DESIGN AND SYNTHESIS OF NEAR-IR EMITTING FLUORESCENT CHEMOSENSORS FOR TRANSITION METAL IONS

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DESIGN AND SYNTHESIS OF NEAR-IR EMITTING

FLUORESCENT CHEMOSENSOR FOR TRANSITION METAL IONS

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ABSTRACT

DESIGN AND SYNTHESIS OF NEAR-IR EMITTING

FLUORESCENT CHEMOSENSOR FOR TRANSITION METAL IONS

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Supramolecular chemistry is an emerging field of chemistry which has attracted much attention in recent years as a result of its broad applicability in many areas. Thus, the design of functional supramolecular systems is strongly in demand in this field. For this purpose, we have developed near-IR emitting ratiometric fluorescent chemosensors for transition metal ions. Judicious placement of dithiodioxaazamacrocycles on the BODIPY chromophore generates this chemosensor which is selective for Hg(II) ions and both absorption and emission spectra display large changes that would allow ratiometric sensing.

Keywords: supramolecular chemistry, boradiazaindacene, fluorescent chemosensor, ion sensing

ÖZ

YAKIN KIZILÖTESİ BÖLGEDE GEÇİŞ METALLERİ İÇİN ORANTILAMALI FLORESAN MOLEKÜLER ALGILAYICILARIN TASARIMI VE SENTEZİ

Kütük, İlker
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Haziran 2008, 53 sayfa

Süpramoleküler kimya geniş uygulama alanlarından dolayı, son yıllarda kimyanın, hızla gelişen bir dalı haline gelmiştir. Bunun sonucu olarak da, fonksiyonel supramoleküler yapıların dizaynına ve geliştirilmesine oldukça fazla ihtiyaç vardır. Bu amaçla, yakın kızılötesi bölgede emisyonu olan orantılamalı floresan moleküler algılayıcılar geliştirmiş bulunuyoruz. BODIPY kromoforuna bağlı ditiodiokzaaza makrosiklik ligandın uygun pozisyonu, Hg(II) iyonuna seçici moleküler algılayıcı oluşturmaktadır. Absorpsiyon ve emisyon spektrumları büyük değişimlerin olduğunu göstermektedir ve bu da orantılamalı moleküler algılayıcı olarak kullanıma imkan verecektir.

Anahtar kelimeler: süpramoleküler kimya, boradiazaindasen, floresan moleküler algılayıcı, iyon algılanması

To my parents

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I was extraordinarily fortunate in having such a wonderful mother, father and sister. I can not forget my little niece Beren. Her smile always forced me to finish this thesis.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

PET: Photoinduced electron transfer

PCT: Photoinduced charge transfer

TFA: Trifluoroacetic acid

BODIPY: Boradizaindacene

DCM: Dichloromethane

$CHCl_3$: Chloroform

CHAPTER 1

INTRODUCTION

1.1 What is Supramolecular Chemistry ?

Supramolecular Chemistry is the area of the chemistry that focuses on the noncovalent bonding interactions of the molecules [1]. Traditional chemistry focuses on the covalent bond, on the other hand, supramolecular chemistry examines the weaker and reversible noncovalent interactions between molecules. These forces include hydrogen bonding, metal coordination, hydrophobic forces, Van der Waals forces, π-π interactions and electrostatic effects. Important concepts in supramolecular chemistry are molecular self-assembly, folding, molecular recognition, host guest chemistry, mechanically interlocked molecular architectures and dynamic covalent chemistry [2].

Supramolecular Chemistry is a multidisciplinary field which has strong interactions with other disciplines. For instance, organic chemistry and inorganic chemistry are used for the synthesis of the receptor molecules for the desired analyte. Furthermore, physical chemistry is used to understand the properties of supramolecular systems. A functional supermolecule would be formed when a receptor molecule interacts with the proper analyte. This formed supermolecule can perform mainly three operations, these are recognition, transformation and translocation. As a result, functional molecular and supramolecular devices can be obtained with the help of self-assembly, self-organization and appropriate functional components.

Supramolecular Chemistry can be divided into two main categories: these are host-guest chemistry and self-assembly. Host-guest chemistry is the study of large host molecules that are capable of enclosing smaller guest molecules by means of non-covalent interactions (Figure 1). If one molecule is significantly larger than another and can wrap around it, then it is termed as 'host' and the smaller molecule as its 'guest', which is surrounded by host. One definition of hosts and guests was given by Donald Cram, who said 'The host component is defined as an organic molecule or ion whose binding sites converge in the complex'[3].

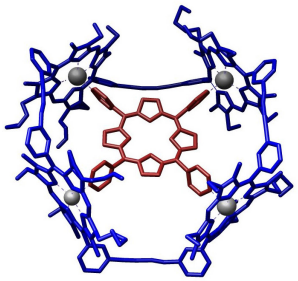


Figure 1. An example of host-guest chemistry reported by Sanders

The second category of the supramolecular chemistry is self-assembly which is the spontaneous and reversible association of two or more components to form larger supermolecules with the help of non-covalent interactions (Figure 2). Molecular self-assembly is very important concept in supramolecular chemistry because assembly of the molecules is directed through noncovalent interactions, such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, and/or electrostatic effects.

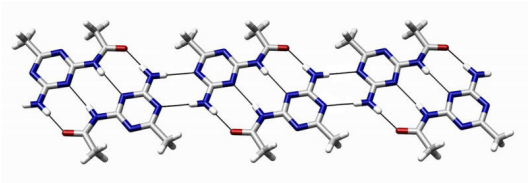


Figure 2. An example of molecular self assembly reported by Meijer

1.2 Supramolecular Interactions

Supramolecular chemistry concerns noncovalent bonding interactions which are considerably weaker than covalent interactions, which can range between 150 kJ mol^{-1} to 450 kJ mol^{-1} for single bonds. They can be classified as follows;

1.2.1 Ionic and dipolar interactions

These interactions can be divided into three categories such as (i) ion-ion interactions (ii) ion-dipole interactions and (iii) dipole-dipole interactions which are based on the Coulumbic attraction between opposite charges (Figure 3). Ion-ion interactions can occur in any orientation, therefore they are the strongest interactions compared with ion-dipole and dipole-dipole interactions.

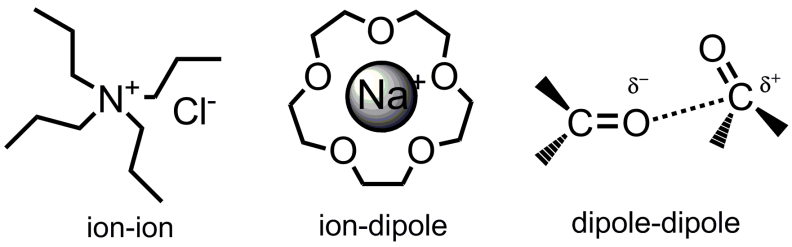


Figure 3. Examples of electrostatic interactions

1.2.2 Hydrogen bonding

Hydrogen bonding is a type of dipole-dipole force between an electronegative atom and a hydrogen atom bonded to another electronegative atom (Figure 4). Hydrogen bonding is very important interaction for supramolecular chemistry because it has relatively strong and high directional nature. The bond strength range between 4 and 120 kJ mol^{-1}. Hydrogen bonds are responsible for the overall shape of many proteins, recognition of substrates by numerous enzymes, and for the double helix structure of DNA [4].

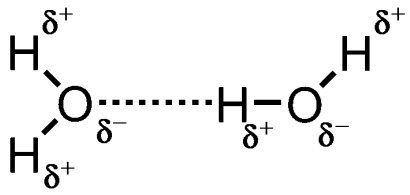


Figure 4. Hydrogen bonding in water

1.2.3 π- interactions

π-interactions can be classified into 2 categories such as cation-π interactions [5] and π-π interactions [6] in supramolecular systems. Cation-π interaction is a noncovalent molecular interaction between the face of an electron-rich π system with an adjacent cation (Figure 5).

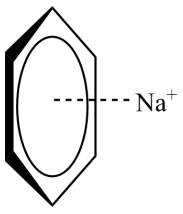


Figure 5. Cation-π interaction between benzene and Na^+

Cation-π interaction energies are of the same order of magnitude as hydrogen bonds or salts bridges and play an important role in molecular recognition [7]. π-π interaction is a noncovalent interaction between organic compounds containing aromatic moieties. π-π interactions are caused by intermolecular overlapping of p-orbitals in π-conjugated systems, so they become stronger as the number of π-electrons increases. π-π interactions are face to face, whereby parallel ring systems, separated by ca. 3.5Å, are offset and the interaction is between the centre of one ring and the corner of another, and edge-to-face, whereby a hydrogen atom from one ring interacts in a perpendicular orientation with respect to the centre of another ring (Figure 6).

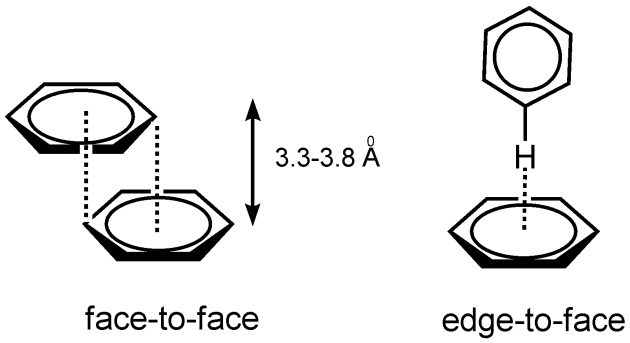


Figure 6. Face-to-face and edge-to-face structures of benzene

1.2.4 Van der Waals forces

Van der Waals forces include momentary attractions between molecules, diatomic free elements, and individual atoms. They differ from covalent and ionic bonding in that they are not stable. They are caused by momentary polarization of particles. Because electrons have no fixed position in the structure of an atom or molecule, but rather are distributed in a probabilistic fashion based on quantum probability, there is a non-negligible chance that the electrons are not evenly distributed

and thus their electrical charges are not evenly distributed. Van der Waals interactions may be divided into dispersion (London) and exchange-repulsion terms. The dispersion interaction is the attractive component that results from the interactions between fluctuating multipoles in adjacent molecules. The attraction decreases very rapidly with distance and is additive with every bond in the molecule contributing to the overall interaction energy [8].

1.2.5 Hydrophobic effects

Hydrophobic effects arises from the exceptionally high energy needed to form a cavity in a solvent. Water has an exceptionally high internal cohesion energy density, resulting in a large vaporization enthalpy and high surface tension [9]. Hydrophobic interactions play an important role in supramolecular chemistry such as the binding of organic molecules by cyclophanes and cyclodextrins in water. Hydrophobic effects can be divided into two components, namely an enthalpic hydrophobic effect and entropic hydrophobic effect. Enthalpic hydrophobic effect occurs when a guest replaces the water within the cavity and entropic hydrophobic effect comes about when there are two or more organic aqueous solution, the combination of host with the guest molecule replaces the water molecules which placed interior of host molecule. There is initially two species in the medium. When supramolecular complex forms, displacement of the water molecules both around the guest and inside host will increase the number of independent species, thus, it will increase the total entropy of the system [10].

1.3 Chemical Sensors

Chemical sensors are usually understood to be devices that transform chemical information into analytically useful signals. The term chemosensor can be defined as a molecule of inanimate origin that signals the presence of matter or energy. A key requirement of chemosensor function is that analyte binding must occur reversibly, therefore analyte concentration can be measured at equilibrium by optical detection of either the chemosensor-bound species or the analyte-free chemosensor. It also permits continuous measurements to be made with dynamic optical response to changing analyte concentrations.

Reversible association between the chemosensor and its analyte is very applicable phenomena. The chemosensors of interest bind their guest analytes by noncovalent interactions, hence they can be termed supramolecular chemosensors.

An optical chemosensor consists of a molecule incorporating a binding site, a chromophore or fluorophore, and a mechanism for communication between the two. When analyte binds, there is a change in chemosensor optical properties (absorption or fluorescence)(Figure 7). The chemosensor binding site by definition cannot be biological in origin (e.g., an enzyme or antibody); it must be an artificial receptor. Biotic receptors in biosensors may have high affinity and selectivity for biological analytes, but artificial receptors have many potential advantages. Biomolecules are sensitive to pH, oxidizing agents and heat, while inanimate receptors can be synthesized from more strong components. In principle, artificial receptors can be adapted for various analytes, and their physical properties can be adjusted to meet specific sensor requirements [11].

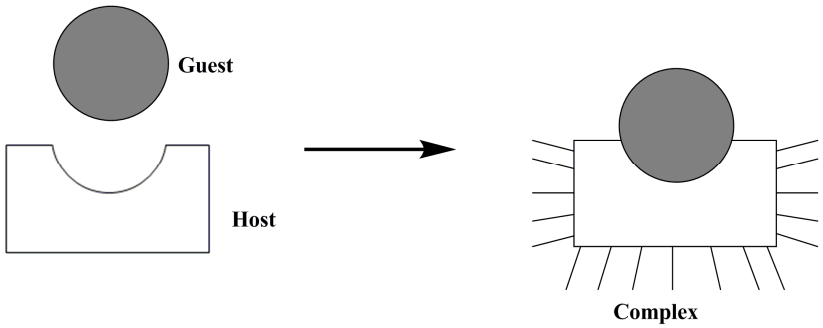


Figure 7. Cartoon showing binding of an analyte (guest) by a chemosensor (host) , producing a complex with high fluorescence.

1.4 Fluorescence Theory

Fluorescence is a luminescence that is mostly found as an optical phenomenon in cold bodies. The energy difference between the absorbed and emitted photons ends up as molecular vibrations or heat. Usually the absorbed photon is in the ultraviolet range, and the emitted light is in the visible range, but this depends on the absorbance curve and Stokes shift of the particular fluorophore. The fluorescence phenomenon may also be explained in another way. The absorption of a quantum light by a molecule results in the elevation of an electron from the molecule's ground electronic state (S_0) to one of the several vibrational levels in the an excited state. In solution, the excited state molecule rapidly relaxes to the lowest vibrational level of the lowest electron state(S_1). Therefore, the energy stored in the excited state may be released in several ways. The electron may return to the electronic ground state with only release of heat (radiationless relaxation). After relaxing thermally to the lowest vibrational level of the S_1 state, the electron may return to the state S_0 state with light emission (fluorescence) (Figure 8).If the molecule is sufficiently long-lived in the S_1 state, it may cross into a lower energy triplet state(T_1). Relaxation from the T_1 to the state S_0 state can also occur with light emission in solids (phosphorescence), by the release of energy (raditionless transition) or by chemical reaction [12].

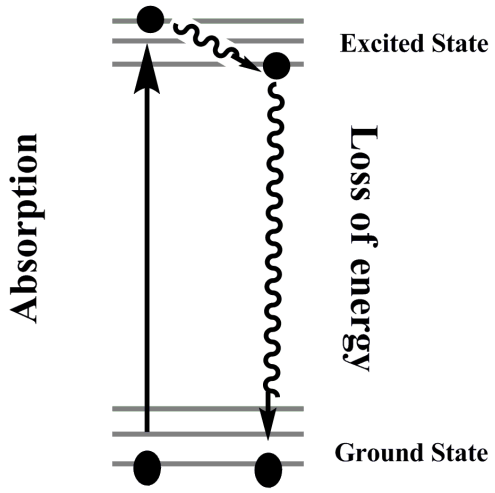


Figure 8. Schematic representation of fluorecence theory

1.5 Fluorescent Chemosensors

The fluorophore and the receptor are the parts of a fluorescent sensor (Figure 9). These fluoroionophors can signal various analytes such as anions, cations and neutral species. The recognition and signaling moieties are the most important part in the design of sensors. When there is a change in the photophysical characteristics of the fluorophore, the information is converted into an optical signal since the signaling moiety acts as a signal transducer. These changes are due to the perturbation of photoinduced processes such as electron transfer, charge transfer, energy transfer, excimer or exciplex formation or disappearance, etc. these aspects are relevant to the field of photophysics. Regarding the recognition moiety, it is responsible for selectivity and efficiency of binding which depend on the ligand topology, on the characteristics of the analyte (charge, size, shape, ….), and on the nature of the solvent (pH, ionic strength, …). These aspects are relevant to the field of supramolecular chemistry. There are two different ways that the signaling moiety and the receptor can be attached. First

way is that the receptor is either directly linked to the signaling unit (integrated) and the second one is that it is linked to the signaling unit via a spacer [13].

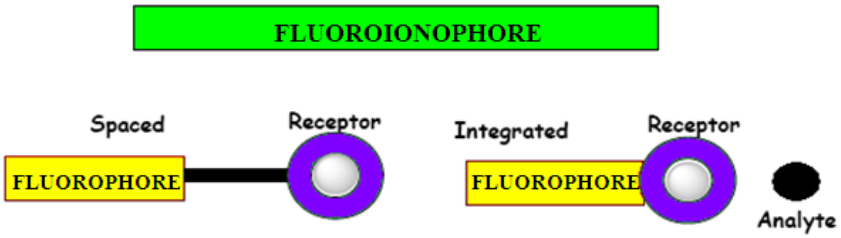


Figure 9. Main aspects of fluorescent molecular sensors

1.5.1 Photoinduced electron transfer (PET)

Photoinduced electron transfer is an electron transfer which occurs when certain photoactive materials interact with light [14]. It plays a major role in photosynthesis and in artificial systems for the conversion of solar energy based on photoinduced charge separation. Furthermore, PET is widely used in sensors for the fluorescent sensing of various analytes such as cations, anions, neutral molecules. Fluorescent signaling via the PET strategy is distinguished by its supramolecular nature since distinct components perform each one of the necessary functions. A fluorophore unit is the site of both photonic transactions of excitation and emission. A receptor unit is responsible for guest complexation and decomplexation. A spacer unit holds the fluorophore and the receptor close to, but separate from, each other. This means that true molecular engineering applies, the optical, guest-binding, and redox properties of the components to allow the quantitative prediction of the signaling parameters of the supramolecular system.

Further, PET signaling systems have natural switchability: guest-induced "off-on" and "on-off" fluorescence are both designable [15].

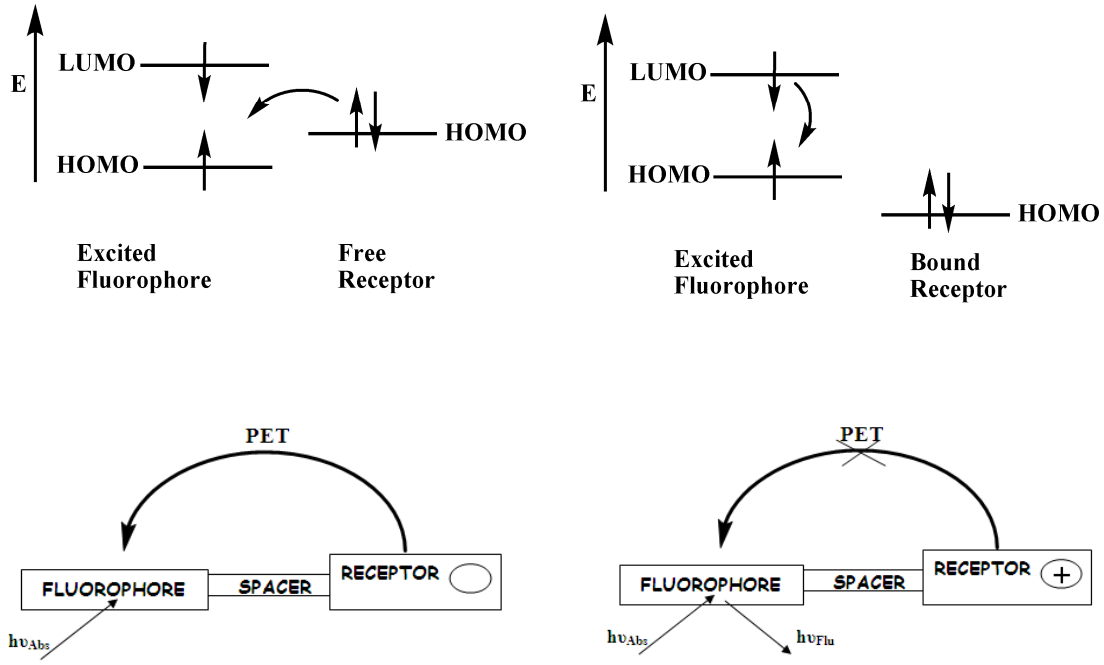


Figure 10. Schematic representation of photoinduced electron transfer (PET)

Figure 10 shows how PET mechanism can be controlled in a fluoroionophore by an analyte. The receptor unit is an electron donor group such as amino group and fluorophore plays the role of an acceptor. As seen in Figure 9, the designed system is an "off-on" type signaling system. An electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO) upon excitation of the fluorophore. Therefore, this enables PET from the HOMO of the donor (which belongs to the free receptor) to that of the fluorophore. As a result, the

fluorescence is quenched. On the other hand, the redox potential of the donor is raised so that the relevant HOMO becomes lower in energy than that of fluorophore upon analyte binding; consequently, fluorescence enhancement occurs. PET may sometimes occur from acceptor to donor. Then it is called reverse PET (or oxidative PET). Excitation energy is transferred from the fluorophore, through the ligand to another bound cation after blocking PET by cation binding. It is also known that coordination of anions to the metal center blocks oxidative PET mechanism and as a result of this emission intensity is restored [16].

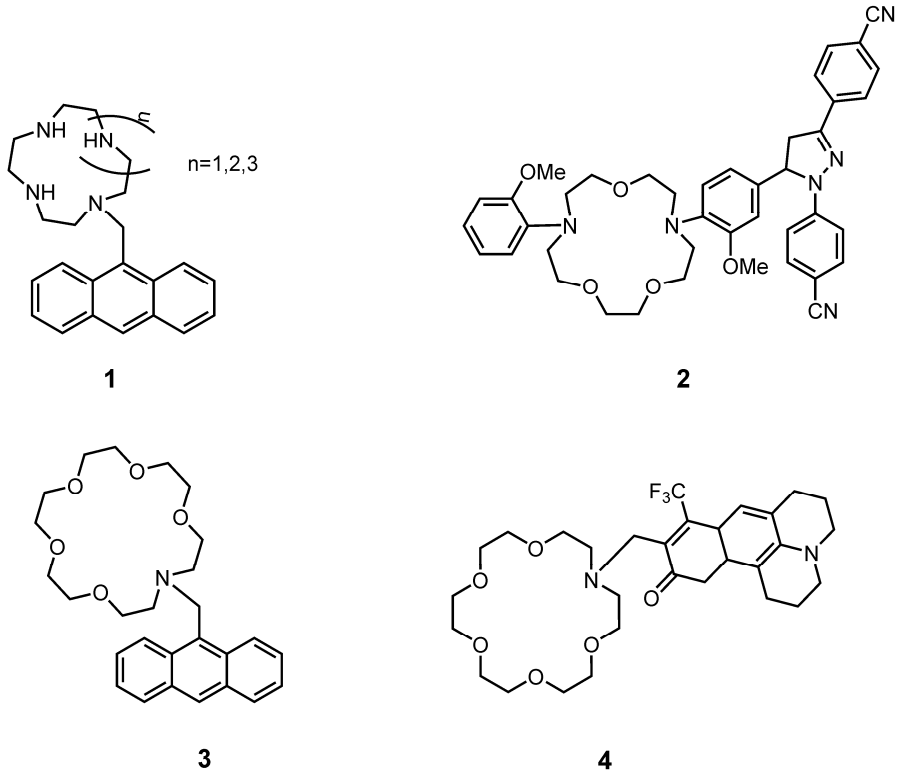


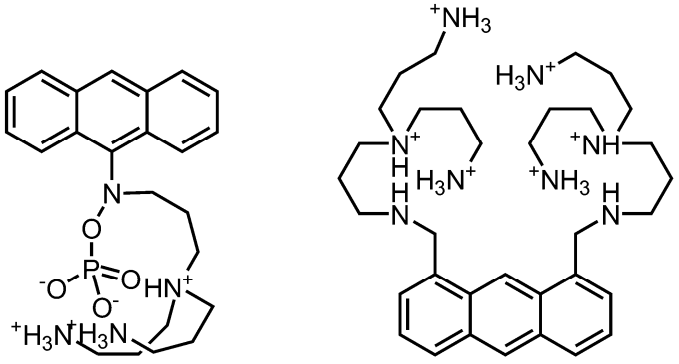
Figure 11. Fluorescent PET sensors containing crown

There are some examples of PET sensors for cation recognition are given in Figure 11. Compound **3** [17] is the first PET sensor which has been synthesized by de Silva and his coworkers. In methanol solution, its fluorescence quantum yield increases from 0.003 to 0.14 upon binding of K^+. Furthermore, for the recognition of soft metal ions like

Zn^{2+}, compound **1** [18] was designed by Czarnik et al.. In **2** [19], strong Na^+ binding is achieved. The methoxy groups in ortho position with respect to the nitrogen atoms of the crown participate in the complexation. Na^+ causes decoupling of the nitrogen centers from the rest of the π-electron system of the receptor. Thus, the deceleration of PET causes Na^+-induced switching `on` of the fluorescence. Compound **4** [20] responds to a variety of metal ions from various regions of the Periodic Table.

Charge density control of the metal ion-induced fluorescence enhancement is evident. There is another interesting point of compound **4**. When metal ion bound the receptor, the ICT excited state of the fluorophore is stabilized; as a result, red shift was observed in the emission.

In literature, some PET sensors for anion recognition are also synthesized. For example, Czarnik et. al. synthesized a partially protonated polyamine as a receptor whose PET channel is blocked upon arrival of HPO_4^- guest which can be seen in figure 12 [21]. Two of these receptor units can be placed on the 1- and 8- positions of an anthracene fluorophore as in **6** [22] in order to create a signaling PET system for pyrophosphate in the form of $H_2P_2O_5^{2-}$.



5 6

Figure 12. Polycationic PET sensors for anion recognition

1.5.2 Photoinduced charge transfer (PCT)

The spacer part between receptor unit and signaling unit is removed in the case of PCT. In PET, a flurophore is not directly bound with the receptor. In contrast to PET, a fluorophore is directly integrated with the receptor so that their orbitals overlap. Therefore, one terminal tends to be electron rich and the other electron poor. This is a kind of "push-pull" cases. Excitation leads to a redistribution of electron density, so that a considerable dipole is created in such pull-push cases. As a result, intramolecular charge transfer from donor to the acceptor is caused by this excitation. When a target species, especially a charged one, binds into the receptor, an interaction is caused with this excited state dipole. This interaction energy shows up in the emission signature.

The excited state of the fluorophore looks like the resonance form of the ground state. The consequent change in dipole moment results in a Stokes shift that depends on the microenvironment of the fluorophore. It can be expected that cations will change the photophysical properties of the fluorophore when they are in close interaction with the donor or the acceptor moiety; because the complexed cation affects the efficiency of intramolecular charge transfer.

When a group such as an amino group, which plays the role of an electron donor within the fluorophore, interacts with a cation, the latter reduces the electron-donating character of this group; owing to the resulting reduction of conjugation. Therefore, a blue shift of the absorption spectrum and a decrease of the extinction coefficient are expected. The photophysical changes upon cation binding can also be described in terms of charge dipole interactions.

Excited state will be electronically similar to the resonance forms of the ground state, where the amino group will be positively charged. Therefore, the excited state will be destabilized because of the interaction between this moiety and the cation. As a result, the energy gap between S_0 and S_1 energy levels increase. Increasing energy will cause decrease in the wavelength, as a result of this interaction a blue-shift occurs and

the desired analyte can be signaled in this way. On the other hand, if a cation interacts with the acceptor group (like a carbonyl group), the cation raises the electron-withdrawing character of this group. Consequently, the absorption spectrum is red-shifted and the molar absorption coefficient is increased. Moreover, this change can be described in terms of a charge-dipole interaction. When the cation interacts with the acceptor group, the excited state is more stabilized by the cation than the ground state, and this phenomenon leads to a red shift of the absorption and emission spectra. If we consider the resonance structure of the acceptor group, carbonyl oxygen is negatively charged in the excited state, this situation will increase the interaction between cation and the acceptor group. As a result of this interaction, the excited state is stabilized, and the energy gap between S_0 and S_1 will decrease, thus a red-shift is observed in the spectrum.

In principle, the fluorescence spectra are shifted in the same direction as those of the absorption spectra. In addition, photophysical effects such as quantum yields and lifetimes are often observed. All of them obviously depend on the charge and the size of the cation, and selectivity of these effects are expected (Figure 13)[23].

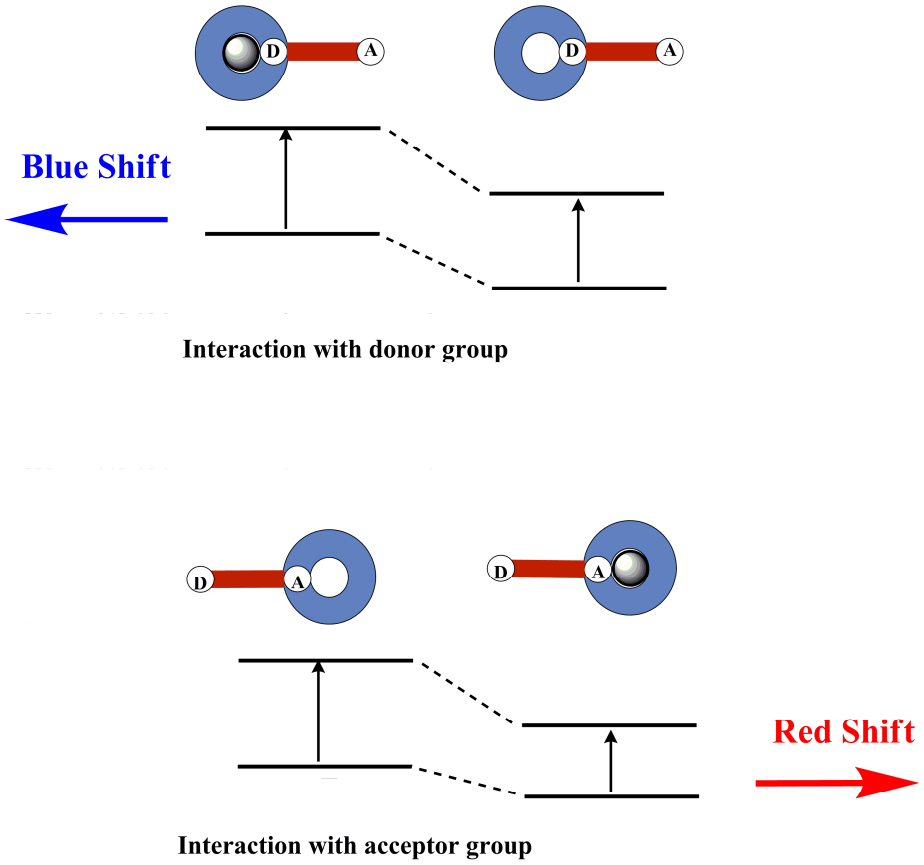


Figure 13. Spectral displacements of PCT sensors resulting from interaction of a bound cation with an electron-donating or electron-withdrawing group.

Many fluoroionophores have the same principle during the design. The cation receptor is an azacrown containing a nitrogen atom and it is conjugated to an electron-withdrawing group. As seen in Figure 14, compounds **7** to **10** show common properties; the blue shift of the absorbtion spectrum is much larger than that of the emission spectrum on cation binding. **7** [24], **8** [25], **9**[26], **10** [27], showed similar affinities towards calcium ion.

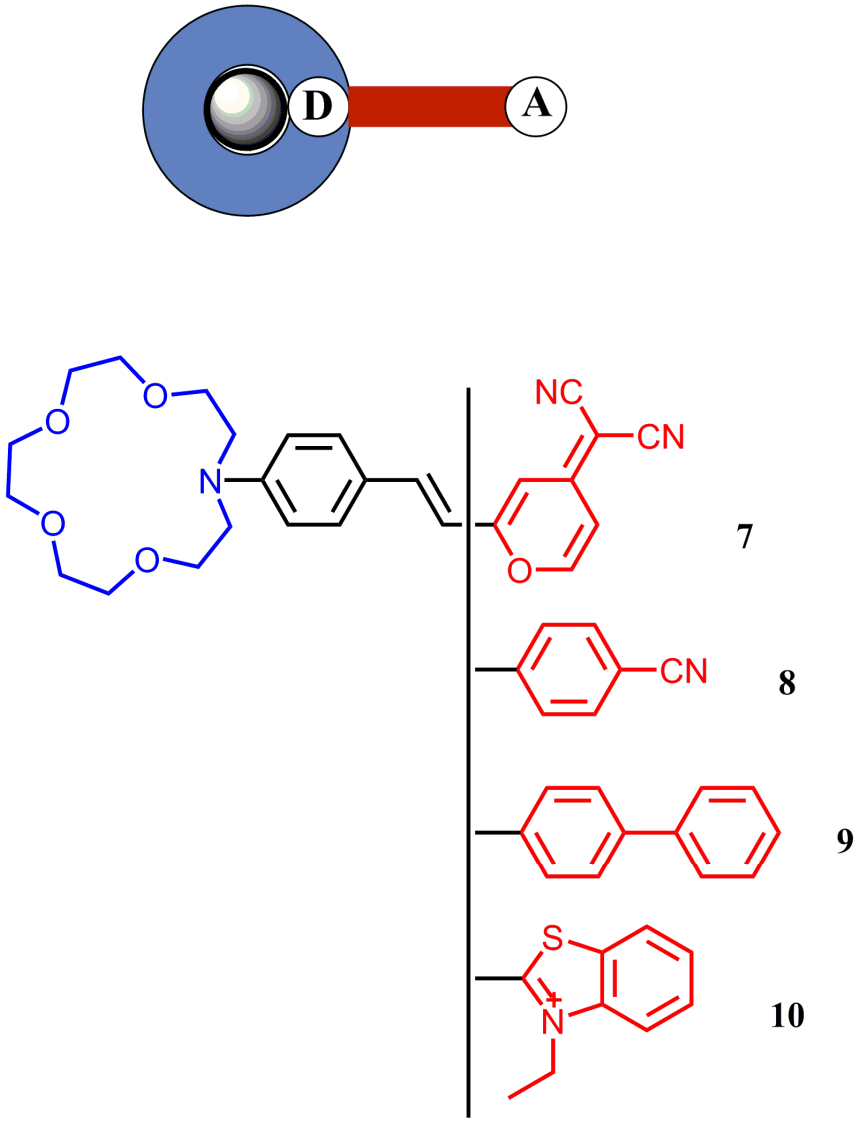


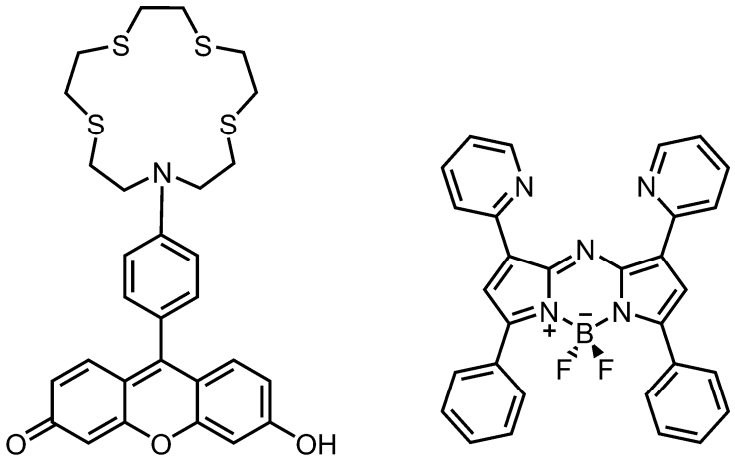
Figure 14. Crown containing PCT sensors

The electron density on the nitrogen atom of the crown is reduced by the PCT and this nitrogen atom becomes a noncoordinating atom since it is positively charged. Therefore, excitation induces a photodisruption of the interaction between the cation and the nitrogen atom of the crown. The fluorescence spectrum is slightly affected because most of the fluorescence originates from species in which the interaction between the cation and the fluorophore is non-existent or much weaker. Also the quantum yield of this type of fluorophores is quite low on account of the excited state rotation around the double bond between donor and acceptor units.

1.6 Cation Binding

Cations have many important roles in biological processes. Metal cations are present at the active sites of many enzymes playing catalytic roles. Furthermore, some metal complexes include complexes of paramagnetic lanthanides (e.g., Gd(III)) used as contrast agents in magnetic resonance imaging (MRI) of soft issue. Besides this, zinc and aluminum are known to have possible implications in Alzheimer`s disease.

As known some cations, such as Pb(II), Cd(II), Hg(II), have toxicity and pollutant effects. The early detection of them in the environment is very important for these reasons. Therefore, there has been a great deal of effort aimed at producing selective receptors for cationic guest species. Compound **11** [28], developed by Chang et al., is a sulfur containing macrocyle. It is known to be selective to soft metal cations. (Figure 15). The ligand shows exclusive selectivity towards Hg(II) ion depending on the size and the shape.



11 **12**

Figure 15. Hg(II) selective fluorescent crown containing PCT sensors

This compound has been used to detect the mercury levels in fish. The method provides a useful starting point for developing new mercury contamination screens for a wide range of biological, toxicological, and environmental samples. The chemosensor **12** [29] is selective for Hg(II) ions, and according to absorbtion and the emission spectra large changes are displayed. Therefore, that would allow ratiometric sensing. It is seen that proper derivatization of these novel fluorophores would lead to satisfactory chemosensors for other molecular or ionic species of interest. Furthermore, such boratriazaindacenes would have very long wavelength emissions in the electromagnetic spectrum.

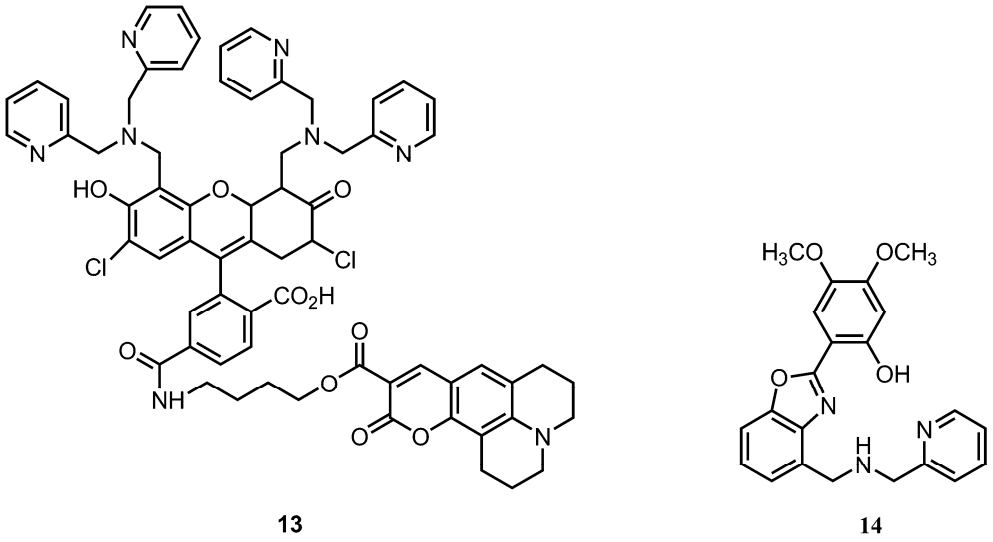


Figure 16. Selective fluorescent chemosensors for Zn(II) ion.

Selective and sensitive signaling of Zinc(II) ion is quite important due to its effects on Alzeimer`s disease and human metabolism. Compound **13** [29] is a ratiometric chemosensor for Zn(II). Coumazin group which is attached to fluorescein via ester linkage is hydrolyzed within the cell by esterase enzyme. After hydrolysis, Zn(II) sensitive fluorescein and insensitive coumazin fluorophore is created and Zn(II) concentration can be signaled from the emission signal at 534 nm from fluorescein, emission from hydrolyzed coumazin fluorophore from the emission signal at 488 nm. Thus, ratiometric sensing of Zn(II) is provided. Furthermore, O`Halloran et al. developed **14** [30], this benzoxazole type fluorescent chemosensor shows great selectivity towards Zn(II) in aqueous media. Emission ration imaging experiments of **14** reveals changes in intracellular zinc availability (Figure 16).

1.7 BODIPY Dyes

Among the large variety of known fluorescent dyes, the boradiazaindacene family has gained recognition as being one of the most versatile reagents and has found great popularity with chemists, biochemists and physicists. It has many advantages. For example, BODIPY dye has a high molar extinction coefficient and high fluorescence quantum efficiency ($\mathbf{\Phi_{fl}}$). Furthermore, it shows lower sensitivity to solvent polarity and pH than fluorescein-based probes, and its excitation and emission wavelengths can be changed due to the modification of its structure. As a result, BODIPY-based fluorescence probes have been studied in depth. They were first discovered by Treibs and co-workers in 1968, [31] BODIPY dyes have many application areas and uses such as biomolecular labels, chromogenic probes and cation sensors, drug delivery agents, fluorescent switches, electroluminescent films, laser dyes, light harvesters and sensitizers for solar cells. High fluorescence yield, the excellent stability, good solubility, negligible triplet state formation, intense absorption profile, and chemical strength have added to the general attractiveness of these dyes. There are many research groups working on the derivatization of BODIPY dyes, these groups are those of Akkaya, Rurack, Burgess, Ziessel, Nagano, Boens et al.

1.7.1 Applications of BODIPY dyes

BODIPY® dyes have excellent thermal and photochemical stability, high fluorescence yield, negligible triplet state formation, intense absorption profile, good solubility, therefore it is very attractive area. Moreover, it can be functionalized from many ways.

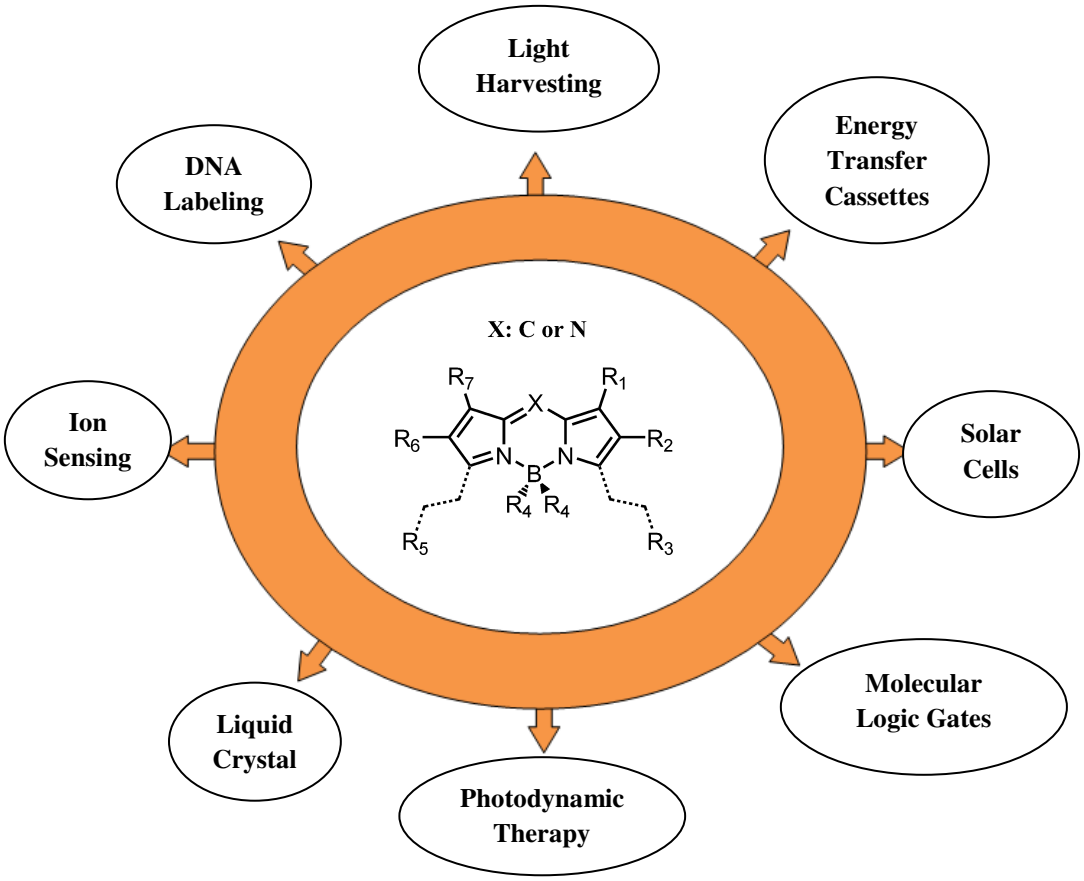


Figure 17. Application of BODIPY® dyes.

The labeling of proteins is one of the first applications of BODIPY dyes [32]. Furthermore, not only the labeling of proteins, but also photodynamic therapy and singlet oxygen generation are the interesting applications of BODIPY dyes. Distyryl-boradiazaindacenes **15** can be obtained by the condensation of 3,5-dimethyl derivatives with corresponding aldehyde.[33] (Figure 18).

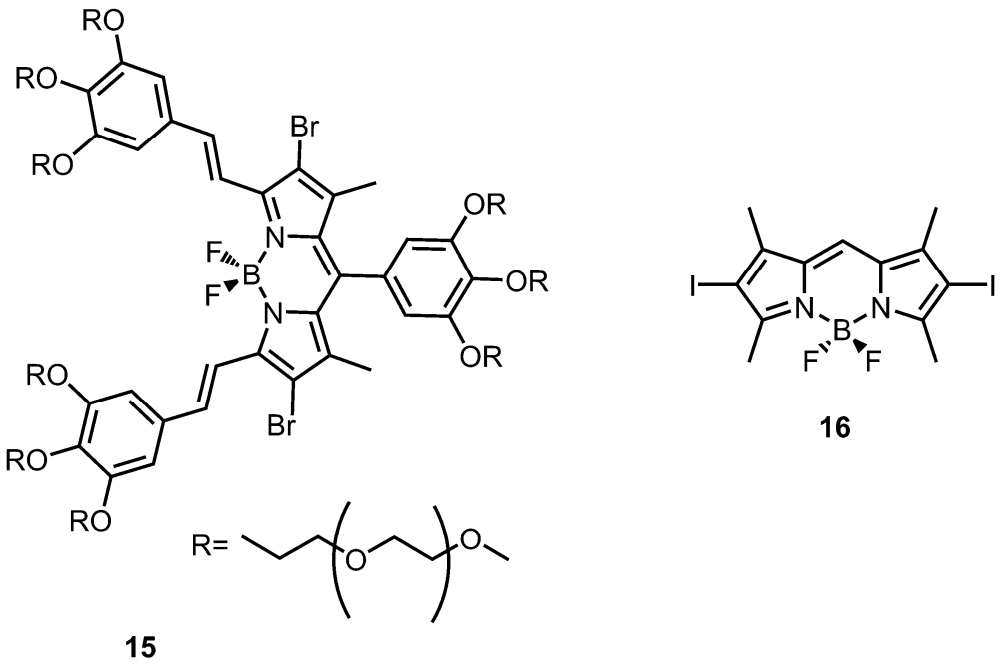


Figure 18. BODIPY based sensitizers

BODIPY dyes can be extended from different parts of its structure. As a result, the longer wavelength emitting dye can be obtained. In order to get them soluble in water, it can be functionalized with oligoethyleneglycol groups. These materials show good permeability into biological cells and have tumor targeting characteristics that make them interesting candidates for sensitizers in photodynamic therapy.

Attachment of heavy atoms to the 2 and 6 positions increase the triplet yields of these dyes and these heavy atoms favors intersystem crossing to the triplet state. Compound **16,** synthesized by Nagano et al., was compared to the reference dye lacking iodine groups. The fluorescence efficiency drops from 70% to 2%. Under aerobic

conditions, $^1O_2$ is generated with modest efficiency on illumination and cellular toxicity has been reported. [34]

There are also many chemosensors which are built by using BODIPY dyes. Compound **17** were synthesized as an "off-on" fluorescent chemosensor by Yoon et al. It displayed selective and large chelation enhanced fluorescence effects with Pb^{2+} [35]. (Figure 18). On the other hand, highly selective and sensitive ratiometric fluorescent chemosensor **18** for Ag(I) were introduced by Akkaya et al. [36]. The design allows a facile attachment and derivatization of boradiazaindacene fluorophores geared for efficient energy transfer and modulation of the emission signal. Furthermore, compound **19** [37] was synthesized with a high selectivity of K^+ over other alkali metals. The probe absorbs and emits light in the visible spectral region and shows a large wavelength shift and a considerable conformational change upon K^+ binding.

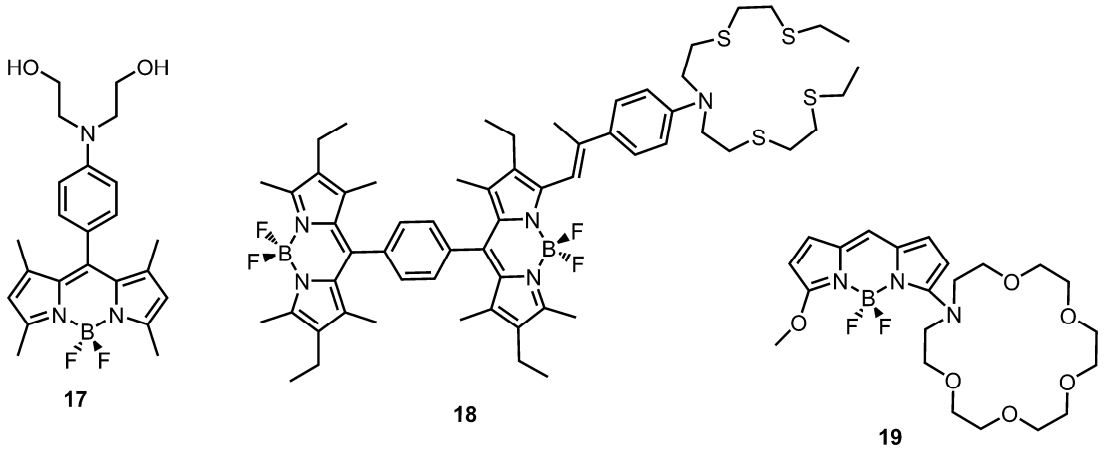


Figure 19. BODIPY based chemosensors

1.8 Mercury Sensing Fluorescent Chemosensor with BODIPY dyes

The design and construction of chemosensors with high selectivity and sensitivity for heavy metal cations has received considerable attention, as such metal ions can cause severe risks for human health and the environment.[12][13]. Among them, mercury ion (Hg^{2+}) is considered as one of the most dangerous cations for the environment because it is widely distributed in air, water, and soil. Mercury can accumulate in the human body and affects a wide variety of diseases even in a low concentration, such as prenatal brain damage, serious cognitive and motion disorders [38]. Therefore, it is very important to develop highly sensitive and selective assays for Hg^{2+} ions. In recent years many efforts have been made to design various chemosensors specific for Hg^{2+} ion detection [39].

Among the reported sensing molecules, fluorophores with a fluorescent switching property driven by photoinduced electron transfer are often employed to signal metal ion binding. However, the sensitivity and selectivity of the Hg^{2+} detection based on the PET mechanism are still unsatisfactory because of their poor fluorescent switching and the interference with other cations, such as Ag^{+} and Pb^{2+} ions. Boradiazaindacenes (BODIPY) are very attractive functional groups for construction of molecular sensors because of their advantageous characteristics, such as sharp absorption and fluorescence bands, high extinction coefficients, high fluorescence quantum yields, and high stability against light and chemical reactions.[40]

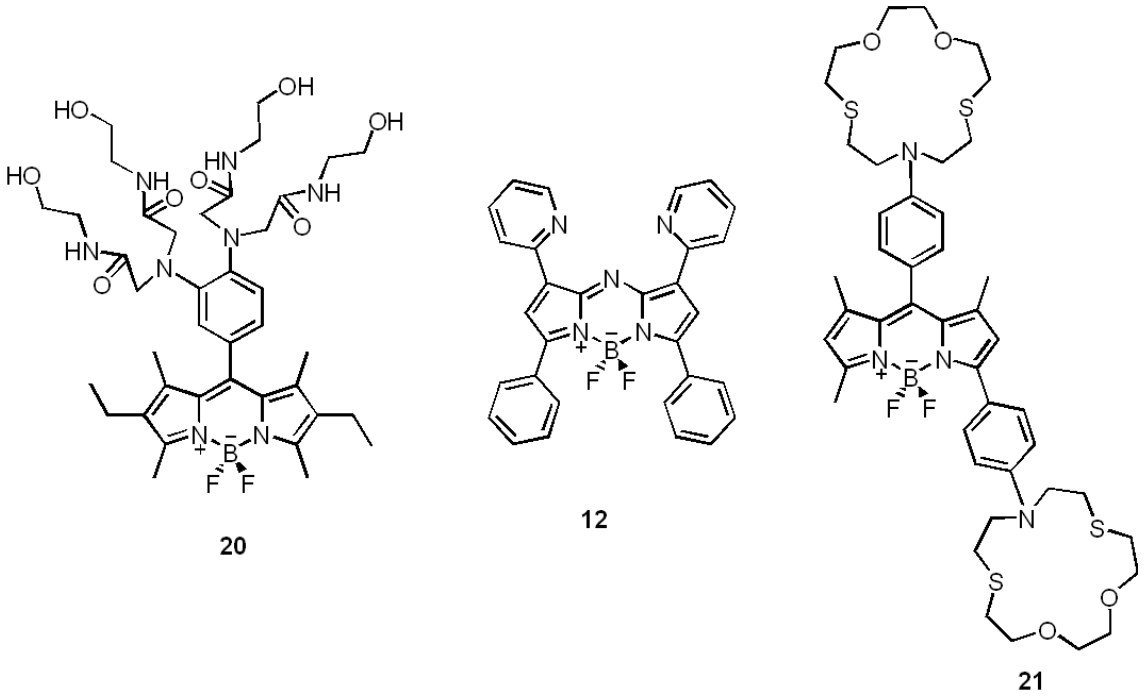


Figure 20. Mercury sensing fluorescent chemosensors with BODIPY dyes

Some examples from the literature are given on Figure 20. Compound **20** displays a super sensitivity unparalleled by earlier reported polyamide fluorescent sensor molecules. A clear emission turn-on response is observed when as low as 2 ppb of Hg^{2+} ions are present.[41] There are numerous examples of boradiazaindacene (BODIPY)-based chemosensors, but compound **12** [29] is the first demonstration of boratriazaindacenes acting as chemosensors for Hg^{2+}. As a bonus, it would have very long wavelength emission (719 nm) in otherwise silent region of the electromagnetic spectrum. Li et. al. developed a new class of colorimetric and fluorometric probe for Hg^{2+} detection with high selectivity and sensitivity on the basis of the tuning of PET and ICT processes on a single molecule (compound **21**). A highly Hg^{2+}-selective fluorescence enhancing property (>7-fold) in conjunction with a visible colorimetric change from purple to red-pink can be observed [42].

CHAPTER 2

EXPERIMENTAL

2.1 Instrumentation

All chemicals and solvents purchased from Aldrich were used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker DPX-400 in $CDCl_3$ or DMSO-d_6 with TMS as internal reference. Absorption spectrometry was performed using a Varian spectrophotometer. Steady state fluorescence measurements were conducted using a Varian Eclipse spectrofluorometer. Column chromatography of all products was performed using Merck Silica Gel 60 (particle size: 0.040–0.063 mm, 230–400 mesh ASTM). Reactions were monitored by thin layer chromatography using fluorescent coated aluminum sheets. Solvents used for spectroscopy experiments were spectrophotometric grade.

2.2 Syntheses

2.2.1 Synthesis of N,N-bis(bromomethyl)aniline (22)

(Phenylazanediyl)dimethanol (compound **21**) (23.7 mmol, 4.3g) was dissolved in a small amount of DCM and PBr_3 (68.25 mmol, 5.6 mL) was added dropwise in an ice bath for an hour. After an hour, ice was added to quench the excess PBr_3. K_2CO_3 was used for neutralization. Then the product was extracted with DCM (3 x 100ml). In addition, it was dried under vacuum and the final product was purified by silica gel chromotography ($CHCl_3$). Brownish solid (5.23 gr, 80 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (m, 2H), 6.72 (t, *J*=7.48 Hz, 1H), 6.64 (d, *J*=8.3 Hz, 2H), 3.70 (t, *J*=7.50 Hz,4 H), 3.34 (t, *J*=7.48, 4H).

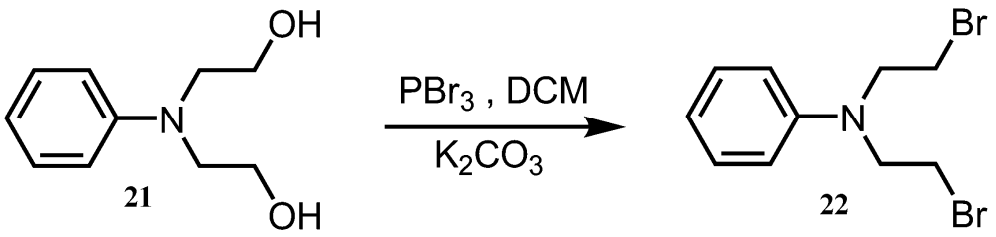


Figure 21. Synthesis of Compound **22**

2.2.2 Synthesis of 10-phenyl-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (23)

Cs_2CO_3 (3.58 mmol, 1.17 g) was dissolved in 150 mL THF. After 30 minutes compound **22** (1.63 mmol, 500 mg) and 2,2'-(ethane-1,2diylbis(oxy))diethanethiol (1.63 mmol, 297 mg) were dissolved in a small amount of THF and then added dropwise in 3 hours. The reaction was refluxed for 3 days. In order to understand that the reaction was finished or not, it was monitored by TLC (2 hexane/ 1 EtOAc). The final compound was filtered and the liquid part was concentrated in vacuo. Finally, the product was purified by silica gel chromotography (2 hexane/ 1 EtOAc). The orange fraction was collected. (400 mg, 75 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ; 7.20 (t, *J*=7.48 Hz, 2H), 6.64 (m, 3H), 3.73 (t, *J*=5.05 Hz, 4H), 3.60 (m, 8H), 2.84 (t, *J*=5.1 Hz, 4H), 2.69 (t, *J*=5.04 Hz, 4H).

^{13}C NMR (100 MHz, $CDCl_3$) δ; 146.9, 129.4, 116.2, 111.8, 76.8, 70.7, 51.8, 31.2, 29.5

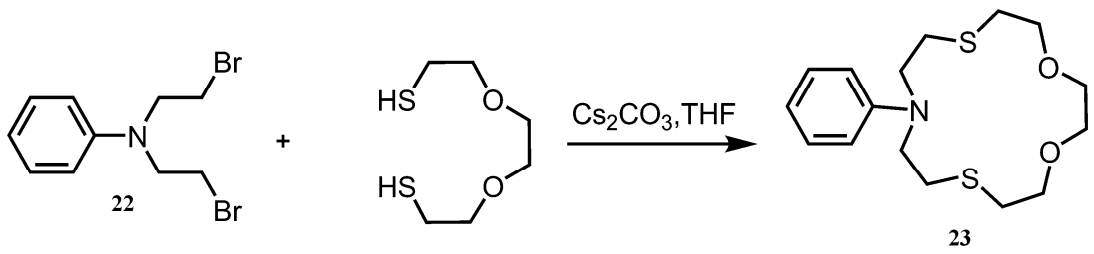


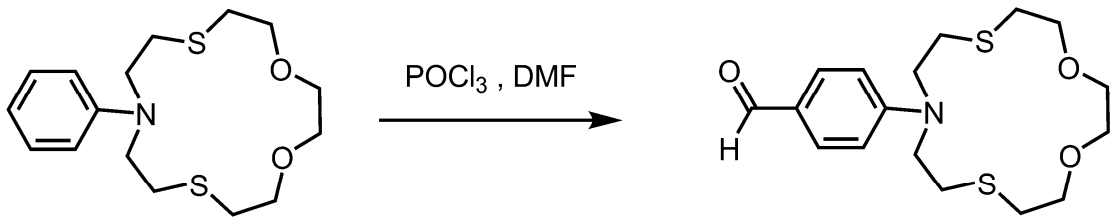
Figure 22. Synthesis of Compound **23**

2.2.3 Synthesis of 4-(1,4-dioxa-7,13-dithia-10-azacyclopentadecan-10-yl) benzaldehyde (24)

To a 5°C solution of DMF (12 mL) was added $POCl_3$ (1.5 mmol, 0.15 mL) within 5 minutes. The mixture was stirred for 30 min; then compound **23** (1.22 mmol, 400 mg) was added and the resulting mixture was heated for 3h at 80°C under argon. The mixture was hydrolized by slow addition of ice-cold water and then neutralized with 5M NaOH. The product was extracted with diethyl ether. It was concentrated under vacuum and purified by silica gel chromotography (3 Hexane / 1 EtOAc). (259 mg, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ; 9.80 (s, 1H), 7.70 (d, *J*=8.01 Hz, 2H), 6.62 (d, *J*=8.01 Hz, 2H), 3.73 (t, *J*=5.05 Hz, 4H), 3.60 (m, 8H), 2.84 (t, *J*=5.1 Hz, 4H), 2.69 (t, *J*=5.04 Hz, 4H).

^{13}C NMR (100 MHz, $CDCl_3$) δ; 190.9, 153.2, 134.4, 126.2, 114.2, 76.4, 70.2, 53.7, 32.2, 29.5



23 **24**

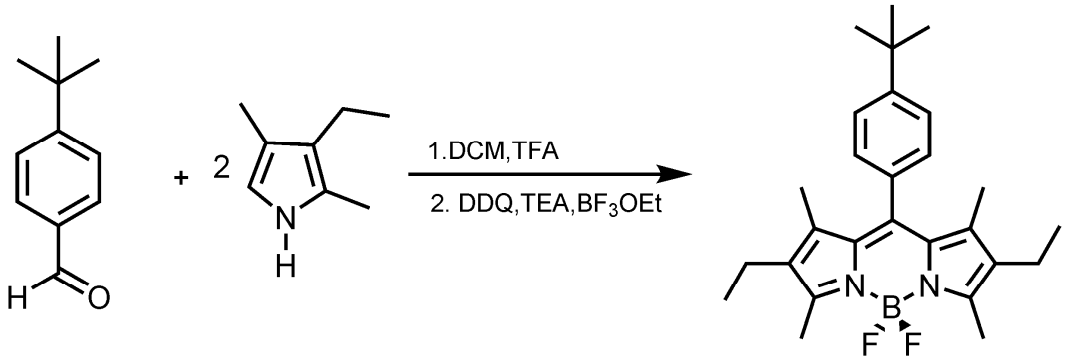
Figure 23. Synthesis of Compound **24**

2.2.4 Synthesis of 2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-(4-terti-arybutyl phenyl)-4-bora-3a,4a-diaza-s-indacene (25)

4-*tert*-butyl benzaldehyde (3.1 mmol, 0.5 mL) and 3-ethyl-2,4-dimethyl pyrrole (6.2 mmol, 0.85 mL) were dissolved in CH_2Cl_2 and refluxed for 3 hours. After 3 h, TFA was added (2-3 drops). Then brownish color was observed. In the second day, DDQ (3.1 mmol, 500 mg), Et_3N (5 mL) and BF_3OEt_2 (5 mL) were added and dark green color was observed. Crude product was washed with water 3 times and dried over Na_2SO_4 and concentrated in vacuum. The product was purified by silica gel column chromotography (2 $CHCl_3$/ 1 Hexane). Orange solid (540 mg, 40%).

^{1}H NMR (400 MHz, $CDCl_3$) δ; 7.39 (d, *J*=8.1 Hz, 2H), 7.11 (d, *J*=8.2 Hz, 2H), 2.40 (s, 6H), 2.22 (m, *J*=7.89 Hz, 4H), 1.29 (s, 9H), 1.18 (s, 6H), 0.9 (t, *J*=7.6 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ; 153.2, 152.2, 140.4, 138.2, 132.2, 130.0, 126.4, 124.0, 34.2, 30.9, 16.7, 16.5, 14.0, 11.9, 11.0



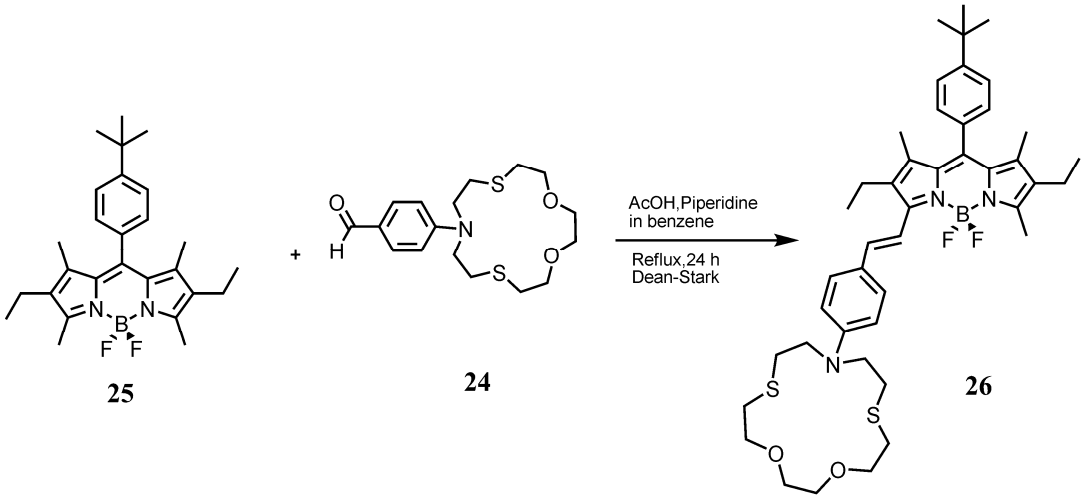
25

Figure 24. Synthesis of Compound **25**

2.2.5 Synthesis of monostyryl BODIPY (26):

Compound **25** (0.25 mmol, 110 mg) and compound **24** (0.26 mmol, 92 mg) were dissolved in 500 ml CH_2Cl_2, 0.3 ml glacial acetic acid and 0.3 ml piperidine and refluxed for a day. Water from the reaction was removed azeotrapically by heating overnight in a Dean-Stark apparatus. Solvent was removed and the product was concentrated. The purification of the product was done with 1 MeOH / 99 $CHCl_3$. The blue fraction was isolated. (44 mg, 24%)

^{1}H NMR (400 MHz, CDCl3) δ; 7.49 (d, *J*=16.65 Hz, 1H), 7.43-7.39 (m, 4H), 7.24-7.21 (m, 2H), 7.10 (d, *J*=16.65 Hz, 1H), 6.58 (m, 2H), 3.74 (t, *J*=4.70 Hz, 4H), 3.63-3.58 (m, 8H), 2.84 (m, 4H), 2.69 (t, *J*=4.70 Hz, 4H), 2.52-2.45 (m, 5H), 2.22 (q, *J*=7.50 Hz, 2H), 1.32 (s, 9H), 1.23 (s, 3H), 1.19 (s, 3H), 1.07 (t, *J*=7.50 Hz, 3H), 0.92(t, *J*=7.50 Hz, 3H);
^{13}C NMR (100 MHz, $CDCl_3$) δ; 153.2, 137.7, 137.4, 136.6, 135.2, 134.7, 132.1, 131.8, 131.0, 130.5, 128.0, 127.9, 127.6, 125.3, 115.6, 114.9, 111.7, 74.2, 71.7, 51.1, 35.2, 31.4, 31.3, 29.6, 18.4, 16.3, 13.6, 13.0, 11.6, 10.6, 10.3;



25 **24** **26**

Figure 25. Synthesis of Compound **26**

2.2.6 Synthesis of distyryl BODIPY: (27)

Compound **25** (0.25 mmol, 110 mg) and compound **24** (0.50 mmol, 184 mg) were dissolved in 500 ml CH_2Cl_2, 0.5 ml glacial acetic acid and 0.5 ml piperidine and refluxed for a day. Any water from the reaction, was removed azeotrapically by heating overnight in a Dean-Stark apparatus. Solvent was removed and the product was concentrated. The purification of the product was done with 1 MeOH / 99 $CHCl_3$ and then EtOAc. The green fraction was isolated. (60 mg, 25%)

^{1}H NMR (400 MHz, CDCl3) δ; 7.54 (d, *J*=16.65 Hz, 2H), 7.41 (d, *J*=8.1 Hz, 4H), 7.40 (d, *J*=8.6 Hz, 2H), 7.14 (d, *J*=8.2 Hz, 2H), 7.13-7.06 (m, 2H), 6.61 (d, *J*=8.6 Hz, 4H) 3.74 (t, *J*=4.92 Hz, 4H), 3.70-3.52 (m, 16 H), 2.84 (t, *J*=8.0 Hz ,8H), 2.69 (t, *J*=5.0 Hz, 8H), 2.52-2.45 (q, *J*=7.2 Hz 4H), 1.30 (s, 9H), 1.23 (s, 6H), 1.09 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ; 152.2, 150.3, 147.2, 138.1, 136.9, 135.5, 133.4, 133.2, 133.0, 129.0, 128.5, 126.1, 125.6, 116.1, 111.9, 74.2, 70.7, 52.0, 34.7, 31.4, 31.3, 30.8, 29.6, 18.4, 14.0, 11.6

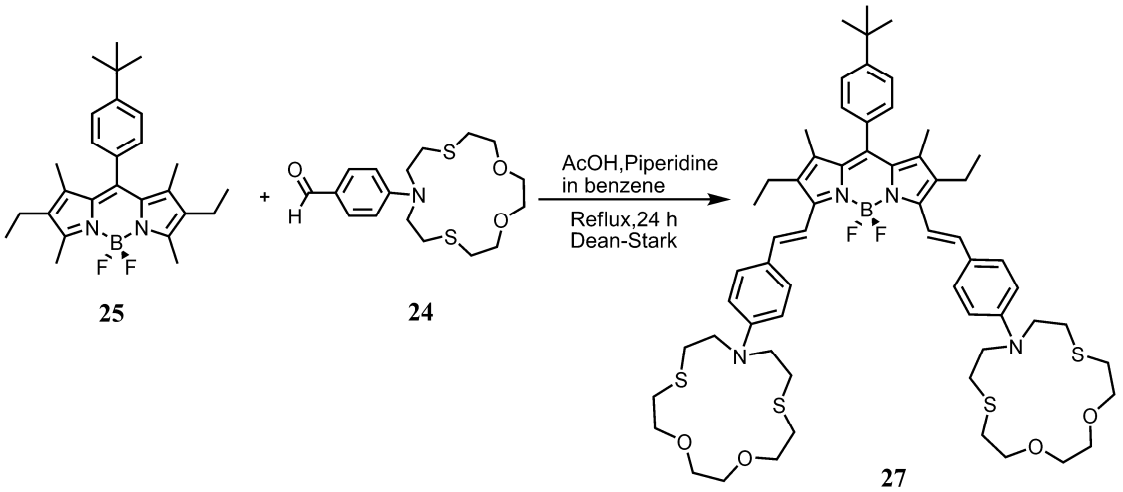


Figure 26. Synthesis of Compound **27**

CHAPTER 3

RESULTS AND DISCUSSION

Designing of fluorescent chemosensor is an active field of supramolecular chemistry since it has many benefits in analytical and environmental chemistry and in cell physiology. Moreover, chemosensors have selectivity and sensitivity when they contact with the target molecular or ionic species. Thus, boradiazaindacenes have become the fluorophore of choice in many chemosensor designs recently. They have exceptional properties as fluorophores and also they have a remarkable rich chemistry.

In order to obtain a cation responsive chemosensor, we chose dithiaazacrown substituted benzaldehyde which is selective for Hg(II) ions in a range of solvents. We made titration experiments for compound **27**. Stock solutions are prepared in $CHCl_3$ solution, the concentration of the dye are kept at 10 μM. Stock solutions of the cations are prepared by using the perchlorate salts as their $CHCl_3$ solutions, then diluted with $CHCl_3$ to obtain desired concentration of the cation. Bodipy dye is titrated with Hg(II) at increasing concentrations (0, 10, 20, 50, 250, 500, 1000, 2500 μM).

Our compound **27** has two distyryl units. As expected, the absorbance spectra of compound **27** show two prominent peaks in the bound form; these are 560 nm and 650 nm respectively (Figure 27). Compound **27** was saturated with Hg(II) ion; therefore, binding of it to the 2 receptor parts gave absorbance at 560 nm. On the other hand, mono binding of Hg (II) ion to the dithiaazacrown part gave absorbance at 650 nm. As a result, binding of Hg (II) ion to receptor parts of the dye causes approximately 160 nm blue shift.

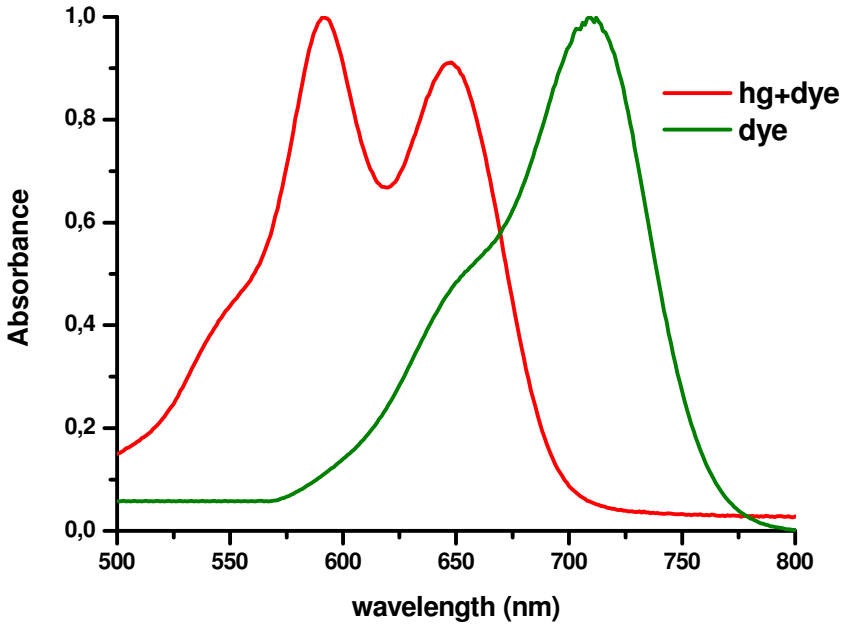


Figure 27. Normalized absorbance spectra of compound **27** with Hg and without Hg in $CHCl_3$; the concentration of chemosensor is 10.0 μM and the concentration of the Hg(II) is 250 μM .

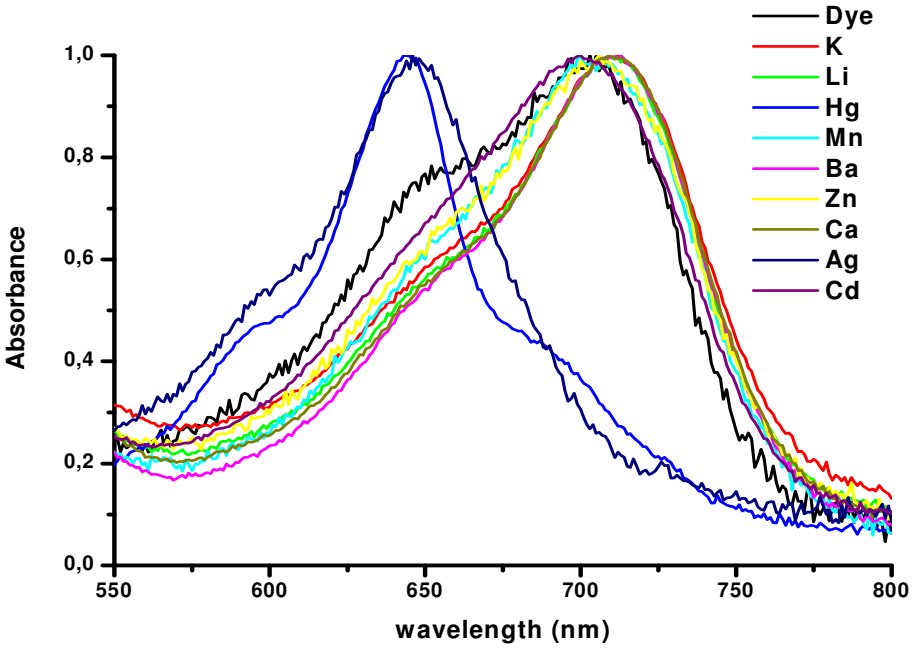


Figure 28. Absorbance spectra of compound **27** in the presence of various cations. (Cation concentration is 1.0 mM).

Among the cations tested, the compound **27** showed high affinity towards Hg(II). Interestingly, Ag(I) shows a similar affinity with the designed dye (Figure 28). This is due to the hard-soft interaction and the shape of the macrocycle. Except for Ag(I) and Hg(II) ions, the cations did not cause any change in the spectra. However, similar responses of Ag(I) and Hg(II) to the dye could not be seen in emission spectra. Dye concentration is kept constant at 10.0 μM, and cation concentration in each solution is 1 mM in the fluorescence measurement (Figure 29).

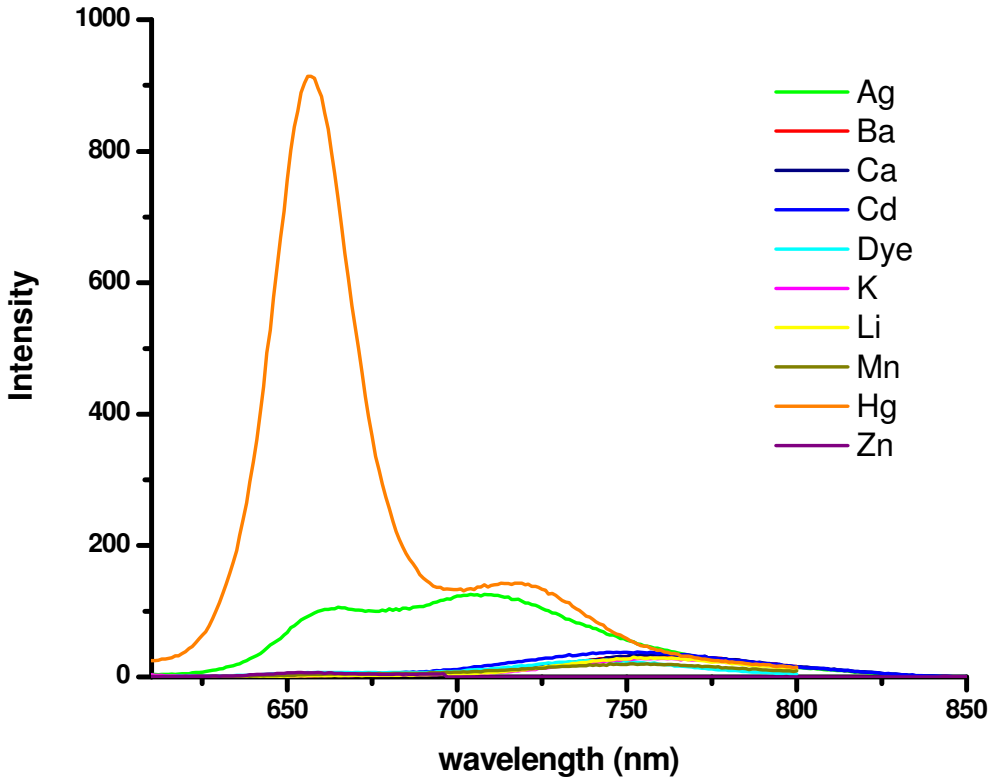


Figure 29. Emission spectra of compound **27** in the presence of various cations (cation concentrations 1.0 mM). Excitation wavelength 600 nm with 5 nm slit widths, and spectra were corrected. The concentration of the chemosensor was 10.0 μM.

According to Figure 29; Hg(II) gave a remarkable fluorescence ; however, Ag (I) did not increase the intensity.

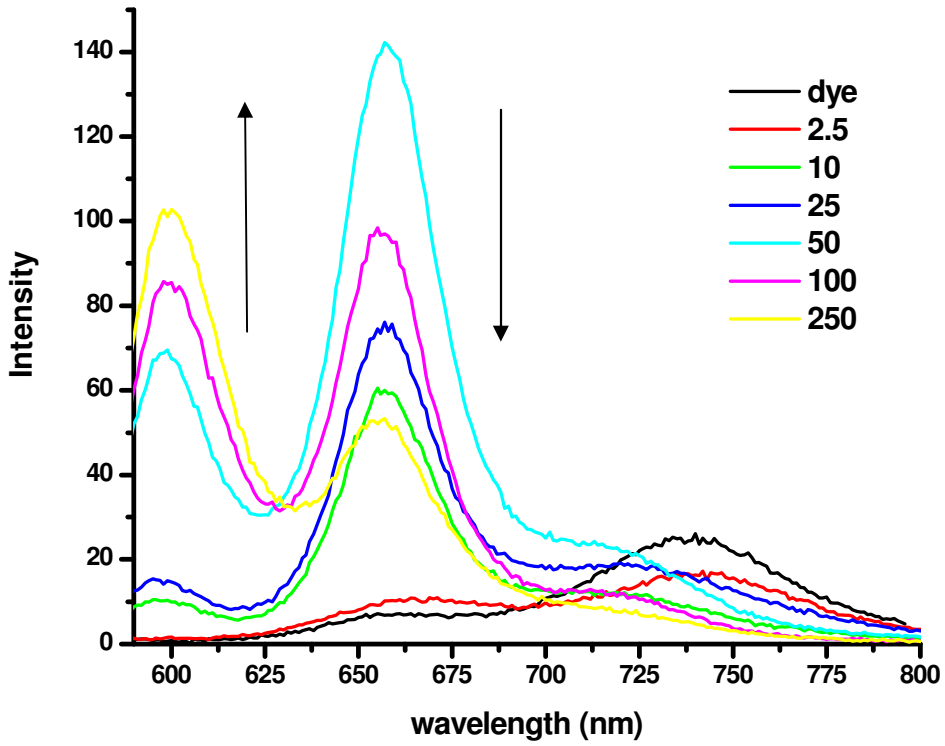


Figure 30. Emission spectra of compound **27** in the presence of increasing Hg(II) concentrations (0, 25, 100, 250, 500, 1000, 2500 μM). Excitation wavelength 580 nm with 5 nm slit widths, and spectra were corrected. The concentration of the chemosensor was 10.0 μM.

As seen in Figure 30, the emission of the chemosensor without Hg(II) is approximately at 730 nm. Increasing the concentration of the Hg (II) ions causes blue shift. 2.5 mM of Hg (II) gave the most blue shift. The intensity of the chemosensor is directly proportional to the increase in the concentration of the cation. It is observed that the intensity at 600 nm increases whereas the intensity at 650 nm firstly increases then decreases.

CHAPTER 4

CONCLUSION

Our aim was to synthesize and characterize a near-IR emitting fluorescent chemosensor for Hg (II) ion. Considering the selectivity of the metal ligands, a thio based crown ether was chosen. In literature, there are many examples of rationally designed fluorescent chemosensors where selectivity of certain ligands was expoited. However; such near-IR fluorescent chemosensors are very rare. Near-IR emission is an important property for this study; it is because light at this wavelength, is much less scattered by biological media, and there are especially sensitive detectors for photons in this region of the spectrum. In addition, autofluorescence (a.k.a., background fluorescence) is much weaker, because there are very few biomolecules emitting in this region. Until this study, there was no example of any near-IR fluorescent chemosensors for any kind of metal ions, with BODIPY unit as a fluorescent part. Another property of the target chemosensors is its selectivity for Hg ions. Hg^{2+} is considered as one of the most dangerous cations for the environment, because it is widely distributed in air, water, and soil. Furthermore, mercury can accumulate in human body and initiates a wide variety of diseases even in a low concentration. The absorbance and fluorescence spectrum of the chemosensor shows that we reached our goal. The addition of Hg(II) to the chemosensor shifts the dye absorption peak wavelength towards to the blue region by approximately 160 nm, and results in an increase the emission intensity of the dye at this wavelength. Since chemosensor has two metal binding arms, stoichiometric addition of the metal ions to the chemosensor shows different shifts 1:1 addition shows a 630 nm emitting complex and 1:2 addition results in a complex of different stoichiometry with a peak emission wavelength of 580 nm.

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APPENDIX

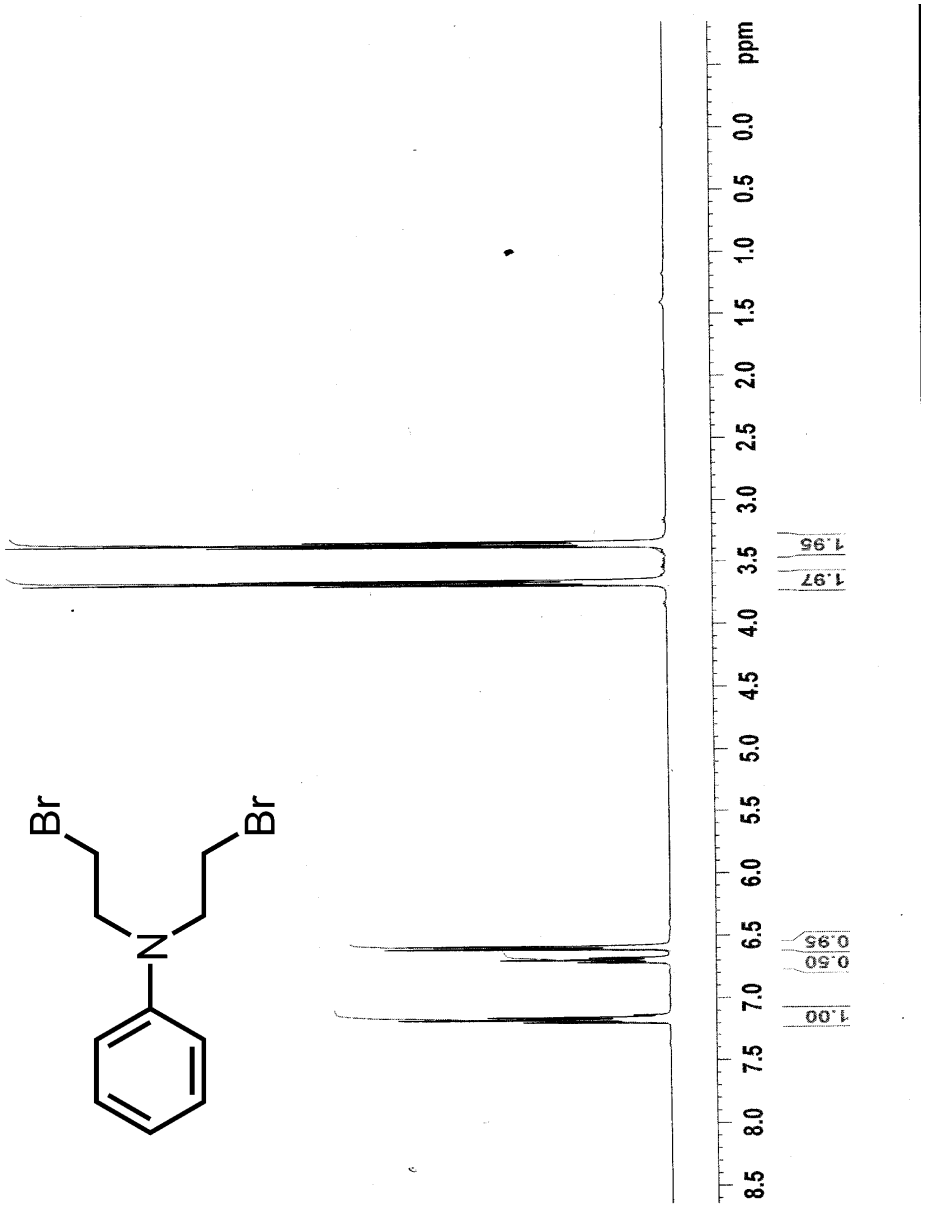

Figure 31. ^{1}H spectrum of compound **22**

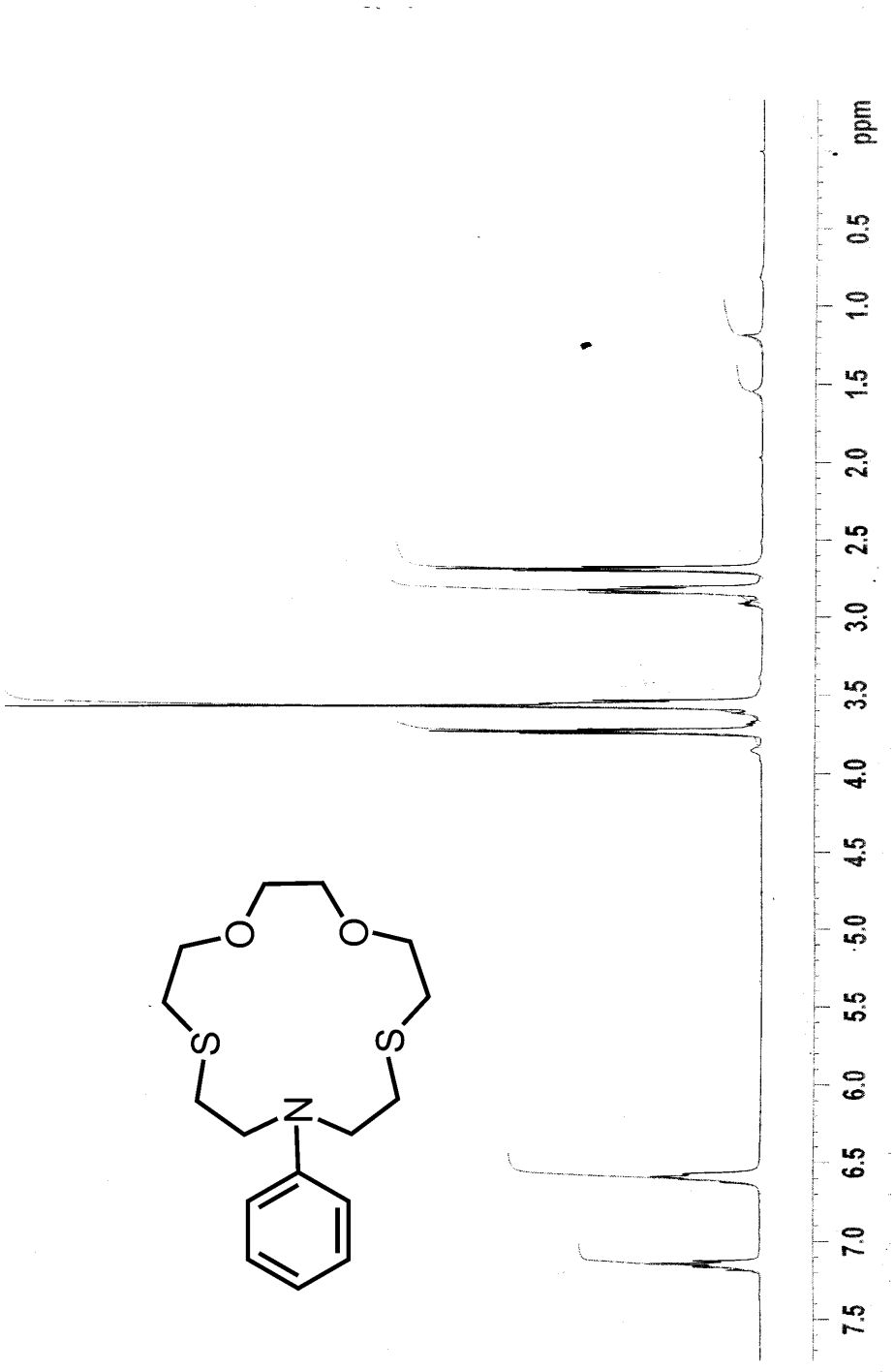

Figure 32. ^{1}H spectrum of compound **23**

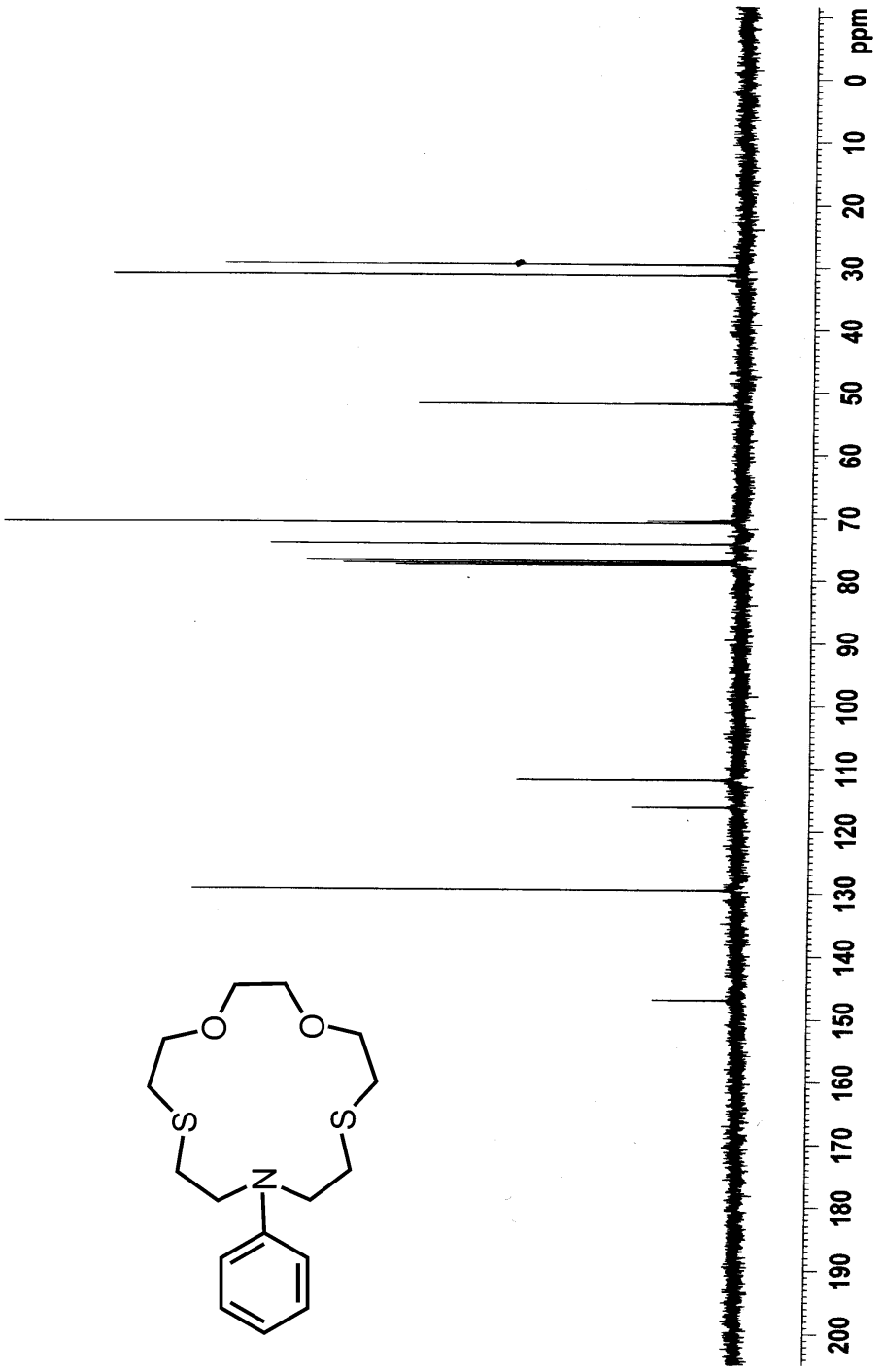

Figure 33. ^{13}C spectrum of compound **23**

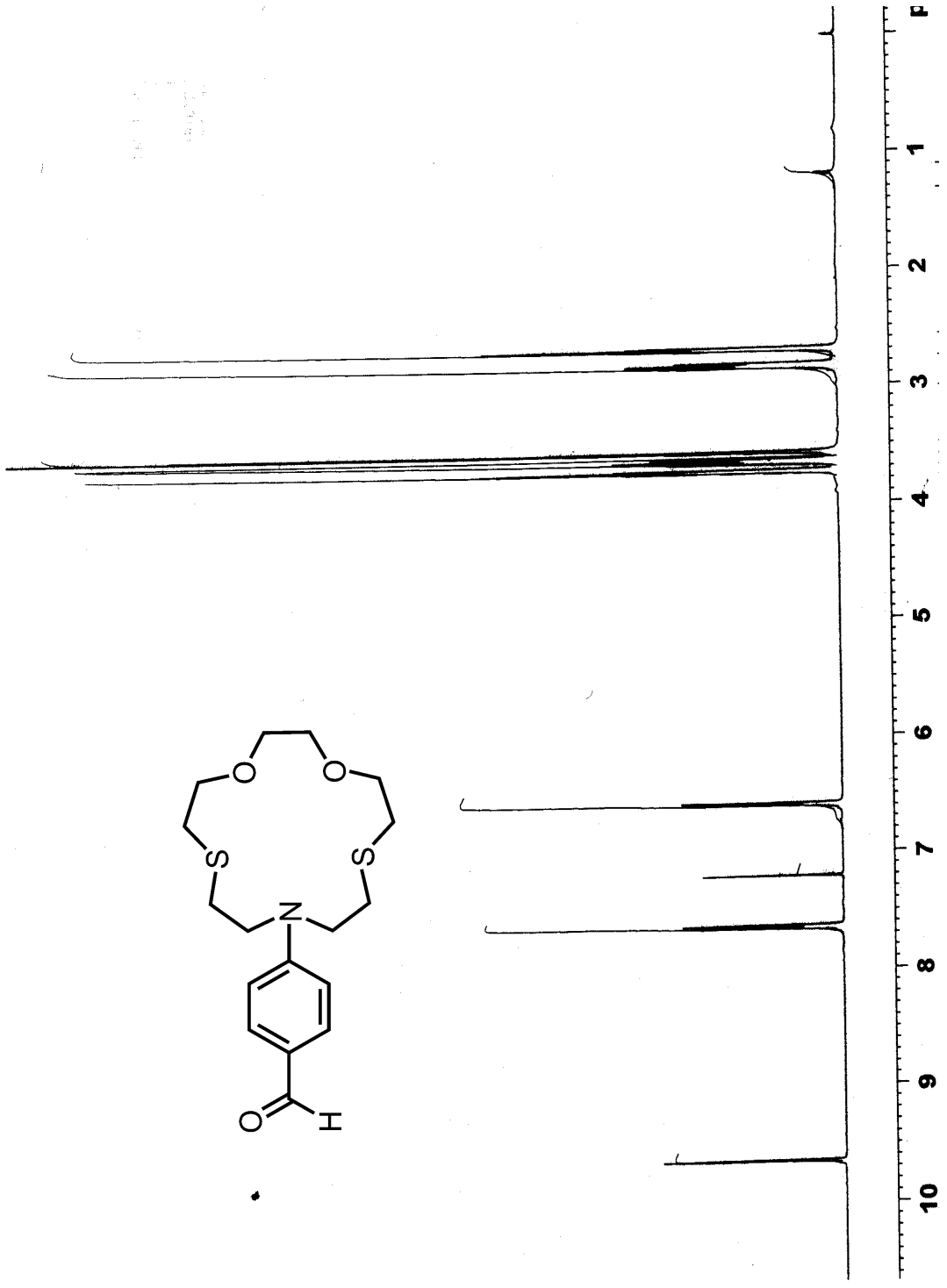

Figure 34. ^{1}H spectrum of compound **24**

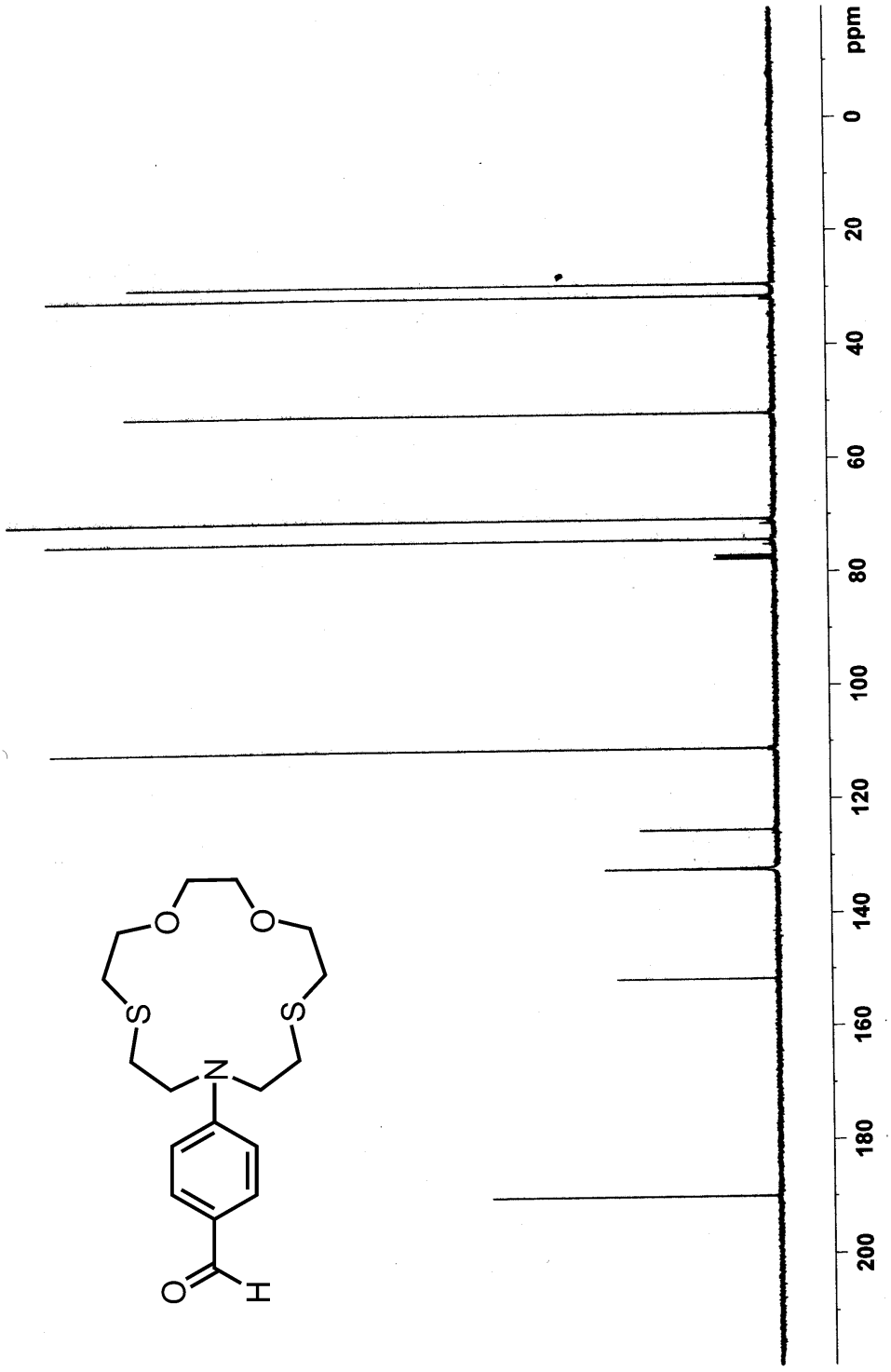

Figure 35. ^{13}C spectrum of compound **24**

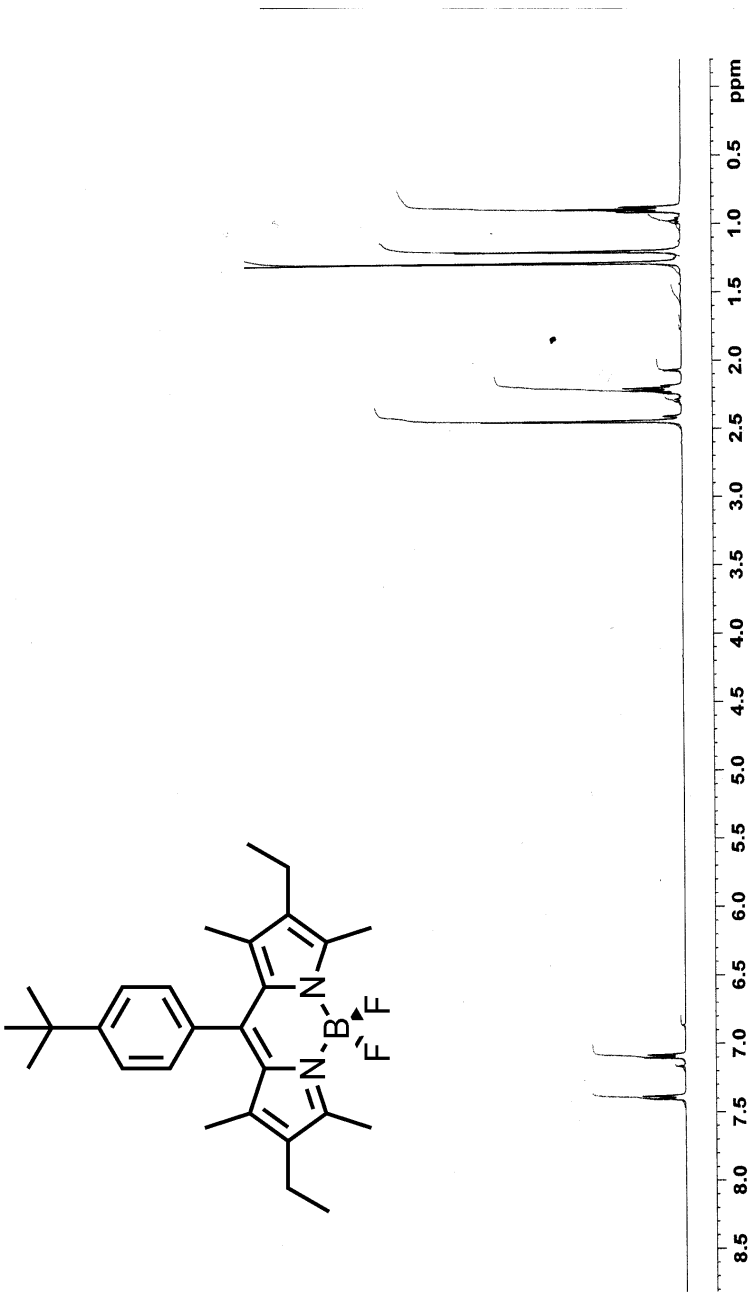

Figure 36. ^{1}H spectrum of compound **25**

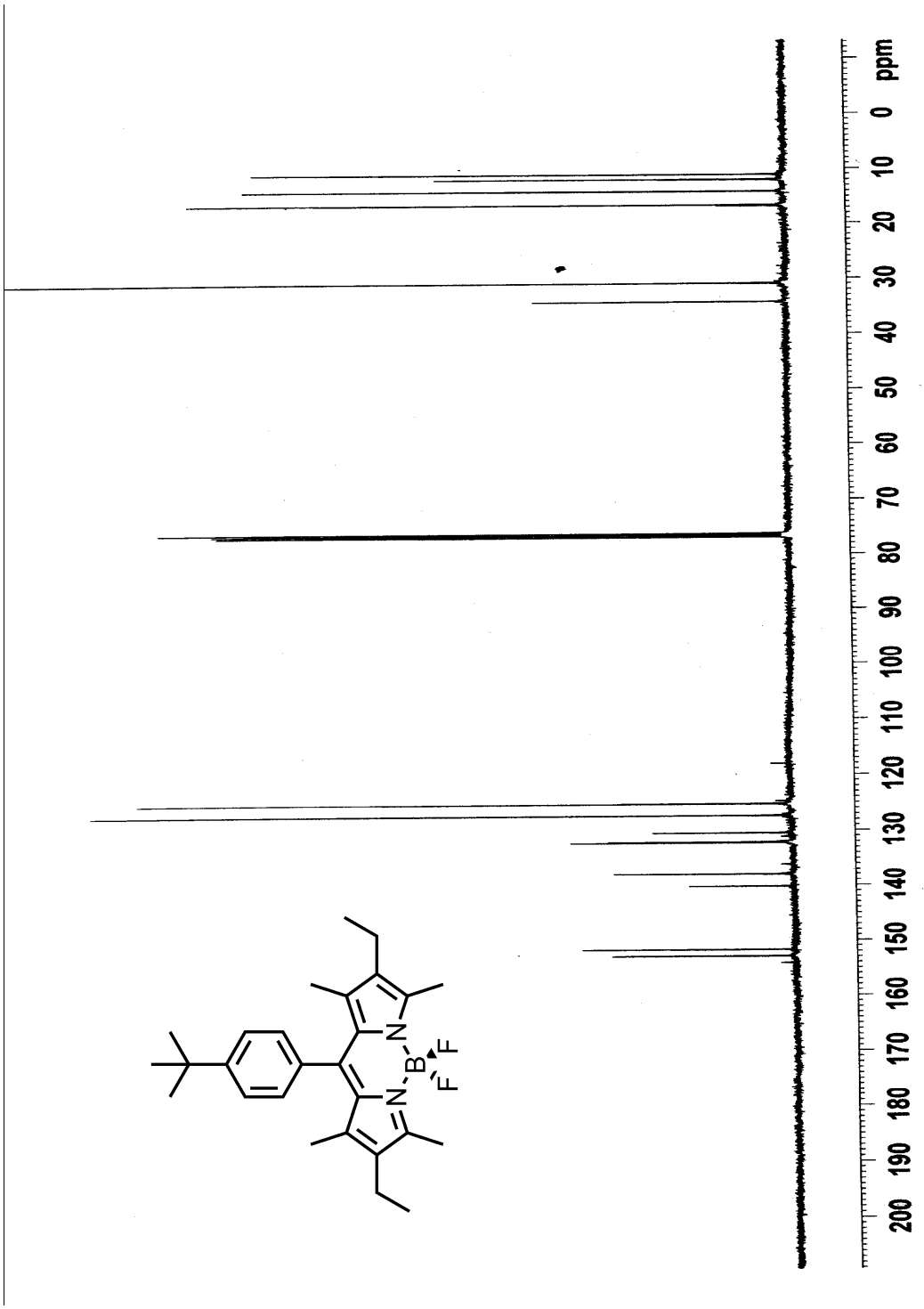

Figure 37. ^{13}C spectrum of compound **25**

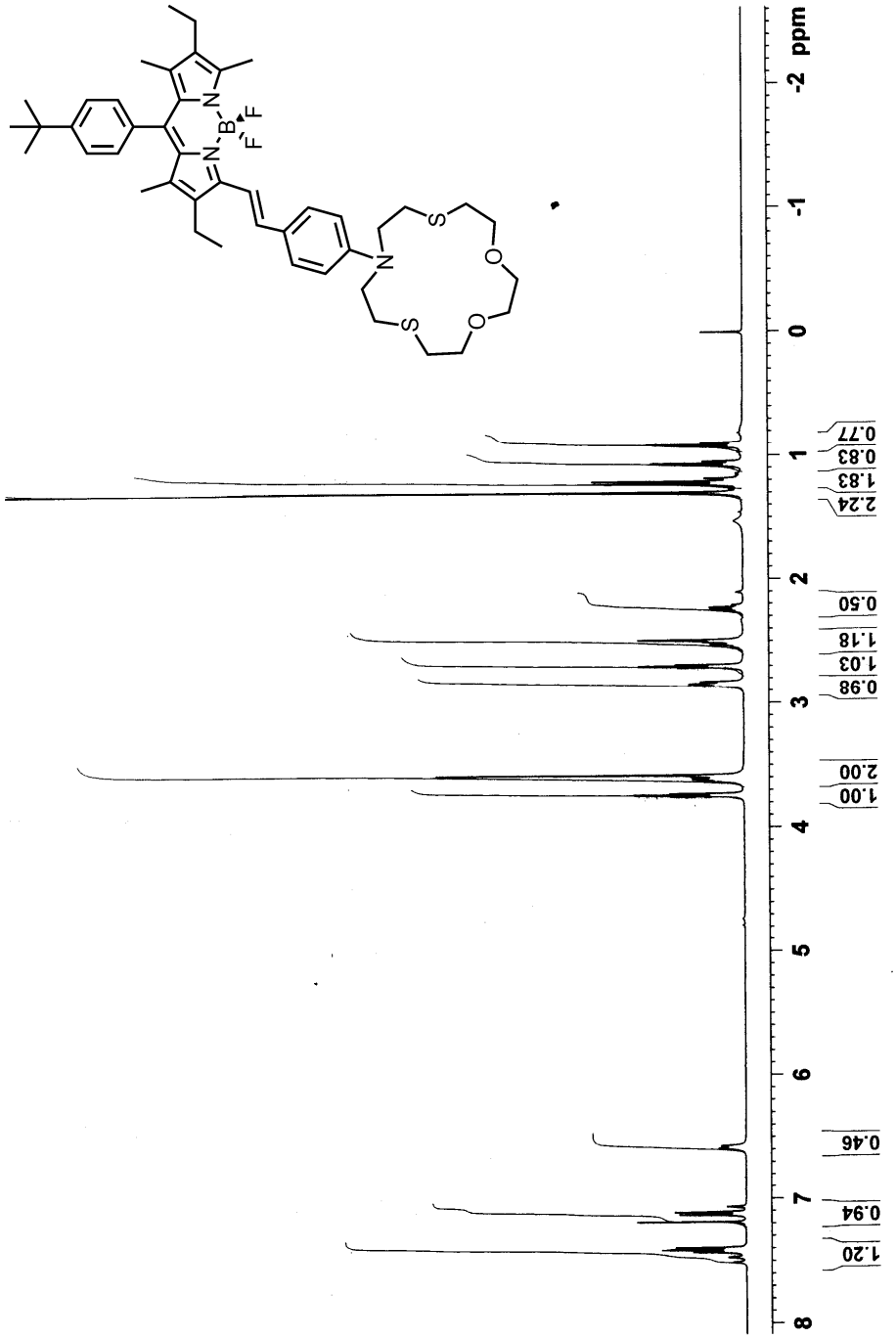

Figure 38. ^{1}H spectrum of compound **26**

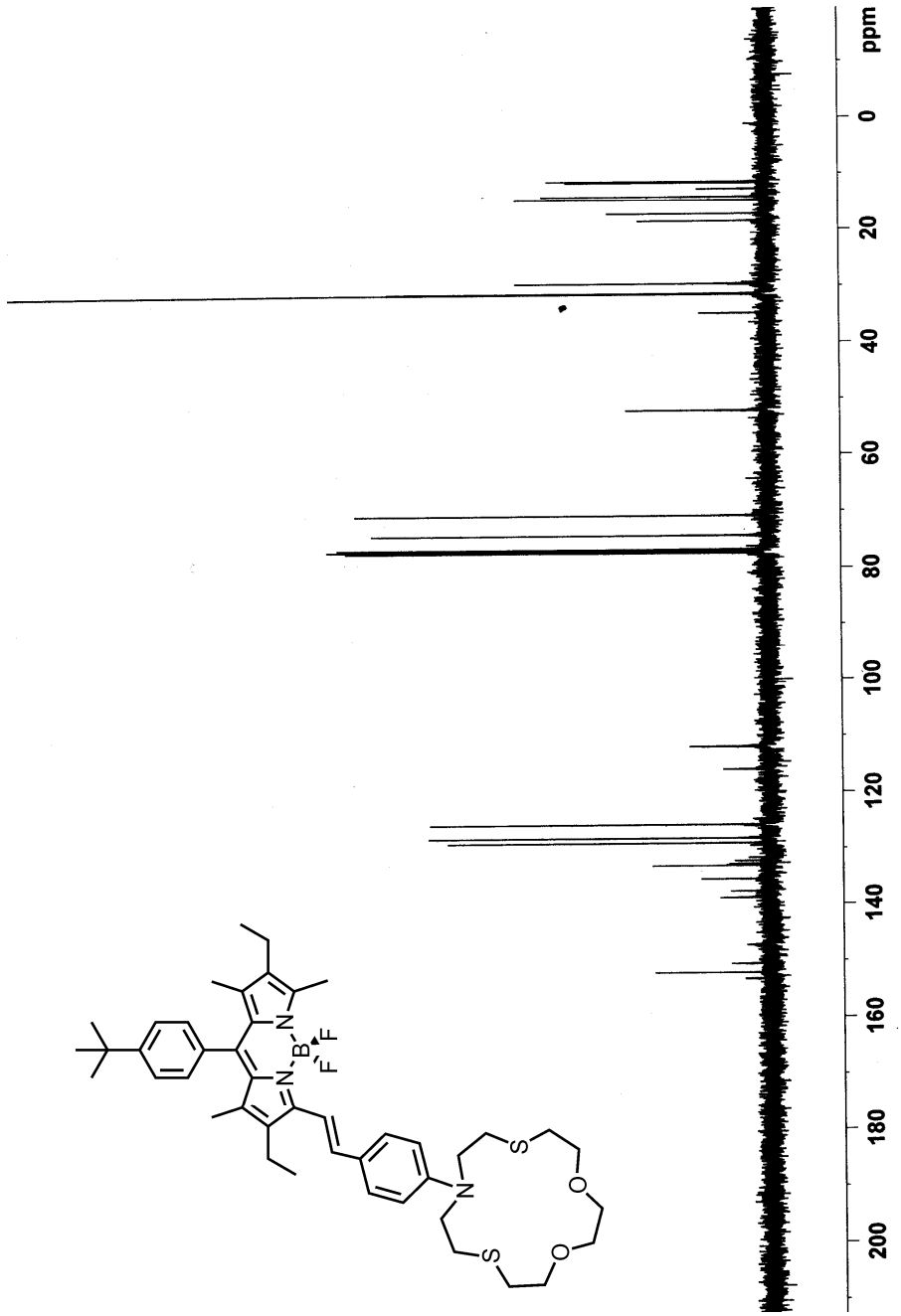

Figure 39. ^{13}C spectrum of compound **26**

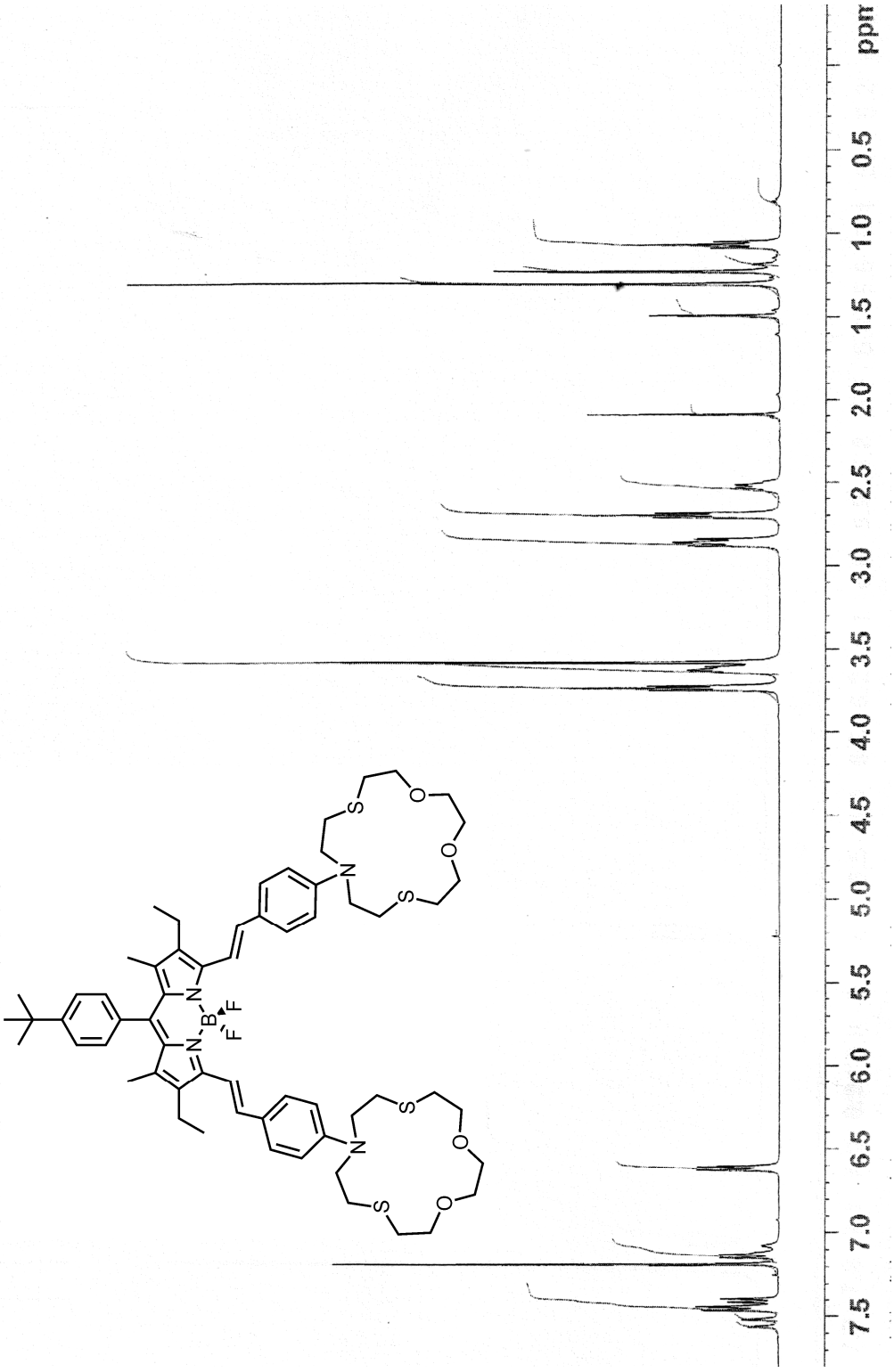

Figure 40. ^{1}H spectrum of compound **27**

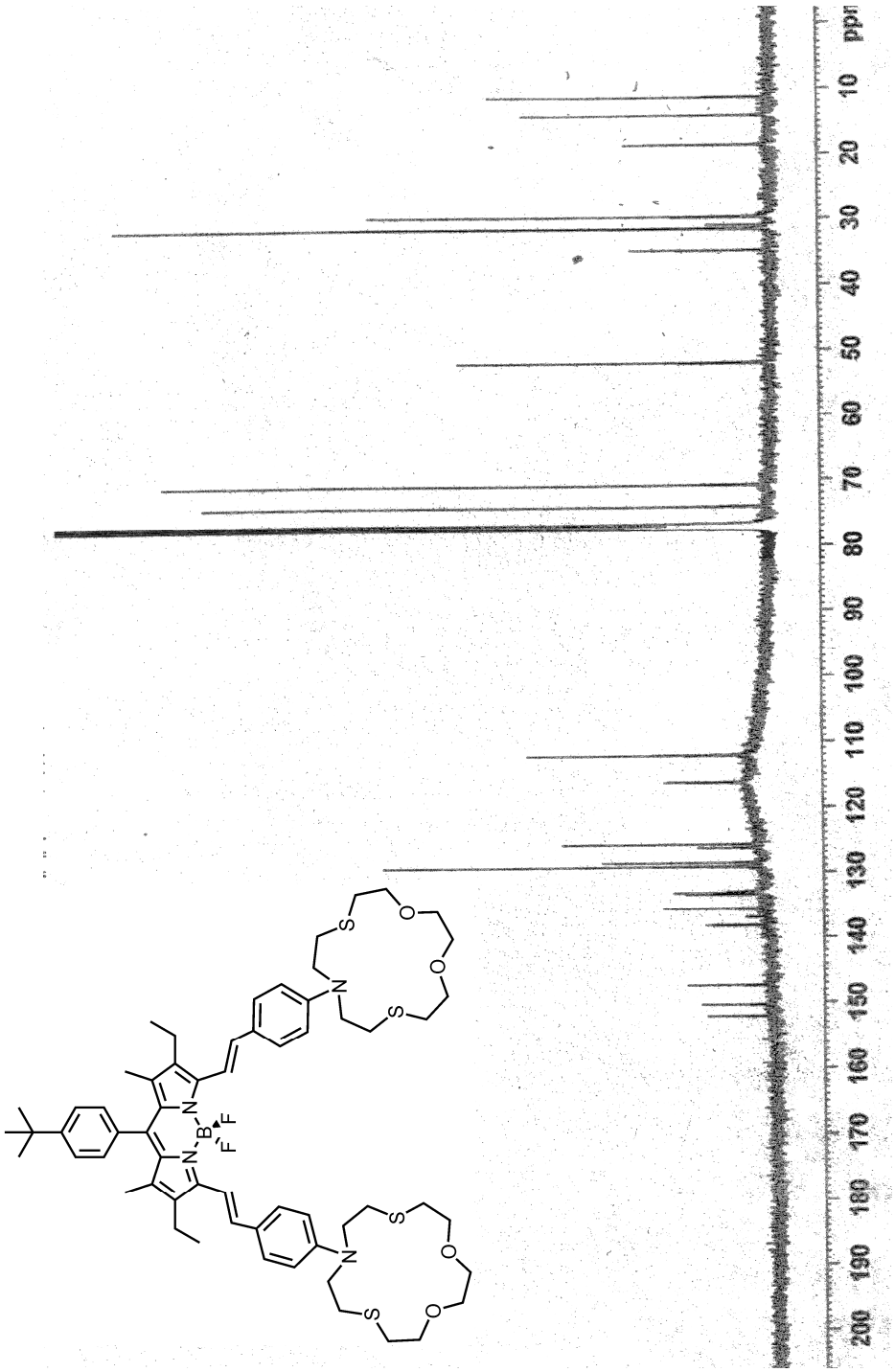

Figure 41. ^{13}C spectrum of compound **27**