

**SYNTHESIS AND CHARACTERIZATION OF NOVEL ION
SENSOR BASED ON 2, 6-FUNCTIONALIZED BODIPY
STRUCTURE**

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MASTER OF SCIENCE

By

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September 2012

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF NOVEL ION SENSOR BASED ON 2, 6-FUNCTIONALIZED BODIPY STRUCTURE

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M.S. in Materials Science and Nanotechnology

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Latest developments in chemistry of BODIPY make available synthesis of novel BODIPY based fluorescent chemosensors. Therefore, the design of novel chemosensors is attracting great attention in recent years. In this study, we functionalized 2 and 6 positions of BODIPY chromophore to understand that binding of different metal ions how affect the absorption and emission spectrums of BODIPY. We used three metal ions which are important in environment and biological systems and these are Hg (II), Zn (II) and Ca ions. Binding of these metal ions to azo crown ether is which attached BODIPY caused large changes in absorption and emission spectrums according to photoinduced electron transfer (PET) and internal charge transfer (ICT) mechanism.

Keywords: BODIPY, fluorescent chemosensor, ion sensing

ÖZET

2,6- FONKSİYONLANDIRILMIŞ BODIPY YAPISI TEMELLİ YENİ İYON SENSÖRÜNÜN SENTEZİ VE KARAKTERİZASYONU

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BODIPY kimyasındaki son gelişmeler, BODIPY tabanlı yeni floresan kemosensörlerin sentezini mümkün kılmıştır. Bundan dolayı da son yıllarda yeni kemosensörlerin sentezi büyük ilgi görmektedir. Biz bu çalışmada bu kromoforun 2 ve 6 pozisyonlarını türevlendirdik ve farklı metal iyonlarının bağlanmasının BODIPY' nin absorbans ve emisyon spektrumlarını nasıl etkilediğini anlamaya çalıştık. Çevre ve biyolojik sistemlerde önemli olan Hg (II), Zn (II) ve Ca iyonları olmak üzere 3 metal iyonu kullandık. Bu metal iyonlarının BODIPY' ye bağlı bulunan azo taç eterine bağlanması absorbans ve emisyon spektrumlarında büyük değişikliğe neden oldu. Bu değişiklikler ışıkla indüklenen elektron transferi (PET) ve molekül içi yük transferine (ICT) bağlı olarak gerçekleşti.

Anahtar kelimeler: BODIPY, floresan kemosensör, iyon algılanması

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LIST OF ABBREVIATIONS

ASTM : American Society for Testing and Materials

BODIPY : Boradiazaindacene

DDQ : Dichlorodicyanoquinone

DMF : Dimethylformamide

HOMO : Highest Occupied Molecular Orbital

ICT : Internal Charge Transfer

LUMO : Lowest Unoccupied Molecular Orbital

MS : Mass Spectroscopy

NMR : Nuclear Magnetic Resonance

PET : Photoinduced Electron Transfer

TFA : Trifluoroacetic Acid

THF : Tetrahydrofuran

TLC : Thin Layer Chromotography

CHAPTER 1

INTRODUCTION

1.1. Supramolecular Chemistry

Supramolecular chemistry is one of the new research areas of the chemistry and growing rapidly.¹⁻⁵ It has described as ‘chemistry beyond molecule’ and consists of the systems which hold together by reversible non-covalent interactions.⁶ Another description of supramolecular chemistry is ‘legoTM chemistry’ and each molecule which is like legoTM brick build the supramolecular structure by the intermolecular interactions.

Supramolecular chemistry is highly interdisciplinary area. Organic and inorganic chemistry is used for synthesis of molecules, physical chemistry, to explain properties of supramolecular structures, computational chemistry, modeling of structures and understanding of behavior of supramolecular systems. Supramolecular chemistry is also important for biology to mimic of natural systems such as enzymes.^{7,8}

Application areas of supramolecular chemistry are very wide. It has been used for non-covalent synthesis and catalysis^{9,10} beside typical organic synthesis. It is also important for molecular recognition by using luminescence^{11,12} and electrochemistry^{13,14}. Moreover, rising of molecular devices is another hot topic of supramolecular chemistry^{15,16}. Other application areas are light harvesting systems,^{17,18} solar cells^{19,20} and logic gates^{21,22}, and surface

studies^{23,24}. Finally, supramolecular chemistry makes possible the artificial biological agents²⁵ such as enzymes, healing agents²⁶ for particular diseases.

Supramolecular chemistry consists of two main groups; *host-guest chemistry* and *self-assembly*.²⁷ That classification is related to size and shape. In host-guest chemistry, one of the molecules much larger than the other one and that is called 'host'. The molecule which is smaller and surrounded by host is called 'guest'. There are many examples for host-guest interactions. In biological systems, enzymes and their substrates are examples for host-guest chemistry. Also in coordination chemistry, large ligands are hosts and metals are guests and interaction between them is electrostatic interaction. Binding sites are very important in host-guest chemistry; their size, geometry and chemical natures must be proper for complementarity. The other category in the supramolecular chemistry is self-assembly. If the sizes of the host and guest are close to each other, that is called self-assembly system. The self-assembly structures can involve two or more components and formation of the supramolecular structures usually spontaneous and reversible processes.

All of supramolecular structures are hold together by non-covalent interactions. These are electrostatic interactions, hydrogen bonding, π interactions, Van der Waals forces and hydrophobic effects.

1.1.1. Electrostatic Interactions

These interactions are consists of three groups such as (i) ion-ion interactions (ii) ion-dipole interactions and (iii) dipole-dipole interactions. The Coulombic attractions which are between positive and negative charges play role in these interactions. The strongest one is ion-ion interactions between them. It is as strong as covalent bond and non-directional that means it can occur in any orientation. Ion-dipole and dipole-dipole interactions are orientation-dependant and effective in only one direction. They are weaker compared to ion-ion interactions.

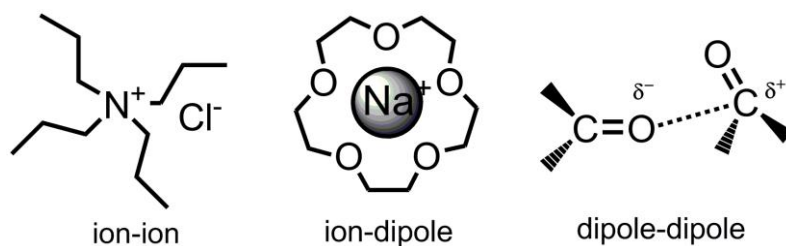


Figure 1. Examples of electrostatic interaction.

1.1.2. Hydrogen Bonding

It is actually a dipole-dipole interactions but different from other dipole-dipole interactions aspects of high strength and directionality. It is very important for supramolecular systems because of these properties. Hydrogen bond forms between a proton donor and a proton acceptor. For proton donor, hydrogen atom must be bound to strong electronegative atoms such as oxygen, nitrogen or fluor to form positively charged hydrogen atom. There must be again oxygen, nitrogen or fluor atoms in acceptor group. There are many examples for hydrogen bonding in nature such as in shape of proteins and helix structure of DNA²⁸.

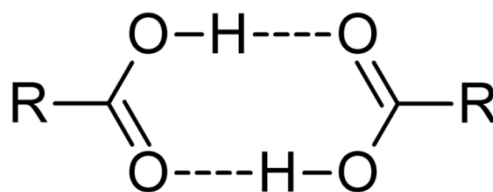


Figure 2. In vapor phase, carboxylic acid usually form dimers by hydrogen bonding.

1.1.3. π -Interactions

π interactions can be divided into two types such as (i) cation- π interactions and (ii) π - π interactions.^{29,30} Cation- π interactions occur between electron-rich

aromatic ring and cation. It is especially important in coordination chemistry.

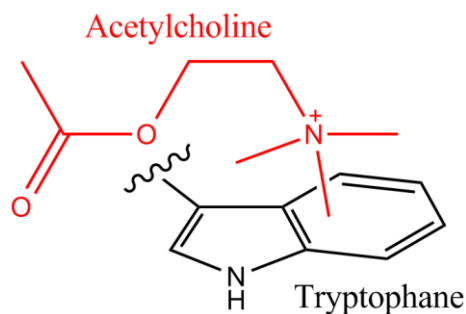


Figure 3. Cation- π interactions.

The other type of non-covalent π -interactions is π - π interactions are also divided into two groups those are face-to-face and edge-to-face. It is formed by intermolecular overlapping of p-orbitals. One of examples for face-to-face π -interactions is structure of graphite. There are weak π -interactions between layers.

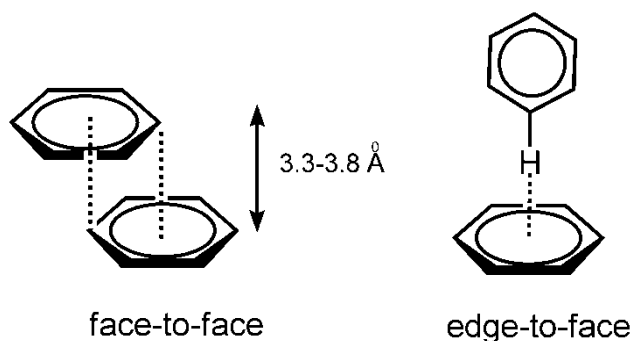


Figure 4. Face-to-face and edge-to-face interaction of benzene.

1.1.4. Van der Waals Forces

Van der Waals interactions occur between induced-dipole and induced-dipole. They are instantaneous dipoles and caused by movement of electron clouds of atoms. Distribution of electrons around the each molecule changes very fastly and by this way temporary dipoles form and that results weak non-covalent interactions. These interactions are too general so these are cannot be used for

designing of supramolecular structures. Van der Waals interactions are much dependant on distance and rapidly diminish by increasing of distance.

1.1.5. Hydrophobic Effects

There is a simple rule that polar dissolves in polar and non-polar dissolves in non-polar. Reason of the hydrophobic effect is that exclusion of non-polar groups from polar solvents. This more favorable aspect of energy because polar molecules prefer to interact with other polar ones due to being of dipole-dipole interaction is stronger than dipole-non dipole. Because of that reasons, water and organic solvents such as dichloromethane, chloroform are immiscible. Hydrophobic effect is important in supramolecular chemistry; binding of organic molecules to cavity of cyclodextrins in water is example for that.

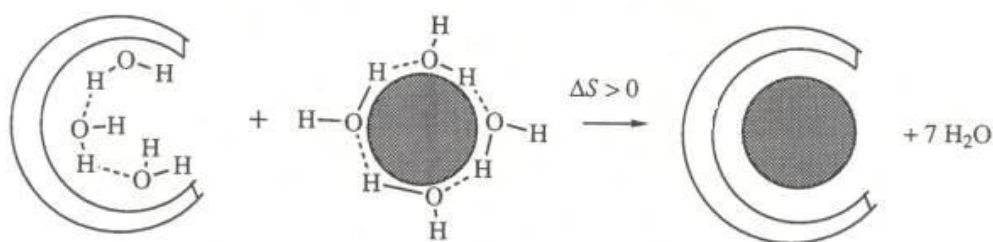
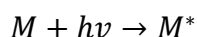


Figure 5. Entropic hydrophobic effect.

Hydrophobic effects are divided into two type according to energy; they are enthalpic hydrophobic effect and entropic hydrophobic effect. Binding of organic molecules instead of water to hydrophobic cavity is related to the enthalpic hydrophobic effect. Since interaction between organic molecules and hydrophobic cavity is stronger. Explanation for the entropic hydrophobic effect is that organic molecules create hole or cage in water and that supramolecular aggregation increases entropy.³¹

1.2. Fluorescence

At room temperature, most of elementary particles are in their ground states. When the particles absorb the light which has suitable energy, electrons move to excited state from the ground state. That process is called absorption.



Luminescence is relaxation of the excited particles to the ground state with an emission of light. Luminescence is separated to two types, fluorescence and phosphorescence. Average life time fluorescence is 10^{-8} s and relaxation of excited electron to ground state is spin-allowed. Also there are relaxation types those do not have emission for de-excitation. These are internal conversion, intersystem crossing, intramolecular charge transfer and conformational change. Also, excited molecule can interact other molecules such as electron transfer, energy transfer, excimer formation.

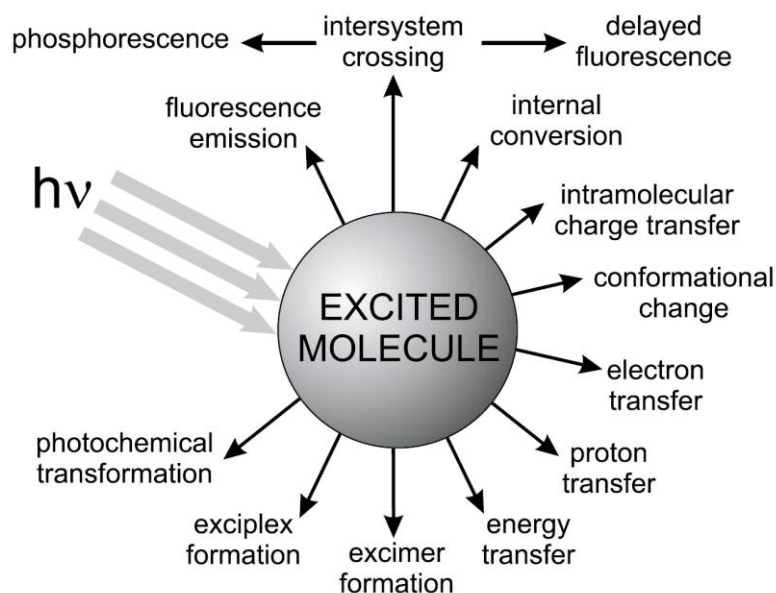


Figure 6. De-excitation pathways of excited molecules³².

The Jablonski diagram (Figure 7) shows that possible pathways after photon absorption. S_0 shows the ground state. Singlet energy levels are represented with S_1 , S_2 and S_n and triplet energy levels are T_1 and T_2 . Difference between

singlet and triplet states is spin of electron. Energy levels which are between singlet energy levels and also triplet energy levels are vibrational levels. When molecule is irradiated with photon, electron is excited from S_0 to upper singlet energy levels (S_n). Then excited electron relaxes to lowest vibrational level of S_1 non-radiatively and the name that process is internal conversion. At this situation, there are two possibilities. First way is that, excited electron at S_1 relaxes to S_0 , ground state, and that relaxation is named fluorescence and radiative process. The other option is intersystem crossing. In that process, spin of the electron changes because electron moves S_1 , singlet excited state, to T_1 , triplet excited state. That is also non-radiative process. After the intersystem crossing, relaxation of the electron occurs from triplet excited state to ground state and that is called phosphorescence. In solution, non-radiative relaxation from T_1 is more than phosphorescence at room temperature.

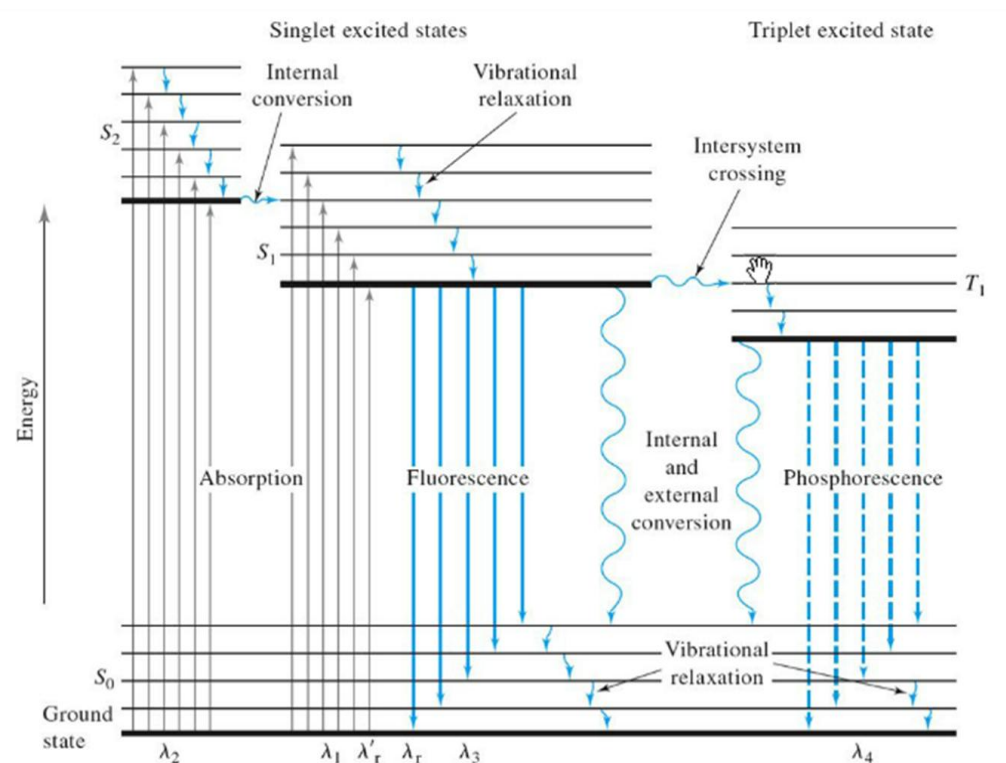


Figure 7. The Jablonski Diagram.

Jablonski diagram clearly shows that emitted light has less energy from absorbed one. It means that, emission occurs in longer wavelength and the difference between excitation and emission wavelength is called Stokes'

shift.³³ The reason of the Stokes' shift is that rapid relaxation of excited electron to lowest vibrational state of S_1 non-radiatively. In some situations, fluorescence emission can be irrespective of excitation wavelength and that is named as Kasha's rule.³⁴

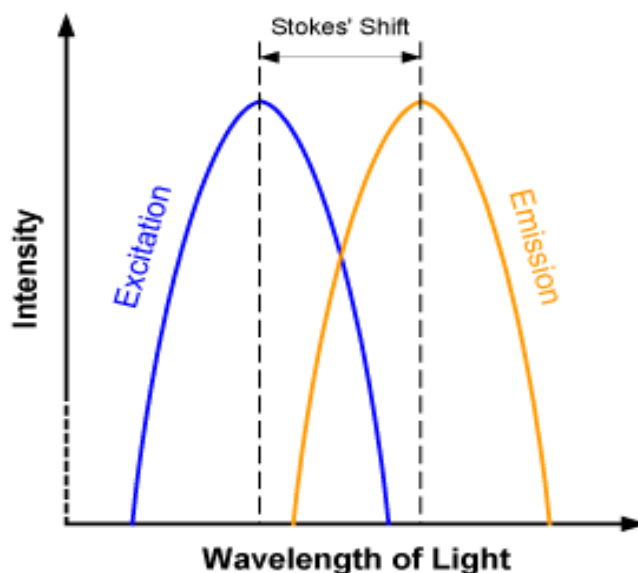


Figure 8. Stokes' Shift.

1.2.1. Fluorescent Dyes

All of traditional dyes absorb light and remaining wavelengths are reflected. An observer sees remaining part of the light which is reflected and that is the color of the matter. For instance, a dye absorbs blue part of the visible light and reflects yellow which is complementary light of blue. However, fluorescent dyes are different from the traditional dyes. They have fluorescence property which is explained before.

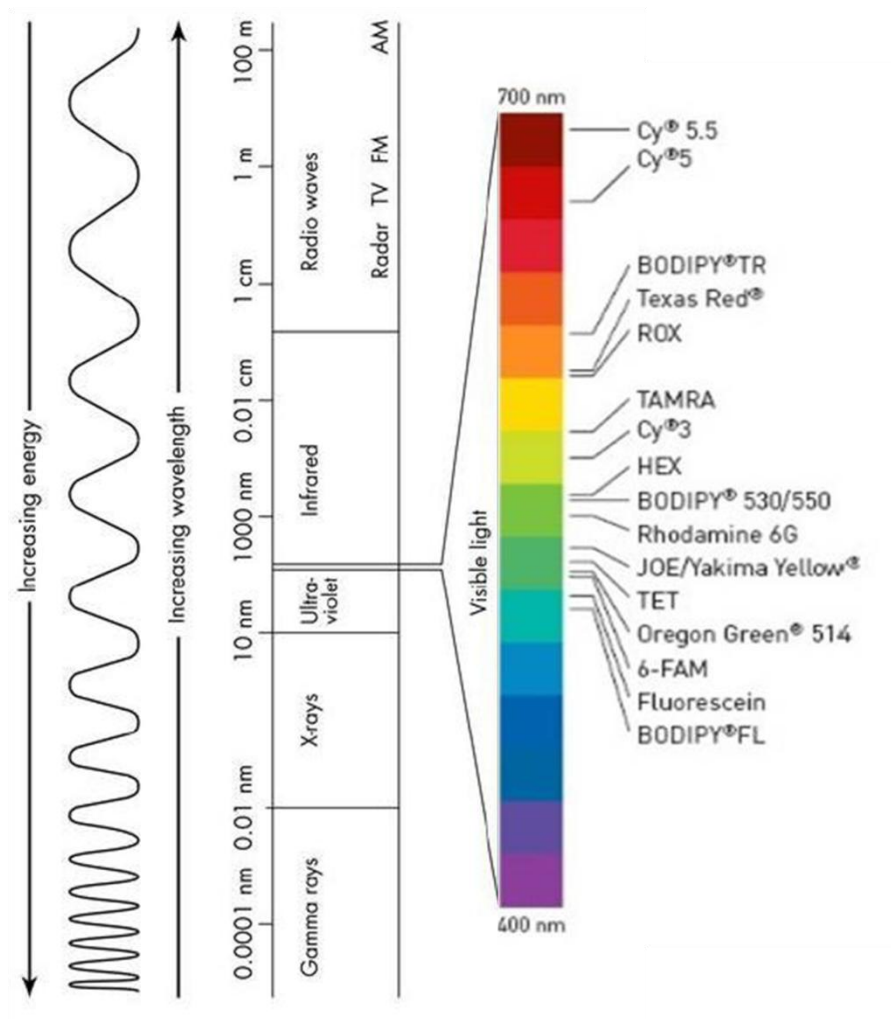


Figure 9. Common dyes in visible region.

Organic dyes in UV-Vis area cover almost all of the UV-Vis area and have wide application areas. In the Figure 9, the most common dyes are shown. These dyes are generally rhodamine, pyrene, naphthalene, coumarin, fluorescein, rhodamine, and cyanine based structures.

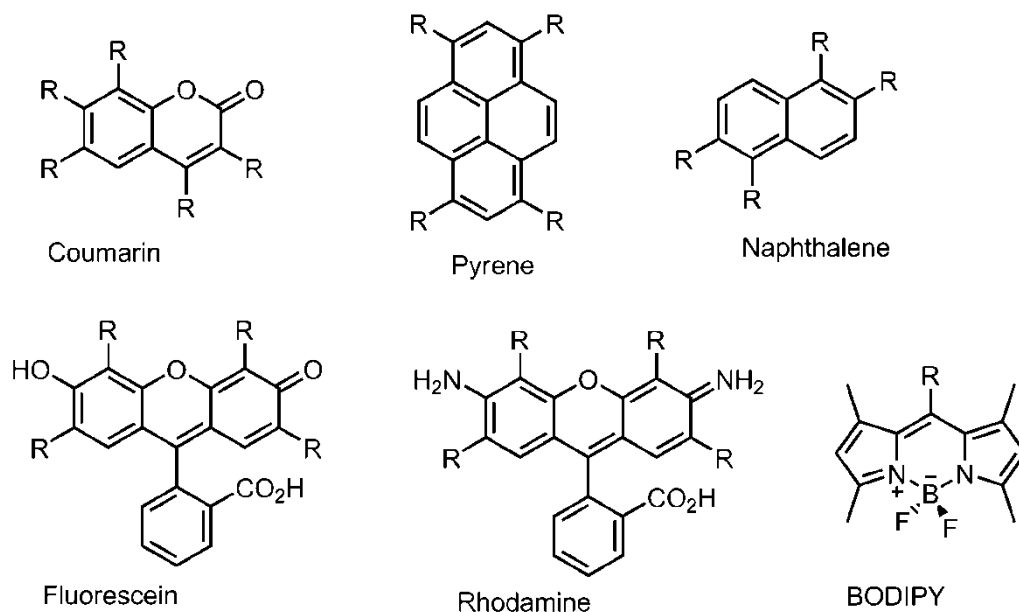


Figure 10. Structure of common dyes.

All of the dyes have some advantages and drawbacks. High quantum yield, bioconjugation and solubility can be example for the advantages; on other hand self-quenching and easy photobleaching are disadvantages. One of the most important members of fluorescent dyes family is boradiazaindacene (BODIPY) which will be explained at following parts.

1.3. Molecular Sensors

A receptor can be used as a sensor when some physical changes occur after the binding of analyte. Selectivity is also important for sensors. Sensor should be selective for a specific guest and it makes possible to monitor amount of guest besides reporting of presence of the guest molecule. This is important in many application areas such as medicinal and environmental analyses. For example, a sensor can be used for detecting of pollutant levels and understanding of presence of drug.

There are several analytical methods for detection of many analytes but they are usually expensive and their detection limit is not low. Those reasons increase importance of molecular sensors. There two types of molecular

sensors; these are electrochemical and optical sensors. Electrochemical sensors consist of a redox active unit and a receptor. Binding of analyte to the receptor changes charge separation of the sensor. This change can be monitored by an electrochemical technique such as cyclic voltametry (CV).

The other type of sensors is optical sensors. Fluorescent sensors are most common types of the optical sensors because of the advantages such as high sensitivity, safety, and high speed. The most important advantage of fluorescent sensors over other light-based methods such as absorbance is high sensitivity. The sensitivity of fluorescent is related the difference between the excitation and the emission wavelength. It can be used for biological systems that very small concentration changes such as pico and even femtomolar ranges can be important. On the other hand, absorbance which is different expression of transmittance of light is used for measurements of concentration of micromolar ranges and it is not as sensitive as fluorescent as it can be seen. Another advantage is safety. There is no hazardous product in the process and samples are not affected. The last advantage is high speed. Fluorescence is very fast process therefore; changes in concentration can be displayed rapidly. On the contrary, fluorescent sensors has a drawback which is they can be used mainly in organic solvents. In the aqueous media they do not respond.

Figure 11 shows that some of examples for fluorescent sensors. For example, compound **2** involves Lariat ether and it is used for K^+ sensing. On the other hand, there are also fluorescent sensors for anions. Compound **1** is one of the F^- sensors.³⁵

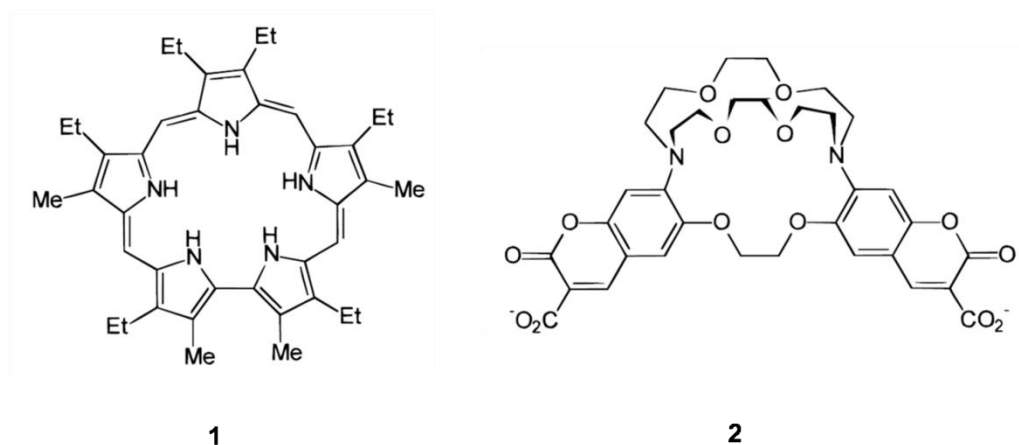


Figure 11. Examples of molecular sensors for anion and cation.

1.3.1. Fluorescent Sensors

Fluorescent sensors are simply designed that receptor which is attached to fluorophore. In the fluorescent sensors, selectivity and signaling is very important. When analyte binds to receptor, changes occur in the fluorescence of fluorophore. Those changes are converted to signals and monitored. The fluorescent sensors can be used for many analytes such as neutral molecules, cations and anions. For the all of these species, quantitative or qualitative measurements can be possible. When analyte binds to the receptor, fluorescence can be increased or decreased and quenched according to type of analyte and fluorescent sensor.³² There are two types of fluorescent sensors. First one is fluorophore-spacer-receptor, as it can be seen fluorophore and receptor is separated by spacer such as alkyl chain. In other type, receptor is linked directly to the fluorophore and because of that receptor participates in π -system of fluorophore.³⁶

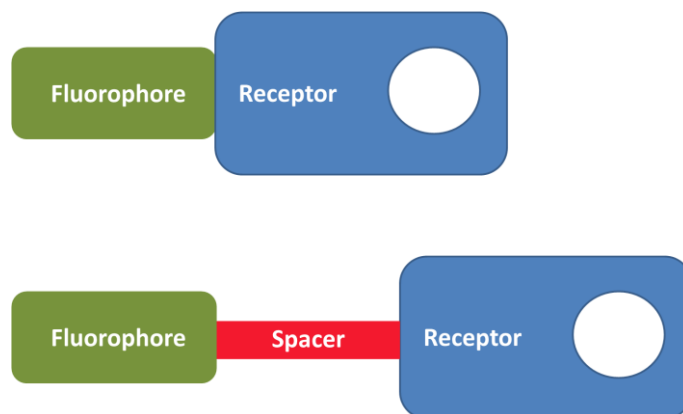


Figure 12. Schematic representations of fluorescent sensors.

1.3.1.1. Photoinduced Electron Transfer (PET)

In photoinduced electron transfer, the system consists of fluorophore, spacer and receptor which includes a electronegative donor atom. There is no conjugation between fluorophore and receptor but they are close enough for electronic interaction by the spacer. Absorbing of light causes excitation of electron of fluorophore then electron of receptor jumps that hole to reduce its energy.

In the Figure 13, working principal of PET is shown. Firstly, electron of fluorophore is excited from the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). Energy level of HOMO of donor atom (usually amine groups) which is part of receptor is between HOMO and LUMO of the fluorophore. After excitation, an electron from HOMO of the donor atom moves to HOMO of the fluorophore and that causes quenching of fluorophore. However, when analyte such as metal cation binds to the receptor that results decreasing energy of donor atom's HOMO. By this way PET is prevented and quenching is finished.³⁷

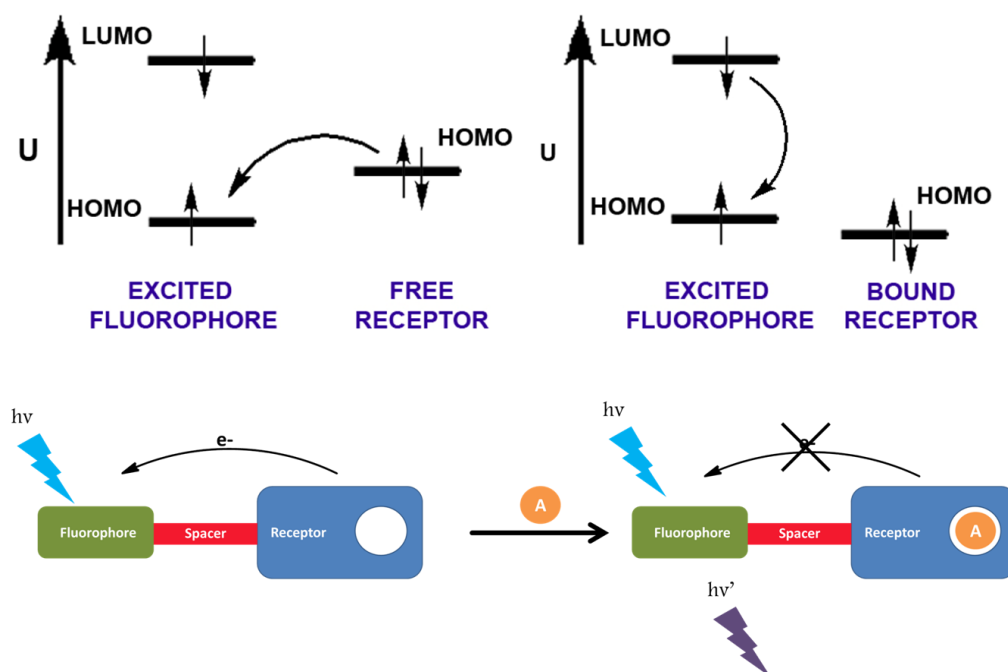


Figure 13. Schematic representation and mechanism of PET.

There are many fluorescent chemosensors work with PET mechanism. Recognition moiety can change in systems from crown ether to cryptand as it can be seen in Figure 14. There are many PET sensors for the specific metal ions such as sodium, magnesium, potassium, calcium, and transition metal ions.

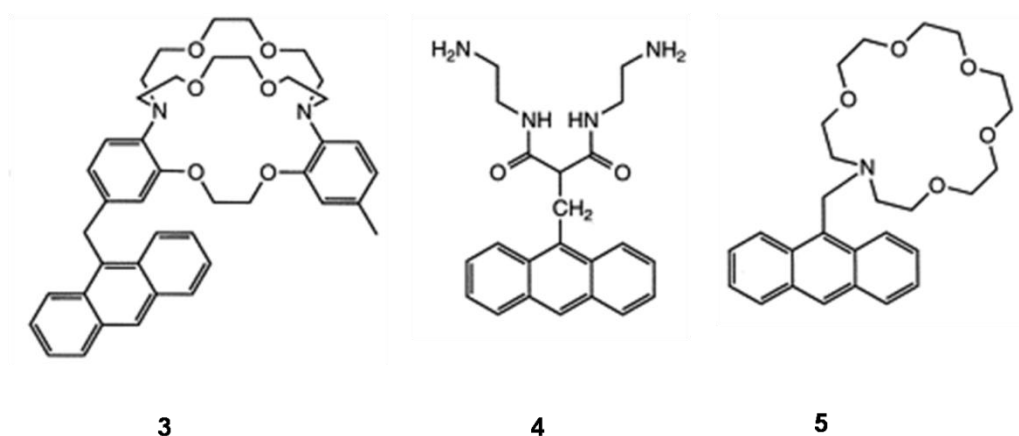


Figure 14. Examples for PET based fluorescent sensors^{38–40}.

In some systems, PET may occur from acceptor to donor. It is called reverse PET or oxidative PET. Figure shows mechanism of reverse PET. In the absence of metal cation emission occurs normally. When analyte binds to receptor, energy level of LUMO of donor atom decreases and takes place between HOMO and LUMO of fluorophore. After excitation fluorophore, excited electron in LUMO goes to LUMO of receptor instead HOMO of fluorophore while relaxation. That causes quenching of fluorophore.

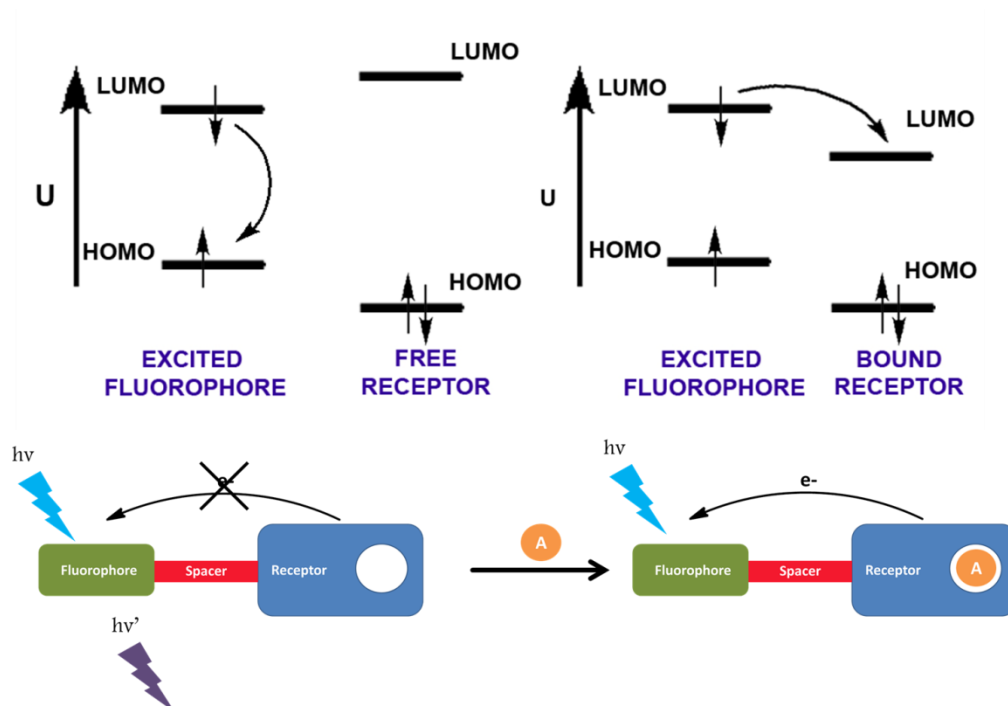


Figure 15. Schematic representation and mechanism of reverse PET.

Compound **6**⁴¹ which is synthesised by Akkaya et al is good example for reverse PET. Fluorophore and receptor are BODIPY and 2, 2-bipyridine respectively. After the binding of zinc ion, emission is quenched by oxidative PET mechanism.

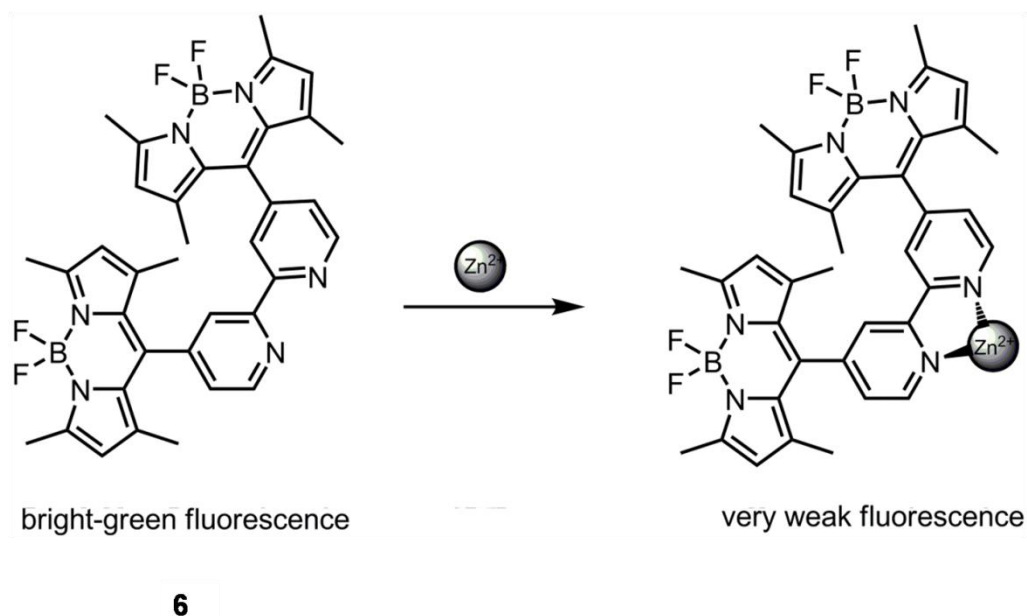


Figure 16. An example of oxidative PET.

1.3.1.2. Internal Charge Transfer (ICT)

The system includes fluorophore and receptor only. In PET systems, there is a spacer between fluorophore and receptor. On the other hand, fluorophore is directly bound to receptor in internal charge transfer systems. Therefore, there is orbital overlapping in this conjugated system and because of that reason internal charge transfer occurs. Binding of analyte to the receptor actually causes excited state dipole and affects emission spectrum.

There are two types of stoke shifts in the emission spectrum of the molecule; these are blue shift and red shift. If there is an electron donating group that binds the fluorophore, receptor-cation interaction reduces electron density in system and that causes decreasing in conjugation. That results in a blue shift in the absorbance spectrum of fluorophore. Excited state of molecule is used for understanding of changes in emission spectrum. There is a positive charge on donor group such as amine at the excited state. The interaction of two positively charged groups causes destabilizing of excited state. Therefore, energy gap between HOMO and LUMO increases by this way analyte can be determined.

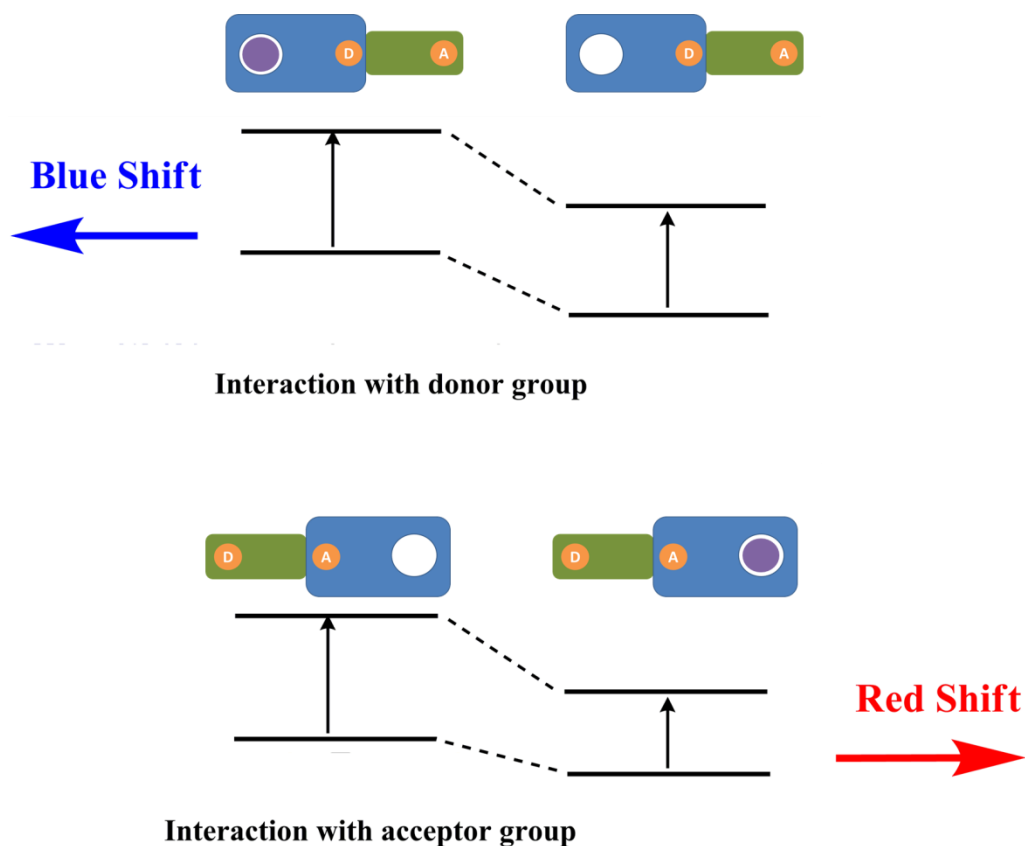


Figure 17. Shifts of ICT based sensors.

On the other hand, interaction between cation and acceptor group like a carbonyl results enhancement of electron withdrawing property of acceptor. It can be also explained with the excited state interactions. Interaction between cation and the negatively charged acceptor group stabilize the excited state of course. As a result of this interaction, the energy gap between HOMO and LUMO decreases and that means red shift is observed in the emission spectrum.

There are many examples in literature and compound **7**⁴² and **8**⁴³ can be seen in the Figure 18. These ICT based sensors show blue shift when cation binds to their receptors.

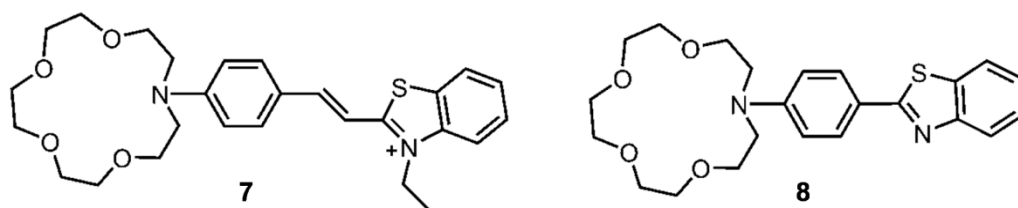


Figure 18. Some examples of ICT based sensors.

1.3.2. Sensing of Particular Metal Ions

1.3.2.1. Mercury Ion Sensors

Heavy and transition metals have important positions in biological and environmental systems. Therefore, detection and monitoring of them is gaining great importance day by day. Mercury is one of them and has significant negative effects on health of human beings.⁴⁴

Mercury is presented in nature both ionic and elemental forms. Mercury releases to nature from many sources which are natural and industrial. Industrial sources of mercury are gold and coal mines, fossil fuel combustion, chemical production, and burning of solid wastes. For example, nearly 80% of mercury comes from fossil fuel combustion and burning of solid wastes in United States. Natural sources are volcanoes, oceans and forest fires.⁴⁵

Elementary mercury vapors which are emitted from especially oceans are finally oxidized to Hg (II). That causes deposition of Hg (II) on plants and waters. After the all of them, Hg (II) is converted to Hg (0) by microorganisms and turns back to the atmosphere. Bacteria which live in fish gills and gut and some prokaryotes produces methylmercury from the mercury. Methylmercury is the most dangerous one in the mercury species and almost all of them come from seafood consumption. An example for that is many poisonings in Minamata, Japan. Methylmercury threats especially brain, kidneys, central nervous system, and immune system.⁴⁶ Affecting of these systems by mercury can result in brain damage, vision and hearing loss, cognitive and motion

disorders, and death. The long-term exposure of methylmercury is also great concern for human embryos, the developing fetus, and children. Because of the reasons which are mentioned in above, sensing of mercury species has vital importance.⁴⁵

Advantages that are mentioned in molecular sensors part also available for Hg (II) sensors. By using mercury chemosensors, detection can be made by naked eye without any instrument. Simple design, selectivity and strong signal are other important properties of Hg chemosensors.

It is known that interaction between hard acid and hard base or soft acid and soft base is better than hard and soft interaction. Usually sulphur is used in Hg (II) chemosensors because of the softness of mercury ion.

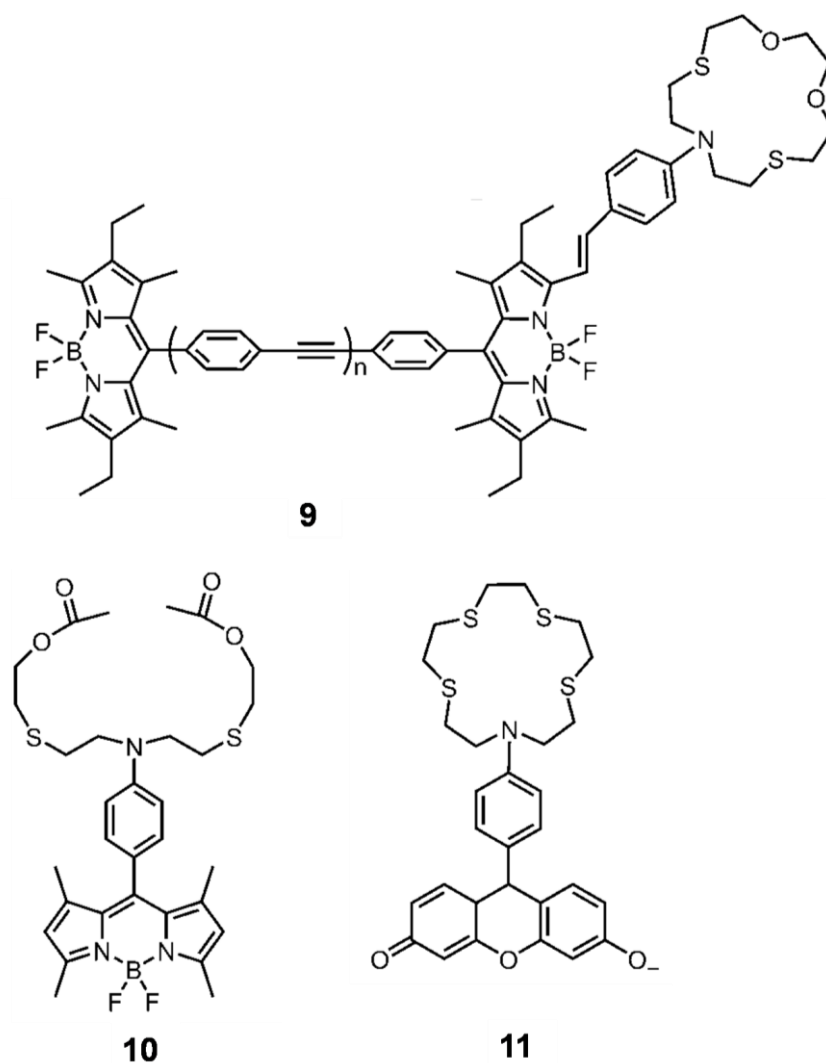


Figure 19. Hg (II) selective sensors.

The Figure 19 shows some examples for mercury chemosensors from literature. Compound **10**⁴⁷ which was developed by Shiguo Sun et al is photoinduced electron transfer (PET) based chemosensor because it is not planar due to steric hindrance. There is no emission without Hg (II) then by the presence of Hg (II), emission occurs.

The most important characteristic of compound **11**⁴⁸ is that it works in aqueous media. That provides applicability in biological systems.

Compound **9**⁴⁹ which was published Akkaya group is another example for Hg (II) chemosensors. That is ICT based chemosensor and it uses energy transfer

mechanism as well. Binding of Hg (II) causes blue shift in acceptor part while donor part remains same. As a result of that, increasing spectral overlap between the absorbance of the acceptor and the emission of the donor parts causes more effective FRET.

1.3.2.2. Zinc Ion Sensors

In human body, zinc is the second most abundant transition metal. Many proteins and enzymes involve Zn (II). Zinc play important role in many neurological diseases such as Alzheimer's disease, Parkinson's disease, epilepsy, and amyotrophic lateral sclerosis (ALS). Moreover, zinc has a important role in insulin absorption. Zinc also affects the body functions such as immune, gastroenterological, and endocrine systems besides growth of children.⁵⁰

In recent years, heavy metal ion pollution is one the most controversial environmental problems. They are great concern for human health because of their toxic properties. Zinc ion is one the heavy metals. Production of brass and bronze alloys and galvanization are main sources of zinc in addition to paints, rubber, plastics, cosmetics and pharmaceuticals. There is limit which is 5.0 mg/L for zinc in drinking water and beyond this limit it is toxic anymore.⁵¹

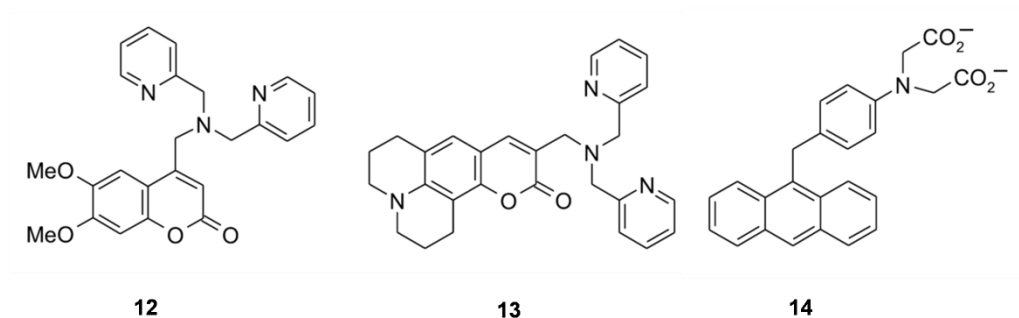


Figure 20. Some examples of Zn (II) sensors from literature.

Detection and monitoring of Zn (II) is very important in biological and environmental systems because of the reason which is explained above. Here are the some examples⁵² for zinc sensors in the Figure 20.

1.3.2.3. Calcium Ion Sensors

Recent studies show that some ions such as Ca^{2+} and Mg^{2+} have essential roles in physiological processes.⁵³ Calcium ion plays critical role as intercellular messenger in body. Intercellular Ca^{2+} concentration is important for excitation-contraction coupling in muscle and secretory cells. In many cellular processes, Ca ion participated in as second messenger, therefore; many scientist show great attention to calcium probes.⁵⁴ In recent years, studies on monitoring of Ca^{2+} concentration increases rapidly because of insufficiency of other monitoring techniques.⁵⁵

In Figure 21, it can be seen an example for caged Ca^{2+} chelator.⁵³ This probe is nitrobenzyl-based and by this way its affinity can be changed with light. It has a bond which is photosensitive and bond can be broken with UV light. After bond cleavage, affinity of sensor to calcium ion decreases dramatically.

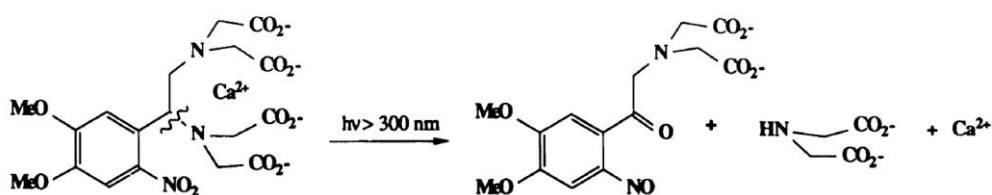


Figure 21. Photocleavage reaction of caged Ca^{2+} compound.

1.4. BODIPY

Boradiazaindacene (BODIPY) dyes were first discovered by Treibs and Kreuzer in 1968.⁵⁶ Popularity of BODIPY is increasing day by day since 1968. Today, BODIPY has a significant place among the fluorescent dyes. In many research areas such as ion sensing, molecular logic gates, sensitizers for solar cells, biomolecular labeling, drug delivery reagents and light harvesting systems, BODIPY is used by chemists, biologists and physicists.

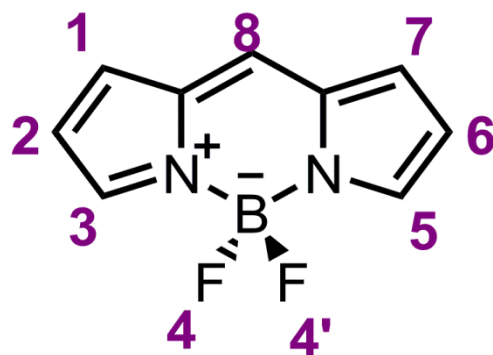


Figure 22. BODIPY has many positions for functionalization.

BODIPY dyes have great properties for the many application areas which are mentioned above. They have high molar extinction coefficients, high quantum yields and fluorescence emission in visible range.^{57,58} Also, its sensitivity to pH and solvent polarity is lower so it is more stable than many other dyes in hard physical conditions. Emission range of BODIPY dyes is very wide from 500 nm to 900 nm. Solubility in organic solvents is good. Maybe, the most significant property of the BODIPY is easy functionalization. All of the positions (1 to 8) in BODIPY skeleton are suitable for chemical modifications. Many BODIPY derivatives were synthesised until today.^{59–63} There are several research groups which are working on BODIPY and derivatives. These research groups are Akkaya, Burgess, Nagano, Rurack, and Ziessel.

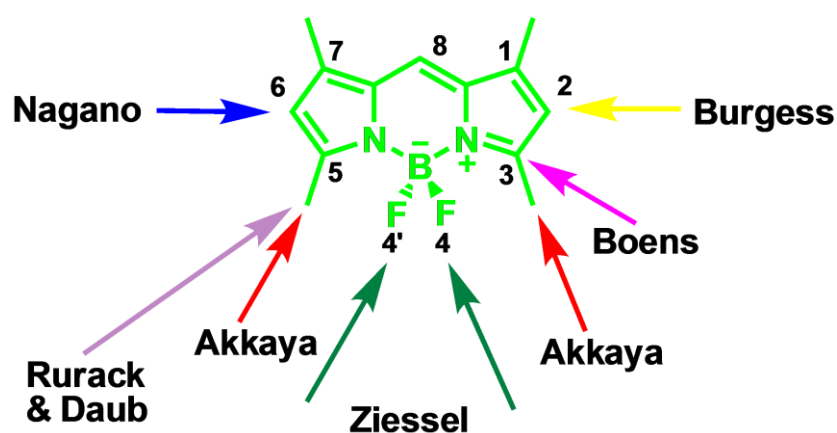


Figure 23. Research groups that working on BODIPY.

1.4.1. Applications of BODIPY

There are many application areas of BODIPY (Figure 24) because of the properties which are mentioned before. Some of the BODIPY derivatives which are fluorescent sensors, photosensitizers, and photodynamic therapy agents will be mentioned with examples from literature.

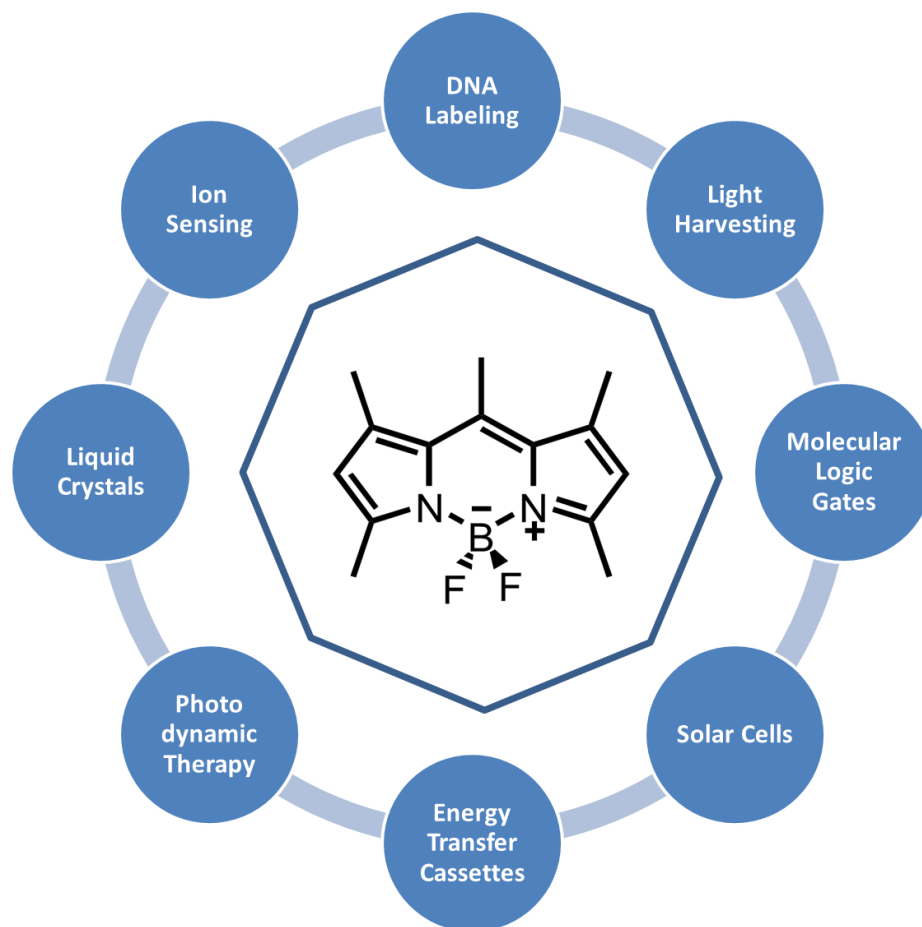


Figure 24. Application areas of BODIPY.

Because of the easy functionalization, photostability and high quantum yields, there are many BODIPY based chemosensors in literature. The first example for BODIPY based chemosensors was synthesised by Daub and Rurack in 1997⁶⁴. Then innumerable BODIPY derivatives have been synthesised for sensing until today.

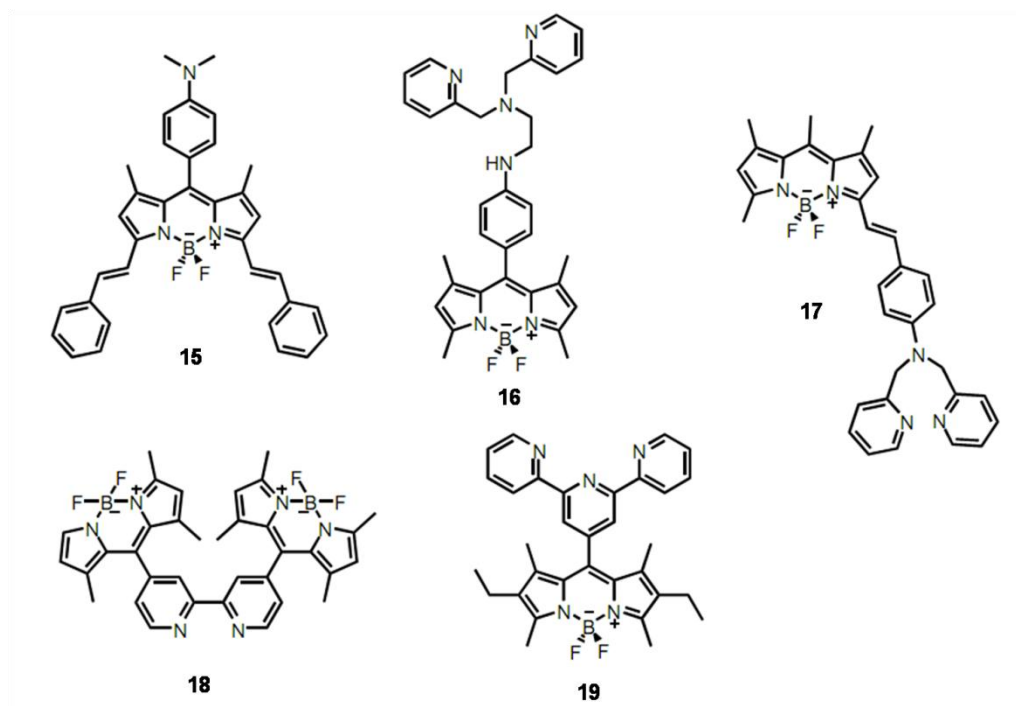


Figure 25. BODIPY based chemosensors.

In the Figure 25, there are examples of PET and ICT based BODIPY sensors. Compound **15** is proton sensor.⁶⁵ Dimethylamino group is strong electron donor group and causes ICT. However, in the presence of proton, dimethylamino group is protonated and ICT is blocked. Compound **16**⁶⁶ and **17**⁶⁷ are respectively zinc (II) and cadmium (II) sensors and their receptor parts are anilino groups. Compound **18**⁶⁸ and **19**⁶⁹ have bipyridine and terpyridine groups as recognition sites.

Another application area of BODIPY dyes is photodynamic therapy which is a kind of treatment for cancer. For the PDT, a dye has the absorbance band between 650 nm to 850 nm. The reason is that the most suitable wavelength range for penetrating of light to body is between 650-800 nm. After localization of PDT agents, tissue is irradiated with light to produce singlet oxygen for destroying of cancer cells. There are many dyes used for PDT. In recent years, BODIPY dye is also used for PDT. In the Figure 26, examples for BODIPY based PDT agents. Solubility in water is very important for PDT because living systems mainly consist of water. Hydrophilic groups can be attached to increase solubility in water as it can be seen in the figure 26.

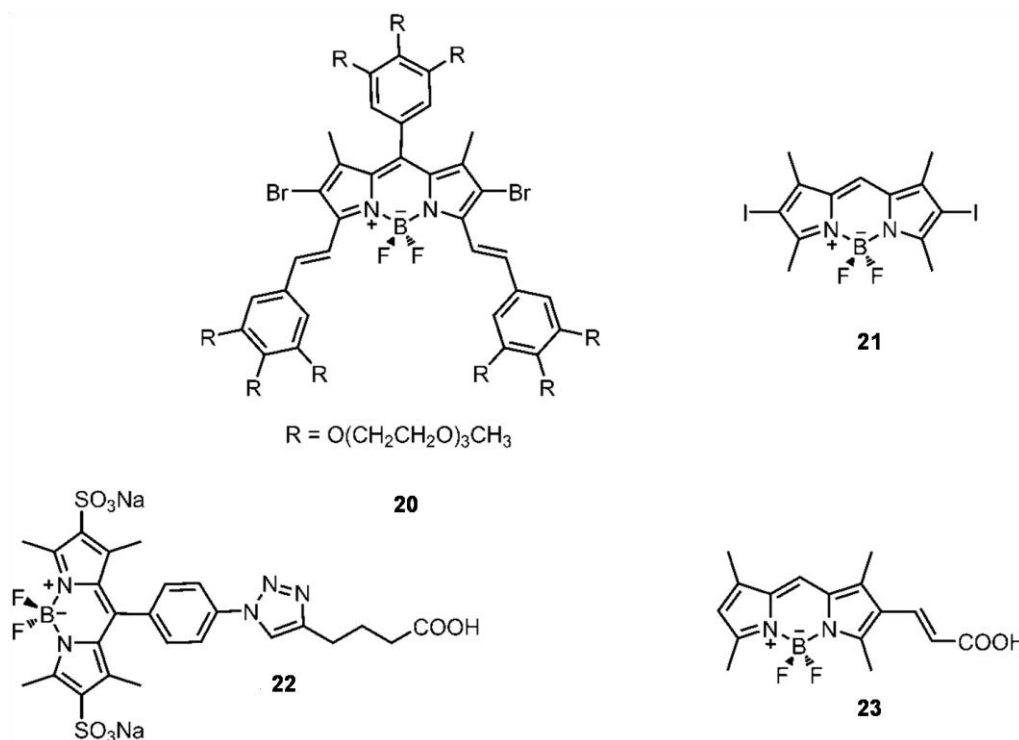


Figure 26. Photosensitizers for photodynamic therapy.

There are some examples of BODIPY based sensitizers in above figure. Compound **20**⁷⁰ which was developed by Akkaya et al is good example of water soluble BODIPY based sensitizer. Increasing of conjugation provides longer absorption wavelength. Heavy atoms which were attached to BODIPY core increase intersystem crossing and by this way singlet oxygen production yield of molecule. Compound **21**⁷¹ is synthesised by Nagano et al and its quantum efficiency is 0.02 that is very low because of the heavy atoms. **22**⁷² and **23**⁷³ are other examples of water soluble BODIPY based sensitizers.

Light harvesting systems and energy transfer cassettes are also application areas of BODIPY. These systems consist of donor and acceptor parts and energy transfer occurs from donor to acceptor. They are actually mimicking of nature because some living systems are used energy transfer system efficiently. There are two types of energy transfer which are through space and through bond.

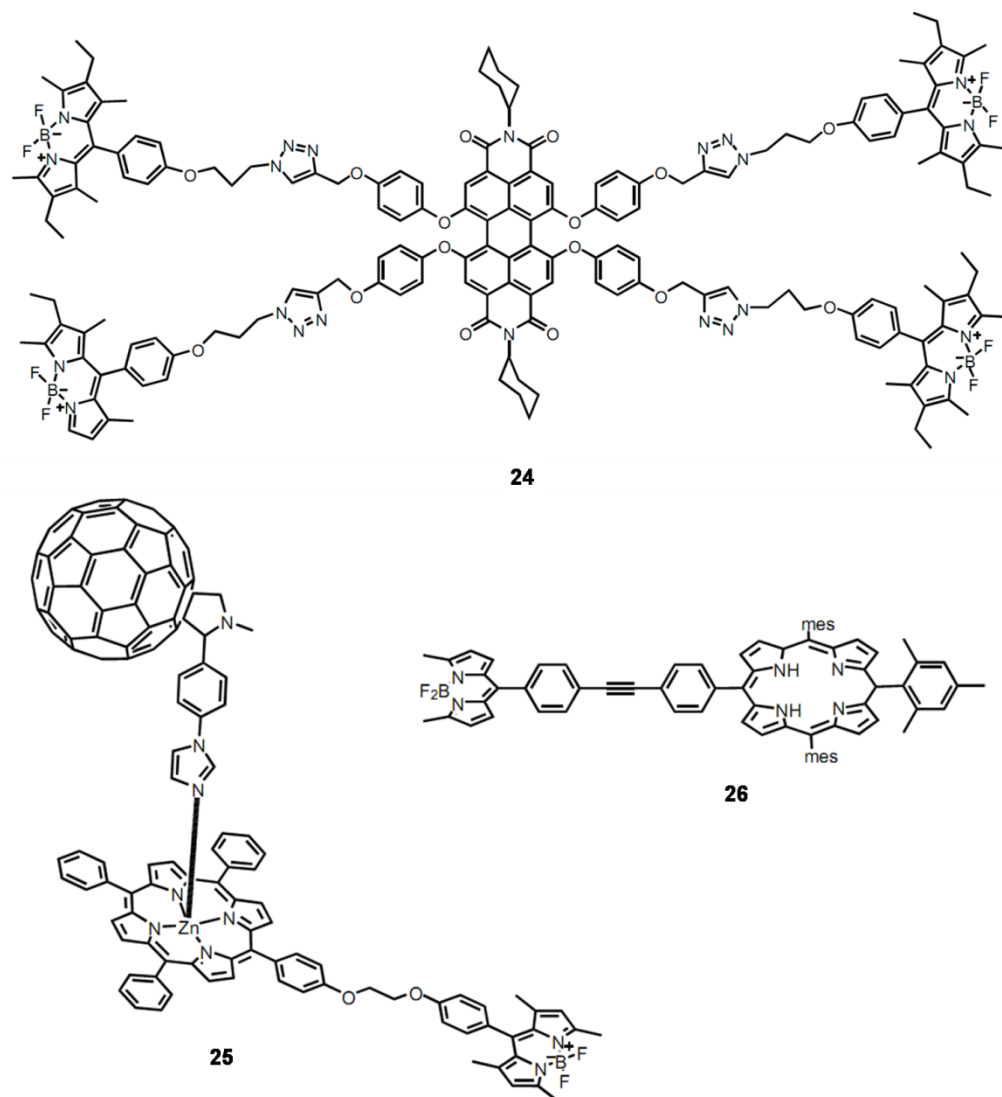


Figure 27. Examples of light harvesting systems and energy transfer cassettes.

In the figure 27, compound **24** which was synthesized by Akkaya et al is an example for dendritic light harvesting systems.⁷⁴ The core of molecule is perylene bisimide as acceptor part and molecule has BODIPY based donor parts. **25**⁷⁵ and **26**⁷⁶ are examples of energy transfer cassettes and through space and through bond types respectively.

Interest to dye-sensitized solar cells (DSSC) increases rapidly in recent years. Many research groups believe that DSSC can be alternative for more expensive solar cell technologies and they try to maximize efficiency of DSSC. First using of BODIPY as photosensitizer was realized by Nagano. In DSSC systems,

carboxylate (-COOH) group on BODIPY derivative is attached TiO₂ surface and electron transfer occurred by irradiation of sunlight. In the figure, an example of BODIPY based photosensitizer can be seen.

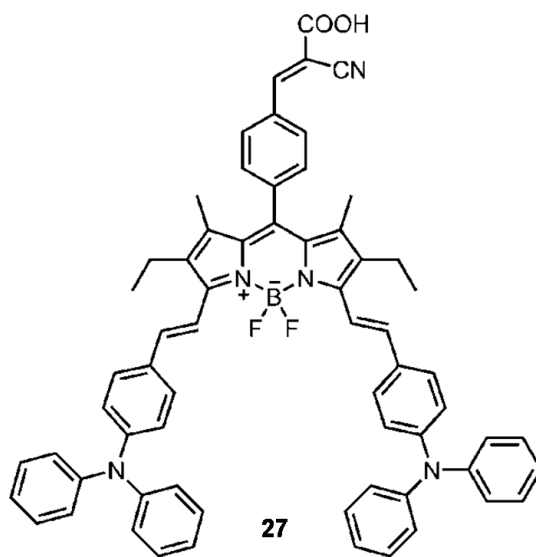


Figure 28. An example of BODIPY based photosensitizer.

As it can be seen in the above figure, compound **27** which was developed by Akkaya group is novel example of BODIPY based photosensitizer. Its reported conversion efficiency was 1.66%.⁷⁷

CHAPTER 2

EXPERIMENTAL

2.1. General

All chemicals and solvents purchased from Aldrich were used without further purification. Column chromatography of all products was performed using Merck Silica Gel 60 (particle size: 0.040–0.063 mm, 230–400 mesh ASTM). Thin layer chromatography by Merck TLC Silica gel 60 F254 was used to monitor reactions.

^1H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 (operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) in CDCl_3 with tetramethylsilane as internal standard. All spectra were recorded at 25 °C and coupling constants (J values) are given in Hz. Chemical shifts are given in parts per million (ppm). Splittings in the spectra are shown as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet).

Absorption spectrometry was performed using a Varian spectrophotometer. Fluorescence spectra were determined on a Varian Eclipse spectrofluorometer. All spectroscopy experiments were performed using spectrophotometric grade solvents.

Mass spectroscopy measurements were conducted using MSBQTOF at Bilkent University, UNAM, Mass Spectrometry Facility.

2.2. Synthesis of Compound 28

K₂CO₃ (53.5 mmol, 7.40 g) was added to solution of 4-hydroxybenzaldehyde (16.4 mmol, 2.00 g) in 40 ml of acetonitrile. Catalytic amount of 18-crown-6 was added. 1.5 equivalent of 1-bromooctane (24.6 mmol, 4.75 g) was added to the reaction. Then it was stirred and refluxed over night. The solvent was evaporated and extracted with CH₂Cl₂. Organic layers were dried over Na₂SO₄. The crude product was purified with silica gel column chromatography (CHCl₃/Hexane, 1:1) . White solid was acquired (3.54 g, 93%).

¹H NMR (400 MHz, CDCl₃) δ 9.74 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 3.97 (t, *J* = 3.2 Hz, 2H), 1.67 (m, 2H), 1.18 (m, 11H), 0.77 (t, *J* = 4.7 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 190.72, 164.26, 131.94, 129.76, 114.73, 68.41, 31.75, 29.30, 29.27, 29.05, 25.96, 22.61, 14.07.

HRMS-ESI: calculated for M+H 235.1698, found 235.1674, Δ= -7.68 ppm.

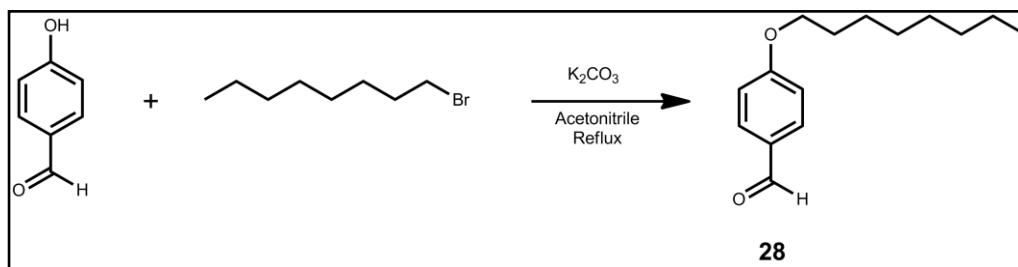


Figure 29. Synthesis of compound 28.

2.3. Synthesis of Compound 29

2,4-dimethyl pyrrole (8.40 mmol, 0.80 g) and compound 28 (4.00 mmol, 0.94 g) were dissolved in 200 mL of CH₂Cl₂ which was bubbled with argon gas for 25 min. One drop of Trifluoroacetic acid was added and the solution was stirred for 1 day at room temperature. A solution of DDQ (4.00 mmol, 0.90 g)

in 50 ml of CH₂Cl₂ was added to reaction and stirring was continued for 45 min. After addition of 5 ml of NEt₃ and 5 ml of BF₃.OEt₂, the reaction was monitored by TLC (CHCl₃). After stirring for 1 hour, the reaction mixture was washed with water (3 x 200 ml) then it was dried with Na₂SO₄. The solvent was evaporated under vacuum and the residue was purified with silica gel column chromatography (CHCl₃). Brown solid was obtained (0.47 g, 26%).

¹H NMR (400 MHz, CDCl₃) δ 7.06 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 5.89 (s, 2H), 3.93 (t, *J* = 6.6 Hz, 2H), 2.47 (s, 6H), 1.73 (m, 2H), 1.36 (s, 6H), 1.41 (m, 2H), 1.06 (m, 10H), 0.83 (t, *J* = 6.9 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 159.76, 155.18, 143.18, 142.04, 131.88, 131.50, 129.14, 126.79, 115.09, 68.17, 31.83, 29.41, 29.40, 29.27, 26.08, 22.65, 14.56, 14.54, 14.11.

HRMS-ESI: calculated for M-H 451.2733, found 451.2767, Δ= 3.23 ppm.

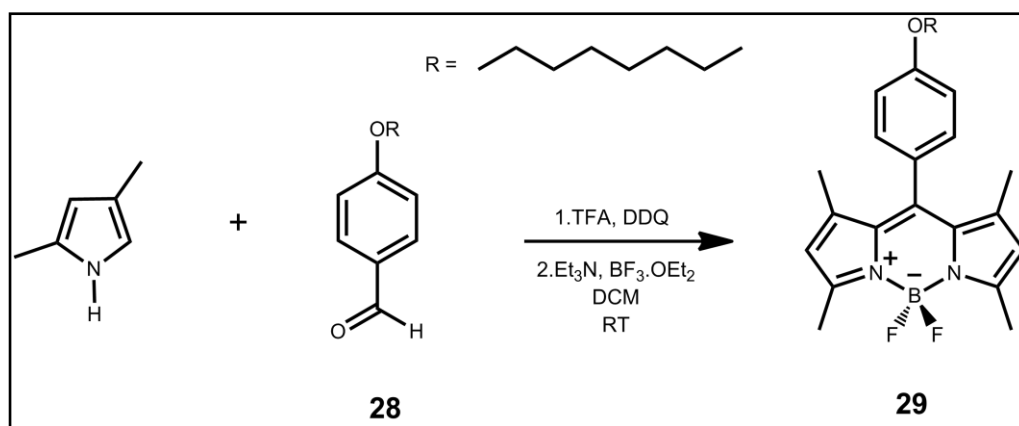


Figure 30. Synthesis of compound **29**.

2.4. Synthesis of Compound 30

Iodic acid (2.00 mmol, 0.35 g) in 2 ml water was added to the mixture of I₂ (2.50 mmol, 0.63 g) and compound **29** (1.04 mmol, 0.47 g). Then 50 ml of ethanol was added to reaction and solution was stirred for 2 hours at 60 °C.

Reaction was monitored by TLC using EtOAc as eluent. After consuming of all starting materials, saturated Na₂S₂O₃ solution in water was added to solution and product was extracted into CH₂Cl₂. Then solvent was evaporated and silica gel column chromatography (EtOAc) was used for further purification. Red solid was acquired (0.72 g, 98%).

¹H NMR (400 MHz, CDCl₃) δ 7.02 (d, *J* = 8.5 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 3.94 (t, *J* = 6.5 Hz, 2H), 2.56 (s, 6H), 1.75 (m, 2H), 1.43 (m, 2H), 1.36 (s, 6H), 1.25 (m, 10H), 0.82 (t, *J* = 6.9 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 160.19, 156.47, 145.34, 141.77, 131.79, 129.05, 126.38, 115.44, 85.64, 68.29, 31.89, 29.48, 29.32, 29.29, 26.15, 22.76, 17.29, 16.09, 14.26.

HRMS-ESI: calculated for M-H 703.0665, found 703.0713, Δ = 1.85 ppm.

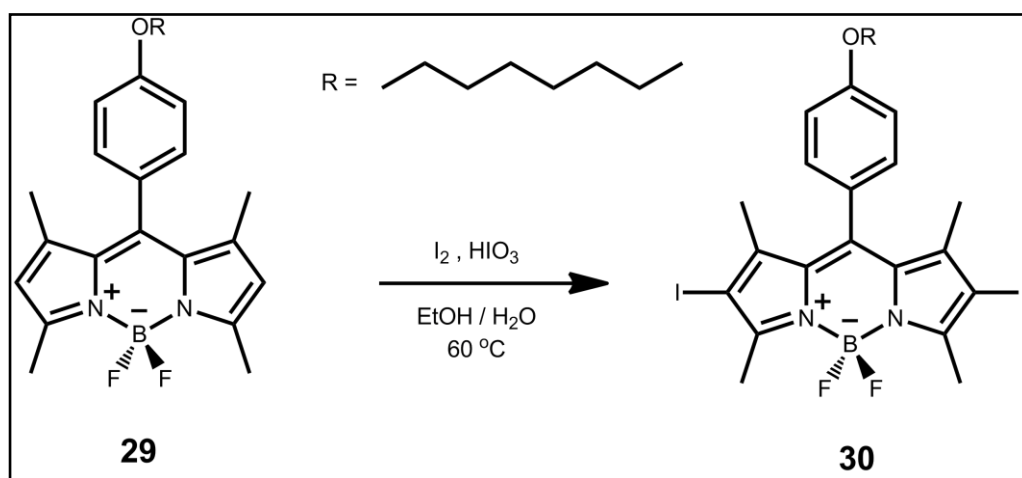


Figure 31. Synthesis of compound 30.

2.5. Synthesis of Compound 31

To a 1L round-bottomed flask were added NaOH (0.25 mol, 10.0 g) in 100 ml of water and tetraethylene glycol (0.088 mol, 17.0 g) in 150 ml of THF and the mixture was cooled on an ice-bath. p-toluenesulfonyl chloride (0.18 mol, 32.5

g) in 150 ml of THF was added dropwise to the mixture with continuous stirring for 2 hours at 0 °C. After stirring of solution an additional 2 hours, the reaction mixture poured into ice-water (100 ml). After the extraction (CH₂Cl₂/water), the crude product was purified with silica gel column chromatography (EtOAc). Yellowish liquid was obtained (31.8 g, 72%).

¹H NMR (400 MHz, CDCl₃) δ 7.64 (d, *J* = 8.3 Hz, 4H), 7.25 (d, *J* = 8.3 Hz, 4H), 4.02 (t, *J* = 4.7 Hz, 4H), 3.53 (t, *J* = 4.7 Hz, 4H), 3.43 (m, 8H), 2.30 (s, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 144.86, 132.94, 129.85, 127.90, 70.64, 70.48, 69.34, 68.61, 21.58.

HRMS-ESI: calculated for M+Na 525.1223, found 525.1205, Δ= -3.46 ppm.

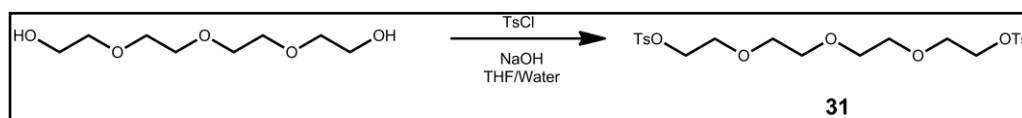


Figure 32. Synthesis of compound **31**.

2.6. Synthesis of Compound 32

To a two-necked flask which was purged with argon was added NaH (60% suspension in paraffin oil) (40.0 mmol, 1.61 g) in 200 ml of dry THF and refluxed. N,N-bis(2-hydroxyethyl)aniline (20.0 mmol, 3.66 g) and tetraethylene glycole ditosylate (20.0 mmol, 10.1 g) were dissolved in 200 ml of THF and added dropwise to the reaction mixture in 3 hours. Stirring and refluxing was continued for additional 5 h. After completing of the reaction, mixture was extracted into THF three times and then solvent was evaporated under reduced pressure. Silica gel column chromatography (2 EtOAc : 1 Hex) was used for purification of the residue. Yellowish liquid was acquired (3.73 g, 55%).

^1H NMR (400 MHz, CDCl_3) δ 7.07 (t, J = 7.7 Hz, 2H), 6.55 (m, 3H), 3.52 (m, 24H).

^{13}C NMR (100 MHz, CDCl_3) δ 147.81, 129.26, 115.85, 111.61, 70.83, 70.78, 70.75, 70.67, 68.72, 51.27.

HRMS-ESI: calculated for $\text{M}+\text{H}$ 340.2119, found 340.2104, Δ = -4.29 ppm.

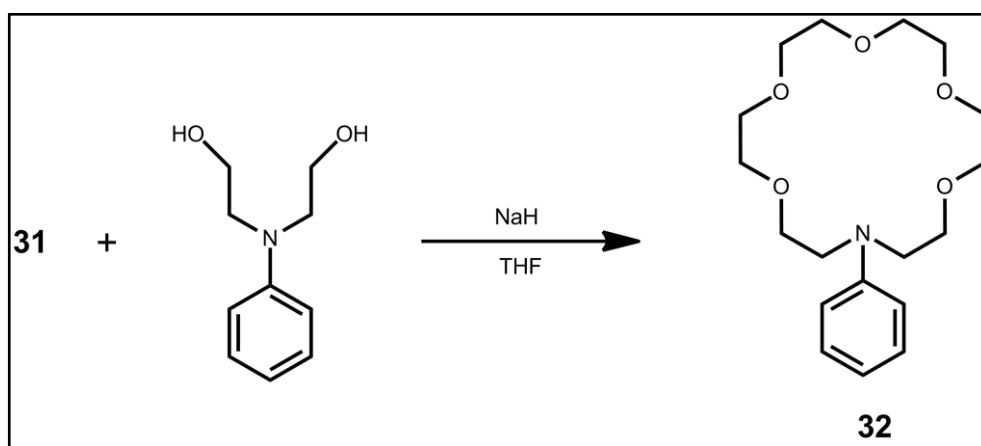


Figure 33. Synthesis of compound 32.

2.7. Synthesis of Compound 33

Compound 32 (1.47 mmol, 0.50 g) was dissolved in 10 ml of DCM. ICl (2.21 mmol, 0.36 g) in 10 ml of DCM was added dropwise to solution at room temperature. After stirring 1 h, saturated $\text{Na}_2\text{S}_2\text{O}_3$ solution in water was added to reaction mixture. Then mixture was extracted into CH_2Cl_2 and it was dried over Na_2SO_4 . The residue was purified by silica gel column chromatography (EtOAc). Brown liquid was obtained (0.55 g, 80%).

^1H NMR (400 MHz, CDCl_3) δ 7.31 (d, J = 8.6 Hz, 2H), 6.37 (d, J = 8.7 Hz, 2H), 3.57 (m, 24H).

^{13}C NMR (100 MHz, CDCl_3) δ 147.52, 137.72, 129.84, 114.12, 70.80, 70.77, 70.70, 70.54, 68.42, 51.36.

HRMS-ESI: calculated for M+H 466.1085, found 466.1060, Δ = -5.22 ppm.

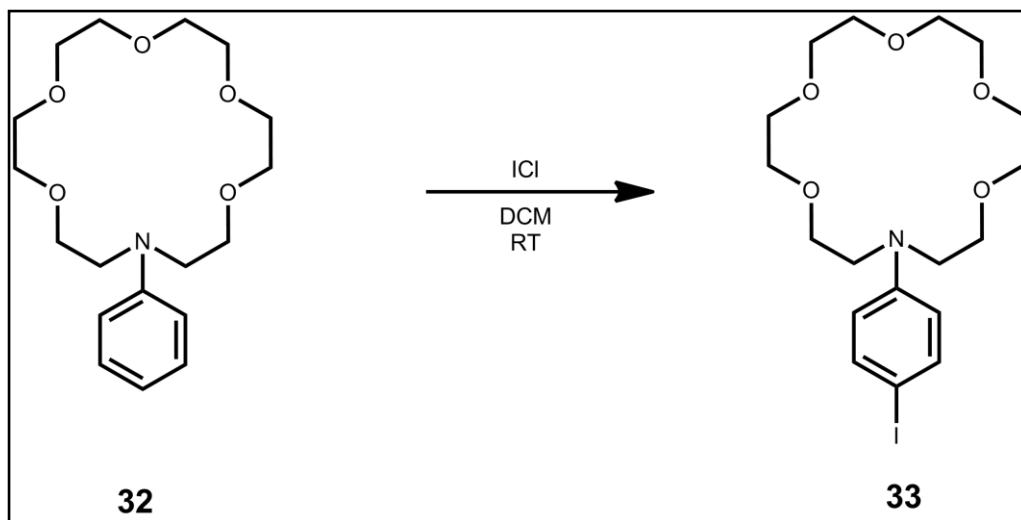


Figure 34. Synthesis of compound **33**.

2.8. Synthesis of Compound **34**

Compound **33** (1.07 mmol, 0.50 g), Pd(PPh₃)Cl₂ (0.07 mmol, 49.1 mg) and CuI (0.13 mmol, 24.4 mg) were dissolved in mixture of 20 ml of THF and 10 ml of DIPA (diisopropylamine) which was purged with Argon for 20 minutes at the beginning. Then trimethylsilylacetylene (2.14 mmol, 0.21 g) was added to reaction mixture and it was stirred overnight at 50 °C. After the extraction with THF and water, solvent was evaporated under vacuum. TMS attached compound **34** was acquired. Saturated K₂CO₃ solution was prepared with MeOH. TMS attached compound **34** (0.92 mmol, 0.40 g) was dissolved in 10 ml of DCM. 2 ml of saturated solution was added to reaction mixture and stirred for 1 h at room temperature. Organic layers were extracted into DCM and organic layers were dried with Na₂SO₄ then solvent was evaporated. . Silica gel column chromatography (EtOAc) was used for further purification. Brown gummy product was acquired (0.34 g, 100%).

¹H NMR (400 MHz, CDCl₃) δ 7.15 (d, J = 8.8 Hz, 2H), 6.43 (d, J = 8.9 Hz, 2H), 3.47 (m, 24H), 2.84 (s, 1H).

^{13}C NMR (100 MHz, CDCl_3) δ 147.99, 133.29, 131.94, 128.56, 111.18, 108.34, 84.80, 75.02, 70.78, 70.73, 70.69, 70.67, 68.46, 51.19.

HRMS-ESI: calculated for $\text{M}+\text{H}$ 364.2119, found 364.2070, $\Delta = -13.23$ ppm.

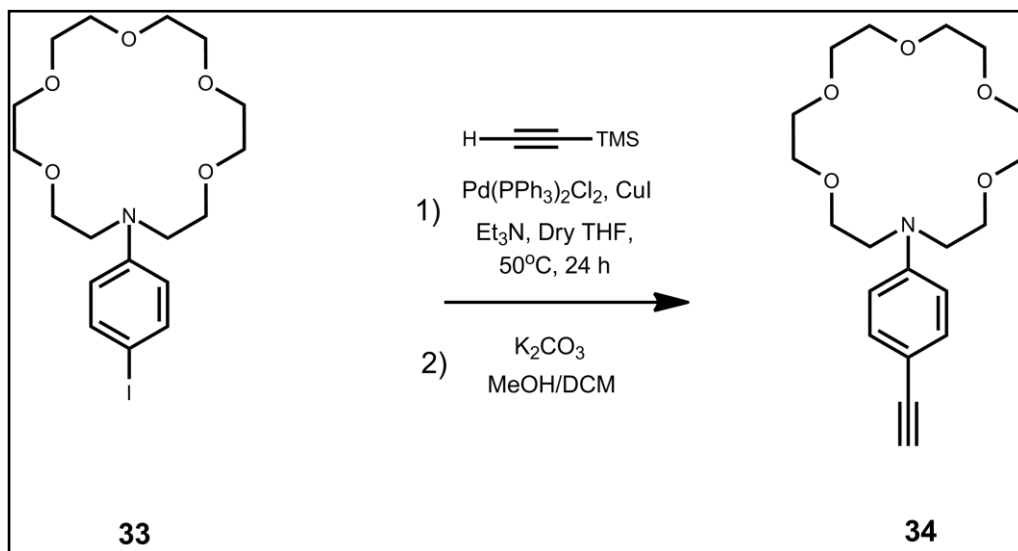


Figure 35. Synthesis of compound **34**.

2.9. Synthesis of Compound 35

Compound **30** (0.71 mmol, 0.50 g), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.085 mmol, 59.7 mg) and CuI (0.043 mmol, 8.1 mg) were added to mixture of 20 ml of THF and 10 ml of DIPA which was bubbled with Argon for 20 minutes. Compound **34** (1.42 mmol, 0.52 g) was added to reaction mixture. The same procedure, which was applied in the first part of synthesis of compound 34, was followed. 2 EtOAc: 1 Hex mixture was used as eluent. Blue solid was obtained (0.65 g, 78%).

^1H NMR (400 MHz, CDCl_3) δ 7.22 (d, $J = 8.1$ Hz, 4H), 7.08 (d, $J = 7.6$ Hz, 2H), 6.94 (d, $J = 7.7$ Hz, 2H), 6.54 (d, $J = 7.7$ Hz, 4H), 3.95 (t, $J = 6.3$ Hz, 2H), 3.59 (m, 48H), 2.61 (s, 6H), 1.81 (m, 2H), 1.48 (s, 6H), 1.33 (m, 10H), 0.82 (t, $J = 6.3$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ 159.91, 157.84, 147.67, 142.95, 142.03, 132.65, 131.59, 129.19, 126.48, 116.74, 115.19, 111.51, 110.04, 97.26, 79.39, 70.77, 70.72, 70.69, 68.68, 68.23, 51.24, 31.81, 29.69, 29.41, 29.24, 26.06, 22.66, 14.12, 13.66, 13.58.

HRMS-ESI: calculated for $\text{M}+\text{H}$ 1175.6662, found 1175.6597, $\Delta = -6.41$ ppm.

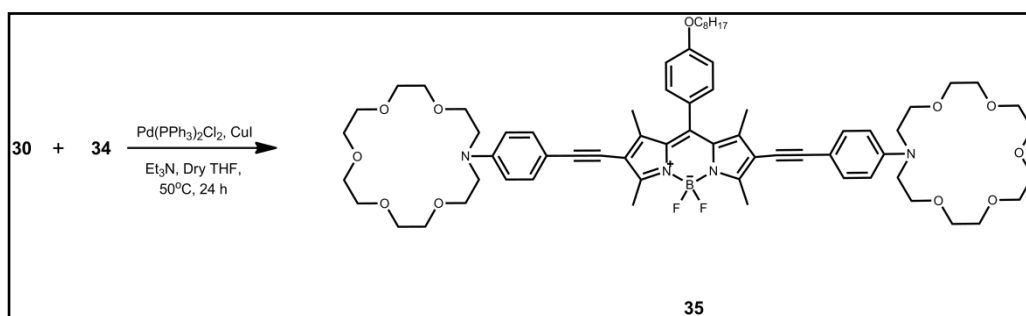


Figure 36. Synthesis of compound **35**.

CHAPTER 3

RESULTS AND DISCUSSIONS

Fluorescent chemosensors are very beneficial for environmental and biological systems and therefore designing of fluorescent chemosensors are promising area of supramolecular chemistry. Although there are some techniques for detection of particular ions or compounds, fluorescent chemosensors have significant advantages. They are cheap besides high sensitivity and selectivity.

In recent years, fame of BODIPY has been increased rapidly between fluorescent dyes because of the great photophysical properties of BODIPY. There a lot of BODIPY based chemosensors in literature. Especially chemosensors for heavy and transition metals such as Hg (II), Zn (II) and Ag (I) are remarkable between scientist. Moreover, sensing of metals which play important role in living systems such as Ca and Mg ions is important.

Fluorescent chemosensors have two type of working mechanism and these are photoinduced electron transfer (PET) and internal charge transfer (ICT). Majority of chemist prefer PET based chemosensors. In ICT based sensors, receptor is directly attached to fluorophore. In PET based chemosensors, there is spacer between fluorophore and receptor. We designed our chemosensor as ICT based. It can be seen in the figure 37, receptors were directly attached to BODIPY core. Receptors are part of the conjugation and there is no spacer.

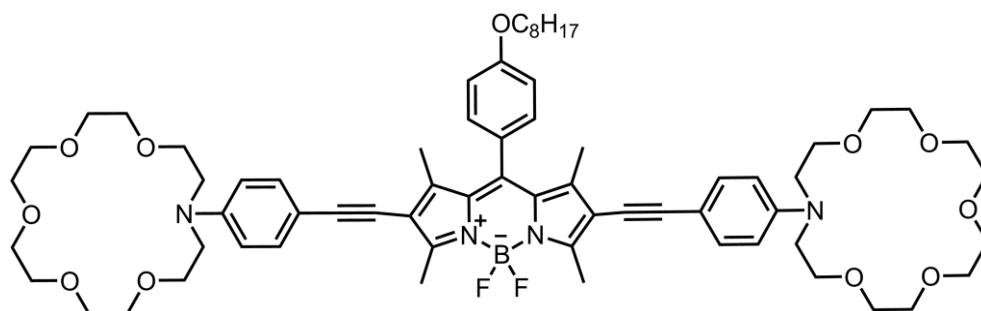


Figure 37. Structure of compound **35**.

In this study, we designed and synthesized 2, 6-functionalized BODIPY based chemosensor. In literature, there are many examples BODIPY based chemosensors but almost all of them 1, 3, 5, 7 and 8-functionalized. Therefore, we synthesized 2, 6-functionalized BODIPY based chemosensor and examined spectral properties of this molecule.

We used azo crown ether as receptor because it can sense wide range of metal ions. We did not choose selective receptor since we wanted to investigate how different metals affect the emission and absorption spectrum of 2, 6-functionalized BODIPY. We used Hg^{2+} , Zn^{2+} and Ca^{2+} ions as analytes because of the different roles in environment and living systems. Then we determined detection limit, metal-ligand ratio and binding constants for these metal ions.

In this study, 8 compounds were synthesized. Compound **28** was synthesized by using simple substitution nucleophilic reaction. In Argon atmosphere from 2,4-dimethyl pyrrole and compound **28**, compound **29** which is a BODIPY derivative was synthesized. $\text{BF}_3 \cdot \text{OEt}_2$ was used to form BF_2 bridge. In the synthesis of the compound **30**, ionic acid and I_2 were used for iodination. Synthesis of compound **31** was completed by tosylation of tetraethylene glycol in THF. Reaction of N,N-bis(2-hydroxyethyl)aniline and tetraethylene glycole ditosylate in very diluted THF solution made possible the synthesis of compound **32**. For the iodination of phenyl azo crown, iodic acid was used firstly but it did not work efficiently. Therefore, ICl was used for iodination of phenyl ring and synthesis was occurred with almost 100 % efficiency. Sonagashira coupling was used to attach trimethylsilylacetylene to compound

33. Then TMS was removed by saturated K_2CO_3 solution. Finally to synthesize compound **35** again Sonagashira coupling was used and yield was 78 %.

As it can be seen in absorption spectrums (Figure 38), there were blue shifts for three metal ions. Shift in absorption spectrum means that our chemosensor is ICT based and worked for all of three metal ions. If receptor is part of conjugative system of the fluorophore, this system is ICT based fluorescent chemosensor. Because of the orbital overlapping, internal charge transfer occurs and that causes shifts in absorption and emission spectrums. In ICT based system, there is no increasing; decreasing or quenching of emission different from PET based chemosensors.

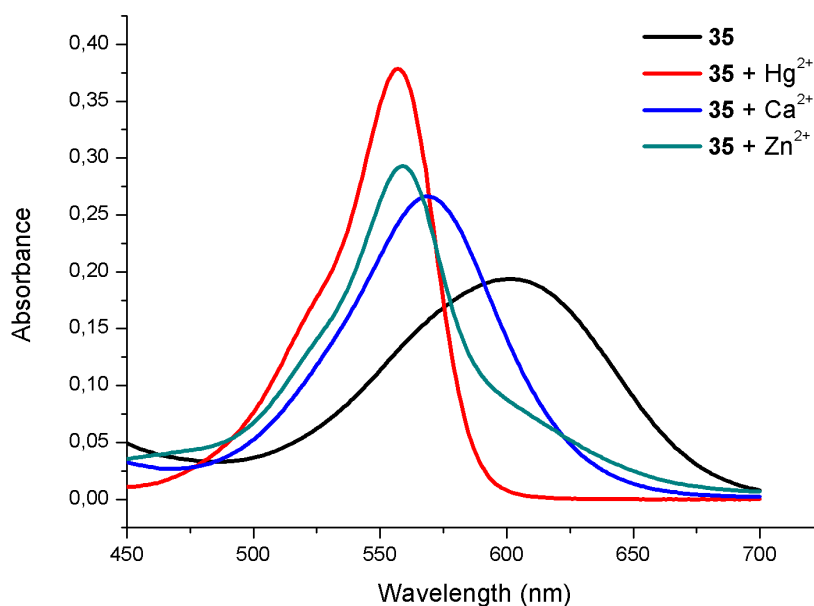


Figure 38. Absorption spectrums of **35** and complexes.

Absorption wavelengths shifted from 601 nm to 560 nm for Hg^{2+} and Zn^{2+} . That is blue shift and nearly 40 nm. However, that is 30 nm for calcium ion. The reason is related to interaction between metal ion and donor atoms especially nitrogen in crown ether. Interaction with Hg and Zn ions are stronger, so internal charge transfer occurs better.

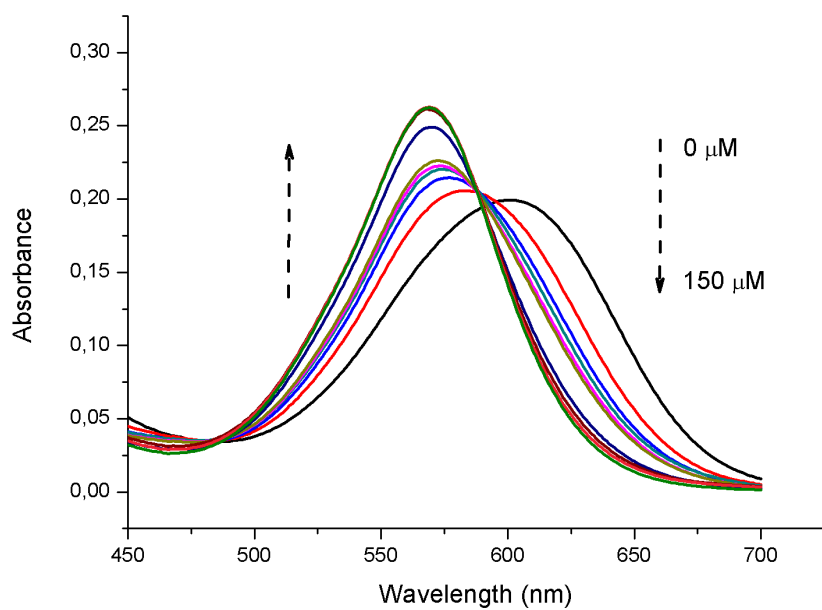


Figure 39. Titration of 35+Ca (5 μM) with increasing Ca ion (perchlorate salt) concentration (0-150 μM) in acetonitrile.

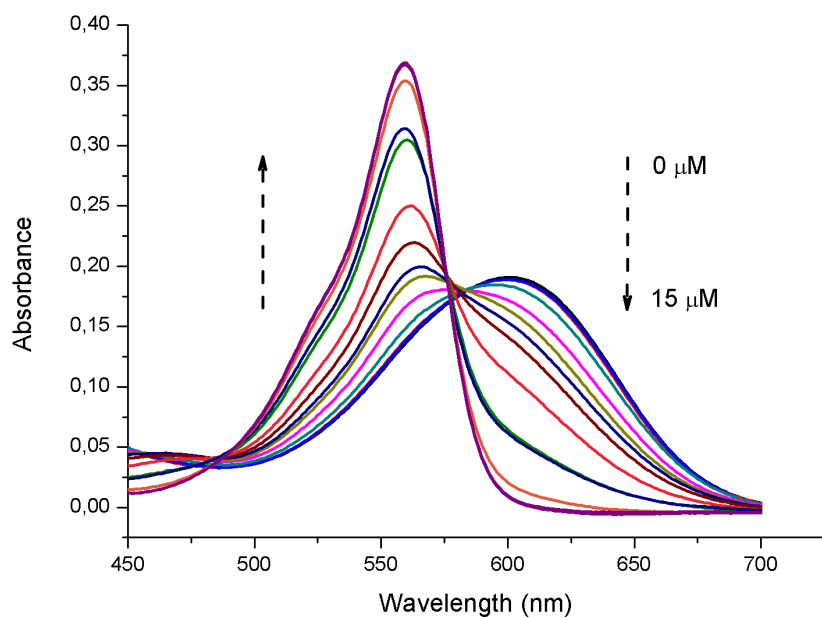


Figure 40. Titration of 35+Hg (5 μM) with increasing Hg ion (perchlorate salt) concentration (0-15 μM) in acetonitrile.

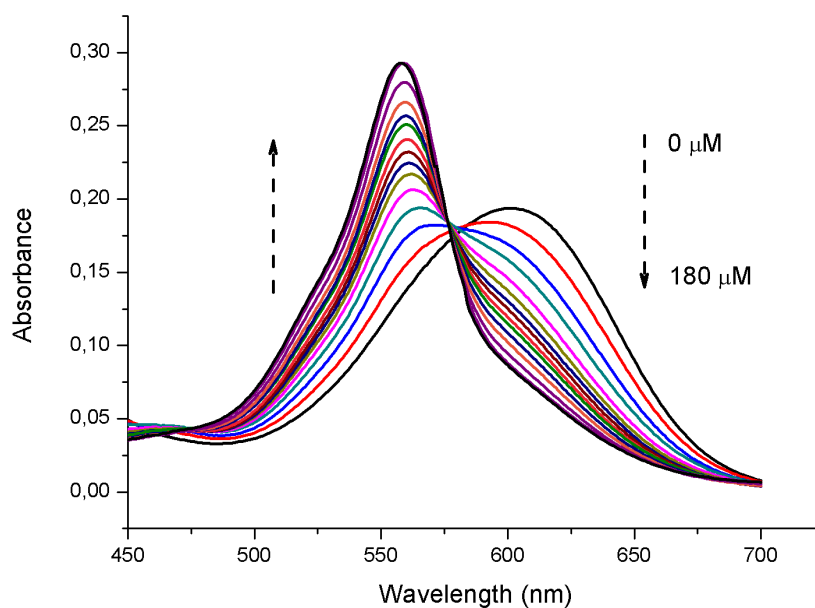


Figure 41. Titration of **35**+Zn (5 μ M) with increasing Zn ion (perchlorate salt) concentration (0-180 μ M) in acetonitrile.

The figure 41 shows the emission spectrums of dye and dye-metal complexes. As it can be seen in the emission spectrum, only dye has no emission. Binding of Ca ion did not change this situation. On the other hand, binding of Hg (II) and Zn (II) resulted in strong emission.

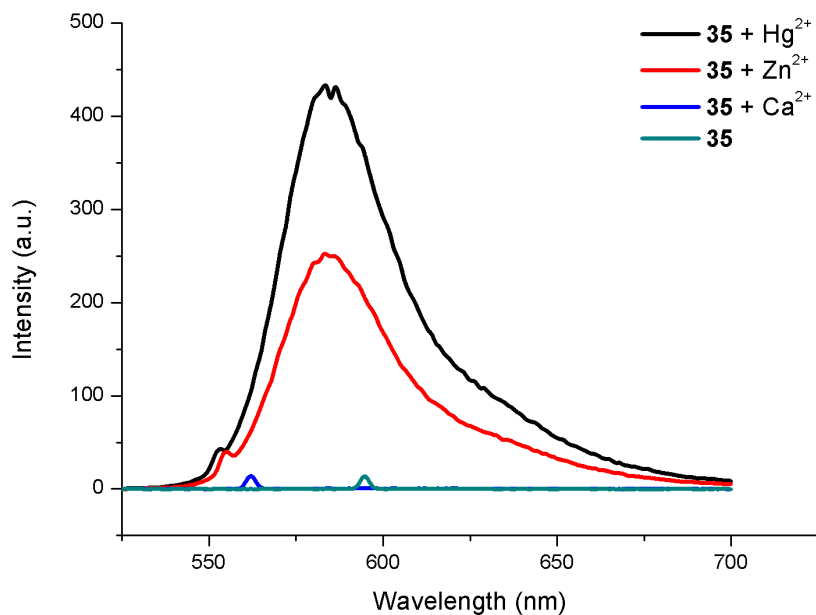


Figure 42. Emission spectrums of **35** and complexes.

There are two interesting things at this point. First, our chemosensor, compound **35**, designed as ICT based sensor. In this compound, receptor is directly attached to fluorophore, so it can be claimed that dye should have emission in the absence of metal ions. However, our chemosensor behaved as PET based chemosensors. There is nitrogen atom in the structure of azo crown and probably electron pair on the nitrogen atom quenched fluorescent emission of dye. However, nitrogen atom can quench emission if it is not part of the conjugation. Compound **35** is huge molecule so staying in planar form can be hard for this molecule. Its probable structure can be seen in figure 43. In this situation, overlapping of p orbitals decrease and conjugation is not provided fully. Because of that reason, the electron pair on the nitrogen atom can quench the emission and compound **35** can behave as PET based chemosensors. Therefore, our chemosensors is not only ICT based but also PET based chemosensor.

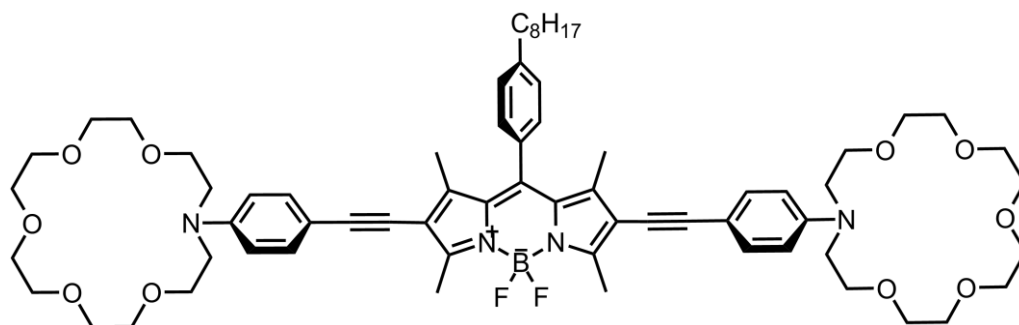


Figure 43. Probable structure of **35** according to absorption and emission spectra.

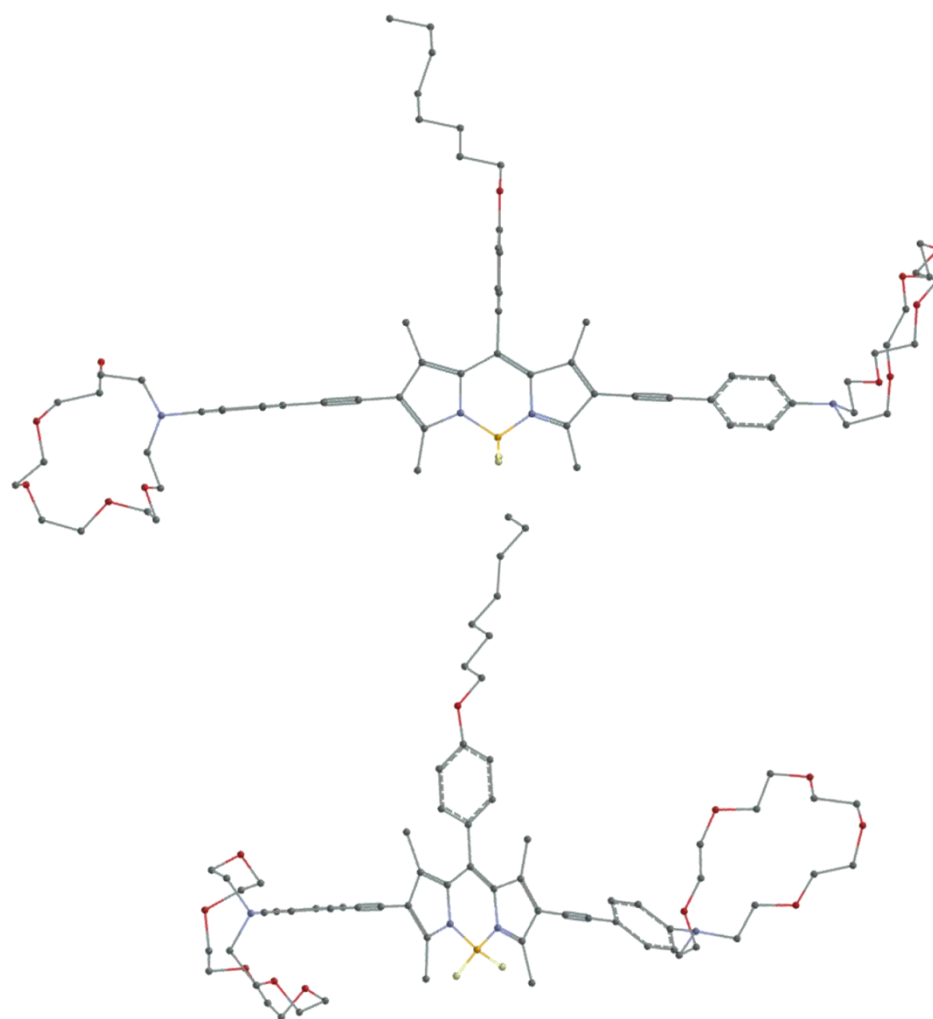


Figure 44. Energy minimized (Spartan'08 geometry optimization) structure of compound **35**.

The other point is that binding of Hg (II) and Zn (II) caused emission while binding of Ca ion did not. That can be explained hard and soft acid-base theory. “Hard” means high charge and small size in contrast “soft” has small charge and large size. According to this theory, hard acid prefers to interact with hard base instead of soft base. The theory explains this situation by using energy levels of HOMO and LUMO of acids and bases. We know that Ca ion is hard acid while Zn (II) and Hg (II) are softer. Also, nitrogen atom is softer than oxygen atom because of the larger size. Therefore, Zn (II) and Hg (II) interact stronger with nitrogen atom and that block the PET. On the other hand, Ca ion mostly interacts with oxygen atoms so PET continues.

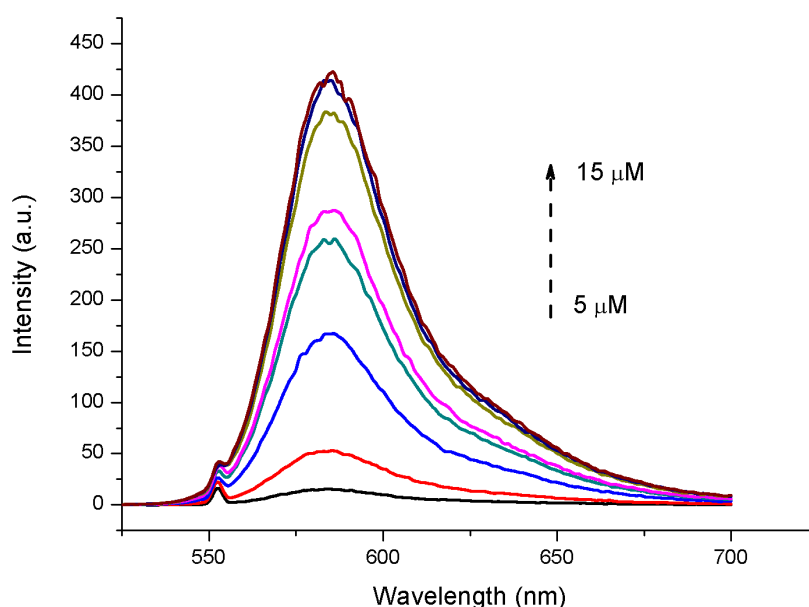


Figure 45. Emission spectrum (555 nm) of 35+Hg (5 μM) with increasing Hg ion (perchlorate salt) concentration (5-15 μM) in acetonitrile.

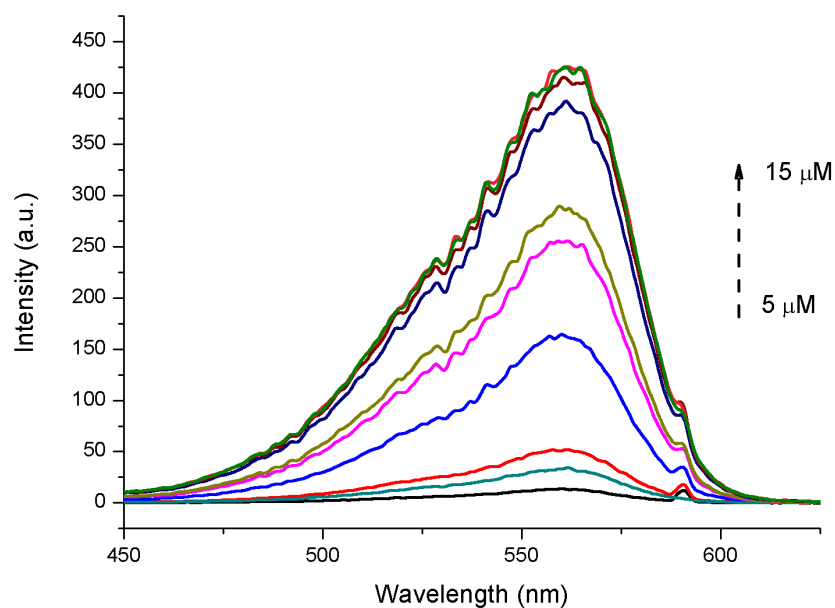


Figure 46. Excitation spectrum (590 nm) of 35+Hg (5 μM) with increasing Hg ion (perchlorate salt) concentration (5-15 μM) in acetonitrile.

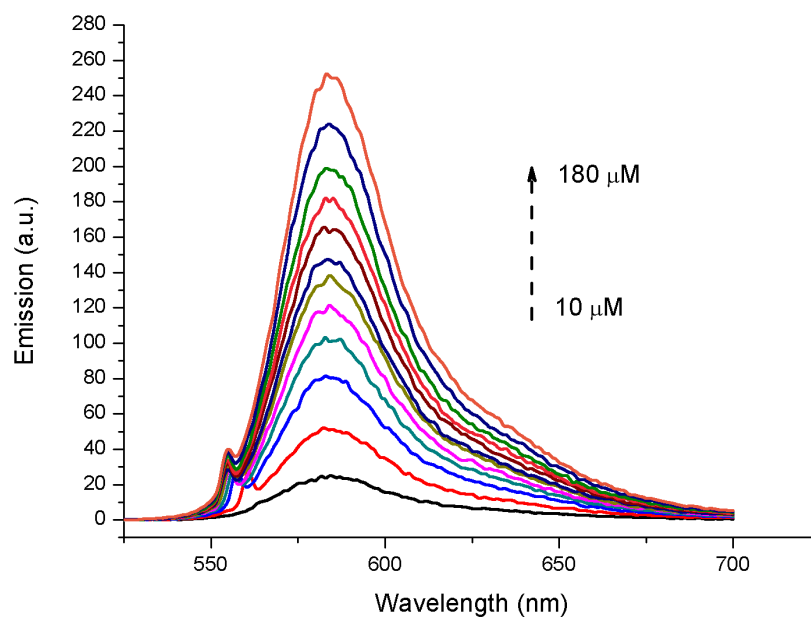


Figure 47. Emission spectrum (555 nm) of 35+Zn (5 μM) with increasing Zn ion (perchlorate salt) concentration (10-180 μM) in acetonitrile.

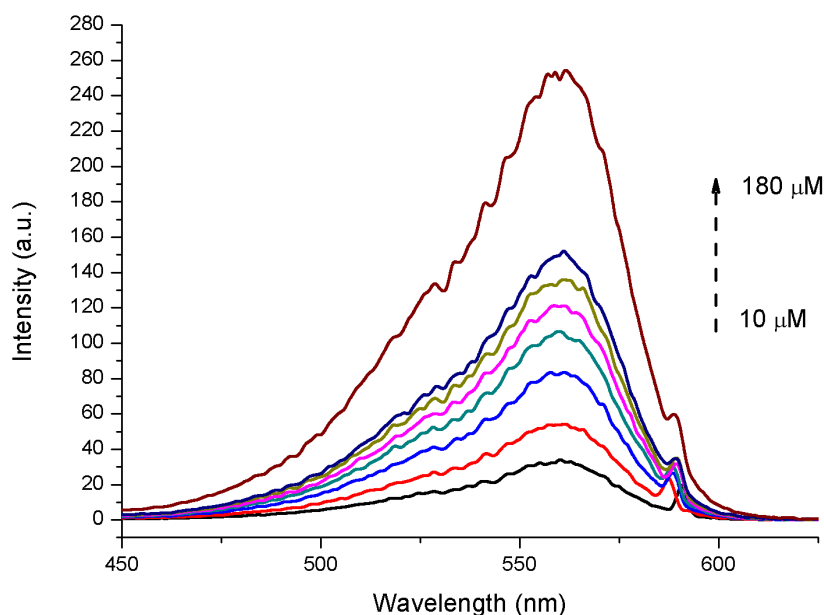


Figure 48. Excitation spectrum (590 nm) of **35**+Zn (5 μ M) with increasing Zn ion (perchlorate salt) concentration (10-180 μ M) in acetonitrile.

Job's plot⁷⁸ is used for to determine stoichiometry of binding. In this method, the mole fraction is changed while the total molar concentration of two components is constant. For example, absorbance against mole fraction graph is plotted in complex formation reactions. Binding ratio is determined by using maximum or minimum point in graph. For Job's plot, it is essential that absorbance of complex must predominate at the chosen wavelength. In our graph, there was some overlapping in chosen wavelength so we made some corrections. For the complex of Hg (II) and **35**, 560 nm is the wavelength which complex has highest absorption. However, only **35** also has some absorption at this wavelength. We calculated approximate absorption coefficient constant of **35** from the wavelength which the complex does not absorb. By this way, mole fraction against absorption of only the complex graph was plotted.

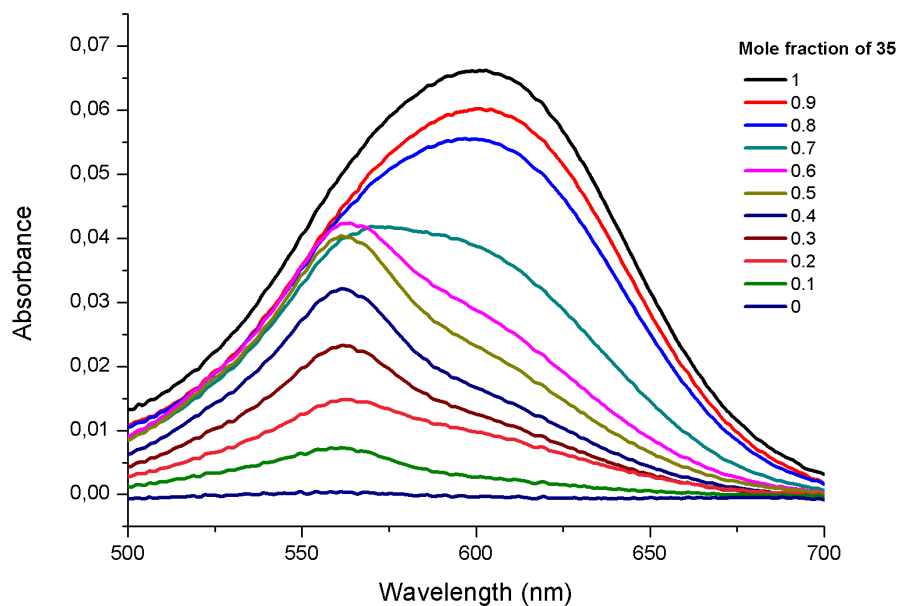


Figure 49. Absorption spectrums in different mole fractions of **35**.

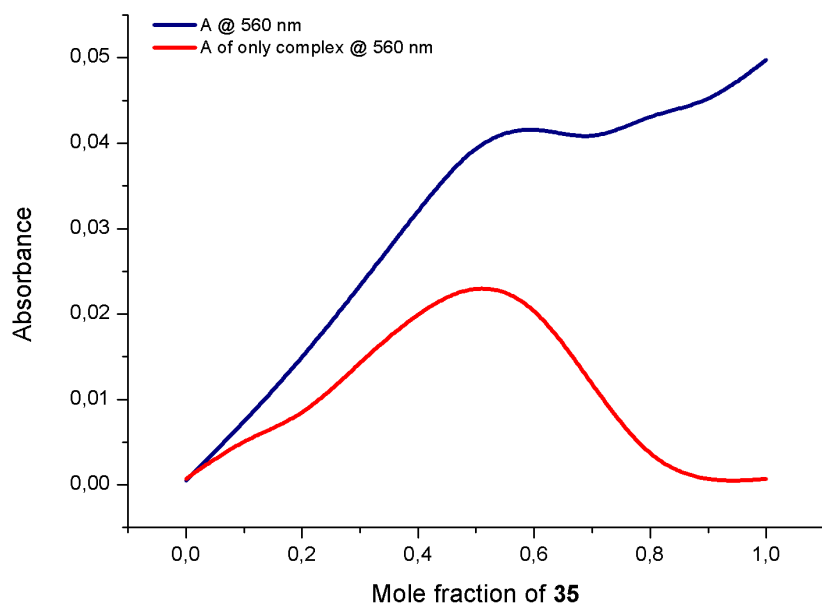


Figure 50. Job's plot of complexe of **35** with Hg (II).

The graph (Figure 50) shows that binding ratio is 1 to 1 for Hg (II) complex. The result is interesting because compound **35** has two receptor group so it is

expected that two Hg (II) binds to one compound **35**. In the figure 51, there is the structure which is predicted for one to one complex. We will use X-ray instrument to find out 3D structure of the complex.

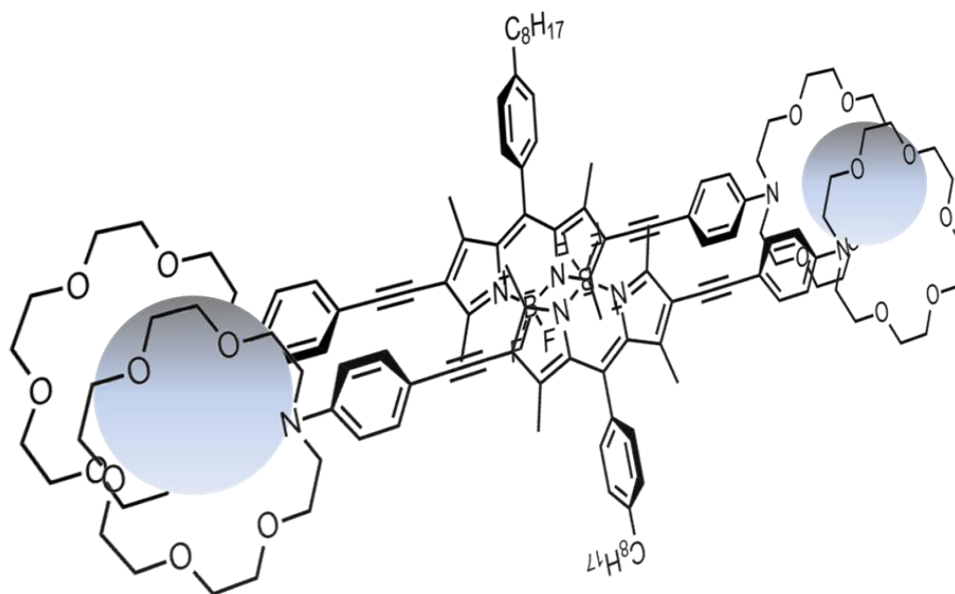


Figure 51. Predicted structure of complex of **35** with Hg.

CHAPTER 4

CONCLUSION

In this study, we functionalized 2 and 6 positions of BODIPY and characterized different complexes of this chemosensor. We used three metal ions which are important in environment and biological systems. We analyzed all of absorption and emission spectrums of the chemosensor and it was determined that our chemosensor worked successfully. Our chemosensor have both PET (photoinduced electron transfer) and ICT (internal charge transfer) properties while almost all of the chemosensors have only of them.

Actually this project is proof of principle and after this study, many chemosensor can be synthesesed based on 2, 6-functionalized BODIPY structure. We show that 2, 6-functionalized BODIPY structures can be used for sensing in some biological and environmental applications.

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APPENDIX A

NMR SPECTRA

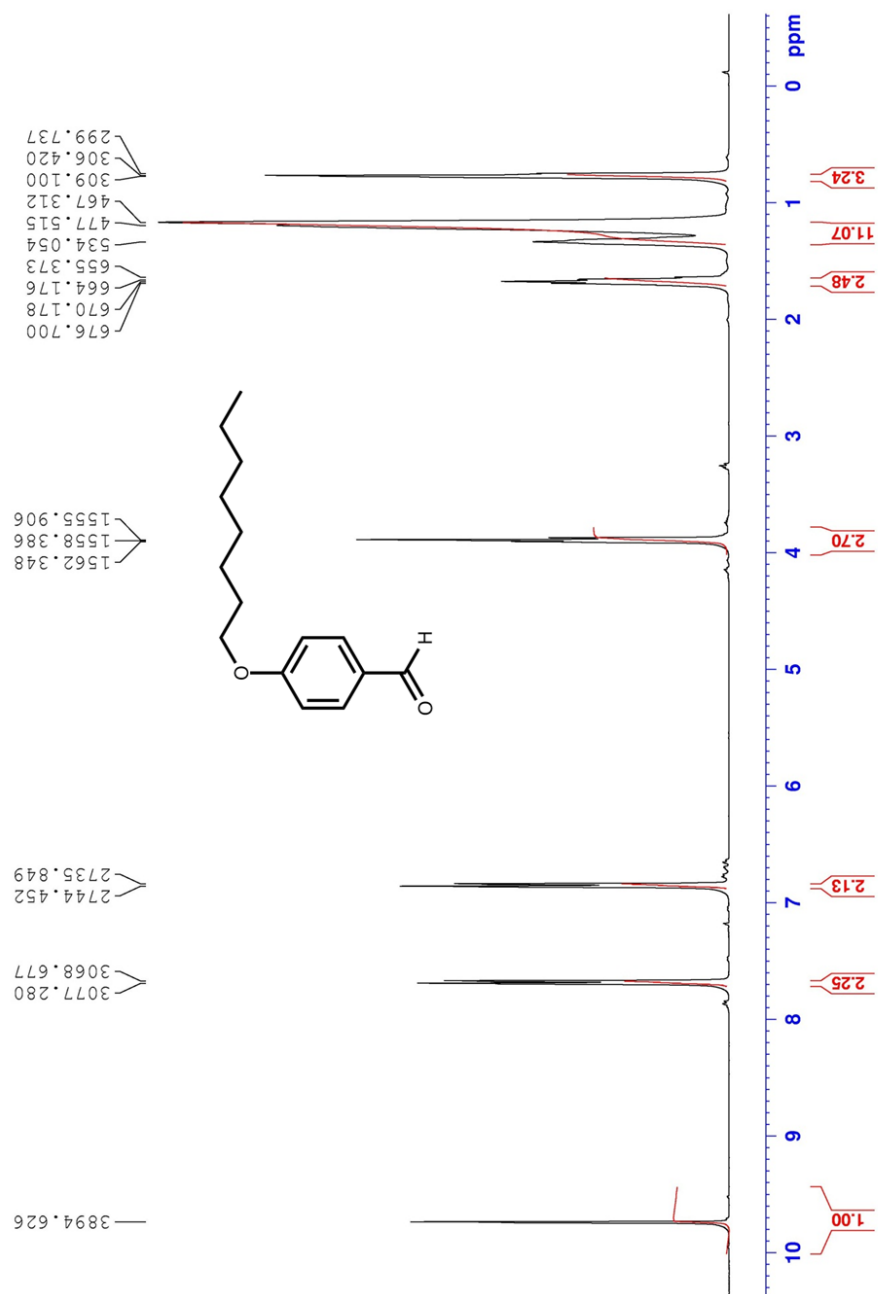


Figure 52. ¹H spectrum of compound 28

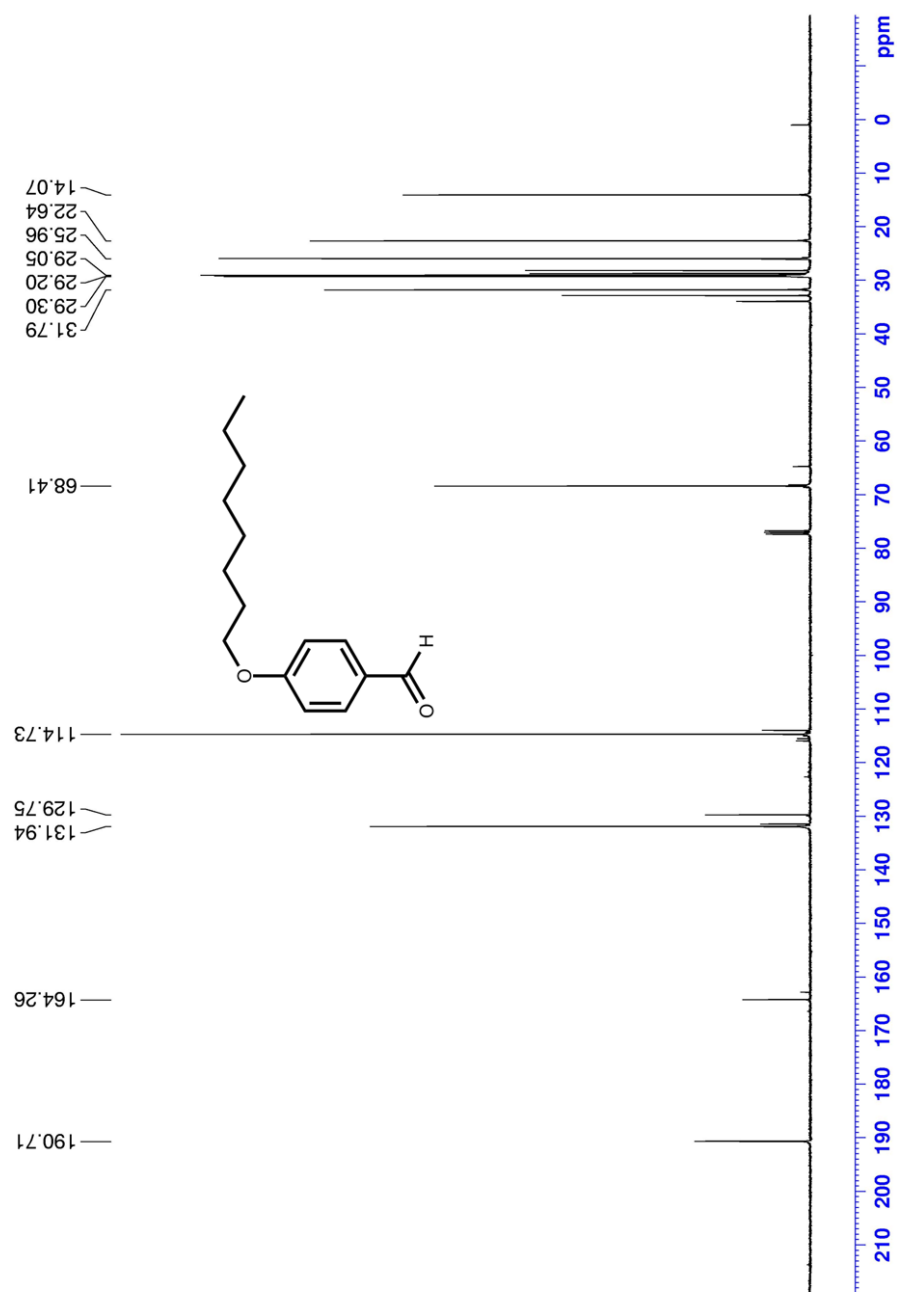


Figure 53. ^{13}C spectrum of compound 28

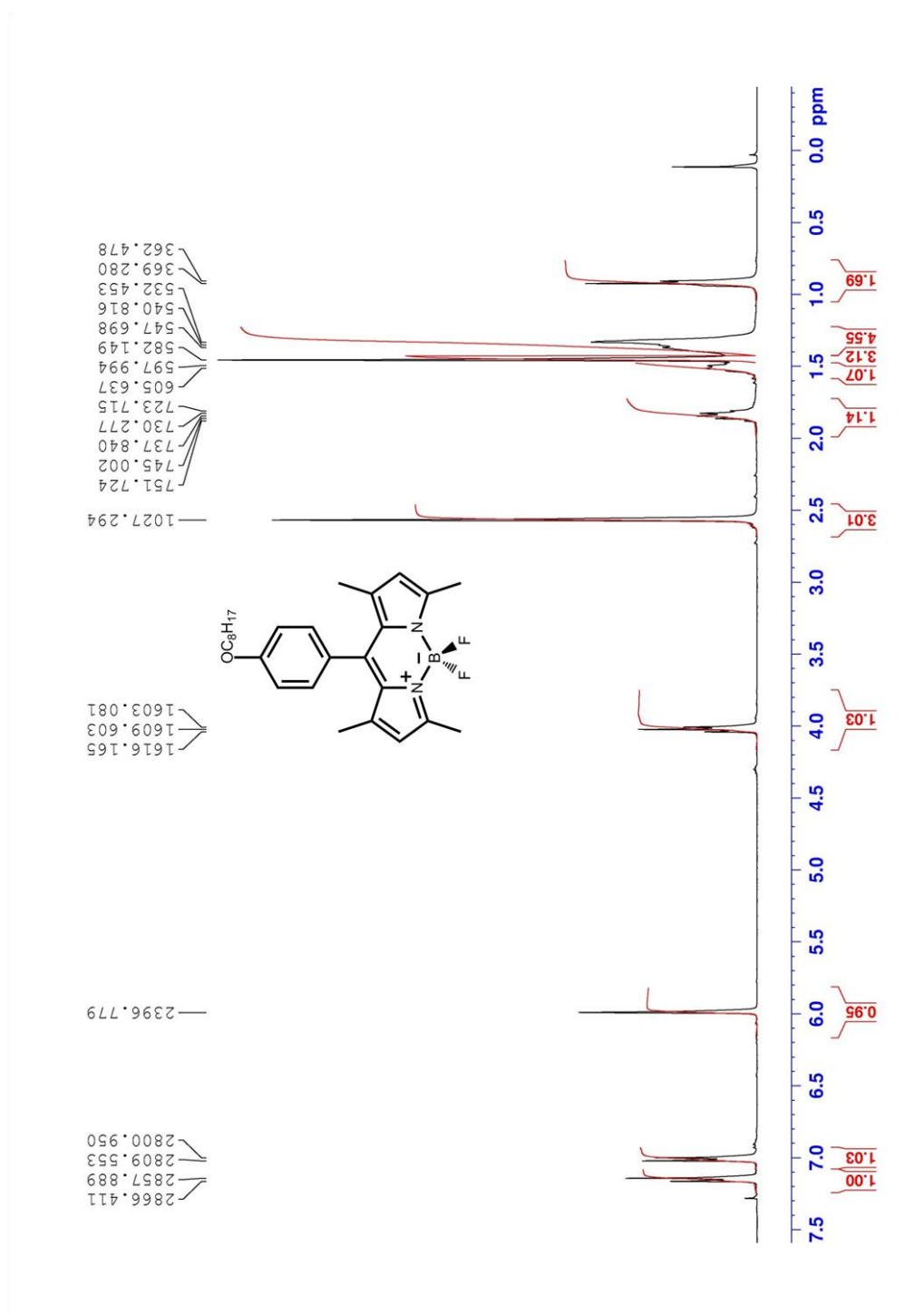


Figure 54. ¹H spectrum of compound 29

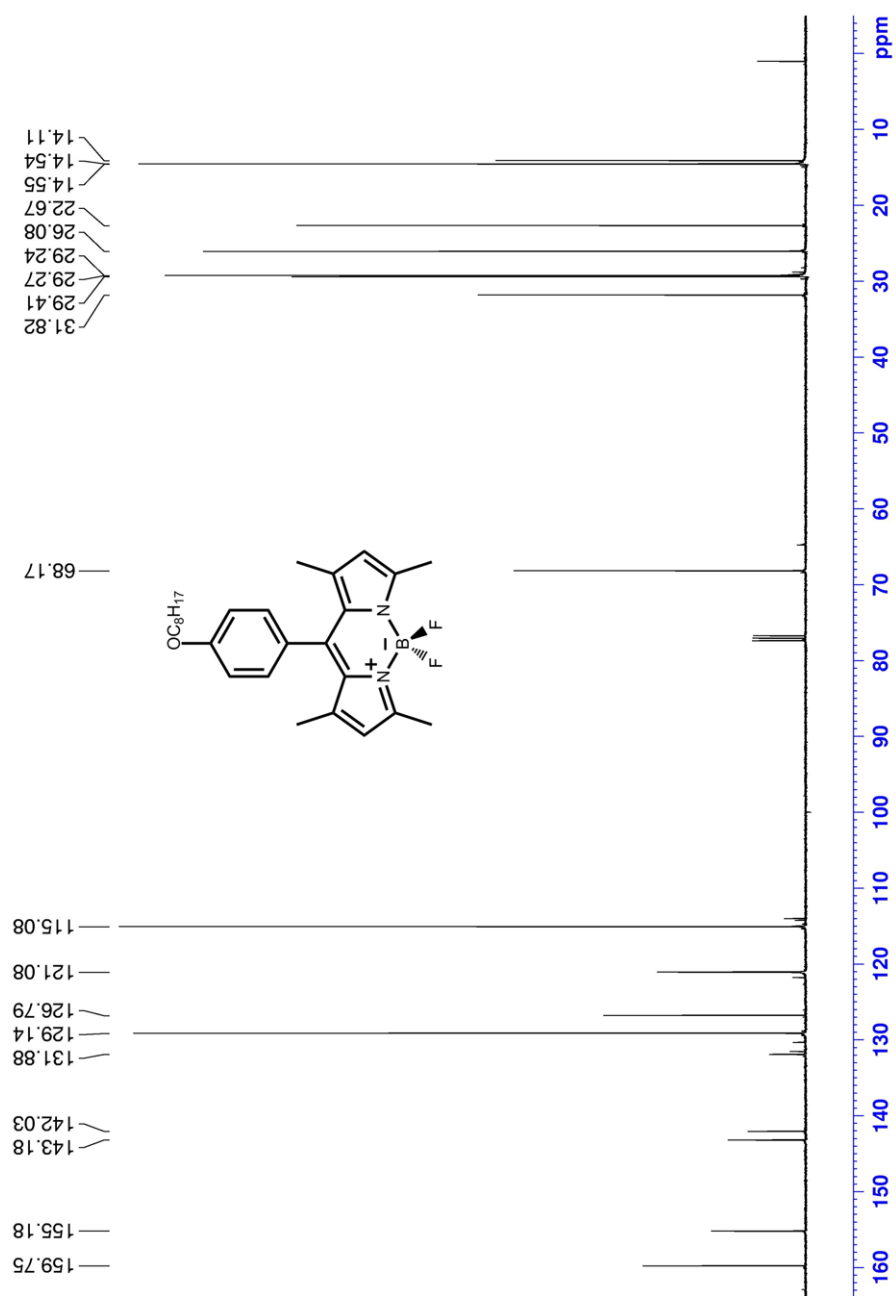


Figure 55. ¹³C spectrum of compound 29

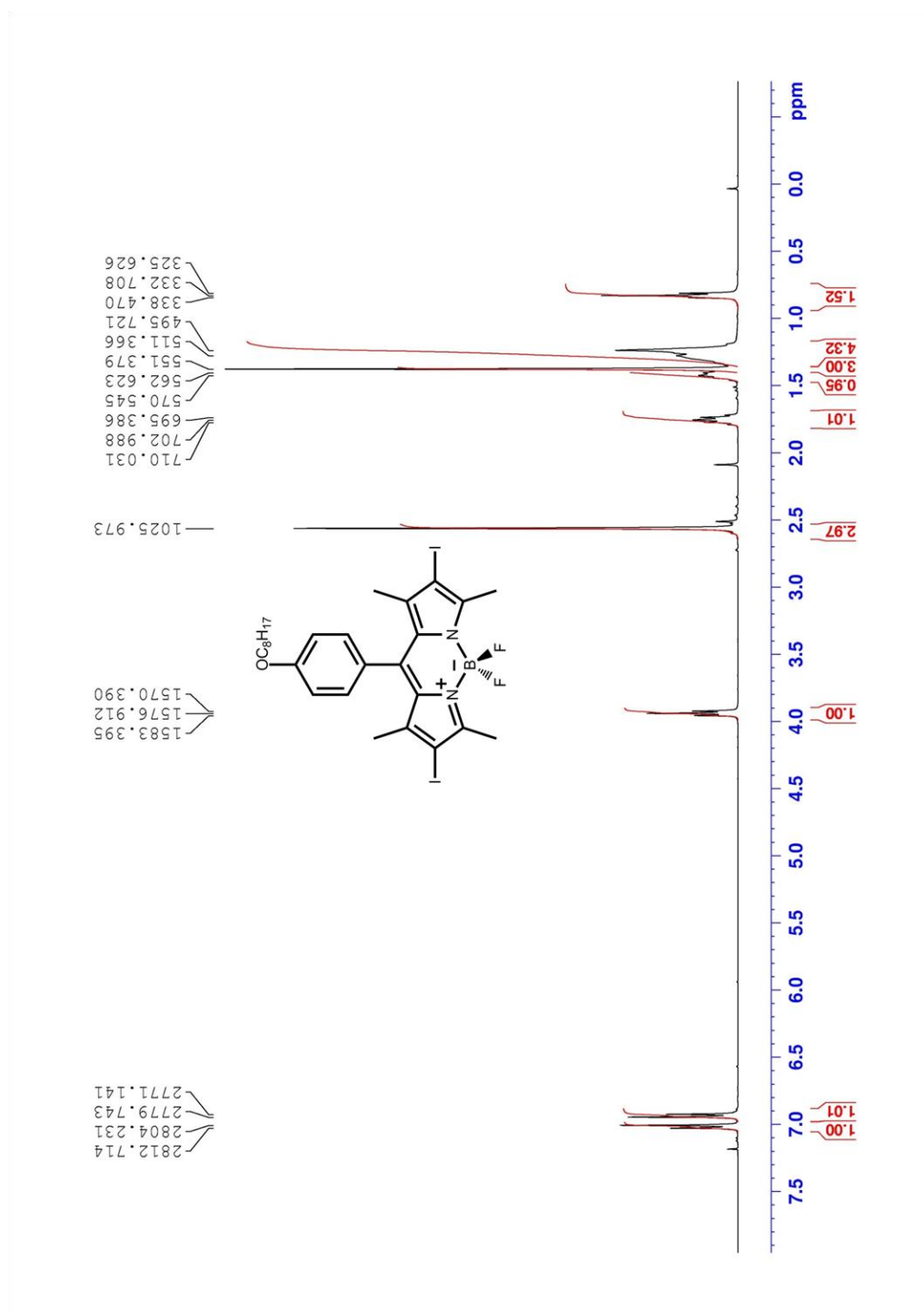


Figure 56. ¹H spectrum of compound 30

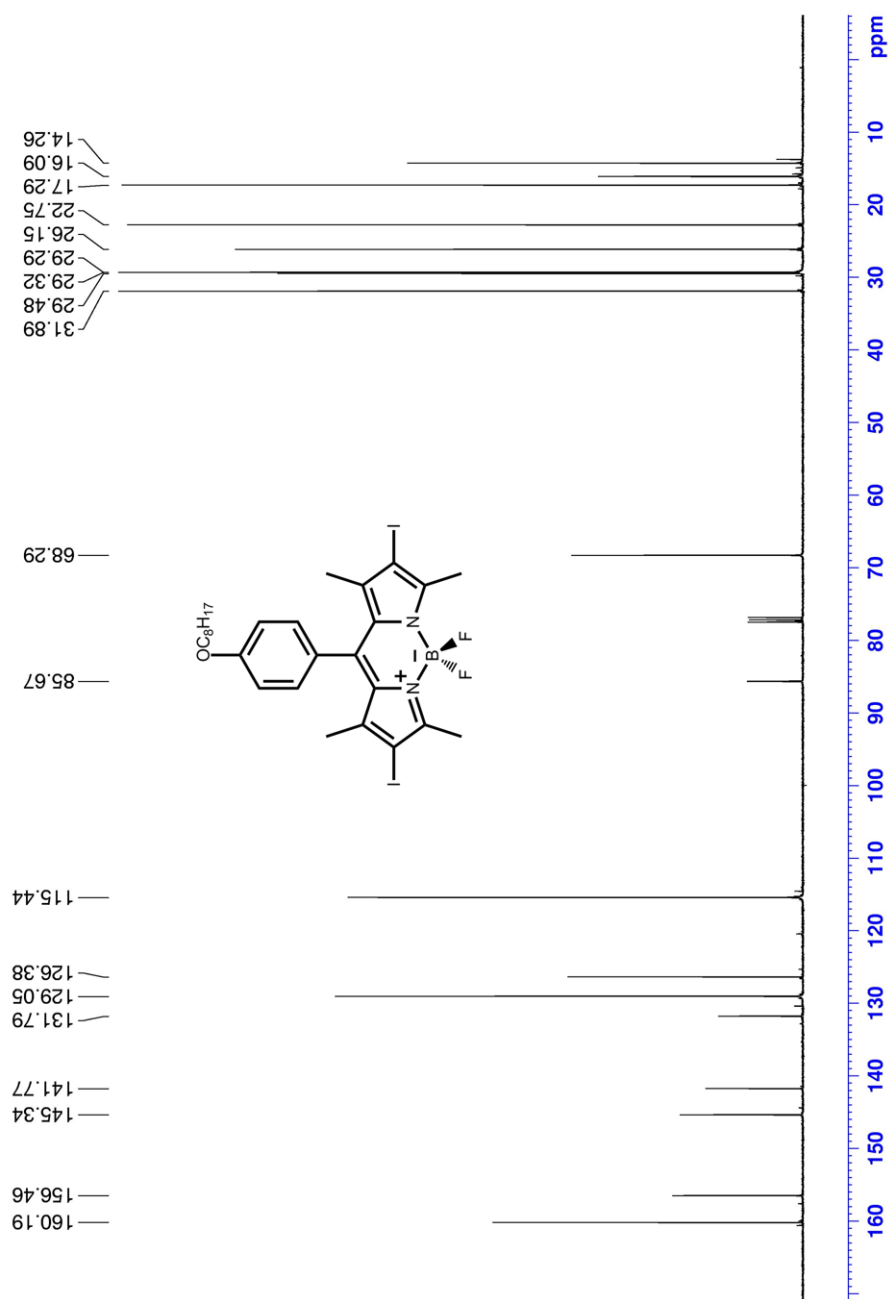


Figure 57. ^{13}C spectrum of compound 30

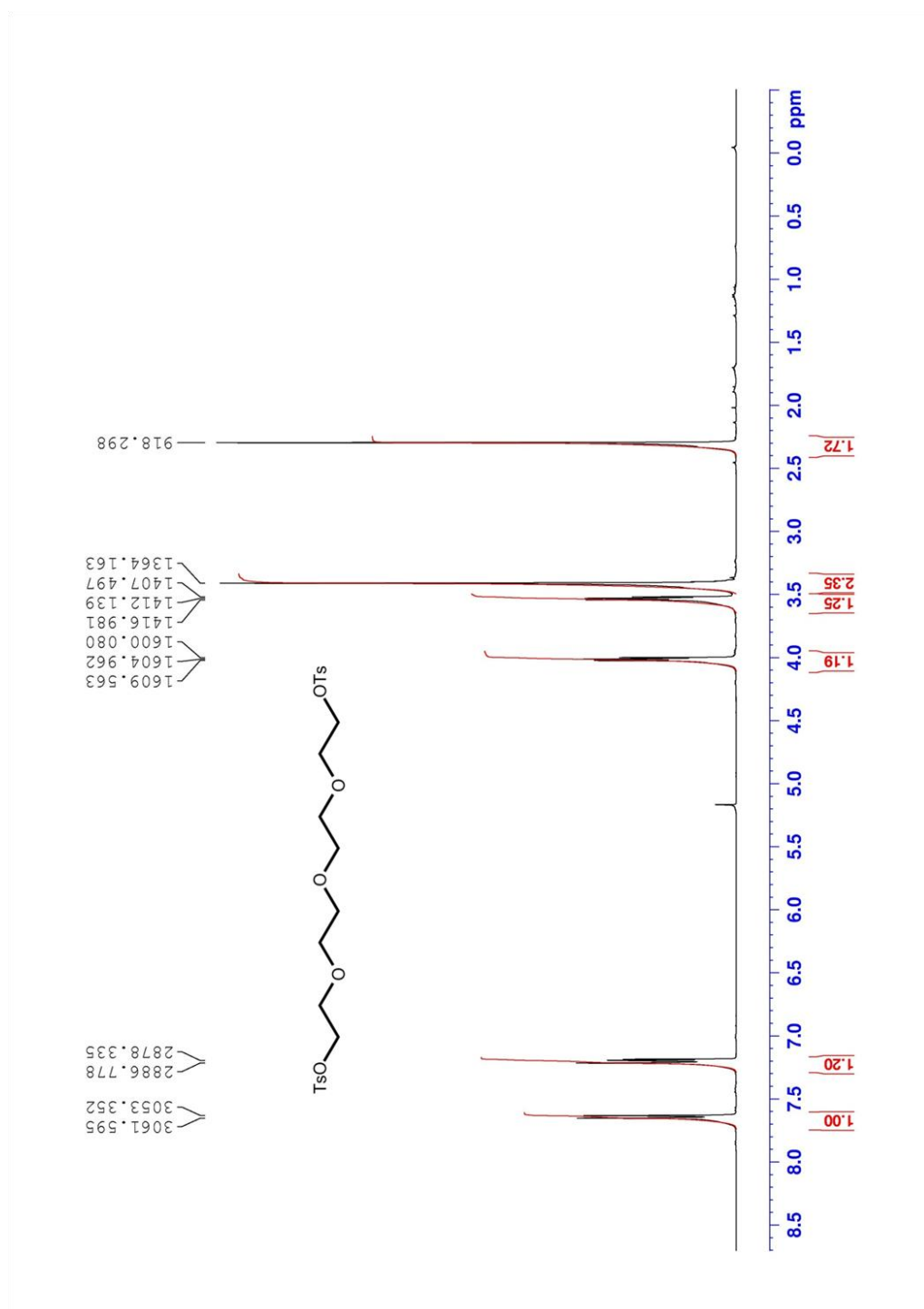


Figure 58. ¹H spectrum of compound **31**

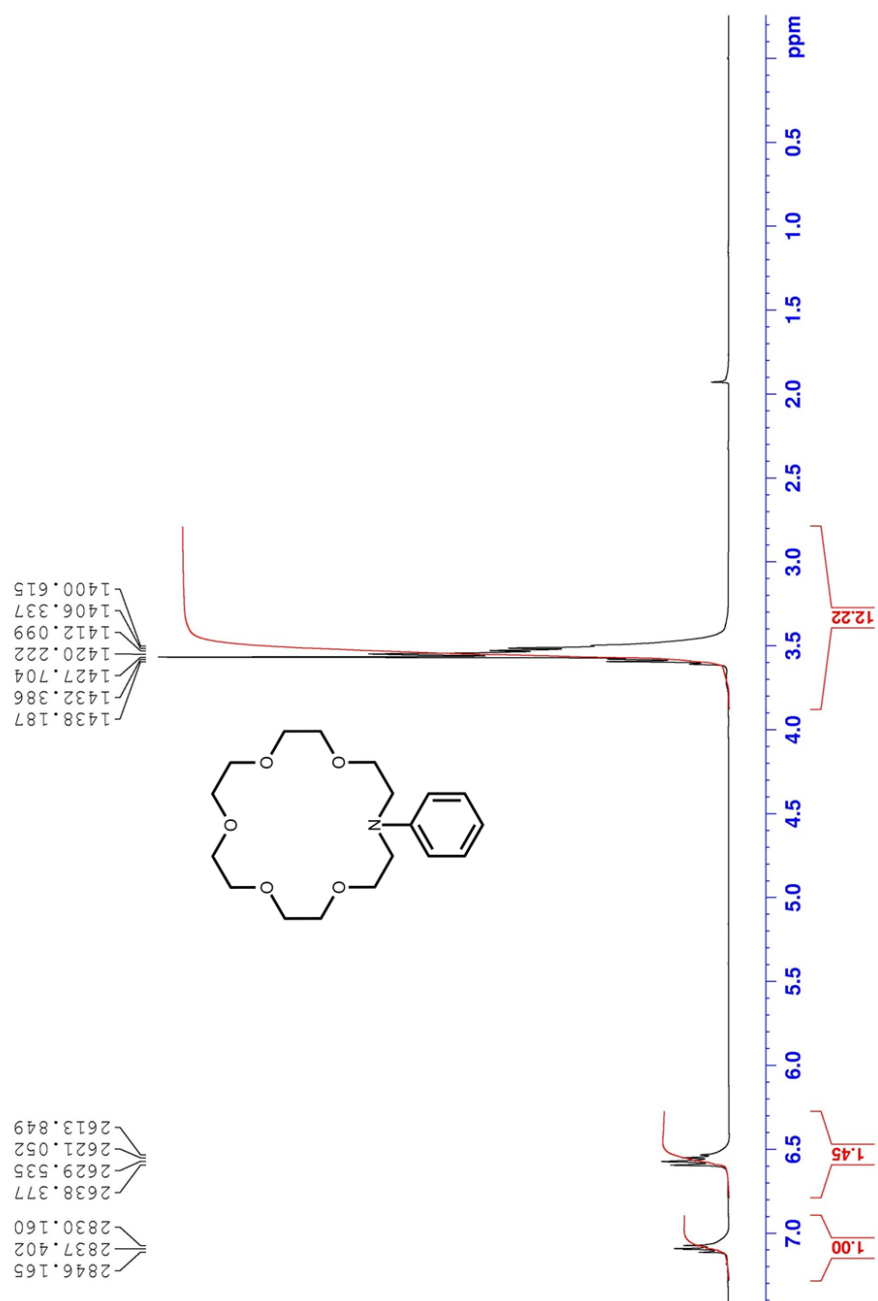


Figure 60. ¹H spectrum of compound 32

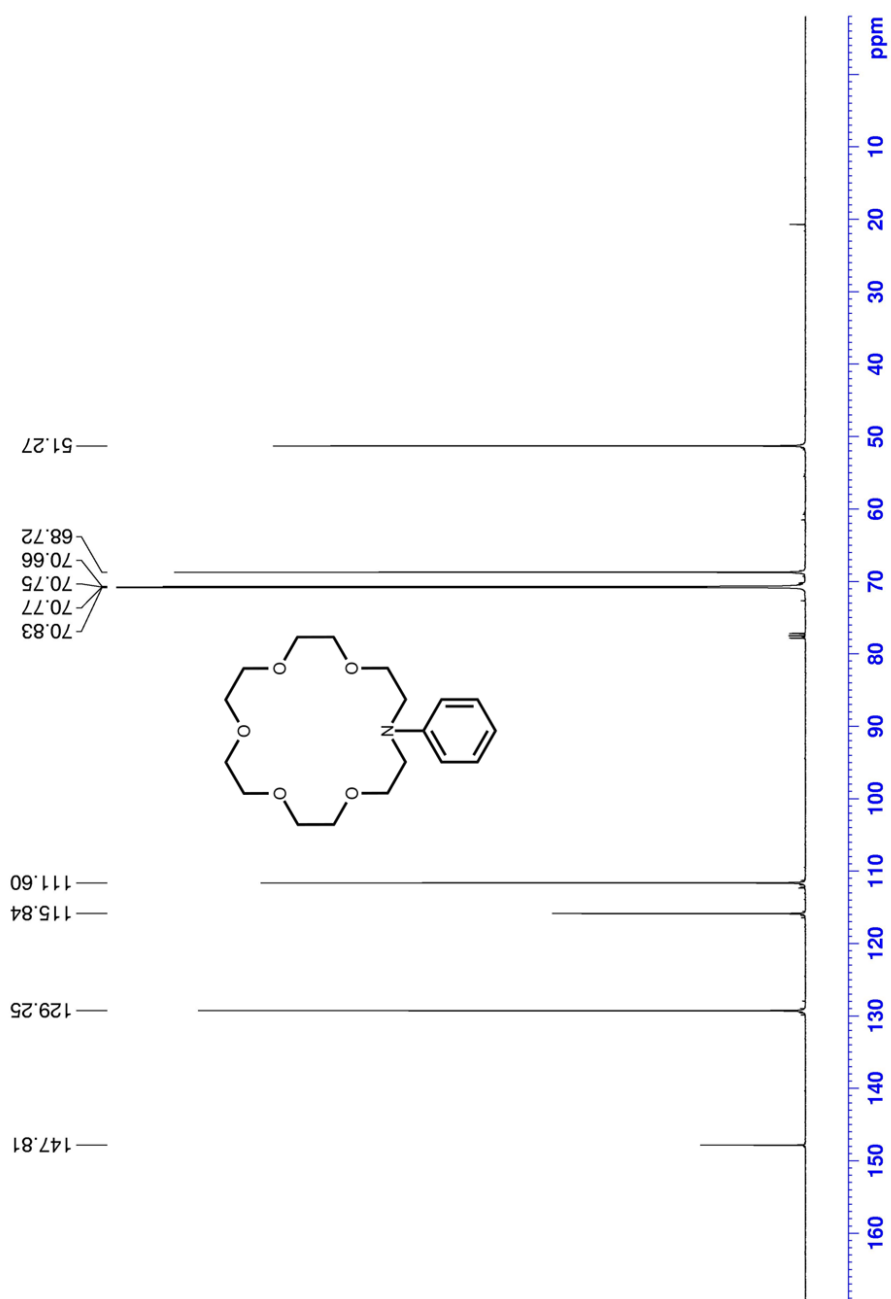


Figure 61. ^{13}C spectrum of compound 32

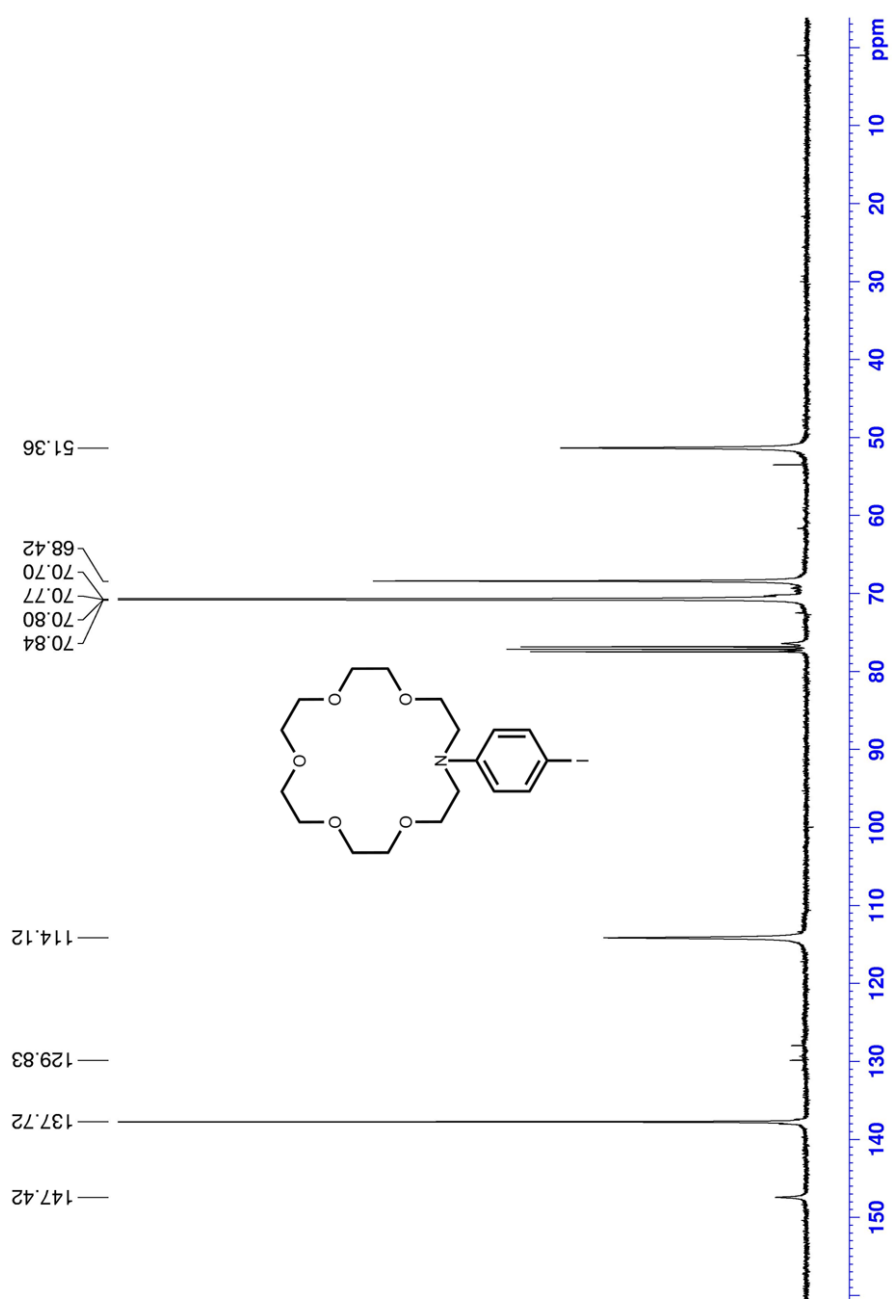


Figure 63. ^{13}C spectrum of compound 33

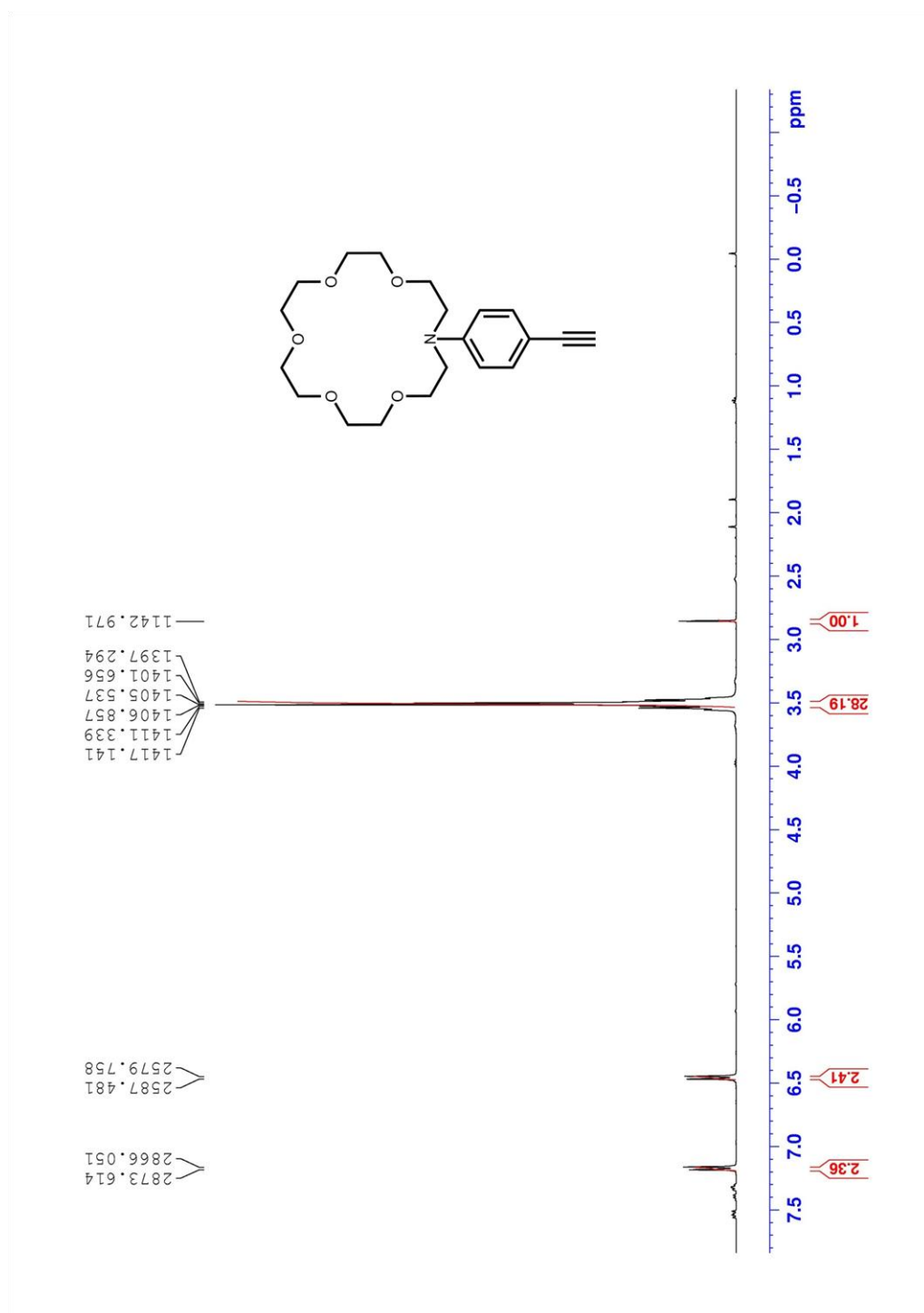


Figure 64. ¹H spectrum of compound 34

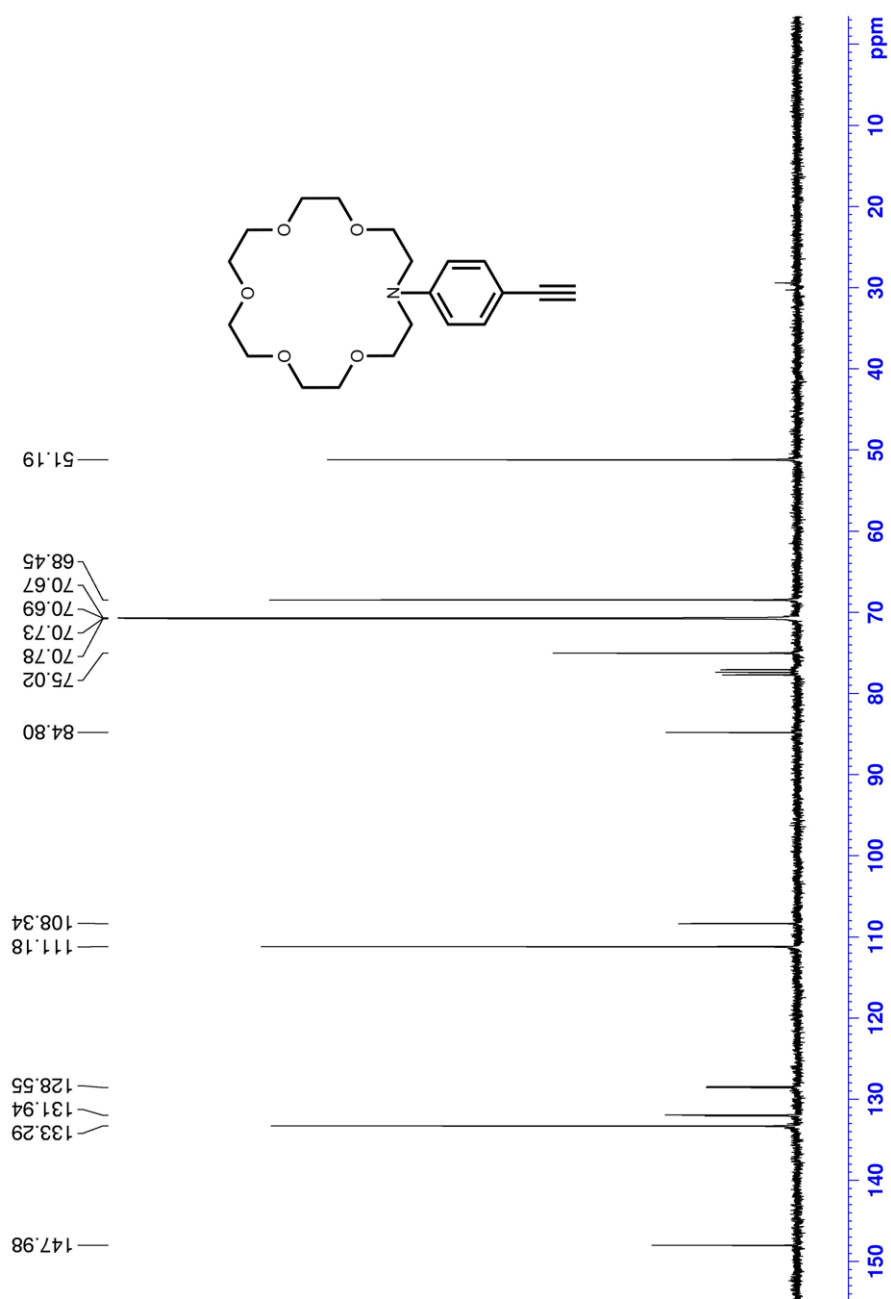


Figure 65. ^{13}C spectrum of compound 34

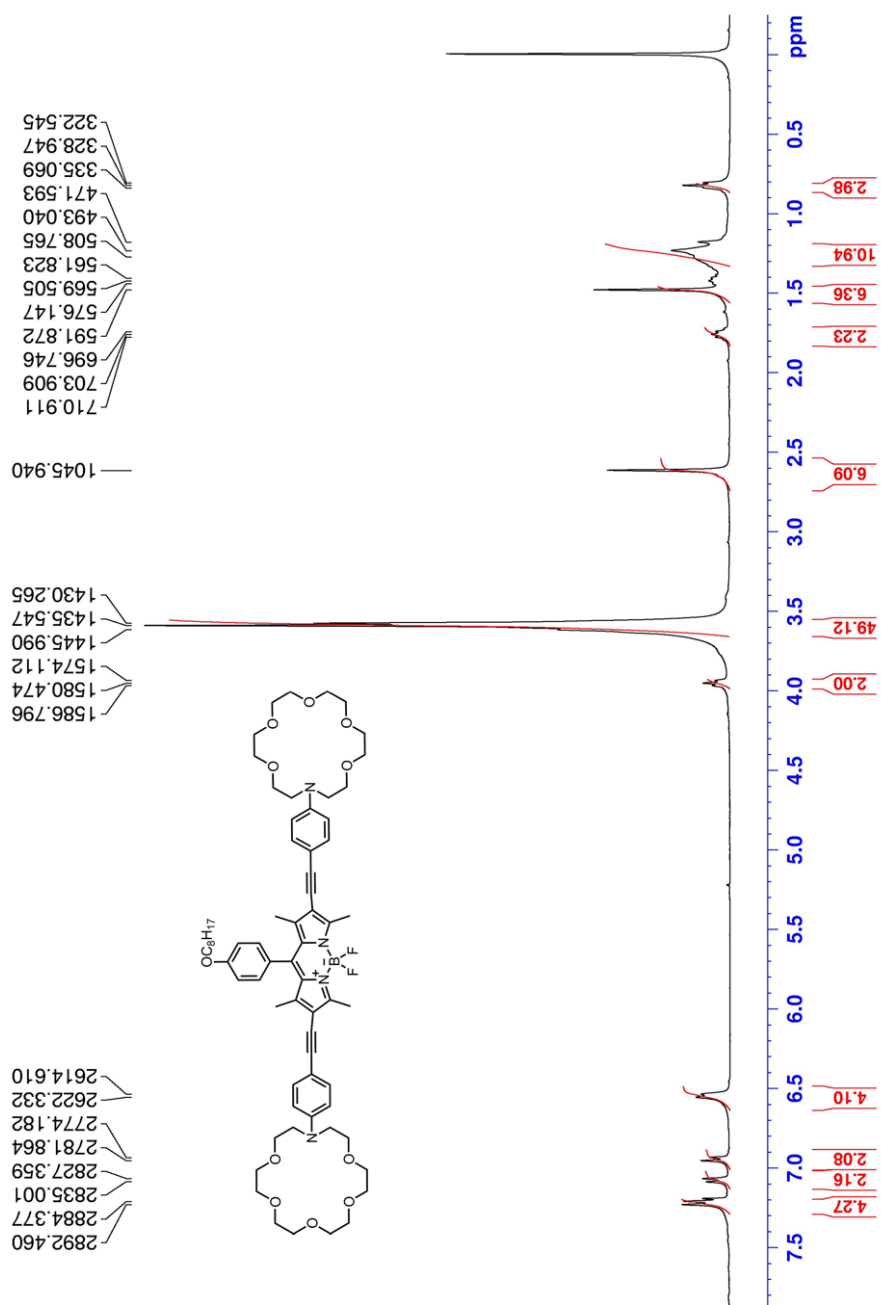


Figure 66. ¹H spectrum of compound 35

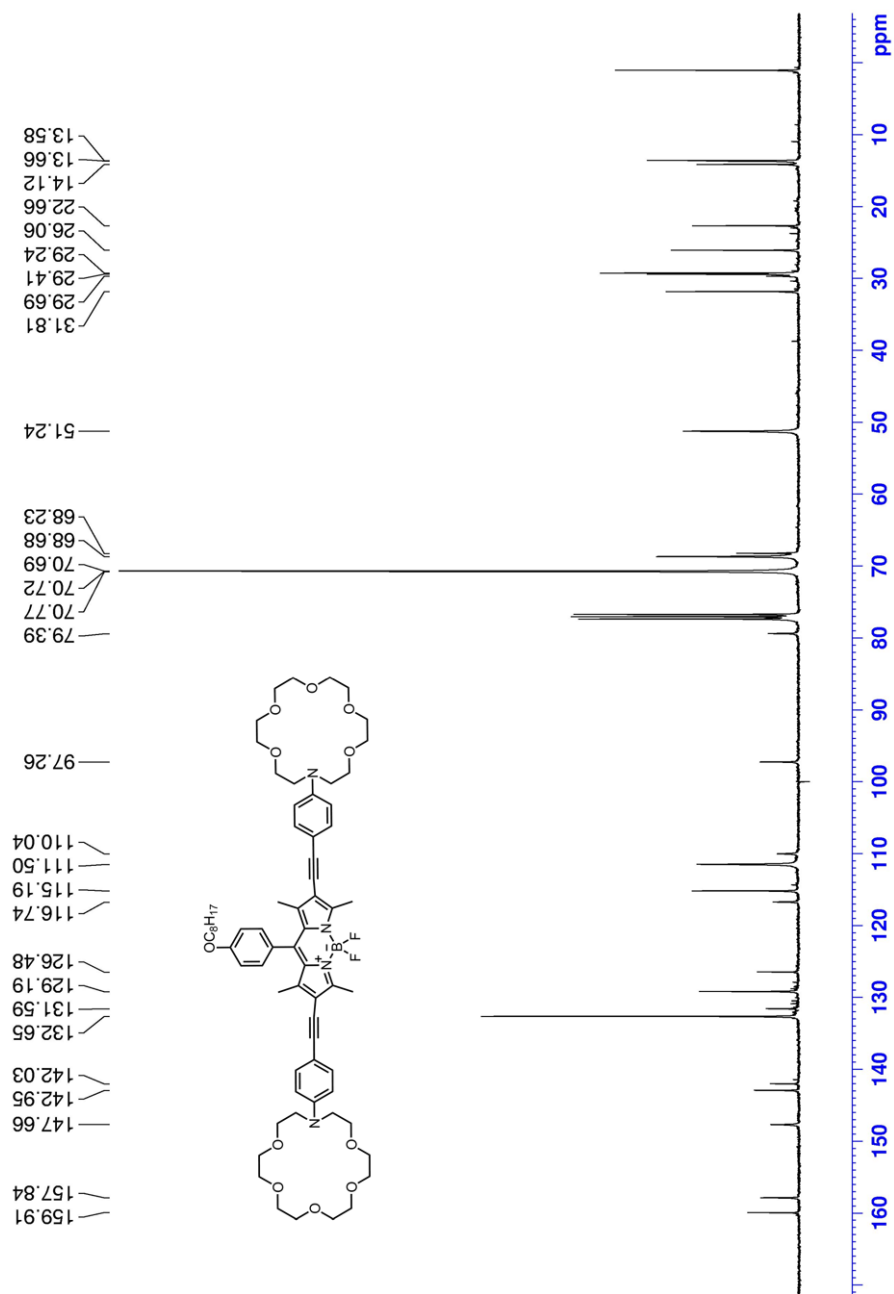


Figure 67. ¹³C spectrum of compound 35

APPENDIX B

MASS SPECTRA

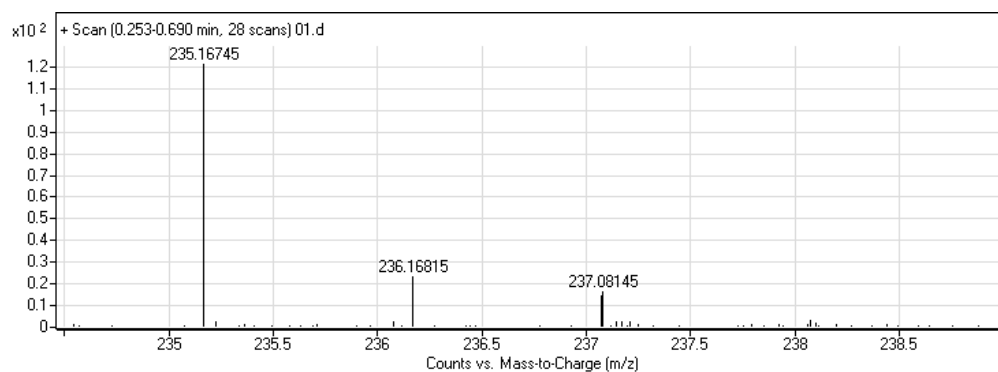


Figure 68. ESI-HRMS of compound **28**

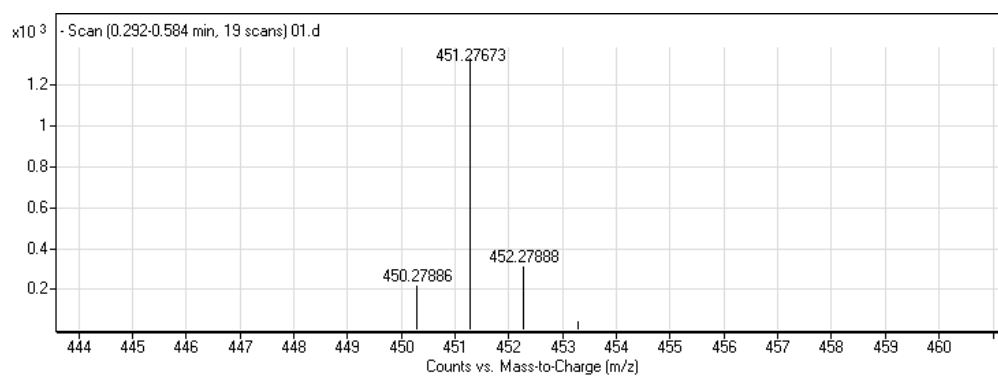


Figure 69. ESI-HRMS of compound **29**

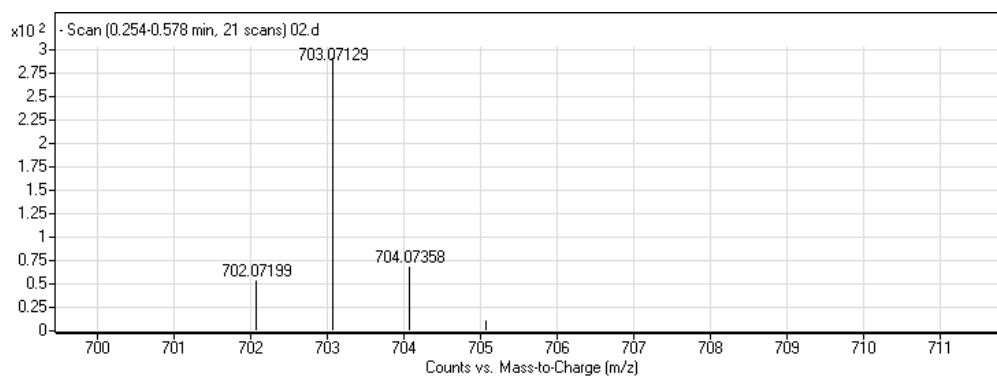


Figure 70. ESI-HRMS of compound **30**

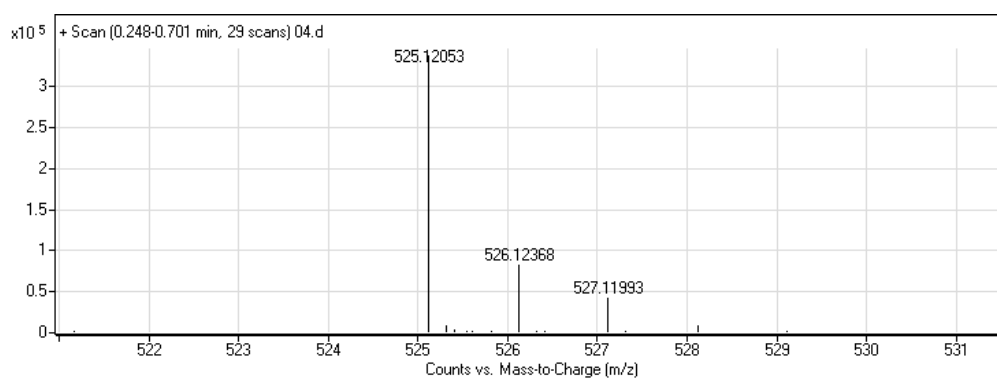


Figure 71. ESI-HRMS of compound **31**

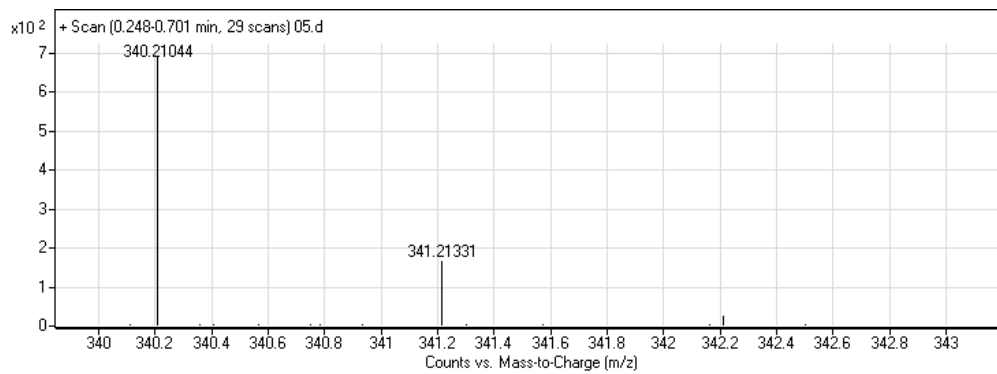


Figure 72. ESI-HRMS of compound **32**

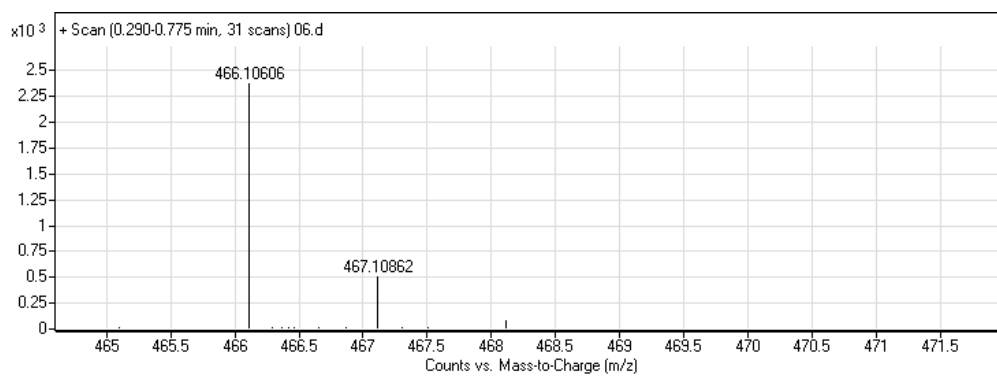


Figure 73. ESI-HRMS of compound **33**

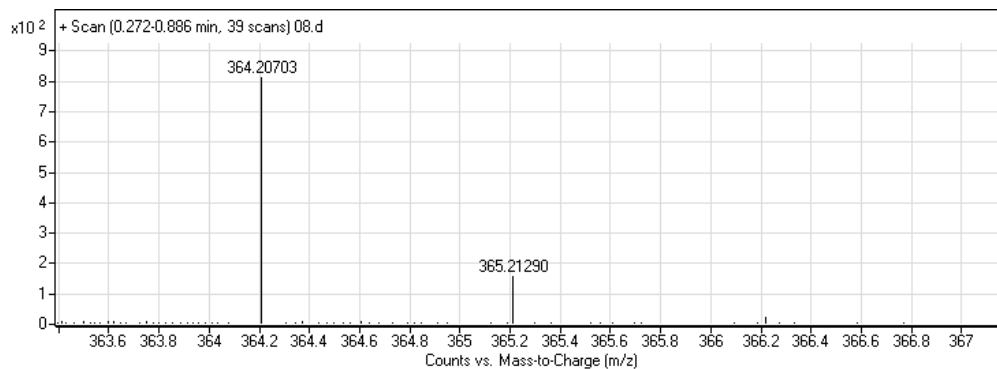


Figure 74. ESI-HRMS of compound **34**

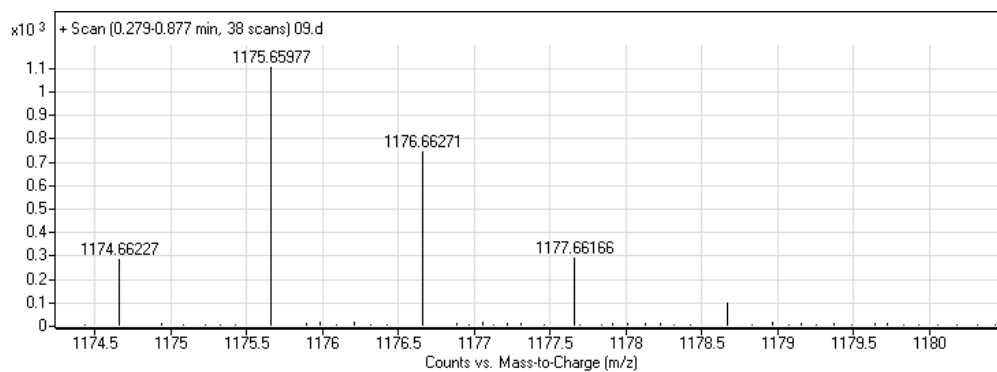


Figure 75. ESI-HRMS of compound **35**