)	C(6)	1.397(3)			

Table A. 5 Bond angles(°) for BODIPY-5i

C(24) O(2) C(27)	118.1(2)	C(8) C(9) C(12)	128.1(3)
C(1) N(1) C(4)	108.39(19)	C(15) C(14) C(5)	119.8(2)
C(1) N(1) B(1)	126.0(2)	C(19) C(14) C(5)	121.8(2)
C(4) N(1) B(1)	125.5(2)	C(19) C(14) C(15)	118.2(2)
C(6) N(2) B(1)	125.64(19)	C(16) C(15) C(14)	120.9(2)
C(9) N(2) C(6)	108.0(2)	C(17) C(16) C(15)	121.2(2)
C(9) N(2) B(1)	126.3(2)	C(16) C(17) N(3)	116.8(2)
C(20) N(3) C(17)	121.6(2)	C(16) C(17) C(18)	118.6(2)
N(1) C(1) C(2)	109.1(2)	C(18) C(17) N(3)	124.4(2)
N(1) C(1) C(10)	123.1(2)	C(17) C(18) C(19)	120.0(2)
C(2) C(1) C(10)	127.8(3)	C(14) C(19) C(18)	121.1(2)
C(3) C(2) C(1)	109.0(2)	N(3) C(20) C(21)	122.4(2)
C(2) C(3) C(4)	106.4(2)	C(22) C(21) C(20)	120.1(2)
C(2) C(3) C(11)	123.9(2)	C(22) C(21) C(26)	118.4(2)
C(4) C(3) C(11)	129.7(2)	C(26) C(21) C(20)	121.5(2)
N(1) C(4) C(3)	107.2(2)	C(23) C(22) C(21)	121.6(2)
C(5) C(4) N(1)	120.2(2)	C(22) C(23) C(24)	119.6(2)
C(5) C(4) C(3)	132.5(2)	O(2) C(24) C(23)	114.7(2)
C(4) C(5) C(6)	121.7(2)	O(2) C(24) C(25)	124.7(2)
C(4) C(5) C(14)	118.0(2)	C(25) C(24) C(23)	120.6(2)
C(6) C(5) C(14)	120.2(2)	C(24) C(25) C(26)	119.0(2)
N(2) C(6) C(7)	107.7(2)	O(1) C(26) C(21)	121.4(2)
C(5) C(6) N(2)	119.7(2)	O(1) C(26) C(25)	117.9(2)
C(5) C(6) C(7)	132.6(2)	C(25) C(26) C(21)	120.7(2)
C(6) C(7) C(13)	129.1(2)	F(1) B(1) N(1)	110.3(2)
C(8) C(7) C(6)	105.7(2)	F(1) B(1) N(2)	110.2(2)
C(8) C(7) C(13)	125.1(3)	F(2) B(1) F(1)	109.2(2)
C(7) C(8) C(9)	109.6(3)	F(2) B(1) N(1)	110.0(2)
N(2) C(9) C(8)	109.0(2)	F(2) B(1) N(2)	110.1(2)
N(2) C(9) C(12)	122.9(3)	N(2) B(1) N(1)	107.02(18)

Table A. 6 Hydrogen bond parameters for BODIPY-5i

D-H·····A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O(1)-H(1)·····N(3)	0.82	1.89	2.614(3)	147.3
C(10)-H(10A)·····F(1)	0.96	2.55	3.208(3)	126.1
C(12)-H(12A)·····F(2)	0.96	2.48	3.077(4)	120.0
C(18)-H(18)·····O(2)*	0.93	2.70	3.353(3)	127.6

*-x,2-y,1-z

Table A. 7 Bond lengths(Å) for **BODIPY-5d**

F(1)	B(1)	1.383(3)	C(5)	C(6)	1.398(4)
F(2)	B(1)	1.397(3)	C(5)	C(14)	1.487(3)
N(1)	C(1)	1.343(3)	C(6)	C(7)	1.418(4)
N(1)	C(4)	1.400(3)	C(7)	C(8)	1.381(4)
N(1)	B(1)	1.536(4)	C(7)	C(13)	1.508(4)
N(2)	C(6)	1.401(3)	C(8)	C(9)	1.392(4)
N(2)	C(9)	1.347(3)	C(9)	C(12)	1.492(3)
N(2)	B(1)	1.534(4)	C(14)	C(15)	1.381(3)
N(3)	C(17)	1.416(3)	C(14)	C(19)	1.378(3)
N(3)	C(20)	1.264(3)	C(15)	C(16)	1.377(3)
N(4)	C(21)	1.376(4)	C(16)	C(17)	1.382(4)
N(4)	C(24)	1.319(3)	C(17)	C(18)	1.382(4)
C(1)	C(2)	1.383(4)	C(18)	C(19)	1.377(3)
C(1)	C(10)	1.498(4)	C(20)	C(21)	1.446(4)
C(2)	C(3)	1.373(3)	C(21)	C(22)	1.362(4)
C(3)	C(4)	1.428(4)	C(22)	C(23)	1.399(4)
C(3)	C(11)	1.499(3)	C(23)	C(24)	1.379(5)
C(4)	C(5)	1.393(3)			

Table A. 8 Bond angles(°) for **BODIPY-5d**

C(1)	N(1)	C(4)	108.1(2)	C(7)	C(8)	C(9)	108.9(3)
C(1)	N(1)	B(1)	126.7(2)	N(2)	C(9)	C(8)	108.9(2)
C(4)	N(1)	B(1)	125.1(2)	N(2)	C(9)	C(12)	123.5(3)
C(6)	N(2)	B(1)	125.2(2)	C(8)	C(9)	C(12)	127.6(3)
C(9)	N(2)	C(6)	108.3(2)	C(15)	C(14)	C(5)	120.9(2)
C(9)	N(2)	B(1)	126.4(2)	C(19)	C(14)	C(5)	121.1(2)
C(20)	N(3)	C(17)	118.4(3)	C(19)	C(14)	C(15)	118.0(2)
C(24)	N(4)	C(21)	110.6(3)	C(16)	C(15)	C(14)	121.1(2)
N(1)	C(1)	C(2)	109.2(2)	C(15)	C(16)	C(17)	120.6(2)
N(1)	C(1)	C(10)	122.8(3)	C(16)	C(17)	N(3)	117.1(2)
C(2)	C(1)	C(10)	127.9(3)	C(18)	C(17)	N(3)	124.2(2)
C(3)	C(2)	C(1)	109.4(3)	C(18)	C(17)	C(16)	118.6(2)
C(2)	C(3)	C(4)	105.8(2)	C(19)	C(18)	C(17)	120.4(2)
C(2)	C(3)	C(11)	124.4(3)	C(18)	C(19)	C(14)	121.4(2)
C(4)	C(3)	C(11)	129.7(3)	N(3)	C(20)	C(21)	123.0(3)
N(1)	C(4)	C(3)	107.5(2)	N(4)	C(21)	C(20)	123.7(3)
C(5)	C(4)	N(1)	120.5(2)	C(22)	C(21)	N(4)	106.7(3)
C(5)	C(4)	C(3)	132.0(2)	C(22)	C(21)	C(20)	129.5(3)
C(4)	C(5)	C(6)	121.2(2)	C(21)	C(22)	C(23)	107.7(3)
C(4)	C(5)	C(14)	119.8(2)	C(24)	C(23)	C(22)	106.9(3)
C(6)	C(5)	C(14)	119.0(2)	N(4)	C(24)	C(23)	108.1(3)
N(2)	C(6)	C(7)	107.7(2)	F(1)	B(1)	F(2)	108.5(2)
C(5)	C(6)	N(2)	120.1(2)	F(1)	B(1)	N(1)	110.4(2)
C(5)	C(6)	C(7)	132.1(2)	F(1)	B(1)	N(2)	110.8(2)
C(6)	C(7)	C(13)	129.5(3)	F(2)	B(1)	N(1)	110.2(2)
C(8)	C(7)	C(6)	106.2(2)	F(2)	B(1)	N(2)	109.4(2)
C(8)	C(7)	C(13)	124.4(3)	N(2)	B(1)	N(1)	107.5(2)

Table A. 9 Hydrogen bond parameters for **BODIPY-5d**

D-H·····A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N(4)-H(4)·····F(2)*	0.86	2.18	2.988(3)	157.5
C(12)-H(12C)·····F(2)	0.96	2.67	3.233(4)	117.7
C(19)-H(19)·····F(1)**	0.93	2.71	3.357(3)	127.7
C(24)-H(24)·····F(1)***	0.93	2.59	3.255(3)	128.5

*1+x,3/2-y,1/2+z; **+x,3/2-y,1/2+z; ***1+x,+y,1+z

Table A. 10 Bond lengths(Å) for **BODIPY-5f**

S1	C21	1.695(5)	C5	C6	1.389(6)
S1	C24	1.713(5)	C5	C14	1.487(5)
F1	B1	1.383(6)	C6	C7	1.427(6)
F2	B1	1.387(6)	C7	C8	1.377(7)
N1	C1	1.343(6)	C7	C13	1.484(7)
N1	C4	1.397(5)	C8	C9	1.387(7)
N1	B1	1.540(7)	C9	C12	1.493(7)
N2	C6	1.398(5)	C14	C15	1.367(6)
N2	C9	1.352(6)	C14	C19	1.379(6)
N2	B1	1.547(7)	C15	C16	1.398(6)
N3	C17	1.419(5)	C16	C17	1.378(6)
N3	C20	1.262(6)	C17	C18	1.394(6)
C1	C2	1.396(7)	C18	C19	1.377(6)
C1	C10	1.506(6)	C20	C21	1.465(6)
C2	C3	1.372(6)	C21	C22	1.348(7)
C3	C4	1.426(6)	C22	C23	1.416(7)
C3	C11	1.504(6)	C23	C24	1.353(8)
C4	C5	1.405(6)			

Table A. 11 Bond angles(°) for **BODIPY-5f**

C21	S1	C24	91.3(3)	C7	C8	C9	109.7(5)
C1	N1	C4	108.7(4)	N2	C9	C8	108.9(4)
C1	N1	B1	126.4(4)	N2	C9	C12	122.7(5)
C4	N1	B1	124.8(4)	C8	C9	C12	128.4(5)
C6	N2	B1	125.1(4)	C15	C14	C5	119.6(4)
C9	N2	C6	107.9(4)	C15	C14	C19	119.2(4)
C9	N2	B1	126.7(4)	C19	C14	C5	121.2(4)
C20	N3	C17	121.0(4)	C14	C15	C16	120.1(4)
N1	C1	C2	108.6(4)	C17	C16	C15	120.9(4)
N1	C1	C10	123.7(5)	C16	C17	N3	115.5(4)
C2	C1	C10	127.7(5)	C16	C17	C18	118.4(4)
C3	C2	C1	109.2(4)	C18	C17	N3	126.1(4)
C2	C3	C4	106.2(4)	C19	C18	C17	120.1(4)
C2	C3	C11	125.0(5)	C18	C19	C14	121.2(4)
C4	C3	C11	128.8(4)	N3	C20	C21	120.7(5)
N1	C4	C3	107.3(4)	C20	C21	S1	121.8(4)
N1	C4	C5	120.5(4)	C22	C21	S1	112.0(4)
C5	C4	C3	132.2(4)	C22	C21	C20	126.2(5)
C4	C5	C14	118.9(4)	C21	C22	C23	113.1(5)
C6	C5	C4	121.4(4)	C24	C23	C22	111.3(5)
C6	C5	C14	119.6(4)	C23	C24	S1	112.2(4)
N2	C6	C7	108.1(4)	F1	B1	F2	108.6(4)
C5	C6	N2	119.9(4)	F1	B1	N1	110.8(4)
C5	C6	C7	131.9(4)	F1	B1	N2	109.7(4)
C6	C7	C13	129.6(4)	F2	B1	N1	110.5(4)
C8	C7	C6	105.4(4)	F2	B1	N2	110.1(4)
C8	C7	C13	125.0(5)	N1	B1	N2	107.2(3)

Table A. 12 Hydrogen bond parameters for **BODIPY-5f**

D-H·····A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C10-H10C·····F1	0.96	2.49	3.077(7)	119.3
C12-H12A·····F1	0.96	2.58	3.015(8)	107.5
C12-H12C·····F2	0.96	2.79	3.294(7)	113.4
C23-H23·····F1*	0.93	2.41	3.193(5)	141.5
C23-H23·····F2**	0.93	2.86	3.449(7)	122.5
C24-H24·····F2*	0.93	2.65	3.445(5)	143.8

*-1+x,+y,-1+z; **-1+x,3/2-y,-1/2+z

Table A. 13 Bond lengths (Å) for **BODIPY-5g**

F1	B1	1.3984(17)	C5	C6	1.3917(18)
F2	B1	1.3828(16)	C5	C14	1.4888(17)
N1	C1	1.3476(18)	C6	C7	1.4260(19)
N1	C4	1.3980(16)	C7	C8	1.3805(19)
N1	B1	1.5409(18)	C7	C12	1.500(2)
N2	C6	1.4002(16)	C8	C9	1.399(2)
N2	C9	1.3453(17)	C9	C13	1.4901(19)
N2	B1	1.5426(19)	C14	C15	1.3886(18)
N3	C17	1.4154(17)	C14	C19	1.3885(19)
N3	C20	1.2625(19)	C15	C16	1.3831(18)
N4	C21	1.3350(19)	C16	C17	1.3876(19)
N4	C25	1.3324(19)	C17	C18	1.3910(19)
C1	C2	1.396(2)	C18	C19	1.3870(19)
C1	C10	1.488(2)	C20	C21	1.4722(18)
C2	C26	1.380(2)	C21	C22	1.3876(19)
C26	C4	1.4241(18)	C22	C23	1.379(2)
C26	C11	1.4966(19)	C23	C24	1.375(2)
C4	C5	1.3977(19)	C24	C25	1.378(2)

Table A. 14 Bond angles (°) for **BODIPY-5g**

C1	N1	C4	108.28(11)	N2	C9	C8	109.17(12)
C1	N1	B1	125.98(11)	N2	C9	C13	123.51(13)
C4	N1	B1	125.41(11)	C8	C9	C13	127.32(13)
C6	N2	B1	125.56(11)	C15	C14	C5	120.04(12)
C9	N2	C6	108.25(11)	C19	C14	C5	120.61(12)
C9	N2	B1	126.16(11)	C19	C14	C15	119.25(12)
C20	N3	C17	119.94(12)	C16	C15	C14	120.41(12)
C25	N4	C21	116.95(13)	C15	C16	C17	120.28(12)
N1	C1	C2	109.20(12)	C16	C17	N3	116.80(12)
N1	C1	C10	122.75(13)	C16	C17	C18	119.59(12)
C2	C1	C10	128.04(13)	C18	C17	N3	123.53(12)
C26	C2	C1	108.51(13)	C19	C18	C17	119.90(13)
C2	C26	C4	106.37(12)	C18	C19	C14	120.54(12)
C2	C26	C11	124.21(13)	N3	C20	C21	120.62(13)
C4	C26	C11	129.41(13)	N4	C21	C20	115.10(12)
N1	C4	C26	107.64(11)	N4	C21	C22	122.94(13)
C5	C4	N1	120.30(11)	C22	C21	C20	121.95(13)
C5	C4	C26	132.06(12)	C23	C22	C21	119.00(14)
C4	C5	C14	118.61(11)	C24	C23	C22	118.49(14)
C6	C5	C4	121.24(12)	C23	C24	C25	118.61(14)
C6	C5	C14	120.14(12)	N4	C25	C24	123.98(15)
N2	C6	C7	107.79(11)	F1	B1	N1	109.51(11)
C5	C6	N2	120.26(12)	F1	B1	N2	110.20(11)
C5	C6	C7	131.81(12)	F2	B1	F1	108.66(11)
C6	C7	C12	128.68(13)	F2	B1	N1	111.00(11)
C8	C7	C6	106.12(12)	F2	B1	N2	110.66(11)
C8	C7	C12	125.15(13)	N1	B1	N2	106.80(10)
C7	C8	C9	108.67(13)				

Table A. 15 Hydrogen bond parameters for **BODIPY-5g**

D-H···A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C10-H10A···F1	0.98	2.56	3.1908(19)	121.8
C13-H13C···F1	0.98	2.58	3.2775(17)	128.1
C19-H19···F2 [*]	0.95	2.49	3.2352(16)	134.8

^{*}2-x,-y,1-z

Table A. 16 Bond lengths (Å) for **BODIPY-Sq**

F1	B1	1.390(3)	C5	C6	1.397(3)
F2	B1	1.384(3)	C5	C14	1.491(3)
O1	C21	1.219(3)	C6	C7	1.425(3)
O2	C22	1.217(2)	C7	C8	1.379(3)
O3	C23	1.317(3)	C7	C12	1.495(3)
O3	C24	1.458(2)	C8	C9	1.393(3)
N1	C1	1.344(3)	C9	C13	1.495(3)
N1	C4	1.400(3)	C14	C15	1.383(3)
N1	B1	1.541(3)	C14	C19	1.384(3)
N2	C6	1.399(3)	C15	C16	1.378(3)
N2	C9	1.343(3)	C16	C17	1.392(3)
N2	B1	1.549(3)	C17	C18	1.380(3)
N3	C17	1.414(3)	C18	C19	1.375(3)
N3	C20	1.339(3)	C20	C21	1.463(3)
C1	C2	1.399(3)	C20	C23	1.394(3)
C1	C10	1.488(3)	C21	C22	1.517(3)
C2	C3	1.378(3)	C21	C23	2.037(3)
C3	C4	1.423(3)	C22	C23	1.462(3)
C3	C11	1.491(3)	C24	C25	1.497(3)
C4	C5	1.399(3)			

Table A. 17 Bond angles (°) for **BODIPY-Sq**

C23	O3	C24	117.48(17)	C19	C14	C5	119.75(19)
C1	N1	C4	107.92(19)	C16	C15	C14	120.5(2)
C1	N1	B1	126.8(2)	C15	C16	C17	120.1(2)
C4	N1	B1	124.71(19)	C16	C17	N3	117.92(19)
C6	N2	B1	124.7(2)	C18	C17	N3	122.46(19)
C9	N2	C6	108.0(2)	C18	C17	C16	119.6(2)
C9	N2	B1	126.8(2)	C19	C18	C17	119.5(2)
C20	N3	C17	127.30(19)	C18	C19	C14	121.5(2)
N1	C1	C2	109.2(2)	N3	C20	C21	128.4(2)
N1	C1	C10	122.5(2)	N3	C20	C23	140.6(2)
C2	C1	C10	128.3(2)	C23	C20	C21	90.95(17)
C3	C2	C1	108.9(2)	O1	C21	C20	134.9(2)
C2	C3	C4	105.8(2)	O1	C21	C22	136.2(2)
C2	C3	C11	125.2(2)	O1	C21	C23	177.58(19)
C4	C3	C11	129.1(2)	C20	C21	C22	88.88(17)
N1	C4	C3	108.24(19)	C20	C21	C23	43.16(12)
C5	C4	N1	120.1(2)	C22	C21	C23	45.73(12)
C5	C4	C3	131.7(2)	O2	C22	C21	136.2(2)
C4	C5	C14	119.7(2)	O2	C22	C23	137.5(2)
C6	C5	C4	121.7(2)	C23	C22	C21	86.28(16)
C6	C5	C14	118.6(2)	O3	C23	C20	131.0(2)
N2	C6	C7	108.13(19)	O3	C23	C21	176.76(18)
C5	C6	N2	119.8(2)	O3	C23	C22	135.1(2)
C5	C6	C7	132.0(2)	C20	C23	C21	45.89(12)
C6	C7	C12	130.1(2)	C20	C23	C22	93.86(18)
C8	C7	C6	105.6(2)	C22	C23	C21	47.98(12)
C8	C7	C12	124.3(2)	O3	C24	C25	105.71(18)
C7	C8	C9	109.1(2)	F1	B1	N1	109.7(2)
N2	C9	C8	109.2(2)	F1	B1	N2	110.0(2)
N2	C9	C13	122.4(2)	F2	B1	F1	109.2(2)
C8	C9	C13	128.4(2)	F2	B1	N1	110.5(2)
C15	C14	C5	121.65(19)	F2	B1	N2	110.6(2)
C15	C14	C19	118.6(2)	N1	B1	N2	106.79(19)

Table A. 18 Hydrogen bond parameters for **BODIPY-Sq**

D-H····A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C18-H18····O3	0.95	2.29	2.975(3)	128.3
C41-H41····O6	0.95	2.33	2.961(3)	123.4
N9-H9····O1	0.88	2.06	2.864(2)	150.9